Encyclopedia of VIROLOGY

Third Edition



Editors-in-Chief
B.W.J. Mahy
M.H.V. van Regenmortel



Encyclopedia of
VIROLOGY

THIRD EDITION

ENCYCLOPEDIA OF
VIROLOGY

THIRD EDITION

EDITORS-IN-CHIEF

Dr BRIAN W J MAHY
and
Dr MARC H V VAN REGENMORTEL



ELSEVIER

AMSTERDAM • BOSTON • HEIDELBERG • LONDON • NEW YORK • OXFORD
PARIS • SAN DIEGO • SAN FRANCISCO • SINGAPORE • SYDNEY • TOKYO
Academic Press is an imprint of Elsevier



ACADEMIC
PRESS

Academic Press is an imprint of Elsevier
Linacre House, Jordan Hill, Oxford, OX2 8DP, UK
525 B Street, Suite 1900, San Diego, CA 92101-4495, USA

Copyright © 2008 Elsevier Inc. All rights reserved

The following articles are US government works in the public domain and are not subject to copyright: Bovine Viral Diarrhea Virus, Coxsackieviruses, Prions of Yeast and Fungi, Human Respiratory Syncytial Virus, Fish Rhabdoviruses, Varicella-Zoster Virus: General Features, Viruses and Bioterrorism, Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus, Metaviruses, Crimean-Congo Hemorrhagic Fever Virus and Other Nairoviruses, AIDS: Global Epidemiology, Papaya Ringspot Virus, Transcriptional Regulation in Bacteriophage.

Nepovirus, Canadian Crown Copyright 2008

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means electronic, mechanical, photocopying, recording or otherwise without the prior written permission of the publisher

Permissions may be sought directly from Elsevier's Science & Technology Rights Department in Oxford, UK: phone (+44) (0) 1865 843830; fax (+44) (0) 1865 853333; email: permissions@elsevier.com. Alternatively you can submit your request online by visiting the Elsevier web site at (http://elsevier.com/locate/permission), and selecting *Obtaining permission to use Elsevier material*

Notice
No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the material herein. Because of rapid advances in the medical sciences, in particular, independent verification of diagnoses and drug dosages should be made

British Library Cataloguing in Publication Data
A catalogue record for this book is available from the British Library

Library of Congress Catalog Number: 200892260

ISBN: 978-0-12-373935-3

For information on all Elsevier publications
visit our website at books.elsevier.com

PRINTED AND BOUND IN SLOVENIA
08 09 10 11 10 9 8 7 6 5 4 3 2 1

Working together to grow
libraries in developing countries
www.elsevier.com | www.bookaid.org | www.sabre.org
ELSEVIER BOOK AID International Sabre Foundation

EDITORS-IN-CHIEF

Brian W J Mahy MA PhD ScD DSc
Senior Scientific Advisor,
Division of Emerging Infections and Surveillance Services,
Centers for Disease Control and Prevention,
Atlanta GA, USA

Marc H V Van Regenmortel PhD
Emeritus Director at the CNRS,
French National Center for Scientific Research,
Biotechnology School of the University of Strasbourg,
Illkirch, France

ASSOCIATE EDITORS

Dennis H Bamford, Ph.D.
Department of Biological and Environmental Sciences
and Institute of Biotechnology, Biocenter 2,
P.O. Box 56 (Viikinkaari 5),
00014 University of Helsinki,
Finland

Charles Calisher, B.S., M.S., Ph.D.
Arthropod-borne and Infectious Diseases Laboratory
Department of Microbiology, Immunology and Pathology
College of Veterinary Medicine and Biomedical Sciences
Colorado State University
Fort Collins
CO 80523
USA

Andrew J Davison, M.A., Ph.D.
MRC Virology Unit
Institute of Virology
University of Glasgow
Church Street
Glasgow G11 5JR
UK

Claude Fauquet
ILTAB/Donald Danforth Plant Science Center
975 North Warson Road
St. Louis, MO 63132

Said Ghabrial, B.S., M.S., Ph.D.
Plant Pathology Department
University of Kentucky
201F Plant Science Building
1405 Veterans Drive
Lexington
KY 4050546-0312
USA

Eric Hunter, B.Sc., Ph.D.
Department of Pathology and Laboratory Medicine, and
Emory Vaccine Center
Emory University
954 Gatewood Road NE
Atlanta Georgia 30329
USA

Robert A. Lamb, Ph.D., Sc.D.
Department of Biochemistry,
Molecular Biology and Cell Biology
Howard Hughes Medical Institute
Northwestern University
2205 Tech Dr.
Evanston
IL 60208-3500
USA

Olivier Le Gall
IPV, UMR GDPP, IBVM,
INRA Bordeaux-Aquitaine, BP 81,
F-33883 Villenave d'Ornon Cedex
FRANCE

Vincent Racaniello, Ph.D.
Department of Microbiology
Columbia University
New York, NY 10032
USA

David A. Theilmann, Ph.D., B.Sc., M.Sc
Pacific Agri-Food Research Centre
Agriculture and Agri-Food Canada
Box 5000, 4200 Highway 97
Summerland
BC V0H 1Z0
Canada

H. Josef Vetten, Ph.D.
Julius Kuehn Institute, Federal Research Centre for
Cultivated Plants (JKI)
Messeweg 11-12
38104 Braunschweig
Germany

Peter J Walker, B.Sc., Ph.D.
CSIRO Livestock Industries
Australian Animal Health Laboratory (AAHL)
Private Bag 24
Geelong
VIC 3220
Australia

PREFACE

This third edition of the *Encyclopedia of Virology* is being published nine years after the second edition, a period which has seen enormous growth both in our understanding of virology and in our recognition of the viruses themselves, many of which were unknown when the second edition was prepared. Considering viruses affecting human hosts alone, the worldwide epidemic of severe acute respiratory syndrome (SARS), caused by a previously unknown coronavirus, led to the discovery of other human coronaviruses such as HKU1 and NL63. As many as seven chapters are devoted to the AIDS epidemic and to human immunodeficiency viruses. In addition, the development of new molecular technologies led to the discovery of viruses with no obvious disease associations, such as torque-teno virus (one of the most ubiquitous viruses in the human population), human bocavirus, human metapneumovirus, and three new human polyomaviruses.

Other new developments of importance to human virology have included the introduction of a virulent strain of West Nile virus from Israel to North America in 1999. Since that time the virus has become established in mosquito, bird and horse populations throughout the USA, the Caribbean and Mexico as well as the southern regions of Canada.

As in the two previous editions, we have tried to include information about all known species of virus infecting bacteria, fungi, invertebrates, plants and vertebrates, as well as descriptions of related topics in virology such as antiviral drug development, cell- and antibody-mediated immunity, vaccine development, electron microscopy and molecular methods for virus characterization and identification. Many chapters are devoted to the considerable economic importance of virus diseases of cereals, legumes, vegetable crops, fruit trees and ornamentals, and new approaches to control these diseases are reviewed.

General issues such as the origin, evolution and phylogeny of viruses are also discussed as well as the history of the different groups of viruses.

To cover all these subjects and new developments, we have had to increase the size of the Encyclopedia from three to five volumes.

Throughout this work we have relied upon the 8th Report of the International Committee on Taxonomy of Viruses published in 2005, which lists more than 6000 viruses classified into some 2000 virus species distributed among more than 390 different genera and families. In recent years the criteria for placing viruses in different taxa have shifted away from traditional serological methods and increasingly rely upon molecular techniques, particularly the nucleotide sequence of the virus genome. This has changed many of the previous groupings of viruses, and is reflected in this third edition.

Needless to say, a work of this magnitude has involved many expert scientists, who have given generously of their time to bring it to fruition. We extend our grateful thanks to all contributors and associate editors for their excellent and timely contributions.

Brian W J Mahy
Marc H V van Regenmortel

HOW TO USE THE ENCYCLOPEDIA

Structure of the Encyclopedia

The major topics discussed in detail in the text are presented in alphabetical order (see the Alphabetical Contents list which appears in all five volumes).

Finding Specific Information

Information on specific viruses, virus diseases and other matters can be located by consulting the General Index at the end of Volume 5.

Taxonomic Groups of Viruses

For locating detailed information on the major taxonomic groups of viruses, namely virus genera, families and orders, the Taxonomic Index in Volume 5 (page. . .) should be consulted.

Further Reading sections

The articles do not feature bibliographic citations within the body of the article text itself. The articles are intended to be a first introduction to the topic, or a 'refresher', readable from beginning to end without referring the reader outside of the encyclopedia itself. Bibliographic references to external literature are grouped at the end of each article in a Further Reading section, containing review articles, 'seminal' primary articles and book chapters. These point users to the next level of information for any given topic.

Cross referencing between articles

The "See also" section at the end of each article directs the reader to other entries on related topics. For example. The entry *Lassa, Junin, Machupo and Guanarito Viruses* includes the following cross-references:

See also: Lymphocytic Choriomeningitis Virus: General Features.

CONTRIBUTORS

S T Abedon
The Ohio State University, Mansfield, OH, USA

G P Accotto
Istituto di Virologia Vegetale CNR, Torino, Italy

H-W Ackermann
Laval University, Quebec, QC, Canada

G Adam
Universität Hamburg, Hamburg, Germany

M J Adams
Rothamsted Research, Harpenden, UK

C Adams
University of Duisburg–Essen, Essen, Germany

E Adderson
St. Jude Children's Research Hospital, Memphis, TN, USA

S Adhya
National Institutes of Health, Bethesda, MD, USA

C L Afonso
Southeast Poultry Research Laboratory, Athens, GA, USA

P Ahlquist
University of Wisconsin – Madison, Madison, WI, USA

G M Air
University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

D J Alcendor
Johns Hopkins School of Medicine, Baltimore, MD, USA

J W Almond
sanofi pasteur, Lyon, France

I Amin
National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

J Angel
Pontificia Universidad Javeriana, Bogota, Republic of Colombia

C Apetrei
Tulane National Primate Research Center, Covington, LA, USA

B M Arif
Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada

H Attoui
Faculté de Médecine de Marseilles, Etablissement Français Du Sang, Marseilles, France

H Attoui
Université de la Méditerranée, Marseille, France

H Attoui
Institute for Animal Health, Pirbright, UK

L Aurelian
University of Maryland School of Medicine, Baltimore, MD, USA

L A Babiuk
University of Alberta, Edmonton, AB, Canada

S Babiuk
National Centre for Foreign Animal Disease, Winnipeg, MB, Canada

A G Bader
The Scripps Research Institute, La Jolla, CA, USA

S C Baker
Loyola University of Chicago, Maywood, IL, USA

T S Baker
University of California, San Diego, La Jolla, CA, USA

J K H Bamford
University of Jyväskylä, Jyväskylä, Finland

Y Bao
National Institutes of Health, Bethesda, MD, USA

M Bar-Joseph
The Volcani Center, Bet Dagan, Israel

H Barker
Scottish Crop Research Institute, Dundee, UK

A D T Barrett
University of Texas Medical Branch, Galveston, TX, USA

J W Barrett
The University of Western Ontario, London, ON, Canada

T Barrett
Institute for Animal Health, Pirbright, UK

R Bartenschlager
University of Heidelberg, Heidelberg, Germany

N W Bartlett
Imperial College London, London, UK

S Basak
University of California, San Diego, CA, USA

C F Basler
Mount Sinai School of Medicine, New York, NY, USA

T Basta
Institut Pasteur, Paris, France

D Baxby
University of Liverpool, Liverpool, UK

P Beard
Imperial College London, London, UK

M N Becker
University of Florida, Gainesville, FL, USA

J J Becnel
Agriculture Research Service, Gainesville, FL, USA

K L Beemon
Johns Hopkins University, Baltimore, MD, USA

E D Belay
Centers for Disease Control and Prevention, Atlanta, GA, USA

M Benkő
Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary

M Bennett
University of Liverpool, Liverpool, UK

M Bergoin
Université Montpellier II, Montpellier, France

H U Bernard
University of California, Irvine, Irvine, CA, USA

K I Berns
University of Florida College of Medicine, Gainesville, FL, USA

P Biagini
Etablissement Français du Sang Alpes-Méditerranée, Marseilles, France

P D Bieniasz
Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY, USA

Y Bigot
University of Tours, Tours, France

C Billinis
University of Thessaly, Karditsa, Greece

R F Bishop
Murdoch Childrens Research Institute Royal Children's Hospital, Melbourne, VIC, Australia

B A Blacklaws
University of Cambridge, Cambridge, UK

C D Blair
Colorado State University, Fort Collins, CO, USA

S Blanc
INRA–CIRAD–AgroM, Montpellier, France

R Blawid
Institute of Plant Diseases and Plant Protection, Hannover, Germany

G W Blissard
Boyce Thompson Institute at Cornell University, Ithaca, NY, USA

S Blomqvist
National Public Health Institute (KTL), Helsinki, Finland

J F Bol
Leiden University, Leiden, The Netherlands

J-R Bonami
CNRS, Montpellier, France

L Bos
Wageningen University and Research Centre (WUR), Wageningen, The Netherlands

H R Bose Jr.
University of Texas at Austin, Austin, TX, USA

H Bourhy
Institut Pasteur, Paris, France

P R Bowser
Cornell University, Ithaca, NY, USA

D B Boyle
CSIRO Livestock Industries, Geelong, VIC, Australia

C Bragard
Université Catholique de Louvain, Leuven, Belgium

J N Bragg
University of California, Berkeley, Berkeley, CA, USA

R W Briddon
National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

M A Brinton
Georgia State University, Atlanta, GA, USA

P Britton
Institute for Animal Health, Compton, UK

J K Brown
The University of Arizona, Tucson, AZ, USA

K S Brown
University of Manitoba, Winnipeg, MB, Canada

J Bruenn
State University of New York, Buffalo, NY, USA

C P D Brussaard
Royal Netherlands Institute for Sea Research, Texel, The Netherlands

J J Bugert
Wales College of Medicine, Heath Park, Cardiff, UK

J J Bujarski
Northern Illinois University, DeKalb, IL, USA and Polish Academy of Sciences, Poznan, Poland

R M Buller
Saint Louis University School of Medicine, St. Louis, MO, USA

J P Burand
University of Massachusetts at Amherst, Amherst, MA, USA

J Burgyan
Agricultural Biotechnology Center, Godollo, Hungary

F J Burt
University of the Free State, Bloemfontein, South Africa

S J Butcher
University of Helsinki, Helsinki, Finland

J S Butel
Baylor College of Medicine, Houston, TX, USA

M I Butler
University of Otago, Dunedin, New Zealand

S Bühler
University of Heidelberg, Heidelberg, Germany

P Caciagli
Istituto di Virologia Vegetale – CNR, Turin, Italy

C H Calisher
Colorado State University, Fort Collins, CO, USA

T Candresse
UMR GDPP, Centre INRA de Bordeaux, Villenave d'Ornon, France

A J Cann
University of Leicester, Leicester, UK

C Caranta
INRA, Montfavet, France

G Carlile
CSIRO Livestock Industries, Geelong, VIC, Australia

J P Carr
University of Cambridge, Cambridge, UK

R Carrion, Jr.
Southwest Foundation for Biomedical Research, San Antonio, TX, USA

J W Casey
Cornell University, Ithaca, NY, USA

R N Casey
Cornell University, Ithaca, NY, USA

S Casjens
University of Utah School of Medicine, Salt Lake City, UT, USA

R Cattaneo
Mayo Clinic College of Medicine, Rochester, MN, USA

D Cavanagh
Institute for Animal Health, Compton, UK

A Chahroudi
University of Pennsylvania School of Medicine, Philadelphia, PA, USA

S Chakraborty
Jawaharlal Nehru University, New Delhi, India

T J Chambers
Saint Louis University School of Medicine, St. Louis, MO, USA

Y Chang
University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

J T Chang
Baylor College of Medicine, Houston, TX, USA

D Chapman
Institute for Animal Health, Pirbright, UK

D Chattopadhyay
University of Calcutta, Kolkata, India

M Chen
University of Arizona, Tucson, AZ, USA

J E Cherwa
University of Arizona, Tucson, AZ, USA

V G Chinchar
University of Mississippi Medical Center, Jackson, MS, USA

A V Chintakuntlawar
University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

W Chiu
Baylor College of Medicine, Houston, TX, USA

J Chodosh
University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

I-R Choi
International Rice Research Institute, Los Baños, The Philippines

P D Christian
National Institute of Biological Standards and Control, South Mimms, UK

M G Ciufolini
Istituto Superiore di Sanità, Rome, Italy

P Clarke
University of Colorado Health Sciences, Denver, CO, USA

J-M Claverie
Université de la Méditerranée, Marseille, France

J R Clayton
Johns Hopkins University Schools of Public Health and Medicine, Baltimore, MD, USA

R J Clem
Kansas State University, Manhattan, KS, USA

C J Clements
The Macfarlane Burnet Institute for Medical Research and Public Health Ltd., Melbourne, VIC, Australia

L L Coffey,
Institut Pasteur, Paris, France

J I Cohen
National Institutes of Health, Bethesda, MD, USA

J Collinge
University College London, London, UK

P L Collins
National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

A Collins
University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

D Contamine
Université Versailles St-Quentin, CNRS, Versailles, France

K M Coombs
University of Manitoba, Winnipeg, MB, Canada

J A Cowley
CSIRO Livestock Industries, Brisbane, QLD, Australia

J K Craigo
University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

M St. J Crane
CSIRO Livestock Industries, Geelong, VIC, Australia

J E Crowe, Jr.
Vanderbilt University Medical Center, Nashville, TN, USA

H Czosnek
The Hebrew University of Jerusalem, Rehovot, Israel

T Dalmay
University of East Anglia, Norwich, UK

B H Dannevig
National Veterinary Institute, Oslo, Norway

C J D'Arcy
University of Illinois at Urbana-Champaign, Urbana, IL, USA

A J Davison
MRC Virology Unit, Glasgow, UK

W O Dawson
University of Florida, Lake Alfred, FL, USA

L A Day
The Public Health Research Institute, Newark, NJ, USA

J C de la Torre
The Scripps Research Institute, La Jolla, CA, USA

X de Lamballerie
Faculté de Médecine de Marseille, Marseilles, France

M de Vega
Universidad Autónoma, Madrid, Spain

P Delfosse
Centre de Recherche Public-Gabriel Lippmann, Belvaux, Luxembourg

B Delmas
INRA, Jouy-en-Josas, France

M Deng
University of California, Berkeley, CA, USA

J DeRisi
University of California, San Francisco, San Francisco, CA, USA

C Desbiez
Institut National de la Recherche Agronomique (INRA), Station de Pathologie Végétale, Montfavet, France

R C Desrosiers
New England Primate Research Center, Southborough, MA, USA

A K Dhar
Advanced BioNutrition Corp, Columbia, MD, USA

R G Dietzgen
The University of Queensland, St. Lucia, QLD, Australia

S P Dinesh-Kumar
Yale University, New Haven, CT, USA

L K Dixon
Institute for Animal Health, Pirbright, UK

C Dogimont
INRA, Montfavet, France

A Domanska
University of Helsinki, Helsinki, Finland

L L Domier
USDA–ARS, Urbana, IL, USA

L L Domier
USDA-ARS, Urbana-Champaign, IL, USA

A Dotzauer
University of Bremen, Bremen, Germany

T W Dreher
Oregon State University, Corvallis, OR, USA

S Dreschers
University of Duisburg–Essen, Essen, Germany

R L Duda
University of Pittsburgh, Pittsburgh, PA, USA

J P Dudley
The University of Texas at Austin, Austin, TX, USA

W P Duprex
The Queen's University of Belfast, Belfast, UK

R E Dutch
University of Kentucky, Lexington, KY, USA

B M Dutia
University of Edinburgh, Edinburgh, UK

M L Dyall-Smith
The University of Melbourne, Parkville, VIC, Australia

J East
University of Texas Medical Branch – Galveston, Galveston, TX, USA

A J Easton
University of Warwick, Coventry, UK

K C Eastwell
Washington State University – IAREC, Prosser, WA, USA

B T Eaton
Australian Animal Health Laboratory, Geelong, VIC, Australia

H Edskes
National Institutes of Health, Bethesda, MD, USA

B Ehlers
Robert Koch-Institut, Berlin, Germany

R M Elliott
University of St. Andrews, St. Andrews, UK

A Engel
National Institutes of Health, Bethesda, MD, USA and
D Kryndushkin
National Institutes of Health, Bethesda, MD, USA

J Engelmann
INRES, University of Bonn, Bonn, Germany

L Enjuanes
CNB, CSIC, Madrid, Spain

A Ensser
Virologisches Institut, Universitätsklinikum, Erlangen, Germany

M Erlandson
Agriculture & Agri-Food Canada, Saskatoon, SK, Canada

K J Ertel
University of California, Irvine, CA, USA

R Esteban
Instituto de Microbiología Bioquímica CSIC/University de Salamanca, Salamanca, Spain

R Esteban
Instituto de Microbiología Bioquímica CSIC/University of Salamanca, Salamanca, Spain

J L Van Etten
University of Nebraska–Lincoln, Lincoln, NE, USA

D J Evans
University of Warwick, Coventry, UK

Ø Evensen
Norwegian School of Veterinary Science, Oslo, Norway

D Falzarano
University of Manitoba, Winnipeg, MB, Canada

B A Fane
University of Arizona, Tucson, AZ, USA

R-X. Fang
Chinese Academy of Sciences, Beijing, People's Republic of China

D Fargette
IRD, Montpellier, France

A Fath-Goodin
University of Kentucky, Lexington, KY, USA

C M Fauquet
Danforth Plant Science Center, St. Louis, MO, USA

B A Federici
University of California, Riverside, CA, USA

H Feldmann
National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada

H Feldmann
Public Health Agency of Canada, Winnipeg, MB, Canada

F Fenner
Australian National University, Canberra, ACT, Australia

S A Ferreira
University of Hawaii at Manoa, Honolulu, HI, USA

H J Field
University of Cambridge, Cambridge, UK

K Fischer
University of California, San Francisco, San Francisco, CA, USA

J A Fishman
Massachusetts General Hospital, Boston, MA, USA

B Fleckenstein
University of Erlangen – Nürnberg, Erlangen, Germany

R Flores
Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Valencia, Spain

T R Flotte
University of Florida College of Medicine, Gainesville, FL, USA

P Forterre
Institut Pasteur, Paris, France

M A Franco
Pontificia Universidad Javeriana, Bogota, Republic of Colombia

T K Frey
Georgia State University, Atlanta, GA, USA

M Fuchs
Cornell University, Geneva, NY, USA

S Fuentes
International Potato Center (CIP), Lima, Peru

T Fujimura
Instituto de Microbiología Bioquímica CSIC/University of Salamanca, Salamanca, Spain

R S Fujinami
University of Utah School of Medicine, Salt Lake City, UT, USA

T Fukuhara
Tokyo University of Agriculture and Technology, Fuchu, Japan

D Gallitelli
Università degli Studi and Istituto di Virologia Vegetale del CNR, Bari, Italy

F García-Arenal
Universidad Politécnica de Madrid, Madrid, Spain

J A García
Centro Nacional de Biotecnología (CNB), CSIC, Madrid, Spain

R A Garrett
Copenhagen University, Copenhagen, Denmark

S Gaumer
Université Versailles St-Quentin, CNRS, Versailles, France

R J Geijskes
Queensland University of Technology, Brisbane, QLD, Australia

T W Geisbert
National Emerging Infectious Diseases Laboratories, Boston, MA, USA

E Gellermann
Hannover Medical School, Hannover, Germany

A Gessain
Pasteur Institute, CNRS URA 3015, Paris, France

S A Ghabrial
University of Kentucky, Lexington, KY, USA

W Gibson
Johns Hopkins University School of Medicine, Baltimore, MD, USA

M Glasa
Slovak Academy of Sciences, Bratislava, Slovakia

Y Gleba
Icon Genetics GmbH, Weinbergweg, Germany

U A Gompels
University of London, London, UK

D Gonsalves
USDA, Pacific Basin Agricultural Research Center, Hilo, HI, USA

M M Goodin
University of Kentucky, Lexington, KY, USA

T J D Goodwin
University of Otago, Dunedin, New Zealand

A E Gorbalenya
Leiden University Medical Center, Leiden, The Netherlands

E A Gould
University of Reading, Reading, UK

A Grakoui
Emory University School of Medicine, Atlanta, GA, USA

M-A Grandbastien
INRA, Versailles, France

R Grassmann
University of Erlangen – Nürnberg, Erlangen, Germany

M Gravell
National Institutes of Health, Bethesda, MD, USA

M V Graves
University of Massachusetts–Lowell, Lowell, MA, USA

K Y Green
National Institutes of Health, Bethesda, MD, USA

H B Greenberg
Stanford University School of Medicine and Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA

B M Greenberg
Johns Hopkins School of Medicine, Baltimore, MD, USA

I Greiser-Wilke
School of Veterinary Medicine, Hanover, Germany

D E Griffin
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

T S Gritsun
University of Reading, Reading, UK

R J de Groot
Utrecht University, Utrecht, The Netherlands

A J Gubala
CSIRO Livestock Industries, Geelong, VIC, Australia

D J Gubler
John A. Burns School of Medicine, Honolulu, HI, USA

A-L Haenni
Institut Jacques Monod, Paris, France

D Haig
Nottingham University, Nottingham, UK

F J Haines
Oxford Brookes University, Oxford, UK

J Hamacher
INRES, University of Bonn, Bonn, Germany

J Hammond
USDA-ARS, Beltsville, MD, USA

R M Harding
Queensland University of Technology, Brisbane, QLD, Australia

J M Hardwick
Johns Hopkins University Schools of Public Health and Medicine, Baltimore, MD, USA

D Hariri
INRA – Département Santé des Plantes et Environnement, Versailles, France

B Harrach
Veterinary Medical Research Institute, Budapest, Hungary

P A Harries
Samuel Roberts Noble Foundation, Inc., Ardmore, OK, USA

L E Harrington
University of Alabama at Birmingham, Birmingham, AL, USA

T J Harrison
University College London, London, UK

T Hatziioannou
Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY, USA

J Hay
The State University of New York, Buffalo, NY, USA

G S Hayward
Johns Hopkins School of Medicine, Baltimore, MD, USA

E Hébrard
IRD, Montpellier, France

R W Hendrix
University of Pittsburgh, Pittsburgh, PA, USA

L E Hensley
USAMRIID, Fort Detrick, MD, USA

M de las Heras
University of Glasgow Veterinary School, Glasgow, UK

S Hertzler
University of Illinois at Chicago, Chicago, IL, USA

F van Heuverswyn
University of Montpellier 1, Montpellier, France

J Hilliard
Georgia State University, Atlanta, GA, USA

B I Hillman
Rutgers University, New Brunswick, NJ, USA

S Hilton
University of Warwick, Warwick, UK

D M Hinton
National Institutes of Health, Bethesda, MD, USA

A Hinz
UMR 5233 UJF-EMBL-CNRS, Grenoble, France

A E Hoet
The Ohio State University, Columbus, OH, USA

S A Hogenhout
The John Innes Centre, Norwich, UK

T Hohn
Basel university, Institute of Botany, Basel, Switzerland

J S Hong
Seoul Women's University, Seoul, South Korea

M C Horzinek
Utrecht University, Utrecht, The Netherlands

T Hovi
National Public Health Institute (KTL), Helsinki, Finland

A M Huger
Institute for Biological Control, Darmstadt, Germany

L E Hughes
University of St. Andrews, St. Andrews, UK

R Hull
John Innes Centre, Colney, UK

E Hunter
Emory University Vaccine Center, Atlanta, GA, USA

A D Hyatt
Australian Animal Health Laboratory, Geelong, VIC, Australia

T Hyypiä
University of Turku, Turku, Finland

T Iwanami
National Institute of Fruit Tree Science, Tsukuba, Japan

A O Jackson
University of California, Berkeley, CA, USA

P Jardine
University of Minnesota, Minneapolis, MN, USA

J A Jehle
DLR Rheinpfalz, Neustadt, Germany

A R Jilbert
Institute of Medical and Veterinary Science, Adelaide, SA, Australia

P John
Indian Agricultural Research Institute, New Delhi, India

J E Johnson
The Scripps Research Institute, La Jolla, CA, USA

R T Johnson
Johns Hopkins School of Medicine, Baltimore, MD, USA

W E Johnson
New England Primate Research Center, Southborough, MA, USA

S L Johnston
Imperial College London, London, UK

A T Jones
Scottish Crop Research Institute, Dundee, UK

R Jordan
USDA-ARS, Beltsville, MD, USA

Y Kapustin
National Institutes of Health, Bethesda, MD, USA

P Karayiannis
Imperial College London, London, UK

P Kazmierczak
University of California, Davis, CA, USA

K M Keene
Colorado State University, Fort Collins, CO, USA

C Kerlan
Institut National de la Recherche Agronomique (INRA), Le Rheu, France

K Khalili
Temple University School of Medicine, Philadelphia, PA, USA

P H Kilmarx
Centers for Disease Control and Prevention, Atlanta, GA, USA

L A King
Oxford Brookes University, Oxford, UK

P D Kirkland
Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia

C D Kirkwood
Murdoch Childrens Research Institute Royal Children's Hospital, Melbourne, VIC, Australia

R P Kitching
Canadian Food Inspection Agency, Winnipeg, MB, Canada

P J Klasse
Cornell University, New York, NY, USA

N R Klatt
University of Pennsylvania School of Medicine, Philadelphia, PA, USA

R G Kleespies
Institute for Biological Control, Darmstadt, Germany

D F Klessig
Boyce Thompson Institute for Plant Research, Ithaca, NY, USA

W B Klimstra
Louisiana State University Health Sciences Center at Shreveport, Shreveport, LA, USA

V Klimyuk
Icon Genetics GmbH, Weinbergweg, Germany

N Knowles
Institute for Animal Health, Pirbright, UK

R Koenig
Biologische Bundesanstalt für Land- und Forstwirtschaft, Brunswick, Germany

R Koenig
Institut für Pflanzenvirologie, Mikrobiologie und biologische Sicherheit, Brunswick, Germany

G Konaté
INERA, Ouagadougou, Burkina Faso

C N Kotton
Massachusetts General Hospital, Boston, MA, USA

L D Kramer
Wadsworth Center, New York State Department of Health, Albany, NY, USA

P J Krell
University of Guelph, Guelph, ON, Canada

J Kreuze
International Potato Center (CIP), Lima, Peru

M J Kuehnert
Centers for Disease Control and Prevention, Atlanta, GA, USA

R J Kuhn
Purdue University, West Lafayette, IN, USA

G Kurath
Western Fisheries Research Center, Seattle, WA, USA

I Kusters
sanofi pasteur, Lyon, France

I V Kuzmin
Centers for Disease Control and Prevention, Atlanta, GA, USA

M E Laird
New England Primate Research Center, Southborough, MA, USA

R A Lamb
Howard Hughes Medical Institute at Northwestern University, Evanston, IL, USA

P F Lambert
University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

A S Lang
Memorial University of Newfoundland, St. John's, NL, Canada

H D Lapierre
INRA – Département Santé des Plantes et Environnement, Versailles, France

G Lawrence
The Children's Hospital at Westmead, Westmead, NSW, Australia and
University of Sydney, Westmead, NSW, Australia

H Lecoq
Institut National de la Recherche Agronomique (INRA), Station de Pathologie Végétale, Montfavet, France

B Y Lee
Seoul Women's University, Seoul, South Korea

E J Lefkowitz
University of Alabama at Birmingham, Birmingham, AL, USA

J P Legg
International Institute of Tropical Agriculture, Dar es Salaam, Tanzania,
UK and
Natural Resources Institute, Chatham Maritime, UK

P Leinikki
National Public Health Institute, Helsinki, Finland

J Lenard
University of Medicine and Dentistry of New Jersey (UMDNJ), Piscataway, NJ, USA

J C Leong
University of Hawaii at Manoa, Honolulu, HI, USA

K N Leppard
University of Warwick, Coventry, UK

A Lescoute
Université Louis Pasteur, Strasbourg, France

D-E Lesemann
Biologische Bundesanstalt für Land- und Forstwirtschaft, Brunswick, Germany

J-H Leu
National Taiwan University, Taipei, Republic of China

H L Levin
National Institutes of Health, Bethesda, MD, USA

D J Lewandowski
The Ohio State University, Columbus, OH, USA

H-S Lim
University of California, Berkeley, Berkeley, CA, USA

M D A Lindsay
Western Australian Department of Health, Mount Claremont, WA, Australia

R Ling
University of Warwick, Coventry, UK

M L Linial
Fred Hutchinson Cancer Research Center, Seattle, WA, USA

D C Liotta
Emory University, Atlanta, GA, USA

W Ian Lipkin
Columbia University, New York, NY, USA

H L Lipton
University of Illinois at Chicago, Chicago, IL, USA

A S Liss
University of Texas at Austin, Austin, TX, USA

J J López-Moya
Instituto de Biología Molecular de Barcelona (IBMB), CSIC, Barcelona, Spain

G Loebenstein
Agricultural Research Organization, Bet Dagan, Israel

C-F Lo
National Taiwan University, Taipei, Republic of China

S A Lommel
North Carolina State University, Raleigh, NC, USA

G P Lomonossoff
John Innes Centre, Norwich, UK

M Luo
University of Alabama at Birmingham, Birmingham, AL, USA

S A MacFarlane
Scottish Crop Research Institute, Dundee, UK

J S Mackenzie
Curtin University of Technology, Shenton Park, WA, Australia

R Mahieux
Pasteur Institute, CNRS URA 3015, Paris, France

B W J Mahy
Centers for Disease Control and Prevention, Atlanta, GA, USA

E Maiss
Institute of Plant Diseases and Plant Protection, Hannover, Germany

E O Major
National Institutes of Health, Bethesda, MD, USA

V G Malathi
Indian Agricultural Research Institute, New Delhi, India

A Mankertz
Robert Koch-Institut, Berlin, Germany

S Mansoor
National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

L Moser
University of Wisconsin – Madison, Madison, WI, USA

B Moury
INRA – Station de Pathologie Végétale, Montfavet, France

J W Moyer
North Carolina State University, Raleigh, NC, USA

R W Moyer
University of Florida, Gainesville, FL, USA

E Muller
CIRAD/UMR BGPI, Montpellier, France

F A Murphy
University of Texas Medical Branch, Galveston, TX, USA

A Müllbacher
Australian National University, Canberra, ACT, Australia

K Nagasaki
Fisheries Research Agency, Hiroshima, Japan

T Nakayashiki
National Institutes of Health, Bethesda, MD, USA

A A Nash
University of Edinburgh, Edinburgh, UK

N Nathanson
University of Pennsylvania, Philadelphia, PA, USA

C K Navaratnarajah
Purdue University, West Lafayette, IN, USA

M S Nawaz-ul-Rehman
Danforth Plant Science Center, St. Louis, MO, USA

J C Neil
University of Glasgow, Glasgow, UK

R S Nelson
Samuel Roberts Noble Foundation, Inc., Ardmore, OK, USA

P Nettleton
Moredun Research Institute, Edinburgh, UK

A W Neuman
Emory University, Atlanta, GA, USA

A R Neurath
Virotech, New York, NY, USA

M L Nibert
Harvard Medical School, Boston, MA, USA

L Nicoletti
Istituto Superiore di Sanità, Rome, Italy

N Noah
London School of Hygiene and Tropical Medicine, London, UK

D L Nuss
University of Maryland Biotechnology Institute, Rockville, MD, USA

M S Oberste
Centers for Disease Control and Prevention, Atlanta, GA, USA

W A O'Brien
University of Texas Medical Branch – Galveston, Galveston, TX, USA

D J O'Callaghan
Louisiana State University Health Sciences Center, Shreveport, LA, USA

W F Ochoa
University of California, San Diego, La Jolla, CA, USA

M R Odom
University of Alabama at Birmingham, Birmingham, AL, USA

M M van Oers
Wageningen University, Wageningen, The Netherlands

M B A Oldstone
The Scripps Research Institute, La Jolla, CA, USA

G Olinger
USAMRIID, Fort Detrick, MD, USA

K E Olson
Colorado State University, Fort Collins, CO, USA

A Olspert
Tallinn University of Technology, Tallinn, Estonia

G Orth
Institut Pasteur, Paris, France

J E Osorio
University of Wisconsin, Madison, WI, USA

N Osterrieder
Cornell University, Ithaca, NY, USA

S A Overman
University of Missouri – Kansas City, Kansas City, MO, USA

R A Owens
Beltsville Agricultural Research Center, Beltsville, MD, USA

M S Padmanabhan
Yale University, New Haven, CT, USA

S Paessler
University of Texas Medical Branch, Galveston, TX, USA

P Palese
Mount Sinai School of Medicine, New York, NY, USA

M A Pallansch
Centers for Disease Control and Prevention, Atlanta, GA, USA

M Palmarini
University of Glasgow Veterinary School, Glasgow, UK

P Palukaitis
Scottish Crop Research Institute, Invergowrie, Dundee, UK

A A Marfin
Centers for Disease Control and Prevention, Atlanta, GA, USA

S Marillonnet
Icon Genetics GmbH, Weinbergweg, Germany

G P Martelli
Università degli Studi and Istituto di Virologia vegetale CNR, Bari, Italy

M Marthas
University of California, Davis, Davis, CA, USA

D P Martin
University of Cape Town, Cape Town, South Africa

P A Marx
Tulane University, Covington, LA, USA

W S Mason
Fox Chase Cancer Center, Philadelphia, PA, USA

T D Mastro
Centers for Disease Control and Prevention, Atlanta, GA, USA

A A McBride
National Institutes of Health, Bethesda, MD, USA

L McCann
National Institutes of Health, Bethesda, MD, USA

M McChesney
University of California, Davis, Davis, CA, USA

J B McCormick
University of Texas, School of Public Health, Brownsville, TX, USA

G McFadden
University of Florida, Gainesville, FL, USA

G McFadden
The University of Western Ontario, London, ON, Canada

D B McGavern
The Scripps Research Institute, La Jolla, CA, USA

A L McNees
Baylor College of Medicine, Houston, TX, USA

M Meier
Tallinn University of Technology, Tallinn, Estonia

P S Mellor
Institute for Animal Health, Woking, UK

X J Meng
Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

A A Mercer
University of Otago, Dunedin, New Zealand

P P C Mertens
Institute for Animal Health, Woking, UK

T C Mettenleiter
Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

H Meyer
Bundeswehr Institute of Microbiology, Munich, Germany

R F Meyer
Centers for Disease Control and Prevention, Atlanta, GA, USA

P de Micco
Etablissement Français du Sang Alpes-Méditerranée, Marseilles, France

B R Miller
Centers for Disease Control and Prevention (CDC), Fort Collins, CO, USA

C J Miller
University of California, Davis, Davis, CA, USA

R G Milne
Istituto di Virologia Vegetale CNR, Torino, Italy

P D Minor
NIBSC, Potters Bar, UK

S Mjaaland
Norwegian School of Veterinary Science, Oslo, Norway

E S Mocarski
Emory University School of Medicine, Atlanta, GA, USA

E S Mocarski, Jr.
Emory University School of Medicine, Emory, GA, USA

V Moennig
School of Veterinary Medicine, Hanover, Germany

P Moffett
Boyce Thompson Institute for Plant Research, Ithaca, NY, USA

T P Monath
Kleiner Perkins Caufield and Byers, Menlo Park, CA, USA

R C Montelaro
University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

P S Moore
University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

F J Morales
International Center for Tropical Agriculture, Cali, Colombia

H Moriyama
Tokyo University of Agriculture and Technology, Fuchu, Japan

T J Morris
University of Nebraska, Lincoln, NE, USA

S A Morse
Centers for Disease Control and Prevention, Atlanta, GA, USA

I Pandrea
Tulane National Primate Research Center, Covington, LA, USA

O Papadopoulos
Aristotle University, Thessaloniki, Greece

H R Pappu
Washington State University, Pullman, WA, USA

S Parker
Saint Louis University School of Medicine, St. Louis, MO, USA

C R Parrish
Cornell University, Ithaca, NY, USA

R F Pass
University of Alabama School of Medicine, Birmingham, AL, USA

J L Patterson
Southwest Foundation for Biomedical Research, San Antonio, TX, USA

T A Paul
Cornell University, Ithaca, NY, USA

A E Peaston
The Jackson Laboratory, Bar Harbor, ME, USA

M Peeters
University of Montpellier 1, Montpellier, France

J S M Peiris
The University of Hong Kong, Hong Kong, People's Republic of China

P J Peters
Centers for Disease Control and Prevention, Atlanta, GA, USA

M Pfeffer
Bundeswehr Institute of Microbiology, Munich, Germany

H Pfister
University of Köln, Cologne, Germany

O Planz
Federal Research Institute for Animal Health, Tuebingen, Gemany

L L M Poon
The University of Hong Kong, Hong Kong, People's Republic of China

M M Poranen
University of Helsinki, Helsinki, Finland

K Porter
The University of Melbourne, Parkville, VIC, Australia

A Portner
St. Jude Children's Research Hospital, Memphis, TN, USA

R D Possee
NERC Institute of Virology and Environmental Microbiology, Oxford, UK

R T M Poulter
University of Otago, Dunedin, New Zealand

A M Powers
Centers for Disease Control and Prevention, Fort Collins, CO, USA

D Prangishvili
Institut Pasteur, Paris, France

C M Preston
Medical Research Council Virology Unit, Glasgow, UK

S L Quackenbush
Colorado State University, Fort Collins, CO, USA

F Qu
University of Nebraska, Lincoln, NE, USA

B C Ramirez
CNRS, Paris, France

A Rapose
University of Texas Medical Branch – Galveston, Galveston, TX, USA

D V R Reddy
Hyderabad, India

A J Redwood
The University of Western Australia, Crawley, WA, Australia

M Regner
Australian National University, Canberra, ACT, Australia

W K Reisen
University of California, Davis, CA, USA

T Renault
IFREMER, La Tremblade, France

P A Revill
Victorian Infectious Diseases Reference Laboratory, Melbourne, VIC, Australia

A Rezaian
University of Adelaide, Adelaide, SA, Australia

J F Ridpath
USDA, Ames, IA, USA

B K Rima
The Queen's University of Belfast, Belfast, UK

E Rimstad
Norwegian School of Veterinary Science, Oslo, Norway

F J Rixon
MRC Virology Unit, Glasgow, UK

Y-T Ro
Konkuk University, Seoul, South Korea

C M Robinson
University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

G F Rohrmann
Oregon State University, Corvallis, OR, USA

M Roivainen
National Public Health Institute (KTL), Helsinki, Finland

L Roux
University of Geneva Medical School, Geneva, Switzerland

J Rovnak
Colorado State University, Fort Collins, CO, USA

D J Rowlands
University of Leeds, Leeds, UK

P Roy
London School of Hygiene and Tropical Medicine, London, UK

L Rubino
Istituto di Virologia Vegetale del CNR, Bari, Italy

R W H Ruigrok
CNRS, Grenoble, France

C E Rupprecht
Centers for Disease Control and Prevention, Atlanta, GA, USA

R J Russell
University of St. Andrews, St. Andrews, UK

B E Russ
The University of Melbourne, Parkville, VIC, Australia

W T Ruyechan
The State University of New York, Buffalo, NY, USA

E Ryabov
University of Warwick, Warwick, UK

M D Ryan
University of St. Andrews, St. Andrews, UK

E P Rybicki
University of Cape Town, Cape Town, South Africa

K D Ryman
Louisiana State University Health Sciences Center at Shreveport, Shreveport, LA, USA

K D Ryman
Louisiana State University Health Sciences Center, Shreveport, LA, USA

K H Ryu
Seoul Women's University, Seoul, South Korea

M Safak
Temple University School of Medicine, Philadelphia, PA, USA

M Salas
Universidad Autónoma, Madrid, Spain

S K Samal
University of Maryland, College Park, MD, USA

J T Sample
The Pennsylvania State University College of Medicine, Hershey, PA, USA

C E Sample
The Pennsylvania State University College of Medicine, Hershey, PA, USA

R M Sandri-Goldin
University of California, Irvine, Irvine, CA, USA

H Sanfaçon
Pacific Agri-Food Research Centre, Summerland, BC, Canada

R Sanjuán
Instituto de Biología Molecular y Cellular de Plantas, CSIC-UPV, Valencia, Spain

N Santi
Norwegian School of Veterinary Science, Oslo, Norway

C Sarmiento
Tallinn University of Technology, Tallinn, Estonia

T Sasaya
National Agricultural Research Center, Ibaraki, Japan

Q J Sattentau
University of Oxford, Oxford, UK

C Savolainen-Kopra
National Public Health Institute (KTL), Helsinki, Finland

B Schaffhausen
Tufts University School of Medicine, Boston, MA, USA

K Scheets
Oklahoma State University, Stillwater, OK, USA

M J Schmitt
University of the Saarland, Saarbrücken, Germany

A Schneemann
The Scripps Research Institute, La Jolla, CA, USA

G Schoehn
CNRS, Grenoble, France

J E Schoelz
University of Missouri, Columbia, MO, USA

L B Schonberger
Centers for Disease Control and Prevention, Atlanta, GA, USA

U Schubert
Klinikum der Universität Erlangen-Nürnberg, Erlangen, Germany

D A Schultz
Johns Hopkins University School of Medicine, Baltimore, MD, USA

S Schultz-Cherry
University of Wisconsin – Madison, Madison, WI, USA

T F Schulz
Hannover Medical School, Hannover, Germany

P D Scotti
Waiatarua, New Zealand

B L Semler
University of California, Irvine, CA, USA

J M Sharp
Veterinary Laboratories Agency, Penicuik, UK

M L Shaw
Mount Sinai School of Medicine, New York, NY, USA

G R Shellam
The University of Western Australia, Crawley, WA, Australia

D N Shepherd
University of Cape Town, Cape Town, South Africa

N C Sheppard
University of Oxford, Oxford, UK

F Shewmaker
National Institutes of Health, Bethesda, MD, USA

P A Signoret
Montpellier SupAgro, Montpellier, France

A Silaghi
University of Manitoba, Winnipeg, MB, Canada

G Silvestri
University of Pennsylvania, Philadelphia, PA, USA

T L Sit
North Carolina State University, Raleigh, NC, USA

N Sittidilokratna
Centex Shrimp and Center for Genetic Engineering and Biotechnology, Bangkok, Thailand

M A Skinner
Imperial College London, London, UK

D W Smith
PathWest Laboratory Medicine WA, Nedlands, WA, Australia

G L Smith
Imperial College London, London, UK

L M Smith
The University of Western Australia, Crawley, WA, Australia

E J Snijder
Leiden University Medical Center, Leiden, The Netherlands

M Sova
University of Texas Medical Branch – Galveston, Galveston, TX, USA

J A Speir
The Scripps Research Institute, La Jolla, CA, USA

T E Spencer
Texas A&M University, College Station, TX, USA

P Sreenivasulu
Sri Venkateswara University, Tirupati, India

J Stanley
John Innes Centre, Colney, UK

K M Stedman
Portland State University, Portland, OR, USA

D Stephan
Institute of Plant Diseases and Plant Protection, Hannover, Germany

C C M M Stijger
Wageningen University and Research Centre, Naaldwijk, The Netherlands

L Stitz
Federal Research Institute for Animal Health, Tuebingen, Gemany

P G Stockley
University of Leeds, Leeds, UK

M R Strand
University of Georgia, Athens, GA, USA

M J Studdert
The University of Melbourne, Parkville, VIC, Australia

C A Suttle
University of British Columbia, Vancouver, BC, Canada

N Suzuki
Okayama University, Okayama, Japan

J Y Suzuki
USDA, Pacific Basin Agricultural Research Center, Hilo, HI, USA

R Swanepoel
National Institute for Communicable Diseases, Sandringham, South Africa

S J Symes
The University of Melbourne, Parkville, VIC, Australia

G Szittya
Agricultural Biotechnology Center, Godollo, Hungary

M Taliansky
Scottish Crop Research Institute, Dundee, UK

P Tattersall
Yale University Medical School, New Haven, CT, USA

T Tatusova
National Institutes of Health, Bethesda, MD, USA

S Tavantzis
University of Maine, Orono, ME, USA

J M Taylor
Fox Chase Cancer Center, Philadelphia, PA, USA

D A Theilmann
Agriculture and Agri-Food Canada, Summerland, BC, Canada

F C Thomas Allnutt
National Science Foundation, Arlington, VA, USA

G J Thomas Jr.
University of Missouri – Kansas City, Kansas City, MO, USA

J E Thomas
Department of Primary Industries and Fisheries, Indooroopilly, QLD, Australia

H C Thomas
Imperial College London, London, UK

A N Thorburn
The University of Melbourne, Parkville, VIC, Australia

P Tijssen
Université du Québec, Laval, QC, Canada

S A Tolin
Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

L Torrance
Scottish Crop Research Institute, Invergowrie, UK

S Trapp
Cornell University, Ithaca, NY, USA

S Tripathi
USDA, Pacific Basin Agricultural Research Center, Hilo, HI, USA

E Truve
Tallinn University of Technology, Tallinn, Estonia

J-M Tsai
National Taiwan University, Taipei, Republic of China

M Tsompana
North Carolina State University, Raleigh, NC, USA

R Tuma
University of Helsinki, Helsinki, Finland

A S Turnell
The University of Birmingham, Birmingham, UK

K L Tyler
University of Colorado Health Sciences, Denver, CO, USA

A Uchiyama
Cornell University, Ithaca, NY, USA

C Upton
University of Victoria, Victoria, BC, Canada

A Urisman
University of California, San Francisco, San Francisco, CA, USA

J K Uyemoto
University of California, Davis, CA, USA

A Vaheri
University of Helsinki, Helsinki, Finland

R Vainionpää
University of Turku, Turku, Finland

A M Vaira
Istituto di Virologia Vegetale, CNR, Turin, Italy

N K Van Alfen
University of California, Davis, CA, USA

R A A Van der Vlugt
Wageningen University and Research Centre, Wageningen, The Netherlands

M H V Van Regenmortel
CNRS, Illkirch, France

P A Venter
The Scripps Research Institute, La Jolla, CA, USA

J Verchot-Lubicz
Oklahoma State University, Stillwater, OK, USA

R A Vere Hodge
Vere Hodge Antivirals Ltd., Reigate, UK

H J Vetten
Federal Research Centre for Agriculture and Forestry (BBA), Brunswick, Germany

L P Villarreal
University of California, Irvine, Irvine, CA, USA

J M Vlak
Wageningen University, Wageningen, The Netherlands

P K Vogt
The Scripps Research Institute, La Jolla, CA, USA

L E Volkman
University of California, Berkeley, Berkeley, CA, USA

J Votteler
Klinikum der Universität Erlangen-Nürnberg, Erlangen, Germany

D F Voytas
Iowa State University, Ames, IA, USA

J D F Wadsworth
University College London, London, UK

E K Wagner
University of California, Irvine, Irvine, CA, USA

P J Walker
CSIRO Australian Animal Health Laboratory, Geelong, VIC, Australia

A L Wang
University of California, San Francisco, CA, USA

X Wang
University of Wisconsin – Madison, Madison, WI, USA

C C Wang
University of California, San Francisco, CA, USA

L-F Wang
Australian Animal Health Laboratory, Geelong, VIC, Australia

R Warrier
Purdue University, West Lafayette, IN, USA

S C Weaver
University of Texas Medical Branch, Galveston, TX, USA

B A Webb
University of Kentucky, Lexington, KY, USA

F Weber
University of Freiburg, Freiburg, Germany

R P Weir
Berrimah Research Farm, Darwin, NT, Australia

R A Weisberg
National Institutes of Health, Bethesda, MD, USA

W Weissenhorn
UMR 5233 UJF-EMBL-CNRS, Grenoble, France

R M Welsh
University of Massachusetts Medical School, Worcester, MA, USA

J T West
University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

E Westhof
Université Louis Pasteur, Strasbourg, France

S P J Whelan
Harvard Medical School, Boston, MA, USA

R L White
Texas A&M University, College Station, TX, USA

C A Whitehouse
United States Army Medical Research Institute of Infectious Diseases, Frederick, MD, USA

R B Wickner
National Institutes of Health, Bethesda, MD, USA

R G Will
Western General Hospital, Edinburgh, UK

T Williams
Instituto de Ecología A.C., Xalapa, Mexico

K Willoughby
Moredun Research Institute, Edinburgh, UK

S Winter
Deutsche Sammlung für Mikroorganismen und Zellkulturen, Brunswick, Germany

J Winton
Western Fisheries Research Center, Seattle, WA, USA

J K Yamamoto
University of Florida, Gainesville, FL, USA

M Yoshida
University of Tokyo, Chiba, Japan

N Yoshikawa
Iwate University, Ueda, Japan

L S Young
University of Birmingham, Birmingham, UK

R F Young,III
Texas A&M University, College Station, TX, USA

T M Yuill
University of Wisconsin, Madison, WI, USA

A J Zajac
University of Alabama at Birmingham, Birmingham, AL, USA

S K Zavriev
Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

J Ziebuhr
The Queen's University of Belfast, Belfast, UK

E I Zuniga
The Scripps Research Institute, La Jolla, CA, USA

CONTENTS

Editors-in-Chief v

Associate Editors vii

Preface ix

How to Use the Encyclopedia xi

Contributors xiii

VOLUME 1

A

Adenoviruses: General Features *B Harrach* 1

Adenoviruses: Malignant Transformation and Oncology *A S Turnell* 9

Adenoviruses: Molecular Biology *K N Leppard* 17

Adenoviruses: Pathogenesis *M Benkő* 24

African Cassava Mosaic Disease *J P Legg* 30

African Horse Sickness Viruses *P S Mellor and P P C Mertens* 37

African Swine Fever Virus *L K Dixon and D Chapman* 43

AIDS: Disease Manifestation *A Rapose, J East, M Sova and W A O'Brien* 51

AIDS: Global Epidemiology *P J Peters, P H Kilmarx and T D Mastro* 58

AIDS: Vaccine Development *N C Sheppard and Q J Sattentau* 69

Akabane Virus *P S Mellor and P D Kirkland* 76

Alfalfa Mosaic Virus *J F Bol* 81

Algal Viruses *K Nagasaki and C P D Brussaard* 87

Allexivirus *S K Zavriev* 96

Alphacryptovirus and *Betacryptovirus* *R Blawid, D Stephan and E Maiss* 98

Anellovirus *P Biagini and P de Micco* 104

Animal Rhabdoviruses *H Bourhy, A J Gubala, R P Weir and D B Boyle* 111

Antigen Presentation *E I Zuniga, D B McGavern and M B A Oldstone* 121

Antigenic Variation *G M Air and J T West* 127

Antigenicity and Immunogenicity of Viral Proteins *M H V Van Regenmortel* 137

Antiviral Agents *H J Field and R A Vere Hodge* 142
Apoptosis and Virus Infection *J R Clayton and J M Hardwick* 154
Aquareoviruses *M St J Crane and G Carlile* 163
Arboviruses *B R Miller* 170
Arteriviruses *M A Brinton and E J Snijder* 176
Ascoviruses *B A Federici and Y Bigot* 186
Assembly of Viruses: Enveloped Particles *C K Navaratnarajah, R Warrier and R J Kuhn* 193
Assembly of Viruses: Nonenveloped Particles *M Luo* 200
Astroviruses *L Moser and S Schultz-Cherry* 204

B

Baculoviruses: Molecular Biology of Granuloviruses *S Hilton* 211
Baculoviruses: Molecular Biology of Mosquito Baculoviruses *J J Becnel and C L Afonso* 219
Baculoviruses: Molecular Biology of Sawfly Baculoviruses *B M Arif* 225
Baculoviruses: Apoptosis Inhibitors *R J Clem* 231
Baculoviruses: Expression Vector *F J Haines, R D Possee and L A King* 237
Baculoviruses: General Features *P J Krell* 247
Baculoviruses: Molecular Biology of Nucleopolyhedroviruses *D A Theilmann and G W Blissard* 254
Baculoviruses: Pathogenesis *L E Volkman* 265
Banana Bunchy Top Virus *J E Thomas* 272
Barley Yellow Dwarf Viruses *L L Domier* 279
Barnaviruses *P A Revill* 286
Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus *R Jordan and J Hammond* 288
Bean Golden Mosaic Virus *F J Morales* 295
Beet Curly Top Virus *J Stanley* 301
Benyvirus *R Koenig* 308
Beta ssDNA Satellites *R W Briddon and S Mansoor* 314
Birnaviruses *B Delmas* 321
Bluetongue Viruses *P Roy* 328
Border Disease Virus *P Nettleton and K Willoughby* 335
Bornaviruses *L Stitz, O Planz and W Ian Lipkin* 341
Bovine and Feline Immunodeficiency Viruses *J K Yamamoto* 347
Bovine Ephemeral Fever Virus *P J Walker* 354
Bovine Herpesviruses *M J Studdert* 362
Bovine Spongiform Encephalopathy *R G Will* 368
Bovine Viral Diarrhea Virus *J F Ridpath* 374
Brome Mosaic Virus *X Wang and P Ahlquist* 381
Bromoviruses *J J Bujarski* 386

Bunyaviruses: General Features *R M Elliott* 390
Bunyaviruses: Unassigned *C H Calisher* 399

C

Cacao Swollen Shoot Virus *E Muller* 403
Caliciviruses *M J Studdert and S J Symes* 410
Capillovirus, *Foveavirus*, *Trichovirus*, *Vitivirus* *N Yoshikawa* 419
Capripoxviruses *R P Kitching* 427
Capsid Assembly: Bacterial Virus Structure and Assembly *S Casjens* 432
Cardioviruses *C Billinis and O Papadopoulos* 440
Carlavirus *K H Ryu and B Y Lee* 448
Carmovirus *F Qu and T J Morris* 453
Caulimoviruses: General Features *J E Schoelz* 457
Caulimoviruses: Molecular Biology *T Hohn* 464
Central Nervous System Viral Diseases *R T Johnson and B M Greenberg* 469
Cereal Viruses: Maize/Corn *P A Signoret* 475
Cereal Viruses: Rice *F Morales* 482
Cereal Viruses: Wheat and Barley *H D Lapierre and D Hariri* 490
Chandipura Virus *S Basak and D Chattopadhyay* 497
Chrysoviruses *S A Ghabrial* 503
Circoviruses *A Mankertz* 513
Citrus Tristeza Virus *M Bar-Joseph and W O Dawson* 520
Classical Swine Fever Virus *V Moennig and I Greiser-Wilke* 525
Coltiviruses *H Attoui and X de Lamballerie* 533
Common Cold Viruses *S Dreschers and C Adams* 541
Coronaviruses: General Features *D Cavanagh and P Britton* 549
Coronaviruses: Molecular Biology *S C Baker* 554
Cotton Leaf Curl Disease *S Mansoor, I Amin and R W Briddon* 563
Cowpea Mosaic Virus *G P Lomonossoff* 569
Cowpox Virus *M Bennett, G L Smith and D Baxby* 574
Coxsackieviruses *M S Oberste and M A Pallansch* 580
Crenarchaeal Viruses: Morphotypes and Genomes *D Prangishvili, T Basta and R A Garrett* 587
Crimean–Congo Hemorrhagic Fever Virus and Other Nairoviruses *C A Whitehouse* 596
Cryo-Electron Microscopy *W Chiu, J T Chang and F J Rixon* 603
Cucumber Mosaic Virus *F García-Arenal and P Palukaitis* 614
Cytokines and Chemokines *D E Griffin* 620
Cytomegaloviruses: Murine and Other Nonprimate Cytomegaloviruses *A J Redwood, L M Smith and G R Shellam* 624
Cytomegaloviruses: Simian Cytomegaloviruses *D J Alcendor and G S Hayward* 634

VOLUME 2

D

Defective-Interfering Viruses *L Roux* 1
Dengue Viruses *D J Gubler* 5
Diagnostic Techniques: Microarrays *K Fischer, A Urisman and J DeRisi* 14
Diagnostic Techniques: Plant Viruses *R Koenig, D-E Lesemann, G Adam and S Winter* 18
Diagnostic Techniques: Serological and Molecular Approaches *R Vainionpää and P Leinikki* 29
Dicistroviruses *P D Christian and P D Scotti* 37
Disease Surveillance *N Noah* 44
DNA Vaccines *S Babiuk and L A Babiuk* 51

E

Ebolavirus *K S Brown, A Silaghi and H Feldmann* 57
Echoviruses *T Hyypiä* 65
Ecology of Viruses Infecting Bacteria *S T Abedon* 71
Electron Microscopy of Viruses *G Schoehn and R W H Ruigrok* 78
Emerging and Reemerging Virus Diseases of Plants *G P Martelli and D Gallitelli* 86
Emerging and Reemerging Virus Diseases of Vertebrates *B W J Mahy* 93
Emerging Geminiviruses *C M Fauquet and M S Nawaz-ul-Rehman* 97
Endogenous Retroviruses *W E Johnson* 105
Endornavirus *T Fukuhara and H Moriyama* 109
Enteric Viruses *R F Bishop and C D Kirkwood* 116
Enteroviruses of Animals *L E Hughes and M D Ryan* 123
Enteroviruses: Human Enteroviruses Numbered 68 and Beyond *T Hovi, S Blomqvist, C Savolainen-Kopra and M Roivainen* 130
Entomopoxviruses *M N Becker and R W Moyer* 136
Epidemiology of Human and Animal Viral Diseases *F A Murphy* 140
Epstein–Barr Virus: General Features *L S Young* 148
Epstein–Barr Virus: Molecular Biology *J T Sample and C E Sample* 157
Equine Infectious Anemia Virus *J K Craigo and R C Montelaro* 167
Evolution of Viruses *L P Villarreal* 174

F

Feline Leukemia and Sarcoma Viruses *J C Neil* 185
Filamentous ssDNA Bacterial Viruses *S A Overman and G J Thomas Jr.* 190
Filoviruses *G Olinger, T W Geisbert and L E Hensley* 198
Fish and Amphibian Herpesviruses *A J Davison* 205
Fish Retroviruses *T A Paul, R N Casey, P R Bowser, J W Casey, J Rovnak and S L Quackenbush* 212

Fish Rhabdoviruses *G Kurath and J Winton* 221
Fish Viruses *J C Leong* 227
Flaviviruses of Veterinary Importance *R Swanepoel and F J Burt* 234
Flaviviruses: General Features *T J Chambers* 241
Flexiviruses *M J Adams* 253
Foamy Viruses *M L Linial* 259
Foot and Mouth Disease Viruses *D J Rowlands* 265
Fowlpox Virus and Other Avipoxviruses *M A Skinner* 274
Fungal Viruses *S A Ghabrial and N Suzuki* 284
Furovirus *R Koenig* 291
Fuselloviruses of Archaea *K M Stedman* 296

G

Gene Therapy: Use of Viruses as Vectors *K I Berns and T R Flotte* 301
Genome Packaging in Bacterial Viruses *P Jardine* 306
Giardiaviruses *A L Wang and C C Wang* 312

H

Hantaviruses *A Vaheri* 317
Henipaviruses *B T Eaton and L-F Wang* 321
Hepadnaviruses of Birds *A R Jilbert and W S Mason* 327
Hepadnaviruses: General Features *T J Harrison* 335
Hepatitis A Virus *A Dotzauer* 343
Hepatitis B Virus: General Features *P Karayiannis and H C Thomas* 350
Hepatitis B Virus: Molecular Biology *T J Harrison* 360
Hepatitis C Virus *R Bartenschlager and S Bühler* 367
Hepatitis Delta Virus *J M Taylor* 375
Hepatitis E Virus *X J Meng* 377
Herpes Simplex Viruses: General Features *L Aurelian* 383
Herpes Simplex Viruses: Molecular Biology *E K Wagner and R M Sandri-Goldin* 397
Herpesviruses of Birds *S Trapp and N Osterrieder* 405
Herpesviruses of Horses *D J O'Callaghan and N Osterrieder* 411
Herpesviruses: Discovery *B Ehlers* 420
Herpesviruses: General Features *A J Davison* 430
Herpesviruses: Latency *C M Preston* 436
History of Virology: Bacteriophages *H-W Ackermann* 442
History of Virology: Plant Viruses *R Hull* 450
History of Virology: Vertebrate Viruses *F J Fenner* 455

Hordeivirus *J N Bragg, H-S Lim and A O Jackson* 459
Host Resistance to Retroviruses *T Hatziioannou and P D Bieniasz* 467
Human Cytomegalovirus: General Features *E S Mocarski Jr. and R F Pass* 474
Human Cytomegalovirus: Molecular Biology *W Gibson* 485
Human Eye Infections *J Chodosh, A V Chintakuntlawar and C M Robinson* 491
Human Herpesviruses 6 and 7 *U A Gompels* 498
Human Immunodeficiency Viruses: Antiretroviral Agents *A W Neuman and D C Liotta* 505
Human Immunodeficiency Viruses: Molecular Biology *J Votteler and U Schubert* 517
Human Immunodeficiency Viruses: Origin *F van Heuverswyn and M Peeters* 525
Human Immunodeficiency Viruses: Pathogenesis *N R Klatt, A Chahroudi and G Silvestri* 534
Human Respiratory Syncytial Virus *P L Collins* 542
Human Respiratory Viruses *J E Crowe Jr.* 551
Human T-Cell Leukemia Viruses: General Features *M Yoshida* 558
Human T-Cell Leukemia Viruses: Human Disease *R Mahieux and A Gessain* 564
Hypovirulence *N K Van Alfen and P Kazmierczak* 574
Hypoviruses *D L Nuss* 580

VOLUME 3

I

Icosahedral dsDNA Bacterial Viruses with an Internal Membrane *J K H Bamford and S J Butcher* 1
Icosahedral Enveloped dsRNA Bacterial Viruses *R Tuma* 6
Icosahedral ssDNA Bacterial Viruses *B A Fane, M Chen, J E Cherwa and A Uchiyama* 13
Icosahedral ssRNA Bacterial Viruses *P G Stockley* 21
Icosahedral Tailed dsDNA Bacterial Viruses *R L Duda* 30
Idaeovirus *A T Jones and H Barker* 37
Iflavirus *M M van Oers* 42
Ilarvirus *K C Eastwell* 46
Immune Response to Viruses: Antibody-Mediated Immunity *A R Neurath* 56
Immune Response to Viruses: Cell-Mediated Immunity *A J Zajac and L E Harrington* 70
Immunopathology *M B A Oldstone and R S Fujinami* 78
Infectious Pancreatic Necrosis Virus *Ø Evensen and N Santi* 83
Infectious Salmon Anemia Virus *B H Dannevig, S Mjaaland and E Rimstad* 89
Influenza *R A Lamb* 95
Innate Immunity: Defeating *C F Basler* 104
Innate Immunity: Introduction *F Weber* 111
Inoviruses *L A Day* 117
Insect Pest Control by Viruses *M Erlandson* 125
Insect Reoviruses *P P C Mertens and H Attoui* 133
Insect Viruses: Nonoccluded *J P Burand* 144

Interfering RNAs *K E Olson, K M Keene and C D Blair* 148
Iridoviruses of Vertebrates *A D Hyatt and V G Chinchar* 155
Iridoviruses of Invertebrates *T Williams and A D Hyatt* 161
Iridoviruses: General Features *V G Chinchar and A D Hyatt* 167

J

Jaagsiekte Sheep Retrovirus *J M Sharp, M de las Heras, T E Spencer and M Palmarini* 175
Japanese Encephalitis Virus *A D T Barrett* 182

K

Kaposi's Sarcoma-Associated Herpesvirus: General Features *Y Chang and P S Moore* 189
Kaposi's Sarcoma-Associated Herpesvirus: Molecular Biology *E Gellermann and T F Schulz* 195

L

Lassa, Junin, Machupo and Guanarito Viruses *J B McCormick* 203
Legume Viruses *L Bos* 212
Leishmaniaviruses *R Carrion Jr, Y-T Ro and J L Patterson* 220
Leporipoviruses and Suipoxviruses *G McFadden* 225
Luteoviruses *L L Domier and C J D'Arcy* 231
Lymphocytic Choriomeningitis Virus: General Features *R M Welsh* 238
Lymphocytic Choriomeningitis Virus: Molecular Biology *J C de la Torre* 243
Lysis of the Host by Bacteriophage *R F Young III and R L White* 248

M

Machlomovirus *K Scheets* 259
Maize Streak Virus *D P Martin, D N Shepherd and E P Rybicki* 263
Marburg Virus *D Falzarano and H Feldmann* 272
Marnaviruses *A S Lang and C A Suttle* 280
Measles Virus *R Cattaneo and M McChesney* 285
Membrane Fusion *A Hinz and W Weissenhorn* 292
Metaviruses *H L Levin* 301
Mimivirus *J-M Claverie* 311
Molluscum Contagiosum Virus *J J Bugert* 319
Mononegavirales *A J Easton and R Ling* 324
Mouse Mammary Tumor Virus *J P Dudley* 334
Mousepox and Rabbitpox Viruses *M Regner, F Fenner and A Müllbacher* 342
Movement of Viruses in Plants *P A Harries and R S Nelson* 348

Mumps Virus *B K Rima and W P Duprex* 356
Mungbean Yellow Mosaic Viruses *V G Malathi and P John* 364
Murine Gammaherpesvirus 68 *A A Nash and B M Dutia* 372
Mycoreoviruses *B I Hillman* 378

N

Nanoviruses *H J Vetten* 385
Narnaviruses *R Esteban and T Fujimura* 392
Nature of Viruses *M H V Van Regenmortel* 398
Necrovirus *L Rubino and G P Martelli* 403
Nepovirus *H Sanfaçon* 405
Neutralization of Infectivity *P J Klasse* 413
Nidovirales *L Enjuanes, A E Gorbalenya, R J de Groot, J A Cowley, J Ziebuhr and E J Snijder* 419
Nodaviruses *P A Venter and A Schneemann* 430
Noroviruses and Sapoviruses *K Y Green* 438

O

Ophiovirus *A M Vaira and R G Milne* 447
Orbiviruses *P P C Mertens, H Attoui and P S Mellor* 454
Organ Transplantation, Risks *C N Kotton, M J Kuehnert and J A Fishman* 466
Origin of Viruses *P Forterre* 472
Orthobunyaviruses *C H Calisher* 479
Orthomyxoviruses: Molecular Biology *M L Shaw and P Palese* 483
Orthomyxoviruses: Structure of Antigens *R J Russell* 489
Oryctes Rhinoceros Virus *J M Vlak, A M Huger, J A Jehle and R G Kleespies* 495
Ourmiavirus *G P Accotto and R G Milne* 500

VOLUME 4

P

Papaya Ringspot Virus *D Gonsalves, J Y Suzuki, S Tripathi and S A Ferreira* 1
Papillomaviruses: General Features of Human Viruses *G Orth* 8
Papillomaviruses: Molecular Biology of Human Viruses *P F Lambert and A Collins* 18
Papillomaviruses of Animals *A A McBride* 26
Papillomaviruses: General Features *H U Bernard* 34
Paramyxoviruses of Animals *S K Samal* 40
Parainfluenza Viruses of Humans *E Adderson and A Portner* 47
Paramyxoviruses *R E Dutch* 52
Parapoxviruses *D Haig and A A Mercer* 57

Partitiviruses of Fungi *S Tavantzis* 63

Partitiviruses: General Features *S A Ghabrial, W F Ochoa, T S Baker and M L Nibert* 68

Parvoviruses of Arthropods *M Bergoin and P Tijssen* 76

Parvoviruses of Vertebrates *C R Parrish* 85

Parvoviruses: General Features *P Tattersall* 90

Pecluvirus *D V R Reddy, C Bragard, P Sreenivasulu and P Delfosse* 97

Pepino Mosaic Virus *R A A Van der Vlugt and C C M M Stijger* 103

Persistent and Latent Viral Infection *E S Mocarski and A Grakoui* 108

Phycodnaviruses *J L Van Etten and M V Graves* 116

Phylogeny of Viruses *A E Gorbalenya* 125

Picornaviruses: Molecular Biology *B L Semler and K J Ertel* 129

Plant Antiviral Defense: Gene Silencing Pathway *G Szittya, T Dalmay and J Burgyan* 141

Plant Reoviruses *R J Geijskes and R M Harding* 149

Plant Resistance to Viruses: Engineered Resistance *M Fuchs* 156

Plant Resistance to Viruses: Geminiviruses *J K Brown* 164

Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes *P Moffett and D F Klessig* 170

Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes *C Caranta and C Dogimont* 177

Plant Rhabdoviruses *A O Jackson, R G Dietzgen, R-X Fang, M M Goodin, S A Hogenhout, M Deng and J N Bragg* 187

Plant Virus Diseases: Economic Aspects *G Loebenstein* 197

Plant Virus Diseases: Fruit Trees and Grapevine *G P Martelli and J K Uyemoto* 201

Plant Virus Diseases: Ornamental Plants *J Engelmann and J Hamacher* 207

Plant Virus Vectors (Gene Expression Systems) *Y Gleba, S Marillonnet and V Klimyuk* 229

Plum Pox Virus *M Glasa and T Candresse* 238

Poliomyelitis *P D Minor* 242

Polydnaviruses: Abrogation of Invertebrate Immune Systems *M R Strand* 250

Polydnaviruses: General Features *A Fath-Goodin and B A Webb* 256

Polyomaviruses of Humans *M Safak and K Khalili* 261

Polyomaviruses of Mice *B Schaffhausen* 271

Polyomaviruses *M Gravell and E O Major* 277

Pomovirus *L Torrance* 282

Potato Virus Y *C Kerlan and B Moury* 287

Potato Viruses *C Kerlan* 296

Potexvirus *K H Ryu and J S Hong* 310

Potyviruses *J J López-Moya and J A García* 313

Poxviruses *G L Smith, P Beard and M A Skinner* 322

Prions of Vertebrates *J D F Wadsworth and J Collinge* 330

Prions of Yeast and Fungi *R B Wickner, H Edskes, T Nakayashiki, F Shewmaker, L McCann, A Engel and D Kryndushkin* 336

Pseudorabies Virus *T C Mettenleiter* 341
Pseudoviruses *D F Voytas* 352

Q

Quasispecies *R Sanjuán* 359

R

Rabies Virus *I V Kuzmin and C E Rupprecht* 367
Recombination *J J Bujarski* 374
Reoviruses: General Features *P Clarke and K L Tyler* 382
Reoviruses: Molecular Biology *K M Coombs* 390
Replication of Bacterial Viruses *M Salas and M de Vega* 399
Replication of Viruses *A J Cann* 406
Reticuloendotheliosis Viruses *A S Liss and H R Bose Jr.* 412
Retrotransposons of Fungi *T J D Goodwin, M I Butler and R T M Poulter* 419
Retrotransposons of Plants *M-A Grandbastien* 428
Retrotransposons of Vertebrates *A E Peaston* 436
Retroviral Oncogenes *P K Vogt and A G Bader* 445
Retroviruses of Insects *G F Rohrmann* 451
Retroviruses of Birds *K L Beemon* 455
Retroviruses: General Features *E Hunter* 459
Rhinoviruses *N W Bartlett and S L Johnston* 467
Ribozymes *E Westhof and A Lescoute* 475
Rice Tungro Disease *R Hull* 481
Rice Yellow Mottle Virus *E Hébrard and D Fargette* 485
Rift Valley Fever and Other Phleboviruses *L Nicoletti and M G Ciufolini* 490
Rinderpest and Distemper Viruses *T Barrett* 497
Rotaviruses *J Angel, M A Franco and H B Greenberg* 507
Rubella Virus *T K Frey* 514

S

Sadwavirus *T Iwanami* 523
Satellite Nucleic Acids and Viruses *P Palukaitis, A Rezaian and F García-Arenal* 526
Seadornaviruses *H Attoui and P P C Mertens* 535
Sequiviruses *I-R Choi* 546
Severe Acute Respiratory Syndrome (SARS) *J S M Peiris and L L M Poon* 552
Shellfish Viruses *T Renault* 560
Shrimp Viruses *J-R Bonami* 567

Sigma Rhabdoviruses *D Contamine and S Gaumer* 576
Simian Alphaherpesviruses *J Hilliard* 581
Simian Gammaherpesviruses *A Ensser* 585
Simian Immunodeficiency Virus: Animal Models of Disease *C J Miller and M Marthas* 594
Simian Immunodeficiency Virus: General Features *M E Laird and R C Desrosiers* 603
Simian Immunodeficiency Virus: Natural Infection *I Pandrea, G Silvestri and C Apetrei* 611
Simian Retrovirus D *P A Marx* 623
Simian Virus 40 *A L McNees and J S Butel* 630
Smallpox and Monkeypox Viruses *S Parker, D A Schultz, H Meyer and R M Buller* 639
Sobemovirus *M Meier, A Olspert, C Sarmiento and E Truve* 644
St. Louis Encephalitis *W K Reisen* 652
Sweetpotato Viruses *J Kreuze and S Fuentes* 659

VOLUME 5

T

Taura Syndrome Virus *A K Dhar and F C T Allnutt* 1
Taxonomy, Classification and Nomenclature of Viruses *C M Fauquet* 9
Tenuivirus *B C Ramirez* 24
Tetraviruses *J A Speir and J E Johnson* 27
Theiler's Virus *H L Lipton, S Hertzler and N Knowles* 37
Tick-Borne Encephalitis Viruses *T S Gritsun and E A Gould* 45
Tobacco Mosaic Virus *M H V Van Regenmortel* 54
Tobacco Viruses *S A Tolin* 60
Tobamovirus *D J Lewandowski* 68
Tobravirus *S A MacFarlane* 72
Togaviruses Causing Encephalitis *S Paessler and M Pfeffer* 76
Togaviruses Causing Rash and Fever *D W Smith, J S Mackenzie and M D A Lindsay* 83
Togaviruses Not Associated with Human Disease *L L Coffey,* 91
Togaviruses: Alphaviruses *A M Powers* 96
Togaviruses: Equine Encephalitic Viruses *D E Griffin* 101
Togaviruses: General Features *S C Weaver, W B Klimstra and K D Ryman* 107
Togaviruses: Molecular Biology *K D Ryman, W B Klimstra and S C Weaver* 116
Tomato Leaf Curl Viruses from India *S Chakraborty* 124
Tomato Spotted Wilt Virus *H R Pappu* 133
Tomato Yellow Leaf Curl Virus *H Czosnek* 138
Tombusviruses *S A Lommel and T L Sit* 145
Torovirus *A E Hoet and M C Horzinek* 151
Tospovirus *M Tsompana and J W Moyer* 157
Totiviruses *S A Ghabrial* 163

Transcriptional Regulation in Bacteriophage *R A Weisberg, D M Hinton and S Adhya* 174
Transmissible Spongiform Encephalopathies *E D Belay and L B Schonberger* 186
Tumor Viruses: Human *R Grassmann, B Fleckenstein and H Pfister* 193
Tymoviruses *A-L Haenni and T W Dreher* 199

U

Umbravirus *M Taliansky and E Ryabov* 209
Ustilago Maydis Viruses *J Bruenn* 214

V

Vaccine Production in Plants *E P Rybicki* 221
Vaccine Safety *C J Clements and G Lawrence* 226
Vaccine Strategies *I Kusters and J W Almond* 235
Vaccinia Virus *G L Smith* 243
Varicella-Zoster Virus: General Features *J I Cohen* 250
Varicella-Zoster Virus: Molecular Biology *W T Ruyechan and J Hay* 256
Varicosavirus *T Sasaya* 263
Vector Transmission of Animal Viruses *W K Reisen* 268
Vector Transmission of Plant Viruses *S Blanc* 274
Vegetable Viruses *P Caciagli* 282
Vesicular Stomatitis Virus *S P J Whelan* 291
Viral Killer Toxins *M J Schmitt* 299
Viral Membranes *J Lenard* 308
Viral Pathogenesis *N Nathanson* 314
Viral Receptors *D J Evans* 319
Viral Suppressors of Gene Silencing *J Verchot-Lubicz and J P Carr* 325
Viroids *R Flores and R A Owens* 332
Virus Classification by Pairwise Sequence Comparison (PASC) *Y Bao, Y Kapustin and T Tatusova* 342
Virus Databases *E J Lefkowitz, M R Odom and C Upton* 348
Virus Entry to Bacterial Cells *M M Poranen and A Domanska* 365
Virus Evolution: Bacterial Viruses *R W Hendrix* 370
Virus-Induced Gene Silencing (VIGS) *M S Padmanabhan and S P Dinesh-Kumar* 375
Virus Particle Structure: Nonenveloped Viruses *J A Speir and J E Johnson* 380
Virus Particle Structure: Principles *J E Johnson and J A Speir* 393
Virus Species *M H V van Regenmortel* 401
Viruses and Bioterrorism *R F Meyer and S A Morse* 406
Viruses Infecting Euryarchaea *K Porter, B E Russ, A N Thorburn and M L Dyall-Smith* 411
Visna-Maedi Viruses *B A Blacklaws* 423

W

Watermelon Mosaic Virus and Zucchini Yellow Mosaic Virus *H Lecoq and C Desbiez* 433
West Nile Virus *L D Kramer* 440
White Spot Syndrome Virus *J-H Leu, J-M Tsai and C-F Lo* 450

Y

Yatapoxviruses *J W Barrett and G McFadden* 461
Yeast L-A Virus *R B Wickner, T Fujimura and R Esteban* 465
Yellow Fever Virus *A A Marfin and T P Monath* 469
Yellow Head Virus *P J Walker and N Sittidilokratna* 476

Z

Zoonoses *J E Osorio and T M Yuill* 485

Taxonomic Index 497
Subject Index 499

A

Adenoviruses: General Features

B Harrach, Veterinary Medical Research Institute, Budapest, Hungary

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Adenoviruses are middle-sized, nonenveloped, icosahedral, double-stranded DNA viruses of animals. The prefix adeno comes from the Greek word ἀδήν (gland), reflecting the first isolation of a virus of this type from human adenoid tissue half a century ago. Adenoviruses have since been isolated from a large variety of hosts, including representatives of every major vertebrate class from fish to mammals. Using polymerase chain reaction (PCR) technology, a large variety of putative novel adenoviruses have been detected, but isolation of such viruses and *in vitro* propagation is hampered in most cases by the lack of appropriate permissive cell cultures. Some human and animal adenoviruses can cause diseases or even death, but most are not pathogenic in non-immuno-compromised, healthy individuals. Adenoviruses have been used as model organisms in molecular biology, and important findings of general relevance have emerged from such studies, including splicing in eukaryotes. Adenoviruses have become one of the most popular vector systems for virus-based gene therapy and vaccination and have potential as antitumor tools. Wide prevalence in diverse host species and a substantially conserved genome organization make adenoviruses an ideal model for studying virus evolution.

Taxonomy

Adenoviruses belong to the family *Adenoviridae*. No higher taxonomical level has yet been established, despite the fact that certain bacteriophages (*Tectiviridae*), the green alga virus *Paramecium bursaria Chlorella virus 1* (*Phycodnaviridae*), and a virus of Archaea living in hot springs (sulfolobus turreted icosahedral virus) seem to have common evolutionary roots with adenoviruses.

There are four official genera in the family. Two genera (*Mastadenovirus* and *Aviadenovirus*) comprise adenoviruses that have probably co-evolved with mammals and birds, respectively. The other two genera (*Atadenovirus* and *Siadenovirus*) have a broader range of hosts. Atadenoviruses were named after a bias toward high A+T content in the genomes of the initial representatives, which infect various ruminant and avian hosts, as well as a marsupial. Every known reptilian adenovirus also belongs to the atadenoviruses, although their genomes do not show the same bias toward high A+T content. The very few known siadenoviruses were isolated from or detected by PCR in birds and a frog. This genus was named in recognition of the presence of a gene encoding a potential sialidase in the viruses concerned. The single confirmed fish adenovirus falls into a separate group that may eventually found a fifth genus; adenovirus-like particles have been described in additional fish species.

Within each genus, the viruses are grouped into species, which are named according to the host first described and supplemented with letters of the alphabet (**Table 1**). Host origin is only one of several criteria that are used to demarcate the species. Phylogenetic distance is the most significant criterion, with species defined as separated by more than 5–10% amino acid sequence divergence of hexon and DNA polymerase (pol), respectively. Further important characteristics come into play, especially if DNA sequence data are not available: DNA hybridization, restriction fragment length polymorphism, nucleotide composition, oncogenicity in rodents, growth characteristics, host range, cross-neutralization, ability to recombine, number of virus-associated (VA) RNA genes, hemagglutination properties, and organization of the genome. However, all of these ancillary data are expected to accord with the results of phylogenetic calculations. Thus, for example, chimpanzee adenoviruses are classified into human adenovirus species. Adenoviruses of humans have been studied far more intensively than those of other animals, and the six species (*Human adenovirus A* to *Human adenovirus F*; abbreviated informally to HAdV-A to HAdV-F) correspond to substantial 'groups' or 'subgenera' defined previously. Each human adenovirus serotype is abbreviated to HAdV hyphenated to a number.

Table 1 The taxonomy of family *Adenoviridae*[a]

Genus/species	*Serotype*	*Strain*	*Genome*
Mastadenovirus			
Bovine adenovirus A	BAdV-1		G
Bovine adenovirus B	BAdV-3		G
Bovine adenovirus C	BAdV-10		
Canine adenovirus	CAdV-1, 2		G
Equine adenovirus A	EAdV-1		
Equine adenovirus B	EAdV-2		
Human adenovirus A	HAdV-12, 18, 31		12
Human adenovirus B	HAdV-3, 7, 11, 14, 16, 21, 34, 35, 50		G
	Simian adenovirus 21 (SAdV-21)		G
Human adenovirus C	BAdV-9, HAdV-1, 2, 5, 6		1, 2, 5
Human adenovirus D	HAdV-8–10, 13, 15, 17, 19, 20,		9, 17
	22–30, 32, 33, 36–39, 42–49, 51		26, 46, 48, 49
Human adenovirus E	HAdV-4, SAdV-22–25		G
Human adenovirus F	HAdV-40, 41		G
Murine adenovirus A	MAdV-1		G
Ovine adenovirus A	BAdV-2, OAdV-2–5		BAdV-2
Ovine adenovirus B	OAdV-1		
Porcine adenovirus A	PAdV-1, 2, 3		3
Porcine adenovirus B	PAdV-4		
Porcine adenovirus C	PAdV-5		G
Tree shrew adenovirus	TSAdV-1		G
Goat adenovirus (ts)	GAdV-2		
Guinea pig adenovirus (ts)	GPAdV-1		
Murine adenovirus B (ts)	MAdV-2		
Ovine adenovirus C (ts)	OAdV-6		
Simian adenovirus A (ts)	SAdV-3		G
Squirrel adenovirus (ts)	SqAdV-1		
?	HAdV-52		G
?	SAdV-1–2, 4–20		1, 6, 7, 20
Aviadenovirus			
Fowl adenovirus A	FAdV-1	CELO	G
Fowl adenovirus B	FAdV-5	340	
Fowl adenovirus C	FAdV-4, 10	KR95, CFA20	
Fowl adenovirus D	FAdV-2, 3, 9, 11	P7-A, 75, A2-A, 380	9
Fowl adenovirus E	FAdV-6, 7, 8a, 8b	CR119, YR36, TR59, 764	
Goose adenovirus	GoAdV-1–3		
Duck adenovirus B (ts)	DAdV-2		
Pigeon adenovirus (ts)	PiAdV-1		
Turkey adenovirus B (ts)	TAdV-1, 2		
Psittacine adenovirus?	Psittacine adenovirus 1		
Falcon adenovirus?	Falcon adenovirus 1		
Atadenovirus			
Bovine adenovirus D	BAdV-4, 5, 8, strain Rus		4
Duck adenovirus A	DAdV-1		G
Ovine adenovirus D	GAdV-1, OAdV-7		7
Possum adenovirus	PoAdV-1		
Bearded dragon adenovirus (ts)	BDAdV-1		
Bovine adenovirus E (ts)	BAdV-6		
Bovine adenovirus F (ts)	BAdV-7		
Cervine adenovirus (ts)	Odocoileus adenovirus 1 (OdAdV-1)		
Chameleon adenovirus (ts)	ChAdV-1		
Gecko adenovirus (ts)	GeAdV-1	Fat-tailed gecko	
Snake adenovirus (ts)	SnAdV-1	Corn snake, python	G
Gekkonid adenovirus(?)	Tokay gecko adenovirus		
Helodermatid adenovirus(?)	Gila monster adenovirus		
Scincid adenovirus(?)	Blue-tongued skink adenovirus		
Genus Siadenovirus			
Frog adenovirus	FrAdV-1		G
Turkey adenovirus A	TAdV-3		G
?	Raptor adenovirus 1	Harris hawk	

Continued

Table 1 Continued

Genus/species	*Serotype*	*Strain*	*Genome*
Unassigned Viruses in the Family			
?	White sturgeon adenovirus 1 (WSAdV-1)		
?	Crocodile adenovirus		

[a]Official genus and species names as published in the Eighth Report of the ICTV are in italics, and tentative species (ts), proposed species (marked by a query) or single isolates are not. Because of confusion in the serotype numbering in some cases (e.g. among fowl adenoviruses), certain characteristic strains are shown for easier identification. Available full genome sequences are noted by G or by the number of the sequenced serotype(s) if those listed are not all available.

To illustrate the need to proceed carefully in developing adenovirus taxonomy, the case of the newest adenovirus isolated from human samples (HAdV-52) is illuminating. This virus seems to be sufficiently different from other human adenoviruses to merit the erection of a new species. However, it is very similar to some previously characterized Old World monkey adenoviruses (simian adenoviruses 1 and 7 (SAdV-1, SAdV-7) plus others). One taxonomical proposal would be to establish a new species, *Human adenovirus G*, containing HAdV-52 and the related monkey adenoviruses. Clearly, this would depend on epidemiological data demonstrating that HAdV-52 is properly a human virus and not an occasional, opportunistic transfer from monkeys. For similar reasons and others, classification of many nonhuman adenoviruses into species is not yet resolved.

Virion Morphology and Properties

The icosahedral capsid is 70–90 nm in diameter and consists of 240 nonvertex capsomers (called hexons), each 8–10 nm in diameter, and 12 vertex capsomers (pentons), each with a protruding fiber 9–77.5 nm in length. The members of genus *Aviadenovirus* that have been studied have two fiber proteins per vertex. Fowl adenovirus 1 (FAdV-1) even has two, tandem fiber genes of different lengths, resulting in two fibers of different sizes at each vertex. Members of species *Human adenovirus F* (and HAdV-52 and the related monkey viruses) also have two fiber genes of different lengths, but the fibers are distributed in single copies alternately on the vertices. The main capsomers (hexons) are formed by the interaction of three identical polypeptides (designated hexon, and also as polypeptide II, after a Roman numeral system based on the relative mobilities of structural proteins under reducing conditions in sodium dodecyl sulfate-polyacrylamide gel electrophoresis). Each hexon has two characteristic parts: a triangular top with three 'towers', and a pseudohexagonal base with a central cavity. Hexons, or more exactly their bases, are packed tightly to form a protein shell that protects the inner components of the virion.

In members of the genus *Mastadenovirus* 12 copies of polypeptide IX are found between the nine hexons in the center of each facet. However, polypeptide IX is not present in the members of any other genus. Two monomers of polypeptide IIIa penetrate the hexon shell at the edge of each facet, and multiple copies of polypeptide VI form a ring underneath the peripentonal hexons. Penton bases are formed at the vertices by the interaction of five copies of polypeptide III, and are tightly associated with one (or, in the aviadenoviruses, two) fibers, each consisting of three copies of polypeptide IV in the form of a shaft of characteristic length with a distal knob. Polypeptide VIII is situated at the inner surface of the hexon shell. Polypeptides VI and VIII appear to link the capsid to the virion core, which consists of a single copy of the DNA genome complexed with four polypeptides (V, VII, X, TP). Polypeptide V exists only in mastadenoviruses.

Adenoviruses are stable on storage in the frozen state. They are stable to mild acid and insensitive to lipid solvents. Heat sensitivity varies among the genera.

Nucleic Acid, Genome Organization, and Replication

The adenovirus genome is a linear molecule of double-stranded DNA (26 163–45 063 bp) containing an inverted terminal repeat (ITR) of 36–368 bp at its termini, with the 5′ ends of the genome linked covalently to a terminal protein (TP). The nucleotide composition is 33.7–63.8% G+C. The genetic organization of the central part of the genome is conserved throughout the family, whereas the terminal parts show large variations in length and gene content (**Figure 1**). Splicing was first discovered in adenoviruses, and is a common means of expressing mRNAs in this virus family. In the conserved region, most late genes are expressed by splicing from the rightward-oriented major late promoter located in the pol gene. The early genes encoding pol, the precursor of TP (pTP), and the DNA-binding protein (DBP) are spliced from leftward-oriented promoters. Where it has been examined, splicing is also a common feature of genes in the nonconserved regions.

Replication of various human adenoviruses has been studied in detail, in particular with HAdV-2. Virus entry takes place via interactions of the fiber knob with specific receptors on the surface of a susceptible cell followed by internalization via interactions between the penton base

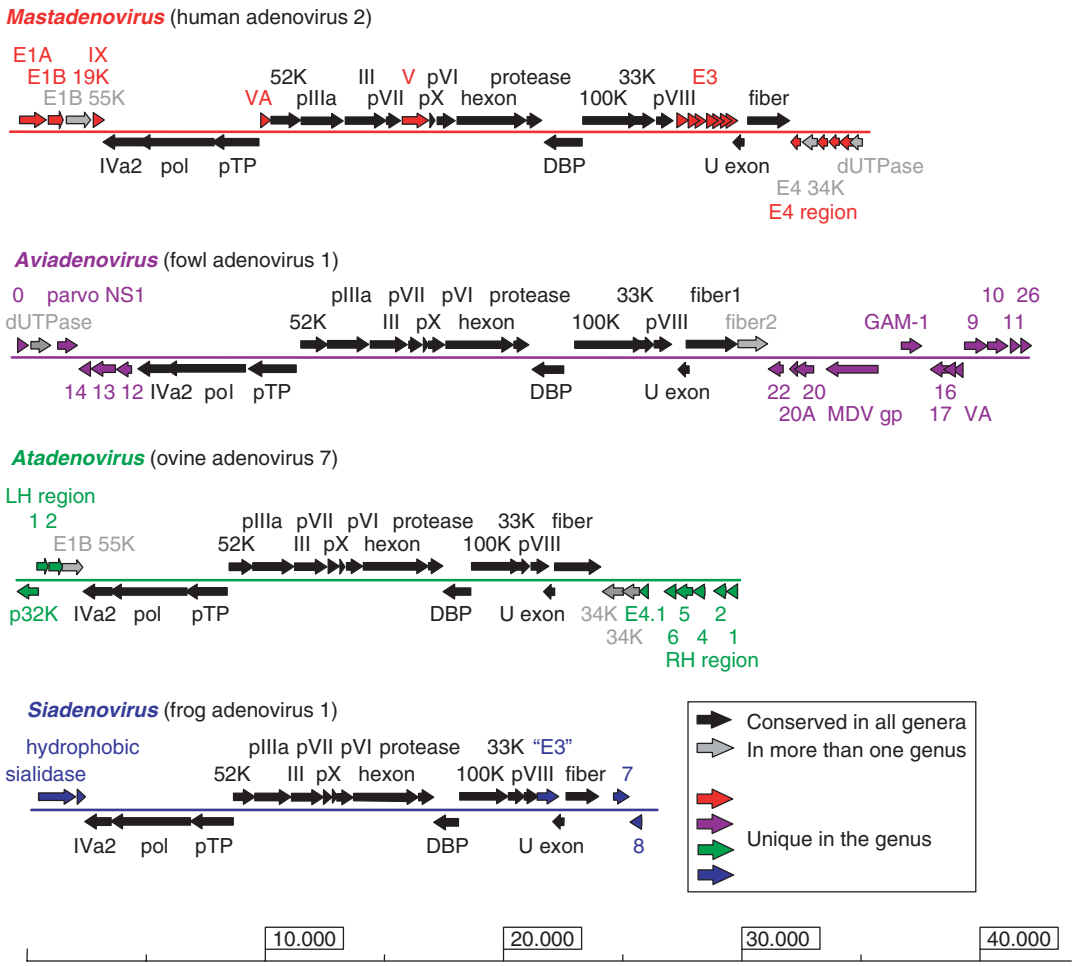


Figure 1 Schematic illustration of the different genome organizations found in representative members of the four adenovirus genera. Black arrows depict genes conserved in every genus, gray arrows show genes present in more than one genus, and colored arrows show genus-specific genes. Reprinted from Benkö M, Harrach B, Both GW, *et al.* (2005) Family *Adenoviridae* In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 213–228. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

and cellular α_v integrins. After uncoating, the virus core is delivered to the nucleus, which is the site of virus transcription, DNA replication, and assembly. Virus infection mediates the shutdown of host DNA synthesis and later RNA and protein synthesis. Transcription of the adenovirus genome by host RNA polymerase II involves both DNA strands of the genome and initiates (in HAdV-2) from five early (E1A, E1B, E2, E3, and E4), two intermediate, and the major late (L) promoter. All primary transcripts are capped and polyadenylated, with complex splicing patterns producing families of mRNAs. In primate adenoviruses, one or two VA RNA genes are usually present upstream from the main pTP coding region. These are transcribed by cellular RNA polymerase III and facilitate translation of late mRNAs and blocking of the cellular interferon response. Corresponding VA RNA genes have not been identified in nonprimate adenoviruses, although a nonhomologous VA RNA gene has been mapped in some aviadenoviruses near the right end of the genome. More generally, the replication of aviadenoviruses has been shown to involve significantly different pathways from those characterized in human adenoviruses. This is not unexpected, given the considerable differences in gene layout between nonconserved regions of the genome.

Proteins

About 40 different polypeptides (the largest number being in fowl adenoviruses and the smallest in siadenoviruses) are produced. Almost a third of these compose the virion, including a virus-encoded cysteine protease, which processes a number of precursor proteins (these are prefixed with p). With the exception of polypeptides V and IX, the other structural proteins are conserved in every genus. Products of the four early regions of mastadenoviruses modulate the host cell's transcriptional machinery (E1 and E4), assemble the virus DNA replication complex

(E2), and subvert host defense mechanisms (E3). The E2 region (encoding DBP, pol, and pTP) is well conserved throughout the family, while the E3 and E4 regions show great variation in length and gene content among the mastadenoviruses. E1A, E1B 19K, and the E3 and E4 regions (with the exception of 34K) occur only in mastadenoviruses. Genes encoding proteins related to 34K are also present in atadenoviruses, sometimes in duplicate. Intermediate (IX, only in mastadenoviruses, and IVa2) and late gene products (in some mastadenoviruses expressed from five transcription units, L1–L5) are concerned with assembly and maturation of the virion. Late proteins include: 52K (scaffolding protein) and pIIIa (L1); III (penton base), pVII (major core protein), V (minor core protein, only in mastadenoviruses) and pX (L2); pVI, hexon (II) and protease (L3); 100K, 33K (and an unspliced version, 22K) and pVIII (L4); and fiber (IV) (L5). Seemingly, there are no lipids in adenovirus particles. However, the fiber proteins and some of the nonstructural proteins are glycosylated.

Antigenic Properties

Adenovirus serotypes are differentiated on the basis of neutralization assays. A serotype is defined as either exhibiting no cross-reaction with others or showing a homologous/heterologous titer ratio of greater than 16 (in both directions). For homologous/heterologous titer ratios of 8 or 16, a serotype assignment is made either if the viral hemagglutinins are unrelated (as shown by lack of cross-reaction in hemagglutination-inhibition tests), or if substantial biophysical, biochemical, or phylogenetic differences exist. Antigens at the surface of the virion are mainly type specific. Hexons are involved in neutralization, and fibers in neutralization and hemagglutination inhibition. Soluble antigens associated with virus infection include surplus capsid proteins that have not been assembled. The genus-specific antigen is located on the basal surface of the hexon, whereas serotype-specific antigens are located mainly on the tower region. Practical problems may arise during serotyping (and in phylogenetic calculations) from the occasional occurrence of homologous recombination in the hexon gene (e.g., HAdV-16 and SAdV-23). Under natural circumstances, recombination occurs only between members of the same species.

Biological Properties

The natural host range of adenovirus types is usually confined to one animal species or to closely related species. This also applies for cell cultures. Some human adenoviruses (mainly in species *Human adenovirus C*) cause productive infection in various animal (rodent or ruminant) cells. Several viruses cause tumors in newborn rodents. Subclinical infections are frequent in various virus–host systems. Transmission occurs from the throat, feces, the eye, or urine, depending on the virus serotype. Certain human adenovirus types (given in parentheses below) are predominantly associated with a specific pathology, such as adenoidal–pharyngeal conjunctivitis (3, 4, 7, and 14), acute respiratory outbreaks (4, 7, 14, and 21), epidemic kerato-conjunctivitis (8, 19, and 37), or venereal disease (3). HAdV-40 and HAdV-41 can be isolated from the feces of young children with acute gastroenteritis, and only the rotaviruses are known to cause more cases of infantile viral diarrhea. HAdV-11 is associated with hemorrhagic cystitis (mostly in immuno-suppressed patients after organ transplantation). The newer types of human adenovirus (42–51) were isolated from acquired immune deficiency syndrome patients. In mammals, mastadenovirus infection is common, but disease is usually manifested only if predisposing factors (such as management problems, crowding, shipping, or concurrent bacterial infections) are also present. Canine adenovirus, which can cause hepatitis or respiratory disease in dogs, seems to be an exception and has caused epizootics in foxes, bears, wolves, coyotes, and skunks.

Certain siadenoviruses and atadenoviruses that may have undergone host switches during their evolution seem to be more pathogenic in general in their new hosts: birds, ruminants, and a marsupial. Egg drop syndrome virus (duck adenovirus 1, DAdV-1) or turkey hemorrhagic enteritis virus (turkey adenovirus 3, TAdV-3) can cause serious economical losses for the poultry industry. An atadenovirus (Odocoileus adenovirus) caused a hemorrhagic epizooty and killed thousands of mule deers in California.

Adenoviruses infecting susceptible cells cause similar cytopathology consisting of early rounding of cells, aggregation or lysis of chromatin, followed by the later appearance of characteristic eosinophilic or basophilic nuclear inclusions.

HAdV-5 has been engineered and used extensively as a gene delivery vector. Other human adenoviruses such as HAdV-35, or even nonhuman serotypes (ovine adenovirus 7 and canine adenovirus 1), are being tested as a means of overcoming the problem posed by preexisting neutralizing antibodies in the human population, and also to achieve targeting to specific organs and tissues. Bovine, porcine, canine, and fowl adenovirus types have also been tested as novel antigen delivery vectors for immunizing the cognate animal species.

Mastadenoviruses

Mastadenoviruses occur only in mammals, and in general, mammals are host to only mastadenoviruses. However, half of the adenoviruses found in ruminant species are atadenoviruses and the only marsupial adenovirus identified so far is also an atadenovirus. Mastadenoviruses were originally distinguished from aviadenoviruses on the basis of different genus-specific complement-fixing antigens.

Virus infectivity is inactivated by heating at 56 °C for more than 10 min.

The mastadenovirus genomes that have been sequenced range in size between 30 288 bp and 37 741 bp, and in nucleotide composition from 40.8% to 63.8% G+C. The ITR is considerably longer (93–368 bp) and more complex (containing a variety of cellular factor-binding sites) than in members of the other genera. Proteins encoded only by the mastadenoviruses are polypeptides V and IX, and most of those from the E1A, E1B, E3, and E4 regions. Polypeptide IX, besides cementing the hexons on the outer surface of the capsid, has been demonstrated to act as a transcriptional activator, and also takes part in nuclear reorganization during infection. Polypeptide V is a core protein that, in association with cellular protein p32, seems to be involved in the transport of viral DNA into the nucleus of the infected cell. The E3 and E4 regions can differ markedly even among different mastadenovirus species. The E3 region is considerably shorter and simpler in nonprimate adenoviruses than in monkey, ape, and human adenoviruses. The simplest layout is found in murine adenovirus 1, where it consists of a single 12.5K gene. In the E4 region, only the 34K gene seems to be conserved in all mastadenoviruses. This gene is duplicated in bovine adenovirus 3 and porcine adenovirus 5.

The entry processes of human mastadenoviruses into the cell are well characterized. The coxsackievirus and adenovirus receptor (CAR) is the most common, but not the only, receptor for the attachment to the cell.

Aviadenoviruses

Members of the genus *Aviadenovirus* occur only in birds and possess a common genus-specific complement-fixing antigen. However, birds can also harbor siadenoviruses and atadenoviruses. Aviadenovirus virions contain two fibers per vertex. Fowl adenovirus 1 (FAdV-1) has two fiber genes, and two projections of considerably different lengths from each penton base. Other fowl adenoviruses also have two fibers per vertex, but apparently only one fiber gene, and the projections are therefore of similar lengths. For attachment of FAdV-1 to the cell, the long, but not the short, fiber utilizes CAR. The aviadenovirus genomes are considerably larger (20–45%) than those of mastadenoviruses. FAdV-1 and FAdV-9, with genomes of 43 804 and 45 063 bp, respectively, represent the longest adenovirus DNA molecules known to date. The nucleotide composition of the partial or full genome sequences characterized so far is between 53.8% and 59% G+C. The length of the ITR is 51–72 bp.

The genes encoding polypeptides V and IX are absent from aviadenovirus genomes, as well as the genes in the mastadenovirus E1 and E3 regions (**Figure 1**). A dUTP pyrophosphatase (dUTPase) gene is situated next to the left end of the genome in the aviadenoviruses studied to date; a dUTPase gene is also present in some mastadenoviruses, but it is at the right genome end. The right end of aviadenovirus genomes contains a large number of transcription units that are confined to this genus. The majority of genes and proteins from this region have not yet been characterized in detail. The GAM-1 protein of FAdV-1 has been demonstrated to have an anti-apoptotic effect, and to activate the heat-shock response in the infected cell. GAM-1, in synergy with the protein encoded by ORF22, binds the retinoblastoma protein and can activate the E2F pathway. Some as yet uncharacterized aviadenovirus gene products are similar to proteins of other viruses, such as the nonstructural protein NS1 (Rep protein) of parvoviruses, or triacylglycerol lipase, a homolog of which occurs in an avian herpesvirus (Marek's disease virus).

Aviadenoviruses have been isolated from various poultry species including turkey, goose, and Muscovy duck, as well as a number of wild birds, such as falcon and parrots. However, chicken adenoviruses have been studied in most detail. Twelve fowl adenovirus serotypes have been classified into species *Fowl adenovirus A* to *Fowl adenovirus E* (FAdV-A to FAdV-E). Unfortunately, the type numbering of fowl adenoviruses became inconsistent when different systems were adopted in Europe and North America. The resulting confusion was partially resolved by the introduction of FAdV-8a and FAdV-8b types, though their distinctness could not be fully confirmed because of the ambiguous results of serum neutralization tests.

Avian adenoviruses have been associated with diverse disease patterns, including body hepatitis, bronchitis, pulmonary congestion, edema, and gizzard erosion in various bird species, but they generally seem to be less pathogenic than siadenoviruses and atadenoviruses in birds. An exception to this is FAdV-4, the causative agent of hydropericardium syndrome in chickens, which causes considerable losses mainly in Asia. FAdV-1 (chick embryo lethal orphan virus), FAdV-9, and FAdV-10 are studied extensively for their potential feasibility as gene delivery vectors.

Atadenoviruses

As opposed to the mastadenoviruses and aviadenoviruses, which have clear host origins, the members of genus *Atadenovirus* represent a much broader host range spanning several vertebrate classes. This genus was originally established to cope with the classification of certain exceptional bovine and ovine adenoviruses with unusual characteristics. The name refers to the nucleotide composition of the first members of this genus, which is biased toward high A+T content. The large phylogenetic distance between the ruminant atadenoviruses and the mastadenoviruses and aviadenoviruses (**Figure 2**) inspired a hypothesis that they may have originated via tranfers of adenoviruses with lower vertebrate hosts. Indeed, recent studies

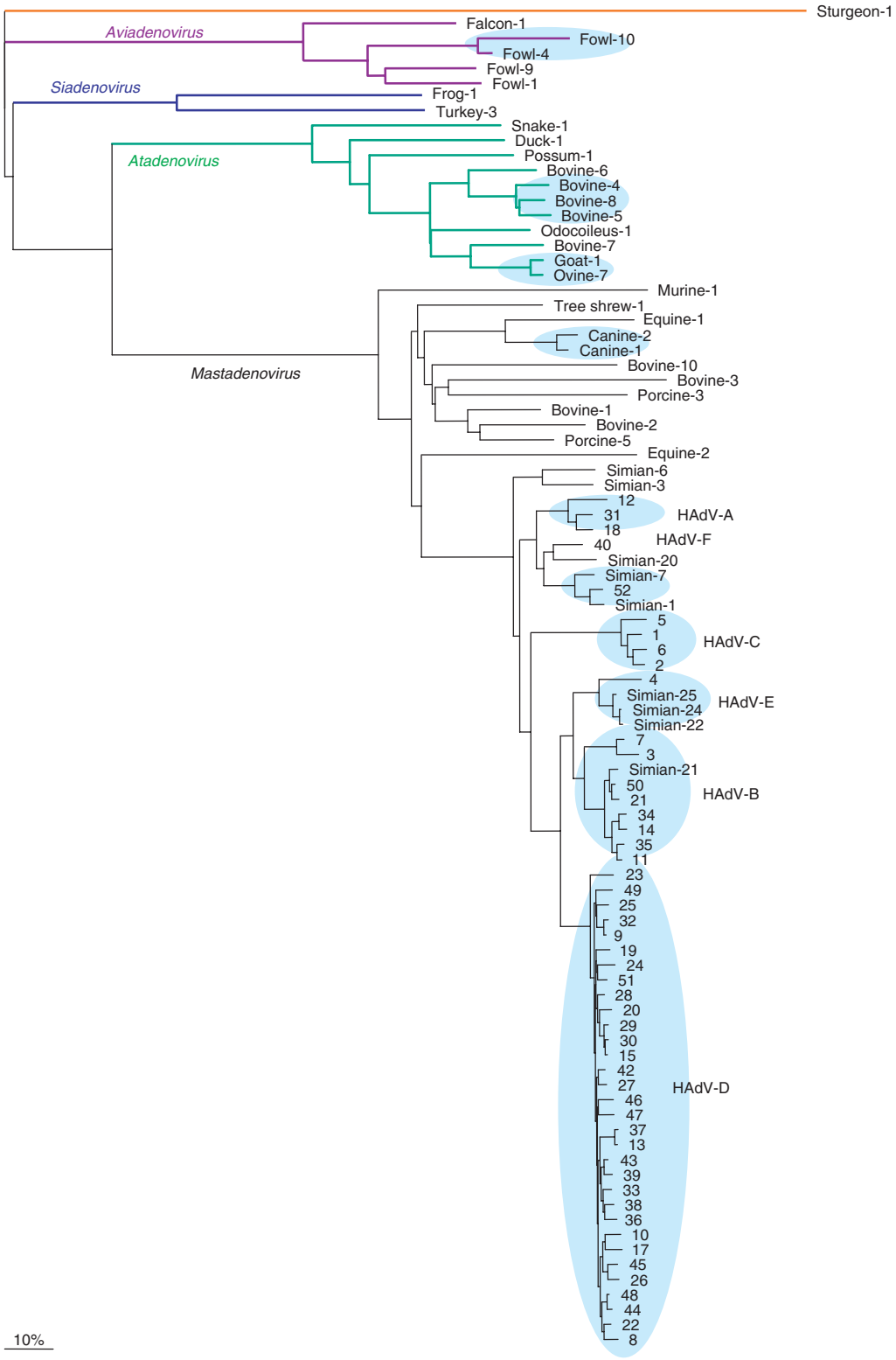


Figure 2 Phylogenetic tree of adenoviruses based on distance matrix analysis of hexon amino acid sequences. The analysis was performed using the Protdist and Fitch programs of the PHYLIP 3.65 package. The unrooted tree was visualized with white sturgeon adenovirus as the outgroup. Adenoviruses are denoted by the name of the host and the serotype number, or only by the serotype number for human adenoviruses. Viruses that belong to the same species are grouped by light-blue ovals, and human adenovirus species are indicated by their informal abbreviations. Data for HAdV-16, HAdV-41, and SAdV-23 were excluded, as evolution of their hexon genes may have involved homologous recombination events. The bar indicates 10% difference between two neighboring sequences.

have confirmed that every reptilian adenovirus for which data are available belongs to this genus. However, atadenoviruses found in reptiles seem to have a balanced nucleotide content. Additional atadenovirus types have been detected in various species of birds and mammals, including duck, domestic and wild ruminants, as well as a marsupial, the brushtail possum. DAdV-1, which was originally isolated from flocks of laying hens that showed a sharp decrease in egg production, was moved to the genus *Atadenovirus* from the genus *Aviadenovirus*.

The virions of bovine atadenoviruses are more heat stable than those of mastadenoviruses. They retain substantial infectivity after treatment for 30 min at 56 °C.

The size of sequenced atadenovirus genomes ranges from 27 751 bp (snake adenovirus 1, SnAdV-1) to 33 213 bp (DAdV-1) with an ITR of 46 (OAdV-7) to 118 bp (SnAdV-1). For ruminant, marsupial, and avian atadenoviruses, the nucleotide composition ranges between 33.7% (OAdV-7) and 43% G+C (DAdV-1).

The common ancestry of the atadenoviruses is supported by their shared genomic organization. Putative early regions (LH and RH) on the genome ends occupy the places of the mastadenovirus E1 and E4 regions (**Figure 1**). The functions of the novel genes in these regions have been elucidated only partially. The LH region includes a gene encoding a novel structural protein (p32K), and genes LH1 (not present in DAdV-1), LH2, and LH3. LH3 seems to be weakly related to the E1B 55K gene of mastadenoviruses, and its product has also been shown to be a structural protein. In the RH region, E4.3 and E4.2 seem to be homologs of the E4 34K gene of mastadenoviruses. E4.1 is absent from DAdV-1. Closer to the right end, different numbers of RH genes are found in different viruses. Many genes seem to be the result of duplication events, with one family encoding putative F-box proteins. There is only one copy of an F-box gene in SnAdV−1, whereas five are present in ovine adenovirus 4. Surprisingly, SnAdV−1 also encodes a protein that is homologous to the gene 105R product found in tree shrew adenovirus, a mastadenovirus. The DAdV-1 genome has an extension at the right end containing seven additional genes, which are so far unique to this virus. These genes are likely to be host specific in their function. This region of the DAdV-1 genome also encodes a VA RNA gene that is seemingly homologous to that of FAdV-1. In atadenoviruses, no immuno-modulatory genes such as those in the mastadenovirus E3 region have yet been identified, though it is likely that they exist.

Siadenoviruses

The fourth genus has been established for two adenoviruses that are of very distant host origin and yet share a similar genome organization and are phylogenetically closely related. These are frog adenovirus 1 (FrAdV-1), which was isolated from the renal tumor of a leopard frog, and TAdV-3, which was isolated from birds. Additional strains of TAdV-3, originating from turkey, pheasant and chicken, as well as avirulent (vaccine) strains, show little or no diversity in terms of nucleotide sequence or serology, respectively. The genomes of FrAdV-1 and TAdV-3 are collinear and represent the minimal gene content recognized among adenoviruses to date (**Figure 1**). In addition to the set of genes occupying the central genome region and conserved throughout the whole family, only five additional genes have been identified. The protein encoded next to the left ITR seems to be related to bacterial sialidases. Adjacent to this is a gene predicted to code for a highly hydrophobic protein. The E3 gene takes this name solely because of its position between the pVIII and fiber genes; it is not homologous to any of the mastadenovirus E3 genes, or to any other known genes. Similarly, ORF7 and ORF8, which are situated at the right end of the genome, have no recognizable homologs elsewhere. The genes encoding polypeptides V and IX are absent, as well as those in the mastadenovirus E1, E3 and E4 regions. FrAdV-1 and TAdV-3 have the shortest adenovirus genomes, 26 163 and 26 263 bp in size, respectively, with ITRs of 36 and 39 bp and nucleotide compositions of 38% and 35% G+C.

The evolutionary history of siadenoviruses awaits elucidation. In the same way that it is possible that atadenoviruses originated with reptiles (see above), the siadenoviruses may represent adenoviruses that have co-evolved with amphibians, with TAdV-3 the result of an interclass host switch.

FrAdV-1 is supposedly nonpathogenic, whereas TAdV-3 is associated with serious diseases in various hosts, including hemorrhagic enteritis in turkeys, marble spleen disease in pheasants, and splenomegaly in chickens. Recently, a putative novel siadenovirus has been detected in fatally diseased birds of prey.

Phylogeny

The five clusters of adenoviruses defined by gene organization and sequence-based phylogenetic calculations correspond to the four recognized genera (*Mastadenovirus*, *Aviadenovirus*, *Atadenovirus*, and *Siadenovirus*) plus one proposed genus for fish adenoviruses (**Figure 2**). The evolutionary distances among the adenoviruses seem to be generally proportional to those among their hosts, supporting co-evolution of virus and host. However, there are some exceptions where very distantly related viruses infect the same host, for example, the bovine mastadenoviruses and atadenoviruses. Thus, in addition to co-evolution, several host switches might have occurred. According to this hypothesis, the mastadenoviruses and aviadenoviruses represent co-evolution, whereas reptilian adenoviruses

may have switched to ruminants, birds, and marsupials. Indeed, genome organization and phylogenetic analysis indicate that SnAdV-1 most closely represents the most ancient common ancestor of the atadenoviruses. Siadenoviruses may have an amphibian origin, though the small number of viruses in this group limits analyses of this possibility. More recent host switches can also be identified within genera, for example between humans and other primates, or vice versa.

See also: Adenoviruses: Malignant Transformation and Oncology; Adenoviruses: Molecular Biology; Adenoviruses: Pathogenesis; Electron Microscopy of Viruses; Evolution of Viruses; Gene Therapy: Use of Viruses as Vectors; Phylogeny of Viruses; Recombination; Virus Databases; Virus Species.

Further Reading

Benkö M, Harrach B, Both GW, *et al.* (2005) Family *Adenoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 213–228. San Diego, CA: Elsevier Academic Press.

Burnett RM (1997) The structure of adenovirus. In: Chiu W, Burnett RM, and Garcea RL (eds.) *Structural Biology of Viruses*, pp. 209–238. Oxford: Oxford University Press.

Davison AJ, Benkö M, and Harrach B (2003) Genetic content and evolution of adenoviruses. *Journal of General Virology* 84: 2895–2908.

Doerfler W and Böhm P (eds.) (2003) Adenoviruses: Model and vectors in virus host interactions. In: *Current Topics of Microbiology and Immunology,* vol. 272. Berlin: Springer.

Farkas SL, Benkö M, Élö P, *et al.* (2002) Genomic and phylogenetic analyses of an adenovirus isolated from a corn snake (*Elaphe guttata*) imply common origin with the members of the proposed new genus *Atadenovirus*. *Journal of General Virology* 83: 2403–2410.

Kovács GM, LaPatra SE, D'Halluin JC, and Benkö M (2003) Phylogenetic analysis of the hexon and protease genes of a fish adenovirus isolated from white sturgeon (*Acipenser transmontanus*) supports the proposal for a new adenovirus genus. *Virus Research* 98: 27–34.

Russell WC (2000) Update on adenovirus and its vectors. *Journal of General Virology* 81: 2573–2604.

Wold WSM and Tollefson A (eds.) (2006) Methods in molecular medicine. In: *Adenovirus Methods and Protocols*, 2nd edn., vol. 131, pp. 299–334. Totowa, NJ: Humana Press.

Zsivanovits P, Monks DJ, Forbes NA, *et al.* (2006) Presumptive identification of a novel adenovirus in a Harris hawk (*Parabuteo unicinctus*), a Bengal eagle owl (*Bubo bengalensis*), and a Verreaux's eagle owl (*Bubo lacteus*). *Journal of Avian Medicine and Surgery* 20: 105–112.

Adenoviruses: Malignant Transformation and Oncology

A S Turnell, The University of Birmingham, Birmingham, UK

© 2008 Elsevier Ltd. All rights reserved.

Transforming and Oncogenic Properties of Human Adenoviruses

Human adenoviruses (Ads) are small, nonenveloped DNA viruses with linear, double-stranded genomes of about 35 kbp that are associated with a broad range of infections, but are most commonly linked with acute infections of the upper respiratory and gastrointestinal tracts. The clinical manifestations of an adenovirus infection are dependent on the adenovirus and the host cell type infected. The oncogenic capacity of adenovirus was first established by John Trentin and colleagues in 1962 when they showed that human adenovirus 12 (Ad12) was tumorigenic when injected into newborn hamsters. Adenovirus tumorigenicity was shown to be dependent upon various factors, such as virus serotype, virus dose, host age at infection time, and host genetic and immune status. Indeed, the tumorigenic potential of various adenoviruses was attributed to their ability to evade the host immune system, as nontumorigenic adenoviruses were shown to induce tumors in immunocompromised hosts. Adenovirus tumorigenicity in this regard is associated with nonproductive infections of human viruses in rodent cells. It is generally believed that a productive lytic infection in human cells precludes human adenoviruses from being tumorigenic in humans (see later). Consistent with the ability of nontumorigenic adenoviruses to promote tumors in nude mice, tissue culture studies later revealed that rodent cells could be transformed by both tumorigenic and nontumorigenic adenoviruses. Adenovirus serotypes have been classified into species (*Human adenovirus A* to *Human adenovirus F*) according to various criteria and correlated broadly with oncogenic potential (**Table 1**).

Transfection studies in primarily rodent, but also human, cells have revealed that various combinations of adenovirus DNA can transform primary cells in tissue culture, and this has greatly facilitated the study of the transformation properties of specific adenovirus genes. As with other DNA tumor viruses, the oncogenic properties of the various adenovirus genes become apparent when the mechanics of the productive lytic cycle are considered. Adenoviruses normally infect quiescent

epithelial cells. In order to replicate the viral genome, the virus must first create a cellular environment conducive for DNA replication. It does this by reactivating the host cell cycle to create an S-phase-like environment. Additionally, it prevents the host cell from undergoing premature apoptosis, allowing for the assembly and production of progeny virions. Thus viral genes have evolved to circumvent the normal growth restrictions placed upon the cell. It is therefore perhaps no surprise that many viruses, in addition to adenovirus, target the tumor suppressor gene products pRB and p53 and proteins involved in DNA damage response/repair pathways (see later) to overcome normal cell cycle checkpoints. The focus of the rest of this article is a consideration of the biological functions of adenovirus-transforming genes.

Transforming and Immortalizing Properties of E1A

E1A expression is essential for a fully productive adenovirus infection and for adenovirus-mediated transformation. The Ad5 *E1A* gene encodes two major proteins of 289 and 243 amino acid residues (R) that differ only by an internal sequence of 46 residues present in the larger protein, namely, conserved region 3 (CR3), which is required specifically for adenovirus early gene expression during infection (**Figure 1**). As for E1A species from other Ad serotypes, the Ad5E1A 289R and 243R proteins possess transforming and immortalizing activity, although the smaller 243R protein is more efficient than the 289R protein in this regard. In isolation, however, E1A displays

Table 1 Classification and oncogenicity of human adenoviruses

Species	*Serotype*	*Oncogenicity in rodents*	*Transformation* in vitro
Human adenovirus A	12,18,31	High	+
Human adenovirus B	3, 7, 11, 14, 16, 21, 34, 35, 50	Moderate	+
Human adenovirus C	1, 2, 5, 6	Low or none	+
Human adenovirus D	8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51	Low or none	+
Human adenovirus E	4	Low or none	+
Human adenovirus F	40, 41	Not reported	?

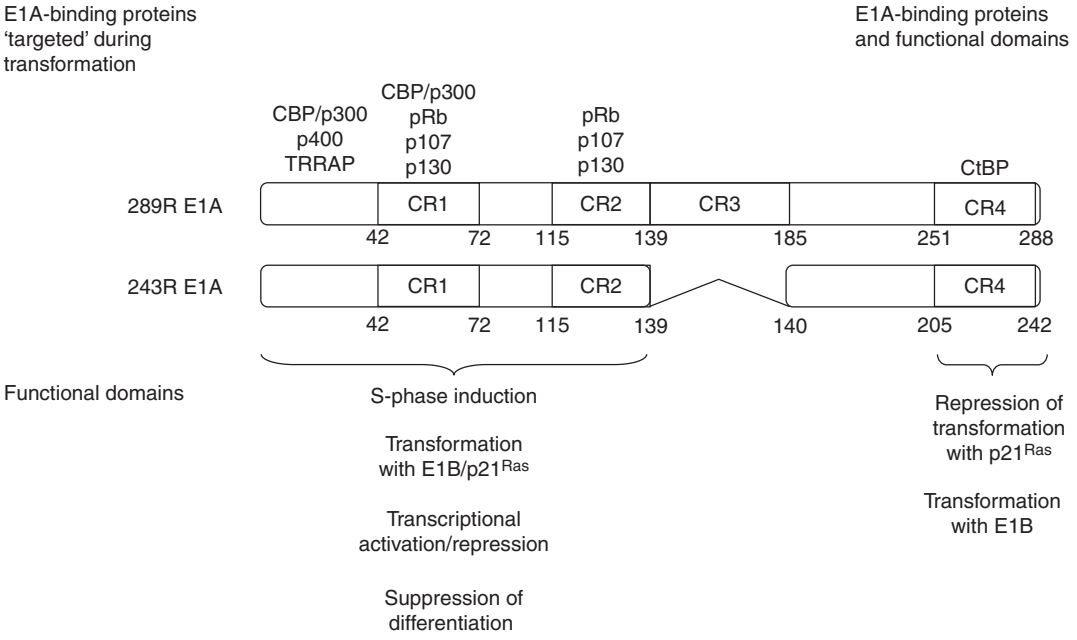


Figure 1 Linear depiction of the Ad5 243R and 289R E1A proteins. The regions conserved between serotypes are labeled as CR1, CR2, CR3, and CR4; amino acid ordinates show CR boundaries. The E1A-binding proteins considered important for E1A-mediated transformation are listed and their relative binding sites depicted. E1A regions important in cell cycle control and cellular transformation are also shown.

only poor transforming activity. This is due specifically to E1A's potent proapoptotic activity. E1A's transforming capacity only becomes apparent upon its expression with co-operating oncogenes such as adenovirus *E1B*, adenovirus *E4*, or activated, mutant $p21^{ras}$. Sequence comparisons of the largest E1A proteins of several adenovirus serotypes have identified four regions of sequence conservation, designated CR1, CR2, CR3, and CR4, which are largely responsible for many of E1A's biological activities, although the less well conserved N-terminal region also participates in E1A function (**Figure 1**). The transforming properties of E1A are mainly attributable to E1A's ability, through the reorchestration of host cell transcription programmes, to force quiescent cells to enter, and, for rodent cells at least, pass through the cell cycle; E1A does not promote mitosis in human cells. E1A also blocks cellular differentiation programmes.

The N-terminal region and CR1 of E1A promote transformation by targeting the transcriptional co-activators CBP and p300 (CBP/p300); E1A mutants unable to bind CBP/p300 are transformation defective. Specifically, E1A binding to CBP/p300 allows for quiescent G_0 rodent cells to enter the cell cycle and progress into S-phase. CBP and p300 are highly conserved lysine acetyltransferases. In this regard, the cellular activities of sequence-specific transcription factors such as p53, c-Myc, and NF-κB are all regulated by CBP/p300-acetyltransferase activity. CBP/p300 also acetylate the core histone proteins H2A, H2B, H3, and H4 to regulate transcription. It is as yet unclear, however, whether E1A utilizes CBP/p300 acetyltransferases during transformation to reprogram cellular transcription through altered acetylation, or, alternatively, promotes transformation by inhibiting CBP/p300 acetyltransferase activities. Indeed, E1A generally inhibits CBP/p300 transcription function in transient gene reporter assays. A model has been proposed, however, whereby E1A utilizes cellular acetyltransferases to alter cellular histone acetylation status, in order to stimulate E2F-regulated transcription programmes and induce S-phase. It is suggested that E1A first facilitates the demethylation of K9 of histone H3 to promote the release of repressor E2F transcription factors from transcriptionally repressed promoters, whereupon it subsequently promotes the acetylation of K9 of histone H3 and the recruitment of activator E2F transcription factors to these promoter elements to activate transcription. The specific role played by CBP/p300 in the acetylation of K9 in this particular situation awaits clarification. Additionally, the ability of E1A to modulate CBP/p300 binding to transcription factors, independent of acetyltransferase activity, might also affect transforming activity. Theoretically, E1A could utilize other cellular acetyltransferases to promote G1–S progression. Specifically, it is possible that E1A could utilize the acetyltransferase P/CAF, to which it also binds, to regulate transcription programmes during transformation. Unfortunately, E1A point mutants have not yet been identified that unambiguously distinguish between CBP/p300 and P/CAF binding *in vivo*, such that the contribution of P/CAF in E1A-mediated transformation is unclear. It seems unlikely from the literature published thus far, however, that the interaction of other proteins with the N-terminal region of E1A spanning residues 4 and 25 is necessary to promote transformation.

Recent evidence suggests that the APC5 and APC7 components of the anaphase-promoting complex (APC/C) interact with CBP/p300 through protein interaction domains evolutionarily conserved in the N-terminal region, and CR1 ($Fx^{D}/_{E}xxxL$ motif) of E1A. E1A specifically targets APC/C-CBP/p300 complexes in order to promote the cellular transformation of rat embryo fibroblasts. Thus, exogenous expression of wild-type (w.t.) APC5 or APC7 suppresses the ability of E1A to cooperate with either E1B or activated $p21^{Ras}$ in the transformation process, whereas the expression of APC/C mutants unable to bind CBP/p300 does not suppress E1A-induced transformation. E1A also targets endogenous CBP/p300-APC/C complexes during transformation, such that RNAi-mediated knockdown of either APC5 and/or APC7 restores transforming activity to E1A species unable to bind CBP/p300, such as R2G.

Interestingly, the N-terminal region of E1A possesses additional transforming capacity. At limiting concentrations of E1A, it has been determined that residues 26–35 also promote transformation in cooperation with activated $p21^{Ras}$. It is yet to be established whether this region also cooperates with *E1B*, or other cellular or viral oncogenes, in transformation. It has been determined, however, that this region of E1A binds specifically to two cellular proteins, p400 and TRRAP, in order to promote transformation. p400 is a member of the SWI2/SNF2 chromatin-remodeling family, which associates specifically with TRRAP, the DNA helicases TAP54α/β, actin-like proteins, and the human homolog of the *Drosophila* enhancer of polycomb protein, EPc, while TRRAP is a Myc-binding protein and also a component of distinct GCN5/SAGA and Tip60/NuA4 acetyltransferase complexes. *In vitro* binding studies have indicated that E1A binds to p400 through a central region (residues 951–2048) of the protein, which also encompasses the SWI2/SNF2 homology domain and TAP54-binding site. Interestingly, E1A can also associate with p400 at a distinct C-terminal site *in vivo* (residues 2033–2484), through its interaction with TRRAP. Similar binding studies indicate that E1A binds TRRAP through residues 1360–2260. It has been determined that E1A utilizes p400 complexes to promote transformation, as co-expression of p400 fragments with E1A enhances E1A-dependent transformation. However, co-expression of a TRRAP fragment that binds E1A suppresses

E1A-dependent transformation. Collectively, these data suggest that multiple and distinct E1A/p400/TRRAP complexes exist within cells which contribute differently to the transformation process. It has been proposed that during transformation E1A perturbs normal p400 and TRRAP function by forming E1A/p400 and E1A/TRRAP complexes with distinct subunit compositions to regulate chromatin structure and function.

The ability of CR1 and CR2 of E1A to promote cellular transformation resides in its capacity to interact with the protein product of the pRB tumor suppressor gene. Similar to its ability to bind CBP/p300, it has been established that E1A binding to pRB allows for quiescent G_0 rodent cells to enter the cell cycle and progress into S-phase; however, E1A must bind both CBP/p300 and pRB in order to promote mitosis in primary rodent cells. The integrity of the core DLxCxE motif in CR2 as well as CR1 is essential for E1A interaction with pRB and pRB family members p107 and p130. E1A binds specifically to the A and B pocket domains of these proteins. Like E1A's interaction with CBP and p300, any specific requirement for E1A binding to particular pRB family members during transformation is currently unknown. It has been proposed that E1A interaction with pRB and either p107 and/or p130 is important in driving quiescent cells into S-phase. In a temporally coordinated manner, E1A disrupts p130–E2F4 transcriptional repression complexes, allowing for chromatin remodeling, histone demethylation, and histone acetylation. E1A's ability to mimic cyclin-dependent kinase (CDK)-dependent phosphorylation of pRB through binding to pRB disrupts pRB–E2F1 complexes and allows the recruitment of activator E2Fs to promoters. This recruitment induces E2F-regulated genes important in S-phase induction, such as CDK2, CDC6, and cyclins E and A. The ability of E1A to disrupt pRB–E2F1 complexes also allows for E1A to overcome pRB-induced senescence programs initiated by CDK inhibitors, $p21^{CIP1/WAF1}$ and $p16^{INK4}$.

Interestingly, E1A has been found to reorganize, and associate with, PML-containing nuclear oncogenic bodies (PODs), nuclear structures implicated in regulating apoptosis, tumor suppression, antiviral responses, DNA repair, and transcriptional regulation. E1A recruitment to PODs is dependent on the pRB-interaction domain (pRB can be found associated with PODs). Whether E1A similarly associates with CBP in PODs is unclear. The precise requirement for E1A–POD interaction in the transformation process is unclear, though it is suspected that E1A might be associated with multiple POD functions.

E1A has been shown to target the CtBP family of transcriptional co-repressors through a conserved PxDLS motif located toward the C-terminal region of the protein. E1A's interaction with CtBP is believed to be important in the transformation process. The role of the region encoded by exon 2 in cellular immortalization and cellular transformation is, however, context dependent. In cooperation with exon 1, exon 2 enhances the E1A-dependent immortalization of rodent cells and E1A-dependent transformation with E1B, such that certain exon 2 deletion mutants have reduced immortalization/transformation capacities relative to w.t. 12S (243R) E1A. In contrast, exon 2 suppresses 12S E1A/$p21^{Ras}$-mediated immortalization and transformation, such that exon 2 deletion mutants enhance the E1A-dependent immortalization and transformation of primary cells in culture, and, moreover, their tumorigenicity and metastatic potential in nude mice. These differences in exon 2 functions presumably reside in the differential capacities of E1B and $p21^{Ras}$ to activate/suppress cellular pathways involved in promoting immortalization and/or transformation.

It has been suggested that exon 2 promotes immortalization and transformation with E1B through its ability to complement exon 1 immortalization functions and evade Mortality stage 2 (crisis). It has also been proposed that the ability of exon 2, in the context of w.t. 12S E1A, to function as a tumor suppressor and suppress $p21^{Ras}$-mediated transformation resides in its ability to promote mesenchymal–epithelial cell transitions through the regulation of specific epithelial-promoting transcription programs. The ability of exon 2 mutants to promote hypertransformation, enhanced tumorigenicity and metastatic potential, relative to w.t. 12S E1A, of both epithelial and fibroblastic cells is believed to be mediated, in part, by the activation of the small Ras-related proteins, Rac and Cdc42, suggesting that w.t. 12S E1A may normally serve to suppress these pathways. The current literature suggests that the ability of exon 2 to regulate oncogenesis is mediated at least in part by its ability to bind to CtBP. A more thorough examination however of exon 2-binding proteins and exon 2 deletion mutants is required to determine whether E1A binding to CtBP accounts for all of these cellular phenotypes.

Oncogenicity of E1A

E1A from oncogenic serotypes specifically regulates pathways involved in immune regulation and contributes toward immune evasion – a major determinant of adenovirus oncogenicity. It is well established that E1A from the tumorigenic serotype, Ad12, downregulates the presentation of major histocompatibility complex class I (MHC-I) antigens in rodent cells, whereas E1A from the nontumorigenic serotype, Ad5, does not. MHC-I downregulation allows for adenovirus-infected cells to avoid clearance by cytotoxic T-lymphocytes (CTLs). A major difference between oncogenic and nononcogenic serotypes in this regard is in their ability to affect transcription mediated through the MHC-I enhancer sites R1 and R2. Oncogenic serotypes repress transcription mediated through the MHC-I enhancer by upregulating the transcriptional

repressor COUP-TFII, which binds to the R2 site, while concomitantly reducing the level of NF-κB binding to the R1 site by reducing the level of phosphorylation of the p50 subunit of NF-κB. Nononcogenic serotypes do not upregulate COUP-TFII levels, and NF-κB binds to the R1 site with high affinity. Oncogenic serotypes have also been shown to interfere with MHC-I presentation by downregulating components of the immune proteasome, such as LMP2 and LMP7, and downregulating the transporter proteins associated with antigen presentation, TAP1 and TAP2. A further feature of Ad12 E1A that facilitates immune evasion is that it does not produce immunological epitopes recognized by CTLs, whereas Ad5 E1A does. Ad12-infected cells are also resistant to natural killer cell lysis. These functions of E1A are largely attributed to an alanine-rich 20-residue spacer region present in Ad12 E1A between CR2 and CR3, which is not present in the nontumorigenic adenovirus serotypes. Other regions of Ad12 E1A do possess tumorigenic properties, but how these manifest mechanistically awaits further investigation.

Transforming Properties of Adenovirus E1B and E4 Regions

In the absence of cooperating oncogenes, such as $p21^{ras}$, *E1B*, or *E4*, *E1A* has little or no capacity to transform and immortalize embryonic human cells in tissue culture systems and only limited capacity to transform and immortalize embryonic rodent cells; E1A-immortalized clones tend to harbor mutations in the p53 tumor suppressor gene. Although the *E1B* and *E4* genes have no transforming capacity alone, they can cooperate with *E1A* in the transformation process through their ability to neutralize E1A-induced, p53-dependent and p53-independent, apoptosis. The biological properties of E1B and E4 proteins, in this regard, will be discussed in turn.

E1B-19K

The *E1B* gene expresses two principle proteins: E1B-19K and E1B-55K. E1B-19K was initially identified as a homolog of the cellular antiapoptotic protein, BCL-2. Despite sequence conservation between the two proteins, it is of interest to note that E1B-19K and BCL-2 differ in the methods they employ to neutralize the proapoptotic functions of BCL-2 family members BAX and BAK. Specifically, BCL-2 inhibits the activation of BAX/BAK by binding to BID and preventing its caspase-8 dependent cleavage to active tBID, which, under normal circumstances, promotes BAX/BAK heterooligomerization at mitochondrial membranes and the subsequent induction of apoptosis. E1B-19K, on the other hand, inhibits apoptosis by binding directly to tBID-activated BAX and/or BAK, preventing BAX/BAK heterooligomerization and the subsequent downstream events that lead to apoptosis (**Figure 2**). Significantly, the ability of E1A to induce p53-independent apoptosis relies, in part, upon its ability to specifically activate BAK/BAX. In this context, and akin to ultraviolet (UV)-induced DNA damage-induced apoptotic pathways, E1A can promote apoptosis by specifically downregulating the BCL-2 antiapoptotic

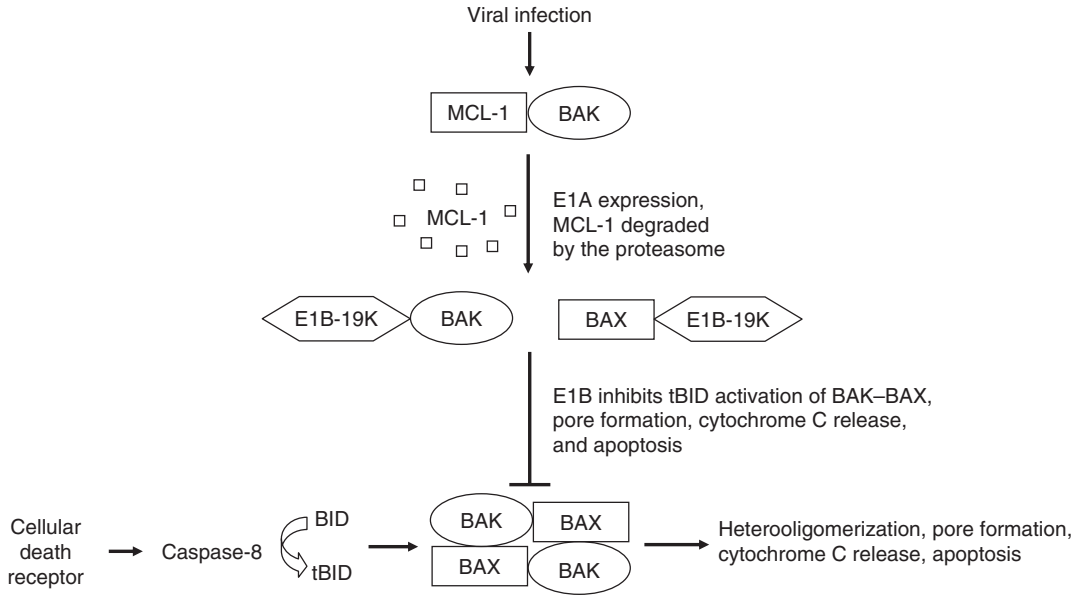


Figure 2 Role of E1B-19K in antiapoptotic signaling pathways. E1B-19K inhibits E1A-induced, p53-independent apoptosis by binding tBID-bound BAK and BAX, and specifically inhibiting the mitochondrial-release apoptosis pathway. See text for details.

family member MCL-1 through proteasomal-mediated degradation. MCL-1 normally serves to functionally inhibit BAK, but upon E1A-mediated degradation of MCL-1, BAK can activate apoptosis. In this regard, E1B-19K cooperates with E1A in the transformation process by inhibiting E1A-activated BAK (**Figure 2**).

E1B-55K and E4orf6

The Ad5 E1B-55K and 34K E4orf6 proteins functionally cooperate during viral infection to regulate p53, the MRE11-RAD50-NBS1 (MRN) complex (**Figure 3**), and potentially late-phase viral mRNA nuclear export and translation. For this reason, it is perhaps best to consider their roles in cellular transformation together. The current perception is that the independent, and combined, abilities of both E1B-55K and E4orf6 to repress p53-transactivation of proapoptotic genes underlies their respective, and synergistic, capacities to co-operate with E1A in the transformation process. The requirement for E1B-55K and E4orf6 binding and regulation of the MRN complex in the transformation process awaits clarification.

Specifically, the central region of E1B-55K encompassing residues 250–310, and a small region around residue 180, bind to the N-terminal transactivation domain of p53, blocking co-activator recruitment and further inhibiting p53 transcription function through specific C-terminal transcriptional repression domains. In Ad5 E1A/E1B-55K transformed cells, E1B-55K and p53 are sequestered in cytoplasmic 'phase-dense' aggresome structures that also contain microfilaments, centrosomal proteins, hsp70, and WT1. The cytoplasmic location of E1B-55K is mediated in part by a leucine-rich nuclear export signal (NES) located between residues 83 and 93; the nuclear export of E1B-55K is dependent upon the CRM1 cellular export receptor. Significantly, mutation of the critical leucine residues in the E1B-55K NES potentiates E1A/E1B-mediated transformation through enhanced inhibition of p53 transactivation function and the accumulation of mutant E1B-55K and p53 in PODs. Consistent with a role for E1B-55K localized to PML-containing nuclear bodies in p53 repression and cellular transformation, it has been demonstrated through mutational studies that SUMOylation of K104 in E1B-55K not only promotes its recruitment to PML bodies, but augments p53 transcriptional repression and enhances cellular transformation. Other functions of E1B-55K might also contribute toward its transforming potential. Indeed, the Ad12 large E1B protein (equivalent to Ad5 E1B-55K) can extend the lifespan of human embryo fibroblasts, bypassing Mortality stage 1 through ALT maintenance of telomere length.

The C-terminal region of E4orf6 can, independently of E1B-55K, bind to the C-terminal oligomerization domain (residues 318–360) of p53 to repress specifically the p53 N-terminal transactivation function; E4orf6 disrupts p53 interaction with TFIID component TAFII31. In contrast to E1B-55K, however, E4orf6 also possesses an independent and distinct ability to relieve C-terminal p53 transcriptional repression through binding. Akin to E1B-55K, E4orf6 cooperates with E1A in the transformation of primary baby rat kidney (BRK) cells. E1A/E4orf6 transformants grow more slowly than E1A/E1B-55K transformants, but are essentially morphologically indistinguishable. As already indicated, E4orf6 will also synergize with E1B-55K,

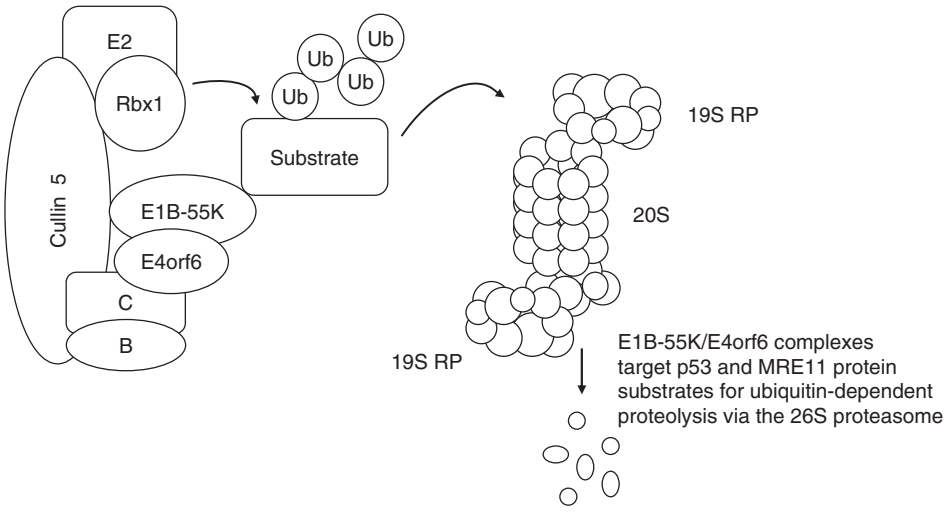


Figure 3 Role of E1B-55K/E4orf6 complexes in the regulation of p53 and MRE11. E1B-55K/E4orf6 form complexes with the Cullin 5-based E3 ubiquitin ligase to promote the poly-ubiquitylation, and hence 26S proteasomal-mediated destruction, of p53 and MRE11. See text for more detail. B, C, Elongin B, C; E2, ubiquitin conjugating enzyme; Ub, ubiquitin.

or E1B-55K and E1B-19K, to enhance the frequency of E1A-dependent transformation; E1A/E1B/E4orf6 mutants express lower levels of p53 than E1A/E1B and E1A/E4orf6 transformants. Interestingly, E1A/E1B/E4orf6 transformants arise more rapidly than E1A/E1B and E1A/E4orf6 transformants, and when injected subcutaneously into the nude mouse promote more rapid tumor formation. In support of the tumorigenic properties of the E4orf6 protein, it also converts human 293 cells from nontumorigenic to tumorigenic in nude mice. Interestingly, and in stark contrast to E1B-55K, E4orf6 does not cooperate with E1A and E1B-19K in the transformation process; in fact, E4orf6 suppresses E1A/E1B-19K mediated transformation. It has been suggested that the additional ability of E4orf6 to relieve p53 transcriptional repression contributes toward its ability to suppress transformation in this instance.

As indicated earlier, E1B-55K and E4orf6 also regulate the activity of the MRN complex. Functionally, the MRN complex is integral to both the ATM- and ATR-DNA damage response/repair signaling pathways initiated in response to ionizing and UV irradiation, respectively. It has been suggested that adenovirus circumvents these checkpoint controls through the ability of E1B-55K and E4orf6 to target the MRE11 component of the MRN complex for ubiquitin-mediated, proteasome-dependent degradation. Inactivation of MRE11 by E1B-55K and E4orf6 ensures, in the context of viral infection, that linear viral DNA is not concatemerized by double-strand break–repair pathways initiated by the MRN complex.

The functional cooperativity displayed by E1B-55K and E4orf6 is mediated through protein–protein interaction. It has been suggested that the N-terminal 55 residues of E4orf6 are required for its binding to E1B-55K, although others suggest that the amphipathic α-helical region toward the C-terminus governs the interaction; the central region of E1B-55K encompassing residues 262–326, and a region around residue 143, are required for interaction with E4orf6. Although the E4orf6-binding site on E1B-55K overlaps with the p53-binding site, E1B-55K and E4orf6 bind independently of p53. Crucial to establishing the biochemical basis of this functional cooperativity between the two proteins, mass spectrometric identification of cellular proteins bound to E4orf6 in p53-null H1299 cells has revealed that E4orf6 can bind to the Cullin-containing, Cul5-Elongin B/C-Rbx1 E3 ubiquitin ligase complex (**Figure 3**). Significantly, E1B-55K is only found in complexes with E4orf6 bound to the Cul5-Elongin B/C-Rbx1 complex, suggesting that E1B-55K and E4orf6 might not bind directly. E4orf6 contains within its primary sequence two functional Elongin B/C-interaction (BC) boxes located between residues 46–55 and 121–130, which define its direct interaction with Elongin C. E1B-55K also contains a putative BC-box located between residues 179 and 188, though this region does not bind Elongin B or C directly. It has been postulated that E4orf6 might also bind Cul5 directly, though this awaits confirmation.

Functional studies have indicated that E4orf6–E1B-55K utilizes the Cul5-Elongin B/C-Rbx1 ubiquitin ligase to promote the poly-ubiquitylation and hence 26S proteasomal-mediated degradation of p53 and MRE11 during viral infection and cellular transformation. E4orf6 is postulated to recruit the functional E3 ligase complex, whereas E1B-55K is proposed to interact with the substrate protein targeted for degradation. The absolute depletion of functional p53, MRE11, and potentially other cellular proteins by the E4orf6–E1B-55K cellular ubiquitin ligase complexes might explain why E4orf6 and E1B-55K are functionally more adept at enhancing the frequency of E1A-dependent transformation than either protein in isolation.

E1B-55K binds the hnRNP, RNA-binding protein E1B-AP5 independently of E4orf6. The central region of E1B-55K encompassing residues 262–326, and regions around residues 180, 380, and 484 all participate in E1B-AP5 binding; E4orf6 can modulate E1B-AP5 binding to E1B-55K. However, E1B-AP5 is not targeted for degradation during viral infection by E1B-55K and E4orf6, suggesting that E1B-55K is more than just a substrate adaptor for the E4orf6–Cul5-containing E3 ubiquitin ligase. The E1B-55K/E1B-AP5 complex is proposed to regulate the shutdown of host cell mRNA export during infection. Interestingly, overexpression of E1B-AP5 suppresses E1A/E1B-55K-mediated transformation, suggesting that E1B-55K might target E1B-AP5 directly to facilitate transformation.

E4orf3

The 11K E4orf3 gene product operates in redundant pathways with E4orf6 and E1B-55K during viral infection to regulate RNA processing and viral DNA replication. Mechanistically, E4orf3 cooperates with E4orf6 in the inhibition of DNA damage repair, presumably through binding DNA-PK and inactivation of the MRN complex. Indeed, Ad5 E4orf3 redistributes the MRN complex to nuclear PODs away from viral replication centers. Ad4 and Ad12 E4orf3 do not cause relocalization of the MRN complex, suggesting serotype-specific modulation of MRN function. E4orf3 similarly binds E1B-55K and targets it to PODs. Akin to E4orf6, E4orf3 will co-operate with E1A and E1A/E1B-55K to transform primary BRK cells in tissue culture and increase the tumorigenicity of E1A/E1B-55K transformed BRKs in nude mice. E4orf3 will also co-operate with E1A/E4orf6 and E1A/E1B-55K/E4orf6 in transformation. Like E4orf6, E4orf3 increases the frequency of transformation, the rate of cell growth, and the saturation densities to which transformants grow.

Ad9 E4orf1

Ad9 is unique among the adenovirus family in that it promotes estrogen-dependent mammary tumors in rodents. The oncogenic capacity of Ad9 has been mapped to a single protein, E4orf1. Although conserved among different adenoviruses (45–51% identity), only the Ad9 protein is oncogenic, although other adenovirus E4orf1 proteins do display some transforming characteristics (e.g., anchorage-independent growth). It has been suggested that the oncogenic capacity of the Ad9 protein resides in its differential ability to bind the first PDZ domain of the PDZ-containing, MAGUK homology protein, and tumor suppressor protein, ZO-2. The C-terminal PDZ-binding domain of Ad9 E4orf1 selectively targets ZO-2 and redistributes it to the cytoplasm. Consistent with a role in oncogenicity, ZO-2 inhibits Ad9 E4orf1 transformation of the rat embryo fibroblast cell line, CREF. Activation of phosphoinosite 3-kinase (PI3K) by the PDZ-binding domain of E4orf1 at the plasma membrane is also crucial in the transformation process. PI3K activation presumably allows for the specific initiation of downstream survival signaling pathways, and the activation of other potential oncoproteins (e.g., $p21^{Ras}$).

'Hit and Run' Transformation

As outlined earlier, the prevailing, generally held view is that adenovirus infection is not a causative agent of human malignancy. Intriguingly, however, and consistent with early studies looking at human simplex virus- and Abelson leukemia virus-mediated transformation, studies have suggested that certain adenovirus genes may promote transformation by a 'hit and run' mechanism whereby adenovirus DNA, mRNA, or protein are only rarely retained in the adenovirus-transformed cells. Incidentally, this makes it very difficult to tell whether adenovirus infection actually promotes human tumorigenesis, or whether 'hit and run' transformation is merely an *in vitro* phenomenon of no clinical relevance. It has been suggested that adenovirus genes are required for the initiation of transformation, but are not required for the maintenance of the transformed phenotype. Although mechanistically ill-defined, it is suggested that adenovirus genes could promote gene mutation and/or direct genetic instability through interaction with cellular proteins. Specifically, studies have shown that when E4orf3 and/or E4orf6 cooperate with E1A in transformation, in the majority of instances the transformed cells do not retain E1A, E4orf6, and/or E4orf3, though there are clear exceptions where these proteins can be detected. Interestingly, co-expression of these proteins with E1B-55K subsequently facilitates their detection in transformed cells, suggesting that E1B-55K antagonizes the genetic instability promoted by E1A, E4orf6, and E4orf3. Significantly, a role for these proteins in promoting genetic instability is already well established. The N-terminal region of E1A and CR3 have been previously implicated in causing both random and nonrandom host cell chromosome aberrations, as well as abnormal mitoses generating both aneuploid and polyploid cells in human cells and rodent cells, whereas E4orf3 and E4orf6 have been implicated in promoting genetic instability through the interaction with MRN and DNA-PK and inhibiting the repair of damaged DNA.

Conclusions

Adenovirus has long served as a faithful model system for investigating the molecular mechanisms of cellular immortalization, transformation, and tumorigenicity. During this time, studies on adenovirus E1A and E1B have identified the functions of key tumor suppressor proteins, not to mention the functions of proteins not directly involved in oncogenesis. In more recent years, the roles of E4 proteins in the transformation process have become increasingly apparent. It is reasonable to anticipate that future studies investigating adenovirus-mediated transformation will enhance further our understanding of the molecular mechanisms underlying oncogenesis.

See also: Adenoviruses: General Features; Adenoviruses: Molecular Biology; Adenoviruses: Pathogenesis.

Further Reading

Berk AJ (2005) Recent lessons in gene expression, cell cycle control, and cell biology from adenovirus. *Oncogene* 24: 7673–7685.

Chinnadurai G (2004) Modulation of oncogenic transformation by the human adenovirus E1A C-terminal region. *Current Topics in Microbiology and Immunology* 273: 139–161.

Endter C and Dobner T (2004) Cell transformation by human adenoviruses. *Current Topics in Microbiology and Immunology* 273: 163–214.

Frisch SM and Mymryk JS (2002) Adenovirus-5 E1A: Paradox and paradigm. *Nature Reviews. Molecular Cell Biology* 3: 441–452.

Gallimore PH and Turnell AS (2001) Adenovirus E1A: Remodelling the host cell, a life or death experience. *Oncogene* 20: 7824–7835.

White E (2006) Mechanisms of apoptosis regulation by viral oncogenes in infection and tumorigenesis. *Cell Death and Differentiation* 13: 1371–1377.

Williams JF, Zhang Y, Williams MA, Hou S, Kushner D, and Ricciardi RP (2004) E1A-based determinants of oncogenicity in human adenovirus groups A and C. *Current Topics in Microbiology and Immunology* 273: 245–288.

Adenoviruses: Molecular Biology

K N Leppard, University of Warwick, Coventry, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction and Classification

Adenoviruses were first discovered during the 1950s, in studies of cultures from human adenoids and virus isolation from respiratory secretions. Their study accelerated dramatically in the 1960s after it was demonstrated that a human adenovirus could cause tumors in experimental animals, leading to the hope that such work would lead to a better understanding of human cancer. What has been learned subsequently, as well as making a significant contribution in this area, has also had a major influence on our understanding of fundamental eukaryotic cell processes such as RNA splicing and apoptosis.

The adenoviruses are classified in the family *Adenoviridae*. Historically, this family was divided into two genera, *Aviadenovirus* and *Mastadenovirus*, containing viruses that infected avian and mammalian hosts, respectively, but the advent of large-scale DNA sequencing has led to two further genera being established, based on comparisons of genome sequences. Thus, for example, the genus *Atadenovirus* contains two of five species of bovine adenovirus and one of four species of sheep adenovirus, the others being members of the genus *Mastadenovirus*. The fourth genus, *Siadenovirus*, so far contains a frog adenovirus and a turkey adenovirus.

There are six human adenovirus species, *Human adenovirus A* through *F*, all of which are classified in the genus *Mastadenovirus*. These species correspond to the subgroups of human adenovirus established earlier on the basis of the hemagglutination characteristics of their virions, their genome nucleotide composition, and their oncogenicity in rodents. There are 51 known human adenovirus serotypes that are distributed between these species. Each causes characteristic disease symptoms in man. Although there has been an increasing level of interest in other adenoviruses in recent years, the majority of what is known about adenovirus molecular biology comes from the study of human adenoviruses 2 and 5, two very closely related members of species *Human adenovirus C*. The following account is therefore based on these two viruses; important differences from this picture that pertain to other adenoviruses are pointed out where appropriate.

Particle Structure

Adenovirus particles have icosahedral capsids containing a core that is a complex of proteins and DNA (**Figure 1**). There is no lipid envelope and no host proteins are found in the virion; thus all components of the particle are encoded by the virus (see the section of titled 'Gene expression'). The structural proteins of the particle are known by roman numerals in order of decreasing apparent size on polyacrylamide gels.

The capsid shell is built of 252 capsomers. Twelve of these are known as pentons. They occupy the vertices of the shell and are formed of two components, penton base, which is a pentamer of the penton polypeptide

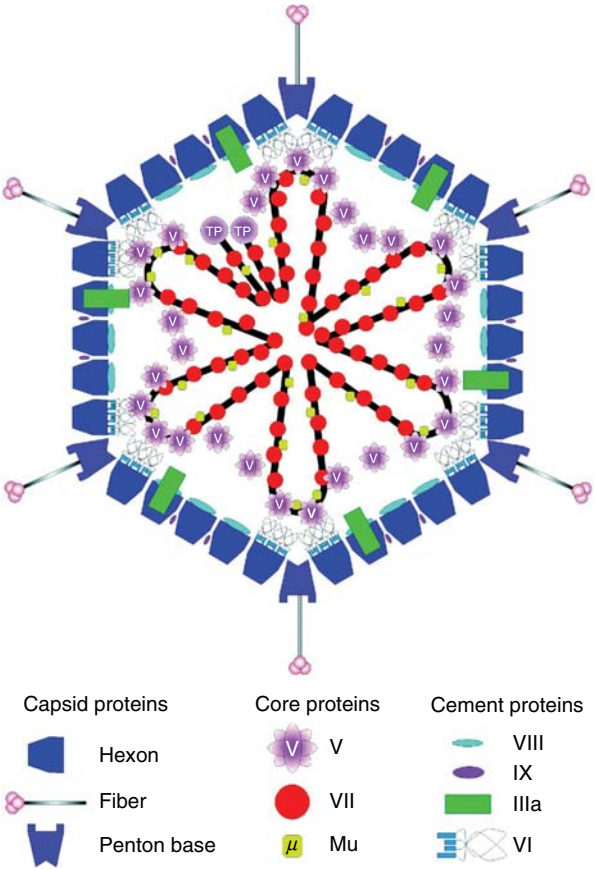


Figure 1 A cut-through diagram of a mature adenovirus particle. The positions of proteins within the shell of the particle are defined based on cryoelectron microscopy analyses. However, the indicated structure of the DNA core is largely hypothetical; the core appears disordered in structural analyses. Several of these proteins (IIIa, VI, VII, μ, and TP) are synthesized in precursor form (pIIIa, pVI, pVII, pX, and pTP, respectively) and assembled as such into immature particles before processing by the adenovirus protease. Reproduced from Russell WC (2000) Update on adenovirus and its vectors. *Journal of General Virology* 81: 2573–2604, with permission from Society for General Microbiology.

(protein III), and fiber, which is a trimer of the fiber polypeptide (protein IV). This fiber unit projects outward from each vertex of the particle; it has a long shaft, formed of a repeating β-sheet module, and a distal knob domain. Both the penton base and fiber components play specific roles in attachment and internalization (see the section of titled 'Attachment and entry'). Each of the remaining 240 capsomers, which are known as hexons, are trimers of the hexon polypeptide (protein II), which is thus the major capsid protein of the virus.

Inside the shell, the viral core comprises the genome in complex with three proteins: VII, V and μ, the latter being a processed form of polypeptide X. The capsid shell is stabilized by proteins IIIa, VI, VIII, and IX, some of which bridge between the shell and the core. Protein IX trimers occupy crevices on the external faces of the shell; when IX is absent, the particle is less stable and hence its capacity for DNA is reduced. Finally, the particle carries a virus-coded, sequence-specific protease of 23 kDa that is essential both for the maturation of newly formed particles to an infectious state and for particle uncoating after infection. Thus, adenovirus particles are considerably more complex than those of the smaller icosahedral DNA viruses of the families *Polyomaviridae* and *Papillomaviridae*.

Genome Organization

All adenovirus genomes are linear, double-stranded DNA molecules. They vary in length from genus *Siadenovirus* members at ~26 kbp to genus *Aviadenovirus* members at ~45 kbp; genomes of members of the genera *Mastadenovirus* and *Atadenovirus* are 30–36 kbp in length. The genomes are all thought to have a virus-coded protein, known as the terminal protein (TP), covalently attached to each genome 5′-end. This linkage is a consequence of the mechanism of DNA replication (see below). Also at each end of the linear genome is an inverted terminal repeat sequence varying in length from ~40 to 200 bp; the terminal repeats of human adenovirus 5 are 103 bp.

Among the human adenoviruses, there is a high level of conservation of genome organization. Both genome strands are used to encode proteins from genes that are designated E1A, E1B, E2, E3, E4, IX, IVa2, and the major late transcription unit (MLTU), while two small noncoding RNAs are expressed from the VA genes (**Figure 2**). The E2 and E3 genes are divided into regions A and B while the MLTU is divided into five regions, L1–L5, each with a distinct polyadenylation site. Within each gene/region there are typically multiple proteins encoded. This genome organization applies to all human adenoviruses with only subtle variations, such as the presence of one rather than two VA genes, or the presence of a second fiber coding region in L5, in a few types. Considering adenoviruses in general, the organization of the center

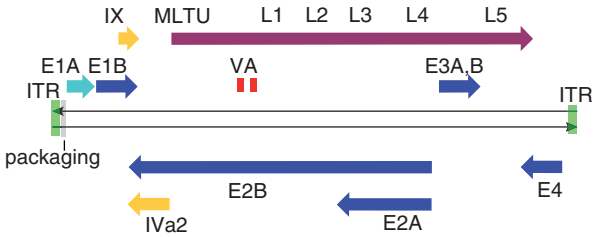


Figure 2 A schematic representation of the genes and major genome features of human adenoviruses (not to scale). The duplex DNA is represented by black lines, with arrowheads indicating 3′-ends. Inverted terminal repeats (ITRs) and the packaging sequence are indicated as green and gray boxes, respectively. Protein-coding genes are indicated as colored arrows, the color indicating the phase of infection when expression commences (see also **Figure 3(a)**), and RNA pol III transcripts as red boxes.

of the genome (where the structural proteins and replication proteins are coded) is conserved but the regions closer to the genome termini do not necessarily contain recognizable E1A, E1B, and E4 sequences. However, it is presumed that the products encoded here perform the same types of function as these genes supply in human adenoviruses. Unlike the other adenovirus genes, E3 is highly divergent in both sequence and the number and nature of its protein products, even between different human adenoviruses. This may be responsible for aspects of viral pathogenicity and tropism that differ between adenovirus types.

Attachment and Entry

Primary attachment of particles to the target cell surface is thought to be mediated, for all adenoviruses, by the knob domains at the distal ends of the fibers that project from the virion vertices. For human viruses in species *Human adenovirus A*, *C*, *E*, and *F*, the cell receptor for attachment appears to be a molecule designated CAR (coxsackie adenovirus receptor), a cell-surface protein of the immunoglobulin superfamily that is present on a variety of cell types. CAR mediates cell-to-cell interactions, being present at tight junctions and other types of intercellular contact, and is also used as a receptor by members of the unrelated human coxsackie viruses (members of the genus *Enterovirus*, family *Picornaviridae*). The location of CAR has consequences for virus entry and exit (see the section titled 'Assembly and release'). Other adenoviruses, such as members of species *Human adenovirus D* and certain animal adenoviruses, may also use this receptor. However, members of species *Human adenovirus B* clearly use a distinct receptor, the CD46 molecule. CD46 is involved in regulating complement activation and is widely distributed on human cells. Other

cell surface molecules may also serve as receptors for some or all of these viruses under particular circumstances.

Following primary attachment, there is a secondary interaction between the penton base component of the particle and cell-surface integrins. These are a family of heterodimeric cell adhesion molecules, several of which can bind the penton base polypeptide via a conserved RGD peptide sequence motif in the viral protein. The interaction between fiber and CAR or CD46 and between the penton base and integrin together lead to internalization of the particle in an endocytic vesicle. Acidification of the endosome then causes conformational changes in the virion that lead to the loss of several proteins from the particle vertices, including fiber and VI. It is this latter protein that is now thought to cause lysis of the endosomal membrane, permitting the residual particle to enter the cytoplasm. The ability of the particle to undergo these uncoating steps depends crucially on the prior action of the encapsidated viral protease, which cleaves several particle proteins during final maturation (see the section titled 'Assembly and release'). The function of protein VI in particular is controlled by sequential specific cleavage events. Cleavage of particle proteins is thought to render the particle metastable, meaning that it is primed for uncoating during the subsequent round of infection.

Once the residual virion has escaped from the vesicle, it is rapidly transported along microtubules toward the nucleus. During this process, there is further loss of virion proteins. Ultimately, the core of the particle is delivered into the nucleus where all the remaining steps of the replication cycle, excepting actual protein synthesis, take place.

Gene Expression

Once the incoming genome has reached the nucleus, it is used as template for transcription. The principal enzyme involved is host RNA polymerase II, although the VA genes are transcribed by RNA polymerase III. A program of viral gene expression is established, during which essentially the entire length of each genome strand is transcribed. However, not all the regions of the genome are expressed at the same time during infection. Instead, gene expression is tightly regulated, resulting in a temporal cascade of gene activation and hence protein production (**Figure 3**(**a**)). Key to this is the action of a combination of viral and host proteins as specific activators of viral genes. Thus, initially upon infection, the E1A gene is the only one to be transcribed. If protein production from these mRNAs is prevented with metabolic inhibitors, little further gene expression is seen, indicating that E1A proteins are important activators of the remainder of viral gene expression. One of the E1A proteins, known as the 13 S RNA product or the 289 residue protein, has a potent transactivation function although it lacks specific DNA-binding activity. Instead, its recruitment to promoters is thought to be through protein–protein interactions. The action of this protein, together with host transcription factors that E1A activates indirectly, leads to activation of the remaining early gene promoters. Among the proteins produced from these genes are the ones required for DNA replication. Other early proteins modulate the host environment (see the section titled 'Effects on the host cell'). The production of large numbers of replicated DNA templates, together with the action of the intermediate gene product IVa2 and L4 33 kDa/22 kDa proteins working in a feed-forward activation mechanism, dramatically activates the MLTU, ultimately resulting in high levels of transcription of the genes that encode the structural proteins needed to form progeny particles.

Figure 3(**b**) shows a detailed transcript map of human adenovirus 5. There are only nine promoters for RNA polymerase II in the genome, one for each of the eight genes excepting E2, which has distinct promoters for the early and late phases of infection. Of these eight protein-coding genes, only the one encoding protein IX has a single product. Extensive use of alternative splice sites and polyadenylation sites within the primary transcripts of the other genes leads to multiple mRNAs, each with distinct coding potential; in total, at least 50 different proteins are made. The MLTU, for example, encodes around 18 proteins, each with a unique and essential role as either a virion component, assembly factor, or late gene expression regulator.

As well as allowing the expression of a wide range of proteins, the use by adenovirus of differential RNA processing also opens up the possibility of further controls on the temporal pattern of gene expression during the course of infection. For all the genes that show alternative splicing, there is a trend toward the removal of larger or greater numbers of introns as infection proceeds. A well-documented example is the MLTU L1 region. This limited segment of the MLTU is actually expressed at low level even in advance of replication beginning, but only the 5′-proximal reading frame (encoding the 52/55 kDa proteins) is accessed by splicing. Later in infection, the production of L1 mRNA switches to access the distal reading frame encoding protein IIIa. This is achieved through virus-directed modification of host-splicing components and the direct involvement of L4 33 kDa protein; this protein is also needed to generate the full pattern of protein expression from other regions of the MLTU.

DNA Replication

Adenoviral DNA replication follows a mechanism that is completely distinct from that of other animal DNA viruses,

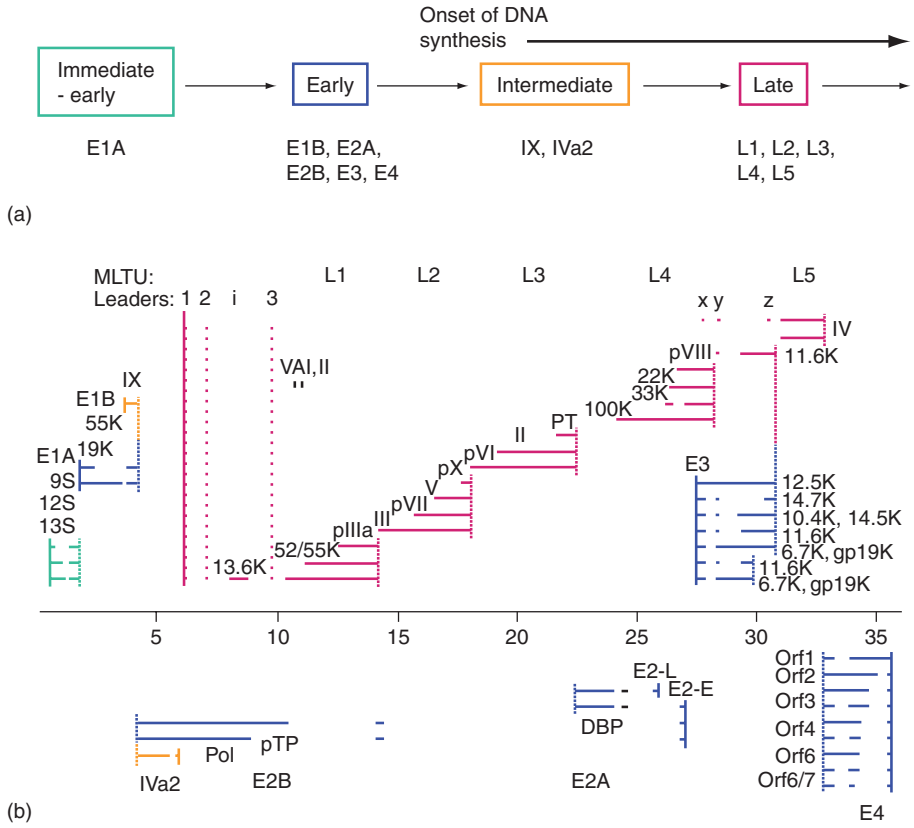


Figure 3 Gene expression by human adenovirus 5. (a) The phases of gene expression. The numbers E1A, L1, etc. refer to regions of the viral genome from which transcription takes place. (b) A transcription map of the genome. The genome is represented at the center of the diagram as a line scale, numbered in kbp from the conventional left end, with rightward transcription shown above and leftward transcription below. Genes or gene regions are named in boldface. Promoters of RNA polymerase II transcription are shown as solid vertical lines and polyadenylation sites as broken vertical lines. VAI and VAII are short RNA polymerase III transcripts. Individual mRNA species are shown as solid lines, color-coded according to the temporal phase of their expression in (a), with introns indicated as gaps. The protein(s) translated from each mRNA is indicated above or adjacent to the RNA sequence encoding it. Structural proteins are shown by roman numerals; PT, 23 kDa virion proteinase; DBP, 72K DNA-binding protein; pTP, terminal protein precursor; Pol, DNA polymerase. (a) Reproduced from Dimmock NJ, Easton AJ, and Leppard KN (2006) *Introduction to Modern Virology*, 6th edn., figure 9.6. Oxford: Blackwell Scientific, with permission from Blackwell Publishing. (b) Adapted from Leppard KN (1998) Regulated RNA processing and RNA transport during adenovirus infection. *Seminars in Virology* 8: 301–307. Academic Press, with permission from Elsevier.

and from that of its host organism. To achieve this, it encodes three proteins that function directly in replication: a DNA polymerase (DNApol), the terminal protein precursor (pTP), and a single-stranded DNA-binding protein (DBP), all from the E2 gene. Three host proteins also contribute to the replication process: two transcription factors and topoisomerase I. Aside from this, none of the host enzymes that function in DNA replication are needed.

The requirements for adenovirus replication were established using a cell-free replication system in which accurate initiation and elongation were obtained from the viral origin of replication. This is a sequence of 51 bp that is present at the two ends of the genome as part of the inverted terminal repeat. Within this sequence, only the terminal 18 bp (the core origin) is essential for initiation of replication; these bind a complex of DNApol and pTP. The remainder of the 51 bp origin is accessory sequences that bind the two host transcription factors. This binding alters the conformation of the DNA so as to facilitate binding of the DNApol/pTP complex to the adjacent core origin. Once bound at the origin, DNApol uses a serine residue hydroxyl side chain in pTP as a primer for DNA synthesis. The 5′-residue is almost invariably a cytosine, templated by the guanidine residue at the fourth position from the 3′-end of the template strand. After initiation has occurred, the complex then slips back to pair with the residue at the 3′-end of the template strand (also a guanidine), and synthesis then proceeds 5′ to 3′ away from the origin. DBP and topoisomerase I are not needed for initiation of replication, but are essential for elongation.

A scheme for the complete replication of adenoviral DNA is shown in **Figure 4**. A key feature of the process is that there is no synchronous replication of the leading and lagging strands of the template duplex at a single

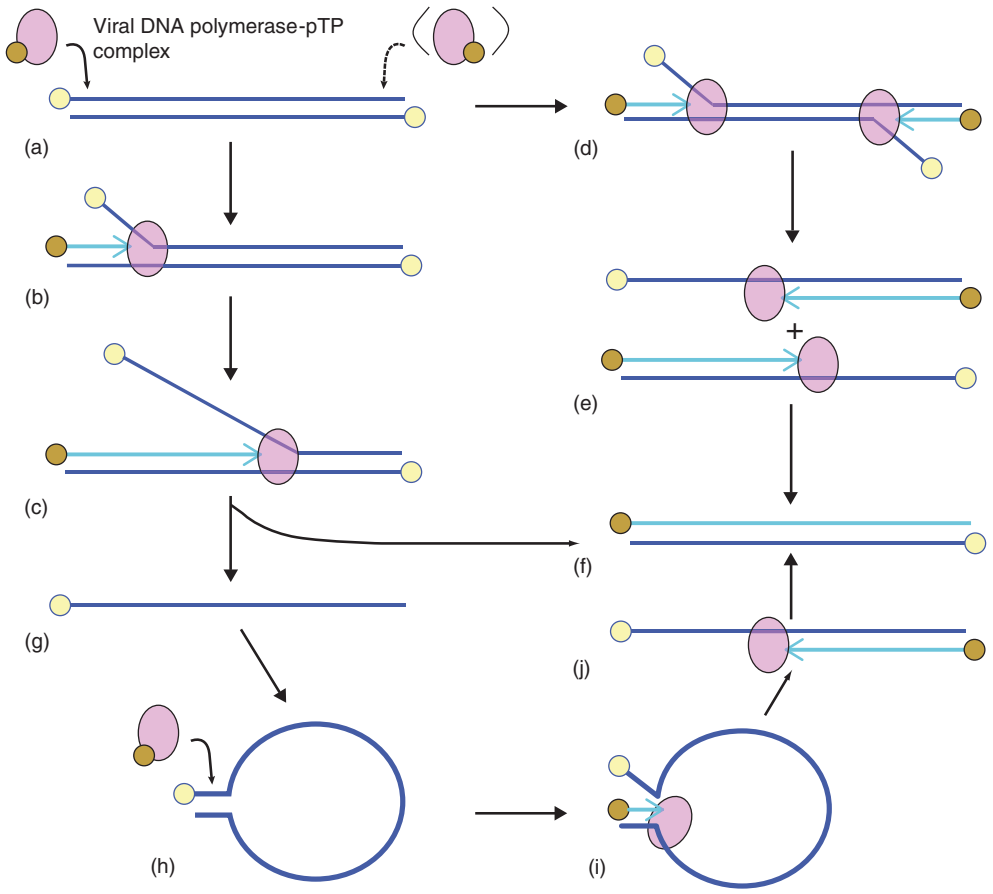


Figure 4 A general scheme for adenovirus DNA replication. Dark blue, parental DNA; light blue, new DNA. Arrowheads on new DNA strands represent 3′-ends. Yellow circles represent the terminal protein (TP) attached to parental DNA 5′-ends and brown circles the terminal protein precursor molecules (pTP) which prime new DNA synthesis. The viral DNA polymerase is represented in pink. Reproduced from Dimmock NJ, Easton AJ, and Leppard KN (2006) *Introduction to Modern Virology*, 6th edn., figure 6.7. Oxford: Blackwell Scientific, with permission from Blackwell Publishing.

replication fork, as is seen in eukaryotic DNA replication. Instead, the complementary strand of the template DNA duplex is displaced until it can be replicated later. This requires large amounts of DBP to stabilize it. Replication of this displaced single strand is achieved in one of two ways. Either its replication will already have been initiated, via the second origin at the other genome end, prior to its displacement from the duplex by the advancing replication fork, or else it can form a pan-handle structure in which its two ends come together to form a functional origin on which its replication can be initiated subsequently.

Assembly and Release

Electron microscopy performed on nuclei of cells late in the infectious cycle reveals the formation of paracrystalline arrays of adenovirus particles; this is the site of adenovirus assembly. The initial step in particle formation is the bringing together of the necessary components. Progeny genomes are generated within prominent replication centers in the nucleus while the proteins necessary to package this DNA are produced in the cytoplasm and then transported back to the nucleus. Hexon polypeptides require the assistance of a large 100 kDa protein, encoded in the MLTU L4 region, and protein pVI, to form trimers and reach the nucleus efficiently. It is generally believed that immature adenovirus particles assemble with the aid of scaffolding proteins (proteins that appear in intermediate virion forms but not the mature virion), prior to the insertion of the DNA genome. DNA packaging into these particles requires specific sequences in the genome that lie between the left-end copy of the inverted terminal repeat and the E1A gene (**Figure 2**). Packaging also involves the viral IVa2, L1 52/55 kDa, and L4 22 kDa proteins, and probably other host factors, to achieve efficient encapsidation of the DNA, which is accompanied by the ejection of scaffolding proteins. Sequence-specific binding of IVa2 to elements in the packaging sequence is

important in this process. The final step in particle maturation is the cleavage of a number of the component proteins, including the genome-attached pTP, by the virion 23 kDa protease, an L3 gene product. The protease is stimulated by a peptide cleaved from pVI. Maturation cleavage is essential for the particles to acquire infectivity (see the section titled 'Attachment and entry').

In immortalized cell lines such as HeLa, infected cells show severe cytopathic effect, with loss of cytoskeleton leading to rounding and detachment from the monolayer within 1–2 days at high multiplicity of infection. However, the release of virus particles is rather inefficient, with considerable amounts of virus remaining cell associated. One of the E3 gene products, an 11.6 kDa protein (ADP, an abbreviation for adenovirus death protein) that is produced late in infection, facilitates loss of cell viability and particle release. In its absence, although cytopathic effect occurs normally, cell death is delayed and virus release is even less effective. However, this protein is not widely conserved, even among human adenoviruses, so the general significance of this function is uncertain.

The time taken from the initial addition of virus to the point of maximum progeny yield in cultures of HeLa cells is 20–24 h. However, in cultures of normal fibroblasts, this period is considerably longer, at around 72 h. The precise factors that determine the rate of adenovirus replication in different cell types are not known, but the availability of host functions needed for adenovirus replication may be higher in established cell lines than in normal cells.

The tissues that are targeted by adenovirus *in vivo* are epithelial cell sheets. Individual cells are linked by tight junctions that define distinct apical and basolateral membrane surfaces (externally facing and internally facing, respectively; **Figure 5**). In a culture model of such epithelia, virus infection leads to initial virus release via the basolateral surface. However, fiber–CAR interactions (see the section titled 'Attachment and entry') break down the CAR–CAR adhesion between adjacent cells, leading to increased permeability across the epithelium and escape of virus to the apical surface through gaps between the cells. The location of CAR on the basolateral surface of the cell means that when virus first enters the body and encounters the apical surface of an epithelium, it cannot access the receptors necessary to initiate infection. The initial round of infection must depend either on a break in epithelial integrity exposing the basolateral surface or on an alternative receptor that the virus can use to enter the first target cell in the epithelium.

Effects on the Host Cell

The interaction between adenovirus and its host is complex and subtle. Infection leads to a variety of effects on the molecular and cell biology of the host. These effects can usually be rationalized as favoring the production of progeny virus. A very large number of such effects have been studied; this section mentions some of those that are best understood.

One area of intervention by viral proteins is in reacting to aspects of the host response to infection. Several viral proteins act to block the pro-apoptotic signaling that is activated early in the infectious process. These include the E1B 19 kDa and 55 kDa proteins, the E3 14.7 kDa protein, and the E3 RID protein complex. The adaptive immune response is also impaired by the E3 gp19 kDa protein, which prevents export to the cell membrane of mature major histocompatibility complex class I antigens. A third intervention is by the VA RNA, which blocks activation of protein kinase R, an aspect of the interferon response that is triggered by double-stranded RNA produced during the infection by hybridization between

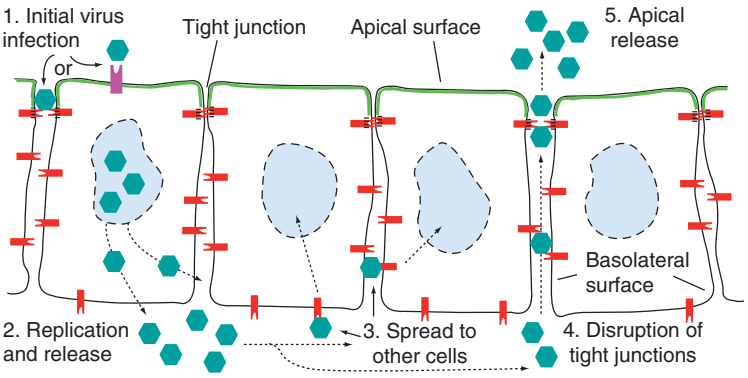


Figure 5 A schematic diagram of a polarized epithelial cell sheet, showing the sequence of events (1–5) by which adenovirus is thought to infect the epithelium and then spread both locally and to distant sites. CAR receptor molecules are shown in red, and a hypothetical alternative receptor in pink. See text for further details.

transcripts produced from the two genome strands; this would otherwise stop protein synthesis. Susceptibility of adenovirus infections to interferon is also apparently blocked via the action of E4 Orf3 protein on PML nuclear bodies (promyelocytic leukemia protein-containing structures also known as ND10). Finally, the host cell stress and DNA damage sensor, p53, is inactivated and targeted for degradation, through the action of E1B 55 kDa and E4 Orf6 proteins, while these same viral proteins (as well as E4 Orf3 in species *Human adenovirus C*) also block the double-stranded DNA break repair pathway, which otherwise leads to end-to-end joining of progeny genomes and a substantial failure of replication.

Adenovirus proteins also have effects on the host gene expression apparatus that impact on viral gene expression. First, through the action of E1A and E4 Orf6/7 proteins, the host transcription factor E2F is released from inhibitory complexes with Rb (retinoblastoma protein) family members so that it can activate the E2 promoter; this also has the effect of activating cell-cycle progression. Second, the activity of protein phosphatase 2A is altered through the action of E4 Orf4 protein, leading to a variety of effects including changes in the activity of SR splicing proteins and consequent changes in viral mRNA splicing pattern during the course of infection. Third, the E1B 55 kDa and E4 Orf6 proteins cause a block to the export of host cell mRNA from the nucleus during the late phase of infection and instead favor the export of viral mRNA. Finally, the L4 100 kDa protein is involved in allowing efficient translation of viral mRNA while the normal process of cap-dependent recognition of mRNA by ribosomes is inhibited.

Adenoviruses as Vectors

Several features of human adenoviruses have led to interest in them as potential gene delivery vectors for human cells. Applications are under development in which such vectors are designed to elicit a specific immune response to a delivered gene product (i.e., a recombinant vaccine), to give tumor-specific cell killing for cancer therapy, or to give long-term persistence and expression of a delivered gene (a gene therapy approach). Initial attention focused on human adenovirus 5 because of its experimental tractability (ease of making recombinants, ability to grow virus to very high titers in cell culture, and high level of genetic characterization), its ability to deliver genes into a variety of cell types whether or not they were actively dividing, and its mild disease course in a natural context. Difficulties experienced with these vectors, including the relative nonaccessibility of the CAR receptor on target tissues (see the section titled 'Assembly and release'), have since led to the development of vectors based on several other adenoviruses.

To convert human adenovirus 5 into a passive gene delivery vector, genome modifications are required to render it replication-defective and to create sufficient spare capacity for the insertion of foreign DNA; otherwise, only an additional 1–1.5 kbp of DNA can be accommodated. The E3 region is usually deleted, as its functions are dispensable for growth in cell culture, and the E1A and E1B genes are also removed. This latter deletion renders the virus largely replication defective, but the deficiency can be readily complemented in widely available cell lines to allow growth of the vector in the laboratory. Adding further deletions in other essential genes, such as E2 and E4, further impairs replication, giving even lower levels of late gene expression. Ultimately, all the viral genes can be stripped from the vector genome to leave only the replication origins and packaging sequence from the termini. These vectors have given the best performance among adenovirus vectors for long-term gene delivery but are more difficult to complement for growth in culture and hence much lower yields of vector particles are achieved.

See also: Adenoviruses: General Features; Adenoviruses: Malignant Transformation and Oncology; Adenoviruses: Pathogenesis; Coxsackieviruses; Gene Therapy: Use of Viruses as Vectors.

Further Reading

Akusjärvi G and Stévenin J (2003) Remodelling the host cell RNA splicing machinery during an adenovirus infection. *Current Topics in Microbiology and Immunology* 272: 253–286.

Berk AJ (2005) Recent lessons in gene expression, cell cycle control, and cell biology from adenovirus. *Oncogene* 24: 7673–7685.

Berk AJ (2006) *Adenoviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 5th edn., ch. 63. Philadelphia: Lippincott Williams and Wilkins.

Davison AJ, Benkö M, and Harrach B (2003) Genetic content and evolution of adenoviruses. *Journal of General Virology* 84: 2895–2908.

Dimmock NJ, Easton AJ, and Leppard KN (2006) *Introduction to Modern Virology*, 6th edn. Oxford: Blackwell Scientific.

Fabry CMS, Rosa-Calatrava M, Conway JF, *et al.* (2005) A quasi-atomic model of human adenovirus type 5 capsid. *EMBO Journal* 24: 1645–1654.

Leppard KN (1998) Regulated RNA processing and RNA transport during edenovirus infections. *Seminars in Virology* 8: 301–307.

Liu H, Naismith JH, and Hay RT (2003) Adenovirus DNA replication. *Current Topics in Microbiology and Immunology* 272: 131–164.

Meier O and Greber UF (2003) Adenovirus endocytosis. *Journal of Gene Medicine* 5: 451–462.

Ostapchuk P and Hearing P (2005) Control of adenovirus packaging. *Journal of Cellular Biochemistry* 96: 25–35.

Russell WC (2000) Update on adenovirus and its vectors. *Journal of General Virology* 81: 2573–2604.

Zhang Y and Bergelson JM (2005) Adenovirus receptors. *Journal of Virology* 79: 12125–12131.

Adenoviruses: Pathogenesis

M Benkő, Veterinary Medical Research Institute, Hungarian Academy of Sciences, Budapest, Hungary

© 2008 Elsevier Ltd. All rights reserved.

History

Adenoviruses were first recognized as distinct agents in 1953, although certain disease conditions that were later clarified as being caused by adenoviruses had been described previously in humans as well as in animals. These include epidemic keratoconjunctivitis, the viral etiology of which had been suggested as early as 1930. It had also been recognized before the discovery of adenoviruses that the so-called fox encephalitis and dog hepatitis were caused by the same virus. Also, the first avian adenovirus (chicken embryo lethal orphan (CELO) virus) isolate had erroneously been obtained when bovine samples of lumpy skin disease were examined for an infectious etiological agent by inoculation into embryonated eggs.

The actual discovery of human adenoviruses was the result of targeted investigations into the etiology of an acute, respiratory illness frequently affecting young military recruits in the USA. The first isolates (those assigned low type numbers) were obtained either from diseased soldiers or from adenoid tissues removed by tonsillectomy (hence the name adenovirus). Morphologically indistinguishable viruses were classified as new serotypes on the basis of the results of cross-neutralization tests. The number of human adenovirus serotypes grew rapidly during the 1960s and 1970s. There are 51 human adenovirus serotypes known to date, and an additional candidate has been discovered recently. Nonhuman animal adenoviruses were initially described largely in domesticated species of economic importance. Although a considerable number of new human or animal adenoviruses were isolated from diseased individuals, many were recovered from primary tissue cultures, such as simian adenoviruses from Vero cells and bovine adenoviruses from calf kidney or testicle cell cultures.

The striking diversity of human adenoviruses was recognized early on, and various biological properties, most of which have obvious influences on pathogenicity, were used to establish a basis for subclassification (**Table 1**). The criteria used for grouping human adenoviruses included differences in hemagglutination properties, nucleotide composition of the genomic DNA (i.e., G+C content), and oncogenicity *in vivo* and *in vitro* (the latter indicated by malignant transformation in tissue culture). Four hemagglutination groups (I–IV) and six human adenovirus subgroups (A–F) were established. In the most recent taxonomy, the latter groups, which were often referred to as subgroups or subgenera, are now correctly termed species *Human adenovirus A* (abbreviated informally to HAdV-A) through *Human adenovirus F* (HAdV-F). Phylogenetic analyses of nucleotide and protein sequences and characteristic features of genome organization fully support this classification. Each species consists of a collection of serotypes; for example, human adenovirus 12 (HAdV-12) is a member of species HAdV-A. Species are also being erected for animal adenoviruses in a similar manner.

Table 1 Features of human adenoviruses

Species *Serotype*	*Hemagglutination group*	*Tumors in animals*	*Transformation in tissue culture*	*G+C content in DNA*[a]	*Associated disease and affected organs*
Human adenovirus A HAdV-12, 18, 31	IV (no or weak hemagglutination)	High	Positive	45–47 (48–49)	Cryptic enteric infection
Human adenovirus B HAdV-3, 7, 11, 14, 16, 21, 34, 35, 50	I (complete agglutination of monkey erythrocytes)	Moderate	Positive	48–52 (50–52)	Conjunctivitis Acute respiratory disease Hemorrhagic cystitis Central nervous system
Human adenovirus C HAdV-1, 2, 5, 6	III (partial agglutination of rat erythrocytes)	Low or negative	Positive	55–56 (57–59)	Endemic infection Respiratory symptoms
Human adenovirus D HAdV-8–10, 13, 15, 17, 19, 20, 22–30, 32, 33, 36–39, 42–49, 51	II (complete agglutination of rat erythrocytes)	Low or negative (mammary tumors)	Positive	48–57 (57–61)	Keratoconjunctivitis In immunocompromised and AIDS patients
Human adenovirus E HAdV-4	III	Low or negative	Positive	57 (57–59)	Conjunctivitis Acute respiratory disease
Human adenovirus F HAdV-40, 41	IV	Negative	Negative	51	Infantile diarrhea

[a]Values are based on complete genome sequences. Estimates made previously using other methods are shown in parentheses.

Prevalence and Spread

Adenoviruses are abundant and widespread, and in principle every vertebrate species could host at least one species. Endemic or enzootic infections that are frequently inapparent seem to be very common. In contrast, epidemic or epizootic occurrence is relatively rare and normally has much more significant consequences. The nonenveloped virions shed by infected individuals are rather stable in the environment and can survive for long periods. Virions are not only able to tolerate adverse environmental effects, such as drought and moderate temperature or pH changes, but are also resistant to lipid solvents and simple disinfectants. There is evidence that human adenoviruses can persist and perhaps even retain infectivity in natural or communal water sources. Moreover, freshwater and marine bivalves have also been found to accumulate adenoviruses. Because of their stability, adenoviruses have become a major indicator of viral pollution in the environment. Vertical transmission has also been documented in several host species, including birds and cattle.

Although different species or types of adenovirus have different affinities for various organs and tissues, primary virus shedding is likely through the intestines. Adenoviruses are often transmitted by person-to-person contact, particularly among young children where fecal–oral spread is common. Aerosol transmission is also possible and is probably not rare in crowded populations. Adenovirus DNA may be found in tonsillar tissue, peripheral blood lymphocytes, and lung epithelial cells long after clinical disease has abated. Swimming pool-related outbreaks, particularly of strains causing keratoconjunctivitis or pharyngitis, are not uncommon. In poultry and other farm animals, transportation, crowding, and mixing of different populations can lead to mass infections.

Epidemiology

The tropism and pathogenicity of human adenoviruses are largely type- and species-dependent. Respiratory pathogens are common, although infections of a large variety of different organs have been described. The fiber protein is likely to mediate primary tissue tropism, but the specific determinants, epitopes, and receptors have been mapped only preliminarily. The most common clinical manifestations connected to each human adenovirus species are listed below (also see **Table 1**). HAdV-B contains two phylogenetic lineages, which are informally termed subspecies HAdV-B1 and HAdV-B2.

HAdV-A

HAdV-12, HAdV-18, and HAdV-31 can replicate efficiently in the intestines, and, based on serological surveys, are common in the population, especially in children with gastrointestinal disease. However, their role in the etiology of infant diarrhea is yet to be determined. Serotypes belonging to this species are generally difficult to isolate and culture.

HAdV-B1

Respiratory pathogens HAdV-3, HAdV-7, HAdV-16, and HAdV-21 belong here. Seasonal outbreaks of febrile respiratory diseases, mainly during winter, can be caused in infants by HAdV-7 in most parts of the world. Similar diseases, usually with a less severe outcome, can be seen among school children. In some countries, HAdV-3 is the dominant serotype, whereas HAdV-7 seems to have a number of different genotypes that shift occasionally in certain geographic areas. HAdV-4 (from species HAdV-E) and HAdV-14 (from subspecies HAdV-B2) are most often implicated in the etiology of acute respiratory outbreaks among freshly enlisted military recruits in the USA, in addition to HAdV-7 and HAdV-21.

HAdV-B2

HAdV-11, HAdV-34, and HAdV-35 cause persistent interstitial infection in the kidney and hemorrhagic cystitis. These, along with one of the most recently described human adenoviruses (HAdV-50), are most often shed in the feces or urine of acquired immune deficiency syndrome (AIDS) patients and organ or tissue transplantation recipients. Severe respiratory infections caused by a novel variant of HAdV-14 with seemingly elevated pathogenicity have emerged in the USA during 2006 and 2007.

HAdV-C

The low serotype designations (HAdV-1, HAdV-2, HAdV-5, and HAdV-6) of viruses in this group reflect the relative ubiquity of the species. They can easily be isolated and, indeed, comprise approximately half of all human adenovirus serotypes reported to the World Health Organization. HAdV-1, HAdV-2, and HAdV-5 are known to maintain endemic infections, and most teenagers will have had infections with more than one serotype. The site of persistence is lymphoid tissue, and shedding can last for a couple of years after primary infection.

HAdV-D

This species contains 32 serotypes and therefore encompasses well over one half of all known human adenoviruses. HAdV-8, HAdV-19, and HAdV-37 cause epidemic keratoconjunctivitis, especially in dry climates or densely populated areas. Other serotypes are rarely isolated except from immunocompromised patients.

HAdV-E

This is the only species that has a single serotype, namely HAdV-4, although different genotypes of this virus have been described. HAdV-4 strains have been most often implicated in respiratory diseases among military recruits in the USA. In Japan, HAdV-4 is the second most important cause of adenovirus-associated eye disease after HAdV-8.

HAdV-F

The so-called enteric or fastidious HAdV-40 and HAdV-41 are classified here. The genetic distance between these two serotypes comes close to meriting separation into different species, but, for practical reasons including indistinguishable pathology, they remain in a single species.

These viruses are a major cause of infantile diarrhea all over the world. In Europe, a shift in dominance from HAdV-40 in the 1970s to HAdV-41 after 1992 has been observed. Because of a deletion in the E1 region near the left end of the genome, HAdV-40 and HAdV-41 can be isolated and cultured only in complementing, transformed cell lines such as 293 or A547. The most recently described human adenovirus serotype, namely HAdV-52, was also recovered from patients with diarrhea. HAdV-52 has not yet been classified into a species.

Epizootiology

Simian Adenoviruses

A series of simian viruses (termed SVs) were isolated from various tissue cultures prepared from apes and monkeys. These were numbered serially, irrespective of the virus family to which they belong, since identification and allocation to a family were performed later. A considerable number of the SV isolates have been identified as adenoviruses. In many cases, unfortunately, the original SV numbers have been retained as the numbers of the adenovirus type, and thus a somewhat confusing system of SAdV numbering can still be encountered in publications as well as in the records of the American Type Culture Collection. To date, there are 25 simian adenoviruses (SAdV-1 through SAdV-25). SAdV-1 to SAdV-20 originate from Old World monkeys, whereas SAdV-21 to SAdV-25 are from chimpanzees. There are no known examples of New World monkey adenoviruses, though it is likely that they exist.

There are few data concerning prevalence or pathogenicity of simian adenoviruses in their natural hosts. An interesting mixing and grouping can be observed among the primate adenoviruses, suggesting that they can sometimes be less host specific, apparently readily infecting closely related hosts.

Canine Adenoviruses

Only two serotypes of adenovirus have been isolated from carnivores, both termed canine adenoviruses. CAdV-1 is the causative agent of infectious canine hepatitis, a life-threatening disease of puppies. Regular vaccination worldwide has decreased the number of clinical cases. Serologically indistinguishable viruses have been shown to cause encephalitis in numerous other carnivore species such as foxes, raccoons, bears, and skunks. The disease caused by a genetically closely related but serologically distinct virus, CAdV-2, is called kennel cough and is common among breeder stocks.

Interestingly, in spite of a limited number of inclusion-body-hepatitis-like conditions in felids, focused attempts to find a distinct feline adenovirus remain unsuccessful. It is likely, however, that cats living in close proximity with humans can harbor HAdV-1 or HAdV-5 from species HAdV-C.

Porcine Adenoviruses

At least five porcine adenovirus types (PAdV-1 to PAdV-5) are recognized. These represent three different species. Species PAdV-A contains three serotypes (PAdV-1 to PAdV-3) that are fairly similar to each other and cause no specific diseases. PAdV-4 (in species PAdV-B) has been described as associated with neurological disease, and PAdV-5 (in species PAdV-C) is most distantly related to PAdV-A. The pathogenic roles of porcine adenoviruses need further investigation.

Equine Adenoviruses

Two equine adenovirus serotypes (EAdV-1 and EAdV-2) were described several decades ago, but their genetic characterization has lagged behind that of other adenoviruses. Only very short, partial sequences from the hexon gene are available, and apparently these contain poorly resolved areas that will need thorough revisiting. Certain Arabian horse lineages carry a genetic defect that, when present in the homozygous state, causes severe combined immunodeficiency disease. Affected animals are incapable of mounting an immune response, and foals usually die of pneumonia due to equine adenovirus, which is harmless in immunocompetent animals.

Bovine, Ovine, Caprine, and Other Ruminant Adenoviruses

There is an amazing diversity of adenoviruses that can infect ruminant hosts. The adenoviruses described above all belong to various species in genus *Mastadenovirus*, as do

a number of ruminant adenoviruses. Unusual bovine adenoviruses that are substantially different from mastadenoviruses, as judged for example by the absence of the genus-specific antigen, were described almost four decades ago. Out of the roughly one dozen bovine adenovirus serotypes (in the BAdV series) recognized to date, six are now classified in a separate genus, *Atadenovirus*. One ovine (OAdV-7) and one goat (GAdV-1) adenovirus are also classified as atadenoviruses.

Bovine mastadenoviruses were recognized early on, and were found occasionally to cause enzootic bronchopneumonia or calf pneumo-enteritis. Oddly enough, experimental infection of young calves with the isolated adenoviruses seldom, if at all, resulted in convincing reproduction of disease. BAdV-10 is one of the most interesting ruminant mastadenoviruses in that all five isolates originated from diseased or dead animals. Genomic analysis of these isolates, one from New Zealand and four from Northern Ireland, revealed genetic variations in and around the fiber gene, suggesting that the virus might be undergoing some kind of adaptation process. Propagation of BAdV-10 strains has been difficult, with most success achieved on either primary testicle cells or a pulmonary alveolar cell line. It is noteworthy that BAdV-10 not only clusters together with mouse adenovirus (MAdV-1) on phylogenetic trees but also that it has a very simple E3 region. BAdV-10 has been proposed as an example of an adenovirus that may be in the process of switching from one host to another. Crossing the host barrier has also been suggested for BAdV-2, with two subtypes, one of which is capable of infecting calves and the other sheep. Based on evolutionary relationships, BAdV-2 is presently classified into species OAdV-A together with OAdV-2 to OAdV-5.

Bovine atadenoviruses have been found more often in the intestines, and disease reproduction was more frequently successful. Serological evidence is available for the occurrence of mastadenoviruses and atadenoviruses in a large number of free living and wild ruminant hosts. A novel atadenovirus was recently recovered from mule deer during an epizootic in California causing high mortality.

Avian Adenoviruses

Adenoviruses isolated from poultry and waterfowl were initially classified into genus *Aviadenovirus*. In addition to the criterion of host origin, aviadenoviruses can be distinguished from mastadenoviruses on the basis of a lack of the genus-common complement-fixing antigen. A large number of serotypes have been described from chicken. Some of these viruses have been isolated from other species as well. For example, strains serologically identical to CELO virus have also been found to cause bronchitis in quails. The pathologic roles of aviadenoviruses are not fully understood. The results of experimental infections have been ambiguous in the majority of cases. A specific condition referred to as hydro-pericardium syndrome has been clearly associated with fowl adenovirus 4 (FAdV-4) from species FAdV-C. Another specific disease called gizzard erosion, however, could not be linked to a definite aviadenovirus type, and experimental infections gave contradictory results.

Duck adenovirus 1 (DAdV-1) is the causative agent of egg drop syndrome (EDS), which results in depressed egg production accompanied by the production of abnormal (soft-shelled or deformed) eggs. The disease was first experienced in Europe in 1976, and soon became known worldwide. Retrospective serological studies confirmed the presence of hemagglutination inhibitory antibodies to EDS virus in archive sera originating from a large number of wild and domestic bird species, with a predominance in waterfowl, which are now considered as the main reservoir. The virus was initially assigned to genus *Aviadenovirus*, but was recorded as an exception because of its lack of common complement-fixing antigens with other fowl adenoviruses. Genome analysis of the EDS virus revealed its relatedness to atadenoviruses, and DAdV-1 has consequently been moved into this genus, which is hypothesized to be the adenovirus lineage of reptilian hosts.

Turkey adenovirus 3 (TAdV-3), better known as turkey hemorrhagic enteritis virus, shows cross-reactivity with neither aviadenoviruses nor DAdV-1, and used to be viewed as another exception among the aviadenoviruses. Serologically indistinguishable strains have been associated with various pathological entities in turkey, pheasant, and chicken. Based on genome analyses and phylogenetic calculations, TAdV-3 is a member of genus *Siadenovirus*, along with the single known adenovirus from an amphibian host, frog adenovirus 1 (FrAdV-1). This lineage has been tentatively proposed to represent adenoviruses that have coevolved with amphibians. Interestingly, a recently described sensitive polymerase chain reaction (PCR) method led to the detection of a novel type (and likely new species) of siadenovirus in diseased birds of prey. A novel avian adenovirus has also been isolated from falcons.

Reptilian Adenoviruses

Every adenovirus found in reptiles thus far is an atadenovirus. Adenovirus infections in various snake and lizard species have been described repeatedly, but the number of virus strains isolated is very limited. In Germany, captive boid snakes from multiple collections and breeders seem to be infected with the same type of adenovirus, and also frequently by a parvovirus. Neutralizing antibodies to the adenovirus have been found in 13% of more than 100 serum samples originating from free-living and captive

snakes of miscellaneous species. Bearded dragons, a reptile pet of growing popularity in North America and Europe, seem frequently to have an adenovirus infection. Partial sequence analyses have revealed that identical viruses are present on both continents. At least six additional atadenovirus types have been detected by PCR in various lizard species.

Frog and Fish Adenoviruses

Data concerning adenovirus infection of aquatic vertebrates are scarce. In fact, only a single adenovirus has been isolated from each of the two host classes, amphibians and fish. Interestingly, FrAdV-1 was isolated from a renal tumor of a leopard frog on a reptilian cell line (TH1) prepared from turtle heart tissue. FrAdV-1 has a genome organization similar to that of TAdV-3, and the common evolutionary origin of these two viruses in genus *Siadenovirus* is supported by phylogenetic calculations. The only adenovirus isolate available from fish was obtained from an ancient chondrostei species, the white sturgeon. Partial genomic characterization implies a likely new genus. Nuclear inclusion bodies typical of adenoviruses and adenovirus-like particles have been observed by light and electron microscopy, respectively, in damaged tissues of a couple of other fish species, but molecular confirmation of adenovirus infection has not yet been successful.

Pathogenesis

Adenovirus infections of man generally occur in childhood, and the outcome varies in severity from asymptomatic to explosive outbreaks of upper or lower respiratory tract manifestations. Less commonly, adenoviruses cause gastrointestinal, ophthalmic, urinary, and neurological diseases. The vast majority of adenovirus-caused diseases are self-limiting. However, immunocompromised patients, above all organ transplant recipients, individuals infected with human immunodeficiency virus (HIV) developing AIDS, and those receiving radiation and chemotherapy against tumors, represent special populations that are prone to experience grave, frequently fatal consequences of adenovirus infection. In numerous cases, the organ to be transplanted itself proves to be the source of invasive adenoviruses. Sporadic fatal infections may occasionally occur in healthy, immunocompetent individuals. In such cases, the presence of certain predisposing immunogenetic factors cannot be excluded. Cellular immune responses are also important for the recovery from acute adenovirus infection. Peripheral blood mononuclear cells have been found to exhibit proliferative responses to HAdV-2 antigen. This function is mediated by CD4+ T cells, which seem to recognize conserved antigens among different human adenovirus serotypes.

The incubation period from infection to clinical symptoms is estimated to be 1–7 days and may be dose dependent. Clinical symptoms during the initial viremia are dominated by fever and general malaise. Recovery from infection is associated with the development of serotype-specific neutralizing antibodies that protect against disease or reinfection with the same serotype of the virus but do not cross-react with other serotypes.

Pathology by adenoviruses is partially the consequence of viral replication and cell lysis. Correspondingly, in various tissues and organs, such as bronchial epithelium, liver, kidney, and spleen, disseminated necrotic foci can be observed upon necropsy or histopathological examination. Characteristic intranuclear inclusion bodies and so-called smudge cells contain large amounts of adenovirus capsid proteins. Besides the lesions caused by virus replication, direct toxic effects of high doses of structural proteins, as well as the host's inflammatory respond, may contribute to the aggravation of pathology. Experimental infection of animals with various adenovirus types seldom results in a pathology similar to that experienced with the same virus under natural circumstances. One of the few exceptions is turkey hemorrhagic enteritis, the pathogenesis of which has been studied in detail and is due to TAdV-3, which causes intestinal hemorrhages and immunosuppression. By *in situ* DNA hybridization and PCR, the presence of virus-specific DNA as evidence for virus replication has been demonstrated in the immunoglobulin M-bearing B lymphocytes and macrophage-like cells, but not in CD4+ or CD8+ T lymphocytes. Interestingly, fewer virions were present in the intestines, which are the principal site of pathology, than in the neighboring lymphoid organs including spleen and cecal tonsils. This finding strongly suggests that the intestinal lesions induced by TAdV-3 are mediated by the immune system. Systemic or intestinal hemorrhagic disease of ruminants seems to be related to virus replication in endothelial cells.

Detection and Identification

With the development of modern techniques, especially PCR and direct DNA sequencing, the number of adenoviruses detected in various organ samples of human or animal origin has increased rapidly. However, there is no official agreement or convention on the criteria that would be prerequisites for the approval of new serotypes. The conventional methods, including virus isolation in tissue culture, raising antisera, and performing a large set

of cross-neutralization tests, are cumbersome, and the majority of medical and veterinary diagnostic laboratories do not possess appropriate prototype strain and serum collections. Full genomic sequences should validate new types even in the absence of isolated virus strains. However, the value of short sequences from PCR fragments is still a topic of debate. In principle, PCR and sequencing should be able to replace serotyping if appropriate targets are identified.

Human medical laboratories use commercially available tests, such as complement fixation and enzyme immunoassay, to detect adenovirus-specific antibodies that cross-react with all serotypes. Nearly all adults have serologic evidence of past infection with one or more adenoviruses. For the detection of human or animal adenovirus-specific DNA, the most common target in PCR methods was initially the gene encoding the major capsid protein, the hexon. With subsequent restriction enzyme digestion, typing systems for human adenoviruses have also been elaborated. Recently, a novel nested PCR method targeting the most conserved region of the adenovirus DNA polymerase gene has been published. The highly degenerate consensus primers seem to be capable of facilitating amplification of DNA from every adenovirus known, irrespective of genus affiliation. A major drawback of this exceptionally sensitive method is the relaxed specificity required. Although its application cannot be recommended for routine diagnostic purposes, it may come in handy for finding novel adenoviruses, especially in cases where adenovirus involvement is strongly suspected from other evidence, such as electron microscopy.

Prevention and Therapy

In the USA, orally administrable, live, enteric-coated vaccines against HAdV-4, HAdV-7, and HAdV-21 were used in military units for a couple of decades. After the cessation of vaccine production in 1996, the impact of adenovirus infection among military recruits increased, and re-emergence of HAdV-7 and especially HAdV-4 has been verified. Since 1999, 12% of all recruits were affected by adenovirus disease. Efforts to resume vaccination are in progress.

In the veterinary practice, dog vaccination schedules all over the world invariably include a live or killed CAdV-1 component against dog hepatitis. Inactivated vaccine for horses against equine adenoviruses has been prepared in Australia. In farm animals, inactivated bivalent vaccines (containing one mastadenovirus and one atadenovirus) have been in use in several countries for controlling enzootic calf pneumonia or pneumo-enteritis. In poultry practice, commercially available or experimental vaccines for the prevention of EDS or turkey hemorrhagic enteritis are applied occasionally. There are several attempts ongoing for the production of recombinant subunit vaccines, which should be safer than vaccines derived from infected birds or tissue culture.

No specific anti-adenovirus therapy has yet been established. Recent advances in understanding the pathophysiology of fulminant adenovirus diseases in immunocompromised patients have prompted the consideration of applying donor lymphocyte infusions after transplantation.

Cidofovir is a monophosphate nucleotide analog that, after undergoing cellular phosphorylation, competitively inhibits incorporation of dCTP into virus DNA by the virus DNA polymerase. Incorporation of the compound disrupts further chain elongation. Cidofovir demonstrates activity *in vitro* against a number of DNA viruses, including adenoviruses. There are a limited number of experiences with using this drug against adenovirus infections, and its clinical utility remains to be determined.

See also: Adenoviruses: General Features; Adenoviruses: Malignant Transformation and Oncology; Adenoviruses: Molecular Biology; Gene Therapy: Use of Viruses as Vectors.

Further Reading

Barker JH, Luby JP, Sean Dalley A, *et al.* (2003) Fatal type 3 adenoviral pneumonia in immunocompetent adult identical twins. *Clinical Infectious Diseases* 37: 142–146.

Jones MS, Harrach B, Ganac RD, *et al.* (2007) New adenovirus species found in a patient presenting with gastroenteritis. *Journal of Virology* 81: 5978–5984.

Kojaoghlanian T, Flomenberg P, and Horwitz MS (2003) The impact of adenovirus infection on the immunocompromised host. *Reviews in Medical Virology* 13: 155–171.

Leen AM, Bollard CM, Myers GD, and Rooney CM (2006) Adenoviral infections in hematopoietic stem cell transplantation. *Biology of Blood and Marrow Transplantation* 12: 243–251.

Leen AM, Myers GD, Bollard CM, *et al.* (2005) T-cell immunotherapy for adenoviral infections of stem-cell transplant recipients. *Annals of the New York Academy of Sciences* 1062: 104–115.

Neofytos D, Ojha A, Mookerjee B, *et al.* (2007) Treatment of adenovirus disease in stem cell transplant recipients with cidofovir. *Biology of Blood and Marrow Transplantation* 13: 74–81.

Schrenzel M, Oaks JL, Rotstein D, *et al.* (2005) Characterization of a new species of adenovirus in falcons. *Journal of Clinical Microbiology* 43: 3402–3413.

Tang J, Olive M, Pulmanausahakul R, *et al.* (2006) Human CD8+ cytotoxic T cell responses to adenovirus capsid proteins. *Virology* 350: 312–322.

Wellehan JFX, Johnson AJ, Harrach B, *et al.* (2004) Detection and analysis of six lizard adenoviruses by consensus primer PCR provides further evidence of a reptilian origin for the atadenoviruses. *Journal of Virology* 78: 13366–13369.

African Cassava Mosaic Disease

J P Legg, International Institute of Tropical Agriculture, Dar es Salaam, Tanzania, UK and Natural Resources Institute, Chatham Maritime, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Pandemic An outbreak of a disease over a whole country or large part of the world.
Pseudo-recombinant A viral infection involving complementary genome components from different virus species.

History

Cassava (*Manihot esculenta* Crantz) is a root crop that is grown widely throughout the tropics, primarily for its value as a starchy staple food. From its origins in Latin America, cultivated cassava was introduced to Africa in the sixteenth century by Portuguese seafarers, and subsequently spread through much of Africa south of the Sahara. Fortuitously perhaps, none of the viruses that affect cassava in the Americas seems to have been co-introduced with the crop. Over time, however, the crop became infected by indigenous viruses. The first report of a virus-like disease in African cassava was made in 1894 from what is now northeastern Tanzania. The original German descriptor, 'Krauselkrankheit', made reference to the characteristic mosaic symptoms elicited in affected plants. It was not until just over a decade later, however, that the first firm indication was given that the disease had a viral etiology. In spite of these early reports, there seems to have been little concern about the impact of cassava mosaic disease (CMD) until the 1920s. Between 1929 and 1937, however, numerous reports were made of the spread and damaging effect on cassava crops of CMD from diverse locations throughout the continent, from the island of Madagascar off the southeastern shores of the African mainland, to Sierra Leone in West Africa. These developments provided the stimulus for the earliest concerted efforts to develop approaches to controlling the viruses that caused this damaging crop disease. Although substantial progress was made in the development of cassava varieties that were resistant to cassava mosaic-causing viruses, in the 1930s and 1940s, the viruses themselves remained poorly understood, and it was not until 1983 that the first definitive study confirming the viral etiology of CMD was published. Geminate virions were shown to encapsidate a bipartite genome of single-stranded circular DNA, leading to the designation of these viruses as geminiviruses in the genus *Begomovirus*. The group is now commonly referred to as the cassava mosaic geminiviruses (CMGs). Although the earliest studies of diversity indicated the occurrence of two species in Africa, based on serological characterization, more detailed genetic studies conducted from 1993 up to the present day have provided evidence for the occurrence of seven species, albeit with varying levels of geographic coverage. In common with all begomoviruses, CMGs are transmitted by the whitefly vector, *Bemisia tabaci*, a co-evolutionary partnership that has been a key factor in the success of this group of viruses.

Properties of the Virion, Its Genome, and Replication

Structural Features

CMGs comprise two single-stranded circular DNA molecules (DNA-A and DNA-B) each encapsidated within a twinned or geminate icosahedral coat, approximately 22 nm × 35 nm in size. The three-dimensional structure has been resolved for one of the CMGs, African cassava mosaic virus (ACMV) (genus *Begomovirus*; family *Geminiviridae*), based on comparisons with related viruses and through electron cryomicroscopy and image reconstruction. Through these analyses, the structure has been shown to resemble that of virions of maize streak virus (MSV) (genus *Mastrevirus*; family *Geminiviridae*) in having two $T = 1$ symmetry icosahedra joined at a position at which one subunit is missing from each. This gives a total of 22 capsomers made up of 110 30.2 kDa coat protein molecules. Capsomers of the two halves of CMG particles are twisted to each other by 20° so that capsomers of one half are apposed to gaps between two capsomers of the other half. A six-residue insertion in the βD/βE loop protrudes from the coat protein and has been shown to be required for whitefly transmission. This appears to be an important structural feature of the *B. tabaci* transmitted begomoviruses. By contrast, the leafhopper-transmitted MSV and viruses of the species *Beet curly top virus* (genus *Curtovirus*; family *Geminiviridae*) have their own characteristic 14-residue insertion in the βF/βG loop.

The Genome

Analyses of the two single-stranded DNA (ssDNA) molecules that make up the CMG genome have revealed the presence of six open reading frames (ORFs) on DNA-A and two on DNA-B. Of the two virion sense ORFs on

DNA-A, AV1 (*CP*) codes for the coat protein and plays an important role in vector specificity, while AV2 is involved in virus movement. Complementary sense DNA-A ORFs include AC1 (*Rep*) which plays a central role in virus replication, AC2 (*TrAP*) which transactivates virion-sense genes and acts as a suppressor of post-transcriptional gene silencing (PTGS), AC3 (*REn*) which enhances virus replication and AC4, whose function is yet to be fully elucidated, but which, like AC2, seems to function in suppressing host plant PTGS. On DNA-B, BV1 (*NSP*) is involved in shuttling virus between the nucleus and cell cytoplasm, while BC1 (*MP*) is implicated in longer distance intercellular movements of CMGs. All CMGs have a conserved intergenic 'common' region of *c.* 200 bp which is also shared between the two genome components.

Replication

Most of the processes of CMG replication take place within the nuclei of infected cells. In common with other geminiviruses, CMGs multiply using a combination of rolling circle and recombination mediated replication. The mechanisms behind the initiation of negative-strand synthesis have yet to be elucidated, although it is thought that wounding responses at the point of primary infection may lead to the induction of host genes required for cell cycle reentry and DNA replication. Positive-strand nicking followed by synthesis has been mapped to the conserved nonanucleotide TAATATT/AC within the intergenic region (IR). The nonanucleotide sequence sits within the loop of a hairpin-loop structure in the 3′ IR, a feature common to all geminiviruses. Strand nicking and positive-strand synthesis are catalyzed by oligomerized *Rep* in concert with host factors. *Rep* binds specifically to repeated 'iteron' sequences within the IR. Importantly, *Rep* also binds to retinoblastoma related protein (*pRBR*), a process which catalyzes a series of intracellular reactions that move the cell from G to S phase and in so doing mobilizes host factors essential for DNA replication. Double-stranded DNA (dsDNA) produced through the replication process is then thought to assemble into transcriptionally active mini-chromosomes, under the control of *TrAP* and *REn*. Nuclear export is mediated by *NSP*, movement out of the cell through plasmodesmata, and longer-distance transport through *MP*. These processes ultimately lead to the multiplication of viral DNA within initially infected cells, and subsequent systemic movement through phloem tissue. The life cycle is then sustained both through the multiplication of CMG virions in the young newly emerged cassava leaves most favored by adult *B. tabaci*, as well as by the distribution of CMG particles throughout the length of mature stems (in susceptible cultivars), ensuring that when cuttings are taken, the presence of CMG components will ensure the continued infection of the newly sprouting plant.

Diversity and Distribution

Distribution of the African CMGs

The earliest studies of CMG diversity and distribution used serological techniques to characterize variability, and then utilized that variability to develop diagnostic tests based on the use of monoclonal antibodies in the enzyme-linked immunosorbent assay (ELISA). Using these methods, two principal groups of CMGs were recognized that were subsequently confirmed as distinct species following the sequencing of DNA-A molecules. These were ACMV and East African cassava mosaic virus (EACMV) (genus *Begomovirus*; family *Geminiviridae*). Two other viruses have been shown to cause CMD in south Asia, namely: Indian cassava mosaic virus (ICMV) and Sri Lankan cassava mosaic virus (SLCMV). The earliest distribution maps of the African CMGs showed EACMV to occur in the coastal east African areas of Kenya, Tanzania, and Madagascar, as well as Malawi and Zimbabwe. ACMV, by contrast, occurred throughout the remainder of the cassava-growing areas of Africa, from South Africa and Mozambique in the southeast, to Senegal in the northwest. Significantly, at this time there was no reported zone of co-occurrence of the two virus species. With the increased use of polymerase chain reaction (PCR)-based diagnostics from 1990s onward, it became possible to identify differences not solely associated with the coat protein. This led to two important developments in the understanding of CMGs in Africa. First, several new species were identified, all of which were more closely related to EACMV than to ACMV. Second, it was shown that virus mixtures belonging to different species occurred frequently. A notable consequence of this finding was the concomitant evidence for the more widespread distribution of the EACMV-like viruses than had hitherto been recognized. New virus identifications included: South African cassava mosaic virus (SACMV) (1998), East African cassava mosaic Malawi virus (EACMMV) (1998), East African cassava mosaic Cameroon virus (EACMCV) (2000), East African cassava mosaic Zanzibar virus (EACMZV) (2004), and East African cassava mosaic Kenya virus (EACMKV) (2006). Significantly, all of the CMGs occur in different parts of East and Southern Africa, while ACMV predominates in West Africa (**Figure 1**). EACMCV, the only EACMV-like virus occurring in West Africa, is nevertheless infrequent and generally occurs in mixed infections together with ACMV. ACMV is absent from coastal areas of Kenya and Tanzania. Although there has been very little CMG characterization in many of the cassava-growing countries of Africa, and it seems clear that much of the variability of this group of viruses still remains to be revealed, an assessment of the data currently available has led to the conclusion that East Africa is the center of diversity for the EACMV-like CMGs and is probably the home for yet-to-be-identified wild hosts of the proto-CMGs that

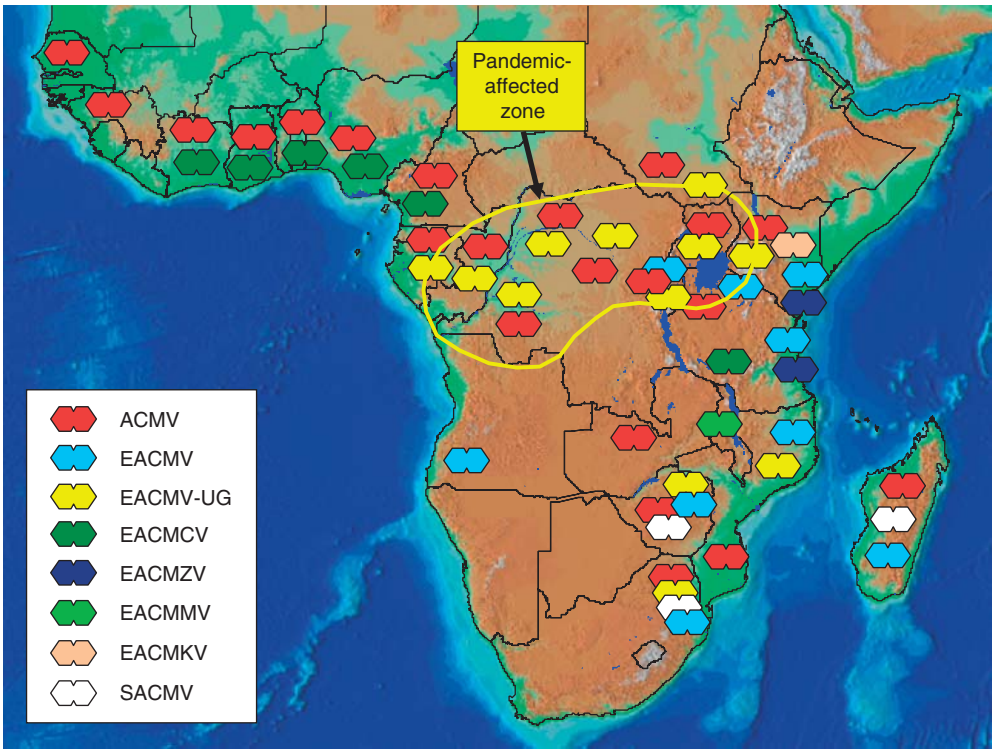


Figure 1 Diagrammatic representation of the distribution of CMGs in Africa, 2006.

were first introduced to cassava by *B. tabaci* sometime between the earliest introductions of cassava to this part of Africa in the eighteenth century, and the first report of CMD in 1894.

Recombination and the CMD Pandemic

The CMGs represent a very dynamic group of viruses, and evidence has been presented for the occurrence of both pseudo-recombinants, in which the DNA-A of one virus species co-replicates with the DNA-B of another species, as well as true recombinants, in which portions of the DNA-A or -B of one species have been spliced into the DNA-A or -B of another. In fact, all of the EACMV-like viruses other than EACMV show evidence for recombination events either with known or as yet unknown begomoviruses. One of the most important developments in the study of CMGs in Africa in recent years was the recognition that an unusually damaging strain, referred to as EACMV-UG, had arisen through a recombination event between EACMV and ACMV in which a 340 nt portion of ACMV AV1, had replaced the equivalent portion of the DNA-A of EACMV. The obvious consequence of this was that ELISA-based diagnostic tests erroneously identified this strain as ACMV. This is one of the reasons why PCR-based diagnostic methods are now used almost exclusively for CMG monitoring work. EACMV-UG has been associated with the rapid epidemic-like spread of CMD in East and Central Africa through the latter part of the twentieth and into the current century. Its continually expanding distribution currently includes: Burundi, Democratic Republic of Congo (DRC), Gabon, Kenya, Republic of Congo, Rwanda, southern Sudan, Tanzania, and Uganda (**Figure 1**). Additional countries in which it is likely to be widespread, but has yet to be formally identified, include Central African Republic and Angola. Localized identifications have also been made from South Africa, Swaziland, and Zimbabwe.

The dynamic character of CMG diversity and distribution cannot be overemphasized, and the propensity that this group of viruses shows to produce both naturally occurring pseudo-recombinants, as well as novel true recombinants, ensures that the patterns of diversity and distribution will continue to evolve rapidly.

Transmission

CMGs, in common with all other members of the genus *Begomovirus*, are transmitted by the whitefly vector *B. tabaci*. Transmission can also be achieved through grafting, and relatively inefficiently through mechanical inoculation of indicator plants, but there is no seed-borne transmission. Cassava is normally propagated through the use of vegetative cuttings, and this is perhaps the most frequent source of infection in new crops under field conditions. *Bemisia tabaci* adults feed, mate, and

reproduce preferentially on the upper newly emerged leaves of cassava plants, and almost all transmission occurs here. Transmission is persistent, and ACMV has been shown to be retained by *B. tabaci* adults for up to 9 days. Transtadial, but not transovarial transmission, has been demonstrated, although the larval instars are unimportant in the epidemiology of CMGs in view of their sessile nature. Minimum periods for each of the stages of transmission for ACMV by *B. tabaci* adults are: acquisition (3 h), latent period (3 h), and inoculation (10 min). Inoculated plants begin to show symptoms of infection after 3–5 weeks. Varying levels of transmission efficiency have been reported, ranging from 0.3% to 10%, depending primarily on the nature of the CMG infection in the plants from which virus is acquired. There is evidence for a limited degree of co-evolutionary adaptation between the whitefly vector and CMGs, as Indian whiteflies are significantly better at vectoring Indian than African CMGs and vice versa. However, within Africa, there is currently no indication that whiteflies from a particular location are more efficient at vectoring locally occurring CMGs than they are at vectoring CMGs from another part of the continent. There is, however, an important balance between the pattern of transmission and the nature of the virus infection. More severe infections, caused either by more virulent virus species/strains or by mixed virus infections, lead to a greater frequency of whitefly-borne infection but diminished propagation through cuttings by farmers. Conversely, more moderate or mild infections, caused by less virulent virus species/strains occurring in single infections, lead to less frequent whitefly-borne infection and increased propagation through cuttings by farmers. This balance dictates the epidemiological characteristics of CMG infection in any given location, area, or region.

Virus–Plant Interactions

Viral Infection Mechanisms and Host Plant Responses

Following initial infection of a previously uninfected cassava plant by a CMG, viral DNA moves through the phloem to the newly developing tissues immediately behind the meristem where rapid multiplication of virus particles takes place. Plants have developed defense responses to virus multiplication through the process of PTGS, but in response, CMG species/strains have developed various effective mechanisms to overcome this, and the degree of this effectiveness seems to be the main factor in determining the severity of disease resulting from infection. Both AC2 and AC4 have been shown to act as suppressors of PTGS. Significantly, in mixed ACMV + EACMV-like virus infections, the responses of the two viruses to host plant PTGS seem to be complementary, leading to a synergistic interaction between the two viruses, greatly increased titers of both, and a concomitant increase in severity of the disease symptoms expressed in the plant. Molecular studies of these interactions have shown that the abundance of short interfering RNA (siRNA) molecules associated with the host plant PTGS response increases over time in pure ACMV infections, yet remains low over similar time periods for ACMV + EACMV-UG co-infections. Synergism has been described for ACMV + EACMCV mixtures in Cameroon, but its significance is greatest in the widely occurring ACMV + EACMV-UG mixed infections that cause severe CMD in much of East and Central Africa.

Field-Level Epidemiology

Epidemiological studies of the CMGs can be broadly categorized into two groups: those that describe patterns of infection at the field level and those that relate to area or region-wide spread. In the case of the former, external sources of infection have been shown to be most important for determining the rate and quantity on infection in initially CMD-free plantings in the normal field environment. Gradients of infection occur in which new infections are most frequent on the windward sides of cassava crops and these gradients are matched by similar patterns of vector distribution. Multiple regression relationships have been used to describe the association between measures of inoculum pressure and final CMD incidence in test plots. Gompertz curves model patterns of infection increase in plots of CMG-susceptible cassava cultivars, and incidence increases rapidly to 100% over the first 3–6 months of growth. For resistant varieties, however, under similar conditions of inoculum pressure, rates of infection increase are much lower and final incidences typically range from 0% to 50%.

Regional Epidemiology

Following the earliest 'first colonization' descriptions of CMD epidemics in the 1920s and 1930s, a few reports were made of rapid area-wide spread of severe CMD at other times during the twentieth century, some of the most notable of which were epidemics in Cape Verde and south-eastern Nigeria in the 1990s. Of much greater importance, however, has been the African CMD pandemic that was first reported from the northern-central part of Uganda in the late 1980s. CMD associated with the epidemic was unusually severe and was being rapidly spread by super-abundant populations of *B. tabaci*. During the 1990s it became apparent that the zone affected by this severe CMD was expanding southwards at a rate of 20–30 km per year, and in 1997 molecular studies revealed that the severe disease phenotype was associated with the occurrence and

spread of an 'invasive' recombinant CMG, EACMV-UG, commonly in mixed infection with the locally occurring, but now synergized ACMV. Regular monitoring surveys conducted throughout the East and Central African region through the 1990s and early part of the new century have used PCR-based diagnostics to map the spread of the EAMCV-UG associated with this 'pandemic' of severe CMD. This work has given rise to first reports of EACMV-UG and resulting severe CMD in eight additional countries in East and Central Africa: Kenya (1996), Sudan (1997), Tanzania (1999), DRC (1999), Republic of Congo (1999), Rwanda (2001), Burundi (2003), and Gabon (2003). Significantly, the pandemic 'front' advanced through north-western Tanzania from the Uganda border a distance of *c.* 400 km between 1999 and 2004. If these rates of virus spread are sustained, EACMV-UG might be expected to arrive in Zambia by 2012 and in Malawi by 2015. Similarly, westwards spread of the EACMV-UG-associated pandemic threatens both Cameroon and Nigeria in the near future.

Economic Importance

In common with many viruses causing plant disease, the main features of the damage caused by CMGs are a mosaic-like chlorosis on the leaf lamina, leaf puckering, twisting and distortion, and an overall reduction in plant size. These effects lead to a reduction in the quantity of photosynthetic assimilates channeled into the tuberous roots, and through this, a reduction in yield. The degree of yield loss varies greatly, depending primarily on the susceptibility of the cassava cultivar, the virulence of the CMG species/strain, and the stage of growth at which infection occurred (being most severe for cutting-infected plants). Typically, however, individual plants sustain yield losses ranging from 20% to 100%. The only study of the effects of specific viruses on yield showed average yield losses to be 42% for ACMV alone, 12% for EACMV-UG mild, 68% for EACMV-UG severe, and 82% for mixed ACMV + EACMV-UG.

The relatively moderate losses attributable to ACMV infection, coupled with moderate to low incidences, have been the reasons for the apparent lack of concern through large parts of cassava-growing Africa about the impact of CMGs and CMD. This outlook has changed markedly, however, following the emergence and regional spread of the CMD pandemic. Extensive surveys of CMD incidence, CMD severity, and the occurrence of the causal viruses have made it possible to make continent-wide yield loss estimates for CMD. The most recent of these (2006) provides estimates for losses in pandemic-recovered (16% loss), pandemic-affected (47%), and as yet unaffected (18%) countries in sub-Saharan Africa, leading to an overall loss figure of 34 million tons per year, roughly a third of total African production of fresh cassava roots. Significantly, as the pandemic-affected area of the continent increases beyond the current estimate of 2.6 million sq. km, losses are likely to continue to rise through the first quarter of the twenty-first century.

Control

The most widely practiced approaches to controlling the CMGs that cause CMD include the deployment of host plant resistance and the use of cultural methods, particularly phytosanitation. More recently, considerable attention has been directed toward the use of genetic engineering techniques to produce transgenic virus-resistant cassava plants.

Host Plant Resistance

The potential value of introgressing virus resistance genes from wild relatives of cassava was recognized from the earliest days of CMD research in the 1920s/30s, and interspecific crosses combining cultivated cassava with Ceara rubber (*Manihot glaziovii*) were developed independently through breeding programs in modern-day Tanzania and Madagascar. F1 progeny were triple backcrossed with cultivated cassava to produce plants that combined acceptable food quality with significantly enhanced resistance to CMGs. Germplasm developed in this way formed the basis for the later continental breeding program run from the Nigeria-based International Institute of Tropical Agriculture (IITA), which from its establishment in 1967, to the present day, has developed thousands of CMD-resistant cassava clones. Many of these have been sent to cassava-producing countries in Africa for use either specifically for CMD management programs, or more generally for cassava development. Germplasm derived from the initial interspecific crosses uses the name prefix 'TMS' for 'tropical Manihot species'. This resistance source is multigenic and has provided high levels of resistance which have been very durable when used against all CMGs and CMG combinations. Four distinct mechanisms of resistance are recognized: resistance to infection, resistance to virus multiplication, resistance to virus movement (leading to incomplete systemicity), and resistance of normal plant function to the effects of virus infection. During the 1990s, resistant landraces from West Africa, given the name prefix 'TME' for 'Tropical Manihot Esculenta', were incorporated into the breeding program. These have been shown to possess alternative sources of resistance, one of the most important of which has been characterized through genetic analyses as a single dominant gene designated *CMD2*. Molecular marker approaches have been used subsequently to combine the multigenic *M. glaziovii*-derived resistance with *CMD2*. Looking further into the future, there is hope that genomics approaches will lead to the elucidation of

additional CMG-resistance genes that can be jointly incorporated into cassava germplasm through pyramiding.

CMG-resistant varieties have been widely disseminated throughout the cassava-growing areas of sub-Saharan Africa. However, their adoption by farming communities has been most widespread in CMD pandemic-affected countries, where they have provided the only effective means of restoring cassava production to pre-pandemic levels.

Cultural Methods

A range of cultural methods has been proposed for the control of CMGs. The methods most widely recommended have been the removal of infected plants (roguing) or the selection of disease-free planting material for the establishment of a new crop (selection). Crop isolation, adjusting crop disposition in relation to the prevailing wind, varying planting date, varietal mixtures, and intercropping cassava with other 'putative' protective crops have all been suggested at various times as potentially useful control options for CMGs. No convincing experimental evidence has yet been presented to confirm the value of any of these methods, however, and current field practice is restricted to selection and occasional roguing. Roguing is considered to be of value within the framework of institutional programs for the multiplication of CMD-resistant germplasm, in view of the requirement for the production of high-quality planting material. Experiments conducted in 'post-epidemic' areas of East Africa, first affected by the CMD pandemic 5 or more years previously, have provided clear evidence for the value of selection of CMD-free stems when choosing planting material. Local cultivars treated in this way provided equivalent yields to those of CMD-resistant varieties after two cropping cycles. A key drawback to the wider adoption of this approach, however, is the variability in effectiveness of the approach in relation both to the virus inoculum pressure of the location, as well as the relative susceptibility of the cultivar.

Transgenic Resistance

There are four strategies that are currently being explored for transgenic resistance to geminiviruses. These include: the expression of viral proteins, the expression of nonviral proteins, DNA interference, and RNA interference.

Expression of viral proteins

Coat protein transformation has been widely used as a means of interfering with coat protein assembly and also inter- and intracellular transport, but this approach seems to be less effective for the bipartite geminiviruses, such as the CMGs, in which movement functions are controlled by BC1 and BV1. The most thoroughly tested approach has been transformation with AC1 (*Rep*). AC1 transformed plants were shown to have high levels of siRNAs homologous to ACMV AC1 prior to virus challenging and AC1 mRNA levels were reduced by up to 98% postinfection compared to controls. This PTGS-associated mechanism also seemed to be broadly active against other CMG species and strains. Current (2006) information, however, suggests that this resistance has been lost over time, apparently through methylation of the transgene. Further studies are currently underway to determine how to prevent this acquired interference.

Expression of nonviral proteins

A number of nonviral proteins have been investigated for their potential use as transgenes conferring resistance through enhancing the hypersensitive response at the initial site of infection. These include: barnase and barstar genes under the control of the ACMV A bidirectional promoter, and the gene for the ribosome-inactivating protein *dianthin*, sited downstream of an ACMV transactivatable promoter. 'Antibodies' that interfere with the protein and DNA-binding epitopes of viral proteins also offer promise as potential transgenes, and one such example is the development of artificial zinc-finger proteins (AZPs) which have a high affinity and selectivity for the *Rep* dsDNA binding site.

DNA interference

Naturally occurring defective interfering (DI) subgenomic (*c.* 1500 bp) DNA molecules of CMGs have been shown to ameliorate symptoms in plants infected by wild-type CMGs. Although virus symptom reduction has also been achieved in plants transformed with tandem repeats of DI ACMV DNA-A, potential problems with this approach include virus specificity and the possibility of deleterious effects following the integration of DI virus DNA into the host genome.

RNA interference

RNA interference (RNAi) results from the expression of artificial dsRNAs homologous to viral sequences which when processed into siRNAs direct silencing complexes to target RNA or DNA sequences. For ACMV, transgenic plants expressing dsRNAs homologous to the DNA A promoter have reduced levels of virus replication. An alternative RNAi approach uses antisense RNA (asRNA) constructs. Transgenic cassava plants have been produced expressing AC1, AC2, and AC3 in the antisense direction, and both *in vitro* assays and infection experiments have shown greatly reduced ACMV replication and symptom expression.

Although virus-resistant transgenic cassava plants have yet to be tested under field conditions in Africa, this approach to control, based as it is on a detailed and fundamental understanding of CMG function, offers much promise for the future.

Other Control Methods

Although ACMV and EACMV-like CMGs interact synergistically, there is evidence that EACMV-like CMGs may hinder infection by other EACMV-like viruses and interfere with their replication. Studies in Uganda showed that plants initially infected by mild strains of EACMV-UG were much less likely to become severely diseased when exposed in the field than plants initially CMG-free. This cross-protective effect seems to be an important cause of symptom amelioration in post-CMD pandemic areas of East and Central Africa. A thorough understanding of the molecular mechanisms underlying this phenomenon will be required, however, before an assessment can be made of the potential utility of cross protection for CMG management.

Biological control and whitefly resistance are being investigated for their potential to reduce the impact of *B. tabaci* that comes from its transmission of CMGs as well as the physical damage it causes to cassava plants. The latter is a particularly important feature of the superabundant *B. tabaci* populations found in many of the pandemic-affected areas. Biological control efforts are hindered by the fact that *B. tabaci* is considered to be indigenous to Africa, and already has a well-developed (albeit ineffective), natural enemy fauna. Cassava cultivars are variably attractive and susceptible to *B. tabaci*, however, there is a poor correlation between these characters and patterns of CMG infection. Alternative potential resistance sources are currently being sought, both from Latin American cassava germplasm (some of which is highly resistant to non-*Bemisia* whitefly pests) and from wild relatives. Much remains to be done, however, before effective vector control tactics are ready for incorporation into CMG management strategies.

Future Perspectives

Rapid advances within the field of molecular biology, from the latter part of the twentieth century onwards, have enabled researchers to make significant progress in furthering understanding of how geminiviruses interact with both their plant host and insect vector in causing CMD. This is particularly important in view of the great and increasing economic and social impact that this disease has on the more than 300 million people in sub-Saharan Africa who depend on cassava for their subsistence. Much progress has been made in the development and deployment of conventional sources of resistance to CMGs, and this work is playing a central role in managing the effects of the EACMV-UG-associated CMD pandemic that continues to spread in East and Central Africa. Although diverse approaches have been used to develop transgenic resistance, and CMG-resistant transgenic cassava plants have been produced, none has yet been evaluated under field conditions. Although transgenics offer great promise for the future, regulatory concerns in many African countries are likely to slow the progress of this work in the near term. The relative effectiveness of conventionally bred resistance also means that CMG-resistant transgenic cassava plants will almost certainly also have to carry with them additional transgene-derived traits such as improved nutritional characteristics and resistance genes to other key pests and diseases. Set against this current and possible future progress in developing control approaches to CMGs is the remarkable ability of this group of viruses to adapt to a changing agro-ecological environment through the processes of virus–virus cooperation, pseudo-recombination, and true recombination. There can be few more dramatic examples of the impact of virus evolution on agricultural systems than that of the recombinant virus-driven African CMD pandemic. Clearly therefore, a sustained commitment to all stages of the research to development continuum will be essential if the 'balance of power' between host plant and pathogenic virus is to be tipped back in favor of the cassava host. Only when this happens will cassava be able to fulfill its true potential in sub-Saharan Africa as a key component in the continent's agricultural and broader economic development.

See also: Plant Resistance to Viruses: Geminiviruses; Plant Virus Diseases: Economic Aspects; Satellite Nucleic Acids and Viruses.

Further Reading

Bock KR and Woods RD (1983) The etiology of African cassava mosaic disease. *Plant Disease* 67: 994–995.

Fauquet CM and Stanley J (2003) Geminivirus classification and nomenclature: Progress and problems. *Annals of Applied Biology* 142: 165–189.

Hanley-Bowdoin L, Settlage SB, Orozco BM, Nagar S, and Robertson D (1999) Geminiviruses: Models for plant DNA replication, transcription and cell cycle regulation. *Critical Reviews in Plant Sciences* 18: 71–106.

Legg JP and Fauquet CM (2004) Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology* 56: 585–599.

Legg JP, Owor B, Ndunguru J, and Sseruwagi P (2006) Cassava mosaic virus disease in East and Central Africa: Epidemiology and management of a regional pandemic. *Advances in Virus Research* 67: 356–419.

Ndunguru J, Legg JP, Aveling TAS, Thompson G, and Fauquet CM (2005) Molecular biodiversity of cassava begomoviruses in Tanzania: Evolution of cassava geminiviruses in Africa and evidence for East Africa being a center of diversity of cassava geminiviruses. *Virology Journal* 2: 21.

Storey HH and Nichols RFW (1938) Studies on the mosaic of cassava. *Annals of Applied Biology* 25: 790–806.

Thresh JM and Cooter RJ (2005) Strategies for controlling cassava mosaic virus disease in Africa. *Plant Pathology* 54: 587–614.

Zhou X, Liu Y, Calvert L, *et al.* (1997) Evidence that DNA-A of a geminivirus associated with severe cassava mosaic disease in Uganda has arisen by interspecific recombination. *Journal of General Virology* 78: 2101–2111.

African Horse Sickness Viruses

P S Mellor and P P C Mertens, Institute for Animal Health, Woking, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ascites Abnormal collection of fluid in the abdominal cavity.
Cecum The first part of the large intestine.
Choroid plexus A highly vascular membrane and part of the roof of the brain that produces cerebrospinal fluid.
Culicoides Genus of blood-feeding dipterous insects also known as biting midges.
Cyanosis Bluish discoloration of the skin or mucus membranes caused by lack of oxygen in the blood.
Ecchymotic Diffuse type of hemorrhage larger than a petechia.
Fascial A band of fibrous tissue that covers the muscles and other organs.
Hydropericardium Excessive collection of serous fluid in the pericardial sac.
Hydrothorax Excessive collection of serous fluid in the thoracic cavity.
Petechiae Pinpoint to pinhead-sized red spots under the skin that are the result of small bleeds.
Purpura hemorrhagica Hemorrhages in skin, mucous membranes and other tissues. First shows red then darkening into purple, then brownish-yellow.
Supraorbital fossae Holes in the skull situated above the eye socket.
$TCID_{50}$ 50% tissue culture infective dose.

Introduction

African horse sickness virus (AHSV) causes a noncontagious, infectious, insect-borne disease of equids (African horse sickness – AHS) that was first recognized in Africa in the sixteenth century. The effects of the disease, particularly in susceptible populations of horses, can be devastating with mortality rates often in excess of 90%. Although AHS is normally restricted to Africa (and possibly north Yemen), the disease has a much wider significance as a result of the ability of AHSV to spread, without apparent warning, beyond the borders of that continent. For these reasons the virus has been allocated OIE 'serious notifiable disease' status (i.e., communicable diseases which have the potential for very rapid spread, irrespective of national borders, which are of serious socioeconomic or public health consequence and which are of major importance in the international trade of livestock or livestock products).

Taxonomy, Properties of the Virion and Genome

African horsesickness virus is a species of the genus *Orbivirus* within the family *Reoviridae*. The virus is nonenveloped, approximately 90 nm in diameter and has an icosahedral capsid that is made up of three distinct concentric protein layers (**Figure 1**), and which is very similar to the structure of bluetongue virus (the prototype orbivirus). Nine distinct serotypes of AHSV have been identified by the specificity of interactions between the more variable viral proteins that make up the outermost layer of the virus capsid (VP2 and VP5), and neutralizing antibodies that are normally generated during infection of a mammalian host. The outer capsid layer surrounds the AHSV core particle (~70 nm diameter), which has a surface layer composed of 260 trimers of VP7 attached to the virus subcore. These VP7 trimers form a closed icosahedral lattice, which is made up of five- and six-membered rings that are visible by electron microscopy, giving rise to the genus *Orbivirus* (from the Latin '*orbis*' meaning ring or cycle – **Figure 2**). The VP7 trimers synthesized in infected cells sometimes form into large hexagonal crystals, composed entirely of six-membered rings, which can be observed by both electron and light microscopy. The VP7 lattice on the core surface helps to stabilize the thinner and more fragile subcore layer, which is composed of 120 copies of VP3 arranged as 12 dish-shaped decamers that interact, edge to edge, to form the complete innermost capsid layer. This subcore shell also contains the three minor viral proteins (VP1, VP4, and VP6) that form approximately 10 transcriptase complexes, associated with the 10 linear segments of dsRNA that make up the virus genome.

The five viral proteins present in the AHSV core particle and two of the nonstructural proteins (NS1 and NS2) that are also synthesized within the cytoplasm of infected cells are relatively more conserved than the outer capsid proteins. NS1 forms long tubules within the infected cell cytoplasm that are characteristic of orbivirus infections. NS2 is a major component of the granular matrices (viral inclusion bodies or VIBs) that represent the major site of viral RNA synthesis and particle assembly during the replication of AHSV and other orbiviruses (**Figure 3**). These more conserved AHSV proteins contain serogroup-specific epitopes, which cross-react between different AHSV serotypes and can therefore be used as a basis for serological assays to distinguish AHSV from the members of other *Orbivirus* species (e.g., *Equine encephalosis virus* (EEV)).

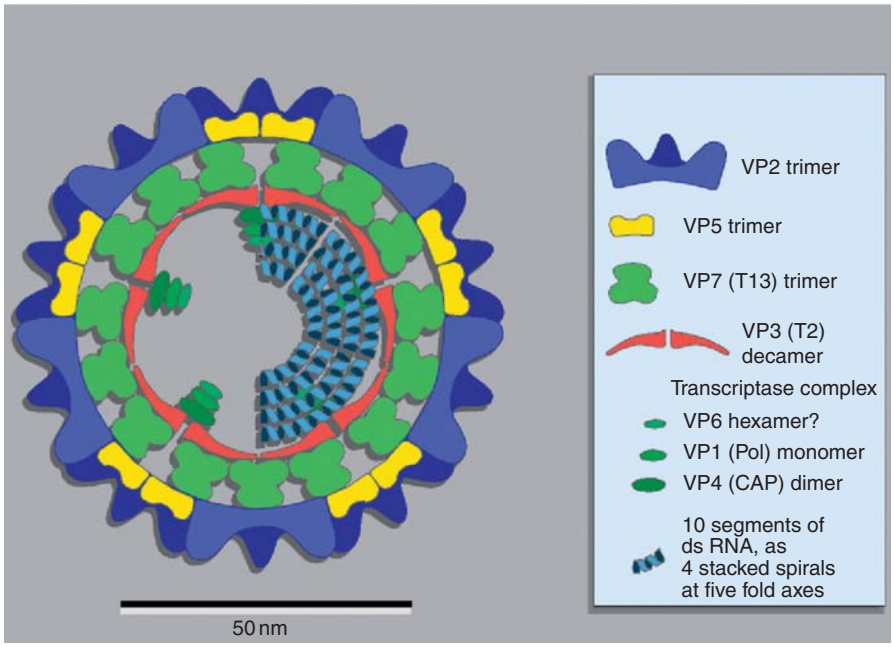


Figure 1 Diagram of the African horse sickness virus particle structure, constructed using data from biochemical analyses, electron microscopy, cryo-electron microscopy, and X-ray crystallography. Courtesy of P.P.C. Mertens and S. Archibald – reproduced from Mertens PPC, Maan S, Samuel A, and Attoui H (2005) Orbivirus, Reoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 466–483. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

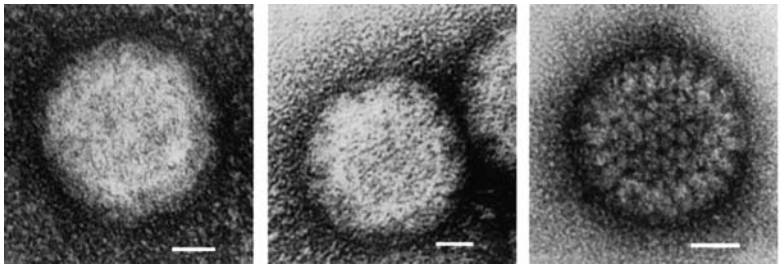


Figure 2 Electron micrographs of African horse sickness virus (AHSV) serotype 9 particles stained with 2% aqueous uranyl acetate (left) virus particles, showing the relatively featureless surface structure; (center) infectious subviral particles (ISVP), containing chymotrypsin cleaved outer capsid protein VP2 and showing some discontinuities in the outer capsid layer; (right) core particles, from which the entire outer capsid has been removed to reveal the structure of the VP7(T13) core-surface layer and showing the ring-shaped capsomeres (line represents 20 nm). Reproduced from Mertens PPC, Maan S, Samuel A, and Attoui H (2005) Orbivirus, Reoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 466–483. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

AHSV genome segment 10 encodes two small but largely similar proteins, NS3 and NS3a, that are translated from two in-frame start codons near the upstream end of the genome segment (see **Figure 4**). These proteins, which (by analogy with bluetongue virus) are thought to be involved in the release of virus particles from infected cells, are also highly variable in their amino acid sequence, forming into three distinct major clades. The biological significance of sequence variation in NS3/3a is uncertain, although it is clearly independent of virus serotype.

AHSV serotypes 1–8 are typically found only in restricted areas of sub-Saharan Africa while serotype 9 is more widespread and has been responsible for virtually all epizootics of AHS outside Africa. The only exception is the 1987–90 Spanish–Portuguese outbreak that was due to AHSV serotype 4.

AHSV is relatively heat resistant; it is stable at 4 and −70 °C but is labile between −20 and −30 °C. It is partially resistant to lipid solvents. At pH levels below 6.0 the virus loses its outer capsid proteins, reducing its infectivity for mammalian cell systems, although the

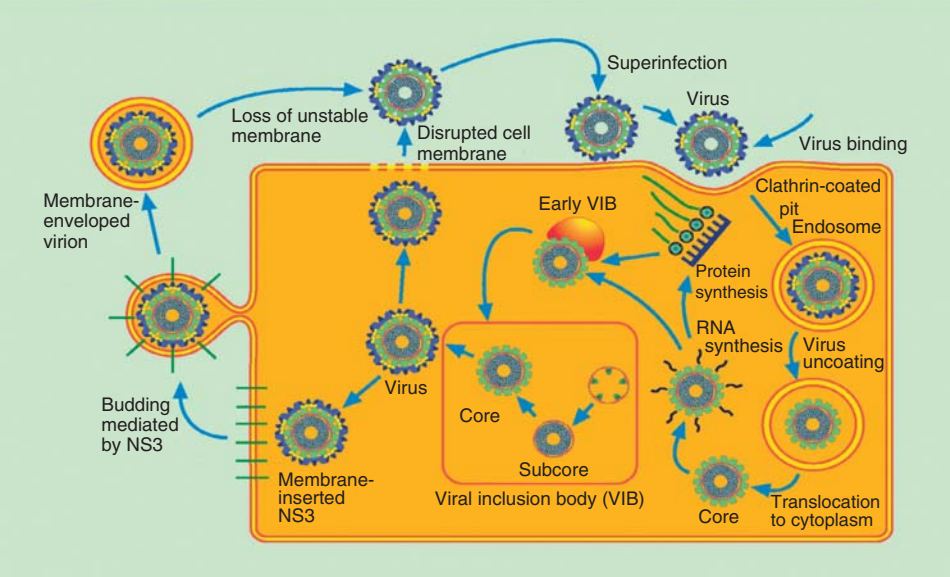


Figure 3 Diagram of the AHSV replication cycle, based primarily on that of BTV and other members of the family *Reoviridae*. Virus adsorption involves components of the outer capsid, although cell entry may also involve VP7(T13). VP2 (possibly also VP5) is involved in cell attachment. VP5 may be involved in penetration of the cell membrane (release from endosomes into the cytoplasm) and the expressed protein can induce cell fusion. The outer capsid layer is lost during the early stages of replication, which activates the core-associated transcriptase complexes. These synthesize mRNA copies of the 10 genome segments, which are then translated into the viral proteins. These mRNAs are also thought to combine with newly synthesized viral proteins, during the formation and maturation of progeny virus particles. The viral inclusion bodies (VIBs) are considered to be the sites of viral morphogenesis and viral RNA synthesis. Negative RNA strands are synthesized on the mRNA templates, within nascent progeny particles, reforming the dsRNA genome segments. The smallest particles containing RNA that are observed within VIBs are thought to represent progeny subcore particles. The outer core protein (VP7(T13)) is added within the VIB and the outer CP at the periphery of the VIB. Reproduced from Mertens PPC, Maan S, Samuel A, and Attoui H (2005) Orbivirus, Reoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 466–483. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

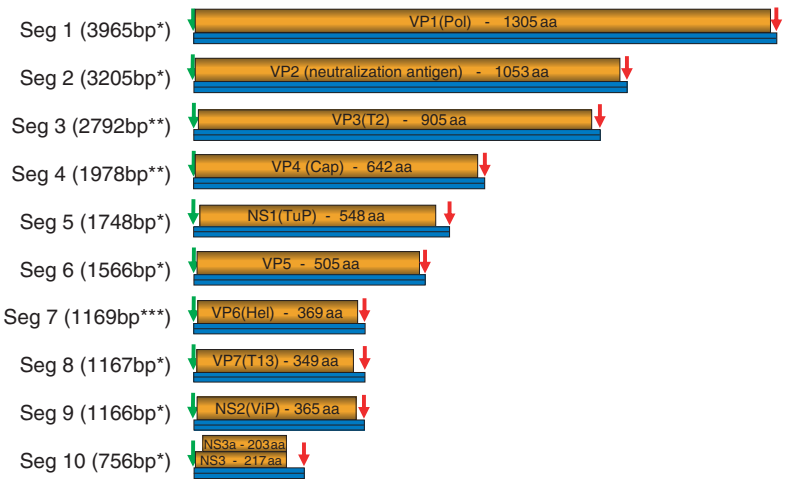


Figure 4 The genome organization of the 10 linear dsRNA genome segments of AHSV. Each genome segment encodes a single viral protein, with the exception of genome segment 10 which has two in-frame and functional initiation codons near the upstream end of the segment. *Data derived from AHSV-9. **Data derived from AHSV-4. ***Data derived from AHSV-6 (see: www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/AHSV.htm). Like other members of the family *Reoviridae,* each AHSV genome segment contains conserved terminal sequences immediately adjacent to the upstream and downstream termini (+ve strand (green arrow) 5′-GUU$^{A}/_{U}$A$^{A}/_{U}$. . . . . ..AC$^{A}/_{U}$UAC-3′ (red arrow)) (www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/CPV-RNA-Termin.htm).

core particle retains a lower level of infectivity until it is disrupted at ~pH 3.0.

Vertebrate Hosts

Equids are by far the most important vertebrate hosts of AHSV and the horse is the species most susceptible to disease, with mules and European donkeys somewhat less so. African donkeys are fairly resistant to clinical AHS, while zebra are usually only affected subclinically.

Occasionally, dogs or wild carnivores may become infected with AHSV by ingesting virus-contaminated equid meat and can die from the disease. Some reports also suggest that they can be infected by insect bite but most authorities believe that they play little or no part in the epidemiology of AHS and are merely dead-end hosts.

AHS is not a zoonosis. Although at least four human cases of severe disease have been documented, these were all infections acquired in an AHSV vaccine plant under conditions unlikely to be duplicated elsewhere.

Clinical Signs

AHSV can cause four forms of disease in equids and these are discussed in ascending order of severity.

Horse sickness fever is the mildest form of disease involving only a rise in temperature and possibly, edema of the supraorbital fossae; there is no mortality. It occurs following the infection of horses with less virulent strains of virus, or when some degree of immunity exists. It is usually the only form of disease exhibited by the African donkey and zebra.

The cardiac or subacute form of disease has an incubation period of about 7–14 days and then the first clinical sign is fever. This is followed by edema, first of the supraorbital fossae and surrounding ocular tissues (which may also exhibit hemorrhage), then extending to other areas of the head, neck, and chest. Petechial hemorrhages may appear in the conjunctivae and ecchymotic hemorrhages on the ventral surface of the tongue. Colic is also a feature of the disease. The mortality rate in horses from this form of disease may be as high as 50% and death usually occurs within 4–8 days of the onset of fever.

The next most severe is the mixed form of AHS which is a combination of the cardiac and pulmonary forms with mortality rates in horses as high as 80%.

The pulmonary form is peracute and may develop so rapidly that an animal can die without prior indication of disease. Usually, there will be marked depression and fever (39–41 ^{o}C) followed by onset of respiratory distress. Coughing spasms may also occur, the head and neck tend to be extended, and severe sweating develops. There may be periods of recumbence and terminally, frothy fluid or foam may be discharged from the nostrils. Death is from congestive heart failure or asphyxia and the mortality rate in horses is frequently over 90%. During epizootics in naive populations of horses all forms of disease can occur but the mixed and pulmonary forms usually predominate, so mortality rates well in excess of 80% are likely, making AHS one of the most lethal of all horse diseases.

Pathogenesis

On entry into the vertebrate host, initial multiplication of AHSV occurs in the regional lymph nodes. This is followed by dissemination throughout the body via the blood (primary viremia) and subsequent infection of the lungs, spleen, and other lymphoid tissues, and certain endothelial cells. Virus multiplication in these tissues and organs gives rise to secondary viremia, which is of variable duration and titer dependent upon a number of factors including host species. Under natural conditions, the incubation period to the commencement of secondary viremia is less than 9 days, although experimentally it has been shown to vary between 2 and 21 days. In horses, a virus titer of up to $10^{5.0}$ $TCID_{50}\,ml^{-1}$ may be recorded but viremia usually lasts for only 4–8 days and has not been detected beyond 21 days. In zebra, viremia occasionally extends for as long as 40 days but peaks at a titer of only $10^{2.5}$ $TCID_{50}\,ml^{-1}$. Viremia in donkeys is intermediate between that in horses and zebra in titer and duration, while in dogs it is considered to be very low level and transitory.

In experimentally infected horses, high concentrations of AHSV accumulate in the spleen, lungs, caecum, pharynx, choroid plexus, and most lymph nodes. Subsequently, virus is found in most organs, probably due to their blood content. In the blood, virus is associated with the cellular fraction (both red blood cells and the buffy coat) and very little is present in the plasma. This may be similar to the situation that occurs with bluetongue virus, in infected ruminants where virus is sequestered in the cell membrane of infected red blood cells and is thereby protected from the effects of humoral antibody. This leads to both virus and antibody circulating in the system together. In ruminants, this leads to extended viremia. This seems not to occur with AHSV in horses although viremia in the presence of circulating antibody has been reported in zebra. For AHSV, the onset of viremia usually corresponds with the appearance of fever and persists until it disappears.

In experimentally infected horses, exhibiting the peracute form of disease, antigen is found primarily in the cardiovascular and lymphatic systems and to a lesser extent throughout the body. In animals with horse sickness fever, antigen is concentrated in the spleen, with lesser amounts elsewhere. The main locations of antigen

are endothelial cells (suggesting that they are a primary target for the virus) and large cells of the red pulp of the spleen. The presence of antigen in large mononuclear cells and surrounding lymphoid follicles suggests that these cells might also be involved in virus replication and in the transport of viral protein to the lymphoid follicles.

Pathology

Macrolesions

These vary in accordance with the type of disease. In the pulmonary form, the most conspicuous lesions are interlobular edema of the lungs and hydrothorax. The subpleural and interlobular tissues are infiltrated with a yellowish gelatinous exudate and the entire bronchial tree may be filled with a surfactant, stabilized froth. Ascites can occur in the abdominal and thoracic cavities and the stomach mucosa may be hyperemic and edematous.

In the cardiac form, the most prominent lesions are gelatinous exudate in the subcutaneous, subfascial and intramuscular tissues, and lymph nodes. Hydropericardium is seen and hemorrhages are found on the epicardial and/or endocardial surfaces. Petechial hemorrhages and/or cyanosis may also occur on the serosal surfaces of the cecum and colon. In these instances, a distinct demarcation can often be seen between affected and unaffected parts. This may be due to a selective involvement of endothelial cells. As in the pulmonary form, ascites may occur but edema of the lungs is usually absent.

In the mixed form of AHS, lesions common to both the pulmonary and cardiac forms of the disease occur.

Microlesions

The histopathological changes are a result of increased permeability of the capillary walls and consequent impairment in circulation. The lungs exhibit serous infiltration of the interlobular tissues with distension of the alveoli and capillary congestion. The central veins of the liver may be distended, with interstitial tissue containing erythrocytes and blood pigments while the parenchymous cells show fatty degeneration. Cellular infiltration can be seen in the cortex of the kidneys while the spleen is heavily congested. Congestion may also be seen in the intestinal and gastric mucosae, and cloudy swelling in the myocardial and skeletal muscles.

Epidemiology and Transmission

AHSV is widely distributed across sub-Saharan Africa. It is enzootic in a band stretching from Senegal and Gambia in the west to Ethiopia and Somalia in the east, and reaching as far south as northern parts of South Africa. The virus is probably also enzootic in northern Yemen, the only such area outside the African continent. From these zones, the virus makes seasonal extensions both northward and southward in Africa. The degree of extension is dependent mainly upon the climatic conditions and how these affect the abundance, prevalence, and seasonal incidence of the vector insects. More rarely, the virus has spread much more widely and has extended as far as Pakistan and India in the east and Spain and Portugal in the west. However, prior to the 1987–91 Spanish, Portuguese, and Moroccan outbreaks, AHSV had been unable to persist for more than 2–3 consecutive years in any area outside sub-Saharan Africa or Yemen.

AHSV is transmitted between its vertebrate hosts almost exclusively via the bites of hematophagous arthropods. Various groups have been implicated over the years, ranging from mosquitoes to ticks, but certain species of *Culicoides* biting midge are considered to be by far the most significant vectors. Biting midges act as true biological vectors and support virus replication by up to 10 000-fold. Subsequent to feeding upon a viremic equid, susceptible species of *Culicoides* become capable of transmission after an incubation period of 8–10 days at 25 °C. This period lengthens as the temperature falls, and becomes infinite below 15–18 °C. The incubation or prepatent period in the vector is the time interval necessary for ingested virus to escape from the gut lumen by entering and replicating in the mid-gut cells, and then for progeny virus particles released into the hemocoel to reach and replicate in the salivary glands. Transovarial or vertical transmission of AHSV by biting midge vectors does not occur.

Culicoides imicola, a widely distributed species found across Africa, southern Europe, and much of Asia, is the major vector of AHSV and has long been considered to be the only important field vector. However, a closely related species, *C. bolitinos,* has recently been identified as a second vector in Africa, and the North American *C. sonorensis* (= *variipennis*) is a highly efficient vector in the laboratory. The identification of additional vectors is likely.

In general, *Culicoides* species have a flight range of less than a few kilometers. However, in common with many other groups of flying insects, they have the capacity to be transported as 'aerial plankton' over much greater distances. In this context, a considerable body of evidence suggests that the emergence of AHSV from its enzootic zones may sometimes be due to long-range dispersal flights by infected vectors carried on the prevailing winds.

Diagnosis

In enzootic areas, the typical clinical features of AHS (described earlier) can be used to form a presumptive

diagnosis. Laboratory confirmation should then be sought. The specimens likely to be required are:

1. *Blood for virus isolation.*
2. *Tissues for virus isolation (or for antigen detection by ELISA or RT-PCR-based assays)*: Spleen is best, followed by lung, liver, heart, and lymph nodes.
3. *Serum for serological tests*: Preferably, paired samples should be taken 14–28 days apart.

Confirmation of AHS is by one or more of the following:

1. Identification of the virus in submitted samples by the group specific, antigen detection ELISA or RT-PCR–based assays. AHSV RNA can be identified by RT-PCR assays using virus-species-specific oligonucleotide primers. This identification can be confirmed by sequence analyses of the resulting cDNA products and comparison to sequences previously determined for reference strains of AHSV and other orbiviruses.
2. Isolation of infectious virus in suckling mice or embryonating hens' eggs identification first by the group-specific antigen-detection ELISA, and then by the serotype-specific, virus neutralization or RT-PCR tests.
3. Identification of AHSV-specific antibodies by the group-specific antibody detection ELISA, CF, or the serotype-specific virus neutralization tests.

Differential Diagnosis

The clinical signs and lesions reported for AHS can be confused with those caused by the closely related EEV. Many aspects of the epidemiology of the diseases caused by these two viruses are also similar. They have a similar geographical distribution and vertebrate host range and the same vector species of *Culicoides*. As a result, both can occur simultaneously in the same locations and even in the same animal. Fortunately, rapid, sensitive, and specific ELISAs are available to enable the detection of the antigen and antibody of both the AHSV and EEV, and if used in conjunction can provide a rapid and efficient differential diagnosis.

Several other diseases may also be confused with one or other of the forms of AHS. The hemorrhages and edema reported in cases of purpura hemorrhagica and equine viral arteritis may be similar to those seen in the pulmonary form of AHS, although with AHS the edema tends to be less extensive and the hemorrhages are less numerous and widespread. The early stages of babesiosis (*Babesia equi* and *B. caballi*) can be confused with AHS, particularly when the parasites are difficult to demonstrate in blood smears.

Treatment

Apart from supportive treatment, there is no specific therapy for AHS. Affected animals should be nursed carefully, fed well, and given rest as even the slightest exertion may result in death. During convalescence, animals should be rested for at least 4 weeks before being returned to light work.

Control

Importation of equids from known infected areas to virus-free zones should be restricted. If importation is permitted, animals should be quarantined for 60 days in insect-proof accommodation prior to movement

Following an outbreak of AHS in a country or zone that has previously been free of the disease, attempts should be made to limit further transmission of the virus and to achieve eradication as quickly as possible. It is important that control measures are implemented as soon as a suspected diagnosis of AHS has been made and without waiting for confirmatory diagnosis. The control measures appropriate for outbreaks of AHS in enzootic and epizootic situations are described in Mellor and Hamblin.

See also: Orbiviruses; Reoviruses: General Features; Reoviruses: Molecular Biology.

Further Reading

Coetzer JAW and Guthrie AJ (2004) African horsesickness. In: Coetzer JAW and Tustin RC (eds.) *Infectious Diseases of Livestock,* 2nd edn, pp. 1231–1246. Cape Town: Oxford University Press.

Hess WR (1988) African horse sickness. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. 2, pp. 1–18. Boca Raton, FL: CRC Press.

Howell PG (1963) African horsesickness. In: *Emerging Diseases of Animals,* pp. 71–108. Rome: FAO Agricultural Studies.

Lagreid WW (1996) African horsesickness. In: Studdert MJ (ed.) *Virus Infections of Equines,* pp. 101–123. Amsterdam: Elsevier.

Meiswinkel Venter GJ and Nevill EM (2004) Vectors:*Culicoides* spp. In: Coetzer JAW and Tustin RC (eds.) *Infectious Diseases of Livestock,* 2nd edn, pp. 93–136. Cape Town: Oxford University Press.

Mellor PS (1993) African horse sickness: Transmission and epidemiology. *Veterinary Research* 24: 199–212.

Mellor PS (1994) Epizootiology and vectors of African horse sickness virus. *Comparative Immunology, Microbiology and Infectious Diseases* 17: 287–296.

Mellor PS, Baylis M, Hamblin C, Calisher CH, and Mertens PPC (eds.) (1998) *African Horse Sickness.* Vienna: Springer.

Mellor PS and Hamblin C (2004) African horse sickness. *Veterinary Research* 35: 445–466.

Mertens PPC and Attoui H (eds.) (2006) Phylogenetic sequence analysis and improved diagnostic assay systems for viruses of the family *Reoviridae*. http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/ReoID/AHSV-isolates.htm (accessed July 2007).

Mertens PPC and Attoui H (eds.) (2006) The dsRNA genome segments and proteins of African horse sickness virus (AHSV).

http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/AHSV.htm (accessed July 2007).
Mertens PPC, Attoui H, and Bamford DH (eds.) (2007) The RNAs and proteins of dsRNA viruses. http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/orbivirus-accession-numbers.htm (accessed July 2007).
Mertens PPC, Duncan R, Attoui H, and Dermody TS (2005) *Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 447–454. San Diego, CA: Elsevier Academic Press.
Mertens PPC, Maan S, Samuel A, and Attoui H (2005) *Orbivirus, Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 466–483. San Diego, CA: Elsevier Academic Press.
Sellers RF (1980) Weather, host and vectors: Their interplay in the spread of insect-borne animal virus diseases. *Journal of Hygiene Cambridge* 85: 65–102.
Walton TE and Osburn BI (eds.) (1992) *Bluetongue, African Horse Sickness and Related Orbiviruses.* Boca Raton, FL: CRC Press.

Relevant Website

http://www.oie.int – OIE data on AHSV outbreaks.

African Swine Fever Virus

L K Dixon and D Chapman, Institute for Animal Health, Pirbright, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Multigene family Genes that are derived by duplication and therefore related to each other.
Hemadsorption Binding of red blood cells around infected cells.

History and Geographical Distribution

African swine fever virus (ASFV) infection has been established over very long periods in areas of eastern and southern Africa, specifically in its wildlife hosts the warthog (*Phacochoerus aethiopicus*), the bushpig (*Potamochoerus porcus*), and the soft tick vector (*Ornithodoros moubata*). The virus is well adapted to these hosts, in which it causes inapparent persistent infections.

The disease caused by the virus, African swine fever (ASF), was first reported in the 1920s when domestic pigs came into contact with infected warthogs. Since then, ASF has spread to most sub-Saharan African countries. The first trans-continental spread of the virus occurred in 1957 to Portugal, via infected pig meat. Following a reintroduction of virus in 1960, ASF remained endemic in Spain and Portugal until the 1990s. During the 1970s and 1980s, ASF spread to other European countries, as well as Brazil and the Caribbean. Outside Africa, ASF is now endemic only in Sardinia, but within Africa ASF continues to cause major economic losses and has spread to countries such as Madagascar which were previously free from infections.

Analysis of the genomes of different virus isolates showed that those from wildlife sources in eastern and southern Africa are very diverse, reflecting long-term evolution in geographically separated host populations. Isolates from domestic pigs in western and central Africa, Europe, the Caribbean, and Brazil obtained over a 40 year period were all very closely related, suggesting that they were derived from a few introductions from wildlife reservoirs that have spread through pig populations. It is possible that these virus strains have been introduced into previously uninfected wildlife reservoirs in western and central Africa. Domestic pig isolates are more diverse in eastern and southern Africa. This suggests that several introductions of virus from wildlife hosts into domestic pigs have occurred in these regions.

Transmission

In its sylvatic cycle, ASFV is maintained by a cycle of infection involving warthogs and the soft tick vector *O. moubata*. Ticks are thought to become infected by feeding on young warthogs, which develop transient viremia. Virus replicates to high titers in ticks and can be transmitted between different developmental stages, sexually between males and females, and transovarially. Warthogs can become infected by bites from infected ticks. Although virus is present in adult warthog tissues, high viremia is not detected, and direct transmission between adult warthogs may therefore be limited. For this reason the tick vector is thought to play an important role in the transmission cycle involving these hosts (**Figure 1**).

In many African countries, ASF has become established as an enzootic disease in domestic pigs and is maintained in the absence of contact with warthogs. Within pig populations, virus can spread by direct contact between

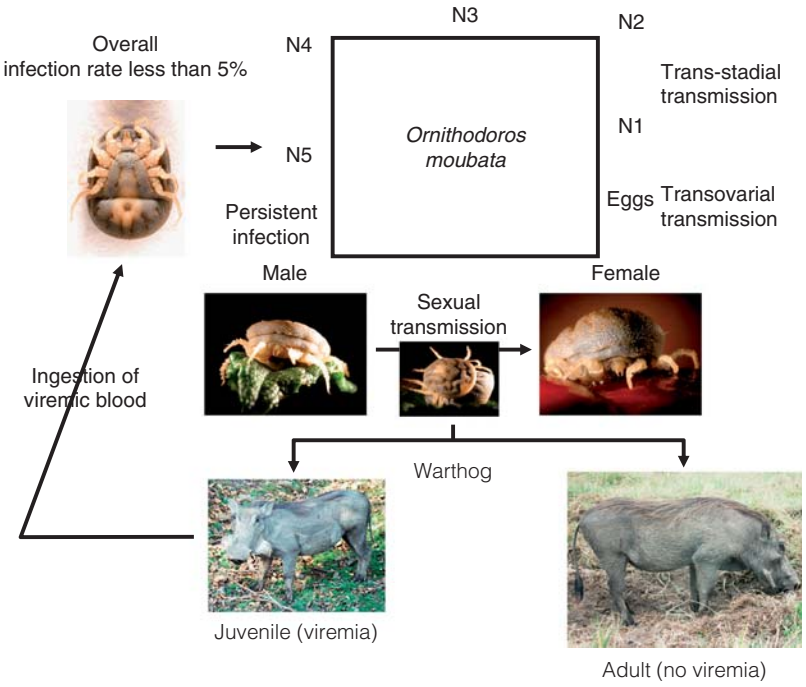


Figure 1 Transmission of ASFV between warthogs and *O. moubata* ticks. Ticks become infected by feeding on young warthogs, which develop a transient viremia. Virus replicates in ticks and is transmitted between different nymphal stages (N1–N5), transovarially, and transexually between males and females. Ticks remain infected over long time periods and can transmit virus to the warthogs on which they feed. Direct transmission between warthogs is thought to be infrequent since viremia is low in adult warthogs. In contrast, direct transmission between infected pigs occurs readily.

pigs, which develop a high viremia, or by ingestion of infected meat or other material. However, tick vectors may also play an important role in maintaining infection in areas where they are present. The virus can replicate in other species of *Ornithodoros* including *O. erraticus*, which played a role in maintaining infection in southern Spain and Portugal. There is no vaccine available, and disease control relies on rapid diagnosis and implementation of quarantine.

Pathogenesis

Most ASFV isolates cause an acute hemorrhagic fever with mortality approaching 100% in domestic pigs and wild swine (*Sus scrofa domesticus* and *S. s. ferus*) within 8–14 days post infection. Some moderately virulent isolates have been described that have a reduced mortality of around 30–50%. Low virulence isolates, which cause few disease signs and very low mortality, were also identified in the Iberian peninsula.

In pigs infected with virulent or moderately virulent ASFV isolates, viremia can peak at over 10^8 hemadsorption units $50\,ml^{-1}$. However, pigs that recover generally have lower levels of viremia and reduced replication in tissues. Only sporadic low viremia is observed in pigs infected with low virulence isolates, although moderate levels of virus replication are detected in lymphoid tissues. Persistence for long periods in recovered pigs demonstrates that the virus has effective mechanisms to evade host defense systems.

Virus replication in macrophages is observed at early times post infection, and only at later stages of disease has infection been reported in a variety of cell types, including endothelial cells, megakaryocytes, platelets, neutrophils, and hepatocytes. Thus, virus infection of macrophages is probably the primary event leading to the hemorrhagic pathology.

In common with other viral hemorrhagic fevers, ASF is characterized by damage involving induction of apoptosis in vascular endothelial cells, and this contributes to vascular permeability. This is thought to be caused by factors released from virus-infected macrophages in the early stages of disease. In the later stages of disease, the appearance of fibrin degradation products and the presence of numerous fibrin thrombi in blood indicate the development of disseminated intravascular coagulation.

Massive apoptosis of lymphocytes is observed in lymphoid tissues in pigs infected with virulent ASFV isolates. Since neither T nor B lymphocytes are infected directly by the virus, apoptosis is presumed to be caused by factors released from or on the surface of virus-infected macrophages. Observations that ASFV can act as a B-cell mitogen have suggested a model to explain lymphocyte apoptosis. This model proposes that dramatic depletion of T cells occurs by apoptosis induced by factors from

infected macrophages early in infection. B cells are activated by virus infection but, because of T-cell depletion, do not receive survival signals from T cells (such as CD154 interaction), and this results in B-cell apoptosis. This dramatic depletion of T and B lymphocytes impairs the immune response to infection.

Virus Structure

Virus particles are approximately 200 nm in diameter and have a complex multilayered structure (**Figure 2**). The nucleoprotein core contains the virus genome and enzymes and other proteins that are packaged into virus particles and used in the early stages of infection following virus entry. These include a DNA-dependent RNA polymerase, mRNA capping and polyadenylation enzymes, and other factors required for early gene transcription. The core is surrounded by a core shell and an internal envelope onto which the icosahedral capsid is assembled. Although earlier reports suggested that this internal membrane consists of a collapsed double membrane layer, it has also been suggested that only one membrane layer is present. Extracellular virions contain a loosely fitting external envelope which is derived by budding through the plasma membrane.

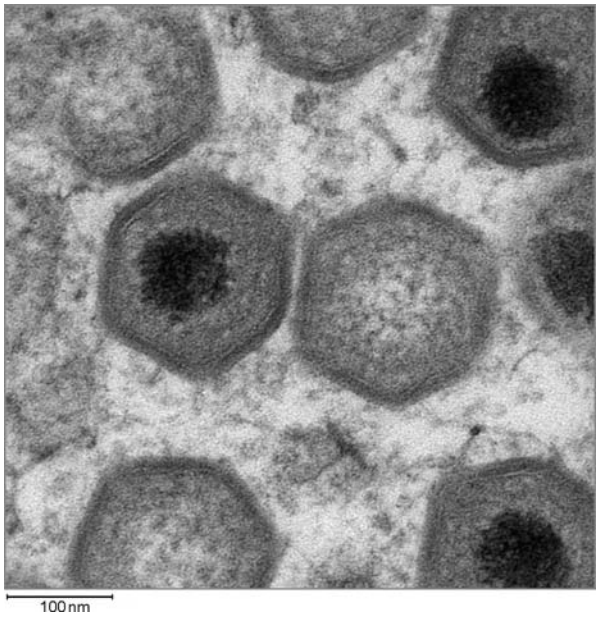


Figure 2 ASFV structure. An electron micrograph of mature and immature virions is shown. Mature particles contain a dense nucleoprotein core containing the DNA genome and proteins that include enzymes required for early gene transcription. This is surrounded by an internal envelope derived from the endoplasmic reticulum on which the icosahedral capsid is assembled. Immature particles differ in that the nucleoprotein core has not condensed to a dense structure and the icosahedral structure is not complete. Extracellular virus particles contain an additional envelope layer derived from the plasma membrane. Electron micrographs were kindly provided by Dr. Paul Monagahan and Pippa Hawes, Institute for Animal Health, Pirbright.

The capsid consists of a hexagonal arrangement of capsomers that appear as 13 nm long hexagonal prisms each with a central hole. The intercapsomer distance is about 8 nm, and the triangulation number has been estimated at between 189 and 217, corresponding to a capsomer number between 1892 and 2172. The number of virion proteins has been estimated at over 50 by two-dimensional gel electrophoresis. In addition to the virus-encoded enzymes, which are packaged into virions, 16 virus genes that encode virion structural proteins have been identified. Of these, two encode polyproteins (pp220 and pp60) which are processed by proteolytic cleavage by a virus-encoded SUMO-like protease into virion proteins p150, p37, p14, p34, or p35, and p15, respectively. Some of these proteins have been localized in virus particles by immunogold electron microscopy. The products of the pp220 polyprotein are localized in the core shell. Proteins p12, p24, and the CD2-like protein EP402R are present in the external region of virions. Seven of the virion proteins identified contain transmembrane domains.

Genome Structure

The virus genome consists of a single molecule of linear, covalently close-ended double-stranded DNA that is 170–192 kbp in size. The end sequences are present in two flip-flop forms that are inverted and complementary with respect to each other. Adjacent to both termini are inverted repeats, which consist of tandem repeat arrays and vary in length between 2.1 and 2.5 kbp. The complete genome sequences of one tissue-culture adapted ASFV isolate and nine field isolates have been determined. The number of open reading frames (ORFs) encoding proteins ranges between 160 and 175 depending on the isolate, and these are closely spaced and distributed on both DNA strands. The genome is A+T rich (~61%).

Replication

Virus Entry

The main target cells for ASFV replication are macrophages. Those macrophages that express cell surface markers characteristic of intermediate and late stages of differentiation are permissive for infection. Virus enters cells by receptor-mediated endocytosis (**Figure 3**). Recombinant scavenger receptor CD163 has been shown to bind to virus particles and to inhibit virus infection, suggesting that it may act as a virus receptor. Antibodies against recombinant virus proteins p12, p72, and p54 inhibit virus binding to cells, and those against p30 inhibit virus internalization. This suggests that these proteins have a role in these processes. Virus entry requires a fusion event between the viral envelope and the limiting membrane of the endosome at low pH. Following entry, virus cores are transported to perinuclear assembly sites via the microtubule network.

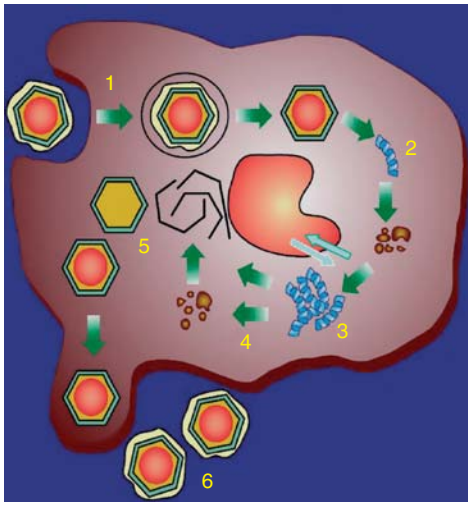


Figure 3 ASFV replication cycle. Virus particles enter cells by receptor-mediated endocytosis (1), and early gene expression begins in the cytoplasm using enzymes and factors packaged in the nucleoprotein core (2). Replication of full-length genomes takes place in cytoplasmic perinuclear factory regions, although an early stage of replication of subgenomic fragments occurs in the nucleus (3). Following the start of DNA replication, late gene expression occurs and virus structural proteins are synthesized (4). Virion assembly takes place in the virus factories (5) and virus particles are released by budding through the plasma membrane, during which process they gain an extra membrane (6).

DNA Replication and mRNA Transcription

Early virus gene transcription begins in the cytoplasm immediately following virus entry, using virus-encoded RNA polymerase, mRNA capping and polyadenylation enzymes, and factors packaged in the nucleoprotein core. Proteins encoded by early genes include enzymes and factors needed for later stages of the replication cycle.

A shift in the pattern of gene transcription occurs after the onset of DNA replication in the cytoplasm. Intermediate and late gene classes have been identified, and transcription of the latter is dependent on the onset of DNA replication. The similarity in the temporal pattern of gene expression suggests that ASFV transcription follows a similar regulation cascade to that of poxviruses. In general, transcription factors required for the next phase of transcription are synthesized in the previous phase. Late genes encode virion structural proteins and enzymes, and include factors such as RNA polymerase and early transcription factors that are packaged into virus particles for use during the next round of infection. Transcription of some early genes continues throughout the replication cycle.

Those virus promoters that have been mapped are short A+T-rich sequences that are located close upstream from the translation initiation codons. Transcription of all gene classes terminates at a sequence consisting of at least seven consecutive T-residues, which are often located within downstream coding regions. Increasing the number of T-residues causes transcription to terminate more efficiently. Virus mRNAs are capped at the 5′ end and polyadenylated at the 3′ end.

Although ASFV transcription is independent of host RNA polymerase II, productive infection requires the presence of the cell nucleus. The reasons for this are unknown, although a role in the early stages of DNA replication has been indicated by data showing replication of subgenomic length DNA fragments in the nucleus. One suggestion is that a nuclear primase may be necessary to initiate virus DNA replication.

Replication of full-length genomes occurs in the cytoplasmic factory regions via head-to-head concatamers, which are resolved to unit-length genomes and packaged into virus particles in the factories. The mechanism of DNA replication and transcription is similar to that of poxviruses, although an early phase of poxvirus DNA replication in the nucleus has not been detected.

Assembly

Virus morphogenesis takes place in perinuclear factory regions that are adjacent to the microtubule organizing center. Virus factories resemble aggresomes since they are surrounded by a vimentin cage and increased numbers of mitochondria. Aggresomes are formed in response to cell stress and function to remove misfolded proteins, and the virus may take advantage of this cellular stress response to form its assembly sites. Progeny virus particles are assembled from precursor membranes that are thought to be derived from the endoplasmic reticulum. These membranes are incorporated as an inner envelope into virus particles and become an icosahedral structure by the progressive assembly of viral capsid protein p72. Assembly of the p72 protein into virions requires a chaperone encoded by the B602L protein. Expression of virus protein p54 (encoded by the E183L gene) is required for recruitment of envelope precursors to factory regions. This transmembrane protein is inserted into the endoplasmic reticulum when expressed in cells. It also binds to the LC8 chain of the microtubule motor dynein via a motif in its cytoplasmic domain, and this may provide a mechanism for the recruitment of membranes to virus assembly sites. The core shell is formed beneath the inner envelope by the consecutive assembly of the core shell and the DNA-containing nucleoid. Envelopment and capsid formation require calcium gradients and ATP. Expression and processing of the p220 polyprotein is required for packaging of the nucleoprotein core, and when its expression is suppressed empty virus particles accumulate in factories and can be observed budding through the plasma membrane. Both ASFV and poxviruses assemble in the reducing environment of the cell cytosol and encode proteins involved in a redox pathway that is involved in formation of disulfide bonds in some

proteins in the factory. The ASFV B119L protein has been demonstrated to be a flavine adenine dinucleotide-linked sulfhydrl oxidase that is not incorporated into virus particles but is required for efficient virion maturation. The B119L protein interacts with the A151R protein, which contains a CXXC motif similar to that found in thioredoxins, and this binds to virion structural protein E248R. Possibly, all these proteins are components of a system for formation of disulfide bonds in virions.

The virus protein E120R binds to DNA and to capsid protein p72, suggesting a possible role in packaging of the genome. This protein is also required for transport from assembly sites to the plasma membrane, which occurs on microtubules using the conventional kinesin motor. Extracellular virus has an additional loose fitting external lipid envelope that is probably derived by budding through the plasma membrane.

Virus-Encoded Proteins

Comparison of the complete genomes of ten virus isolates shows that 109 ORFs are present as single copies.

The known functions of the proteins encoded include enzymes involved in replication and transcription of the virus genome, virion structural proteins, and proteins involved in evading host defenses. Many virus encoded-proteins are not essential for replication in cells but have roles in host interactions, which are important for virus survival and transmission (**Figure 4** and **Table 1**).

Proteins Involved in DNA Replication and Repair and mRNA Transcription and Processing

The virus encodes enzymes involved in nucleotide metabolism, such as thymidine and thymidylate kinases, ribonucleotide reductase and deoxyuridine triphosphatase. Although several of these are nonessential for replication in dividing tissue-culture cells, they are required for efficient replication in macrophages, which are nondividing and have small pools of precursor deoxynucleotide triphosphates required for incorporation into DNA. The virus encodes a DNA polymerase type B and a PCNA-like DNA clamp involved in replication of the genome. ASFV also encodes a putative DNA primase and helicase that is related to enzymes involved in binding to origins of replication and are probably involved in initiating DNA replication. An ERCC4-like nuclease is related to the principle Holliday junction resolvase, Mus81, of eukaryotes, and the virus also encodes a lambda-type exonuclease. These enzymes may be involved in resolution of the concatamers formed during virus replication.

A DNA polymerase type X, which is the smallest known, together with an ATP-dependent-DNA ligase and AP endonuclease comprise the components of a minimalist DNA base excision repair mechanism. The requirement of the AP endonuclease for efficient replication in macrophages, but not tissue-culture cells, supports the hypothesis that this repair system is an adaptation to virus replication in the highly oxidizing environment of the macrophage cytoplasm, which is likely to cause high levels of DNA damage.

Transcription of virus genes does not require the host RNA polymerase II, indicating that the virus encodes all of the enzymes and factors required. Genes with similarity to five subunits of RNA polymerase have been identified. The gene encoding the mRNA capping enzyme contains all three domains required for this function, namely a triphosphatase, a guanyl transferase, and a methyltransferase. Since transcription takes place in the cytoplasm, no introns are present in genes and the virus does not encode enzymes involved in splicing. An FTS-J-like RNA methyltransferase is encoded, which could play a role in stabilizing rRNA in infected cells.

The virus specifies several enzymes that might be involved either in regulating the virus replication cycle or in modulating the function of host proteins or cellular compartments. These enzymes include a serine/threonine protein kinase, a ubiquitin conjugating enzyme, and a prenyl transferase. The latter two enzymes are not encoded by other viruses.

Proteins Involved in Evading Host Defenses

A number of conserved ORFs encode proteins involved in evading host defense systems. The virus replicates in macrophages, which have important roles in activating and orchestrating the innate and adaptive immune responses. By interfering with macrophage function the virus can thus disrupt both of these types of host response. One protein, A238L, inhibits activation of the host transcription factor nuclear factor kappa B (NF-κB) and also inhibits calcineurin phosphatase activity. Calcineurin-dependent pathways, such as activation of NFAT transcription factor, are therefore inhibited. This single protein may prevent transcriptional activation of the wide spectrum of immunomodulatory genes whose expression depends on these transcription factors. So far, A238L has been shown to inhibit transcription of cyclooxygenase 2 (COX2) mRNA, thus inhibiting production of prostaglandins, which have a pro-inflammatory role. A238L has also been shown to inhibit transcription from the tumor necrosis factor (TNF)-α promoter.

In addition, the virus encodes a transmembrane protein (EP402R or CD2v), which has an extracellular domain that resembles the host CD2 protein. The host CD2 protein is involved in stabilizing the interaction between T cells and antigen-presenting cells. CD2v causes binding of red blood cells to infected cells and extracellular virions, and this may help to hide virus particles and infected cells from components of the host immune system. Deletion of the CD2v gene reduces virus dissemination in infected pigs and *in vitro* abrogates the ability of ASFV to inhibit

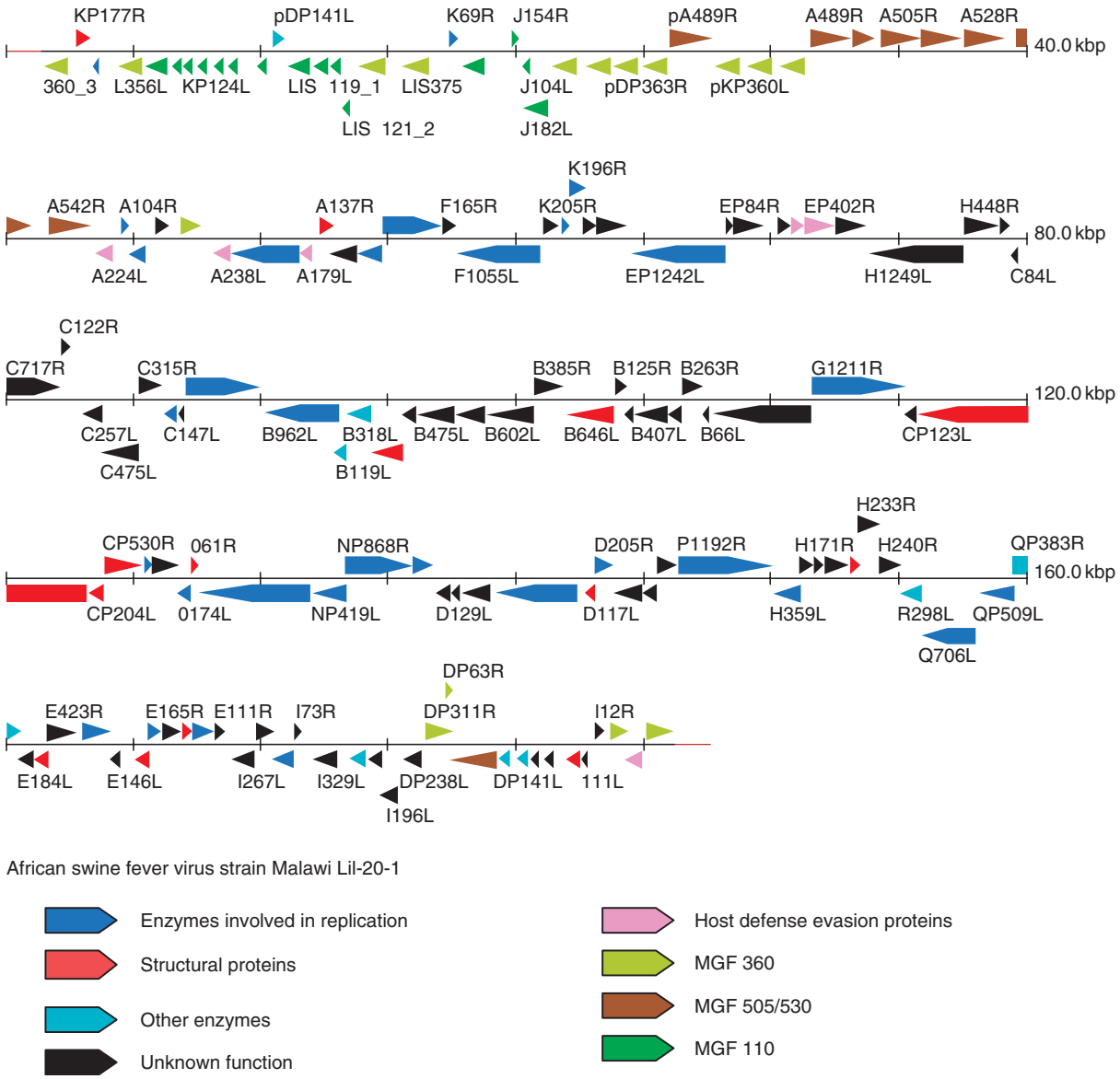


Figure 4 Genome map of the Malawi LIL20/1 isolate. Arrows show the size and direction in which protein-coding regions are transcribed. The colors indicate protein functional class or membership of MGFs.

proliferation of bystander lymphocytes in response to mitogens. The cytoplasmic tail of this protein differs from that of the host protein and binds to a cellular adaptor protein (SH3P7/mabp1), which has roles in endocytosis, transport through the Golgi, and signaling pathways. This interaction may modulate functions of SH3P7/mabp1.

One protein (designated NL-S, l14L, or DP71L) is related in its C-terminal domain to a herpes simplex virus-encoded neurovirulence factor (ICP34.5) and host GADD34 proteins. These proteins act as regulatory subunits of protein phosphatase 1 (PP1). The ASFV protein is required for virus-induced activation of PP1, and its predominantly nuclear location indicates a possible function in regulating host gene transcription.

Three virus proteins are known to inhibit apoptosis and therefore are predicted to prolong survival of infected cells and facilitate virus replication. These include proteins which are similar to the cellular apoptosis inhibitors Bcl-2 and IAP. The Bcl-2 homolog is expressed early and thought to be essential for virus infection, whereas the IAP homolog is nonessential, is expressed late, and packaged into virus particles. The IAP homolog may play a more critical role in the tick vector.

Multigene Families

A large proportion of the ASFV genome encodes multigene families (MGFs) consisting of related protein-coding

Table 1 Functions of ASFV genes

	Gene name in BA71V isolate	*Alternative names*	*Predicted protein size (kDa)*
Nucleotide metabolism, transcription, replication, and repair			
Thymidylate kinase	A240L		27.8
Thymidine kinase	K196R		22.4
dUTPase*	E165R	k1R	18.3
Ribonucleotide reductase (small subunit)	F334L		39.8
Ribonucleotide reductase (large subunit)	F778R		87.5
DNA polymerase β	G1211R		139.8
DNA topoisomerase type II*	P1192R	i7R	135.5
Proliferating cell nuclear antigen (PCNA) like	E301R	j15R	35.3
DNA polymerase family X*	O174L		20.3
DNA ligase*	NP419L	g3L	48.2
Putative DNA primase	C962R		111.2
AP endonuclease class II*	E296R	k4R	33.5
RNA polymerase subunit 2	EP1242L	g2L	139.9
RNA polymerase subunit 6	C147L	g6L	16.7
RNA polymerase subunit 1	NP1450L		163.7
RNA polymerase subunit 3	H359L	j1L	41.3
RNA polymerase subunit 5	D205R	i2R	23.7
Helicase superfamily II	A859L		27.8
Helicase superfamily II similar to origin binding protein	F1055L		123.9
Helicase superfamily II	B962L		109.6
Helicase superfamily II	D1133L	g10L	129.3
Helicase superfamily II	Q706L	j10L	80.4
Helicase superfamily II	QP509L		58.1
Transcription factor SII	I243L	k9L	28.6
Guanyl transferase*	NP868R	g4R	29.9
Poly A polymerase large subunit	C475L		54.7
FTS-J-like methyl transferase domain	EP424R		49.3
ERCC4 nuclease domain	EP364R		40.9
Lambda-like exonuclease	D345L		39.4
Other enzymes			
Prenyl transferase*	B318L		35.9
Serine protein kinase*	R298L	j8L	35.1
Ubiquitin conjugating enzyme*	I215L	k13L	24.7
Nudix hydrolase*	D250R	g5R	29.9
Host cell interactions			
IAP apoptosis inhibitor*	A224L	4CL	26.6
Bcl-2 apoptosis inhibitor*	A179L		21.1
IkB homolog and inhibitor of calcineurin phosphatase*	A238L	5EL	28.2
C-type lectin-like*	EP153R		18.0
CD2-like. Causes hemadsorbtion to infected cells*	EP402R	CD2v, Mw8R	45.3
Similar to HSV ICP34.5 neurovirulence factor	DP71L	I14L, NL	8.5
Nif S-like	QP383R	j11R	42.5
Structural proteins and proteins involved in morphogenesis			
P22	KP177R	P22	20.2
Histone-like	A104R		11.5
P11.5	A137R		21.1
P10	A78R	P10	8.4
P72 major capsid protein. Involved in virus entry	B646L	P72, P73	73.2
P49	B438L	P49	49.3
Chaperone. Involved in folding of capsid	B602L	9RL	45.3
ERV1-like. Involved in redox metabolism*	B119L	9GL	14.4
SUMO 1-like protease. Involved in polyprotein cleavage	S273R		31.6
P220 polyprotein precursor of p150, p37, p14, p34. Required for packaging of nucleoprotein core	CP2475L		281.5
P32 phosphoprotein. Involved in virus entry	CP204L	P30, P32	23.6
P60 polyprotein precursor of p35 and p15	CP530R		60.5
P12 attachment protein	O61R	P12	6.7

Continued

Table 1 Continued

	Gene name in BA71V isolate	*Alternative names*	*Predicted protein size (kDa)*
P17	D117L	i11L	13.1
J5R	H108R	j5R	12.5
P54 (j13L) Binds to LC8 chain of dynein, involved in virus entry	E183L	j13L, p54	19.9
J18L	E199L	j18L	22.0
pE248R	E248R	k2R	
P14.5 DNA binding. Required for movement of virions to plasma membrane	E120R	k3R	13.6

The functions of the virus encoded-genes are shown. Those which have been experimentally confirmed are marked with an asterisk. The designated gene names in the BA71V isolate sequence are shown and alternative names used in the literature. The predicted sizes of the encoded proteins are shown.

regions that are present in multiple copies and vary in number between different isolates. MGF 360 is the largest and contains between 11 and 19 copies in different genomes; MGF 505/530 contains between 8 and 10 copies; MGF 110 between 5 and 13; MGF 300 between 3 and 4; and MGF 100 between 2 and 3. In addition, the virus genomes contain between 1 and 3 copies of one of the virus structural proteins, p22. Comparison of the genomes of high and low virulence isolates has identified a fragment encoding 6 copies of MGF 360 and 2 copies of MGF 530 which are absent from the genome of the nonpathogenic isolate. These genes were also implicated in virulence and control of interferon-alpha production by deletion from the genome of a virulent isolate. The tissue-culture adapted isolate also has a deletion from a region close to the right end of the genome encoding five ORFs. These ORFs may facilitate virus replication in macrophages since the tissue-culture adapted isolate replicates poorly in primary macrophages.

The large investment in MGFs implies that they offer a selective advantage to the virus. However, their roles are largely unknown.

Classification and Relationship with Other Virus Families

ASFV was first classified as a member of the family *Iridoviridae* because of its large size, cytoplasmic location, and double-stranded DNA genome. However, studies of replication strategy and the genome revealed similarities with the *Poxviridae*, although the viruses differ structurally. ASFV was therefore placed as the species *African swine fever virus* into a separate virus family, the *Asfarviridae*, of which it is the sole member of the single genus, the *Asfivirus* genus. ASFV has also been considered to be part of a larger grouping of nucleo-cytoplasmic large DNA viruses (NCLDV) which, apart from the families mentioned above, includes the family *Phycodnaviridae* (large DNA viruses that infect blue-green algae) and the genus *Mimivirus* (which infect amebae). Replication of all families in the NCLDV grouping involves at least some stage in the cytoplasm, although each family has varying requirements for host nuclear functions. For example, both ASFV and poxviruses encode their own RNA polymerase which is packaged into virus particles so that transcription of early genes begins immediately following virus entry. Members of the *Iridoviridae* have a greater requirement for the nucleus since virus particles do not contain an RNA polymerase and early virus gene transcription and replication take place in the nucleus and are initiated by host enzymes. At later stages, virus DNA replication, transcription, and virus assembly take place in the cytoplasm. Less is known about the replication strategies of the *Phycodnaviridae* and *Mimivirus*, although they exhibit a greater involvement of nuclear functions in replication compared to the *Poxviridae* and *Asfarviridae*.

Analysis of the gene complements of different families in the grouping has indicated that the ancestral NCLDV may have encoded at least 40 genes involved in replication, transcription, packaging, and assembly. Each family has evolved to encode genes that represent adaptations to its particular ecological niche. Genome analysis suggests there are two major lineages, one consisting of the *Poxviridae* and *Asfarviridae* and other of the *Iridoviridae*, *Phycodnaviridae*, and *Mimivirus*.

Comparison of the NCLDV families suggests that genes have been acquired by horizontal transfer from eukaryotic and prokaryotic hosts as well as possibly from other viruses. However, there are few genes which show evidence of recent acquisition. Another feature of the NCLDV is the presence of MGFs which have evolved by processes of gene duplication and sequence divergence. The remarkable adaptation of ASFV to replicate in its tick vector suggests that its ancestor may have replicated only in arthropods and later acquired the ability to replicate also in mammalian hosts. The independence of the virus from host transcriptional machinery facilitates virus replication in both mammalian and arthropod hosts since the gene promoters do not have to be recognised by both the mammalian and

arthropod host transcriptional machinery. This could have facilitated the jump from arthropod to mammalian hosts. However, ASFV also encodes proteins (such as CD2v) that are clearly derived from a higher eukaryotic host, suggesting that growth in such hosts has substantially influenced ASFV evolution. Replication in macrophages provides the virus with opportunities to manipulate the host response to infection. This advantage may offset difficulties encountered by replicating in the harsh, microbiocidal environment of the macrophage cytoplasm.

Future Prospects

Over the last decade our knowledge of ASFV-encoded proteins involved in virus entry and assembly, as well as those with roles in evading host defenses and causing virulence, has increased dramatically. Likewise, our knowledge of host protective immune responses and of some of the virus proteins important in their induction has increased. These advances will help the development of effective vaccines to control this economically important disease. As with other large DNA viruses, the ASFV genome may be viewed as a repository of genes that have co-evolved with its hosts and serve to manipulate host defense responses. These genes will continue to provide tools for understanding host antiviral pathways and potential leads for discovery of new immunomodulatory drugs.

Investigations of the unique replication strategy and evolutionary niche of ASFV will aid our understanding of many aspects of virus host interactions and pathogenesis, as well as mechanisms of virus replication and evolution.

See also: Crenarchaeal Viruses: Morphotypes and Genomes; Emerging and Reemerging Virus Diseases of Vertebrates; Enteric Viruses; Epidemiology of Human and Animal Viral Diseases; Poxviruses; Viruses Infecting Euryarchaea.

Further Reading

Afonso CL, Piccone ME, Zaffuto KM, *et al.* (2004) African swine fever virus multigene family 360 and 530 genes affect host interferon response. *Journal of Virology* 78: 1858–1864.

Andres G, Alejo A, Salas J, and Salas ML (2002) African swine fever virus polyproteins pp220 and pp62 assemble into the core shell. *Journal of Virology* 76: 12473–12482.

Borca MV, Carrillo C, Zsak L, *et al.* (1998) Deletion of a CD2-like gene, 8-DR, from African swine fever virus affects viral infection in domestic swine. *Journal of Virology* 72: 2881–2889.

Brun A, Rivas C, Esteban M, Escribano JM, and Alonso C (1996) African swine fever virus gene A179L, a viral homologue of bcl-2, protects cells from programmed cell death. *Virology* 225: 227–230.

Carrascosa JL, Carazo JM, Carrascosa AL, Garcia N, Santisteban A, and Vinuela E (1984) General morphology and capsid fine-structure of African swine fever virus-particles. *Virology* 132: 160–172.

Cobbold C, Whittle JT, and Wileman T (1996) Involvement of the endoplasmic reticulum in the assembly and envelopment of African swine fever virus. *Journal of Virology* 70: 8382–8390.

Dixon LK, Escribano JM, Martins C, Rock DL, Salas ML, and Wilkinson PJ (2005) *Asfarviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 135–143. San Diego, CA: Elsevier Academic Press.

Gomez-Puertas P, Rodriguez F, Oviedo JM, Brun A, Alonso C, and Escribano JM (1998) The African swine fever virus proteins p54 and p30 are involved in two distinct steps of virus attachment and both contribute to the antibody-mediated protective immune response. *Virology* 243: 461–471.

Iyer LM, Balaji S, Koonin EV, and Aravind L (2006) Evolutionary genomics of nucleo-cytoplasmic large DNA viruses. *Virus Research* 117: 156–184.

Miskin JE, Abrams CC, and Dixon LK (2000) African swine fever virus protein A238L interacts with the cellular phosphatase calcineurin via a binding domain similar to that of NFAT. *Journal of Virology* 74: 9412–9420.

Powell PP, Dixon LK, and Parkhouse RME (1996) An IκB homolog encoded by African swine fever virus provides a novel mechanism for downregulation of proinflammatory cytokine responses in host macrophages. *Journal of Virology* 70: 8527–8533.

Revilla Y, Callejo M, Rodriguez JM, *et al.* (1998) Inhibition of nuclear factor κB activation by a virus-encoded IκB-like protein. *Journal of Biological Chemistry* 273: 5405–5411.

Takamatsu H, Denyer MS, Oura C, *et al.* (1999) African swine fever virus: A B cell-mitogenic virus *in vivo* and *in vitro*. *Journal of General Virology* 80: 1453–1461.

Tulman ER and Rock DL (2001) Novel virulence and host range genes of African swine fever virus. *Current Opinion in Microbiology* 4: 456–461.

Yanez RJ, Rodriguez JM, Nogal ML, *et al.* (1995) Analysis of complete nucleotide sequence of African swine fever virus. *Virology* 208: 249–278.

AIDS: Disease Manifestation

A Rapose, J East, M Sova, and W A O'Brien, University of Texas Medical Branch – Galveston, Galveston, TX, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Human immunodeficiency virus (HIV) infection has had a staggering global impact despite its emergence only 25 years ago. It is estimated that there were around 40 million people in the world living with HIV infection in the year 2006; there were approximately 4.5 million new cases, and 3 million individuals died of acquired immunodeficiency syndrome (AIDS). Since the introduction of highly active antiretroviral therapy (HAART) in the USA and in western Europe, around 1996, there has been a significant reduction in mortality and morbidity

among patients with HIV infection. The incidence of the three major opportunistic infections (OIs) associated with AIDS, namely *Pneumocystis carinii* (now called *Pneumocystis jiroveci*) pneumonia, *Mycobacterium avium complex* infection, and C*ytomegalovirus* infection is markedly reduced. Life expectancy for individuals with AIDS has increased from an estimated 4 years in 1997, to more than 24 years in 2004. Mortality rates for patients who are initiated on HAART appropriately are now comparable to populations successfully treated for other chronic conditions like diabetes. This paradigm shift has made OIs less common in populations able to access HAART. Cardiovascular disease, renal diseases, and malignancy are now the more common causes of death.

Since OIs commonly seen before the HAART era are rarely seen in patients with viral suppression, these are discussed only in passing, with citations referring to reviews or treatment guideline documents. This article focuses on clinical manifestations that pose problems in the management of HIV disease in the HAART era, including manifestations and consequences of HIV infection as well as toxic effects of treatment. There is overlap in these manifestations in that some HIV-related syndromes can be exacerbated by some antiretroviral medications. In addition, with prolonged survival, diseases associated with advancing age are becoming more prominent in HIV infection, most notably, the metabolic syndrome, which is associated with increased risk of morbidity and mortality from cardiovascular disease.

Table 1 Common opportunistic diseases in AIDS

Fungal infections
Candidiasis
Pneumocystis jirovecii pneumonia
Cryptococcal meningitis
Disseminated histoplasmosis
Disseminated coccidioidomycosis
Mycobacterial infections
Mycobacterium tuberculosis
Mycobacterium avium complex
Mycobacterium kansasii
Viral infections
Herpes simplex virus (HSV)
Varicella zoster virus (VZV)
Cytomegalovirus (CMV)
Epstein–Barr virus (EBV-associated with oral hairy leukoplakia and lymphoma)
Human papilloma virus (HPV – associated with cervical dysplasia and ano-genital squamous cancers)
Human herpes virus-8 (HHV-8 – associated with Kaposi's sarcoma)
JC polyomavirus (JCPyV – associated with progressive multifocal leukoencephalopathy)
Bacterial infections
Salmonella septicemia
Listeriosis
Bartonella henselae (associated with bacillary angiomatosis)
Parasitic infections
Cerebral toxoplasmosis
Cryptosporidiosis
Isosporiasis
Malignancies
Kaposi's sarcoma (associated with HHV-8)
Primary CNS lymphoma
Other non-Hodgkin's lymphomas
Cervical cancer (associated with HPV)

Opportunistic Infections and Malignancies Associated with HIV Infection

OIs and malignancies in patients with HIV infection emerge as a consequence of immune deficiency related to CD4+ T-lymphocyte depletion. A CD4+ T-lymphocyte count below 200 cells per mm^3 is defined as AIDS even in the absence of other diseases or symptoms since this represents such an increase in risk for OI and malignancy.

OIs and malignancies seen most commonly in HIV patients are listed in **Table 1**, and are discussed in detail in the 'AIDS surveillance case definition'. Although many of these diseases can occur in immunocompetent individuals, they are more common and often more severe in patients with AIDS. Detailed information regarding these diseases can be obtained from the website of the National Institutes of Health and the US National Library of Medicine.

Updated guidelines on the prevention of OIs in patients with HIV are available at 'Relevant website' section. While OIs are rarely seen in patients with viral suppression secondary to HAART, they may still be manifest on initial presentation. *Pneumocystis jirovecii* infection remains an important and serious clinical manifestation as initial presentation of many HIV patients. Since HIV can be a sexually transmitted disease (STD), other STDs also occur and can pose problems in patients with AIDS. These have been recently reviewed by Jeanne Marrazzo. The differential diagnosis of oral lesions in HIV patients is vast; this has been reviewed by Baccaglini L *et al.* Eye involvement in patients with HIV infection in the era of HAART has been studied in the longitudinal study of ocular complications of AIDS (LSOCA) supported by the National Eye Institute.

Malignancies classically associated with HIV infection include Kaposi's sarcoma, primary brain lymphoma, and other non-Hodgkin's lymphomas. These have been reviewed by Mathew Cheung. While some of the AIDS-defining cancers are seen less frequently in patients on HAART, it should be noted that the incidence of anal squamous intraepithelial lesions and squamous cell carcinomas is increasing in HIV-positive individuals receiving HAART.

An important phenomenon to recognize is 'immune reconstitution inflammatory syndrome' (IRIS). Following initiation of HAART, recovery of the immune system can be associated with an apparent worsening of an

HIV-associated OI or malignancy, or less commonly 'uncovering' of previously unrecognized and untreated diseases. Shingles (re-emergence of the chicken pox virus, *Varicella zoster virus*), other *Herpes* viruses, and mycobacterial infections are especially common in IRIS. With better global access to HAART, including Africa and Asia, an increasing number of cases of IRIS are being reported especially in areas with high prevalence of diseases like tuberculosis.

The Metabolic Syndrome in HIV-Infected Individuals

A constellation of laboratory and physical abnormalities, termed the metabolic syndrome, is associated with increased risk of cardiovascular morbidity and mortality (**Table 2**). This syndrome is seen in 24% of the US population overall; its prevalence is increasing in the US. The metabolic syndrome encompasses disturbances in glucose, insulin, and lipid metabolism, associated with abdominal obesity. The presence of the metabolic syndrome roughly doubles cardiovascular disease mortality. In other studies, the risk for cardiovascular disease with metabolic syndrome is even higher.

The metabolic syndrome may be even more common in HIV-infected individuals; there are many possible reasons. HIV infection on its own may exacerbate many of the manifestations of the metabolic syndrome, particularly elevation in serum triglycerides; this was seen in HIV-infected individuals prior to the advent of antiretroviral therapy. In addition, many HIV-infected individuals smoke (50% vs. 25% in the US population overall), and many HIV-infected individuals have hypertension. The metabolic syndrome may be exacerbated by some of the drugs used to treat HIV infection, including thymidine analog reverse transcriptase inhibitors and some protease inhibitors; both tend to increase triglycerides and cholesterol, and may be associated with glucose intolerance.

There are several studies, however, that show a lower incidence of metabolic syndrome in HIV-infected individuals. In a cross-sectional study examining a cohort of 788 HIV-infected adults, metabolic syndrome prevalence was 14% by IDF criteria. Despite this low overall number, many patients in this study (49%) had at least two features of the metabolic syndrome but were not classified as having the metabolic syndrome, typically because waist circumference was not in the metabolic syndrome range. The metabolic syndrome was more common in those individuals currently receiving protease inhibitors. Although the formally defined metabolic syndrome may occur less commonly in HIV-infected individuals, components of this syndrome associated with increased risk of cardiovascular disease certainly are increased in HIV-infected individuals.

Table 2 Universal classification of the metabolic syndrome, as defined by the International Diabetes Foundation (IDF)[41]

Characteristic	*Measurement*	
Waist circumference		
in women	>80 cm	(31.5 in)
in men	>94 cm	(37 in)
Triglycerides	>1.7 mmol l^{-1}	(>150 mg dl^{-1})
HDL	<1.29 mmol l^{-1}	(<50 mg dl^{-1})
Glucose	>5.6 mmol l^{-1}	(>100 mg dl^{-1})
Systolic blood pressure	>130 mm Hg	
or		
Diastolic blood pressure	>85 mm Hg	

In contrast to the decreased risk of OIs seen in HIV-infected individuals who achieved virologic suppression, the metabolic syndrome and lipodystrophy (described below) appear to be more common in patients receiving HAART. The higher presence of cardiovascular disease risk factors and administration of drugs that may induce it, together with the increased survival from improved outcomes from antiretroviral therapy, make this syndrome an important one for primary care of HIV-infected individuals.

Management of the metabolic syndrome initially involves improvement in diet, increase in exercise, and avoidance of drugs that are more likely to cause the perturbation in lipid and glucose metabolism and girth. Unfortunately, switch from medications associated with higher risk of the metabolic syndrome to those that appear to be less toxic, typically, only result in minor, partial reversal of both laboratory abnormalities and abdominal fat accumulation.

HIV-Specific Diseases

Lipodystrophy

Lipodystrophy was identified and characterized by Carr and colleagues in 1998. In addition to laboratory abnormalities associated with the metabolic syndrome, there can also be subcutaneous lipodystrophy, which can involve either fat accumulation in the abdomen, neck and upper back, (**Figure 1**) or lipoatrophy, involving the extremities and the face; (**Figure 2**), and they can both be seen together. The lipoatrophy in the face is highly recognizable and can be stigmatizing. There is deepening of the nasolabial folds and loss of subcutaneous tissue in the temples and cheeks. Lipodystrophy has been associated with treatment with thymidine nucleoside analog-based (stavudine or zidovudine) regimens, and co-administration of a thymidine analog with some protease inhibitors may further accelerate fat loss. Studies investigating thymidine-sparing regimens typically show normal limb fat mass and lower incidence of clinical lipoatrophy, even over prolonged follow-up.

Lipodystrophy is extremely difficult to manage, as specific treatments including rosiglitazone do not appear to be effective. Switching therapy from a thymidine analog to abacavir has shown modest improvement but not resolution of lipodystrophy. Recombinant growth hormone may



Figure 1 Lipodystrophy – fat accumulation. (a) Visceral fat accumulation, (b) flank fat accumulation, and (c) dorsal cervical fat-pad – 'Buffalo hump'.

increase subcutaneous fat but effects may not persist. Abacavir, lamivudine, and newer nucleotide reverse transcriptase inhibitors (NRTIs) do not appear to be associated with development of lipodystrophy, and to prevent development of lipodystrophy, the older NRTIs are now commonly avoided. An exception is zidovudine, which may still retain activity in the presence of drug resistance mutations that render other drugs in this class ineffective. Nonetheless, toxicities of HIV medications are now of paramount importance since there appear to be many ways to effectively reduce viral load.

HIV and Kidney Disease

Kidney disease related to AIDS was described as early as 1984 in reports from New York and Florida. Since then, a wide spectrum of acute and chronic renal syndromes has been reported. HIV-associated kidney disease was initially thought to occur late in the course of the infection, but it is now known that the kidneys may be involved in all the stages of HIV disease including acute infection. Renal glomerular and tubular epithelial cells may be directly infected by HIV. Effective therapies for HIV infection and



Figure 2 Lipodystrophy–lipoatrophy. (a) Facial lipodystrophy with deepening of the nasolabial fold and malar lipoatrophy, (b) partial fat atrophy with prominent veins, and (c) buttocks and leg wasting.

the associated OIs have led to improved patient survival, which in turn has resulted in an increased number of HIV-infected individuals who require renal replacement therapy. Antiretroviral treatment has also resulted in increased reports of drug-related nephrotoxicities. IRIS may also involve the kidneys. Mortality rates for kidney diseases in HIV-infected individuals are increasing, and there is evidence that HAART may slow or prevent progression.Appropriate screening for renal dysfunction and early intervention may reduce the incidence and progression of renal disease in patients with HIV infection.

Acute renal failure

Many of the causes of acute renal failure in HIV-infected patients are the same as for HIV-negative individuals, with a similar incidence of 5.9 cases per 100 patient-years. It is associated with a nearly sixfold increased risk of in-hospital mortality in HIV-infected patients. Factors associated with increased incidence in HIV-infected patients include advanced stage of HIV disease, exposure to antiretroviral therapy, and co-infection with hepatitis C virus. Pre-renal causes include hypovolemia, hypotension, or hypoalbuminemia. Intrinsic kidney diseases including acute tubular

necrosis may occur secondary to hypotension, sepsis, or nephrotoxic drugs. Some agents used for treatment of OIs in HIV-infected patients, such as amphotericin B, aminoglycosides, foscarnet, and trimethoprim-sulphamethoxazole require careful renal monitoring. Post-renal etiologies in the setting of HIV may include outflow obstruction secondary to tumor, lymphadenopathy or fungus balls, and medication related nephrolithiasis as seen with the antiretroviral medications indinavir and atazanavir, and with sulfadiazine and acyclovir. Acute renal failure secondary to interstitial nephritis as a manifestation of IRIS has also been reported.

Chronic kidney disease

Three syndromes of chronic renal disease are associated with HIV infection: (1) HIV-associated nephropathy (HIV-AN), (2) HIV-associated immune complex disease (HIV-ICD), and (3) HIV-associated thrombotic microangiopathies. A kidney biopsy is required to make these diagnoses.

HIV-associated nephropathy

HIV-associated nephropathy (HIV-AN) is directly caused by HIV infection and is the most common form of chronic renal disease in HIV-infected patients. Although this syndrome has been reported in all stages of HIV disease including acute infection, advanced immunosuppression is strongly associated with HIV-AN risk. In one study, 83% of HIV-infected patients with microscopic albuminuria who were biopsied, had HIV-AN. The prevalence of HIV-AN is variable among different ethnic and racial groups suggesting that there may be genetic determinants of the disease. Casanova *et al.* did not find HIV-AN on biopsies in 26 Italian HIV-positive patients with renal disease. Also, none of 26 HIV-infected positive individuals with proteinuria were found to have HIV-AN in a study from Thailand by Praditpornsilpa *et al.* The majority of patients in the U.S. who have HIV-AN are African-American males (more than 85%). However, in one study on Ethiopian HIV patients, none of the patients fulfilled criteria for HIV-AN. This suggests that even among individuals with African heritage there may be genetic differences. Alternatively, factors other than race may play an important role in the epidemiology of this condition. Patients typically present with nephrotic syndrome in which large amounts of protein pass abnormally in the urine. However, peripheral edema, hypertension, and hematuria are often absent. Microalbuminuria is an early marker for HIV-AN and screening for microalbumniuria is recommended for early diagnosis of HIV-AN. Renal biopsy should be considered for HIV-seropositive African-American patients who present with microalbuminuria even if they have normal creatinine clearance. Classic HIV-AN is associated with focal and segmental glomerulosclerosis on histopathology. These patients can exhibit rapid progression to end-stage renal disease and prognosis is poor if left untreated. HAART may reduce the risk of development of HIV-AN and may also reduce progression of HIV-AN to end-stage renal disease, but this is controversial and antiretroviral therapy for patients with HIV-AN who do not otherwise have indications for treatment is not recommended. The best evidence for benefit of HAART in HIV-nephropathy is the reduction in incidence in the HAART era compared with earlier periods.

HIV-associated immune complex disease

HIV-associated immune complex disease (HIV-ICD) occurs less frequently than HIV-AN. There is a higher incidence in Caucasians. Four different categories have been described: (1) immune-complex mediated glomerulonephritis (with diffuse proliferative and crescentic forms), (2) IgA nephritis (with diffuse or segmental mesangial proliferation), (3) mixed sclerotic/inflammatory disease, and (4) lupus-like syndrome. The precise role of HIV infection in the pathogenesis of these entities has not been established, and glomerular inflammation may be due to the abnormal immune responses associated with HIV infection, or secondary to superinfections. The clinical presentation is often very different from HIV-AN. Patients present with hematuria and mild proteinuria. The course is more indolent, with low rates of progression to end-stage renal disease.

Thrombotic microangiopathy

Thrombotic microangiopathy in the setting of HIV infection is being increasingly recognized and has even been proposed as an AIDS-defining illness. It is seen more often in Caucasians as compared with African-Americans or Hispanics. Features include fever, diarrhea, hemolytic anemia, thrombocytopenia, renal failure, and neurological symptoms. Mortality rates are high even in the setting of aggressive treatment like plasma exchange and relapse is often seen in survivors. The pathogenesis of HIV-associated thrombotic microangiopathies is unknown.

Renal disease associated with HAART

In some cases, HAART can reverse or at least control nephropathy associated with HIV infection. However, many antiretroviral medications have been associated with renal toxicity including acute and chronic renal disease. The newer antiretroviral agents commonly in use are associated with few side effects. Adefovir was the first NRTI shown to have variable antiretroviral efficacy. However, it was highly nephrotoxic in doses (60–120 mg per day) used for treatment of HIV infection and for the first time an FDA advisory committee voted against the approval of an antiretroviral drug. It was subsequently used to treat hepatitis B infection, and appears to be safer at the lower dose. The follow-up NRTI tenofovir is associated with a modest decline in renal function, but this did not lead to greater rates of discontinuation of therapy. Also, in several large randomized trials, tenofovir did not show adverse effects on overall renal function.

However as clinical use of tenofovir has widened, there have been reports of tenofovir-induced, acute renal failure, Fanconi's syndrome, renal tubular damage, and diabetes insipidus. The majority of cases have occurred in patients with underlying systemic or renal disease, or in patients taking other nephrotoxic agents including other antiretroviral agents like didanosine, lopinavir-ritonavir, or atazanavir. Patients receiving tenofovir should have creatinin clearance monitored closely. The protease inhibitor indinavir is associated with nephrolithiasis (the drug can crystallize in the urine) but cases of kidney stones associated with saquinavir, nelfinavir, and atazanavir often associated with dehydration have also been reported. The majority of medication-related, adverse events are reversible on discontinuation of the offending drug.

Although HAART can decrease the incidence of some HIV-related kidney diseases, the drugs used may cause renal problems on their own. Hence, patients should be carefully monitored clinically and by laboratory testing for microalbuminuria. A diagnosis of HIV-AN could be an indication for early initiation of HAART in an attempt to prevent further progression and potentially reverse renal disease. Other pathologies such as microangiopathy-associated renal disease carry a poor prognosis in spite of aggressive interventions. Long-term survival in patients on HAART will be associated with increased prevalence of metabolic alterations, diabetes, hypertension, and cardiovascular disease which in turn may be associated with increased secondary renal disease in these patients.

Neurologic Manifestations of HIV

Neurological complications of HIV infection are common, with more than 50% HIV patients ultimately developing some clinical manifestations. The spectrum of the disease is broad. Classical neurologic complications of HIV infection recognized in the 1980s include: (1) aseptic meningitis, (2) HIV-associated dementia (HAD), (3) vacuolar myelopathy, and (4) distal symmetric sensory polyneuropathy. More recently, neurologic manifestations are most commonly associated with the therapeutic agents used in HAART. The most dramatic neurologic manifestations occur in HIV patients not on HAART. These include manifestations secondary to opportunistic diseases like progressive multifocal leukoencephalopathy (PML), cerebral toxoplasmosis, cryptococcal meningitis, tuberculosis, and malignancies like CNS lymphomas.

Aseptic meningitis or encephalitis

Aseptic meningitis or encephalitis may be seen in up to 50–70% of patients who develop acute antiretroviral syndrome. Manifestations may recur during the course of the disease or as part of IRIS. As seen with other acute viral infections, symptoms are nonspecific and may consist of fever, headache, malaise, lymphadenopathy, and skin rash. Sometimes they may be severe enough to require hospitalization. Less common manifestations of early HIV infection include cranial nerve involvement (most commonly facial nerve), brachial plexus, and cauda equina syndromes, Guillian-Barré-like demyelinating polyneuropathy, mononeuritis multiplex, and radiculopathy.

HIV-associated dementia

HIV-associated dementia (HAD) is the most common neurological manifestation of chronic HIV infection and developed in 20–60% HIV patients before effective antiretroviral treatments became available; recently, the incidence of HAD has declined. However, the prolonged life span of individuals with HIV may ultimately lead to an increased prevalence of HAD. Even in the HAART era, HIV-related neuropsychologic deficits have significant influence on the lives of the HIV-infected patients, with rates of unemployment and dependence for activities of daily living being higher among these individuals. Minor forms of cognitive and motor abnormalities that do not progress to severe dementia are also seen. HAD manifests itself in the form of progressive impairment of attention, learning, memory, and motor skills, often accompanied by a variety of behavioral changes. In the early stages, symptoms include poor concentration, mental slowing, and apathy-mimicking depression. As the disease progresses, there is worsening memory loss, personality changes (either reduced emotion or increased irritability, or disinhibition), loss of fine motor control, tremors, slowing and unsteadiness of gait, urinary incontinence, generalized hyperreflexia, and cerebellar and frontal release signs. HAD is characterized by a waxing and waning course over months to years. Neuropsychologic testing is required to make the diagnosis and follow its progression. The effects of HAD on daily activities can be measured using standardized functional evaluations. Cerebrospinal fluid (CSF) levels of HIV RNA are strongly predictive of HIV-related cognitive disorders. Low peripheral CD4+ lymphocyte counts and high levels of HIV viral load in plasma may also predict future dementia. HAD should be a diagnosis of exclusion, meaning that other causes of cognitive impairment like depression, metabolic disorders, thyroid disorders, OIs and malignancies, and drugs should be excluded. Efavirenz, a nonnucleoside reverse transcriptase inhibitor, is associated with neuropsychiatric side effects including dizziness, confusion, impaired concentration, amnesia, hallucinations, and insomnia. Most of these symptoms occur early after initiation of efavirenz and tend to resolve within a few weeks of continued treatment. Delayed onset psychiatric symptoms have also been described. This is readily distinguished from classical HAD. Proximal muscle weakness without sensory changes suggests myopathy. Polymyositis characterized by myalgias and proximal muscle weakness can result from HIV infection itself, or secondary to zidovudine therapy.

Distal symmetric sensory polyneuropathy

Distal symmetric sensory polyneuropathy (DSPN) was recognized as a complication of HIV infection, but now is more commonly seen as a toxicity of some antiretroviral medications. In the pre-HAART era the prevalence rate was about 35% and at autopsy, 95% patients had sural nerve involvement. Low CD4+ lymphocyte counts and high viral load are significant risk factors for the development of DSPN. It is characterized by burning or aching pain, paresthesias, along with numbness or hyperpathia. It is seen more often in the lower extremities, starting in the toes and progressing proximally. On examination, there may be reduced sensation to pain, temperature, and vibration, along with reduced ankle reflexes. Strength is usually preserved. It is important to rule out other causes like diabetes, alcohol, vitamin deficiencies, and drug related injury including HIV medications such as stavudine, didanosine, and the discontinued drug zalcitabine. Medications used to treat OIs such as the TB drug isoniazid can also cause neuropathy. Clinical presentation of neuropathy secondary to antiretroviral agents is similar to that of DSPN, though it is often more painful and may develop rapidly.

See also: Human Immunodeficiency Viruses: Antiretroviral agents; Human Immunodeficiency Viruses: Origin; Human Immunodeficiency Viruses: Pathogenesis.

Further Reading

Baccaglini L, Atkinson JC, Patton LL, Glick M, Ficarra G, and Peterson DE (2007) Management of oral lesions in HIV-positive patients. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics* 103(supplement): S50 e1–e23.

Carr A, Samaras K, Chisholm DJ, and Cooper DA (1998) Pathogenesis of HIV-1-protease inhibitor-associated peripheral lipodystrophy, hyperlipidaemia, and insulin resistance. *Lancet* 351(9119): 1881–1883.

Cheung MC, Pantanowitz L, and Dezube BJ (2005) AIDS-related malignancies: Emerging challenges in the era of highly active antiretroviral therapy. *Oncologist* 10(6): 412–426.

Cho ME and Kopp JB (2004) HIV and the kidney: A status report after 20 years. *Current HIV/AIDS Reports* 1(3): 109–115.

Daugas E, Rougier J-P, and Hill G (2005) HAART-related nephropathies in HIV-infected patients. *Kidney International* 67(2): 393–403.

Friis-Moller N, Reiss P, Sabin CA, *et al.* (2007) Class of antiretroviral drugs and the risk of myocardial infarction. *New England Journal of Medicine* 356(17): 1723–1735.

Kaplan JE, Hanson D, Dworkin MS, *et al.* (2000) Epidemiology of human immunodeficiency virus-associated opportunistic infections in the United States in the era of highly active antiretroviral therapy. *Clinical Infectious Diseases* 30(supplement 1): S5–S14.

Manji H and Miller R (2004) The neurology of HIV infection. *Journal of Neurology, Neurosurgery and Psychiatry* 75(supplement 1): i29–i35.

Marrazzo J (2007) Syphilis and other sexually transmitted diseases in HIV infection. *Topics in HIV Medicine* 15(1): 11–16.

Moyle GJ, Sabin CA, Cartledge J, *et al.* (2006) A randomized comparative trial of tenofovir DF or abacavir as replacement for a thymidine analogue in persons with lipoatrophy. *AIDS* 20(16): 2043–2050.

Palella FJ, Jr., Baker RK, Moorman AC, *et al.* (2006) Mortality in the highly active antiretroviral therapy era: Changing causes of death and disease in the HIV outpatient study. *Journal of Acquired Immune Deficiency Syndromes* 43(1): 27–34.

Roling J, Schmid H, Fischereder M, Draenert R, and Goebel FD (2006) HIV-associated renal diseases and highly active antiretroviral therapy-induced nephropathy. *Clinical Infectious Diseases* 42(10): 1488–1495.

Triant VA, Lee H, Hadigan C, and Grinspoon SK (2007) Increased acute myocardial infarction rates and cardiovascular risk factors among patients with HIV disease. *Journal of Clinical Endocrinology and Metabolism* 92: 2506–2512.

Weisberg LA (2001) Neurologic abnormalities in human immunodeficiency virus infection. *Southern Medical Journal* 94(3): 266–275.

Relevant Website

http://aidsinfo.nih.gov – Guidelines for the Use of Antiretroviral Agents in HIV-infected Adults and Adolescents.

AIDS: Global Epidemiology

P J Peters, P H Kilmarx, and T D Mastro, Centers for Disease Control and Prevention, Atlanta, GA, USA

Published by Elsevier Ltd.

Glossary

Adult prevalence Prevalence among the proportion of the population 15–49 years old (adults of reproductive age).
Antenatal Occurring before birth.
Concentrated epidemic HIV prevalence consistently over 5% in at least one defined subpopulation but below 1% in pregnant women. Implies that the epidemic is not established in the general population.
Generalized epidemic HIV prevalence consistently over 1% in pregnant women, a sentinel population used to assess trends in HIV prevalence and to estimate the adult HIV prevalence. Implies that sexual networking in the general population is sufficient to sustain the epidemic independent of high-risk subpopulations.

Incidence Number of new cases arising in a given time period (usually 1 year).
Nosocomial Relating to a hospital.
Pandemic An epidemic over a wide geographic area that affects a large population.
Prevalence Number of cases in the population at a given time point divided by the total population (a proportion).

Brief History of HIV/AIDS

Although the first cases of acquired immune deficiency syndrome (AIDS) were recognized in the United States in 1981, phylogenetic analysis of human immunodeficiency virus (HIV) sequences suggest that HIV may have been initially transmitted to humans around 1930. By 1985, HIV had been identified in every region of the world and an estimated 1.5 million people were infected globally. Since then, unprecedented scientific advances have been made in the epidemiology, basic science, and treatment of this newly identified virus. Despite these advances, the global HIV pandemic has expanded rapidly. By 2007, an estimated 33.2 million people were living with HIV and greater than 20 million people had died of AIDS. AIDS is now the leading cause of death among people 15–59 years old and the world's most urgent public health challenge. The implementation of effective prevention strategies has proven challenging but there have been notable successes. In the US and Western Europe, extensive prevention programs in the 1980s reduced rates of infection among men who had sex with men, and systematic screening of blood donations since 1985 has virtually eliminated the risk of HIV transmission from blood transfusion. Several middle- and low-income countries have also had successful prevention initiatives. Prevention campaigns in Thailand and Uganda, for example, have resulted in substantial reductions in HIV prevalence since the 1990s. Unfortunately, in many parts of the world, stigma, discrimination, and denial about issues such as sexuality and drug use have hampered attempts to contain this epidemic.

Molecular Epidemiology

HIV is an extremely genetically diverse virus. There are three phylogenetically distinct groups of HIV-1 based on genomic sequencing, groups M (main), O (outlier), and N (non-M, non-O). Each group has likely evolved from independent cross-species transmission events of chimpanzee simian immunodeficiency virus (SIVcpz) to humans. HIV-1 group M has spread to every region of the world and caused the global AIDS pandemic. Group O infections are uncommon and limited to people living in or epidemiologically linked to Central Africa (especially Cameroon). Group N infections have only rarely been described in Cameroon.

HIV-2 is a distinct primate lentivirus related to HIV-1 that is both less pathogenic and less transmissible. HIV-2 evolved from the cross-species transmission of sooty mangabey SIV (SIVsm). HIV-2 is highly concentrated in the West African country of Guinea-Bissau where the adult prevelance has been estimated to be as high as 8–10%. A lower prevalence (<2%) is found in surrounding West African countries. HIV-2 has remained geographically isolated to West Africa and countries with strong links to the region (Portugal, India, Angola, Mozambique, and France). Dual infection with HIV-1 and HIV-2 has been described but the viruses do not appear to recombine with each other.

High rates of viral replication coupled with continuous mutation and recombination events have resulted in the rapid genetic diversification of HIV-1 group M viruses. M group strains have diversified into nine distinct subtypes (or clades) and over 34 circulating recombinant forms (CRFs). The number of described CRFs has grown rapidly and is cataloged at the Los Alamos HIV sequence database. There are also a variety of unique recombinant forms (URFs) that have only been identified in a single person or an epidemiologically linked pair. The precise implications of variation between HIV-1 subtypes on pathogenesis, transmission, drug resistance, and immune control are not well understood. However, HIV diagnostic tests (enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), Western blot) have been able to accurately detect the vast majority of emerging subtypes and CRFs.

The initial genetic diversification of HIV-1 group M viruses likely occurred in Central Africa where the greatest diversity and earliest cases of HIV-1 have been identified. Subsequently, HIV-1 subtypes have spread with a geographically heterogeneous distribution (**Figure 1**). Subtype C, the dominant subtype in Southern Africa, Ethiopia, and India, causes 50% of the HIV infections worldwide. The predominance of subtype C, especially in countries with high-prevalence epidemics driven by heterosexual sex, has led to speculation that it may have an increased fitness for transmission. Subtype A accounts for 12% of infections worldwide and has a broad geographic distribution. CRF01_AE and CRF02_AG are two additional recombinant viruses involving subtype A that are epidemiologically important in Southeast Asia and West Africa, respectively. The emergence of these CRFs has raised concern that recombination may contribute to the selection of viruses with increased fitness, immune escape, or transmissibility. Subtype B predominates in the Americas and Western Europe. Finally, URFs are important components of the epidemics in East Africa, Central Africa, and South America. Undoubtedly, some of these URFs will emerge as important CRFs in the future.

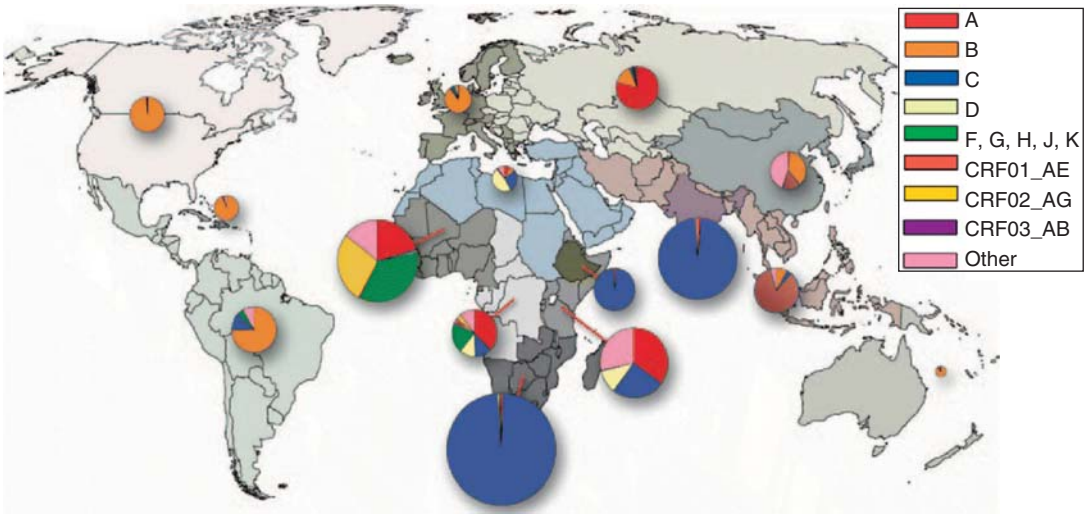


Figure 1 The global distribution of HIV-1 group M subtypes, 2004, by region. The world is subdivided into regions. Countries forming a region are shaded in the same color. Pie charts representing the distribution of HIV-1 subtypes and recombinants are superimposed on or connected by a line to the relevant region. The colors representing the different HIV-1 subtypes are indicated in the legend. The relative surface areas of the pie charts correspond to the relative number of individuals living with HIV in the region. Adapted from Hemelaar J, Gouws E, Ghys PD, and Osmanov S (2006) Global and regional distributions of HIV-1 genetic subtypes and recombinants in 2004. *AIDS* 20(16): W13–W23, with permission from Lippincott Williams and Wilkins.

Modes of Transmission

HIV can be transmitted by sexual contact, exposure to blood, and from mother to child with variable efficiency (**Table 1**). Although HIV has been isolated from a variety of body fluids, only blood, semen, genital fluids, and breast milk have been proven as sources of infection.

Unprotected sexual contact is the predominant mode of HIV transmission throughout the world. Despite a relatively low efficiency of transmission per sexual act, numerous factors can enhance transmission. Receptive anal intercourse often results in microtrauma to the rectal mucosa and therefore facilitates HIV transmission by exposing damaged mucosa to HIV-infected semen. Likewise, receptive vaginal intercourse probably transmits HIV (male-to-female) more efficiently than insertive vaginal intercourse. In general, concurrent sexual partners (and not simply the absolute number of partners) augment HIV's spread in a community. The probability of sexual transmission is also augmented by factors that affect the infectiousness of the source partner and the susceptibility of the recipient partner. A high HIV viral load, genital ulcerative disease (and other sexually transmitted diseases), and blood contact during sex (due to trauma or menstruation) can all increase the probability of transmission. Male circumcision reduces female-to-male transmission of HIV and may reduce male-to-female transmission to a lesser extent. Certain genetic factors can also decrease the probability of HIV transmission.

Among injection-drug users, HIV is transmitted by exposure to HIV-infected blood through shared contaminated needles and other injection equipment. Nosocomial transmission of HIV in hospitals from reuse of syringes and needles has also been documented and the risk of acquiring HIV from a transfusion with HIV-contaminated blood products approaches 100%.

Mother-to-child transmission can take place during pregnancy, labor and delivery, and during breast-feeding. The majority of transmissions (excluding breast-feeding) occur in

Table 1 Estimated per-act risk for acquisition of HIV, by exposure route

Exposure route	*Risk per 100 exposures to an infected source*[a]
Blood transfusion	90
Needle-sharing injection-drug use	0.67
Percutaneous needle stick	0.3
Receptive anal intercourse	0.5
Receptive penile-vaginal intercourse	0.1
Insertive anal intercourse	0.065
Insertive penile-vaginal intercourse	0.05
Receptive oral intercourse	0.01
Insertive oral intercourse	0.005
Mother-to-child transmission (without breast-feeding)	30
Breast-feeding for 18 months	15

[a]Estimates of risk for transmission from sexual exposure assumes no condom use.

Adapted from Smith DK, Grohskopf LA, Black RJ, *et al. (2005)* Antiretroviral postexposure prophylaxis after sexual, injection-drug use, or other nonoccupational exposure to HIV in the United States: Recommendations from the US Department of Health and Human Services. *MMWR Recommendations and Reports.* 54: 1–20 and Kourtis AP, Lee FK, Abrams EJ, Jamieson DJ, and Bulterys M (2007) Mother-to-child transmission of HIV-1: Timing and implications for prevention. *The Lancet Infectious Diseases* 6(11): 726–732.

the short interval during which the placenta detaches, labor occurs, and the infant passes through the birth canal. Overall rates of transmission are 15–40% without preventative interventions.

Epidemiology

An estimated 33.2 million people (2.5 million children) were living with HIV in 2007 (**Figure 2**), which is an increase of 4.2 million people since 2001. Every region of the world has had an increase in the number of people living with HIV from 2001 to 2007. The prevalence of HIV varies dramatically worldwide with a disproportionate number of infections in sub-Saharan Africa (**Figure 3**). Despite these statistics, there are some promising recent developments. The incidence of new HIV infections has peaked in many countries. There has also been a decline in the HIV prevalence among young women attending antenatal clinics (a sentinel population in generalized HIV epidemics) in several high-prevalence countries. These declines have correlated with reductions in high-risk sexual behavior and increased condom usage. General trends should be interpreted cautiously, however, as even within countries there can be tremendous variability in the HIV epidemic. Improvements in surveillance techniques over time, which include expanding surveillance sites to antenatal clinics in rural areas and conducting population-based surveys, can also make trends difficult to interpret. Furthermore, the epidemics in countries with large populations, such as Nigeria, Ethiopia, Russia, India, and China (which together comprise 44% of the world's population), are still evolving and expanding in some populations.

Sub-Saharan Africa

HIV has caused a generalized epidemic in many parts of sub-Saharan Africa. In 2007, almost 22.5 million people were living with HIV in sub-Saharan Africa (68% of the global infections but only 11–12% of the world's population), and although considerable efforts have been made to improve access to anti-retrovirals in recent years,



Figure 2 Adults and children estimated to be living with HIV, 2007. Reproduced from UNAIDS/WHO (2007) AIDS Epidemic update: December 2007. http://www.unaids.org/en/HIV_data/2007Epiupdate/default.asp (accessed November 2007).

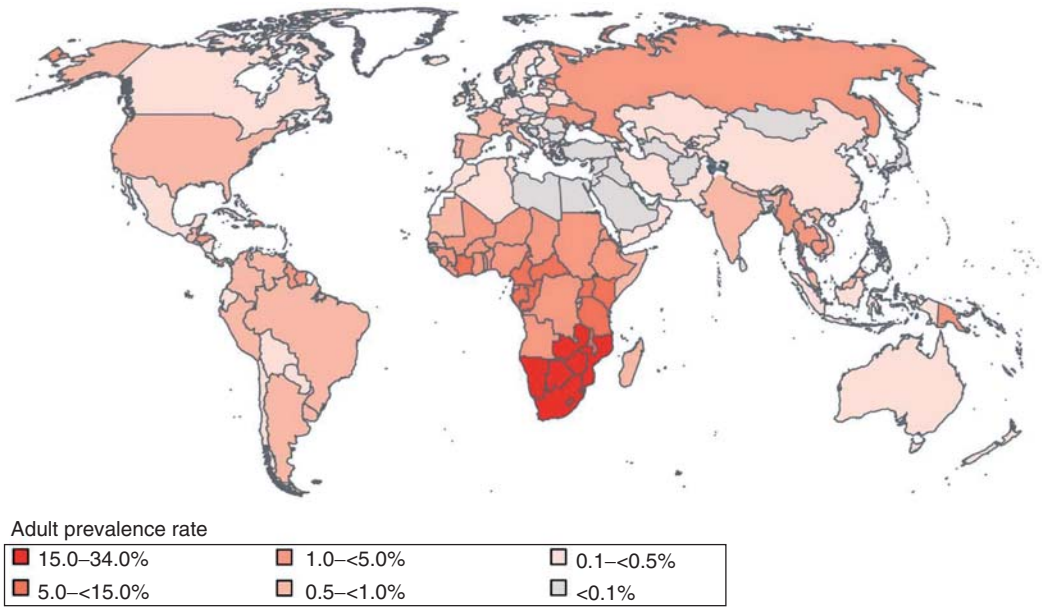


Figure 3 Adult prevalence of HIV, 2005. Reproduced with permission from UNAIDS/WHO (2006) 2006 report on the global AIDS epidemic. Geneva: UNAIDS.

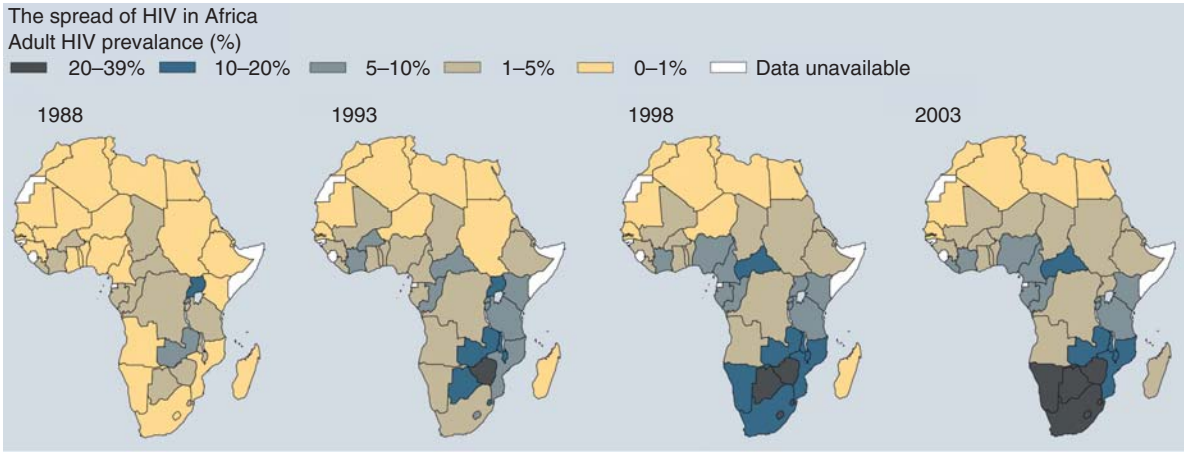


Figure 4 The spread of HIV in Africa, 1988–2003. Reproduced with permission from UNAIDS (2005) *AIDS in Africa: Three Scenarios to 2025*. Geneva: UNAIDS.

1.6 million Africans still died of AIDS in 2007 (76% of the AIDS deaths worldwide). Within Africa, the distribution of HIV is heterogeneous. Although HIV originated in Central Africa, Southern Africa now has the highest HIV prevalence in the world. Conversely, many East African countries have seen declines in their HIV prevalence and most West African countries have maintained a relatively low HIV prevalence.

HIV arrived late in Southern Africa. In 1988, South Africa had an HIV prevalence of less than 1% and the epidemic was centered in East and Central Africa (**Figure 4**). Unfortunately in the ensuing years HIV spread to unprecedented levels. Swaziland now has the most intense HIV epidemic in the world with an estimated 1 in 4 (26%) adults living with HIV in 2007. Several other Southern African countries also had an HIV prevalence greater than 20% in 2005. Various social and biological factors have likely predisposed certain African countries to these massive HIV epidemics. High rates of men migrating for work, concurrent sex partners, and genital herpes, in addition to low rates of male circumcision and gender-based inequalities, combine to fuel the epidemic in this region.

From 2003 to 2005, the adult HIV prevalence has remained stable but high (19–24%) in Botswana, Lesotho, and Namibia and has continued to increase in South Africa from 18.6% (2003) to 18.8% (2005). This stability, however, masks extremely high rates of new infections

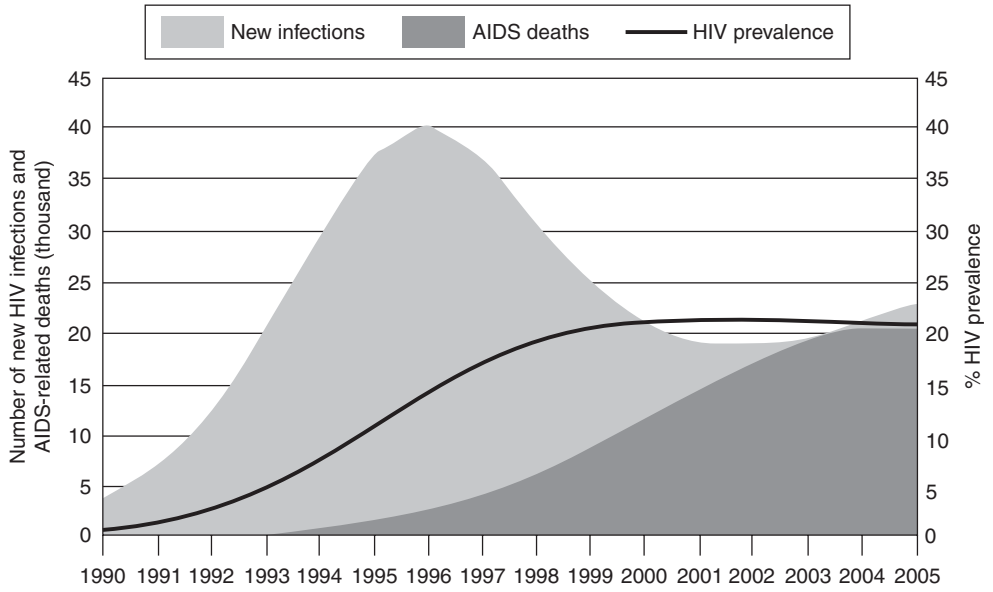


Figure 5 Estimated number of annual new infections and AIDS-related deaths among adults (15+) in relation to the stabilizing trend of estimated prevalence among adults (15–49), Lesotho, 1990–2005. Reproduced with permission from UNAIDS/WHO (2006) AIDS Epidemic Update: December 2006. Geneva: UNAIDS.

that are being balanced by high rates of AIDS deaths (**Figure 5**). The impact of HIV in Southern Africa cannot be overstated. The average life expectancy in Botswana has dropped from 65 years (1985) to 34 years (2006) as a result of HIV. Denial continues to be a factor driving high rates of new infections as many people in the region believe that they are at low risk of infection. In a survey by Shisana *et al.* in 2005 in South Africa, 50% of people who tested HIV-positive stated that they had no risk of acquiring HIV.

East Africa was among the regions most severely affected by HIV in the early 1990s (**Figure 4**), but subsequently the HIV prevalence has dropped. Uganda's HIV epidemic was the first to stabilize and then fall sharply during the 1990s. Strong political leadership and major prevention campaigns have been credited with this success. Uganda's national adult HIV prevalence was still 6.7% in 2005, however, and new evidence of increasing infections in rural areas reinforces the need to adapt prevention campaigns to changes in the epidemic. Kenya provides another encouraging example of a serious HIV epidemic that has declined from a national prevalence of 10% in the late 1990s to 7% in 2003 and 6% in 2006. In Ethiopia, the prevalence of HIV has declined slowly in the capital city of Addis Ababa (4.7% in 2005) since the mid-1990s and has remained low in rural areas. A concerning trend has been a recent increase in the number of new infections in rural parts of the country where 80% of the population lives. Halting the spread of HIV in rural communities will be one of the most important prevention priorities in East Africa.

Although the HIV prevalence in most of West Africa is comparatively low, HIV remains a serious problem in certain communities. The HIV epidemic in Côte d'Ivoire rapidly expanded in the 1980s and the adult prevalence has remained greater than 4% as of 2005. Fortunately, data from 2005 suggest a declining prevalence in the capital city of Abidjan as well as several other West African cities. As of 2005, an estimated 2.9 million people were living with HIV in Nigeria where the national prevalence is 4%. Within the country, there is considerable variation in rates of HIV for reasons not clear. In many low-prevalence West African countries, such as Senegal with a national prevalence of 1%, commercial sex work appears to be driving the epidemic. In Central Africa, incomplete data have hidden the exact nature of the HIV epidemic. Cameroon (5% national prevalence), the Central African Republic (11%), and the Democratic Republic of the Congo (3%), however, all have significant epidemics based on estimates of prevalence from 2005.

Asia

The adult HIV prevalence is lower in Asian countries (**Figure 3**) than sub-Saharan Africa and concentrated in high-risk groups. The epidemic continues to spread considerably, however, with over 400 000 new infections and over 300 000 deaths in 2007.

The highest regional HIV prevalence has occurred in Southeast Asia where commercial sex work, sex between men, and injection-drug use combine to fuel the epidemic. In Thailand and Cambodia, HIV spread rapidly in the late

1980s and early 1990s. Initial cases were seen in men who have sex with men and injection-drug users before spreading to female sex workers. HIV then spread to male clients who transmitted the virus to their wives and girlfriends. Extensive public education and prevention campaigns, however, have had some success in the region. Among young men in Thailand, increased knowledge of HIV and changes in sexual behavior, such as an increase in condom usage and a decrease in visits to sex workers, have correlated with reductions of the HIV prevalence in new military conscripts. Thailand's '100% Condom' campaign, which educated sex workers and promoted condom use, has also decreased HIV transmission from female commercial sex workers to their clients. Encouragingly, the epidemics in Thailand and Cambodia have been declining since the 1990s.

India is experiencing a complex HIV epidemic. Although the precise number of HIV infections in India has been debated, the Indian National AIDS Control Organization estimates that 2.5 million people were living with HIV in 2006 (an adult HIV prevalence of 0.36%). The majority of infections in India result from unprotected heterosexual sex and a large proportion of women are getting infected by their regular partners who became infected during commercial sex. Fortunately, there is evidence of a reduction in the prevalence of HIV among women attending antenatal clinics in south India from 2000 to 2004 which has correlated with increased prevention efforts in several southern Indian states. HIV prevention targeting sex workers could have a dramatic effect on the epidemic but law enforcement barriers and stigma complicate these prevention efforts. Injection-drug use is the major risk factor for HIV in the northeast and has become increasingly important in several major cities (Chennai, Mumbai, New Delhi).

In China, the HIV epidemic has followed an unusual pattern by beginning in certain rural areas, then spreading to cities. HIV was first identified among injection-drug users in the Yunnan Province (near China's southwestern border) in 1989. In the mid-1990s, a major HIV outbreak occurred in paid plasma donors in China's rural east–central provinces (Henan). During plasma donation, blood is taken from the donor and using a technique called apheresis the plasma is separated from the red blood cells. The red blood cells are then reinfused into the donor to prevent anemia. Reuse of tubing and mixing of red blood cells from multiple donors prior to reinfusion led to thousands of HIV infections in these paid donors. Since the mid-1990s, HIV has spread to all 31 of China's provinces and an estimated 650 000 people are living with HIV (0.05% prevalence in 2005). Although injection-drug use has been the predominant mode of transmission, sexual transmission has become increasingly important and accounted for an estimated 50% of new infections in 2005. China's response to its HIV epidemic was initially slow but has accelerated recently to incorporate evidence-based interventions from other countries (such as Australia's needle-exchange program and Thailand's condom campaign among sex workers).

Recently, the HIV epidemic has expanded among injection-drug users in several Asian countries, such as Pakistan, Afghanistan, Indonesia, and Malaysia. Given the overlap between injection-drug users and commercial sex workers, there is concern that the HIV epidemic could spread rapidly in these countries.

Eastern Europe and Central Asia

Until the late 1990s, the prevalence of HIV in Eastern Europe and Central Asia was extremely low. In the last decade, however, this region has experienced the fastest growing HIV epidemic and an estimated 1.7 million people are now living with HIV (over 90% of cases are in the Russian Federation and Ukraine). Injection drug use is the predominant method of HIV transmission but high rates of HIV are also emerging in the sex partners of injection drug users, female sex workers, and prisoners. Although the epidemic is still concentrated in high-risk groups in the Russian Federation and Ukraine, there is evidence that HIV is starting to spread into the general population. In the Ukraine, the proportion of people infected through heterosexual sex increased from an average of 14% from 1999–2003 to 35% in 2006. The Ukraine's HIV epidemic is also yet to peak despite an estimated adult HIV prevalence of 1.5% in 2005. The HIV epidemic is also starting to grow rapidly in the injection-drug user populations of several other Central Asian countries (Uzbekistan, Kazakhstan, and Tajikistan).

Caribbean

The HIV prevalence is high in many Caribbean countries where an estimated 1–4% of adults are living with HIV. The epidemic is fueled by concurrent heterosexual partners and commercial sex work. Sex between men is a hidden phenomenon but may account for 10% of cases. Since 2002, the adult HIV prevalence in urban areas of Haiti and the HIV prevalence among pregnant women in the Bahamas have declined, and these declines have correlated with high rates of condom use by sex workers.

Latin America

Brazil, the most populous country in Latin America, has 620 000 people living with HIV in 2005. In the 1990s, many experts predicted that Brazil's epidemic would rapidly accelerate. Brazil's sustained campaign, however, to promote sex education, condom use, harm reduction,

and HIV testing, and to provide anti-retroviral therapy halted HIV's expansion. Overall, the adult prevalence has remained stable at 0.5%. The majority of HIV infections in South America is occurring among men who have sex with men and injection-drug users. Incarcerated men, in particular, are at high risk for infection. Recently, rates of HIV have also increased among poor women from unprotected heterosexual sex with injection-drug users and men who also have sex with men.

The countries of Central America have the highest estimated HIV prevalences (1–2.5% in 2005) in Latin America and the epidemics appear to be growing. Sex between men and commercial sex are the major risk factors, but there is evidence that the epidemics are generalizing. Mexico has a relatively low (0.3% in 2005) HIV prevalence, but increasing rates of infection in cities along the United States border are being driven by injection-drug use and commercial sex work.

North America

The total number of people living with HIV continues to grow in North America due to the combination of the life-prolonging anti-retroviral treatment (with a corresponding decline in AIDS deaths) and a steady rate of new infections. The main risk factors for HIV are estimated to be unsafe sex between men (44%), unprotected heterosexual sex (34%), and injection-drug use (17%). In the US, women are estimated to account for over 25% of the AIDS cases and minority populations have been disproportionately affected by the epidemic. The rate of new HIV infections in the US was seven times higher in African–American men than white men and 21 times higher among African–American women compared to white women. In Canada, aboriginal people are also disportionately affected by the epidemic. An estimated 250 000 people living with HIV in the United States are not aware of their diagnosis. This large group presents a major obstacle toward controlling the HIV epidemic since people who are unaware of their status may continue to engage in high-risk behaviors.

Western Europe

In the 1980s, HIV spread widely among men who had sex with men and injection-drug users in Western Europe. Subsequent prevention programs have had success in reducing the incidence of HIV in these risk groups although the HIV prevalence in injection-drug users in Southern Europe remains high. The major modes of HIV transmission are now unprotected heterosexual sex (56%), unsafe sex between men (35%), and injection drug use (8%). Although heterosexual transmission has emerged as an important mode of HIV transmission in Western Europe, there is no evidence of a generalized epidemic. In many Western European countries, including Belgium, Sweden, and the United Kingdom, immigrants from sub-Saharan Africa account for the majority of heterosexually acquired infections. Unprotected sex with injection-drug users or men who have sex with men represents the other major risk for heterosexual transmission.

Oceania

There is a serious HIV epidemic in Papua New Guinea with an estimated adult prevalence of 1.8% in 2005. High rates of concurrent sexual partners, transactional sex, and violence against women have allowed the epidemic to grow rapidly. Sex between men is the predominant mode of HIV transmission in Australia and New Zealand.

Middle East and North Africa

Uneven HIV surveillance makes it difficult to gauge the epidemics in this region. Sudan has a generalized epidemic with an HIV prevalence of 1.6% in 2005. High rates of HIV have also been observed in injection-drug users in Iran, Libya, Algeria, Egypt, Morocco, and Lebanon.

Global Response

The world was initially slow to recognize the severity of the HIV pandemic but global efforts have increased dramatically in recent years. Between 1996 and 2005, annual funding for AIDS in low- and middle-income countries increased from $300 million to $8.3 billion. This acceleration of the world's response was prompted by increased human rights advocacy from people living with HIV and a concern that AIDS could destabilize global economic systems and threaten global security. Although these increases in funding are impressive, an effective global response will depend on sustained growth in annual funding of effective prevention and treatment programs until the epidemic can be stopped.

HIV Prevention

Comprehensive and sustained prevention programs have been proven to reduce HIV transmission. Unfortunately, HIV prevention strategies have not reached the majority of people at high risk. The major challenge for prevention has been generating the political will and economic resources to effectively implement proven strategies that address issues such as sex, sexuality, and drug use.

Behavioral interventions to promote safer sexual behavior are essential to reduce HIV transmission.

In Zimbabwe, sexual behavior change (increased condom use, delayed onset of sexual activity, and a reduction in sexual relations with nonregular partners) has contributed to declines in HIV prevalence. Successful behavioral interventions incorporate educational messages with prevention skills such as how to negotiate and use condoms and how to refuse sex. These interventions have been successfully implemented in school-based sex education classes, peer-led small group discussion groups, in the context of HIV counseling and testing, and as national structured interventions, such as Thailand's '100% Condom' program. For successful implementation, prevention programs must identify and engage high-risk populations.

Globally, an estimated 80% of people living with HIV are unaware of their status. Since people who are unaware that they are HIV-infected are more likely to continue transmitting HIV, an urgent priority is to increase access to HIV testing. Botswana has implemented a national, opt-out HIV-testing program in medical settings. Opt-out HIV testing has also been implemented in certain clinical settings in Kenya, Malawi, Uganda, and Zambia. In African countries with a high HIV prevalence a major risk factor for acquiring HIV is having a stable heterosexual partner who is HIV-infected (and often unaware of their status). Couples testing and counseling where both the male and female partners are counseled and HIV tested together has gained increased acceptance in certain African countries (Rwanda, Zambia) as a prevention tool in this high risk population. Universal HIV testing could both reduce transmission of HIV among newly identified infections and reduce the stigma of HIV testing.

An effective HIV vaccine would be a major advance in HIV prevention and is the subject of intense research efforts. Several other biomedical strategies also have the potential to dramatically improve HIV prevention. Male circumcision reduces a man's risk of acquiring HIV heterosexually by 50–60%. Since the highest prevalence of HIV is in countries where men are uncircumcised, large-scale implementation of male circumcision has the potential to prevent millions of new infections if provided in a safe and culturally appropriate way. Herpes simplex virus type 2 (HSV-2) infection is the most common cause of genital ulcers and is associated with an increased risk of HIV transmission. Clinical trials are now evaluating whether antiviral prophylaxis (to suppress HSV-2) will reduce the rate of HIV transmission. Interventions under a woman's control such as barrier diaphragms and vaginal microbicides which incorporate anti-retroviral drugs to block HIV transmission are also being evaluated in clinical trials. Daily oral administration of anti-retroviral drugs to high-risk, HIV-negative individuals, a strategy called pre-exposure prophylaxis (PrEP), is also being assessed. Successful biomedical interventions must still be packaged with effective behavioral prevention strategies to avoid behavioral disinhibition (an increase in high-risk behavior driven by a perceived decrease in risk for HIV infection).

Prevention of mother-to-child transmission (PMTCT) strategies have almost eliminated pediatric HIV in high-income countries. Treating the HIV-infected mother with anti-retrovirals and replacing breast milk with formula reduces the rate of mother-to-child HIV transmission from as high as 41% to less than 2%. Even a single dose of nevirapine given to the mother in labor and to the infant at birth significantly decreases the rate of HIV transmission. Despite these effective prevention strategies, there are still an estimated 1800 new mother-to-child HIV infections per day. These pediatric infections are occurring predominantly in sub-Saharan Africa due to a lack of access to PMTCT services. Breast-feeding also presents a difficult problem in sub-Saharan Africa since substituting formula for breast milk results in higher mortality among both HIV-infected and HIV-negative infants born to HIV-infected mothers. Treating HIV-infected breast-feeding women and/or their children with anti-retrovirals to prevent breast milk transmission is an innovative strategy being investigated.

Treatment of drug addiction, education about safe injection practices, and access to clean needles are effective prevention strategies in injection-drug users. In hospitals, infection-control programs that prohibit the reuse of injection equipment are necessary to prevent nosocomial HIV transmission. Finally, maintaining the safety of the blood supply by screening voluntary donors for HIV risk factors, testing each unit of blood for HIV, and reducing unnecessary transfusions, is another critical intervention to prevent HIV transmission.

HIV Care and Treatment

HIV mortality dropped precipitously in high-income countries after the introduction of highly active antiretroviral therapy (HAART) in 1996, but HAART remained unaffordable for many low- and middle-income countries. Brazil, however, made the pioneering decision to initiate a highly successful universal HIV treatment program which has inspired similar efforts in low-income countries. Since 2001, global financing for HIV care and treatment in low-income countries has increased markedly driven by funding from the Global Fund to Fight AIDS, Tuberculosis, and Malaria, the US President's Emergency Plan for AIDS Relief (PEPFAR), and the World Bank. By December 2006, over 2 million people living with HIV/AIDS were receiving treatment in low- and middle-income countries which represents 28% of the estimated 7.1 million people in need of treatment (**Table 2**). Treatment coverage remains strongest in Latin America (72%) driven by Brazil's leadership. The most encouraging improvements have been in sub-Saharan

Table 2 Estimated number of people receiving anti-retroviral therapy, people needing anti-retroviral therapy, and percentage coverage in low- and middle-income countries according to region, Dec. 2003–Dec. 2006

Geographical region	*Estimated number of people receiving antiretroviral therapy, Dec. 2006 (range)*[a]	*Estimated number of people needing antiretroviral therapy, 2006 (range)*[a]	*Antiretroviral therapy coverage, Dec. 2006 (range)*[b]	*Estimated number of people receiving antiretroviral therapy, Dec. 2005 (range)*[a]	*Estimated number of people receiving antiretroviral therapy, Dec. 2003 (range)*[a]
Sub-Saharan Africa	1 340 000 (1 220 000–1 460 000)	4 800 000 (4 100 000–5 600 000)	28% (24–33%)	810 000 (730 000–890 000)	100 000 (75 000–125 000)
Latin America and the Caribbean	355 000 (315 000–395 000)	490 000 (370 000–640 000)	72% (55–96%)	315 000 (295 000–335 000)	210 000 (160 000–260 000)
East, South, and South-east Asia	280 000 (225 000–335 000)	1 500 000 (1 000 000–2 100 000)	19% (13–28%)	180 000 (150 000–210 000)	70 000 (52 000–88 000)
Europe and Central Asia	35 000 (33 000–37 000)	230 000 (160 000–320 000)	15% (11–22%)	21 000 (20 000–22 000)	15 000 (11 000–19 000)
North Africa and the Middle East	5000 (4000–6000)	77 000 (43 000–130 000)	6% (4–12%)	4000 (3 000–5000)	1000 (750–1250)
Total	2 015 000 (1 795 000–2 235 000)	7 100 000 (6 000 000–8 400 000)	28% (24–34%)	1 330 000 (1 200 000–1 460 000)	400 000 (300 000–500 000)

Note: Some numbers do not add up due to rounding.
[a]Data on children (when available) are included.
[b]The coverage estimate is based on the estimated numbers of people receiving and needing antiretroviral therapy.
Reproduced with permission from the World Health Organization. Data from World Health Organization (2007) Towards Universal Access. Scaling up Priority HIV/AIDS Interventions in the Health Sector. Progress Report, April 2007. Geneva: WHO.

Africa where the number of people being treated has increased from 100 000 in 2003 to 1.3 million by December 2006. Sustained international funding from high-income countries and political will in low-income countries will be necessary to maintain high medication adherence, to monitor for drug toxicities and drug resistance, and to improve treatment regimens.

The HIV epidemic intersects with other important diseases. By weakening the immune system, HIV predisposes to opportunistic infections such as tuberculosis (TB). Globally, more than 21 million people are co-infected with TB and HIV. Although TB infection remains latent (neither contagious nor symptomatic) in most people without HIV, with TB/HIV co-infection the risk of progression to active, contagious disease increases 100-fold. TB and HIV synergistically worsen the burden of both diseases. The global TB incidence has increased, fueled by a rapid increase in HIV-associated TB in Africa. TB is also a leading cause of death among people living with HIV. Increased collaboration between TB-elimination and HIV-treatment programs is necessary to control both of these diseases.

Although there has been debate regarding the relative cost-effectiveness of prevention strategies compared to treatment programs, combined prevention and treatment strategies have synergistic benefits. Increased treatment access, for example, enhances awareness, reduces stigma, and increases the use of HIV testing and counseling services. Increased HIV testing, in turn, identifies HIV-infected people who can be counseled to prevent further transmission and treated with HAART when indicated, which also decreases infectiousness. Only effective prevention programs can reduce the incidence of new infections and thereby reduce the number of people who will need treatment in the future.

Future Prospects

In the past 25 years, HIV has emerged as the world's most serious public health problem. Every region of the world has been affected by AIDS, but sub-Saharan Africa has been disproportionately affected. Recent successful efforts to provide HIV care and treatment in low-income countries must now be expanded and sustained. In addition, prevention programs need to expand in a similar manner into low-income countries. These prevention strategies must include evidence-based interventions such as safer sex education programs with access to condoms and HIV-testing and counseling services. These programs must also be able to incorporate new biomedical prevention techniques (such as male circumcision) without compromising existing effective prevention services. Most importantly, sustained financial and political commitments will be required from both high- and low-income countries to control the HIV pandemic.

See also: AIDS: Disease Manifestation; AIDS: Vaccine Development; Human Immunodeficiency Viruses: Antiretroviral agents; Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Origin.

Further Reading

Centers for Disease Control, Prevention (CDC) (2006) The Global HIV/AIDS Pandemic, 2006. *Morbidity Mortality Weekly Report* 55(31): 841–844.

Galvin SR and Cohen MS (2004) The role of sexually transmitted diseases in HIV transmission. *Nature Reviews Microbiology* 2(1): 33–42.

Global HIV Prevention Working Group (2006) New Approaches to HIV Prevention: Accelerating Research and Ensuring Future Access, Aug. 2006. http://www.kff.org/hivaids/hiv081506pkg.cfm (accessed August 2007).

Gupta K and Klasse PJ (2006) How do viral and host factors modulate the sexual transmission of HIV? Can transmission be blocked? *PLoS Medicine* 3(2): e79.

Hemelaar J, Gouws E, Ghys PD, and Osmanov S (2006) Global and regional distributions of HIV-1 genetic subtypes and recombinants in 2004. *AIDS* 20(16): W13–W23.

Kourtis AP, Lee FK, Abrams EJ, Jamieson DJ, and Bulterys M (2007) Mother-to-child transmission of HIV-1: Timing and implications for prevention. *Lancet Infectious Diseases* 6(11): 726–732.

Sawires SR, Dworkin SL, Fiamma A, Peacock D, Szekeres G, and Coates TJ (2007) Male circumcision and HIV/AIDS: Challenges and opportunities. *Lancet* 369(9562): 708–713.

Smith DK, Grohskopf LA, Black RJ, *et al.* (2005) Antiretroviral postexposure prophylaxis after sexual, injection-drug use, or other nonoccupational exposure to HIV in the United States: Recommendations from the US Department of Health and Human Services. *MMWR Recommendations and Reports* 54: 1–20.

UNAIDS (1998) Partners in Prevention: International Case Studies of Effective Health Promotion Practice in HIV/AIDS. http://data.unaids.org/Publications/IRC-pub01/JC093-PartnersInPrevention_en.pdf (accessed August 2007).

UNAIDS (2005) *AIDS in Africa: Three Scenarios to 2025*. Geneva: UNAIDS.

UNAIDS/WHO (2006) 2006 report on the global AIDS epidemic. http://www.unaids.org/en/HIV_data/2006GlobalReport/default.asp (accessed August 2007).

UNAIDS/WHO (2006) AIDS Epidemic Update: December 2006. http://www.unaids.org/en/HIV_data/epi2006/default.asp (accessed August 2007).

UNAIDS/WHO (2007) AIDS Epidemic update: December 2007. http://www.unaids.org/en/HIV_data/2007Epiupdate/default.asp (accessed November 2007).

Valdiserri RO, Ogden LL, and McCray E (2006) Accomplishments in HIV prevention science: Implications for stemming the epidemic. *Nature Medicine* 9(7): 881–886.

World Health Organization (2007) Towards Universal Access. Scaling up Priority HIV/AIDS Interventions in the Health Sector. Progress report, April 2007. http://www.who.int/hiv/mediacentre/universal_access_progress_report_en.pdf (accessed August 2007).

Relevant Website

http://www.hiv.lanl.gov – The Circulating Recombinant Forms (CRFs), HIV Sequence Database, Los Alamos National Laboratory.

AIDS: Vaccine Development

N C Sheppard and Q J Sattentau, University of Oxford, Oxford, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Adjuvants Immune modulators designed to increase immune responses to vaccine antigens. These are of particular use for nonreplicating vaccine antigens such as killed microorganisms or antigenic subunits of microorganisms.

AIDS (acquired immunodeficiency syndrome) A pathological state that can be defined by the emergence of opportunistic infections or neoplasms in HIV seropositive patients, which coincides with a decline in the peripheral blood CD4 T-cell count below 200 cells μl^{-1}.

Antigen presenting cells Dendritic cells, macrophages, and B-cells are specialized cells capable of internalizing antigen and presenting it to lymphocytes together with the co-stimulatory molecules necessary to induce immune responses.

Breakthrough infection/vaccine failure Denotes that recipients of candidate vaccines have become infected and/or that viremia has escaped control by vaccine-induced immune responses.

CD4+ T cells A subset of T lymphocytes that coordinate the adaptive immune response, including antibody production and cytotoxic T-cell activation. They are also the principal target cell for HIV-1 replication *in vivo*.

Cytotoxic T cells (CTL) A subset of T lymphocytes that have the ability to kill virally infected cells, thus controlling viral replication and spread. They may also have additional functions that reduce the replication of viruses, such as production of soluble inhibitory factors, for example, cytokines and chemokines.

Envelope glycoproteins The HIV-1 envelope glycoproteins are used by the virus to gain entry into receptor-bearing target cells. The surface glycoprotein, gp120, binds the primary receptor CD4 and the co-receptor, usually CCR5. The transmembrane glycoprotein gp41 mediates fusion of the viral membrane with the cell membrane, allowing infection. The envelope glycoproteins are the principal targets of neutralizing antibodies (nAbs).

Exposed-uninfected (EU) individuals Those that are highly exposed to HIV-1 and therefore at high risk of infection, but fail to seroconvert and have undetectable viral RNA load.

Long-term nonprogressors (LTNP) Individuals that have been infected, normally for at least 10–12 years without ever experiencing HIV-related disease or requiring antiretroviral therapy. The CD4 T-cell count remains stable and $>600\,\mu l^{-1}$.

Neutralizing antibodies Strictly defined as antibodies (Abs) that reduce viral infectivity without additional factors such as complement. These Abs usually function by preventing virus entry into permissive cells. The activity of nAbs may be augmented by factors such as complement, and non-neutralizing antibodies may become antimicrobial in the presence of complement.

Primary isolates HIV-1 strains that have undergone minimal *in vitro* culture in primary leukocytes and are thus not adapted to cell culture. Adaptation to continued cell culture often increases the growth rate of a virus while dramatically reducing its resistance to neutralizing antibodies. An exaggerated phenotype of this kind is seen with viruses adapted to growth in immortalized lymphocytic cell lines.

SIV/SHIV challenge models Rhesus macaques can be used to model HIV/AIDS by experimental infection with simian Immunodeficiency Virus (SIV) or chimeric SIV recombinant for the envelope glycoprotein from HIV-1 (SHIV). A rapid loss of CD4 T cells often results in comparatively early onset of an AIDS-like syndrome in these models.

Introduction

Since the discovery of HIV-1, the causative agent of AIDS in 1983, researchers have attempted to develop a prophylactic vaccine to control the pandemic. Early attempts to achieve protective immunity made use of recombinant viral envelope glycoproteins (Env) to attempt to induce neutralizing antibody (nAb) responses. To date such approaches have not been successful, probably due to the inability of such subunit proteins to induce antibody (Ab) responses capable of cross-neutralizing the innumerable number of viral strains encountered in the field. Nevertheless, some seropositive cohorts have Ab responses capable of neutralizing diverse isolates and broadly neutralizing monoclonal antibodies (NmAbs) have been isolated from such patients. When these NmAbs are infused into animal models such as rhesus macaques prior to virus challenge, sterilizing immunity is often afforded. These observations reveal that the induction of nAbs could form a component of a protective

vaccine strategy. A second component that has received much attention is the induction of CD8 cytotoxic T-lymphocyte (CTL) responses. Such responses play a major role in the control of HIV-1 replication *in vivo* and, when sufficiently induced by vaccination, can suppress viremia and delay clinical progression in macaques challenged with virus. If such reductions in viremia are achieved in humans, viral transmission between infected and uninfected individuals might be prevented or reduced. Results from a plethora of Ab and CTL strategy-based vaccine trials conducted to date suggest that poor immunogenicity is often an obstacle to achieving robust responses; thus, novel adjuvants and immunogenic recombinant vectors are likely to be required for a successful vaccine.

Lessons from Successful Vaccines against Other Pathogens

Studies of the immune correlates of protection associated with existing vaccines reveal that Ab responses play a dominant role. Only BCG vaccination against *Mycobacterium tuberculosis* induces immunity in which Abs appear to have no role. Since CTL responses are likely to be required for an effective HIV-1 vaccine, traditional vaccine technologies are likely to be suboptimal, or counter-indicated due to safety concerns. Many existing vaccines are based on live-attenuated microorganisms. Such vaccines have potential hazards such as transmission to unvaccinated persons and the risk of reversion to pathogenicity, both of which have been observed with the Sabin oral polio vaccine. Killed microorganism vaccines do not have these drawbacks. However, they are occasionally associated with disease when the inactivation process is incomplete, and are inefficient at inducing CTL responses. Due to these factors, neither the live-attenuated nor killed vaccine approaches appear to be suitable for the development of a prophylactic vaccine against HIV-1. Therefore, the majority of HIV-1 vaccine candidates are based on variations of the subunit approach whereby one or more of the viral proteins is produced for delivery either as a protein, or alternatively encoded in a DNA plasmid or a live-attenuated vector.

Although there are now successful vaccines against persistent viral infections (HBV and HPV) and even one against a virus capable of latency (VZV), retroviruses such as HIV-1 present new challenges. Probably the most challenging is the unparalleled mutation rate of HIV-1. The enormous genetic diversity of this virus means that if the first rounds of replication are not aborted by vaccine-induced immune responses, the virus will escape the immune response and establish long-term reservoirs of latent infection refractory to immune clearance. If HIV-1 infection cannot be prevented by vaccination, then reducing the rapid diversification of HIV-1 in a new host by controlling replication to the lowest possible levels is a second major challenge.

Clinical Trials of HIV-1 Vaccine Candidates

In 2003, VaxGen announced that phase III efficacy trials of its gp120-based AIDSVAX candidate, aimed at inducing nAb responses, had failed to protect volunteers. The inadequacy of AIDSVAX and emergence of data suggesting CTLs play the major role in the control of HIV-1 heralded a paradigm shift away from the induction of nAb responses toward inducing CTL responses. Reflecting this, most of the vaccines currently on trial are based on strategies that have, as their primary or sole aim, the induction of CTL and T-helper responses against HIV-1. Thus, the main hypothesis currently being tested in clinical trials is that CTL responses can be induced safely in humans and will impact upon viral load and disease progression as they have done in primate models of simian-human immunodeficiency virus (SHIV) infection. In 2007, the phase IIb trials of the MRKAd5 trivalent vaccine developed by Merck & Co, which aimed to give 'proof of concept' for the CTL-based approach in man were discontinued. An interim investigation revealed that the vaccine did not reduce the likelihood of infection or impact on viral load after infection. Therefore, the CTL-based approach remains to be validated in man. The vaccine pipeline will likely respond to these disappointing findings by testing vaccine candidates that induce CTL responses to additional viral targets with a further paradigm shift putting equal emphasis on nAb and CTL-based approaches.

Defining the Correlates of Protection

A major difficulty in HIV-1 vaccine research has been in determining which host immunological factors, if any, correlate with protection from infection or protection from disease progression. The influence of human leukocyte antigen (HLA) genotype on HIV progression is a clear indicator that the adaptive immune response is implicated in the control of infection. However, only rare cohorts of individuals seem to resist HIV-1 infection despite frequent exposure to the virus, or control an established infection robustly. Groups of highly exposed seronegative (ES) individuals display great heterogeneity. They may have increased natural killer (NK) cell activity, HIV-1-specific CTL responses, HIV-specific CD4 T-cell production of MIP-1β, IFN-γ, and IL-2, neutralizing IgA responses at the mucosae or no detectable responses. So far, the most frequent finding is the presence of CTL responses to HIV-1 antigens in ES cohorts. Where CTL activity exists, it shows marked differences in epitope specificity from responses seen in infected individuals and is often restricted only by HLA alleles associated with reduced risk of infection. Findings of neutralizing IgA responses are controversial due to the difficulty in ruling out contamination of the mucosal samples of ES

study subjects with Env-specific IgA from an infected partner's seminal fluid.

Although ES cohorts are defined as lacking serum IgG responses and not having detectable virus by PCR, it remains to be determined whether these individuals have never been infected, have had abortive infections; or are persistently infected at extremely low levels. Studies have detected low levels of HIV provirus in peripheral blood mononuclear cells (PBMCs) taken from seronegative infants born to seropositive mothers, high-risk seronegative homosexual men, and individuals several years prior to their seroconversion. These findings favor a model of early control of infection prior to seroconversion. In the cohorts of ES homosexual men, lower T-cell responsiveness and immune activation were seen when compared with homosexual men who later seroconverted, suggesting T-cell hypo-responsiveness might be a correlate of protection, potentially by depriving HIV-1 of susceptible target cells (activated CD4 T cells upregulate CCR5, the principal co-receptor for HIV-1 and become more permissive for viral replication).

A second group of individuals who have attracted intense scrutiny are the long-term nonprogressors (LTNPs), who maintain high CD4 T-cell counts and low-to-undetectable viral loads for many years after HIV-1 infection. Again, these people are not a homogeneous group but consist of: those of certain HLA types; groups co-infected with hepatitis G (GB virus C); cohorts who have poly-functional CTL responses, capable of lytic function as well as releasing antiviral cytokines, β-chemokines, and/or CD8 antiviral factor (CAF), which suppresses HIV-1 replication at the level of transcription; those possessing TCR clones capable of tolerating mutation in the target epitope; a few individuals infected with naturally attenuated viruses that are mutated in regions such as *nef* and *vpr*; and rare individuals who have broad nAb responses.

CTLs probably have the central role in controlling viremia in acute and chronic infection and have been most frequently correlated with natural immunity among ES individuals and LTNPs. The case for nAbs is weaker as their role in controlling established virus infection *in vivo* is not clearly defined. The rapid diversification of Env is known to be driven by nAb responses with CTLs making little contribution. NAbs exert 'soft' selection, with the rate of mounting an autologous nAb response strongly correlated with the rate of phenotypic escape. Multiple mechanisms allow the virus to rapidly escape at minimal fitness cost, meaning that the nAb response is rarely active against contemporaneous isolates. Nevertheless, the presence of nAbs in some ES and LTNP cohorts and the ability of NmAb infusions to provide sterilizing immunity in macaque challenge models supports them as a correlate of protective immunity. In addition, studies in which acutely or chronically infected individuals were transfused with a cocktail of NmAbs showed that viremia was controlled in some cases. These data suggest that NmAbs alone can control established infection, but that the dose required to do so is high and the virus can still escape, especially in patients who harbor a greater diversity of viral strains. Therefore, nAb responses are likely to be more important at preventing infection or controlling early infection, than at reducing later viral replication.

The induction of nAbs to Env and robust CTL responses to multiple components of the virus in resistant cohorts implies that CD4 T-cell responses must also have been induced. It is known that the ability of HIV-1-specific CD4 T-cells to respond to Gag epitopes by secreting IL-2 alone, or together with IFN-γ, is negatively correlated with virus load in HIV-1-infected patients. This implies that vaccines should induce good helper CD4 T-cell responses in order to optimize processes such as affinity maturation and class switching of Abs, proliferation of CTLs, and induction of appropriate memory T- and B-cell responses.

Studies of the 'correlates of disease' rather than 'protection' in nonhuman primates infected with SIV in the wild reveal that unlike their counterparts experimentally infected with SIV viruses isolated from other monkey species, natural SIV infection in the host monkey species rarely leads to an AIDS-like syndrome. Most animals remain healthy with normal CD4 T-cell levels despite a persistently high viral load. Comparative analysis of naturally and experimentally infected animals has demonstrated a lack of generalized activation of the immune response in the naturally infected animals, which consequently avoid the immune-mediated damage observed in pathogenic SIV infection. Gut-associated lymphoid tissue (GALT)-resident memory CD4 T cells are normally lost early in pathogenic SIV/HIV infection, compromising immune integrity of the GALT and resulting in the translocation of bacteria and associated inflammatory mediators from the gut lumen into the circulation. These might contribute to chronic immune activation, which eventually exhausts the homeostatic mechanisms that replenish CD4 T cells. Microbial translocation is not detected in nonpathogenic SIV infection, concurring with the lack of chronic immune activation. Interestingly, similar observations have been made with respect to reduced immune activation in the less-pathogenic HIV-2 infection of humans and T-cell hypo-responsiveness correlates with ES status in several cohorts. As a corollary to the 'immune-activation' hypothesis, activation of SIV-specific CD4 T cells by vaccination leads to exacerbated SIV-mediated disease upon challenge in the natural host. These findings shed light not only on potential mechanisms of immune pathology associated with HIV-1 infection of humans, but also send a warning that suboptimal vaccination protocols might be detrimental by encouraging immune pathology.

Abs and HIV-1

The Role of Abs *In Vivo*

Env is the principal target of Ab-mediated immunity since it is the sole virus antigen expressed on virions and productively infected cells. Neutralization is achieved when Ab binds any exposed epitope on the functional Env spike, thus sterically blocking the gp120–CD4 interaction, or in a few cases, preventing the necessary conformational changes required to induce fusion. Theoretically, all spikes may need to be inactivated since the binding of an nAb to one spike does not affect adjacent spikes and a single fusogenic spike may be sufficient to mediate entry. The entropy and kinetics of nAb–Env association affect neutralization potency as they determine which of many conformations that Env samples can be recognized, and the stability of the interaction. Neutralization is the most important, but not the only, antiviral mechanism mediated by Env-specific Abs. Both Ab-dependent complement-mediated inactivation (ADCMI) and Ab-dependent cellular cytotoxicity (ADCC) correlate with positive clinical indicators in some studies. In addition, Ab-mediated opsono-phagocytosis of virions might also play a significant role.

Tat is not present at the cell/virion surface, but is secreted by infected cells and is believed to transactivate bystander cells increasing HIV-1 replication. Abs that neutralize the ability of Tat to transactivate cells might reduce viral load and prevent disease progression. Clinical trials are in progress to assess whether Tat-based vaccines are capable of inducing Tat-neutralizing Ab responses and whether these correlate with beneficial outcomes.

Env Evolution in Response to NAbs

Over the course of HIV-1 evolution, the unique mutational plasticity of Env has allowed it to accrue multifaceted immunoevasive properties to escape humoral immunity (**Table 1**).

In light of such elegant strategies, it is not surprising that HIV-1 fails to induce high nAb titers in most patients and rapidly escapes narrow-specificity nAbs as they arise. The preferential loss of HIV-1-specific CD4 T cells due to their unique ability to be both activated by and infected with the virus no doubt plays an important role in viral escape by reducing T-cell help for B cells (and CTLs). Nevertheless, in some patients, chronic exposure to Env variants leads to the production of broadly reactive nAbs that target the portions of Env that are highly conserved in order to allow virus–receptor interactions. Thus far, only a handful of such NmAbs have been isolated from patients, namely b12, 2G1, 2F5, and 4E10 (**Table 2**).

These NmAbs have been subject to intense scrutiny and the X-ray crystal structures for b12, 2G12, 2F5, and 4E10 in complex with Env or peptides have been solved. Extraordinary characteristics have been observed, including: extended complementarity-determining region (CDR) H3 loops (b12, 2F5, and 4E10); dimerization of the heavy-chain variable domain V_H (2G12); the use of only the heavy chain in Ab–antigen interactions (b12); and the ability to make contacts with the epitope-utilizing interactions between main-chain atoms (b12). Whether such unusual Abs can be elicited by vaccination is a central question to the field. The different NmAbs act at one or more phases to achieve neutralization; b12 is most

Table 1 Immunoevasion strategies utilized by HIV-1 Env to escape NAb responses

Strategy	*Mechanism*
Decoys	Irrelevant epitope exposure on monomeric gp120, gp4l that has shed gp120, gp120-gp4l monomers, dimers, and tetramers, uncleaved gp160, and alternate trimeric forms.
Entropic masking	Inherent conformational flexibility of Env imposes a significant entropic penalty on Ab binding. Most Abs appear to bind a limited number of conformations and thus are at a kinetic disadvantage.
Glycan shield	Shifting glycans can sterically inhibit Abs from reaching their epitopes.
Protection of the CD4-binding site (CD4bs)	Part of the CD4bs is located in a hydrophobic cavity that limits access to CD4bsAbs. The remainder is induced by CD4 binding and in the unliganded form lies near or within a long cavity formed by portions of the outer and inner domains as well as part of the bridging sheet. The V1N2 loop on one gp120 molecule appears to obscure part of the CD4bs on the inner domain in a neighboring gpl20 in the trimer. Similarly, the V3-loop partly obscures the bridging sheet portion of the CD4bs. Contact with CD4 involves some main-chain atoms and thus mutation is tolerated.
Protection of the co-receptor binding site	The co-receptor binding site is composed by elements of the V3-loop stem and bridging sheet. The necessary conformation is rarely sampled by Env prior to CD4-ligation and afterwards it lies in close apposition to target cell membrane and thus bulky IgG molecules are sterically excluded. The V1N2 loop on one gp120 molecule obstruct elements of co-receptor-binding site on neighboring gp120 molecules in the trimer.
Sequence variability	Five V-loops that are highly tolerant of amino acid substitutions, additions, and deletions induce strain-specific Abs. (NB: V3-loop length is conserved).
Silent face	A hyperglycosylated region of the outer domain of gp120 which imparts low immunogenicity to gp120.

Table 2 The properites of the broadly specific NmAbs

Antibody	*Target*	*Epitope*
b12	gp120	A recessed and conformationally invariant region overlapping the CD4-binding site, which is involved in the metastable interaction with CD4. All three complementarity determining region (CDR) loops of the heavy chain are involved but the light chain is not. Many contacts involve main-chain atoms.
2G12	gp120	Mannose c(1→2) mannose-linked glycans attached to N295 and N332 and stabilized by other proximal glycans. The Fab domains form a unique dimer, which extends the surface for interaction with the dense glycan cluster on gp120.
2F5	gp41	Centered on the core epitope ELDKWAS, which is potentially helical in nature and is situated in the membrane proximal extracellular region (MPER). An extended CDR H3 loop (22 aa) may play a role in destabilizing some proximal structure but interaction with the membrane is not necessary. Polyspecific autoreactivity shown with cardiolipin.
4E10	gp41	Binds the helical epitope WFX(I/L)(T/S)XX(L/I)W, which lies C-terminal side of the 2F5 epitope in the MPER and potentially associated with the membrane. The apex of the long CDR H3 loop extends beyond the peptide and is likely to contact the membrane. Polyspecific autoreactivity shown with cardiolipin.

effective before CD4 binding, whereas 2G12 still neutralizes after CD4 binding and 2F5 and 4E10 are active even after co-receptor engagement. By comparison, sera from LTNPs with high nAb titers have been shown to act mostly before CD4 engagement, 2G12-like Abs correlate with the broadest nAb activity in LTNP sera, whereas 2F5 and 4E10-like Abs appear to be absent. This is in keeping with the finding that 2F5 and 4E10 appear to be examples of polyspecific autoreactive Abs, which may be difficult to induce in most people due to self-tolerance mechanisms.

In a new host, the homogenization of Env sequences is observed during acute infection with evolution toward an ancestral sequence occurring for upto 2–3 years before diversification begins. In this process, Env may adopt shorter variable loops and reduced glycosylation, possibly increasing its fusogenicity. Such virus is unusually sensitive to neutralization. During acute infection, the patient reaches a peak in its infectivity, potentially transmitting neutralization-sensitive strains to new recipients. However, neutralization-resistant virus can also be transmitted since virus is often passed between mother and baby despite the transfer of maternal nAbs. In the context of vaccination, this suggests that acutely infected patients might pass on neutralization-sensitive virus more vulnerable to vaccine-induced nAbs, whereas neutralization-resistant virus is likely to be passed on later in infection. If sterilizing immunity is not achieved, pre-existing humoral immunity might thwart the outgrowth of neutralization-sensitive strains, perhaps limiting the rate of dissemination and magnitude of peak viremia.

Optimizing Antigens to Induce Abs with Antiviral Activity

The problem of inducing Abs of the correct specificities that will have protective roles *in vivo* has proved difficult to solve. It is clear from clinical trials conducted thus far that vaccination with native gp120, gp160, gp41, or Env peptides fails to induce nAbs against more than a handful of isolates. Therefore, improved strategies that aim to circumvent the immuno-evasive properties of Env are being developed. The use of consensus sequence Env reduces the antigenic distance between the vaccine strain and isolates that will be encountered in the field. So far, consensus sequence Env has induced nAb responses of equal or slightly better breadth compared to naturally occurring strains, suggesting at best, only an incremental improvement. Attempts to recapitulate the native structure of the fusogenic virion-associated Env trimeric spike have also produced incremental improvements when compared with monomeric gp120. Since Gag drives the self-assembly of the virion, virus-like particles (VLPs) resembling the native virion structure but devoid of nucleic acid are generated when Gag is expressed along with other structural proteins. The presentation of stabilized trimers in the context of VLPs appears a useful approach since the presence of the cytoplasmic tail of gp160 is known to alter the antigenicity of the external portions of Env and the membrane proximal extracellular region (MPER) epitopes might be appropriately presented only when anchored proximal to the membrane. Soluble gp140 immunogens, containing the gp120 subunit and the extracellular portion of gp41, lack the intracellular portion of gp41 and thus have subtly different antigenic profiles. Studies examining variable loop deletion or truncation and the removal of glycosylation sites have revealed that partial truncation of the V2 loop gives an incremental improvement in the breadth of nAbs, whereas the removal of glycans does not. Multivalent approaches in which several strains are used simultaneously show moderate improvement in the breadth of the nAb response in animal models. However, this increase appears to be due to the additive effect of nAbs specific to each individual component and not due to the induction of Abs with broader neutralizing properties. Although there are undoubtedly fewer neutralization serotypes than genotypes

of HIV-1, it will not be practical to cover all neutralization serotypes in a multivalent cocktail if each component only induces nAbs of narrow specificity. An alternative approach is the development of mimics of the epitopes of NmAbs that might induce similar broadly neutralizing specificities after immunization. Combining approaches that give incremental improvements when used alone might provide substantial additive improvements in the breadth of response.

Inducing Cell-Mediated Immunity by Vaccination

In macaque models, vaccination with DNA plasmids or recombinant viruses bearing SIV/SHIV antigenic inserts leads to significant attenuation of viremia after subsequent infection with the highly pathogenic X4-tropic hybrid isolate SHIV89.6P. Although they do not afford sterilizing immunity under any regimen tested thus far, such vaccines enable long-term suppression of viremia and prevent disease progression. Nonetheless, eventual escape of the challenge virus from CTL control after acquisition of single amino acid mutations in critical immunodominant epitopes is sometimes observed. This results in rapid rebound of viremia and normal disease progression, and underlies the necessity of targeting the most conserved epitopes critical for viral fitness, as well as targeting multiple epitopes if 'breakthrough' replication is to be avoided. A critical caveat of the work in macaques with the frequently used SHIV-89.6P is that this virus appears to be unusually sensitive to vaccine-induced immunity. Experience using R5-tropic SIVmac239 or SIVmac251 challenge models demonstrates that viremia is often controlled only transiently and no control of HIV-1 infection was observed in the clinical trials of MRKAd5 vaccine despite earlier trials showing promising immunogenicity of this candidate.

HIV-1 evades T-cell responses in a number of ways (**Table 3**). Once an epitope has mutated to a form not recognized by a particular CTL clone, a new population of antigenic variants can rapidly replace those sensitive to that clone. This occurs if the mutation is less detrimental to viral fitness than remaining sensitive to the CTL clone. Even in vaccinated and challenged macaques, a degree of viral replication remains despite strong CTL responses. From this small pool of viral variants, escape mutants can arise. The current challenge is for vaccination to induce optimal cellular immunity to HIV-1, including sufficient breadth of responses to decrease the chances of viral escape. Since an individual's CTL responses are entirely governed by their HLA class I alleles, vaccines must include enough material from the virus to allow the majority of the population to make multiple responses. This might be achieved by expressing strings of epitopes, or whole or truncated HIV-1 proteins, or even fusions of several proteins from DNA plasmids or recombinant vectors. However, the process is not straightforward and, to date, the immune responses induced by experimental vaccines have been disappointingly narrow and often dominated by a response to a single epitope.

Selecting the Best Target Antigens

The obvious advantage of CTL-based vaccines over those that aim to induce nAbs is that all HIV-1 proteins are targets for cellular immunity and some of these are considerably less tolerant of mutation than Env. CTLs escape mutations with a high cost to viral fitness and revert after transmission to a new host where the epitope is not targeted. Conversely, CTL epitopes restricted by common HLA class I alleles that are not critical to viral fitness will drive the accumulation of escape mutants in a population, since the chance of mutant virus being transmitted to individuals with the same restricting HLA

Table 3 Immunoevasive strategies used by HIV-1 to escape T-cell response

Strategy	*Mechanism*
Bystander apoptosis	HIV can induce bystander apoptosis of CD4 and CD8 T-cells. Activated CD4 T-cells are especially vulnerable to apoptosis induced by highly pathogenic X4-tropic isolates in a CXCR4-Env dependent manner, whereas the CD8 T-cells are killed by soluble factors released by CD4 T-cells. Fas-mediated mechanisms and virion-associated MHC class II and CD86 have been implicated.
Dysregulation of T-cell migration	The V2- and V3-loops of X4 using gp120 have been shown to repel T-cells, including CTL via a CD4-independent, receptor-mediated mechanism. CTLs also accumulate in the blood rather than lymphatics in acute infection, suggesting that non-X4-tropic virus mechanisms are also active.
Mutation	Amino acid substitutions can prevent anchoring to MHC molecules, can prevent recognition by or antagonize responding TCRs, or can alter proteolytic processing and peptide transport preventing surface expression of peptide-MHC complexes.
Nef-mediated downregulation of MHC class I	One of the functions of Nef is the downregulation of MHC class I molecules and subsequent reduction in the ability of infected cells to display any MHC class I epitopes from HIV-1. This results in increased resistance to CTL-mediated killing in an epitope-independent fashion.

class I allele is high. This necessitates the rational design of vaccine immunogens to provide epitopes for the less common HLA alleles that may prove more effective at combating HIV-1 and delineating which epitopes of a mutant virus may still be presented by the common HLA alleles. Targeting early antigens such as Tat, Rev, and Nef allows CTLs the greatest amount of time to act on an infected cell. By contrast, targeting highly expressed structural proteins such as Gag would increase the chances of the CTLs recognizing infected cells given that HLA class I expression is downregulated by Nef. The question of whether CTL responses to highly conserved epitopes exist in HIV-1-infected patients is one of some controversy since such responses are only detected in some studies. It is possible that vaccination will induce such responses, but it is more likely that the most highly conserved regions of HIV-1 protein have evolved to be weakly immunogenic and poorly processed in infected cells. At present, a strategy that induces multiple responses, perhaps to consensus or mosaic sequences constructed to optimize CTL epitope coverage across clades, seems most likely to invoke prolonged control of viremia independent of virus clade.

Achieving Strong Immune Responses to Candidate HIV-1 Vaccines

Class of Immunogen

One of the major obstacles facing the design of HIV-1 vaccine candidates is poor immunogenicity. The methods of delivery, vectors, and adjuvants used require considerable optimization in order to induce robust and persistent immune responses. Env is considered poorly immunogenic in absolute terms, requiring high doses and repeat-boosting simply to induce substantial titers of binding Abs. Factors such as glycosylation, restricted numbers of CD4 T-cell epitopes, and tertiary structure limit the immunogenicity of Env. The low innate immunogenicity of Env and other HIV-1 antigens can be overcome by optimizing delivery and providing adjuvants. Each delivery method comes with distinct advantages and disadvantages. DNA vaccines are conceptually simple vaccine candidates that can induce both T-cell and Ab responses, but immune responses in humans and other primates have proved disappointing when compared to small animal models. Despite their promise at inducing CTL responses, DNA vaccines are expensive, requiring doses of 2–5 mg to induce weak CD4 T-cell responses in macaques. However, DNA immunization has shown promise at priming immune responses that can later be boosted by recombinant vectors and benefit from expressing only the target antigen and not multiple vector antigens. Due to the expense and poor results achieved thus far with DNA vaccines, some researchers are looking to replace DNA priming immunizations with recombinant viral or bacterial vectors, or protein. However, since the immunogenicity of DNA vaccines can be substantially improved *in vivo* by electroporation or transfection reagents, more efficient use of DNA may be eminently feasible.

Recombinant vectors, mostly based on attenuated viruses, are among the lead HIV-1 vaccine candidates currently on trial. They show particular promise at inducing T-cell responses and are especially immunogenic when used in heterologous prime/boost regimens. Vectors based on poxviruses and adenoviruses show good immunogenicity but may be sensitive to pre-existing antivector immunity, which is common in many populations. The development of vectors with low seroprevalence should counter this problem. Other vectors with potential for greater immunogenicity are under development, including: measles virus, vesicular stomatitis virus, Venezuelan equine encephalitis virus, and poliovirus.

Protein immunogens are generally excellent at inducing Ab responses, but poor at inducing CTL responses unless adjuvants that favor cross-presentation are included. However, with the correct adjuvant they can be used to induce Abs, CD4, and CD8 T-cell responses. Protein immunogens have the disadvantage of being relatively expensive to produce and, like recombinant vectors, require cold-chain storage.

Adjuvants

Adjuvants fall into two broad classes: (1) Immune potentiators, including host factors such as cytokines, chemokines, heat-shock proteins, and complement components or pathogen associated molecular patterns (PAMPs) such as modified lipopolysaccharide (LPS), peptidoglycans, nucleic acids, or bacterial toxin subunits. (2) Delivery systems, including liposomes, synthetic microparticles, mineral salts, and oil-in-water emulsions. Adjuvants can be co-administered with, or incorporated into all the vaccine types discussed. For example, genes for pro-inflammatory cytokines can be cloned into DNA or recombinant vectors or they can be given in protein form together with the vaccine. Importantly, the direct conjugation of adjuvant to antigen appears to optimize its effects by ensuring co-delivery to antigen-presenting cells. Novel delivery systems can be combined with the novel immune potentiators to optimize their effects. Defining which combinations of these immune modulators, PAMPs, and novel delivery systems will synergize to impart the greatest magnitude and duration of immune responses is a problem that needs considerable attention.

See also: AIDS: Disease Manifestation; AIDS: Global Epidemiology; Antigenic Variation.

Further Reading

Brenchley JM, Price DA, and Douek DC (2006) HIV disease: Fallout from a mucosal catastrophe? *Nature Immunology* 7(3): 235–239.

Burton DR, Stanfield RL, and Wilson IA (2005) Antibody vs. HIV in a clash of evolutionary titans. *Proceedings of the National Academy of Sciences, USA* 102(42): 14943–14948.

Douek DC, Kwong PD, and Nabel GJ (2006) The rational design of an AIDS vaccine. *Cell* 124(4): 677–681.

Pantaleo G and Koup RA (2004) Correlates of immune protection in HIV-1: What we know, what we don't know, what we should know. *Nature Medicine* 10: 806–810.

Relevant Website

http://www.iavireportonline.org – New! Vax Special Issue – 2004: Year in Review.

Akabane Virus

P S Mellor, Institute for Animal Health, Woking, UK
P D Kirkland, Elizabeth Macarthur Agricultural Institute, Menangle, NSW, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arthrogryposis (AG) Rigid fixation of the joints, usually in flexion but occasionally in extension.
Culicoides Blood-feeding dipterous insects also known as biting midges.
Dystocia Abnormal or difficult birth.
Encephalomyelitis Inflammation both of the brain and the spinal cord.
Porencephaly Disease of the brain with the formation of small cavities in the brain substance.
Hydranencephaly (HE) A condition in which the brain's cerebral hemispheres are almost or completely absent and replaced by sacs filled with cerebrospinal fluid.
Kyphosis Outward curvature of the spine causing a humped back.
Nystagmus Rapid involuntary oscillation of the eyes.
Scoliosis Lateral deviation of the normal vertical line of the spine.
Torticollis Twisting of the neck that causes the head to rotate or tilt.

Introduction

Akabane virus was originally isolated from mosquitoes in Japan, in the summer of 1959, 'Akabane' being the name of the village where the virus was first isolated. Subsequently, it has been shown to occur widely in Africa, Australia, throughout Asia and the Middle East, its distribution being determined by the occurrence of its insect vectors which are predominantly biting midges from the genus *Culicoides*.

The virus is important in veterinary pathology because it is able to cross the placenta of cattle, sheep, and goats causing a range of congenital defects, principally arthrogryposis (AG) and hydranencephaly (HE), and abortion. The virus can be widespread without evidence of disease because it usually produces asymptomatic infections in adult animals. It is only when pregnant, serologically naive adults are infected early in pregnancy that the virus may cross the placenta to cause damage to the fetus. Evidence of the damage only becomes apparent some months later with the birth of the affected young – by which time the viruses have been eliminated from both mother and offspring and so cannot be isolated from them. This meant that for many years Akabane virus was not connected to these clinical manifestations, until careful epidemiological observations and the development of appropriate serological tests confirmed the association.

Taxonomy, Properties of the Virion and Genome

Akabane virus is a member of the genus *Orthobunyavirus* in the family *Bunyaviridae*. Several related viruses (Aino, Cache Valley, Peaton, and Tinaroo) have been shown under experimental conditions to have the potential to cross the ruminant placenta but only Aino in Australia and Japan and Cache Valley in the USA have been recognized as pathogens in the field.

The Akabane virion is spherical in shape and is *c.* 90–100 nm in diameter. A lipid envelope with projecting glycoprotein peplomers surrounds the viral genome which consists of three separate segments of single-stranded RNA. Each segment is of a different length. The large segment encodes the viral transcriptase, the medium

segment two glycoproteins, and the small segment the internal nucleoprotein. Each of the segments also encodes a nonstructural protein.

The virus is acid labile and is readily inactivated by chloroform, ether, and trypsin. It is also very heat labile and is inactivated at 56 °C in a few minutes and loses about 0.3 log of infectivity per hour at 37 °C. However, it remains viable in blood samples kept at 4 °C for several months and can be stored indefinitely at –80 °C or lower.

Vertebrate Hosts

The virus has been isolated from cattle, sheep, and goats. Antibodies have also been detected in horses, pigs, camel, red deer, and a wide selection of African wildlife ranging from various species of antelope to hippopotamus, elephant, and giraffe.

There are no known cases of human disease caused by Akabane virus even though some of the mosquito species from which the virus has been isolated (*Aedes vexans, Culex tritaeniorhynchus*, and *Anopheles funestus*) regularly bite humans. However, the role of mosquitoes as vectors remains unclear and limited serological surveys have not found evidence of human infection. Further, the *Culicoides* species that are known vectors of the virus rarely bite humans.

Clinical Signs

When Akabane virus infects pregnant cattle, sheep, or goats, the virus is able to replicate in and cross the ruminant placenta causing a variety of congenital abnormalities in the fetus. The range and severity of these abnormalities are dependent upon the stage of gestation at infection. In adult animals, however, infection is usually subclinical and in endemic areas most breeding age animals will have acquired an active immunity during early life sufficient to prevent the virus from reaching the developing fetus. In these situations, the virus exists as a 'silent' infection and no evidence of disease is seen. The pathogenic effects of Akabane infection are usually observed only when the vector spreads beyond the limits of its endemic areas under favorable conditions, to enter regions where many adult animals are still susceptible and therefore able to be infected during pregnancy. In such situations, an epidemic in cattle, sheep, or goats may be noticed by an increased incidence of abortions and premature births in late autumn or early winter. Calves infected close to term may be born with encephalitis, often apparent clinically as a flaccid paralysis. Some strains of Akabane virus may also cause encephalitis in newborn calves and, infrequently, in older animals. This is followed by the birth of calves, lambs, or kids with a range of congenital defects, principally AG and HE (see **Figures 1** and **2**). Young with these defects may be stillborn or delivered alive at term. AG is characterized by fixation of the joints in flexion (most frequently) or extension. The birth of some arthrogrypotic animals is associated with dystocia, necessitating embryotomy or cesarean section to save the dam. Young born with HE may show blindness, nystagmus, deafness, dullness, slow suckling, paralysis, and lack of coordination. They may survive for several months if hand-reared but often die from misadventure. In cattle herds with animals at different stages of pregnancy, there will be a succession of defects, initially with cases of AG and later calves with HE. In sheep and goats, due to the shorter period of gestation and narrower range at which the fetus is susceptible, affected progeny born may show one or more of the defects of AG and HE concurrently.



Figure 1 Calf with AG and torticollis due to Akabane virus infection.

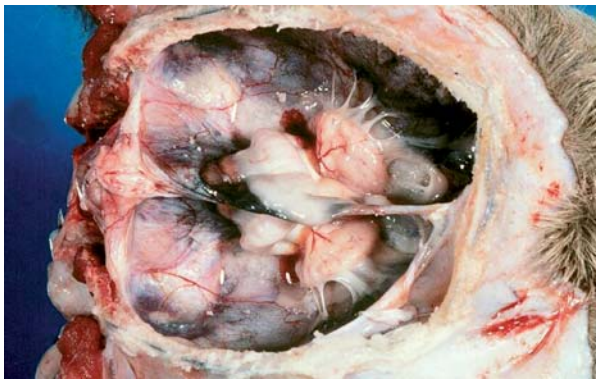


Figure 2 Akabane virus-induced HE in a calf.

Epidemics of AG/HE disease due to Akabane virus have been recorded in Japan, Australia, Israel, and Turkey, the most severe involving 30 000 calves in Japan and approximately 8000 calves in Australia. The incidence of affected progeny varies depending on the strain of virus. In naturally infected cattle, the incidence of AG and HE

varies from 25% to 50% but in sheep that have been experimentally infected with different virus strains, the incidence of abnormal lambs can vary from 15% to 80%.

Pathogenesis

In naturally infected cattle, there is an incubation period of a few days followed by a viremia of 3–6 days duration. This is not usually associated with clinical signs in the adult animal. From about day 12 post infection, virus-neutralizing antibodies begin to be detected in the circulation.

The sequence of events leading to fetal infection involves virus replication in the endothelial cells of the placenta, then the trophoblastic cells, and finally in the fetus itself. The ability of the virus to produce congenital damage depends upon the stage of gestation at which fetal infection occurs. Fetal Akabane virus infection is characterized by a predictable chronological sequence of congenital defects directly referable to the fetal age at which infection has occurred. The bovine fetus is susceptible from about 90 days through to term while the ovine and caprine are most susceptible between 28 and 56 days of gestation. In sheep and goats, defects are less common than in cattle because of both the limited gestational range and the fact that small ruminants are less likely to be pregnant during the virus transmission period.

In cattle, the defects occur over a longer period and are seen in consistent sequence. The first abnormalities to appear in live neonates are nonsuppurative encephalitis and encephalomyelitis, the outcome of infection late in gestation. Arthrogrypotic neonates follow as a result of infection in the fifth and sixth months of gestation, then more severe malformations including porencephaly and HE reflecting infection in the third and fourth months. When AG is first observed, only one joint on a single limb might be affected, but in cases that are born later (infected earlier) the abnormalities are more severe with changes in several joints on all limbs. Calves affected with severe AG after infection early in the fifth month of gestation may show early changes in the brain with small cystic lesions of porencephaly. The lesions of HE then develop as a result of infection in the third and fourth months of gestation. As calves are born later in an outbreak, the lesions of HE will progress in severity, initially being seen as small focal cystic cavities but soon progressing to large fluid-filled cavities. In calves with severe lesions, the cerebral hemispheres are completely absent and only fluid-filled meninges remain but the brainstem and cerebellum remain unaffected.

The replication of Akabane virus and its tissue tropisms in the fetus are determined by fetal age, the presence of rapidly dividing cells, and the development of immunocompetence. The most severe lesions occur in fetuses infected during the early stages of organogenesis. In cattle the most severe lesions occur after infection between 70 and 90 days of gestation and in sheep and goats between 28 and 36 days. Immunocompetence develops in calves from about 90 days and in lambs from 65–70 days of gestation, and subsequent to these times virus is rapidly eliminated from the fetal tissues. As a result, virus cannot be isolated from calves and lambs delivered at term but may be isolated from a fetus that is aborted soon after infection.

Pathology

AG and HE are the main gross lesions of Akabane disease but cervical scoliosis, torticollis, and kyphosis also occur. In sheep and goats, there may also be pulmonary hypoplasia. Arthrogrypotic calves exhibit restrictions to movements of the limb joints exclusively as a result of changes to the soft tissues. The joint surfaces are normal and the bones are unaffected. In affected limbs, the muscles are usually reduced in size and paler in color than normal. This is a result of both neurogenic muscular atrophy and also from primary infection of the muscles.

Microscopically, there is a severe loss of myelinated fibres in the lateral and ventral funiculi of the spinal cord, and of ventral horn neurons and nerve fibers in the ventral spinal nerves. However, white matter is unaffected.

In HE calves, the cerebral cortex is represented only by a thin shell of brain tissue, perhaps only the meninges, filled with fluid (**Figure 2**). The meninges may be thickened. In most cases, the brainstem is intact and the cerebellum appears normal.

During the early stages of an outbreak of Akabane disease, there may be calves which are incoordinate or unable to stand at birth. No gross pathological lesions are found in such animals but microscopically there is a mild to moderate nonsuppurative acute encephalomyelitis, most evident in the gray matter of the mid- and posterior brain.

Epidemiology and Transmission

Most of Africa, the Middle East, southern Asia, Japan, Korea, and Australia may be regarded as being endemic for Akabane virus. Papua New Guinea and the island countries of the Pacific, however, are free from infection. The geographical distribution of this virus is controlled completely by the distribution, seasonal activity, and abundance of its insect vectors.

Akabane virus was first isolated in Japan in 1959 from mosquitoes of the species *Ae. vexans* and *Cx. tritaeniorhynchus*. Then, in 1968, it was isolated in Australia from the biting midge *Culicoides brevitarsis*. More recently, isolations have also been made from *An. funestus* in Kenya, and from *Culicoides* species such as *C. oxystoma* in Japan,



Figure 3 *Culicoides imicola*, a vector of Akabane virus.

C. imicola (**Figure 3**) and *C. milnei* in Zimbabwe, *C. imicola* in Oman, *C. brevitarsis* and *C. wadai* in Australia, and a mixed pool consisting mainly of *C. imicola* in South Africa. The virus has been shown to replicate in *C. sonorensis* (=*C. variipennis*) by up to 1000-fold, and transmission occurs after 7–10 days incubation at 25 °C. As *C. sonorensis* is widely distributed and abundant in North America, this suggests that should Akabane virus gain entry to that continent, transmission would be likely. The virus has also been shown to replicate in *C. brevitarsis* and reaches the salivary glands of infected individuals after 10 days incubation. In Australia, *C. brevitarsis* is the only biting insect whose distribution correlates closely with the distribution of Akabane antibodies in cattle. The development of antibodies also coincides with the seasons when *C. brevitarsis* is active. Furthermore, *C. brevitarsis* feeds upon sheep and horses in Australia, in which species antibodies to Akabane virus have been found. These findings suggest that in Australia *C. brevitarsis* is the principal vector of Akabane virus. No close correlation has yet been reported between any mosquito species and Akabane virus distribution, and as replication and transmission of the virus have not been demonstrated in any mosquito species, this suggests that *Culicoides* species are likely to be the major vectors of Akabane virus and mosquitoes are of lesser importance. Akabane virus persists in nature by alternate cycling of the virus between the midges and its mammalian hosts after biting of the mammalian host by the insects. Transovarial (vertical) transmission of Akabane virus has not demonstrated.

In Australia, the principal vector *C. brevitarsis* spreads Akabane virus throughout an endemic area that extends across the north of the country and southward along the east coast. Within this area, virus transmission occurs annually, so breeding animals are usually infected early in life before becoming pregnant and few Akabane cases occur. Under harsh climatic conditions, usually drought, the distribution of the midge may retreat northward and toward the coast, reducing the extent of infection for one or perhaps two seasons. On resumption of the usual pattern of spread, a susceptible population of pregnant females will be infected at the margins of the range of the midge, resulting in small disease outbreaks. Under the influence of very favorable conditions of higher rainfall and mild temperatures, the insect may spread further south and inland than is usual and result in virus transmission in highly susceptible livestock populations. Large outbreaks will then occur with abortions noticed in late autumn and calves with AG/HE begin to appear in midwinter and spring, from July through to September.

Outbreaks of Akabane disease in Japan also show seasonal and geographic clustering, with most cases occurring from September to March. In Israel, disease outbreaks have occurred from November to June and in western Turkey during early spring (March). The differences in timing are presumably related to the abundance of the vector insects as influenced by climate.

Diagnosis

Sporadic cases of AG/HE due to Akabane virus often remain undiagnosed. However, when there is a cluster of cases, a teratogenic virus should be considered as a possible cause.

Because Akabane virus does not persistent in the fetus, attempts to isolate virus from affected newborn calves, lambs, and kids are uniformly unsuccessful. Nevertheless, virus may be isolated from the tissues (e.g., brain, spinal cord, skeletal muscle) of fetuses that are aborted early in pregnancy. Virus isolation is usually conducted by inoculation of a continuous cell line and the polymerase chain reaction (PCR) assay has been used to reliably detect viral RNA in fetal tissues.

Akabane infection is most frequently confirmed serologically. Serum from affected calves, lambs, and kids should be collected prior to suckling to enable detection of specific antibodies to Akabane virus. As virus-specific antibodies are produced in fetuses after they become immunocompetent, the detection of antibodies to this virus in serum and body fluids prior to suckling is considered to confirm an *in utero* infection. An examination of the abomasum for milk curd should be made to confirm that the calf has not suckled. As a further check, a serum sample from the dam should also be taken and the two samples tested in parallel for antibodies to Akabane and other common viruses. If the serum of the neonate contains a similar range of antibodies to the dam, it would suggest that suckling has taken place. If only antibodies to Akabane virus are detected in the serum of the neonate but there are antibodies to several in its dam's serum then the evidence is strong that it has been infected *in utero*.

The virus neutralization test is the most specific serological test available but enzyme-linked immunosorbent assays (ELISAs) have also been developed that are either Akabane specific or Simbu serogroup specific.

Differential Diagnosis

Other viral teratogens may be associated with *in utero* infections and the delivery of neonates with congenital defects. Of the bunyaviruses, Aino virus has been linked with congenital AG/HE in cattle, though the lesions of the cerebrum are less symmetrical than those due to Akabane virus and the cerebellum may also be affected. In sheep in the USA (where Akabane virus is exotic), Cache Valley virus is a possible cause. Other viral agents that may cause defects include bluetongue, Rift Valley fever, Wesselsbron, border disease, and bovine viral diarrhea viruses. An important differential feature of Akabane infection in cattle is that the cerebellum is unaffected. Individual agents may be confirmed by using virus-specific serology to test precolostral serum or fluid from body cavities (e.g., pericardial or pleural fluid).

A range of noninfectious causes of congenital AG and HE in calves, lambs, and kids should also be considered and include maternal intoxication (e.g., by ingestion of toxic plants) or an inherited defect. The detection of elevated immunoglobulin G (IgG) levels in precolostral serum or fluids will differentiate between infectious and noninfectious causes of congenital defects.

Prophylaxis and Control

There are two main approaches to the prophylaxis of Akabane virus infections: vaccination and management strategies to control or avoid vectors.

Vaccination

Vaccination is the main method of prophylaxis and Akabane vaccines have been produced in Japan and Australia.

In Japan and Australia, inactivated vaccines have been developed that induce high antibody titers after two doses given with a 4 week interval. In trials, these vaccines prevented development of a viremia and fetal infection on challenge or after natural exposure and were safe when used in pregnant animals. As antibody titers decline fairly rapidly, annual revaccination is recommended. In Japan, a live attenuated vaccine has also been produced by serial passage of Akabane virus in cell culture. When used in cattle this vaccine induces high titers of neutralizing antibodies without pyrexia, leucopoenia, or viremia and virus was not recoverable from the organs or the fetuses of vaccinated animals. It also prevented infection of the bovine fetus on challenge with virulent Akabane virus. However, in pregnant ewes, the vaccine induced a viremia and frequently caused intrauterine infection of the fetus, so this vaccine is not recommended for use in sheep.

Vector Control and Management Strategies

At present, vector control is considered to be impractical partly because of inadequate knowledge of vector biology over much of the global range of Akabane virus and partly because vector control can be prohibitively expensive. Further, absolute control of vectors is impossible and animals only need to be bitten by a single virus-infected vector for infection to occur. Possible control measures involve elimination or reduction in the vector-breeding sites (damp organically enriched soil or large herbivore dung) and direct insecticide attack on adult vectors. Susceptible hosts may also be protected from the bites of vectors by the use of insect repellents or by screening animal housing with insect-proof nets or mesh.

An important measure to minimize losses due to Akabane virus is to avoid the movement of susceptible pregnant animals into virus-endemic areas during the vector season. Such inadvertent movements have resulted in significant losses.

See also: Bunyaviruses: General Features; Vector Transmission of Animal Viruses.

Further Reading

Al-Busaidy SM, Mellor PS, and Taylor WP (1988) Prevalence of neutralising antibodies to Akabane virus in the Arabian Peninsula. *Veterinary Microbiology* 17: 11–149.

Charles JA (1994) Akabane virus. *Veterinary Clinics of North America: Food Animal Practice* 10: 525–546.

Inaba Y and Matumoto M (1990) Akabane virus. In: Dinter Z and Morein B (eds.) *Virus Infections of Ruminants*, pp. 467–480. Oxford: Elsevier.

Jagoe S, Kirkland PD, and Harper PAW (1993) An outbreak of Akabane virus induced abnormalities in calves following agistment in an endemic region. *Australian Veterinary Journal* 70: 56–58.

Jennings DM and Mellor PS (1988) *Culicoides*: Biological vectors of Akabane virus. *Veterinary Microbiology* 21: 125–131.

Kirkland PD and Barry RD (1986) The economic impact of Akabane virus and cost effectiveness of Akabane vaccine in New South Wales. In: St. George TD, Kay BH, and Blok J (eds.) *Arbovirus Research in Australia*, pp. 229–232. Brisbane: CSIRO.

Kirkland PD, Barry RD, Harper PAW, and Zelski RZ (1988) The development of Akabane virus induced congenital abnormalities in cattle. *Veterinary Record* 122: 582–586.

Matumoto M and Inaba Y (1980) Akabane disease and Akabane virus. *Kitasato Archives of Experimental Medicine* 53: 1–21.

St. George TD and Kirkland PD (2004) Diseases caused by Akabane and related Simbu-group viruses. In: Coetzer JAW and Tustin RC (eds.) *Infectious Diseases of Livestock,* 2nd edn., vol. 2, pp. 1029–1036. Cape Town: Oxford University Press.

St. George TD and Standfast HA (1989) Simbu group viruses with teratogenic potential. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. 4, pp. 145–166. Boca Raton, FL: CRC Press.

Taylor WP and Mellor PS (1994) The distribution of Akabane virus in the Middle East. *Epidemiology and Infection* 113: 175–185.

Alfalfa Mosaic Virus

J F Bol, Leiden University, Leiden, The Netherlands

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Agroinfiltration Infiltration of plant leaves with a suspension of Agrobacterium tumefaciens for transient expression of genes from a T-DNA vector.
T-DNA vector Plasmid with the T-DNA (transferred DNA) sequence of the Ti plasmid of Agrobacterium tumefaciens, which is transferred to plant cells where it can be transiently expressed or become integrated in the plant genome.

History

Alfalfa mosaic virus (AMV) was identified in 1931 by Weimer as the causal agent of an economically important disease in alfalfa. Purification of AMV around 1960 showed that virus preparations contained bacilliform particles of different length. Fractionation of these particles by sucrose-gradient centrifugation revealed that the four major components each contained a specific type of RNA, termed RNAs 1, 2, 3, and 4. Initially, an analysis of the biological activity of the viral nucleoproteins and RNAs resulted in a puzzle. A mixture of the three largest viral particles was fully infectious, but a mixture of RNAs 1, 2, and 3, purified from these particles, was not. At the RNA level, RNA 4 was required in the inoculum to initiate infection. In 1971 it became clear that the AMV genome consisted of RNAs 1, 2, and 3, and that a mixture of these genomic RNAs became infectious only after addition of coat protein (CP) or its subgenomic messenger, RNA 4. High-affinity binding sites for CP were identified in the AMV RNAs in 1972 and could be localized to the 3′ termini of the RNAs in 1978. From 1975 onwards it became clear that, similar to AMV, ilarviruses required CP to initiate infection. Moreover, CPs of AMV and ilarviruses could bind to the 3′ termini of each other's RNAs, and could be freely exchanged in the initiation of infection. Currently, AMV is the type species of the genus *Alfamovirus* and is classified together with the genus *Ilarvirus* in the family *Bromoviridae*.

Taxonomy and Classification

In addition to the genera *Alfamovirus* and *Ilarvirus*, the family *Bromoviridae* contains the genera *Bromovirus*, *Cucumovirus,* and *Oleavirus*. The tripartite RNA genomes of bromo- and cucumoviruses are infectious as such and do not require CP to initiate infection. Although a mixture of the genomic RNAs of AMV and ilarviruses has a low intrinsic infectivity, initiation of infection is stimulated approximately 1000-fold by addition of CP or RNA 4. This phenomenon has been termed 'genome activation'. CP of bromo- and cucumoviruses is unable to initiate infection by AMV or ilarvirus genomic RNAs. Oleaviruses have been studied in less detail but are believed to resemble bromo- and cucumoviruses in their replication strategy. The 3′ termini of the RNAs of bromo- and cucumoviruses contain a tRNA-like structure (TLS) that can be charged with tyrosine, whereas the RNAs of AMV and ilarviruses cannot be charged with an amino acid. Many isolates of AMV are known which are closely related by serology and nucleotide sequence similarity. The complete nucleotide sequence of AMV isolate 425 (Leiden [L] and Madison [M] isolates) has been determined, and partial sequences of the Strasbourg (S) and yellow spot mosaic virus (Y) isolates have been published. The available data indicate that sequence similarity between isolates is over 95%.

Host Range and Economic Significance

AMV occurs worldwide. Strains of this virus have been found in natural infections of about 150 plant species representing 22 families. The experimental and natural host ranges include over 600 species in 70 families. Recently, 68 *Arabidopsis thaliana* ecotypes were analyzed for their susceptibility to AMV infection. Thirty-nine ecotypes supported both local and systemic infection, 26 ecotypes supported only local infection, and three ecotypes could not be infected. Although AMV infects mostly herbaceous plants, several woody hosts are included in its natural host range. AMV is an economically important pathogen in alfalfa and sweet clover and may affect pepper, pea, tobacco, tomato, soybean, and celery. Worldwide, AMV causes calico mosaic in potato but its economic importance in this crop is limited.

Particle Structure and Composition

Figure 1 shows an electron micrograph of AMV. The four major classes of particles in AMV preparations are called bottom component (B), middle component (M), top component *b* (Tb), and top component *a* (Ta). B, M, and Tb are bacilliform and contain the genomic RNAs 1, 2, and 3, respectively. Ta contains two molecules of the subgenomic

RNA 4 and can be subdivided into bacilliform Ta-b and spheroidal Ta-t particles. The bacilliform particles are all 19 nm wide and have lengths of 56 nm (B), 43 nm (M), 35 nm (Tb), and 30 nm (Ta-b). The RNAs are encapsidated by a single type of CP which in the case of strain AMV-L has a length of 220 amino acids (mol. wt 24 280). In solution, AMV CP occurs as dimers which under appropriate conditions of pH and ionic strength form a $T = 1$ icosahedral structure built from 30 dimers. This structure can be crystallized and has been studied by X-ray diffraction, cryoelectron microscopy, and image reconstruction methods. The CP was found to have the canonical eight-stranded β-barrel fold with the N- and C-terminal arms as extended chains. Dimer formation in the $T = 1$ particle is based on the clamping of the C-terminal arms of the subunits.

From particle weight measurements and analysis of electron microscopic images, it has been concluded that the number of CP monomers in the major viral components is $60 + (n \times 18)$, n being 10 (B, 240 subunits), 7 (M, 186 subunits), 5 (Tb, 150 subunits), or 4 (Ta, 132 subunits). By gel electrophoresis at least 13 minor components have been resolved which probably represent other n values and contain monomers of genomic RNAs, multimers of genomic RNAs or RNA 4, or specific degradation products of RNA 3. Although details of the arrangement of the protein monomers have not been established, electron microscopical studies indicate that the cylindrical parts of the bacilliform particles have a hexagonal surface lattice with dimers of CP associated with the twofold symmetry axes. The cylindrical part is believed to be capped by two halves of an icosahedron by changing the axes from sixfold symmetry in the cylinder into axes of fivefold symmetry. Neutron scattering data suggested that the capsid structure of spheroidal Ta-t particles is represented by a deltahedron with 52-point group symmetry built from 120 subunits. The percentage of RNA in the virions decreases from 16.3 in B to 15.2 in Ta-b. The buoyant density in CsCl of the major components fixed with formaldehyde varies from 1.366 (Ta) to 1.372 (B) g cm^{-3}. The protein shell has an inner radius of 6.5 nm and an outside radius of 9.4 nm. The RNA is uniformly packed within the 6.5 nm radial limit, occupies about 20% of the interior volume available, and slightly penetrates the protein shell. The particles are mainly stabilized by protein–RNA interactions. The RNA is easily accessible to ribonucleases A and T1 through holes in the protein shell. At slightly alkaline pH the particles unfold reversibly.

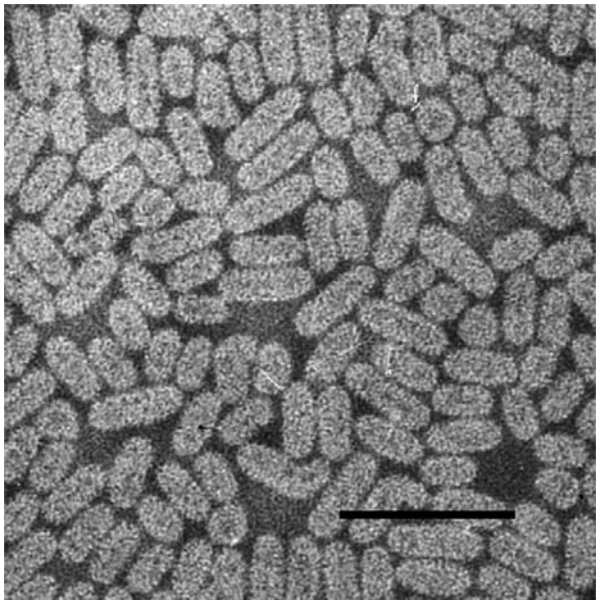


Figure 1 Electron micrograph of alfalfa mosaic virus. Scale = 100 nm.

Genome Structure

The genome structure of the L isolate of AMV is shown in **Figure 2**. RNAs 1 and 2 encode the replicase proteins P1 and P2, respectively. P1 contains an N-terminal methyltransferase-like domain and C-terminal helicase-like domain, whereas P2 contains a polymerase-like domain. RNA 3 is dicistronic and encodes the 5′-proximal movement protein (MP) gene and the 3′-proximal CP gene. MP and CP are both required for cell-to-cell

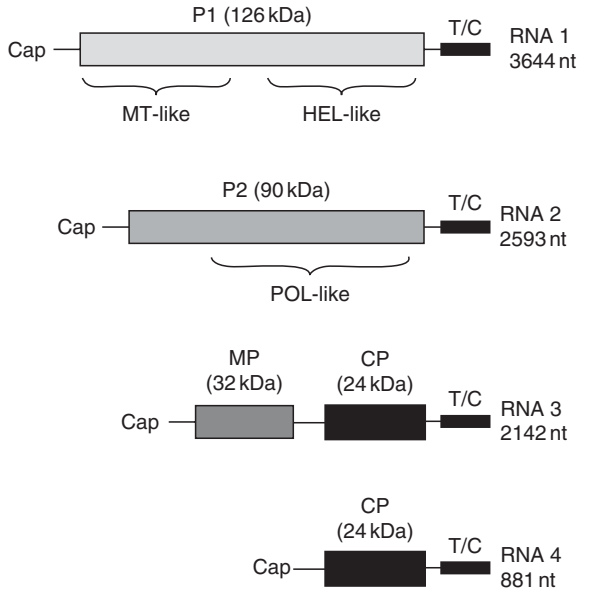


Figure 2 Structure of the AMV genome (isolate 425). RNAs 1 and 2 encode the replicase proteins P1 and P2; RNA 3 encodes the movement protein (MP) and coat protein (CP). CP is translated from subgenomic RNA 4. The bar labeled T/C represents the 3′-terminal 112 nt of the RNAs, which can adopt either a tRNA-like structure (TLS) or a structure with a high affinity for CP (CPB).

transport of the virus. CP is translated from RNA 4, which is identical in sequence to the 3′-terminal 881 nucleotides (nt) of RNA 3. The length of the intercistronic region in RNA 3 is 52 nt including the leader sequence of RNA 4 of 36 nt. At the 5′ end, all four AMV RNAs are capped. The organization of the leader sequence of RNA 3 varies between strains. For strains M, S, L, and Y the length of this leader sequence is 240, 313, 345, and 391 nt, respectively. The increased length of the last three strains is due to the presence of direct repeats of 56 (S), 75 (L), or 149 (Y) nt. At their 3′ termini, the viral RNAs contain a homologous sequence of 145 nt. The 3′ 112 nt of this sequence can adopt two mutually exclusive conformations, one representing a strong CP binding site (CPB) and the other representing a TLS resembling the TLS of bromo- and cucumoviruses. In the CPB structure, the 112 nt sequence consists of four hairpins (designated A, B, C, and D from 3′ to 5′ end) flanked by tetranucleotide sequences AUGC (or a UUGC derivative). Base-pairing between a four nucleotide sequence in the loop of the 5′-proximal hairpin D and a four nucleotide sequence in the stem of the 3′-proximal hairpin A results in a pseudoknot interaction that generates the TLS structure. Structures similar to the AMV CPB and TLS conformations have been identified at the 3′ termini of the RNAs of prunus necrotic ring-spot ilarvirus. Although the TLS of AMV and ilarviruses cannot be charged with an amino acid, the 3′ end of AMV RNAs is specifically recognized by the host enzyme that adds CCA to the 3′ termini of cellular tRNAs (CTP/ATP:tRNA nucleotidyl transferase). The CCA-adding enzyme was able to adenylate the AMV TLS structure *in vitro*, but not the CPB structure. The AMV CPB and TLS structures have been shown to be required for translation of viral RNA and minus-strand promoter activity, respectively.

Interaction between Viral RNA and Coat Protein

In vitro, the 3′-untranslated region (UTR) of AMV RNAs can bind several dimers of CP. A minimal CP binding site consists of the 3′-terminal 39 nt of the RNAs with the structure 5′-AUGC-[hairpin B]-AUGC-[hairpin A]-AUGC-3′. This structure contains two overlapping binding sites for the N-terminal peptides of the two subunits of a CP dimer. The consensus binding site in the RNA is UGC-[hairpin]-RAUGC (in which R is a purine). In addition to dimers of native CP, N-terminal peptides of CP can bind the 39 nt RNA fragment in a 2:1 ratio. The N terminus of CP contains basic residues at positions 5, 6, 10, 13, 16, 17, 25, and 26, but only arginine-17 appeared to be critical for binding of CP to RNA. This Arg residue is part of a Pro-Thr-x-Arg-Ser-x-x-Tyr (PTxRSxxY) RNA binding domain conserved among AMV and ilarvirus CPs.

The complex of the 3′-terminal 39 nt RNA fragment and a peptide corresponding to the N-terminal 26 amino acids of CP has been crystallized and its structure was solved to 3 Å resolution. Co-folding altered the structure of both the peptide and the RNA. In the co-crystal, hairpins A and B are oriented at approximately right angles and each hairpin is extended by 2 bp formed between nucleotides from adjacent AUGC sequences. If the AUGC motifs in the 39 nt fragment are numbered 1–3, starting from the 3′ end of the RNA, hairpin B is extended by a duplex formed by base-pairing between the U and C residue of motif 3 and the A and G residue of motif 2. Similarly, hairpin A is extended by a duplex formed by base-pairing between the U and C residue of motif 2 and the A and G residue of motif 1. The two peptides in the co-crystal each form an α helix with residues 12–26 ordered in peptide 1 and residues 9 to 26 ordered in peptide 2. The data provided insight into the role of the PTxRSxxY-motif in RNA binding. *In vitro* selection of RNA fragments with a high affinity for full-length AMV CP from a pool of randomized RNAs yielded fragments that maintained the unusual inter-AUGC base pairs observed in the crystal structure, although the primary sequences diverged from the wild-type RNA.

Translation of Viral RNA

Extension of the 3′ end of AMV genomic RNAs with an artificial poly(A)-tail increased the basal level of infectivity of these RNAs 50-fold, compared to a 1000-fold increase caused by binding of CP to the RNAs. A role of CP in translation of viral RNAs was investigated by extension of the 3′ end of a luciferase reporter RNA with the AMV 3′-UTR [Luc-AMV], a poly(A)-tail [Luc-poly(A)], or a plasmid-derived sequence [Luc-control]. Transfection of plant protoplasts with these transcripts in the absence or presence of AMV CP showed that CP did not affect translation of Luc-control or Luc-poly(A), but stimulated translational efficiency of Luc-AMV 40-fold. Moreover, CP had only a minor effect on the half-life of Luc-AMV. GST-pull-down assays and Far Western blotting revealed that AMV CP specifically interacted with the eIF4G-subunit from the eIF4F initiation factor complex from wheat germ (and with the eIFiso4G-subunit from the eIFiso4F complex that is present in plants). eIF4F consists of the helicase eIF4A, the cap-binding protein eIF4E, and the multifunctional scaffold protein eIF4G. **Figure 3** illustrates that translational efficiency of cellular messengers is strongly enhanced by the formation of a closed-loop structure, due to interactions of the poly(A) binding protein (PABP) with the poly(A)-tail and with the eIF4G subunit of the eIF4F complex. Due to its binding to the CPB structure at the 3′ end of viral

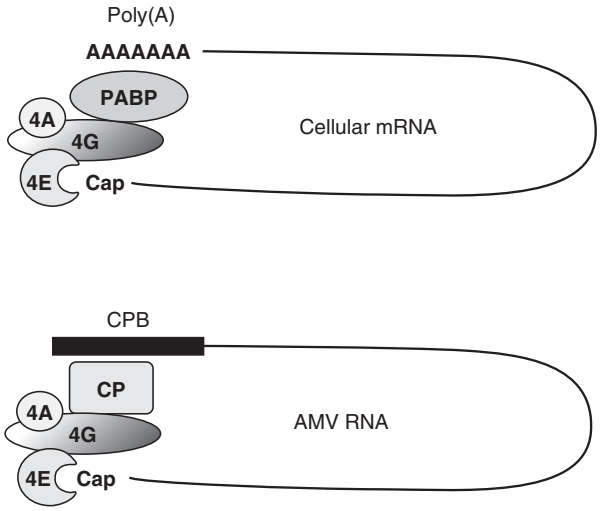


Figure 3 Model for the role of coat protein in translation of AMV RNAs. Translation of cellular mRNAs is strongly enhanced by the formation of a closed-loop structure by interactions of the poly(A) binding protein (PABP) with the 3′ poly(A) tail and with the eIF4G subunit of the eIF4F complex of initiation factors bound to the 5′ cap structure (upper panel). AMV coat protein (CP) enhances translational efficiency of viral RNAs 40-fold by binding to the CP binding site (CPB) at the 3′ end of the viral RNAs. The finding that CP also interacts with eIF4G indicates that CP mimics the function of PABP in formation of the closed-loop structure (lower panel).

RNAs and its interaction with eIF4G, AMV CP could stimulate translation of viral RNAs by a similar mechanism (**Figure 3**).

Protoplasts transfected with wild-type AMV RNA 4 accumulated CP at a detectable level, although accumulation was 100-fold lower than in productively infected protoplasts. However, translation of the RNA 4 mutant R17A was below the detection level. This mutant encodes CP with arginine-17 replaced by alanine, and this CP is unable to bind to the 3′ end of AMV RNAs. Translation of mutant R17A could be rescued to wild-type levels by expression in *trans* of CP that was functional in RNA binding. Also, translation of mutant RNA 4 was rescued to wild-type levels by replacing the 3′-UTR of this mutant by the 3′-UTR of brome mosaic virus (BMV) RNA 4. BMV is the type species of the genus *Bromovirus* in the family *Bromoviridae*. Apparently, the 3′-UTR of AMV requires binding of CP to stimulate translation, whereas the BMV 3′-UTR stimulates translation independently of CP, possibly by the binding of host factors.

Replication of Viral RNA

In tobacco, AMV replication complexes are associated with vacuolar membranes. Template-dependent replicase preparations have been purified from infected plants, which specifically accept exogenous AMV RNA as template. These preparations have been used to study viral minus-strand and plus-strand RNA synthesis *in vitro*.

The identification in 1978 of high-affinity binding sites for CP at the 3′-termini of AMV RNAs led to the hypothesis that binding of CP was required to permit initiation of minus-strand RNA synthesis. However, all experiments done to test this hypothesis showed that CP is not required for the synthesis of AMV minus-strand RNA *in vivo* or *in vitro*: (1) expression of AMV RNAs 1 and 2 from a T-DNA vector in agroinfiltrated leaves results in wild-type levels of minus-strand RNA synthesis in the complete absence of CP; (2) a CP-free replicase, purified from these agroinfiltrated leaves, transcribes AMV RNAs *in vitro* into minus-strand RNAs as efficiently as does the replicase purified from infected leaves; (3) in an *in vitro* replicase assay, addition of CP inhibits minus-strand RNA synthesis; (4) mutation of the 3′-terminal AUGC-motifs in AMV RNAs interferes with binding of CP *in vitro* but does not affect minus-strand RNA synthesis by the purified viral replicase. However, transcription of such mutant RNA by purified AMV replicase is no longer inhibited by CP.

An analysis of sequences in AMV RNAs which direct minus-strand RNA synthesis by the purified viral replicase *in vitro* showed that the entire 3′-terminal homologous sequence of 145 nt is required for minus-strand promoter activity. This promoter consists of the TLS structure formed by the 3′-terminal 112 nt and a hairpin structure, termed hairpin E, between nt 112 and 145 from the 3′ end of the RNAs. Hairpin E is probably the primary element recognized by the viral replicase, and the TLS serves to direct the bound replicase to the very 3′ end of the template. If the TLS structure is disrupted by mutations affecting the pseudoknot interaction or if the TLS is completely deleted, hairpin E directs initiation of minus-strand synthesis to a position located 5′ from the hairpin. In the absence of the TLS, the mechanism of RNA synthesis directed by hairpin E is very similar to that directed by the subgenomic promoter hairpin. This subgenomic promoter is located in minus-strand RNA 3 and directs plus-strand RNA 4 synthesis. The finding that the subgenomic promoter hairpin could be replaced by hairpin E without loss of infectivity illustrates the functional equivalence between the two hairpins.

Although a knockout mutation of the CP gene does not affect AMV minus-strand RNA synthesis in infected protoplasts or in agroinfiltrated leaves, such a mutation results in a 100-fold drop in the accumulation of viral plus-strand RNAs. Initially, it was proposed that this reflected a role of CP in *de novo* synthesis of plus-strand RNA. The observation that CP stimulated RNA 4 synthesis on a minus-strand RNA 3 template by the purified replicase *in vitro* supported this hypothesis. However, this observation could not be reproduced in later experiments. Recently, it was shown that expression of CP in agroinfiltrated leaves not only enhances the accumulation of

replication-competent RNAs but also the accumulation of replication-defective viral RNAs. Thus, it is possible that the stimulation of the accumulation of plus-strand RNAs by CP in infected cells reflects a role of CP in protection of the RNAs from degradation.

RNA 3 can be replicated in *trans* by P1 and P2 proteins expressed from replication-defective RNAs 1 and 2 in agroinfiltrated tobacco leaves or by P1 and P2 expressed from nuclear transgenes in transgenic P12 tobacco. However, RNAs 1 and 2 are unable to make use of these transiently or transgenically expressed replicase proteins and are dependent for replication on their encoded proteins in *cis*. This requirement in *cis* explains why RNA 3 can initiate replication in protoplasts of transgenic P12 plants without a requirement for CP in the inoculum, whereas initiation of replication of RNA 1 or RNA 2 in this system requires CP to permit efficient translation of these RNAs into their encoded proteins. Moreover, replication of RNAs 1 and 2 is strictly coordinated. If RNAs 1, 2, and 3 replicate in plant cells in the presence of a replicase transiently expressed from a T-DNA vector, a mutation in RNA 1 that is lethal to the function of the encoded P1 protein blocks the replication of both mutant RNA 1 and wild-type RNA 2, but does not affect the replication of RNA 3 by the transiently expressed replicase. Similarly, a mutation in RNA 2 that affects the conserved GDD polymerase sequence in the P2 protein blocks replication of RNAs 1 and 2 in this system, but not replication of RNA 3. Apparently, replication of RNA 1 controls replication of RNA 2 and vice versa.

Virus Encapsidation and Movement

Expression of mutant RNAs 1 and 2 with their 3′-UTRs deleted and wild-type RNA 3 in agroinfiltrated leaves results in replication of RNA 3 by the transiently expressed replicase proteins and encapsidation of all three viral RNAs into virions. This indicates that the high-affinity binding sites for CP in the 3′-UTRs of RNAs 1 and 2 are dispensable for encapsidation of these RNAs. Possibly, assembly of virions initiates on internal CP binding sites that have been identified in AMV RNAs.

The RNA 3 encoded MP and CP are both required for cell-to-cell movement of AMV in infected plants. The MP is able to form tubular structures on the surface of infected protoplasts, suggesting that virus movement in plants involves transport of virus particles through tubules, which traverse the cell wall through modified plasmodesmata. Such a mechanism has been found for viruses of the genera *Comovirus*, *Caulimovirus*, and *Badnavirus*. However, tubular structures filled with virus particles have not been observed in AMV infected leaf tissue. Moreover, a CP mutant has been reported that is defective in the formation of virions but is able to move cell-to-cell at a reduced level. Possibly, AMV moves cell-to-cell as viral ribonucleoprotein complexes that structurally differ from virions.

Role of Coat Protein in the AMV Replication Cycle

A natural infection with AMV will be initiated by viral particles B, M, and Tb, containing the genomic RNAs 1, 2, and 3, respectively. Inoculation of plants in the laboratory with a mixture of these three genomic RNAs results in very low, barely detectable levels of infection and infectivity of this mixture is increased 1000-fold by addition of four to ten molecules of CP per RNA molecule. Alternatively, infectivity can be increased by addition of RNA 4, the subgenomic messenger for CP. It is assumed that AMV infection starts with the three genomic RNAs, each associated with one or more dimers of the viral CP. These RNA/CP complexes can be generated by partial disassembly of particles B, M, and Tb in inoculated cells, by mixing purified viral RNAs and CP *in vitro*, or by translation of RNA 4 in the inoculated cells and subsequent binding of *de novo* synthesized CP to the viral RNAs. Infection can be initiated independently of CP when the three genomic RNAs are extended with an artificial 3′-terminal poly(A) tail or are transcribed in the plant nucleus from viral cDNA by polymerase II. It has been shown that binding of CP to the 3′ end of AMV RNAs strongly stimulates translation of the RNAs in plant cells, and that CP specifically binds to the eIF4G subunit of the eIF4F complex of plant initiation factors. Based on these observations, it has been proposed that the role of CP in the inoculum is to stimulate translation of AMV (and ilarvirus) RNAs by mimicking the function of the poly(A) binding protein in translation of cellular mRNAs (**Figure 3**). A similar function in translation of nonpolyadenylated viral RNA has been reported for the NSP3 protein of rotaviruses (family *Reoviridae*). Efficient translation of AMV RNA 4 in protoplasts requires the ability of its encoded CP to bind to the 3′ end of its own messenger. Available data support the notion that after inoculation of plants with AMV RNAs 1, 2, 3, and 4, RNA 4 is initially translated with low efficiency until translation of this messenger is stimulated by its own translation product.

In vitro, binding of CP to the 3′ end of AMV RNAs blocks minus-strand RNA synthesis by the purified viral replicase. It is possible that early in infection the binding of CP to the 3′ end of the RNAs not only promotes translation but also blocks premature initiation of replication to prevent a collision between ribosomes and replicase, traveling along the RNA in opposite directions. However, this possibility is not supported by experimental evidence. The switch from translation to replication requires dissociation of parental CP from the 3′ end of

the RNAs to allow the formation of the TLS structure, as the TLS conformation has been shown to be required for minus-strand promoter activity. Replicase proteins translated from inoculum RNAs 1 and 2 may target the viral RNAs to vacuolar membranes where replication complexes are formed and may bind to minus-strand promoter hairpin E (between nt 112 and 145 from the 3′ end of these RNAs) to promote dissociation of CP. Another possibility is that embedding of viral ribonucleoprotein complexes in the vacuolar membrane promotes dissociation of CP.

The mechanism of the switch from minus-strand to plus-strand RNA synthesis is not yet clear. Although the promoter for plus-strand subgenomic RNA 4 synthesis is structurally similar to hairpin E in the minus-strand promoter, the promoters for plus-strand genomic RNA synthesis have not yet been characterized. The requirement of CP for efficient accumulation of plus-strand RNA in infected cells may reflect a role of CP in protection of the RNAs from degradation, but a role in plus-strand RNA synthesis has not yet been ruled out. There is growing evidence for AMV and other viruses in the family *Bromoviridae* that many steps in the viral replication cycle are tightly linked.

Virus–Host Relationships

The AMV group is a large conglomerate of strains infecting a high number of susceptible hosts. This accounts for the tremendous range of symptoms displayed by AMV-infected plants. Mutations in the coat protein gene and 5′-UTR of RNA 3 have been shown to affect symptom formation in tobacco. Cytological modifications in AMV-infected plants occur only in cells of organs showing symptoms. In these cells fragmentation of the ground cytoplasm and an increased accumulation of membrane-bound vesicles has been observed. Sometimes the lamellar system of chloroplasts is affected and invaginations of the nuclear membrane have been reported. The MP protein has been localized in the middle lamella of walls of those cells that had just been reached by the infection front and in which viral multiplication had just begun. The P1 protein is exclusively localized at the tonoplast, whereas P2 was found both at the tonoplast and other locations in the infected cell. Virus particles are mainly found in the cytoplasm with a few records of particles in the nucleus. Depending on the strain, different types of intracellular aggregates of virus particles may occur.

Transmission

AMV is easily transmissible manually. Field spread occurs predominantly by aphid transmission. At least 15 aphid species are known to transmit the virus in the stylet-borne or nonpersistent manner. Acquisition of the virus occurs within 10–30 s and is followed by immediate transmission without a latent period. The ability to continue transmission is lost by the aphid within 1 h. The variability of individual aphid species in their capacity to transmit different AMV strains suggests a specific virus–vector relationship which is probably governed by the structural properties of the CP. Seed transmission of AMV has been reported for alfalfa and seven other plant species with rates of transmission varying from 0.1% to 50%. Transmission of the virus between plants by parasitic dodder has been observed with five *Cuscuta* species.

Epidemiology and Control

Although there have been reports on resistance and tolerance of alfalfa to AMV, control of the virus in this crop can be done mainly by using virus-free seed and avoiding reservoir hosts of the virus. Because the virus occurs naturally in many different plant species, this is practically impossible.

Tobacco plants transformed with the CP gene of AMV were found to be highly resistant to the virus when infection was done by mechanical inoculation. Resistance to transmission of virus by aphids has not yet been tested. The resistance was clearly protein mediated as plants with the highest level of CP accumulation were the most resistant. Plants with the highest level of CP were resistant to infection with inocula consisting of either viral particles or RNAs, whereas plants with lower levels of CP were resistant to infection with particles only. A mutation in the transgene that affected the N-terminal sequence of the encoded CP destroyed resistance to the wild-type virus but the mutant transgene conferred resistance to virus expressing the mutant CP.

See also: Ilarvirus.

Further Reading

Balasubramaniam M, Ibrahim A, Kim B-S, and Loesch-Fries S (2006) *Arabidopsis thaliana* is an asymptomatic host of alfalfa mosaic virus. *Virus Research* 121: 215–219.

Bol JF (2005) Replication of alfamo- and ilarviruses: Role of the coat protein. *Annual Review of Phytopathology* 43: 39–62.

Boyce M, Scott F, Guogas LM, and Gehrke L (2006) Base-pairing potential identified by *in vitro* selection predicts the kinked RNA backbone observed in the crystal structure of the alfalfa mosaic virus RNA–coat protein complex. *Journal of Molecular Recognition* 19: 68–78.

Guogas LM, Filman DJ, Hogle JM, and Gehrke L (2004) Cofolding organizes alfalfa mosaic virus RNA and coat protein for replication. *Science* 306: 2108–2111.

Hull R (1969) Alfalfa mosaic virus. *Advances in Virus Research* 15: 365–433.

Jaspars EMJ (1985) Interaction of alfalfa mosaic virus nucleic acid and protein. In: Davies JW (ed.) *Molecular Plant Virology,* vol.1, pp. 155–230. Boca Raton, FL: CRC Press.
Krab IM, Caldwell C, Gallie DR, and Bol JF (2005) Coat protein enhances translational efficiency of alfalfa mosaic virus RNAs and interacts with the eIF4G component of initiation factor eIF4F. *Journal of General Virology* 86: 1841–1849.
Kumar A, Reddy VS, Yusibov V, *et al.* (1997) The structure of alfalfa mosaic virus capsid protein assembled as a $T = 1$ icosahedral particle at 4.0-Å resolution. *Journal of Virology* 71: 7911–7916.
Olsthoorn RCL, Haasnoot PC, and Bol JF (2004) Similarities and differences between the subgenomic and minus-strand promoters of an RNA plant virus. *Journal of Virology* 78: 4048–4053.
Olsthoorn RCL, Mertens S, Brederode FT, and Bol JF (1999) A conformational switch at the 3′ end of a plant virus RNA regulates viral replication. *EMBO Journal* 18: 4856–4864.

Algal Viruses

K Nagasaki, Fisheries Research Agency, Hiroshima, Japan
C P D Brussaard, Royal Netherlands Institute for Sea Research, Texel, The Netherlands

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Algal viruses Viruses infecting eukaryotic algae.
HaRNAV A positive-sense single-stranded RNA virus infecting the bloom-forming microalga *Heterosigma akashiwo* (Raphidophyceae).
VLPs Virus-like particles that are identified by transmission electron microscopy.

Introduction

With the realization during the last two decades that viruses are highly abundant in various aquatic environments (both marine and freshwater), interest in aquatic viruses significantly increased. Viruses are now recognized as important biological agents not only regulating population dynamics, succession, and diversity of the host organisms in marine systems, but also influencing the functioning of aquatic food webs and biogeochemical cycling (energy and matter fluxes). Organisms within the microbial food web can become infected by viruses, including eukaryotic algae, cyanobacteria, and heterotrophic protists. Indeed, viruses or virus-like particles (VLPs) have been found in more than 60 algal species for 12 recognized classes of eukaryotic algae; to date, about 40 algal viruses have been isolated and characterized (**Table 1**). Although their classification and nomenclature has been improved on, only two algal virus families are officially established to date: *Phycodnaviridae* and *Marnaviridae*. The family *Phycodnaviridae* comprises large genome-sized double-stranded DNA (dsDNA) viruses infecting eukaryotic algae, and the family *Marnaviridae* was just recently established based on the analysis of a positive-sense single-stranded RNA (ssRNA) virus infecting a raphidophyte *Heterosigma akashiwo*.

Most recently, four algal viruses were discovered that do not belong to the above two virus families – RsRNAV, HcRNAV, MpRV and CsNIV; they are infectious to the single-celled marine phytoplankton species *Rhizosolenia setigera*, *Heterocapsa circularisquama*, *Micromonas pusilla*, and *Chaetoceros salsugineum*, respectively. In this article, we summarize the characteristics of these algal viruses, and discuss their putative classification.

ssRNA Viruses Infecting Eukaryotic Algae

RsRNAV

Rhizosolenia setigera RNA virus (RsRNAV) is the first diatom-infecting virus that was isolated and characterized. It is a positive-sense ssRNA virus infecting the bloom-forming diatom *R. setigera*. Viral replication occurs within the host cytoplasm, the virus particle is icosahedral, lacking a tail, and 32 nm in diameter (**Figure 1**).

The RsRNAV genome is a 3′ polyadenylated linear RNA lacking a cap structure at the 5′ terminus. Although the genome size was initially estimated to be 11.2 kbp using denaturing gel electrophoresis, full genome sequencing later revealed that the genome is 8877 nt in length, excluding the polyA tail (DDBJ accession number: AB243297). This may be due to the additional length of the 3′ poly(A) tail or the addition of a viral genome-linked protein (VPg). Further analysis is required to explain this disagreement in genome length estimates. RsRNAV genome has two open reading frames (ORFs): ORF-1 coding for a putative polyprotein containing the RNA helicase domain and the RNA-dependent RNA polymera (RdRp) domain, and ORF-2 encoding at least three major

Table 1 Viruses infecting eukaryotic algae

Virus	*Host*	*Size (nm)*	*Genome type*	*Genome size (kbp)*
Viruses infecting unicellular algae				
BtV	*Aureococcus anophagefferens*	140	dsDNA	
CbV	*Chrysochromlina brevifilum*	145–170	dsDNA	
CdDNAV[a]	*Chaetoceros debilis*	30	ssDNA (fragmented?)	
CeV	*Chrysochromlina ericina*	160	dsDNA	510
CgNIV[a]	*Chaetoceros* cf. *gracilis*	30	ssDNA (?)	3–4
Chlorella virus (e.g., ATCV-1, -2)	*Chlorella* SAG 3.83 (symbiont of *Acanthocystis turfacea*)	140–190	dsDNA	288
Chlorella virus (e.g., PBCV-1, NY-2A, AR158)	*Chlorella* NC64A (symbiont of *Paramecium bursaria*)	190	dsDNA	331–369
Chlorella virus (e.g., MT325, FR483)	*Chlorella* Pbi (symbiont of *Paramecium bursaria*)	140–150	dsDNA	314–321
Chlorella virus (e.g., HVCV)	*Chlorella*-like alga (symbiont of *Hidra viridis*)	170–180	dsDNA	200
CnRNAV[a]	*Chaetoceros neogracilis*	31	ssRNA	
CsNIV	*Chaetoceros salsugineum*	38	(ss + ds)DNA	6
CspNIV	*Chaetoceros* cf. *gracilis*	25		
CsRNAV[a]	*Chaetoceros socialis*	~30	RNA (?)	
EhV	*Emiliania huxleyi*	170–200	dsDNA	410–415
HaNIV[a]	*Heterosigma akashiwo*	30	ssDNA	
HaV	*Heterosigma akashiwo*	202	dsDNA	294
HaRNAV	*Heterosigma akashiwo*	25	ssRNA	9.1
HcRNAV	*Heterocapsa circularisquama*	30	ssRNA	4.4
HcV	*Heterocapsa circularisquama*	197	dsDNA	356
MpRV (originally MpRNAV-01B)	*Micromonas pusilla*	50–60	dsRNA	24.6
MpV (e.g., MpV-PL1, PB7)	*Micromonas pusilla*	115	dsDNA	200
MpV[a] (e.g., MpV-02T,-03T)	*Micromonas pusilla*	~110–120	dsDNA	191–217
MpV[a] (e.g., MpV-01T, 15T)	*Micromonas pusilla*	~70–90		
PgV Group I	*Phaeocystis globosa*	150	dsDNA	466 ± 4
PgV Group II	*Phaeocystis globosa*	100	dsDNA	177 ± 3
PgV-102P	*Phaeocystis globosa*	98	dsDNA	176
PoV	*Pyramimonas orientalis*	180–220	dsDNA	560
PpV	*Phaeocystis pouchetii*	130–160	dsDNA	485
RsRNAV	*Rhizosolenia setigera*	32	ssRNA	11.2
Viruses infecting multicellular algae				
EsV	*Ectocarpus siliculosus*	130–150	dsDNA	336
EfasV	*Ectocarpus fasciculatus*	135–140	dsDNA	340
FlexV	*Feldmannia simplex*	120–150	dsDNA	170
FirrV	*Feldmannia irregularis*	140–170	dsDNA	180
FsV	*Feldmannia* sp.	150	dsDNA	170
HincV	*Hincksia hinckiae*	140–170	dsDNA	220
MclaV	*Myriotrichia clavaeformis*	170–180	dsDNA	340
PlitV	*Pilayella littoralis*	161	dsDNA	280

[a]Y Tomaru, Y Eissler, Y Shirai, CPD Brussaard, JE Lawrence, personal communication.

capsid proteins (MCPs) with molecular mass of 41.5, 41.0, and 29.5 kDa (**Figure 2**). Although a significant similarity in amino acid sequences of the nonstructural and structural proteins between RsRNAV and HaRNAV was found by BLAST search, these viruses differ in the number of ORFs (two and one, respectively) and AU content (63.7% and 53.1%, respectively); hence, RsRNAV was concluded not to be a member of the family *Marnaviridae*. Phylogenetic analysis of the amino acid sequences of the RNA helicase and RdRp domains suggests that RsRNAV belongs to a new previously unrecognized virus group (**Figure 3**). Smaller unidentified RNA molecules ranging in size of 0.6, 1.2, and 1.5 kbp were occasionally included in RsRNAV virions; one possibility is that they are subgenomic RNAs.

RsRNAV has a high degree of strain specificity. One-step growth experiment using an exponentially growing host culture showed 3.1×10^3 infectious units are released from an infected host cell within 48 h post infection. When a stationary phase culture was used as host for the one-step growth experiment, the burst size decreased to 1.0×10^3 infections units per cell. This shows that viral propagation

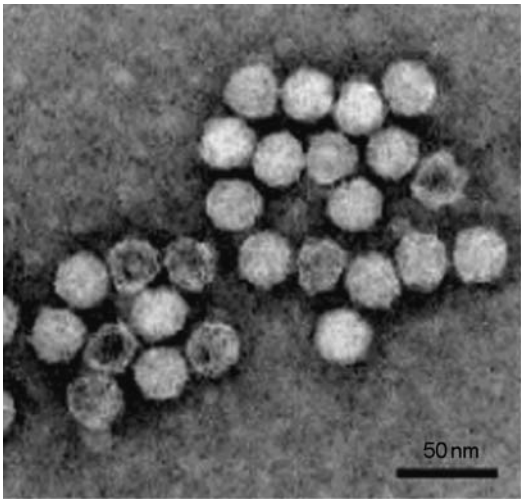


Figure 1 Negatively stained RsRNAV particles. Reprinted from Nagasaki K, Tomaru Y, Katanozaka N, *et al*. (2004) Isolation and characterization of a novel single-stranded RNA virus infecting the bloom-forming diatom *Rhizosolenia setigera*. *Applied and Environmental Microbiology* 70: 704–711, with permission from American Society for Microbiology.

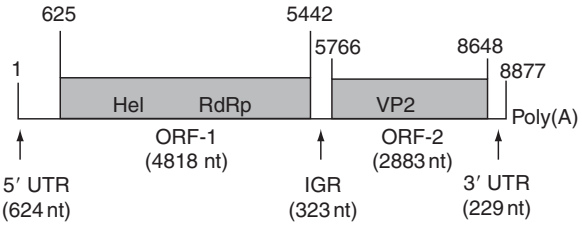


Figure 2 Schematic genome structure of RsRNAV. Numbers indicate base positions from the 5′ terminus in the nucleotide sequence. Hel, RNA helicase domain; RdRp, RNA-dependent RNA polymerase domain; UTR, untranslated region; IGR, intergenic region. Reprinted from Shirai Y, Takao Y, Mizumoto H, *et al*. (2006) Genomic and phylogenetic analysis of a single-stranded RNA virus infecting Rhizosolenia setigera (Stramenopiles: Bacillariophyceae). *Journal of the Marine Biological Association of the United Kingdom* 86: 475–483, with permission from The Marine Biological Association of the UK.

is considerably affected by the physiological condition of the host cells. Although the entry mechanism of RsRNAV into the host cell is unknown, the host's frustule pores (ellipses; 91 nm × 73 nm) are considered to be a possible route of infection.

HcRNAV

Heterocapsa circularisquama RNA virus (HcRNAV) is a positive-sense ssRNA virus infecting the bivalve-killing bloom-forming dinoflagellate *H. circularisquama*. This virus is icosahedral, ~30 nm in diameter, and propagates in the host cytoplasm often forming a crystalline array (**Figure 4**). Its genome is linear positive-sense ssRNA ~4.4 kbp long, lacking 5′ cap structure and 3′ polyA tail, and has a strong stem–loop structure at the 3′ end (**Figure 5**).

HcRNAV clones can be divided into two ecotypes (types UA and CY) based on their intraspecies host specificity patterns. Each type shows its own strain-specific infectivity that is complementary to each other; that is, the *H. circularisquama* strains sensitive to HcRNAV type UA were resistant to HcRNAV type CY, and vice versa, showing that HcRNAV is not species specific, but strain specific. These two HcRNAV ecotypes can coexist in natural water. Typical HcRNAV clones of type UA and CY (HcRNAV34 and 109, respectively) were fully sequenced (DDBJ accession numbers: AB218608 and AB218609, respectively); they are ~97% identical at the nucleotide sequence level. Each genome has two ORFs (**Figure 5**). ORF-1 encodes a putative polyprotein having at least the serine protease domain and the RdRp domain, but no RNA helicase domain was identified. A specific 15 nt deletion was found in about half of the tested virus clones. This, however, is not involved in determining the intraspecies host specificity. ORF-2 coding for the single MCP is unlikely a polyprotein gene because of the molecular mass directly estimated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis is in good agreement with the value predicted by the deduced amino acid sequence of ORF-2. Between the two virus clones tested, the stem–loop structures at the 3′ end are different in stem length and loop size; this may affect the replication efficiency.

Similarity analysis for the deduced amino acid sequences of ORF-1 revealed that HcRNAV is evolutionarily quite distant from any of the land and aquatic viruses that have been genetically studied. This is also supported by a phylogenetic analysis of the RdRp amino acid sequence (**Figure 3**(b)). Although HcRNAV shares some characteristics with the typical marnavirus HaRNAV in infectivity (lytic to marine eukaryotic microalgae) and genome structure (a linear positive-sense ssRNA), they still differ in the number of ORFs (two and one, respectively) and 3′ polyadenylation of the genome RNA (polyadenylated and nonpolyadenylated, respectively). Hence, HcRNAV is not a member of the family *Marnaviridae*.

Genomic comparison revealed complementary host ranges of the two HcRNAV ecotypes (UA and CY: mentioned above) which may be related to the amino acid substitution patterns in ORF-2, the MCP-encoding gene. In addition, the tertiary structure of the MCPs predicted by using computer modeling indicated many of the amino acid substitutions were located in regions on the outside of the viral capsid proteins exposed to the ambient water environments. This suggests that the intraspecies host specificity of HcRNAV is determined by small structural differences of the viral surface that may affect its binding affinity to the host cell and the uncoating process. The results of the transfection experiment also supported this idea: (1) the intraspecies host specificity of HcRNAV

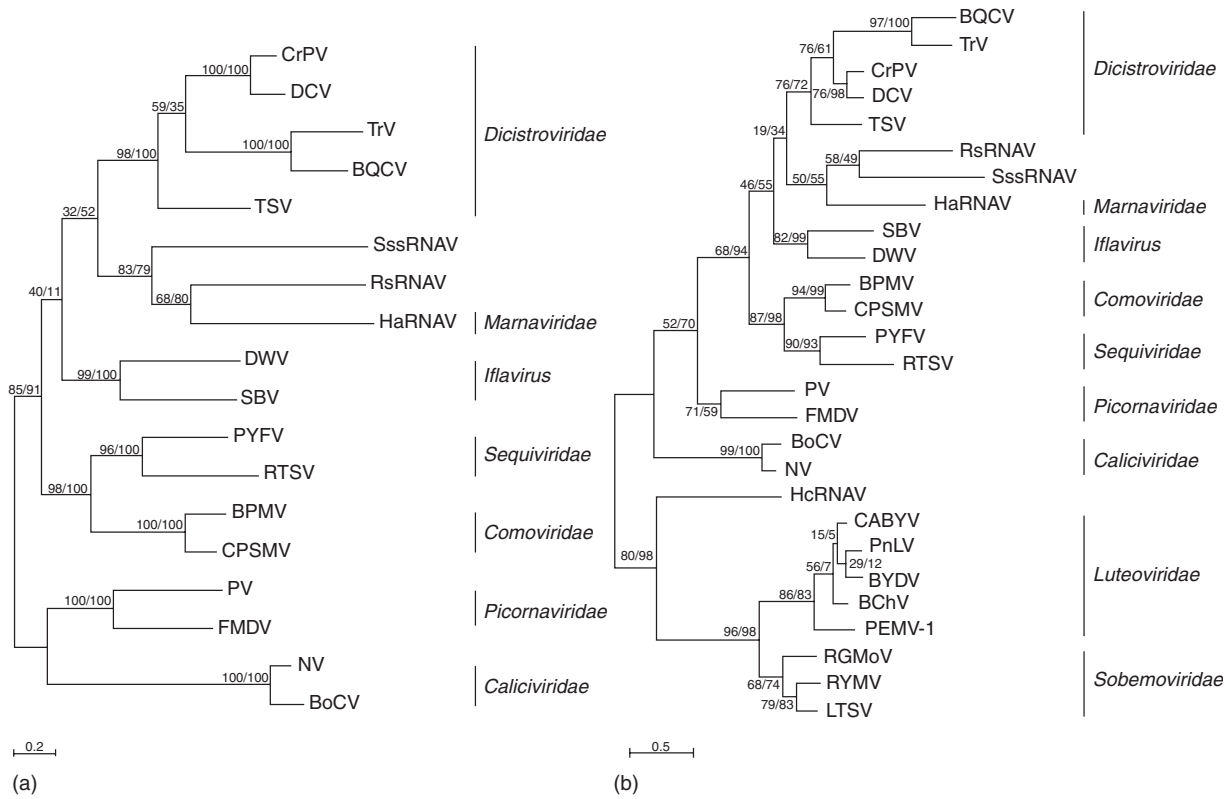


Figure 3 Maximum likelihood (ML) trees calculated from confidently aligned regions of amino acid sequences of concatenated amino acid sequences of RNA helicase domain and RNA-dependent RNA polymerase (RdRp) domain (a), and of RdRp whole domain (b). ML bootstrap values (%) from 100 samples are shown at the nodes followed by bootstrap values based on neighbor-joining analysis (%) from 100 samples. The ML distance scale bars are shown. Amino acid sequences used for comparison in the analyses are as follows with the organism's scientific names, with abbreviations in parentheses if necessary, and the database accession numbers (referring to the US National Center for Biotechnology Information (NCBI) unless otherwise stated): beet chlorosis virus (BChV), AAK49964; bovine enteric calicivirus (BoCV), AJ011099; bean pod mottle virus (BPMV), NC__003496; black queen cell virus (BQCV), NC__003784; barley yellow dwarf virus (BYDV), BAA01054; cucurbit aphid-borne yellows virus (CABYV), CAA54251; cowpea severe mosaic virus (CPSMV), M83830; cricket paralysis virus (CrPV), NC__003924; drosophila C virus (DCV), NC__001834; deformed wing virus (DWV), NC__004830; foot-and-mouth disease virus (FMDV), P03306; heterosigma akashiwo RNA virus (HaRNAV), NC__005281; heterocapsa circularisquama RNA virus 109 (HcRNAV), DDBJ accession number: AB218609; lucerne transient streak virus (LTSV), NP__736596; Norwalk virus (NV), M87661; pea enation mosaic virus 1 (PEMV-1), AAA72297; poinsettia latent virus (PnLV), CAI34771; human poliovirus 1 Mahoney (PV), V01149; parsnip yellow fleck virus (PYFV), D14066; ryegrass mottle virus (RGMoV), NP__736587; rhizosolenia setigera RNA virus (RsRNAV), DDBJ accession number: AB243297; rice turgo spherical virus (RTSV), AAA66056; rice yellow mottle virus (RYMV), CAE81345; sacbrood virus (SBV), NC__002066; schizochytrium single-stranded RNA virus (SssRNAV), BAE47143; triatoma virus (TrV), NC__003783; taura syndrome virus (TSV), NC__003005. Reprinted with minor modification from Shirai Y, Takao Y, Mizumoto H, *et al*. (2006) Genomic and phylogenetic analysis of a single-stranded RNA virus infecting Rhizosolenia setigera (Stramenopiles: Bacillariophyceae). *Journal of the Marine Biological Association of the United Kingdom* 86: 475–483, with permission from The Marine Biological Association of the UK.

is determined by the upstream events of virus infection; (2) the host intracellular condition is permissive for HcRNAV replication even in incompatible host–virus combinations.

By the cross-reactivity test, *H. circularisquama* clones are also divided into two ecotypes according to the sensitivity spectra to the two HcRNAV ecotypes; however, the two host ecotypes are indistinguishable when comparing their morphology or the sequences of the internal spacer regions of the ribosomal RNA genes. These two host ecotypes coexist in natural water. Thus, there are at least two distinct (and independent) host/virus systems between *H. circularisquama* and HcRNAV; that is, multiple ecotypes of host and virus coexist within natural blooms of *H. circularisquama* and their combinations are regulated with exquisite molecular mechanisms.

dsRNA Viruses Infecting Eukaryotic Algae

MpRV

The micromonas pusilla reovirus (MpRV, originally abbreviated as MpRNAV) is a dsRNA virus infecting the cosmopolitan picoprasinophyte *M. pusilla* (**Figure 6**).

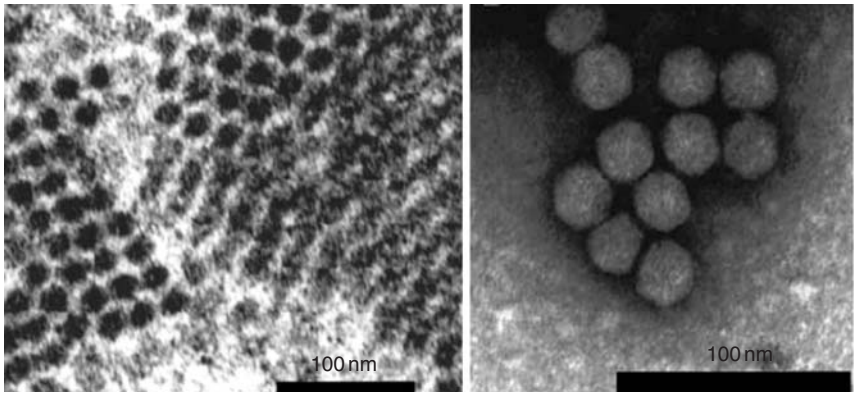


Figure 4 Transmission electron micrographs of intracellular crystalline array formation of HcRNAV (a) and negatively stained HcRNAV particles (b). Reprinted from Tomaru Y, Katanozaka N, Nishida K, *et al*. (2004) Isolation and characterization of two distinct types of HcRNAV, a single-stranded RNA virus infecting the bivalve-killing microalga Heterocapsa circularisquama. *Aquatic Microbial Ecology* 34: 207–218, with permission from Inter-Research Publication.

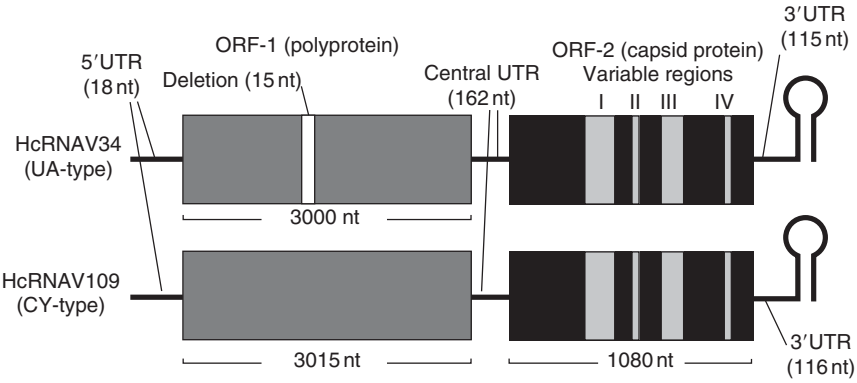


Figure 5 Schematic genome structure of HcRNAV34 and HcRNAV109. Note that the variable regions in ORF-2 are remarkably different between these two virus clones. Reprinted from Nagasaki K, Shirai Y, Takao Y, *et al*. (2005) Comparison of genome sequences of single-stranded RNA viruses infecting the bivalve-killing dinoflagellate *Heterocapsa circularisquama*. *Applied and Environmental Microbiology* 71: 8888–8894, with permission from American Society for Microbiology.

This is the first algal virus having a dsRNA genome that was isolated and characterized. The virus can coexist with a large genome-sized dsDNA virus infecting the same *M. pusilla* strain. It has a narrow host range, a latent period of 36 h (based on a one-step lytic virus growth cycle and transmission electron microscopy (TEM) enumeration of the virus particles), and sensitivity to temperatures $>35\,^{\circ}C$. Its genome is composed of 11 segments ranging between 741 and 5792 bp in length, with a total size of 25563 bp (**Figure 7** and **Table 2**). The polysegmented dsRNA genome of MpRV identified it as a member of the family *Reoviridae*, which was confirmed by sequence analysis, morphological and physiochemical properties. The size of intact virus particles (90–95 nm) is, however, large. The subcore particles size of 50 nm and its smooth surface indicate that MpRV belongs to non-turreted *Reoviridae*.

Comparison of the genome sequence of MpRV to those of characterized members of the family *Reoviridae* indicated that MpRV could not be classified within any of the existing genera of the family (including *Rotavirus* and *Aquareovirus* that both contain viruses with 11-segmented dsRNA genomes). The maximum amino acid identity with other *Reoviridae* proteins was 21%, which is compatible with differences existing between distinct genera.

Within the phylogenetic tree built with reovirus RdRp sequences, the branch of MpRV dissects the tree, separating the group of turreted and nonturreted viruses (**Figure 8**). As *M. pusilla* is evolutionarily older than the hosts of other members of the family *Reoviridae*, the topology of the tree suggests that the branch of MpRV could be ancestral. An interesting feature is the unusual length of segment 1, encoding a protein of 200 kDa (VP1). The many repeats within this sequence suggest that VP1 may have arisen from amino acid fragment duplication, followed by diversification of the sequence. The mechanism and the constraints, which have driven such an evolution, are not clear, although

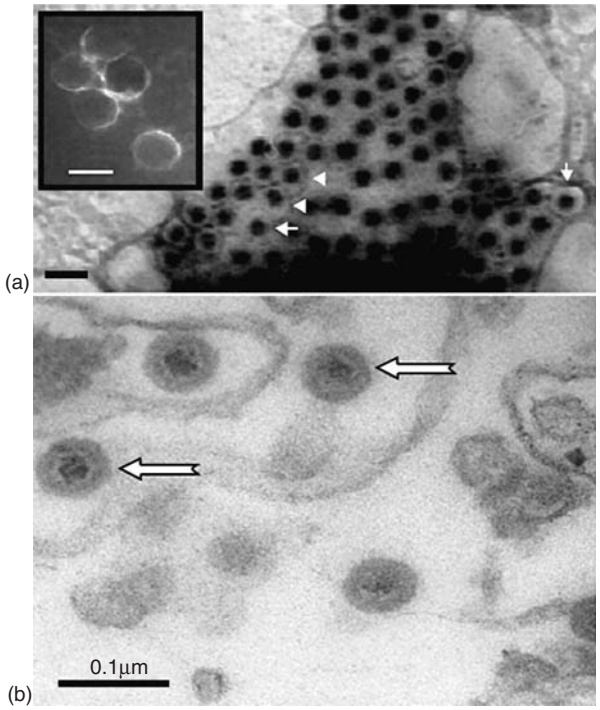


Figure 6 (a) Electron micrographs of MpRV. Particles pelleted from the clarified lysate of infected *M. pusilla*. Some particles (indicated by arrowheads) have a larger diameter. At the upper left corner (inset), core particles treated with 1.5 M $CaCl_2$ are shown to have a smooth outline (turrets are absent). Scale = 100 nm (main image); 50 nm (inset). (b) A TEM image of thin-sectioned *M. pusilla* cells infected with MpRV. The arrows point at intracellular virus particles consisting of a thick outer layer and a smaller electron-dense inner core. (a) Reprinted from Attoui H, Jaafar FM, Belhouchet M, *et al*. (2006) *Micromonas pusilla* reovirus: A new member of the family *Reoviridae* assigned to a novel proposed genus (*Mimoreovirus*). *Journal of General Virology* 87: 1375–1383, from the Society for General Microbiology. (b) Reprinted from Brussaard CP, Noordeloos AA, Sandaa RA, *et al*. (2004) Discovery of a dsRNA virus infecting the marine photosynthetic protist *Micromonas pusilla*. *Virology* 319: 280–291, with permission from Elsevier.

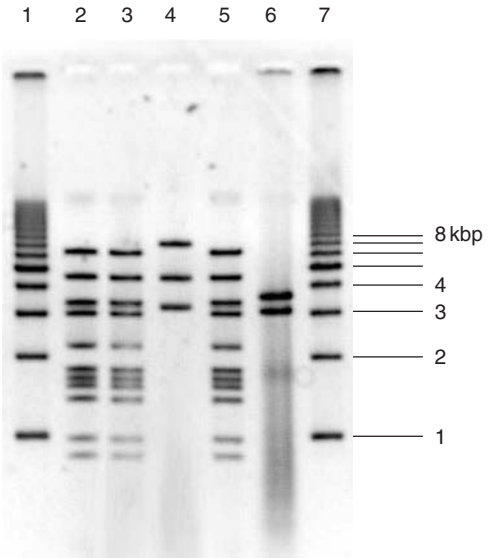


Figure 7 Total nucleic acid patterns of MpRV (lanes 2, 3, and 5). Molecular size standards: 1 kbp dsDNA Molecular Ruler (lanes 1 and 7), dsRNA bacteriophage Phi-6 (lane 4; segments of 6374, 4074, 2948 bp), and dsRNA Infectious Bursal Disease Virus IBDV-V4 (lane 6; segments of 3260 and 2827 bp). Precise lengths of the 11 dsRNA segments are shown in **Table 2**. Reprinted from Brussaard CP, Noordeloos AA, Sandaa RA, *et al*. (2004) Discovery of a dsRNA virus infecting the marine photosynthetic protist *Micromonas pusilla*. *Virology* 319: 280–291, with permission from Elsevier.

Table 2 Lengths of the dsRNA segments 1–11 of MpRV

Segment	*Segment length (bp)*
1	5792
2	4175
3	3129
4	2833
5	2027
6	1687
7	1556
8	1449
9	1296
10	878
11	741
Total	25 563

Adapted from Attoui H, Jaafar FM, Belhouchet M, *et al*. (2006) *Micromonas pusilla* reovirus: A new member of the family *Reoviridae* assigned to a novel proposed genus (*Mimoreovirus*). *Journal of General Virology* 87: 1375–1383, from the Society for General Microbiology.

very recently a model of stem–loop formation (based on the crystal structure of the polymerase of a mammalian orthoreovirus) was proposed for explaining such duplications.

The structural proteins in the purified particles were analyzed and a protein having a molecular weight of approximately 200 kDa was identified in relatively intact particles. This size is compatible with the VP1 which should represent an additional coat protein layer. It is noteworthy that the sequence of VP1 bears many glycosylation sites and matched the envelope proteins of many bacteria and viruses.

Based on particle morphology and sequence analysis, MpRV was classified as the representative of a new genus within the family *Reoviridae*, for which the name *Mimoreovirus* has been proposed.

ssDNA Viruses Infecting Eukaryotic Algae

CsNIV

Chaetoceros salsugineum nuclear inclusion virus (CsNIV) infects the small-sized (2.0–9.5 μm wide) diatom *C. salsugineum*, which forms short or long straight chains.

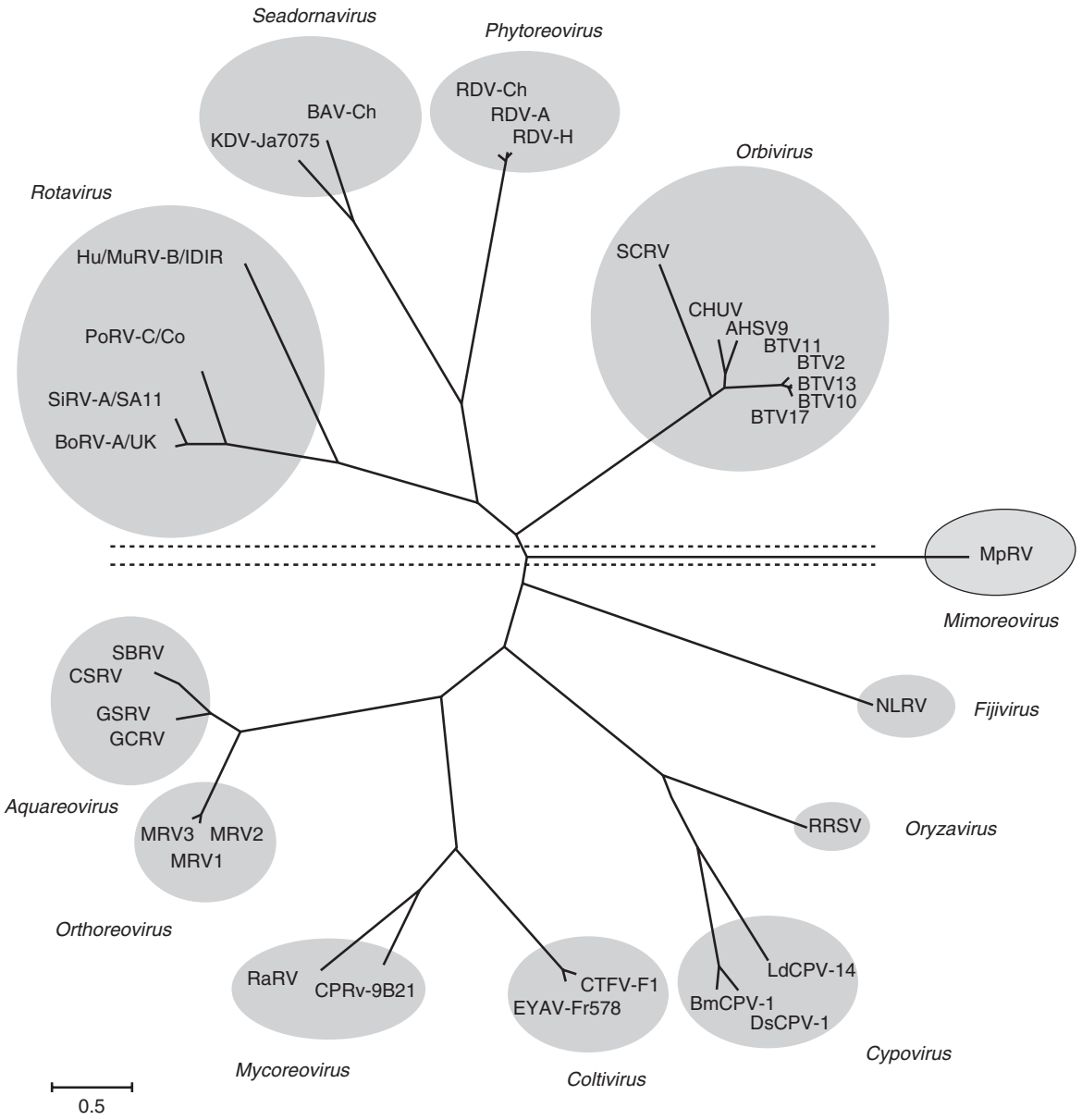


Figure 8 Phylogenetic relationships of MpRV to other members of the family *Reoviridae* based on the sequence of the putative RdRp. Neighbor-joining phylogenetic tree built with available polymerase sequences (using the Poisson-correction or gamma-distribution algorithms) for representative members of 11 genera of the family *Reoviridae*. Scale bar represents the number of substitutions per site. Reprinted from Attoui H, Jaafar FM, Belhouchet M, *et al*. (2006) Micromonas pusilla reovirus: A new member of the family Reoviridae assigned to a novel proposed genus (Mimoreovirus). *Journal of General Virology* 87: 1375–1383, with permission from the Society for General Microbiology.

Its bloom occurs in brackish lakes and estuarine waters. CsNIV is a ~38 nm icosahedral virus that replicates within the host nucleus (**Figure 9**). The genome structure is unlike that of other viruses that have been described to date; that is, it consists of a single molecule of covalently closed circular ssDNA (6000 nt (previously reported as 6005 nt)) as well as a segment of linear ssDNA (997 nt); the linear segment is complementary to a portion of the closed circle creating a partially double-stranded genome (**Figure 10**) (DDBJ accession number: AB193315). Sequence analysis revealed, within one of the six ORFs identified in the genome, only a low similarity to the replicase of circoviruses that have a covalently closed circular ssDNA genome. One-step growth experiment showed that ~330 infectious units are released from an infected cell within 24 h; however, considering that

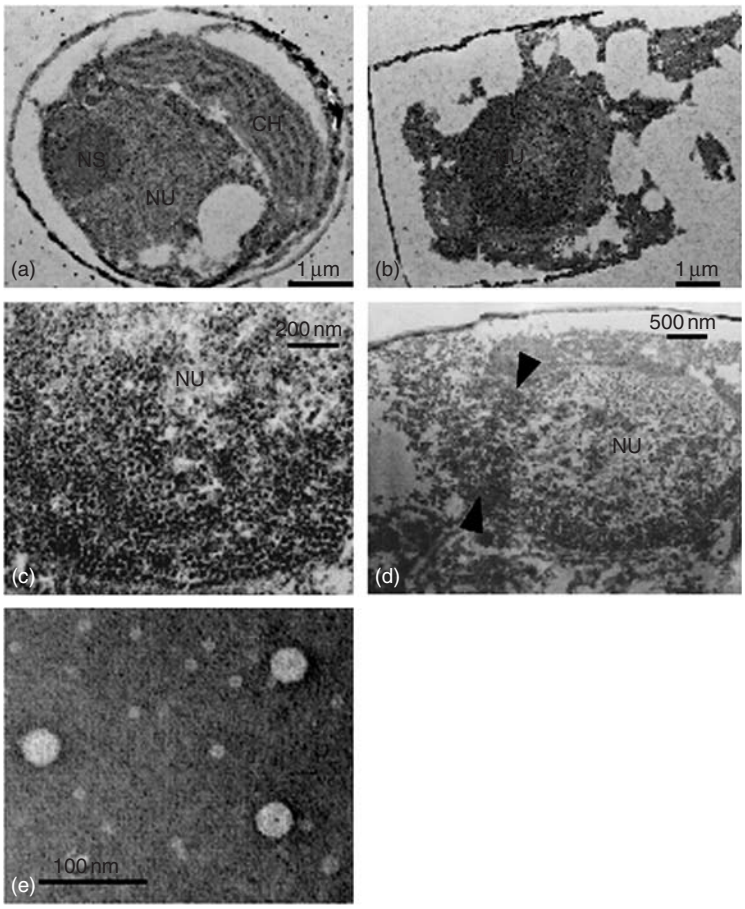


Figure 9 Transmission electron micrographs of *Chaetoceros salsugineum*. (a) Thin section of a healthy cell; (b) thin section of a cell 24 h after inoculation with CsNIV; (c) close-up view of intranuclear CsNIV particles in (b); (d) thin section of a CsNIV-infected cell in which the nuclear envelope was partially ruptured (arrowheads); (e) negatively stained CsNIV particles in the culture lysate. CH, a chloroplast; NU, a nucleus; NS, a nucleolus. Reprinted from Nagasaki K, Tomaru Y, Takao Y, *et al*. (2005) Previously unknown virus infects marine diatom. *Applied and Environmental Microbiology* 71: 3528–3535, with permission from American Society for Microbiology.

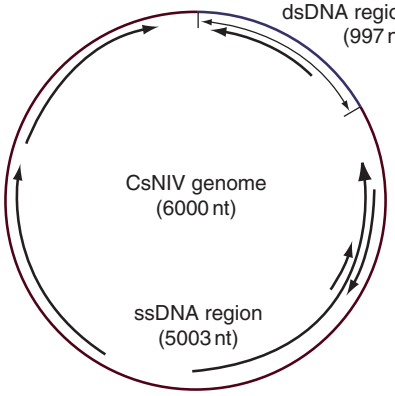


Figure 10 Schematic genome structure of CsNIV. Bold arrows indicate putative ORFs. Adapted from Nagasaki K, Tomaru Y, Takao Y, *et al*. (2005) Previously unknown virus infects marine diatom. *Applied and Environmental Microbiology* 71: 3528–3535, with permission from American Society for Microbiology.

several hundreds of virus particles were included even in a thin section for TEM observation, the resulting burst size is assumed to be underestimated. This may reflect aggregation of virions or dominance of defective particles.

Implications

Phytoplankton is important in maintaining oxygen levels in the atmosphere and sustaining the primary nutritional production of the aquatic environment. Its ecological dynamics is affected at different levels by various factors: physical factors (e.g., temperature, salinity, and irradiation), chemical factors (e.g., nutrients and metals), and biological factors (e.g., grazing, virus infection, and algicidal effects of bacteria). Among them, viruses are considered significant mortality agents, resulting in cell loss comparable

to grazing and/or sedimentation. Whether algae sink out, are grazed upon, or die due to viral-induced cell lysis has different implication for the flow of matter and energy in the aquatic ecosystems. Viral-mediated mortality will force the food web toward a more regenerative system as a result of enhanced production of dissolved organic matter.

Viral control of the algal host's population occurs for *M. pusilla*, a eukaryotic marine phytoplankter that is globally abundant but almost never forms massive blooms. *M. pusilla* belongs to the Prasinophyceae, a class of phytoplankton that is considered to have given rise to green algae and land plants. The dsRNA virus MpRV described earlier in this chapter has been found to coexist with large genome-sized dsDNA viruses, of which some infect the same clone of *M. pusilla*. The various different virus model systems infecting *M. pusilla* that are available in culture permit to investigate virus competition, intraspecies diversity, co-evolution, and genetic flux in more detail.

A good example of viral control on a finer scale, that is, the clonal composition of the algal host population, is the relationship between HcRNAV and its host *H. circularisquama*. Occurrence of *H. circularisquama* blooms in natural waters is accompanied by increased abundance of HcRNAV. Its abundance in the water column rapidly decreases following the termination of host blooms, but the viruses that are supplied to the sediment show only a very gradual decrease. Dynamics of the type UA viruses and the type CY viruses having complementary host ranges are different, supporting the idea that there are two independent host/virus systems between *H. circularisquama* and HcRNAV. Changes in abundance of each HcRNAV ecotype are considered to reflect the fluctuation of its suitable host ecotype *in situ*. By comparing the dynamics of HcRNAV and *H. circularisquama*, the amount of HcRNAV accumulated in the sediment just prior to the host's blooming season is suggested to be a significant factor in determining the size or term length of *H. circularisquama* blooms; that is, when concentration of HcRNAV is high in sediments, occurrence of dense *H. circularisquama* blooms appears suppressed. HcRNAV infection seems, thus, to affect the population dynamics of *H. circularisquama* not only in quantity (biomass) but also quality (clonal composition).

In contrast, ecological implication of viruses infecting diatoms has not been sufficiently understood. Considering that diatoms are one of the most widespread plant groups on earth, the impact viruses have on the diatom's population dynamics should be more intensively studied.

In this article, algal viruses having ssRNA, dsRNA, or ssDNA genomes are described. Given the large variety of algae and the diversity of viruses isolated and characterized to date, marine and freshwaters provide a treasury of undiscovered viruses.

See also: Marnaviruses; Phycodnaviruses.

Further Reading

Attoui H, Jaafar FM, Belhouchet M, *et al.* (2006) *Micromonas pusilla* reovirus: A new member of the family *Reoviridae* assigned to a novel proposed genus (*Mimoreovirus*). *Journal of General Virology* 87: 1375–1383.

Bettarel Y, Kan J, Wang K, *et al.* (2005) Isolation and characterisation of a small nuclear inclusion virus infecting the diatom *Chaetoceros* cf. *gracilis*. *Aquatic Microbial Ecology* 40: 103–114.

Brussaard CP (2004) Viral control of phytoplankton populations – a review. *Journal of Eukaryotic Microbiology* 51: 125–138.

Brussaard CP, Noordeloos AA, Sandaa RA, *et al.* (2004) Discovery of a dsRNA virus infecting the marine photosynthetic protist *Micromonas pusilla*. *Virology* 319: 280–291.

Lang AS, Culley AI, and Suttle CA (2004) Genome sequence and characterization of a virus (HaRNAV) related to picorna-like viruses that infects the marine toxic bloom-forming alga *Heterosigma akashiwo*. *Virology* 320: 206–217.

Lawrence JE, Chan AM, and Suttle CA (2001) A novel virus (HaNIV) causes lysis of the toxic bloom-forming alga *Heterosigma akashiwo* (Raphidophyceae). *Journal of Phycology* 37: 216–222.

Mizumoto H, Tomaru Y, Takao Y, *et al.* (2007) Intraspecies host specificity of a single-stranded RNA virus infecting a marine photosynthetic protist is determined at the early steps of infection. *Journal of Virology* 81: 1372–1378.

Nagasaki K, Shirai Y, Takao Y, *et al.* (2005) Comparison of genome sequences of single-stranded RNA viruses infecting the bivalve-killing dinoflagellate *Heterocapsa circularisquama*. *Applied and Environmental Microbiology* 71: 8888–8894.

Nagasaki K, Tomaru Y, Katanozaka N, *et al.* (2004) Isolation and characterization of a novel single-stranded RNA virus infecting the bloom-forming diatom *Rhizosolenia setigera*. *Applied and Environmental Microbiology* 70: 704–711.

Nagasaki K, Tomaru Y, Nakanishi K, *et al.* (2004) Dynamics of *Heterocapsa circularisquama* (Dinophyceae) and its viruses in Ago Bay, Japan. *Aquatic Microbial Ecology* 34: 219–226.

Nagasaki K, Tomaru Y, Shirai Y, *et al.* (2006) Dinoflagellate-infecting viruses. *Journal of the Marine Biological Association of the United Kingdom* 86: 469–474.

Nagasaki K, Tomaru Y, Takao Y, *et al.* (2005) Previously unknown virus infects marine diatom. *Applied and Environmental Microbiology* 71: 3528–3535.

Shirai Y, Takao Y, Mizumoto H, *et al.* (2006) Genomic and phylogenetic analysis of a single-stranded RNA virus infecting *Rhizosolenia setigera* (Stramenopiles: Bacillariophyceae). *Journal of the Marine Biological Association of the United Kingdom* 86: 475–483.

Tomaru Y, Hata N, Masuda T, *et al.* (2007) Ecological dynamics of the bivalve-killing dinoflagellate *Heterocapsa circularisquama* and its infectious viruses in different locations of western Japan. *Environmental Microbiology* 9: 1376–1383.

Tomaru Y, Katanozaka N, Nishida K, *et al.* (2004) Isolation and characterization of two distinct types of HcRNAV, a single-stranded RNA virus infecting the bivalve-killing microalga *Heterocapsa circularisquama*. *Aquatic Microbial Ecology* 34: 207–218.

Allexivirus

S K Zavriev, Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

© 2008 Elsevier Ltd. All rights reserved.

History

The genus *Allexivirus* of plant viruses belongs to a new family of plant viruses, *Flexiviridae*, which also includes the genera *Capillovirus*, *Carlavirus, Citrivirus, Foveavirus, Potexvirus*, *Trichovirus*, *Vitivirus*, and *Mandarivirus*. The first publication on an allexivirus as a member of a new plant virus group appeared in 1992 after analysis of the complete genome structure of a filamentous virus isolated from shallot plants in the Institute of Agricultural Biotechnology (Moscow, Russia). The virus thus discovered and described was named shallot virus X (ShVX). Allexiviruses acquired their name by fusion of the host (family Alliaceae) and the type member (ShVX) names. The allexivirus-associated diseases are usually mild and in many cases symptomless. Very often, allexiviruses persist in the infected plants as a part of multiple infection induced by viruses with similarly restricted host ranges. Since allexiviruses do not cause serious plant diseases (as well as the majority of related carlaviruses), they do not attract special attention of phytopathologists, but they are quite interesting for taxonomy studies, genome structure, and relationships between genera, species, and subspecies of distinct viruses.

Taxonomy and Classification

Table 1 lists definite and possible or insufficiently characterized members of the genus *Allexivirus*. Practically, all viruses have been included in the group according to the characteristics of their genome structure (primarily, a unique virus-specific protein encoded by ORF4) as well as the serological relatedness to ShVX and the common host range.

Poorly characterized viruses serologically related to ShVX have been found in several *Allium* species, as well as in tulip and narcissus plants, but it is unclear whether these should be regarded as strains of ShVX or as distinct viruses. The serological relationships of ShVX to other well-characterized allexiviruses or to the viruses in other genera have not yet been studied.

Viral Structure and Composition

Virions

Virions of allexiviruses are highly flexible filamentous particles, about 800 nm in length and 12 nm in diameter. They resemble potyviruses in size and closteroviruses in flexibility and cross-banded substructure (**Figure 1**).

Virus preparations obtained from individual *Allium ascalonicum* plants infected only with ShVX were found to contain virions of two morphological types: (1) typical allexivirus particles with characteristic cross-striation; and (2) thinner (6 nm), more flexible, and aggregation-prone particles. The latter were minor in most preparations, but constituted the bulk of the viral population in about 5% of plants. Particles of both types were formed of homologous genomic RNAs and serologically close coat proteins (CPs). Sequence analysis of the 2500 3′-terminal nucleotides of the genomic RNA of type 1 and 2 particles showed 87% homology; the amino acid sequences of the coat and the 15 kDa (OPF6) proteins had a single difference each, whereas the 42 kDa (ORF4) proteins had about 15% changes.

Table 1 Definite and tentative species of the genus *Allexivirus*

Species in the genus	*GenBank accession no*	
Garlic mite-borne filamentous virus	[X98991, AY390254]	GarMbFV
Garlic virus A	[AB010300, F478197]	GarVA
Garlic virus B	[AB010301, F543829]	GarVB
Garlic virus C	[AB010302, D49443]	GarVC
Garlic virus D	[AB010303, AF519572, L38892]	GarVD
Garlic virus E	[AJ292230]	GarVE
Garlic virus X	[AJ292229, U89243]	GarVX
Shallot virus X (397[a])	[M97264, L76292]	ShVX
Tentative species in the genus		
Garlic mite-borne latent virus		GarMbLV
Onion mite-borne latent virus		OMbLV
Shallot mite-borne latent virus		ShMbLV

[a]Number of the CMI/AAB Plant Virus Description (see http://www.dpvweb.net).

Nucleic Acid

Virions contain a single molecule of linear single-stranded RNA (ssRNA), about 9.0 kb in length, with a 3′ poly(A) tract. ShVX RNA preparations, besides genomic ssRNA, contain molecules of 1.5 kb double-stranded RNA (dsRNA), whose genesis and function(s) are unknown. The complete nucleotide sequences of the genomic RNA of ShVX, garlic viruses A (GarVA), C (GarVC), E (GarVE), and X (GarVX), and the partial sequences of the RNA of garlic miteborne filamentous virus (GarMBFV) and garlic virus B (GarVB) and D (GarVD) have been determined.

Proteins

Virions are composed of a single polypeptide of 28–37 kDa. It was reported that the ORF6 protein is a minor component in ShVX virions.

Physicochemical and Physical Properties

The sedimentation constant of the ShVX virion is about 170S (0.1 M Tris-HC1, pH 7.5 at 20 °C). The buoyant density in CsCl is 1.33 g cm^{-3}.

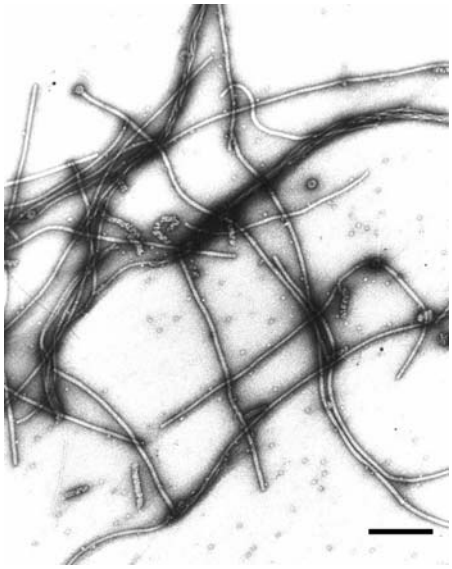


Figure 1 Electron micrograph of ShVX virions. Scale = 200 nm. Courtesy of V. Vishnichenko.

Transmission

Allexiviruses are supposed to be mite-borne; the only vector known is the eriophyd mite *Aceria tulipae,* which was proved to transmit GarVC and GarVD. All allexiviruses are manually transmissible by sap inoculation of healthy host plants. None could be transmitted by aphids or any other insects.

Genome Organization and Replication

The genome organization of allexiviruses resembles that of carlaviruses, with the major exception of an 'additional' ORF between the triple gene block (TGB) and the CP ORF. The genomic RNA of allexiviruses contains six large ORFs and noncoding sequences of about 100 nucleotides at the 5′ terminus and about 100 nucleotides followed by a poly(A) tail at the 3′ terminus (**Figure 2**). Determination of the complete genomic RNA sequence of several allexiviruses proves their genome organization to be almost identical. The type member, ShVX, codes for polypeptides of 195, 26, 11, 42, 28, and 15 kDa, respectively, from the 5′ end to the 3′ end. Gene arrangement of other incompletely sequenced allexiviruses is similar. The 195 kDa polypeptide is a viral RNA polymerase, and most probably it is the only virus-encoded protein required for replication. In comparison of the amino acid sequences of methyltransferase, helicase, or RNA-dependent RNA polymerase (RdRp) motifs, these conserved domains of allexiviruses are most similar to those of potexviruses. The 26 and 11 kDa proteins are similar to the first two proteins encoded by the TGB of potexviruses and carlaviruses and are probably involved in cell-to-cell movement of the virus. There is a coding sequence for a small (7–8 kDa) TGB protein but it lacks the initiation AUG codon. The 42 kDa polypeptide has no significant homology with any proteins known, though it is expressed in plants infected with ShVX in relatively large amounts. Immunoelectron microscopy using polyclonal antisera against the recombinant ShVX 42 kDa protein showed reaction with certain regions of the virions, with the immune complexes nonuniformly distributed along the particle. The 42 kDa protein was supposed to act as a cofactor to provide proper interaction of the CP with the genomic RNA in the virion assembly process. The 28 kDa polypeptide is the CP. In polyacrylamide gel electrophoresis (PAGE)

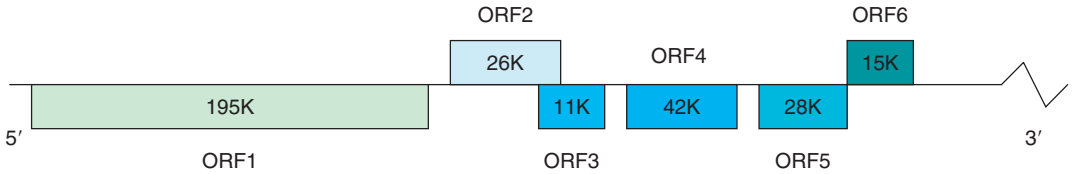


Figure 2 Genome organization of shallot virus X (genus *Allexivirus*).

it migrates as a 32–36 kDa protein, which can be due to its high hydrophilicity evident from amino acid sequence. The 15 kDa protein is similar to the 11–14 kDa proteins encoded by the 3′-proximal ORFs of carlaviruses, and has a zinc-binding-finger motif with affinity for nucleic acids. The exact function of this polypeptide is unknown, but it may be involved in virion assembly.

At least two subgenomic mRNAs (sgRNA) are used for translating the 5′-distal ORFs: one for the movement proteins (ORF2 and ORF3) and the other for the CP (ORF5) and the 3′-distal nucleic acid-binding protein (ORF6). Although the ORF4 protein may be expressed in the infected plants in relatively large amounts, no evidence is available for an ORF4-specific sgRNA. It is suggested that the ORF4-encoded protein is translated from the sgRNA for the ORF2 and ORF3 proteins.

Geographical Distribution

Allexiviruses have been identified in Russia, Japan, France, Germany, the UK, The Netherlands, Korea, China, Taiwan, Thailand, and Argentina. Most probably, they are all distributed across the world, especially in the regions where bulbous plants are widespread.

Phylogenetic Information

Phylogenetic trees based on the nucleotide sequence depend on the genome region used for analysis. In the family *Flexiviridae*, the allexiviruses in general occupy an intermediate position in putative phylogeny between carlaviruses, potexviruses, and mandariviruses.

See also: Capillovirus, Foveavirus, Trichovirus, Vitivirus; *Carlavirus*; Cytomegaloviruses: Murine and Other Nonprimate Cytomegaloviruses; Flexiviruses; Vegetable Viruses.

Further Reading

Adams MJ, Antoniw JF, Bar-Joseph M, *et al.* (2004) The new plant virus family Flexiviridae and assessment of molecular criteria for species demarcation. *Archives of Virology* 149: 1045–1060.

Arshava NV, Konareva TN, Ryabov EV, and Zavriev SK (1995) The 42K protein of shallot virus X is expressed in the infected *Allium* plants. *Molecular Biology* (Russia) 29: 192–198.

Barg E, Lesemann DE, Vetten HJ, and Green SK (1994) Identification, partial characterization and distribution of viruses infecting crops in south and south-east Asian countries. *Acta Horticulturae* 358: 251–258.

Chen J, Chen J, and Adams MJ (2001) Molecular characterization of a complex mixture of viruses in garlic with mosaic symptoms in China. *Archives of Virology* 146: 1841–1853.

Kanyuka KV, Vishnichenko VK, Levay KE, Kondrikov DYu, Ryabov EV, and Zavriev SK (1992) Nucleotide sequence of shallot virus X RNA reveals a 5′-proximal cistron closely related to those of potexviruses and a unique arrangement of the 3′-proximal cistrons. *Journal of General Virology* 73: 2553–2560.

Song SI, Song JT, Kim CH, Lee JS, and Choi YD (1998) Molecular characterization of the garlic virus X genome. *Journal of General Virology* 79: 155–159.

Sumi S, Tsuneyoshi T, and Furuntani H (1993) Novel rod-shaped viruses isolated from garlic, possessing a unique genome organization. *Journal of General Virology* 74: 1879–1885.

Van Dijk P and van der Vlugt RA (1994) New mite-borne virus isolates from Rakkyo, shallot and wild leek species. *European Journal of Plant Pathology* 100: 269–277.

Vishnichenko VK, Konareva TN, and Zavriev SK (1993) A new filamentous virus in shallot. *Plant Pathology* 42: 121–126.

Vishnichenko VK, Stelmashchuk VY, and Zavriev SK (2002) The 42K protein of the Shallot virus X participates in formation of virus particles. *Molecular Biology* (Russia) 36: 1080–1084.

Yamashita K, Sakai J, and Hanada K (1996) Characterization of a new virus from garlic (*Allium sativum* L.), garlic mite-borne virus. *Annals of Phytopathology Society of Japan* 62: 483–489.

Alphacryptovirus and *Betacryptovirus*

R Blawid, D Stephan, and E Maiss, Institute of Plant Diseases and Plant Protection, Hannover, Germany

© 2008 Elsevier Ltd. All rights reserved.

History

The common symptoms induced by plant virus infections are most easily recognized as mosaics, mottles, and ringspots. However, in 1968 and 1969, Pullen showed that all apparently healthy plants of seven beet species (*Beta* sp.) contained isometric virus-like particles (VLPs) of *c.* 29–30 nm in diameter with unusual properties. The particles could not be transmitted to other herbaceous plants and were not eliminated by heat therapy. At that time VLPs were supposed to be of plant origin until a few beet plants were found not containing VLPs. In 1977, Kassanis *et al.* rediscovered these virus particles and showed that they were present in 90% of commercially grown sugar beet plants. They referred to the VLPs as beet cryptic virus (BCV) and were the first to develop a purification method of BCV particles. Nevertheless, evidence from serology, analytical ultracentrifugation, and CsCl isopycnic centrifugation suggested that more than one type of particles might have been present. This was the first time that the name 'cryptic' virus was suggested. The name 'cryptic' comes from Greek and means 'hidden, secret' as no symptom can be visualized from plants carrying cryptic viruses.

During the 1980s, two other VLPs were discovered, with properties similar to those of BCV. These were named vicia cryptic virus (VCV) and carnation cryptic virus (CarCV). In 1978, Kenten *et al.* showed that VCV was up to 88% seed borne in some *Vicia faba* cultivars but was not transmitted to VLP-free plants by aphids, dodder, sap inoculation, or grafting. Furthermore, VCV was not eliminated from seedlings by heat treatment. In field trials, VCV infection had little or no effect on plant growth and yield. Abou-Elnasr *et al.* purified isometric particles of VCV from *Vicia faba* seedlings. In 1981, Lisa *et al.* identified the nucleic acids of CarCV as double-stranded RNA (dsRNA) using serological methods, electron microscopy, and DNase and RNase treatments. In 1988, Marzachi *et al.* purified CarCV particles, which subsequently were shown to possess RNA-dependent RNA polymerase (RdRp) activity. Analysis of the RNAs synthesized *in vitro* showed that the CarCV putative RdRp enzyme acts as a replicase, catalyzing the synthesis of dsRNA. In the mid-1980s, Natsuaki and colleagues found also VLPs in alfalfa, beet, and white clover that had common characteristics to those referred to as cryptic viruses. These 'temperate' viruses cause little or no symptoms; the virus concentration in plants was often very low and they were not graft-transmitted or mechanically transmissible but highly seed-transmitted, showing 80–100% transmission rates through ovules and pollen to the seed. It became clear that 'temperate' and 'cryptovirus' were belonging to the same group of viruses.

In 1985, Boccardo *et al.* presented striking evidence that cryptic viruses are in fact viruses of plants. They purified from apparently healthy plants white clover cryptic virus (WCCV) 1, WCCV 2, and WCCV 3 with yields of about 200 µg of virus per kg of tissue. After isopycnic centrifugation in CsCl, WCCV 1 and 2 formed homogeneous bands at densities of 1.392 and 1.375 g ml^{-1}, respectively, whereas WCCV 3 was not recovered. Particles of WCCV 1 and 3 were about 34 nm in diameter, whereas WCCV 2 particles were 38 nm in diameter, with prominent morphological subunits. Each of the three viruses contained two segments of dsRNA (molecular weight from 1.03 to 1.49×10^6 Da). Again, particles of WCCV 1 and 2 were neither transmitted by grafting nor by mechanical inoculation. Boccardo *et al.* showed also that the incidence and concentration of WCCV 1 and 2 in white clover plants were unaffected by high levels of systemic and surface fungicides and also that the viruses were already present in minute amounts in plantlets grown from surface-sterilized seeds, indicating that the virus does not reside in fungal hyphae, which are able to colonize the interior and surface of a plant.

Until recently, only little work on the molecular characterization of cryptic plant viruses was done. In 1993, Xie *et al.* determined the nucleotide sequence of the BCV 3 dsRNA 2 that encodes a putative RdRp. In spring 2006, only the complete nucleotide sequences of two assigned members of the genus *Alphacryptovirus*, namely VCV and WCCV 1, were available in GenBank.

Taxonomy and Classification

Cryptic viruses belong to the family *Partitiviridae*, which includes three genera: *Partitivirus*, *Alphacryptovirus*, and *Betacryptovirus*. Viruses of the last two genera in this family infect plants. The *VIII Report of the International Committee on Taxonomy of Viruses* (ICTV) listed 16 species of plant cryptic viruses and ten species that are unassigned members in the genus *Alphacryptovirus*. The genus *Betacryptovirus* contains only four species and one tentative, whereas no nucleotide sequence is available up to now. Cryptoviruses have been found in different plants like, sugar beet, ryegrass, carnation, alfafa, radish, spinach, fire trees, green and brown algae, red pepper, carrot, hop trefoil, and broad beans. **Table 1** shows a list of species in the genera *Alphacryptovirus* and *Betacryptovirus* and tentative species, virus abbreviations, and accession numbers. In addition, **Table 1** shows a list of unassigned plant partitiviruses found in GenBank.

Virion Properties

Viruses with dsRNA genomes are classified into seven families, namely *Birnaviridae*, *Cystoviridae*, *Hypoviridae*, *Partitiviridae*, *Reoviridae*, *Totiviridae*, and *Chrysoviridae*. Only the families *Partitiviridae* and *Reoviridae* include plant-infecting viruses. In addition, only recently the ICTV accepted the *Endornavirus* as a new genus of plant dsRNA virus.

The family *Partitiviridae* is characterized by morphological, physical, and physicochemical properties. All candidate viruses consist of small isometric particles, 37–38 nm in diameter. The virion buyant density in CsCl is in the range of 1.34–1.39 g cm^{-3}. Virions are stable in butanol and chloroform. *In vitro* transcription/replication occurs by a semiconservative mechanism and virions accumulate mainly in the cytoplasm and rarely in nuclei and nucleoli of parenchyma cells.

All cryptic viruses that have been analyzed to date possess at least two classes of dsRNAs with molecular weights between 0.8 and 1.6×10^6 Da. In 1990, Accotto *et al.* purified and characterized from *Medicago sativa* the alfalfa cryptic virus (ACV). The genome of ACV-1 consists of two dsRNAs, one with an estimated molecular weight of 1.27×10^6 Da (RNA 1) and the other of 1.17×10^6 Da (RNA 2). In a different paper, ACV was designated as ACV_M, showing besides the two earlier described dsRNA segments a third larger dsRNA of an estimated molecular weight of 2.70×10^6 Da. The RdRp, able to replicate the genomic dsRNAs *in vitro*, was associated with purified ACV particles. In addition, further examples

Table 1 Definite members (italics) and tentative members (Roman) in the genera *Alphacryptovirus* and *Betacryptovirus*

Genus	*Abbreviation*	*Accession number*
Alphacryptovirus		
Official virus species names		
Alfalfa cryptic virus 1	(ACV-1)	
Beet cryptic virus 1	(BCV-1)	
Beet cryptic virus 2	(BCV-2)	
Beet cryptic virus 3	(BCV-3)	(S63913)
Carnation cryptic virus 1	(CCV-1)	
Carrot temperate virus 1	(CTeV-1)	
Carrot temperate virus 3	(CTeV-3)	
Carrot temperate virus 4	(CTeV-4)	
Hop trefoil cryptic virus 1	(HTCV-1)	
Hop trefoil cryptic virus 3	(HTCV-3)	
Radish yellow edge virus	(RYEV)	
Ryegrass cryptic virus	(RGCV)	
Spinach temperate virus	(SpTV)	
Vicia cryptic virus	(VCV)	(NC_007241)
White clover cryptic virus 1	(WCCV-1)	(NC_006275)
White clover cryptic virus 3	(WCCV-3)	
Tentative virus species names		
Carnation cryptic virus 2	(CCV-2)	
Cucumber cryptic virus	(CuCV)	
Fescue cryptic virus	(FCV)	
Garland chrysanthemum temperate virus	(GCTV)	
Mibuna temperate virus	(MTV)	
Red pepper cryptic virus 1	(RPCV-1)	
Red pepper cryptic virus 2	(RPCV-2)	
Rhubarb temperate virus	(RTV)	
Santosai temperate virus	(STV)	
Betacryptovirus		
Official virus species names		
Carrot temperate virus 2	(CTeV-2)	
Hop trefoil cryptic virus 2	(HTCV-2)	
Red clover cryptic virus 2	(RCCV-2)	
White clover cryptic virus 2	(WCCV-2)	
Tentative virus species names		
Alfafa cryptic virus 2	(ACV-2)	
Unassigned virus in the family		
Pyrus pyrifolia cryptic virus	(PyrR-1)	(AB012616)
Fragaria chiloensis cryptic virus		(DQ093961) Partial sequence
Pepper cryptic virus 1		(DQ361008) Partial sequence
Pinus sylvestris partitivirus NL-2005		(AY973825) Partial sequence
Raphanus sativus cryptic virus 1		(DQ181926) (AY949985)
Raphanus sativus cryptic virus 2		(DQ218036) (DQ218037) (DQ218038)

exist, where three or more classes of dsRNAs have been found, namely from *Beta vulgaris*, *Festuca pratensis*, *Vicia faba*, *Medicago lupulina*, and *Trifolium repens*. These plant species may contain mixtures of two or more viruses.

Genome Organization, Molecular Biology, and Replication

The partitivirus genome consists of two monocistronic dsRNA segment. One open reading frame (ORF) of dsRNA 1 codes for the RdRp, whereas the other of dsRNA 2 codes for the coat protein (CP).

Figure 1 shows a schematic representation of the genome organization of VCV. Sequence analysis of dsRNA 1 revealed two ORFs. The larger ORF initiation codon AUG at nucleotide position 93–95 is probably the start codon of the VCV polymerase. The termination codon UAA is located at position 1941–1943. Therefore, the larger ORF consists of 1848 nucleotides and translates into a protein of 616 amino acids with a calculated molecular mass of 72.9 kDa. In addition, an internal ORF was found which translates into a putative protein with a calculated molecular mass of 18 kDa. No significant hits were obtained using Blastp to any other protein, therefore its synthesis and function in the plant remains unclear.

Sequence analysis of dsRNA 2 of VCV revealed also two ORFs. The larger ORF consists of 1461 nucleotides and translates into a protein of 487 amino acids with a calculated molecular mass of 53.9 kDa. The smaller ORF located on the complementary strand translates into a putative protein with a calculated molecular mass of 16 kDa. Like the small ORF of dsRNA 1, no information is available on its synthesis and function in plants.

Figure 2 shows the 5′ nontranslated region (NTR) of the dsRNA 1 and dsRNA 2 of VCV and WCCV 1. The 5′ NTR consists of 92 nucleotides up to the initiation codon of the RdRp ORF, which is longer in comparison with the 5′ NTR of WCCV 1. The 5′ NTRs of VCV and WCCV 1 genomes share 52 nucleotides that are conserved between both genomes (**Figure 2**).

One interesting feature concerning the NTR of several cryptic viruses is the presence of 'cytosine-adenine-adenine (CAA)' repeats, which are thought to be associated with initiation and enhancement of translation. It appears that the 5′ end conserved regions are involved in replication and packaging of the respective dsRNAs as well as in translation of the RdRp and CP genes. These 'CAA' repeats are highly conserved and can be found in RdRp and/or CP 5′ NTRs of partitiviruses like gremmeniella abietina virus, heterobasidion annosum virus, discula destructiva virus 2, and fusarium poae virus 1. Many partitiviruses contain interrupted poly(A) tails at their 3′ ends. The VCV dsRNA 1 reveals 33 A residues out of 40 bases at its 3′ end and the dsRNA 2 34 A residues. The polyadenylation signal AAUAAA was identified in the 3′ NTRs of both VCV dsRNAs.

The calculated molecular mass of RdRps of viruses belonging to the genus *Alphacryptovirus* varies from 55 to 73 kDa. Amino acid comparisons of RdRp's from plant cryptic viruses showed 12 different conserved motifs. Five slightly different motifs out of the eight standard motifs found in RdRp sequences of single-stranded and double-stranded RNA viruses are present in the RdRp's of cryptic viruses: motif I 'L', motif III 'KXR3XG', motif IV 'DW2XFD', motif V 'SG3XT4XS2XN', and motif VI 'GDD'.

Currently, there are not many nucleotide sequences of plant cryptic virus CPs available at the GenBank. A Blast search using the CP of VCV identified no well-known conserved domains. However, multiple alignments using CPs of different partitiviruses revelead two major conserved domains (SQLY and PGPL3XF), but it is still unknown if these motifs have any essential function for the virus. Even if there is little information on how replication and encapsidation of plant cryptic viruses occur,

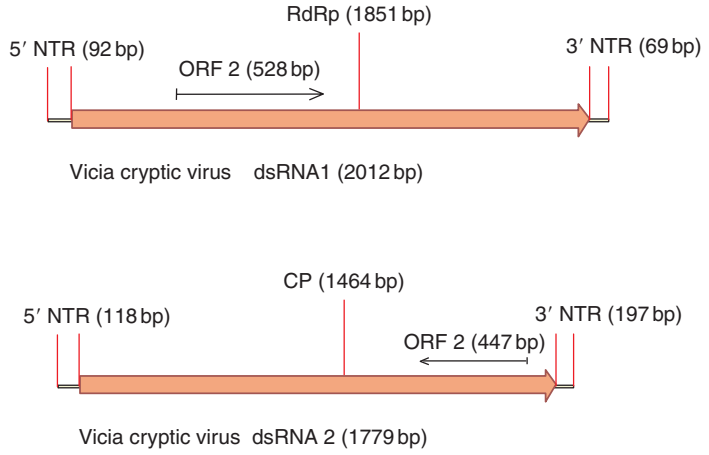


Figure 1 Genome organization of vicia cryptic virus (VCV). The RdRp ORF and the CP ORF are represented by orange arrows. Thin black arrows indicate putative ORFs.

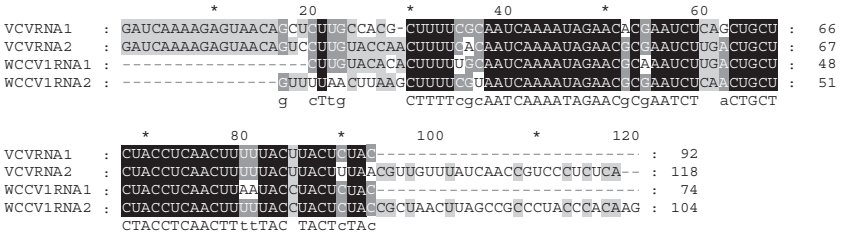


Figure 2 Alignment of the 5′ NTRs of the dsRNA 1 and dsRNA 2 of VCV and WCCV 1. Adapted from Blawid R, Stephan W, and Maiss E (2007) Molecular characterization and detection of Vicia cryptic virus in different *Vicia faba* cultivars. *Archives of Virology* 152(8): 1477–1488.

CsCl buoyant density values may indicate that only one dsRNA molecule can be encapsidated in each particle. The genome replication is catalyzed by the virus-encoded RdRp. It is very likely that plant cryptic viruses replicate inside the capsid, as known for others dsRNA viruses, to protect themselves from dsRNA-induced host defense mechanisms. RdRp activity has been associated with purified VLPs of CarCV, WCCV, and ACV. But it is still not clear if the same RdRp that acts in viral replication and assembly is also responsible for transcription. It is likely that plant factors are also involved in the process of transcription of plant cryptic viruses. The model of replication and transcription of plant cryptic viruses might be very similar to those described for members of the genus *Partitivirus* that infect fungi. In this model, a newly synthesized positive strand displaces the parental strand (semiconservative replication). The RdRp catalyzes the synthesis of newly negative strands that later will serve as template for synthesis of the positive strand.

Transmission

In the past, many unsuccessful attempts have been made to transmit cryptic viruses either by mechanical inoculation or by grafting to virus-free plants. Attempts to transmit BCV by mechanical inoculation of sugar beet sap to healthy sugar beet or other plant species like *Nicotiana tabacum* cv. Xanthi-nc, *Nicotiana clevelandii*, *Cucumis sativus*, *Petunia hybrida*, and *Chenopodium* ssp. failed. In addition, different cultivars of *Vicia faba* (Beryl, Maris Bead, and Minica) that were not containing VCV dsRNA were inoculated with sap from plants containing VCV. No VCV dsRNA could be extracted from these inoculated plants. Experiments involving transmission of WCCV to different plant species (*Chenopodium*, *Glycine max*, *Gomphrena globosa*, *N. clevelandii*, *N. tabacum*, and *Vigna unguiculata*) by grafting or mechanical inoculation also failed.

However, experiments involving BCV transmission through seed were successful. It was shown that cryptic viruses are transmissible through pollen as well as through the female plant. Cross-pollination experiments performed by Kassanis *et al.*, Kenten *et al.*, and Natsuaki *et al.* demonstrated that the percentage of progeny infection differed if the cryptic virus was obtained from the female parent or from the pollen donor. The progenies derivate from female carriers were presenting higher infection percentages (80% carrying BCV) in comparison to those originated from pollen carriers (40%).

Virus–Host Relationships

It is known that cryptic viruses do not induce symptoms in their hosts. Plants carrying cryptic viruses are symptomless. However, in 2005, Nakatsukasa-Akune *et al.* reported that TrEnodDR1 encoding the CP gene of WCCV 1 expressed in *Lotus japonicus* after *Agrobacterium* spp. transformation increased the concentration of endogenous abscisic acid and thereby suppresses root nodulation. Plant cryptic viruses can affect the growth and development of the host plant in mixed infections. In 1989, Kühne and Stanarius measured the interaction between mixed infections of BCV 2 and beet mild yellowing virus (BMYV) as well as BCV 1 and beet yellows virus (BYV). The amount of BCV 2 increased significantly 23 weeks after infection with BMYV. The same was observed if BCV 1 carrying plants were co-infected with BYV. Similar results are reported for ryegrass mosaic virus (RgMV) and ryegrass cryptic virus (RGCV) mixed infection, meaning that RGCV particle concentration increased after infection with RgMV.

As cryptic viruses are present in very low concentrations in plants, localization in different plant tissues is difficult. Kühne and Stanarius observed that BCV 1 was present mainly in the mesophyll, whereas BVC 2 was found in the vascular bundle. In addition, BCV 1 and BCV 2 might occur separately or together in different cultivars of *Beta vulgaris* L. Many different plant cultivars of carnation, white clover, radish, and *Lolium* species were found to be free or containing only sparse amounts of cryptic virus particles. Abou-Elnasr group detected dsRNAs from VCV in extracts from leaves, stems, roots, flowers, seeds, and mesophyll protoplasts from different *Vicia* cultivars. Although they could extract dsRNA from stems and flowers, we were not able to extract dsRNA from stems of *Vicia faba* 'Hangdown'. It is still uncertain, if cryptic viruses are present in all organs and tissues of their hosts in equal amounts. However, it is clear that different serological unrelated cryptic viruses might be present in the same plant species.

No serological relationship has been detected between cryptic viruses isolated from plants in different families, but relationships have been found between cryptic viruses in related host species. Thus, an antiserum to RYEV did not react with ACV, BCV 1, CTeV, GCTV, MTV, RTV, STV, and SpTV (for abbreviations, see **Table 1**). Other reports show that cryptic viruses from plant species belonging to different families are not serologically related, for example, WCCV 1 and WCCV 2 did not react when tested with antisera to CarCV and RCV. Even CarCV was not detected with antisera obtained with BCV and RCV as well as many others. In contrast, serological relationships have been demonstrated between HTCV1 and ACV1, between HTCV2 and RCCV2, and finally between HTCV 3 and VCV. In addition, cryptic viruses from red clover appeared to be serologically related to WCCV 1 and WCCV 2.

Evolutionary Relationships

During cloning and analysis of the VCV genome, seven different cultivars ('Hangdown', 'Dreifach Weisse', 'Frühe Weisskeimige', 'Osnabrück', 'The Sutton', 'Divine', and 'Major') were investigated for the presence of VCV. dsRNA molecules were extracted from all cultivars and the presence of RdRp and CP of VCV was confirmed by reverse transcription-polymerase chain reaction (RT-PCR) in all cultivars, with exception of the cultivar 'Major'. For a better understanding of the virus–host relationship between VCV and *Vicia faba* plants, we sequenced the complete ORFs of VCV RdRp and CP from four different cultivars of *Vicia faba*. Interestingly, comparisons based on nucleic acid as well as amino acid level showed a high degree of identity (98–99%), indicating that these cultivars are infected probably by one virus, which may have a common cryptic virus ancestral, thereby demonstrating the high evolutionary relationship between VCV and *Vicia faba* varieties.

VCV RdRp shows high amino acid identity (84%) to WCCV 1, also a member of the genus *Alphacryptovirus*. In 2005, Chen *et al.* isolated dsRNA from radish plants. Although radish yellow edge virus (RYEV) and raphanus sativus cryptic virus 1 and 2 were isolated from the same plant species (*Raphanus sativus*), no significant similarity was found between the deduced amino acid sequence of the putative RdRp's from raphanus sativus cryptic virus 1 (RasR 1) or raphanus sativus cryptic virus 2 to VCV or WCCV 1 (**Figure 3**). A Blastp search using RasR 1 delivered an identity of 51% and 32% to the RdRp of helicobasidium mompa virus V1–2 and WCCV 1, respectively. However, this might change when more plant partitiviruses will be characterized at the molecular level. Osaki *et al.* detected dsRNA from Japanese pear (*Pyrus pyrifolia*) that presented conserved RdRp motifs found in genes that encode putative RdRp of RNA viruses. Amino acid analysis revealed an identity of 53% with BCV 3 dsRNA 2 and 38% with raphanus sativus cryptic virus 2. In 2006, Veliceasa *et al.* isolated dsRNA from *Pinus sylvestris*. Analysis of partial amino acid sequences showed 41% and 40% of identity to the unassigned pyrus pyrifolia cryptic virus and BCV 3, respectively.

RdRp's and CPs of VCV, WCCV1, and raphanus cryptic virus 1 form a separate branch within the *Partitiviridae* (**Figures 3** and **4**). However, the question of the origin of cryptic viruses and their relationship to fungal infecting viruses still remains open. Further molecular cloning and characterization of cryptic viruses might help in obtaining a better view on their relatedness. Therefore, the current phylogenetic trees based on the amino acid sequences of RdRp's and CPs may change in future when additional sequence information becomes available, resulting probably in a different relationship among these viruses.

See also: Partitiviruses: General Features; Partitiviruses of Fungi.

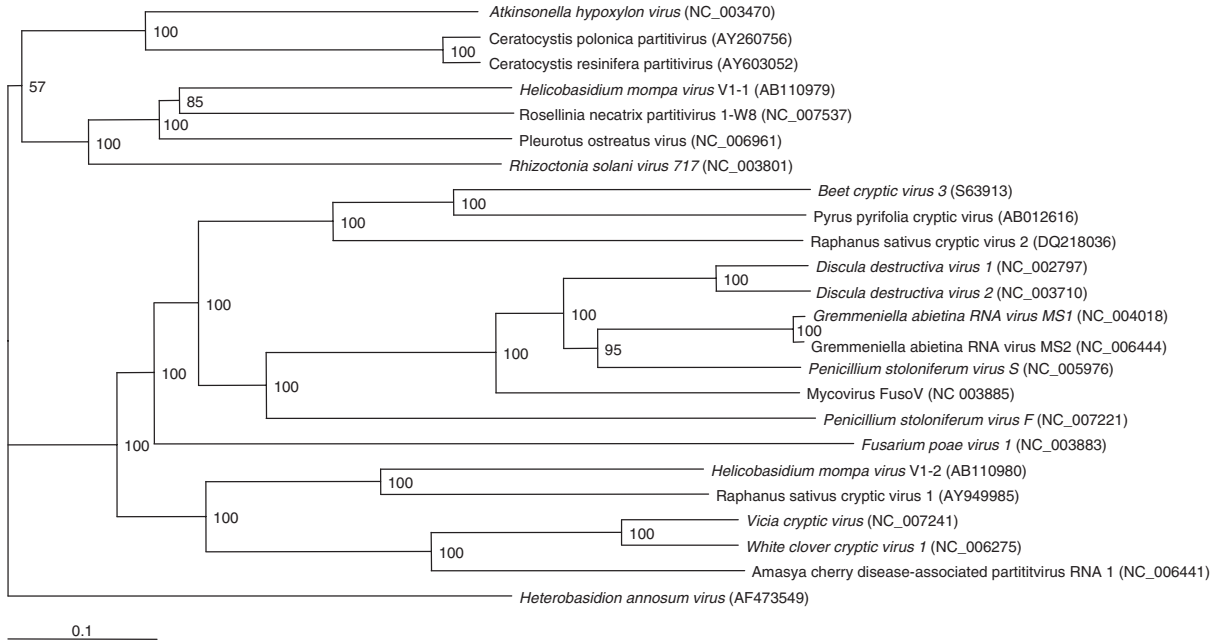


Figure 3 Phylogenetic tree based on RdRp amino acid sequences of definitive members (italics) and tentative members (Roman) of the family *Partitiviridae*. Numbers at nodes indicate bootstrap values for 100 replicates. Accession numbers are given in brackets.

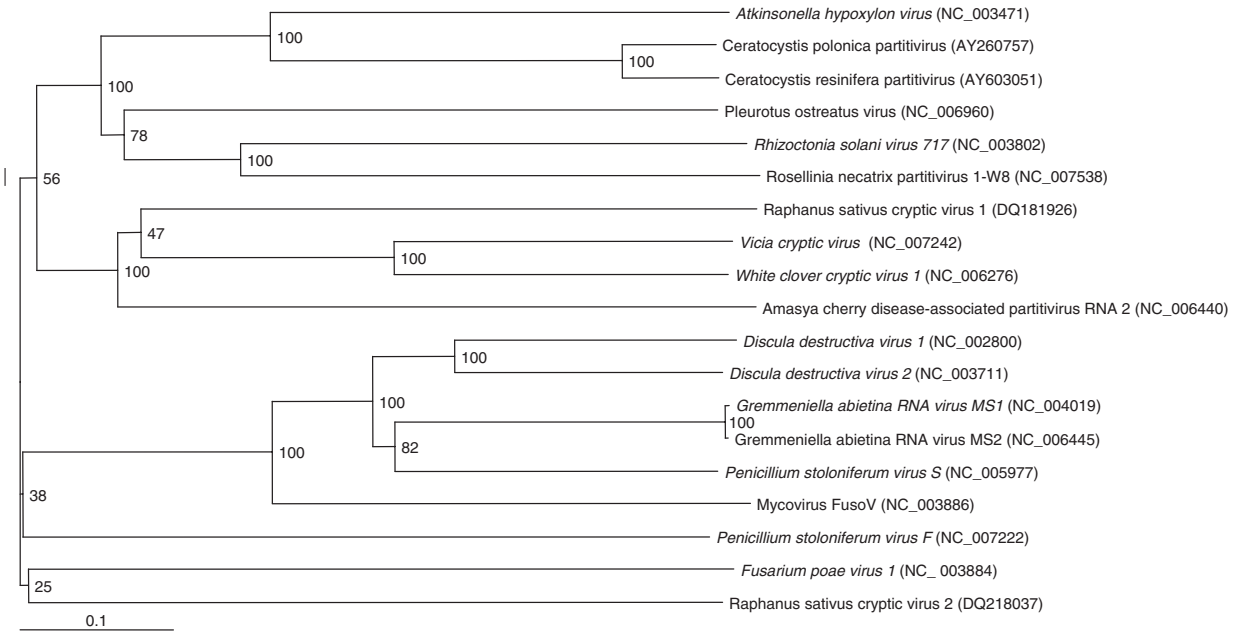


Figure 4 Phylogenetic tree based on CP amino acid sequences of definitive members (italics) and tentative members (roman) of the family Partitiviridae. Numbers at nodes indicate bootstrap values for 100 replicates. Accession numbers are given in brackets. Adapted from Blawid R, Stephan W, and Maiss E (2007) Molecular characterization and detection of Vicia cryptic virus in different *Vicia faba* cultivars. *Archives of Virology* 152(8): 1477–1488.

Further Reading

Antoniw JF, White RF, and Xie WS (1990) Cryptic viruses of beet and other plants. In: Frasser RSS (ed.) *Recognition and Response in Plant–Virus Interactions*, pp. 273–285. Heidelberg: Springer.

Boccardo G and Candresse T (2005) Complete sequence of the RNA 1 of an isolate of White clover cryptic virus 1, type species of the genus *Alphacryptovirus*. *Archives of Virology* 150: 399–402.

Boccardo G and Candresse T (2005) Complete sequence of the RNA 2 of an isolate of White clover cryptic virus 1, type species of the genus *Alphacryptovirus*. *Archives of Virology* 150: 403–405.

Boccardo G, Lisa V, Luisoni E, and Milne RG (1987) Cryptic plant viruses. *Advances in Virus Research* 32: 171–214.

Chen L, Chen JS, Liu L, *et al.* (2006) Complete nucleotide sequences and genome characterization of double-stranded RNA 1 and RNA 2 in the *Raphanus sativus*-root cv. *Yipinghong*. *Archives of Virology* 151: 849–859.

Alphaviruses *See:* Togaviruses: Alphaviruses.

Anellovirus

P Biagini and P de Micco, Etablissement Français du Sang Alpes-Méditerranée, Marseilles, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Apoptosis Mechanism that allows cells to self-destruct when stimulated by the appropriate trigger.
Hepatitis Disease or condition marked by inflammation of the liver.
Rhinitis Inflammation of the mucous membrane of the nose.
Tamarin American monkey that is related to the marmoset.
Tupaia Small mammal native to the tropical forests of Southeast Asia, also known as the tree shrew.
Unassigned genus Genus that is not assigned taxonomically to any existing virus family.

Introduction

In 2004, the International Committee on Taxonomy of Viruses (ICTV) officially created the genus *Anellovirus* (from latin 'Anello', the ring) to accommodate circular single-stranded DNA viruses isolated from humans and some other animal species. The genus *Anellovirus* is an unassigned genus and its members are thereby distinguished from viruses belonging to other families with circular single-stranded DNA genomes that infect bacteria, plants, and vertebrates, such as circoviruses, nanoviruses, and geminiviruses.

The genus *Anellovirus* officially includes one species *Torque teno virus* (TTV), one tentative species Torque teno mini virus (TTMV) and several yet unclassified animal viruses. Recently, a third group of anelloviruses infecting humans was further identified and called 'small anellovirus' in anticipation of its official designation.

This group of viruses is characterized by a very high genomic variability, a high prevalence in human populations, a still unknown significance for host health and the absence of well-defined mode(s) of transmission.

Historical Overview of Anelloviruses

TTV was the first virus with circular single-stranded DNA genome identified in humans. It was initially discovered in 1997 by means of a subtractive technique (representational difference analysis, RDA) in the serum of a Japanese patient (initials T.T.) with post-transfusion non-A–G hepatitis. The short nucleotide sequence obtained (~500 nt) was initially extended to ~3700 nt; subsequently, the resolution of an additional GC-rich region of about 120 nt permitted to complete the TTV genome sequence and highlighted the circular nature of the viral genome.

TTMV was discovered at the end of 1999 during a study of TTV prevalence in blood donors. Some unexpected amplification products were sequenced and identified as highly divergent when compared to known TTV sequences. A circular genome of about 2900 nt was further characterized and initially designated TTV-like mini virus (TLMV) by analogy with TTV. Following a taxonomic re-evaluation of the status of this virus, it has been officially named TTMV by ICTV.

A putative third member of the genus *Anellovirus*, the 'small anellovirus', has been identified in 2005 using a sequence-independent polymerase chain reaction (PCR) amplification method. Two highly divergent circular sequences (~2200 and ~2600 nt) were initially characterized in human plasma samples by this approach.

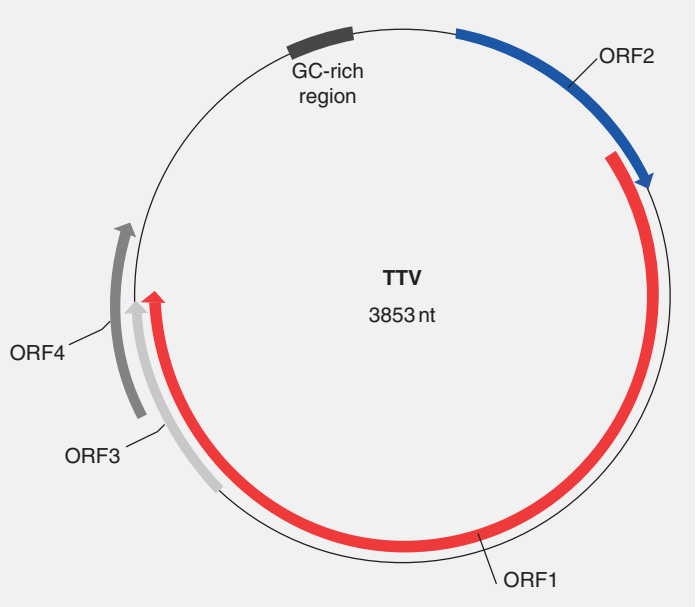
Using a primer system located on a relatively well-conserved region of the genome, extremely divergent anellovirus sequences were also characterized in several animal species: examples were found in cats (~2100 nt), in dogs (~2800 nt), in pigs (~2900 nt), and in tupaia (~2200 nt). Circular genomes, highly divergent or similar to those identified in humans, were also identified in nonhuman primates.

Virion Properties, Genome, and Replication

Members of the genus *Anellovirus* are nonenveloped viruses, with an estimated diameter of about 30–32 nm for TTV and slightly less than 30 nm for TTMV. The buoyant density of virions in CsCl is 1.31–1.33 g cm^{-3} for TTV and 1.27–1.28 g cm^{-3} for TTMV. The genomes of anelloviruses are negative stranded. This has been demonstrated by hybridization studies using sense or reverse nucleic acid probes. They are hydrolyzed by DNase I and Mung Bean nuclease as well.

The genome organization shows: (1) a coding region with at least two main open reading frames (ORF1 (long) and ORF2 (short)) deduced directly from the nucleotide sequence, generally overlapping, and (2) a noncoding region containing a GC-rich zone that forms a stem–loop structure (**Figure 1**). Respective sizes differ widely depending on the isolates studied. On the basis of the TTV prototype 1a, the coding region is about 2600 nt long, with the ORF1 and ORF2 composed of 770 and 202 amino acids respectively, while the noncoding region is composed of about 1200 nt with a short zone (~110 nt) of high GC content (~90%). Sequences analysis reveals a variable G+C content for anelloviruses: calculated values from full-length sequences are ~52%, ~38%, and ~39% for TTV, TTMV, and the 'small anellovirus', respectively.

The replication mechanism of anelloviruses is not well known. However, some studies highlighted the presence of TTV mRNA forms and double-stranded DNA in bone marrow and in various human tissues and organs (including the liver), suggesting an active replication in these locations. The presence of double-stranded DNA forms would suggest, as for other circular single-stranded DNA viruses, a rolling-circle mechanism for replication. Three types of TTV mRNAs (2.9, 1.2, and 1.0 kbp), generated by an alternative splicing, have been detected in bone marrow cells and were also obtained following transfection of a permuted whole-genome into African green monkey COS cells. Identification of these mRNAs permitted to establish the functionality of both ORF1 and ORF2 and confirmed the implication of additional ORFs. Transfection into the 293 cell line (human embryonic kidney cells) of a full-length TTV clone not only confirmed the existence of the three mRNA classes but also further demonstrated the expression of six different proteins following an alternative translation strategy. Importance of the untranslated region (UTR) of TTV was highlighted by the identification of a basal promoter ~110 nt upstream the transcription initiation site,



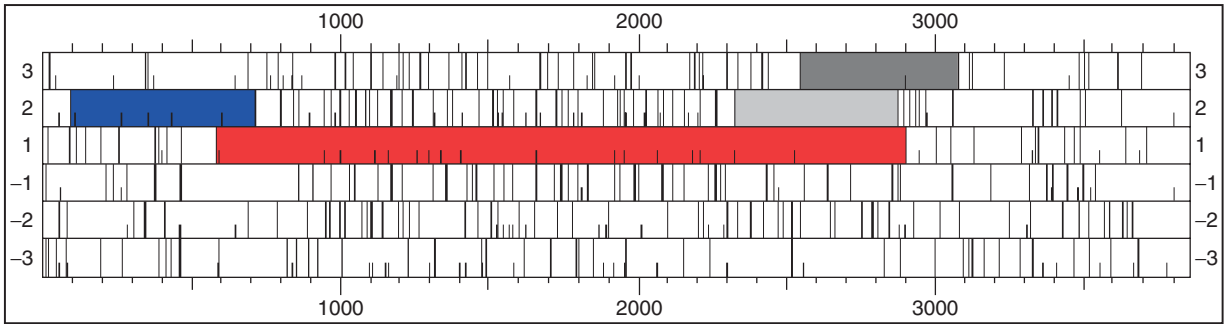


Figure 1 Genome organization and ORF map of *Torque teno virus* prototype (isolate TTV1a).

and by the presence of enhancer elements in a ~490 nt region upstream this promoter.

The absence of a tissue culture system that would support efficient replication of anelloviruses hindered for a long time the studies of virus–host cell interactions. Interestingly, a human liver cell line (the Chang Liver cell line) does support TTV replication following infection with contaminated sera, and releases significant and persistent levels of infectious viral particles into culture fluid supernatants.

Information concerning the functions of the various proteins expressed during the anellovirus cell-cycle infection is fragmentary. The ORF1 might encode a single polypeptide combining structural and functional roles, that is, the capsid and replication functions of the virus. The presence of conserved motifs related to the Rep protein in the ORF1 would confirm the involvement of a rolling-circle mechanism for the anellovirus replication. Expression studies identified TTMV ORF2 as a dual-specificity protein phosphatase which could be involved in mechanisms of immune evasion and virus persistence. The TTV ORF3 gene would also generate two variants of a protein with a different serine-phosphorylation state which could be involved in the virus-replication cycle. Finally, a putative TTV protein located upstream the ORF1 was shown to induce apoptosis in hepatocellular carcinoma-derived cells.

Phylogenetic and Taxonomic Aspects

The genetic diversity among anelloviruses is far larger than within any other defined group of ssDNA viruses. The considerable genetic heterogeneity is exemplified by the large number of highly divergent full-length sequences progressively identified as TTV, TTMV, and 'small anellovirus' genomes.

Historically, primer extension of the initial sequence (~500 nt, N22 clone) to about 3700 nt (TA278 clone) primarily suggested a distant resemblance of TTV to parvoviruses, based on the apparent linear nature of the characterized genome. The circular nature of the genome

was subsequently elucidated (TTV-1a clone), leading to the possible assignment of TTV members to the families of viruses possessing a circular single-stranded DNA genome. This initiated studies on circular single-stranded DNA viruses infecting humans.

Concomitant comparisons of short nucleotide sequences obtained by PCR in the N22 region allowed to identify three distinct genotypes (differing by 27–30% nucleotide divergence) describing TTV genetic diversity in early 1999. The progressive characterization of partial and complete nucleotide sequences had not only demonstrated the existence of a large number of genotypes but has also allowed to classify these genotypes into five distinct clusters (~50% nucleotide divergence) representing the TTV major phylogenetic groups, as defined in 2002 (**Figure 2**). The creation of the genus *Anellovirus* by ICTV in 2004 has officially presented such classification, but it is possible that the next ICTV report will bring significant changes to the taxonomic status of anelloviruses, such as the creation of a specific family hosting several genera accommodating many species, and modify phylogenetic clusters due to the description of new genomic sequences.

Despite the fact that genomic sequences from TTMV and 'small anellovirus' are not as well described as those of TTV, they revealed a high genetic heterogeneity, at least of the same magnitude of that identified for TTV or greater. In 2005, the available full-length TTMV sequences clustered in four major phylogenetic groups (~40% nucleotide divergence), whereas the only two 'small anellovirus' complete sequences described exhibited a nucleotide divergence reaching 46%. Extremely divergent isolates (as compared to human TTV, TTMV, and 'small anellovirus') have been also identified in nonhuman primates and low-order mammals.

A low degree of sequence homology exists between TTV, TTMV, the 'small anellovirus', and animal isolates. However, there is a ~130 nt long sequence that is relatively well conserved between the viral groups, which is located within the UTR downstream of the GC-rich region (**Figure 3**). Moreover, the genome organization of anelloviruses globally appears similar. It includes a coding region containing at least two main ORFs, and a UTR generally having a GC-rich stretch. The respective sizes of each of these components is however variable between isolates.

Accurate phylogenetic analyses inside each viral group are feasible by comparing full-length ORF1, and to a lesser extent ORF2 nucleotide sequences. By contrast, phylogenetic analysis of short nucleotide sequences located in the N22 region or on the UTR proved to be unreliable. The latter approach is also biased by the occurrence of recombination events which are statistically more frequent in this location than in the rest of the genome.

The amino acid sequence comparisons of translated ORF1 or ORF2 proved to be reliable for phylogenetic analyses, and is the only approach for taxonomic studies combining all *Anellovirus* members which markedly differ in sequence and size. Such comparisons at the amino acids level have also highlighted that most, if not all, of the anelloviruses possess an ORF1 with an arginine-rich N-terminal part and motifs related to the Rep protein, while the ORF2 exhibits the well-conserved motif $WX_7HX_3CX_1CX_5H$. Interestingly, the same features are found in the chicken anemia virus (CAV), the type species of the genus *Gyrovirus* of the family *Circoviridae*. CAV possesses a negative-sense genome, a similar genome organization to anelloviruses, with overlapping ORFs, and several viral proteins with functions supposedly similar to their counterparts in members of the genus *Anellovirus*.

Distribution, Epidemiology, and Transmission

In 1997, the identification of TTV DNA in the blood of Japanese patients with hepatitis of unknown etiology was the starting point of the research on this new group of viruses. Despite its initial identification in populations with liver disorders, further epidemiological studies not only identified the virus in populations with parenteral risk exposure, including intravenous drug users, hemophiliacs, or HIV infected patients, but also in populations without proven pathology like blood donors. It was also clearly demonstrated that TTV is distributed worldwide, as the virus was detected in rural or urban populations in Africa, Americas, Asia, Europe, and Oceania.

Due to the enormous genetic variability characterizing anelloviruses, the choice of the viral DNA target for PCR amplification proved to be highly important for the sensitivity of PCR assays. It was in direct correlation with the progressive determination of full-length genomic sequences and the identification of highly conserved regions between viral isolates. So, early estimated prevalence values for TTV DNA ranged from 1% to 5% in the general population but increased dramatically within 1 year to ~80% in blood donor cohorts, revealing a wide and intriguing dispersion of anelloviruses in human populations without any apparent disease. Higher prevalence values (~90%) are generally identifiable in cohort of patients with health disorders, such as cancer, diabetes mellitus, HIV infection, or under hemodialysis treatment. It was also shown that the prevalence of viremia increased slightly with age. Data relating to TTMV and the 'small anellovirus' tend to reveal similar features concerning their prevalence in human populations.

Subsequent information concerning anellovirus infection in humans has been obtained by the analysis of the distribution of the five major TTV phylogenetic groups in blood donors: interestingly, it revealed a nonrandom pattern of group distribution and a predominant prevalence

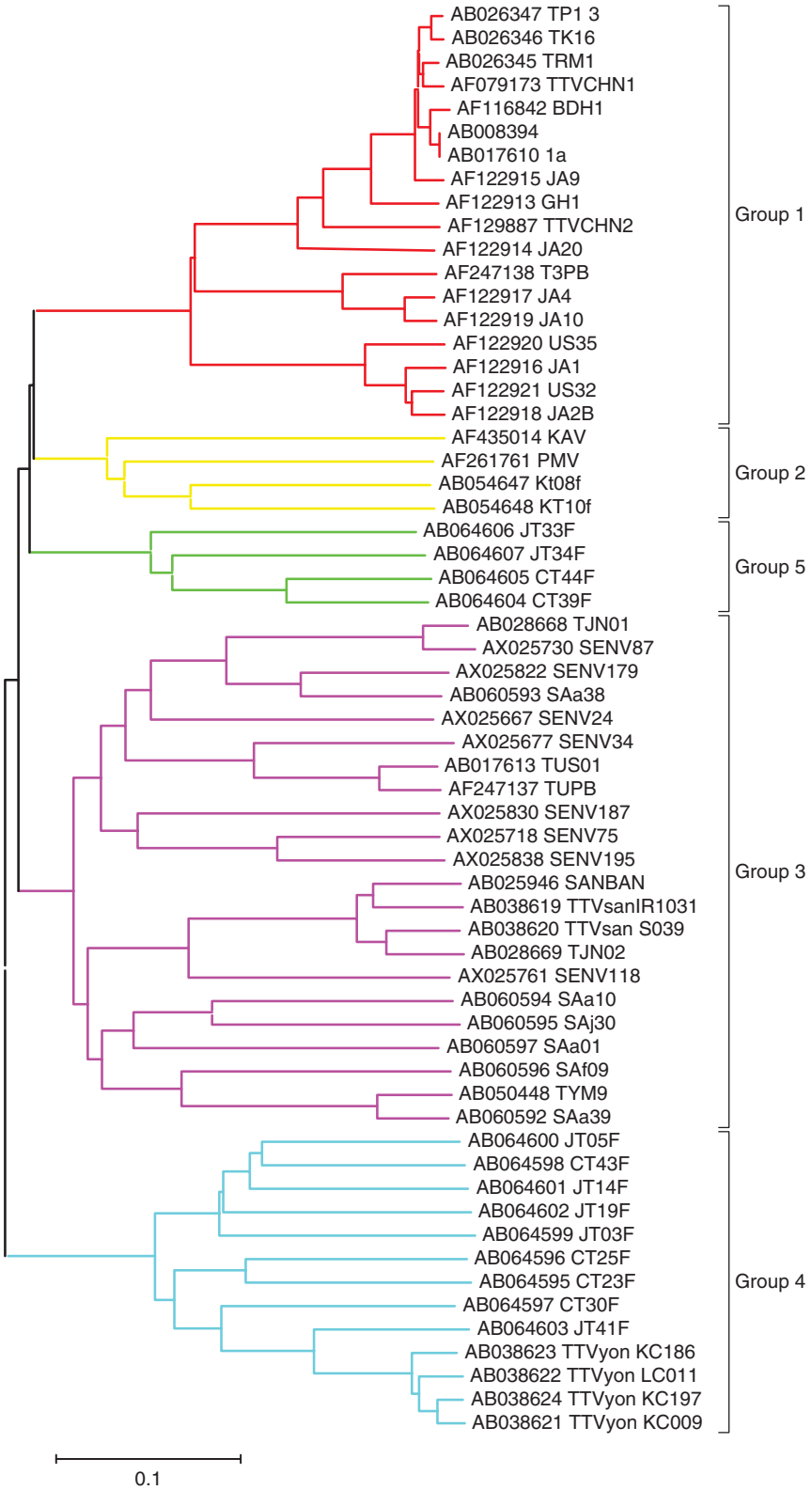


Figure 2 Phylogenetic relations among members of species *Torque teno virus* (neighbor-joining tree built with full-length nucleotide sequences).



Figure 3 Alignment of the nucleotide sequence of TTV, TTMV, and 'small anellovirus' representative isolates identified in humans (conserved zone, UTR region).

of phylogenetic groups 1, 3, and 5 in Brazilian and French cohorts. For the latter, the first study of TTMV distribution revealed a predominance of phylogenetic group 1 over the three other groups considered.

Mixed infections appeared as an important characteristic of the *Anellovirus*–host relationship. The possible co-infection of an individual by multiple viral strains of TTV, TTMV, or 'small anellovirus', even highly divergent, is frequent. As demonstrated for TTMV with the analysis of multiples clones of a single PCR, intra-individual viral genetic diversity could reach values above 40%.

Initially described in plasma and serum samples, TTV, TTMV, and 'small anellovirus' DNA have been subsequently isolated in various biological specimens such as peripheral blood mononuclear cells (PBMCs), saliva and nasal secretions, feces, and in other body fluids including semen and tears. Identification of anelloviruses in breast milk, in cord blood samples, and in the blood of newborns, was also described.

These data would suggest that the high rates of prevalence of anelloviruses in various body fluids of healthy subjects might be due to a possible transfusion, fecal–oral, saliva droplet, sexual, or mother-to-child route(s) of transmission.

The presence of anelloviruses members is not limited to human hosts since they were also detected in nonhuman primates such as chimpanzees and tamarins, in tupaias, in popular pets like cats and dogs, and in domesticated farm animals such as pigs and cows. Viral genomic sequences identified in these hosts were either highly divergent or very similar when compared to those obtained from humans. The fact that TTV partial sequences already identified in human blood were found in the blood of farm animals suggests that interspecies transmission could occur. In the same way, intravenous inoculation of TTV-positive human serum or infected fecal supernatant into chimpanzees demonstrated that TTV can be transmitted to primates.

Detection Approaches/Viral Load

Genomic DNA detection has been the mostly developed approach for the identification of anelloviruses in biologic samples. Historically, due to the limited knowledge of TTV genomic sequences, initial PCR systems were restricted to a short portion of the genome in the center of the ORF1. This region was extensively targeted in nested or hemi-nested PCR protocols, but despite improvements in the design of degenerate primers or optimization of the PCR protocols, detection of TTV DNA appeared restricted to certain genotypes and revealed reduced sensitivity when the virus content in samples was low. Extension of the TTV genomic sequence allowed the design of alternative PCR systems located in different parts of the viral genome and their use in combined detection approaches; such strategy permitted to significantly improve the estimated TTV prevalence in the general population. The growing knowledge of virus genome diversity, as revealed by the characterization of highly divergent full-length genomic sequences, permitted to identify a short and relatively well-conserved zone suitable for optimum primer design. The current 'gold' method for viral DNA detection is based on the use of highly conserved primers, genotype-independent, located in the UTR of the genome upstream the ORF1. This PCR system increased the positive detection rates of samples for TTV DNA and also proved to be applicable to the efficient detection of TTMV, 'small anellovirus' DNA, and highly divergent genomes identified in animals as well.

Real-time PCR assays were developed using such conserved PCR systems in order to gain insights on quantitative aspects of viral infection; such an approach demonstrated that plasma viral loads vary widely, ranging generally between $\sim 10^3$ and $\sim 10^8$ genomes ml^{-1} in individuals.

Distinct amplification systems, designed using a representative dataset of nucleotide sequences specific to the major phylogenetic groups of TTV and TTMV, have also been used for the analysis of the genetic distribution of members of the genus *Anellovirus* in some human cohorts, including blood donors.

Alternative strategies in the diagnosis of viral infection were proposed in a few studies describing serologic (IgG and IgM detection tests, Western blot test using partial ORF1 recombinant protein) and *in situ* hybridization approaches.

Clinical Significance

Since their discovery, the question of a possible implication of anelloviruses in a particular disease is still a matter to debate.

Historical presentation of TTV as associated with elevated transaminase levels in post-transfusion hepatitis of unknown etiology suggested that the virus was able to induce non-A–G hepatitis. Therefore, TTV was suspected as a possible cause of some forms of acute and chronic hepatitis and fulminant liver failure, and could be involved in liver diseases. The identification of TTV in the general population seems to refute this interpretation and led to the suggestion that the virus may cause only occasional liver injury, either by the implication of hepatotropic variants or by the presence of host determinants enhancing the pathogenicity of TTV.

The further identification of TTMV and 'small anellovirus' DNA in humans has increased the number of hypotheses concerning the impact of anelloviruses in human health. Hence, based on studies involving patient cohorts with well-defined health disorders, it has been suggested that this class of viruses may also be implicated in various diseases such as pancreatic cancer, systemic lupus eythematosus, idiopathic inflammatory myopathies, or chromosomal translocations. The implication of anelloviruses in respiratory diseases has also been proposed following studies involving cohorts of children suffering from asthma or rhinitis. It has also been suggested that the respiratory tract could be a site of primary infection in young children and a site of continual replication of TTV and related viruses.

Other studies suggested a possible link between the viral load and the immune status of the host because of high TTV or TTMV titers in plasma samples from immunocompromised patients, but this remains hypothetical since the loads of TTV and TTMV can differ extensively among individuals in the general population.

To overcome the limitations of a diagnosis of anellovirus infection based only on highly conserved amplification systems, it has been suggested that it would be useful to compare individuals with specific diseases with a reference population such as blood donors. Such an approach may lead to the recognition of a possible pathogenic role of these viruses.

Alternatively, anelloviruses may be considered as part of the 'normal' human flora.

Further Reading

Bendinelli M, Pistello M, Maggi F, *et al.* (2001) Molecular properties, biology, and clinical implications of TT virus, a recently identified widespread infectious agent of humans. *Clinical Microbiology Reviews* 14: 98–113.

Biagini P, Charrel RN, de Micco P, and de Lamballerie X (2003) Association of TT virus primary infection with rhinitis in a newborn. *Clinical Infectious Diseases* 36: 128–129.

Biagini P, Gallian P, Attoui H, *et al.* (2001) Genetic analysis of full-length genomes and subgenomic sequences of TT virus-like mini virus human isolates. *Journal of General Virology* 82: 379–383.

Biagini P, Gallian P, Cantaloube JF, *et al.* (2006) Distribution and genetic analysis of TTV and TT MV major phylogenetic groups in French blood donors. *Journal of Medical Virology* 78: 298–304.

Biagini P, Todd D, Bendinelli M, *et al.* (2005) Anellovirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press.

Hino S and Miyata H (2007) Torque teno virus (TTV): Current status. *Reviews in Medical Virology* 17: 45–57.

Jones MS, Kapoor A, Lukashov VV, *et al.* (2005) New DNA viruses identified in patients with acute viral infection syndrome. *Journal of Virology* 79: 8230–8236.

Maggi F, Pifferi M, Fornai C, *et al.* (2003) TT virus in the nasal secretions of children with acute respiratory diseases: Relations to viremia and disease severity. *Journal of Virology* 77: 2418–2425.

Nishizawa T, Okamoto H, Konishi K, *et al.* (1997) A novel DNA virus (TTV) associated with elevated transaminase levels in posttransfusion hepatitis of unknown etiology. *Biochemical and Biophysical Research Communications* 241: 92–97.

Okamoto H, Nishizawa T, Kato N, *et al.* (1998) Molecular cloning and characterization of a novel DNA virus (TTV) associated with posttransfusion hepatitis of unknown etiology. *Hepatology Research* 10: 1–16.

Okamoto H, Nishizawa T, Takahashi M, *et al.* (2001) Heterogeneous distribution of TT virus of distinct genotypes in multiple tissues from infected humans. *Virology* 288: 358–368.

Okamoto H, Takahashi M, Nishizawa T, *et al.* (2002) Genomic characterization of TT viruses (TTVs) in pigs, cats and dogs and their relatedness with species-specific TTVs in primates and tupaias. *Journal of General Virology* 83: 1291–1297.

Pifferi M, Maggi F, Caramella D, *et al.* (2006) High torquetenovirus loads are correlated with bronchiectasis and peripheral airflow limitation in children. *Pediatric Infectious Disease Journal* 25: 804–808.

Takahashi K, Hoshino H, Ohta Y, Yoshida N, and Mishiro S (1998) Very high prevalence of TT virus (TTV) infection in general population of Japan revealed by a new set of PCR primers. *Hepatology Research* 12: 233–239.

Takahashi K, Iwasa Y, Hijikata M, and Mishiro S (2000) Identification of a new human DNA virus (TTV-like mini virus, TLMV) intermediately related to TT virus and chicken anemia virus. *Archives of Virology* 145: 979–993.

Animal Rhabdoviruses

H Bourhy, Institut Pasteur, Paris, France
A J Gubala, CSIRO Livestock Industries, Geelong, VIC, Australia
R P Weir, Berrimah Research Farm, Darwin, NT, Australia
D B Boyle, CSIRO Livestock Industries, Geelong, VIC, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Dipterans Insects having usually a single pair of functional wings (anterior pair) with the posterior pair reduced to small knobbed structures and mouth parts adapted for sucking or lapping or piercing.
Hematophagous Feeding on blood.
Homopterans Insects having membranous forewings and hind wings.
Orthopterans Insects having leathery forewings and membranous hind wings and chewing mouthparts.

Introduction

RNA viruses of the family *Rhabdoviridae* comprise arthropod-borne agents that infect plants, fish, and mammals, as well as a variety of non-vector-borne mammalian viruses. The *Rhabdoviridae* presently comprises six genera, and members of three of these genera – *Vesiculovirus*, *Lyssavirus*, and *Ephemerovirus* – have been obtained from a variety of animal hosts and vectors, including mammals, fish, and invertebrates. The remaining three rhabdovirus genera are more taxon-specific in their host preference. Novirhabdoviruses infect numerous species of fish, while cytorhabdoviruses and nucleorhabdoviruses are arthropod-borne and infect plants. Rhabdoviruses are the etiological agents of human diseases that cause serious public health problems. Some of them can also cause important economic loss in plants and livestock. Other than the well-characterized rhabdoviruses that are known to be important for agriculture and public health, there is also a constantly growing list of rhabdoviruses (presently 85), isolated from a variety of vertebrate and invertebrate hosts, that are partially characterized and are still awaiting definitive genus or species assignment.

History and Classification

Commencing in the 1950s, monitoring programs have been established in tropical regions of Africa, America, Southeast Asia, and northern Australia to detect and identify arboviruses of medical or veterinary importance. In Australia, the use of sentinel cattle herds monitoring associated with insect and vertebrate trapping has provided a means of monitoring the ecology of endemic arboviruses that infect livestock and humans, and an early warning system for incursions of exotic arboviruses and insect vectors. In most other regions, these programs have been based on the collection of viruses from terrestrial vertebrates and hematophagous arthropods. As a result, many new and uncharacterized rhabdoviruses have been isolated. The considerable range of likely vectors and hosts and the wide geographical distribution seen among these isolates highlights the diversity of animal rhabdovirus evolution and ecological adaptation.

The isolation of viruses in the early years was primarily performed in suckling mice by intracranial inoculation with clarified supernatant fluid obtained after grinding of pools of collected arthropods. The mice were then observed daily for at least 14 days and those that showed signs of illness were euthanized and the brains were removed for extraction and subsequent passage in mice to amplify the virus. In more recent times, virus isolations have been made by routine propagation through embryonated eggs or mosquito (*Aedes albopictus* C6/36 or *Aedes pseudoscutellaris* AP 61), baby hamster kidney (BHK-21), African green monkey kidney epithelial (Vero), hamster kidney (CER), or swine kidney (PS) cell lines. Monolayers are observed daily until cytopathic effect (CPE) is observed, at which point the cell culture medium is clarified at low-speed centrifugation and aliquots of virus are stored frozen at −70 °C. Generally, the presence of virus has been verified by indirect immunofluorescent assays (IFAs) and electron microscopy.

Unknown viruses have been classified as members of the *Rhabdoviridae* by electron microscopy, based on their bullet-shaped morphology – a characteristic trait of members of this family (**Figure 1**). Subsequently, the assessment of antigenic relationships between these unknown viruses and other viruses worldwide has been performed using serological tests. Immune reagents have been developed for tentative assignment of the unclassified viruses by IFA, complement fixation (CF), virus neutralization (VNT) assays, hemagglutination inhibition (HI), and more recently enzyme-linked immunosorbent assays (ELISAs). However, many of these isolates have revealed no relationship with any known rhabdovirus. Up to seven different antigenic groups have been defined. Gene sequencing and phylogenetic relationships have been progressively applied to complete this initial virus taxonomy.

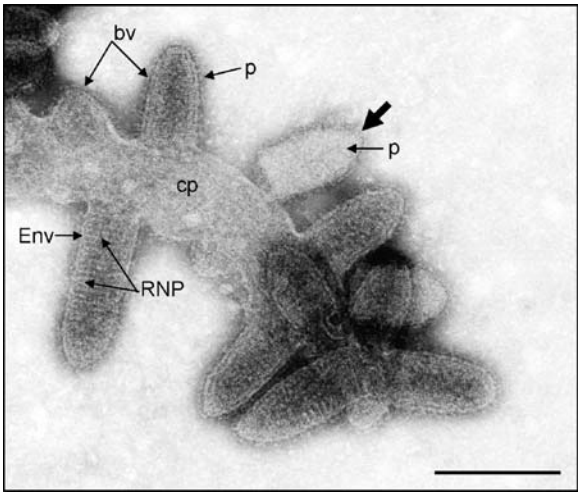


Figure 1 Electron micrograph of a negative-stained cell infected with Wongabel virus, representative of the nonassigned rhabdoviruses. Viruses are budding (bv) from a cell process (cp). The viruses are bullet-shaped, possess an envelope (Env), surface projections (peplomers) (p), and an internal helical ribonucleocapsid (RNP). The peplomers (p) consisting of trimers of viral glycoprotein can also be observed on the surface of the virus not penetrated by stain (short thick arrow). Scale = 200 nm. Electron micrograph courtesy 'Electron Microscopy and Iridoviruses Group', CSIRO-AAHL.

Other than the approved species, tentative species have been proposed in each of the four currently recognized genera of animal rhabdoviruses: 19 in the genus *Vesiculovirus*, 5 in the genus *Lyssavirus*, 3 in the genus *Ephemerovirus*, and 2 in the genus *Novirhabdovirus*. There remain six serogroups of animal rhabdoviruses (altogether comprising 20 different viruses) that have not yet been assigned to an existing genus: the Bahia Grande group, Hart Park group, Kern Canyon group, Le Dantec group, Sawgrass group, and Timbo group. A further 43 unassigned animal rhabdoviruses are listed in the *Eighth Report of the International Committee on Taxonomy of Viruses* (Eighth ICTV Report). Serological surveys conducted using sera collected from livestock, various wildlife and humans from Australia, Asia, and the Pacific region have revealed potential animal hosts and the geographical distribution for some of these viruses. However, for most of the uncharacterized rhabdoviruses information is limited. Although links between disease and the uncharacterized rhabdoviruses have not been made, recent studies have provided insights into their genetic composition and have revealed that wide genetic diversity exists among them, beckoning more intensive study of this family of viruses.

Virion Properties

Morphology

Rhabdovirus virions are 100–400 nm long and 50–100 nm in diameter (**Figure 1**). Viruses appear bullet-shaped. From the outer to the inner side of the virion, one can distinguish the envelope covered with viral glycoprotein spikes and, internally, the nucleocapsid with helical symmetry consisting of the nucleoprotein tightly bound to genomic RNA.

Genome Organization and Genetics

All rhabdoviruses contain a genome consisting of a nonsegmented single-stranded negative-sense RNA molecule with a size in the range of approximately 8.9–15 kbp. This RNA molecule contains at least five open reading frames (ORFs) encoding five virion proteins in the order (3′–5′): nucleoprotein (N); phosphoprotein (P); matrix protein (M); glycoprotein (G); and polymerase (L). Viruses in the genus *Ephemerovirus* contain several additional ORFs between the G and L genes, which encode a second glycoprotein (G_{NS}) and several other nonstructural proteins. Similarly, in the genus *Novirhabdovirus*, a sixth functional cistron between the G and L genes encodes a nonstructural protein (NV) of unknown function. The unclassified rhabdoviruses, sigma virus infecting flies (*Drosophila* spp.) and plant rhabdoviruses in the genera *Cytorhabdovirus* and *Nucleorhabdovirus* also contain an additional ORF which is located between the P and M genes. Flanders virus from mosquitoes (*Culista melanura*) has a complex arrangement of genes and pseudogenes in the same genome region. Nucleotide sequence analysis of Tupaia virus from the tree shrew (*Tipiai belangeri*) has identified an additional gene encoding a small hydrophobic protein between the M and G genes, and genome sequence analysis of Wongabel virus, an unassigned rhabdovirus isolated from biting midges (*Culicoides austropalpalis*), has revealed that it contains five additional genes that appear to be novel. The function of these other proteins (including additional glycoproteins) is not yet known. Therefore, despite preservation of a characteristic particle morphology, the *Rhabdoviridae* includes viruses that display a wide genetic diversity (**Figure 2**).

Relatively low sequence identities across the *Rhabdoviridae* prevent the construction of a family phylogeny. One approach to determining the phylogenetic relationships among the rhabdoviruses, as well as the identification of new viral species, is to utilize the conserved regions that have been identified in alignments of the N and L genes.

Evolutionary Relationships

A molecular phylogenetic analysis of 56 rhabdoviruses, including 20 viruses which are currently unassigned or assigned as tentative species within the *Rhabdoviridae*, has been reported by using the sequences from a region of block III of the L polymerase (**Table 1** and **Figure 3**). Block III is predicted to be essential for RNA polymerase function because it is conserved among all L proteins, and mutations

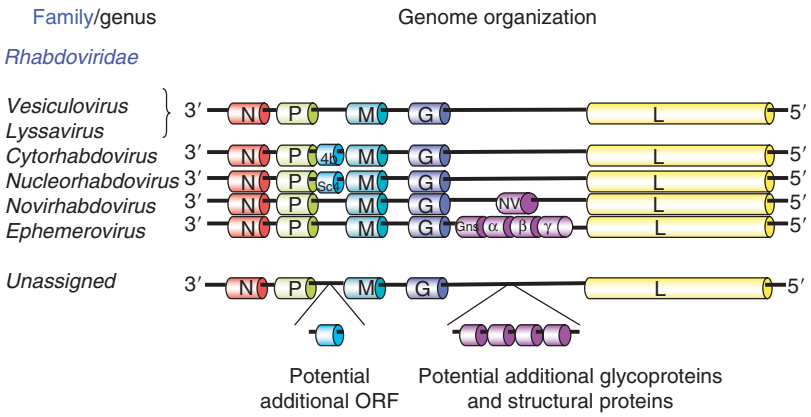


Figure 2 Genome organization of rhabdoviruses.

Table 1 Unassigned rhabdoviruses from Australia and Papua New Guinea

Virus name	*Isolated from*	*Neutralizing antibody detected*	*Year of isolation*	*Location*
Almpiwar	*Ablepharus boutonii virgatus* (skink)	Skink, cattle, horse, sheep, kangaroo, bandicoot, various birds, human	1966	Mitchell River, Queensland
Charleville	*Phlebotomus* spp. (sand fly) *Gehyra australis* (gecko) *Lasiohelea* spp. (biting midge)	Human	1969	Charleville, Queensland
Coastal Plains	Cattle	Buffalo, cattle (Australia and Papua New Guinea)	1981	Beatrice Hill[a], Northern Territory
Humpty Doo	*C. marksi*, *Lasiohelea* spp. (biting midge)	Unknown	1975	Beatrice Hill, Northern Territory
Joinjakaka	Mixed Culicines (mosquito)	Cattle	1966	Sepik District, Papua New Guinea
Koolpinyah	Cattle (*Bos indicus* x *Bos taurus*)	Cattle	1985	Berrimah Farm, Northern Territory
Kununurra	*Ad. catasticta* (mosquito)	Unknown	1973	Kununurra, Western Australia
Ngaingan	*C. brevitarsis* (biting midge)	Wallaby, kangaroo, cattle	1970	Mitchell River, Queensland
Oak Vale	*Cx. edwardsi* (mosquito)	Ferral pigs	1981	Peachester, Queensland
Parry Creek	*Cx. annulirostris* (mosquito)	Unknown	1973	Kununurra, Western Australia
Tibrogargan	*C. brevitarsis* (biting midge)	Buffalo, cattle	1976	Peachester, Queensland
Wongabel	*C. austropalpalis* (biting midge)	Sea birds	1979	Wongabel, Queensland
CSIRO75 (Harrison Dam virus)	*Cx. annulirostris* (mosquito)	Unknown	1975	Beatrice Hill, Northern Territory
CSIRO1056	*C. austropalpalis* (biting midge)	Unknown	1981	Samford, Queensland
DPP1163 (Holmes Jungle virus)	*Cx. annulirostris* (mosquito)	Cattle, buffalo, humans	1987	Darwin, Northern Territory
OR559 (Little Lilly Creek virus)	*Cx. annulirostris* (mosquito)	Unknown	1974	Kununurra, Western Australia
OR1023 (Ord River virus)	*Cx. annulirostris* (mosquito)	Unknown	1976	Kununurra, Western Australia

[a]Beatrice Hill, NT has in the past been known as Coastal Plains.

in this region abolish polymerase activity. This phylogenetic analysis produced an evolutionary tree that generally, although not entirely, conforms to accepted serological groupings and taxa within the *Rhabdoviridae*. In particular, members of four genera – *Lyssavirus*, *Novirhabdovirus*, *Cytorhabdovirus*, and *Nucleorhabdovirus* – obtained from a variety of host species, including mammals, fish, arthropods, and plants, can be easily distinguished and fall into

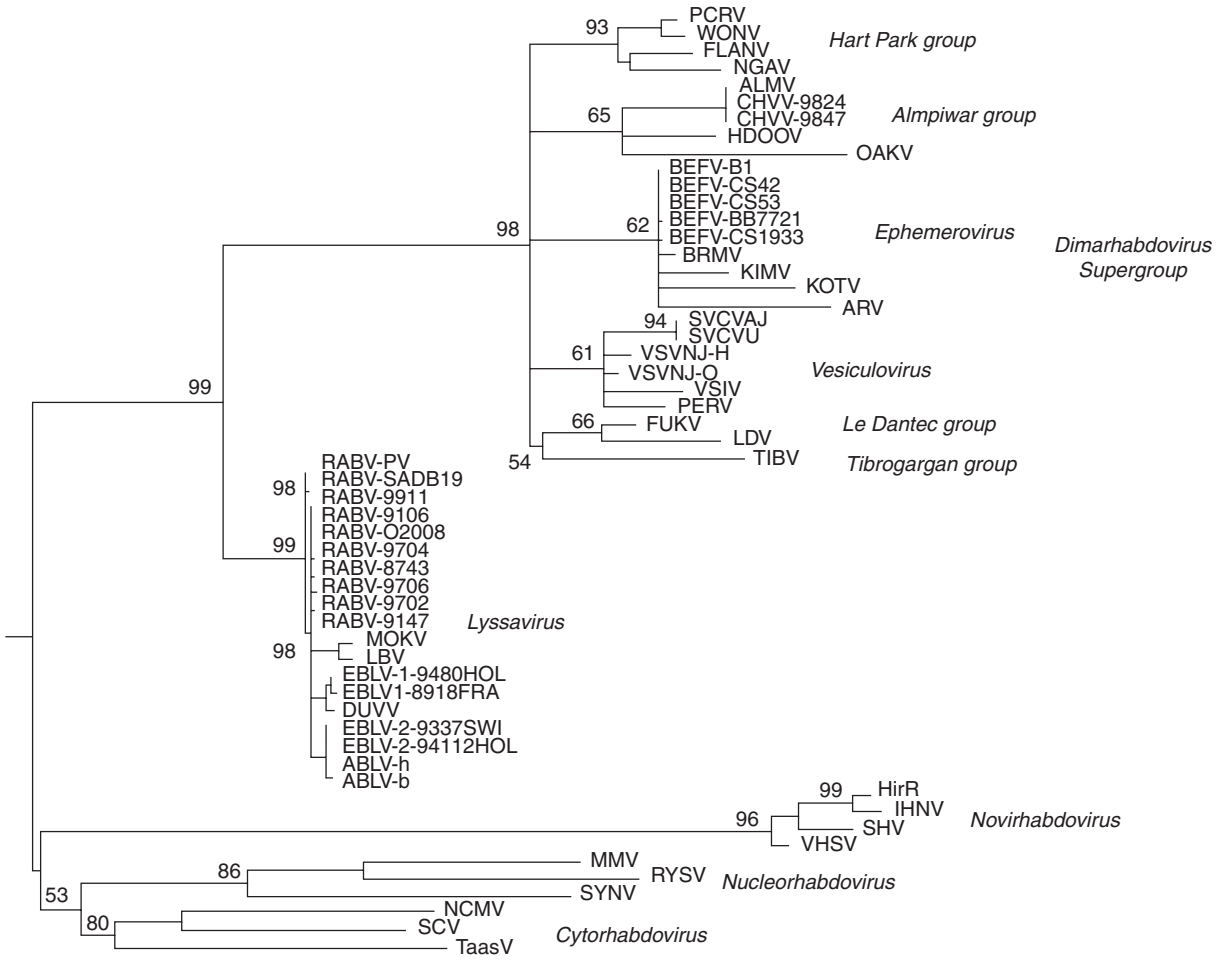


Figure 3 Phylogenetic relationships of members of the *Rhabdoviridae* based on a maximum likelihood analysis of a 158-amino-acid residue alignment of the L polymerase region. The established rhabdovirus genera as well as the newly proposed groups are indicated. Horizontal branches are drawn to scale and quartet puzzling frequencies are shown for key nodes (values in italics are for genera, groups, and supergroups, while all other quartet puzzling frequencies are shown in normal font). The tree is midpoint rooted for purposes of clarity only and all potential outgroup sequences were deemed too divergent to include in the analysis. Adapted from Bourhy H, Cowley JA, Larrous F, Holmes EC, and Walker PJ (2005) Phylogenetic relationships among rhabdoviruses inferred using the L polymerase gene. *Journal of General Virology* 86: 2849–2858.

relatively well-supported clades. Although the vesiculoviruses and ephemeroviruses also fall into clear monophyletic groups, they are less well supported, and each genus contains some unclassified viruses. Furthermore, kotonkan virus, which causes clinical ephemeral fever in cattle but has previously been classified as a lyssavirus, is very clearly clustered with members of the genus *Ephemerovirus*. Lastly, there is some evidence that the two groups of plant rhabdoviruses – the cytorhabdoviruses and nucleorhabdoviruses – form a distinct clade. Taastrup virus which remains unassigned is related to cytorhabdoviruses.

Strikingly, this phylogenetic analysis has also identified four more monophyletic groups of currently unclassified rhabdoviruses, which have variable support values. First, Wongabel, Parry Creek, Flanders, and Ngaingan viruses formed a distinct cluster, with high levels of support. We refer to this as the Hart Park group, based on the serologic grouping of Flanders virus in the Hart Park serological group. Second, a tentatively named Almpiwar group containing Almpiwar, Humpty Doo, Charleville, and Oak Vale viruses was also identified. Although this grouping had a lower support, the Almpiwar and Charleville viruses possess almost indistinguishable sequences in the L gene region and each has been associated with infection in lizards. Another group, consisting of the Le Dantec and Fukuoka viruses, and herein referred to as the Le Dantec group, was also seen to form a distinct cluster. Finally, the phylogenetic position of Tibrogargan virus was ambiguous.

Common rhabdovirus sequence motifs have also been found in the central region of the N gene. Phylogenetic analysis of partial N gene sequences indicated that two viruses isolated from bats from various regions of the world, Oita virus and Mount Elgon bat virus, were grouped

in a monophyletic cluster. Kolongo virus (Africa) and Sandjimba virus (Asia) also formed a distinct clade together with Tupaia virus (Asia). In this analysis, Flanders virus, sea trout virus, sigma virus, Kern Canyon virus, and Rochambeau virus remained isolated on the phylogenetic tree.

Phylogenetic analysis of both partial N and L gene sequences of Obodhiang virus and kotonkan virus has indicated that they should be classified as members of the genus *Ephemerovirus*.

Host Range and Virus Propagation

Transmission

Many of the rhabdoviruses replicate in and are transmitted by insect vectors. Plant-infecting rhabdoviruses are transmitted by vectors including aphids, planthoppers, and leafhoppers. Several animal rhabdoviruses (e.g., ephemeroviruses and vesiculoviruses) are known to be transmitted by hematophagous insects such as mosquitoes and biting midges. The widespread ability of rhabdoviruses to infect insects has led to the hypothesis that this virus family has evolved from an ancestral insect virus and that the host range is largely determined by the insect host. Sigma virus (SIGMAV) does not appear to have any vertebrate host but can be transmitted congenitally in flies (*Drosophila* spp.).

A number of important biological conclusions can be drawn from the rhabdovirus phylogeny based on the L gene. First, assuming a midpoint rooting of the tree, there is major split between those viruses that infect fish (novirhabdoviruses) or infect plants and employ arthropods as vectors (cytorhabdoviruses and nucleorhabdoviruses) – and those viruses that infect both mammals or lizards and dipterans (dimarhabdoviruses, sigla for dipteran-mammal-associated rhabdovirus). Such a division illuminates the biology of a number of key rhabdoviruses. For example, although vesicular stomatitis virus (VSV) is responsible for a disease of horses, cattle, and pigs, and can be transmitted directly by transcutaneous or transmucosal routes, the virus replicates in a wide range of hosts, including insects, and there is good evidence that VSV may employ insect vectors as at least one of its mechanisms of transmission. Similarly, bovine ephemeral fever virus, which is frequently found in Australasia and Africa, is also dipteran-transmitted, using vectors such as biting midges and culicine and anopheline mosquitoes. Finally, viruses assigned by the L-based phylogenetic analysis to the four new groups (Le Dantec, Tibrogargan, Hart Park, and Almpiwar groups) have all been found to infect dipterans and in some cases also mammals (Tibrogargan, Le Dantec, and Ngaingan viruses) or lizards (Charleville virus).

Importantly, there is, as yet, no evidence for a virus making the link between plant rhabdoviruses, novirhabdoviruses of fish, or dimarhabdoviruses. Furthermore, the uncertainty over branching order at the root of the tree makes it difficult to determine whether the ancestral mode of transmission in rhabdoviruses was vector or nonvector transmission. However, the major phylogenetic division between these groups indicates that the biology of the rhabdoviruses could be strongly influenced by mode of transmission and by the host (plant, fish, or mammals) and vector species (orthopterans, homopterans, or dipterans).

Clinical Features and Pathology

For most of the uncharacterized rhabdoviruses, links with disease have not yet been made. The only virus isolated from a natural infection in humans is Le Dantec virus. As indicated above, kotonkan virus causes clinical ephemeral fever in cattle and deer. Fukuoka virus has also been isolated from the blood of febrile calves.

Serogroups of Nonassigned Rhabdoviruses (As Recognized in the Eighth ICTV Report)

Hart Park Group

Phylogenetic analyses of the L gene have indicated that Flanders virus (FLANV), Ngaingan virus (NGAV), Parry Creek virus (PCV), and Wongabel virus (WONV) cluster with Hart Park virus (HPV) and are possible members of the Hart Park group. FLANV was isolated in 1961 from a pool of engorged female *Culista melanura* mosquitoes that were collected in Long Island, New York, USA. Similar viruses have been collected from different mosquito species in various parts of the United States. FLANV has also been isolated from the blood of house sparrows, red-winged blackbirds, and from the spleen of an oven bird. HPV was isolated in 1955 from a pool of female *Culex tarsalis* collected at Hart Park, California. NGAV (strain MRM14556) was isolated in 1970 from a pool of biting midges (*Culicoides brevitarsis*) that were collected at Mitchell River Aboriginal community in northern Queensland. This arbovirus has also been found to multiply in experimentally infected mosquitoes (*Aedes aegypti*). Serological surveys have indicated that NGAV infects wallabies, and possibly kangaroos and cattle, although its role in disease in these animals is unknown. IFA, CF, and VNT results place NGAV in the Tibrogargan antigenic group.

PCV (strain OR189) was isolated in 1973 from mosquitoes (*Culex annulirostris*) that were collected at Parry Creek near Kununurra, Western Australia. WONV (strain CSIRO264) was isolated in 1979 from biting midges (*Culicoides austropalpalis*) collected at Wongabel on the Atherton Tableland of northern Queensland. Morphological examination has revealed bullet-shaped particles (80–90) × (160–180) nm in dimension (**Figure 1**). This

species of biting midge has been observed to have a feeding preference for birds. Although no link has been established between WONV and disease, neutralizing antibodies were detected in sea birds collected off the Great Barrier Reef. No neutralizing antibodies were detected in human sera from island residents in this region. Two other viruses, Mossuril virus (MOSV) and Kamese virus (KAMV), have been classified with the Hart Park group based on antigenic relationships only, as they were not included in any phylogenetic analysis. MOSV was first identified in 1959 in Mozambique and later in Central African Republic. It was isolated from mosquitoes (*Culex sitiens*, *Culex decens*, *Culex perfusus*, *Culex pruina*, *Culex telesilla*, *Culex Weschei*, and *Culex tigripes*) and birds (*Andropadus virens* and *Cliuspassrer maccrourus*). KAMV was first identified in Uganda in 1967 and then in Central African Republic. It has been isolated from culicine mosquitoes (*Culex annulioris*, *Aedes africanus*, *Culex decens*, *Culex perfuscus*, *Culex pruina*, and *Culex tigripes*).

Le Dantec Group

At the L gene amino acid level, Fukuoka virus (FUKAV) and Le Dantec virus (LDV) appear to be related, although FUKAV was previously classified in the Kern Canyon group on the basis of its antigenic properties (see below). LDV was originally recovered in 1965 from a patient with a febrile illness, headaches, and spleen and liver hypertrophy in Senegal. In CF tests with other known rhabdoviruses, Le Dantec virus was found to be antigenically related to Keuraliba virus, a previously ungrouped agent isolated from rodents (*Tatera kempi*) in Senegal in 1968. FUKAV was first isolated from biting midges, *Culicoides punctalis* and *Culex tritaeniorhynchus* in 1986, and subsequently isolated from blood of calves with fever and leucopoenia.

Bahia Grande Group

Bahia Grande (BGV) (prototype strain TB4–1054), Reed Ranch (RRV)(TB4–222), and Muir Springs (MSV) viruses (76V-23524) were first obtained from salt-marsh mosquitoes (*Culex*, *Aedes*, *Anopheles*, and *Psorophora* spp.) collected between 1972 and 1979 in west Texas, New Mexico, Louisiana, Colorado, North Dakota, and south Texas. Structural analysis of the prototype strain of BGV from Texas has revealed five proteins. Comparative oligonucleotide fingerprint maps has shown 51–86% sharing of the large oligonucleotides between BGV (strain TB4–1054) and 11 other antigenically related isolates but not with MSV (strain 76V-23524), an antigenically distinct isolate from mosquitoes collected in Colorado. A serological survey for antibody to BGV has shown that humans, cattle, sheep, reptiles, and wild mammals from south Texas have neutralizing antibodies to this virus.

Timbo Group

Chaco virus (CHOV) was isolated from *Ameiva ameiva ameiva* and *Kentropyx calcaratus* lizards in Brazil in 1962. Timbo virus (TIMV) was isolated from *Ameiva ameiva ameiva* lizards in Brazil. The optimal growth temperature of these viruses is approximately 30 °C.

Sawgrass Group

New Minto virus (NMV) was isolated from *Haemaphysalis leporis-palustris* (Packard) ticks removed from snowshoe hares (*Lepus americanus* Erxleben) in east central Alaska in 1971. This virus is serologically (complement fixation and neutralization tests) related to Sawgrass virus. Sawgrass virus (SAWV) was isolated for the first time in Florida in 1964 from *Dermacentor variabilis* ticks removed from a raccoon, and later from *Haemophysalis leporis-palustris* ticks. Connecticut virus (CNTV) was first isolated in 1978 from a pool of nymphal *Ixodes dendatus* ticks removed from eastern cottontail rabbits (*Sylvilagus floridanus*) trapped in Connecticut, USA. Neutralizing antibodies have been detected in the eastern cottontail population in Connecticut, suggesting tick–rabbit maintenance cycle.

Kern Canyon Group

Kern Canyon virus (KCV) was first isolated in 1956 from a pool of spleen and heart tissues from *Myotis yumanensis* bats in Kern County, California. KCV is not related to other groups or viruses classified in established genera according to the N gene phylogeny. Barur virus (BARV) was isolated from rodents (*Mus booduga*) and from ixodid ticks (*Haemaphysolis intermedia*) in 1961 in Mysore State, India and later, in 1971, from a pool of *Mansonia uniformis* mosquitoes in Kono Plain, Kenya. FUKAV was first grouped with KCV based on its antigenic properties. According to recent phylogenetic studies, it seems more related to the Le Dantec group. Nkolbisson virus (NKOV) was first isolated from *Aedes* sp., *Culex* sp., and *Eretmapodites* sp. mosquitoes in Cameroon in 1965. It was later isolated from Culicidea in the Ivory Coast, and from humans in the Republic of Central Africa.

Other Groups as Proposed by Phylogenetic Analyses but Not Yet Recognized in the Eighth ITCV Report

Almpiwar Group

Recent analysis of the L gene of different rhabdoviruses has suggested that Almpiwar virus (ALMV), Charleville virus (CHVV), Oak Vale virus (OVRV), and Humpty Doo virus (HDOOV) share a high genetic similarity, and they have been proposed to constitute the Almpiwar group.

ALMV (strain MRM4059) was isolated in 1966 from the skink *Ablepharus boutonii virgatus* at the low-lying plains

of the Mitchell River Aboriginal community on the Gulf of Carpentaria in northern Queensland (**Figure 4**). Antibody to this virus has been detected in this species of skink. The virus optimally replicates at 30 °C in cell culture, which further supports the presumption that it is a reptilian virus. Although ALMV has never been isolated from arthropods, evidence of multiplication and passage of the virus in experimentally infected mosquitoes (*Culex fatigans*) supports the assumption that it is an arbovirus. No antigenic relationship has been found by complement fixation or neutralization test to any other known or suspected arboviruses. Although evidence indicates the presence of neutralizing antibodies to ALMV in sera from several different vertebrates, including humans, the significance of these results is considered uncertain. While ALMV and Charleville virus (CHVV) (see below) were each isolated from lizards (of different species) captured at Mitchell River, the viruses appear to share no serological relationship.

CHVV (strain Ch9824) was initially isolated in 1969 from sandflies (*Phlebotomus* spp.) collected near Charleville in southern Queensland. The following year, CHVV was isolated from the heart, liver, and lung of a lizard (*Gehyra australis*), and from a pool of biting midges (*Lasiohelea* spp.), each collected at Mitchell River in northern Queensland. Multiplication of this virus was demonstrated in experimentally infected mosquitoes (*A. aegypti*), supporting the possibility that it may be an arbovirus. CHVV was analyzed by complement-fixation test against all known Australian arboviruses (including ALMV, see above) but no relationships were found. Limited evidence suggests the presence of neutralizing antibody to CHVV in humans (1 of 30 sera tested).

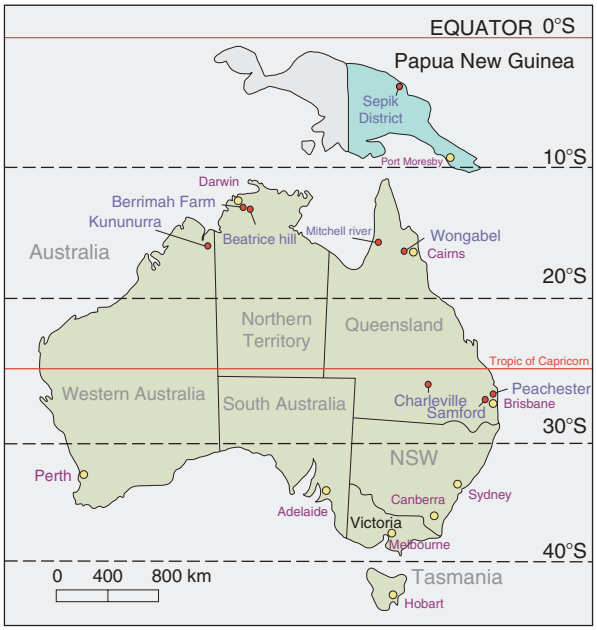


Figure 4 Map of Australia and Papua New Guinea showing locations from which unassigned animal rhabdoviruses were isolated.

OVRV (strain CSIRO1342) was isolated on nine occasions in 1984 from mosquitoes (*Culex edwardsi*) that were collected near a sentinel cattle herd located at Peachester near Brisbane in Queensland. OVRV was also isolated once from mosquitoes (*Aedes vigilax*) in Darwin, Northern Territory in January 1984. OVRV is not neutralized by any antiserum prepared against known Australian arboviruses. However, antiserum to this virus has been shown to cross-react with Kimberley virus (genus *Ephemerovirus*) by IFA. No antibodies to OVRV were detected in cattle sera collected at the same time from the same area, but neutralizing antibodies were found in feral pigs. Although it has been suggested that OVRV may be cycling between mosquitoes and an undefined avian host, this virus did not successfully replicate in cattle egrets, unlike Kununurra virus (KNAV) which is also suspected to have an avian host.

Humpty Doo virus (strains CSIRO79 and 80) was isolated between the years 1974 and 1976 from biting midges (*Culicoides marksi* and *Lasiohelea* spp.) that were collected at Beatrice Hill, near Darwin in the Northern Territory.

Tibrogargan Group

At the L amino acid level, Tibrogargan virus (TIBV) is likely to be a member of the proposed 'dimarhabdovirus supergroup'. However, it appears to be considerably different from the other members of this supergroup. Complement fixation tests have indicated that Coastal Plains virus (CPV) is serologically related to TIBV, but cross-neutralization tests using rabbit antisera prepared to each of these viruses indicated that they are distinct.

TIBV (prototype strain CSIRO132) was isolated from a pool of biting midges (*Culicoides brevitarsis*) that were collected during a 2-week summer period in 1976 at a farm near Peachester, near Brisbane, Queensland. Shortly after isolation, analysis of the virus by complement fixation and hemagglutination inhibition tests did not reveal any relationships with other known or suspected arboviruses from Australia, Papua New Guinea, or elsewhere in the world. However, further studies have indicated that TIBV and CPV are antigenically related, but distinguishable by VNT tests. Subsequent tests performed using sera collected during the mid-1970s indicated the presence of neutralizing antibodies to TIBV in cattle from New Guinea, and in a region from northern Australia spanning as far south as the central coast of New South Wales. This distribution mirrors the geographical distribution of the biting midge *C. brevitarsis*. Some sentinel cattle herds have been found to be up to 100% seropositive for neutralizing antibodies. Neutralizing antibodies were also found in water buffalo in far north Australia, but no evidence of neutralization has been found in sera from a range of other animal species including humans. Despite the high prevalence of neutralizing antibodies in the tested cattle and water buffalo, there are no records of the direct isolation of

this virus from any vertebrate, and it has not been directly linked with disease.

CPV (strain DPP53) was isolated from the blood of a healthy steer (*Bos taurus*) in 1981 at Coastal Plains Research Station near Darwin in the Northern Territory. Although TIBV was isolated from biting midges, CPV was isolated directly from cattle and it is therefore not conclusively an arbovirus. However, the geographic distribution of neutralizing antibodies to CPV in cattle sera in Australia and Papua New Guinea appears to correspond to the distribution of the biting midge *C. brevitarsis*, which is the known vector of TIBV. Neutralizing antibody to CPV has also been detected in the sera of water buffalo, dogs, and one horse, but not in sera from humans, deer, pigs, or wallabies collected in the same areas.

Mount Elgon Bat Virus Group

Nucleoprotein gene sequence analysis has indicated that Mount Elgon Bat virus (MEBV) and Oita virus (OITAV) form a distinct clade. Although they do not group by this analysis with the other vesiculoviruses, MEBV is currently classified as a tentative species of the genus *Vesiculovirus*. OITAV (296/1972) was isolated in 1972 from the blood of a wild horseshoe bat *Rhinolophus cornutus* (*Temminck*) in Japan. This virus causes lethal encephalitis in mice through the intracerebral route. MEBV was first isolated in 1964 in Kenya from bats (*Rhinolophus hildebrandtii*).

Kolongo and Sandjimba Group

Kolongo virus (KOLV) and Sandjimba virus (SJAV) form a monophyletic clade based on analysis of the central region of the N gene. KOLV and SJAV were isolated in 1970 in Central African Republic from birds (*Euplected afra* and *Acrocephalus schoenbaeus*, respectively). Tupaia virus (TUPV) (a tentative species of the genus *Vesiculovirus* according to the Eighth ITCV Report) has been shown to join the same clade, although quite distantly.

Other Nonassigned Rhabdoviruses

'Sigma virus' (SIGMAV) is the agent responsible for CO_2 sensitivity in *Drosophila melanogaster*. It is a noncontagious rhabdovirus which is transmitted through gametes.

Rochambeau virus (RBUV) was first isolated from mosquitoes (*Coquillettidia albicosta*) in French Guiana in 1973. It is classified as a tentative species of the genus *Lyssavirus* in the Eighth ICTV Report and is not related to any other dimarhadovirus, according to the phylogenetic analysis of the central region of the nucleoprotein.

Joinjakaka virus (strain MK7837) (JOIV) was isolated in 1966 from a pool of mixed mosquitoes (*Culicines*) that were aspirated from human bait at Joinjakaka, Sepik River District of Papua New Guinea. No relationship has been found between JOIV and any other known or putative arboviruses by complement fixation tests. Evidence suggests that cattle in Queensland have tested positive for antibody to JOIV by serum neutralization tests, but this study is not well documented.

Koolpinyah virus (KOOLV) was isolated from heparinized blood collected from two bulls (*Bos indicus-taurus* cross) at Berrimah Farm near Darwin in the Northern Territory in 1985 (strain DPP819) and 1986 (strain DPP883). In 1985, three additional bulls in the herd located at Berrimah developed neutralizing antibody to KOOLV, as did a sheep experimentally inoculated with blood from one of the bulls. KOOLV has been reported to be related serologically to Parry Creek virus (PCRV) and kotonkan virus (KOTV) by cross-neutralization tests, and to KOTV, PCRV, Obhodiang virus, and SJAV by indirect immunofluorescent antibody tests.

Kununurra virus (strain OR194) (KNAV) was isolated in 1973 from a pool of mosquitoes (*Aedeomyia catasticta*) that were collected in the Ord River Valley near Kununurra, Western Australia. Although KNAV is accepted as a new virus, no other serologically related rhabdovirus has since been identified. Under experimental conditions, KNAV has been shown to multiply in cattle egrets. Additionally, the mosquito host from which KNAV was isolated has a bird-feeding preference. Collectively, these results suggest that this virus may circulate in birds.

Several other apparently novel viruses with morphological characteristics of rhabdoviruses have been isolated from hematophagous insects in Australia but little or no information is available on their serological relationships to other viruses (**Table 2** and **Figure 1**). Isolate CS1056 was obtained in 1981 from a pool of biting midges (*C. austropalpalis*) collected at Samford near Brisbane in southeast Queensland. Isolate DPP1163 (tentatively named Holmes Jungle virus) was obtained in 1987 from mosquitoes (*C. annulirostris*) collected at Palm Creek near Darwin in the Northern Territory. Neutralizing antibody to this virus has been detected in cattle, buffalo, and humans, but there is no known association with disease. Isolate CSIRO75 (tentatively named Harrison Dam virus) was isolated in 1975 from mosquitoes (*C. annulirostris*) at Beatrice Hill, near Darwin in the Northern Territory. No information is available on the prevalence of antibodies in domestic or native animals. Isolate OR559 (tentatively named Little Lily Creek virus) was isolated from mosquitoes (*C. annulirostris*) collected at Kununurra in the Ord River region of Western Australia in 1974. Isolate OR1023 (tentatively named Ord River virus) was obtained in 1976, also from mosquitoes (*C. annulirostris*) collected at Kununurra. No other information is yet available about these viruses. Recent evidence suggests that KNAV might not be a rhabdovirus, but further studies are required to confirm this.

Two unassigned rhabdoviruses were isolated from birds during surveillance for arboviral encephalitis in the

Table 2 Representative isolates of the genera and groups of rhabdoviruses used for the phylogenetic analysis of the L gene

Genus	*Name*	*UA /TS*[a]	*Abbreviation*	*Species from which it was isolated and from which neutralizing antibodies were identified*	*Origin*[b]	*Year of first isolation*
Nucleorhabdovirus	Rice yellow stunt virus		RYSV	Leafhopper		
	Sonchus yellow net virus		SYNV	Aphid		
	Maize mosaic virus		MMV	Leafhopper (*Peregrinus maidis*)		
Cytorhabdovirus	Northern cereal mosaic virus		NCMV	Leafhopper (*Laodelphax striatellus*)	Japan	
	Strawberry crinkle virus		SCV	Aphid (*Fragaria* spp.)	Chile	
	Taastrup virus	UA	TaasV	Leafhopper (*Psammottetix alienus*)	Denmark	
Novirhabdovirus	Infectious hematopoietic necrosis virus		IHNV	Rainbow trout (*Onchorynchus mykiss*)/ Invertebrate reservoirs?		
	Viral hemorragic septicemia virus		VHSV	Rainbow trout (*Onchorynchus mykiss*)/ Invertebrate reservoirs?		
	Snakehead rhabdovirus	TS	SHV	Sneakhead fish (*Ophicephalus striatus*)		
	Hirame rhabdovirus	UA	HirR			
Ephemerovirus	Adelaide River virus		ARV	*Bos taurus*	Australia	1981
	Berrimah virus		BRMV	*Bos taurus*	Australia	1981
	Kimberley virus	TS	KIMV	*Bos taurus*	Australia	1980
	Kotonkan virus	UA	KOTV	*Culicoides* species	Nigeria	1967
	Bovine ephemeral fever virus		BEFV	*Bos taurus*, *An. Bancrofti*	Australia, China	1968
	Obodhiang virus	UA	OBOV	*Mansonia uniformis*	Sudan	1963
Almpiwar Group	Humpty Doo virus	UA	HDOOV	*Lasiohelea* species, *Culicoides marski.* cattle	Australia	1975
	Charleville virus	UA	CHVV	*Phlebotomus* species (sand fly), *Lasiohelea* species (biting midge) lizard (*Gehyra australis*), human	Australia	1969
	Almpiwar virus	UA	ALMV	*Ablepharus boutonii virgatus,* skink, cattle, horse, sheep, kangaroo, bandicoot, birds, human	Australia	1966
	Oak-Vale virus	UA	OVRV	*Culex* species, *Culex edwardsi*, *Aedes vigilax*, ferral pigs	Australia	1981
Tibrogargan Group	Tibrogargan virus	UA	TIBV	*Culicoides brevitarsis*, *water* buffaloes, cattle	Australia	1976
	Coastal Plains	UA	CPV	Cattle	Australia, Papua New Guinea	1981
Hart Park Group	Parry Creek virus	UA	PCRV	*Culex annulirostris*	Australia	1972
	Hart Park virus	UA	HPV	*Culex tarsalis,* birds	USA	1955
	Wongabel virus	UA	WONV	*Culicoides austropalpalis,* sea birds	Australia	1979
	Flanders virus	UA	FLANV	*Culiseta melanura, Culex pipiens quinquefasciatus, Cx. salinarus, Cx. territans, Cx. restuans, Cx. tarsalis, Seiurus aurocapillus,* birds	New York, USA	1961

Continued

Table 2 Continued

Genus	*Name*	*UA /TS*[a]	*Abbreviation*	*Species from which it was isolated and from which neutralizing antibodies were identified*	*Origin*[b]	*Year of first isolation*
	Ngaingan virus	UA	NGAV	*Culicoides brevitarsis*, wallaby, kangaroo, cattle	Australia	1970
	Mossuril virus	UA	MOSV	*Culex sitiens*, *Culex decens*, *Culex perfusus*, *Culex pruina*, *Culex telesilla*, *Culex weschei*, *Culex tigripes*, birds (*Andropadus virens* and *Cliuspassrer maccrourus*)	Mozambique, Central African Republic	1959
	Kamese virus	UA	KAMV	*Culex annulioris*, *Aedes africanus*, *Culex decens*, *Culex perfuscus*, *Culex pruina and Culex tigripes*	Uganda, Central African Republic	1967
Le Dantec and	Le Dantec virus	UA	LDV	Human	Senegal	1965
Kern Canyon Group	Fukuoka virus	UA	FUKV	*Culicoides punctatus*, *calves*	Japan	1982
	Keuraliba virus	UA	KEUV	*Rodents* (*Tatera kempi, taterillus* sp.)	Senegal	1968
Vesiculovirus	Perinet virus	TS	PERV	Mosquitoes: *Anopheles coustani*, *Culex antennatus*, *Culex gr pipiens, Mansonnia uniformis* Others: *Phlebotomus berentensis*	Madagascar	1978
	Vesicular stomatitis virus New Jersey		VSNJV	*Sus scrofa* ; *Bos taurus*, *Culex nigripalpus*, *Culicoides species*, *Mansonia indubitans*	USA	1949
	Vesicular stomatitis virus Indiana		VSIV	*Bos taurus*	USA	1925
	Spring viremia of carp virus	TS	SVCV	*Cyprinus carpio*	Yougoslavia	1971
Lyssavirus	Mokola virus		MOKV	Cat	Zimbabwe	1981
	Lagos bat virus		LBV	Bat (*Eidolon helvum*)	Nigeria	1956
	European Bat Lyssavirus subtype 1		EBLV-1	Bat (*Eptesicus serotinus*)		
	European Bat Lyssavirus subtype 2		EBLV-2	Bat (*Myotis daubentonii, Myotis dasycneme*)		
	Duvenhage virus		DUVV	Human	Rep. South Africa	1986
	Australian bat lyssavirus		ABLV	Human, bat (*Pteropus* species)	Australia	1996
	Rabies virus		RABV			

[a]UA, unassigned species and unclassified viruses; TS, tentative species.
[b]Precise location of isolates from Australia and Papua New Guinea is given on **Figure 4.**

northeastern United States. Rhode Island virus strains RI-166 and RI-175 were each isolated from brain tissue of dead pigeons (*Columba livia*) collected at two localities in Rhode Island in summer 2000. Farmington virus designated CT-114 was originally isolated from an unknown wild bird captured in central Connecticut in 1969. Both viruses infect birds and mice, as well as monkey kidney cells in culture.

Future Perspectives

The list of viruses described here is not complete and more viruses will certainly be characterized in the near future. Although there is strong phylogenetic support for the dimarhabdovirus supergroup, the precise branching order within this group cannot be resolved on the L or

N gene data. Indeed, there is a clear need for further phylogenetic studies within the dimarhabdovirus supergroup, particularly with respect to the demarcation of genera, which currently is influenced more by genome structure than host/vector relationships. There is some evidence that some of these viruses contain additional genes that are not present in lyssaviruses and vesiculoviruses. Although the functions of these additional proteins are not understood, revealing the evolution of genome complexity may be an important factor in resolving the taxonomy of this supergroup.

See also: Chandipura Virus; Rabies Virus.

Further Reading

Bourhy H, Cowley JA, Larrous F, Holmes EC, and Walker PJ (2005) Phylogenetic relationships among rhabdoviruses inferred using the L polymerase gene. *Journal of General Virology* 86: 2849–2858.

Calisher CH, Karabatsos N, Zeller H, *et al.* (1989) Antigenic relationships among rhabdoviruses from vertebrates and hematophagous arthropods. *Intervirology* 30: 241–257.

Fu ZF (2005) Genetic comparison of the rhabdoviruses from animals and plants. In: Fu ZF (ed.) *Current Topics in Microbiology and Immunology, Vol. 292: The World of Rhabdoviruses*, p. 1. Berlin: Springer.

Hogenhout SA, Redinbaugh MG, and Ammar ED (2003) Plant and animal rhabdovirus host range: A bug's view. *Trends in Microbiology* 11: 264–271.

Karabatsos N (1985) *International Catalogue of Arboviruses Including Certain Other Viruses of Vertebrates*. San Antonio, TX: American Society of Tropical Medicine and Hygiene.

Kuzmin IV, Hughes GJ, and Rupprecht CE (2006) Phylogenetic relationships of seven previously unclassified viruses within the family *Rhabdoviridae* using partial nucleoprotein gene sequences. *Journal of General Virology* 87: 2323–2331.

Relevant Websites

http://www.pasteur.fr – CRORA Report. This report involves all the data collected by Pasteur Institute and ORSTOM, since 1962, more than 6000 isolated strains of 188 arboviruses or mixed arboviruses.For each virus identified by the CRORA, all the observed hosts or vectors are given, with the number of collected strains in each country, viral properties of collected strains, and bibliographical references concerning them.

http://www.ncbi.nlm.nih.gov – ICTV database on *Rhabdoviridae*, International Committee on Taxonomy of Viruses, NCBI.

Antigen Presentation

E I Zuniga, D B McGavern, and M B A Oldstone, The Scripps Research Institute, La Jolla, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The immune system is responsible for the tremendous task of fighting a wide range of pathogens to which we are constantly exposed. This system can be broadly subdivided in innate and adaptive components. The innate immune system exists in both vertebrate and invertebrate organisms and represents the first barrier against microbial invasion. This arm of the immune system rapidly eliminates the vast majority of microorganisms that we daily encounter and is responsible for limiting early pathogen replication. The adaptive response is a more sophisticated feature of vertebrate animals involving a broad repertoire of genetically rearranged receptors that specifically recognize microbial antigens (antigen is a generic term for any substance that can be recognized by the adaptive immune system). The hallmark of the adaptive response is the generation of a potent and long-lasting defense specifically directed against the invading pathogen.

B and T lymphocytes represent the effector players of adaptive immunity and carry on their surface antigen-specific receptors, B-cell receptors (BCRs) and T-cell receptors (TCRs), respectively. There are two major classes of T lymphocytes: CD8 cytotoxic and CD4 helper T cells. Upon antigen encounter, lymphocytes undergo clonal expansion and differentiation of their unique functional features. B cells differentiate into plasma cells and secrete antibodies that specifically bind the corresponding antigen. CD8 T cells directly kill infected cells or release cytokines that interfere with viral replication, while CD4 T cells activate other cells such as B cells and macrophages. Unlike B cells, which can directly bind native free antigen, T cells only recognize antigen-derived peptides displayed on cell surfaces in the context of major histocompatibility complex (MHC) class I (MHC-I, CD8 T cells) or class II (MHC-II, CD4 T cells) molecules.

Different pathogens preferentially replicate in distinct cellular compartments. While viruses and intracellular bacteria replicate in the cytosol, microbes such as mycobacterium and protozoan parasites are intravesicular and colonize the endosomal and/or lysosomal compartments. In addition, extracellular bacteria release antigens, such as toxins, that are engulfed by antigen-presenting cells (APCs) to also reach the endosomal pathway. Antigenic

peptides derived from these sources are exhibited on cell surfaces by MHC molecules. This process, which represents the major focus of this article, is named 'antigen presentation' and is a fundamental pillar of antimicrobial host defense.

Antigen-Presenting Cells

For initiation of an immune response, naive T cells need to be activated or 'primed'. For that, they require both the recognition of the specific MHC–peptide complex (signal 1) and simultaneous co-stimulation (signal 2). Although all nucleated cells express MHC-I and can potentially display MHC-I –microbial peptide complexes after infection, only a specialized group of leukocytes, named APCs, expresses both MHC-I and MHC-II as well as co-stimulatory molecules. The best-characterized co-stimulatory molecules are B7-1 and B7-2, which bind to the CD28 molecule on the T-cell surface. In addition, T cells express CD40 ligand, which interacts with CD40 on APC further enhancing co-stimulation and enabling T-cell response. Finally, there is another group of adhesion molecules such as lymphocyte function-associated antigen-1 (LFA-1) on APCs which binds to ICAM-1 on T cells that seal the APC–T-cell interface. During APC–T-cell interactions, all these molecules cluster together forming a highly organized supramolecular adhesion complex (SMAC), enabling the intimate contact between the two cells that is referred to as the immunological synapse (**Figure 1**).

APCs are composed of macrophages, B cells, and dendritic cells (DCs). They differ in location, antigen uptake, and expression of antigen-presenting and co-stimulatory molecules. Macrophages are localized in connective tissues, body cavities, and lymphoid tissues. Within the secondary lymphoid tissues, macrophages are mainly distributed in the marginal sinus and medullary cords. They specialize in phagocytosis and engulf particulate antigens through scavenger germline receptors such as the mannose receptor. On the other hand, B cells form follicular structures within secondary lymphoid organs and recirculate through the blood stream and lymph seeking their specific antigen. B cells recognize antigens specifically through a rearranged BCR. DCs are the most professional and robust of the APCs. They are widely distributed through the body at an 'immature' stage of development, acting as sentinels in peripheral tissues. They continuously sample the antigenic environment by both phagocytosis and macropinocytosis,

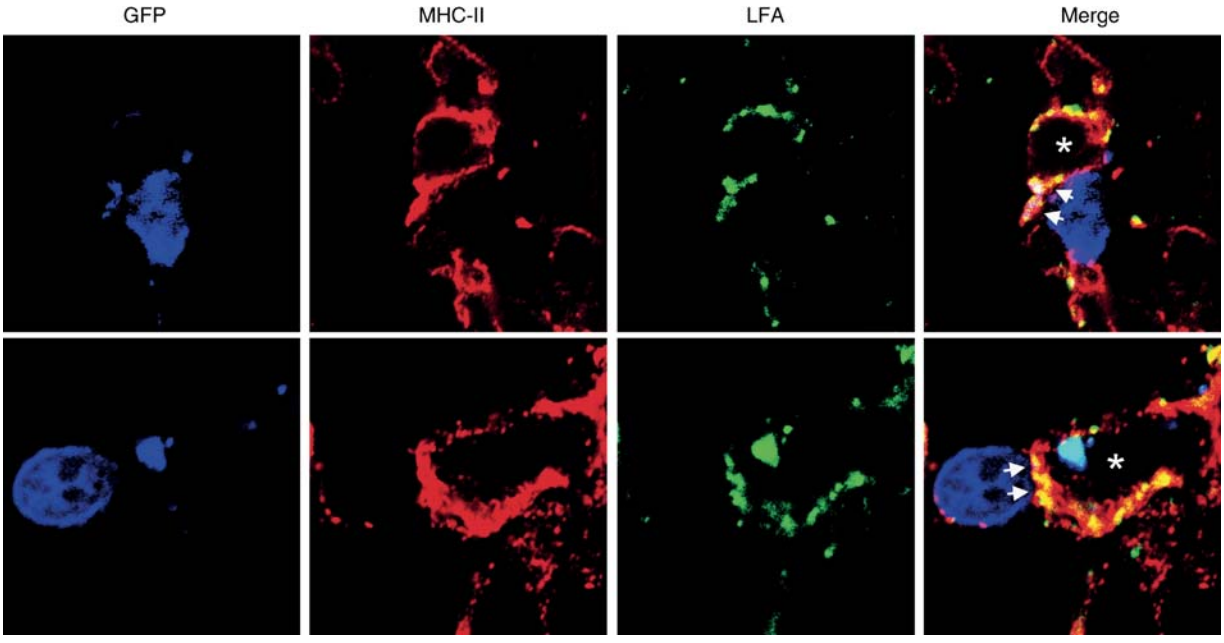


Figure 1 Interactions between virus-specific T cells and APCs. Three-color confocal microscopy was used to demonstrate immunological synapse formation between lymphocytic choriomeningitis virus-specific T cells (blue) and MHC-II^{+} APCs (red) in the central nervous system. Immunological synapses were indicated by the polarization of the adhesion molecule LFA-1 (green) between the CTL and APC. Asterisks denote the engaged APC, and arrows denote the contact point between the two cells. LFA-1 is expressed on both CTLs and APCs, but note that all of the CTL-associated LFA-1 is focused toward a contact point at the CTL–APC interface. Reproduced from Lauterbach H, Zúñiga EI, Truong P, Oldstone MBA, and McGavern DB (2006) Adoptive immunotherapy induces CNS dentritic cell recruitment and antigen presentation during clearance of a persistent viral infection. *Journal of Experimental Medicine* 203 (8): 1963–1975, with permission from Rockefeller University Press.

which is the engulfment of large volume of surrounding liquid. Within the secondary lymphoid organs, some DCs strategically localize within T-cell areas where they can optimally encounter circulating naive T lymphocytes that actively scan the DC network.

APCs are able to detect components of invading pathogens which trigger their activation/maturation. Specifically, pathogen-associated molecular patterns (PAMPs), as these components are termed, range from lipoproteins to proteins to nucleic acids carried by potential invaders. These PAMPs are recognized by evolutionary conserved 'pattern recognition receptors' (PRRs) on APCs. Among PRRs, the Toll-like receptors (TLRs) have emerged as critical players in determining APC imprinting on the ensuing immune response. TLR triggering has pleiotropic effects on APCs, promoting survival, chemokine secretion, expression of chemokine receptors, migration, cytoskeletal and shape changes, and/or endocytic remodeling. After interacting with these pathogen signatures, the microbial antigens are processed and presented as peptides associated with MHC molecules and activated APCs upregulate both antigen-presenting and co-stimulatory molecules initiating a 'maturation' process. As part of this process, APCs in peripheral tissues change their chemokine receptors and initiate migration to secondary lymphoid organs where the adaptive immune response is initiated.

The strategic migration and location of DCs into T-cell areas of the secondary lymphoid organs coupled to their superior antigen-presenting capacity make them the most powerful APCs. Indeed, DCs are about 1000 more efficient than B cells or macrophages in stimulating naive T cell. This has been shown by several experiments in which elimination of DCs prevented the initiation of antigen-specific T-cell responses. Interestingly, DCs are a heterogeneous cell population composed of different subtypes which present unique and overlapping functions. As many as six different subsets of DCs occupy the lymph nodes. Three major defined populations of DCs have been recognized in mouse spleen and humans: CD8+ conventional DCs (cDCs), CD11b+ cDCs, and plasmacytoid DCs. These subpopulations differ not only in surface phenotype but also in functional potential and localization. In this regard, cDCs are potent activators of naive T lymphocytes, as CD8+ DCs are believed to be specialized in cross-presentation of exogenous antigens. A recent study suggests that CD8+ and CD11b+ cDCs differ from each other in their intrinsic antigen-processing capacity being specialized in MHC-I and MHC-II antigen presentation, respectively. In contrast, plasmacytoid DCs are poorer activators of T cells, even after stimulation *in vitro*. They likely play a more protagonist role during innate immunity by secreting specific cytokines and chemokines, such as type 1 interferons (IFNs an important antiviral mediator), and activation of a broad range of effector cells, such as natural killer (NK) cells. Thus, the heterogeneity inherent to DC populations significantly influences the varieties of immune responses to different pathogens, which are subsequently amplified by cross talk between the various subsets.

Major Pathways of Antigen Presentation

Although in all healthy individuals MHC molecules play the same crucial role of antigen presentation, these molecules are highly polymorphic. There are hundreds of different alleles encoding the MHC molecules in the whole population and each individual exhibits only few of them. The major allelic variants of MHC are found in key amino acids forming the peptide-binding cleft. Thus, although a given MHC-I molecule can bind several different peptides; particular amino acids are preferred in certain positions of the peptide resulting in differential peptide sets for particular MHC variants. Importantly, T-cell specificity involves co-recognition of a particular antigenic peptide together with a particular MHC variant, a feature known as T-cell MHC restriction.

Like other polypeptide chains of proteins destined to arrive at the cell surface, MHC molecules are translocated to the lumen of the endoplasmic reticulum (ER) during synthesis. In this compartment, the subunits of MHC molecules are assembled together and the peptide-binding groove or cleft is formed. However, MHC molecules are unstable in the absence of bound peptide. In the following sections, we will consider how MHC molecules are folded and generated peptides are bound to MHC-I or MHC-II molecules. After binding, MHC–peptide complexes travel to the cell surface where they are recognized by antigen-specific T cells. Although not discussed in this article, it should be noted that other MHC-like molecules (i.e., CD1) also display peptide and lipid antigens contributing to antigen presentation, especially during mycobacteria infections.

MHC-I Antigen Presentation

MHC-I molecules are expressed in most if not all nucleated cells. MHC-I molecules are heterodimers of a highly polymorphic α-chain (43 kDa) that binds noncovalently to β2-microglobulin (12 kDa), which is nonpolymorphic. The α-chain contains three domains. The α3 domain crosses the plasma membrane while the α1 and α2 domains constitute the antigen-binding site. The peptides that bind the MHC-I molecule are usually 8–10 amino acids long and contain key amino acids at two or three positions that anchor the peptide to the MHC pocket and are called anchor residues.

As mentioned above, the peptide-binding site of MHC molecules is formed in the ER. However, all proteins,

including viral-derived antigens, are synthesized in the cytosol. Numerous studies in the last years outlined the molecular events connecting the antigen generation in the cytosol with the peptide binding to the MHC-I molecule in the ER. A highly conserved multicatalytic proteasome complex is in part responsible for cytosolic protein degradation into small peptides. The proteasome contains 28 subunits forming a cylindrical structure composed of four rings, each of seven units. Under normal conditions, the proteasome complex exists in a constitutive form. During viral infections, IFNs released by cells of the innate immune system induce the synthesis of three different proteasome subunits, which replace their constitutive counterparts to form the immunoproteasome. This inflammatory form of the proteasome favors the production of peptides with a higher chance of MHC binding. Moreover, IFNs can also enhance the rate of proteasome peptide degradation increasing the availability of peptides and reducing their excessive cleavage. It is important to highlight that other cytosolic proteases also contribute to MHC-I peptide generation and further cleavage can occur within the ER before MHC binding. The source of peptides for MHC-I complexes still holds its secrets. Proteasome substrates may encompass *de novo* synthesized, mature stable, and/or defective proteins. It is believed that defective ribosomal products (DRiPs), which are proteins targeted for degradation due to premature termination or misfolding, constitute an important source of MHC-I peptides.

Peptides available in the cytosol are transported into the lumen of the ER by ATP-dependent transporters-associated antigen-processing 1 and 2 (TAP-1 and TAP-2) proteins. TAP proteins are localized in the ER membrane forming a channel through which peptides can pass. Within the ER, the newly synthesized MHC-I α-chain binds to a chaperone molecule called calnexin, which retains the incomplete MHC molecule in the ER. After binding to the β2-microglobulin, calnexin is displaced and the emerging MHC molecule binds to a loading complex composed by the chaperone protein calreticulin, TAP, the thiol oxidoreductase Erp57, and tapasin, which bridges MHC-I molecule and TAP. After peptide binding, the fully folded MHC-I molecule and its bound peptide are released from the complex and transported to the cell membrane. Importantly, under steady-state conditions, the MHC-I molecules in ER are in excess with respect to peptides allowing the rapid appearance of microbial peptides onto the cell surface during infection. However, since MHC-I molecules are unstable without bound peptide, they also present self antigens under normal conditions. Because of the absence of microbial signatures, antigen presentation of self peptides by inactivated/immature APCs leads to T-cell tolerance rather than activation. This is one of the important ways anti-self or autoimmune responses are controlled.

For several years, intracellular peptides were believed to be the only source of MHC-I molecules. However, it is now clear that exogenous proteins also have access to the cytosolic compartment and bind MHC-I in the ER. This mechanism is known as cross-presentation and is believed to be particularly important for enabling MHC-I presentation by cells that are not directly infected by the virus but instead are engulfing viral particles by phagocytosis or micropinocytosis. The molecular mechanism by which MHC-I molecules access exogenous peptides is of considerable interest. Different nonexclusive possibilities have been proposed, including sampling of phagosome-generated peptides by MHC-I molecules, transference of ER molecules (including MHC-I and its loading complex) into phagosomes, re-entry of plasma membrane MHC-I into recycling endosomes with the subsequent peptide exchange, and finally the acquisition of peptides from other cells through GAP junctions.

MHC-II Antigen Presentation

The MHC-II molecule is composed by two noncovalently bound transmembrane glycoprotein chains, α (34 kDa) and β (29 kDa). Each chain has two domains and altogether form a four-domain heterodimer similar to the MHC-I molecule. α1 and β1 domains form the peptide-binding cleft resulting in a groove which is open at the ends, which is different from the MHC-I groove in which the extremes of the peptide are buried at the ends. Peptides that bind to MHC-II are larger than those that bind to MHC-I molecules, being 13–17 amino acids long or even much longer.

Since MHC-I is a surface protein, its biosynthesis is initiated in the ER. To prevent newly synthesized MHC-II molecules from binding cytosolic peptides that are abundant in the ER, its peptide-binding cleft is covered by a protein known as MHC-II-associated invariant chain (Ii). Through a targeting sequence in its cytoplasmic domain, the Ii also directs MHC-II molecules to acidified late endosomal compartments, where Ii is cleaved leaving only the Ii pseudopeptide (CLIP) covering the peptide-binding groove. MHC-II molecules bound to CLIP cannot bind other peptides, indicating that CLIP must be dissociated or displaced by the antigenic peptide.

Proteins that enter the cell through endocytosis or are derived from pathogens that replicate in vesicles are degraded by endosome proteases. These proteases become activated as the endosome pH progressively decreases. The final set of peptides available in the endosomal compartment is a result of antigen processing by several acid proteases that exist in endosomes and lysosomes. For instance, the cathepsin S is a very predominant acid protease and mice deficient in this enzyme have a compromised antigen-processing capacity. Vesicles carrying

peptides fuse with the vesicles carrying MHC-II molecules, achieving CLIP dissociation and the incorporation of antigenic peptides to MHC-II molecules. An MHC-II-like molecule that is predominant in the endosome facilitates this process. This molecule contributes to 'peptide editing', removing weakly bound peptides and assuring that the emerging MHC-II–peptide complexes are stable enough to be scanned by CD4 T cells.

MHC-II molecules seem to be in excess and are rapidly degraded unless microbial peptides become available to fill the groove. This excess is important to permit MHC-II availability upon infection. However, during infection, APCs are exposed to both self and microbial peptides. How APCs discriminate between self and non-self represents a fundamental question in immune biology. Recent evidence suggests that the efficiency of presenting antigens from phagocytosed cargo is dependent on the presence of a TLR ligand within the cargo. Thus, TLR signaling would mark a particular phagosome for an inducible mode of maturation dictating the fate of the cargo-derived peptides and favoring their presentation by MHC-II molecules in a phagosome autonomous fashion.

Because they travel through the endocytic pathway, which can be considered a topological continuation of the extracellular space, MHC-II molecules were believed to be specialized in the presentation of exogenous antigens. However, the analysis of MHC-II peptidome revealed many peptides of cytosolic or even nuclear origin. Autophagy or 'self-eating' explains MHC-II access to cytosolic peptides. This highly conserved pathway could be accomplished by several mechanisms including microphagy (when lysosomal invagination sequesters cytosolic componets), macrophagy (when a double membrane structure that encloses and isolates cytoplasmic components and eventually fuses with lysosome), and chaperone-mediated autophagy (when cytosolic proteases generate peptides that are transported into lysosomes).

Viral Subversion of Antigen Presentation

Considering the crucial role of antigen presentation for host defense, it is not surprising that many viruses have evolved maneuvers to evade or divert this process. Particularly, the essential role played by APCs in host defense to pathogens makes them an ideal target for viruses to suppress the immune response, thereby maximizing their chances of survival, replication, and transmission. Indeed, many viruses that cause major health problems are able to interfere with the ability of APCs to prime an efficient and effective antiviral immune response. In fact, many viruses have developed different mechanisms to subvert each stage of APC biology. Furthermore, with the greater understanding of antigen presentation pathways comes the discovery of novel viral immune-evasion strategies. In this section, we illustrate selective viral strategies to subvert antigen presentation by describing particular cases.

An interesting example of virus blockade of antigen presentation from very initial steps is the ability of the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV), to dramatically block DC development from early hematopoietic progenitors. Fms-like tyrosine kinase 3 ligand (Flt3L) is known to induce the expansion of undifferentiated progenitors into DCs within the spleen and bone marrow (approximate 20-fold increase), both in mice and humans. In contrast, LCMV-clone (CL)-13 that suppresses the immune response and causes a persistent infection in mice is associated with DC early progenitors that become refractory to the stimulatory effects of Flt3L.

TLRs function in APCs as an early sensor against pathogens; therefore, impairment in TLR signaling confers another selective advantage to certain infectious agents. As an example, vaccinia virus (VV) blocks TLR signaling and the subsequent maturation of APCs. Specifically, two proteins of VV suppress intracellular signaling of interleukin-1 (a potent pro-inflammatory host factor) and TLR-4.

Migration of DCs is a crucial step in initiating the adaptive immune response. Examples of viruses that have developed mechanisms to prevent migration of infected DCs to lymphoid organs are herpes simplex virus (HSV) 1 and human cytomegalovirus (HCMV). In both cases, there is an inhibition of complete DC maturation and subsequent expression of chemokine homing receptors. In addition, HCMV inhibits DC migration one step further by preventing APCs from arriving at a site of infection by producing homologs of chemokines that interfere with host pro-inflammatory chemokine gradients.

Another effective immune-evasion strategy used by viruses to disrupt APCs is the prevention of or interference with antigen-specific T-cell activation. The ability to disrupt MHC–peptide binding has evolved in many different virus species including adenovirus and human immunodeficiency virus (HIV). Herpesviruses have also evolved to block host cell antigen presentation. Some mechanisms utilized by herpesviruses to disrupt the antigen-presentation pathway include blocking peptide transport to the ER through interference with TAP proteins (HSV ICP47, HCMV US6), transport of particular MHC-I heavy chains from the ER to the cytosol where they are destroyed (HCMV US11, HCMV US2), retention of specific MHC-I heavy chains in the ER (HCMV US3, murine CMV–MCMV-m152), and disruption of T-cell recognition of MHC-I on the cell surface (MCMV m04). That viruses have independently evolved numerous mechanisms to disrupt MHC–peptide presentation indicates the effectiveness and importance of this strategy to the survival of viruses with different infectious life cycles.

Maturation of APCs results in upregulation of co-stimulatory molecules and expression of cytokines that enable them to stimulate naive T cells. Viruses that can impair T-cell stimulation by preventing the upregulation of co-stimulatory molecules include Ebola virus, Lassa fever virus (LFV), HSV-1, and HIV. Additionally, a number of viruses (hepatitis C virus (HCV), HIV, measles virus (MV), and dengue virus (DV)) are also able to inhibit interleukin (IL)-12 production, which is often required for effective T-cell response. HCV does this through the action of its core and nonstructural protein 3 (NS3), which induces production of IL-10. DV, on the other hand, is able to inhibit IL-12 production through an IL-10 independent mechanism. In addition, compelling evidence showed that *in vivo* persistent infection of mice with LCMV, as well as persistent HCV infection in humans, induces IL-10 production by APCs resulting in the blunting of the CD8 T-cell response and chronic viral infection. Remarkably, antibodies blocking IL-10/IL-10R interactions correct T-cell exhaustion by restoring T-cell function, which results in purging of virus from mice persistently infected with LCMV.

Finally, a novel immunosuppressive molecule, programmed cell death-1 (PD-1), is upregulated in nonfunctional CD8 T cells during chronic infections (LCMV, HIV, HCV). Interaction of PD-1 with PD-ligands on APCs (or parenchymal cells) inhibits lymphocyte activation. As for IL-10 blockade, antibodies interfering with PD-1/PDL interactions also promote viral clearance from a persistently infected host.

The fact that not all viruses are able to block APC maturation does not necessarily represent a failure of the pathogen or a success for the host. A good example of this is observed following MV infection that exploits the ability of DCs to mature and migrate to lymphoid organs in response to infection. MV benefits greatly by having infected DCs home to lymphoid compartments where the infected cells are able to actively suppress T-cell proliferation (mediated through T-cell contact with surface viral glycoproteins) and also facilitate virus spread to more lymphoid cells. Therefore, the full understanding of the virus–host relationship requires not only studying the active mechanisms that viruses use to disable the immune system, but by also asking how a virus benefits by not altering a particular immune function.

Concluding Remarks

Co-evolution of certain hosts and pathogens for millions of years has resulted in a fine-tuned equilibrium that enables survival of both. Antigen presentation is one of the critical elements in this balance. While antigen presentation is an essential process for long-term effective host defense, targeting APCs represents a common maneuver of many viruses to avoid host surveillance and establish a chronic or persistent infection. A major challenge in biomedical research is to thwart microbial APC subversion to promote eradication of the pathogen. A better understanding of the mechanisms used by APC to display microbial antigens as well as the virus strategies to subvert APC functions during immune responses will provide new tools for designing novel vaccination approaches and immunotherapeutic treatments for human infectious diseases.

Acknowledgments

This is publication no. 18909 from Molecular and Neuroscience Integrative Department, The Scripps Research Institute (TSRI). This work was supported by NIH grants AI 45927, AI 05540, and AI 09484.

E. I. Zuniga is a Pew Latin American Fellow.

See also: Cytokines and Chemokines; Immune Response to viruses: Antibody-Mediated Immunity; Immune Response to viruses: Cell-Mediated Immunity; Immunopathology; Persistent and Latent Viral Infection; Vaccine Strategies.

Further Reading

Bevan MJ (2006) Cross-priming. *Nature Immunology* 7: 363–365.

Blander JM and Medzhitov R (2006) On regulation of phagosome maturation and antigen presentation. *Nature Immunology* 7: 1029–1035.

Dudziak D, Kamphorst AO, Heidkamp GF, *et al.* (2007) Differential antigen processing by dendritic cell subsets *in vivo*. *Science* 315: 107–111.

Itano AA and Jenkins MK (2003) Antigen presentation to naive CD4 T cells in the lymph node. *Nature Immunology* 4: 733–739.

Janeway CA, Travers P, Walport M, and Shlomchik MJ (2005) *Immunobiology: The Immune System in Health and Disease.* New York: Garland Science Publishing.

Lauterbach H, Zúñiga EI, Truong P, Oldstone MB, and McGavern DB (2006) Adoptive immunotherapy induces CNS dendritic cell recruitment and antigen presentation during clearance of a persistent viral infection. *Journal of Experimental Medicine* 203(8): 1963–1975.

Menendez-Benito V and Neefjes J (2007) Autophagy in MHC class II presentation: Sampling from within. *Immunity* 26: 1–3.

Norbury CC and Tewalt EF (2006) Upstream toward the 'DRiP'-ing source of the MHC class I pathway. *Immunity* 24: 503–506.

Oldstone MB (2007) A suspenseful game of 'hide and seek' between virus and host. *Nature Immunology* 8: 325–327.

Reis e Sousa C (2006) Dendritic cells in a mature age. *Nature Reviews Immunology* 6: 476–483.

Shen L and Rock KL (2006) Priming of T cells by exogenous antigen cross-presented on MHC class I molecules. *Current Opinion in Immunology* 18: 85–91.

Shortman K and Liu YJ (2002) Mouse and human dendritic cell subtypes. *Nature Reviews Immunology* 2: 151–161.

Strawbridge AB and Blum JS (2007) Autophagy in MHC class II antigen processing. *Current Opinion in Immunology* 19: 87–92.

Yewdell JW and Nicchitta CV (2006) The DRiP hypothesis decennial: Support, controversy, refinement and extension. *Trends in Immunology* 27: 368–373.

Antigenic Variation

G M Air and J T West, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Envelope protein (Env) The surface spike of HIV-1.
Gp120 The subunit of HIV-1 Env that binds to receptors on cells to initiate infection.
Gp41 The subunit of HIV-1 Env that carries fusion activity.
Hemagglutinin Influenza surface glycoprotein that binds cell surface receptors to initiate infection.
Neuraminidase Influenza surface glycoprotein that cleaves sialic acid receptors to spread virus to new cells.
Neutralizing antibody An antibody that blocks virus infection.

Manifestations of Antigenic Variation

Antigenic variation refers to the observation that different isolates of a single virus species may show variable cross-reactivity when tested with a standard serum. The homologous virus (the isolate that was used to raise the antiserum) usually shows the highest reactivity. Cross-reactivities with other viruses of the same species may vary from high to zero. While zero cross-reaction is due to a different type or subtype of the species, intermediate reactivities define antigenic groups or serotypes.

Immune response to viruses is generally thought of as negative selection – that is, elimination of the pathogen. There are a few examples where immune recognition has a positive influence on the continued proliferation of the recognized pathogen, due to viral mechanisms to exploit immune elimination. Some viruses can use Fc receptors or mannose receptors to internalize antibody-bound virus into a replication mode instead of a destructive one. Alternatively, the virus may sabotage the immune response by changing antigenicity. A primary basis of antigenic variation is selection of virus mutants by antibodies. These are known as escape mutants, and since the escaped virus is resistant to antibody neutralization it possesses a fitness advantage in the presence of antibody. Escape mutants may also be selected by CD4+ or CD8+ T cells. There is certainly variation in the T-cell epitopes, but a lack of definitive examples that these have been selected by T cells as an immune evasion mechanism in the same way as occurs with antibody selection.

There are several mechanisms that allow mutations to occur with sufficiently high frequency to be selected by antibodies. Viruses with an RNA genome show the highest degrees of antigenic variation. The mutations are not induced by antibody but are present in the population and can be selected out by antibodies when wild-type virus particles are neutralized. The origin of this rather high rate of mutation is the viral replicase. RNA polymerases have no 3′ editing function and so insertion of an occasional mismatched base is not corrected and quasispecies with random mutations are present in any virus population. The mystery of RNA viruses is not why they show so much antigenic variation, but why some RNA viruses are antigenically almost invariant. Rhinoviruses exist in over 100 serotypes while poliovirus, another picornavirus, is stable enough that the vaccine did not need updating during the WHO Global Eradication campaign. Dengue virus exists in four serotypes and shows large variations in antigenic cross-reactivity within each serotype, but another flavivirus, yellow fever virus, is antigenically stable. Respiratory syncytial virus exists as two distinct antigenic groups with high antigenic diversity within each group, but other paramyxoviruses, such as measles, mumps, and rubella, are stable and therefore amenable to easier vaccination protocols.

It is clear from the above observations that vaccine success is inversely related to the degree of antigenic variation in the targeted virus. The measles-mumps-rubella vaccine has reduced those diseases almost to zero in the developed world and where vaccine compliance is high, but there is still no vaccine against respiratory syncytial virus. It does not follow that an antigenically stable virus necessarily leads to a successful vaccine, since viruses have evolved many different mechanisms to evade the immune system. Some viruses remain hidden from antibodies – for example, in neural tissues. Some make cytokine mimics that block immune signaling, or code for proteins that inhibit signaling pathways. But it is antigenic variation that causes as yet insurmountable barriers to making effective vaccines for many pathogens where the diversity is high. If all antigenic groups are represented in the vaccine, the dose becomes extremely large with consequent danger of adverse side effects. The recently licensed human papillomavirus vaccine contains the three genotypes considered oncogenic, but difficulties in culturing human papillomaviruses has precluded a serological classification of the more than 100 genotypes that exist and it remains unclear how broadly effective the

current vaccine will be in areas where different or unique subtypes co-circulate.

DNA viruses do not generally incorporate mutations during replication. DNA polymerases can only extend from a base that is correctly hydrogen bonded to its partner on the template strand. A mismatched nucleotide causes synthesis to stop until the wrong base is excised by the 3′ nuclease activity. Nevertheless, DNA viruses have accumulated mutations during evolution, and some have a high rate of recombination that leads to antigenic diversity. Some small DNA viruses are quite diverse in sequence (papillomaviruses) and canine parvovirus has evolved different antigenic properties since it first appeared in the 1970s. There is evidence that the Epstein–Barr and Kaposi's sarcoma herpesviruses undergo some degree of antigenic variation.

There are distinct patterns of antigenic diversity among viruses. Most exist in several serotypes that 'rotate' in the human population, resulting in waves of disease. As antibodies accumulate in the population against one serotype, another can move in because it is not neutralized by those antibodies. Presumably, there is little antigenic memory against these viruses, because even with >100 serotypes of rhinoviruses, a person would eventually become exposed to all of them. Alternatively, the lack of immunity against all serotypes could be because the immune response focuses on single or a few immunodominant but noncross-reactive epitopes (a phenomenon known as original antigenic sin) or rapid clearance of the antigen may result in failure to achieve affinity maturation of the antibody response. Influenza viruses, on the other hand, are constantly evolving into new antigenic variants and the old ones never seem to return. It is not clear if other viruses, even very variable RNA viruses, undergo the same style of antigenic drift as influenza: a progressive, unidirectional evolution. The most variable virus of all is human immunodeficiency virus (HIV), the cause of AIDS. During HIV infection each infected individual generates a unique swarm of virus variants known as a quasispecies that diversify in a radial fashion. For many virus species, the pattern of variation is not well understood, due to infrequent epidemics, geographic isolation, or lack of sufficiently extensive molecular analysis. Here we compare and contrast the antigenic variability of the most-studied viruses, influenza virus and HIV.

Antigenic Variation in Influenza Viruses

Influenza viruses are classified as types A, B, or C based on cross-reactivity of internal antigens. Type A influenza viruses, those most commonly associated with human infection, show two distinct mechanisms of antigenic variation: antigenic shift and antigenic drift. Antigenic shift is a replacement of one subtype of surface antigen with another. There are two surface glycoprotein antigens, hemagglutinin (HA) and neuraminidase (NA) that exist in multiple serotypes. To date there are 16 subtypes of HA (H1–H16) and nine subtypes of NA (N1–N9), based on lack of antigenic cross-reactivity. H1, H2, H3, N1, and N2 have been found in human epidemic viruses. All subtypes are found in avian influenza viruses and thus birds are considered the natural viral reservoir, providing new antigens that occasionally are transferred into human viruses. The segmented influenza genome facilitates this replacement, since a new antigen gene can be readily exchanged into an existing human virus during a mixed infection without the need for RNA recombination. However, avian viruses do not easily infect mammals, so one hypothesis is that such mixing can only occur in a species that can host both of the parental viruses, such as the pig. Pigs have not been implicated in the transmission of avian influenza H5N1 to humans, which continues to be a rare event, and so far no reassortant viruses with H5 HA have been found. Antigenic shifts are shown in **Figure 1**. In recent years influenza viruses circulating in humans have been H1N1, H3N2, and type B.

Antigenic drift is a less dramatic form of antigenic variation but is responsible for annual epidemics of influenza. The term refers to a gradual change in serological cross-reactivity when compared to the original pandemic virus. Drift has been continuous in H3 HA since 1968, in N2 NA since 1957, and in H1 and N1 since 1977. Drift is detected using ferret antiserum, which discriminates better than antisera of other species, raised against a particular H3N2 strain. There is some degree of cross-reactivity to viruses isolated before and after the appearance of that strain, typically over a period of about 10 years, but for better protection, the vaccine strain is changed as soon as cross-reactivity is decreased.

The progressive, unidirectional changes in antigenic cross-reactivity correlate with progressive accumulation of changes in amino acid sequence. **Figure 2** shows the sequence changes that have occurred in the H3 HA since it appeared in the human population in 1968. Comparison of isolates in any given year shows the same antigenic properties and virtually the same sequence worldwide. This is commonly attributed to air travelers carrying the virus across and between continents, but it is also possible that the same mutations have been independently selected in different places.

Mechanism of Antigenic Drift

The mechanism of antigenic drift in influenza viruses is relatively well understood, although it does not allow one to predict the next epidemic strains. The development of monoclonal antibodies, and the demonstration that these can be used to select antigenic variants that escape from antibody neutralization by a single amino acid change, led to the mapping of neutralizing epitopes on both HA and

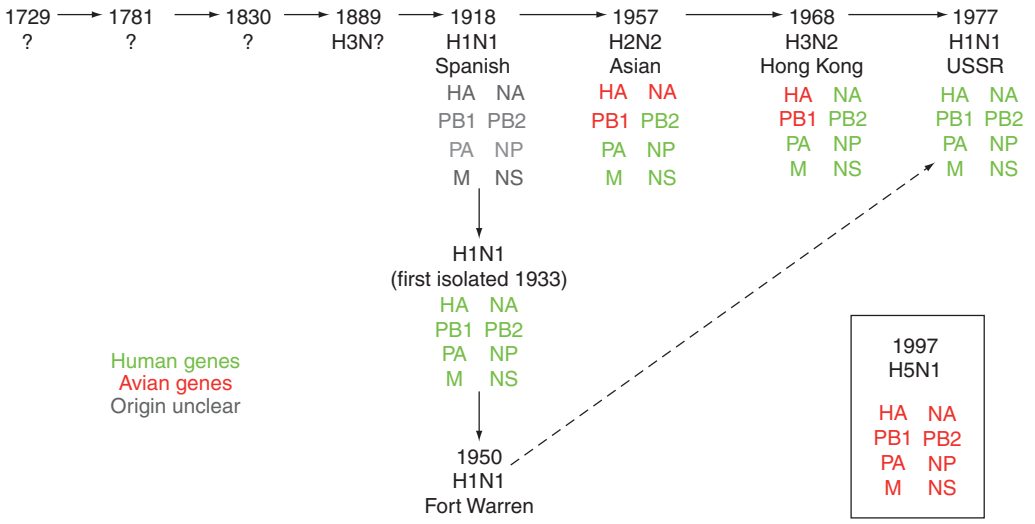


Figure 1 Influenza pandemics are very distinctive in their epidemiology and descriptions can be found back to ancient times. The figure shows some well-described pandemics (antigenic shifts) of more recent times. Retrospective serology of samples taken before the H3N2 pandemic of 1968 from people who had lived through the 1889 pandemic showed the presence of H3 antibodies. The virus was first isolated in 1933 and antibodies raised against it showed no break back to the pandemic of 1918, classified as H1N1. The H2 HA and N2 NA introduced in 1957 were both of avian origin, as was the H3 HA in 1968. The return of H1N1 in 1977 is not regarded as a pandemic since people over the age of 20 still had antibodies. The genes have been sequenced from the 1918 virus and many authors say they were of avian origin, but in the absence of an essentially identical avian influenza sequence from the same time period, this conclusion is not substantiated. The H5N1 virus that has occasionally infected people since 1997 is wholly avian in its genes. Potentially it could provide new antigens to human influenza viruses, or mutate to spread more widely into the human population.

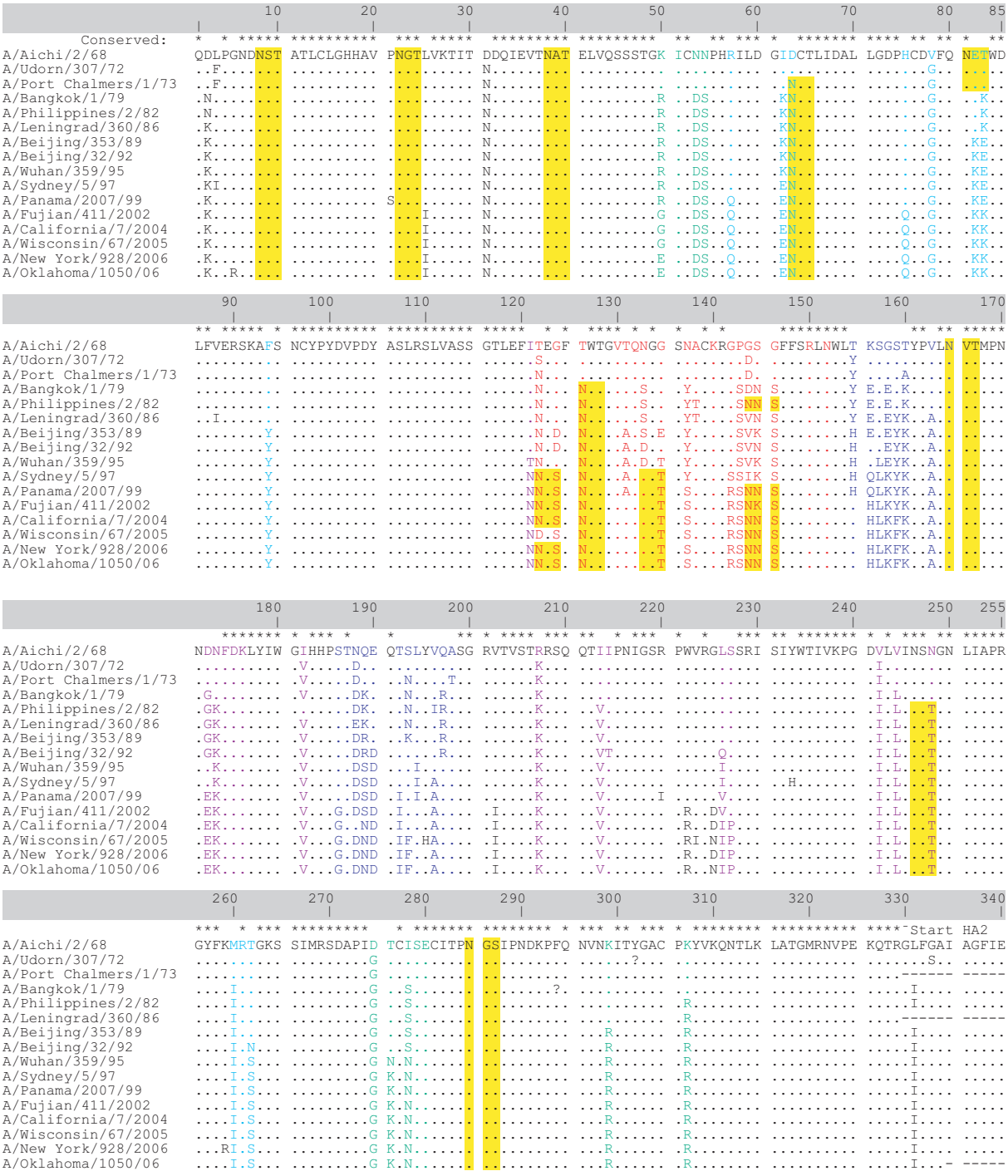
NA. Crystal structures of HA and NA bound to neutralizing antibodies show that the amino acids that change in escape mutants are within the epitope as defined by amino acids that contact the antibody. Potentially, escape mutants could have amino acid sequence changes outside the epitope causing a conformational change that is transmitted to the epitope, but this has not been observed. The reason is that a widespread conformational change would almost certainly affect the function of the HA or NA, debilitating the virus, and the selection of escape mutants can only occur if the mutants are close to wild type in fitness.

The neutralizing epitopes, determined crystallographically, consist of 11–18 amino acids that are separated in the linear sequence but come together in the three-dimensional structure to form a binding surface that exists only when the antigen is folded into its native structure (**Figure 2**). The footprint of an antibody on HA and NA is quite large and so it is not surprising that antibodies contact more than one linear segment of the protein. Many years of effort have not yielded a peptide-based vaccine for influenza and it seems unlikely that this will be possible. In contrast, for HIV there is still very active research ongoing to develop peptide-based vaccines or therapeutic antibodies that bind to linear peptides, because the surface antigen gp160 may be more dynamic than influenza HA or NA and linear segments may become transiently accessible to antibodies (see below).

Mechanisms of Neutralization

The basis of antibody-mediated neutralization of influenza virus (and HIV, see below) is generally considered to be blocking of biological function rather than Fc-mediated mechanisms such as phagocytosis or complement activation. The most commonly used assay to assess effectiveness of vaccination is the hemagglutination inhibition (HAI) test, which measures blocking of the receptor-binding site by antibodies. This might seem to be an oversimplification of many potential mechanisms of neutralization, but the correlation between HAI and neutralization or protection has held up for several decades. Similarly, although NA antibodies are not as much studied, monoclonal antibodies do not neutralize or protect from infection unless they inhibit the NA activity. Thus, neutralizing antibodies against influenza bind close enough to the sialic acid binding site (HA) or the enzyme active site (NA) to inhibit binding to receptors or substrates. The binding sites themselves have constraints on tolerating changes because they must retain function, but the polypeptide loops surrounding them are able to mutate so that the neutralizing antibody can no longer bind, or binds with such low affinity that it no longer inhibits function. Site-directed mutagenesis of all side chain contacts of the epitope has shown that a subset contributes the major energy of the interaction. These two or three critical contacts are the ones that are found to change in escape mutants, and are the only changes in site-directed mutants that abolish antibody inhibition. In a few cases, the

N-linked glycosylation: yellow
Antigenic sites: A red, B blue, C green, D magenta, E cyan



(a)

Figure 2 Continued

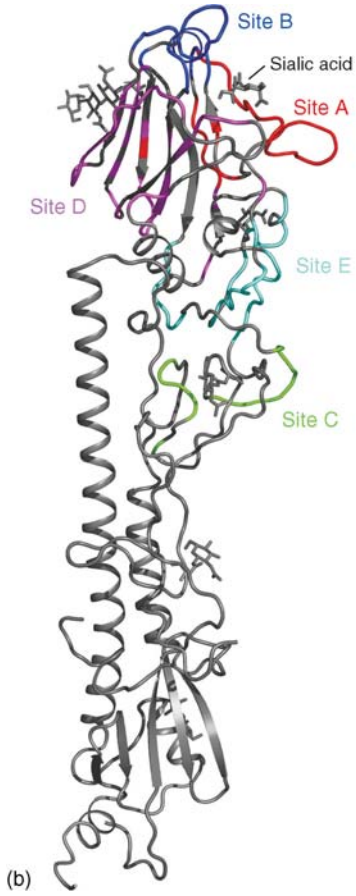


Figure 2 (a) The amino acid sequence of A/Aichi/2/68, representative of the first human H3N2 isolates. The HA precursor peptide is cleaved into two polypeptides called HA1 and HA2. Only HA1 is shown here since there are no known antigenic sites on HA2 and it shows very little sequence drift. Changes in HA1 of later viruses are shown, with dots where there is no change. Mutations that map in the five antigenic regions are colored: red, site A; blue, site B; green site C, magenta, site D; cyan, site E. The assignment of residues to antigenic sites is accessible at the Influenza Sequence Database (ISD). Sequons that predict N-linked glycosylation are shown as yellow bands. The progressive nature of antigenic (and genetic) drift is clearly seen; previous mutations are generally retained in later isolates. The sequences chosen were in most cases used as vaccine components, and essentially the same sequence (and antigenic character) was found worldwide. Two 2006 isolates are included for comparison. (b) Alpha carbon trace of a monomer of HA showing one or more sugars at each site of N-linked glycosylation and a bound sialic acid in the receptor binding site. Each peptide segment of the antigenic sites in (a) are seen to come together in the three-dimensional structure. Antigenic sites A through E are colored as in (a). Site D is partly buried in the trimer interface when the whole molecule is present. Site C is some distance from the receptor binding site but the crystal structure of a complex with antibody shows that the constant domain of the antibody reaches to and obscures the sialic acid binding site.

antibody still binds but does not inhibit function, but most commonly there is no detectable binding of antibody to a mutant that has a change in a critical contact. This explains how a change in only one out of the 11–19 amino acids that make up the epitope can eliminate the antibody inhibition, and greatly reduces the number of changes needed for a new antigenic variant.

How Does Antigenic Drift Occur?

The surface area of the HA accommodates a multitude of epitopes that have been grouped into antigenic sites A through E (**Figure 2**) by determining if binding of one antibody blocks the binding with another. If it does, the two antibodies are considered to belong to the same site. In the face of a human polyclonal antibody response, it would seem necessary to mutate each of the five distinct sites to generate an escape mutant. The early H3 variants show changes in all sites, but more recently the changes have been confined to fewer sites. The difference is likely to be in immunogenicity, and suggests that the human antibody response to recent viruses is not very polyclonal. The possible reasons include previous substitutions rendering a site nonimmunogenic, or blocking of sites by the addition of N-linked carbohydrates. A/Aichi/68 HA1 contained six carbohydrate chains but by 1999 there were 11 sites for carbohydrate addition. Site A may now be completely shielded from antibodies. The mobilization

and addition of carbohydrate is a primary mechanism of immune avoidance in HIV (see below). The antigenically significant transition from California/04 to Wisconsin/05 was due to changes only in site B.

Is There Significant Antigenic Drift in NA?

Antibodies against NA have been shown to contribute to protection. They do not inhibit entry of human viruses into cells but they inhibit spread of progeny viruses because sialic acid is not removed from the N-linked carbohydrates of the surface glycoproteins, causing aggregation of virus particles by HA–HA interaction. The rate of amino acid substitution in NA is about half that in HA, suggesting a lesser antigenic selection. Ferret antisera that show clear differences in HA antigenicity from 1 year to the next may not discriminate between drifted NAs. However, the crystal structure of the NA of A/Memphis/31/98 complexed with a neutralizing antibody suggests that NA may be contributing significantly to antigenic drift in H3N2 viruses. Of the 11 amino acids that make contact through side chains to the antibody, five have changed in isolates up to 2006 (**Figure 3**).

Antigenic Variation in HIV

Antibody and Antigenic Variation in HIV-1 Infection

Nearly all HIV-1-infected individuals develop antibodies capable of mediating some level of neutralization. The presence of strong, broadly neutralizing responses in some long-term nonprogressors suggests that neutralizing antibodies may be a correlate of protection, but their absence during acute infection, when the escalating HIV-1 viral load is reduced, suggests they play a minor role in initial immune control and that cellular immune responses are more effective. HIV-1 neutralizing responses take months or even years to develop in patients but during a long and chronic infection, antibody may be critical in limiting both virus-to-cell and cell-to-cell spread of virus. It is possible that non-neutralizing responses such as antibody-dependent cell-mediated cytotoxicity (ADCC) are important in acute infection.

In contrast to influenza, there seems to be no basis for a serological definition of subtypes in HIV-1. Instead, subtypes are defined based on genetic relatedness. HIV-1 exhibits intra-subtype sequence differences of up to 20% in the envelope glycoprotein gene (*env*) and differences of up to 35% between subtypes. Only antibodies directed against the viral envelope glycoprotein that limit or prevent virus entry into CD4-bearing target cells are effective in reducing viral replication. HIV-1 is also selected by cellular immune mechanisms, and recent studies suggest that CTLs target Env more often than previously appreciated; however, here we will focus on the antigenic variation in Env and its interplay with humoral immune selection.

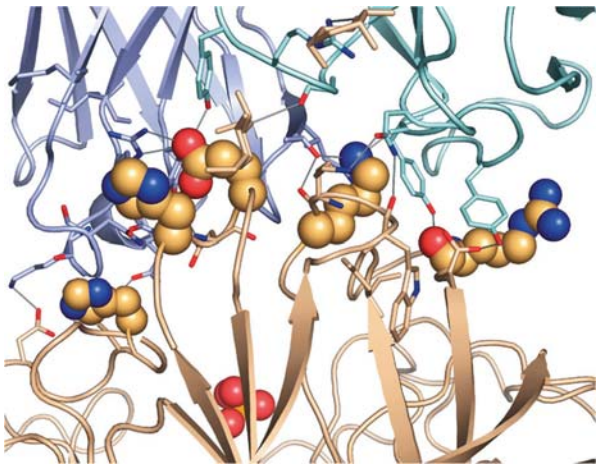


Figure 3 Changes in the epitope of a 1998 N2 NA up to 2006. The NA is brown, Fab H chain is dark blue, and L chain is light blue. Amino acid side chains that are in contact in the interface are shown as stick models, except for those on NA that have changed in later isolates, which are shown in space-filling form. Reproduced from Venkatramani L, Bochkareva E, Lee JT, *et al*. (2006) An epidemiologically significant epitope of a 1998 human influenza virus neuraminidase forms a highly hydrated interface in the NA-antibody complex. *Journal of Molecular Biology* 356(3): 651–663 (cover picture), with permission from Elsevier.

HIV-1 Evolves to Create Global and Intra-Patient Antigenic Variation

HIV-1, a member of the genus *Lentivirus* of the subfamily *Orthoretrovirinae* of the family *Retroviridae*, is one of the most rapidly evolving pathogens ever studied. Through a combination of rapid production of highly genetically diverse progeny and the extreme plasticity of many of its viral proteins, HIV-1 has managed to elude efforts aimed at control through drug treatment or vaccination. The most recent common ancestor of the HIV-1 pandemic subtypes came into the human population less than a century ago. Over the course of that relatively short interval, the virus has become not only one of the most significant human pathogens, but also one of the most diverse. The pandemic HIV-1 can be segregated into subtypes A–K (minus E, I, and K) (**Figure 4**). Most subtypes co-circulate in West-Central Africa pointing to this region, where the geographical habitat of chimpanzees and humans overlap, as a nexus for the phylogenetically inferred initial transmission events into humans. Circulating recombinant forms (CRFs) are rapidly emerging in areas where more than one subtype co-circulates. These recombinants pose a serious concern, as they can be thought of as analogous to reassortants in influenza and could rapidly obviate the effectiveness of vaccination unless sufficiently broad cross-reactivity can be generated. Antigenic

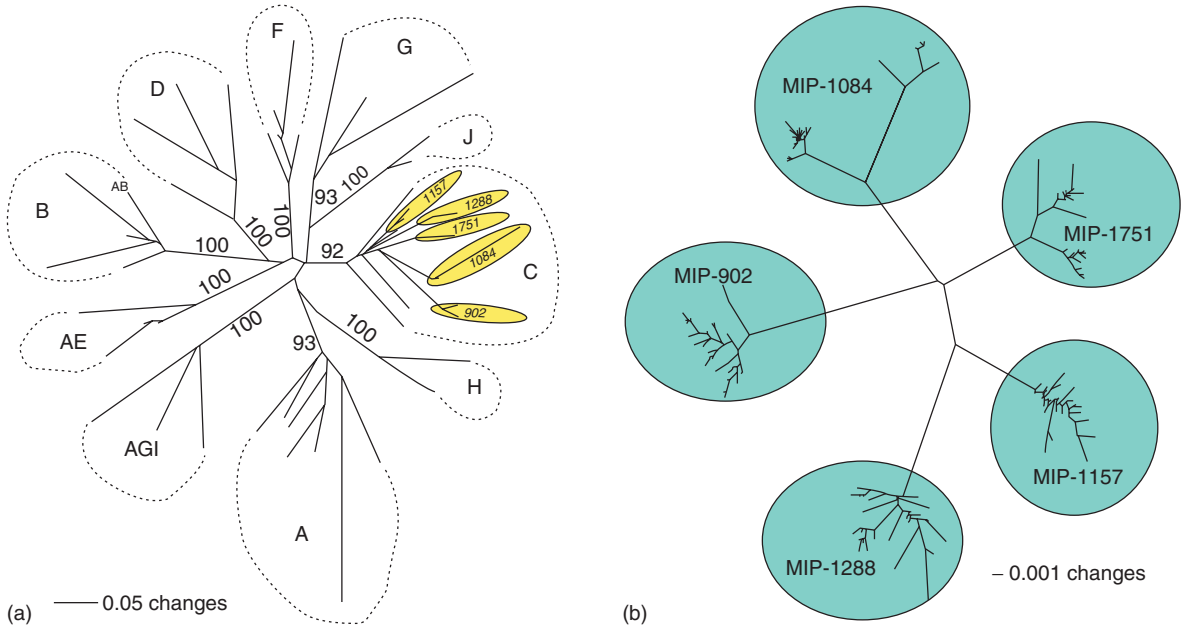


Figure 4 (a) Shows that the various HIV-1 subtypes segregate one from another when compared using envelope glycoprotein sequences and that individual viruses cluster within the subtype into distinct branches. (b) An unrooted phylogenetic tree shows that each of five mother–infant pair's glycoprotein sequences form an independent lineage despite all being subtype C viruses isolated from the same geographic region. Over time, infant and maternal sequences will also segregate as they undergo independent viral antigenic variation and selection. HIV-1 diversification appears perpetual.

variation within the global HIV-1 population, as well as within each infected individual, is a primary mechanism underlying the success of HIV-1 as a human pathogen.

The HIV-1 Replicase and the Generation of Diversity/Variation

In order to understand the mechanisms that produce variation, it is necessary to become familiar with some of the basic processes of retroviral replication. HIV-1 contains two copies of message (+)-sense RNA. Replication of the HIV-1 genome proceeds through a dsDNA intermediate that becomes permanently integrated into the target cell genome. The HIV-1 replicase is an RNA-dependent DNA polymerase or reverse transcriptase (RT). The HIV-1 RT lacks any proofreading capacity. The rate of misincorporation of nucleotides by HIV-1 RT is between 10^{-3} and 10^{-4}/position/round of replication. Given a genome size of approximately 10 000 base pairs this suggests that HIV-1 introduces at least one mutation with each genome copy.

HIV-1 Diversity Influences Its Population Genetics

The viral loads in plasma for individuals undergoing acute infection are as high as 10^8 copies ml^{-1}. This means that the virus has the potential to produce all possible single mutations each day, as well as a population containing multiple mutations. The replication rate is accompanied by rapid viral and infected cell turnover, pressure to avoid immune recognition and elimination, all coupled with fitness maximization. The population of closely related but distinct viruses generated by replication is referred to as a quasispecies. For HIV, this term refers to the dynamic group of viral genotypes found in an infected individual at a given sampling. It is the creation of this swarm of subtly different progeny viruses and the resultant selection of those progeny that drives HIV natural selection and antigenic variation.

In addition to single-base mutations, HIV readily undergoes recombination but this does not occur through reassorting genomic segments as in influenza. HIV-1 does not possess a segmented genome, but instead packages two copies of viral genomic RNA. If co-infection of target cells and co-packaging of distinct RNA genomes occurs, reverse transcriptase can switch viral RNA templates during synthesis of DNA. The result is the creation of large insertions and deletions that introduce antigenic changes in a single replication cycle. While co-infection has proved difficult to monitor *in vivo*, the globally increasing number of HIV-1 CRFs suggests that co-infection occurs with relatively high frequency. In the absence of a vaccine the abundance of such recombinants will only increase given the continuation of human cultural, medical, and sexual practices that promote virus transmission, and the occurrence of infection rates in the developing world as high as 35%. Both vaccine development and drug treatment efforts will be further complicated as these recombinants become more widely intermixed and distributed.

The HIV-1 Envelope Glycoprotein: Variable Loops and Decoys

The target of HIV-neutralizing responses is the envelope glycoprotein. The glycoprotein complex exists in the virion membrane as a trimer of noncovalently associated heterodimers of surface protein (SU) and transmembrane protein (TM). The gp120 component is the most exposed component of the complex and contains five hypervariable loops (V1–V5) and five relatively invariant or constant regions (C1–C5). Vaccination with gp120 peptides, glycoprotein, or glycoprotein-expression constructs has met with only limited success, as defined by elicitation of broadly neutralizing antibody responses. Neutralization of HIV-1 correlates with blocking virus entry into cells. The diversity of HIV sequences means that the vast majority of epitopes are not shared and thus the only hope for vaccination is to target conserved epitopes. Much of what we know about Env epitopes that are cross-reactive comes from studies of a handful of human monoclonal antibodies that have broad neutralizing activity for multiple viral strains and subtypes. Structural studies of some of these antibodies in complex with gp120 have revealed their binding sites and it is now clear that they do not bind to linear epitopes but to conserved structural or conformational epitopes that the virus has evolved to rarely reveal to the immune system (**Figures 5** and **6**).

HIV-1 entry is a series of steps mediated through interactions between Env and receptors on the target cell

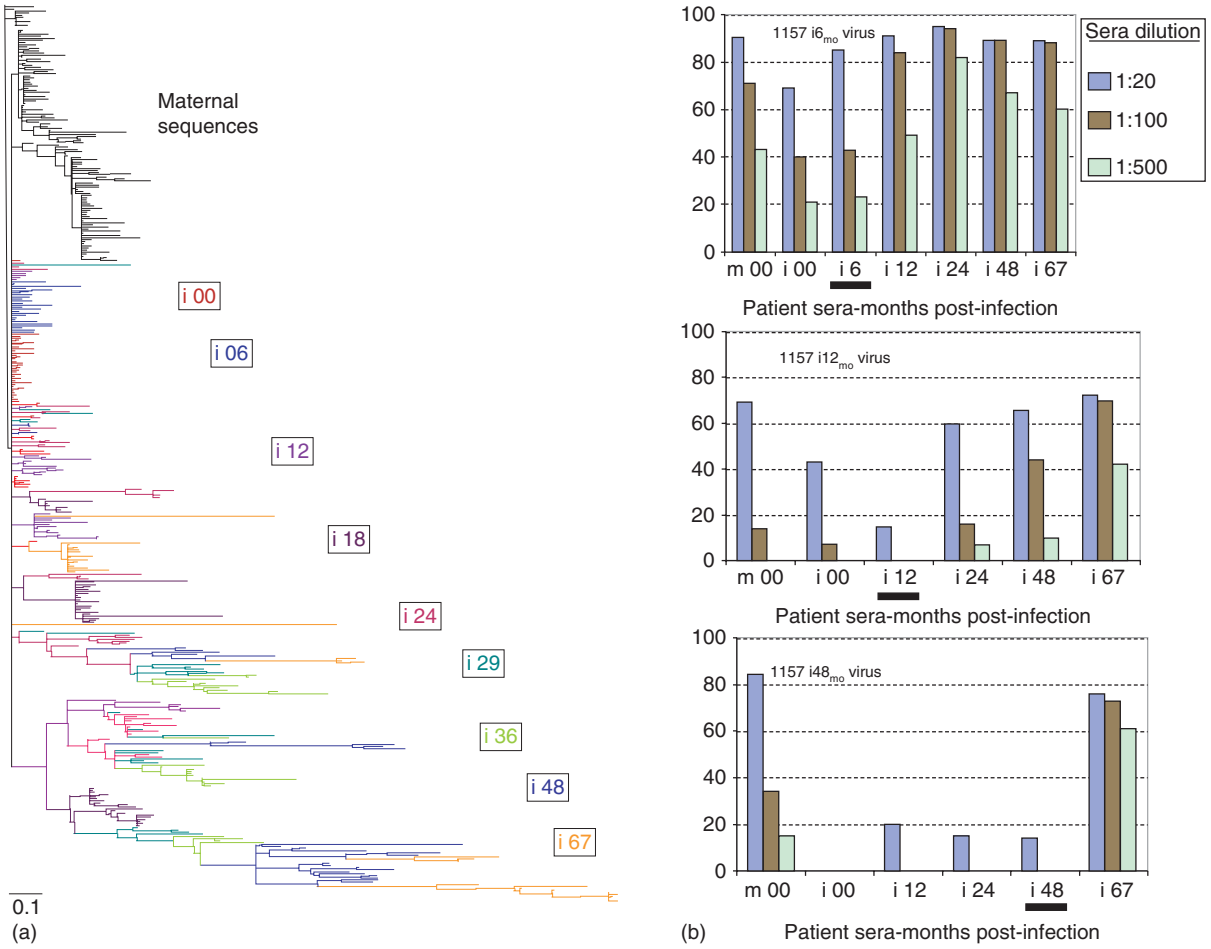


Figure 5 (a) A maximum-likelihood phylogenetic tree displaying the diversifying evolution of HIV-1 Env in a single individual. The numbers in color indicate the months post infection when samples were obtained for sequence analysis. Note that divergence from a maternal root sequence continues with time and that at later time points the virus appears to resample earlier genotypes. (b) Neutralization of viruses derived from some of the time points in (a) by autologous contemporaneous and noncontemporaneous sera suggests that recognition of early (6 month) viruses is maintained even though the genotype is clearly replaced, and that the neutralizing response generated early is incapable of neutralizing virus generated after selection (12 month serum is ineffective on 12 month virus or subsequent time points). In total, these data suggest that diversity generates an antibody response that is never 100% effective, not broadly reactive and perhaps mistargeted by 'original antigenic sin' as discussed in the text.

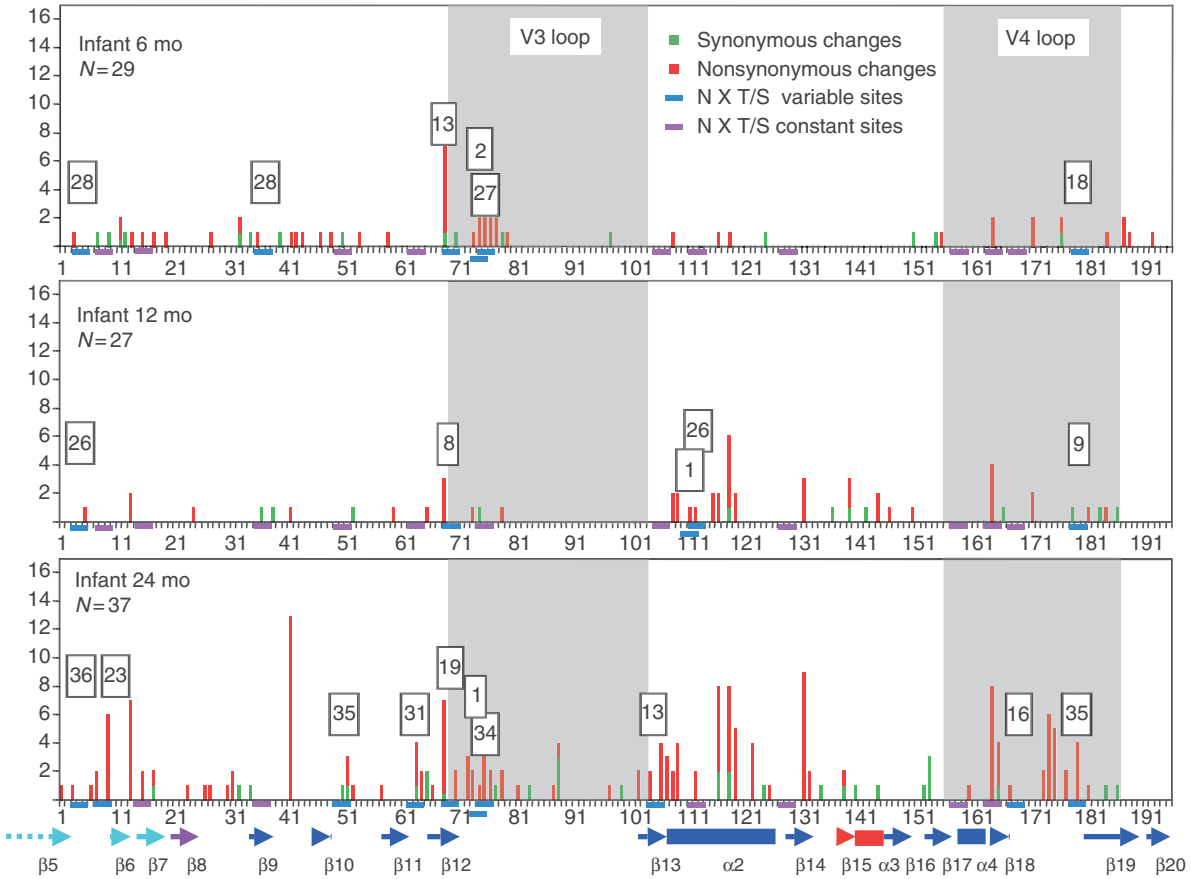


Figure 6 Amino acid sequence changes found in the HIV-1 subtype C Envelope glycoprotein in a newly infected child. C2-V4 diversity (amino acids 228–427 in HXB2 numbering) for sequences in a single patient (*N* = number of sequences per time point) in relation to potential glycosylation sites that are either constant in the population at that time point (blue) or show variability (pink). The proportion of the glycans maintained, for variable sites, is shown in the boxes above the sites. Red bars indicate the presence of nonsynonymous changes (codon level) and their height indicates the abundance in the population. Green bars indicate synonymous polymorphism. Note that variability increases with time, sites undergoing positive selection (red bars) change with time in some instances but not in others, and that glycan addition sites change both in abundance and position over the course of infection. Designations at the bottom of the figure shows relationships to structural elements identified in the unliganded SIV envelope glycoprotein compared with HIV-1 structures PDB id 2BF1.

plasma membrane. The first of these steps is a high-affinity interaction between gp120 and the primary receptor CD4. Thus, antibodies that interfere with CD4 binding would be anticipated to be effective at inhibiting infection. The interaction with CD4 induces substantial conformational changes in Env that lead to exposure of the chemokine co-receptor (usually CCR5 or CxCR4) binding site in the V3 loop and other induced structures. The CD4-induced conformation is another step at which it is thought that neutralizing antibodies might exert a selective and protective influence. The interaction with CD4 and chemokine co-receptor induces further changes in the gp120 structure that promote exposure of gp41 and insertion of its N-terminal fusion domain into the target cell membrane. This final step is also potentially a target for recognition of epitopes and the development of fusion-inhibiting antibody responses. However, despite considerable antigenic diversity, the HIV-1 glycoprotein has evolved to be an exceedingly poor neutralization immunogen; at least it is a very poor inducer of broadly neutralizing responses particularly at conserved sites most pertinent to the functional processes of Env defined above.

Some of the hypervariable loops of gp120 tolerate extensive sequence variation as well as insertion and deletion, and are excellent inducers of antibody, but rarely neutralizing antibody. In the event a loop-induced neutralizing response is generated, it is isolate specific and not cross-reactive; the virus utilizes the diversity it generates through replication to escape the neutralizing response and repopulate. Thus such loop-targeted antibodies are unlikely to have durable neutralizing capacity even in the individual. It is therefore likely that at least one function

of the loops is to decoy the immune response, misdirecting it to portions of the protein that can continually support variation to no discernable functional detriment for the virus. In support of this concept, HIV-1 with deletion of V1–V2 is still replication competent but is more susceptible to neutralization.

The CD4 binding site is conserved in all HIV subtypes and is contained within a deep and inaccessible pocket of the protein that is inaccessible to antibodies (the canyon hypothesis). Ab that could interact with Env to prevent CD4 association would be neutralizing. However, it appears that the loops also serve to mask important targets for neutralization such as the CD4 binding site and CD4-induced epitopes such as the co-receptor binding regions by sterically inhibiting the access of antibody to potential epitopes or by limiting the affinity with which such Ab can associate. The loops are also extensively glycosylated and this also impacts recognition as discussed below.

Env Glycosylation and Antibody Recognition

HIV-1 Env gp120 and gp41 have been shown to be extensively modified by an average of ~30 carbohydrate additions for both proteins, and glycosylation of the HIV-1 Env is essential for folding and oligomerization. Moreover, glycosylation participates in protecting HIV-1 from recognition by antibodies. This concept, termed glycan shielding, suggests that the concentration of carbohydrate additions on gp120 block antibody access to the protein domains beneath that are critical for CD4 recognition, co-receptor binding, and exposure of the fusion peptide. Highly conserved glycans often play important structural and functional roles, but others are highly polymorphic between subtypes and within infected individuals. Thus, variability in the protein sequence in or around glycosylation sites affects immune recognition by subtly altering the primary sequence, the fold of the structure, and/or accessibility to epitopes. The continual diversification leads to a dynamic of appearing and disappearing glycosylation sites followed by humoral selection and outgrowth of the escape mutant.

It is important to realize that the nature of the shield itself is also a component of protection. Unlike the highly immunogenic cell wall components in prokaryotes (e.g., lipopolysaccharide), the sugars in HIV-1 are synthesized and modified as host components and, therefore, indistinguishable from carbohydrate additions to a multitude of other membrane proteins. Vaccine strategies that stimulate recognition of glycans will need to take into consideration targeting what is essentially 'self' carbohydrate. Some broadly neutralizing anti-HIV-1 envelope monoclonal antibodies have polyspecific or self-reactivity to host antigens but are not routinely made due to elimination by host B-cell tolerance mechanisms.

Concluding Remarks

Though influenza virus and HIV both show significant antigenic variation in response to neutralizing antibody, the mechanisms they use to generate variation are both similar and distinct. Influenza virus diversification by drift is unidirectional whereas HIV tends to generate radial diversity. Both viruses undergo shift to induce completely novel antigens; influenza virus by reassorting genomic segments and HIV by RT-mediated recombination. A new influenza variant spreads through the human population whereas a new HIV variant merely repopulates the infected individual. Both viruses utilize carbohydrate additions to avoid the antibody response, but in HIV the number of modifications is more substantial. In influenza, variation is utilized to avoid antibody binding whereas HIV diversity promotes recognition of epitopes lacking functional significance, and therefore non-neutralizing responses.

See also: AIDS: Vaccine Development; Antigenicity and Immunogenicity of Viral Proteins; Immune Response to viruses: Antibody-Mediated Immunity; Influenza; Neutralization of Infectivity; Orthomyxoviruses: Structure of antigens; Quasispecies; Vaccine Strategies.

Further Reading

Air GM, Laver WG, and Webster RG (1987) Antigenic variation in influenza viruses. *Contribution Microbiology and Immunology* 8: 20–59.

Hammond AL, Lewis J, May J, Albert J, Balfe P, and McKeating JA (2001) Antigenic variation within the CD4 binding site of human immunodeficiency virus type 1 gp120: Effects on chemokine receptor utilization. *Journal of Virology* 75: 5593–5603.

Knipe DM, Howley PM, Griffin DE, *et al.* (2001) *Field's Virology,* 4th edn. Philadelphia: Lippincott Williams and Wilkins.

Korber B, Gaschen B, Yusim K, Thakallapally R, Kesmir C, and Detours V (2001) Evolutionary and immunological implications of contemporary HIV-1 variation. *British Medical Bulletin* 58: 19–42.

Skehel JJ and Wiley DC (2000) Receptor binding and membrane fusion in virus entry: The influenza hemagglutinin. *Annual Review of Biochemistry* 69: 531–569.

Venkatramani L, Bochkareva E, Lee JT, *et al*. (2006) An epidemiologically significant epitope of a 1998 human influenza virus neuraminidase forms a highly hydrated interface in the NA-antibody complex. *Journal of Molecular Biology* 356(3): 651–663.

Wilson IA and Cox NJ (1990) Structural basis of immune recognition of influenza virus hemagglutinin. *Annual Review of Immunology* 8: 737–771.

Yusim K, Kesmir C, Gaschen B, *et al.* (2002) Clustering patterns of cytotoxic T-lymphocyte epitopes in human immunodeficiency virus type 1 (HIV-1) proteins reveal imprints of immune evasion on HIV-1 global variation. *Journal of Virology* 76: 8757–8768.

Relevant Websites

http://www.hiv.lanl.gov – A series of freely accessible reviews on HIV-1 diversity, drug resistance and immunology.

http://www.flu.lanl.gov – The Influenza Sequence Database (ISD). To see the sequence diversity in influenza viruses.

Antigenicity and Immunogenicity of Viral Proteins

M H V Van Regenmortel, CNRS, Illkirch, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

B-cell epitope Surface region of a native protein antigen that is specifically recognized by the binding sites of free and membrane-bound antibody molecules. The membrane-bound antibodies are the B-cell receptors that recognize the antigen during the immunization process.

Cryptotope Antigenic site or epitope hidden in polymerized proteins and virions because it is present on the surfaces of subunits that become buried. Cryptotopes are antigenically active only after dissociation of protein aggregates and virions.

Mimotope A peptide possessing similar binding activity as that of a peptide epitope but showing little or no sequence similarity with it. Originally, mimotopes were defined as peptides able to bind an antibody but showing no sequence similarity with the protein antigen used to induce the antibody, usually because the antibody was directed to a discontinuous epitope.

Neotope Antigenic site or epitope specific for the quaternary structure of polymerized proteins or virions. Neotopes arise from the juxtaposition of residues from neighboring subunits or through conformational changes in the monomers resulting from intersubunit interactions.

Paratope Binding site of an antibody molecule that binds specifically to an epitope of the antigen. Paratopes are constituted of residues from six complementarity-determining regions (CDRs) located on the heavy and light chains of immunoglobulins. The CDRs vary greatly in sequence and in length in individual antibodies.

Virus Antigenicity

The antigenic reactivity or antigenicity of viruses corresponds to their capacity to bind specifically to the functional binding sites of certain immunoglobulin molecules. Once such binding has been observed experimentally, the particular immunoglobulin becomes known as an antibody specific for the virus.

The antigenicity of nonenveloped viruses resides in the viral proteins that form the viral capsid, whereas the antigenicity of enveloped viruses resides mostly in the exposed proteins and glycoproteins that are anchored in the viral, lipid membrane. The oligosaccharide side chains of viral glycoproteins contribute significantly to the antigenic properties of enveloped viruses.

The antigenic sites or B-cell epitopes of viral proteins correspond to those parts of viral capsids and envelope proteins that are specifically recognized by the binding sites or paratopes of free and membrane-bound antibody molecules. Antibody molecules anchored in the outer membrane of B cells correspond to the receptors of B cells that recognize the antigen when it is administered to a vertebrate host during immunization. The B-cell receptors recognize the native tertiary and quaternary structure of viral proteins and the antibody molecules that are released when the B cells have matured into plasmocytes, also recognize the native conformation of the proteins.

During a natural viral infection or after experimental immunization, the immune system of the host may also encounter dissociated viral protein subunits and it will elicit antibodies specific for these components. In addition, during a viral infection, nonstructural viral proteins that are not incorporated into the virions will also induce the production of specific antibodies in the infected host. Diagnostic tests that detect antibodies to nonstructural viral proteins are useful for differentiating animals infected with, for instance, foot-and-mouth disease virus from vaccinated animals that possess only antibodies directed to the capsid proteins of the virus. In this case, the ability to differentiate vaccinated animals from infected animals by a suitable immunoassay is an important prerequisite for convincing trading partners that a cattle-exporting country is free of foot-and-mouth disease.

Viral Antigenic Sites

In the absence of further qualification, the term epitope used in the present article refers to an antigenic site of a protein recognized by B cells. Immune responses are also mediated by T cells, that is, by lymphocytes that recognize protein antigens through T-cell receptors, after the antigen has been processed into peptide fragments. T-cell responses to viral antigens which involve the so-called T-cell epitopes will not be discussed here.

Because of the additional antigenic complexity that arises in viral proteins as a result of the quaternary structure of virions, it is useful to distinguish two categories of viral epitopes known as cryptotopes and neotopes. Cryptotopes are epitopes that are hidden in the intact, assembled capsid. They are located on the surfaces of viral

subunits that become buried when the subunits associate into capsids, and they are antigenically active only after dissociation or denaturation of virions. Neotopes are epitopes that are specific for the quaternary structure of virions and which are absent in the dissociated protein subunits. Neotopes arise either through conformational changes in the monomers induced by intersubunit interactions or result from the juxtaposition of residues from neighboring subunits. These terms are useful when it is important to distinguish between the epitopes carried by different aggregation states of viral proteins. The availability of monoclonal antibodies has made it easy to identify neotopes and cryptotopes in many viruses whose antigenic structure has been analyzed in detail. There is evidence, for instance, that the trimeric form of the gp160 protein of human immunodeficiency virus (HIV) possesses neotopes that are not present in the monomeric form but are important for the induction of neutralizing antibodies.

Another important category of viral epitope is the neutralization epitopes that are recognized by antibodies able to neutralize viral infectivity. Since it is the antibodies that bring about neutralization, it is appropriate to talk of neutralizing antibodies and not of neutralizing epitopes. These epitopes are best referred to as neutralization epitopes.

Infectivity neutralization depends on properties of the virus, the antibody, and the host cell. The ability of an antibody to interfere with the process of infection always takes place in a specific biological context and it cannot be described adequately only in terms of a binding reaction between virus and antibody. In many cases loss of infectivity occurs when the bound antibody molecules inhibit the ability of the virus to attach to certain receptors of the host cell. It can happen that the antibody prevents the virus from infecting one type of host cell but not another type.

Some picornaviruses such as the rhinoviruses occur in the form of as many as 100 different variants known as serotypes, each serotype being neutralized by its own antibodies but not by antibodies specific for other serotypes. In contrast, another picornavirus, that is, poliovirus, exists only as three serotypes. The structural basis for this difference in the number of neutralization serotypes between different picornaviruses has not yet been clarified.

Antibodies to Viral Antigens

The capacity of antibodies to recognize viral epitopes resides in their functional binding sites or paratopes. The most common type of antibody is an immunoglobulin known as IgG which possesses two identical binding sites. These paratopes are constituted of three complementarity-determining regions (CDRs) located in a heavy chain and three CDRs in a light chain. The CDRs comprise a total of about 50–70 amino acid residues and form six loops that vary greatly not only in sequence but also in length from one antibody to another. Each individual paratope is made up of atoms from not more than 15–20 CDR residues which make contact with a specific epitope. This means that as many as two-thirds of the CDR residues of an antibody molecule do not participate directly in the interaction with an individual epitope. These residues remain potentially capable of binding to other epitopes that may have little structural resemblance with the first epitope, a situation which gives rise to antibody multispecificity. The relation between an antibody and its antigen is thus never of an exclusive nature and antigenic cross-reactivity will be observed whenever it is looked for. The so-called antibody footprint corresponds to an area on the surface of the protein antigen of about 800 $Å^2$.

Continuous and Discontinuous Epitopes

Protein epitopes are usually classified as either continuous or discontinuous depending on whether the amino acids included in the epitope are contiguous in the polypeptide chain or not (**Figure 1**). This terminology may give the impression that the elements of recognition operative in epitope–paratope interactions are individual amino acids, whereas it is in fact at the level of individual atoms that the recognition occurs. The distinction between continuous and discontinuous epitopes is not clear-cut since discontinuous epitopes often contain stretches of a few contiguous residues that may be able, on their own, to bind to antibodies directed to the cognate protein. As a result, such short stretches of residues may sometimes be given the status of continuous epitopes. On the other hand, continuous epitopes often contain a number of indifferent residues that are not implicated in the binding interaction and can be replaced by any of the other 19 amino acids without impairing antigenic activity. Such continuous epitopes could then be considered to be discontinuous.

Since discontinuous epitopes consist of surface residues brought together by the folding of the peptide chain, their antigenic reactivity obviously depends on the native conformation of the protein. When the protein is denatured, the residues from distant parts of the sequence that collectively made up the epitope are scattered and they will usually no longer be individually recognized by antibodies raised against the discontinuous epitope.

Discontinuous epitopes are often called conformational epitopes because of their dependence on the intact conformation of the native protein. However, this terminology may be confusing since it seems to imply that continuous epitopes, also called sequential epitopes, are conformation independent. In reality the linear peptides that constitute continuous epitopes necessarily also have a

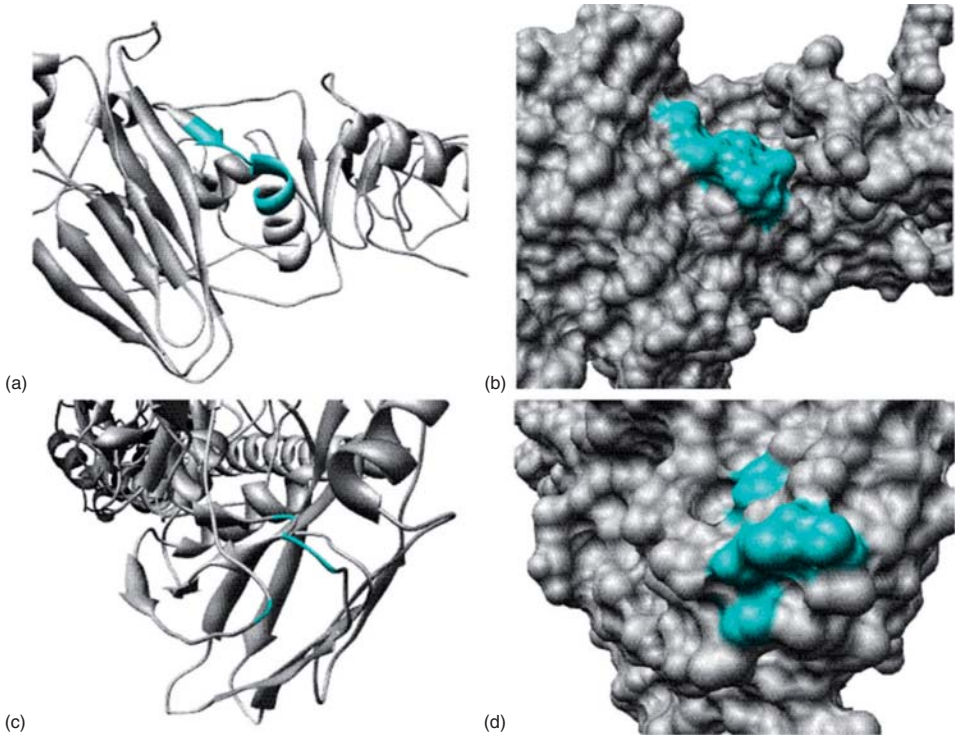


Figure 1 Illustration of the difference between a continuous (a, b) and discontinuous epitope (c, d) in the hemagglutinin protein of influenza virus. The residues that are part of epitopes are colored in blue ; the remaining residues are gray. (a) Ribbon representation of a continuous epitope. (b) Surface representation of the epitope shown in (a). (c) Ribbon representation of a discontinuous epitope. Residues distant in the sequence are brought close together by the folding. (d) Surface representation of the epitope in (c). Reproduced from Greenbaum JA, Andersen PH, Blythe M, *et al*. (2007) Towards a consensus on datasets and evaluation metrics for developing B cell epitope prediction tools. *Journal of Molecular Recognition* 20: 75–82, with permission from Wiley.

conformation which, however, is mostly different from that of the corresponding region in the intact protein.

Most of our knowledge of the structure of discontinuous epitopes has been obtained from a small number of X-ray crystallographic studies of antibody–antigen complexes. It is important to realize that the structure of epitopes and paratopes seen in the complex may be different from the structure of the respective binding sites in the free antigen and antibody molecules, that is, before they have been altered by the mutual adaptation or induced fit that occurs during the binding interaction. For this reason, the structure of epitopes after complexation tends to be an unreliable guide for identifying the exact epitope structure that was recognized by the B-cell receptors during the immunization process. Crystallographic studies of antigen–antibody complexes have shown that the vast majority of protein epitopes are discontinuous and consist of residues from between two and five segments of the polypeptide chain of the antigen.

Most of our knowledge of protein antigenicity has not been obtained by X-ray crystallography but was derived from studies of the antigenic cross-reactivity between the intact protein and peptide fragments. In such studies, antibodies raised against the virus or isolated viral proteins are tested for their ability to react in various immunoassays with natural or synthetic 6–20-residue peptides derived from the protein sequence. Any linear peptide that is found to react in such an assay is labeled a continuous epitope of the protein. It is customary to test peptides of decreasing size and to give the status of epitope to the smallest peptide that retains a measurable level of antigenic reactivity. Usually this leads to the identification of continuous epitopes with a length of 5–8 residues, although the lower size limit tends to remain ill-defined. It is not unusual for certain di- or tripeptides to retain a significant binding capacity in particular types of solid-phase immunoassays.

On the other hand, increasing the length of peptides does not always lead to a higher level of cross-reactivity with antiprotein antibodies since longer peptides may adopt a conformation that is different from the one present in the intact protein. There is also no reason to assume that short peptides will have a unique conformation mimicking that of the corresponding region in the protein. Cross-reactivity of peptides with antiprotein antibodies is commonly observed because of antibody multispecificity and of the induced fit and mutual adaptation capacity of the two partners.

Many investigators believe that the majority of so-called continuous epitopes described in the literature

and listed in immunological databases actually correspond to unfolded regions of denatured protein molecules, that is, that they are not genuine epitopes of native proteins. They argue that there is usually little experimental evidence to show that short peptides are actually able to bind to antibodies specific for the native state of the cognate protein. It is indeed very difficult to demonstrate that the protein sample used in an immunoassay does not contain at least some denatured molecules that could be responsible for the observed binding reaction. Furthermore, claims made in the early 1980s that immunization with peptides always leads to a high frequency of induction of antibodies that recognize the native cognate protein are no longer considered valid. It is now accepted that such claims arose because the ability of antipeptide antibodies to recognize proteins was tested in solid-phase immunoassays in which the proteins had become denatured by adsorption to plastic.

Mimotopes

The multispecificity of antibody molecules is illustrated by the existence of so-called mimotopes. The term mimotope was coined in 1986 by Mario Geysen and was originally defined as a peptide that is able to bind to a particular antibody, but is unrelated in sequence to the protein antigen used to induce that antibody, usually because the antibody is directed to a discontinuous epitope. Currently, the term mimotope is applied to any epitope mimic, irrespective of whether the epitope being mimicked is continuous or discontinuous. It is indeed possible to mimic a continuous epitope with a cross-reactive mimotope peptide that shows little sequence similarity with the original epitope. This cross-reactivity of mimotopes is due to the fact that dissimilar amino acid residues may actually contain a sufficient number of identical atomic groups to allow the peptide to cross-react with atoms of the antibody-combining site.

To qualify as a mimotope, a peptide should not only be able to bind a particular antibody but it should also be capable of eliciting antibodies that recognize the epitope being mimicked. This requirement stems from the fact that a single immunoglobulin always harbors a number of partly overlapping or nonoverlapping paratopes, each one capable of binding related or unrelated epitopes. When different subsites in the immunoglobulin-binding pocket partly overlap, binding to one epitope may prevent a second unrelated epitope from being accommodated at a nearby location. Therefore, when a peptide is labeled a mimotope of epitope A because of its capacity either to bind to an anti-A antibody or to inhibit the binding of epitope A to the antibody, it cannot be excluded that the putative mimotope actually binds to a different subsite from the one that interacts with epitope A. This is why it is necessary to show that a putative peptide mimotope is also able to induce antibodies that cross-react with epitope A, thereby demonstrating that it really is a mimotope of epitope A.

Nowadays, mimotopes are often identified by testing combinatorial peptide libraries, obtained by chemical synthesis or phage display, for their capacity to bind monoclonal antibodies specific for viral proteins. It is also possible to screen a phage library by means of sera collected from individuals that recovered from a viral infection and have seroconverted. Mimotopes identified in this manner may find applications in peptide-based diagnostic assays. It is also believed that mimotopes could be used for developing peptide-based vaccines.

The antigenic reactivity of viral proteins discussed so far is based on chemical reactions between epitopes and paratopes and it can be described using parameters such as the structural and chemical complementarity of the two partners, electrostatic and hydrogen bond interactions between them, the kinetics and equilibrium affinity constants of the interaction, the discrimination potential of individual antibody molecules, etc. Such immunochemical descriptions take the existence of antibodies for granted and do not ask questions about the biological origin, synthesis, or maturation of antibody molecules. The situation is different when it comes to investigations of the immunogenicity of viral proteins since this property cannot be analyzed outside of the biological context of an immunized host.

Immunogenicity

Whereas the antigenicity of proteins is a purely chemical property, their immunogenicity is a biological property that has a meaning only in the context of a particular host. Immunogenicity is the ability of a protein to give rise to an immune response in a competent host and it depends on extrinsic factors such as the host immunoglobulin repertoire, self-tolerance, the production of cytokines, and various cellular and regulatory mechanisms definable only in a given biological context.

When a number of continuous epitopes of a protein have been identified, this knowledge does not provide information on which particular immunogenic structure, present in the antigen used for immunization, was recognized by B-cell receptors and initiated the production of antibodies. Our ignorance of the exact immunogenic stimulus is often referred to as the black box conundrum and this makes the study of immunogenicity very much an empirical endeavor.

Although any peptide identified as a continuous epitope will readily elicit antipeptide antibodies, it is only rarely able to induce antibodies that also recognize the cognate, native protein antigen. In an immunoassay, an antibody raised against the native protein may be able to select one conformation in a peptide or it may induce a reactive conformation by an induced fit or mutual

adaptation mechanism, the result in both cases being the occurrence of a cross-reaction between the peptide and the antiprotein antibody. On the other hand, when the same peptide meets a variety of B-cell receptors during the immunization process, it may not be able to bind preferentially to those rare paratopes in the receptors that, in addition to recognizing the peptide, also cross-react with the native protein. As a result most elicited antipeptide antibodies will only recognize the peptide and will not cross-react with the cognate protein.

As far as the immunogenicity of discontinuous epitopes is concerned, the situation is even more uncertain since the residues belonging to the epitope were identified by crystallographic analysis at the end of a process of mutual adaptation and conformational change in the two binding partners. The discontinuous epitope with its unique conformational features cannot be dissected out of the three-dimensional assembly of residues in the native protein and its immunogenic potential cannot therefore be studied independently of the rest of the protein antigen. As a result, the exact three-dimensional structure of the immunogen which was recognized by B-cell receptors and initiated the immune response cannot be known with certainty. Furthermore, reconstituting a discontinuous epitope in the form of a linear peptide that would include all the epitope residues from distant parts of the antigen sequence and would assemble them in the correct conformation appears to be an extremely difficult task.

Implications for Vaccine Development

Our increasing knowledge of the structure of viral epitopes has given rise in some quarters to the expectation that it should be possible to develop peptide-based viral vaccines. However, the results so far have been disappointing. In spite of the hundreds of viral epitopes that have been identified by studying the antigenicity of peptide fragments of viral proteins, no commercial peptide vaccine has yet reached the marketplace.

Although most peptide fragments are immunogenic in the sense that they readily induce antibodies that react with the peptide immunogen, this type of immunogenicity is irrelevant for vaccination purposes. What is required is the induction of antibodies which, on the one hand, recognize the cognate, native viral antigen (so-called cross-reactive immunogenicity) and which in addition also neutralize the infectivity of the virus (so-called cross-protective immunogenicity). Unfortunately, very few of the continuous epitopes of viruses that have been described possess the required cross-reactive and cross-protective immunogenicity. Many attempts have been made to increase the conformational similarity between peptide and intact protein, for instance, by constraining the peptide conformation by cyclization but this has not resulted in peptides possessing adequate cross-protective immunogenicity. There is also some evidence that the intrinsic disorder in certain loop regions of viral proteins may be responsible for the finding that peptides corresponding to such disordered regions are sometimes better vaccine immunogens than peptides with a constrained conformation.

The difficulties that must be overcome to transform a continuous epitope into an effective vaccine immunogen are illustrated by the many studies of the peptide ELDKWAS corresponding to residues 662–668 of the gp41 protein of HIV-1. This peptide which is recognized by the anti-HIV-1 broadly cross-reactive neutralizing monoclonal antibody (Mab) 2F5 has, for a long time, been regarded as a promising vaccine candidate because it is located in a conserved region of gp41 necessary for envelope-mediated fusion of the virus.

The ELDKWAS peptide has been incorporated into a variety of immunogenic constructs in an attempt to have it elicit antibodies with the same neutralizing capacity as Mab 2F5. When additional gp41-derived flanking residues were added to the peptide or when its conformation was constrained, peptides were obtained which had a tenfold higher affinity for the 2F5 antibody than the unconstrained peptide. However, in spite of their improved antigenicity, the peptide constructs, when used as immunogens, were still unable to induce antibodies with detectable neutralizing capacity.

In an attempt to ascertain which structural elements close to the ELDKWAS residues in the gp41 immunogen may have influenced the induction of the neutralizing 2F5 antibody, the crystal structure of the 2F5 antibody in complex with various gp41 peptides was determined. The conformation of the bound peptides was found to differ significantly from the corresponding region in the gp41 protein, indicating that the putative ELDKWAS epitope was able to assume various conformations depending on the fusogenic state of gp41. However, it was not clear which conformation should be stabilized in the peptide constructs intended for vaccination.

It seems that the viral epitopes involved in the immunogenic stimulus may in fact be dynamic structures with variable conformations and it has been suggested that such epitopes should be referred to as transitional epitopes. Such a label is an appropriate reminder that it is necessary to include the fourth dimension of time in the description of antigenic specificity.

The findings obtained with the ELDKWAS peptide suggest that these residues are part of a more complex discontinuous epitope that elicited the neutralizing Mab 2F5. There is also evidence that the hydrophobic membrane environment close to the ELDKWAS sequence played a role in the induction of the neutralizing antibody 2F5.

Unfortunately, only some of the antibodies induced by viral antigens possess neutralizing activity and it is not known which structural features in the immunogen are responsible for the appearance of neutralizing rather than

non-neutralizing antibodies. In recent years, attempts to find out have relied mainly on the crystallographic analysis of viral proteins complexed with neutralizing Mabs. Although such studies reveal the structure of the epitopes present in the complexes, they do not provide information on which transitional immunogenic epitopes were able to induce neutralizing antibodies. It seems likely that this type of information will only be obtained by systematic empirical trials in which numerous candidate immunogens are tested for their ability to induce protective immune responses.

See also: AIDS: Vaccine Development; Antigenic Variation; Immune Response to viruses: Antibody-Mediated Immunity; Neutralization of Infectivity; Vaccine Strategies.

Further Reading

Clavijo A, Wright P, and Kitching P (2004) Developments in diagnostic techniques for differentiating infection from vaccination in foot-and-mouth disease. *Veterinary Journal* 167: 9–22.

Geysen HM, Rodda SJ, and Mason TJ (1986) *A priori* delineation of a peptide which mimics a discontinuous antigenic determinant. *Molecular Immunology* 23: 709–715.

Greenbaum JA, Andersen PH, Blythe M, *et al*. (2007) Towards a consensus on datasets and evaluation metrics for developing B cell epitope prediction tools. *Journal of Molecular Recognition* 20: 75–82.

Klenk H-D (1990) Influence of glycosylation on antigenicity of viral proteins. In: Van Regenmortel MHV and Neurath AR (eds.) *Immunochemistry of Viruses II*, pp. 25–37. Amsterdam: Elsevier.

Sheppard N and Sattentau Q (2005) The prospects for vaccines against HIV-1: More than a field of long-term nonprogression? *Expert Review Molecular Medicine* 7(2): 1–21.

Uversky VN, Oldfield CJ, and Dunker AK (2005) Showing your ID: Intrinsic disorder as an ID for recognition, regulation and cell signaling. *Journal of Molecular Recognition* 18: 343–384.

Van Regenmortel MHV (1996) Mapping epitope structure and activity: From one dimensional prediction to four-dimensional description of antigenic specificity. *Methods: Companion to Methods in Enzymology* 9: 465–472.

Van Regenmortel MHV (1998) From absolute to exquisite specificity. Reflections on the fuzzy nature of species, specificity and antigenic sites. *Journal of Immunological Methods* 216: 37–48.

Van Regenmortel MHV (1999) Molecular design versus empirical discovery in peptide-based vaccines: Coming to terms with fuzzy recognition sites and ill-defined structure-function relationships in immunology. *Vaccine* 18: 216–221.

Van Regenmortel MHV (2006) Immunoinformatics may lead to a reappraisal of the nature of B cell epitopes and of the feasibility of synthetic peptide vaccines. *Journal of Molecular Recognition* 19: 183–187.

Van Regenmortel MHV and Muller S (1999) In: *Synthetic Peptides as Antigens*, pp. 1–381. Amsterdam: Elsevier.

Wyatt R and Sodroski J (1998) The HIV-1 envelope glycoproteins: Fusogens, antigens and immunogens. *Science* 280: 1884–1888.

Yuan W, Bazick J, and Sodroski J (2006) Characterization of the multiple conformational states of free monomeric and trimeric human immunodeficiency virus envelope glycoproteins after fixation by cross-linker. *Journal of Virology* 80: 6725–6737.

Zolla-Pazner S (2004) Identifying epitopes of HIV-1 that induce protective antibodies. *Nature Reviews Immunology* 4: 199–210.

Zwick MB, Jensen R, Church S, *et al.* (2005) Anti-human immunodeficiency virus type 1 (HIV-1) antibodies 2F5 and 4E10 require surprisingly few crucial residues in the membrane-proximal external region of glycoprotein gp41 to neutralize HIV-1. *Journal of Virology* 79: 1252–1261.

Antiviral Agents

H J Field, University of Cambridge, Cambridge, UK
R A Vere Hodge, Vere Hodge Antivirals Ltd., Reigate, UK

© 2008 Published by Elsevier Ltd.

Introduction

There have been two previous articles in *Encyclopedia of Virology,* the latter being in 1999. That review, by A. K. Field and C. A. Laughlin, gave a good update, especially in the treatment of HIV. In order to avoid repetition, we have focused on antiviral targets. We have not attempted to cover vaccines nor immunomodulating agents, such as interferons, except when these are mentioned briefly in those cases in which they are the therapy of choice. These approaches which utilize a cellular target are outside the scope of this article but the reader is referred to a recent review by L. Schang.

The term 'virucidal' has long been used to describe an agent that destroys virions while outside the host cell. A few compounds inactivate viruses by highly specific mechanisms but many, such as alcohol wipes, have a broad spectrum of activity against many infectious entities. Virucidal agents have an important role, especially in hospitals, in preventing the transmission of viruses and may be useful in reducing the risk of virus transmission between individuals, for example, during sexual contact. These types of agent will not be considered further in this article.

The focus of this article is those antiviral compounds which target virus replication selectively. After a brief historical overview, we discuss the concepts and challenges which have guided and stimulated progress with antiviral therapy – selectivity, spectrum, viral targets, prodrugs, resistance and drug combinations, viral fitness, latency. A summary of the most important antiviral agents is given in **Table 1**. This article outlines the principles

Table 1 List of important current antiviral agents

(a) Primarily active against Herpes viruses

Generic name (abbreviation) Trade name (company)	*Structural information*	*Mechanism viral target*	*Fig 1 stage*	*Target viruses*
Valaciclovir (VACV) Valtrex® (GSK)	Prodrug of acyclovir (ACV) Zovirax®	Activated by viral TK, inhibits viral polymerase	VI	HSV-1 and -2 VZV (CMV)

Notes: ACV is extremely safe and, apart from some problems related to high doses and crystallization in the kidney, no serious side effects have been encountered. Drug-resistance in immunocompetent patients has remained rare ($< 1\%$) in over two decades of clinical experience. In immunocompromised patients, resistant variants (generally in TK and occasionally in DNA polymerase) occur in up to 5% patients. Fortunately, TK-deficient HSV appears to be less pathogenic and less able to reactivate from latency than wt HSV.

VACV is a valine ester of ACV. In addition to the main target viruses, VACV is sometimes used to prevent CMV infections in transplant patients. During suppressive therapy for genital herpes, VACV reduced the HSV-transmission rate by about 50%.

Famciclovir (FCV) Famvir® (Novartis)	Prodrug of penciclovir (PCV) Denavir®/ Vectavir®	Activated by viral TK, inhibits viral polymerase	VI	HSV-1 and -2 VZV

Notes: This was the first antiviral agent to be developed as the prodrug and stimulated the search for an ACV prodrug. FCV was shown to be highly effective, vs. placebo, against HSV-1 and -2 and VZV. In the only clinical trial comparing FCV and VACV for the treatment of shingles, they appeared to be equally effective. Interestingly, PCV has a higher affinity than ACV for HSV TK but is a less potent inhibitor of herpes DNA polymerase. However, the intracellular half-life of PCV triphosphate is significantly longer than ACV triphosphate and this may help to explain its potent antiviral activity. The dynamics of resistance selection may differ subtly from ACV but clinical resistance in immunocompetent patients remains extremely rare.

Foscarnet (PFA) Foscavir® (Astra Zeneca)	Pyrophosphate analog	Polymerase inhibitor	VI	HSV-1 and -2 VZV

Notes: PFA is an effective inhibitor of HSV replication. However, the compound must be administered iv and suffers from toxicity (e.g., tendency to accumulate in bone). Therefore it is used in immunocompromised patients with herpes infections resistant to ACV and PCV.

Valganciclovir (VGCV) Valcyte® (Roche)	Prodrug of ganciclovir (GCV) Cymmevene®	Activated by kinase encoded by UL 97, polymerase inhibitor	VI	CMV

Notes: VGCV has become the drug of choice for the prevention and treatment of CMV in immunocompromised patients.

(b) Primarily active against RNA viruses

Generic name Trade name (company)	*Structural information*	*Mechanism viral target*	*Fig 1 stage*	*Target viruses*
Zanamivir Relenza® (GSK)	Sialic acid analog	Neuraminidase inhibitor	X	Influenza A and B

Notes: Zanamivir prevents the influenza virions leaving the infected cell. It can be used either for the treatment or prophylaxis of influenza infections. It has a good safety record but its use is limited by the need for inhalation. It is currently being stockpiled, as the second line agent, in preparation for a possible outbreak with H5N1 virus.

Oseltamivir Tamiflu® (Roche)	Sialic acid analog	Neuraminidase inhibitor	X	Influenza A and B

Notes: Like zanamivir, oseltamivir has good, selective activity against influenza viruses. In contrast to zanamivir, it has good bioavailability which has led to it becoming the first line agent for treatment of influenza. In adult patients, resistance is rare and it appears that those resistant strains are partially disabled. It is the main agent for stockpiling for an H5N1 outbreak.

An inhibitor of influenza RNA polymerase, T-705, has demonstrated excellent activity in cell culture assays and animal models. It is expected to be entering clinical trials soon. With the threat of an H5N1 outbreak, it will be useful to have another treatment option, possibly used in combination with oseltamivir.

Continued

Table 1 Continued

(b) Primarily active against RNA viruses

Generic name *Trade name* *(company)*	*Structural information*	*Mechanism viral target*	*Fig 1 stage*	*Target viruses*
Ribavirin	Nucleoside analog	Possibly, no direct viral target	?	HCV
Virazole® (Schering-Plough)				RSV

Notes: HCV – Current therapy is with one of several interferon forms, usually with ribavirin which seems to enhance the chance for long-term response. Being immune modulators, these are outside the scope of this review. However, antiviral agents, targeting either HCV protease or polymerase, are being developed.

RSV – Although ribavirin has been used to treat serious cases of RSV, its efficacy has been questioned. Palivizumab and RSV immune globulin (RSV-IGIV) are FDA approved.

(c) Primarily active against Hepadnaviruses (HBV)

Generic name *(abbreviation)* *Trade name*	*Structural information*	*Mechanism viral target*	*Fig 1 stage*	*Company*
Lamivudine (3TC) Zeffix®, Heptovir®	NA inhibitor	Polymerase	VI	GSK

Notes: Lamivudine has become the first line choice for therapy of chronic HBV although viral resistance appears in about 50% of patients after two years monotherapy. Lamivudine is unusual as it is in routine use for both HBV and HIV.

Adefovir dipivoxil (ADV) Hepsera®	Prodrug of adefovir NA inhibitor	Polymerase	VI	Gilead

Notes: In comparison with lamivudine, the rate of appearance of resistance is much slower, in about 2.5% patients after 2 years monotherapy.

Entecavir Baraclude®	NA inhibitor	Polymerase	VI	BMS

Notes: Entecavir was approved by FDA for HBV in 2006. It is very active with tablet sizes of 0.5 and 1 mg.

Telbivudine Tyzeka®	NA inhibitor	Polymerase	VI	Idenix/Roche

Notes: Telbivudine is the most recent (2007) of FDA approved agents for HBV. Telbivudine has an excellent safety record in animal toxicity tests. Although the tablet size (600 mg) is larger than with the other compounds, it is perhaps the most effective in reducing HBV DNA levels but it should not be used to treat patients failing on lamivudine therapy. It may replace lamivudine as the first line therapy.

(d) Primarily active against Retroviruses (HIV)

Generic name *(abbreviation)* *Trade name*	*Structural information*	*Mechanism viral target*	*Fig 1 stage*	*Company*
Zidovudine/Lamivudine (AZT/3TC) Combivir®	Two NRTIs	Polymerase	VI	GSK

Notes: As single agents, the trade names are Retrovir®/Epivir® respectively.

AZT/3TC/abacavir Trizivir®	Three NRTIs	Polymerase	VI	GSK

Notes: The trade name for abacavir as a single agent is Ziagen® and, when combined with lamivudine, Epzicom® or Kivexa®.

Emtricitabine/tenofovir/ efavirenz Atripla®	Two NNRTIs and one NNRTI	Polymerase	VI/VI/VII	Gilead & BMS (Jointly)

Notes: The trade name for the combination emtricitabine/tenofovir is Truvada®. As single agents, the trade names are Emtrive®/Viread®/Sustiva® respectively.

Continued

Table 1 Continued

(d) Primarily active against Retroviruses (HIV)

Generic name (abbreviation) Trade name	*Structural information*	*Mechanism viral target*	*Fig 1 stage*	*Company*
Nevirapine Viramune®	NNRTI	Polymerase	VII	Boehringer
Notes: Should be used with at least two other agents, one of which should be an NRTI.				
Fosamprenavir Lexiva®	PI	Protease	V	GSK
Notes: Fosamprenavir is the oral prodrug of amprenavir (Agenerase®) and is replacing it in clinical use.				
Saquinavir mesylate Invirase® Fortovase® (Roche)	PI	Protease	V	Roche
Notes: Invirase capsules and tablets should be used only with ritonavir which significantly inhibits the metabolism of saquinavir to provide plasma saquinavir levels at least equal to those achieved with Fortavase capsules which should be use, if at all, as the sole PI in a regimen.				
Lopinavir/ritonavir Kaletra®	2 PIs	Protease	V	Abbott Lab
Notes: Lopinavir is not marketed separately but combined with ritonavir (ratio 4:1) which enhances the plasma levels of lopinavir. The trade name for ritonavir is Norvir®.				
Indinavir Crixivan®	PI	Protease	V	Merck
Notes: Indinavir should be used only with other agents.				
Darunavir Prezista®	PI	Protease	V	Tibotec
Notes: Darunavir was given fast track status and was approved in 2006. Darunavir has less than tenfold decreased susceptibility in cell culture against 90% of 3309 clinical isolates resistant to other PIs.				
Efuviritide (T-20) Fuzeon® (Roche)	Fusion inhibitor	gp41 envelope protein	II	Roche
Notes: Administered sc bid. Mainly used in salvage therapy. It is the only FDA approved fusion inhibitor.				

Further information, structures, and references may be obtained from AVCC FactFile and company web sites.

underlying the successful development of antiviral compounds for the many clinical applications available today. Some prospects for the future are discussed.

Historical Perspective

The First Uncertain Steps

Following the discovery of antibiotics for treating bacterial infections, for several decades it was thought that 'safe' antiviral chemotherapy would be difficult if not impossible. The earliest antiviral compounds only emphasized the problems. Marboran was introduced in the early 1960s to treat smallpox and vaccinia; its effectiveness was equivocal and its use short-lived. Amantadine and rimantadine were used to treat influenza with relatively little side effects but the influenza virus became resistant within a few days of treatment and the resistant virus spread readily to contacts. The first nucleoside analogs, with antiviral activity, emerged during the search for drugs to treat cancer. The first of these, idoxuridine (IDU), was discovered to be active against herpes viruses by Dr. William (Bill) Prusoff in 1959. However, its toxicity limited its use to topical treatments only (e.g., infections of the eye). Vidarabine (Ara A) was slightly more selective; its main systemic use was to treat herpes encephalitis. No convincing selective antiviral compound had yet been discovered.

The Major Breakthrough

The discovery of the antiherpes drug, acyclovir (ACV) in 1978, was the major milestone in antiviral therapy. For the first time, it was demonstrated that an effective, nontoxic, antiviral drug is an achievable aim. Moreover,

chronic suppressive acyclovir therapy for several years, to prevent the misery of recurrent herpes, is possible without adverse effects. More than two decades of worldwide use has proved that acyclovir is one of the safest drugs in clinical therapy. Acyclovir proved that not all nucleoside analogs had to be mutagenic and/or carcinogenic. The early doubts, about the potential for discovering safe antiviral agents, were dispelled for ever.

The selectivity of acyclovir for the herpes viruses is dependent on it being activated only in herpes virus-infected cells. The critical initial step in that activation is its phosphorylation to ACV-monophosphate by the viral thymidine kinase. Cellular enzymes convert the monophosphate to the triphosphate which inhibits selectively herpes viral DNA polymerase. Following the discovery of acyclovir, other selective antiherpes virus compounds were discovered, including BVDU, BvaraU, and penciclovir (PCV). Currently, ACV and PCV, and their prodrugs valaciclovir (VACV) and famciclovir (FCV), respectively, are the most commonly used drugs to treat infections with HSV-1 and -2 and VZV. Another compound, ganciclovir (GCV), was discovered to have good activity against CMV. Like ACV and PCV, GCV is activated specifically in virus-infected cells but by the kinase encoded by the CMV UL97 gene. The prodrug of GCV, valganciclovir (VGCV), is the preferred therapy for CMV prevention and treatment in immunocompromised patients.

Impetus from the Challenge of HIV

Following the emergence of AIDS, an enormous research effort worldwide was directed at the search for inhibitors of HIV, resulting in a large number of new drugs. Initially, these inhibitors were nucleoside analogs. These had to be phosphorylated (activated) to their triphosphate entirely by cellular enzymes; HIV does not encode a thymidine kinase. Therefore, the selectivity was due entirely to the greater inhibition of HIV reverse transcriptase than the cellular polymerases. Because the medical need was so great, several inhibitors were brought quickly into clinical use even though they were associated with long-term toxicity problems, for example, mitochondrial toxicity with didanosine (ddI) and zalcitibine (ddC) and nephrotoxicity with cidofovir (HPMPC), used to treat CMV in AIDS patients. More recently, great progress has been made in selecting nucleoside and nucleotide analogs (**Table 1**) with much less potential for toxicity and so long-term therapies became normal practice. Once HIV protease had been shown to be essential for HIV replication, protease inhibitors (PIs) were discovered. Although these inhibitors have a high degree of selectivity for the HIV protease, they are not without some side effects, including the development or redistribution of fatty masses. However, one of the PIs is commonly included in the combination therapy now known as HAART (highly active anti-retroviral therapy).

Expansion into Therapies for Other Viruses

A spin-off from this work with HIV has led to inhibitors of other families of viruses, particularly hepatitis B virus (HBV). Lamivudine and adefovir have become the treatments of choice for HBV. Meanwhile, there seemed to be progress discovering compounds active against picornaviruses (which include rhinoviruses causing the common cold). These compounds bind into a pocket within the viral capsid. The best example is pleconaril although unacceptable side effects stopped its development. However, the viruses quickly became resistant and some strains even became dependent on the 'antiviral' compound. Therefore, this approach has not resulted in any clinically useful drugs.

Once the structure of influenza virus neuraminidase was known, new inhibitors of influenza viruses were discovered. Recent advances with replication systems for hepatitis C virus (HCV) have allowed the discovery of anti-HCV compounds. Currently, the threat of bio-terrorism has prompted the successful search for drugs active against poxviruses and, with the prospect of the next influenza pandemic looming, there is renewed research effort directed to anti-influenza drugs. There are at least 25 (**Table 1**) widely used licensed antiviral agents and that number looks set to increase rapidly in the coming years.

Key Concepts

Definition of Selective Activity Index

Viruses are obligate intracellular parasites. Thus, inhibitors of virus replication must do so without toxic effects on the cells, tissues, and organs of the host. This is the concept of 'selective activity' against viruses.

For those viruses which form plaques in a cell monolayer, reduction in plaque formation has long been regarded as the 'gold standard' assay. Antiviral activity is usually defined as the concentration of the inhibitor which reduces viral plaques by 50% (50% effective concentration; EC_{50}). Alternatively, the reduction in the yield of infectious virus can give useful information by using different multiplicities of infection (MOI); in such assays, the concentration of inhibitor to reduce replication by 99% (EC_{99}) is usually reported. For some viruses, for example, HBV, reduced production of a measurable virus product (e.g., nucleic acid or protein) is the only way to assess viral replication. For inhibition of a viral enzyme, the 50% inhibitory concentration (IC_{50}) is commonly reported.

Irrespective of the antiviral assay, it is necessary to assess cellular toxicity of the test compound. This is essential to eliminate the possibility that the lack of virus replication is due to the destruction of the cells. However, the ratio of the concentration to inhibit the replication of the virus to the concentration to destroy nondividing cells in a monolayer is *not* an indication of selective activity.

To determine selective activity, it is essential to compare like with like, replicating virus with replicating cells. To test for an effect on replicating cells, it is necessary to aim for a tenfold increase (just over three doublings) so that at the 50% cytotoxic concentration (CC_{50}), at least one doubling has occurred and the second round of replication has started. The ratio of 50% viral inhibitory concentration to 50% cytotoxic concentration is defined as the selective index (SI).

In the literature, authors may not abide by the above definition of selective index. Therefore, published values for selectivity should be considered with some skepticism unless the methodology is fully described. We urge editors of antiviral articles to be aware of this difficulty.

The determination of CC_{50} values in a single assay may be sufficient to select compounds in a primary screen. For assessing compounds worthy of progression into development, it is prudent to assess the compounds in a variety of cytotoxicity tests using different cells. For example, inhibition of mitochondrial DNA can be indicated by using granulocyte-macrophage (CFU-GM) and erythroid (BFU-E) cells. Human bone marrow stem cells in primary culture are a good predictor for potential hematoxicity. A particular class of compounds may be known to have a potential for a particular toxicity, such as phosphonates for nephrotoxicity. In this case, the established pattern of nephrotoxicity in the clinic was reflected in a cell culture assay using primary human renal proximal tubule cells (RPTCs).

When antiviral agents are evaluated against virus infections *in vivo*, the ratio of the effective dose (ED_{50}) to the minimum toxic dose is sometimes referred to as the 'therapeutic ratio'. These measurements are widely used during the preclinical development of new antiviral agents and are often helpful in making comparisons between alternative compounds.

Toxicity of Antiviral Compounds

As mentioned above, SIs provide a numerical estimate of the degree of selectivity for antiviral compounds and may be useful in proceeding with structure–activity relationships (SARs) from a lead compound. However, these results must be treated with great caution when extrapolating to man. Although a high (>1000) SI value may be encouraging, a low SI should not be ignored. During preclinical evaluation, many potential toxic effects can be assessed. For example, pharmacokinetic studies can identify drug–drug interactions. For nucleoside analogs, it is not sufficient to study the possible interactions in plasma; it is also necessary to study drug–drug interactions on phosphorylation within cells. There are well-established tests for indicating potential genetic toxicity of nucleosides and nucleotides and toxicity for mitochondrial enzymes. However, there always remains the possibility for unexpected effects such as lipodystrophy which may not become apparent until the drug is used in man – possibly for several years. In some cases, these complications once identified can be avoided or managed. In other cases, for example, ddI and ddC, the development of safer options eclipses the older compounds. For those infections requiring long-term therapies, over a year for HBV, several years for suppression of recurrent genital herpes, over 10 years for HIV, the test for clinical safety is very exacting. It is a credit to research workers in antiviral chemotherapy that compounds, which have stood the test of time, have been brought into clinical practice.

Spectrum of Antiviral Action

There are few, if any, truly broad spectrum antivirals. Given the highly specific nature of the mechanism of action of most antivirals, this is unsurprising. Most antiviral compounds affect a narrow range of viruses – often restricted to a single virus family or particular subfamily. Telbivudine is highly specific for the HBV (and the related duck and woodchuck viruses) and is inactive against all other viruses tested. Acyclovir is typical in being active against some viruses within one family, the herpes viruses. Lamivudine is unusual in becoming a common treatment for both HIV and HBV.

Broad-spectrum activity against all enveloped viruses does seem to be a possibility. Rep 9 is a phosphorothioate oligonucleotide with a random order of bases (A, C, G, and T), the antiviral activity being dependent not on the base sequence but on the size, the optimal being about 40–50 bases. This oligonucleotide has both hydrophobic (along the backbone) and hydrophilic (bases) surfaces which seem to be essential for activity. Various enveloped viruses have a surface protein (e.g., HIV gp41, influenza hemagglutin) with an alpha-helix which matches the length of the 40 nucleotides in REP 9. The viruses that have been shown to be sensitive to REP 9 include members of the *Herpes-*, *Orthomyxo-*, *Paramyxo-*, and *Retroviridae*.

Virus Replication Cycle Presents Antiviral Targets

The Virus Replication Cycle

All viruses are dependent upon a host cell for protein synthesis. Thus, all viruses replicate via a broadly similar sequence of events (**Figure 1**). The virus must first attach ('adsorb') to the cell. The virus or virus nucleic acid genome then enters ('penetrates') the cytoplasm. The genome is liberated from the protective capsid ('uncoats'). In some cases the viral genome enters the cell nucleus; in all cases the genome is transcribed and thus the viral mRNA directs protein synthesis. Virus products include proteins that regulate further transcription; enzymes

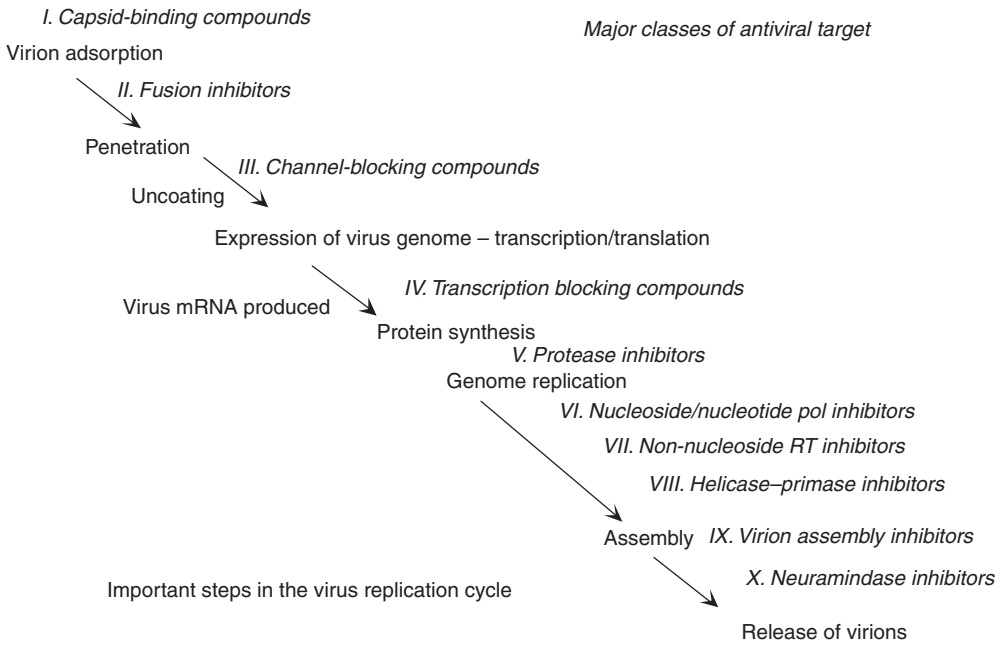


Figure 1 Key steps in the virus replication cycle that provide antiviral targets.

including those involved in genome replication; proteases that process other virus-induced proteins; and many proteins that subvert host-defense mechanisms within and without the infected cell. The virus undergoes 'genome replication' and together with virus structural proteins form new virions ('assembly') which are 'released' from the cells. In some cases release is associated with budding through cellular membranes that have been decorated with virus glycoproteins enabling the virus to acquire its envelope. All stages are highly regulated both temporarily and quantitatively, and vary for each particular virus.

Important Classes of Antiviral Targets and Compounds

The major targets and compounds are illustrated in **Figure 1**.

Virus attachment

Currently, there are no major drugs which prevent virus attaching to the host cell. The problem has been illustrated by the picornavirus capsid-binding compounds such as pleconaril. Other compounds are being tried for HIV but with little success so far. It seems that many sites on the surface of a virus can undergo changes without great loss of viral viability; the compounds present too low a genetic barrier (see below).

HIV fusion inhibitors

To date, only one entry inhibitor has been approved by the US Food and Drug Administration (FDA). This is efuviritide (T-20), a synthetic peptide that targets the HIV gp41 envelope protein to prevent fusion. The broad-spectrum antiviral agent, Rep 9, is progressing through development.

Influenza channel-blocking compounds

Amantadine and rimantadine act by binding to M2 and thus interfering with the penetration of hydrogen ions into the virion, a process essential for uncoating the single-strand RNA genome. Key resistant mutations map to the *M2* gene (and to a lesser extent, HA gene). Such resistant variants are selected quickly and are fully viable. This has severely limited the clinical usefulness of these drugs. During the 2005/06 season in the USA, 109/120 (91%) of H3N2 isolates were resistant to amantadine and rimantadine. Combination with other drugs now seems unlikely to be a viable option and so their future role may be minimal.

Antisense binding to viral mRNA

The antisense approach has the potential for great selectivity due to the sequence of bases in the oligonucleotide being complementary to a particular viral sequence. Conceptually, it may be possible to interfere with latent virus, even when the HIV genome has integrated into the DNA of a long-lived human cell. In practice, there have been many difficulties in progressing antisense compounds to the clinic. So far, only formivirsen has been licensed by the FDA. Its use is limited to intravitreal inoculation in patients with CMV retinitis.

Protease inhibitors

Many viruses encode one or more enzymes involved in the cleavage of virus or cellular proteins. HIV induces an enzyme that specifically cleaves protein precursors in the maturation of gag and pol polyproteins. Peptide

mimetics were designed specifically to target HIV protease. The resulting compounds, several of which are now standard HIV drugs, are among the most active antiviral compounds known and inhibit HIV in the nanomolar range. Like other HIV antivirals, drug-resistant mutants arise and so the compounds are used in combination with reverse transcriptase inhibitors. Other families of viruses encode proteases; except for HCV, the search for effective inhibitors has yielded few useful compounds.

Viral DNA/RNA polymerase inhibitors

Nucleoside and nucleotide analogs play a pivotal role in antiviral chemotherapy. The ultimate target is the viral DNA/RNA polymerase which is well conserved within different strains of a particular virus and sometimes within a virus family. For those nucleosides which are activated initially to their monophosphates by a viral enzyme, there is an additional potential for selectivity. In general, the viral enzymes are less stringent in their structural requirements than the corresponding host (e.g., human) equivalents. Because the viral polymerase is crucial to the replication of that virus, there are few potential mutations which allow the polymerase to retain full function but reject the antiviral compound. This results in a relatively high genetic barrier (see below).

Nucleoside/nucleotide analogs are the treatment of choice for herpes viruses and HBV. They are included as two or three components of HAART for HIV. For influenza, T-705 is a good candidate progressing through development. For HCV, viral polymerase inhibitors, such as R1626, are being developed.

Non-nucleoside RT inhibitors

In the search for new inhibitors of retrovirus reverse transcriptase (RT), several chemically unrelated compounds emerged from screens that bind to RT at sites other than the nucleotide binding site. Some are highly selective and inhibit HIV-1 RT at nanomolar concentrations. Enzyme studies showed that these compounds inhibit RT non-competitively. The compounds are generally thought to be allosteric inhibitors. An early problem associated with these compounds was the rapid emergence of drug-resistant strains. Because the drug-resistant mutations do not directly involve the active sites on the enzyme, they are less likely to compromise enzyme function. Therefore, the genetic barrier is lower than that corresponding to nucleosides targeting the catalytic site. However, adding non-nucleoside RT inhibitor (NNRTI) to NRTI(s) raises the genetic barrier and so the NNRTIs play an important role in HIV combination therapy (see below).

Helicase inhibitors

A new target for effective inhibition of HSV has been discovered. These compounds are being developed by Bayer AG Pharma (e.g., BAY 57–1293) Boehringer-Ingelheim (e.g., BILS 22 BS) and Tularik Inc. (e.g., T157602), and others. To date, they are all aminothiazole derivatives and interact with the helicase–primase complex. This comprises a group of three proteins concerned with unwinding the dsDNA and priming the daughter strand during DNA replication. The specific target is thought to be the product of the HSV UL5 gene, the helicase proteins. The compounds appear to be highly potent in tissue culture and in animal models where they appear to be superior to the nucleosides, ACV, and PCV, especially their efficacy upon delayed therapy. However, it remains to be seen whether toxicity will be an issue; furthermore, preliminary evidence suggests that the development of drug resistance may impede their development.

Virus assembly

As yet, there are no FDA-approved drugs targeting virus assembly but ST-246 is progressing through development. It was discovered as part of the program to prepare for bioterrorism, pox viruses being one of the perceived threats. Investigations with ST-246 resistant virus indicated that the mechanism involves the F13L gene which codes for a major virus envelope protein, p37. Intracellular enveloped viruses (IEVs) are formed from intracellular mature viruses (IMVs) and p37 participates in this wrapping process. ST-246 inhibits this stage and so IEVs are not available to be transported to the cell surface to produce extracellular virus. Exactly how ST-246 acts is being elucidated. Deleting F13L from vaccinia resulted in a virus (delta F13L-Vac) which replicates in cell culture although producing smaller plaques than wild-type (wt) virus. In mice infected with delta F13L-Vac, no lesions were produced but there was a good immune response which protected mice from subsequent challenge with wt virus.

Neuraminidase inhibitors

Mature virions of influenza virus comprise enveloped particles. The envelope membrane is decorated with two glycoproteins which form morphologically distinct 'spikes'. The most prominent of these are hemagglutinin (HA) molecules. Much less numerous but with a distinctive long-stalked 'mushroom' shape are the neuraminidase (NA) molecules. The function of the latter is to cleave sialic acid from sialylated proteins. Sialic acid is the receptor for the influenza virus on mammalian cells. Thus, NA is thought to help the release of virus from the host cell, preventing re-adsorption to the same cell; the enzyme may also aid the passage of virus through mucus to facilitate the colonization of respiratory or intestinal tissues.

During the early 1990s, the crystal structures of NA and its binding to sialic acid were solved. The NA active site was shown to contain well-formed large and relatively rigid pockets. Molecules were designed to interact with this pocket leading to a series of potent inhibitors. Two compounds with this mechanism of action are zanamivir and oseltamivir. Both are potent inhibitors of influenza

A virus replication attesting to the important functional role of NA in virus replication. Other compounds targeting this function are currently in development.

Challenges for Antiviral Therapies

Antiviral Prodrugs

The efficacies of several important antiviral drugs (including the nucleoside analogs acyclovir and penciclovir) are severely limited by poor oral bioavailability. One approach to this has been the synthesis of chemically modified derivatives that are rapidly converted to the nucleosides by the host metabolic enzymes. Valaciclovir, the prodrug for acyclovir, and famciclovir, the prodrug for penciclovir, are readily absorbed yielding high blood levels of the parent compound. Both are used widely for treating herpes simplex and varicella zoster infections. Similarly, other oral prodrugs have been developed: the influenza drug oseltamivir, the HIV drugs tenofovir disoproxil fumarate and fosamprenavir, the HBV drug adefovir dipivoxil and the HCV drug R1626. When new compounds are identified with antiviral activity, this kind of approach may be considered at an early stage of development. For example, famciclovir, rather than penciclovir, was used in the clinical trials and currently R1626, the oral prodrug of R1479, is being developed for HCV therapy.

Besides considering prodrugs for improving oral bioavailability, there are many other potential aims. In order to improve cell permeability of GS9148, a prodrug (GS9131) was selected. GS9131 showed potent anti-HIV activity with mean EC_{50} of 50 nM against multiple HIV-1 isolates. After oral administration to dogs at $3\,mg\,kg^{-1}$, GS9131 effectively delivered the active metabolite, GS9148-DP, at high levels for a prolonged period into PBMCs ($C_{max} = 9.2\,\mu M$, $T_{1/2} > 24$ h).

A new approach was reported in the *Prusoff Award Lecture* by Dr. Tomas Cihlar (Gilead Sciences, Foster City, CA, USA) at ICAR 2006. The concept is well established that nucleoside analogs, as their triphosphates, are trapped within the cell and therefore give prolonged activity against virus replication. Could the same strategy be applied to HIV protease inhibitors (PI)? Is it possible to make a phosphonate prodrug of a known PI so that, after entering the cell, it is converted to a charged active phosphonate metabolite with enhanced intracellular retention? This approach is currently under investigation.

Potential for Mutations Leading to Drug Resistance

Most antiviral compounds are highly selective and target a single virus protein. A natural consequence is that point mutations in the virus genome can result in drug-resistant variants. Double-stranded DNA viruses have a relatively low intrinsic error rate (1×10^{-7} to 1×10^{-8} errors per nucleotide) and a 3′–5′ exonuclease proofreading function can edit out errors. In contrast, some RNA viruses have very high intrinsic mutation rates with no proofreading. This high rate of mutation ($\geq 1 \times 10^{-4}$), coupled with a large population of virions, can quickly lead to enormous genetic diversity within a single infected host. For example, HIV has a single-strand RNA genome of approximately 9000 nucleotides. The replication rate in an infected individual has been estimated to be approximately 10^9 daily; thus, $10^{-4} \times 9000 \times 10^9 = 9 \times 10^8$ mutants occur each day. This means that, in theory, every point mutation occurs 10^5 times per day in an HIV-infected individual and every double mutant 10 times per day! As a result, HIV actually exists as a 'quasispecies' or 'swarm' around a particular consensus sequence. Similarly, HCV exists as 'quasispecies'; it has the fastest known daily replication rate of 10^{12} virions daily. HBV, which has a reverse transcriptase step within its replication cycle, mutates readily but it has overlapping reading frames so that a change in the gene encoding the surface protein may also change the gene for the polymerase. This may limit the number of viable mutations. Influenza virus has an additional mechanism for the spread of resistance. The influenza virion contains eight separate segments of single-stranded RNA genome. When two influenza strains co-infect a single cell, the segments can re-assort and pass a resistance mutation from one strain to another.

Based upon virus mutation rates alone, viruses would evolve to resist any specific inhibitor if it were not highly effective in reducing virus replication, ideally to zero. In practice, this is, almost, achieved with drug combinations but patients have a responsibility for their own therapy – any missed dose gives the virus a chance. Also, resistance mutations usually have a biological price; virus fitness may be compromised.

Mutations, that give rise to amino acid substitutions at the interaction site and cause reduced binding of the inhibitor, are termed 'primary mutations'. Such mutations usually arise early upon exposure to the inhibitor. Further mutations, termed 'secondary mutations', may accumulate and contribute to the level of resistance. Yet further mutations may appear which are apparently unrelated to the interaction site and may have no effect on the resistance level. However, these can increase enzyme efficiency so as to compensate for the deleterious effects of the primary and secondary mutations. Many compensating mutations are suspected but often their precise role has yet to be elucidated.

Drug Resistance

Genetic barrier

When a virus is being inhibited by an antiviral compound, resistance mutations are selected but the ease with which this is done depends upon how many potential mutations can give resistance without compromising viral fitness.

This has become known as the genetic barrier. With monotherapies, resistance appears quickly with NNRTIs but more slowly with NRTIs which target the same reverse transcriptase (RT) but at the catalytic site. For PIs, which target the protease catalytic site, the rate of appearance of resistance is about comparable to that for NRTIs. It was thought that combining one NRTI and one PI would delay the appearance of resistance greatly, but clinical practice showed that the delay was modest. It is more effective to combine two or three compounds which target the same HIV enzyme but have differing mutation patterns. One high genetic barrier is more effective in delaying resistance than two low genetic barriers.

The anti-influenza M2 channel blockers and anti-picornarvirus capsid binding compounds are examples of agents which appear to create too low a genetic barrier to become useful clinical therapies.

Mechanism of Antiviral Action

The most important classes of virus inhibitors are shown in **Figure 1**. This defines the mechanism of action which may be determined by various approaches. The time-of-addition of the compound in relation to its activity may give important clues. Patterns of cross-resistance to previously characterized agents give useful information. Drug-resistant mutants may be selected by passage of virus in suboptimal concentrations of the inhibitor. The location of drug resistance, found by virus genome sequencing followed by site-directed mutagenesis or marker transfer, provides direct evidence for the mechanism and may give information about the precise interaction between compound and the target protein; this may be at or near the active site of an enzyme. In the search for new antiviral compounds, inhibition of a viral enzyme may be the test screen. Computer modeling of compounds binding to their target proteins may aid further optimization and define the mechanism more precisely.

Virus Fitness

Drug-resistant mutant viruses are often attenuated or 'less fit'. The efficiency of virus replication can be reduced leading to smaller plaque size in cell cultures or reduced pathogenicity when tested in suitable animal infection models. The TK^- strains of herpes viruses resistant to ACV and PCV are usually less pathogenic and less able to reactivate from latency than wt HSV. The His274Tyr influenza virus, resistant to oseltamavir, is less pathogenic in ferrets than wt influenza. In chronic HBV patients, continued therapy with lamivudine, even after the appearance of the YMDD resistant strain, used to be better than no treatment although now patients would be switched to alternative therapy. In HIV patients being treated with PIs, the initial resistant mutations give HIV protease with reduced catalytic activity. Later, a constellation of mutations gives rise to more successful drug-resistant viruses that will then dominate the population.

Antiviral Combination Therapy

The problem of antiviral resistance led directly to the introduction of antiviral combination therapy as a crucial feature to control chronic virus infections, notably those caused by HIV and probably will be with HBV and HCV. Initially, there was much opposition to the introduction of antiviral combinations, probably best explained by an underlying fear of enhanced toxicity: this was the problem that dominated the early phase of HIV therapy but eased as newer, better tolerated drugs became available. HIV presented a new urgency; very high levels of HIV genome RNA turnover in the patient and the high mutation rate in the HIV genome meant that antiviral resistance inevitably led to the failure of monotherapies. The concept of genetic barrier was developed at this time. The aim is to create the highest possible genetic barrier and stop all virus replication so that the virus has no chance to overcome that barrier by mutation. This ultimately led to HAART involving the use of triple and quadruple combinations of active compounds. Experience has shown that sequential use of monotherapies is undesirable. Switching from one drug to another enables the sequential development of multiple resistant strains. Current guidelines recommend that at least two RT inhibitors are included in any HAART regimen. This has been very successful in controlling the disease but put a heavy burden on patients who had to take many pills at varying times through the day. So companies developed combinations of their drugs, the first being Combivir (**Table 1(d)**). Recently, two major pharmaceutical companies have been cooperating in the development of a joint formulation in which three antivirals are combined within a single pill (Atripla) leading to a greatly improved convenience and, hopefully, compliance. This first example has started a trend. A similar approach is considered applicable to HBV. For example, telbivudine and valtorcitabine together are being evaluated in clinical trials.

Favorable drug/drug interaction is another rationale for combining drugs. Nearly all current selective antiviral compounds are considered to be virustatic agents. This concept has had major implications in the treatment of HIV. Continual antiviral blood levels of drugs, with intervening troughs, are not sufficient for control of HIV replication. There must be continuous high levels which well exceed the antiviral concentration for that drug. Ritonavir, by blocking the elimination of other PIs, enhances both the concentration and duration of the PI in patients. Lopinavir is co-formulated with ritonavir in a 4:1 ratio primarily to enhance the pharmacokinetics of lopinavir (**Table 1(d)**).

When the symptoms of an infection require treatment, then combining an antiviral agent with an immunomodulator, anesthetic, or anti-inflammatory substance may give added benefit. Thus, acyclovir has been combined with cortisone to simultaneously reduce pain and irritation of the herpes lesion at the same time reducing the replication of infectious virus which would otherwise be prolonged by the anti-inflammatory component. This concept is likely to be widened to other cases in which the inflammatory response plays an important role in the disease, for example, influenza.

Virus Latency

Virus latency remains an obstacle to successful antiviral chemotherapy for which no solution appears to be in sight. Members of the *Herpesviridae* are notable for being able to establish latent infections; with HSV and VZV, latency is established in neuronal cells. During latent HSV-1 infection, the latency-associated transcript (LAT) of HSV-1 is the only known viral gene expressed. A micro-RNA (miR-LAT), which is generated from the exon 1 region of the LAT gene, reduces the induction of apoptosis and so contributes to the survival of that latently infected neuron. Few, if any, HSV proteins are expressed, so affording little chance for either the immune system or antiviral drugs to control the infection. Latency can be established despite ongoing antiviral chemotherapy although there is some evidence from animal models that famciclovir may be more effective than valaciclovir in reducing the load of latent virus when given very early during experimental infections. It still remains to be demonstrated whether or not this has any practical value in patients where therapy shortly after primary exposure is unlikely to be possible. While long-term therapy using nucleoside analogs such as valaciclovir or famciclovir are very successful in suppressing recrudescent lesions in a latently infected individual, on termination of suppressive therapy reactivation leading to recurrent disease is prone to occur. Other viruses, notably HIV, also establish latency in a few long-lived cells, thus making the complete eradication of these infections especially difficult to achieve.

Future Prospects

Herpes

Some bicyclic nucleoside analogs (BCNAs) had been found to be exceptionally active and selective for VZV. A lead compound is Cf 1743. Like ACV and PCV, Cf 1743 is activated by VZV TK but, unlike ACV and PCV, it seems not to act via its triphosphate. Therefore, it will be particularly interesting to evaluate this highly active compound for its ability to reduce postherpetic neuralgia (PHN) in patients with shingles. Cf 1743 as its valyl ester prodrug (FV 100) has recently entered development and progress has been reported on large-scale synthesis and initial toxicity testing.

Helicase-primase inhibitors (see above) represent a new class of compounds active against the herpes viruses. During the last decade, there has been a notable lack of new classes of antiherpes compounds. It will be interesting to see how they progress.

HBV

Since 1999, four drugs have received FDA approval, lamivudine, adefovir dipivoxil, entecavir, and telbivudine (**Table 1(c)**). Although entecavir is the most active compound on a weight basis, the greatest reductions in HBV DNA levels have often been achieved with the most recently (2007) approved compound, telbivudine. In addition, some interesting compounds are progressing through development.

Telbivudine, in phases IIb and III, has been consistently more active than lamivudine against HBV. Because HBV strains (with rtM204I and rtL180M mutations in the polymerase) are cross-resistant to both telbivudine and lamivudine, telbivudine should not be used to treat patients with HBV resistant to lamivudine. However, it may be expected that the greater control of HBV replication with telbivudine than with lamivudine would lead to a lower proportion of patients with resistant virus. Telbivudine, in both pre-clinical toxicological tests and clinical trials, has shown a remarkably good safety record. Therefore, telbivudine may become the first line drug of choice, replacing lamivudine. Since telbivudine and valtorcitabine showed synergy in both cell culture assays and in the woodchuck hepatitis model, these two compounds are being evaluated together in phase IIb trials. Only time will tell if this combination is also able to reduce the rate of appearance of resistance. Telbivudine preferentially inhibits the second strand of HBV DNA synthesis and valtorcitabine inhibits both the first and second strand of HBV DNA synthesis; HBV would have to overcome two stages in its replication cycle and the genetic barrier (especially for the second strand synthesis) would be increased.

With most HBV therapies, although there may be a short lag period after treatment, HBV DNA levels return to baseline. This is typical for drugs which are virustatic. In contrast, clevudine showed a prolonged effect. In a phase I/II trial, at a daily dose of 100 mg, HBV DNA levels were reduced by 3.0 $\log_{10}$ at the end of 4 weeks of therapy and were still 2.7 $\log_{10}$ below baseline at 6 months. However, until an antiviral mechanism has been elucidated for this prolonged effect, one is not sure if this is a great benefit or a warning sign. If there is an antiviral mechanism, then clevudine clearly differs from other anti-HBV drugs and cannot be regarded as a virustatic drug. We suggest that an antiviral compound, which acts

via inhibition of the replication cycle and destroys the ability of the virus to continue replication, should be described by a new term, a virureplicidal drug.

HCV

Progress has been severely restricted by the lack of suitable test systems. It seems ironic that, in patients, the production rate of HCV is 10^{12} virions/day, the highest known rate of any virus to date and yet it has taken so long to discover a replication system in cell culture. In 1999, an HCV replicon system became available. No infectious particles are formed but viral RNA synthesis can be followed. Alternatively, HCV pseudoparticles can be obtained by adding the HCV genes for E1(GP35) and E2(gp66) to a partial HIV. The resulting hybrid virus can generate pseudoparticles which incorporate E1 and E2 as heterodimers. These pseudoparticles can be used to raise neutralizing antibody against HCV.

Only in 2005 was full HCV virus replication achieved by Wakita *et al.* JHF1 cDNA was cloned from an individual with fulminant HCV, strain 2a. After transfection into human hepatoma cell line (Huh7), the JFHI genome replicates and leads to the secretion of viral particles. These virions were then used to infect Huh7 cells and chimpanzees. Essentially, only this combination of JHF1 and Huh7 cells has been successful although some chimeric viruses, with the E1 and E2 genes of JFH1 replaced by those from another strain, can also replicate in Huh7 cells.

The availability of the HCV replicon system allowed screening for HCV RNA polymerase inhibitors. One example is R1479 which has progressed through to phase I/II trials. Its activity in HCV-infected patients was sufficient to encourage the development of the oral prodrug, R1626. An alternative approach is the inhibition of the HCV NS3/NS4A protease. Two compounds in phase II studies are VX-950 and SCH 503034. Both gave reductions in HCV RNA levels when used as monotherapies and both appear to have additive or synergistic activity with interferons. A potential limitation of these compounds is the requirement to be dosed 3 times daily but the addition of ritonavir improves the pharmacokinetics such that less frequent dosing may be possible, just as for the HIV protease inhibitors.

Influenza

Since 1999, two neuraminidase inhibitors, zanamivir and oseltamivir, have received FDA approval. Further development of other compounds became slow due to lack of interest but the threat of avian influenza A (H5N1) has stimulated renewed interest. Zanamivir dimer has a long duration of activity, about a week. Peramivir failed to lessen the duration of symptoms in a phase III trial but an oral prodrug may be worth trying.

T-705 (6-fluoro-3-hydroxy-3-pyrazine carboxamide), as its ribo-triphosphate (T-705RTP), inhibits the influenza virus polymerase in a GTP-competitive manner. In cell culture assays, it is active against many strains of A, B, and C influenza viruses. In mice infected with an H5N1 strain, T-705 was highly effective and, on rechallenge with 100-fold more virus on day 21, all the T-705 treated mice survived. T-705 seems to be a likely future therapy.

HIV

Over the last decade, there have been some big gains (reductions in morbidity and mortality) and harsh realities (replication rate 10^{10} virions/day, long-lived latent pool, side effects and viral resistance). Prospects for a vaccine remain poor but new compounds are being developed, including entry and integrase inhibitors. Great efforts have been made to make HIV therapies available to resource-limited areas. Because so many factors differ between areas, outcomes are most likely to differ. Most importantly, research, studying clinical outcomes in these resource-limited settings, should be continued. Otherwise resistant virus may be unknowingly spread around. Several firms have agreed to support the creation of centers of excellence. A great challenge is to investigate the role that therapeutics might play in preventing transmission.

A novel therapeutic concept was described by Jan Balzarini (Rega Institute for Medical Research). HIV gp120 is heavily glycosylated with about 11 'high-mannose' type glycans/gp120 molecule. Pradimicin A (PRM-A) can be considered as a prototype. A high genetic barrier is created by several PRM-A molecules binding to each HIV gp120 protein; resistance can occur in cell culture but only when there have been a large number (>5) of mutations leading to the loss of glycosylation sites. In HIV-infected patients, every loss of a glycosylation site presumably exposes part of the HIV gp120 protein surface to the immune system. Thus, the more resistant the HIV becomes toward PRM-A, the more immunogenic it may become. This is a 'hard choice' which has not yet been presented to HIV with any current therapy. Could the same approach be successful with HBV and HCV?

Conclusions

Niche Targets for Antivirals

Many viruses are well controlled by vaccines; the most outstanding instances are smallpox and polio. Cases of rubella, munps, and measles have been greatly reduced by routine vaccination. Vaccines, mainly used to protect travellers, are available for hepatitis A, rabies, yellow fever, and others. New vaccines can prevent those cervical cancers caused by papillomavirus types 16 and 18. Although these two types account for the majority of cervical cancers, activity against many other types would be required to give full protection. An antiviral could

possibly be active against many strains and be effective in an established infection. However, the papillomavirus genome is so small, not even encoding a DNA polymerase, there are few targets. New infections with VZV and HBV are preventable with vaccines but there remains a large pool of infected patients for whom antiviral therapy is required. The threat of avian influenza has led to the stockpiling of anti-influenza drugs. The lack of effective vaccines for HSV, HIV, and HCV has encouraged antiviral therapies. West Nile virus is an example of a newly emerging virus for which there is neither vaccine nor specific antiviral.

Broad Spectrum Antivirals?

Antiviral chemotherapy has come a long way since the first faltering steps with marboran, amantadine and rimantadine, idoxuridine (IDU), and vidarabine (Ara A). Acyclovir set the standard for effectiveness together with clinical safety. For over two decades, clinical safety has been associated with high selectivity for a single virus or a few closely related viruses. So far, there are no FDA-approved antiviral drugs, targeting viral replication, which are active against a broad spectrum of viruses. However, Rep 9 may be the first example.

See also: Human Immunodeficiency Viruses: Antiretroviral agents.

Further Reading

Anderson RM and May RM (1992) *Infectious Diseasese of Humans Dynamics and Control.* New York: Oxford University Press.
De Clercq E (2005) Recent highlights in the development of new antiviral drugs. *Current Opinion in Microbiology* 5: 552–560.
De Clercq E (2005) Antiviral drug discovery and development where chemistry meets with biomedicine. *Antiviral Research* 67: 56–75.
De Clercq E, Branchale A, Vere Hodge A, and Field HJ (2006) Antiviral chemistry and chemotherapy's current antiviral agents FactFile 2006. *Antiviral Chemistry and Chemotherapy* (1st edition) 17: 113–166.
De Clercq E and Field HJ (2006) Antiviral prodrugs – the development of successful prodrug strategies for antiviral chemotherapy. *British Journal Pharmacology* 147: 1–11.
Field AK and Loughlin CA (1999) Antivirals. In: Granoff A and Webster RG (eds.) *Encyclopedia of Virology*, 2nd edn., pp. 54–68. New York: Academic Press.
Field HJ and De Clercq E (2004) Antiviral drugs: A short history of their discovery and development. *Microbiology Today* 31: 58–61.
Field HJ and Whitley RJ (2005) Antiviral chemotherapy. In: Mahy BWJ and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections,* 10th edn., vol. 2, pp. 1605–1645. London: Hodder Arnold.
Schang LM, St. Vincent MR, and Lacasse JJ (2006) Five years of progress on cyclin-dependent kinases and other cellular proteins as potential targets for antiviral drugs. *Antiviral Chemistry and Chemotherapy* 17(6): 293–320.
Wakita T, Pietschmann T, Kato T, *et al.* (2005) Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nature Medicine* 11(7): 791–796.
Wutzler P and Thust R (2001) Genetic risks of antiviral nucleoside analogues – a survey. *Antiviral Research* 49: 55–74.

Apoptosis and Virus Infection

J R Clayton and J M Hardwick, Johns Hopkins University Schools of Public Health and Medicine, Baltimore, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Bcl-2 Founding member of anti-apoptotic Bcl-2 protein family; homologs found in cells and viruses.
Caspase Family of cysteine proteases that cleave after aspartate residues.
DD/DED/CARD Structurally related protein–protein interaction domains found in a variety of different proteins (e.g., death receptors, adaptor molecules, and caspases).
FLICE Caspase-8 (original name: Fas-associated death domain protein interleukin-1beta-converting enzyme (ICE)-like).
FLIP Family of caspase-8 (FLICE)-like inhibitory proteins encoded by viruses (vFLIPs) and cells (cFLIPs).
IAP (inhibitor of apoptosis) Family of proteins with BIR (baculovirus IAP repeats) and a RING finger; homologs found in cells and viruses.
Micro-RNA Small hairpin RNAs that regulate mRNAs through RNAi or translational inhibition.
NF-κB Transcription factor that when activated translocates to the nucleus and regulates a variety of cellular and viral functions.
RNAi (RNA interference) Mechanism by which RNA is targeted for degradation in a sequence-specific manner.
TRAILR TRAIL (tumor necrosis factor-related apoptosis inducing ligand) receptor.

Introduction

Programmed cell death is any process by which cells participate in their own death. Cell suicide programs are facilitated by the actions of gene products encoded by the cell destined to die. These death-promoting genes evolved, at

least in part, for the purpose of orchestrating cell autonomous death. The term apoptosis describes the morphology of naturally occurring programmed cell death during development and following certain pathological stimuli. In contrast to this deliberate cell death, the term necrosis describes cell morphology resulting from accidental cell death caused by acute injury. However, necrosis is less well defined, and is also used to describe the appearance of cells undergoing nonapoptotic programmed cell death. In addition, the term apoptosis can be used as a mechanistic term that refers to all types of programmed cell death. In multicellular organisms, programmed cell death is essential for development, tissue homeostasis, and regulating immune responses. In humans, it is estimated that billions of cells normally die per day by programmed death. Insufficient or excessive cell death characterizes most human disease states, including virus infections.

Classical apoptosis is characterized by a number of morphological and biochemical changes, including condensation of chromatin, cleavage of DNA between nucleosomes (DNA laddering), plasma membrane blebbing, exposure of phosphatidyl-serine on the outer leaflet of the plasma membrane (detected by annexin V binding), and fragmentation/division of mitochondria. These classic apoptotic events are caused by the activity of a subset of intracellular proteases known as caspases. Caspases are a family of cysteine proteases that cleave after specific aspartate residues in selected cellular proteins. A second subset of caspases includes the proteases that activate inflammatory mechanisms and may promote nonapoptotic programmed cell death. Other nonapoptotic programmed cell death pathways involve other types of proteases such as lysosomal enzymes, but less is known about these pathways, and little is known about their role in virus infections.

Any perturbation of the cell can potentially lead to activation of programmed cell death as a host defense mechanism to eliminate aberrant or damaged cells. Similarly, virus infection of a cell usually triggers the activation of programmed cell death. The ability of cells to recognize intruding viruses and activate cell suicide provides an important host defense mechanism for eliminating viruses by eliminating virus-infected cells prior to mounting host immune responses. However, viruses have developed myriad mechanisms to adapt to and regulate cellular death processes. As a consequence, many viruses cause disease primarily by inducing host cell death.

Cellular Death Pathways

Of the multiple pathways leading to cell death, caspase-dependent apoptosis is the best characterized. There are two general pathways for activating caspases, the receptor-activated (extrinsic) pathway, and the mitochondrial (intrinsic) pathway. Different viruses regulate multiple steps in both of these pathways.

Receptor-mediated pathways for the activation of caspases can be further subdivided into two groups: cell surface death receptors of the tumor necrosis factor receptor (TNFR) superfamily, and the intracellular pattern recognition receptors that activate primarily inflammatory caspases. The cytoplasmic portions of death receptors recruit and activate long pro-domain caspases (e.g., caspase-8). In turn, caspase-8 cleaves and activates the short pro-domain caspases (e.g., caspase-3). Caspase-3 is responsible for cleaving most of the known cellular substrates to facilitate cell death. Death receptor pathways are regulated by both viral and cellular factors that bind the receptor or other associated components in a manner that prevents caspase activation (**Figure 1**). The second receptor subgroup includes the pattern recognition receptors (PRRs), which can be further subdivided into three groups: the plasma membrane toll-like receptor family and two intracellular receptor families, the NOD-like receptors (NLRs), and the RIG-like helicases (RLHs), which are capable of detecting intracellular invaders. NLRs are activators of inflammatory caspases. Of particular relevance to virus infections, the intracellular RLH receptors recognize viral nucleic acids and trigger host defense mechanisms.

The mitochondrial pathway for activating caspases is triggered when cytochrome *c* is released from the intermembrane space of mitochondria into the cytoplasm. Released cytochrome *c* and ATP bind to Apaf-1 resulting in oligomerization of Apaf-1 and its binding partner caspase-9. Like the oligomerization-induced activation of caspase-8 by death receptors, oligomerization of the DED-like caspase-recruitment domain (CARD) motifs of caspase-9 also serves to activate the protease. Thus, initiator caspases (e.g., caspases-8 and-9) are activated by oligomerization, while effector caspases (e.g., caspases-3 and -7) are activated by proteolytic processing. Like caspase-8, activated caspase-9 also cleaves and activates caspase-3 to mediate apoptotic cell death. The mitochondrial pathway is regulated by pro- and anti-apoptotic cellular Bcl-2 family proteins that promote or inhibit cytochrome *c* release. Pro-apoptotic Bcl-2 family members Bax and Bak form homo-oligomers in the mitochondrial outer membrane that directly or indirectly release cytochrome *c* and other mitochondrial factors. This function of Bax and Bak is inhibited directly or indirectly by anti-apoptotic Bcl-2 family members Bcl-2, Bcl-x_L, and others. A more diverse subclass of Bcl-2-related proteins, the BH3-only proteins, inhibits antideath family members and/or promotes the functions of Bax and Bak. Cross-talk between the mitochondrial pathway and the receptor-activated pathway occurs when receptor-activated caspase-8 cleaves and activates the BH3-only protein Bid, leading to Bax- or Bak-mediated cytochrome *c* release. Cross-talk serves to amplify the apoptotic death pathway. Viruses also modulate multiple steps in the mitochondrial cell death pathway (**Figure 1**). For example, many viruses encode homologs of cellular anti-apoptotic Bcl-2 family proteins or other antagonists of

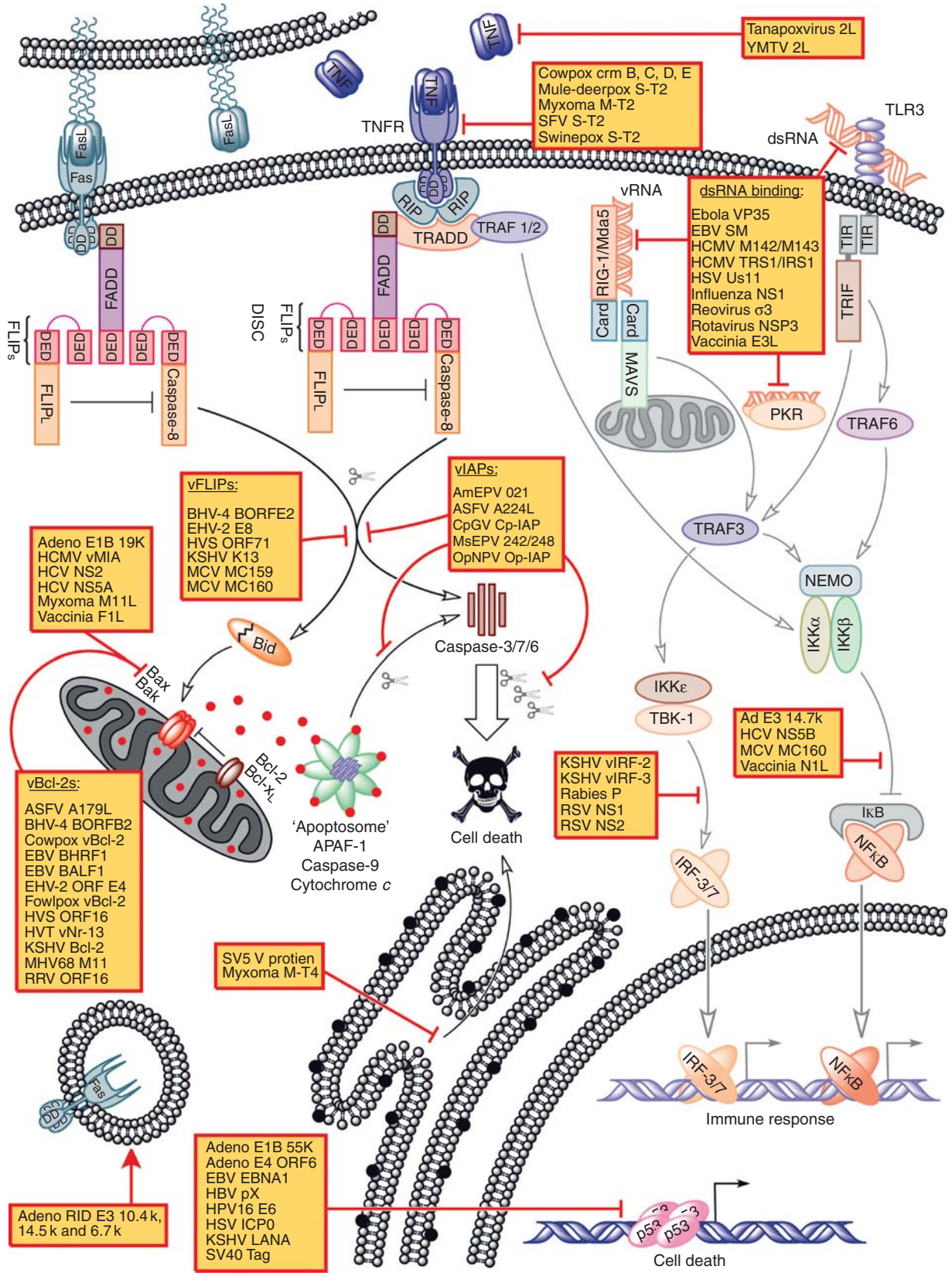


Figure 1 Cell death signalling during virus infection. Abbreviations are as in **Table 1**. Grayed areas indicate innate immunity signalling. Viral cell death regulators (yellow boxes, red arrows); cellular death regulators (black arrows); proteolytic cleavage events (scissors); ribosomes (black circles); cytochrome *c* (red circles).

Bax/Bak-mediated cell death. Still other viruses encode direct inhibitors of caspases, both pro-apoptotic and pro-inflammatory caspases.

Viruses also cause nonapoptotic cell death, but the mechanisms are less well characterized. Nonprogrammed necrotic cell death may simply be a consequence of extensive virus-induced cell injury after the virus has successfully inhibited apoptosis. Nonapoptotic cytopathologies induced by viruses include the formation of membranous vacuoles (often the sites of virus replication), activation of autophagy (lysosome-mediated degradation), and plasma membrane rupture/lysis. Viruses also trigger inflammation, which is mediated in part by caspases that cleave and activate pro-inflammatory cytokines, such as caspase-1 cleavage and activation of IL-1β. However, even if nonapoptotic death mechanisms are facilitated by cellular gene products, they may or may not cause programmed cell death. For example, cell death caused by lysosomal proteases that are released accidentally in damaged cells is not programmed if the pro-death function of lysosomal proteases did not evolve, at least in part, for the purpose of promoting cell suicide (to benefit the whole organism). However, this pathway is programmed if cathepsin cleavage sites in the pro-apoptotic BH3-only protein Bid, or other substrates, were selected during evolution to facilitate purposeful cell death during infection. Both programmed and non-programmed cell death mechanisms induced by viruses can be useful targets for therapeutic intervention.

Death Receptor Signaling

Death-inducing receptors on the cell surface can be placed into two subgroups, one containing Fas/CD95 and the other containing tumor necrosis factor receptor 1 (TNFR1). Fas recruits caspase-8 to directly and potently activate cell death. TNFR1 recruits different factors to form a distinct complex to inefficiently induce apoptosis or to activate other cellular signaling pathways.

Binding of Fas ligand (FasL) to Fas, or binding of TRAIL to death receptors 4 and 5 (DR4 and DR5), triggers the cytoplasmic tails of these receptors to form a complex known as the death-inducing signaling complex (DISC). The death domain (DD) in the cytoplasmic tail of the receptor binds directly to the DD domain of the small adaptor protein Fas-associated death domain (FADD). The DD-related death-effector domain (DED) of FADD binds directly to the DED domains in the N-terminus of caspase-8 to oligomerize caspase-8 in the DISC. In the other subgroup of death-inducing receptors, tumor necrosis factor-alpha (TNF-α) binds to TNFR1, allowing TNFR1 to recruit intracellular adaptor molecules receptor-interacting protein (RIP) kinase, TNFR-associated factors (e.g., TRAF2), and TNFR1-associated death domain (TRADD) protein to assemble a complex that dissociates from the membrane, recruits FADD and caspase-8 that in turn cleaves and activates the effector caspases (e.g., caspase-3,-7) to cause cell death. Both cellular and viral factors regulate cell death and other functions of TNFR1 and related receptors. TNFR1 can recruit additional factors, including the cellular inhibitors of apoptosis (e.g., cIAP1 and cIAP2), and can cause pleiotropic effects on cells, including the activation of the pro-inflammatory transcription factor NF-κB.

Viral Regulators of Receptor Signaling

Viruses encode a plethora of molecules that interfere with signaling by extracellular ligands and their respective receptors. In some cases, these viral factors mimic TNFR1, such as the cytokine response modifier (CRM) proteins of cowpox virus, M-T2 protein of myxoma virus, and S-T2 orthologs encoded by several poxviruses. These soluble or membrane-bound receptor mimics act as TNFα-sinks, thereby diminishing the frequency of the interaction between TNFα and the host receptor. Similarly, the viral TNF-binding protein (vTNF-BP) 2L from tanapox (and several other poxviruses) sequesters TNF directly, but bears amino acid similarity to MHC class I rather than TNFR. Another TNFR-superfamily receptor mimic found in cowpox and mousepox viruses is vCD30, which diminishes signaling to the host cell and the proliferation of B and T cells via CD30L, dampening the immune response to infection (**Table 1**).

Other viruses alter intracellular mechanisms of death receptor signaling. The latency membrane protein 1 (LMP1) of Epstein–Barr virus (EBV) acts like a constitutively active (ligand-independent) TNFR-superfamily receptor capable of interacting with multiple TRAFs to inhibit apoptosis. Herpes simplex virus (HSV) glycoprotein D binds a TNFR-related protein as a receptor (HVEM) for entry into the cell, resulting in the activation of NF-κB. Wild strains of human cytomegalovirus (HCMV) encode a HVEM-like molecule UL144 that is missing in laboratory strains. Adenovirus encodes proteins E3–10.4k, E3–14.5k, and E3–6.7k that can form the 'receptor internalization and degradation' or RID complex that binds to the cytoplasmic portion of several different death receptor-superfamily members (including TNFR1, Fas, and TRAILR). Several different viruses target the adaptor TRAF2 to redirect cell death signals toward activation of NF-κB. Herpesvirus saimiri (HVS) StpC, KSHV K15, and rotavirus VP4 also activate NF-κB. Similarly, hepatitis C virus (HCV) nonstructural protein 5A (NS5A) can redirect the death signal by interacting with TRADD to inhibit its association with FADD, thus inhibiting death signaling, while HCV core protein selectively recruits FADD to TNFR1 to promote cell death. NS5A is also known to inhibit TRAF2, further directing the death signal away from NF-κB activation and survival signaling. These are only a few of the many examples

Table 1 Virus-encoded cell death modulators, listed alphabetically by virus family

Family	*Name*	*Viral gene*	*Function*
Adenoviridae	Adenovirus	E1A	Downregulates $cFLIP_S$, induces TNF-mediated caspase-8 activation, inhibits NF-κB
		E1B-19k	Binds and inhibits Bax/Bak
		E1B-55k	Ubiquitination of p53
		E3 (10.4k, 14.5k, 6.7k)	Internalization of TNF-related death receptors and their subsequent degradation
		E3-14.7k	Binds IKK and p50, suppresses NF-κB; inactivates caspase-8
		E4-ORF6	Ubiquitination of p53
		VAI	Inhibits Dicer as pseudo-substrate
		VAI	Inhibits PKR as pseudo-substrate
Asfarviridae	ASFV[a]	A179L	Bcl-2 homolog, inhibits MMP and cell death
		A224L	vIAP, activator of IKK
		A238L	Non-phosphorylatable IκB ortholog
Baculoviridae	AcMNPV[b]	P35	Broad spectrum caspase inhibitor
	BmNPV[c]	P35	Broad spectrum caspase inhibitor
	CpGV[d]	Cp-IAP	Binds caspases; Ub-ligase
	OpNPV[e]	Op-IAP	Binds caspases, Ub-ligase
	SlNPV[f]	P49	P35-related caspase inhibitor
Bunyaviridae	LAC[g]	NSs	Suppresses RNAi
	SAV[h]	NSs	Inhibits transcription/translation; *Drosophila* reaper-like
Filoviridae	Ebola virus	VP35	Binds dsRNA
Flaviviridae	HCV[i]	Core	Enhances Bid cleavage and TRAIL signaling, binds TNFR1
		E2	PKR pseudo-substrate, interacts with PKR
		IRES	vRNA; competitive inhibitor of PKR
		NS2	Interacts with CIDE-B and prevents MMP
		NS3/4A	Cleaves MAVS, inhibits interferon response; cleaves TRIF, inhibits recognition by TLR3
		NS5A	Direct interaction with PKR; inhibits TRADD/FADD association; interacts with TRAF2, inhibits NF-κB; binds Bax
		NS5B	IKKα-specific inhibitor of IKK complex
Hepadnaviridae	HBV[j]	pX	Direct binding to and inhibition of p53, activation of Src, MAPK, and NF-κB
Herpesviridae	BHV-4[k]	BORFB2	Bcl-2 homolog, inhibits MMP and cell death
		BORFE2	vFLIP, modulates caspase-8 activation
	EBV[l]	BALF1	Suppresses BHRF1 indirectly
		BHRF1	Bcl-2 homolog; inhibits cell death
		BZLF1	Downregulates TNFR1 expression
		EBER1,2	PKR pseudoactivator
		EBNA1	Transcriptionally downregulates HAUSP, leads to p53 poly-Ub
		LMP1	Constitutively active CD40, important for transformation
		miR-BHRF1	Latency-related
		SM	Binds dsRNA, interacts with PKR
	EHV-2[m]	E8	vFLIP, modulates caspase-8 activation
		ORF E4	Bcl-2 homolog, inhibits MMP
	HCMV[n]	IE1	Transcriptional regulator of NF-κB effectors
		IE1/IE2	Affects TNFR trafficking
		M142/M143	Binds dsRNA
		TRS1, IRS1	Binds dsRNA
		UL144	TNFR Superfamily; NF-κB activation
		UL36/vICA	Non-FLIP caspase-8 inhibitor
		UL37/vMIA	Binds ANT, GADD45; promotes mitochondria fragmentation but inhibits cell death
	HSV[o]	γ(1)34.5	eIF2α phosphatase
		ICP0	Sequesters the deubiquitinating enzyme HAUSP, inactivates p53
		LAT	Micro-RNA of TGF-β and SMAD3, suppresses stress response
		Us11	Binds dsRNA, interacts with PKR
		gD	Binds TNFR-related HVEM, activates NF-κB
		US3	vSer/Thr Kinase; modifies Bad, blocks Bad induced death
	HVS[p]	ORF 16	Bcl-2 homolog, inhibits MMP
		ORF 71	vFLIP, modulates caspase-8 activation
		StpC	Interacts with TRAF2, activates NF-κB

Continued

Table 1 Continued

Family	*Name*	*Viral gene*	*Function*
		Tip	Lck Adaptor; NF-κB activation
	HVT[q]	vNr-13	Bcl-2 homolog, inhibits MMP
	KSHV[r]	K1	vTyrosine kinase, constitutive Lyn activation
		K13	vFLIP, modulates caspase-8 activation
		K15	Interacts with TRAF2, activates NF-κB
		K7	Complexes with Bcl-2/caspase-3; regulates Ca^{2+} levels
		KSBcl-2	Bcl-2 homolog, inhibits MMP
		LANA	Suppresses p53 transcription
		vIRF-2	Direct interaction with PKR
		vIRF-3	Inhibits IKKβ and NF-κB activation
	Marek's disease virus	MEQ	Transcriptional inhibitor of apoptosis through TNFR, ceramide, UV and serum withdrawal
	MCMV[s]	Unknown	Disregulates TNFR levels
	MHV68[t]	M11	Bcl-2 homolog, inhibits MMP
Iridoviridae	RRV[u]	ORF16	Bcl-2 homolog, inhibits MMP
	ATV[v]	ReIF2H	v-eIF2α; not phosphorylatable
Nodaviridae	FHV[w]	B2	Binds dsRNA
			Orthomyxoviridae
Influenza virus	NS1	Binds dsRNA	
		NS2	Triggers RIG-I
		PB1-F2	Mitochondrial fragmentation, MMP loss and cytochrome *c* release
Papillomaviridae	HPV16[x]	E6	eIF2α phosphatase; targets p53 for ubiquitination and degradation
		E6/E7	Interferes with DISC formation
Paramyxoviridae	Hendra virus	V	Inhibits Mda5
	Mumps virus	V	Inhibits Mda5
	RSV[y]	M2-1	Associates with RelA, activates NF-κB
		NS1, NS2	Interferes with IRF3 activation
	Sendai virus	C	Abolishes IFN/STAT signaling
		V	Inhibits Mda5
	SV5[z]	V	Inhibits Mda5; suppressor of ER-stress-mediated cell death
Picornaviridae	Coxsackie B3	Unknown	Degradation of Cyclin D1, p53, and β-catenin
	Poliovirus	3A	Affects TNFR trafficking
		3C	Cleaves RelA
Polydnaviridae	MdBV[aa]	H4, N5	vIκB, inhibit NF-κB translocation
Polyomaviridae	SV40[bb]	miR-S1	Suppresses TAg and early transcripts in late stages; immune evasion
		TAg	eIF2α phosphatase; binds and inactivates p53
Poxviridae	AmEPV[cc]	021	vIAP; IAP-binding motif antagonist
	Cowpox virus	CrmA/B12R	Serpin and caspase-1, 8, 10 inhibitor
		CrmB,C,D,E	TNFR/death receptor mimic
		vBcl-2	Bcl-2 homolog, inhibits MMP
		vCD30	CD30 homolog that dampens proliferative signal to B and T Cells
	DPV[dd]	S-T2-like	TNF-receptor decoy; sequesters TNF
	Fowlpox virus	vBcl-2	Bcl-2 homolog, inhibits MMP
	MCV[ee]	MC066L	vGlutathione-peroxidase; protects from UV and peroxide stress
		MC159	vFLIP, interacts with FADD, TRAF3 and caspase-8; promotes NF-κB activation
		MC160	IKKα-specific inhibitor of IKK complex and NF-κB activation; vFLIP; inhibits Fas signaling; binds FADD and caspase-8
	Mousepox virus	P28/012	Anti-apoptotic ubiquitin ligase
		vCD30	CD30 homolog that dampens proliferative signal to B and T Cells
	MsEPV[ff]	242	vIAP; IAP-binding motif antagonist
		248	vIAP; IAP-binding motif antagonist
	Myxoma virus	E13L	Interacts with ASC; inhibits caspase-1 activation
		M11L	Inhibits MMP and cell death
		MNF	vIκB, inhibits NF-κB translocation
		M-T2	TNF-receptor decoy; sequesters TNF
		M-T4	Inhibits apoptosis, localizes to ER
		Serp2/M151R	Caspase and granzyme B inhibitor
	SFV[gg]	P28/N1R	Anti-apoptotic ubiquitin ligase
		S-T2	TNF-receptor decoy; sequesters TNF

Continued

Table 1 Continued

Family	*Name*	*Viral gene*	*Function*
	Swinepox virus	S-T2-like	TNF-receptor decoy; sequesters TNF
	Tanapox virus	2L	vTNF-BP; sequesters TNF
	Vaccinia virus	A46R	TLR3 decoy receptor, TIR domains only
		CrmA/B13R	Serpin and caspase-1, 8, 10 inhibitor
		E3L	Binds dsRNA, independently inhibits PKR
		F1L	Interacts with Bim and Bak to inhibit MMP
		K1L	Prevents IκB degradation, inhibits NF-κB
		K3L	v-eIF2α, inhibits PKR function
		N1L	Inhibits TRAF2/6 and IKKα/β
	YMTV[hh]	2L-like	vTNF-BP; sequesters TNF
Reoviridae	Reovirus	σ3	Binds dsRNA
		Unknown	Prevents IκB degradation
	Rotavirus	NSP3	Binds dsRNA
		VP4	TRAF2 binding, IKK activating impairs caspase-8 activation
Retroviridae	BLV[ii]	Unknown	Disregulation of TNFR cell surface expression
	HIV[jj]	gp120	Interacts with CXCR4, causes apoptosis in CTLs; cross-links CD4, induces Fas expression in uninfected cells
		miR-N367	Suppresses Nef, suppresses its own replication
		Nef	Enhances NF-κB signaling and TNF-mediated apoptosis; reduces MHC-I, CD4 expression; phosphorylation and inhibition of Bad
		Protease	Cleaves caspase-8, degradation of Bcl-2
		Tar	vRNA binds TRBP; suppresses RISC
		Tat	PKR pseudo-substrate; enhances NF-κB signaling, TNF-apoptosis
		Vpr	G2/M arrest; glucocorticoid-like; Bax-dependent apoptosis
		Vpu	Suppresses NF-κB activation
	HTLV-1[kk]	Tax	IKKγ adaptor ; enhancer of Bcl-x_L, repressor of Bax,
	REV-T[ll]	v-Rel	Activated c-Rel
Rhabdoviridae	Rabies virus	P	Interferes with IRF3 activation

[a]African swine fever virus; [b]*Autographa californica* multiple nucleopolyhedrovirus; [c]*Bombyx mori* nucleopolyhedrovirus; [d]*Cydiapomonella* granulovirus; [e]*Orgyia pseudotsugata* multiple nucleopolyhedrovirus; [f]*Spodoptera litura* nucleopolyhedrovirus; [g]La Crosse virus; [h]San Angelo virus; [i]Hepatitis C virus; [j]Hepatitis B virus; [k]Bovine herpesvirus-4; [l]Epstein Barr virus; [m]Equid herpesvirus-2; [n]Human cytomegalovirus; [o]Herpes simplex virus; [p]Herpesvirussaimiri; [q]Herpesvirus of turkeys; [r]Kaposi's sarcoma-associated herpesvirus; [s]Mouse cytomegalovirus; [t]Mouse herpesvirus strain 68; [u]Regina ranavirus; [v]*Ambystoma tigrinum stebbensi* virus; [w]Flock house virus; [x]Human papillomavirus 16; [y]Respiratory syncytial virus; [z]Simian virus 5; [aa]*Microplitis demolitor* bracovirus; [bb]Simian virus 40; [cc]*Amsacta moorei* entomopoxvirus; [dd]Mule-deer poxvirus; [ee]*Molluscum contagiosum* virus; [ff]*Melanoplus sanguinipes* entomopoxvirus 'O'; [gg]Shope fibroma virus; [hh]Yaba monkey tumorvirus; [ii]Bovine leukemia virus; [jj]Human immunodeficiency virus; [kk]Human T-lymphotropic virus 1; [ll]Reticuloendotheliosis virus strain T.

where viral factors alter death receptor signaling apparently to alter programmed cell death and other signaling pathways (**Figure 1**).

FLIP Proteins Interfere with Receptor-Induced Death

Viral FLIP proteins (vFLIPs), identified in herpesviruses, modulate the activation of caspase-8 by competitively binding with the DED domain of FADD and preventing the oligomerization of preformed caspase-8-containing complexes that otherwise activate cell death (**Figure 1**). Cellular counterparts of cFLIPs were later identified and take the form of catalytically inactive caspase-8 or shorter forms that, like viral FLIPs, consist of only two DED domains, thereby mimicking the pro-domain of caspase-8. Thus, vFLIPs function more as suppressors than as modulators of caspase activation. Many different viruses encode FLIP proteins, including several herpesviruses and the molluscum contagiosum virus (MCV) that encodes two distinct vFLIPs, MC159 and MC160. The HCMV gene product vICA (inhibitor of caspase activation) encodes an unrelated caspase-8 inhibitor (**Table 1**). In contrast, adenovirus E1A induces tumor formation and cell death, perhaps in part by downregulating $cFLIP_S$. However, just as cytochrome *c* has both cell survival (mitochondrial electron carrier) and cell death (apoptosome formation) functions, cellular caspases also have alternative functions, including cell proliferation, at least at low activity levels. Therefore, caspase activation by E1A may have dual functions.

Viral Caspase Regulators

The P35 protein of baculoviruses is a caspase pseudosubstrate that effectively inhibits caspases by binding into the caspase active site. In addition to being the first molecule of its kind to be discovered, P35 has proved to be exceptional in its ability to inhibit a wide variety of

caspases. The related viruses BmNPV and SlNPV encode P35 homologs. To date, there are no cellular homologs of these viral proteins that directly inhibit caspases. Viral IAP proteins related to cellular cIAPs are another class of caspase regulators discovered in baculoviruses. IAPs are characterized by their baculovirus inhibitory repeat (BIR) domains and by a C-terminal RING finger domain with ubiquitin ligase activity that targets its substrates for proteasomal degradation. Exemplars from this class of caspase inhibitor include Cydiapomonella granulovirus (CpGV) Cp-IAP and Orgyia pseudotsugata nuclear polyhedrosis virus (OpNPV) Op-IAP. Viral IAPs are also encoded by poxviruses; these include Amsacta moorei entomopoxvirus (AmEPV) gene 021 and Melanoplus sanguinipes entomopoxvirus genes 242 and 248. Viral and cellular IAP proteins were suggested to directly inhibit caspases, but this model is currently challenged by evidence suggesting more indirect mechanisms to explain their inhibitory effects. Other poxviruses encode RING finger proteins that are suggested to have E3 ubiquitin ligase activity, though they lack overall amino sequence similarity to the IAP family.

Another important poxvirus caspase inhibitor produced by vaccinia and the closely related cowpox virus is CrmA, a member of the serine protease inhibitor (serpin) superfamily. CrmA inhibits both serine and cysteine proteases, including caspases-1,-8, and -10. Myxoma virus (Serp-2) can inhibit caspases as well as the related cysteine protease granzyme B. Myxoma virus E13L also acts at the level of caspase activation by binding and inhibiting the apoptosis-associated speck-like protein containing a CARD (ASC) protein, a component of the caspase-1-activating inflammasome complex (**Table 1**).

Regulatory Viral RNAs and RNA-Binding Proteins

Virus Recognition by Cellular PRRs

Early recognition of several viruses is mediated by PRRs and results in the activation of interferon and NF-κB. Recent advances have been made in this area through the discovery of RIG-I and Mda5 as well as their downstream signaling partner, the CARD-containing mitochondrial antiviral signaling protein (MAVS). The protease NS3/4A from the flavivirus hepatitis C virus (HCV) cleaves and inactivates MAVS, thereby short-circuiting virus recognition and signaling through the CARD. Meanwhile, the same protease also cleaves the TIR domain-containing adaptor-inducing IFN-β (TRIF) and short-circuits TLR signaling as well. Vaccinia virus uses a clever trick to dampen TLR signaling; A46R contains a TIR domain to sequester TRIF away from TLR3, thus preventing the recognition of viral antigens. Other virus factors modulate RIG-I and Mda5 by alternative mechanisms, including NS2 from Influenza virus and V proteins of paramyxoviruses. B2 protein of the betanodavirus flock house virus (FHV) is an important determinant of virulence that acts by sequestering double-strand RNA and preventing its recognition by the host. RNA-binding proteins encoded by both RNA viruses (influenza, Ebola virus, and reovirus) and DNA viruses (vaccinia, CMV, and HSV) have also been implicated in modulating cellular strategies for controlling RNA levels.

The nonstructural protein (NSs) encoded on the small genome segment of a subset of mosquito-borne bunyaviruses (e.g., La Crosse virus and San Angelo virus) shares limited sequence similarity with the *Drosophila* pro-death molecule Reaper, and suppresses host cell gene expression, perhaps through an RNA interference (RNAi) mechanism, in both mammalian and cell extracts. These related proteins are also known to inhibit translation, suggesting that they act at multiple levels to direct cell death. The virus-associated interfering RNA (VAI) encoded by adenovirus acts at least in part by forming secondary structures that competitively inhibit the dsRNA binding by the endonuclease Dicer. Similarly, the RNA element TAR of HIV can bind TRBP (TAR RNA-binding protein), a cellular component of the Dicer-containing dsRNA-induced silencing complex (RISC), to inhibit RNA degradation activity. Therefore, post-transcriptional gene silencing through RNAi is involved in regulating host cell responses to RNA virus infections.

Virus-Encoded Micro-RNAs

Micro-RNAs (miRNAs) are small hairpin-forming RNAs that are processed to mature forms both in the nucleus by the nuclease Drosha and in the cytoplasm by Dicer. Just as endogenous cellular miRNAs regulate cellular protein expression, the number of known virus-encoded miRNAs is growing rapidly. HIV miR-N 367 targets and suppresses the translation of Nef and negatively regulates virus replication. The polyomavirus simian virus 40 (SV40) produces an miRNA that suppresses the T-antigen (TAg), effectively evading immune surveillance. The Bcl-2 homolog BHRF-1of EBV is regulated by three distinct miRNAs that are encoded in the 3′-UTR of BHRF1 on the opposite strand. The HSV latency-associated transcript (LAT) also encodes an miRNA, whose targets were recently identified as components of the TGF-β signaling pathway, including TGF-β itself and the downstream adapter SMAD3, the regulation of which results in the suppression of cellular stress responses.

Viral Regulators of the Mitochondrial Pathway

The mitochondrial cell death pathway is regulated by viral and cellular Bcl-2 proteins. Cellular Bcl-2 proteins

are generally divided into three functional subgroups, anti-apoptotic (Bcl-2, Bcl-x_L, Mcl-1, Bcl-w), pro-apoptotic Bcl-2 family members (Bax and Bak), and the more distantly related pro-apoptotic BH3-only proteins (Bad, Bid, and others). While these classifications generally apply, there are definitive examples where the endogenous functions of these proteins are opposite to expected. For example, Bax, Bak, and Bad protect neurons from Sindbis virus-induced apoptosis, while Bcl-2 and Bcl-x_L can promote cell death when they are cleaved by caspases.

Viral Bcl-2 Family Proteins

Viral homologs of the cellular anti-apoptotic protein Bcl-2 are encoded by the γ-herpesviruses, including Epstein–Barr virus (EBV) and Kaposi's sarcoma-associated herpesvirus (KSHV/HHV8), and several different poxviruses including the myxoma virus M11L protein and fowlpox virus-Bcl-2. Viral Bcl-2 proteins have the same three-dimensional structure as their cellular counterparts, but differ from the cellular factors in that vBcl-2 proteins are resistant to normal cellular regulatory mechanisms, such as cleavage by caspases, which can convert cellular Bcl-2 into a pro-death factor. Like cellular Bcl-2, vBcl-2 prevents permeabilization of the outer mitochondrial membrane by Bax and Bak, thereby preventing cytochrome *c* release and caspase activation (**Figure 1**).

Other Viral Proteins That Target Pro-Death Cellular Bcl-2 Proteins

Adenovirus E1B-19K, vaccinia virus F1L, cytomegalovirus vMIA (viral mitochondrial inhibitor of apoptosis) and vICA (viral inhibitor of caspase-8 activation) proteins also function at the same step as Bcl-2 as they bind and regulate cellular Bax and/or Bak. vMIA is encoded by exon 1 of immediate early gene UL37. vMIA localizes to mitochondria and causes mitochondrial fragmentation/division that is generally associated with apoptotic cell death, yet vMIA potently suppresses apoptosis triggered by diverse stimuli (**Table 1**).

Yeast Viruses Induce Yeast Programmed Cell Death

Programmed cell death was assumed to arise during evolution with the origin of multicellular organisms. However, unicellular species such as yeast and bacteria also undergo programmed cell death. In mammals, virus-induced apoptosis can be responsible for the pathogenesis caused by viruses such as HIV, mosquito-borne encephalitis viruses, and many others. Most wild yeast strains are persistently infected with viruses, such as the dsRNA L-A virus and associated satellite dsRNA M viruses. These viruses are transmitted to a new host cell by cell–cell fusion. Yeast viruses are more analogous to sexually transmitted human viruses such as retroviruses and herpesviruses that remain in latently infected cells throughout the life of the host organism.

The M1 and M2 viruses of *Saccharomyces cerevisiae* encode a preprotoxin that is cleaved by the Kex proteases to produce the α- and β-chains of active M1- and M2-encoded toxins. Interestingly, yeast M1 virus preprotoxin is organized analogously to mammalian preproinsulin. The M1-encoded K1 toxin induces programmed cell death in neighboring susceptible cells by activating the cell death function of mitochondrial factors encoded by the cells destined to die. Therefore, mitochondrial factors may regulate an evolutionarily conserved cell death pathway that provides single cell organisms with a type of host defense or innate immunity. This virus–host relationship in yeast is analogous to pathogenic human viruses that persist throughout life and can occasionally reactivate to cause disease.

Concluding Remarks

There are nearly as many strategies by which viruses interface with the cell death/survival machinery of host cells as there are viruses, from initial recognition of a virus to the ultimate proteolytic dismantling of the cell by proteases. A large number of virus-encoded cell death modulators have been reported in recent years (partial list in **Table 1**), and many more are likely to be discovered. The importance of viral regulators of cell death is clear from the number of these regulators encoded by a single virus, particularly DNA viruses with large genomes, while small RNA viruses must develop additional strategies. Common themes uncovered through the study of virus-modulated cell death pathways have proved to be broadly applicable to biology, as well as to the study of virus infection, pathogenesis, and evolution.

See also: Capillovirus, Foveavirus, Trichovirus, Vitivirus.

Further Reading

Benedict CA, Norris PS, and Ware CF (2002) To kill or be killed: Viral evasion of apoptosis. *Nature Immunology* 3(11): 1013–1018.

Chen YB, Seo SY, Kirsch DG, Sheu T-T, Cheng W-C, and Hardwick JM (2006) Alternate functions of viral regulators of cell death. *Cell Death and Differentiation* 13: 1318–1324.

Flint SJ, Enquist LW, Racaniello VR, and Skalka AM (eds.) (2004) *Principles of Virology: Molecular Biology, Pathogenesis, and Control of Animal Viruses,* 2nd edn. Washington, DC: ASM Press.

Hiscott J, Nguyen T-LA, Arguello M, Nakhaei P, and Paz S (2006) Manipulation of the nuclear factor-κB pathway and the innate immune response to viruses. *Oncogene* 25: 6844–6867.

Rahman MM and McFadden G (2006) Modulation of tumor necrosis factor by microbial pathogens. *PLoS Pathogens* 2(2): e4 0066–0077.

Aquareoviruses

M St. J Crane and G Carlile, CSIRO Livestock Industries, Geelong, VIC, Australia

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Aquareovirus, as the name implies, is a genus (one of 12 genera) of the family *Reoviridae*. Species in this genus infect aquatic animals – finfish, crustaceans, and mollusks – from both marine and freshwater environments. The name *Reoviridae* is derived from *r*espiratory *e*nteric *o*rphan viruses, and 'orphan' viruses are those viruses that are not associated with any known disease. Although, originally, reoviruses commonly may have been associated with subclinical or asymptomatic infections, it is now known that many are associated with disease. Reo-like viruses have been reported from aquatic animals since the 1970s and, as for other reoviruses, the majority of aquareoviruses are of low pathogenicity and have not attracted much attention. However, others have been isolated from populations with mortality rates higher than normal and some with mortality rates as high as 80%. There are at least six (A–F) recognized aquareovirus species based on RNA–RNA blot hybridization, RNA electrophoresis, antigenic properties, and nucleic acid sequence analysis (**Table 1**). More than 40 aquareoviruses have been isolated to date, with the majority classified within species groups A and B. However, some aquatic reoviruses such as the marine crab reoviruses, P and W2, do not fit this classification and these and several other aquareoviruses are yet to be classified.

Taxonomy and Classification

The genus *Aquareovirus* forms one of 12 genera (*Orthoreovirus*; *Orbivirus*; *Rotavirus*; *Coltivirus*; *Seadornavirus*; *Aquareovirus*; *Idnoreovirus*; *Cypovirus*; *Fijivirus*; *Phytoreovirus*; *Oryzavirus*; *Mycoreovirus*) that make up the family *Reoviridae*. The *Reoviridae* is one of the six families of viruses possessing a double-stranded RNA (dsRNA) genome. Of these, the *Reoviridae* is one of only two families that infect vertebrates and the only family that infects mammals. Within the family, each genus can be placed in a subset based on whether the virion is turreted (*Orthoreovirus*; *Aquareovirus*; *Idnoreovirus*; *Cypovirus*; *Fijivirus*; *Oryzavirus*; *Mycoreovirus*) or unturreted (*Orbivirus*; *Rotavirus*; *Coltivirus*; *Seadornavirus*; *Phytoreovirus*). The reovirus genome is made up of 10–12 segments of dsRNA. Each segment encodes one to three (usually one) proteins. Aquareovirus genomes are made up of 11 segments of dsRNA. Except for segment 11, which usually encodes two proteins, each segment encodes a single protein. Interestingly, segment 11 of chum salmon reovirus is tricistronic. Aquareoviruses have been reported from finfish, crustaceans, and mollusks and from both freshwater and marine host species.

For aquareoviruses, the primary species demarcation criteria are RNA cross-hybridization and antibody-based cross-neutralization. Currently, six genogroups or species (A–F) have been identified with several aquareoviruses remaining unclassified (**Table 1**). Ultrastructural similarity between orthoreoviruses and aquareoviruses has been noted and the relatively high level of sequence homology between some isolates of these two genera indicates a common evolutionary lineage. Despite this relatedness, other properties, such as host range, different numbers of genome segments, and absence of antigenic similarity, support classification into two genera.

Virion Structure and Morphology

The structure and morphology of aquareovirus virions are similar to other members of the family *Reoviridae*, particularly members of the genus *Orthoreovirus*. Virions are spherical in appearance, have icosahedral symmetry, and are *c.* 80 nm in diameter (**Figure 1**). The capsid consists of two concentric shells made up of three layers of protein. The outer capsid surrounds the inner core which is approximately 60 nm in diameter. For members of the genus *Aquareovirus*, the core is turreted – turrets or spikes project from the surface of the inner core and interconnect with the outer capsid layers. The inner protein layer of the core surrounds the 11 segments of the dsRNA.

A study of the turbot aquareovirus has indicated that its replication and morphogenesis generally follow that typical of other reoviruses. Virions were observed to be internalized by direct penetration of the host cell plasma membrane. In infected cells, two sizes of virus particles were observed in the cytoplasm: particles, 30 nm in diameter, which were probably inner cores; and single-shelled particles, 45 nm in diameter, located in the endoplasmic reticulum. Viral replication was observed to occur in the cytoplasm and immature virions (45 nm in diameter) formed complete, mature, double-shelled virions (75–80 nm in diameter) by budding through the plasma membrane so that complete viral particles were only observed outside host cells.

Table 1 Aquareovirus species, host species, and geographical distribution

Virus	*Host species*	*Pathogenicity*	*Mortality rate*	*Country*
Aquareovirus A				
Geoduck clam aquareovirus (CLV)	*Panope abrupta*	NS	0%	USA
Herring aquareovirus (HRV)	*Clupea harengus*	NS	0%	USA
Striped bass aquareovirus (SBRV)	*Morone saxatilis*	L	NR	USA
American oyster reovirus (13_{p2}RV)	*Crassostrea virginica*	NS	0%	USA
	Lepomis macrochirus	H	44%	Exp
	Oncorhynchus mykiss	H	60%	Exp
Angelfish aquareovirus (AFRV)	*Pomacanthus semicirculatus*	L	L	USA
Chum salmon aquareovirus (CSRV)	*Oncorhynchus keta*	L	0% to L	Japan
Smelt aquareovirus (SRV)	*Osmerus mordax*	H	H	Canada
Atlantic salmon reovirus (ASRV)	*Salmo salar*	NS	NR	Canada
Atlantic salmon reovirus (TSRV)	*S. salar*	L	L	Australia
Atlantic salmon reovirus (HBRV)	*S. salar*	NS	NR	USA
Chinook salmon reovirus (DRCRV)	*Oncorhynchus tshawytscha*	NS	NR	USA
Guppy aquareovirus (GRV)	*Poecilia reticulata*	L	L	Singapore
Aquareovirus B				
Coho salmon aquareovirus (CSRV)	*Oncorhynchus kisutch*	L	L	USA
Coho salmon reovirus (LBS)	*O. kisutch*	NS	NR	USA
Chinook salmon reovirus (YRCV)	*O. tshawytscha*	NS	NR	USA
Chinook salmon reovirus (ICRV)	*O. tshawytscha*	NS	NR	USA
Coho salmon reovirus (GRCV)	*O. kisutch*	NS	NR	USA
Coho salmon reovirus (CSRV)	*O. kisutch*	NS	NR	USA
Coho salmon reovirus (ELCV)	*O. kisutch*	NS	NR	USA
Coho salmon reovirus (SSRV)	*O. kisutch*	NS	NR	USA
Aquareovirus C				
Golden shiner aquareovirus (GSRV)	*Notemignous crysoleucas*	L	L	USA
	Semotilus atromaculatus	L	NR	USA
	Pimephales promelas	M	M	USA
Grass carp reovirus (GCRV) (GCHV)	*Ctenapharyngodon idellus*	H	70–95%	China
Aquareovirus D	*Mylopharyngodon piceus*	H	70–80%	China
Channel catfish reovirus (CCRV)	*Ictalurus punctatus*	L	<5%	USA
Aquareovirus E				
Turbot aquareovirus (TRV)	*Scophthalmus maximus*	L	<5%	Spain
Aquareovirus F				
Chum salmon reovirus (PSRV)	*O. keta*	NR	NR	NR
Chinook salmon reovirus (SCRV)	*O. tshawytscha*	NR	NR	NR
Unclassified aquareoviruses				
Haddock reovirus	*Melanogrammus aeglefinus*	NR	0%	UK
Golden ide reovirus (GIRV)	*Leuciscus idus*	NR	0%	Germany
Gilthead seabream reovirus	*Sparus aurata*	M	M	Spain
Red grouper reovirus	*Plectropomus maculatus*	H	30–90%	Singapore
Landlocked salmon virus	*Oncorhynchus masou* (Brereoort)	L	L	Taiwan
Tench reovirus (TNRV)	*Tinca tinca*	NS	0%	Germany
Japanese eel reovirus	*Anguilla japonica*	NR	NR	Japan
Fancy carp reovirus	*Cyprinus carpio*	NR	NR	Japan
Halibut reovirus	*Hippoglossus hippoglossus*	H	H	Canada, UK
Chub reovirus (CHRV)	*Leuciscus cephalus*	NS	0%	Germany
Marine threadfin fish reovirus	*Eleutheronema tetradactylus*	H	Up to 100%	Singapore
	Lates calcarifer	H	60%	Exp
Brown trout reovirus	*Salmo trutta*	L	0%	UK
	O. mykiss	L	0%	Exp
Snakehead reovirus (SKRV)	*Channa striata*	NS	0%	Thailand
Mediterranean shore crab reovirus (RC84)	*Carcinus mediterraneus*	L	0%	France
Mediterranean shore crab reovirus (W2)	*C. mediterraneus*	M	NR	France
Mediterranean swimming crab reovirus (P)	*Macropipus depurator*	L	NR	France
Chinese mitten crab reovirus	*Eriocheir sinensis*	NR	NR	China
Tiger shrimp reovirus	*Penaeus japonicus*	M	M	France
Tiger prawn reovirus	*Penaeus monodon*	H	95%	Malaysia

Exp, Experimental infections; L, low; M, moderate; H, high; NS, no significant pathology; NR, not reported.

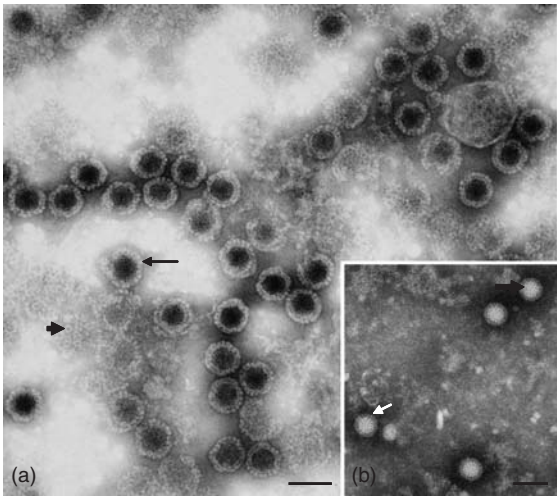


Figure 1 Transmission electron micrographs of negative-stained preparations of Tasmanian Atlantic salmon aquareovirus (TSRV). (a) Image of a purified preparation where the stain has penetrated the inner capsid. (b) Image of similar aquareovirus particles derived from the supernatant of infected cells. Thin arrows (black and white): surface projections associated with the outer capsid. Thick arrows indicate locations of hexameric (or tetrameric) clusters of surface projections (thin arrows). Scale = 100 nm (a, b). Courtesy of Electron Microscopy Group, Australian Animal Health Laboratory, CSIRO Livestock Industries, Geelong, VIC, Australia.

Genome Organization and Transcription Strategy

The genus *Aquareovirus* is relatively diverse, being made up of at least six species. The genomes of viruses in each species consist of 11 segments of dsRNA which migrate as three size classes in 1% agarose gels. Segments 1–3 are the large-size class, segments 4–6 medium, and segments 7–11 small. The complete genomic sequence of golden shiner reovirus (GSRV), comprising 23 696 bp, and grass carp reovirus (GCRV), comprising 23 695 bp, have been determined. The sequences of some segments of other aquareovirus isolates have also been determined. Apart from segments 7 and 11, each segment is monocistronic, containing a single open reading frame (ORF). For most aquareoviruses, segment 11 encodes two proteins (**Table 2**); segment 11 from chum salmon reovirus (CSRV) encodes three proteins. Thus, there are seven structural proteins (VP1–VP7) and five nonstructural proteins (NS1, NS2, NS4, NS15, and NS29). There is variation in the estimated molecular mass of each polypeptide for different aquareoviruses. For example, when compared by electrophoresis in a single polyacrylamide gel, the estimated molecular masses of the five major structural viral proteins for four aquareoviruses (13_{p2}, GSRV, CSRV, channel catfish reovirus (CCRV)) varied in the ranges 132–137 kDa (VP1), 126–130 kDa (VP2), 63–72 kDa (VP5), 43–45 kDa (VP6), and 32–36 kDa (VP7). **Table 2** shows the data reported for *Aquareovirus A*. As stated above, segment 11 of CSRV has been reported to be tricistronic encoding three polypeptides with estimated masses of 16.9, 15.1, and 13.0 kDa, and segment 7 of GCRV/GSRV appears to be bicistronic encoding two nonstructural proteins.

Table 2 *Aquareovirus A* genome segments and encoded polypeptides with the estimated mass and location

Genome segment	*Protein*	*Estimated Mass (kDa)*	*Protein location*
1	VP1	130	Core turret
2	VP2	127	Inner capsid
3	VP3	126	Inner capsid
4	NS1	97	NS
5	VP5	71	Inner capsid
6	VP4	73	Outer capsid
7[a]	NS4	28	NS
8	VP6	46	Inner capsid
9	NS2	39	NS
10	VP7	34	Major outer capsid protein
11[b]	NS29	29	NS
	NS15	15	

[a]Segment 7 of GCRV and GSRV has been reported as bicistronic encoding two nonstructural proteins with estimated masses of 16 (NS5) and 31 kDa (NS4).
[b]Segment 11 of CSRV is tricistronic encoding three nonstructural proteins with estimated masses of 13, 15, and 16 kDa.

Putative functions of the aquareovirus proteins have been predicted based on the functions established for the equivalent mammalian orthoreovirus proteins. Accordingly, VP1 is the homologue of λ2 protein, pentamers of which form the turrets. This protein has important functions in particle assembly, as well as guanylyltransferase and methyltransferase activities in mRNA capping. VP2, equivalent to λ3, is located in the inner capsid or core and has RNA-dependent RNA polymerase activity associated with transcription and replication. VP3 (λ1) is the main component of the inner capsid and possesses NTPase, RTPase, and helicase activities. VP4 (μ1) is located in the outer capsid and is believed to function in penetration of the cellular membrane to allow virus entry into the cell. The function of VP5 (μ2) is not fully understood but it is known to bind RNA and possess NTPase activity, indicating that VP5 may be an RNA polymerase cofactor. VP6 (σ2), together with VP3, forms the core shell. VP6 functions are poorly understood; it binds to RNA and is thought to be involved in replicase-particle assembly. VP7 (σ3) is the major outer capsid protein and is thought to play important roles in outer capsid assembly and in stabilizing virions in extracellular environments. VP7 also binds dsRNA and functions in translational control in infected cells. The nonstructural proteins NS1 and NS2 are likely to be involved in RNA replication and packaging during virus particle assembly and the function of NS4 is unclear thought to be the cell attachment

protein. The functions of the nonstructural proteins encoded by RNA segment 11 are also unknown.

Geographic Distribution and Host Range

Most aquareoviruses have been isolated from healthy aquatic animals and are likely to be of low pathogenicity. Thus, the presence of aquareoviruses is likely to be under-reported. Moreover, it is unlikely that routine surveillance for aquareoviruses has been undertaken in any systematic manner. Many isolations of aquareoviruses have occurred incidentally during surveillance activities for other, more significant, pathogens. It is during these surveillance activities that several aquareoviruses have been isolated from apparently healthy aquatic animals. **Table 1** lists most of the known aquareoviruses, their hosts, and country of isolation, cited in the readily accessible scientific literature. Some of the reoviruses listed have not been fully characterized and it may eventuate that some of the viruses will be classified outside of the currently recognized taxa.

Clearly, both the host range and the geographical distribution of aquareoviruses are broad and it is probable that, with ongoing surveillance, the known host range and geographical distribution will be extended. As aquaculture expands and the interaction between wild and farmed aquatic animal species increases, it can be expected that the number of known viruses, including reoviruses, will increase. A good example is virus 13_{p2}, originally isolated from healthy American oysters has been shown to infect and cause disease in more than one finfish species, indicating that the primary host and wild reservoir of this virus are unclear.

While *Aquareovirus A* appears to be the most diverse species with virus isolates obtained from several host species from very different parts of the world, it appears that *Aquareovirus B* has very limited host and geographical ranges, based on the several available isolates. The isolates of *Aquareovirus B* listed in **Table 1** may be the same virus merely isolated on different occasions. However, such a conclusion may be premature since many of the aquareoviruses have not been fully characterized and a large number of aquareoviruses remain unclassified with respect to species assignment.

Pathology

As stated above, the majority of known aquareoviruses are of low pathogenicity and have been isolated from apparently healthy animals. Nevertheless, some of the known aquareoviruses are highly pathogenic. For example, GCRV and others that have been isolated from normal animals may, under certain environmental conditions, be pathogenic. There are several examples in which stocking density and water temperature influence the pathogenicity of the virus. A good example is GSRV. Mortality rates associated with GSRV are normally around 5% but, under crowded conditions and high temperatures, acute epizootics with mortality rates of 50–75% have been reported.

It is also of note that isolation of aquareoviruses often occurs during investigations of conditions with apparently mixed etiology, involving either other viruses or a concomitant bacterial infection. In mixed infections with bacteria, treatment with antibiotics does not always resolve the condition, suggesting that the reovirus may play some role in the disease, if not as the primary pathogen. In other cases of mixed infections, the respective roles of each agent are not at all clear and it is difficult to ascribe disease signs to either agent.

Several reoviruses isolated from apparently healthy finfish with no gross external signs have been shown to cause low-level pathology in experimental infections. While these viruses have been shown to replicate in experimental fish, mortality is rare. The pathology is characterized by a diffuse multifocal necrosis of the liver which, in some cases, subsequently resolves.

For those aquareoviruses that have been isolated from disease outbreaks in finfish hosts, external signs are those typically found for systemic infections and include lethargy, inappetance, anorexia, abnormal swimming behavior, petechial hemorrhages on the body surface, lateral recumbency, distended abdomen, and high mortality rates. Internal signs included discoloration of the liver. Histological examination may reveal hepatic lesions with varying degrees of severity. Syncytial giant cell formations of hepatocytes have been reported.

GCRV is one of two highly pathogenic aquareoviruses isolated to date. The virus, first reported in 1984, is responsible for an acute hemorrhagic disease affecting grass carp (*Ctenopharyngodon idella*) and black carp (*Mylopharyngodon piceus*) in China. Disease outbreaks occur in the summer with mortality rates in the range of 70–95%. As with most viral diseases of finfish, younger age classes are more susceptible than older fish. External signs include exophthalmia and hemorrhages at the fin bases and gill covers. Internally, hemorrhages have been reported to occur in all the major organs – intestinal tract, liver, spleen, kidneys, and throughout the musculature. Recent studies indicate that GCRV and GSRV are variants of the same virus.

The other highly pathogenic reovirus was isolated in 1998 from cultured threadfin (*Eleutheronema tetradactylus*) fingerlings undergoing a mass mortality at a farm in Singapore. Following isolation, the virus was used in

experimental infections of threadfin fingerlings and sea bass (*Lates calcarifer*). Clinical signs (dark pigmentation, lethargy, recumbency with sudden bursts of swimming) developed within 1 day post infection (d.p.i.), resulting in 100% cumulative mortality in the infected threadfin within 4 d.p.i. and 60% cumulative mortality in the sea bass within 7 d.p.i.

Several reoviruses or reo-like viruses have been isolated from crustaceans such as shrimp and crabs, some of which harbored mixed infections with other agents. As commonly reported for other viral diseases, external signs have included discoloration and abnormal behavior such as lethargy and inappetance. While virus particles have been observed in different tissues/organs (hepatopancreas, gills, digestive tract, lymphoid organ), a common feature appears to be involvement of the hepatopancreas where the most obvious lesions occur.

Host Response to Infection

There are no detailed studies of the immune response of finfish to infection by aquareoviruses but, from observations that have been reported, it is clear that cellular and humoral immune reactions are stimulated. While not consistent, local infiltration of host inflammatory cells, particularly in the liver of infected fish, has been observed during aquareovirus infections. Moreover, in studies in which sera have been collected from infected fish, specific neutralization titers of >1:4000 have been obtained in neutralization tests. Further evidence of an immune response comes from studies with a vaccine produced in China for use against GCRV. A crude inactivated GCRV vaccine was produced in the late 1970s and was reported to provide good protection (70% survival of fingerlings).

Of further interest is a study reporting aquareovirus interference-mediated resistance of rainbow trout to infectious hematopoietic necrosis. Pre-exposure of rainbow trout to coho salmon reovirus (CSRV) induced a protective response to subsequent challenge with the rhabdovirus infectious hematopoietic necrosis virus. Protection appeared to be mediated by innate immune factors.

Transmission

The natural transmission cycle has not been studied in detail for any of the aquareoviruses. Experimentally, infection and disease can be transmitted horizontally by injection and by immersion in diluted tissue culture supernatants from reovirus-infected fish cell lines. It is not known whether vertical transmission occurs.

Genetic Diversity

Within the family *Reoviridae*, viruses in only three genera, *Mycoreovirus*, *Rotavirus*, and *Aquareovirus*, possess an 11-segmented dsRNA genome. Other properties such as host range, RNA sequence, and serological differences justify the classification of the aquatic reoviruses into a separate genus. Interestingly, there are some relatively high sequence identities in some proteins of aquareoviruses and orthoreoviruses, indicating these two genera share a common ancestral lineage. It is also noteworthy that some reoviruses, or reo-like viruses, that infect aquatic animals, such as viruses P and W2, isolated from marine crabs, possess 12 segments of dsRNA and may represent a new reovirus genus.

Currently, there are six genogroups/species (A–F) within the genus *Aquareovirus*. This classification is based on RNA–RNA blot hybridization, RNA electrophoresis, antigenic properties, and nucleotide sequence analysis (**Table 1**). While reciprocal RNA–RNA hybridization and cross-neutralization assays provide useful data for the classification of aquareoviruses, genomic sequence provides more precise data. For example, based on RNA–RNA hybridization, it had been suggested that GCRV may represent a seventh species group (G) but subsequent nucleotide sequence analysis has clearly indicated that it should be placed within genogroup C.

In a study that analyzed the relative electrophoretic mobility of the 11 RNA segments of 19 aquareoviruses, distinct electropherotypes were observed. Whilst there was no correlation with the host species from which the viruses were isolated, there was a correlation with geographical location. Further studies are required to determine whether, as for other members of the *Reoviridae*, electrophoretic mobility will be useful for strain identification within the aquareoviruses.

A comparison of deduced amino acid sequences of segment 10 (encoding major outer capsid protein VP7) of viral isolates representing the species *Aquareovirus A*, *Aquareovirus B*, and *Aquareovirus C*, has indicated sequence identities in the range 19–100%. Within a species, sequence identities were >80% and, for some, >99%. Between viruses in different species, identities were in the range 19–22%. Similar results have been obtained in other studies.

With the current level of knowledge, it is difficult to determine any correlation between *Aquareovirus* species, host and geographical ranges, and pathogenicity.

Diagnosis and Disease Management

External signs of disease associated with infection by highly pathogenic aquareoviruses, including lethargy,

inappetance, anorexia, abnormal swimming behavior, petechial hemorrhages, lateral recumbency, distended abdomen, high mortality rates, are not pathognomonic and laboratory investigation is required for definitive diagnosis. Several of the known aquareoviruses have been detected by virus isolation on any of a number of fish cell lines in common use in diagnostic laboratories. Depending on the aquareovirus isolate and local conditions, cell lines used for viral isolation and replication have included bluegill fry cell line (BF-2), chinook salmon embryo cell line (CHSE-214), fathead minnow cell line (FHM), *Epithelioma papillosum cyprini* cell line (EPC), channel catfish ovary cell line (CCO), brown bullhead cell line (BB), grass carp kidney cell line (CIK), Asian seabass cell line (SB), rainbow trout mesothelioma cell line (RTM), striped snakehead cell line (SSN-1), and rainbow trout gonad cell line (RTG-2). The appearance of the cytopathic effect (CPE) caused by aquareoviruses in fish cell lines is quite variable, depending on the aquareovirus isolate and the cell line used for isolation. Examples of CPE caused by Tasmanian Atlantic salmon reovirus (TSRV) in two cell lines are shown in **Figure 2**. Where pathogenic aquareoviruses are endemic, more rapid, sensitive, and specific diagnostic tests are highly desirable. Species-specific antisera are currently not available but would provide useful diagnostic reagents in a variety of immunoassays. A rapid test, based on a reverse-transcription polymerase chain reaction (RT-PCR), has recently been developed for local pathogenic strains of threadfin aquareovirus and grass carp reovirus that are endemic in China and Singapore. Tentative assignment of species/genogroup can be achieved by comparing isolates with known aquareovirus species by reciprocal RNA–RNA hybridizations and cross-neutralization assays. Precise genogroup assignment can only be achieved by RT-PCR, subsequent sequence analysis, and comparison with sequences of other known genogroup isolates.

Apart from the GCRV vaccine, no vaccines or therapeutics are currently available for the control of diseases

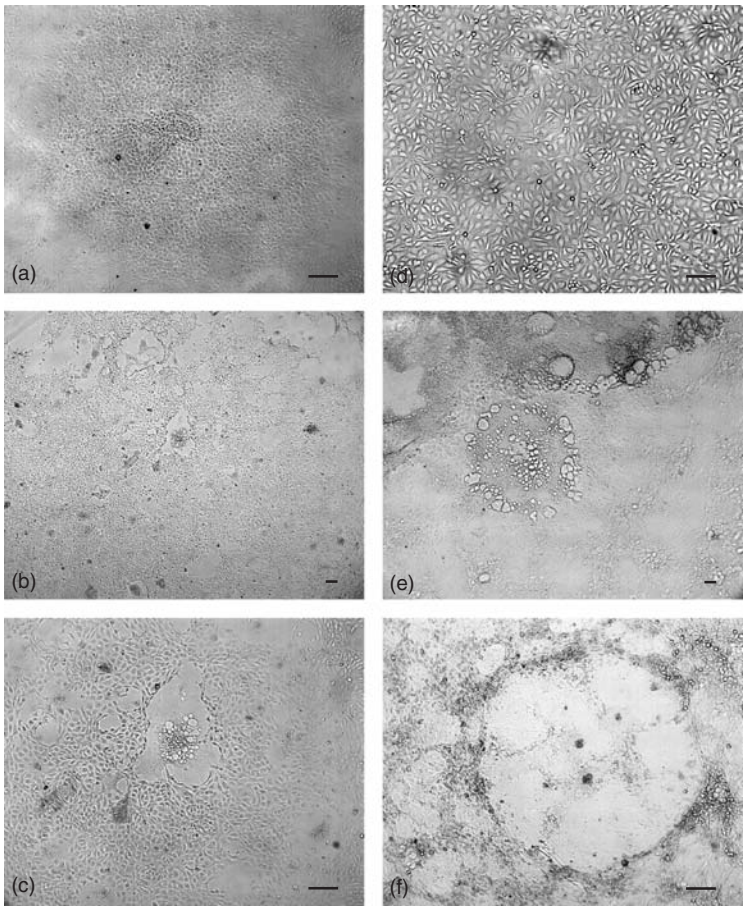


Figure 2 Photomicrographs of cytopathic effect (CPE) produced by infection by TSRV. (a–c) CHSE-214 and (d–f) EPC cell cultures. (a, d) Uninfected cultures; (b, e) low magnification image; (c, f) high magnification image. Scale = 100 μm (a–f). Courtesy of Nette Williams, Australian Animal Health Laboratory, CSIRO Livestock Industries, Geelong, VIC, Australia.

associated with aquareovirus infections. As for other viral diseases of aquatic animals, avoidance is the preferred strategy. Thus, when available, pathogen exclusion can be attempted using good biosecurity and sanitary protocols in hatcheries and on farms. These can include the selection of specific pathogen-free (SPF) broodstock using virus isolation on cell lines and RT-PCR as screening tools, disinfection of fertilized eggs, and rearing water with, for example, ozone. In addition, stress reduction by maintaining high water quality and low stocking density is important. As aquareoviruses appear to have broad host and geographical ranges, the farming of aquatic animals where they are exposed to wild host species could present risks. Vaccines, when available, are likely to provide an additional level of protection.

Current Status

The classification of aquareoviruses is not fully resolved, with many viral isolates remaining unclassified, and this is an important area for further research. For many of the species/genogroups, there are insufficient numbers of available isolates to allow determination of the key biological properties within each species. Thus, there does not appear to be correlation between host species, geographical range, pathogenicity, and species group. Many of the aquareoviruses, including the pathogenic threadfin aquareovirus, remain unclassified. As sequence data are available for only a few of the aquareoviruses, phylogenetic analysis has been limited. Further research is required not only to clarify the phylogenetic relationships within the genus *Aquareovirus* but also to assist in a better understanding of the role each segment plays in viral pathogenesis. Furthermore, the classification of the crustacean reo-like viruses and their relationship to other reoviruses, including aquareoviruses, requires clarification.

The isolation of different *Aquareovirus* species from both healthy and diseased fish indicates that, as for many other viruses of fish, pathogenicity is influenced by host (e.g., species, concomitant infections, immunocompetency) and environmental factors (e.g., water temperature, water quality, stocking density). In addition to elucidation of virulence factors (virus factors), further investigation on the respective roles of host and environmental factors on the pathogenicity of aquareoviruses is required. Based on knowledge to date, it seems likely that, for some *Aquareovirus* species at least, disease outbreaks will occur under adverse conditions of water quality/stocking density/temperature, and possibly in new host species. Thus, as the aquaculture industry expands globally, the interactions between farmed species and wild aquatic animal species will become more numerous, increasing the chance of virus transfer between wild and farmed animals. With this situation, taken together with their broad geographic range, it appears inevitable that aquareoviruses will produce disease in an increasing number of aquatic animal species in the future.

See also: Fish Viruses; Reoviruses: General Features; Reoviruses: Molecular Biology.

Further Reading

Attoui H, Billoir F, Cantaloube JF, Biagini P, de Micco P, and de Lamballerie X (2000) Strategies for the sequence determination of viral dsRNA genomes. *Journal of Virological Methods* 89: 147–158.

Attoui H, Fang Q, Mohd Jaafar F, *et al.* (2002) Common evolutionary origin of aquareoviruses and orthoreoviruses revealed by genomic characterization of golden shiner reovirus, grass carp reovirus, striped bass reovirus and golden ide reovirus (genus *Aquareovirus*, family *Reoviridae*). *Journal of General Virology* 83: 1941–1951.

Dopazo CP, Bandin I, Rivas C, Cepeda C, and Barja JL (1996) Antigenic differences among aquareoviruses correlate with previously established genogroups. *Diseases of Aquatic Organisms* 26: 159–162.

Goodwin AE, Nayak DK, and Bakal RS (2006) Natural infections of wild creek chubs and cultured fathead minnow by Chinese grass carp reovirus (golden shiner virus). *Journal of Aquatic Animal Health* 18: 35–38.

Kim J, Tao Y, Reinisch KM, Harrison SC, and Nibert ML (2004) Orthoreovirus and aquareovirus core proteins: Conserved enzymatic surfaces, but not protein–protein interfaces. *Virus Research* 101: 15–28.

Lupiani B, Hetrick FM, and Samal SK (1993) Genetic analysis of aquareoviruses using RNA–RNA blot hybridization. *Virology* 197: 475–479.

Lupiani B, Subramanian K, and Samal SK (1995) Aquareoviruses. *Annual Review of Fish Diseases* 5: 175–208.

McEntire ME, Iwanowicz LR, and Goodwin AE (2003) Molecular, physical and clinical evidence that golden shiner virus and grass carp reovirus are variants of the same virus. *Journal of Aquatic Animal Health* 15: 257–263.

Meyers TR (1980) Experimental pathogenicity of reovirus 13_{p2} for juvenile American oysters *Crassostrea virginica* (Gmelin) and bluegill fingerlings *Lepomis macrochirus* (Rafinesque). *Journal of Fish Diseases* 3: 187–201.

Rivas C, Noya M, Cepeda C, Bandin I, Barja JL, and Dopazo CP (1998) Replication and morphogenesis of the turbot aquareovirus (TRV) in cell culture. *Aquaculture* 160: 47–62.

Samal SK, Attoui H, Mohd Jaafar F, and Mertens PPC (2005) *Reoviridae – Aquareovirus*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 511–516. San Diego, CA: Elsevier Academic Press.

Seng EK, Fang Q, Chang SF, *et al.* (2002) Characterisation of a pathogenic virus isolated from marine threadfin fish (*Eleutheronema tetradactylus*) during a disease outbreak. *Aquaculture* 214: 1–18.

Seng EK, Fang Q, Lam TJ, and Sin YM (2004) Development of a rapid, sensitive and specific diagnostic assay for fish *Aquareovirus* based on RT-PCR. *Journal of Virological Methods* 118: 111–122.

Subramanian K, Hetrick FM, and Samal SK (1997) Identification of a new genogroup of aquareovirus by RNA–RNA hybridization. *Journal of General Virology* 78: 1385–1388.

Subramanian K, McPhillips TH, and Samal SK (1994) Characterization of the polypeptides and determination of genome coding assignments of an aquareovirus. *Virology* 205: 75–81.

Arboviruses

B R Miller, Centers for Disease Control and Prevention (CDC), Fort Collins, CO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arbovirus A virus that is biologically transmitted to vertebrates by infected hematophagous arthropods.
Hematophagous arthropods Arthropods, including mosquitoes, ticks, sandflies, blackflies, biting-midges, etc., that gain nourishment by feeding on vertebrate blood.
M_r The relative molar mass of a virion.
Transovarial transmission Transmission of an arbovirus from an infected arthropod female to her progeny via viral infected ovaries.
Vertical transmission Transmission of an arbovirus from an infected female vector to her progeny.
Zoonoses An infectious disease transmissible from vertebrate animals to humans under natural conditions.

Introduction

The term 'arbovirus' is used to define viruses transmitted by blood-feeding arthropods. The word was coined by virologists investigating taxonomically diverse viruses that shared this biological feature. These agents are maintained in nature in various transmission cycles, from simple to complex, by replicating in blood-feeding arthropods that transmit the virus in their saliva to vertebrates. The virus then replicates or is 'amplified' in the vertebrate where it becomes accessible in peripheral blood to other blood-feeding arthropods, completing the cycle. These viruses thus are, 'arthropod-borne viruses', or arboviruses.

The *International Catalog of Arboviruses Including Certain Viruses of Vertebrates* lists 545 viruses that are known or suspected to be arthropod-borne as well as viruses with 'no known vector'. Many of the viruses in the catalog infect and replicate in vertebrates other than humans and livestock. Arboviruses circulate in nature, going largely unnoticed until they produce disease in humans and/or domestic animals or wildlife. Of the 545 viruses listed in the catalog, 134 are known to cause human disease. Arboviruses including *Yellow fever virus* (genus *Flavivirus*, family *Flaviviridae*), *Crimean-Congo hemorrhagic fever virus* (genus *Nairovirus*, family *Bunyaviridae*), and *Japanese encephalitis virus* (genus *Flavivirus*, family *Flaviviridae*) are capable of producing severe disease in humans; while other arboviruses including *Rift Valley fever virus* (genus *Phlebovirus*, family *Bunyaviridae*), *Venezuelan equine encephalitis virus* (genus *Alphavirus*, family *Togaviridae*), and *West Nile virus* (genus *Flavivirus*, family *Flaviviridae*) can cause clinical disease in humans and domestic animals.

Although arboviruses are found throughout the world, the majority is found in the tropics, where arbovirus transmission can occur without interruption, thus increasing opportunities for these viruses to evolve. The biological characteristic of arthropod transmission has evolved independently in diverse virus lineages; for instance, in the virus family, *Flaviviridae*, some members are transmitted by ticks, others by mosquitoes, others have no known vector and may be exclusively viruses of vertebrates, and still others appear to have been transmitted by vectors but secondarily have lost this ability over evolutionary time. Viruses in another primitive lineage in the family only replicate in mosquitoes.

Taxonomy and Classification

The most important human and veterinary arboviral pathogens listed in the catalog are found primarily in select genera of three virus families: the *Bunyaviridae* (three of five genera), the *Flaviviridae* (one of three genera), and the *Togaviridae* (one of two genera). Arboviruses also belong to other virus families: the *Rhabdoviridae* (two of six genera), the *Reoviridae* (two of 12 genera), and the *Orthomyxoviridae* (one of five genera) (**Table 1**). The viruses in these families all have genomes consisting of RNA molecules in various configurations; there is only a single arbovirus, *African swine fever virus* (genus *Asfivirus*), in the family *Asfarviridae* that has a DNA genome. Viruses have been classified by using serological and molecular methodologies.

Virion: Physical Properties, Composition, and Genome Organization

African swine fever virus has a sedimentation coefficient $S_{20,W}$ of about 3500 S and a buoyant density of 1.095 g cm^{-3} in Percoll and 1.19–1.24 g cm^{-3} in CsCl. Virions are susceptible to irradiation, ether, deoxycholate, and chloroform, and they are inactivated at 60 °C for 30 min. Virions can survive for years at 4 °C and they are stable over a wide range of pH. The asfivirus particle consists of a nucleoprotein core that is 70–100 nm in diameter, surrounded by two internal lipid layers, and a 170–190 nm icosahedral capsid ($T = 189$–217) with an external, lipid-containing envelope. The mature virion has 1892–2172 capsomers and a diameter of 175–215 nm. The genome of African swine

Table 1 Representative arboviruses associated with human and livestock illnesses

Virus	*Genus*	*Family*	*Host/associated illness*	*Arthropod vector*	*Primary host*	*Distribution*
African swine fever	*Asfivirus*	*Asfarviridae*	Swine, bush pigs/hemorrhagic fever	Soft ticks, *Ornithodoros*	Wild boar	Africa, Spain, Portugal
Crimean-Congo hemorrhagic fever	*Nairovirus*	*Bunyaviridae*	Human/hemorrhagic fever	Hard ticks, *Hyalomma*	Wild and domestic animals	Africa, Asia, Europe
La Crosse	*Orthobunyavirus*	*Bunyaviridae*	Human/encephalitis	Mosquitoes, *Aedes triseriatus*	Chipmunks, squirrels	North America
Rift Valley fever	*Phlebovirus*	*Bunyaviridae*	Human/hemorrhagic fever, hepatitis, retinitis, encephalitis Livestock/abortion, fever, hepatitis	Mosquitoes, *Culex* and *Aedes*	Cattle and sheep	Africa, Arabian Peninsula
Dengue 1–4	*Flavivirus*	*Flaviviridae*	Human/fever, hemorrhagic fever	Mosquitoes, *Aedes aegypti*, *Aedes albopictus*	Humans, primates	Worldwide in tropics
Japanese encephalitis	*Flavivirus*	*Flaviviridae*	Human/fever, encephalitis	Mosquitoes, *Culex*	Wading birds, pigs	Asia, Pacific Islands
Kyasanur Forest disease	*Flavivirus*	*Flaviviridae*	Humans, primates/fever, encephalitis, hemorrhagic fever	Hard ticks, *Hemaphysalis*	Camels, rodents	India, Saudi Arabia
Murray Valley encephalitis	*Flavivirus*	*Flaviviridae*	Humans/fever, encephalitis	Mosquitoes, *Culex*	Birds	Australia
Rocio	*Flavivirus*	*Flaviviridae*	Humans/fever, encephalitis	Mosquitoes, *Culex*	Birds	South America
West Nile	*Flavivirus*	*Flaviviridae*	Humans/fever, encephalitis	Mosquitoes, *Culex*	Birds	Africa, Asia, Europe, Americas
Yellow fever	*Flavivirus*	*Flaviviridae*	Humans, New World primates/fever, hemorrhagic fever, hepatitis	Mosquitoes, *Aedes*, *Hemagogus*, *Sabethes*	Primates	Africa, South America
Thogoto	*Thogotovirus*	*Orthomyxoviridae*	Humans/fever, optic neuritis, encephalitis	Hard ticks	Wild and domestic vertebrates	Africa, Europe
Colorado tick fever	*Coltivirus*	*Reoviridae*	Humans/fever, myalgia, encephalitis	Ticks, *Dermacentor*	Ground squirrels	Western North America
Banna	*Seadornavirus*	*Reoviridae*	Humans/fever, myalgia, encephalitis	Mosquitoes, *Culex, Aedes, Anopheles*	Pigs, cattle	Asia
Vesicular stomatitis Indiana	*Vesiculovirus*	*Rhabdoviridae*	Humans/fever Domestic animals/ fever, vesicles in the mucosa	Sandflies, blackflies, mosquitoes	Wild and domestic vertebrates	Americas
Chikungunya	*Alphavirus*	*Togaviridae*	Humans/fever, arthralgias, rarely encephalitis	Mosquitoes, *Aedes*	Humans, primates	Africa, Asia
O'nyong-nyong	*Alphavirus*	*Togaviridae*	Humans/fever, arthralgias, lymphadenopathy	Mosquitoes, *Anopheles*	Unknown	Africa
Ross River	*Alphavirus*	*Togaviridae*	Humans/fever, arthralgia	Mosquitoes, *Aedes, Culex*	Macropods	Australia, Pacific Islands
Sindbis	*Alphavirus*	*Togaviridae*	Humans/fever, arthralgia	Mosquitoes, *Culex*, *Culiseta, Aedes*	Passerine birds	Europe, Africa, Asia, Australia
Mayaro	*Alphavirus*	*Togaviridae*	Humans/fever, arthralgia	Mosquitoes, *Haemagogus*	Primates	Americas
Eastern equine encephalitis	*Alphavirus*	*Togaviridae*	Humans, equines, pheasants /fever, encephalitis	Mosquitoes, *Culiseta, Culex*	Birds	Americas
Western equine encephalitis	*Alphavirus*	*Togaviridae*	Humans, equines/fever, encephalitis	Mosquitoes, *Culex*	Birds	Americas
Venezuelan equine encephalitis	*Alphavirus*	*Togaviridae*	Humans, equines/fever, encephalitis	Mosquitoes, *Culex* (*Melanoconion*)	Rodents	Americas

fever virus is a single molecule of double-stranded DNA that is covalently close-ended and ranges from 170 to 190 kbp in size. Mature virions contain more than 50 proteins, including an inhibitor of apoptosis homolog protein, guanyltransferase, poly A polymerase, protein kinase, and RNA polymerase. There are 150 open reading frames (ORFs) that are read from both strands. Early mRNA synthesis begins in the cytoplasm of the infected cell using enzymes in the virus core; viral DNA replication and assembly take place in perinuclear areas. Viral transcripts are capped at the 5′-end, and the 3′-end is polyadenylated. Formation of the capsid takes place on two layers of membranes derived from the endoplasmic reticulum (ER); extracellular virus acquires a membrane by budding through the cellular plasma membrane.

In the *Bunyaviridae*, virion M_r is $(3–4) \times 10^8$ with sedimentation coefficients (S_{20W}) in the range of 350–500 S. Buoyant densities in sucrose and CsCl are 1.16–1.18 and 1.20–1.21 g cm^{-3}, respectively. Virions contain 50% protein, 20–30% lipid, and they are sensitive to heat, irradiation, formaldehyde, and lipid solvents. Bunyavirus virions are spherical with a diameter of 80–120 nm and have a lipid bilayer that contains glycoprotein projections (5–10 nm). Virion envelopes are obtained from Golgi membranes and the ribonucleocapsids exhibit helical symmetry. The three arboviral genera in the family *Bunyaviridae* (*Nairovirus*, *Orthobunyavirus*, and *Phlebovirus*) have slightly different genome organizations; however, all have segmented, single-stranded, negative-sense RNA genomes with an L-segment that codes for the viral polymerase, an M-segment that codes for two envelope glycoproteins, and an S-segment that codes for the viral nucleocapsid protein. Synthesis of viral mRNA takes place in the cytoplasm using host cellular capped primers ('cap-stealing' or 'cap-snatching'). Mature virions are produced by budding into the Golgi cisternae, although *Rift Valley fever virus* has been observed to bud at the cell surface.

Flavivirus virion M_r is about 6×10^7; virions have sedimentation coefficients of approximately 200 S and have buoyant densities of about 1.19 g cm^{-3} in sucrose. Virions are stable at weakly alkaline pH but they are inactivated at acidic pH values, at heat above 40 °C, in organic solvents, and on irradiation. Virions are spherical with icosahedral symmetry and about 50 nm in diameter. There are two glycoproteins in the lipid bilayer: M and E in mature, extracellular virions and prM and E in immature virions; prM is cleaved to M by host-cell enzymes during maturation. The E-glycoprotein is a rod-shaped, dimeric structure that does not form spike-like projections; rather, it lies parallel to the viral membrane in a neutral pH environment. The flavivirus genome is capped and consists of single-stranded, positive-sense RNA that codes (5′→3′) for three structural proteins and seven nonstructural proteins contained in a single ORF; the 5′- and 3′-ends are noncoding regions (NCRs) necessary for viral replication and translation. A nascent polyprotein is transcribed and cleaved co- and post-translationally by cellular and viral proteases. Viral RNA synthesis, virion assembly, and acquisition of a lipid envelope take place in the ER; mature virions are released by exocytosis.

Orthomyxovirus virion M_r is 250×10^6 and buoyant density is 1.19 g cm^{-3} in sucrose. $S_{20,W}$ is 700–800 S and virions are sensitive to irradiation, solvents, heat, and detergents. Virions are spherical (80–120 nm) and sometimes pleomorphic with surface glycoproteins projecting 10–14 nm from the surface. The nucleocapsid is segmented and has helical symmetry. Within the genus, *Thogotovirus*, the negative-sense, single-stranded, segmented RNA genome is comprised of six segments for *Thogoto virus* (family *Orthomyxoviridae*) and seven segments for *Dhori virus* (family *Orthomyxoviridae*); the genome is about 10 kb in size. Interestingly, the glycoprotein, coded by the fourth segment, shares sequence similarity with a surface glycoprotein of insect baculoviruses. Thogoto virus nulceocapsids are transported to the nucleus where viral enzymes synthesize 5′-capped mRNA that are polyadenylated. Protein synthesis occurs in the cytoplasm, although early on in infection certain proteins are found in the nucleus and later transported to the cytoplasm. Viral membrane proteins are transported through the Golgi cisternae to the cellular plasma membrane; nulceocapsids bud through the plasma membrane in regions populated by viral membrane proteins.

In the *Reoviridae*, coltiviruses and orbiviruses have a buoyant density of 1.38 and 1.36 g cm^{-3}, respectively, in CsCl. Viruses are sensitive to low pH, heat, and detergent but are stable for long periods at 4 °C in the presence 50% fetal bovine serum. Coltivirus virions are 60–80 nm in diameter consisting of two concentric capsid shells with a core structure that is about 50 nm in diameter while the orbivirus, bluetongue virus is 90 nm in diameter with core particles 73 nm in diameter. Virus particles have icosahedral symmetry and are closely associated with granular matrices and filamentous formations in the cytoplasm. The mosquito-borne seadornaviruses and tick-borne coltiviruses have genomes composed of 12 double-stranded (dsRNA) segments that are, respectively, 21 000 and 29 000 bp in size; each genome segment is flanked 5′ and 3′ by similar noncoding sequences. Coltivirus VP1 (the largest segment) encodes the viral RNA-dependent RNA polymerase. There is no indication of virus release prior to cell death; more than 95% of coltivirus progeny virions and 60% of seadornavirus progeny virions remain cell associated. Orbivirus core particles are arranged as hexameric rings composed of capsomeres. The viral genome is composed of ten segments of dsRNA surrounded by the inner capsid shell. Inclusion bodies and tubules are produced during viral replication. Flat hexagonal crystals formed from the major outer core protein may also be produced during orbivirus replication. Unpurified virus is associated with cellular membranes although mature virus lacks a lipid envelope. Virions can leave the host cell by budding through the plasma membrane and transiently acquiring an envelope that is unstable and is rapidly lost.

Rhabdovirus virion M_r is $3–10 \times 10^8$ and $S_{20,W}$ is 550–1045 S. Buoyant density in sucrose is 1.7–1.9 g cm^{-3} and 1.19–1.20 g cm^{-3} in CsCl. Heat above 56 °C, lipid solvents, and irradiation will inactivate virions. Rhabdoviruses are 'bullet-shaped' or 'cone-shaped', 100–430 nm in length, and 45–100 nm in diameter. Trimers of the viral glycoprotein (G), 10 nm long and 3 nm in diameter, known as 'peplomers' cover the outer surface of the virus. The nucleocapsid is 30–70 nm in diameter and shows helical symmetry. It is composed of RNA and N (nucleocapsid proteins) collectively with the L-(transcription and replication factors) and P-(polymerase cofactor) proteins that interact with the envelope G-protein via the M-(binds to N and G) protein. The genomes are negative-sense, single-stranded RNA molecules that encode five major polypeptides. Viral genes are transcribed as monocistronic mRNAs that are capped and polyadenylated. After the nucleocapsid is released into the cytoplasm, the viral genomic RNA is transcribed by the virion transcriptase. Genome replication occurs in the cytoplasm by full-length positive-strand RNA synthesis followed by full-length negative-strand RNA synthesis. Nucleocapsids are synthesized in the cytoplasm and viral membranes are acquired as viruses bud from the host-cell plasma membranes.

Togavirus virion M_r is approximately 52×10^6. Virions have a buoyant density of 1.22 g cm^{-3} in sucrose and an $S_{20,W}$ is of 280 S. Virions are sensitive to heat, acidic pH, solvents, and irradiation. Togavirus viruses are spherical, 70 nm in diameter, and they have heterodimer spikes in the envelope consisting of two virus glycoproteins, E1 and E2. The envelope surrounds a 40 nm nucleocapsid that has icosahedral symmetry ($T = 4$). Togaviruses have a genome that is positive-sense, single-stranded RNA; the mRNA is capped and polyadenylated. There are four genes coding for nonstructural proteins situated at the 5′-end of the genome and five genes coding for structural proteins located at the 3′-end of the genome. Negative-sense RNA, synthesized during RNA replication, is utilized as the template for production of genome-length, positive-sense RNA as well as for creation of a 'subgenomic' 26S mRNA that is capped and polyadenylated and is translated to make the viral structural proteins. The polyprotein is cleaved by a combination of cellular and viral proteases. Noncoding sequences located at the 5′- and 3′-termini of the genome are required for both negative and positive RNA synthesis. The nucleocapsids are assembled in the cytoplasm; viral surface glycoproteins are processed in the ER and translocated through the Golgi apparatus to the cellular plasma membrane where they are acquired as the nucleocapsids bud through the membrane.

Evolutionary Relationships Among Arboviruses

The biological characteristic of viral replication in arthropods followed by transmission to vertebrates in the course of blood feeding has arisen independently in seven families of viruses. Taxonomic relationships within the arboviruses were originally based on analyses of antigenic cross-reactivity data obtained from neutralization, complement fixation, and hemagglutination tests. Virion morphology, determined by electron microscopy, was also important in taxonomic classification.

The availability of viral, genomic, nucleic acid sequences and sophisticated nucleic acid analyses and phylogenetic software has enabled hypothesis testing of arboviral evolution. Defining the deeper nodes in evolutionary trees has been difficult because of the limited number of sequences from different virus species in each virus family. Predictably, the arboviruses that are most well-characterized are those associated with disease. It will be difficult to formulate an accurate and robust theory of arboviral evolution until this sampling bias is resolved by obtaining data from other viruses, those not necessarily associated with disease. Brief summaries of the best-studied arbovirus groups are presented below.

Evolutionary Relationships of the Flaviviruses

The genus, *Flavivirus* is currently composed of about 70 different viruses that infect a wide variety of vertebrate hosts from birds to bats, transmitted by ticks and mosquitoes; some have no known vector associations and others are found only in mosquitoes.

Flavivirus-related sequences have been detected in the genomes of *Aedes* mosquitoes suggesting flavivirus sequence integration into an eukaryotic genome. Another curious finding is the discovery of defective Dengue virus 1 (genus *Flavivirus*, family *Flaviviridae*) virions that are transmitted by *Aedes aegypti* to humans over long periods of time. The defective genomes have acquired a stop codon in the envelope glycoprotein gene resulting in a truncated E-protein. This defective lineage persists through complementation by co-infection of host cells with functional viruses. The relevance of these findings on virulence and pathogen transmission are unknown.

The detection of diverse quasispecies populations of Dengue virus 3 in *Aedes aegypti* mosquitoes and in humans suggests that flaviviral mutation frequencies are similar to those of other RNA viruses. Estimates of flavivirus evolutionary rates suggest they are generally less than those for single-host RNA viruses, reflecting the evolutionary constraints of obligatory replication in vertebrate and invertebrate hosts. Approximation of the degree of amino acid divergence between mosquito-borne and tick-borne viruses indicates that flaviviruses in the tick-borne group evolve two to three times more slowly than do the mosquito-borne viruses. This is probably the result of limited virus replication and the lengthy tick life cycle, which can be measured in years. Ixodid (hard) ticks feed only three times during their life cycle (larva→nymph→adult) with months passing between ecdysis (molting) and

feeding on a subsequent vertebrate host, thus allowing virus lineages to persist for long periods of time in a quiescent arthropod. Also, an infected tick can directly infect another tick co-feeding on the same uninfected animal host, eliminating the need for a viremic vertebrate as had been demonstrated for tick-borne encephalitis virus and *Ixodes ricinus* ticks. This 'nonviremic' transmission may result in viruses persisting for long periods of time in the tick, further constraining rates of evolutionary change. This is in marked contrast to mosquitoes where viral replication occurs rapidly and to high titers over a life span measured in days. The increased amount of RNA replication in mosquitoes generates more virus variation, thereby increasing opportunities for viral evolution.

Data sets from alignments of sequences from the nonstructural genes NS3 and NS5 and whole-genome alignments have been examined for phylogenetic signal in determining evolutionary relationships among most viruses in the family. In general, data alignments from the NS3 genes and from the full-length genomes have proven to be the most useful in elucidating the basal divergence of derived lineages. Hypotheses based on recent studies suggest that the absence of a vector is the ancestral condition for the family as a whole (genera *Hepacivirus*, *Pestivirus*, and *Flavivirus*); within this family, arboviruses occur only in the *Flavivirus* genus.

Within the genus *Flavivirus*, determination of the divergence of the three main groups (no known vector, mosquito-borne, and tick-borne) is equivocal. Phylogenetic analysis of the NS5 data set suggests that the no-known vector group diverged before the arthropod-borne viruses while analysis of the NS3 and full-genome data sets imply that the mosquito-borne flaviviruses diverged first and form a sister clade to the no-known vector and tick-borne viruses. If the mosquito-borne viruses were the first divergent group, we would expect the existence of a lineage of unknown flaviviruses occurring in mosquitoes. The recent discovery of the flavivirus, *Kamiti River virus*, which replicates in mosquitoes exclusively, adds support to the speculation that there may be a large number of unidentified flaviviruses existing in nature that are the descendants of a primitive, mosquito–host lineage.

Attempts have been made to associate epidemiology, disease pattern, and biogeography with the major virus clades using either partial sequences of the NS5 gene or of the E-gene. The mosquito-borne flaviviruses partition into two groups: viruses that cause neuroinvasive disease (Japanese encephalitis virus and West Nile virus) and are transmitted by *Culex* vectors to bird amplification reservoirs, and viruses that cause hemorrhagic disease (Yellow fever virus and dengue viruses) and are transmitted by *Aedes* vectors to primate amplification reservoirs. The tick-borne viruses also formed two groups, one associated with seabird hosts and another associated with rodents. The no-known vector viruses split into three groups, two associated with bats and one with rodents. The above associations undoubtedly reflect the complex interactions and selection pressures between arboviruses, arthropod vectors, and vertebrate hosts over evolutionary time.

Regardless of which group diverged first, the flaviviruses probably originated in the Old World. Within the *Aedes* mosquito-borne clade, only Yellow fever virus, the four dengue viruses, and West Nile virus are established in the Americas; the presence of these viruses represents relatively recent introductions from Africa or Asia. With the exception of the *Powassan virus* (family *Flaviviridae*), all the tick-borne flaviviruses occur in the Old World. Evidence for the Old World origin of the no-known vector group is less convincing. The rodent-borne viruses are only found in the New World with the exception of *Apoi virus* (family *Flaviviridae*); the bat-borne group has representatives that occur worldwide. These observations could be explained by a single dispersion event of an ancestral virus from the Old World into the New World that resulted in infection of resident rodents. Rodents generally occupy a small geographic range and are less likely to widely disperse viruses, increasing the likelihood of founder effects, genetic drift, and viral allopatric speciation. Migratory bats on the other hand are capable of transporting viruses over long distances.

Evolutionary Relationships of the Alphaviruses

The genus *Alphavirus* in the family *Togaviridae* is comprised of 29 described species. Within the genus, viruses have been grouped into eight antigenic complexes by classic serological techniques. Phylogenetic relationships in the genus have been estimated using nucleic acid and amino acid alignments from the E1, nsP1, and nsP4 codons. Phylogenetic trees produced from analyses of alignments of nucleic acids and amino acids generally support the antigenic groups as monophyletic clades. The antigenic complexes are also associated with disease syndromes, as described for the flaviviruses with the exception of hemorrhagic fevers. For example, members of the Eastern equine encephalitis and Venezuelan equine encephalitis complexes produce neuroinvasive disease in humans and equines, while members of the *Semliki Forest virus* complex commonly cause mild to severe arthralgias. Interestingly, the Western encephalitis virus complex contains viruses capable of causing both disease syndromes; arthralgias in the *Sindbis virus* clade and neuroinvasive disease caused by Highlands J and Western equine encephalitis viruses. This dichotomy in disease associations is the probable result of an ancient, genomic recombination event. Two of the viruses in this complex, Highlands J and Western equine encephalitis, are descendents of a recombinant ancestral alphavirus; presumably, the genetic material responsible for the potential to cause encephalitis came from an Eastern equine encephalitis virus-like ancestor and a Sindbis-like virus.

Molecular studies using phylogenetic analyses of alphaviruses have shown that the majority of genome sequence divergence results in synonymous substitutions, indicating that rates of evolutionary change are constrained by purifying selection. This conservation is revealed in the 26S subgenomic promoter sequences; they are functionally interchangeable between Sindbis virus and other alphaviruses. Again, the hypothesis to explain the lower rates of evolution in these viruses in contrast to nonarboviral RNA viruses is the restraint imposed by required replication in hosts with and hosts without a backbone.

An examination of alphavirus phylogenetic divergence demonstrates the importance of host switching and host mobility. An intriguing example is the relationship between the closely related Chikungunya and O'nyong-nyong viruses. *Chikungunya virus* is thought to have originated in Africa in a sylvan, *Aedes*–primate transmission cycle. *O'nyong-nyong virus* (family *Togaviridae*) is genetically very similar to Chikungunya virus yet it has become adapted to *Anopheles funestus* and *Anopheles gambiae* moquitoes. Molecular studies with chimeric Chikungunya–O'nyong-nyong viruses indicate that an ancestor of Chikungunya virus became adapted to replication in anopheline mosquitoes. This is significant because *Anopheles* species as arboviral vectors are the exception, rather than the rule.

In general, alphavirus divergence is highest when virus transmission occurs focally, usually in small vertebrate hosts, such as rodents which have restricted geographic ranges. Virus transmission in these isolated foci can produce new genotypes as has been elegantly demonstrated for viruses in the Venezuelan equine encephalitis complex. Conversely, genomic divergence is less for viruses that are amplified in wide-ranging vertebrates like birds where genotypic mixing and selection maintains a dominant genotype.

Disease Associations

Although arboviruses are represented in diverse viral taxa, the disease syndromes they produce in humans can be arbitrarily classified into three major groups: systemic febrile illness, meningitis and/or encephalitis, and hemorrhagic fever. It is important to note that these syndromes represent a spectrum of disease severity: from sub-clinical infections to fatal outcomes. The factors responsible for differential disease severity are unknown but age and the immune status of the host are important as well as the amount of virus inoculated (dose) and the relative pathogenicity of the infecting arbovirus strain. Disease is most often correlated with infection of dead-end hosts including humans and their domestic animals; there is little evidence of these viruses causing harm in their natural hosts.

Febrile illness caused by infection with dengue viruses (serotypes 1–4), West Nile, Chikungunya, and other viruses consists of fever, headache, muscle and joint pain, and sometimes with rash. In a certain subset of cases, infection with West Nile virus progresses to encephalitis, resulting in fatalities or neurological sequelae in survivors, whereas infection with the dengue viruses rarely results in encephalitis or a hemorrhagic syndrome. Age-associated disease syndromes have been noted with West Nile virus infection: inapparent infection in children, typical fever syndrome in adults, and neuroinvasive disease in the elderly. Other arboviruses that can produce a febrile illness associated with polyarthritis are the alphaviruses, Chikungunya, O'nyong-nyong, Sindbis, Ross River, Barmah, and Mayaro. These febrile illnesses are impossible to differentiate on a clinical basis and are often confused with malaria and other common viral infections, including influenza. No fatalities are associated with uncomplicated febrile illnesses although recovery time can be prolonged and sequelae are commonly observed.

More serious illnesses are caused by arboviruses that have a tropism for the central nervous system, including Japanese encephalitis, Saint Louis encephalitis, West Nile, Eastern equine encephalitis, Western equine encephalitis, Venezuelan equine encephalitis, and *LaCrosse* (genus *Orthobunyavirus*, family *Bunyaviridae*) viruses. Illness begins with fever, chills, headache, malaise, drowsiness, anorexia, myalgia, and nausea. The syndrome can progress to confusion, stupor, and coma. These infections can be life-threatening and survivors may suffer from severe neurological sequelae.

Another serious manifestation of infection by some arboviral pathogens is hemorrhagic fever. Infection with the flavivirus Yellow fever virus can produce a clinical spectrum ranging from a mild febrile illness to fulminating fatal disease. Flaviviruses in the mammalian tick-borne virus group that can cause hemorrhagic fevers include Kyasanur Forest disease and Omsk hemorrhagic fever viruses. Infection with Rift Valley fever and Crimean-Congo hemorrhagic fever viruses can also result in hemorrhagic fever.

The above-mentioned examples are all for human infections. As might be expected, domestic animals may exhibit similar disease syndromes following arboviral infection. Infections that can produce febrile illness in animals include the alphavirus *Getah* in horses, *Bovine ephemeral fever virus* (genus *Ephemerovirus*, family *Rhabdoviridae*) in cattle, and Crimean-Congo hemorrhagic fever virus in cattle, sheep, and goats. More severe disease with encephalitis results from infection with West Nile, Eastern equine encephalitis, Western equine encephalitis, and Venezuelan equine encephalitis viruses in horses. Infection with the flavivirus *Wesselsbron virus*, *Bluetongue virus* (genus *Orbivirus*, family *Reoviridae*) and Nairobi sheep disease virus (genus *Nairovirus*, family *Bunyaviridae*) in sheep, and African swine fever virus in pigs can result in hemorrhages. Infection of sheep, goats, and cattle with Rift Valley fever virus can result in abortions while infection with the orthobunyavirus *Akabane virus* (family *Bunyaviridae*) can result in congenital malformations.

Transmission Cycles

Human and livestock encroachment into natural transmission cycles is the most common means of exposure to arboviral infections. This virus transmission scenario is sometimes termed 'spillover' because the principal enzootic vector(s) has a wide host feeding range not limited to the natural, amplifying, vertebrate hosts. Arboviruses in this category of transmission include, but are not limited to, West Nile, sylvan Yellow fever, Western equine encephalitis and La Crosse viruses. African swine fever virus is maintained in sub-Saharan Africa in a cycle involving *Ornithodoros* ticks and warthogs. Infected ticks infesting warthog burrows can also transmit the virus to their progeny by transovarial transmission. Infected warthogs do not show signs of the disease; however, domestic swine are severely affected with mortality rates approaching 100%. In addition to tick bite, transmission between domestic pigs can occur through direct contact and the ingestion of infected offal.

Domestication of animals for human use has provided arboviruses with new and abundant hosts. For example, Rift Valley fever virus circulates in extensive areas of Africa and is transmitted by certain species of *Aedes* and *Culex* mosquitoes to various native vertebrates. The virus is thus maintained by horizontal transmission in the vertebrates and by transovarial and/or vertical transmission of the virus in mosquito eggs during periods of drought. El Nino events can lead to sustained rainfall and flooding, resulting in the production of massive numbers of other species of *Aedes* and *Culex* mosquitoes that enter into the existing, endemic transmission cycle. Infected vectors disperse and bloodfeed on livestock, initiating a secondary amplification cycle. Viremic livestock become the source for a dramatic increase in the numbers of infected vectors, which in turn engenders human epidemics and livestock epizootics.

The third pattern of arboviral transmission is one in which humans serve as both the reservoir and amplification hosts. Arboviruses maintained in human→vector→human cycles are generally transmitted by domestic mosquito vectors, most importantly, *Aedes aegypti.* The dengue viruses, the most important arboviruses in terms of human disease, are the classic example of an arbovirus that has adapted to the human host. Other examples include Chikungunya and Yellow fever viruses which can cause spectacular outbreaks in areas infested with *Aedes aegypti* vectors. These latter viruses generally disappear after a sufficient numbers of humans become immune, but persist in sylvan transmission cycles until nonimmune human hosts become available or when human herd immunity wanes.

See also: Akabane Virus; Crimean-Congo Hemorrhagic Fever Virus and Other Nairoviruses.

Further Reading

Calisher C (2005) A very brief history of arbovirology focusing on contributions by workers of the Rockefeller Foundation. *Vector Borne and Zoonotic Diseases* 5: 202.

Cook S and Gould E (2006) A multigene analysis of the phylogenetic relationships among the flaviviruses (Family: *Flaviviridae*) and the evolution of vector transmission. *Archives of Virology* 151: 309.

Karabatsos N (1985) *International Catalog of Arboviruses Including Certain Other Viruses of Vertebrates,* 3rd edn. San Antonio: American Society of Tropical Medicine and Hygiene.

Knipe DM and Howley PM (eds.) (2001) *Fields Virology,* 4th edn. 2 vols. Philadelphia: Lippincott.

Monath TP (ed.) (1988) *The Arboviruses: Epidemiology and Ecology.* 5 vols. Boca Raton: CRC.

Theiler M and Downs WG (1973) *The Arthropod-Borne Viruses of Vertebrates.* New Haven, CT: Yale University Press.

Weaver SC and Barrett ADT (2004) Transmission cycles, host range, evolution and emergence of arboviral disease. *Nature Reviews Microbiology* 2: 789.

Arteriviruses

M A Brinton, Georgia State University, Atlanta, GA, USA
E J Snijder, Leiden University Medical Center, Leiden, The Netherlands

© 2008 Elsevier Ltd. All rights reserved.

History

Equine arteritis virus (EAV) was first isolated in 1953 in Bucyrus, Ohio from the lung tissues of an aborted fetus during an epidemic of abortions and arteritis in pregnant mares. However, an equine disease similar to that caused by EAV was first observed in the late 1800s. At the time of its discovery, EAV was distinguished from equine (abortion) influenza virus.

Lactate dehydrogenase-elevating virus (LDV) was discovered by accident in 1960 during a study to find methods for early detection of tumors in mice. A five- to tenfold increase in lactate dehydrogenase (LDH) levels in serum 4 days after inoculation of mice with either Ehrlich carcinoma cells or cell-free extracts suggested that an infectious agent was responsible for the observed LDH elevation.

Porcine respiratory and reproductive syndrome was first observed in North America in 1987 and in Europe

in 1990. This disease has also been referred to as porcine epidemic abortion and respiratory syndrome (PEARS), swine infertility and respiratory syndrome (SIRS), and mystery swine disease (MSD). The causative agent of the disease is now referred to as porcine respiratory and reproductive syndrome virus (PRRSV).

Simian hemorrhagic fever virus (SHFV) was isolated in 1964 during outbreaks of a fatal hemorrhagic fever disease in macaque colonies in the US, Russia, and Europe. A number of additional SHFV outbreaks in macaque colonies have occurred since the 1960s. The most 'famous' of these was the one in Reston, Virginia, which occurred in conjunction with an Ebola virus outbreak in the same facility.

Taxonomy and Classification

On the basis of virion size and morphology as well as the positive polarity of the RNA genome, LDV and EAV were originally classified within the family *Togaviridae*. In 1996, following the sequence analysis of their genomes, EAV, LDV, SHFV, and PRRSV were classified as species within a new family, *Arteriviridae*, genus *Arterivirus*. EAV was designated the prototype of this family. At the same time, the family *Arteriviridae* was classified together with the family *Coronaviridae* in the order *Nidovirales*. This order also includes two additional virus groups, the toroviruses (a genus in the *Coronaviridae* family) and the family *Roniviridae*. The arterivirus genome shares similar general organizational features and conserved replicase motifs with corona-, toro-, and ronivirus genomes, but is only about half their size. In addition, arterivirus particles are smaller than those of other nidoviruses, differ from them morphologically, and are the only ones to have an isometric nucleocapsid structure.

Geographic Distribution

Viruses with biological properties identical to those of LDV have been isolated from small groups of wild mice (*Mus musculus*) in Australia, Germany, the US, and UK. Natural infections with EAV and EAV-induced disease in horses and donkeys have been documented in North America, Europe, and Japan and anti-EAV antibodies have been detected in horse sera from Africa and South America, indicating that EAV infection is geographically widespread. Natural PRRSV infections in pigs have been reported in North America, Europe, and Asia. SHFV infection in captive patas monkeys has been documented and this virus has also been detected in the blood of wild-caught patas and African green monkey as well as baboons, suggesting that these African primates are the natural hosts for SHFV.

Host Range and Virus Propagation

Natural infections with EAV occur only in horses and donkeys. Field isolates of EAV can be readily obtained from field samples (semen, fetal tissues, and buffy coats) using RK-13 cells. Laboratory strains of EAV have been successfully grown in primary cultures of horse macrophages and kidney cells, rabbit kidney cells, and hamster kidney cells and also in cell lines, such as BHK-21, RK-13, MA-104, and Vero.

LDV replicates efficiently in all strains of laboratory and wild *Mus musculus* and somewhat less efficiently in the Asian mouse *Mus caroli*. Numerous attempts to infect other rodents such as rats, hamsters, guinea pigs, rabbits, deer mice (*Peromyscus maniculatus*), and dwarf hamsters (*Phodopus sungorus*) with LDV have not been successful. LDV replicates only in primary murine cell cultures that contain macrophages, such as spleen, bone marrow, embryo fibroblast, and peritoneal exudate cell cultures. Although peritoneal cultures prepared from starch-stimulated adult mice contain 95% phagocytic cells, only 6–20% of these cells support LDV replication as demonstrated by autoradiographic, *in situ* hybridization, immunofluorescence and electron microscopic techniques, suggesting that LDV infects an as yet uncharacterized subpopulation of macrophages. A much higher percentage of cells in peritoneal exudate cells obtained from infant mice are susceptible to virus infection.

Natural infections with PRRSV were thought to be restricted to pigs. However, one report suggested that chickens and mallard ducks may be susceptible to the virus. PRRSV can replicate in primary cultures of porcine alveolar macrophages and macrophages from other tissues. Some, but not all, isolates of PRRSV can be adapted to replicate in a subclone of the MA-104 cell line.

Natural infections with SHFV occur in several species of African primates, namely *Erythrocebus patas*, *Cercopithecus aethiops*, *Papio anuibus* and *Papio cynocephalus*. SHFV infection of members of the genus *Macaca* has occurred in primate facilities in a number of countries and was associated with a fatal hemorrhagic fever. Isolates of SHFV can replicate in primary cultures of rhesus aveolar lung macrophages or peripheral macrophages and some isolates can replicate efficiently in the MA-104 cell line.

Maximum arterivirus yields after infection of susceptible cell cultures are observed by 10–15 h after infection. The maximum titers obtained for LDV and PRRSV are 10^6–10^7 ID_{50} ml^{-1} and can exceed 10^8 PFU ml^{-1} for EAV and SHFV.

Properties of the Virion

Arterivirus particles are spherical, enveloped, and 40–60 nm in diameter (**Figures 1(a)** and **1(b)**). Unfixed

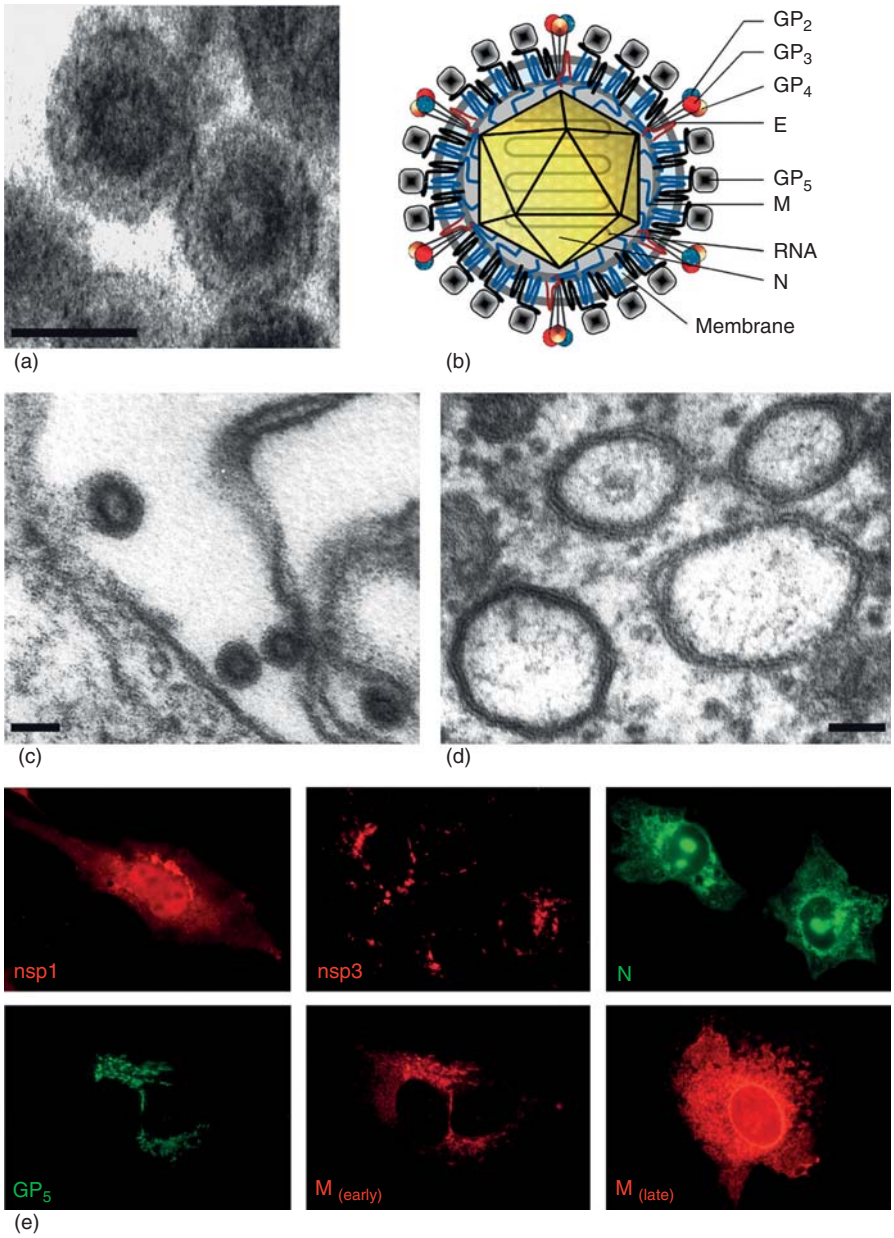


Figure 1 (a) An electron micrograph of extracellular PRRSV particles. (b) Schematic representation of an arterivirus particle and its seven structural proteins. (c) An electron micrograph of EAV particles budding from smooth membranes in (or close to) the Golgi region of infected baby hamster kidney cells (BHK-21) cells. (d) An electron micrograph of typical double-membrane vesicles found in the cytoplasm of arterivirus-infected cells that have been implicated in replication complex formation and viral RNA synthesis. (e) Localization of selected EAV nonstructural and structural proteins in infected BHK-21 cells by immunofluorescence microscopy. In contrast to all other nsps (e.g., nsp3), the N-terminal replicase subunit nsp1 only partially localizes to the perinuclear region and is partially targeted to the nucleus. The double-labeling for nsp3 and the N protein shows that a considerable part of the latter co-localizes with the viral replication complex, whereas another fraction of the N protein is targeted to the nucleus. Early in infection, double-labeling for the major glycoprotein GP_5 and the M protein showed almost complete co-localization of the two proteins in the Golgi complex, in the form of the heterodimer that has been found to be critical for virus assembly. Later in infection, the M protein accumulates in the endoplasmic reticulum. Scale = 50 nm (a, c–d). (a) Reprinted from Snijder EJ and Meulenberg JM (1998) The molecular biology of arteriviruses. *Journal of General Virology* 79: 961–979. (b) Reprinted from Snijder EJ, Siddell SG, and Gorbalenya AE (2005) The order Nidovirales. In: Mahy BWJ and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections*, *Vol. 1: Virology*, 10th edn., pp. 390–404. London: Hodder Arnold. (c, d) Reprinted from Snijder EJ and Meulenberg JM (1998) The molecular biology of arteriviruses. *Journal of General Virology* 79: 961–979. (e) Images courtesy of Yvonne van der Meer and Jessika Zevenhoven, Leiden University Medical Center, The Netherlands.

virions undergo distortion and disintegration during standard negative staining procedures. The virion surface appears rather smooth. The virion capsid is icosahedral and about 25–35 nm in diameter. Buoyant densities of 1.13–1.17 g cm^{-3} and sedimentation coefficients of 214S to 230S have been reported for arteriviruses. Virions can be stored indefinitely at −70 °C but are heat labile. For instance, the infectivity of LDV samples in plasma decreased by half after 4 weeks at −20 °C and by about 3.5 logs after storage for 32 days at 4 °C. Virus in media supplemented with 10% serum is stable for 24 h at room temperature, but completely inactivated by heating at 58 °C for 1 h. Virions are fairly stable between pH 6 and pH 7.5, but are rapidly inactivated by high or low pH. Virus is efficiently disrupted by low concentrations of nonionic detergent.

The locations of the seven structural proteins in an EAV virion are indicated schematically in **Figure 1(b)**. The icosahedral capsid is composed of the nucleocapsid (N) protein. The major envelope glycoproteins, GP_5 and M, form a disulfide-linked heterodimer. The minor glycoproteins GP_2, GP_3, and GP_4 form a disulfide-linked heterotrimer. GP_2–GP_4 heterodimers have also been detected in EAV virions. Although all six of these proteins were shown to be required for EAV and PRRSV infectivity, not all of the minor structural proteins have been identified so far in the other arteriviruses and the nomenclature for the SHFV structural proteins differs due to an insertion in the 3′ region of the SHFV genome. Virions bud into the lumen of cytoplasmic vesicles (**Figure 1(c)**).

Properties of the Genome

Arterivirus genomes are single-stranded RNAs of positive polarity that contain a 3′ poly(A) tract of approximately 50 nucleotides in length and a type I cap at the 5′ end. The genome lengths are 12.7 kb for EAV, 14.1 kb for LDV, 15.1 kb for PRRSV, and 15.7 kb for SHFV.

The large nonstructural or 'replicase' polyproteins are encoded by open reading frames (ORFs) 1a and 1b and occupy the 5′ three-fourths of the genome. ORF 1b is translated only when a −1 ribosomal frameshift occurs in the short ORF 1a/ORF 1b overlap region. A 'slippery sequence' upstream of a pseudoknot directs frameshifting and for EAV, a frameshifting efficiency of 15–20% has been reported. ORF 1a encodes three or four proteases that post-translationally cleave the pp1a and pp1ab polyproteins at multiple sites into the mature viral nonstructural proteins (**Figure 2**). The lengths of the ORF 1a regions of the different arteriviruses vary. ORF 1b encodes major conserved domains, in particular an RNA-dependent RNA polymerase, a putative zinc-binding domain, an RNA helicase, and a nidovirus uridylate-specific endoribonuclease. The multiple 3′-proximal ORFs (**Figure 2**) encode the structural proteins. There are six such ORFs in the genomes of EAV, PRRSV, and LDV, while SHFV contains nine ORFs downstream of ORF 1b. Limited sequence homology suggests that the SHFV ORFs 2a, 2b, and 3 may be duplications of ORFs 4, 5, and 6, respectively. In most cases, adjacent structural protein genes of arteriviruses are in different reading frames and overlap. Conserved

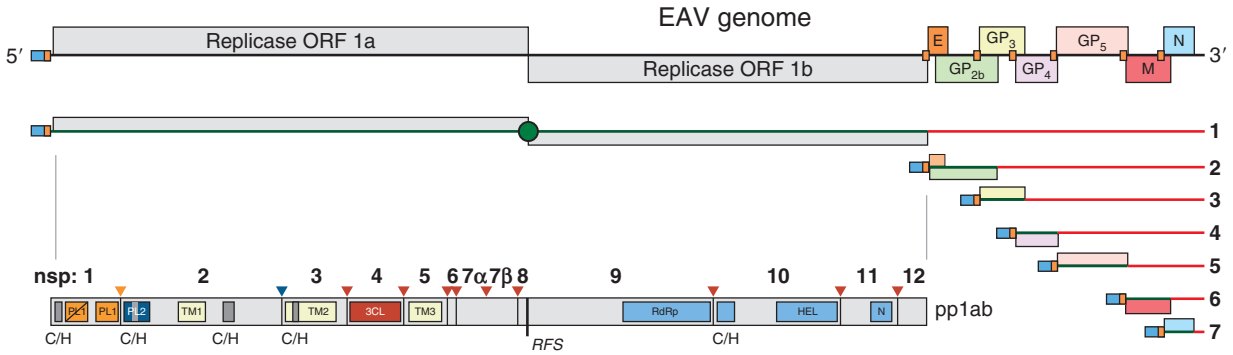


Figure 2 Arterivirus genome organization and expression are illustrated using the family prototype EAV as an example. The genomic open reading frames are indicated and the names of the corresponding proteins are given. Below the genome, the nested set of mRNAs found in infected cells is depicted, with RNA1 being identical to the viral genome and subgenomic RNAs 2–7 being used to express the structural protein genes located in the 3′-proximal quarter of the genome. With the exception of the bicistronic mRNA2, the subgenomic mRNAs are functionally monocistronic. The EAV replicase gene organization is depicted in the polyprotein pp1ab from the replicase (pp1a is identical to the nsp1–8 region of pp1ab). Ribosomal frameshift (RFS) delineates the boundary between amino acids encoded in ORF 1a and ORF 1b and arrows represent sites in pp1ab that are cleaved by papain-like proteases (yellow and blue) or the main (3CL) protease (red). The proteolytic cleavage products (nsps) are numbered and the locations of various conserved domains are highlighted. These include domains with conserved Cys and His residues (C/H), putative transmembrane domains (TM), protease domains (PL1, PL2, and 3CL), the RNA-dependent RNA polymerase domain (RdRp), helicase (HEL), and uridylate-specific endoribonuclease (N). Adapted from Siddell SG, Ziebuhr J, and Snijder EJ (2005) Coronaviruses, toroviruses, and arteriviruses. In: Mahy BWJ and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections*, *Vol. 1: Virology*, 10th edn., pp. 823–856. London: Hodder Arnold.

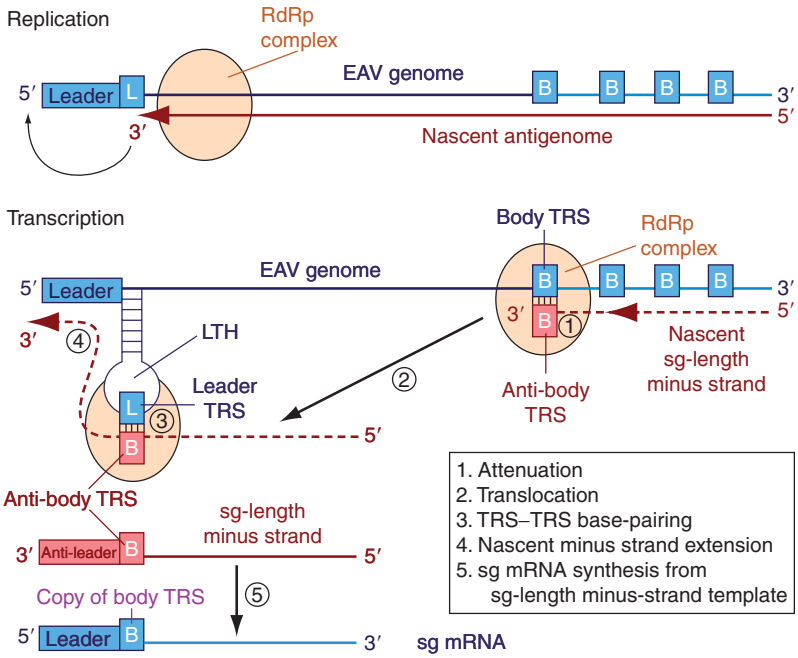


Figure 3 Arterivirus RNA synthesis (using a hypothetical virus that produces four subgenomic (sg) mRNAs). Both replication (top panel) and transcription (bottom panel) are depicted. In the 'replication mode', the RdRp produced a full-length minus-strand RNA (antigenome) that serves as the template for synthesis of new genomic RNA. In the 'transcription mode', minus-strand RNA synthesis is thought to be discontinuous and regulated by transcription-regulating sequences (TRSs). Body TRSs (B) in the genome would act as attenuators of minus-strand RNA synthesis. Subsequently, the nascent minus strand, with an anti-body TRS at its 3′ end, would be redirected to the 5′-proximal region of the genomic template by a base-pairing interaction with the leader TRS (L) that (for EAV) has been shown to reside in a leader TRS hairpin (LTH) structure. Following the addition of the anti-leader to the nascent minus strands, the sg-length minus strands would then serve as templates for sg mRNA synthesis.

transcription-regulating sequences (TRSs; **Figure 3**) are located at the 3′ end of the genomic leader sequence (leader TRS) and upstream of each of the 3′-proximal ORFs (body TRSs). RNA hairpin structures are located near the 5′ end of the genome (including a leader TRS-presenting hairpin) and also in the 3′ NTR.

Properties of the Viral Proteins

Arterivirus proteins that are encoded at the 5′ end of the genome are translated directly from the genomic RNA as polyproteins (pp1a and pp1ab; **Figure 2**). The proteins generated from these ORFs contain all functions required for viral RNA synthesis. Both pp1a/pp1ab contain multiple papain-like cysteine proteases and a chymotrypsin-like (or '3C-like') serine protease (**Figure 2**). The EAV papain-like cysteine proteases each cleave at a single site. The nsp4 serine protease, or main protease, cleaves at six sites in the pp1a region and at three additional sites in the ORF 1b-encoded part of pp1ab. Due to the existence of two alternative processing cascades (minor and major pathways) a variety of processing intermediates and mature proteins are generated from the C-terminal half of pp1a. In total, 13 (EAV) or 14 (PRRSV/LDV) mature proteins are (predicted to be) generated from the arterivirus replicase polyproteins. Three hydrophobic regions in pp1a are thought to be important for membrane association of the viral replication–transcription complexes. With the exception of nsp1, which is partly found in the nucleus, the rest of the nonstructural proteins localize to endoplasmic reticulum-derived double-membrane structures (**Figure 1(d)**) in the perinuclear region (**Figure 1(e)**). The mature ORF 1b-encoded proteins (nsp9–nsp12) are thought to be the primary enzymes of the viral replication–transcription complexes that direct viral RNA synthesis.

The proteins encoded in the 3′-proximal quarter of the genome are expressed from six (nine for SHFV) overlapping subgenomic mRNAs (a 3′ co-terminal nested set; **Figure 2**). Although the subgenomic mRNAs, with the exception of the smallest one, are structurally polycistronic, in general only the 5′ terminal ORF is translated. An exception is mRNA 2, which is bicistronic encoding GP_{2b} and E. The nucleocapsid (N) protein is encoded by ORF 7 (ORF 9 in SHFV). Analysis of the crystal structure of the C-terminal domain of the PRRSV N protein suggests that arteriviruses have a unique capsid-forming domain. The M protein which is encoded by ORF 6 (ORF 8 in SHFV), is a triple-membrane-spanning protein and the major nonglycosylated envelope protein. The major envelope glycoprotein is encoded by ORF 5 (ORF 7 in SHFV). GP_{2b}, GP_3, and GP_4 are each about

20 kDa in size and are minor envelope glycoproteins (EAV, **Figure 2**). GP_{2b} and GP_4 (GP_{4b} and GP_6 in SHFV) are class I integral membrane proteins. A soluble, non-virion-associated form of PRRSV GP_3 is released from infected cells. E is an unglycosylated small hydrophobic minor envelope protein. The PRRSV E protein has been shown to possess ion channel protein-like properties.

The nonstructural protein nsp1 and the N protein have been detected in the nucleus as well as the cytoplasm (**Figure 1(e)**). However, the biological significance of the nuclear localization of these two viral proteins is currently not known.

Replication

Cell tropism is determined in part at the level of a receptor on the cell surface, since in some cases cells that are nonpermissive for an arterivirus have been reported to be productively infected after transfection of viral genomic RNA. Evidence for a specific saturable, but as yet unidentified receptor for LDV on a subpopulation of murine macrophages has been reported. LDV-immune complexes are also infectious and can infect macrophages via Fc receptors. Sialoadhesin (sialic acid-dependent lectin-like receptor 1), a macrophage-restricted, cell surface protein, has been shown to mediate the internalization of PRRSV by alveolar macrophages. Heparin sulphate on the cell surface and sialic acid on the virion are also thought to play a role in entry. PRRSV has been reported to enter cells via a low pH-dependent endocytic pathway. Soon after infection, both EAV and LDV particles have been observed in small vesicles that appear to be clathrin-coated. The existence of an additional level of host restriction at the endosomal membrane fusion or uncoating steps was suggested by the observation that nonsusceptible cells expressing recombinant sialoadhesin could internalize virus but rarely became productively infected.

The arterivirus replication cycle occurs in the cytoplasm of infected cells. After the incoming genomic RNA is uncoated, it is translated to produce polyproteins pp1a (1727–2502 amino acids) and pp1ab (3175–3959 amino acids) and then becomes the template for minus-strand synthesis (**Figure 3**). Either a full-length minus-strand RNA, that then serves as a template for genomic RNA synthesis, or subgenomic minus strands, that then serve as the templates for subgenomic mRNA synthesis, can be produced. The subgenomic RNAs are thought to be produced by a mechanism of discontinuous minus-strand RNA synthesis that utilizes conserved primary (TRSs) and higher-order RNA structures as signals for producing a subgenomic minus-strand template for each subgenomic mRNA. Subgenomic mRNAs are 3′-coterminal and contain a common 5′ leader sequence that is identical to the 5′ terminus of the genomic RNA. *cis*-Acting regulatory signals required for arterivirus replication have been mapped to the ~300 nt at each end of the genome. Host proteins also appear to be involved in the regulation of arterivirus RNA synthesis.

The co-localization of N with replicase complexes (**Figure 1(e)**) suggests that genome encapsidation may be coordinated with genome synthesis. New virions form via budding of preformed capsids into the lumens of smooth endoplasmic reticulum and/or Golgi complex membranes (**Figure 1(c)**). Arterivirus envelope proteins localize to intracellular membranes and recent data suggest that the formation of the GP_5–M heterodimer is required for budding (**Figure 1(e)**). Mature virions in the lumens of these vesicles are then transported to the exterior of the cell and released.

The formation of cytoplasmic double-membrane vesicles (**Figure 1(d)**), which have been implicated in viral RNA synthesis, is characteristic of arterivirus-infected cells. Infection of primary macrophages by EAV, SHFV, PRRSV, and probably also LDV is cytocidal. Laboratory strains of EAV, SHFV, and PRRSV cause obvious cytopathology in the continuous cell cultures that they infect, such as MA-104 cells. Infected cells become rounded by 24–36 h after infection and release from the tissue culture flask. Apoptosis has been reported in PRRSV-infected primary porcine alveolar macrophages and MA-104 cell cultures as well as in testicular germ cells in pigs. However, other studies with PRRSV showed that necrosis, not apoptosis, was the main cause of death of infected cells.

Genetics

Evidence for virulence variants of all arteriviruses has been obtained. One strain of LDV isolated from C58 tumor-bearing mice and designated LDV-C was shown to efficiently induce neurologic disease in a few susceptible inbred mouse strains, such as AKR and C58, both of which are homozygous for the *Fv-1*^{n} allele. Subsequent studies showed that neuropathogenic and non-neuropathogenic isolates coexist in most LDV pools. The number of glycosylation sites in the ectodomain of GP_5 varies in different LDV strains and it has been postulated that antibodies bind less efficiently to virions with extensive glycosylation in this region. A neurovirulent strain of PRRSV has also been reported. Virulent and avirulent mutants of EAV have been identified on the basis of the severity of the diseases they cause. Attenuated vaccine strains of EAV and PRRSV and a number of temperature-sensitive mutants of EAV have been selected. SHFV isolates that produce acute asymptomatic infections and ones that cause persistent, asymptomatic infections in patas monkeys have been reported. EAV and PRRSV infectious cDNA clones have been constructed and provide a means for analyzing the virulence determinants via reverse genetics.

Evolution

Evidence of RNA recombination has been obtained by genome sequencing for both LDV and PRRSV after co-infections with different strains of the same virus type and it is thought that RNA recombination is the mechanism responsible for the observed gene duplication in the SHFV genome. Sequence comparisons of various field isolates of either PRRSV or EAV indicate that the sequences of the M and N proteins are more conserved than those of the virion glycoproteins. The extent of the divergence of the sequences of European and North American PRRSV isolates indicates that these two virus populations represent subspecies and also suggests that the ability to cause porcine disease arose independently in geographically separated virus populations. Phylogenetic analysis of the arteriviruses indicated that PRRSV is most closely related to LDV and that SHFV is more closely related to both of these viruses than to EAV. Although the host specificity of LDV has experimentally been shown to be restricted to mouse species, it has been postulated that PRRSV arose when wild boars became infected with LDV after they ate infected wild mice and that wild boars then introduced a divergent 'LDV' virus into domestic pigs independently in North America and Europe.

The nidoviruses represent a distinct evolutionary lineage among positive-strand RNA viruses. Although the organization of the conserved replicase motifs in the arterivirus genome is very similar to that of the other nidoviruses (coronaviruses, toroviruses, and roniviruses), the structural protein genes of these viruses are apparently unrelated (**Figure 1**). This level of divergence may be related to a high frequency of RNA recombination that appears to be a characteristic of nidovirus replicases and may be a byproduct of the mechanism of discontinuous RNA synthesis utilized by these viruses for subgenomic RNA production. The ancestral nidovirus has been postulated to have had an icosahedral capsid. If via a recombination event with another type of virus, the progenitor of the coronavirus/torovirus lineage acquired an N protein that could form a helical nucleocapsid, then packaging restrictions on genome size would have been lost, allowing genome size expansion via further recombination events and divergence from the arterivirus branch.

Serologic Relationships and Variability

Attempts to demonstrate antigenic cross-reactivity between EAV, LDV, PRRSV, and SHFV have not been successful with one exception. Antibodies produced to a single linear LDV neutralization site located in GP_5 neutralized both LDV and PRRSV. Monoclonal antibodies elicited by one strain of LDV did not bind to the proteins of most other LDV isolates in Western blots, suggesting that serologic variants of LDV exist. Variation in PRRSV N epitopes has been observed between North American and European virus isolates and a high degree of heterogeneity has been observed between strains of PRRSV within the ectodomain of GP_4, which contains a secondary neutralization epitope.

Transmission

There is no evidence for transmission of any of the arteriviruses via insect vectors. Horizontal transmission of both EAV and PRRSV occur via the respiratory route as well as via the venereal route by virus in the semen of persistently infected 'carrier' males. Vertical transmission of PRRSV *in utero* has been reported.

Nothing is currently known about the incidence of transmission of LDV in wild mouse populations. In the laboratory, unless the cage mates are fighting males, LDV is rarely transmitted between mice housed in the same cage, even though infected mice excrete virus in their feces, urine, milk, and saliva. However, transmission of LDV from mother to the fetus via the placenta and to pups via breast milk/saliva has been documented within the first week after infection of the mother. Since LDV in mice and SHFV in patas monkeys is produced throughout the lifetime of persistently infected animals, the transfer of fluids or tissues from an infected animal to an uninfected one results in the inadvertent transfer of infection. Historically the most frequent mode of transmission of LDV among laboratory mice and SHFV from patas monkeys to macaques has been through experimental procedures such as the use of the same needle for sequential inoculation of several animals. Currently, the most frequent sources of LDV contamination are pools of other infectious agents or tumor cell lines that have been repeatedly passaged in mice, especially those first isolated in the 1950s. Such materials should be checked for the presence of LDV. Infectious agent stocks can be 'cured' of LDV by passage in a continuous cell line or a different animal species. Tumor cell stocks can be 'cured' by *in vitro* culture for several passages. It has been suggested but not proven that SHFV can be transmitted between macaques via the respiratory route.

Tissue Tropism

The primary target cells for all four arteriviruses are macrophages. Measurement of the amount of virus in various tissues during natural EAV infections indicated that lung macrophages and endothelial cells were the first host cells to be infected. Bronchial lymph nodes subsequently became infected and then the virus spread throughout the body via the circulatory system. In fatally infected horses, lesions are found in subcutaneous tissues, lymph nodes, and viscera. The progression of PRRSV

infection in pigs is likely to be similar to that observed with EAV. However, although PRRSV is thought to be naturally transmitted by aerosols, experimental transmission by this route has been difficult to achieve. PRRSV has been reported to replicate in testicular germ cells which could result in excretion of virus into the semen. LDV replicates in an uncharacterized subpopulation of murine macrophages. Virus target cells are located in tissues as well as in the blood. Cells containing LDV-specific antigen have been identified in sections of liver and spleen by indirect immunofluorescence. In spleen, the virus-infected cells are nucleated and located in the red pulp. In liver, only Kupffer cells contained LDV-specific antigen. In C58 and AKR mice infected with a neurotropic strain of LDV, virus replication was demonstrated in ventral motor neurons by *in situ* hybridization.

Pathogenicity and Clinical Features of Infection

Serological evidence indicates that even though EAV is widespread in the horse population, it rarely causes clinical disease. Both EAV and PRRSV can cause either persistent asymptomatic infections or induce various disease symptoms such as respiratory disease, fever, necrosis of small muscular arteries, and abortion. The severity of disease caused by EAV and PRRSV depends on the strain of virus as well as the condition and age of the animal. The most common symptoms of natural EAV infections in horses are anorexia, depression, fever, conjunctivitis, edema of the limbs and genitals, rhinitis, enteritis, colitis, and necrosis of small arteries. If clinical symptoms occur, they are most severe in young animals and pregnant mares. Infections in pregnant mares are often inapparent, but result in a high percentage (50%) of abortions. Young animals occasionally develop a fatal bronchopneumonia after infection, but natural infections are not usually life-threatening. In contrast, about 40% of pregnant mares and foals experimentally inoculated with EAV die as a result of the infection. Horses infected with virulent EAV isolates develop a high fever, lymphopenia, and severe disease symptoms. Symptoms observed in PRRSV-infected pigs include fever, anorexia, labored breathing, and lymphadenopathy. Lesions are observed in the lungs and infected pregnant sows produce weak or stillborn piglets.

Mice infected with LDV usually display no overt symptoms of disease. A distinguishing feature of LDV infections is the chronically elevated levels of seven serum enzymes, LDH (eight- to tenfold), isocitrate dehydrogenase (five- to eightfold), malate dehydrogenase (two- to threefold), phosphoglucose isomerase (two- to threefold), glutathione reductase (two- to threefold), aspartate transaminase (two- to threefold), and glutamate-oxaloacetate transaminase (two- to threefold). A decrease in the humoral and cellular immune response to non-LDV antigens is observed during the first 2 weeks following LDV infection. Thereafter, the immune response to other antigens is normal. In immunosuppressed C58 and AKR mice, neurovirulent isolates of LDV can induce a sometimes fatal poliomyelitis. In these mice, immunosuppression is required to delay antibody production until after virus has reached the central nervous system (CNS) and infected susceptible ventral motor neurons. LDV-infected neurons become the targets of an inflammatory response. In mice 6 months of age or older, paralysis of one or both hindlimbs and sometimes a forelimb is observed. In younger C58 mice, poliomyelitis is usually subclinical.

Isolates of SHFV that induce persistent, asymptomatic infections and ones that cause acute, asymptomatic infections of patas monkeys have been reported. All SHFV isolates cause fatal hemorrhagic fever in macaque monkeys. Infected macaques develop fever and mild edema followed by anorexia, dehydration, adipsia, proteinuria, cynosis, skin petechia, bloody diarrhea, nose bleeds, and occasional hemorrhages in the skin. The pathological lesions consist of capillary-venous hemorrhages in the intestine, lung, nasal mucosa, dermis, spleen, perirenal and lumbar subperitoneum, adrenal glands, liver, and periocular connective tissues. These signs and symptoms are not unique to SHFV-infected animals, since they are also observed after infection of macaques with other types of hemorrhagic fever viruses such as Ebola virus. Although the SHFV-induced lesions are widespread in infected animals, the level of tissue damage is not severe. Even so, mortality in macaques infected with SHFV approaches 100% and occurs within 1 or 2 weeks after infection.

Pathology and Histopathology

In horses experimentally or fatally infected with EAV, the most common gross lesions are edema, congestion, and hemorrhage of subcutaneous tissues, lymph nodes, and viscera. Microscopic investigation of tissues from chronically infected horses, which had mildly swollen lymph nodes and slightly increased volumes of pleural and peritoneal fluids, revealed extensive lesions consisting of generalized endothelial damage to blood vessels of all sizes as well as severe glomerulonephritis. Both types of lesions are thought to be caused by the deposition of viral immune complexes. Extensive capillary necrosis leads to a progressive increase in vascular permeability and volume, hemoconcentration, and hypotension. During the terminal stages of the disease, lesions are also found in the adrenal cortex, and degenerative changes are observed in the bone marrow and liver. Focal myometritis is observed in infected pregnant mares and is thought to be the cause of deficiencies in the fetal and placental blood supply. The resulting anoxia is probably the cause of abortion.

Although most LDV infections are inapparent in mice, some histopathogenic changes are observed in infected animals. As described above, the serum levels of seven enzymes are chronically elevated in LDV-infected mice. Normally, an increase in serum levels of tissue enzymes is the result of tissue damage, but in LDV-infected animals little tissue damage is observed. Although there are five naturally occurring LDH isozymes in mouse plasma, only the level of isozyme LDVV is elevated in LDV-infected mice. Studies have indicated that the increase in enzyme levels is primarily the result of a decreased rate of enzyme clearance. A subpopulation of Kupffer cells involved in receptor-mediated endocytosis of LDH is severely diminished in mice by 24 h after LDV infection. It has been postulated that LDV replication in these cells causes their death and results in increased LDH serum levels. Splenomegaly, characterized by a greater than 30% increase in spleen weight, occurs in about 40% of the mice infected with LDV. The increase in spleen weight is observed by 24 h after infection and persists for up to a month. A marked necrosis of lymphocytes in thymic-dependent areas occurs during the first 4 days after LDV infection together with a transient decrease in the number of circulating T lymphocytes between 24 and 72 h after infection. A transient decrease in peritoneal macrophages is also observed between the first and tenth day of infection. Despite the lifelong presence of circulating viral immune complexes and the demonstration of some LDV antibody deposits in the kidneys of LDV-infected mice as early as 7 days after infection, these animals do not develop kidney disease. It has been suggested that nephritis does not develop in these chronically infected mice because of the inability of the majority of the LDV–antibody complexes to bind C1q. Low levels of C1q-binding activity can only be detected between days 10 and 18 after LDV infection. LDV infection can alter the outcome of concomitant autoimmune disease, probably through modulation of the host-immune responses. LDV infection can also trigger the spontaneous production of different types of autoantibodies, possibly as a result of polyclonal B-lymphocyte activation.

The CNS lesions in neurovirulent LDV-infected C58 and AKR mice are located in the gray matter of the spinal cord and, occasionally, in the brainstem and consist of focal areas of inflammatory mononuclear cell infiltrates in the ventral horn. Virus-specific protein and nucleic acid have been detected in ventral motor neurons, and maturing virions in these neurons have been observed by electron microscopy.

Immune Response

Antibodies in sera obtained from animals infected with EAV or PRRSV recognize virion proteins N, M, GP_5, and GP_2. Neutralizing antibodies are primarily directed to GP_5 and the neutralization epitopes of EAV, LDV, and PRRSV have been mapped to the ectodomain of this protein. For EAV, there are four major GP_5 conformational neutralization sites that are interactive. Also, for EAV, interaction between GP_5 and M is required for neutralization. For LDV and PRRSV, the major neutralization site is located in the N-terminus of GP_5. A secondary neutralization site for PRRSV has been mapped to the ectodomain of GP_4. The neutralization epitopes of SHFV have not yet been studied.

Anti-EAV antibodies can be detected in horses 1–2 weeks after infection with virulent or avirulent strains of the virus. Complement-fixing, antiviral antibodies peak 2–3 weeks after the initiation of infection and then decline. Neutralizing antibody peaks between 2 and 4 months after infection. An increase in neutralizing antibodies usually leads to virus clearance. Often after 8 months, anti-EAV antibody can no longer be detected by complement-fixation or neutralization assays. However, in some animals the virus persists and viral immune complexes continue to circulate.

The first month of infection of pigs with PRRSV is characterized by high viremia and disease symptoms. A vigorous antiviral antibody response can be detected by ELISA beginning 7–9 days after infection but these antibodies have little neutralizing activity. Beginning at about a month after infection, neutralizing antibody can be detected and peaks between 1 and 2 months after infection. Viremia is reduced to very low levels but virus continues to be produced from infected cells in tissues for at least 5 months. Usually, the infection eventually is completely cleared, but in some cases it continues to persist.

In LDV-infected mice, which always become persistently infected, antiviral GP_5 and N antibody that is primarily of the IgG2a subclass is produced as early as 6–10 days after infection. The production of this antibody is dependent on functional T helper cells. Plasma from LDV-infected mice has a much higher nonspecific binding activity than plasma from uninfected mice; virus-specific binding measured by enzyme-linked immunosorbent assay (ELISA) usually cannot be detected until the plasma has been diluted at least 1:400. Some virus neutralization by this early antibody has been demonstrated but is incomplete due to the presence of virus quasispecies that are resistant to neutralization. Anti-LDV antibody that is not complexed to virus can be detected by 15 days after infection, indicating that antibody is present in excess of virus in chronically infected mice. Although the presence of anti-LDV antibodies does not prevent infection of macrophages, it does effectively neutralize neurovirulent LDV strains and so protects motor neurons from becoming infected. LDV-infected mice display a polyclonal humoral response and anti-LDV antibody apparently accounts for only a small portion of this polyclonal response. The mechanism by which LDV

infection activates B cells polyclonally is currently not known, but mice immunized with inactivated virus do not develop a polyclonal response. Autoantibodies to a variety of cellular components (autoimmune antibodies) have been detected in mice chronically infected with LDV.

SHFV isolates that produce acute infections in patas monkeys induce high levels of neutralizing antibody, whereas SHFV isolates that induce persistent infections induce low titers of non-neutralizing antibody. Antibodies to virus that causes acute infection do not cross-neutralize virus that causes persistent infection. In macaques, death from an SHFV infection occurs before an effective adaptive immune response can be elicited.

LDV-infected animals develop cytotoxic T cells that can specifically recognize and lyse virus-infected macrophages. However, this cytotoxic response is not able to clear the infection. Whether anti-LDV cytotoxic T cells persist indefinitely in chronically infected mice or eventually disappear due to clonal exhaustion is disputed. A cytotoxic T-cell response as indicated by IFNγ-producing T cells can be detected in PRRSV-infected pigs starting about a month (the same time that neutralizing antibody appears) after infection and lasts at least a year.

Although the production of neutralizing antibody and cytotoxic T cells is delayed in EAV- and PRRSV-infected animals, these responses are usually effective in clearing the infection. However, in some EAV and PRRSV infections and in all LDV infections, persistent infections develop even though good levels of neutralizing antiviral antibodies and helper and cytotoxic T-cell responses are elicited. The mechanisms by which these viruses evade clearance by the adaptive immune system include extensive glycosylation of the major glycoprotein GP_5 that masks the major neutralization epitope of some strains of the virus, and enhanced infection of macrophages by infectious viral immune complexes via cell surface Fc receptors. Neutralization escape virus variants have been reported to arise during persistent LDV infections and may also arise during persistent infections with other arteriviruses. An immunodominant decoy epitope located just upstream of the neutralization epitope in PRRSV GP_5 induces a strong non-neutralizing antibody response and may be responsible for the weak/absent induction of neutralizing antibodies during the first month of infection.

Prevention and Control

Avirulent and virulent strains of EAV and PRRSV have been isolated. A number of live attenuated vaccines and killed vaccines are commercially available for both EAV and PRRSV. The live vaccines are more efficacious in providing protection and induce a longer-lasting immunity than the killed vaccines. Although these vaccines induce immunity against disease, immunized animals are not completely protected from reinfection. Animals immunized with live vaccines can spread virus and can become persistently infected. Outbreaks of disease due to reversion of live PRRSV vaccines have been reported. To allow discrimination between natural and vaccine infections, markers have been engineered into one EAV live vaccine. Recent vaccine development efforts have focused on the utilization of recombinant virus vectors, such as Venezuelan equine encephalitis virus and pseudorabies, or DNA vectors to express GP_5 or both GP_5 and M.

The current lack of rapid diagnostic assays for the detection of LDV and SHFV in persistently infected animals means that it is still a time-consuming task to identify animals with inapparent infections. Care should be taken not to inadvertently transfer arteriviruses from a persistently infected animal to other susceptible animals. Cells and infectious agent pools obtained from animals that might be persistently infected with an arterivirus should be checked for viral contamination before they are injected into a susceptible animal.

Future Perspectives

Arteriviruses have so far been isolated from mice (LDV), horses (EAV), pigs (LV), and monkeys (SHFV). It seems likely that other host species, including humans, harbor additional members of this virus family. However, such viruses will be difficult to find if the natural hosts develop asymptomatic infections. Little is yet known about the functional roles of the arterivirus proteins in the virus life cycle. Recent studies suggest that the arterivirus nucleocapsid and envelope proteins have unique properties. The intense current interest in dissecting the structure and function of the SARS-coronavirus replicase may provide new insights for similar analyses of the arterivirus replicase. The availability of reverse genetic systems for several of the arteriviruses will not only aid the further molecular characterization of these viruses but will also facilitate the study of viral pathogenesis and antiviral immunity as well as the development of improved vaccines.

Further Reading

Balasuriya UB and Maclachlan NJ (2004) The immune response to equine arteritis virus: Potential lesions for other arteriviruses. *Veterinary Immunology and Immunopathology* 102: 107–129.

Coutelier J-P and Brinton MA (2006) Lactate dehydrogenase-elevating virus. In: Fox JG, Newcomer C, Smith A, Barthold S, Quimby F, and Davidson M (eds.) *The Mouse in Biomedical Research,* 2nd edn., pp. 215–234. San Diego, CA: Elsevier.

Delputte PL, Costers S, and Nauwynck HJ (2005) Analysis of porcine reproductive and respiratory syndrome virus attachment and internalization: Distinctive roles for heparan sulphate and sialoadhesin. *Journal of General Virology* 86: 1441–1445.

Godeny EK, de Vries AAF, Wang XC, Smith SL, and de Groot RJ (1998) Identification of the leader–body junctions for the viral subgenomic

mRNAs and organization of the simian hemorrhagic fever virus genome: Evidence for gene duplication during arterivirus evolution. *Journal of Virology* 72: 862–867.
Gorbalenya AE, Enjuanes L, Ziebuhr J, and Snijder EJ (2006) Nidovirales: Evolving the largest RNA virus genome. *Virus Research* 117: 17–37.
Lopez OJ and Osorio FA (2004) Role of neutralizing antibodies in PRRSV protective immunity. *Veterinary Immunology and Immunopathology* 102: 155–163.
Murtaugh MP, Xiao ZG, and Zuckermann F (2002) Immunological responses of swine to porcine reproductive and respiratory syndrome virus infection. *Viral Immunology* 15: 533–547.
Pasternak AO, Spaan WJM, and Snijder EJ (2006) Nidovirus transcription: How to make sense. . .? *Journal of General Virology* 87: 1403–1421.
Plagemann PGW and Moennig V (1992) Lactate dehydrogenase-elevating virus, equine arteritis virus, and simian hemorrhagic fever virus: A new group of positive-strand RNA viruses. *Advances in Virus Research* 41: 99–192.
Siddell SG, Ziebuhr J, and Snijder EJ (2005) Coronaviruses, toroviruses, and arteriviruses. In: Mahy BWJ and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections, Vol. 1: Virology*, 10th edn., London: Hodder Arnold.
Snijder EJ and Meulenberg JM (1998) The molecular biology of arteriviruses. *Journal of General Virology* 79: 961–979.
Snijder EJ, Siddell SG, and Gorbalenya AE (2005) The order Nidovirales. In: Mahy BWJ and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections, Vol. 1: Virology*, 10th edn., London: Hodder Arnold.
Snijder EJ and Spaan WJM (2007) Arteriviruses. In: Knipe DM, Howley PM, Griffin DE, *et al*. (eds.) *Fields Virology*, 5th edn., pp. 1337–1356. Philadelphia, PA: Lippincott Williams and Wilkins.
Wieringa R, de Vries AAF, van der Meulen J, *et al.* (2004) Structural protein requirements in equine arteritis virus assembly. *Journal of Virology* 78: 13019–13027.
Ziebuhr J, Snijder EJ, and Gorbalenya AE (2000) Virus-encoded proteinases and proteolytic processing in the *Nidovirales*. *Journal of General Virology* 81: 853–879.

Ascoviruses

B A Federici, University of California, Riverside, CA, USA
Y Bigot, University of Tours, Tours, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Apoptosis Genetically programmed cell death.
Apoptotic bodies Cell vesicles resulting from apoptosis.
Caspase Protease that activates a major portion of programmed cell death.
Endoparasitic wasps Species of insect parasites belonging to the order Hymenoptera, which lay their eggs in insects where the wasp larvae develop.
***Per os* infection** Infection by feeding.
Programmed cell death Genetically programmed cascade proteases and nucleases that cleave DNA and proteins within a cell leading to its death.
Reniform Shaped like a kidney.
Transovarial transmission Transmission of virus inside the egg.
Virion-containing vesicles Vesicles containing virions formed by ascoviruses by rescue of apoptotic bodies induced by ascovirus infection.

Introduction

The family *Ascoviridae* is one of the newest families of viruses, established in 2000 to accommodate several species of a newly recognized type of DNA virus that attacks larvae of insects of the order Lepidoptera. Viruses of this family produce large, enveloped virions, measuring 130 nm in diameter by 300–400 nm in length, and when viewed by electron microscopy have a reticulated appearance. They are typically bacilliform or reniform in shape, and contain a circular double-stranded DNA genome that, depending on the species, ranges from ~120 to 185 kbp. Whereas the virions of ascoviruses are structurally complex like those of other large DNA viruses that attack insects, such as those of iridoviruses (family *Iridoviridae*) and entomopoxviruses (family *Poxviridae*), they differ from these in two significant aspects. First, ascoviruses are transmitted from diseased to healthy lepidopteran larvae or pupae by female endoparastic wasps when these lay eggs in their hosts. Second, ascoviruses have a unique cell biology and cytopathology in which shortly after infecting a cell, they induce apoptosis and then rescue the developing apoptotic bodies and convert these into virion-containing vesicles. This aspect of viral reproduction apparently evolved to disseminate virions to the larval blood where they could contaminate the ovipositors of female wasps so that the virus could be transmitted to new hosts. Ascoviruses appear to occur worldwide, wherever there are endoparasitic wasps and larvae of species belonging to the lepidopteran family Noctuidae. However, as these viruses have been discovered relatively recently and their signs of disease are not commonly known in the scientific community, relatively few ascovirus species have been described.

History

The first ascoviruses were discovered during the late 1970s in southern California where they were found causing disease in larvae of moths belonging to the lepidopteran family Noctuidae. Diseased larvae were recognized by the presence of blood that was very white and opaque, in marked contrast to the blood of healthy larvae which is translucent and slightly green (**Figure 1**). The color and opacity of the blood in diseased larvae was shown to be due to the presence of high concentrations of vesicles that contained virions (**Figure 2**). The white blood and virion-containing vesicles are diagnostic for the disease, and the name for this group, ascoviruses (derived from the Greek *asco* meaning 'sac'), was chosen on the basis of the latter characteristic. Since the discovery of the first ascovirus, ascoviruses have been isolated as the cause of disease in many species of noctuid larvae. In addition, an ascovirus that attacks the pupal stage of a species belonging to the family Yponomeutidae was discovered in the 1990s in France.

Distribution and Taxonomy

With respect to distribution, ascoviruses have been reported from the United States, Europe, Australia, and Indonesia, and it is highly probable that they occur worldwide. This is because their most common hosts, larvae of lepidopteran species belonging to the family Noctuidae, the largest family of the order Lepidoptera, as well as their most common vectors, endoparasitic wasps of the families Braconidae and Ichnuemonidae, are distributed throughout the world. Although only a few ascovirus species have been described to date, there are probably many, including variants, that occur worldwide. Thus, given the common occurrence of their hosts and vectors, it is possible that ascoviruses are very common insect viruses. That they have not been discovered in large numbers, for example, like baculoviruses (family *Baculoviridae*), is probably because they cause a chronic disease with few easily detectable signs, making it difficult for individuals not familiar with the disease to recognize diseased larvae in field populations.

At present, five species of ascoviruses are officially recognized based on a combination of properties including the relatedness of key genes coding for the DNA polymerase and major capsid protein, the degree to which their genomic DNA cross-hybridizes under conditions of low stringency, their lepidopteran host range, and their tissue tropism (**Table 1**). The type species is the *Spodoptera frugiperda ascovirus* (SfAV-1a), with the other species being *Tricoplusia ni ascovirus* (TnAV-2a), *Heliothis virescens ascovirus* (HvAV-3a), and *Diadromus pulchellus ascovirus* (DpAV-4a). The Arabic numeral reflects the order in which each species was formally recognized, whereas the lower case letter indicates the type species of the variants. Variants from the type species are recognized by different consecutive lower case numbers; for example, TnAV-2b and 2c would represent two different variants of TnAV-2a recognized subsequently. Herein, these viruses are referred to by their acronyms without the numerical and lower case suffix.

Ascoviruses have been isolated from many more insect species than those listed above, but these isolates have turned out to be variants of known ascoviruses, and therefore they have not been named after the host from which they have been isolated. For example, ascoviruses related to TnAV and HvAV have been isolated from noctuid species such as *Autographa precationis*, *Helicoverpa zea*, *Helicoverpa armigera*, and *Helicoverpa punctigera*; however, they do not bear the name of their host of isolation. What this implies is that ascoviruses belonging to the TnAV and HvAV species have a broad and overlapping host range among different noctuid species, although this has only been tested experimentally to a limited extent.

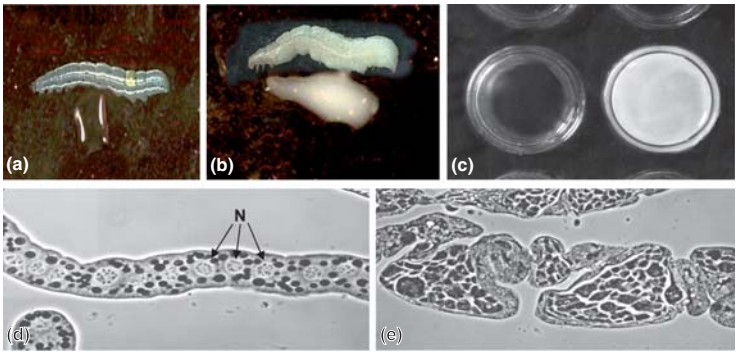


Figure 1 Major characteristics of the disease typically caused by ascoviruses in lepidopteran larvae. (a) and (b) Healthy and ascovirus-infected larvae, respectively, of the cabbage looper, *Trichoplusia ni*, infected with TnAV. Note the opaque white blood in the infected larva. (c) Spot plate containing blood from healthy (left) and infected larvae (right). (d, e) Sections through lobes of fat body from a healthy and infected larva, respectively, of the fall armyworm, *Spodoptera frugiperda*, infected with SfAV ascovirus. Note the greatly hypertrophied cells in the fat body of the infected larva. The cells in most of this tissue have already cleaved into viral vesicles. N, nuclei.

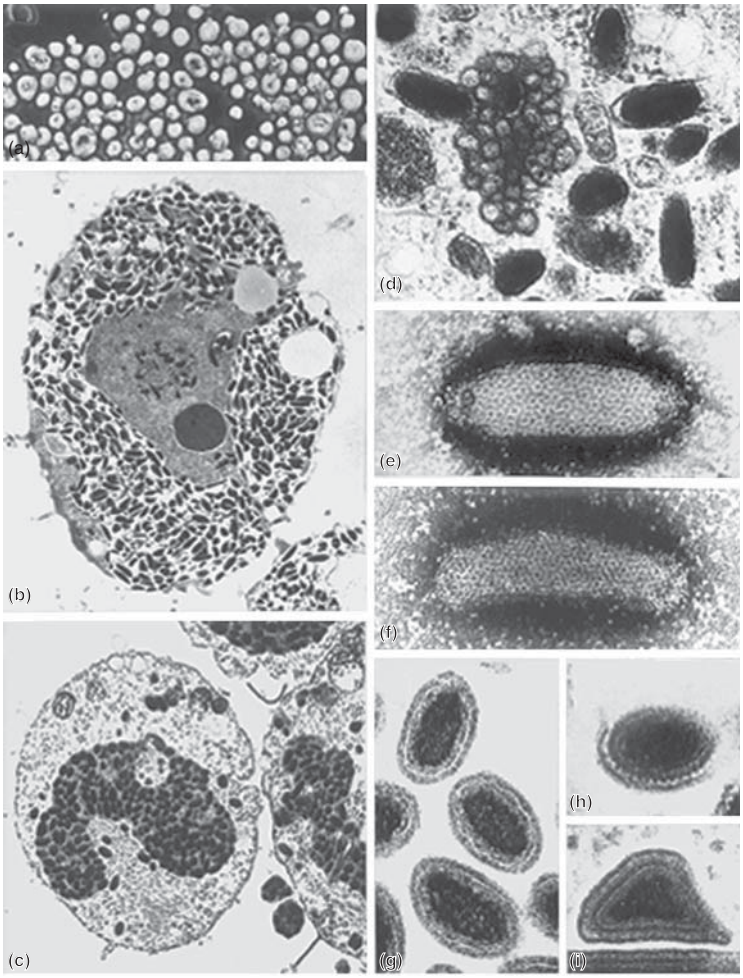


Figure 2 Structural and morphological characteristics of ascovirus virions and virion-containing vesicles. (a) Wet mount preparation viewed with phase microscopy of blood from a *Spodoptera frugiperda* larva infected with SfAV. The spherical refractile bodies are virion-containing vesicles. (b, c) Electron micrographs of ultrathin sections through viral vesicles produced by the trichoplusia (TnAV) and spodoptera (SfAV) ascoviruses, respectively. (d) Matrix of the occlusion body produced by the spodoptera (SfAV) ascovirus. The occlusion consists of virions, protein, and small spherical vesicles. (e, f) Negatively stained virions of the spodoptera (SfAV) and trichoplusia (TnAV) ascoviruses, respectively. Note the reticulate appearance of the virions. (g, h) Electron micrographs of ultrathin cross sections through inner particles of the spodoptera (SfAV) ascovirus after formation (g) and during envelopment (h). (i) Ultrathin cross section through a fully developed virion of the trichoplusia (TnAV) ascovirus.

Table 1 Members in the genus *Ascovirus* belonging to the family *Ascoviridae*

Species name	*Virus abbreviation*	*Accession number*
Recognized species		
Spodoptera frugiperda ascovirus 1a	SfAV-1a	[AM3988432]
Trichoplusia ni ascovirus 2a	TnAV-2a	[AJ312707]
Heliothis virescens ascovirus 3a	HvAV-3a	
Diadromus pulchellus ascovirus 4a	DpAV-4a	[AJ279812]
Tentative species		
Spodoptera exigua ascovirus 5a	SeAV-5a	
Spodoptera exigua ascovirus 6a	SeAV-6a	
Helicoverpa armigera ascovirus 7a	HaAV-7a	
Helicoverpa punctigera ascovirus 8a	HpAV-8a	

Virion Structure and Composition

Depending on the species, the virions of ascoviruses are either bacilliform or reniform in shape, with complex symmetry, and very large, measuring about 130 nm in diameter by 300–400 nm in length. The virion consists of an inner particle surrounded by an outer envelope (**Figure 2**). The inner particle is complex containing a DNA/protein core as well as an apparent internal lipid bilayer surrounded by a distinctive layer of protein subunits. Thus, the virion appears to contain two lipid membranes: one associated with the inner particle and the other forming the lipid component of the envelope. In negatively stained preparations, virions have a distinctive reticulate appearance, which is thought to be due to superimposition of subunits on the surface of the internal particle with those in the envelope.

As indicated by the size and complexity of the virions, the genome of ascoviruses is large, and consists of a single molecule of double-stranded circular DNA. The genomes of three species have been sequenced, the type species SfAV, TnAV, and HvAV. The SfAV genome is 157 kbp and codes for at least 120 proteins (**Figure 3**), whereas the TnAV 2a genome is slightly larger, 174 kbp, and codes for at least 134 proteins. The genome of HvAV is 186 kbp and codes for approximately 180 potential proteins. Based on gel analyses, ascovirus virions contain at least 12 structural polypeptides ranging in size from 12 to 200 kDa. In addition to proteins and the DNA genome, the presence of an envelope as detected by electron microscopy, as well as experiments with detergents and organic solvents, indicate that virions contain a substantial lipid component. And, as in other enveloped viruses of eukaryotes, it is likely that the virion also contains carbohydrate in the form of glycoproteins, though none have been identified.

Transmission and Ecology

One of the most interesting features of ascoviruses is that their transmission from host to host appears to be dependent on their being vectored by female endoparasitic wasps belonging to the families Braconidae and Ichneumonidae (order Hymenoptera). Ascoviruses are extremely difficult to transmit *per os*, with typical infection rates averaging less than 15% even when larvae are fed as many as 10^5 virion-containing vesicles in a single dose. In contrast to this, infection rates for caterpillars injected with as few as 10 virion-containing vesicles are typically greater than 90%. Moreover, experiments with parasitic wasps show that they can effectively transmit ascoviruses to their noctuid hosts. For example, when females are allowed to lay eggs in ascovirus-infected noctuid caterpillars, thereby contaminating their ovipositor, and then allowed to lay eggs in healthy larvae, the majority of the latter contract ascovirus disease. Interestingly, though the parasite eggs hatch in their infected noctuid hosts, the parasite larvae die as the ascovirus disease develops in the caterpillar. Under field conditions, the prevalence of ascovirus disease in caterpillars is correlated with high rates of parasitization by endoparasitic wasps. When wasps from these populations are collected in the field and allowed to oviposit in healthy caterpillars reared in the laboratory, the latter often exhibit ascovirus disease within a few days. Thus, laboratory and field studies provide sound evidence that the primary mechanism for the transmission of ascoviruses attacking noctuid larvae is through being vectored mechanically by parasitic wasps. No evidence has been found in the lepidopteran hosts for transovum or transovarial transmission.

In the case of DpAV, the association of the virus with its wasp and caterpillar hosts is much more intimate. DpAV DNA is carried in wasp nuclei as a circular molecule, and small numbers of virions are produced in the oviducts of females. However, the virus does not cause noticeable pathology in the wasp host. The females lay eggs in the pupal stage of the lepidopteran host, *Acrolepiopsis assectella*, introducing small numbers of ascovirus virions along with the wasp eggs. These virions invade lepidopteran host cells, replicate, and initiate destruction of major host tissues. The wasp larva then emerges from the egg and feeds on the host tissues and ascovirus virions. The DpAV genome is carried by both male and female wasps, where it is apparently transmitted from generation to generation transovarially. These observations make ascoviruses the only known group of viruses pathogenic to insects primarily dependent on vectors for their transmission.

Now that the characteristics of the disease are known, field studies in the southeastern United States and California are beginning to show that ascoviruses are probably the most common type of virus to occur during most of the year in populations of several important noctuid pests, including the cabbage looper, *T. ni.*, fall armyworm, *S. frugiperda*, and the corn earworm, *H. zea.* Prevalence rates range from 10% to 25%, depending on the species and time of the year, with the highest rates of infection, as noted above, being correlated with high levels of parasitization. In South Carolina, ascovirus infection rates as high as 60% have been reported in populations of noctuid larvae at the end of summer.

Host Range

The experimental host range of ascoviruses varies with the viral species. TnAV, HvAV, and SeAV have a broad host range and are capable of replication in a variety of noctuid

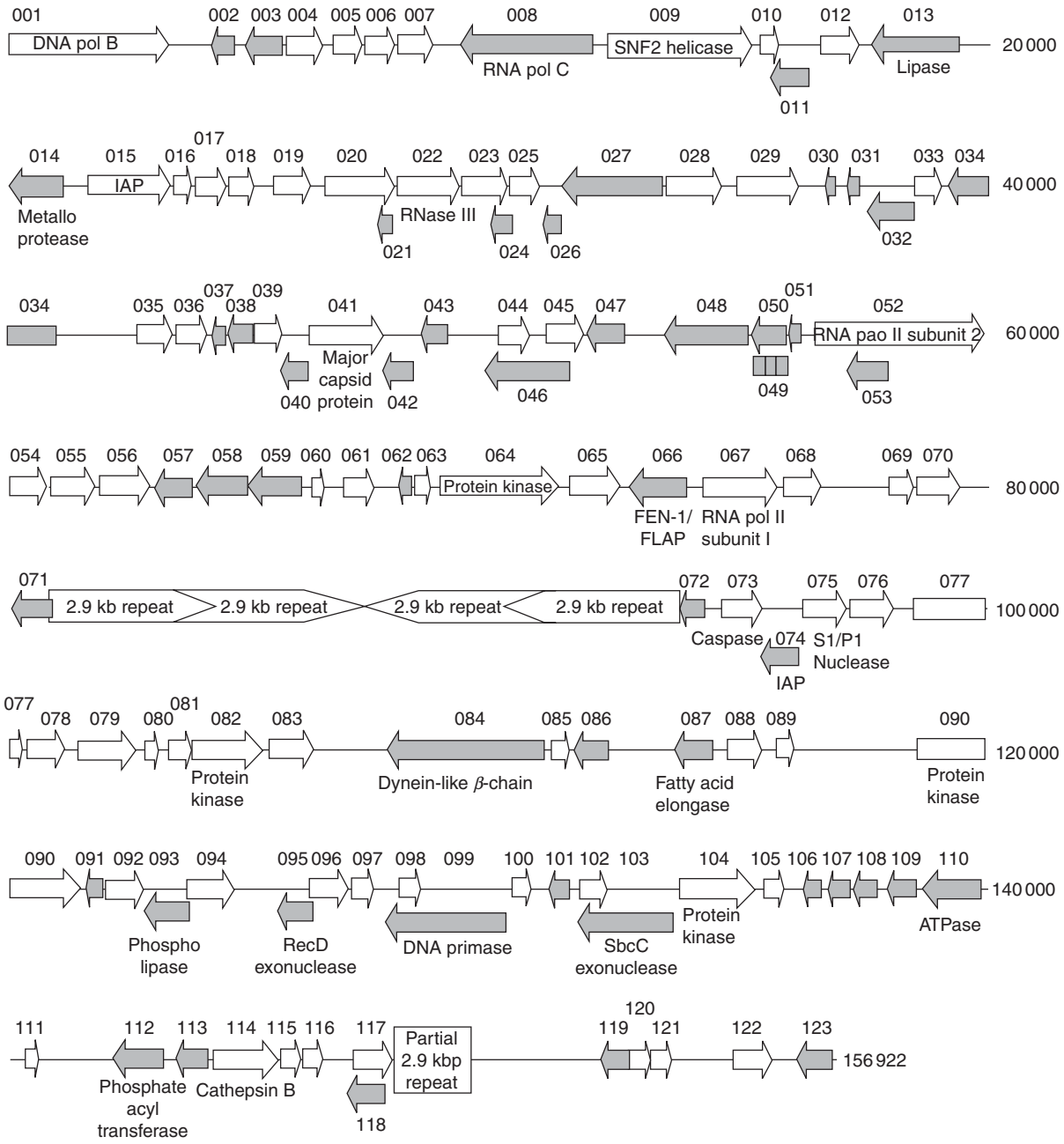


Figure 3 Schematic illustration in linear form of the circular DNA 156 922 base pair genome of the SfAV 1a ascovirus, the ascovirus type species, isolated from a larva of the fall armyworm, *Spodoptera frugiperda*. The illustration identifies the relative positions of key genes including those coding for the DNA polymerase, major capsid protein, executioner caspase, as well as several genes coding for proteins involved in lipid metabolism. White and dark arrows represent, respectively, ORFs in forward and reverse orientations along the genome. From Bideshi DB, Demattei MV, Rouleux-Bonnin F, *et al*. (2006) Genomic sequence of spodoptera frugiperda ascovirus 1a, an enveloped, double-stranded DNA insect virus that manipulates apoptosis for viral reproduction. *Journal of Virology* 80: 11791.

species, as well as in selected species belonging to other families of the order Lepidoptera. Alternatively, the experimental host range of SfAV is limited to other species of the genus *Spodoptera*. DpAV can replicate in hymenopteran and lepidopteran hosts closely related to its natural host species, *A. assectella*. To propagate virus in the laboratory, all ascoviruses can be grown in their larval or pupal hosts. To infect caterpillars, they are injected with virus in the fourth or early fifth instar, and virion-containing vesicles are harvested from the blood 5–7 days later.

Pathology and Pathogenesis

Signs of Disease

The signs of ascovirus disease are very subtle, and this probably accounts for why ascoviruses were discovered only recently. The most obvious sign of disease within 24 h of infection is a decrease in the normal rate of feeding. The feeding rate continues to slow as the disease progresses, and as a result larvae fail to gain weight or advance in development. Healthy larvae, particularly in the early stages of development, will easily quadruple their weight and size in a period of 3 or 4 days, whereas ascovirus-infected larvae cease to grow and may actually lose weight. This feature of ascovirus disease is almost impossible to detect in infected larvae in the field. However, it is easily noticed under laboratory conditions when infected and healthy larvae are reared side by side over a period of a few days. A second feature easily noted in the laboratory is that ascovirus diseases are chronic, though usually fatal. When infected during early stages of development, ascovirus-diseased larvae often survive for 2 or 3 weeks beyond the time at which most healthy larvae have completed their development and pupated. Signs of disease other than these are minor, but include the inability to completely cast the molted cuticle, a bloated thoracic region, and a white or creamy discoloration and hypertrophied appearance of the larval body at advanced stages of disease development.

Cytopathology and Cell Biology

In comparison to all other known viruses, the most unique property of ascoviruses is the unusual cytopathology that leads to the formation of the virion-containing vesicles. This process resembles apoptosis, and recent studies of the SfAV genome have shown that it encodes an executioner caspase, synthesized 9 h after infection, which by itself is capable of inducing apoptosis (**Figure 4**).

At the cellular level *in vivo*, the disease begins with extraordinary hypertrophy of the nucleus accompanied by invagination of sections of the nuclear envelope, followed by a corresponding enlargement of the cell. Cells typically grow from 5 to 10 times the diameter of uninfected cells. As the nucleus enlarges, the nuclear envelope ruptures and disintegrates into fragments. At about this stage, the cell plasmalemma begins to invaginate along 'planes' toward the now anucleate cell center. Concomitantly, sheets of membrane form closely adjacent to mitochondria that accumulate along the planes. As this process continues, the membrane sheets coalesce and join the invaginating plasmalemma, thereby cleaving the cell into a cluster of 20 to more than 30 vesicles, ranging in size from 5 to 10 µm in diameter. This aspect of ascovirus cellular pathology resembles the formation of apoptotic bodies during apoptosis. However, rather dissipate as the cell dies, the developing apoptotic bodies are rescued by the virus and progress to form vesicles in which virions continue to assemble. These virion-containing vesicles, also referred to as viral vesicles, typically remain in the tissue until the basement membrane ruptures, though on occasion cell hypertrophy can be so great that the enlarging cell erupts out through the basement membrane of the infected tissue, releasing large fragments of the infected cell directly into the blood. Analysis of both the SfAV and TnAV genomes shows that, unlike many other large DNA viruses, ascoviruses encode several lipid-metabolizing enzymes that are likely involved in the process of converting developing apoptotic bodies into virion-containing vesicles.

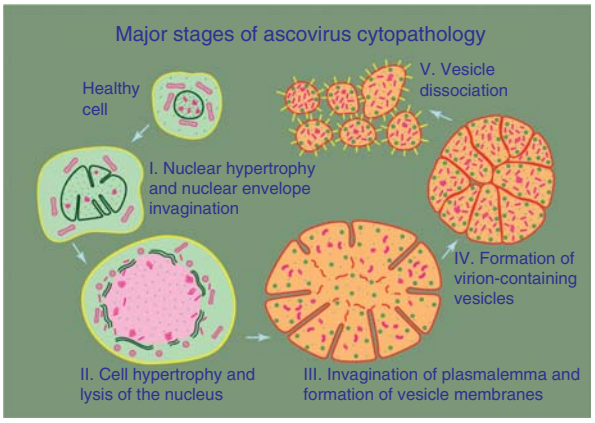


Figure 4 Major stages of cellular pathogenesis caused by a typical ascovirus, a process that resembles apoptosis. After infection, the nucleus enlarges and the nuclear membrane invaginates, and then lyses. Subsequently, the plasma lemma of the cell invaginates and coalesces with cytoplasmic membranes, apparently formed *de novo*, thereby dividing the cell into a cluster of virion-containing vesicles. These vesicles dissociate and are liberated into the blood as the basement membrane of infected tissues degenerates. Virion assembly becomes apparent as the nuclear membrane lyses, and continues throughout all subsequent stages of vesicle formation.

Although the process by which viral vesicles are cleaved from cells varies among different ascoviruses, the histopathology is similar among virtually all viruses. Vesicles accumulate in the tissues where they are formed, but as these tissues degenerate during disease progression, the basement membrane of infected tissues deteriorates and ruptures, allowing the vesicles to spill out into the blood. There they accumulate reaching concentrations as high as 10^7–10^8 vesicles ml^{-} within 3–4 days of infection. There is some evidence that viral replication proceeds within the vesicles as they circulate in the blood, and thus this tissue must also be considered one of the tissues attacked by ascoviruses. If fact, because such high concentrations of viral vesicles are found in the blood, this tissue

could be considered a major site of infection, particularly if it is eventually shown that these viruses continue to replicate in the vesicles as they circulate in the blood.

Despite the chronic nature of the disease caused by ascoviruses, virion-containing vesicles are present in the blood within 2 or 3 days of infection. When the virus replicates in cells *in vitro*, the vesicles are formed within 12–16 h of infection. The rapid development and circulation of the viral vesicles in the blood probably evolved to enhance transmission of the virus by parasitic wasps.

Tissue Tropism

The cytopathology of ascoviruses is consistent among different viral species; however, considerable variation occurs with respect to the tissues attacked, that is, in which replication occurs. TnAV, HvAV, and SeAV exhibit a relatively broad tissue tropism infecting the tracheal matrix, epidermis, fat body, and connective tissue. Differences exist between these species in that some HvAV variants infect the epidermis much more extensively than TnAV variants, whereas some of the latter can also replicate more extensively in fat body cells, but appear only to do this when larvae are infected early in their development. Alternatively, the type species, SfAV, and its variants have a very narrow tissue tropism, with the fat body being the primary site of infection. DpAV occurs in the nuclei of all tissues of its wasp host, but appears to only produce progeny in ovarial tissues. In its lepidopteran pupal host, it attacks and replicates in a wide variety of tissues.

Replication and Virion Assembly

Although there have been few biochemical studies of viral DNA replication or protein synthesis, studies carried out with ascoviruses *in vivo* and *in vitro* show that progeny virions first appear about 12 h after infection. Virion assembly is initiated after the nucleus ruptures, and occurs prior to and during the cleavage of the cell into viral vesicles. The first recognizable structural component of the virion to form is the multilaminar layer of the inner particle. Based on its ultrastructure, this layer consists of a unit membrane and an exterior layer of protein subunits. As the multilaminar layer assembles, a dense nucleoprotein core aggregates on the interior surface. This process continues until the inner particle is complete. After formation, the inner particle is enveloped by membranes within the cell or vesicle. These membranes are apparently synthesized *de novo*. Thus, the assembly of the virions is reminiscent of that in other viruses with complex virions, such as the ridoviruses, herpesviruses, and poxviruses, where the virions differentiate after association of the precursors of virion structural components.

After formation, the virions of the TnAV ascovirus accumulate toward the periphery of the vesicle where they often form inclusion bodies, that is, aggregations of virions (**Figure 2**). In SfAV, occlusion bodies are formed in which the virions are actually occluded in a 'foamy' vesicular matrix that consists of a mixture of protein and minute spherical vesicles. When viewed with phase microscopy, these viral inclusion and occlusion bodies are phase bright, and are largely responsible for the highly refractile appearance of the vesicles. Ascoviruses do not typically form the types of occlusion bodies characteristic of other types of DNA insect viruses, such as baculoviruses and entomopoxviruses.

Origin and Evolution

The subject of viral evolution over millions of years has received relatively little study due to the lack of a fossil record. Moreover, viruses are considered polyphyletic, and thus most of the more than 70 families of viruses are thought to have originated independently. In this regard, ascoviruses may provide a unique opportunity to obtain insights into virus evolution over long periods. Phylogenetic comparisons of ascovirus genes sequenced to date including those coding DNA polymerase and major capsid protein as well as several enzymes indicate that these viruses evolved from a lepidopteran iridovirus (family *Iridoviridae*). Iridoviruses, in turn, appear to have originated from phycodnavirsues (family *Phycodnaviridae*), which attack certain ciliates and algae. On the other end of the evolutionary scale, ascovirus virions are structurally and morphologically similar to the particles formed by ichnoviruses of the family *Polydnaviridae*. Ichnovirus particles are produced in the reproductive tracts of endoparastic wasps of the family Ichneumonidae, and the wasp vector and host of DpAV is a member of this family. Thus, there is a reasonable possibility that ascoviruses and ichnoviruses are related phylogenetically, and share a common ancestor. This possibility is currently under investigation, and should be resolved over the next several years through a comparative analysis of the molecular evolution of genes of ascoviruses and ichnoviruses, after more structural genes from the latter viruses have been cloned and sequenced. A major question to be addressed is whether the DpAV represents an early ascovirus branch that evolved from an iridovirus or is representative of an ascovirus branch that eventually led to the origin of ichnovirus particles. With respect to the ichnoviruses and bracoviruses, recent data on the DNA contained by the particles of these putative viruses suggest that these are not viruses after all, but rather are an unusual highly evolved type of organelle that evolved from DNA viruses, which are used by endoparasitic wasps to suppress the internal defense responses of their insect hosts.

Future Perspectives

As present, too little is known about ascoviruses to assess whether they are or will turn out to be of economic importance. Their poor infectivity *per os* makes it highly unlikely they will ever be developed as viral insecticides, especially given the successful advent of insect-resistant transgenic crops. However, as more entomologists become familiar with the disease caused by ascoviruses, it may be shown that in habitats rarely treated with chemical insecticides, such as transgenic crops, these viruses are responsible for significant levels of natural pest suppression, particularly where parasitic wasps are abundant. Such findings would encourage even greater emphasis on the development of biological control and other more environmentally sound methods of pest control. With respect to the cell biology of viral vesicle formation, ascoviruses provide an interesting model for how apoptosis can be manipulated at the molecular level. Additionally, study of the unusual process by which ascoviruses rescue the developing apoptotic bodies to form viral vesicles could lead to insights into how cells manipulate the cytoskeleton and mitochondria. Finally, it is possible that viral vesicles will provide a unique anucleate cellular system for studying the replication of a complex type of enveloped DNA virus *in vitro*.

See also: Baculoviruses: Apoptosis Inhibitors; Baculoviruses: General Features; Entomopoxviruses; Phycodnaviruses; Polydnaviruses: General Features.

Further Reading

Asgari S (2006) Replication of Heliothis virescens ascovirus in insect cell lines. *Archives of Virology* 151: 1689.

Asgari S, Davis J, Wood D, Wilson P, and McGrath A (2007) Sequence and Organization of the Heliothis virescens ascovirus genome. *Journal of General Virology* 88: 1120–1132.

Bideshi DB, Demattei MV, Rouleux-Bonnin F, *et al.* (2006) Genomic sequence of spodoptera frugiperda ascovirus 1a, an enveloped, double-stranded DNA insect virus that manipulates apoptosis for viral reproduction. *Journal of Virology* 80: 11791.

Bideshi DB, Tan Y, Bigot Y, and Federici BA (2005) A viral caspase contributes to modified apoptosis for virus transmission. *Genes and Development* 19: 1416.

Bigot Y, Rabouille A, Doury G, *et al.* (1997) Biological and molecular features of the relationships between Diadromus pulchellus ascovirus, a parasitoid hymenopterna wasp (*Diadromus pulchellus*) and its lepidopteran host, *Acrolepiopsis assectella*. *Journal of General Virology* 78: 1149.

Cheng XW, Wang L, Carner GR, and Arif BM (2005) Characterization of three ascovirus isolates form cotton insects. *Journal of Invertebrate Pathology* 89: 193.

Federici BA (1983) Enveloped double-stranded DNA insect virus with novel structure and cytopathology. *Proceedingsof the National Academy of Sciences, USA* 80: 7664.

Federici BA and Bigot Y (2003) Origin and evolution of polydnaviruses by symbiogenesis of insect DNA viruses in endoparasitic wasps. *Journal of Insect Physiology* 49: 419.

Federici BA, Bigot Y, Granados RR, *et al.* (2005) Family *Ascoviridae*. In: Fauquest CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 269–274. San Diego, CA: Elsevier Academic Press.

Federici BA and Govindarajan R (1990) Comparative histopathology of three ascovirus isolates in larval noctuids. *Journal of Invertebrate Pathology* 56: 300.

Federici BA, Vlak JM, and Hamm JJ (1990) Comparison of virion structure, protein composition, and genomic DNA of three ascovirus isolates. *Journal of General Virology* 71: 1661.

Govindarajan R and Federici BA (1990) Ascovirus infectivity and the effects of infection on the growth and development of Noctuid larvae. *Journal of Invertebrate Pathology* 56: 291.

Pellock BJ, Lu A, Meagher RB, Weise MJ, and Miller LK (1996) Sequence, function, and phylogenetic analysis of an ascovirus DNA polymerase gene. *Virology* 216: 146.

Stasiak K, Renault S, Demattei MV, Bigot Y, and Federici BA (2003) Evidence for the evolution of ascoviruses from iridoviruses. *Journal of General Virology* 84: 2999.

Wang L, Xue J, Seaborn CP, Arif BM, and Cheng XW (2006) Sequence and organization of the Trichoplusia ni ascovirus 2c (*Ascoviridae*) genome. *Virology* 354: 167.

Assembly of Viruses: Enveloped Particles

C K Navaratnarajah, R Warrier, and R J Kuhn, Purdue University, West Lafayette, IN, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Core-like particle (**CLP**) Subviral particles assembled from recombinantly expressed capsid protein are referred to as core-like particles. They are morphologically similar to authentic cores isolated from viruses or infected cells.

Cryo-EM Cryo-electron microscopy and image reconstruction techniques are used to elucidate the structures of viruses and other macromolecular structures.

Immature virion Viruses usually produce noninfectious particles which require a maturation step in order to form the infectious, mature virus. The

maturation step often involves the proteolytic cleavage of a precursor protein.
Nucleocapsid core (**NC**) Capsid protein packages the nucleic acid genome to form a stable protein–nucleic acid complex which is then enveloped with a lipid bilayer.

Introduction

Viruses have long been distinguished by their physical features, usually visualized by electron microscopy or analyzed biochemically. One feature that has been frequently used to categorize viruses is the presence or absence of a lipid bilayer. Many animal viruses are surrounded by a lipid bilayer that is acquired when the nucleocapsid buds through cell membranes, usually at a late stage of virus assembly. While the protein coat of nonenveloped viruses plays a crucial role in protecting the genome from the environment, for enveloped viruses the lipid membrane partially fulfills this role. The lipid membranes are decorated with virus-encoded envelope proteins that are important for the subsequent infectivity of the virus, although some viruses also incorporate cellular proteins in their membrane. Virus envelopment can take place after the assembly of an intact nucleocapsid structure (betaretroviruses) or capsid assembly and envelopment can occur concomitantly (orthomyxovirus). Specific or nonspecific interactions between the viral envelope glycoproteins and the proteins that make up the nucleocapsid mediate the envelopment of the core or nucleoprotein–nucleic acid complex. Enveloped viruses acquire their lipid bilayer from a variety of locations within the cell, but a given virus will usually bud from one specific cellular membrane (**Table 1**). Enveloped viruses can take advantage of the cellular secretory pathway in order to assemble and bud out of the cell. In contrast, nonenveloped viruses usually exit infected cells by disrupting the plasma membrane. Thus, budding provides enveloped viruses with a nonlytic method of exiting infected cells, and they must do so while the cell is still alive.

Viral Envelope

The main component of the viral envelope is the host-derived lipid bilayer. The precise composition of this lipid membrane varies, as different viruses acquire their envelopes from different cellular membranes. The choice of membrane from which the virus buds is often determined by the specific targeting and accumulation of the envelope proteins at a particular site in the secretory pathway (**Table 1**). There are examples of viruses that bud from the plasma membrane (togaviruses, rhabdoviruses, paramyxoviruses, orthmyxoviruses, and retroviruses), endoplasmic reticulum (ER) (coronaviruses and flaviviruses) and the Golgi complex (bunyaviruses). There are also examples of viruses that undergo transient envelopment and reenvelopment (herpesvirus).

Table 1 List of enveloped virus families and the origin of the envelope

Virus family[a]	*Membrane*
Arenaviridae	Plasma membrane
Arterivirus	Endoplasmic reticulum
Asfarviridae	Endoplasmic reticulum and plasma membrane
Baculoviridae	Plasma membrane
Bunyaviridae[b]	Golgi complex
Coronaviridae	ER Golgi intermediate compartment
Cystoviridae[c]	Plasma membrane
Deltavirus	Endoplasmic reticulum
Filoviridae	Plasma membrane
Flaviviridae	Endoplasmic reticulum
Fuselloviridae[d]	Plasma membrane
Hepadnaviridae	Endoplasmic reticulum
Herpesviridae	Nuclear envelope
Hypoviridae	Plasma membrane
Iridoviridae	Plasma membrane
Lipothrixviridae[d]	Plasma membrane
Orthomyxoviridae	Plasma membrane
Paramyxoviridae	Plasma membrane
Plasmaviridae[c]	Plasma membrane
Polydnaviridae	Plasma membrane
Poxviridae	ER Golgi intermediate compartment
Retroviridae	Plasma membrane
Rhabdoviridae[b]	Plasma membrane
Togaviridae	Plasma membrane

[a]Animal viruses unless otherwise noted.
[b]This group also includes plant viruses.
[c]Bacteriophage.
[d]Archaea.

Viral proteins are found embedded in the lipid membrane. The majority of these proteins are transmembrane glycoproteins. The viral envelope glycoproteins mediate the interaction of the virus with cell receptors and promote the fusion of the viral and cellular membranes during infection of susceptible cells. Viral glycoproteins are also crucial for the assembly of the virion. They can make important lateral contacts with each other, thus driving oligomerization and also capturing other viral components such as the capsid or matrix protein. The majority of enveloped viruses contain one or more glycoproteins that are usually found as oligomers embedded within the lipid membrane. High-resolution structural information is available for many glycoproteins such as the hemagglutinin and neuraminidase proteins of influenza A virus, the gp120 of HIV, and the E protein of dengue virus. Based on these structural and biochemical studies, it has been shown that most glycoproteins are primed for the conformational changes that are required in order to gain entry to the host cell during an infection.

Some enveloped viruses contain integral membrane proteins that have multiple membrane-spanning regions that oligomerize to form channels in the membrane. The influenza A virus M2 protein forms an ion channel and plays an important role in the assembly and entry of the virus particle. Some viruses such as the retroviruses also incorporate cellular membrane proteins into the viral envelope. In a majority of the cases the host proteins that are present at the sites of assembly or budding are incorporated in a passive, nonselective manner. However, there are examples where the virus actively recruits specific host proteins that may help in evading the defenses of the immune system or enhance infectivity.

Icosahedral Enveloped Viruses: Alphaviruses and Flaviviruses

Alphaviruses, and more recently, flaviviruses have served as model systems to study the assembly and budding of simple enveloped viruses. These positive-strand RNA viruses consist of a single RNA genome that is encapsidated by multiple copies of a capsid protein to form the nucleocapsid core (NC). The envelopment of the NC is mediated by the interaction between the envelope glycoproteins and this core. The assembly and budding of these two simple enveloped viruses will be described in detail in order to present common themes in the assembly of icosahedral enveloped viruses.

Alphavirus Assembly

Alphavirus life cycle

Alphaviruses are members of the family *Togaviridae*, which also includes the genus *Rubivirus*. Alphaviruses enter the host cell by receptor-mediated endocytosis via the clathrin-coated endocytic pathway. Following fusion at low pH with the endosomal membrane, the NC is released into the cytoplasm. The NC has been proposed to uncoat by transfer of capsid proteins (CPs) to ribosomes. This releases the genome RNA into the cytoplasm which is translated to produce the nonstructural proteins. The nonstructural proteins transcribe a negative-sense copy of the genome RNA. This RNA serves as template for genomic and subgenomic RNA. The subgenomic RNA, which is synthesized in greater amounts than the genomic RNA, codes for the structural proteins of the virus. CP is found at the N-terminus of the structural polyprotein, followed by proteins PE2 (E3+E2), 6K, and E1. Two hundred and forty copies of CP, E2, and E1 assemble to form the alphavirus virion (**Figure 1(a)**). The transmembrane E1 glycoprotein functions during entry to mediate the fusion of the viral membrane with that of the endosomal membrane, while the transmembrane E2 glycoprotein is responsible for cell receptor binding. E1 and PE2, a precursor of E2 and E3, are processed together as a heterodimer in the ER and Golgi, and are transported to the cell surface in the form of spikes that are each composed of three heterodimers of E1/E2. E3 serves as a chaperone to promote the correct folding of E2, as well as to prevent the premature fusion of E1 in the acidic environment of the late Golgi. The maturation cleavage of PE2 to generate E3 and E2 by a furin-like protease in a late Golgi compartment primes the glycoprotein spike complex for subsequent fusion during virus entry. The function of 6K is unclear but it does promote infectivity of the particle. A single copy of the genome RNA is packaged by 240 copies of the CP to form an icosahedral NC in the cytoplasm of infected cells. The NC interaction with the E1/E2 trimeric spikes at the plasma membrane results in the budding of the mature virus from the cell membrane.

Alphavirus virion structure

Cryo-electron microscopy (cryo-EM) and image reconstruction techniques have revolutionized the understanding of the molecular architecture of alphaviruses (**Figure 1(a)**). Studies with Ross River, Semliki Forest, Venezuelan equine encephalitis, Aura, and Sindbis have shown that these viruses consist of an outer protein layer made up of the glycoproteins E1 and E2 (**Figures 1(a)** and **1(c)**). The membrane spanning regions of these glycoproteins traverse a host-derived lipid bilayer that surrounds the NC of the virus. The CP and glycoprotein layers interact with one another and are arranged symmetrically in a $T=4$ icosahedral configuration. Fitting of the atomic coordinates of the crystal structures of the ectodomain of E1 and amino acids 106–264 of the CP into the cryo-EM density of Sindbis virus allowed a pseudo-atomic model of the virus to be generated. The fitting of the E1 structure into the cryo-EM density reveals that E1 forms an icosahedral scaffold on the surface of the viral membrane. E1 is positioned almost tangential to the lipid bilayer, whereas E2 has a more radial arrangement. The bulk of E2 lies on top of E1 and caps the fusion peptide, thereby preventing premature fusion with cell membranes. This arrangement of the glycoproteins is in agreement with the function of each protein, where the surface-exposed E2 interacts with cellular receptors and protects E1 until it is required for fusion. The fusion peptide is only exposed when the E1–E2 heterodimer dissociates in the presence of low pH in the endosome. Fitting of amino acids 106–264 of the CP into the cryo-EM density of Sindbis virus showed that each subunit of the projecting pentamers and hexamers (known as capsomeres) observed in the NC layer is made up of the CP protease domain consisting of amino acids 114–264. There is very little interaction between amino acids 114–264 of the CP either within the capsomere or in between capsomeres. Thus, the major contributors to the stability of the NC in the absence of glycoproteins are

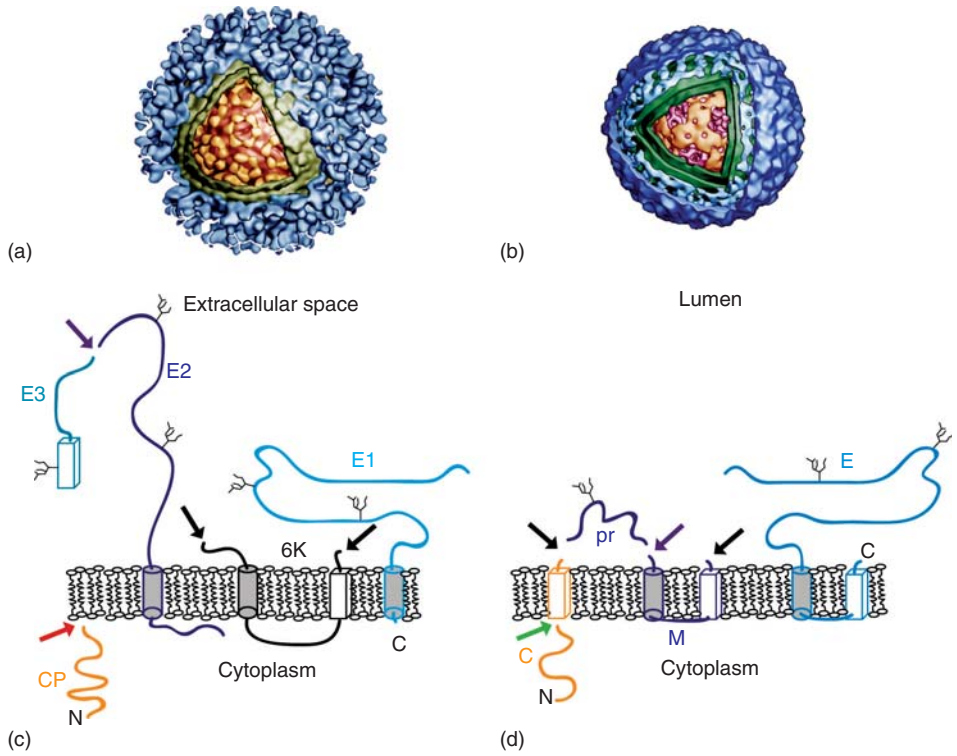


Figure 1 Cryo-EM reconstructions and glycoprotein topology of alphaviruses and flaviviruses. (a) Surface shaded view of a Cryo-EM reconstruction of Ross River virus (alphavirus). The glycoprotein envelope shown in blue has been cut away to reveal the lipid bilayer (green) and the nucleocapsid core (orange). (b) Cryo-EM reconstruction of dengue virus (flavivirus). The glycoprotein envelope shown in blue has been cut away to reveal the lipid bilayer (green) and the nucleocapsid core (orange/purple). (c) The topology of the alphavirus glycoproteins is shown. The rectangular cubes represent signal sequences while the cylinders represent stop transfer sequences. The glycosylation sites are represented by branched structures. The arrows indicate cleavage sites. The black arrows indicate signalase cleavage sites, the purple arrow indicates the furin cleavage site, and the red arrow indicates the CP cleavage site. (d) The topology of the flavivirus structural proteins is shown. The rectangular cubes represent signal sequences while the cylinders represent stop transfer sequences. The glycosylation sites are represented by branched structures. The arrows indicate cleavage sites. The black arrows indicate signalase cleavage sites, the purple arrow indicates the furin cleavage site, and the green arrow indicates the NS2B/NS3 (viral protease) cleavage site.

CP–RNA and RNA–RNA interactions that take place in the RNA–protein layer below the projecting capsomeres.

Alphavirus assembly and budding

Alphavirus virions always contain an NC and it is likely that this promotes and is required for budding through direct interactions with the glycoproteins. Thus, the first step in assembly is for the alphavirus CP to specifically recognize and encapsidate the genome RNA to form NCs in the cytoplasm of infected cells (**Figure 2**). The N-terminus of the CP (amino acids 1–80, SINV numbering) is largely basic and thought to be involved in charge neutralization of the genome RNA. Amino acids 38–55 are conserved uncharged residues that form a coiled-coil alpha-helix (helix I) important in dimerization of the CP during the assembly process. While the process of virus assembly is difficult to study in the complex cellular environment, the development of an *in vitro* assembly system based on bacterial expression of CP has led to advances in understanding NC assembly. These studies suggest that the initial event of NC assembly is the binding of CP amino acids 81–112 to the encapsidation signal on the genome RNA corresponding to nucleotides 945–1076 (SINV numbering) (**Figure 2**). This interaction exposes a second site on the encapsidation signal where another molecule of CP binds and forms a dimer with the first CP molecule. Amino acids 114–264 constitute the previously mentioned chymotrypsin-like serine protease that autoproteolytically cleaves CP from the nascent structural polyprotein. This region is involved in binding residues from the cytoplasmic domain of E2, thus linking the outer icosahedral glycoprotein shell with the NC across the lipid bilayer (**Figure 1(a)**).

Other lines of evidence support the dimer model of NC assembly. Helix I of CP, which is required for core accumulation in infected cells, may be functionally substituted by a GCN4 helix that forms dimeric coiled–coil interactions but not by a GCN4 helix that has a propensity to form trimeric coiled–coil interactions. In addition, helix I acts as a checkpoint in NC assembly whereby incompatible helices

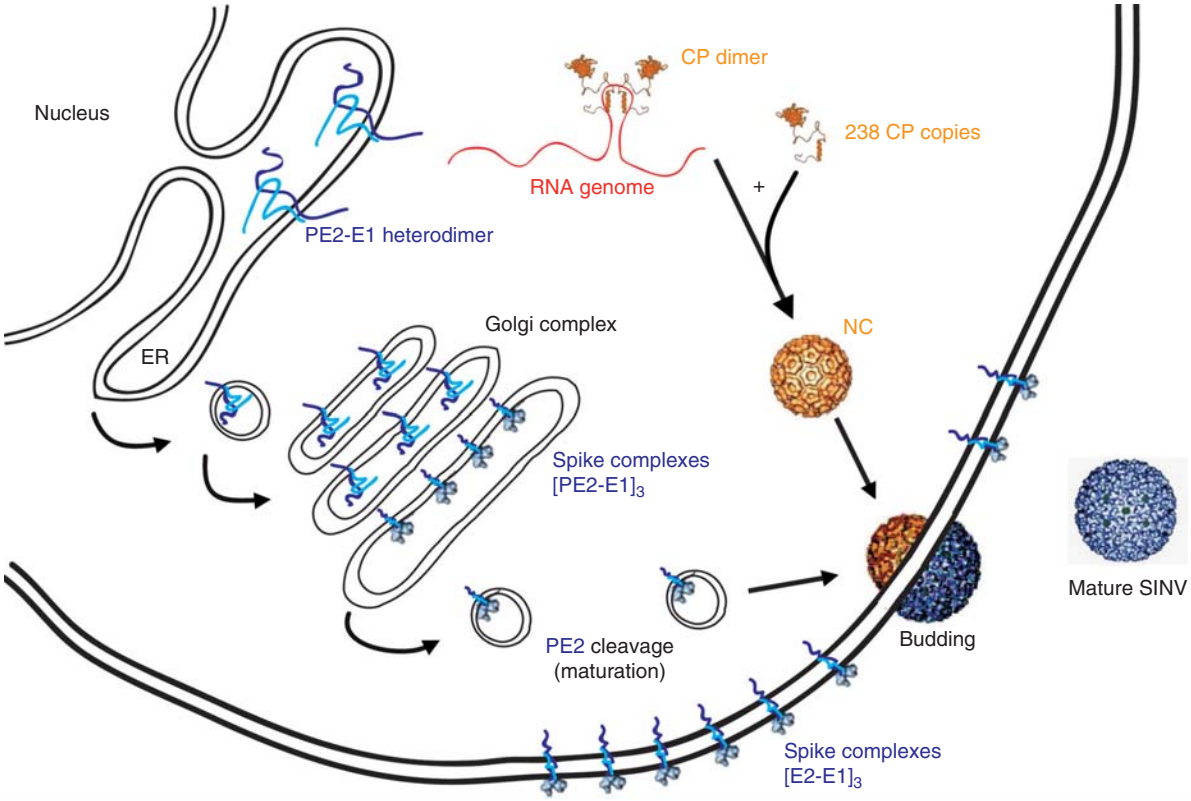


Figure 2 Alphavirus assembly. Two capsid proteins (CPs) bind the encapsidation signal of the genome RNA to form a CP dimer-RNA complex in the cytoplasm. The CP dimer is stabilized by coiled–coil helix I interactions. The subsequent steps of nucleocapsid core assembly have not been elucidated but cores form and accumulate in the cytoplasm. The glycoproteins PE2 and E1 form heterodimers in the endoplasmic reticulum (ER). The glycoproteins are folded, glycosylated, and palmitoylated as they are transported through the ER and Golgi. The PE2–E1 heterodimers form spike complexes $[PE2\text{-}E1]_3$ in the Golgi. E3 is cleaved from the spikes by a furin-like protease before they are transported to the plasma membrane. The NC interacts with the cytoplasmic domain of E2 in the spike complexes, driving the budding of the mature virus from the plasma membrane.

prevent the formation of core-like particles (CLPs) *in vitro*. Furthermore, a portion of CPs in either NCs or CLPs may be cross-linked into dimers by DMS, a lysine specific cross-linker with a 12 Å cross-linking distance. Cross-linking enabled an assembly deficient helix mutation of CP to assemble into NCs, suggesting that the cross-link can functionally replace the helix interaction.

While assembly of the CP into NCs proceeds in the cytoplasm, the processing and assembly of the glycoproteins occur in the ER and Golgi (**Figures 1(c)** and **2**). The autocatalytic cleavage of the CP reveals a signal sequence on the N-terminus of the newly cleaved structural polyprotein that directs it to the ER (**Figure 1(c)**). PE2 is translocated into the ER until it reaches a 26-amino-acid stop transfer signal which anchors PE2 in the membrane. The C-terminal 33 residues of E2 then act as a second signal sequence to direct the next protein, 6K (55 amino acids), into the ER. 6K possesses a stop transfer sequence which anchors it in the membrane, and the C-terminus of 6K acts as signal sequence for the translocation of E1. E1 is anchored in the ER membrane by a final stop transfer sequence close to its C-terminus. The release of PE2 and E1 by cellular signalase cleavage allows the formation of PE2-E1 heterodimers in the ER (**Figure 2**). PE2 and E1 are each glycosylated in all alphaviruses, but the number and location of the modifications vary. In addition to glycosylation, the glycoproteins are palmitoylated in the Golgi apparatus. As the heterodimers are processed and transported through the ER and Golgi, they undergo a series of folding intermediates that are mediated by disulfide exchange and chaperones. Ultimately, they associate to form spikes which are composed of trimers of PE2-E1 dimers (**Figure 2**).

The final maturation event is the cleavage of PE2 into E3 and E2 by a furin-like protease (**Figure 2**). This cleavage occurs in a late Golgi or post-Golgi compartment and results in the destabilization of the heterodimer enabling the mature virus to fuse more readily with the target membrane. In most alphaviruses including Sindbis, E3 is released and not found in the mature virion. The final destination for the spike complexes is the plasma membrane, where the cytoplasmic domain of E2 (cdE2) recruits NCs assembled in the cytoplasm (**Figure 2**). Structural studies show that cdE2 residues Tyr400

and Leu402 bind into a hydrophobic pocket of the CP. Mutation of Tyr400 negatively impacted virus budding, while protein translation and core accumulation were at wild-type levels. This interaction of cdE2 with the hydrophobic pocket in the CP is thought to drive the budding of the mature virus at the cell membrane.

Flavivirus Assembly

Flavivirus life cycle

Flaviviruses belong to the family *Flaviridae* of positive-strand RNA viruses which also consist of the pestiviruses and the hepaciviruses. The flaviviruses comprise more than 70 members including important human pathogens such as yellow fever virus, dengue virus, and West Nile virus. Flaviviruses enter cells via receptor-mediated endocytosis. The low pH environment of the endosomal membrane triggers the conformational change of the envelope glycoprotein which results in the fusion of the viral and endosomal membranes releasing the genome into the cytoplasm. The viral proteins are translated from the RNA genome as a single polyprotein. Signal sequences and stop transfer sequences result in the translocation of the nascent polyprotein to the ER membrane. The polyprotein is processed by a combination of cellular and viral proteases to produce the mature structural and nonstructural proteins. Genome replication and virion assembly occur in ER membrane-bound vesicles. The structural proteins and the genome bud into the lumen of the ER to form the immature virion which is transported through the secretory pathway. Prior to secretion of the virion, a furin cleavage converts the immature virus into the mature, infectious form of the virus.

Flavivirus virion structure

The flavivirus virion is made up of three structural proteins: capsid (C), pre-membrane (prM), and envelope (E) that are translated from the 5′ one-third of the RNA genome (**Figures 1(b)** and **1(d)**). Signal sequences at the C-terminus of the C protein and prM serve to translocate prM and E respectively into the ER (**Figure 1(d)**). The role of the highly basic C protein (12 kDa) is to encapsidate the viral genome during virion assembly. In contrast to the alphavirus CP which exhibits no membrane association, the flavivirus C protein is anchored to the membrane, at least transiently (**Figure 1(d)**). However, Rubella virus, the sole member of the genus *Rubivirus* within the family *Togaviridae*, has a membrane anchored CP, perhaps indicating a common origin for the *Togaviridae* and *Flaviviridae*. prM is a glycoprotein that associates with the E protein and serves as a chaperone to facilitate the proper folding of E. The immature virions that bud into the ER consist of prM-E heterodimers. prM prevents premature fusion from occurring in the acidic environment of the ER and Golgi. Thus, prM has several functions analogous to the E3 glycoprotein of the alphaviruses. Cleavage of prM into pr and M by a furin-like protease triggers the rearrangement of the prM-E heterodimers into E–E homodimers, resulting in a radical change in size and shape required for the formation of the mature virus particle. The E glycoprotein is responsible for host cell receptor binding and for fusion of the viral and cellular membranes. The E glycoprotein is also critical for the assembly of the virion. High-resolution structures of the ectodomains of several flavivirus E proteins are available. The ectodomain is divided into three domains. Domain II constitutes the dimerization domain as it contains most of the intradimeric contacts between E–E homodimers. Domain II also contains the fusion peptide, a glycine-rich hydrophobic sequence that initiates fusion by insertion into the target cell membrane. Domain III comprises the immunoglobulin-like domain responsible for receptor binding. In addition to the dramatic conformational and translational changes that the E protein undergoes during the virion maturation process, it also changes conformation during membrane fusion. The low pH of the endosome during infection triggers a conformational change which results in the formation of E homotrimers. In this arrangement, the fusion peptides are exposed and available to insert into cellular membranes. Interestingly, the structure of the E protein was found to be very similar to the structure of the Semliki Forest virus E1 protein, the fusion protein of the alphaviruses.

The structures of two flaviviruses, dengue and West Nile virus, have been solved by cryo-EM and image reconstruction techniques and have been shown to be similar (**Figure 1(b)**). The mature virion is ∼50 nm in diameter and exhibits a smooth outer surface in contrast to the alphaviruses which have distinctive spike structures (cf. **Figures 1(a)** and **1(b)**). The E proteins are arranged parallel to the surface of the virus, with 90 E dimers arranged in groups of three to form a 'herringbone' pattern on the viral surface. This arrangement of the E proteins completely covers the surface of the virus, thus rendering the lipid bilayer inaccessible. Domain III of E protrudes slightly from the viral surface, allowing interaction with cell receptors. The membrane-spanning regions of E and M proteins form antiparallel helices while the stem regions are arranged parallel to the membrane.

The immature virus particle exhibits a dramatically different glycoprotein organization compared to the mature virion. Cryo-EM and image reconstruction of dengue and yellow fever virus immature virions have revealed that these particles are larger (∼60 nm) and have spikes that protrude from the surface of the virus. These spikes are composed of trimers of prM-E heterodimers. The pr peptide covers the fusion peptide of E in this arrangement, similar to E2 covering E1 in alphaviruses, thus protecting it from premature fusion as the immature particle is transported through the acidic environment of the secretory pathway.

The NC is found below the viral envelope and is composed of a single copy of the genome RNA and multiple copies of the C protein. Cryo-EM reconstructions of the virion have shown that in contrast to the alphaviruses, there is no apparent organization to the flavivirus NC. This may be because there is no direct interaction between the C proteins in the core and the glycoproteins in the viral envelope since the E and M proteins do not penetrate below the inner leaflet of the membrane. Furthermore, no NCs have been observed in the cytoplasm of infected cells and attempts to establish an *in vitro* assembly system analogous to the alphavirus *in vitro* assembly system have failed. The lack of coordination between the C protein and the viral envelope proteins suggests that the assembly of virions is driven by the lateral interactions of the E and M proteins in the viral envelope and not by the C protein. This is supported by the observation that flavivirus infections result in the production of noninfectious subviral particles which are composed of just the viral envelope (E and M) and the lipid bilayer. Thus, the flavivirus glycoproteins are sufficient to induce particle budding.

Flavivirus assembly and budding

Virus-induced membrane structures called vesicle packets, which are continuous with the ER membrane, are the sites of flavivirus replication and assembly (**Figure 3**). Within these structures the structural proteins are in intimate contact with the genome RNA. The C protein associates with the genome RNA via interactions between the positive charges distributed throughout the protein and the negatively charged phosphate backbone of the RNA. It is not yet clear how the C protein specifically recognizes the genome RNA; unlike for alphaviruses, a packaging signal has not been conclusively identified for flaviviruses. Coupling between genome replication and assembly within the vesicle packets has been proposed as a mechanism to ensure the specific encapsidation of the genome RNA. It has been shown that one or more nonstructural proteins (NS2A and NS3) are involved in genome packaging and NC assembly. The NC lacks a defined icosahedral structure as described above. Therefore, core formation is probably concomitant with the association of the C protein and RNA genome with the viral glycoproteins and budding into the ER lumen, thus giving rise to the immature particle (**Figure 3**). The immature virion is transported from the ER to the Golgi where the viral glycoproteins are post-translationally modified. The cleavage of the prM protein in the trans-Golgi network triggers the dramatic reorganization of the viral glycoproteins that results in the formation of the mature virion (**Figure 3**). The mature virion is then released from the host cell by exocytosis.

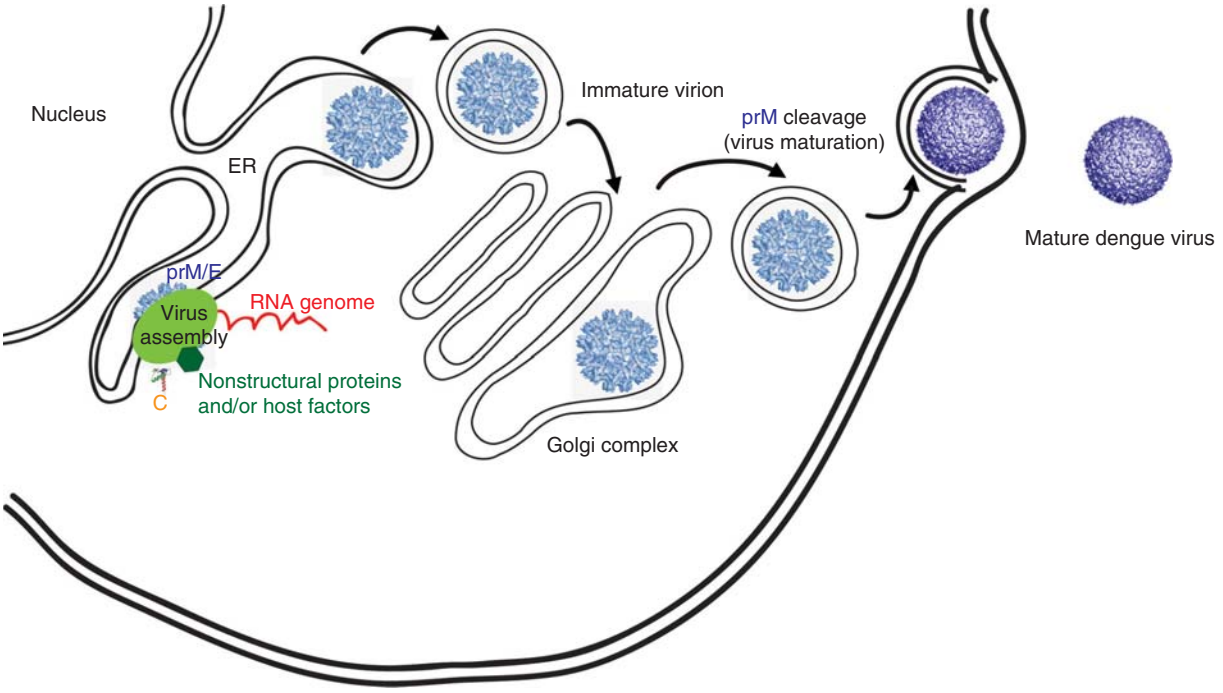


Figure 3 Flavivirus assembly. Flavivirus assembly occurs on ER-associated membranes known as vesicle packets. Assembly and genome replication are coupled and the sites of assembly consist of the capsid proteins (C), the glycoproteins prM and E, the RNA genome, and one or more nonstructural proteins and/or host factors. The immature virion buds into the ER and is transported to the Golgi and trans-Golgi network. The glycoproteins are post-translationally modified as the immature virus is transported through the secretory pathway. Furin cleavage of prM results in the formation of the mature virus which then exits the cell by exocytosis.

Conclusion

Following from the discussion of alphavirus and flavivirus assembly, it is apparent that the assembly of even the simplest enveloped viruses requires the complex interaction of viral and host factors in order to produce a virus particle which is at once stable and at the same time primed for disassembly. The whole range of the cell's machinery including the translation apparatus, polymerases, chaperones, and post-translational modification enzymes are co-opted by viruses in order to replicate the viral components necessary for assembly. Enveloped viruses have evolved to utilize different cellular membranes and cellular compartments for assembly and they take advantage of the secretory pathway to produce their viral glycoproteins. A majority of viruses bud from the plasma membrane (**Table 1**). This is the case with alphaviruses, where NC assembly occurs in the cytoplasm and the final assembly of the mature virion occurs at the plasma membrane. The high concentration of viral proteins, often concentrated at specific sites allows for the efficient interaction and assembly of virions. In contrast to alphaviruses, NC assembly and glycoprotein assembly is coupled in the flaviviruses and occurs in vesicle packets associated with the ER. Thus, the whole flavivirus virion is transported through the ER and Golgi while in the case of the alphaviruses only the glycoproteins are transported through the secretory pathway. These exit strategies are not unique and thus serve as model systems to study enveloped virus assembly and release.

Proteolytic cleavage of glycoproteins in order to convert them from stable oligomeric structures to metastable structures primed for fusion are common themes in enveloped virus structure and assembly. Cleavage of PE2 into E3 and E2 by a furin-like protease primes the alphavirus spike complex for fusion. A similar cleavage of prM triggers a dramatic conformational change of the flavivirus glycoproteins resulting in the formation of the mature virion which is now infectious.

Alphavirus budding requires the specific interaction of the NC with the E1–E2 spike complexes at the plasma membrane, thus ensuring that all virions have a genome packaged into them. However, the flaviviruses only require the interaction of the envelope proteins for budding, giving rise to subviral particles devoid of the C protein and genome RNA. Thus, the flavivirus envelope proteins alone are sufficient to drive budding of virus particles and the close coupling of genome replication and the C protein (perhaps mediated by replication proteins and host factors) is required to package the genome into virus particles. A third strategy for budding is exhibited by the retroviruses where capsid assembly has been shown to be sufficient to drive budding of the virus. In this case, targeting of the envelope proteins to these sites of CP assembly is essential to ensure the incorporation of the glycoproteins into the virion.

Although much has already been discovered about enveloped virus assembly, there are still many processes yet to be described. There is an increasing interest in the assembly pathway of viruses partly fueled by the potential to develop successful therapeutic agents targeting virus specific assembly processes. Advances in the field of structural biology will further help attempts to understand the assembly pathway of this important class of viruses.

See also: Assembly of Viruses: Nonenveloped Particles.

Further Reading

Garoff H, Hewson R, and Opstelten DJ (1998) Virus maturation by budding. *Microbiology and Molecular Biology Reviews* 62(4): 1171–1190.

Harrison SC (2006) Principles of virus structure. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 53–98. Philadelphia, PA: Lippincott Williams and Wilkins.

Hunter E (2006) Virus assembly. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 141–168. Philadelphia, PA: Lippincott Williams and Wilkins.

Mukhopadhyay S, Kuhn RJ, and Rossmann MG (2005) A structural perspective of the flavivirus life cycle. *Nature Reviews Microbiology* 3(1): 13–22.

Assembly of Viruses: Nonenveloped Particles

M Luo, University of Alabama at Birmingham, Birmingham, AL, USA

© 2008 Elsevier Ltd. All rights reserved.

Architecture of Viruses

Nonenveloped viruses have two essential components: protein and nucleic acid. The protein forms a coat called 'capsid' that packages the nucleic acid, which may be DNA or RNA. This complex constitutes a virion or a virus particle. The nucleic acid is the viral genome that encodes all the virus-specific genes required for viral replication. The protein capsid packages the viral genome during replication, and transmits it for the next round of infection. When the virion reaches the host cell, the capsid usually recognizes a specific receptor that helps the

virion to enter. Once inside the host cell, the capsid has to release the viral genome so replication can begin. The size of a viral genome is usually limited, so only a few genes can be encoded. It is more efficient if only one or a small number of genes encode for capsid proteins that can self-assemble into a complete shell by use of many copies of the same proteins. The assembly of the capsid proteins follows a specific type of symmetry that allows a small protein unit to assemble into a large particle. The protein capsid can have a helical (filamentous virus) or icosahedral (spherical virus) symmetry. Helical symmetry is described by the diameter, *d*, the pitch, *P*, and the number of subunits per turn. There are as many capsid proteins as necessary for completely covering the nucleic acid genome. Icosahedral symmetry is defined by 12 fivefold axes, 20 threefold axes, and 30 twofold axes. A number, *T*, called the 'triangulation number', indicates how many quasi-symmetrical subunit interactions are within one asymmetrical region of the icosahedron. There are a total of 60 T copies of the capsid proteins in one icosahedral capsid.

For example, a picornavirus (pico-(small)-RNA virus) has a single positive-stranded RNA genome of about 8000 nucleotides. The RNA genome encodes a long polyprotein that is processed into individual viral proteins after translation directly from the positive RNA genome. Three of the 10 proteins are capsid proteins: viral protein 1 (VP1), viral protein 3 (VP3), and viral protein 0 (VP0). VP0 is processed to VP2 and VP4 after virus assembly. VP1, VP2, and VP3 are the three capsid proteins that form the body of the coat while VP4 is entirely inside the coat. There are 60 copies of each capsid protein in the coat. A picornavirus is, therefore, a $T=1$ particle. However, the structure of VP1, VP2, and VP3 is highly homologous. If the small differences in each major capsid protein are ignored, the three proteins can be considered as the same building block. A picornavirus is thus called a pseudo-$T=3$ particle (**Figure 1**). The interactions between the capsid proteins in the coat are similar despite their different symmetry locations. For instance, VP1 (blue) interacts around the fivefold symmetry axis, whereas VP2 (green) and VP3 (red) interact with each other around the threefold symmetry axis. Since VP2 and VP3 are similar, the symmetry around the threefold axis is like a sixfold symmetry axis. This symmetry axis is therefore called a quasi-sixfold axis. The interactions between the neighboring VP1 proteins are considered quasi-equivalent (means more or less similar) to the interactions between VP2 and VP3 even though VP1 subunits are around a fivefold axis, and VP2/VP3 subunits are around a quasi-sixfold axis. This quasi-equivalence allows the closed capsid to be assembled using the same building block, the capsid proteins. It should be emphasized, however, that the true differences that exist in the capsid proteins are critically correlated with functions such as virus entry and release or packaging the viral genome.

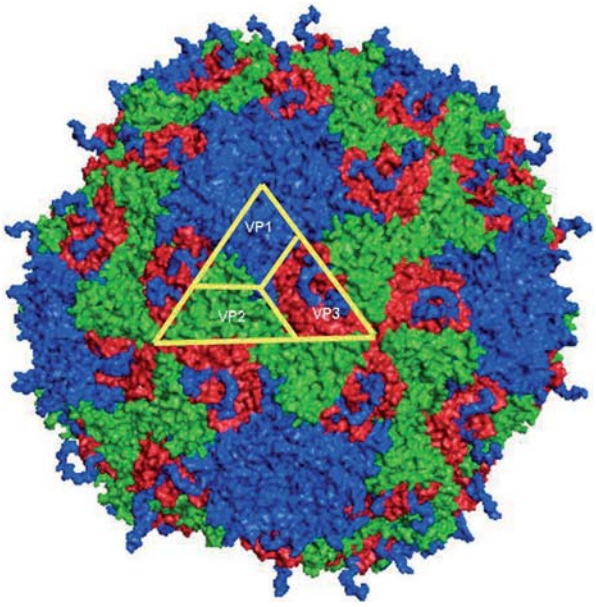


Figure 1 The capsid of Theiler's murine encephalomyelitis virus (TMEV), a picornavirus. The triangle outlines one set of 60 copies of each capsid protein.

Methods of Structure Determination

X-ray diffraction is the common technique used for studying the atomic structure of proteins and nucleic acids. When X-rays strike on the electrons of the atoms in a stationary specimen, a diffraction pattern of spots with different intensities is generated and recorded. By analysis of the diffraction pattern and the spot intensities, a three-dimensional electron density map (EDM) can be calculated by Fourier transformation. A three-dimensional chemical structure could be built based on the interpretation of the EDM. Two types of X-ray diffraction experiments are useful for virus structure studies: fiber diffraction (for filamentous viruses) and crystallography (for spherical viruses and globular viral proteins).

Another common technique is electron microscopy. Recent advances in electron microscopy allow researchers to determine relatively high-resolution three-dimensional structures of viral particles by use of image reconstruction of cryo-electron micrographs and electron tomography. This technique is particularly suitable for large viral particles that are difficult to crystallize. Electron microscopy and X-ray crystallography are therefore complementary to each other.

Atomic Structure of Helical Viruses

The disk of the tobacco mosaic virus (TMV) coat protein has been crystallized and its atomic structure resolved by

X-ray crystallography. The intact TMV structure containing its nucleic acid could only be determined by X-ray fiber diffraction experiments, as also was that of Pf2 phage. The coat proteins of TMV and Pf2 contain mainly α-helices and the nucleic acid interacts with the coat protein by one base (Pf2) or three bases (TMV) per protein unit. The protein subunit is arranged in a super-helical structure coincident with a super-helical structure of the nucleic acid. The protein subunits form the outer layer of the helix to protect the nucleic acid from the environment. The nucleic acid is embedded between the protein layers that are stacked up in the spiral structure. The axis of the coat protein helix coincides with that of the nucleic acid. Since the symmetry of the super-helix puts no limit on the length of the helix, the virus particle can grow as long as the length of the nucleic acid genome; in this way all bases in the genome are covered by the coat protein. The coat proteins of TMV have many aggregation forms, depending on pH or ionic strength. In natural conditions, the TMV coat protein forms a disk with two layers of 17 protein subunits in each. The subunit of the TMV capsid protein contains four antiparallel α-helices with connecting long loops. Each protein subunit takes the shape of a shoe. When the disks of the capsid proteins are stacked, the RNA fits into the grooves formed by the protein subunits. The backbone of the RNA has charged interactions with the side chains of the amino acids while the bases of the RNA are accommodated by the hydrophobic space. The TMV RNA genome is inserted into the center of the coat protein disk to begin virus assembly. More and more disks are added to the top of the growing virus particle to pull the RNA through the center of the super-helix. The assembly is completed when the complete RNA is pulled into the virus particle. The coat protein of Pf2 has a different shape that contains an extended α-helix. The coat protein is added one by one to the DNA helix emerging from the bacterial membrane. The length of the α-helix is in the direction of the virus particle.

Atomic Structure of Spherical Viruses

Nonenveloped spherical viruses form large single crystals under proper conditions. Their atomic structure can be determined by X-ray crystallography with aid of fast computers and synchrotron X-ray sources. Since 1978, there are numerous atomic structures of viruses reported. Today, atomic structures have been determined for every major family of nonenveloped viruses, especially human pathogens.

Most capsid proteins of these viruses contain an antiparallel, eight-stranded β-barrel folding motif (**Figure 2**). The motif has a wedge-shaped block with four β-strands

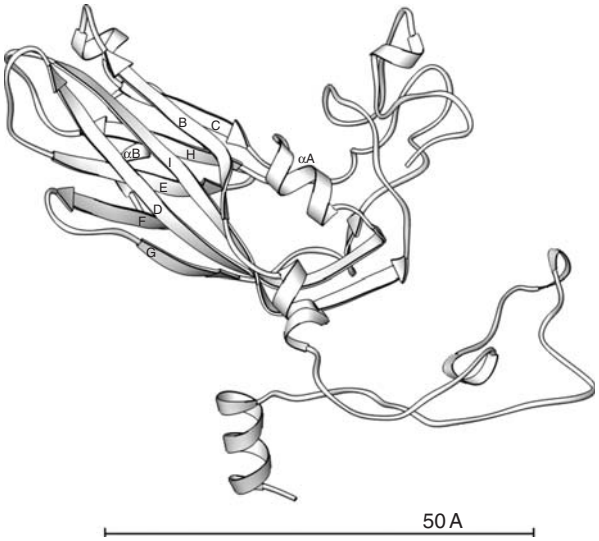


Figure 2 The β-barrel fold found in spherical viruses. The fold contains eight essential β-strands: BIDG on one side and CHEF on the other side. The figure was generated with rhinovirus 16 VP1 (PDB code 1AVM).

(BIDG) on one side and another four (CHEF) on the other. There are also two conserved α-helices (A and B): one is between βC and βD, and the other between βE and βF. In animal viruses, there are large loops inserted between the β-strands. These loops form the surface features of individual viruses. The common presence of the β-barrel motif in viral capsid proteins is the result of structural requirements for capsid assembly. It also points to a common ancestor of different virus families.

A virus capsid may contain multiple copies of the β-barrel fold with the same amino acid sequence (such as $T = 3$ calicivirus or $T = 1$ canine parvovirus) or with different amino acid sequences (such as pseudo $T = 3$ picornavirus). In some cases, there are two β-barrel folds in a single polypeptide (such as adenovirus hexon). Capsid proteins of spherical viruses can have other motifs such as α-helices in reovirus and hepadnavirus.

Nucleic Acid–Protein Interaction

The viral nucleic acid genome is always packaged inside the protein capsid. Positively charged patches formed by the side chains of arginines and lysines are found on the interior surface of the protein capsid. These positively charged areas are the preferred binding sites for the nucleotides. Usually the structure of the nucleic acid cannot be observed in a single-crystal X-ray diffraction experiment because of the random orientation of the icosahedral particles in the crystal. However, in rare cases, the nucleic acid might assume icosahedral symmetry by

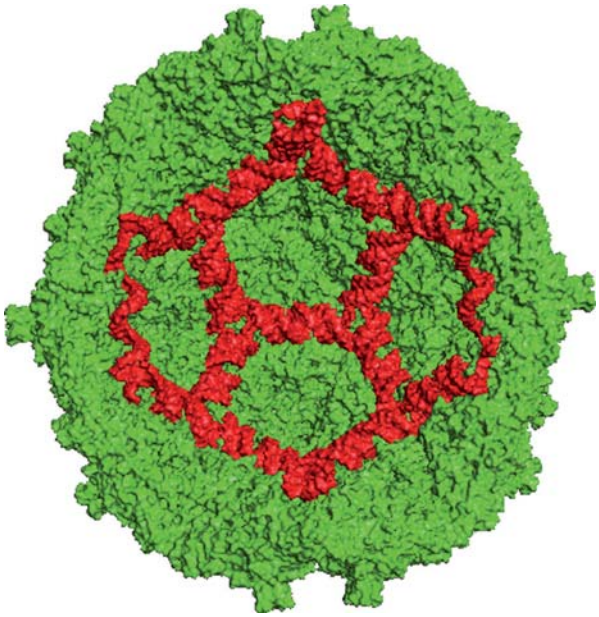


Figure 3 The interior of the pariacoto virus capsid (green). The structured RNA genome is shown in red. The figure was generated with coordinates of pariacoto virus (PDB code 1F8V).

interacting with the protein capsid. Fragments of the complete genome assume the same conformation, although with different nucleotide sequences, at locations related by icosahedral symmetry. Such structures have been seen in bean pod mottle virus, tobacco mosaic virus, pariacoto virus and flock house virus (RNA viruses), and canine parvovirus (DNA virus). The bases are stacked either as an A-type RNA helix or form a coiled conformation to fit the interactions with the protein capsid. In the case of pariacoto virus, the RNA genome forms a cage that reassembles the icosahedral symmetry of the capsid (**Figure 3**). These viruses readily form empty virus particles and have a hydrophobic pocket on the interior surface of the capsid. The nucleic acid generally interacts nonspecifically with the capsid protein.

Evolution

The highly conserved β barrel motif of the viral capsid protein indicates that many viruses must have evolved from a single origin. The unique three-dimensional structure of this motif is required for capsid assembly and it is generally conserved over a longer period of time than the amino acid sequence. The superposition of the capsid proteins from different viruses can be used to estimate the branch point in the evolutionary tree for each virus group. The structure alignment not only relates plant viruses to animal viruses, RNA viruses to DNA viruses, but also viruses to other proteins such as concanavalin A that has a similar fold and competes with poliovirus for its cellular receptor. The evolutionary relationship of these viruses is supported by amino acid sequence alignment of more conserved viral proteins such as the viral RNA polymerase. The structural similarities of the capsid proteins support the notion of a common evolutionary origin among nonenveloped viruses.

Assembly

The icosahedral capsid is assembled from smaller units made of several protein subunits. For picornaviruses, a protomeric unit is first formed with one copy of each polypeptide after translation. The termini of the subunits are intertwined with each other to hold the subunits together in the protomer. The protomers are then associated as pentamers which in turn form the complete icosahedral virion while encapsidating the viral RNA. In $T=3$ or $T=1$ plant RNA viruses, the pentamers are formed by dimers of the capsid proteins. In adenovirus and SV40, the capsid proteins form hexon units (three polypeptides, each has two β-barrels) or pentamers before they assemble into an icosahedral shell. The nucleic acid could be packaged at different stages of assembly.

Host Receptor Recognition Site

Animal viruses have to recognize a specific host cellular receptor for entry during infection. Host receptor binding is the initial step of virus life cycle and could be an effective target for preventing virus infection. Based on the atomic structure of animal viruses, it was found that the receptor recognition site is located in an area surrounded by hyper-variable regions of the antigenic sites. Usually, the area is in a depression (called the 'canyon') on the viral surface that may be protected from recognition by host antibodies. This structural feature is, for instance, present in human rhinovirus (also known as the common cold virus), and the active site of influenza virus hemagglutinin (HA). The receptor-binding site on influenza virus HA does not have a deep depression, but it is surrounded by antigenic sites. The receptor-binding area on the surface of the viral capsid is conserved for recognition by the receptor, whereas the sites recognized by antibodies are distinct from the receptor-binding site and keep changing from strain to strain. By this mechanism, the virus can escape the host immune system by mutating the antibody epitopes, and at the same time maintain a constant receptor-binding site to continue its infection of the host cells. Evidence supports that this is a general mechanism that viruses use to evade the host immune defense.

Antigenic Sites

Antibodies are the first line of defense by the immune system against a viral infection. The epitopes combined with the neutralizing antibodies are mapped on a few isolated locations on the surface of viral proteins. The structure of human rhinovirus complexed with Fab fragments showed that the antibody makes contact with an area about 6 nm^2 and that the epitope spans different discontinuous polypeptides. Therefore, an effective vaccine usually needs to include a complete viral protein or a large fragment. The binding of the antibodies does not significantly change the structure of the antigen. The exact mechanism by which antibodies neutralize antigens is dependent upon the binding site and processes of the virus replication.

Antiviral Agents

Viral infectious diseases can be cured if an agent can be administered to stop viral infection. Such agents have been synthesized and shown to bind to the capsid of rhinovirus in the crystal structure. The compounds were inserted into the hydrophobic pocket within the β-barrel of the major capsid protein VP1. Binding of the compounds stops uncoating of the virion and the receptor binding, which resulted in the failure of releasing the viral RNA into the cytoplasma. These compounds inhibit infections of several other RNA viruses and may be effective against other viruses after modification since the β-barrel structure exists in many viruses.

The most successful antiviral drugs are the HIV protease inhibitors which are developed based on the atomic structure of the protease. Through iterative cycles of computer modeling, chemical synthesis and structural studies of the protein-inhibitor complexes, a panel of clinical effective drugs has been brought to the market and has shown great benefits to patients. Inhibitors of influenza virus neuraminidase have also been developed by the same method and marketed as antiviral drugs.

See also: Assembly of Viruses: Enveloped Particles; Theiler's Virus.

Further Reading

Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) (2002) *Fields Virology,* 4th edn. Philadephia, PA: Lippincott Williams and Wilkins.
Rossmann MG and Johnson JE (1989) Icosahedral RNA virus structure. *Annual Review of Biochemistry* 58: 533–573.
Schneemann A (2006) The structural and functional role of RNA in icosahedral virus assembly. *Annual Review of Microbiology* 60: 51–67.

Astroviruses

L Moser and S Schultz-Cherry, University of Wisconsin – Madison, Madison, WI, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Enterocytes Epithelial cells lining the intestines.
Intussusception Obstruction of the intestine.
Interstitial nephritis Inflammation of the kidney.
Poult Young turkey.
Villi Finger-like intestinal projections lined with enterocytes.

Introduction

Astroviruses are enteric viruses first identified in the feces of children with diarrhea. Detection was originally based on a five- to six-pointed star morphology of virions by electron microscopy (EM). However, only about 10% of viral particles display these structures; the remaining 90% of particles have a smooth surface and a size similar to other small, round-structured viruses like picornaviruses and caliciviruses. Thus, accurate diagnostics were difficult to obtain and the true prevalence of astrovirus within a population was difficult to assess. Development of much more sensitive detection techniques like real time reverse transcription-polymerase chain reaction (RT-PCR), cell culture RT-PCR, and astrovirus-specific enzyme-linked immunosorbent assays (ELISAs) have made detection more accurate and specific, even allowing diagnosis of specific serotypes. Utilizing these techniques, astroviruses have been found in approximately 3–8% of children with diarrhea. Astroviruses can also be isolated in a subset of asymptomatic individuals, suggesting that a proportion of infected individuals shed the virus asymptomatically or for some time after the

resolution of other symptoms of infection. Asymptomatic carriers may be a major reservoir for astroviruses in the environment and could contribute to dissemination of the virus.

The release of astroviruses into the environment is a concern due to the extreme stability of the virus. Astroviruses are resistant to inactivation by alcohols (propanol, butane, and ethanol), bleach, a variety of detergents, heat treatment including 50 °C for an hour or 60 °C for 5 min, and UV treatment up to 100 mJ cm^{-2}. Human astroviruses are known to survive up to 90 days in both marine and tap water, with survival potential increasing in colder temperatures. Studies have described the isolation of infectious virus from water treatment facilities. Furthermore, astroviruses can be concentrated by filter-feeding shellfish like oysters and mussels in marine environments. Astroviruses are transmitted fecal–orally, and contaminated food and water have been linked to astrovirus outbreaks.

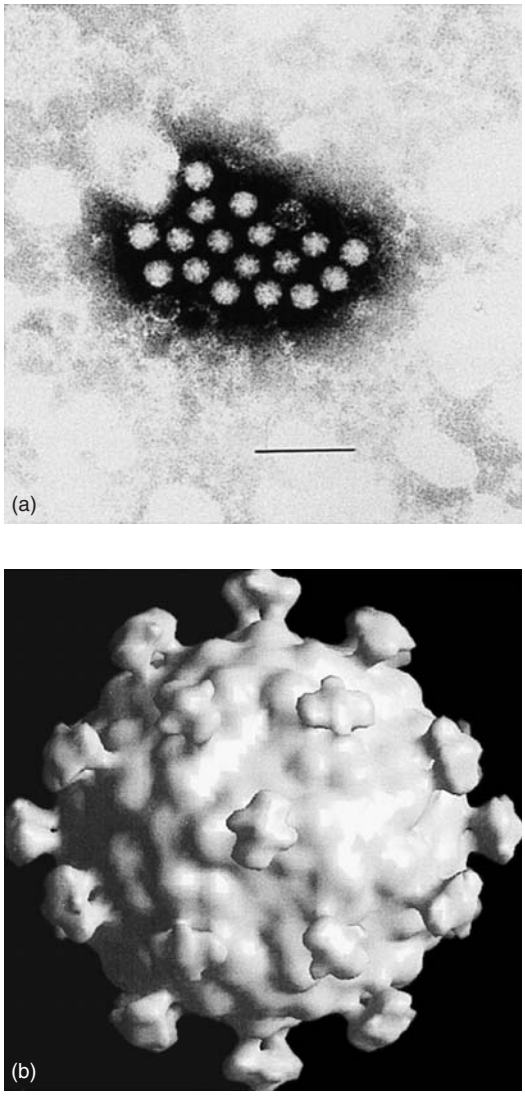


Figure 1 (a) Astroviruses have historically been identified by the five- to six-pointed star morphology visible by electron microscopy. Scale = 100 nm. (b) A reconstruction of the astrovirus virion, based on cytoelectron microscopy, along the twofold axis of symmetry. Reprinted from Matsui SM and Greenberg HB (1996) Astroviruses. In: Fields BN, Knipe DM, and Howley PM (eds.) *Fields Virology*, 3rd edn., pp. 875–893. Philadelphia: Lippencott-Raven.

History and Classification

Astroviruses were originally observed by Appleton and Higgins in 1975 as a small round virus in stools. Later that year, Madeley and Cosgrove identified the virus in association with diarrhea in children and bestowed the name astrovirus (from the Greek *astron*, meaning star) for the star-like morphology of a proportion of viral particles seen by EM (**Figure 1(a)**). Because of genomic similarities, astroviruses were originally thought to belong to either the families *Picornaviridae* or *Caliciviridae*. However, the lack of a helicase and use of a frameshifting event during replication (discussed below) distinguish astroviruses so completely that, in 1993, the International Committee on Taxonomy of Viruses (ICTV) classified astroviruses as a unique family, *Astroviridae*, composed of a single genus *Astrovirus*. Continued investigation into newly discovered astroviruses led to the division of the family into two genera, *Mamastrovirus* and *Avastrovirus*, by the ICTV in 2005. ICTV nomenclature abbreviates astrovirus AstV, with a single-letter abbreviation for the species type (i.e., human astrovirus; HAstV; turkey astrovirus; TAstV, etc.). Successive, serologically distinct isolates of astroviruses are named sequentially within that species (i.e., HAstV-1 through HAstV-8).

Epidemiology

Humans

Astroviruses have been detected throughout the world. While the exact incidences of infection vary from study to study, community-acquired astroviruses are found in 3–6% of children with infectious gastroenteritis. In some developing countries, infection rates as high as 20% have been observed. In many cases, astroviruses are the second most commonly detected viral pathogen in young children after rotavirus. Astrovirus infections are identified in up to 2% of asymptomatic individuals. These data may underrepresent actual astrovirus infections, as studies generally survey individuals visiting medical care centers. Because astrovirus disease is generally mild in humans (see the section discussing pathogenesis), hospital cases may represent only a slight proportion of actual infections in the community. In support of this, serological studies have demonstrated that up to 90% of children have been exposed to at least one strain of astrovirus by age 9.

Eight serotypes of human astrovirus have been identified to date, with all eight circulating within the global population to various levels. HAstV-1 is by far the most prevalent serotype, comprising 25–100% of astroviruses in a region, and the most prevalent reactivity of antibodies detected, although serological surveys of all serotypes have not been undertaken. HAstV-6, -7, and -8 are the least frequently detected, although three to four serotypes of HAstV are often detected in a region at any given time. The differing prevalence of serotypes could be a reflection of severity; perhaps HAstV-1 infection results in a higher frequency of hospital visits than other serotypes and is therefore overrepresented in hospital-based epidemiological studies. Alternatively, serotypes may be restricted by region. For example, one Mexican study identified HAstV-1 as the predominant serotype throughout the country, but HAstV-3 and -8 were prominent in select regions.

Viral infection occurs with equal frequency in boys and girls and predominantly in children under the age of 2. Infection is not restricted to young children, however, and has been noted in individuals of all ages, including immunocompetent adults and the elderly. Immunodeficient individuals, particularly those that are HIV-positive, appear to be at an increased risk of astrovirus infection.

Astrovirus infection occurs year-round, but with the highest frequency during the autumn and early winter months. In tropical climates, infection correlates with the rainy season. These seasonal correlations likely reflect the indoor confinement of the population as well as the increased stability of astroviruses in cold, damp conditions. Astrovirus outbreaks have also been associated with high-density environments, including childcare centers, primary and junior high schools, military recruiting centers, elderly care centers, and swimming pools. Astrovirus as a cause of hospital-acquired viral diarrhea in young children is second only to rotavirus, occurring at rates of 4.5–6%, and, in some studies, surpasses rotavirus in rates of nosocomial infections.

Interestingly, astrovirus infection occurs quite frequently (up to 50%) as a co-infection with other enteric pathogens. The most frequent co-pathogens are noroviruses and rotaviruses, but infections with adenoviruses, parasites, and enteric bacteria are often detected as well. The importance of this in humans is not entirely clear. In a study specifically examining co-infections, astrovirus co-infection with rotavirus increased the duration of diarrhea and vomiting over either virus alone, although whether this difference was statistically significant is unknown.

Animals

Most animals are not routinely screened for astrovirus infection, so our knowledge of the prevalence of infection is limited to surveillance studies. Astroviruses have been found in association with most animals examined, although the effect of infection varies with species (see below). While astroviruses were originally identified in humans, they have since been identified in both mammalian and avian species, including rabbits, mice, calves, sheep, piglets, dogs, red-tailed deer, kittens, mink, turkeys, ducklings, chicken, and guinea fowl. At least three serotypes of bovine astroviruses are postulated to exist based on distinct neutralizing antibodies (one in the United States and two in the United Kingdom). In addition, astroviruses have been isolated from mink across Scandinavia, and serological studies have demonstrated that astroviruses were prevalent in chicken flocks in the 1980s as well as in 2001. Interestingly, two very different manifestations of chicken astrovirus infection have been described (see the section on pathogenesis), suggesting that distinct chicken astroviruses may circulate; however, this is yet to be proven. The best epidemiologically characterized animal astroviruses are the turkey astroviruses. Surveillance of turkey flocks in the 1980s isolated astrovirus from 78% of diseased flocks, but only 29% of normal flocks. Astroviruses were the first pathogen detected in many flocks and were most commonly detected in birds less than 4 weeks of age. Similar to human infections, turkey astrovirus was frequently isolated with other pathogens, most commonly rotavirus-like viruses. The early age of infection and the prevalence of co-infections led one group to postulate that astrovirus infection may predispose birds to infection by other viruses.

Virus Propagation

Attempts at *in vitro* propagation of astroviruses have been met with varying degrees of success. The most successful techniques utilize cultured cells from the host species and provide exogenous trypsin in the culture. Successful propagation of human astroviruses was originally achieved by repeated passage through primary human embryonic kidney cells; it was later discovered that direct passage through the human intestinal cell line Caco-2 would also yield infectious virus. Propagation of porcine, bovine, and chicken astroviruses has been successful in their respective host cells *in vitro*. However, many astroviruses still have not been adapted to propagation *in vitro* for unknown reasons, while others lose infectivity with subsequent passages and therefore cannot be maintained continuously. This problem has been circumvented in some systems by passing the virus through an animal system, as is the case for the turkey astrovirus, in which highly concentrated virus can be obtained from infected turkey embryos *in ovo*.

Molecular Virology and Protein Expression

Astroviruses contain one copy of positive-sense, single-stranded RNA. The genome is approximately 6.8 kb long and contains three open reading frames (ORFs), ORF1a, -1b, and -2, as well as 5′ and 3′ untranslated regions (UTRs) (**Figure 2**). The RNA is polyadenylated, but lacks a 5′ cap structure. The 5′ and 3′ UTRs are highly conserved and are believed to contain signals important for genome replication.

Astroviruses initiate infection by binding to an unknown receptor and entering the cell via receptor-mediated endocytosis. The plus-strand genome is released into the cytoplasm by unknown mechanisms and ORF1a and -1b are immediately translated by the host machinery. ORF1a is 2.8 kb and encodes a polypeptide of approximately 110 kDa. This polypeptide contains a variety of conserved motifs, including several putative transmembrane domains, a bipartite nuclear localization sequence (NLS), and a serine protease motif. The translated polypeptide is cleaved by both cellular protease(s) and the viral protease into at least five peptides. The actual function of each protein remains largely unknown. The transmembrane domains may localize to the endoplasmic reticulum (ER) membrane to facilitate replication, as all plus-strand RNA viruses have been shown to replicate in association with a membrane. One peptide, NSP1a/4, colocalizes with the viral RNA at the ER membrane; mutations in NSP1a correlate with increased viral titers *in vitro* and *in vivo*, suggesting a role for this protein in viral replication. The role for the NLS remains unclear; some reports suggest viral antigen is observed in the nucleus, while others find that it is excluded.

The second reading frame, ORF1b, overlaps ORF1a by 70 nucleotides and has no detectable start codon. Intensive research has determined that ORF1b is translated by a frameshift into the −1 frame. This frameshifting event is unique among plus-strand animal RNA viruses and requires a highly conserved shifty heptameric sequence (A_6C) as well as a downstream hairpin structure. This event, which occurs with frequencies up to 25% in cells, results in an ORF1a/1b fusion peptide. Cleavage near the 1a/1b border releases the ORF1b gene product: the viral RNA-dependent RNA polymerase (RdRp). Astrovirus polymerase is a supergroup 1 RdRp, a group which generally utilizes a VPg to initiate transcription. Although a VPg is postulated to exist and a putative VPg genomic linkage site has been identified, its existence is yet to be empirically proven.

Expression of the RdRp results in production of a minus-strand viral template. This generates multiple copies of the plus-strand genome as well as a polyadenylated subgenomic RNA (sgRNA) containing short 5′ and 3′ UTRs and ORF2. ORF2 is in the 0 frame and overlaps ORF1b slightly (four nucleotides) in human astroviruses. Production of the capsid protein from a sgRNA not only temporally restricts capsid production to later in the viral replication cycle, but also allows for massive capsid protein expression; it is estimated that sgRNA is produced in tenfold excess of the viral genome by 12 h post infection (hpi). The sgRNA is about 2.4 kb and encodes the single structural protein of approximately 87 kDa. This peptide is cleaved by an intracellular protease to approximately 79 kDa; mutational analyses suggest that this 8 kDa stretch is required for efficient expression of the capsid protein. Individual capsid proteins multimerize spontaneously to form icosahedral structures of about 32 nm (**Figure 1**(**b**)).

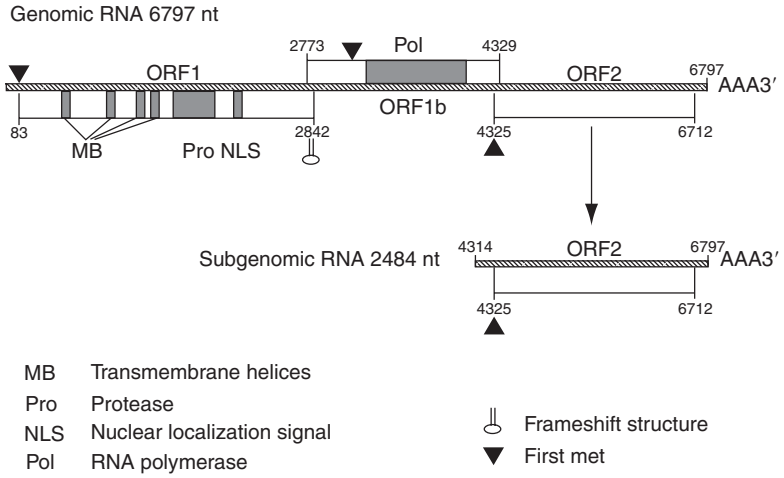


Figure 2 The genomic organization of astroviruses (based on HAstV-1), including open reading frames and encoded protein features, is shown. Reprinted from *Virus Taxonomy*: *Sixth Report of the International Committee on Taxonomy of Viruses*, 1995, p. 365, *Astroviridae*, Murphy FA, Fauquet CM, Bishop DHL, *et al*. (eds.), copyright 1995, with kind permission of Springer Science and Business Media.

Positive-sense genomes are packaged into these viral-like particles (VLPs), possibly through interactions with the first 70 amino acids of the capsid protein. The virions are released by an unknown mechanism, which may involve cellular caspases, after which the capsid undergoes an extracellular trypsin-mediated maturational cleavage. This increases infectivity up to 10^5 fold, condenses the virion to approximately 28 nm, and transforms the 79 kDa capsid protein into at least three smaller peptides of approximately 34, 29, and 26 kDa. Computational predictions suggest that VP34 may comprise the core of the virion while VP29 and VP26 form spike-like projections that may be important for viral tropism and receptor binding. This is corroborated by studies suggesting that VP26 is only loosely associated with the virion. These spikes are also thought to be responsible for the star morphology visible by EM (**Figure 1**(**a**)).

Evolution

Examination of nucleotide changes and nonsynonymous amino acid changes from the whole genome and across species suggests that an ancient divergence between avian and mammalian astroviruses occurred approximately 310 million years ago. Mammalian astroviruses split more recently into two distinct clades: human astroviruses and feline/mink-associated astroviruses. Phylogenetic clustering of the human astroviruses together argues against continual human–animal interspecies transmission. It is hypothesized that at least two interspecies transmission events (avian to porcine, porcine to feline) led to the current division of viruses. Further comparison of synonymous mutations by codon usage generates an astrovirus evolutionary pattern which mirrors the evolution of respective hosts, suggesting that recent evolution of the virus may have been in adaptation to the host. As RNA viruses, astroviruses are expected to undergo frequent genetic changes. However, nucleotide changes occur at rates of approximately 5% in human viruses over time, despite the co-circulation of muptiple serotypes within a region. Nucleotide and amino acid comparisons of ORF1a of human astroviruses demonstrate two distinct lineages, known as genogroup I (HAstV-1 to -5) and genogroup II (HAstV-6 and -7). Comparisons of ORF1b or ORF2 lack these distinct groups, leading investigators to postulate that a recombination event at the ORF1a/1b junction occurred before HAstV-6 or -7 diverged.

Clinical Features, Pathology, and Pathogenesis

Mammalian Astroviruses

Astrovirus infection in mammals presents clinically as gastroenteritis. Disease has been most closely studied in humans and, in volunteer studies, astrovirus-infected individuals develop diarrhea, the most prominent symptom, as well as vomiting, nausea, anxiety, headache, malaise, abdominal discomfort, and fever. Onset of symptoms at 2–3 days post infection (dpi) correlates with shedding of the virus in feces, although shedding can continue after resolution of other symptoms. Astrovirus infection has also been associated with intussusception, although a causative role has not been established.

The earliest studies of astrovirus pathogenesis utilized gnotobiotic sheep and calves as models. In calves, astrovirus infection was localized to the dome epithelial cells overlying Peyer's patches. These cells appeared flat or rounded and released cells were identified in the intestinal lumen. Astrovirus infection in calves was shown to be specifically targeted to M cells and led to the sloughing of necrotic M cells into the intestinal lumen. Enterocytes were never observed to be infected. Specific tropism of the virus for immune cells suggests that astrovirus may have an immunomodulatory role in calves. While the virus replicated in these animals and could be detected in their feces, the calves displayed no clinical signs. In most bovine studies, viral infection is asymptomatic, although changes in the feces from solid and brown to soft and yellow were noted in one study. Mild villus atrophy and slight changes in villus-to-crypt ratios have been noted but no changes in xylose absorption were observed. Despite the lack of symptoms, viral shedding continued until the termination of the experiment.

Studies in sheep have shed more light on histological changes associated with infection. Astrovirus-infected sheep developed a transient diarrhea as early as 2 dpi, but virus was detected at early as 14 hpi and initially confined to the lumenal tips of the intestinal villi. By 23 hpi, virus was observed coating the microvilli and infection had spread to the apical two-thirds of the villi. This correlated with sloughing of degenerate cells from the apical portion of the villi, which continued through 38 hpi. At this time, villus blunting was apparent in the ileum and midgut. Furthermore, normal epithelial cells lining the villi were replaced with immature, cuboidal cells reminiscent of crypt cells. Neither these immature cells nor crypt cells were ever observed to be infected, suggesting that only mature enterocytes are susceptible to infection. By 5 dpi, viral infection had cleared and intestinal histology had returned to normal.

Volunteer studies in humans have not explored the underlying causes of astrovirus pathogenesis; our knowledge is therefore limited to intestinal biopsies taken for other reasons, but generally support the observations described above. In a biopsy from a child shedding large quantities of astrovirus, slight histological changes, including mild villous blunting and irregular epithelial cells, were observed. Infection increased distally through the small intestine. Similarly to animal models, astrovirus

infection was restricted to the apical two-thirds of intestinal villi and could be identified in infected cells.

Avian Astroviruses

In avian species, astrovirus infection has a much broader range of disease than in mammals. While astrovirus does cause gastroenteritis in turkeys and chickens, it can also cause nephritis in chickens and a severe, often fatal, hepatitis in young ducklings.

Turkey astrovirus was the first discovered avian astrovirus and remains the best characterized in terms of pathogenesis, due in part to the development of the turkey as a small animal model. In these animals, virus could be detected from 1 to 12 dpi in the intestines. Viral replication was limited to the enterocytes on the apical portion of the villi, but the virus could be detected throughout the body, including the blood. The development of viremia is rare among enteric viruses and its function remains unclear. Infected turkeys developed a yellow, frothy, gas-filled diarrhea from 1 to 12 dpi. Diarrhea occasionally contained undigested food, but never blood. The intestines of infected birds became thin walled, flaccid, and distended. Despite these changes, histological examination suggested that only mild changes occur during infection. A mild crypt hyperplasia and shortening of the villi were noted from 4 or 5 to 9 dpi, and single degrading enterocytes could be identified. However, TUNEL staining suggested that the amount of cell death in infected intestines is similar to control birds. D-xylose absorption, a measure of intestinal absorption, was significantly decreased from 2 to 5 dpi in one study and up to 13 dpi in another. This effect was exacerbated in the presence of another enteric pathogen, turkey coronavirus. Astrovirus infection also caused a significant growth depression in turkey poults by 5 dpi; infected birds never recovered from this, leading to flock unevenness. Infected birds also demonstrated a transient (3–9 dpi) reduction of the thymus, which returned to normal by 12 dpi.

Avian infection by astroviruses can present with nonenteric symptoms as well. Infection of ducklings with duck astrovirus causes a severe hepatitis. Infected birds develop liver hemorrhage, swollen kidneys, and hepatocyte necrosis. On farms, infection leads to mortality rates of 10–25% in adult (4–6-week-old) ducks, but can reach 50% in ducklings under 14 days of age. In chickens, infection with the astrovirus avian nephritis virus (ANV) results in discoloration of the kidney, development of renal lesions, and interstitial nephritis by 3 dpi. Pathogenesis is age dependent, with 1-day-old chicks the most susceptible and adult birds the least. ANV infection can result in mortality rates of up to 33%, although rates appear to be strain specific.

Immune Response

The immunological response to astrovirus infection is poorly defined; however, observations in humans and animal models suggest that both the adaptive and innate responses play important roles in controlling and eliminating the virus.

The humoral immune response likely plays a major role in astrovirus immunity. The biphasic infection pattern of young children and the elderly suggests that antibodies are protective during the middle of life. Indeed, serological studies have indicated that approximately 50% of neonates have maternally acquired antibody to HAstV, which wane by 4–6 months of age. Children then acquire anti-HAstV antibodies rapidly due to astrovirus exposure. By the age of 9, up to 90% of the population has been exposed to HAstV-1. Furthermore, volunteer experiments demonstrate that astrovirus exposure generally leads to an increase in anti-astrovirus antibody titer. While astrovirus antibodies protected individuals from symptoms associated with infection, virus was identified in the feces, suggesting that such antibodies do not necessarily prevent viral replication. Additionally, immunoglobulin treatment has been attempted as a treatment for severe or chronic astrovirus infection. The results have been mixed and difficult to interpret, as the presence of astrovirus-specific antibodies in the immunoglobulin treatment was not always confirmed.

Cellular immunity may also play a role in controlling and/or preventing astrovirus infection. Studies have demonstrated that most individuals possess HLA-restricted, astrovirus-specific T cells. When stimulated with astrovirus *in vitro*, these cells produce tumor necrosis factor, interferon gamma, and occasionally interleukin (IL)-5 but not IL-2 or IL-4. These cytokines are typical of the T-helper-type response thought to be important in controlling viral infections. Individuals deficient in T and B-cell functions are unable to control infection, shedding virus to very high titers ($\geq 10^{14}$ particles ml^{-1}) and for extended periods of time (up to 18 months), further supporting the importance of cellular immunity.

Experiments in a turkey model demonstrate that the adaptive response is not the only important immunological response. In this model, no increase in T cells ($CD4^+$ or $CD8^+$) could be demonstrated after TAstV-2 infection. Moreover, while infected turkeys produced a slight increase in antibody production, these antibodies were not neutralizing and did not prevent against future infection. However, it was noted that macrophages from TAstV-2 infected turkeys produced significantly higher levels of nitric oxide (NO) both *in vivo* and upon stimulation *ex vivo*. Inhibition of NO *in vivo* led to a significant increase in viral production, while addition of exogenous

NO decreased viral production to below the detection limit, suggesting that NO is an important factor in controlling astrovirus infection. The importance of macrophages and their role in astrovirus infection has been corroborated by observations in astrovirus-infected lambs, where EM showed virions within macrophages. Furthermore, it is possible that astroviruses have a mechanism to combat this response, as macrophages in astrovirus-infected turkeys demonstrate a reduced ability to phagocytose.

Treatment, Prevention, and Control

Because astrovirus infection is generally mild and self-limiting in humans, treatment is generally restricted to fluid rehydration therapy. This can often be accomplished at home; thus, hospital admittance is rare. No vaccine is yet available for humans, and as noted above, immunoglobulin treatment for immunocompromised individuals has been met with varying degrees of success. Additionally, no treatment for astrovirus-infected animals exists. The best solution, therefore, is prevention of transmission, which is best done in humans by conscientious hand and food washing. The stability of astroviruses and their resistance to inactivation make them difficult to eliminate after introduction. This is a significant problem in hospitals, where individuals are generally immunocompromised and therefore more susceptible to infection. One outbreak in a bone marrow transplant ward prompted the hospital to scrub the entire ward with warm, soapy water. However, surveillance of the subsequent inhabitants demonstrated fecal shedding of astroviruses, underscoring the difficulty in removing the virus. This is also a significant problem in commercial farming, where astrovirus infection of animals significantly decreases productivity. Its introduction and maintenance in this environment can mean drastic financial losses. In each of these environments, early detection and thorough disinfection are keys to limiting transmission and controlling infection.

See also: Caliciviruses; Enteric Viruses; History of Virology: Vertebrate Viruses; Picornaviruses: Molecular Biology; Replication of Viruses; Rotaviruses; Viral Pathogenesis; Virus Particle Structure: Nonenveloped Viruses.

Further Reading

Koci MD (2005) Immunity and resistance to astrovirus infection. *Viral Immunology* 18: 11–16.

Koci MD and Schultz-Cherry S (2002) Avian astroviruses. *Avian Pathology* 31: 213–227.

Matsui SM and Greenberg HB (1996) Astroviruses. In: Fields BN, Knipe DM, and Howley PM (eds.) *Fields Virology,* 3rd edn., pp. 875–893. Philadelphia: Lippincott-Raven.

Monroe SS, Carter MJ, Herrmann JE, Kurtz JB, and Matsui SM (1995) *Astroviridae*. In: Murphy FA, Fauquet CM, Bishop DHL, *et al.* (eds.) *Virus Taxonomy: Sixth Report of the International Committee on Taxonomy of Viruses*, pp. 364–367. Vienna: Springer.

Monroe SS, Jiang B, Stine SE, Koopmans M, and Glass RI (1993) Subgenomic RNA sequence of human astrovirus supports classification of *Astroviridae* as a new family of RNA viruses. *Journal of Virology* 67: 3611–3614.

B

Baculoviruses: Molecular Biology of Granuloviruses

S Hilton, University of Warwick, Warwick, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Defective interfering particle Virus particles that are missing part of their genome. These deletions in their genome mean that they cannot sustain an infection by themselves and depend on coinfection with a suitable helper virus.
Occlusion body A crystalline protein matrix which surrounds the virions of some insect viruses.
Origin of replication A unique DNA sequence at which DNA replication is initiated.
Palindrome A sequence of DNA equal to its complementary sequence read backwards.

Introduction

The *Baculoviridae* are a family of invertebrate viruses with large circular, double-stranded DNA genomes (80–180 kbp). The genomes are packaged into nucleocapsids, which are enveloped and embedded in proteinaceous occlusion bodies. Baculoviruses are divided taxonomically into two genera, *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV). GVs and NPVs show major differences, not only in the morphology of their occlusion bodies but also in their tissue tropism and cytopathology. NPVs have large polyhedral-shaped occlusion bodies measuring 0.15–15 μm, comprised predominantly of a single protein called polyhedrin and typically have many virions embedded. GVs have small ovicylindrical occlusion bodies, which average about 150 nm × 500 nm. These are comprised predominantly of a single protein called granulin, which is related in amino acid sequence to polyhedrin. GVs normally have a single virion consisting of one nucleocapsid within a single envelope (**Figure 1**(**a**)). They infect both agricultural and forest pests, making them important as potential biological insecticides. To date, eight complete GV genomes have been sequenced (**Table 1**).

Infection Cycle

Baculoviruses produce two distinct virion phenotypes, occlusion-derived virus and budded virus, which are responsible for infection of insects (*per os* infection) and insect cells (cell-to-cell spread), respectively. Infection for both GVs and NPVs begins with the ingestion and solubilization of the occlusion bodies in the host larval midgut. The released occlusion-derived virus then fuses with the microvilli of midgut epithelial cells, and nucleocapsids pass through the cytoplasm to the nucleus. At the nuclear pore, the GV DNA is thought to be injected into the nucleus leaving the capsid in the cytoplasm. As replication proceeds, the nucleus enlarges and the nucleoli and chromatin move to its periphery (**Figure 1**(**b**)). The nuclear membrane ruptures early in infection in GVs, following the production of limited numbers of nucleocapsids. This is followed by extensive virogenesis, nucleocapsid envelopment and the formation of occlusion bodies in the mixed nuclear-cytoplasmic contents. Some nucleocapsids continue to form and pass out of the cell, forming budded virus which initiates secondary infection (**Figure 1**(**c**)). Baculoviruses encode two different major budded virus envelope glycoproteins, GP64 for group I NPVs and F protein for group II NPVs and GVs, which mediate membrane fusion during viral entry. Other nucleocapsids are enveloped and occluded within granulin-rich sites throughout the cell. As the number of occlusion bodies increase, the cell greatly enlarges and eventually lyses (**Figure 1**(**d**)). When the larvae die, the remaining cells lyse and the occlusion bodies are released back into the environment.

Taxonomy and Classification

GVs have been isolated only from the insect order Lepidoptera (butterflies and moths). They have been isolated from over 100 species belonging to at least 10 different host families within Lepidoptera, mainly Noctuidae and

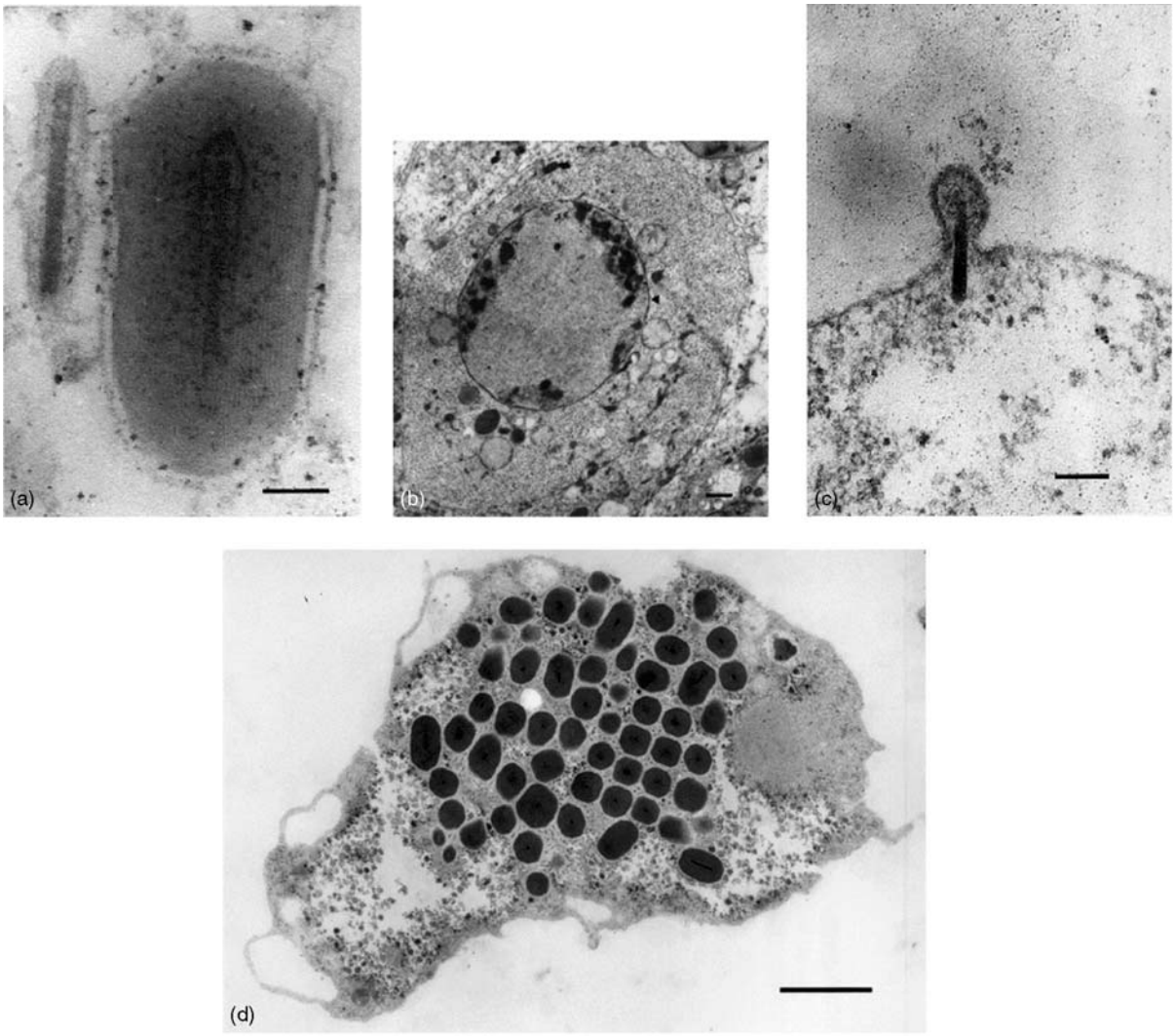


Figure 1 GV morphology. (a) Enveloped GV virion and adjacent occluded virion. (b) Early stages of GV replication. Nucleocapsids are present throughout the nucleus prior to disintegration of the nuclear membrane. The nuclear membrane is indicated by an arrowhead. (c) virus budding from plasma membrane. (d) *In vitro* replication of CpGV. *C. pomonella* cell late in infection containing occluded virus particles. Scale = 100 nm (a, c); 1 μm (b, d). (a–c) Reprinted from Winstanley D and Crook NE (1993) Replication of *Cydia pomonella* granulosis virus in cell cultures. *Journal of General Virology* 74: 1599–1609, with permission from Society for General Microbiology.

Tortricidae. There has been no formal classification of subgroups within the GVs. However, it is recognized that there are three types of GVs based on their tissue tropism, although these groups are not supported by phylogenetic analyses.

The first type of GV is slow-killing and predominantly infects larvae belonging to the family Noctuidae, but also includes the Torticidae-specific virus adoxophyes orana GV (AdorGV). They infect only the fat body of the larvae after the virus has passed through the midgut epithelium. Slow-killing GVs tend to kill in the final instar regardless of the instar in which the larvae are infected. These infected larvae tend to take longer to die than other GV- or NPV-infected larvae, typically from 10 to 35 days. A possible explanation for this is that important tissues such as the tracheal matrix and the epidermis are not infected.

The second type of GV includes the GV type species, *Cydia pomonella* GV (CpGV). They are relatively fast-killing, taking only 5–10 days to kill. The fat body is infected along with the epidermis, malpighian tubules, tracheal matrix, hemocytes, and many other tissues to a lesser extent. The faster speed of kill could be due to the infection of a wider range of body tissues. Fast-killing GVs tend to kill in the instar in which the larvae are infected or within the following instar. These viruses infect larvae from a variety of families of Lepidoptera including Tortricidae, Pieridae, Yponomeutidae, Pyralidae, and Gelechiidae.

The third type of GV contains only one species to date, *Harrisina brillians* GV (HabrGV). HabrGV replicates only

in the midgut epithelium cells of *H. brillians* larvae, from the family Zygaenidae. The larvae develop diarrhoea which consists of a discharge containing occlusion bodies. This can lead to a rapid spread of infection. The younger the larvae are infected, the longer they take to die, which is also typical of the slow-killing GVs.

Genome Organization

Phylogenetic analysis of the completely sequenced baculoviruses (**Figure 2**) shows that there is one clade which contains all of the Tortricidae-specific GVs. Although there are no other clearly defined clades, it is clear that the GVs tend to group based on the family of their host insect and not on their tissue tropism type. The only known type 3 GV, HabrGV, which infects insects from the family Zygaenidae, has not been completely sequenced and so was not included in these analyses. Previous phylogenetic analysis using the granulin gene placed HabrGV in a clade with the Torticidae-specific viruses. However, baculovirus relationships based solely on occlusion body protein sequence can disagree with other

Table 1 Features of the eight completely sequenced granuloviruses

Granulovirus	*Abbreviation*	*Length (nt)*	*AT content (%)*	*Number of ORFs*	*Host family*
Adoxophyes orana granulovirus	AdorGV	99 657	65.5	119	Tortricidae
Agrotis segetum granulovirus	AgseGV	131 680	62.7	132	Noctuidae
Choristoneura occidentalis granulovirus	ChocGV	104 710	67.3	116	Tortricidae
Cryptophlebia leucotreta granulovirus	CrleGV	110 907	67.6	128	Tortricidae
Cydia pomonella granulovirus	CpGV	123 500	54.8	143	Tortricidae
Phthorimaea operculella granulovirus	PhopGV	119 217	64.3	130	Gelechiidae
Plutella xylostella granulovirus	PlxyGV	100 999	59.3	120	Plutellidae
Xestia c-nigrum granulovirus	XecnGV	178 733	59.3	181	Noctuidae

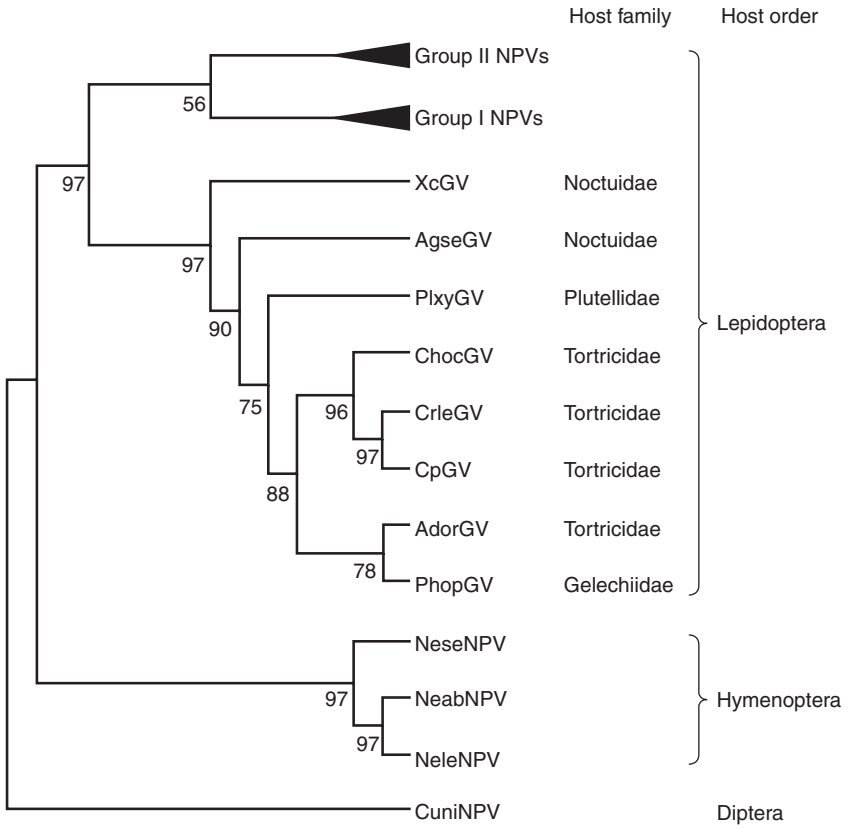


Figure 2 Baculovirus phylogeny (maximum pasimony) of completely sequenced baculoviruses based on the LEF-8 and LEF-9 concantenated sequences. Bootstrap percentage support values are indicated.

gene phylogenies, and further HabrGV genes need to be analyzed before evolutionary conclusions can be made.

The genomic organization of the GVs has been compared using gene parity plot analysis (**Figure 3**). Gene parity plots compare the relative positions of homologous genes in different genomes and illustrate conservation between baculovirus genomes. Gene arrangement in baculoviruses reflects their evolutionary history, with the more closely related viruses sharing a higher degree of gene collinearity. The gene order among the sequenced GVs is virtually identical, with between only one and five common genes in different positions along the genome. One of these, the *iap-3* gene, is in three different positions in the five genomes which contain it. This suggests that this gene may be a more recent acquisition. The GVs appear to be far more collinear than the NPVs. There are many inversions and rearrangements among the NPV genomes relative to each other. The only GV genome to show any inversion of a block of genes is phthorimaea operculella GV (PhopGV) which has a block of six genes (Phop113–118) inverted relative to the other GVs. This block of genes flanks a putative homologous repeat region, which in NPV genomes, are sites where most major rearrangements, insertions, and deletions are found. The gene order among GVs is quite different from that of NPVs (**Figure 3**). GVs share only one main region of collinearity with NPVs, although this is inverted with respect to the granulin/polyhedrin gene. This region is from autographa californica NPV (AcMNPV) open reading frames (ORFs) Ac103-61.

The gene content of CpGV is shown in **Figure 4** and **Table 2**. There are 62 genes common to all Lepidopteran baculoviruses sequenced to date. There are a further 28 genes common to all GVs, 22 of which are absent from NPVs. A further 22 GV genes are found in more than one GV, but not in NPVs. The GV-specific genes could be responsible for the biological differences between these two baculovirus groups. The relationship between biological differences and gene content of GVs and NPVs awaits the characterization of further genus specific genes. CpGV also contains 11 CpGV-specific genes, which may encode host-specific factors. There are also 20 genes which are present in some GVs and some NPVs, these are likely to be auxiliary genes, which are not essential for viral replication, but provide the virus with some selective advantage. These include *chitinase, cathepsin, iap-3, pe38, bro, ptp-2, rr1* and *rr2a, lef-10*, and egt. The GV auxiliary genes which have been assigned putative functions are shown in **Table 3.**

GV Replication *In Vitro*

Several NPVs replicate well in cell culture and some cell lines are commercially available. In contrast, there are only a few laboratories worldwide that have managed to maintain GV-permissive cell lines, which yield low virus

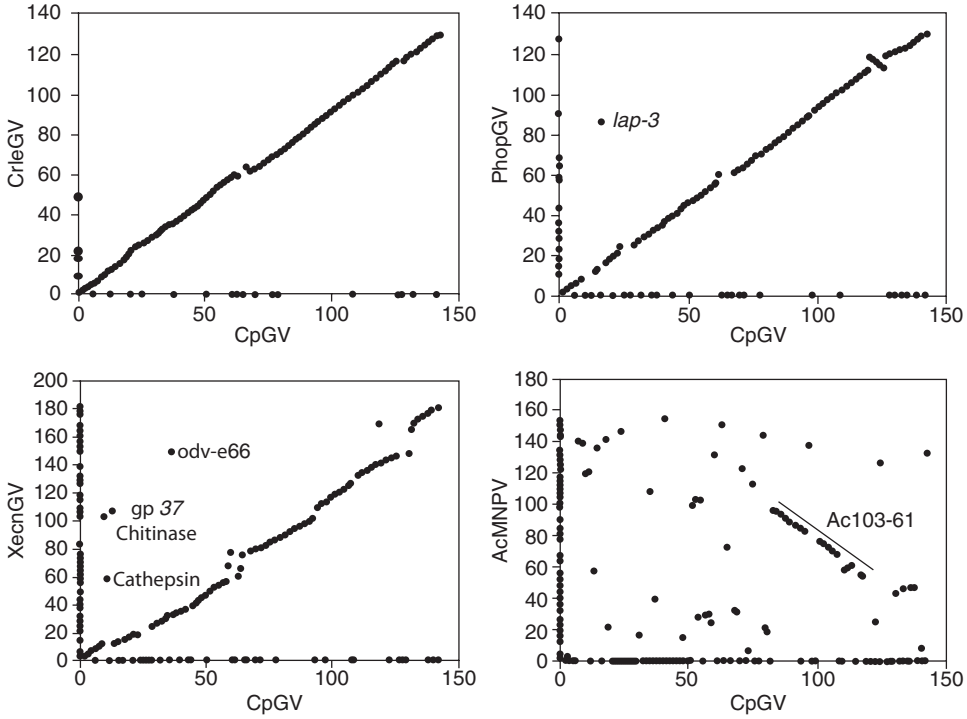


Figure 3 Gene parity plots of CpGV gene organization versus CrleGV, PhopGV, XecnGV, and AcMNPV.

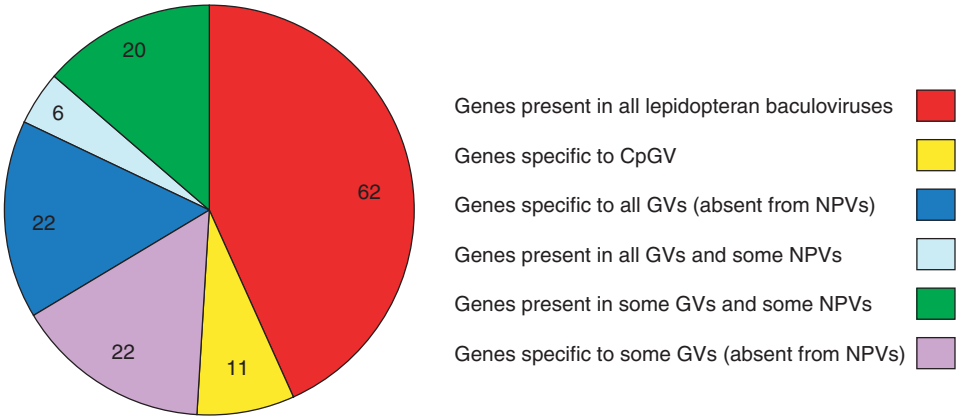


Figure 4 CpGV gene content. The number of CpGV genes (from a total of 143) are shown in each section of the pie chart.

Table 2 Genes present in all lepidopteran baculoviruses and those present in all GVs

	Genes present in all lepidopteran baculoviruses	*Additional genes present in all GVs (genes in bold italic are absent from NPVs)*
Transcription	*39K, p47, lef-5, lef-4, lef-6, lef-8, lef-9, lef-11, vlf-1*	
Replication	*lef-1, lef-2, dnapol, helicase, dbp1, lef-3, ie-1, me53,*	*Cp120 (dnaligase), Cp126 (helicase-2)*
Structural	*F protein, gp41, odv-ec27, odv-e56, p6.9, p74, vp91, vp39, vp1054, pif, fp25K, odv-e18, odv-e25, odv-e66, pk1, polh*	***pep/p10 (Cp22)**, Cp71(p24capsid)*
Auxiliary	*alk-exo, fgf, ubiquitin*	***mp-nase (Cp46), fgf-1 (Cp76), iap-5 (Cp116), fgf-3 (Cp140)**, Cp59(sod)*
Unknown	*38K, ac22, ac29, ac38, ac53, ac66, ac68, ac75, ac76, ac78, ac81, ac82, ac92, ac93, ac96, ac106, ac109, ac110, ac115, ac142, 38.7K, ac145, ac146, p40, p12, p45*	***Cp2, Cp4, Cp5, Cp20, Cp23, Cp29, Cp33, Cp39, Cp45**, Cp47(p13), **Cp50, Cp56**, Cp79(Ac150), **Cp99, Cp100, Cp115, Cp122, Cp135, Cp136***
Total	62	28 (***22***)

Table 3 Auxiliary gene content of GVs

	egt	*enhancin*	*rr1*	*rr2a*	*pe38*	*ptp-2*	*chitinase*	*cathepsin*	*Cp94 (iap)*	*iap-3*	*bro*	*lef-10*
AdorGV	✓	×	×	×	×	×	×	×	×	✓	×	×
AgseGV	✓[a]	✓	×	×	×	×	✓	✓	×	✓	×	✓
ChocGV	✓	×	×	×	×	×	×	×	×	✓	×	✓
CrleGV	✓	×	×	×	✓	✓	✓[b]	✓	✓	✓	×	✓
CpGV	✓	×	✓	✓	✓	✓(2)	✓	✓	✓	✓	✓[a]	✓
PhopGV	✓	×	×	×	×	×	×	×	✓	×	✓	✓
PlxyGV	✓	×	×	×	×	×	×	×	×	×	×	×
XecnGV	×	✓(4)	×	×	×	×	✓	✓	×	×	✓(7)	✓

Numbers in parenthesis indicate gene copy number if greater than one.
[a]Likely not functional due to truncation.
[b]Likely not functional due to chitinase active site absent.

titers. As a result, there are very few gene expression studies and little biochemical characterization of the GV genes. Currently, little is known regarding the molecular basis for phenotypic differences between GVs and NPVs. Consequently, some GV genes have been studied using NPV systems. These include the CpGV *cathepsin* and *iap-3* genes, trichoplusia ni GV (TnGV) *helicase* and *enhancin* genes and the xestia c-nigrum GV (XecnGV) *mmp* gene. However, the study of GV genes in their native virus and host cells is preferable. Only three GVs have

replicated in cell culture. TnGV was reported to infect *Trichoplusia ni* cells but their permissive character was not maintained. Complete viral replication of PhopGV has been reported in a *P. operculella* cell line but has not led to any molecular studies. A *C. pomonella* embryonic cell line permissive for CpGV has enabled molecular studies on GVs to progress and recombinant CpGV viruses to be produced.

Gene Expression

The gene expression of NPVs has been found to be sequential and coordinated. It is essentially split into two stages. Early gene expression precedes DNA replication and late/very late gene expression occurs after the onset of DNA replication. The successive stages of viral gene expression are dependent on the previous gene products. Early genes are thought to be transcribed using the host RNA polymerase II. Therefore, promoters of NPV early genes tend to mimic promoters of eukaryotic genes. Late and very late genes are transcribed by virus-induced RNA polymerase that recognizes the late promoter motif (A/T/G)TAAG. Temporal studies on GV gene expression are limited due to a lack of highly permissive cell lines. However, GV genes possess promoters similar to the early and late promoters described above, and transcription of a small number of genes has been shown to initiate from them. It is therefore likely that GVs follow the same cascade of gene expression as NPVs.

AT Content

The sequenced GV genomes are AT-rich, ranging from 54.8% in CpGV, to 67.6% in cryptophlebia leucotreta GV (CrleGV), with an average of 62.6% (**Table 1**). CpGV and CrleGV are the most closely related sequenced GVs, although their AT content is highly divergent. The difference in AT content is mainly due to the base composition of the third nucleotide position within the codon of the coding regions. The host-cell machinery has to provide the virus with sufficient suitable t-RNA to replicate. The viruses may therefore have adapted to the base composition of the host. CrleGV is only infective to *C. leucotreta*, whereas CpGV is infective to both hosts, *C. pomonella* and (to a lesser degree) *C. leucotreta.* However, the AT content of these hosts is not yet known. CpGV is the only sequenced GV to contain the genes encoding the large and small subunits of ribonucleotide reductase (*rr1* and *rr2a*), which are also found in several NPVs and other DNA virus genomes. These enzymes are involved in nucleotide metabolism and catalyze the reduction of host cell rNTPs to dNTPs. It may be advantageous for CpGV to maintain these genes in order to alter the composition of dNTPs, allowing for replication in hosts with a divergent AT composition.

GV Specific Genes

Metalloproteinase

Baculovirus genomes have been shown to contain a number of proteases. Those studied so far are classified as nonessential auxiliary genes. Enhancin is a metalloproteinase which digests the peritrophic membrane in the insect midgut to facilitate virus infection. It has so far been found in all Noctuidae-specific GVs and one Tortricidae-specific GV (choristoneura fumiferana GV) and also in several group II NPVs. A cysteine proteinase (cathepsin) is involved in postmortem degradation of the infected host, in conjunction with a viral chitinase homolog. Cathepsin is found in most lepidopteran NPVs and four of the sequenced GVs (**Table 3**). A third group of proteases has been identified exclusively in the GVs, with homology to the matrix metalloproteinases (MMP). MMPs are active against components of the extracellular matrix and are usually secreted. All eight GV genomes possess an MMP homolog, suggesting it plays an important role in GV infection. Very little is known about MMP activity in infected larvae. A role for MMP in the breakdown of infected host tissue, potentially facilitating virus dispersal, and stimulation of postmortem melanization, has been proposed for XecnGV MMP. This study was performed using XecnGV MMP, overexpressed in occlusion-negative bombyx mori NPV (BmNPV), and may not be representative of its function in GV infection. However, results suggested that XecnGV MMP did encode a functional MMP. Putative signal peptide sequences are found in all of the GV MMP sequences except XecnGV. This suggests that these proteins, in keeping with MMPs, may enter the secretory pathway. Analysis of the predicted GV MMP amino acid sequences shows that residues predicted to be essential for metalloproteinase activity are conserved between all of the GV MMP sequences. The greatest conservation is in the region of the predicted zinc-binding active site of the enzyme. The conservation of MMP in all sequenced GVs implies that MMP plays an important role during GV infection, possibly in the breakdown of the basement membrane. As MMP is found exclusively in the GVs, its activity may lead to a difference in the infection process between GVs and NPVs.

Inhibitors of Apoptosis

Baculoviruses possess two families of genes that suppress apoptosis, the P35/P49 family and the inhibitor of apoptosis (IAP) family. The IAP-3 protein of CpGV was the first member of the baculovirus IAP family of proteins to

be identified. It has been shown to block apoptosis in diverse systems and substitute for the *p35* gene in blocking AcMNPV-induced apoptosis in SF21 cells. Since then all of the GVs (and lepidopteran NPVs) have been found to contain IAP homologs. The GVs contain between one and three IAP homologs. Phylogenetic analyses have demonstrated that there are three clades of GV IAPs suggesting that they are not gene duplications. The first group contains the previously characterized IAP-3, which is found in CpGV, CrleGV, choristoneura occidentalis GV (ChocGV), AdorGV, and agrotis segetum GV (AgseGV). A second group includes Cp94 which has homologs, in the same genome position, in CrleGV and PhopGV. This IAP has so far been shown not to have any anti-apoptotic activity. A third group contains IAP-5 which is specific to all of the GVs. All GV genomes sequenced to date contain IAP-5, which is absent from NPV genomes. The IAP-5 genes are located in the same positions on the GV genomes suggesting an ancient acquisition before the GVs diverged. This is in contrast to IAP-3 which is one of the only GV genes found in different positions within the GV genomes, suggesting a more recent acquisition. The CpGV IAP-5 has not been shown to have anti-apoptotic activity by itself, but it can stimulate the anti-apoptotic activity of CpGV IAP-3. However, its function in genomes lacking IAP-3 is unknown. The P35/P49 family was thought to be NPV specific but recently a P35/P49 homolog has been identified in ChocGV, although functionality has yet to be ascertained.

Fibroblast Growth Factors

Fibroblast growth factors, or FGFs, are a family of growth factors involved in tissue repair and cell proliferation and differentiation. It has been suggested that baculovirus FGFs may be required for efficient spread of infection beyond the midgut. FGFs are conserved among vertebrate and invertebrate organisms, but within the viruses they have only been identified in baculoviruses. All of the GV genomes contain three *fgf* genes, whereas there is only one *fgf* gene present in the NPV genomes (homologs of AcMNPV Ac32). Ac32 is transcribed both early and late in infection and is secreted when expressed in insect cells, which suggests that it acts as an extracellular ligand. An Ac32-null mutant was constructed and compared to wild-type virus in cell culture. No obvious differences were observed in protein or DNA synthesis, although its pathogenesis *in vivo* has not yet been evaluated.

All of the GV FGF proteins show homology to the FGF domain in Ac32. Phylogenetic analyses have demonstrated that there are three clades of GV FGFs suggesting that they are not gene duplications. The NPV FGFs did not group clearly with any GV FGF clade, but FGF-2 is usually annotated as the Ac32 homolog. It has not been determined whether all copies are functional and the advantage of three GV *fgf* genes with respect to pathogenesis is not yet known.

DNA Ligase and Helicase-2

All GV genomes contain the genes, *DNA ligase* and *helicase-2*. The *helicase-2* gene is part of the helicase superfamily I which includes PIF1 from eukaryotes and RecD from *Escherichia coli*. These enzymes are typically involved in DNA metabolism, such as replication, recombination, and repair. The only NPVs to contain either of these genes are lymantria dispar (LdMNPV) which contains a *DNA ligase* and a *helicase-2*, and mamestra configurata NPV (MacoNPV) and spodoptera litura NPV (SpltNPV) which both contain a *helicase-2*. The LdMNPV DNA ligase displays catalytic properties of a type III DNA ligase. However, in LdMNPV, neither the *helicase-2* nor the *DNA ligase* gene stimulates DNA replication in transient assays. The roles of *DNA ligase* and *helicase-2* in viral replication are unknown but based on their homology to genes from other organisms, it is likely that they are involved in DNA recombination or repair systems.

PEP/P10

Three GV genes form part of a highly conserved GV gene family. These are the homologs of the CpGV genes, Cp20, Cp22, and Cp23. Two of the genes, Cp20 and Cp23, are 35% identical to each other. Phylogenetic analyses of Cp20 and Cp23 and their homologs in other GVs clearly suggest that they are likely to be paralogous genes which were duplicated in a common ancestor before the GVs differentiated. The three genes show a significant similarity to domains of the baculovirus polyhedron envelope/calyx protein (PEP). PEP is thought to be an integral part of the polyhedron envelope. It is concentrated at the surface of polyhedra, and is considered important for the proper formation of the periphery of polyhedra. It is thought that PEP may stabilize polyhedra and protect them from fusion or aggregation. Electron microscopic evidence exists for a GV calyx and PEP may play a similar role in GVs.

The GV homologs of Cp22 share a number of motifs with P10, including a proline-rich domain and a heptad repeat sequence. In NPV-infected cells, P10 forms fibrillar structures in the nucleus and cytoplasm. These structures have yet to be identified in electron micrographs of GVs. It is possible that GV fibrous bodies are smaller, more granular structures, which may have been overlooked. The P10 protein is implicated in occlusion body morphogenesis and disintegration of the nuclear matrix, resulting in the dissemination of occlusion bodies. P10 is also crucial for the proper formation of the polyhedron envelope. The Cp22 homologs are over twice the size of most NPV P10 proteins and much of the sequence identity is between sequences of low complexity. The large size of the Cp22 homologs may

be due to the presence of domains similar to the calyx/pep protein of NPVs. It has been suggested that in GVs the PEP and P10 may be conserved in a single protein (Cp22) and that the other members of the gene family (Cp20 and Cp23) may also be involved in the formation of the occlusion body envelope.

Origins of Replication

Within the genomes of NPVs, sequences have been identified that function as origins of replication (*ori*s) when cloned into plasmids and transfected into infected cells. In some NPVs, these also function as enhancers of early gene transcription. These regions, called homologous regions (*hr*s), typically contain one or more copies of an imperfect palindrome sequence and are located in several positions on the genome. They have been identified in most NPVs sequenced to date and have been found to act as *ori*s in several NPVs using infection-dependent DNA replication assays. In addition, complex structures containing multiple direct and inverted repeats of up to 4000 bp have also been identified that act as *ori*s. These have similar structural characteristics to eukaryotic *ori*s and are found only once per genome; they are therefore called non-*hr ori*s. The discovery that serial passage of some NPVs results in the appearance of defective interfering particles (DIs) containing reiterations of a non-*hr ori*, is further evidence that non-*hr ori*s may be involved in genome replication. It has also been suggested that the expansion of non-*hr ori*-like regions is not restricted to DI particles. A 900 bp region within the non-*hr ori*-like region of CrleGV was amplified in submolar populations in CrleGV *in vivo,* resulting in the expansion of the hypervariable region.

The CpGV genome contains 13 imperfect palindromes of 74–76 bp (**Figure 5**), which are found in 11 regions of the genome. Six of these palindromes are within putative genes. The 13 palindromes have been characterized using an infection-dependent replication assay in *C. pomonella* cells and all have been found to replicate. The palindromes are most conserved at their ends and have a highly AT-rich center. The entire 76 bp palindrome is required for replication, with no replication occurring when 10 bp from each end of the palindrome are removed. The specific flanking DNA of each palindrome is required for optimal replication, even though there was no homology between the flanking sequences. A region reminiscent of the non-*hr* type *ori* in NPVs has also been identified in the CpGV genome. However, this does not replicate in the infection-dependent replication assay and its function in GVs is not yet known.

Singleton imperfect palindromes are also found in CrleGV, ChocGV, PhopGV, and AdorGV. These GVs group in a clade with CpGV and it is likely that these palindromes descended from a common viral ancestor. The palindromes range in size from 63 bp in CrleGV to 320 bp in PhopGV and are often found in putative *hr*s which also consist of short direct repeats. The number of palindromes in each putative *hr* ranges from typically one up to four, which is less than the number of repeated palindromes in most NPV *hr*s. The ends of the palindromes are conserved in all of these GVs having a consensus of 13 bp (A(C/T)GAGTCCGANTT). The centers of the palindromes differ in sequence but all are AT-rich. These palindromes may have the potential to form secondary structures, such as cruciform (stem-loop or hairpin) structures through intrastrand base pairing. These structures may allow the initiation of DNA replication via the binding of specific protein factors. They may also remain in the linear state, acting as binding sites for protein dimers. This appears to be the case for the AcMNPV *hr*5 palindrome and the regulatory protein IE-1. NPV *hr*s have been demonstrated to be *cis*-acting enhancers of transcription of baculovirus early genes including *39K*, *ie-2*, *p35*, and *helicase.* It is not yet known if CpGV *hr*s act as enhancers of early gene transcription.

The other GVs which do not group in the CpGV clade (PlxyGV, XecnGV, and AgseGV) have quite different putative *hr* regions. PlxyGV has large *hr* regions of up to 2383 bp consisting of direct repeats of 101–105 bp, repeated up to 23 times with a 15 bp palindrome near the center. XecnGV contains direct repeats of about 120 bp, repeated 3–6 times without any palindromes. So far been no putative *hr*s have been identified in the AgseGV genome. The only shared



Figure 5 The 13 CpGV imperfect palindromes that acts as origins of replication.

feature between all of the GV *hr*s is the relative positions of some of the *hr*s within the genome. For example, there is often a putative *hr* between the *desmoplakin* and *lef-3* genes, between *sod* and *p74* and flanking or within the *vp91* gene. Within the NPVs, *hr* regions are also often found flanking *sod* and *vp91*, which suggests that some of the *hr*s may have originated before the GVs and NPVs diverged.

See also: Baculoviruses: Apoptosis Inhibitors; Baculoviruses: Expression Vector; Baculoviruses: General Features; Baculoviruses: Molecular Biology of Mosquito Baculoviruses; Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Baculoviruses: Molecular Biology of Sawfly Baculoviruses; Baculoviruses: Pathogenesis.

Further Reading

Escasa SR, Lauzon HAM, Mathur AC, Krell PJ, and Arif BM (2006) Sequence analysis of the *Choristoneura occidentalis* granulovirus genome. *Journal of General Virology* 87: 1917–1933.

Hashimoto Y, Hayakawa T, Ueno Y, Fujita T, Sano Y, and Matsumoto T (2000) Sequence analysis of the *Plutella xylostella* granulovirus genome. *Virology* 275: 358–372.

Hayakawa T, Ko R, Okano K, Seong S-I, Goto C, and Maeda S (1999) Sequence analysis of the *Xestia c-nigrum* granulovirus genome. *Virology* 262: 277–297.

Herniou EA, Olszewski JA, Cory JS, and O'Reilly DR (2003) The genome sequence and evolution of baculoviruses. *Annual Review of Entomology* 48: 211–234.

Hess RT and Falcon LA (1987) Temporal events in the invasion of the codling moth *Cydia pomonella* by granulosis virus: An electron microscope study. *Journal of Invertebrate Pathology* 50: 85.

Hilton SL and Winstanley D (2007) Identification and functional analysis of the origins of DNA replication in the *Cydia pomonella* granulovirus genome. *Journal of General Virology* 88: 1496–1504.

Jehle JA (2002) The expansion of a hypervariable, non-*hr ori*-like region in the genome of *Cryptophlebia leucotreta* granulovirus provides *in vivo* evidence for the utilization of baculovirus non-*hr ori*s during replication. *Journal of General Virology* 83: 2025–2034.

Jehle JA (2006) Molecular identification and phylogenetic analysis of baculoviruses from lepidoptera. *Virology* 346: 180–193.

Lange M and Jehle JA (2003) The genome of the *Cryptophlebia leucotreta* granulovirus. *Virology* 317: 220–236.

Luque T, Finch R, Crook N, O'Reilly DR, and Winstanley D (2001) The complete sequence of *Cydia pomonella* granulovirus genome. *Journal of General Virology* 82: 2531–2547.

Miller LK (ed.) (1997) *The Baculoviruses.* New York: Plenum.

O'Reilly DR and Vilaplana L (2003) Functional interaction between *Cydia pomonella* granulovirus IAP proteins. *Virus Research* 92: 107–111.

Winstanley D and Crook NE (1993) Replication of *Cydia pomonella* granulosis virus in cell cultures. *Journal of General Virology* 74: 1599–1609.

Wormleaton S, Kuzio J, and Winstanley D (2003) The complete sequence of the *Adoxophyes orana* granulovirus genome. *Virology* 311: 350–365.

Baculoviruses: Molecular Biology of Mosquito Baculoviruses

J J Becnel, Agriculture Research Service, Gainesville, FL, USA
C L Afonso, Southeast Poultry Research Laboratory, Athens, GA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Epizootiology The study of the causes and forms of diseases at all levels of intensity in an animal population.
Hypertrophy Denotes greater bulk through an increase in the size of a cell but not in the number of individual tissue elements.
Occlusion body Proteinaceous bodies that occlude the virions of certain insect viruses expecially baculoviruses (DNA) and cypoviruses (RNA).
Patent infection External signs of infection with a pathogen that are obvious at the macro level of diagnostics.

Introduction

Perhaps the first scientific description of a baculovirus was made in 1808 from silkworms in France but it was more than 150 years later before the first baculovirus was isolated from a mosquito. Mosquito baculoviruses are of growing interest as they may represent a separate branch within the family *Baculoviridae* that existed prior to the split of lepidopteran nucleopolyhedroviruses (NPVs) and granuloviruses (GVs). They may also be ancestral to the baculoviruses from hymenoptera which form a branch distinct from the lepidopteran baculoviruses. Mosquitoes are also important vectors of numerous human and veterinary diseases and baculoviruses offer the opportunity to investigate specific virus–host interactions at the molecular level. This possibility has been facilitated by the

availability of the complete genomes for one mosquito baculovirus and several important mosquito vectors, especially the malaria vector *Anopheles gambiae*.

History and Classification

The family *Baculoviridae* currently contains the genera *Nucleopolyhedrovirus* and *Granulovirus*. Members of the genus *Nucleopolyhedrovirus* have occlusion bodies (OBs) that contain many virions while members of the genus *Granulovirus* have OBs that contain one or rarely two virions. The baculoviruses found in mosquitoes thus far have been mainly assigned to the *Nucleopolyhedrovirus* group although it has been proposed that they represent a new genus in the family *Baculoviridae*. (Jehle and collaborators have recently proposed that a new genus, *Deltabaculovirus*, should be created in the family *Baculoviridae* for dipteran-specific baculoviruses.) The first baculovirus (OcsoNPV) to be isolated from mosquitoes was from *Ochlerotatus sollicitans* (formerly *Aedes sollicitans*) in Louisiana, USA. Since then, naturally occurring NPVs have been isolated from 11 additional mosquito species (**Table 1**) in the genera *Aedes, Anopheles, Culex, Ochlerotatus, Psorophora, Uranotaenia*, and *Wyeomyia*. More than 20 mosquito species have been found to be susceptible to NPV infections.

Before 2001, the only detailed studies on development and transmission of mosquito NPVs were conducted with an isolate from *O. sollicitans* (OcsoNPV) found in several different mosquito hosts in Louisiana. Recently, a great deal of new information has been compiled for a mosquito NPV from *Culex nigripalpus* (CuniNPV) and to a lesser extent for an NPV from *Uranotaenia sapphirina* (UrsaNPV). Therefore, information presented here will draw heavily on CuniNPV to describe current knowledge of the biology, morphology, and genomics of mosquito baculoviruses.

Virion and OB Properties

Mature OBs are relatively large (5–20 μm) and polyhedral in shape or small (0.5 μm) and globular in shape (**Figure 1**). The OBs lack an envelope (the polyhedron envelope of NPVs), a characteristic feature of most other baculoviruses. OBs can contain a variable number of virions depending on the species, ranging from very few (1–2) in *Anopheles crucians* to many (50+) in some *Ochlerotatus* spp. (**Table 1**). Nucleocapsids are singly enveloped and the resulting virions are approximately 200 nm × 40 nm. Each virion consists of the nucleocapsid, intermediate layer, and an outer envelope. In some species (*Ochlerotatus sollicitans nucleopolyhedrovirus*, *Uranotaenia sapphirina nucleopolyhedrovirus*) early OBs are irregularly shaped and seem to subsequently coalesce to form large polyhedra with many virions while in other species (*Culex nigripalpus nucleopolyhedrovirus*) the OBs remain as relative small globular particles with 4–8 virions. Mature OBs of UrsaNPV have a unique dumbbell shape, lack the polyhedron envelope, and measure up to 10–15 μm in length and 2–3 μm in diameter.

Occlusion bodies of CuniNPV have a density of 1.14–1.18 g ml^{-1} on a Ludox continous gradient. Dissolution of the OBs requires a $pH > 12.0$. Temperatures below 50 °C have a minimal effect on infectivity. Temperatures above 55 °C result in a total loss of activity.

Pathology and Histopathology

Mosquito NPVs are highly specific for midgut tissues (primarily midgut epithelium) of mainly larval mosquitoes but infections have been found in midguts of adult mosquitoes. Patent infections are detected by the hypertrophied nuclei of midgut cells that appear white due to the accumulation of occlusion bodies (**Figure 2**).

CuniNPV affects the development, behavior, and appearance of infected *C. quinquefasciatus* and *C. nigripalpus*

Table 1 Mosquito baculovirus names and corresponding abbreviations

Virus	*Abbreviation*	*Accession number*
Anopheles crucians nucleopolyhedrovirus	AncrNPV	
Culex nigripalpus nucleopolyhedrovirus	CuniNPV	AF403738
Culex pipiens nucleopolyhedrovirus	CupiNPV	
Culex salinarius nucleopolyhedrovirus	CusaNPV	
Ochlerotatus sollicitans nucleopolyhedrovirus	OcsoNPV	
Ochlerotatus taeniorhynchus nucleopolyhedrovirus	OctaNPV	
Ochlerotatus triseriatus nucleopolyhedrovirus	OctrNPV	
Psorophora confinnis nucleopolyhedrovirus	PscoNPV	
Psorophora ferox nucleopolyhedrovirus	PsfeNPV	
Psorophora varipes nucleopolyhedrovirus	PsvaNPV	
Uranotaenia sappharina nucleopolyhedrovirus	UrsaNPV	
Wyeomyia smithii nucleopolyhedrovirus	WysmNPV	

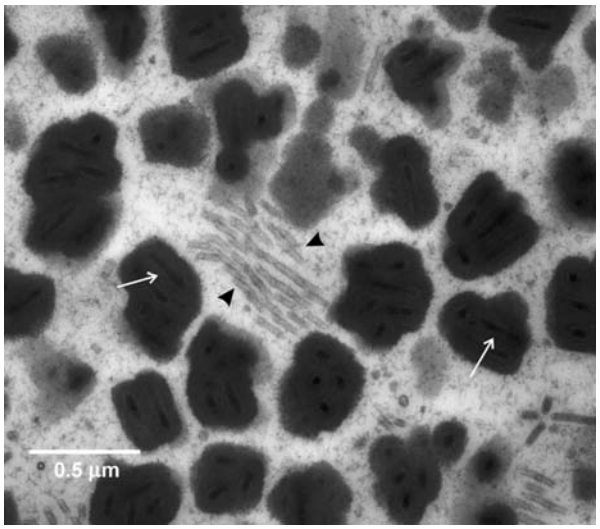


Figure 1 Ultrastructural features of CuniNPV OBs and virions. Arrow indicates rod-shaped virons within an OB. Arrowhead indicates empty capsids.

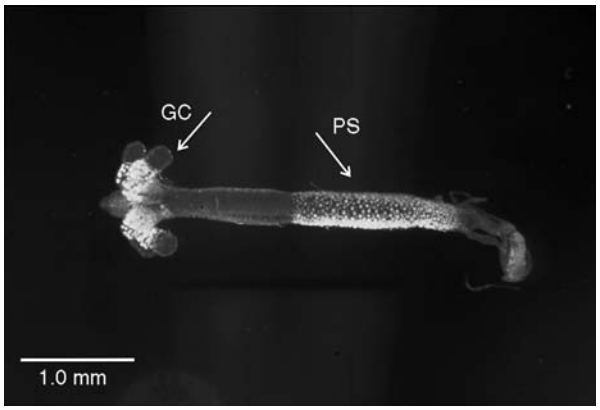


Figure 2 Gross pathology of CuniNPV in a dissected midgut of a *Culex quinquefasciatus* larvae. Arrows indicate infected nuclei in the gastric caecae and the posterior stomach.

larvae. Within 24 h postinfection (p.i.), infected larvae are typically stunted in size when compared to unexposed individuals, indicating a failure to molt or problems with nutrient uptake. Larvae actively feed through about 48 h p.i., but by 72 h p.i. larvae are lethargic and often remain suspended at the water surface. By 48 h p.i., nuclei of most cells in the gastric ceca and posterior stomach (**Figure 2**) are opaque to white in color due to the proliferation of OBs. Cells in the anterior stomach rarely support viral development of CuniNPV but the entire midgut may support infection in some species as occurs in OcsoNPV. Death of the larvae usually occurs within 72–96 h p.i., at which time most susceptible nuclei in midgut and gastric ceca are infected.

Life Cycle

A cascade of events is required for the invasion, replication, and spread of CuniNPV in the mosquito host. Infections are initiated when larval mosquitoes ingest OBs together with the appropriate divalent cation, usually magnesium (see next section). Occlusion-derived virions (ODVs) are released from OBs due to the alkaline conditions of the midgut together with other possible factors. The released ODVs attach and pass through the peritrophic matrix (PM) followed by attachment to the membrane of the microvilli and entry into the cytoplasm of midgut epithelial cells. Invasion of the nucleus occurs when nucleocapsids attach to the nuclear envelope where DNA is released into the nucleoplasm to initiate viral replication. This process of ingestion of infectious particles to the invasion of the nucleus occurs within 2–4 h post exposure to OBs. The next phase of the process involves the rapid spread of CuniNPV to other midgut cells. The initial viral replication produces nucleocapsids that are released from the nucleus that form extracellular virions (the budded virion, BV), which are released out of the midgut cells into the ectoperitrophic space. The BVs can then attach to microvilli of other midgut cells and make their way to the nucleus to initiate another round of replication. Newly synthesized nucleocapsids acquire *de novo* envelopes in the nucleus and become occluded by the OB protein. The nuclei become packed with OBs which are released upon death of the host and cause infection when ingested by a susceptible mosquito larva. The spread within the mosquito midgut and production of OBs occurs within 14–48 h post exposure.

Transmission and Host Range

Initial attempts to transmit CuniNPV to larval mosquitoes in the laboratory were unsuccessful. Studies using deletion analysis of the most abundant cations present in the field water were used to determine the water-borne factors critical for transmission of CuniNPV. Assays of *C. quinquefasciatus* larvae exposed to CuniNPV were conducted in an artificial salt mixture based on the analysis of the ion composition of field water. The addition of salts to deionized water significantly improved the infection levels in larvae. Salt mixtures without magnesium resulted in less than 1.0% infections. Salts with magnesium or salts without calcium increased infection levels to 80–100%. Further investigations revealed conclusively that transmission is mediated by divalent cations: magnesium is essential, whereas the presence of calcium inhibits the magnesium's activity to mediate transmission. In addition, other divalent cations can function as activators and inhibitors of CuniNPV transmission. Activators include barium, cobalt, nickel, and strontium, while additional

inhibitors of transmission are copper, iron, and zinc. Transmission of CuniNPV has only been successful to mosquitoes within the genus *Culex*, subgenus *Culex*. These include *C. nigripalpus, C. quinquefasciatus*, *C. salinarius*, *C. pipiens*, *C. pipiens* f. *molestus*, and *C. restuans*. *Culex territans* (subgenus *Neoculex*) is the only *Culex* species tested that is not susceptible to CuniNPV. No infections have been found with any species of *Aedes*, *Anopheles*, *Ochlerotatus, Culiseta*, or *Toxorhynchites* exposed in laboratory assays. Transmission of UrsaNPV is also driven by cations similar to that found for CuniNPV with a host range restricted to species of *Uranotaenia*. The very restricted host range of CuniNPV and UrsaNPV differs from transmission studies conducted with OcsoNPV, showing that *Aedes*, *Ochlerotatus*, and *Psorophora* spp. are susceptible but not *Culex* and *Anopheles* spp.

Field Epizootiology

Mosquito baculoviruses have been less commonly reported from field populations than other baculoviruses and generally at very low prevalence rates (<1.0%). Recently, natural epizootics of CuniNPV have been studied at two field sites in Florida: one, a swine wastewater site with *C. nigripalpus* dominant during the warmer months and *C. quinquefasciatus* dominating during the cooler months. Regular and extended epizootics of CuniNPV in *C. nigripalpus* were documented at this site with an average infection rate of 20.1% and a maximum rate of 60%. *C. quinquefasciatus* larvae have an average CuniNPV infection rate of 8.6% and a maximum infection rate of 20%. The second site was a dairy wastewater facility where *C. quinquefasciatus* was the dominant species. Although high larval populations of *C. quinquefasciatus* were present, CuniNPV infected larvae were collected rarely and never at epizootic levels (0.08% infection). The Mg^{2+}/Ca^{2+} ratios at the swine wastewater site are 1.9/0.8 mM while these ratios at the dairy wastewater site were 3.7/3.0 mM. It appears that the favorable Mg^{2+}/Ca^{2+} ratios at the swine wastewater site mediated transmission of CuniNPV while the high calcium levels at the dairy wastewater site were unfavorable for transmission.

Genome Structure

CuniNPV contains a circular double-stranded DNA genome packaged into rod-shaped singly enveloped nucleocapsids. Complete nucleotide sequencing (GenBank accession no. AF403738) has determined a size of 108 252 base pairs encoding densely arranged bidirectional nonoverlapping clusters of two to ten open reading frames (ORFs). CuniNPV encodes at least 109 putative proteins, some of which are homologous to those from other baculoviruses involved in early and late gene expression, DNA replication, structural and auxiliary functions. Only 36 of the 109 putative CuniNPV predicted proteins demonstrate clear homology to proteins from other baculoviruses, and 72 of the CuniNPV ORFs show no homology to any other known baculovirus ORFs. Amino acid conservation between CuniNPV and other baculovirus proteins is low (18–44% to hymenopteran, and 18–54% to lepidopteran viruses), with an average of 28% identity to autographa californica multiple NPV (AcMNPV). CuniNPV genes display clusters of only two to four genes in similar transcriptional orientation or genomic position in comparison to other known baculoviruses, but 46 putative genes contain typical early and late baculovirus promoters upstream from the initiation codons. The CuniNPV genome contains distinctive baculovirus features such as homologous regions (*hr*s) that have been implicated as enhancers of viral transcription and also as origins of DNA replication. Four *hr*s located in intergenic regions are composed of 64–85-bp repeats but lack sequence similarity to *hr*s from other baculoviruses.

RNA Transcription

Baculovirus infections are initiated by the host RNA polymerases that transcribe early promoters in conjunction with the baculovirus transcriptional transactivators. Following DNA replication, baculovirus RNA polymerases transcribe genes containing late promoters. The large number of CuniNPV homologs of baculovirus genes involved in RNA transcription and DNA replication suggests that CuniNPV may utilize similar mechanisms, but with species-specific differences. CUN096 (*lef-4*), CUN026 (*lef-8*), CUN059 (*lef-9*), and CUN073 (*p47*) are homologs of genes that encode the multi-subunit RNA polymerases involved in late transcription. In addition, CUN088 (*lef-5*) has been implicated in late gene transcription. CUN018 is similar to very late transcription factor-1 (*vlf-1*), the major transactivator of very late gene expression. Although CuniNPV contains the minimal complex necessary for late polymerase activity (*lef-4*, *lef-8*, *lef-9*, and *p47*), it lacks homologs of other lepidopteran baculovirus expression factors which are required for optimum levels of late transcription (*lef-6*, *lef-10*, *lef-11*, *lef-12*, and *pp31* (*39k*)) in other baculovirus–host systems. Some of the transcription factors absent in CuniNPV are species specific (*lef-7*, *lef-10*, *lef-12*, and *host cell specific factor-1*), but others are conserved in many completely sequenced lepidopteran baculoviruses (*lef-6*, *lef-11*, *pk-1*, and *pp31* (*39k*)). Since promoters of early baculovirus genes are transcribed by a combination of host RNA polymerase II and virally encoded transcription factors, the absence of *ie-0*, *ie-1*, *ie-2*, and *pe38* homologs suggests that CuniNPV is either completely dependent on host factors for early transcription, or that unique CuniNPV genes are involved in these functions. Alternatively, sequence

conservation of certain CuniNPV genes may be extremely low, thus making identification of homologs from other baculoviruses impossible.

DNA Replication

CuniNPV replicates in the nuclei of the infected host. Of the six virally encoded AcMNPV genes implicated as being essential for replication, four homologs are present in CuniNPV (CUN045 (*lef-1*), CUNO25 (*lef-2*), CUN089 (*helicase-1*) and CUN091 (*dna-pol*)). In addition, CUN054 (*alk-exo*) and CUN018 (*vlf-1*) are also likely involved in DNA replication. The CuniNPV genome contains six baculovirus-repeated ORFs (*bro* ORFs) (CUN001, CUN004, CUN005, CUN095, CUN108, and CUN109). All six CuniNPV *bro* ORFs contain the sequence for the 41-amino-acid motif common to all *bro* proteins and two additional amino acid motifs conserved in group I and III *bro* proteins. Some *bro* proteins have been shown to bind DNA and may be involved in viral DNA replication. CuniNPV is distinguished from other hymenopteran and lepidopteran baculoviruses by the existence of significant amino acid differences in proteins involved in viral replication and by the absence of a number of genes considered essential and nonessential for DNA replication. CuniNPV lacks recognizable homologs for *lef-3*, which has the properties of a single-stranded DNA-binding protein (*dbp*), for *lef-7*, proliferating cell nuclear antigen (*pcna*), for *DNA ligase*, *rnr1*, and *rnr2* (large and small subunits of ribonucleotide reductase), and for *dUTPase.*

Virion and Other Structural Functions

CuniNPV has two virion phenotypes: an occluded form (ODV) which initiates infection in the midgut, and a budded form (BV) which spreads the infection within the midgut. CuniNPV contains only 8 of the 17 virion structural protein genes conserved among lepidopteran baculoviruses (*vp39*, *vp91*, *vp1054*, *odv-ec27*, *odv-e56*, *p6.9*, *gp41*, and *p74*). CUN024 is similar to the *vp39* capsid protein gene, CUN035 resembles the *vp91*gene encoding a protein found in both the capsid and envelope of ODVs, and CUN008 resembles the *vp1054* gene for a virion-associated protein that functions in nucleocapsid formation. Homologs of the genes encoding capsid-associated proteins p87, p24, and orf1629 are absent or not identifiable in CuniNPV. Interestingly, orf1629 has been shown to be an AcMNPV essential protein that is associated with the basal structure of the capsid. While CuniNPV lacks a homolog of AcMNPV *gp64*, it does encode for a homolog of the gene for LdMNPV envelope protein Ld130 (CUN104), which is also a fusion protein and can functionally replace *gp64.* The CUN104 also demonstrates relatedness to gene for the envelope fusion protein Se8, of spodoptera exigua multiple NPV (SeMNPV), and CG4715, a *Drosophila* gene product related to Ld130. CUN032 is the homolog of *odv-ec27*, which encodes a protein present in ODV nucleocapsids and envelopes and may function as a cyclin. CUN074 is similar to the gene for p74, an ODV protein required for oral infectivity. CUN102 is the homolog of *odv-e56*, which encodes a protein associated with both viral-induced intranuclear vesicles and envelopes. CUN033 is the homolog of the gene for the ODV tegument protein gp41. The protein gp41 is required for the production of polyhedra and the egress of nucleocapsids from the nucleus is blocked. The absence of multiple lepidopteran baculovirus gene homologs suggests involvement of another set of viral or perhaps cellular proteins in CuniNPV ODV assembly and cellular entry functions. Structural genes absent or not identifiable in CuniNPV include *orf1629*, *p87*, *gp64*, *p24*, *odv-e18*, *odv-e25*, *odv-e66*, *p10*, *pp34*, *polyhedrin/granulin*, and *fp25k.*

Virion Occlusion

CuniNPV OBs are globular, lack a polyhedron envelope and calyx structure, and typically contain about four individually enveloped virions. In addition, although they appear to be composed of a peptide similar in size to lepidopteran polyhedrin or granulin (about 30 kDa), N-terminal amino acid sequence analysis did not reveal homology to other baculovirus OB proteins. However, the sequence did match positions 693–709 of CUN085, an ORF with no homology to any other known baculovirus gene. The large size of the CUN085 product (882 amino acids) suggests that it is cleaved to produce components of CuniNPV OBs. CuniNPV lacks homologs of the genes encoding polyhedrin, granulin, or the polyhedron-associated proteins p10, FP25 (a conserved protein involved in polyhedra formation), and pp34, the polyhedron envelope and calyx protein. The lack of a *pp34* homolog is reflected in the absence of a polyhedron envelope and calyx surrounding the OB. The small size of the CuniNPV OBs, the limited number of virions occluded, and the lack of an envelope and calyx structure are reminiscent of the structure of the OBs of GVs. The absence of a P10 gene in the CuniNPV genome is supported by observed morphological differences from cells infected with lepidopteran NPVs. In infected lepidopteran cells, p10 is expressed as an abundant protein that is associated with nuclear and cytoplasmic fibrillar bodies during the terminal stages of infection. Fibrillar material produced during CuniNPV infection, however, does not resemble the fibrillar bodies associated with other lepidopteran baculovirus infections. Most notably,

the cytoplasmic features of CuniNPV-infected cells are microtubule bundles and irregular cisternae of smooth endoplasmic reticulum.

Auxiliary Functions

Auxiliary genes are nonessential for viral replication in cell culture, although they likely provide selective advantages in insects. CuniNPV contains one gene (CUN075) with homology to the baculovirus anti-apoptosis *p35* gene, but it lacks homologs of the inhibitor of apoptosis (*iap*) family of genes. CUN075 lacks sequence identity to *p35* in the 110-amino-acid C-terminal region, which in AcMNPV p35 mediates anti-apoptotic activity, suggesting a different mode of action or function for this gene. CUN039 shows relatedness to genes for insulin-binding proteins of *Spodoptera frugiperda*. It is also related to a *Caenorhabditis elegans* gene of unknown function and the *Drosophila* gene IMP-L2. Insulin and related peptides are very important hormones for the regulation of host growth and metabolism. The insulin-related peptide binding protein secreted from *S. frugiperda* cells is composed of two immunoglobulin-like C2 domains, is capable of binding human insulin, and inhibits insulin signaling through the insulin receptor. The closest homolog of the *S. frugiperda* binding protein is the essential protein IMP-L2 found in *Drosophila melanogaster*. IMP-L2 also binds insulin and related peptides and is implicated in neural and ectodermal development. CUN039 may have a similar role in modifying host metabolism and/or development. CuniNPV lacks homologs of at least 14 other baculovirus auxiliary genes, including those encoding for superoxide dismutase, ubiquitin, inhibitor of apoptosis, protein kinase 1, and viral enhancing factors, but the existence of a large number of genes of unknown functions in the CuniNPV genome suggest that other auxiliary genes of unknown function may exist.

Phylogenetic Relationships to Other Baculoviruses

Striking differences in genome organization, the lack of conservation in gene order, the low level of amino acid conservation for homologous genes, and the absence of many genes that are conserved in other baculoviruses suggest that CuniNPV is a distant relative to lepidopteran and hymenopteran baculoviruses (**Figure 3**). Phylogenetic analyses using DNA polymerase, p74, and concatenated gene sequences place CuniNPV in a baculovirus lineage distinct from the three other lineages (lepidopteran NPVs and GVs and hymenopteran NPVs). The most striking differences with other baculoviruses are the absence of homologs of genes that have been found to be essential for or stimulatory to both DNA replication and gene transcription in lepidopteran baculoviruses and the lack of homologs of genes involved in the formation of virogenic stroma, nucleocapsid, envelope of occluded

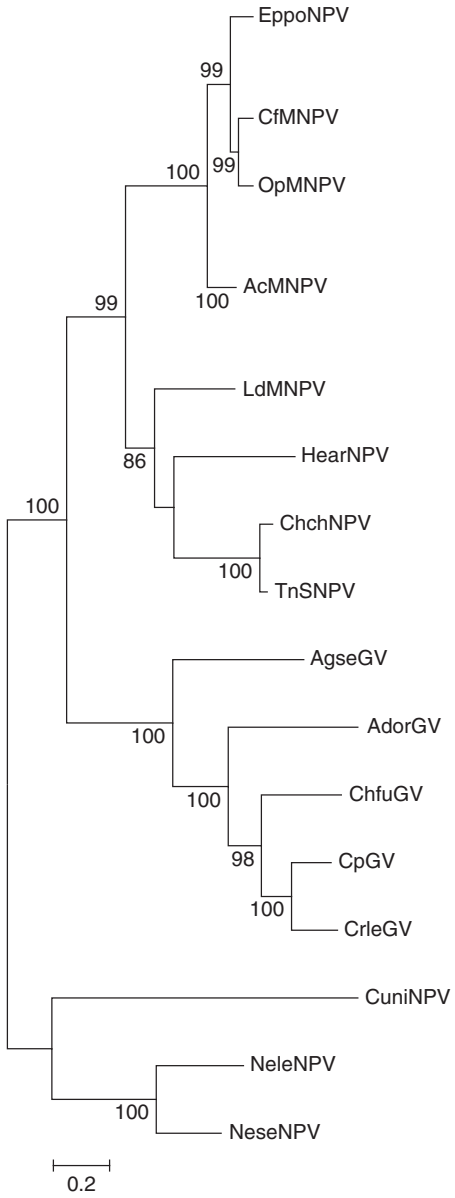


Figure 3 Maximum likelihood phylogenetic tree for the p74 gene of CuniNPV and baculoviruses representative of different lineages (obtained using Phyml discrete gamma model and 100 bootstraps). Abreviations are as follows: EppoNPV, epiphyas postvittana NPV; CfMNPV, choristoneura fumiferana MNPV ; OpMNPV, orgyia pseudotsugata MNPV; AcMNPV, autographa californica MNPV; LdMNPV, lymantria dispar MNPV; HearNPV, helicoverpa armigera NPV. ChchNPV, chrysodeixis chalcites NPV; TnSNPV, trichoplusia ni SNPV; AgseGV, agrotis segetum GV; AdorGV, adoxophyes orana GV; ChfuGV, choristoneura fumiferana granulovirus; CpGV, cydia pomonella GV; CrleGV, cryptophlebia leucotreta GV; CuniNPV, culex, nigripalpus NPV; NeleNPV, neodiprion lecontei NPV; NeseNPV, neodiprion sertifer NPV.

virions, and polyhedral formation. It has been suggested that some viruses, including baculoviruses, may co-evolve with their hosts in a process known as host-dependent evolution. Molecular evidence indicates that the ancestral Lepidoptera and Diptera separated about 280 million years ago and the extent of the genomic differences between the lepidopteran baculoviruses and CuniNPVs may reflect this ancient separation.

See also: Baculoviruses: General Features; Baculoviruses: Molecular Biology of Granuloviruses; Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Baculoviruses: Pathogenesis.

Further Reading

Afonso CL, Tulman ER, Lu Z, *et al.* (2001) Genome sequence of a baculovirus pathogenic for *Culex nigripalpus*. *Journal of Virology* 75: 11157–11165.

Andreadis TG, Becnel JJ, and White SE (2003) Infectivity and pathogenicity of a novel baculovirus, CuniNPV from *Culex nigripalpus* (Diptera : Culicidae) for thirteen species and four genera of mosquitoes. *Journal of Medical Entomology* 40: 512–517.

Anthony DW, Hazard EI, and Crosby SW (1973) A virus disease in *Anopheles quadrimaculatus*. *Journal of Invertebrate Pathology* 22: 1–5.

Becnel JJ, White SE, Moser BA, *et al.* (2001) Epizootiology and transmission of a newly discovered baculovirus from the mosquitoes *Culex nigripalpus* and *C. quinquefasciatus*. *Journal of General Virology* 82: 275–282.

Becnel JJ, White SE, and Shapiro AM (2003) Culex nigripalpus nucleopolyhedrovirus (CuniNPV) infections in adult mosquitoes and possible mechanisms for dispersal. *Journal of Invertebrate Pathology* 83: 181–183.

Federici BA (1980) Mosquito baculovirus: Sequence of morphogenesis and ultrastructure of the virion. *Virology* 100: 1–9.

Federici BA (1985) Viral pathogens of mosquito larvae. *Bulletin of the American Mosquito Control Association* 6: 62–74.

Federici BA and Anthony DW (1972) Formation of virion-occluding proteinic spindles in a baculovirus disease of *Aedes triseriatus*. *Journal of Invertebrate Pathology* 20: 129–138.

Federici BA and Lowe RE (1972) Studies on the pathology of a baculovirus in *Aedes triseriatus*. *Journal of Invertebrate Pathology* 20: 14–21.

Herniou EA, Olszewski JA, Cory JS, and O'Reilly DR (2003) The genome sequence and evolution of baculoviruses. *Annual Review of Entomology* 48: 211–234.

Jehle JA, Blissard GW, Bonning BC, *et al.* (2006) On the classification and nomenclature of baculoviruses: A proposal for revision. *Archives of Virology* 151: 1257–1266.

Moser BA, Becnel JJ, White SE, *et al.* (2001) Morphological and molecular evidence that Culex nigripalpus baculovirus is an unusual member of the family *Baculoviridae. Journal of General Virology 82: 283–297.*

Perera OP, Valles S, Green TB, *et al.* (2006) Molecular analysis of an occlusion body protein from Culex nigripalpus nucleopolyhedrovirus (CuniNPV). *Journal of Invertebrate Pathology* 91: 35–42.

Shapiro AM, Becnel JJ, and White SE (2004) A nucleopolyhedrovirus from *Uranotaenia sapphirina* (Diptera: Culicidae). *Journal of Invertebrate Pathology* 86: 96–103.

Stiles B, Dunn PE, and Paschke JD (1983) Histopathology of a nuclear polyhedrosis infection in *Aedes epactius* with observations in four additional mosquito species. *Journal of Invertebrate Pathology* 41: 191–202.

Stiles B and Paschke JD (1980) Midgut pH in different instars of three *Aedes* mosquito species and the relation between pH and susceptibility of larvae to a nuclear polyhedrosis virus. *Journal of Invertebrate Pathology* 35: 58–64.

Baculoviruses: Molecular Biology of Sawfly Baculoviruses

B M Arif, Great Lakes Forestry Centre, Sault Ste. Marie, ON, Canada

© 2008 Published by Elsevier Ltd.

Glossary

Anopheles gambiae Mosquito from the genus Anopheles.
Apis mellifera Western or European honeybee.
Gene parity plot A plot of the sequential organization and collinearity of homologous genes within two genomes.
Gilpinia hercyniae European spruce sawfly.
Hymenoptera Large order of insects comprising of sawflies, ants, bees, and wasps.
Neodiprion abietis Balsam fir sawfly.
Neodiprion lecontei Red-headed pine sawfly.
Neodiprion sertifer European pine sawfly.
Synteny Preserved order of genes between related species.

Introduction

Hymenoptera having existed since the Triassic period ($205–248 \times 10^6$ years ago) represent a more ancient order of insect than Lepidoptera, which appeared on earth much later during the Cretaceous period of approximately $65–144 \times 10^6$ years ago. Sawflies are considered primitive

Hymenoptera and have existed since the Mesozoic period. Nucleopolyhedroviruses (NPVs) that infect sawflies have always been known to be excellent biological control agents. For example, the European spruce sawfly, *Gilpinia hercyniae*, was introduced to North America early in the twentieth century and became a serious forest pest of spruce in northeastern Canada and the United States. A singly embedded NPV infecting *Gilpinia hercyniae* was imported from Finland and upon introduction to infested stands, the virus spread rapidly in the population and caused the collapse of the infestation. Viruses of sawflies are considered highly host specific and efficacious pathogens. One of the main reasons for its efficacy is the insect habitat. Sawflies are gregarious in nature and the virus causes what has been described as infectious diarrhea which is the sloughing off of infected cells causing rapid spread of infection in the population. The use of sawfly viruses in the control of infestations in Canada and Europe has been well documented. Recently, it has been shown that a decline in infestation of the balsam fir sawfly, *Neodiprion abietis*, was consistently associated with the presence of an NPV of this insect (NeabNPV). It was later proved that an epizootic by NeabNPV initiated population decline of the balsam fir sawfly.

In the laboratory of Dr. James Maruniak at the University of Florida, we became interested in the genomics of sawfly viruses for three main reasons. First, since sawflies represent a more ancient order of insects, we wondered if their viruses actually represent ancestors to the more recent baculoviruses of Lepidoptera. Second, restriction endonuclease analyses have shown that these viruses contain a much smaller genome than baculoviruses of Lepidoptera. Third, while lepidopteran baculoviruses generally infect a wide variety of larval tissues, tropism of sawfly viruses is limited to the larval midgut and we wondered whether there is a relationship between tissue tropism and the small number of genes in the viral genome.

In order to reveal the gene content and organization in the genomes of sawfly viruses, the DNAs of two viruses were fully sequenced; namely those of the *Neodiprion lecontei* and the *Neodiprion sertifer* NPVs (NeleNPV and NeseNPV, respectively). In this brief review we highlight features that distinguish them from baculoviruses of Lepidoptera and show how these differences might play a role in the biology of the virus.

Gene Content and Organization

The open reading frame (ORF) organization in the genome of NeleNPV is shown in **Figure 1**. The genome of this virus contains 89 predicted ORFs while that of NeseNPV contains 90 ORFs. These are the smallest baculovirus genomes sequenced so far. The G+C content is quite low of the order of 33%. Both viruses have 44 ORFs that are homologous to known baculovirus genes and additional 43 that are only shared with each other. Alignment of the two genomes reveals that they are mostly collinear but a noticeable difference is seen between the genes encoding polyhedrin (ORF1) and DNA binding protein (*dbp*, ORF14) **Figure 2**. This region has been termed nonsyntenic region (NSR) due to the lack of conserved synteny. Within the NSR, NeseNPV contains 15 ORFs that are absent from the genome of NeleNPV. The latter has eight ORFs within this region that are absent from the genome of NeseNPV. Also within the NSR, *nese18* and *nese19* encode proteins that are homologs and share 71.2% amino acid identity. They lack an identifiable promoter, are in the opposite orientation, and are flanked either by homologous repeated sequences (*hrs*) or direct repeated sequences (*drs*). The region also contains the NeseNPV methyltransferase homolog (NeseNPV ORF 5), which is absent from the genome of NeleNPV, and the inhibitor of apoptosis (*iap*) homologs (*nese17* and *Nele11*). It has also been noted that the closest BLAST matches of these ORFs were to insect proteins rather than to baculovirus proteins. The NeleNPV NELE11 had its highest match to an IAP from *Sprodoptera frugiperda* and the NeseNPV NESE5 had its highest match to a honeybee protein. The NeseNPV NESE17 was closest to a *Bombyx mori* IAP. The NSR also has homologs to trypsin-like proteases from insects (*nese7* and *nele6*). It has been hypothesized that the NSR probably arose by horizontal transfer of a cluster of ORFs from an insect host or hosts and only those genes that survived selection processes remained in the genomes.

Specific Genes

Inhibitors of Apoptosis

NeseNPV and NeleNPV contain iap genes that have their closest identity to insect-specific *iap* genes indicating that the viral genes were acquired by a transfer from an insect host. This hypothesis has been previously suggested for other baculoviruses. Both *iap* homologs (*nese17* and *nele11*) were closest to insect IAPs than to each other or to IAPs from other baculoviruses. The NESE17 contained one IAP repeat (BIRs) and a zinc finger while the NELE11 contained two BIRs and lacked a ring-finger motif.

Trypsin-Like Serine Protease

Until the sequencing of the NeleNPV and the NeseNPV genomes, no trypsin-like serine protease has been reported in the genomes of baculoviruses. The predicted protein of *nele6* has the trypsin catalytic triad of histidine, aspartic, and serine and the six conserved cysteines in NESE7. Not surprisingly these ORFs share maximum identity with insect trypsin-like serine proteases. Phylogenetic analysis

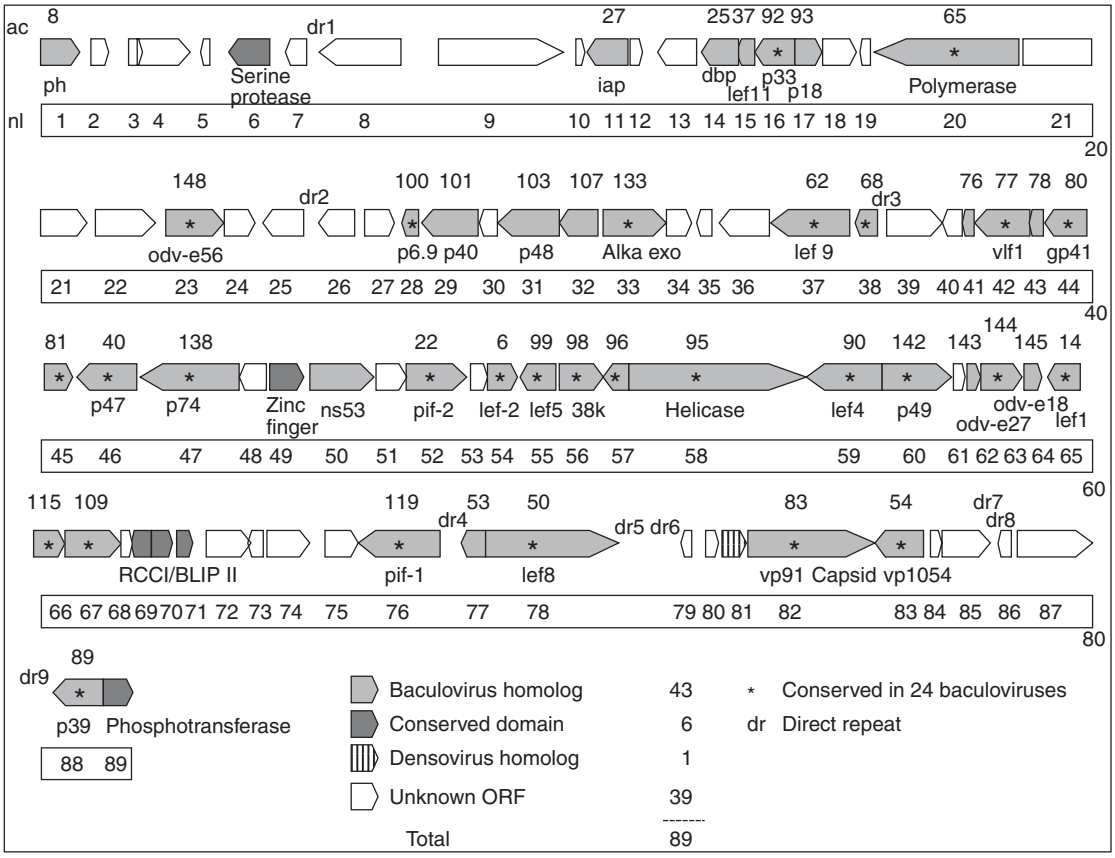


Figure 1 A linearized map of the NeleNPV genome. Arrows indicate ORF orientation and the ORF numbers are indicated below the arrows. Reprinted from Lauzon HAM, Lucarotti CJ, Krell PJ, Feng Q, Retnakaran A, Arif BM (2004) Sequence and organization of the *Neodiprion lecontei* nucleopolyhedrovirus genome. *Journal of Virology* 78: 7023–7035.

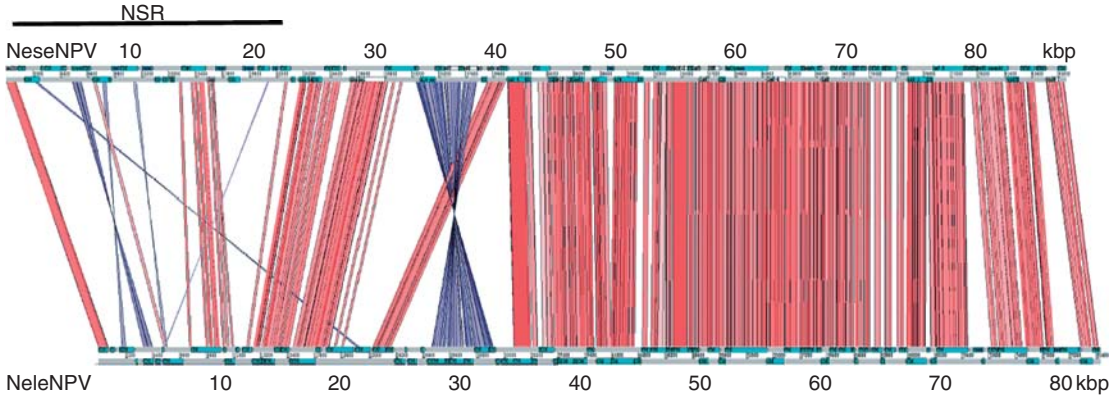


Figure 2 Alignments of the NeseNPV and NeleNPV genomes. The genomes are mostly collinear, as indicated by the red lines. Blue lines show inversions. The nonsyntenic region (NSR) is indicated. Reprinted from Lauzon HAM, Garcia-Maruniak A, Zanotto PMA, *et al*. (2006) Genomic comparison of neodiprion sertifer and *Neodiprion lecontei* nucleopolyhedroviruses and identification of potential hymenopteran baculovirus-specific open reading frames. *Journal of General Virology* 87: 1477–1489.

showed that NELE6 and NESE7 grouped with the homolog from *Apis mellifera* and exhibited relatedness to other insect trypsin-like serine proteases. It was suggested that NeleNPV and NeseNPV acquired the gene from an ancestral baculovirus by a horizontal transfer from a host.

Phosphotransferase

Phosphotransferases have been reported in eukaryotes and in limited numbers of eubacteria and archaeal organisms. To date, they have not been reported in viruses

until the sequencing of the two sawfly viruses. This enzyme is involved in tRNA splicing. Phylogenetic analysis showed that the hymenopteran baculovirus homologs grouped with the eukaryotic enzymes. The closest identity of the nele89 was to a phosphotransferase from *D. melanogaster.*

Regulator of Chromosome Condensation Proteins (RCC1)

These proteins play a role in chromosome condensation and are associated with chromatin or bind directly to DNA. Both NeleNPV and NeseNPV genomes contain three RCC1 homologs that are not found in other baculoviruses. In fact, these are the only viruses that have RCC1/BLIP domains mentioned in the InterPro taxonomic treatment for RCC1 proteins. Predicted proteins from *nese74* and *nele71* have strong matches to insect RCC1 proteins but they share very low amino acid identity with each other.

Zinc-Finger Protein

Zinc fingers are domains known to bind nucleic acids and comprise a varied superfamily. The proteins encoded by *nese52* and *nele49* contain four C2H2 zinc-finger domains and share an amino acid identity of 35.6%. The C2H2 zinc-finger domains contain 25–30 amino acids including two cysteines and two histidines that are conserved in a motif of C2C12H3H. Top matches for the proteins from *nese52* and *nele49* were to *Anopheles gambiae* (36% aa identity) to *D. melanogaster* zinc-finger protein (36% aa identity), respectively.

Membrane Fusion Proteins (MFPs)

Baculoviruses encode either a GP64 (group I NPVs) or an F protein which is a homolog of the *Lymantria dispar* NPV LD130 (group II and in some group I NPVs). A homolog to LD130 was also reported in the mosquito, *Culex nigripalpus,* NPV (CuniNPV), which is a baculovirus of mosquitoes. These proteins are a diagnostic feature of the budded virus phenotype and mediate the fusion of the virus to cell membrane and facilitate the release of nucleocapsids into the cell. The budded virus phenotype is responsible for the dissemination of infection within larval tissues and in tissue culture cells. These proteins have a distinct structure of a signal peptide, transmembrane domain, conserved cysteines, and a furin cleavage site. Thorough searches of all the potential ORFs in the genomes of NeseNPV and NeleNPV revealed the absence of an MFP. Several ORFs were found to contain transmembrane domains but lacked the other features of MFPs. It, therefore, appears that the budded virus phenotype may not play a role in the biology of sawfly baculoviruses and, indeed, this phenotype may not be needed by these viruses).

Immediate Early Proteins (IE1)

IE1 is a main transcriptional transactivator that activates early and late baculovirus gene expression. The *ie-1* gene itself is transactivated by host cell factors. It has been known that this is an absolutely essential gene in baculoviruses that basically initiates the infection process within the cell. Thorough searches of ORF sequence and structure in the genomes of NeleNPV and NeseNPV did not expose the presence of this gene. It appears that these two viruses may not need an IE-1 or utilize a hitherto unknown protein to substitute its function.

Auxiliary Genes

Normally, baculoviruses contain a number of auxiliary genes that are not needed for infectivity but appear to give the virus a selective advantage in nature. Practically none of the baculovirus genes classified as auxiliary have been found in the genome of NeleNPV and NeseNPV. Only the gene encoding an alkaline exonuclease (*alk-exo*) was located in these genomes.

Densovirus Capsid Protein

Densoviruses contain a linear single-stranded 4–6 kbp DNA genome that can be either positive or negative sense. Interestingly, ORFs *nele81* and *nese83* had strong BLAST matches to densovirus structural proteins. Highest matches were to proteins 1–4 from *Casphalia extranea* densovirus to a capsid protein from *Bombyx mori* densovirus. It is interesting to note that densoviruses infect a variety of larval tissues; however, the *Bombyx mori* densovirus is restricted to the midgut. It is not possible at the present time to ascertain if these proteins are functional in the hymenopteran baculoviruses.

Gene Parities and Phylogeny

Gene parity plots, have shown that genes are basically organized in a collinear manner in closely related baculovirus genomes. Many viruses have distinct rearrangements such as deletions, inversions, insertions, etc. that are revealed when comparing two genomes. Data have shown that in comparisons of the NeleNPV genome with those from representatives group I NPVs (AcMNPV) and group II NPVs (HaSNPV), a GV (PxGV) and CuniNPV, the gene order was not generally conserved. Except for a central portion of four genes (*lef-5, 38k, ac96, helicase*), there was no conservation of gene order (**Figure 3**).

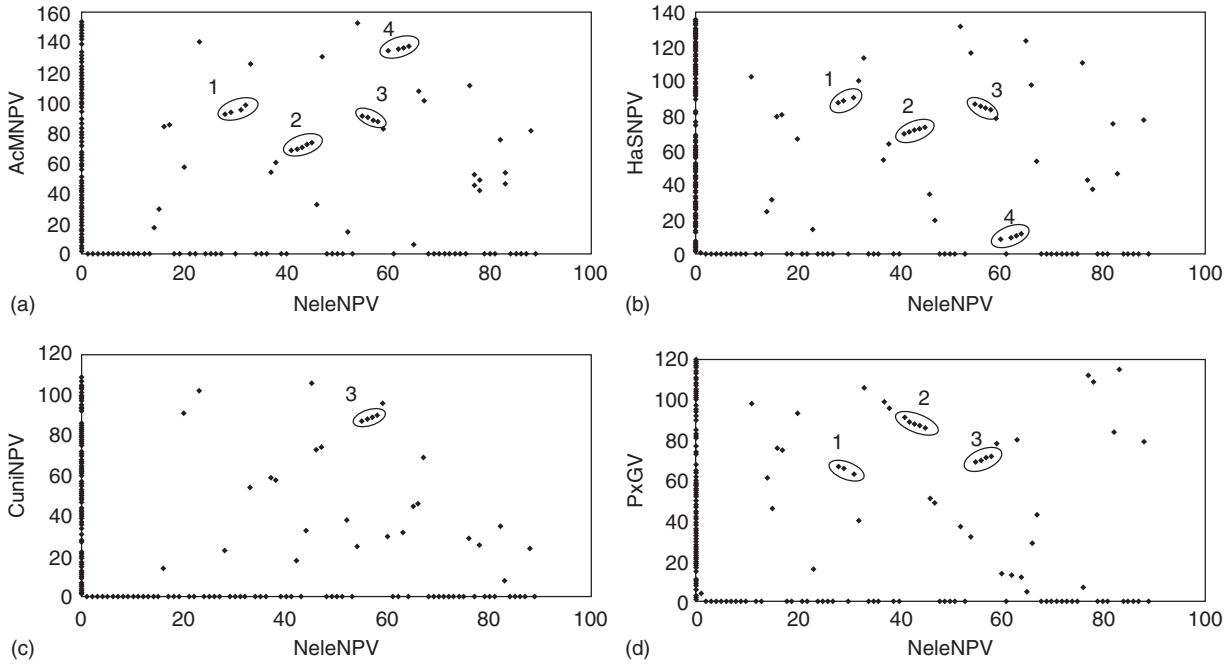


Figure 3 Gene parity plots. Pairwise comparisons of gene order of (a) NeleNPV/AcMNPV; (b) NeleNPV/HaSNPV; (c) NeleNPV/CuniNPV, and (d) NeleNPV/PxGV. The most conserved clusters have been circled. 1. *p6.9, p40, p48*; 2. *ac76, vlf-1, ac78, gp41*; 3. *lef-5,38k, ac96, helicase*; 4. *p49, odv-ec27, ac145*. Reprinted from Lauzon HAM, Lucarotti CJ, Krell PJ, Feng Q, Retnakaran A, Arif BM (2004) Sequence and organization of the *Neodiprion lecontei* nucleopolyhedrovirus genome. *Journal of Virology* 78: 7023–7035.

Phylogenies based on single genes have generally been found to be less robust than those based on combined sequences of several shared genes. With the complete sequencing of two hymenopteran baculoviruses, the total number of conserved genes among all baculoviruses sequenced so far is at least 29. A maximum parsimony tree was generated by using a combined data set of the 29 conserved proteins in 24 sequenced baculovirus genomes (**Figure 4**). The tree demonstrates that the hymenopteran and dipteran baculoviruses do not fit into the NPV groups I or II and, in fact, do not fit into the present classification genera of baculoviruses. This led us to suggest a new classification scheme to accommodate the dipteran and hymenopteran baculoviruses and any other future baculovirus. The authors suggested adding two new genera to the classification system and changing the names of the *Baculoviridae* genera to *Alphabaculovirus*, *Betabaculovirus*, *Gammabaculovirus*, and *Deltabaculovirus*. This system will accommodate the hymenopteran and dipteran baculoviruses and has the flexibility to accept more genera and is consistent with other groups of viruses such as the *Herpesviridae* and *Entomopoxvirinae*.

DNA Repeat Regions

Most baculovirus genomes contain what has been described as homologous regions (*hrs*) that consist of multiple tandem repeats of perfect or imperfect palindromes within direct repeated sequences. They have been implicated as origins of DNA replication and as enhancers of gene expression. A major difference between NeleNPV and NeseNPV is in the structure of the repeated sequences. The NeseNPV genome contains baculoviral *hrs* interdispersed in the genome as well as direct repeats (*drs*). The latter repeats do not bear resemblance to baculoviral *hrs*. NeleNPV genome contains only *drs*. Most of the repeated regions are located in the NSR of both genomes. Within the NSR, the NeseNPV had five *hrs* and two *drs* and NeleNPV had one *dr* and three ORFs that contained repeats (*nele2, 7,* and *8*).

Biology of the Virus

The tissue tropism of sawfly viruses is quite restricted and they appear to replicate only in midgut cells. As a defense mechanism against invaders, the insect sloughs off infected cells resulting in infectious diarrhea. Since these insects are gregarious in nature (**Figure 5**), the infected sloughed-off cells usually result in rapid dissemination of the virus within the larval population causing collapse within a few days to a week. The infection process within midgut cells is still not totally clear. For example, it is not known if progeny virus spreads from one cell to another. Since the virus does not contain a MFP, it is unlikely that a

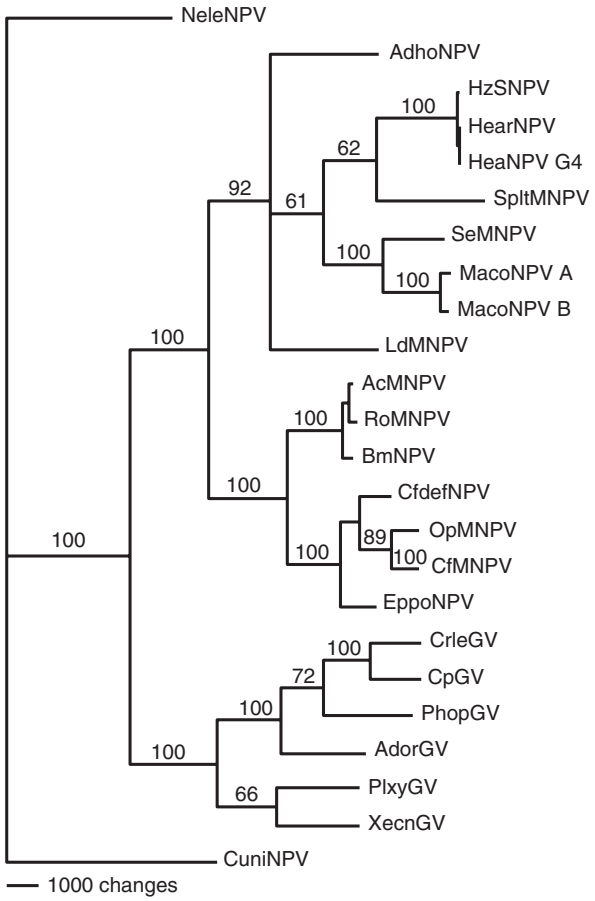


Figure 4 A phylogenetic map based on a data set of 29 conserved baculovirus genes. The dipteran CuniNPV and the hymenopteran NeleNPV do not branch with NPV of Lepidoptera.

budded virus phenotype is produced and spreads infection from one cell to another. It is interesting to note that these viruses encode a GP41 protein that has been termed an egress protein, which allows the virus to exit from an infected cell. It is conceivable that these viruses spread by crossing over to adjacent cells but this is yet to be demonstrated. The need for a complement of auxiliary genes may also not be necessary in this case. For example, expression of chitinase and v-cathepsin at the end of baculovirus replication cycle causes dissolution of the integument and melting of the insect. Restricted replication to midgut cells precludes the need for these two enzymes. Baculoviruses also encode an ecdysteroid UDP-glucosyltransferase that conjugates the hormone ecdysone with sugars and renders it inactive. In this way the virus prevents larval molting and prolongs the life within a certain instar, resulting in a more prolific replication of the virus in various larval tissues. Again, because of their midgut restriction, hymenopteran viruses do not need to inactivate ecdysone in the larval hemolymph.



Figure 5 The gregarious nature of the red-headed pine sawfly, *Neodiprion lecontei.*

See also: Baculoviruses: Molecular Biology of Granuloviruses; Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Insect Pest Control by Viruses.

Further Reading

Afonso CL, Tulman ER, Lu Z, *et al.* (2001) Genome sequence of a baculovirus pathogenic for *Culex nigripalpus*. *Journal of Virology* 75: 11157–11165.

Büchen-Osmond C (ed.) (2003) *Densovirus*. In: *ICTVdB – The Universal Virus Database*, version 3, chapter 00.050.2.01. ICTVdB Management, Columbia University, New York, USA. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB/index.htm (accessed July 2007).

Cunningham JC and Entwistle PF (1981) Control of sawflies by baculoviruses. In: Burges HD (ed.) *Microbial Control of Pests and Plant Diseases 1970–1981*, pp. 379–408. Academic Press: New York.

Garcia-Maruniak A, Maruniak JE, Zanotto PMA, *et al.* (2004) Sequence analysis of the genome of the *Neodiprion sertifer* nucleopolyhedrovirus. *Journal of Virology* 78: 7036–7051.

Guarino LA, Gonzales MA, and Summers MD (1986) Complete sequence and enhancer function of the homologous DNA regions of *Autographa californica* nuclear polyhedrosis virus. *Journal of Virology* 60: 224–229.

Herniou E, Olszewski JA, O'Reilly DR, and Cory JS (2004) Ancient coevolution of baculoviruses and their insect hosts. *Journal of Virology* 78: 3244–3251.

Herniou EA, Olszewski JA, Cory JS, and O'Reilly DR (2003) The genome sequence and evolution of baculoviruses. *Annual Review of Entomology* 48: 211–234.

Hughes AL and Friedman R (2003) Genome-wide survey for genes horizontally transferred from cellular organisms to baculoviruses. *Molecular Biology and Evolution* 20: 979–987.
Kool M, Ahrens CH, Vlak JM, and Rhormann GF (1995) Replication of baculovirus DNA. *Journal of General Virology* 76: 2103–2118.
Kuzio J, Pearson MN, Harwood SH, *et al.* (1999) Sequence and analysis of the genome of a baculovirus. *Virology* 253: 17–34.
Lauzon HAM, Garcia-Maruniak A, Zanotto PMA, *et al.* (2006) Genomic comparison of neodiprion sertifer and *Neodiprion lecontei* nucleopolyhedroviruses and identification of potential hymenopteran baculovirus-specific open reading frames. *Journal of General Virology* 87: 1477–1489.
Lauzon HAM, Lucarotti CJ, Krell PJ, Feng Q, Retnakaran A, and Arif BM (2004) Sequence and organization of the *Neodiprion lecontei* nucleopolyhedrovirus genome. *Journal of Virology* 78: 7023–7035.
Moreau G, Lucarotti CJ, Kettela EG, *et al.* (2005) Aerial application of nucleopolyhedrovirus induces decline in increasing and peaking populations of *Neodiprion abietis*. *Biological Control* 33: 65–73.
Ohtsubo M, Okazaki H, and Nishimoto T (1989) The RCC1 protein, a regulator for the onset of chromosome condensation locates in the nucleus and binds to DNA. *Journal of Cell Biology* 109: 1389–1397.
Pearson MN and Rohrmann GF (2002) Transfer, incorporation, and substitution of envelope fusion proteins among members of the *Baculoviridae*, *Orthomyxoviridae*, and *Metaviridae* (insect retrovirus) families. *Journal of Virology* 76: 5301–5304.
Theilmann D and Stewart S (1991) Identification and characterization of the IE-1 gene of *Orgyia pseudotsugata* multicapsid nuclear polyhedrosis virus. *Virology* 180: 492–508.

Baculoviruses: Apoptosis Inhibitors

R J Clem, Kansas State University, Manhattan, KS, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

AcMNPV Autographa californica multiple nucleopolyhedrovirus, the most studied baculovirus.
Annihilator mutant The first *p35* mutant of AcMNPV described.
Apoptosis A type of programmed cell death commonly observed in multicellular animals that involves cell shrinkage, plasma membrane blebbing, nuclear fragmentation, and chromatin degradation. Important in many different physiological and pathological situations.
Apoptosome A large protein complex consisting of multiple copies of an initiator caspase (DRONC in *Drosophila*, caspase-9 in mammals) bound to multiple copies of adaptor protein (DARK in *Drosophila*, Apaf-1 in mammals). In mammals, cytochrome *c* is also bound to Apaf-1. Apoptosome formation allows for activation of the initiator caspase.
BIR domain Baculovirus IAP repeat, the signature domain of IAP proteins.
Blebbing A term used to describe the outward bulging of plasma membrane often observed during apoptosis. Blebbing results from changes in the cytoskeleton that occur during apoptosis, and eventually leads to the cell breaking up into membrane-bound fragments called apoptotic bodies.
Caspase A family of cysteine proteases that are centrally involved in the execution of apoptosis.
Effector caspase One of several caspases that are activated by initiator caspases. Responsible for cleavage of numerous substrates that lead directly to apoptosis.
Iap *inhibitor of apoptosis* gene.
Initiator caspase One of the several caspases that are the first to be activated following an apoptotic signal. Activate effector caspases.
RING domain A type of zinc finger domain found in many different proteins, including some IAP proteins. Important in E3 ubiquitin ligase activity.

Introduction

In order to successfully replicate and survive, viruses must evade the immune responses of their hosts. One aspect of the innate immune response that was largely ignored by virologists until relatively recently is apoptosis. Apoptosis is a type of programmed cell death that is important in the clearance of unwanted or potentially harmful cells, including pathogen-infected cells. We now appreciate that many viruses carry genes that can inhibit apoptosis, indicating that host cell apoptosis is an important evolutionary pressure that viruses have had to overcome. Members of the virus family *Baculoviridae* were some of the first viruses that were shown to carry apoptosis-inhibiting genes, and these insect viruses continue to provide an excellent model for understanding the

role of apoptosis in antiviral defense in both invertebrates and higher animals.

Apoptosis Pathways in Insects

The ability to commit suicide appears to be a universal characteristic of all cells, whether they be prokaryotic, single-celled eukaryotic, plant, or animal cells. As it is strictly defined morphologically, the specific type of cell death known as apoptosis is confined to metazoans (multicellular animals), and is just one type of programmed cell death found in these organisms. The biochemical pathways that lead to apoptosis are more or less conserved in metazoans, and can be triggered by a wide variety of different stimuli. While a myriad of interconnected pathways are involved in transmitting the death signal to the core apoptotic machinery, the core machinery itself is well conserved. What follows is a highly simplified description of the basic apoptotic pathway in the only insect where it has been examined in detail, *Drosophila melanogaster*. For more in-depth information, the reader is encouraged to consult recent reviews on the subject.

The engine that runs the apoptotic core machinery consists of a family of proteases called caspases (**Figure 1**). Caspases are cysteine proteases that cleave their target substrates at highly specific recognition sites. Cleavage involves recognition and proteolysis following specific aspartate residues. The amino acids immediately surrounding the aspartate cleavage site are crucial in determining caspase substrate specificity. Caspases are present in cells as pro-enzymes which need to undergo proteolytic cleavage to become active. Caspases can be roughly divided into two groups, the initiator caspases and the effector caspases. Initiator caspases are the first to be activated following an apoptotic stimulus. In most cases, caspase activation involves caspase-mediated cleavage, whether it is autocatalytic, in the case of initiator caspases, or mediated by other caspases, as with effector caspases. An apoptotic stimulus causes the aggregation of adaptor proteins, which bind to pro-initiator caspases and cause them to self-associate and autoactivate. Once activated, initiator caspases then recognize and cleave pro-effector caspases. Activated effector caspases then cleave a number of cellular proteins that directly cause the morphological changes associated with apoptosis, including plasma membrane blebbing, nuclear condensation and fragmentation, chromatin degradation, and cellular disintegration.

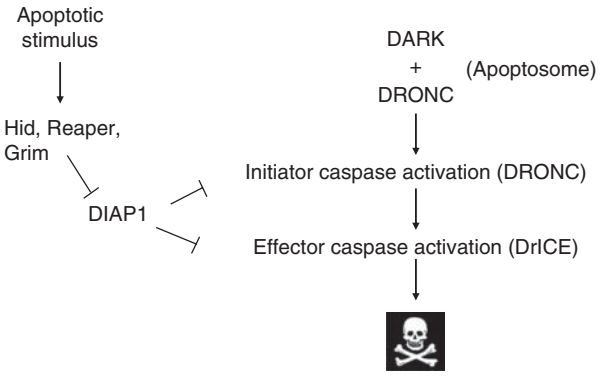


Figure 1 A simplified apoptosis pathway in *Drosophila*. Unlike mammalian cells, where it is thought that initiator caspases are not activated until the cell receives a death signal, in *Drosophila* cells the initiator caspase DRONC appears to spontaneously autoactivate all of the time, even in unstimulated cells. In order to become activated, DRONC must bind to the adapter protein DARK, forming a structure known as the apoptosome. DRONC activity is normally kept at very low levels because of the ability of the DIAP1 protein to bind and ubiquitinate activated DRONC, resulting in DRONC degradation by the proteasome. Interruption of the DIAP1/DRONC interaction by IAP antagonists such as Hid, Reaper, or Grim results in accumulation of activated DRONC, which leads to cleavage and activation of effector caspases such as DrICE. Effector caspase activity then leads to death of the cell.

The existence of proteases that are capable of autoactivation and whose activity leads to death is obviously a dangerous situation for the cell and must be tightly regulated. There are many checks and balances in the pathways that lead to caspase activation, but the main proteins that directly oppose caspase activity are the Inhibitor of apoptosis (IAP) proteins, some of which are able to directly bind and inhibit the activity of initiator and effector caspases. In *Drosophila*, one IAP protein in particular, DIAP1, is crucial for cell survival, as down-regulation of DIAP1 causes rapid, spontaneous apoptosis (**Figure 1**). The main target of DIAP1 appears to be an initiator caspase called DRONC. DRONC undergoes continuous autoactivation in *Drosophila* cells. DRONC activation requires association with a protein called DARK, which is homologous to the Apaf-1 protein in mammals. Together, DRONC and DARK form a structure called the apoptosome. In mammals, apoptosome formation requires the release of cytochrome *c* from mitochondria, and binding of cytochrome *c* to DARK. In *Drosophila*, however, cytochrome *c* does not appear to play a role in formation of the apoptosome. DIAP1 can directly bind to activated DRONC and cause DRONC to be ubiquitinated and degraded by proteasomes. Thus, as long as DIAP1 levels remain constant, the continuous DRONC activation that occurs in normal cells is kept in check.

In order for *Drosophila* cells to die, DIAP1 inhibition of DRONC must be removed. This is often accomplished by upregulation of proteins which antagonize the DIAP1-DRONC interaction, called Reaper, Hid, Grim, and Sickle (RGH proteins). RGH proteins bind to DIAP1 at the same site as DRONC, allowing active DRONC to accumulate, which leads to apoptosis through the activation of effector caspases. However, signals that disrupt

DIAP1 expression, such as metabolic inhibitors or stress signals, also trigger spontaneous apoptosis in *Drosophila* cells by allowing DRONC activation.

Induction of Apoptosis by Baculoviruses

The first realization that a baculovirus could stimulate apoptosis arose from characterization of a spontaneous mutant of the baculovirus autographa californica multiple nucleopolyhedrovirus (AcMNPV). This mutant, which was called the annihilator mutant, causes rapid and widespread apoptosis in Sf-21 cells, a cell line derived from the lepidopteran insect *Spodoptera frugiperda* (**Figure 2**). Mapping of the mutation that caused this apoptotic phenotype revealed that the mutation was a 754 bp deletion, which resulted in premature truncation of an open reading frame (ORF) in the AcMNPV genome called the 35K ORF. This gene, which was renamed *p35*, turned out to encode a highly effective inhibitor of effector caspases from not only insects but also other organisms including nematodes and mammals.

Infection of Sf-21 cells with wild-type AcMNPV does not result in apoptosis, but rather in passive cell lysis. Permissive baculovirus infections are most often lytic, but whether cell lysis occurs by apoptosis or by a passive mechanism is a complicated matter that is determined by several factors. In a typical permissive infection, such as Sf-21 cells infected with wild-type AcMNPV, the infected cells produce large amounts of budded virus over the first day or so, and then switch to producing occluded virus particles. The cells usually survive for several days before lysing. By this time, the cell has been largely subverted by the virus, and many cellular processes such as host mRNA and protein synthesis have long since declined or ceased. The mechanism of death does not appear to be apoptotic, but rather passive lysis, presumably due to impaired cellular functions.

However, things are not always so simple. Even in a normal infection, some of the initial stages of apoptosis can occur. For example, in Sf-21 cells infected with AcMNPV, a transient blebbing of the plasma membrane similar to what is observed in early apoptosis is observed at approximately 9–12 h post infection, and effector

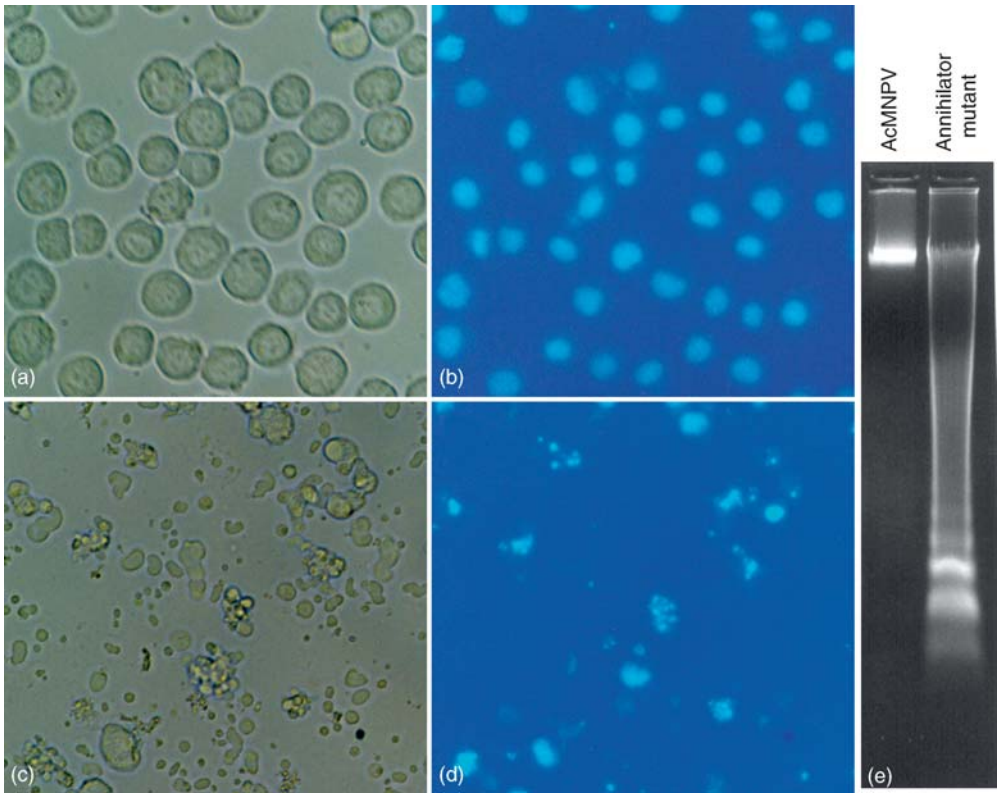


Figure 2 The apoptotic morphology of Sf-21 cells infected with the annihilator mutant of AcMNPV, a mutant virus lacking the *p35* gene. In panels (a)–(d), cells were stained with a fluorescent dye that binds to DNA and viewed by phase contrast ((a) and (c)) or by fluorescence microscopy ((b) and (d)). Panels (a, b) are uninfected Sf-21 cells, while panels (c) and (d) are Sf-21 cells infected with the annihilator mutant. Note the extensive cell blebbing, chromatin condensation, and nuclear fragmentation in the annihilator-infected cells. Panel (e) shows the oligonucleosomal DNA fragmentation associated with apoptosis of Sf-21 cells infected with the annihilator mutant. Cellular DNA was isolated from infected cells and analyzed by agarose gel electrophoresis.

caspases become activated. Effector caspase activity is kept low, however, by the P35 protein.

In some cases, full-blown apoptosis can occur even though the virus encodes one or more functional apoptosis inhibitors. Whether or not apoptosis occurs depends on the particular baculovirus and cell line, but is usually observed in nonpermissive or semipermissive situations. One example is seen with wild-type AcMNPV, which causes apoptosis in nonpermissive *Spodoptera littoralis* SL2 cells and *Choristoneura fumiferana* CF-203 cells, despite the presence of *p35*. In the case of SL-2 cells infected with AcMNPV, apoptosis has been shown to be due to low expression of the *p35* gene, and apoptosis can be prevented by expressing *p35* using a stronger promoter. The complicated nature of the interactions between virus and host is further illustrated by the fact that infection of another *S. littoralis* cell line results in extensive cytopathic effect, but little apoptosis. Also, nonpermissive infection does not always result in apoptosis. Infection of *Drosophila* cell lines with wild-type AcMNPV does not result in virus replication, and apoptosis is not observed. However, apoptosis does occur in *Drosophila* cells if the infecting strain of AcMNPV lacks the *p35* gene. Thus, the response of cells to baculovirus infection varies widely, depending on the interaction between the gene products expressed by the virus and the particular cellular environment.

The mechanisms by which baculoviruses induce apoptosis are incompletely understood, and our knowledge is entirely based on studies with AcMNPV in Sf-21 cells. What is clear from these studies is that mere attachment of the virus to a cellular receptor is not sufficient to induce apoptosis. Rather, the virus must enter the cell and express at least some of its early genes. The viral transcriptional activator *ie-1* has been reported to be capable of inducing apoptosis when expressed in Sf-21 cells, and another transactivator, *pe-38*, can enhance the ability of *ie-1* to stimulate apoptosis, although the ability of these genes to cause apoptosis is somewhat controversial. Even so, expression of these transactivators may affect the transcription of cellular genes that lead to triggering of apoptosis, or at least making the cells more sensitive to apoptotic stimuli.

While these early viral gene products may play a role in apoptosis, full induction of apoptosis appears to also require late events in the virus replication cycle. The transition from the early to late phases of gene expression involves several inter-related processes including the onset of viral DNA replication, a block in the cell cycle at the G2 phase, the shut-off of host mRNA and protein synthesis, and the initiation of transcription of viral late genes by a viral RNA polymerase. Evidence exists implicating each of these events in triggering apoptosis, so it appears that there are multiple stimuli that contribute to the onset of the apoptotic cascade during baculovirus infection. This probably explains why most DNA viruses need to carry apoptosis inhibitors in their genomes: apoptosis may be an unavoidable consequence of DNA virus replication.

Baculovirus Anti-Apoptotic Genes: P35 and Its Relatives

The P35 protein is a remarkable molecule. This baculovirus protein is the most widely acting apoptosis inhibitor known; it can inhibit apoptosis in nematodes, insects, and mammals. As such, P35 has proven to be a highly useful tool to investigators studying apoptosis in a wide variety of systems ranging from developmental events in *Drosophila* to immune system function in mice. Thanks to structural biology, we know a great deal about how P35 functions as a stoichiometric caspase inhibitor.

The crystal structure of P35 was published in 1999 and revealed a teapot-like structure with a body, neck, and handle (**Figure 3**). The most interesting aspect of the structure was the handle, or reactive site loop, which contains a caspase cleavage site at its apex. Further studies demonstrated that cleavage at this site by caspases results in a conformational shift in the P35 protein, causing the N-terminus of P35 to interact with the caspase active site, and part of the reactive site loop to swing down and interact with a β-sheet in the body of the protein. A thioester bond is formed between P35 and the caspase, resulting in the P35 cleavage products becoming covalently

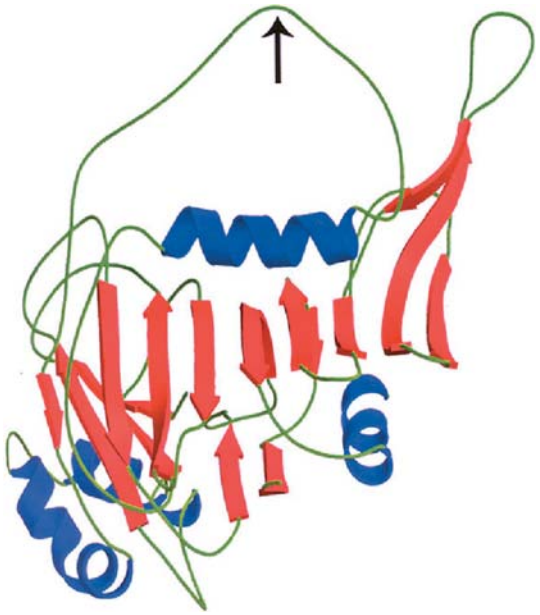


Figure 3 The crystal structure of AcMNPV P35 reveals a teapot-like structure, with a solvent-exposed loop that contains the caspase cleavage site (arrow). Reproduced from Clarke TE and Clem RJ (2003) Insect defenses against virus infection: The role of apoptosis. *International Reviews of Immunology* 22: 401–424, figure 3, with permission from Taylor & Francis.

bound to the caspase, permanently inactivating the caspase. While somewhat reminiscent of the mechanism used by serpins, this represents a unique mechanism of protease inhibition, and one that may have application in designing novel therapeutic drugs.

Although it is often referred to as a pan-caspase inhibitor, AcMNPV P35 actually prefers to inhibit effector caspases, and has little inhibitory activity against most initiator caspases. However, a homolog of P35 found in spodoptera littoralis M nucleopolyhedrovirus called P49 is able to inhibit both effector and initiator caspases. The mechanistic basis for this difference in specificity between P35 and P49 is not known at this time. While other baculovirus P35 homologs have not been characterized biochemically, recently the first homolog of *p35* outside of the baculoviruses was characterized in the entomopoxvirus of *Amsacta moorei*. This gene, called *p33*, encodes a protein with low but significant homology to P35 and P49. P33 appears to function more similarly to P35, as it also preferentially inhibits effector caspases.

P35 Proteins: More Than Just Caspase Inhibitors?

While the ability of P35 to inhibit caspases is well documented, evidence also exists that hints at additional functions for this protein. P35 has been reported to bind to RNA polymerase II and have effects on gene transcription in both insect and human cells. P35 has also been reported to promote transformation of mouse embryo fibroblasts by a mechanism that does not appear to be due to inhibiting apoptosis. Similarly, P35 expression is able to stimulate translation arrest in an AcMNPV-infected cell line, but chemical caspase inhibitors do not. Finally, recent unpublished data indicate that *p35* mutant virus is less efficient than wild-type virus at initiating infection in TN-368 cells from *Trichoplusia ni*, which do not undergo apoptosis when infected with either virus. Together, these observations suggest that P35 may do more than just inhibit caspases, although exactly what else it does remains unclear.

The IAPs: Apoptosis Inhibitors Found in Both Viral and Cellular Genomes

Shortly after the discovery of *p35*, a genetic complementation assay was used in an attempt to identify *p35* homologs in two other baculoviruses, orgyia pseudotsugata M nucleopolyhedrovirus (OpMNPV) and cydia pomonella granulovirus (CpGV). While this assay did identify genes in both OpMNPV and CpGV that could complement the *p35* mutation in AcMNPV (i.e., inhibit AcMNPV-induced apoptosis), the complementing genes turned out not to be homologs of *p35*. They were, however, related to each other. These genes were named *inhibitor of apoptosis* (*iap*) genes, and the genes from OpMNPV and CpGV were named Op-*iap* and Cp-*iap*, respectively.

The sequencing of baculovirus genomes (more than 30 to date) has revealed that *iap* genes are widespread among the baculoviruses. Almost all baculoviruses carry at least one, and usually more, *iap* homologs. This includes viruses that also carry *p35* homologs, such as AcMNPV. Phylogenetic analysis has classified the baculovirus *iap* genes into five groups, *iap*-1 through *iap*-5. The *iap*-5 group has so far been found only in granuloviruses, while the other four groups are present in both NPVs and GVs. None of the viruses sequenced to date have more than one member of each family.

Many of the baculovirus *iap* genes do not seem to have anti-apoptotic function. While less than half of known baculovirus *iap* homologs have been tested, most of the ones that inhibit apoptosis are found in the *iap*-3 group, including the original Op-*iap* and Cp-*iap*. For example, the Ac-*iap* gene does not have the ability to inhibit apoptosis, at least not in any of the situations where it has been tested, and a study of four *iap* homologs from epiphyas postvittana nucleopolyhedrovirus demonstrated that only two of the four genes could inhibit apoptosis, and another one was only able to delay apoptosis. The question of what role these 'nonfunctional' *iap* homologs play during a viral infection is an interesting one. As viruses do not normally retain genes that fail to confer a selective advantage, presumably they have some type of function. It is possible that they may inhibit apoptosis in specialized tissues or insect hosts that have not been tested yet, or they may have other, as yet unknown functions.

The distinguishing feature of *iap* genes is that the proteins they encode contain between one and three copies of a type of domain known as a BIR (baculovirus IAP repeat) (**Figure 4**). BIR domains are approximately 70 amino acids in length and are always found at the N-terminus of IAP proteins. They contain several highly conserved residues, including cysteines and histidines. Based on this, it was predicted that BIR domains were a type of zinc finger domain, and structural studies have borne this out, with each BIR coordinating one atom of zinc. Many, but not all, IAP proteins also contain a second type of zinc finger domain at the C-terminus known as a RING domain. RING domains, which are also found in many other types of proteins besides IAPs, have been shown to be involved in ubiquitin ligase activity. In fact, in every case to date where a RING-containing protein has been tested, including two baculovirus IAPs, they have been shown to possess this activity.

Expression of Op-*iap* or Cp-*iap* inhibits apoptosis triggered by various stimuli, in both lepidopteran and mammalian cells. For some unknown reason, Op-IAP does not inhibit apoptosis in *Drosophila* cells, and actually

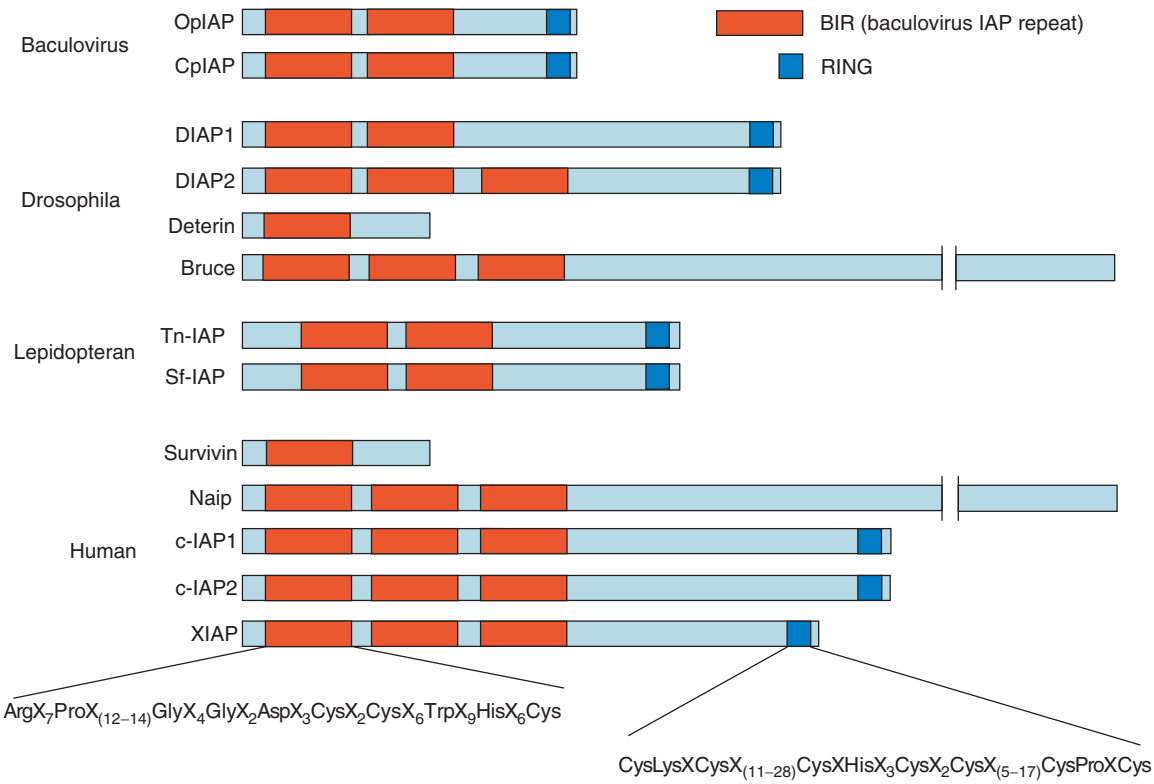


Figure 4 Schematic representation of IAP proteins from select baculoviruses, insects, and humans. The consensus sequences of BIR and RING domains are shown below the schema.

has been shown to sensitize *Drosophila* S2 cells to AcMNPV-induced apoptosis. However, in most cells, Op-IAP is an efficient apoptosis inhibitor. Exactly how Op-IAP functions is not known, and may be different in insect versus mammalian cells. The available evidence indicates that Op-IAP does not act by directly binding to caspases and inhibiting their activity, but may simply be a sink for IAP antagonists such as Reaper, Hid, and Grim in insects or Smac/DIABLO in mammals. However, mutants of Op-IAP exist that still appear to bind to Hid normally, but fail to inhibit Hid-induced apoptosis, indicating that binding to Hid is necessary but not sufficient to inhibit apoptosis induced by Hid. Thus, questions remain about how baculovirus IAP proteins inhibit apoptosis.

Genes with homology to the baculovirus *iap* genes are found in the genomes of a few other invertebrate viruses such as entomopoxviruses, iridoviruses, and African swine fever virus (which has an obligate stage in ticks). Homologs of *iap* genes are also found in cellular genomes including those of yeast, nematodes, insects, and mammals. The *iap* genes in yeast and nematodes appear to be involved in regulation of cytokinesis, while those in higher animals are primarily involved in regulating apoptosis. However, the mammalian *survivin* gene, which is most similar in structure to the yeast and nematode *iap* genes, also regulates cytokinesis. Other mammalian IAP proteins also appear to act in other types of signal transduction pathways, suggesting that IAPs are more pleiotropic in function than their name implies.

Apoptosis as an Antiviral Immune Response

Like all other living organisms, insects are constantly challenged by bacteria, fungi, and viruses. In vertebrate animals, immunity can be broken down into innate and adaptive immunity. Insects do not possess the adaptive immune response that is characteristic of vertebrates including antibodies and T cells. That does not mean, however, that insects are defenseless against invading microorganisms. Insects have robust innate immune systems that are in many ways similar to the innate immune mechanisms found in vertebrates. Over the past 10–15 years, enormous strides have been made in understanding the molecular basis for insect innate immunity against bacteria and fungi. Despite these advances, the understanding of how insects defend themselves against viruses has lagged behind. Work with baculoviruses has demonstrated that apoptosis can serve as an innate immune mechanism in insects.

As soon as the annihilator mutant was discovered, it was suspected that apoptosis would have negative consequences

for baculovirus replication. This was because the mutant virus replicated poorly in Sf-21 cells, which became apoptotic when infected with the mutant, but the mutant replicated normally in TN-368 cells, which did not die by apoptosis. Growth curve experiments revealed that the replication of AcMNPV mutants that lack the *p35* gene is reduced by 100-fold or more (depending on the multiplicity of infection) in Sf-21 cells, but not in TN-368 cells. In addition, viral late and very late gene expression was substantially reduced in Sf-21 cells infected with *p35* mutants, but was normal in TN-368 cells.

These cell culture results predicted that *p35* mutant viruses might also be less able to infect insect larvae. Indeed, successful infection of *S. frugiperda* or *Spodoptera exigua* larvae by feeding of occluded virus (the natural route of infection) or by intrahemocoelic injection of budded virus requires much higher doses of *p35* mutant virus than control viruses containing *p35*, and analysis of infected tissues reveals widespread apoptosis in infected tissues of the *p35* mutant-infected insects. However, *T. ni* larvae are equally susceptible to *p35* mutant and wild-type infection, and apoptosis is not observed in *p35* mutant-infected tissues. Additional evidence that the defect in infectivity is due to apoptosis comes from the demonstration that insertion of a different anti-apoptotic gene, Cp-*iap*, into a *p35* mutant background rescues the infectivity defect in *S. frugiperda* larvae.

The importance of apoptosis in antiviral immunity in insects is only beginning to be understood, and many questions remain. For example, apoptosis has thus far been examined in only a small number of virus–host combinations. Also, there is evidence suggesting that apoptosis can be more effective when combined with other types of immune responses, but almost nothing is known about these other responses. Whether certain tissues within the insect are more important than others is also not known. What is apparent, however, is that the apoptosis inhibitors carried by baculoviruses are important virulence factors, and that apoptosis probably plays a role in determining the host range of these viruses.

See also: Apoptosis and Virus Infection; Baculoviruses: General Features; Baculoviruses: Molecular Biology of Granuloviruses; Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Baculoviruses: Pathogenesis; Innate Immunity: Defeating; Innate Immunity: Introduction; Polydnaviruses: Abrogation of Invertebrate Immune Systems.

Further Reading

Clarke TE and Clem RJ (2003) Insect defenses against virus infection: The role of apoptosis. *International Reviews of Immunology* 22: 401–424.

Clem RJ (2001) Baculoviruses and apoptosis: The good, the bad, and the ugly. *Cell Death Differentiation* 8: 137–143.

Clem RJ (2005) The role of apoptosis in defense against baculovirus infection in insects. *Current Topics in Microbiology and Immunology* 289: 113–130.

Clem RJ, Fechheimer M, and Miller LK (1991) Prevention of apoptosis by a baculovirus gene during infection of insect cells. *Science* 254: 1388–1390.

Fisher AJ, Cruz W, Zoog SJ, Schneider CL, and Friesen PD (1999) Crystal structure of baculovirus P35: Role of a novel reactive site loop in apoptotic caspase inhibition. *EMBO Journal* 18: 2031–2039.

Hay BA and Guo M (2006) Caspase-dependent cell death in *Drosophila*. *Annual Review of Cell and Developmental Biology* 22: 623–650.

Kornbluth S and White K (2005) Apoptosis in *Drosophila*: Neither fish nor fowl (nor man, nor worm). *Journal of Cell Science* 118: 1779–1787.

Okano K, Vanarsdall AL, Mikhailov VS, and Rohrmann GF (2006) Conserved molecular systems of the *Baculoviridae*. *Virology* 344: 77–87.

Salvesen GS and Duckett CS (2002) IAP proteins: Blocking the road to death's door. *Nature Reviews. Molecular Cell Biology* 3: 401–410.

Wang L and Ligoxygakis P (2006) Pathogen recognition and signaling in the *Drosophila* innate immune response. *Immunobiology* 211: 251–261.

Xu G, Cirilli M, Huang Y, *et al.* (2001) Covalent inhibition revealed by the crystal structure of the caspase-8/p35 complex. *Nature* 410: 494–497.

Baculoviruses: Expression Vector

F J Haines, Oxford Brookes University, Oxford, UK
R D Possee, NERC Institute of Virology and Environmental Microbiology, Oxford, UK
L A King, Oxford Brookes University, Oxford, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Bacterial artifical chromosome (BAC) An artificial chromosome based on the bacterial fertility (F) plasmid which permits replication of large DNA molecules within bacterial cells.

Co-transfection Introduction of both transfer vector and viral DNA into eukaryotic cells to mediate

homologous recombination and ultimately recombinant virus production.
Homologous recombination The process by which the exchange or replacement of a DNA region occurs between homologous flanking regions of genetic material.

Introduction

Baculoviruses are a diverse group of insect-specific viruses, predominantly infecting insect larvae of the order Lepidoptera. By far the most widely studied member of this family is autographa californica multiple nucleopolyhedrovirus (AcMNPV) for which the complete genome sequence has been determined. AcMNPV has a circular, double-stranded, super-coiled DNA genome of *c.* 130 kbp packaged in a rod-shaped nucleocapsid. These nucleocapsids can be extended lengthways and thus the virus genome can effectively accommodate large insertions of foreign DNA. Such insertions of foreign genes into the AcMNPV genome have resulted in production of baculovirus expression vectors, that is, recombinant viruses genetically modified to contain a foreign gene of interest, which can be expressed in insect cells under the control of a baculovirus gene promoter. The baculovirus expression vector system is now one of the most popular methods of protein production, largely due to the high yields of good-quality protein that are obtained with a wide variety of different protein types.

AcMNPV has a biphasic replication cycle resulting in the production of two virus phenotypes: budded virus (BV) and occlusion-derived virus (ODV) (**Figure 1**). BVs consist of a single, rod-shaped nucleocapsid enveloped in a host-derived membrane, enriched in a virally encoded membrane fusion protein, GP64, which is incorporated into the BV particle during virus budding and release. The budded form of the virus is responsible for the cell-to-cell transmission of the virus both *in vivo* and *in vitro*. During the later stages of infection, large numbers of occlusion bodies (OBs) or polyhedra are formed. The major component of the OB matrix is polyhedrin, a virus-encoded protein produced by the powerful transcriptional activity of the polyhedrin (*polh*) gene promoter. This protein protects the virus in the environment and allows virus particles to survive outside of their natural host.

Insect Cell Culture

The traditional cell lines for baculovirus-mediated expression studies are Sf-21 and Sf-9 cells. Both cell lines were originally derived from the pupal ovarian cells of *Spodoptera frugiperda* (fall army worm) termed IPLB-Sf-21 cells, with Sf-9 cells (IPLB-Sf-21-AE) being a clonal isolate of Sf-21 cells. High-Five cells (BTI-TN-5B14), a clonal isolate from *Trichoplusia ni* (cabbage looper) cell lines, are also used for expression of recombinant proteins using the baculovirus expression system and have been found to produce greater levels of recombinant protein compared to Sf-21 and Sf-9 cells in some circumstances. In general, Sf-21 or Sf-9 cell lines are used for co-transfections, virus amplification, and plaque assays while *T. ni* cell lines are employed for protein expression.

Insect cell lines are generally considered simple to sustain compared to mammalian cell lines and can be maintained as either suspension cultures, in shake or stirred flasks, or in monolayer cultures in T-flasks or culture dishes. Most insect cell culture media utilize a phosphate buffering system rather than the carbonate-based buffers commonly used for mammalian cell lines, negating the requirement for CO_2 incubators. The range for growth and infection of most cultured insect cells is between 25 and 30 °C, although 28 °C is generally considered the optimal growth temperature.

Certain insect cells, for example, Sf-21 cell lines, require serum supplement. Fetal bovine serum (FBS) is most frequently used as the primary growth supplement in insect cell culture and although it promotes cell growth and provides shear force protection important in shake and stirrer cultures, it can also cause excessive foaming, interfere with transfection reagents and is costly. For this reason, a number of commercially available cell lines have now been adapted and optimized for growth in serum-free media, such as Sf-900 II SFM and EXPRES-FIVE SFM for Sf-9 cell growth. Such media contain amino acids, carbohydrates, vitamins, and lipids essential for insect cell growth that reduce the effect of rate-limiting nutritional restrictions or deficiencies present within serum-supplemented media. Serum-free media also tend to support faster cell-doubling times and permit cell growth to higher densities than serum-supplemented media, which, when used for recombinant virus and/or protein production, ultimately results in higher virus titers or protein yields.

Baculovirus Expression Vector Systems

Since the application of baculovirus expression systems for the safe and abundant expression of foreign proteins in insect cells in the early 1980s, this expression system has become one of the most popular methods for the production of large quantities of recombinant proteins within eukaryotic cells. The majority of baculovirus expression systems exploit the *polh* promoter to drive high-level production of foreign proteins. The baculovirus *polh* gene is nonessential for virus replication in insect cell cultures and therefore can be removed from the virus genome with no detrimental effect to BV production.

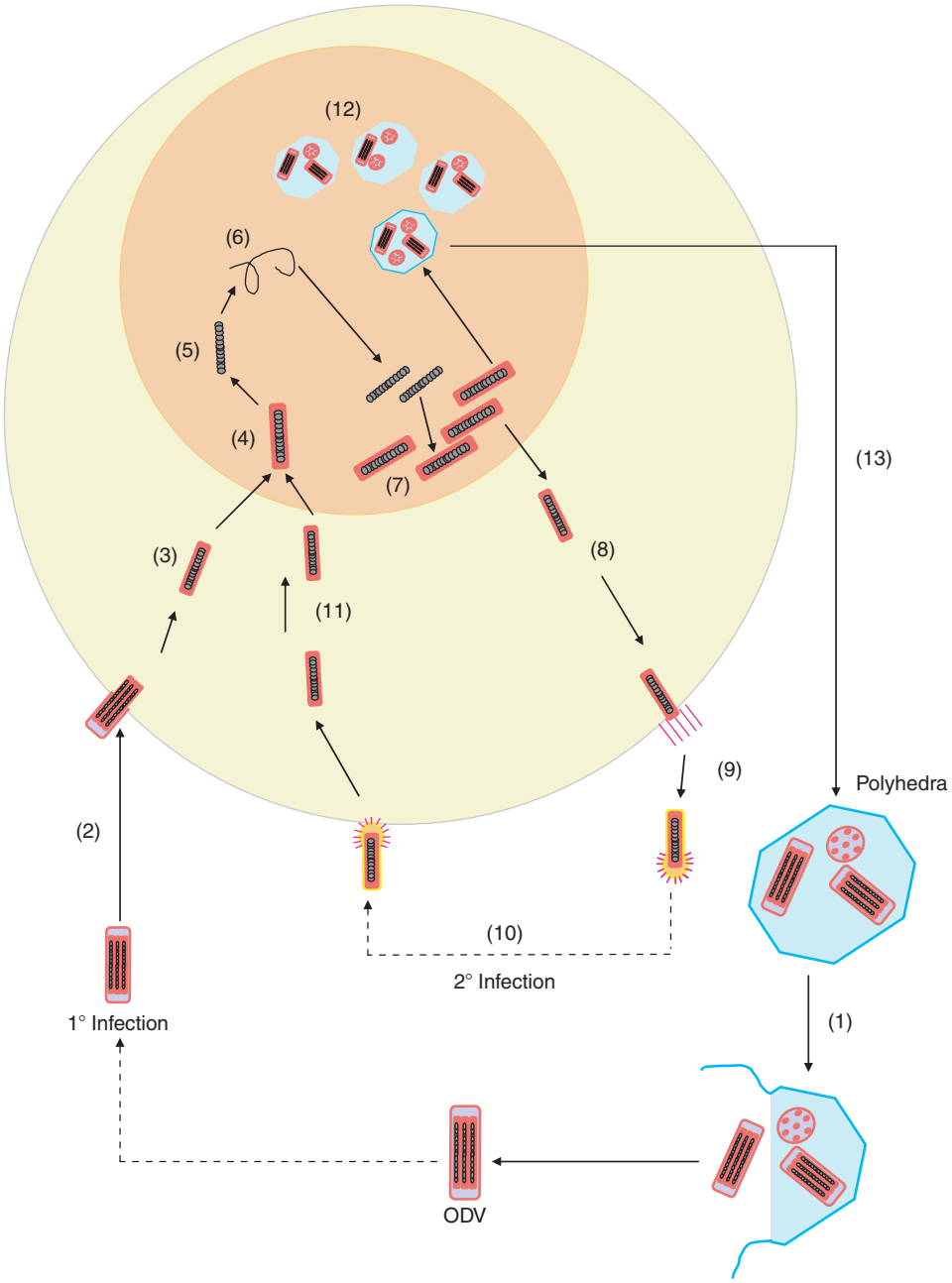


Figure 1 Schematic of the biphasic AcMNPV replication cycle. Polyhedra persist in the environment until ingested by a susceptible larval host. The alkaline environment of the insect midgut dissolves the polyhedra (1) to release occlusion-derived virions (ODVs). ODVs then fuse with midgut epithelial cells (2) and enter the cell. Nucleocapsids (NCs) then travel to the nucleus (3), enter the nucleus most likely via the nuclear pore (4), where the NC is uncoated (5) and viral transcription initiated (6). *De novo* production of NCs ensues (7) and NCs leave the host cell nucleus (8). NCs are then transported to the plasma membrane where they bud from the host cell acquiring an envelope derived from the host cell membrane, to produce budded virus (BV) (9). BVs then attach to further susceptible cells (10), are uncoated within the cell cytoplasm, and NCs then traffic to the nucleus (11), following which translation, production of *de novo* NCs and additional BV occurs (4–10). During the later stages of infection, NCs remaining within the nucleus are occluded within polyhedra (13) which are released from the cell on cell death and host liquefaction.

As the baculovirus genome is generally considered too large for direct insertion of foreign genes, the gene of interest is first cloned into a transfer vector containing sequences that flank the *polh* gene in the viral genome. Virus DNA and transfer vector are co-transfected into the host insect cell and homologous recombination between the flanking sequences common to both DNA molecules occurs. This causes the insertion of the gene of interest

into the viral genome at the *polh* locus, resulting in the production of a recombinant virus genome. The genome then undergoes replication within the host nucleus, generating recombinant BV containing the foreign gene under the control of the strong, late viral polyhedrin promoter. Polyhedra are not produced as the *polh* gene is no longer functional, having been replaced by the gene of interest.

Recombinant baculovirus expression vectors were originally produced using the highly inefficient homologous recombination process between a transfer vector containing the gene of interest and parental virus DNA. Insect cells were co-transfected with the baculovirus and transfer plasmid DNA, producing mixture of both recombinant and parental viruses, with recombinant viruses comprising only *c.* 0.1% of total virus produced. Isolation of recombinant virus then required plaque purification where recombinant clones were identified by their characteristic occlusion-negative plaque phenotypes.

To improve the efficacy of recombinant virus production, a unique *Bsu*361 restriction enzyme site was engineered into the *polh* locus of baculovirus DNA to permit linearization of the viral genome prior to co-transfection, giving rise to a higher frequency of recombinant virus production. Further improvements employed multiple *Bsu*361 - sites, with digestion of the viral DNA resulting in a partial deletion within an essential gene, ORF1629. The deletion within this gene prevents replication of parental virus, increasing the yield of recombinant virus to more than 90%. Insertion of the *Escherichia coli lacZ* gene into the *polh* locus, replacing the *polh* gene, produced the commercially available BacPAK6 (**Figure 2**). A *Bsu*361 restriction enzyme site is located within the *lacZ* gene along with two additional sites situated in the two flanking genes either side of *lacZ*. Digestion of BacPAK6 with *Bsu*361 removes the *lacZ* gene and a fragment of ORF1629 (**Figure 2**(**b**)), resulting in linear virus DNA incapable of replicating within insect cells.

Co-transfection of insect cells with BacPAK6 DNA and a transfer vector containing the gene of interest restores the deletion in ORF1629 and re-circularizes the virus

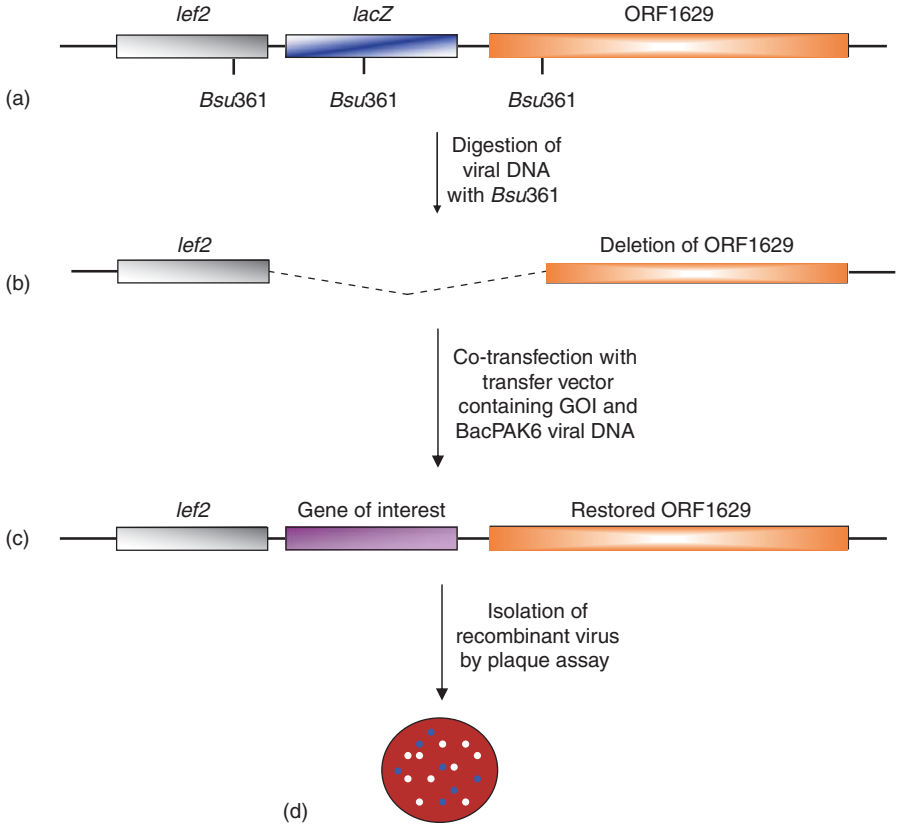


Figure 2 Schematic of recombinant virus production using BacPAK6 DNA. (a) The commercially available BacPAK6 baculovirus DNA contains the *E. coli lacZ* gene inserted at the polyhedrin (*polh*) locus. A *Bsu*361 restriction enzyme site is located within the *lacZ* gene in addition to 2 BSU36q sites found in the flanking *lef2* and ORF1629 coding regions. (b) Restriction enzyme digestion of the viral DNA results in the removal of the *lacZ* gene and partial deletion of the ORF1629 coding region, a gene essential in viral replication, resulting in linear DNA incapable of replication within insect cells. (c) Co-transfection of insect cells with linear BacPAK6 DNA and a transfer vector containing a gene of interest results in insertion of the foreign gene into the virus DNA and restoration of the ORF1629 deletion, circularizing the DNA and permitting virus replication within insect cells and recombinant virus production. (d) Isolation of recombinant virus is achieved via plaque assay. GOI, gene of interest.

DNA by allelic replacement (**Figure 2(c)**). This restoration of the essential virus gene permits replication within insect cells, followed by assembly of nucleocapsids within the nucleus and ultimately production of recombinant viruses. Although the additional *Bsu*361 sites in BacPAK6 lead to an increased proportion of recombinants compared to previous baculovirus expression systems by reducing parental background, restriction enzyme digestion of virus DNA is never 100% efficient and co-transfection still results in the mixture of both parental and recombinant virus. Isolation of solely recombinant virus also requires plaque purification. The presence of *lacZ,* however, allows the selection of colorless, recombinant virus plaques against a background of parental, nonrecombinant, blue plaques in the presence of X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopranoside).

Efforts to remove the requirement for plaque purification and increase both the speed and ease of recombinant virus production have resulted in the development of a number of unique baculovirus expression systems that involve various mechanisms to transfer foreign genes into the viral genome and shall be reviewed here.

Bac-to-Bac

The Bac-to-Bac baculovirus expression system (invitrogen) provides a rapid and efficient method to generate recombinant baculoviruses (**Figure 3**). The system is based on *in vivo* bacterial site-specific transposition of an expression cassette into a baculovirus shuttle vector, also known as a bacmid, propagated in *E. coli*. The bacmid contains a low-copy number mini-F replicon, a kanamycin-resistance gene, and the *lacZ*α gene. Located at the N-terminus of the *lacZ*α gene is an attachment site for the bacterial transposon Tn7 that does not interrupt the reading frame of the *lacZ*α peptide. The bacmid is propagated in *E. coli* cells as a large plasmid resistant to kanamycin that demonstrates blue selection in the presence of X-gal and isopropyl-beta-D-thiogalactopyranoside (IPTG).

Prior to the production of a recombinant baculovirus using the Bac-to-Bac system, the gene of interest is cloned into a donor plasmid containing either a *polh* or *p10* promoter region (**Figure 3(a)**). The expression cassette is flanked by the left and right arms of the Tn7 transposon and contains a gentamicin-resistance gene and an SV40 polyadenylation signal to form a mini-Tn7. The recombinant transfer vector is then transformed into *E. coli* cells containing the bacmid, and a helper plasmid encoding a transposase (**Figure 3(b)**). The mini-Tn7 element from the recombinant transfer vector is transposed to the mini-*att*Tn7 attachment site on the bacmid DNA aided *in trans* by helper plasmid-encoded transposase. Insertion of the gene of interest into the bacmid disrupts the *lacZ*α peptide resulting in white recombinant colonies when exposed to X-gal and IPTG compared to blue parental colonies containing the unaltered bacmid DNA.

Having created the recombinant bacmid, the viral DNA must then be extracted from bacterial cells (**Figure 3(d)**) followed by transfection into insect cells (**Figure 3(e)**). The recombinant virus DNA undergoes transcription and translation, resulting in the production of recombinant BV particles which are then harvested and amplified through repeated rounds of infection to generate a high-titer baculovirus working stock in large volume.

Using such site-specific transposition of genes into bacmid DNA has a number of advantages over using homologous recombination within insect cells for the production of recombinant baculoviruses. Primarily, the viral DNA isolated from selected bacterial colonies is not mixed with parental DNA, eliminating the need to isolate recombinant from nonrecombinant virus and multiple rounds of plaque purification. This substantially reduces the time taken to isolate and purify recombinant virus from 4–6 weeks to 7–19 days. Second, the Bac-to-Bac system easily permits simultaneous production and isolation of several recombinant viruses by one person. In addition to this, the Bac-to-Bac expression system is compatible with the Invitrogen Gateway cloning vectors, a system comprising a multitude of plasmids permitting both single and multiple insertions of genes into a single baculovirus genome.

BaculoDirect

The BaculoDirect baculovirus expression system (Invitrogen) uses the Gateway technology to permit the direct transfer of a gene of interest into the baculovirus genome, without the need for production of recombinant bacmid DNA. Gateway technology is a universal cloning method which provides a rapid cloning step, allowing the efficient transfer of DNA sequences into multiple vector systems. The technology is based on the site-specific recombination properties of the bacteriophage lambda, which facilitates the integration of lambda into the *E. coli* chromosome (**Figure 4(a)**). This integration is mediated by a mixture of enzymes, the lambda recombinase, termed integrase, and the *E. coli*-encoded integration host factor (IHF) and occurs via intermolecular DNA recombination at specific attachment (*att*) sites.

During lambda integration into the *E. coli* chromosome, recombination occurs between *att*B and *att*P sites, present in the *E. coli* chromosomal and lambda phage DNA, respectively, to give rise to *attL* and *att*R sites which flank the integrated phage DNA. This reaction can also be reversed utilizing another lambda-encoded enzyme, excisionase, and recombination of *att*L and *att*R sites, resulting in the excision of the lambda DNA from the *E. coli* chromosome, recreating the *att*B site in *E. coli* and the *att*P site present in lambda phage.

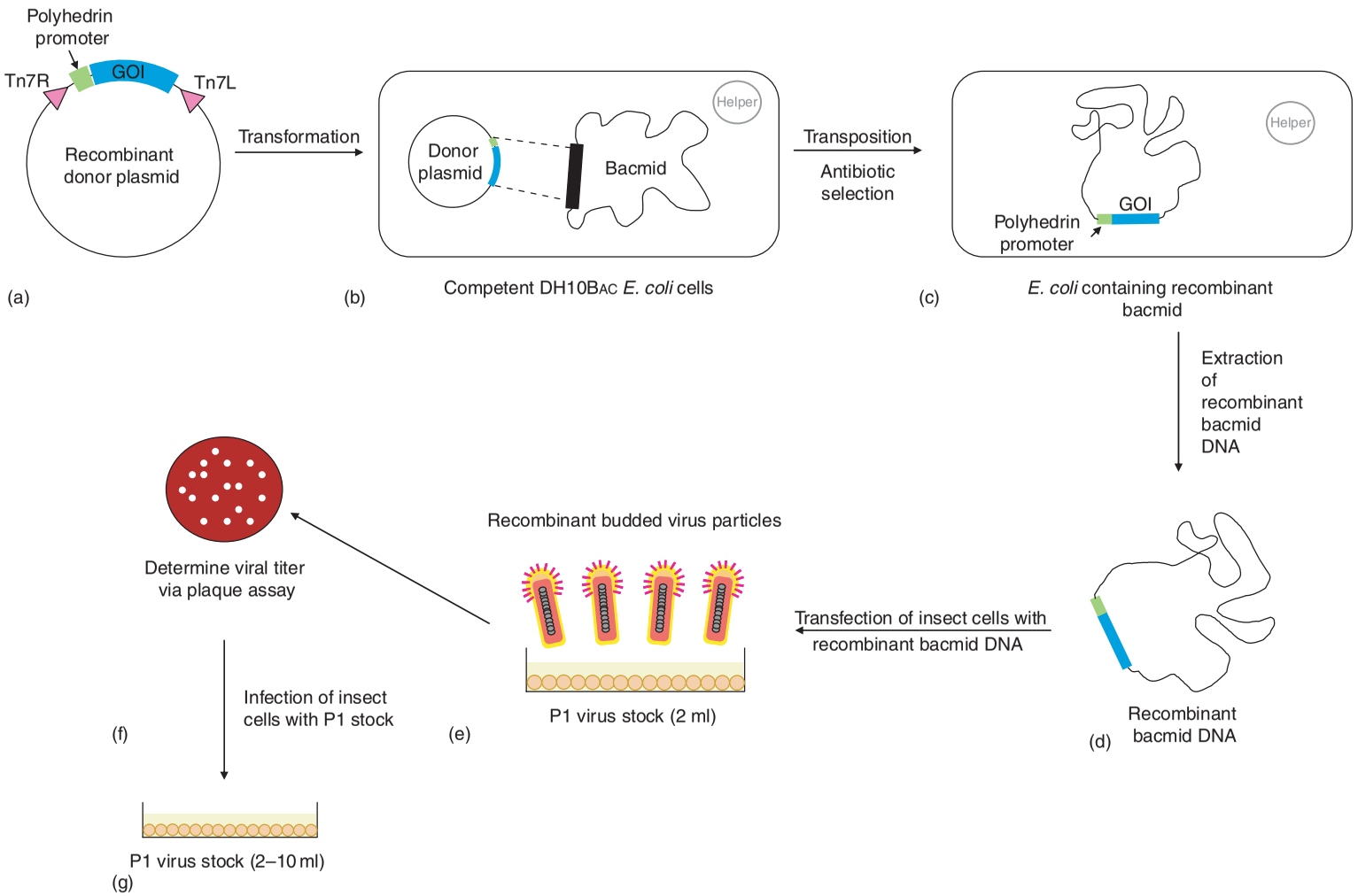


Figure 3 Schematic representation of recombinant virus generation using the Bac-to-Bac system. (a) The gene of interest is first cloned into a suitable donor plasmid containing a polyhedrin (*polh*) promoter and a mini-Tn7 element. (b) The recombinant donor plasmid is then transformed into *E. coli* cells which contain a bacmid with a mini-*att*Tn7 target site and a helper plasmid. The mini-Tn7 element on the recombinant donor plasmid facilitates transposition of the gene of interest (GOI) into the target site on the bacmid along with transposition proteins provided by the helper plasmid, resulting in insertion of the GOI into the bacmid DNA (c). Recombinant virus DNA is then extracted and purified (d) following which insect cells are transfected with the recombinant bacmid DNA (e) to produce a P1 virus stock. (f) The virus titer is determined via plaque assay and further insect cell cultures infected with the P1 stock to create a P2 stock of 2–10 ml (g). GOI, gene of interest.

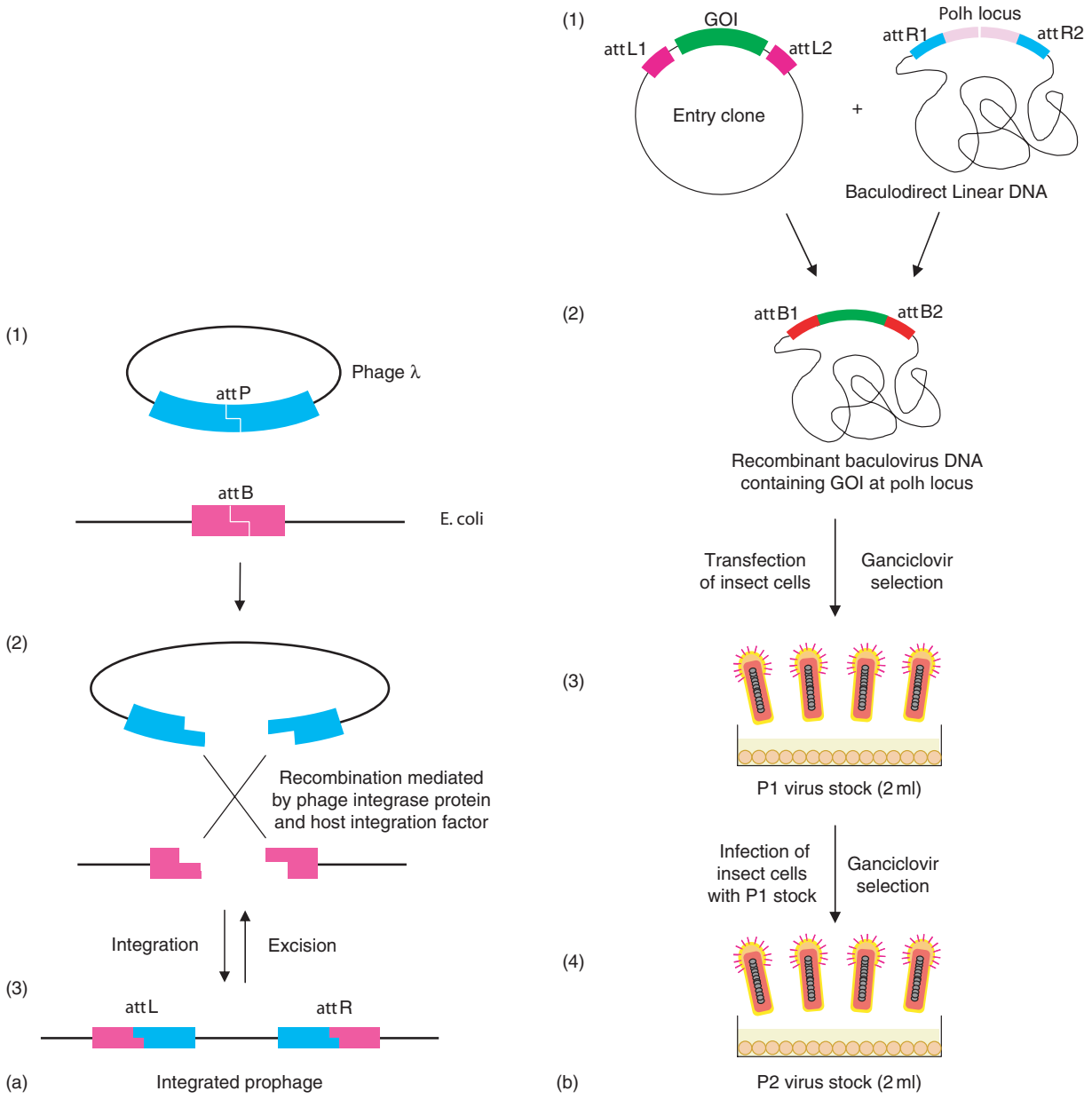


Figure 4 (a) Schematic representation of lambda bacteriophage site-specific transposition in *E. coli.* Following infection of *E. coli* cells, the lambda phage DNA integrates into the host genome between integration sites *att*P and *att*B found in the phage and *E. coli* DNA, respectively (1). The integration reaction is mediated by both phage (intergase) and host (host integration factor) proteins (2) and results in the production of two new sites, termed *att*L and *att*R and the integration of phage DNA into the host genome (3). The integration reaction is reversible mediated by the protein integrase, host integration factor, and excisionase, resulting in the removal of the phage DNA from the viral genome, recreating the *att*B and *att*P sites in *E. coli* and phage DNA, respectively. (b) Schematic representation of recombinant baculovirus production using the BaculoDirect system. Following successful production of an entry clone containing a gene of interest (GOI), an LR reaction between the entry clone and the BaculoDirect linear DNA (1) generates recombinant baculovirus DNA (2) containing the GOI at the polyhedrin (*polh*) locus. Insect cells are then transfected with the recombinant baculovirus DNA and recombinant BV selected via the addition of ganciclovir, resulting in the production of a 2 ml P1 recombinant virus stock (3). This P1 virus stock is then used to infect further insect cell cultures in the presence of the selective compound ganciclovir to produce a high titer, 2 ml P2 virus stock (4).

The Gateway system mimics the lambda integration system in an *in vitro* environment and combined with the BaculoDirect system, provides a suitable system for high-throughput production of multiple recombinant viruses (**Figure 4(b)**). First, the gene of interest must be cloned into a suitable vector, termed an entry clone, with the foreign DNA flanked by two recombination sites, *att*L1 and *att*L2. BaculoDirect linear DNA has been modified to

contain *att*R1 and *att*R2 recombination sites located at the *polh* locus, along with a herpes simplex virus thymidine kinase gene (HSV1 tk) and a *lacZ* gene located between *att*R sites. To create a recombinant baculovirus, it is necessary to perform the recombination reaction utilizing the Gateway LR clonase solution, an enzyme mix comprised of integrase, IHF, and excisionase, to transfer the gene of interest into the baculovirus DNA. The *att*R sites present within the virus DNA undergo recombination with the *att*L sites within the entry clone, resulting in the integration of the foreign gene into the virus DNA to create an *att*B-containing expression virus.

Having performed the LR reaction, baculovirus DNA is then used to directly transfect insect cells. Purification of recombinant viruses ensues following addition of medium containing the selective nucleoside analog, ganciclovir. Ganciclovir is enzymatically phosphorylated by HSV1 tk present in non-recombinant genomes, and results in the incorporation of this nucleoside into DNA, thereby inhibiting their DNA replication. Recombinant viruses that have lost the counterselectable marker via homologous recombination are capable of replication within insect cells, promoting the amplification of recombinant viruses and production of a low-titer, P1 viral stock. Screening of P1 stocks can then be undertaken to ensure recombinant protein expression and the absence of parental background. Alternatively, the P1 stock can be used to directly infect insect cells in the presence of ganciclovir to produce a larger volume P2 working stock.

The high-throughput application of BaculoDirect for the production of multiple recombinant viruses appears to offer significant time savings compared to other systems such as Bac-to-Bac. However, this system is currently restricted to the production of recombinant single gene baculoviruses preventing co-expression of multiple proteins. In addition, the necessity to selectively isolate recombinant from parental virus via the addition of ganciclovir is detrimental to total cell numbers and therefore consequently negative to final viral titers, ultimately requiring multiple virus amplification steps before a high-titer working virus stock is produced.

*flash*BAC

A new platform technology for the production of recombinant baculoviruses has recently emerged that has significant advantages over the other vector systems described above. The *flash*BAC expression system (Oxford Expression Technologies, Oxford, UK) combines the advantages of the traditional homologous recombination system within insect cells with those of the more recent bacterial-based systems. *Flash*BAC has been developed to remove the requirement for isolation of recombinant from parental virus via plaque purification, resulting in a one-step procedure for recombinant virus production and ultimately significantly reducing the time taken to produce a working high-titer recombinant virus stock.

The *flash*BAC expression system is based around a modified AcMNPV genome that contains a bacterial artificial chromosome (BAC) at the *polh* locus replacing the *polh* gene. The presence of the BAC within the virus genome allows the virus genome to be maintained and propagated within bacterial cells from which the circular DNA is then isolated and purified. Additionally, the modified genome contains a deletion in the essential gene ORF1629 which renders the virus inactive and unable to replicate within insect cells (**Figure 5(a)**).

Homologous recombination between *flash*BAC DNA and a suitable transfer vector containing the gene of interest, following transfection into insect cells, restores the deletion within, and thus the function of, the essential ORF1629 gene (**Figure 5(b)**). Simultaneously, the gene of interest is inserted into the viral genome at the *polh* locus under the control of the *polh* promoter, concomitantly removing the BAC replicon. The recombinant virus genome, with the restored essential gene, is then capable of replication within insect cells, producing recombinant BV particles that can be harvested from the culture medium of the transfected cells (**Figure 5(c)**). The deletion present within ORF1629 of *flash*BAC DNA prevents replication of any nonrecombinant, parental virus; therefore, there is no requirement for recombinant virus isolation. Following transfection, virus can be added directly to insect cells to produce a high-titer working recombinant virus stock.

The *flash*BAC system is back-compatible with all baculovirus transfer vectors based on homologous recombination in insect cells at the *polh* locus, including vectors using the *polh* promoter, dual triple and quadruple expression vectors, and those that use other gene promoters such as *p10*, *ie1*, or *gp64*. However, vectors such as pFastBac which are designed for site-specific transposition in *E. coli* systems such as Bac-to-Bac are not compatible with the *flash*BAC system.

The *flash*BAC system is unique among baculovirus expression systems as it maximizes protein secretion and membrane protein targeting. Within the baculovirus genome are a number of auxiliary genes which are nonessential in the replication and production of BV particles *in vitro*. One such gene codes for chitinase (*chiA*), an enzyme with exo- and endochitinase activity, essential in the host-to-host transmission of the virus. Following infection of a host insect, chitinase works in synergy with another virally encoded protein, termed cathepsin, to break down the host cuticle, ultimately resulting in tissue liquefaction and release of polyhedra to infect more hosts. Confocal and electron microscopy analysis has shown the localization of chitinase within the endoplasmic reticulum during baculovirus infection. This severely compromises

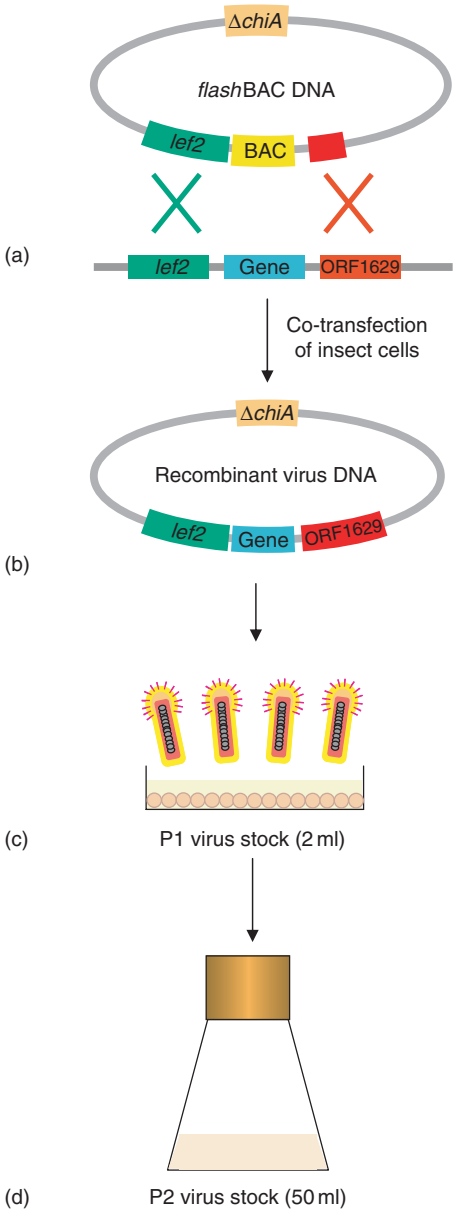


Figure 5 Schematic representation of recombinant virus production using the *flash*BAC system. The *flash*BAC expression system relies on a modified baculovirus genome containing a BAC at the polyhedrin (*polh*) locus and a partial deletion of the essential ORF1629 viral gene (a). Homologous recombination within insect cells between the *flash*BAC DNA and a transfer vector containing a gene of interest (GOI) flanked by *lef2* and the complete ORF1629 gene coding regions results in the insertion of the GOI at the *polh* locus and restoration of ORF1629 gene (b). The deletion within ORF1629 results in a P1 stock containing only recombinant baculovirus production with no parental background (c). This P1 stock is then used to infect a larger insect cell culture resulting in the production of a high-titer, high-volume (50 ml) P2 working recombinant virus stock (d).

the function efficiency of the secretory pathway, consequently affecting the levels of recombinant protein production. Deletion of *chiA* from *flash*BAC has substantially improved the efficacy of the secretory pathway for producing recombinant proteins in insect cells, enhancing the yield of secreted of membrane-bound proteins in comparison to proteins produced in recombinant viruses that synthesize chitinase.

The one-step *flash*BAC technology facilitates the use of robotic systems for the generation of multiple recombinant viruses without the requirement for plaque purification. It has been demonstrated that co-transfection mixtures from multiple recombinant viruses can be used to infect insect cells for automated high-throughput protein screening in 96-well format. Additionally, this process can be performed solely in insect cells, removing the risk of cross-contamination between insect and bacterial cell cultures.

Baculovirus-Mediated Gene Delivery in Mammalian Cells

The baculovirus expression system has been extensively used for the expression of recombinant proteins within insect cells for a number of years. More recently, recombinant baculovirus vectors have been developed to permit transient and stable gene delivery into a number of mammalian cell lines. Such vectors contain baculovirus promoters alongside mammalian cell-active expression cassettes to permit amplification of recombinant viruses in insect cells and expression of recombinant proteins in mammalian cells, respectively.

Baculovirus-mediated gene delivery into mammalian cells, known as BacMam technology, was first demonstrated in hepatic cell lines and subsequent studies have reported transduction of human cell lines including HeLa, pancreatic β-cells and primary neural cells, Chinese hamster ovary and porcine kidney cell lines. The transduction efficiencies of different cell lines vary considerably, with baby hamster kidney cells having a transduction efficiency of 95% while mouse NIH 3T3 cells demonstrate only 10% efficiency. Transduction efficiency can be enhanced by the addition of various compounds such as trichostain A or sodium butyrate that act as histone deacetylase inhibitors; however, these drugs have cytotoxic effects on cell cultures. The majority of recombinant viruses used to transduce mammalian cell lines contain hybrid promoters consisting of a chicken β-actin gene promoter and a cytomegalovirus immediate early gene enhancer element along with a *p10* baculovirus promoter for expression of the gene in insect cell lines.

One significant advantage of BacMam technology is that successful gene delivery of foreign DNA into mammalian cells is possible by simply adding recombinant baculovirus inoculum to a culture of mammalian cells. The entry mechanism of baculovirus into mammalian cell is poorly understood; however, it is thought that the viral surface glycoprotein, GP64, plays a role in viral entry and

endosomal release. Following viral entry, nucleocapsids induce actin filament formation within mammalian cells, a process also known to occur within insect cells involved in the propulsion of virus particles to the nucleus. Reports have demonstrated an increase in nuclear localization of nucleocapsids in human hepatocytes following the disruption of the microtubule network. This suggests that microtubules constitute a barrier to baculovirus transport toward the nucleus and thus disruption of these filaments may provide a simple method with which to increase nuclear localization of recombinant nucleocapsids and ultimately increase foreign gene expression within mammalian cell lines. Transduction of mammalian cells with recombinant baculovirus is generally considered nontoxic and has no apparent effect on cell growth, even at high multiplicities of infection.

The inability of baculoviruses to replicate within mammalian cells provides an attractive vector for *in vivo* applications, including gene function studies and gene therapy. However, baculovirus is rapidly inactivated by human serum complement, destroying the ability of the recombinant virus to transfer genes *in vivo*. A number of methods have been developed to alleviate this problem, including production of baculovirus particles pseudotyped with the vesicular stomatitis virus (VSV)-G protein, which provides a virus more resistant to complement than unmodified virus. Additionally, the use of baculoviruses to express antigens under the control of mammalian promoters has been shown to elicit an immune response *in vivo*. Recombinant baculoviruses expressing the gB protein from pseudorabies produced an immune response in mice intramuscularly inoculated with the modified baculovirus. Additionally, recombinant baculoviruses expressing the influenza hemagglutinin protein elicited immune responses in mice when delivered by intramuscular injection and provided immunity to further infection following subjection to a lethal dose of influenza virus. The possibility of utilizing baculoviruses for gene therapy and vaccine applications is still a very new, albeit extremely promising, technology and much research continues in this area to optimize baculoviruses as potential gene therapy vectors.

Conclusions and Future Perspectives

Manipulation of the baculovirus genome provides a powerful tool for the expression of recombinant proteins in both insect and mammalian cell lines. Advances in vector design and the various commercially available baculovirus expression systems permit simple, recombinant virus production, with some systems negating the requirement of virus isolation and purification. This, coupled with the automation and high-throughput possibilities of virus production using robotic systems for simultaneous multiple-virus production, has resulted in more laboratories employing baculoviruses as their expression system of choice.

One of the main disadvantages of the baculovirus expression system is the dissimilarity of the insect cell protein-processing pathways compared to those of higher eukaryotes, for example, the N-glycosylation pathway. The production of transgenic insect cell lines expressing humanized protein glycosylation pathways offers a way to overcome this potential problem, enabling the production of recombinant polypeptides which demonstrate greater similarity to their native mammalian proteins.

Recombinant baculoviruses have become a widely used system for the production of recombinant proteins within insect cells. The availability of the entire baculovirus sequence will continue to enable further manipulation of the virus genome to increase and further optimize recombinant protein expression in both insect and mammalian cell lines. It is hoped that the use of recombinant baculoviruses as gene delivery vectors for higher eukaryotic cell lines will become as routine as the use of such viruses for recombinant protein expression within insect cells and that advances in knowledge and technology will continue to expand the possibilities and applications of the baculovirus expression system.

See also: Baculoviruses: General Features; Baculoviruses: Molecular Biology of Mosquito Baculoviruses; Baculoviruses: Pathogenesis.

Further Reading

Hu Y (2005) Baculovirus as a highly efficient expression vector in insect and mammalian cells. *Acta Pharmacologica Sinica* 26: 405–416.

Hunt I (2005) From gene to protein: A review of new and enabling technologies for multi-parallel protein expression. *Protein Expression and Purification* 40: 1–22.

King LA and Possee RD (1992) *Baculovirus Expression System – A Laboratory Guide.* London: Chapman and Hall.

Kitts PA and Possee RD (1993) A method for producing recombinant baculovirus expression vectors at high frequency. *Biotechniques* 14: 810–817.

Kost TA and Condreay JP (2002) Recombinant baculovirus as mammalian cell gene-delivery vectors. *Trends in Biotechnology* 20: 173–180.

Kost TA, Condreay JP, and Jarvis DL (2005) Baculovirus as versatile vectors for protein expression in insect and mammalian cells. *Nature Biotechnology* 23: 567–575.

Pennock GD, Shoemaker C, and Miller LK (1984) Strong and regulated expression of *Escherichia coli* beta-galactosidase in insect cells with a baculovirus vector. *Molecular and Cellular Biology* 4: 399–406.

Smith GE, Summers MD, and Fraser MJ (1983) Production of human beta interferon in insect cells infected with a baculovirus expression vector. *Molecular and Cellular Biology* 3: 2156–2165.

Baculoviruses: General Features

P J Krell, University of Guelph, Guelph, ON, Canada

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Apoptosis Programmed cell death.
Caspase Protease involved in the apoptotic response.
Enhancin A baculovirus protein which enhances the infectivity of another virus.
Granulin The major protein in GV granules.
IAP A baculovirus inhibitor of apoptosis.
P35 A baculovirus inhibitor of apoptosis.
Polyhedrin The major protein in NPV polyhedra.
VLF Very late expression factor.

Historical Perspectives

Diseases reminiscent of baculovirus infections appear in the historical record going back to at least 1527 when Bishop Marco Girolamo Vida in Italy wrote a verse which referred to a jaundice disease of the silkworm *Bombyx mori*. In 1856, Maestri and Cornalia independently described refractile bodies, reminiscent of the polyhedra of nucleopolyhedroviruses (NPVs), within cells of infected silkworm larvae demonstrating a 'polyhedrosis' disease. Glaser, Paillot, and colleagues, between 1913 and 1928, demonstrated that the polyhedral disease of caterpillars (a polyhedrosis) was due to a filterable agent and therefore must have a viral etiology. With the perfection of insect tissue culture methodology, William Trager in 1935 reported on the ability of the Grasserie virus (now known as bombyx mori NPV (BmNPV)) to replicate in silkworm tissue culture cells. This led to the opportunity of studying the replication of these viruses under more controlled conditions and free of other microbial agents.

Nomenclature, Taxonomy, and Classification

The family *Baculoviridae* consists of insect viruses with a large, 80–180 kbp circular dsDNA genome and a distinct rod-shaped virion morphology. It is composed of two genera, *Nucleopolyhedrovirus* for NPVs with *Autographa californica nucleopolyhedrovirus* as the type species and *Granulovirus* for the granuloviruses (GVs), with *Cydia pomonella granulovirus* (CpGV) as the type species (refer to **Table 1** for virus abbreviations). Though not an accepted taxonomic designation, but based on comparison of genomic sequences, the NPVs can further be subdivided into group I, which encode *gp64* but not *Fusion protein* (F) or *p13*, and group II, which encode *F* and *p13* but not *gp64*.

The family *Baculoviridae,* infecting invertebrate, mostly insect, hosts, owes its name to the stick-shaped morphology of the virions (*baculum*, Latin for stick). The rod-shaped double-stranded DNA (dsDNA)-containing nucleocapsids measuring around 30 nm × 100 nm are surrounded by a membrane envelope. The *Baculoviridae* are unique in having two morphologically distinct forms and a biphasic replication cycle. Early in infection, budded viruses are produced by nucleocapsids budding through the cell membrane. This budded form is responsible for cell-to-cell transmission within the infected insect or in tissue culture. Later in infection, nucleocapsids remain in the nucleus where they are enveloped either singly or in multiples in a nuclear-derived envelope and are subsequently embedded in a polyhedral (*Nucleopolyhedrovirus*) or granular (*Granulovirus*)-shaped proteinacious, alkaline-sensitive, crystalline matrix that is highly resistant to environmental influences. The occluded form is responsible for the horizontal, insect-to-insect spread.

In the past, the baculovirus family was a repository for many arthropod viruses with a similar rod-shaped viral morphology including the polydnaviruses of parasitic wasps and the whispoviruses of shrimp that now have their own taxa, families *Polydnaviridae* and *Nimaviridae*, respectively. Oryctes virus (OrV; formerly named oryctes baculovirus) and Hz-1 baculovirus are also baculovirus-like but lack an occlusion body whereby the genus *Nudivirus* has been suggested for them. As the characteristics needed to define the *Baculoviridae* have crystallized, the number of species recognized has become more manageable. For example, in the VIth Report of the International Taxonomy of Viruses, a total of 15 species and an amazing 372 tentative species were assigned to the genus *Nucleopolyhedrovirus*, while the genus *Granulovirus* numbered four species and a tentative species list of 65. As many of these were too poorly characterized, original stocks were not available, and some may have simply been the same virus but isolated from a different host, most of the tentative species have been dropped. In the VIIIth report, there are now 24 *Nucleopolyhedrovirus* species recognized with only five tentative species and 15 *Granulovirus* species but no tentative species.

Analysis of complete baculovirus genome sequences provides a powerful basis for refining this classification from two to four genera to reflect the differences in biology and genome phylogeny of members of the *Baculoviridae*.

Table 1 Taxonomy, names, abbreviations, and genome sizes of of baculoviruses used in the text

Subgroup/host	*Species name*[a]	*Abbreviation for virus name*	*Genome size (bp)*
Genus *Nucleopolyhedrovirus*			
Lepidopteran			
Group I	*Anticarsia gemmatalis M nucleopolyhedrovirus*	AgMNPV	132 239
	Autographa californica M nucleopolyhedrovirus	AcMNPV	133 894
	Bombyx mori nucleopolyhedrovirus	BmNPV	128 413
	Choristoneura fumiferana M nucleopolyhedrovirus	CfMNPV	129 593
	Epiphyas postvittana nucleopolyhedrovirus	EppoNPV	118 584
	Hyphantria cunea nucleopolyhedrovirus	HycuNPV	132 959
	Orgyia pseudotsugata M nucleopolyhedrovirus	OpMNPV	131 995
Group II	*Adoxophyes honmai nucleopolyhedrovirus*	AdhoNPV	113 220
	Helicoverpa armigera nucleopolyhedrovirus	HearNPV	130 759
	Leucania separata nucleopolyhedrovirus	LeseNPV	168 041
	Lymantria dispar M nucleopolyhedrovirus	LdMNPV	161 046
	Spodoptera exigua M nucleopolyhedrovirus	SeMNPV	135 611
	Spodoptera littoralis nucleopolyhedrovirus	SpliNPV	
	Spodoptera litura nucleopolyhedrovirus	SpltNPV	139 342
Dipteran	*Culex nigripalpus nucleopolyhedrovirus*	CuniNPV	108 252
Hymenopteran	*Neodiprion abietis nucleopolyhedrovirus*	NeabNPV	84 264
	Neodiprion lecontei nucleopolyhedrovirus	NeleNPV	81 755
	Neodiprion sertifer nucleopolyhedrovirus	NeseNPV	86 462
Genus *Granulovirus*			
Lepidopteran	*Adoxophyes orana granulovirus*	AdorGV	99 657
	Choristoneura occidentalis granulovirus	ChocGV	104 710
	Cydia pomonella granulovirus	CpGV	123 500
	Phthorimaea operculella granulovirus	PhopGV	119 217
	Xestia c-nigrum granulovirus	XecnGV	178 733

[a]Species names in italics are approved species, names in Roman script are tentative or as yet an unassigned species according to *Eighth Report of the International Committee on Taxonomy of Viruses*.

The four genera suggested are lepidopteran-specific NPVs (*Alphabaculovirus*), dipteran-specific NPVs (*Deltabaculovirus*), hymenopteran-specific NPVs (*Gammabaculovirus*), and lepidopteran-specific GVs (*Betabaculovirus*).

Ever since their earliest discovery, baculoviruses have been named according to the insect from which the virus was derived and the type of occlusion body associated with it. While this might cause some confusion, since the same virus might occur in different insect species, this approach is still applied today. Originally baculovirus abbreviations used the first letter of the genus and species name of the host insect followed by NPV or GV depending on the type of occlusion body. Hence the multiple-embedded NPV (MNPV) from the alfalfa looper *Autographa californica* is abbreviated as AcMNPV and the MNPV from *Choristoneura fumiferana* is abbreviated CfMNPV. Those baculoviruses which have historically had a two-letter species code continue to maintain those abbreviations. However, as more baculoviruses were being described, a two-letter species designation system was untenable and now a four-letter species identification based on the first two letters of each of the genus and species names of the host insect from which the virus was first isolated is used. Hence the more recently described NPV from *Helicoverpa armigera* is abbreviated as HearNPV and that from *Neodiprion lecontei* is abbreviated as NeleNPV. If identical letterings might result, the third or later letters are used instead of the second as for the NPV from *Spodoptera litura* which is abbreviated SpltNPV since an NPV from *Spodoptera littoralis* is abbreviated SpliNPV. Although the S and M are useful morphological descriptors in the names indicating if nucleocapsids exist either singly or in multiples within virions, they do not appear to have any taxonomic relevance and so in recently described baculoviruses these designations have been dropped but have been maintained for those baculoviruses in which there is a strong historical precedence.

Morphological Characteristics

The *Baculoviridae* are currently comprised of two genera, *Nucleopolyhedrovirus* and *Granulovirus*, based initially on different morphological characteristics. Viruses in both genera have dual phenotypes and biphasic replication cycles, though with similar virion and genomic features. Nucleocapsids are rod shaped with diameters of 30–60 nm and lengths, dependent on the size of the dsDNA genome,

of 250–300 nm. Nucleocapsids are structurally polar with a flat base plate on one end and a nipple at the other. The nucleocapsids are enveloped in either *de novo*-derived membrane in the nucleus for occlusion-derived viruses or viral-modified plasma membrane of the infected cell for budded viruses. The budded virions of NPVs also have polarity with the nipple end showing a distinct peplomeric structure consisting of viral glycoproteins such as GP64 or F proteins.

For the genus *Nucleopolyhedrovirus*, several virions containing either single (SNPVs) or multiple nucleocapsids within a single envelope (MNPVs) are embedded in a proteinaceous polyhedral-shaped occlusion body measuring 0.5–15 μm in diameter but most measure around 1 μm in diameter. The major structural protein in the polyhedra is aptly named polyhedrin.

The *Granulovirus* virions contain only a single nucleocapsid per virion and only one virion is embedded in a much smaller proteinaceous ovocylindrical occlusion body referred to as a granule which measures *c.* 120–300 nm × 300–500 nm. The major structural protein in the granules is aptly named granulin but is, however, evolutionarily related to polyhedrin. GVs tend to have a more restricted host range and limited tissue tropism compared to the NPVs.

Genomes, Gene Content, Organization, and Evolution

Of 41 full-length baculovirus genome sequences deposited in the NCBI Virus Genome database, the genome sizes of the GVs range from 99 657 bp for adoxophyes orana granulovirus to 178 733 for xestia c-nigrum granulovirus, which is also the largest genome of any baculovirus; and for the NPVs range from 81 755 bp for neodiprion lecontei nucleopolyhedrovirus to 168 041 bp for leucania separata nucleopolyhedrovirus. The total number of computational open reading frames (ORFs, usually defined as those encoding proteins of at least 50 amino acids) identified among baculoviruses ranges from 89 (neodiprion lecontei nucleopolyhedrovirus) to 181 (xestia c-nigrum granulovirus) per genome. The guanine–cytosine (GC) content of baculovirus genomes is very broad ranging for NPVs from 33% for neodiprion lecontei nucleopolyhedrovirus to 58% for lymantria dispar M nucleopolyhedrovirus, and for GVs from 32% for choristoneura occidentalis granulovirus to 45% for cydia pomonella granulovirus.

Over 500 unique genes have been identified in baculoviruses but all genomes sequenced to date encode a common set of 29 core genes including those involved in DNA replication like *dnapol* and *hel*; transcription, such as for the four proteins making up the late viral RNA polymerase and late expression factor-4 (LEF-4), a protein which might be involved in capping; structural proteins such as the small basic P6.9 protein associated with the genomic DNA, the major capsid protein VP39, and proteins VP1054 and VP91 associated with both budded and occluded virus; auxiliary proteins like alkaline exonuclease and the two *per os* infectivity factors; and some unknown proteins identified only by an ORF sequence such as the AcMNPV ac98 ORF.

Transcription is fairly equally distributed to both strands. There is no particular clustering of genes on the genome on the basis of, for example, function or temporal transcription class although there is some conservation of gene order among closely related viruses.

Baculovirus genomes can contain multiple homologous repeat regions (*hr*'s) containing differing numbers of imperfect palindromic sequences. The analysis of *Eco*R1 digestion patterns in AcMNPV DNA initially led to the discovery of *hr*'s. AcMNPV has 8 *hr*'s, with each *hr* having two to eight highly conserved regions of *c.* 72 bp. AcMNPV *hr*5 has six, 60–70 bp direct repeats, most with an *Eco*R1 restriction site within a 28 bp imperfect palindrome. *Hr*'s act as enhancers of transcription and as replication origins for transient plasmid DNA replication assays.

The availability of baculovirus complete genome sequences, currently 41, along with a better understanding of the biology of the baculoviruses allowed for the modeling of potential evolutionary pathways. Throughout evolution, individual genes doubtless underwent mutations, but there also appeared to be extensive horizontal gene transfer between virus and host and between virus and virus in shared environments. Moreover, most viruses in nature exist as a mixture of genotypes. It is this genotype plasticity in concert with horizontal gene transfer that complicates efforts at defining a clear evolutionary pathway for the baculoviruses.

Evolutionary models were based initially on phylogeny of individual core genes such as polyhedrin and granulin, and then, of 'fused' core protein sequences as well as conservation of gene order. This phylogeny results in four evolutionary groups which is in concert with the proposal for creating four genera. The most ancient baculoviruses are thought to be the dipteran ones, with culex nigripalpus nucleopolyhedrovirus as the sole member. The next most ancient are hymenopteran baculoviruses, like neodiprion sertifer NPV (NeseNPV) and NeleNPV which infect sawflies and have the smallest genomes of all baculoviruses. The lepidopteran NPVs represent a more recent lineage with initial branches separating the GVs such as CpGV and xestia c-nigrum granulovirus (XecnGV) from the NPVs and the NPVs separating easily into two groups, group II including lymantria dispar MNPV (LdMNPV) and spodoptera exigua MNPV (SeMNPV) and the most recent group, group I NPVs, including AcMNPV and CfMNPV.

Virus Replication Cycle

Attachment, Entry, and Uncoating

The initial steps leading to virus entry in insects differ somewhat from that in cell culture. In insects, following *per os* ingestion of polyhedra, the polyhedra dissolve in the alkaline environment of the insect gut releasing the occlusion-derived virions. In order to access the target columnar midgut epithelial cells, the released virus must first penetrate the peritrophic membrane lining the gut. Some viruses, like some GVs, encode enhancin or virus-enhancing factor (VEF), which increases the infectivity in larvae of other baculoviruses. As a metalloproteinase which degrades mucin, VEF is thought to disrupt the peritrophic membrane barrier to the midgut epithelium, allowing easier penetration by the virus. VEF is also thought to increase the fusion efficiency between the viral and cellular membranes during infection. The membrane of the virus then fuses with the membranes of the microvilli of the epithelial cell, releasing the nucleocapsid into the cytoplasm. The host cell receptor is not known and may be a generalized one. The attachment and fusion require the highly conserved baculovirus envelope protein p74 and proteins such as *per os* infectivity factors 1 or 2 (PIF-1, PIF-2).

In insect cell cultures, virus attachment is facilitated by a viral attachment protein, for example, either the group I NPV glycoprotein GP64 or its homologs, or the group II NPV F (fusion) protein or its homologs. The host cell receptor is not known, though it is likely a glycoprotein. Following virus entry by receptor-mediated endocytosis, virus is released from the acidified endosome, presumably as a consequence of acid-mediated fusion between the viral and the endosomal membranes.

The released nucleocapsids, either in insects or in tissue culture cells, migrate to the nuclear pore, perhaps via actin cables induced by the nucleocapsids. Some nucleocapsids which enter midgut epithelial cells bypass the nucleus and bud out into the hemocoel through the basal cell membrane. The nucleocapsid is uncoated either at the nuclear pore or as it enters the nuclear pore to release the DNA core into the nucleus.

Temporal Regulation of Transcription

Baculovirus transcription is temporally regulated into at least three classes, early, late, and very late, in which expression of genes of one temporal phase is dependent on the expression of genes in the preceding phase. The expression of some genes is not clearly delineated and extend through several phases. Early genes are expressed prior to DNA replication and late genes are defined as those starting at or after the onset of viral DNA replication.

Early gene expression

Early genes can be divided into immediate early and delayed early genes. The early genes can have promoters containing TATA-like or CAGT and initiator motifs and some have enhancers allowing them to be transcribed by the host transcription. Immediate early genes, like *ie1*, *ie2*, and *ie0*, the only baculovirus gene yet shown to produce spliced RNAs that result in different protein products, are transcribed within minutes of entry by host RNA pol II. IE0, IE1, and IE2 act as transregulators of downstream genes. For example, IE1 transregulates early genes such as *dnapol* and *hel* for viral DNA replication and genes important for suppression of apoptosis, such as *p35* or inhibitor of apoptosis (*iap*). Some baculoviruses, such as those from sawflies, do not have identifiable early transregulatory genes and may have alternate mechanisms for early gene regulation. Another set of early genes are those for auxiliary proteins important for modifying the cell or host insect environment to the benefit of the virus including the production of ecdysteroid UDP glucosyl transferase (EGT), which prevents larval molting and pupation.

Transcription of some of these early genes is increased by *hr* enhancer sequences, allowing for enhanced transcription of genes under conditions early in infection when only a small amount of input viral DNA is available.

DNA replication

Once viral proteins involved in viral DNA replication, like DNApol, helicase, and some of the late expression factors (LEFs), are produced, viral DNA replication can initiate. The timing varies with the virus and with the host insect or host cell. In AcMNPV-infected *Spodoptera frugiperda* cells, for example, DNA replication begins at around 6 h post infection and increases until about 18 h post infection. In comparison in CfMNPV-infected *C. fumiferana* cells, DNA replication does not initiate until about 24 h post infection. Little is known about the *cis*-sequences which might act as origins of DNA replication. The baculovirus *hr*'s act as DNA *ori*'s in transient, plasmid DNA replication assays. Other *non hr ori*'s have also been implicated as origins of DNA replication, for example, the *Hin*dIII K fragment of AcMNPV and the *Xba*I-F2 fragment of SeMNPV. Several viral genes, such as AcMNPV *ie-1*, *ie-2*, *hel*, *dnapol*, *lef-1*, *lef-2*, *lef-3*, and *p35*, are essential for NPV viral DNA replication and replication is augmented by *pe38*. LEF-1 is a DNA primase, LEF-2 might help stabilize binding of LEF-1 to the DNA, LEF-3 is a single-stranded DNA (ssDNA) binding protein and acts to transport the viral helicase into the nucleus, and P35 is an apoptotic suppressor which might act both by inhibiting apoptosis and by activating expression of the *hel*, *lef-3*, and *DNA pol* genes among others. It is not currently known if all baculoviruses require similar virally encoded genes for viral DNA replication.

In NPV-infected cells, viral DNA and at least four viral proteins (IE1, LEF3, DNA helicase and another viral ssDNA binding protein, DBP) in association with *hr*'s accumulate in discrete areas within the nucleus, forming a

virogenic stroma in which viral DNA replication, occluded virus morphogenesis, and occlusion body formation are thought to occur.

Late gene expression

The late phase of baculovirus transcription begins after the initiation of viral DNA replication and appears to be at least in part dependent on DNA replication. The late genes are mostly for the virion structural proteins including the AcMNPV major capsid protein VP39, a basic protein P6.9, and the virion envelope glycoprotein GP64. Chitinase and viral cathepsin, both involved in the liquefaction of the infected insects, are also synthesized late in infection.

All baculovirus late and very late genes are distinguished from the early genes by their promoters, which have a canonical DTAAG as an essential element, and their expression by a viral RNA polymerase. These promoters are differentially recognized by a viral RNA polymerase comprised of four late proteins, LEF-4, LEF-8, LEF-9, and P47. LEF-4 has an essential guanyltransferase activity and so is presumably responsible for 5′ capping of viral transcripts. LEF-8 and LEF-9 have motifs suggestive of an active site for RNA polymerase. The role of P47 is still unknown. Coincident with late gene expression is an almost global decrease in host gene expression. This depression appears to be due to a downregulation of host mRNA synthesis such as transcripts for initiation factors eIF2, eIF2α, eIF4E, and eIF5A, ribosomal protein L15 (rpL15), histone, and actin. However, a few host genes, such as the heat shock cognate Sf Hsc70 gene, appear to be upregulated, at least transiently, following infection with AcMNPV.

p10 and *polyhedrin* (or *granulin*) are expressed very late in infection. In addition to the DTAAG element typical of late gene promoters, very late promoters have a 'burst element' downstream of the DTAAG site. In addition, a very late expression factor 1 (*vlf-1*) gene product, VLF1, preferentially stimulates very late gene expression. P10 is involved in cellular lysis and is associated with a nuclear/cytoplasmic fibrillar structure in the area of polyhedral morphogenesis in the intranuclear virogenic stroma and is thought to stabilize them. Though expendable, P10 might mediate production of tubular structures found in infected cells. As both polyhedrin for NPVs and granulin for GVs are needed in high amounts and only very late in infection after budded virus is produced, their genes are expressed both at high levels and very late in infection.

Morphogenesis and Release

During viral morphogenesis, nucleocapsids start forming in the nuclear virogenic stroma, first by the production of capsid sheaths later filled in by core material in a polar manner with a cap structure at one end. The virus then undergoes two different morphogenetic pathways, one leading to budded virus, the other leading to enveloped virions, which become occluded to form polyhedra (NPVs) or granules (GVs). The budded viruses are responsible for cell-to-cell transmission *in vitro* or *in vivo*, while the occluded form is responsible for insect-to-insect spread.

Early in infection, some of these intranuclear nucleocapsids follow the budding route. The NPV nucleocapsids first bud through the nuclear membrane by synhymenosis, acquiring a double membrane. By an unknown mechanism (e.g., disintegration of the membrane, or fusion of inner and outer membranes), the nucleocapsids shed that membrane to release the nucleocapsids into the cytoplasm. In GV-infected cells, the nuclear envelope disintegrates and the nucleocapsids are released directly into the cytoplasm. For NPV and possibly GV, nucleocapsids align in a polar fashion with the nipple end of the nucleocapsids aligned with the plasma membrane. They then bud through the plasma membrane such that the peplomers, that may contain GP64 or F, cover only the apical end, providing polarity to the virions.

For some baculovirus species, such as the sawfly NPVs and some GVs, the infection remains within the midgut. However, for most baculovirus species, virus disseminates from the midgut epithelial cells, throughout the insect to infect all tissues. For these, the virus buds through the basal layer of the midgut epithelial cells and then must pass through the basal lamina to the hemolymph in the open circulatory system of the larvae where it is circulated to other tissues. Baculoviruses are also thought to be disseminated via the tracheal system.

Later in infection, the intranuclear nucleocapsids become enveloped in a *de novo*-derived membrane. Some baculovirus nucleocapsids become enveloped singly (SNPVs and GVs) and for some, multiple nucleocapsids are enveloped within a common envelope (MNPVs). The SNPVs and the GVs generally have only a single nucleocapsid per virion. For the MNPV, several nucleocapsids are found within each virion. The virions then become occluded within polyhedra (NPVs) or granules (GVs). The systemic infection is so effective that following death, up to 25% of the larval mass is due to polyhedra.

Also, late in infection, chitinase and cathepsin, which have been produced in an inactive form, are activated. They are interdependent for activation, release, and mediating liquefaction, which is critical to release, spread, and transmission of the infection in nature. If either of these proteins is missing, as in a knockout virus or viruses that lack one or both of these genes, there is no liquefaction. These enzymes are not encoded by certain GVs such as adoxophyes orana granulovirus (AdorGV), phthorimaea operculella granulovirus (PhopGV), and XecnGV, or the dipteran, mosquito baculovirus culex nigripalpus nucleopolyhedrovirus (CuniNPV), and the hymenopteran sawfly viruses NeleNPV, NeseNPV, and neodiprion abietis

nucleopolyhedrovirus (NeabNPV). For these, much of the virus is released by sloughing of infected gut epithelial cells and excretion of viruses, so there is no need for liquefaction to spread the virus.

Modulation of Host Insect Defenses and Physiology

Many viruses are now known to modulate host defenses and host physiology through specific viral proteins which disrupt the normal signaling pathways. One of these is the inhibition of apoptosis (cellular suicide) and another is manipulation of the ecdysone-mediated insect molting program.

Apoptosis and Baculovirus Defense against It

Apoptosis, also known as programmed cell death, can be activated in response to detection of cell damage and infection with viruses. Apoptosis is a regulated process in response to an apoptotic signal and effected through the activation of initiator caspases which in turn activate effector caspases. The protease activities of these activated caspases, in concert with that of certain enzymes, like caspase-activated DNase (CAD), activated by them, leads to DNA fragmentation, membrane blebbing, and disruption of the cellular architecture resulting in the formation of apoptotic bodies and cell death. Though apoptosis is a normal part of development and tissue homeostasis, during a virus infection, a cell often initiates an apoptotic response, preventing the cell from supporting virus replication and halting further virus spread. However, many viruses are now known to have an anti-apoptotic defense to prevent this. As shown in 1991, one of the first viral proteins demonstrated to have this anti-apoptotic ability is the P35 protein of AcMNPV.

The AcMNPV P35 is a pancaspase inhibitor which targets one of the *S. fugiperda* host cell effector caspases, Sf-caspase-1, thereby preventing completion of the apoptotic cascade. AcMNPVs lacking P35, as in the annihilator mutant, do not replicate efficiently due to premature apoptosis of infected cells. Interestingly, the AcMNPV P35 also protects *Ceanorhabditis elegans*, *Drosophila melanogaster*, and mammalian cells against apoptotic stimuli, thereby increasing the stature of this baculovirus P35 in the wider apoptosis community. Although BmNPV has a *p35* homolog, it is not functional. However, *p49*, the SpliNPV *p35* homolog, can inhibit apoptosis and moreover can rescue *p35* deletion mutants of AcMNPV and inhibits the *Drosophila* initiator caspase DRONC which P35 is unable to.

The inhibitor of apoptosis proteins (IAPs) are another class of baculovirus apoptosis inhibitors. Some act by binding to and inhibiting the function of various pro-apoptotic factors as well as more directly inhibiting caspase activity. First discovered in CpGV and orgyia pseudotsugata nucleopolyhedrovirus (OpMNPV), as many as 20 other *iap*'s have been described among both GVs and NPVs. IAPs were shown to be functional in CpGV, OpMNPV, and epiphyas postvittana nucleopolyhedrovirus (EppoNPV). IAPs are metalloproteins with an essential amino-terminal zinc-binding motif, BIR (baculovirus IAP repeat), and a carboxy-terminal zinc-binding RING motif. As for P35, some IAPs, like Op-IAP from OpMNPV, also target Sf caspase-1. Op-IAP binds HID and GRIM, two *Drosophila* cell death activators, through the BIR domains. The RING domain appears to be involved with ubiquination, but whether the RING is functional in inhibition of apoptosis is not yet known.

Baculovirus EGT and Inhibition of Molting

While many of the virus–host interactions occur at the cellular level, baculoviruses can also influence the overall physiology of infected insects to their advantage. One example is the disruption of the hormonally regulated metamorphosis through larval molts and pupation by modification of the hormone, ecdysone, which regulates this process. Many baculoviruses produce EGT, a UDP ecdysteroid glucosyltransferase resulting in glycosylation of ecdysone, thereby disrupting its normal regulatory function. Though overly simplistic, essentially the insect remains in the larval stage and continues to grow during infection, resulting in a higher yield of virus than would otherwise be possible and resulting in even more extensive feeding damage. *Egt* is nonessential for growth in cell culture and so is a common site for foreign gene insertion. Depending on the virus–host system, deletion of *egt* enhances virus infectivity. For example, deleting the *egt* of HearNPV decreased the LT_{50} for this virus by 27% compared to nonmodified HearNPV.

Baculovirus Host Range Determinants

Depending on the virus species, different baculoviruses have host ranges from one or only a few host insect species, like many of the GVs and the CfMNPV attacking the spruce budworm, up to a very broad host range such as for AcMNPV. Although the host range and tissue tropism of many viruses is dependent on interaction with specific host receptors, this does not appear to be the case for baculoviruses and no specific cell receptors have yet been identified. There are several other factors which influence the host range of different baculoviruses. In some cases, the exchange of as little as a single gene (or even only a small part of the gene) is sufficient to alter the host range. For example, AcMNPV can replicate in

S. frugiperda cells but very poorly in *B. mori* cells. However, if the AcMNPV helicase gene is exchanged for the helicase from BmNPV, the host range of AcMNPV is expanded to include *B. mori* larvae. In order for AcMNPV to replicate in TN368 cells, it requires a host-cell-specific factor gene 1 (*hcf-1*); however, *hcf-1* is dispensable for replication in Sf21 cells. HCF-1 is thought to act at the level of gene repression requiring a RING finger domain. Similarly, a host range factor gene, *hrf-1*, was identified in LdMNPV, which allowed replication of LdMNPV in Gypsy moth *Lymantria dispar* cells and insects. When *hrf-1* was introduced into three NPVs – AcMNPV, hyphantria cunea nucleopolyhedrovirus (HycuNPV), and BmNPV – all of which normally cannot replicate in an *L. dispar*-derived cell line (Ld652Y), all three expanded their host range to include cell type. The mechanism of action of HRF-1 to expand the host range is not yet known.

The relative ability of anti-apoptotic proteins, like P35 and IAPs or their homologs to inhibit apoptosis in different species of insects, might also affect the host range. For example, P35 deletion mutants of AcMNPV impairs the ability of that virus to grow in Sf21 cells but not in TN368 cells. Similarly, the ability of viruses to replicate in cells of certain insect species depends in part on the types of caspases encountered. For example, AcMNPV can replicate in Sf9 cells due to its ability to inactivate the effector caspase, Sf caspase-1; however, in Sl2, a cell line from *S. littoralis*, AcMNPV actually induces apoptosis presumably as a result of the upregulation of a different effector caspase, Sl caspase-1. Consequently, the AcMNPV host range does not extend to Sl2 cells.

Baculovirus-Revolutionized Expression Vector Technology

Early work on the molecular biology and the sequence of the polyhedrin gene, the first baculovirus gene ever sequenced, led to the discovery, in Max Summers' group in 1981, that the polyhedrin promoter is a powerful promoter. The first heterologous gene tested by a recombinant baculovirus based on the *polh* promoter was the human beta interferon which was expressed to very high levels. Since this pioneering work on the baculovirus expression vector system (BEVS), it has become the basis for the expression of many thousands of proteins for use as research reagents, as antigens, in diagnostics, as vaccines, and as therapeutic proteins like monoclonal antibodies. As a baculovirus is a eukaryotic expression vector, many of the common post-translational modifications that occur to produce mammalian proteins also occur in the BEVS system. However, whereas the glycosylation in insect cells involves simpler N-glycans with terminal mannose residues, in mammalian cells, more complex N-glycans with terminal sialic acids are made. Nevertheless, biological activity is retained in most BEVS-expressed proteins, even if the glycosylation is not that of the native protein. Attempts are now underway to 'mammalianize' the glycosylation pathway in insect cells to improve the authenticity of baculovirus-expressed mammalian proteins even more. Since baculoviruses are also capable of transduction into mammalian cells, they are also being studied for their potential in gene therapy.

Baculoviral Insecticides

The major impetus for the study of baculoviruses has been and continues to be their real and theoretical value as potent, biologically based insect pesticides. As these viruses already occur in nature, their use should leave less of an environmental imprint compared to the use of synthetic chemicals in the management of agricultural and forest pest insects. There are many examples of insect baculoviruses being effective in controlling pest populations. In Canada, for example, during the late 1930s, the 12 000 mi^2 infestation of the European sawfly *Gilpinia hercyniae* was brought completely under control by an NPV specific to this pest, brought in from Finland, and by 1945, the pest was totally eradicated. In Brazil, and elsewhere, the velvetbean caterpillar anticarsia gemmatalis is a major pest of soybeans. However, use of the native baculovirus anticarsia gemmatalis MNPV (AgMNPV) reduced the larval populations by 80%, the same level as for insecticide treatment and reduced the need for and costs of chemical insecticides. Up to 1.2 million ha has been treated on an annual basis in Brazil, with excellent results using virus produced by farm cooperatives and commercial sources. In the case of Brazil, the extension services informing farmers of this opportunity to reduce the use of chemical pesticides and the clear success shown by the use of AgMNPV was critical to the overall implementation of this form of biological control. Some of the major drawbacks on the commercialization of baculoviruses for pest control remain the relatively high cost of production, the extensive safety documentation needed for registration, the slow speed of kill, and the reluctance to implement new insect pest control strategies. Nevertheless, the development of pesticide resistent insects combined with the decreased public acceptance of chemical control methods, the biologically based baculovirus alternatives should become more attractive. Much work is also ongoing to improve the efficacy of a variety of baculoviruses in controlling pest insects. Some of these strategies include introduction of entomotoxic protein genes into the baculovirus genome, and other genetic manipulations, such as deletions of genes, like *egt* or exchange of

genes among different baculoviruses and reorganization of the genome.

See also: Baculoviruses: Apoptosis Inhibitors; Baculoviruses: Expression Vector; Baculoviruses: Molecular Biology of Granuloviruses; Baculoviruses: Molecular Biology of Mosquito Baculoviruses; Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Baculoviruses: Molecular Biology of Sawfly Baculoviruses; Baculoviruses: Pathogenesis.

Further Reading

Arif BM (2005) A brief journey with insect viruses with emphasis on baculoviruses. *Journal of Invertebrate Pathology* 89: 39–45.

Clem RJ (2005) The role of apoptosis in defense against baculovirus infection in insects. *Current Topics in Microbiology and Immunology* 289: 113–129.

Cory JS and Myers JH (2003) The ecology and evolution of insect baculoviruses. *Annual Review of Ecology, Evolution, and Systematics* 34: 239–272.

Harrison RL and Jarvis DL (2006) Protein N-glycosylation in the baculovirus-insect cell expression system and engineering of insect cells to produce 'mammalianized' recombinant glycoproteins. *Advances in Virus Research* 68: 159–191.

Hu YC (2006) Baculovirus vectors for gene therapy. *Advances in Virus Research* 68: 287–320.

Inceoglu AB, Kamita SG, and Hammock BD (2006) Genetically modified baculoviruses: A historical overview and future outlook. *Advances in Virus Research* 68: 323–360.

Jehle JA, Blissard GW, Bonning BC, *et al.* (2006) On the classification and nomenclature of baculoviruses: A proposal for revision. *Archives of Virology* 151: 1257–1266.

Miller LK (ed.) (1997) *The Baculoviruses.* New York: Plenum Press.

Moscardi F (1999) Assessment of the application of baculoviruses for control of Lepidoptera. *Annual Review of Entomology* 44: 257–289.

Summers MD (2006) Milestones leading to the genetic engineering of baculoviruses as expression vector systems and viral pesticides. *Advances in Virus Research* 68: 3–73.

Theilmann DA, Blissard GW, Bonning B, *et al.* (2005) Baculoviridae. In: Fauquet CM, May MA, Maniloff J, Desselberger U and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, San Diego, CA: Elsevier Academic Press.

Van Oers MM (2006) Vaccines for viral and parasitic diseases produced with baculovirus vectors. *Advances in Virus Research* 68: 193–253.

Baculoviruses: Molecular Biology of Nucleopolyhedroviruses

D A Theilmann, Agriculture and Agri-Food Canada, Summerland, BC, Canada
G W Blissard, Boyce Thompson Institute at Cornell University, Ithaca, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Budded virus The type of baculovirus virion that is formed by budding from the cell plasma membrane and mediates the systemic spread of infection in the infected insect.

Homologous repeat element A DNA sequence that is typically comprised of a series of imperfect palindromes and repeated at various locations around a baculovirus genome. Homologous repeat elements function as origins of DNA replication and as transcriptional enhancers.

Occlusion derived virus The type of baculovirus virion that is assembled in the nucleus and becomes occluded with the paracrystalline matrix of the occlusion body.

Occlusion body A crystalline protein matrix which surrounds the virions of some insect viruses.

Polyhedrin The major occlusion body protein of NPV occlusion bodies.

Introduction

The family *Baculoviridae* currently consists of two genera, *Nucleopolyhedrovirus* and *Granulovirus*. The nucleopolyhedroviruses (NPVs) currently include all baculoviruses that are not classified as a granulovirus (GV), including those isolated from hymenopterans (sawflies) and dipterans (mosquitoes). In recent years, it has been recognized that the hymenopteran and dipteran baculoviruses are distinct from all other baculoviruses and it has been proposed that the *Baculoviridae* be subdivided into four new genera: the alphabaculoviruses (which include the current lepidopteran NPVs), betabaculoviruses (which include the current GVs), gammabaculoviruses (which include the current hymenopteran NPVs), and deltabaculoviruses (which include the current dipteran NPVs). This article focuses primarily on the lepidopteran NPVs (the proposed alphabaculoviruses).

Lepidopteran NPVs are the most widely studied of the *Baculoviridae*, primarily due to the availability of insect cell culture systems that are permissive for infection.

A number of lepidopteran NPVs have been developed as bioinsecticides, and in addition lepidopteran NPVs are used widely as protein expression vectors and more recently as vectors for mammalian cell transduction. Also, there is a great deal of recent interest in developing certain lepidopteran NPVs as mammalian gene therapy vectors.

Life Cycle

Like all baculoviruses the lepidopteran NPVs contain genomes of double-stranded circular DNA, with genomes ranging in size from ~111 to 168 kbp. Two distinct types of virions are produced in the life cycle: the budded virus (BV) and the occlusion-derived virus (ODV) (**Figure 1**). BVs generally contain a single nucleocapsid and obtain an envelope by budding from the plasma membrane of the infected cell. In contrast, ODVs are formed in the nucleus, acquire a membrane that is derived from the inner nuclear membrane, and virions consist of either single (S) or multiple (M) nucleocapsids per envelope. The S and M designation (SNPV and MNPV) does not appear to hold any taxonomic significance above the species level but this characteristic is clearly associated with certain viral species. As the infection progresses, ODVs are assembled in the nucleus and become embedded in a paracrystalline proteinaceous matrix to form occlusion bodies (OBs) (also known as polyhedra) that can range in size from 0.15 to 15 μm. A single matrix protein called polyhedrin makes up the majority of the mass of the polyhedra or OB. The OB as a whole is surrounded by a carbohydrate-protein layer known as the polyhedral envelope.

Because the ODVs and BVs have lipid bilayer envelopes that are derived from different sources (nuclear vs. plasma membranes, respectively), the envelopes of ODV and BV have a significantly different protein composition. Proteomic analyses of ODV from the archetype NPV, autographa californica MNPV (AcMNPV), and from helicoverpa armigera NPV (HearNPV) have resulted in the identification of up to 46 viral proteins of which eight or more may be found in the ODV envelope (summarized in **Figure 1**). ODV-specific proteins include P74, PIF-1 and -2, ODV-E18, ODV-EC27, ODV-E56 (ODVP-6E), ODV-E66, and P96. Such detailed proteomic analyses have not been performed on BV but the BV virion

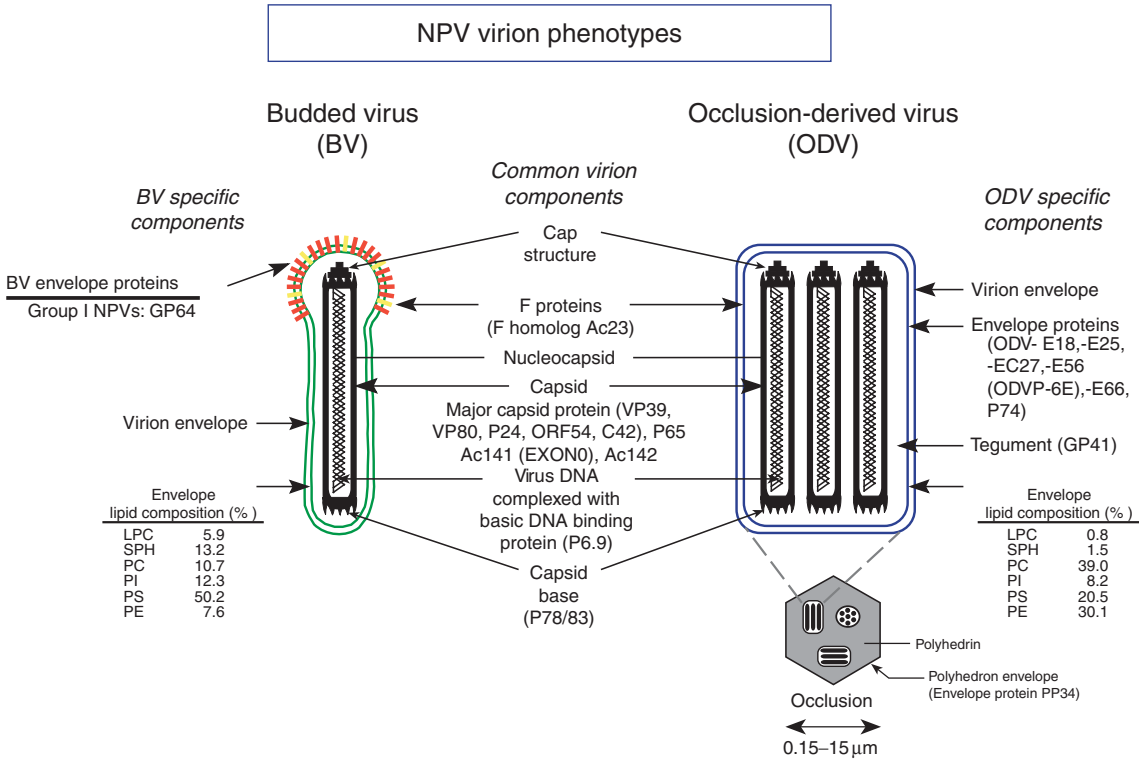


Figure 1 Schematic diagram of NPV virion phenotypes showing BV and ODV structures. Components common to both virion phenotypes are shown in the center and components unique to each phenotype are indicated on the left and right. The lipid compositions of the BV and ODV envelopes are indicated. LPC, lysophosphatidylcholine; SPH, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine. Adapted from Cytotechnology, vol. 20(1), 1996, pp. 73–93, Baculovirus–insect cell interactions, Blissard GW, Copyright 1996. With kind permission from Springer Science and Business Media.

phenotype is known to contain BV-specific genes such as GP64. The NPV BV envelope appears to contain fewer viral proteins than ODV envelopes.

Infection Cycle

The infection cycle of NPVs is initiated when insects ingest polyhedral OBs (**Figure 3**(**a**)). The OBs pass through the foregut, enter the midgut, and dissolve or disassemble in response to the alkaline environment of the lepidopteran insect midgut. This process releases the ODVs which then traverse the peritrophic membrane (a protein–chitin structure that lines the gut) and infect the columnar epithelial cells of the lepidopteran midgut. This infection process is aided by virally encoded metalloproteases called enhancins that degrade the peritrophic membrane, thus helping virions cross this barrier and interact with the microvilli of the midgut epithelial cells. At the microvillar surface ODVs bind to the cell surface and are believed to enter the cell by direct fusion of the ODV envelope with the plasma membrane at the cell surface. ODV entry has not been examined in great detail although ODV-specific protein P74 appears to be necessary for ODV entry.

Upon entry into the cell, nucleocapsids are transported to the nucleus where they appear to interact with the nuclear pore complex enter the nucleus, and deliver the DNA genome to the interior of the nucleus. The interaction of baculovirus nucleocapsids with the nuclear pore complex and genome delivery to the nucleus is not clearly understood and requires further study. After the initial uncoating of the viral genome, transcription of viral early genes is initiated by host RNA polymerase II, resulting in early transcription and the subsequent production of proteins associated with viral DNA replication and late gene expression. A variety of additional regulatory and even structural protein genes are also transcribed in the early phase. Production of early gene products results in the initiation of viral DNA replication, and the assembly and activity of a virus-encoded RNA polymerase. This viral 'late' RNA polymerase recognizes unique viral late promoters resulting in the transcription of viral structural and other genes. With only rare exceptions, NPV late promoters contain the sequence 'DTAAG' at the late transcription start site. The DTAAG motif and the sequences immediately flanking it comprise the late promoter. Viral DNA replication and nucleocapsid assembly occurs within the nucleus in a viral structure known as the virogenic stroma (**Figure 2**(**a**)). Nucleocapsids are transported from the cell nucleus to the plasma membrane, where they bud from the cell surface to form the BVs, which are responsible for the systemic spread of the viral infection to other tissues within the insect (**Figure 3**(**b**)). Nucleocapsids are also retained in the nucleus and become singly or multiply enveloped to form the ODV, which become embedded in polyhedrin to form the OBs (**Figure 2**). Many or most cells and tissues of the host insect are permissive for viral infection and become infected and filled with OBs, which are released when the cells lyse. At least two viral proteins, chitinase and cathepsin, aid in the disruption of infected cells and tissues and the release of OBs into the environment. OBs released into the environment can remain stable for months or even many years if protected from ultraviolet (UV) light. Over 1×10^8 OBs can be produced from an individual infected larva depending on the host species, the virus, and the larval instar. The dissemination of OBs is believed to be aided by viral modification of host behavior. Many NPV-infected insects will exhibit a behavior of climbing to the upper regions of plants during the late stages of infection. Release of the OBs from such elevated locations is believed to enhance the spread of the virus by making the released OBs more accessible to other larvae feeding on lower regions of the plant.

Genome Organization (Genomes, Gene Content, Organization, Evolution)

To date the complete genomic sequences have been determined for 30 lepidopteran NPVs. The smallest genome is that from the maruca vitrata NPV (MaviNPV) at 111 953 bp and the largest from the leucania separata NPV (LeseNPV) at 168 041 bp. The archetype and best-studied lepidopteran NPV, AcMNPV, has a genome size of 133 894 bp (**Figure 4**). The numbers of predicted genes (open reading frames (ORFs) of 50 amino acids or greater) range from 126 (MaviNPV) to 169 (LeseNPV). Taxonomic analysis of lepidopteran NPV genomes has led to the subdivision of the NPVs into two distinct clades: group I and group II NPVs (**Figure 5**). The group I clade appears to represent a more cohesive and well-defined phylogeny, whereas the group II clade represents a less-homogenous group of viruses (**Table 1**).

A distinct feature of nearly all lepidopteran NPVs is regions of homologous repeats (*hr*'s) distributed throughout the genome. The *hr* sequences can vary significantly in size. Each *hr* region contains a series of repeated palindromic sequences. In the AcMNPV genome, for example, seven *hr*'s are present and they all contain repeats of an imperfect palindrome that contains an *Eco*RI restriction site at its core (**Figure 4**). Transient transcription and plasmid replication assays have shown that the *hr* sequences function as transcription enhancers and as replication origins. Single copy non-*hr* replication origins have also been identified in the genomes of AcMNPV, orgyia pseudotsugata MNPV (OpMNPV), and spodoptera exigue MNPV (SeMNPV). The non-*hr* origins appear more similar to eukaryotic cellular replication

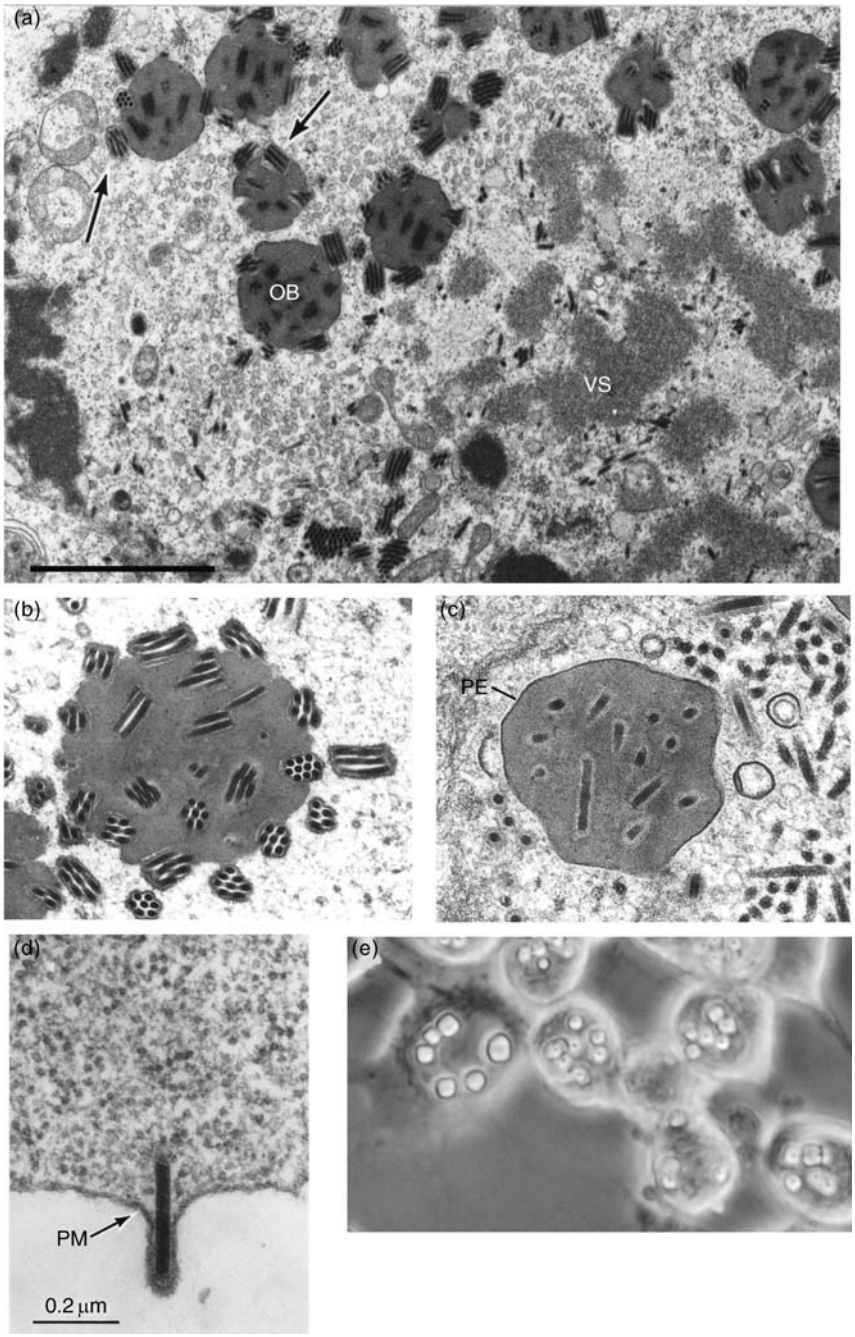


Figure 2 Electron and light micrographs of NPV-infected insect cells. (a) An insect cell infected with AcMNPV showing the nuclear virogenic stroma (VS) that contains developing nucleocapsids and the OBs showing ODV in the occlusion process (arrows). Scale = 1 μm. (b) An OB of the MNPV type, showing the process of occlusion of ODV-containing multiple nucleocapsids in each virion. Many virions are found in an OB. (c) A mature OB of the SNPV type, showing a single nucleocapsid in each virion (ODV) and many ODVs per occlusion body. Also note the formation of the polyhedral envelope (PE). (d) An NPV nucleocapsid budding from the plasma membrane (PM) of an infected cell. (e) Tn5b1–4 cells infected with AcMNPV producing large relatively uniform OBs. (a–d) Courtesy of R. Granados.

origins and contain multiple short repeated elements. The AT content of the lepidopteran NPVs can be quite variable, ranging from 42.4% (lymantria dispar MNPV; LdMNPV) to 64.4% (adoxophyes hommai NPV; AdhoNPV) and the significance of this variation is not currently known.

DNA Replication

Baculovirus DNA replication has been best characterized in the lepidopteran NPVs. Transient assays or the use of gene knockout viruses have shown that a number of genes are

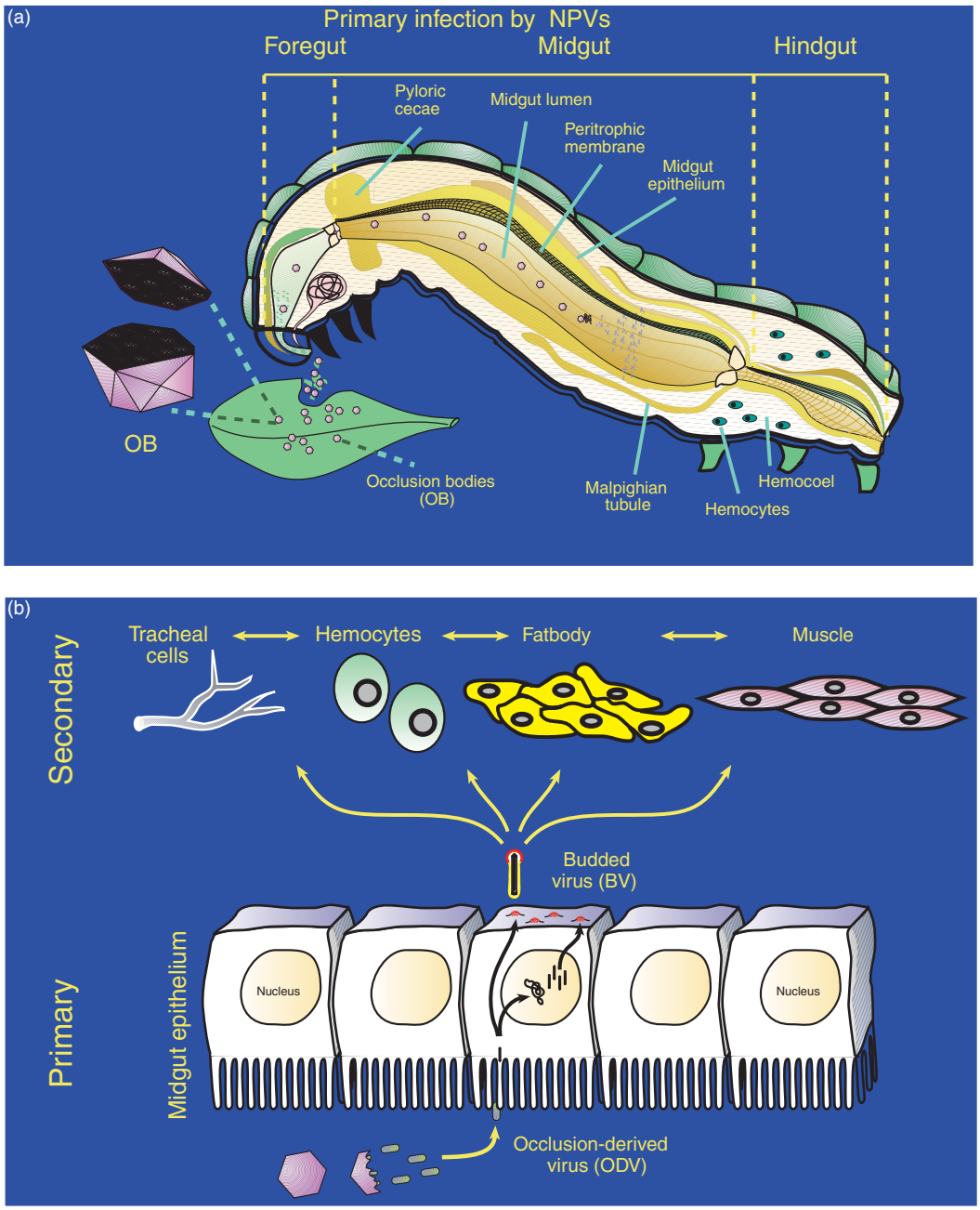


Figure 3 Schematic diagrams illustrating the infection process in a lepidopteran larva after ingestion of NPV OBs from a contaminated food source. (a) Within the midgut, OBs dissolve or 'disassemble' in the alkaline environment and the embedded ODVs are released into the lumen of the midgut. ODVs traverse the lining of the midgut, the peritrophic membrane, aided by virally encoded proteins and establish primary foci of infection in the midgut epithelial cells. (b) Upon infection of midgut epithelial cells by ODV interactions with microvilli at the apical surfaces of midgut epithelial cells, nucleocapsids are transported to the nucleus and initiate a primary round of viral replication, producing BVs that bud from the basal surface and subsequently infect cells of secondary tissues including tracheal cells, hemocytes, fatbody, and muscle. Alternatively, there is evidence that nucleocapsids can traverse the midgut cell and bud directly from the basal surface without uncoating or replicating in the nucleus, potentially avoiding cellular defense mechanisms associated with the midgut and accelerating the infection of secondary tissues. (a) Reproduced from Slack J and Arif BM (2006) The baculoviruses occlusion-derived virus: Virion structure and function. *Advances in Virus Research* 69: 99–165, with permission from Elsevier.

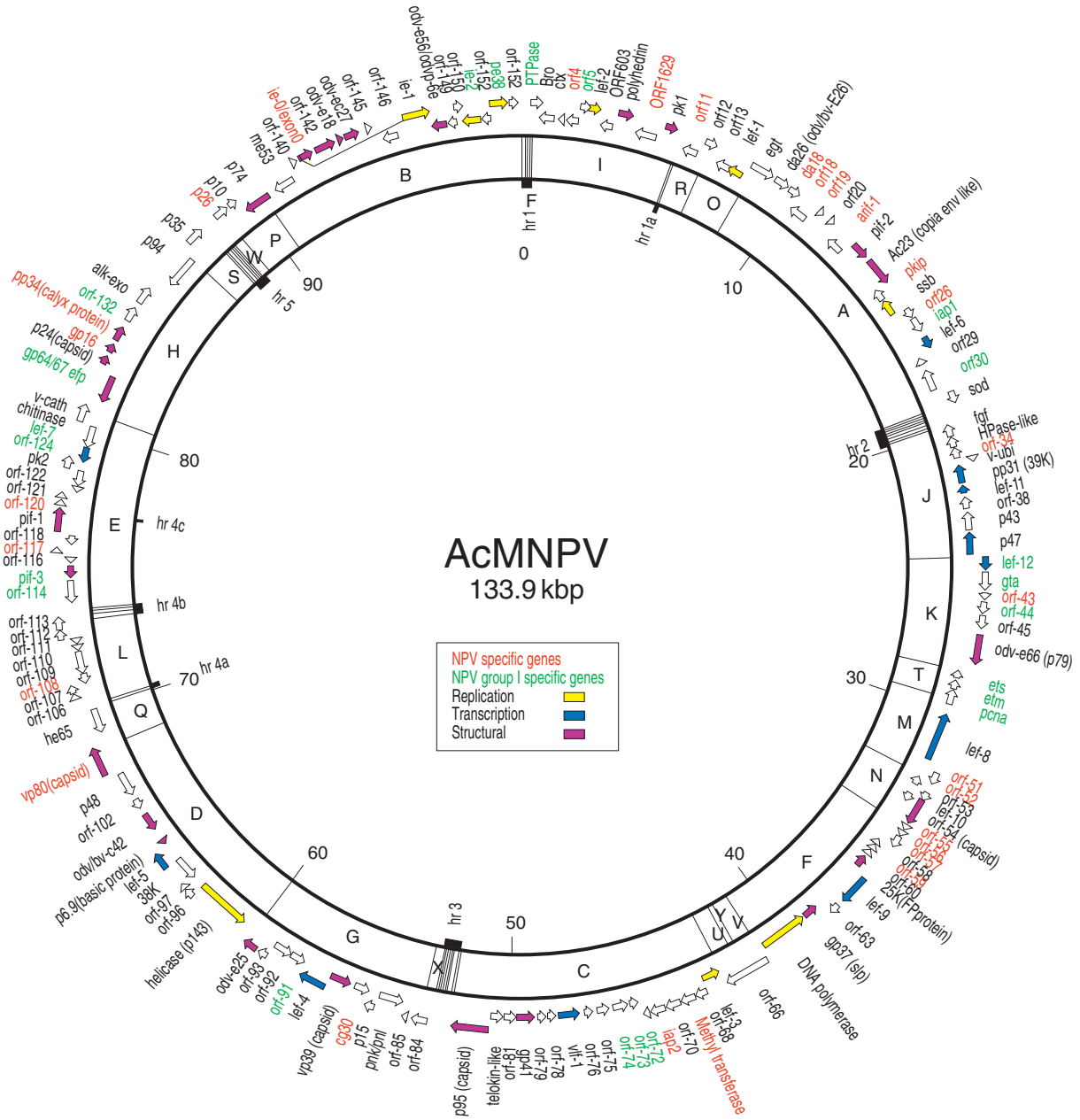


Figure 4 Genomic map of the archetype lepidopteran NPV, AcMNPV, which contains 154 open reading frames that encode predicted proteins of 50 or more amino acids. Functional groups of genes are highlighted (colored arrows) as well as genes specific to lepidopteran NPVs and group I lepidopteran NPVs (text colors). The inner circle shows the *Eco*RI restriction map of the C6 strain of AcMNPV. Locations of homologous repeat or *hr* regions (which contain repeats of *Eco*RI restriction sites) are indicated.

involved in viral DNA replication and viral genome processing. This includes *lef-1*, *lef-2*, *lef-3*, *lef-11*, *DNA polymerase*, *helicase*, *vlf-1*, *dna binding protein* (*dbp*), *alkaline exonuclease* (*ae*), *me53*, and *ie0* or *ie1*. *lef-1* has been shown to have primase activity and potentially to interact with *lef-2* which has an unknown function. The LEF-3 protein has a single-stranded DNA-binding (SSB) activity, forms homotrimers, and is required for transport of helicase to the nucleus. Helicase is believed to be involved in unwinding of viral DNA. DNA polymerase has homology to other known polymerases and is essential when assayed within the context of a viral infection. In addition to LEF-3, DBP has also been shown to have SSB activity and localizes at sites of viral DNA replication. In the absence of DBP, viral DNA is not processed into full-length genomes and is not packaged correctly into nucleocapsids. In transient replication assays, LEF-11 is not required for viral DNA replication; however, a *lef-11* knockout virus is unable to replicate DNA. LEF-11 has been shown to be a nuclear

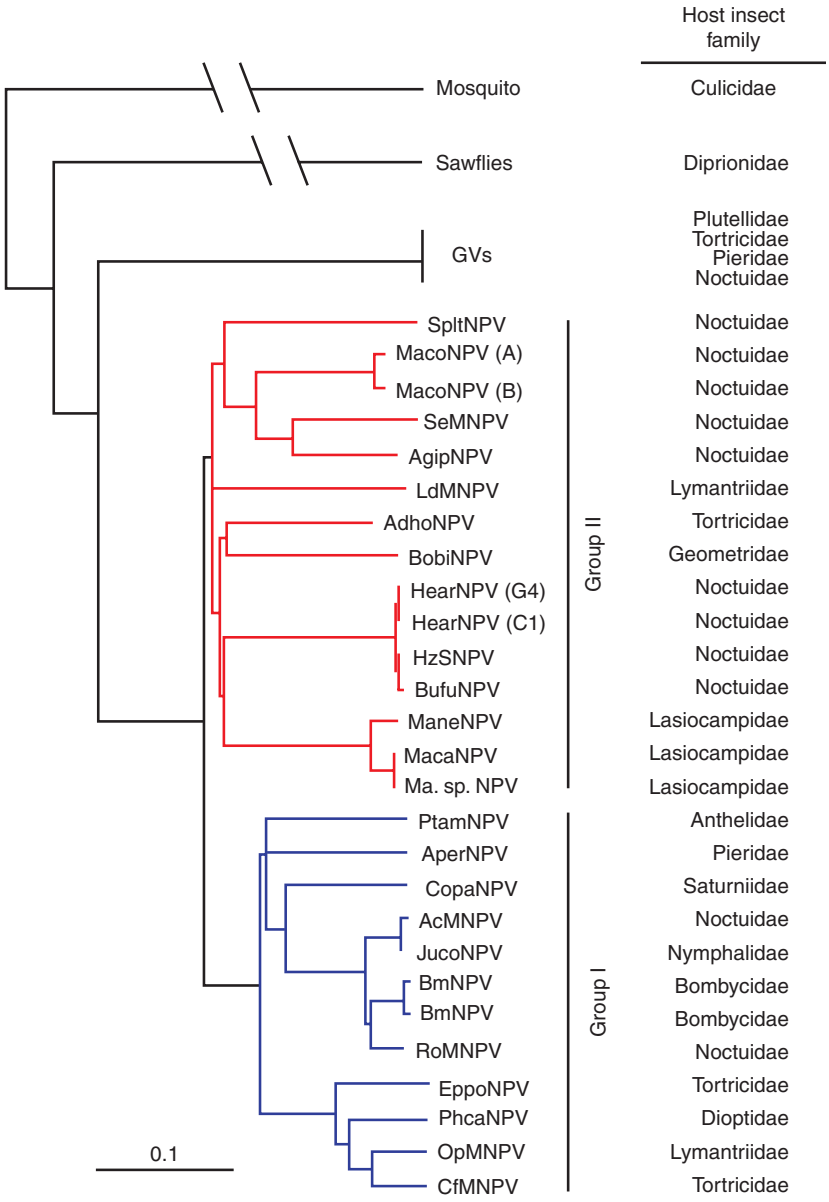


Figure 5 Phylogram showing the evolutionary relationships among lepidopteran NPVs and their lepidopteran hosts. A concatenated partial amino acid sequence of the common proteins polyhedrin/granulin, lef-8, and lef-9 was used to generate the neighbor-joining (NJ) distance tree and CuniNPV was used as outgroup. Blue branches identify group I NPVs, red identify group II NPVs, and green identify GVs. The lepidopteran family which is the known host of each respective baculovirus is shown on the right. Reprinted from Lange M, Wang H, Zhihong H, and Jehle JA (2004) Towards a molecular identification and classification system of lepidopteran-specific baculoviruses. *Virology* 325: 36–47, with permission from Elsevier.

protein but its role in NPV DNA replication is unknown. Similar to *lef-11*, the deletion of *me53* also results in a virus that is unable to replicate viral DNA. VLF-1 was originally identified due to its impact on baculovirus very late gene transcription. However, in the absence of VLF-1, viral DNA is not packaged into nucleocapsids correctly. In transient assays or recombinant viruses, either of the viral transactivators IE0 or IE1 can support viral DNA replication but both are required to achieve full levels of viral replication. IE-1 and presumably IE-0 bind *hr* sequences and therefore may function as origin binding proteins.

A number of viral proteins have been shown to augment viral DNA replication but are not essential. They include *iap-1* and the group I specific NPV genes *pe38* and *ie2*. All of these genes are RING finger proteins and PE38 and IE2 have ubiquitin ligase activities. However, it is not known whether this enzymatic activity plays a role in DNA replication.

Table 1 Summary of genes specific to lepidopteran NPVs, and the group I and group II NPV clades

Gene	*Gene function*
Lepidopteran NPV specific	
arif-1 (ac21)	Rearrangement of cellular actin
iap-2 (ac71) iap3	RING domain protein-possible apoptosis inhibition
orf1629 (ac9)	Essential structural gene of the nucleocapsid
pkip (ac24)	Stimulates the activity of the viral protein kinase-1 *in vitro*
pp34 (ac131)	Polyhedral envelope-associated phosphoprotein
vp80 (ac104)	Capsid protein
cg30 (ac88)	RING domain protein
exon0 (ac141)	BV nuclear egress
ie0 (ac141 and ac147)	Transcriptional transactivator, DNA replication
ac4, ac11, ac17, ac18, ac19, ac26, ac34, ac43, ac51, ac52, ac55, ac56, ac57, ac59, ac69, ac108, ac117, ac120, gp16(ac130), p26 (ac136)	Unknown function
Group I NPV specific	
ptp1 (ac1)	Protein tyrosine/serine phosphatase, affects OB formation
odve26 (ac16)	ODV envelope protein
iap1 (ac27)	RING domain protein-possible apoptosis inhibition
ie2 (ac151)	RING domain protein, transcriptional activation
lef7(ac125)	Late gene expression
vp80a (ac87)	Capsids protein
lef12 (ac41)	Late gene expression
gp64 (ac128)	BV specific glycoprotein required for virion entry
pe38 (ac153)	RING domain protein, transcriptional activation
ac5, ac30, ac72, ac73, ac114, ac124, ac132, gta (ac42), etm (ac48), ets (ac47), ac44, ac74, ac91	Unknown function
Group II NPV specific	
orf4PE	Polyhedral envelope protein
rr2b (ld120)	Ribonucleotide Reductase, R2/beta subunit
rr1 (ld148)	Ribonucleotide Reductase small subunit
parg (ld141)	Possible poly (ADP-ribose) glycohydrolase
ld55, ld129, ld111, ld124, ld127, ld142, ld144 , ld138	Unknown function

The mechanism by which the viral genome replicates is not known but high molecular weight DNAs that are suggestive of genomic concatemers have been reported. It has therefore been suggested that NPV DNA may replicate as a rolling circle. More recently however, due to presence of high molecular weight non-unit lengths of viral DNA in infected cells, it has been proposed that baculoviruses may use a recombination-dependent mechanism of viral DNA replication.

Temporal Regulation of Transcription

Transfected NPV DNA is infectious indicating that no viral proteins are required to initiate or mediate early transcription from the viral genome. Baculovirus genes are expressed in a temporal cascade beginning with early gene expression and followed by late gene expression. Late gene transcription requires prior or concomitantly with the onset of DNA replication. Very late gene expression occurs at the terminal part of the replication cycle and includes hyperexpressed viral genes such as polyhedrin.

Early Gene Expression

Early NPV expression is dependent upon the host cell RNA polymerase II complex and is sensitive to α-amanitin. NPV early genes have been divided into two categories, immediate early (IE) and delayed early (DE) genes. IE genes require only cellular factors for expression whereas DE genes are either dependent on, or substantially upregulated by prior viral gene expression. Early genes are primarily involved in gene regulation, host modification, DNA replication, and factors required for late gene expression.

Many IE genes have a common motif, TATA-$N_{24–26}$-CAGT, at their transcription start site similar to many motifs found in insect genomes. The promoters of IE and DE genes resemble typical eukaryotic RNA Pol II promoters and contain host cell transcription factor binding sites.

The primary viral regulatory protein for early transcription is the IE-1/IE-0 complex. The *ie0* gene is the only known spliced baculovirus gene that produces two viral protein products. IE0 contains the entire IE1 coding sequence but in addition has an N-terminal extension that is variable in length depending on the NPV species. IE0

has peak expression prior to viral DNA replication whereas IE1 continues to increase in steady-state levels until the final stages of the infection cycle. IE0 appears to be specific to the lepidopteran NPV as bioinformatics analysis has been unable to identify homologs in non-lepidopteran NPVs or GVs.

Viral gene knockouts have shown that either IE1 or IE0 is essential for viral replication and in the absence of this gene no viral infection is initiated. IE1/0 is an acidic domain transcriptional transactivator, similar to the herpesvirus VP16 protein; and activates viral gene transcription by both enhancer-dependent and independent mechanisms. IE1/0 forms dimers that bind to the *hr* sequences which serve as the transcription enhancers. IE1/0 is also essential for the viral replication complex where it is believed to play the role of an origin binding protein. In support of this, it has been shown that a transcriptionally inactive OpMNPV IE1 is able to support transient viral DNA replication.

Additional IE genes include *ie2* and *pe38*, which have been shown to augment viral transcription and, as indicated previously, viral DNA replication. Unlike IE1/0, both *ie2* and *pe38* can be deleted from the genome and the virus remains capable of replicating. In both cases however, dependent on the cellular environment, BV production and viral DNA replication are reduced. Some of the most highly expressed viral IE genes are *he65*, *me53*, and *gp64*. The localization of G-actin within the nucleus has been shown to be in part due to HE65 function, whereas ME53 is essential for DNA replication. Overall the IE genes play crucial regulatory roles that coordinate the NPV infection cycle.

DE expression requires additional viral factors to achieve nonbasal levels of expression. An example of a DE promoter is the AcMNPV *pp31* promoter which contains a TATA-CAGT sequence at the transcription start site and is dependent on IE1 for activated transcription (above basal-level) expression. The promoters of DE genes have not been clearly defined and it is not clear whether IE1/0 or other regulatory proteins may bind directly to the DE gene promoter.

Late Gene Expression

Baculovirus late genes are transcribed by a viral-encoded DNA-dependent α-amanitin-insensitive RNA polymerase that recognizes baculovirus late promoters. With only very rare exceptions, the baculovirus late promoters contain the sequence DTAAG and late transcription initiates within this seqeunce. The DTAAG sequence is considered to be at the core of the late promoter. In model late promoters that have been examined, only very short sequences immediately up- and downstream of the DTAAG sequence were identified as components of the late promoter. Regions farther downstream have been shown to affect levels of very late gene expression. Using transient assays 20 NPV genes or late expression factors (*lef*'s) were identified as important or necessary for late gene expression (*lef-1 to -12*, *pp31*, *p47*, *DNApol*, *vlf-1*, *helicase*, *p35*, *ie1/0*, *ie-2*). Many of the *lef* genes have been shown to be required for DNA replication, a prerequisite for late gene expression. Three of these genes are only found in the group I lepidopteran NPVs (*lef-7*, *lef-12*, and *ie-2*). Studies of purified NPV RNA polymerase have shown that it is comprised of four major proteins, LEF-4, LEF-8, LEF-9, and P47, and homologs of all these genes have been identified in all baculovirus genomes sequenced to date. LEF-4 has been shown to have enzymatic activity for RNA capping and LEF-8 and LEF-9 contain conserved motifs found in known RNA polymerases.

At very late times post infection, transcription of the hyperexpressed late genes (*polyhedrin* and *p10*) is upregulated, resulting in expression of those gene products at extremely high levels. The burst of very late gene transcription is mediated by the viral protein VLF-1, which binds to an A/T-rich region called a burst sequence located downstream of the DTAAG motif. This hyperexpression during the very late phase forms the basis of the baculovirus expression system. The *vlf-1* gene is a core baculovirus gene found in all sequenced baculovirus genomes.

Morphogenesis

Viral Structures – Nucleocapsids

The NPV life cycle is biphasic and produces two virion phenotypes, the BV and the ODV. Nucleocapsids of both virion phenotypes are believed to be structurally identical. NPV nucleocapsids are rod-shaped and approximately 30–60 nm by 250–300 nm (**Figures 1** and **2**) and are assembled in the ring zone of the nuclear virogenic stroma. DNA is densely packed within the nucleocapsid and small highly basic protein P6.9 is associated with the viral DNA. P6.9 is a core baculovirus protein that contains *c.* 40% arginine and 30% serine/threonine. The highly basic nature of this protein functions in neutralizing the positive charges of the nucleic acid and aids in the condensation and packaging of the viral DNA. Surrounding the viral DNA is the major capsid protein, VP39 (AcMNPV *orf89*). In the virus AcMNPV, the following proteins are associated with the nucleocapsid: P24, VP80, ORF54, BV/ODV-C42, ORF142, P95, ORF1629, and EXON0. ORF1629 is a phosphoprotein that is essential and is found associated with the basal end of the nucleocapsid. EXON0 is required for efficient egress of nucleocapsids from the nucleus. Interestingly, the very late gene transcription factor VLF-1 has also been shown to associate with the ends of the nucleocapsids and the data

suggest that it facilitates the packaging of viral DNA and the formation of correctly sized nucleocapsids. Deletion of BV/ODV-C42 showed that this gene is essential and BV is not produced though viral DNA replication is not affected.

Viral Structures – BV and ODV Envelopes

The virion envelopes of BV and ODV are obtained by different mechanisms and contain arrays of proteins that are generally specific to each virion type. Nucleocapsids are believed to acquire an initial envelope when they egress from the nucleus to the cytoplasm. By an unknown mechanism, the envelope is lost and nucleocapsids are transported to the cell surface, potentially utilizing cytoskeletal structures such as microtubules. At the cell surface, the nucleocapsids bud from the plasma membrane acquiring the modified plasma membrane as the BV envelope. Nucleocapsids destined to be incorporated into ODV are retained in the nucleus and are enveloped by a membrane that is derived from the inner nuclear membrane. Analysis of the lipid composition of AcMNPV ODV and BV envelopes show that they differ significantly, reflecting the differences of their origins.

The BV envelope of group I NPVs contains the major envelope glycoprotein, GP64, which is essential for viral attachment and membrane fusion during entry (see below). GP64 is not present in ODV and is therefore a BV-specific protein. GP64 proteins are not found in group II NPVs but a protein that is functionally equivalent, the F (fusion) protein, is present in group II NPVs and GVs. The best-studied GP64 proteins are those of AcMNPV and OpMNPV. F proteins have been studied most extensively from SeMNPV (ORF8), HaNPV (ORF133), and LdMNPV (ORF133). All viruses that encode a GP64 protein (group I NPVs) also encode a homolog of the F protein, although the F homolog in group I NPVs is not essential for virion entry or viral replication (see below). Unlike GP64, F proteins (and homologs in the group I NPVs) may not be specific to BVs. Proteomic analyses have identified F proteins in the ODVs of AcMNPV, HearNPV, and CuniNPV. More detailed studies will be necessary to understand any potential role of F proteins in the ODV.

Viral entry by budded virions of the NPVs is mediated primarily by the major envelope protein, GP64 or F. The GP64 proteins are highly conserved, with approximately 80% amino acid sequence identity among all GP64 protein ectodomains examined. Structurally, GP64 is a type I integral membrane glycoprotein that is phosphorylated, palmytoylated, and heavily glycosylated. It is found on the cell surface and on the virion as a disulfide-linked trimer of GP64 monomers. GP64 functions in both viral entry and exit. In viral entry, GP64 is important for host cell receptor binding and membrane fusion. Little is known of the details of GP64 interactions with host cell receptor(s) and the specific molecule(s) that serves as the host cell receptor for virion binding is unknown. However, GP64 binding appears to be highly promiscuous and this feature has been exploited in the use of baculoviruses as mammalian transduction vectors, as potential gene therapy vectors, and in the use of GP64 for pseudotyping other viruses such as retroviruses and paramyxoviruses. F proteins from group II NPVs presumably serve a similar role in host receptor binding although they may recognize a different host cell receptor. After host cell binding, BVs of viruses such as AcMNPV are internalized via endocytosis and the low pH of the endosome triggers a conformational change in the GP64 protein, resulting in activation of membrane fusion. Unlike many other membrane fusion proteins, the GP64 protein does not require a prior internal cleavage (within the ectodomain) for maturation or activation of the functional fusion protein. Studies of GP64-mediated membrane fusion indicate that large short-lived complexes of approximately 10 or more GP64 trimers form immediately prior to membrane merger and are likely the unit structure of the membrane fusion mechanism. In addition, the opening of the fusion pore occurs rapidly after triggering.

F proteins from group II NPVs such as SeMNPV, HaNPV, and LdMNPV are also low-pH-activated membrane fusion proteins. These F proteins require an internal cleavage by a cellular proprotein convertase, for fusion activity. F proteins from group II NPVs share general structural features with paramyxovirus F proteins.

Studies of a *gp64* gene knockout AcMNPV virus showed that GP64 is also necessary for efficient budding of progeny BV. Because GP64 is found on the surface of infected cells, concentrated in discrete areas, it is thought that these concentrations of GP64 represent the sites of virion budding. GP64 is found on the virion in a polarized manner with the GP64 spikes found concentrated at the end of the virion that corresponds to the end where budding initiated (**Figure 2**). An important question in the biology of this virus is whether GP64 accumulation determines the sites of BV budding, or whether it is simply targeted to the same sites. Like the VSV G protein, GP64 is required for efficient budding. However, the precise domains required for virion assembly or budding are not yet known.

Group I NPVs encode both GP64 and an F homolog (Ac23 in AcMNPV, Op21 in OpMNPV). In AcMNPV, GP64 is essential whereas the F homolog (Ac23) is not and Ac23 can be deleted with no substantial effect in cell culture infections. However, the conservation of F homologs in the genomes of the group II NPVs suggests an important function. Deletion of the Ac23 gene from the AcMNPV genome results in delayed mortality of infected larvae. Thus, while F proteins found in group II NPVs are essential entry proteins, the F homologs in

group I NPVs are not essential but appear to serve an accessory role that may be important in the pathogenicity or virulence of the virus.

The GP64 protein has been used for several biotechnological applications, including peptide display on baculovirus particles, and pseudotyping by replacing the envelope protein of another virus with GP64. Gene therapy vectors derived from retroviruses can be effectively pseudotyped with AcMNPV GP64 or other GP64 proteins. Further study of these viral envelope proteins should yield important and useful new tools for applications in biotechnology, agriculture, and medicine.

ODV envelopes have been shown to contain a number of specific proteins that are not present in BV. They include the following AcMNPV proteins (or their homologs): ODV-E18, ODV-E25, ODV-EC27, ODV-E56 (ODVP-6E), ODV-E66, P74, and ORF142. Viruses containing gene knockouts have shown that AcMNPV ORF142 and ODV-EC27 are essential for successful virus assembly and production of infectious virus.

Four genes encode ODV envelope proteins that are required for oral infectivity. *Per os infectivity factor-1*, *-2* and *-3* (*pif-1*, *pif-2*, *pif-3*) and *p74* have been shown to be required for oral infectivity of SeMNPV and AcMNPV. ODVs produced from viruses that do not express P74 are not orally infectious and evidence suggests that P74 is required for receptor binding on the midgut cell surface. Similar results have been obtained with PIF-1, -2, and -3. None of these proteins is required for infection of cells by BV in tissue culture.

Viral Structures – Tegument Proteins and Polyhedra

GP41 is an ODV-specific glycoprotein that does not fractionate with either the nucleocapsid fraction or the envelope fraction and is therefore believed to be a so-called tegument protein. GP41 is a core baculovirus gene, conserved in all baculovirus genomes examined. A temperature-sensitive mutant of the AcMNPV GP41 protein shows that it plays a critical role in viral development. When grown at the nonpermissive temperature, *ts* mutants of *gp41* fail to produce ODVs and polyhedra, and in addition nucleocapsids fail to egress from the nucleus to form BV. GP41 therefore appears to play a key role in the assembly of both virion phenotypes even though it is a component of only the ODV. Tegument proteins appear to be acquired within the nucleus when ODV nucleocapsids are enveloped. BVs do not contain GP41 as it is believed that all nuclear proteins surrounding nucleocapsids are lost when the nucleocapsids migrate from the nucleus to the plasma membrane.

OBs represent a feature that is common to all the currently classified baculoviruses (**Figure 2**) but the shape and size of the OB can vary substantially. Lepidopteran NPVs produce some of the largest OBs (polyhedra) and, unlike the GVs or the dipteran or hymenopteran NPVs, the embedded ODV can contain single or multiple nucleocapsids per envelope (**Figure 2**). The major component of the OBs of NPVs is the OB matrix protein, polyhedrin, which forms the bulk of the paracrystalline array (**Figure 2**). Surrounding the OB is a structure known as the calyx or envelope which is thought to be comprised of carbohydrate and protein. A lepidopteran NPV-specific phosphoprotein, PP34, is the major protein associated with this structure. To date no specific function has been attributed to the polyhedra calyx in the infection cycle of NPV. However, the OBs of an AcMNPV virus with a *pp34* deletion were found to have increased sensitivity to alkali disruption and enhanced virulence in fourth instar *Spodoptera exiguae* larvae, suggesting that the polyhedra calyx may stabilize the OB.

NPV-Specific Genes

The lepidopteran NPVs have 28 genes that are specific to all members of this genus and a further 13 genes specific to group II NPVs and 21 genes specific to group I NPVs (**Figure 4**). A number of these genes have been characterized and are known to impart specific functionality on these viruses but many remain to be investigated to determine their role in NPV biology.

See also: Baculoviruses: Apoptosis Inhibitors; Baculoviruses: Expression Vector; Baculoviruses: General Features; Baculoviruses: Molecular Biology of Granuloviruses; Baculoviruses: Molecular Biology of Mosquito Baculoviruses; Baculoviruses: Molecular Biology of Sawfly Baculoviruses; Baculoviruses: Pathogenesis; Insect Pest Control by Viruses.

Further Reading

Blissard GW (1996) Baculovirus–insect cell interactions. *Cytotechnology* 20: 73–93.

Clem RJ (2005) The role of apoptosis in defense against baculovirus infection in insects. *Current Topics in Microbiology and Immunology* 289: 113–129.

Guarino LA, Xu B, Jin J, and Dong W (1998) A virus-encoded RNA polymerase purified from baculovirus-infected cells. *Journal of Virology* 72: 7985–7991.

Herniou EA, Olszewski JA, Cory JS, and O'Reilly DR (2003) The genome sequence and evolution of baculoviruses. *Annual Review of Entomology* 48: 211–234.

Jehle JA, Blissard GW, Bonning BC, *et al.* (2006) On the classification and nomenclature of baculoviruses: A proposal for revision. *Archives of Virology* 151: 1257–1266.

Lange M, Wang H, Zhihong H, and Jehle JA (2004) Towards a molecular identification and classification system of lepidopteran-specific baculoviruses. *Virology* 325: 36–47.

Miller LK (ed.) (1997) *The Baculoviruses.* New York: Plenum.

Okano K, Vanarsdall AL, Mikhailov VS, and Rohrmann GF (2006) Conserved molecular systems of the *Baculoviridae*. *Virology* 344: 77–87.

Oomens AGP and Blissard GW (1999) Requirement for GP64 to drive efficient budding of autographa californica multicapsid nucleopolyhedrovirus. *Virology* 254: 297–314.

Slack J and Arif BM (2006) The baculoviruses occlusion-derived virus: Virion structure and function. *Advances in Virus Research* 69: 99–165.

Smith GE, Fraser MJ, and Summers MD (1983) Molecular engineering of the *Autographa californica* nuclear polyhedrosis virus genome: Deletion mutations within the polyhedrin gene. *Journal of Virology* 46: 584–593.

Stewart TM, Huijskens I, Willis LG, and Theilmann DA (2005) The *Autographa californica* multiple nucleopolyhedrovirus *ie0–ie1* gene complex is essential for wild-type virus replication, but either IE0 or IE1 can support virus growth. *Journal of Virology* 79: 4619–4629.

Theilmann DA, Blissard GW, Bonning B, *et al.* (2005) The *Baculoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 177–185. San Diego, CA: Elsevier Academic Press.

Baculoviruses: Pathogenesis

L E Volkman, University of California, Berkeley, Berkeley, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Viral Architecture, Phenotypes, and Host Distribution

The family *Baculoviridae* is comprised of large, enveloped, bacilliform, double-stranded DNA viruses with juvenile insect hosts. The family currently contains the genera *Granulovirus* and *Nucleopolyhedrovirus* though others are proposed. Granuloviruses (GVs), with exclusively lepidopteran hosts, and nucleopolyhedroviruses (NPVs) with hymenopteran, dipteran, and lepidopteran hosts, fall into distinct clusters phylogenetically. The lepidopteran NPVs are the most abundant of the baculoviruses, and fall into two clades, groups I and II. GVs are packaged as single nucleocapsids per envelope, and the NPVs are packaged either as single nucleocapsids per envelope (the single nucleopolyhedroviruses, SNPVs), or one through multiple nucleocapsids per envelope (the multiple nucleopolyhedroviruses, MNPVs) (**Figure 1**). The latter feature, referred to as the M trait in this article, is unique to lepidopteran NPVs, and occurs in both groups I and II. The M and S traits have not been linked to any viral genes, and nothing is known about factors that regulate nucleocapsid distribution, but the M and S traits reflect differences in strategies for establishing systemic infection.

Baculovirus-enveloped virions are occluded within protein matrices that protect them, enabling them to remain viable in soil for years. The *granulin* gene encodes the major occluding protein for the GVs, and the *polyhedrin* gene does the same for the NPVs. These homologous genes, along with the NPVs' *p10*, are hyperexpressed during the very late phase of infection as late gene expression subsides. Occlusion formation is not unique to baculoviruses. It evolved independently at least three times among insect viruses as a viability-maintenance mechanism. Entomopox viruses in the family *Poxviridae* and cytoplasmic polyhedrosis viruses in the family *Reoviridae* both form viral occlusions that persist in the environment.

GVs pack a single enveloped virion per occlusion while the NPVs can pack hundreds of virions per occlusion. NPV occlusions range from 1 to 15 μm in diameter and can have over 1000-fold the volume of the much smaller GV occlusions. All of the GVs and most of the NPVs infect lepidopteran larvae. Close to 300 lepidopteran species are known to support NPV infections, and 150 species support GV infections. The GVs are known to have very narrow host ranges, and are limited to one or two species within a family. The SNPVs tend to have narrow host ranges as well. In contrast, some MNPVs have impressive arrays of alternative hosts. Autographa californica multiple nucleopolyhedrovirus (AcMNPV) has an extremely wide host range and mortally infects at least 43 species within 11 families of lepidopteran insects.

Less than 10% of the NPVs have nonlepidopteran hosts, and all that do, have the SNPV phenotype. The hosts of sequenced NPVs in this category are sawflies and mosquitoes representing orders Hymenoptera and Diptera, respectively, all of which evolutionarily predate the Lepidoptera. Most baculoviruses appear to have radiated along with the Lepidoptera, the most recent of the insect orders. Infection of nonlepidopteran hosts is limited to midgut cells, and midgut cells produce occlusions containing infectious virus. These cells are shed as new midgut cells are infected, and the cycle continues until the death of the insect.

Infections are initiated when viral occlusions, consumed by a susceptible insect host, dissolve and release occlusion-derived virus (ODV) into the midgut lumen. In lepidopteran hosts, occlusion dissolution is triggered by the high pH (8–11) of the digestive fluids. Primary infection

of lepidopteran larvae occurs in cells of the columnar epithelial lineage. Fully mature cells typically are targeted but differentiating and regenerative cells also can be infected. Secondary (systemic) infection is established by budded virus (BV), a second phenotype consisting of a single nucleocapsid (even for the MNPVs) with an envelope derived from the basal plasma membrane of the infected columnar cell (**Figure 2**). The BV envelope contains a newly synthesized, viral-encoded fusion protein not present in ODV. BV infects a variety of tissues within the host and uses the fusion protein for productive entry. GVs and most NPVs are thought to use F proteins for BV fusion, but AcMNPV and other group I NPVs use GP64 instead. GP64 is thought to have entered the group I lineage subsequent to F, and to have taken over its fusion function.

NPV Pathogenesis in Lepidopteran Larvae

Primary Infection

Occlusion dissolution in the gut lumen is a host range factor that begins a deadly race for the body of the caterpillar. Both virus and insect want to use the larval mass for the same ultimate purpose: reproduction. However, virus and insect reproduction are mutually exclusive events; hence, it is a life-or-death struggle.

The larval protection strategy of covering most of its epithelium with cuticle gives a virus little opportunity for achieving contact with a living cell. The midgut is accessible via ingestion, however, and it is free of cuticle. Moreover, the load maximizing feeding behavior of larval lepidopteran hosts means ingestion is likely to occur if occlusions contaminate their food supply, plant tissue. Even though the midgut is free of cuticle, it secretes a gel-like structure called the peritrophic membrane (PM) that acts as a protective barrier. Some GVs and a few NPVs contain enhancins, viral-encoded metalloproteases, thought to improve access to target cells by compromising the PM. Most baculoviruses also contain products of the '11K gene family' that improve primary infection as well, but by an unknown mechanism.

ODV initiates infection by binding at or near the tips of apical columnar cell microvilli where they fuse with the microvillar membranes. Evidence suggests that receptor binding on midgut cells is mediated by three ODV proteins. For AcMNPV ODV, the viral attachment proteins are *per os* infectivity factors PIF1, PIF2, and P74. The proteins are encoded by genes conserved among all

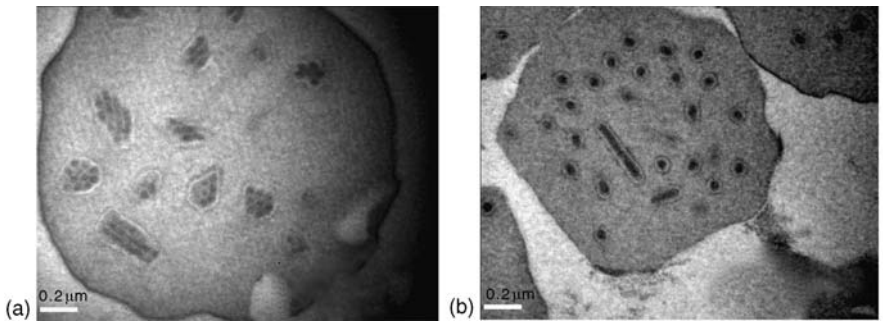


Figure 1 Comparison of M and S traits. (a) Autographa californica multiple nucleopolyhedrovirus (AcMNPV) and (b) Helicoverpa zea single nucleopolyhedrovirus (HzSNPV) occlusions. Note that HzSNPV occlusions are smaller and more densely packed with virions, but the virions have only a single nucleocapsid compared to multiple nucleocapsids for AcMNPV.

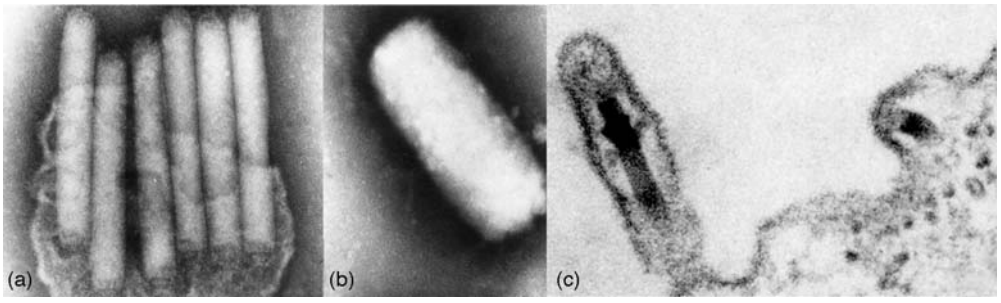


Figure 2 Comparison of ODV and BV phenotypes of AcMNPV. (a) ODV multiple nucleocapsids and (b) intact virion showing typical smooth envelope. (c) BV in the process of budding showing envelope spikes composed of GP64.

baculoviruses (regardless of the order of their host), suggesting that a conserved mechanism exists among baculoviruses for establishing primary infection. The virus attachment proteins and PIF3, another highly conserved protein, are essential for ODV infectivity but dispensable for BV infectivity. The role of PIF3 is not known, but it probably does not include ODV primary binding or fusion. Curiously, fusion is unimpaired by the absence of any one of these four proteins, suggesting that a novel fusion mechanism may exist for primary infection, or that the ODV fusion protein has not yet been identified.

Columnar cell microvilli of larval lepidopterans may be well over 10 μm long, but typically they are only 100 nm across. Both GV and NPV nucleocapsids, by comparison, are large, measuring 30–60 nm × 250–300 nm. A single nucleocapsid, therefore, occupies about one-third the cross-sectional volume of an infected columnar cell microvillus. Moreover, the MNPVs pack several nucleocapsids within the same virion, a trait with a demonstrated advantage for achieving mortal infection. A virion containing five nucleocapsids (an average number) fused with a single microvillus would greatly exceed the normal cross-sectional volume of the microvillus, and, indeed, this uncomfortable situation has been observed by electron microscopy. Transport of the nucleocapsids down the microvillus would seem improbable as well because of the bulky microvillar F-actin core and cross filaments that seemingly would prevent or severely impair nucleocapsid transport. Because microvilli have no microtubules, nucleocapsid transport must be actin-based and directed toward the minus end of the microvillar F-actin filaments. Only one class of minus-end-directed actin-based motors has been described to date – myosin VI – but its involvment remains to be demonstrated. For the moment, the existing data present an interesting conundrum and suggest that modulation of microvillar architecture to accommodate nucleocapsid transport could be a likely activity of the highly conserved *p74* and *pif* gene products.

Systemic Infection

Tracheal cells are the primary targets of AcMNPV BV produced in midgut cells in six hosts with widely varying susceptibilities to mortal infection, including *Trichoplusia ni*, *Heliothis virescens*, *Helicoverpa zea*, *Manduca sexta*, *Spodoptera frugiperda*, and *Spodoptera exigua.* Tracheal cells produce cytoplasmic extensions that breach basal laminal barriers to achieve close proximity to the cells they service for efficient gas exchange (**Figure 3**). BV contact with these extensions is key for systemic infection because basal laminae are effective barriers to direct BV passage. In fully permissive hosts, BVs produced in midgut cells infect tracheal cells, and tracheal cell-produced BVs, in turn, bud into the hemolymph where they have direct access to hemocytes, the only insect tissue without a protective basal lamina. Infected hemocytes produce most of the BVs that accumulate within the hemolymph. High titers are needed to infect tracheal cells at distant sites so that they, in turn, can transmit infection to the tissues they are servicing. In this manner, tracheal cell infection is key for viral passage through basal laminal barriers. Sequential infection of midgut cells, tracheal cells, and hemocytes is required for hosts to be fully permissive. Some insects have permissive hemocytes but not permissive midgut cells, while others have permissive midgut and tracheal cells but not permissive hemocytes. Such insects are semipermissive hosts under laboratory conditions, but of doubtful significance to the baculovirus in question in the field.

BVs are much more efficient units of infection than ODVs and have just one nucleocapsid per particle. Intrahemocoelically, in four different species, an LD_{50} dose of AcMNPV BV is less than 0.1 PFU. AcMNPV BV has a physical to infectious particle ratio of 128:1 in cell culture; therefore, very few intrahemocoelic particles (less than 12 and possibly only 1) are needed to achieve fatal infection in fully permissive hosts. Such hosts have no resistance to infection beyond the midgut (see below), so the payoff for infecting even 1 tracheal cell is enormous. Other hosts, such as *H. zea* and *M. sexta,* can mount effective immune responses to systemic infection by AcMNPV. Such responses increase the BV concentration required for an LD_{50} dose considerably.

Primary Host Defense and Virus Counter Response

The first line of defense of lepidopteran larvae against baculovirus infection is shedding ODV-infected midgut cells. As larvae age, the rate of midgut cell sloughing increases, and, at the end of the instar in which infection is initiated, any remaining infected midgut cells are shed into the gut lumen and voided during the molt. As a consequence of this behavior, larvae become increasingly resistant to fatal infection by NPVs as they age. This phenomenon is known as developmental resistance, and it is a widespread characteristic among lepidopteran larvae. This behavior also leads to a healthy midgut in the instar following the molt that can process food needed for maximizing host size and virus yield (**Figure 3(e)**).

The easily distinguishable M trait observed in some lepidopteran NPVs and nowhere else among extant viruses represents a counter response to developmental resistance. The M trait is one component of a two-component strategy used to minimize the time required to establish systemic infection via midgut cells. AcMNPV cheats the clock by bringing in extra parental nucleocapsids that can be repackaged within midgut cells as BV. Some of the entering nucleocapsids are targeted directly

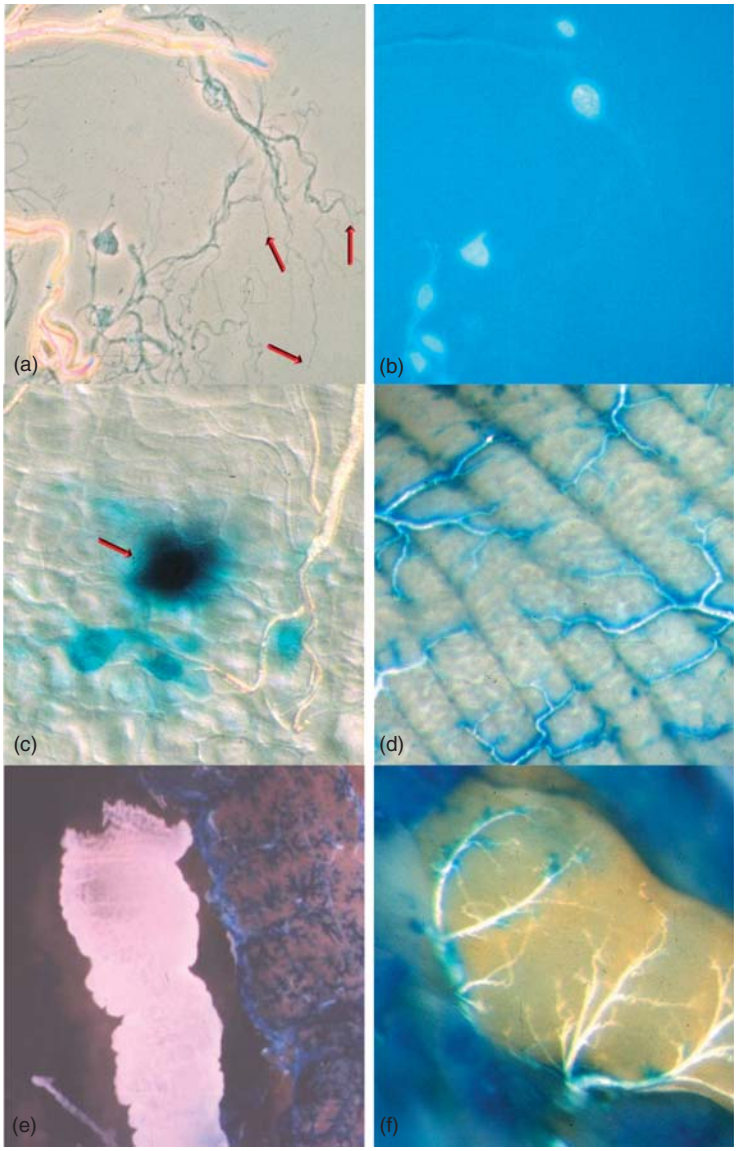


Figure 3 Tracheal cell infection is key to NPV systemic infection. (a, b) Trachea and tracheal cells, uninfected. Note the long cytoplasmic branches (arrows) relative to the 4′,6-diamidino-2-phenylindole (DAPI)-stained nuclei (b) of the cells. (c) Establishing secondary (systemic) infection in *M. sexta* by AcMNPV expressing the reporter gene β-galactosidase. Focus of infection involves central midgut cell (arrow) and surrounding tracheal cells. (d, e) *Heliothis virescens*-infected tracheal cells against a backdrop of uninfected midgut post clearance. (f) Blue foci of infection of *H. virescens* testis are associated with infected trachael cells, demonstrating their importance in overcoming basal lamina barriers.

through the cell to the basal plasma membrane where they bud as soon as other nucleocapsids targeted to the nucleus begin early gene expression. The genes encoding GP64 and a surprising number of F proteins have early and late promoter elements. Importantly, early expression enables ODV nucleocapsids to bud as BV before late gene expression even begins. In five of the six previously listed insect species, AcMNPV establishes infection of tracheal cells in 4 h or less after having established primary infection in midgut cells. Because both multiple ODV nucleocapsids and early expression of the BV fusion protein are required for this pass-through strategy to work optimally, this mechanism cannot be used by GVs and SNPVs, a factor that may contribute to the relatively wider host ranges enjoyed by MNPVs compared to those of GVs and SNPVs.

GV Pathogenesis

GVs can be divided into three types, according to their tissue tropisms. The first type, Trichoplusia ni granulovirus (TnGV), can replicate in both the midgut and the

fat body. However, nothing is known about how infection is transmitted from the midgut to the fat body, or whether tracheal cells may be involved. TnGV-infected larvae take 10–14 days to die, giving the feeding host time to increase its mass so more virus can be produced. At death, the cadaver does not liquefy. Type II GVs, such as Cydia pomonella granulovirus (CpGV), have the widest tissue tropism of the GVs, and can infect midgut, hemocytes, tracheal cells, fat body, and epidermis similar to the NPVs. Midgut infection for these GVs is transient, and the gross pathology associated with type II GV mortal infections is similar to that seen with lepidopteran NPV mortal infections (see below).

Infection by harrisinia brillians (Western grapeleaf skeletonizer) granulovirus (HabrGV), as typical of type III GVs, is confined to the midgut and is not cleared by molting. Interestingly, HabrGV is more pathogenic in older instars than younger instars, the exact opposite pattern of pathogenesis induced by NPVs and type I and II GVs. First instars, for example, may die after 17 days of infection compared to 8 days for third instars. This pathology is consistent with increased midgut cell sloughing and increased organismal growth observed in later instars. The need to generate many centers of differentiating columnar cells could be problematic if stem and/or early differentiating midgut cells are widely infected. *Harrisinia brillians* are gregarious feeders but the later instars develop a watery diarrhea when infected, and change from gregarious to solitary 'wandering' behavior. As they wander, they leave trails of highly infectious watery excrement. In a plexiglass cage, the effect is very striking. In healthy colonies, the caterpillars stay on the plants almost exclusively, whereas in infected colonies, the cage walls become completely coated with excrement.

Wandering Behavior and Terminal Disease

Wandering behavior, or enhanced locomotory activity, is normal behavior for lepidopteran larvae anticipating pupation. Wandering behavior induced by baculovirus infection is symptomatic of the terminal stages of infection and serves to spread the virus in the environment. The induction of wandering behavior by bombyx mori nucleopolyhedrovirus (BmNPV), a group I NPV, is caused by the expression of a virus-encoded protein tyrosine phosphatase (*ptp*). *Bombyx mori* larvae infected with BmNPV *ptp* null mutants, do not wander, making *ptp* an unusual and exciting example of a virus gene that controls host behavior.

Infected larvae eventually cease feeding, but not before infected tissues, especially the fat body, are replete with occlusions. Infected larvae in this stage of disease respond more slowly to touch, appear to be swollen, and are more fragile. Within a day of taking on this appearance, the larvae liquefy, an astonishing sight to witness (**Figure 4**). The entire infection process can take 5–12 days. Under laboratory conditions, AcMNPV-infected larvae begin liquefaction 5–7 days post infection. This process is dependent on expression of V-CATH, a cathepsin-like cysteine protease that is activated at cell death. V-CATH cannot be activated, however, if expressed in the absence of viral-encoded chitinase, CHI-A, encoded by a gene found in the same baculovirus genomes that harbor *v-cath.*

There is only one way a fully susceptible larval lepidopteran host can possibly escape death after having been systemically infected, and that is to pupate. Pupae and adults are much less susceptible to infection than larvae, and occlusion yields correspondingly drop precipitously. It is in the best interest of the virus, therefore, to control host development, and baculoviruses do just that. Baculoviruses counter the escape route pupation offers by secreting ecdysteroid UDP glucosyltransferase (EGT) into the hemolymph, an enzyme that catalyzes the transfer of galactose to ecdysome, thereby inactivates it. Ecdysone is a hormone needed to signal the beginning of the molting process, and in lower concentrations, the beginning of pupation. Secretion of EGT, therefore, masks the hormone signal and thereby prevents both pupation and molting. Interestingly, viral mutants lacking functional EGT kill hosts faster than wild-type viruses do. The earlier death is thought to be related to the degeneration of malpighian tubules, but increased anoxic stress might also be a factor. Infected tracheal cells are no doubt incapable of replacing chitinous tracheae lost during the molting process; thus, hosts that molt likely experience increased anoxic stress compared to hosts that do not molt and retain the use of existing trachae.

Pathogenesis *In Vitro*

Columnar epithelial cells are dead-end cells that experience two extremes of pH at once. Apically, they are bathed in highly alkaline gut fluids and basally they are bathed in slightly acidic hemolymph. These conditions are difficult to mimic in cell culture, a fact that may account for the paucity of information available on primary infection.



Figure 4 The cadaver of an *H. virescens* larva killed by AcMNPV.

Cell lines that support GV replication are rare and currently only exist for CpGV. Consequently, we know little more about mechanisms of GV infection than we do about ODV infection. Even so, we know that during GV nucleocapsid morphogenesis, the nuclear membrane of the host cell disintegrates, an event that does not disrupt nucleocapsid processing. The disappearance of the nuclear membrane is distinctive and does not occur in NPV-infected host cells.

A number of insect cell lines have been established that support NPV infections. Most of the cell lines are derived from caterpillar ovaries or embryos. AcMNPV can replicate in cell lines derived from several insect species, a fact that contributes to its being the best-studied baculovirus. In a standard one-step growth curve, AcMNPV BVs typically are produced 12–20 h post infection (hpi), and occlusions, 20–48 hpi. Replication of most other NPVs in cell culture takes hours to days longer.

AcMNPV BV is 1000-fold more infectious than ODV, primarily due to the presence of its fusion protein, GP64. BVs enter their target cells by clathrin-mediated endocytosis. BV nucleocapsids penetrate deep into the cytoplasm of target cells by being released from endosomes. Using this strategy, nucleocapsids bypass the possibly hazardous environment that lies just beneath the plasma membrane and enter the cell closer to the nucleus than fusion at the plasma membrane would allow. The pH-sensitive conformational shifts experienced by the BV fusion proteins serve to trigger a fusion event between the viral envelope and endosomal membrane.

AcMNPV nucleocapsid's escape from the endosome is immediately followed by its association with F-actin cables. Cable formation is transient, and occurs during the time viral nucleocapsids move to and enter into the nucleus. During transit, viral nucleocapsids co-localize with one end of an actin cable, possibly via P78/83, an F-actin-binding protein thought to be located at the base of nucleocapsids. The association of nucleocapsids with F-actin cables and the delay of reporter gene expression in the presence of drugs that disrupt actin/myosin function suggest that the cables may facilitate the transport of nucleocapsids to the nucleus, or mediate their passage through nuclear pores.

NPV and some GV nucleocapsids enter nuclei via nuclear pores. Some GVs uncoat at the nuclear pores but most baculovirus uncoating occurs within the nucleus. Early gene transcription begins immediately, mediated by host RNA polymerase II (Pol II). Among the early genes expressed is a subset whose expression leads to efficient G-actin transport to and accumulation within the nucleus (**Figure 5**). This spectacular manipulation of actin localization is critical for NPV progeny production and has not been described for any other pathogen. The genes involved in the nuclear localization of actin, identified in transient transfection experiments, include *ie1*, *pe38*, *Ac004*, *Ac152*, *he65*, and *Ac102*. Two of the genes, *ie1* and *Ac102*, are conserved among all lepidopteran NPVs and GVs, and both are essential.

Transition to the late stage of infection is the beginning of the BV progeny production period; cellular activities are geared toward synthesizing viral components at maximal rates, assembling them into nucleocapsid products and then exporting them. Host macromolecular synthesis is shut down during this period but host chromatin structure remains intact until cell death. Late and very late viral genes are expressed by an RNA polymerase encoded by the virus.

Microtubules, reorganized during the early phase of infection, are depolymerized by factors produced during the late phase leading to cell rounding (**Figure 6**). Similarly, G-actin, accumulated within the nucleus during the early phase, polymerizes during the late phase, concurrent with nuclear swelling and disappearance of visible host nuclear structures (**Figure 7**). The virogenic stroma, the site of viral DNA synthesis, forms at the center of the nucleus and is interspersed and surrounded by an electron-lucent zone called the 'ring zone', a place where capsids are assembled and tethered during genome loading. Nuclear F-actin co-localizes with the major AcMNPV capsid protein in the ring zone (**Figure 7**).

Nuclear F-actin is required for BV production. In the presence of F-actin-disrupting drugs, viral capsids are

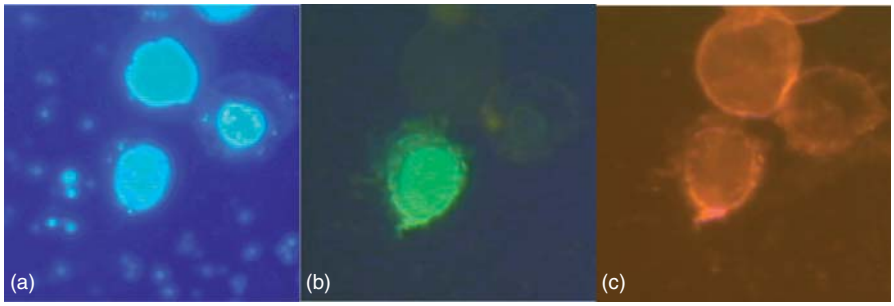


Figure 5 Nuclear G-actin in AcMNPV-infected cells. (a) 4′,6-Diamidino-2-phenylindole (DAPI)-stained infected cells; (b) infected cell expressing transfected green fluorescent protein (GFP)-actin. (c) Lack of nuclear rhodamine phalloidin staining indicates that the nuclear actin is monomeric.

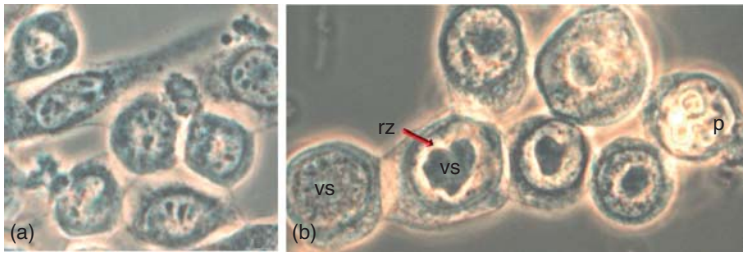


Figure 6 AcMNPV-infected Sf21 cells demonstrating different phases of cytopathic effect (CPE). (a) Uninfected cells. Note spindle shape and presence of nucleochromatin structures. (b) AcMNPV-infected cells showing early and late CPE. Cells are rounded due to depolymerizing microtubules, nuclei are larger and cleared of host structures, and virogenic stroma (vs) is surrounded by ring zone (rz). Virogenic stromas become denser as the late stage of infection progresses. Polyhedra fill the nucleus during the very late stage of infection (p).

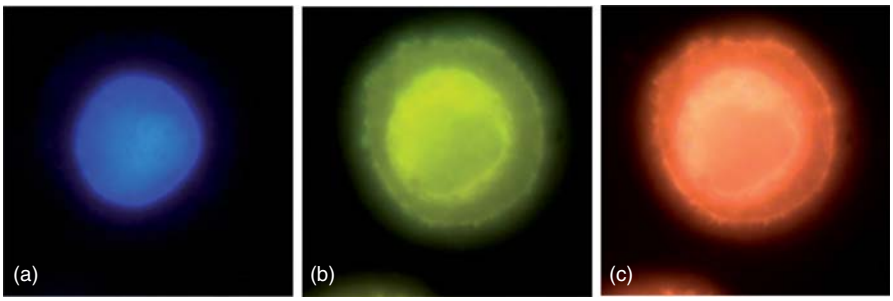


Figure 7 Nuclear F-actin in AcMNPV-infected cell during late gene expression. (a) DAPI stain indicates location of the nucleus. (b) Green fluorescence indicates capsid protein is primarily localized in the ring zone of the nucleus. (c) Rhodamine phalloidin staining shows that F-actin co-localizes with capsid in the ring zone.

malformed and appear as long tubular structures juxtaposed to the inner nuclear membrane with infrequent patches of electron-dense material. Base plates and cap structures normally present are not evident, and an excess of membranous profiles are produced. Viral DNA synthesis occurs at the normal rate but genomes are not packaged, and the virogenic stroma has a 'relaxed' appearance compared to normal stroma. Interestingly, the phenotype for the absence of AcMNPV very late factor-1 (VLF-1) is similar, suggesting that VLF-1 may be involved in tethering capsids to the virogenic stroma, and that F-actin is a component of the stroma to which capsids are attached, directly or indirectly.

P78/83, a minor capsid protein expressed late during infection, is essential for AcMNPV viability. This feature was noted over 30 years ago and exploited in the first commercial baculovirus expression kits. P78/83 (78 kDa when unphosphorylated and 83 kDa when phosphorylated) is thought to be part of the base plates of both BV and ODV capsids. P78/83 is an F-actin-binding protein, and this activity may help tether capsids to the nuclear matrix during assembly. Interestingly, P78/83 contains another activity that explains why it is essential; it promotes actin polymerization within the nucleus. P78/83 contains domains conserved in Wiskott–Aldrich syndrome protein (WASP) family members, factors that promote nucleation of actin filaments. WASP family members positively regulate the actin-nucleating activity of the Actin Related Protein (Arp)-2/3 complex. The seven-subunit complex, conserved across eukaryotes, is translocated to the nucleus in AcMNPV-infected cells and is activated by P78/83. Mutations in P78/83 that lead to decreased ability in promoting actin nucleation correspondingly lead to decreased ability in producing infectious BV.

Immediately upon entry into the nucleus, AcMNPV DNA adopts a nucleosomal-like structure and uses nucleosomes and nucleosome-related processes in genome replication. A component of the viral replication strategy, therefore, appears to be hijacking components of, if not the entire, host chromatin remodeling capacity. Recent evidence suggests the BRO (baculovirus repeated orf) family of proteins are likely participants in this process. The BRO proteins are expressed early during infection, bind single-stranded DNA (ssDNA) and core histones, and partition with histones in fractionation experiments. BmNPV, orgyia pseudotsugata multiple nucleopolyhedrovirus, and lymantria dispar multiple nucleopolyhedrovirus all have multiple *bro* genes. AcMNPV carries only one *bro* gene, related to BmNPV *bro-d*, which is essential.

AcMNPV-encoded P6.9, the highly basic genome packaging protein, begins accumulating during the late stage of infection and an alternate chromatin structure emerges.

The P6.9–DNA interactions are controlled by the phosphorylation state of P6.9. During genome packaging, the genomic DNA from the virogenic stroma binds with P6.9 as P6.9 is dephosphorylated, condensing into a preformed capsid sheath through an opening in a conical end structure. The conical structures lie proximal to the virogenic stroma with capsid sheaths capped by base plates extending distally into a less-electron-dense space, the ring zone. F-actin and capsid protein co-localize in the ring zone, along with P78/83 and Arp2/3 complex. It is this stage of replication that is affected both by drugs that disrupt F-actin and the absence of VLF-1.

With the onset of the very late phase of infection there is a reduction in BV production and there is an onset of ODV production. Newly assembled nucleocapsids stay within the nucleus where they are wrapped in envelopes thought to be derived from the inner nuclear membrane. Enveloped virions, but not unenveloped nucleocapsids, become occluded in a protein matrix to form capsules or polyhedra. The cells eventually lyse, releasing occlusions into the medium.

See also: Baculoviruses: Apoptosis Inhibitors; Baculoviruses: General Features; Baculoviruses: Molecular Biology of Granuloviruses; Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Baculoviruses: Molecular Biology of Sawfly Baculoviruses.

Further Reading

Adams JR and Bonami JR (eds.) (1991) *Atlas of Invertebrate Viruses.* Boca Raton, FL: CRC Press.

Bonning BC (2005) Baculoviruses: Biology, biochemistry and molecular biology. In: Gilbert LI, Iatrou K, and Gill SS (eds.) *Comprehensive Molecular Insect Science*, pp. 233–270. Oxford: Pergamon.

Goley ED, Ohkawa T, Woodruff JB, D'Alessio JA, Volkman LE, and Welch M (2006) Nuclear actin assembly by Arp2/3 complex and a viral WASP-like protein is essential for nucleopolyhedrovirus replication. *Science* 314: 464–467.

Kamita SG, Nagasaka K, Chuya JW, *et al.* (2005) A baculovirus-encoded ptotein tyrosine phosphatase gene induces enhanced locomotory activity in a lepidopteran host. *Proceedings of the National Academy of Sciences, USA* 102: 2584–2589.

Miller LK (ed.) (1997) *The Baculoviruses.* New York: Plenum.

Ohkawa T and Volkman LE (1999) Nuclear F-actin is essential for AcMNPV nucleocapsid morphogenesis. *Virology* 264: 1–4.

Ohkawa T, Washburn JO, Sitapara R, Sid E, and Volkman LE (2005) Specific binding of AcMNPV ODV to midgut cells of *Heliothis virescens* larvae is mediated by products of *pif* genes *Ac119* and *Ac022*, but not *Ac115*. *Journal of Virology* 79: 15258–15264.

Okano K, Vanarsdall AL, Mikhailov VS, and Rohrmann GF (2006) Conserved molecular systems of the *Baculoviridae*. *Virology* 334: 77–87.

Trudeau D, Washburn JO, and Volkman LE (2001) Central role of hemocytes in Autographa californica M nucleopolyhedorivrus pathogenesis in *Heliothis virescens* and *Helicoverpa zea*. *Journal of Virology* 75: 996–1003.

Washburn JO, Chan EY, Volkman LE, Aumiller JJ, and Jarvis DL (2003) Early synthesis of budded virus envelope fusion protein gp64 enhances Autographa californica multicapsid nucleopolyhedrovirus virulence in orally infected *Heliothis virescens*. *Journal of Virology* 77: 280–290.

Banana Bunchy Top Virus

J E Thomas, Department of Primary Industries and Fisheries, Indooroopilly, QLD, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cell-cycle link protein A plant virus protein which most probably subverts the cell-cycle control of the host, forcing cells into DNA synthesis or S phase favorable to viral replication.

Circulative transmission Mode of transmission whereby the virus is acquired from plant sieve tubes, via the insect's stylet, and traverses a number of specific barriers as it passes from the hindgut, into the hemocoel and then to the salivary glands, from where it can be reinjected into a plant during feeding. It is characterized by a latent period before which re-transmission cannot occur and by retention of infectivity by the vector for periods ranging from several days to the entire life span of the insect. The virus does not replicate in the insect.

Nuclear shuttle protein A virus-encoded protein that transports the viral single-stranded DNA, as a complex with the viral movement protein, to and from its site of replication in the nucleus and into adjacent cells.

Introduction

Banana bunchy top disease is the most economically important virus disease of banana and plantain (*Musa* spp.) worldwide, due to its devastating effect on crop yield, and the importance of banana and plantain as both a staple food and a major export commodity in much of the developing world. The causal agent is banana bunchy top virus (BBTV). Edible bananas are derived from wild progenitors including *M. acuminata*, *M. balbisiana*, and

M. schizocarpa, which have a center of origin in the South and Southeast Asian–Australasian region, and it is likely that BBTV also originated within this area. BBTV has a multipartite circular single-stranded (ssDNA) genome, encapsidated in small isometric virions, and is transmitted by the banana aphid, *Pentalonia nigronervosa.*

Disease Symptoms

The symptoms of bunchy top disease in banana are characteristic, especially in the Cavendish subgroup of cultivars, and easily distinguished from all other virus diseases of banana. Plants can become infected at any stage of growth, and the initial appearance of symptoms can depend on the manner of infection. BBTV is systemic within the banana plant, and following aphid inoculation, symptoms do not appear until at least two more new leaves have been produced (bananas produce single new leaves sequentially from a basal meristem). The first symptoms comprise a few dark green streaks and dots on the lower part of the lamina and on the petiole, becoming more general on subsequent leaves. These streaks form hooks as they enter the midrib, and are best viewed from the underside of the leaf, with transmitted light (**Figure 1**). However, these dark streaks can be rare or absent in some cultivars. Successive leaves become shorter and narrower, and have a brittle lamina with upturned, chlorotic, ragged margins. Leaves fail to emerge fully, giving the plant a bunched appearance (**Figure 2**). Plants derived from infected planting material (suckers, bits) develop severe symptoms from the first leaf to emerge.

Infected plants seldom produce a bunch, though if infected late in the current cropping cycle, a small, distorted bunch may result. With very late infections, the only symptoms to appear in the current season may be a few dark streaks on the tips of the flower bracts. No fruit is produced in subsequent years, and plants generally die within a couple of years.

Histological examination suggests that BBTV is restricted to the phloem tissue, which shows hypertrophy and hyperplasia and a reduction in the development of the fibrous sclerenchyma sheaths surrounding the vascular bundles. The cells surrounding the phloem contain abnormally large numbers of chloroplasts, resulting in the macroscopic dark green streak symptom.

Using RNA probes and polymerase chain reaction (PCR), it has been demonstrated that BBTV replicates briefly at the site of aphid inoculation, then moves down the pseudostem to the basal meristem, subsequently infecting the newly formed leaves, the corm, and the roots. The virus apparently does not replicate in leaves formed prior to infection, consistent with the lack of symptoms on these leaves and an inability to recover the virus from them via the aphid vector.



Figure 1 Dark green dot-dash, hooking and vein clearing fleck symptoms on a Cavendish banana leaf



Figure 2 Young BBTV-infected banana plants, showing stunting, and successively shorter narrow leaves with upturned, chlorotic margins. Leaves have failed to emerge fully, giving the plant a bunched appearance.

From Taiwan, symptomless strains of BBTV, and mild strains that produce only limited vein clearing and dark green streaks have been reported. Also, some plants of the Cavendish subgroup cultivar Veimama from Fiji have been observed to initially show severe symptoms, then to recover and display few if any symptoms.

Host Range

Confirmed hosts of BBTV are confined to the family Musaceae. Known susceptible hosts include *Musa* species, cultivars in the Eumusa and Australimusa series of edible banana and *Ensete ventricosum.* Susceptible *Musa* species include *M. balbisiana*, *M. acuminata* ssp. *banksii*, *M. textilis*, *M. velutina*, *M. coccinea*, *M. jackeyi*, *M. ornata*, and *M. acuminata* ssp. *zebrina.* There are some reports of hosts outside the Musaceae, though in independent tests none has been confirmed.

Causal Agent

The viral nature of bunchy top disease was established by C. J. P. Magee, in Australia, in the 1920s. However, it was not until 1990 that the virus particles were first isolated, in part due to lack of a suitable herbaceous experimental host, its low titer in infected plants, and its restriction to the phloem in the fibrous vascular tissue of *Musa*. BBTV has icosahedral particles, 18–20 nm in diameter (**Figure 3**), a buoyant density of 1.29–1.30 in cesium sulfate, and a sedimentation coefficient of 46S.

BBTV has a multicomponent genome comprising six transcriptionally active components (**Figure 4**; **Table 1**), each *c.* 1 kbp in size. DNA-R encodes the master replication initiation protein (Rep), which can initiate and terminate replication, and a potential second internal ORF of unknown function, for which a transcript only has been identified. All other components are monocistronic; DNA-S encodes the coat protein, DNA-C the cell-cycle link protein, DNA-M the cell-to-cell movement protein, DNA-N the nuclear shuttle protein, and the function of the protein potentially encoded by DNA-U3 is unknown. The untranslated regions of all six components share two areas of high sequence identity, both concerned with the rolling-circle mode by which BBTV replicates. A stem–loop common region (CR-SL) of 69 nt includes a nonanucleotide sequence (TAT/GTATTAC) which is shared between plant-infecting circular ssDNA nanoviruses and geminiviruses, and which is the site of the origin of viral replication. It also contains iterated sequences, thought to act as recognition sites for the replication initiation protein. The major common region (CR-M) varies between 66 and 92 nt in length among the various components. Virions contain a heterogeneous population of DNA primers, *c.* 80 nt in length, which bind to the CR-M and prime complementary strand synthesis.

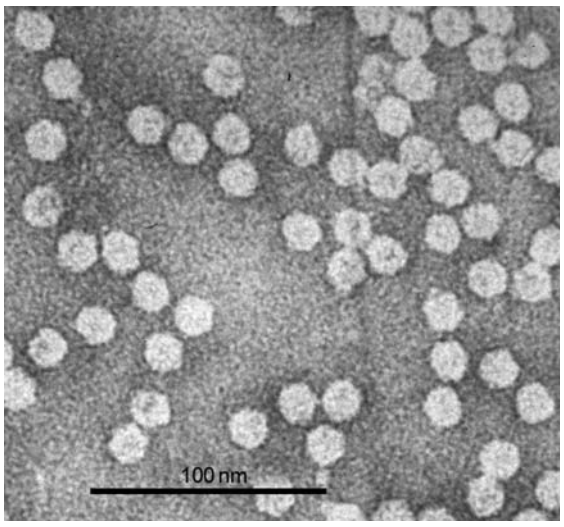


Figure 3 Electron micrograph of BBTV virions, negatively contrasted with 1% ammonium molybdate.

The coat protein gene of BBTV is highly conserved, with a maximum difference of 3% at the amino acid level between isolates. No serological differences have been detected between any isolates using polyclonal or monoclonal antibodies.

The intergenic regions of all six integral components of BBTV have been shown to have promoter activity. The highest activity in banana embryonic cells was shown by promoters from DNA-C and DNA-M, components thought to be intrinsic to the infection process. Studies on the DNA-N promoter demonstrated that expression in banana embryonic cells is limited to phloem tissue, consistent with the circulative mode of transmission by the aphid vector and observations by Magee in the 1920s that histological effects were confined to the phloem and phloem parenchyma cells. Circulative transmission by aphid vectors usually involves specific feeding on the phloem tissue for virus acquisition. The CR-M and CR-SL are not essential for promoter activity. All essential elements of the promoter are located 3′ of the stem–loop and within 239 bp of the translation start codon and within this region are an ASF-1 motif (TGACG), a hexamer motif (ACGTCA), *rbc*S-I-box (GATAAG), G-boxcore, and the TATA box, all associated with promoter activity in other genomes.

Some isolates of BBTV from Taiwan and Vietnam contain additional Rep-encoding DNAs that are thought to be capable only of self-replication and to behave like satellite molecules. These molecules have a CR-SL, though the stem sequence is not conserved with the six integral DNA components. Unlike DNA-R, the putative satellites lack the internal ORF, their TATA boxes are 5′ of the stem loop, and they generally lack the CR-M. Interestingly, the amino acid sequences of satellite Reps BBTV-S1, BBTV-S2, and BBTV-Y1 are actually more closely related phylogenetically to the Reps encoded by nanovirids outside the genus *Babuvirus* than they are to DNA-R of BBTV. Similar molecules have been detected by Southern hybridization in isolates from the Philippines, Tonga, and Western Samoa, but not from Australia, Egypt, Fiji, and India.

Taxonomy and Phylogenetic Relationships

BBTV is a member of the genus *Babuvirus*, in the family *Nanoviridae*. Other members of this family are classified in the genus *Nanovirus*, and include faba bean necrotic yellows virus, milk vetch dwarf virus, and subterranean clover stunt virus.

BBTV isolates worldwide fall into two broad phylogenetic groups (**Figure 5**), called the South Pacific group

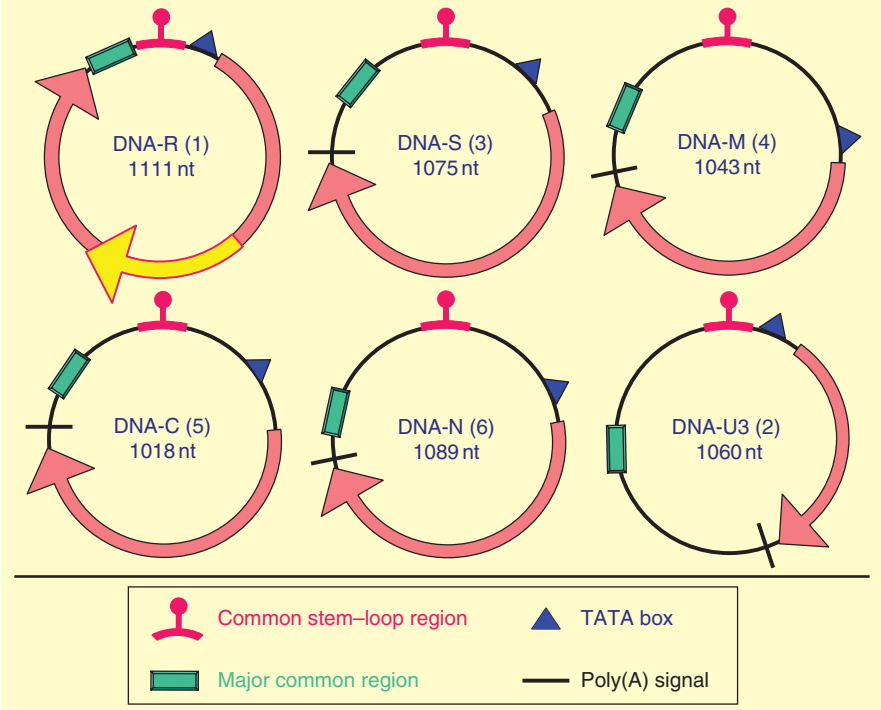


Figure 4 Diagram of the integral genome components of BBTV, showing transcribed ORFs (black arrows) and other main genome features. Reproduced from Vetten HJ, Chu PWG, Dale JL, *et al*. (2005) *Nanoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy*: *Eighth Report of the International Committee on Taxonomy of Viruses*, p. 349, figure 3. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

Table 1 Size and function of the six integral components of BBTV

Genome component	*Size (nt)*	*Size of encoded protein(s) (kDa)*	*Function*
DNA-R	1111	(1) 33.6 (2) 5.2[a]	(1) Replication initiation protein (2) Unknown
DNA-S	1075	20.1	Capsid protein
DNA-M	1043	13.7	Movement protein
DNA-C	1018	19.0	Cell-cycle link protein
DNA-N	1089	17.4	Nuclear shuttle protein
DNA-U3	1060	10.4[b]	Unknown

[a]Expression has not been demonstrated for the transcript of this internal ORF.
[b]Most isolates of BBTV do not encode a functional ORF on DNA-U3, and where present there is no evidence for its expression.

(isolates from Australia, Fiji, Hawaii, Western Samoa, Tonga, India, Pakistan, Burundi, Egypt, Malawi) and the Asian group (China, Philippines, Japan, Vietnam, Taiwan). Sequence differences between the two groups have been demonstrated across all genome components. Most striking is the variation in the CR-M of DNA-R, where the mean sequence difference between the groups was 30%, and up to a maximum of 55% for individual pairs of isolates. Within group sequence diversity is much greater for the Asian group, suggesting a longer evolutionary period and possible evolutionary origin for the virus. The presence of the virus in many countries from the South Pacific group can be traced to introductions within the last century. A Taiwanese isolate (TW4) causing only mild symptoms was recently identified. Interestingly, it apparently lacks DNA-N and is clearly a chimeric isolate, having DNA-M of the Asian group and DNA-S, -C, and -U3 of the South Pacific group. It also contains two DNA-R molecules, one from each group.

Recently, two new viruses have been identified which are clearly distinct members of the genus *Babuvirus* (**Figure 5**). Abaca bunchy top virus has homologous components for

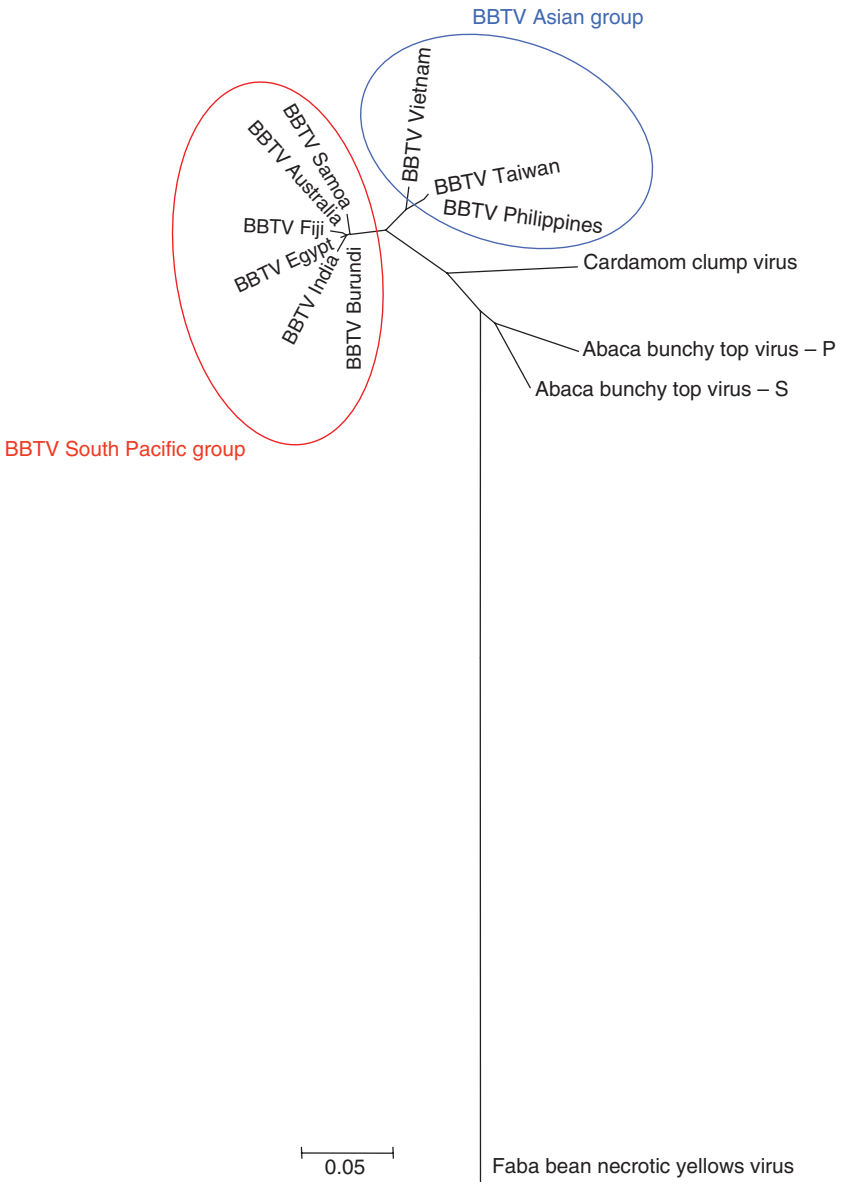


Figure 5 Phylogenetic tree based on the amino acid sequences of babuvirus Rep proteins, using the nanovirus faba bean necrotic yellows virus as an outgroup. The Asian and South Pacific groups of BBTV are circled in blue and red, respectively.

all six genome components of BBTV and also infects *Musa* in Southeast Asia. The sequence and organization of DNA-R of cardamom clump virus (syn. cardamom bushy dwarf virus), associated with Foorkey disease of cardamom in India, also suggests that it is also a genome component of a novel babuvirus.

Geographical Distribution

Historical Records and Possible Origins

The origin of BBTV is unclear. The first records were from Fiji in 1889, and though reports and photographs clearly indicate that it was present at least 10 years prior, soon after the establishment of an export industry based on Cavendish cultivars, evidence suggests that the disease did not originate there. Other early records are from Egypt (1901 – source unknown) and Australia and Sri Lanka (both in 1913, and probably from planting material imported from Fiji).

The wild progenitors of modern edible bananas originated in the South and Southeast Asian–Australasian region, and the Cavendish cultivars of international trade, associated with early outbreaks of bunchy top disease, are thought to have originated in Vietnam. These factors lead to speculation that BBTV also originated and evolved in

this region. The recent discovery of a chimeric BBTV isolate from Taiwan containing genome components of both geographic groups could support this hypothesis.

Current Distribution

BBTV has a widespread, but scattered distribution in many of the banana-growing countries of the Asia-Pacific regions and Africa, but at present is not found in the Americas. In some countries, the occurrence is localized, probably due to geographic isolation. For example, in Australia, it is present in southern Queensland and northern New South Wales, but not in the major production area of north Queensland. Banana bunchy top disease has been recorded in the following countries, and for those marked with asterisk (*), the presence of the virus has been confirmed by serological or molecular assays:

Asia. China*, Japan (Okinawa*, Bonin Is.), India*, Indonesia*, Iran*, Myanmar, Malaysia (Sarawak*), Pakistan*, Philippines*, Sri Lanka*, Taiwan*, Vietnam*.

Pacific. Australia*, Fiji*, Guam, Kiribati (formerly Gilbert Is.), New Caledonia*, Samoa (American, Western*), Tonga*, Tuvalu (formerly Ellice Is.), USA (Hawaii*), Wallis Is.

Africa. Angola, Burundi*, Central African Republic, Congo* Democratic Republic of the Congo (formerly Zaire), Egypt*, Gabon*, Malawi*, Rwanda, Zambia*.

Virus Transmission

The banana aphid (*Pentalonia nigronervosa*) has a worldwide distribution, and in 1925 was shown to be a vector of BBTV. It remains the only known insect vector of this virus. Hosts of the aphid include species in the family Musaceae and several related families, including Araceae, Heliconiaceae, Strelitzeaceae, and Zingiberaceae, though the aphid shows a degree of host preference and can be difficult to transfer between host species. On banana, they commonly colonize the base of the pseudostem at soil level and for several centimeters below the soil surface, beneath the outer leaf sheaths and newly emerging suckers.

Transmission by *P. nigronervosa* is of the persistent, circulative, nonpropagative type, and individuals from areas where the virus is endemic and from where it is absent, both transmit the virus with equal efficiency. There is a minimum acquisition access period of 4 h, a minimum latent period of a few hours, and a minimum inoculation access period of 15 min. Aphids retain infectivity, after removal from a virus source, for at least 20 days and probably for life. Both nymphs and adults can acquire the virus, though more efficiently by the former, and reported transmission rates for individual aphids are in the range 46–67%. There is no evidence for transmission of BBTV to parthenogenetic offspring or for replication of the virus in the aphid.

BBTV is also efficiently transmitted in vegetative planting material, both conventional corms, corm pieces (bits) and suckers, and through micropropagation. All meristems from an infected corm will eventually become infected.

Epidemiology and Control of BBTV

Epidemiology

Outbreaks of bunchy top disease can have a devastating effect on banana production, especially industries based on Cavendish cultivars. Production in Fiji fell by more than 80% from 1892 to 1895, primarily due to bunchy top disease. By 1925, nearly 10 years since the introduction of BBTV to Australia, the banana industry in northern New South Wales and southern Queensland had collapsed, with most plantations affected and production decreased by 90–95%. Magee noted at the time "It would be difficult for anyone who has not visited these devastated areas to visualize the completeness of the destruction wrought in such a short time by a plant disease."

More recently, a severe outbreak of banana bunchy top disease occurred in Pakistan. From 1991 to 1992, production area fell by 55% and total production by 90%, as a direct result of the disease. The disease has also recently appeared in Hawaii, New Caledonia, Angola, Zambia, and Malawi.

The epidemiology of banana bunchy top disease is simplified by the occurrence of a single insect vector species and a limited host range for the virus, usually cultivated or feral edible bananas. Long-distance spread is usually via infected planting material, and local spread via aphids and planting material.

Analysis of actual outbreaks of bunchy top disease in commercial banana plantations in Australia showed that the average distance of secondary spread by aphids was only 15.5–17.2 m, with nearly two-thirds of new infections less than 20 m from the nearest source of infection and 99% less than 86 m. Isolation of new plantations has a marked effect on reducing the risk of infection. New plantations situated adjacent to affected plantations had an 88% chance of recording infections in the first year. This was reduced to 27% if the plantations were separated by 50–1000 m, and to 5% if separated by more than 1000 m. The disease latent period (i.e., period from inoculation of a plant until an aphid can transmit the virus from this plant to another) is equivalent to the time taken for 3.7 new leaves to emerge from the plant. The actual time varies seasonally.

Control

Control strategies were devised by C. J. P. Magee, in the 1920s, and these measures still form the basis of the very successful control program in Australia today. The two

major elements of the strategy are (1) exclusion of the disease from unaffected and lightly affected areas and (2) eradication of infected plants from both lightly and heavily affected areas.

These measures require the participation of all growers and are unlikely to succeed if left to the goodwill of growers alone and are thus enforced by legislation in Australia. The measures include:

- registration of all banana plantations;
- establishment of quarantine zones;
- restrictions on the movement and use of planting material;
- regular inspections of all banana plantations for bunchy top;
- prompt destruction of all infected plants; and
- ongoing education and extension programs for growers.

When adopted, these measures allowed the complete rehabilitation of the Australian banana industry. Occasionally, total eradication of BBTV from a district has been achieved, but in most cases, incidence has been reduced to very low, manageable levels. Such successful control of bunchy top is rarely achieved in other countries, in most cases due to an inability to enforce an organized control program across whole districts.

Detection Assays

Polyclonal and monoclonal antibodies to BBTV are used in enzyme-linked immunosorbent assay (ELISA) to detect the virus in field and tissue culture plants and can detect the virus in single viruliferous aphids. PCR was shown to be about a thousand times more sensitive than ELISA or dot blots with DNA probes. Substances in banana sap inhibitory to PCR can be circumvented by simple extraction procedures or by immunocapture PCR.

BBTV has been detected in most parts of the banana plant, including the leaf lamina and midrib, pseudostem, corm, meristems, roots, fruit stalk, and fruit rind.

Resistance

There are no confirmed reports of immunity to BBTV in *Musa*. However, it has frequently been observed that there are differences in susceptibility between cultivars to both field and experimental infection. Edible bananas have diploid, triploid, or tetraploid genomes containing, predominantly, elements of the *M. acuminata* (A) or *M. balbisiana* (B) genomes. Cultivars in the Cavendish subgroup (AAA genome), which dominates the international export trade, and many other A genome cultivars are highly susceptible and show severe disease symptoms. By contrast, Gros Michel (AAA) displays resistance to the disease under both experimental inoculation and field conditions. Compared with highly sensitive cultivars such as Cavendish, the cultivar is less susceptible to aphid inoculation, contains a lower level of virions in infected plants, and symptoms are less severe and develop more slowly. These factors may contribute to a reduced rate of aphid transmission and field spread in plantations of Gros Michel, and introduction of this cultivar may explain the partial recovery of the Fijian banana industry after devastation of the Cavendish-based industry in the early 1900s. Field observations and glasshouse inoculations suggest that some B genome-containing cultivars are less susceptible to infection and/or display more limited symptoms, but this needs to be further investigated.

Despite concerted efforts to generate transgenic resistance to BBTV, no successful glasshouse or field results have yet been reported. However, some promising strategies are being developed at Queensland University of Technology involving the following steps.

1. Transdominant negative strategies to interfere with replication, by constitutive overexpression of mutated Rep proteins, are employed. Single mutations in either of two motifs involved with rolling-circle replication render the Rep inactive, and in transient assays with constitutive overexpression, virus replication is significantly reduced, but not abolished.
2. Rep-activated cell death is carried out using a so-called suicide gene. DNA-R intergenic sequence is cloned within an intron and flanked by a split barnase gene construct. The suicide gene is only activated in the presence of the Rep protein, resulting in cell death and containment of the virus.

See also: Nanoviruses.

Further Reading

Allen RN (1987) Further studies on epidemiological factors influencing control of banana bunchy top disease, and evaluation of control measures by computer simulation. *Australian Journal of Agricultural Research* 38: 373–382.

Bell KE, Dale JL, Ha CV, Vu MT, and Revill PA (2002) Characterisation of Rep-encoding components associated with banana bunchy top nanovirus in Vietnam. *Archives of Virology* 147: 695–707.

Burns TM, Harding RM, and Dale JL (1995) The genome organization of banana bunchy top virus: Analysis of six ssDNA components. *Journal of General Virology* 76: 1471–1482.

Dugdale B, Becker DK, Beetham PR, Harding RM, and Dale JL (2000) Promoters derived from banana bunchy top virus DNA-1 to -5 direct vascular-associated expression in transgenic banana (*Musa* spp.). *Plant Cell Reports* 19: 810–814.

Geering ADW and Thomas JE (1997) Search for alternative hosts of banana bunchy top virus in Australia. *Australasian Plant Pathology* 26: 250–254.

Hafner GJ, Harding RM, and Dale JL (1995) Movement and transmission of banana bunchy top virus DNA component one in bananas. *Journal of General Virology* 76: 2279–2285.

Hu J-M, Fu H-C, Lin C-H, Su H-J, and Yeh H-H (2007) Reassortment and concerted evolution in banana bunchy top virus genomes. *Journal of Virology* 81: 1746–1761.

Hu JS, Wang M, Sether D, Xie W, and Leonhardt KW (1996) Use of polymerase chain reaction (PCR) to study transmission of banana bunchy top virus by the banana aphid (*Pentalonia nigronervosa*). *Annals of Applied Biology* 128: 55–64.
Karan M, Harding RM, and Dale JL (1994) Evidence for two groups of banana bunchy top virus isolates. *Journal of General Virology* 75: 3541–3546.
Magee CJ (1953) Some aspects of the bunchy top disease of banana and other *Musa* spp. *Journal and Proceedings of the Royal Society of New South Wales* 87: 3–18.
Magee CJP (1927) *Investigation on the Bunchy Top Disease of the Banana. Bulletin No. 30*. Melbourne: Council for Scientific and Industrial Research.
Thomas JE and Dietzgen RG (1991) Purification, characterization and serological detection of virus-like particles associated with banana bunchy top disease in Australia. *Journal of General Virology* 72: 217–224.
Thomas JE and Iskra-Caruana ML (2000) Bunchy top. In: Jones DR (ed.) *Diseases of Banana, Abaca and Enset*, pp. 241–253. Wallingford: CABI Publishing.
Thomas JE, Smith MK, Kessling AF, and Hamill SD (1995) Inconsistent transmission of banana bunchy top virus in micropropagated bananas and its implication for germplasm screening. *Australian Journal of Agricultural Research* 46: 663–671.
Vetten HJ, Chu PWG, Dale JL, *et al.* (2005) *Nanoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses,* pp. 343–352. San Diego, CA: Elsevier Academic Press.
Wu R-Y and Su H-J (1990) Purification and characterization of banana bunchy top virus. *Journal of Phytopathology* 128: 153–160.

Barley Yellow Dwarf Viruses

L L Domier, USDA-ARS, Urbana-Champaign, IL, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Hemocoel The primary body cavity of most arthropods that contains most of the major organs and through which the hemolymph circulates.
Hemolymph A circulatory fluid in the body cavities (hemocoels) and tissues of arthropods that is analogous to blood and/or lymph of vertebrates.

Introduction

Barley yellow dwarf (BYD) is the most economically important virus disease of cereals, and is found in almost every grain growing region in the world. Widespread BYD outbreaks in cereals were noted in the United States in 1907 and 1949. However, it was not until 1951 that a virus was proposed as the cause of the disease. The causal agents of BYD are obligately transmitted by aphids, which probably delayed the initial classification of BYD as a virus disease. Subsequently, BYD was shown to be caused by multiple viruses belonging to the species barley yellow dwarf virus (BYDV) and cereal yellow dwarf virus (CYDV). Depending on the virulence of the virus strain, infection may contribute to winter kill in regions with harsh winters, induce plant stunting, inhibit root growth, reduce or prevent heading, or increase plant susceptibility to opportunistic pathogens and other stresses. Yield losses to wheat in the United States alone are estimated at 1–3% annually, exceeding 30% in certain regions in epidemic years. The effects of BYD in barley and oats typically are more severe than in wheat; sometimes resulting in complete crop losses. The existence of multiple strains of viruses that are transmitted in strain-specific manner has made BYDV and CYDV model systems to study interactions between viruses and aphid vectors in the circulative transmission of plant viruses. In addition, the compact genomes of the viruses have provided useful insights into the manipulation of host translation machinery by RNA viruses.

Taxonomy and Classification

The viruses that cause BYD are members of the family *Luteoviridae*, and were first grouped because of their common biological properties. These properties included persistent transmission by aphid vectors, the induction of yellowing symptoms in grasses, and serological relatedness. Different viruses are transmitted more efficiently by different species of aphids, a fact that was originally used to distinguish the viruses. Around 1960, the viruses were separated into five 'strains' (now recognized as distinct species) based on their primary aphid vector(s). BYDVs transmitted most efficiently by *Sitobion* (formerly *Macrosiphum*) *avenae* were assigned the acronym MAV, for *Macrosiphum avenae* virus. Similarly, viruses transmitted most efficiently by *Rhopalosiphum maidis* and *Rhopalosiphum padi* were assigned the acronyms RMV and RPV, respectively. Viruses transmitted most efficiently by *Schizaphis graminum* were assigned the acronym SGV. Finally vector-nonspecific viruses, that is, viruses transmitted efficiently by both *R. padi* and *S. avenae* were assigned the acronym PAV.

Based on genome organization and predicted amino acid sequence similarities, BYDV-MAV, -PAS, and -PAV

have been assigned to the genus *Luteovirus*, and CYDV-RPS and -RPV to the genus *Polerovirus*. The RNA- dependent RNA polymerases (RdRps) encoded by open reading frames (ORFs) 1 and 2 of BYDVs resemble those of members of the family *Tombusviridae* (**Figure 1**). In contrast, the predicted amino acid sequence of the RdRps encoded by ORFs 1 and 2 of CYDVs resemble those of viruses in the genus *Sobemovirus*. The two polymerase types are distantly related in evolutionary terms. For this reason, viruses for which RdRp sequences have not been determined (BYDVs GPV, RMV, and SGV) have not been assigned to a genus. These observations suggest that the genomic RNAs of BYDVs and CYDVs resulted from recombination between RNAs expressing a common set of structural and movement proteins and RNAs expressing two different sets of replication proteins. Because of these differences, it has been suggested that BYDVs should be placed in the family *Tombusviridae* and CYDVs in the genus *Sobemovirus*.

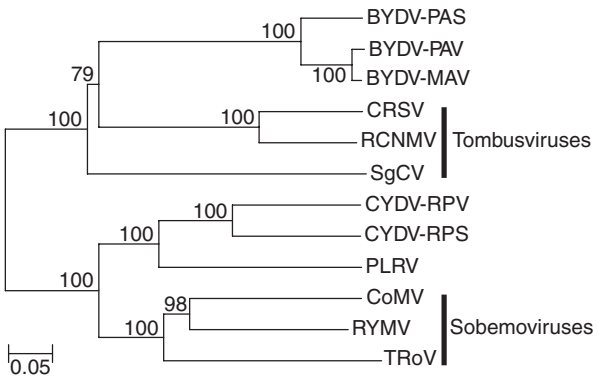


Figure 1 Phylogenetic relationships of the predicted amino acid sequences of RNA-dependent RNA polymerases (RdRps; ORFs 1 and 2) of barley yellow dwarf viruses (BYDVs) and cereal yellow dwarf viruses (CYDVs) and members of the genus *Sobemovirus* and family *Tombusviridae*. The RdRps of BYDV-MAV, -PAS, and -PAV are more similar to those of members of the family *Tombusviridae* (carnation ringspot virus (CRSV), red clover necrotic mosaic virus (RCNMV), and saguaro cactus virus (SgCV)) than to those of CYDVs. The RdRps of CYDV-RPS and -RPV and potato leaf roll virus (PLRV, type member of the genus *Polerovirus*) are more similar to those of members of the genus *Sobemovirus* (cocksfoot mottle virus (CoMV), rice yellow mottle virus (RYMV), and turnip rosette virus (TRoV)) than to those of the BYDVs. The resulting consensus tree from 1000 bootstrap replications is shown. The numbers above each node indicate the percentage of bootstrap replicates in which that node was recovered.

Virion Properties and Composition

All BYDVs and CYDVs have nonenveloped icosahedral particles with diameters of 25–28 nm (**Figure 2**). Capsids are composed of major (22 kDa) and minor (65–72 kDa) coat proteins (CPs), which is formed by a carboxy-terminal extension to the major CP called the read-through domain (RTD). According to X-ray diffraction and molecular mass analysis, virions consist of 180 protein subunits, arranged in $T = 3$ icosahedra. Virus particles do not contain lipids or carbohydrates, and have sedimentation coefficients $s_{20,w}$ (in Svedberg units) that range from 115–118S. Buoyant densities in CsCl are approximately $1.4\,g\,cm^{-3}$. Virions are moderately stable, insensitive to freezing, and are insensitive to treatment with chloroform or nonionic detergents, but are disrupted by prolonged treatment with high concentrations of salts.

The single encapsidated genomic RNA molecule is single-stranded, positive-sense, and lacks a 3′-terminal poly(A) tract. A small protein (VPg) is covalently linked to the 5′-terminus of CYDV RNAs. CYDV-RPV also encapsidates a 322-nucleotide satellite RNA that accumulates to high levels in the presence of the helper virus. Complete genome sequences have been determined for BYDV-MAV, -PAS, and -PAV and CYDV-RPS and -RPV (**Table 1**). For several viruses, notably BYDV-PAV, genome sequences have been determined from multiple isolates.

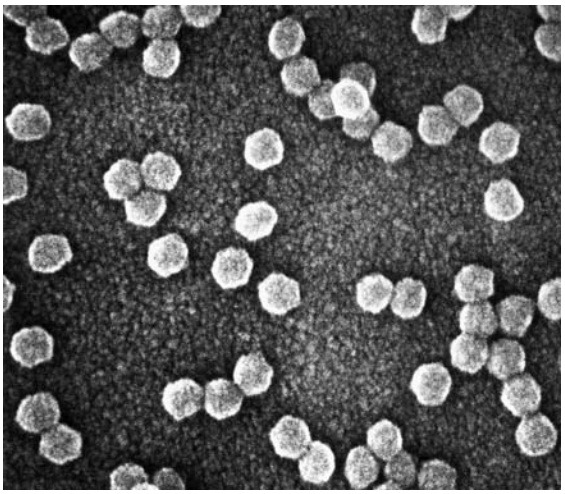


Figure 2 Scanning electron micrograph of barley yellow dwarf virus-PAV particles, magnified 200 000×. Virions are *c*. 25 nm in diameter, hexagonal in appearance, and have no envelope.

Genome Organization and Expression

Genomic RNAs of BYDVs and CYDVs for which complete nucleotide sequences are available contain five to six ORFs (**Figure 3**). ORFs 1, 2, 3, and 5 are shared among all BYDVs and CYDVs. BYDVs lack ORF0. Genomic sequences of some BYDVs contain one or two small ORFs, ORFs 6 and 7, downstream of ORF5. In CYDVs, ORFs 0 and 1 and ORFs 1 and 2 overlap by more than 600 nucleotides. In BYDVs, ORF1 overlaps ORF2 by less than 50 nucleotides. In BYDV and CYDV genome sequences, ORF4 is contained within ORF3. An amber (UAG) termination codon separates ORFs 3 and 5.

Table 1 Viruses causing barley yellow dwarf in cereals

Genus	*Virus (alternative name)*	*Abbreviation*
Luteovirus	Barley yellow dwarf virus-MAV	BYDV-MAV
	Barley yellow dwarf virus-PAS	BYDV-PAS
	Barley yellow dwarf virus-PAV	BYDV-PAV
	(Barley yellow dwarf virus-RGV) (rice giallume)	
Polerovirus	Cereal yellow dwarf virus-RPS	CYDV-RPS
	Cereal yellow dwarf virus-RPV	CYDV-RPV
Unassigned	Barley yellow dwarf virus-GAV	BYDV-GAV
	Barley yellow dwarf virus-GPV	BYDV-GPV
	Barley yellow dwarf virus-RMV	BYDV-RMV
	Barley yellow dwarf virus-SGV	BYDV-SGV

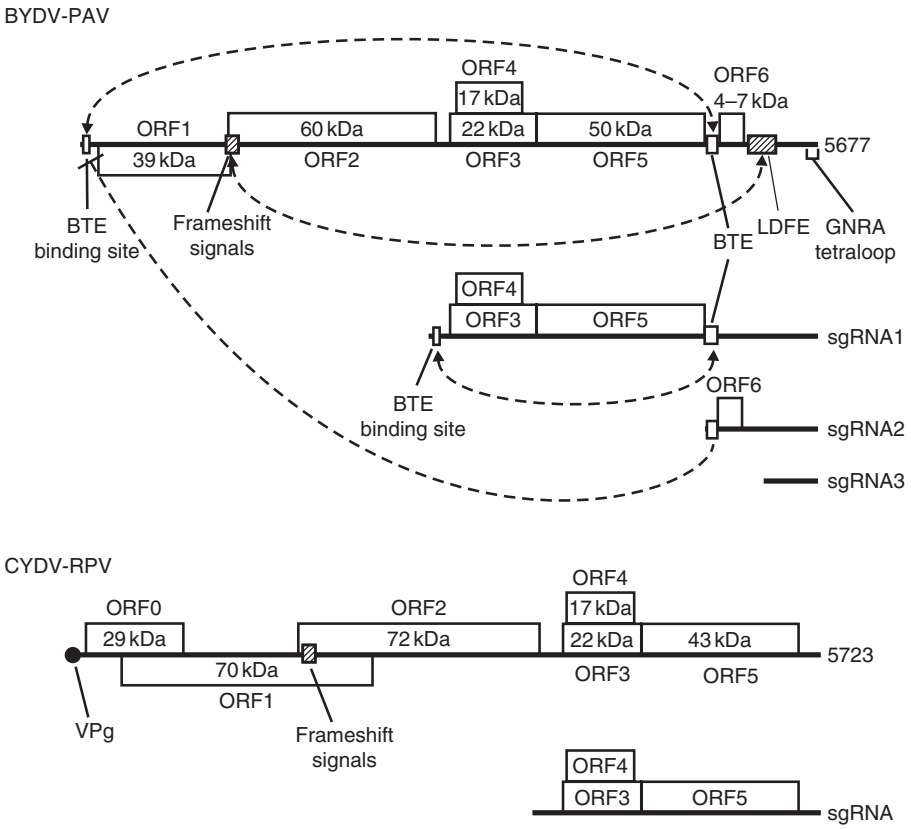


Figure 3 Genome organizations of barley yellow dwarf virus-PAV (BYDV-PAV) and cereal yellow dwarf virus-RPV (CYDV-RPV). Individual open reading frames (ORFs) are shown as staggered open boxes. The predicted sizes of the protein products are indicated. The genome-linked protein (VPg) attached to the 5′ terminus of CYDV RNA is indicated by a solid circle. Based on homology to other viruses ORF0 encodes a silencing suppressor and ORFs 1 and 2 encode replication-related proteins. ORFs 3 and 5 encode the major coat protein and readthrough domain, respectively. ORF4 encodes a protein required for virus cell-to-cell movement. The BYDV translation enhancer (BTE) facilitates translation initiation of BYDV-PAV genomic RNA and subgenomic RNA1 (sgRNA1). In both BYDV-PAV and CYDV-RPV, ORF2 is expressed as a translational fusion with the product of ORF1 via a −1 frameshift. In BYDV-PAV, frameshifting requires interaction between the 5′ frameshift signals and the long-distance frameshift element (LDFE). Dashed lines indicate long-distance RNA–RNA interactions.

BYDVs and CYDVs have relatively short 5′ and intergenic noncoding regions. ORFs 2 and 3 are separated by about 200 nucleotides in BYDVs and CYDVs. The lengths of noncoding sequences downstream of ORF5 are very different between BYDVs and CYDVs. BYDV-PAV contains over 860 nucleotides downstream of ORF5 compared to just 170 nucleotides for CYDV-RPV.

The expression of BYDV-PAV RNA has been studied in detail and has revealed a complex set of RNA–RNA and RNA–protein interactions that are employed to express and replicate the virus genome. Less experimental data are available for CYDVs. However, expression and replication strategies and gene functions can be inferred from those of closely related poleroviruses, particularly

beet western yellows virus (BWYV) and potato leaf roll virus (PLRV). ORFs 0, 1, and 2 are expressed directly from genomic RNAs. Downstream ORFs are expressed from subgenomic RNAs (sgRNAs) that are transcribed from internal initiation sites by virus-encoded RdRps from negative strand RNAs and are 3′-coterminal with the genomic RNA. Since the initiation codon for ORF0 of CYDVs is upstream of that of ORF1, translation of ORF1 is initiated by 'leaky scanning' in which ribosomes bypass the AUG initiation codon of ORF0 and continue to scan the genomic RNA until they reach the initiation codon of ORF1. The protein products of ORF2 are expressed only as a translational fusion with the product of ORF1. At a low frequency during the expression of ORF1, translation continues into ORF2 through a –1 frameshift that produces a large protein containing sequences encoded by both ORFs 1 and 2 in a single polypeptide. In BYDV-PAV, frameshifting between ORFs 1 and 2 is dependent upon the interaction of RNA sequences close to the site of frameshifting and a long-distance frameshift element (LDFE) located 4000 nucleotides downstream in the 3′ noncoding region of genomic RNAs. Mutations that disrupt the interactions between these two distal regions suppress frameshifting and abolish RNA replication.

ORFs 3, 4, and 5 are expressed from sgRNA1, the 5′ terminus of which is located about 200 nucleotides upstream of ORF3, and extends to the 3′ terminus of the genome. BYDVs produce a second sgRNA that contains ORF6. BYDV-PAV also produces a third sgRNA, which does not appear to encode a protein. ORF3 is translated from the 5′ terminus of sgRNA1. ORF4 of BYDVs and CYDVs, which encodes a 17 kDa protein, is contained within ORF3 and is expressed from the same sgRNA as ORF3 through a leaky scanning mechanism much like that used to express ORF1 of CYDVs. In BYDVs and CYDVs, ORF5 is expressed only as a translational fusion with the products of ORF3 by readthrough of the UAG termination codon at the end of ORF3. This produces a protein with the product of ORF3 at its amino terminus and the product of ORF5 at its carboxyl terminus.

While genomic RNAs of CYDVs contain 5′ VPgs that interact with translation initiation factors, BYDV-PAV RNA contains only a 5′ phosphate. Unmodified 5′ termini usually are recognized poorly for translation initiation. To circumvent this problem, a short sequence located in the noncoding region just downstream of ORF5 in the BYDV-PAV genome, called the BYDV translation enhancer (BTE), interacts with sequences near the 5′ termini of the genomic and subgenomic RNAs to promote efficient cap-independent translation initiation.

Functions BYDV and CYDV proteins have been ascribed based on homology to virus proteins with known functions and mutational characterization of protein coding regions. Similarity to proteins encoded by BWYV and PLRV suggests that the 28–29 kDa proteins encoded by ORF0 of CYDVs are inhibitors of post-transcriptional gene silencing (PTGS). PTGS is an innate and highly adaptive antiviral defense found in all eukaryotes that is activated by double-stranded RNAs (dsRNAs), which are produced during virus replication. The ORF1-encoded proteins of CYDVs contain the VPg and a chymotrypsin-like serine protease that is responsible for the proteolytic processing of ORF1-encoded polyproteins. The protease cleaves the ORF1 protein in *trans* to liberate the VPg, which is covalently attached to genomic RNAs. ORF2s of BYDVs and CYDVs, which are expressed as translational fusions with the product of ORF1, have coding capacities of 59–72 kDa and predicted amino acid sequences that are very similar to known RdRps and hence likely represent the catalytic portion of the viral replicase.

ORF3 encodes the major 22 kDa CP for both BYDVs and CYDVs. ORF5 has a coding capacity of 43–50 kDa, which is expressed only as a translational fusion with the product of ORF3 when translation reads through the termination codon at the end of ORF3 and continues through to the end of ORF5. The ORF5 portion of this readthrough protein has been implicated in aphid transmission and virus stability. Recombinant viruses that do not express ORF5 produce virions assembled from the major CP alone, which are not transmitted by aphid vectors and are less efficient in systemic infection of host plants than wild-type viruses. The amino-terminal portions of ORF5 proteins are highly conserved among BYDVs and CYDVs while the carboxyl termini are much more variable.

ORF4 of both BYDVs and CYDVs is contained within ORF3 and encodes a 17 kDa protein. Viruses that contain mutations in ORF4 are able to replicate in isolated plant protoplasts, but are deficient or delayed in systemic movement in whole plants. Hence, proteins encoded by ORF4 are thought to facilitate intra- and intercellular virus movement.

Some BYDV genomic sequences contain small ORFs (ORF6) downstream of ORF5. The predicted sizes of the proteins expressed by ORF6 range from 4 to 7 kDa. The predicted amino acid sequences of the proteins encoded by ORF6 are poorly conserved among BYDV-PAV isolates. Repeated attempts to detect protein products of ORF6 have been unsuccessful. In addition, BYDV-PAV genomes into which mutations have been introduced that disrupt ORF6 translation are still able to replicate in protoplasts. Based on these observations, it has been concluded that ORF6 is not translated *in vivo*.

Host Range and Transmission

BYD-causing viruses infect over 150 species of annual and perennial grasses in five of the six subfamilies of the Poaceae. The feeding habits of vector aphids have a

major impact on the host ranges of virus species. Hence the number of species naturally infected by the viruses is much lower than the experimental host range. As techniques for infecting plants with recombinant viruses have improved, the experimental host ranges of BYDVs and CYDVs have been expanded to include plants on which aphid vectors would not normally feed. For example, BYDV-PAV and CYDV-RPV have been shown to infect *Nicotiana* species when inoculated using *Agrobacterium tumefaciens* harboring binary plasmids containing infectious copies of the viruses, which had not been described previously as experimental hosts for the viruses.

Viruses that cause BYD are transmitted in a circulative strain-specific manner by at least 25 aphid species. Circulative transmission of the viruses is initiated when the piercing–sucking mouthparts of aphids acquire viruses from sieve tubes of infected plants during feeding. Aphids that do not probe into and feed from the vascular tissues of infected plants do not transmit the viruses. The virions of BYDVs and CYDVs travel up the stylet, through the food canal, and into the foregut (**Figure 4**). After 12–16 h, virions then are actively transported across the cells of the hindgut into the hemocoel in a process that involves receptor-mediated endocytosis of the viruses and the formation of tubular vesicles that transport viruses through epithelial cells and into the hemocoel. Virions then passively diffuse through the hemolymph to the accessory salivary gland where virions must pass through the membranes of accessory salivary gland cells in a similar type of receptor-mediated transport process to reach the lumen of the gland. The accessory salivary gland produces a watery saliva, containing few or no enzymes, that is thought to prevent phloem proteins from clogging the food canal. Once in the salivary gland lumen, virions are expelled with the watery saliva into vascular tissues of host plants. Typically hindgut membranes are much less selective than those of the accessory salivary glands. Consequently, viruses that are not transmitted by a particular species of aphid often are transported across gut membranes and accumulate in the hemocoel, but do not traverse the membranes of the accessory salivary gland. The specificity of aphid transmission and gut tropism has been linked to the RTD of the minor capsid protein. Even though large amounts of virions can accumulate in the hemocoel, there is no evidence for virus replication in their aphid vectors. Aphids may retain the ability to transmit virus for several weeks.

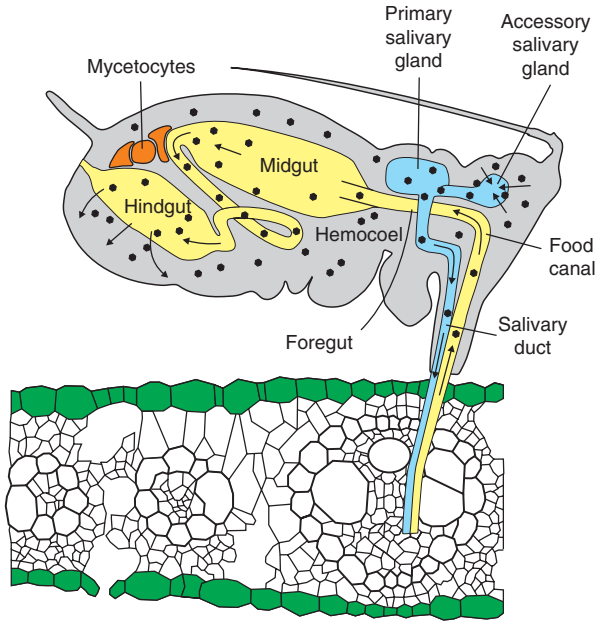


Figure 4 Circulative transmission of BYDVs and CYDVs by vector aphids. While feeding from sieve tubes of an infected plant, an aphid (shown in cross section) acquires virions, which travel up the stylet, through the food canal, and into the foregut. Virions are actively transported across cells of the hindgut into the hemocoel. Virions then passively diffuse through the hemolymph to the accessory salivary gland where they are again actively transported into the lumen of the gland. Once in the salivary gland lumen, the virions are expelled with the saliva into the vascular tissue of host plants. Viruses that are not transmitted by a particular species of aphid often accumulate in the hemocoel, but do not traverse the membranes of the accessory salivary gland.

Genetic and biochemical studies have been conducted to identify aphid determinants of strain-specific transmission of BYDV-MAV and BYDV-PAV. Protein–protein and protein–virus interaction experiments were used to isolate two proteins from heads of vector aphids that bind BYDV-MAV that were not detected in nonvector aphids. These two proteins are good candidates for the cell-surface receptors that are thought to be involved in strain-specific transport of viruses into accessory salivary gland lumens. In addition, endosymbiotic bacteria that reproduce in specialized cells called mycetocytes in abdomens of aphids express chaperonin-like proteins that bind BYDV particles and the amino-terminal region of recombinant BYDV-PAV RTD proteins. However, the role of these proteins in aphid transmission is unclear since they are found in both vector and nonvector aphid species. Interactions of virus particles with these proteins seem to be essential for persistence of the viruses in aphids. The proteins may protect virus particles from degradation by aphid immune systems.

Replication

Like other viruses of the family *Luteoviridae*, BYDVs and CYDVs infect and replicate in sieve elements and companion cells of the phloem and occasionally are found in phloem parenchyma cells (**Figure 5**). BYDVs and CYDVs induce characteristic ultrastructural changes in infected cells. BYDV-MAV, -PAV, and -SGV induce

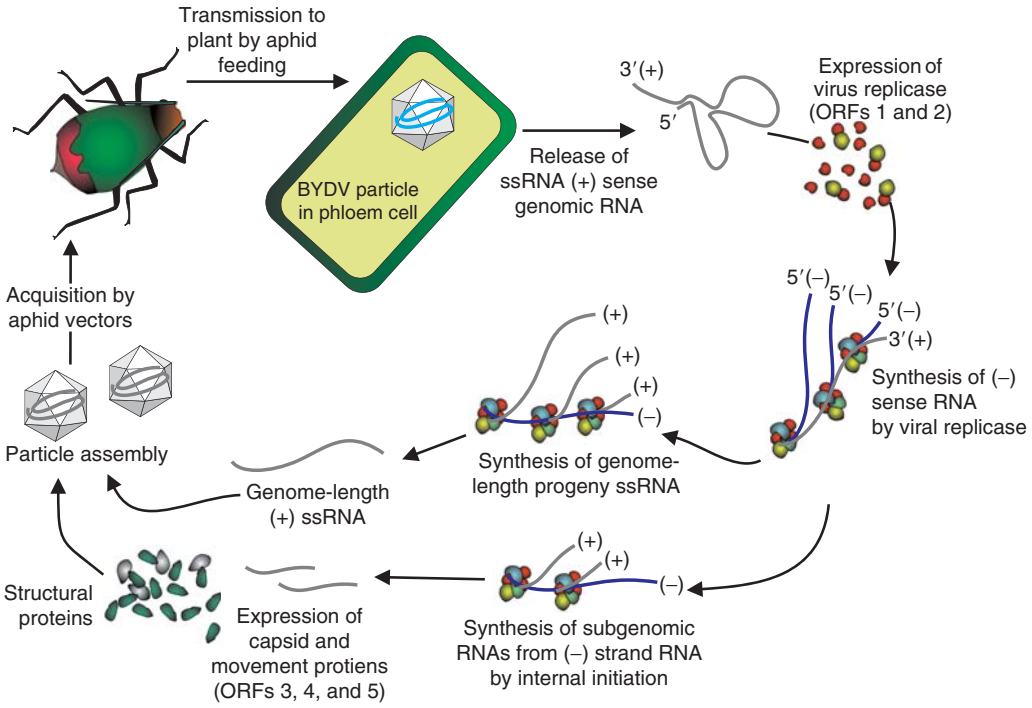


Figure 5 Barley yellow dwarf virus-PAV life cycle. Virus particles are deposited in sieve elements by aphid vectors. By a yet unknown process, single-stranded messenger-sense genomic RNA is released from virus particles and translated by host translation machinery, which is facilitated by long-distance RNA–RNA interactions. Open reading frames (ORFs) 1 and 2, which encode the viral replicase, are expressed first because of their proximity to the 5′ termini of genomic RNAs. Virus encoded replicase then synthesizes negative-sense RNAs that are used as templates for the production of new full-length positive-sense genomic RNAs and subgenomic RNAs. Production of subgenomic RNAs results in synthesis of structural and cell-to-cell movement proteins. Subgenomic RNA2 suppresses translation from genomic RNAs, furthering the switch from early to late gene expression. Full-length positive-sense genomic RNAs and structural proteins then assemble into virions in cells of phloem tissues where they can be ingested by aphid vectors to start the process again.

single-membrane-bound vesicles in the cytoplasm near plasmodesmata early in infection. Subsequently, filaments are observed in nuclei, and virus particles are first observed in the cytoplasm. In contrast, BYDV-RMV and CYDV-RPV induce double-membrane-bound vesicles in the cytoplasm that are continuous with the endoplasmic reticulum. Later, filaments and tubules form in the cytoplasm, and BYDV-RMV and CYDV-RPV particles are first observed in nuclei.

The subcellular location of viral RNA replication has not been determined unequivocally. However, early in infection, negative-strand RNAs of BYDV-PAV are first detected in nuclei and later in the cytoplasm, which suggests that at least a portion of the BYDV-PAV replication occurs in the nucleus. A nuclear location for replication is supported by the observation that the movement protein encoded by ORF4, which also binds single-stranded RNA, localizes to the nuclear envelope and is associated with virus RNA in nuclei of infected cells. Synthesis of negative-strand RNA, which requires tetraloop structures at the 3′ end of BYDV-PAV genomic RNAs, is detected in infected cells before the formation of virus particles. Because tetraloops have been implicated in RNA–protein interactions, these structures could be binding and/or recognition sites for BYDV replication proteins. BYDV-PAV sgRNAs are synthesized by internal initiation of RNA synthesis on negative-strand RNAs from three dissimilar subgenomic promoters. Late in infection, the BTE near the 5′ terminus of BYDV-PAV sgRNA2 inhibits translation from genomic RNA, which may promote a switch from translation to replication and packaging of genomic RNAs. In addition to genomic RNAs, CYDV-RPV replicates a satellite RNA by a rolling-circle mechanism that generates multimeric satellite RNAs that self-cleave to unit length.

Virus–Host Relationships

Visible symptoms induced by BYDVs and CYDVs vary greatly depending on the host and strain of the virus. The most common symptoms are stunting and chlorosis. While some infected plants display no obvious symptoms, most BYDVs and CYDVs induce characteristic symptoms that include stunting, leaves that become thickened, curled or serrated, and yellow, orange or red

leaf discoloration, particularly of older leaves of infected plants. These symptoms result from phloem necrosis that spreads from inoculated sieve elements and causes symptoms by inhibiting translocation, slowing plant growth, and inducing the loss of chlorophyll. Symptoms may persist, may vary seasonally, or may disappear soon after infection. Temperature and light intensity often affect symptom severity and development. In addition, symptoms can vary greatly with different virus isolates or strains and with different host cultivars. Yield losses caused by BYD are difficult to estimate because the viruses are so pervasive and symptoms often are overlooked or attributed to other agents. In Australia alone, losses in barley production have been valued at over 100 million US dollars annually. Plants infected with BYD at early developmental stages suffer the most significant yield losses, which often are linearly correlated with the incidence of virus infection.

Epidemiology

BYD infections have been reported from temperate, subtropical, and tropical regions of the world. Even though the incidence of infections of individual viruses varies from year to year and can differ among annual and perennial hosts, BYDV-PAV usually is the most prevalent of the viruses causing BYD in small grains worldwide followed by CYDV-RPV or BYDV-MAV. The remaining BYD-causing viruses are typically much less prevalent. BYDVs and CYDVs must be reintroduced into annual crops each year by their aphid vectors. Alate, that is, winged aphids may transmit viruses from local cultivated, volunteer, or weed hosts. Alternatively, alate aphids may be transported into crops from distant locations by wind currents. These vectors may bring the virus with them, or they may first have to acquire virus from locally infected hosts. In temperate regions of Europe and North America moderate and long-distance migration of viruliferous aphids is important to development of BYD epidemics. In Australasia, and other regions with Mediterranean climates, alate aphids usually transmit viruses from relatively close infected plants. Secondary spread of the viruses is often primarily by apterous, that is, wingless aphids. The relative importance of primary introduction of viruses by alate aphids and of secondary spread of viruses by apterous aphids in disease severity varies with the virus, aphid species, crop, and environmental conditions.

Diagnosis

Accurate diagnosis of infections has been important in understanding the transmission and epidemiology of BYDVs and CYDVs and developing control strategies for BYD. Because BYD symptoms resemble those caused by other biotic and abiotic factors, visual diagnosis is unreliable and other methods have been developed. Initially, infectivity, or biological, assays were used to diagnose infections. In bioassays, aphids are allowed to feed on infected plants and then are transferred to indicator plants. These techniques have also been used to determine vector specificities of viruses causing BYD and to identify viruliferous vector aphids in epidemiological studies. These techniques are very sensitive, but they can require several weeks for symptoms to develop on indicator plants. The viruses causing BYD are strongly immunogenic, which has facilitated development of genus- and even strain-specific antibodies that have been used extensively in BYD diagnosis. Because the viruses causing BYD are present in infected tissues at very low levels, mice have been used to produce monoclonal antibodies against the viruses. Mice typically require much less viral antigen per immunization than rabbits, and hybridoma cell lines that produce monoclonal antibodies can be stored for extended periods and used for many years, which further reduces the amount of antigen needed to produce diagnostic antibodies. Techniques have also been developed to detect viral RNAs from infected plant tissues by reverse transcription polymerase chain reaction, which can be more sensitive and discriminatory than serological diagnostic techniques. Even so, serological tests are the most commonly used techniques for the detection of infections because of their simplicity , speed, and relatively low cost.

Control

Planting of insecticide-treated seeds that protect emerging seedlings from aphid infestation has been shown to reduce losses caused by BYD in North America, Australasia and Africa. Foliar applications of insecticides on older plants typically have been less effective. Alternatively, planting of tolerant or resistant cereals has proved to be a much more cost-effective and sustainable management strategy for BYD. Breeding programs have successfully integrated genes conferring high levels of tolerance into barley and oat and to a lesser extent in wheat. Even though a limited number of single genes for BYD resistance/tolerance have been identified in cultivated barley and rice, in most instances, tolerance to BYD is conditioned by multiple genes in a quantitative fashion, which has made moving BYD tolerance into new plant lines challenging. Particularly in barley, molecular markers have begun to facilitate the process of breeding for BYD tolerance. Because of a lack of effective single-gene resistance in cultivated wheat, some researchers have moved BYD resistance genes from wheat grasses (*Thinopyrum intermedium* and *Thinopyrum ponticum*) into wheat, which have provided high levels of resistance. The lack of naturally occurring resistance in cereals to BYD has made transgene-mediated resistance very attractive. Even though the expression of CP sequences in transgenic plants has conferred resistance in several other

plant-virus systems, it has not provided significant resistance to BYD in barley, oat, or wheat. In contrast, transgenic barley and oat plants have been produced that express either intact or inverted copies of BYDV-PAV replicase genes, which conferred high levels of resistance to BYDV-PAV and closely related viruses.

In many small grain growing regions, viruliferous aphids arrive at similar times each spring and fall even though sizes of the aphid populations can vary significantly from year to year. In these areas, it is sometimes possible to plant crops so that young, highly susceptible plants are not in the field when the seasonal aphid migrations occur. However, crops planted later typically do not yield as well as those planted early in the growing season. Consequently, growers must weigh the probability of obtaining higher yields against possible yield losses caused by BYD. In some instances, biological control agents such as predatory insects and parasites have reduced aphid populations significantly.

See also: Cereal Viruses: Wheat and Barley; Luteoviruses.

Further Reading

Crasta OR, Francki MG, Bucholtz DB, *et al.* (2000) Identification and characterization of wheat-wheatgrass translocation lines and localization of barley yellow dwarf virus resistance. *Genome* 43: 698–706.

D'Arcy CJ and Burnett PA (1994) *Barley Yellow Dwarf Virus, Forty Years of Progress.* St. Paul, MN: American Phytopathological Society Press.

Falk BW, Tian T, and Yeh HH (1999) Luteovirus-associated viruses and subviral RNAs. *Current Topics in Microbiology and Immunology* 239: 159–175.

Figueira AR, Domier LL, and Darcy CJ (1997) Comparison of techniques for detection of barley yellow dwarf virus PAV-IL. *Plant Disease* 81: 1236–1240.

Gray S and Gildow FE (2003) Luteovirus–aphid interactions. *Annual Review of Phytopathoogy* 41: 539–566.

Koev G, Mohan BR, Dinesh-Kumar SP, *et al.* (1998) Extreme reduction of disease in oats transformed with the 5′ half of the barley yellow dwarf virus PAV genome. *Phytopathology* 88: 1013–1019.

Miller WA, Liu SJ, and Beckett R (2002) Barley yellow dwarf virus: *Luteoviridae* or *Tombusviridae? Molecular Plant Pathology* 3: 177–183.

Miller WA and Rasochova L (1997) Barley yellow dwarf viruses. *Annual Review of Phytopathology* 35: 167–190.

Miller WA and White KA (2006) Long-distance RNA–RNA interactions in plant virus gene expression and replication. *Annual Review of Phytopathology* 44: 447–467.

Nass PH, Domier LL, Jakstys BP, and D'Arcy CJ (1998) *In situ* localization of barley yellow dwarf virus PAV 17-kDa protein and nucleic acids in oats. *Phytopathology* 88: 1031–1039.

Ordon F, Friedt W, Scheurer K, *et al.* (2004) Molecular markers in breeding for virus resistance in barley. *Journal of Applied Genetics* 45: 145–159.

Zhu S, Kolb FL, and Kaeppler HF (2003) Molecular mapping of genomic regions underlying barley yellow dwarf tolerance in cultivated oat (*Avena sativa* L.). *Theoretical and Applied Genetics* 106: 1300–1306.

Barnaviruses

P A Revill, Victorian Infectious Diseases Reference Laboratory, Melbourne, VIC, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Casing A layer of peat moss placed on top of the growing beds to encourage sporophore formation.
Sporophore Mushroom fruiting body.

Introduction

In the USA in 1948, a disease of the cultivated mushroom *Agaricus bisporus* was discovered on a property in Pennsylvania that had a major impact on the mushroom industry and fungal pathology in general. It was characterized by poor colonization of compost by mycelium and misshapen sporophores with long thin stipes and small globular caps producing a drumstick-like appearance, or they were thickened with a barrel-like appearance. The poor colonization of the compost and casing by infected mycelium often produced characteristic bare patches on the growing beds and reduced yields. The disease was named La France disease and a virus was implicated as a possible cause in 1960, after it was shown that the disease could be transmitted to healthy cultures by hyphal anastamosis. In 1962, three different virus-like particles were identified, two of which were spherical (25 and 29 nm), and the third was a 19 nm × 50 nm elongated or bacilliform particle with rounded ends. Subsequently a 34–36 nm particle with a double-stranded (ds)RNA genome (La France infectious virus (LFIV)) has been identified as the causal agent of La France disease.

The bacilliform virus particle was of particular interest as almost all mycoviruses identified to that point had a spherical or isometric morphology. Bacilliform 19 nm × 48 nm virus-like particles were subsequently identified in the ascomycete *Microsphaera mougeotti*, and

17 nm × 35 nm bacilliform particles were also observed in the deuteromycete *Verticilium fungicola*, itself a pathogen of *A. bisporus*. However no relationship with the mushroom bacilliform virus (see below) and these bacilliform particles has been established.

Originally named mushroom virus 3 (MV3), the virus was subsequently renamed mushroom bacilliform virus (MBV). The viral genome was identified as positive-sense single-stranded (ss)RNA, and the virus was classified as the type member of the genus *Barnavirus* in the family *Barnaviridae*. The family name derives its roots from Bacilliform RNA virus. MBV remains the only barnavirus identified to date.

Virion Properties

The MBV virion M_r is 7.1×10^6, with a buoyant density in Cs_2SO_4 of 1.32 g cm^{-3}. Virions are stable between pH 6 and 8 and ionic strength of 0.01 to 0.1 M phosphate.

Virion Structure and Composition

MBV has a bacilliform or bullet-shaped morphology, with particles generally 19 nm × 50 nm in size (**Figure 1**). Virions contain a single major capsid protein (CP) of 21.9 kDa and there are approximately 240 molecules in each capsid.

Virions encapsidate a single linear molecule of a positive-sense ssRNA, 4.0 kb in size. The complete 4009 nt sequence is available (GenBank accession No. U07551). The RNA has a 5′-linked VPg and lacks a poly(A) tail. RNA constitutes about 20% of virion weight.

Genome Organization and Expression

The RNA genome (4009 nt) contains four major and three minor open reading frames (ORFs) and has 5′- and 3′-untranslated regions (UTRs) of 60 and 250 nt, respectively (**Figure 2**). ORFs 1–4 encode polypeptides of 20, 73, 47, and 22 kDa, respectively. The deduced amino acid sequence of ORF2 contains three conserved chymotrypsin-related serine protease sequence motifs. Blast searches of the deduced ORF2 amino acid sequence show similarity to serine proteases encoded by plant sobemoviruses. ORF2 also encodes the VPg. ORF3 contains the **$GX_3TX_3NX_nGDD$** amino acid sequence shared by the putative RNA-dependent RNA polymerases (RdRps) of positive-sense ssRNA viruses and has similarity to the RdRps of sobemoviruses, enamoviruses, and poleroviruses. ORF4 encodes the CP. ORFs 5–7 encode 8, 6.5, and 6 kDa polypeptides, respectively. The polypeptides potentially encoded by ORFs 1, 5, 6, and 7 show no significant similarity to known polypeptides. The negative strand of MBV contains seven small ORFs of unknown significance. These potentially encode polypeptides ranging from M_r 6·5K to M_r 10·5K.

The genome arrangement and transcription/translation strategies of MBV are strikingly similar to those of a number of plant viruses, particularly poleroviruses and sobemoviruses. MBV probably also uses similar strategies to express its gene products, including leaky ribosomal scanning for expression of ORF2, ribosomal frameshifting for expression of the RdRp, and subgenomic RNA for expression of the CP. Of these, only subgenomic RNA has been confirmed *in vivo*. In a cell-free system, genomic-length RNA directs the synthesis of major 21 and 77 kDa polypeptides and several minor polypeptides of 18–60 kDa. The full-length genomic RNA and a sgRNA (0.9 kb) encoding ORF4 (CP) are found in infected cells. Virions accumulate singly or as aggregates in the cytoplasm. However the MBV life cycle has yet to be determined.

Evolutionary Relationships

The MBV genome sequence has no similarity with any other mycovirus genome characterized to date. However the deduced ORF2 and ORF3 amino acid sequences share striking similarity with those of plant viruses, particularly sobemoviruses, poleroviruses, and enamoviruses (**Figure 3**). This, together with the similarity of the MBV and sobemovirus/polerovirus genome arrangements, suggests that MBV may have shared a common ancestor with these plant virus groups.

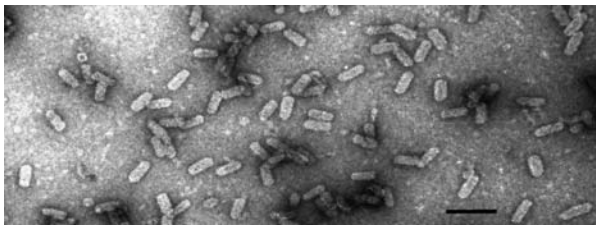


Figure 1 Electron micrograph *Mushroom bacilliform virus* particles. Scale = 100 nm. Reprinted from *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, Wright PJ and Revill PA, The *Barnaviridae*, copyright 2004, with permission from Elsevier.

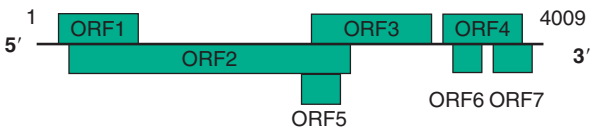


Figure 2 The MBV genome arrangement.

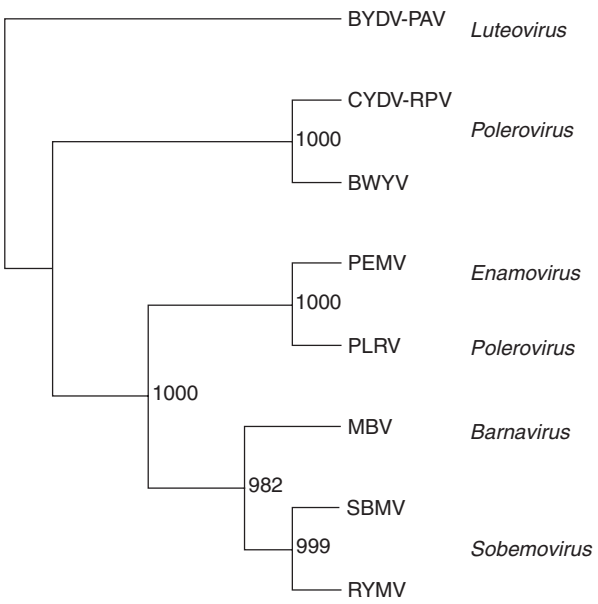


Figure 3 Neighbor-joining tree of the MBV RdRp compared to RdRps of a number of plant viruses. Sequences were aligned using Clustal X (1000 bootstrap replicates) and the tree was constructed with Treeview. The tree was rooted using the outgroup (BYDV-PAV, barley yellow dwarf virus). CYDV-RPV, cereal yellow dwarf virus-RPV; SBMV, southern bean mosaic virus; RYMV, rice yellow mottle virus; PEMV, pea enation mosaic virus; BWYV, beet western yellows virus; PLRV, potato leafroll virus. The GenBank accession numbers of the sequences used in the analysis were PLRV (D00530), CYDV-RPV (NC004751), BWYV (NC004756, PEMV (NC003629), RYMV (NC001575), SBMV (DQ875594), MBV (U07557), BYDV-PAV (EF043235).

Transmission and Host Range

MBV is transmitted horizontally through infected mycelium and it is yet to be determined if the virus can be transmitted in spores. There is no known insect vector. Although morphologically similar viruses to MBV have been identified in the field agaric, *A. campestris*, it is unknown whether these particles are related to MBV. Consequently MBV remains the only barnavirus identified to date.

See also: Fungal Viruses; Luteoviruses; *Sobemovirus*.

Further Reading

Goodin MM, Schlagnhaufer B, and Romaine CP (1992) Encapsidation of the La France disease specific double-stranded RNAs in 36 nm isometric virus-like particles. *Phytopathology* 82: 285–290.

Moyer JW and Smith SH (1976) Partial purification and antiserum production to the 19 × 50 nm mushroom virus particle. *Phytopathology* 66: 1260–1261.

Moyer JW and Smith SH (1977) Purification and serological detection of mushroom virus-like particles. *Phytopathology* 67: 1207–1210.

Revill PA, Davidson AD, and Wright PJ (1994) The nucleotide sequence and genome organization of mushroom bacilliform virus: A single-stranded RNA virus of *Agaricus bisporus* (Lange) Imbach. *Virology* 202: 904–911.

Revill PA, Davidson AD, and Wright PJ (1998) Mushroom bacilliform virus RNA: The initiation of translation at the 5′-end of the genome and identification of the VPg. *Virology* 249: 231–237.

Revill PA, Davidson AD, and Wright PJ (1999) Identification of a subgenomic mRNA encoding the capsid protein of mushroom bacilliform virus, a single-stranded RNA mycovirus. *Virology* 260: 273–276.

Romaine CP and Schlagnhaufer B (1991) Hybridization analysis of the single-stranded RNA bacilliform virus associated with La France disease of *Agaricus bisporus*. *Phytopathology* 81: 1336–1340.

Tavantzis SM, Romaine CP, and Smith SH (1980) Purification and partial characterization of a bacilliform virus from *Agaricus bisporus*: A single-stranded RNA mycovirus. *Virology* 105: 94–102.

Tavantzis SM, Romaine CP, and Smith SH (1983) Mechanism of genome expression in a single-stranded RNA virus from the cultivated mushroom *Agaricus bisporus*. *Phytopathology* 106: 45–50.

Wright PJ and Revill PA (2004) The *Barnaviridae*. In: Fauquet CM (ed.) *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, London: Elsevier Academic Press.

Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus

R Jordan and J Hammond, USDA-ARS, Beltsville, MD, USA

Published by Elsevier Ltd.

Glossary

Pathogroup A series of isolates of the same strain or pathotype.

Pathotype An isolate or strain of a virus that is biologically distinct from other isolates of the same virus by virtue of differential host reactions.

Serogroup A series of isolates or strains of the same serotype.

Serotype An isolate or group of isolates that are distinguished from biologically related isolates by reaction (or lack of reaction) with key serological reagents such as defined polyclonal antisera or monoclonal antibodies.

History

Bean common mosaic virus (BCMV) was first reported from the US in 1917, and the associated disease initially known as bean mosaic; it was renamed bean common mosaic in 1934 to differentiate it from bean yellow mosaic, caused by bean yellow mosaic virus (BYMV). Several pathotypes or strains of BCMV were distinguished in the 1970s by differential reactions of a number of bean cultivars, and in the early 1980s strains were further divided by serology into serotype A and serotype B. Serogroup A isolates were also biologically differentiated by temperature-insensitive induction of necrosis in bean cultivars carrying the dominant I gene. Peptide profiling of the coat protein (CP) and sequence analysis of the CP gene and 3′-untranslated region (UTR) demonstrated that the serotypes represented distinct viruses. Serotype A became known as bean common mosaic necrosis virus (BCMNV), while serotype B retains the name BCMV. 'Bean necrosis mosaic virus' was used for serogroup A isolates prior to acceptance of the species name *Bean common mosaic necrosis virus*. BCMV and BCMNV also differ in particle length, CP size, and biological properties (see below). Complete genome sequences support the distinction between BCMV and BCMNV proposed using CP sequences and peptide profiles.

Taxonomy and Classification

The current criteria for differentiation of potyvirus species are CP amino acid identity of less than *c.* 80%, and nucleotide identity of less than 85% over the complete genome; different species also often have distinct polyprotein cleavage sites. Using these criteria, *Bean common mosaic virus* and *Bean common mosaic necrosis virus* are distinct potyviral species; BCMV also now includes strains previously described by other names, including azuki bean mosaic virus, blackeye cowpea mosaic virus, dendrobium mosaic virus, guar green sterile virus, peanut chlorotic ring mottle virus, peanut mild mottle virus, and peanut stripe virus. At least 19 different strains of BCMV have been biologically authenticated as distinct on the basis of host range and host response, whereas only four biologically distinct strains of BCMNV have been recognized.

BCMV and BCMNV, together with CerMV, CABMV, DsMV, FrVY, IFBV, PWV, SaVY, SMV, TrVY, WMV, WVMV, ZaMMV, and ZYMV, form the BCMV subgroup of the genus *Potyvirus* within the family *Potyviridae* (for full virus names, see **Table 1**). Although 'subgroup' has no formal taxonomic meaning, it is a useful concept to describe a subset of virus species that cluster together in a phylogenetic tree (see **Figure 1**) and also has common serological properties. Some confusion exists in the literature over isolates described as CABMV; true CABMV isolates are distinct from BCMV, but some isolates initially described as CABMV were later shown to be synonymous with BlCMV, which is now recognized as a strain of BCMV.

Table 1 The viruses included in the bean common mosaic virus subgroup

Virus species recognized as the bean common mosaic virus subgroup	*Acronym*
Bean common mosaic virus[a]	**BCMV**
Azuki bean mosaic virus[b]	(AzMV)
Bean common mosaic virus serotype B	BCMV
Blackeye cowpea mosaic virus	(BlCMV)
Dendrobium mosaic virus	(DeMV)
Guar green sterile virus	(GGSV)
Peanut chlorotic ring mottle virus	
Peanut mild mottle virus	(PMMV)
Peanut stripe virus	(PStV)
Bean common mosaic necrosis virus	**BCMNV**
Bean common mosaic necrosis virus	BCMNV
Bean common mosaic virus serotype A (Bean necrosis mosaic virus)[c]	(BNMV)
Ceratobium mosaic virus	**CerMV**
Cowpea aphid-borne mosaic virus	**CABMV**
Cowpea aphid-borne mosaic virus	CABMV
Sesame mosaic virus	
South African passiflora virus	(SAPV)
Dasheen mosaic virus	**DsMV**
(Vanilla mosaic virus)[d]	(VanMV)
Fritillary virus Y[e]	***FrVY***
Impatiens flower break virus	***IFBV***
Passionfruit woodiness virus	**PWV**
Sarcochilus virus Y	**SaVY**
Soybean mosaic virus	**SMV**
Tricyrtis virus Y	***TrVY***
Watermelon mosaic virus	**WMV**
Watermelon mosaic virus	WMV
Watermelon mosaic virus 2	(WMV-2)
Vanilla necrosis virus	(VNV)
Wisteria vein mosaic virus	**WVMV**
Zantedeschia mild mosaic virus	***ZaMMV***
Zucchini yellow mosaic virus	**ZYMV**

[a]Names in bold italic font are species currently recognized by the ICTV.
[b]Names in regular font are no longer recognized as potyvirus species, but are indented to indicate their position as strains of the species listed above. Acronyms in parentheses are no longer valid as species, but refer to strains of the virus in bold font.
[c]'Bean necrosis mosaic virus' was a name used for strains of the A serotype prior to ICTV designation of these isolates as strains of BCMNV.
[d]Vanilla mosaic virus is recognized in the ICTV Eighth Report as a tentative species in the genus *Potyvirus*; recent reports suggest that it is an isolate of DsMV.
[e]Viruses with names in bold font appear to meet the criteria for distinct virus species, but have not yet been recognized by the ICTV; their acronyms are indicated in bold italics.

Geographic Distribution

BCMV probably originated in Latin America, the center of diversity of *Phaseolus vulgaris*, and is distributed

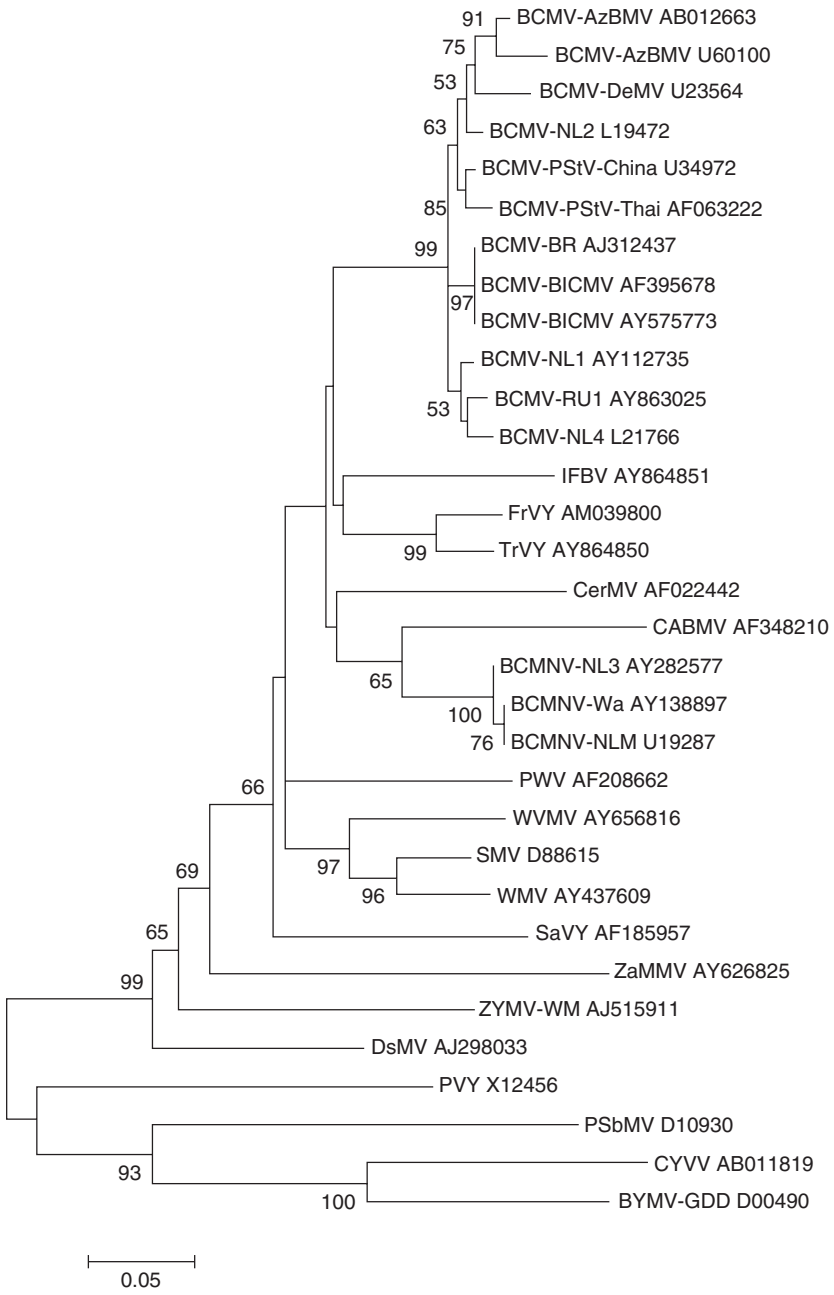


Figure 1 Phylogenetic relationships of the bean common mosaic virus subgroup. Phylogenetic relationship based on analysis of coat protein amino acid sequences of bean common mosaic virus and bean common mosaic necrosis virus with members of the BCMV subgroup, other legume-infecting potyviruses, and the genus *Potyvirus* type member potato virus Y (PVY). The tree was constructed by the neighbor-joining algorithm based on calculations from Clustal W pairwise amino acid sequence distances. The horizontal branch lengths are proportional to the genetic distance. The data set was subjected to 1000 bootstrap replicates. All nodes supported by >50% confidence values are shown. GenBank sequence sources are listed. Virus acronyms are as listed in **Table 1**. BYMV, bean yellow mosaic virus; CYVV, clover yellow vein virus; PSbMV, pea seed-borne mosaic virus.

worldwide wherever beans are grown, and in association with other bean types, as well as soybean, pea, and cowpea. Blackeye cowpea mosaic isolates are also found worldwide. Peanut stripe isolates are found in peanuts in Asia and the United States, and also infect lupin, soybean, and sesame. In many areas of the world BCMV is the most important potyvirus affecting beans, although in some areas BCMNV or BYMV may predominate. Before major efforts were made to eradicate BCMV from US germplasm collections, more

than 60% of germplasm accessions were found to be infected.

BCMNV is presumed to originate in Africa and is more important than BCMV in much of eastern, central, and southern Africa, where BCMNV is common in a wide variety of wild and forage legumes as well as beans grown for human consumption. BCMNV has also been distributed worldwide, largely through germplasm introductions, and has increased in importance in the United States partly as a consequence of deployment of resistance genes against BCMV (see below).

The increase in diversity and severity of bean-infecting geminiviruses in many regions has displaced BCMV and BCMNV as the most important viruses infecting beans in some areas.

Host Range and Transmission

BCMV naturally infects *P. vulgaris* (kidney bean), *P. acutifolius* (tepary bean), *P. atropurpureus, P. coccineus* (runner bean), *P. mungo* (black gram), *Glycine max* (soybean), *Macroptilium lathyroides* (horse gram), *Pisum sativum* (pea), *Rhynchosia minima*, *Vicia faba* (broad bean), *Vigna radiata* (mung bean, green gram), *V. angularis* (azuki bean), and *V. unguiculata* (cowpea). Peanut stripe isolates naturally infect *Arachis hypogea* (peanut), *Dolichos lablab* (hyacinth bean), *Indigofera amoena, G. max*, *Lupinus albus* (lupin), *Pueraria phaseoloides, Sesamum* spp. (sesame), *Stylosanthes capitata,* and *S. craba.* Soybean isolates are adapted to soybean, and rarely infect peanuts. Dendrobium mosaic isolates infect *Dendrobium* orchids, while guar green sterile isolates infect guar (*Cyamopsis tetragonoloba*). In addition to its natural hosts, BCMV has a wide experimental host range, infecting about 100 species from 44 genera over nine families including Amaranthaceae, Chenopodiaceae, Fabaceae, Solanaceae, and Tetragoniaceae, although individual isolates may be much more restricted.

BCNMV naturally infects *P. vulgaris*, *P. lunatus*, *Centrosema pubescens*, *Crotalaria incana*, *Lablab purpureus, Senna bicapsularis, S. hirsuta, S. sophora,* and *V. vexillata*, and has been detected using a BCMNV-specific monoclonal antibody in naturally infected plants of *Albizia coriaria, Desmodium intortum, D. uncinatum, Rhynchosia resinosa, Tephrosia barbigera,* and *T. paniculata.* Experimental hosts include several other leguminous species, such as *Canavalia ensiformis, Crotalaria spinosa, Macroptilium lathyroides, Rhynchosia minima*, and *V. radiata.*

Both BCMV and BCMNV are transmitted by aphids in a nonpersistent manner typical of viruses in the genus *Potyvirus*; the most important vector species in many countries are *Acyrthosiphon pisum, Aphis fabae*, and *Myzus persicae. Aphis craccivora* may be the most important vector in India. Minimum acquisition access and inoculation access periods are less than 1 min; no latent period is required between acquisition and inoculation. Aphid transmission is the cause of secondary transmission within the crop, but the primary means of introduction to the crop is through infected seed. Aphid transmission and infection rates tend to be higher when beans are grown under irrigation in dry regions.

Both BCMV and BCMNV are also seed-transmitted. Seed transmission varies with isolates and host species, as well as the timing of infection, but rates of up to 93% of infected seed may result from diseased plants, with erratic distribution of infected seed within individual pods. Plants infected after flowering typically do not yield infected seed, probably because the virus is not found in the embryo and cotyledons, and the virus may not be able to spread into these locations if flowering occurs prior to infection of the mother plant. Infection of the seed, and perhaps of the mother plant, can occur through infected pollen. Infectivity of the virus is retained during prolonged storage of seed.

Dendrobium mosaic (a strain of BCMV) is transmitted by aphids and by vegetative propagation of *Dendrobium* orchids.

Properties of the Virion and Genome

Properties of Particles

BCMV and BCMNV are typical of viruses in the genus *Potyvirus* in that particle preparations contain a single sedimenting (154–158S) and buoyant density component (1.31–1.32 g cm^{-3} in CsCl). Virions are nonenveloped flexuous filaments, 12–15 nm wide, and 847–886 nm (BCMV) or 810–818 nm (BCMNV) long. Virions are composed of one single-stranded RNA of *c.* 9600 nt for BCMNV and *c.* 10 000 nt for BCMV (accounting for 5% of the particle weight) encapsulated in about 1700–2000 monomer units of one CP polypeptide species with a molecular mass of *c.* 30 kDa for BCMNV, and *c.* 33 kDa for BCMV (comprising 95% of the particle weight). In gel electrophoresis the CP of BCMNV migrates with apparent M_r 33 kDa, and that of BCMV of M_r 34.5–35 kDa. Virus preparations that have undergone limited proteolysis also contain lower-molecular-weight peptides of apparent M_r 29–34 kDa.

Serological Relationships

BCMV and BCMNV particles are strongly immunogenic and can be easily distinguished using polyclonal antisera and monoclonal antibodies (McAbs). Polyclonal antisera show only a very distant serological relationship between strains of these two viruses. BCMV- and BCMNV-specific antisera are available commercially for use in standard serological tests such as enzyme-linked

immunosorbent assay (ELISA). Early work with BCMV and homologous antisera showed that BCMV was closely related serologically to AzMV, BlCMV, PStV, and several soybean infecting-isolates from Taiwan. As noted above, these viruses are now recognized as strains of BCMV. BCMV is distantly related to BYMV, ClYVV, CABMV, PVY, PMV, PWV, SMV, TEV, and WVMV.

Several broad-spectrum McAbs have been identified that detect all of the strains of BCMV, including AzMV, BlCMV, DeMV, and PStV, as well as the BCMV subgroup member CABMV, but not with any strains of BCMNV. Other McAbs are reported which display various degrees of specificity for these viruses (e.g., can only detect CABMV or BCMV-PStV). While these McAbs can be used to discriminate among the BCMV isolates, biological diversity among these viruses may limit the use of many of these antibodies for diagnostic purposes. McAbs produced to BCMNV are specific only for strains of BCMNV (i.e., have no cross-reactivity with any strains of BCMV, nor other potyviruses).

Another series of McAbs were raised against three strains of BCMV (AzMV, BlCMV, and PStV). Results of ELISA, immunosorbent electron microscopy, and western-blot analyses indicated that these McAbs are specific for a series of epitopes located entirely on the virion surface. From western-blot analyses of untreated and trypsin- as well as endopeptidase Lys-C-treated CPs, these virus-specific epitopes appear to be located exclusively on the amino-terminus of the CP. These McAbs were found to discriminate between strains of BCMV. These and other N-terminal targeted antibodies often enable clear distinction of strains in mixed infections, but a BCMV-PStV-specific McAb that reacts well with most blotch isolates failed to detect a necrotic isolate from Taiwan and several blotch isolates from Georgia, while a BCMNV-specific McAb misdiagnosed a naturally occurring genomic recombinant between BCMV and BCMNV.

Properties of the Genome and Replication

The single-stranded RNA genome, which has a VPg protein covalently linked to its 5′ end, is about 10 000 nt for BCMV and 9600 nt for BCMNV. The organization of the BCMV and BCMNV genomes is similar to other potyviruses, consisting of short untranslatable sequences at the 5′ and 3′ ends, and a single, long open reading frame (ORF). The ORF is translated into a single polyprotein: *c.* 3222 aa for BCMV and *c.* 3071 aa for BCMNV. The polyprotein undergoes co- and post-translational proteolytic processing by three viral-encoded proteinases to form ten individual gene products. Most of the polyprotein cleavage sites differ between BCMV and BCMNV, with the exception of the HC-Pro/P3 cleavage. The ten viral proteins (see **Figure 2**) include, in order from the 5′ end of the genome, P1 proteinase; helper component-proteinase (HC-Pro); P3; a 6 kDa protein; cylindrical inclusion (CI); a second 6 kDa protein; the nuclear inclusion 'a' (NIa)-VPg protein; NIa-proteinase; nuclear inclusion 'b' (NIb); and the CP. Genomic RNA replicates via production of a full-length negative-sense RNA.

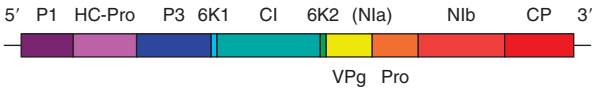


Figure 2 Genome organization of BCMV and BCMNV. 5′, 5′-UTR; P1, P1 proteinase; HC-Pro, helper component-proteinase; P3, P3 protein; 6K1, a 6 kDa protein; CI, cylindrical inclusion; 6K2, a second 6 kDa protein; NIa, nuclear inclusion 'a', cleaved into VPg and Pro; VPg, genome-linked protein; Pro, proteinase; NIb, nuclear inclusion 'b'; CP, coat protein; 3′,3′-UTR.

Pathogenicity, Pathology, and Resistance Genes

Isolates of BCMV and BCMNV can be differentiated into at least ten pathotypes (out of 16 theoretical pathotypes) based on their reactions on a series of differential bean cultivars. BCMV normally produces only mosaic symptoms in susceptible genotypes; BCMNV isolates induce lethal systemic necrosis and plant death in bean cultivars carrying the incompletely dominant '*I*' gene. In most such cultivars the systemic necrosis develops at or above 20 °C; in hypersensitive cultivars the response is restricted to necrotic local lesions at normal temperatures, and systemic or 'black root' symptoms only at temperatures above 30 °C. Hypersensitivity results in field resistance, and is genetically dominant; no seed transmission of BCMV occurs in genotypes with the dominant '*I*' gene. Other resistance genes (*bc-1, bc-1*^{2}, *bc-2, bc-2*^{2}, *bc-3,* and *bc-u*) condition resistance to particular pathotypes in various combinations; the combination of the dominant *I* gene with the strain-specific recessive genes, *bc-1*^{2}, *bc-2*^{2}, or *bc-u* protects against systemic infection and seed transmission of BCMNV. In the absence of the recessive strain-specific resistance genes effective against necrosis-inducing isolates of BCMNV, the *I* gene alone is not sufficient to protect against systemic infection by BCMNV in many regions of Africa. The viruses carry various combinations of six pathogenicity determinants (*P*0, *P*1, *P*1^2, *P*2, *P*2^2, and *P*x), which to date have not been correlated with particular viral genes. Pathotype is not correlated with either CP or 3′-UTR phylogeny, nor is there evidence for determinants controlling systemic movement in the 3′-terminal region. Evidence from natural recombinants suggests that the P1 gene carries pathogenicity determinants.

The incompletely dominant *I* gene was first identified in the cultivar 'Corbett Refugee', and the first recessive

genes in 'Robust' and 'Great Northern 1' in the 1930s; strain-specificity of the recessive genes was identified in the 1950s, and the strain-unspecific gene *bc-u* shown in the 1970s to be necessary for expression of the strain-specific genes. No isolates have yet been identified that break resistance conferred by *bc-3*; however, the gene originated in a single European breeding line, and it took many years to introgress *bc-3* into genotypes adapted to the tropics. Cultivars carrying the dominant *I* gene may have increased susceptibility to cowpea severe mosaic comovirus.

There is genetic linkage between certain seed colors and BCMV susceptibility, including red-mottled seed coat; the dominant *I* gene is closely linked to darker purple-mottled seed color. The lighter seed color associated with susceptibility is preferred in many cultures and commercial uses of the beans; this linkage can be partially avoided by selecting resistant types with commercially acceptable intermediate seed colors. Introgression of *bc-3* can be problematic, as lines carrying the gene can be symptomless; serological or infectivity assays may be necessary to differentiate resistant and susceptible lines. Introgression of *I* and *bc-3* has become simpler with development of molecular markers associated with each gene, allowing marker-assisted selection without virus challenge and phenotypic evaluation. The *I* gene is closely linked to a multigene family of TIR-NBS-LRR resistance genes conferring resistance to several related potyviruses and a comovirus.

In Latin America most black-seeded bean cultivars carry the *I* gene, and many additional bean lines bred at Centro Internacional de Agricultura Tropical and associated national programs also carry resistance. Many land races grown by traditional farmers, and particularly beans with light-colored seed coats, are susceptible; infection rates of up to 100%, and yield losses of 35–98% are reported. In North America many bean varieties have effective resistance to BCMV, although many pinto, navy, and red-seeded types have only strain-specific resistance. However, recent breeding has yielded pinto and navy bean cultivars carrying both *I* and *bc-3*. BCMNV was previously rare in the US, but combined resistance to BCMV and BCMNV is now available in some cultivars of most bean types.

Many of the beans grown by small farmers in eastern and southern Africa are susceptible to both BCMV and BCMNV. The presence of necrosis-inducing strains of BCMNV, also found in wild legumes and species grown for fodder, means that the *I* gene cannot be deployed independent of recessive resistance genes, as infection by necrosis-inducing isolates could result in death of a significant proportion of plants in a field. In India infections of up to 100% are reported in bean, urdbean, mungbean, and cowpea, resulting in mottle, mosaic, blistering, stunting, and poor pod set. Many Asian bean types also lack resistance, due to linkage between susceptibility and desirable seed colors.

Peanut stripe strains of BCMV causing symptoms described as blotch, blotch-CP-N, blotch stripe, chlorotic line pattern, chlorotic ring mottle, mild mottle, necrosis, and stripe have also been differentiated on the basis of disease reactions on specific host genotypes. Losses of 23–38% to peanut mild mottle have been documented in China. Peptide profiles of five symptom variants from Thailand that were not serologically distinct confirmed close relationships. No resistance was identified among over 8000 cultivated peanut genotypes in the germplasm collection at the International Crops Research Institute for the Semi-Arid Tropics. Some wild *Arachis* species have been identified as immune or highly resistant, including accession PI 475998 of *A. cardenasii*, which could not be infected.

Resistance genes have been identified and incorporated into resistant cultivars of *Pisum sativum, Lupinus angustifolius, Lablab purpureus,* and *Macroptilium lathyroides*.

Transgenic resistance has been demonstrated in peanut transformed with the CP gene of a peanut stripe isolate; a high level of resistance was observed in lines expressing either untranslatable or N-terminally truncated forms of the CP gene, suggesting that resistance resulted from RNA silencing. While beans have been transformed for resistance to bean golden mosaic geminivirus, and soybean for resistance to soybean mosaic potyvirus, there are no reports of transgenic BCMV- or BCMNV-resistant bean lines.

Recombination and Variability

Although recombination between different isolates of BCMV had been reported for some years, no recombinants between BCMV and BCMNV were reported until recently. No recombinants were recovered from deliberate mixed infections of BCMV strain US-5 (pathogroup IV) and BCMNV strain NL-8 (pathogroup III) following many serial passages in either of two susceptible hosts. However, multiple recombinants were recovered within 28 days when the same strains were inoculated on opposite primary leaves of beans that were susceptible to one virus and resistant to the other; these recombinants fell into five different classes based on combinations of serotype (A, BCMNV; or B, BCMV) and pathogroup (IV, V, or VI). Subsequently, a naturally occurring strain of BCMNV (NL-3 K) with atypical responses on differential hosts was sequenced and shown to have a 5′-UTR and 5′ region of the P1 gene almost identical to BCMV strain RU1, while the rest of the genome was almost identical to BCMNV strain NL-3 D. The P1 gene of the recombinant was larger than that of BCMNV, and similar to the BCMV P1. These results suggest that the P1 gene may play a significant role in pathogenicity and virulence, as previously suggested for BYMV.

The observed phenotypes of recombinants indicate that serotype, pathogenicity, and symptom expression are independent traits with separate determinants in the genome. The occurrence of recombination in bean genotypes with only recessive, strain-specific resistance genes suggests that this may be the primary cause of emergence of new resistance-breaking strains.

Much of the variability between isolates of either BCMV or BCMNV appears to be within the 5′ portion of the genome, as has been shown both within and between other potyviruses. The P1 and P3 genes, and the N-terminal region of the CP gene are the most variable regions of the potyvirus genome, and may be involved in host–virus interactions. Differences in the 5′-UTR and N-terminus of P1 of both BCMV and BCMNV have been associated with symptom differences in common bean (*P. vulgaris*), and for BCMV in asparagus bean (*V. sesquipedalis*). Variability in the P1 gene is extensive, with as little as 61% identity at the amino acid level between BCMV strains, with most of the differences in the N-terminal domain. Differences in the CP gene result in clustering of BCMV isolates according to host species (bean, cowpea, peanut, asparagus bean, guar, *Dendrobium*, etc.) and to some extent by geographic origin within host clusters; peanut stripe isolates from China, Indonesia, and Thailand form separate clusters.

Diagnosis

Host Range and Symptomatology

Potyviruses can be detected and identified by a variety of techniques that are based on biological, cytological, biochemical, antigenic, and structural properties. With respect to BCMV and BCMNV, host range and symptomatology are not very useful because there are many viruses that infect bean, and significant differences in symptoms may be reproduced depending on the virus pathotype and host genotype. Morphology of cytoplasmic inclusions has been useful in the identification of many potyviruses since inclusions can be virus-specific. However, inclusion morphology is not reliable for detection and differentiation of BCMV and BCMNV. BCMNV induces pinwheel and scroll types of cytoplasmic inclusions (subdivision I) in infected tissue. Most strains of BCMV, including BCMV-AzMV and BCMV-BlCMV, also induce subdivision I type inclusions. However, PStV and Taiwan soybean isolates induce subdivision IV cylindrical inclusions.

Serological Techniques

BCMV- and BCMNV-specific polyclonal antisera and McAbs are useful for the detection and differentiation of these seed-borne viruses, and several methods have been used for detection in bean seed. When indirect ELISA and a dot immunoassay (DIA) with both McAbs and polyclonal antisera were evaluated, ELISA with McAbs proved the most sensitive method. However, BCMV antigen was found to be erratically distributed within the seed, with more than 50% of infected samples having detectable levels of BCMV antigen only in the seed coat. Tests on individual seeds proved unreliable and it is recommended that flour from a bulked sample of eight seeds be used when testing seed lots. Other assays include immunosorbent electron microscopy to detect BCMV in bean seed; or double antibody sandwich (DAS)-ELISA and bioassay on *Chenopodium quinoa* to test hydrated mature cowpea seed for presence of BCMV-BlCMV. In these tests infectious virus was detected in cotyledons and sometimes in the embryo axis of infected seed. Viral antigen was found in or on the testae, but very little infectious virus was present. Others have used direct antigen-coated-ELISA to detect BCMV-BlCMV in cowpea seed.

Reverse Transcription-Polymerase Chain Reaction

The sensitivity, capacity, and potential of the PCR technique has been applied to BCMV and BCMNV. Several sets of BCMV- and BCMNV-specific RT-PCR primers have been designed and utilized to sensitively and accurately detect, differentiate, and characterize the two viral species. In one study using virus-specific CP primers, the specific geographical distribution of these viruses in Mexico was determined. In addition, the alignment of nine nucleotide sequences from cloned amplicons from the RT-PCR reactions for each viral species confirmed the identities of the viruses and was helpful in assigning them tentatively to pathogroups. Other primer sets have been developed and utilized, for example, in the successful detection and differentiation of the necrotic and blotch isolates of BCMV-PStV, and in the differentiation of naturally occurring genomic BCMV-BCMNV recombinants.

Considering the seed-borne nature of these viruses, RT-PCR has potential in quarantine programs for screening imported seed material and germplasm.

Prevention and Control

It is possible to eradicate BCMV and BCMNV from a particular production region, or at least to significantly reduce primary inoculum, by eliminating susceptible genotypes and planting only resistant varieties. There are few important noncrop reservoirs of inoculum, and infected seed are responsible for primary infections in the crop.

Rogueing of symptomatic plants is not recommended as a means of control, as it is highly likely that systemically infected plants without significant symptoms will

remain to act as significant reservoirs for secondary infection by aphid transmission.

Exclusion of the virus is highly effective in areas where the virus is not present, but this may preclude introduction of new germplasm or cultivars without effective resistance. One or both viruses are almost universally present in seed lots of varieties that do not carry the *I* gene, and the majority of Latin American land race types do not possess any of the strain-specific recessive resistance genes. Most cultivars derived through extensive breeding programs carry the dominant *I* gene for resistance to BCMV and BCMNV. Increasingly *bc-3* is being combined with *I* to yield cultivars with effective resistance to both BCMV and BCMNV.

Certified seed can be an effective means of control. A certified seed program in California with a limit of <0.5% BCMV has aided in the control of the disease, as resistant varieties are not yet available for all bean classes currently grown in California. Monitoring of BCMV for certification also aided in identification and containment of an outbreak of BCMNV introduced in seed of a navy bean cultivar.

Vector exclusion may aid in control, but as aphids rarely colonize common beans, migratory aphids are responsible for most transmission in many areas. As BCMV and BCMNV can be acquired and transmitted in less than 1 min access to plants, insecticide treatment is unlikely to prevent, and may even encourage vector movement and transmission. Vector control through insecticide application may be effective in parts of Africa and Asia where aphids do colonize beans. Oil sprays are known to reduce transmission of stylet-borne viruses such as BCMV and BCMNV, but their lack of systemic action, and thus need for frequent reapplication to a growing crop, reduces the cost-effectiveness of their use.

See also: Potyviruses.

Further Reading

Adams MJ, Antoniw JF, and Fauquet CM (2005) Molecular criteria for genus and species discrimination within the family *Potyviridae*. *Archives of Virology* 150: 459–479.

Berger PH, Wyatt SD, Shiel PJ, *et al.* (1997) Phylogenetic analysis of the *Potyviridae* with emphasis on legume-infecting potyviruses. *Archives of Virology* 142: 1979–1999.

Dijkstra J and Khan JA (1992) A proposal for a bean common mosaic subgroup of potyviruses. *Archives of Virology* (supplement 5): 389–395.

Drijfhout E (1978) Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. *Agricultural Research Reports No. 872*. Wageningen: Center for Agricultural Publishing and Documentation.

Higgins CM, Hall RM, Mitter N, Cruickshank A, and Dietzgen RG (2004) Peanut stripe potyvirus resistance in peanut (*Arachis hypogea* L.) plants carrying viral coat protein gene sequences. *Transgenic Research* 13: 59–67.

Larsen RC, Miklas PN, Druffel KL, and Wyatt SD (2005) NL-3 K strain is a stable and naturally occurring interspecific recombinant derived from *Bean common mosaic necrosis virus* and *Bean common mosaic virus*. *Phytopathology* 95: 1037–1042.

McKern NM, Ward CW, and Shukla DD (1992) Strains of bean common mosaic virus consist of at least two distinct potyviruses. *Archives of Virology* (supplement 5): 407–414.

Milne RG (ed.) (1988) *The Plant Viruses, Vol. 4: The Filamentous Plant Viruses.* New York: Plenum.

Mink GI and Silbernagel MJ (1992) Serological and biological relationships among viruses in the bean common mosaic virus subgroup. *Archives of Virology* (supplement 5): 397–406.

Morales FJ (1998) Present status of controlling bean common mosaic virus. In: Hadidi A, Kheterpal RK, and Koganezawa H (eds.) *Plant Virus Disease Control*, pp. 524–533. St. Paul: APS Press.

Provvidenti R and Hampton RO (1992) Sources of resistance to viruses in the *Potyviridae*. *Archives of Virology* (supplement 5): 189–211.

Sengooba TN, Spence NJ, Walkey DGA, Allen DJ, and FemiLana A (1997) The occurrence of bean common mosaic necrosis virus in wild and forage legumes in Uganda. *Plant Pathology* 46: 95–103.

Shukla DD, Ward CW, and Brunt AA (1994) *The Potyviridae.* Wallingford: CAB International.

Vallejos CE, Astua-Monge G, Jones V, *et al.* (2006) Genetic and molecular characterization of the *I* locus of *Phaseolus vulgaris*. *Genetics* 172: 1229–1242.

Vetten HJ, Lesemann D-E, and Maiss E (1992) Serotype A and B strains of bean common mosaic virus are two distinct potyviruses. *Archives of Virology* (supplement 5): 415–431.

Bean Golden Mosaic Virus

F J Morales, International Center for Tropical Agriculture, Cali, Colombia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

3′ or 5′ end Chemical convention of naming carbon atoms numerically in the nucleotide sugar ring.
Bipartite Viruses made up of two particles containing different nucleic acids.
Complementary sense Nucleic acid that has its sequence in the opposite direction in which nucleic acids usually replicate.
DNA probes DNA molecule converted into single strands and then labeled (radioactively with 32P or otherwise) that will hybridize with complementary nucleic acid strands, detecting their presence due to the label attached.
ELISA Enzyme-linked immunosorbent assay, a serological test that uses an enzyme to increase the sensitivity of the assay by inducing a colorimetric reaction.

Gene silencing Switching off a gene.
GMO Genetically modified organism that has received a foreign gene from other organism.
Immunity Lack of interaction between a pathogen and an organism.
Infectious pseudo-recombinants Mixture of A and B molecules from related strains of a begomovirus or begomovirus species, which can induce disease when inoculated in a susceptible plant.
Inoculation Bringing a pathogen into contact with a potentially susceptible organism.
Isometric Round particle with similar dimensions in all directions.
MAS Use of molecular markers that detect specific genes present in plants to select desirable plant genotypes under laboratory conditions.
ORF Any sequence of DNA or RNA that can be translated into a protein.
PCR (polymerase chain reaction) The reproduction of a nucleic acid segment in a DNA molecule, using short nucleic acid strands ('primers') that serve as the starting point for DNA replication.
Polyadenylation Addition of adenosine residues to the end of an mRNA.
Polyclonal antiserum An animal serum that has different antibodies produced against different antigenic determinants.
Replication A nucleic acid makes a copy of itself.
Resistance Relative ability of an organism to attenuate the effects of a pathogen.
Sequence identity Same nucleotide or amino acid sequence in a nucleic acid molecule.
Sieve-tube cells Cells that belong to phloem tissue; do not have a nucleus; have a few vacuoles and some organelles, such as ribosomes.
Single-stranded Viruses consisting of only one strand of viral RNA or DNA.
Translational codons Tri-nucleotide units coding for a single amino acid.
Viroplasm Amorphous aggregation of viral components.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes in the world. This species was domesticated in its center of origin, Latin America, where different races exist according to their origin (Mexico, Central America, and the Andean region of South America). Common bean has been one of the major staples of most Latin American countries since pre-Columbian times, and it has also become a vital food crop in East Africa. The largest producer of common beans in the world is Brazil, with total areas planted with this legume ranging from 4 to 5 million hectares. The second producer of common bean in the world is Mexico, with a total area of approximately 1 900 000 ha. Common bean is also a major staple in Central America (*c.* 700 000 ha) and the rest of South America (*c.* 600 000 ha), excluding Brazil.

Bean golden mosaic was first observed in 1961 by the pioneer Brazilian plant virologist Dr. Alvaro Santos Costa in the state of São Paulo, Brazil. Common bean (*P. vulgaris* L.) plants affected by this disease generally develop striking yellowing symptoms that affect the entire foliage in a truly systemic manner, unlike other partial yellowing diseases incited by different viruses in this legume species. The affected leaves of most common bean varieties also show downward curling and various degrees of malformation. Golden mosaic-affected common bean plants are usually stunted, and some highly susceptible common bean genotypes may not grow at all when infected at the early stages of plant development. Flower abortion has also been observed in golden mosaic-affected plants, particularly in hot environments. Pods formed by systemically infected plants are usually noticeably underdeveloped and malformed, and produce few small and badly damaged seeds.

Although Costa reported in 1965 that bean golden mosaic was 'not currently of sufficient economic importance', he noted its epidemiological potential. A decade later, Costa informed that bean golden mosaic was present in the two main common bean-producing states of Brazil, São Paulo and Parana. The role of *Bemisia tabaci* as a vector of the 'Abutilon mosaic virus' suspected to cause the 'infectious chlorosis of the Malvaceae' had already been demonstrated in Brazil in 1946, and Costa rapidly associated the occurrence of bean golden mosaic with the presence of *B. tabaci* in affected common bean plantings. He attributed the rapid dissemination of this disease to the noticeable increase in the population of the whitefly *B. tabaci* Gennadius (Homoptera: Aleyrodidae) , as a result of the exponential growth in the area planted with soybean, a reproductive host of this whitefly species. Soybean plantings in Brazil had increased from 1.3 million hectares in the early 1970s, to almost 6 million hectares by 1975; and bean golden mosaic had disseminated to the remaining common bean-growing states: Minas Gerais, Goiás, and Bahia in the same period.

In Central America, a 'golden mosaic' of common bean had been observed to occur in the late 1960s from Guatemala to Panama. In 1966, a 'yellow mottle' severely affected common bean plantings in the Pacific coastal lowlands of Guatemala, El Salvador, and Nicaragua, mostly in regions where *B. tabaci* had previously been reported as a major pest of cotton in the 1950s. Based on the research conducted in Brazil on bean golden mosaic, and the similarities between the bright yellowing symptoms observed in

Central America and Brazil, it was assumed that the causal virus was the same. The golden mosaic disease of common bean became economically important in the mid-1970s, in southeastern Guatemala and the Pacific region of El Salvador. In Honduras, Nicaragua, and Costa Rica, the disease took longer to affect common bean production, and it was only in the 1980s that the disease became economically important in these countries.

In the Caribbean region, the name 'bean golden yellow mosaic' was used for the first time in 1973 to describe a disease of common bean in Puerto Rico. A 'golden mosaic' of common bean had also been reported in the Dominican Republic and Jamaica, in 1970 and 1975, respectively. Similar symptoms were observed in Cuba in the early 1970s, particularly in the province of Velasco, where farmers referred to this disease as 'amachamiento' (female sterility), because the pods of affected common bean plants did not produce seeds or the seeds were underdeveloped. The emergence of this disease was associated with an increase in the population of the whitefly *B. tabaci*. In Haiti, the first association between the presence of whiteflies and bean golden yellow mosaic in the lowlands was made in 1973. By 1978, the presence of the disease had been registered in all major bean-producing regions of the country. In Central America, other *Phaseolus* species, such as *P. acutifolius, P. coccineus*, and particularly *P. lunatus* (lima bean), are commonly found affected by this disease under field conditions.

In 1974, a 'yellow mosaic' was observed in common bean fields in the Valley of Culiacan, Sinaloa, north western Mexico. The disease was later observed in the Valley of Mochis, Sinaloa, and the Valley of Santo Domingo, Baja California Sur. The disease was referred to as 'bean golden mosaic', again, due to the similarities with the yellowing diseases of common bean in Brazil and Central America, and their association with the whitefly vector *B. tabaci*.

Etiology

Until 1975, Costa in Brazil reported that "the causal agent of the golden mosaic disease of beans is assumed to be a virus and is considered to belong to the Abutilon mosaic virus complex. It probably represents an evolved variant of this complex that became adapted epidemiologically to certain species of the legume family." Attempts to visualize the causal agent in infected plant extracts or purified preparations by electron microscopy yielded negative results. However, ultrathin sections of vascular tissue obtained from golden mosaic-affected bean plants in Brazil revealed the presence of isometric particles (20–25 nm) in sieve tube elements. The causal agent of bean golden mosaic in Brazil could not be transmitted by mechanical means or sexual seed, and it did not infect species of *Sida* that are known hosts of the Abutilon mosaic virus.

The first successful isolation of a whitefly transmitted virus that induced golden mosaic-like symptoms in common bean was achieved in 1975 by Galvez and Castaño at CIAT, Colombia, using a bean golden yellow mosaic virus (BGYMV) isolate from El Salvador, Central America. The isolated virus had quasi-isometric twin particles *c.* 32 nm × 19 nm (**Figure 1**). Using the same methodology, similar particles were isolated from common bean plants infected with a Puerto Rican isolate of the bean golden yellow mosaic pathogen. This isolate was used to characterize the viral nucleic acid in 1977, as a single-stranded DNA molecule. In 1978, the bean golden mosaic-like viruses and other similar single-stranded DNA viruses transmitted by *B. tabaci* or leafhoppers (Homoptera: Cicadellidae) were included in the newly created Geminivirus group. In 1981, the Puerto Rican bean golden yellow mosaic geminivirus isolate was shown to possess a divided ssDNA genome of approximate M^r $(7–8 \times 10^5)$ (~2510 nt) consisting of two different molecules separately encapsidated in each paired particle. In 1989, 'bean golden mosaic virus' (BGMV) was considered a 'subgroup II geminivirus' (whitefly transmitted viruses with a bipartite ssDNA genome); and in 1995, a member of 'subgroup III of the Geminivirus group', to further differentiate the whitefly borne geminiviruses from similar viruses having different homopteran vectors.

In 1991, a Brazilian isolate of BGMV was cloned in order to prepare broad-spectrum DNA probes that detected all of the selected whitefly borne geminivirus isolates tested from Brazil, Colombia (Bean dwarf mosaic virus), Central America (Guatemala), and the Caribbean (Dominican Republic). Specific DNA probes were also prepared in this study, which detected only individual

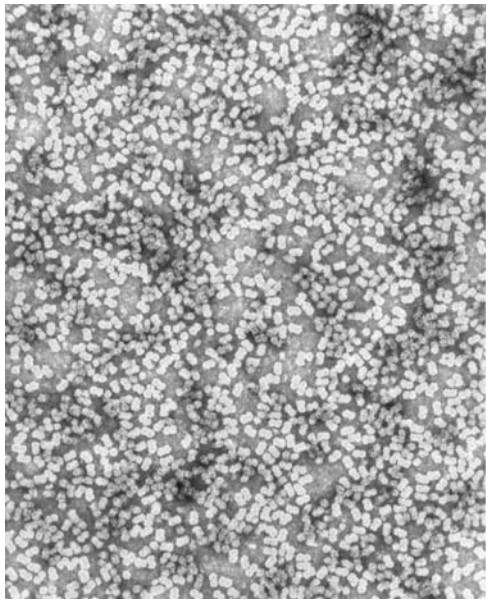


Figure 1 Characteristic geminate particles of BGYMV.

viruses, with the exception of the Central American and Caribbean viruses that were detected by a single DNA probe. These assays demonstrated the genetic variability of the 'BGMV' isolates tested. In the same year, the polymerase chain reaction (PCR) method was used to produce nucleotide sequence of a hypervariable region (intergenic region between BC1 and the common region of the B component). Nucleotide sequence identities between different isolates from the Dominican Republic (95% sequence identities among them); 'BGMV' isolates from Guatemala (86%), Puerto Rico (75%); and BGMV from Brazil (46%), further suggested the existence of significant genetic variability, particularly between the Mesoamerican geminiviruses and the Brazilian BGMV isolate. In 1993, the complete nucleotide sequences of the Brazilian and Puerto Rican 'BGMV' isolates were available for comparison. The percent nucleotide identities between these two viruses ranged between 71% and 82%; the latter value corresponding to the coat protein gene (AV1), which is the most conserved gene among whitefly transmitted geminiviruses. These results clearly suggested that BGMV-Brazil (BR) was significantly different from BGMV-Puerto Rico (PR) and, consequently, BGMV-BR was considered as a type I BGMV, and BGMV-PR as a type II BGMV. In the same year, the Central American 'BGMV' isolates from Guatemala and Dominican Republic were sequenced and shown to belong to the BGMV type II cluster with the BGMV isolate from Puerto Rico.

In 2000, the family *Geminiviridae* was created to host four genera of single-stranded DNA plant viruses, including the genus *Begomovirus* (sigla from BGMV). However, the name of the type species from Puerto Rico was changed from BGMV to BGYMV, leaving BGMV to designate the Brazilian virus, thus recognizing these two viruses as distinct species of the genus *Begomovirus*.

The bean golden mosaic disease observed in north western Mexico was shown in 1992, to be caused by yet another distinct begomovirus originally isolated in 1981 from squash in southwestern United States: Squash leaf curl virus (SLCV). Some SLCV isolates sampled from diseased common beans in Mexico had already changed significantly and were thus considered a separate species named *Bean calico mosaic virus* (BcaMV). However, SLCV can still be found in northwestern Mexico causing bean calico mosaic, a severe disease that often progresses from the characteristic systemic yellowing of bean golden/yellow mosaic into severe foliar bleaching and plant death.

Molecular Characterization of Bean Golden/Yellow Mosaic Viruses

The first nucleotide sequence was produced for the two genomic DNA molecules of the original BGYMV-PR isolate in 1985. DNA-A and DNA-B were 2646 and 2587 nt long, respectively. The only region that showed a high degree of homology in the two molecules, was the 'common region' (CR), approximately 205 nt long. The CR had direct and inverted repeats that could form a stem–loop structure. Other direct and inverted repeats were present in A+T-rich regions adjacent to G + C regions in DNA-A. The highly conserved CR contains critical sequences for the replication of begomoviruses. Six open reading frames (ORFs) were found in BGYMV-PR that could encode six proteins >10 kDa (15.6, 19.6, 27.7, 29.7, 33.1, and 40.2 kDa). By 1993, nine different begomoviruses had been fully sequenced, including a Brazilian isolate of BGMV. The DNA-A of BGMV-BR had 2617 base pairs (bp) and its DNA-B 2580 bp, with little sequence identity except for the CR of 181 nt. DNA-A had four ORFs encoding proteins >10 kDa: one in the viral sense (AV1) and three in the complementary sense (AC1, AC2, and AC3). The complementary sense ORFs were in different frames, and the 3′ end of AC1 overlapped the 5′ end of AC2, and AC2 overlapped all but the 3′ end of AC3. A shorter complementary sense ORF (AC4) that encodes a 9.5 kDa protein overlaps (nt 2227–2464) AC1. The four AC genes are involved in tasks associated with DNA replication (AC1), and possibly trans-activation and virus movement (AC2), virus replication enhancement (AC3), and gene silencing (AC4). AV1 and AC3 overlapped in opposite directions in an AT-rich region and shared four nucleotides at their C-termini, which contained their translational stop codons. AV1 encodes the coat protein, also necessary for whitefly transmission. On DNA B of BGMV-BR, two non-overlapping ORFs were detected: one in the viral sense (BV1), the nuclear shuttle protein (NSP), and one in the complementary sense (BC1), the cell-to-cell movement protein (MP). These genes code for proteins required for systemic movement of susceptible plants. All ORFs had one or more possible regulatory sequences (TATA boxes) within 100 nt of the putative start codon, except for the AC3 and BC1 ORFs. Putative polyadenylation signals (AATAAA motif) were located within the AT-rich regions at or near the 3′ ends of AV1 and AC3 ORFs and BV1 ORFs. BGMV ORFs AC1, AC2, and AC3 appeared to share a single polyadenylation signal located inside the 3′ end of AC3. Bipartite begomoviruses seem to utilize both rolling circle replication and recombination-dependent replication strategies. A specific intergenic region contains the origin of replication and the signature stem–loop structure that contains a nona-nucleotide motif involved in the rolling circle replication. The replication-associated protein (Rep) functions as a specific DNA binding protein that acts as both nuclease and ligase in the initiation and termination of the rolling circle replication process (**Figure 2**).

Sequence comparisons between BGYMV-PR and BGMV-BR showed nucleotide identities of 82%, 71%, and 72% for their respective AV1, AC1, and BV1 ORFs,

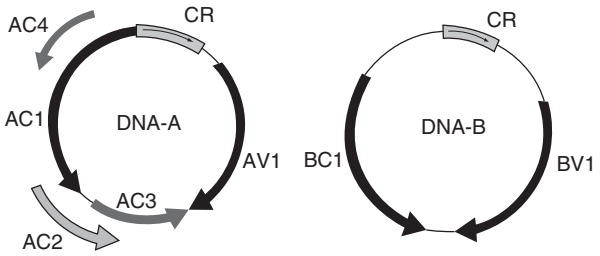


Figure 2 Genomic structure of a typical bipartite begomovirus: CR, common region; BC1 and AC1–4, genes encoded in the complementary sense in the B and A components, respectively; AV1 and BV1, virion sense genes.

which indicates that these two viruses are genetically distinct. In 1993, the complete nucleotide sequences of BGMV isolates from Guatemala and Dominican Republic were obtained. Comparisons of the complete DNA-A sequence of the Guatemalan isolate with the Dominican Republic isolate, and with BGYMV-PR and BGMV-BR yielded identities of 97%, 96%, and 74%, respectively. For DNA-B, the nucleotide identities were 92%, 87%, and 65%, respectively. These results demonstrate that the Central American and the Caribbean viruses form one cluster (BGYMV), whereas the Brazilian BGMV belongs to a different clade. The genome organization of the BGMV and BGYMV isolates sequenced to date is quite similar in terms of number, location, and direction of their DNA-A and -B components, and presence of large intergenic regions between the CR and AV1 in DNA-A, and between the CR and BC1 and BV1 in DNA-B. Infectious pseudo-recombinants between the DNA-A and -B components were only produced between the BGYMV isolates, but not between BGYMV and BGMV. However, infectious pseudo-recombinants have been produced between distinct begomovirus species, and symptom expression depends on the components selected.

Diagnosis

The fully systemic and intense yellowing induced by BGMV and BGYMV in most susceptible common bean genotypes is very characteristic of the diseases induced by these viruses. However, as mentioned above, other begomoviruses, such as SLCV and BCaMV, may also induce similar symptoms. Other viruses that may induce similar symptoms (intense yellowing) in common bean are alfalfa mosaic virus, bean yellow mosaic virus, and cowpea chlorotic mottle virus (which causes 'bean yellow stipple' in common bean). The most practical method available to detect the presence of BGMV or BGYMV is serology, using the enzyme-linked immunosorbent assay (ELISA) technique and suitable broad-spectrum polyclonal and/or monoclonal antibodies. Specific monoclonal antibodies have also been developed to differentiate between BGMV and BGYMV, but mutations in their coat protein genes have rendered the monoclonal antibodies useless. Nucleic acid hybridization tests have also been implemented to develop universal and specific probes for BGMV, BGYMV, and other bean begomoviruses. This technique, however, is more demanding in materials, time and facilities than serology. Finally, the PCR can be used to detect begomoviruses and produce nucleotide sequence for phylogenetic analyses and proper identification of begomovirus species and strains. A simple diagnostic characteristic of BGMV and BGYMV is that the former species is not manually transmissible, whereas BGYMV can be manually transmitted, although with some difficulty.

Pathogenicity and Disease Resistance

Begomoviruses, including BGMV and BGYMV, induce major changes in the nuclear structure of infected cells. One of the earliest changes observed is the hypertrophy of the nucleolus, which eventually occupies most of the nuclear volume. The nucleolar components also segregate into granular and fibrillar regions composed of ribonucleoprotein. Characteristic, electron dense fibrillar rings appear next, which have been shown to contain DNA. Viroplasms and virus particle aggregates are only observed in the nucleus of infected phloem cells in the case of single infections of BGMV or BGYMV.

The first reports on BGMV in Brazil already suggested that BGMV was a highly pathogenic virus capable of systemically infecting a wide range of dry and snap common bean genotypes possessing different grain types and origins. BGMV was also observed to infect lima bean (*P. lunatus*). In the first evaluation of 28 different common bean (*P. vulgaris*) varieties conducted in Brazil in 1965, none of the genotypes inoculated by viruliferous *B. tabaci* individuals was immune to BGMV. The virus was not seed-borne in any of the common bean genotypes inoculated. The search for sources of resistance to BGMV and BGYMV continued in the 1970s, when over 4500 different common bean genotypes were evaluated and found to be susceptible to these viruses. In the late 1970s, Brazilian breeders made individual selections of symptomless common bean plants found under field conditions among BGMV-susceptible varieties, only to find out that their progenies were also susceptible. Some of these individual selections were further used to produce atomic mutants, with equally disappointing results. In 1975, a large common bean improvement project was launched in Central America to breed for resistance to BGYMV. Out of 7000 common bean accessions evaluated under natural virus pressure, only few black-seeded genotypes of Mesoamerican origin showed a moderate level of resistance to BGYMV, but none was immune to the virus. These materials were used as parents to increase the level of resistance

of the main common bean cultivars grown in bean golden/yellow mosaic-affected regions of Latin America. The first BGYMV-resistant varieties obtained were derived from crosses made between the best parental materials selected (Porrillo Sintetico, ICA-Pijao, Turrialba 1). Of these resistant varieties, line DOR 41 (Porrillo Sintetico x ICA Pijao) became not only a cultivar in Central America, and in Argentina, South America, but one of the most frequently used sources of BGYMV and BGMV resistance in Latin America. In the 1980s, over 90% of all common bean genotypes possessing (partial) resistance to these viruses had DOR 41 or Porrillo Sintetico in its pedigree. The limitation with these sources of resistance was that the improved cultivars were all black-seeded, and many of the commercial common bean cultivars grown in bean golden/yellow mosaic virus-prone regions had other seed colors that were changed when crossed with the black-seeded parental sources of begomovirus resistance. Moreover, the level of virus resistance of the improved black-seeded varieties depended on the populations of viruliferous *B. tabaci*. Under high virus pressure, significant yield losses were to be expected. Consequently, a search for different sources of BGMV/BGYMV resistance was initiated in the mid-1980s.

The first breakthrough occurred when an advanced common bean line, A 429, developed for its superior growth habit (not for virus resistance), was observed to show low levels of BGYMV expression under field conditions in Guatemala, Central America. An evaluation of the parental materials involved in the development of this genotype revealed the presence of a 'pinto' (cream with brown stripes) genotype originally from Mexico (race 'Durango'). This genotype was fully susceptible and, in fact, severely affected by BGYMV in terms of arrested plant growth, plant malformation, and flower abortion, but it did not manifest the characteristic yellowing induced by BGYMV. The use of this genotype in crosses with virus susceptible common bean varieties led to the development of highly resistant genotypes possessing seed colors other than black. Eventually, the resistance was traced to a recessive gene known as *bgm-1*, for which a molecular marker is already available. The search for new sources of resistance eventually led to the identification of other useful mechanisms of resistance to BGMV and BGYMV. The most important mechanisms of resistance currently used to pyramid different bean golden/yellow mosaic resistance genes are: 'tolerance', defined as the ability of a genotype to yield acceptably despite its systemic infection and expression of characteristic disease symptoms. This mechanism is often found in genotypes belonging to race 'Nueva Granada' of Andean origin. Another recessive gene, *bgm-2*, was found to be associated with this trait. Some Andean genotypes also possessed the ability to grow normally despite their systemic infection by these viruses, that is, they showed resistance to the 'dwarfing' commonly induced by these viruses in susceptible common bean genotypes. The gene responsible for the dwarfing reaction in Andean genotypes was identified as *dwf*. Some varieties that posses this gene tend to escape infection under low-to-moderate disease pressure, but, in early infections, these genotypes do not grow at all. Nevertheless, some of these genotypes have been effectively used as source of resistance, as in the case of two Andean genotypes identified in the Dominican Republic as 'Pompadour J, and G', and line DOR 303. Highly susceptible cultivars possessing the *dwf* gene include the highly prized, large, white-seeded 'Alubia' grain type of Argentina. Another useful trait found in some Andean genotypes was the resistance to flower abortion and, more important, to pod and seed malformation, one of the most damaging effects of these begomoviruses in common bean. A dominant gene, *Bgp*, has been associated with this desirable trait. The resistance originally identified in Mesoamerican, black-seeded common bean genotypes is still useful and effective in combination with the genes mentioned above. The mechanism of resistance present in 'Porrillo Sintetico' is associated with its ability to 'escape infection' and, also, with a mechanism known as 'adult plant resistance'. That is, the longer plants grow in the field without being infected by these viruses, the greater the chance they have to escape disease. Most of the cultivated species of *Phaseolus* are also susceptible to BGMV and BGYMV, and those accessions that exhibit adequate levels of resistance have not been widely used in crossing programs due to the undesirable agronomic traits associated with the inter-specific hybridization of cultivated plants.

Bean Golden/Yellow Mosaic Disease Management

Bean golden yellow mosaic is currently a severe disease of common bean in southern Mexico, Central America, the Caribbean (including Florida in the continental USA), and northern South America, mainly Colombia. Bean golden mosaic is restricted to Brazil, Argentina, and Bolivia in South America. Although these two viruses belong to different species, most of the sources of resistance found in *P. vulgaris* germplasm collections have proved to be equally effective as parental materials to increase the levels of resistance to either BGMV or BGYMV. Unfortunately, the recent emphasis on 'marker-assisted selection' (MAS) and 'genetically modified organisms' (GMOs) has greatly reduced the output of BGMV/BGYMV resistant cultivars possessing the necessary agronomic and commercial characteristics demanded by farmers and consumers alike. In the absence of a constant supply of new cultivars, the resistance present in the early common bean cultivars improved for their resistance to BGMV and

BGYMV starts to decline due to the gradual adaptation of begomoviruses to common bean cultivars exposed to these viruses for over a decade. The new generation of systemic insecticides currently used to control whitefly pests may prolong the life span of some of the golden/yellow mosaic resistant common bean varieties grown in Latin America. These systemic insecticides have some time to act in the plant against viruliferous whiteflies, because begomoviruses are transmitted in a semipersistent manner that require an average of 20 min for the viruses to be inoculated into the vascular (phloem) tissues of susceptible plants. However, in the presence of high populations of viruliferous *B. tabaci* individuals, the effectiveness of these new insecticides is significantly reduced. Hence, some cultural practices, such as avoidance of continuous planting of virus/vector susceptible crops, are also important components of integrated disease management programs designed to reduce the incidence of BGYMV and BGMV.

See also: Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus.

Further Reading

Bird J, Sanchez J, and Vakili NG (1973) Golden yellow mosaic of beans (*Phaseolus vulgaris*) in Puerto Rico. *Phytopathology* 63: 1435.

Brown JK, Ostrow KM, Idris AM, and Stenger DC (1999) Biotic, molecular, and phylogenetic characterization of Bean calico mosaic virus, a distinct begomovirus species with affiliation in the Squash leaf curl virus cluster. *Phytopathology* 89: 273–280.

Costa AS (1965) Three whitefly-transmitted diseases of beans in the State of São Paulo, Brazil. *FAO Plant Protection Buletin* 13: 121–130.

Costa AS (1975) Increase in the populational density of *Bemisia tabaci*, a threat of widespread virus infection of legume crops in Brazil. In: Bird J and Maramorosch K (eds.) *Tropical Diseases of Legumes*, pp. 27–49. New York: Academic Press.

Faria JC, Gilbertson RL, Hanson SF, *et al.* (1994) Bean golden mosaic geminivirus type II isolates from the Dominican Republic and Guatemala: Nucleotide sequences, infectious pseudorecombinants, and phylogenetic relationships. *Phytopathology* 84: 321–329.

Gamez R (1970) Los virus del frijol en centroamérica. I. Transmisión por moscas blancas (*Bemisia tabaci* Gen.) y plantas hospedantes del virus del mosaico dorado. *Turrialba* 21: 22–27.

Gilbertson RL, Faria JC, Ahlquist P, and Maxwell DP (1993) Genetic diversity in geminiviruses causing bean golden mosaic disease: the nucleotide sequence of the infectious cloned DNA components of a Brazilian isolate of Bean golden mosaic geminivirus. *Phytopathology* 83: 709–715.

Goodman RM and Bird J (1978) Bean golden mosaic virus. Commonwealth Mycological Institute/Association of Applied Biologists. *Descriptions of Plant Viruses* 192: 1–4.

Lazarowitz SG (1987) The molecular characterization of geminiviruses. *Plant Molecular Biology Reporter* 4: 177–192.

Morales FJ and Anderson PK (2001) The emergence and dissemination of whitefly-transmitted geminiviruses in Latin America. *Archives of Virology* 146: 415–441.

Morales FJ and Jones PG (2004) The ecology and epidemiology of whitefly-transmitted viruses in Latin America. *Virus Research* 100: 57–65.

Morales FJ and Niessen AI (1988) Comparative responses of selected *Phaseolus vulgaris* germplasm inoculated artificially and naturally with bean golden mosaic virus. *Plant Disease* 72: 1020–1023.

Morales FJ and Singh SP (1991) Genetics of resistance to bean golden mosaic virus in *Phaseolus vulgaris* L. *Euphytica* 52: 113–117.

Beet Curly Top Virus

J Stanley, John Innes Centre, Colney, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Enation Virus-induced swelling of the plant tissue.
Endoreduplication DNA replication in the absence of cell division.
Etiology The cause of a disease.
Hemolymph The insect circulatory system.
Hyperplasia Unregulated cell division.
Hypertrophy Cell enlargement.
Nonpropagative transmission The virus does not replicate in the insect.
RFLP Restriction fragment length polymorphism, an analytical tool to distinguish DNA viruses.
Transovarial transmission Transmission of virus through the insect egg.

History

Curly top disease of sugar beet was first reported in the USA in 1888. With the establishment and growth of the sugar beet industry, it was soon realized that the disease

was widespread throughout the western states where it also affected a variety of crops including tomato, bean, and potato. The disease spread eastward during the middle of the twentieth century and similar diseases were reported to occur in western Canada, northern Mexico, Brazil, Argentina, the Caribbean Basin, Turkey, and Iran, although in most cases the etiology still remains to be established. A relationship between the disease and the beet leafhopper *Circulifer tenellus* (Baker) (Homoptera: Cicadellidae) was established in 1909. Although the leafhopper transmission characteristics suggested viral etiology, the low level of accumulation of the pathogen in plants hampered its isolation, and it was not until 1974 that a virus was eventually isolated from tobacco and shown to adopt a twinned particle morphology characteristic of a geminivirus infection. The viral genomic component was eventually cloned from viral DNA isolated from symptomatic sugar beet and characterized by sequence analysis in 1986. Infectivity studies using the cloned genomic component and transmission of the clone progeny by *C. tenellus* confirmed that the disease was caused by the geminivirus beet curly top virus (BCTV).

Taxonomic Classification and Phylogenetic Relationships

Members of the family *Geminiviridae* are divided into the genera *Begomovirus*, *Curtovirus*, *Topocuvirus*, and *Mastrevirus* on the basis of genome organization, host range, and insect vector characteristics. Originally collectively referred to as strains of *Beet curly top virus*, the genus *Curtovirus* currently includes BCTV as the type species and *Beet mild curly top virus*, *Beet severe curly top virus*, *Horseradish curly top virus*, and *Spinach curly top virus* (**Figure 1**). The names given to beet

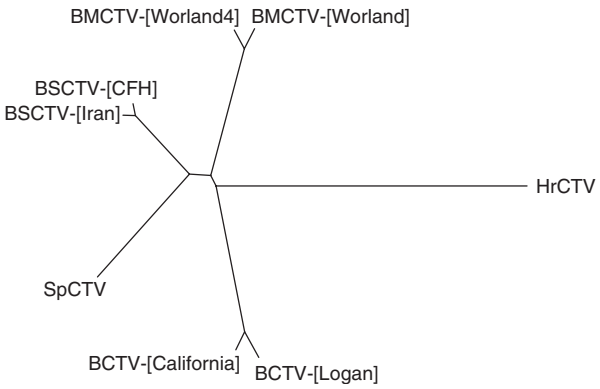


Figure 1 Phylogenetic relationships between members of the genus *Curtovirus*. The unrooted tree is based on the alignment of the complete nucleotide sequences of isolates of beet curly top virus (BCTV), beet mild curly top virus (BMCTV), beet severe curly top virus (BSCTV), horseradish curly top virus (HrCTV), and spinach curly top virus (SpCTV).

mild curly top virus (BMCTV) and beet severe curly top virus (BSCTV) reflect the symptom severity of these viruses in sugar beet although their phenotypes vary in other hosts. Species demarcation within the genus is based primarily on an 89% nucleotide sequence identity threshold for the entire genomic DNA while other biological factors such as host range, pathogenicity, and replication compatibility help to distinguish species and variants. The organization of BCTV complementary-sense genes resembles that of whitefly-transmitted begomoviruses while the virion-sense coat protein gene is more closely related to that of leafhopper-transmitted mastreviruses, suggesting that a curtovirus ancestor may have evolved by recombination between members of distinct genera. Phylogenetic analysis of horseradish curly top virus (HrCTV) suggests that a recombination event involving a region within the complementary-sense genes has also occurred between an ancestral curtovirus and begomovirus. Phylogenetic analysis of spinach curly top virus (SpCTV) indicates that its complementary-sense genes most closely resemble those of BMCTV and BSCTV while its virion-sense genes are more closely related to those of HrCTV, suggesting that it may have originated by recombination between members of the genus. However, because recombination frequently occurs between geminiviruses and undoubtedly plays a major role in their evolution, it is extremely difficult to establish an exact lineage for a particular virus and whether it represents the parent or progeny of a recombination event.

Geographic and Seasonal Distribution

Curly top disease occurs widely throughout arid and semiarid regions of western USA that are favored by the leafhopper vector, and it is here that the epidemiology of the disease has been most closely monitored. Early investigations of host range, symptom induction, and virulence suggested that curly top disease was caused by a complex of viral pathogens, a notion borne out by the identification of distinct species and variants with overlapping geographic distributions. Adult leafhoppers overwinter on perennial weeds from which they acquire the virus. Viruliferous adults migrate to cultivated areas during spring where they undergo several generations while feeding on weeds and crops to which they transmit the disease. In autumn, the adult leafhoppers migrate back to their overwintering grounds. Using restriction fragment length polymorphism (RFLP) analysis, a field survey of sugar beet growing in Texas during 1994 showed BSCTV to be the predominant species. Both BMCTV and BSCTV were detected in a more comprehensive survey of sugar beet from California, Colorado, Idaho, New Mexico, Oregon, Washington, Wyoming, and Texas in 1995. Plants frequently contained genotypic variants of these species and occasionally maintained mixed infections of both

species. Although BCTV was detected in samples maintained in laboratories and nurseries for analytical purposes, it was not recovered from field samples. This might reflect limitations of the sampling and screening procedures although a change in the population structure in which BCTV is no longer prevalent in the field seems likely. This is supported by a more recent survey of beet, pepper, and tomato as well as native weed species growing in California between 2002 and 2004, which again indicated the presence of BMCTV and BSCTV and the absence of BCTV. HrCTV was isolated in 1990 from horseradish originating from Illinois that exhibited brittle root disease, and SpCTV was isolated in 1996 from spinach growing in southwest Texas. The lack of RFLP patterns diagnostic of these two species in other surveys suggests they may have rather restricted geographic distributions.

A variant of BSCTV is also associated with curly top disease in the Mediterranean Basin, implying a common origin for the New and Old World viruses. Interbreeding experiments indicated that the leafhopper populations in these two parts of the world are closely related, although leafhopper genetic diversity in the Old World is much greater than in the USA. Coupled with the fact that sugar beet is native to Europe, it has been suggested that disease originated in the Old World and was introduced into the USA from the Mediterranean Basin as a result of movement of infected plants and accompanying viruliferous insects.

Genome Organization and Gene Expression

In common with all geminiviruses, BCTV has a genome of circular single-stranded DNA (ssDNA) that is encapsidated in twinned quasi-isometric particles approximately 20×30 nm in size. The BCTV genome comprises a single component of *c.* 3000 nt that encodes seven genes distributed between the virion-sense (V) and complementary-sense (C) strands (also referred to as rightward (R) and leftward (L) strands in the literature), separated by an intergenic region (IR) that contains the origin of replication. A nonanucleotide motif (TAATATTAC) that is highly conserved in geminiviruses is located within the IR, between inverted repeat sequences with the potential to form a stem–loop structure. Short repeat sequences, termed iterons, occur upstream of the stem–loop (**Figure 2**).

The complementary-sense ORFs encode the replication-associated protein (Rep) required for the initiation of viral DNA replication (ORF C1), a replication-enhancer protein (REn) (ORF C3), and two proteins that contribute to viral pathogenicity (ORFs C2 and C4), all of which may be considered to be required during the early stages of infection. The virion-sense ORFs encode proteins that

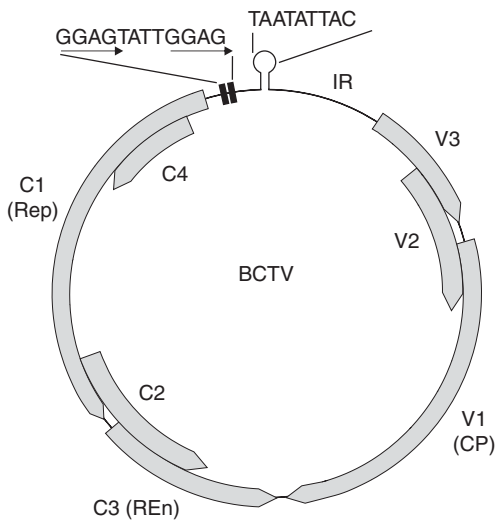


Figure 2 The genome organization of BCTV. The position and orientation of virion-sense (V) and complementary-sense (C) ORFs are shown in relation to the intergenic region (IR) that contains the invariant nonanucleotide motif TAATATTAC and iterons (repeat sequences indicated by arrows) involved in the initiation of viral DNA replication. CP, coat protein; Rep, replication-associated protein; REn, replication-enhancer protein.

are required later in infection, namely the coat protein (ORF V1) and proteins involved in the regulation of the relative levels of viral ssDNA and double-stranded DNA (dsDNA) (ORF V2) and virus movement (ORF V3).

The identification of BCTV virion- and complementary-sense transcripts is consistent with a bidirectional transcription strategy. The two most abundant complementary-sense transcripts are similar in size to their begomovirus counterparts, the larger transcript mapping across all four ORFs and the smaller transcript across ORFs C2 and C3. However, the most abundant transcripts map across the virion-sense ORFs, downstream of two consensus eukaryotic promoter sequences, and are suitably positioned for the expression of ORFs V2 and V3, while a less abundant transcript maps across ORF V1. Precisely how the viral proteins are expressed from these transcripts is not yet understood although the overlapping nature of both the virion-sense and complementary-sense ORFs may provide a means for their temporal control during the infection cycle.

Replication

Fractionation of viral DNA forms by a combination of chromatography and two-dimensional gel electrophoresis has identified BCTV intermediates consistent with both rolling-circle and recombination-dependent replication strategies. At the onset of infection, the viral ssDNA is uncoated and converted to a circular dsDNA intermediate by host factors. During rolling-circle replication, Rep

interacts with iterons located in the IR and introduces a nick into the virion-sense strand of the dsDNA within the nonanucleotide motif (TAATATT$^{\downarrow}$AC). By analogy with the replication strategy of begomoviruses, Rep then forms a covalent bond with the 5′ terminus of the nicked virion-sense strand. However, as Rep does not have polymerase activity, the 3′ terminus must be extended by a host polymerase. The full-length nascent strand is nicked and relagated by Rep to produce circular ssDNA that either reenters the replication cycle or is encapsidated. BCTV ORF C3 mutants produce severe symptoms and accumulate to wild-type levels in *Nicotiana benthamiana*, although they induce only a mild infection in sugar beet associated with significantly reduced levels of viral DNA accumulation, consistent with the proposed role for REn in viral DNA replication. Interestingly, HrCTV does not contain an intact ORF C3.

The interaction between Rep and the origin of replication, defined by the composition of the N-terminal region of Rep and the iteron sequence, is highly specific and determines whether distinct curtovirus species and variants are compatible for *trans*-replication. For example, BCTV Rep can only functionally interact with its own iteron (GGAGTATTGGAG; **Figure 2**) and not those of BMCTV and BSCTV (GGTGCTATGGGAG and GGTGCTTTGGGTG, respectively). Conversely, BMCTV and BSCTV Reps will not functionally interact with the BCTV iteron. However, the BMCTV and BSCTV iterons are sufficiently similar to allow mutual Rep recognition and, hence, *trans*-replication compatibility. Comparison of the Rep N-terminal sequences has identified amino acid residues conserved between BMCTV and BSCTV but differing in BCTV that may participate in iteron recognition, but this awaits experimental confirmation. While replication incompatibility between *cis*- and *trans*-acting elements serves to maintain the integrity of a particular species, this constraint may be overcome by recombination whereby the functional module comprising the 5′ terminus of the Rep ORF and the origin of replication is exchanged between incompatible viruses, as has been suggested to have occurred during the evolution of SpCTV.

Although replication intermediates consistent with a rolling-circle strategy for BCTV replication have been observed, the production of other viral DNA forms can be explained readily by a recombination-dependent replication mechanism. Recombination-dependent replication is generally considered to occur later in the infection cycle than rolling-circle replication whereby a ssDNA byproduct recombines with homologous sequences within a circular or multimeric dsDNA template to initiate replication, a process mediated by a recombination protein and often triggered by a double-stranded break. It remains to be established which, if any, viral proteins contribute to this process, although it is possible that Rep helicase activity could participate in both replication strategies. The nick site for rolling-circle replication has been shown to be a recombinational hot spot. However, recombination-dependent replication may explain the propensity of geminiviruses to undergo recombination throughout their genomes.

Subgenomic-sized viral DNAs of diverse size (ranging from 800 to 1800 nt) and complexity are rapidly produced from the cloned BCTV genomic component in sugar beet, tomato, and *N. benthamiana*. Deletions occur within all ORFs and only the IR is retained, consistent with its participation in viral DNA replication. Field-affected sugar beet frequently contains such defective DNAs, indicating that they are also produced under natural conditions. They occur as both single- and double-stranded DNA forms but it is not known if they are encapsidated or have the ability to move systemically in the plant. However, in view of their rapid appearance, it is likely that they are produced *de novo* in every infected cell. Short repeat sequences of 2–6 nt at the deletion boundaries may specify the deletion endpoints and suggest that the defective DNAs are produced by recombination or as a consequence of errors during replication. Many geminiviruses produce subgenomic-sized DNAs, several of which, including those associated with BCTV, have been shown to interfere with infection. Hence, they may have a biological role in modulating pathogenicity of the helper virus, thus conferring a selective advantage by slowing down infection of the plant to encourage leafhopper feeding and virus transmission.

Virus Movement and Insect Transmission

The coat protein is a multifunctional protein that is targeted to the nucleus where virions accumulate, and is essential for systemic infection and leafhopper transmission. BMCTV coat protein mutants that retain the ability to form virions (mainly N-terminal mutations) are generally able to produce a systemic infection while those unable to form virions (mainly C-terminal mutations) cannot, despite being competent for replication. This serves to reinforce the proposal that long-distance movement of the virus throughout the plant occurs in the form of virions, consistent with the observation that high levels of virus-like particles accumulate in the phloem. It further suggests that C-terminal amino acids play an important role in determining the structure of the coat protein during virion assembly. ORF V2 mutants accumulate high levels of dsDNA and greatly reduced levels of ssDNA compared to the wild-type virus, implicating V2 protein in the regulation of the relative levels of these viral DNA forms. The function of V2 protein may be to ensure that ssDNA is available in sufficient amounts for encapsidation and systemic movement in the latter stages of the infection cycle. ORF V3 mutants remain

competent for replication but produce only sporadic and asymptomatic infections in sugar beet and *N. benthamiana* associated with low levels of viral DNA accumulation, suggesting a role for V3 protein in virus movement.

All curtoviruses are transmitted in nature by the leafhopper *C. tenellus* (**Figure 3**) in a persistent circulative manner, and there is no evidence to suggest that there are any significant differences in transmission characteristics between curtovirus species. *Circulifer opacipennis* is reported to be an additional vector of the disease in the Mediterranean Basin, but this leafhopper does not occur in the USA. The leafhopper feeds primarily in the phloem from where it rapidly acquires the virus. Using a PCR-based approach, BMCTV has been detected in the digestive tract of the insect after an acquisition access period (AAP) of 1 h, in the hemolymph after 3 h, and the salivary glands after 4 h, from where it is reintroduced into plants through saliva during feeding. This is consistent with earlier reports estimating the minimum time between virus acquisition and the leafhopper becoming infective (the latent period) to be 4 h. The minimum feeding time necessary for transmission of the virus can be as short as 1 min. Depending on the length of the AAP, leafhoppers can remain infective for most of their lifetime, although the amount of virus they retain and their ability to transmit the disease decline with time when maintained on plants that are not hosts for the virus, implying that transmission is nonpropagative. There is no evidence for transovarial transmission of the virus.

Several lines of evidence demonstrate that coat protein composition defines leafhopper transmission specificity. First, BMCTV coat protein mutants that are unable to produce virions are not transmitted by *C. tenellus*. Second, recombinant virus in which the coat protein coding sequence of the whitefly-transmitted begomovirus African cassava mosaic virus has been replaced with that of BCTV produces virions that are transmissible by the leafhopper. Finally, the autonomously replicating nanovirus-like DNA-1 component, normally associated with the whitefly-transmitted begomovirus ageratum yellow vein virus, can be maintained in systemically infected sugar beet by BCTV and is encapsidated in BCTV coat protein, conferring on DNA-1 the ability to be transmitted by *C. tenellus*. This implies that surface features of the virion interact in a highly specific manner during circulative transmission, possibly by receptor-mediated endocytosis



Figure 3 (a) Adult *Circulifer tenellus* (Baker) leafhopper. Symptoms of BCTV infection in (b) sugar beet and (c) *Nicotiana benthamiana*. Plants show upward leaf roll and vein-swelling symptoms typical of curly top disease.

during virus acquisition across the gut wall and movement into the salivary glands.

BCTV and other members of the genus are generally poorly transmitted by mechanical inoculation, reflecting their phloem limitation. Infection can be achieved using a fine-gauge needle (pin-pricking) to introduce virus or viral DNA inoculum directly into the phloem, although infection rates are dramatically improved using either biolistic delivery under pressure or *Agrobacterium tumefaciens*-mediated inoculation (agroinoculation) of cloned DNA. Agroinoculation exploits the ability of *A. tumefaciens* to introduce binary vector T-DNA, containing partially or tandemly repeated cloned genomic DNA, into the plant cell nucleus. To initiate an infection, circular viral DNA is resolved from the T-DNA, either by recombination between the repeat sequences or by a more efficient replicative mechanism if two copies of the origin of replication are present.

Host Range and Pathogenesis

In contrast to most other geminiviruses which tend to have a limited host range, BCTV is reported to have an extremely wide host range confined to dicotyledonous plants, including over 300 species in 44 plant families, particularly members of the Chenopodiaceae, Compositae, Cruciferae, Leguminosae, and Solanaceae. Although the wide host range is not in doubt, these largely early observations were conducted using diseased plants of undefined etiology and, hence, the exact host range of BCTV remains to be confirmed. BMCTV, BSCTV, and SpCTV also have wide host ranges, although HrCTV has an atypically narrow host range. Curly top disease was originally described in association with agricultural crops showing severe symptoms, and virus tends to accumulate to higher levels in sugar beet and tomato compared to weeds. However, hosts include a wide variety of annual and perennial weed species which act as a reservoir for the disease and in which the virus frequently produces a mild or asymptomatic infection.

The products of BCTV complementary-sense ORFs C2 and C4 have been implicated in pathogenesis. ORF C2 mutants induce severe symptoms and accumulate to wild-type virus levels in sugar beet and *N. benthamiana*. However, plants infected with the mutants have a greater propensity for recovery from severe symptoms than those infected with wild-type virus, suggesting that C2 protein suppresses a plant stress or defense mechanism. Consistent with this, transgenic *N. benthamiana* and *N. tabacum* plants constitutively expressing C2 protein exhibit enhanced susceptibility to BCTV infection, manifested by a decrease in the inoculum concentration required to elicit an infection and a reduction in latent period before the onset of infection, although they do not develop more severe symptoms or accumulate higher levels of viral DNA than nontransformed plants. The transgenic plants are also more susceptible to the begomovirus tomato golden mosaic virus (TGMV) and the unrelated RNA virus tobacco mosaic virus, indicating that C2 protein impacts on a nonspecific host response. C2 protein has been shown to suppress post-transcriptional gene silencing (PTGS) in *N. benthamiana*, and in this respect resembles its positional counterpart (AC2) in the begomovirus TGMV. However, the two proteins share only limited homology and BCTV C2 protein does not appear to have transcriptional activator activity for virion-sense gene expression shown by its TGMV counterpart. Despite this, both BCTV C2 and TGMV AC2 proteins bind to, and inactivate, adenosine kinase (ADK), an enzyme required for 5′-adenosine monophosphate (AMP) synthesis. ADK activity is also reduced in BCTV-infected plants and transgenic plants expressing C2 protein. Regulation of adenosine levels by ADK plays a key role in the control of intermediates required for methylation, providing a possible link between C2 protein activity and suppression of transcriptional gene silencing (TGS) of the viral genome. Both BCTV C2 and TGMV AC2 proteins also bind to, and inhibit, SNF1-related nucleoside kinase. Reduction of SNF1 expression in *N. benthamiana* results in an enhanced susceptibility phenotype resembling that associated with the expression of C2 and AC2 proteins, while SNF1 overexpression produces plants exhibiting enhanced resistance. This activity is not directly linked to silencing suppression, suggesting that C2 protein inhibition of SNF-1 activity affects a distinct plant defense pathway.

BCTV typically induces upward curling of the leaves associated with vein clearing and the development of enations on the lower surface of veins (**Figure 3**). *In situ* localization studies have shown that BCTV is tightly phloem-limited in sugar beet and *N. benthamiana*. Although the virus cannot access apical meristematic tissues, it is possible that it exploits undifferentiated cambium cells in the vascular bundles. Infected tissues exhibit hyperplasia and hypertrophy within the phloem and adjacent parenchyma, and the dividing cells differentiate into sieve-like elements. In older infected tissues, the affected phloem cells eventually become necrotic and collapse. Pathogenic effects occur in developing tissues only after mature sieve elements have developed, suggesting that the virus moves from source to sink cells with the flow of metabolites. Infected sugar beet leaves accumulate enhanced levels of sucrose, attributed to impaired transport resulting from disruption of the phloem. This is associated with a reduction in chlorophyll content, reduced activity of key photosynthetic enzymes and concomitant reduction in the rate of photosynthesis, and altered turgor pressure that causes an increase in mesophyll cell size.

Geminiviruses control the plant cell cycle to produce an environment suitable for their replication. This is achieved by the interaction of Rep with the plant homolog of retinoblastoma-related tumor-suppressor protein (pRBR), which relieves the constraint imposed by pRBR binding to E2F transcription factor and allows the expression of E2F-responsive genes. In most cases, infected cells enter S phase and may undergo endoreduplication but do not divide. In contrast, BCTV infection induces cell division within vascular tissues, attributed to the action of C4 protein. Sugar beet infected with ORF C4 mutants remain asymptomatic and *N. benthamiana* shows an altered phenotype, and neither host develops hyperplasia of the phloem and enations. Furthermore, ectopic expression of C4 protein in transgenic *N. benthamiana* results in abnormal plant development and tumorigenic growths. Consistent with this phenotype, C4 protein interacts with BIN2, a negative regulator of transcription factors in the brassinosteroid signaling pathway that controls cell division and tissue development. The reason for the induction of cell division remains obscure, particularly as the hyperplastic tissues often do not contain detectable levels of virus.

The host range of BCTV includes *Arabidopsis thaliana* which represents an important resource for the study of virus–host interactions. Most susceptible ecotypes become stunted and develop enations on affected tissues although the Sei-O ecotype is hypersusceptible to BCTSV and develops callus-like structures containing high levels of the virus, suggesting virus-induced hormonal imbalance. Symptom development correlates with enhanced expression of the cell cycle gene *cdc2* and small auxin-upregulated RNA gene (*saur*). Hence, disruption of the phloem in infected tissues may affect auxin transport, causing localized increases that result in cell proliferation.

Disease Control

As young plants are most susceptible to infection, a significant reduction in disease incidence may be achieved by protecting seedlings using either a physical barrier to prevent leafhopper access or by timing the emergence of the crop to avoid predicted leafhopper spring migration from the overwintering grounds. Breeding resistant varieties of sugar beet has been very successful although the basis of resistance is not understood. Plants remain susceptible to infection but are sufficiently productive, particularly when used in combination with an integrated pest-management scheme. Breeding program have provided better uniformity of resistance with improved yield and sugar content, which has reinvigorated the sugar beet industry in the western USA. Resistant bean varieties are also available, although breeding for resistance to curly top disease in tomato has not been so successful. Systemic insecticides, for example, soil treatment with imidacloprid and dimethoate foliar sprays, have been used to control the leafhopper vector on crops and on weeds located in the breeding areas, both with some success, particularly when applied to young susceptible plants, although this approach is both costly and damaging to the environment. Parasites and predators of the leafhopper are numerous, but it has proved difficult to assess their impact on leafhopper populations under natural conditions.

The subgenomic-sized DNAs produced during BCTV infection are known to adversely affect virus proliferation, for which reason they are termed defective interfering (DI) DNAs. Transgenic *N. benthamiana* plants containing an integrated tandem copy of the DI DNA are less susceptible to BCTV infection. The DI DNA is mobilized from the transgene and amplified to high levels during infection. Competition between the helper virus and DI DNA results in a significant reduction in both viral DNA accumulation and symptom severity. DI DNA amplification is dependent on *trans*-replication by the helper virus, governed by the specific interaction between Rep and the origin of replication. However, as BMCTV and BSCTV are the predominant species in the western USA and are mutually competent for *trans*-replication, genetic modification of sugar beet with the appropriate DI DNA transgene may provide a viable alternative for the production of resistant plants.

See also: Defective-Interfering Viruses; Plant Resistance to Viruses: Geminiviruses; Plant Virus Diseases: Fruit Trees and Grapevine; Plant Virus Diseases: Ornamental Plants; Replication of Viruses; Vector Transmission of Plant Viruses.

Further Reading

Bennet CW (1971) *American Phytopathology Society Monograph No. 7: The Curly Top Disease of Sugar Beet and Other Plants.* St. Paul, MN: American Phytopathology Society.

Esau K and Hoefert LL (1978) Hyperplastic phloem in sugarbeet leaves infected with the beet curly top virus. *American Journal of Botany* 65: 772–783.

Hanley-Bowdoin L, Settlage SB, Orozco BM, Nagar S, and Robertson D (1999) Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. *Critical Reviews in Plant Sciences* 18: 71–106.

Stanley J, Bisaro DM, Briddon RW, *et al.* (2005) Geminiviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 301–326. San Diego, CA: Elsevier.

Stanley J, Markham PG, Callis RJ, and Pinner MS (1986) The nucleotide sequence of an infectious clone of the geminivirus beet curly top virus. *EMBO Journal* 5: 1761–1767.

Benyvirus

R Koenig, Institut für Pflanzenvirologie, Mikrobiologie und biologische Sicherheit, Brunswick, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Benyvirus Siglum derived from *Beet necrotic yellow vein virus*.
Rhizomania, root beardedness (**root madness**) Extensive proliferation of often necrotizing secondary rootlets at the expense of the main tap root.

History

Beet necrotic yellow vein virus is the type species of the genus *Benyvirus*. Originally it was classified as a possible member of the tobamovirus group, because its rod-shaped particles resemble those of tobamoviruses. In 1991, the fungus-transmitted rod-shaped viruses, all of which have multipartite genomes, were separated from the monopartite tobamoviruses to form a new group, named furovirus group. Soil-borne wheat mosaic virus (SBWMV) became the type member of this new group; beet necrotic yellow vein virus (BNYVV) and rice stripe necrosis virus (RSNV) were listed as possible members. Molecular studies performed in the following years revealed that the genome organization of many of these furoviruses greatly differed from that of SBWMV. Eventually, four new genera were created, that is, the genus *Furovirus* with *Soil-borne wheat mosaic virus* as the type species, the genus *Benyvirus* with *Beet necrotic yellow vein virus* as the type species, the genus *Pomovirus*, and the genus *Pecluvirus*. The genus *Benyvirus* presently comprises two definitive species, viz. *Beet necrotic yellow vein virus* and *Beet soil-borne mosaic virus*, and two tentative species, viz. RNSV and burdock mottle virus (BdMV).

Host Ranges and Diseases

BNYVV is the causal agent of rhizomania, one of the most damaging diseases of sugar beet. Fodder beets, Swiss chard (*Beta vulgaris* var. *cicla*), red beets, and spinach may also become infected naturally. Infections of sugar beet are mainly confined to the root system. Susceptible sugar beet varieties show an extensive proliferation of nonfunctional, necrotizing secondary rootlets, a condition described by the names 'root beardedness', 'root madness', or rhizomania. The tap roots are stunted, their shape tends to be constricted, and their sugar content is low. A brownish discoloration of the vascular system is often seen. Leaves may become pale and have an upright position. Under dry conditions, wilting is often observed due to the disturbances in the root system. The upper parts of the plants are invaded only rarely by the virus. In infected leaves, the veins turn yellow, and occasionally become necrotic, a condition after which the virus has been named. In susceptible varieties, rhizomania may cause yield losses of 50% and more. Like other diseases caused by soil-borne organisms, rhizomania often occurs in patches, especially in recently infested fields. BNYVV can be mechanically transmitted to many species in the Chenopodiaceae and to some species in the Aizoacea (e.g., *Tetragonia expansa*), Amaranthaceae, and Caryophyllaceae. *Beta macrocarpa* becomes infected systemically. Recently obtained isolates also infect *Nicotiana benthamiana* systemically, causing slight mottling and growth reduction.

The symptoms caused by beet soil-borne mosaic virus (BSBMV) on its natural host *B. vulgaris* are more variable than those produced by BNYVV. Infected roots may remain symptomless or show rhizomania-like symptoms. Infections of the upper parts of the plants occur somewhat more frequently than with BNYVV. Leaves may develop vein banding or faint mottling. When plants are dually infected with BSBMV and BNYVV, foliar symptoms appear more frequently. In general, BSBMV causes much less damage to sugar beets than BNYVV. BSBMV can be transmitted mechanically to *Chenopodium quinoa, Chenopodium album*, and *Tetragonia tetragonioides,* all of which become infected locally, and to *Beta maritima,* which becomes infected systemically.

RSNV was first described in 1983 in West Africa (Ivory Coast) as the causal agent of a long-known disease named 'rice crinkling disease'. The percentage of infection was found to vary greatly according to the year and to the variety. In 1991, the virus was first noticed in Columbia where it causes a severe disease of rice named 'entorchamiento' that is characterized by seedling death, foliar striping, and severe plant malformation. The virus can be transmitted mechanically to *C. quinoa, C. album*, and *Chenopodium amaranticolor* where it produces local lesions, but not to rice. *Nicotiana benthamiana* does not become infected.

BdMV has been isolated from the leaves of naturally infected *Arctium lappa* L. Leaves as well as roots of this plant are common vegetables in Japan. The virus often occurs in mixed infections with other viruses and produces only mild symptoms which often become masked in older plants. BdMV can be transmitted mechanically to *C. quinoa*, *Chenopodium murale*, *Nicotiana clevelandii*, and *Nicotiana rustica,* which are infected systemically, and to

B. vulgaris var. *cicla* and var. *rapa*, *Spinacia oleracea, Cucumis sativus*, and *Tetragonia expansa,* which are infected only locally, some of them only with difficulty.

Geographic Distribution and Epidemiology

BNYVV is now found worldwide in sugar beet-growing areas. Molecular analyses have revealed the existence of different genotypes (A type, B type, P type) that cannot be distinguished serologically, but their RNAs differ in *c.* 1–5% of their nucleotides. The A type is most common and occurs in Southern, Eastern, and parts of Northern Europe, in the USA, and – with a number of nucleotide exchanges – also in East Asia. The B type is prevalent in Germany, France, and other central European countries. B-type-like BNYVV is also found in East Asian countries. The P type, which usually has a fifth RNA species and causes especially severe symptoms, has only been found in limited areas of France (Pithiviers), of the UK, and in Kazakhstan. In the UK, a new RNA5-containing virus source (FF) has recently been described. Its RNA5 occupies an intermediate position between East Asian and European forms of RNA5. In the UK, where rhizomania was observed later than in most other European countries, a larger number of different BNYVV types have now been identified than in any other country in the world. Originally it has been assumed that rhizomania, which was first observed in Italy in 1952, has spread from that country to central and later to Northern Europe. It is unlikely, however, that all BNYVV types have originated in Italy where only the A type has so far been observed. It seems more likely that naturally infected local hosts in different geographic regions may harbor various types of BNYVV which may be transmitted to beets only with difficulty, perhaps due to vector/host compatibility problems or to their occurrence in different habitats. BNYVV is not commonly found in weeds in sugar beet fields. Once a transmission from a native host to sugar beet has been successful, further spread in beet-growing areas by machinery, irrigation, or infested soil may be very rapid.

BSBMV is widely distributed in the USA, but has so far not been found in other parts of the world. Single-strand conformation analyses of polymerase chain reaction (PCR) products indicated that this virus is genetically much more variable than BNYVV. This may explain the variability in the symptoms produced by this virus. RSNV occurs throughout West Africa and several countries in South America, that is, Colombia, Ecuador, Brazil, and Panama. It is assumed that it has been introduced to these countries from West Africa with seed contaminated at the surface with RSNV-harboring *Polymyxa graminis* resting spores. BdMV has been reported only from Japan.

Transmission

In nature, the assigned species of the genus *Benyvirus*, viz. *Beet necrotic yellow vein virus* and *Beet soil-borne mosaic virus*, are transmitted by the zoospores of *Polymyxa betae,* a soil-borne ubiquitous plasmodiophorid protozoan. *P. betae* and the related *P. graminis* that has always been found in natural RSNV infections had formerly been considered to belong to the fungi. *Polymyxa*-transmitted viruses (or their RNAs?) are taken up by the plasmodia of the vector in infected root cells, but there is no evidence that they multiply in the vector. The multinucleate plasmodia which are separated from the host cytoplasm by distinct cell walls may either develop into zoosporangia from which secondary viruliferous zoospores are released within a few days. Alternatively, the plasmodia may form cystosori which act as resting spores and may survive in the soil for many years even under extreme conditions. They may be distributed on agricultural equipment, by irrigation, or even by blowing of wind, and upon germination they release primary zoospores transmitting the virus. Zoospores inject their contents into the cytoplasm of root cells where new plasmodia are formed. With the furovirus SBWMV, immunolabeling and *in situ* RNA hybridization tests have recently revealed the presence of viral movement protein and RNA but not of viral coat protein in the resting spores of *P. graminis.* This might suggest that the vector does not transmit virions but rather a ribonucleoprotein complex possibly formed by the viral movement protein and the viral RNAs. It remains to be shown whether benyviruses are transmitted in a similar manner. The RNA-binding ability of P42 movement protein and further properties of the other two benyviral movement proteins as well as the additional involvement in the transmission process of two transmembrane regions found in the coat protein read-through proteins of benyviruses and other *Polymyxa*-transmitted viruses are described in the section 'Organization of the genome and properties of the encoded proteins'.

RSNV-harboring cystosori may be carried in soil adhering to the surface of rice seeds, but true seed transmission has not been observed. The natural mode of transmission of BdMV is unknown. *P. betae* and the aphid species *Myzus persicae* and *Macrosiphum gobonis* failed to transmit a BdMV isolate obtained from leaves of *Arctium lappa.*

Under experimental conditions, benyviruses are readily transmitted mechanically from test plants to test plants. However, attempts to transmit BNYVV mechanically from sugar beet rootlets to test plants may not always be successful.

Control

Polymyxa-transmitted plant viruses may survive in soil in the long-living resting spores of the vector for many years,

probably even decades. The diseases caused by these viruses are, therefore, more difficult to control than those caused by, for example, insect-transmitted viruses. Chemical control of these soil-borne diseases, for example, by soil treatment with methyl bromide, is neither efficient nor acceptable for economic and ecological reasons. Growing resistant or tolerant varieties currently represents the only practical and environmentally friendly means to lower the impact of theses diseases on yield. BNYVV-tolerant or partially resistant sugar beet varieties are now available which enable good yields also on infested fields. Their resistance is based mainly on the *Rz1* gene from a sugar beet line ('Holly' resistance) and the *Rz2* gene from the *Beta maritima* line WB42. In some locations, there are indications of a breakdown of the *Rz1*-mediated resistance. Genes which would confer immunity against BNYVV to sugar beet have not been found so far. Vector resistance has been detected in *Beta patellaris* and *Beta procumbens*, but attempts to develop agronomically acceptable sugar beet cultivars resistant to *Polymyxa* have so far failed. The degree of resistance or tolerance to RSNV differs in different rice cultivars. A high degree of resistance is found in *Oryza glaberrima.* The genes responsible for this resistance have been transferred to cultivated rice in breeding programs.

Genetically modified sugar beets expressing various portions of the BNYVV genome have been shown to be highly resistant to BNYVV, but have not yet been utilized commercially. Genome recombinations have not been observed when A-type BNYVV coat protein gene-expressing beets were grown in soil infested with B-type BNYVV-carrying *P. betae.*

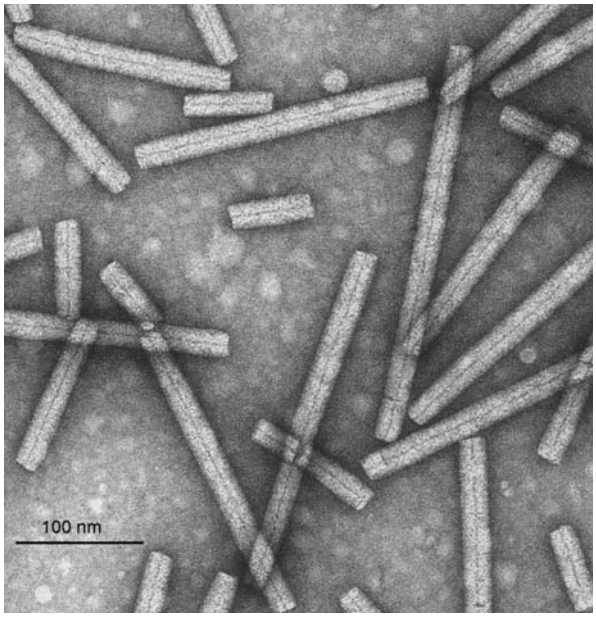


Figure 1 Particles of *Beet necrotic yellow vein virus* in a purified preparation negatively stained with uranyl acetate. Courtesy D. E. Lesemann and J. Engelmann, BBA, Braunschweig, Germany.

Particle Properties and Relations of Particles with Cells

Benyvirus virions are nonenveloped rods which have a helical symmetry (**Figure 1**). The diameter of the particles is *c.* 20 nm and they usually show several length maxima ranging from *c.* 80 to 400 nm depending on the RNA species encapsidated. Additional length maxima may be due to end-to-end aggregation or breakage of particles. The right-handed helix of BNYVV particles has a 2.6 nm pitch with an axial repeat of four turns involving 49 subunits of the *c.* 21 kDa major coat protein which consists of 188 amino acids. Each coat protein subunit occupies four nucleotides on the RNAs of BNYVV. The 75 kDa coat protein readthrough protein (**Figure 2**) has been detected by immunogold-labeling on one end of particles in freshly extracted plant sap. It is believed to act as a minor coat protein which initiates the encapsidation process.

Green fluorescent protein (GFP)-labeled particles of BNYVV were shown to localize to the cytoplasmic surface of mitochondria early during infection, but later they are relocated to semi-ordered clusters in the cytoplasm. In ultrathin sections, BNYVV particles are found scattered throughout the cytoplasm or occur in aggregates. More or less dense masses of particles arranged in parallel or angle-layer arrays may be formed. Membranous accumulations of endoplasmic reticulum may also be found.

Antigenic Properties

Benyviruses are moderately to strongly immunogenic. Polyclonal antibodies have been obtained for BNYVV, BSBMV, and RSNV. BNYVV and BSBMV are only very distantly related serologically – some antisera may fail to detect this relationship. Particles of RSNV and BdMV do not react with antisera to BNYVV. Monoclonal antibodies to BNYVV have been used for diagnostic purposes and for determining the accessibility of stretches of the coat protein amino acid chain on the virus particles. The C-terminal amino acids 182–188 which are readily cleaved off by treatment with trypsin are exposed along the entire surface of the particles whereas amino acids 42–51 and 156–121 are accessible only on one end of the particles; amino acids 125–140 are located inside and are exposed only after disrupting the particles. Antibody single-chain fragments (scFv's) have been expressed in *Escherichia coli* and *N. benthamiana.*

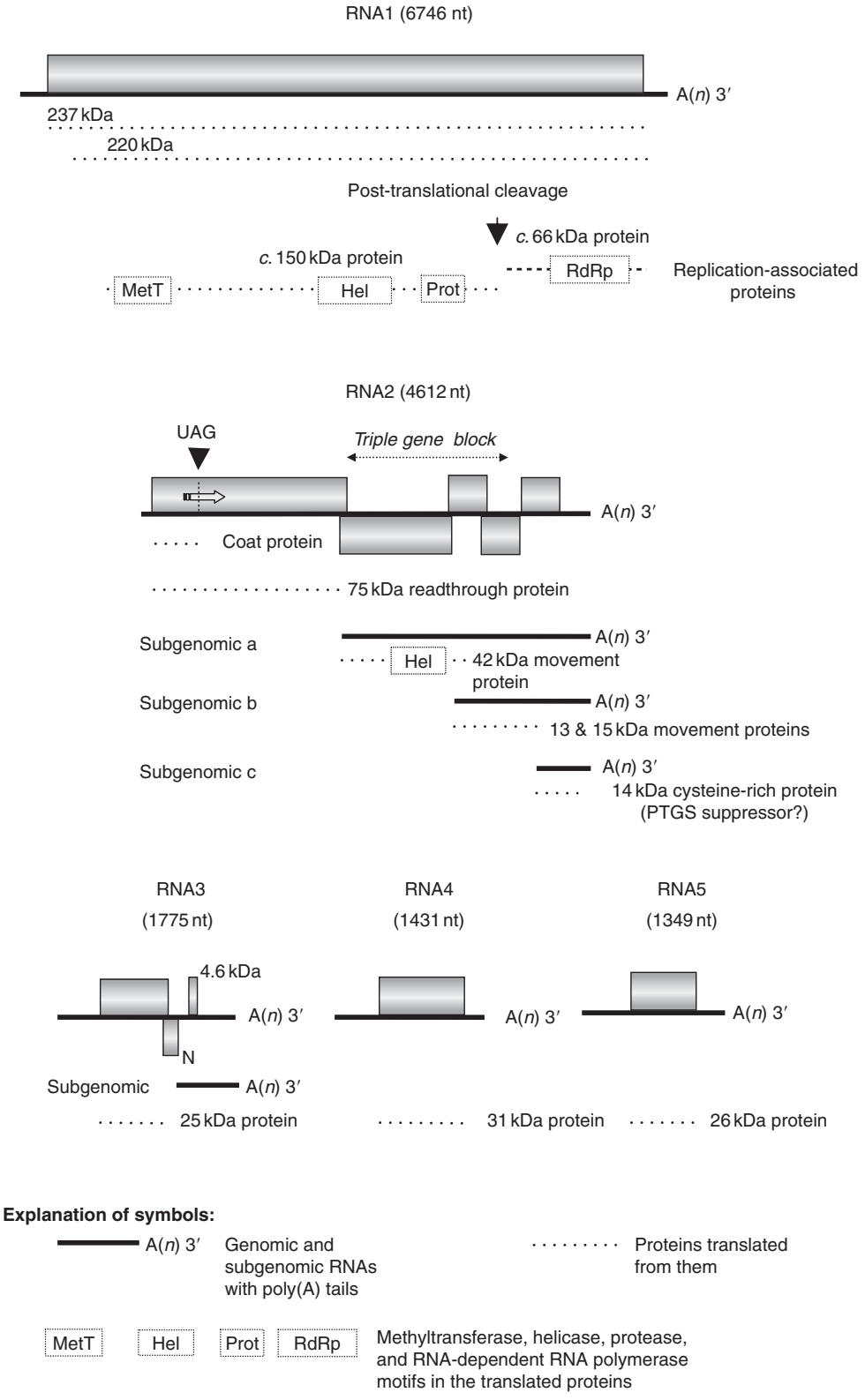


Figure 2 Organization of the BNYVV genome and expression of BNYVV genes.

Nucleic Acid Properties and Interrelationships between Benyviruses

Benyvirus genomes consist of two to five molecules of linear positive-sense single-stranded RNAs (ssRNAs) which terminate in a 3′ poly(A) tail. In naturally infected sugar beets, the BNYVV genome consists of four and in some isolates five RNAs of *c.* 6.7, 4.6, 1.8, 1.4, and 1.3 kb (**Figure 2**). Complete or partial nucleotide sequences have been determined for several isolates. After repeated mechanical transfers to local lesion hosts, BNYVV isolates often contain only partially deleted forms of their RNAs 3, 4, and/or 5, or they lose these small RNAs altogether. RNA1 and RNA2 contain all the genetic information necessary to enable multiplication, encapsidation, and cell-to-cell movement on local lesion hosts (see below); the additional presence of RNA3 and RNA4, however, is essential for vector-mediated transmission and disease development in sugar beet roots. RNA5 which is found only in limited areas increases the virulence of the virus.

Four genomic RNA species have also been identified for BSBMV and RSNV, but only two for BdMV. The BdMV isolate might possibly have lost additional small RNAs after prolonged cultivation on *C. quinoa* as do isolates of BNYVV. The complete sequence has been published for all four RNAs of an isolate of BSBMV. The complete sequences of RNA1 (*c.* 7.0 kb) and RNA2 (*c.* 4.3 kb) of BdMV and a partial sequence of RSNV RNA1 (2239 nt) have also been determined but not yet released.

Available data indicate that the genome organization is similar for all four viruses. Highest nucleotide sequence identities are found in the RNA1-encoded replication-associated proteins (*c.* 84% for the pair BNYVV/BSBMV) and the RNA2-encoded second triple gene block (TGB) proteins (*c.* 82% for the pair BNYVV/BSBMV; *c.* 50% for the pair BNYVV/BdMV), but the lowest ones in the RNA2-encoded cysteine-rich proteins, which are presumably RNA-silencing suppressors (*c.* 38% for the pair BNYVV/BSBMV; *c.* 20% for the pair BNYVV/BdMV).

Organization of the Genome and Properties of the Encoded Proteins

The genome organization of BNYVV is outlined in **Figure 2**. BNYVV RNA1 contains one large open reading frame (ORF) for a replication-associated protein which is cleaved autocatalytically by a papain-like proteinase. In *in vitro* systems, its translation may start either at the first AUG at position 154 or at a downstream AUG at position 496. The resulting proteins of 237 and 220 kDa, respectively, contain in their N-terminal part methyltransferase motifs (MetT), in their central part helicase (Hel) and papain-like protease motifs (Prot), and in their C-terminal part RNA-dependent RNA polymerase (RdRp) motifs. BNYVV RNA2 contains six ORFs, viz. the coat protein gene which is terminated by a suppressible UAG stop codon, the coat protein readthrough protein gene, a TGB coding for proteins of 42, 13, and 15 kDa involved in viral movement, and a gene coding for a 14 kDa cysteine-rich protein, a putative RNA silencing suppressor. The N-terminal part of the 75 kDa coat protein readthrough protein is apparently necessary for initiating encapsidation (see section 'Particle properties and relations of particles with cells'), the C-terminal part for enabling transmission by *P. betae*. A KTER motif in positions 553–556 of the 75 kDa coat protein readthrough protein is essential for efficient transmission of the virus by *P. betae*. After prolonged cultivation on a local lesion host, the area containing this motif may be lost. Computer analyses have revealed the presence of two complementary transmembrane domains (TM1 and TM2) in the coat protein readthrough proteins not only of benyviruses, but also of the likewise *Polymyxa*-transmitted furoviruses and pomoviruses and in the P2 proteins of the *Polymyxa*-transmitted bymoviruses. The second domain is absent or disrupted in deletion mutants which are not vector transmitted. The TM helices are apparently tightly packaged with ridge/groove arrangements between the two helices and strong electrostatic associations. It has been suggested that they facilitate the movement of the virus across the membrane surrounding the plasmodia in plant cells. Benyvirus TM1 and TM2 helices are identical in length, each consisting of 23 amino acids. The E in the KTER motif is highly conserved in position −1 of TM 2.

Three subgenomic RNAs are derived from BNYVV RNA2. The first TGB protein (p42) is translated from subgenomic RNA2a, the second and third TGB proteins (P13 and P15) from the bicistronic subgenomic RNA2b, and the 14 kDa cysteine-rich protein from subgenomic RNA2c (**Figure 2**). The three TGB proteins can be functionally substituted in *trans* by the 30 kDa movement protein of tobacco mosaic virus or by the three TGB proteins of peanut clump virus when these are supplied together but not when they are substituted for their BNYVV counterparts one by one. This suggests that highly specific interactions among cognate TGB proteins are important for their function and/or stability *in planta*. The N-terminal part of the first (42 kDa) TGB protein has nucleic acid-binding activity; its C-terminal part contains helicase motifs. P42 labeled with GFP on its N-terminus is targeted by the second and third TGB proteins (P15 and P13) to punctuate bodies associated with plasmodesmata. It has been speculated that P15 and P13 provide a docking site for a P42–viral RNA complex at the plasmodesmata where P42 alters the plasmodesmatal size exclusion limit and potentiates transit of viral RNA.

The typical rhizomania symptoms in beet are produced only in the presence of BNYVV RNA3. The 25 kDa protein (P25) encoded by this RNA acts as a movement protein in beet roots and *Beta macrocarpa* and is also

responsible for the production of bright yellow rather than pale green local lesions on *C. quinoa*. P25 has an amino acid tetrad in positions 67–70 which is highly variable in virus isolates originating from different geographical areas, especially in A-type BNYVV. A connection between the composition of this tetrad and the severity of symptoms in beets has been discussed, but not yet proven. P25 is found in the cytoplasm as well as in the nuclei of infected cells. A nuclear localization signal has been identified on its amino acids 57–62 and a nuclear export sequence within the region of amino acids 169–183. The four valine residues in positions 169, 172, 175, and 178 are all necessary for nucleocytoplasmic shuttling. Mutants that lack this shuttling ability fail to produce yellow lesions on *C. quinoa*. P25 has also a zinc-finger motif in positions 73–90. The small N gene (**Figure 2**) is not detectably expressed from full-length RNA3, but it is translationally activated by the deletion of upstream sequences which positions it closer to the 5′ end of RNA3. Expression of the N gene induces the formation of necrotic lesions in BNYVV infections and also when it is expressed by another plant virus (i.e., cauliflower mosaic virus). The function of the RNA3-encoded 4.6 kbp ORF, which is presumably expressed from an abundantly present subgenomic RNA, is not known. RNA5 enhances symptom expression in sugar beet. Scab-like symptoms rather than root proliferation have been observed with a recombinant virus containing RNA5, but lacking RNA3. Addition of P-type RNA5 to RNA1 and -2 results in the formation of necrotic local lesions in *C. quinoa*. The RNA5-encoded P26 apparently occurs mainly in the nucleus as suggested by studies with fluorescent fusion proteins. BNYVV RNA4 greatly increases the transmission rate of the virus by *P. betae*. RNA3 and RNA4 or RNA5 and RNA4 may act in a synergistic way. Some authors have classified BNYVV RNA5 as a satellite RNA and RNAs 3 and 4 as satellite-like RNAs.

Diagnosis

Symptoms caused by benyviruses in the field can easily be confused with those produced by other causes, for example, nematodes, soil-borne fungi, or nitrogen deficiency. Enzyme-linked immunosorbent assay, immunoelectron microscopy, and PCR techniques, such as immunocapture reverse transcription-PCR, are useful for diagnosing benyvirus infections in plant parts likely to be infected (e.g., root beards of sugar beets). Infestation of soil may be detected by means of bait plants which are tested for the presence of virus by the above techniques. PCR also allows the detection of genome deletions and specific primers can be designed to differentiate the various BNYVV types (A type, B type, etc.), which are undistinguishable serologically. Even more detailed information is obtained by sequence analyses of PCR products. The sensitivity of BNYVV detection has been increased by using nested primers and real-time PCR.

Similarities and Dissimilarities with Other Taxa

The morphology of benyviruses resembles that of other rod-shaped viruses, that is, of furoviruses, pecluviruses, pomoviruses, hordeiviruses, tobraviruses, and tobamoviruses. The CPs of these viruses have a number of conserved residues, for example, RF and FE in their central and C-terminal parts, respectively, which are presumably involved in the formation of salt bridges. The benyvirus genomes – with the possible exception of the BdMV genome – consist of at least four RNA species, whereas the tobamoviruses have monopartite, the furoviruses, pecluviruses, and tobraviruses bipartite, and the pomoviruses and hordeiviruses tripartite genomes. The fact that the RNAs of benyviruses are polyadenlylated differentiates them from the RNAs of the other viruses listed above. Benyviruses have a single large ORF on their RNA1: it codes for a polypetide which is cleaved post-translationally to yield two replication-associated proteins (**Figure 2**). This also differentiates the benyviruses from the other viruses listed above which have their replication-associated proteins encoded by two ORFs located either on two different RNA species (hordeiviruses) or on the same RNA where an ORF-1 is terminated by a leaky stop codon and extends into an ORF-2 (tobamoviruses, tobraviruses, furoviruses, pecluviruses). Benyviruses like pomo-, peclu-, and hordeiviruses, but unlike furo-, tobamo-, and tobraviruses, have their movement function encoded on a TGB. Sequence identities in the first and second TGB-encoded proteins reveal affinities not only to pomo- and hordeiviruses, but also to potex- and carlaviruses. The methyltransferase, helicase, and RdRp motifs in the putative replication-associated proteins of benyviruses show a higher degree of similarity to those of hepatitis virus E (genus *Hepevirus*) and rubella virus (family *Togaviridae*) than to those of other rod-shaped plant viruses.

See also: Cereal Viruses: Rice; *Furovirus*; *Pomovirus*; Satellite Nucleic Acids and Viruses; Vector Transmission of Plant Viruses.

Further Reading

Adams MJ, Antoniw JF, and Mullins JG (2001) Plant virus transmission by plasmodiophorid fungi is associated with distinctive transmembrane regions of virus-encoded proteins. *Archives of Virology* 146: 1139–1153.

Erhardt M, Vetter G, Gilmer D, *et al.* (2005) Subcellular localization of the triple gene block movement proteins of beet necrotic yellow vein virus by electron microscopy. *Virology* 340: 155–166.

Harju VA, Skelton A, Clover GR, *et al.* (2005) The use of real-time RT-PCR (TaqMan) and post-ELISA virus release for the detection of beet necrotic yellow vein virus types containing RNA 5 and its comparison with conventional RT-PCR. *Journal of Virological Methods* 123: 73–80.
Koenig R and Lennefors BL (2000) Molecular analyses of European A, B and P type sources of beet necrotic yellow vein virus and detection of the rare P type in Kazakhstan. *Archives of Virology* 145: 1561–1570.
Koenig R and Lesemann D-E (2005) Benyvirus. In: Fauquet CM, Mayo M, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1043–1048. San Diego, CA: Elsevier Academic Press.
Lee L, Telford EB, Batten JS, Scholthof KB, and Rush CM (2001) Complete nucleotide sequence and genome organization of beet soil-borne mosaic virus, a proposed member of the genus *Benyvirus*. *Archives of Virology* 146: 2443–2453.
Link D, Schmidlin L, Schirmer A, *et al.* (2005) Functional characterization of the beet necrotic yellow vein virus RNA-5-encoded p26 protein: Evidence for structural pathogenicity determinants. *Journal of General Virology* 86: 2115–2125.
Meunier A, Schmit JF, Stas A, Kutluk N, and Bragard C (2003) Multiplex reverse transcription-PCR for simultaneous detection of beet necrotic yellow vein virus, beet soil-borne virus, and beet virus Q and their vector *Polymyxa betae* KESKIN on sugar beet. *Applied and Environmental Microbiology* 69: 2356–2360.
Ratti C, Clover GR, Autonell CR, Harju VA, and Henry CM (2005) A multiplex RT-PCR assay capable of distinguishing beet necrotic yellow vein virus types A and B. *Journal of Virological Methods* 124: 41–47.
Rush CM (2003) Ecology and epidemiology of benyviruses and plasmodiophorid vectors. *Annual Review of Phytopathology* 41: 567–592.
Rush CM, Liu HY, Lewellen RT, and Acosta-Leal R (2006) The continuing saga of rhizomania of sugar beets in the United States. *Plant Disease* 90: 4–15.
Tamada T (2002) Beet necrotic yellow vein virus. *AAB Descriptions of Plant Viruses* 391. http://www.dpvweb.net/dpv/showadpv.php?dpvno=391 (accessed July 2007).
Valentin C, Dunoyer P, Vetter G, *et al.* (2005) Molecular basis for mitochondrial localization of viral particles during beet necrotic yellow vein virus infection. *Journal of Virology* 79: 9991–10002.
Vetter G, Hily JM, Klein E, *et al.* (2004) Nucleo-cytoplasmic shuttling of the beet necrotic yellow vein virus RNA-3-encoded p25 protein. *Journal of General Virology* 85: 2459–2469.
Ward L, Koenig R, Budge G, *et al.* (2007) Occurrence of two different types of RNA 5-containing beet necrotic yellow vein virus in the UK. *Archives of Virology* 152: 59–73.

Beta ssDNA Satellites

R W Briddon and S Mansoor, National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Koch's postulates Criteria designed to establish a causal relationship between a causative microbe and a disease.
Post-transcriptional gene silencing (PTGS) An epigenetic process of gene regulation that results in the mRNA of a particular gene being destroyed. PTGS is believed to protect the organism's genome from, among other things, transposons and viruses.
Rolling-circle replication A process of DNA replication that synthesizes multiple copies of circular molecules of DNA. Rolling-circle replication is initiated by an initiator protein which nicks one strand of the double-stranded, circular DNA molecule. The initiator protein remains bound to the 5′-phosphate end of the nicked strand, and the free 3′-hydroxyl end is released to serve as a primer for DNA synthesis using the un-nicked strand as a template; replication proceeds around the circular DNA molecule, displacing the nicked strand as single-stranded DNA.

Introduction

The DNA β components are a diverse group of symptom-modulating, single-stranded DNA (ssDNA) satellites associated with begomoviruses. These begomovirus–DNA β complexes cause some of the economically damaging diseases of crops across Asia including cotton leaf curl disease (CLCuD, an epidemic of which decimated cotton production in Pakistan and India during the 1990s, and is making a resurgence in recent times) and leaf curl diseases of tomato, tobacco, peppers, and papaya, as well as affecting numerous ornamental plants and weeds. Now, the DNA β components are classified as satellite nucleic acids in the satellite subviral agents.

Satellites

Satellites are defined as viruses or nucleic acids that depend on a helper virus for their replication but do not have extensive nucleotide sequence similarity to their helper virus and are dispensable for its proliferation. Satellite viruses encode a structural protein that encapsidates its own nucleic acid while satellite nucleic acids rely

on the helper virus structural protein for encapsidation and do not necessarily encode additional nonstructural proteins. A third type of agent, referred to as satellite-like nucleic acid, also depends on the helper virus for its replication but provides a function that is necessary for the biological success of the helper virus and is therefore considered as being part of the helper virus genome. The vast majority of these satellites consists of RNA and is associated with viruses which have genomes of RNA. Most satellites do not encode functional proteins but can nevertheless have a dramatic effect on the symptoms induced by their helper viruses, ranging from symptom amelioration to an increase in symptom severity.

The DNA β Satellites of Begomoviruses

Identification of the Begomovirus Satellites

The first begomovirus satellite discovered, referred to as tomato leaf curl virus-sat (ToLCV-sat), was identified in tomato plants infected with the monopartite begomovirus tomato leaf curl virus (ToLCV) originating from Australia. The component is a small (682 nt) circular ssDNA with no extensive open reading frames (ORFs) and sequence similarity to its helper virus limited to sequences within the apex of two stem–loop structures. The first contains the ubiquitous geminivirus nonanucleotide (TAATATTAC) motif and is thus similar, in both structure and sequence, to the origin of replication of geminiviruses. The second is unique to this satellite and contains a putative ToLCV replication-associated protein (Rep) binding motif. ToLCV-sat is not required for ToLCV infectivity and has no effect on the symptoms induced by the helper virus but is dependent on the helper begomovirus for its replication and encapsidation and hence has the hallmarks of a satellite DNA.

During the early 1990s, there was some astonishment within the geminivirus community with the revelation that some begomoviruses could apparently dispense with the second genomic component, DNA B. Two whitefly-transmitted viruses causing tomato leaf curl disease, a disease of significant economic importance and at the time confined to the Mediterranean Basin, were shown to have genomes consisting of only a single component. These viruses are now known as tomato yellow leaf curl virus (TYLCV) and tomato yellow leaf curl Sardinia virus (TYLCSV). Up to this point, all characterized begomoviruses – including African cassava mosaic virus, tomato golden mosaic virus, and bean golden mosaic virus (now known as bean golden yellow mosaic virus) – were shown to have genomes consisting of two components, both of which are essential for infection of hosts. There was some reluctance to accept this finding until Koch's postulates were conclusively satisfied, which ultimately they were.

Subsequent to the identification of TYLCV and TYLCSV, numerous apparently monopartite begomoviruses were identified. However, for a number of these viruses Koch's postulates could not be satisfied at the time. Although viruses such as ageratum yellow vein virus (AYVV) and cotton leaf curl Multan virus (CLCuMV) were infectious to experimental hosts, they were not, or only poorly, infectious to the hosts from which they were isolated (*Ageratum conyzoides* and *Gossypium hirsutum* (cotton), respectively) and did not induce the symptoms characteristic of the diseases with which they are associated. This inability to fulfill Koch's postulates was the first indication that, for some monopartite begomoviruses, a single component was not the entire infectious unit. All attempts to identify a DNA B component were unsuccessful and the hunt for additional components was initiated.

Plants infected with geminiviruses frequently contain less than unit length (less than 2800 nt) circular molecules derived from the virus component(s). These are deletion mutants which retain the origin of replication and are *trans*-replicated by the virus. Their presence in some cases leads to symptom attenuation, due to competition for cellular resources, and these components are for this reason known as defective interfering DNAs (diDNAs). diDNAs are typically either one-half or one-quarter the size of the virus' genomic component(s), due to stringent size selection for encapsidation or, more likely, movement within the host plant. Since they are virus derived and play no part in the etiology of the disease (other than to attenuate symptoms), less-than-unit-length molecules encountered in plants were frequently disregarded. However, in the late 1990s, a less-than-unit-length molecule was identified with some unusual characteristics. Associated with CLCuD, this molecule was half unit length, encoded a Rep (a rolling-circle replication initiator protein), and was capable of autonomous replication. Surprisingly, the molecule, named DNA 1, showed no significant sequence similarity to geminiviruses (other than the presence of a hairpin structure containing the nonanucleotide sequence) but was instead related to the Rep-encoding components of a second group (now family) of phytopathogenic DNA viruses, the nanoviruses. Nanoviruses are multicomponent, ssDNA viruses that are transmitted by aphids. Each virus is believed to have a genome consisting of six to eight components, the majority of which encode only a single product. All the *bona fide* virus components are *trans*-replicated by the Rep encoded on one component known as the master Rep (mRep) component. In addition to the mRep component, many nanovirus isolates are also associated with a varying number of other Rep-encoding components. These satellite-like Rep-encoding components (slReps) are not required by the virus, are sequence-distinct from the mRep component, and capable of autonomous replication. It is likely that DNA 1 evolved from a nanovirus-associated slRep by a process of component capture, following infection of a plant with both

a geminivirus and a nanonovirus. The major differences between DNA 1 and the slRep are a slightly larger Rep-encoding gene and the presence of an adenine-rich (A-rich) region of sequence, which is likely required to raise the slRep from the size of a nanovirus component (~1000 nt) to half the size of a begomovirus component (~1400 nt). Subsequent to the identification of DNA 1 in CLCuD-affected cotton, this class of molecule was shown to be associated with many other monopartite begomoviruses, including AYVV. Unfortunately, DNA 1 proved to be an interesting 'red herring' in the search for the etiology of CLCuD and a number of other monopartite begomovirus-associated diseases. It was shown to play no part in symptom induction and to be dispensable to the helper begomovirus with which it is associated.

Encouraged by the identification of DNA 1, efforts were renewed to identify additional components among the less-than-unit-length DNAs associated with begomoviruses. The breakthrough came with the identification of a second ~1400-nt component associated with AYVV. This component was subsequently shown to be associated with numerous other monopartite begomoviruses that induce a wide range of symptoms across a diverse range of plant species (**Figure 1**). This group of molecules is now known as satellite DNA β.

Structure of Satellite DNA β

DNA β molecules have a highly conserved structure (**Figure 2**). They are typically in the region of 1350 nt in length, roughly half the size of their helper begomoviruses. Their sequences encode multiple small ORFs in both the virion and complementary-sense orientations. Of these, only one is conserved in both position and

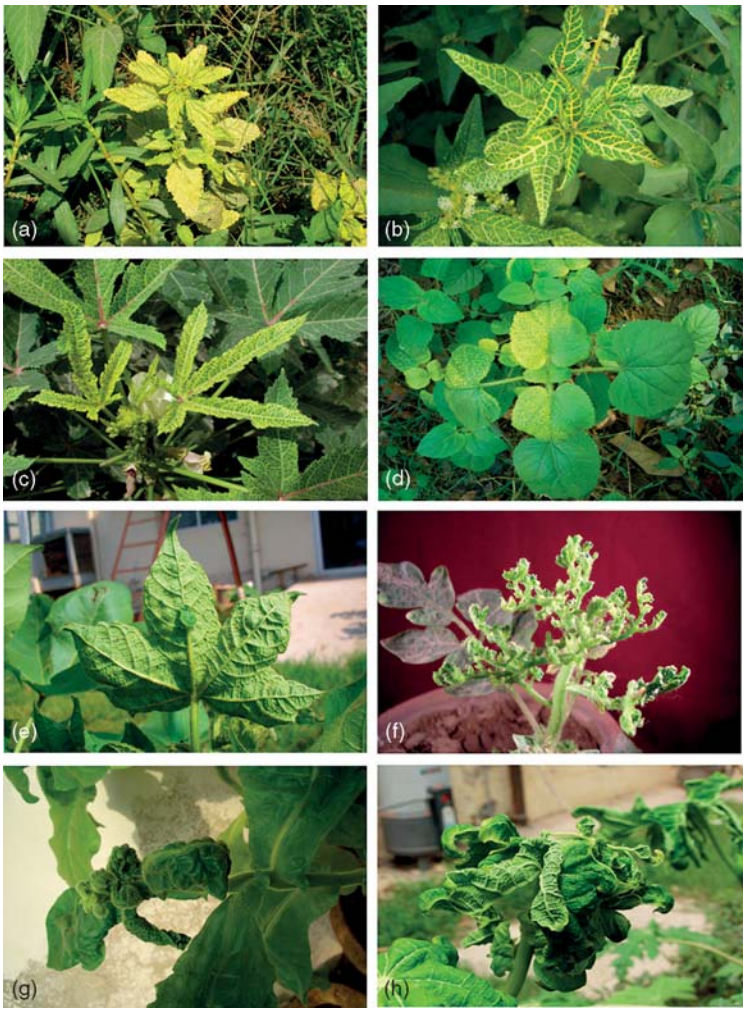


Figure 1 Typical symptoms induced by begomovirus associated with DNA β satellites. These fall essentially into two types: the yellow vein type, represented here by (a) *Malvastrum coromandelianum;* (b) *Croton* sp.; (c) okra; and (d) *Ageratum conyzoides*, and the leaf curl type illustrated by (e) cotton (*G. hirsutum)*; (f) tomato; (g) tobacco; and (h) papaya. Note the leaf-like enation on the cotton which is characteristic of CLCuD.

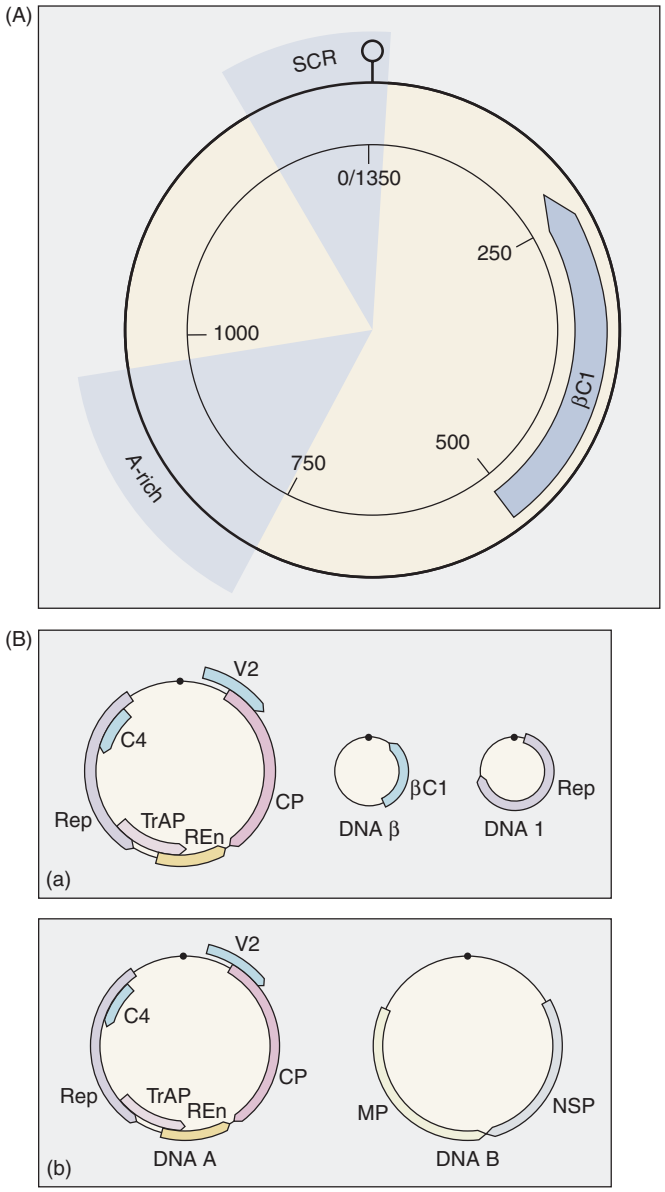


Figure 2 Features of the DNA β satellites (A) and components of begomovirus DNA β complexes (B, a) and bipartite begomoviruses (B, b). Approximate nucleotide positions of the features are shown within the circle in (A). The position and orientation of genes are indicated with filled arrows. These are labeled as the DNA β C1 gene (βC1), replication-associated protein (Rep), transcriptional activator protein (TrAP), replication enhancer protein (REn), coat protein (CP), movement protein (MP), and nuclear shuttle protein (NSP). The functions of the V2 and C4 genes remain unclear. Also shown are the A-rich and satellite conserved region (SCR) of DNA β. The DNA-β-associated begomoviruses frequently are also associated with the satellite-like DNA 1 (B, a). Monopartite begomoviruses lack the DNA β component present in bipartite viruses (B, b). The black dot (B) and small black circle (A) indicate the position of the predicted stem–loop structure, containing the nonanucleotide motif (TAA/GTATTAC), that marks the origin of replication for the geminiviruses and likely also for DNA β and DNA 1 (although this is yet to be confirmed experimentally).

sequence. Transcript mapping of the AYVV and CLCuD-associated DNA β molecules has identified a single major transcript spanning the conserved ORF; this is now known as the βC1 gene.

A sequence of *c.* 80–90 nucleotides highly conserved between all identified DNA β components occurs adjacent to the putative stem loop. This is referred to as the satellite conserved region (SCR). A set of 'universal primers', designed to the sequence of the SCR, for polymerase chain reaction (PCR)-mediated amplification of the components, has provided a simple and reliable diagnostic test for the presence of DNA β. These primers have also, in the majority of cases, been shown to produce clones suitable for infectivity studies and are a major factor in the rapid

identification of the large number of DNA β components. The SCR contains the stem–loop structure that, for the majority of the DNA β, contains the TAATATTAC motif. A small number have the nonanucleotide sequence TAGTATTAC, more typical of the nanoviruses. For the geminiviruses, the nonanucleotide marks the origin of virion-strand DNA replication that is nicked by virus-encoded Rep to initiate rolling-circle replication. For the DNA β components, this is yet to be confirmed.

DNA β components share, in addition to the nonanucleotide-containing hairpin structure and βC1, one further feature, a region of sequence rich in adenine. The function (if any) of this sequence to the satellite remains unclear. Deletion of the sequence does not unduly disable the component; it remains capable of *trans*-replication and maintenance by the helper begomovirus. The most plausible hypothesis for the presence of the A-rich sequence is that, like DNA 1, this may be required to increase the size of a preexisting component to half that of a begomovirus. This, in turn, has implications for the possible origins of DNA β, as discussed below.

Functions Provided by Satellite DNA β to Its Helper Begomovirus

Early studies with the DNA β components associated with AYVV and CLCuMV showed that these satellites make an important contribution to the disease phenotype. Stable integration of a dimeric construct of the DNA β component associated with AYVV, and subsequently with that associated with CLCuMV, into *Nicotiana benthamiana* resulted in transgenic plants with severe developmental abnormalities, demonstrating that the components encode a pathogenicity determinant that is active in the absence of the helper virus. That the pathogenicity of the satellite is attributable to the βC1 ORF was initially provided by the analysis of naturally occurring mutants in which the entire βC1 coding sequence was deleted. The mutants were capable of *trans*-replication and systemic movement by the helper virus but did not induce the severe disease phenotype of the intact component. Transcript mapping of both the AYVV and CLCuMV DNA β satellites subsequently confirmed that only a single gene, on the complementary-sense strand, was present. More direct evidence for the activity of βC1 was provided by studies involving the disruption of the coding sequence and the production of transgenic *N. benthamiana* constitutively expressing the gene. For AYVV DNA β, replacement of the first in-frame methionine codon with a nonsense codon resulted in attenuated symptoms. It is likely that in this case initiation of translation occurs from a downstream methionine codon. Constitutive expression of βC1 resulted in transgenic plants with severe developmental abnormalities, resembling the plants transformed with dimeric DNA β constructs.

Significant advances have been made in determining the function of the only gene encoded by the DNA β components. A general feature of DNA β components is that they enhance the systemic accumulation of their helper begomoviruses. However, at least for AYVV DNA β, no significant increase in the accumulation of AYVV is seen in *N. benthamiana* leaf disk assays. This may suggest that βC1 is involved in virus movement, possibly in a host-specific manner, and is supported by the finding that CLCuMV DNA β can complement the DNA B functions of the bipartite tomato leaf curl New Delhi virus (ToLCNDV), as well as mutants of the V1 and C4 genes of ToLCV (the V1 and C4 genes have been implicated in virus movement). However, the nuclear localization of βC1, demonstrated by green fluorescent protein (GFP)-tagging the βC1 of the DNA β associated with tomato yellow leaf curl China virus (TYLCCNV), would seem to disagree with a role for βC1 in mobilizing viral DNA across the plasma membrane. βC1 does appear to have a role in overcoming host defenses. The βC1s encoded by the TYLCCN- and CLCuM-encoded DNA β components have been shown to suppress post-transcriptional gene silencing (PTGS). PTGS is a double-stranded RNA (dsRNA)-induced response that targets and degrades foreign nucleic acid sequences and is believed to be part of the plant's defense against viruses and other pathogens. The TYLCCNV DNA βC1 has additionally been shown to bind both ssDNA and double-stranded DNA (dsDNA) in a sequence-nonspecific manner.

Diversity and Geographic Distribution of the DNA β Components

More than 200 full-length DNA β sequences have been deposited in the databases since they were first identified – an indication of the widespread nature, agricultural importance, and ease with which the components can be cloned and sequenced. So far, no DNA β components have been identified in association with viruses originating from the New World where monopartite begomoviruses are not known to occur. Similarly no DNA β components have been identified in Australia, although the identification of the DNA β remnant, ToLCV-sat, may indicate that at some time in the past they were present in this region. With the exception of these two areas, DNA β components have been identified in all tropical and subtropical areas, including Africa, the Middle East, Asia, and the Far East. Based on presently available numbers, the center of diversity for DNA β appears to lie either in South Asia (southern China and the Indian subcontinent) or in Southeast Asia, possibly pinpointing these regions as the center of origin.

The DNA β components fall into two major groups, those isolated from plant species of the family

Malvaceae and those associated with plants of other families. Although it is common to encounter malvaceous DNA βs in non-malvaceous hosts in the field, it is rare to identify non-malvaceous DNA β components in plants of the Malvaceae. The most prominent example of this is the DNA β associated with Sida yellow mosaic China virus in southern China. This is a typical non-malvaceous DNA β that occurs in the malvaceous species *Sida acuta* in the field (**Figure 3**). However, some doubt remains as to whether this begomovirus–DNA β pair can cause disease in *S. acuta*, since infectivity of the virus and DNA β clones to *S. acuta* was not shown experimentally.

The DNA β molecules identified from Africa differ significantly from their Asian counterparts. So far only satellites of the malvaceous type have been identified from this continent, possibly indicating that all DNA βs present in Africa originated from malvaceous plants. However, the relatively small number of DNA β sequences available from Africa may be misleading in this respect. Nevertheless, this finding does indicate that DNA β molecules have diversified due to geographical isolation.

It is likely that DNA β components also have a degree of host adaptation. For the DNA β components causing CLCuD on the Indian subcontinent, the satellite prevalent in *G. hirsutum* is distinct from that identified in *G. barbadense* (77% overall nucleotide sequence identity and 80.5% amino acid sequence similarity of the βC1 gene product). Neither of these cotton species is indigenous to the subcontinent, suggesting that the DNA β components and their helper begomoviruses evolved from other, possibly weed-infecting progenitors. The relatively close relationship between the DNA β components of *G. hirsutum* and *G. barbadense* to components isolated from *Malvastrum coromandelianum*, a pantropical weed, suggests that this species

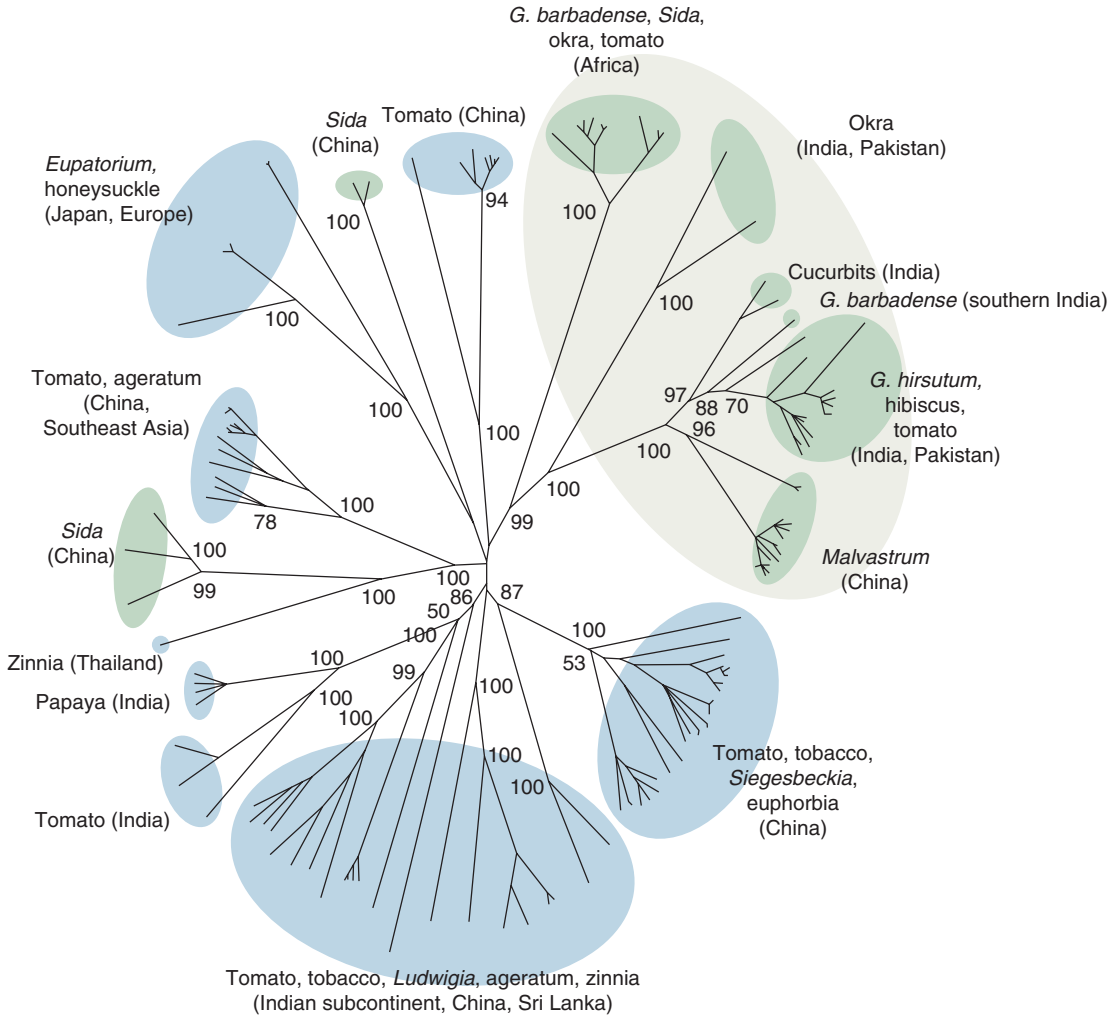


Figure 3 Phylogenetic dendrogram based upon an alignment of 170 selected full-length DNA β sequences. The geographic origins and hosts from which the DNA β components were isolated are indicated. Clusters of DNA βs that include malvaceous hosts are highlighted in dark green, those that do not are highlighted in blue. The 'Malvaceae' group of DNA βs are shown in a light green oval. Numbers at major nodes indicate percentage bootstrap confidence scores (1000 replicates).

may have been the host for the satellite which adapted to infect cotton. It is also interesting to note that the species of cotton native to the subcontinent (*G. arboreum* and *G. herbaceum*) are immune to CLCuD, likely indicative of a long association with the disease.

Complexity of DNA β Interaction with the Helper Begomoviruses

The first DNA β satellites to be isolated, those associated with AYVV and CLCuMV, have an obligate association with their helper begomoviruses. Although these viruses are capable of infecting plants in the absence of their satellite, in the field the virus is not found without the satellite. We can take this to indicate that these viruses are lacking in some way, and that the satellite provides an important, if not strictly essential, function to overcome this deficit in the competitive and demanding conditions of the 'wild'.

Recently, some begomoviruses have been identified which have a facultative association with their DNA β component. A recent study has shown that some isolates of tobacco curly shoot virus (TbCSV) are associated with a satellite whereas others are not. Cloned viruses from these isolates were shown to behave similarly, each being able to associate with DNA β component (yielding a more severe infection but not elevated viral DNA levels) as well as induce a symptomatic infection in the absence of the satellite. It is possible that TbCSV represents an evolutionary intermediate between the obligate satellite-associated begomoviruses and those that appear to be entirely solitary. However, it is unclear whether it is evolving to gain or lose the requirement for a DNA β component.

Even some strains of the archetypal monopartite begomovirus TYLCV have been shown to associate with DNA β in the field. The interaction in this case has not been analyzed in detail and it is as yet unclear whether this is a productive association – whether the DNA β concerned is contributing to the disease or is merely maintained as a nonfunctional satellite. Nevertheless, this is a disturbing finding. TYLCV is a global problem in tomatoes, having been spread by human hands from its probable place of origin (the Middle East/Mediterranean basin area) to the Far East, Australia, North Africa, Southern Europe, the Caribbean, and North and Central America. Few truly monopartite begomoviruses, for which no association with a DNA β has been shown, remain. These include TYLCSV and its close relatives in Southern Europe. It is likely that, in future, even these viruses will be shown, at some point during their evolution, to have had a relationship with this important group of satellites.

DNA β satellites, however, are not confined to monopartite begomoviruses. Isolates of the bipartite begomoviruses ToLCNDV and mungbean yellow mosaic India virus (MYMIV) have recently been identified in association with DNA β in the field. Both these viruses are prevalent in the Indian subcontinent, causing major losses to solanaceous (particularly tomato) and leguminous crops, respectively. Experimentally, the DNA A components have been shown to be complemented (for movement) by DNA β, and the presence of both the DNA B and DNA β results in a distinctive and more severe symptom phenotype.

A major question that remains to be answered is how DNA β satellites are *trans*-replicated by their helper begomoviruses. Current dogma maintains that Rep interaction with its cognate recognition sequences is highly specific. Thus the integrity of bipartite begomoviruses is maintained due to the presence, in both the DNA A and DNA B components, of a region of high sequence similarity (known as the 'common region' (CR)) which spans the origin of replication – encompassing both the nonanucleotide-containing hairpin structure and the Rep recognition sequences. However, DNA β components lack the Rep recognition sequences of their helper begomoviruses which raises the question as to how the recognition between helper virus-encoded Rep and DNA β genomic sequences functions. The presence of the SCR, which occurs in all DNA βs at a position equivalent to the CR of bipartite begomoviruses, may indicate that this has a function in Rep recognition. The specificity for *trans*-replication of DNA β components is also more relaxed than between cognate DNA A and DNA B components of a bipartite virus. The monopartite begomoviruses associated with CLCuD (eight identified to date), each having distinct predicted Rep-binding specificities, are all able to *trans*-replicate a single DNA β (CLCuD DNA β), and a single monopartite begomovirus, AYVV, has been shown to be able to *trans*-replicate the majority of DNA β in the model host *N. benthamiana*. Whether this is an adaptation by the DNA-β-associated viruses (by encoding a Rep with a more relaxed specificity) or an adaptation by DNA β (by encoding Rep-binding sequences recognized by a greater range of helper begomoviruses – a possible 'universal Rep recognition sequence') remains unclear. Whatever the underlying basis for the phenomenon, the ability to easily exchange components undoubtedly provides these pathogens with an evolutionary advantage.

Potential Origins of Satellite DNA β

While begomovirus-associated DNA 1 components undoubtedly derive from nanoviruses, the evolutionary origin of DNA β remains unclear. Like DNA 1 components, DNA β components contain an A-rich region (**Figure 2**), suggesting that they may have originated as a component of another, as-yet-unidentified or extinct, pathogenic agent prior to being captured by a begomovirus. If the

A-rich sequence is a 'stuffer', required to increase the size of the component, this would suggest that the pathogenic agent from which DNA β was captured had circular ssDNA and a genome (or genomic components) <1400 nt in size. The putative pathogenic agent need not necessarily have originated in plants. For example, ToLCV has been shown to replicate in *Agrobacterium*, a soil-borne prokaryote that can transfer exogenous DNA into plants where it becomes integrated into the nuclear genome, an observation that prompted the suggestion that geminiviruses may have originated from prokaryotic episomal replicons that undergo rolling-circle replication. The genomic components of the bipartite begomovirus abutilon mosaic virus (AbMV) have been shown to be associated with plastids as well as the nucleus, an observation that may reflect a past functional relationship with these prokaryotic-like endosymbionts. Phylogenetic analysis provides compelling evidence to suggest that vertebrate circoviruses (a family of circular ssDNA viruses) may have originated from nanoviruses, possibly facilitated by arthropod vector intermediaries. Hence, it is not inconceivable that genetic material can also be transferred in the opposite direction, to plants from animals or arthropods that feed on plants.

Interestingly, further symptom-modulating satellite molecules have recently been identified in cassava mosaic disease-affected cassava in East Africa. These are apparently unrelated to DNA β but are yet to be fully characterized.

See also: African Cassava Mosaic Disease; Bean Golden Mosaic Virus; Cotton Leaf Curl Disease; Nanoviruses; Satellite Nucleic Acids and Viruses; Tomato Yellow Leaf Curl Virus; Tomato Leaf Curl Viruses from India.

Further Reading

Briddon RW, Bull SE, Amin I, *et al.* (2003) Diversity of DNA β: A satellite molecule associated with some monopartite begomoviruses. *Virology* 312: 106–121.

Briddon RW and Stanley J (2006) Sub-viral agents associated with plant-infecting single-stranded DNA viruses. *Virology* 344: 198–210.

Dry IB, Krake LR, Rigden JE, and Rezaian MA (1997) A novel subviral agent associated with a geminivirus: The first report of a DNA satellite. *Proceedings of the National Academy of Sciences, USA* 94: 7088–7093.

Mansoor S, Zafar Y, and Briddon RW (2006) Geminivirus disease complexes: The threat is spreading. *Trends in Plant Science* 11: 209–212.

Tao X and Zhou X (2004) A modified satellite that suppresses gene expression in plants. *Plant Journal* 38: 850–860.

Birnaviruses

B Delmas, INRA, Jouy-en-Josas, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

LexA repressor Protease involved in the bacterial SOS response.
Lon protease ATP-dependent bacterial protease involved in cell homeostasis.
Rotifera Phylum of planktonic animals.

Introduction

Birnaviruses form a distinct family of double-stranded (ds) RNA viruses infecting animals as different as vertebrates (birds, fish), mollusks, insects, and rotifers. No birnavirus has been reported to infect mammals, including humans. The name 'birna' highlights two important features of these viruses: the *bi*segmented nature of their genome and *rna* the ribonucleic acid they contain. Due to their economic impact, mainly two birnaviruses have been studied: the infectious bursal disease virus (IBDV) that is responsible for important losses in the poultry industry, and the infectious pancreatic necrosis virus (IPNV) that infects salmonids and represents a main concern for the aquaculture of North European and North American countries and Japan. The first characterizations of these two viruses were carried out in the early 1960s and the family was established as a distinct entity of classification in 1986. Several other viruses with bisegmented dsRNA genomes have been isolated from rats, rabbits, and humans in the 1980s, and due to their genomic organization and specific morphological features, they have recently (2006) been reclassified from a genus of the *Birnaviridae* into a family of their own, the *Picobirnaviridae*. Picobirnaviruses will be described at the end of this article.

The *Birnaviridae*

Taxonomy and Classification

The family *Birnaviridae* contains four genera (**Table 1**): *Aquabirnavirus* (type species: IPNV), *Avibirnavirus* (type species: IBDV), *Blosnavirus* (type species: blotched snakehead

Table 1 Virus members in the family *Birnaviridae*

Genus	*Virus*	*Abbreviation*	*GenBank sequence accession number*	*PDB accession number*
Aquabirnavirus	Infectious pancreatic necrosis virus	IPNV	U56907, M58757	2PNM
	Tellina virus 2	TV-2	AF342730	
	Yellowtail ascites virus	YTAV	AB006783, AY129662	
	Marine birnavirus[a]	MABV	AY123970	
Avibirnavirus	Infectious bursal disease virus	IBDV	X92761, X92760	1 WCE, 1WCD, 2DF7, 2GSY, 2PGG, 2IMU
Blosnavirus	Blotched snakehead virus	BSNV	AJ459383, AJ459382	2GEF
Entomobirnavirus	Drosophila X virus	DXV	U60650, AF196645	
Unassigned viruses	Tellina virus 1	TV1	AJ920335, AJ920336	
	Rotifer birnavirus			

[a]Tentative member in the genus.

virus (BSNV) infecting blotched snakehead fish (*Channa lucius*)), and *Entomobirnavirus* (type species: drosophila X virus (DXV) of the fruit fly). While complete nucleotide sequences have been reported for a large number of IBDV and IPNV strains and isolates, only a single set of nucleotide sequences has been determined for the two genomic segments of each DXV and BSNV. Several representative GenBank and PDB accession numbers are listed in **Table 1**. Due to the lack of genome sequence information, several other tentative birnavirus species (tellina virus 1 (TV-1) and rotifer birnavirus (RBV)) have not yet been assigned to these genera. Cross-neutralization assays indicate that TV-1 and RBV are antigenically distant from other birnaviruses.

Virion Structure and Composition

The birnaviruses have nonenveloped isometric particles, approximatively 60 nm in diameter, with icosahedral symmetry (**Figure 1**). The IBDV capsids are single shelled and consist of 260 VP2 trimers (441 amino acids $\times$ 3), arranged in $T = 13laevo$ lattices. The crystal structure of IBDV virions has been determined to a resolution of 7 Å. Purified particles also contain another abundant protein, VP3 (257 amino acids) and several peptides that are not visible in the icosahedral shell. Different ratios of VP3 over VP2 have been reported in virions from different birnavirus species. VP1, the viral RNA-dependent RNA polymerase (RdRp, 879 amino acids), is covalently linked to the two genomic segments. In addition, free VP1 molecules are present within viral particles. The buoyant density of particles in cesium chloride gradients is 1.33 g ml^{-1}, a low value compared to 1.43 and 1.44 g ml^{-1} for the cores of other dsRNA viruses like reovirus and rotavirus, respectively, which enclose a similar volume for a genome three to four times bigger (see further ahead).

Genome Organization and Gene Expression

The genome organization and expression strategy of the birnaviruses are similar; each virus genome contains two dsRNA segments (segments A and B) that range in size from 2.9 to 3.4 kbp (**Figure 2**). Segment B contains a single open reading frame that encodes the viral polymerase VP1. Segment A contains two open reading frames: a large one encoding a polyprotein precursor pVP2-VP4-VP3 (in N- to C-terminal order) and an overlapping (in IPNV and IBDV, designated VP5) or internal (in DXV or BSNV) small reading frame encoding a 17–27 kDa protein. For BSNV, an additional polypeptide, designated [X], is encoded in frame between pVP2 (the VP2 capsid protein precursor) and VP4. VP4 is a protease that cleaves its own N- and C-termini in the polyprotein, releasing pVP2 and VP3 within the infected cell. This primary cleavage occurs co-translationally. Subsequently, serial cleavages are catalyzed by VP4 at the C-terminus of pVP2, yielding the mature VP2 protein and several (three or four) peptides that remain associated with the virion. This cleavage can be incomplete; pVP2 may also be found in purified virus, although VP2 predominates.

Virion Proteins and Peptides

The RdRp VP1

All the RdRps and many of the DNA-dependent polymerases employ a fold whose organization has been compared to the shape of a cupped right hand with three domains, termed fingers, palm, and thumb. Only the palm domain, composed of a four-stranded antiparallel β-sheet with two α-helices packed beneath, is well conserved among all of these enzymes. This domain contains several ordered sequence motifs, with motifs A, B, and C being the most prominent. The C motif contains the Asp-Asp dipeptide signature often preceded by a Gly. The VP1 molecule of birnaviruses and of two positive-strand RNA ('tetra-like') insect viruses share a unique

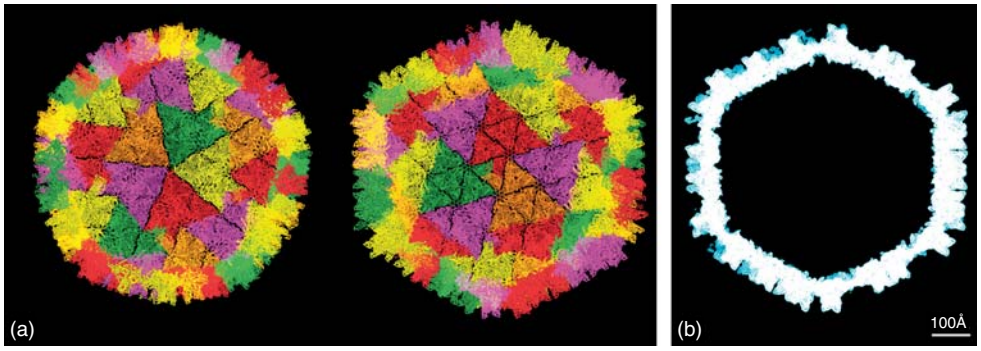


Figure 1 The IBDV virion. (a) Half portion of an IBDV particle viewed down the fivefold (left) and the threefold (right) axes. Five different colors (yellow, orange, red, green, and magenta) were used for the 60 icosahedral asymmetric units, distributed such that immediate neighbors are colored differently. A flat face of the icosahedron is formed by three colors. For simplicity, the front half of the virion has been removed, showing the concave internal face of the particle. Threefold and fivefold axes are located where three and five colors meet, respectively. (b) Vertical 200 Å thick electron density slab through the center of the IBDV particle, viewed down the threefold axis. Reproduced from Coulibaly F, Chevalier C, Gutsche I, *et al.* (2005) The birnavirus crystal structure reveals structural relationships among icosahedral viruses. *Cell* 120: 761, with permission from Elsevier.

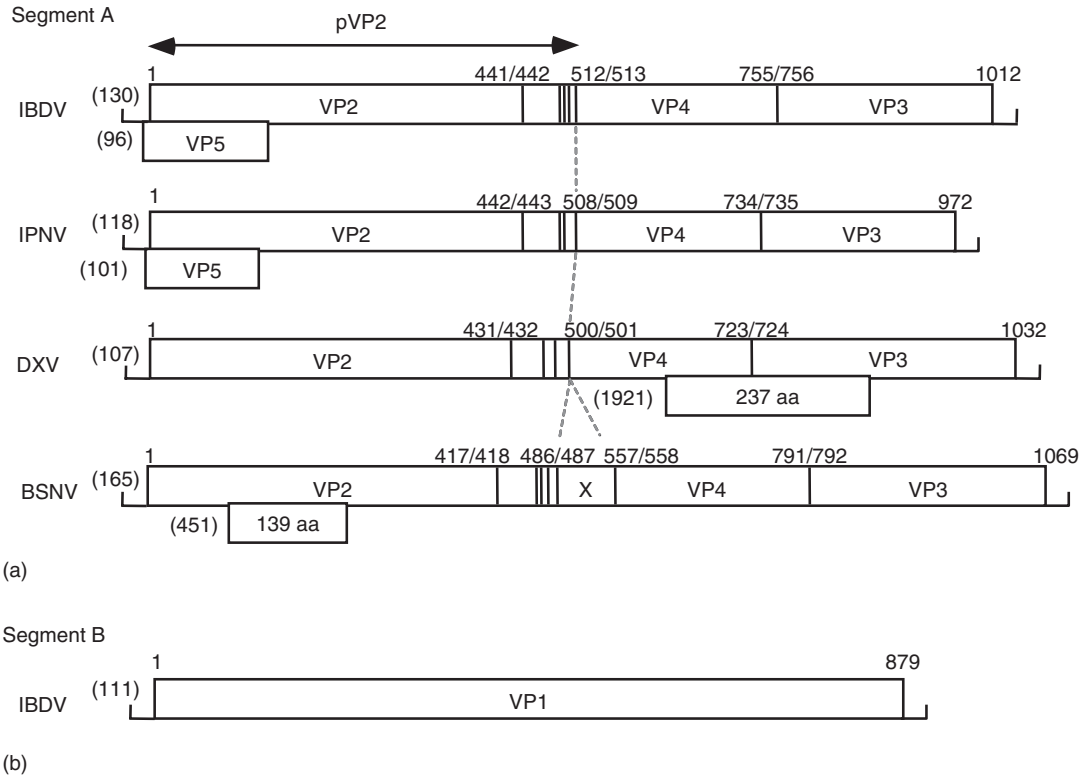


Figure 2 Genome organization of birnaviruses. (a) Schematic representation of the gene arrangement of genomic segment A of infectious bursal disease virus (IBDV), infectious pancreatic necrosis virus (IPNV), drosophila X virus (DXV) and blotched snakehead virus (BSNV). (b) Schematic representation of the genomic segment B of IBDV. The B segments of others birnaviruses have the same organization.

characteristic polymerase motif rearrangement not found in other viral RdRps. Computer-assisted sequence analysis and resolution of the 2.5 Å structure of the IBDV VPI demonstrated that the birnavirus RdRp displays its catalytic motifs arranged in the permutated order C-B-A in the primary sequence of the palm domain. In addition, the conventional motif C (Gly-Asp-Asp) is missing and has been replaced by Ala-Asp-Asn in all birnavirus VP1s.

VP1 initiates RNA synthesis via a protein-priming strategy. Whilst the protein primer and the polymerase activity are carried by separate entities in many viruses (e.g., in picornaviruses and caliciviruses), the VP1 molecules

of birnaviruses possess both RNA polymerase and protein priming functions. VP1 has a self-guanylyltransferase activity to produce VP1-pG and VP1-pGpG.

The capsid protein VP2

The VP2 protein carries the immune determinants that control antibody-mediated neutralization and protection. While IBDV displays only two serotypes, nine different serotypes of IPNV have been described to reflect their antigenic diversity. The crystal structure of the IBDV VP2 trimer has been determined at 2.6 Å resolution. The VP2 subunit is folded into three distinct domains disposed radially in the virus particle. They are designated base (B), shell (S), and projection (P) (**Figure 3**). The B and P domains are both structured as barrels with the jelly roll topology, orientated in such a way that the strands, respectively, run tangentially and radially, to the spherical particle. There are ions bound at the trimer axis, a calcium ion at a distance of 11 Å at the inside of VP2, and a chloride ion at a distance of 17 Å from the calcium ion. The calcium ion is tightly bound by three pairs of symmetry-related conserved aspartic acid residues. Sequence alignment analysis of birnavirus VP2 molecules shows that domains S and B are relatively well conserved but that domain P is more variable. The amino acid differences between the two IBDV serotypes, and among the pathogenic serotype 1 isolates, map mainly in the P domain. Antibody neutralization–escape mutations are located at only a few residues, all in the outmost loops of the P jelly roll. Using the reverse-genetics approach, it was shown that IBDV adaptation to cell culture and virulence is also controlled by changes in these most exposed loops of domain P, suggesting that these residues may engage directly in contacts with a cell surface receptor which has not yet been identified.

Domains S and B together display a very high structural homology with the capsid protein of positive-strand RNA viruses of the families *Nodaviridae* and *Tetraviridae*. The coat proteins of these $T = 3$ and $T = 4$ icosahedral viruses exhibit the same topology features, including the α-helical N- and C-terminal extensions making up the domain B. In contrast, the domain P of birnavirus VP2 is most similar to the corresponding domain of the rotavirus VP6 protein, and its homolog forms the $T = 13$ layer in members of the family *Reoviridae*, containing 10–12 segments of dsRNA as their genome.

The peptides derived from pVP2

In the infected cells, the processing of pVP2 generates VP2 and three or four peptides that remain associated with the virus particles. In IBDV virions, the four peptides do not follow the icosahedral symmetry of the particles,

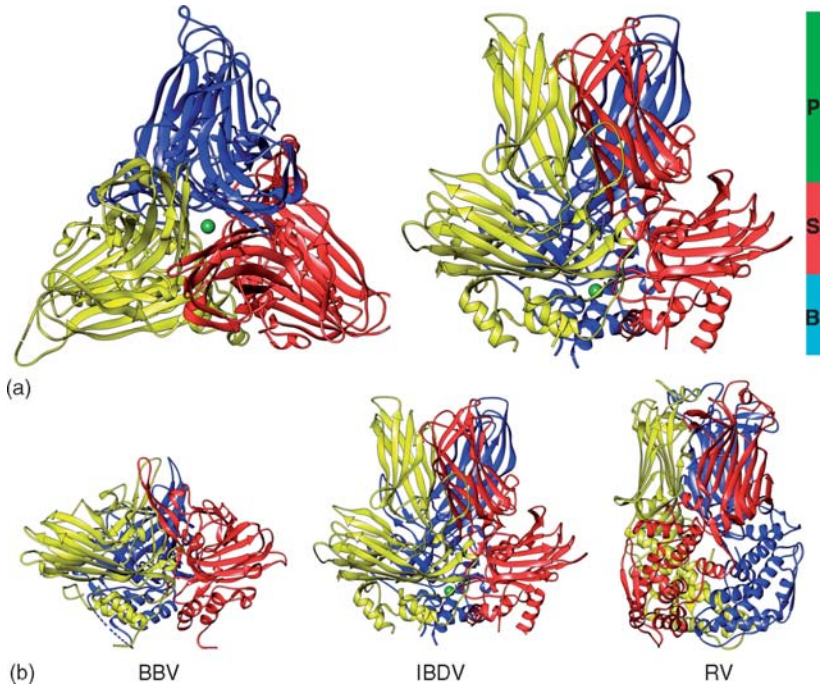


Figure 3 The VP2 trimer. (a) Ribbon diagram of the IBDV VP2 trimer (left, top view; right, side view). Each subunit is colored differently. The sphere in green color indicates the calcium ion. The domains P, S, and B are indicated at the right. (b) The VP2 structure is a link between two categories of RNA viruses. Ribbon diagrams of the capsid proteins of black beetle virus (BBV, an alphanodavirus; protein β, PDB code 2bbv), IBDV (protein VP2), and rotavirus (RV, protein VP6, PDB code 1qhd). The DALI score between VP2 and BBV protein β, 15.3, and the rms is 3.3 Å for 211 α–carbons (out of 250 residues from domains B and S). The DALI score between VP2 and RV VP6 is 5.1, with 3.5 Å rms for 105 αcarbons (out of 140 residues from domain P).

and they are accessible to proteases or biochemical labeling. The largest peptide, pep46 (46 amino acids long), was shown to induce large structural rearrangements in liposomes and to destabilize biological membranes, suggesting its implication in cell entry.

The internal viral protein VP3

There is only little information available on the three-dimensional structure of VP3. This abundant virion protein is able to form stable complexes with VP1 in the cytoplasm of IBDV-infected cells, suggesting that VP1 is incorporated in the virions through interaction with VP3. The VP3 domain interacting with VP1 was mapped to its 10 C-terminal amino acids. VP3 also interacts with viral dsRNA, suggesting a key role for VP3 in birnavirus assembly. In IPNV particles, VP3 and the genomic RNA have been shown to form thread-like ribonucleoprotein complexes.

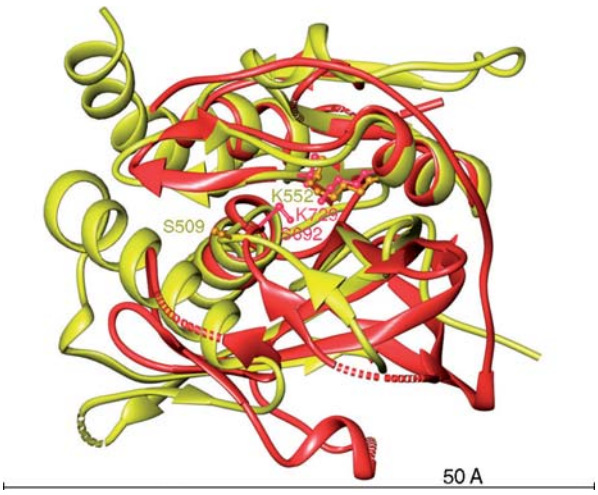


Figure 4 The VP4 protease. A comparison of the protein folds of the BSNV VP4 protease (red) and the proteolytic domain of *Archaeoglobus fulgidus* Lon protease (yellow, PDB code 1zob). The catalytic Ser/Lys dyads are indicated.

The Nonstructural Polypeptides

The viral protease VP4

Using bioinformatics tools and mutagenesis analyses, it was demonstrated that the VP4s of IBDV and IPNV are proteases that utilize a serine/lysine dyad to process the birnavirus polyprotein. The catalytic residues of the VP4 of BSNV have also been experimentally ascertained. This catalytic dyad represents a so far unrecognized feature among viral proteases. On the other hand, this dyad has been described in several proteases of different origin, such as bacterial signal peptidase (SP), LexA repressor, and Lon protease. The VP4 thus belongs to the clan SJ and family S50 of proteases in the MEROPS databank. During polyprotein maturation, VP4 first autoprocesses the pVP2-VP4-VP3 polyprotein (*cis*-cleavage) and then cleaves pVP2 at its C-terminus (*trans*-cleavage) during virus assembly.

The crystal structure of the VP4 of BSNV has been solved at a resolution of 2.2 Å. These data confirmed the presence of a lysine in the catalytic site (**Figure 4**). The topology of the substrate's binding site is consistent with the substrate specificity and a nucleophilic attack from the *si*-face of the substrates scissile bond. Despite low scores of sequence identity, VP4 shows structural similarities in its active site with other serine/lysine proteases such as *E. coli* SP, LexA and Lon proteases (**Figure 4**). The binding pocket of VP4 is significantly larger and more extended than that of the *E. coli* Lon protease, the structure of which is most similar in the domain of the active site.

The nonstructural protein encoded by the small segment A open reading frame

This protein has been designated VP5 for IBDV and IPNV. Reverse genetic studies on these two viruses have shown that VP5 is dispensable for virus viability. VP5 plays a role in the modulation of apoptosis and pathogenesis. In contrast to its parental strain, an IBDV VP5 knockout mutant in which the initiation codon was mutated does not induce bursal lesions in susceptible infected chicken. Comparisons of the replication kinetics and markers for apoptosis indicate that the mutant induces increased DNA fragmentation, elevated caspase activity and NF-KB activation earlier in the replication cycle, allowing for the conclusion that VP5 has antiapoptotic functions at early steps of viral replication.

The VP5 of IBDV has been shown to be a class II membrane protein with a cytoplasmic N-terminal domain. VP5 is targeted to the plasma membrane and its expression without other viral components results in the alteration of the cell morphology and a reduction of the cell viability. VP5 has been proposed to play an important role in the release of the virions from the infected cell.

Other proteins

The proteins encoded by the small segment A open reading frame of BSNV and DXV have not yet been characterized.

Replication Cycle and Virus Assembly

There is still little information available about the early events of birnavirus replication. Investigations to identify their cellular receptors have so far not revealed the nature of the molecule(s) involved. Entry of IPNV does not seem to depend on endosomal acidification. Like all the other dsRNA viruses, birnaviruses have been shown to become

transcriptionally active *in vitro* in the presence of nucleotides, extruding nonpolyadenylated mRNAs through channels in the capsid. It is presently not known if these mRNAs are capped. The single-layered capsid architecture raises questions concerning the particle translocation across the cell membrane for transcription. The two mRNAs can be detected by 3–4 h post infection and are synthesized at equal molar ratio throughout the replication cycle. Similarly, viral polypeptides are present in similar relative proportions until the end of the replication cycle.

Birnavirus morphogenesis is a complex process that is controlled by interactions of VP3 with both pVP2 and VP1 and by the regulated proteolytic activity of VP4. In IBDV, expression of the pVP2-VP4-VP3 polyprotein gene in the absence of VP1 results in the assembly of tubes displaying helical symmetry and composed of unprocessed pVP2. While expression of the gene segment corresponding to the mature VP2 (441 amino acids) in the absence of other viral components leads to the formation of $T=1$ subviral particles with diameter 25 nm, expression of the VP2 precursor, pVP2 (512 amino acids), results in the formation of irregular particles. Co-expression of the polyprotein and VP1 genes allows the sequential maturation of pVP2 to proceed and the concomitant pVP2/VP2/VP3/VP1 assembly into $T=13$ laevo virus-like particles (VLP) that are morphologically identical to the virions. Whereas the VLP shell is constituted by 260 VP2 trimers, the subviral particles contain only 20 trimers. VLP formation requires VP1 to screen for the negative charges at the C-terminus of VP3, as in the virus particle. Any exogenous peptide fused at the C-terminus of VP3 or simply a deletion of its last C-terminal residues similarly allows VLP assembly. Whereas VP3 plays a pivotal role in virus morphogenesis, it is not closely associated with VP2 in the virus particle. Moreover, the cleavage of pVP2 into VP2 and several peptides only occurs when the particle assembles. Two of the four structural peptides, pep11 and pep46 (11 and 46 amino acids long, respectively) also control virus assembly. Deletion of pep11 or substitution of most of its residues by other amino acids blocks capsid morphogenesis. Removal of pep46 also prevents capsid assembly but leads to the formation of $T=1$ particles which consist of an unprocessed VP2 species. Structural analysis of these particles showed that the presence of uncleaved pVP2 causes a steric hindrance at the vertices, blocking fivefold axis formation. Furthermore, addition of an exogenous sequence at the N-terminus of a VP2 form containing part of pep46 allows assembly of this species into $T=13$ and $T=7$ VLPs, in part mimicking the assembly controlled by VP3. Thus, the structural polymorphism observed with the various VP2 assemblies represents a paradigm in the analysis of nonequivalent interactions among identical subunits.

Evolutionary Relationship between Birnaviruses and Other RNA Icosahedral Viruses

Due to the many similarities between birnaviruses and several positive-strand RNA viruses, it has been argued that birnaviruses represent an evolutionary link between dsRNA and positive-strand RNA viruses. The structural homologies among the coat proteins of nodaviruses, tetraviruses and VP2 (**Figure 3**) and the characteristic polymerase motif rearrangement not found in viruses other than birnaviruses and two positive-strand RNA viruses are the main features pointing to the existence of an evolutionary relationship among these viruses. Additional similarities among birna-, noda-, and tetraviruses are found in the organization of their bisegmented genomes, one RNA segment coding for the polymerase and the other for a capsid precursor, the latter undergoing proteolytic maturation at its C-terminus with a peptide of about 45 amino acids in length remaining associated with the virus particle. The mRNAs are not polyadenylated. The birnavirus VPg-linked genome replication strategy, which has been observed elsewhere only in a subset of positive-strand RNA viruses, including the picornaviruses, is a further common feature bringing positive-strand RNA viruses and birnaviruses together. Besides their dsRNA genomes, the only bridging element between birnaviruses with the other dsRNA viruses is the $T=13$ laevo protein layer and the structural homology of the VP2 P domain with the proteins forming the $T=13$ layer of the Reoviridae.

To better assess the evolutionary link between birnaviruses and positive-strand RNA viruses, it should be of interest to characterize additional dsRNA viruses having a bisegmented genome, such as picobirnaviruses and insect dsRNA and positive-strand RNA viruses.

The Disease Induced by IBDV and IPNV

IBDV

IBDV causes a highly contagious disease to young chickens, namely infectious bursal disease or 'Gumboro disease'. Due to its worldwide distribution, IBDV poses an important threat to the commercial poultry industry. Lymphoid cells in the bursa of Fabricius are the target cells of IBDV serotype-1 strains. Infection results in lymphoid depletion and the final destruction of the bursa. In the classical form of the outbreaks, the mortality rate ranges from 1% to 50%. In addition to mortality, IBDV is immunosuppressive and infected chicken become more susceptible to other pathogens and refractory to vaccines. In 1986, very virulent (vv) strains of IBDV, which can cause up to 70% mortality have emerged in Europe. These strains cause lesions typical of IBDV and are antigenically similar to 'classical' strains. Remarkably, vvIBDV can establish infection in the face of maternally derived antibodies that were

previously protective against 'classical' strains. vvIBDV infections have been observed in Africa, Asia, and South America.

Using reverse-genetics systems, it was shown that IBDV virulence (and cell culture adaptation) is determined by changes in a few amino acid positions in the capsid protein VP2. These residues may engage directly in contacts with a receptor of the target cell. It is also believed that VP1, the RdRp, plays a role in virulence.

IPNV

IPNV is the causative agent of infectious pancreatic necrosis in juvenile salmonid fish worldwide. Clinical signs observed in diseased fish are distended abdomen, aberrant swimming, darkened pigmentation and focal necrosis in the exocrine pancreatic tissue. Mortality observed in an outbreak varies considerably, thus probably revealing differences in genetic susceptibility of the host. Virulence determinism is also due to variations at discrete amino acid positions in the primary sequence of VP2.

The *Picobirnaviridae*

Picobirnaviruses were first detected in fecal specimens from human and rats in 1988, and later also in other mammalian species and in chicken. They have been found in both healthy and diarrheic human patients. An association of their presence with diarrhea is particularly apparent in patients with immunodeficiencies. In a search for RNA viruses in feces of healthy humans, a metagenomic analysis has established that the most common animal viruses identified in RNA libraries generated from feces are picobirnaviruses. (This study also revealed that 97% of the virus-like sequences were homologous to plant virus sequences.) The study also revealed that the picobirnavirus population in the same individual can drastically change over time, highlighting the dynamic nature of picobirnavirus infections in humans. Picobirnaviruses have not been adapted to *in vitro* cell culture and are therefore mainly characterized by partial nucleotide sequence analyses of the RdRp gene.

Picobirnaviruses have recently been proposed to be reclassified from a genus of the *Birnaviridae*, based on the singularity of their genomic sequences and highly singular morphological features. Picobirnavirus particles are 35–41 nm in diameter with a triangulation number of 1, 3, or 4. They are nonenveloped and have a buoyant density of 1.38–1.40 g ml^{-1}. Their genome consists of two dsRNA segments of 2.3–2.6 and 1.5–1.9 kbp, respectively. While the smaller segment encodes the viral RdRp, the larger appears to code for the capsid protein. Only two sequences of the larger segment of a rabbit and a human picobirnavirus are available in the sequence database (**Table 2**). Two or three nonoverlapping open reading frames are present in the larger segment. Comparison of the amino acid sequences of the RdRp of human picobirnaviruses of different origins showed that they may differ by as much as 60%.

Table 2 Virus members in the family *Picobirnaviridae*

Genus	*Virus*	*GenBank sequence accession number*
Picobirnaviruses	Human picobirnavirus	NC_007026, NC_007027
	Rabbiit picobirnavirus	AJ244022
Unassigned viruses	Rat picobirnavirus	
	Chicken picobirnavirus	
	Calf picobirnavirus	

The RdRp of picobirnavirus is about 500 amino acids long, with the polymerase motifs of the palm domain arranged in the conventional A-B-C motifs order. Therefore, picobirnaviruses probably do not represent the missing link between +ssRNA viruses and birnaviruses.

See also: Taxonomy, Classification and Nomenclature of Viruses; Reoviruses: General Features; Reoviruses: Molecular Biology; Nodaviruses; Viral Pathogenesis.

Further Reading

Birghan C, Mundt E, and Gorbalenya AE (2000) A non-canonical Lon proteinase lacking the ATPase domain employs the ser–lys catalytic dyad to exercise broad control over the life cycle of a double-stranded RNA virus. *EMBO Journal* 19: 114.

Chevalier C, Galloux M, Pous J, *et al.* (2005) Structural peptides of a nonenveloped virus are involved in assembly and membrane translocation. *Journal of Virology* 79: 12253.

Coulibaly F, Chevalier C, Gutsche I, *et al.* (2005) The birnavirus crystal structure reveals structural relationships among icosahedral viruses. *Cell* 120: 761.

Delmas B, Kibenge FSB, Leong JC, *et al.* (2005) *Birnaviridae*. In: Fauquet C, Mayo M, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 561–569. San Diego, CA: Elsevier Academic Press.

Feldman AR, Lee J, Delmas B, *et al.* (2006) Crystal structure of a novel viral protease with a serine/lysine catalytic dyad mechanism. *Journal of Molecular Biology* 358: 1378.

Giordano MO, Martinez LC, Espul C, *et al.* (1999) Diarrhea and enteric emerging viruses in HIV-infected patients. *AIDS Research and Human Retroviruses* 15: 1427.

Gonzalez GG, Pujol FH, Liprandi F, *et al.* (1998) Prevalence of enteric viruses in human immunodeficiency virus seropositive patients in Venezuela. *Journal of Medical Virology* 55: 288.

Gorbalenya AE, Pringle FM, Zeddam J-L, *et al.* (2002) The palm subdomain-based active site is internally permuted in viral RNA-dependent RNA polymerases of an ancient lineage. *Journal of Molecular Biology* 324: 47.

Liu M and Vakharia VN (2006) Nonstructural protein of infectious bursal disease virus inhibits apoptosis at the early stage of virus infection. *Journal of Virology* 80: 3369.

Lombardo E, Maraver A, Espinosa I, *et al.* (2000) VP5, the nonstructural polypeptide of infectious bursal disease virus, accumulates within the host plasma membrane and induces cell lysis. *Virology* 277: 345.
Mundt E and Vakharia VN (1996) Synthetic transcripts of double-stranded birnavirus genome are infectious. *Proceedings of the National Academy of Sciences, USA* 93: 11131.
Pan J, Vakharia VN, and Tao YJ (2007) The structure of a birnavirus polymerase reveals a distinct active site topology. *Proceedings of the National Academy of Sciences, USA* 104: 7385.
Saugar I, Luque D, Ona A, *et al.* (2005) The structural polymorphism of the major capsid protein of a double-stranded RNA virus: An amphipathic helix as a molecular switch. *Structure* 13: 1007.
Tacken MG, Peeters BP, Thomas AA, *et al.* (2002) Infectious bursal disease virus capsid protein VP3 interacts both with VP1, the RNA-dependent RNA polymerase, and with viral double-stranded RNA. *Journal of Virology* 76: 11301.
Zhang T, Breitbart M, Lee WH, *et al.* (2006) RNA viral community in human feces: Prevalence of plant pathogenic viruses. *PLoS Biology* 4: 108.

Relevant Website

http://merops.sanger.ac.uk – MEROPS, Peptidase Database.

Bluetongue Viruses

P Roy, London School of Hygiene and Tropical Medicine, London, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arthrogryposis Persistent flexure or contracture of a joint.
Campylognathia A condition marked by misalignment of the jaws leading to a twisted appearance of the face.
Hemoconcentration An increase in the concentration of red blood cells in the circulating blood.
Hemorrhagic Related to bleeding.
Hydranencephaly Related to the abnormal buildup of cerebrospinal fluid in the ventricles of the brain.
Hydropericardium A noninflammatory accumulation of fluid within the linings surrounding the heart.
Hydrothorax An accumulation of serous fluid in the cavity of the chest.
Hypotension Abnormally low blood pressure.
Mucosal edema The presence of abnormally large amounts of fluid in the intercellular tissue spaces of the connective tissue lining the internal cavities of the body.
Prognathia A condition marked by abnormal protrusion of the lower jaw.
Pulmonary edema A state of increased interstitial fluid within the lung that leads to flooding of the lung alveoli with fluid.
Serosal hemorrhages Bleeding in the delicate membranes of connective tissue which line the internal cavities of the body.
Thrombotic Related to formation of thrombus; that is, an aggregation of blood factors, frequently causing vascular obstruction at the point of its formation.
Triskelions A shape consisting of three protruding branches radiating from a common center.

History

Bluetongue disease (initially known as 'malarial catarrhal fever') was first observed in the late eighteenth century in sheep, goats, cattle, and other domestic animals, as well as in wild ruminants (e.g., white-tailed deer, elk, and pronghorn antelope) in Africa. A distinctive lesion in the mouths of the infected animals with severely affected, dark blue tongues was a characteristic symptom. That the disease was caused by a filterable agent was discovered in 1905. The first confirmed outbreak outside of Africa occurred in sheep in Cyprus in 1924 and this was followed by a major outbreak in 1943–44 with 70% mortality. The disease was recognized subsequently in the USA in 1948 and in Southern Europe in 1956 where approximately 75% of the affected animals died. The outbreaks of bluetongue disease in the Middle East, Asia, Southern Europe, and the USA in the early 1940s and 1950s led to its subsequent description as an 'emerging disease'. To date, based on serum-neutralization tests, 24 different serotypes have been isolated in tropical, semitropical, and temperate zones of the world including Africa, North and

South America, Australia, Southeast Asia, the Middle East, and, more recently, Southern and Central Europe. An important factor in the distribution of bluetongue virus (BTV) worldwide is the availability of suitable vectors, usually biting midges (gnats) of the genus *Culicoides*.

Properties of the Virion

Bluetongue virus is the type species of the genus *Orbivirus* within the family *Reoviridae*. Details of the structure, genetics, and molecular properties of orbiviruses have been gleaned largely from studies of BTV. BTV and other orbiviruses are nonenveloped with two concentric protein shells and a genome consisting of ten double-stranded RNA (dsRNA) segments. BTV virions (550S) are architecturally complex icosahedral structures and are composed of seven discrete proteins that are numbered VP1 to VP7 in order of their decreasing size. When viewed by electron microscopy (EM), negative-stained, 550S BTV particles exhibit a poorly defined surface structure with a 'fuzzy' appearance. Complete virions are relatively fragile and the infectivity of BTV is lost easily in mildly acidic conditions. The outer capsid of BTV consists of two major proteins (VP2 and VP5) which constitute approximately 40% of the total protein content of the virus. Both proteins are removed shortly after infection to yield a transcriptionally active core (470S) particle (see schematic, **Figure 1**) which, in contrast to virions, is fairly robust. They are composed of two major proteins (VP7 and VP3), three minor proteins (VP1, VP4, and VP6), and the dsRNA genome. The cores may be further uncoated to form subcore particles (390S) that lack VP7. The 470S cores can be derived from virions *in vitro* by physical or proteolytic treatments that remove the outer capsid and cause activation of the BTV transcriptase. Considerable information on the three-dimensional (3-D) structures of BTV particles and proteins has been generated in recent years.

Virion Structure and Outer Capsid Proteins

In contrast to negative staining, cryoelectron microscopy (cryo-EM) of intact BTV particles shows the icosahedral morphology of the mature particle, with a diameter of 86 nm. The outer layer consists of 60 sail-shaped, spike-like structures made up of VP2 (110 kDa) trimers and of 120 globular structures made up of VP5 (59 kDa) trimers (**Figure 2**). The most external part of the outer capsid is the propeller-shaped triskelion blade of VP2, the tip of which bends upward, perpendicularly to the plane of the virus. These bent tips give the entire virion a diameter of ~88 nm and extend from the main body of the particle by 3 nm. Interspersed between the triskelions and lying more internally are the globular VP5 trimers, each with a ~6 nm diameter. These are also entirely exposed in the virion, not covered by VP2, and both proteins make extensive contacts with the core VP7 layer underneath.

Core Particle and Proteins

The core particles derived from purified virus by proteolytic treatment have been analyzed by cryo-EM and by X-ray crystallography. The icosahedral core has a diameter of 73 nm and a triangulation number of 13 ($T = 13$) with the surface layer made up of 260 VP7 (38 kDa) trimers. Trimers are arranged around 132 distinctive channels (three types: I, II, and III) as six-member rings, with five-member rings at the vertices of the icosahedrons (**Figure 3**). The five quasiequivalent trimers form a visible protomeric unit (P, Q, R, S, T). Each trimer consists of two distinct domains, 'upper' (an antiparallel β-sandwich) and 'lower' (mainly α-helical), which are twisted in such a

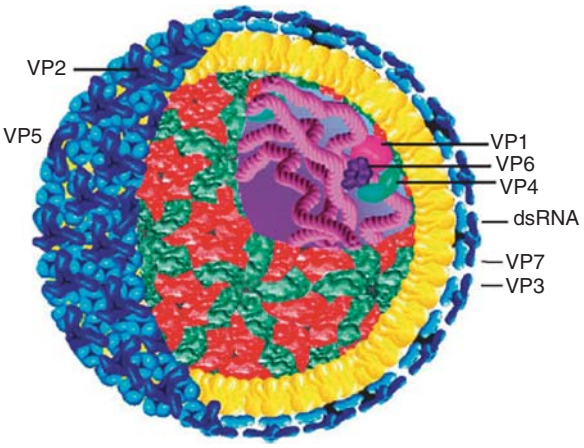


Figure 1 A schematic diagram of bluetongue virus (BTV) showing the positions and structural organizations of BTV components. Reproduced courtesy of professor David Ian Stuart.

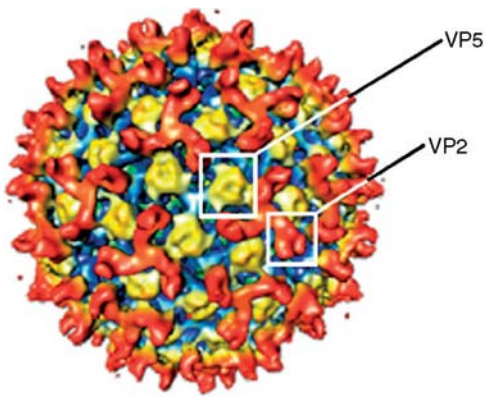


Figure 2 Surface representations of the 3-D cryo-EM structures (22 Å resolution) of BTV. Whole particle showing sail-shaped triskelion propellers (VP2 trimers) in red and globular domains (VP5 trimers) in yellow.

way that the top domain of one monomer rests upon the lower domain of an adjacent monomer and the interaction between monomers is extensive. The lower domains are attached to an inner shell (59 nm in diameter) made up of 120 VP3 (103 kDa) molecules arranged as 60 dimers with $T = 2$ symmetry (**Figure 4**). Each molecule consists of three distinct domains: a rigid 'carapace' domain, an 'apical' domain, and a 'dimerization' domain. Five of the VP3 dimers form a decamer with fivefold axes of symmetry, and 12 decamers, each a convex disk shape, are arranged together to form the complete VP3 shell.

Much of the genomic RNA can also be detected as an electron-dense region within the central core space. The dsRNA appears to be highly ordered, and approximately 80% of the entire genome can be modeled as four distinct concentric layers that have center-to-center spacing between RNA strands of 26–30 Å.

The arrangement of three internal minor proteins within the VP3 layer is not discernible in the core structure. However, cryo-EM of core-like particles (CLPs) consisting of VP3 and VP7 together with two minor proteins, VP1 (150 kDa) and VP4 (78 kDa), though lacking the genome, and VP6 has revealed a flower-shaped complex formed by VP1 and VP4, directly beneath the icosahedral fivefold axes. The exact position of the smallest minor protein VP6 (35 kDa) remains unclear although it forms a stable hexamer *in vitro* in the presence of BTV RNA, indicating that it is closely associated with the genomic RNA.

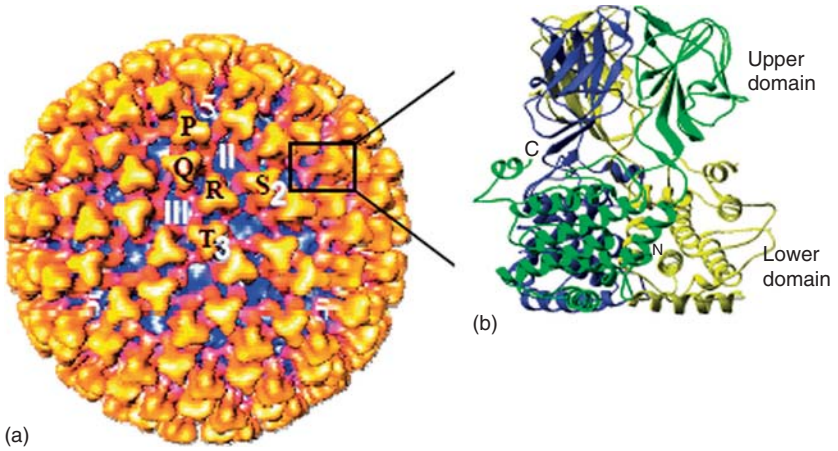


Figure 3 Three-dimensional structures of BTV core and VP7 protein. (a) Surface representations of the 3-D cryo-EM structures of BTV core (700 Å in diameter) viewed along the icosahedral threefold axes showing the 260 trimers of VP7 (in blue). The five quasiequivalent trimers (P, Q, R, S, and T) and the locations of channels II and III are marked. (b) The trimer image of the VP7 atomic structure solved at 2.8 Å resolution. Two domains of the molecule are indicated. The view is shown from the side. Note the flat base of the trimer lies in a horizontal plane in this view.

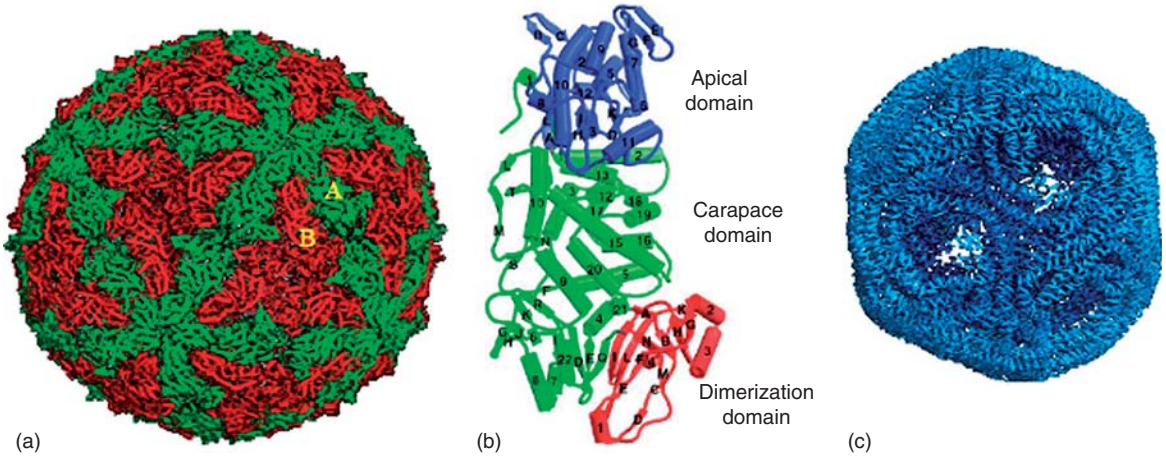


Figure 4 The X-ray structure of the VP3 shell. (a) The structure of the VP3 layer showing the arrangement of 120 copies of two conformationally distinct types of VP3 molecules, 'A' shown in green and 'B' shown in red. (b) Structure of VP3 molecule shown as a thin triangular plate consisting of three domains as indicated. (c) The model diagram of genomic dsRNA that has been built into the four layers of electron density in the core. Reproduced courtesy of professor David Ian Stuart.

The Virus Genome

The BTV genome comprises ten dsRNA segments in equimolar amounts which appear to be organized in an orderly fashion as four distinct layers within the core. The RNA segments, numbered 1–10 in order of migration by polyacrylamide gel electrophoresis (PAGE), were historically referred to as large, medium, and small segments (i.e., L1–L3, M4–M6, and S7–S10). Their relative order of mobility may vary according to the electrophoretic conditions used (agarose gel vs. PAGE) and the dsRNA profiles of members representing each orbivirus serogroup are generally distinctive and different from those of other members of the family *Reoviridae*.

The complete sequences of all ten dsRNA segments of a number of BTV serotypes (BTV-10 being completed as early as in 1989) are available. Each segment has six nucleotides at the 3′ end and eight nucleotides at the 5′ end conserved in each messenger sense RNA (mRNA) strand. For BTV-10, the genome is 19 218 bp in length with a molecular weight of 1.3×10^7 Da and the individual sizes of BTV-10 RNAs range from 3954 bp (segment 1, molecular weight 2.7×10^6 Da) to 822 bp (segment 10, molecular weight 5×10^5 Da). The 5′ noncoding regions range from 8 nt (segment 4) to 34 nt in length (segment 6) while the 3′ noncoding sequences are generally longer, that is, from 31 nt (segment 5) to 116 nt (segment 10) in length.

The 5′ and 3′ terminal sequences of the ten mRNA strands of BTV have inverted complementarity capable of forming intramolecular hydrogen bonds (i.e., end-to-end hydrogen bonding) and share some common features (e.g., a looped-out sequence proximal to the 3′ termini). Apart from segment 2 and segment 5 (segment 6 in agarose gels) that code for the two outer capsid proteins (VP2 and VP5), all other eight RNA segments are highly conserved. Despite this sequence conservation, some genetic diversity exists for each RNA segment representing the various BTV serotypes as well as for various isolates within a single serotype. In addition, high-frequency segment reassortment occurs between different BTV serotypes in cell cultures, vertebrate hosts, or *Culicoides* vectors to generate new genotype combinations. Thus, both genetic drift and genetic shift contribute to BTV evolution.

Apart from segment 10, each of the AUG codons on the positive-sense mRNA strand of each of the segments initiates a single long open reading frame (see **Table 1**). There are two methionine codons in the same reading frame in the segment-10 RNA sequences, one at triplet 20–22 and the other at triplet 59–61, encoding two overlapping proteins. Thus, segment 10 codes for two nonstructural proteins, NS3 and NS3A. In addition to the seven structural proteins, two major nonstructural proteins (NS1, NS2) are also synthesized in infected cells. **Table 1** summarizes the coding assignments of the ten genome segments of BTV-10.

Viral Replication

The basic features of the BTV replication cycle, including the transcription process and the conservative mode of genome replication, are similar to those of reoviruses and rotaviruses. Unlike reoviruses or rotaviruses, however, BTV and other orbiviruses multiply in arthropods as well as in vertebrate hosts. Also, in view of the structural differences, it is likely that some stages of BTV replication and morphogenesis are unique.

Attachment and Entry into Cells

BTV adsorbs rapidly to susceptible cells at both 4 and 37 °C. The cell receptor for BTV is not known although it binds to a sialoglycoprotein via the outer capsid protein VP2 in mammalian cells. VP2 is the serotype determinant and viral hemagglutinin protein, elicits serotype-specific virus-neutralizing antibody, and is the most variable protein among different serotypes. Although protease-treated particles with VP2 cleavage products attached are fully infectious, removal of VP2 results in loss of virus infectivity

Table 1 Coding assignments and function of the BTV-10 viral RNA segments

Genome segment[a]	*Size (kbp)*	*Protein*	*Protein size (kDa)*	*Location*	*Function*
Seg. 1 (L1)	3.954	VP1	149.5	Core	Polymerase
L2	2.926	VP2	111	Outer shell	Attachment protein
L3	2.772	VP3	103	Core	Structural
M4	2.011	VP4	764	Core	Capping enzyme
M5	1.770	NS1	644	Tubules	Trafficking?
M6	1.639	VP5	591	Outer shell	Fusion protein
S7	1.156	VP7	385	Core	Insect cell attachment
S8	1.123	NS2	409	Nonstructural	RNA selection
S9	1.046	VP6	357	Core	Helicase
S10	0.822	NS3	256	Nonstructural	Egress

[a]Genome segment nomenclature based on order of migration by electrophoresis. In some gel systems the migration of segments 5 and 6 is frequently, but not always, reversed.

in mammalian cells. Core particles, however, are infectious only for invertebrate cells.

The attachment of VP2 leads to receptor-mediated endocytosis by clathrin-coated vesicles which are subsequently lost when large endocytic vesicles form. Only the early endosomes are involved in BTV entry. BTV entry into the cytoplasm requires endosomal acidic pH which allows the second outer capsid protein VP5 to permeabilize the endosomal membrane. This occurs via the function of the N-terminal 40 hydrophobic residues which act as a 'pore-forming' peptide, analogous to the fusion peptides of envelope viruses. Thus, VP2 makes an initial contact with the host cell and VP5 mediates the penetration of the host cell membrane by destabilizing the endosomal membrane. Core particles lacking both VP2 and VP5 proteins then enter the cytoplasm.

Transcription

In the cytoplasm, core particles do not disassemble further but initiate the transcription of the viral genome, and newly synthesized viral mRNAs, capped but not polyadenylated, are extruded into the cytoplasm. When intact cores, isolated from virus particles, are activated *in vitro* by the presence of magnesium ions and nucleoside triphosphate (NTP) substrates, distinct conformational changes can be seen around the fivefold axes of the core, forming pores in the VP3 and VP7 layers through which mRNAs are extruded. The role of each internal minor protein in genome replication activity has been established using individual purified proteins.

The smallest minor protein VP6, which is rich in basic amino acids (Arg, Lys, and His), binds both single-stranded RNAs (ssRNAs) and dsRNAs and, in isolation, has the ability to unwind dsRNA substrates *in vitro*. The protein exhibits physical properties characteristic of other helicases, including being hexameric, and has the ability to form ring-like structures in the presence of BTV RNAs. Mutation of amino acid residues in the active site destroys the catalytic activity of the protein.

The replication of viral dsRNA occurs in two distinct steps. First, plus-strand RNAs (mRNAs) are transcribed and extruded from the core particle. Second, the plus-strand RNAs serve as templates for the synthesis of new minus-strand RNAs. The largest core protein VP1 (150 kDa), in soluble form, has the ability to both initiate and elongate minus-strand synthesis *de novo*, but the catalytic activity is lost when a GDD motif (amino acids 287–289), the polymerase signature motif, is mutated.

The third minor protein VP4 is the mRNA-capping enzyme. The purified, soluble, recombinant VP4 alone has the ability to synthesize type 1-like 'cap' structures on uncapped BTV transcripts *in vitro*, and 'cap' structures are identical to those found on authentic BTV mRNAs. Thus, VP4 possesses methyltransferase, guanylyltransferase, and RNA triphosphatase activities. The recently resolved atomic structure has shown how distinct domains of this single protein perform each of these catalytic activities consecutively.

In summary, each of the three minor proteins of the BTV core has the ability to function on its own. Together they constitute a molecular motor that can unwind RNAs, synthesize ssRNAs of both polarities, and modify the 5′ termini of the newly synthesized mRNA molecules. The transcripts are not produced in equimolar amounts from the ten segments of BTV; the smaller genome segments are generally the most frequently transcribed, although segment-6 RNA (encoding NS1) is synthesized more abundantly than segment-10 (the smallest) RNA (encoding NS3). The molar ratios of the ten different BTV mRNAs remain the same throughout the infection cycle. The ratios of mRNAs synthesized *in vivo* and *in vitro* are similar.

Much less is known about the *in vivo* RNA replication mechanisms of BTV. It is believed that, like reoviruses and rotaviruses, the packaged plus-strand RNA serves as a template for synthesis of a minus strand, and once the minus strand is synthesized, the dsRNA remains within the nascent progeny particle. As discussed, VP1 acts as the replicase enzyme but the roles of other proteins in minus-strand synthesis remain undefined.

Protein Synthesis and Replication

In tissue culture, the first BTV-specific proteins are detectable 2 h post infection and the rate of protein synthesis increases rapidly until 12–13 h post infection, after which it slows down but continues until cell death. BTV infection of mammalian cells, in contrast to insect cells, leads to a rapid inhibition of cellular macromolecular synthesis and the induction of a robust apoptotic response triggered via multiple apoptotic pathways.

Two NS proteins, NS1 (64 kDa) and NS2 (41 kDa), are synthesized abundantly early in BTV infection and coincide with two virus-specific intracellular structures, tubules, and viral inclusion bodies (VIBs), respectively. By contrast, synthesis of NS3 and NS3A (26 and 25 kDa) varies from barely detectable to highly expressed, depending on the host cells, and may correlate with virus release.

Tubules are present in large numbers, mostly in perinuclear locations, and are made up of helically coiled NS1 dimers, on average 52.3 nm in diameter and ~1 μm long. The exact role of tubules or NS1 dimers in virus replication is not known. During BTV infection, VIBs are found in the infected cells, predominantly near the nucleus. VIBs act as the nucleation site for newly synthesized proteins that form the core structure and transcripts as well as the subsequent assembly of subviral particles. The major component of VIBs is the phosphoprotein NS2. Phosphorylation plays a key role in the formation of

VIBs and may be involved in stabilizing NS2 folding. The cellular protein kinase, casein kinase II (CKII), is responsible for NS2 phosphorylation via two serine residues.

Soluble NS2 and NS2 in VIBs have a strong affinity for ssRNA. NS2 preferentially binds BTV transcripts via specific hairpin structures, an indication of its role in the recruitment and selection of BTV mRNA during virus replication and RNA packaging. Since phosphorylation of NS2 is not necessary for recruitment of core components, but important for VIB formation, phosphorylation and dephosphorylation of NS2 is plausibly a dynamic process that controls the assembly and release of core particles.

Capsid Assembly

The assembly of BTV capsids requires a complex and highly ordered series of protein–protein interactions. The use of CLPs and virus-like particles (VLPs), together with structure-based mutagenesis, has revealed the key principles that drive the assembly process. The VP3 shell appears to play the major role in the initiation of core assembly with formation of VP3 decamers and the complex formed with VP1 and VP4. These assembly intermediates subsequently recruit the viral RNA, and possibly VP6, prior to completion of the assembly of the VP3 subcore and the addition of VP7 trimers. Initially, multiple sheets of VP7 trimers form around different nucleation sites and thus it is likely that a number of strong VP7 trimer–VP3 contacts act as multiple equivalent initiation sites and that a second set of weaker interactions then 'fill the gaps' to complete the outer core layer. The VP7 layer gives increased rigidity and stability to the core particle. Assembly of the core takes place entirely within VIBs and assembled core is released from VIBs prior to the simultaneous addition of VP2 and VP5, which most likely occurs within the vimentin component of the cytoskeleton.

Egress from Host Cells

The majority of mature virus particles remain cell-associated in mammalian cells, causing substantial cytopathic effect. However, some particles bud through the cell membrane or move in groups through a local disruption of the plasma membrane. NS3 (229 aa) and its shorter form NS3A (216 aa) are the only BTV glycoproteins and are associated with smooth-surfaced, intracellular vesicles. NS3/NS3A proteins have long N-terminal and shorter C-terminal cytoplasmic domains connected by two transmembrane domains and a short extracellular domain. A single glycosylation site is present in the extracellular domain of BTV NS3. The NS3/NS3A proteins are synthesized at very low levels in infected mammalian cells but at very high levels in invertebrate cells where there is nonlytic virus release. The NS3 protein is localized at the site of the membrane where viruses or VLPs are released. NS3 may cause local disruption of the plasma membrane, allowing virus particles to be extruded through a membrane pore without acquiring a lipid envelope. The N-terminal residues of NS3 interact with the calpactin light chain (p11) of the cellular annexin II complex, itself involved in membrane secretory pathways. The interaction of p11 with NS3 may direct NS3 to sites of active cellular exocytosis, or NS3 could become part of an active extrusion process. NS3 is also capable of interaction with Tsg101, a cellular protein implicated in the intracellular trafficking and release of a number of enveloped viruses. Interactions of both p11 and Tsg101 with NS3 appear to impact the nonlytic release of virions from infected cells. The significance of these interactions for BTV egress becomes more apparent in the light of the observation that the other cytoplasmic domain of the protein, situated at the C-terminal end, interacts specifically with the BTV outer capsid protein VP2. Current data suggest that NS3 makes use of host proteins and acts as an intermediate to facilitate the release of newly synthesized progeny virus across the cell membrane.

Pathogenesis

BTV produces a spectrum of conditions from subclinical infection to severe and fatal disease, depending on virus strain and host species. Virus is transmitted to vertebrate hosts through blood-feeding insect vectors and infectious particles migrate to lymph nodes where they initially replicate and subsequently spread to spleen, thymus, and other lymph nodes. In the final phase of infection, the virus begins circulating in the bloodstream and can persist for several months. In certain vertebrate hosts (e.g., cattle), BTV can induce a prolonged viremia. BTV binds to glycophorins on the surface of bovine and ovine erythrocytes where it may remain in an infectious state as invaginations of the erythrocyte cell membrane for prolonged periods of time. This precludes contact with antibody and T cells and thus provides multiple opportunities for transmission by infection of blood-sucking midges.

The role of leukocytes in cell-associated viremia is less certain, although BTV has been recovered from bovine mononuclear cells during the early stage of infection. A characteristic feature of BTV pathogenesis is the ability of viruses to replicate in and to damage endothelial cells with tropisms for cells representing distinct anatomic sites. Infection of endothelial cells is followed by infection of vascular smooth muscle cells and pericytes. These then undergo lytic infections, resulting in virus-induced vascular injury and a cascade of pathophysiologic events characterized by capillary leakage, hemorrhage, and disseminated intravascular coagulation. Clinically, these events

are manifested by mucosal edema, hemoconcentration, pulmonary edema, hydrothorax, hydropericardium, serosal hemorrhages, other hemorrhagic and thrombotic phenomena, hypotension, and shock. A curious feature of BTV infection in sheep is hemorrhage at the base of the pulmonary artery. Generally, sheep develop potentially fatal hemorrhagic disease and have extensive endothelial cell infections, whereas cattle typically develop subclinical infections and have only minimal endothelial infections. Thus, BTV and other related orbiviruses in their respective large animal hosts have pathophysiologic features resembling other viral hemorrhagic fevers.

Infection of pregnant cattle and sheep with BTV can result in maternal death, abortion and fetal death, or congenital anomalies including runting, blindness, deafness, hydranencephaly, arthrogryposis, campylognathia, and prognathia.

Epidemiology

In many countries, the prevalence of BTV infection is high in ruminants although clinical disease is often not recorded. The epidemiology of bluetongue disease can be considered in the context of three major zones of infection: an endemic zone (generally subclinical infection), an epidemic zone (disease occurs at regular intervals), and an incursive zone (disease occurs usually only at extended intervals, but when it does occur, the epidemic is often extensive). Transmission of BTV is influenced by both the distribution and biology of insect vectors and the weather pattern, and different strains of the virus are perpetuated within distinct ecosystems and in separate geographic regions by different vector species. This has been emphasized by the application of molecular genotyping techniques which have given further insights into the epidemiology of BTV and the extent of BTV evolution. Indeed molecular epidemiology studies have demonstrated the basis of the recent European emergence of BTV and how changes in BTV epidemiology coincide with climate changes which appear to have increased both the distribution and size of vector insect populations in the region. It is also evident that bluetongue viruses have crossed from the original insect vector species (e.g., *Culicoides imicola*) and are now being transmitted by novel vectors (e.g., *C. obsoletus* and *C. pulicaris*) that are abundant across Central and Northern Europe. As a result, many of the serotypes associated with the initial outbreaks have persisted and spread across much of Southern and Central Europe.

Immunity

Neutralizing antibodies, which play major role in protection against reinfection, appear within 8–14 days of virus infection. Although BTV infection results in the production of antibodies to all ten proteins, the outer capsid protein VP2 alone is sufficient to induce protective neutralizing antibodies. Cell-mediated immunity (CMI) also plays an important role in recovery from infection and protection against reinfection. Cytotoxic (CD8+) cells responsive *in vitro* to BTV antigens have been demonstrated in the blood of cattle and sheep during the first week of infection and observed to peak at 2 weeks post infection. Both cross-reactive and serotype-specific cytotoxic T-lymphocyte (CTL) responses occur in BTV-infected animals. The role of CMI in protection against BTV infection has been demonstrated in sheep in which possible transfer of specific CD8+ cells conferred protection on challenge with live virus. Immunity was major histocompatibility complex (MHC) restricted and thus was host specific.

Prevention and Control

Despite the high morbidity and mortality associated with BTV infection, little attention has been given to the development of efficacious vaccines. Early vaccination attempts included the use of virus in the serum of sheep which had recovered from the disease. This mild strain of the virus, which had been serially propagated in sheep, was used as an attenuated vaccine for more than 50 years despite evidence that it was not entirely safe and the resultant immunity was not adequate.

The only vaccines currently in use are live-attenuated vaccines developed by serial passage of BTV in embryonated chicken eggs. These are administered as polyvalent vaccines consisting of a total of 15 serotypes. Sheep develop BTV antibodies by 10 days post-vaccination. The antibody response reaches a maximum at 4 weeks and may persist up to 1 year. There is a temporal relationship between the increase in neutralizing antibody titer and clearance of virus from the peripheral circulation. However, there are several problems associated with these vaccines including incomplete protection and reversion to virulence. Attenuated virus can also be transmitted by insects from vaccinated sheep to other animals. This poses risks since live virus vaccine is teratogenic in pregnant sheep and may cause fetal death and abnormalities.

To avoid the problems encountered by live virus vaccines, a number of inactivated vaccines have been developed in various countries and are used locally. Many experimental trials have also been undertaken using VP2 antigen and VLPs as candidate vaccines. VP2 administered at high dose (>100 μg per dose) alone or together with VP5 (~20 μg per dose) has been demonstrated to be protective against virulent virus challenge in sheep. VLPs (containing the four major proteins) appear to be highly protective at a lower dose (10 μg per dose) and have generated protective

immunity for up to 15 months. However, despite their high protective efficacy and safety, no subunit vaccines are yet available commercially. One of the likely reasons for lack of interest in these vaccines is the lower demand of BTV vaccine in the Western countries, but this may change following the recent emergence of BTV in Europe.

Future Perspectives

BTV has been a major focus for understanding of the molecular biology of the *Reoviridae* and has served as a model system for the other members of the genus *Orbivirus*. Significant recent advances have been made in understanding the structure–function relationships of BTV proteins and their interactions during virus assembly. By combining structural and molecular data, it has been possible to make progress on the fundamental mechanisms used by the virus to invade, replicate in, and escape from susceptible host cells. Evidence has been obtained for the role of cellular proteins in non-enveloped virus entry and egress.

Despite these advances, some critical questions remain unanswered. In particular, host–virus interactions during virus trafficking is one area needing intense attention in the future. Exactly how each genome segment is packaged into the progeny virus is another outstanding question. One of the major drawbacks of research with BTV and other members of *Reoviridae* has been the lack of availability of a suitable system for genetic manipulation of the virus and this has limited our understanding of the replication processes. However, in a recent major development in BTV research, live virus has been rescued by transfection of BTV transcripts. There is no doubt that this will soon be extended to establish *in vitro* manipulative genetic systems and will allow molecular and structural studies of individual BTV proteins to be placed in the context of the whole virus.

See also: Reoviruses: General Features; Reoviruses: Molecular Biology.

Further Reading

Boyce M and Roy P (2007) Recovery of infectious bluetongue virus from RNA. *Journal of Virology* 81: 2179–2186.

Eaton BT, Hyatt AD, and Brookes SM (1990) The replication of bluetongue virus. In: Roy P and Gorman BM (eds.) *Current Topics in Microbiology, Vol. 162: Bluetongue Viruses*, pp. 89–118. Berlin: Springer.

Huismans H and Verwoerd DW (1973) Control of transcription during the expression of the bluetongue virus genome. *Virology* 52: 81–88.

MacLachlan NJ (1994) The pathogenesis and immunology of bluetongue virus infection of ruminants. *Comparative Immunology, Microbiology, and Infectious Diseases* 17: 197–206.

Mertens PPC, Maan S, Samuel A, and Attoui H (2005) *Reoviridae* – Orbivirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 466–483. New York: Academic Press.

Noad R and Roy P (2006) Bluetongue virus assembly and morphogenesis. In: Roy P and Gorman BM (eds.) *Current Topics in Microbiology and Immunology, Vol. 309: Reoviruses: Entry, Assembly and Morphogenesis*, pp. 87–116. Berlin: Springer.

Parsonson IM (1990) Pathology and pathogenesis of bluetongue infections. In: Roy P and Gorman BM (eds.) *Current Topics in Microbiology and Immunology,* Vol. 162: *Bluetongue Viruses*, pp. 119–141. Berlin: Springer.

Roy P (2005) Bluetongue virus proteins and particles and their role in virus entry, assembly and release. In: Roy P (ed.) *Virus Structure and Assembly, Vol. 64,* pp. 69–114. New York: Academic Press.

Roy P (2006) Orbiviruses and their replication. In: Knipe DM and Howley PM (eds.) *Field's Virology,* 5th edn., pp 1975–1997. Philadelphia, PA: Lippincott–Raven Publishers.

Stott JL and Osburn BI (1990) Immune response to bluetongue virus infection. In: Roy P and Gorman BM (eds.) *Current Topics in Microbiology and Immunology, Vol. 162: Bluetongue Viruses*, pp. 163–178. Berlin: Springer.

Verwoerd DW, Huismans H, and Erasmus BJ (1979) Orbiviruses. In: Fraenkel-Contrat H and Wagener RR (eds.) *Comprehensive Virology,* vol. 14, pp. 163–178. New York: Plenum.

Border Disease Virus

P Nettleton and K Willoughby, Moredun Research Institute, Edinburgh, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cytopathic effect A deleterious change in the microscopic appearance of cultured cells.
Genotype Individual or groups of viruses which form a significant branch in a phylogenetic tree generated using computer-assisted nucleotide sequence analysis.

Persistent infection (PI) A lifelong infection with noncytopathic BDV resulting from *in utero* infection during the first 60 days of gestation. Infected fetuses have an immune tolerance of the virus which lasts long after birth. PI sheep can live for at least 5 years and constantly excrete high levels of infectious virus.

Introduction

Border disease virus (BDV) is one of the four species of pestivirus classified within the family *Flaviviridae*. The genus *Pestivirus* comprises BDV, classical swine fever virus (CSFV), and the two distinct species of bovine viral diarrhea virus (BVDV), BVDV 1 and BVDV 2. The viruses were named after the diseases they cause, so traditionally pestiviruses isolated from sheep or goats were termed BDV, those from swine CSFV, and those from cattle BVDV. It is now recognized, however, that domestic ruminants and pigs can all be infected by more than one pestivirus. Pestiviruses also have been recovered from wild animals of various species, and modern methods of antigenic and genetic analysis have identified BDV from sheep, goats, pigs, cattle, reindeer, European bison, and chamois.

BDV is principally recognized as a significant cause of congenital disease in sheep, resulting in barren ewes, abortion, stillbirths, and the birth of small weak lambs some of which show tremor and hairy fleece. Many such lambs are persistently infected (PI) with BDV all their lives, and because they constantly excrete virus they are very efficient spreaders of infection. The ability of BDV to produce PI animals following *in utero* infection is a fascinating biological phenomenon of virological and immunological interest. Control of the disease in a sheep flock relies on the identification of PI sheep and the prevention of infection of susceptible ewes during pregnancy.

History

First Reports of Border Disease

Although shepherds in the border region of England and Wales had been seeing weak, rough-coated, and trembling lambs for many years, it was not until 1959 that workers at the then newly opened Veterinary Laboratory at Worcester described a clinico-pathological condition they named border or 'B' disease. Recognition of the disease in New Zealand, Australia, and the United States soon followed and affected sheep were aptly named 'hairy-shaker' or 'fuzzy' lambs. In the early years, when the condition was only recognized in two closely related breeds of sheep, a genetic etiology was seriously considered. As more reports appeared in other sheep breeds, an infectious cause was thought more likely and the transmissibility of the condition was confirmed in 1967 by Dickinson and Barlow in Edinburgh and Shaw and colleagues at Worcester.

Isolation of Virus

Evidence for a viral etiology came from workers at the Moredun Research Institute in Edinburgh who reported in 1972 that, antibiotic-treated, cell-free filtrates of tissue homogenates could cause infection. They showed the agent to be ether sensitive, to be destroyed by heating to 60 °C for 90 min, and to have an estimated size of about 27 nm. Similar experiments in Australia, Ireland, and the USA confirmed the transmissibility findings and added the valuable information that ewes receiving inocula derived from border disease (BD)-affected lambs developed antibodies against BVDV, so that by the mid-1970s the serological relationship between BDV, BVDV, and CSFV was established. Throughout the 1970s there were reports that BVDV or BDV from sheep could be isolated in cell culture. The first reports were from Australia, where a BVD virus caused abortion but no other signs of BD. The full clinical picture of BD was subsequently reproduced with noncytopathic cell culture isolates by Harkness at Weybridge, UK, Plant in Australia, and Terpstra in the Netherlands. Meanwhile, Vantsis at Moredun had isolated a virus in fetal lamb kidney cells, a virus that was cytopathic and reproduced BD at the 12th and 29th cell culture passages.

Understanding the Pathogenesis of BD

In all the early transmission experiments clinical disease was rarely detected in recently infected animals. Slight fever and mild leukopenia was detected 1–2 weeks post infection, after which serum neutralizing antibodies were demonstrable. Inoculation of pregnant ewes, however, resulted in BD-affected offspring. In a series of experiments at Moredun, Barlow and Gardiner studied the effects on the ewe and the fetus of different inocula given at different stages of gestation. They showed that the main effect on the ewe was a necrotizing placentitis which varied from a mild focal lesion to a diffuse lesion, sometimes serious enough to contribute to fetal death and abortion. In affected fetuses and newborn lambs BDV infection interfered with pre- and postnatal development of the viscera, for example, thymus, spleen, skeleton, and muscles. The abnormal coarseness of the fleece was shown to be due to increased size and altered structure of the primary wool fibers (**Figure 1**), while the neurological signs predominantly resulted from a myelin deficiency throughout the central nervous system (CNS), with occasional brain malformations being seen. In all these outcomes the strain of virus and the timing of infection were critical.

Persistent Viremia

Current understanding of the timing of infection hinges on the onset of immune competence by the fetal lamb. The ovine fetus can first respond to an antigenic stimulus between 60 and 85 days of its 150-day gestation period. In



Figure 1 (a) A group of clinically affected BD lambs showing variable ability to stand. With careful nursing all these lambs survived and their neurological signs were no longer evident by 6 months old. (b) Hairy-shaker lamb in the foreground with breed matched normal lamb behind. The fleece of the hairy-shaker lamb is less curly with long fibers most prominent over the top of the head and neck. Reproduced by permission of Moredun Research Institute.

fetuses infected before the onset of immune competence, viral replication is uncontrolled and it is common to observe that half these fetuses die. Lambs that survive infection in early gestation are tolerant to the virus, which is widespread in all organs. Such PI lambs are born with a persistent viremia, so that a precolostral blood sample is virus positive and antibody negative. In such lambs there are no inflammatory changes. At all levels in the CNS there is a deficiency of myelin. This may be slight in lambs with only mild trembling of the head, ears, and tail, but can be pronounced in lambs unable to stand due to violent rhythmic contractions of their hind leg muscles. In lambs that suck colostrum containing anti-BDV antibodies, viremia may be masked for up to 2 months but after that lambs have high virus titers in their blood and continue to excrete virus, usually for the rest of their lives. With careful nursing a proportion of BD lambs can be reared although deaths may occur at any age. The neurological signs gradually become less noticeable and may have disappeared by 3–6 months. Weakness, swaying of the hind quarters, and fine trembling of the head may reappear at times of stress. Affected lambs can grow slowly and many die at or around weaning. However, a small proportion of viremic sheep appear normal and can survive to breeding age. Lambs born to viremic dams will always be PI. PI sheep are a continual source of infectious virus and their identification is a major factor in any control program. Sheep being traded for breeding should be screened for the absence of BDV viremia.

Fetal Infection Later in Gestation

Fetal infections occurring just as the immune system is developing, around mid-gestation, can result in lambs with severe nervous signs, locomotor disturbances, and abnormal skeletons. Such lambs have destructive lesions of cerebellar hypoplasia and dysplasia, hydranencephaly, and porencephaly resulting from necrotizing inflammation. The severe lesions appear to be immune-mediated and such lambs usually have high levels of anti-BDV antibody at birth. Lambs infected in late gestation mount an immune response to the virus and are born apparently healthy, free of virus but with antibody to BDV. Such lambs, however, can be stillborn or weak and losses would be above average in early life.

Late-Onset Disease in PI Sheep

In the early 1980s, experiments at Moredun investigating the nature of immune tolerance to BDV unexpectedly resulted in the deaths of PI sheep, which had been challenged 2–3 weeks earlier with live, cytopathic BDV. The sheep developed intractable scour, wasting, and excessive ocular and nasal discharges, sometimes with respiratory distress. At necropsy there was gross thickening of the distal ileum, cecum, and colon resulting from focal hyperplastic enteropathy. Cytopathic BDV was recovered from the guts of dying lambs and the syndrome had several similarities with bovine mucosal disease. Similar lesions also had been seen in PI sheep that had been housed away from other animals and from which noncytopathic BDV had been repeatedly recovered since birth. At postmortem of these spontaneous cases of late-onset disease, cytopathic virus was consistently recovered from gut tissues. With no obvious outside source of cytopathic virus, it was believed that such virus originated from the lamb's own virus pool.

Virtually, all pestivirus isolates from sheep and goats are noncytopathic in cell culture. The NS2–3 genes of two cytopathic sheep strains (Moredun and Cumnock) have been studied, and, in both these, it has been shown that they contain insertions of cellular sequences within

the NS2–3 encoding region, which results in its cleavage to NS2 and NS3. This is analogous to the similar process in BVDV, which is associated with the development of mucosal disease in cattle. Such cattle are PI with noncytopathic (NCP) BVDV following *in utero* infection. Mutation of the persisting virus RNA in the region coding for NS2–3 can result in overproduction of NS3, which correlates with the development of mucosal disease. While it has proved possible to isolate and biologically clone pairs of noncytopathic and cytopathic viruses from individual cattle dying of mucosal disease, this has so far proved not to be the case with the analogous condition in sheep. Thus, no cytopathic isolate of BDV has ever been successfully biologically cloned and shown not to also contain noncytopathic virus.

Physico-Chemical Properties of BDV

Pestiviruses are enveloped, spherical particles approximately 46 nm in diameter with a 20–25 nm core. They consist of an outer lipid envelope surrounding an inner capsid protein that contains the 12.3 kbp long, single-stranded, positive-oriented RNA viral genome, which comprises two terminal flanking untranslated regions and a single open reading frame (ORF), encoding approximately 4000 amino acids. The lipid envelope is pleiomorphic which makes it difficult to purify infectious particles by ultracentrifugation or to visualize virions by electron microscopy. The M_r of a virion is estimated at 6×10^7 and the buoyant density in sucrose of BDV is 1.09–1.15 g ml^{-1}, with peak infectivity at 1.115 g ml^{-1}. Virions are stable within a pH range of 5.7–9.3, but are inactivated by lipid solvents (e.g., 5% chloroform for 10 min at room temperature), detergents, and treatment with 0.05% trypsin for 60 min at 37 °C. Viruses in cell culture fluids are inactivated by heating at 56 °C for 30 min, but this is not a reliable method for the inactivation of viral infectivity in fetal calf serum. At lower temperatures the virus is more stable; but even at 37 °C reductions in infectivity can be detected after 24 h. Inactivation of pestiviruses in fetal bovine serum is reliably achieved using gamma irradiation (20–30 kGy) or ethyleneimine (10 mM at 37 °C for 2 h).

Antigenic and Genetic Relationships among Pestiviruses

The *Eighth Report of the International Committee on Taxonomy of Viruses* recognized four species of pestiviruses, namely BDV, CSFV, and BVDV-1 and-2 with a fifth virus originating from a giraffe as a tentative species. All pestiviruses are antigenically related but criteria for virus species demarcation include: nucleotide sequence relatedness, antigenic relatedness as measured in cross-neutralization or cross-protection assays, and host of origin. While nucleotide sequences can be generated quickly from new viruses and easily compared with sequences from known viruses, comprehensive antigenic relatedness studies are more demanding and often lacking. As pestiviruses are increasingly identified in wild animals, the natural host of origin becomes less certain. Among pestiviruses, rapid sequencing of nucleic acids coupled with computer-assisted phylogenetic analysis can be readily used to identify viruses of the four recognized species plus the giraffe pestivirus. Nearly all isolates can be fitted into these species, but as more isolates are examined, groups of viruses which form significant branches within each pestivirus species are becoming recognized. Thus, recent studies of ovine pestivirus isolates from Germany and Switzerland by Becher and colleagues at Giessen in Germany led to the proposal for three such genogroups among BDVs. BDV-1 contains many of the traditional well-characterized BDV isolates, BDV-2 contains recent German isolates plus a reindeer virus, while BDV-3 contains a single recent German isolate. Since that work a fourth genogroup BDV-4 has been proposed following analysis of Pyrenean chamois and Iberian sheep isolates. Furthermore, pestiviruses isolated from Tunisian sheep may represent an additional BDV genotype and an Italian goat isolate may represent a putative novel pestivirus close to BDV. Thus, it would appear that BDV may contain the widest spectrum of virus subtypes of all the pestiviruses.

Molecular Biology of BDV

Viral Genome

The complete genomic sequences of two sheep BDVs have been published. Strain BD31 (GenBank accession number U70263) is an American hairy-shaker lamb isolate which is available from the American Type Culture Collection. Strain X818 (GenBank accession number AF037405) is an Australian isolate also derived from a lamb clinically affected with BD. Both BD31 and X818 belong within genogroup BDV-1. The only other complete BDV genome sequence published is that of an isolate from a reindeer (GenBank accession number NC003678 or AF144618), a representative strain of the BDV-2 genotype. The structures of these three genomes are comparable and are similar to those of other pestiviruses. Thus, the 12.3 kbp single-stranded genome of BDV codes for one long ORF of 3895 codons bracketed by a 5′ untranslated region (UTR) of 356–372 bp and a 3′ UTR of 223–273 bp. When compared with other pestiviruses, BDVs unexpectedly have closer sequence homology to strains of CSFV than to some BVD viruses.

The 5′ UTR is recognized as having the highest overall nucleic acid sequence identity among pestiviruses. Conservation of 5′ UTR sequences suggests a strong functional pressure likely to be related to the formation of tertiary structures required for the internal ribosomal entry site (IRES) from which translation is initiated. Within the highly conserved 5′ UTR, there are two short variable regions located between nucleotide positions 210–230 and 300–320. The 5′ UTR region thus lends itself to the design of panpestivirus polymerase chain reaction (PCR) primers which allow the amplification of all pestiviruses, as well as primers which allow the speciation of pestiviruses.

The 3′ UTR region of BDVs is of interest since it is longer than those found in other pestiviruses and contains a conserved sequence motif at more than one location, also found in CSFVs but absent from BVDV viruses.

Viral Proteins

The identification of BDV-encoded proteins together with the predictions of conserved cleavage sites strongly suggests that the polyprotein of BDV is processed similarly as those of the other pestiviruses.

Structural Proteins

The large ORF is translated as a single polyprotein with the order of individual proteins being N^{pro}, C, E^{rns}, E1, E2, p7, NS 2/3, NS4A, NS4B, NS5A, NS5B (**Figure 2**). The polyprotein is processed during and after translation by viral and host proteases. There are four structural proteins in the mature virion: C, E^{rns}, E1, and E2. C is the nucleocapsid protein and the other three are glycosylated envelope proteins. E2 is the outer immunodominant envelope glycoprotein against which the host produces a significant neutralizing antibody response. As E2 is the most variable protein among pestiviruses, monoclonal antibodies against E2 can be useful for discriminating pestivirus isolates. There is some evidence that E^{rns} also stimulates protective host immune responses, but there is no evidence as yet that E1, the inner envelope protein, stimulates protective antibody. E^{rns} is less conserved than is E1 and is a protein of fascination to virologists, since it has ribonuclease activity, has anti-interferon activity, and is present in the serum of PI animals at levels high enough for diagnostic detection.

Nonstructural Proteins

The first viral protein of the pestivirus ORF is the nonstructural protein, N^{pro}. Like E^{rns}, this protein is unique to pestiviruses and is crucial in the establishment and maintenance of persistent infection. It has protease activity and cleaves itself from the polyprotein. The tiny nonstructural protein p7 follows E2 in the polyprotein and can remain associated with it, so that two forms of E2 with different C-termini (E2 and E2-p7) are found in infected cells. However, p7 alone or as E2-p7 is not found associated with infectious virions and its role is unknown. The next nonstructural protein, NS2–3, has attracted considerable attention because of its role in the cytopathogenicity of pestiviruses. The success of pestiviruses is due to the unique biological properties of the noncytopathic virus. Only the noncytopathic viruses are biologically able to ensure they are maintained. When cytopathic viruses evolve from noncytopathic viruses by nonhomologous RNA recombination, they signal the end of the life of the PI animal and the removal of that source of biologically competent noncytopathic virus. The NS2–3 in noncytopathic viruses contains a serine protease that is essential for the release of the NS proteins located downstream of NS3. In cells infected with cytopathic BDV, two antigenically related polypeptides of apparent M_r 80 kDa and 130 kDa are detectable, whereas only a single polypeptide of 120 kDa is recognized in cells infected with noncytopathic BDV. The 120 kDa and the 130 kDa proteins represent NS2–3, with the polypeptide from the cells infected with cytopathic virus containing a cellular insertion. The 80 kDa polypeptide in the cells infected with cytopathic BDV is NS3. NS2 appears to be nonimmunogenic and little is known about its function. On the other hand, NS3 produced by cytopathic pestiviruses is of considerable interest. It is highly conserved across the pestiviruses and contains immunogenic epitopes. Purified NS3 possesses serine protease, RNA helicase, and RNA-stimulated NTPase activities. A catalytically active NS3 serine protease is essential for pestivirus replication.

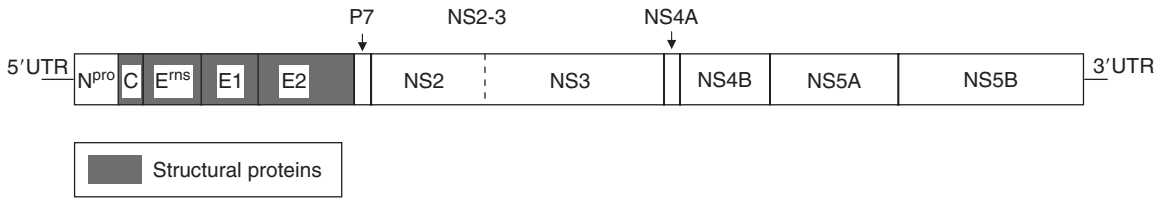


Figure 2 Diagram of the proteins formed from the polyprotein translated from the genome of a noncytopathic pestivirus. In a cytopathic pestivirus a protease cleavage site results in NS2–3 being cleaved into NS2 and the conserved, immunodominant NS3. Reproduced by permission of Moredun Research Institute.

It appears to be produced in large amounts in cells infected with cytopathic viruses and its buildup coincides with cell death.

Less is known about the NS proteins downstream of NS3. The NS3 protease depends on a cofactor domain in the central region of NS4A and there is evidence that duplications of codons in NS4B and an insertion of viral sequences between NS4A and NS4B are well tolerated and do not interfere with efficient viral replication. This has led to the suggestion that the NS4A–NS4B region in pestiviruses and related viruses may be suited to the insertion of heterologous sequences.

The NS5A is the least conserved protein when BDV X818 is compared to other pestiviruses. No information is available on the role of this protein in BDVs. Work with BVDV, CSFV, and the related hepatitis C virus (HCV, genus *Hepacivirus*, family *Flaviviridae*) shows that pestivirus NS5A contains an essential zinc-binding site similar to that of HCV. Also structural conservations within NS5A point toward conserved roles of the terminal in-plane membrane anchor in the replication complex formation in these viruses.

The pestivirus NS5B protein possesses RNA-dependent RNA polymerase activity and is involved in the assembly of infectious viruses. It has attracted attention as a target for antiviral drug discovery.

Viral Replication

BDV has rarely been used to study pestivirus replication. It is appropriate to assume that BDV replicates in the same way as do other pestiviruses. Studies on the replication of pestiviruses have received added impetus because their mechanisms of virion maturation and assembly seem to be equivalent to those of HCV. BVDV has consequently been used as a surrogate model of HCV for the evaluation of antiviral agents.

Viral Attachment

The cell-surface receptors for pestiviruses require further clarification. There is good evidence that bovine CD46 serves as a cellular receptor for BVDV, and some evidence that heparin sulfate also does. E2 and E^{rns} are the viral glycoproteins essential for attachment and they appear to use different cell-surface receptors. A chimeric BVDV with the E2 coding region of a BDV has been used to establish a role for E2 in determining different tropisms of a pestivirus for bovine or ovine cell cultures. It may be, however, that such results are virus strain and cell line dependent since another study showed that CSFV E2 and BVDV E2 share an identical receptor on porcine and bovine cells. In the same study several BVDV strains isolated from cattle were not inhibited by the presence of CSFV E^{rns} suggesting that E^{rns} also plays a role in host tropism.

Virus Replication

After binding to a cellular receptor, BVDV is internalized via clathrin-dependent endocytosis. Virus-membrane fusion occurs in the endosomal compartment and release of viral RNA follows pH-dependent breakdown of the envelope glycoproteins. The viral RNA serves as mRNA and, using its own and cellular enzymes, undergoes translation into viral proteins. Viral RNA must also serve as the template for RNA replication through the synthesis of complementary negative strands. These, in turn, serve as templates for the synthesis of genome length positive strands for incorporation into new virions. Electron micrographs comparing BDV- and BVDV-infected cells showed mature virions of both viruses to be similar. Virus replication takes place totally within the cytoplasm in association with structures formed from modified endoplasmic reticulum (ER). Free virus-like particles were not seen in the cytoplasm, nor were any seen budding through internal host cell membranes or passing through the cytoplasmic membrane. Virus release is presumed to occur when remnants of ER are released or when cells disintegrate. Pestiviruses have a surprisingly large number of unique properties, for example, N^{pro}, E^{rns}, NS2–3 cleavage, and RNA recombination, all of which contribute to its replication and fascinating biology.

Detection and Control

The presence of BDV in an infected flock is most easily determined by detection of the virus in dead or otherwise clinically affected lambs at birth, and by demonstrating the presence of antibody in ewes with BD-affected lambs. Virus detection can be by virus isolation, immunohistochemistry, viral antigen detection by the enzyme-linked immunosorbent assay (ELISA), or by nucleic acid detection by reverse transcriptase-polymerase chain reaction (RT-PCR). Antibody to BDV can be detected in sheep sera using virus neutralization or ELISA.

The control of BDV in a sheep flock has two essential requirements: the identification of PI sheep and the prevention of infection of susceptible pregnant ewes especially during the first half of gestation. One commercial vaccine has been produced, a killed adjuvanted vaccine containing representative strains of BDV and BVDV-1. It is administered to young animals before they reach breeding age in order to maximize their immunity during early pregnancy. An annual booster dose is required. Further vaccine development is required, with candidate vaccines being tested for efficacy in pregnant sheep.

See also: Bovine Viral Diarrhea Virus; Classical Swine Fever Virus.

Further Reading

Barlow RM and Patterson DSP (1982) Border disease of sheep: A virus-induced teratogenic disorder. *Advances in Veterinary Medicine* (supplement to the *Journal of Veterinary Medicine*) No. 36: 1–90.

Nettleton PF, Gilray JA, Russo P, and Dlissi E (1998) Border disease of sheep and goats. *Veterinary Research* 29: 327–340.

Thiel H-J, Collett MS, Gould EA, *et al.* (2005) *Flaviviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 981–998. San Diego, CA: Elsevier Academic Press.

Vilcek S and Nettleton PF (2006) Pestiviruses in wild animals. *Veterinary Microbiology* 116: 1–12.

Bornaviruses

L Stitz and O Planz, Federal Research Institute for Animal Health, Tuebingen, Gemany
W Ian Lipkin, Columbia University, New York, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Borna disease (BD) is one of the oldest known viral infections of animals. The name was adopted in 1895 after an epidemic among horses of a cavalry regiment in the town of Borna in Saxony near Leipzig. However, descriptions of natural infection in horses and sheep were reported in the veterinary literature as early as 1767. Synonyms such as disease of the head (Kopfkrankheit), brain fever, subacute meningitis, or hypersomnia of horses have been known for more than 200 years and reflect the restriction of the disease to the central nervous system (CNS). A viral etiology was proposed in 1925 by Zwick and Seyfried based on filtration experiments. The causative agent, Borna disease virus (BDV), was identified as a unique, nonsegmented negative-strand RNA virus in the late 1980s following the first successful application of subtractive cloning in microbiology.

Properties of the Virion

Viral particles have not been visualized in infected tissues. Thus, morphological descriptions are based on observations in cultured cells. BDV particles are enveloped, spherical, 70–130 nm in diameter, and contain a 60 nm diameter helical ribonucleoprotein. BDV does not differ in its physical properties from other known enveloped viruses. It is sensitive to lipid solvents such as chloroform or acetone and to detergents. BDV is stable at low temperature and at pH 5–12; however, infectivity is destroyed by high temperatures and pH less than 5.

Properties of the Genome

Data on the nature of the BDV genome have been obtained from complementary DNA clones using libraries prepared either from the brain of BDV-infected rats or from cell cultures. The genomic single-stranded RNA (ss RNA) of 8.9 kb with complementary 3′- and 5′-termini has been shown to be of negative polarity and represents the smallest genome of all *Mononegavirales* (**Figure 1**). Investigations of the organization of the genome have revealed six open reading frames (ORFs) on the antiviral genome (3′-N-X/P-M-G-L). Several unusual aspects for an ssRNA virus have been recognized in molecular biological studies, in particular an overlap of reading frames, a post-translational processing of subgenomic RNAs (RNA splicing), and nuclear phases indispensable for virus replication. Results of sequence analyses of viruses obtained from various animal species, man, or tissue culture, revealed a remarkable conservation of coding sequences.

Properties of the Viral Proteins

Infected brains and cultured cells contain BDV proteins with an approximately relative molecular masses of 56, 38/40, 24, 14, and 10 kDa can be detected in cultured cells and brains after BDV infection. The most abundant viral proteins in infected cells as well as in viral particles have a molecular mass of 38/40 and 24 kDa. Together these comprise what is known as the soluble antigen (S-antigen). The BDV genome encodes six major BDV proteins. ORF I, which is the prominent 3′-ORF, encodes the nucleoprotein (NP) in its two isoforms (38 and 40 kDa proteins). ORF II codes a 24 kDa protein, a protein phosphorylated in its serine residues, representing the viral phosphoprotein (P). An additional ORF X has been identified that overlaps ORF II, encoding a nonglycosylated BDV protein designated X or p10. ORF III encodes the 14 kDa matrix protein (M). ORF IV encodes a 57 kDa precursor that is found in its N-glycosylated form at approximately 90 kDa (described as gp84 and gp94) and represents the glycoprotein of the virus (G); post-translational cleavage

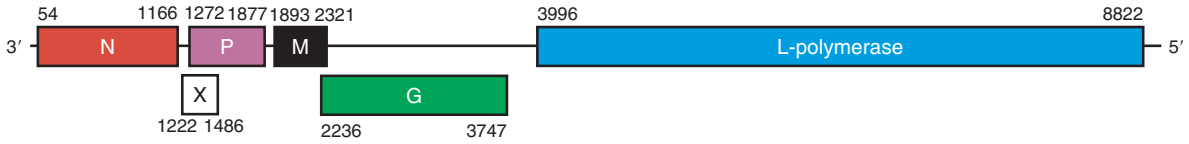


Figure 1 Organization of the 8.9 kb BDV genome.

products of this G have been reported to result in molecular masses of approximately 40 kDa by the subtilisin-like cellular protease furin, corresponding to the N- and C-terminal regions of G. ORF V, which is localized at the 5′-end of the viral antigenome, has been identified as the 180 kDa RNA-dependent RNA polymerase (L) of BDV. It contains conserved regions characteristic for *Mononegavirales* and three RGD motifs which are generally important in protein–protein interactions.

Proteins X, P, and N are found mainly in the nucleus of infected cells. Colocalization of P and X on the one hand and N and X on the other hand might be explained by the fact that sequences within the X sequence have been identified which are typical for nuclear export trafficking. X itself has been demonstrated both in the nucleus and the cytoplasm. In addition, evidence has been presented that indicates the presence of a nuclear targeting sequence in the 40 kDa isoform of N which is not present in the 38 kDa isoform. This results in accumulation of the 40 kDa protein in the nucleus, whereas the 38 kDa isoform is found in both the nucleus and the cytoplasm. The heavily N-glycosylated gp84 has been seen both intracytoplasmically in the ER as well as on the cell surface, and its function is seen in context with receptor-mediated viral entry, assembly, and budding of BDV. The gp84 and gp94 forms of G as well as the M contain neutralization epitopes.

Replication and Life Cycle

Both neural and non-neural cell lines support BDV replication. However, replication is more efficient in neural cell lines. Even in neural lines a single cell produces less than 10 infectious particles and titers rarely exceed 10^6 TCID50/ml. This observation is unexplained as large amounts of viral antigen can be demonstrated immunohistologically.

After receptor-mediated endocytosis the BDV genome is replicated and transcribed in the nucleus. The cytoplasmic-nuclear shuttle is mediated by specific nuclear localization signals (NLSs) located in the N (p40), X, P, and L proteins. X contains a nuclear export signal (NES); although export activity has not formally been shown, X is proposed to participate in the subcellular localization of N and P. A variety of mechanisms are used to regulate gene expression. The use of cellular splicing machinery to process primary RNA transcripts is unique by BDV among the *Mononegavirales*. Other intriguing unusual aspects of BDV molecular biology include overlapping reading frames, overlapping transcription units, alternate RNA splicing, and leaking scanning of ribosomes during translation. The replication cycle seems to be completed within 24 h. Little, if any, infectious virus is released from infected cells. Additional information concerning the role of individual gene products in the virus life cycle will be forthcoming with the recent introduction of reverse genetic systems.

The distribution of BDV *in vivo* has been studied in rat models of experimental infection using immunohistochemical and molecular probes (**Figure 2**). Early in infection of adult immunocompetent animals, viral gene products are confined to the CNS and are found predominantly in neurons. At later phases, virus is found in astrocytes, oligodendroglia, ependymal cells, and Schwann cells. In animals that are immunocompromised or tolerant of the virus because of infection within 24 h of birth, BDV may also replicate in cells of peripheral organs. In recent years, it could be demonstrated that BDV interacts with intracellular signaling pathways and with the cell cycle of the host cell.

Geographic Distribution

BD was originally described as a disease restricted to certain endemic areas in eastern and southern Germany and Switzerland. More recent seroepidemiological studies, however, have shown that natural infection may be more widely distributed than previously anticipated. Seropositive horses have been found throughout Germany, in other European countries, and in North America and Japan. The potential impact for agriculture of BDV infection has almost certainly led to under-reporting. One of the authors performed serological tests for an agricultural ministry that revealed evidence of infection in horses, sheep, and cattle but was not provided with the information needed to publish the results.

Host Range and Virus Propagation

Natural infection with BDV was first described in horses, sheep, cattle, and rabbits. Later, cats, ostriches, and various

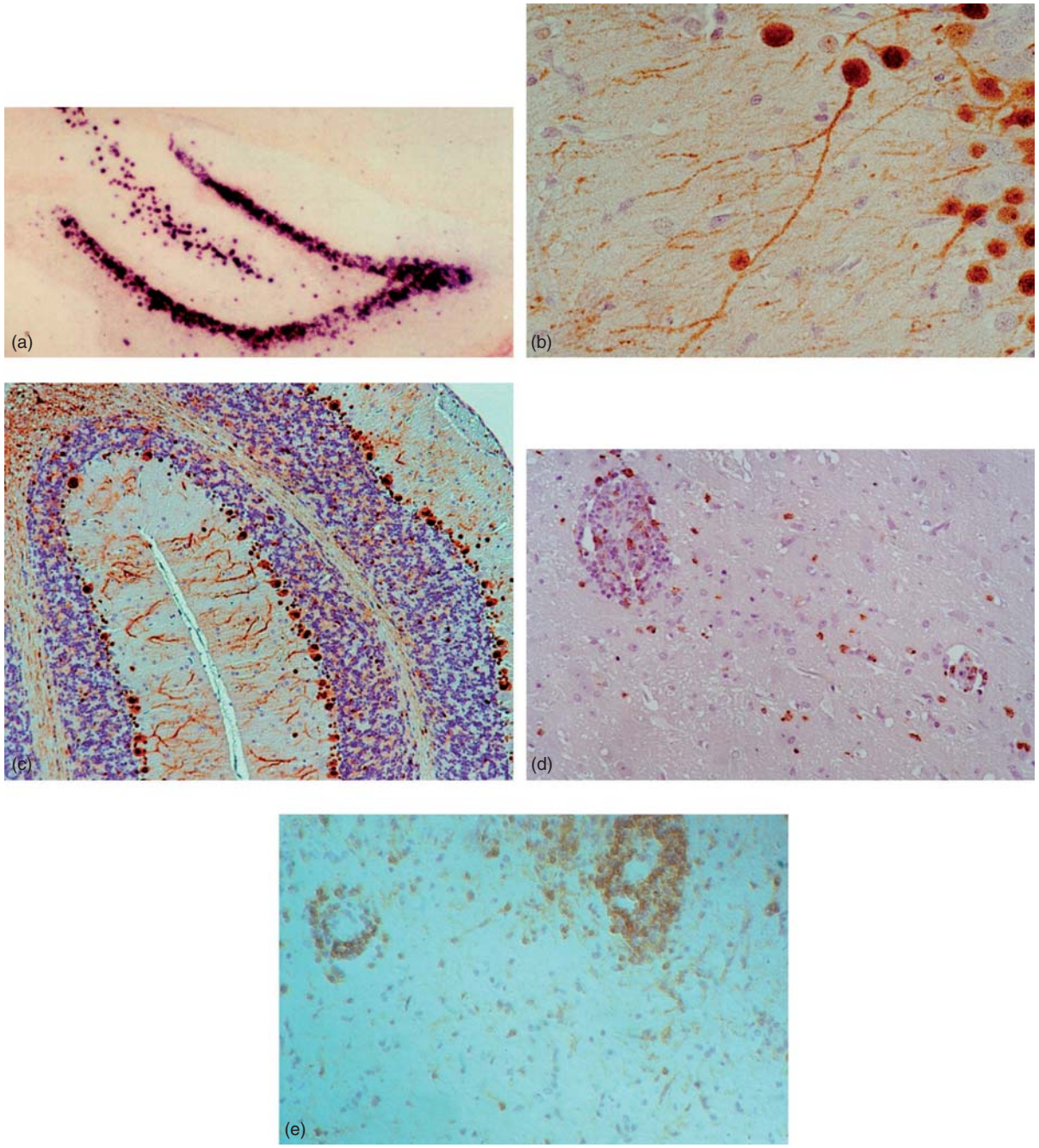


Figure 2 (a) Presence of BDV RNA in the hippocampus of an infected rat (*in situ* hybridization); (b) detection of BDV p40 in neurons of rat brain cortex by specific monoclonal antibody (immunohistochemistry); (c) presence of BDV p40 in rat cerebellum (immunohistochemistry); (d) $CD8^+$ T cells in the parenchyma of BDV-infected rat; and (e) presence of $CD4^+$ T cells in perivascular cuffing of infected animal. Courtesy to Dr. H. J. Rziha, Tuebingen.

zoo ruminants were identified as natural hosts. Reports of infection in goats, deer, and donkeys have not been proved unequivocally, but are plausible in view of the broad experimental host range. The role of BDV in human disease remains controversial. Seropositive reactions have been reported in patients with psychiatric disorders from Germany, the US, and Japan. The presence of virus-specific nucleic acid has also been reported in postmortem brain samples of patients with schizophrenia, affective disorders, and hippocampal sclerosis. Virus has been recovered from the brain of one schizophrenic patient. Some investigators have also reported BDV nucleic acid in monocytes of

patients with chronic fatigue syndrome, affective disorders, and schizophrenia. However, other investigators have been unable to independently replicate experiments showing human infection. The route for human infection (if true) is unknown as no convincing correlation has been established between the distribution of potential cases and infected individual animals or herds.

Experimental hosts comprise a wide variety of species ranging from birds to nonhuman primates. The most thoroughly investigated experimental model of BDV infection is the Lewis rat. This model has contributed considerably to the elucidation of BD pathogenesis, because the manifestations of infection are similar to those observed in naturally infected horses. Mice can be experimentally infected and have advantages with respect to genetic studies; however, the utility of murine models has been limited to immunopathology because behavioral consequences do not accurately represent what is observed in natural infection. Infection of adult mice can result in persistent CNS infection, but only rarely results in clinical disease. Infection of suckling mice can result in profound disease dependent on the mouse strain.

Serological Relationship and Variability

Serologically, no distinction can be made between different virus isolates by using polyclonal antibodies. Variable detectability of viral antigens in brain specimens by monoclonal antibodies indicates, however, that some strain variation may exist. Using phylogenetic analyses virus isolates can be clustered corresponding to the geographical regions from which the viruses originated. In addition to the natural isolates and to the so-called laboratory strains, a BDV vaccine strain (Dessau) exists. Nevertheless, no conclusive data are available on the occurrence of biologically different virus strains in natural hosts. A recently described BDV strain (No/98) originated from an area in Austria where BD is nonendemic, is highly divergent to all field and laboratory strains, as well as to the vaccine strain.

It remains to be seen whether inapparent infections in horses and sheep might be due to variant strains. All virus strains used for infection of experimental animals originate from brain tissue of naturally infected diseased horses. These isolates mostly underwent serial passages in rabbits and were adapted thereafter to different animals. Different biological properties of virus isolated at various times after infection from rats could also point to the existence of variants. In this context it should be emphasized that virus isolates exist that cause extreme obesity syndrome or behavioral alterations in infected rats. Sequence analyses confirmed the general view of highly conserved regions in the BDV genome.

Epidemiology

Originally, BD was considered an endemic disease occurring sporadically in Central Europe. Seroepidemiological investigations in horses have revealed virus-specific antibodies in 12% of randomly collected horse sera from Germany, Switzerland, and other European countries. Most of these animals were without clinical signs of BD, but some exhibited disease symptoms within an observation period of 1 year. Since the chance of contact between horses has increased over the past few decades through worldwide horse trading and international sport activities, it cannot be excluded that BD has been distributed worldwide. Investigations in endemic areas in Germany, Switzerland, Austria, and Liechtenstein have supported the notion of regional virus reservoirs. In this context, the finding that shrews harbored a virus with 99.9% sequence identity to a virus sequence derived from a diseased horse in the same area of Switzerland might be of great importance. However, it cannot be excluded that virus is shed during an inapparent infection of horses and sheep which also could lead to further propagation of the infection. Virus-specific RNA has been demonstrated in leukocytes, saliva, conjunctival and nasal fluids, and in urine of clinically healthy horses in stables with BD history. If one takes into account that persistent infections can be established in tolerant infected rats and that these animals continuously shed virus, this mode of infection – in addition to other reservoirs as mentioned – appears to also be a possibility for maintaining BDV in the horse population.

Transmission and Tissue Tropism

Intranasal infection seems to be the most likely route of natural infection. Nerve endings in the nasal mucosa are readily accessible to the virus and pathological features observed in naturally infected horses such as edema of the bulbus olfactorious can be found in experimentally infected animals only after intranasal infection. An oral route via the trigeminal nerve is also plausible. In its natural hosts and in adult experimentally infected animals the virus shows a strict neurotropism. After inoculation in the periphery, BDV migrates centripetally to the brain where it replicates in neurons and astrocytes, especially in neurons of the hippocampus. Various routes of infection have been shown to result in clinical disease in susceptible animals. The most common route of experimental infection is intracerebral inoculation; however, intranasal, intraperitoneal, and intravenous routes are also effective. In rats infected as adults the virus shows the same strict neurotropism as in the natural hosts. Infectious virus is found in the brain as early as 7 days after infection and reaches titers of 10^6 TCID50/ml. Infectious virus can also be detected in the spinal cord, in ganglia of

the autonomic nervous system, in peripheral nerves, in the adrenal glands and neural cells of the retina although at considerably lower titers. No infectivity is found in the eyes late during the course of infection, possibly because degeneration of neurons in the retina eliminates cells in which the virus replicates most efficiently. In other nervous system tissues infectivity can be found throughout the life span. In sharp contrast to rats infected as adults, neonatally infected rats harbor virus not only in the CNS but also in peripheral organs, including salivary and lachrymal glands and the urinary bladder. Indeed, virus can be found in saliva, lachrymal fluid, and in urine. A similar pattern of distribution of BDV was found in long-term immunosuppressed adult rats.

Pathogenicity

To date virus strains isolated from naturally infected animals have been equally pathogenic. However, because the isolates have been obtained from animals with active disease, it is conceivable that these strains do not reflect the full spectrum of pathogenicity. Support for the existence of less pathogenic virus variants can be found in experimentally infected rats, where some strains are associated with mild or inapparent disease. Some species appear to be infectible but resistant to disease. Although adult rabbits and rats develop severe meningoencephalitis following inoculation with virus isolated from diseased horses or sheep, adult mice, hamsters, and tree shrews can have inapparent infection or mild disease. The basis for differences in susceptibility to disease is still unknown. Some experimentally infected rats become obese. Although this has been linked to anatomical abnormalities in hypothalamus, the basis for this tropism is unknown.

Immune Response

The immune response to BDV has been shown to represent the basis of the pathogenesis of the disease. Although experimentally infection of adult immunocompetent animals regularly results in disease, infection of newborn or athymic rats or animals rendered immunodeficient by drugs leads neither to encephalitis nor to overt disease despite the persistence of the virus. These findings in conjunction with the property of BDV being a noncytolytic virus that persists in the brain of immunocompromised hosts at titers comparable to those in immunocompetent animals provide all characteristics of an immunopathological disease. Rats infected as adults have both a T- and B-cell response to BDV (**Figure 2**). The role of the latter is unclear because BDV-specific antibodies adoptively transferred to immunoincompetent recipients do not induce pathological changes or disease. In contrast, adoptive transfer of T cells does induce BD in infected immunosuppressed recipients. The role of specific T-cell subsets has been investigated. Passive transfer of *in vitro* established homogeneous BDV-specific CD4+ T-cell lines can elicit BD in persistently infected asymptomatic rats; CD4+ T cells do not cause disease by themselves but rather induce activity of cytolytic CD8+ T cells. In the absence of CD8+ T cells, no disease or immunopathology is seen even if CD4+ T cells can be detected in the brain. One mechanism for the activity of CD8+ T cells may be their capacity to express perforin, the major molecule involved in cytolysis of target cells by CD8+ T cells. Other mechanisms are under investigation, such as pro-inflammatory cytokines or free radicals. Interestingly, virus-specific MHC class II-restricted T cells, when applied before infection, prevent the manifestation of clinical signs in rats. Antibodies to virus-specific antigens are synthesized early after infection in all BDV-infected animals; however, neutralizing antibodies do not appear until infection is well established in the CNS and never reach high titers.

Clinical Features of Infection

Incubation time in natural infection ranges from weeks to months. If the infection becomes manifest, the disease is characterized during the early phase by hyperexcitability or somnolence, impairment of posture and balance, hyperesthesia, visual disturbance, anorexia, fever, and colic. Most naturally infected animals die 1–2 weeks after onset of the disease, but recoveries or recurrences of disease can also be observed. Manifestations of disease are similar in natural and experimental infection; however, the time course and features of experimental infection have been mapped in greater detail. In rats, disease starts with alertness and loss of fear. Later, rats show incoordination, occasionally with increasing hyperactivity and aggressiveness. Thereafter, most rats enter a stage of disease where passiveness and hypersomnia dominate. These symptoms are at least in part attributable to the onset of blindness. In the chronic stage of the disease, signs of dementia and chronic debility predominate.

Pathology and Histopathology

Pathological changes in BDV are restricted to the brain, spinal cord, and the retina. In acute disease in naturally infected hosts, a massive, perivascular as well as parenchymal inflammation is found, composed of infiltrated macrophages, T and B cells (**Figure 2**). The sequence and development of pathological changes have been extensively studied in rats. BDV-infected rats develop a

severe nonpurulent disseminated mononuclear meningoencephalitis with most intense inflammatory reaction restricted to the gray matter of cortex and diencephalon (**Figure 3**). Macrophages and T lymphocytes are the most numerous cell populations present in the inflammatory lesions during the earlier phases of the local reaction in both experimentally infected rats and in naturally infected horses and sheep; B lymphocytes predominate at later stages. After intracerebral infection in rats, the initial reaction is characterized by a focal accumulation of mononuclear cells in the leptomeninges at day 8–10. Thereafter, perivascular and parenchymal infiltrations are observed which intensify during the course of the disease and consist of massive accumulations of inflammatory cells in perivascular spaces and the neuropil between day 20 and 30 post infection. Beyond day 30 p.i. the intensity of the inflammatory reaction decreases and late after infection (>60 days) the number and intensity of the inflammatory infiltrates is significantly reduced although the level of infectious virus remains constant. Simultaneously with the development of the encephalitis, degenerative lesions can be found in hippocampal and cortex regions. One of the consequences of the necrotic process is a dilatation of the lateral ventricles resulting in a marked hydrocephalus *ex vaccuo*. Reactive astrogliosis is found throughout the brain. Infected neurons contain intranuclear acidophilic inclusion bodies (Joest–Degen inclusion bodies) that are regarded as pathognomonic for BD. In the brain the virus is predominantly found in neurons, but viral antigen is also detected in astrocytes, oligodendrocytes, and ependymal cells. Histopathological lesions in the eye of infected rats and rabbits are seen almost exclusively in the retina resulting in a degeneration of rods and cones and ultimately in a progressive disappearance of neurons from the inner and outer nuclear layers. The loss of photoreceptors results in blindness. Edema of the bulbus olfactorious is observed in naturally infected horses and in rats infected intranasally. These findings support the hypothesis that natural infection occurs via the nasal route.

Prevention and Control

Although vaccines have shown efficacy in animal models, no vaccine is approved for use in veterinary medicine. Knowledge of the epidemiology of infection is critical to control viral diseases; nonetheless, studies in this vein have been initiated only recently. With the exception of

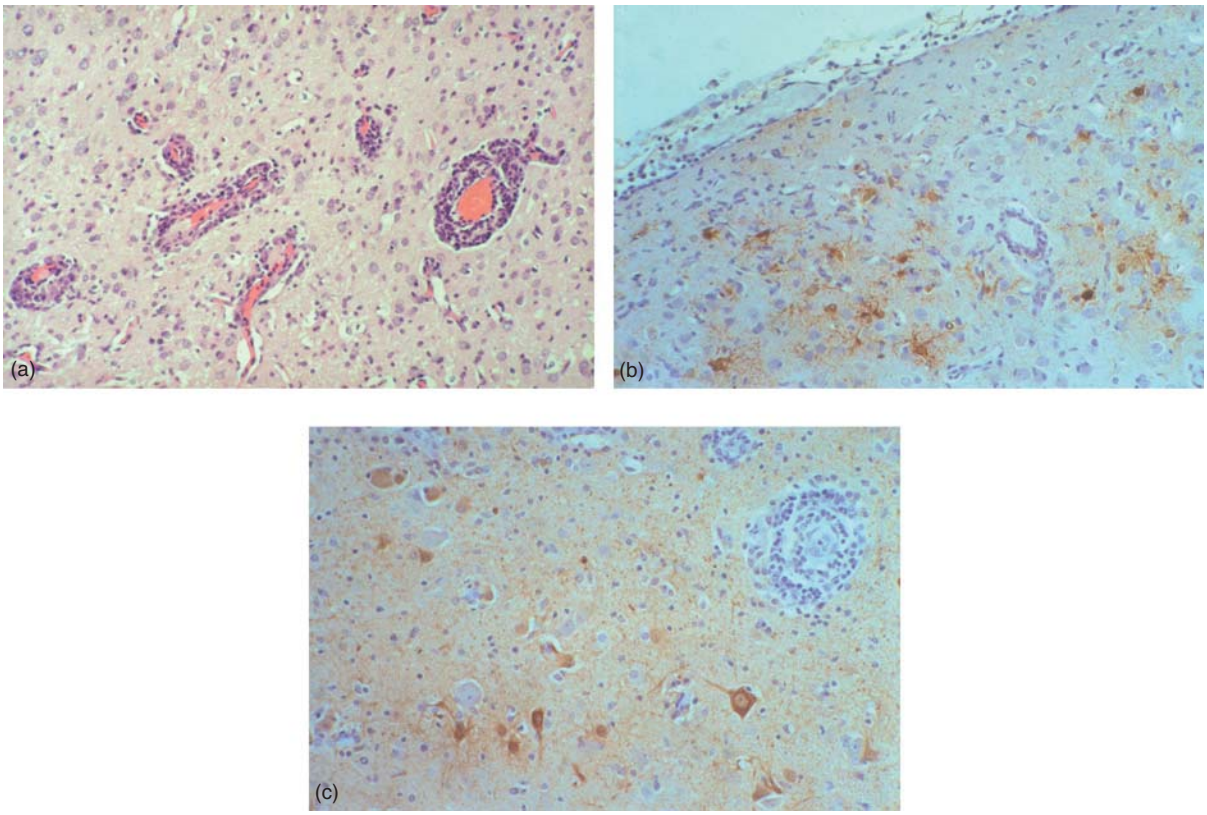


Figure 3 Histology of natural cases of Borna disease. (a) Horse (severe inflammatory reaction; HE-staining); (b) sheep (inflammatory perivascular cuff and presence of viral p40 antigen; immunohistology counterstained); and (c) cattle (inflammatory reaction and presence of p40 antigen; immunohistology counterstained). Courtesy to Prof. T. Bilzer, Düsseldorf.

virus isolation or postmortem analysis of brain using immunohistochemical or *in situ* hybridization techniques, there are no standardized tests for BDV infection. Many investigators continue to use a labor-intensive and subjective immunofluorescence assay for serology in vitam diagnosis. However, more sensitive assays are gaining acceptance such as enzyme-linked immunosorbent assay (ELISA) in its various modifications (classical, capture, competitive, reverse-type sandwich) and Western blots. The advent of viral sequence information has allowed development of gene amplification technologies such as PCR and ligase chain reaction. While these methods are sensitive, results obtained have been complex to interpret. Analyses of some amplified sequences have revealed similarities to laboratory strains. Whether signal in these assays represents *bona fide* natural infection or contamination is unclear. Control of the disease will depend on identification of potential reservoirs of infection, including asymptomatic carriers.

Future Perspectives

Of urgent importance is the refinement of reliable methods for routine diagnosis. These should include assays for the detection of BDV-specific antibodies as well as detection of the virus and viral gene products. Without such assays it is impossible to pursue rigorous epidemiology. Molecular biology characterization and pathogenesis also merit investment. This is a unique agent and investigating the mechanisms by which it maintains a broad host range, targets the nervous system, and establishes a persistent infection will almost certainly lead to seminal insights in microbiology, neuroscience, and medicine.

See also: Epidemiology of Human and Animal Viral Diseases.

Further Reading

de la Torre JC (2002) Bornavirus and the brain. *Journal of Infectious Disease* 186(supplement 2): S241–S247.
Gonzalez-Dunia D, Volmer R, Mayer D, and Schwemmle M (2005) Borna disease virus interference with neuronal plasticity. *Virus Research* 111(2): 224–234.
Lipkin WI and Briese T (2006) Bornaviridae. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 5th edn., pp. 1829–1851. Philadelphia: Lippincott Williams and Wilkins.
Lipkin WI and Hornig M (2004) Psychotropic viruses. *Current Opinion in Microbiology* 7: 420–425.
Planz O and Stitz L (2005) The role of Borna disease virus in neuropsychiatric disorders. In: Fatemi SH (ed.) *Infectious Etiologies of Neuropsychiatric Disorders*, pp. 123–134. Oxford: Taylor and Francis.
Staeheli P (2002) Bornaviruses. *Virus Research* 82(1–2): 55–59.
Stitz L, Bilzer T, and Planz O (2002) The immunopathogenesis of Borna disease virus infection. *Frontiers in Bioscience* 7: d541–d555.

Bovine and Feline Immunodeficiency Viruses

J K Yamamoto, University of Florida, Gainesville, FL, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Since the isolation of human immunodeficiency virus (HIV) in 1983, the search for its counterpart in domestic and laboratory animals was initiated in a number of laboratories. Bovine immunodeficiency virus (BIV) was isolated in 1969 from a Louisiana dairy cow (called R29) during an intensive search for bovine leukemia virus (BLV). However, the viral sequencing of the frozen cell preparation from this cow was not performed until 1987, when this isolate (BIV_{R29}) was determined to be a lentivirus. Unlike the long history of BIV discovery, feline immunodeficiency virus (FIV) was isolated in 1986 from two laboratory cats inoculated with tissues derived from stray cats found in Petaluma, California. The stray cats were from a private cattery with high incidence of mortality and immunodeficiency-like syndrome. The isolated virus, called feline T-lymphotropic virus, resembled HIV in biochemical and morphological characteristics and was renamed FIV in 1988. Viral sequencing in 1989 confirmed this isolate (FIV-Petaluma, FIV_{Pet}) to be a lentivirus. BIV and FIV infections are prevalent worldwide and each has a distinctive impact on the survival of its host.

Taxonomy and Classification

Based on nucleotide sequence analysis, both BIV and FIV belong to the genus *Lentiviruses* in the family *Retroviridae*. Phylogenetic tree analyses of Pol sequences indicate that BIV is related to caprine arthritis encephalitis virus (CAEV), whereas FIV is related to the equine infectious anemia virus (EIAV). Both BIV and FIV are distantly related to the primate lentiviruses, HIV and SIV. Serosurvey for BIV has identified a bovine lentivirus, called Jembrana disease virus (JDV), which causes an acute

disease with 17% mortality in Balinese cattle. The full nucleotide sequence of JDV demonstrates that JDV is more closely related to BIV than to other lentiviruses.

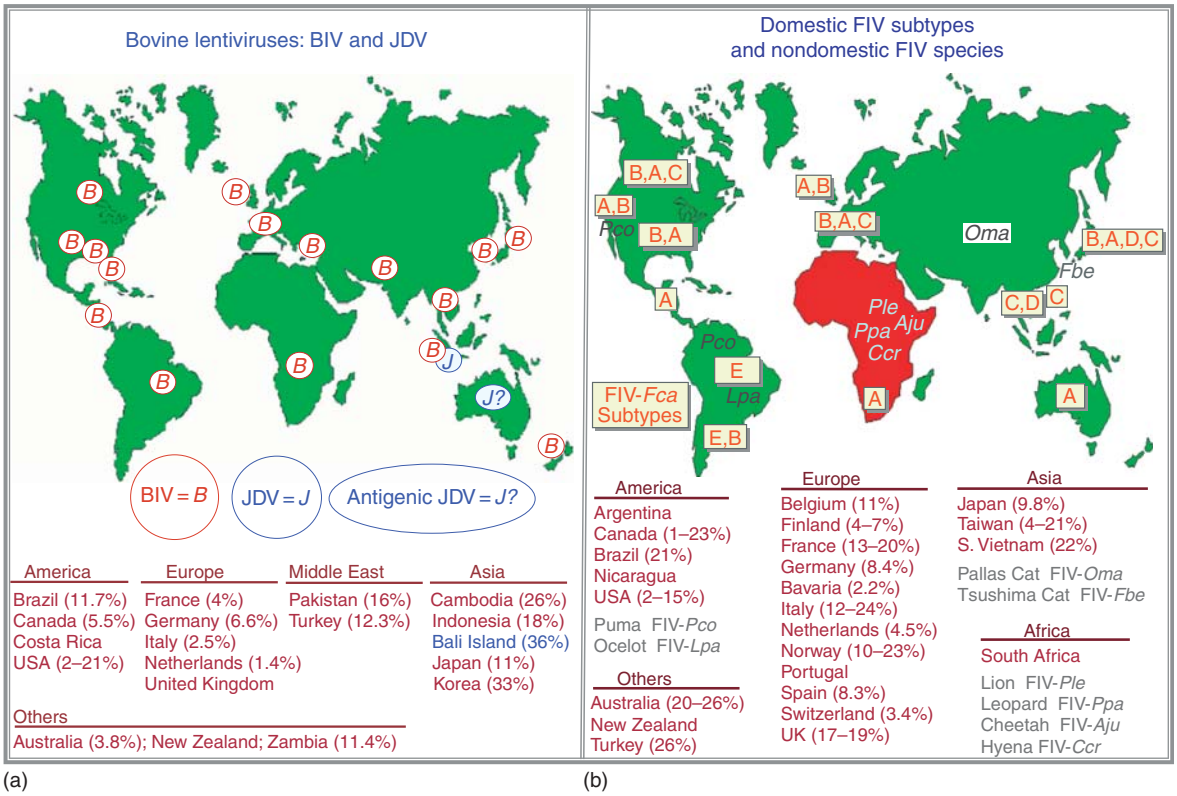
FIV has been classified into five clades or subtypes (A–E) based on *env* and *gag* sequence analyses. Both serological and sequence analyses suggest that wild cats are infected with species-specific FIV. Eighteen of 37 nondomestic feline species, including lions, pumas, ocelots, leopards, cheetah, and Pallas cats (**Figure 1(b)**), are infected with species-specific FIV. Lion FIV-Ple and puma FIV-Pco are currently classified into three subtypes (A–C) and two subtypes (A, B), respectively. Based on *pol-RT* sequence, domestic FIV (FIV-Fca) is more closely related to puma FIV-Pco and ocelot FIV-Lpa than to other nondomestic FIV. Recent serological and *pol-RT* sequence analyses demonstrated species-specific FIV infection in Hyaenidae species (spotted hyena FIV-Ccr), the closest relative to the feline family.

Geographic Distribution

The global distribution of BIV infection was determined by serological survey. BIV infection was found in Europe; North, Central, and South America; Asia; Middle East; Australia; New Zealand; and Africa (**Figure 1(a)**). In the USA, BIV infection was more prevalent in cattle herds from Southern states than those from Northern states. Standard serological survey has difficulty distinguishing BIV infection from JDV infection due to antibody cross-reactivity in the capsid protein (CA). JDV infection was reported on the island of Bali and neighboring districts in Indonesia.

A recent survey concluded that cattle in Australia have antibodies to a lentivirus antigenically more closely related to JDV than BIV.

The worldwide prevalence of FIV infection in domestic cats was determined by serosurvey and confirmed by sequence analysis (**Figure 1(b)**). In general, FIV subtypes are distributed according to the geographic location. Subtype B FIV was the predominant global subtype with higher prevalence in USA, Canada, eastern Japan, Argentina, Italy, Germany, Austria, and Portugal. Subtype A was the next predominant subtype with prevalence in western USA, western Canada, Argentina, Nicaragua, northern Japan, Australia, Germany, Italy, Netherlands, France, Switzerland, United Kingdom, and South Africa. Subtype C was detected predominantly in Vietnam,



(a) (b)

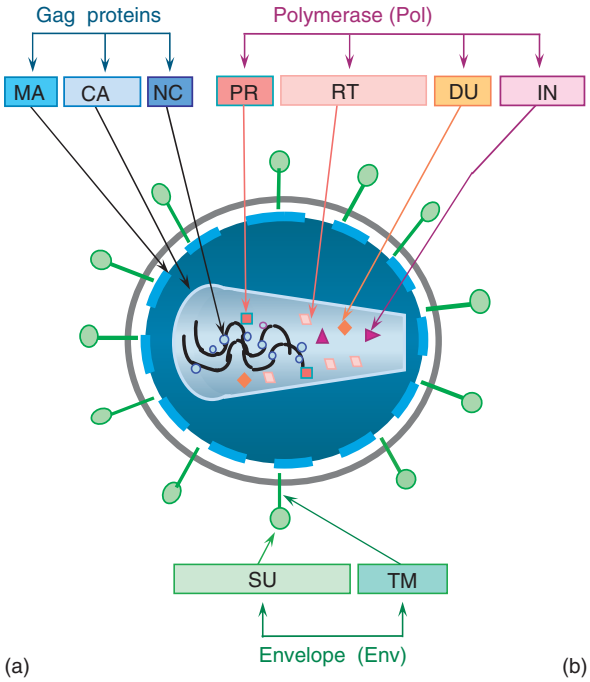
Figure 1 Global distribution of (a) bovine lentiviruses and (b) domestic FIV subtypes and nondomestic FIV species. Seroprevalence rates of BIV, JDV, and domestic FIV-Fca (prevalence in high risk group) are described in the parentheses next to the corresponding country. Nomenclatures of the nondomestic FIV in wild cats and hyena are shown in the prevalent country or continent.

northern Taiwan, Japan, southern Germany, and British Columbia; subtype D in southern Japan and Vietnam; and subtype E in Brazil and Argentina.

Virion and Genome Structure

BIV, JDV, and FIV are RNA viruses with two identical single-stranded RNA molecules of approximately 8.5, 7.7, and 9.5 kb per genome, respectively. The viral genomes, viral enzymes, and nucleoproteins (NC) involved in viral assembly are all packaged within the nucleocapsid core (**Figure 2(a)**). The mature core is shaped like a cone, typical of primate lentiviruses. Viral enzymes are protease (PR), reverse transcriptase (RT), and integrase (IN) encoded by the *pol* gene. FIV also produces a deoxyuridine triphosphatase (dUTPase or DU), which is unique to certain ungulate lentiviruses and important for replication in nondividing macrophages or resting T lymphocytes. The virus core, composed of CA, is surrounded by the matrix protein (MA), which coats the inner surface of viral envelope membrane. FIV has a myristoylated MA, similar to that of HIV and SIV. In contrast, BIV has a nonmyristoylated MA, typical of ungulate lentiviruses (EIAV, maedi-visna virus (MVV)). Myristoylation is important in targeting Gag to the inner surface of plasma membrane. The viral envelope (Env) consists of surface (SU) and transmembrane (TM) glycoproteins, projecting from the lipid bilayer derived from host plasma membrane.

BIV, JDV, and FIV have a genomic organization of 5′-*LTR-gag-pol-env-LTR*-3′, like all retroviruses. The viral RNA is reverse-transcribed into double-stranded DNA in the cell cytoplasm, which are transported into the cell nucleus and then integrated into the host genome as viral provirus using viral IN. A genome-length mRNA, transcribed from the provirus, is translated into the Gag and Gag-Pol precursor polyproteins. These polyproteins are cleaved by the viral protease into MA, CA, and NC from Gag and PR, RT, DU (only FIV), and IN from Pol (**Figure 2(b)**). Similarly, the spliced *env* mRNA is translated into the Env precursor polyprotein, which is subsequently cleaved by a cellular protease into SU and TM. Like other lentiviruses, the BIV, JDV, and FIV genomes are more complex in organization than other retroviruses by containing regulatory genes that are found in the open reading frames (*orfs*) overlapping or flanking the 5′ and 3′ ends of the *env* gene. These viruses do not possess a *nef* gene or produce Nef protein. BIV *orfs* contain *tat*, *rev*, *vpy*, *vpw*, *vif*, and *tmx* genes but JDV *orfs* have no *vpy* and *vpw*. Tat is a spliced gene product that binds to viral transcripts to enhance transcribing polymerase activity. Rev is a spliced gene product that is important in production and transport of viral RNA transcripts. Vpy and Vpw are speculated to have similar function as HIV-1 Vpu and Vpr. Vpu is involved in increasing virion release, while



(a)

(b)

Viral components	*FIV*	*BIV*
Gag proteins		
Capsid (CA)	p24	p26
Matrix (MA)	p15	p16
Nucleoprotein (NC)	p7	p13
Pol proteins		
Protease (PR)	p13*	p11*
Reverse Transcriptase (RT)	p61*	p64
Integrase (IN)	p31	p32*
Env glycoproteins		
Surface Env (SU)	gp95–gp100	gp100
Transmembrane Env (TM)	gp39	gp45
Regulatory proteins		
Regulator of expression of viral protein (Rev)	p23	p23
Virus infectivity factor (Vif)	p29	Vif**
Transactivator (Tat)	?***	p14
Virus protein y (Vpy)	None	p10*
Virus protein w (Vpw)	None	p7*
Transmembrane x (Tmx)	None	p19

* Predicted value; ** Predicted; *** 79 aa Tat-like protein

Figure 2 General structure of (a) BIV and FIV with (b) the size designations of structural proteins, enzymes, and regulatory proteins.

Vpr promotes the transport of the DNA pre-integration complex into the cell nuclei. Tmx has been detected in infected cells but its function remains unknown. FIV has four main *orfs* (*orf 1–4*). FIV *orf 1* appears to be analogous to the HIV-1 *vif* gene. Vif acts late in the viral life cycle and is needed for optimal production of virions. FIV *orf 2* (also called *orf A*) was speculated to be the first exon of the *tat* gene (encoding a 79 aa Tat-like protein) but recently was reported to be related to the *vpr* gene found in primate lentiviruses. FIV *orf 3* (3′ end of *orf A* or *orf 2)* and *orf 4 (orf H)* are analogous to the exons of the *rev* gene found in HIV and SIV.

Host Range and Virus Propagation

BIV has been isolated from dairy and beef cattle, and buffaloes. In addition, rabbits, goats, and sheep can be experimentally infected with BIV. BIV infects primary cells from bovine embryonic tissues (lung, brain, thymus, and spleen) and established cell lines of epithelial bovine trachea, canine fetal thymus, and embryonic rabbit epidermis, but does not infect human cell lines. Monocytes and macrophages are the major cell types infected by BIV. Recent studies show low-level infection in T cells and B cells from BIV_{R29}-infected cattle.

Domestic FIV has limited host range with productive infection found only in felid species. In contrast, nondomestic FIV sequence was recently identified in Hyaenidae species. Puma FIV-pco and lion FIV-Ple cause persistent infection in laboratory cats without clinical disease. Domestic FIV has been found in the wild cat population, Tsushima cat (*Felis bengalensis euptilura*), and may present problems for the conservation of this endangered species. FIV of domestic cats infects macrophages, endothelial cells, glial cells, B cells, $CD8^+$ T cells, and $CD4^+$ T cells. *In vitro* infection has been demonstrated in feline astrocytes, fibroblastic Crandell feline kidney cells, feline T cell lines, macaque peripheral blood mononuclear cell (PBMC), and low-level-to-defective infection in human cell lines. Experimental inoculation with domestic FIV grown in macaque PBMC resulted in FIV infection of macaques. However, there has been no evidence of zoonotic transmission and productive infection of humans with FIV.

Genetics and Evolution

Due to the difficulty of isolating BIV from infected cattle, only a few full sequences, all from the USA (Louisiana, Florida, and Oklahoma), are available. Genetic variability of different USA strains shows as much as 10% variation in the *pol* and considerably more at the *env*. Genetic variability was also assessed by analyzing polymerase chain reaction (PCR)-amplified short sequences of *env* and *pol*. Phylogenetic analysis of the *SU* gene showed clustering of Asian isolates distinct from USA isolates. Moreover, inter- and intra-animal *env* variations were observed in BIV_{R29}-infected rabbits, suggesting the potential development of quasispecies. Only one Bali JDV isolate has been fully sequenced and therefore, little is known about the genetics of JDV.

Due to the wide genetic variability between worldwide isolates, FIV is classified into five subtypes (A–E). Studies on mutation rates in FIV *env* and *gag* show positive selective pressure for *env* mutations, consistent with reports that HIV *env* has a high mutation rate compared to *gag* and *pol*. PCR analyses of FIV variants isolated within individual cats over a 3 year period indicate that sequence variation in *env* increases over time, with later isolates showing divergence of 0.5–1.5%. Hence, the divergence of the variants within individual cats appears to be twofold less than those variants from an HIV-1-positive individual. Superinfections and recombinations have been reported for FIV much like those reported for HIV-1. Phylogenetic analyses suggest domestic FIV originated from a lineage associated with the wild cat lentivirus (see the section titled 'Taxonomy and classification').

Serologic Relationships and Variability

Sera from BIV-infected cattle reacted to CA from HIV-1, SIV, EIAV, and JDV, and vice versa. Strongest serum cross-reactivity to CA was observed between BIV- and JDV-infected cattle. Sera from BIV_{R29}-infected cattle contained virus-neutralizing antibodies (VNAs) to BIV_{R29} throughout 5 years post infection (pi), but these VNAs did not cross-neutralize Florida BIV_{FL112}. The *env* sequences between BIV and JDV and among BIV, HIV-1, SIV, and EIAV are less conserved. Consequently, cross-neutralization of HIV-1, SIV, and EIAV with sera from BIV-infected cattle is not expected.

Sera from cats infected with different FIV strains reacted to prototype FIV_{Pet}. The cross-reactivity of the infected sera has been the basis for the commercial FIV diagnostic kit. Sera from wild cats infected with nondomestic lentivirus cross-reacted predominantly to CA p24 and lesser degree to MA p15 of domestic FIV, and vice versa. Sera from HIV-1-positive subjects cross-reacted to domestic FIV p24 and vice versa. Rabbit polyclonal antibodies to ungulate lentiviruses, MVV and CAEV, also reacted to domestic FIV at p24 and p15, demonstrating the evolutionarily conserved epitopes on p24 and p15. In general, FIV-specific VNAs can neutralize closely related strains but not divergent strains. Preliminary studies suggest that a loose correlation exists between the genotype based on *env* sequence and the VNAs elicited by infected cats.

Epidemiology

Global distribution of BIV infection in dairy and beef cattle has a seroprevalence of 1.4–33% (**Figure 1**(**a**)). Clinical signs were more frequently observed in BIV-infected dairy cattle than in BIV-infected beef cattle. This may be due to fewer stress factors in beef cattle than in dairy cattle. In some countries, BIV infection was higher in the dairy cattle than the beef cattle, because of the management practices (hand-feeding pooled colostrums/milk to dairy calves) and the longer lifespan (more risk of exposure) of the dairy cattle than the beef cattle. The global prevalence of FIV infection is 1–26% in the high-risk populations (symptomatic cats) (**Figure 1**(**b**)) and 0.7–16% in the healthy (minimal to no-risk) populations. FIV infection is found more frequently in cats >5 years of age than in younger cats and rarely in cats <1 year of age. Furthermore, free-roaming cats have the highest incidence of infection as compared to indoor cats. Male cats are 2–3 times more likely to be infected than female cats. Since male cats are territorially aggressive and have higher incidence of wounds and bite abscesses, the high prevalence of FIV infection in male free-roaming cats is consistent, with the major mode of FIV transmission being via biting.

Transmission and Tissue Tropism

BIV has been detected in the spleen, liver, brain, lymph nodes, and PBMC of infected cattle. Based on PCR analysis of purified cell populations from BIV_{R29}-infected cattle, BIV is pantropic and infects αβ T cells, γδ T cells, B cells, and monocytes/macrophages. The cellular receptor for BIV is still unknown. Transmission of BIV is by exposure to contaminated blood, contaminated milk/colostrum, and possibly by sexual contact. *In utero* vertical transmission was reported. BIV was detected by PCR in embryo and occasionally in semen from infected cattle. Controversy exists about the BIV PCR detection in the semen and on the viability of BIV from the PCR-positive frozen semen and embryos, which are the sources for artificial insemination. Transmission by arthropod vector has been suggested but the evidence for this mode is lacking. Transmission by ingesting contaminated colostrum/milk was also suspected since BIV was detected in milk. Moreover, iatrogenic transmission (reused hypodermic syringe and equipment for pregnancy examination, de-horning, artificial insemination, and castration) of contaminated blood is suspected to be the main source of passing the infection to large numbers of uninfected cattle.

Although significant $CD4^+$ T-cell loss occurs during FIV infection, the feline CD4 molecule is not the receptor for FIV. The primary cellular receptor for domestic FIV was recently identified to be feline CD134, also called OX40. CD134 is a member of tumor necrosis factor receptor family that is transiently expressed on activated T cells. FIV cannot use human CD134 as receptor to infect human cells, but can use both feline and human CXCR4, a chemokine receptor, as co-receptor. It has been speculated that CXCR4 can also serve as primary cellular receptor for FIV. Based on experimental transmission studies, the major route of FIV transmission is through bites from infected cats. This transmission route is consistent with the epidemiological studies and with the fact that cats shed significant amounts of virus in saliva. FIV transmission by ingestion of virus via grooming and licking bleeding wounds of an infected cat cannot be excluded, since oral administration of infected blood can cause infection. FIV infection was demonstrated by experimental vaginal and rectal inoculation, and FIV was isolated from vaginal swab and semen of infected cats. Another route of transmission is by ingesting contaminated colostrum/milk of infected queens. Experimental studies suggest the possibility of transplacental infection and transmission during birth through a contaminated birth canal. In conflict with these experimental observations is the low incidences of FIV infection in cats <1 years of age and in kittens born to chronically infected queens, suggesting that vertical transmission of FIV may be rare in nature.

Clinical Features and Infection

Experimental BIV_{R29} infection of dairy cattle caused lymphocytosis, hypertrophic regional lymph nodes, and hypertrophic hemal lymph nodes, which was identical to the lymphoid changes observed in cow R29. Seroconversion and virus recovery also demonstrated BIV infection. Unlike cow R29, clinical disease was not observed in the experimentally infected cattle. Naturally infected dairy cows had lymphoid changes similar to the experimentally infected cattle, and a portion of the population displayed postparturition problems. These included foot problems, mastitis, diarrhea, pneumonia, neuropathy, and decreased milk production. Clinical signs in beef cattle were less frequent when compared to dairy cattle. Infected beef cattle grew normally and gained weight but displayed dullness, lumbering gait, and enlarged subcutaneous hemal lymph nodes. The low pathogenicity of BIV has been described in a number of studies. In contrast, JDV infection of Bali (*Bos javanicus*) cattle had pronounced clinical disease with mortality of 17% and was more pathogenic than BIV or JDV infection of Ongole (*Bos taurus*) and Friesian (*Bos indicus*) cattle.

The immunological hallmark of FIV infection is depletion of peripheral $CD4^+$ T cells and reduced CD4:CD8 ratios, leading to B- and T-cell dysfunctions and hypergammaglobulinemia. The clinical stages of FIV infection are similar to human AIDS in several ways. The acute

stage of experimental FIV infection was characterized by immunological abnormalities followed by depression, fever, diarrhea, neutropenia, and persistent generalized lymphadenopathy. FIV was primarily detected in lymphoid tissues followed by dissemination of the virus into nonlymphoid organs. Both antibodies against FIV and virus recovery from PBMC persisted throughout infection. The FIV load in the blood was lower and $CD4^+$ T-cell decline was slower at the asymptomatic stage than acute stage. By late symptomatic stage, the animals were severely immunosuppressed and displaying wasting syndrome, neurological disorders, and persistent secondary opportunistic infections. The virus load was extremely high at this stage, and FIV was readily isolated from nonlymphoid tissues and organs, such as kidney, saliva, and central nervous system (CNS). Lymphomas with and without FIV proviral integration were reported in naturally and experimentally infected cats, including B-cell lymphoma of more unusual extranodal forms (predominantly in the neck and head). The major clinical manifestations observed in naturally infected cats were a progressively degenerative immune disorder, neurological disorders, wasting syndrome, and persistent secondary opportunistic infections. The specific signs were chronic oral diseases, chronic upper respiratory tract disease, chronic enteritis, chronic conjunctivitis, anorexia, fever of unspecified origin, and recurrent cystitis. Abnormal behavioral problems, lymphosarcoma, and myeloproliferative disease were observed in a small proportion of affected cats.

Pathology and Histopathology

Experimental BIV_{R29} infection of dairy cattle caused B-cell lymphocytosis, hypertrophic regional lymph nodes with lymphoid hyperplasia, and primary hypertrophic hemal lymph nodes with follicular lymphoid hyperplasia. The lymph nodes and hemal lymph nodes during natural infection showed hyperplasia followed by follicular exhaustion and dysfunction, and follicular hypoplasia with atrophy and central follicular depletion especially of T-dependent zones in severe cases. Infected cattle developed early atypical proliferation of lymphocytes in lymphoid tissue followed by recurrent opportunistic infections, loss of circulating monocytes, poor body condition, and weight loss. Encephalitis was diagnosed with lesions of lymphocytic infiltration into the meninges and perivascular spaces, and foci of microglia and astrocytosis. Overall, the primary lesions were in the brain, lymphoid tissue, and feet (lymphocyte infiltration of hoof tissues). Secondary diseases included nerve paralysis, persistent mastitis, septicemia, laminitis, secondary pododermatitis (plasmacytic inflammation), gastrointestinal diseases, bronchopneumonia, and subcutaneous and intramuscular abscesses.

Major histopathological changes during acute stage of experimental FIV infection were observed in the lymphoid tissues. In the first three weeks pi, lymphoid hyperplasia was observed in the lymph nodes, tonsils, spleens, and gut-associated lymphoid tissues. A majority of the FIV-infected cells were found in the germinal centers of lymphoid follicles of these tissues. Some infected cells were observed in the paracortex and medullary cords of lymph nodes, and in periarterial lymphoid sheaths and red pulp of the spleen. Shortly after, cats developed myeloid hyperplasia in the bone marrow and cortical involution, thymitis, and follicular hyperplasia of the medulla in the thymus. Both T cells and monocytes/macrophages were infected with FIV at an early phase of the acute stage, followed by infection of B cells. By early symptomatic stage, lymph nodes displayed follicular hyperplasia, involution, and lymphoid depletion, and by late phase, destruction of nodal architecture with involution and depletion of lymphoid follicles.

The CNS disease of FIV resembles those induced by HIV, and includes perivascular mononuclear cell infiltrates, glial nodules and diffuse gliosis of gray and white matter, and neuronal loss. Similar to HIV, neurotropic FIV strains infect microglia, astrocytes, and brain microvascular endothelial cells, but do not infect neuronal cells. Both anti-FIV antibodies and FIV virions have been isolated from cerebral spinal fluid (CSF) of infected cats, in addition to the elevated IgG index detected in the CSF. Like HIV, the level of FIV infection in the CNS cannot account for the cognitive/motor function abnormalities observed in these cats, suggesting that cytokines induced during CNS infection may play a key role in FIV neuropathogenesis. Neurological abnormalities included limb paresis, delayed righting and pupillary reflexes, behavioral changes, delayed visual and auditory evoked potentials, decreased spinal and peripheral nerve conduction velocities, and sleep abnormalities (e.g., increased awake time with decreased rapid eye movement) similar to sleep disturbances described in AIDS patients.

Immune Response

Antibodies to p26 followed by antibodies to Env developed during early BIV_{R29} infection, while a transient CD4/CD8 ratio decrease developed 2–7 weeks pi. This decrease was attributed to the greater $CD8^+$ cell increase than the slight $CD4^+$ cell increase. No significant $CD4^+$ cell or CD4/CD8 ratio decreases were observed throughout 5 years pi, even though persistent infection was demonstrated by the continued presence of TM-specific antibodies and VNAs. High VNA titers resulted in faster detection of virus, indicating high viral load. The role that VNAs play during natural pathogenesis of BIV is still unclear. It has been speculated that cellular immunity

such as BIV-specific cytotoxic T lymphocytes (CTLs) may control the infection from progressing into clinical disease. However, decreased proliferation responses to T-cell mitogen and depressed T-dependent antibody responses to recall antigens at 4 and 5 years pi suggest loss in functional T-cell responses. In another study, B-cell lymphocytosis but no changes in $CD4^+$ cells and $CD8^+$ cells were observed in cattle infected with slightly pathogenic BIV_{FL112}. Although BIV infection does not cause $CD4^+$ cell loss, signs of T-cell dysfunction appear to develop upon prolonged infection.

Both the exposure dose and the FIV strain infecting the cats determine the nature of humoral and cellular immunity generated against the virus. Some strains do not elicit high or even significant VNAs even though high antibody titers to SU and TM are produced. Upon experimental infection with a moderate dose of FIV, anti-FIV antibodies were detected as early as 3 weeks pi, followed by VNAs starting 6–9 weeks pi. A decrease in primary proliferative response to only foreign antigen (none to T-cell mitogens and recall antigen) was observed at 5 weeks pi, the earliest time point tested. Hence, selective defects in primary antigen-specific response of naive $CD4^+$ T-helper cells are the early signs of T-cell dysfunction. Meanwhile, PBMC developed CTL responses to FIV Gag and Env at 7–9 and 16 weeks pi, respectively. The time of CD4/CD8 inversion depended greatly on the FIV strain used, with a moderate dose of pathogenic strains causing CD4/CD8 inversion as early as 4–6 weeks pi. In general, the $CD4^+$ T-cell loss accounted for the CD4/CD8 ratio inversion with most strains, but $CD8^+$ T-cell increases also contributed to this inversion in a number of strains. A defect in proliferative response of memory T cells to recall antigen developed at about 19 weeks pi. Decreased T-cell mitogen responses developed upon prolonged infection when considerable CD4/CD8 ratio inversion prevailed. B-cell dysfunctions were milder and were decreased primary antibody responses to T-dependent antigens and increased serum IgG levels, indicative of virus-specific B-cell hyperactivity. In addition, functional abnormalities in macrophages, neutrophils, and natural killer cells were observed in the FIV-infected cats.

Prevention and Control

The control of BIV infection in animal food relies on the practice of testing and preventing the spread of BIV infection by decreasing iatrogenic transmission through improved management practices. Public health concern appears to be minimal, since there is no evidence of BIV zoonosis and the virus in milk is readily inactivated by pasteurization. The importance of BIV infection to the cattle industry depends on the severity of the economic losses resulting from lower milk production and poor beef quality. Similarly, the need for developing a BIV vaccine will hinge on the perception of the cattle industry rather than the significance this virus has as an animal model for AIDS.

Experimental FIV vaccine trials, ranging from inactivated single-subtype virus vaccine to proviral DNA vaccine, were performed with minimal-to-no success against homologous (identical to vaccine strains) and heterologous strains. A commercial dual-subtype FIV vaccine, consisting of inactivated subtype A and D viruses, was released in USA in 2002. This vaccine was effective against homologous strains, heterologous subtype A strains, and subtype B strains. This vaccine induced VNAs to closely related FIV strains from subtypes A and D. The vaccine protection against subtype B viruses was achieved in the absence of VNAs to challenge viruses, suggesting the importance of vaccine-induced cellular immunity. Both the prototype and commercial dual-subtype vaccines induced strong FIV-specific $CD4^+$ T-helper and $CD8^+$ CTL responses. Since there is no evidence of FIV zoonosis, the efforts to develop FIV-specific antiretroviral drugs have been limited. Only few antiretroviral drugs for HIV-1 therapy have been tested in infected cats. These include azidothymidine (AZT), 9-(2-phosphonylmethoxyethyl) adenine, dideoxycytidine 5′-triphosphate, dideoxycytidine, lamivudine (3TC), cyclosporine A, interferon-α, and commercial HIV-1 protease inhibitors. Prophylactic 2 week therapy with a high-dose AZT/3TC combination, started either 3 days before or on the day of FIV inoculation, resulted in 100% and 67% protection of cats from infection, respectively. Although the prophylaxis with nucleoside analogs was remarkable, the therapeutic use of the aforementioned drugs, including nucleoside analogs, was somewhat disappointing. Like HIV-1 drug therapy, FIV therapy will require multiple drug combination with each drug inhibiting different stages of FIV replication cycle.

Future

The importance of BIV infection to the cattle industry is still unclear. The current strategy is to contain BIV infection by methodical testing and imposing management practices that prevent the spread of BIV. More information about the genetic and pathogenic evolution of BIV is needed to assess whether fatal pathogenic strains can evolve from current low-pathogenic strains. Overall, the policies set by cattle industry and government agencies will influence the extinction or survival of BIV infection in cattle.

FIV infection causes an important disease in domestic pet cats. The commercial vaccine was effective against strains from global subtypes A and B, and should be able to contain the global spread of FIV. However, the inability of current FIV diagnostics (enzyme-linked immunosorbent assay (ELISA) and immunoblot assay) to distinguish

vaccinated cats from FIV-infected cats has caused a dilemma in the use of this vaccine. This problem can be resolved by developing sensitive molecular diagnostics or a vaccine that does not conflict with current FIV diagnostics. Identifying the protective vaccine epitopes should assist in designing a vaccine that is devoid of diagnostic epitopes. Moreover, FIV research on vaccines will provide new insights to HIV vaccine development for humans. The recent discovery of fatal pathogenic FIV strains (10% acute mortality) demonstrates the pathogenic evolution of FIV similar to HIV-1 immunopathogenesis. Hence, FIV infection is not only important for feline medicine but serves as an important small animal model for testing novel antiretroviral drugs, immunotherapy, and vaccine approaches for HIV/AIDS.

See also: Equine Infectious Anemia Virus; Human Immunodeficiency Viruses: Antiretroviral agents; Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Origin; Human Immunodeficiency Viruses: Pathogenesis; Simian Immunodeficiency Virus: Animal Models of Disease; Simian Immunodeficiency Virus: General Features; Simian Immunodeficiency Virus: Natural Infection; Visna-Maedi Viruses.

Further Reading

Bachmann MH, Mathiason-Dubard C, Learn GH, *et al.* (1997) Genetic diversity of feline immunodeficiency virus: Dual infection, recombination, and distinct evolutionary rates among envelope sequence clades. *Journal of Virology* 71: 4241–4253.

Burkhard MJ and Dean GA (2003) Transmission and immunopathogenesis of FIV in cats as a model for HIV. *Current HIV Research* 1: 15–29.

Evermann JE, Howard TH, Dubovi EJ, *et al.* (2000) Controversies and clarifications regarding bovine lentivirus infections. *Journal of the American Veterinary Medical Association* 217: 1318–1324.

Podell M, March PA, Buck WR, and Mathes LE (2000) The feline model of neuroAIDS: Understanding the progression towards AIDS dementia. *Journal of Psychopharmacology* 14: 205–213.

Snider TG, Hoyt PG, Jenny BF, *et al.* (1997) Natural and experimental bovine immunodeficiency virus infection in cattle. *The Veterinary Clinics of North America. Food Animal Practice* 13: 151–176.

St-Louis M-C, Cojocariu M, and Archambault D (2004) The molecular biology of bovine immunodeficiency virus: A comparison with other lentiviruses. *Animal Health Research Reviews* 5: 125–143.

Troyer JL, Pecon-Slattery J, Roelke ME, *et al.* (2005) Seroprevalence and genomic divergence of circulating strains of feline immunodeficiency virus among Felidae and Hyaenidae species. *Journal of Virology* 79: 8282–8294.

Uhl EW, Heaton-Jones TG, Pu R, and Yamamoto JK (2002) FIV vaccine development and its importance to veterinary and human medicine: A review. *Veterinary. Immunology and Immunopathology* 90: 113–132.

Wilcox GE, Chadwick BJ, and Kertayadnya G (1995) Recent advances in the understanding of Jembrana disease. *Veterinary Microbiology* 46: 249–255.

Bovine Ephemeral Fever Virus

P J Walker, CSIRO Livestock Industries, Geelong, VIC, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Epicardium The outer layer of heart tissue.
Hypocalcemia A low level of calcium in the circulating blood.
Leucopenia A decreased total number of white blood cells in the circulating blood.
Pericardial fluid Fluid within a double-walled sac that contains the heart and the roots of the great blood vessels.
Sternal recumbency Reclined in a position of comfort on the chest bone.
Synovial membranes Connective tissue membranes lining the cavities of the freely movable joints.
Thoracic fluid Fluid in the chest cavity.

Introduction

Bovine ephemeral fever virus (BEFV) is an arthropod-borne rhabdovirus that causes a disabling and sometimes lethal disease of cattle (*Bos taurus*, *Bos indicus,* and *Bos javanicus*) and water buffaloes (*Bubalus bubalis*). Unapparent infections can also occur in cape buffalo, hartebeest, waterbuck, wildebeest, deer, and possibly goats. Bovine ephemeral fever (BEF) was first recorded in East Africa and Egypt during the late nineteenth century. However, BEF (which is also variously called three-day sickness, bovine enzootic fever, bovine influenza, and stiffsiekte) is thought to have been endemic since antiquity in much of tropical and subtropical Africa, and Asia. As the name suggests, BEF is often characterized by the rapid onset of and recovery from clinical signs that can include a bi- or multiphasic fever, ocular and nasal discharge,

muscle stiffness, anorexia, rumenal stasis, lameness, and sternal recumbency. Although mortality rates rarely exceed 1–2%, a particularly severe outbreak in Taiwan in 1996 has been reported to have resulted in the death or culling of 11.3% of a population of 110 247 dairy cattle on 516 farms. Severe infections commonly occur in larger, more valuable animals. Morbidity rates may approach 100% with significant economic impacts including loss of milk production, temporary infertility in bulls, abortion, loss of condition in beef herds, and disablement of draft animals at the time of harvest. The economic consequences of BEF can be significant. An outbreak of BEF in Israel in 1999 has been estimated to have cost, on average $280 per lactating cow and $112 per nonlactating cow. In Australia, sweeping epidemics in the 1970s have been estimated to have caused industry-wide losses exceeding $200 million in today's values. Due to limitations on the importation of livestock and semen from animals with evidence of BEFV infection, the disease can also have significant impact on international trade.

Taxonomic Classification

BEFV is classified in the order *Mononegavirales*, family *Rhabdoviridae* as the type species of the genus *Ephemerovirus*. Ephemeroviruses have morphological and genetic characteristics common to all rhabdoviruses, including an enveloped, bullet-shaped virion containing a nonsegmented, negative-sense, single-stranded (ss) RNA genome. However, unlike viruses classified in other established rhabdovirus taxa, ephemeroviruses share the unusual characteristic of two type 1 transmembrane glycoproteins (G and G_{NS}) that are related in amino acid sequence and appear to have arisen by gene duplication. Other recognized species in the genus include *Berrimah virus* (BRMV) and *Adelaide River virus* (ARV). These viruses have each been isolated from cattle in northern Australia and also appear to be transmitted by biting insects. Tentative species in the genus include Kimberley virus (KIMV), Malakal virus (MALV), and Puchong virus (PUCV), each of which is closely related serologically to BEFV. KIMV has been isolated from *Culex annulirostris* mosquitoes and *Culicoides brevitarsis* midges in Australia, and from healthy cattle. MALV was isolated in Sudan and PUCV in Malaysia, each from *Mansonia uniformis* mosquitoes. Potential vertebrate hosts for these viruses are yet to be identified. Although BEFV is the only ephemerovirus known to be associated with disease, Kotonkan virus (KOTV), which was originally isolated from *Culicoides* spp. in Nigeria, does cause an ephemeral fever-like illness in cattle. Recent phylogenetic studies using the N gene and segments of the L gene suggest that KOTV, as well as Obodhiang virus (OBOV) which was isolated from *Mansonia uniformis* mosquitoes in Sudan, should also be classified as ephemeroviruses. It has also been suggested that all arthropod-borne rhabdoviruses, including the ephemeroviruses, vesiculoviruses, and a large number of other unclassified rhabdoviruses share a common ancestor and should form a larger taxonomic group for which the name 'dimarhabdovirus' (dipteran-mammalian rhabdovirus) has been proposed (**Figure 1**).

Geographic Distribution

BEFV is known to occur in most tropical and subtropical regions of Africa, Asia, and Australia. The disease occurs throughout much of Africa and in Asian countries south of a line that includes Israel, Iraq, Iran, Syria, India, Pakistan, Bangladesh, southern and central China, and east to Taiwan, and southern Japan. It occurs throughout Southeast Asia, parts of New Guinea, and in most of northern and eastern Australia. It does not occur in New Zealand or the islands of the Pacific, or in the Americas, or Europe, where it is considered an important exotic pathogen. There is reported serological evidence of infection in southern Russia but the disease has not been described. The distribution of BEFV is determined by the geographic range of available insect vectors and is limited by international trade restrictions on live animals and semen showing evidence of infection.

Epizootiology

Bovine ephemeral fever is a seasonal disease. It occurs principally in the summer and early autumn, and with the onset of the monsoon season in Asia. Outbreaks are usually associated with periods of high rainfall which precipitate the emergence of insect vectors in large numbers. BEFV can also spread in epizootics that follow the pattern of prevailing winds with a general southward movement in the Southern Hemisphere and northward movement above the Equator. Wind-borne movement of insect vectors is the likely mechanism of spread. Vectors include biting midges (*Culicoides* spp.) and mosquitoes, in which the virus replicates. BEFV has been isolated from *Culicoides brevitarsis*, *Culicoides coarctatus*, *Anopheles bancroftii*, a mixed pool of mosquito species in Australia, and from a mixed pool of biting midges in Africa. The virus has also been recovered from several other species of biting midge and mosquito following experimental infection. The abundance and distribution of insects from which BEFV has been isolated suggests that several major vectors may be involved in transmission. There is no evidence of direct transmission of BEFV between cattle, even when encouraged by smearing nasal or ocular discharges on mucosal surfaces.

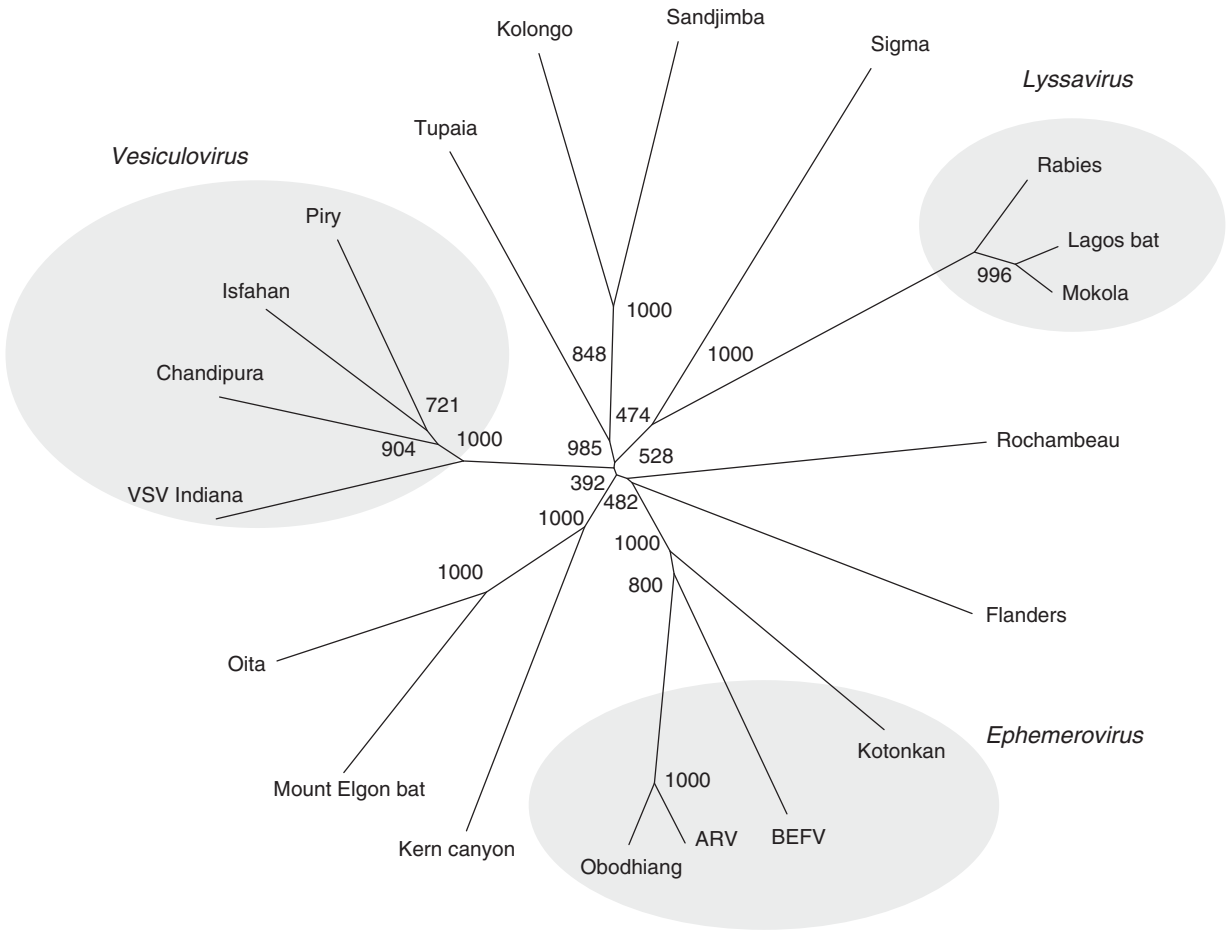


Figure 1 Phylogenetic tree of partial N gene sequences of 20 rhabdoviruses infecting mammals and/or insects. The tree was generated from a Clustal X alignment of the sequences by the neighbor-joining method and presented graphically using Treeview software. The viruses include representatives of three rhabdovirus genera: *Ephemerovirus* (BEFV, ARV, Obodhiang virus, and Kotonkau virus); *Vesiculovirus* (VSV Indiana, Chandipura, Isfahan, and Piry viruses); and *Lyssavirus* (rabies, Mokola, and Lagos bat viruses). Unclassified rhabdoviruses include Sandjimba, Rochambeau, Flanders, Kern Canyon, Mount Elgon bat, Oita, Tupaia and Kolongo, and sigma viruses. Confidence in branch nodes was determined by bootstrap analysis on 1000 replicates (indicated as numbers).

Pathology and Pathogenesis

Bovine ephemeral fever is principally an inflammatory disease. The incubation period is normally 2–4 days. Viremia usually persists for 1–3 days and peaks approximately 24 h before the onset of fever. The initial sites of infection are not known but the virus has been isolated from neutrophils and reticuloendothelial cells of the lungs, spleen, and lymph nodes. There is also evidence of infection in synovial membranes, epicardium and aorta, and in cells derived from synovial, pericardial, thoracic, and abdominal fluids. There is not widespread tissue damage. The primary lesion is a vasculitis affecting the endothelium of small vessels of synovial membranes, tendon sheaths, muscles, facia, and skin. The onset of fever and other clinical signs is accompanied by marked leucopenia, relative neutrophilia, elevated plasma fibrinogen, and elevated levels of cytokines including interferon α, interleukin 1, and tissue necrosis factor. There is also a significant hypocalcemia that is thought to be responsible for sternal recumbency. The major clinical signs can be treated very effectively with anti-inflammatory drugs.

Virion Structure and Morphogenesis

BEFV virions are enveloped, bullet-shaped particles (approximately 70×180 nm) containing a precisely coiled, helical nucleocapsid with 35 cross-striations at an interval of 4.8 nm. Virions have a prominent axial channel intruding from the base and typically are more cone-shaped than commonly observed for viruses in other genera of animal rhabdovirus (e.g., lyssaviruses and vesiculoviruses). The envelope contains a single 81 kDa class

1 transmembrane glycoprotein (G) that forms visible projections on the virion surface. The G protein mediates cell attachment and entry, is the target for virus-neutralizing antibodies, and induces protective antibodies in cattle. Nucleocapsids contain a negative-sense ssRNA genome, a 52 kDa nucleoprotein (N) which is tightly bound to the genome, a large 250 kDa replicase protein (L), and a highly charged 43 kDa replicase cofactor (P). Virions also contain a 29 kDa matrix protein (M) which is a major structural component and appears to lie between nucleocapsids and the inner surface of the lipid envelope (**Figure 2**).

Viral replication is cytoplasmic and morphogenesis occurs primarily at the plasma membrane in association with accumulations of a filamentous, granular, intracytoplasmic matrix. However, late in infection, there is a proliferation of plasma membrane, cells become highly vacuolated, and virions are observed both at the plasma membrane and within cytoplasmic vacuoles. Following budding as cone-shaped extrusions, virions accumulate in extracellular spaces. The general characteristics of viral morphogenesis appear to be similar in infected mammalian cell cultures and mouse neurons.

BEFV Genome Organization and the Encoded Proteins

The 14 900 nt BEFV genome is the largest known for any rhabdovirus and one of the largest and most complex genomes for any nonsegmented, negative-sense RNA virus. The genome comprises 12 genes, flanked by terminal, partially complementary leader (*l*) and trailer (*t*) sequences, and arranged in the order 3′-*l*-N-P/C-M-G-G_{NS}-α1/α2/α3-β-γ-L-*t*-5′. By analogy with other rhabdoviruses, the 3′ leader (50 nt) and 5′ trailer (70 nt) sequences are likely to have important roles in the initiation and control of replication and transcription, and in nucleoprotein assembly and packaging. The structural protein genes (N, P, M, G, and L) are arranged in the same order as for all other known rhabdoviruses and encode proteins with similar functional characteristics (**Figure 3**).

The N Protein

The BEFV N gene encodes the 431 amino acid nucleoprotein (N). The N protein is a highly hydrophilic, RNA-binding protein containing 14.4% basic residues

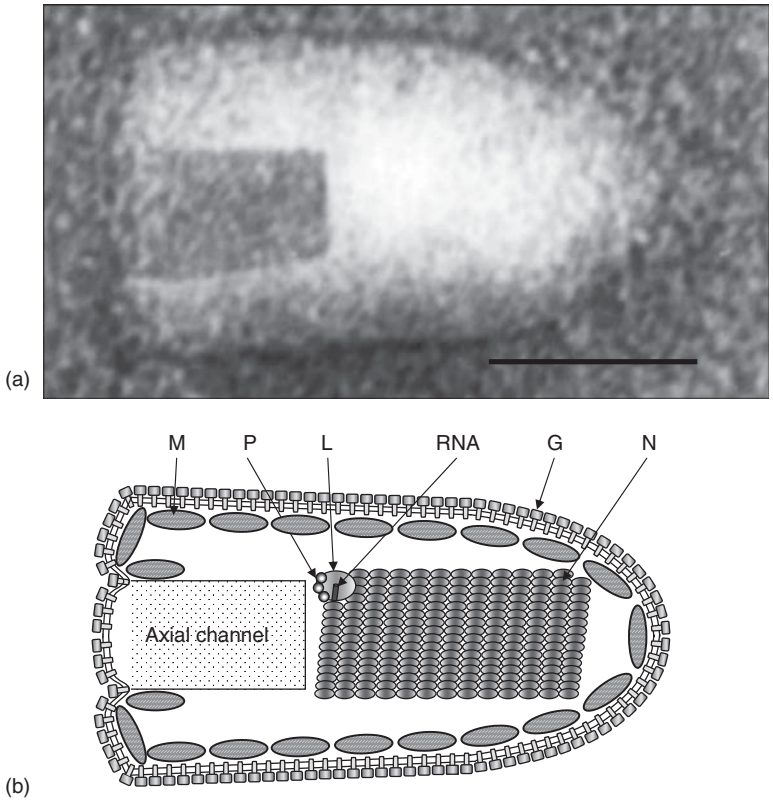


Figure 2 (a) Negative-contrast electron micrograph of a purified BEFV virion. (b) Schematic representation of the BEFV virion. Structural proteins N, P, M, G, and L, and the negative-sense ssRNA genome are indicated. The axial channel is also depicted. The size and relative quantities of the proteins do not accurately reflect the content in virions. Scale = 50 nm (a).

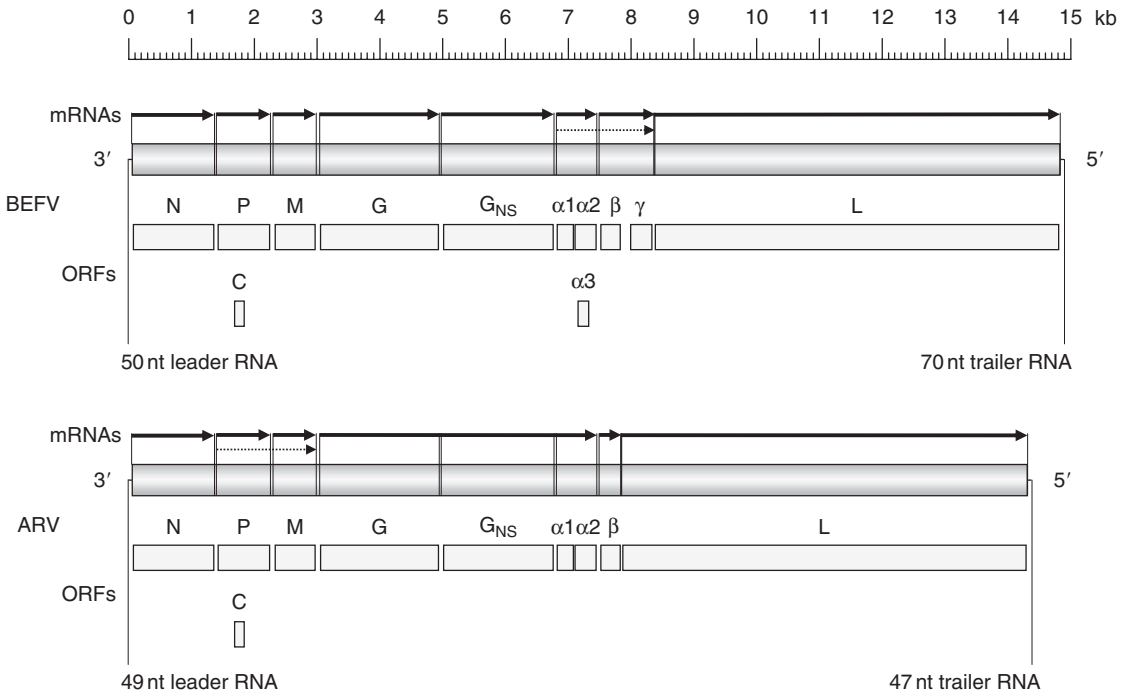


Figure 3 Illustration of the genome organization and transcription strategy of BEFV and ARV. Solid arrows indicate the major transcriptional products. Minor transcripts are indicated by dotted arrows. The length of the ARV L protein is estimated from the alignment of available sequences with those of BEFV.

(lysine, arginine, and histidine) distributed relatively evenly throughout the molecule. Seven of these residues are highly conserved in rhabdoviruses and are involved in RNA-binding and stabilizing the interaction. The N protein also contains 14.6% acidic residues (glutamate and aspartate) that are less evenly distributed with significant clustering in a short domain near the C terminus. A similar acidic domain in the rabies N protein is a phosphorylation site involved in binding P protein to nucleocapsids. The BEFV N protein is also phosphorylated when packaged in virions and contains a similar phosphorylation site in this acidic domain.

The P Protein

The P gene encodes the 278 amino acid, highly hydrophilic P protein. It corresponds to the polymerase-associated phosphoproteins of rabies virus (RV) and vesicular stomatitis virus (VSV) which are components of nucleocapsids and act as essential cofactors to the L protein during transcription and replication. The BEFV P protein has not been observed to be phosphorylated when extracted from virions but is phosphorylated when expressed from a recombinant baculovirus in insect cells. The BEFV P gene also contains an alternative open reading frame (ORF) encoding a 48 amino acid, highly basic 5.8 kDa polypeptide. This protein has not been detected in BEFV-infected cells and it is not known if it is expressed. However, alternative ORFs in the P gene occur in vesiculoviruses and are a common feature of many viruses in the *Mononegavirales*. As in VSV, the BEFV C protein has two potential initiation codons, suggesting it could be expressed in two different forms.

The M Protein

The M gene encodes the 691 amino acid, basic, hydrophilic protein that corresponds to the matrix protein (M) of rabies and VSV. The M protein is a major component of rhabdovirus virions and has been shown to have important functions in regulation of viral replication and transcription, inhibition of host cell protein synthesis and induction of apoptosis, and in budding of nucleocapsids at cytoplasmic membranes. The BEFV M protein has been shown to be phosphorylated when extracted from virions but not when expressed from a recombinant baculovirus in insect cells. This is consistent with observations that phosphorylation of the VSV M protein occurs at a late stage in viral assembly. The BEFV M protein also contains a 'late domain' sequence motif (PPSY) which, in VSV and several other RNA viruses, is essential for efficient budding from infected cells.

The G Protein

The BEFV G gene encodes the 623-amino-acid virion transmembrane glycoprotein (G). The G protein is a

class 1 membrane protein. It shares significant amino acid sequence identity with other animal rhabdovirus G proteins and contains a core of conserved cysteine residues suggesting preservation of fundamentally similar secondary structure. The G protein contains five potential N glycosylation sites, three of which appear to align with similar sites in VSV and/or rabies virus. The G protein is responsible for cell docking and entry, and is the target of virus-neutralizing antibodies for which the major binding sites have been defined (see below).

The L Protein

The L gene encodes the 2144-amino-acid RNA-dependent RNA polymerase (L protein). The L protein is a structural component of nucleocapsids that, in cooperation with the N and P proteins, forms the ribonucleoprotein (RNP) complex that is responsible for replication and transcription of the viral genome. The BEFV L protein shares a high level of sequence similarity with other rhabdovirus L proteins and contains all of the conserved sequence motifs associated with the major functional domains; these include the polymerase catalytic site and other regions involved in replication, transcription, initiation, elongation, and termination, 3′ polyadenylation, 5′ capping, and cap methylation.

The G_{NS} Protein

The BEFV nonstructural protein genes are located in a 3442 nt region of the genome between the G gene and L gene. Immediately downstream of the G gene is a second gene encoding a class 1 transmembrane glycoprotein (G_{NS}). The 90 kDa G_{NS} protein is abundant in infected cells but has not been detected in virions. It is related in structure and sequence to the BEFV virion G protein and other rhabdovirus G proteins and the evidence suggests that it has arisen by gene duplication. The G_{NS} protein contains eight potential N-glycosylation sites and, as the size by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is approximately 21 kDa in excess of the calculated molecular weight of the unmodified polypeptide, it appears to be highly glyscosylated. Sequence alignments indicate that it shares 10 of 12 cysteine residues that appear to form the core of secondary structure common to all animal rhabdoviruses. However, the G_{NS} protein does not share antigenic sites with the BEFV G protein and antibody to G_{NS} does not neutralize the infectivity of BEFV produced in either mammalian or insect cells. It has been shown to accumulate at the cell surface in association with amorphous structures but not with budding or mature virions. The function of the G_{NS} is currently unknown.

The Small Nonstructural Proteins

Downstream of the G_{NS} gene is a complex region of the genome-encoding proteins that appear to be unique to BEFV and other ephemeroviruses. None of these proteins has yet been detected in virions or BEFV-infected cells. The α-gene coding region contains three long ORFs (α1, α2, and α3). The α1 ORF encodes an 88 amino acid, 10.6 kDa protein. It features a central transmembrane domain comprising 16 hydrophobic amino acids bounded by arginine residues, and a highly basic C-terminal domain in which 12 of 18 amino acids are lysine or arginine residues. This structure suggests α1 may function as a viroporin, a class of proteins that causes cytopathic effects by increasing membrane permeability. The BEFV α1 protein has been shown to be cytotoxic when expressed in insect cells from a recombinant baculovirus vector. The α2 ORF encodes a 116 amino acid, 13.7 kDa polypeptide. It overlaps the α3 ORF which encodes a 51 amino acid, 5.7 kDa polypeptide that contains an unusual triple repeat of isomers of the sequence KLMEE at intervals of four residues. The β-gene encodes a 107 amino acid, 12.3 kDa polypeptide. The γ-gene encodes a 114 amino acid, 13.5 kDa polypeptide. The α2, α3, β, and γ products share no sequence homology with known viral proteins (other than ARV, see below) and their functions are yet to be determined.

BEFV Transcription

The RNP complex, comprising the RNA genome and the N, L, and P proteins, is the active replication and transcription unit of the virus. As for other rhabdoviruses, BEFV transcription from the (−) RNA genome generates 5′ methylated, capped, and polyadenylated mRNAs by a progressive mechanism that initiates and terminates at short, conserved sequences flanking each gene. For each of the structural protein genes (N, P, N, G, and L), and the nonstructural glycoprotein gene (G_{NS}), transcription initiates at the sequence UUGUCC and terminates at the polyadenylation signal GUAC $[U]_7$. Transcription of the α-, β-, and γ-coding regions is more complex. The α-coding region is translated as an α1-α2-α3 polycistronic mRNA that initiates at UUGUCC but terminates at the variant polyadenylation signal GUUC $[U]_7$. This variant signal appears to cause incomplete termination and partial read-through of a longer tri-cistronic α-β-γ mRNA. The β gene is also immediately followed by a variant polyadenylation signal (GUAC $[U]_6$). However, the truncated (U_6) palindrome does not allow transcription termination, and reinitiation does not occur at a UUGUCC sequence located immediately in advance of the γ gene. As a result, the β- and γ-coding regions are transcribed as a bi-cistronic mRNA that initiates at UUGUCC upstream of the β ORF and terminates at the functional GUAC $[U]_7$

polyadenylation signal downstream of the γ ORF. This polyadenylation signal overlaps the L gene initiation sequence by 21 nt, requiring an upstream repositioning of the polymerase to commence L gene transcription. A similar arrangement for L gene transcription has been observed for several other nonsegmented (−) RNA viruses.

ARV Genome Organization and Transcription

The genome organization and transcription strategy of ARV are similar to those of BEFV. However, there are subtle differences that reveal aspects of genome evolution and the control of gene expression in ephemeroviruses. The ARV genome includes the five structural protein genes that are common to all rhabdoviruses (N, P, M, G, and L). Each of the proteins encoded in these genes is similar in size and shares a high level of sequence identity with the corresponding BEFV proteins. Like BEFV, the ARV P gene also contains an alternative ORF that encodes a basic protein of similar size (7.4 kDa) to the BEFV C protein. The ARV 3′ leader sequence (49 nt) is similar in size to the BEFV leader RNA and shares a high level of sequence identity (21/22 nt) in the U-rich terminal domain. The ARV 5′ trailer sequence (47 nt) is shorter than the BEFV trailer RNA (70 nt), primarily due to the absence of a 26 nt direct repeat of the BEFV leader sequence that occurs in the BEFV trailer RNA. The function (if any) of this direct repeat is not known. The A-rich 5′ terminal region of the ARV trailer RNA shares only moderate sequence identity (15/21 nt) with the BEFV trailer, and is partially complementary (18/21 nt) to the U-rich ARV 3′ leader. This complementarity reflects the specificity of interaction of the polymerase with both the (−) RNA genome and (+) RNA antigenome during replication.

Like BEFV, the ARV genome encodes a second, class 1 transmembrane glycoprotein (G_{NS}) immediately downstream of the G gene. ARV G_{NS} is also nonstructural and shares significant amino acid sequence identity with the BEFV G_{NS} protein, as well as the G proteins of BEFV and other animal rhabdoviruses. ARV G_{NS} has eight potential N-glycosylation sites, four of which appear to align with sites in BEFV G_{NS}. There is a high level of preservation of cysteine and proline residues with BEFV G_{NS}, suggesting a similar folded secondary structure. The ARV and BEFV G_{NS} glycoproteins also appear to have preserved a core of cysteine residues conserved in rhabdovirus G proteins and is crucial for maintaining a fundamentally similar secondary structure. A proline-rich motif that, in VSV, forms a crucial 'P' helix in the membrane fusion domain, is also present in the BEFV and ARV G proteins. The 'P' helix motif is also present in BEFV G_{NS} but it is absent from the ARV G_{NS} protein. However, unlike the BEFV G protein, BEFV G_{NS} does not induce cell fusion when expressed in insect cells from a recombinant baculovirus vector. The biological significance of these observations will not be clear until further studies are conducted to better define the functions of ephemerovirus G_{NS} proteins.

Between the G_{NS} and L genes, ARV also contains a complex region encoding several proteins of uncertain function. The genes in this region are arranged in the order $-G_{NS}$-α1/α2-β-L-*t*-5′. The α1 ORF encodes a membrane-spanning, nonstructural protein with a highly basic C-terminal domain which is similar to the BEFV α1 protein and may well also function as a viroporin. The ARV α2 and β ORFs encode proteins similar in size to the corresponding BEFV proteins but, although the overall sequence similarity is relatively high, there is no significant sequence identity. A 17 kDa protein reported in purified ARV virions is similar in size to that predicted for the β protein. The α3 and γ ORFs are not present in the ARV genome.

The ARV transcription strategy is somewhat different from that of BEFV. Only the N-gene, L-gene, and β-gene are transcribed solely as monocistronic mRNAs. For the N-gene and L-gene, transcription initiates and terminates at standard UUGUCC and GUAC $[U]_7$ signals flanking each gene. Transcription of the β-gene initiates at the variant UUGUCU sequence and terminates at GUAC $[U]_7$. The P-gene and the M-gene are transcribed both as high abundance monocistronic mRNAs and a lower abundance (approximately 10%) bicistronic P/M mRNA. Each initiates at UUGUCC and the M-gene terminates at GUAC $[U]_7$. However, the P-gene terminates at the leaky variant signal GCAC $[U]_7$. The G-, G_{NS}-, and α-genes are transcribed primarily as a long polycistronic mRNA that initiates at UUGUCG upstream of the G-gene and terminates at GUAC $[U]_7$ following the α-gene. Corrupted termination/polyadenylation signals following the G-gene (GUAC $[U]_4$C $[U]_2$) and the G_{NS}-gene (GUGC $[U]_2$C $[U]_4$) appear to allow a very low level of termination and transcription initiation at UUGUCC signals immediately preceding both the G_{NS}- and α-genes. As for the BEFV γL junction, there is an overlap (22 nt) of the β-L gene junction in ARV, highlighting the importance of polymerase repositioning in the control of L gene expression. There is also a high level of nucleotide sequence identity between the α-β and β-γ gene junctions in BEFV, and between the ARV β-L gene junction and the BEFV γL gene junction. This suggests that the BEFV γ-gene may have evolved as a consequence of β-gene duplication. It appears, therefore, that gene duplication may have an important role in ephemerovirus evolution.

Antigenic Variation

As defined either by cross-protection experiments in cattle, or by cross-neutralization tests in mice, or in cell

cultures, BEFV exists as a single serotype globally. The relative antigenic stability of BEFV is most likely due to the occurrence of viremia and vector-borne transmission several days prior to the appearance of significant levels of virus-neutralizing antibodies. The BEFV virion G protein is the target of neutralizing antibody and four major neutralization sites have been identified and mapped to the amino acid sequence. Antigenic site G1 is a linear site that maps as two minimal B-cell epitopes at each end of the sequence spanning amino acids Y^{487} to K^{503} in the 'stem' domain of the G protein. This domain appears to be a unique feature of ephemeroviruses. Antigenic site G2 is conformational. It is located adjacent to two cysteine residues (C^{172} and C^{182}) that appear to form a disulfide bridge linking a tight glycosylated loop structure in the folded G protein. Site G3 is the major conformational site comprising two partially overlapping elements (G3a and G3b). The site encompasses three different domains of the cysteine-rich 'head' structure of the folded G protein spanning Q^{49} to D^{57}, K^{215} to E^{229}, and Q^{265}. Similarly complex antigenic sites map to corresponding regions of other animal rhabdoviruses, again supporting the view that essential elements of G protein secondary structure are preserved. Site G4 is a linear site. It has not yet been mapped to the G-protein sequence but it is known to be conserved in BRMV and KIMV which are also neutralized by site G4 monoclonal antibodies. (Amino acid residues are numbered here to include the N-terminal signal peptide that is cleaved during maturation of the G protein; **Figure 4**).

Limited natural antigenic variation has been reported between BEFV isolates. Variations in sites G3a and G3b have been identified among 70 Australian BEFV isolates collected from diverse locations between 1956 and 1992. There appears to be a temporal basis for the shift in site G3a which is present in most strains isolated after 1973. Comparison of prototype Australian and Chinese BEFV isolates has also indicated variations in site G3a. In Taiwan, variations have been reported in sites G1 and G3. The pattern of amino acid substitutions indicates that the isolates cluster into those which included the 1984 Taiwanese vaccine strain (Tn73) and those which were isolated after 1986. It is possible that incomplete protection provided by available BEF vaccines is contributing to antigenic instability in the G protein.

Immune Response and Vaccination

Natural BEFV infection induces a strong neutralizing antibody response and apparently durable immunity. Following experimental infection, neutralizing IgG antibody appears 4–5 days after the onset of clinical signs and peaks within 1–4 weeks. Although there are some reports that cattle with high levels of neutralizing antibody can be susceptible to experimental challenge, other evidence

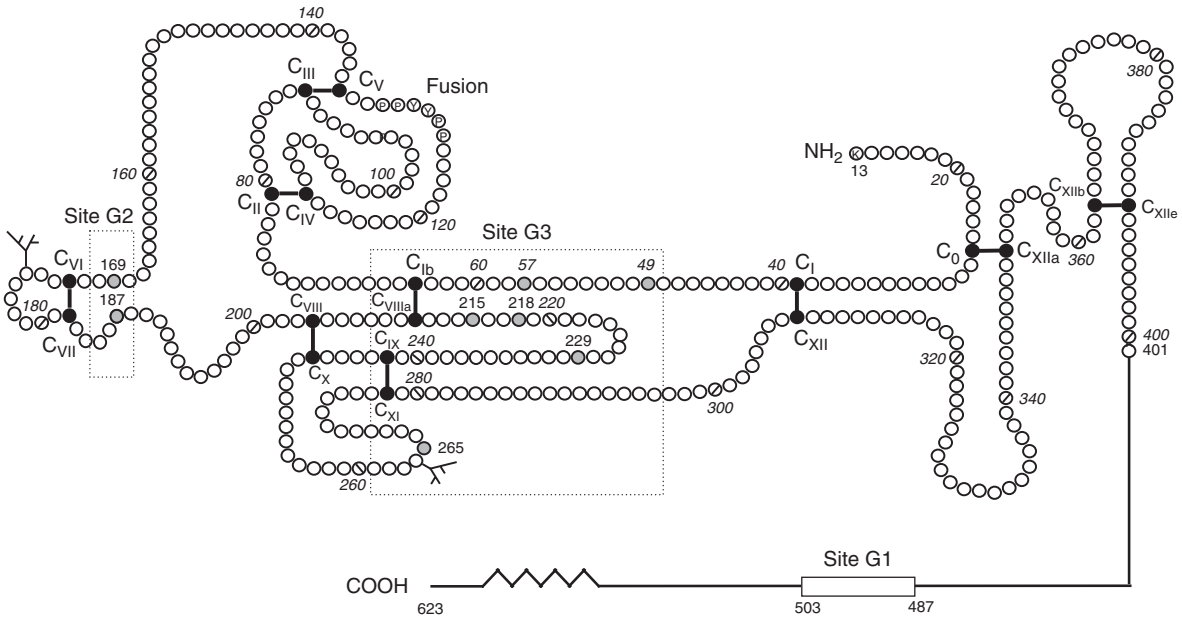


Figure 4 Schematic illustration of the structure of the BEFV G protein showing the locations of the major neutralization sites (G1, G2, and G3) and the predicted fusion domain including the highly conserved poly-proline helix (PPYYPP). Amino acids known to be located in the major antigenic sites are indicated as shaded circles. Disulfide bridges are assigned according to previous predictions from sequence alignments with other rhabdoviruses and from the known crystallographic structure of the low-pH form of the VSV G protein. Amino acids are numbered from the first residue of the translated protein, prior to removal of the N-terminal signal peptide.

suggests a good correlation between protection and neutralizing antibody. Colostral antibody has also been shown to protect calves against experimental challenge. High levels of cytokines circulate during the acute phase of infection but little is known of the role of innate or adaptive cell-mediated immunity in recovery from infection or protection against natural or experimental challenge.

Several forms of live-attenuated, inactivated, subunit, and recombinant BEFV vaccines have been reported and vaccines of varying format are produced for commercial use. Live-attenuated vaccines have been produced in mice and in cell cultures. In general, live vaccines are relatively effective in inducing protection but require at least two doses in adjuvant to generate durable immunity. Inactivated vaccines have been produced by treatment of BEFV with formalin or β-propiolactone, but have generally poor efficacy. Consecutive vaccinations with live-attenuated and killed preparations have also been used with some success. A purified G-protein subunit vaccine delivered in Quil A adjuvant has been shown to provide reliable protection following a two-dose treatment at an interval of 21 days. Recombinant BEFV vaccines employing the BEFV G protein delivered in vaccinia and capripox viral vectors have also been trialed.

See also: Animal Rhabdoviruses; Chandipura Virus; Fish Rhabdoviruses; Rabies Virus; Vesicular Stomatitis Virus.

Further Reading

Inaba Y, Kurogi H, Takahashi A, *et al.* (1974) Vaccination of cattle against bovine ephemeral fever with live attenuated virus followed by killed virus. *Archiv fur die Gesamte Virusforschung* 44: 121–132.

Kirkland PD (2002) Akabane and bovine ephemeral fever virus infections. *Veterinary Clinics of North America: Food Animal Practice* 18: 501–514.

Kongsuwan K, Cybinski DH, Cooper J, and Walker PJ (1998) Location of neutralizing epitopes on the G protein of bovine ephemeral fever rhabdovirus. *Journal of General Virology* 79: 2573–2578.

Kuzmin IV, Hughes GJ, and Rupprecht CE (2006) Phylogenetic relationships of seven previously unclassified viruses within the family *Rhabdoviridae* using partial nucleoprotein gene sequences. *Journal of General Virology* 87: 2323–2331.

McWilliam SM, Kongsuwan K, Cowley KA, Byrne KA, and Walker PJ (1997) Genome organization and transcription strategy in the complex G_{NS}-L intergenic region of bovine ephemeral fever rhabdovirus. *Journal of General Virology* 78: 1309–1317.

Nandi S and Negi BS (1999) Bovine ephemeral fever: A review. *Comparative Immunology, Microbiology, and Infectious Diseases* 22: 81–91.

St. George TD (1990) Bovine ephemeral fever virus. In: Dinter Z and Morein B (eds.) *Virus Infections of Vertebrates, Vol. 3: Virus Infections of Ruminants*, pp. 405–415. Amsterdam: Elsevier.

Theodoridis A, Giesecke WH, and Du Toit IJ (1973) Effects of ephemeral fever on milk production and reproduction of dairy cattle. *The Onderstepoort Journal of Veterinary Research* 40: 83–92.

Tomori O, Fagbami A, and Kemp G (1974) Kotonkan virus: Experimental infection of Fulani calves. *Bulletin of Epizootic Diseases of Africa* 22: 195–200.

Tordo N, Benmansour A, Calisher C, *et al.* (2005) *Rhabdoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 623–644. San Diego, CA: Elsevier Academic Press.

Uren MF, St. George TD, and Zakrzewski H (1989) The effect of anti-inflammatory agents on the clinical expression of bovine ephemeral fever. *Veterinary Microbiology* 19: 99–111.

Venter GJ, Hamblin C, and Paweska JT (2003) Determination of the oral susceptibility of South African livestock-associated biting midges, *Culicoides* species, to bovine ephemeral fever virus. *Medical and Veterinary Entomology* 17: 133–137.

Walker PJ (2005) Bovine ephemeral fever in Australia and the World. In: Fu Z and Kaprowski H (eds.) *The World of Rhabdoviruses. Current Topics in Microbiology and Immunology,* vol. 292, pp. 57–80. Berlin: Springer.

Walker PJ, Byrne KA, Riding GA, *et al.* (1992) The genome of bovine ephemeral fever rhabdovirus contains two related glycoprotein genes. *Virology* 191: 49–61.

Wang YH, McWilliam SM, Cowley JA, and Walker PJ (1994) Complex genome organization in the G_{NS}-L intergenic region of Adelaide River rhabdovirus. *Virology* 203: 63–72.

Bovine Herpesviruses

M J Studdert, The University of Melbourne, Parkville, VIC, Australia

© 2008 Elsevier Ltd. All rights reserved.

History

Although some of the clinical diseases caused by herpesviruses in members of the family Bovidae have been recognized for centuries, it was not until the first and probably most important alphaherpesvirus now called bovine herpesvirus 1 (BHV1) was isolated in the late 1950s from the genital disease coital exanthema (also called infectious pustular vulvovaginitis (IPV) in the female) and from the respiratory disease infectious bovine rhinotracheitis (IBR) that any of these diseases was confirmed to be caused by a herpesvirus. Historically, IPV and its male counterpart infectious pustular balanoposthitis (collectively the male and female diseases are termed coital exanthema or *blaschenausschlag*) were commonly described diseases in central Europe throughout the nineteenth century. It was common for a single bull in a village to serve all the female cattle in that village and, where distances were small, also in nearby villages, and *blaschenausschlag* was a frequently observed sequel to mating. The isolation

of IBR virus in 1957 and IPV virus in 1958, and subsequent work that established that the two viruses were essentially identical, led to the designation of BHV1.

Around 1970, a distinctly different, but BHV1-related, alphaherpesvirus was recognized as a cause of encephalitis. This virus is designated BHV5 pending identification of its definitive host, which may not be European cattle.

The alphaherpesvirus BHV2 was recognized as a cause of pseudolumpy skin disease and, as an independent syndrome, mammilitis, in about 1960, although both diseases were known clinically well before this time.

An alphaherpesvirus with a natural history similar to that of BHV1 was isolated from goats in the mid-1960s. An increasing number of alphaherpesviruses, again assumed to have a natural history similar to BHV1, have been isolated from various deer and other wild ruminant species. Curiously, no alphaherpesvirus has been isolated from sheep.

A slowly growing, highly cell-associated gammaherpesvirus, uncertainly associated with a number of disease syndromes in cattle, is designated BHV4.

The disease bovine malignant catarrhal fever (MCF) has been described for at least a century and the causative gammaherpesvirus of the African form of the disease, formerly designated BHV3 (sometimes BHV4), was first isolated in 1968. However, the natural host for the best-characterized MCF gammaherpesvirus, acquired by European cattle, as originally reported in southern Africa, is the wildebeest (*Connochaetes gnu*) and this virus is now termed alcelaphine herpesvirus 1 (AlHV1). A poorly characterized gammaherpesvirus (OvHV1) associated with ovine pulmonary adenomatosis has been described and a second ovine gammaherpesvirus (OvHV2) is the cause of sheep-associated bovine MCF.

An increasing number of gammaherpesviruses have been identified in normally free-ranging ruminant species when they are farmed or held in zoological collections. Several of these viruses have caused MCF-like syndromes when transmitted to other in-contact ruminant species.

Classification

Members of the family *Herpesviridae* (in the proposed order *Herpesvirales*) that infect certain members of the family *Bovidae* are classified in the subfamilies *Alphaherpesvirinae* or *Gammaherpesvirinae*. Some of the viruses are listed in **Table 1**, which also lists their respective genera, together with some other salient properties, where known, including nucleotide composition and genome size. All of the ruminant alphaherpesviruses are classified in the genus *Varicellovirus* with the notable exception of BHV2, which is classified in the genus *Simplexvirus* because it is most closely related to human herpes simplex virus. All of the ruminant gammaherpesviruses have been proposed as members of the new genus *Macavirus* (*maca* is short for MCF) with the exception of BHV4, which remains in the genus *Rhadinovirus*.

Structure

Each of the viruses listed in **Table 1** has a typical herpesvirus morphology. Virions are enveloped and about 150 nm in diameter. The double-stranded DNA genome is spooled within the capsid. There is an icosahedral nucleocapsid 100 nm in diameter composed of 162 hollow capsomers (150 hexamers and 12 pentamers). The nucleocapsid is surrounded by a layer of globular material called the tegument that is enclosed by a typical bilayer lipoprotein envelope in which are embedded glycoproteins, which generally appear as projecting spikes in negatively stained electron micrographs. There are about 12 distinct glycoproteins associated with the envelope spikes. Though the size of the DNA genome varies (**Table 1**), there is evidence that there are up to 76 open reading frames (genes) minimally coding for a corresponding number of individual proteins. About 40 of these proteins are structural (i.e., associated with the virion), while the remainder are nonstructural, being found only in infected cells. Repeat DNA sequences are found in the genomes of all bovine herpesviruses. For the alphaherpesviruses a set of two inverted repeats bracket the so-called short region of the genome, and in the case of BHV2 only a second set of inverted repeats bracket the so-called long region. The gammaherpesviruses have a set of terminal repeat structures, within each of which a variable number of tandemly repeated sequences is found.

Replication

Virus replication occurs in the nucleus of cells, and in the case of alphaherpesviruses typically results in the production of a rapid cytopathic effect, with characteristic large intranuclear, eosinophilic inclusion bodies present in appropriately stained preparations. Some of the gammaherpesviruses can be cultivated in monolayer cell cultures, where they produce a cytopathic effect, but others have not been isolated in cell cultures. The replication cycle involves at least three classes of genes termed α, β, and γ or immediate early, early, and late, the synthesis of which is coordinately regulated in a cascade manner during the replication cycle.

Herpesviruses transcribe sets of micro (mi)RNAs that add complexity to understanding the replication cycle and the host–virus relationship and change views of the antiviral roles of RNA interference (RNAi), also known as gene silencing. Rather than being inhibited, many herpesviruses appear to be able to usurp or divert

Table 1 Herpesviruses of the family Bovidae and some other ruminant species

Virus	*Abbreviation*	*Disease names/synonyms*	*Subfamily*	*Genus*[a]	*Genome*	
					Size (kbp)	*G+C (mol.%)*
Bovine herpesvirus 1	BHV1	Infectious bovine rhinotracheitis; infectious pustular vulvovaginitis; coital exanthema	*Alphaherpesvirinae*	*Varicellovirus*	135	72
Bovine herpesvirus 2	BHV2	Bovine mammilitis; Allerton virus; pseudolumpy skin disease	*Alphaherpesvirinae*	*Simplexvirus*	133	64
Bovine herpesvirus 4	BHV4	Movar virus	*Gammaherpesvirinae*	*Rhadinovirus*	160[b]	41
Bovine herpesvirus 5	BHV5	Bovine encephalitis	*Alphaherpesvirinae*	*Varicellovirus*	135	74
Bovine herpesvirus 6	BHV6	Bovine lymphotropic HV	*Gammaherpesvirinae*	*Macavirus*		
Bubaline herpesvirus 1	BuHV1	Buffalo HV	*Alphaherpesvirinae*	*Varicellovirus*		
Elk herpesvirus 1	ElHV1	Elk HV related to BHV1				
Caprine herpesvirus 1	CapHV1	Goat HV1	*Alphaherpesvirinae*	*Varicellovirus*		
Caprine herpesvirus 2	CapHV2	Goat HV2	*Gammaherpesvirinae*	*Macavirus*		
Ovine herpesvirus 1	OvHV1	Sheep pulmonary adenomatosis-associated HV				
Ovine herpesvirus 2	OvHV2	Cause of sheep-associated bovine malignant catarrhal fever	*Gammaherpesvirinae*	*Macavirus*	160[b]	52[b]
Cervid herpesvirus 1	CerHV1	Red deer HV	*Alphaherpesvirinae*	*Varicellovirus*		
Cervid herpesvirus 2	CerHV2	Reindeer HV	*Alphaherpesvirinae*	*Varicellovirus*		
Alcelaphine herpesvirus 1	AlHV1	Wildebeest HV, cause of malignant catarrhal fever of European cattle	*Gammaherpesvirinae*	*Macavirus*	155–160[b]	46[b]
Alcelaphine herpesvirus 2	AlHV2	Barbary red deer HV, cause of malignant catarrhal fever of Jackson's hartebeest	*Gammaherpesvirinae*	*Macavirus*		

[a]Viruses listed in proposed genus *Macavirus* are currently in genus *Rhadinovirus* or are unclassified.
[b]Sizes shown include the terminal repeats, which vary in total size around 25–30 kbp. G + C contents exclude the terminal repeats.

the host RNA silencing machinery to their advantage. Herpesvirus-encoded miRNAs can act in *cis* to ensure accurate expression of viral genomes or in *trans* to modify the expression of host RNA transcripts.

Geographic Distribution

In general, each of the bovine alphaherpesviruses occurs worldwide, paralleling the distribution of the host species. The bovine alphaherpesviruses, with minor exceptions, have a restricted host range. None is known to infect nonbovine species; most are restricted to the primary host species.

Increasing numbers of gammaherpesviruses have been identified in normally free-ranging (exotic) ruminant species that have been farmed or held in zoological collections, and several of these viruses have caused MCF-like syndromes when transmitted to other in-contact ruminant species. At least six members of the MCF virus group of ruminant gammaherpesviruses have been identified thus far. Four of these viruses are clearly associated with clinical disease: alcelaphine herpesvirus 1 (AlHV-1) carried by wildebeest (*Connochaetes* spp.); ovine herpesvirus 2 (OvHV-2), ubiquitous in domestic sheep; caprine herpesvirus 2 (CapHV-2), endemic in domestic goats; and the virus of unknown origin that caused classic MCF in white-tailed deer (*Odocoileus virginianus*; MCFV-WTD). Gammaherpesviruses in the MCF virus group have been found in musk ox (*Ovibos moschatus*), Nubian ibex (*Capra nubiana*), and gemsbok (South African oryx, *Oryx gazella*). Gammaherpesviruses have also been found in bighorn sheep, bison, black-tailed deer, mule deer, fallow deer, elk, and addax.

Antigenic Relationships

Bovine herpesviruses are genetically stable, with only a single antigenic type described for each species and no major changes in antigenicity over time recognized. Some intratypic (within species) differences are detectable using restriction endonuclease DNA fingerprinting, but these differences have not been correlated with major antigenic differences in the proteins coded for by the regions where variable sequences have been identified.

Epidemiology

In general, transmission of the alphaherpesviruses requires close contact, particularly the kinds of physical contact that bring moist epithelial surfaces into apposition (e.g., coitus, or licking and nuzzling as between mother and offspring). In large, closely confined populations such as cattle feedlots or zoo collections, short-distance aerosol is an important mode of transmission.

Most of the bovine gammaherpesviruses are recognized when they are transmitted to heterologous hosts. For example, MCF in European cattle is acquired from wildebeest, sheep, or other nondomestic (exotic) ruminant species. Where exotic species are the source of infection, more often than not the transmissions occur outside of the natural geographic habitat of the transmitting host.

The gammaherpesviruses are probably transmitted primarily via nasal secretions in both the natural host and to the heterologous hosts that develop MCF. The latter transmission cycle is probably much less efficient, at least for some of the viruses, since many cases of MCF are sporadic. It is only recently that cell-free OvHV2 virions have been demonstrated in nasal secretions of sheep, which is a reflection of the highly cell-associated nature of gammaherpesviruses.

Pathogenesis

Alphaherpesviruses typically cause localized lesions, particularly of mucosal surfaces of the respiratory and genital tracts or, less commonly, the skin. Progression is characterized by the sequential production of vesicles, pustules, and shallow ulcers that become covered by a pseudomembrane and heal after 10–14 days, usually without scar formation (**Figure 1**).

Generalized alphaherpesvirus infections may occur in very young calves or in a fetus prior to abortion. Encephalitis produced by bovine encephalitis herpesvirus (BHV5) occurs as a consequence of spread of virus from the nasal cavity to the brain, via trigeminal nerve branches.

The gammaherpesvirus BHV4 is associated with low-grade clinical infection. MCF is a uniformly fatal disease associated with mucosal erosions, ophthalmia, and encephalitis that appear to be immune mediated. Lesions are characterized by infiltration and proliferation of lymphocytes. It is still not clear which lymphocyte population is the site of latency; both B and T lymphocytes have been implicated for different gammaherpesviruses. Immune complexes of viral antigen are probably also produced and contribute to the pathology.

Latency is a hallmark of bovine herpesviruses. The genome, probably as a circularized episome, persists in ganglion cells, typically the trigeminal and sciatic in the case of alphaherpesviruses, and in white blood cells in the case of gammaherpesviruses. From these sites of latency, virus is periodically shed to give rise to recurrent disease, shedding, and transmission to in-contact animals.

BHV1 establishes latency in sensory neurons of trigeminal ganglia, and in germinal centers of pharyngeal tonsil and similar sites related to the genital tract. BHV1 reactivates



(a) (b) (c)

Figure 1 As examples of alphaherpesvirus genital disease, three naturally occurring cases (a (early case), b, c) of acute, primary, infectious pustular vulvovaginitis in 8-month-old does caused by caprine herpesvirus 1 are shown. The extent of the individual vesicular/pustular lesions on the vaginal mucosa is not well shown because of the swelling, and the pain associated with closer examination. Reproduced from Piper KL, Fitzgerald CJ, Ficorilli N, and Studdert MJ (2008) Isolation of caprine herpesvirus 1 from a major outbreak of infectious pustular vulvovaginitis in goats. *Australian Veterinary Journal* 86: 136–138, with permission.

periodically from latency, virus is shed, and consequently virus transmission occurs. Two RNA transcripts, the latency-related RNA and ORF-E RNA, are abundantly expressed in trigeminal ganglia of latently infected cattle, and these transcripts probably regulate the BHV1 latency–reactivation cycle.

Clinical Diseases

The alphaherpesvirus BHV1 causes coital exanthema and IBR. Both diseases are a consequence of lesions of the mucous membrane at the two sites. The extent and severity of the lesions may vary from mild, subclinical disease to acute, complicated and severe clinical disease that is more likely to occur in the case of IBR in feedlot cattle, where the disease is complicated by secondary bacterial infections. Complicated cases of IBR extending to pneumonitis in feedlot cattle may be fatal.

BHV1 may occasionally cause enteritis in calves. Encephalitis caused by BHV1 has not been confirmed; all cases of alphaherpesvirus encephalitis in cattle have been caused by the distinctly different virus BHV5. In groups of young calves the mortality caused by BHV5 encephalitis may approach 100%.

Mammilitis caused by BHV2 may be acute, leading to loss of skin from the teats, udder, and perineal regions following vesicle and pustule formation. Pseudolumpy skin disease caused by BHV2 appears to be a consequence of viremic spread, possibly as a cell-associated viremia with localization of the virus in the skin resulting in large golf-ball-sized subcutaneous swellings. These eventually resolve after a course of 3–4 weeks.

BHV6 was identified in bovine B-lymphoma cells and peripheral blood mononuclear cells.

The natural history of caprine herpesvirus 1 is similar to that of BHV1, with disease characterized by a variety of clinical signs including conjunctivitis and lesions of the genital tracts and sometimes the respiratory and gastrointestinal tracts. Abortion may occur.

The red deer and reindeer alphaherpesviruses probably cause clinical disease and have a similar natural history to BHV1.

Bovine MCF in European cattle is caused by alcelaphine herpesvirus 1 or 2 or ovine herpesvirus 2. The disease follows an incubation period of 3 weeks and is

characterized by fever, depression, leucopenia, profuse nasal and ocular discharge, generalized lymphadenopathy, extensive mucosal erosions, central nervous system signs, and bilateral ophthalmia that begins as a keratoconjunctivitis and extends to a panophthalmitis. Death, which is invariable, occurs about 1 week after the onset of clinical signs.

Caprine herpesvirus 2 causes clinical MCF when transmitted to farmed white-tailed deer. The transmission pattern of caprine herpesvirus 2 in goats is similar to that of ovine herpesvirus 2 in sheep, with nasal secretions believed to be the major mode of transmission.

Ovine herpesvirus 2 is a noncultivable, lymphotropic gammaherpesvirus that asymptomatically infects most sheep, but causes MCF in cattle, bison, and, somewhat surprisingly, pigs and deer. A uniformly fatal enteric form of MCF caused by ovine herpesvirus 2 was identified in American bison (*Bison bison*) at a large feedlot in the American Midwest in 1998. An estimated 150 bison died. Clinical onset was acute, and most affected bison died within 1–3 days following the onset of clinical disease.

Immune Response

Both antibody and cell-mediated immune responses are generated during herpesvirus infections. Neutralizing antibody primarily directed against envelope glycoproteins is probably important in long-term immunity. Viral antigens, some of which may be nonstructural immediate early and early proteins, are incorporated into the cell membrane and serve as targets for cytotoxic T lymphocytes. The immune response associated with infection does not prevent the establishment of latency and its role in regulating reactivation of latent virus and recurrent disease and shedding is debated. A central contradiction of herpesvirus immunity is that following natural infection immune animals are also animals that are infected for life.

Prevention and Control

BHV1 genital disease can be controlled by eliminating carrier cattle identified either serologically or by reactivation and isolation of virus following the administration of corticosteroids such as dexamethasone. Alternatively, where it is important to do so, such as for bulls in artificial breeding centers, a two-herd system may be established. IBR is often associated with stress of transport, intercurrent disease, overcrowding and the mixing together of cattle from different sources, all of which are typically associated with feedlot operations. Awareness and minimization of these predisposing factors can reduce the severity of clinical disease. In an increasing number of countries, test and slaughter programs have achieved total eradication of BHV1 from national herds.

In many parts of the world, including North America, control of BHV1 is achieved by vaccination with conventional live attenuated or inactivated vaccines. With parts of Europe being BHV1 free, the ability to differentiate infected from vaccinated animals has become critical for trade. Live and killed glycoprotein E-deleted marker vaccines are now widely used in Europe, in combination with glycoprotein-based diagnostic tests to monitor cattle. There is debate about the cost and sustainability of eradication programs other than in limited settings such as artificial insemination centers or small countries. Conventional inactivated and attenuated vaccines are less efficacious in neonates because of interference by virus-specific, passively derived maternal antibody. Alternative vaccine types, such as those incorporating CpG oligonucleotides as adjuvant for recombinant protein vaccines or DNA vaccines, are being explored.

Vaccines are not generally available for the control of other bovine or other ruminant herpesvirus diseases.

The epidemiology of BHV2 is not well understood and a possible approach to prevention and control would be to consider removal of known infected cattle.

Since MCF is acquired from a heterologous host (wildebeest, sheep, goats, or other ruminant species), it is clearly preventable by avoiding such contacts. The often-sporadic nature of the disease and the lack of detailed knowledge of the putative sheep-associated virus make avoidance difficult. In zoological collections, bovid species known to harbor alcelaphine herpesviruses 1 and 2 or any of the other gammaherpesviruses should not be cohabited with those species known to be susceptible to MCF.

Future

Continued progress in understanding the molecular biology of the bovine herpesviruses, including full genome sequencing such as already reported for some viruses including BHV1, BHV4, BHV5, and AlHV1, will occur. Characterization of the transcripts and proteins of the viruses will be taken forward. Progress in developing better vaccines and diagnostic reagents for BHV1 including those based on recombinant DNA technologies, including DNA vaccines, will continue. How effective these new vaccines and diagnostic tests will be in national and international eradication programs is a question of will and financial commitment. The unusual epidemiologies of BHV2 and BHV5 are matters for future inquiry. Further progress in the unusual pathogenesis of MCF and the characterization of the sheep-associated virus responsible for many cases of MCF in the Western world are a part of ongoing work. It may be anticipated that the number of new heterologous host transmission cycles of gammaherpesviruses leading to highly fatal MCF outbreaks will continue to increase, and definition of these

should lead to better control measures where ruminant species cohabit. There are many members of the family *Bovidae* for which neither alphaherpesviruses nor gammaherpesviruses have been identified, and over time it may be expected that more of these viruses will be isolated and characterized.

See also: Herpes Simplex Viruses: General Features; Herpes Simplex Viruses: Molecular Biology; Herpesviruses: Discovery; Herpesviruses: Latency; Pseudorabies Virus; Taxonomy, Classification and Nomenclature of Viruses.

Further Reading

Jones C, Geiser V, Henderson G, *et al.* (2006) Functional analysis of bovine herpesvirus 1 (BHV-1) genes expressed during latency. *Veterinary Microbiology* 113: 199–210.

Li H, Gailbreath K, Flach EJ, *et al.* (2005) A novel subgroup of rhadinoviruses in ruminants. *Journal of General Virology* 86: 3021–3026.

Murphy FA, Gibbs EPJ, Horzinek MC, and Studdert MJ (eds.) (1999). *Herpesviridae*. In: *Veterinary Virology*, 3rd edn., ch.18, pp. 301–325. New York: Academic Press.

O'Toole D, Li H, Sourk C, Montgomery DL, and Crawford TB (2002) Malignant catarrhal fever in a bison (*Bison bison*) feedlot, 1993–2000. *Journal of Veterinary Diagnostic Investigation* 14: 183–193.

Pfeffer S, Sewer A, Lagos-Quintana M, *et al.* (2005) Identification of microRNAs of the herpesvirus family. *Nature Methods* 2: 269–276.

Piper KL, Fitzgerald CJ, Ficorilli N, and Studdert MJ (2008) Isolation of caprine herpesvirus 1 from a major outbreak of infectious pustular vulvovaginitis in goats. *Australian Veterinary Journal* 86: 136–138.

Rovnak J, Quackenbush SL, Reyes RA, Baines JD, Parrish CR, and Casey JW (1998) Detection of a novel bovine lymphotropic herpesvirus. *Journal of Virology* 72: 4237–4242.

Taus NS, Oaks JL, Gailbreath K, Traul DL, O'Toole D, and Li H (2006) Experimental aerosol infection of cattle (*Bos taurus*) with ovine herpesvirus 2 using nasal secretions from infected sheep. *Veterinary Microbiology* 116: 29–36.

Thiry J, Keuser V, Muylkens B, *et al.* (2006) Ruminant alphaherpesviruses related to bovine herpesvirus 1. *Veterinary Research* 37: 169–190.

Thonur L, Russell GC, Stewart JP, and Haig DM (2006) Differential transcription of ovine herpesvirus 2 genes in lymphocytes from reservoir and susceptible species. *Virus Genes* 32: 27–35.

van Drunen Littel-van den Hurk S (2006) Rationale and perspectives on the success of vaccination against bovine herpesvirus-1. *Veterinary Microbiology* 113: 275–282.

Bovine Spongiform Encephalopathy

R G Will, Western General Hospital, Edinburgh, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Bovine spongiform encephalopathy (BSE) is a prion disease of cattle, which was first identified in 1986, and has subsequently become a source of widespread concern for policymakers and public health. To date, more than 184 000 cases have been identified in the UK and more than 5000 cases in other countries, primarily, but not exclusively, in Europe. The origin of BSE is unknown, but may have been related to scrapie contamination of cattle feed, with amplification of the epidemic through within-species recycling of infection. Legislative measures, including restrictions on the feeding of ruminant protein to ruminants, has led to a decline in annual numbers of identified cases in most countries, and there is a possibility that the introduction and implementation of appropriate control measures will lead to the eradication of BSE.

In 1996, a new form of human prion disease, variant Creutzfeldt–Jakob disease (vCJD), was identified in the UK, and epidemiological and laboratory data indicate that this disease is a zoonosis caused by infection with BSE, probably through past dietary exposure to infection. The human population in the UK and many European countries were exposed to significant titers of BSE infectivity over a period of years from about 1980, but the possibility of an extensive epidemic of vCJD has not, as yet, materialized. There has been a relatively limited and declining annual mortality rate in the UK and, with the exception of France, only isolated cases in other, mainly European, countries. However, future outbreaks of vCJD, perhaps related to polymorphisms in the human prion protein gene (*PRNP*), cannot be excluded and concern for public health has increased with the demonstration of transmission of vCJD through blood transfusion. Accurate predictions of future numbers of cases are hampered by many uncertainties including the mean incubation period of human BSE infection and the prevalence of sub- or preclinical infection in exposed populations.

BSE

Clinical and Subclinical Infection

All prion diseases are degenerative conditions of the central nervous system and present with progressive and fatal neurological disorders. The clinical features of BSE include weight loss, reduced milk yield, ataxia and hyperesthesia, progressing to recumbency and death. Although

there is a wide age range of affected cattle, the majority of cases are aged 4–6 years. Identification of clinically affected animals is critical to analysis of the epidemiology of BSE and for protection of public health, but depends on recognition of the clinical phenotype or active testing in abattoirs. As in other prion diseases, BSE has a protracted incubation period, in BSE a mean of about 5 years prior to the onset of clinical signs, and infectivity may be present in some tissues, particularly in the pre-terminal stages. However, the tissue distribution of infectivity in BSE is relatively restricted in comparison to other prion diseases such as sheep scrapie. Effective protection of public health depends on accurate case identification in the field or testing for the presence of disease in the abattoir to prevent clinically unrecognized cases entering the human food chain.

Passive surveillance for BSE has proved to be a relatively inefficient strategy for case identification and varies by country according to available skills and resources and the size of the cattle population. Active testing for BSE in abattoirs by examination of the obex region of the brain stem for disease-associated prion protein (PrP^{Sc}) has proved to be a reliable method of identifying infected animals. This has allowed more precise information on the course of the BSE epidemic, although the costs of systematic testing of cattle populations are significant.

Infectivity in prion diseases is not restricted to the central nervous system and may involve peripheral tissues, particularly in the lymphoreticular system, in which the agent replicates during the incubation period prior to neuroinvasion and clinical disease. In order to minimize human exposure to infection from preclinical or unrecognized clinical cases, many countries have introduced a ban on certain bovine tissues from entering the human food chain from apparently healthy cattle. A 'specified bovine offal' ban was introduced in the UK in 1989 and an extended list of tissues, the 'specified risk materials' in other European countries in 2000. Implementation and enforcement of these measures is essential to protect public health in countries with a significant risk of human exposure to BSE.

The original list of proscribed tissues was based on information from previous studies in sheep scrapie, but experimental pathogenesis studies have subsequently provided information on the tissue distribution of infectivity in BSE during the incubation period and in the clinical phase. Infectivity in BSE can be identified in tonsil at 10 months after challenge, in terminal ileum after 6–18 months, in the dorsal root ganglia at 32 months, with clinical onset and involvement of brain at 35 months. Many tested tissues have been negative by bioassay in mice and a restricted range of tissue by similar studies in cattle, indicating that the anatomical distribution of BSE is relatively restricted in comparison to other prion diseases such as sheep scrapie. A more extensive tissue involvement in BSE, including involvement of sciatic nerve, has been suggested by the development of more sensitive techniques for the identification of either PrP^{Sc} or infectivity.

The Origin of BSE

BSE was first identified in the UK in 1986 and epidemiological investigation indicated that the disease was a common source epidemic caused by infection in cattle feed in the form of meat and bone meal. This hypothesis has been strongly supported by the decline in the BSE epidemic in the UK about 5 years after a ban on feeding ruminant protein to cattle and the effectiveness of similar measures in other countries.

The original hypothesis was that the initial source of infection was sheep scrapie, which had been inadvertently included in cattle feed, and that sufficient levels of infection had been present to cross the 'species barrier' between sheep and cattle. Circumstantial evidence of a change in the production methods for meat and bone meal in the 1970s provided an explanation for the timing of the initial cases and the subsequent extensive epidemic was attributed to subsequent recycling of infected cattle tissues to cattle. An alternative hypothesis for the origin of BSE is that this was due to the development of spontaneous disease in a single animal, which was used in the production of meat and bone meal and recycling of infection within the cattle population resulted in an epidemic.

The true origin of BSE is unknown and will probably never be established with certainty. However, any hypothesis must be consistent with the origin of BSE in the UK rather than any other country. The UK had a large sheep population, a high incidence of scrapie, and a practice of feeding meat and bone meal to calves. If spontaneous BSE actually occurs, by analogy with sporadic CJD, the probability of a spontaneous case will be proportionate to the size of the cattle population and that in the UK was smaller than some other countries such as the USA, Australia, and New Zealand, and the latter two countries are believed to be free of scrapie and BSE.

Epidemiology

The first cases of BSE in the UK probably occurred in the early 1980s and the subsequent epidemic peaked in 1992 and then declined as a result of the ban on feeding ruminant protein to ruminants, introduced in the UK in 1988 (**Figure 1**). At its peak, more than 30 000 clinical cases were identified annually by passive surveillance, although it is likely that there was underascertainment of cases, particularly in the early years of the epidemic and before the introduction of an active abattoir testing program. In addition, mathematical models suggest that at least 1 000 000 preclinically infected cattle may have entered the human food chain in the

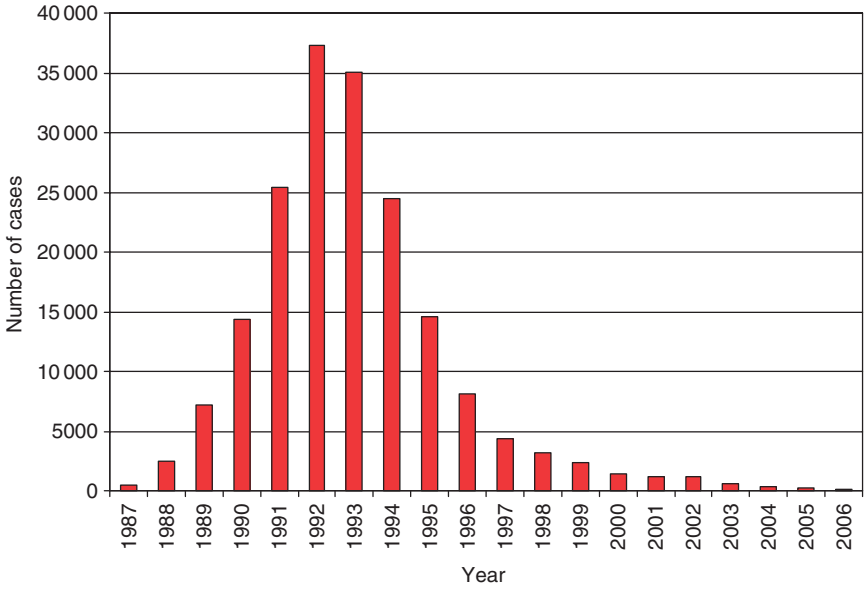


Figure 1 Annual number of cases of BSE identified in the UK. Data from OIE Website.

1980s prior to the introduction of measures to minimize human exposure to BSE.

To date, more than 40 000 affected cattle born after the introduction of the ruminant feed ban have been identified, indicating that this measure was not fully enforced. There is no good evidence of alternative vertical or lateral routes of transmission in BSE. A likely explanation for these cases is that meat and bone meal was still being fed to other species such as pigs and poultry and that cross contamination in feed mills resulted in continuing cattle exposures. A reinforced feed ban, prohibiting the feeding of meat and bone meal to any farmed species, was introduced in 1996 and only 140 cases of BSE born after this date have been identified, possibly linked to importation of animal feed. An experimental challenge study in BSE has shown that as little as 1 mg of infected brain is sufficient to cause infection by the oral route. It is of note, however, that the BSE epidemic continues to decline and in 2006 there were only 114 cases in the UK.

Live cattle and bovine products, including cattle feed, were exported from the UK in the 1980s and early 1990s and from other European countries in later years. It is likely that the risk of cattle exposure to BSE has a widespread geographical distribution. BSE was identified in Ireland in 1989, in Portugal and Switzerland in 1990, in France in 1991, and has subsequently been identified in all original member states of the European Union (EU). A ban on feeding ruminant protein to ruminants was introduced in the EU in 1994 and an SRM ban in 2000, although some countries introduced these measures earlier. In contrast to the UK, the passive surveillance system appears to have been relatively inefficient in identifying cases of BSE in most countries (with the exception of Switzerland), and it was with the introduction of a mandatory abattoir testing program in 2000–01 that some countries first identified indigenous BSE (e.g., Denmark, Germany, Italy, and Spain), with resulting extensive public concern. It is likely that cases of BSE may not have been identified in preceding years and the true size of BSE outbreaks in some counties, although limited in relation to the size of the UK epidemic, is unknown. In recent years, there has been a decline in the number of cases in almost all European countries (**Figure 2**), underlining the importance of introducing and enforcing measures to prevent the recycling of infection within cattle populations.

Cases of BSE have been found in small numbers in non-European countries, including Canada (9), Israel (1), Japan (31), and the USA (2, including 1 that originated in Canada). Despite the limited numbers of these cases, their identification has had important implications for trade. The possibility that risk of BSE may have been widely disseminated has resulted in a recommendation that all countries carry out a risk assessment, taking into account the possibility of importation of relevant risk materials and the possibility of recycling infection within cattle populations. Active abattoir testing for BSE can be an efficient means of identifying cases of BSE, but the precise populations to be tested, that is, normal slaughter, fallen stock, casualty animals, etc., and the numbers of required tests in specific populations are controversial. Over 10 000 000 cattle are currently tested per annum in the EU at a cost of 45 euros per test, with 561 positives in 2005.

New and atypical forms of BSE have been identified through the active testing programs, Cases of a novel form of BSE defined by a differential neuropathology and biochemical prion protein characteristics, bovine

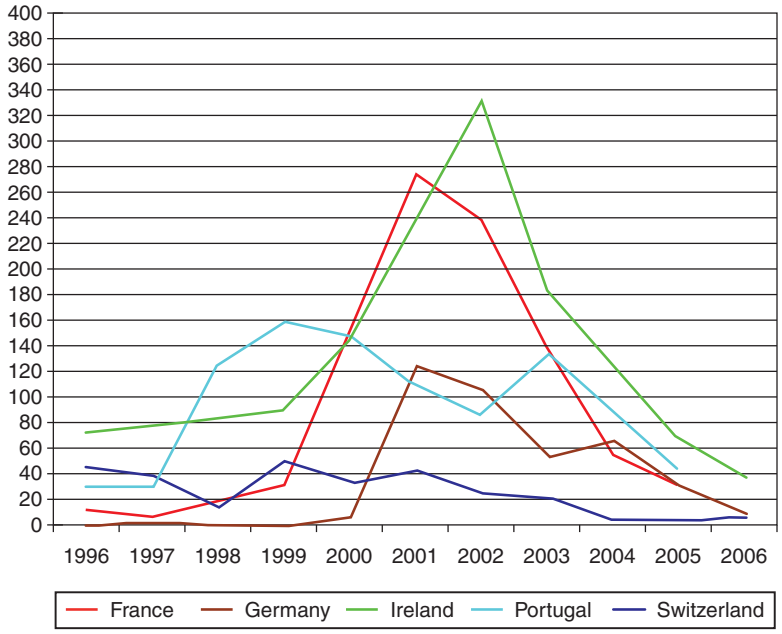


Figure 2 Annual number of cases of BSE identified in some European countries. Data from OIE Website.

amyloidotic spongiform encephalopathy, were first recognized in Italy, and subsequently a small number of cattle with brain prion protein characteristics different from both the Italian cases and BSE itself have been found, mainly in Europe. The total number of atypical BSE cases identified is currently18 and the majority are in the older age groups (>10 years) and most had no clinical signs. The origin of these cases is unknown (curiously no cases have yet been identified in the UK) and the implications for human health, if any, are uncertain.

Variant CJD

Clinical and Subclinical Infection

vCJD presents clinically with psychiatric symptoms, including depression and withdrawal, followed after a mean of 6 months by progressive ataxia and cognitive impairment, associated with involuntary limb movements. The mean survival is 14 months. vCJD affects younger age groups than in sporadic Creutzfeldt–Jakob disease (sCJD) with a mean age at death of 29 years (range 16–74 years), and it has been proposed that this may be related to either age-related susceptibility or variation in dietary exposure by age. All tested clinical cases have been methionine homozygotes at codon 129 of the human prion protein gene (*PRNP*).

The pathogenesis of vCJD is distinct from other human prion diseases as there is evidence of significant involvement of peripheral tissues and in particular the lymphoreticular system, including lymph nodes, spleen, appendix, and tonsil, in addition to the central nervous system. Infectivity is also present in peripheral nerves and large intestine and PrP^{Sc} in enteric plexus, adrenal, ileum, and skeletal muscle. Infectivity may be present in some peripheral tissues during the incubation period and act as a source of potential secondary iatrogenic infection, for example, through blood transfusion.

The prevalence of sub- or preclinical infection has not been established with certainty in any population, but anonymized screening of appendectomy and tonsillectomy specimens in the UK has led to estimates that there may be a minimum prevalence of infection of 237 per million, translating to about 4000 individuals in the age group 10–30 years who are currently infected, taking account of the age distribution of those from whom specimens were sourced. Two out of the three positive appendix specimens were analyzed for codon 129 genotype and both were valine homozygotes, suggesting that individuals with this genetic background may be susceptible to infection with BSE.

The Origin of vCJD

The hypothesis of a causal link between BSE and vCJD is supported by a range of evidence. The clinical and pathological phenotypes are remarkably consistent and distinct from previous experience. The characteristic neuropathological findings, including widespread deposition of florid plaques of PrP^{Sc}, have not been recognized previously in human prion disease, and review of archive tissues in a number of countries has failed to identify any case with the typical pathological phenotype prior to the identification of vCJD in the UK. Retrospective review of deaths certified under a range of rubrics and review of

neuropathology in a limited number of these cases have failed to identify past cases of unrecognized vCJD. This evidence strongly suggests that vCJD is a new disease.

Laboratory studies have demonstrated that the infectious agent in vCJD is almost identical to the BSE agent in terms of incubation period and brain lesion distribution in experiments carried out on wild-type and transgenic mice. The biochemical characteristics of the PrP^{Sc} deposited in the brain in vCJD are similar to BSE and distinct from other human prion diseases. Macaque monkeys inoculated experimentally with BSE develop florid plaques similar to those in vCJD. These studies indicate that the BSE agent is the cause of vCJD.

It has been proposed that BSE-infected humans through past dietary exposure, probably to high-titer bovine tissues and, in particular, spinal cord, dorsal root ganglia, and products containing mechanically recovered meat. Direct evidence of this hypothesis is lacking, not least because of the difficulties in investigating exposures that may have taken place years or even decades in the past. Furthermore, details of dietary history are necessarily obtained from surrogate witnesses because of the cognitive impairment that develops in vCJD. A case-control study comparing dietary exposures in cases of vCJD and age-matched population controls is consistent with increased risk through past oral intake of food products likely to have contained high levels of BSE infectivity, but the potential biases in this study compromise any firm conclusions. It is however of note that the mortality rate of vCJD is approximately double in the north when compared to the south of the UK, and this may reflect regional differences in past dietary exposures. Neither descriptive analyses nor case-control studies have provided evidence of any plausible alternative route of BSE exposure in vCJD cases, including past occupation or previous surgery.

The occurrence of a novel form of human prion disease, vCJD, in a country with a potentially new risk factor, BSE, first raised the possibility that these conditions were linked. Importantly, data from a harmonized system for surveillance of CJD in Europe indicated, in 1996, at the time vCJD was first found in the UK, that similar cases had not been identified in other countries. Improved efficiency of surveillance in the UK was therefore unlikely to explain the identification of this new disease. Subsequently, cases of vCJD have been found in other countries, but the fact that some of the cases occurred in countries with a very limited risk of exposure to indigenous BSE and had a history of residence in the UK during the time of maximal human exposure to BSE supports the concept that BSE is indeed the cause of vCJD.

Epidemiology

Up to February 2007, 165 cases of vCJD have been identified in the UK, all but three of which are presumed to be related to past dietary exposure to BSE. The annual number of deaths from vCJD in the UK peaked in 2000 with 28 cases and has subsequently declined to 5 deaths in both 2005 and 2006 (**Figure 3**). Fears of a large epidemic have receded, but there remains the possibility of further outbreaks of cases related to BSE infection in individuals with a heterozygous or valine homozygous genotype at codon 129 of *PRNP* and it is possible that such cases may occur with an extended incubation period and perhaps with a different clinical and pathological phenotype. It is also likely, by analogy with other human prion diseases such as kuru, that there will be an extended tail to the epidemic with a low annual number of deaths for years or even decades.

Cases of vCJD have been found in a number of other countries, mainly, but not exclusively, in Europe (**Table 1**). To date, 21 cases have been identified in France with a peak in annual deaths some 5 years later than in the UK, consistent with a mathematical model which attributes the French cases to exposure to exports of BSE-infected materials from the UK rather than to indigenous BSE.

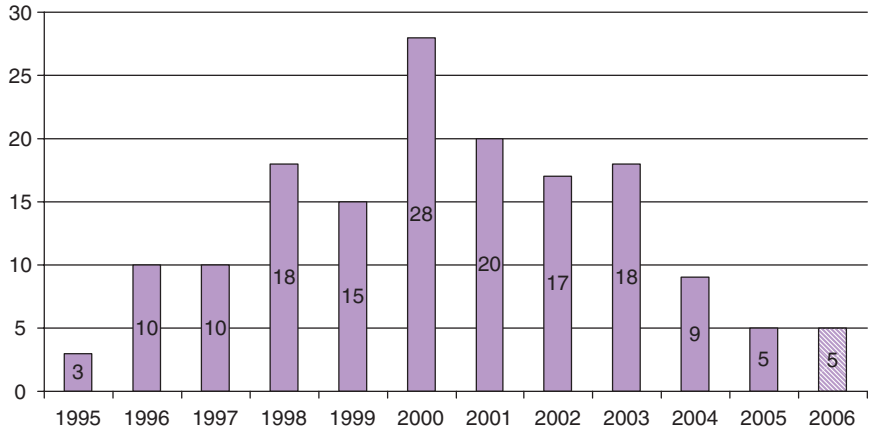


Figure 3 Number of vCJD deaths per annum (UK).

Table 1 Number of vCJD cases per country (Feb. 2007)

Country	*Total number of primary cases (number alive)*	*Total number of secondary cases: blood transfusion (number alive)*	*Residence in UK >6 months during period 1980–96*
UK	162 (6)	3 (1)	165
France	21 (1)		1
Rep. of Ireland	4 (1)		2
Italy	1 (0)		0
USA	3[a] (0)		2
Canada	1 (0)		1
Saudi Arabia	1 (1)		0
Japan	1[b] (0)		0
Netherlands	2 (0)		0
Portugal	1 (1)		0
Spain	1 (0)		0

[a]The third US patient with vCJD was born and raised in Saudi Arabia and has lived permanently in the United States since late 2005. According to the US case report, the patient was most likely infected as a child when living in Saudi Arabia.
[b]The case from Japan had resided in the UK for 24 days in the period 1980–96.

Cases of vCJD are attributed by country according to the country of normal residence at the time of the onset of clinical symptoms. This does not necessarily correlate with the country in which exposure to BSE took place and it is of note that 2/3 US cases, 2/4 Irish cases, and the single Canadian case all had a history of extended residence in the UK during 1900–96 and were probably exposed to BSE in the UK rather than the country of attribution. The third US case had lived for most of his life in the country of origin, Saudi Arabia, and a further case has been identified from this country, which is not known to have BSE. One possibility is that human BSE exposure was related to exports from the UK, a matter of concern as exports from the UK and other European countries were distributed worldwide. The single case of vCJD in Japan could be related to this factor, although the individual had also spent a short period of time in the UK. It is of note that, although Italy did import bovine material from the UK and has indigenous BSE, only a single Italian case of vCJD has been identified and that was in 2001, with no new cases for 6 years. Although measures to minimize human exposure to high-titer bovine tissues were introduced in continental Europe more than 10 years after the UK, it is likely that the total number of vCJD cases in other countries will be significantly less than in the UK.

Concerns about the public health implications of BSE and vCJD have increased with the identification of transfusion transmission of vCJD. Four recipients of non-leucodepleted red cells, donated by individuals who later developed vCJD, have either developed vCJD ($n = 3$) or have become sub- or preclinically infected ($n = 1$). In the latter case, an individual who died of an intercurrent illness was found to have immunostaining for PrP^{Sc} in spleen and one lymph node. The three clinical cases were methionine homozygotes and the preclinical case a heterozygote at codon 129 of *PRNP*, thus indicating that methionione homozygotes are not the only ones susceptible to secondary infection. In all four instances of transmission of infection by blood transfusion, the donation had been given months to years prior to clinical onset in the donor, indicating that infection is present in blood during the incubation period. The three clinical cases developed symptoms between 6 and ~9 years after transfusion, and it is of note that the four infections developed out of a total cohort of 26 individuals who survived at least 5 years after transfusion, indicating that this route is an efficient mechanism of transmitting vCJD infection from person to person. Although transfusion transmission of vCJD has only been identified in the UK, individuals with vCJD who had previously donated blood have been found in France, Spain, Ireland, and Saudi Arabia.

There is no evidence, to date, of secondary transmission of vCJD through plasma-derived products, contaminated surgical instruments, or vertically from mother to child and, although risk assessments suggest that the risks by some of these routes are limited, the period of observation is currently too short to exclude the possibility of alternative routes of transmission in the future, taking account of the potentially extended incubation periods in these diseases. A range of measures have been introduced in many countries to limit the risks of secondary transmission of vCJD, including, for example, deferral of blood donors with a history of extended residence in the UK.

See also: Viral Pathogenesis.

Further Reading

Bradley R (1998) An overview of the BSE epidemic in the UK. *Developments in Biological Standard* 93: 65–72.

Brown P, McShane LM, Zanusso G, and Detwiler L (2007) On the question of sporadic or atypical bovine spongiform encephalopathy and Creutzfeldt–Jakob disease. *Emerging Infectious Diseases* 12(12): 1816–1821.

Collee JG and Bradley R (1997) BSE: A decade on – Part 1. *Lancet* 349: 636–641.
Collee JG and Bradley R (1997) BSE: A decade on – Part 2. *Lancet* 349: 715–721.
Cousens S, Everington D, Ward HJT, Huillard J, Will RG, and Smith PG (2003) The geographical distribution of variant Creutzfeldt–Jakob disease in the UK: What can we learn from it? *Statistical Methods in Medical Research* 12: 235–246.
Hewitt PE, Llewelyn CA, Mackenzie J, and Will RG (2006) Creutzfeldt–Jakob disease and blood transfusion: Results of the UK Transfusion Medicine Epidemiology Review study. *Vox Sanguins* 91: 221–230.
Hilton DA, Ghani AC, Conyers L, *et al.* (2004) Prevalence of lymphoreticular prion protein accumulation in UK tissue samples. *Journal of Pathology* 203: 733–739.
Kimberlin RH (1996) Speculations on the origin of BSE and the epidemiology of CJD. In: Gibbs CJ, Jr. (ed.) *Bovine Spongiform Encephalopathy: The BSE Dilemma*, pp. 155–175. New York: Springer.
Valleron A-J, Boelle P-Y, Will R, and Cesbron J-Y (2001) Estimation of epidemic size and incubation time based on age characteristics of vCJD in the United Kingdom. *Science* 294: 1726–1728.
Ward HJT, Everington D, Cousens SN, *et al.* (2006) Risk factors for variant Creutzfeldt–Jakob disease: A case-control study. *Annals of Neurology* 59: 111–120.
Wells GAH, Scott AC, Johnson CT, *et al.* (1987) A novel progressive spongiform encephalopathy in cattle. *Veterinary Record* 121: 419–420.
Wilesmith JW, Ryan JBN, and Atkinson MJ (1991) Bovine spongiform encephalopathy: Epidemiological studies of the origin. *Veterinary Record* 128: 199–203.
Wilesmith JW, Wells GAH, Cranwell MP, and Ryan JB (1988) Bovine spongiform encephalopathy: Epidemiological studies. *Veterinary Record* 123: 638–644.
Will RG, Ironside JW, Zeidler M, *et al.* (1996) A new variant of Creutzfeldt–Jakob disease in the UK. *Lancet* 347: 921–925.
World Health Organisation, Food and Agricultural Organisation, Office International des Epizooties (2002) Technical Consultation on BSE: Public health, animal health, and trade.

Relevant Website

http://www.oie.int – OIE website.

Bovine Viral Diarrhea Virus

J F Ridpath, USDA, Ames, IA, USA

Published by Elsevier Ltd.

Glossary

Biotype Designation based on expression of cytopathic effect in cultured epithelial cells.
Cytopathic effect Alteration in the microscopic appearance of cultured cells following virus infection.
Genotype Group designation determined by genetic sequence comparison.
Hemorrhagic syndrome Form of severe acute bovine viral diarrhea (BVD) characterized by high morbidity and mortality as high as 50%. Clinical signs include hemorrhages throughout digestive system, high fevers, and bloody diarrhea. While similar in clinical presentation to mucosal disease it differs in that it is the result of an uncomplicated acute infection, only one biotype is present (noncytopathic) and it is not 100% fatal.
Mucosal disease (MD) Form of BVD characterized by low morbidity and 100% mortality. Clinical signs include severe and bloody diarrhea, sores in the mouth, and rapid wasting. MD occurs in animals born persistently infected with a noncytopathic BVDV that are subsequently superinfected with a cytopathic bovine viral diarrhea virus (BVDV). While similar in clinical presentation to hemorrhagic syndrome it differs in that it only occurs in persistently infected animals, two biotypes of virus are present, and it is 100% fatal.
Persistent infection Results from infection with noncytopathic virus during the first 125 days of gestation. The infected fetus develops an immune tolerance to the virus and sheds virus throughout its subsequent lifetime. Cytopathic BVDV are not able to establish persistent infections.
Species A fundamental category of taxonomic classification, ranking below a genus or subgenus and consisting of related organisms grouped by virtue of their common attributes and assigned a common name.

Introduction

Bovine viral diarrhea viruses (BVDVs) present the researcher and veterinary clinician with arguably the most complicated combination of clinical presentation, pathogenesis, and basic biology of all bovine viral pathogens (**Figure 1**). There are two distinct species of BVDV (BVDV1 and BVDV2), two distinct biotypes (cytopathic and noncytopathic), two states of infection (acute and persistent), five recognized forms of clinical acute presentation (acute BVDV infection, severe acute BVDV infection, hemorrhagic BVDV infection, acute BVDV infection/bovine respiratory disease (BRD), and acute BVDV infection/immunosuppression), and one clinical

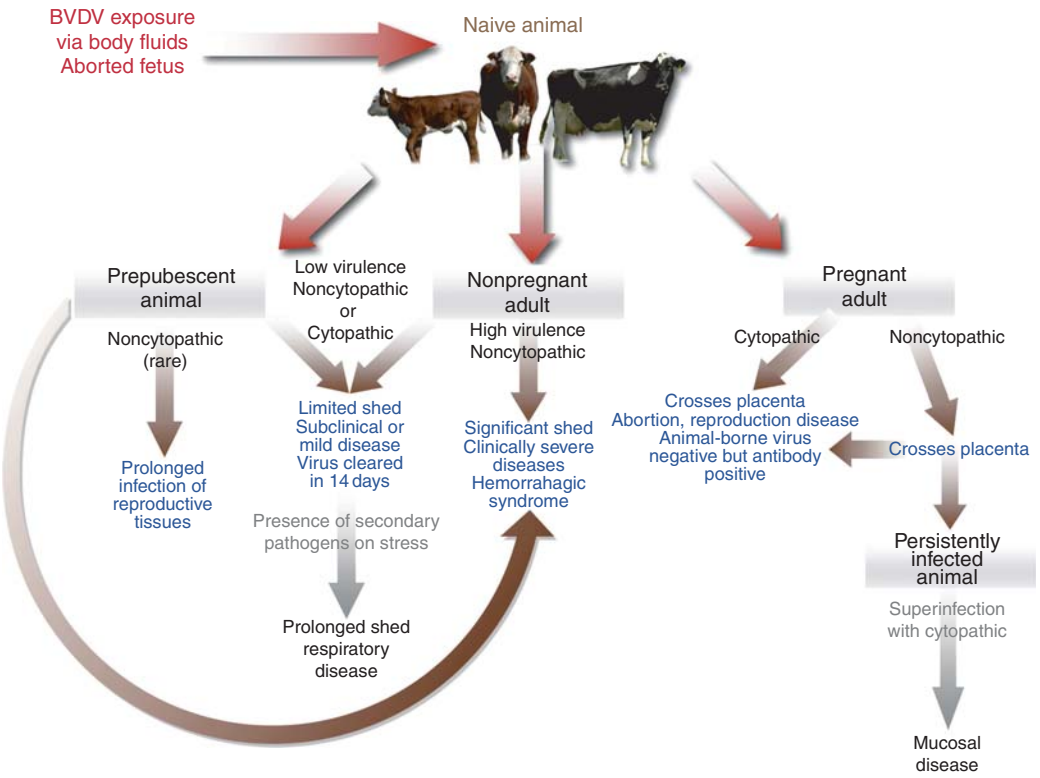


Figure 1 BVDV infections. Clinical presentation following BVDV infection is dependent on viral strain, viral biotype, viral virulence, age of host, reproductive status of host, and presence of other pathogens. Acute uncomplicated BVDV infections are always accompanied by a loss of immune tissue and circulating lymphocytes.

presentation that is the sequeale of a persistent infection with one biotype followed by an acute infection with the other biotype. While BVDV infections are most commonly associated with cattle, they also occur in a wide variety of domesticated and wild ruminants including white tail and mule deer, bison, elk, and sheep.

In the last 10 years a host of advances in BVDV research and diagnostics have led to the development of improved diagnostics and burgeoning eradication/control programs. While the economic impact of BVDV is largely due to affects of acute infections, persistently infected animals are the most frequent vector. Thus, identification and removal of persistently infected animals is key to effective control strategies.

History

First Reports of BVD and MD

Bovine viral diarrhea (BVD) was first reported as a 'new' disease of cattle observed in New York dairies in 1946. The first report, by Dr. Francis Fox of Cornell University, described a 'rinderpest like' disease characterized by leukopenia, high fever, depression, diarrhea and dehydration, anorexia, salivation, nasal discharge, gastrointestinal erosions, and hemorrhages in various tissues. In the five initial herds in which it was observed, morbidity rates ranged from 33% to 88% and mortality rates ranged from 4% to 8%. In addition, fetal abortions were observed 10 days to 3 months following infection. It was shown that this disease could be transmitted experimentally. Rinderpest virus was ruled out as a causative agent because sera from convalescent animals did not neutralize rinderpest virus and cattle that had recovered from BVD were not resistant to rinderpest virus infection.

In 1953 another disease was reported in the US. Given the name mucosal disease (MD), it was characterized by severe diarrhea, fever, anorexia, depression, profuse salivation, nasal discharge and gastrointestinal hemorrhages, erosions, and ulcers. The gut-associated lesions were similar to, but more severe, than those reported for BVD. However, unlike BVD, MD could not be transmitted experimentally. In addition, while BVD outbreaks were marked by high morbidity but low mortality, MD usually only infected a small number of animals in the herd but once contracted was invariably fatal. Based on differences in lesions, transmissibility, and morbidity/mortality rates MD and BVD were initially thought to have different causative agents.

Isolation of Virus

In 1957 Dr. James Gillespie of Cornell University isolated a noncytopathic virus as the causative agent of BVD. Three years later, in 1960, he isolated a cytopathic virus from a MD case. Cross-neutralization studies demonstrated that the viral agents associated with BVD and MD were the same and led to the realization that BVD and MD were different disease manifestations of infection with the same agent. The agent was termed bovine viral diarrhea virus (BVDV). While BVDV was identified as the causative agent, the etiology of MD, now referred in the literature as BVD-MD, remained a puzzle. In the late 1960s several research groups reported that animals succumbing to MD had persistent BVDV infections. It was also noted that persistently infected animals did not mount a serological immune response to the virus that they carried. The observations that fetal bovine serum was frequently contaminated with BVDV and that BVDV infections could be detected in newborn and one-day-old calves suggested that persistent infection might arise from *in utero* exposure.

Release of First MLV Followed by Reports of pvMD

Because cytopathic strains could be more easily detected, quantitated, and studied in tissue culture than noncytopathic strains, the discovery of cytopathic BVDV was a boon to the study of BVDV. In 1964, the first cytopathic BVDV strain discovered (Oregon C24 V) was incorporated into a modified live multivalent vaccine. Soon it was reported that subsequent to use of this vaccine a minority of animals became sick with MD-like symptoms and died. These cases were referred to as postvaccinal MD (pvMD).

Further investigation revealed that the vaccinated animals that succumbed to pvMD responded with serum antibodies to the other components of the vaccine but did not respond to the BVDV component. This suggested that the susceptibility to pvMD might be correlated with failure of the immune system to recognize BVDV. Questions raised by pvMD lead to the elucidation of the etiology of MD.

Unraveling the Etiology of MD

By the early 1970s the prevailing wisdom was that calves with persistent BVDV infections were uniformly unthrifty and usually died within the first few months of life. In a series of papers published in the late 1970s and early 1980s, Arlen McClurkin of the USDA's National Animal Disease Center (then known as the National Animal Disease Laboratory or NADL) reported persistent infection and immune tolerance in apparently healthy adult animals. Calves born to persistently infected (PI) cows were persistently infected at birth indicating maternal transmission. Further, McClurkin was able to generate persistently infected calves by exposing seronegative cows to noncytopathic BVDV between 42 and 125 days gestation. He followed the fate of the PI calves he generated and observed the following:

1. while many PI animals appeared weak and had congenital malformations, some appear apparently normal;
2. while that majority of PI animals died soon after birth, some lived to breeding age;
3. PI lines of cattle could be generated by breeding PI animals; and
4. PI animals spontaneously developed MD.

Using the McClurkin studies as a springboard, Joe Brownlie (Institute for Animal Health, Compton Laboratory, UK) and Steve Bolin (NADC/ARS/USDA) in separate but nearly concurrent studies experimentally reproduced MD in PI cattle. In the Brownlie study a cytopathic virus isolated from an animal that died from MD was inoculated into healthy PI herd mates. Both animals succumbed to MD. In the Bolin study, PI cattle were infected with noncytopathic and cytopathic strains of BVDV. Only those animals receiving cytopathic BVDV developed MD. Both studies concluded that cattle born persistently infected with a noncytopathic BVDV succumb to MD later in life when they are superinfected with a cytopathic BVDV. Follow-up studies revealed that the noncytopathic and cytopathic viruses isolated from individual MD cases were antigenically similar. At this point the origin of the cytopathic virus remained a mystery.

Discovery of the Molecular Basis for Biotype

The first two BVDV strains to be sequenced were the cytopathic strains Osloss (European origin, sequence published in 1987 by L. De Moerlooze) and NADL (North American origin, sequence published in 1988 by M. Collett). Studies of proteins associated with BVDV replication in cultured cells, done during this same time period, demonstrated that cytopathic BVDV could be distinguished from noncytopathic BVDV by the production of an extra nonstructural protein (now known as NS3). This protein is a smaller version of a nonstructural now known as NS2-3 (**Figure 2**). Comparison of the sequences of the cytopathic viruses Osloss and NADL to noncytopathic viruses from the genus *Pestivirus* revealed insertions in the region of the genome coding for the NS2-3. Subsequent studies, in which the NS2-3 coding region of cytopathic and noncytopathic viral pairs isolated from MD cases were sequenced, revealed that nearly all

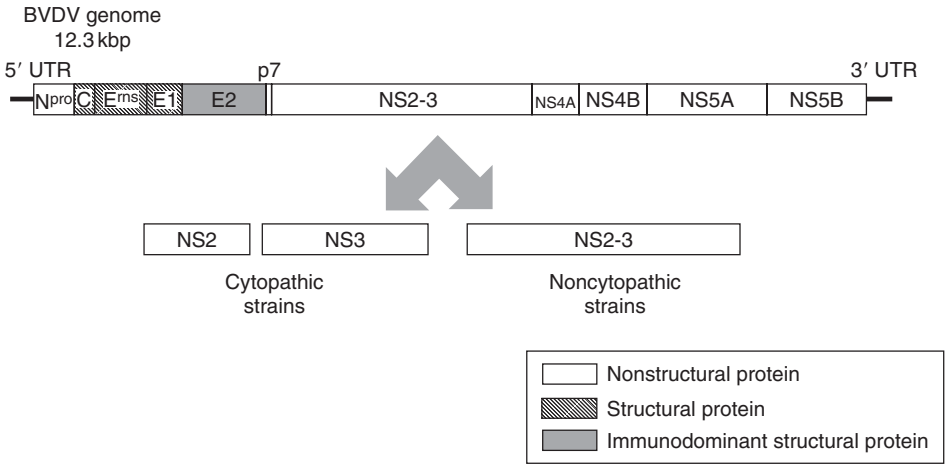


Figure 2 BVDV genome. The organization of the single-stranded RNA genome of BVDV is shown. The cleavage of the NS2-3 protein to NS2 and NS3 is observed with cytopathic, but not noncytopathic, isolates.

cytopathic viruses had host-cell genetic sequences or duplicated BVDV genetic sequences inserted into the NS2-3 region when compared to their noncytopathic counterparts. These studies suggested that cytopathic viruses arise from noncytopathic viruses by a recombinational event.

Segregation of BVDV into Two Genotypes

Studies done in the late 1980s and early 1990s, comparing vaccination cross-protection and monoclonal antibody binding, revealed antigenic variability among BVDV strains. While these studies indicated that there was considerable variation among BVDV strains, no standard means of grouping viruses based on these variations was generated. Meanwhile, based on hybridization analysis and sequence comparison, several groups evaluated the 5′ untranslated region (UTR) as a target for polymerase chain reaction (PCR) tests designed to detect the wide range of BVDV strains or to differentiate BVDV strains from other pestiviruses.

Concurrent to this research a highly virulent form of BVD, termed hemorrhagic syndrome, was reported in Canada and the US. While the first report of BVDV in 1946 described a severe acute disease, the most commonly reported form of acute uncomplicated BVD between the 1950s and late 1980s was a mild or subclinical infection. Acute BVDV infections came to be regarded as clinically unimportant and textbooks of the day stated that the transmission of the virus between healthy immunocompetent cattle was probably insignificant. The potential for infection with BVDV to result in clinical disease was downplayed and the research focused on the consequences of transplacental infections and the pathogenesis of BVD-MD. However, the recognition of hemorrhagic syndrome in the late 1980s and early 1990s brought the concept of severe acute BVD once more to the forefront. Case records of cattle admitted to the Cornell College of Veterinary Medicine for the years 1977–87 revealed that 10% of clinically acute BVD infections in adult cattle were associated with thrombocytopenia. During this period an outbreak in the state of New York resulted in 50 of 100 animals in a milking herd becoming ill and 20 of them subsequently dying. Clinical signs included high temperatures, bloody diarrhea, hemorrhages, and prolonged bleeding from venipuncture sites. This disease came to be considered as a distinct form of severe acute BVD termed hemorrhagic syndrome. Severe acute BVD cases were reported with increasing frequency in North America in the early 1990s. These outbreaks were particularly devastating in the Canadian provinces of Quebec and Ontario. The disease in some ways resembled MD, but differed in that it was transmissible to normal non-PI cattle and that only one virus, from the noncytopathic biotype, was present. In 1994, in separate studies, Charles Pellerin (Institut Armand-Frappier, University of Quebec, Canada) and Julia Ridpath (NADC/ARS/USDA) performed phylogenetic analysis of BVDV strains, isolated from animals suffering from hemorrhagic syndrome and arrived at the same conclusion. The BVDV strains associated with the outbreaks of hemorrhagic syndrome belong to a genetic group (genotype) clearly distinct from the BVDV strains commonly used, at that time, in vaccine production, diagnostic tests, and research. The newly recognized group of BVDV was designated BVDV genotype II, while the group containing the strains used in vaccines, detection, and research was termed BVDV genotype I. The names of these two genotypes were later modified to BVDV1 and BVDV2 in keeping with taxonomic

conventions in use with other viruses. It was further noted that viruses from the BVDV2 genotype were also isolated from PI calves born to dams that had been vaccinated with vaccines based on BVDV1 isolates. In 2005 the Eighth Report of the International Committee on Taxonomy of Viruses officially classified BVDV1 and BVDV2 as separate and distinct species within the genus *Pestivirus*.

In the late 1990s there was some speculation that BVDV2 strains represented newly emerging viruses that originated in the US as a result of use of vaccines by US producers and were then transferred to Europe. While BVDV2 strains were first recognized in 1994, retrospective characterization of strains collected from BVDV outbreaks in Ontario that occurred between 1981 and 1994 demonstrated that BVDV2 were present in North America at least since the early 1980s. However, the first isolate, retrospectively identified as BVDV2, described in the literature was isolated in Europe prior to 1979. Interestingly, this strain was isolated from a pig and was referred to as an atypical classical swine fever virus (CSFV).

Emergence of BVDV Reduction/Eradication Programs

In the decades after the first description of BVD and the discovery of its causative agent, systematic reduction/eradication programs were not considered. This was partially due to an underestimation of the economic impact of BVDV infections and the lack of suitable diagnostics. In the mid-1990s studies began to appear showing the sizable economic impact BVDV infections have on beef and dairy industries worldwide. Initially, veterinarians and producers assumed that vaccination alone could substantially reduce the incidence of BVDV infections. However, by the late 1990s it became apparent that four decades of vaccination had not reduced the incidence of BVDV. At this time, better diagnostics, particularly for the detection of PI animals, began to be developed. Concurrently, in the Scandinavian countries, programs designed around a strict testing and removal program for PI accompanied by movement restrictions for infected herds resulted in near eradication of BVDV in those countries by 2005. The success of these efforts encouraged other European countries and US producers to implement reduction/eradication programs. The Scandinavian programs, which were conducted in countries with a relatively low incidence of both BVD and cattle densities, eschewed the use of vaccines and were based solely on the test and removal of PI cattle. In areas of the world in which cattle densities are high and BVDV is endemic, current control programs are focused on a combination of test and removal of PI's, systematic vaccination, and consistent biosecurity.

Characteristics of BVDV

Characteristics Common to Both BVDV1 and BVDV2

All BVDV belong to the genus *Pestivirus* within the family *Flavivirus*. The BVDV virion is an enveloped, spherical particle 40–50 nm in diameter that consists of an outer lipid envelope surrounding an inner protein shell or capsid that contains the viral genome. The capsid appears as an electron-dense inner core with a diameter of approximately 30 nm. The lipid envelope that surrounds the virion is pleomorphic which impedes purification of infectious particles by banding in sucrose gradients and identification by electron microscopy. The M_r of the virion is estimated as 6.0×10^7 and the buoyant density in sucrose is 1.10–1.15 gm cm^{-3}.

The viral genome consists of a single strand of positive-sense RNA, that in the absence of insertions is about 12.3 kbp long and codes for a single open reading frame (ORF). The ORF is preceded and followed by relatively long UTRs on the 5′ and 3′ ends of the genome (**Figure 2**). Similar to other members of the genus *Pestivirus*, BVDV1 and BVDV2 viruses encode two unique proteins, N^{pro} and E^{rns}. The nonstructural protein N^{pro} is encoded at the very beginning of the ORF and is a proteinase, whose only known function is to cleave itself from the viral polypeptide. The E^{rns} is an envelope glycoprotein that possesses an intrinsic RNase activity.

All *Pestivirus* species, including BVDV1 and BVDV2, are antigenically related. However, neutralizing antibody titers found in convalescent sera are typically several-fold higher against viruses from the same species as compared to viruses from other *Pestivirus* species. Both BVDV1 and BVDV2 viruses may exist as one of two biotypes, cytopathic and noncytopathic. The noncytopathic biotype is the predominant biotype in both BVDV species. Noncytopathic viruses from both the BVDV1 and the BVDV2 species can cross the placenta and establish persistent infections. All BVDV strains, regardless of species or biotype, are lymphotrophic and acute infection always results in destruction of immune tissues. The extent of the loss of immune tissue and the accompanying immunosuppression is dependent on viral strain.

Virions are stable within a pH range of 5.7–9.3. Infectivity is not affected by freezing but decreases at temperatures above 40 °C. Like other enveloped viruses, BVDV are inactivated by organic solvents and detergents. Other methods of inactivation include trypsin treatment (0.5 mg ml^{-1}, 37 °C, 60 min), ethylenimine (reduction of 5 log10 units using 10 mM at 37 °C for 2 h), electron beam irradiation (4.9 and 2.5 kGy needed to reduce virus infectivity 1 log10 unit for frozen and liquid samples, respectively), and gamma irradiation (20–30 kGy).

Differences between BVDV1 and BVDV2

While severe disease and death loss have been reported in association with both BVDV1 and BVDV2 strains, severe acute BVD and hemorrhagic syndrome have only been reproduced experimentally with BVDV2 strains. Aside from severe acute BVDV, which is only caused by a small proportion of BVDV2 strains, it is difficult to distinguish BVDV1 infections from BVDV2 infections based on clinical signs. Further, while virulence is strain dependent, clinical presentation may also be affected by immune status, reproductive status, stress, and the presence of secondary pathogens.

BVDV1 and BVDV2 strains are antigenically distinct as demonstrated by serum neutralization using polyclonal sera and monoclonal antibody binding. The practical significance of antigenic differences is indicated by the birth of BVDV2 PI animals to dams that had been vaccinated against BVDV1 strains. While modified live BVDV1 vaccines may induce antibodies against BVDV2 strains, the titers average one log less than titers against heterologous BVDV1 strains. These observations have lead to the inclusion of both BVDV species in BVDV vaccines.

While the first studies segregating BVDV strains into two different genotypes were based on comparison of the 5′ UTR, differences between BVDV1 and BVDV2 strains are consistently found throughout the genome. Based on complete genomic sequence comparisons, the genetic sequences of BVDV1 and BVDV2 differ from each other as much as other member species of the genus *Pestivirus*, such as CSFV and border disease virus, differ from each other. The level of the genetic difference was the basis for declaring BVDV1 and BVDV2 to be two separate and distinct species.

Molecular Biology of BVDV

Viral Genome

The single-stranded RNA genome of BVDV codes for one long ORF (approximately 4000 codons in the absence of insertions). The ORF is bracketed by relatively large 5′ (360–390 bp) and 3′ (200–240 bp) UTRs. The 5′ terminus does not contain a cap structure and there is no poly(A) tract present at the 3′ end. Similar to the genomes of other pestiviruses, both BVDV1 and BVDV2 genomes terminate at the 3′ end with a short poly(C) tract. Sequence identity is highest between BVDV1 and BVDV2 strains in the 5′ UTR region. It is thought that conservation of 5′ UTR sequences is related to formation of tertiary structures required for internal ribosomal entry-mediated initiation of translation. While sequence conservation between BVDV1 and BVDV2 is high in the 5′ UTR, there are two short regions that are notable for their variability. These are located between nucleotides 208–223 and nucleotides 294–323 (nucleotide position numbers based on the sequence of BVDV1-SD-1). (Although BVDV1a-NADL and BVDV2-890 are the type virses for genotypes BVDV1 and BVDV2, respectively, both genomes have inserted sequences. Insertions can cause confusion when indicating genomic location based on nucleotide number. For this reason BVDV1a-SD-1 is used as the reference for nucleotide position. It was the first noncytopathic BVDV1 sequenced and does not have an insertion. The accession number for BVDV1a-SD-1 is M96751.) Sequence variations in these regions have been exploited in PCR-based tests designed to differentiate BVDV1 strains from BVDV2 strains.

Viral Proteins

Structural proteins

The large ORF is translated as a polyprotein. The order of the individual viral proteins within the polyprotein is as follows: N^{pro}-C-E^{rns}-E1-E2-p7-NS2/3-NS4A-NS4B-NS5A-NS5B (**Figure 2**). The polyprotein is processed co- and post-translationally by host and viral proteases. The proteins associated with the mature virion (structural proteins) are C, E^{rns}, E1, and E2. C is the virion nucleocapsid protein. E^{rns}, E1, and E2 are associated with the outer envelope of the BVDV virion. These three proteins are highly glycosylated and possess the antigenic determinants of the virus. It is not known whether the E^{rns} and E1 possesses neutralizing epitopes that are important in disease control. The E2 protein is the immunodominant structural protein and possesses neutralizing epitopes that function in disease control. Protective antibodies induced by killed vaccines are predominantly against the E2. Monoclonal antibodies (Mab's) produced against the E2 have been used to differentiate between BVDV1 and BVDV2 strains.

Nonstructural proteins

The first viral protein encoded by the BVDV ORF is the nonstructural protein, N^{pro}. This protein, as discussed above, is unique to the genus *Pestivirus*. Its only known function is to cleave itself from the polyprotein. The next nonstructural protein, p7, follows the structural protein E2 in the polyprotein. While the role of this cell-associated protein is unknown, it is hypothesized that it is required for production of infectious virus but not for RNA replication. The p7 protein is inefficiently cleaved from the E2 during processing of the polyprotein. This leads to two intracellular forms of E2 with different C termini (E2 and E2-p7). However, neither p7 or E2-p7 are found associated with infectious virus.

Following p7 is the serine protease, NS2-3. As discussed above, in BVDV strains from the cytopathic biotype the NS2-3 is cleaved to NS2 and NS3 (**Figure 2**). Both the uncleaved NS2-3 and the cleaved NS3 act as

serine proteases that cleave the remaining nonstructural proteins from the polyprotein. The function of the NS2 is unknown. It is not required for RNA replication and its cleavage from the NS2-3 does not affect serine protease activity. Purified BVDV NS3 also possesses RNA helicase and RNA-stimulated NTPase activities and all three activities (serine protease, RNA helicase, and RNA-stimulated NTPase) are essential to virus viability. While antibodies to the NS2-3 and NS3 do not neutralize infectivity, these proteins possess immunodominant epitopes. The NS2-3 and NS3 (but not the NS2), are strongly recognized by polyclonal convalescent sera and animals vaccinated with modified live vaccines have as nearly a strong antibody response to the NS2-3 and/or NS3 protein as to the E2 structural protein. In contrast, animals vaccinated with inactivated (killed) vaccines primarily react with structural proteins and not the NS2-3 or NS3. The difference in recognition of NS2-3 or NS3 may be useful in differentiating between immune responses to inactivated vaccines and immune responses to natural infection.

The NS4A and NS4B proteins are similar in size, composition, and hydrophobicity to the NS4A and NS4B proteins of other flaviviruses. NS4A acts as a cofactor for the NS2-3 and NS3 serine protease activity. NS4B and NS5A probably are replicase complex components. RNA polymerase activity has been demonstrated for the NS5B protein.

Viral Replication

Viral uptake

Uptake of virus appears to be a multistep process that occurs by endocytosis. In the initial step, the virus attaches to the cell surface through interaction of E^{rns} envelope protein and a docking glycosaminoglycan receptor molecule. The next step is mediated by attachment of the E2 envelope protein to the low-density lipoprotein receptor (LDLR) followed by internalization via endocytosis.

Release of genomic RNA, translation, and replication

The mechanism of release of genomic RNA into the cell cytoplasm is unknown but probably involves acidification of endocytic vesicles. Following release, the genomic RNA must act as mRNA, directing the translation of viral proteins. The translated viral proteins provide functions necessary for RNA replication, protein processing (protease cleavages), and protein trafficking, but are insufficient to perform all or perhaps even most of the functions required. Thus, the virus relies on host cell machinery to provide many functions required for virus replication. The most important of these host-provided functions is protein synthesis. After translation to produce viral proteins, RNA replication begins with the synthesis of complementary negative strands. It has been proposed that a secondary structure motif in the 5′ UTR enables the switch of viral RNA from a template for translation to a template for replication. Using these negative strands as templates, genome-length positive strands are synthesized by a semiconservative mechanism involving replicative intermediates and replicative forms. Because viral proteins are not detected on the surface of infected cells, it is thought that virions mature in intracellular vesicles and are released by exocytosis. A substantial fraction of the infectious virus remains cell associated.

Detection and Control

BVDV diagnostics have focused on the detection of PI animals. Virus isolation on cultured bovine cells remains the gold standard. However, due to ease and lower expense, antigen detection by either immunohistochemistry or antigen capture ELISA or nucleic acid detection by RT-PCR are gaining favor. Both killed and modified live vaccines are available for the prevention of BVD. Control by vaccination alone is compromised by the heterogeneity observed among BVDV strains, lack of complete fetal protection afforded by vaccination, and the failure to remove PI animals from cattle populations.

See also: Border Disease Virus; Classical Swine Fever Virus; Flaviviruses: General Features.

Further Reading

Dubovi ED, Brownlie J, Donis R, *et al.* (eds.) (1996) *International Symposium on Bovine Viral Diarrhea Virus: A 50 Year Review.* Ithaca, NY: Cornell University.

Goyal SM and Ridpath JF (eds.) (2005) *Bovine Viral Diarrhea Virus: Diagnosis, Management and Control.* Ames, IA: Blackwell Publishing.

Houe H, Brownlie J, and Steinar Valle P (eds.) (2005) *Bovine Virus Diarrhea Virus (BVDV) Control, Vol. 72: Preventive Veterinary Medicine.* Special Issue. Amsterdam: Elsevier Academic Press.

Smith RA (consulting ed.) and Brock KV (guest ed.) (2004) *Veterinary Clinics of North America Food animal practice. Vol. 20: Bovine Viral Diarrhea Virus: Persistence is the Key.* Philadelphia, PA: Saunders.

Thiel H-J, Collett MS, Gould EA, *et al.* (2005) Family *Flaviviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberget U, and Ball LA (eds.) *Virus Taxonomy Classification and Nomenclature of Viruses,* 8th edn., pp. 981–998. Amsterdam: Elsevier Academic Press.

Brome Mosaic Virus

X Wang and P Ahlquist, University of Wisconsin – Madison, Madison, WI, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Brome mosaic virus (BMV) is a positive-strand RNA virus that infects cereal plants, causing mosaic symptoms and stunting. BMV is the type member of the genus *Bromovirus* in the family *Bromoviridae*, which belong to the alphavirus-like superfamily of human, animal, and plant viruses. BMV has been used as a model for studying gene expression, RNA replication, host–virus interactions, recombination, and encapsidation by positive-strand RNA viruses. Results produced in these areas by many researchers have revealed insights and principles that extend beyond BMV to many other viruses and to general cellular biology. This article reviews selected aspects of this work.

Genome Structure, Expression, and Sequence

In 1971, Lane and Kaesberg used buoyant density gradients to separate BMV virions into three classes having identical capsids but different RNAs (**Figure 1**). Heavy virions contain a single copy of RNA1 (3.2 kbp), medium-density virions contain one copy each of RNA3 (2.1 kbp) and RNA4 (0.9 kbp), and light virions contain a single copy of RNA2 (2.9 kbp). Productive infections require all three virions, but not all four RNAs: infectivity is abolished by omitting RNA1, RNA2, or RNA3, but not RNA4.

Interest in characterizing the protein(s) encoded by each of the four BMV virion RNAs motivated early *in vitro* translation studies with purified BMV virion RNAs. Shih and Kaesberg found that RNAs 1, 2, and 4 are monocistronic while RNA3 is dicistronic. Although early infectivity studies showed that RNA3 encodes coat protein (CP), trypsin degradation analysis showed that the principal translation product of RNA3 was unrelated to CP, while RNA4 served as an excellent template for CP. Moreover, when mixtures of all BMV RNAs were added to wheat germ extracts in increasing amounts, RNA4 inhibited translation of genomic RNAs 1–3. These results showed that RNA4 is a subgenomic CP mRNA derived from RNA3, and implied an elegant system of gene regulation by translational competition: early in infection when viral RNA concentrations are low, all viral proteins including nonstructural RNA replication factors are translated, while after the virion RNAs are sufficiently amplified, CP is preferentially translated to encapsidate these RNAs.

By the early 1970s, RNA bacteriophage studies had provided valuable information on prokaryotic translation initiation sites, including the finding that the first AUG initiation codon usually was 100 or more nucleotides from the RNA 5′-end. In 1975, Dasgupta and Kaesberg characterized the first eukaryotic translation initiation site by isolating two fragments of BMV RNA4 that were efficiently bound by wheat germ ribosomes. RNA sequencing (a challenging, chromatography-based process at the time) revealed that the two fragments were 5′-terminal, overlapping, and encoded the first 4 and 14 amino acids of CP, respectively. The most distinguishing feature was that the initiating AUG codon for CP began only 10 nucleotides (nt) from the $m^7G^{5'}ppp^{5'}$Gp-capped RNA4 5′-end, presaging the now well-known mechanistic linkage between most eukaryotic translation initiation and 5′-mRNA caps.

The ~8.2 kbp BMV genome sequence was completed in 1984. In good agreement with *in vitro* translation results, RNA1 and RNA2 encode single proteins 1a (109 kDa) and 2a (94 kDa), respectively, the 5′-half of RNA3 encodes protein 3a (35 kDa), and the 3′-half of RNA3 encodes RNA4, the subgenomic mRNA for CP (20 kDa) (**Figure 1**). Later work discussed below showed that 3a is required for infection movement in plants. Comparisons with other emerging viral RNA and protein sequences quickly revealed that the BMV 1a and 2a proteins, already implicated in RNA replication by protoplast experiments, shared extensive amino acid sequence similarities with proteins encoded by an outwardly diverse set of plant and animal positive-strand RNA viruses. These similarities initially were recognized between BMV, alfalfa mosaic virus, tobacco mosaic virus, and Sindbis virus, and subsequently were found to extend to many other viruses now grouped together as the alphavirus-like superfamily. Similarities shared by these viruses include a polymerase domain in BMV 2a (hereafter $2a^{pol}$) and RNA capping and RNA helicase-like domains in 1a (**Figure 1**).

Infectious *In Vitro* Transcripts and Foreign Gene Expression

Also in 1984, BMV was used to produce the first infectious transcripts from cloned RNA virus cDNA. Specially designed BMV cDNA clones were transcribed to produce capped *in vitro* transcripts of genomic RNAs 1–3, each with the natural viral RNA 5′-end and only a few extra nucleotides at the 3′-end. Mixtures of all three BMV

RNA transcripts, but not their parent cDNA clones, were infectious to barley plants, a natural BMV host.

This ability to engineer the expression of infectious transcripts provided a means to manipulate the viral RNA genome at the cDNA level using recombinant DNA technology, which has subsequently proved applicable to many other RNA viruses. In one of the first applications of these new reverse genetics approaches, French and co-workers demonstrated that foreign genes could be inserted into the viral genome while retaining the ability to replicate and express genes. Using a transcribable BMV RNA3 cDNA clone, the CP open reading frame (ORF) was replaced with the ORF of the bacterial reporter gene chloramphenicol acetyltransferase (CAT). When *in vitro* transcribed and inoculated onto barley protoplasts with RNA1 and RNA2 transcripts, this RNA3 derivative was replicated and produced CAT activity at higher levels than previously achieved by DNA-based transformation. This first demonstration that RNA viruses can be engineered at the cDNA level showed that the viral RNA genome functions in a sufficiently flexible and modular fashion to tolerate even large changes such as whole gene replacements without substantial optimization, which has significant implications for virus evolution, basic research and biotechnology applications such as development of additional gene expression vectors.

In Vitro and *In Vivo* Replication Studies

Positive-strand RNA viruses like BMV replicate their genomes in a completely RNA-dependent manner, producing a negative-strand RNA replication intermediate for each genomic RNA. Studies on BMV RNA replication were greatly advanced by the development and subsequent use of such tools as *in vitro* RNA-dependent RNA polymerase (RdRp) extracts and cultured plant protoplasts. In 1979, Hall and colleagues developed a virus-specific *in vitro* RdRp extract from BMV-infected barley leaves that synthesized full-length negative-strand RNAs using added BMV virion RNAs as templates. This was the first eukaryotic *in vitro* RdRp preparation exhibiting a high level of template specificity, with other viral RNAs having less than 20% of the template activity of BMV RNAs. As noted in part below, this and similar BMV *in vitro* systems have been utilized by the groups of Hall, Quadt and Jaspars, Kao, and others to make many advances regarding promoters for positive- and negative-strand RNA synthesis, initiation mechanisms, and other issues.

In parallel to *in vitro* systems, cultured plant protoplasts provided a valuable substrate for *in vivo* replication studies due to their ability to be infected or transfected with nearly 100% efficiency with virions, virion RNAs, or *in vitro* transcripts from cloned viral cDNAs. For BMV, barley protoplast systems developed and refined by the groups of Okuno and Furusawa, Hall and others in the late 1970s have allowed studies of all aspects of BMV RNA replication, subgenomic RNA synthesis, progeny RNA encapsidation, and the like. The highly synchronized infections obtained also allowed detailed kinetic studies.

In the early 1990s, it was demonstrated that BMV also would direct RNA replication, subgenomic RNA synthesis, selective viral RNA encapsidation, RNA recombination and the like in the well-studied yeast, *Saccharomyces cerevisiae*. This host provides some of the same advantages as plant protoplasts together with rapid growth, a particularly small genome, well-characterized cell biology, and powerful classical and molecular genetic tools including genome-wide arrays of isogenic yeast strains with each gene systematically modified by deletion, GFP-tagging, etc. Yeast expressing BMV RNA replication factors 1a and $2a^{pol}$ support the replication of BMV genomic RNAs introduced by transfection or DNA-dependent transcription from plasmids or chromosomally integrated expression cassettes, duplicating nearly all features of replication in plant cells. To facilitate yeast genetic studies, BMV RNA replicons can express selectable or screenable markers in yeast and are transmitted to yeast daughter cells during cell division with 85–90% efficiency, rivaling the transmission of yeast DNA plasmids.

BMV Proteins in RNA Replication

Protoplast studies showed that BMV RNA replication and subgenomic RNA4 synthesis require the viral 1a and $2a^{pol}$ proteins but not 3a or CP. As mentioned above, 1a and $2a^{pol}$ share sequence similarity with proteins encoded by other viruses in and beyond the alphavirus-like superfamily. The conserved central domain of $2a^{pol}$ shows similarity to RdRps encoded by picornaviruses and some other RNA viruses. The N-terminal 1a protein domain is related to alphavirus protein nsp1 and contains m^7G methyltransferase and m^7GMP covalent binding activities required for capping viral RNA *in vivo*. The C-terminal 1a domain has sequence similarity to superfamily I NTPase/helicases and NTPase activity that is required for RNA template recruitment and RNA synthesis.

In plant and yeast cells, BMV RNA replication occurs on endoplasmic reticulum (ER) membranes, predominantly in the perinuclear region. In both cell types, 1a localizes to ER membranes in the absence of other viral factors. This localization depends on the N-terminal 1a domain and brings 1a to the cytoplasmic face of ER membranes as a peripheral, not transmembrane, protein. The C-terminal 1a NTPase domain recruits $2a^{pol}$ to ER membranes by interacting with $2a^{pol}$ N-proximal sequences.

In yeast cells replicating BMV RNAs, the ER membrane is modified by numerous 50–60 nm spherular invaginations into the ER lumen. Similar membrane invaginations, designated spherules, are seen in plant cells infected by any of several bromoviruses and in animal cells infected by viruses in and beyond the alphavirus-like superfamily. Immunogold labeling and electron microscopy localized 1a, $2a^{pol}$, and nascent BMV RNAs to the spherule interiors, which remain connected to the cytoplasm by a narrow neck. Protein 1a is sufficient to induce these spherules, and immunogold and biochemical studies show that each spherule contains from one to a few hundred 1a proteins and ~25-fold fewer $2a^{pol}$ proteins. These and other features imply that the structure, assembly, and operation of these spherule replication complexes have functional and perhaps evolutionary links to the replicative cores of retrovirus and dsRNA virus virions, which sequester viral RNA replication templates and their polymerases in a protein shell.

Noncoding Regions and *cis*-Signals

The first region of BMV RNAs to attract attention as a potential regulatory element was the 3′-end. Synergistic work by multiple groups showed that the 3′-noncoding regions of BMV RNAs are highly conserved, multifunctional domains that direct negative-strand RNA synthesis, contribute to RNA encapsidation, translation, and stability, and possess multiple tRNA-like features and functions.

Limited early sequence data showed that BMV RNAs 1–4 share a tRNA-like CCA_{OH} 3′-end. In 1972, Hall and colleagues showed that all four BMV RNAs were selectively aminoacylated *in vitro* with tyrosine. Their further studies showed that tyrosylated BMV RNAs interacted with translation elongation factor 1a and that BMV RNAs were tyrosylated *in vivo* during infection of barley protoplasts. BMV RNA 3′-ends were also found to interact with (ATP, CTP):tRNA nucleotidyl transferase, which can add 3′-CCA_{OH} ends to mature or maintain incomplete BMV RNAs, thus acting as a primitive telomerase. Beginning in the 1970s with work by Dasgupta and Kaesberg, further sequencing, enzymatic structure probing, and three-dimensional computer modeling showed that the 3′ ~200 nt of all BMV RNAs were strongly conserved and folded into an extended, tRNA-like structure with at least two alternate forms that differed in pairing nt near the 3′ with local or distal partners. Similarly conserved, highly structured 3′-regions with related alternate forms were also found in other members of the family *Bromoviridae*.

In vitro and *in vivo* studies using RNA fragments and mutations showed that sequences within the 3′-terminal tRNA-like structure direct negative-strand RNA initiation for RNA replication. Recent results have also implicated the tRNA-like sequence in translation and encapsidation (see below). Involvement in all of these functions led to early and continuing suggestions that the 3′-region mediates co-regulation of the varied uses of BMV positive-strand RNAs to minimize conflicts between multiple essential processes.

A second class of elements combining tRNA-like features, replication signals, and possible interaction with translation are the BMV template recruitment elements, first recognized in RNA3. Deletion analysis revealed that, in addition to 3′- and 5′-sequences required for negative- and positive-strand RNA initiation, BMV RNA3 replication *in vivo* requires a segment of ~150 nt in the 5′-half of the intergenic noncoding region between the 3a and CP ORFs (**Figure 1**). Subsequent studies

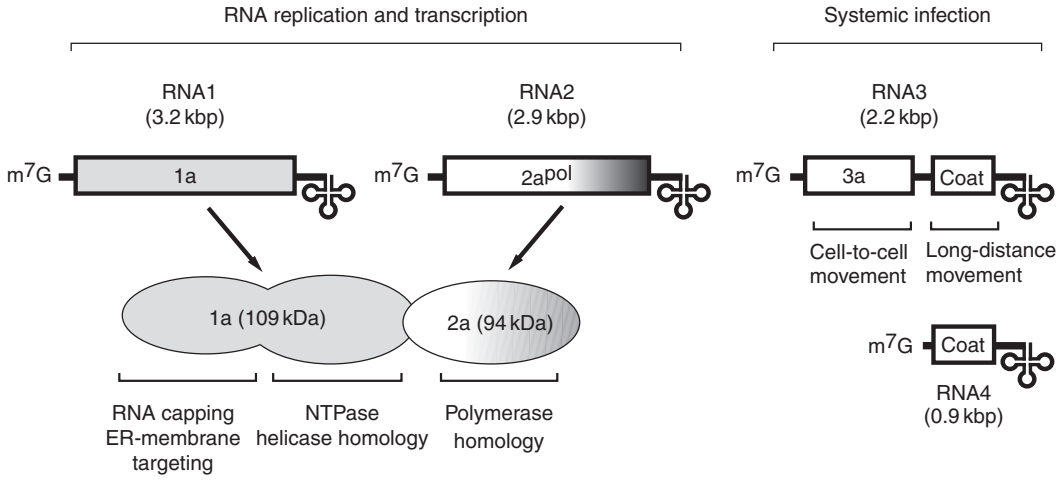


Figure 1 Organization of the brome mosaic virus (BMV) RNA genome. BMV has three genomic RNAs, RNA1, RNA2, and RNA3, and a subgenomic mRNA, RNA4, encoded by the 3′-portion of RNA3 as shown. Each of these four BMV positive-strand RNAs bears a 5′ m^7G cap and 3′ tRNA-like structure (cloverleaf). Open boxes represent the ORFs for the viral proteins 1a and $2a^{pol}$, which are required for RNA replication, and 3a and CP, which are required for systemic movement in host plants. Some specific functions and features of each viral protein are listed.

showed that this region is required for a step prior to negative-strand RNA synthesis, and is necessary and sufficient for 1a to recognize and recruit an RNA to a membrane-associated, translationally inaccessible, nuclease-resistant state that appears to be the spherule interior. Structure probing studies show that this intergenic RNA3 sequence folds into a long, bulged stem loop, which presents at its apex the invariant sequence and structure of tRNA TΨC stem loops. In plant and yeast cells, the appropriate BMV residues in this consensus are modified to T and Ψ, showing that, like the 3′-end, this sequence interacts *in vivo* with tRNA-specific enzymes. Moreover, any mutations to this TΨC stemloop mimicry abolish 1a-mediated template recruitment. Similar stem loops with apical TΨC stem loop regions are found at the extreme 5′-ends of BMV RNA1 and RNA2, where they similarly direct 1a-mediated template recruitment.

As one important aspect of *cis*-signals in BMV replication, the Kao group in particular has defined and dissected minimal core promoters for negative-strand, positive-strand, and subgenomic RNA synthesis, using a variety of approaches. Among other results, their mutational studies imply that the BMV RdRp–promoter interaction has the characteristics of an induced fit, wherein the RdRp has some tolerance to adjust its binding to a range of promoter variants as long as some key sequence features remain. This model potentially reconciles the specificity of BMV RNA synthesis with the ability of the RdRp to synthesize different forms of viral RNA from separate promoters with distinct primary sequences and secondary structures. Kao and associates further found that DNA or DNA/RNA hybrid templates containing the key BMV promoter sequences can be recognized *in vitro* by BMV RdRp extracts and copied into RNA. Although the efficiency of copying DNA templates is ~15-fold lower than for BMV RNA templates, these results have significant potential implications for virus evolution.

Subgenomic mRNA Synthesis

Early observations that the nature of CP in BMV infections is dictated by RNA3 rather than RNA4, and that RNA4 was regenerated when omitted from BMV inoculum, were partially explained when sequencing revealed that RNA4 was encoded by the 3′-portion of RNA3. Nevertheless, whether RNA4 was produced from RNA3 by cleavage or any of several possible RNA synthesis pathways remained uncertain. In 1984, Miller, Hall, and colleagues showed that a BMV RdRp extract produced subgenomic RNA4 *in vitro* when supplied with negative-strand RNA3 templates, and that the product RNA4 could be labeled by γ-^{32}P-GTP incorporation, demonstrating *de novo* initiation. This first elucidation of a pathway for subgenomic mRNA synthesis appears to provide a meaningful precedent for similar subgenomic mRNA synthesis by many positive-strand RNA viruses, and an important foundation for understanding the diversity of alternate mechanisms that has begun to emerge with the demonstration of distinctly different subgenomic mRNA synthesis pathways used by coronaviruses, nodaviruses, and some other positive-strand RNA viruses.

In vivo and *in vitro* analyses of the BMV subgenomic mRNA promoter have complemented well to reveal a core promoter within the 20 nt immediately upstream of the RNA4 start site, which directs low level but accurate initiation of subgenomic mRNA. *In vivo*, the activity of this core promoter is greatly enhanced by upstream sequences that include an oligo(A) tract of variable, ~16–22 nt length in the viral population as well as upstream, partially conserved repeats of core promoter sequences. The important role of the oligo(A) tract suggests that while negative-strand ssRNA can serve as a subgenomic mRNA template *in vitro*, the natural *in vivo* template might be a dsRNA within which the oligo(A) provides a melting site to facilitate internal initiation.

Host Factors in RNA Replication

As for many other viruses, the small size of the 8.2 kbp BMV genome relative to the complexity of BMV replication suggests that many, if not most, steps in BMV RNA replication involve contributions from host factors. Since most of the viral genome is devoted to RNA replication functions, host factors appear particularly likely to be involved in that process as well. Biochemical and genetic results support these ideas and have begun to elucidate some of the relevant host factors and contributions. In 1990, for example, Quadt and Jaspars used anti-1a antiserum to precipitate a complex of BMV 1a, 2a^{pol}, and approximately five host proteins from an active BMV RdRp extract from barley cells. Subsequently, the 41 kDa subunit of cellular translation initiation factor-3 (eIF-3) was found tightly associated with and capable of stimulating BMV RdRp activity.

The ability of BMV to direct RNA replication in yeast further facilitated analysis of host contributions to these processes. Classical yeast genetic approaches identified a number of host genes important to BMV RNA replication, such as *DED1*, a translation factor helicase involved in selective regulation of 2a^{pol} translation; multiple components of the *LSM1–7/PAT1/DHH1* complex, which facilitates recruitment of BMV genomic RNA templates into translation and from translation into RNA replication; *YDJ1*, encoding a chaperone required to activate the RNA replication complex, likely through action on 2a^{pol}; and *OLE1*, encoding a fatty acid desaturase required to produce a membrane lipid profile compatible with membrane-associated viral RNA replication. More

recently, systematic screening of a genome-wide array of yeast knockout strains identified nearly 100 host genes that, when deleted, inhibited or enhanced BMV RNA replication by 3- to >25-fold. These include host genes in RNA, protein, or membrane modification pathways and genes of unknown function, which are shedding further light on BMV replication, virus–host interactions, and cell processes.

RNA Recombination

In 1986, Bujarski and Kaesberg used BMV to provide an early demonstration of RNA recombination in a plant virus. Subsequent work by the Bujarski group and others demonstrated many forms and features of inter- and intramolecular, homologous, and nonhomologous RNA recombination in BMV. Among other findings, Bujarski and collaborators showed that mutations in BMV RNA replication factors 1a and $2a^{pol}$ could alter the frequencies and distributions of crossover sites, implying that at least a significant portion of such recombination was a byproduct of RNA replication, as by template switching. These and other results show that RNA recombination is a major force for repairing BMV genomes damaged by the high mutation rates of viral RNA replication and other events, thereby contributing to BMV survival and adaptability.

Virion Structure and RNA Encapsidation

BMV forms nonenveloped virions ~28 nm in diameter. The outer capsid is composed of 180 copies of CP arranged with $T = 3$ quasi-icosahedral symmetry. Cryo-electron microscope reconstructions by Baker and collaborators and subsequently X-ray crystallography by McPherson and co-workers showed that the BMV capsid structure is extremely similar to that of the related bromovirus, cowpea chlorotic mottle virus (CCMV), whose capsid structure was solved by Johnson and colleagues in 1995. Intriguingly, some features of these capsids are dissimilar from other known isometric RNA virus capsids, but similar to capsids of the DNA-containing papovaviruses. These features include orientation of the core β-barrel nearly perpendicular to the capsid surface to form distinctively prominent hexameric and pentameric capsomeres, and linking of adjacent capsomere clusters by exchange of invading C-terminal arms. These shared features suggest that BMV CP and the CPs of polyoma- and papillomaviruses likely share a common ancestor. BMV virion RNA is arranged as an interior subshell inside the capsid, leaving a hollow virion center. The N-terminal 26 amino acids of the CP, which are highly basic and required for RNA packaging, interact with the RNA to neutralize its charge.

In addition to the predominant 180-subunit capsid, BMV CP can also assemble *in vivo* into a 120-subunit capsid, composed of 60 CP dimers, first discovered in yeast and subsequently confirmed in infected plants. This assembly polymorphism is controlled *in vivo* by the RNA packaged, with BMV RNA2 packaged in 180-subunit capsids, while a small chimeric mRNA containing the CP ORF as its only BMV sequence is packaged in 120-subunit capsids. Structural features shared by 120- and 180-subunit capsids imply that a common pentamer of CP dimers is an important intermediate in BMV virion assembly.

In vitro and *in vivo* encapsidation studies by the Rao group and by Mise, Okuno, Furusawa, and colleagues identified portions of the BMV 3a coding region whose deletion blocked RNA3 encapsidation and interfered with normal co-encapsidation of RNA3 and RNA4. Studies by the Rao group also implicated the 3′ tRNA-like structure of BMV RNAs as a facilitator of encapsidation in *cis* or *trans*, possibly by nucleating CP interactions into productive assemblies such as pentamers of dimers, and showed that BMV RNA replication promotes selective encapsidation of viral RNAs, possibly by inducing coupled synthesis of viral RNA and CP in close proximity.

Infection Spread and Host Range

BMV replicates and encapsidates its RNAs in directly inoculated cells from a wide variety of plants, but has a fairly restricted host range for systemic infection of whole plants. The effective host range for BMV infection thus appears to be determined at the level of initiating or sustaining infection spread from the sites of primary infection. Work by De Jong, Mise, Okuno, Furusawa, Rao, their colleagues, and others have elucidated many features of such spread and its viral determinants.

BMV 3a and CP are dispensable for RNA replication but required for systemic spread. Disruption of the 3a gene blocks cell-to-cell movement, limiting infection to individual, directly inoculated cells. The 3a protein shares multiple properties with cell-to-cell movement proteins of other viruses, including cooperative binding to single-stranded RNA, localization to the plasmodesmatal connections, and induction of virion-containing tubules from the surface of BMV-infected protoplasts. Disruption of the CP gene stops virus spread to noninoculated leaves. Whether local cell-to-cell spread occurs in the absence of CP depends on several factors including the 3a allele and the host plant.

Exchanging genomic RNAs, individual genes, and gene segments among BMV strains and between BMV and other viruses shows that adaptation for infection spread in particular host plants depends not only on 3a

and CP but also on features of RNA1 and RNA2. Host adaptation of 3a generally exerts the predominant effects on infection spread, and only a few amino acid changes in 3a are required to extend BMV host range from monocotyledonous to dicotyledonous plants. However, changes modulating the efficiency of systemic spread also map to 1a and $2a^{pol}$. Such changes may alter systemic spread through host-specific effects on RNA replication, as by influencing the ability of the virus to replicate and spread faster than host defense responses. Alternatively, 1a and $2a^{pol}$ may possess additional functions, as for some C-terminal $2a^{pol}$ sequences that are dispensable for RNA replication but required for efficient systemic infection.

Future Perspectives

Through a variety of intrinsic features and the work of many investigators, research on BMV not only has advanced understanding of bromoviruses, but also has contributed significantly to general virology and molecular biology. Some of the challenges for the future include improved definition and analysis of distinct substeps in viral RNA synthesis including initiation, elongation, termination, and capping; better characterization at molecular, cellular, and tissue levels of the pathways and mechanisms involved in infection spread and the interplay of virus-directed processes and host defenses; and improved understanding of the linkages between different infection processes, including regulated gene expression, RNA replication, encapsidation, and spread.

See also: Alfalfa Mosaic Virus; Bromoviruses; Cucumber Mosaic Virus; *Ilarvirus*; Recombination.

Further Reading

Bujarski JJ and Kaesberg P (1986) Genetic recombination between RNA components of a multipartite plant virus. *Nature* 321: 528–531.

Dreher TW, Bujarski JJ, and Hall TC (1984) Mutant viral RNAs synthesized *in vitro* show altered aminoacylation and replicase template activities. *Nature* 311: 171–175.

French R, Janda M, and Ahlquist P (1986) Bacterial gene inserted in an engineered RNA virus: Efficient expression in monocotyledonous plant cells. *Science* 231: 1294–1297.

Hardy SF, German TL, Loesch-Fries LS, and Hall TC (1979) Highly active template-specific RNA-dependent RNA polymerase from barley leaves infected with brome mosaic virus. *Proceedings of the National Academy of Sciences, USA* 76: 4956–4960.

Johnson JE and Speir JA (1997) Quasi-equivalent viruses: A paradigm for protein assemblies. *Journal of Molecular Biology* 269: 665–675.

Lane LC and Kaesberg P (1971) Multiple genetic components in bromegrass mosaic virus. *Nature New Biology* 232: 40–43.

Lucas RW, Larson SB, and McPherson A (2002) The crystallographic structure of brome mosaic virus. *Journal of Molecular Biology* 317: 95–108.

Miller WA, Dreher TW, and Hall TC (1985) Synthesis of brome mosaic virus subgenomic RNA *in vitro* by internal initiation on (–)-sense genomic RNA. *Nature* 313: 68–70.

Nagano H, Okuno T, Mise K, and Furusawa I (1997) Deletion of the C-terminal 33 amino acids of cucumber mosaic virus movement protein enables a chimeric brome mosaic virus to move from cell to cell. *Journal of Virology* 71: 2270–2276.

O'Reilly EK and Kao CC (1998) Analysis of RNA-dependent RNA polymerase structure and function as guided by known polymerase structures and computer predictions of secondary structure. *Virology* 252: 287–303.

Rao ALN (2006) Genome packaging by spherical plant RNA viruses. *Annual Review of Phytopathology* 44: 61–87.

Schwartz M, Chen J, Janda M, Sullivan M, den Boon J, and Ahlquist P (2002) A positive-strand RNA virus replication complex parallels form and function of retrovirus capsids. *Molecular Cell* 9: 505–514.

Shih DS and Kaesberg P (1976) Translation of the RNAs of brome mosaic virus: The monocistronic nature of RNA1 and RNA2. *Journal of Molecular Biology* 103: 77–88.

Siegel R, Bellon L, Beigelman L, and Kao CC (1999) Use of DNA, RNA and chimeric templates by a viral RNA-dependent RNA polymerase: Evolutionary implications for the transition from the RNA to the DNA world. *Journal of Virology* 73: 6424–6429.

Bromoviruses

J J Bujarski, Northern Illinois University, DeKalb, IL, USA and Polish Academy of Sciences, Poznan, Poland

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The family *Bromoviridae* represents one of the most important families of plant viruses, infecting a wide range of herbaceous plants, shrubs, and trees. Several of them are responsible for major epidemics in crop plants. The *Bromoviridae* include the spherical icosahedral viruses with a tripartite positive-sense RNA genome. Since these viruses usually accumulate to a high level in the infected tissue, they have been a convenient subject of molecular studies. The type members of different genera, such as cucumber mosaic virus (CMV), brome mosaic virus (BMV), and alfalfa mosaic virus (AMV), constitute excellent molecular models for basic research on viral gene expression, RNA replication, virion assembly, and the role of cellular genes in basic virology.

The genus *Bromovirus* (**Table 1**) comprises not only the best-characterized RNA viruses of the family such as

Table 1 Main characteristics of the RNA genome in members of the genus *Bromovirus*

Species	*Particle size (nm)*	*RNA1*[a]	*RNA2*	*RNA3*	*3′end*[b]	*sgRNAs*[c]/DI RNAs[d]
BMV	26	3234	2865	2117	tRNA	2/yes
BBMV	26	3158	2799	2293	tRNA	1/yes
CCMV	26	3171	2774	2173	tRNA	1/n.a.
CYBV	26	3178	2720	2091	n.a.	1/n.a.
MYFV	25	n.a.	n.a.	n.a.	n.a.	1/n.a.
SBLV	28	3252	2898	2213	n.a.	1/n.a.

[a]Sequences of RNAs 1 through 3 are available in GenBank.
[b]For these bromoviruses tRNA-like structures were analyzed.
[c]Shows the number of identified sgRNAs.
[d]n.a. – not analyzed.

brome mosaic virus (BMV), broad bean mottle virus (BBMV), or cowpea chlorotic mottle virus (CCMV), but also melandrium yellow fleck virus (MYFV), spring beauty latent virus (SBLV), and cassia yellow blotch virus (CYBV). BMV infects cereal grains and numerous members of the Graminae, and is commonly distributed throughout the world. BBMV and CCMV infect legume species, while other bromoviruses infect selected members of several plant families. Interestingly, BMV, MYFV, and SBLV were found to infect *Arabidopsis thaliana*, a model plant host.

Bromoviruses are mostly related to cucumoviruses, the agriculturally important genus of *Bromoviridae*. Both bromo- and cucumoviruses share such properties like the molecular and genetic features of their tripartite RNA genome, the number of encoded proteins, and similar virion structure. The computer-assisted comparisons of amino acid sequences revealed similarity among their RNA replication proteins. More broadly, the replication factors share amino acid sequence similarity within the alphavirus-like replicative superfamily of positive-strand RNA viruses, which includes numerous plant and important animal/human viruses.

Phylogeny and Taxonomy of *Bromoviridae*

The current taxonomy divides the family *Bromoviridae* into five genera, named after their best-known members: *Alfamovirus* (one member, type virus: *Alfalfa mosaic virus*, AMV), *Bromovirus* (five members, type virus: *Brome mosaic virus*), *Cucumovirus* (three members, type member: *Cucumber mosaic virus*), *Ilarvirus* (16 viruses, type virus: *Tobacco streak virus*), and *Oleavirus* (one member: *Olive latent virus* 2, OLV-2). However, the evaluation of the phylogenetic relationships based on integrating information on the entire proteome of viruses of the *Bromoviridae* revealed that (1) AMV should be considered a true ilarvirus instead of forming a distinct genus *Alfamovirus*; (2) one virus, pelargonium zonate spot virus (PZSV), should probably constitute a new genus; (3) the genus *Ilarvirus* should be divided into three subgroups; and (4) the exact location of OLV-2 within the *Bromoviridae* remains unresolved.

Virion Properties and Structure

The bromovirus virions are nonenveloped, possess a $T = 3$ icosahedral symmetry, measure about 28 nm in diameter, and are composed of 180 molecules of a 20 kDa coat protein (CP) clustered into 12 pentamers and 20 hexamers. Three types of icosahedral particles of identical diameters (28 nm) and similar sedimentation coefficients (*c.* 85 S) encapsidate different RNA components: RNA1 (mol. wt. *c.* 1.1×10^6), RNA2 (mol. wt. *c.* 1.0×10^6), and RNA3 plus sgRNA4 (mol. wt. *c.* 0.75×10^6 and 0.3×10^6). The crystal structures of both BMV and CCMV have been resolved showing very similar organization (**Figure 1**). The structure of CCMV serves as a model bromovirus structure. Similar to other icosahedral viruses, its CP subunits, folded into a β-barrel core, are oriented vertically to the capsid surface and organized within both the protruding pentameric and hexameric capsomers. The interactions among hydrophobic amino acid residues stabilize the capsomers, and the hexameric subunits are further stabilized via interactions between N-terminal portions, where six short β-strands form a hexameric tubule called β-hexamer. Mutational analysis demonstrated that this structure was not required for virion formation but modulated virus spread *in planta*. In addition, the capsomers are held together by interactions through C-terminal portions that extend radially from the capsid. The C-termini are anchored between the β-barrel core and the N-proximal loop, and this interaction might be responsible for initiation of assembly of CCMV capsids.

The molecular replacement using the CCMV structure as a model revealed that distinct portions of the BMV capsids can also form the pentameric and the hexameric capsomers. The CP has the canonical β-barrel topology with extended N-terminal polypeptides where a significant fraction of the N-terminal peptides is cleaved. Overall, the virion appears to assemble loosely among the hexameric capsomers. This is likely responsible for virion swelling at neutral pH. The structure also coordinates metal (Mg^{2+}) ions. Interestingly, $T = 1$ imperfect icosahedral particles of BMV virions can be created by treatment

of $T = 3$ virus, with particles composed of a loose arrangement of reoriented pentameric capsomers.

The single-stranded viral RNA is located inside the capsid, as a separate torus-shaped subshell, where the basic N-terminal amino acids of the CP interact with the RNA and neutralize the phosphate groups. Other sites of RNA interaction localize to the internally proximal basic amino acids of the CP subunits. CCMV capsid undergoes well-studied reversible structural transitions where shifting pH from 5.0 to 7.0 causes capsid expansion. The swollen forms can be completely disassembled at 1 M salt concentration. Some CP mutations can further stabilize the capsids by a new series of bonds that are resistant to high ionic strength. Other mutations in the CP subunits show that capsid geometry is flexible and may adapt to new requirements as the virus evolves. Capsids of bromoviruses are also stabilized by metals. In CCMV, there are 180 unique metal-binding sites that coordinate five amino acids from two adjacent CP subunits. Some metals have less affinity for the binding sites than the others. The biological significance of capsid swelling is not completely understood.

The detailed knowledge about the structure of bromoviral virions has found applications in nanotechnology. The pH-dependent structural transitions of CCMV capsids provide opportunities for the reversible pH-dependent gating, useful during the regulation of size-constrained biomimetic mineralization. The interior surface of CCMV capsids can be genetically engineered to act as a ferritin surrogate that spatially constrains the formation of iron oxide nanoparticles, whereas the exterior surfaces have been functionalized for ligand presentation. The latter can be tuned by asymmetric reassembly of differentially functionalized CCMV CP subunits. The electrostatically driven adsorption behavior of CCMV on Si and amine-functionalized Si as well as the fabrication of multilayer CCMV films have been reported.

Genome Organization and Expression

High-resolution density gradients as well as gel electrophoresis under denaturing conditions reveal three classes of BMV virion particles, each encapsidating separate plus-sense components of the viral genome. One class encapsidates one copy of RNA1, another class one copy of RNA2, and the third class one copy of RNA3 and one copy of subgenomic RNA4 (**Figure 2**). All three virion

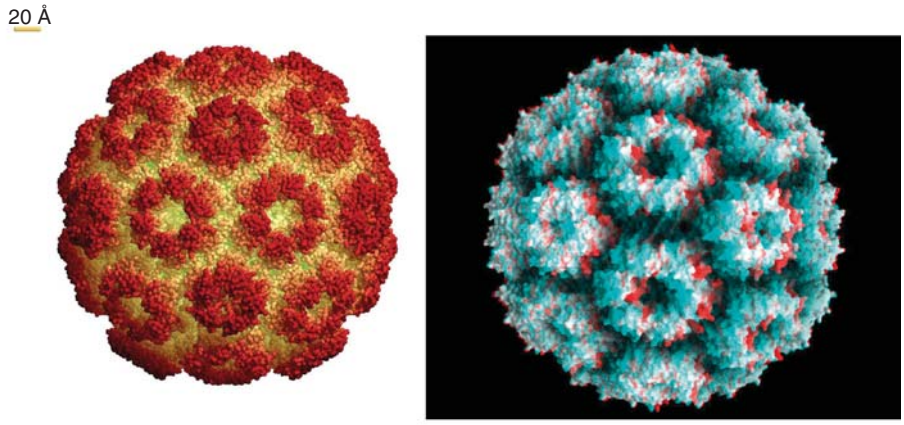


Figure 1 Surface structure of the (a) BMV and (b) CCMV capsids. The hexameric and the pentameric structural elements are visible. See also text for further description (a, b). From the virion picture collection at the web-site of the Institute for Molecular Virology at the University of Wisconsin–Madison). Reproduced by permision of Institute for Molecular Virology.

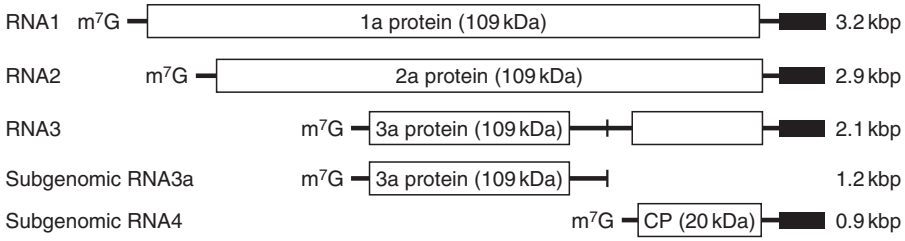


Figure 2 Molecular organization of a typical bromovirus RNA genome. The RNA components 1 and 2 encode two replicase polypeptides (1a and 2a) while RNA3 encodes the movement protein (3a) and the coat protein (CP). The open reading frames are boxed and labeled. The 3′ terminal sequences, which are common among all four RNA components, are marked as black solid boxes on the right. The oligo(A) tract is shown as a small vertical rectangle within the intercistronic region of RNA3 or at the 3′ end of sgRNA3a. The existence of sgRNA3a has been observed, so far, only for BMV.

classes must infect a cell to initiate viral infection. All BMV RNAs are 5′-capped with RNA1 and RNA2 coding for viral replicase proteins 1a and 2a, respectively, while RNA3 encodes for two proteins, the 5′ movement protein 3a and the 3′ CP. The CP is translated from the 3′ subgenomic RNA4. Recent studies reveal that 3a may be translated from another 5′ subgenomic RNA3a (sgRNA3a). *In vitro* data demonstrate that sgRNA3a is a more efficient RNA template for translation of 3a than full-length RNA3, suggesting a separation of translation (sgRNA3a and RNA4) from replication (whole RNA3) functions.

The purified bromovirus genomic RNAs are directly infectious. By using recombinant DNA technology, the *in vitro* transcripts from complete cDNA clones of RNAs 1, 2, and 3 can be synthesized, and such combined RNAs are also infectious to the bromovirus hosts. In addition, transient expression of DNA constructs carrying bromovirus cDNAs adjacent to the transcription promoter can directly induce bromovirus infection.

RNA Replication

Replication of bromovirus RNAs completely relies on RNA templates without known DNA intermediates. Both positive and negative RNA components accumulate in the infected tissue but the plus strands reach a 100-fold excess over the minus strands. In addition to whole plants, bromovirus RNAs can replicate in single cells (protoplasts) isolated from various plants (including nonhosts) or even in yeast cells. The complete BMV RNA replication cycle occurs in yeast, demonstrating the presence of compatible host factors.

Only viral proteins 1a and 2a, but not proteins 3a or CP, are required from bromovirus RNA replication cycle, as demonstrated for BMV. These proteins form the active RNA replicase complex that localize to the endoplasmic reticulum membranes called spherules. Computer-assisted comparisons reveal two large domains in protein 1a and a central domain in protein 2a that are conserved with viral proteins of other members of the alphavirus-like superfamily (**Figure 2**). Protein 2a is actual RdRp enzyme whereas 1a has both helicase and methyltransferase activities, and both function cooperatively inside the spherular structures.

Bromoviral RdRp enzyme preparations can be extracted from virus-infected plants or from yeast cells. These activities are more or less virus RNA-specific and in case of BMV, the enzyme can copy full-length bromoviral RNAs and can generate *in vitro* both sgRNAs (sgRNA4 and sgRNA3a) on (−)-RNA3 templates. The well-studied promoter of (−)-strand synthesis comprises the last 200 nt of the 3′ noncoding region in all genomic BMV RNAs. In addition, it participates in several tRNA-specific activities, including adenylation or aminoacylation of the 3′ CAA terminus. The promoters of (+)-strand synthesis have also been mapped to the 5′ proximal noncoding region in BMV RNAs.

The sgp promoter in (−)-strand of BMV RNA3 of CCMV RNA3 includes a core region that binds the RdRp complex, an upstream polyU tract, and an A/U-rich enhancer, both increasing the level of RNA4 synthesis (transcription). Apparently the sgp is responsible not only for the initiation of sgRNA4 but also for premature termination of sgRNA3a (as demonstrated recently for BMV).

The RNA encapsidation signals have been mapped for BMV RNAs. The 3′ tRNA-like structure has been shown to function as a nucleating element of CP subunits. In addition, a *cis*-acting, position-dependent 187 nt region present in the 3a open reading frame (ORF) is essential for efficient packaging of RNA3. The co-packaging of sgRNA4 is contingent upon both RNA replication and translation of CP.

Homologous and Nonhomologous Recombination of Bromoviral RNAs

Genetic RNA recombination in plant RNA viruses has been first demonstrated by noting the crossovers between BMV RNAs during infection. Both homologous and nonhomologous crossovers have been reported to occur during BMV replication cycle and the restoration of CCMV infectious virus from genomic RNA3 fragments has been reported.

Most importantly, homologous crossing-over has been demonstrated between the same BMV RNA components, and a high-frequency hot spot was observed within the sgp region in BMV RNA3. This type of crossing-over is analogous to the well-studied meiotic DNA crossing-over in DNA-based organisms.

Bromoviruses are capable of generating the defective-interfering (DI) RNAs. In particular, strains of BBMV accumulate RNA2-derived deletion variants that tend to exacerbate the severity of symptoms. The *de novo* generation of DI-like RNAs was demonstrated during serial passages of BBMV in broad bean, and the importance of sequence features for BBMV DI RNA accumulation has been demonstrated. In BMV, both replicating and nonreplicating truncated RNA2-derived artificial DI RNAs have been shown to interfere with accumulation of BMV RNAs.

Host Factors Involved in Replication of Bromoviral RNAs

Numerous results, including the role of tRNA-specific enzymes, the composition of BMV RdRp preparations, or the dependence of bromovirus infectivity on the host plant,

all suggest possible functions of cellular host factors during replication of bromovirus RNAs. More recently, the use of powerful yeast genetics has demonstrated a direct involvement of several host genes in BMV replication, including those related to RNA, protein, or membrane modification pathways. Also, some of the bromoviruses, including BMV or SBLV, have been shown to infect *Arabidopsis thaliana*, and preliminary data reveal the role of host genes in BMV replication in *Arabidopsis*.

Transmission and Virus–Host Relationship

Mechanical inoculation has been used to efficiently transmit bromoviruses from plant to plant. Also in the field, mechanical transmission by human activity can spread bromoviruses among plant hosts. In general, seed transmission has not been reported in case of bromoviruses, although there is one report of seed transmission of BBMV. Beetles can reproducibly but inefficiently transmit bromoviruses. Aphids are generally negative in transmission whereas inefficient transmission of BMV by nematodes has been reported.

Bromoviruses have restricted host range for systemic spread, with BMV generally infecting monocotyledonous plants whereas BBMV and CCMV infect dicotyledonous plants. BMV requires the CP for both the cell-to-cell and long-distance movement in barley. The interactions between BMV CP and certain host genes (e.g., an oxidoreductase) have been reported during viral infection in barley. The use of interstrain pseudorecombinants has demonstrated the involvement of RNA3 genetic information in virus spread and in symptom formation for BMV and CCMV, and during the breakage of CCMV resistance in soybean. Certain changes in RNAs 1 and 2 also confirmed their roles as determinants of systemic spread and symptom formation in BMV and CCMV. Similarly, the genome exchange experiments suggest that the adaptations of bromoviruses to their hosts rely on movement protein and on both the replicase proteins. Also, various strains of BBMV cause different symptom intensity in their host plants. However, there is no direct correlation between bromovirus yield and symptom severity, but rather the symptoms seem to be associated with changes in the CP gene or in the subgenomic promoter.

See also: Alfalfa Mosaic Virus; Brome Mosaic Virus; Cucumber Mosaic Virus; *Ilarvirus*; Recombination.

Further Reading

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses,* 2nd edn., 1162pp. San Diego, CA: Elsevier: Academic Press.

Figlerowicz M and Bujarski JJ (1998) RNA recombination in brome mosaic virus, a model plus strand RNA virus. *Acta Biochimica Polonica* 45(4): 847–868.

Johnson JE and Speir JA (1997) Quasi-equivalent viruses: A paradigm for protein assemblies. *Journal of Molecular Biology* 269: 665–675.

Noueiry AO and Ahlquist P (2003) Brome mosaic virus RNA replication: Revealing the role of the host in RNA virus replication. *Annual Review of Phytopathology* 41: 77–98.

Wooley RS and Kao CC (2004) Brome mosaic virus. Descriptions of Plant Viruses, No. 405. http://www.dpvweb.net/dpv/showdpv.php?dpvno=405 (accessed Jul. 2007).

Bunyaviruses: General Features

R M Elliott, University of St. Andrews, St. Andrews, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ambisense Coding strategy used by some bunyaviruses in which a genome segment encodes proteins in both positive and negative sense orientations. This strategy also is employed by arenaviruses.

Bunyavirus Any member of the family *Bunyaviridae*.

Cap snatching Cleavage of the capped 5′-end of a cellular mRNA to produce short oligonucleotides that are used to prime viral mRNA transcription. The cellular sequences are incorporated into the viral mRNAs.

Reassortment Exchange of genome segments during the course of a mixed infection; generates new viruses.

Reverse genetics Rescue of infectious virus from cloned cDNA copies of the viral genome.

Vector competence Ability of an arthropod to transmit a pathogen.

Introduction

Currently more than 300 mainly arthropod-transmitted viruses are classified within the family *Bunyaviridae*. The majority of these viruses are classified into one of five genera: *Orthobunyavirus*, *Hantavirus*, *Nairovirus*, *Phlebovirus*, and *Tospovirus*, though more than 40 remain unassigned. The basic features that unite these viruses are similar virion morphology, negative-sense tripartite single-stranded RNA genome, and cytoplasmic site of viral replication with intracellular maturation in the Golgi. However, considering the large number of viruses within this one taxonomic grouping, it is not surprising that considerable diversity occurs in terms of biological behavior, and in genome coding and replication strategies. In this article, genus-specific traits are indicated with the terms orthobunyavirus, hantavirus, etc., while the term bunyavirus is reserved for familial traits. Bunyaviruses in all five genera impinge on human health and well-being (**Table 1**), either directly in causing diseases ranging from encephalitis (e.g., La Crosse virus) to hemorrhagic fever (e.g., Crimean-Congo hemorrhagic fever virus), or indirectly by causing disease in animals (e.g., Cache Valley and Akabane viruses) or crop plants (e.g., tomato spotted wilt virus). Furthermore, this family contains prime examples of emerging viruses such as Sin Nombre and related hantaviruses that have been identified in the Americas since 1993.

Bunyavirus Biology

Viruses in each genus are associated with a principal arthropod vector, with the exception of hantaviruses, which have no arthropod involvement in their life cycle. Gross generalizations can be made that orthobunyaviruses are mainly transmitted by mosquitoes, nairoviruses primarily by ticks, and phleboviruses by phlebotomine flies, ticks, and, notably for Rift Valley fever virus, mosquitoes as well. Tospoviruses are plant-infecting bunyaviruses that are transmitted by thrips. In all cases, the virus can replicate efficiently in the vector without doing overt damage to it, though behavioral changes to mosquito feeding patterns have been noted with some of them. Hantaviruses cause persistent infections in rodents and usually one particular hantavirus is associated with rodents of one particular species. Humans are infected by inhaling aerosolized rodent excretions. Human-to-human transmission is rare but has been reported for Andes hantavirus.

Table 1 Selected important pathogens in the family *Bunyaviridae*

Genus/ virus	*Disease*	*Vector*	*Distribution*
Orthobunyavirus			
Akabane	Cattle: abortion and congenital defects	Midge	Africa, Asia, Australia
Cache Valley	Sheep, cattle: congenital defects	Mosquito	N. America
La Crosse	Human: encephalitis	Mosquito	N. America
Oropouche	Human: fever	Midge	S. America
Tahyna	Human: fever	Mosquito	Europe
Hantavirus			
Hantaan	Human: severe hemorrhagic fever with renal syndrome (HFRS), fatality 5–15%	Field mouse	Eastern Europe, Asia
Seoul	Human: moderate HFRS, fatality 1%	Rat	Worldwide
Puumala	Human: mild HFRS, fatality 0.1%	Bank vole	Western Europe
Sin Nombre	Human: hantavirus cardiopulmonary syndrome, fatality 50%	Deer mouse	N. America
Nairovirus			
Crimean–Congo hemorrhagic fever	Human: hemorrhagic fever, fatality 20–80%	Tick, culicoid fly	Eastern Europe, Africa, Asia
Nairobi sheep disease	Sheep, goat: fever, hemorrhagic gastroenteritis, abortion	Tick, culicoid fly, mosquito	Africa, Asia
Phlebovirus			
Rift Valley fever	Human: encephalitis, hemorrhagic fever, retinitis, fatality 1–10% Domestic ruminants: necrotic hepatitis, hemorrhage, abortion	Mosquito	Africa
Sandfly fever	Human: fever	Sandfly	Europe, Africa
Tospovirus			
Tomato-spotted wilt	Plants: over 650 species, various symptoms	Thrips	Worldwide

The maintenance and transmission cycles of orthobunyaviruses have been studied in more detail than for other bunyaviruses, and involve both horizontal and vertical transmission between a restricted number of mosquito and vertebrate hosts (**Figure 1**). The integrity of the cycles is maintained even when the geographic distributions of the different hosts overlap in nature. Horizontal transmission usually refers to transmission to susceptible vertebrate hosts via the biting arthropod, with the resulting vertebrate viremia a source for infection for another feeding arthropod. Humans are often dead end hosts in that they rarely function as a source for further vector infection. Horizontal transmission can also refer to venereal transmission between mosquitoes, and has been reported for orthobunyaviruses. Vertical transmission is a major maintenance mechanism, particularly in periods of unfavorable climatic conditions. Virus can be maintained in mosquito eggs over winter or during periods of drought for months if not years, and when conditions allow, mosquito larvae emerge infected with the virus.

Vector competence is the term used to describe the ability of a particular arthropod species to transmit a virus, and is governed by both host and viral factors. After ingestion of virus in a blood meal, the virus infects the midgut cells, and then escapes into the hemocoel to allow disseminated infection of all organs. High level replication in the salivary glands provides the viral inoculum for transmission at the next feeding occasion. For orthobunyaviruses in the California serogroup, experiments with reassortant viruses (see later) determined that the viral factors associated with efficient infection of midgut cells and subsequent transmission mapped to the medium (M) RNA genome segment.

Virion Proteins and RNA Segments

The spherical bunyavirus particle is about 100 nm in diameter and electron microscopy reveals a fringe of spikes on the surface (**Figure 2**). The virion consists of four structural proteins: two internal proteins (N and L) and two external glycoproteins, termed Gn and Gc, which are inserted in the Golgi-derived viral membrane (**Figure 3**). The N protein (2100 copies per particle) encapsidates the RNA genome segments to form ribonucleoprotein complexes termed RNPs or nucleocapsids, to which the L protein (25 copies per particle) associates. There is no equivalent of a matrix protein to stabilize the virion structure, and it is presumed that interaction between the cytoplasmic tail(s) of either or both of the glycoproteins and the N protein in the RNP is important for structural integrity of the virion.

The three genomic RNA segments, which are designated L (large), M (medium), and S (small), characteristically have complementary terminal sequences that are similar for the three segments of viruses within a genus, but differ between genera; the genus-specific consensus sequences are shown in **Table 2**. A consequence of the terminal complementarity is that the ends of the RNAs may base-pair, and circular or panhandle bunyavirus RNAs have been seen in the electron microscope. Bunyavirus nucleocapsids are also circular, and the ends of the RNA segments are base-paired within the RNP. It is probable

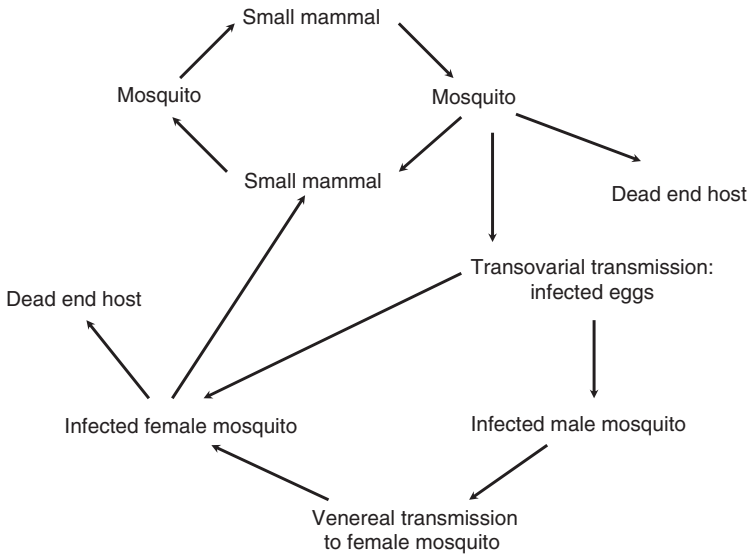
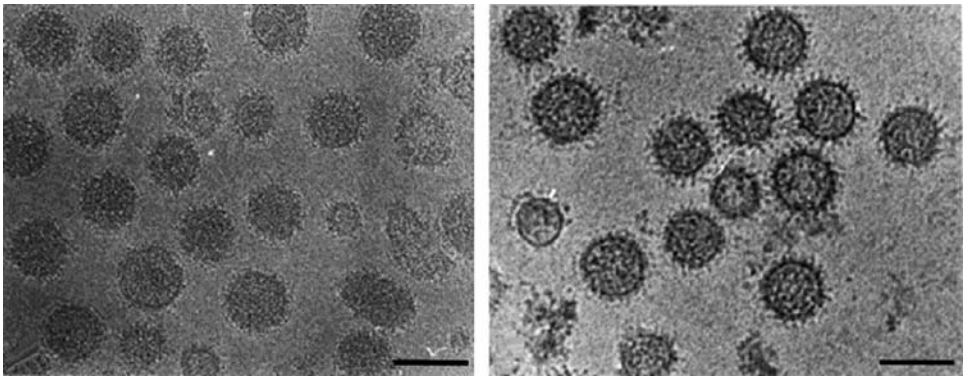


Figure 1 Life cycle of mosquito-transmitted bunyaviruses. From Elliott RM and Koul A (2004) *Bunyavirus/Mosquito Interactions*, symposium 63, pp. 91–102. Society for General Microbiology.



(a) (b)

Figure 2 Electron micrographs of vitrified-hydrated La Crosse orthobunyavirus virions. (a) Small defocus value which demonstrates the membrane bilayer; (b) large defocus value which demonstrates the glycoprotein spikes (see Elliott (1990) for further details). Scale = 100 nm. The photographs were generously provided by Dr. B. V. V. Prasad. From Elliott RM (1990) Molecular biology of *Bunyaviridae*. *Journal of General Virology* 71(Pt. 3): 501–522.

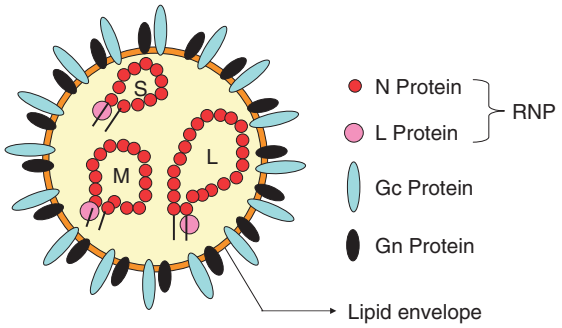


Figure 3 Schematic of bunyavirus particle.

Table 2 Consensus 3′ and 5′ terminal nucleotide sequences of bunyaviral genome RNAs

Orthobunyavirus	3′ UCAUCACAUGA. . .UCGUGUGAUGA 5′
Hantavirus	3′ AUCAUCAUCUG. . . . . .AUGAUGAU 5′
Nairovirus	3′ AGAGUUUCU. . . . . .AGAAACUCU 5′
Phlebovirus	3′ UGUGUUUC. . . . . .GAAACACA 5′
Tospovirus	3′ UCUCGUUAG. . . . . .CUAACGAGA 5′

that the encapsidation signal for the N protein is at the 5′-end of the RNA.

Coding Strategies of the Viral Genomes

Complete nucleotide sequences have been determined for at least one representative of each genus in the *Bunyaviridae* which has allowed the coding strategies of the individual genome segments to be elucidated. These are shown schematically in **Figure 4**.

The L RNA encodes the L protein using a conventional negative-strand strategy, that is, in a complementary positive-sense mRNA. The L protein contains motifs found in all RNA polymerases, and expression of recombinant L proteins, via a variety of systems, demonstrated the L had RNA synthesis activity. The L protein is therefore at least a component of the virion-associated transcriptase.

The M segment encodes in the complementary sense mRNA the virion glycoproteins in the form of a precursor polyprotein. The M segment gene products have been implicated in many biological attributes of the virus including hemagglutination, virulence, tissue tropism, neutralization, and cell fusion. By convention, the glycoprotein of greater molecular weight (or slower electrophoretic mobility in sodium dodecyl sulfate–polyacrylamide gels) was termed G1, and the smaller glycoprotein G2. However, functional analyses of glycoproteins of viruses in different genera have revealed some commonality between the glycoproteins according to whether they are encoded at the N- or C-terminal region on the polyprotein. Therefore, the glycoproteins are now referred to as Gn or Gc. In the case of orthobunyaviruses, hantaviruses, and phleboviruses, the precursor is co-translationally cleaved to yield the mature proteins, which are characteristically rich in cysteine residues.

Orthobunyaviruses encode an additional, nonstructural protein called NSm between Gn and Gc, while some phleboviruses encode an NSm protein upstream of the glycoproteins, also as part of the precursor. Only the N-terminal domain of NSm is required for orthobunyaviruses to replicate in cell cultures, while the entire NSm region of Rift Vally fever phlebovirus can be deleted without compromising replication in cultured cells. However, the function of these NSm proteins remains unknown.

Tospoviruses encode an NSm protein, using an ambisense strategy. The NSm coding region is contained in the 5′ terminal part of the viral genomic RNA, but is translated from a subgenomic mRNA. This mRNA is transcribed from the full-length complement of the

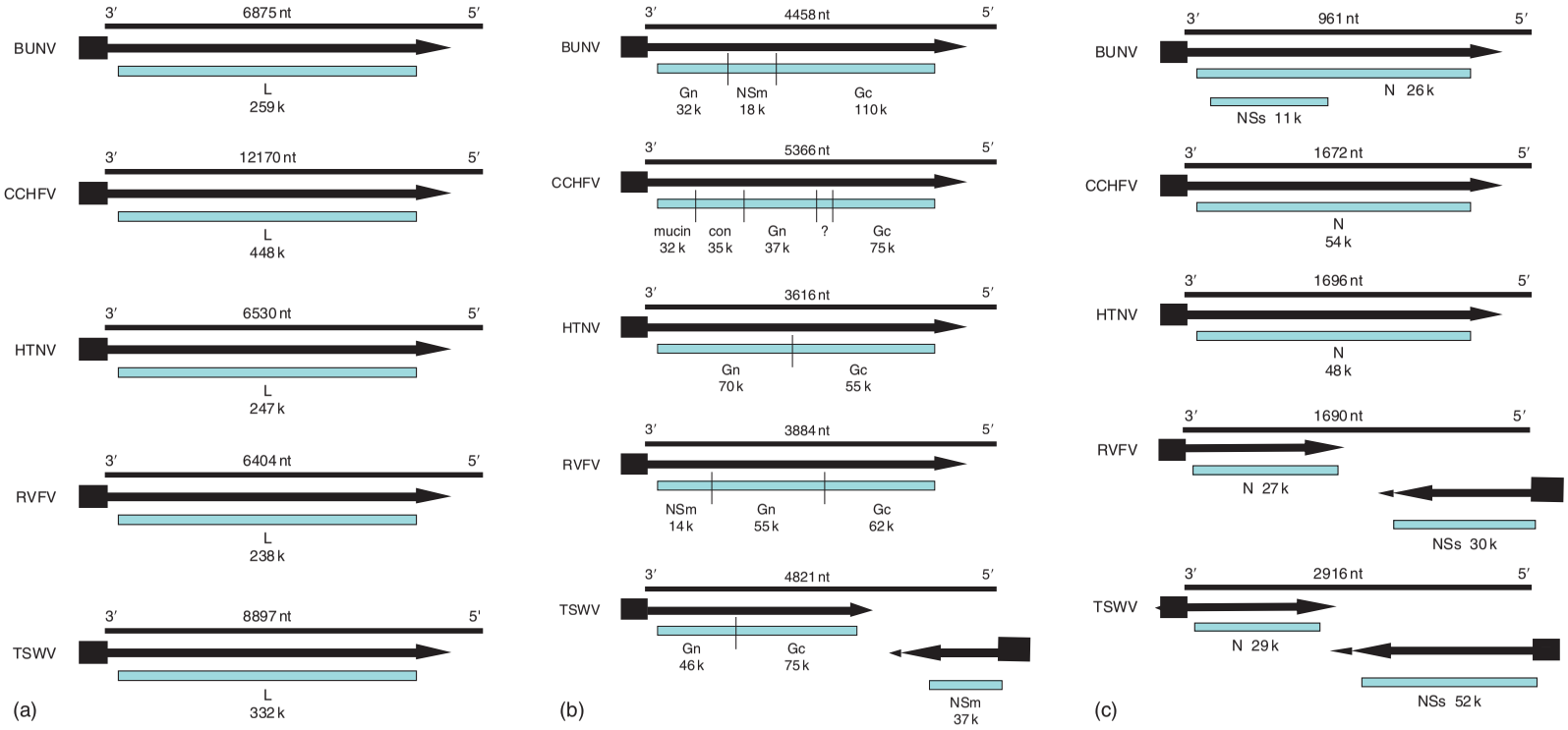


Figure 4 Coding strategies of bunyavirus genome segments. (a) L segment; (b) M segment; (c) S segment. Genomic RNAs are represented by thin lines (the length in nucleotides is given above each segment) and the mRNAs are shown as arrows (filled square indicates host-derived sequences at 5′-end). Gene products, with their apparent M_r, are represented by light colored boxes. Abbreviations: BUNV, Bunyamwera orthobunyavirus; CCHFV, Crimean-Congo hemorrhagic fever nairovirus; HTNV, Hantaan hantavirus; k, mole weight of protein 2; RVFV, Rift Valley fever phlebovirus; TSWV, tomato spotted wilt tospovirus. In (b), CCHFV M segment, mucin represents mucin-like region; con the connector region; and ? unidentified protein product.

genomic RNA. The tospovirus NSm functions as a movement protein by forming tubular structures that penetrate through plasmadesmata and allow cell-to-cell transport of viral nucleocapsids.

Processing of the nairovirus M segment-encoded polyprotein is more complex, occurs over a period of several hours and involves the production of a number of intermediate proteins. Upstream of Gn are two domains, a highly variable region at the N-terminus (resembling the mucin-like domain observed in other viral glycoproteins) followed by a second domain (connector domain), both of which are cleaved in separate events from Gn, while a further domain is cleaved from between mature Gn and Gc (**Figure 3**). The function(s) of the released domains awaits further investigation.

The NSs protein of orthobunyaviruses and phleboviruses is dispensable for replication and is regarded as an accessory virulence factor. Although these NSs proteins are of different sizes, show no obvious sequence similarity, and are encoded by different mechanisms, they appear to have a similar function in antagonizing the host interferon (IFN) response. Studies on Bunyamwera and La Crosse orthobunyaviruses, engineered by reverse genetics (see below) to ablate the NSs open reading frame, and on an attenuated variant of Rift Valley fever phlebovirus that expresses a truncated NSs protein, showed that these mutant viruses induce IFN, whereas wild-type viruses do not. Further work revealed that the NSs proteins inhibited RNA polymerase II-mediated host transcription, thus preventing synthesis of IFN mRNA.

The S RNA segments also show great diversity in their coding strategies. The bunyavirus S segment encodes two proteins, N, and a nonstructural protein, NSs, in different overlapping reading frames in the complementary-sense RNA. The two proteins are translated from the same mRNA species, the result of alternative initiation at different AUG codons. For hantaviruses and nairoviruses, a single open reading frame, encoding the N protein, is found in the S segment complementary-sense RNA. The S segments of phleboviruses and tospoviruses employ an ambisense coding strategy; the N protein is encoded in the complementary-sense RNA corresponding to the 3′ half of the genomic S segment, whereas the coding sequence for the NSs protein is contained in the 5′ half of the genomic RNA. The proteins are translated from separate subgenomic mRNAs.

Genome Segment Reassortment

In common with other viruses with segmented genomes, bunyaviruses can reassort their genome segments during co-infection (**Figure 5**), and this phenomenon has been extensively studied for orthobunyaviruses in both cell cultures and in mosquitoes. There are barriers to the extent to which reassortment occurs in that it is restricted to antigenically closely related viruses within a genus, and certain segment combinations seem favored over others. In mosquitoes, reassortment of orthobunyavirus RNA segments occurs following ingestion of two viruses in a single blood meal or in separate feedings (mimicking interrupted feeding due to the host's physical reaction to the mosquito) provided the two events are temporally close. If the time between the infections is greater than 2 or 3 days, there is resistance to infection with the second virus. Reassortant viruses were used to map viral proteins and biological functions to these proteins with individual genome segments before the advent of nucleotide sequencing. Reassortment has been detected in nature, for instance, among various orthobunyaviruses, and of significance is the discovery that Ngari (originally called Garrissa) virus, an orthobunyavirus associated with human hemorrhagic fever, is a reassortant between the relatively innocuous viruses Bunyamwera and Batai.

Viral Replication Cycle

Attachment, Entry, and Uncoating

Infection of the target cell is mediated by one or both of the virion glycoproteins interacting with the cellular receptor. Receptors have not been formally identified for any bunyavirus, though cellular entry of hantaviruses involves β1-integrins (apathogenic hantaviruses) or β3-integrins (pathogenic hantaviruses). The relative importance of either of the glycoproteins in attachment has not been fully elucidated and may differ between the genera. For orthobunyaviruses, it has been suggested that Gc is the major attachment protein for vertebrate cells, whereas Gn may contain the major determinants for attachment to mosquito cells. Neutralization and hemagglutination-inhibition sites have been mapped to both glycoproteins encoded by hantaviruses and phleboviruses, suggesting that for these viruses both Gn and Gc may be involved in attachment. In common with many other enveloped viruses bunyaviruses can fuse cells at acidic pH; at least for orthobunyaviruses, this is accompanied by a conformational change in Gc. Based on electron microscopic studies of phleboviruses, entry into cells is by endocytosis. It is probable that uncoating occurs when endosomes become acidified, thus initiating fusion of the viral membrane and endosomal membrane, followed by release of the nucleocapsids into the cytoplasm.

Transcription

The classical scheme for replication of a negative-strand RNA virus is that the infecting genome is first transcribed into mRNAs by the virion-associated RNA polymerase or transcriptase (**Figure 6**). This process, termed primary

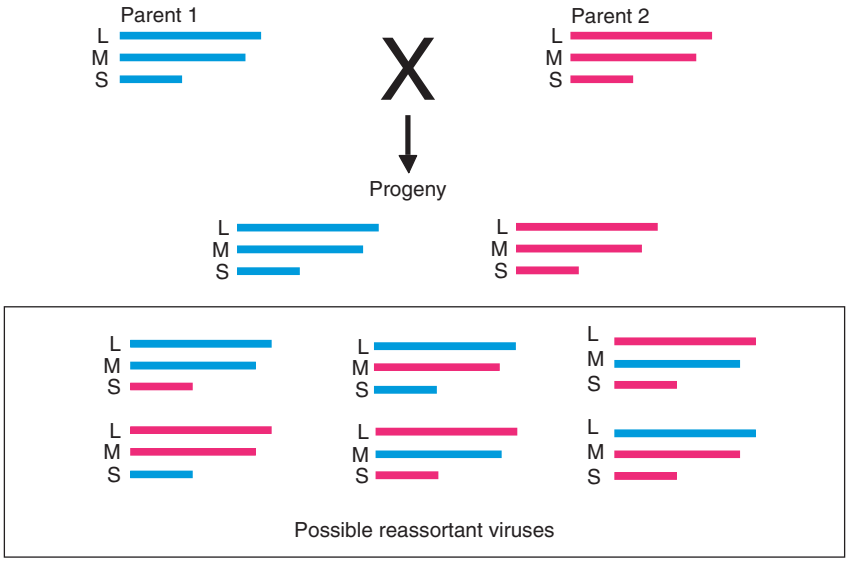


Figure 5 Generation of reassortant bunyaviruses following coinfection of the same cell. From Elliott RM and Koul A (2004) *Bunyavirus/Mosquito Interactions*, symposium 63, pp. 91–102. Society for General Microbiology.

transcription, is independent of ongoing protein synthesis. Following translation of the primary transcripts into viral proteins, the genome is replicated via a complementary full-length positive-strand RNA, the antigenome, and then further mRNA synthesis (secondary transcription) ensues. Transcriptase activity has been detected in detergent-disrupted virion preparations of representatives of most *Bunyaviridae* genera. The enzymatic activity was weak compared to, for example, the transcriptase of vesicular stomatitis virus, which has hampered extensive biochemical characterization of the enzyme. However, the bunyavirus transcriptase was shown to be stimulated by oligonucleotides of the (A)nG series, cap analogs (e.g., mGpppAm) and natural mRNAs, such as alfalfa mosaic virus RNA 4. These appeared to act as primers for transcription. Further support for this notion was provided by sequencing studies of the 5′-ends of both *in vivo* and *in vitro* synthesized mRNAs, which showed they contained an additional 10–18 nontemplated nucleotides; a cap structure was present at the 5′ terminus. *In vitro*, an endonuclease activity which specifically cleaved methylated capped mRNAs was detected. Taken together, these data indicate that bunyavirus transcription is markedly similar to that of influenza viruses in using a cap-snatch mechanism to prime transcription. In contrast to influenza viruses, bunyavirus transcription is not sensitive to actinomycin D or α-amanitin, and occurs in the cytoplasm of infected cells. The apparent reiteration of viral terminal sequences at the junction between the primer and viral sequence itself suggests that the polymerase may slip during transcription; further analysis of hantavirus RNAs suggests that this may occur during replication as well, and has been dubbed prime-and-realign.

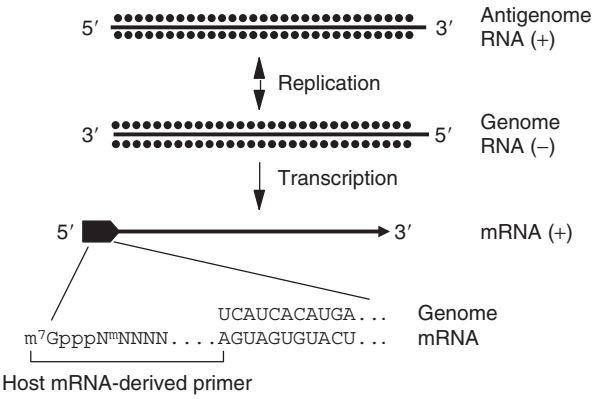


Figure 6 Transcription and replication scheme of negative-sense bunyavirus genome segments. The genome RNA and the positive-sense complementary RNA known as the antigenome RNA are only found as ribonucleoprotein complexes and are encapsidated by N protein (filled square). The mRNA species contain host-derived primer sequences at their 5′-ends (filled square) and are truncated at the 3′-end relative to the vRNA template; the mRNAs are neither polyadenylated nor encapsidated by N protein. The sequence at the 5′-end of an orthobunyavirus mRNA is shown. From Elliott RM (2005) Negative strand RNA virus replication. In: Mahy B and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections*, 10th edn., vol. 1, pp. 127–134. London: Hodder Arnold.

Analysis of bunyavirus primary transcription *in vivo*, however, appeared initially to produce results incompatible with the presence of a virion transcriptase, in that no mRNA synthesis could be detected in the presence of protein synthesis inhibitors in certain virus-cell systems. Further work showed that only short transcripts were produced in the absence of protein synthesis *in vivo*; subsequent gel electrophoresis analyses of the *in vitro*

transcriptase products showed that these too were short transcripts. If the *in vitro* reaction was supplemented with rabbit reticulocyte lysate, however, full-length RNAs were synthesized. The translational requirement was not at the level of mRNA initiation, but rather during elongation or, more precisely, to prevent the transcriptase from terminating prematurely. A model to account for these observations proposes that in the absence of ribosome binding and protein translation the nascent mRNA chain and its template can base-pair, thereby preventing progression of the transcriptase enzyme. This translational requirement is not ubiquitous, however, since concurrent translation is not needed for efficient readthrough of premature termination sites in some strains of BHK cells or in C6/36 mosquito cells. Reconstitution and mixing experiments suggest that the translational requirement is mediated by a host cell factor, present in some BHK cells, which may promote interaction between the nascent mRNA and its template.

The 3′-ends of the bunyavirus mRNAs are not co-terminal with their genome templates. For the nonambisense segments, mRNAs terminate 50–100 nucleotides before the end of the template, though there does not appear to be a universal termination signal in the bunyaviruses. The pentanucleotide sequence UGUCG has been mapped as the transcription-termination signal in the Bunyamwera orthobunyavirus S segment, and the same or highly related motifs have been identified in the Bunyamwera virus L segment and in the S segments of other orthobunyaviruses. However, the motif is not present in orthobunyavirus M segments nor in the genomes of viruses in other genera.The subgenomic mRNAs transcribed from ambisense S segments terminate within the noncoding intergenic sequences in the RNA (**Figure 7**); for some but not all viruses, the intergenic region has the potential to form a stable hairpin structure, though the role of secondary structure in transcription termination is unclear. Bunyavirus mRNAs are not demonstrably 3′ polyadenylated, but many have the potential to form stem–loop structures which may confer stability.

Genome Replication

In order to replicate the negative-sense genome RNA, a full-length complementary, positive-sense RNA, the antigenome, must be synthesized (**Figures 6** and **7**). This molecule differs from the positive-sense mRNA in that it does not have the 5′ primer sequences and the 3′-end extends to the 5′ terminus of the genomic RNA template. Experiments with minigenome systems show that only the viral L and N proteins are required for replication, but it is not known what controls the switch from transcriptive to replicative mode of the polymerase. Two events differ between transcription and replication: initiation, which does not require a primer, and readthrough of the mRNA termination signal. The difference in initiation may be because the RNA polymerase is modified by a cellular protein. In the infected cell antigenomes are only found assembled into nucleocapsids; therefore, encapsidation of the nascent antigenome RNA may prevent its interaction with the template, thereby overcoming the mRNA termination signal.

Assembly and Release

Maturation of bunyaviruses characteristically occurs at the smooth membranes in the Golgi apparatus, and hence is inhibited by monensin, a monovalent ionophore. The viral glycoproteins accumulate in the Golgi complex and cause a progressive vacuolization. However, the morphologically altered Golgi complex remains functionally active in its ability to glycosylate and transport glycoproteins destined for the plasma membrane. Using recombinant expression systems, it has been shown that the targeting of the bunyavirus glycoproteins to the Golgi is a property of the glycoproteins alone, and does not require other viral proteins or virus assembly. Electron microscopic studies revealed that viral nucleocapsids condense on the cytoplasmic side of areas of the Golgi vesicles, whereas viral glycoproteins are present on the luminal side. The absence of a matrix-like protein in bunyaviruses, which for other viruses may function as a bridge between the nucleocapsid and the glycoproteins, suggests that direct transmembrane interactions between the bunyavirus nucleocapsid and the glycoproteins may be a prerequisite for budding. After budding into the Golgi cisternae, vesicles containing viral particles are transported to the cell surface via the exocytic pathway, eventually releasing their contents to the exterior.

There are important exceptions, however, to the above maturation scheme; Rift Valley fever phlebovirus has been observed to bud at the surface of infected rat hepatocytes, and it appears that a characteristic of the newly described American hantaviruses which cause hantavirus pulmonary syndrome is assembly and maturation occurring at the plasma membrane.

Persistent Infections

Arboviruses share a common biological property in their capacity to replicate in both vertebrate and invertebrate cells. The outcomes of these infections can be markedly different; whereas infection of vertebrate cells is often lytic, leading to cell death, infection of invertebrate cells is often asymptomatic, self-limiting, and leads to a persistent infection. For bunyaviruses this has been demonstrated both at the organismic level and in cultured cells. Studies of persistent infections of mosquito cells with orthobunyaviruses showed no inhibition of host cell

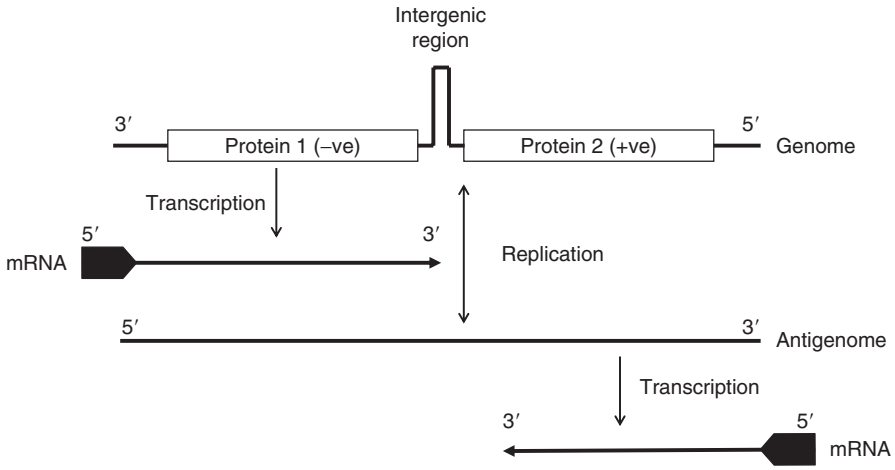


Figure 7 Transcription and replication scheme of ambisense-sense bunyavirus genome segments. The genome RNA encodes proteins in both negative- and positive-sense orientations, separated by an intergenic region that can form a hairpin structure. The proteins are translated from subgenomic mRNAs, with the mRNA encoding protein 2 transcribed from the antigenome RNA after the onset of genome replication. From Elliott RM (2005) Negative strand RNA virus replication. In: Mahy B and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections*, 10th edn., vol. 1, pp. 127–134. London: Hodder Arnold.

protein synthesis, in sharp contrast to replication in mammalian cells. A feature of the persistently infected cells was the excess amount of S segment RNA they contained, but although the level of S mRNA remained high the amount of N protein translated declined. Blockage of N protein synthesis was shown to be because N was able to encapsidate its own mRNA, thereby preventing its translation. Defective L segment RNAs were also found in the persistently infected cells, but these were not packaged into virions. In contrast, a novel type of defective interfering particle was produced which contained only S segment RNA. It is highly probable that host cell factors contribute to these events, but the identity of these factors awaits further investigation.

Reverse Genetics

Reverse genetic approaches to the study of bunyavirus RNA synthesis have been described, in which transiently expressed recombinant N and L proteins transcribe and replicate synthetic RNA transcripts containing a reporter gene. A system to recover infectious Bunyamwera orthobunyavirus entirely from cloned cDNA copies of the three genome segments was developed in 1996, and improvements to this system paved the way for the subsequent recovery of La Crosse virus and Rift Valley fever viruses. These represent significant accomplishments in bunyavirology and will allow future in-depth studies of the functions of all the viral proteins as well as detailed investigation of biological properties such as virulence, tissue tropism, and vector competence. It is expected that similar systems will be developed for viruses in the other *Bunyaviridae* genera. In the longer term, the design and recovery of specifically modified viruses having potential as vaccines may be feasible. For instance, a recombinant Bunyamwera virus containing a modified L RNA segment (L coding region flanked by M segment noncoding sequences) was found to be attenuated for replication in cell cultures and in mice.

See also: Akabane Virus; Crimean-Congo Hemorrhagic Fever Virus and Other Nairoviruses; Hantaviruses.

Further Reading

Billecocq A, Spiegel M, Vialat P, *et al.* (2004) NSs protein of Rift Valley fever virus blocks interferon production by inhibiting host gene transcription. *Journal of Virology* 78: 9798–9806.

Bridgen A and Elliott RM (1996) Rescue of a segmented negative-strand RNA virus entirely from cloned complementary DNAs. *Proceedings of the National Academy of Sciences USA* 93: 15400–15404.

Bridgen A, Weber F, Fazakerley JK, and Elliott RM (2001) Bunyamwera bunyavirus nonstructural protein NSs is a non-essential gene product that contributes to viral pathogenesis. *Proceedings of the National Academy of Sciences USA* 98: 664–669.

Elliott RM (ed.) (1996) *The Bunyaviridae*, 337pp. New York: Plenum Press.

Elliott RM (1990) Molecular biology of *Bunyaviridae*. *Journal of General Virology* 71: 501–522.

Elliott RM (2005) Negative strand RNA virus replication. In: Mahy BWJ and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections*, 10th edn., vol. 1, pp. 127–134. London: Hodder Arnold.

Elliott RM and Koul A (2004) *Bunyavirus/Mosquito Interactions*, symposium 63, pp. 91–102. Society for General Microbiology.

Lowen AC, Boyd A, Fazakerley JK, and Elliott RM (2005) Attenuation of bunyavirus replication by rearrangement of viral coding and noncoding sequences. *Journal of Virology* 79: 6940–6946.

Lowen AC, Noonan C, McLees A, and Elliott RM (2004) Efficient bunyavirus rescue from cloned cDNA. *Virology* 330: 493–500.

Nichol ST, Beaty BJ, Elliott RM, *et al.* (2005) *Bunyaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 695–716. San Diego, CA: Elsevier Academic Press.
Nichol ST, Spiropoulou CF, Morzunov S, *et al.* (1993) Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science* 262: 914–917.
Sanchez AJ, Vincent MJ, Erickson BR, and Nichol ST (2006) Crimean-Congo hemorrhagic fever virus glycoprotein precursor is cleaved by Furin-like and SKI-1 proteases to generate a novel 38-kilodalton glycoprotein. *Journal of Virology* 80: 514–525.
Schmaljohn C and Hooper JW (2001) *Bunyaviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology* 4th edn., pp. 1581–1602. Philadephia: Lippincott Williams and Wilkins.
Weber F, Bridgen A, Fazakerley JK, *et al.* (2002) Bunyamwera bunyavirus non-structural protein NSs counteracts the induction of type I interferon. *Journal of Virology* 76: 7949–7955.
Won S, Ikegami T, Peters CJ, and Makino S (2006) NSm and 78-kilodalton proteins of Rift Valley fever virus are nonessential for viral replication in cell culture. *Journal of Virology* 80: 8274–8278.

Bunyaviruses: Unassigned

C H Calisher, Colorado State University, Fort Collins, CO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arbovirus A virus transmitted to vertebrates by hematophagous (blood-feeding) insects.
Arthralgia Joint pain.
Myalgia Muscle pain.
Orthobunyavirus, hantavirus, phlebovirus, nairovirus, tospovirus Any virus in the genus *Orthobunyavirus, Hantavirus, Phlebovirus, Nairovirus,* or *Tospovirus*.
Polyarthritis Simultaneous inflammation of several joints.
Taxon A taxonomic category or group, such as a phylum, order, family, genus, or species.
Unassigned bunyavirus A virus placed in a family *Bunyaviridae* but not in a genus within that family.
Unclassified A virus not yet placed in a taxon.
Ungrouped A virus not yet shown to be related to any other virus.
Viremia Virus in the blood.

Introduction

Although most viruses in the family *Bunyaviridae* have been placed in one of the five established genera (*Orthobunyavirus*, *Phlebovirus*, *Nairovirus*, *Hantavirus*, or *Tospovirus*), some have not. The original studies of all these viruses were done by serologic or other methods and were meant only to determine whether a virus was a newly recognized one or simply an additional isolate of a known virus. When two or more viruses were shown to be related antigenically, they were considered members of a 'group', nothing more. However, when results of further molecular studies provided definitive genetic information, it became possible to place a virus, or a group of viruses, in a particular taxon. Still other viruses, for which we lack data that would allow placement in a genus, were shown by electron microscopy (morphology of the virus and morphogenesis), or by other physical or biochemical characteristics to be analogous to recognized members of the family *Bunyaviridae* and so were placed in the family as 'unassigned' members. Many of these viruses are known to be related to each other and so are assigned to groups of unassigned virus and the rest are considered as ungrouped and unassigned. The unassigned viruses of the family *Bunyaviridae* (i.e., 'bunyaviruses') are listed in **Table 1**.

Certain of these viruses cause human illness but have not been adequately characterized nor, with few exceptions, have their epidemiologies been studied methodically and we do not have information regarding their prevalences.

Bhanja Virus

This virus, first isolated from *Haemaphysalis intermedia*, was collected from a paralyzed goat in India. It was subsequently isolated from ticks of other species collected in Senegal, Central African Republic, Nigeria, Cameroon, Somalia, Armenia, Bulgaria, Croatia (former Yugoslavia), and Italy. Infection of a laboratory worker provided the first evidence that this virus is pathogenic for humans. The patient had a mild illness characterized by myalgias and arthralgias, moderate frontal headache, slight photophobia, slight elevation of temperature, all lasting less than 2 days. Subsequently, a few additional human infections, including one fatal case, were recorded. Clearly, this virus is widespread geographically and its significance underreported.

Table 1 Unassigned viruses in the family *Bunyaviridae*

Group	*Viruses*
Bhanja	Bhanja, Forecariah, Kismayo
Kaisodi	Kaisodi, Lanjan, Silverwater
Mapputta	Gan Gan, Mapputta, Maprik, Trubanaman
Tanga	Okola, Tanga
Resistencia	Antequera, Barranqueras, Resistencia
Upolu	Aransas Bay, Upolu
Yogue	Kasokero, Yogue

Ungrouped: Bangui, Belem, Belmont, Bobaya, Caddo Canyon, Chim, Enseada, Keterah (= Issyk-Kul), Kowanyama, Lone Star, Pacora, Para, Razdan, Salanga, Santarem, Sunday Canyon, Tai, Tamdy, Tataguine, Wanowrie, Witwatersrand, Yacaaba.

Kasokero Virus

Kasokero virus was first isolated from the bloods of Egyptian rousette bats (*Rousettus aegyptiacus*) in Uganda. It subsequently was isolated from bloods of three people working in the laboratory at the time the bats and the virus were being manipulated and from the blood of a driver who only occasionally entered the laboratory, suggesting the possibility of aerosol transmission of the virus. Patients suffered from fever, headache, abdominal pain, diarrhea, and from some or all of nausea, abdominal pain, chest pain, hyperactive reflexes, coughing, and severe myalgias and arthralgias, lasting 5–14 days, but followed by complete recovery.

Tataguine Virus

Tataguine virus was first isolated from mosquitoes aspirated from dwelling places near Tataguine, Senegal, and subsequently from mosquitoes collected in Nigeria, Central African Republic, Burkina Faso, Cameroon, and Senegal. Clinical manifestations of Tataguine virus infection in humans are mild, characterized by fever and rash, and can be confused with malaria and fevers of unknown origin in West Africa. When active surveillance for this virus was done in Nigeria and Senegal, it was found to be one of the most prevalent viruses in blood samples taken from febrile humans.

Wanowrie Virus

Wanowrie virus, first isolated from *Hyalmomma marginatum* ticks in India, has been isolated from other ticks in Egypt and Iran and from mosquitoes in India. It has also been isolated from the brain of a child in Sri Lanka, suggesting that further studies of this ungrouped pathogen are warranted.

Keterah Virus (Also Known As Issyk-Kul Virus)

This virus was first isolated from *Argas pusillus* ticks collected from bats in Malaysia and from the bloods of those bats (*Scotophilus temmencki*), and has been isolated from ticks and mosquitoes that had fed on infected bats. It was subsequently isolated from pooled brain, liver, spleen, and kidney tissues of other bats in Kyrgyzstan and Tadzhikistan. The virus also was isolated from a staff member of a virology institute in Tadzhikistan who had contracted the infection during field work with bats. Sporadic human cases of this disease have been recognized for more than 20 years in central Asia, particularly in Tadzhikistan, and in Malaysia, and the virus may occur in parts of Iran, Afghanistan, India, and Pakistan. The nonfatal illness caused by this virus is characterized by fever, headache, and myalgias, which are sufficiently nonspecific to be generally undiagnosed or mistaken for diseases caused by other pathogens.

Experimental infections of African green monkeys (*Cercopithecus aethiops*), golden hamsters (*Mesocrictus auratus*), and laboratory mice with the Issyk-Kul strain of this virus demonstrated virus in blood and organs of all animals. Histological studies revealed inflammatory and dystrophic changes in the central nervous system, lungs, liver, and kidneys and pronounced morphological changes in the spleen. The virus is pantropic, causing generalized infection in all animals, irrespective of the route of infection. In monkeys, asymptomatic infection was accompanied by marked organ damages and viremia.

Other Unassigned Orthobunyaviruses Causing Disease in Humans

Gan Gan virus, isolated thus far only from Australian mosquitoes, has been associated with a few cases of acute epidemic polyarthritis-like illness, and may be confused with other viruses causing this syndrome in Australia, such as Ross River virus and Barmah Forest viruses (*Togaviridae*, *Alphavirus*). Similarly, Trubanaman virus, also isolated only from Australian mosquitoes, is suspected of being pathogenic. These viruses may employ marsupials as principal vertebrate hosts. Finally, Tamdy virus, isolated from *Hyalomma* spp. ticks in Uzbekistan and Turkmenistan, also has been shown to be a human pathogen. Clearly, many more studies are needed, if we are to understand the natural cycles of these viruses, and their prevalences in humans and in wild animals.

See also: Baculoviruses: Molecular Biology of Granuloviruses; Hepatitis B Virus: Molecular Biology.

Further Reading

Calisher CH and Goodpasture HC (1975) Human infection with Bhanja virus. *American Journal of Tropical Medicine and Hygiene* 24: 1040–1042.

Dandawate CN, Shah KV, and D'Lima LV (1970) Wanowrie virus: A new arbovirus isolated from *Hyalomma marginatum isaaci*. *Indian Journal of Medical Research* 58: 985–989.

Fagbami AH, Monath TP, Tomori O, Lee VH, and Fabiyi A (1972) Studies on Tataguine infection in Nigeria. *Tropical and Geographic Medicine* 24: 298–302.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 713–714. San Diego, CA: Elsevier Academic Press.

Kalunda M, Mukwaya LG, Mukuye A, *et al.* (1986) Kasokero virus: A new human pathogen from bats (*Rousettus aegyptiacus*) in Uganda. *American Journal of Tropical Medicine and Hygiene* 35: 387–392.

L'vov DK, Sidorova GA, Gromashevskii VL, Skvortsova TM, and Aristova VA (1984) Isolation of Tamdy virus (*Bunyaviridae*) pathogenic for man from natural sources in Central Asia, Kazakhstan and Transcaucasia (in Russian). *Voprosy Virusologii* 29: 487–490.

L'vov DK, Terskikh II, Abramova LN, Savosina NS, Skvortsova TM, and Gromashevskii VL (1991) An experimental infection caused by the Issyk-Kul arbovirus (in Russian). *Meditsinskaia Parazitolologiia I Parazitarnye Bolezni* 4: 15–16.

Mackenzie JS, Lindsay MD, Coelen RJ, Broom AK, Hall RA, and Smith DW (1994) Arboviruses causing human disease in the Australasian zoogeographic region. *Archives of Virology* 136: 447–467.

Moore DL, Causey OR, Carey DE, *et al.* (1975) Arthropod-borne viral infections of man in Nigeria, 1964–1970. *Annals of Tropical Medicine and Parasitology* 69: 49–64.

Pavri KM, Anandarajah M, Hermon YE, Nayar M, Wikramsinghe MR, and Dandawate CN (1976) Isolation of Wanowrie virus from brain of a fatal human case from Sri Lanka. *Indian Journal of Medical Research* 64: 557–561.

C

Cacao Swollen Shoot Virus

E Muller, CIRAD/UMR BGPI, Montpellier, France

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Cacao swollen shoot virus (CSSV) is a member of the genus *Badnavirus*, family *Caulimoviridae*. The importance and diversity of badnaviruses has only been recognized relatively recently and particularly on tropical plants, due to progress made in molecular diagnostic techniques.

In addition to CSS3V, other badnaviruses commonly reported are banana streak virus (BSV) on banana, dioscorea alata bacilliform virus (DaBV) on yam, taro bacilliform virus (TaBV) on taro, sugarcane bacilliform virus (SCBV) on sugarcane, citrus mosaic bacilliform virus (CMBV) on citrus species, and pineapple bacilliform virus (PBV) on pineapple, but these viruses also infect various ornamental plants such as Aucuba, Commelina, Kalanchoe, Yucca, Mimosa, and Schefflera. Some of the above are only tentative species of the genus *Badnavirus* due to insufficient molecular data. There are regularly new reports of badnaviruses on other plants.

CSSV is naturally transmitted to cacao (*Theobroma cacao)* in a semipersistent manner by several mealybug species, the vector of most badnaviruses. Cacao swollen shoot disease occurs in all the main cacao-growing areas of West Africa, where it has caused enormous damage. It was highlighted for the first time in Ghana in 1922 but was described and named in 1936, then in Nigeria in 1944, in Ivory Coast in 1946, in Togo in 1949, and in Sierra Leone in 1963. The disease was also described in Trinidad and Tobago but currently seems to have disappeared. Cacao swollen shoot disease is present in Sri Lanka and in Indonesia (Java and North Sumatra).

Host Range and Symptomatology

Experimental host range is limited to species of the families of Sterculiaceae, Malvaceae, Tiliaceae, and Bombaceae but the principal host of the virus is *Theobroma cacao.* Symptoms are mostly seen in leaves, but stem and root swellings, as well as pod deformation also occur. In some varieties of cocoa, particularly Amelonado cocoa, reddening of primary veins and veinlets in flush leaves is characteristic (**Figure 1**(**a**)). This red vein banding later disappears. There can be various symptoms on mature leaves, depending on the cocoa variety and virus strain. These symptoms can include: yellow clearing along main veins; tiny pin-point flecks to larger spots; diffused flecking; blotches or streaks. Chlorotic vein flecking or banding is common and may extend along larger veins to give angular flecks.

Stem swellings may develop at the nodes, internodes, or shoot tips. These may be on the chupons, fans, or branches (**Figure 1**(**a**)). Many strains of CSSV also induce root swellings. Stem swellings result from the abnormal proliferation of xylem, phloem, and cortical cells.

Infected trees may suffer from partial defoliation initially due to the incompletely systemic nature of the infection. Ultimately, in highly susceptible varieties, severe defoliation and dieback occurs.

Smaller, rounded to almost spherical pods may be found on trees infected with severe strains. Occasional green mottling of these pods is seen and their surface may be smoother than the surface of healthy pods. Various isolates were described in Ghana as in Togo with a variability and a gradation in the type of symptoms observed.

A few avirulent strains occur in limited, widely scattered outbreaks, usually inducing stem swellings only, and having little effect, if any, on growth or yield. Moreover, there are periods of remission during which symptoms are not visible.

The natural host range of the virus includes *Ceiba pentandra*, *Cola chlamydantha*, *Cola gigantea* var. *glabrescens*, *Sterculia tragacantha*, and *Adansonia digitata* with associated symptoms of transient leaf chlorosis or conspicuous leaf chlorosis.



Figure 1 (a) Symptoms of red-vein banding observed on young flush leaves of cocao tree in Togo, Kloto area. (b) Symptoms of swellings on chupons of cocao tree, in Togo, Litimé area. (c) Symptoms of swollen shoot observed on *Theobroma cacao* plantlets four months after agroinoculation with the Togolese Agou 1 isolate. From left to right: A plantlet inoculated with the wild strain *Agrobacterium tumefaciens* LBA4404 and two plantlets inoculated with the recombinant *A. tumefaciens* bacteria LBA4404 (pAL4404, pBCPX2) containing CSSV insert.

Transmission

Fourteen species of mealybugs (*Pseudococcidae* spp.), including *Planococcoides njalensis, Planococcus citri, Planococcus kenyae, Phenacoccus hargreavesi, Planococcus* sp. *Celtis, Pseudococcus concavocerrari, Ferrisia virgata, Pseudococcus longispinus, Delococcus tafoensis*, and *Paraputo anomalus*, have been reported to transmit CSSV. Only the nymphs of the first, second, and third larval stages and the adult females are able to transmit the virus. The virus does not multiply in the vector and is not transmitted to its progeny. CSSV is transmitted neither by seed nor by pollen. CSSV can infect cocoa at any stage of plant growth. The virus is transmitted experimentally to susceptible species by grafting, particle bombardment, by agro-infection using transformed *Agrobacterium tumefaciens* and with difficulty by mechanical inoculation. Seedlings usually produce acute red vein banding within 20–30 days and, 8–16 weeks later, swellings on shoots and tap roots (**Figure 1(c)**).

As this disease appeared soon after the introduction of cacao to West Africa, it is likely that CSSV came from indigenous hosts. The species *Adansonia digitata*, *Ceiba pentandra, Cola chlamydantha*, and *Cola gigantea* were identified as reservoir hosts in Ghana.

Economic Importance

CSSV is a serious constraint to cocoa production in West Africa, particularly in Ghana. Severe strains of this virus can kill susceptible cocoa trees within 2–3 years. They affect Amelonado cocoa, widely considered to give the best-quality cocoa, more seriously than Upper Amazon cocoa and hybrids which have been selected for resistance to the virus.

The disease was first recognized in 1936 but almost certainly occurred in West Africa in the 1920s. Estimates of annual yield losses due to this virus vary from about 20 000 tonnes to approximately 120 000 tonnes of cocoa from the Eastern Region of Ghana alone. The average annual loss between 1946 and 1974 in Ghana was estimated to be worth over £3 650 000.

Attempts at CSSV control in Ghana have required substantial financial and manpower inputs. The 'cutting-out' policies in place in Ghana since the early 1940s which attempted to control cocoa swollen shoot disease resulted in the removal of over 190 million trees up to 1988. Over ten million infected trees which still required 'cutting out' were identified in the field by 1990.

CSSV is currently confined to West Africa, Sri Lanka, and Sumatra. The disease does not pose a real economic problem for the culture of cocoa in Sri Lanka and Indonesia. However, the devastation which has occurred in West Africa has serious implications for germplasm movement. International attempts at crop improvement are hampered by the need to index cocoa germplasm for this virus, particularly if the germplasm is to be moved to where highly susceptible varieties are grown. Although seed transmission of this virus is not known to occur, there is often the need to move germplasm as stem cuttings, which must then pass through intermediate quarantine for indexing.

Molecular Characterization

Characteristics of the Virions

The viral origin was shown in 1939. CSSV possesses small nonenveloped bacilliform particles and a double-stranded DNA genome of 7–7.3 kbp. The bacilliform particles

measure 130 nm × 28 nm in size and have been shown by dot–blot hybridization to occur in the cytoplasm of phloem companion and xylem parenchyma cells.

Description of Full-Length Sequence

Molecular characterization of CSSV had a boost in 1990, thanks to the improvement of the techniques of purification.

The first complete sequence of a CSSV isolate (Agou1 from Togo) was determined in 1993. Five putative open reading frames (ORFs) are located on the plus strand of the 7.16 kbp CSSV genome. ORF1 encodes a 16.7 kDa protein whose function is not yet determined. The ORF2 product is a 14.4 kDa nucleic acid-binding protein. ORF3 codes for a polyprotein of 211 kDa which contains, from its amino- to carboxyl-terminus, consensus sequences for a cell-to-cell movement protein, an RNA binding domain of the coat protein, an aspartyl proteinase, a reverse transcriptase (RTase), and an RNase H. The last two ORFs X (13 kDa) and Y (14 kDa) overlap ORF3 and encode proteins of unknown functions. CSSV, CMBV, and TaBV are the only badnaviruses which, to date, are known to code for more than three ORFs, CMBV, and TaBV encode six and four ORFs respectively.

Variability of the CSSV

A more extensive study of the molecular aspects of CSSV variability is relevant for three reasons. First, the knowledge of molecular variability will allow the improvement and the validation of a PCR diagnostic test for better virus-indexing procedures. Second, the variability of the virus must be taken into account for resistance screening of new cocoa varieties to CSSV. Finally, a better understanding of the genetic diversity of CSSV in West Africa and elsewhere will in turn help to provide a better understanding of the development of the epidemics and their eradication, and of the evolution of viral populations.

Badnaviruses are highly variable at both the genomic and serological level, a feature which complicates the development of both molecular and antibody-based diagnostic tests. Moreover, CSSV isolates were for a long time classified according to the variability of the symptoms expressed on *T. cacao*, but it is not known if there is a correspondence between this variability of symptoms and the intrinsic molecular variability of the virus.

A first study analyzed the molecular variability of the area of the ORF3 coding for N-terminal coat protein for 1A-like isolates from Ghana and recently the analysis of new whole sequences made it possible to have a better idea of the variability of the CSSV.

Figure 2 presents the circular genome maps of the six isolates of CSSV sequenced to date, and these isolates come from Togo (Agou1, Nyongbo2 from Kloto area, and Wobe12 from Litimé area) and Ghana. The size of the total genome varies from 7024 bp for isolate N1A to 7297 bp for the isolate Wobe12. The overall organization of ORFs is the same for all the isolates but differs by the partial disappearance of the ORFX for the three Ghanaian isolates, and by the existence of an additional ORF for the Peki isolate, which codes for a putative protein of 11 kDa. **Table 1** shows the nucleotide and amino acid sequence percentage identity and similarity between the different ORFs of isolates. ORF1 is the most conserved ORF coding region (81 synonymous mutations versus 101 mutations among the six isolates) and the amino acid dissimilarity of pairwise combinations of CSSV isolates ranges from 1.4% to 8.4%. The maximum amino acid dissimilarity between isolates is 25.5% for ORF2, 23.8% for ORF3, and slightly higher for ORFY (33.6%). The value of overall variability calculated for ORF3 corresponds to a succession of more or less variable regions that can be identified on alignment. In agreement with the division of the ORF3 polyprotein in three regions, we observed that the first region (amino acids 1–350), which corresponds to the movement protein region, is highly conserved as already observed for other pararetroviruses. Region 2 is far less conserved (particularly amino acids 370–500 and 1010–1070). Region 3 has an intermediary level of variability. ORFX is the least conserved ORF.

Maximum nucleotide sequence variability between pairwise combinations of complete genomic sequences of CSSV isolates was 29.4% (between Wobe12-CSSV and Peki-CSSV).

The alignments of the nucleotide and protein sequences of the ORFs (**Table 1**) and the phylogenetic trees built from these sequences make it possible to separate the isolates into three groups according to their geographical origin, rather than their aggressiveness (CSSV-N1A are CSSV-Peki are considered as mild isolates).

A study of sequence variability was made on the level of the first region of the ORF3 on isolates taken in two areas different from Togo having distinct epidemiologic histories. The swollen shot disease was observed for the first time in 1949 in Togo in the area of Kloto, and then spread only in this area. It is only toward the end of the 1990s that the disease started to be observed in Litimé. The phylogenetic tree built from the sequences of isolates of these two areas distinguishes three groups (A, B, and C), as does the analysis based on the whole sequences (**Figure 3**). One of these groups, A, containing Wobe12 is more distantly related to the others and contains only isolates coming from the area of Litimé, Togo. Moreover, the amplified sequence of isolates from group A (724 bp instead of the 721 bp amplified for the other isolates) code for an additional amino acid. Group A of isolates cannot therefore originate from the area of Kloto, infected before and probably originate from Ghana. In Kloto, only one group of isolates is present (C), whereas in the area of Litimé the three groups of diversity coexist.

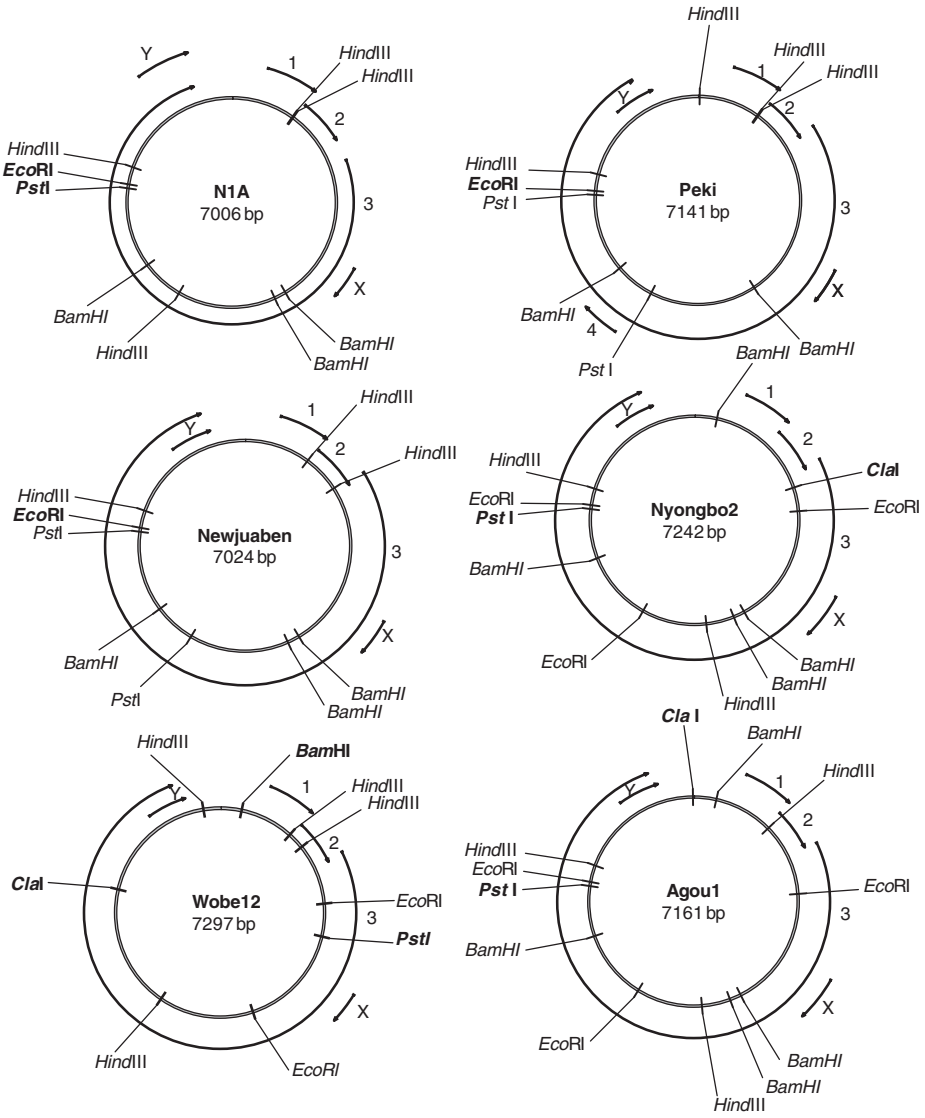


Figure 2 Organization of the circular genomes of new CSSV isolates compared to Agou 1. Arrows indicate the deduced ORFs 1–4, X and Y capable of encoding proteins larger than 9 kDa. *Bam*HI, *Cla*I, *Eco*RI, *Hind*III, and *Pst*I restriction sites are shown. Unique restriction sites are in bold. Reprinted from Muller E and Sackey S (2005) Molecular variability analysis of five new complete cacao swollen shoot virus genomic sequences. *Archives of Virology* 150: 53–66, with permission from Springer Wien.

It is probable that the group B of isolates of Kloto, that are present also in Litimé, come from a contamination of Litimé by isolates of Kloto.

Homology with Other Viruses

A phylogenetic tree was built from all the available full-length sequences of badnaviruses (**Figure 3**). CSSV isolates are closer to each other than to badnaviruses and to members of the genus *Tungrovirus*. In addition, among the badnaviruses sequenced to date, CSSV has the closest relationship with CMBV whose host is also a tree crop. The majority of the hosts of the badnavirus are indeed plants with vegetative multiplication.

However, one group of BSV-like sequences recently detected in Uganda are closer to CSSV sequences than to other BSV sequences and one sequence found integrated in *Musa acuminata* germplasm has good homology with CSSV.

The maximum variability level observed between the six CSSV isolates is slightly higher than the one observed between the three rice tungro bacilliform virus (RTBV) isolates but much lower than between the various isolates of BSV sequenced until now.

Table 1 Nucleotide sequence identity and amino acid sequence similarity of pairwise combinations of CSSV isolates for the ORFs 1, 2, 3, X, and Y

percentage of nucleotide sequence identity
percentage of amino acid sequence similarity

	ORF1						*ORF2*						*ORF3*						*ORFX*						*ORFY*					
	Ag1	*Nb2*	*NJ*	*Peki*	*N1A*	*w12*	*Ag1*	*Nb2*	*NJ*	*Peki*	*N1A*	*w12*	*Ag1*	*Nb2*	*NJ*	*Peki*	*N1A*	*w12*	*Ag1*	*Nb2*	*NJ*	*Peki*	*N1A*	*w12*	*Ag1*	*Nb2*	*NJ*	*Peki*	*N1A*	*w12*
Agou1	100	98.8	80	80.9	80.7	95.6	100	88.3	73.3	73.6	73.6	70.7	100	98.2	85.5	84.7	81.8	74.4	100	97.1	58.7	58.4	59.5	40.1	100	98.5	89.3	87.3	89.1	71
	100	**98.6**	**93.7**	**94.4**	**93**	**97.2**	**100**	**89**	**82.8**	**82.1**	**82.1**	**74.5**	**100**	**98.2**	**90.6**	**90.2**	**86.5**	**79.8**	**100**	**92.9**	**41.9**	**45.3**	**42.7**	**22.4**	**100**	**97.7**	**92.4**	**87.8**	**89.3**	**69.5**
Nyongbo2		100	80.2	81.1	80.9	94.9		100	81.8	81.8	81.8	77.6		100	85.1	84.4	81.5	74.2		100	58.7	58.1	60.1	41.3		100	88.3	86.3	88	71
		100	**93.7**	**94.4**	**93**	**97.2**		**100**	**89.7**	**88.3**	**88.3**	**76.5**		**100**	**90.3**	**89.8**	**86.**3	**79.**7		**100**	**41.9**	**43.6**	**44.4**	**22.4**		**100**	**90.1**	**85.5**	**87**	**71**
New Juaben			100	97.2	96.7	80.9			100	96.6	96.3	76.7			100	96.4	93.3	73.3				96	96.3	47.3			100	92.9	94.9	71.8
			100	**97.9**	**97.9**	**92.3**			**100**	**96.6**	**96.6**	**77.9**			**100**	**96.2**	**93.6**	**79.5**				**89**	**91.2**	**14.3**			**100**	**90.1**	**92.4**	**69.5**
Peki				100	97.7	81.4				100	96.6	76.5				100	91.8	73.5				100	94.1	46.9				100	93.4	69.7
				100	**97.2**	**93.7**				**100**	**95.9**	**77.2**				**100**	**92**	**80**				**100**	**89**	**20.5**				**100**	**88.5**	**65.6**
N1A					100	80.7					100	77					100	70.4					100	51.3					100	70.7
					100	**91.6**					**100**	**78.5**					**100**	**76.2**					**100**	**20.5**					**100**	**66.4**
Wobe12						100						100						100						100						100
						100						**100**						**100**						**100**						**100**

Ag1, Agou1; Nb2, Nyongbo2; NJ, New Juaben; w12, Wobe12.
Reproduced from Muller and a Sockey (2005) Molecular variability analysis of five new complete cacao swollen shoot virus genomic sequences. *Archives of Virology* 150: 53–66, with permission from Springer Wien.

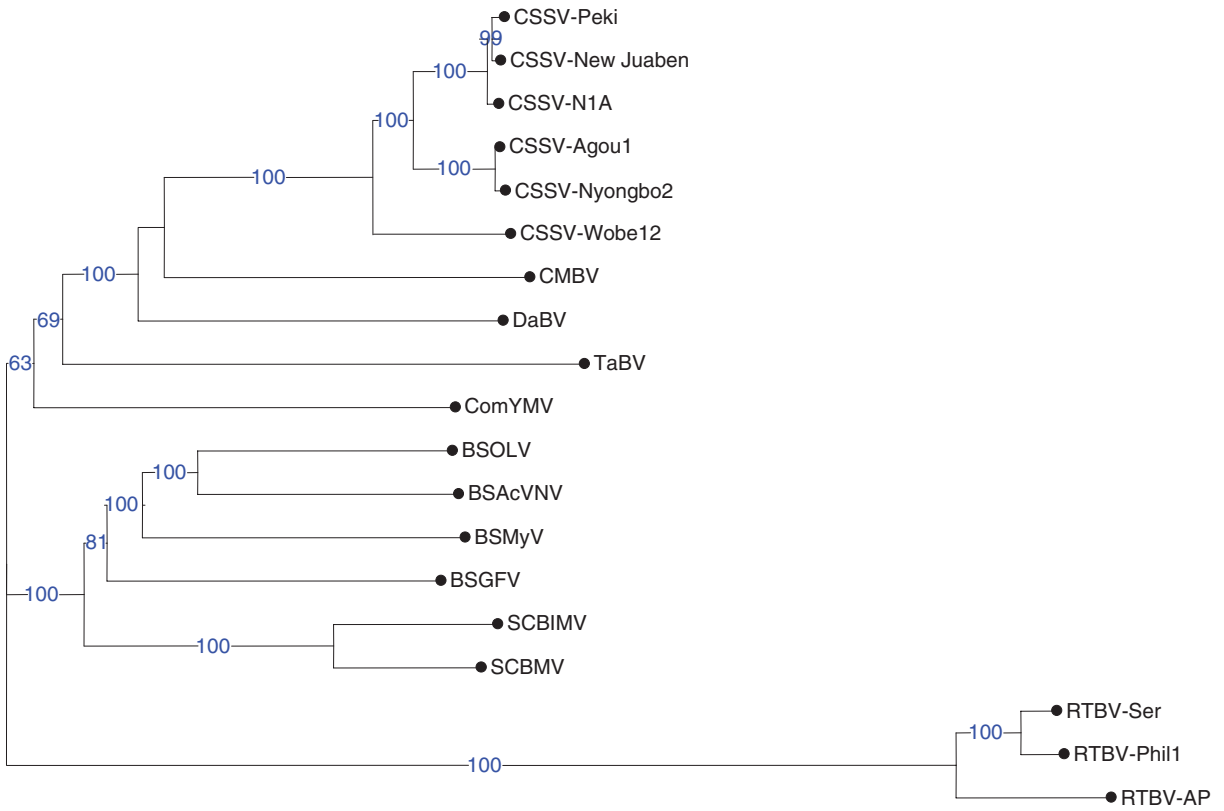


Figure 3 Neighbor-joining tree generated by the Darwin 4 program based on complete nucleotide sequences of badnaviruses and rice tungro bacilliform virus (RTBV). Numbers at the nodes of the branches represent percentage bootstrap values (1000 replicates) when superior to 60. The GenBank accession numbers of sequences are L14546 (CSSV-Agou1), AJ534983 (CSSV-Nyongbo2), AJ608931 (CSSV-New Juaben), AJ609019 (CSSV-Peki), AJ609020 (CSSV-NIA), AJ781003 (CSSV-Wobe12), AJ002234 (banana streak OL virus – BSOLV), AY805074 (banana streak mysore virus – BSMyV) AY750155 (banana streak acuminate vietnam virus – BSAcVNV), AY493509 (banana streak GF virus – BSGFV), X52938 (commelina yellow mottle virus – comYMV), AF347695 (citrus mosaic bacilliform virus – CMBV), X94576 (dioscorea alata bacilliform virus – DaBV), M89923 (sugarcane bacilliform Mor virus – SCBMV), AJ277091 (sugarcane bacilliform IM virus – SCBMIV), AF357836 (taro bacilliform virus – TaBV), AJ292232 (RTBV-AP), O76470 (RTBV-Ser), X57924 (RTBV-Phill).

Molecular Epidemiology

A systematic molecular characterization of viral isolates should make it possible to study the molecular epidemiology of CSSV. Moreover, studies on CSSV in adventitious plants or insect vectors would make it possible to better understand the epidemiologic factors that lead to the rapid expansion of the disease in some plots.

Diagnosis

Serological Diagnostics

The virus is not strongly immunogenic; however, several antisera have been raised and shown to react with CSSV. Enzyme-linked immunosorbent assay (ELISA) has been used to detect CSSV but high background values were obtained and difficulties were found in detecting CSSV in plants suspected of having only a low virus titer. Immunosorbent electron microscopy has been used for detection and comparison of some isolates of CSSV in Ghana. Both of these techniques cannot detect latent infection.

The virobacterial agglutination (VBA) test has been found to be a useful test for detecting CSSV in leaf tissue from infected trees. Using this assay, CSSV can be detected in trees showing symptoms as well as in infected, but symptomless, trees. The immunocapture polymerase chain reaction (IC-PCR) technique was adapted to the only detection of CSSV-1A isolates from Ghana.

There is considerable strain variation among the many recognized CSSV isolates, some of which react only

weakly with certain antisera. Using monoclonal antibodies, four serotypes of CSSV have been distinguished by ELISA analysis of 31 samples of the virus from different geographical locations in Ghana. The efficiency of serological diagnosis depends on the use of polyvalent antiserum able to detect all serotypes.

PCR Diagnosis

For a reliable PCR diagnosis, it is necessary to design primers from conserved regions of the genome. Until recently, only few badnaviruses were sequenced in full, and only the end of the ORF3 which contains the conserved motifs coding for RTase and RNaseH made it possible to obtain primers useful for diagnosis. PCR-based diagnosis is able to detect CSSV not only in symptomless leaves of symptomatic plants, but also in symptomless plants as early as one week post-inoculation.

The alignment of the six full-length sequences of CSSV allowed conserved regions to be identified and polyvalent primers to be designed for the diagnosis of CSSV. These primers can detect all the isolates tested to date from Togo and Ghana. These primers are located in the first part of the ORF3 and amplify a fragment from 721 to 724 bp, including from isolates having only 70% nucleotide identity (CSSV-Wobe12 and CSSV-Peki). The extraction method is based on a buffer containing MATAB (mixed alkyltrimethylammonium bromide), which reduces the co-extraction of PCR-inhibitory substances, as well as being less costly and generates a higher DNA yield.

These primers for CSSV diagnosis may require modifications as more sequence data from other CSSV isolates are generated. As the sensitivity of diagnosis is better when young symptomatic host plant leaves are tested, it is recommended that validation of the diagnostic test is done using this type of material in the first instance, especially when testing isolates from different areas.

Control of the Disease

Control of the swollen shoot disease based only on 'cutting-out' campaigns has not been successful due to several factors including political and socioeconomic problems. The better strategy for dealing with the disease is to develop a combination of control measures in an integrated approach. Moreover, this approach should implicate cocoa farmers as much as possible.

Intermediate quarantine facilities are at present hampered by the lack of suitable indexing methods for the virus. The early PCR diagnosis could be easily tested in intermediate quarantine facilities and compared with the grafting procedure used for indexation on Amelonado cocoa seedlings.

Mild strain protection is a possibility which is being investigated. Mild strains which appear to confer some protection against the severe strains are available and are being tested in the field in Ghana. However, the degree of protection afforded so far is not sufficient and further research is necessary.

The possibility of isolating new cocoa plantings from infected cocoa by using barriers of CSSV-immune crops could be considered. These crops would form a barrier to the movement of vectors. Examples of possible barrier crops include oil palm, coffee, cola, and citrus. The 'cutting out' of the adventitious plants of the type Commelina and Taro could be a possible strategy if it is confirmed that these plants harbor CSSV.

The use of resistant cocoa is advocated as many of the new hybrids available in West Africa do have some resistance to CSSV and because it seems to be the most sustainable method. Replanting with resistant cacao trees, however, requires the installation of a protocol of effective screening for resistance. Severe isolates representative of the different molecular groups should be used as well as a suitable screening method. However, a standardized inoculation method is not yet available because particle bombardment is difficult to develop on a large scale and agroinoculation needs biosafety confinement. The screening for CSSV resistance for two types of severe isolates has been initiated in 2003 in Togo.

See also: Caulimoviruses: General Features; Caulimoviruses: Molecular Biology; Virus Classification by Pairwise Sequence Comparison (PASC).

Further Reading

Brunt AA (1970) *CMI/AAB Descriptions of Plant Viruses No 10. Cacao Swollen Shoot Virus.* Wellesbourne: Association of Applied Biologists.

CABI, Crop Protection Compendium(2002) *Cacao Swollen Shoot Virus.* Wallingford: CABI Publishing.

Castel C, Amefia YK, Djiekpor EK, Partiot M, and Segbor A (1980) Le swollen shoot du cacaoyer au Togo. Les différentes formes de viroses et leurs conséquences économiques. *Café, Cacao, Thé* 24(2): 131–146.

Fauquet C, Mayo M, Maniloff J, Desselberger U, and Ball L (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.* San Diego, CA: Elsevier Academic Press.

Hagen LS, Jacquemond M, Lepingle A, Lot H, and Tepfer M (1993) Nucleotide sequence and genomic organization of cacao swollen shoot virus. *Virology* 196(2): 619–628.

Hagen LS, Lot H, Godon C, Tepfer M, and Jacquemond M (1994) Infection of *Theobroma cacao* using cloned DNA of cacao swollen shoot virus and particle bombardment. *Molecular Plant Pathology* 84: 1239–1243.

Lot H, Djiekpor E, and Jacquemond M (1991) Characterization of the genome of cacao swollen shoot virus. *Journal of General Virology* 72: 1735–1739.

Muller E and Sackey S (2005) Molecular variability analysis of few new complete cacao swollen shoot virus genomic sequence. *Archives of Virology* 150: 53–66.

Caliciviruses

M J Studdert and S J Symes, The University of Melbourne, Parkville, VIC, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Chimeric virus A virus containing genetic material from at least two genetically different parents.
Genetic recombination The molecular process of forming a chimeric virus and in the caliciviruses is likely to involve a copy-choice mechanism, whereby the viral RNA polymerase switches template during replication.
Histo-blood group antigens Molecules with carbohydrate epitopes, first described on the surface of red blood cells but subsequently shown to be expressed on the surface of many cells and some of which are receptors for some calciviruses.
Pinniped Marine mammals including sea lions, walruses, fur seals, and true seals.
Splenomegaly Enlargement of the spleen.
Vesicular disease A disease characterized by a fluid-filled sac arising within an epithelial surface (blister).

Introduction

The members of the family *Caliciviridae* are classified in four genera: *Vesivirus*, *Lagovirus*, *Norovirus*, and *Sapovirus* (**Table 1**). Caliciviruses produce diseases that are important in both human and veterinary medicine.

History

Vesiviruses

In 1932 there occurred in southern California, in pigs fed uncooked garbage, a vesicular disease that was provisionally diagnosed as foot and mouth disease. The disease was eradicated by slaughter and quarantine. However, in 1933 the disease recurred and at that time it was realized that it was not foot and mouth disease but a new disease that was named vesicular exanthema of swine. Between 1932 and 1951, the disease continued to occur in southern California and more than 2.5 million pigs representing 21% of the total pig population in southern California were involved in the outbreaks. There was a clear linkage between the disease and the feeding of raw, that is, uncooked garbage containing, it was assumed, pork scraps. Outbreaks of the disease continued to occur despite the introduction of laws requiring that all garbage fed to pigs be cooked. In late 1951 a train left San Francisco and offloaded in Wyoming, to a pig rancher, garbage containing pork scraps that were fed to his pigs. When a few of the pigs in the herd developed vesicular lesions, the owner shipped the pigs for sale. Following this, between 1952 and 1956, outbreaks of vesicular exanthema occurred in 40 of the 48 states. The last outbreak occurred in New Jersey in 1951. By 1956 rigid enforcement of laws that required all garbage fed to pigs be cooked, or a total ban on feeding garbage, resulted in the disappearance of the disease from the national swineherd. In 1959 vesicular exanthema of swine virus was declared eradicated from the United States, the only country in which it has occurred.

In 1972 a calicivirus was isolated from sea lions during an investigation of an outbreak of abortion on San Miguel Island off the southern Californian coast. This virus, called San Miguel sea lion virus, closely resembled vesicular exanthema virus and was transmissible to pigs. It is believed that the origin of vesicular exanthema virus of swine was a consequence of feeding uncooked sea lion carcasses that were washed up on the beaches of southern California, to pigs. Subsequently, caliciviruses have been isolated from other pinniped species including Northern fur seals inhabiting the Pribilof Islands, Alaska.

Acute upper respiratory disease of cats is common and in 1957 the first feline calicivirus, originally identified as a picornavirus, was isolated. It was shown to be one of the two major viral causes of respiratory disease in cats, the other virus being feline herpesvirus 1.

Lagoviruses

In 1984, a new, highly infectious disease of the European rabbit, *Oryctolagus cuniculus*, was identified in China. It was characterized by hemorrhagic lesions, particularly affecting the lungs and liver. The virus was eventually called rabbit hemorrhagic disease virus. It killed some 470 000 rabbits in the first 6 months and by 1985 had spread throughout China. By 1988, it had spread throughout eastern and western Europe and had reached North Africa. In December 1988, cases occurred in Mexico City. Both wild and domestic *O. cuniculus* were affected, but all other species of mammals, except the European hare, appear to be resistant to infection. The disease was unknown in Europe before 1984; however, a very similar disease called European brown hare syndrome had been recognized in the early 1980s affecting *Lepus europaeus* and subsequently some other *Lepus* species.

Table 1 Overview of the family *Caliciviridae*

Genus	*Virus*	*Acronym*	*Primary host*	*Cultivable*	*Disease*
Vesivirus	Vesicular exanthema of swine virus	VESV	Pig	Yes	Vesicular lesions of mouth, snout, hooves
	San Miguel sea lion virus	SMSV	San Miguel sea lion	Yes	Vesicular lesions of flippers, abortions
	Feline calicivirus	FCV	Cat	Yes	Upper respiratory tract and oral cavity disease
Lagovirus	Rabbit hemorrhagic disease virus	RHDV	Rabbit	No	Generalized hemorrhagic disease, liver necrosis, disseminated intravascular coagulation
	European brown hare syndrome virus	EBHSV	European brown hare	No	Hemorrhagic disease, liver necrosis
Norovirus	Norwalk virus	NV	Human	No	Gastroenteritis
Sapovirus	Sapporo virus	SV	Human	No	Gastroenteritis

Rabbit hemorrhagic disease virus was imported into Australia in 1991 to a high security laboratory. Australian native animal species susceptibility studies were conducted prior to determining whether the virus would be a safe and effective biocontrol agent. In Australia, rabbits are in plague numbers, perhaps as many as 100 million. Rabbits are estimated to cause $600 million in annual losses; some of these losses, including native species and habitat destruction, may be permanent. The virus was transferred from the high security laboratory to Wardang Island for further pen trials. During these trials it escaped to the mainland, possibly by insect vector transmission (mosquitoes, bush flies) or carrion-eating birds (crows, hawks, eagles), and subsequently spread to many areas prior to 'official' release as a biocontrol agent. In New Zealand in mid-1997, the virus was illegally introduced and spread, probably, by farmers, irate, following a decision of the government not to allow legal importation of the virus until more was known about its host range, including native animal species susceptibility, particularly of the kiwi.

Noroviruses and Sapovirues

Noroviruses are a major cause of epidemic nonbacterial gastroenteritis outbreaks in humans. Norwalk virus, the prototype strain of the genus *Norovirus*, was identified in 1972 by negative stain electron microscopy of fecal specimens obtained from human volunteers. These volunteers were exposed to filtrates of feces collected during an outbreak of gastroenteritis at a school in Norwalk, Ohio in 1968. Noroviruses lack the typical calicivirus morphology and were initially and awkwardly referred to as 'small round-structured viruses'.

Noroviruses have not been cultivated in conventional monolayer cell cultures. Noroviruses have been cultured in a physiologically relevant three-dimensional (3D) organoid model of human small intestinal epithelium. Determination of the genomic sequences of Norwalk virus and of Southampton virus (a norovirus detected in the United Kingdom) in the early 1990s allowed the development of diagnostic tests, including reverse transcription–polymerase chain reaction for virus detection and enzyme-linked immunosorbant assays using recombinant proteins for antibody detection. The detection and sequencing of a large number of norovirus genomes revealed significant genetic variability.

Sapporo virus, the prototype of the genus *Sapovirus*, was identified as a cause of gastroenteritis in human infants following an outbreak in a children's home in Japan in 1982. Unlike the noroviruses, sapoviruses possess the classic calicivirus morphology when viewed by electron microscopy. Historically, the noroviruses and sapoviruses have taken their name from the location of the outbreak.

Some caliciviruses of veterinary importance are still to be officially classified. Bovine enteric caliciviruses including Newbury agent 2 and Jena viruses, and a murine virus are proposed members of the genus *Norovirus*, while a porcine enteric calicivirus is a proposed member of the genus *Sapovirus*. The recent genomic characterization of another bovine enteric calicivirus, Newbury agent 1, shows that phylogenetically this virus does not fall within any of the four defined calicivirus genera and may represent a fifth genus, within the family *Caliciviridae*.

Classification and Properties

Caliciviruses were initially classified as picornaviruses with which they share a number of properties. However, the distinctive properties of caliciviruses led to the creation of a new family *Caliciviridae*. Human hepatitis E virus was originally considered to be a calicivirus because of apparent similarity in morphology but is now classified in the genus *Hepevirus* in an undefined family.

The family name *Caliciviridae* derives from the cup-shaped (*calix* = cup) surface depressions that give the virion its unique appearance. By cryoelectron microscopy, virions are 40.5 nm with 32 cup-shaped surface structures

comprising 90 arch-like capsomers arranged in $T = 3$ icosahedral symmetry (**Figure 1**). The individual capsomers are dimers of the ~60 kDa capsid protein and have two main structural domains: the protruding (P) domain, which consists of the P1 and P2 subdomains, which are joined to the shell (S) domain by a flexible binge (**Figure 1**). The capsid protein has six regions (A–F) that probably form hypervariable, loop-like structures on the surface of the folded protein and are sites of antigenic variation. Noroviruses are exceptional in that they lack the characteristic cup-shaped depressions and have a fuzzy edge.

Virion buoyant density is 1.33–1.41 g^{-1} ml in CsCl. Physicochemical properties have not been fully established for all members of the family and may not be common to all. Rabbit hemorrhagic disease virus is reported to be stable over a wide range of pH values (4.5–10.5). Noroviruses are acid, ether, and relatively heat stable. Vesiviruses are inactivated at pH 3–5, thermal inactivation is accelerated in high concentrations of Mg^{2+} ions, and virions are insensitive to treatment with ether, chloroform, or mild detergents.

Complete genomic sequences have been determined for a least one member of each genus. The phylogenetic

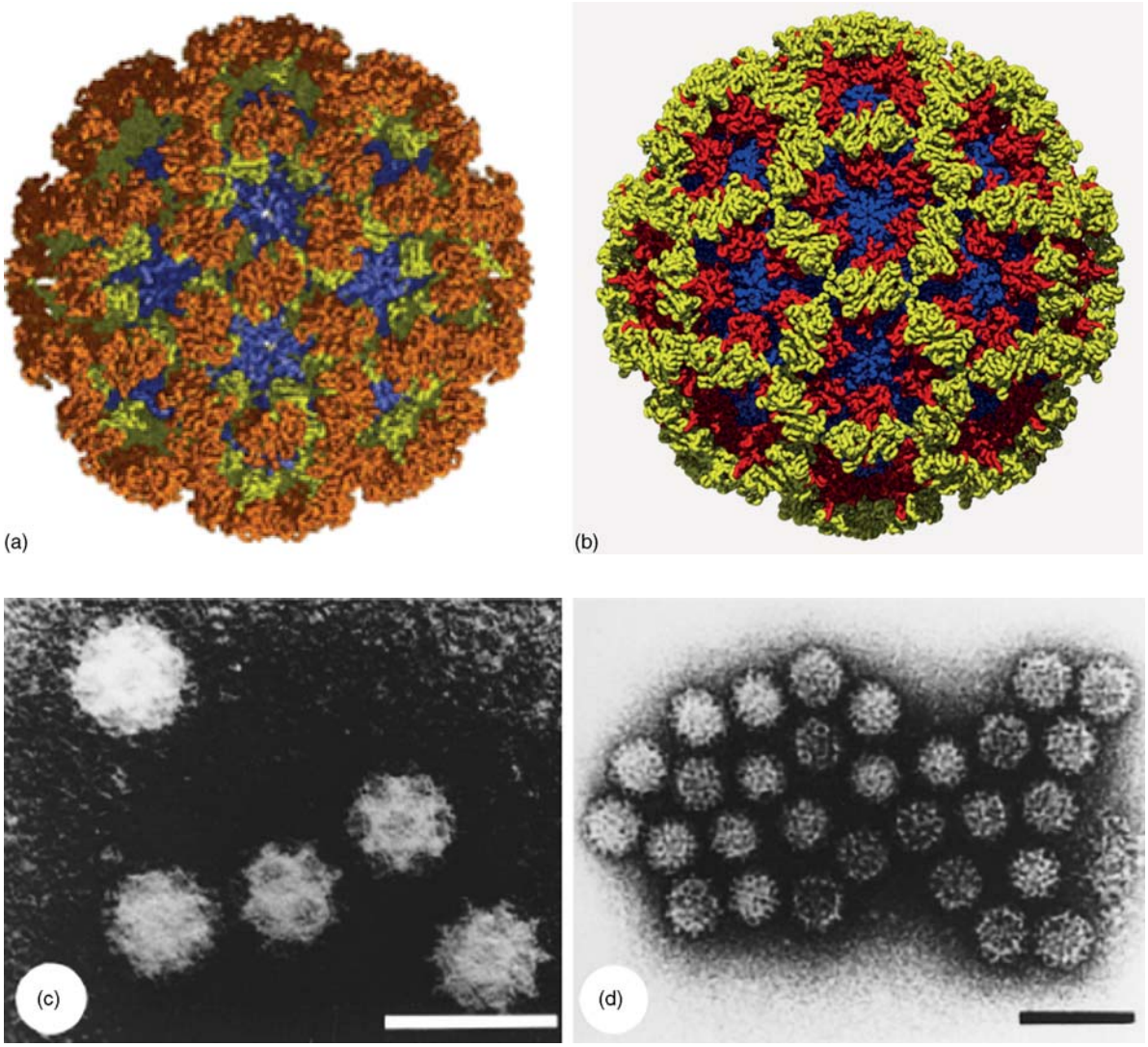


Figure 1 (a) X-ray structure of the capsid of San Miguel Sea Lion virus. The S, P1, and P2 domains of the capsid protein are colored in blue, yellow, and darkish orange respectively. (b) X-ray structure of the capsid of Norwalk virus. The S, P1, and P2 domains of the capsid proteins are colored in blue, red, and yellow respectively. (c) Negative contrast electron micrograph of Norwalk virus in a human fecal sample. (d) Negative contrast electron micrograph of rabbit hemorrhagic disease virus in a rabbit liver homogenate. Scale = 100 nm (c,d). Images (a, b) provided by Dr. B. V. Venkataram Prasad, Department of Biochemistry and Molecular Biology, Baylor College of Medicine. Images (c, d) from Dr. A. Z. Kapikian (Norwalk) and M. König and H.-J. Thiel (RHDV), respectively and are adapted from the *seventh Report, International Committee on the Taxonomy of Viruses*, Academic Press, 2000.

relationships among the genera and viruses are shown in (**Figure 2**). The RNA genomes (**Figure 3**) consist of a linear, positive-sense, ssRNA molecule of 7.3–8.6 kb. A 10–15 kDa protein, designated VPg, is covalently attached to the 5′-end of the genomic RNA and the 3′-end is polyadenylated. Viruses of each genus have a characteristic open reading frame (ORF) arrangement but differences are found between the genera. In general, the nonstructural proteins are encoded at the 5′-end of the genome and the structural proteins at the 3′. For lagoviruses and sapoviruses the nonstructural proteins and the major structural protein are expressed as a single polyprotein encoded by ORF1, while for the vesiviruses and noroviruses the nonstructural polyproteins and the structural capsid protein are separately encoded by ORF1 and ORF2, respectively.

The nonstructural proteins 2C NTPase, 3C trypsin-like serine protease, and 3D RNA-dependent RNA polymerase are somewhat similar in both sequence identity and relative position, to similar proteins found in picornaviruses. There are helicase motifs at the 5′-end of the genome but no helicase activity has been detected. The individual nonstructural proteins are released from the polyprotein by specific viral protease cleavage in a cascade similar to that of picornaviruses. Viruses from all four genera also possess an ORF at the 3′-end of the genome, which is ORF3 for vesiviruses and noroviruses and ORF2 for lagoviruses and sapoviruses. The 3′ encoded protein is a small basic protein believed to be a minor structural protein. Some sapoviruses also possess an additional ORF (designated ORF3) contained completely within the capsid-encoding gene. However, the biological significance of this ORF is unclear.

Vesiviruses have two polyadenylated RNA species, one corresponding to the genome of 7.6 kb and the second, a subgenomic species of 2.4 kb, that is bicistronic encoding ORF2 and ORF3. The subgenomic RNA, like the full length genomic RNA, is VPg-linked. Feline calicivirus subgenomic RNA can be packaged into virions. Similar polyadenylated RNA species of 7.5 and 2.2 kb are recognized for rabbit hemorrhagic disease virus and both species are packaged into virions, either the same or separate virions. The 5′-ends of the genomic and subgenomic RNAs show high levels of sequence conservation. The 5′ untranslated region (UTR) of the caliciviruses is relatively short comprising 4 to 21 nucleotides.

Geographic Distribution

As noted, vesicular exanthema was eradicated in 1956 from the United States. Other marine vesiviruses, including San Miguel sea lion virus, are endemic in pinniped species and have been particularly described as occurring

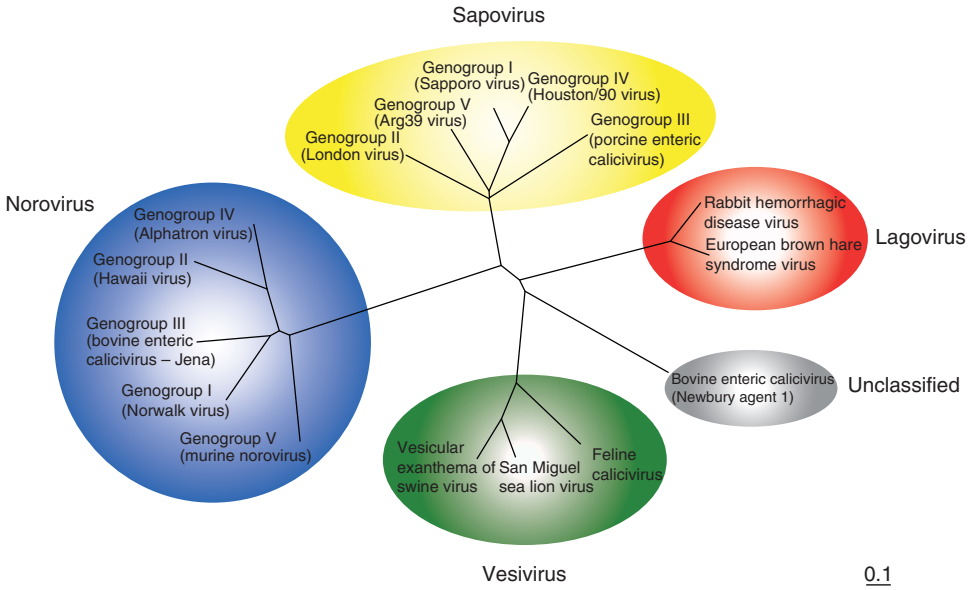


Figure 2 Phylogenetic relationships among members of the family *Caliciviridae*. Phylogenetic analysis was performed using nucleotide sequences of the full capsid encoding region of representative caliciviruses. This unrooted maximum-likelihood tree is drawn with branch lengths to scale, and the scale bar indicating 0.1 substitutions per site. Shading indicates genera. Assignment into genera is supported by high bootstrap values (not shown). The following viruses were used in the analysis: vesicular exanthema of swine virus (A48, GenBank accession U76874), San Miguel sea lion virus (serotype 1, M87481), feline calicivirus (F9, Z11536), rabbit hemorrhagic disease virus (FRG, M67473), European brown hare syndrome virus (Z69620), Norwalk virus (M87661), Hawaii virus (U07611), bovine enteric calicivirus (Jena, AJ011099), Alphatron virus (AF195847), murine norovirus (AY228235), Sapporo virus (U65427), London virus (U95645), porcine enteric calicivirus (AF182760), Houston/90 virus (U95644), Arg/39 virus (AY289803), bovine enteric calicivirus (Newbury agent 1, NC_007916).

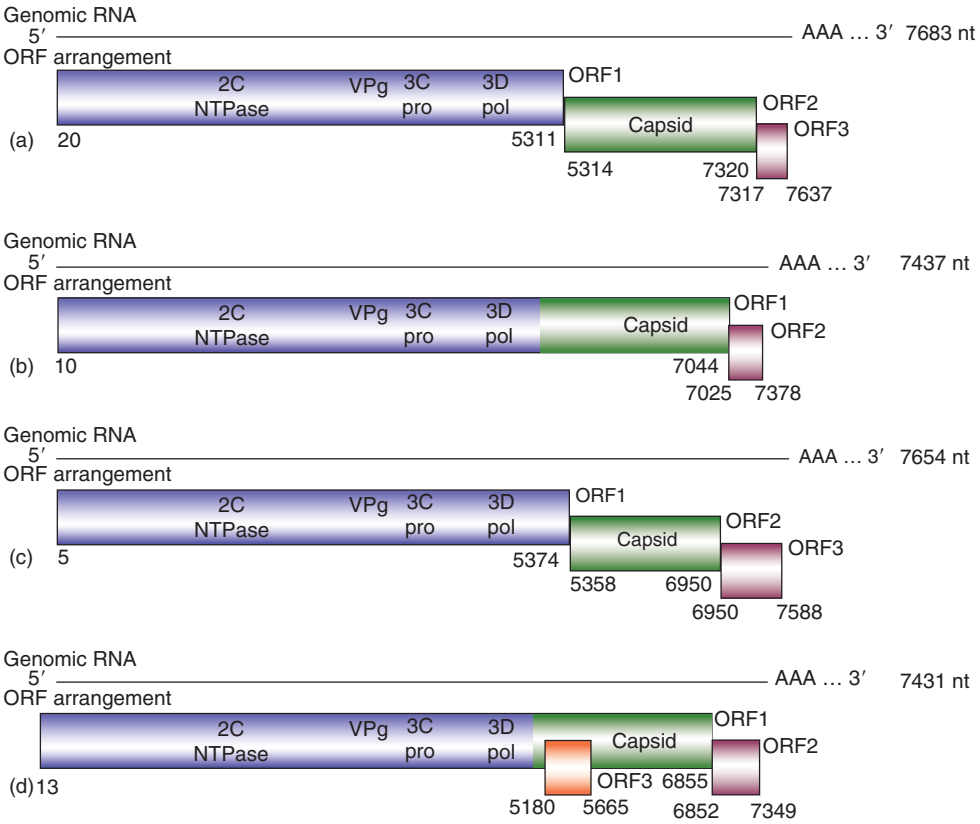


Figure 3 Comparative genome organization of the caliciviruses. The four calicivirus genera illustrated are: (a) *Vesivirus* represented by feline calicivirus (Urbana, GenBank accession L40021); (b) *Lagovirus* represented by rabbit hemorrhagic disease virus (FRG, M67473); (c) *Norovirus* represented by Norwalk virus (M87661); and (d) *Sapovirus* represented by Manchester virus (X86560). Numbers below the ORFs correspond to the first and last nucleotides. Amino acid motifs in the nonstructural polyprotein correspond to the 2C NTPase, VPg, 3C protease (pro), and 3D RNA-dependent RNA polymerase (pol). The regions of the genomes are shown as follows: blue region – nonstructural polyprotein; green region – capsid protein; purple region – 3′ terminal ORF; orange region – sapovirus ORF3.

along the western seaboard of North America. Feline calicivirus is a very common cause of respiratory disease of cats throughout the world. Rabbit hemorrhagic disease has occurred in China, Eastern and Western Europe, North Africa, Korea, Mexico, Cuba and is now endemic in Australia and New Zealand.

Human noroviruses have a worldwide distribution and have been detected in association with outbreaks and sporadic cases of gastroenteritis on all permanently inhabited continents. Sapoviruses also have a global distribution but are less frequently detected than the noroviruses and are often linked to gastroenteritis in pediatric patients.

Host Range and Virus Propagation

Caliciviruses infect a broad range of animals that include pigs, rabbits, hares, cats, pinnipeds, cetaceans, mice, cattle, reptiles, skunks, chimpanzees, and humans. Although individual calicivirus species generally have a natural host restriction, vesicular exanthema of swine virus appears an exception and has been isolated from several marine animal species (including fish), birds, reptiles, and land mammals. The geographic distribution of each calicivirus species usually reflects the host distribution. A probable calicivirus has been isolated from dogs with a vesicular genital disease.

Pigs are a significant alternative host for at least some of the pinniped caliciviruses. The extent to which pinniped and perhaps other marine species share caliciviruses as hosts has not been fully defined. Feline calicivirus is restricted to members of the family *Felidae* although most commonly, infections are recognized in domestic cats. Rabbit hemorrhagic disease virus, in addition to infecting rabbits, *O. cuniculus* and other *Oryctolagus* species, is known to infect and cause disease in the European brown hare, *Lepus europaeus* and some other *Lepus* species. Noroviruses and Sapoviruses are primarily associated with human gastroenteritis, but infections of other animal species including pigs and cattle occur. It is unlikely that filter-feeding bivalve marine species (mollusks) such as oysters and clams, which are common sources of human infection,

usually as a consequence of contamination with human feces, support virus replication.

The pig, pinniped, and feline viruses grow readily and rapidly in monolayer cell cultures derived from pig and feline tissues respectively; most strains of vesicular exanthema virus grow in Vero cells. Rabbit hemorrhagic disease virus, human noroviruses, and human sapoviruses have not been cultivated in conventional cell cultures. However, the porcine enteric sapovirus has been cultivated in cell cultures when gnotobiotic pig intestinal contents are added to the growth medium and a 3D model of human intestinal epithelium has been shown to support norovirus replication.

The construction of an infectious cDNA clone of Norwalk virus has been described. When Norwalk virus cDNA and a replication-deficient vaccinia virus were co-transfected into mammalian cells, Norwalk viral RNA was replicated and packaged into virus particles. Norwalk virus subgenomic RNA was transcribed from genomic RNA using nonstructural proteins expressed from genomic RNA and was translated into the major capsid protein VP1. An infectious clone for rabbit hemorrhagic disease virus has also been described.

Serologic Relationships and Variability

Based on serum neutralization assays and cross-protection studies in pigs, vesicular exanthema virus has a very high number of antigenic types. It was not uncommon for more than one type to be isolated during a single outbreak of disease or indeed for more than one virus type to be isolated from a single pig. The exact number of types cannot be recorded with certainty since some of the early collections of viruses were lost, although at least 13 distinct antigenic types exist in one collection of viruses.

A similar pattern of antigenic variation (types) was initially recognized among feline caliciviruses. When rabbit antiserums were used in serum neutralization assays, a large number of different antigenic types were identified in collections examined by the few individual laboratories that attempted these studies; no single set of typing criteria has been used to examine all feline caliciviruses. Curiously, however, when antisera raised in specific pathogen-free cats were used in virus neutralization assays to examine reasonably large collections of feline caliciviruses, while considerable antigenic variation was recognized, extensive cross-reactions were identified essentially between all viruses. It was concluded that feline caliciviruses were related as a single antigenic type and these findings paved the way for the development of monotypic, later bivalent, vaccines.

Antigenic variation among rabbit hemorrhagic disease virus isolates has not been much studied primarily because of the lack of a cell culture system; however, monotypic vaccines appear to be fully protective. Rabbit hemorrhagic disease and European brown hare syndrome viruses are antigenically distinct.

Several attempts have been made to define antigenic types for noroviruses and sapoviruses using methods including cross-challenge studies in human volunteers, immune electron microscopy, and ELISA using antisera made to virus-like particles (VLPs). Originally, four antigenic types of human noroviruses were proposed: Norwalk, Hawaii, Snow Mountain, and Taunton viruses; other schemes have also been proposed, but a unified system for serotyping based on neutralization is not available.

The most comprehensive information about variability of noroviruses and sapoviruses comes from the sequencing of various regions of genomic RNA from a large number of strains. As might be expected for a single-stranded RNA virus, much sequence variation is observed. Available data suggest the division of both genera into several distinct 'genogroups'. Noroviruses have been divided into at least five genogroups, with the human viruses in genogroups I, II, and IV, the bovine noroviruses in genogroup III, and murine norovirus in genogroup V. Sapoviruses fall into at least five genogroups. Within the genogroups, viruses have been further divided into 'clusters'. Because of the lack of appropriate cell culture systems or standardized serological reagents, the correlation between the genogroup and cluster designation of a virus and its antigenic type has not been completely established.

Genetic recombination resulting in chimeric viruses has been observed for noroviruses, sapoviruses, and feline calicivirus. For noroviruses the most commonly identified recombination site is the ORF1–ORF2 junction. There are two features of this site that predispose it to recombination. First, the site is highly conserved among different viruses, increasing the likelihood of homologous recombination between separate viruses. Second, it is the putative beginning of subgenomic RNA, which may itself play a role in recombination. Recombination is believed to occur by a copy-choice mechanism. A similar form of recombination was originally described for picornaviruses, specifically poliovirus. Copy choice occurs during viral replication when the viral RNA-dependent RNA polymerase switches templates from the RNA of one parent virus to the RNA of a second parent.

Epidemiology

Vesicular exanthema was initially and on an ongoing basis transmitted to pigs by the feeding of uncooked pinniped carcass meat and additional transmission occurred by feeding uncooked garbage containing pork scraps. Within a herd, pig-to-pig transmission occurred since

ruptured vesicles shed large quantities of virus into the environment such that transmission would readily occur by contact or via fomites.

Feline caliciviruses are transmitted by contact and particularly by sneezing when cats are closely confined as in multiple cat households, breeding and boarding establishments, cat shelters, and veterinary hospitals. Recovered cats remain persistently infected with virus for many months or years presumably as a consequence of low-grade infection in pharyngeal tonsillar tissues. True long-term persistent infection in individual cats is relatively rare. Progressive genetic variation following primary infection and perhaps even more importantly, in persistently infected cats leads to a gradual increase in the diversity of a given strain of virus, both in the individual and in the population, until eventually new strains emerge.

Rabbit hemorrhagic disease virus is readily transmitted between rabbits via a fecal–oral route and over longer distances apparently by fomites including contaminated vehicles. Insects were believed to have played a role as mechanical vectors in the escape and subsequent dissemination of rabbit hemorrhagic disease virus from an offshore island location to the mainland of Australia. Infected rabbit carcass meat could be carried over considerable distances by predatory and carrion-eating mammals and birds.

Noroviruses and sapovirues are transmitted via a fecal–oral route. Transmission often results from consumption of fecally contaminated water or food; bivalve filter-feeding mollusks (oysters, clams) are commonly implicated. However, transmission from person to person in semiclosed communities such as nursing homes, hospitals, and cruise ships is also common. Short-distance aerosol transmission following vomiting has been suggested. Common source outbreaks associated with food and water contamination have involved up to 2000 individuals. In more recent years, cruise ships have emerged as sites for many outbreaks, causing major problems for the cruise ship industry and ruining holidays for passengers. Hospital ward closures following norovirus outbreaks cause major disruptions.

Currently, noroviruses belonging to genogroup II, especially cluster 4, are the predominant type circulating worldwide. However, other types continue to circulate concurrently. Several variants of the genogroup II cluster 4 viruses, correlated with an increase in the detection of norovirus-associated gastroenteritis in various regions around the world, have been described.

Pathogenesis

Vesicular exanthema virus gains entry via abrasions usually around the snout and mouth or on the feet. Secondary vesicles may occur as a result of direct local spread or following viremia. Abortion may occur and death of baby pigs from agalactia in their dams also occurs.

Feline calicivirus produces vesicular lesions on the muzzle, and within the oral cavity and the respiratory tract. These tend to rupture quickly. During the past decade a more severe disease termed virulent-systemic (VS) feline calicivirus disease has been recognized. No genetic or *in vitro* diagnostic methods to distinguish viruses isolated from cases of VS disease from other isolates have been described. Phylogenetic analyses and alignments of the capsid and protease–polymerase regions of the genome did not reveal any conserved changes that correlated with virulence and the VS isolates did not segregate into a distinct cluster. These results suggest that VS isolates arise independently and at multiple sites. VS isolates spread more efficiently in cell culture than other isolates when cultures are inoculated at low multiplicity, although the overall growth kinetics of both standard strains and VS isolates are very similar.

Rabbit hemorrhagic disease is a generalized infection in which viremic spread results in lesions in a wide range of tissues. At postmortem lesions are particularly evident as hemorrhagic necrosis of the liver and lung. The key to the pathogenesis is massive liver necrosis leading to disseminated intravascular coagulation.

For noroviruses the incubation period is 24–48 h (range 10–77 h) and illness usually lasts 24–48 h. Virus shedding as detected by immune electron microscopy coincides with the onset of symptoms and stops at about 72 h. By reverse transcription polymerase chain reaction (RT-PCR) virus shedding has been detected for up to 7 days, although, in one case, an otherwise healthy child excreted norovirus for 60 days. Excretion times are even longer in immunocompromized individuals, 156 days in one instance. Asymptomatic virus excretion occurs in some individuals.

Virions are acid stable and hence safely traverse the stomach to reach the site of primary replication, which is assumed to be the upper intestinal tract. Based on human volunteer studies biopsy samples from the jejunum show that there is a broadening and blunting of the intestinal villi although curiously the epithelial cells remain intact; virus has not been detected in these cells by thin section electron microscopy. There is a shortening of microvilli, infiltration of mononuclear cells, and cytoplasmic vacuolation.

There is evidence to indicate that carbohydrate histo-blood group antigens are receptors for noroviruses. The histo-blood group antigens, first described on the surface of red blood cells, are also expressed on the surface of epithelial cells. First, a correlation has been observed between the ABO histo-blood group and susceptibility. Second, norovirus particles, either native virions or VLPs, directly bind to the blood group antigens *in vitro*. Different norovirus strains may utilize histo-blood group

antigens encoded for by different alleles as receptors. Therefore, susceptibility alleles for infection with one strain are likely to be different to susceptibility alleles for infection with another strain, unless the strains are closely related.

There is evidence that rabbit hemorrhagic disease virus may also use histo-blood group antigens as receptors. Hemagglutination of human erythrocytes and binding to synthetic blood group oligosaccharides was demonstrated for rabbit hemorrhagic disease virus using both native virions and recombinant VLPs. Rabbits less than 2 months of age are reported to express negligible amounts of histo-blood group antigens on the surface of epithelial cells. This observation may explain the resistance of young rabbits to fatal infection. Feline junctional adhesion molecule 1 (JAM-1), a glycoprotein belonging to the immunoglobulin superfamily, was shown to act as a receptor for feline calicivirus.

Clinical Signs

Vesicular exanthema of pigs is clinically indistinguishable from the other three, so-called vesicular diseases that affect pigs, that is, foot and mouth disease, vesicular stomatitis, and swine vesicular disease. Following an incubation period of 12–48 h there is a marked febrile response, anorexia, and listlessness. Primary vesicles are blanched, raised areas of epithelium up to 3 cm in diameter and up to 1 cm high filled with a serous virus-rich fluid. They easily rupture leaving raw bleeding ulcers that subsequently become covered with a fibrinous pseudomembrane. Secondary vesicles appear 48–72 h post infection. Notably these appear on the soles of the feet and in the interdigital space and at the coronary band. There is severe four-footed lameness. Secondary bacterial infection of lesions, particularly of the feet, occurs and prolongs recovery where slaughter and eradication are not pursued.

Feline calicivirus infection may produce subclinical, acute, or subacute disease. Symptoms include conjunctivitis, rhinitis, tracheitis, pneumonia (usually in young kittens) and vesiculation/ulceration of the epithelium of the oral cavity and muzzle. There is fever, anorexia, lethargy, stiff gait, and usually a profuse ocular and nasal discharge. Morbidity is high and mortality in untreated cases may reach 30%. The virulent systemic disease is characterized by high, persistent fever, anorexia, depression, facial and limb edema, sores or alopecia on the face, pinnae and paws, pulmonary edema, coagulation abnormalities, pancreatitis, and hepatic necrosis.

Intriguingly, rabbit hemorrhagic disease does not generally affect rabbits less than 2 months of age even where there is no maternal antibody protection. As indicated earlier, there is evidence that rabbits less than 2 months of age do not express histo-blood group antigens that are believed to be the receptors for the virus. The disease is often peracute, characterized by sudden death following a 6–24 h period of depression and fever. Infection is via a fecal–oral route. Morbidity rates of 100% and mortality rates of 90% are observed in rabbits older than 2 months. At postmortem there is congestion and hemorrhage in the lungs, with accentuated lobular markings, necrosis of the liver, and splenomegaly. Massive blood clots are present throughout the vasculature.

Norovirus and sapovirus gastroenteritis usually lasts 24–48 h although more severe episodes requiring medical intervention occur. Clinical signs may include nausea, vomiting, diarrhea, abdominal cramps, headache, fever, chills, and myalgia.

Immunity

Recovered pigs are immune to the particular antigenic types of vesicular exanthema virus with which they were infected but not to other types. Since slaughter and eradication policies are pursued, the questions of long-term immunity and vaccine development are not at issue.

Cats recovered from feline calicivirus infection or immunized with feline calicivirus vaccine appear to remain relatively free of disease following subsequent challenges. This appears to be the case despite the considerable degree of antigenic variability recognized among feline caliciviruses. It is practice to recommend annual vaccination.

Vaccines have been developed for rabbit hemorrhagic disease and are widely used particularly in those many countries such as China and Italy where rabbit farming for meat and pelt production are major industries.

The basic features of calicivirus immunity have been best studied for feline calicivirus. Antibody and cell-mediated immune responses, including the generation of cytotoxic T-lymphocyte responses, have been described. Chromium release assays for cytotoxic T lymphocytes in which autologous feline fetal kidney monolayer cell cultures were used as target cells have been reported. Using these assays the nature and kinetics of the cytotoxic T-cell responses following vaccination and challenge in kittens were described.

Immunity to norovirus and sapovirus is not well understood since there is no cell culture system for assaying neutralizing antibody. Much of the early understanding of immunity to these viruses comes from volunteer studies. There is evidence that immunity may not be durable and that the presence of serum antibody does not invariably correlate with protective immunity. As the putative histo-blood group antigen receptors are variable within the human population and different virus strains bind to different allelic forms of these molecules, innate

resistance to infection with certain strains may occur. It has been suggested that the distribution of susceptibility alleles among human populations may go some way to explain the recent predominance of the genogroup II, cluster 4 noroviruses globally. Due to their preference for carbohydrate attachment molecules, it appears that these viruses have a wider host range among humans and may therefore spread more efficiently.

The recently identified murine norovirus has been used as a model for studying norovirus immunity. These studies point to a critical role of the innate immune response, an observation that may help explain the relatively short clinical course of a norovirus disease. However, clearance of the virus requires an adaptive immune response. Shedding norovirus may occur for some time after clinical symptoms have resolved.

Prevention and Control

Vesicular exanthema is effectively controlled by slaughter and is now considered an extinct virus disease. A real time PCR that detects a wide range of available mammalian and pinniped vesiviruses has been described.

Feline calicivirus infection is most difficult to control in large open cat populations. Vaccination is an important means of control. Clinically ill cats should be isolated and incoming cats of uncertain status should be held in isolation for at least a week before being introduced into the general colony. Although recovered cats remain persistently infected, the amount of virus spread is not usually large, so they do not pose the same threat to in-contact cats as cats with obvious clinical disease.

Preventing entry of rabbit hemorrhagic disease virus into commercial rabbitries, either via fomites or via infected wild rabbits, creates a major challenge in control. Where feed and other supplies are delivered to multiple farms in a common delivery run, special care is required. Feeding pellets that have been sterilized should minimize transmission. Vaccines for the control of the disease are prepared as inactivated, adjuvanted homogenates of infected rabbit tissues. VLPs produced by recombinant DNA technology in baculovirus, yeast, or plant expression systems are effective as vaccines following parenteral or oral administration but are not available commercially.

As noted, norovirus and sapovirus produce mild self-limiting gastroenteritis that normally resolves without complications or the need for treatment. Oral rehydration and electrolyte replacement delivered orally may be required. Complications, sometimes with fatal outcomes, are seen in elderly or immunocompromised patients. A key to prevention is meticulous attention to hygiene, which should include effective hand washing and disposal or disinfection of contaminated materials. Hygiene in food preparation is important. Depuration of oysters is not adequate to clear them of norovirus. Therefore, prevention of contamination of oyster beds with human feces or vomitus is of critical importance. More simply, oysters and other shellfish should be cooked before human consumption and detailed protocols for such procedures have been developed by the US Food and Drug Administration. Vaccines based on VLPs are in various stages of development but none is at present licensed for human use. Such vaccines would have important applications in settings such as the cruise ship industry, hospitals, nursing homes, military and various other institutions as well as in reducing the incidence of diarrhea in young children in both the developed and developing world.

Future

Vigilance is required to avoid the reemergence of vesicular exanthema of swine either from marine sources or from laboratory escape sources. The host range of the virus strains currently known requires constant monitoring, as does the emergence of variant strains. A clearer understanding of the molecular basis for virulence remains a major challenge. The molecular basis for antigenic variation and variation in virulence among feline caliciviruses remain important objectives. There is a sense that currently used vaccines appear effective but require ongoing validation. The nature of the carrier state and its distinction from reinfection requires further understanding.

Further details of the replication cycle of caliciviruses in general should continue to emerge in the coming few years. Further comparative sequence analysis among caliciviruses will be as interesting and fascinating as it has been thus far. The taxonomic status of the unclassified bovine Newbury agent 1, which may require the establishment of a fifth calicivirus genus, should be resolved. In addition, a universally accepted classification scheme for the noroviruses and sapoviruses needs to be established. Such a scheme would need to reconcile the phylogenetic ambiguities resulting from recombinant viruses.

The need to cultivate rabbit hemorrhagic disease virus is a high priority although this has been somewhat abrogated by the availability of an infectious cDNA clone.

For those countries such as Australia and New Zealand where wild rabbits are a plague upon the nations, reducing profits from farming and degrading the land, continuing assessment and enhancement of the effectiveness of rabbit hemorrhagic disease virus to control rabbits should be an ongoing priority. The emergence of a ‘smooth’ virion, possibly nonvirulent, form of rabbit hemorrhagic disease virus in Europe, presumably as mutation rather than a phenotypic change due to enzymatic digestion of outer peptide residues of the virion surface, may be one of many factors that in the long term diminish the effectiveness of

rabbit hemorrhagic disease virus as a biocontrol agent. In Australia in many areas rabbit numbers have declined precipitously (>60%) and there is evidence for the restoration of original habitats and species in unfarmed areas. It is too early to assess the long-term effectiveness and benefits following the introduction of the virus into Australia and New Zealand but it is likely that it, together with other established methods of control including myxomatosis (in Australia), will bring long-term benefit. The use of rabbit hemorrhagic disease virus as a biocontrol agent has caused conflict among farming groups and animal welfare groups on the one hand and those other greens among us concerned with the preservation and return of the environment to its more natural state as was before the arrival of the rabbit in Australia and New Zealand. The paradox created by the use of the virus as a lethal biocontrol agent and the recovery of some environments to near pristine state with the reappearance of native plant and animal species that were either much depleted or considered extinct will continue to be discussed. As in much of the rest of the world it is now essential in Australia and New Zealand to have effective vaccination programs in place to protect farmed, pet, and laboratory rabbits. In contrast to the situation in the Antipodes, the preservation of wild rabbit populations for hunting purposes throughout all of Europe has been a contentious political issue and the mass immunization of these wild populations will be considered.

The availability of infectious cDNA clones to study the replication of the noncultivable norovirus, sapovirus, and rabbit hemorrhagic disease virus will bring a better understanding of the biology of these viruses.

Further Reading

Asanaka M, Atmar RL, Ruvolo V, Crawford SE, Neill FH, and Estes MK (2005) Replication and packaging of Norwalk virus RNA in cultured mammalian cells. *Proceedings of the National Academy of Sciences, USA* 102: 10327–10332.

Chen R, Neill JD, Estes MK, and Prasad BV (2006) X-ray structure of a native calicivirus: Structural insights into antigenic diversity and host specificity. *Proceedings of the National Academy of Sciences, USA* 103: 4048–4053.

Coyne KP, Gaskell RM, Dawson S, Porter CJ, and Radford AD (2006) Evolutionary mechanisms of persistence and diversification of a calicivirus within endemically infected natural host populations. *Journal of Virology* 81: 1961–1971.

Dingle KE, Lambden PR, Caul EO, and Clarke IN (1995) Human enteric *Caliciviridae:* The complete genome sequence and expression of virus-like particles from a genetic group II small round structured virus. *Journal of General Virology* 76: 2349–2355.

Green KY, Chanock RM, and Kapikian AZ (2001) Human caliciviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 841–874. New York: Lippincott Williams and Wilkins.

Guo M, Chang KO, Hardy ME, Zhang Q, Parwani AV, and Saif LJ (1999) Molecular characterization of a porcine enteric calicivirus genetically related to Sapporo-like human caliciviruses. *Journal of Virology* 73: 9625–9631.

Jiang X, Wang M, Wang K, and Estes MK (1993) Sequence and genomic organization of Norwalk virus. *Virology* 195: 51–61.

Karst SM, Wobus CE, Lay M, Davidson J, and Virgin HW (2003) STAT1-dependent innate immunity to a Norwalk-like virus. *Science* 299: 1575–1578.

Koopmans MK, Green KY, Ando T, *et al.* (2005) Family *Caliciviridae*. In: Fauquet CM, Maniloff J, D'esselberger U, and Ball DA (eds.) *Virus Taxonomy: Eigth Report of the International Committee on Taxonomy of Viruses*, pp. 843–851. San Diego, CA: Elsevier Academic Press.

Oliver SL, Asobayire E, Dastjerdi AM, and Bridger JC (2006) Genomic characterization of the unclassified bovine enteric virus Newbury agent-1 (Newbury1) endorses a new genus in the family *Caliciviridae*. *Virology* 350: 240–250.

Ossiboff RJ, Sheh A, Shotton J, Pesavento PA, and Parker JS (2007) Feline caliciviruses (FCVs) isolated from cats with virulent systemic disease possess in vitro phenotypes distinct from those of other FCV isolates. *Journal of General Virology* 88: 506–527.

Prasad BV, Hardy ME, Dokland T, Bella J, Rossmann MG, and Estes MK (1999) X-ray crystallographic structure of the Norwalk virus capsid. *Science* 286: 287–290.

Studdert MJ (1978) Caliciviruses. Brief review. *Archives of Virology* 58: 157–191.

Tham KM and Studdert MJ (1987) Antibody and cell-mediated immune responses to feline calicivirus following inactivated vaccine and challenge. *Zentralbl Veterinarmed B* 34: 640–654.

Wirblich C, Thiel HJ, and Meyers G (1996) Genetic map of the calicivirus rabbit hemorrhagic disease virus as deduced from *in vitro* translation studies. *Journal of Virology* 70: 7974–7983.

Capillovirus, *Foveavirus*, *Trichovirus*, *Vitivirus*

N Yoshikawa, Iwate University, Ueda, Japan

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Alfavirus A genus in the family *Togaviridae*. The type species is *Sindbis virus*.

Helical symmetry A form of capsid structure found in many RNA viruses in which the protein subunits which interact with the nucleic acid form a helix.

Methyltransferase Enzyme activity involved in capping of viral mRNAs.

Movement protein A virus-encoded protein which is essential for the cell-to-cell movement of the virus in plant tissues.

Semipersistent manner The relationship between a plant virus and its arthropod vector which is

intermediate between nonpersistent manner and persistent manner. It has the features of short acquisition feed and no latent period found in nonpersistent manner, but the vector remains able to transmit the virus for periods of hours to days which is longer than the nonpersistent manner.

Stem grooving Deformation of the normally smooth surface of a trunk cased by its furrowing.

Stem pitting A plant disease characterized by the formation of larger or smaller depression in the old wood, between the phloem and the xylem of the tree trunk.

Subgenomic RNA A species of RNA less than genomic length found in infected cells. Viral genomic RNA codes for several proteins but all the 3′ open reading frames will effectively be closed for translation. The formation of subgenomic RNAs overcomes this problem as each species has a different cistron at its 5′ end, thus opening it for translation.

Genus *Capillovirus*

The genus *Capillovirus* contains three species – *Apple stem grooving virus* (ASGV, the type species), *Cherry virus A* (CVA), and *Lilac chlorotic leafspot virus* (LiCLV) – and a tentative species – Nandina stem pitting virus (NSPV) (**Table 1**). Citrus tatter leaf virus (CTLV) from citrus and lily is indistinguishable from ASGV from Rosaceae fruit trees biologically, serologically, in genome organization, and in nucleotide sequence, and these days CTLV is regarded as an isolate of ASGV.

Biological Properties

ASGV occurs worldwide in Rosaceae fruit trees, including apple, European pear, Japanese pear, Japanese apricot and cherry, and it is usually symptomless. However, the virus causes stem grooving, brown line, and graft union abnormalities in Virginia Crab, and it causes topworking disease of apple trees grown on Mitsuba kaido (*Malus sieboldii*) in Japan. ASGV is also widespread in citrus, and it induces bud union abnormalities of citrus trees on trifoliate orange. It also infects lily. CVA occurs in cherry trees in Germany and Japan, but it probably is not associated with any disease. LiClV and NSPV occur in England and the United states, respectively.

No vectors of any of these viruses have been reported. ASGV has been known to be transmitted through seeds to progeny seedlings of lily (1.8%) and *Chenopodium quinoa* (2.5–60%).

Particle Structure

Virions are flexuous filamentous particles 670–700 nm long and 12 nm in diameter, with obvious cross-banding and helical symmetry and a pitch of *c.* 3.8 nm (**Figure 1**). Virus particles are composed of a linear positive-sense ssRNA, 6.5–7.4 kbp, and a single polypeptide species of M_r 24–27 kDa. The 3′ terminus of the RNA has poly A-tail and the 5′ terminus probably has a cap structure.

Genome Organization and Replication

The complete nucleotide sequences (6496 bases) of the single RNA genome of three ASGV isolates have been determined: isolate P-209 from apple, and isolates L and

Table 1 Virus species in the genus *Capillovirus, Foveavirus, Trichovirus, Vitivirus* in the family *Flexiviridae*

Genus	*Species*	*Sequence accession numbers*
Capillovirus	*Apple stem grooving virus* (ASGV)	D14995, D16681, D16368, D14455, AB004063
	Cherry virus A (CVA)	X82547
	Lilac chlorotic leafspot virus (LiCLV)	
	Nandina stem pitting virus (NSPV)[a]	
Foveavirus	*Apple stem pitting virus* (ASGV)	D21829, AB045731, D21828
	Apricot latent virus (ApLV)	AF057035
	Rupestris stem pitting-associated virus (RSPaV)	AF026278, AF057136
Trichovirus	*Apple chlorotic leaf spot virus* (ACLSV)	M58152, D14996, X99752, AJ243438
	Cherry mottle leaf virus (CMLV)	AF170028
	Grapevine berry inner necrosis virus (GINV)	D88448
	Peach mosaic virus (PMV)	
Vitivirus	*Grapevine virus A* (GVA)	X75433, AF007415
	Grapevine virus B (GVB)	X75448
	Grapevine virus D (GVD)	Y07764
	Heracleum latent virus (HLV)	X79270
	Grapevine virus C (GVC)[a]	

[a]Tentative species.

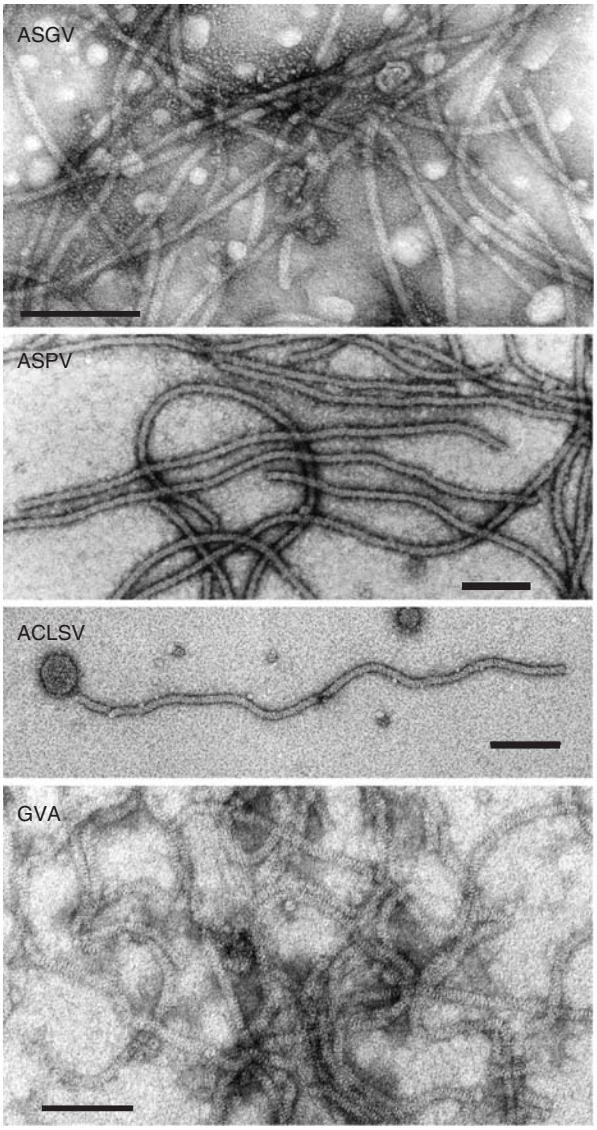


Figure 1 Electron micrographs of particles of *Apple stem grooving virus* (ASGV), the type species of the genus *Capillovirus*; *Apple stem pitting virus* (ASPV), the type species of the genus *Foveavirus* (courtesy of H. Koganezawa); *Apple chlorotic leaf spot virus* (ACLSV), the type species of the genus *Trichovirus*; and *Grapevine virus A* (GVA), the type species of the genus *Vitivirus* (courtesy of J. Imada). Scale = 100 nm.

Li-23 from lilies. Identities of the nucleotide sequences are 82.9% (P-209/L), 83.0% (P-209/Li-23), and 98.4% (L/Li-23). The genomic RNA has the same structural organization and two overlapping open reading frames (ORFs) in the positive strand (**Figure 2**). ORF1 (bases 37–6341) encodes a 241–242 kDa polyprotein (2105 aa) containing the consensus motifs of methyltransferase (Met), papain-like protease (P-pro), nucleotide triphosphate-binding helicase (Hel), RNA polymerase (Pol), and coat protein (CP) in the C-terminal region. The protein has homologies with putative polymerase of the 'alphavirus-like' supergroup of RNA viruses. ORF2 (bases 4788–5747) encodes a 36 kDa putative movement protein (320 aa). A region (amino acid (aa) position 1585–1868) of the ORF1-encoded protein between the polymerase and the CP, that encodes ORF2 in another frame has none of the other functional motifs found in other known plant virus genomes. This region (designated the V-region) shows high variability among isolates and sequence variants. The genome organization of CVA is composed of a 266 kDa polyprotein (ORF1) and a 52 kDa protein (ORF2) located within ORF1. ORF1 encodes the CP (24 kDa) in the C-terminal region. The overall nucleotide sequence identity between CVA and ASGV is 57.6%.

Although the ASGV-CP is located in the C-terminal region of the ORF1-encoded polyprotein and genomic RNA directs the synthesis of a polypeptide of *c.* 200 kDa as a major product in *in vitro* translation, and is immunoprecipitated by antiserum to virus particle preparations, the following evidence suggests that the CP is expressed from a subgenomic RNA. Analysis of double-stranded (ds) RNA from infected *C. quinoa* tissues indicates that all ASGV isolates tested contain five virus-specific dsRNAs (6.5, 5.5, 4.5, 2.0, and 1.0 kbp). The 6.5 kbp species represents the double-stranded form of the full-length genome, whereas the 2.0 and the 1.0 kbp species may be the double-stranded forms of subgenomic RNAs coding for the putative movement protein and the CP, respectively. The 5.5 and 4.5 kbp species are thought to be 5′ co-terminal with the genome. The size of the *Escherichia coli*-expressed protein corresponding to the C-terminal region of the ORF1-encoded protein, which starts with the methionine at aa position 1869, agrees with that of the CP. The single-stranded subgenomic RNAs for movement protein (MP) and CP have also been reported in infected tissues.

In infected *C. quinoa* leaves, the particles occur singly or as aggregates in the cytoplasm of mesophyll and phloem parenchyma cells, suggesting that the replication of the genome and the assembly of the particles may occur in the cytoplasm, although no virus-specific inclusion bodies, such as pinwheels, viroplasmas, or vesicles, have been observed.

Serology

Polyclonal antisera were prepared in rabbits against purified virus or CP expressed in *E. coli*. Enzyme-linked immunosorbent assay was used to detect the virus in fruit trees. Monoclonal antibodies were produced in mice against an isolate from citrus and three selected monoclonal antibodies reacted with all isolates tested, including nine isolates from citrus trees in Japan, four isolates from citrus in the USA, six isolates from Chinese

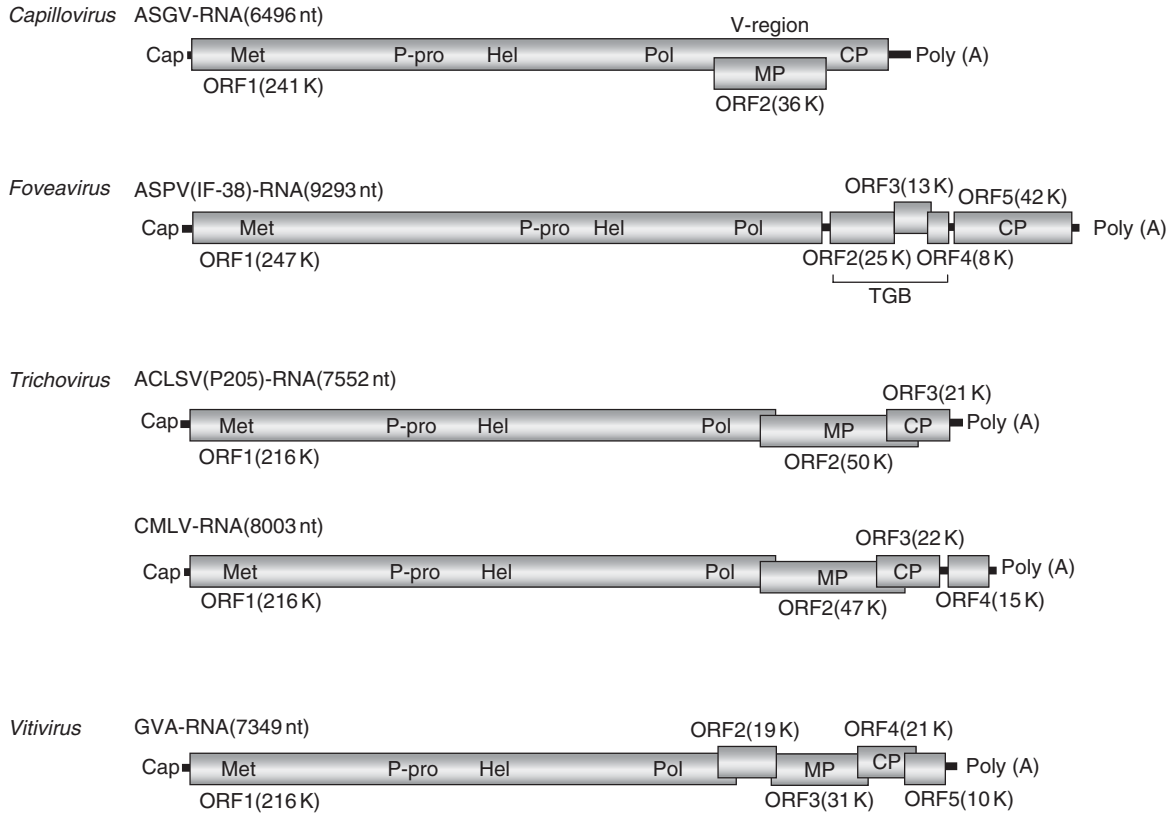


Figure 2 Gemone organization of virus species in the genera *Capillovirus*, *Foveavirus*, *Trichovirus*, and *Vitivirus*. ASGV, apple stem grooving virus; ASPV, apple stem pitting virus; ACLSV, apple chlorotic leaf spot virus; CMLV, cherry mottle leaf virus; GVA, grapevine virus A; Met, methyltransferase; P-pro, papain-like protease; Hel, nucleotide triphosphate-binding helicase; Pol, RNA-dependent RNA polymerase; CP, coat protein; MP, movement protein; and V-region, variable region.

citrus, and isolates from lily, apple, and Japanese apricot. ASGV is serologically unrelated to all known virus species in the genus *Capillovirus*.

Strains and Genome Heterogeneity

Many isolates have been reported from apple, Japanese pear, European pear, Japanese apricot, lily, and citrus plants, but most have not been characterized. Some isolates have been differentiated only on symptomatology. Virus isolates from apple, Japanese pear, and European pear trees comprise at least two to four variants that differ considerably from each other in nucleotide sequence. The composition of sequence variants within a tree differs among leaves from different branches, showing that each sequence variant is distributed unevenly within an individual tree.

Genus *Foveavirus*

The genus *Foveavirus* consists of three species: *Apple stem pitting virus* (ASPV, the type species), *Apricot latent virus* (ApLV), and *Rupestris stem pitting-associated virus* (RSPaV) (**Table 1**).

Biological Properties

ASPV is one of the causative agents of apple topworking disease in Japan and induces lethal decline in apple trees grown on *Malus sieboldii* (Regel) Rehd. rootstock. The virus is usually latent in apple cultivars and is distributed widely in many apple trees. Pear vein yellows is also thought to be caused by this virus. ApLV infects apricot, peach, and sweet cherry, and may be the causal agents of the peach asteroid spot disease and of the peach sooty ringspot diseases. RSPaV is thought to be an agent of rupestris stem pitting, that is probably the most common component of the Rugose wood complex on grapevines. No vectors have been reported for viruses in the genus *Foveavirus,* and the viruses are probably spread in nature by graft-transmission.

Particle Structure

ASPV (isolate B-39) has flexuous filamentous particles, approximately 800 nm in length and 12–15 nm in width (**Figure 1**). Virus particles readily form end-to-end aggregates with four prominent peaks appearing at 800, 1600, 2400, and 3200 nm in length. ASPV is comprised of a single species of RNA of M_r 3.1 $\times$ 10^6 and a major CP of M_r 48 kDa.

Genome Organization and Replication

The genomes of two ASPV isolates (PA66 and IF 38) have been completely determined and found to consist of 9306 and 9237 nucleotide (nt), respectively, excluding the 3′ poly A-tail. The base composition of ASPV genome is 27.6% A, 20.0% C, 23.4–23.8% G, and 28.6–29.0% T. Analysis of the putative ORFs of the nucleotide sequence in both positive and negative strands showed that ASPV genome contains five ORFs in the positive-strand, encoding proteins with M_r's of 247K (ORF1), 25K (ORF2), 13K (ORF3), 7–8K (ORF4), and 42–44K (ORF5) (**Figure 2**). The 5′-noncoding regions of the genome have been reported to be 33 nt (PA66) and 60 nt (IF38). ORF1 encodes a protein (247K) with motifs associated with Met, P-pro, Hel, and Pol (**Figure 2**). ORF2 encodes a protein (25K) containing a helicase motif (GSGKT, aa positions 31–35). ORF3 and ORF4 encode proteins with 13K and 7–8K, respectively. The ORFs 2, 3, and 4 constitute the triple gene block (TGB) found in allexi-, carla-, potex-, and mandariviruses. ORF5 encodes a protein with an M_r of 42–44K, and the sequence of this protein contains the conserved amino acids (R and D) potentially involved in a salt bridge formation that is typical of the CPs of rod-shaped and filamentous plant viruses. The 3′-noncoding region of both genomes of PA66 and IF38 isolates consists of 132 nucleotides, excluding the poly A-tail. The comparison of the nucleotide sequence of the PA66 genome with that of the IF38 genome showed a high level of divergence (76% identity) between the two isolates. Comparisons of aa sequences of five proteins between PA66 and IF38 show the identities of 87% (247K), 94% (25K), 87% (13K), 77% (7–8K), and 81% (42–44K). A hypervariable region was found between the MET and P-Pro domains in the 247K protein. This hypervariable region between MET and P-Pro domains of the 247K protein was also found in the apple chlorotic leaf spot virus (ACLSV) 216K protein, indicating that this region has not undergone severe evolutionary constraint. The 25K protein was mostly conserved between the two isolates. Another striking variability between PA66 and IF38 was found in the N-terminal region of ASPV-CP, in which there were many deletions compared with PA66. This resulted in an aa number of IF38-CP which is 18 aa fewer than that of PA66.

The genome of RSPaV consists of 8726 nt excluding the 3′ poly A-tail and has five potential ORFs on its positive strand which have the capacity to code for the replicase (ORF1; 244 K), the TGB (ORF2–4; 24K, 13K, and 8K), and the CP (ORF5; 28K). The identities of aa sequences of five proteins between RSPaV and ASPV (PA66) are 39.6% (ORF1), 38.0% (ORF2), 39.3% (ORF3), 27.1% (ORF4), and 31.3% (ORF5). Partial sequences of the 3′-terminal regions of ApLV have been reported.

In Northern blot hybridization analysis of virus-specific dsRNAs and ssRNAs in ASPV (IF38)-infected tissues by use of three negative-sense RNA probes complementary to nt positions 6207–6683 (ORF1 region), nt positions 6678–7447 (ORF2 region), nt positions 8717–9293 (CP and the 3′-noncoding regions) of the IF38 genome showed the presence of five dsRNAs (9, 7.5, 6.5, 2.6, and 1.6 kbp) and three ssRNAs (9, 2.6, and 1.6 kbp) in infected tissues (**Figure 3**). The slowest migrating RNA (*c.* 9 kbp) was equivalent to that of the ASPV genome, and other two RNAs (2.6 and 1.6 kbp) are thought to be subgenomic RNAs of ORF2–ORF4 proteins and CP (ORF5), respectively.

In electron microscopy of infected leaves, virus particles were found in mesophyll, epidermal, and vascular

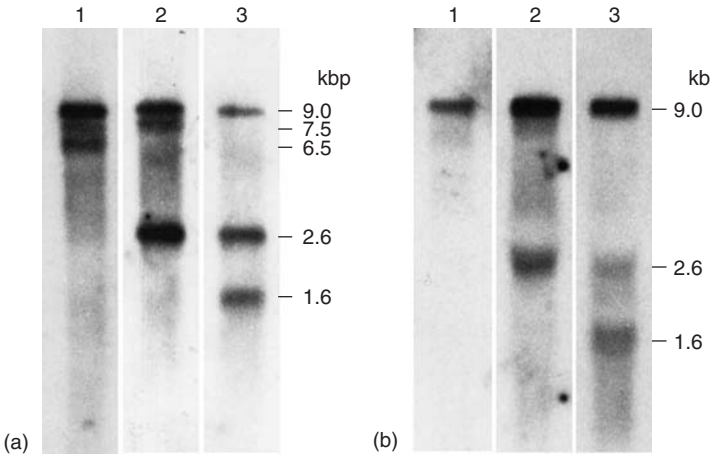


Figure 3 Northern blot analysis of double-stranded (a) and single-stranded (b) RNAs in leaves of *Nicotiana occidentalis* infected with apple stem pitting virus (IF-38) using three different RNA probes specific to different sequences within the genomic RNA. Lane 1, a probe complementary to nt positions 6207–6683 (ORF1 region); lane 2, nt positions 6678–7447 (ORF2 region); and lane 3, nt positions 8717–9293 (CP and the 3′-noncoding regions).

parenchyma cells. The particles were observed often as large aggregates in the cytoplasm, but not in the vacuole, the nucleus, or in other cellular organelles.

Strains and Genome Heterogeneity

Restriction fragment length polymorphism (RFLP) analysis of the cDNA clones from four ASPV isolates from apple (B39, B12, IG39, and IF38) showed that three different patterns were found in cDNA clones from isolate B39, two patterns from isolates B12 and IG39, and one pattern from isolate IF38. Sequence analysis of the 3′-terminal 600 nt of the genomes indicated that sequence identities among cDNA clones showing the same RFLP pattern were more than 99%, and in contrast, considerable sequence variations were found among the cDNA clones showing a different RFLP pattern. These results indicated that isolate B39 is composed of at least three sequence variants (SVs), and isolates B12 and IG39 at least two SVs. The nucleotide sequence identity among nine isolates and SV including PA66 was 77.9–95.7% in the 3′-terminal 600 nt regions. The aa number of CP varied from 396 to 415 among nine isolates and SV, and their identities ranged from 69.4% to 92.7%.

Genus *Trichovirus*

The genus *Trichovirus* consists of four viral species: *Apple chlorotic leaf spot virus* (ACLSV, the type species), *Cherry mottle leaf virus* (CMLV), *Grapevine berry inner necrosis virus* (GINV), and *Peach mosaic virus* (PcMV) (**Table 1**).

Biological Properties

ACLSV occurs in woody plants of the family Rosaceae including apple, pear, peach, plum, cherry, apricot, and prune. Though ACLSV infection is symptomless in most commercial apple varieties, the virus causes topworking disease of apple grown on Maruba kaido (*Malus prunifolia* var. *ringo*) rootstocks in Japan. ACLSV may cause plum bark split, plum pseudopox, pear ring pattern mosaic, and apricot pseudopox disease. CMLV occurs naturally in sweet cherry, peach, and apricot, and causes mottle leaf disease of cherry in some regions of North America. GINV is the causal virus of grapevine berry inner necrosis disease, one of the most important virus diseases of grapevine in Japan.

The viruses can be transmitted by mechanical inoculation, grafting, and through propagating materials. No vectors have been reported for ACLSV. On the other hand, CMLV and GINV are transmitted by the peach bud mite *Eriophyes insidiosus* and the grape erineum mite *Colomerus vitis*, respectively.

Particle Structure

Virus particles are very flexuous filaments 680–780 nm long and 9.25–12 nm in width (**Figure 1**), with obvious cross-banding and helical symmetry; the pitch of the helix is about 3.47–3.8 nm. ACLSV has a buoyant density of 1.27 g ml^{-1} in $CsSO_4$ gradients, and the particles are degraded in CsCl gradients. The A_{260}/A_{280} of purified ACLSV preparation is 1.85–1.89. The particles require the presence of divalent cations to maintain the integrity of the quaternary structure.

Virus particles are composed of a linear positive-sense ssRNA, 7.2–8.0 kbp, and a single polypeptide species of M_r 21–22 kDa. The genomic RNA is about 5% of particle weight. The 3′ terminus of the RNA has a poly A-tail, and the 5′ terminus probably has a cap structure. Nucleotide base ratios are 32% A, 18% C, 23% G, and 27% U for ACLSV-RNA.

Genome Organization and Replication

The complete nucleotide sequences of the genomes of ACLSV (four isolates from plum, apple, and cherry), CMLV, and GINV have been determined. The genomes of ACLSV consist of 7549–7555 nt in length excluding the 3′ poly A-tail. The complete genomes of CMLV and GINV are 8003 nt and 7243 nt long excluding the poly A tail, respectively.

The genomic RNAs of ACLSV and GINV have three slightly overlapping ORFs in the positive strand (**Figure 2**). ORF1 encodes a replication-associated protein of M_r 214–216 kDa containing the consensus motifs of Met, P-pro, Hel, and Pol of the alphavirus-like supergroups of ssRNA. ORF2 encodes a putative MP of M_r 39–50 kDa. The 50 kDa protein encoded by ORF2 of ACLSV genome is an MP, which has the following characteristics: (1) it is localized to plasmodesmata of infected and transgenic plant cells; (2) it can spread from the cells that initially produce it into neighboring cells; (3) it enables cell-to-cell trafficking of green fluorescent protein (GFP) when the 50 kDa protein and GFP are coexpressed in leaf epidermis; (4) it induces the production of tubular structures protruding from the surface of protoplast; and (5) it binds to single-stranded nucleic acids. Additionally, transgenic plants expressing the 50 kDa protein can complement the systemic spread of mutants of an infectious cDNA clone that are defective in ORF2. ORF3 encodes a 21–22 kDa CP.

The genomic RNA of CMLV has four putative ORFs encoding 216 kDa (ORF1), 47 kDa (ORF2), 22 kDa (ORF3), and 15 kDa (ORF4) proteins (**Figure 2**). The ORF4 protein that is not present in the genomes of ACLSV and GINV may be a nucleic acid-binding protein because similarities have been found between ORF4 of CLMV and ORF5 of grapevine virus B.

In analysis of the dsRNAs of ACLSV-infected plants, six dsRNA species of *c.* 7.5, 6.4, 5.4, 2.2, 1.1, and 1.0 kbp were found in infected tissues. The 7.5 kbp species corresponding to the full-length genome may be a replicative form of the ACLSV genome, and the 2.2 and 1.1 kbp species are thought to be the double stranded forms of subgenomic RNAs coding for the MP and the CP, respectively. The 6.4 and 5.4 kbp species were found to be 5′ co-terminal with the genomic RNA. In the model for the expression of the genome of ACLSV, only the 5′-proximal ORF (216 kDa) coding for the putative viral replicase is translated directly, and the two other ORFs encoding MP and CP are expressed through two subgenomic RNAs.

In infected plant leaves, ACLSV and GINV particles occur as aggregates in the cytoplasm. ACLSV was also observed in nucleoplasm of a mesophyll cell in the infected *C. quinoa*. Replication is presumed to occur in the cytoplasm although no virus-specific inclusions, such as vesicles and viroplasm, were observed.

Serology

No serological relationships were found among ACLSV, CMLV, and GINV. Polyclonal and monoclonal antibodies (MAbs) of CMLV cross-reacted with all isolates of PcMV tested, indicating that CMLV and PcMV are closely related viruses. MAbs against ACLSV particles were produced and used to investigate the antigenic structure of the virus. Epitope studies using these MAbs identified seven independent antigenic domains in ACLSV particles.

Strains

Many isolates have been reported from apple, cherry, peach, plum, and prune. Several strains could be differentiated serologically as well as by symptoms in indicator plants. Sequence comparisons among isolates indicate large molecular variability, that is, sequence conservation rates vary between 77.4% and 99.4%, with most of the isolates differing by 10–20% from any other given isolates.

Genus *Vitivirus*

The genus *Vitivirus* consists of four viral species – *Grapevine virus A* (GVA, the type species), *Grapevine virus B* (GVB), *Grapevine virus D* (GVD), and *Heracleum latent virus* (HLV) – and a tentative species – Grapevine virus C (**Table 1**).

Biological Properties

GVA, GVB, and GVD naturally infect only grapevines and induce severe diseases, the rugose wood complex of grapevine, characterized by pitting and grooving of the wood. HLV occurs in hogweed (*Heracleum* sp. family Apiaceae) without causing obvious symptoms. Herbaceous plant species, *Nicotiana benthamiana* and *N. occidentalis*, are used for propagation hosts of GVA and GVB, respectively, and *Chenopodium* species are used for diagnostic and propagation species of HLV.

All virus species are transmitted by mechanical inoculation. Transmission by grafting and dispersal through propagating materials is common with infected grapevines. GVA and GVB are transmitted in nature by several species of the pseudococcid mealybug genera *Planococcus* and *Pseudococcus* in a semipersistent manner, whereas HLV is transmitted from naturally infected hogweed plants by the aphids in a semipersistent manner, which depends on a helper virus present in naturally infected plants. No seed transmission of HLV is found in *C. quinoa*, chervil, coriander, or hogweed.

Particle Structure

Virus particles are flexuous filaments 725–825 nm long and 12 nm in width, showing obvious cross-banding and helical symmetry with a pitch of 3.5 nm. Particles sediment as a single peak in sucrose or Cs_2SO_4 density gradients with a sedimentation coefficient (S_{20w}) of about 96S and a buoyant density in Cs_2SO_4 of 1.24 g cm^{-3} for HLV. The A_{260}/A_{280} of purified virus preparation is 1.5–1.52 for GVB and HLV.

Virus particles are composed of a linear positive-sense ssRNA, 7.3–7.6 kbp, and a single polypeptide species of M_r 18–21.5 kDa. The genomic RNA is about 5% of the particle weight. The 3′ terminus of the RNA has a poly A tail, and the 5′ terminus probably has a cap structure. The overall A+U and G+C content of GVB-RNA is 53.5% and 46.5%, respectively.

Genome Organization and Replication

The complete nucleotide sequences of the genomes of GVA and GVB have been determined. The genomes of GVA and GVB consist of 7349 and 7598 nt in length excluding the 3′-poly A tail, respectively.

The genomic RNAs of GVA and GVB have five slightly overlapping ORFs in the positive strand (**Figure 2**). ORF1 of both viruses encodes a replication-associated protein of M_r 194–195 kDa containing the consensus motifs of methyltransferase, papain-like protease, nucleotide triphosphate-binding helicase, and RNA polymerase of the alphavirus-like supergroups of ssRNA. ORF2 encodes a protein of M_r 19 kDa (GVA) or 20 kDa (GVB) that does not show any significant sequence homology with protein sequences from the databases. The biological function of these proteins has not been determined yet. ORF3 encodes an MP with M_r 31 kDa (GVA) and 36.5 kDa (GVB), possessing the G/D motif of the '-30K' superfamily movement protein. ORF4 encodes

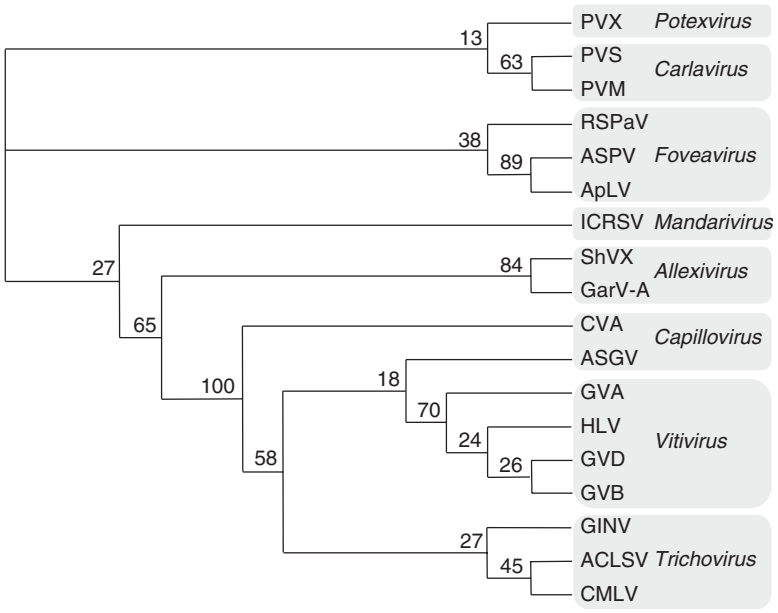


Figure 4 Dendrogram showing the relationships among virus species in the family *Flexiviridae* using the aa sequences of the coat protein. The tree was produced and bootstrapped using CLUSTAL W. PVX, potato virus X; PVS, potato virus S; PVM, potato virus M; RSPaV, ruspestris stem pitting-associated virus; ASPV, apple stem pitting virus; ApLV, apricot latent virus; ICRSV, Indian citrus ringspot virus; ShVX, shallot virus X; GarV-A, garlic virus A; CVA, cherry virus A; ASGV, apple stem grooving virus; GVA, grapevine virus A; HLV, heracleum latent virus; GVD, grapevine virus D; GVB, grapevine virus B; GINV, grapevine berry inner necrosis virus; ACLSV, apple chlorotic leaf spot virus; CMLV, cherry mottle leaf virus.

a CP with M_r 21.5 kDa (GVA) and 21.6 kDa (GVB). ORF5 encodes a protein with M_r 10 kDa (GVA) and 14 kDa (GVB). A 14 kDa protein encoded by GVB-ORF5 shares weak homologies to proteins with nucleic acid-binding properties.

The gene expression strategy may be based on proteolytic processing of ORF1 protein and subgenomic RNA production for translation of ORF2 to ORF5 proteins. In analysis of the dsRNAs of infected plants, four major dsRNA bands with a size of 7.6, 6.48, 5.68, and 5.1 kbp for GVA and GVD, and 7.6, 6.25, 5.03, and 1.97 kbp for GVB were found in infected tissues. In *N. benthamina* plants infected with GVA, three 3′-terminal subgenomic RNAs of 2.2, 1.8, and 1.0 kbp were also detected and thought to serve for the expression of ORF2, ORF3, and ORF4, respectively.

In electron microscopy of leaves infected with GVA and GVB, tonoplast-associated membranous vesicles containing finely fibrillar materials were found in phloem parenchyma cells, and virus particles occurred only in phloem tissues. Thus, replication may occur in the cytoplasm, possibly in association with vesicles protruding from the tonoplast of phloem cells.

Serology

In immunoelectron microscopy, the antiserum against GVB-NY clearly decorated homologous (GVB-NY) and heterologous (GVB-CAN) virus particles, but did not decorate GVA particles, indicating no serological relationships between GVA and GVB.

Phylogenetic Relationships

Sequences from viruses within the genus *Foveavirus* clustered into a branch different from other TGB-containing viruses (allexi, carla, potex, and mandariviruses) (**Figure 4**). Sequences for viruses within the genera *Capillovirus*, *Trichovirus*, and *Vitivirus* also clustered into branches different from each other (**Figure 4**).

See also: Allexivirus; *Carlavirus*; Flexiviruses; Plant Virus Diseases: Fruit Trees and Grapevine; *Potexvirus*.

Further Reading

Adams MJ, Antoniw JF, Bar-Joseph, *et al.* (2004) The new plant virus family *Flexiviridae* and assessment of molecular criteria for species demarcation. *Archives of Virology* 149: 1045–1066.

Adams MJ, Accotto GP, Agranovsky AA, *et al.* (2005) Family *Flexiviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1089–1124. San Diego, CA: Elsevier Academic Press.

Boscia D, Savino V, Minafra A, *et al.* (1993) Properties of a filamentous virus isolated from grapevines affected by corky bark. *Archives of Virology* 130: 109–120.

German S, Candresse T, Lanneau M, *et al.* (1990) Nucleotide sequence and genome organization of apple chlorotic leaf spot closterovirus. *Virology* 179: 1104–1112.
Jelkmann W (1994) Nucleotide sequence of apple stem pitting virus and of the coat protein of a similar virus from pear associated with vein yellows disease and their relationship with potex- and carlaviruses. *Journal of General Virology* 75: 1535–1542.
Koganezawa H and Yanase H (1990) A new type of elongated virus isolated from apple trees containing the stem pitting agent. *Plant Disease* 74: 610–614.
Magome H, Yoshikawa N, Takahashi T, Ito T, and Miyakawa T (1997) Molecular variability of the genomes of capilloviruses from apple, Japanese pear, European pear, and citrus trees. *Phytopathology* 87: 389–396.
Martelli GP and Jelkmann W (1998) *Foveavirus*, a new plant virus genus. *Archives of Virology* 143: 1245–1249.
Martelli GP, Minafra A, and Saldarelli P (1997) *Vitivirus*, a new genus of plant viruses. *Archives of Virology* 142: 1929–1932.
Meng B, Pang S-Z, Forsline PL, McFerson JR, and Gonsalves D (1998) Nucleotide sequence and genome structure of grapevine rupestris stem pitting associated virus-1 reveal similarities to apple stem pitting virus. *Journal of General Virology* 79: 2059–2069.
Minafra A, Saldarelli P, and Martelli GP (1997) Grapevine virus A: Nucleotide sequence, genome organization, and relationship in the *Trichovirus* genus. *Archives of Virology* 142: 417–423.
Yanase H (1974) Studies on apple latent viruses in Japan. *Bulletin of the Fruit Tree Research Station, Japan, Series* C1: 47–109.
Yoshikawa N (2000) Apple stem grooving virus. AAB Descriptions of Plant viruses 376. http://www.dpvweb.net/dpv/showdpv.php?dpvno=376 (accessed January 2008).
Yoshikawa N (2001) Apple chlorotic leaf spot virus. AAB Descriptions of Plant viruses 386. http://www.dpvweb.net/showdpv.php?dpvno=386 (accessed January 2008).
Yoshikawa N, Sasaki E, Kato M, and Takahashi T (1992) The nucleotide sequence of apple stem grooving capillovirus geneme. *Virology* 191: 98–105.

Capripoxviruses

R P Kitching, Canadian Food Inspection Agency, Winnipeg, MB, Canada

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Abomasal Pertaining to the fourth stomach of ruminants.
Agalactia Shortage of milk supply.
Myiasis Infestation with maggots.
Hydropic Accumulating water.

History

Sheeppox and goatpox are malignant pox diseases of sheep and goats easily recognizable by their characteristic clinical signs, and described in the earliest texts on animal diseases. Lumpy skin disease (Neethling) of cattle (LSD), however, was first described in 1929 in Northern Rhodesia (Zambia), having apparently been absent from domestic cattle until that time. From Zambia, LSD spread south to Botswana and Zimbabwe, and by 1944 the disease appeared in South Africa, where it caused a major epizootic, affecting over 6 million cattle. In 1957, LSD was first diagnosed in Kenya, and was thought at the time to be associated with the introduction of a flock of sheep affected with sheeppox on the farm. Since then LSD has been present in most of the countries of sub-Saharan Africa, often associated with large epizootics followed by periods in which the disease is only rarely reported. In 1988, LSD caused a major outbreak in Egypt, and in 1989 it spread from Egypt to a village in Israel. This was the first time that a diagnosis of LSD outside of Africa had been supported by laboratory confirmation.

Taxonomy and Classification

The viruses that cause sheeppox, goatpox, and LSD are all members of the genus *Capripoxvirus*, in the subfamily *Chordopoxvirinae* of the family *Poxviridae*, and have morphological, physical, and chemical properties similar to vaccinia virus. Originally, the viruses were classified according to the species from which they were isolated, but comparisons of their genomes indicate that the distinction between them is not so clear, and that recombination events occur naturally between isolates from different species. This is reflected in the ability of some strains to cause disease in both sheep and goats and in experimental results which show that all the sheep isolates examined could infect goats, and that goat isolates could infect sheep.

The epidemiological relationship between sheeppox and goatpox isolates and cattle isolates is less clear, apparent in differences in the geographical distribution of sheeppox and goatpox and LSD (see below). However, some isolates recovered from sheep and goats in Kenya have genome characteristics very similar to cattle isolates. It has been proposed that confusion can be reduced by referring to the malignant pox diseases of sheep, goats, and cattle, including Indian goat dermatitis and Kenyan sheep and goatpox, as capripox. It is envisaged that when sufficient isolates have been examined biochemically, no clear distinction will be possible between sheep, goat, and cattle isolates, but a spectrum will emerge in which some strains have clear host preferences while others will be less defined and will naturally infect the host with which they come into contact.

Geographical and Seasonal Distribution

Capripox of sheep and goats is enzootic in Africa north of the equator, the Middle East and Turkey, Iran, Afghanistan, Pakistan, India, Nepal, and parts of the People's Republic of China, and, in 1986, Bangladesh. Sheeppox was eradicated from Britain in 1866, and from France, Spain, and Portugal in 1967, 1968, and 1969, respectively. Sporadic outbreaks still occur in Europe, for instance in Italy in 1983 and Greece and Bulgaria both in 1995 and 1996, and Greece in 1997 and 2000. In 2005, goatpox was first reported in Vietnam, following its introduction from China.

LSD is enzootic in the sub-Saharan countries of Africa and is still present in Egypt. The single outbreak in Israel was eradicated by slaughter of affected and in-contact cattle.

There is no clear seasonality to outbreaks of capripox in sheep and goats. In enzootic areas, lambs and kids are protected against infection with capripoxvirus for a variable time dependent on the immunity of the mother. However, the spread of LSD is related to the density of biting flies and consequently major enzootics have been associated with humid weather when fly activity is greatest.

Host Range and Virus Propagation

Among domestic species, capripoxvirus is restricted to cattle, sheep, and goats. Experimentally, it is possible to infect cattle, sheep, or goats with isolates derived from any of these three species, although clinically the reaction following inoculation may be indiscernible. Viral genome analysis using restriction endonucleases has identified fragment size characteristics by which it is possible to classify strains into cattle, sheep, or goat isolates. However, the identification of strains that have intermediate characteristics between typical sheep and goat isolates does suggest the movement of strains between these species. Analysis of some Kenyan isolates derived from sheep and goats shows very close homology with cattle LSD isolates.

The involvement of the African buffalo (*Syncerus caffer*) in the maintenance of LSD has not been clearly established. Some surveys have shown the presence of capripoxvirus antibody in buffalo, while others have failed to show its presence. Buffaloes clinically affected with LSD have not been described. Experimental infection of giraffe (*Giraffe camelopardalis*), impala (*Aepyceros melampus*), and gazelle (*Gazella thomsonii*) has resulted in the development of clinical disease.

Bos indicus cattle are generally less susceptible to LSD and develop milder clinical disease than *Bos taurus*, of which the fine-skin Channel Island breeds are particularly susceptible. Similarly, breeds of sheep and goats indigenous to capripoxvirus enzootic areas appear less susceptible to severe clinical capripox than do imported European or Australian breeds.

Capripoxvirus will grow on the majority of primary and secondary cells and cell lines of ruminant origin. Primary lamb testes cells are considered the most sensitive system for isolation and growth of capripoxvirus. The virus produces a characteristic cytopathic effect (cpe) on these cells which can take up to 14 days for field isolates, but can be as short as 3 days for well-adapted strains.

Isolates of capripoxvirus derived from cattle have been adapted to grow on the chorioallantoic membrane of embryonated hens' eggs, although attempts to grow isolates from sheep and goats in eggs have been unsuccessful. Vaccine strains of capripoxvirus have been adapted to grow on Vero cells. Capripoxvirus will not grow in any laboratory animals.

Genetics

Less is known concerning the specific genetics of capripoxvirus than is known about the orthopoxvirus genome. Studies on field isolates taken from cattle suggest that the virus is very stable, as *Hin*dIII restriction endonuclease digest patterns of isolates from the 1959 Kenya outbreak of LSD are identical to those obtained from 1986 LSD isolates. However, recombination has been shown to occur between cattle and goat isolates and this could be the natural method by which the virus evolves. By analogy with the orthopoxviruses, it is also likely that sequences are deleted or repeated within the genome in the normal replicative cycle.

The genomes of those capripoxvirus isolates that have been sequenced, representing isolates from cattle, sheep, and goats, have a 96% nucleotide homology along their entire length of approximately 150 kbp.

Sheep and goat isolates have 147 putative genes and LSD isolates an additional nine. However, the sheep and goat isolates have these nine in a disrupted form, suggesting that the LSD virus is the more ancient progenitor. This is clearly not consistent with the apparent first appearance of LSD in 1929. Nevertheless, the published sequences have shown a range of genes coding for host cell protein homologs, in common with many identified in the orthopoxviruses. In fact, in the central region of the genome, there is a high degree of similarity (*c.* 65%) with amino acid sequences found in other poxvirus species, in particular suipoxvirus, yatapoxvirus, and leporipoxvirus. These sequence studies and those reported from India suggest that distinction can be made between sheep and goat isolates, but limited numbers were studied; however, some isolates examined from the Middle East show less evidence of host-species-specific sequences.

Evolution

The capripoxviruses have evolved into specific cattle, sheep, and goat lines, but, as has been described above, intermediate strains exist, particularly those with cattle and goat genome characteristics. In Kenya, there is evidence of movement of strains between all three species, but the absence of sheeppox or goatpox in LSD enzootic areas in southern Africa, and the absence of LSD outside of Africa, would suggest that host-specific strains are being maintained and presumably are continuing to evolve.

Serologic Relationships and Variability

Polyclonal sera fail to distinguish in the virus neutralization test between any of the isolates of capripoxvirus so far examined. Sheep, goats, or cattle that have been infected with any of the isolates are totally resistant to challenge with any of the other isolates. On this basis, it has been possible to use the same vaccine strain to protect all three species. No monoclonal antibodies are as yet available against capripoxvirus, but it can be expected that differences will emerge between strains using these reagents.

Capripoxviruses share a precipitating antigen with parapoxviruses, but no cross-immunity has been shown between these two genera, or between capripoxvirus and any other poxvirus genera.

Epizootiology

In sheeppox and goatpox enzootic areas the distribution of disease is frequently a reflection of the traditional form of husbandry. For instance, in the Yemen Arab Republic, the sheep and goat flocks kept on the grassland of the central plateau and better irrigated regions of the coastal plain move about in search of food, frequently mixing with flocks from neighboring villages at water holes, and in this situation disease is restricted to the young stock. Animals over 1 year of age have a solid immunity. The animals belonging to villages in the more mountainous regions and the arid areas of the coastal plain are isolated by terrain or semidesert from mixing with animals from other villages. It is not known what is the critical number of animals required to maintain capripoxvirus within a single population but it is over a thousand adult animals, which is the approximate village sheep and goat populations. In these villages, disease is usually only seen following the introduction of new animals, typically from market, and generally affects animals of all age groups. The disease spreads through the village, usually within 3–6 months, and then disappears in the absence of more susceptible animals. Occasionally, even within areas of high sheep and goat density, it is possible to encounter animals that have been kept totally isolated in the confines of a domestic residence, and these may remain susceptible to infection until adult.

In Sudan, large numbers of sheep and goats are trekked from the west to the large collecting yards and markets of Omdurman, outside Khartoum. Here also, it is possible to see capripox infection in adult animals. Many of the flocks originate in villages which, like in the Yemen, are isolated from their neighbors. Capripoxvirus does not persist in these villages, and as a result the animals acquire no resistance, and are fully susceptible when they first encounter disease on the long journey across Sudan. Animals being exported from countries that are free of capripoxvirus may suffer a similar fate when they arrive in a capripoxvirus enzootic area, as often seen in Australian or New Zealand sheep imported into the Middle East.

In a study of 49 outbreaks of capripox in the Yemen, only 8 were reported to affect both sheep and goats, the remaining 41 causing clinical disease in either sheep or goats. It is possible that both sheep and goats could have been involved in more than the eight outbreaks, but that the disease was inapparent in one species; whether, therefore, the species in which the disease was inapparent could transmit virus and become a vector for disease has not been determined. In Kenya, capripox is frequently encountered in both sheep and goats within the same flock, and there is the possibility that the same strain of capripoxvirus could also cause LSD in cattle.

The epidemiology of sheeppox, goatpox, and LSD is similar; the severity of outbreaks depends on the size of the susceptible population, the virulence of the strain of capripoxvirus, the breed affected (indigenous animals tending to be less susceptible to clinical disease than imported), and, with LSD, the presence of suitable insect vectors. Morbidity rates vary from 2% to 80%, and mortality rates may exceed 90%, particularly if the infection is in association with other disease or bad management.

Transmission and Tissue Tropism

Under natural conditions, capripoxvirus is not transmitted very readily between animals, although there are circumstances when transmission appears very rapid; for example, in association with factors that damage the mucosae, such as peste des petits ruminants infection or feeding on abrasive forage. Animals are most infectious soon after the appearance of papules and during the 10-day period before the development of significant levels of protective antibody. High titers of virus are present in the papules, and those papules on the mucous membranes quickly ulcerate and release virus in nasal, oral, and lachrymal secretions, and into milk, urine, and semen. Viremia may last up to 10 days, or in fatal cases until death. Those animals that die of acute infection before

the development of clinical signs and those that develop only very mild signs or single lesions rarely transmit infection, while those that develop generalized lesions produce considerable virus and are highly infectious. Aerosol infection over a few meters only, as with other poxvirus infections, is probably the usual form of transmission. Contact transmission of LSD virus under experimental conditions in the absence of insect vectors has only rarely been reported. Biting flies are significant in the mechanical transmission of LSD, and *Stomoxys calcitrans* and *Biomyia fasciata* have been implicated. There are probably a number of insects capable of mechanically transmitting LSD virus, but insects such as mosquitoes, which preferentially feed on hyperemic sites such as papules and if interrupted inoculate a new host intravenously, are considered the most likely to be involved in outbreaks characterized by large numbers of affected animals with generalized infections. Experimentally, *S. calcitrans* has also been shown to be capable of transmitting sheeppox and goatpox, and mosquitoes have transmitted LSD virus under experimental conditions.

During the recovery phase following infection, the papules on the skin become scabs. It is relatively easy to demonstrate virions in the scab, but difficult to isolate virus on tissue culture, probably because of the complexing of antibody and virus within the scab. Capripoxvirus is reported to remain viable in wool for 2 months and in contaminated premises for 6 months, and is reported to remain infectious in skin lesions of cattle for 4 months. The true epidemiological significance of the virus within the scab, and ultimately the environment, is not clear. It has been suggested that the protein material that envelops the virus within the type A intracytoplasmic inclusion bodies of infected cells protects the virus in the environment.

There is no evidence for the existence of animals persistently infected with capripoxvirus. Transplacental transmission of capripoxvirus may be possible in association with simultaneous pestivirus infection, as may occur with pestivirus-contaminated capripox vaccine.

Capripoxvirus can be isolated from the leukocytes during viremia, and has been isolated from lesions in the liver, urinary tract, testes, digestive tract, and lungs; however, the cells of the skin and skin glands and the internal and external mucous membranes appear to be the major sites of virus replication.

Pathogenicity

There is considerable variation in the pathogenicity of strains of capripoxvirus. Little is known concerning the genes responsible in the capripoxvirus genome for virulence or host restriction; some preliminary results have been published.

Clinical Features of Infection

The incubation period of capripox infection, from contact with virus to the onset of pyrexia, is approximately 12 days, although it frequently appears longer as transmission is often not immediate between infected and susceptible animals. Following experimental inoculation of virus, the incubation period is approximately 7 days, and this is similar to that shown experimentally using biting flies to transmit virus.

The clinical signs of malignant disease are similar in sheep, goats, and cattle. Twenty-four hours after the development of pyrexia of between 40 and 41 °C, macules (2–3 cm diameter areas of congested skin) can be seen on the white skin of sheep and goats, particularly under the tail. Macules are not seen on the thicker skin of cattle, and are frequently missed on skin of pigmented sheep and goats. After a further 24 h, the macules swell to become hard papules of between 0.5 and 2 cm diameter, although they may be larger in cattle. In the generalized form of capripox, papules cover the body, being concentrated particularly on the head and neck, axilla, groin, and perineum, and external mucous membranes of the eyes, prepuce, vulva, anus, and nose. In cattle, these papules may exude serum, and there may be considerable edema of the brisket, ventral abdomen, and limbs. The papules on the mucous membranes quickly ulcerate, and the secretions of rhinitis and conjunctivitis become mucopurulent (**Figure 1**). Keratitis may be associated with the conjunctivitis.

All the superficial lymph nodes, particularly the prescapular, are enlarged. Breathing may become labored as the enlarged retropharyngeal lymph nodes put pressure on the trachea. Mastitis may result from secondary infection of the lesions on the udder.

The papules do not become vesicles and then pustules, typical of orthopoxvirus infections. Instead, they become necrotic, and if the animal survives the acute stage of the disease, change to scabs over a 5–10 day period from the first appearance of papules. The scabs can persist for up to



Figure 1 Sheeppox showing rhinitis and conjunctivitis.

a month in sheep and goats, whereas in cattle the necrotic papules that penetrate the thickness of the skin may remain as 'sitfasts' for up to a year.

Severe disease is accompanied by significant loss of condition, agalactia, possibly secondary abortion, and pneumonia. Eating, drinking, and walking may become painful, and death from dehydration is not uncommon. Secondary myiasis is also a major problem in tropical areas.

Pathology and Histopathology

The lesions of capripox are not only restricted to the skin, but also may affect any of the internal organs, in particular the gastrointestinal tract from the mouth and tongue to the anus, and the respiratory tract. In generalized infections, papules are prominent in the abomasal mucosa, trachea, and lungs. Those in the lungs are approximately 2 cm in diameter, and papules may coalesce to form areas of gray consolidation (**Figure 2**).

In affected skin, there is an initial epithelial hyperplasia followed by coagulation necrosis as thrombi develop in the blood vessels supplying the papules. Histiocytes accumulate in the areas of the papules, and the chromatin of the nuclei of infected cells marginates. The cells appear stellate as their boundaries become poorly defined, and many undergo hydropic degeneration with the formation of microvesicles. Intracytoplasmic inclusion bodies are present in infected cells of the dermis and also in the columnar epithelial cells of the trachea where frequently gross lesions may not be apparent. These are initially type B inclusions at the sites of virus replication (**Figure 3**), but later in infection they are replaced by type A inclusions (see above). The maximum titer of virus is obtained from papules approximately 6 days after their first appearance.

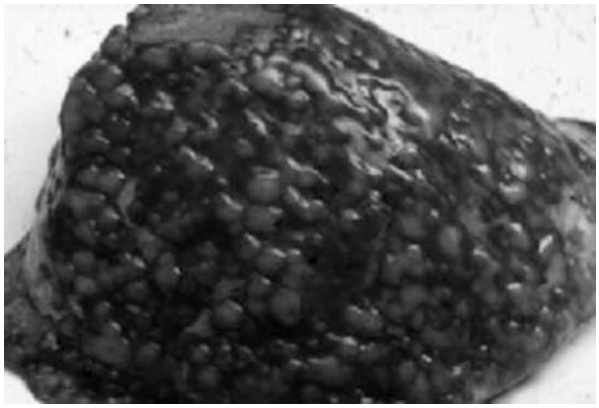


Figure 2 Sheeppox showing severe lung lesions.

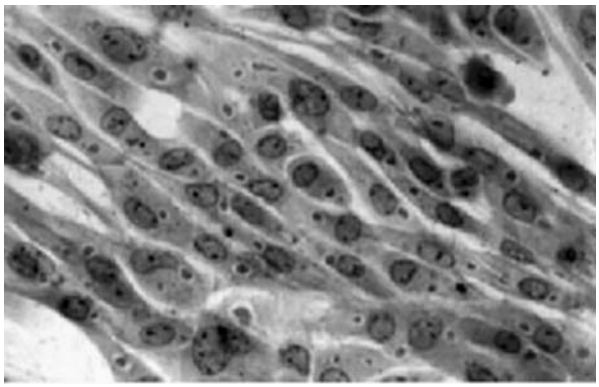


Figure 3 Capripoxvirus growing in lamb testis cells showing many intracytoplasmic inclusion bodies. Magnification ×400.

Immune Response

Capripoxvirus, like orthopoxvirus, is released from an infected cell within an envelope derived from modified cellular membrane. The enveloped form of the virus is more infectious than the nonenveloped form, which can be obtained experimentally by freeze–thawing infected tissue culture. By analogy with orthopoxvirus, antigens on the envelope and on the tubular elements of the virion surface may stimulate protective antibodies. Animals immune to nonenveloped virus are still fully susceptible to the enveloped form. Passively transferred antibody, either colostral or experimentally inoculated, will protect susceptible animals against generalized infection; however, in the vaccinated or recovered animal, there is no direct correlation between serum levels of neutralizing antibody and immunity to clinical disease. Antibody may limit the spread of capripoxvirus within the body, but it is the cell-mediated immune response that eliminates infection. In sheep, major histocompatibility complex-restricted cytotoxic T-lymphocytes are required in the protective immune response to orthopoxvirus infection, and therefore probably also capripoxvirus infection.

Immune animals challenged with capripoxvirus by intradermal inoculation develop a delayed-type hypersensitivity reaction at the challenge site. This may not be apparent in animals with high levels of circulating antibody. It has been suggested that the very severe local response shown by some cattle at the site of vaccination against LSD may be a hypersensitivity reaction due to previous contact with the antigens of parapoxvirus.

There is total cross-immunity between all strains of capripoxvirus, whether derived from cattle, sheep, or goats.

Prevention and Control

In temperate climates, capripox can be effectively controlled by slaughter of affected animals, and movement control of all susceptible animals within a 10 km radius for

6 months. In tropical climates, particularly in humid conditions when insect activity is high, movement restrictions are not sufficient and vaccination of all susceptible animals should be considered. In outbreaks of LSD, it is not considered necessary to vaccinate sheep and goats, although theoretically cattle strains of virus could infect them. Similarly, in outbreaks of capripox in sheep and goats, cattle are not normally vaccinated.

Countries in which capripoxvirus is absent can maintain freedom by preventing the importation of animals from infected areas. There is always a possibility that skins from infected animals could introduce infection into a new area, although there have been no proven examples of this. The insect transmission of capripoxvirus into Israel from Egypt over a distance of between 70 and 300 km would indicate that it is impossible for countries neighboring enzootic areas to totally secure their borders.

In enzootic areas, annual vaccination of susceptible animals with a live vaccine will control the disease. Calves, kids, and lambs up to 6 months of age may be protected by maternal antibody, but this would only occur if the mother had recently been severely affected with capripox. Although maternal antibody will inactivate the vaccine, it is advisable to vaccinate all stock over 10 days of age. No successful killed vaccines have been developed for immunization against capripoxvirus infection, other than those that give only very short-term immunity.

Future Perspectives

Capripox of sheep and goats is present in most of Africa and Asia, whereas LSD is restricted to Africa. There is no good explanation as to why LSD has not spread into the Middle East and India, carried by the considerable trade in live cattle. Unless there is a reservoir host in Africa which is required for the maintenance of the cattle-adapted capripoxvirus, it can be anticipated that LSD will spread out of Africa, with major economic consequences.

While considerable attention has been given to vaccinia virus as a vector of other viral genes for development as a recombinant vaccine, little attention has been given to capripoxvirus as a potential vector vaccine. Although its use would be restricted to the not inconsiderable capripoxvirus enzootic area, it would have the advantage of not being infectious to humans, and be a useful vaccine in its own right.

The high degree of homology between the genomes of capripoxvirus isolates from different species and the apparent differences between their virulence and host preference would make them good candidates for studying the genetic basis of virulence and host specificity.

See also: Leporipoviruses and Suipoxviruses; Poxviruses.

Further Reading

Black DN, Hammond JM, and Kitching RP (1986) Genomic relationship between capripoxvirus. *Virus Research* 5: 277–292.

Carn VM and Kitching RP (1995) An investigation of possible routes of transmission of lumpy skin disease (Neethling). *Epidemiology and Infection* 114: 219–226.

Gershon PD, Ansell DM, and Black DN (1989) A comparison of the genome organization of capripoxvirus with that of the orthopoxviruses. *Journal of Virology* 63: 4703–4708.

Gershon PD, Kitching RP, Hammond JM, and Black DN (1989) Poxvirus genetic recombination during natural virus transmission. *Journal of General Virology* 70: 485–489.

Kitching RP, Bhat PP, and Black DN (1989) The characterization of African strains of capripoxvirus. *Epidemiology and Infection* 102: 335–343.

Kitching RP, Hammond JM, and Taylor WP (1987) A single vaccine for the control of capripox infection in sheep and goats. *Research in Veterinary Science* 42: 53–60.

Tulman ER, Afonso CL, Lu Z, Zsak L, Kutish GF, and Rock DL (2001) Genome of lumpy skin disease virus. *Journal of Virology* 75: 7122–7130.

Tulman ER, Afonso CL, Lu Z, *et al.* (2002) The genomes of sheeppox and goatpox viruses. *Journal of Virology* 76: 6054–6061.

Capsid Assembly: Bacterial Virus Structure and Assembly

S Casjens, University of Utah School of Medicine, Salt Lake City, UT, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Coat protein The protein which is the structural building block for the nucleic acid container part of the virion.

DNA encapsidation or DNA packaging The process of enclosing a nucleic acid inside the protein coat of a virion.

Head The icosahedrally symmetric portion of *Caudovirales* virions that contains the nucleic acid.

Portal protein The *Caudovirales* protein through which DNA enters and exits the coat protein shell; it is present at only one of the 12 otherwise identical icosahedral vertices.
Procapsid or prohead The virion precursor particle that contains no nucleic acid; it is the preformed container into which the nucleic acid is packaged.
Scaffolding protein A protein that directs the proper assembly of the coat protein, but is not present in the completed virion.
Tail The portion of *Caudovirales* virions that binds to cells bearing the correct surface receptor and through which DNA traverses during delivery from the virion into the cell. The tail is attached to the portal protein of the head in the complete virion.
Triangulation (T) number Formally, T is the number of smaller equilateral triangles (facets) into which each of the 20 icosahedral faces is subdivided in an icosadeltahedron. In viruses, this is directly related to the number of coat protein subunits (S) per virion ($S = 60T$), since there are typically three subunits per facet and 20 faces in icosahedral viruses.
Virion The stable virus particle that is released from infected cells and is capable of binding to and infecting other sensitive cells.

History and Overview

The structure of bacterial viruses (bacteriophages or phages) remained a mystery for over a quarter of a century after their original discovery in about 1910, and even then early electron micrographs of phage T4 showed only a vague outline of its complex features. The invention in the late 1950s of negative staining with heavy metal salts and high-resolution metal evaporation techniques allowed electron microscopy to be used to visualize virions with a resolution of a few nanometer – a big step forward, but most individual protein components could still not be resolved. Meanwhile, viruses were shown to be made up primarily of protein on the outside and nucleic acid inside. It is worth noting that phage T2 was a major participant in the proof that DNA is in fact the genetic material, when Hershey and Chase used it to show that during an infection the virion's DNA entered the cell and the bulk of the protein remained outside. After their discovery that DNA is the genetic material, in 1953, Crick and Watson also recognized that virus chromosomes could not encode a single protein molecule large enough to enclose their nucleic acid molecules, and they correctly postulated that virions had to be constructed with repeating arrays of protein molecules. They also noted that there are only two ways of building virions with symmetric arrays of coat proteins that enclose a space suitable for a virion's nucleic acid, and those are arrays built with cubic or helical symmetry (the former includes icosahedral, octahedral, and tetrahedral arrays which would be built from 60, 24, and 12 identical protein subunits, respectively). In 1962, Caspar and Klug deduced that many viruses must be built from icosahedral shells that contain more than 60 subunits, and developed a geometric theory for such arrangements if the subunits were given some flexibility in bonding to their neighbors (referred to as 'quasi-equivalence', since the flexibility gives rise to subunits that are not quite identical in their conformation and/or bonding properties). Some viruses, especially the tailed phages (*Caudovirales*), which are in large part the subject of this article, were found not to be this 'simple' and their repeating arrays of proteins are embellished with parts that are not icosahedrally symmetric. These asymmetric parts can be very complex; for example, phage T4 virions contain over 40 different protein species, of which a substantial majority are not icosahedrally arranged like the coat protein. Recent determination of the complete atomic structure of virions, procapids, or parts of them by X-ray diffraction (usually 2–4 Å resolution) and three-dimensional (3-D) reconstruction by superposition of electron micrographs of many particles and averaging of the resulting structure about the icosahedral rotational symmetry axes (current best is 6–8 Å resolution) has given rise to much detailed information about the structure of protein arrays in viruses in general and phages in particular, and very recent advances have allowed structural determination of virions' asymmetric parts to below 20 Å in advantageous cases (**Figure 1**).

As structural information about virus particles and other macromolecular assemblies accumulated, scientists naturally wondered how such structures were built. Animal and plant viruses were difficult to study inside infected cells, and so the assembly of the bacterial viruses in the easier-to-study bacterial cells became an important model for understanding the mechanisms utilized in the assembly of many macromolecular structures in addition to the viruses themselves. Critical in these studies was the determination of 'parts lists' for the bacteriophages under study, and these were determined both biochemically by delineation of the different protein components and genetically by identification of the genes that encode the protein components; both approaches were crucial in understanding the true nature of phage virions and how they assemble. Although some eukaryotic virions do include host encoded proteins, this has not been found to be the case for phages; all of their protein components are encoded by their own genomes. Although small organic molecules (e.g., putrescine) have been found in phage virions, none have been shown to be essential structural components.

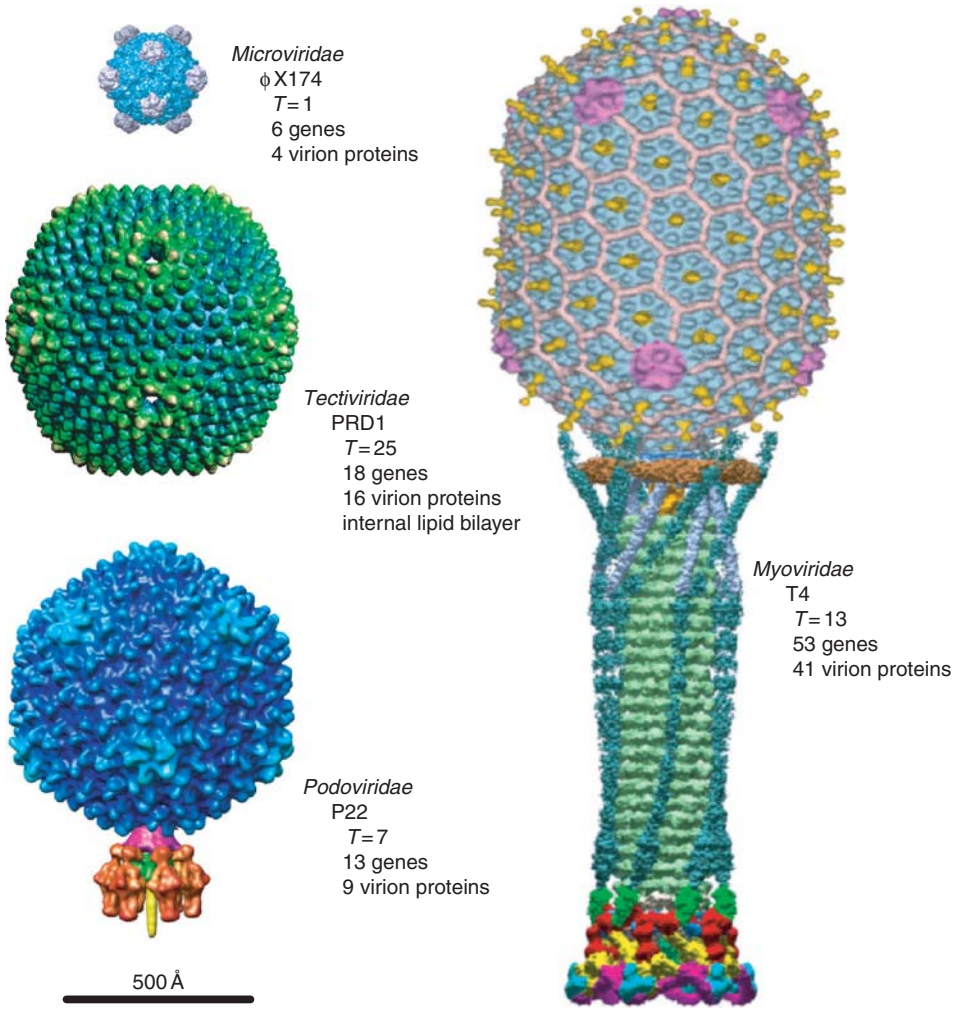


Figure 1 Bacterial virus structures. The structures of representatives from four bacteriophage families are shown at the same scale. The images are shown at 20 ± 5 Å resolution. The 'number of genes' indicated in the figure is the number of genes currently known to be involved in virion assembly and the 'virion proteins' is the number of these present in the completed virion. In ϕX174, DNA packaging into the virion is dependent upon DNA replication but the genes that encode the replication machinery are not included. In each case, different structural proteins visible from the outside are shown as different colors. The PRD1 structure shown is not the actual virion but only the arrangement of coat proteins. The T4 virion is shown in its 'tail fiber up' conformation, in which the tail fibers (dark green) are folded up and the whiskers are folded down, and both are lying along the body of the tail. The T4 image was provided by V. Kostyuchenko, A. Fokine, and M. Rossmann, the P22 image was provided by G. Lander and J. Johnson, and the other two images are from the Virus Particle Explorer website (http://viperdb.scripps.edu/index.php).

Phages are known that contain any one of the four types of nucleic acid, single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), single-stranded RNA (ssRNA), and double-stranded RNA (dsRNA), and they are built with icosahedral symmetry, helical symmetry, and a combination of the two. **Table 1** lists the eight formally named phage orders which appear to be largely unrelated to one another, and one of these, the *Caudovirales*, has been divided into three families with different tail types. Note that there are poorly studied phages that infect the bacteria class Mollicutes that have not yet been formally classified, and viruses that infect the Archeas are not discussed here. Given this great diversity of phage structures and assembly mechanisms, it is not possible to describe them all in detail here, and the reader is urged to consult other articles in this volume for more specific information on the various bacteriophage systems. In this article, we explore phage virion structure and the strategies and mechanisms by which virions assemble. Focus is largely upon the *Caudovirales*, since their assembly is the best understood, but other phage phyla will be mentioned as they are relevant.

Assembly Pathways

A major theme that emanates from the early study of phage assembly is the idea of an 'assembly pathway'.

Table 1 Types of bacteriophages

Phage type	*Best-studied phages*	*Major virion symmetry*	*Virion nucleic acid*	*Comments*
Microviridae	ϕX174	Icosahedral; protein fold A[a]	Circular ssDNA; 4–6 kbp	$T = 1$
Inoviridae	f1, M13	Helical	Circular ssDNA; 5–9 kbp	Special proteins at both ends
Caudovirales Myoviridae	T4, P2	Icosahedral head; protein fold B[a]; long helical contractile tail	Linear dsDNA; 30–500 kbp[b]	*Caudovirales* heads range from $T = 3$ to $T = 52$ and can be isometric or elongated
Caudovirales Siphoviridae	λ, HK97, SPP1	Icosahedral head; protein fold B[a]; long noncontractile tail	Linear dsDNA; 22–140 kbp[b]	Heads can be isometric or elongated
Caudovirales Podoviridae	P22, T7, ϕ29	Icosahedral head; protein fold B[a]; short tail	Linear dsDNA; 18–75 kbp[b]	Heads can be isometric or elongated
Tectiviridae	PRD1	Icosahedral; protein fold A[a]	Linear dsDNA; 13–15 kbp	$T = 25$; contains internal lipid bilayer
Corticoviridae	PM2	Icosahedral	Circular dsDNA; 10 kbp	$T = 21$; contains internal lipid bilayer
Plasmaviridae	L2	Pleomorphic	Circular dsDNA; 2 kbp	Contains lipid bilayer
Leviviridae	MS2, R17	Icosahedral; protein fold C[a]	Linear ssRNA; 3.5–4.3 kbp	$T = 3$
Cystoviridae	ϕ6	Icosahedral; protein fold D[a]	Linear dsRNA; 13–14 kbp	$T = 13$; three different dsRNA molecules in virion; contains external lipid bilayer

[a]The protein folds (named arbitrarily in the table) of typical *Microviridae*, *Caudovirales* (*Siphoviridae*), *Leviviridae*, and *Tectiviridae* coat proteins have been determined by X-ray diffraction; that of *Cystoviridae* is suspected from its arrangement in virions which is similar to other dsRNA viruses.
[b]Approximate size ranges – *Caudovirales* phages are extremely diverse and the limits of genome sizes are not fully known.

The complex phages (*Caudovirales*), which can have as many as 50 different proteins in their virions, typically do not synthesize these different proteins in the temporal order of their assembly. Instead, the different proteins are all synthesized simultaneously, and the inherent properties of the proteins dictate their order of assembly. Thus, virion assembly proceeds via a specific 'pathway' in which the components assemble in an orderly manner. How is such a program of assembly achieved? The generally accepted mechanism for this type of control, which is now known to occur in the construction of many macromolecular structures, not just phages, is that the component proteins change conformation upon binding to the growing structure. Thus, once an initiator complex (of say protein A) is generated, protein B binds to it and changes its conformation so that protein C can bind, protein C in turn changes its conformation so protein D can bind, etc. In some cases, the precise juxtaposition of proteins B and C on the growing structure may also contribute to the creation of a binding site for D. In such a scheme, the unassembled proteins B, C, and D have no affinity for each other and so do not assemble in alternate ways. Thus, an ordered 'assembly pathway' of protein A first creating an initiation complex, then B, then C and then D binding in this defined order is determined solely by the properties of A, B, C, and D proteins. Because of these properties, when one component is missing, in most cases the assembly intermediate to which the missing component would have bound accumulates, and downstream components remain unassembled. This has allowed the examination of many such intermediates in the characterization of phage assembly pathways. The isolation in relevant genes of conditionally lethal nonsense mutations, which are particularly tractable in phage systems, made this approach extremely productive. Several issues regarding the assembly pathway strategy remain poorly understood, and these include such questions as: What controls initiation of the assembly process? Control could simply be the synthesis and proper folding of a limiting amount of protein A in the example above, but in some situations such as procapsid assembly (below) it appears to be more complex. And what is the exact nature of the conformational changes that occur upon binding? These remain fertile ground for research.

Assembly Strategies and Mechanisms

Branched Pathways, Subassemblies, and Quality Control

Phages have apparently evolved ways of maintaining quality-control systems in virion assembly. One such system is assembly though 'subassemblies' or discrete assembly intermediates that are unable to join with other subassemblies unless they are properly assembled. Thus, if an incorrectly built subassembly (say missing one of 10 protein molecules) is defective and unusable, it is

much less costly than if a complete virion (containing hundreds to thousands of protein molecules) were rendered defective due to one missing or defective protein molecule. Thus in the *Caudovirales*, for example, head precursors called procapsids are built first and most likely only package DNA if they are correctly assembled, tails are built independently (though a series of smaller subassemblies), and DNA-filled heads and tails only join when both are successfully completed. A second quality-control system may be that phage proteins have evolved to minimize assembly problems by ensuring that the participating proteins do not assemble unless their C-terminus is intact. Such a strategy ensures that prematurely terminated polypeptides (arguably the most probable translation error) are unable to participate, since they are missing their assembly site. Although this is an evolutionary argument, the fact that among the many phage assembly genes and proteins that have been studied, very few of the N-terminal fragments have 'dominant negative' genetic characteristics or are incorporated into the assembling virion.

Assembly of Symmetrical Protein Arrays

Assembly and maturation of icosahedral arrays

The use of an icosahedral protein shell to enclose phages' virion chromosomes appears to have evolved independently at least four times. Phage coat proteins are sufficiently ancient that even those with common ancestors are often not recognizably similar in amino acid sequence. We are thus left with comparison of their folded (secondary and tertiary) structures to deduce common or separate ancestries, and such structural information is not known for most phages. Nonetheless, high-resolution atomic structures are known by X-ray diffraction for a few members of several groups and those from the *Caudovirales*, *Tectiviridae*, *Microviridae*, and *Leviviridae* form three very different coat protein folding groups that are certainly not related. But do coat proteins from all members within each of these groups have a common ancestor? Our current limited knowledge suggests that this may well be true. High-resolution 3-D reconstructions of electron micrographs of several members of the *Podoviridae* and *Myoviridae* strongly suggest that all *Caudovirales* have the same unusual protein fold that has been determined for phage HK97. Finally, the *Cystoviridae* are thought to have a fourth type of subunit that is related to dsRNA viruses that infect eukaryotes.

Although much is understood about the actual structures of the phage (and other virus) icosahedral arrays that encase the nucleic acid in so many virions, in different viruses the size and shape of these arrays can vary greatly and control of these variations is poorly understood. No octahedral or tetrahedral viruses have been discovered. The geometry of icosahedral arrays is well understood, and if strict conformational and bonding identity is maintained among the participating chemically identical coat proteins, only exactly 60 subunits can be accommodated in the shell. However, if some flexibility (quasi-equivalence, above) is allowed, then the number of subunits (S) allowed in an isometric icosahedral shell is equal to $60T$ where $T = h^2 \times hk \times k^2$, where h and k are any integer including zero. This gives rise to the infinite series $T = 1, 3, 4, 7, 9, 12, \ldots$ and $S = 60, 180, 240, 420, 540, 720, \ldots$, respectively. Such shells can be thought of as having pentamers of the coat protein at each of the 12 icosahedral vertices and different numbers of hexamers making up the 20 triangular faces in different-sized shells. Phage heads are known with T numbers ranging from 1 to 52. Little is understood about exactly how the assembly of such shells is initiated or how their size is controlled. In many cases, the coat protein by itself will assemble into several sizes of shell as well as long tubes and irregular structures. In some cases (notably phages T4 and ϕ29), the icosahedral shell is elongated along the tail axis, and evidence from the systems under study suggests that both portal protein and scaffolding protein (see below) can be involved in this control, so factors beyond the innate coat protein properties clearly participate in the control of the spatial aspect of shell assembly.

Coat protein shells of the dsDNA phages assemble into a metastable 'immature' procapsid shell which undergoes a maturation that consists of a conformational transition to its final more stable state at about the time of DNA packaging. This maturation transition is typically accompanied by a significant expansion of the shell, the release of the scaffolding protein, and any proteolytic cleavage of the head proteins that might occur. This transition is surprisingly complex, and in the case of phage HK97, where it has been studied at the highest resolution, it proceeds through at least five separable coat protein conformational/organizational stages, in which there is little or no refolding of the main structural domain of coat protein, but there are significant changes in the orientations of the coat protein and the conformations of surface parts of the protein that are not within the central domain (**Figure 2**). The trigger(s) for this transition is not fully understood, but it clearly requires the successful completion of procapsid assembly. DNA entry and/or head protein proteolysis may be the natural signal for the transition to occur. This transition is universal in the assembly of tailed phages, and it has been suggested that topological difficulties in the construction of a closed shell with building units that have uneven surfaces might demand building a loosely bonded shell first, which subsequently rearranges into a more tightly bonded structure. The transition could also be part of a quality-control mechanism to ensure the structure is correctly built, and the concomitant expansion increases the available internal space for occupation by the DNA.

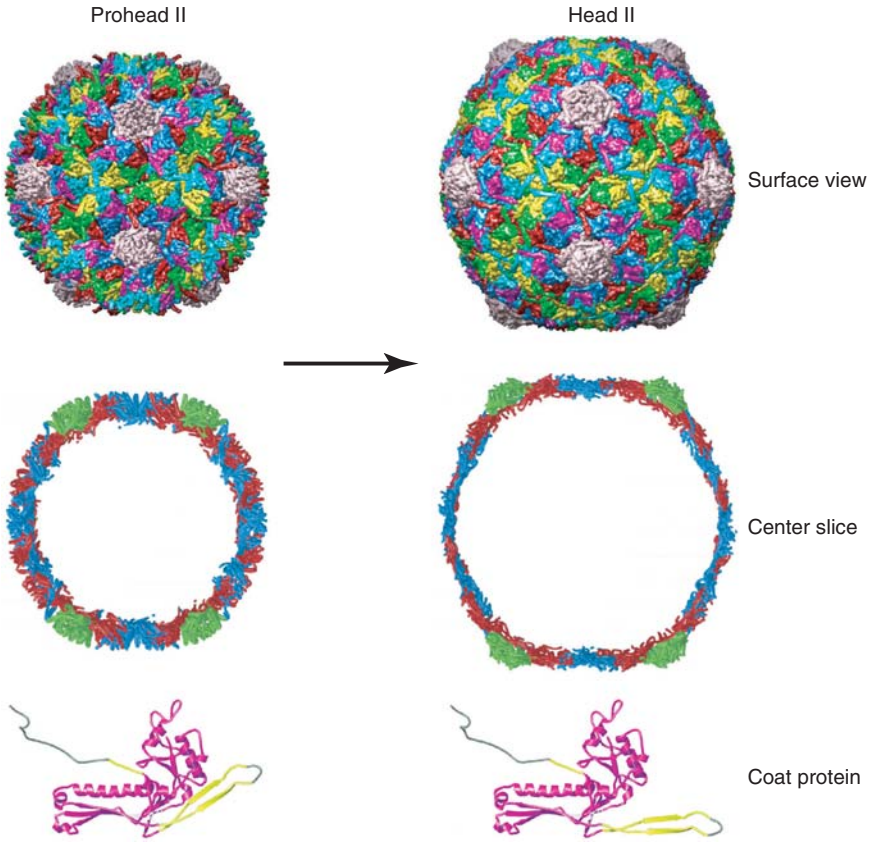


Figure 2 The maturation transition of the bacteriophage HK97 head. HK97 coat protein assembles into procapsids, its N-terminal 102 residues are removed by proteolytic cleavage, and the resulting structure is called prohead II. This coat protein shell undergoes a several-stage transition to become head II, which has expanded in diameter and has a thinner shell. In the surface view, the seven different 'quasi-equivalent' coat subunit locations are given different colors. In the slice view, the penton subunits are green and hexon subunits red or blue. In addition to subunit reorientation during the transition, the loop (called the E loop) shown at the lower right of the coat protein ribbon diagrams (the bottom tier) undergoes a major change in conformation during the transition. The images were provided by Lu Gan, Jack Johnson, and Roger Hendrix.

Symmetry disruptions and symmetry mismatches

The tails of the tailed phages (*Caudovirales*) and the portal of the *Tectiviridae* disrupt the icosahedral symmetry of coat protein shells of these virions, by being present at only one of the 12 otherwise identical vertices. How does this occur? Where it is known (phages P22, T4, T7, ϕ29), this 'disruption' replaces exactly one of the coat pentamers and it is present in the procapsid before the tail joins to the head. In procapsids, a dodecameric (12-mer) ring of portal protein occupies the position of the coat protein pentamer and tails will eventually bind to this portal protein. A single unique vertex is most simply explained if the portal protein forms the site that initiates coat shell assembly, and, in agreement with this idea, it has been shown that the absence of portal protein affects the spatial aspects of coat protein assembly in several phages (e.g., T4, λ, ϕ29). However, in some other phages, such as P22, coat assembles quite normally in the absence of portal protein. In all these systems, other factors, especially scaffolding protein (below), clearly play an as yet imprecisely defined role in initiation of coat protein assembly and in creating the unique portal vertex. The 12-fold symmetry of the portal ring and the fivefold symmetry of the capsid at the portal vertex means that there is a 'symmetry clash' between these two parts of the virion, and thus there cannot be identical interactions between the individual coat and portal subunits; it has been suggested that this could allow rotation of the portal with respect to the coat shell, but this has not yet been shown experimentally to be the case.

Helical arrays

Cylindrically shaped helical and stacked-ring protein arrays are present in the long tails of *Myoviridae* and *Siphoviridae*. In both types of viruses, these arrays assemble (in the cases studied) from the tail tip up to the head proximal end to give rise to the tail subassembly. Both types of tails have a complex baseplate (or tail tip) structure at their head-distal ends. Baseplates assemble first,

and when they are complete, they form the site at which the helical tail shaft begins to assemble. But helical arrays can be indefinitely long; so how are the discrete tail lengths programmed? Phage tails use a protein template (discussed in more detail below) to determine tail length, and when this length is attained, other proteins bind to the growing tip to block further growth. There are three types of helix (or stacked ring) building proteins, the *Myoviridae* tail tube (inner) and sheath (outer) proteins and the *Siphoviridae* tail shaft protein. Of these three, the sheath proteins are most highly conserved, and in many (but not all) tailed phages they are recognizably related in amino acid sequence. The other two are extremely variable, and with no structural information it is impossible to know whether or not they form discrete groups of distantly related proteins. Thus, there is no convincing evidence that the *Myoviridae* tail core and sheath and the *Siphovirdiae* tail shaft subunits have a common ancestor, and their different functionalities and roles suggests that they may have independent origins.

The *Inoviridae* or filamentous phages, unlike most other bacterial viruses, do not escape the host cell by lysing it. Their helical coat is assembled from subunits embedded in the cell membrane as the DNA is extruded through a complex assembly machine built into the membranes of the infected cell. Since they assemble in a such a different manner from the other phages under discussion here, and the detailed mechanism of their assembly is not understood in detail, they will not be considered in depth here.

Catalysis and Assembly

Scaffolds, templates, and jigs

Scaffolding proteins are proteins that assemble in fairly large numbers into procapsids but are not present in the completed virion. They act transiently to help in the proper assembly of coat protein shells and in the tailed-phage cases in the determination of the one unique vertex (above). The precise mechanisms by which scaffolding proteins perform these functions is unknown, but typically the interior space of *Caudovirales* procapsids contains several hundred molecules of scaffolding protein that are essential for proper procapsid assembly. They are then either proteolytically destroyed or released intact before DNA is packaged. Among the latter type, the scaffold of phage P22 has been shown to participate in an average of five rounds of procapsid assembly and so clearly acts catalytically in the assembly process. Stable oligomeric complexes between coat and scaffold molecules have not been found, yet scaffold 'nucleates' coat assembly (in the absence of all other proteins) at concentrations at which coat fails to assemble by itself. Scaffold remains as small oligomers in the absence of coat protein. It is not clear whether scaffold is forming a template whose surface guides coat assembly or is transiently binding and modifying the coat conformation so that it assembles correctly. *Microviridae*, in spite of their 'simple' $T=1$ coat protein shell, have both an internal and external scaffolding protein. In addition to roles in both temporal and spatial control of coat protein assembly, scaffolds may also function to fill the interior and thus exclude cellular macromolecules from the procapsid interior.

Perhaps the best example of an assembly template is the assembly of the *Caudovirales* tail shafts. *Siphoviridae* tail shaft subunits require two things to assemble, an initiation site on the completed baseplate and a template along which to assemble. This template has been best studied in phage λ, but even there tail shaft assembly is complex and not yet fully understood. It has been shown that the phage λ gene *H* (or 'tape measure') protein serves as a template for the assembly of the shaft subunit outward from the distal tip structure, and it is the length of the extended tape measure protein that determines the length of the tail shaft; shortening the tape measure protein causes the formation of a commensurately shorter tail. Interestingly, in these virions, the tape measure is thought to occupy the lumen of the tail, through which DNA must pass during injection, and the tape measure protein is ejected from the virion along with the DNA during the infection process. A second type of tape measure has been suggested for assembly of the phage PRD1 $T=25$ coat protein shell. The protein product of PRD1 gene *30* is stretched along the face edges of the icosahedral structure and may determine the length of the face and thus the size of the icosahedron.

A third type of spatial control of assembly is the 'jig', of which the phage T4 whisker is the prime example. This fiber protrudes from the head–tail junction, and its distal end binds to the elbow of the bent tail fiber, holding it in the correct position for joining of the tail fiber to the baseplate. The use of such a jig here may reflect some inherent difficulty in successfully attaching the tail fibers (see below).

Assembly enzymes and chaperonins

Aside from the scaffolding proteins, there are other well-studied examples of proteins that act catalytically during phage virion assembly. Perhaps the most curious of these is the gene *63* product of phage T4. This protein is required for the addition of tail fibers to the otherwise completed virion, but it is not present in virions. Its exact mechanism of action is not known, but it has been suggested that it and the jig described above are required to allow the creation of a flexible ball-and-socket joint between the fiber and the rest of the virion, which in turn might allow the fiber to 'search' more efficiently for its binding site on the surface of bacterial cells. In addition, T4 gene *38* protein appears to bind to and catalyze efficient trimerization of the subunit that forms the outer half of the bent tail fiber, but then it is released and not found in virions. Chaperonins are required in the folding of many proteins and so can be critical to phage assembly in that the proteins must be properly folded to participate

in the assembly process. It is interesting to note that the chaperonin protein-folding function was discovered during the genetic analysis of the host's role in phage λ head assembly, and that some phages (e.g., T4) encode replacement subunits for the GroE chaperonin to ensure that their proteins are folded correctly.

Other catalytic actions during phage assembly are the ATP cleavage-dependent action of DNA translocases that move the DNA into the procapsid through the portal ring and the cleavage of overlength replicated DNA to virion length by the phage-encoded enzyme called 'terminase' in the *Caudovirales* (the nucleic acid packaging process is less well understood in other phages). Both of these enzymatic functions are essential for tailed-phage assembly, and the reader is referred to the article in this volume on nucleic acid encapsidation for a more detailed discussion. In addition, enzymatic proteolytic modification of virion proteins is often essential during assembly (below).

Nucleic Acid Encapsidation

The packaging of nucleic acid within virions is covered in more depth in other articles of this volume, but it is noted here that different phages utilize very different strategies to build a virion with nucleic acid on the inside and protein (and lipid in some cases) surrounding that nucleic acid. The packaging of an ssRNA within the virion molecule by members of the *Leviviridae* family occurs by concomitant assembly of coat protein molecules into a $T = 3$ icosahedral structure and condensation of the RNA (which is predicted to be rather compact due to extensive secondary structure) by a process that is not understood in detail. Here, as apparently in all phages, recognition of a particular sequence in the viral nucleic acid is required to initiate packaging, thereby ensuring that only virus-specific nucleic acid is encapsidated. The other phage types either assemble a procapsid into which the nucleic acid is inserted by an energy-consuming nucleic acid translocase (*Caudovirales*, *Tectiviridae*, *Cystoviridae*) or assemble the virion around the ssDNA as it is extruded from the cell (*Inoviridae*).

Covalent Protein Modifications during Assembly

Protein cleavage

A common, but not universal, feature of phage assembly is the controlled proteolytic cleavage of some protein participants. This is best studied in the *Caudovirales*, and so they will be discussed here. It was discovered early that an N-terminal portion of all of phage T4's coat protein molecules are removed by proteolytic cleavage during assembly, and that this cleavage is dependent upon proper assembly of the coat protein shell. It has been suggested that the role of such coat protein trimming, which is common, is to make assembly irreversible, allow coat protein to find a more stable folded state, to alter coat protein function between virion assembly and DNA delivery, to help create room inside for the DNA, to be a kind of quality control, and/or to simply to remove part of the virion that is no longer needed after successful assembly. These of course are not mutually exclusive roles. Assembly-dependent cleavage of other virion assembly proteins such as portal proteins, scaffolding proteins and tape measure proteins, as well as the phage-encoded protease itself, have also been observed. Where it has been studied, a phage-encoded protease co-assembles into the procapsid and subsequently proteolyzes the head proteins, including itself. Tail protein cleavage is less well understood, and the protease that trims, for example, the C-terminus of phage λ tape measure protein has not been identified, but a host protease is responsible for the essential but non-assembly-dependent removal of the N-terminal region of a phage P22 virion protein that is injected with the DNA.

Protein cross-linking

Escherichia coli phage HK97 typifies a subset of *Siphoviridae* that are known to covalently cross-link all of their coat proteins as the final step in head shell maturation. Other covalent cross-links have been described between a few of the coat protein molecules of phage λ and a fragment of its putative head protease, but their role in assembly is not known. In HK97, each coat subunit is joined to its neighbors through lysine–asparagine side-chain isopeptide bonds. The topology of these cross-links is such that they form covalent rings of five and six subunits that are interlocked, thus making a sort of molecular 'chain mail'. This cross-linking is catalyzed by the coat protein itself after the shell has expanded, and the cross-links contribute to the stability of the head.

Lipid Membrane Acquisition

Several bacterial virus families have lipid bilayers in their virions (**Table 1**). These have been studied most in the *Tectiviridae* phage PRD1, which has a lipid bilayer between the icosahedral capsid shell and the internal dsDNA. This layer may aid in the protection of the intravirion nucleic acid, but also participates in DNA delivery into sensitive cells. Members of the *Tectiviridae*, *Cystoviridae*, and *Corticoviridae* all appear to acquire their lipid membrane in the cytoplasm from the host's membrane by as yet poorly understood mechanisms, after virus-encoded proteins have been inserted into the host membrane. Information is available on some of the virion proteins that interact with the lipid bilayers, but little is known about the detailed mechanism of the assembly of the membrane into these phages' virions. The *Plasmaviridae* phage L2, which infects a member of the bacterial *Mycoplasma* family, appears to bud from the host cell membrane. This kind of release may only be possible in the mycoplasmas, since they are the only bacteria that have no cell wall, but the morphogenesis of L2 has not been studied in detail.

Summary

Clearly, there is no single 'mechanism of bacteriophage capsid assembly'. Bacterial viruses are varied and complex, and they utilize many different mechanisms on the road to assembling completed virions. Two major take home lessons regarding phage assembly are (1) the ubiquitous use of obligate pathways in assembly processes and (2) the use of proteins that are essential for assembly but which are not present in the completed virion. The latter means that assembly cannot be understood by simply examining the properties of the components of the completed virion. In addition, the protein and lipid parts of virions are not simple containers designed solely for the protection of the nucleic acid inside. They are also sophisticated molecular devices that are designed to deliver their nucleic acid payloads into sensitive cells by mechanisms that are as varied as the ways in which they are assembled. No doubt, this requirement that virions be metastable 'spring-loaded' structures, that can spontaneously release their nucleic acid when the right external signal is received, is largely responsible for the complexity of phage virion structure and assembly. Because of this diversity and complexity, the study of phage assembly has shed considerable light on the mechanisms of many other macromolecular assembly processes.

Acknowledgments

The author thanks Lu Gan, Roger Hendrix, Jack Johnson, Victor Kostyuchenko, and Michael Rossmann for material for the figures.

See also: Assembly of Viruses: Nonenveloped Particles; Filamentous ssDNA Bacterial Viruses; Genome Packaging in Bacterial Viruses; Icosahedral dsDNA Bacterial Viruses with an Internal Membrane; Icosahedral Enveloped dsRNA Bacterial Viruses; Icosahedral ssDNA Bacterial Viruses; Icosahedral ssRNA Bacterial Viruses; Inoviruses; Virus Particle Structure: Nonenveloped Viruses; Virus Particle Structure: Principles.

Further Reading

Abrescia N, Cockburn J, Grimes J, *et al.* (2004) Insights into assembly from structural analysis of bacteriophage PRD1. *Nature* 432: 68–74.

Calendar R (ed.) (005) *The Bacteriophages,* 2. New York: Oxford University Press.

Casjens S (1985) An introduction to virus structure and assembly. In: Casjens S (ed.) *Virus Structure and Assembly*, pp. 1–28. Boston: Jones & Bartlett.

Casjens S and Hendrix R (1988) Control mechanisms in dsDNA bacteriophage assembly. In: Calendar R (ed.) *The Bacteriophages,* vol. 1, pp. 15–91. New York: Plenum.

Caspar D and Klug A (1962) Physical principles in the construction of regular viruses. *Cold Spring Harbor Symposium on Quantitative Biology* 27: 1–24.

Chiu W, Burnett R, and Garcea R (eds.) (1997) *Strucutral Biology of Viruses.* New York: Oxford University Press.

Fane B and Prevelige P , Jr. (2003) Mechanism of scaffolding-assisted viral assembly. *Advances in Protein Chemistry* 64: 259–299.

Fokine A, Chipman P, Leiman P, Mesyanzhinov V, Rao V, and Rossmann M (2004) Molecular architecture of the prolate head of bacteriophage T4. *Proceedings of the National Academy of Sciences, USA* 101: 6003–6008.

Hendrix R (2005) Bacteriophage HK97: Assembly of the capsid and evolutionary connections. *Advances in Virus Research* 64: 1–14.

Horn W, Tars K, Grahn E, *et al.* (2006) Structural basis of RNA binding discrimination between bacteriophages Qβ and MS2. *Structure* 14: 487–495.

Kostyuchenko V, Chipman P, Leiman P, Arisaka F, Mesyanzhinov V, and Rossmann M (2005) The tail structure of bacteriophage T4 and its mechanism of contraction. *Nature Structural and Molecular Biology* 12: 810–813.

Lander G, Tang L, Casjens S, *et al.* (2006) A protein sensor for headful viral chromosome packaging is activated by spooled dsDNA. *Science* 312: 1791–1795.

Poranen M, Tuma R, and Bamford D (2005) Assembly of double-stranded RNA bacteriophages. *Advances in Virus Research* 64: 15–43.

Steven A, Heymann J, Cheng N, Trus B, and Conway J (2005) Virus maturation: Dynamics and mechanism of a stabilizing structural transition that leads to infectivity. *Current Opinion in Structural Biology* 15: 227–236.

Wikoff W, Conway J, Tang J, *et al.* (2006) Time-resolved molecular dynamics of bacteriophage HK97 capsid maturation interpreted by electron cryo-microscopy and X-ray crystallography. *Journal of Structural Biology* 153: 300–306.

Cardioviruses

C Billinis, University of Thessaly, Karditsa, Greece
O Papadopoulos, Aristotle University, Thessaloniki, Greece

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Artiodactyls Even-toed hoofed plant-eating mammals, including pigs, deer, antelope, cows, camel, giraffe, and hippopotamus.

Autogenous vaccine A vaccine manufactured from virus isolated from the same animal or herd of animals.
Myocardiotropic Virus tropism to the myocardium.
Myocarditis Inflammation of the myocardium.

Neurotropic Virus tropism to the nervous system.
Phylogeny The evolutionary relationships between groups of organisms.

History

The genus *Cardiovirus* contains two species, *Encephalomyocarditis virus* and *Theilovirus* (**Table 1**). The first report of a cardiovirus was by Theiler in 1934. He reported that a neurological disease of mouse could be transmitted by intracerebral or intranasal inoculation, and was caused by a virus, which persisted in the central nervous system (CNS). This virus is known as Theiler's murine encephalomyelitis virus (TMEV). The disease was described as a polio-like infection with destruction of neurons of the anterior horns of spinal cord. Later, similar agents were isolated from the intestinal contents of normal mice and other rodents. Initially, some of these viruses were confused with poliomyelitis virus. In the early 1950s, Daniels *et al.* reported that a recently isolated strain of Theiler's virus, the DA strain, was responsible for a chronic white matter disease characterized by focal demyelination. A comprehensive picture was finally given in the mid-1970s by Lipton who showed that the DA strain, as well as the strains originally isolated by Theiler, cause a biphasic disease characterized by an initial polio encephalomyelitis with destruction of neurons followed by a chronic inflammatory and demyelinating disease of white matter resembling multiple sclerosis.

The first strain of encephalomyocarditis virus (EMCV), Columbia SK, was isolated from cotton rats (*Sigmodon hispidus*) by Jungeblut and Sanders in 1940. Immunological studies showed that this agent, which caused a flaccid paralysis of the hind limbs of mice followed by death, was a new and different neurotropic virus. This first description was followed by the isolation of MM virus in 1943, of EMCV in 1945, and Mengo virus in 1946. A last member of this serological group was isolated in 1949 by Gronnert, from the intestinal tissues of a sick mouse; this was called mouse-encephalomyelitis or maus-Elberfeld (ME) virus. A close relationship between all these viruses was soon recognized. Warren and Smadel in 1948 reported that sera from humans or animals containing neutralizing antibodies against EMCV would also neutralize Columbia SK and MM viruses. In 1949, Dick demonstrated cross-neutralization between Mengo and EMC viruses. Since the late 1960s, EMCV infection in laboratory animals has been used extensively as a research tool and model for human diseases. Extensive work has

Table 1 Cardiovirus strains

Serotype	*Isolate*	*Origin*	*Species*	*Year of isolation*	*Pathogenicity*	*Accession no. for P1*
Encephalomyocarditis virus						
EMCV	VR-129B	USA	Chimpanzee	1945	Myocardial	AJ617356
EMCV	Rueckert	USA	Chimpanzee	1945	Myocardial	M81861
EMCV	Mengo	Uganda	Rhesus monkey	1946	Paralysis	L22089
EMCV	B	Panama	Pig	1958	Myocardial	M22457
EMCV	MN-30	USA	Pig	1987	Reproductive	AY296731
EMCV	GRE-424	Greece	Pig	1990	Myocardial	AJ617362
EMCV	BEL-2887A	Belgium	Pig	1991	Reproductive	AF356822
EMCV	CYP-108	Cyprus	Pig	1995	Myocardial	AJ617359
EMCV	BEL-279	Belgium	Pig	1995	Myocardial	AJ617361
EMCV	BEL-440	Belgium	Pig	1995	Reproductive	AJ617360
EMCV	ITL-136	Italy	Pig	1986	Reproductive	AJ617358
EMCV	ITL-001	Italy	Pig	1996	Reproductive	AJ617357
Theilovirus						
TMEV	GDVII	USA	Mouse	1937	Neurovirulent	M20562
TMEV	GDVII	USA	Mouse	1937	Neurovirulent	X56019
TMEV	FA	USA	Mouse	1937	Neurovirulent	U32924
TMEV	TO4	USA	Mouse	1937	Biphasic	U33045
TMEV	TO Yale	USA	Mouse	1943	Biphasic	U33047
TMEV	WW	USA	Mouse	1977	Biphasic	U33046
TMEV	DA	USA	Mouse	1952	Biphasic	M20301
TMEV	BeAn8386	Brazil	Mouse	1957	Biphasic	M16020
TMEV	M2	Brazil	BeAn variant	1957	Biphasic	AF030574
TLV	NGS910		Rat	1991		AB090161
VHEV	Siberia-55	Siberia	Human	1963	Encephalitis	M94868

EMCV, encephalomyocarditis; TMEV, Theiler's murine encephalomyelitis virus; TLV, Theiler's-like virus of rats; VHEV, Vilyuisk human encephalomyelitis virus.

been carried out using animal models for diabetes mellitus, cardiovascular diseases, polymyositis, and diseases of the CNS. More recently, EMCV has been recognized as a viral pathogen of domestic animals, especially swine. Clinical outbreaks of EMCV infection in pig herds were first encountered in Panama in 1958 and in Florida in 1960. Subsequently, clinical outbreaks in pigs have been reported in North America, New Zealand, Australia, Brazil, Cuba, Greece, Italy, Belgium, and Cyprus. In addition, EMC virus or antibodies have been detected in several species, including rodents, domestic and wild mammals, birds, and humans, often without evidence of clinical disease.

Properties of the Virion and Genome

The virion of all cardioviruses contains single-stranded positive-sense RNA of 2.6×10^6 Da enclosed in an icosaedral capsid. The genome varies in length from 7800 to 8000 nt and is composed of the leader protein followed by the four structural proteins (VP1–4) and seven nonstructural proteins (2A, 2B, 2C, 3A, 3B, 3C, 3D). The coding region is surrounded by two untranslated regions (UTRs) at the 5′ and 3′ ends. The 5′ UTR comprises an internal ribosome entry site (IRES) from which viral protein translation is initiated. EMC viruses, but not theiloviruses, contain an unusual polypyrimidine (poly C) tract within the distal region of their 5′ UTRs. This tract could be involved in viral virulence, although its exact role remains unclear, and can vary in length from 60 to 420 residues, depending upon the strain of virus. In all cardioviruses, the 3′ UTRs terminate with a heterogeneous poly (A) tail, being involved in the binding process of the viral RNA-dependent RNA polymerase, whose encoding gene is located upstream to the 3′ UTR.

Cardioviral proteins and their precursors take their names (L, P1, P2, P3) from their sequential locations within the polyprotein (**Figure 1**). The leader or L proteins are present only in the EMC viruses and the persistent strains of TMEV. The EMC L proteins play an undefined role in host or tissue tropism and may also be involved in translational regulation of the IRES. The four P1 peptides are the capsid proteins 1A, 1B, 1C, and 1D. The middle portion of the polyprotein (P2) contains peptides 2A, 2B, and 2C. Protein 2C is an ATPase, not a polymerase, and its contribution to the replication cycle remains unclear. The P3 peptides, 3A, $3B^{VPg}$, $3C^{pro}$, and $3D^{pol}$ are more closely associated with genome replication. Protein 3B is VPg, the peptide covalently linked to the 5′ end of the genome. VPg sequences are rich in basic, hydrophilic amino acids and have only one tyrosine residue (the attachment site) at position 3 from the amino end of the peptide. Protease $3C^{pro}$ is the central enzyme in the viral cleavage cascade.

Taxonomy and Classification

The cardioviruses constitute a separate genus, *Cardiovirus*, within the family *Picornaviridae*. They are distinguished from other picornaviruses by their pathological properties, genome organization, and the dissociability of their virions at pHs between 5 and 7 (in 0.1 M NaCl). The genus *Cardiovirus* consists of two species, *Encephalomyocarditis virus* and *Theilovirus*. Comparative analysis of RNA sequencing of different isolates has shown a clear difference between the two species (**Table 1**; **Figures 2** and **3**).

Encephalomyocarditis virus is represented by a single serotype of the same name. Mengovirus, Columbia SK virus, and Maus Elberfeld virus are strains of EMCV, based on serological cross-reaction and sequence identity. The species *Theilovirus* consists of three viruses (which are probably distinct serotypes): TMEV, Vilyuisk human encephalomyelitis virus (VHEV), and rat Theiler's-like virus (TLV). A fourth virus, rat encephalomyelitis virus (REV), is probably a strain of TMEV, although conclusive sequence data are awaited. Theiloviruses are also divided into two subgroups, based on neurovirulence (see the section titled 'Pathogenicity').

Virus Propagation

Cardioviruses replicate in primary or continuous cell lines originating from a variety of species, including murine, bovine, porcine, human, primate, guinea pig, and hamster. Baby hamster kidney (BHK-21) and Vero cells are most commonly used. The virus also replicates in baby mice and chicken embryos and is pathogenic to many laboratory animals. EMC virus hemagglutinates guinea pig, rat, horse, and sheep erythrocytes. Serial passages of EMC viruses in cell culture can alter *in vitro* growth characteristics, reduce virulence, and affect hemagglutinating activity.

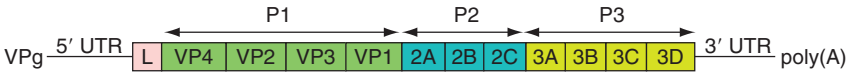


Figure 1 Diagram of the cardiovirus RNA genome, showing the genome-linked VPg at the 5′ end, the 5′ untranslated region (5′ UTR), the leader protein (L), the three viral protein coding regions P1 (VP4, VP3, VP2, VP1), P2 (2A, 2B, 2C), and P3 (3A, 3B, $3C^{pro}$, $3D^{pro}$), the 3′ untranslated region (3′ UTR), and the poly (A) tail.

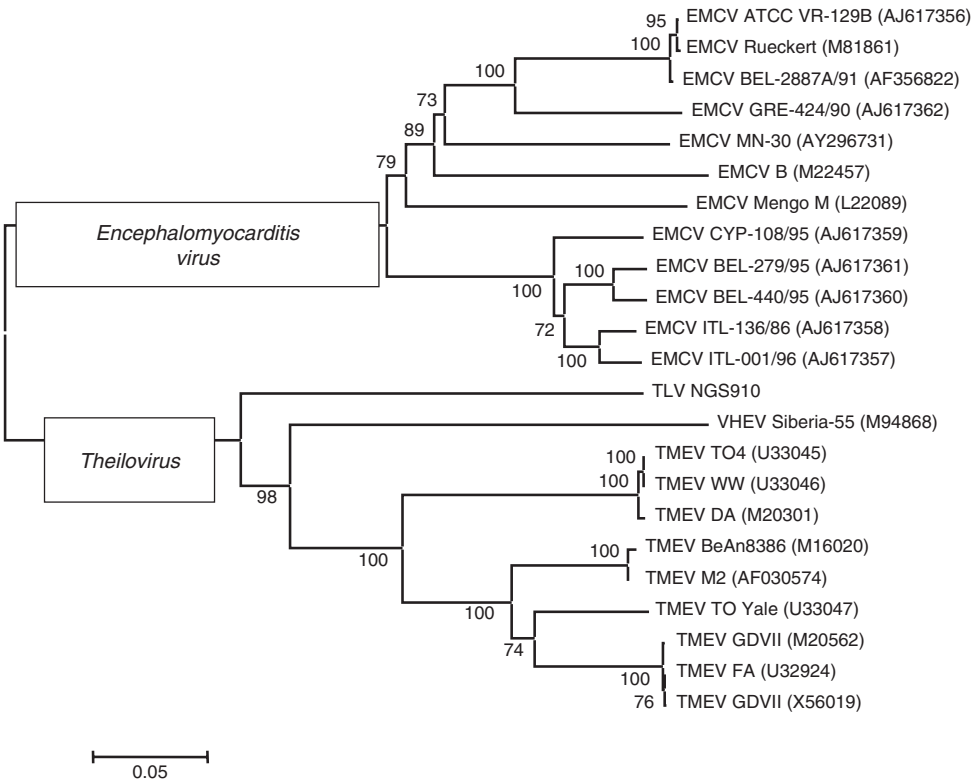


Figure 2 Neighbor-joining tree based on a comparison of nucleotide similarities of P1 capsid polypeptides of EMCVs and theiloviruses. The tree was generated with the MEGA 3.1 program. Neighbor-joining trees were constructed from a different matrix employing the Kimura two-parameter correction. One thousand bootstrap pseudoreplicates were used to test the branching (shown as percentages). For abbreviations, see **Table 1**. Data provided by Dr. N. J. Knowles, Institute for Animal Health, Pirbright Laboratory, UK.

Replication

The virus particle attaches to a cell receptor and is taken into the cell. The RNA genome is then uncoated and translated into a single polyprotein which is cleaved in a cascade of events catalyzed by the viral protease $3C^{pro}$, and possibly by protein 2A in the case of the 2A/2B cleavage. The viral RNA is replicated by the RNA polymerase $3D^{pol}$ through a complementary negative strand. Notably, proteins L and 2A are not required for viral replication in BHK-21 cells, although they may play important roles *in vivo*. Provirions are assembled by packaging RNA within capsid pentamers to form infectious particles. The replication takes place in the cell cytoplasm (**Figure 7**). However, in the case of EMCV, dense and empty viral particles have also been found in the nucleus of cardiac muscle cells (**Figure 8**).

Evolution

The complete genome sequences of P1 capsid-coding region (CCR) of 12 EMC viruses, nine TMEVs, a Theiler's-like virus of rats, and a Vilyuisk human encephalomyelitis virus have been deposited in GenBank databases. Phylogenetic and molecular evolutionary analyses using MEGA 3.1 confirmed that two distinct species exist: EMCVs and theiloviruses. The 12 EMCVs formed one cluster, while the second cluster comprised all theiloviruses. These clusters differed by an average of approximately 42%. The maximum nucleotide variation among EMCVs was 21.70%, whereas within Theilovirus group it was 29.70%. The tree constructed from amino acids shows how viruses within a serotype are more closely related than they appear on the tree constructed from nucleotides, while members of different serotypes are still more distantly related (**Figures 2** and **3**).

Genetic analysis of European pig EMC viruses, in comparison with Mengo virus, have been performed with sequences of the $3D^{pol}$ gene and the VP3/VP1 junction. These studies showed clustering of all the European isolates in two lineages, A and B, with a similar tree topology for both genome regions. A correlation between the phylogeny and the geographic origin was demonstrated, but no link was established with the clinical picture induced in pigs. Phylogenetic analyses of nucleotide sequences of the coding regions (the Leader gene, the CCR, and the end of $3D^{pol}$ gene) also confirmed the A and B subgroups of

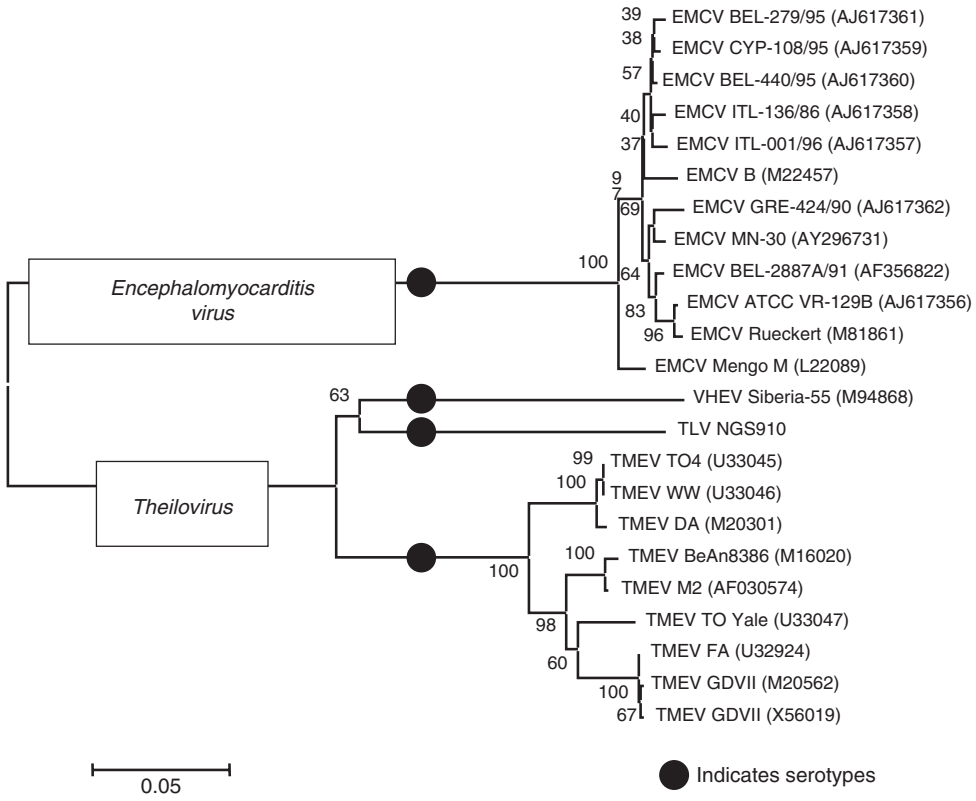


Figure 3 Neighbor-joining tree based on a comparison of amino acid similarities of P1 capsid polypeptides of EMCVs and theiloviruses. The tree was generated with the MEGA 3.1 program. Neighbor-joining trees were constructed from a different matrix employing the Kimura two-parameter correction. One thousand bootstrap pseudoreplicates were used to test the branching (shown as percentages). For abbreviations, see **Table 1**. Data provided by Dr. N. J. Knowles, Institute for Animal Health, Pirbright Laboratory, UK.

EMCV. In addition, the topology of the tree constructed on the 5′ UTR showed that a Greek isolate, with complex clinical picture, was different, and most closely related to Mengo, in comparison to the others. The divergence of this isolate was also observed for the sequence of the 3′ UTR reduced by the 14 nt upstream to the poly (A) tail. This short adenosine-rich region involved in RNA polymerase binding influenced considerably the topology of the tree. It also appeared that the length of the poly (C) tract was not related to the difference in virulence observed between various EMCV isolates as it has been described for other EMCV strains in the past.

Serial passages *in vivo* of an EMCV pig isolate had a limited impact on the nt and aa sequence of the regions targeted, with an exception of the 2A gene and VP1, but it had a critical effect on the virulence of the virus by inducing its attenuation. A large deletion of 115 aa was found within the 2A gene which encodes a protein able to affect a co-translational break in the polyprotein chain at its own C-terminus releasing P1–2A. The N-terminal cleavage of 2A from P1 CCR is achieved by the virus-encoded proteinase $3C^{pro}$. The EMCV 2A protein also contains a nuclear localization signal, which allows it to migrate to nuclei and nucleoli where it is involved in inhibition of cellular mRNA transcription and inhibition of cap-dependent mRNA translation, respectively. The deletion, which had been reported in the *in vitro*-passaged EMCV, removed all of 2A except the 18 aa necessary for the N- and C-terminal protein processing; thus, this deletion could, at least in part, be responsible for the attenuation observed experimentally. In addition, the mutation located at position 62 of VP1, which is adjacent to the aa responsible for the ability of certain EMCV strains to induce diabetes in mice, could also be involved in attenuation. Moreover, the low variability that has been detected after *in vivo* passages revealed that EMCV is stable in pigs.

Epidemiology (Geographic Distribution, Host Range, and Transmission)

Cardioviruses have a worldwide distribution and are associated with a wide variety of species. Rodents, especially rats, are considered to be the natural host and reservoir of EMCV. In wild rodents, the virus usually persists without causing disease, whereas infection in laboratory rodents,

especially baby mice, commonly produces fatal encephalitis and/or myocarditis. In pigs, natural infections with EMCV have different clinical characteristics, probably depending on different EMCV strains, which act with different pathogenicity. The infection has been recognized either as a cause of mortality in young pigs, due to acute myocarditis, or reproductive failure in sows. Clinical outbreaks of EMCV infection in pig herds were reported in Europe, Australia, New Zealand, and America. In Europe, the myocardial form has been reported in Greece, Italy, Cyprus, and Belgium. The reproductive form has only been reported in Belgium. In addition, apparently subclinical infections were reported in British and Austrian pigs, with a serological prevalence of 28% and 5%, respectively.

Two major routes of infection are suggested for the introduction and/or subsequent spread of the virus within a pig farm. The first infection route is ingestion of feces or carcasses of infected rodents, as many outbreaks have been linked with rodent plagues. The second route is horizontal pig-to-pig transmission through virus excretion by acutely infected pigs for 1–4 days. The virus can also be transiently excreted from persistently infected pigs, after reactivation of the infection. Transplacental infection has also been described as a potential route of virus spread. Other factors, such as infectious dose, route of infection, and age of pigs, have been found to be important in the spread of the virus under experimental conditions.

Clinical outbreaks in zoological parks and research institutions, involving several animal species, including elephants, artiodactyls, marsupials, mongooses, porcupines, and primates, have also been reported. In addition, EMCV virus or antibodies have been detected in domestic and wild mammals, birds, and humans, often without evidence of clinical disease.

There have been reports that humans are susceptible to EMCV. However, most of the evidence has been indirect, based on the presence of antibodies. In the 1950s, only a few documented cases of EMCV infection in humans were associated with fever, neck stiffness, lethargy, delirium, and headache. Human cases have been reported in Australia, in an area with a high incidence of EMCV in pigs. An EMCV outbreak in a zoo in the US, involving multiple animal species, did not result in illness in humans, although a zoo attendant who cared for EMCV-infected primates demonstrated a high antibody titer. Recent serological surveys indicate that human EMCV infections are relatively common in certain areas of the world (i.e., Austria, Greece), but generally are asymptomatic or unrecognized. Recent advances toward using pig tissues as a means of overcoming the acute shortage of transplantation tissues and organs for humans have made it necessary to determine the risk of transmitting this zoonotic virus from pigs to humans. The experimental infection of mice by transplanting pig organs infected with EMCV demonstrates that the risk is real.

TMEV is a natural enteric pathogen of mice that causes asymptomatic infection, but on rare occasions the virus will invade the CNS. TMEV shares many biological characteristics in common with poliovirus, another member of the picornavirus family, that may include spread from host to host via the fecal–oral route or the oropharyngeal–oral route.

Pathogenicity, Clinical Features, and Pathology

Encephalomyocarditis Virus

The expression of clinical disease in animals depends upon both viral and host factors. In susceptible species, strains of EMCV vary in virulence and/or tissue tropism. In the natural hosts, wild rodents, the virus usually persists without causing disease. Among laboratory animals, clinical manifestations and pathogenesis of EMCV are variable. Certain virus strains cause predominantly fatal encephalitis, widespread myocardial damage, or specific destruction of pancreatic β-cells. The course of infection varies in rats, mice, guinea pigs, hamsters, gerbils, rabbits, and monkeys, depending on the age of the animals and the virus strain used.

On the basis of organ tropism in mice, Graighead in 1966 classified EMCV into two variants, E (neurotropic) and M (myocardiotropic). EMCV-M also shows tropism for the β-cells of the pancreas, producing a disease syndrome similar to insulin-dependent diabetes mellitus. In 1980, Yoon *et al.* established a highly diabetogenic D variant and a nondiabetogenic B variant by repeated purification of EMCV-M. In addition, EMCV strains vary in pathogenicity and tissue tropism. In adult rats and mice experimentally infected with two myocardial EMCV strains of pig origin, no clinical signs or gross lesions were observed. Regardless, virus was isolated from several tissues from the third day post infection until the end of the observation, at 62 days post infection. EMCV was most frequently isolated from Peyer's patches and thymus. The results suggested that these tissues represent a site of persistence. Furthermore, EMCV antigen was detected in various organs but consistently in the heart and thymus.

In zoo animals, the disease is characterized by sudden death due to acute myocarditis. With the exception of pigs, domestic animals are generally resistant to overt clinical EMCV infection. In pigs, disease due to EMCV may take one of two main forms, depending on the tropism of the viral strain: an acute myocarditis (usually in piglets) or reproductive failure in sows. Each form of the disease in pigs (myocardial or reproductive) seems to be restricted to certain geographical areas, probably reflecting the character of viral strains originating from local rodent populations. Pig age at the time of infection is an important determinant of clinical severity. Extremely

high mortality, approaching 100%, is limited to pigs of pre-weaning age. Infections in pigs from post-weaning age to adulthood are usually subclinical, although some mortality may be observed even in adult pigs. The myocardial form is most commonly characterized by acute disease with sudden death due to myocardial failure. Other clinical signs may be observed, including lethargy, inappetence, trembling, staggering, paralysis, vomiting, and dyspnea. Persistent infection has been demonstrated, the virus persisting in lymphoid tissues and transiently excreted in the feces following reactivation. Macroscopical lesions vary in severity and consist of multiple white-gray linear or circular areas, which are visible in the wall of the ventricles, especially those of the right ventricle (**Figure 4**). Histopathological lesions are characterized mainly by interstitial mononuclear cell infiltration, degeneration, and necrosis of cardiac muscle cells and occasionally calcium deposits (**Figures 5** and **6**). Electron microscope examination reveals presence of the virus intracytoplasmically in cardiac muscle cells, Purkinje fibers, and endothelial cells of the capillaries and intranuclearly in cardiac muscle cells (**Figures 7** and **8**). In breeding females, clinical signs may vary from no obvious illness to various forms of reproductive failure, including poor conception rates, embryo resorption, mummification, stillbirths, abortions, and neonatal death.

Theiler's Virus

Theiloviruses are divided into two subgroups, based on neurovirulence following intracerebral inoculation of mice: GDVII and Theiler's Original (TO). The GDVII group includes GDVII and FA strains; it is extremely neurovirulent and induces acute fatal polioencephalomyelitis without demyelination. The TO group is less

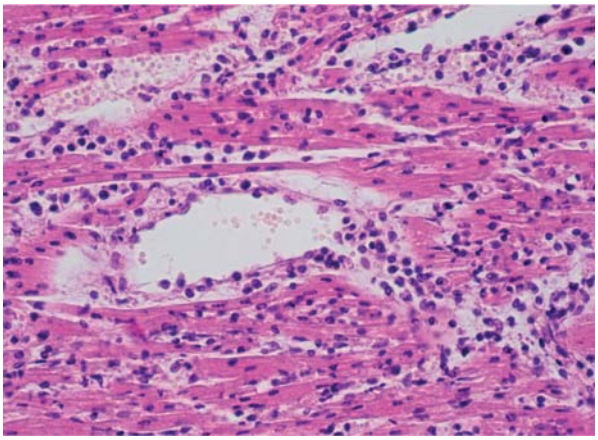


Figure 5 Pig myocardium. Interstitial mononuclear cell infiltration, consisting of lymphocytes and macrophages. Courtesy of Dr. V. Psychas, Faculty of Veterinary Medicine, Aristotle University, Thessaloniki, Greece.

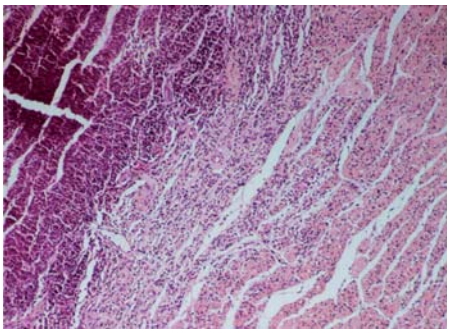


Figure 6 Pig myocardium. Diffuse interstitial mononuclear cell infiltration accompanied by calcium deposits (toward left). Courtesy of Dr. V. Psychas, Faculty of Veterinary Medicine, Aristotle University, Thessaloniki, Greece.

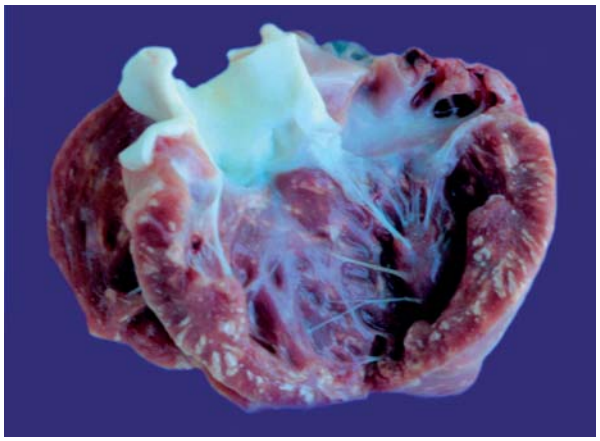


Figure 4 Heart of a pig with EMCV infection showing multiple white-gray linear and circular calcified necrotic areas in the myocardium also visible under the endocardium. Courtesy of Dr. V. Psychas, Faculty of Veterinary Medicine, Aristotle University, Thessaloniki, Greece.

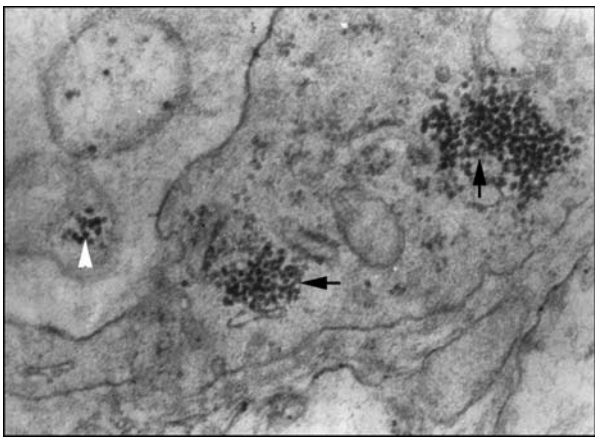


Figure 7 Electron photomicrograph from myocardium of a pig infected with EMCV. Notice the numerous virus particles (arrows) in the cytoplasm of an endothelial cell and the membranous vesicles (arrowhead) containing virus particles free in the lumen of the capillary. Courtesy of Dr. V. Psychas, Faculty of Veterinary Medicine, Aristotle University, Thessaloniki, Greece.

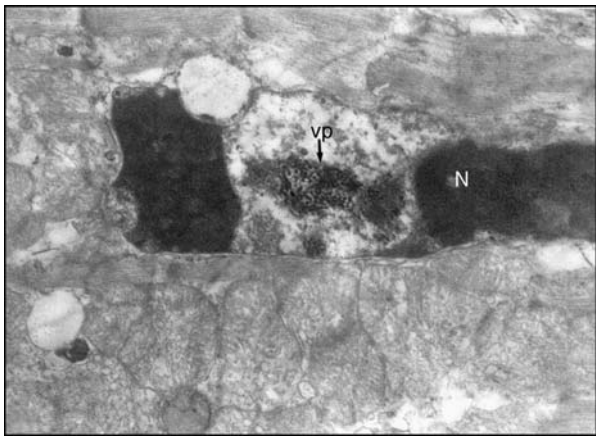


Figure 8 Electron photomicrograph from myocardium of a pig infected with EMCV. Notice a pyknotic nucleus (N) with aggregations of dense virus particles (vp). Courtesy of Dr. V. Psychas, Faculty of Veterinary Medicine, Aristotle University, Thessaloniki, Greece.

neurovirulent and includes the TO4, TO-Yale, BeAn, WW, and DA strains. These strains cause a biphasic disease in which the early phase is characterized by an acute encephalomyelitis. If the virus persists, the animals will develop a chronic demyelinating disease in the CNS.

A large number of detailed pathogenesis studies in mice have been reported and reviewed. Generally, no clinical signs are observed, but on rare occasions the virus may invade the CNS and cause neurological disease. After ingestion, the virus replicates in the pharynx or the lower gastrointestinal tract, spreads to local and regional lymphatic tissue, and then invades the blood stream before penetrating and replicating in the nervous system, resulting in unilateral or bilateral flaccid paralysis of the hind limbs and rarely other neurological signs. It is postulated that invasion of the CNS is taking place via the circulation and crossing the blood–brain barrier (BBB) or through lymphatic channels. The BBB is composed of specialized cerebrovascular endothelial cells, which restrict vesicular transport and protect the CNS from harmful substances circulating in the blood. Hematogenous virus entry into the CNS may involve endothelial cells and/or macrophages. TMEV viral RNA has been detected in vascular endothelial cells, neurons, and glial cells in the brain and spinal cord. Moreover, mononuclear cells isolated directly from the CNS inflammatory infiltrates of TMEV-infected mice were found to contain infectious viral antigens, and TMEV was found to replicate in brain macrophages. Several studies also suggest that the main viral dissemination route for Theiler's virus may occur by infection of peripheral organs, such as muscle, followed by viral entry from these secondary sites via peripheral neural routes.

Diagnosis

Encephalomyocarditis Virus

In piglets, the disease is often characterized by sudden death without any prior clinical signs. The most striking lesions are white-gray linear or circular areas under the endocardium and epicardium. All age categories may be affected, but mostly pigs 20–40 days are involved. In sows, a clinical history of reproductive failure and pre-weaning piglet mortality is suggestive of EMCV infection. EMCV-induced reproductive problems should be differentiated from other pathogens, such as porcine reproductive and respiratory syndrome, Aujeszky's disease, and porcine parvovirus.

Histopathological examination may contribute to diagnosis. A variable degree of nonsuppurative interstitial myocarditis is characteristic of EMCV infection. The presence of virus antigen both in the cytoplasm and in the nucleus of cardiac muscle cells by immunohistochemical examination with the use of monoclonal antibodies further strengthens the diagnosis. A conclusive diagnosis of EMCV should be demonstrated by virus isolation in sensitive cell culture (e.g., BHK-21) and identification by cross-serum neutralization with a reference antiserum. Virus isolation should be attempted mainly from heart and spleen tissues of acutely infected piglets. Experimental infection in piglets demonstrated that the virus could be easily isolated during the period of viremia, that is, from 1 to 5 days post infection. Nucleic acid probes and reverse transcription-polymerase chain reaction (RT-PCR) may provide sensitive and specific methods of diagnosis.

The commonly used serological test is virus-neutralization test. Enzyme-linked immunosorbent assay (ELISA) may also be used, if available. However, serology should not be used alone, as EMCV antibodies have been detected in areas with no clinical disease, presumably due to nonpathogenic EMCV strains.

Theiler's Virus

Molecular detection by PCR is highly sensitive and specific for TMEV. It is used for monitoring laboratory mouse colonies. Serological detection of the virus is inadequate, especially in nude or immunocompromised mice.

Prevention and Control

There is no specific treatment for EMC. In pigs, avoiding stress or excitement in affected animals may minimize mortality. EMCVs appear to persist in rodents and are most likely to affect pigs and zoo animals when rodent populations are high. Elimination or reduction of feral rodent populations is recommended in the prevention

and control of EMCV in order to minimize environmental contamination with virus. Prompt and proper disposal of animals that have died of the disease is also recommended. EMCV is inactivated by the use of disinfectants labeled for use against nonenveloped viruses. Autogenous and commercial killed vaccines or a genetically engineered vaccine have been used for the prevention of the myocardial form in pigs or zoo animals.

Recent studies demonstrated that the vaccination of mice with cDNA encoding the capsid proteins of TMEV could alter the course of TMEV-induced demyelinating disease. A single vaccination with cDNA encoding VP1 led to increased pathology in the CNS and increased clinical expression of disease. In another study, vaccinating mice three times with cDNA encoding the capsid proteins VP2 and VP3 reduced TMEV-induced disease, as demonstrated by a decrease in clinical expression of disease and reduce CNS pathology.

See also: Phylogeny of Viruses; Picornaviruses: Molecular Biology; Theiler's Virus.

Further Reading

Brahic M and Bureau JF (1998) Genetics of susceptibility to Theiler's virus infection. *BioEssays* 20: 627–633.

Denis P, Liebig HD, Nowotny N, *et al.* (2006) Genetic variability of encephalomyocarditis virus (EMCV) isolates. *Veterinary Microbiology* 113: 1–12.

Koenen F (2006) Encephalomyocarditis virus. In: Straw BE, Zimmerman JJ, D'Allaire S, and Taylor DJ (eds.) *Diseases of Swine*, p. 331. Ames, IA: Blackwell.

Lipton HL and Jelachich ML (1997) Molecular pathogenesis of Theiler's murine encephalomyelitis virus-induced demyelinating disease in mice. *Intervirology* 40: 143–152.

Lipton HL, Kumar MAS, and Trottier M (2005) Theiler's virus persistence in the central nervous system of mice is associated with continuous viral replication and a difference in outcome of infection of infiltrating macrophages versus oligodendrocytes. *Virus Research* 111: 214–223.

Racaniello V (2001) *Picornaviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 685–722. Philadelphia: Williams and Wilkins.

Scraba DG and Palmenberg A (1999) Cardioviruses (*Picornaviridae*). In: Webseter RG and Granoff A (eds.) *Encyclopedia of Virology*, pp. 229–238. London: Academic Press.

Zimmerman JJ (1994) Encephalomyocarditis. In: Beran GW and Steele JH (eds.) *Handbook of Zoonoses*, pp. 423–436. Boca Raton, FL: CRC Press.

Carlavirus

K H Ryu and B Y Lee, Seoul Women's University, Seoul, South Korea

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Symptomless Having no apparent symptoms of disease.

Triple gene block (TGB) A specialized evolutionarily conserved gene module of three partially overlapping ORFs involved in the cell-to-cell and long-distance movement of some plant viruses.

Introduction

The genus *Carlavirus* derived from the name of the type species *Carnation latent virus* is one of nine genera in the family *Flexiviridae* and contains a large number of members and tentative members. The carlavirus-infected natural host plants usually have very mild symptoms or remain symptomless, and thus the term 'latent' appears in the names of many species of the genus *Carlavirus*. The natural host range of individual species is restricted to a few plant species. This tendency toward mild or latent (symptomless) infection, a characteristic feature of carlaviruses, has led to carlaviruses and carlavirus-associated diseases failing to attract any special attention from phytopathologists and phytovirologists. However, many members of the genus have been identified in the last few decades and most of them have been associated with more serious diseases when plants are co-infected with other viruses. Most species are transmitted by aphids in a nonpersistent manner but two species (*Coupea mild mottle virus* and *Melon yellowing-associated virus*, CPMMV and MYaV) are transmitted by whiteflies (*Bemisia tabaci*).

Taxonomy and Classification

The genus *Carlavirus* belongs to the family *Flexiviridae*. The type species of the genus is *Carnation latent virus*. The name carlavirus was derived from the type species. The genus contains a large number of members. In total, 68 members of the genus *Carlavirus* (39 definitive species and 29 tentative species) are listed in **Table 1**. Some members listed as species and all members listed

Table 1 Virus species in the genus *Carlavirus*

Mode of transmission	*Virus species name*	*Abbreviation*	*Accession number*
Species			
Aphid-transmitted	*American hop latent virus*	AHLV	
	Blueberry scorch virus	BlScV	NC_003499
	Cactus virus 2	CV-2	
	Caper latent virus	CapLV	
	Carnation latent virus	CLV	X52627, AJ010697
	Chrysanthemum virus B	CVB	NC_009087
	Cole latent virus	CoLV	AY340584
	Dandelion latent virus	DaLV	
	Daphne virus S	DVS	AJ620300
	Elderberry symptomless virus	ESLV	
	Garlic common latent virus	GarCLV	AB004805
	Helenium virus S	HVS	D10454
	Honeysuckle latent virus	HnLV	
	Hop latent virus	HpLV	NC_002552
	Hop mosaic virus	HpMV	AB051109
	Hydrangea latent virus	HdLV	
	Kalanchoe latent virus	KLV	AJ293570-1
	Lilac mottle virus	LiMoV	
	Lily symptomless virus	LSV	NC_005138
	Mulberry latent virus	MLV	
	Muskmelon vein necrosis virus	MuVNV	
	Nerine latent virus	NeLV	DQ098905
	Passiflora latent virus	PLV	NC_008292
	Pea streak virus	PeSV	AF354652, AY037925
	Potato latent virus	PotLV	AY007728
	Potato virus M	PVM	NC_001361
	Potato virus S	PVS	NC_007289
	Red clover vein mosaic virus	RCVMV	
	Shallot latent virus	SLV	NC_003557
	Sint-Jan's onion latent virus	SJOLV	
	Strawberry pseudo mild yellow edge virus	SPMYEV	
Whitefly-transmitted	*Cowpea mild mottle virus*	CPMMV	DQ444266
	Melon yellowing-associated virus	MYaV	AY373028
Unknown vector	*Aconitum latent virus*	AcLV	NC_002795
	Narcissus common latent virus	NCLV	NC_008266
	Poplar mosaic virus	PopMV	NC_005343
	Potato rough dwarf virus	PRDV	AJ250314
	Sweetpotato chlorotic fleck virus	SPCFV	NC_006550
	Verbena latent virus	VeLV	AF271218
Tentative species			
Aphid-transmitted	Anthriscus latent virus	AntLV	
	Arracacha latent virus	ALV	
	Artichoke latent virus M	ArLVM	
	Artichoke latent virus S	ArLVS	
	Butterbur mosaic virus	ButMV	
	Caraway latent virus	CawLV	
	Cardamine latent virus	CaLV	
	Cassia mild mosaic virus	CasMMV	
	Chicory yellow blotch virus	ChYBV	
	Coleus vein necrosis virus	CVNV	DQ915963
	Cynodon mosaic virus	CynMV	
	Dulcamara virus A	DuVA	
	Dulcamara virus B	DuVB	
	Eggplant mild mottle virus	EMMV	
	Euonymus mosaic virus	EuoMV	
	Fig virus S	FVS	
	Fuchsia latent virus	FLV	
	Gentiana latent virus	GenLV	
	Gynura latent virus	GyLV	
	Helleborus mosaic virus	HeMV	

Continued

Table 1 Continued

Mode of transmission	Virus species name	Abbreviation	Accession number
	Hydrangea chlorotic mottle virus	HCMV	DQ412999
	Impatiens latent virus	ILV	
	Lilac ringspot virus	LiRSV	
	Narcissus symptomless virus	NSV	NC_008552
	Plantain virus 8	PlV-8	
	Potato virus P	PVP	DQ516055
	Prunus virus S	PruVS	
	Southern potato latent virus	SoPLV	
	White bryony mosaic virus	WBMV	

as tentative species have no sequence data, and therefore the taxonomic status of these members is yet to be verified.

Carlaviruses are either closely or distantly related to each other and these relationships have been confirmed by phylogenetic analysis of some viral proteins. The phylogenetic tree analysis of sequenced carlaviruses is presented in **Figure 1**.

Species demarcation criteria in this genus include the following:

1. distinct species have less than about 72% nucleotides or 80% amino acids identical between their coat protein (CP) or replicase genes;
2. distinct species are readily differentiated by serology, and strains of individual species are often distinguishable by serology;
3. distinct species do not cross-protect in infected common host plant species, and each distinct species usually has a specific natural host range and distinguishable experimental host ranges.

Virus Structure and Composition

Virions are slightly flexuous filaments that are not enveloped, and measure 610–700 nm in length and 12–15 nm in diameter. Nucleocapsids appear longitudinally striated. Virions have helical symmetry with a pitch of 3.3–3.4 nm. Virion M_r is about 60×10^6 with a nucleic acid content of 5–7%. Virions contain a monopartite, linear, single-stranded, positive-sense RNA that has a size range of 7.4–9.1 kbp in length. The 3′ terminus has a poly(A) tract and the 5′ terminus occasionally has a methylated nucleotide cap, or a monophosphate group. CP subunits are of one type, with size 31–36 kDa. The genome is encapsidated in 1600–2000 copies of CP subunit. Purified preparations of carlaviruses such as potato virus S and helinium virus S (PVS, HVS) contain small amounts of encapsidated subgenomic RNAs.

Physicochemical Properties

Virus particles in purified preparations have a sedimentation coefficient of 147–176 S. Isoelectric point of virions is about pH 4.5. The UV absorbance spectra of carlaviruses have maxima at 258–260 nm and minima at 243–248 nm, with A_{max}/A_{min} ratios of 1.1–1.3, and the A_{260}/A_{280} ratio of purified preparation is 1.08–1.40. Physical properties of viruses in this genus are: thermal inactivation point (TIP) 50–85 °C, longevity *in vitro* (LIV) 1–21 days, and dilution endpoint (DEP) 10^{-2}–10^{-7}. Infectivity of sap does not change on treatment with diethyl ether. Infectivity is retained by the virions despite being deproteinized by proteases and by phenols or detergents.

The CPs of the carlaviruses are partly degraded during purification and storage of virus preparations. The carlavirus virions are susceptible to and can be broken down by denaturing agents such as sodium dodecyl sulfate (SDS), urea, guanidine hydrochloride, acetic acid, and alkali.

Genome Structure and Gene Expression

The genome is a single-stranded RNA (ssRNA), 7.4–9.1 kbp in size, which contains six open reading frames (ORFs), encoding the replicase, three putative protein components of movement proteins called triple gene block (TGB), the CP, and a putative nucleic acid-binding regulatory protein, from the 5′–3′-end in that order (**Figure 2**). In genome organization, the genus *Carlavirus* particularly resembles the genera *Allexivirus*, *Foveavirus*, *Mandarivirus*, and *Potexvirus* but is distinguished from them by its six ORFs and a large replication protein. The genome RNA is capped at the 5′ end and polyadenylated at the 3′ end. The 223 kDa viral replicase ORF is translated from the full-length genomic RNA. For expression of its 3′-proximal viral genes, the virus utilizes at least two subgenomic RNAs. The genome structure of potato virus M (PVM) (8553 nt) is represented in **Figure 2**. PVM RNA has 75 nt 5′ untranslated region (UTR) at the 5′ terminus and 70 nt 3′ UTR followed by

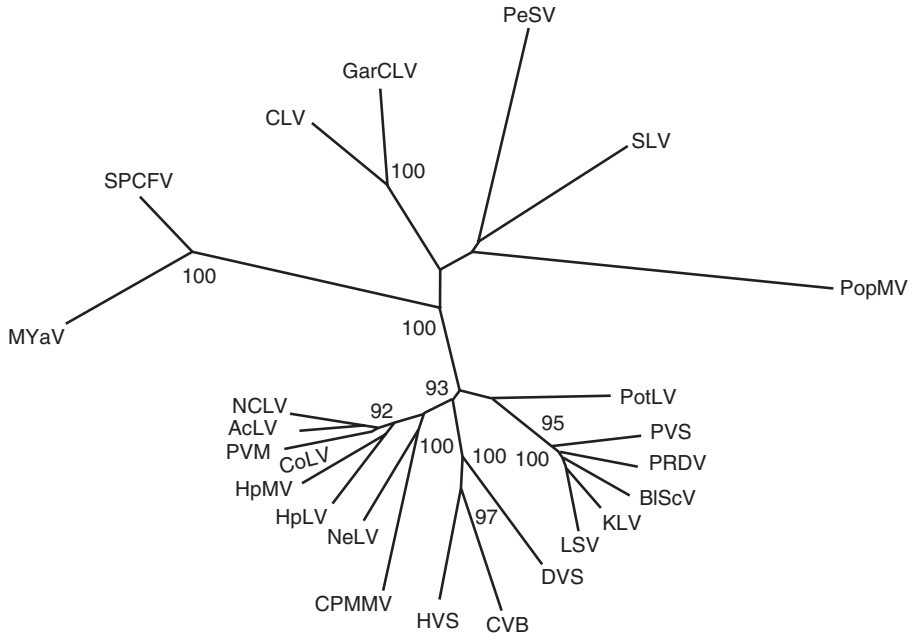


Figure 1 Phylogenetic tree of carlaviruses derived from multiple-aligned deduced amino acid sequences of CP. See **Table 1** for abbreviations of carlavirus names.

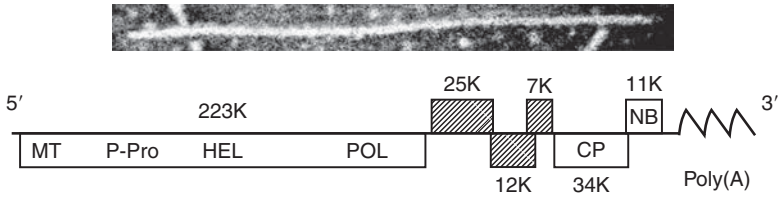


Figure 2 Particle morphology and genome organization of potato virus M (PVM), showing a typical genome structure for the genus *Carlavirus* in the family *Flexiviridae*. The 5′-proximal one large ORF encodes an RNA-dependent RNA polymerase (viral replicase), three overlapping ORFs encode the putative MPs (TGBs), and two ORFs encode CP and nucleic acid binding protein (NB). Motifs in the replicase are methyltransferase (MT), papain-like protease (P-Pro), helicase (HEL), and RNA-dependent RNA polymerase (POL).

a poly(A) tail at the 3′ terminus and intergenic UTRs of 38 and 21 nt between three large blocks of coding sequences. Carlavirus-infected plants contain double-stranded RNA (dsRNA) whose molecular mass corresponds to that of genomic RNA.

ORF1 encodes a 223 kDa polypeptide that is presumed to be the viral replicase. It contains motifs of methyltransferase, a papain-like protease, helicase, and RNA-dependent RNA polymerase. With blueberry scorch virus (BLScV) and probably other carlaviruses, the 223 kDa viral replicase is proteolytically processed by papain-like protease activity, which results in about 30 kDa being removed. ORFs 2–4 form the TGB encoding polypeptides of 25, 12, and 7 kDa, respectively, which facilitate virus cell-to-cell movement. ORF5 encodes the 34 kDa CP, and ORF6 encodes a cysteine-rich 11 kDa protein. The function of the 3′-proximal 11 kDa polypeptide has yet to be elucidated, but its ability to bind nucleic acid and agroin-filtration-mediated transient expression studies suggest that it may facilitate vector transmission, may be involved in viral RNA transcription, or may be a viral pathogenicity determinant for plant defense system.

Putative promoters for the subgenomic RNA synthesis have been identified for several carlaviruses, C/UUUAGGU, 19–43 residues upstream from both putative subgenomic RNA initiation sites.

Complete genomic sequences have recently been obtained for some carlaviruses: BLScV (8512 nt), chrysanthemum virus B (CVB) (8870 nt), daphne virus S (DVS) (8739 nt), hop latent virus (HpLV) (8612 nt), lily symptomless virus (LSV) (8394 nt), PVM (8553 nt), PVS (8478 nt), shallot latent virus (SLV) (8363 nt), aconitum latent virus (AcLV) (8657 nt), poplar mosaic virus (PopMV) (8741 nt), passiflora latent virus (PLV) (8386 nt), narcissus symptomless virus (NSV) (8281 nt),

sweetpotato chlorotic fleck virus (SPCFV) (9104 nt), and narcissus common latent virus (NCLV) (8539 nt).

Infectious cDNA clones have been reported for two carlaviruses, BlScV and PopMV.

Viral Transmission

Carlaviruses are spread by mechanical contact, by horticultural and agricultural equipment, and transmitted in a nonpersistent or semipersistent manner by aphids with varying efficiencies, or by whiteflies. Some members are not transmitted by mechanical inoculation. Seed transmission may occur with some legume-infecting carlaviruses, but is not common. Transmission by root grafts may occur in the case of PopMV. Those viruses that infect vegetatively propagated hosts persist in the propagated parts.

Cytopathology

The distribution of carlaviruses in the infected plant is not tissue specific. Virions are usually found in the cytoplasm, or sometimes in chloroplasts or in mitochondria of infected tissues, or may occur in membrane-associated bundle-like or plate-like aggregates. Inclusions are present in infected cells of some members. They occur as crystals in the cytoplasm, as amorphous X-bodies, membranous bodies, and viroplasms and they sometimes contain virions.

Host Range

Infection by most members of this genus is symptomless in the natural host. The individual natural host ranges are, usually narrow, although a few species can infect a wide range of experimental hosts.

Symptomatology

Carlavirus infection often results in no apparent symptoms in natural hosts. Symptoms vary cyclically or seasonally or may disappear soon after infection. If symptoms are evident, more severe symptoms such as mosaics appear in early stages of infection. Some carlaviruses such as PVM, PVS, and BlScV cause diseases that are of economic importance on their own; however, most of them are associated with more serious diseases when the plants are co-infected with other viruses.

Serology

Carlavirus virions are good immunogens. Serological relationships among carlaviruses may be close or distant or sometimes undetectable: some species are serologically interrelated but others apparently distinct. A number of carlaviruses are more or less closely interrelated serologically, with serological differentiation indices (SDIs) ranging from about 3.5 to 6.5.

Geographical Distribution

Some carlaviruses are found wherever their natural hosts are grown, but the geographical distribution of many species is restricted to only certain parts of the world. Those infecting vegetatively propagated crops are usually widely distributed. Most species commonly occur in temperate regions, but whitefly transmitted carlaviruses are restricted to tropical and subtropical regions.

Viral Epidemiology and Control

Most carlavirus-associated diseases are usually very mild or symptomless and few efforts are made to control them. However, crops such as potatoes, certain legumes, and blueberries that may contain more damaging carlaviruses require suitable control measures. Seed potatoes and vegetatively propagated materials must be screened continuously to certify a virus-free status. Rapid removal of infected plants is particularly important for plants associated with aphid- or whitefly-vectored carlaviruses. PVS is the only carlavirus for which transgenic plants (potato and *Nicotiana debney*), which are resistant to virus infection, have been reported so far.

See also: Allexivirus; *Capillovirus, Foveavirus, Trichovirus, Vitivirus*; Flexiviruses; Plant Virus Vectors (Gene Expression Systems); *Potexvirus*; Vector Transmission of Plant Viruses.

Further Reading

Adams MJ, Antoniw JF, Bar-Joseph M, *et al.* (2004) The new plant virus family *Flexiviridae* and assessment of molecular criteria for species demarcation. *Archives of Virology* 149: 1045–1060.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy, Classification and Nomenclature of Viruses, Eighth Report of the International Committee on the Taxonomy of Viruses,* p. 1101. San Diego, CA: Elsevier Academic Press.

Hillman BI and Lawrence DM (1994) Carlaviruses. In: Singh RP, Singh US, and Kohmoto K (eds.) *Pathogenesis and Host Specificity in Plant Diseases,* vol. 3, 35pp. New York: Plenum.

Lee BY, Min BE, Ha JH, Lee MY, Paek KH, and Ryu KH (2006) Genome structure and complete sequence of genomic RNA of Daphne virus S. *Archives of Virology* 151: 193–200.

Martelli GP, Adams MJ, Kreuze JF, and Dolja VV (2007) Family Flexiviridae: A case study in virion and genome plasticity. *Annual Review of Phytopathology* 45. 73–100.

Carmovirus

F Qu and T J Morris, University of Nebraska, Lincoln, NE, USA

© 2008 Elsevier Ltd. All rights reserved.

Taxonomy, Classification and Evolutionary Relationships

Carmovirus is one of eight genera in the family *Tombusviridae*. Members of this family all have icosahedral virions of about 30 nm in diameter with $T = 3$ symmetry that consists of 180 coat protein (CP) subunits of about 38–43 kDa and a single-stranded (ss) RNA genome ranging in size from 4.0 to 4.8 kbp. Carmoviruses share recognizable yet varied sequence similarity with members of other genera of *Tombusviridae*.

Carmoviruses contain a single-component positive-sense genome of about 4.0 kbp. The genome, as exemplified by turnip crinkle virus (TCV) in **Figure 1**, consists of five definitive open reading frames (ORFs) which encode proteins of about 28, 88, 8, 9, and 38 kDa from the 5′ to the 3′ end, respectively. Some carmoviruses such as hibiscus chlorotic ringspot virus (HCRSV) have additional ORFs of unknown functions. The virions are icosahedral in symmetry and consist of 180 CP subunits of approximately 38 kDa. The genus name is derived from the first member of the genus to be sequenced, carnation mottle virus (CarMV). Much more detailed knowledge about virus structure and genome function is, however, known for TCV because its crystal structure has been determined and it was the first carmovirus for which infectious transcripts were produced from a cDNA clone of the genome.

To date, the nucleotide sequences of 12 definitive carmoviruses have been determined (see **Table 1**). These sequenced members share similar morphological and physicochemical properties with about a dozen other viruses listed in **Table 1** that are recognized as species or tentative species depending on the level of detail of the molecular characterization of the viral genomes. Various carmoviruses are sufficiently distant from each other that they do not cross-react in standard RNA hybridization or serological tests.

Carmoviruses share properties with viruses belonging to other genera of the family *Tombusviridae*. Their particle structure and CP sequences are closely related to tombus-, aureus-, diantho-, and avenaviruses. Their RNA-dependent RNA polymerase (RdRp) genes share significant homology with viruses of the following genera: *Machlomovirus*, *Panicovirus*, *Necrovirus*, *Aureusvirus*, and *Tombusvirus*. Carmovirus RdRp genes also share similarity with more distantly related viruses outside of *Tombusviridae*, such as umbraviruses and luteoviruses. In a broader context, phylogenetic comparisons of viral RNA polymerase genes have identified the *Tombusviridae* as a representative plant virus cluster for one of three RNA virus supergroups with relatedness to animal viruses of *Flaviviridae* and small RNA phage (*Leviviridae*).

Distribution, Host Range, Transmission, and Economic Significance

Carmoviruses occur worldwide and are generally reported to cause mild or asymptomatic infections on relatively restricted natural host ranges. Most accumulate to high concentrations in infected tissues and are mechanically transmitted. Beetle transmission has been reported for some carmoviruses as has transmission in association with soil and/or irrigation water, and in some cases in association with fungal zoospores.

A number of carmoviruses have been identified in association with ornamental hosts and have been widely distributed in such hosts by vegetative propagation. CarMV is the most noteworthy, being widespread in cultivated carnations, and recognized as one of the more important components of viral disease complexes in this crop worldwide. It accumulates to high concentrations without producing severe symptoms and spreads primarily by contact transmission and vegetative propagation. It has a broad experimental host range that includes over 30 species in 15 plant families. Pelargonium flower break virus (PFBV) is widespread in vegetatively propagated *Pelargonium* species causing disease in association with other viruses. The incidence of narcissus tip necrosis virus (NTNV) in narcissus cultivars and HCRSV in hibiscus primarily reflects distribution of infected nursery stock.

Numerous small RNA viruses have been reported to naturally infect cucurbits causing significant disease problems. Several of these viruses are recognized tombusviruses while others such as melon necrotic spot virus (MNSV) and cucumber soil-borne virus (CSBV) have been identified as carmoviruses based on sequence and genome organization properties. MNSV occurs worldwide in greenhouse cucurbits and is both soil and seed transmitted, while CSBV has been primarily restricted to infrequent outbreaks around the Mediterranean. Both have been reported to be transmitted in association with the fungus *Olpidium bornovanus*.

Several carmoviruses have been discovered in natural leguminous hosts, with glycine mottle virus (GMoV) being potentially the most important, causing serious disease losses in legumes in Africa. Bean mild mosaic virus (BMMV) has been reported to be a latent virus

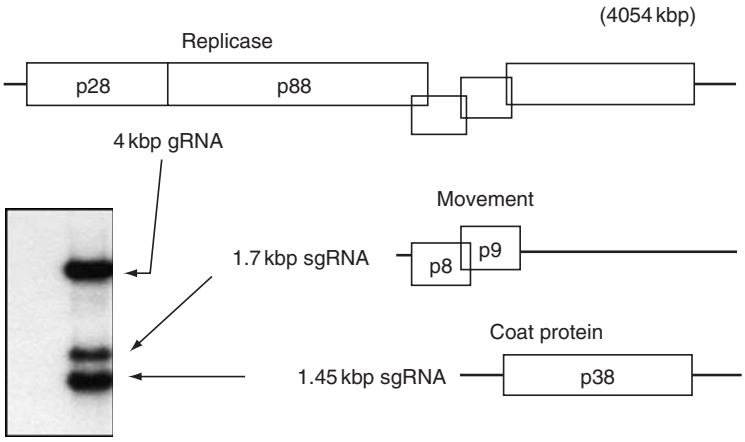


Figure 1 Genome organization of a typical carmovirus as represented by turnip crinkle virus (TCV). The boxes represent open reading frames with the sizes of the encoded proteins indicated within the boxes, in kilodaltons. The two proteins required for replication (p28 and p88) are translated from the 4 kbp genomic RNA with the p88 protein translated by readthrough of an amber codon at the end of the p28 gene. Two small proteins involved in cell-to-cell movement (p8 and p9) are translated from a 1.7 kbp subgenomic RNA. The viral coat protein (p38) is translated from a 1.45 kbp subgenomic RNA. The panel on the left is a Northern blot showing the typical pattern of accumulation of genomic and subgenomic RNAs in carmovirus-infected cells.

Table 1 Virus members in the genus *Carmovirus*

Sequenced viruses	
Angelonia flower break virus	(AnFBV)
Cardamine chlorotic fleck virus	(CCFV)
Carnation mottle virus	(CarMV)
Cowpea mottle virus	(CPMoV)
Galinsoga mosaic virus	(GaMV)
Hibicus chlorotic ringspot virus	(HCRSV)
Japanese iris necrotic ring virus	(JINRV)
Melon necrotic spot virus	(MNSV)
Pea stem necrosis virus	(PSNV)
Pelargonium flower break virus	(PFBV)
Saguaro cactus virus	(SCV)
Turnip crinkle virus	(TCV)
Unsequenced viruses	
Ahlum water-borne virus	(AWBV)
Bean mild mosaic virus	(BMMV)
Cucumber soil-borne virus	(CSBV)
Weddel water-borne virus	(WWBV)
Viruses assigned to tentative species	
Blackgram mottle virus	(BMoV)
Calibrachoa mottle virus	(CbMV)
Elderberry latent virus	(ELV)
Glycine mottle virus	(GMoV)
Narcissus tip necrosis virus	(NTNV)
Plaintain virus 6	(PlV-6)
Squash necrosis virus	(SqNV)
Tephrosia symptomless virus	(TeSV)

widely distributed in bean cultivars in El Salvador, and backgram mottle virus (BMoV) has been found in *Vigna* species in Asia. Beetle vectors have been identified for these viruses, but seed transmission may also be an important factor in their distribution.

TCV is neither common nor widespread in nature in spite of the fact that it is reportedly beetle transmitted. It has a relatively wide experimental host range in some 20 plant families including experimentally useful species such as *Arabidopsis* and *Brassica* in which it accumulates to extremely high concentrations, often approaching a level equivalent to 0.5% of the fresh weight of the plant tissue. Cardamine chlorotic fleck virus (CCFV) was first discovered in the Mount Kosiusko alpine region of Australia in *Cardamine lilacina*, a wild perennial of the family Brassicaceae. It has also been shown to infect *Arabidopsis* and other Brassicaceae species.

Other carmoviruses have been isolated worldwide from natural hosts with little apparent disease and are presumably of little agricultural concern. These include tephrosia symptomless virus (TeSV) from legumes in Kenya, GMoV from glycine in Australia, saguaro cactus virus (SCV) from saguaro cactus in Arizona galinsoga mosaic virus (GaMV) from potato weed in Australia, and plaintain virus 6 (PlV-6) from plantain weed in England. The infrequent isolation of these genetically similar viruses in remote locations around the world has prompted the speculation that ancestor carmoviruses may have been introduced into their natural hosts well before the last Ice Age and have since co-evolved in isolation in their diverse host plants.

Virion Structure and Assembly

Four carmoviruses (TCV, CarMV, cowpea mottle virus (CPMoV), and HCRSV) have now been analyzed in structural detail, with TCV being the first and most thoroughly studied by high-resolution X-ray crystallography. The detailed information about CP structure and

intersubunit interactions established that TCV and tomato bushy stunt tombusvirus (TBSV) show marked structural conservation. In this regard, the common structural features shared by other members of the family *Tombusviridae* have been primarily deduced from alignment of the amino acid sequence of the coat proteins of TBSV and TCV. TCV consists of a $T = 3$ icosahedral capsid of 180 subunits of the 38 kDa CP. The individual CP subunit folds into three distinct domains typical of CP subunit of tombusviruses. The relatively basic N-terminal R domain extends into the interior of the virus particle and presumably interacts with viral RNA. The R domain is connected by an arm to the S domain which constitutes the virion shell. The S domain is attached through a hinge to the P domain which projects outward from the virion surface. The protein subunits are believed to form dimers in solution and during assembly.

TCV is also the only carmovirus on which detailed *in vitro* assembly studies have been performed. The virion has been shown to dissociate at elevated pH and ionic strength to produce a stable RNA–CP complex (rp-complex) and free CP subunits. Reassembly under physiological conditions in solution could be demonstrated using the isolated rp-complex and the soluble CP subunits. This rp-complex, consisting of six CP subunits tightly attached to viral RNA, could be generated *in vitro* and was shown to be important in selective assembly of TCV RNA. A model for assembly was proposed in which three sets of dimeric CP interact with a unique site on the viral RNA to form an initiation complex to which additional subunit dimers could rapidly bind. Preliminary characterization of the 'origin of assembly' for this virus identified two possible sites based on the identification of RNA fragments in the rp-complex protected from RNase digestion by CP. Further *in vivo* studies narrowed the assembly origin site to a bulged hairpin-loop of 28 nt within a 180 nt region at the 3′ end of the CP gene.

Genome Structure

Complete nucleotide sequences have been determined for 12 carmoviruses as listed in **Table 1**. Comparative studies of the deduced ORFs revealed that all of these viruses encode a similar set of genes that are closely related and in the same gene order as illustrated for TCV in **Figure 1**. The genome organization of the carmoviruses is quite compact with most of the identified ORFs overlapping each other. Both the product of the most 5′ proximal ORF (26–28 kDa) and its readthrough product (86–89 kDa) are essential for replication of the TCV genome. The 3′ proximal gene encodes the viral CP which varies from 37 to 42 kDa for the different viruses. All of the sequenced carmoviruses characteristically encode two small ORFs in the middle of the genome that both have been shown in TCV to be indispensable for cell-to-cell movement (movement proteins or MP). Although the genome organizations of all sequenced carmoviruses are quite similar, there are some unique features evident in the individual carmoviruses. For example, the two small central ORFs in MNSV (p7a and p7b) are connected by an in-frame amber codon that could result in the production of a 14 kDa fusion protein of the two ORFs by a readthrough mechanism. The CPMoV as well as HCRSV are predicted to encode a sixth ORF nested within the 3′ proximal CP gene. HCRSV has also been reported to encode another novel protein that is nested within the RdRp gene.

The 5′ end of the genome is not capped. The 5′ noncoding region varies from 34 nt in CPMoV to 88 nt in MNSV. No extensive sequence homology was observed within this region. The 3′ noncoding region of carmoviruses varies from about 200 to 300 nt in length and possesses neither a poly-A tail nor a tRNA-like structure.

Replication and Gene Expression

Carmoviruses replicate to very high concentrations in protoplasts, with the genomic RNA accumulating to levels approaching that of the ribosomal RNAs. Upon infection of susceptible plants, carmoviruses transcribe two 3′ coterminal subgenomic RNAs (sgRNAs) for expression of the MP and CP genes. The smaller sgRNA (*c.* 1.5 kbp) is the mRNA for CP. The larger sgRNA (*c.* 1.7 kbp) presumably functions as the mRNA for the two MP genes utilizing a leaky scanning mechanism. Results involving transgenic expression of the p8 and p9 gene products of TCV in *Arabidopsis* plants have demonstrated that both of them are essential for viral cell-to-cell movement and that they function by in *trans*-complementation in the same cell.

Carmoviruses are thought to replicate through a (−) strand intermediate because virus-specific double-stranded RNAs (dsRNAs) corresponding in size to the genomic RNA and sgRNAs characteristically accumulate in infected plant tissue. The product of the 5′ proximal ORF (p28 in TCV) and its readthrough product (p88 in TCV) are the only virus-encoded components of the polymerase complex. When expressed from two separate mRNAs, p28 and p88 complemented *in trans* to enable the genome replication. The only host factor found to augment TCV replication so far is the eukaryotic translation initiation factor 4G (eIF4G) of *Arabidopsis*, presumably through more efficient translation of viral genes. Recently, a membrane-containing extract prepared from evacuolated protoplasts of uninfected *Arabidopsis* plants has been shown to faithfully produce both genomic and subgenomic RNAs from a full-length TCV RNA template. Such an extract should be useful in

characterizing viral as well as host elements required for virus replication.

Recent studies have demonstrated that, besides the virus-encoded proteins, a plethora of structural elements in the viral RNA play critical roles in the genome replication of carmoviruses. These elements are located throughout the entire viral genome and occur in both the (+) and (−) strands. Their roles range from promoters, enhancers, or repressors for RNA replication and transcription, enhancers for translation, and specificity determinants for virus assembly. The current knowledge of these RNA structures suggests that different secondary structural motifs, some of them mutually exclusive, are formed at the different stages of virus multiplication, and the highly coordinated nature of their formation ensures optimal utilization of the compact viral genome.

Satellites, Defective-Interfering RNAs

TCV is the only carmovirus in which replication of associated small subviral RNAs in infected plants has been characterized, and the situation for this virus is curiously complex. TCV infections are associated with defective interfering (DI) RNAs derived totally from the parent genome (e.g., RNA G of 342–346 nt), satellite RNAs of nonviral origin (e.g., RNAs D, 194 nt and F, 230 nt), and chimeric RNAs (e.g., RNA C of 356 nt) with a 5′ region derived from sat RNA D and a 3′ region derived from the 3′ end of the TCV genome. All three types of small RNAs depend on the helper virus for their replication and encapsidation within the infected plant. The different satellite and DI RNAs have been shown to affect viral infections in different ways. Both RNA C and G intensify viral symptoms while interfering with the replication of the helper virus, while RNAs D and F seem to produce no detectable effects on either expression of symptoms or helper virus replication.

Virus–Host Interaction

Considerable progress has been made in the last decade in our understanding of the molecular mechanisms of virus–plant interactions with significant contributions coming from studies utilizing TCV and its host plant *Arabidopsis*. Most notably, one ecotype of *Arabidopsis thaliana* has been determined to be resistant to TCV infection and the resistance gene (*HRT*) and its encoded protein have been characterized. Additional host factors that contribute to the resistance response have also been elucidated including the identification of a novel transcription factor (TIP) whose interaction with TCV CP is critical for the initiation of the resistance response. The determination that TCV CP is targeted by HRT resistance protein suggests that TCV CP plays a key role in combating antiviral defense mechanisms of the plant host, in addition to being required for virus assembly. Interestingly, recent studies have also established that TCV CP is a strong suppressor of RNA silencing, a host defense mechanism that targets invading RNA. RNA silencing is a potent defense mechanism that is conserved in nearly all eukaryotic organisms. Accordingly, TCV CP is an effective silencing suppressor in both plant and animal cells. Silencing suppressor activity has also been associated with several other carmovirus CPs, establishing this activity as a conserved feature of the structural subunit.

TCV CP is also important in satellite RNA C interactions in the host plant. Normally, the presence of RNA C results in symptom intensification in TCV infections. However, when the TCV CP ORF is either deleted or replaced by the CCFV CP ORF, RNA C attenuates symptoms caused by the helper virus suggesting that CP either downregulates the replication of RNA C or enhances its own competitiveness.

Finally, the replicase gene has also been implicated in the symptom modification by satellite RNA C by two independent groups. The 3′ end of the TCV genome, a sequence common in TCV RNA, RNA C, and DI RNA G, was also suggested to be a symptom determinant. Environmental conditions also affect the extent of resistance of *Arabidopsis* plants to TCV. In conclusion, it is clear that recent intensive studies of TCV have established this small RNA virus as an ideal model for unraveling the complicated processes involved in viral pathogenesis.

See also: Legume Viruses; Luteoviruses; Machlomovirus; *Necrovirus*; Plant Virus Diseases: Ornamental Plants; Tombusviruses.

Further Reading

Hacker DL, Petty ITD, Wei N, and Morris TJ (1992) Turnip crinkle virus genes required for RNA replication and virus movement. *Virology* 186: 1–8.

Kachroo P, Yoshioka K, Shah J, Dooner HK, and Klessig DF (2000) Resistance to turnip crinkle virus in *Arabidopsis* is regulated by two host genes, is salicylic acid dependent but NPR1, ethylene and jasmonate independent. *Plant Cell* 12: 677–690.

Komoda K, Naito S, and Ishikawa M (2004) Replication of plant RNA virus genomes in a cell-free extract of evacuolated plant protoplasts. *Proceedings of the National Academy of Sciences, USA* 101: 1863–1867.

Lommel SA, Martelli GP, Rubino L, and Russo M (2005) *Tombusviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 907–936. San Diego, CA: Elsevier Academic Press.

Morris TJ and Carrington JC (1988) Carnation mottle virus and viruses with similar properties. In: Koenig R (ed.) *The Plant Viruses: Polyhedral Virions with Monopartite RNA Genomes,* vol. 3, pp. 73–112. New York: Plenum.

Nagy PD, Pogany J, and Simon AE (1999) RNA elements required for RNA recombination function as replication enhancers *in vitro* and *in vivo* in a plus-strand RNA virus. *EMBO Journal* 18: 5653–5665.
Qu F and Morris TJ (2005) Suppressors of RNA silencing encoded by plant viruses and their role in viral infections. *FEBS Letters* 579: 5958–5964.
Ren T, Qu F, and Morris TJ (2000) HRT gene function requires interaction between a NAC protein and viral capsid protein to confer resistance to turnip crinkle virus. *Plant Cell* 12: 1917–1925.
Russo M, Burgyan J, and Martelli GP (1994) Molecular biology of *Tombusviridae*. *Advances in Virus Research* 44: 381–428.
Yoshii M, Nishikiori M, Tomita K, *et al.* (2004) The *Arabidopsis cucumovirus multiplication 1* and *2* loci encode translation initiation factors 4E and 4G. *Journal of Virology* 78: 6102–6111.

Caulimoviruses: General Features

J E Schoelz, University of Missouri, Columbia, MO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Agroinoculation An inoculation technique in which *Agrobacterium tumefaciens* is used to deliver a full-length infectious clone of a virus into a plant cell.
Plasmodesmata A narrow channel of cytoplasm that functions as a bridge between two plant cells to facilitate movement of macromolecules.
Reverse transcriptase An enzyme that utilizes an RNA template for synthesis of DNA.
Ribosome shunt A translational mechanism in which ribosomes enter the RNA at the 5′ end and scan for a short distance before being translocated to a downstream point.
Semipersistent transmission Vector acquires the virus in minutes to hours, and can transmit to other plants for hours after the initial feeding.
Transgene A gene introduced into an organism through any one of a number of genetic engineering techniques.

Introduction

The members of the family *Caulimoviridae* are plant viruses that replicate by reverse transcription of an RNA intermediate and whose virions contain circular, double-stranded DNA (dsDNA). They replicate by reverse transcription, but unlike the true retroviruses, integration into the host chromosomes is not required for completion of their replication cycle. The circular DNA encapsidated in virions is not covalently closed, as it contains at least one discontinuity in each DNA strand, and these discontinuities occur as a consequence of the replication strategy of the virus. There are six genera in this family and they can be divided into two groups based on virion morphology; the members of the genera *Caulimovirus*, *Petuvirus*, *Cavemovirus*, and *Soymovirus* contain viruses that form icosahedral particles that are largely found within amorphous inclusion bodies in the cell (**Figures 1(a)** and **1(b)**). In contrast, the members of the genera *Badnavirus* and *Tungrovirus* form bacilliform particles that are not associated with inclusion bodies (**Figure 1(c)**). Their virions are found in the cytoplasm either individually or clustered in palisade-like arrays.

Cauliflower mosaic virus (CaMV), a member of the type species *Cauliflower mosaic virus* of the genus *Caulimovirus*, was the first of the plant viruses to be shown to contain dsDNA in its icosahedral virion by Shepherd in 1968. This discovery led to an extended investigation into its replication strategy throughout the 1970s and early 1980s, culminating in Pfeiffer and Hohn's study in 1983 that showed that CaMV replicates through reverse transcription of an RNA intermediate. Perhaps because the genome of CaMV is composed of dsDNA, it was also the first of the plant viruses to be completely sequenced and cloned into bacterial plasmids in an infectious form.

In the early 1980s, CaMV was thought to have some promise as a vector for foreign genes in plants. However, the effort to convert CaMV into a vector was scaled back when it was shown that the virus genome could tolerate only small insertions of up to a few hundred basepairs of DNA. A few small genes, such as dihydrofolate reductase and interferon, were eventually expressed in plants via a CaMV vector, but other viruses have been shown to be much more versatile as vectors for foreign genes. Although the caulimoviruses have had only limited utility as plant virus vectors, they continue to have a great impact on plant biotechnology. The 35S promoter of CaMV is capable of directing a high level of transcription in most types of plant tissues. This promoter was used to drive expression of one of the first transgenes introduced into transgenic plants, and it is still widely used for expression of transgenes for both research and commercial applications. The promoter regions of several other

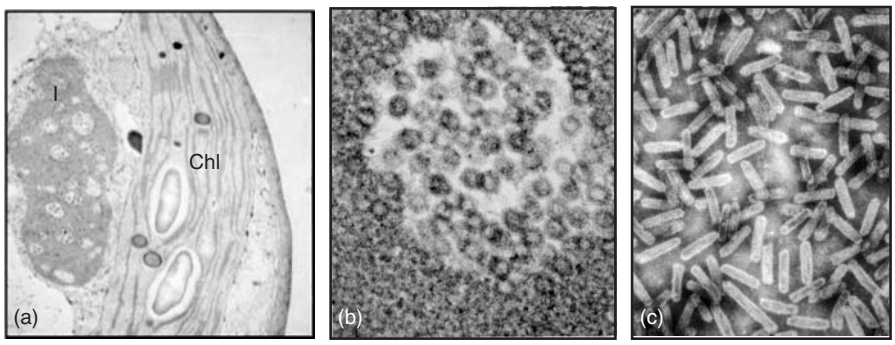


Figure 1 Inclusion bodies and virions of the family *Caulimoviridae*. (a) Amorphous inclusion body of CaMV (I) adjacent to a chloroplast (Chl). Individual virions can be seen in the vacuolated regions of the inclusion body. (b) Icosahedral virions of CaMV visualized within an inclusion body. (c) Purified bacilliform virions of a badnavirus. (a) Reproduced with permission from the *Encyclopedia of Virology*, 2nd edn. (c) Photo courtesy of Ben Lockhart (University of Minnesota).

caulimoviruses have also been evaluated for expression of transgenes in both monocots and dicots, and they can be good alternatives to the CaMV 35S promoter.

The feature that was used initially to characterize the caulimoviruses was the presence of circular dsDNA encapsidated into icosahedral virions of approximately 50 nm diameter. However, as icosahedral viruses continued to be isolated from a variety of hosts and their genomes sequenced, it was discovered that their genome structure diverged from that of CaMV. These differences were significant enough for the International Committee on Taxonomy of Viruses (ICTV) to create three new genera in its VIII ICTV Report. Consequently, *Soybean chlorotic mottle virus*, *Cassava vein mosaic virus*, and *Petunia vein clearing virus* became the type species of the genera *Soymovirus*, *Cavemovirus*, and *Petuvirus*, respectively.

For many years, only plant viruses that had icosahedral virions of 50 nm in diameter were thought to have genomes composed of dsDNA. However, in 1990, Lockhart showed that the bacilliform virions of commelina yellow mottle virus (CoYMV) contained circular, dsDNA, and *Commelina yellow mottle virus* became the type species for the genus *Badnavirus*. Lockhart showed that nucleic acid isolated from the bacilliform virions was resistant to RNase and degraded by DNase. Furthermore, DNA treated with S1 nuclease revealed that the CoYMV genome contained at least two single-stranded discontinuities, a hallmark of the plant viruses that replicate by reverse transcription. In addition to CoYMV, Lockhart also purified DNA from banana streak virus, kalanchoe top-spotting virus (KTSV), and canna yellow mottle virus, providing evidence that they should also be placed in the new genus *Badnavirus*. There are now 18 species in the genus *Badnavirus* (**Table 1**), many of which cause economically important diseases in the tropics. However, new badnaviruses have been characterized since the publication of the VIII Report of the ICTV, so this number will almost certainly be revised upward in the near future.

Soon after the discovery that CoYMV was a DNA virus, the bacilliform component of rice tungro disease was also shown to contain circular, dsDNA. Rice tungro is the most important virus disease of rice in South and Southeast Asia, with annual losses approaching $1.5 billion dollars. The disease is caused by a complex of an RNA virus, called rice tungro spherical virus (RTSV) coupled with the bacilliform dsDNA virus called rice tungro bacilliform virus (RTBV). The genus *Tungrovirus* is distinguished from the genus *Badnavirus* because RTBV has one more open reading frame (ORF) than CoYMV and overall, the RTBV genome has only 20–25% nucleotide sequence identity with members of the genus *Badnavirus*. There is only one species in the genus *Tungrovirus*, although several isolates of the species *Rice tungro bacilliform virus* have been collected and sequenced.

Taxonomy and Classification

The family *Caulimoviridae* consists of six genera (**Table 1**), and they can be conveniently divided into two groups based on virion morphology. Viruses with icosahedral virions (**Figure 1(b)**) include the genera *Caulimovirus*, *Petuvirus*, *Soymovirus*, and *Cavemovirus*. Viruses with bacilliform virions (**Figure 1(c)**) include the genera *Badnavirus* and *Tungrovirus*. Genera can be further distinguished because of differences in genome organization and nucleotide differences in common genes such as the reverse transcriptase. All members of the family *Caulimoviridae* infect only plants. There are no animal or insect viruses in this family.

Virion Structure and Composition

The viruses in the genera *Caulimovirus*, *Cavemovirus*, *Soymovirus*, and *Petuvirus* form nonenveloped, isometric particles that vary in size from 43 to 50 nm. The virion is

Table 1 Virus members in the family *Caulimoviridae*

Genus	*Virus*	*Abbreviation*	*Host (family)*	*Geographic distribution*	*Representative accession number*
Caulimovirus	Carnation etched ring virus	CERV	Carnation (Caryophyllaceae)	Worldwide	X04658
	Cauliflower mosaic virus[a]	**CaMV**	***Brassica* sp.** (**Crucifereae**)	**Worldwide**	**V00140**
	Dahlia mosaic virus	DMV	Dahlia (Compositae)	Worldwide	
	Figwort mosaic virus	FMV	Figwort (Scrophulariaceae)	USA	X06166
	Horseradish latent virus	HRLV	Horseradish (Crucifereae)	Denmark	
	Mirabilis mosaic virus	MiMV	Mirabilis (Nyctaginaceae)	USA	AF454635
	Strawberry vein banding virus	SVBV	Strawberry (Rosaceae)	Worldwide	X97304
	Thistle mottle virus	ThMoV	Thistle (Compositae)	Europe	
	Aquilegia necrotic mosaic virus[b]	ANMV	Columbine (Ranunculaceae)	East Asia, Japan	
	Plantago virus 4[b]	PlV-4	Plantain (Plantaginaceae)	United Kingdom	
	Sonchus mottle virus[b]	SMoV	*Sonchus* sp. (Asteraceae)		
Petuvirus	**Petunia vein clearing virus**	**PVCV**	**Petunia** (**Solanaceae**)	**Worldwide**	**U95208**
Soymovirus	Blueberry red ringspot virus	BRRSV	Blueberry (Ericaceae)	USA	AF404509
	Peanut chlorotic streak virus	PCSV	Peanut (Leguminosae)	India	U13988
	Soybean chlorotic mottle virus	**SbCMV**	**Soybean** (**Leguminosae**)	**Japan**	**X15828**
	Cestrum yellow leaf curling virus[b]	CmYLCV	*Cestrum* sp. (Solanaceae)	Italy	AF364175
Cavemovirus	**Cassava vein mosaic virus**	**CsVMV**	**Cassava** (**Euphorbiaceae**)	**Brazil**	**U59751**
	Tobacco vein clearing virus	TVCV	*Nicotiana* sp. (Solanaceae)	Worldwide	AF190123
Badnavirus	Aglaonema bacilliform virus	ABV	*Aglaonema* sp. (Araceae)	Southeast Asia	
	Banana streak GF virus	BSGFV	Banana (Musaceae)	Worldwide	AY493509
	Banana streak MYsore virus	BSMyV	Banana (Musaceae)	Worldwide	AY805074
	Banana streak OL virus	BSOLV	Banana (Musaceae)	Worldwide	AJ002234
	Cacao swollen shoot virus	CSSV	*Theobroma* sp. (Sterculiaceae)	Africa	NC001374
	Canna yellow mottle virus	CaYMV	Canna (Cannaceae)	Japan, USA	
	Citrus mosaic virus	CMBV	*Citrus* sp. (Rutaceae)	India	AF347695
	Commelina yellow mottle virus	**ComYMV**	***Commelina*** (**Commelinaceae**)	**Caribbean**	**X52938**
	Dioscorea bacilliform virus	DBV	Yam (Dioscoreaceae)	Africa	X94576
	Gooseberry vein banding-associated virus	GVBAV	Ribes sp. (Grossulariaceae)	Worldwide	AF298883
	Kalanchoe top-spotting virus	KTSV	*Kalanchoe* (Crassulaceae)	UK, USA	AY180137
	Piper yellow mottle virus	PYMoV	Pepper (Piperaceae)	Brazil, India, Asia	
	Rubus yellow net virus	RYNV	Raspberry (Rosaceae)	Eurasia, USA	AF468454
	Schefflera ringspot virus	SRV	*Schefflera* (Araliaceae)	Worldwide	
	Spiraea yellow leaf spot virus	SYLSV	Spiraea (Rosaceae)		AF299074
	Sugarcane bacilliform IM virus	SCBIMV	Sugarcane (Poaceae)	USA, Cuba, Morocco	AJ277091
	Sugarcane bacilliform Mor virus	SCBMV	Sugarcane (Poaceae)	USA, Cuba, Morocco	M89923
	Taro bacilliform virus	TaBV	Taro (Araceae)	South Pacific	AF357836

Continued

Table 1 Continued

Genus	*Virus*	*Abbreviation*	*Host (family)*	*Geographic distribution*	*Representative accession number*
	Aucuba bacilliform virus[b]	AuBV	*Aucuba* sp. (Garryaceae)	Japan, UK	
	Mimosa bacilliform virus[b]	MBV	*Mimosa* sp. (Fabaceae)	USA	
	Pineapple bacilliform virus[b]	PBV	Pineapple (Bromeliaceae)	Australia	Y12433
	Stilbocarpa mosaic bacilliform virus[b]	SMBV	Stilbocarpa (Araliaceae)	Subantarctic islands	AF478691
	Yucca bacilliform virus[b]	YBV	Yucca (Agavaceae)	South America, Italy	AF468688
Tungrovirus	**Rice tungro bacilliform virus**	**RTBV**	**Rice (Poaceae)**	**East Asia, China**	**X57924**

[a]The entry for type member of each genus is highlighted in bold font.
[b]Tentative member.

composed of 420 subunits with a $T = 7$ structure. The viruses in the genera *Badnavirus* and *Tungrovirus* form nonenveloped, bacilliform particles that are 30 nm in width, but can vary in length from 60 to 900 nm. The length most commonly observed is 130 nm. Their structure is based on an icosahedron, in which the ends are formed from pentamers and the tubular section is made up of hexamers.

The virions of the family *Caulimoviridae* contain a single, circular dsDNA that is 7.2–8.3 kbp in length. Complete and partial nucleotide sequences of members of the family *Caulimoviridae* are listed in **Table 1**. Only a single accession number is given for CaMV and RTBV, although multiple strains of each have been sequenced.

Genome Organization and Expression

Several genome features are common to all members of the family *Caulimoviridae*, in addition to their circular dsDNA genomes. For example, the dsDNA is not covalently closed; it has at least two discontinuities, but may have up to four. Furthermore, all of the ORFs are found on only one of the DNA strands and all of the viruses have at least one strong promoter that drives the expression of a terminally redundant mRNA. In addition, all of the viruses have a reverse transcriptase, and in most cases it is located downstream from the coat protein. Several active sites can be identified within the reverse transcriptase protein, including a protease, the core reverse transcriptase, and RNaseH activity. The reverse transcriptase of PVCV is distinguished from all other members of the family *Caulimoviridae* because it has the core features of an integrase function, in addition to other functions. The most significant difference among the caulimoviruses concerns the arrangement and number of ORFs, and this is illustrated in an examination of the genome organization and expression strategies of CaMV and CoYMV (**Figure 2**).

The genome of CaMV is approximately 8000 bp in size and it contains three single-stranded discontinuities (**Figure 2(a)**). One discontinuity occurs in the negative-sense DNA strand and, by convention, this is the origin of the DNA sequence. Two other discontinuities occur in the positive-sense strand, one at nucleotide position 1600 and a second at approximately nucleotide position 4000. The virus genome consists of seven ORFs. ORF1 encodes a protein necessary for cell-to-cell movement (P1). ORF2 and -3 (proteins P2 and P3) are both required for aphid transmission. P2 is responsible for binding to the aphid stylet, whereas P3 is a virion-associated protein. To complete the bridge for aphid transmission of virions, the C-terminus of P2 physically interacts with the N-terminus of P3. Recent evidence also indicates that P3 may have an additional role in cell-to-cell movement. ORF4 encodes the coat protein, and ORF5 encodes a reverse transcriptase that also has protease and RNaseH domains. With the exception of PVCV, none of the reverse transcriptases of the *Caulimoviridae* have evidence for an integrase function. The ORF6 product (P6) was originally described as the major inclusion body protein, but it also has a function as a translational transactivator (TAV). It physically interacts with host ribosomes to reprogram them for reinitiation of translation of the polycistronic 35S RNA. Furthermore, P6 is an important symptom and host range determinant. No protein product has been found for ORF7. Its nucleotide sequence appears to play a regulatory role in aligning ribosomes for translation of other CaMV gene products.

Two transcripts are produced from the CaMV genomic DNA. The 19S RNA serves as the mRNA for P6, whereas the terminally redundant 35S RNA serves as a template for reverse transcription and as a polycistronic mRNA for ORFs 1–5. One feature common to many of the caulimoviruses is the ribosomal shunt mechanism of translation. The ribosomal shunt has undoubtedly evolved over time to compensate for the complexity and length of the leader sequence of the genomic RNA. In the case of CaMV, this

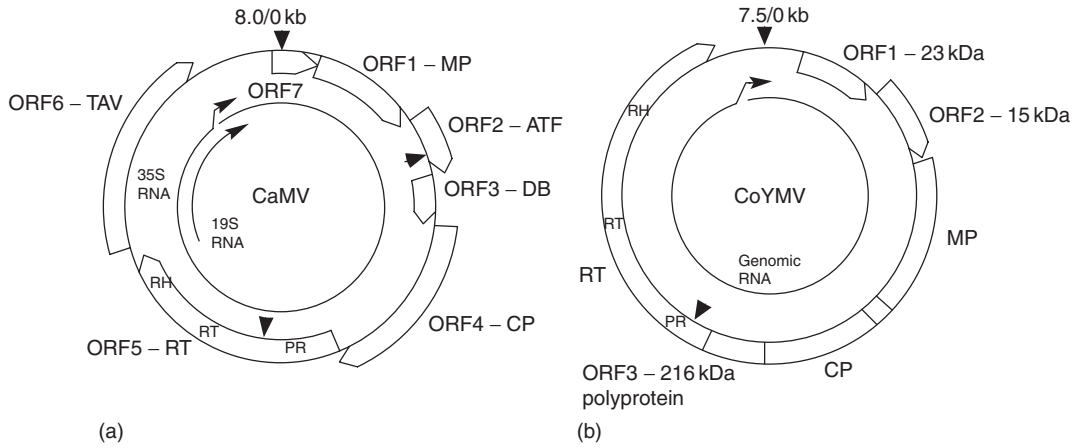


Figure 2 Genomic maps of (a) CaMV and (b) CoYMV. The single-stranded discontinuity in the negative-sense DNA strand is indicated by the triangle outside of the circle, whereas single-stranded discontinuities in the positive-sense DNA strand are indicated by triangles inside the circle. The mRNAs for each virus are represented by the inner circles and the 3′ end of the RNA is indicated by the arrowhead. The functions for each of the ORFs are MP, cell-to-cell movement protein; ATF, aphid transmission factor; DB, DNA-binding protein, which also has role in aphid transmission; CP, coat protein; RT, reverse transcriptase; TAV, translational transactivator and major inclusion body protein. The domains within the reverse transcriptase are: PR, protease; RT, reverse transcriptase domain; and RH, ribonucleaseH activity. ORF3 of CoYMV encodes a 216 kDa polyprotein that is cleaved to produce the MP, CP, and RT proteins.

leader sequence is approximately 600 bp in length and it contains up to nine short ORFs that vary in size from 9 to 102 nt. The complexity of the CaMV 35S RNA leader sequence is bypassed through the formation of a large stem–loop structure, which allows ribosomes to bypass most of the leader and to initiate translation at ORF7. CaMV utilizes several other strategies for expression of the 35S RNA, including splicing and reprogramming of host ribosomes by the TAV for reinitiation of translation.

The genome of CoYMV is approximately 7500 bp in size, and the circular DNA contains two single-stranded discontinuities, one in each strand (**Figure 2(b)**). As with CaMV, the discontinuity in the negative-sense DNA strand serves as the origin of the DNA sequence, whereas the discontinuity in the positive-sense strand is found at approximately nucleotide position 4800. CoYMV encodes a single transcript that is terminally redundant, with 120 nucleotides reiterated on the 5′ and 3′ ends. The virus genome consists of three ORFs, encoding proteins of 23, 15, and 216 kDa, respectively. Both the 23 and 15 kDa proteins are associated with the virions. The 23 kDa protein contains a coiled-coil motif and by analogy with the P3 protein of CaMV, may be necessary for cell-to-cell movement. The 216 kDa P3 protein is a polyprotein that contains motifs for a movement protein, coat protein, aspartate protease, reverse transcriptase, and RNase H.

Replication

Caulimoviruses replicate by reverse transcription of an RNA intermediate, but integration of the viral DNA into host chromosomes is not required to complete the replication process. **Figure 3** illustrates the replication of an icosahedral caulimovirus, but the same steps are applicable for the bacilliform viruses. After virions enter a plant cell, the viral DNA becomes unencapsidated and is transported into the nucleus. The viral DNA contains two to four single-stranded discontinuities, which form as a consequence of the reverse transcription process. Once in the nucleus, the single-stranded discontinuities are covalently closed and the DNA is associated with histones to form a minichromosome. The host RNA polymerase II is responsible for synthesizing an RNA that is terminally redundant; the sequence on the 5′ end is reiterated on the 3′ end. In the case of CaMV, the terminal redundancy is 180 nt in size, whereas the terminal redundancy in the CoYMV RNA is 120 nt. The terminally redundant RNA is transported out of the nucleus into the cytoplasm where it can either serve as a template for translation of viral proteins or as a template for reverse transcription. Reverse transcription is thought to occur in nucleocapsid-like particles.

First strand DNA synthesis is primed by a methionine (Met) tRNA that binds to a complementary sequence near the 5′ end of the terminally redundant RNA. In the case of CaMV, the Met tRNA binds to a sequence approximately 600 nt from the 5′ end of its 35S RNA. The virally encoded reverse transcriptase synthesizes DNA up to the 5′ end of the terminally redundant RNA and the RNase H activity of the reverse transcriptase degrades the 5′ end of the RNA. The terminal redundancies present in the genomic RNA provide a mechanism for a template switch, as the reverse transcriptase is able to switch from the 5′ end of the genomic RNA to the same sequences on the 3′ end of the genomic RNA and continue to synthesize the first strand of DNA.

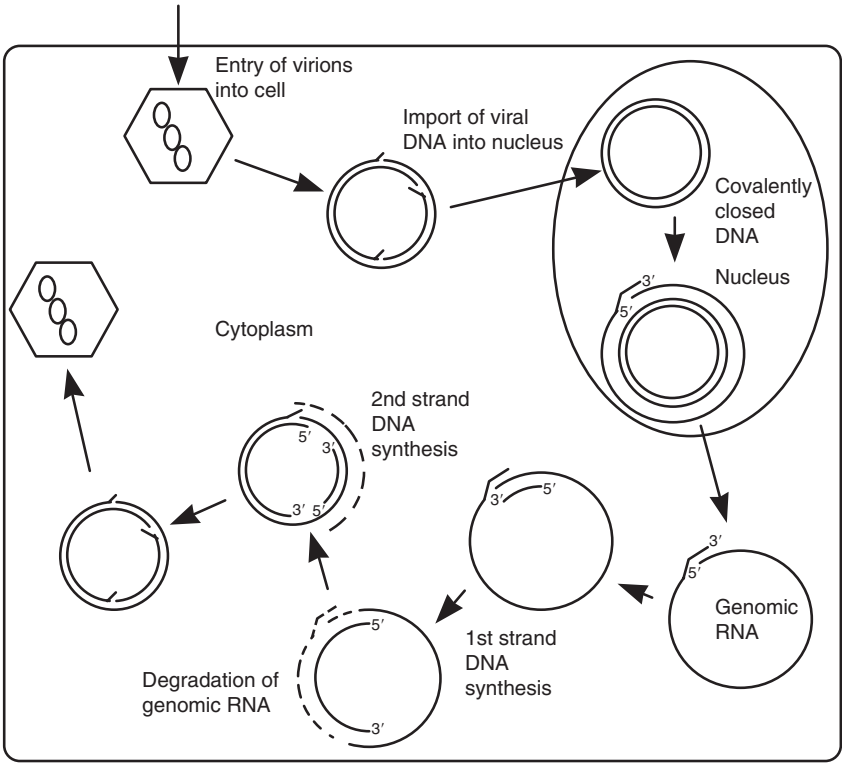


Figure 3 Replication strategy of the family *Caulimoviridae*.

The RNase H activity of the reverse transcriptase degrades the viral RNA template and small RNA fragments serve as the primers for second strand DNA synthesis. The RNA fragments bind to guanosine-rich tracts present in the first DNA strand, and these priming sites determine the positions of the discontinuities in the second DNA strand. A second template switch is required to bridge the gap from the 5′ to the 3′ end of the first DNA strand. The completion of the second DNA strand results in the formation of a dsDNA molecule that contains the characteristic single-stranded discontinuities.

Integration of Some Petuviruses, Cavemoviruses, and Badnaviruses into Host Chromosomes

Although caulimoviruses do not integrate into the host as part of their replication strategy, the sequences of several viruses have been detected in the genomes of their hosts. Furthermore, the integrated copies of several of these viruses, the banana streak viruses, TVCV and PVCV, can be activated to yield episomal infections. All three viruses can form virions once episomal infections are initiated, but they differ in their capacity to be transmitted to other plants. Banana streak virus can be transmitted by mealybugs, whereas PVCV is transmitted only by grafting. TVCV and PVCV are transmitted vertically, through seed, but this probably involves only the integrated copies of the virus rather than the episomal forms.

The sites of integration are complex, as the viral sequences have undergone rearrangements, and multiple copies are arranged in tandem. Banana streak virus is integrated at two loci in *Musa* chromosomes, TVCV is integrated at multiple loci in *Nicotiana edwardsonii* chromosomes, and PVCV is integrated into four loci in *Petunia hybrida* chromosomes. The mechanism that results in episomal infections remains to be elucidated, but there are some features common to all three viruses. First, the infections arise in interspecific hybrids. Consequently, each parent plant must contribute some factor to activate the integrated virus. Second, the infections arise after the hybrid has been exposed to some sort of stress. PVCV is activated when plants are exposed to water or nutrient stress, or the plants are wounded. TVCV infections arise in *N. edwardsonii* in the winter months in the greenhouse and are thought to be related to changes in light quality or duration. Integrated banana streak virus sequences are activated in otherwise healthy *Musa* species when they are subjected to tissue culture. In each case, the virus is likely released through a series of recombination events or through reverse transcription of an RNA template.

Portions of other DNA viruses have been detected in plant chromosomes, but they have not been associated with episomal infections. This has led to speculation that plant DNA viruses might be capable of recombination in every

infected plant, likely through a mechanism involving nonhomologous recombination. However, since episomal forms of the caulimoviruses and badnaviruses have not been found in tissues fated for seed formation, the integrated forms would not appear in the next generation of plants. Consequently, the banana streak viruses, TVCV, and PVCV must have gained access to germline cells at some point such that their integrants would be passed through seed. This is likely to have been a relatively rare event.

Transmission and Host Range

Most members of the genus *Caulimovirus* are transmitted by as many as 27 species of aphids in a semipersistent manner. The virions are acquired rapidly by the aphid and can be transmitted immediately upon acquisition. Virions can be maintained by the aphid for as little as 5 h up to 3 days, but are not retained after the aphid molts and are not passed on to aphid progeny. The protein products encoded by ORF2 (P2) and ORF3 (P3) are both required for aphid transmission. P3 forms a tetramer that binds to both the virion and to the C-terminus of P2. P2 is responsible for the binding of this complex to the aphid, as the N-terminus of P2 binds to a site in the aphid foregut. No insect vectors have been identified for species in the genera *Petuvirus*, *Soymovirus*, or *Cavemovirus*.

None of the virions of the icosahedral viruses in the genera *Caulimovirus*, *Petuvirus*, *Soymovirus*, or *Cavemovirus* are transmitted through seed or pollen. Most are transmitted after mechanical inoculation, but there are a few exceptions, as PVCV, TVCV, blueberry red ringspot virus, and strawberry vein banding virus cannot be mechanically inoculated. In addition, most of these viruses are transmitted by grafting. In particular, dahlia mosaic virus and blueberry red ringspot virus infections in the field may be initiated through vegetative propagation or through grafting of infected plant material.

Badnaviruses are transmitted primarily by mealybugs, but a few are transmitted by aphids. Badnaviruses transmitted by mealybugs include the banana streak virus species, the sugarcane bacilliform viruses, cacao swollen shoot virus, dioscorea bacilliform virus, kalanchoe top spotting virus (KTSV), piper yellow mottle virus, taro bacilliform virus, and schefflera ringspot virus. These viruses are transmitted in a semipersistent manner and can be retained after molts, but do not multiply in the mealybug and are not transmitted to progeny. The badnaviruses shown to be transmitted by aphids include gooseberry vein banding-associated virus, rubus yellow net virus, and spiraea yellow leafspot virus.

Other modes of transmission of the badnaviruses vary with the species. For example, CoYMV and KTSV can be mechanically inoculated, cacao swollen shoot virus and piper yellow mottle virus are mechanically inoculated with some difficulty, and rubus yellow net virus and taro bacilliform virus have not been shown to be mechanically inoculated. The preferred method for inoculation of infectious badnavirus clones is agroinoculation. Some of the badnaviruses are transmitted through seed, including the banana streak virus, KTSV, and mimosa bacilliform virus. KTSV is very efficiently transmitted by seed, with transmission rates from 60% to 90%, and is also transmitted in pollen.

RTBV is dependent on the RNA virus, RTSV, for its transmission. RTSV is vectored by a leafhopper, but causes only very mild symptoms. RTBV is only transmitted by the leafhopper in the presence of RTSV, but is responsible for the severe symptoms associated with the rice tungro disease. RTBV is not mechanically transmitted or carried in seed or pollen.

The host range of most caulimoviruses is fairly narrow, as in nature they generally infect plants within a single family (**Table 1**). Their experimental host range may extend to members of one or two other families, but in many instances, they may only infect a single genus of plants. There are a few exceptions. For example, the sugarcane bacilliform viruses have a broader host range than most badnaviruses, as they can infect *Sorghum*, *Rottboellia*, *Panicum*, rice, and banana. A limited host range can also be associated with similar limitations in the geographic distribution of the virus. For example, soybean chlorotic mottle virus has only been recovered from a few samples in Japan. Perhaps the virus with the smallest geographic distribution is stilbocarpa mosaic bacilliform virus, which has only been found on a single, small island in the Subantarctic, midway between Tasmania and Antarctica. Other viruses, such as CaMV, carnation etched ring virus, and dahlia mosaic virus are found worldwide, wherever their hosts are grown. Furthermore, the distribution of viruses that originate from integrated copies in their host's genomes, PVCV, TVCV, and the banana streak virus species, are also closely aligned with the locations of their hosts. Interestingly, the icosahedral viruses of the caulimoviruses tend to infect hosts in temperate climates, whereas the bacilliform viruses of the badnavirus group are more likely to infect hosts in tropical or subtropical climates.

Virus–Host Relationships

Caulimoviruses induce a variety of systemic symptoms in their hosts, from chlorosis, streaking, and mosaics, to necrosis. The best-characterized pathogenicity determinant is the P6 protein of CaMV, as it has been shown to play a key role in the formation of chlorotic symptoms in turnips. This virulence function was first associated with P6 through gene-swapping experiments between CaMV isolates. It was confirmed when P6 was transformed into

several species of plants, and in most cases, they exhibited virus-like symptoms. The P6 protein is also responsible for triggering systemic cell death in *Nicotiana clevelandii*, as well as a non-necrotic resistance response in *N. glutinosa*, and a hypersensitive resistance response in *N. edwardsonii*.

One feature that distinguishes the icosahedral viruses from the bacilliform viruses is that the former have the capacity to aggregate into amorphous inclusion bodies (**Figure 1(a)**), whereas the latter do not form inclusions. The inclusion bodies formed by the icosahedral viruses are not bound by a membrane, can range in size from 5 to 20 μm, and occur in virtually all types of plant cells. The inclusions can be visualized with a light microscope in strips of epidermal tissue that has been stained with phloxine B. Close examination of CaMV inclusion bodies by electron microscopy reveals that there are actually two types. One type contains many vacuoles and consists of an electron-dense, granular matrix that is composed primarily of the P6 protein (**Figure 1(a)**). A second, electron translucent type is made up of the P2 protein, a protein required for aphid transmission. Both types of inclusions are thought to have a role in the biology of the virus. The vacuolated inclusion bodies may be considered pathogen organelles, as they are thought to serve as the sites for replication of the viral nucleic acid, as well as translation of the 35S RNA and assembly of the virions. The electron translucent inclusions are considered to have a role in aphid transmission.

A second feature characteristic of the caulimoviruses is that the plasmodesmata of infected cells are enlarged enough to accommodate the 50 nm virions, as electron micrographs have revealed the presence of CaMV and CoYMV virions in the enlarged plasmodesmata. For both CaMV and CoYMV, the alteration in size is mediated by their proteins required for cell-to-cell movement. In infected protoplasts, the CaMV P1 protein has been shown to induce the formation of tubular structures that extend away from the protoplast surface. It is hypothesized that virions are assembled in the cell and then are escorted to the enlarged plasmodesmata by the cell-to-cell movement proteins.

See also: Caulimoviruses: Molecular Biology.

Further Reading

Calvert LA, Ospina MD, and Shepherd RJ (1995) Characterization of cassava vein mosaic virus: A distinct plant pararetrovirus. *Journal of General Virology* 76: 1271–1278.

Geering ADW, Olszewski NE, Harper G, *et al.* (2005) Banana contains a diverse array of endogenous badnaviruses. *Journal of General Virology* 86: 511–520.

Harper G, Hull R, Lockhart B, and Olszewski N (2002) Viral sequences integrated into plant genomes. *Annual Review of Phytopathology* 40: 119–136.

Hasagewa A, Verver J, Shimada A, *et al.* (1989) The complete sequence of soybean chlorotic mottle virus DNA and the identification of a novel promoter. *Nucleic Acids Research* 17: 9993–10013.

Hay JM, Jones MC, Blakebrough ML, *et al.* (1991) An analysis of the sequence of an infectious clone of rice tungro bacilliform virus, a plant pararetrovirus. *Nucleic Acids Research* 19: 2615–2621.

Hohn T and Fütterer J (1997) The proteins and functions of plant pararetroviruses: Knowns and unknowns. *Critical Reviews in Plant Sciences* 16: 133–161.

Hull R (1996) Molecular biology of rice tungro viruses. *Annual Review of Phytopathology* 34: 275–297.

Hull R, Geering A, Harper G, Lockhart BE, and Schoelz JE (2005) *Caulimoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 385–396. San Diego, CA: Elsevier Academic Press.

Lockhart BEL (1990) Evidence for a double-stranded circular DNA genome in a second group of plant viruses. *Phytopathology* 80: 127–131.

Pfeiffer P and Hohn T (1983) Involvement of reverse transcription in the replication of cauliflower mosaic virus: A detailed model and test of some aspects. *Cell* 33: 781–789.

Qu R, Bhattacharya M, Laco GS, *et al.* (1991) Characterization of the genome of rice tungro bacilliform virus: Comparison with Commelina yellow mottle virus and caulimoviruses. *Virology* 185: 354–364.

Richert-Pöggeler KR and Shepherd RJ (1997) Petunia vein-clearing virus: A plant pararetrovirus with the core sequences for an integrase function. *Virology* 236: 137–146.

Schoelz JE, Palanichelvam K, Cole AB, Király L, and Cawly J (2003) Dissecting the avirulence and resistance components that comprise the hypersensitive response to cauliflower mosaic virus in *Nicotiana*. In: Stacey G and Keen N (eds.) *Plant–Microbe Interactions,* vol. 6, pp. 259–284. St. Paul, MN: The American Phytopathological Society.

Shepherd RJ, Wakeman RJ, and Romanko RR (1968) DNA in cauliflower mosaic virus. *Virology* 36: 150–152.

Skotnicki ML, Selkirk PM, Kitajima, *et al.* (2003) The first subantarctic plant virus report: Stilbocarpa mosaic bacilliform badnavirus (SMBV) from Macquarie Island. *Polar Biology* 26: 1–7.

Caulimoviruses: Molecular Biology

T Hohn, Institute of Botany, Basel university, Basel, Switzerland

© 2008 Elsevier Ltd. All rights reserved.

Viruses of the Genus *Caulimovirus*

Several viruses have been assigned to the genus *Caulimovirus*, characterized by their content of open circular DNA, their icosahedral capsid, and their arrangement of seven open reading frames (ORFs) (**Table 1**). They have a narrow host range, usually restricted to one of the plant families. The genus *Caulimovirus* is one of six genera of the family *Caulimoviridae*, or plant pararetroviruses, which include three more genera of icosahedral viruses,

Table 1 Caulimoviruses species

Species	*Abbr.*	*Host range*	*Sequence*
Cauliflower mosaic virus	CaMV	Cruciferae	V00141; X02606; J02046
Carnation etched ring virus	CERV	Caryophyllaceae	X04658
Figwort mosaic virus	FMV	Scrophulariaceae	X06166
Strawberry vein banding virus	SVBV	Rosaceae	X97304
Horseradish latent virus	HRLV	Cruciferae	AY534728 - 33
Dahlia mosaic virus	DMV	Compositae	
Mirabilis mosaic virus	MiMV	Nyctaginacea	NC_004036
Thistle mottle virus	ThMoV	*Cirsium arvense*	

Note that *Blueberry ringspot virus and Cestrum yellow leaf curl virus*, originally classified as caulimoviruses, are not included because they belong to the genus *Soymovirus*.

Soymovirus, *Cavemovirus*, and *Petuvirus*, and two genera of bacilliform viruses, *Badnavirus* and *Tungrovirus*. Besides the differences in capsid structure of the two categories, the genera are distinct in the details of their genome arrangement and expression strategies (**Figure 1**).

Although members of the family *Caulimoviridae* (plant pararetroviruses), unlike retroviruses, do not integrate obligatorily into the host genome, 'illegitimately' integrated sequences have been found for several genera to date, that is, caulimo, petu-, cavemo-, badna-, and tungroviruses. These have been named commonly 'endogenous plant pararetroviruses' (EPRVs). EPRVs can be found in the pericentromeric region of chromosomes, are passively replicated together with the host DNA, and are inherited from generation to generation.

Properties of the Virion and Inclusion Bodies

Members of the genus *Caulimovirus* are icosahedral ($T = 7$), 45–50 nm in diameter and sediment between 200 and 250S. The main capsid components of Cauliflower mosaic virus (CaMV) are proteins with mobilities of 37 and 44 kDa on sodium dodecyl sulfate polyacrylamid electrophoresis. They are derived from ORF IV. The capsid is further decorated with 420 subunits of loosely bound 15 kDa virus-associated protein (VAP) derived from ORF III. In addition, CaMV particles contain minor amounts of other polypeptides: protease and reverse transcriptase derived from ORF V, and host casein kinase II. In cells infected with CaMV or a number of other caulimoviruses large numbers of virus particles accumulate in typical stable inclusion bodies, the matrix of which is the virus-encoded transactivator/viroplasmin (TAV) protein of 62 kDa, derived from ORF VI. Inclusion bodies are visible in the light microscope and can be stained with phloxin.

Properties of the Genome

The genome of the members of the family *Caulimoviridae* is built from 7200 to 8200 bp. It exists (1) in infected plant nuclei as supercoiled DNA bound to nucleosomes (minichromosome), (2) in infected cytoplasm as RNA with a ~180 nt redundancy, and (3) in virus particles as open circular dsDNA. The open form is due to nicks at specific sites in both strands with short 5′ overlaps (**Figure 2**), remnants of the reverse transcription process, which are apparently removed in the infected nucleus. The sequences of several of the caulimoviruses are known (**Table 1**). They contain seven ORFs in the order VII (dispensable unknown function), I (cell-to-cell movement), II (aphid transmission factor), III (VAP), IV (capsid protein precursor), V (protease, reverse transcriptase, RNase H), and VI (multifunctional protein, transactivator of translation/viroplasmin).

Properties of the Proteins

Movement Protein

CaMV and probably all the other members of the family *Caulimoviridae* with icosahedral capsid move from cell to cell as particles through tubular structures spanning the cell walls between adjacent cells. In CaMV the 37 kDa movement protein (MOV), coded for by ORF I, is responsible for the formation of these tubules and also provides their main component.

Aphid Transmission Factor

In general, aphids transmit viruses of the genus *Caulimovirus* and probably other genera of icosahedral (but not bacilliform) Caulimoviruses. In the case of CaMV, ORF II codes for the 18 kDa aphid transmission factor (ATF). Some of the viral inclusion bodies in CaMV-infected cells consist mainly of ATF. ATFs interact with the virus particles via their VAP and with tubulin and also with the cuticulum lining the tip the aphid's stylet. ATF mutants can still be transmitted by mechanical inoculation and by aphids that had previously be in contact with wild-type ATF either by feeding on plants infected with a related virus or on nutrient solutions supplied with artificially produced ATF.

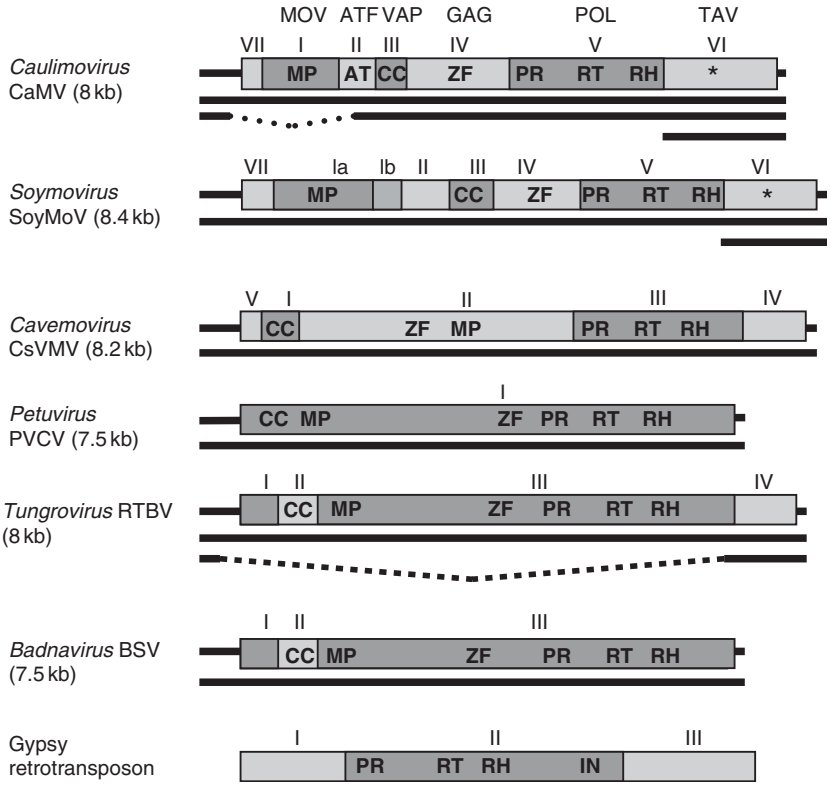


Figure 1 Open reading frame (ORF) arrangement in *Caulimovirus* and the other genera of the family *Caulimoviridae*. Examples of each of the six genera are shown, as well as the retrotransposon 'Gypsy' for comparison. For CaMV, the ORFs code for dispensable unknown function (VII), movement protein (I, MOV), aphid transmission factor (II, ATF), virion-associated protein (III, VAP), capsid protein precursor (IV, GAG), Pol polyprotein (V, POL), and transactivator/viroplasmin (VI, TAV). Although the number of final proteins might be similar for the other genera, the number of original ORFs differs, with soymoviruses having the most ORFs and petuviruses the least, namely only one original ORF, meaning more work for the protease to produce individual proteins. Key motifs are conserved in the family *Caulimoviridae*, movement protein motif (MP), coiled-coil domain (CC), zinc-finger (ZF), protease domain (PR), reverse transcriptase domain (RT), RNase H domain (RH), transactivator domain (*). In contrast to retroviruses and retrotransposons, an integrase domain (IN) is missing in members of the family *Caulimoviridae*.

Virion-Associated Protein

The VAP provides for coupling between virions and the movement and insect transmission factors and is therefore required for both insect transmission and cell-to-cell movement.

MOV, ATF, and VAP have no counterpart in retroviruses, reflecting the differences in cell-to-cell movement and infection routes used by animal and plant viruses. However, the movement proteins of plant pararetroviruses are related to a corresponding class of genes in most other plant viruses. In animals, viruses usually move from cell to cell by endocytosis and budding, while in plants, passage through modified plasmodesmata is used.

Capsid Proteins and Their Precursor

The product of CaMV ORF IV has a molecular weight of 56 kDa and an electrophoretic mobility of 80 kDa. It is flanked by very acidic regions, which are removed from the mature capsid proteins, which have a mobility corresponding to proteins of 44 and 37 kDa, although their molecular weight is probably smaller. The sequence of p37 is included within p44 and thus antibodies against p37 react also with p44. p44, but not p37, is phosphorylated. It is not known whether both proteins are required for full infection, or whether only one of them is the true functional capsid protein. It might be that p44 is the mature form and p37 a degradation product. Alternatively, p37 might be the mature form and p44 still a precursor. Both these proteins include a Zn-finger motif, which is implicated in RNA binding. Furthermore, large stretches of basic amino acids at the C-termini of both proteins constitute DNA- and RNA-binding motifs.

The precapsid protein has a nuclear localization signal which is masked by the N-terminal acidic domain. Upon virus assembly and removal of the acidic regions, the NLS becomes exposed and virus particles are transported to the nuclear pore where they release the DNA into the nucleus.

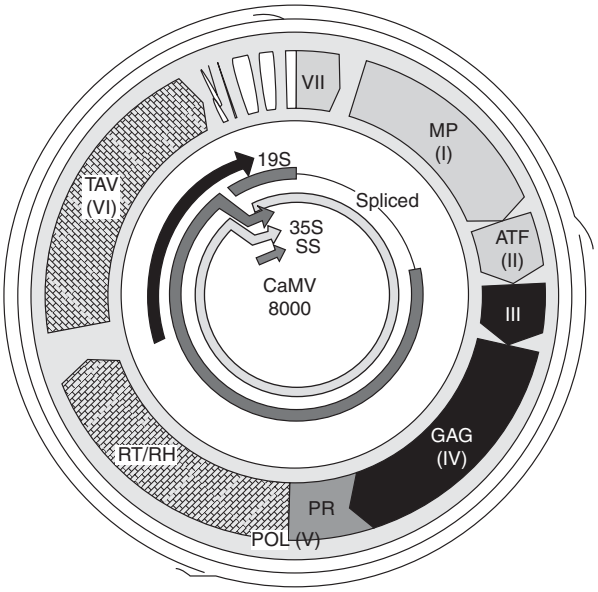


Figure 2 Map of cauliflower mosaic virus. On the outside the open circular double-stranded viral DNA with its overhangs is symbolized. The main circle shows the arrangement of the open reading frames (ORFs). ORF I encodes movement protein (MP or MOV), ORF II aphid transmission factor (ATF), ORF III the VAP, ORF IV the capsid protein precursor (GAG, in analogy to the corresponding protein in retroviruses), ORF V the POL polyprotein consisting of protease (PR), reverse transcriptase (RT), and RNase H (RH), and ORF VI the transactivator/viroplasmin (TAV). On the inside, the primary transcripts and the essential spliced RNAs are shown.

POL Polyprotein, Protease, Reverse Transcriptase/RNase H

CaMV ORF V corresponds to the POL ORF of retroviruses. It produces a polyprotein which is cleaved during virus production into a 15 kDa aspartic proteinase and a 60 kDa reverse transcriptase/RNase H. It is not known whether the RNase H functions as part of the 60 kDa protein or whether it is released by another cleavage reaction. One has to assume that the proteinase also functions as part of the polyprotein, that is, by releasing itself. Members of the family *Caulimoviridae* do not have an integrase and consequently their DNA is not or only accidentally integrated into the host chromatin.

Transactivator of Translation/Viroplasmin

The 62 kDa transactivator of TAV, encoded by ORF VI, has no significant homologies to any other known viral or host genes. It has been implicated in virus assembly, reverse transcription, host range determination, symptom severity, control of polycistronic translation, and seclusion of virus functions and silencing suppression. Perhaps as a consequence of the role of the ORF VI protein in translation, all CaMV proteins including foreign proteins carried by a CaMV vector can be found within the inclusion bodies. A subdomain in its center has been assigned to the translational transactivation and other domains have been found to unspecifically bind single- and double-stranded RNA, respectively. Furthermore, the protein interacts with capsid protein. TAV also has properties of a nuclear shuttle protein and might be involved in viral RNA transport.

Virus Stability

Both, the CaMV inclusion bodies and the virus particles, are very stable. Dissolution of the inclusion bodies to obtain virus particles requires treatment with 1 M urea over a long period. Virus particles aggregate at acidic pH and disintegrate at very high pH (0.1 M NaOH). Virus particles can also be disintegrated by boiling in sodium dodecyl sulfate/dithiothreitol or by treatment with proteinase K and phenol extraction. During these treatments, VAP is released and therefore usually escapes detection as virus component.

Genome Replication

As in retroviruses, replication occurs by production of a terminally redundant RNA by transcription in the nucleus and its reverse transcription in the cytoplasm. Also as in retroviruses, and unlike in hepadnaviruses, a tRNA primer (met-initRNA) is used as the primer for (−)-strand DNA synthesis from the RNA template. The RNA template is digested by RNase H following the reverse transcriptase. However, oligo(G) stretches are resistant to this digestion and remain bound to the (−)-strand DNA where they act as primers for (+)-strand DNA synthesis. The number of (+)-strand synthesis initiation events varies between one and three major events in different members of the family *Caulimoviridae* and even within strains from a particular virus. Minor initiation events also occur. The synthesis of both the strands overshoots, creating short overhangs that can only be repaired by repair exonuclease and ligase after transport into the nucleus. Accordingly, the packaged viral DNA is open circular, while the nuclear one is closed and supercoiled. The latter interacts with histones and forms minichromosomes. Caulimovirus DNA does not integrate into the host chromatin obligatorily. The template for transcription is the supercoiled circular DNA.

Transcription

Transcription in caulimoviruses is unidirectional. All caulimoviruses produce a transcript covering the total genome plus about 180 nt, such that the RNA is terminally

redundant. This RNA is called 35S RNA due to its sedimentation behavior. The terminal redundancy is caused by the polyadenylation signal located on the circular DNA 180 bp downstream of the transcription start site being ignored by the polymerase at its first passage (see below). CaMV-like caulimoviruses produce a second transcript, the 19S RNA, covering ORF VI and encoding the inclusion body protein/translation transactivator.

The 35S promoter of CaMV, FMV, and probably other caulimoviruses is very strong and quasi-constitutive, that is, it is expressed in nearly all types of cells and at all developmental stages. This constitutivity is caused by a number of different enhancer elements, each with some specificity for certain cell types. Some of the corresponding transcription factors have been identified, for example, ASF-1, ASF-2, and CAF recognizing a TGACG motif, a GATA motif, and CA-rich region, respectively.

RNA Processing

Full-length RNA made from the circular DNA template includes a polyadenylation signal. If this is used on the first encounter, a short-stop RNA of 180 nt is formed; if used on the second encounter full-length terminally redundant RNA is formed. The role of the short-stop RNA, if any, is not known.

The CaMV polyadenylation signal consists of an AAUAAA sequence, which determines the cleavage site 13 nt downstream of it. Occasionally cryptic signals are used in addition. In contrast to the animal system, single point mutations in the AAUAAA signal are partially tolerated by the plant system. Polyadenylation enhancers are located upstream of the AAUAAA signal and not downstream as in most animal cases. In CaMV and FMV, these elements are tandem repeats of UAUUUGUA.

In addition to the primary CaMV 35S transcript, alternatively spliced versions of it have been detected. By removing an intron extending from close to the end of the leader to a position within ORF II, an mRNA is created in which ORF III is the first ORF and ORF IV the second. Additional splicing events were found using the same splice acceptor but using donors within ORF I. These led to ORF I–ORF II in-frame fusions, the function of which is not known. Whether the ratio of spliced to unspliced RNA is controlled, for example by nuclear export of unspliced RNA as in human immunodeficiency virus, is not known.

Translation Mechanism

The 35S RNA of caulimoviruses and also its spliced derivatives serve as polycistronic mRNAs for the viral proteins. The ORFs of these viruses closely follow each other, are opened by efficiently recognized start codons, and usually also contain internal start codons. In plant protoplasts and in transgenic plants most of the downstream ORFs are poorly expressed in the absence of TAV interestingly the only ORF that is translated from a monocistronic mRNA. Transactivation activity has been demonstrated for CaMV and FMV, and probably resides also in the corresponding ORFs of the other CaMV-like and PCSV-like caulimoviruses. Transactivation is thought to be based on a reinitiation mechanism.

Another special feature of the caulimovirus (and also the badnavirus) 35S RNAs is their 600–700-nt-long leader, which is rather large for eukaryotic RNAs. These leaders contain several small ORFs and include a large hairpin structure. These are features that usually make an RNA a poor messenger, since initiation factors and/or 40S ribosomes initiate scanning at the cap of an RNA moving in the 3′ direction until an AUG codon is encountered and translation proper begins, whereas translation from AUGs further downstream is precluded. To overcome this problem caulimoviruses employ a shunting mechanism for translation initiation, whereby scanning is initiated at the cap as usual, but the scanning complex bypasses (shunts) most of the leader to reach the first longer ORF. This mechanism is unlike the internal initiation mechanism used by enteroviruses.

Protein Processing

Many of the caulimovirus proteins are processed, that is, in CaMV-like (and by analogy probably the other) caulimoviruses the precapsid protein is cleaved at both ends removing its very acidic termini. This process might be coupled to virus assembly and maturation. The capsid protein is both methylated and glycosylated. The Pol protein is cleaved to yield the aspartic proteinase and reverse transcriptase/RNase H. At least some of these cleavages occur by the action of the viral proteinase. In contrast, the ORF III product is cleaved by a host cysteine proteinase.

Silencing and Silencing Suppression

Another level of expressional control is provided by transcriptional and post-transcriptional silencing, which lead to strong inhibition of transcription and destabilization of the transcript, respectively. Silencing is viewed as a plant defense mechanism directed against viruses and transposons. On the other hand, viruses have developed silencing suppressors to counteract this plant defence strategy. The response of *Brassica napus* to systemic infection with cauliflower mosaic virus that results first in enhancement followed by subsequent suppression of viral gene

expression in parallel with changes in symptom formation can be explained by the battle being waged between silencing suppression and silencing.

Silencing is autocatalytic and systemic, and hence the silencing of CaMV can also lead to the silencing in *trans* of transgenes driven by the CaMV 35S promoter. In a special case, herbicide resistance in oilseed rape conferred by expression of a 35S promoter-driven bialaphos tolerance transgene can be silenced due to the host response to CaMV infection.

See also: Caulimoviruses: General Features; Endogenous Retroviruses; Legume Viruses; Plant Virus Vectors (Gene Expression Systems); Rice Tungro Disease; Viral Suppressors of Gene Silencing; Virus Induced Gene Silencing (VIGS).

Further Reading

Blanc S (2002) Caulimoviruses. *Virus–Insect–Plant Interactions.* New York: Academic Press.

Covey SN and Al Kaff NS (2000) Plant DNA viruses and gene silencing. *Plant Molecular Biology* 43(special issue): 307–322.

Goldbach R and Hohn T (1996) Plant viruses as gene vectors. In: Bryant JA (ed.) *Methods in Plant Biochemistry 10b: Molecular Biology*, pp. 103–120. San Diego: Academic Press.

Haas M, Geldreich A, Bureau M, *et al.* (2005) The open reading frame VI product of cauliflower mosaic virus is a nucleocytoplasmic protein: Its N terminus mediates its nuclear export and formation of electron-dense viroplasms. *Plant Cell* 17: 927–943.

Hohn T (2007) Plant virus transmission from the insect point of view. *Proceedings of the National Academy of Sciences, USA* 104: 17905–17906.

Hohn T, Park HS, Guerra-Peraza O, *et al.* (2001) Shunting and controlled reinitiation: The encounter of cauliflower mosaic virus with the translational machinery. *Cold Spring Harbor Symposia on Quantitative Biology* 66: 269–276.

Hohn T and Richert-Poeggeler K (2006) Caulimoviruses. In: Heffron KL (ed.) *Recent Advances in DNA Virus Replication*, pp. 289–319. Kerala: Research Signpost Transworld.

Kiss-László Z and Hohn T (1996) Pararetro- and retrovirus RNA: Splicing and the control of nuclear export. *Trends in Microbiology* 4: 480–485.

Lam E (1994) Analysis of tissue specific elements in the CaMV 35S promoter. In: Nover L (ed.) *Results and Problems in Cell Differentiation, #20*, pp. 181–196. Berlin: Springer.

Plisson C, Uzest M, Drucker M, *et al.* (2005) Structure of the mature P3-virus particle complex of cauliflower mosaic virus revealed by cryo-electron microscopy. *Journal of Molecular Biology* 346: 267–277.

Rothnie HM, Chapdelaine Y, and Hohn T (1994) Pararetroviruses and retroviruses: A comparative review of viral structure and gene expression strategies. *Advances in Virus Research* 14: 1–67.

Ryabova L, Pooggin MM, and Hohn T (2005) *Translation Reinitiation and Leaky Scanning in Plant Viruses. Virus Research (Translational Control during Virus Infection).* Oxford: Elsevier.

Staginnus C and Richert-Poggeler KR (2006) Endogenous pararetroviruses: Two-faced travelers in the plant genome. *Trends in Plant Science* 11: 485–491.

Central Nervous System Viral Diseases

R T Johnson and B M Greenberg, Johns Hopkins School of Medicine, Baltimore, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Most viral infections of the nervous system represent serious and potentially life-threatening complications of systemic viral infections. With the possible exception of rabies, viruses are not neurotropic in the literal sense of having a specific affinity for the nervous system. Some viruses frequently invade the nervous system yet seldom cause serious disease; for example, mumps virus may cause meningitis but, even during uncomplicated mumps parotitis, cerebrospinal fluid (CSF) changes in over 50% of patients indicate probable nervous system infection. Other viruses, such as herpes simplex virus, rarely infect the central nervous system (CNS), but when they do, they often cause fatal disease. Thus, both mumps and herpes simplex viruses are regarded as neurotropic; mumps is highly neuroinvasive but has limited neurovirulence; herpes simplex has low neuroinvasiveness but is highly neurovirulent. Such variations are dependent on the structural and functional determinates of nervous system invasion, on the particular neural cells that specific viruses infect, on the effect of this infection on the host cells, and on the immune response or immunopathologic responses to the infection. Several terms are defined as follows:

- Neurotropic: able to infect neural cells.
- Neuronotropic: able to infect neurons in contrast to other nervous system cells.
- Neuroinvasive: able to enter the nervous system.
- Neurovirulent: able to cause neurologic disease.

Anatomic and Physiologic Considerations

Both structural and functional features of the CNS present a unique milieu for viral replication. The blood–brain barrier and the compact structure of the brain and cord

pose formidable barriers to the entry or dissemination of viruses within the nervous system. Yet, the multitude of dense dendritic connections among neurons provide a unique environment for cell-to-cell spread of pathogens. Furthermore, neurons are unique cells with high metabolic rates, intense membrane specialization, and no regenerative capacity. The same barriers that exclude viruses also limit access of immunocompetent cells and antibodies, and the nervous system lacks an intrinsic lymphatic system and has a paucity of phagocytic cells. Thus, the barriers that inhibit virus invasion also deter viral clearance. Therefore, many persistent infections involve the CNS.

The blood–brain barrier was originally conceptualized from the observation that dyes, such as Trypan Blue, stain all tissues except the brain and spinal cord after injection into the systemic circulation. The barrier at the cerebral capillary level consists of tight junctions between the capillary endothelial cells (beyond which most dyes do not pass), a dense basement membrane around the cells and tightly opposed astrocytic footplates. In the choroid plexus, the blood–CSF barrier is structurally different. The capillaries of the plexus are fenestrated, lack a basement membrane, and are surrounded by a loose stroma. Dyes and particles readily pass into the choroid plexus but are prevented from entering the CSF by tight junctions located at the apices of the secretory epithelial cells of the choroid plexus. Tight junctions between the arachnoid cell over the surface of the brain complete the barrier.

There is no comparable barrier between the brain and the spinal fluid. The ependymal cells are not joined by tight junctions and, therefore, there is a free exchange between the extracellular space of the brain and the CSF. However, the intracellular gap between neural cells measures only 10–15 nm, less than the diameter of even the smallest virus, so that free movement of virus particles or inflammatory cells within the extracellular space is relatively restricted.

Neurons have specialized membranes for the transmission and receipt of specific messages; they also have axonal extensions to carry signals to and from distant neuronal populations, motor endplates, and sensory endings. In humans these cytoplasmic extensions may exceed a meter in length. These features are important in viral infections, since different subpopulations of neurons have different receptors usurped by viruses to permit entry into cells. Furthermore, viruses in some cases can be carried by axoplasmic transport systems either into the nervous system or within the nervous system where axonal processes link functionally related neurons.

Antibodies found in the normal CNS are derived entirely from the serum. Antibody levels of immunoglobulin G (IgG) are approximately 0.4% of the serum levels. Since diffusion of macromolecules across the barrier is largely size-dependent, immunoglobulin M (IgM) is present in even lower levels. Complement is largely excluded. There is also no lymphatic system in the usual sense and few phagocytic cells. When inflammation disrupts the blood–brain barrier, antibody molecules leak into the nervous system along with other serum proteins. When a mononuclear inflammatory response is mounted against infection, T lymphocytes usually enter the nervous system first followed by macrophages and B lymphocytes. These B cells from the peripheral circulation move into the perivascular space and can generate immunoglobulins intrathecally.

The postmitotic nature of neurons is one the most important features when considering viral infections of the nervous system. Unlike most organ systems, the fundamental component of the nervous system lacks the ability to regenerate. Thus, by definition, infections that cause cell death (directly or indirectly via the elicited immune response) create irreversible damage. In this setting, neurons have developed various strategies for suppressing viral replication, clearing virus infections, and for creating environments suitable for latent infections.

Pathways of the CNS Invasion

Viruses have been shown to enter the nervous system both along nerves and from the blood. The first experimental studies of viral invasion employed rabies, herpes simplex, or polioviruses, all of which, under experimental circumstances, can penetrate the nervous system along peripheral nerves. The precise mechanisms of neural spread remained a mystery for many years, since it was thought that the axoplasm slowly oozed in an anterograde direction. It was proposed that virus might move in perivascular lymphatics, by ascending infection of the supportive cells within the peripheral nerve, or even by replication in axons, a speculation that is now untenable because of the observed lack of ribosomes or protein synthesis within axons. In the 1960s active anterograde and retrograde axon transport systems were found. Viruses or other particles can be taken up in vesicles at the nerve terminals and transported to the cell body of the sensory or motor neuron (**Figure 1**). This neural route of entry is important in primary viral infections such as rabies and possibly poliomyelitis. Retrograde transport also moves herpes simplex and varicella-zoster viruses from mucous membranes or skin into sensory ganglia at the time of primary infection. Subsequently, anterograde transport carries the reactivated virus from the ganglia to the periphery during exacerbations. Anterograde transport of herpes simplex virus by nerves innervating the dura from the trigeminal ganglia may explain the unique temporal lobe localization of herpes simplex virus encephalitis.

The olfactory spread of virus is a variation of neural spread. In the olfactory mucosa, neural fibers provide a

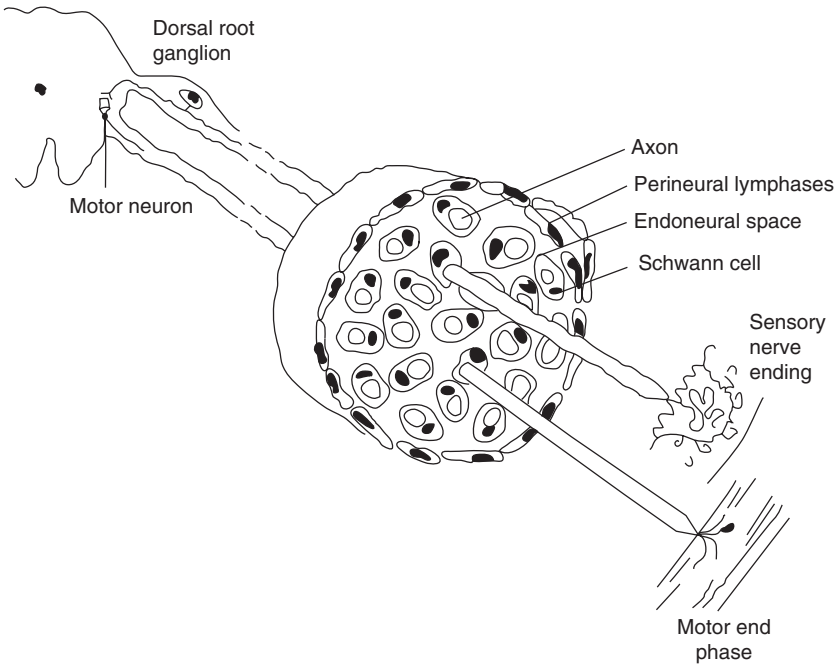


Figure 1 Schematic diagram of possible routes of neural spread of viruses to the nervous system. Reproduced with permission from Johnson RT (1982) *Viral Infections of the Nervous System.* New York: Raven Press.

unique pathway; the apical processes of receptor cells extend beyond the free surface of the epithelium as olfactory rods and the central processes synapse in the olfactory bulb. These are the only nerve cells with processes that link the CNS and ambient environment. Indeed, some colloidal particles placed on the olfactory mucosa can be found in the olfactory bulbs within 1 h. Experimental studies show that viruses can enter through this route, and this may occur in some aerosol infections in humans such as laboratory accidents or rabies virus infections in bat-infested caves. Also, the olfactory pathway has been postulated as a possible route of herpes simplex virus entry into the nervous system as an alternative explanation for the orbital–frontal and medial–temporal lobe localization of herpetic encephalitis. Nevertheless, despite the apparent ease of spread along this route, it appears to be a rare route of natural infection.

In most experimental and natural infections, viruses invade the brain from the blood. Historically, the blood–brain barrier was believed to be impervious to viruses. This belief was based in part on the fact that viruses experimentally inoculated directly into brain cause disease after a brief inoculation period, whereas the incubation period after intravenous inoculation is longer and comparable to that following cutaneous or peritoneal inoculation. The reason for this delay in infection is that virus in the blood is rapidly removed by the reticuloendothelial system; therefore, intravenous inoculation is, in fact, an inoculation primarily of the Kupffer cells of the liver and other reticuloendothelial cells. Therefore, virus must establish a nidus of peripheral replication that can effectively seed a viremia of sufficient magnitude and duration to allow invasion across the blood–brain barrier (**Figure 2**). Thus, some viruses grow in lymphatics and seed into the blood directly via the thoracic duct, others grow in vascular epithelial cells, and others replicate in highly vascular tissue such as muscle.

A persistent viremia can be achieved by several mechanisms. Rate of clearance is dependent upon particle size; small particles such as togaviruses and flaviviruses can maintain high-titer plasma viremias with sufficient rapid replication in peripheral tissue. Other viruses adsorb to red blood cells and thus evade clearance. Many large viruses such as measles and herpes viruses infect white blood cells thus evading clearance and replicating at the same time.

Some viruses enter the nervous system either across the capillary endothelium and others across the choroid plexus. Some viruses infect the capillary endothelial cells and simply grow into the brain while others are able to transit across endothelial cells despite their paucity of endopinocytotic vesicles. Entry in infected leukocytes is a theoretical possibility but leukocyte traffic into the nervous system is limited, although trauma or inflammation due to other causes may predispose the nervous system to infection with viruses that infect white blood cells. Although there are areas of increased blood–brain barrier permeability, no viral infection has been shown to infect these areas selectively. Other viruses, such as mumps

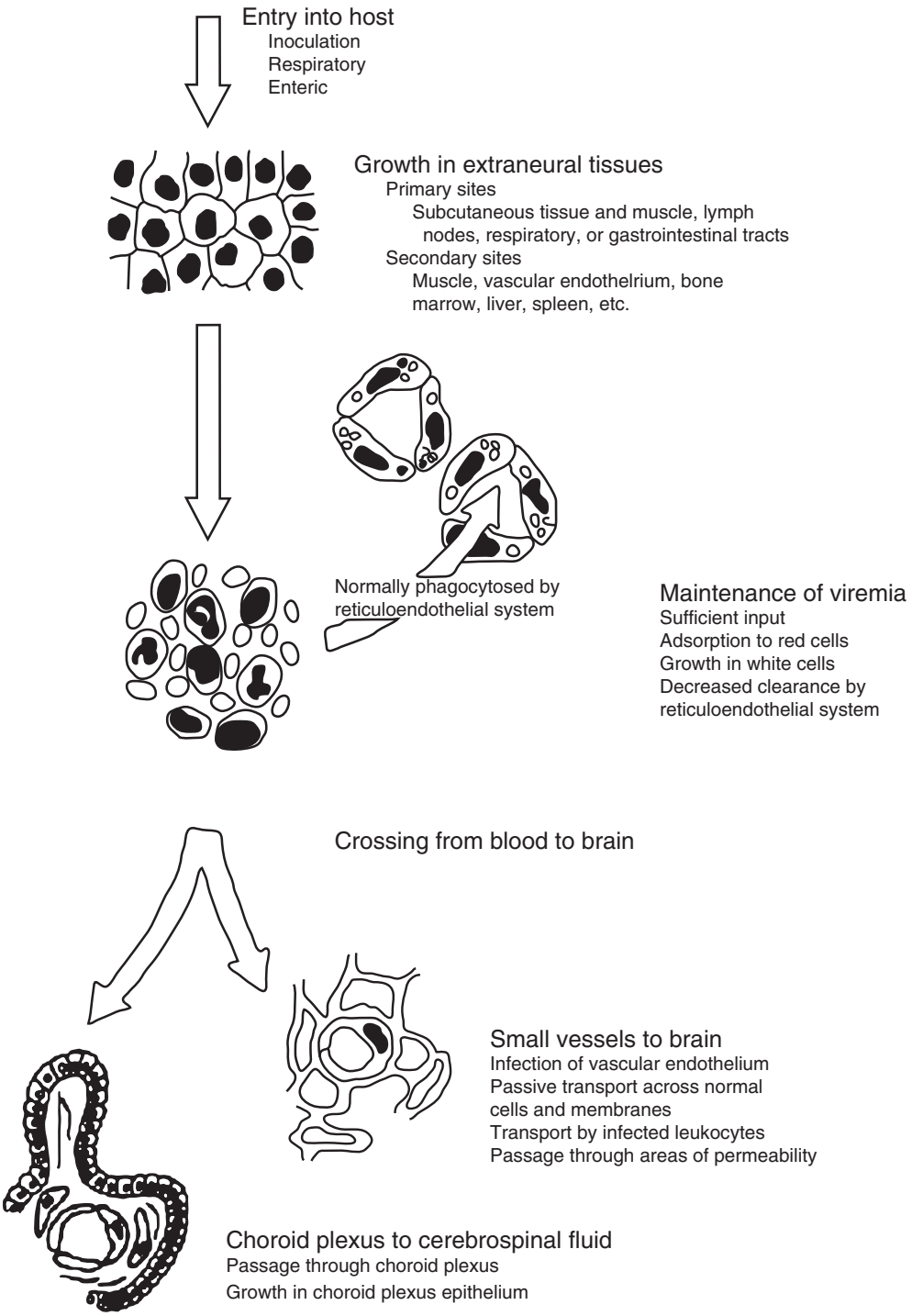


Figure 2 Schematic diagram of steps in hematogenous spread of viruses to the nervous system. Reproduced with permission from Johnson RT (1982) *Viral Infections of the Nervous System*. New York: Raven Press.

virus, grow in choroid plexus epithelium and seed into CSF. Thus, the clearance of particles by the reticuloendothelial system, barriers of nonsusceptible extraneural cells, production of interferon and other nonspecific inhibitors, and the physical barriers of the nervous system itself probably explain why viral infections of the brain are rare, even though systemic infections with the potential pathogens are very common.

Infections of Neural Cells

Once a virus has penetrated into the nervous system it must contact a susceptible cell and spread through the compact neuropil which is a theoretical problem. The fact that some viruses can be neutralized by extracellular antibody even after CNS invasion shows that viruses such as togaviruses and flaviviruses do spread in extracellular space, but this is not true of larger viruses. Conversely, the compact neuropil may facilitate the contiguous cell-to-cell spread of viruses. For example, in subacute sclerosing panencephalitis, a chronic brain infection of humans with measles, extracellular enveloped virus is never seen, and there are enormous titers of extracellular antibody. Apparently, the fusion protein allows measles virus to move from cell to cell through the brain.

Cell-to-cell spread may also involve axoplasmic flow causing infection of functionally linked cells; for example, in poliovirus infections the virus is rapidly spread through the motor system. Some viruses infect only neuronal populations such as rabies, polioviruses, and the arthropod-borne viruses (arboviruses), and some infect selective neuron populations. Other viruses such as herpes simplex virus appear to infect neurons and glial populations with little selectivity.

Infection limited to vascular endothelial cells is found with rickettsial infections but is not recognized in any viral infection of the nervous system. Infection limited to choroid plexus and meningeal cells appears to occur with those viruses that cause benign meningitis. In experimental studies with a number of viruses, widespread lytic infection of ependymal cells can lead to closure or stenosis of the aqueduct of Sylvius and resultant hydrocephalus. Similar aqueductal stenosis and hydrocephalus have been described in children after mumps virus meningitis.

The selective infection of oligodendrocytes has been recognized in nature in the disease progressive multifocal leukoencephalopathy caused in humans by the JC virus and in monkeys by SV-40 virus. In the course of immunosuppression, now seen most frequently with acquired immune deficiency syndrome (AIDS), a selective lytic infection of oligodendrocytes causes multifocal areas of demyelination in the brain. This usually fatal condition has also been seen in patients treated with immunomodulating medical regimens that included natalizumab (a monoclonal antibody against alpha 4 integrin). This drug causes a release of lymphocytes from lymph nodes and prevents the trafficking of lymphocytes across the blood–brain barrier. Thus, this medication may have interfered with the normal immune surveillance of the CNS, leading to the unabated emergence of JC virus within the brain.

With changes in age, the specificity of infection and vulnerability of neural cells may change. For example, bluetongue virus infection of fetal sheep destroys the precursors of neurons and glia of the subependymal plate which leads to hydranencephaly or porencephaly dependent on the age of fetal development, whereas the virus fails to infect the mature postmigratory cells in the late gestational or postnatal animal. Similarly, the external granular cells of the cerebellum in fetal or newborn animals are selectively infected by parvoviruses, and destruction of these mitotic cells leads to the granuloprival cerebellar degeneration seen in both natural and experimental animal infections. Alphaviruses which cause encephalomyelitis have more profound effects in young hosts including mice and humans.

Mechanisms of Cell Damage

Lytic infections of neural cells cause regional destruction of brain or lysis of specific cell populations. Noncytopathic infections of neural cells also occur and lead to persistent infection with no disease or disorders without obvious histological changes. For example, neuroblastoma cells in culture infected with noncytopathic viruses such as rabies can show normal morphology growth rates and protein synthesis, but reduced synthesis of specific neurotransmitters or receptors. These have been termed 'luxury functions', although *in vivo* the ability of neurons to synthesize transmitters or receptors would hardly be considered a luxury. Analogous noncytopathic infection has been demonstrated in mice congenitally infected with lymphocytic choriomeningitis virus. Congenitally infected mice are usually 'runts', but recent studies have shown selective infection of cells of the anterior pituitary which normally generates growth hormone. The animals are actually pituitary dwarfs responsive to growth hormone therapy.

Alternatively, the infected cell may not be damaged by virus replication but destroyed by the immune responses, as seen in adult mice infected with lymphocytic choriomeningitis virus. Indirect cell damage can occur in viral infections that leads to sensitization of the host to neural antigens. This has recently been demonstrated in rats infected with coronaviruses, where the infection of neural cells leads to a cell-mediated autoimmune response to myelin proteins and to subsequent demyelination. In postmeasles encephalomyelitis of humans, autoimmune demyelination appears to occur in the absence of infection of neural cells. Infection of lymphoid tissue leads to disruption of normal immune regulation, and about 1 per 1000 persons develop a symptomatic autoimmune reaction to myelin basic protein.

Other indirect mechanisms of neural cell damage have been postulated to explain the diverse neurological diseases seen in the course of human immunodeficiency virus (HIV) infection. The virus does not appear to cause significant or readily documented infections of

neuronal or glial cells. The primary cells that are infected are macrophages and the microglia of the brain derived from macrophage populations. Possibly viral proteins produced by these cells or cytokines released by these cells interfere with neuronal function or are toxic to neurons or glial cells. For example, tumor necrosis factor, a lymphokine often increased in brains of neurologically affected AIDS patients has been shown *in vitro* to induce demyelination.

Clinical Features

Acute Infections

The varied clinical features of viral infections of the nervous system can be explained in large part by the factors discussed above. Thus, a virus may infect only meningeal or ependymal cells, and cause a clinical syndrome known as viral meningitis or acute aseptic meningitis. This clinical syndrome is characterized by fever, headache, and nuchal rigidity secondary to meningeal irritation but without clinical signs suggesting parenchymal disease. The commonest causes of acutely viral meningitis are enteroviruses and mumps virus.

Encephalitis is a clinical syndrome in which in addition to fever, headache, and stiff neck, there is paralysis, altered mental status, seizures, or other evidence of parenchymal disease of the brain. The commonest causes of severe encephalitis in man are herpes simplex virus and the arboviruses. The former infects neurons and glia and causes diffuse necrotizing encephalitis in the newborn but focal encephalitis in the immune adult presumably because diffuse virus spread is contained by immune responses. Focal signs, hallucinations, behavioral abnormalities, and aphasia are more common with herpes encephalitis because of the localization to temporal lobes. Arboviruses have a propensity to infect neurons, and some flaviviruses tend to infect basal ganglia and brainstem neurons causing movement disorders and sudden respiratory failure. If signs of spinal cord involvement accompanies encephalitis, the term encephalomyelitis is often used. However, the term encephalomyelitis is also used to distinguish an acute postinfectious demyelinating disease of assumed autoimmune origin from acute viral encephalitis. Postinfectious encephalomyelitis (or acute disseminated encephalomyelitis) usually occurs 3–14 days after exanthems (measles, varicella, or rubella) or respiratory infections (mumps, influenza, and others) and clinically is characterized by the abrupt onset of fever, obtundation, seizures, and multifocal neurological signs.

The clinical syndromes of rabies and poliomyelitis are the most distinctive of viral infections because of the selective infection of specific populations of neurons. Polioviruses selectively infect motor neurons which lead to flaccid paralysis. Rabies causes early infection of the limbic system neurons with a relative sparing of cortical neurons which leads to behavioral abnormalities. Rabies infections represent a diabolical adaptation of virus to animal host, causing the animal to remain alert but to lose timidity and develop aggressive behavior to transmit the virus. The advantage of this selectivity is evident considering that strains of rabies that cause the so-called 'dumb' or passive rabies are seldom transmitted in nature.

Slow Infections

Slow infections are characterized by long incubation periods of months to years followed by an afebrile progressive disease. The term was originally coined in veterinary medicine to describe several transmissible diseases in sheep. The prototype slow infections are scrapie, a chronic noninflammatory spongiform encephalopathy due to a transmissible agent in which no nucleic acid has been identified (a prion), and visna, a chronic inflammatory encephalomyelitis caused by a lentivirus. The first slow infection identified in man was 'kuru', a progressive ataxia of a tribal group in New Guinea where the agent, resembling the agent of scrapie, was apparently transmitted by ritual cannibalism. Creutzfeldt–Jakob disease, a subacute dementia with myoclonus, is a worldwide disease due to prion agents. In some cases, transmission is genetic, in others, transmission is by transplants or administration of contaminated growth hormone, but in the vast majority the means of transmission is unknown. In both of these human spongiform encephalopathies, the clinical disease progresses irrevocably to death in about 6 months, but without fever or other clinical or histological findings to suggest infection.

Dementia, a chronic deterioration of cognitive function, can also be caused by several conventional viruses. Subacute sclerosing panencephalitis is a chronic dementing illness caused by measles virus. One per million otherwise healthy children develop this chronic illness at an average of 7 or 8 years after uncomplicated measles. Dementia evolves slowly, associated with myoclonic movements and massive levels of measles antibody in serum and CSF. Children usually die a year or less after infection, but survival may vary from 6 weeks to 6 years. The disease is due to a subacute, slowly progressive diffuse infection of neurons and glial cells. Another viral cause of dementia, HIV, has become a common problem as patients are surviving longer while on highly active antiretroviral therapy.

Clinical symptoms associated with HIV are very diverse (**Figure 2**). This virus now represents the commonest viral infection of the nervous system. From prospective studies of CSF changes, it appears that the majority of people infected with the virus have early invasion of the nervous system, that is, the virus is highly neuroinvasive. However, early during this infection

neurological disease is rare. A presumed autoimmune disorder occasionally causes a demyelinating peripheral neuropathy (Guillian–Barre syndrome); acute meningitis is occasionally seen at the time of seroconversion or during early asymptomatic infection. An asymptomatic pleocytosis is often found. Therefore, the virus is, at this stage, not highly neurovirulent. However, after the onset of immunodeficiency the infection is neurovirulent; at least 50% of the patients develop progressive dementia with cerebral involvement, myelopathies, or painful sensory neuropathies. The pathogenesis of these complications is unknown, but they are thought to be due to some viral protein or lymphokine incited by the virus because of the relative paucity of the virus in the nervous system and its localization to the microglial and macrophage populations.

Tropical spastic paraparesis complicating human T-cell leukemia virus type 1 infections is a recently recognized slow nervous system infection. Less than 1% of the patients infected with this virus develop either acute T-cell leukemia or neurological disease. Since many of those infected are infected by breast milk and the onset of tropical spastic paraparesis is usually in the fourth or fifth decade of life, the incubation period is extraordinarily long. A subacute disease develops with progressive paralysis of the lower extremities associated with impotence, incontinence, and sensory symptoms, but usually minimal sensory findings. Disease progresses slowly until the patients are wheelchair bound, but the disorder remains primarily at the level of the thoracic spinal cord. The involvement of the upper extremities is minimal with hyperreflexia but usually good function, and there is usually little, if any, indication of cerebral involvement. Early pathology found in a biopsy of the spinal cord in a single case showed vasculitis. In late cases, hyalinization of vessels with necrosis and demyelination of spinal cord is found, and findings are most prominent in the thoracic cord. Whether virus replicates in any cells other than T lymphocytes is unknown. These observations lead to the questions of why less than 1:100 who are infected develop disease; why the incubation period is as long as 40 years; why the disease localizes to the thoracic spinal cord; and why over years the disease becomes relatively quiescent despite the fact that there is ongoing high levels of intrathecal antibody synthesis, suggesting that there is still antigenic stimulation by virus within the nervous system.

Identification of viruses or virus-like agents (prions) in a variety of chronic neurological diseases has led to speculation of a viral etiology for multiple sclerosis, Parkinson's disease, amyotrophic lateral sclerosis, Alzheimer's disease, schizophrenia, and other illnesses. Experimental evidence for viruses in these chronic diseases is still tenuous.

See also: Herpesviruses: Latency; Measles Virus; Mumps Virus; Persistent and Latent Viral Infection; Rabies Virus; Viral Receptors; Visna-Maedi Viruses.

Further Reading

Johnson RT (1982) *Viral Infections of the Nervous System*. New York: Raven Press.

Cereal Viruses: Maize/Corn

P A Signoret, Montpellier SupAgro, Montpellier, France

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Maize is the main cereal crop in the world regarding total yield; it is grown on about 120 Mha. Maize has been grown for millennia in Central America. From a plant mainly used as human food, maize is now a main component for feeding animals but is still a major staple food crop in sub-Saharan Africa and America.

Viruses can cause important diseases of maize worldwide. While some viruses are widespread, others are localized. Some maize viruses can be sporadic and devastating causing severe yield losses, others may occur each year but losses are relatively minor. The average yield of maize in several African countries is only about a third of the world's average, and some viral diseases of maize are one of the major factors responsible for this low productivity. When available, resistant or tolerant hybrids provide the most effective means to control maize viruses. A pathogen-derived resistance strategy was developed for some viruses such as maize dwarf mosaic virus (MDMV) and maize streak virus (MSV). The major viruses affecting maize are transmitted by leaf- or planthoppers (16) but four viruses have aphids, two have mites, and one has beetles as vector. Populations of leafhopper and

planthopper vectors can be controlled by systemic insecticides applied as seed dressing or at sowing time. Herbicide eradication of Johnson grass, an overwintering host of some viruses, had a significant impact on disease control. In some cases, crop rotation and selection of planting date result in significant decreases in virus infection.

Maize Virus Diseases

Barley Yellow Dwarf (MAV-PAS-PAV), Cereal Yellow Dwarf (GPV-RPS-RPV), RMV-SGV

All the virus species of the family *Luteoviridae* in this subsection cause similar symptoms (see **Table 1** for a list of relevant virus names). Usually infected cultivars express discoloration of the leaves, reddening and yellowing, but some present no symptoms. More or less red or yellow bands are observed mainly on the borders of the older leaves. Compared to the healthy plants, possible grain yields are lower by 15–20%. Seeds coated with a systemic insecticide have to be used in areas where the disease is frequently encountered. A few maize inbred lines tolerant to barley yellow dwarf virus are available. Maize hybrids are in general tolerant.

Chloris Striate Mosaic Virus

This virus found in 1963 in Australia causes striate mosaic in *Chloris gayana* Kunth and other grass species and in cereals including barley, maize, and oats. It is transmitted by the leafhopper *Nesoclutha pallida* (Evans). Natural hosts are

Table 1 Taxonomic position of maize viruses

Virus names	*Genus*	*Family*
Barley yellow dwarf-MAV virus	*Luteovirus*	*Luteoviridae*
Barley yellow dwarf-PAS virus	*Luteovirus*	*Luteoviridae*
Barley yellow dwarf-PAV virus	*Luteovirus*	*Luteoviridae*
Barley yellow striate mosaic virus	*Cytorhabdovirus*	*Rhabdoviridae*
Brome mosaic virus[b]	*Bromovirus*	*Bromoviridae*
Cereal yellow dwarf-GPV virus	*Polerovirus*	*Luteoviridae*
Cereal yellow dwarf-RPS virus	*Polerovirus*	*Luteoviridae*
Cereal yellow dwarf-RPV virus	*Polerovirus*	*Luteoviridae*
Chloris striate mosaic virus	*Mastrevirus*	*Geminiviridae*
Corn lethal necrosis complex		
Corn stunt complex		
Cynodon chlorotic streak[a]		
High Plains virus[a]		
Indian peanut clump virus	*Pecluvirus*	
Johnson grass mosaic virus	*Potyvirus*	*Potyviridae*
Maize chlorotic dwarf virus	*Waikavirus*	*Sequiviridae*
Maize chlorotic mottle virus	*Machlomovirus*	*Tombusviridae*
Maize dwarf mosaic virus	*Potyvirus*	*Potyviridae*
Maize fine streak virus	*Nucleorhabdovirus*	*Rhabdoviridae*
Maize Indian Fiji-like virus[a]		
Maize Iranian mosaic virus[a]		
Maize mosaic virus	*Nucleorhabdovirus*	*Rhabdoviridae*
Maize mottle/chlorotic stunt virus[a]		
Maize necrotic streak virus[a]		
Maize rayado fino virus	*Marafivirus*	*Tymoviridae*
Maize rough dwarf virus	*Fijivirus*	*Reoviridae*
Maize sterile stunt virus[a]		
Maize streak virus	*Mastrevirus*	*Geminiviridae*
Maize stripe virus	*Tenuivirus*	*Tenuiviridae*
Mal de Rio Cuarto virus	*Fijivirus*	*Reoviridae*
Pennisetum mosaic virus[a]		
Rice black-streaked dwarf virus	*Fijivirus*	*Reoviridae*
RMV		*Luteoviridae*
SGV		*Luteoviridae*
Sugarcane mosaic virus	*Potyvirus*	*Potyviridae*
Wheat American striate mosaic virus	*Cytorhabdovirus*	*Rhabdoviridae*
Wheat streak mosaic virus	*Tritimovirus*	*Potyviridae*

[a]Unassigned virus.
[b]Not described, virus with low or no agronomical importance.

several grasses, and barley, oats, maize, and wheat. Infected plants develop chlorotic grayish white striation on leaves; notching and curling of leaves may also occur. Maize is occasionally infected but most hybrids are resistant.

Cynodon Chlorotic Streak

Cynodon chlorotic streak virus (CCSV) can cause a serious disease in the Mediterranean region. Symptoms on maize are characterized by the development on the youngest leaves of yellow streaks which become larger and can coalesce to form yellow stripes (**Figure 1(e)**). Finally the whole leaf turns yellow with some parts becoming reddish. If contamination occurs at a young stage, the plants are dwarfed and die early. The virus is transmitted by planthoppers and occurs naturally in Bermuda grass (*Cynodon dactylon* (L.) Pers.) and maize. The level of CCSV infection in maize is directly related to the level of CCSV infection and planthopper vector populations in Bermuda grass. As many as 70–84% virus-infected plants have been observed for some years in southern France. Seed dressing with imidachloprid allowed a good control of the vector population.

High Plains Virus

High Plains virus (HPV), a previously unknown pathogen, was found in 1993 to infect maize in US High Plains states. It has also been found in other countries. The virions are flexible, thread-like particles. Analysis of the nucleic acid shows four bands of double-stranded RNA (dsRNA) after electrophoresis. A great variety of symptoms may be expressed in maize, including spots or flecks along vascular bundles, purpling or reddening of the leaf margins or sectors of the leaf, and stunting. The virus is transmitted obligately by the wheat curl mite *Aceria tosichella* (Keifer) that also transmits wheat streak mosaic virus (WSMV). HPV can be seed transmitted. The host range includes barley, maize, and wheat in addition to several weed species. Three dominant genes for resistance have been identified and mapped in maize, and they provide a high degree of resistance.

Johnson Grass Mosaic Virus

Johnson grass mosaic virus (JGMV) was first reported in Australia in maize and sorghum and later in the US and South America. Johnson grass (*Sorghum halepense* (L.) Pers.), maize, and some other plants of the Poaceae are natural hosts. The virus causes mosaic, mottling, and ringspot symptoms on maize and grass hosts, which is particularly evident on young leaves. Since the symptoms of JGMV and MDMV are very similar, serology or polymerase chain reaction (PCR) may be necessary to distinguish between the two viruses. The type strain from Australia can infect oats, which is not susceptible to the other graminaceous potyviruses. The perennial host Johnson grass allows virus survival between seasons in rhizomes. Resistant or tolerant hybrids provide the best control option.

Maize Chlorotic Dwarf Virus

Confirmed reports of maize chlorotic dwarf virus (MCDV) infection are limited to the US, where the virus was discovered in 1969. The extent of distribution of MCDV in the US is second to that of MDMV. The virus is associated with the occurrence of indigenous Johnson grass (*S. halepense* (L.) Pers.), its overwintering host, and the black-faced leafhopper, *Graminella nigrifrons* (DeLong and Mohr), its principal vector. Symptoms on susceptible maize are shortening of the upper internodes or proportionate stunting of the internodes, chlorotic clearing or banding of the tertiary leaf veins, and red or yellow discoloration of upper leaves. Infected leaves are also more difficult to tear off a plant. On resistant maize, MCDV induces mainly chlorotic clearing or banding of tertiary veins without marked stunting or discoloration. The diagnostic symptom of MCDV infection is vein banding. MCDV's host range is limited to species of the Poaceae. MCDV may complex with MDMV in infections of maize and Johnson grass in the same field or plant and with sugarcane mosaic virus strain MDB in maize. Biological tests for MCDV include transmission by the leafhopper in a semipersistent manner and vascular-puncture inoculation (VPI) to susceptible plants with expression of the diagnostic symptoms of vein clearing or banding of the tertiary leaf veins. Confirmation by serological methods is often necessary. No molecular tests for MCDV have been developed.

Agronomical control of MCDV is possible by early planting of maize to escape peak vector populations and by herbicide eradication of overwintering Johnson grass. However, the latter treatment may result in increased MCDV incidence in susceptible hybrids due to mass migration of viruliferous leafhoppers from dying Johnson grass. Maize inbreds with high levels of tolerance to MCDV have been developed. Virus resistance appears inherited mostly as a dominant to partially dominant trait. Resistant Caribbean germplasm has been identified and the inbred line Oh1VI highly resistant to MCDV has been developed. Commercial hybrids with various degrees of resistance or tolerance and with agronomically satisfactory characteristics are available. The systemic insecticide carbofuran which remains toxic to the leafhopper for up to 55 days after application at planting time is very effective and can control the disease. As a result the economic impact of the virus has decreased and it no longer poses a significant threat to US maize production.



Figure 1 (a) Long continuous stripes along the veins of sweet corn caused by maize mosaic virus. (b) Severe dwarfing, stripping of upper leaves and 'leek aspect' of maize rough dwarf virus-infected maize plant. (c) Symptoms of maize streak virus on maize. (d) Maize plants infected with Mal de Rio Cuarto virus showing severe dwarfing and leaves distorted and ragged. (e) Yellow stripe and dwarfing on maize infected by cynodon chlorotic streak virus.

Maize Chlorotic Mottle Virus

Maize chlorotic mottle virus (MCMV) was first reported from Peru in 1973, and was later found in central USA, Hawaii, Mexico, and Argentina. Symptoms vary from a mild chlorotic mottling to severe yellowing, necrosis, and plant death depending on the maize variety and plant age when infected. Plants may be stunted with short, malformed, partially filled ears, and male inflorescences may be shortened with sparse spikes. Under natural infection, 10–15% yield reductions can be observed. Maize is the only natural host of MCMV, and the experimental host range is limited to Poaceae. Laboratory transmission of MCMV can be obtained with six species of chrysomelid beetles, but only *Diabrotica* spp., which have a New World distribution and are mostly tropical, are natural vectors. In areas of South America where maize is grown continuously, MCMV spreads from older plants to younger plants via adult and larval beetles. Control would require a halt in continuous cultivation or management of the beetles. Most maize cultivars are susceptible to MCMV but a new variety (N211) is resistant. Transgenic maize shows milder symptoms from MCMV infection. MCMV is sometimes found as part of the corn lethal necrosis complex.

Maize Dwarf Mosaic Virus

MDMV was first reported on corn in 1965 from Ohio, USA. It is considered to be distributed worldwide. Symptoms of MDMV in corn vary with the stage of host development at time of infection, genotype, and virus strain. Initial leaf symptoms are chlorotic spots and short streaks followed by typical mosaic or mottle. When the plants develop, flecks, streaks, and rings appear on new leaves. Ear formation and development are arrested, resulting in losses in grain yield. MDMV is widely adapted to species of the Poaceae infecting over 66% of 293 species tested. Maize and sorghum are important agricultural hosts. More than 15 species of aphids transmit this virus in a nonpersistent manner. MDMV can be mechanically transmitted; it survives by overwintering in the rhizomes of infected Johnson grass. Crop loss due to MDMV ranges from 9% to 72% for susceptible dent corn hybrids. Losses are generally important in seed production or sweet corn hybrid fields.

Many commercial corn MDMV-resistant hybrids are available. A major gene for resistance to MDMV is a dominant gene (*mdm1*) located on chromosome 6. The control of Johnson grass helps to control disease.

Maize Fine Streak Virus

Maize fine streak virus (MFSV) was isolated in 1999, from sweet corn grown in Georgia, USA. Symptoms in the field include fine chlorotic streaks along the major leaf veins, similar to symptoms associated with maize rayado virus (**Figure 1(c)**). Maize is so far the only identified natural host of MFSV. The virus is transmitted by the leafhopper *Graminella nigrifons* (DeLong & Mohr) in a persistent manner. MFSV is readily transmitted mechanically by kernel vascular-puncture inoculation. Maize lines resistant to maize mosaic virus (MMV: Hi31 and Hi34) are also resistant to MFSV.

Maize Indian Fiji-Like Virus

Symptoms include severe plant dwarfing, dark green leaves, enations on the lower leaf surface, and small malformed ears. All of these symptoms are similar to those produced by Mal de Rio Cuarto virus (MRCV) and maize rough dwarf virus (MRDV) on maize. This unreported reovirus was found in southern India, where its incidence ranged from 4% to 61%.

Maize Iranian Mosaic Virus

First observed in the Fars Province of Iran, it is now distributed in other parts of the country. Maize Iranian mosaic virus (MIMV) symptoms consist of long chlorotic lines and stripes along the veins and sheaths, stunting of the plants, and abortion of ears when infected early. MIMV is serologically distinct from others rhabdoviruses infecting Poaceae plants. In polyacrylamide gel electrophoresis, six proteins corresponding to proteins L, G, N, NS, M, and M2 were detected. MIMV is a new virus in the genus *Nucleorhabdovirus* that may be distantly related to MMV. Delphacids are vectors of MIMV. The natural host range includes maize, oat, rice, and wheat. The disease incidence in maize fields can be as high as 80% depending on sowing date and variety. Late planting effectively controls the virus.

Maize Mosaic Virus

MMV causes a serious disease of maize in tropical and subtropical areas of Africa, the Americas, and Australia. It was first reported in 1914 in Hawaii. MMV can occur in mixed infections with maize stripe virus (MSpV). Initial leaf symptoms on sweet corn inoculated with MMV are long stripes, colored light green to yellow, along the midrib (**Figure 1(a)**). These stripes elongate to form distinct, chlorotic stripes between and along the veins extending on the whole leaf. Stripes can appear on sheath and husk. Stunting of plants is common. Mosaic patterns do not occur despite the disease name. Only species of the Poaceae are known to be hosts of MMV. It occurs naturally in itchgrass, maize, and *Setaria vulpiseta* (Lam.) Roem. & Schult. MMV is transmitted in a persistent-propagative manner by the corn planthopper *Peregrinus maidis* (Ashmead), the sole vector. Transmission is by both nymphs and adult males and females. MMV has been shown to be inoculated by vascular puncture of maize kernels (VPI). Several contact and systemic insecticides can control *P. maidis*. Host-plant resistance is likely to be the most effective means of control. Tropically adapted parental inbred lines of maize resistant to MMV have been developed and sweet corn cultivars have been bred and released, both by the University of Hawaii. From Caribbean and Mascarene germ plasm, high resistance was found in line Hi40 and resistance to some levels was found in lines 37–2, A211, and Mp705.

Maize Mottle Chlorotic Stunt Virus

This virus was first reported, in 1937, in East Africa and called maize mottle virus (MMotV). Maize mottle chlorotic stunt virus (MMCSV) is restricted to tropical African countries and the adjacent islands. The virions are spherical, *c.* 40 nm in diameter. MMCSV can be found in mixed infections with maize mosaic and maize streak viruses. Symptoms on maize appear as chlorotic mottling with mild to severe chlorosis of tertiary veins and stunting. Young mottled leaves fail to support themselves in a normal upright position. Tassel abortion can occur in severe cases. Maize is the only known natural host of the virus which is transmitted by several *Cicadulina* spp. in a

persistent manner. Disease control can be achieved by vector control or by growing resistant cultivars, which are now available. Most maize streak virus-resistant cultivars have also moderate to high levels of resistance to MMCSV.

Maize Necrotic Streak Virus

Maize necrotic streak virus (MNeSV) was discovered in 2000 in maize samples from the US state of Arizona. The virus is of little agronomic importance. Virions are isometric, *c.* 32 nm in diameter. MNeSV appears to be a member of the family *Tombusviridae*. Distinctive symptoms of MNeSV infection are initially chlorotic spots and streaks that become spindle shaped. They later coalesce into long chlorotic bands that become translucent and necrotic around the edges. Plant stalks show a chlorosis that becomes necrotic, a symptom especially distinctive for MNeSV. There is no known aerial vector – the virus is transmitted through the soil. Maize lines Oh 1VI OSU23i and Mo17 are highly resistant to MNeSV.

Maize Rayado Fino Virus

Maize rayado fino virus (MRFV) was first described in 1969 in Costa Rica and El Salvador. MRFV is widespread and becoming increasingly important in tropical areas of the Americas. It is the type member of the genus *Marafivirus*, and is the only known indigenous virus of maize in Mesoamerica. Initially, infection causes fine chlorotic dots or stipples, which coalesce into chlorotic stripes at the base and along the veins of young leaves. As the plant grows older, the symptoms become less conspicuous and may disappear when the plant reaches maturity. In locally adapted Central American varieties, the virus incidence varies from 0% to 20%, but may reach 100% in more susceptible foreign or newly developed cultivars. Maize, teosinte, and the perennial *Zea* spp. are the only known natural hosts. In nature, MRFV is transmitted exclusively by the corn leafhopper vector *Dalbulus maidis* (DeLong and Wolcott) in a persistent manner. The natural host range of MRFV may be limited by the ability of the vector to feed on different species. It has been proposed that MRFV, its insect vector, and the maize host co-evolved in a triad in which the parasitic members (insect and virus) displayed highly specialized interactions. It has been suggested that MRFV originated in Mexico or Guatemala, from where it spread in the region.

MRFV is one of the three major components of the corn stunt disease complex. MRFV is endemic in many areas of Central and South America, where the virus and *D. maidis* reach epidemic levels. The use of systemic insecticides does not reduce virus incidence. Crop rotation, mixed plantings, and selection of planting dates result in significant decreases in virus infection. The Guatemalteco variety introduced in Ecuador in 1962 is used as a source of virus tolerance. Production of transgenic plants resistant to MRFV and their evaluation for their susceptibility are in progress in Costa Rica.

Maize Rough Dwarf Virus

This virus was discovered in Europe when corn hybrids were introduced from the US after World War II. In 1949, in Italy, a severe outbreak threatened the maize cultivation, lowering the yield by ~40%. Later, MRDV was reported in several European countries, where it had the potential to be economically damaging. In China, a similar disease was found to be caused by rice black-streaked dwarf virus (RBSDV), not by MRDV. In young field-grown corn, symptoms caused by MRDV are dark green color of leaves, stunting and irregular swellings of veins (enations) along the lower surfaces of leaves and sometimes also of leaf sheaths, ligules, and husks. The enations are rough to the touch, hence the disease name. Plants are stunted with increased girth giving the plant a 'leek' aspect (**Figure 1(b)**). Short chlorotic streaks develop on mature leaves and coalesce into yellowish green stripes parallel to the veins. Later the leaves can turn reddish. Tassels are sterile. The root system is reduced, roots develop swellings, resulting in their frequent splitting. MRDV has a fairly wide experimental host range including only a few species of Poaceae, among which maize is the only one of economic importance. Oats, wheat, and several grasses can be infected naturally. The planthopper *Laodelphax striatellus* (Fallén) is the natural vector and the only known winter host of MRDV. However, in northern Italy, oats were reported as the first overwintering plant. Planthoppers can be controlled either by spraying grasses around the fields with an insecticide in the spring, by seed treatment with imidachlopride, or by row application of an insecticide at sowing time (e.g., carbofuran). Some maize hybrids are less susceptible to MRDV, but the resistance can be overcome if the vector population is very high.

Maize Sterile Stunt Virus

Maize sterile stunt virus (MSSV) was first reported from Australia in 1977. It is a strain of barley yellow striate mosaic virus (BYSMV). The virus causes severe stunting, sterility, purple coloration, and top necrosis in a small number of maize genotypes. MSSV is transmitted in a persistent manner by the delphacid planthopper *Sogatella longifurcifera* Esaki and Ishihara and inefficiently by *Sogatella kolophon* (Kirk) and *P. maidis* (Ashm.). Natural hosts of MSSV are barley, maize, triticale, and wheat.

Maize Streak Virus

Maize streak was first recorded in 1901 in South Africa and is the most widespread and important disease of maize in sub-Saharan Africa and adjacent islands. Maize streak symptoms are characterized by the development of chlorotic spots and streaks in longitudinal lines on leaves. There is a progressive increase in the number and length of streaks that occur on new leaf tissue. The streaks often fuse laterally, resulting in narrow broken chlorotic stripes, which can in some cases cover the entire leaf. In very susceptible maize cultivars, severe chlorosis occurs leading to stunted plant and premature death. MSV is transmitted by several species of leafhoppers in the genus *Cicadulin*a, with *C. mbila* (Naudé) being the most common vector. It transmits MSV in a circulative nonpropagative manner. Streak symptoms have been described in numerous species of wild grasses. Late plantings are generally more severely infected than early ones. The major source of MSV infection on maize is a previously infected maize field. Therefore, planting of maize close to a previous maize field must be avoided. Carbamate insecticides (such as carbofuran) can effectively prevent MSV transmission but prices of chemicals and equipment for spraying are often prohibitive for smallholder farmers. The development of insect resistant plants is under study.

Maize Stripe Virus

MSpV has often been confused with MMV because of their similarity in vector specificity, mode of transmission, symptomatology, and host range. It often occurs in mixed infection with MMV. The virus is commonly found in most parts of the subtropics and the tropics where the vector occurs. MSpV is found to infect maize, sorghum, and itchgrass (*Rottboellia exaltata* L.). It can be transmitted experimentally to barley, oats, rye, and triticale. Initial symptoms on sweet corn are fine chlorotic stipplings and narrow stripes similar to that of MMV. Later, continuous chlorotic stripes develop with varying width and intensity which show a 'brushed out' appearance, and apical bending occurs. Infected plants are often stunted and die prematurely. The virus is transmitted by *P. maidis* (Ashm.), also a vector of MMV, in a persistent-propagative manner. Both nymphs and adults transmit the virus. Control of the maize stripe disease is largely dependent on chemical control of *P. maidis*.

Mal de Rio Cuarto Virus

The Mal de Rio Cuarto was first reported in maize fields of the Rio Cuarto County in Argentina, at the end of the 1960s. MRCV is a major constraint to maize production in Argentina. It spread in the endemic area of the province of Cordoba, but also appeared in the central region. MRCV initially reported as a strain of MRDV but is now considered to be a separate species within the genus *Fijivirus*. In field-infected maize, the symptoms show up within 4–5 weeks. Plants are dwarfed, showing fine chlorotic flecks on secondary and tertiary veins. Stems are usually flattened with shortened internodes and leaves from the upper third may be stiff, distorted, and ragged (**Figure 1(d)**). The root system is fragile, and greatly reduced. The ears are reduced and malformed. Natural dispersion of the virus occurs by the planthopper *Delphacodes kuscheli* Fennah in a propagative manner. Apart from maize, MRCV can infect several monocots, winter small grains (barley, oats, rye, and wheat), spring–summer grains (millet and sorghum), and several spring–summer annual and perennial weeds. The initial inoculum source is its vector that feeds on winter cereals like oat and wheat, where it acquires the virus and migrates to maize when these cereals become senescent. Early sowing of maize helps to prevent high virus infection. Systemic insecticides (carbofuran, imidachlopride) applied as seed coating protect maize seedlings from the vector. Some commercial hybrids with high tolerance levels are now available.

Pennisetum Mosaic Virus

A potyvirus was isolated from whitegrass (*Pennisetum flaccidum* Griseb) in North China, in the early 1980s and was later named pennisetum mosaic virus (PenMV). This virus infects maize and sorghum naturally. Symptoms on maize are not specific when compared to other members of the sugarcane mosaic virus (SCMV) subgroup in the genus *Potyvirus*. The virus is less important, in terms of yield loss, than the other prevalent viruses of the SCMV subgroup.

Rice Black-Streaked Dwarf Virus

The causal agent of characteristic symptoms on maize (stunting and rough white line veins of leaves) was initially identified as MRDV in China in the late 1970s. Subsequently, molecular studies demonstrated that rice black-streaked virus was the cause of the disease. These two viruses are quite similar in host range, serology, morphology, and the genome sequence. Reverse transcription-polymerase chain reaction can discriminate between RBSDV and MRDV. Host range is limited to species in the Poaceae: oats, hordeum, oryza, triticum, and zea. This virus is transmitted by the leafhopper *L. striatellus* in a persistent manner. RBSDV is managed mainly by control of leafhoppers.

Sugarcane Mosaic Virus

SCMV was first detected in sugarcane in 1919 and in maize in 1963, both in the US. SCMV occurs throughout the world. Infected maize plants develop distinct mosaic symptoms which are especially clear on the lower part of

the younger leaves. Very susceptible cultivars react with a strong chlorosis, sometimes together with a red striped pattern. It is often impossible to distinguish SCMV and MDMV infections on the basis of symptoms. In Southern Europe, MDMV is the most prevalent virus. Maize inbred lines resistant to the virus have been described and transgenic maize expressing the coat protein gene of SCMV is also available.

Wheat Streak Mosaic Virus

The symptoms consist of linear arrays of small, yellow-ringed eyespots. On some germplasm, the spots and streaks later form mosaics, or mottle patterns. WSMV is mite transmitted. Three independent genes controlling resistance to WSMV have been identified in maize. Resistance in certain maize lines is not effective against all strains of WSMV. Late planting of corn adjacent to maturing wheat should be avoided.

Maize Diseases Caused by Complex of Pathogens

Corn Lethal Necrosis Complex

This disease is caused by a synergistic interaction between MCMV and certain members of the family *Potyviridae* (MDMV-A, SCMV-MDB, JGMV, and WSMV). Maize plants infected at early stages develop leaf chlorosis followed by necrosis. Plants are stunted, produce small deformed ears, and die prematurely. Only terminal leaves show symptoms with a late infection. Crop losses of up to 90% have been reported.

Corn Stunt Complex

Corn stunt disease complex, also known by the names achaparramiento, maize stunt, and red stunt, is caused by two or more of the following agents: MRFV, corn stunt spiroplasma (CSS), and maize bushy stunt phytoplasma (MBSP). The corn stunt complex pathogens appear to be restricted to the Americas. Early symptoms of CSS consist of yellowish streaks in the youngest leaves. Later, much of the leaf turns purple. Infected plants are stunted and will often have more ears, but the ears are smaller. Maize plants infected with MBSP show stunted growth and reduced grain production, ear proliferation, and greater tillering. The leafhopper vector *D. maidis* (DeLong & Wolcott) can simultaneously transmit CSS, MBS, and MRFV. Although *D. maidis* is the primary vector, other leafhoppers transmit one or more of the corn stunt complex pathogens under experimental conditions.

See also: Cereal Viruses: Rice; Cereal Viruses: Wheat and Barley; Maize Streak Virus.

Further Reading

Lapierre H and Signoret PA (2004) *Viruses and Virus Diseases of Poaceae Gramineae,* 857pp. Versailles, France: INRA.

Lübberstedt T, Ingvardsen C, Melchinger AE, *et al.* (2006) Two chromosome segments confer multiple potyvirus resistance in maize. *Plant Breeding* 125: 352–356.

Redinbaugh MG, Jones MW, and Gingery RE (2004) The genetics of virus resistance in maize (*Zea mays* L.). *Maydica* 49: 183–190.

Cereal Viruses: Rice

F J Morales, International Center for Tropical Agriculture, Cali, Colombia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ambisense A viral nucleic acid that can be 'read' in both directions to be translated into viral encoded proteins.
Capsid Protein envelope of the viral genome (nucleic acid).
Circulative A virus that passes through the intestine of an insect vector into the hemolymph, and eventually reaches the salivary glands of the insect to be injected back into a plant.
Etiology Studies the cause of disease.
Inclusion Virus particles or viral components found in infected plant cells.
Incubation Period of time that a virus needs inside its vector before it can be transmitted to a susceptible host.
ORF Any sequence of DNA or RNA that can be translated into a protein.
Persistent A virus that is not lost by a vector after feeding in a series of healthy plants, without having access to a virus-infected plant (virus source).

Pinwheel A cytoplasmic protein inclusion induced by potyviruses.
Propagative A virus that multiplies in its vector.
Semi-persistent Virus is lost after a vector feeds on a series of virus-free plants.
Single-stranded Viruses consisting of only one strand of viral RNA or DNA.
Transovarial transmission Of a virus from a female insect vector to her progeny.
Transtadial A virus that is retained by an insect vector after each developmental stage until it reaches adulthood.
Virion A mature virus particle (capsid protein, nucleic acid, and any other constituents).
Viroplasm Amorphous aggregation of viral components.

Introduction

Rice (*Oryza sativa* L.) is the second most extensive food crop grown in the world (*c.* 154 million ha) after wheat, and is the main food crop grown in the tropics. Half of the world's population (over 3 billion people) depends on rice, particularly in Asia, where annual per capita rice consumption may be as high as 170 kg in countries such as Vietnam, as compared to 9 kg in the United States. Asia produces almost 90% of all the rice cultivated in the world (136 million ha), mainly China and India, where rice cultivation and domestication seemed to have started over 5000 years ago. Africa cultivates approximately 9 million ha, and Latin America 6.8 million ha of rice. Nigeria and Brazil are the main producers of rice in Africa and Latin America, respectively, with average cultivated areas of 3.8 million ha, each.

Given the history and extensive cultivation of rice around the world, including Europe (565 000 ha) and the United States (1 355 000 ha), it is not surprising to note that this crop has several pest and disease problems of significant socioeconomic importance. Among the various biotic constraints of rice production, viruses constitute an important group of plant pathogens, particularly in Asia. Viral diseases are also considered major constraints to rice production in Africa and Latin America, even though only two viruses are considered pathogens of economic importance outside Asia.

Rice is also affected in the tropics by numerous insect pests, particularly by several species of leafhoppers and planthoppers (Homoptera:Auchenorrhyncha) that cause direct feeding damage and, more important, transmit economically important plant viruses. This article describes the main viral diseases of rice in the world; their causal viruses and vectors; and the different disease management strategies implemented to date.

Rice Dwarf

Rice dwarf was first described in Japan, in 1883. A Japanese rice grower was the first person to demonstrate in 1894, the relation between this disease and the presence of leafhoppers in affected rice fields. Rice dwarf is also known to occur in Korea, China, Nepal, and the Philippines. In 1885, K. Takata reported that the leafhopper *Recilia dorsalis* was 'responsible' for the disease. In 1900, the leafhopper *Nephotettix cincticeps* was thought to be the cause of rice dwarf. In 1899, *N. cincticeps* individuals captured in a disease-free area could not induce disease in healthy rice plants, unless these individuals had been previously allowed to feed on rice dwarf-affected rice plants. Despite this evidence suggesting the existence of an unknown causal agent, it was not until 1960 that the first electron micrographs of an isometric virus *c.* 70 nm in diameter were obtained. The suspected virus was shown in 1966–70, to contain double-stranded (ds) RNA, divided in 12 different segments (4.354–0.828 kb) with a total genome size of 25.13 kb. The causal virus was classified in 1972 as *Rice dwarf virus*, a species of the genus *Phytoreovirus,* in the family *Reoviridae.* The virus replicates in the cytoplasm of infected cells, and the dsRNA genome is encapsidated in super-coiled form.

Rice dwarf virus (RDV) is transmitted by the leafhoppers *N. cincticeps* (main vector in Japan, Korea and China), *Nephotettix nigropictus* (formerly *N. apicalis*; the main vector in Nepal and the Philippines), *Nephotettix virescens,* and *R. dorsalis* (formerly *Inazuma dorsalis*). Few individuals of these species are active or potential vectors, and they acquire the virus by feeding for up to a day on infected plants. The incubation period of the virus in a potential vector ranges from 12 to 25 days ('propagative' virus), and the leafhopper vector remains viruliferous for life ('persistent' mode of transmission). Viruliferous individuals require from a few minutes to half an hour to transmit the virus to a healthy plant, and there is transovarial transmission of RDV to the progeny of viruliferous female vectors. Some nymphs infected through the egg die prematurely. The average lifespan of viruliferous *N. cincticeps* females is 12.1 days, and of virus-free females is 16.6 days. RDV is not mechanically transmissible and it is not transmitted by sexual seed or pollen.

RDV induces stunting and chlorotic specks in infected rice plants. RDV's host range is restricted to the Gramineae, including the genera *Alopecurus, Avena, Echinochloa, Oryza, Panicum, Paspalum, Poa,* and some cultivated species, namely, barley, millet, oats, rye, and wheat. The disease usually appears in the field after transplanting, suggesting an early infection in the seedbed stage. Early infection generally results in severe stunting, shortening of internodes, and small, rosette-like tillers. The root system of diseased plants does not develop well either, and infected plants usually do not produce panicles or seed. Diseased plants remain green

in contrast to virus-free plants, which naturally turn yellow when mature. Virions are found in phloem, cytoplasm, and in cell vacuoles. Viroplasms can be observed in infected cells. Genetic resistance to RDV has been observed in some rice varieties, but current efforts are directed toward the development of genetically modified (transgenic) rice plants possessing resistance to the virus. Complementary cultural practices and other integrated disease management strategies are also recommended.

Rice Black Streaked Dwarf

Rice black streaked dwarf was first reported to affect rice in Japan in the early 1950s, and it also spreads in China and Korea. This disease was shown to be different from rice dwarf, and to be associated with the planthopper *Laodelphax striatellus*. The causal agent was isolated in 1969 and further characterized in 1974 as a spherical virion *c.* 60 nm in diameter, belonging to the reovirus group. Rice black streaked dwarf virus (RBSDV) is currently classified as a member of a species of the genus *Fijivirus*, family *Reoviridae*. It has 10 dsRNA segments coding for the RNA polymerase, core protein, nonstructural components, spikes, major outer shell, and other proteins of unknown function. Three major proteins (130, 120, and 56 kDa) and three minor proteins (148, 65, and 51 kDa) have been detected in RBSDV virions. The 56 kDa protein is the main component of the outer capsid shell. Most of the genome segments are monocistronic, and replication occurs in the cytoplasm of phloem cells. Inclusion bodies of unknown nature are observed in malformed tissue. Some degree of genomic variability among RBSDV isolates has been observed.

RBSDV is transmitted by the delphacid vector *L. striatellus,* and occassionally by *Unkanodes sapporona* and *Unkanodes albifascia*, in a persistent, circulative, and propagative manner. The proportion of active transmitters of *L. striatellus* is about 30%. Shortest acquisition feeding period was 30 min; and incubation periods ranging from 4 to 35 days in the vector *L. striatellus* have been reported. The virus is not transmitted congenitally. *Laodelphax striatellus* breeds on rice, wheat, and barley, but not on maize; whereas *U. sapporona* favors maize, wheat, and barley, but does not breed on rice. Besides rice, RBSDV infects oats, barley, wheat, maize, rye, and species of *Agrostis, Alopecurus, Digitaria, Echinochloa, Eragrostis, Glyceria, Lolium, Panicum,* and *Poa* in the Gramineae. Perpetuation of the virus between seasons occurs through overwintering planthoppers. Main symptoms associated with this disease are: stunting, darkening of leaves, leaf malformation, and waxy swellings along veins of the abaxial leaf surface, which later form dark tumors. Infected plants usually produce excessive tillers.

Resistance to RBSDV has been observed under field conditions. There is at least one dominant gene involved in the resistance to RBSDV, but some modifying genes may also be involved in the outcome of the interaction between the plant genotype and the amount of virus inoculated. Some wild rice species have exhibited moderate resistance to the virus. Cultural practices aimed at reducing vector populations, such as sowing dates that do not coincide with peak populations of the vector, are recommended.

Rice Gall Dwarf

Rice gall dwarf was first observed in Thailand, in the early 1980s. This disease is also present in China, Malaysia, and Thailand. The causal virus belongs to a species in the genus *Phytoreovirus,* family *Reoviridae*. Virions are icosahedral (60–70 nm) and possess 12 dsRNA genome segments and up to seven structural proteins (45–160 kDa). Rice gall dwarf virus (RGDV) is confined to phloem tissues of the plant host. Largest genome segment has 4.4 kb, and the smallest, 0.6 kb. Virions are found in the phloem, cytoplasm, and cell vacuoles in low concentrations, together with viroplasms containing immature virions.

RGDV is transmitted by the leafhoppers *N. nigropictus, R. dorsalis, N. cincticeps, N. malayanus,* and *N. virescens,* in a persistent, circulative, and propagative manner. RGDV is transmitted transovarially, is acquired rapidly from infected rice plants, and has an incubation period of 10–20 days in the insect vector. Main symptoms in rice are stunting, translucent gall formation on the underside of the leaves and on leaf sheaths (enations), and permanent dark green leaves. Infected plants produce few tillers, and form poorly developed panicles. Other grasses susceptible to this virus include oats, barley, rye, wheat, Italian grass (*Lolium* sp.), and *Oryza rufipogon*. Japanese grass (*Alopecurus aequalis*) is an important reservoir of RGDV; and maize also seems to be a host of RGDV in China.

Cultural practices aimed at breaking the cycle of the leafhopper vectors that move from spring to autumn plantings; and the elimination of ratoon plantings from over-wintered rice are recommended.

Rice Ragged Stunt

Rice ragged stunt was first reported from the Philippines and Indonesia in 1976. It is currently distributed in Bangladesh, China, India, Japan, Malaysia, Sri Lanka, Taiwan, and Thailand. The causal virus was isolated in the early 1980s, and shown to belong to a species of the genus *Oryzavirus,* family *Reoviridae*. Rice ragged stunt virus (RRSV) has icosahedral, double-shelled particles (75–80 nm) with conspicuous spikes. Oryzaviruses have a genome of 10 linear dsRNA segments with a total size of

26.6 kb. RRSV particles are composed of five major structural proteins (33–120 kDa) and three nonstructural proteins (31, 63, and 88 kDa). The dsRNA segments contain a single ORF each, except S4, which contains two ORFs. RRSV is serologically related to Echinochloa ragged stunt virus.

RRSV is transmitted by the delphacid planthoppers *Nilaparvata lugens* (brown planthopper) and *N. bakeri*, in a persistent, circulative, and propagative manner. RRSV is not transovarially transmitted. RRSV infects rice and other *Oryza* species, causing stunting, enations, ragged (serrated) leaves, and suppression of reproductive structures. Infected rice plants remain green when healthy plants have senesced. RRSV also infects maize and wheat. Virions are found in phloem enations; and infected cells show the presence of viroplasms and cell wall proliferation.

RRSV persists in rice and in the brown planthopper, and, consequently, continuous rice planting perpetuates the virus and its vectors. Planthoppers migrate from the tropics to temperate areas in the summer time. Resistance to RRSV has been identified in some breeding lines; but molecular breeding techniques have been preferred to genetically modify rice genotypes for resistance to the virus.

Rice Tungro

Tungro means 'degenerate growth' in Ilocano, a Philippine language. Tungro-like symptoms were first observed in the Philippines, Indonesia, Malaysia, and Thailand in the early 1960s, under different names, such as 'mentek', 'penyakit habang', 'penyakit mera', and 'yellow orange leaf'. Severe outbreaks of tungro disease occurred in the late 1960s, following the introduction of early maturing, fertilizer-responsive, and high-yielding rice varieties. The disease has been reported from other Asian countries, namely, India, Bangladesh, and China. Tungro was first associated with the presence of leafhoppers in affected rice fields, but one of the causal agents could only be visualized in 1967, as isometric particles, 30–35 nm in diameter. Between 1967 and 1970, at least four different strains of the tungro 'virus' were identified, and this number increased to eight strains by 1976. In 1978, Indonesian scientists reported the observation of both isometric (30 nm) and bacilliform (35×350 nm) particles in rice plants affected by the 'penyakit habang' disease (tungro) in Indonesia. Severe symptoms were associated with both particles, whereas rice plants infected only by the bacilliform virus showed moderate symptoms. Plants infected only by the isometric virus remained symptomless. The isometric particles were transmitted by the leafhopper *N. virescens*, but the bacilliform virus could not be transmitted in the absence of the isometric virus. This constituted the first demonstration that tungro is a synergistic disease, and that the isometric virus acts as an 'assistor' or 'helper' virus for the bacilliform virus that causes main symptoms associated with tungro disease. In 1979, *N. nigropictus* was also shown to be an occasional vector of the isometric virus, but not of the bacilliform virus. The shortest acquisition feeding for *Nephotettix impicticeps* is 5–30 min, and there is no incubation period. Therefore, the virus does not persist in the vector; and there is no trans-stadial or transovarial passage of the virus in the insect vector.

Rice tungro disease is thus caused by two different viruses: rice tungro spherical virus (RTSV), a positive-sense RNA virus, and rice tungro bacilliform virus (RTBV), a circular dsDNA virus. The nucleotide sequence of rice tungro bacilliform badnavirus (RTBV) DNA was determined in 1991. The circular genome has 8.3 kb and one strand contains four open reading frames (ORFs). One ORF encodes a protein of 24 kDa (P24). The other three ORFs potentially encode proteins of 12, 194, and 46 kDa. Comparative analyses with retroviruses suggested that the 194 kDa polyprotein is proteolytically cleaved to yield the virion coat protein, a protease, and replicase (reverse transcriptase and RNase H), characteristic of retro-elements. The DNA sequence of RTBV suggests that RTBV is a pararetrovirus. RTBV is currently classified as a member of a species of the genus *Badnavirus*, family *Caulimoviridae*.

The 12 433 nucleotide (nt) sequence of RTSV contains a large ORF, capable of encoding a viral polyprotein of 390.3 kDa. Two viral subgenomic RNAs of *c.* 1.2 and 1.4 kb, respectively, were detected in RTSV-infected leaf tissues. There are at least three capsid protein subunit cistrons near the N-terminus of the large ORF. The C-terminal half of the large ORF revealed conserved protein sequence motifs for a viral RNA polymerase, proteinase, and a putative nucleoside triphosphate (NTP)-binding protein. The sequence motifs are arranged in a manner that resembles those of picorna-like viruses. RTSV is the type species of the genus *Waikavirus*, family *Sequiviridae*.

Rice tungro bacilliform virus isolates from Bangladesh, India, Indonesia, Malaysia, and Thailand were compared with the type isolate from the Philippines. Restriction endonuclease maps revealed differences between the isolates, and cross-hybridization showed that they formed two groups, one from the Indian subcontinent and a second one from Southeast Asia.

Diseased leaves become yellow or orange-yellow, and malformed, starting with the tip of the lower leaves. Young infected leaves may show mottling and chlorotic stripes parallel to the veins. Root development is poor and the reproductive stage may be delayed. Affected panicles are usually small and sterile. Symptom expression varies considerably depending on the variety affected and environmental conditions. Yield losses depend upon plant age at the time of infection, and the plant genotype. *Eleusine indica*, *Echinochloa colonum, E. crusgalli, Oryza* spp., *Paspalum* sp.,

Setaria sp., sorghum, and wheat have also shown to be susceptible to tungro disease.

Several sources of resistance to RTSV have been identified at the International Rice Research Institute (IRRI). Promising lines have been identified and some of the superior lines resistant to tungro have been released as varieties. IR20, IR26, and IR30 were the first tungro-resistant cultivars released by IRRI followed by IR28, IR29, IR34, IR36, IR38, and IR40 during the 1970s. Some cultivars resistant to *N. virescens*, such as IR54, IR56, IR60, and IR62, were released in the 1980s, followed by PSBRc4, PSBRc10, PSBRc18, and PSBRc28 during the 1990s. Recently, five tungro-resistant lines showing resistance to RTSV were released as cultivars in Indonesia and the Philippines. Utri Merah, Balimau Putih, Habiganj DW8, and *O. rufipogon* served as donors of resistance genes.

Rice Stripe

Rice stripe is the second oldest viral disease of rice, having been first observed in the 1890s, in Japan. In 1931, the disease was associated with the presence of the leafhopper *L. striatellus*, but its causal agent could not be isolated. The disease continued to spread in Japan in the early 1950s, due to the introduction of new cultural practices (seeding of rice in wheat fields and early plantings) that expanded the temporal availability of young rice plants to the leafhopper vector. Rice stripe eventually emerged in Korea, Taiwan, China, and southwestern regions (Vladivostok) of the former USSR. The causal agent eluded detection until 1975, when Japanese scientists isolated thin (3–8 nm), coiled, filamentous particles of different lengths (2110–510 nm) from rice stripe-affected plants. In 1989, rice stunt virus (RSV) was shown to consist of four ssRNA components, similar to the genome of maize stripe virus (MSpV) isolated in 1981 in the United States. These viruses, together with rice hoja blanca virus (RHBV), isolated in Colombia in 1982, formed the 'rice stripe virus group' in 1988. Further molecular work led to the creation of the genus *Tenuivirus* in 1995. Tenuiviruses consist of up to six segments of linear, negative-sense, and ambisense ssRNA. RSV is believed to have four to five segments, depending on the isolate. Sizes are *c.* 9 kb for RNA 1 (negative polarity); 3.3–3.6 kb for RNA 2 (ambisense), 2.2–2.5 kb for RNA 3 (ambisense); 1.9–2.2 kb for RNA 4 (ambisense); and 1.2 kb for RNA 5 (negative polarity). The viral genome encodes structural (32–35 kDa) and nonstructural proteins (230 kDa), both of which are useful for diagnostic purposes. The nucleocapsid protein is encoded by the 5′ proximal region of the virion-complementary sense strand of RNA 3. Some proteins are translated from subgenomic RNAs.

RSV is transmitted by *L. striatellus*, the 'smaller brown planthopper', *Terthron albovittatum*, *Unkanodes sapporonus,* and *U. albifascia* (formerly *Ribautodelphax albifascia*). The virus multiplies in the insect vector (propagative) and there is transovarial passage of the virus to the progeny. The proportion of active vectors of *L. striatellus* can be as high as 54%. The shortest virus acquisition feeding period may be less than 30 min, and the incubation period in the vector is 5–10 days, although longer incubation periods have been reported for *L. striatellus* (21 days) and *U. albifascia* (26 days). The virus persists in its planthopper vectors for life ('persistent' transmission). RSV is not transmitted manually or through seed. The main hosts of *L. striatellus* are wheat, barley, and Italian rye in spring, and upland rice, *Echinochloa crusgalli,* and *Digitaria* spp. in summer.

Initial symptoms induced by RSV are failure of emerging leaves to unfold normally. Affected leaves may show chlorosis, and chlorotic stripes running parallel to the leaf veins. Affected leaves may eventually develop necrosis and die. In infected, older leaves, a chlorotic mottle may appear. The tillering capacity is often reduced, and panicles may fail to produce seed. RSV also infects at least 35 other species of plants in the Gramineae, including oats, barley, rye, sorghum, wheat, and maize.

All the Japanese paddy rice cultivars tested as seedlings proved susceptible, whereas most Japanese upland rice cultivars are highly resistant or immune. Outside Japan, most *indica* rice cultivars were resistant, but *japonica* rice cultivars were susceptible. Resistance in Japanese upland varieties was found to be controlled by two complementary dominant genes (St_1 and St_2). Multiple alleles of the latter gene may be responsible for the resistance associated with *indica* cultivars. Partial dominant resistance has been identified, linked to another dominant gene (St_3). Resistance to the virus is not necessarily linked to resistance to the vector. Transgenic rice cultivars (coat protein mediated resistance) possessing resistance to RSV have already been developed.

Rice Grassy Stunt

Rice grassy stunt was first observed in 1963 in the Philippines, where the characteristic stunting was initially referred to as 'yellow dwarf' or 'rice rosette'. This disease is now present in China, India, Indonesia, Japan, Malaysia, Sri Lanka, Taiwan, and Thailand. The association of this disease with the brown planthopper, *N. lugens,* was made in 1964. The causal agent of rice grassy stunt was isolated in the early 1980s, as a member of a distinct species of the genus *Tenuivirus*. Hence, rice grassy stunt virus (RGSV) shares most of its physicochemical characteristics with RSV. However, RGSV has an additional (sixth) ssRNA segment (ambisense), and its RNA 3 (3.1 kb) and RNA 4 (2.9 kb) are different from the corresponding segments of other tenuiviruses. RGSV

RNA 1 (9.8 kb), 2 (4.1 kb), 5 (2.7 kb), and 6 (2.6 kb) are homologous to RNAs 1, 2, 3, and 4, respectively, of other tenuiviruses. Both the capsid and non-structural proteins of RSV and RGSV are serologically related. A tentative tenuivirus, rice wilted stunt virus, originally reported in 1981 from Taiwan, is often considered a synonym of RGSV.

RGSV is transmitted by the planthoppers *Nilaparvata bakeri, N. lugens*, and *N. muiri,* in a persistent manner. RGSV multiplies in the vector, but it is not transmitted congenitally. Main symptoms consist of severe stunting, excessive tillering, erect growth, and short, narrow chlorotic leaves. Young leaves may show mottling and/or stripes. Inclusions are present in infected cells. Other hosts of RGSV include: *E. colonum, Cynodon dactylon, Cyperus rotundus, Leersia hexandra*, and *Monochoria* sp.

Genetic resistance to RGSV has been identified in some rice varieties. A single dominant gene conferring resistance to RGSV was identified in *Oryza nivara*. Some sources of RGSV resistance are susceptible to the planthopper vectors.

Rice Hoja Blanca

Rice hoja blanca ('white leaf') was first observed in the Cauca Valley of Colombia, South America, in 1935. By 1940, the disease was present in most rice-producing regions of Colombia. The disease was detected in Panama in 1952, in Cuba in 1954, in Venezuela in 1956, in Costa Rica in 1958, in El Salvador in 1959, in Guatemala in 1960, and in Nicaragua, Honduras, and the Dominican Republic in 1966. In South America, rice hoja blanca also occurs in Ecuador and Peru. The virus was detected in southern United States in the late 1950s, but it is not a problem there. Until the end of the twentieth century, hoja blanca was the only known viral disease of rice in the Americas.

The etiology of rice hoja blanca remained elusive until 1983, when virus-like particles isolated from infected rice plants in Colombia, were shown to be similar to those described in Japan for RSV. The causal virus was finally characterized as a member of the rice stripe virus group created in 1988, which also include MSpV. In 1995, these viruses became species of the genus *Tenuivirus*. The main physicochemical characteristics of these viruses have been described above, but RHBV virions consist of four species of ssRNA, with a total genome of 17.6 kb. RNA 1 is 9.8 kb and encodes the viral polymerase. RNA 2 (3.5 kb) has the capacity to code for two proteins in an ambisense manner, and RNA 3 (2.3 kb) encodes two proteins in an ambisense manner, including the capsid protein (35 kDa). RNA 4 (1.9 kb) has the same basic arrangement as RNAs 2 and 3, and codes for a nonstructural protein (23 kDa). Echinochloa hoja blanca virus (EHBV) was initially considered a strain of *Rice hoja blanca virus*, because it coexists with this virus in most affected rice fields, but it is now considered a distinct, serologically related tenuivirus.

RHBV is transmitted in a persistent, circulative, and propagative manner by the planthopper *Tagosodes orizicolus* (previously *Sogatodes oryzicola*). The virus also infects the insect vector and passes transovarially to the progeny of viruliferous females (80–95% efficiency). However, only 5–15% of the wild *T. orizicolus* population has the ability to transmit the virus in nature. Incubation periods of 6–36 days have been reported for RHBV in its planthopper vector. *Tagosodes cubanus*, the planthopper vector of EHBV, has been reported to transmit RHBV from rice to *E. colonum* but not from rice to rice, or from *Echinochloa* to rice, unless the viruliferous insects are forced to feed on the test plants. Colonies containing over 90% active vectors can be developed by crossing known male and female vectors. If left unattended, these colonies revert to their natural 5–15% of active vectors, suggesting that viruliferous individuals are at a biological disadvantage with respect to nontransmitters. The acquisition feeding period observed for at least 50% of the potential vectors to transmit RHBV is 15 min, with an optimum of 1 h. The incubation period is 6–9 days for individuals who acquire the virus transovarially, but may be up to 36 days for individuals acquiring the virus as nymphs or adults (males usually have a shorter life span and must acquire the virus transovarially). Nonviruliferous *T. orizicolus* females lay more eggs than viruliferous females, which suggested a deleterious effect of RHBV in its insect vector.

RHBV induces small chlorotic spots at the base of infected leaves, which then show longitudinal chlorotic stripes. The following leaves may be completely chlorotic (hence the name 'white leaf'). Plants affected at an early stage are usually stunted, and may eventually die. Malformation of grains or sterility of the panicle can be observed in systemically infected rice plants that reach the reproductive stage. RHBV has been shown to infect *Digitaria* spp., *Leptochloa* spp., oats, barley, rye, and wheat. Massive screenings of rice cultivars took place in Cuba, Venezuela, and Colombia in the late 1950s and early 1960s. Most commercial long-grain rice varieties are susceptible. Resistance to RHBV was identified in short-grain *japonica* varieties and hybrids with *indica* types. These hybrids have been used to develop resistant rice cultivars, but their level of resistance ultimately depends on virus pressure. Some varieties possessing high levels of RHBV resistance have been developed in recent years in Colombia, which seem to be the result of combining different mechanisms of resistance, not necessarily against the virus. Transgenic plants that show moderate levels of resistance to the causal virus have been developed. RHBV epidemics are cyclic, often separated by several

years of low disease incidence. The deleterious effect of the virus in viruliferous females may account for this cyclical phenomenon.

Rice Yellow Stunt

Rice yellow stunt (previously known as 'rice transitory yellowing') was first reported from southern Taiwan in 1960. The disease was initially believed to be caused by the lack of aeration in paddy fields. The characteristic foliar yellowing, stunting, and root rot were once blamed for the destruction of over 2000 ha of rice in the 1960s. In 1965, a virus was first suspected as the causal agent, and the leafhopper *N. nigropictus* (previously *N. apicalis*) was implicated as the vector. The name 'transitory yellowing' was given to this disease because affected rice plants often recovered from the yellowing symptoms at later stages of growth. The causal virus was isolated in 1986 and shown to be a bullet-shaped rhabdovirus approximately 190×95 nm. *Rice yellow stunt virus* is currently characterized as a species of the genus *Nucleorhabdovirus*, family *Rhabdoviridae*. Nucleorhabdoviruses multiply in the nucleus of infected cells, forming large granular inclusions. Virus maturation occurs at the inner nuclear envelope, where rice yellow stunt viruses (RYSVs) acquire their lipid envelope. The virus contains a single molecule of negative-sense ssRNA about 13.5 kb in size. A modular organization of the genome shows three highly conserved blocks, encoding structural and nonstructural proteins, membrane proteins, movement protein, and the polymerase.

RYSV is transmitted by the leafhoppers *N. nigropictus*, *N. cincticeps,* and *N. virescens* in a persistent and propagative manner. The virus does not pass congenitally to the progeny. Warm winters and continuous rice plantings increase virus incidence. *Japonica* varieties are more susceptible to RYSV than *indica* types. Highly resistant rice varieties have been selected since the early 1960s to manage this disease.

Rice Yellow Mottle

Rice yellow mottle was first observed in 1966 in Kenya and is currently the most important viral disease of rice in sub-Saharan Africa. The disease also affects rice in Madagascar. Yield losses ranging from 25% to 100% are not uncommon in West Africa. The causal agent was described in 1974 as an isometric virus about 30 nm in diameter, possessing positive-sense ssRNA (1.4×10^6 M). The RNA has 4450 nt organized into four ORFs, three of which overlap (except ORF 1). ORF4 codes for the 26 kDa capsid protein. This protein is required for cell-to-cell and long-distance movement. Rice yellow mottle virus (RYMV) is currently classified as a member of the species *Rice yellow mottle virus* in the unassigned genus *Sobemovirus*.

RYMV is transmitted by chrysomelid beetles (Coleoptera): *Sesselia pusilla, Chaetocnema pulla, C. dicladispa, Trichispa sericea, Dicladispa (Hispa) viridicyanea,* and *D. gestroi*, in a semi-persistent manner. The virus is lost when beetles molt; it does not multiply in the vector and is not congenitally transmitted. RYMV is transmitted by mechanical means but is not seed-borne. Rice yellow mottle disease is characterized by stunting, crinkling, mottling, yellow streaks, malformation of panicles, reduced tilllering, and sterility. RYMV also infects other *Oryza* species, and the grasses *Acroceras zizanioides, Dinebra retroflexa, Eragrostis aethipica, E. ciliaris, E. namaquensis, E. tenella, Echinochloa colona, Ischaemum rugosum, Panicum repens, P. subalbidum, Phleum arenarium, Sacciolepsis africana,* and *Setaria longiseta*.

Most African rice cultivars are highly susceptible to RYMV. Two types of resistance have been identified in *Oryza*: polygenic resistance in *O. sativa*, *japonica*, which slows down virus dissemination; and monogenic resistance in *Oryza glaberrima,* which prevents infection. Transgenic rice plants showing 'enhanced' resistance to RYMV have also been developed. The epidemiology of RYMV is poorly understood, specially regarding the identification of primary inoculum sources, and propagation of the virus in the field. Resistance-breaking isolates of RYMV have been recently detected in West and Central Africa.

Rice Stripe Necrosis

Rice stripe necrosis first emerged in Ivory Coast, West Africa, in 1977. The disease induces chlorotic striping, malformation, and necrosis of leaves, besides stunting and reduced tillering. The causal virus was shown to consist of rod-shaped virions of two predominant lengths (110–160 and 270–380 nm), and 20 nm wide. The virus can be mechanically transmitted to *Chenopodium amaranticolor* (local lesions), but is not seed-borne. The virus was transmitted through contaminated soil, which led to the identification of the fungus *Polymyxa graminis* as the vector. The causal virus, rice stripe necrosis virus (RSNV), was tentatively classified as a member of the genus *Furovirus*. However, its relatively low incidence and economic importance, when compared to rice yellow mottle virus, relegated RSNV to the condition of a minor pathogen. In the meantime, RSNV slowly disseminated into the main rice-growing areas of West Africa, where farmers and scientists alike mistook the disease for a soil or physiological problem. It was not until 1991, when a new disease of

rice emerged in the Eastern Plains of Colombia, South America, that research conducted at CIAT, Palmira, Colombia, demonstrated that the new disease was in fact 'rice stripe necrosis', probably introduced from West Africa in contaminated rice seed. The Colombian isolate of RSNV had rod-shaped particles with a bi-modal length of 260 and 360 nm, and a particle width of 20 nm. These particles were observed in the cytoplasm of infected rice cells, affecting mainly mitochondria and the endoplasmic reticulum.

The virus was isolated as four components of ssRNA (6.3, 4.6, 2.7, and 1.8 kb), and a capsid protein subunit of 22.5 kDa. At least two distinct replication-associated domains of RSNV RNA1 show sequence identities of >78% with the corresponding domains of Beet necrotic yellow vein virus (BNYVV-RNA 1). As in the case of BNYVV, the RNAs of RSNV terminate in a 3′-poly(A) tail. RSNV's particle length, number of RNA segments, polyadenylation of the RNAs 3′-end, and partial homology to BNYVV suggest that RSNV is a species of the genus *Benyvirus*. Partial sequence obtained from the Colombian isolate's RNA 1 segment also showed a 51% similarity to the corresponding region of BNYVV, member of the type species of the genus *Benyvirus*. The vector of the Colombian RSNV isolate was molecularly characterized as a type II isolate of the fungus *P. graminis*.

Since the 1970s, when RSNV was first reported from West Africa, it was evident that rice varieties reacted differentially to RSNV. Some IRAT lines (8, 9, and 13) showed low virus incidences or escaped disease. Screening of the main rice varieties grown in Colombia showed that most of these cultivars were susceptible. Some breeding lines and some Asian varieties exhibited moderate resistance levels. *Oryza glaberrima*, a related species planted for human consumption in West Africa, was observed to possess a high level of resistance to RSNV in that region and in Colombia. The disease seems to spread more rapidly in mechanized and irrigated rice plantings, but the main virus-vector dissemination mechanism is undoubtedly the use of seed produced in affected fields, where the fungus and the virus persist for many years. Rice stripe necrosis is currently found throughout West Africa, and in four neighboring countries of Latin America: Colombia, Ecuador, Panama, and Costa Rica.

Rice Necrosis Mosaic

Rice necrosis mosaic was first observed in Japan in 1959. The causal virus was observed in 1968 to have filamentous particles of two different lengths (275 and 550 nm) and 13 nm in width. Rice necrosis mosaic virus (RNMV) is currently classified as a member of the species *Rice necrosis mosaic virus* of the genus *Bymovirus,* family *Potyviridae*. Virions contain two molecules of positive-sense ssRNA. RNA 1 is 7.5–8.0 kb, and RNA 2 is 3.5–4.0 kb, and both RNAs are polyadenylated at their 3′-ends. The capsid protein is located in the 3′-proximal region of RNA 1. Virions have a single capsid protein subunit of *c.* 33 kDa. As other species in the family *Potyviridae*, RNMV induces cylindrical (pinwheels) inclusions in the cytoplasm of infected cells.

RNMV is transmitted by mechanical means and through soil by the vector fungus *P. graminis*. The main symptoms are initial mottling of the lower leaves, which gives rise to streaks and irregular foliar patches. Affected leaves turn yellow. Although susceptible rice plants are not markedly stunted, the number of tillers is usually reduced. Necrotic lesions can appear on leaf sheaths and the base of culms. Grain production is significantly reduced in diseased plants. The virus seems to be limited to *O. sativa*.

Viruses transmitted by *P. graminis* are generally disseminated through contamination of agricultural tools and machinery; irrigation water; and seed contaminated with soil particles containing viruliferous fungal propagules (cystosori). These propagules can remain viable in contaminated soils for over 10 years. Soil used in seedbeds should be kept free of the vector. Resistance to this disease has been identified in some rice varieties.

See also: Cereal Viruses: Maize/Corn; Cereal Viruses: Wheat and Barley; Plant Reoviruses; Plant Rhabdoviruses; Rice Tungro Disease; Rice Yellow Mottle Virus; *Tenuivirus*; Vector Transmission of Plant Viruses.

Further Reading

APS (1992) *Compendium of Rice Diseases,* 62pp. St. Paul, MN: APS Press.

Brunt AA, Crabtree K, Dallwitz MJ, *et al.* (1996) *Viruses of Plants,* 1484pp. Wallingford: CAB International.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) . *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses,* 1259pp. San Diego, CA: Elsevier Academic Press.

IRRI (1969) *The Virus Diseases of the Rice Plant,* 354pp. Los Baños, The Philippines: The International Rice Research Institute.

Ling KC (1972) *Rice Virus Diseases,* 142pp. Los Baños, The Philippines: The International Rice Research Institute.

Morales FJ and Niessen AI (1983) Association of spiral filamentous virus-like particles with rice hoja blanca. *Phytopathology* 73: 971–974.

Morales FJ, Ward E, Castaño M, Arroyave J, Lozano I, and Adams M (1999) Emergence and partial characterization of rice stripe necrosis virus and its fungus vector in South America. *European Journal of Plant Pathology* 105: 643–650.

Wilson MR and Claridge MF (1991) *Handbook for the Identification of Leafhoppers and Planthoppers of Rice,* 142pp. Bristol, UK: Natural Resources Institute and C.A.B. International.

Cereal Viruses: Wheat and Barley

H D Lapierre and D Hariri, INRA – Département Santé des Plantes et Environnement, Versailles, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Guttation Phenomenon linked to the natural movement of the fluid from the apoplastic areas to the leaf surface through stomata under conditions that allow gutate to form.
Pathotype A pathotype is a virus strains that exhibits a differential interaction with the host, usually increased virulence.
Pyramiding genes A combination of different resistance genes introduced in one line or cultivar.
Strain A virus isolate called strain exhibits specific interactions in front of several plant species. A strain may also be charaterized by particular properties of the viral particles.

Introduction

Most of the viruses that infect barley also infect wheat. Of the 57 viruses infecting wheat and or barley (**Table 1**), ten have been discovered less than 30 years ago and new strains or pathotypes are regularly reported. A few apparently nonpathogenic viruses belonging to the genera *Metavirus*, *Pseudovirus*, and *Endornavirus* have been characterized but are not described here. Only the most damaging viruses and some emerging viruses are described.

Agropyron Mosaic Virus

This virus of low agronomical importance on wheat is believed to be transmitted by the cereal rust mite *Abacarus hystrix* (Nalepa). In USA, agropyron mosaic virus (AgMV) may occur in wheat fields infected with wheat streak mosaic virus (WSMV). In the presence of the two viruses, a yield loss of 85% has been estimated.

Arabis Mosaic Virus

In Switzerland, arabis mosaic virus (ArMV) has been detected in fields of winter barley. Partial stunting, without yellowing, can occasionally be observed. Barley infection is achieved by the nematode *Xiphinema diversicaudatum* (Micoletzky).

Aubian Wheat Mosaic Virus

This emergent virus was observed in some winter wheat fields from northern and southern France. A similar virus is present in the UK. The rod-shaped particles of this virus are detected by enzyme-linked immunosorbent assay (ELISA). *Polymyxa graminis* cystosori are detected in the roots of infected plants but aubian wheat mosaic virus (AWMV) is not serologically related to the *Polymyxa*-transmitted viruses already known. The only known natural hosts of this virus belong to the genus *Triticum*. AWMV is mechanically transmitted to wheat and to some eudicots but mosaic symptoms were observed only on wheat. All the cultivars of bread and durum wheat inoculated were infected. In wheat fields, yellow patches of diseased plants are observed from the beginning of the year till heading stage. Most of the cultivars are slightly stunted showing variable mosaic symptoms. Nucleic acid sequences of the genome and natural transmission of the virus are under investigation.

Barley Stripe Mosaic Virus

This virus is no longer a global agronomical problem to barley. In the past, up to 60% yield loss has been reported. Pollen transmission of barley stripe mosaic virus (BSMV) is the main way of contamination. Immunological control of the seeds allows growing virus-free cultivars. BSMV is less frequently found on wheat. Its recent detection in Turkey means this virus should be monitored closely. With its tripartite genome and the high seed transmission, BSMV remains a model for studies of virus/plant interactions.

Barley Yellow Streak Mosaic Virus

This virus has been detected in several US states and Canada. The agronomical importance of barley yellow streak mosaic virus (BaYSMV) has not been evaluated. Nearly all plants in a field may be infected resulting in yield loss up to 100%. Infected plants are stunted and show chlorotic streaks and stripes parallel to the leaf veins. Virus particles are filamentous *c.* 64 nm in diameter and of varying lengths between 127 and 4000 nm. Particles have an outer lipid-like envelope. From purified particles, a dominant protein of ~32 Da and several single-stranded RNA (ssRNA) species of ~13–11 kbp have been isolated. This unassigned virus which resembles some insect and animal viruses probably belongs to a new genus.

Table 1 Viruses infecting naturally wheat and barley in different parts of the world

Viruses	*Genera*	*Families*
Agropyron mosaic virus [a]	*Rymovirus*	*Potyviridae*
Arabis mosaic virus [b]	*Nepovirus*	*Comoviridae*
Aubian wheat mosaic virus [a, l]		
Barley mild mosaic virus [b, c]	*Bymovirus*	*Potyviridae*
Barley stripe mosaic virus [d]	*Hordeivirus*	
Barley yellow dwarf virus-MAV [d, e]	*Luteovirus*	*Luteoviridae*
Barley yellow dwarf virus-PAS [d, e]	*Luteovirus*	*Luteoviridae*
Barley yellow dwarf virus-PAV [d, e]	*Luteovirus*	*Luteoviridae*
Barley yellow mosaic virus [b, c]	*Bymovirus*	*Potyviridae*
Barley yellow streak mosaic virus [d, l]		
Barley yellow striate mosaic virus [d]	*Cytorhabdovirus*	*Rhabdoviridae*
Brazilian wheat spike virus [a, l]		
Brome mosaic virus [d]	*Bromovirus*	*Bromoviridae*
Brome streak mosaic virus [f]	*Ttritimovirus*	*Potyviridae*
Cereal mosaic virus [g, l]		
Cereal pseudorosette virus [g, l]		
Cereal yellow dwarf virus-GPV [d, e]	*Polerovirus*	*Luteoviridae*
Cereal yellow dwarf virus-RPS [d, e]	*Polerovirus*	*Luteoviridae*
Cereal yellow dwarf virus-RPV [d, e]	*Polerovirus*	*Luteoviridae*
Chinese wheat mosaic virus [a, h]	*Furovirus*	
Chloris striate mosaic virus [d]	*Mastrevirus*	*Geminiviridae*
High Plains virus [d, l]		
Indian peanut clump virus [d]	*Pecluvirus*	
Iranian wheat stripe virus [a, l]		
Maize Iranian mosaic virus [a, l]		
Maize rough dwarf virus [a, l]	*Fijivirus*	*Reoviridae*
Maize sterile stunt virus [d, l]		
Maize streak virus [d]	*Mastrevirus*	*Geminiviridae*
Maize white line mosaic virus [a, l]		
Mal de Rio Cuarto virus [d]	*Fijivirus*	*Reoviridae*
Northern cereal mosaic virus [d]	*Cytorhabdovirus*	*Rhabdoviridae*
Peanut clump virus [i]	*Pecluvirus*	
Rice black streaked dwarf virus [d]	*Fijivirus*	*Reoviridae*
RMV [d, e]		*Luteoviridae*
SGV [d, e]		*Luteoviridae*
Soil-borne cereal mosaic virus [a, h]	*Furovirus*	
Soil-borne wheat mosaic virus [a, h]	*Furovirus*	
Soil-borne wheat mosaic virus Marne strain [b, h]	*Furovirus*	
Triticum mosaic virus [a, l]		
Wheat American striate mosaic virus [a]	*Cytorhabdovirus*	*Rhabdoviridae*
Wheat dwarf virus B and W strains [a, b, l]	*Mastrevirus*	*Geminiviridae*
Wheat Eqlid mosaic virus [a, l]		
Wheat rosette stunt virus [g, l]		
Wheat spindle streak mosaic virus [a, j]	*Bymovirus*	*Potyviridae*
Wheat spot mosaic [d, k, l]		
Wheat streak mosaic virus [a]	*Tritimovirus*	*Potyviridae*
Wheat yellow head virus [a, l]		
Wheat yellow leaf virus [d]	*Closterovirus*	*Closteroviridae*
Wheat yellow mosaic virus [a, j]	*Bymovirus*	*Potyviridae*
Winter wheat Russian mosaic virus [g, l]		

[a]Virus infecting wheat.
[b]Virus infecting barley.
[c]See section on bymoviruses of barley.
[d]Virus infecting wheat and barley.
[e]See section on yellow dwarf viruses.
[f]Virus detected in barley volunteers.
[g]See section on Northern cereal mosaic virus.
[h]See section on furoviruses.
[i]See section on Indian peanut clump virus.
[j]See section on bymoviruses of wheat.
[k]See section on High Plains virus.
[l]Unassigned virus.

BaYSMV is transmitted by the brown wheat mite *Petrobia latens* and is retained in eggs of mites which are the overwintering reservoir. No resistant barley cultivars are known.

Barley Yellow Striate Mosaic Virus

The distribution of this virus was first described in the Mediterranean areas. Recently, barley yellow striate mosaic virus (BYSMV) has been detected in East European countries. More than 10% yield loss in susceptible cultivars of durum wheat is suspected. Symptoms consist of leaf chlorotic striations, dwarfing, and excess tillering, and heads may be confined in the boot. Awns are short or broken and show mosaic. Wheat and barley plants die in spring in the case of an early infection in the autumn. BYSMV is transmitted in a persistent manner mainly by the planthopper *Laodelphax striatellus* (Fallén). The virus can be transmitted transovarially. Limited molecular tools have been used to compare BYSMV with other serologically related viruses (digitaria striate virus, maize sterile stunt virus, northern cereal mosaic virus (NCMV), wheat rosette stunt virus (WRSV)). Small grain species and several grasses are hosts of BYSMV. Durum wheat is generally more susceptible to BYSMV than bread wheat. Genetic basis of susceptibility/tolerance in wheat is under study.

Brazilian Wheat Spike Virus

This virus found in Brazil seems to be of low incidence on wheat crops. Young leaves of infected plants are entirely chlorotic, older leaves show bright stripes. Spikes of affected plants are pale yellow to whitish, containing empty grains. Particle morphology and cytology indicate that Brazilian wheat spike virus (BWSpV) could be a tenuivirus.

Brome Mosaic Virus

Brome mosaic virus (BMV) disease has been reported on wheat and triticale. Infected plants show yellow streak and stunting. The virus is present in the guttation fluid of the leaves. Beetles are considered to be the main vectors. Mechanical inoculation by animal and machinery traffic probably also occur.

Bymoviruses of Barley

Barley Yellow Mosaic Virus, Barley Mild Mosaic Virus

Bymoviruses have bipartite ssRNA genomes and each segment (RNA1 and RNA2) carries a single open reading frame (ORF) which encodes a polyprotein. The coat protein (CP) gene is located at the C-terminus of the RNA1.

Barley yellow mosaic virus (BaYMV) and barley mild mosaic virus (BaMMV) are major pathogens of winter barley in Europe and East Asia. Yellow discolored patches of different sizes are often the first sign of field infection in early spring. First symptoms are chlorotic or pale green spots and streaks along the leaf veins and can be observed in December or after snow break in February or March. Generally, the third leaves show as rolled young leaves. The bymoviruses of barley significantly reduce height and number of fertile tillers and losses caused by these viruses vary from 10% to 90%. When temperature rises, the new leaves remain green but show distinct pale green streaks. BaYMV and BaMMV can be found, either separately or together. In general, high yield reductions can be anticipated when the winter is very severe (**Figures 1** and **2**).

Different biological or/and serological variants of BaYMV, BaMMV, and wheat spindle streak mosaic virus (WSSMV) have been reported in Europe. In Europe, the resistant genes *rym4* and *rym5* have been overcome by



Figure 1 Symptoms of barley yellow mosaic virus pathotype 2 (BaYMV 2) in winter barley.



Figure 2 Symptoms of barley mild mosaic virus Sillery pathotype (BaMMV-Sil) in barley.

BaYMV 2 and BaMMV Sillery strain (BaMMV-Sil). In France, the presence of several pathotypes of BaYMV and BaMMV able to overcome at least seven of 15 known genes has been reported. These pathotypes, in slow progression, do not pose an agronomic problem for the moment. In Japan, seven strains of BaYMV and two strains of BaMMV have been described on the basis of pathogenicity toward barley cultivars. A Korean strain of BaMMV (BaMMV-Kor) differing biologically and serologically from the Japanese and European isolates and several biological variants of BaYMV in China have also been recognized.

The vector of BaYMV/BaMMV is *P. graminis* Ledingham, a soil organism belonging to the family Plasmodiophoraceae.

The only effective means of controlling these viruses is through the use of resistant cultivars. Resistant genes have been described and localized in the barley genome. Some of them confer complete immunity against BaYMV and/or BaMMV while others only delay the appearance of symptoms. Pyramiding genes have been introduced in barley cultivars and other genes are studied in various *Hordeum* species.

Bymoviruses of Wheat

Wheat Spindle Streak Mosaic Virus, Wheat Yellow Mosaic Virus

WSSMV and wheat yellow mosaic virus (WYMV) were reported in America/Europe and Asia, respectively. These two viruses are major pathogens of bread and durum wheat, rye, and triticale (**Figure 3**). In the case of WSSMV, it was demonstrated that its genome associated to viral CP is internalized by *P. graminis*. In France, a new serotype (sII) of the WSSMV has been reported. Depending on the wheat cultivars, WSSMV resistance is controlled by a single dominant gene or by two pairs of alleles, which show complementary effects.



Figure 3 Symptoms of wheat spindle streak mosaic virus in winter bread wheat.

Furoviruses

Chinese Wheat Mosaic Virus, Soil-Borne Cereal Mosaic Virus, Soil-Borne Wheat Mosaic Virus

Several wheat furoviruses infecting cereal species have been described in the worldwide: soil-borne wheat mosaic virus (SBWMV) in wheat, rye, and barley; soil-borne cereal mosaic virus (SBCMV) in wheat and rye (**Figure 4**); and Chinese wheat mosaic virus (CWMV) in wheat. These viruses are transmitted by *P. graminis*. SBWMV, the type member of the genus *Furovirus*, occurs mostly on winter wheat in America, Europe, Asia, and Africa. Based on genomic organization and biological characteristics, it was proposed that the American SBWMV, CWMV, European SBCMV, and Japanese SBWMV are four strains of the same virus species. SBWMV is internalized by *P. graminis* in the form of viral RNAs associated to movement proteins. Genomes of these viruses are divided into two ssRNA species that are individually encapsidated. The furoviruses of wheat often causes a 'rosetting' disease, meaning that the plants are severely stunted. The number of tillers and kernel weight are also reduced. The disease usually results in a 10–30% yield loss, but may cause up to 80% yield loss in seriously infested fields. Resistance to SBCMV is controlled by a single locus *Sbm* on 5DL chromosome. Recently a bulk segregant analysis demonstrated that the SBWMV resistance gene was also on chromosome 5DL. The relationship between the two loci is being investigated. This resistance limits the movement of the viruses from the roots to leaves.

Soil-borne wheat mosaic virus Marne strain (SBWMV-Mar) was isolated from barley cv. Esterel in France (Marne department). This furovirus, in contrast to common French isolates of SBCMV, is mechanically transmitted to barley and to oats. Nucleotide and amino acid sequence analyses revealed that the French virus infecting barley is closely related to a Japanese isolate of SBWMV (SBWMV-JT), which was originally isolated from barley.



Figure 4 Symptoms of soil-borne cereal mosaic virus in winter wheat.

High Plains Virus

This virus, discovered in USA in 1993, has been identified in two other continents. High Plains virus (HPV) is similar or identical to wheat spot mosaic virus described since 1952. Severe symptoms are shown by wheat plants when infected by HPV. The host range of HPV is large including barley, maize, and many grasses. The virus is transmitted obligatorily by the mite *Aceria tritici* (Shevtchenko) that also transmits WSMV. Two sources of resistance to WSMV *RonL* and *Wsm1* are not effective on HPV.

Indian Peanut Clump Virus

Found in five states of India and in Pakistan, Indian peanut clump virus (IPCV) is one of the few viruses infecting both Poaceae and Eudicot species. Several Poaceae species are important reservoirs of this virus which induces a severe disease in peanut. Like barley and maize, wheat is severely affected by IPCV. Early infected plants are dark green, stunted, and generally die. Spikes of late infected plants are malformed and produce shriveled seeds. IPCV is transmitted by the soil-borne vector *P. graminis* Ledingham and at a low level by wheat seeds. Peanut clump virus from West Africa is closely related to IPCV. Its agronomical importance on wheat and barley is unknown.

Iranian Wheat Stripe Virus

The agronomical importance of this virus was not estimated but is probably not high in the Fars Province of Iran where it was discovered. Chlorosis, whitening, and striping are observed in infected plants which are dwarfed. Iranian wheat stripe virus (IWSV) is transmitted in a persistent-propagative manner by the planthopper *Unkanodes tanasijevici* (Dlabola). From the comparison of genomic nucleic acid sequences, it has been suggested that a common ancestor between IWSV and RHBV/EHBV tenuiviruses may exist in the New World.

Maize Streak Virus – B Strain

Three strains of MSV have been distinguished on the basis of nucleic acid sequences. Strain A is associated to maize. Strain B (MSV-B) has been characterized in wheat, barley, and grasses in South Africa and in Tasmania. MSV-B is more aggressive on wheat and barley than on maize. Recombinants between strain A (from maize) and strain B have been characterized. Agronomical importance and epidemiology of strain B have to be investigated.

Northern Cereal Mosaic Virus

Several viruses such as WRSV from China, cereal mosaic virus, and winter wheat Russian mosaic virus (WWRMV) from east Russia are probably isolates or strains of the NCMV described in Japan. On winter wheat associated with conditions of high viral inocula in the autumn, yield losses may reach 80%. The infected wheat plants show stunting and prolific tillers. The leaves develop longitudinal yellow and green stripes. Plants infected at the seedling stage die during winter. When infection occurs later, the plant develops thickened stems and foliage. Grains are frequently shriveled. The virus is transmitted in a persistent-propagative manner mainly by *L. striatellus*. Small grains and several grasses are natural hosts of NCMV. Maize has been reported to be resistant to NCMV. Late sowing in autumn and eradication of volunteer plants are recommended.

Particle size, antigenic properties, and vector specificity of these cytorhabdoviruses are very similar or identical. Cereal pseudorosette virus (CPV) is transmitted also by *L. striatellus*. Since its particles are shorter than those of NCMV, CPV is considered a distinct species.

Triticum Mosaic Virus

This virus, discovered in 2006 in Kansas, has been found in areas where WSMV and HPV are present. The gene RonL giving resistance to WSMV is not efficient on triticum mosaic virus (TriMV). The Wsm1 source of resistance is active on TriMV at 19 °C but not at 25 °C. Symptoms induced by TriMV are similar to those of WSMV. In the future, serological diagnosis may become useful to distinguish TriMV from other viruses commoly found in wheat.

Wheat American Striate Mosaic Virus

Reported from Canada and the north-central regions of US, this virus has a limited importance because it is usually present only on the borders of wheat fields. Wheat American striate mosaic virus (WASMV) has a relatively narrow natural host range including barley and maize. Fine chlorotic parallel streaks, brown necrotic streaking of culms and glumes are relatively good indicators of WASMV infection. Susceptible cultivars are stunted and most ears are sterile. *Endria inimica* (Say), the main leafhopper vector, transmits WASMV in a persistent-propagative manner. Early planting favors autumn infection. Several cultivars such as TAM107 are tolerant.

Wheat Dwarf Virus

In continental areas of Europe and its borders, this virus may be very severe on winter wheat, durum wheat, triticale, rye, and barley. Two strains of wheat dwarf virus (WDV) have been characterized. Overall nucleic acid sequences of the wheat-adapted strain (WDV-W) which does not infect barley showed 16% divergence with the barley-adapted strain (WDV-B) which does not infect wheat. The monoclonal antibody MAB 3C10 developed in Germany detects only WDV-B. The two strains have several common hosts. In winter wheat, the leaves show large yellowing bands, the plants are stunted, and an excess of tillering is frequent in contrast to infection by yellow dwarf viruses (YDVs). In winter, barley plants are dwarfed, leaves are shortened, and start yellowing. As in wheat, early infected plants die. *Psammotettix* is the only known leafhopper vector of this virus. Both nymphs and adults of *Psammotettix alienus* (Dahlbom) transmit WDV in a persistent-circulative manner. Only local populations of *P. alienus* carry the viral inoculum to new crops. Depending on the climatic conditions, one, two, or three vector generations may be observed during the warm active periods. In autumn, flying insects are not seen when the maximum temperature is below 10 °C. *Psammotettix alienus* survives the winter in the egg stage on several Poaceae species. Fallows, regrowths, and volunteers play a major role in the constitution of the autumn viral inoculum. Herbicide sprays and tillage of fallows and of preceding susceptible crops reduce the size of *P. alienus* populations. Sowing as late as possible is also recommended. Insecticide-dressed seed offers good protection. Depending on the evolution of *P. alienus* populations, protection of crops can be achieved using one or two pyrethroid sprays.

Wheat Eqlid Mosaic Virus

This emergent virus found in the Fars Province of Iran is transmitted by the root aphid *Forda marginata* (Koch). It has been suggested that wheat Eqlid mosaic virus (WEqMV) should be included in a new genus of the family *Potyviridae*.

Wheat Streak Mosaic Virus

Agronomical importance of this virus is high mainly in European continental areas where analysis of the genome sequence suggested that this virus probably originated in the Fertile Crescent. WSMV-infected plants show mosaic symptoms and develop a rosette appearance. Symptoms on winter wheat usually appear only in spring at temperatures above 10 °C. Infected plants are stunted with sterile heads. Yield losses can be as high as 100%. WSMV is transmitted by the wheat curl mite (WCM) *Aceria tosichella* (Keifer). The virus is acquired during nymphal stages and is retained up to several weeks even following molting. Prevailing winds play an important role in long-distance dispersion of mites. Seed transmission of WSMV in wheat was estimated from 0.5% to 1.5%. The host range of WSMV is very wide including maize. Prevention of the infection of WSMV in winter wheat in the autumn is achieved by elimination of volunteers 2 weeks before planting.

Wsm1 is a gene of *Thinopyrum intermedium* translocated in wheat. Other partial amphiploids (Zhong1, Zhong 2) are resistant to WSMV and WCM. Recently, two wheat origins from Iran (Adl Cross and 4004) have been reported to carry two distinct genes providing complete resistance to WSMV symptoms. Five genes of resistance to WCM detected in *Aegilops* have been transferred to wheat. Introduction of WSMV replicase (NIa) or CP gene into transgenic wheat confers virus resistance (**Figure 5**).

Wheat Yellow Head Virus

This virus detected in USA is of limited agronomical importance. The infected plants show yellow heads and mosaic symptoms of flag leaves. Its vector is not known but wheat yellow head virus (WYHV) can be transmitted by vascular puncture inoculation to maize and to *Nicotiana clevelandii* plants. In infected plants, a major protein of 32–34 kDa is detected. Analysis of the amino acid sequences of this protein demonstrated that WYHV is closely related to rice hoja blanca virus, a tenuivirus.

Wheat Yellow Leaf Virus

Agronomical importance of this virus, detected in Japan, China, and Italy, is limited. The virus causes diffuse chlorotic flecks or interveinal chlorosis and severe yellowing. All small grain crops are susceptible to wheat yellow leaf virus (WYLV). In Japan, the weed *Agropyron*



Figure 5 Symptoms of wheat streak mosaic virus in winter wheat.

tsukushiense (Ohwi) is probably an important reservoir of the virus. *Rhopalosiphum maidis* (Fitch) and *Rhopalosiphum padi* (L.) transmit WYLV in a semi-persistent manner. Resistant Chinese cultivars have been reported.

Yellow Dwarf Viruses

Barley Yellow Dwarf-MAV, -PAS, -PAV; Cereal Yellow Dwarf-GPV, -RPS, -RPV; RMV; SGV

Different viruses of the family *Luteoviridae* infecting Poaceae (barley yellow dwarf-MAV, -PAS, -PAV; cereal yellow dwarf-GPV, -RPS, -RPV) and two other viruses not assigned to a genus are collectively called YDs. The distribution of these viruses is dependent on environmental conditions. BYDV-PAV is probably the most widespread. In fields mainly infected by BYDV-PAV, yield reduction from 5% to 20% and nearly 40% have been reported in susceptible wheat and barley cultivars, respectively. Symptoms in infected fields occur in patches. Yellowing (mostly in barley) or reddening (mostly in wheat) are characteristic of YDV infection. These symptoms appear at the two- to three-leaf stage in barley and often after heading in wheat. YDVs are transmitted in a circulative-persistent manner by one or several aphid species. The capsid of YDVs includes a CP and a read-through protein, both needed for aphid transmission.

In temperate areas, YDV vectors have two main flight periods. At the beginning of autumn, winged aphids leave summer hosts (ripening maize, perennial grasses, regrowths, and volunteers). These aphids are attracted to low plant density areas in young winter cereal fields. Depending on the temperature, one to several cycles of virus infection occur before aphids are killed by low temperatures. In oceanic and in other warm areas, low concentration of apterous aphids is maintained during winter. In spring, aphids leave their hosts and infect spring crops and then maize crops. Perennial grasses maintain a low but permanent reservoir of YDVs and aphids.

Risk assessment systems have been developed. In Australia, forecasts of vector incidence are based on the temperature and rainfall in late summer/early autumn. In France, a decision-support system based on temperature-driven simulations of aphid populations was proposed. In UK, a computer-based decision-support system associating the number of aphids found in suction traps and the numbers of foci of YDV infection per unit area of crop was developed. Using these systems, a risk index allows rationalization of foliar pesticide usage (usually synthetic pyrethroids). Treating seeds with an aphicide is necessary in some areas when early sowing of susceptible cultivars is decided or when the pressure of YDV inocula is known to be regularly high. Imidacloprid (nicotinic agonist) or fipronil (chloride channel agonist) gives a good protection during several weeks.

Only minor sources of resistance are known in wheat. Several sources of tolerance have been detected in other Triticinae genera. Several lines derived from crosses between wheat and *Thinopyrum* sp. are very tolerant to YDVs. The basis for *Thinopyrum*-derived resistance to CYDV-RPV is associated to resistance via inhibition of viral systemic infection.

In barley, a major gene of resistance mapped to chromosome 3H, Ryd2, has been introduced into present-day cultivars. The level of protection conferred by this gene varies according to strains of BYDV-PAV and has a low efficiency for CYDV-RPV. Recently, a new major gene Ryd3 mapped to chromosome 6 has been characterized. In resistant plants, these genes tend to increase seed yield compared to healthy controls.

Recent data on the mechanism and genetics of transmission by aphids and evidence of the glycosylation of virus CP open new avenues to control these viruses in the future.

See also: Barley Yellow Dwarf Viruses; Brome Mosaic Virus; Cereal Viruses: Maize/Corn; Cereal Viruses: Rice; *Furovirus*; *Hordeivirus*; Maize Streak Virus; *Nepovirus*; *Pecluvirus*; Plant Reoviruses; Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes; Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Plant Rhabdoviruses; *Tenuivirus*.

Further Reading

Chain F, Riault G, Trottet M, and Jacquot E (2007) Evaluation of the durability of the barley yellow dwarf virus-resistant Zhong ZH and TC14 wheat lines. *European Journal of Plant Pathology* 117: 35–43.

Hariri D, Meyer M, and Prud'homme H (2003) Characterization of a new barley mild mosaic virus pathotype in France. *European Journal of Plant Pathology* 109: 921–928.
Lapierre H and Signoret P (eds.) (2004) *Viruses and Virus Diseases of Poaceae* (*Gramineae*), 857pp. Versailles, France: INRA.
Li H, Conner R, Chen Q, *et al.* (2005) Promising genetic resources for resistance to wheat streak mosaic virus and the wheat curl mite in wheat – *Thinopyrum* partial amphiploids and their derivatives. *Genetic Resources and Crop Evolution* 51: 827–835.
Malik R, Smith CM, Brown-Guedira GL, Harvey TL, and Gill BS (2003) Assessment of *Aegilops tauchii* for resistance to biotypes of wheat curl mite (Acari: Eriophyidae). *Journal of Economic Entomology* 96: 1329–1333.
Niks RE, Habeku ß A, Bekele B, and Ordon F (2004) A novel major gene on chromosome 6H for resistance of barley against barley yellow dwarf virus. *Theoretical and Applied Genetics* 109: 1536–1543.
Seddas P and Boissinot S (2006) Glycosylation of beet western yellows proteins is implicated in the aphid transmission of the virus. *Archives of Virology* 151: 967–984.
Stein N, Perovic D, Kumlehn J, *et al.* (2005) The eukaryotic translation initiation factor 4E confers multiallelic recessive bymovirus resistance in *Hordeum vulgare* (L.). *Plant Journal* 42: 912–922.
Wiangjun H and Anderson JM (2004) The basis for *Thinopyrum*-derivative resistance to cereal yellow dwarf virus. *Phytopathology* 94: 1102–1106.

Chandipura Virus

S Basak, University of California, San Diego, CA, USA
D Chattopadhyay, University of Calcutta, Kolkata, India

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Vesicular stomatitis has been known for more than a century as a disease that causes severe mouth lesions in cattle, with ruptured vesicles in the gum or tongue and reddish ulcerations. The first report of vesicular stomatitis described the prevalence of the disease in South Africa as early as in 1897, whereas reports of infected horses with symptoms of vesicular stomatitis in the New World date back to an outbreak in the United States in 1916. In spite of multiple outbreaks of the disease in domestic animals that resulted in huge economic loss in different parts of the world, it was only in 1942 that the causative agent was identified as a virus and named vesicular stomatitis virus (Indiana serotype). In the subsequent year, the New Jersey serotype of vesicular stomatitis virus was isolated.

Chandipura virus (CPHV) closely resembles vesicular stomatitis virus but it was readily distinguished by its ability to infect humans. CHPV was first isolated in 1965 in Nagpur, India, from serum samples collected from two febrile patients. This virus isolate was shown to produce cytopathic effects when inoculated into cells in culture. Subsequently, CHPV was isolated from the serum of an encephalopathy patient in 1980 to further confirm its existence and its ability to propagate in the human population. Subsequent serological studies established the existence of this virus in many parts of India in both humans and in a variety of animals.

Taxonomy and Classification

Vesiculoviruses are assigned to the genus *Vesiculovirus* in the family *Rhabdoviridae*, order *Mononegavirales*. Vesiculoviruses are enveloped and contain a nonsegmented, single (mono) strand of genomic RNA of negative sense. Virions display bullet-shaped morphology, which is typical of viruses included in the family *Rhabdoviridae* (rhabdo in Greek means rod-shaped) (**Figure 1(a)**). Other genera of the family *Rhabdoviridae* include *Lyssavirus*, *Ephemerovirus*, *Novirhabdovirus, Cytorhabdovirus*, and *Nucleorhabdovirus*, and these include viruses that are important pathogens of ruminants, fish and plants, and human pathogens such as rabies virus. *Vesicular stomatitis Indiana virus* (VSIV), *Vesicular stomatitis New Jersey virus* (VSNJV), and *Chandipura virus* (CPHV) have been formally recognized as distinct species within the genus *Vesiculovirus*. Virus species that have been classified in the genus *Vesiculovirus* are presented in **Table 1**. Other viruses that have been placed tentatively in the genus *Vesiculovirus* include important viruses such as spring viremia of carp virus, Tupaia virus, and Calchaqui virus.

Viral Epidemiology

Although CHPV was first isolated in 1965, it was only in 2003 that the first evidence for its association in human epidemic was obtained during acute encephalitis outbreaks. One outbreak took place in the state of Andhra Pradesh and affected 329 children, resulting in 183 deaths. Simultaneously, another encephalitis outbreak was reported in the state of Maharashtra, resulting in about 400 encephalitis cases with 115 deaths. Subsequently, another outbreak of encephalitis associated with CHPV infection was reported in 2004 in the eastern state of Gujarat with a fatality rate of more than 75%. These recent outbreaks indicate the emergence of CHPV as a deadly human pathogen in the Indian subcontinent. Phylogenetic analysis has indicated

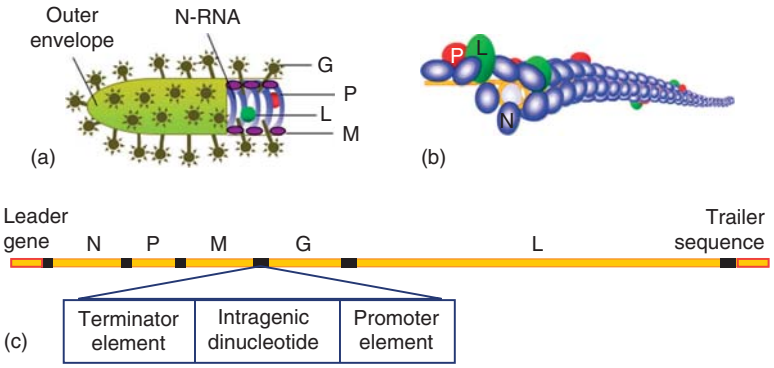


Figure 1 Vesiculoviruses: a general overview. A general overview of the vesiculoviruses with particular reference to Chandipura virus (a) Schematic presentation of bullet-shaped CHPV. Outer membrane envelope and relative organization of N-RNA nucleocapsid, glycoprotein G, phosphoprotein P, large protein L, and matrix protein M within virion structure have been indicated; (b) Nucleocapsid protein enwrapped genome RNA along with associated phosphoprotein P and large protein L forms a helical structure; (c) CHPV genome RNA encoding leader gene, five protein-coding genes, and gene junctions. Relative arrangement of the *cis*-acting elements within the gene junction has been indicated.

Table 1 Species within the genus *Vesiculovirus*

Species name	*Abbreviation*	*Original isolation*	*Natural host(s)*
Vesicular stomatitis Indiana virus	VSIV	USA, 1942; *North Am. Veterinarian* 26: 726–730	Horses, cattle, swine, sandflies,
Vesicular stomatitis New Jersey virus	VSNJV	USA, 1943; *North Am. Veterinarian* 26: 726–730	Horses, cattle, swine, sandflies, mosquitoes
Piry virus	PIRYV	Brazil, 1960; USPHS Publication No. 1760 Government Printing Office 1967	Human, cattle, mosquitoes
Cocal virus	COCV	Trinidad, 1964; *Am. J. Vet. Res.* 1964 Jan; 25: 236–42	Horses, cattle, insects
Chandipura virus	CHPV	India, 1965; *Indian J. Med. Res.* 1967 Dec; 55(12): 1295–305	Human, cattle, sandflies,
Vesicular stomatitis Alagoas virus	VSAV	Brazil, 1967; *Res. Vet. Sci.* 1967, 8; 103–117	Human, sandflies, mosquitoes
Isfahan virus	ISFV	Iran, 1975; *Am. J. Trop. Med. Hyg.* 1977 Mar; 26(2): 299–306	Human, gerbils, sandflies
Maraba virus	MARAV	Brazil, 1984; *Am. J. Trop. Hyg.* 1984 Sep; 33(5): 999–1006	Sandflies
Carajas virus	CJSV	Brazil, 1984; *Am. J. Trop. Hyg.* 1984 Sep; 33(5): 999–1006	Sandflies

circulation in the population during these recent epidemics of multiple strains of CHPV which are subtly different from the original Nagpur strain.

The symptoms of CHPV encephalitis include high fever, generalized convulsions, vomiting, diarrhea, decerebrate posture leading to coma, neurological deficit, and acute encephalitis. During the outbreaks Chandipura encephalitis affected children below 15 years of age and resulted in a high mortality rate. Electron microscopy, complementation fixation and neutralization tests, as well as molecular biological techniques, were performed to examine the existence of Chandipura virus in the sera obtained from patients. The presence of anti-CHPV IgM antibodies and viral RNA was confirmed in the serum to implicate CHPV as an important etiological agent in the outbreak. Subjects infected with CHPV were shown to have an elevated level of cytokines in the blood that is thought to result in the damage to the central nervous system, and CHPV antigen and RNA was found in the brain tissue of the infected patients.

The prevalence of anti-CHPV antibodies in animal sera indicates that domestic animals might serve as a natural reservoir for this pathogen. Indeed, the presence of neutralizing antibodies to Chandipura virus was confirmed in 30.6% of pig, 14.3% of cattle, 17.9% of buffalo, 9.3% of goat, and 7.7% of sheep serum samples collected from the epidemic affected areas. The sandfly has been long recognized as a potential vector for the transmission of CHPV. The virus was isolated from a pool of 253 sandflies of the genus *Phlebotomus* in 1969. Transovarial transmission has

also been established in *Phlebotomus papatasi*. However, investigations carried out in the outbreak-hit areas suggest the involvement of sandflies of the genus *Sergentomyia* in the transmission of the pathogen.

Chandipura Virus Structure and Composition

At the molecular level, VSIV is the most extensively studied member of the family *Rhabdoviridae*. However, research on VSNJV and CHPV has also allowed us to gain further molecular insights into vesiculovirus' replication. The recent association with epidemics have emphasized the necessity to develop better understanding of the mechanism of Chandipura virus pathogenesis. Below, we summarize our current knowledge on the structure and replication of CHPV at the molecular level.

The CHPV virion is a bullet-shaped particle that is ~150 nm long and ~60 nm wide with a ~10 nm distinct surface projection. It has two compartments, the outer envelope and the inner nucleocapsid structure. The helical nucleocapsid is composed of a 11 119-nucleotide (nt) single-stranded genome RNA that is tightly wrapped with ~1200 molecules of nucleocapsid protein N (mol. wt. 50 kDa). In addition, ~50 molecules of large protein L (mol. wt. 241 kDa) and ~500 molecules of phosphoprotein P (mol. wt. 32.5 kDa) remain associated with the N-RNA complex. The envelope is a membranous structure that surrounds the nucleocapsid and contains an embedded transmembrane glycoprotein G that protrudes externally from the membrane surface. The matrix protein M lies at the inner side of the membrane and tethers the nucleocapsid to the envelope (**Figures 1(a)** and **1(b)**).

Genetic Makeup and Gene Structure

Vesiculoviruses exemplify one of the simplest genome architectures among the *Mononegavirales*. The entire CHPV genome has been sequenced and comparison with other vesiculoviruses has confirmed similar genetic makeup among vesiculoviruses. The Chandipura virus RNA genome comprises a 49-nt long leader sequence (l), followed by five transcriptional units coding for viral polypeptides separated by intergenic, nontranscribed spacer regions, and a short nontranscribed trailer sequence (t) arranged in the order 3′ l-N-P-M-G-L-t 5′ (**Figure 1(c)**). The CHPV gene junctions encompass a 23-nt long sequence that is highly conserved among different vesiculoviruses; this conserved sequence is comprised of a 11-nt gene end signal that regulates transcription termination and polyadenylation of viral mRNA, a 2 nt nontranscribed spacer region, and a 10 nt conserved promoter element that has been implicated in the transcription initiation. Comparative sequence analysis has indicated CHPV to be evolutionarily equidistant from the New World vesiculoviruses VSIV and VSNJV and closely related to the Asian vesiculovirus Isfahan.

Replication Cycle

The vesiculovirus replication cycle can be divided into nine discrete steps, namely: (1) adsorption of the virus particle; (2) viral entry into the host cell via receptor-mediated endocytosis; (3) membrane fusion and release of the core RNP into the cytosol from endosomal vesicles; (4) transcription of the genome by viral polymerase; (5) translation of viral mRNA; (6) post-translational modifications of viral proteins; (7) replication of viral genome; (8) assembly of progeny particles; and finally, (9) budding of the mature virion (**Figure 2**). The entire vesiculovirus replication cycle is cytosolic. Genomic RNA enwrapped with nuleocapsid protein N acts as a template during transcription to produce short leader RNA (l) and five monocistronic capped and polyadenylated viral mRNAs. Viral RNA-dependent RNA polymerase (RdRp) is composed of large protein L, which is the catalytic subunit and phosphoprotein P that acts as a regulatory subunit. Translation of viral mRNA results in the accumulation of viral polypeptides within the infected cell to prepare for the onset of genome replication. During replication, the same polymerase copies the entire genomic template into an exact polycistronic complement that acts as replication intermediate to produce multiple copies of negative-sense genome upon further cycles of replication. Subsequently, progeny of negative-sense genomic material is either packaged within the mature virion or subjected to further transcription, often referred to as secondary transcription. Notably, virus-specific genomic analogs always remain encapsidated by N protein; it is believed that the N-mediated enwrapment of the genomic RNA protects RNA from cellular RNases (**Figure 2**).

Viral Invasion

The receptor utilized by Chandipura virus to invade into the host cell has not yet been characterized. However, studies on VSIV have provided valuable information on the entry mechanism used by vesiculoviruses, in general. Consistent with its broad host range, the receptor utilized by VSIV is thought to be phosphatidyl serine, a ubiquitously present membrane lipid. Subsequent to the attachment, clathrin-mediated endocytosis triggers entry of the virus particle into the host cell through the endosomal pathway, which is then followed by G protein-mediated fusion of the virus particle with the endosomal membrane and release of the core nucleocapsid into the host cytoplasm.

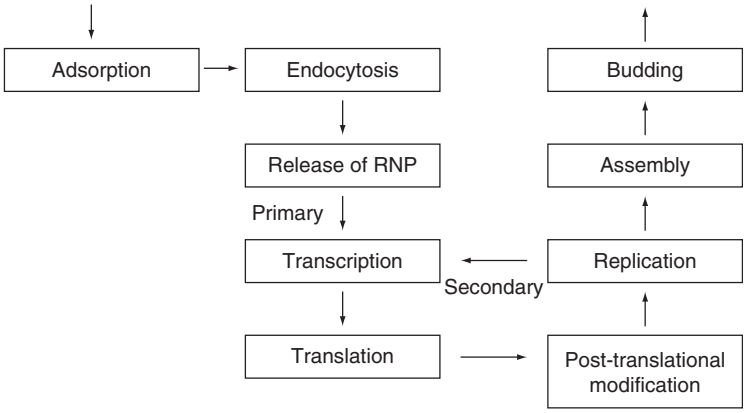


Figure 2 Vesiculovirus replication cycle. Graphical presentation of nine steps of the vesiculovirus replication cycle with particular reference to Chandipura virus. Transcription from the nucleocapsid particle released from the infecting virion, also referred to as primary transcription, as well as transcription from nucleocapsid particles synthesized *de novo* within infected cells, also referred to as secondary transcription, have been indicated in the figure.

Role of Glycoprotein G

Glycoprotein G is the sole spike protein that has been implicated in adsorption as well as budding of Chandipura virus. Studies on CHPV G protein expressed from a cloned DNA have revealed the presence of an N-terminal cleavable signal peptide, an N-terminal ectodomain with two N-linked glycosylation sites, a membrane anchor domain, and a cytosolic domain at the C-terminus. It has been proposed that a low pH-induced conformational change in G protein within the endosomal environment subsequent to viral entry results in membrane fusion to release core nucleocapsid particle into the host cytoplasm. The ectodomain of glycoprotein G mediates critical steps in membrane fusion. It is interesting to note that almost all the mutations in the G protein sequences identified in epidemic-associated virus strains were within this ectodomain.

Role of Matrix Protein M

Matrix protein M glues the core nucleocapsid particle to the outer membrane within the mature virion. The highly basic N-terminal domain of the M protein is primarily responsible for membrane association, although additional domains have been proposed to contribute in membrane binding.

Chandipura virus makes use of the fact that host mRNA is synthesized in the nucleus, whereas viral mRNA synthesis occurs in the cytoplasm to promote preferential translation of viral mRNAs. To achieve this, CHPV utilizes M protein to inhibit nuclear export of host mRNAs and thus channel host machinery towards translating viral mRNAs. Furthermore, M proteins of both VSIV and CHPV have been shown to shut off RNA polymerase I- and II-mediated transcription within infected cells. Not surprisingly, M protein alone was shown to account for cytotoxicity in virus-infected cells.

Gene Expression

Sequential and Polar Transcription

Vesiculovirus gene expression illustrates two distinct well-conserved characteristics; namely sequential and polar transcription. Vesiculovirus mRNAs are synthesized in an obligatory sequential manner after polymerase entry at a single site at the 3′ end of the genome, that is, at the beginning of leader RNA. Also, measurement of molar ratios of different VSIV mRNAs indicated that their abundance decreases with increasing distance from the polymerase entry site in the order $N > P > M > G > L$. To explain this polar and sequential mRNA synthesis, a stop–start model has been postulated for vesiculovirus transcription (**Figure 3**). In this model, subsequent to the entry into the N-RNA template, viral RdRp sequentially transcribes the genome with progressive attenuation at each gene boundary, which results in decreasing amounts of transcripts for genes that are distant from the entry site. Each termination event may cause the polymerase to fall off the template or may allow for re-initiation at the downstream promoter. Therefore, transcription of the downstream genes depends on the termination of the upstream gene transcription and re-initiation processes. Genomic RNA enwrapped with N protein into a nucleocapsid structure and not the naked viral RNA is recognized by the polymerase as a template for RNA synthesis. *In vitro* transcription reconstitution as well as *in vivo* studies have suggested that the proposed mechanism for vesiculovirus transcription is mostly conserved in CHPV system (**Figure 3**(**a**)).

Viral RdRp

CHPV RdRp is composed of the L and P proteins. Catalytic activities for RNA polymerization, capping, and polyadenylation reside within the L protein. The entire ORF

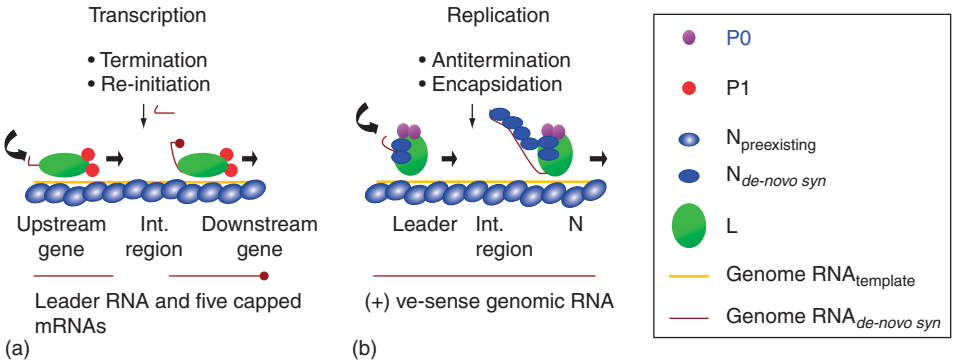


Figure 3 Vesiculovirus gene expression. Schematic diagram to depict Chandipura virus gene expression. (a) Stop–start model to describe vesiculovirus transcription, where, polymerase terminates and re-initiates at each gene junction to sequentially synthesize leader RNA and five mRNAs. (b) Viral replication that is characterized by the antitermination at each gene junction with concomitant encapsidation of the nascent replication product by nucleocapsid protein N. A role for unphosphorylated P protein (P0) has been postulated during replication.

encoding the CHPV L gene has been sequenced. The L protein exhibits a distinct structural module similar to that of a right hand, with palm, thumb, and finger domains. The palm domain structure is particularly conserved among almost all known RdRps. However, L protein only in association with the phosphorylated form of the P protein, and not L alone, acts as viral transcriptase.

Phosphoprotein P in Transcription

The P protein is packaged within the mature virion particle and remains associated with the viral nucleocapsid. Subsequent to the release of core nucleocapsid into host cytoplasm, the P protein plays an essential role in viral transcription. The P protein through its C-terminal domain interacts with both N-RNA template and the L protein, thus assembling transcriptase complex on the genomic template. The P protein utilizes its N-terminal acidic domain to interact with and activate polymerase function during transcription, similar to the activation mediated by classical eukaryotic transcription activators. The CHPV P protein exists in the virus-infected cells in two distinct states; the unphosphorylated form has been designated as P0, while its phosphorylated counterpart is referred to as P1. Phosphorylation of P at a distinct site is required for P to act as a transcriptional activator. Further investigations identified casein kinase II (CKII) as the host kinase responsible for phosphorylating P at specific residues. Biochemical and mutational analysis identified ser62 in the CHPV P protein as a single phosphorylation site both *in vitro* and *in vivo*.

How does phosphorylation regulate P protein function as a transcriptional activator? Studies on CHPV have revealed that the phosphorylated P protein forms a dimer. The N-terminal 46 amino acids were implicated in the dimerization of phosphorylated protein. It has been proposed that the unphosphorylated P protein exists as a monomer, whereas, dimerization upon phosphorylation enables P to act as a transcriptional activator. However, the unphosphorylated form of CHPV P protein has also been shown to form a dimer in a concentration-dependent manner. Recent structural analysis also indicated that a central domain that is conserved among different vesiculoviruses mediates dimer assembly of the VSIV-P protein in its unphosphorylated form. Therefore, dimerization alone is inadequate to explain transcriptional activation by phosphorylated P. Interestingly, phosphorylation at ser62 residue has been shown to promote a major conformational change in the P protein that exposes the N-terminal coiled-coil motif on the surface of protein tertiary structure. In light of the available information, it seems possible that the central domain assembles P into a dimer, whereas, additional interactions mediated through the N-terminal domain may stabilize such a dimer assembly. Conformational changes within the N-terminal domain of P upon phosphorylation and net negative charge attributed by the incorporated phosphate have been proposed to facilitate precise polymerase (L) contact by P1, resulting in optimal transcription.

mRNA Modification by Viral Transcriptase

One unique feature of vesiculovirus gene expression is the utilization of a single protein (L) for mRNA synthesis, 5′ end modification, and 3′ end polyadenylation of the mRNA. With the exception of leader RNA, all other viral transcripts possess a 5′ end cap structure, similar to that observed in cellular mRNAs. Although certain host factors have been postulated to participate in the capping reaction, primary enzymatic activity that mediates viral mRNA capping resides within the L protein. Viral transcriptase is also involved in the polyadenylation of viral mRNA. Polymerase slippage during transcription termination at the U7 tract present in the gene boundaries

results in addition of a poly(A) tail at the 3′ end of viral mRNAs. However, our current knowledge of mRNA modifications mostly relies upon contemporary studies that have been conducted in the VSIV system, due to the lack of a recombinant CHPV L protein expression system.

Replication of the Genome RNA

Transcription–Replication Switch

During transcription, the viral polymerase obeys the termination signals present at the gene boundaries to produce discrete viral mRNAs, whereas the same polymerase antiterminates at the gene boundaries during the replication phase to produce a positive-sense polycistronic complement of the entire genome. Subsequent rounds of replication that utilize the positive-sense replication intermediate as template produce many more copies of the progeny, negative-sense genomic RNA, which, after packaging buds out as a mature virion (**Figure 2**). The switch in polymerase function that allows it to ignore termination signals during replication is known as the transcription–replication switch. Although the same L protein is involved in RNA polymerization during both transcription and replication the phosphorylation state of the regulatory subunit P is proposed to be different in these two processes.

Role of Unphosphorylated P Protein

Recent studies in both CHPV and VSIV systems have indicated a role for unphosphorylated P protein in transcription–replication switch. A tripartite complex composed of a phosphorylation-defective mutant of P, N, and L was shown to efficiently synthesize VSIV replication product *in vitro*. It was postulated that the formation of L–N–P0 complex enables L protein to recognize promoter elements for replication and suppress the transcription termination signal present at the gene boundaries during VSIV replication. Similarly, *in vivo* analysis revealed that the N-terminal phosphorylation of P protein, although necessary for transcription, was dispensable for CHPV or VSIV replication. Indeed, a preexisting pool of phosphorylation-defective mutant of CHPV P protein (Ser62Ala) was shown to boost viral replication with a two-log increase in the virus yield upon subsequent infection with the wild-type virus. Therefore, the form of P protein (P1) engaged in transcription appears to be distinct from the form (P0) that has been implicated in replication.

However, another intriguing observation connected the proposed role of P0 in viral replication with the events on the nascent leader RNA chain. In an *in vitro* RNA-binding assay, it has been shown that P protein in its unphosphorylated form binds to leader RNA, whereas, CKII-mediated phosphorylation abrogated its RNA-binding ability. Leader RNA–P0 interaction was observed to be distinct from the N-mediated encapsidation. Similar phosphoregulation of RNA-binding ability of P protein has also been reported for rinderpest virus, a paramyxovirus. Accordingly, a refined model has been proposed to explain Chandipura virus genome replication. In this proposal, recruitment on the nascent leader chain brings P0 to the vicinity of the transcribing polymerase to promote P0 association with the polymerase. Such P0–L interaction alters the polymerase conformation in a manner that enables read-through at the gene boundaries. Nonetheless, progressive encapsidation of nascent viral RNA by CHPV N has been proposed to maintain processivity of such a replicase complex (L–P0) to allow for the synthesis of ∼11.1 kb positive-sense genome (**Figure 3(b)**). Curiously, recent analysis identified a conserved Glu64Asp mutation in the CKII consensus site within the N-terminal region of P protein in all epidemic isolates. In the light of the proposed function of P0 in viral replication, functional consequences of such mutations need to be further explored.

Genome Encapsidation and Assembly

Genome Encapsidation

Viral replication intermediates or replication products always remain encapsidated by N protein within infected cells and enjoy protection from cellular RNases. Consistently, vesiculovirus replication critically relies upon *de novo* synthesis of N protein. However, N protein is prone to aggregation and this aggregated form is unable to appropriately encapsidate viral RNA. Phosphoprotein P, by virtue of its N-specific chaperone-like activity, prevents self-aggregation of N protein and maintains it in an encapsidation-competent state. *Cis*-acting elements present within the first 20 nucleotides from the 5′ end of the positive-sense anti-genome and also at the 5′ half of the trailer region of the negative-sense genome, have been implicated in the initiation of encapsidation. CHPV N protein, in its monomeric form, possesses intrinsic RNA-binding specificity and is able to recognize *cis*-elements present in the genome RNA. However, this RNA-binding specificity has been shown to be compromised upon homooligomerization of N protein. Accordingly, a model has been proposed to provide mechanistic insight into the encapsidation process. In this model, monomer N initiates encapsidation by recognizing specific sequence element present in the nascent viral RNA. Subsequently, N–N interactions allow oligomer N to continue the elongation phase of the encapsidation process to enwrap diverse RNA sequences into the nucleocapsid structure. It is believed that the ensuing encapsidation of nascent RNA by N protein may also facilitate antitermination during replication by opposing polymerase pausing at the gene boundaries. Nevertheless, the major role of

N protein in viral replication cycle remains to protect viral RNA from cellular RNases and present genomic RNA in a specialized helical form that is recognized by viral polymerase.

Assembly and Budding

Progeny genomic RNA molecules enwrapped in the nucleocapsid structures associate with matrix protein M and are subjected to further M-mediated condensation. Subsequently, M protein participates in recruiting mature nucleocapsid particles to the membrane during assembly and budding in a manner that is dependent on the cellular ubiquitin–proteasome system, at least in the VSIV system. *De novo*-synthesized G protein is organized in the plasma membrane of virus-infected cells into microdomains that serve as assembly sites for the viral components.

Concluding Remarks

Our current understanding of the discrete steps of the CHPV replication cycle relies on the studies being conducted on the CHPV as well as on other vesiculoviruses. Remarkable similarities between CHPV and VSIV in genetic makeup, gene structure, polypeptide composition as well as function allow the postulation of a generalized model to describe vesiculovirus transcription at the molecular detail. While the proposed mechanism for CHPV replication that involves RNA binding by the phosphoprotein needs to be tested in the VSIV system, existence of distinct mechanisms in different serotypes of vesiculoviruses is possible. Nevertheless, information obtained from the molecular analysis of the epidemic-associated strains in conjunction with the large body of information that is available on N–P, N–leader RNA, or P0–leader RNA interactions should be utilized to further understand CHPV pathogenesis. While retaining general academic interest to understand RNA synthesis in rhabdoviruses, future CHPV research should also focus on development of the potential antiviral therapeutic interventions that will allow for specific inhibition of virus multiplication.

See also: Rabies Virus.

Further Reading

Banerjee AK (1987) Transcription and replication of rhabdoviruses. *Microbiological Reviews* 51(1): 66–87.

Barr JN, Whelan SP, and Wertz GW (2002) Transcriptional control of the RNA-dependent RNA polymerase of vesicular stomatitis virus. *Acta Biochimica et Biophysica Hungarica* 1577(2): 337–353.

Basak S, Mondal M, Polley S, Mukhopadhyay S, and Chattopadhyay DJ (2007) Reviewing Chandipura: A vesiculovirus in human epidemics. *Bioscience Reports* 27(4–5): 275–298.

Mishra AC (2007) Chandipura encephalitis: A newly recognized disease of public health importance in India. In: Scheld WH, Hooper DC, and Hughes JM (eds.) *Emerging Infections,* pp. 121–137. Washington, DC: ASM Press.

Chrysoviruses

S A Ghabrial, University of Kentucky, Lexington, KY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Hyphal anastomosis The union of a hypha with another resulting in cytoplasmic exchange.
Mycoviruses Viruses that infect and multiply in fungi.
Viruses with multipartite genomes The essential genome is divided among several genomic segments (segmented genome) that are either separately encapsidated in identical capsids (multicomponent viruses, i.e., chrysoviruses) or jointly enclosed in a single particle (e.g., reoviruses).

Introduction

The discovery in the late 1960s and early 1970s of polyhedral virus particles in many of the industrial strains of *Penicillium chrysogenum* used for penicillin production generated considerable interest in the study of *Penicillium* viruses. It was surmised then that virus infection might be responsible for the instability of some of these strains. *Penicillium chrysogenum virus* (PcV), the type species of the genus *Chrysovirus*, was one of the first mycoviruses to be extensively studied at the biochemical, biophysical, and ultrastructural levels. Although the results of these earlier studies on PcV properties were mostly in agreement, they differed in their explanation of the nature of genome

complexity. Although PcV and the related *Penicillium* viruses penicillium brevicompactum virus (PbV) and penicillium cyaneo-fulvum virus (Pc-fV) have similar isometric particles, 35–40 nm in diameter, and are serologically related, there was confusion as to whether they contain three or four double-stranded RNA (dsRNA) segments. Because none of these viruses was characterized at the molecular level until recently, these three *Penicillium* viruses were originally grouped under the genus *Chrysovirus* and provisionally placed in the family *Partitiviridae* with the assumption that their genomes are bipartite, with dsRNA1 encoding the RNA-dependent RNA polymerase (RdRp) and dsRNA2 encoding the major capsid protein (CP). The additional dsRNAs (dsRNAs 3 and/or 4), like those of some partitiviruses, were presumed to be defective or satellite dsRNAs. The classification of the genus *Chrysovirus* was recently reconsidered because the complete nucleotide sequence and genome organization of each of the four monocistronic dsRNA segments associated with PcV virions and with the chrysovirus Helminthosporium victoriae 145S virus were recently reported. Based on the consistent simultaneous presence of their four dsRNA segments, the existence of extended regions of highly conserved terminal sequences at both ends of all four segments, sequence comparisons and phylogenetic analysis, it became clear that PcV and related viruses should not be classified with the family *Partitiviridae*. This led to the creation of the new family *Chrysoviridae* to accommodate the isometric dsRNA mycoviruses with multipartite genomes. The name *Chryso* is derived from the specific epithet of *Penicillium chrysogenum*, the fungal host of the type species.

Virion Properties

The buoyant densities of virions of members in the family *Chrysoviridae* are in the range of 1.34–1.39 g cm^{-3} and their sedimentation coefficients $S_{20,w}$ (in Svedberg units) are in the range of 145S to 150S. Generally, each virion contains only one of the four genomic dsRNA segments. However, purified preparations of PcV and Pc-fV can contain minor distinctly sedimenting components that include empty particles and replication intermediates.

Chrysovirus particles possess virion-associated RNA-dependent RNA polymerase (RdRp) activity, which catalyzes the synthesis of single-stranded RNA (ssRNA) copies of the (+) strand of each of the genomic dsRNA molecules. The *in vitro* transcription reaction occurs by a conservative mechanism, whereby the released ssRNA represents the newly synthesized plus strand.

Genome Organization

The virions of members of the family *Chrysoviridae* contain four unrelated linear, separately encapsidated, monocistronic dsRNA segments (2.4–3.6 kbp in size; **Table 1**). the largest segment, dsRNA1, codes for the RdRp and

Table 1 List of members and tentative members in the family *Chrysoviridae*

Virus species	*Abbreviation*	*DsRNA segment no. (length in bp, encoded protein; size in kDa)*	*GenBank accession no.*
Helminthosporium victoriae 145S virus	Hv145SV	1 (3612; RdRp,125)	NC_005978
		2 (3134; CP, 100)	NC_005979
		3 (2972; chryso-P4, 93)	NC_005980
		4 (2763; chryso-P3, 81)	NC_005981
Penicillium brevicompactum virus	PbV	Four dsRNA segments; no molecular data	
Penicillium chrysogenum virus	PcV	1 (3562; RdRp,129)	NC_007539
		2 (3200; CP, 109)	NC_007540
		3 (2976; chryso-P3 101)	NC_007541
		4 (2902; chryso-P4, 95)	NC_007542
Penicillium cyaneo-fulvum virus	Pc-fV	Four dsRNA segments; no molecular data	
Tentative members			
Agaricus bisporus virus 1 or LaFrance isometric virus	AbV-L1	L1 (3396; RdRp, 122)	X94361
	LFIV	L5 (2455; unknown, 82)	X94362
Amasya cherry disease associated chrysovirus	ACDACV	1 (3399; RdRp, 124)	AJ781166
		2 (3128; CP, 112)	AJ781165
		3 (2833; chryso-P4, 98)	AJ781164
		4 (2498; chryso-P3, 77)	AJ781163
Cherry chlorotic rusty spot associated chrysovirus	CCRSACV	1 (3399; RdRp, 124)	AJ781397
		2 (3125; CP, 112)	AJ781398
		3 (2833; chryso-P4, 98)	AJ781399
		4 (2499; chryso-P3, 77)	AJ781400

dsRNA2 codes for the major CP. The dsRNA segments 3 and 4 code for proteins of unknown function. The genomic structure of PcV, the type species of the genus *Chrysovirus*, comprising four dsRNA segments, is schematically represented in **Figure 1**. The earlier conflicting reports on whether PcV contains three or four segments were recently explained when studies on cDNA cloning and sequencing of the viral dsRNAs were completed. Although the dsRNA extracted from purified virions is resolved into three bands by agarose gel electrophoresis (**Figure 2**, lane EB), northern hybridization analysis using cloned cDNA probes representing the four dsRNA segments shows clearly that each of the four segment has unique sequences (**Figure 2**). Because dsRNAs 3 and 4 differ in size by only 74 bp (**Table 1**), they co-migrate when separated by agarose gel electrophoresis. Previous studies on sequencing analysis and *in vitro* coupled transcription–translation assays showed that each of the four dsRNAs is monocistronic, as each dsRNA contains a single major open reading frame (ORF) and each is translated into a single major product of the size predicted from its deduced amino acid sequence. Thus, the fact that PcV virions contain four distinct dsRNA segments has clearly been established.

Unlike PcV, dsRNAs 3 and 4 from other chrysoviruses, helminthosporium victoriae 145SV (Hv145SV), amasya cherry disease associated chrysovirus (ACDACV) and cherry chlorotic rusty spot associated chrysovirus (CCRSACV) are clearly resolved from each other when purified dsRNA preparations are subjected to agarose gel electrophoresis. As shown in **Table 1**, dsRNAs 3 and 4 from these viruses are significantly different in size. Assignment of numbers 1–4 to PcV dsRNAs was made according to their decreasing size. Following the same criterion used for PcV, the dsRNAs associated with Hv145SV, CCSRACV, and ACDACV were accordingly assigned the numbers 1–4. Sequence comparisons, however, indicated that dsRNAs 3 of Hv145SV, CCSRACV, and ACDACV are in fact the counterparts of PcV dsRNA4 rather than dsRNA3. Likewise, dsRNA4 of these three chrysoviruses are the counterparts of PcV dsRNA3. Since PcV was the first chrysovirus to be characterized at the molecular level and to avoid confusion, the protein designations P3 and P4 as used for PcV will be adopted and referred to as

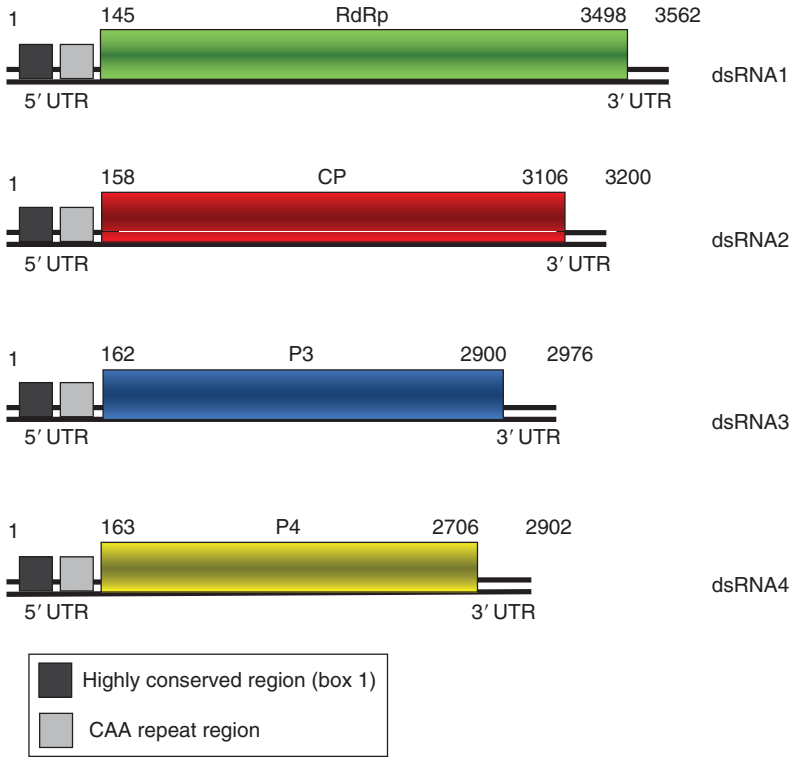


Figure 1 Genome organization of penicillium chrysogenum virus (PcV), the type species of the genus *Chrysovirus*. The genome consists of four dsRNA segments; each is monocistronic. The RdRp ORF (nt positions 145–3498 on dsRNA1), the CP ORF (nt positions 158–3106 on dsRNA2), the p3 ORF (nt positions 162–2900 on dsRNA3), and the p4 ORF (nt positions 163–2706 on dsRNA4) are represented by rectangular boxes. Adapted from Ghabrial SA, Jiang D, and Castón RJ (2005) *Chrysoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses,* pp. 591–595. London: Academic Press, with permission from Elsevier.

chryso-P3 and chryso-P4. Thus, whereas the chryso-P3 protein represents the gene product of PcV dsRNA3, it comprises the corresponding gene product of Hv145SV dsRNA4, and so on.

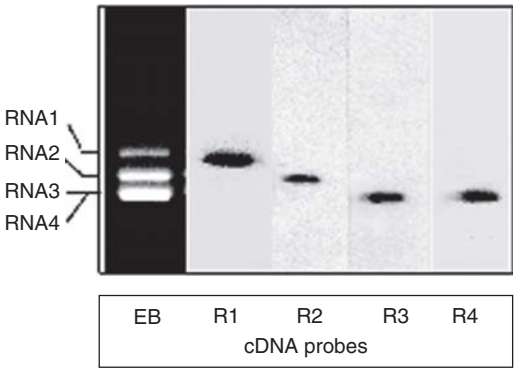


Figure 2 Northern hybridization analysis of PcV dsRNA segments. Virion dsRNAs were separated on a 1.5% agarose gel, transferred onto Hybond-N$^+$ membrane and hybridized with ^{32}P-labeled probes prepared by random-primer labeling of cloned cDNA to PcV dsRNA1 (R1), dsRNA2 (R2), dsRNA3 (R3), and dsRNA4 (R4). EB, ethidium bromide-stained virion dsRNAs separated on a 1.5% agarose gel. Reproduced from Jiang D and Ghabrial SA (2004) Molecular characterization of penicillium chrysogenum virus: Reconsideration of the taxonomy of the genus chrysovirus. *Journal of General Virology* 85: 2111–2121, with permission from Society for General Microbiology.

Except for CCRSACV and ACDACV dsRNAs, the 5′ UTRs of chrysovirus dsRNAs are relatively long, between 140 and 400 nucleotides in length. In addition to the strictly conserved 5′- and 3′-termini, a 40–75 nt region with high sequence identity is present in the 5′ UTR of all four dsRNAs (box 1; **Figure 3**). A second region of strong sequence similarity is present immediately downstream from 'box 1' (**Figure 3**). This consists of a stretch of 30–50 nt containing a reiteration of the sequence 'CAA'. The $(CAA)_n$ repeats are similar to the enhancer elements present at the 5′ UTRs of tobamoviruses. Although the 5′ UTR of CCRSACV and ACDACV contain the 'CAA' repeat region upstream of the translation initiation codon, the 'box 1' region is significantly shortened. Furthermore, these viruses do not share with PcV and Hv145SV the strictly conserved terminal 8 and 7 nucleotides, respectively at the 5′ and 3′ ends (**Table 2**). The discrepancies in the length and features of the 5′ and 3′ UTRs of CCRSACV and ACDACV dsRNAs compared to those of PcV and Hv145SV could be due to the cloning procedure used, which may not have allowed for the exact termini to be cloned. This, however, seems unlikely because of the strong similarities of the termini and internal sequences of the 5′ and 3′ UTRs of all four CCRSACV and ACDACV dsRNA segments. Alternatively, the divergence in the features of the 5′ and 3′ UTRs of ACDACV and CCRSACV may be because

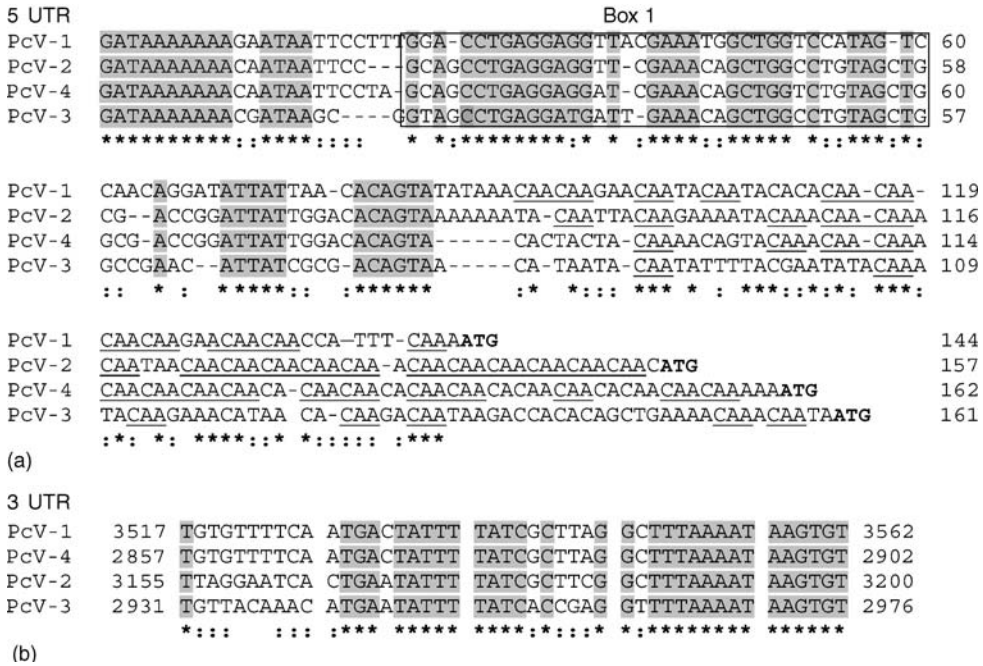


Figure 3 Comparison of the 5′ and 3′ UTRs of the four dsRNA segments of PcV. Multiple sequence alignments were obtained using CLUSTAL X (and some manual adjustments) with the nucleotide sequences of the 5′ UTR (a) and the 3′ UTR (b). The (CAA) repeats are underlined in (a). Asterisks signify identical bases at the indicated position (shaded) and colons specify that three out of four bases are identical at the indicated positions. Reproduced from Jiang D and Ghabrial SA (2004) Molecular characterization of penicillium chrysogenum virus: Reconsideration of the taxonomy of the genus chrysovirus. *Journal of General Virology* 85: 2111–2121, with permission from Society for General Microbiology.

Table 2 Comparison of the nucleotide sequences at the 5′ and 3′ termini of chrysovirus dsRNAs[a]

	5′-terminus	*3′-terminus*
Hv145SV		
dsRNA1	5′-GAUAAAAAGAAAAA-U..	.. UUAGGACUUUAAGUGU-3′
dsRNA2	5′-GAUAAAAACAAAAAAU..	.. UUCGGACUUUAAGUGU-3′
dsRNA3	5′-GAUAAAAACAGAAAAU..	.. UUCGGACUUUAAGUGU-3′
dsRNA4	5′-GAUAAAAACAGAAAAU..	.. UGCGGACUUUAAGUGU-3′
PcV		
dsRNA1	5′-GAUAAAAAAAGAAUAA..	.. GCUUUAAAAUAAGUGU-3′
dsRNA2	5′-GAUAAAAAAACAAUAA..	.. GCUUUAAAAUAAGUGU-3′
dsRNA3	5′-GAUAAAAAAACGAUAA..	.. GCUUUAAAAUAAGUGU-3′
dsRNA4	5′-GAUAAAAAAACGAUAA..	.. GUUUUAAAAUAAGUGU-3′
CCRSACV		
dsRNA1	5′-GAAAUUAUGGUUUUUG..	.. AUUGUCAAUAAUAUGC-3′
dsRNA2	5′-GAAAUUAUGGUUUUUG..	.. GUGUUGAUAUAUAUGC-3′
dsRNA3	5′-GAAAUUAUGGUUUUUG..	.. GGUUAUAACUAUAUGC-3′
dsRNA4	5′-GAAAUUAUGGAUUUUG..	.. AUGUGUAACUAUAUGC-3′

[a]Identical nucleotides in the same position are shaded.

they represent plant rather than fungal chrysoviruses (see section on 'Biological properties').

Genome Expression and Replication

Chrysovirus dsRNA1s Code for RdRp

The largest dsRNA segment (dsRNA1) of the chrysoviruses so far sequenced contains a single large ORF coding for RdRp. The calculated molecular mass of chrysovirus RdRps ranges from 122 to 129 kDa (**Table 1**). These values are consistent with those estimated by SDS-PAGE of the *in vitro* translation products of full-length transcripts derived from cDNAs to dsRNA1s of PcV and Hv145SV. Examination of the deduced amino acid sequence of the RdRp ORF reveals the presence of the eight conserved motifs characteristic of RdRps of dsRNA viruses of simple eukaryotes (**Figure 4**). A comparison of the conserved motifs of chrysovirus RdRps with those of totiviruses and partitiviruses reveals that the RdRps of chrysoviruses are more closely related to those of totiviruses than to those of partitiviruses (**Figure 4**). This conclusion is also supported by published results of phylogenetic analysis of RdRp conserved motifs and flanking sequences of chrysoviruses and viruses in the families *Totiviridae* and *Partitiviridae* (see section on 'Evolutionary relationships').

Chrysovirus dsRNA2s Code for CP

The second largest dsRNA segment (dsRNA2) of chrysoviruses so far sequenced contains a single large ORF coding for CP. The calculated molecular mass of chrysovirus CPs ranges from 100 to 112 kDa (**Table 1**). The predicted size of PcV CP (109 kDa) is similar to that estimated by SDS-PAGE of purified PcV virions as well as that determined for the *in vitro* translation product of full-length transcript of dsRNA2 cDNA. Direct evidence that PcV dsRNA2 encodes CP was provided by amino acid sequencing of a tryptic peptide derived from a gradient-purified PcV capsid.

Chryso-P3 Shares a 'Phytoreo S7 Domain' with Core Proteins of Phytoreoviruses

PcV dsRNA3 codes for its chryso-P3 protein, whereas Hv145SV, ACDACV, and CCRSACV dsRNA4s encode the corresponding chryso-P3s. Although the function of chryso-P3 is not known, sequence analysis and database searches offer some clues. ProDom database searches reveal that chryso-P3 sequences share a 'phytoreo S7 domain' with a family consisting of several phytoreovirus P7 proteins known to be viral core proteins with nucleic acid binding activities. The consensus for the three chrysoviruses is [X(V/I)V(M/L)P(A/M)G(C/H)GK(T/S)T-(L/I)]. Phytoreovirus P7 proteins bind to their corresponding P1 (transcriptase/replicase) proteins, which bind to the genomic dsRNAs. It is of interest, in this regard, that the N-terminal regions of all chryso-P3s (encompassing the amino acids within positions 1–500) share significant sequence similarity with comparable N-terminal regions of the putative RdRps encoded by chrysovirus dsRNA1s. A multiple alignment of a portion of the N-terminal region sequences of chrysovirus P3s and RdRps is shown in **Table 3** to demonstrate the level of similarity among the N-terminal sequences of these proteins. The regions in the dsRNA1-encoded proteins with high similarity to chryso-P3 occur upstream of the eight highly conserved motifs characteristic of RdRps of dsRNA viruses of simple eukaryotes. The significance of this sequence similarity to the function of chryso-P3 is not known for certain, but one may speculate that the N-terminal region of these proteins may play a role in viral RNA binding and packaging.

	1	2	3	4	5
SsRV1	LLGRA(61)	WCVNGSQND(47)	KL-EHG-KTRAIFACDTRSY (47)	LDFDDFNSHHS(45)	TLPSGHRGTTIVNSVLNAAYI(14)
Hv190SV	LQGRY(61)	WCVNGSQNA(42)	KL-ENG-KDRAIFACDTRSY (47)	LDYDNFNSQHS(45)	TLMSGHRATTFTNSVLNAAYI 14)
SsRV2	LQGRA(64)	WAVNGSQSG(46)	KL-EHG-KTRAIFACDTLNY (47)	LDYDDFNSHHS(46)	TLMSGRRGTTYISSVLNEVYL(14)
UmVH1	LYGRG(66)	WLVSGSSAG(55)	KLNETGGKARAIYGVTLWHY (47)	YDYPDFNSMHT(64)	GLYSGDRDTTLINTLLNIAYA(20)
	* **	* *:**:	** * * * ***::::: *	:*:::*** *:	:* ** * ** :::** :*:
Hv145SV	LLGRR(73)	WMTKGSLVS(56)	KLNENGHKDRVLLPGGLLHY (44)	YDWANFNVQHS (49)	GLYSGWRGTTWDNTVLNGCYM(20)
PCV	LVGRG(74)	WLTKGSLVY(60)	K-YEVGKKDRTLLPGTLVHF (44)	YDWADFNEQHS (49)	GLYSGWRGTTWINTVLNFCYV(19)
CCRSACV	LVGRR(73)	WLTKGSTVY(65)	KLNECGYKDRTLLPGSLFHY (44)	FDWANFNAFHS (49)	GLYSGWRGTSFLNSVLNSCYT(19)
	*:**:	*:****:*:	*::* * ***:**** * *:	:***:** :**	*********:: *:*** **
FpV1			SDRDGILKQRPVYAVDDLFL (47)	IDWSGFDQRLP(72)	GVPSGMLNTQFLDSFGNLFLL(19)
RhsV-717			SKRDGTLKVRPVYAVDELFL (47)	IDWSGYDQRLP(71)	GVPSGMLLTQFLDSFGNLYLI(19)
AhV1			SKRD-NLKVRPVYNAPMIYI (47)	IDWSRFDHLAP(92)	GVPSGILMIQFIDSFVNLTIL(19)
HaV			SKIT-KLKVRPVYNAPMLFL (47)	FDYSRFDQLAP(101)	GVPSGIFMTQILDSFVNLFIF(19)
BCV3			ADLREKTKVRGVWGRAFHYI (48)	LDWSSFDSSVT(50)	GIPSGSYYTSIVGSVVNRLRI(15)
FsV1			SPRD-DPKTRLAWIYPSEML (47)	LDFSSFDTKVP(61)	GVPSGSWWTQLVDSVVNWILV(14)
			: :: :*:*::: :	:*:* :* :	*:*** : *: :*::*:

	6	7	8
SsRV1	LHTGDDVYIRA (18)	RINPAKQSVGFGTGEFLRM (8)	GYLARSVASFVSGNW
Hv190SV	LHAGDDVYLRL (18)	RMNPTKQSIGYTGAEFLRL (8)	GYLCRAIASLVSGSW
SsRV2	IHVGDDVYLGV (18)	RMNPMKQSVGHTSTEFLRL (8)	GYLARAVASTISGNW
UmV-H1	LCHGDDIITVH (18)	KGQESKLMIDHKHHEYLRI (9)	GCLARCVATYVNGNW
	:: ***:::	: :: *::: *:**:	*:*:* :*: ::*:*
Hv145SV	DQGGDDVDQEF (18)	EATKSKQMIG-RNSEFFRV (8)	ASPVRGLATFVAGNW
PCV	DHGGDDIDLGL (18)	KANKWKQMFGTR-SEFFRN (8)	ASPTRALASFVAGDW
CCRSACV	DHGGDDIDGGI (18)	EAQKIKQMIGID-SEFFRI (8)	GSATRALARFVSGNW
	*:****:* :	:* * ***:* : *****:	:*::*:** **:*:*
FpV1	FIMGDDNSAFT (26)	SKTKSIITTLRHKIETLSY (8)	RPIGKLVAQLCFPER
RhsV-717	FIMGDDNSIFT (26)	SKTKSVITTLRSKIETLSY (8)	RDVEKLIAQLVYPEH
AhV1	FIMGDDNVIFT (26)	NISKSAVTSIRRKIEVLGY (8)	RSISKLVGQLAYPER
HaV	FIQGDDNLVFY (26)	SPDKSWITRLRTKIEVLGY (8)	RDVSKLIATLAYPER
BCV3	YTQGDDSLIGE (20)	NPDKTEYSTDPGYVTFLGR (8)	RSLDKCLRLLMFPEY
FsV1	RVLGDDS-AFM (21)	SDEKSISVEDATELKLLGV (8)	RETEEWFKLALYPEG
	:: ***: :	: *: : : ::: *::	* ::: : :**

Figure 4 Comparison of the conserved motifs of RdRps of selected isometric dsRNA viruses. Numbers 1–8 refer to the eight conserved motifs characteristic of RdRps of dsRNA mycoviruses. The amino acid positions corresponding to conserved motifs 1 and 2 for the RdRps of viruses in the family *Partitiviridae* are not well defined and, therefore, they are not presented. Multiple sequence alignments were obtained using the CLUSTAL X program with RdRp amino acid sequences of the following viruses. Upper set: viruses in the family *Totiviridae*: sphaeropsis sapinea RNA virus 1 (SsRV-1), SsRV-2, helminthosporium victoriae 190S virus (Hv190SV), and ustilago maydis virus H1 (UmV-H1). Middle set: viruses in the family *Chrysoviridae*: Hv145SV PcV and CCRSACV. Lower set: viruses in the family *Partitiviridae*: fusarium poae virus 1 (FpV1), rhizoctonia solani virus-717 (RhsV-717), atkinsonella hypoxylon virus 1 (AhV1), heterobasidion annosum virus (HaV), beet cryptic virus 3 (BCV3), and fusarium solani virus 1 (FsV1). Asterisks signify identical residues (shaded) at the indicated positions; colons signify highly conserved amino acid residue within a column; numbers in parentheses correspond to the number of amino acid residues separating the motifs.

Chryso-P4 is Virion Associated as a Minor Protein

Present evidence, based on amino acid sequencing of a tryptic peptide derived from gradient-purified PcV virions, strongly supports the conclusion that PcV chryso-P4 is virion-associated as a minor protein. The chryso-P4 encoded by chrysoviruses contains the motif PGDGXCXXHX. This motif (I), along with motifs II (with a conserved K), III, and IV (with a conserved H), form the conserved core of the ovarian tumor gene-like superfamily of predicted cysteine proteases. Multiple alignments showed that motifs I–IV are also present in other viruses including AbV-1, a tentative member of the family *Chrysoviridae*. Whether the RNAs of these viruses indeed code for the predicted proteases remains to be investigated.

Replication of Chrysoviruses

There is very limited information on how chrysoviruses replicate their dsRNAs. The virion-associated RdRp catalyzes *in vitro* end-to-end transcription of each dsRNA to produce mRNA by a conservative mechanism. Purified virions containing both ssRNA and dsRNA have been isolated from *Penicillium* spp. infected with PcV or Pc-fV, which may represent replication intermediates.

Virion Structure

Virions are isometric, nonenveloped, 35–40 nm in diameter (**Figure 5**). The capsid structure of PcV was recently determined at relatively moderate resolutions (~2.5 nm) using cryotransmission electron microscopy combined with three-dimensional image reconstruction. The outer surfaces of full particles of PcV, viewed along a five-, three-, and twofold axis of symmetry are shown in **Figure 6**. The capsid comprises 60 protein subunit monomers arranged on a $T = 1$ icosahedral lattice. The outer diameter of the capsid is 406 Å and the average thickness of the capsid shell is 44 Å. At this low to moderate resolution, some features are distinguished on the relatively smooth

Table 3 Comparison of the amino acid sequences of the N-terminal regions of chryso-P3 and corresponding regions of RdRp proteins

Viral protein	*Amino acid sequence*[a]
PcV-chryso-P3	(102) LYGVVMPMGHGKTTLAQEEGWIDCDSLI (129)
Hv145S-chryso-P3	(82) LYTVVMPAGCGKTTIANEFNCIDVDDLA (109)
ACDACV-chryso-P3	(63) LFAIVLPAGCGKSTLCRKYGYLDIDECA (90)
CCRSACV-chryso-P3	(63) LFAIVLPAGCGKSTLCRKYGYLDIDECA (90)
PcV-RdRp	(84) LFAVIMPSGCGKTTLARTYGMVDVDELV (111)
Hv145S-RdRp	(60) LFAIILPAGTGKTYLAKKYGFIDVDKCV (87)
ACDACV-RdRp	(62) LFAIVMPGGTGKTRWAREYGLVDVDELV (89)
CCRSACV-RdRp	(62) LFAIVMPGGTGKTRWAREYGLVDVDELV (89)
	::*.*.***.:**.** ****...

[a]Asterisks signify identical or similar residues (shaded) at the indicated position; colons signify at least six identical residues within a column; single dots signify 50% identical residues at the indicated position.

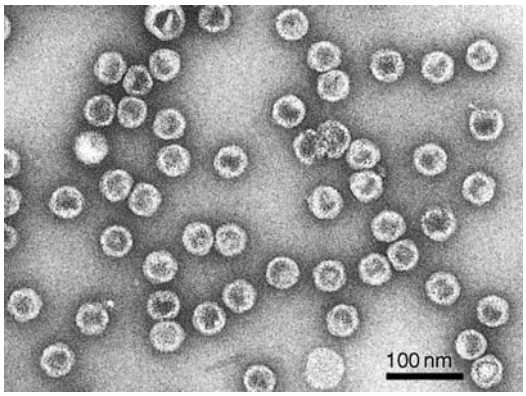


Figure 5 Negative contrast electron micrograph of particles of penicillium chrysogenum virus, the type species of the genus *Chrysovirus* in the family *Chrysoviridae*.

topography of full and empty particles. The $T=1$ full capsid is formed by 12 slightly outward protruding pentamers making an underlying cavity. The pentamers are rather complex; on the outer surface, they are formed by five connected, elongated, ellipsoid-like structures surrounded by another five smaller similar structures that are neither connected to each other, nor intercalated with inner ellipsoid-like structures. No holes are evident in the shell. The outer surface of the empty capsid is essentially identical to that of the full capsid except for the presence of five pores around the inner, elongated, ellipsoid-like structures at the fivefold axis, and three small pores around the threefold axis (not shown). There are significant differences between empty and full capsids on the inner surface around the five- and threefold positions, suggesting conformational changes. Although conformational changes have been characterized in related cores, the structural changes that are observed between empty and full PcV particles are considered unique.

The PcV capsid with its genuine $T=1$ lattice is the exception among dsRNA viruses whose capsids have '$T=2$' layers, which is the typical architecture for dsRNA viruses. PcV has the largest coat protein making a $T=1$ shell. Examination of the PcV coat protein sequence reveals a possible relationship with the canonical $T=2$ layers. Sequence analysis indeed suggests that the PcV capsid subunit falls into two similar domains that are likely to build a similar fold. Even though they are covalently linked, this unusual building unit could resemble the regular 120-subunit capsid. In this situation, PcV capsid might be considered a pseudo-($T=2$) structure. The coat protein of the chrysoviruses Hv145SV and CCRSACV share some of the repeated segments only in their amino-terminal half. The structures of the Hv145SV and CCRSACV capsids have yet to be elucidated.

Biological Properties

There are no known natural vectors for the recognized chrysoviruses PcV, PbV, Pc-fV, and Hv145SV. They are transmitted intracellularly during cell division and sporogenesis (vertical transmission), and following hyphal anastomosis (cell fusion) between compatible fungal strains (horizontal transmission).

Unlike the *Penicillium* chrysoviruses, which are associated with latent infections of their hosts, all other known chrysoviruses occur in mixed infections with other mycoviruses (or possibly plant viruses) and are associated with disease phenotypes of their hosts. The chrysovirus Hv145SV, which together with the totivirus Hv190SV coinfect the plant pathogenic fungus *Helminthosporium* (*Cochliobolus*) *victoriae*, is associated with a debilitating disease of the fungal host. The role of Hv145SV in disease development, however, is not yet clear.

ACDACV and CCRSACV are associated with two diseases of cherry, the Amasya cherry disease (ACD) and

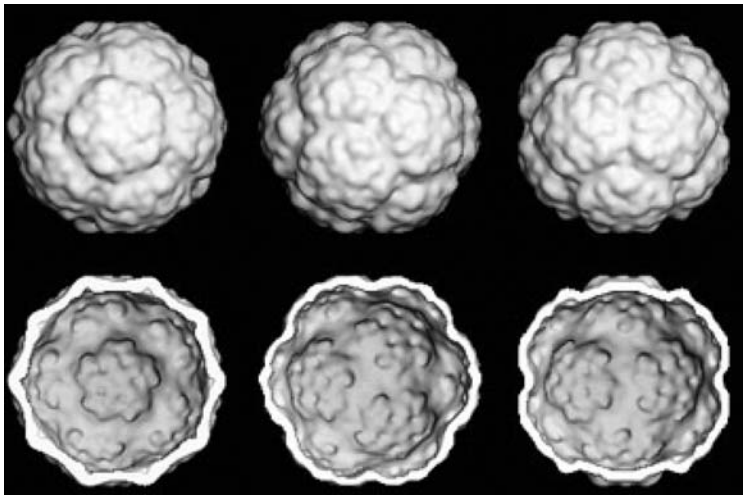


Figure 6 Three-dimensional structures of full PcV capsids. Surface-shaded representations of the outer surfaces of full PcV capsids viewed along a five-, three-, and twofold (left to right, upper row) axis of icosahedral symmetry. Models with the front half of the protein shell removed viewed along the five-, three-, and twofold axis are shown (left to right, lower row). Courtesy of R. J. Castón.

cherry chlorotic rusty spot (CCRS) disease. Both ACD and CCRS diseases are associated with a complex pattern of virus-like dsRNAs. Symptomatologically, both diseases are indistinguishable, a conclusion that was further supported by similar PAGE profiles for their associated dsRNAs. Furthermore, the sequences of at least six of these dsRNAs are essentially identical. Four of the ACD- and CCRS-associated dsRNAs comprise the genomic dsRNAs of the chrysoviruses ACDACV and CCRSACV, respectively. The etiology of these two cherry diseases is unknown and it has yet to be determined whether the dsRNAs associated with the diseases represent the genomes of plant or fungal viruses. In addition to the chrysovirus dsRNAs, the mixture of dsRNAs associated with the disease also contain a partitivirus and and larger dsRNAs with similar sizes to totiviruses. Whether the chrysoviruses alone or in combination with other dsRNA viruses play a role in disease development has yet to be elucidated.

The tentative chrysovirus AbV-1, also designated La France isometric virus (LFIV) induces a serious disease of cultivated mushroom (named La France disease). The AbV1 virions, isolated from diseased fruit bodies and mycelia, are isometric 34–36 nm in diameter and co-purify with nine dsRNA segments (referred to as disease-associated dsRNAs). The sizes of the dsRNA segments vary from 3.6 to 0.78 kbp, at least three of which are believed to be satellites. AbV1 represents a multiparticle system in which the various particle classes appear to have similar densities. Interestingly, phylogenetic analysis of RdRp conserved motifs of AbV1, encoded by dsRNA segment L1, and other dsRNA mycoviruses showed that AbV1 is closely related to the multipartite chrysoviruses. Although there is convincing evidence that infection with AbV-1 alone is essential and sufficient for induction of the La France disease, another mushroom virus, the mushroom bacilliform virus (MBV), the type species of the family *Barnaviridae*, is commonly found in mixed infections with AbV1 in diseased mushrooms. MBV is apparently not essential for disease development since no obvious phenotypic changes were observed in an *Agaricus* culture singly infected by MBV. However, synergistic interactions between AbV1 and MBV in doubly infected mushrooms cannot be ruled out.

Evolutionary Relationships among Chrysoviruses

The *Penicillium* chrysoviruses PcV, Pc-fV, and PbV are serologically related and have similar biochemical and biophysical properties. Although molecular data is only available for PcV, the three viruses could be considered as strains of the same virus for all practical purposes. The fact that these closely related viruses occur in different fungal species suggested that transmission by means other than hyphal anastomosis may occur in nature, since hyphal fusion between different fungal species is doubtful. Horizontal transmission of fungal viruses in nature, however, has yet to be demonstrated, and in the case of viruses of *Penicillium* species may not need to occur since the viruses replicate in parallel with their hosts and are carried intracellularly during the vegetative growth of the host (vertical transmission). Furthermore, the viruses are

efficiently disseminated via the asexual spores (conidia) of *Penicillium* species. It seems feasible, however, that virus infection arose early in the phylogeny of *P. brevi-compactum, P. chrysogenurn*, and *P. cyaneo-fulvum* before they diverged and that the resident virus remained associated with them during their subsequent evolution.

BLAST searches of PcV RdRp amino acid sequence showed that it has significantly high sequence similarity (40% identity and 57% aa sequence similarity) to the RdRps encoded by the chrysoviruses Hv145SV, ACDACV, and CCRSACV. High similarities (BLAST hits of $e^{-}16$ or lower) were also found to the RdRps of the tentative chrysovirus agaricus bisporus virus 1 (AbV-1) as well as to the totivirus ustilago maydis virus H1 (UmV-H1) and the giardiavirus trichomonas vaginalis virus (TVV). Still high similarity hits can be obtained with the RdRps of several members of the family *Totiviridae*. Interestingly, no significant hits were evident with any of the viruses in the family *Partitiviridae,* another validation for the removal of chrysoviruses from the family *Partitiviridae* and their placement in the newly created family *Chrysoviridae*. The conclusion that chrysovirus RdRps are more closely related to those of totiviruses than to those of partitiviruses is consistent with the results shown in **Figure 4**, where the RdRp conserved motifs of members of the three families are compared. Phylogenetic analysis (**Figure 7**), based on the complete nucleotide sequences of the RdRps of members of the three families, provides further confirmation to this conclusion.

BLAST searches of the deduced amino acid sequence of dsRNA2 ORF showed significant high similarity hits ($3e^{-}62$) to Hv145SV CP (29% identity and 50% amino acid sequence similarity) and ACDACV and CCRSACV CPs (24% identity and 46% similarity). It is interesting that the region of high sequence similarity between the CPs of these chrysoviruses is limited to the N-terminal half of the proteins (aa 19–560 of PcV CP; data not shown). This finding may have implications when considering the structural organization of chrysovirus capsids.

Future Perspectives

Functions of Chryso-P3 and Chryso-P4

Present evidence suggests that both chryso-P3 and chryso-P4 are virion associated and that they may play a role in RNA transcription, RNA binding, and packaging. Because infectivity assays are not amenable for chrysoviruses, no direct evidence is currently available in support of the conclusion that chrysovirus segments 3 and 4 and their gene products are essential for virus infection and replication. However, based on the consistent co-presence of the four dsRNA segments, the existence of extended regions of highly conserved terminal sequences at both ends of all four segments, sequence comparisons and phylogenetic analysis, it is abundantly clear that the structural features of chrysoviruses are typical of RNA viruses with multipartite and multicomponent genomes. The dsRNA pattern of PcV virions isolated from different strains of *Penicillium chrysogenum* has remained unchanged throughout the years since PcV was first isolated. This is also true for other chrysoviruses isolated from different *Penicillium* species and from various strains of *Helminthosporium victoriae*. The co-presence of all four segments in different fungal species and strains harboring chrysoviruses and the stability of the dsRNA patterns support the contention that all four segments are essential for infection and that none is defective or satellite in nature.

An alternative approach to infectivity assays that should be applicable to chrysoviruses is to transform virus-free fungal isolates with full-length cDNA clones of viral dsRNAs. In a recent preliminary study involving transformation of virus-free *H. victoriae* protoplasts with individual as well as different combinations of full-length cDNAs of the four dsRNAs of Hv145SV, productive yield of ssRNA transcripts corresponding to all four dsRNA segments was only generated in transformants containing DNA copies of all four dsRNAs. Although dsRNA synthesis was not launched in these transformants, the results suggest that dsRNA segments 3 and 4 and their gene products are necessary for accumulation and stability of the viral transcripts.

Etiology of the CCRS Disease and Potential Occurrence of Plant Chrysoviruses

The etiology of the CCRS disease remains a mystery. Although there is circumstantial evidence for a fungal etiology for the disease, no fungal pathogen was ever isolated from diseased trees. Although it is true that no plant chrysoviruses have been identified to date, there is no reason to exclude this possibility. As a matter of fact, it is not yet determined whether the partitivirus cherry chlorotic rusty spot associated partitivirus (CCRSAPV), which is co-isolated along with CCRSACV from diseased cherry trees, is a plant or a fungal partitivirus. Phylogenetic analysis based on RdRp conserved motifs placed CCRSAPV in a cluster with a mixture of plant and fungal partitiviruses. This finding raised the interesting possibility of horizontal transfer of members of the family *Partitiviridae* between fungi and plants. This possibility is reasonable because some of the viruses in this phylogenetic cluster have fungal hosts that are pathogenic to plants. As more chrysoviruses from a wider range of fungal (and possibly plant) hosts are isolated and characterized, the reality of plant chrysoviruses may become apparent as well as the need to reconsider the taxonomy of the family *Chrysoviridae*.

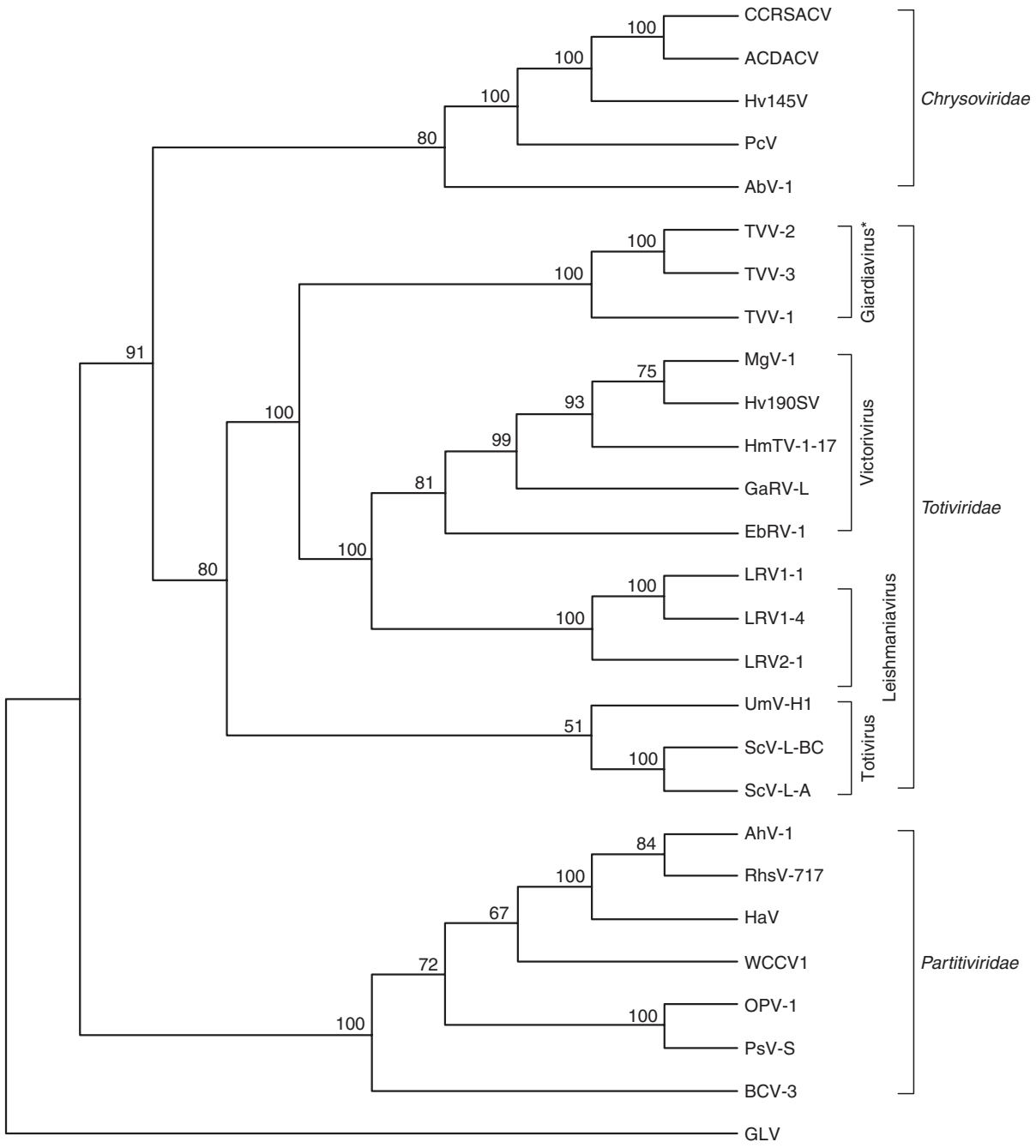


Figure 7 Neighbor-joining phylogenetic tree constructed based on the complete amino acid sequences of RdRps of selected isometric dsRNA viruses. The RdRp sequences were derived from aligned deduced amino acid sequences of members of the families *Chrysoviridae*, *Partitiviridae*, and *Totiviridae* using the program CLUSTAL X. See **Table 1** for names and abbreviations of chrysoviruses. The following viruses in the family *Totiviridae* were included in the phylogenetic analysis (abbreviations in parenthesis): trichomonas vaginalis virus-1 (TVV-1), TVV-2, and TVV-3, magnaporthe grisea virus 1 (MgV-1), helminthosporium victoriae 190S virus (Hv190SV), Helicobasidium mompa *totivirus 1-17* (HmTV-1-17) gremmeniella abietina RNA virus L (GaRV-L), eimeria brunetti RNA virus 1 (EbRV-1), leishmania RNA virus 1-1 (LRV1-1), LRV1-4, LRV2-1, ustilago maydis virus H1 (UmV-H1), saccharomyces cerevisiae virus L-A (ScV-L-A), and ScV-L-BC. The following viruses in the family *Partitiviridae* were included: atkinsonella hypoxylon virus 1 (AhV-1), rhizoctonia solani virus-717 (RhsV-717), heterobasidion annosum virus (HaV), white clover cryptic virus 1 (WCCV-1), ophiostoma partitivirus 1 (OPV-1), penicillium stoloniferum virus S (PsV-S), and beet cryptic virus 3 (BCV-3). The phylogenetic tree was generated using the program PAUP*. Bootstrap numbers out of 1000 replicates are indicated at the nodes. The tree was rooted with the RdRp of Giardia lamblia virus, the type species of the genus *Giardiavirus* in the family *Totiviridae*, which was included as an outgroup. * Note that TVV-1, TVV-2, and TVV-3 are tentative members of the genus *Giardiavirus*.

See also: Fungal Viruses; Partitiviruses: General Features.

Further Reading

Castón JR, Ghabrial SA, Jiang D, *et al.* (2003) Three-dimensional structure of Penicillium chrysogenum virus: A double-stranded RNA virus with a genuine $T = 1$ capsid. *Journal of Molecular Biology* 331: 417–431.

Covelli L, Coutts RHA, Di Serio F, *et al.* (2004) Cherry chlorotic rusty spot and Amasya cherry diseases are associated with a complex pattern of mycoviral-like double-stranded RNAs. Part I: Characterization of a new species in the genus *Chrysovirus*. *Journal of General Virology* 85: 3389–3397.

Ghabrial SA, Jiang D, and Castón RJ (2005) *Chrysoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 581–590. London: Academic Press.

Ghabrial SA, Soldevila AI, and Havens WM (2002) Molecular genetics of the viruses infecting the plant pathogenic fungus *Helminthosporium victoriae*. In: Tavantzis S (ed.) *Molecular Biology of Double-Stranded RNA: Concepts and Applications in Agriculture, Forestry and Medicine*, pp. 213–236. Boca Raton, FL: CRC Press.

Jiang D and Ghabrial SA (2004) Molecular characterization of Penicillium chrysogenum virus: Reconsideration of the taxonomy of the genus *Chrysovirus*. *Journal of General Virology* 85: 2111–2121.

Circoviruses

A Mankertz, Robert Koch-Institut, Berlin, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Apoptosis Programmed self-induced cell death.
Botryoid Grape-like appearance.
Bursa of Fabricius Specialized organ in birds, that is necessary for B-cell development.
Chimera Virus created from two or more different genetic sources.
Koch's postulates Four criteria that must be fulfilled in order to establish a causal relationship between an agent and a disease.
Mono/polycistronic mRNA mRNA is termed polycistronic when it contains the genetic information to translate more than one protein, monocistronic if only one protein is encoded.
Phylogenetic tree Depicts the evolutionary interrelationships among species that are believed to have a common ancestor.
Rolling-circle replication (RCR) Mechanism of replication which takes its name from the characteristic appearance of the replicating DNA molecules, a special feature of RCR is the uncoupling of the synthesis of the two DNA strands.

Circovirus Taxonomy

Circoviruses contain a covalently closed circular single-stranded DNA (ssDNA) genome with sizes between 1759 and 2319 nt. The circular nature of their genomes, which are the smallest possessed by animal viruses, has led to the family being termed *Circoviridae*. Differences in organization of the viral genomes and capsid morphology led to their classification into two different genera.

Chicken anemia virus is the only species member of the genus *Gyrovirus*, while the genus *Circovirus* currently comprises *Porcine circovirus type 1* and *Porcine circovirus type 2*, *Psittacine beak and feather disease virus*, *Pigeon circovirus*, *Canary circovirus*, and *Goose circovirus*. Duck circovirus (DuCV), finch circovirus (FiCV), and gull circovirus (GuCV) are members of tentative species in the genus, while the circoviruses of raven (RaCV) and starling (StCV) have been discovered only recently and are therefore not yet included in the taxonomic classification.

The reported size range for the chicken anemia virus (CAV) virion is 19.1–26.5 nm, the genome is of (−) polarity, and the open reading frames (ORFs) are overlapping. Only one mRNA molecule is produced from a promoter/enhancer region; it encodes three partially overlapping ORFs. CAV shows homology to the newly identified ssDNA viruses in humans, torque teno virus (TTV) and the related torque teno mini virus (TTMV), which are members of the unassigned genus *Anellovirus*. The noncoding regions of CAV and TTV are G/C-rich and show a low level of nucleotide homology. In addition, CAV and TTV specify structural proteins that contain two amino acid motifs with putative roles in rolling-circle replication (RCR) and nonstructural proteins that exhibit protein phosphatase activity. CAV and TTV are separately classified, since their sequence homology is limited and only one mRNA is produced from CAV, while splicing has been detected in TTV and TTMV. Phylogenetic investigation of the family *Circoviridae*

revealed that CAV has no close relationship to the genus *Circovirus* (**Figure 1**).

Members of the genus *Circovirus* differ in several aspects from CAV, because they display a smaller particle size (12–20.7 nm, **Figure 2**), their ambisense genomes (**Figure 3**) are divergently transcribed, and splicing has been reported. The viruses of the genus *Circovirus* show homology in genome organization and protein sequences and function to the plant-infecting viruses of the family *Nanoviridae* and *Geminiviridae*.

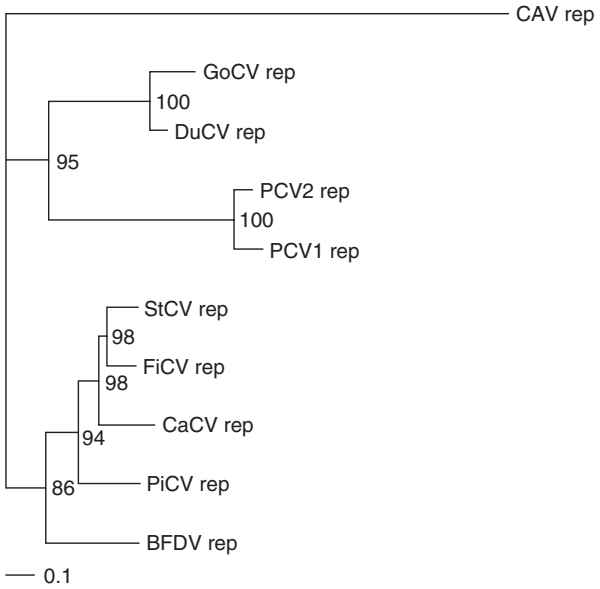


Figure 1 Phylogenetic tree of members of the family *Circoviridae*. The amino acid sequences of the Rep proteins of the members of the genus *Circovirus* (GoCV, DuCV, BFDV, PiCV, StCV, FiCV, CaCV, PCV1, PCV2) and of the only member of the genus *Gyrovirus* (CAV) were compared. Analysis was performed using the MacVector program package by analyzing 100 data sets.

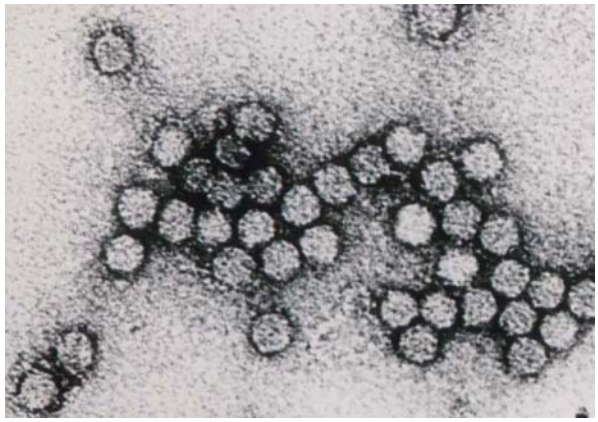


Figure 2 Electron microscope picture of particles of PCV1. PCV1 particles in an immunoaggregation with a PCV1 hyperimmuneserum (180 000-fold magnification, negative contrasting with 1% UAc).

Virion Structure

The size for the CAV virion has been reported as 19.1–26.5 nm and 12–20.7 nm for circoviruses (**Figure 2**).

The virions of CAV, porcine circovirus type 1 (PCV1) and porcine circovirus type 2 (PCV2), are each comprised of one structural protein, for which sizes of 50 (CAV), 30 (PCV1), and 30 kDa (PCV2) have been estimated, respectively. Psittacine beak and feather disease virus (PBFDV) is reported to contain three proteins (26, 24, and 16 kDa). The protein composition of the other avian circoviruses is not known, but putative structural proteins have been identified by homology searches. All capsid proteins have a basic N-terminal region containing several arginine residues, which is expected to interact with the packaged DNA. Virions do not possess an envelope. Investigation of the structures of CAV, PCV1, and PCV2 revealed that all have an icosahedral $T=1$ structure containing 60 capsid protein molecules arranged in 12 pentamer clustered units. PCV2 and BFDV show similar capsid structures with flat pentameric morphological units, whereas chicken anemia virus displayed protruding pentagonal trumpet-shaped units.

Pathogenesis of Circoviruses

Circoviruses and Gyroviruses Induce Diseases

Circoviruses are supposed to be host specific or to have narrow host ranges. The fecal–oral route of transmission is likely, but vertical transmission has been reported in some cases. With the exception of PCV1, all known circoviruses are pathogens, which cause immune suppression and damage in the lymphoreticular tissues.

CAV infections occur mainly in young chicken. The main targets of CAV replication are cells in the bone marrow (hemocytoblasts) and precursor lymphocytes in the thymus. Characteristic symptoms are aplastic anemia and hemorrhagic lesions, watery blood, pale bone marrow, lymphoid depletion, atrophy of thymus and bursa, and swollen and discolored liver. Since macrophages recovered from infected birds produce less interleukin 1 (IL-1) and the pathogenicity of co-infecting viruses such as Marek's disease virus, infectious bursal disease virus, and Newcastle disease virus are enhanced, immune suppression is thought to play a role in CAV-induced pathogenesis.

Another intensively studied circoviral disease is psittacine beak and feather disease (PBFD). PBFD is the most common disease in cockatoos and parrots and is typically detected in young birds. Deformation of beak, claws, and

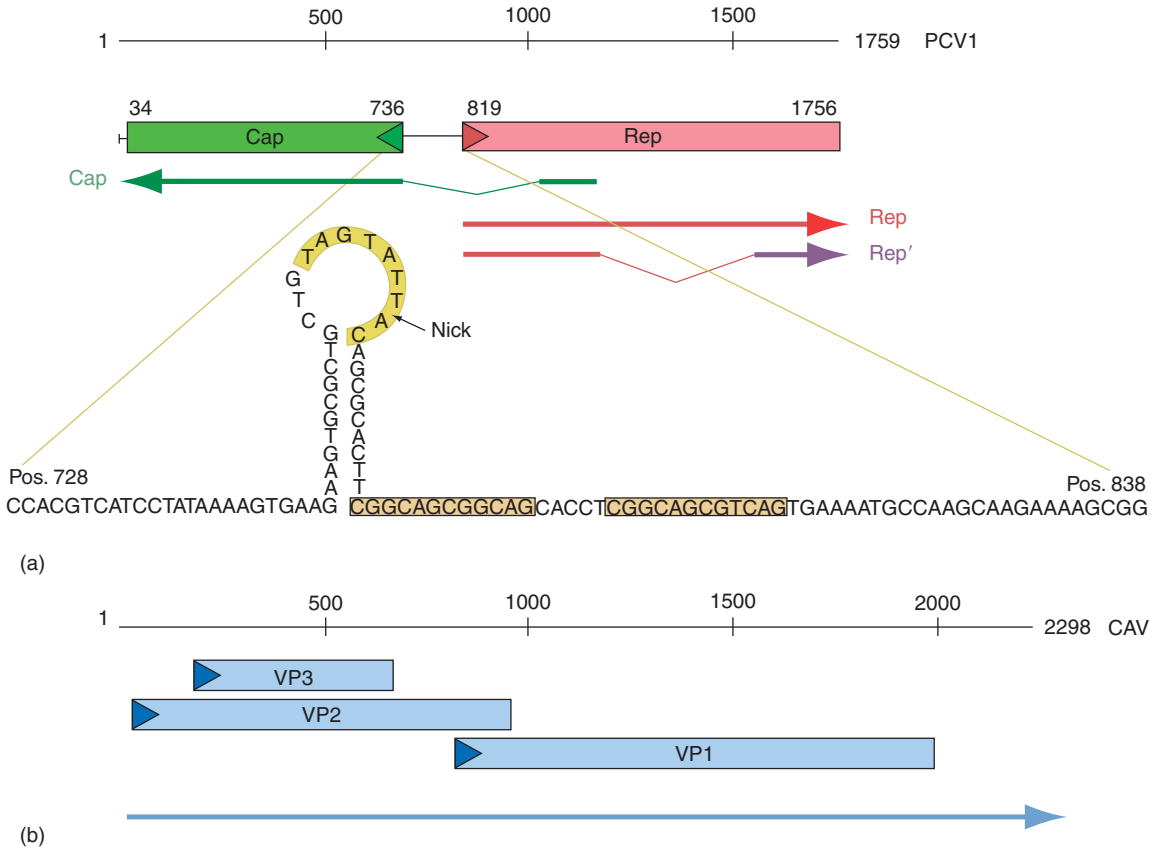


Figure 3 Genomic organization of PCV1 member of the type species of the genus *Circovirus* and CAV member of the type species of the genus *Gyrovirus*. ORFs are shown in open boxes, the direction of transcription is indicated by triangles. Transcripts are indicated by arrows, splice processes by dotted lines. (a) PCV1, the species type of the genus *Circovirus*, is shown. The ambisense genomic organization is outlined, that is, both strands of the replicative form are coding for proteins. Therefore, the two major genes Rep and Cap are divergently transcribed. Between the start points of Rep and Cap, the origin of viral replication is located. This element is drawn to a larger scale, displaying its characteristic features, a putative hairpin, and adjacently located repeats. An arrow indicates the position where Rep and Rep′ restrict the replicative intermediate to initiate the replication.(b) CAV is a member of the type species of the genus *Gyrovirus*. Its genome is of the 'negative-sense' type, that is, the viral strand does not encode genetic information, it has to be converted into a dsDNA version, from which one polycistronic mRNA is produced. Splice processes have not been observed.

feathers, lethargy, depression, weight loss, and severe anemia are the most prominent symptoms.

Young pigeon disease syndrome (YPDS) is a multifactorial disease in which PiCV is assumed to induce immunosuppression in young birds, which suffer from ill-thrift, lethargy, anorexia, and poor race performance. Depletion of splenic and bursal lymphocytes was seen and bacterial agents as *Escherichia coli* and *Klebsiella pneumoniae* were isolated more frequently from PiCV-infected birds. Inclusion bodies were present in various organs, especially the bursa of Fabricius.

In CaCV-infected neonatal canary birds, a condition known as 'black spot' has been reported. It is associated with abdominal enlargement, gall bladder congestion, failure to thrive, dullness, anorexia, lethargy, and feather disorder. Histological changes as lymphofollicular hyperplasia, lymphoid necrosis, cellular depletion, and cystic atrophy are observed in the thymus and the bursa of Fabricius. A general feature of circovirus infection is the formation of globular or botryoid, basophilic inclusion bodies in the cytoplasm, in which the virus may form paracrystalline arrays.

PCV1 and PCV2 seem to be restricted to pigs. PCV2 is the etiological agent of a new disease in swine, the so-called post-weaning multisystemic wasting syndrome (PMWS), and may be involved in several other porcine circoviral diseases (PCVDs) like porcine dermatitis and nephropathy syndrome (PDNS) or porcine respiratory disease complex. PMWS was first recognized in Canada in 1991. Since then it has been described as a major economic concern in virtually all pig-producing areas of the world. PMWS primarily occurs in pigs between 60 and

80 days old. Maternal antibodies confer titer-dependent protection against PCV2 infection – higher titers are generally protective, but low titers are not. PMWS is characterized by wasting, respiratory signs, enlargement of superficial inguinal lymph nodes, diarrhea, paleness of the skin or icterus, but the clinical signs are often variable. The most consistent feature of PMWS is a generalized depletion of lymphocytes. Secondary infections with opportunistic organisms are common. This indicates that the immune system is involved in the pathogenesis of PMWS. On affected farms, mortality may reach up to 40%, but it can be reduced, if special management plans are implemented. In the first attempts, experimental reproduction of the disease according to Koch's postulates has led to an amazing variety of results, since no symptoms were seen as well as histopathological lesions or a full-blown PMWS. The symptoms of the disease were aggravated when piglets were infected in which the immune system had been stimulated either by a prior vaccination or by a co-infection with porcine parvovirus (PPV) or porcine reproductive and respiratory syndrome virus (PRRSV). These and other findings indicate that PMWS is a multifactorial disease, in which not only factors such as the status of the immune system and genetic predisposition but also practical aspects such as nutrition and vaccination policy may influence the onset of the disease and the severity of the symptoms.

Diagnosis of Circoviral Diseases

In general, diagnosis of circoviral diseases is based on detection of the virus by culture, polymerase chain reaction (PCR), immunohistochemistry or *in situ* hybridization, or detection of antibodies against the circovirus by serology. PBFD can also be diagnosed on the basis of feathering abnormalities. PCV2 is a ubiquitous virus and also prevalent in healthy pigs; therefore, diagnosis of PMWS must concurrently meet three criteria: (1) the presence of compatible clinical signs, (2) the presence of moderate to severe characteristic microscopic lymphoid lesions, and (3) the presence of moderate to high amount of PCV2 within these lesions.

PCV1 and PCV2

A striking difference is seen in the pathogenicity of PCV1 and PCV2. No disease is attributed to PCV1, while PCV2 is the etiological agent of PMWS, a new emerging disease of swine. What may be the molecular basis for this distinct feature? The genomes of the two strains are highly conserved, especially the origin of replication (80% sequence homology) and the Rep gene (82%). Exchange of replication factors between PCV1 and PCV2 did not reveal differences, since the Rep protein of PCV1 (Rep/PCV1) replicated its cognate origin as well as the heterotype origin of PCV2 (and vice versa), suggesting that pathogenesis may not be linked to the replication factors. A higher degree of sequence deviation is found in the Cap genes with less than 62% homology between PCV1 and PCV2. Chimeras of PCV1 and PCV2 have been produced and tested for their potential to induce PMWS and to stimulate the immune answer. The chimera PCV2/1, containing the PCV1 capsid gene cloned into the backbone of the pathogenic PCV2 genome, was compared to a chimera PCV1/2, containing the PCV2 capsid gene in the nonpathogenic PCV1 genome. Both variants displayed similar growth characteristics *in vitro*. Gross lesions significant for PMWS were not observed, but PCV1/2 induces protective immunity to wild-type PCV2 challenge in pigs, indicating that it may be an effective vaccine candidate. ORF3 is comprised by the rep gene and may also contribute to the pathogenesis of PCV2, because its sequence differs significantly in PCV1 and PCV2 and induction of apoptosis has been reported.

Interaction with the Immune System

PCV2 provides a valuable model for gaining insight into how ssDNA viruses interact with the host immune system and for understanding their pathogenesis. PCV2 is intriguing in its ability to persist in macrophages and dendritic cells without replication although its infectivity is retained. When natural interferon (IFN)-producing cells responded to an inducer of cytokine synthesis, their co-stimulatory function, which induces myeloid dendritic cell maturation, was clearly impaired in case of a concurrent PCV2 infection. Stimulation of the porcine immune system with IFN-α and IFN-γ causes increased replication of PCV2 *in vivo*, while no changes were observed in IL-1-, IL-6-, tumor necrosis factor alpha (TNF-α)-, or IL-10-treated cells. With the circumstantial evidence compiled over the last years, one may assume that PMWS can be considered as an acquired immunodeficiency syndrome of pigs although direct evidence for this hypothesis is still missing.

Molecular Biology of Circoviruses

Genome Organization of Circoviruses

The genomes of all circoviruses are composed from a circular ssDNA molecule with a size between 1759 and 2319 nt and therefore display the smallest genomes possessed by mammalian viruses. Nevertheless, members of the genera *Circovirus* and *Gyrovirus* show remarkable differences in their genome organization.

The genomes of CAV isolates are either 2298 or 2319 nt in size. Part of the noncoding region of the genome is

G–C-rich and able to form putative hairpin structures. The three genes are encoded by the viral (–)strand, therefore CAV has a negative-sense genome organization. One major polycistronic mRNA (2.0 kbp) is transcribed from the circular double-stranded (ds) replication form (RF), which is produced after infection. The nontranscribed region of the genome contains transcription initiation and termination signals and a tandemly arranged array of four or five 19 nt repeats with which promoter–enhancer activity is associated. Within this sequence, estrogen response element consensus half-sites were found, resembling the arrangement that can be recognized by the nuclear receptor superfamily. Since expression from the CAV promoter was significantly increased with estrogen treatment, members of the nuclear receptor superfamily may provide a mechanism to regulate CAV activity.

The hypothesis that the circular ssDNA genome replicates using the RCR mechanism is supported by the presence of the conserved nonanucleotide motif within the CAV genome, at which RCR is initiated in other circular ssDNA replicons, but it is not located at the apex of a putative hairpin. The presence of two amino acid motifs typical for enzymes involved in RCR within VP1 also suggests that this structural protein possesses DNA replication function, while its basic N-terminus implies that this protein is involved in capsid formation, too. This would be highly unlike the genus *Circovirus*, where two distinctly encoded proteins perform the two most elementary functions of replication and packaging of the genome. Coding regions of the avian and porcine circoviruses are arranged divergently resulting in an ambisense genome organization and creating two intergenic regions, a larger one between the 5′ ends of the two major ORFs rep and cap and a shorter one between their 3′ ends. In case of PCV1 and PCV2, the non-coding regions between the ATGs of the rep and cap gene comprise the origin of viral genome replication. Similar genomic structures are found in members of the families *Geminiviridae* and *Nanoviridae*.

Viral ORFs and Proteins

Synthesis of three virus proteins is directed from the CAV genome. VP1 (52 kDa) is encoded by ORF1 and may combine the function of a structural protein as well as the initiator of replication. VP2 (26 kDa) is a protein phosphatase encoded by ORF2. VP2 protein phosphatase activity is required for efficient replication. It may also have a role as a scaffolding protein during virion assembly. Co-expression of both VP1 and VP2 is necessary for the induction of neutralizing antibodies. VP3 is a 14 kDa virulence factor known to induce apoptosis in transformed cell lines and has been called 'apoptin'.

Two major ORFs are encoded by the genomes of PCV1 and PCV2, encoding the viral functions for replication (Rep and Rep′) and a structural protein (Cap). A similar genomic structure is seen in the avian circoviruses. Several smaller ORFs have been found by computer analyses, but with the exception of ORF3 of PCV, which seems to be involved in pathogenesis and apoptosis, their expression has not been studied yet. The largest ORF of the ambisense organized circoviruses is located on the viral plus-strand (V1). It encodes the Rep protein (312 or 314 aa). Three motifs conserved in enzymes mediating replication in the RCR mode and a dNTP-binding domain have been identified. Both Rep proteins reside in the nucleus of infected cells. Phylogenetic analyses suggest that circovirus Rep proteins may have evolved by a recombination event between the Rep protein of nanoviruses and an RNA-binding protein encoded by picorna-like viruses or a helicase of prokaryotic origin. The second largest ORF of all circoviruses is located on the complementary strand (C1) and encodes the major structural protein Cap protein (234 aa), which displays a basic N-terminus rich in arginine residues. This suggests that this region is involved in binding to viral DNA. Some avian circoviruses use alternative start codons for Cap translation. After expression in bacteria and insect cells, Cap of PCV assembled into virus-like particles when viewed by electron microscopy. Cap has been shown to reside mostly in the nucleoli, but shuttling to the cytoplasm occurs during the infectious cycle. ORF3 is encoded counterclockwisely by ORF1. It encodes a protein that is not essential for PCV replication but has been reported to induce apoptosis.

Transcription of PCV

The promoter of the cap gene of PCV has been mapped to a fragment at 1168–1428, that is, P_{cap} is located within the rep gene. P_{cap} is not regulated by virus-encoded proteins. The cap transcript starts at nucleotide 1238 with an untranslated leader sequence of 119 nt (1238 to 1120) joined to exon 2 of the ORF1 transcript at nucleotide 737, immediately adjacent to the start point of translation. Processing of this RNA has presumably evolved to avoid synthesis of another protein initiated at an internal start codon in the intron. The start of the rep transcript of PCV1 has been mapped to nucleotide 767 ± 10. The promoter of the rep gene, P_{rep}, is comprised within a fragment, nucleotides 640–796. P_{rep} overlaps the intergenic region and the origin of replication. P_{rep} is repressed by the Rep protein by binding to hexamers H1 and H2; these elements are involved in initation of replication, too. Mapping the rep mRNA revealed synthesis of several transcripts in PCV1 and PCV2. A full-length transcript directs synthesis of the Rep protein (312 aa, 35.6 kDa). In a spliced transcript,

removal of an intron (nucleotides 1176 to 1558) results in synthesis of a truncated protein, which has been termed Rep′. Rep′ is truncated to 168 aa (19.2 kDa) and, due to a frameshift, the last 49 aa are expressed in a different reading frame. Comparison of the ratio of Rep and Rep′ transcript with a real-time PCR discriminating between the two transcripts indicated a variation of the ratio of the two transcripts in correlation to time. Replication of PCV is dependent on expression of both proteins. Splicing of Rep proteins is a well-known feature in other small ssDNA viruses (e.g., *Mastrevirus*), but, in contrast to PCV, one Rep protein is sufficient for replication of these viruses.

Replication of Circoviruses

Although the main target for viral replication still remains unknown, PCV2 was seen *in vivo* in a variety of cell types including hepatocytes, enterocytes, epithelial and endothelial cells, lymphocytes, smooth muscle cells, and fibroblasts. This broad range of cells that support PCV2 infection indicates that PCV2 does not enter the cell via a rarely expressed receptor. When binding of PCV2 to monocytic cells was investigated, it became evident that surface proteins and glycosaminoglycans heparan sulfate and chondroitin sulfate B are attachment receptors for PCV2. This result is supported by the finding that the heparan sulfate binding motif (XBBXBX; B = basic amino acid, X = neutral/hydrophobic amino acid) is present on the PCV2 capsid protein. PCV2 enters the cells predominantly via clathrin-mediated endocytosis and requires an acidic environment for infection.

Replication of PCV has been studied in detail. PCVs are supposed to replicate their genomes using a circular, ds RF intermediate, which is produced by host cell DNA polymerases during the S phase of cell division. The origin of replication of PCV has been mapped to a fragment comprising the intergenic region between the start points of the two major ORFs (**Figure 3**).

Replication of these fragments cloned into a vector was observed after co-transfection of porcine kidney cells with plasmids expressing the rep gene. By sequence alignment, analogous elements can be identified for all other circoviruses with the exception of CAV. The origin of replication is characterized by a potential stem–loop structure with a nonamer (5′-TAGTATTAC; **Figure 3**) in its apex. Mutagenesis of the nonamer resulted in inactivation of PCV replication. Adjacent to the nonamer, short repeats are located, which serve as the binding site for the rep gene products. Nonamer, stem–loop, and adjacent short repeats are conserved in all other circoviruses, in the families *Nanoviridae* and *Geminiviridae*, and, although to a lesser extent, in many replicons replicating via RCR. The conserved elements in the *cis*-acting origin of replication as well as RCR signatures in the *trans*-acting Rep protein amino acid (aa) sequence (see below) indicate an RCR-like replication mechanism for the circoviruses.

Functional Analyses of PCV-Encoded Proteins

Truncation of the rep gene as well as site-directed mutagenesis of the four conserved motifs abrogated replication of PCV, indicating that the rep gene products are indispensable. The roles of Rep and Rep′ of PCV have been analyzed in detail.

Binding to DNA. Rep and Rep′ bind *in vitro* to fragments of the origin of replication containing the stem–loop structure plus the conserved nonamer and the four hexamer (H) repeats (5′-CGGCAG; H1 to H4). Proteins bind either the two inner (H1/H2) or the two outer (H3/H4) hexamers. A minimal binding site (MBS) has been identified for Rep and Rep′ protein using truncated substrates: the Rep MBS was mapped to the right leg of the stem–loop plus the two inner hexamer repeats H1/H2, while the MBS of Rep′ was composed of only the two hexamer repeats H1/H2. Gel shift assays also revealed presence of several complexes, indicating that variable amount of proteins may be bound.

Replication. The covalently closed, ssDNA genome of PCV replicates via a dsDNA replicative intermediate. The replication occurs by RCR whereby a single-stranded break is introduced by Rep or Rep′ leading to a free 3′-hydroxyl group serving as a primer for subsequent DNA synthesis. Replication does occur when Rep plus Rep′ protein are expressed in the cells, indicating that both proteins are essential for replication from the PCV origin. This is in contrast to other ssDNA viruses, for example, AAV2 or members of the genus *Mastrevirus*, in which spliced Rep proteins are produced, but are not essential for viral replication. Rep and Rep′ cleave the viral strand between nucleotides 7 and 8 *in vitro* within the conserved nonanucleotide located at the apex of a putative stem–loop structure. In addition, Rep and Rep′ join viral ssDNA fragments, implying that these proteins also play a role in the termination of virus DNA replication. This joining activity is strictly dependent on preceding substrate cleavage and the close proximity of origin fragments accomplished by base pairing of the stem–loop structure. This dual 'nicking/joining' activity associated with Rep and Rep′ are pivotal events underlying the RCR-based replication of porcine circoviruses in mammalian cells. Although presence of the palindrome plus a single H sequence is sufficient for PCV replication, a tandem repeat arrangement is more stable. Within the H sequence, selected nucleotides at specific positions are critical for Rep-associated protein recognition and for viral DNA replication.

Repression of the Rep gene promoter P_{rep}. When the influence of virus-encoded proteins upon P_{rep} was investigated, it

became apparent that P_{rep} is repressed by its own gene product Rep but not by Rep′ or Cap. This finding illustrates that Rep protein initiates replication and controls its own transcription by binding to hexamers H1/H2. Interestingly, Rep′ also binds to H1/H2, but this does not result in repression of P_{rep} activity. Since mutagenesis of H1/H2 decreases but does not inactivate P_{rep} transcription, features other than binding of Rep may be necessary for repression of P_{rep}, for example, interaction of Rep protein with transcription factors. P_{cap} is not regulated by Cap, Rep, and Rep′.

Interaction. Studies investigating the interaction of PCV-encoded proteins are bemusing, because the results were depending on the system used for expression. While two hybrid analysis in bacteria revealed interaction of Rep and Cap, this was not observed in yeast cells, suggesting that post-translational modifications of Rep and Cap may significantly modulate their function. Rep and Rep′ have been observed to interact in yeast cells and this observation was reproduced by immunoprecipitation in mammalian cells, the natural target of PCV.

Localization. Rep and Rep′ protein co-localize in the nucleoplasm of infected cells, but no signal was seen in the nucleoli. The localization did not change during the infection cycle. Rep and Rep′ carry three potential nuclear localization signals (NLSs) in their identical N-termini. Proteins mutated in their NLSs demonstrated that NLS1 and NLS2 mediate the nuclear import, whereas NLS3 enhances the nuclear accumulation of the replication proteins. In contrast to Rep and Rep′, the localization of the Cap protein was restricted to the nucleoli in plasmid-transfected cells. In PCV-infected cells, Cap was localized in the nucleoli in an early stage, while it was seen later on in the nucleoplasm and the cytoplasm. This signifies that Cap is shuttling between distinct cellular compartments during the infection cycle. Since Rep, Rep′, and Cap are all located in the nucleus, this points out that DNA replication and encapsidation of the circular closed ssDNA probably occur in the nucleus and not in cytoplasmic compartments. The biological function of the early localization of Cap to the nucleoli remains unclear. Nucleolar localization has been described for proteins of many DNA and RNA viruses and it has been proposed that virus proteins enter the nucleoli to support viral transcription or influence the cell cycle.

Conclusion

Although the family *Circoviridae* comprises only a relatively small number of viruses, the increasing number of publications demonstrates rising interest. This may not only be related to the fact that circoviruses induce severe multifactorial diseases, which compromise and unbalance the immune system, but also to the fact that the apparent simplicity of the circovirus genome contrasts highly with the complex and poorly understood pathogenesis. Hopefully, this will induce many question-solving studies, enabling us to improve our understanding of these intriguing viruses in the future.

See also: Anellovirus; Nanoviruses; Plant Resistance to Viruses: Geminiviruses.

Further Reading

Cheung AK (2004) Palindrome regeneration by template strand-switching mechanism at the origin of DNA replication of porcine circovirus via the rolling-circle melting-pot replication model. *Journal of Virology* 78: 9016–9029.

Clark EG (1997) Post-weaning wasting syndrome. *Proceedings of the American Association of Swine Practitioners* 28: 499–501.

Crowther RA, Berriman JA, Curran WL, Allan GM, and Todd D (2003) Comparison of the structures of three circoviruses: Chicken anemia virus, porcine circovirus type 2, and beak and feather disease virus. *Journal of Virology* 77: 13036–13041.

Darwich L, Segales J, and Mateu E (2004) Pathogenesis of postweaning multisystemic wasting syndrome caused by porcine circovirus 2: An immune riddle. *Archives of Virology* 149: 857–874.

Fenaux M, Opriessnig T, Halbur PG, Elvinger F, and Meng XJ (2004) A chimeric porcine circovirus (PCV) with the immunogenic capsid gene of the pathogenic PCV type 2 (PCV2) cloned into the genomic backbone of the nonpathogenic PCV1 induces protective immunity against PCV2 infection in pigs. *Journal of Virology* 78: 6297–6303.

Finsterbusch T, Steinfeldt T, Caliskan R, and Mankertz A (2005) Analysis of the subcellular localization of the proteins Rep, Rep′ and Cap of porcine circovirus type 1. *Virology* 343: 36–46.

Gibbs MJ and Weiller GF (1999) Evidence that a plant virus switched hosts to infect a vertebrate and then recombined with a vertebrate-infecting virus. *Proceedings of the National Academy of Sciences, USA* 96: 8022–8027.

Krakowka S, Ellis JA, McNeilly F, Ringler S, Rings DM, and Allan G (2001) Activation of the immune system is the pivotal event in the production of wasting disease in pigs infected with porcine circovirus-2 (PCV-2). *Veterinary Pathology* 38: 31–42.

Mankertz A, Mueller B, Steinfeldt T, Schmitt C, and Finsterbusch T (2003) New reporter gene-based replication assay reveals exchangeability of replication factors of porcine circovirus types 1 and 2. *Journal of Virology* 77: 9885–9893.

Miller MM, Jarosinski KW, and Schat KA (2005) Positive and negative regulation of chicken anemia virus transcription. *Journal of Virology* 79: 2859–2868.

Misinzo G, Delputte PL, Meerts P, Lefebvre DJ, and Nauwynck HJ (2006) Porcine circovirus 2 uses heparan sulfate and chondroitin sulfate B glycosaminoglycans as receptors for its attachment to host cells. *Journal of Virology* 80: 3487–3494.

Segales J, Allan GM, and Domingo M (2005) Porcine circovirus diseases. *Animal Health Research Reviews* 6: 119–142.

Steinfeldt T, Finsterbusch T, and Mankertz A (2006) Demonstration of nicking/joining activity at the origin of DNA replication associated with the Rep and Rep′ proteins of porcine circovirus type 1. *Journal of Virology* 80: 6225–6234.

Tischer I, Gelderblom H, Vettermann W, and Koch MA (1982) A very small porcine virus with circular single-stranded DNA. *Nature* 295: 64–66.

Todd D, Bendinelli M, Biagini P, *et al.* (2005) Circoviridae. In: Fauquet C, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 327–334. San Diego, CA: Elsevier Academic Press.

Citrus Tristeza Virus

M Bar-Joseph, The Volcani Center, Bet Dagan, Israel
W O Dawson, University of Florida, Lake Alfred, FL, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cross-protection Prevention of the symptomatic phase of a disease by prior inoculation with a mild or nonsymptomatic isolate of the same virus.
Defective RNA Subviral RNA molecules lacking parts of the genome while maintaining the signals enabling their synthesis by the viral replication system.
Genome The complete genetic information encoded in the RNA of the virus, including both translated and nontranslated sequences.
Subgenomic RNA Shorter than full-length genomic RNA molecules produced during the replication process sometimes to allow translation of open reading frames (ORFs).
Transgenic pathogen-derived resistance Plants genetically transformed to harbor viral or other pathogen-derived sequences expected to confer resistance against related virus isolates.

History

Over the last 70 years citrus tristeza virus (CTV) has killed, or rendered unproductive, millions of trees throughout most of the world's citrus-growing areas and hence it is rightfully considered as the most important virus of citrus, the world's largest fruit crop, hence the name 'tristeza' which means 'sadness' in Spanish and Portuguese. However, as with many other disease agents, the actual damages of CTV infections and their timings varied considerably at different periods and geographical regions. The origins of CTV infections remain unknown; the virus however existed for centuries in Asia as an unidentified disease agent, but growers in these areas adapted citrus varieties and rootstock combinations with resistance or tolerance to CTV infections. Out of Asia, citrus production moved to the Mediterranean region, primarily through the introduction of fruit and propagation of seed which does not transmit CTV to the resulting plantings; hence, the new cultivation areas starting from seed sources remained free of the virus for centuries. However, the improvement in maritime transportation, allowing long-distance transport of rooted citrus plants, led to the outbreak of the deadly citrus root rot disease caused by *Phytophthora* sp., which was managed by adapting the more tolerant sour orange rootstock. The considerable horticultural advantages offered by this rootstock coincided with the large-scale expansion of plantings throughout the Mediterranean and American countries, and as a result citrus production in many areas was almost entirely based on a single rootstock. Consequently, this decision had grave effects when CTV pandemics swept throughout the world, causing 'quick decline' (death) of trees on this rootstock. Although the CTV problems were first noticed in South Africa and Australia where by the end of the nineteenth century the growers had found that sour orange was an unsuitable rootstock, it was only in the 1930s that the extent of this deadly disease problem manifested itself first in Argentina and shortly later in Brazil and California, with the death of millions of trees.

The virus-like nature of the tristeza-related diseases was demonstrated experimentally by graft and aphid transmission of the disease agent in 1946; however, description of CTV properties began with the seminal findings of Kitajima *et al.* of thread-like particles (TLPs) associated with tristeza-infected trees. These unusually long and thin particles presented a challenging problem for isolation (purification), which was an essential step toward virus characterization. Development of effective purification methods and biophysical characterization of TLP and similar viruses led to their assignment to the closteroviruses, a group of elongated viruses. The association of infectivity with the TLP-enriched preparations was demonstrated first by Garnsey and co-workers; however, the unequivocal completion of Koch's postulates for TLPs was completed only in 2001 with the mechanical infection of citrus plants with TLPs obtained from RNA transcripts of an infectious CTV cDNA clone amplified through serial passaging in *Nicotiana benthamiana* protoplasts.

Developments of TLP purification methods paved the way to antibody preparations and to improved CTV diagnosis by enzyme-linked immunosorbent assay (ELISA), which revealed considerable serological identity among CTV isolates. Later, RNA extracts from CTV-enriched particle preparations were used for molecular cloning of DNA molecules, which when used as probes demonstrated considerable genomic variation among CTV isolates. Nucleic acid probes also demonstrated that plants infected with CTV contained many defective RNAs (dRNAs) in addition to the normal genomic and

subgenomic RNAs. Infected plants also contain unusually large amounts of dsRNAs corresponding to the genomic and subgenomic RNAs and the dRNAs. Because of the ease of purifying these abundant dsRNAs, they often have been the template of choice for producing cDNAs. The advent of cDNA cloning of CTV led to the sequencing of its genome and to description of dRNAs.

Taxonomy and Classification

CTV belongs to the genus *Closterovirus,* family *Closteroviridae*. The family *Closteroviridae* contains more than 30 plant viruses with flexuous, filamentous virions, with either mono- or bipartite (one tripartite) single-stranded positive-sense RNA genomes. A recent revision of the taxonomy of the *Closteroviridae* based on vector transmission and phylogenetic relationships using three proteins highly conserved among members of this family (a helicase, an RNA-dependent RNA polymerase, and a homolog of the HSP70 proteins) led to the demarcation of three genera: the genus *Closterovirus* including CTV and other aphid-borne viruses with monopartite genomes; the genus *Ampelovirus* comprising viruses with monopartite genomes transmitted by mealybugs; and the genus *Crinivirus* that includes whitefly-borne viruses with bipartite or tripartite genomes. Viruses of this family are all phloem-limited viruses. Among the hallmarks of the group is the presence of a conserved five-gene module that includes the four proteins involved in assembly of virions, the major (CP) and minor (CPm) coat proteins, p61, and p64, a homolog of the HSP70 chaperon. In addition, infected phloem-associated cells have clusters of vesicles considered specific to the cytopathology of closteroviruses. Although the evolution of the three genera of the family *Closteroviridae* is unknown, its is interesting to note that dRNAs of CTV analogous to each of the two RNA segments of the crinivirus genome were found in infected plants raising the possibility that criniviruses arose from a closterovirus ancestor.

Geographic Distribution

Citrus is a common fruit crop in areas with sufficient rainfall or irrigation from the equator to about 41° of latitude north and south. CTV is now endemic in most of the citrus-growing areas, with only a few places in the Mediterranean basin and Western USA still remaining free of CTV infections. The spread during the last decade of the brown citrus aphid (*Toxoptera citricida*) to Florida and its recent spread to parts of Portugal and Spain are now threatening most of the remaining CTV-free areas of North America and in the Mediterranean basin. One important aspect of CTV's geographic distribution is that some areas that have endemic isolates of CTV still do not have the most damaging stem-pitting isolates. With the spread of the vector comes the threat of extremely severe CTV isolates which so far have not spread to North America and Mediterranean countries, where only the milder CTV isolates were established in the past.

Host Range and Cytopathology

CTV infects all species, cultivars, and hybrids of *Citrus* sp., some citrus relatives such as *Aeglopsis chevalieri*, *Afraegle paniculata*, *Fortunella* sp., *Pamburus missionis,* and some intergeneric hybrids. Species of *Passiflora* are the only nonrutaceous hosts, infected both naturally and experimentally. The CTV decline strains are associated with the death of the phloem near the bud union, resulting in a girdling effect that may cause the overgrowth of the scion at the bud union, loss of feeder roots, and thus drought sensitivity, stunting, yellowing of leaves, reduced fruit size, poor growth, dieback, wilting, and death. However, other virulent and damaging CTV strains cause stem-pitting (SP), which results in deep pits in the wood under depressed areas of bark and are often associated with severe stunting and considerably reduced fruit production. The seedling-yellow reaction (SY) which includes severe stunting and yellowing on seedlings of sour orange, lemon, and grapefruit is primarily a disease of experimentally inoculated plants but might also be encountered in the field in top-grafted plants.

CTV infection is closely restricted to the phloem tissues which often show strongly stained cells, termed as chromatic. Electron microscopy of infected cells shows that they are mostly filled with fibrous inclusions, consisting of aggregates of virus particles and of membranous vesicles differing in tonicity and containing a fine network of fibrils.

The CTV Virions

The CTV virions are long flexuous particles, 2000 nm long and 10–12 nm in width. The virions have a helical symmetry with a pitch of 3–4 nm, about 8–9 capsids per helix turn, and a central hole of 3–4 nm. Unlike the virions of other elongated plant viruses that possess cylindrical nucleocapsids made of a single coat protein (CP), the CTV virions consist of bipolar helices with a long body and a short tail. Immunoelectron microscopy showed antibodies to CPm attached to only one end of the virions, with the major part (>97%) of the virion encapsidated by CP. Interestingly, the 'tail' corresponds to 5′ end region of the viral genome and the particle tails of other closteroviruses have been associated with small amounts of p61 and p65.

The CTV Genome

Figure 1 shows a schematic presentation of the 19.3 kbp RNA of CTV. Generally, the CTV genome is divided almost equally into two parts, the 5′ part consisting of ORF1a and -1b harboring the viral replication machinery and the 3′ half harboring ten ORFs encoding a range of structural proteins and other gene products involved in virion assembly and host and vector interactions. CTV replicons containing only ORF1a and -1b plus the 5′ and 3′ nontranslated ends (**Figure 1**) fulfill all the requirements for efficient replication in protoplasts.

Interestingly, while the sequences of the 3′ half of all CTV isolates that have been sequenced are 97% and 89% identical when comparing the 3′ nontranslated regions (NTRs) and the rest of the 3′ halves, respectively, the 5′ half sequences often differ considerably. For instance, the isolates T36 and VT show only 60% and 60–70% identities for their 5′ NTR and the remaining 5′ halves, respectively. The considerable deviation of strain T36 from that of the VT group suggests that T36 might have resulted from a recent recombination event involving the 3′ part of a VT-like isolate and the 5′ half derived from a different closterovirus. The common finding of numerous recombination events both within and between CTV isolates and multiple dRNAs supports such a possibility.

The Nontranslated Regions

The remarkable feature of CTV isolates are the close identities (97%) of their 3′ NTR primary sequences and the considerable divergence of their 5′ NTR sequences. Computer-assisted calculations, however, suggest that not only the 3′ NTRs of different isolates fold into similar predicted structures, but surprisingly the dissimilar sequences of the 5′ NTRs also are predicted to fold into similar secondary structures. The 3′ replication signal of several CTV isolates was mapped to 230 nt within the NTR and predicted to fold into a secondary structure composed of ten stem-and-loop (SL) structures. Three of these SL regions and a terminal 3′ triplet, CCA, were essential for replication. Replacement of T36 3′ NTR with 3′ NTRs from other strains allowed replication, albeit with slightly less efficiency, confirming the significance of the primary sequence of this part. The plus-strand sequence of the 5′ NTRs from different strains are predicted to form similar secondary structures consisting of two stem loops (SL1 and SL2) separated by a short spacer region, that were essential for replication. These structures were shown to be *cis*-acting elements involved in both replication and initiation of assembly by CPm.

Genome Organization and Functions

In infected cells, the 12 ORFs of CTV (**Figure 1**) are expressed through a variety of mechanisms, including proteolytic processing of the polyprotein, translational frameshifting, and production of ten 3′-coterminal subgenomic RNAs. The first two mechanisms are used to express proteins encoded by the 5′ half of the genome while the third mechanism is used to express ORFs 2–11. The ORF1a encodes a 349 kDa polyprotein containing

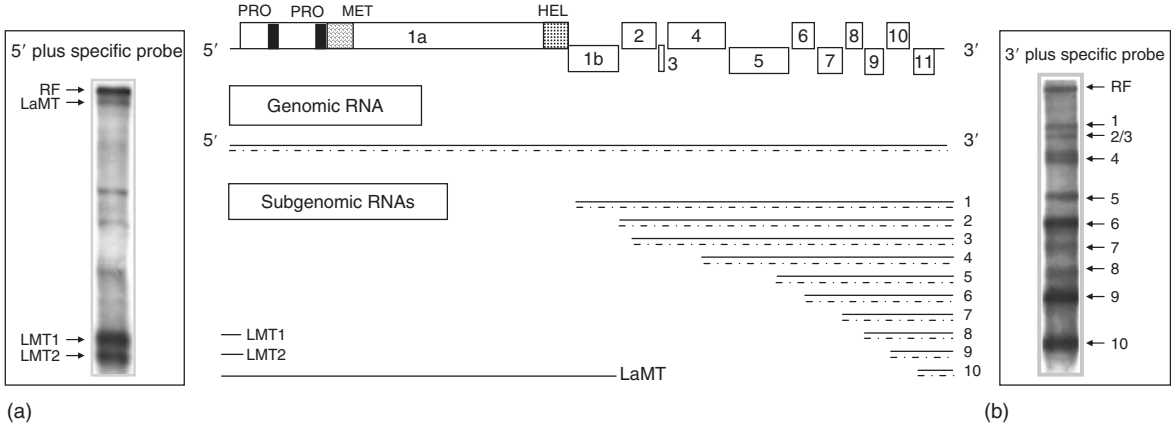


Figure 1 A schematic presentation of the citrus tristeza virus (CTV) genomic (g) and subgenomic (sg) RNAs. The putative domains of papain-like proteases (PRO), methyltransferase (MT), helicase (HEL), and the various open reading frames (ORFs) with their respective numbers are indicated. Lines shown on the left and right side below the genomic map indicate 5′ large molecular single stranded (ss) subgenomic (sg) RNA (LaMT) and two low molecular weight ssRNAs (LMT1 and LMT2) and a nested set of 3′ co-terminal sgRNAs, respectively. Inset (a), shows Northern blot hybridization of dsRNA-enriched extracts from a citrus plant infected with the VT strain of CTV using riboprobes specific to the 5′ end of the viral RNA. Note the presence of the large replicative form (RF) molecules (upper band), LaMT, and two abundant LMT molecules. Inset (b), Hybridization of dsRNA with the 3′ probe. Note the hybridization bands of the different 3′ sgRNAs indicated by arrows.

two papain-like protease domains plus methyltransferase-like and helicase-like domains. Translation of the polyprotein could also continue through the polymerase-like domain (ORF1b) by a +1 frameshift. These proteins along with the signals at the 5′ and 3′ ends of the genome are the minimal requirements for replication of the RNA. The function of p33 (ORF2) is unknown, but is required for infection of a subset of the viral host range. The next five gene products from the 3′ genes include the unique signature block characteristic of closteroviruses, which consist of the small, 6 kDa hydrophobic protein (ORF3), 65 kDa cellular heat-shock protein homolog (HSP70h, ORF4), 61 kDa protein (ORF5), and the tandem pair of p27 (CPm, ORF6) followed by p25 (CP, ORF7). The four latter proteins are required for efficient virion assembly. The small hydrophobic p6 is a single-span transmembrane protein not required for virus replication or assembly but for systemic invasion of host plants. CP is also a suppressor of RNA silencing. The function of p18 (ORF8) and p13 (ORF9) is unknown. Protein p20 (ORF10) accumulates in amorphous inclusion bodies of CTV-infected cells and has been shown to be a suppressor of RNA silencing. p23 (ORF11) has no homolog in other closteroviruses but is a multifunctional protein that (1) binds cooperatively both single-stranded and dsRNA molecules in a non-sequence-specific manner; (2) contains a zinc-finger domain that regulates the synthesis of the plus- and minus-strand molecules and controls the accumulation of plus-strand RNA during replication; (3) is an inducer of CTV-like symptoms in transgenic *C. aurantifolia* plants; (4) is a potent suppressor of intracellular RNA silencing in *Nicotiana tabacum* and *N. benthamiana*; and (5) controls the level of genomic and subgenomic negative-stranded RNAs.

The CTV Subgenomic (sg) RNAs

The replication of CTV involves the production of a large number of less than full-length RNAs. These include ten 3′ coterminal sg mRNAs, and ten negative-stranded sgRNAs corresponding to the ten 3′ sg mRNAs, plus ten 5′-coterminal sgRNAs that apparently are produced by termination just 5′ of each of the ten ORFs (**Figure 1**). The amounts of different sg mRNAs vary with the highest levels for sg mRNAs p23 and p20, located at the distal 3′ end. Infected cells also contain abundant amounts of two other small 5′ coterminal positive-stranded sgRNAs of ~600 and 800 nt designated as low molecular weight tristeza (LMT).

Defective RNAs

Most CTV contain dRNAs, which consist of the two genomic termini, with extensive internal deletions. CTV dRNAs accumulate abundantly even when their genomes contain less than 10% of the viral genome (**Figure 2**).

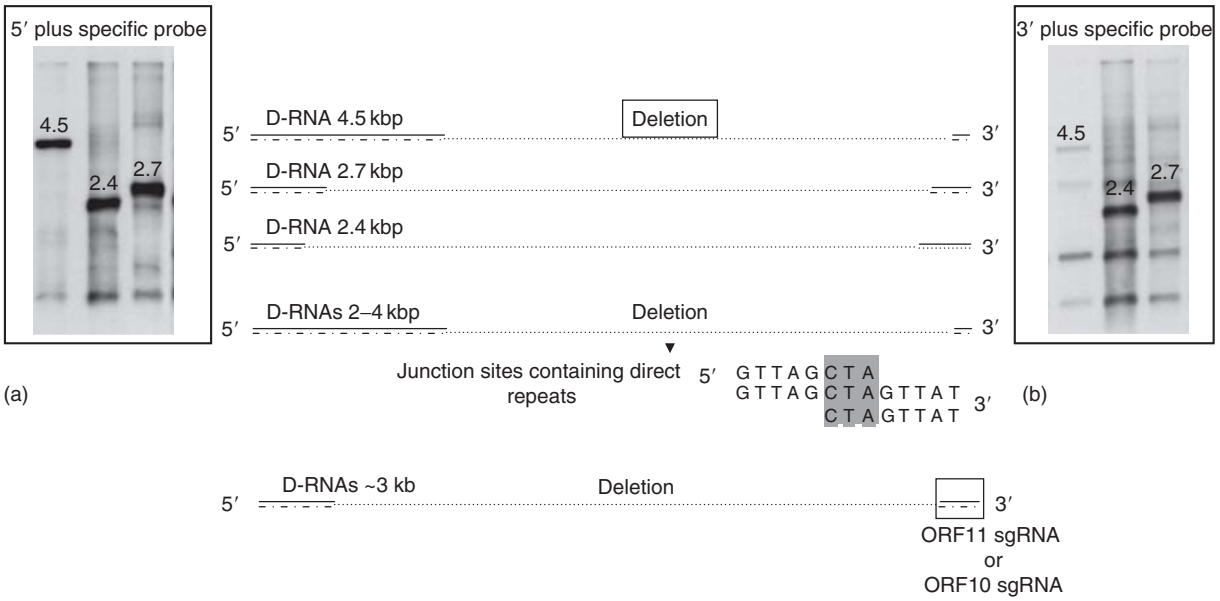


Figure 2 A diagram of class I CTV defective (d) RNAs (=D-RNA) with three different sizes and class II CTV-dRNAs that show 3′ moieties of the size and structure of the full-length sgRNA of ORF11. Insets (a) and (b) show Northern hybridization of class I dsRNAs from three different CTV-VT subisolates, hybridized with riboprobes specific to the 5′ and 3′ ends, respectively, of the genomic RNA of CTV. Note the intense bands resulting from the hybridization of the two probes with the abundantly present dRNAs. Lower part is a diagrammatic presentation of direct repeats at the junction sites of some class I dRNAs. The bottom part shows a schematic presentation of a class II dRNA, with a 3′ terminus corresponding to the ORF11 sgRNA of CTV-VT.

For convenience, the dRNAs were divided into six groups, class I molecules contained different sizes of 5′ and 3′ sequences. Some dRNAs of this class contained direct repeats of 4–5 nt flanking or near their junction sites, supporting the possibility that they were generated through a replicase-driven template-switching mechanism. Class II molecules showed 3′ moieties of the size and structure of the full-length sgRNA of ORF11 (**Figure 2**, lower part). An extra C at the junction sites of several dRNAs of this class corresponded for the extra G reported for 3′ of minus strands from sgRNAs and RF molecules. Class III molecules are large (*c.* 12 kbp) encapsidated dRNAs which are infectious when used to inoculate *N. benthamiana* protoplasts. Class IV consists of dRNAs that retained all or most of the ten 3′ ORFs, analogous to crinivirus RNA 2. Class V includes double recombinants with identical 5′ regions of 948 nt followed with an internal ORF2 sequence and 3′ parts of different sizes, and class VI molecules are closely similar to class I with variable regions from the 5′ and 3′ with junction sites comprised of non-CTV inserts of 14–17 nt.

The biological roles of CTV dRNAs remain obscure as no specific associations have been established for any of the dRNA. Examination of the dRNAs from Alemow plants infected with SY and non-SY inducing isolates revealed mostly a major single dRNA of 4.5 or 5.1 kbp in non-SY plants and two different dRNAs of 2.4 or 2.7 kbp in SY plants. These results suggested the possibility that large dRNAs might play a role in suppression of SY symptoms.

Transmission

Although the virus is phloem limited, mechanical inoculation can be done experimentally at relatively low efficiency by slashing the trunks of small citrus trees with blades containing sap extracts. In commercial groves, the virus is spread naturally by aphid vectors and by vegetative propagation in infected budwood. Long-distance spread of the virus, particularly from country to country, had been through the long-distance transfer of plant propagation material.

Several aphid species including *Aphis gossypii*, *A. spiraecola*, *A. craccivora*, and *T. citricida* transmit CTV semipersistently. The rate of transmissibility varies considerably between different virus isolates and different aphid species. The brown citrus aphid (*T. citricida*) is the most efficient vector, followed by *A. gossypii.* Despite the less efficient transmission by *A. gossypii* and the absence of brown citrus aphid in the Mediterranean region and California, mild strains are spread in these areas. Recently, the brown citrus aphid invaded Central America and Florida, and was also reported to be in the island of Madeira and Portugal.

CTV Control Measures

Strategies to control CTV have varied at different locations and periods and included (1) quarantine and budwood certification programs to prevent the introduction of CTV; (2) costly and ambitious eradication programs to contain situations of virus spread; (3) the use of CTV-tolerant rootstocks and mild (or protective) strain cross-protection, often also named preimmunization; (4) breeding for resistance; and (5) attempts to obtain resistance by genetic engineering. Mild-strain cross-protection has been widely applied for millions of citrus trees in Australia, Brazil, and South Africa to protect against stem pitting of sweet orange and grapefruit trees. However, mild-strain cross-protection has not provided field protection against CTV isolates causing quick decline of trees on the sensitive sour orange rootstock.

Useful resistance to CTV has been found in a citrus relative, *Poncirus trifoliate.* This resistance has been mapped and shown to be controlled primarily by a single dominant gene, *Ctv.* Although the region containing this gene has been identified and sequenced, a specific gene has not been identified by transformation into susceptible citrus with the resistance phenotype.

CTV Diagnosis

The outcome of CTV infections varies considerably depending on the virulence of the prevailing virus isolates and the sensitivity of infected citrus varieties and root stock combinations. Hence, the need of effective means of CTV diagnosis are of utmost importance. Biological indexing of the disease by grafting sensitive citrus indicators has been the definitive assay, although it is costly and time consuming. It has largely been replaced with more rapid immunoassays. ELISA has been widely practiced for almost 30 years for a variety of CTV sanitation programs. Development of recombinant antigens considerably advanced diagnostic possibilities and production of monoclonal antibodies has allowed for more precise differentiation of isolates. PCR and combinations of immunocapture PCR allow more sensitive CTV diagnosis. However, despite the progress in development of better diagnostic tools, none is effective for predicting the biological properties of new CTV isolates.

Economic Costs of CTV

Control strategies varied at different geographic regions and periods, depending on the extent to which CTV was spreading within the newly infested areas, the sensitivity of the specific local varieties, and the economical availability

of alternative crops to replace diseased groves. Most citrus-growing countries that managed to remain free of CTV did so by enforcing sanitation practices to prevent virus spread. These included certification schemes aimed to ensure that citrus nurseries propagate and distribute to growers only CTV-free planting material. Other areas had far more costly and ambitious programs of eradication. These efforts were mostly only temporarily successful, mainly because of lack of long-term grower and governmental commitment to such costly operation. In areas where CTV was endemic, alternative strategies to enable continued commercial citrus production in the presence of CTV were developed. Indeed, long before the viral nature of the tristeza disease was realized, Japanese citrus growers were grafting CTV-tolerant Satsuma mandarins on the cold-tolerant and CTV-resistant trifoliate orange *(P. trifoliate)* rootstocks, thus enabling them to produce quality fruits despite endemic CTV infections. Similarly, the change of rootstocks from the 'quick declining' sour orange rootstocks to rough lemon rootstocks, and to a less extent to mandarin rootstocks, allowed the South African growers to produce good crops of oranges despite the presence of the most efficient aphid vector and infections with severe CTV strains. Similarly, half a century later the Brazilian citrus industry that was completely decimated during 1940s, when all trees were grafted on sour orange rootstocks was saved by adopting CTV-tolerant rootstocks and mild-strain cross-protection.

Historically, the costs of CTV epidemics were estimated to be of the order of tens and even hundreds of millions of dollars. These estimates, however, varied depending on actual market value of the lost production capacity, alternative uses of the land, and the time needed for the CTV-tolerant replants to enter production. There were also indirect costs resulting in poorer performance and/or sensitivity of some CTV-tolerant rootstocks used to replace the widely adapted sour orange rootstocks to other citrus diseases such as citrus blight and citrus sudden death.

Attempts to Apply Transgenic Resistance to CTV

Conventional breeding for CTV resistance is a long, difficult, and inconvenient process mainly because most citrus varieties are hybrids of unknown parentage. Hence, the considerable interest in applying pathogen-derived resistance (PDR) to render both citrus rootstocks and varieties tolerant to stem-pitting isolates and decline causing CTV isolates. Yet, literally hundreds of independent transformations with a range of different types of configuration of CTV sequences have resulted in failure to obtain durable protection against CTV. These failures are especially frustrating since the control of severe stem- pitting isolates is needed in several major citrus production areas. The lack of RNA silencing against CTV in citrus might be due to more effective suppression by the combination of the three suppressors of this virus.

See also: Bromoviruses; Plant Virus Diseases: Fruit Trees and Grapevine.

Further Reading

Bar-Joseph M, Garnsey SM, and Gonsalves D (1979) The *Closteroviruses*: A distinct group of elongated plant viruses. *Advances in Virus Research* 25: 93–168.

Bar-Joseph M, Marcus R, and Lee RF (1989) The continuous challenge of citrus tristeza virus control. *Annual Review of Phytopathology* 27: 291–316.

Dawson WO (in press) Molecular genetics of *Citrus tristeza virus*. In: Karasev AV and Hilf ME (eds.) *Citrus Tristeza Virus and Tristeza Diseases*. St. Paul, MN: APS Press.

Karasev AV (2000) Genetic diversity and evolution of closteroviruses. *Annual Review of Phytopathology* 38: 293–324.

Martelli GP, Agranovsky AA, Bar-Joseph M, *et al.* (2002) The family *Closteroviridae* revised. *Archives of Virology* 147: 2039–2044.

Classical Swine Fever Virus

V Moennig and I Greiser-Wilke, School of Veterinary Medicine, Hanover, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

CPE Viruses can have a cytopathic effect on infected cells, that is, they either kill cells or change their properties.

DI Defective interfering particles lack part(s) of their genome and they depend on complete helper virus for replication; they interfere with the replication of the helper virus by competing for essential enzymes.

ELISA Enzyme-linked immunosorbent assays are widely used for virological and serological diagnosis. Samples can be processed automatically and results are read using optical methods.
IFT Immunofluorescense tests utilize specific antibodies conjugated with fluorescent dye. Viral antigens can be visualized after binding of conjugated antibodies.
MLV Modified live vaccines consist of attenuated viruses that have lost their pathogenic properties and cause an infection without significant clinical signs in the vaccine.
PLA Enzymes like peroxidase can be conjugated to virus-specific antibodies. Binding of these antibodies to viral antigens located in infected cells can be visualized by adding substrate to the antigen–antibody–enzyme complex.
qPCR Real-time PCR allows monitoring of the polymerase chain reaction in real time on a computer screen. In contrast to gel-based PCR systems qPCR yields semi-quantitative results.
VNT Virus neutralization tests are tissue-culture-based assays for the detection and quantification of virus neutralizing antibodies. Of all antibody detection tests they are generally considered the 'gold standard'.

Introduction

Classical swine fever (CSF), formerly known as hog cholera, is a highly contagious viral disease of swine with high morbidity and mortality especially in young animals. Due to its economic impact it is notifiable to the Office International des Epizooties (OIE (World Organisation for Animal Health)). First outbreaks of the disease were observed in 1833 in Ohio, USA. The aetiological agent was long assumed to be a bacterium (hog cholera bacillus), until de Schweinitz and Dorset demonstrated in 1903 that the agent was filterable. CSF has been eradicated in Australia, Canada, the US, and almost all member states of the European Union (EU). Vaccination is banned in these countries. However, outbreaks of CSF still occur intermittently in several European countries in either wild boar or domestic pigs. The latter can cause large economic losses. During the last 16 years in the EU, close to 20 million pigs were euthanized and destroyed because of control measures imposed to combat CSF epizootics, causing total costs of about 5 billion euros. In many countries worldwide, CSF is still a major problem. In 2004, a total of 38 member states of the OIE reported CSF outbreaks.

Virus Properties

Genome Organization and Protein Expression

CSF virus (CSFV) is an enveloped virus with a diameter of *c.* 40–60 nm. The single-stranded RNA genome has a size of 12.3 kb. It has positive polarity with one open reading frame (ORF) flanked by two nontranslated regions (NTRs). The 5′ NTR functions as an internal ribosomal entry site (IRES) for cap-independent translation initiation of the large ORF that codes for a polyprotein of about 3900 amino acids. The polyprotein is co- and post-translationally processed by viral and cellular proteases. From 11 viral proteins, four constitute the structure of the particle, namely three envelope glycoproteins (E^{rns}, E1, and E2) and one core (C) protein. The remaining seven non-structural proteins are N^{pro}, p7, NS2-3, NS4A, NS4B, NS5A, and NS5B. The main target for neutralizing antibodies is the viral envelope glycoprotein E2. To a lesser extent, the host's immune system produces neutralizing antibodies to E^{rns}. This viral envelope glycoprotein occurs as a disulfide-bonded homodimer in the virus particle, and is also secreted by CSFV-infected cells. E^{rns} was shown to be a potent ribonuclease specific for uridine. N^{pro} has an autoproteolytic activity that achieves cleavage from the downstream nucleocapsid protein C. *In vitro*, it had been shown that N^{pro} interferes with the induction of interferon α/β, and *in vivo* CSFV with a deletion in the N^{pro} gene was able to infect pigs but had lost its pathogenicity.

Replication

CSFV replicates in cell lines from different species, for example, pigs, cattle, sheep, and rabbit. For most purposes, the viruses are cultivated in porcine cells. Virus replication is restricted to the cytoplasm of the cell and normally does not result in a cytopathic effect (CPE). The first progeny virus is released from the cells at 5–6 h post infection. Virion assembly occurs on membranes of the endoplasmic reticulum, and full virions appear within cisternae. They are released via exocytosis or cell lysis.

Cytopathic isolates of CSFV occur only sporadically, and most of them are defective interfering (DI) particles. Their genomes have large internal deletions, consisting mainly of the genomic regions coding for the structural proteins. The DI particles are strictly dependent on a complementing helper virus for replication.

Taxonomy and Classification

CSFV belongs together with viruses in the species *Bovine viral diarrhea virus 1* (BVDV-1), BVDV-2, and viruses in the species *Border disease virus* (BDV) to the genus *Pestivirus*, in the family *Flaviviridae*. Classification of CSFV

strains and isolates is currently performed by genetic typing. For this purpose, three regions of the CSFV genome are used, mainly 190 nt of the E2 envelope glycoprotein gene, but also 150 nt of the 5′ NTR, and 409 nt of the NS5B polymerase gene. The phylogenetic tree shows that the CSFV isolates can be divided into three groups with three or four subgroups: 1.1, 1.2, 1.3; 2.1, 2.2, 2.3; 3.1, 3.2, 3.3, 3.4. CSFV groups and subgroups show distinct geographical distribution patterns, which is important for epidemiologists. Whereas isolates belonging to group 3 seem to occur solely in Asia, all CSF virus isolates of the last 15 years isolated in the EU belonged to one of the subgroups within group 2 (2.1, 2.2, or 2.3) and were clearly distinct from historic CSFV of group 1, that are still being used as CSFV laboratory reference viruses. In contrast, current representatives of group 1 viruses continue to cause outbreaks in South America and Central America, and in the Caribbean. Interestingly, CSFVs of all groups have been found in various locations in Asia (**Figure 1**).

Antigenic Relationships and Variability

All pestiviruses were originally classified according to their host origin and the disease they cause. Later, it was found that they are not restricted to their original hosts and that they are serologically related, for example, BVDV and BDV have the capacity to naturally infect many other ruminant species and pigs. The same applies for CSFV, although its extended host range has only been determined experimentally and does not seem to reflect natural conditions. Detailed studies on the antigenic relationship between CSFV and ruminant pestiviruses have been performed using monoclonal antibodies and neutralization studies.

In recent years, progress in molecular biology has allowed a more precise assessment of their relationship. Several genomic regions have been used to study genetic diversity, for example, the 5′ NTR and genes coding for proteins (N^{pro}, C, and E2). Phylogenetic analysis confirmed the classification of the genus *Pestivirus*, comprising CSFV, BVDV-1, BVDV-2, and BDV, and has

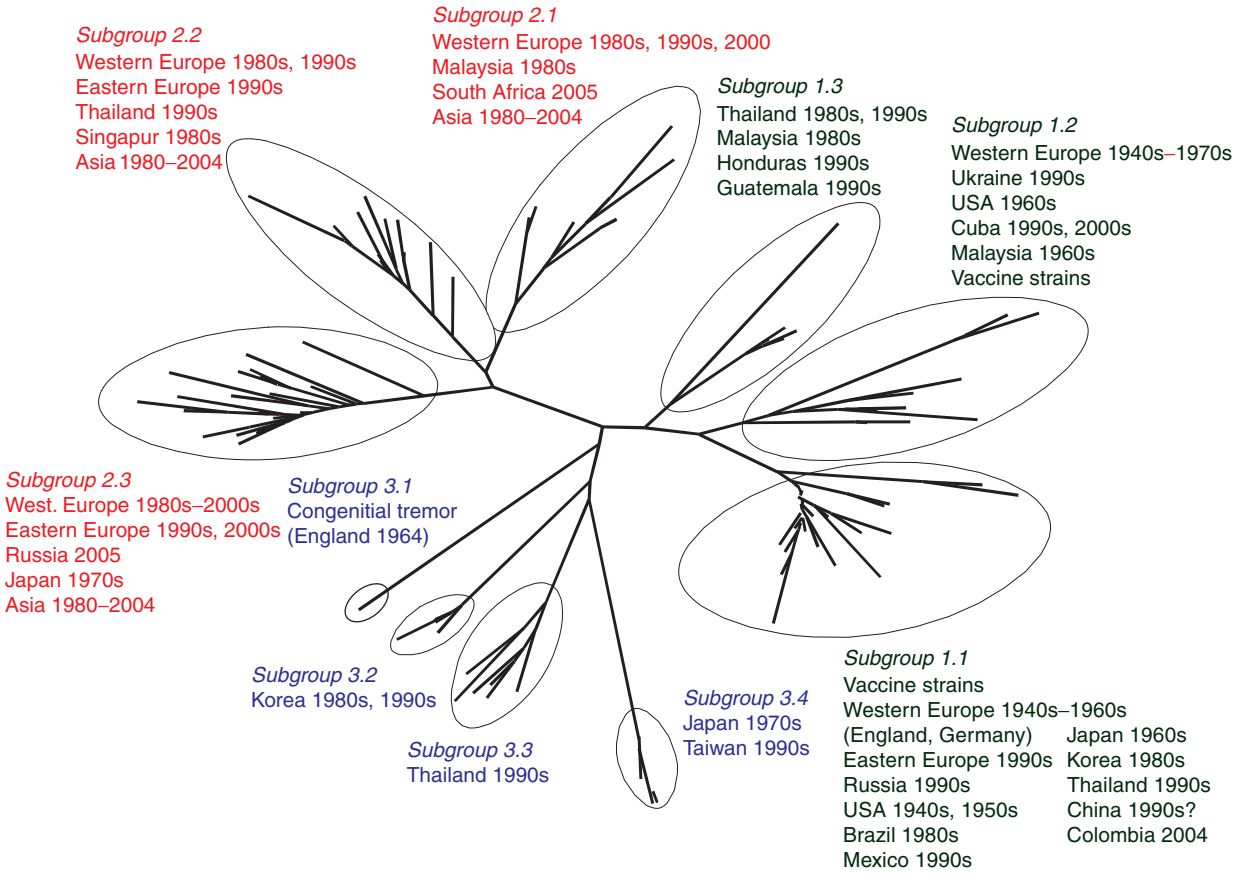


Figure 1 Diversity and geographical distribution of CSF virus isolates. The unrooted neighbor-joining phylogenetic tree was calculated using 190 nt of E2 sequences from 108 CSF viruses. The nomenclature of virus subgroups is as suggested by Paton DJ *et al.* Adapted from Paton DJ, McGoldrick A, Greiser-Wilke I, *et al.* (2000) Genetic typing of classical swine fever virus. *Veterinary Microbiology* 73: 137–157.

revealed at least one additional species, a pestivirus isolated from giraffe.

There is as yet little knowledge concerning the evolution of CSFV, although some analyses have been performed, albeit using short regions of the genome only. These results are difficult to interpret as, in contrast to other RNA viruses, CSFV has little tendency to accumulate mutations. Extensive genomic analyses of isolates obtained between February 1997 and March 1998 in the Netherlands emphasized the genetic stability of CSFV even in the highly variable antigenic region of the E2 gene during a major epidemic that lasted longer than 1 year.

Transmission and Host Range

Members of the family Suidae, in particular domestic pigs (*Sus scrofa domesticus*) and European wild boar (*Sus scrofa scrofa*), are the natural hosts of CSFV. Blood, tissues, secretions, and excretions from an infected animal contain the virus. Transmission occurs mainly by the oral–nasal route, though infection through the conjunctiva, mucous membrane, by skin abrasion, insemination, and percutaneous blood transfer (e.g., common needle, contaminated instruments) may occur. Airborne transmission is not thought to be important in the epizootiology of CSFV, but such transmission could occur between mechanically ventilated units within close proximity to each other.

Experimental transmission of CSFV to goats, sheep, cattle, peccaries (*Tayassu tajacu*), and rabbits was successful, while other vertebrates, for example, racoons, mice, and pigeons did not support the propagation of the virus. Of all species, the rabbit has been of major importance because it was, and in some countries still is, used for the attenuation of CSFV and for large-scale production of live vaccine virus.

Clinical Features

The incubation period in individual animals is 3–10 days. Since transmission from animal to animal may be slow, in large holdings symptoms of CSF may only become evident several weeks after virus introduction ('herd incubation'). The severity of clinical signs mainly depends on the age of the animal and the virulence of the virus. In older breeding pigs, the course of infection is often mild or even subclinical. The virulence of a CSFV isolate is difficult to determine on a rational basis, as the same CSFV isolate can cause different forms of disease depending on age, breed, and immune status of the host animal. Basically acute, chronic, and prenatal forms of CSF can be distinguished, and there is no classical pattern of symptoms that is invariably associated with the disease.

Acute CSF

Most piglets up to 12 weeks of age develop a severe form of acute CSF with high fatalities, whereas older breeding animals may only show mild symptoms. A constant finding in young animals is pyrexia higher than 40 °C. Initial signs are anorexia, lethargy, huddling together, conjunctivitis, respiratory symptoms, and constipation followed by diarrhea. Neurological signs are frequently seen, such as a staggering gait with weakness of hind legs, incoordination of movement, and convulsions. Hemorrhages of the skin are frequently observed on the ear, tail, abdomen, and the lower part of the limbs during the second and third week after infection until death. Virus is shed from the infected animal by all secretions and excretions. CSFV causes severe leukopenia and immunosuppression, which often leads to secondary enteric or respiratory infections. The signs of these secondary infections can mask or overlap the most typical signs of CSF and may mislead the veterinarian. With increasing age of the infected pigs (fattening and breeding animals), clinical signs are less specific and recovery is frequent. The infection is terminated by a strong, predominantly humoral immune response. First neutralizing antibodies against CSFV become detectable 2–3 weeks post infection.

Chronic CSF

The chronic form of CSF is always fatal. It develops in a low percentage of infected animals, when pigs are not able to mount an effective immune response to overcome the virus. Initial signs are similar to those characteristic for the acute infection. Later, predominantly nonspecific signs are observed, for example, intermittent fever, chronic enteritis, and wasting. Animals usually survive for 2–4 months before they die. Until death, CSFV is constantly shed from the onset of clinical signs. Antibodies may be temporarily detected in serum samples, as the immune system begins to produce antibodies, although they are not able to eliminate the virus. Consequently, the antibodies are complexed by circulating virus and cease to be detectable. Since clinical signs of chronic CSF are rather nonspecific, a broad range of other diseases must be considered for differential diagnosis.

Prenatal and Late Onset CSF

Although the course of infection in sows is often subclinical, CSFVs, as do other pestiviruses, cross the placenta of pregnant animals, thereby infecting fetuses during all stages of pregnancy. The outcome of transplacental infection depends primarily on the time of gestation and on viral virulence. Abortions and stillbirths, mummification, and malformations are observed after infection during early pregnancy. In breeding herds, this leads to a reduction

in the fertility index of the affected pig herd. Infection of sows from about 50 to 70 days of pregnancy may lead to the birth of persistently viremic piglets, which may be clinically normal at birth and survive for several months. After birth, they usually show poor growth ('runt'), wasting, or occasionally congenital tremor. This course of infection is referred to as 'late onset CSF' and the outcome is fatal. During their lifetime, these animals constantly shed large amounts of virus and are dangerous virus reservoirs, spreading and maintaining the infection within the pig population. This feature of CSFV infection is comparable to cattle or sheep persistently infected with BVDV and BDV, respectively.

Pathology and Histopathology

After infection, the tonsils are the location of primary virus replication. Thereafter, the agent progresses to neighboring lymphoreticular tissues. Through lymph channels the virus reaches regional lymph nodes, from where it spreads to the blood vascular system. Massive secondary virus replication takes place in spleen, bone marrow, and visceral lymph nodes. Major targets for the virus are cells of the immune system. In the peripheral blood, main target cells for the virus appear to be monocytes. In later stages of the disease, infected lymphocytes as well as granulocytes are found. Early events that are not completely understood play a significant role in the pathogenesis and manifestation of CSF. As early as 24 h post infection with virulent virus, that is, when animals are still asymptomatic and 2–4 days before virus can be detected in the peripheral blood, a progressive lymphopenia is observed resulting in severe immunosuppression. The reason for the massive cell death is not clear; however, a direct interaction with the virus can be ruled out. Viral E^{rns} has been shown to induce apoptosis in lymphocytes of different species. Since this protein is secreted in large quantities from infected cells, it is conceivable that it causes massive destruction of lymphocyte populations. An additional or an alternative mechanism of cell death, respectively, might be triggered by cytokines that are activated shortly after infection, for example, tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-1α, as well as IL-6.

A severe thrombocytopenia develops once infected animals develop fever and virus is detectable in peripheral blood. Currently, there are two explanations for thrombocyte depletion: (1) abnormal peripheral consumption of thrombocytes may be responsible for thrombocytopenia; and (2) progressive degeneration of megakaryocytes, which is observed to begin at day 1 p.i., can result in cell death and shortage of thrombocyte production. It is not clear whether direct or indirect effects are responsible for the latter phenomenon. In analogy to the clinical picture, the severity of pathological lesions depends on time of infection, age of the animal, and virulence of the strain.

In acute cases, pathological changes visible on postmortem examination are observed most often in lymph nodes, spleen, and kidneys. Kidney parenchyma may display a yellowish brown colour. Infarctions of the spleen are considered pathognomonic; however, they are rarely observed. Lymph nodes are swollen, edematous, and hemorrhagic. Hemorrhages of the kidney may vary in size from petechial to ecchymotic. Petechiae can also be observed in the mucous membranes of other organs, for example, urinary bladder, larynx, epiglottis, and heart, and may be widespread over the serosae of the abdomen and chest. Inflammation in the respiratory, gastrointestinal, and urinary tract often are sequelae of secondary infections. Severe pneumonia sometimes is complicated by interstitial edema. Tonsils may display necrotic foci caused by micro infarcts followed by secondary infection. A nonpurulent encephalitis is often present.

In chronically infected animals, pathological changes are less pronounced, especially an absence of hemorrhages on organs and serosae. Instead, thymus atrophy, lymphocyte depletion in peripheral lymphatic organs, and hyperplasia of the renal cortex are often observed. In animals displaying chronic diarrhea, necrotic and ulcerative lesions on the ileum, the ileocaecal valve, and the rectum are found. 'Button' ulcers in the large intestine are pathognomonic, though rare. In cases of congenital infection, a proportion of piglets may show incomplete development of the cerebellum or other developmental abnormalities, such as atrophy of the thymus.

Epizootiology

Primary outbreaks in CSF-free regions usually occur as a consequence of the feeding of swill containing infected pork. Although this practice is officially banned in almost all CSF-free and many other countries, it is still the major risk factor for the importation of the infection into CSF-free populations of domestic pigs or wild boar. The most common route for the spread of the infection among pigs is virus excretion by infected pigs via saliva, feces and urine, and oronasal virus uptake by uninfected animals. Further sources of infection are natural breeding and artificial insemination, since the virus is also excreted in sperm. Trade of live pigs, including at auction sales, has been shown to be the most frequent cause of virus spread from herd to herd. Other farming activities, for example, livestock shows, visits by feed dealers, and rendering trucks are also high-risk factors. Infected pregnant sows ('carrier sows') may give birth to persistently infected piglets that play an important role in the spread of the infection. CSF in small holdings, commonly called 'backyard holdings', is particularly difficult to control. Swill

feeding, lack of animal registration and movement control, poor hygiene and lack of education of pig owners, as well as lack of awareness of private and government veterinarians facilitate the further spread and persistence of the infection in backyard pig populations.

CSF outbreaks in wild boar or feral pigs can be caused by contaminated garbage or 'spillover' from CSFV-infected domestic pigs. The outcome of these outbreaks mainly depends on the size and density of the wild boar populations affected. Outbreaks in small populations that live within natural confines, such as valleys, tend to be self-limiting. In contrast, infections leading to outbreaks in large areas and dense populations often become endemic. Most of the older animals survive the infection and become immune. Piglets become susceptible with waning maternal immunity and then can serve as reservoirs for the perpetuation of the infection. Most fatalities are registered in the young age class. CSFV in wild boar or feral pigs is a threat to any local domestic pig holding and strict measures have to be taken to avoid the spread to domestic pigs.

Diagnosis

The majority of outbreaks of CSF are diagnosed tentatively on clinical grounds, especially in the severe acute form of disease. However, a number of diseases must be considered for differential diagnosis: acute African swine fever leads to a very similar clinical and pathological picture. Erysipelas, porcine reproductive and respiratory syndrome, cumarin poisoning, purpura hemorragica, postweaning multisystemic wasting syndrome, porcine dermatitis and nephropathy syndrome, *Salmonella* or *Pasteurella* infections, or any enteric or respiratory syndrome with fever not responding to antibiotic treatment may display features resembling CSF. Infections of pigs with related ruminant pestiviruses occasionally cause similar symptoms and cross-reactions in diagnostic laboratory tests. In conditions of reduced fertility, CSF, next to parvovirus infections, porcine reproductive and respiratory syndrome, leptospirosis, and Aujeszky's disease (pseudorabies), should be considered in the differential diagnosis. In any case, suspected CSF outbreaks need to be verified using laboratory diagnostic methods. Techniques for the detection of CSFV and virus-specific antibodies are well established and they are described in detail in the Manual of Standards of the OIE as well as in the Diagnostic Manual attached to Decision 2002/106/EC.

Virus Isolation in Cell Culture

Virus isolation using susceptible porcine cell cultures is still considered the standard method for the direct diagnosis of CSFV infection. Suitable samples are whole blood, buffy coat, plasma, serum or clarified organ suspensions from tonsils, spleen, kidney, or gut lymph nodes. Since CSFV does not cause a CPE, the infection must be visualized by fixing and staining the cells. Antigen can then be detected either by direct or indirect immunofluorescence tests (IFTs), or by immunoperoxidase assays (PLAs), using conjugated virus-specific polyclonal or monoclonal antibodies. Virus isolation is time consuming, but the virus isolates can be stored and used for further analyses, for example, genotyping, and it allows the establishment of strain collections.

Direct Antigen Detection

Antigen detection may be carried out on fixed cryosections of organs, using IFT or PLA with polyclonal or monoclonal antibodies. The tests yield results quickly and they are often used for a first laboratory investigation in a suspected case. However, due to limited sensitivity, a CSF suspicion cannot be ruled out in case of a negative result. The correct interpretation of results requires well-trained and experienced personnel.

Commercially available antigen capture enzyme-linked immunosorbent assays (ELISAs) are used for analyzing blood, buffy coat, organ suspension, plasma, or serum samples. Although the method yields quick results (4 h), its usefulness is limited due to its low sensitivity. It is only suitable for herd diagnosis and not for individual animals. In the near future, the ELISA will most probably be replaced by polymerase chain reaction after reverse transcription of the genome (reverse transcription-polymerase chain reaction, RT-PCR) using pools of samples.

Detection of Viral Nucleic Acid by PCR

RT-PCR is becoming an increasingly important tool for the diagnosis of CSFV. Evaluation of RT-PCR can be performed either by agarose gel electrophoresis, or by real-time techniques (RT-qPCR). As laboratory equipment is becoming reliable and also more affordable, coupling of liquid handling robotics for nucleic acid isolation and RT-qPCR is becoming practicable. Another advantage of both the standard gel-based RT-PCR and the RT-qPCR is that, due to their high sensitivities, pooled samples can be tested. In particular, the use of RT-qPCR allows rapid and reliable testing of herds at the perimeter of an outbreak in order to avoid preemptive slaughter. Despite the advantages that RT-PCR methodology may have over conventional diagnostic tests, it is extremely vulnerable to false negative or false positive results. False negative results can arise when the nucleic acid is degraded, or when the reaction mixture contains inhibitors. Due to its high sensitivity, false positive results may arise from contaminations, either from sample to sample or from other sources. This implies that before diagnostic laboratories can replace any test, their

RT-PCR protocols have to be validated, and regular participation in proficiency testing must prove that performance of the methods used is accurate. In addition, specification concerning sensitivity of the detection must be defined. This is important when samples are pooled. In summary, analytical performance must be equal to or better than that of the standard method, that is, isolation of CSFV in permissive cells.

Antibody Detection

CSFV-specific antibodies in pig populations are sensitive indicators for the presence of the infection. Hence, serological tests are valuable tools for diagnosis and surveillance. CSFV infection mainly induces antibodies against viral proteins E2, E^{rns}, and NS3. Detectable levels of antibodies appear 2–3 weeks post infection and persist lifelong in recovered animals. The virus neutralization test (VNT) is the most sensitive and versatile assay. It is very useful for quantifying neutralizing antibodies as well as for discriminating between infections with CSFV or ruminant pestiviruses. This often becomes necessary since, irrespective of its clinical status, a pig herd or single animal seropositive for CSFV is considered as CSFV infected, unless an involvement of CSFV is ruled out. In contrast, in the case of BDV or BVDV infections in pigs, no disease control measures are taken. The choice of test viruses should take into account the local epidemiological situation. While the above-mentioned differentiation is often possible, the VNT is not able to discriminate between antibody titers due to CSFV field infection and antibody titers resulting from immunization with modified live CSFV vaccines. As it relies on cell culture technology, the test is labor intensive and time consuming, and it is not suitable for mass screening of samples. Therefore, it is mainly used for cases where an accurate quantitative and discriminatory assessment of antibody levels is required.

For routine serological investigation, ELISAs are suitable. These tests are either designed as blocking or as indirect ELISAs. They are widely used for screening of antibodies during and after outbreaks, for monitoring of CSFV infections in wild boar, and to test coverage of immunization of wild boars after vaccination. In general, ELISAs are less sensitive than VNTs. However, although ELISAs have some limitations considering specificity and/or sensitivity, they yield results quickly and they are well suited for mass screening of animals, and on a herd basis they are suitable to detect field virus infections.

Immune Response

Live CSFV infection induces a B-cell as well as a T-cell response, while inoculation of inactivated virus elicits only a B-cell response. The cellular immune response has not been thoroughly investigated. Antibodies are produced against viral proteins NS3, E2, and E^{rns}. Antibodies against E2 and to a lesser extent E^{rns} are protective. Neutralizing antibodies against CSFV are regarded as the most important specific defence against infection and disease. This is in accord with the immune responses to other pestivirus infections. There is some antigenic variation among CSFV isolates, but not to the extent observed with ruminant pestiviruses. Therefore, convalescent animals have a stable and long-lasting, if not lifelong, immunity against all variants of CSFV.

Vaccination

First attempts to vaccinate against swine fever date back to the beginning of the last century, when pigs were infected with live virus and simultaneously treated with serum from immune pigs. This high-risk practice was replaced in the 1940s by the use of inactivated and modified live vaccines (MLVs).While the inactivated vaccines proved rather inefficient, MLV turned out to be highly efficacious and safe in pigs of any age, for example, the GPE^{-} and the lapinized Chinese strain (C-strain) of CSFV. The latter is probably the most popular MLV against CSF. When properly used, MLVs are powerful tools for prophylactic protection of domestic pigs against CSF. In countries still struggling with endemic CSF, vaccines are being used in order to limit economical damage. The systematic use of these vaccines often was and is the first step in the eradication of CSF. In countries that have eradicated the infection, prophylactic vaccination is usually banned, mainly because animals vaccinated with conventional MLV (e.g., the C-strain) cannot be distinguished serologically from animals that have recovered from field infection and thus the CSF-free status of the country would not be regained. However, in emergency situations, vaccination may be used, followed by international trade restrictions for vaccinated animals and their products for at least 6 months. For EU member states, provisions have been made in directive 2001/89/EC for limited vaccination of domestic pigs in cases of severe outbreak emergencies.

In order to overcome the severe restrictions after vaccination, novel marker vaccines have been developed. They are based on the concept that the pattern of antibodies against CSFV from a vaccinated animal can be distinguished from that of an animal which has recovered from a field virus infection. The recently developed E2 subunit CSFV marker vaccines induce neutralizing antibodies against the E2 glycoprotein only. Consequently, CSFV antibodies which are not directed against the E2 glycoprotein are indicative of an infection with wild-type CSFV. With the availability of E2 glycoprotein-based subunit marker vaccines against CSFV, two discriminatory

ELISAs were developed as companion tests that detect antibodies directed against the E^{rns} glycoprotein. Positive results are indicative of the exposure to wild-type CSFV. The development of subunit marker vaccines was a major step forward. Compared to MLV, a few shortcomings have to be taken into account when using subunit marker vaccines. In comparison with conventional MLV, full immunity after subunit vaccination is slow (*c.* 21 days). Sometimes, a booster vaccination is needed, and it does not induce sterile immunity, for example, transplacental transmission of CSFV cannot be completely prevented. However, it was shown that the E2 subunit vaccine was able to stop virus spread among pigs that were vaccinated 10 days earlier. The discriminatory tests are suitable for herd diagnosis but they must not be used to assess the serological status of individual animals. Most likely the limitations of the first generation of marker vaccines may be overcome by a second generation of live marker vaccines. Candidates are being developed. Viral vectors carrying the E2 gene of CSFV or chimeras using the genomes of the CSFV C-strain and BVDV are promising candidates.

Prevention and Control

There is no specific treatment for CSF. In countries where CSF occurs endemically, infected animals are killed and destroyed and vaccination is used to prevent further spread of the virus. Countries free of CSF usually implement measures to avoid outbreaks of the disease. The most effective sanction to prevent introduction of CSFV into a free pig population is the ban of swill feeding and the control of trade. Attempts must be made to prevent the illegal importation of meat and meat products. Professional farms must comply with standard biosecurity rules. In order to eradicate the disease, a system for the registration and identification of all holdings and pigs should be in place. This greatly facilitates the traceability of animal movements. In case CSFV has been introduced, eradication programs are based principally on the destruction of infected and serologically positive animals. In order to avoid trade restrictions, vaccination is usually prohibited. Despite continued efforts to control CSF, outbreaks have occurred intermittently in several European countries. In areas with high pig densities very high numbers of pigs had to be culled in order to stop virus spread, and in these cases direct and indirect economical damage was very high. For example, in the course of the 1997 CSF epidemic in the Netherlands, approximately 12 million pigs had to be destroyed and total economic losses amounted to more than 2 billion euro. In severe outbreak situations, an emergency vaccination option is available. Thus far, emergency vaccination has never been used in Western Europe.

Epidemics of CSF in wild boar populations may be long-lasting and difficult to control. Oral vaccination campaigns using MLV sometimes accompanied by specific hunting measures have been shown to be suitable tools to shorten the duration of these epidemics.

Perspectives

The fascinating pathogenesis of CSFV displays similarities with other hemorrhagic diseases. Further elucidation of determinants of viral virulence and virus interaction with the host animal will contribute to the understanding of hemorrhagic diseases.

Control and eradication of CSFV will remain a challenge for many years to come. In CSF-free countries with a highly developed pig industry and densely populated livestock areas, the control of CSF outbreaks will change from the presently practiced excessive culling of pigs to a more sophisticated disease control strategy using sensitive and specific diagnostic tools (e.g., RT-PCR), for the tracing of virus at the perimeters of outbreaks, combined with emergency marker vaccination, preferably with a live marker vaccine, which is yet to be developed.

In countries where CSF is endemic and where a sizable proportion of pigs are held in backyards, extensive vaccination with MLV, in combination with movement controls and epidemiological surveillance, might help to control this pestilence. Of prime importance is thorough education in order to increase knowledge and awareness of all parties involved.

See also: Vaccine Safety; Vaccine Strategies.

Further Reading

Anonymous (2001) Council Directive 2001/89/EC on community measures for the control of classical swine fever. *Official Journal of the European Communities L* 316/5.

Anonymous (2002) Commission Decision 2002/106/EC approving a Diagnostic Manual establishing diagnostic procedures, sampling methods and criteria for evaluation of the laboratory tests for the confirmation of classical swine fever. *Official Journal of the European Communities L* 39/71.

Armengol E, Wiesmüller K-H, Wienhold D, *et al.* (2002) Identification of T-cell epitopes in the structural and nonstructural proteins of classical swine fever virus. *Journal of General Virology* 83: 551–560.

Bouma A, de Smit AJ, de Kluijver EP, Terpstra C, and Moormann RJ (1999) Efficacy and stability of a subunit vaccine based on glycoprotein E2 of classical swine fever virus. *Veterinary Microbiology* 66: 101–114.

Meyers G and Thiel HJ (1996) Molecular characterization of pestiviruses. *Advances in Virus Research* 47: 53–118.

Moennig V, Floegel-Niesmann G, and Greiser-Wilke I (2003) Clinical signs and epidemiology of classical swine fever: A review of new knowledge. *Veterinary Journal* 165: 11–20.

Paton D and Greiser-Wilke I (2003) Classical swine fever – An update. *Research Veterinary Science* 75: 169–178.

Paton DJ, McGoldrick A, Greiser-Wilke I, *et al.* (2000) Genetic typing of classical swine fever virus. *Veterinary Microbiology* 73: 137–157.
Reimann I, Depner K, Trapp S, and Beer M (2004) An avirulent chimeric pestivirus with altered cell tropism protects pigs against lethal infection with classical swine fever virus. *Virology* 322: 143–157.
Ruggli N, Bird BH, Liu L, Bauhofer O, Tratschin D-J, and Hofmann MA (2005) N^{pro} of classical swine fever virus is an antagonist of double-stranded RNA-mediated apoptosis and IFN-α/β induction. *Virology* 340: 265–276.
Stegeman A, Elbers A, de Smit H, Moser H, Smak J, and Pluimers F (2000) The 1997/1998 epidemic of classical swine fever in the Netherlands. *Veterinary Microbiology* 73: 183–196.
Thiel H-J, Plagemann PGW, and Moennig V (1996) Pestivirus. In: Fields B (ed.) *Virology,* 3rd edn., pp. 1059–1073. Philadelphia: Lippincott-Raven Publishers.

Coltiviruses

H Attoui, Faculté de Médecine de Marseilles, Etablissement Français Du Sang, Marseilles, France
X de Lamballerie, Faculté de Médecine de Marseille, Marseilles, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Lagomorph Gnawing herbivorous mammals including rabbits and hares.
Myocarditis Inflammation of the heart muscle.
Nosological From nosology, which is a branch of medical science which deals with disease classification.
Nymph Immature insect or tick.
Orchitis Inflammation of the testicles.
Pericarditis Inflammation of pericardium.
Sarcolemma Thin transparent homogenous sheath enclosing a striated muscle fiber.
***Trans*-ovarial** From parental ticks to progeny ticks through the eggs.
***Trans*-stadial** In-between developmental stage forms of the ticks.

Introduction

The family *Reoviridae* includes 12 recognized and 3 proposed genera. The genera *Coltivirus*, *Seadornavirus,* and *Orbivirus* are those which include causative agents of animal arboviral diseases (including human diseases). In 1991, the International Committee on Taxonomy of Viruses (ICTV) officially created the genus *Coltivirus* (sigla from *Colorado tick fever virus*) for the classification of 12-segmented dsRNA animal viruses. Until recently, this genus contained both tick-borne and mosquito-borne viruses. The tick-borne viruses included two species which are *Colorado tick fever virus* (CTFV) and *Eyach virus* (EYAV). The former species encompasses three serotypes that are the Florio isolate (CTFV-Fl, isolated from humans and ticks), California hare coltivirus (CTFV-Ca, isolated from a hare in northern California), and Salmon River virus (isolated from humans in Idaho). Many mosquito-borne viruses were also considered as tentative species including *Banna virus* (BAV, isolated from humans) and several isolates from mosquitoes. In the Seventh Report of the ICTV, the genus *Coltivirus* was subdivided into coltivirus group A which contained the various strains of CTFV and EYAV (tick-borne isolates), and coltivirus group B which contained the Southeast Asian mosquito-borne isolates. The tick-borne and mosquito-borne viruses of this group have many distinctive features and sequence data led to a re-evaluation of their taxonomic status. In the Eighth Report of the ICTV (2005), the genus *Coltivirus* encompasses only the tick-borne viruses, while the mosquito-borne viruses were assigned to a new genus designated *Seadornavirus*.

Coltiviruses have been implicated in a variety of pathological manifestations including flu-like illness, neurological disorders, and some severe complications as further described in the text below.

Historical Overview of Coltiviruses

Coltiviruses have been isolated from rodents, humans, and various ticks in the family *Ixodidae*. Colorado tick fever virus is endemic in the northwestern part of the American continent. CTFV is responsible for Colorado tick fever (CTF), a human disease that was initially confused with a mild form of Rocky Mountain spotted fever (RMSF), caused by a *Rickettsia* species. The description of fever forms with CTF manifestations was made as early as 1850 and clinical studies of these fevers were initiated in 1880s. In 1930, CTF was recognized as a distinct nosological entity. The causative agent was isolated from human serum in 1944 by Florio using inoculation in adult hamsters, and the disease was reproduced in human volunteers by inoculation with serum from infected patients or

egg-adapted CTFV. Between 1946 and 1950, the virus was adapted to mice, and suckling mice became the routine isolation system for CTFV. This considerably progressed the elucidation of the clinical features, epidemiology, and ecology of CTF. Up to 200 cases of CTF have been reported annually in Colorado State in the USA alone, in the past 10 years. Three isolates of Eyach virus were obtained from ticks in Europe. In 1976, isolate EYAV-Gr was obtained from *Ixodes ricinus* in Eyach village near Stugart, Germany. In 1981, two isolates of the virus designated EYAV-Fr577 and EYAV-Fr578 were obtained from *Ixodes ricinus* and *Ixodes ventalloi* in Mayenne, France. The antigenic relationship of EYAV to CTFV was established by a complement fixation assay.

The recent analysis of the complete genome sequences of both CTFV and EYAV has shown that these two viruses are closely related and confirmed the antigenic observations. The relatedness of EYAV to a well-known human pathogen and the identification of anti-EYAV antibodies in patients' sera indirectly have incriminated EYAV in human neurological diseases. Salmon River virus was isolated from a patient with a moderately severe CTF-like illness in Idaho.

Distribution and Epidemiology

CTF is considered as the one of the most important arboviral diseases of humans in the USA. CTF occurs in mountain forest habitats at an altitude of 1200–3000 m in the Rocky Mountain region of the USA and in Canada (**Figure 1**). The virus distribution closely matches that of its vector, *Dermacentor andersoni*. This region covers British Columbia, Alberta, and Saskatchewan in Canada, and Washington, Montana, Oregon, Idaho, Wyoming, Nevada, Utah, and Colorado in the USA. However, CTFV was also isolated from the dog tick *Dermacentor variabilis* in Long Island, New York, in the late 1940s. California hare coltivirus (CTFV-Ca), identified previously as strain S6-14-03, was isolated from the blood of the white hare *Lepus californicus* in California (outside the range of *Dermacentor andersoni*). Antibodies have been found in hares in Ontario but without any virus isolation or occurrence of human cases. CTFV infection has been contracted from infected ticks between March and September. Interestingly, the presence of antibodies reacting with CTFV antigen have also been detected in sera of humans in South Korea.

Between 1980 and 1988, over 1400 cases were reported in the USA, mainly in Colorado State. The disease is underreported since it has always been confused with other infections. More than 70% of CTF cases occur in adults and a few fatal cases have been reported, mainly in children.

The EYAV isolates EYAV-Gr, EYAV-Fr577, and EYAV-Fr578 were obtained from ticks in Europe. EYAV-Fr577 and EYAV-Fr578 were isolated in France from two ticks, *Ixodes ventalloi* and *Ixodes ricinus,* respectively, feeding on a rabbit (**Figure 1**). However, attempts to isolate these viruses from the rabbit failed. Serological surveys conducted in France identified antibodies to EYAV in rodents. It was found that 0.92% of the tested European rabbit (*Oryctolagus cunniculus*) population in Camargue and Vaucluse had antibodies to EYAV. Similar to CTFV whose principal hosts are rodents, European rabbits could represent the major reservoir for the EYAV. Other studies detected the presence of anti-EYAV antibodies in wild mice in the Sarre region and



Figure 1 The known world distribution of coltiviruses (shaded areas): CTFV, CTFV-Ca, and Salmon River virus in North America and EYAV in Europe (France, Germany, Czech Republic and Slovakia). The geographical localization of the various coltiviruses is within the same range of latitude. The question mark indicates South Korea where antibodies to CTFV have been detected but the virus has not been formally identified by isolation.

in deer, mountain goats, and sheep in the Pyrénées and southern Alps regions of France. Serological surveys in higher mammals, including ovines, deer, and caprines, in the southern half of France, identified anti-EYAV antibodies in 1.35% of tested animals. Following the original isolation of EYAV in France, the serum from a French farmer (0.2% of the tested population) was found to be positive. The presence of the virus is also suspected in Eastern Europe as anti-EYAV antibodies have been identified in patients with neurological disorders. However, the natural cycle of the virus is still unclear and it is not known whether it circulates continuously in Europe. In 2003, the virus was re-isolated from ticks in Baden Wuerttemberg, Germany.

Vectors, Host Range, and Transmission

The vectors of coltiviruses are ticks. Colorado tick fever virus is transmitted by the adult wood tick *Dermacentor andersoni,* but other ticks such as *Dermacentor occidentals, Dermacentor albopictus, Dermacentor parumapertus, Haemaphysalis leporispalustris, Otobius lagophilus, Ixodes sculptus,* and *Ixodes spinipalpis* have been found to transmit the virus. Eyach virus was isolated from the ticks *Ixodes ricinus* and *Ixodes ventalloi. Dermacentor* ticks are three-host ticks, requiring different hosts at the larval, nymph, and adult stages. Ticks become infected with CTFV on ingestion of a blood meal from an infected vertebrate host. Infected nymphs and adults transmit the virus to the host via saliva during feeding.

CTFV is transmitted *trans*-stadially but there is no evidence for *trans*-ovarial transmission. However, it is noteworthy that in other tick-borne viruses in the family *Reoviridae*, such as the orbivirus St. Croix River virus, there is strong suspicion that *trans*-ovarial transmission occurs. CTFV-infected larvae and nymphs can hibernate and both adult and nymphal ticks become persistently infected, providing an overwintering mechanism for the virus. A threshold of 10^2 pfu/ml of blood is required for a tick to become infected. Some rodent species become viremic (as high as $10^{6.5}$ pfu/ml) and this could last more than 5 months, which may also facilitate overwintering and virus persistence in the field. The prevalence of viremic rodents in an endemic area ranges from 3.5% to 25%, producing a tick infection frequency ranging from 10% to 25%. Humans represent accidental hosts and usually become infected with CTFV when bitten by the adult wood tick *Dermacentor andersoni* but most probably do not represent a source of reinfection for other ticks. CTFV has a wide host range including ground squirrels, chipmunks, wild mice, wood rats, wild rabbits and hares, porcupines, marmots, deer, elk, sheep, and coyotes.

Transmission from person to person has been recorded as a result of blood transfusion. In the USA, the virus figures on the list of agents to be screened before bone marrow transplantation. The prolonged viremia observed in humans and rodents is due to the intra-erythrocytic location of virions, protecting them from immune clearance. The maintenance cycle of EYAV in the nature is not known, though the European rabbit *Oryctolagus cunniculus* is suspected to be the primary host.

Virion Properties, Genome and Replication

Coltivirus particles have a total diameter of approximately 80 nm and a core that is about 50 nm in diameter. Electron microscopic studies, have revealed particles with a relatively smooth surface capsomeric structure and icosahedral symmetry (**Figure 2**). The majority of the

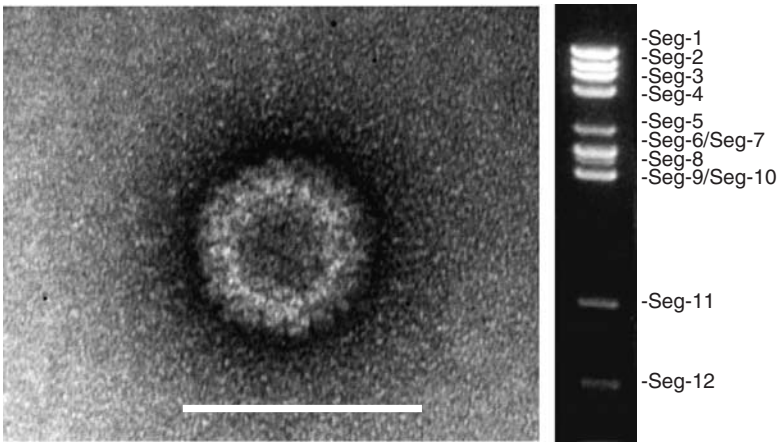


Figure 2 Electron microscopy and the genome of CTFV. (a) Negative contrast electron micrograph of the CTFV Florio strain. The bar represents 100 nm. (b) The agarose gel (1.2%) electopherotype of CTFV genome.

viral particles are non-enveloped, but a few acquire an envelope structure during passage through the endoplasmic reticulum. The buoyant density of CTFV in CsCl is 1.36–1.38 g cm^{-3}. The virus is stable between pH 7.0 and 8.0 and there is total loss of infectivity at pH 3.0. CTFV can be stored at 4 °C for 2–3 months in presence of 50% fetal calf serum in 0.2 M Tris– HC1 pH 7.8, or for years at –80 °C. Upon heating to 55 °C, CTFV rapidly loses infectivity. The virus is stable when treated with nonionic detergents (such as Tween 20) or with organic solvents such as Freon 113 or its ozone-friendly substitute Vertrel XF, but viral infectivity is abolished by treatment with sodium deoxycholate or sodium dodecyl sulfate.

The genome of coltivirus consists of 12 dsRNA segments that are named Seg-1 to Seg-12 in order of reducing molecular weight as observed by agarose and polyacrylamide gel electrophoresis. The genome comprises approximately 29 000 bp and the segment length ranges between 4350 bp and 675 bp. The genomic RNA of CTFV migrates as ten distinct bands during 1% agarose gel electrophoresis (AGE). Two of these bands contain co-migrating segments (**Figure 2**). The genome of the North American California hare coltivirus (CTFV-Ca) has shown a similar electropherotype by AGE. The dsRNA genome of EYAV has not been analyzed by AGE, since this virus is not cultivable. Sequence analysis has shown that the G+C content for coltiviruses ranges between 48% and 51%.

CTFV produces cytophatic effect (CPE) and plaque formation in many mammalian cells including KB (human carcinoma cells), BGM (monkey kidney cells), Vero (monkey kidney cells), BHK-21 (hamster kidney cells), and L-929 (mouse fibroblast). The virus has been found to replicate in a *Dermacentor variabilis* cell line with yields of $10^{6.5}$ pfu ml^{-1}. In cells infected by CTFV, granular matrices, which contain virus-like particles, are produced in the cytoplasm. These structures appear similar to viral inclusion bodies (VIBs) produced during orbivirus infections. In addition, bundles of filaments (tubules) characterized by cross-striations, and kinky threads, are found in the cytoplasm and, in some cases, in the nucleus of infected cells. These may also be comparable to the 'tubules' found in orbivirus infected cells. There is no evidence for virus release prior to cell death and disruption, after which more than 90% of virus particles remain cell-associated. Immunofluorescence has shown that viral proteins accumulate in the cytoplasm and can be detected from 12 h post infection.

The full-length genome sequence of Colorado tick fever virus has been characterized and found to be 29 210 nt long; that of Eyach virus was found to be 29 210 nt in length. In comparison to viruses classified in to other genera of the *Reoviridae,* the EYAV genome is the longest of all members sequenced (genomes range between 18 500 and 28 500 bp). All 12 segments of CTFV and EYAV have conserved sequences which are located at the termini. The conserved motifs 5′-$^{G}/_{C}$ACAUUUG-3′ and 5′-UGCAGU$^{G}/_{C}$-3′ have been found in the 5′ NCR and the 3′ NCR of CTFV, respectively, while in EYAV, these motifs are 5′-GACA$^{A}/_{U}$UU-3′ and 5′-UG$^{C}/_{U}$AGUC-3′. For both viruses, the 5′ and 3′ terminal tri-nucleotides of all segments are inverted complements.

The organization of the genome is well documented. Each genome segment contains a single ORF spanning almost the whole length of the segment (**Figure 3**), except genome segment 9 which has been found to contain a stop codon at position 1054 that is in-frame to a terminal stop codon at position 1846. The in-frame stop codon has been shown to belong to the category of so-called 'leaky stop codons' which allow a partial readthrough, generating two proteins. The first is the short form of the protein designated VP9 (ORF 41–1054) which is structural, and the second, which is the readthrough product designated VP9′ (ORF 41–1846), is nonstructural. The readthrough phenomenon (reading through a stop codon) is well characterized in some plant viruses, retroviruses, and aplhaviruses. None of the members of family *Reoviridae* had been known to use this translational regulatory feature until the identification of readthrough in genome segment 9 of coltiviruses (both CTFV and EYAV). VP9 is supposed to interfere with the action of eukaryotic peptide chain release factors (ERF1 and ERF3), thus facilitating readthrough of the stop codon and synthesis of VP9′.

Segment 6 of CTFV (encoding VP6) is homologous to segment 7 of EYAV (encoding VP7). The amino acid sequence (residues 370–490) of EYAV VP7 shows 50% similarity to the sarcolemmal-associated protein of the European rabbit *Oryctolagus cunniculus*, which is suspected to be its primary host. By comparison, the cognate protein VP6 of CTFV showed no match with this rabbit protein. This is probably due to the insertion of a sequence encoding a lagomorph protein into segment 7 of EYAV.

Antigenic and Genetic Relationship among Coltiviruses

Antigenic variation between CTFV strains has been investigated using immune sera, and antigenic heterogeneity has been observed, especially between strains recovered from humans. However, the variation is not enormous and durable immunity to reinfection appears to be the rule. CTFV from North America and EYAV from Europe show little cross-reaction in neutralization assays. CTFV-Ca cross-reacts with CTFV-Fl but not with EYAV and is considered to be a serotype of CTFV-Fl. Three antigenic variants of EYAV are recognized: EYAV-Gr, EYAV-Fr577, and EYAV-Fr578. RNA cross-hybridization analysis

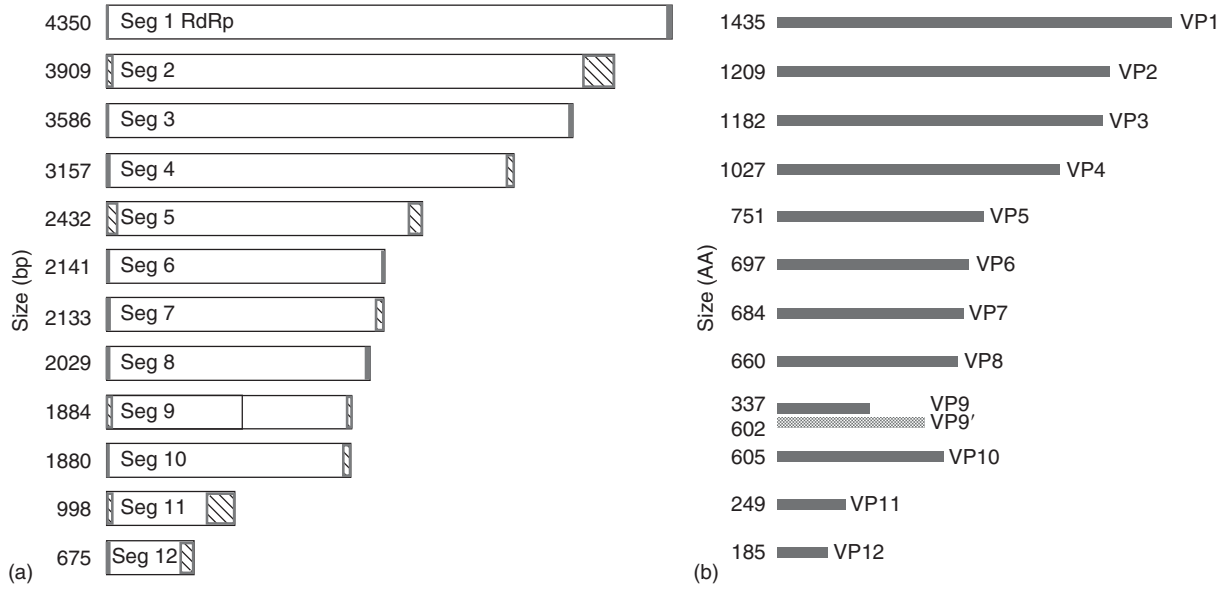


Figure 3 (a) Organization of the genome segments of CTFV and (b) their putative encoded proteins. Shaded areas in A: 5′ and 3′ NCRs. Segment 9 is interrupted by an in-frame stop codon (position 1054) which has been shown to be leaky allowing readthrough, and therefore generating two proteins: a truncated short form designated VP9 (a structural component) and a long readthrough form designated VP9′ (a nonstructural component).

shows that CTFV isolates have remained relatively homogenous (some variation has been reported based on cross-hybridization in genome segments 4 and 6, compared to the prototype strain CTFV-F1). This level of genetic divergence in CTFV is low compared to that found, for example, within the various species in the genus *Orbivirus*, suggesting that the CTFV is sheltered from immunological pressures causing genetic drift. The overall identities between nucleotide sequences of segments 9, 10, 11, and 12 of CTFV strains range between 90% and 100% : 97–100% (segment 9), 96–99% (segment 10), 90–94% (segment 11), and 94–96% (segment 12). The degree of identity between nucleotide sequences of segment 1–12 of CTFV and EYAV isolates ranges from 55% to 88%.

Within family *Reoviridae*, the most conserved gene between the various genera is that encoding the RNA-dependent RNA polymerase. Values lower than 30% amino acid sequence identity distinguish members of distinct genera. A tree constructed from an alignment of polymerase sequences from the various representative members of the *Reoviridae* (**Table 1**) is shown in **Figure 4**. Calculations of the amino acid sequence identity based on this alignment indicate that coltiviruses show only 15% identity with seadornaviruses, confirming the status of coltiviruses and seadornaviruses as members of distinct genera. Amino acid identities as high as 28% occur between coltiviruses and mycoreoviruses (reoviruses of fungi containing 11 or 12 genome segments).

Genome characterization has helped to shed more light on the origin of EYAV. Analysis of the most variable genes (segments 6, 7, and 12) indicates as high as 45% amino acid sequence divergence (37% nucleic acid divergence) as shown in **Figure 5**. Trees displayed in **Figure 5** show that it is possible to distinguish coltivirus species by sequence analysis of their variable genes (segment 6, 7, or 12). It has been proposed that EYAV was derived from CTFV following the introduction into Europe, in 1953 and 1972, of the North American cottontail rabbit *Sylvilagus floridanus* (following the myxoma disease that ravaged the European rabbit *Oryctolagus cuniculus*). EYAV may have then evolved from CTFV under the selective pressure of adaptation to the European rabbit, *Oryctolagus cuniculus,* and European ticks. It follows that the observed genetic divergence of 37% (in segments 6, 7, and 12) occurred over a very short period of time (less than 50 years). This implies a molecular evolutionary rate in the order of 0.5×10^{-2} mutations/nt/year.

Alternatively, it has been also proposed that EYAV might have been derived from an ancestral virus introduced into Europe with the migration of ancestral lagomorphs (hares, rabbits) from North America through Asia. Lagomorph ancestors first appeared during the Eocene epoch (57.8–36.6 MYA) in what was then North America. They are thought to have first migrated into Asia during the Oligocene epoch (34–23 MYA) and by the high Miocene epoch (23–5 MYA) they were common in Europe. This hypothesis implies a molecular evolutionary rate in the order of 10^{-8} to 10^{-9} mutations/nt/year, a rate similar to that of dsDNA genomes. Genetic findings support the second hypothesis.

Table 1 Sequences of RdRp used in phylogenetic analysis of various members of family *Reoviridae*

Species	*Isolate*	*Abbreviation*	*Accession number*
Genus *Seadornavirus* (12 segments)			
Banna virus	Ch	BAV-Ch	AF168005
Kadipiro virus	Java-7075	KDV-Ja7075	AF133429
Genus *Coltivirus* (12 segments)			
Colorado tick fever virus	Florio	CTFV-Fl	AF134529
Eyach virus	Fr578	EYAV-Fr578	AF282467
Genus *Orthoreovirus* (10 segments)			
Mammalian orthoreovirus	Lang strain	MRV-1	M24734
	Jones strain	MRV-2	M31057
	Dearing strain	MRV-3	M31058
Genus *Orbivirus* (10 segments)			
African horse sickness virus	Serotype 9	AHSV-9	U94887
Bluetongue virus	Serotype 2	BTV-2	L20508
	Serotype 10	BTV-10	X12819
	Serotype 11	BTV-11	L20445
	Serotype 13	BTV-13	L20446
	Serotype 17	BTV-17	L20447
Palyam virus	Chuzan	CHUV	Baa76549
St. Croix River virus	SCRV	SCRV	AF133431
Genus *Rotavirus* (11 segments)			
Rotavirus A	Bovine strain UK	BoRV-A/UK	X55444
	Simian strain SA11	SiRV-A/SA11	AF015955
Rotavirus B	Human/murine strain IDIR	Hu/MuRV-B/IDIR	M97203
Rotavirus C	Porcine Cowden strain	PoRV-C/Co	M74216
Genus *Aquareovirus* (11 segments)			
Golden shiner reovirus	GSRV	GSRV	AF403399
Grass carp reovirus	GCRV-873	GCRV	AF260511
Chum salmon reovirus	CSRV	CSRV	AF418295
Striped bass reovirus	SBRV	SBRV	AF450318
Genus *Fijivirus* (10 segments)			
Nilaparvata lugens reovirus	Izumo strain	NLRV-Iz	D49693
Genus *Phytoreovirus* (10 segments)			
Rice dwarf virus	Isolate China	RDV-Ch	U73201
	Isolate H	RDV-H	D10222
	Isolate A	RDV-A	D90198
Genus *Oryzavirus* (10 segments)			
Rice ragged stunt virus	Thai strain	RRSV-Th	U66714
Genus *Cypovirus* (10 segments)			
Bombyx mori cytoplasmic polyhedrosis virus 1	Strain I	BmCPV-1	AF323782
Dendrlymus punctatus cytoplasmic polyhedrosis 1	DsCPV-1	DsCPV-1	AAN46860
Lymantria dispar cytoplasmic polyhedrosis 14	LdCPV-14	LdCPV-114	AAK73087
Genus *Mycoreovirus* (11 or 12 segments)			
Rosellinia anti-rot virus	W370	RaRV	AB102674
Cryphonectria parasitica reovirus	9B21	CPRV	AY277888
Genus *Mimoreovirus* (11 segments)			
Micromonas pusilla reovirus	MPRV	MPRV	DQ126102

The antigenic and genetic relation between CTFV and EYAV is further corroborated by morphological characteristics. Electron microscopic analysis has shown that EYAV and CTFV are morphologically identical.

Clinical Features and Diagnostic Assays

CTF is characterized in humans by the abrupt onset of fever, chills, headache, retro-orbital pains, photophobia, myalgia, and generalized malaise. Abdominal pain occurs in about 20% of patients. Rashes appear in less than 10% of patients. A diphasic, or even triphasic, febrile pattern has been observed, usually lasting for 5–10 days. Severe forms of the disease, involving infection of the central nervous system, or hemorrhagic fever, pericarditis and orchitis have been infrequently observed (mainly in children under 12 years of age). A small number of such cases are fatal. Severity is sufficient to result in hospitalization in about 20% of patients. Congenital infection with CTFV may occur, although the risk of abortion and congenital defects remains uncertain. It has been shown that there is

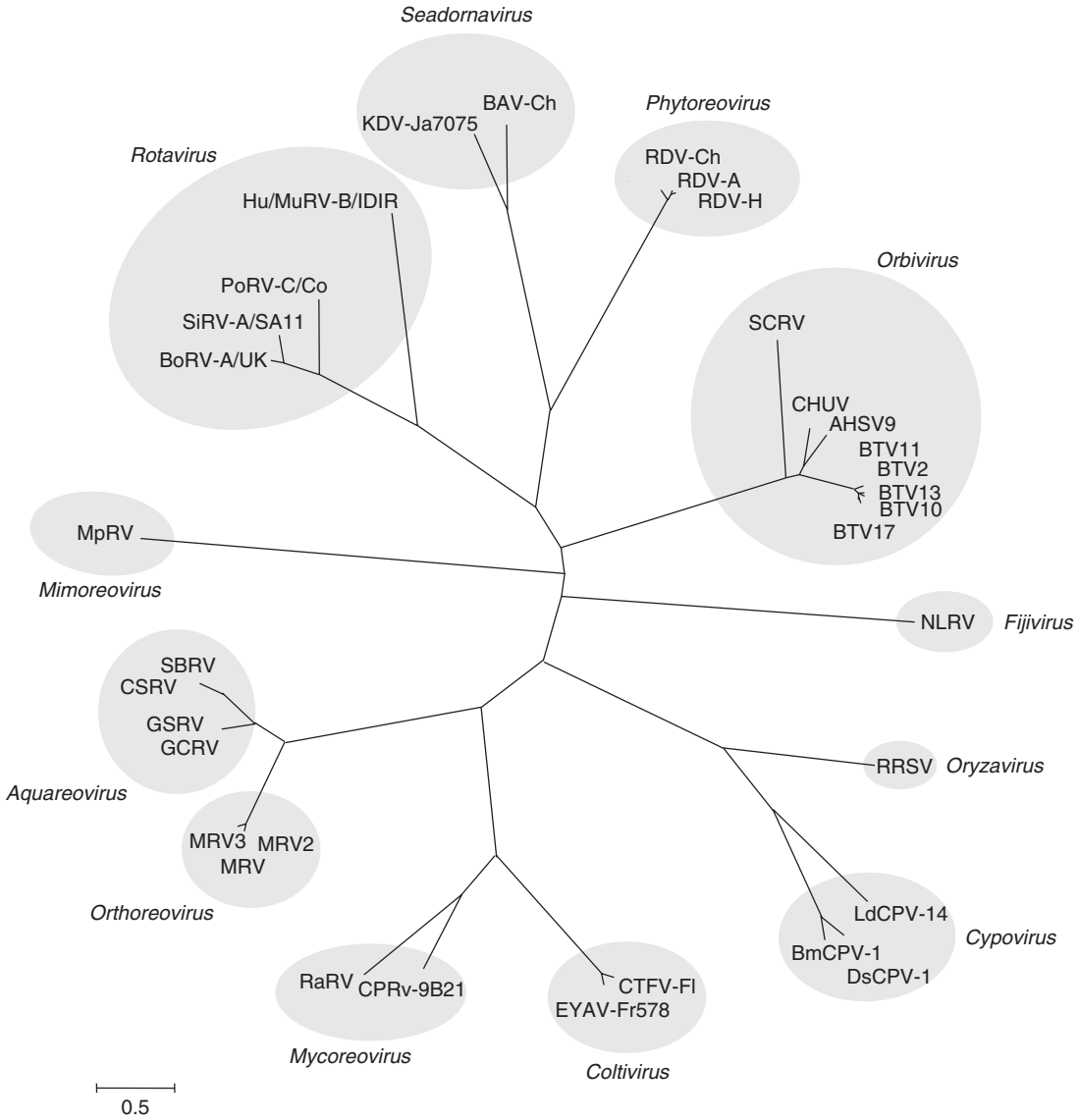


Figure 4 Phylogenetic relations among members of the *Reoviridae* based on the sequences of their putative RdRps. Neighbor-joining phylogenetic tree built with available polymerase sequences (using the Poisson correction or gamma distribution algorithms) for representative members of 12 genera of family *Reoviridae*. The abbreviations and accession numbers are those provided in **Table 1**.

an incidence of teratogenic effects in the offspring of mice experimentally infected with CTFV. Congenital mother to infant transmission has been reported in pregnant women. According to different reports, the incidence of complications has reached up to 7%. CTFV causes leucopoenia (65% of infected humans) with the mean leukocyte count reaching 900 to 3900 mm^{-3} and thrombocytopenia with platelet counts between 20 000 and 95 000. Patients with neurological disorders (meningitis, meningo-encephalitis, and encephalitis) have lymphocyte infiltration of cerebrospinal fluid (CSF) from which the virus has been isolated. Other complications such as pericarditis, myocarditis, and orchitis have been reported. The presence of CNS signs can confuse CTF with other causes of viral meningitis and encephalitis, including enteroviral infections, St. Louis encephalitis and Western equine encephalitis. CTFV infections have also been confused with other tick-borne infections such as Rocky Mountain spotted fever (RMSF: a rickettsial disease), tularemia, relapsing fever, and Lyme disease. Nevertheless, the character of the rash and the presence of leukocytosis distinguish RMSF from CTF.

The virus can be isolated from blood, since it is present in circulating erythrocytes for as long as 4 months after onset. This is because the virus infects hematopoetic progenitor cells and remains sheltered in erythrocytes after maturation. The presence of virus within erythrocytes can be demonstrated by immunofluorescence. Intracerebral

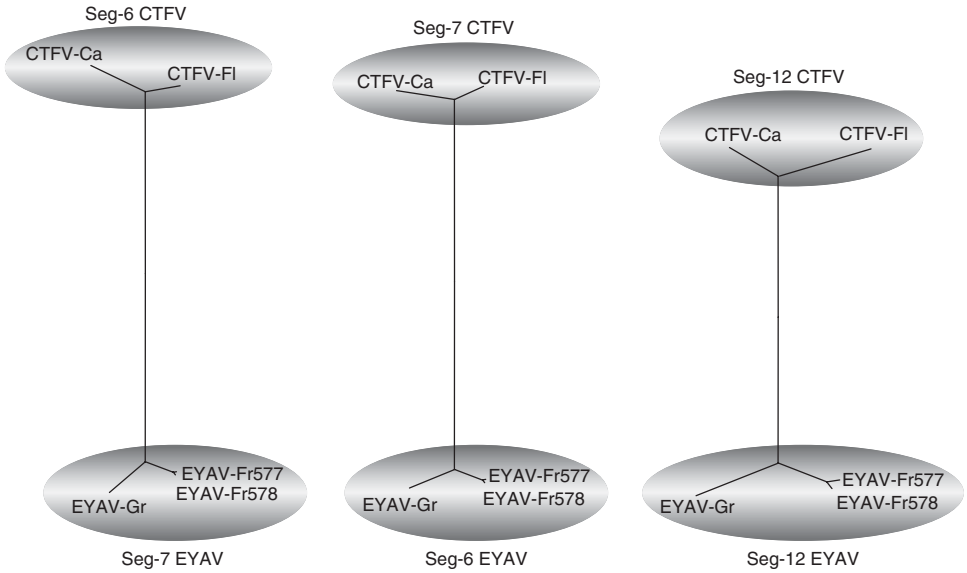


Figure 5 Phylogenetic trees for the most variable genome segments (segments 6, 7, and 12) of different coltiviruses. Unrooted neighbor-joining tree created using MEGA program from Clustal W alignments. The groupings for the deduced amino acid sequence of homologous genes of different coltiviruses. The two species CTFV and EYAV that are currently recognized are labeled. The tree branching is almost identical using the three segments 6, 7, and 12. The genetic distance between species is expressed in percent. These have been calculated for the amino acid sequence of segment 12 and are between species CTFV/EYAV (40%) among isolates of EYAV (<8%); among isolates of CTFV (< 8%).

inoculation of blood in suckling mice has been considered for years to be the most sensitive isolation system. Reverse transcriptase polymerase chain reaction (RT-PCR) has now been developed for the diagnosis of CTFV infection, which permits the detection of human and tick virus isolates as early as 3 days post infection in experimentally infected mice. As little as one genome copy can be detected using the PCR assay.

Serological methods, which utilize infected cell cultures as antigens, have also been developed. These include a complement fixation test, which is relatively insensitive since 25% of patients do not develop CF antibodies, and in 75% of patients CF antibodies appear late. Another test is the virus neutralization test. Neutralizing antibodies appear 14–21 days after onset. An immunofluorescence assay using CTFV-infected BHK-21 or Vero cells offers an easy and rapid means to detect anti-CTFV antibodies. IgM and IgG antibodies can also be detected by enzyme-linked immunosorbent assay (ELISA) and appear concurrently or a few days after neutralizing antibodies (peak at 30–40 days). IgM antibodies decline sharply after day 45. A Western blot assay, which detects antibodies in the acute phase of infection, has also been developed. An ELISA based on the recombinant VP7 of CTFV has been developed and proved efficient and sensitive in detecting various antibodies to CTFV.

A complement fixation assay has been developed for EYAV. In the former Czechoslovakia, a population of 158 patients suffering from encephalitis diagnosed as tick-borne encephalitis (TBE) was tested for TBEV (genus *Flavivirus*), Kemerovo, Lipovnik, or Tribec viruses (all three are tick borne orbiviruses) and EYAV antibodies. Seventeen sera (11%) were found to contain solely anti-EYAV antibodies. Forty-seven patients with polyradiculoneuritis and meningopolyneuritis were tested for the same viruses and eight (17%) were found to have solely anti-EYAV antibodies. Recently, an ELISA based on the recombinant VP6 of EYAV was developed for serological diagnosis of the infection. Antibodies from mice immunized with EYAV and CTFV were used as controls for the assay of the specificity and sensitivity. This test selectively identifies anti-EYAV antibodies. An RT-PCR assay has also been developed for the specific detection of EYAV genome, based on the sequence of genome segment 12.

Treatment, Immunity, and Prevention

There is no specific treatment for CTFV infection. Symptomatic treatment includes relief of fever and pain with paracetamol. Salicylates should be avoided because of thrombocytopenia and occurrence of bleeding disorders. An experimental vaccine (formalin-inactivated, purified CTFV) developed in the 1960s (stopped in l970s) has been used for people at high risk of exposure to infection, and produced long-lasting immunity. After three doses, neutralizing antibodies persist for 5 years. Acaricides or repellents such as permethrin, DEET (*N,N*-diethyl-meta-toluamide), and picaridin may be used to control ticks in rodent niches and as repelling agents to ticks.

See also: Reoviruses: General Features; Reoviruses: Molecular Biology.

Further Reading

Attoui H, Charrel R, Billoir F, *et al.* (1998) Comparative sequence analysis of American, European and Asian isolates of viruses in the genus *Coltivirus*. *Journal of General Virology* 79: 2481–2489.

Attoui H, de Lamballerie X, and Mertens PPC (2005) Coltivirus, Reoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 497–503. San Diego, CA: Elsevier Academic Press.

Attoui H, Mohd Jaafar F, Biagini P, *et al.* (2002) Genus *Coltivirus* (family *Reoviridae*): Genomic and morphologic characterization of Old World and New World viruses. *Archives of Virology* 147: 533–561.

Chastel C, Main AJ, Couatarmanac'h A, *et al.* (1984) Isolation of Eyach virus (*Reoviridae*, Colorado tick fever group) from *Ixodes ricinus* and *I*. ventalloi ticks in France. *Archives of Virology* 82: 161–171.

Emmons RW (1988) Ecology of Colorado tick fever. *Annual Review of Microbiology* 42: 49–64.

Florio L, Miller MS, and Mugrage ER (1950) Colorado tick fever. Isolation of the virus from *Dermacentor andersoni* in nature and laboratory study of the transmission of the virus in the tick. *Journal of Immunology* 64: 257–263.

Karabatsos N, Poland JD, Emmons RW, *et al.* (1987) Antigenic variants of Colorado tick fever virus. *Journal of General Virology* 68: 1463–1469.

Lane RS, Emmons RW, Devlin V, Dondero DV, and Nelson BC (1982) Survey for evidence of Colorado tick fever virus outside of the known endemic area in California. *American Journal for Tropical Medicine and Hygiene* 31: 837–843.

Le Lay-Rogues G, Arthur CP, Vanderwalle P, Hardy E, and Chastel C (1990) Lapin de Garenne, *Oryctolagus cuniculus* L. et arbovirus dans le sud-est de la France résultats de deux enquêtes sérologiques. *Bulletins de la Société de Pathologies Exotiques* 83: 446–457.

Malkova D, Holubova J, Kolman JM, *et al.* (1980) Antibodies against some arboviruses in persons with various neuropathies. *Acta Virologica* 24: 298.

Monath TP and Guirakhoo F (1996) Orbiviruses and coltiviruses. In: Fields BN, Knipe DM, Howley PM, *et al.* (eds.) *Fields Virology,* 3rd edn., pp. 1735–1766. Philadelphia, PA: Lippincott–Raven.

Murphy FA, Coleman PH, Harrison AK, and Gary GW (1968) Colorado tick fever virus: An electron microscopic study. *Virology* 35: 28–40.

Rehse-Küpper B, Casals J, Rehse E, and Ackermann R (1976) Eyach, an arthropod-borne virus related to Colorado tick fever virus in the Federal Republic of Germany. *Acta Virologica* 20: 339–342.

Common Cold Viruses

S Dreschers and C Adams, University of Duisburg–Essen, Essen, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

ASM Acid sphingomyelinase (EC 3.1.4.12), enzyme cleaving sphingomyelin to ceramide and phosphatidylcholine under low pH conditions.
ICAM-1 Intercellular adhesion molecule-1, belongs to the immunoglobulin superfamily.
IRES Internal ribosomal entry site; the sequence enables ribosomes to start translation within a mRNA.
ITAM Immunoreceptor-tyrosine based activation motif; enables proteins to bind to non-receptor tyrosine kinases.
NPDA Niemann–Pick disease type A; patients lack a functional ASM gene and suffer from a malfunctioning lipid storage due to the accumulation of sphingomyelin.
MBCD Methyl-β-cyclodextrin; drug depleting cholesterol out of membranes.

Introduction

Everyone knows the first signs of a common cold. Symptoms usually begin 2–3 days after infection and often include obstruction of nasal breathing, swelling of the sinus membranes, sneezing, cough, and headache. Although the symptoms are usually mild, this (probably the most common illness known to man) exhibits an enormous economic impact due to required medical attention or restriction of activity. According to estimations of the National Center for Health Statistics (NCHS), the common cold caused 157 million days of restricted activity and 15 million days lost from work in 1992. The progress of molecular biology in the twentieth century allowed the isolation and *in vitro* culture of rhinoviruses as well as their ultrastructural and molecular analysis. From the beginning of recorded history to our time people have been interested in how the common cold is spread. Famous ancient investigators, among them Hippocrates and Benjamin Franklin to name two, were the first to suggest inhalation of contaminated air as the origin of infection. This observation still holds its validity, but touching infectious respiratory secretions on skin and on environmental surfaces (desktops, doorknob, etc.) and subsequently touching the eyes or nose is now believed the most common mode of transmission. This article aims to summarize the knowledge about rhinoviral infections and discusses mechanisms of internalization, activation of host-cell receptor molecules, and details of possible therapeutic strategies.

Taxonomic Identification, Genome Organization, and Replication Cycle of Rhinoviruses

The establishment of cell and tissue culture techniques as well as nucleotide sequencing methods allowed for taxonomic classification of rhinoviruses. An overall RNA sequence compilation revealed a close genetic similarity to polio- and coxsackiviruses; hence, rhinoviruses were identified as members of the family *Picornaviridae*.

Genome Organization

The genomic organization is similar in all genera of *Picornaviridae* (**Figure 1**). The 5′ end of the RNA is joined to a small viral protein called VPg, which is a prerequisite for the synthesis of the negative RNA strand as a template for replication. The genomes of *aphtho-*, *erbo-*, *kobu-*, *tescho-*, *cardioviruses* (e.g., the foot and mouth disease virus) contain an additional gene (leader) at the 5′ end of the RNA. The 5′ end also contains the internal ribosomal entry site (IRES) sequence and allows binding to the ribosomal subunits of the host translational machinery. Following the IRES sequence is a single long open reading frame (ORF) encoding the structural (or capsid forming) proteins designated as the P1 precursor and nonstructural proteins (proteases and RNA-dependent RNA polymerase) designated as the P2/P3 precursors(s) (see **Figure 1**). The RNA genome is completed by the 3′ nontranslated region and a poly-A tail.

Translation of the Polyprotein, Processing, and Capsid Maturation

The protease $2A^{pro}$ coded by the P2 precursor was found to have multiple functions. It is required for the inital proteolytic separation of the P1 and P2/P3 precursors in the nascent rhinoviral polyprotein, but also inactivates the cellular *eLF-4F* complex, thereby inhibiting the translation of the cellular mRNA and initiating the virus host shutoff (VHS – the translational machinery of the host cell is producing viral proteins only). Further steps of the post-translational maturation are catalyzed by the protease $3C^{pro}/3CD^{pro}$ (see below). Products of the latter proteolytic activity are the VPg protein and the RNA polymerase. The capsid is composed of four proteins (viral proteins, VP1–VP4, see **Figure 1**), which arise from a single polyprotein generated from the viral RNA genome functioning as an mRNA. The very first cleavage occurs while translation is still in process. This first proteolytic maturation *in cis* is carried out by the protease $2A^{pro}$, which separates structural proteins from those necessary for the replication (designated as P1 and P2/P3, see **Figure 1**). A series of further cleavages form VP1, VP3, and VP0; the latter is finally processed to result in VP2 and VP4. Many picornaviruses undergo VP0 cleavage during virion maturation. Apparently, intact VP0 is necessary for correct assembly of the protomers, whereas processing of VP0 is necessary for the final maturation of the virion. Both viral proteases are targets for an inhibitor screen (see below).

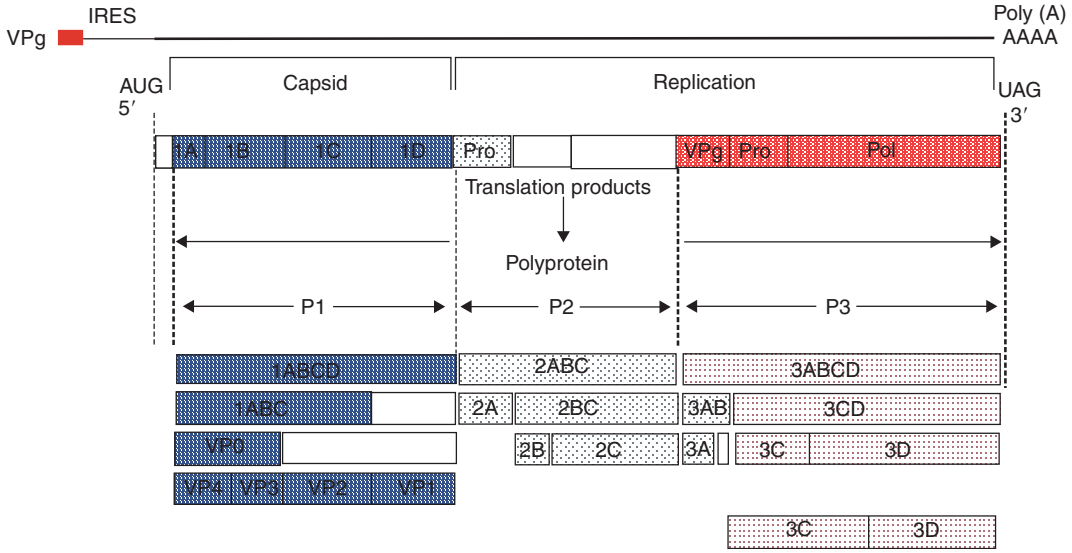


Figure 1 Schematic map of the rhinoviral genome and the encoded proteins. The rhinoviral genome consists of the 5′ IRES sequence (thin black line), followed by one open reading frame (bold black line) and the polyadenylation site. At the 5′ end the VPg protein is linked covalently to the RNA. Below, the regions representing the rhinoviral polyprotein are given. Compounds forming the capsid (structural proteins P1, blue boxes) and nonstructural proteins (P2–P3) such as proteases and the polymerase (light-blue dotted boxes and red dotted boxes, respectively) are translated into one polyprotein. The polyprotein becomes (auto-) proteolytically cleaved until the mature proteins are ready to form new viral particles.

Genome Replication

The following steps of rhinoviral reproduction probably do not differ from other members of the *Picornaviridae*. The RNA genome can be instantly translated; however, for replication of the genome a complementary (–) strand must be synthesized first. Essential for this step is a priming reaction, which is started by the VPg precursor 3AB. The hydrophobic portion of this protein is anchored in endomembranes and is linked to a poly-U tail. After formation of an RNA:RNA duplex at the 3′ end of the (+) strand, the polymerase synthesizes new (–) RNA strands. Only 5–10% of all rhinoviral RNAs are (–) strands. The synthesis of a complete genome takes 45 s to a minute, underlining that the reproduction cyle of picornaviruses in general and of rhinoviruses in particular is a fast process.

Synthesis of rhinoviral RNA in nasal washings could be detected 12 h post infection by quantitative reverse transcription-polymerase chain reaction (RT-PCR). The production of viral RNA and viral particles peaks within 48–72 h and declines thereafter.

Attachment and Entry

Studying any viral reproduction prompts the question of how viruses attach and invade cells. Therefore, the identification of cellular receptors binding to rhinoviruses was a landmark in examining how the infection cycle works. Intercellular adhesion molecule-1 (ICAM-1; CD54) was first detected as the receptor for major group rhinoviruses and later very low density lipoprotein (V)LDL as the receptor for minor group rhinoviruses (**Figure 2**). The receptors were isolated by using monoclonal antibodies directed against cellular surface proteins (see below). The receptors for the minor and major group rhinoviruses are structurally nonrelated, and the molecular reason why rhinoviruses as well as other members of the *Picornaviridae* (e.g., coxsackieviruses) use different receptors is not well understood. One strain (CAV-A21) of the rhinovirus-related family of coxsackieviruses needs to engage both ICAM-1 and decay-accelerating factor (DAF; CD55), whereas other coxsackieviruses require DAF or integrins for binding. HRV binding to ICAM-1 allows a conformational change of the rhinoviral capsid and is a prerequisite for the uncoating of viral RNA. Although binding to (V)LDL receptors is also important for the internalization, the acidification of the endosome seems to be indispensable for proper entry, in particular for minor group rhinoviruses. (V)LDL receptors are well-described molecules, and the uptake of their natural ligands via clathrin-coated vesicles suggested that minor group rhinoviruses enter the host cell via the same pathway. Microscopical studies have detailed the uptake of the minor group rhinovirus HRV2. This viral serotype was shown

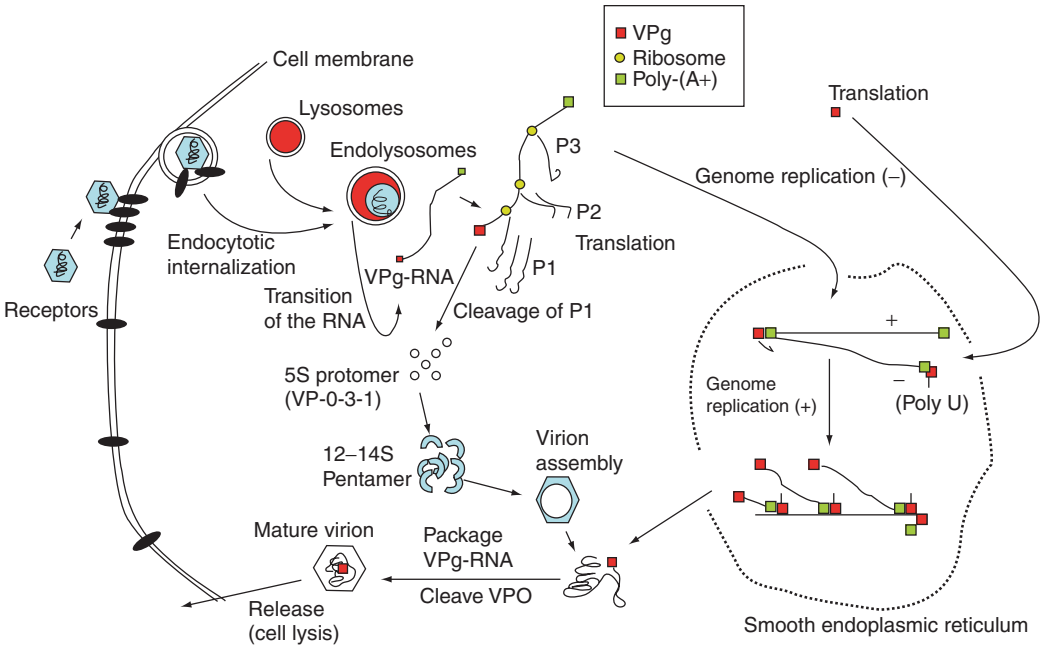


Figure 2 Infection cycle of rhinoviruses. The scheme summarizes the rhinoviral infection cycle from the attachment of the capsid to the release of newly synthesized capsids. Shown are the internalization of the capsids via endocytosis, the transport to the endolysosmal compartment, transition of the RNA to the cytoplasm, synthesis of viral RNA within the smooth endoplasmic reticulum, maturation of the capsid proteins, and self-assembly.

to cluster to membrane domains positive for clathrin, supporting the notion of an uptake mechanism depending on clathrin-coated vesicles. In line with this finding is the blockade of HRV2 uptake by overexpression of dynamin-K44, a transdominant negative isoform of dynamin, which is required for pinching off clathrin-coated vesicles from the plasma membrane. Uptake of rhinoviruses via clathrin-coated vesicles is very fast and does not take longer than 30 min. Moreover, preincubation with methyl-β-cyclodextrin (MBCD) interrupts the uptake of HRV2 at an early stage. Rhinoviral capsids pile up close to the plasma membrane and are not transported to the endolysosomal compartment in cells treated with MBCD. MBCD depletes cholesterol from cellular membranes and leads to destabilization of membrane domains which are thought to play a central role in signal transduction and endocytosis. Recent work indicates that another subgroup of membrane domains termed ceramide-enriched membrane platforms plays a crucial role in the infection cycle of rhinoviruses. These studies demonstrate that pharmacological or genetic inhibition of ceramide-enriched platforms reduces rhinoviral titers in epithelial cells. Membrane domains may not only be involved in the first steps of internalization, but also play a role in transport of vesicles and fusion of transport vesicles, endosomes, and endolysosomal vesicles, finally triggering acidification of the vesicle which is required for uncoating of rhinoviruses and transition of the RNA to the cytoplasm (see below).

Virion/Particle Structure and Phylogenetic Considerations

Human rhinoviruses (HRVs) represent the serologically most diverse group of picornaviruses (over 100 serotypes have already been identified). The reason for this diversity is not known.

The Canyon Hypothesis

Ultrastructural analysis of rhinoviruses by X-ray crystallography resulted in a structural model of rhinoviruses (**Figure 3**). Along the fivefold icosahedral axis of the virion a 2.5 nm depression, the so-called 'canyon', was detected. The genomic region encoding the peptide residues that form this structure is more conserved than regions encoding any other structure on the virion surface. Studies using mutated viral strains showed that point mutations in the genomic region covering the capsid protein VP1 changed the affinity of radiolabeled mutant viruses to purified host cell membranes (**Figure 4** and **Table 1**). Alterations of amino acids at position 103 (Lys),

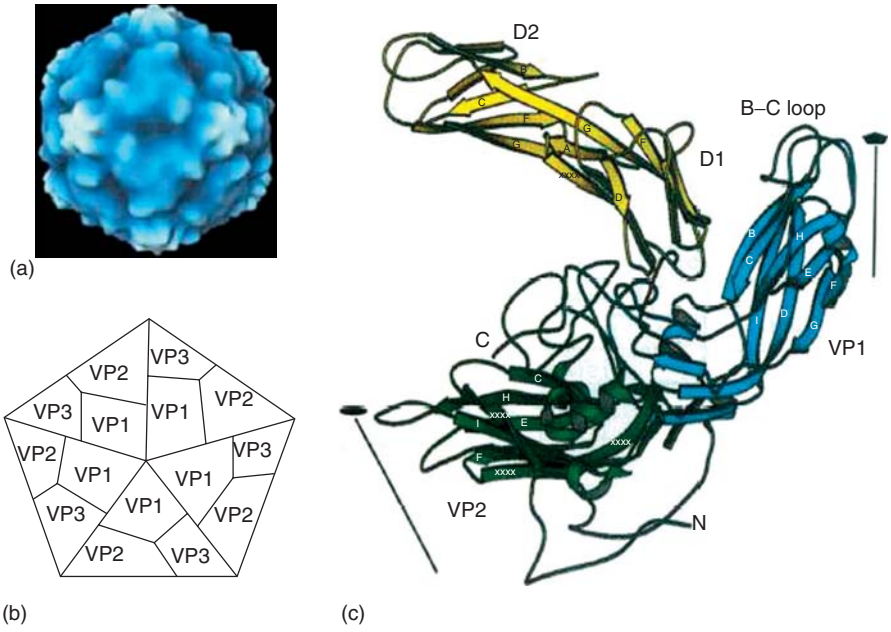


Figure 3 Structural model of rhinoviruses and receptor interaction. (a) The structural model of rhinoviruses was evaluated from cryo-electron microscopic data, the schematic drawing (b) represents the position of the capsid proteins VP1–VP3 in the rhinoviral capsid. (c) Schematic drawing of the viral canyon structure (represented by the capsid compounds VP1 and VP2 given in blue and green, respectively) and the D1 and D2 domains of the receptor ICAM-1. The antiparallel β-strand structures of VP1, VP2, and ICAM-1 are listed in alphabetical order.

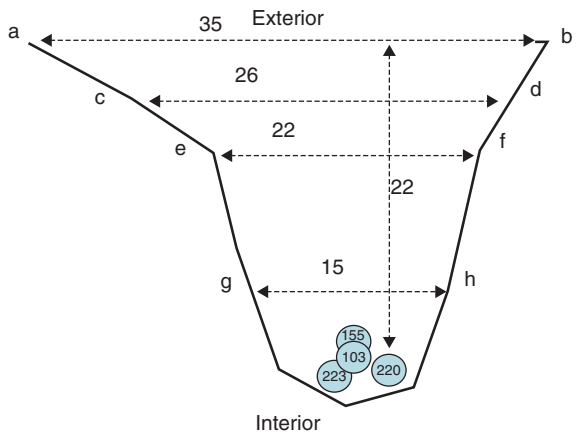


Figure 4 Mutations in the rhinoviral protein VP1 interfere with attachment and growth. The figure displays a scheme of the HRV-14 canyon. Distances are shown in angstrom and circled numbers represent the four amino acids targeted in the studies. Coordinate points used in determining distances are as follows: a, O^{81} of Asp-91; b, O^{e2} of Glu-210; c, O^{81} Asn–92; d, O Glu–210; e, O^{e2} Glu-95; f, N^{e2} Gln–212; g, O^{11} Asp–101; h, C^{81} Ile–215. Modified from Colonno RJ, Condra JH, Mizutani S, Callahan PL, Davies ME, and Murcko MA (1988) Evidence for the direct involvement of the rhinovirus canyon in receptor binding. *Proceedings of the National Academy of Sciences, USA* 85(15): 5449–5453.

Table 1 Mutations in the rhinoviral protein VP1 interfere with attachment and growth

Position of wt amino acids	*Substitutions*	*Virus yield (pfu/cell)*
103 Lys	—	160
	Ile	84
	Arg	54
	Asn	140
155 Pro	—	160
	Gly	1
220 His	—	160
	Ile	10
	Trp	14
223 Ser	—	160
	Ala	8
	Thr	70
	Asn	88

155 (Pro), 220 (His), or 223 (Ser), had the strongest effect regarding the binding affinity of rhinoviral capsids. The structural analysis revealed a juxtaposition of these amino acids at the 'bottom of the canyon'. It is noteworthy that most of the substitutions reduced the binding of capsids to host cell membranes; however, the biochemical property of one amino acid (Pro 155) is of special importance because a change to glycine led to a higher binding affinity and an enhanced yield of mutated virus. Most publications concerning rhinoviral structure described members of the major group rhinoviruses, while only few publications dealt with the structure of minor group rhinoviruses. It was possible to define structural differences between HRV2 (representing minor group rhinoviruses) and HRV14 (representing major group viruses) by analyzing a special subportion of the 'canyon', the 'pocket' region within the VP1 protein. This region binds in particular to hydrophobic compounds – for example, fatty acids supplemented by membranes of the host cell. The interaction of the virus with the fatty acid seems to stabilize the virus during its spread from cell to cell, but it must be removed prior to the uncoating process. For major group viruses the fatty acid is displaced by ICAM-1. The minor group viruses exhibit a higher affinity for the fatty acids and binding of LDL-R is insufficient to expel the fatty acid in the 'pocket' of VP1. The conformational change of the viral capsid is obligatory for the transition of the viral RNA into the cytoplasm. Acidification of the endolysosomal compartment transfers the native virus to a so-called C-antigenic subviral particle in which VP1 forms a channel with a diameter around 10 A – large enough for the RNA to escape. Concomitantly, the N-terminus of VP1 and the entire VP4 are extruded and interact with the endolysosomal membrane. Studies with rhinovirus-related poliovirus identified a 3 kDa peptide from the N-terminus of VP1 capable of integrating into artificial liposomes.

Rhinoviral Antigenicity

Antibodies that neutralize rhinoviruses *in vitro* and *in vivo* permitted the identification of the epitopes in the viral capsid critically important for binding to the receptors and/or release of the RNA into the cytoplasm. Antigenicity in HRVs has been extensively studied in serotypes HRV2 and HRV14. In HRV14 four antigenic sites were identified, designated NIm-IA, NIm-IB, NIm-II, and NIm-III. The sites could be accurately located at the external surface of the virus. In HRV2 three antigenic sites, called A, B, and C could also be mapped. Antigenic site A is located within the B–C loop of VP1 that flanks the rim of the canyon structure, thus, corresponding to the NIm-IA, NIm-IB site of HRV14. Site B encompasses residues from VP1, VP2, and VP3. VP2 residues of site B comprise a continuous epitope that is equivalent to the NIm-II site of HRV14 and mainly defined by the VP2 loop between the β-barrel strands E and F. Antigenic site C includes two amino acids of VP2 which do not correspond to any of the immunogenic sites of major group rhinoviruses.

Phylogenetic Considerations

In addition to the classification based on serotyping, rhinoviruses can be grouped in different ways, on the basis of shared structural and biological properties (e.g., receptor

specificity), sensitivity to antiviral agents or at least genetic similarity. The phylogenetic analysis of the genomic VP4/VP2 interval of 97 different rhinoviral serotypes resulted in two phylogenetic clades, A and B. Surprisingly, some of the 76 serotypes of class A were identified as minor group rhinoviruses such as HRV 1A, 2, etc., whereas class B consists of 25 serotypes, among them major group HRV 14. According to the phylogenetic analysis, two rhinoviral serotypes (HRV 23 and HRV25) which bind to ICAM-1 (major group) constitute an 'intermediate' class between A and B, respectively. Further studies showed that soluble ICAM-1 could not compete with viral binding to cellular ICAM-1. Furthermore, the phylogenetic analysis identified one serotype, namely HRV 87, as closely related to another *Picornaviridae* genus the enteroviruses, namely enterovirus 70. HRV87 does not bind to either ICAM-1 or (V)LDL receptors, but to sialoproteins, which also bind enterovirus 70. Although HRV 87 is acid-labile like all other rhinovirus serotypes, it appears to belong to the enterovirus species D. In general, the analysis shows a greater phylogenetic heterogeneity for minor group rhinoviruses than for major group rhinoviruses, which may indicate that minor group serotypes evolved more recently.

Rhinovirus Infection Models

Rhinoviruses show an almost strict specificity for human cells. Most studies, therefore, have to be carried out in human volunteers or, alternatively, in primates, which necessitates special requirements regarding biosafety of the rhinoviral stock preparations, and raises ethical questions. Some publications reported that host restriction could be overcome by using a method to select rhinovirus strains which are able to infect murine cells. Another possible technique to overcome the host range problem is by expression of human/murine chimeric rhinoviral receptors. Engineered ICAM-1 molecules, stably expressed in murine cell lines, bind rhinoviruses and permit the uptake in murine respiratory cells and L-cells. The latter study underlined that mutations in noncapsid proteins (genomic region P2) are necessary to adapt viral proteins to the new host cell and finally allow an appropriate replication in murine cells. Other researchers developed human ICAM-1 transgenic mice that were recently used in infection studies with coxsackiviruses, and might also be utilized to study rhinoviral infections. Interestingly, a common finding of the field is noteworthy: receptors such as ICAM-1, VCAM-1, and also the (V) LDL play a predominant role in rhinovirus binding, uptake, and associated signal transduction processes. However, these data were determined from infection experiments employing poorly differentiated epithelial cells in culture. In contrast, these molecules are only infrequently expressed *in vivo* on the apical surface, leading to the question of how viruses access these receptors *in vivo*. In addition, the issue whether epithelial cell layers are the primary targets of rhinoviruses remains to be determined, since infections do not result in cytopathic effects in the ciliated epithelial cells as shown for many other respiratory viruses. Many studies focused primarily on the detection of rhinovirus RNA in rhinopharyngeal tissues by means of RT-PCR or *in situ* hybridization showed that cells and tissues (e.g., the germinal layer in the adenoid tissue below the epithelial layer) are positive for rhinoviral RNA. Rhinoviruses can be cultured in WI-38 fibroblasts suggesting that fibroblasts in the rhinopharyngeal tissue can be targets for rhinoviral infection. It is also possible that rhinoviruses attach to and invade the epithelial layer by a mechanism different from that observed in cultured epithelial cells, that is, binding to ICAM-1 and (V)LDL receptors. Further studies are required to address these questions.

Host Cell Reactions upon Rhinoviral Infections – The Molecular Origin of Symptoms

Infection of the upper respiratory tract by HRV16 has been demonstrated to occur at localized portions of the epithelia and does not cause widespread lysis of the infected epithelia. This observation has suggested that the pathology induced by rhinovirus may be due in part to cytokine dysregulation rather than extensive epithelial necrosis observed in other viral infections.

HRV, as well as other respiratory pathogens, have been shown to induce production of many cytokines and chemokines, among them IL-4, IL-6, IL-8, and granulocyte macrophage colony stimulating factor (GMCSF), to name the most frequently published. The induction of chemokines triggers the inflammatory response, which is considered to enhance exacerbations like asthma and chronic obstructive pulmonary disease (COPD). A common feature of asthma and COPD exacerbations is a high level of IL-8 and an increased number of neutrophils in the sputum and nasal secretions of patients suffering an HRV infection.

Little is known, however, about the primary signaling pathways initiated by the rhinoviral infection cycle. Because signal transduction pathways of the LDL receptor family are difficult to understand, the experimental design concentrated on ICAM-1, the receptor for major group rhinoviruses. ICAM-1-inhibiting antibodies abrogated IL-8 production, but infection with UV-inactivated rhinovirus failed to do so, indicating that binding and entry events induced the chemokine response rather than causing effects resulting from viral replication.

HRV ligation to ICAM-1 provokes recruitment of the tyrosine kinase syk to membrane domains in human airway epithelial cells. The tyrosine kinase syk belongs to the family of src receptor tyrosine kinases. The immunoreceptor tyrosine-based activation motif (ITAM) of syk recruits another protein, ezrin, and the syk–ezrin complex finally binds to ICAM-1. Autophosphorylation of syk activates the MAP-kinase p38-K in a biphasic manner, that is, after 1 h and 8–12 h after infection with major group rhinovirus HRV14. This finding is especially interesting because the induction of chemokines is temporally correlating with the second p38-K activation peak. The PI-3 kinase is related to the family of MAP kinases. Again, subsequent studies concentrated on very early phases (5–60 min post infection) of rhinoviral infection. Active PI-3 kinase could be precipitated from lysates of infected cells and isolated plasma membranes showed the presence of metabolic products (i.e., PI (3,4,5)P_3) of the PI-3 kinase. The same studies identified Akt and nuclear factor kappa B (NF-κB) as downstream targets of PI-3 kinase and thus completing this signal transduction pathway showed the binding of NF-κB to the IL-8 promoters.

Interestingly, the activation of PI-3 kinase is functionally connected to the HRV entry. Inhibition of PI-3 kinase by application of the drug Wortmannin reduced the uptake of radiolabeled HRV, due to the retention of HRV2 in endosomal compartments, which results in a reduced transition of the RNA and, therefore, a reduced viral titer.

Inflammatory reactions require effector cells such as macrophages and monocytes. A recent publication addressed the question if the production of chemokines is restricted to the nasal epithelial tissues or if chemokines are synthesized and secreted by lymphocytes. Therefore, alveolar macrophages and monocytes were infected with rhinovirus. After infection with HRV16 the production of the macrophage attractive protein-1 (MCP-1) was shown to be increased in monocytes and macrophages. Surprisingly, HRV16 induces the same signal transduction pathways in lymphocytes as in epithelial cells. Again, the p38-K is activated and engages the nuclear factor ATF-2 involving NF-κB. The signal transduction could be attenuated by preincubation of the lymphocytes with ICAM-1, suggesting that HRV can bind to and enter lymphocytes. However, propagation of rhinoviruses in lymphocytes was not an aim of this study. Finally, these results present the novel idea that cell types other than epithelial cells contribute to viral exacerbations of asthma as well as causing the symptoms of the common cold.

Antirhinoviral Strategies

Although the common cold is usually mild, with symptoms lasting a week or less, it is a leading cause of physician visits and of school/job absence in addition to several more severe exacerbations like asthma (see above). Initial studies that employed monoclonal antibodies to define candidates of viral receptors demonstrated a surprising 'immunological resistance' of rhinoviruses. On the one hand this is due to the fact that over 100 different rhinoviral serotypes have been isolated and antibodies raised against one serotype after immunization are not necessarily protective against a different serotype. However, the identification of the so-called 'canyon' allowed an additional explanation: if this 'canyon' represents the predominant site of receptor interactions, it is difficult to block with antibodies since the canyon is too narrow to enable interactions with the antigen. Both findings seem to rule out vaccination as a standard antirhinoviral treatment. Hence, the canyon structure dominated the development of therapeutic strategies (**Table 2**). Researchers were able to

Table 2 Substances interfering with the rhinoviral propagation

Tested compound	*Functions in or interferes with*
WIN factors	Interaction with the canyon structure, viral adhesion is suppressed
AG 7088	Blocks protease 3 C irreversibly and stops capsid maturation
Soluble ICAM-1	Competition with ICAM-1
Cytochalasin B	Interference with microfilaments and inhibition of vesicle maturation
Bafilomycin	Blocking endosomal acidification
Erythromycin	Reduction of ICAM-1 receptor, blocking endosomal acidification
Impramine	Blocking ASM and inhibiting ceramide-enriched platforms
Desipramine	Blocking ASM and inhibiting ceramide-enriched platforms
Nocodazole	Interference with macrofilaments and inhibiting vesicle maturation
zVAD	Antiapoptotic agent blocking caspases and release of new viruses
Wortmannin	PI-3-kinase inhibitor, blocks vesicle maturation
MBCD	Depletes cholesterol, destroys functional membrane domains, transport of virus is blocked

design chemical structures designated as 'pocket factors', interacting with amino acid residues in the canyon. Because of their chemical similarity to the primarily described drug WIN51711, an isoxazole derivative, such chemicals are generally termed WIN compounds. WIN compounds seem to function both as competitors for binding to the receptor and in a steric blockage interfering with the conformational change of the capsid necessary for the release of the genomic RNA. While WIN compounds have a significant effect in reducing the viral titer *in vitro*, the *in vivo* application was difficult. Structurally related substances like flavinoids, for example 4–6 Dichlorflavane, exhibited a better feasibility regarding application, but caused only little reduction of rhinovirus titers. The chemokines interferon-α/β are produced after viral infection. Host cells detect the presence of double-stranded RNA via activation of the toll-like receptor 3 (TLR-3), and the main function of interferons is to induce IFN-stimulated genes, which attenuate viral replication. Some groups tested the application of interferons and interferon-inducers, respectively, and their interference with rhinoviral replication and production. Although high doses of interferons applied intranasally exhibited a prophylactic effect, these high doses of interferons required for the protective effect caused local erosions and nasal stuffiness.

Therefore, the interest shifted to alternative methods to attenuate or even to block rhinoviral infections and their symptoms. Even though vaccination seemed to be impossible (as pointed out before), some studies dealt with the question of how the human immune system responds to a rhinoviral infection. Rhinovirus inoculation induced both IgG and IgA presence in the serum and the airway, respectively, which neutralized the identical serotype upon reinfection. In studies using several different rhinovirus serotypes the generation of cross-reacting antibodies could be observed. Individuals with preexisting cross-reacting antibodies or cross-reacting antibodies derived from rabbits exhibited a partial immunity when challenged with any new rhinovirus serotype. However, a complete immunity is barely to be expected, because many studies showed that antirhinovirus-protecting IgA titers decreased already 2 months after infection. Nevertheless, a vaccination could attenuate the exacerbations of the infection, that is, reduce asthma and COPD.

Recent work indicates that lipid domains in the plasma membrane of infected cells are functional in signal transduction events caused by rhinoviral infections. Moreover, the lipid composition could influence the interaction of viral proteins with vesicular membranes during the transition of the RNA and replication. For example, rhinovirus uptake is sensitive to treatment with MBCD and blocks p38-K signaling, thus interrupting the release of chemokines and attenuate symptoms. The acid sphingomyelinase (ASM), which was shown to be crucial for generation of ceramide-enriched platforms, a subentity of lipid domains, might be an additional target to prevent HRV infections. Fibroblasts isolated from NPDA patients (Niemann-Pick disease type 1) lacking functional ASM were shown to be resistant to HRV infections. The ASM can be inactivated pharmaceutically by tricyclic antidepressants such as amitryptiline and imipramine. Studies in which the ASM was inhibited by these substances showed a dramatic decrease of viral reproduction, suggesting that the ASM is critically involved in the propagation of rhinoviruses in human cells. Drugs derived from the structure of tricyclic antidepressants might therefore be effective against rhinovirus infections.

Aside from antiviral drugs targeting the attachment and entry processes, antirhinovirus therapy could benefit from the identification of chemicals inhibiting viral maturation. As pointed out earlier, activation of protease 3C is a crucial step in the assembly of the capsid. Tripeptidyl alpha-ketoamides were identified as human rhinovirus protease 3C inhibitors. The protease 3C-inhibiting drug AG7088 (*ruprintivir*) showed a 100-fold reduction of rhinoviral titers and abrogated inflammatory responses as well. Clinical trials indicated that AG7088 could be administered to volunteers without adverse reactions. Subsequent studies are in progress, aiming at another rhinoviral protease (i.e., 2A) as a target for antirhinoviral therapy.

See also: Enteroviruses: Human Enteroviruses Numbered 68 and Beyond; Enteroviruses of Animals; Picornaviruses: Molecular Biology.

Further Reading

Colonno RJ, Condra JH, Mizutani S, Callahan PL, Davies ME, and Murcko MA (1988) Evidence for the direct involvement of the rhinovirus canyon in receptor binding. *Proceedings of the National Academy of Sciences, USA* 85(15): 5449–5453.

Olson NH, Kolatkar PR, Oliveira MA, *et al.* (1993) Structure of human rhinovirus complexed with its receptor molecule. *Proceedings of the National Academy of Sciences USA* 90: 86–93.

Rossman MG (1989) The canyon hypothesis. Hiding the cell receptor attachment site on a viral surface from immune surveillance. *Journal of Biological Chemistry* 264: 14587–14590.

Rossman MG, Arnold E, Erickson JW, *et al.* (1985) Structure of human common cold virus and functional relationship to other picornaviruses. *Nature* 317: 145–153.

Rossman MG, Bella J, Kolatkar PR, *et al.* (2000) Cell recognition and entry by rhino- and enteroviruses. *Virology* 269: 239–247.

Verdaguer N, Blaas D, and Fita I (2000) Structure of human rhinovirus serotype 2 (HRV2). *Journal of Molecular Biology* 300: 1179–1194.

Wang X, Lau C, Wiehler S, *et al.* (2006) Syk is downstream of intercellular adhesion molecule-1 and mediates human rhinovirus activationof p38 MAPK in airway epithelial cells. *Journal of Immunology* 177: 6859–6870.

Coronaviruses: General Features

D Cavanagh and P Britton, Institute for Animal Health, Compton, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Infectious clone A full-length DNA copy of an RNA virus genome from which full-length viral RNA can be generated, leading to production of infectious virus.
Nidovirales (nidoviruses) An order comprising positive-sense RNA coronaviruses, toroviruses, arteriviruses, and roniviruses that have a common genome organization and expression, similar replication/transcription strategies, and form a nested set of 3′ co-terminal subgenomic mRNAs (*nidus*, Latin for nest).
Ribosomal frameshifting Movement (shift) backward by one nucleotide of a ribosome that is on an RNA, caused by particular RNA structures and sequences. Subsequent continuation of the progress of the ribosome is in a different open reading frame.

Introduction

Coronaviruses are known to cause disease in humans, other mammals, and birds. They cause major economic loss, sometimes associated with high mortality, in neonates of some domestic species (e.g., chickens, pigs). In humans, they are responsible for respiratory and enteric diseases. Coronaviruses do not necessarily observe species barriers, as illustrated most graphically by the spread of severe acute respiratory syndrome (SARS) coronavirus among wild animals and to man, with lethal consequences. As a group, coronaviruses are not limited to particular organs; target tissues include the nervous system, immune system, kidney, and reproductive tract in addition to many parts of the respiratory and enteric systems. A great advance in recent years has been the development of systems ('infectious clones') for modifying the genomes of coronaviruses to study all aspects of coronavirus replication, and for the development of new vaccines.

Taxonomy and Classification

The genus *Coronavirus* together with the genus *Torovirus* form the family *Coronaviridae*; members of these two genera are similar morphologically. The *Coronaviridae*, *Arteriviridae*, and *Roniviridae* are within the order *Nidovirales*. Members of this order have a similar genome organization and produce a nested set of subgenomic mRNAs (*nidus*, Latin for nest). To date, coronaviruses have been placed into one of three groups (**Table 1**). Initially, this was on the basis of serological relationships which subsequently have been supported by gene sequencing.

Virion Properties

Virions have a buoyant density of approximately 1.18 g ml^{-1} in sucrose. Being enveloped viruses (**Figure 1**(**a**)), they are destroyed by organic solvents such as ether and chloroform.

Virion Structure and Composition

All coronaviruses have four structural proteins in common (**Figure 1**(**b**)): a large surface glycoprotein (S; *c.* 1150–1450 amino acids); a small envelope protein (E; *c.* 100 amino acids, present in very small amounts in virions); integral membrane glycoprotein (M; *c.* 250 amino acids); and a phosphorylated nucleocapsid protein (N; *c.* 500 amino acids). Group 2a viruses have an additional structural glycoprotein, the hemagglutinin-esterase protein (HE; *c.* 425 amino acids). This is not essential for replication *in vitro* and may affect tropism *in vivo*.

Virions are *c.* 120 nm in diameter, although they can be up to twice that size, and the ring of S protein spikes is approximately 20 nm deep. When present, the HE protein forms a layer 5–10 nm deep. In some species, the S protein is cleaved into two subunits, the N-terminal S1 fragment being slightly smaller than the C-terminal S2 sequence. The S protein is anchored in the envelope by a transmembrane region near the C-terminus of S2. The functional S protein is highly glycosylated and exists as a trimer. The bulbous outer part of the mature S protein is formed largely by S1 while the stalk is formed largely by S2, having a coiled-coil structure. S1 is the most variable part of the S protein; some serotypes of IBV differ from one another by 40% of S1 amino acids. S1 is the major inducer of protective immune responses. Variation in the S1 protein enables one strain of virus to avoid immunity induced by another strain of the same species.

The M glycoprotein is the most abundant protein in virions. In most cases, only a small part (~20 amino acids) at the N-terminus protrudes at the surface of the virus. There are three membrane-spanning segments and the C-terminal half of the M protein is within the lumen of

Table 1 Species of coronavirus[a]

Group 1	*Group 2*	*Group 3*
Subgroup 1a	*Subgroup 2a*	
Transmissible gastroenteritis virus (TGEV)	*Murine hepatitis virus* (MHV)	*Infectious bronchitis virus* (IBV)
Feline coronavirus (FCoV)	*Bovine coronavirus* (BCoV)	*Turkey coronavirus* (TCoV)
Canine coronavirus (CCoV)	*Porcine haemagglutinating encephalomyelitis virus* (HEV)	*Pheasant coronavirus* (PhCoV)
	Equine coronavirus (EqCoV)	
Ferret coronavirus (FeCoV)	Canine respiratory coronavirus (CRCoV)	
	Human coronavirus HKU1	
Subgroup 1b		*Duck coronavirus*[b] (DCoV)
Human coronavirus (HCoV) *229E*	*Human coronavirus* (HCoV) *OC43*	*Goose coronavirus* (GCoV)
Porcine epidemic diarrhoea virus (PEDV)	*Human enteric coronavirus* (HECoV)	*Pigeon coronavirus* (PiCoV)
	Rat coronavirus (RCoV)	
	Puffinosis coronavirus	
Bat coronavirus-61		
Bat coronavirus-HKU2		
Human coronavirus NL63	*Subgroup 2b*	
	Severe acute respiratory syndrome coronavirus (SARS-CoV)	
	Bat-CoV-HKU3-1	

[a]Recognized virus species.
[b]The viruses duck coronavirus, goose coronavirus, and pigeon coronavirus have been recommended by the Coronavirus Study Group for recognition as species by the ICTV.
Official virus species names are in italics. Coronaviruses that have not yet been recognized as distinct species have names that are not italicized. Rabbit coronavirus is considered as a tentative member of the genus. A coronavirus has been isolated from a parrot. On the basis of very limited sequence data, it is not clear in which, if any, of the three groups that this virus would be placed.

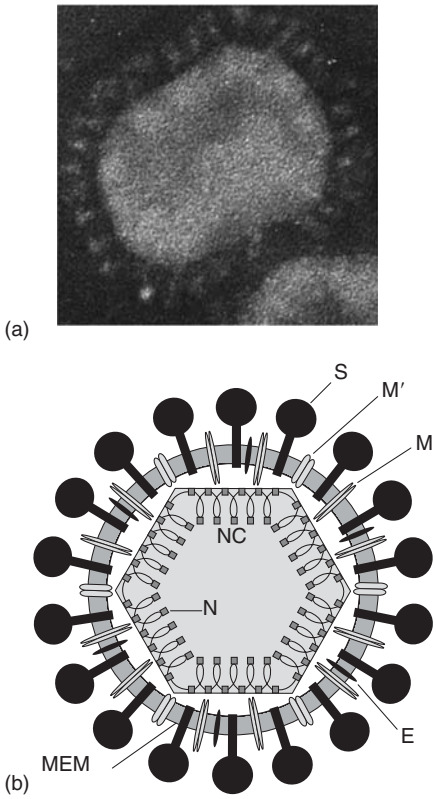


Figure 1 (a) Electron micrograph of an IBV virion, showing the bulbous S protein. (b) Diagrammatic representation of the composition and structure of a coronavirus virion: S, spike glycoprotein; M, M′, integral membrane glycoprotein; E, small envelope protein; N, nucleocapsid protein; NC, nucleocapsid (nucleoprotein) comprising the RNA genome and N protein. Cryoelectron microscopy of TGEV has indicated a core structure comprising the NC and the M protein. Two forms of M protein (M, M′) have been observed for TGEV (see main text). The coronavirus membrane proteins, S, E, M, and M′, are inserted into a lipid bilayer (MEM) derived from internal cell membranes. (b) Reproduced from González JM, Gomez-Puertas P, Cavanagh D, Gorbalenya AE, and Enjuanes L (2003) A comparative sequence analysis to revise the current taxonomy of the family *Coronaviridae*. *Archives of Virology* 148: 2207–2235, with permission from Springer-Verlag.

the virus. In transmissible gastroenteritis virus (TGEV), a proportion of M molecules have four membrane-spanning segments, resulting in the C-terminus also being exposed on the outer surface of the virus (M′ in **Figure 1**(**b**)). The E protein is anchored in the membrane by a sequence near its N-terminus.

Genome Organization and Expression

Coronaviruses have the largest known RNA genomes, which comprise 28–32 kb of positive sense, single-stranded RNA. The overall genome organization is being 5′ UTR–polymerase gene–structural protein genes–3′ UTR, where the UTRs are untranslated regions (**Figure 2**). The first 60–90 nucleotides at the 5′ end form a leader sequence. The structural protein genes are in the same order in all coronaviruses: (HE)–S–E–M–N. Interspersed among these genes are one or more gene (depending on the species; SARS-CoV has four) that encode small proteins of unknown function. Some of these genes encode two or three proteins. In some cases (e.g., gene 3 of IBV and gene

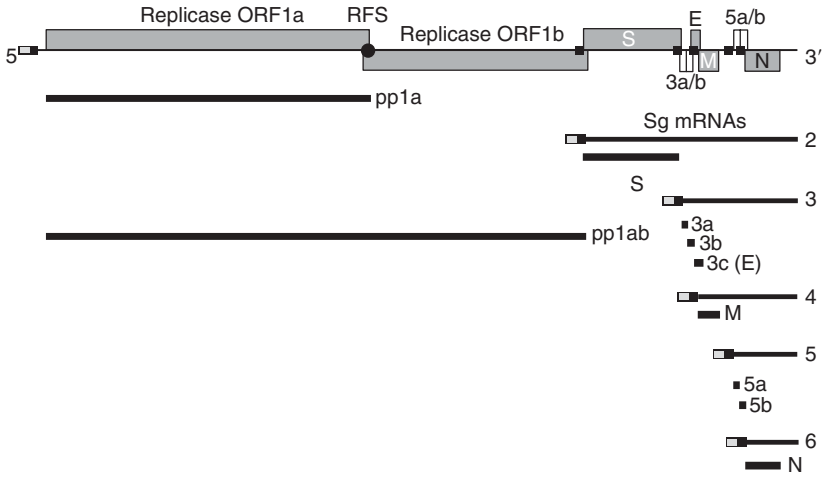


Figure 2 Schematic diagram representing the genomic expression of the avian coronavirus IBV. The upper part of the diagram shows the IBV genomic RNA, with the various genes highlighted as boxed regions. The black boxes represent the transcription regulatory sequences (TRSs) found upstream of each gene and direct the synthesis, via negative-sense counterparts, of the sg mRNAs (2–6 for IBV). The leader sequence, represented by a gray box, is at the 5′ end of the genomic RNA and at the 5′ ends of the sg mRNAs. The genomic RNA is translated to produce two polyproteins, pp1a and pp1ab, that are cleaved by virus-encoded proteases to produce the replicase proteins. The structural proteins, S, E, M, and E, and the accessory proteins, 3a, 3b, 5a, and 5b, produced from IBV genes 3 and 5, respectively, are translated from the sg mRNAs. The proteins produced by the sg mRNAs are represented by lines below the corresponding sg mRNA. All of the sg mRNAs, except the smallest species, are polycistronic but only produce a protein from the 5′-most gene. The ribosome frameshift (RFS) region, denoted as a black circle on the genomic RNA, directs the –1 frameshift event for the synthesis of pp1ab. Translation of the genomic RNA results in the production of pp1a. However, the translating ribosomes undergo the –1 frameshift about 30% of the time resulting in pp1ab. The 5′ and 3′ UTR sequences are represented as single lines downstream of the leader and N gene sequences, respectively.

5 of murine hepatitis virus (MHV)), translation of the third and second open reading frame (ORF), respectively, is effected by the preceding ORFs acting as internal ribosome entry sites. The proteins encoded by these small ORFs are mostly not required for replication *in vitro*; some of them might function as antagonists of innate immune responses, though this has not yet been demonstrated.

Following entry into a cell and the release of the virus ribonucleoprotein (genome surrounded by the N protein) into the cytoplasm, ribosomes translate gene 1, which is approximately 20 kb, into two polyproteins (pp1a and pp1ab). These are cleaved by gene 1-encoded proteases, to generate 15 or 16 proteins (**Figure 3**). Translation of ORF 1b involves ribosomal frameshifting, which has two elements, a slippery site followed by an RNA pseudoknot. At the slippery site (UUUAAAC in IBV), the ribosome slips one nucleotide backward and then moves forward, this time in a –1 frame compared with translation ORF 1a, resulting in the synthesis polyprotein 1ab.

Proteins, including the RNA-dependent RNA polymerase, from gene 1 associate to form the replicase complex, which is membrane associated. Coronavirus subgenomic mRNAs are generated by a discontinuous process. At the beginning of each gene is a common sequence (CUUAACAA in the case of IBV) called a transcription regulatory sequence (TRS). It is believed that when the polymerase producing the nascent negative sense RNA, reaches a TRS, RNA synthesis is attenuated, followed by continuation at the 5′ end of genomic RNA. This results in the addition of a negative copy of the leader sequence to the negative-sense RNA, resulting in a negative-sense copy of an sg mRNA. Of course, progress of the polymerase is not always halted at a TRS. Rather, it sometimes continues, producing a nested set of negative-sense sg mRNAs. These are the templates for the generation of the positive-sense sg mRNAs (**Figure 2**). The amount of each sg mRNA does not necessarily decrease in a linear fashion; the efficiency of termination by a TRS is dependent on adjacent sequences, which are different for each gene. The leader sequence is found at the very 5′ end of the genomic RNA and at the 5′ ends of each sg mRNA.

Replication Cycle

The N-terminal (S1) part of the S protein mediates that mediates attachment to cells. It is a determinant of host species specificity and, in some cases, pathogenicity, by determining susceptible cell range (tissue tropism) within a host. The C-terminal S2 part triggers fusion of the virus envelope with cell membranes (plasma membrane or endosomal membranes), which can occur at neutral or slightly acidic pH, depending on species or even strain. The virus glycoproteins (S, M, and HE, when present) are synthesized at the endoplasmic reticulum. Both subunits

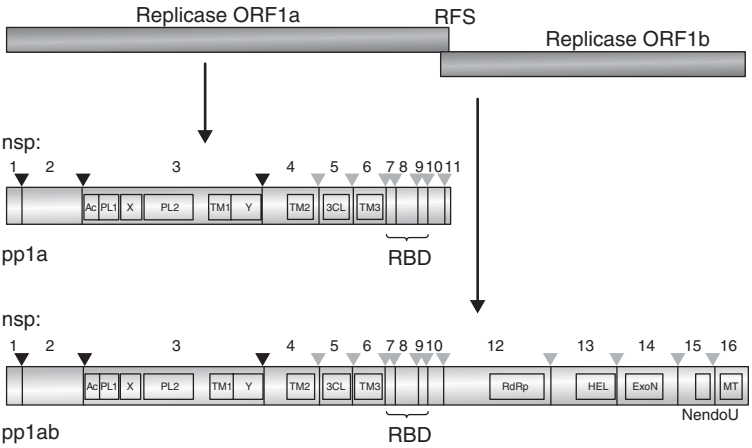


Figure 3 Organization of the coronavirus replicase gene products. Translation of the coronavirus replicase ORF 1a and ORF 1b sequences results in pp1a and pp1ab; the latter is a C-terminal extension of pp1a, following a programmed –1 frameshift event (see legend to **Figure 2**). The two polyproteins are proteolytically cleaved into 10 (pp1a; nsp1–11) and 16 (pp1ab; nsp1–16) products by the papain-like proteinases (PL1^{pro} and PL2^{pro}) and the 3C-like (3CL^{pro}) proteinase. The PL^{pro} proteinases cleave at the sites indicated with a black triangle and the 3CL^{pro} proteinase cleaves at the sites indicated with a gray triangle. The nsp11 product of pp1a is produced as a result of the ribosomes terminating at the ORF 1a translational termination codon, a –1 frameshift results in the generation of nsp12, part of the pp1ab replicase gene product. Various domains have been identified within some of the replicase products: Ac is a conserved acidic domain; X = ADP-ribose 1′-phosphatase (ADRP) domain; PL1 and PL2 the two papain-like proteinases; Y is a conserved domain; TM1, TM2, and TM3 are conserved putative transmembrane domains; 3CL = 3CL^{pro} domain; RdRp, RNA-dependent RNA polymerase domain; HEL, helicase domain; ExoN, exonuclease domain; NendoU, uridylate-specific endoribonuclease domain; MT, 2′-*O*-ribose methyltransferase domain. nsp's 7–9 contain RNA-binding domains (RBDs).

of the S protein are multiply glycosylated, while the M protein has one or two glycans close to its N-terminus. Interestingly, glycosylation of the M protein can be either N- or O-linked, depending on the type of coronavirus, although experiments using reverse genetics showed that conversion of an O-linked glycosylated M protein to an N-linked version had no effect on virus growth.

Early and late in infection, formation of virus particles can occur in the endoplasmic reticulum–Golgi intermediate compartment (ERGIC) and endoplasmic reticulum, but most assembly occurs in the Golgi membranes. The M protein is not transported to the plasma membrane; its location at internal membranes determines the sites of virus particle formation. It interacts with the N protein (as part of the RNP) and C-terminal part of the S protein, retaining some, though not all, of the S protein at internal membranes. The E protein is essential for virus particle formation, though it is not known how it functions. It has a sequence that determines its accumulation at internal membranes, and its interaction with the M protein. The latter interacts with the C-terminus of the S protein, retaining some of it at internal membranes, and with the N protein (itself part of the ribonucleoprotein structure), enabling the formation of virus particles with spikes.

Genome Replication and Recombination

Following infection of a susceptible cell, the coronavirus genomic RNA is released from the virion into the cytoplasm and immediately recognized as an mRNA for the translation of the replicase pp1a and pp1ab proteins. These proteins are cleaved by ORF1a-encoded proteases, after which they become part of replicase complexes for the synthesis of either complete negative-sense copies of the genomic RNA or negative-sense copies of the sg mRNAs. The negative-sense RNAs are used as templates for the synthesis of genomic RNA and sg mRNAs (**Figure 2**). Following synthesis of the sg mRNAs, the structural proteins are produced for the assembly and encapsidation of the *de novo*-synthesized genomic RNA, resulting in the release of new infectious coronavirus virions. The release of new virions starts 3–4 h after the initial infection. As indicated above, the synthesis of the sg mRNAs is the result of a discontinuous process in which the synthesis of a negative-sense copy of an sg mRNA is completed by the addition of the negative-sense leader sequence by a recombination mechanism. If a cell is infected with two related coronaviruses, the polymerase may swap between two RNA templates, in a similar way to addition of the leader sequence. This 'copy-choice' mechanism of genetic recombination results in a chimeric RNA. Such RNAs may give rise to new viruses with modified genomes with a capacity to infect a different cell and, in some cases, new host species.

Evolutionary Relationships among Coronaviruses

Phylogenetic analyses of the structural proteins have resulted in the grouping of coronavirus species in accordance with earlier antigenic groups (**Table 1** and **Figure 4**).

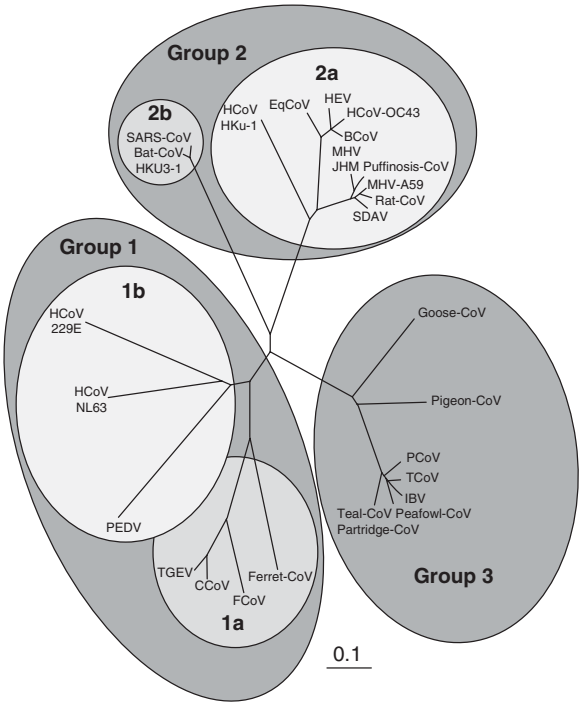


Figure 4 Phylogenetic relationship of aligned coronavirus-derived nucleoprotein amino acid sequences. The complete N protein sequences represent coronaviruses from each of the three groups (**Table 1**). The tree is unrooted and the three main coronavirus groups, 1–3, are highlighted as dark gray ellipsoids. Groups 1 and 2 are divided into two subgroups, a and b, representing some divergence of the sequences within their corresponding groups. Similar relationships are observed when comparing other structural proteins and replicase-derived proteins.

Members of subgroups have higher amino acid sequence identities to each other ($\geq$60%) than to members of another group in the same group (with which they share $\leq$40% identity). Comparing one group with another, protein sequence identities are generally in the range 25–35%. Unlike other members of group 2, SARS-CoV does not have an HE glycoprotein. Phylogenetic analysis using all the encoded proteins indicates that recombination has been a feature of coronavirus evolution. For example, some group 1a viruses are clearly recombinants between a feline and canine group 1 coronavirus.

Diseases and Host Range

Probably all coronaviruses replicate in epithelial cells of the respiratory and/or enteric tracts, though not necessarily producing clinical damage at those sites. Avian IBV not only causes respiratory disease but can also damage gonads in both females and males, and causes serious kidney disease (dependent on the strain of virus, and to some extent on the breed of chicken). IBV is able to replicate at virtually every epithelial surface in the host. Some coronaviruses have their most profound effect in the alimentary tract (e.g., porcine TGEV causes $\geq$90% mortality in neonatal pigs). Human coronaviruses are known to be associated with enteric and respiratory diseases (e.g., diarrhea), in addition to respiratory disease. SARS-CoV was also associated with diarrhea in humans, in addition to serious lung disease. Other coronaviruses, for example, MHV and porcine HEV, spread to cells of the central nervous system, producing disease, for example, acute or chronic demyelination in the case of MHV.

Coronavirus replication and disease are not necessarily restricted to a single host species. Canine enteric CoV and feline CoV can replicate and cause disease in pigs; these two viruses have proteins with very high amino acid identity to those of porcine TGEV. Canine respiratory CoV has proteins, including the S protein (which is the attachment protein and a determinant of host range), with very high amino acid identity ($\geq$95%) to other group 2 viruses Hu CoV-OC43 and BCoV. This raises the possibility of co-infection in these hosts. Bovine CoV causes enteritis in turkeys following experimental oral infection. There is evidence that pheasant CoV can infect chickens, and IBV infect teal (a duck), though without causing disease. The most dramatic demonstration that coronaviruses can have a wide host range was provided by SARS-CoV. This may have had its origin in bats, was transferred to various other species (e.g., civet cat) that were captured for trade, and then caused lethal disease in humans.

Persistent infections *in vivo* are well known for MHV, and less well known for other coronaviruses (e.g., IBV). Following infection of very young chickens, IBV is re-excreted when hens start to lay eggs. The trigger for release is probably the stress of coming into lay.

The S protein is a determinant of both tissue tropism within a host and host range. This has been elegantly demonstrated by genetic manipulation of the genome of MHV, which is unable to attach to feline cells. Replacement of the MHV S protein gene with that of CoV from feline coronavirus resulted in a recombinant virus that was able to attach, and subsequently replicate in, feline cells. However, other proteins can also affect pathogenicity. Research with genetically modified coronaviruses, using targeted recombination or 'infectious clones', has shown that modifications to proteins encoded in ORF1 and the small genes interspersed among the structural protein genes, result in attenuation of pathogenicity. Although the roles of these 'accessory proteins' are not known, this may offer a route to the development of a new generation of live vaccines. Currently, the most widely used prophylactics for control of IBV in chickens include killed vaccines and live vaccines attenuated by passage in embryonated eggs. However, disease control is complicated by extensive variation in the S1 protein which is the inducer of protective immunity.

See also: Nidovirales.

Further Reading

Britton P and Cavanagh D (2007) Avian coronavirus diseases and infectious bronchitis vaccine development. In: Thiel V (ed.) *Coronaviruses: Molecular and Cellular Biology* pp. 161–181. Norfolk, UK: Caister Academic Press.

Britton P and Cavanagh D (2007) Nidovirus genome organization and expression mechanisms. In: Perlman S Gallagher T, and Snijder EJ (eds.) *Nidoviruses*, pp. 29–46. Washington, DC: ASM Press.

Cavanagh D (2003) SARS vaccine development: Experiences of vaccination against avian infectious bronchitis coronavirus. *Avian Pathology* 32: 567–582.

Cavanagh D (2005) *Coronaviridae*: A review of coronaviruses and toroviruses. In: Schmidt A and Wolff MH (eds.) *Coronaviruses with Special Emphasis on First Insights Concerning SARS*, pp. 1–54. Basel: Birkhäuser.

Cavanagh D (2005) Coronaviruses in poultry and other birds. *Avian Pathology* 34: 439–448.

Enjuanes L, Almazán F, Sola I, and Zuñiga S (2006) Biochemical aspects of coronavirus replication: A virus–host interaction. *Annual Reviews in Microbiology* 60: 211–230.

González JM, Gomez-Puertas P, Cavanagh D, Gorbalenya AE, and Enjuanes L (2003) A comparative sequence analysis to revise the current taxonomy of the family *Coronaviridae*. *Archives of Virology* 148: 2207–2235.

Masters PS (2006) The molecular biology of coronaviruses. *Advances in Virus Research* 66: 193–292.

Siddell S, Ziebuhr J, and Snijder E (2005) Coronaviruses, toroviruses and arteriviruses. In: Mahy BWJ and ter Meulen V (eds.) *Topley and Wilson's Microbiology and Microbial Infections, Virology*, pp. 823–856. London: Hodder Arnold.

Coronaviruses: Molecular Biology

S C Baker, Loyola University of Chicago, Maywood, IL, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cell tropism Process that determines which cells can be infected by a virus. Factors such as receptor express can influence the cell type that can be infected.

Discontinuous transcription Process by which the coronavirus leader sequence and body sequence are joined to generate subgenomic RNAs.

Double membrane vesicles (DMVs) Vesicles that are generated during coronavirus replication when viral replicase proteins sequester host cell membranes. These vesicles are the site of coronavirus RNA synthesis.

Transcriptional regulatory sequences (TRSs) Sequences that are recognized by the coronavirus transcription complex to generate leader-containing subgenomic RNAs.

Introduction

Coronaviruses (CoVs) were first identified during the 1960s by using electron microscopy to visualize the distinctive spike glycoprotein projections on the surface of enveloped virus particles. It was quickly recognized that CoV infections are quite common, and that they are responsible for seasonal or local epidemics of respiratory and gastrointestinal disease in a variety of animals. CoVs have been named according to the species from which they were isolated and the disease associated with the viral infection. Avian infectious bronchitis virus (IBV) infects chickens, causing respiratory infection, decreased egg production, and mortality in young birds. Bovine coronavirus (BCoV) causes respiratory and gastrointestinal disease in cattle. Porcine transmissible gastroenteritis virus (TGEV) and porcine epidemic diarrhea virus (PEDV) cause gastroenteritis in pigs. These CoV infections can be fatal in young animals. Feline infectious peritonitis virus (FIPV) and canine coronavirus (CCoV) can cause severe disease in cats and dogs. Depending on the strain of the virus and the site of infection, the murine CoV mouse hepatitis virus (MHV) can cause hepatitis or a demyelinating disease similar to multiple sclerosis. CoVs also infect humans. Human coronaviruses (HCoVs) 229e and OC43 are detected worldwide and are estimated to be responsible for 5–30% of common colds and mild gastroenteritis. Interestingly, HCoV-OC43 and BCoV share considerable sequence similarity, indicating a likely transmission across species (either from cows to humans or vice versa) and then adaptation of the virus to its host. In contrast to the relatively mild infections caused by HCoV-229e and HCoV-OC43, the CoV responsible for severe acute respiratory syndrome (SARS-CoV) causes atypical pneumonia with a 10% mortality rate. Two additional HCoVs, HCoV-NL63 and HCoV-HKU1, have been recently identified using molecular methods and are associated with upper and lower respiratory tract infections in children, and elderly

and immunosuppressed patients. CoVs are grouped according to sequence similarity. CoVs that infect mammals are assigned to group 1 and group 2, whereas CoVs that infect birds are in group 3.

To date, the most infamous example of zoonotic transmission of a CoV is the outbreak of SARS in 2002–03. We now know that the outbreak started with cases of atypical pneumonia in the Guangdong Province in southern China in the fall of 2002. The infection was spread to tourists visiting Hong Kong in February, 2003, resulting in the dissemination of the outbreak to Hong Kong, Vietnam, Singapore, and Toronto, Canada. After attempting to treat cases of atypical pneumonia in Vietnam and acquiring the infection himself, Dr. Carlo Urbani alerted the World Health Organization (WHO) that this disease of unknown origin may be a threat to public health. The WHO rapidly organized an international effort to identify the cause of the outbreak, and within months a novel CoV was isolated from SARS patients and identified as the causative agent. Sequence analysis revealed that the virus was related to, but distinct from, all known CoVs. This led to an intensive search for an animal reservoir for this novel CoV. Initially, the masked palm civet and raccoon dog were implicated in the chain of transmission, since a SARS-CoV-like virus could be isolated from some animals found in wild animal markets in China. However, SARS-CoV-like viruses were never detected in animals captured from the wild, indicating that the civets may have only served as an intermediate host in the chain of transmission. Further investigation revealed that the likely reservoir for SARS-CoV is the Chinese horseshoe bat (*Rhinolophus* spp.), which is endemically infected with a virus, named bat-SARS-CoV, that is closely related to SARS-CoV. The existence of an animal reservoir presents the possibility of re-emergence of this significant human pathogen. By improving our understanding of the molecular aspects of CoV replication and pathogenesis, we may facilitate development of appropriate antiviral agents and vaccines to control and prevent diseases caused by known and potentially emerging CoV infections.

Molecular Features of CoVs

CoV virions (**Figure 1**(**a**)) are composed of a large RNA genome, which combines with the viral nucleocapsid protein (N) to form a helical nucleocapsid, and a host cell-derived lipid envelope which is studded with virus-specific proteins including the membrane (M) glycoprotein, the envelope (E) protein, and the spike (S) glycoprotein. CoV particles vary somewhat in size, but average about 100 nm in diameter. The genomic RNA (gRNA) inside the virion, which ranges in size from 27 to 32 kb for different CoVs, is the largest viral RNA identified to date. CoV gRNAs have a broadly conserved structure which is illustrated by the SARS-CoV genome shown in **Figure 1**(**b**). The gRNA is

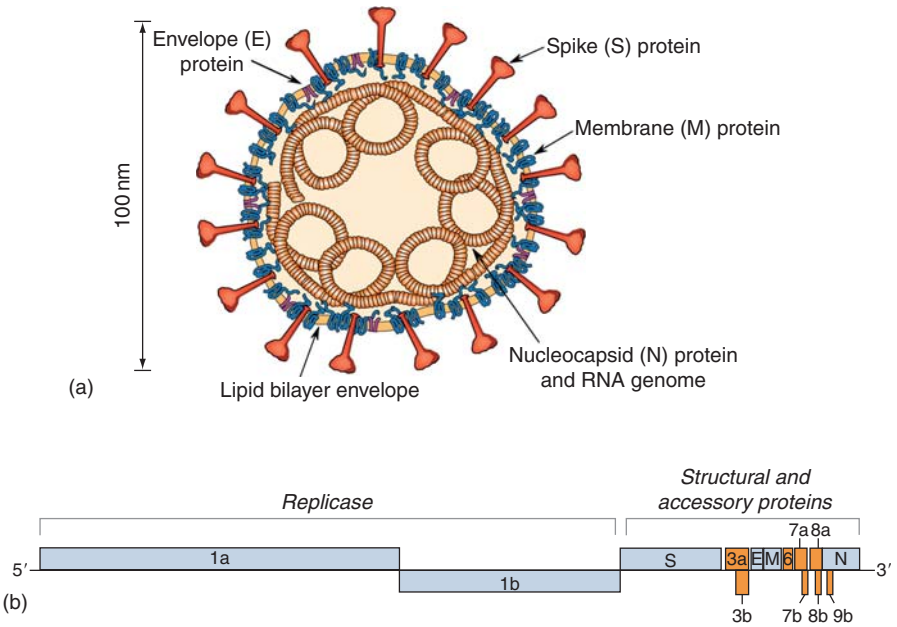


Figure 1 CoV virion and the genome of SARS-CoV. (a) Schematic diagram of a CoV virion with the minimal set of four structural proteins required for efficient assembly of the infectious virus particles: S, spike glycoprotein; M, membrane glycoprotein; E, envelope protein; and N, nucleocapsid phosphoprotein which encapsidates the positive-strand RNA genome. (b) Schematic diagram of the gRNA of SARS-CoV. Translation of the first two open reading frames (ORF1a and ORF1b) generates the replicase polyprotein. ORFs encoding viral structural and accessory (orange) ORFs are indicated at the 3′ end of the genome. (a) Reprinted from Masters PS (2006) The molecular biology of coronaviruses. *Advances in Virus Research* 66: 193–292, with permission from Elsevier.

capped at the 5′ end, with a short leader sequence followed by two long open reading frames (ORFs) encoding the replicase polyprotein. The remaining part of the genome encodes the viral structural and so-called accessory proteins. The structural protein genes are always found in the order S–E–M–N, but accessory protein genes may be interspersed at various sites between the structural genes. SARS-CoV has the most complex genome yet identified, with eight ORFs encoding accessory proteins. The expression of these ORFs is not required for viral replication, but they may play a role in the pathogenesis of SARS. In addition, the products of accessory genes may be incorporated into the virus particle, potentially altering the tropism or enhancing infectivity. For SARS-CoV, the proteins encoded in ORFs 3a, 6, 7a, and 7b have been shown to be incorporated in virus particles, but the exact role of these proteins in enhancing virulence is not yet clear.

The features of CoV structural proteins are shown in **Figure 2**. For each structural protein, a schematic diagram of the predicted structure of the protein is shown on the left and a linear display of the features is shown on the right. The CoV spike glycoprotein is essential for attachment of the virus to the host cell receptor and fusion of the virus envelope with the host cell membrane. CoV spike glycoproteins assemble as trimers with a short cytoplasmic tail and hydrophobic transmembrane domain anchoring the protein into the membrane. The spike glycoprotein is divided into the S1 and S2 regions, which are sometimes cleaved into separate proteins by cellular proteases during the maturation and assembly of virus particles. S1 contains the receptor-binding domain (RBD) and has been shown to provide the specificity of attachment for CoV particles. The cellular receptors and corresponding RBDs in S1 have been identified for several CoVs. MHV binds to murine carcinoembryonic

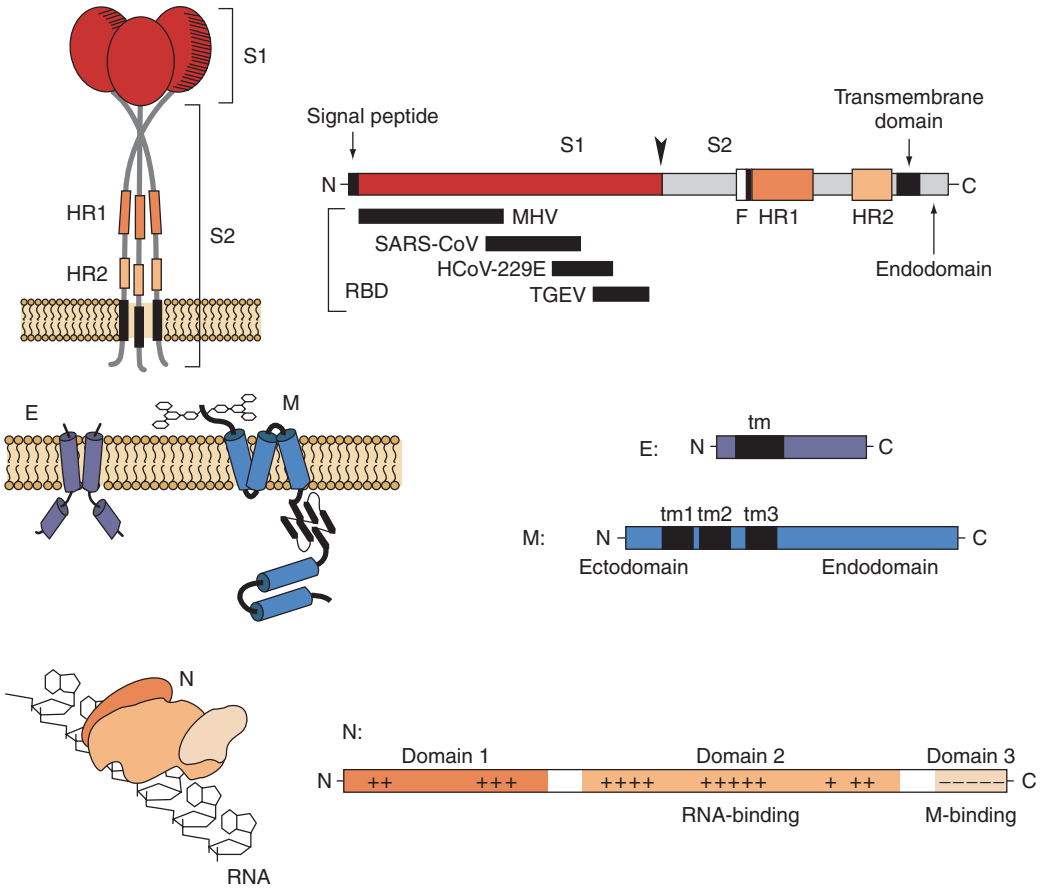


Figure 2 Diagrammatic representation of the spike trimer assembled on membranes, with the S1 receptor binding domain (RBD) and S2 fusion domain indicated. The linear map of spike indicates the location of the RBDs for three CoVs, and the relative location of the heptad repeat domains 1 and 2 (HR1 and HR2) which mediate the conformational changes required to present the fusion peptide (F) to cellular membranes. The membrane (M), envelope (E), and nucleocapsid (N) proteins represented in association with membranes or viral RNA. The linear map of each protein highlights the transmembrane domains of M and E and the RNA-binding and M protein-binding domains of N. Domains 1 and 2 of N are rich in arginine and lysine (indicated by +). Reprinted from Masters PS (2006) The molecular biology of coronaviruses. *Advances in Virus Research* 66: 193–292, with permission from Elsevier.

antigen-related cell adhesion molecules (mCEACAM1 and MCEACAM2); TGEV, FIPV, and HCoV-229e bind to species-specific versions of aminopeptidase N. Interestingly, both HCoV-NL63 and SARS-CoV have been shown to bind to human angiotensin-converting enzyme 2 (ACE2). ACE2 is expressed in both the respiratory and gastrointestinal tracts, consistent with virus replication at both these sites.

Once the S1 portion of the spike has engaged the host cell receptor, the protein undergoes a dramatic conformational change to promote fusion with the host cell membrane. Depending on the virus strain, this can occur at the plasma membrane on the surface of the cell, or in acidified endosomes after receptor-mediated endocytosis. The critical elements in the conformational change are the heptad repeats, HR1 and HR2, and the fusion peptide, F. After engaging the receptor, there is a dissociation of S1 which likely triggers the rearrangement of S2 so that HR1 and HR2 are brought together to form an antiparallel, six-helix bundle. This new conformation brings together the viral and host cell membranes and promotes the fusion of the lipid bilayers and introduction of the nucleocapsid into the cytoplasm. During infection, the spike glycoprotein is also present on the surface of the infected cell where it may (depending on the virus strain) promote fusion with neighboring cells and syncytia formation. The spike glycoprotein is also the major antigen to which neutralizing antibodies develop. The spike protein is a target for development of therapeutics for treatment of CoV infections. Monoclonal antibodies directed against the spike neutralize the virus by blocking binding to the receptor; synthetic peptides that block HR1-HR2 bundle formation have also been shown to block CoV infection.

The membrane (M) and envelope (E) proteins are essential for the efficient assembly of CoV particles. M is a triple-membrane-spanning protein that is the most abundant viral structural protein in the CoV virion. The ectodomain of M is generally glycosylated, and is followed by three transmembrane domains and an endodomain which is important for interaction with the nucleocapsid protein and packaging of the viral genome. The E protein is present in low copy numbers in the virion, but is important for efficient assembly. In the absence of E protein, few or no infectious virus particles are produced. The exact role of the E protein in the assembly of virus particles is still unknown, but recent studies suggest that E may act as an ion channel. The nucleocapsid protein (N) is an RNA-binding protein and associates with the CoV gRNA to assemble ribonucleoprotein complexes. The N protein is phosphorylated, predominantly at serine residues, but the role of phosphorylation is currently unknown. The N protein has three conserved domains, each separated by highly variable spacer elements. Domains 1 and 2 are rich in arginine and lysine residues, which is typical of many RNA-binding proteins. Domain 3 is essential for interaction with the M protein and assembly of infectious virus particles. The N protein has been shown to be an important cofactor in CoV RNA synthesis and is proposed to act as an RNA chaperone to promote template switching, as described below.

Replication and Transcription of CoV RNA

The replication and transcription of CoV RNA takes place in the cytoplasm of infected cells (**Figure 3**). The CoV virion attaches to the host cell receptor via the spike glycoprotein and, depending on the virus strain, the spike mediates fusion directly with the plasma membrane or the virus undergoes receptor-mediated endocytosis and spike-mediated fusion with endosomal membranes to release the viral gRNA into the cytoplasm. Once the positive-strand RNA genome is released, it acts as a messenger RNA (mRNA) and the 5′ end (ORF1a and ORF1b) is translated by ribosomes to generate the viral RNA-dependent RNA polymerase polyprotein, termed the viral replicase. Translation of ORF1b is dependent on ribosomal frameshifting, which is facilitated by a slippery sequence and RNA pseudoknot structure present in all CoV gRNAs. The replicase polyprotein is processed by replicase-encoded proteases (papain-like proteases and a poliovirus 3C-like protease) to generate 16 mature replicase products. These viral replicase proteins sequester host cell membranes to generate distinctive double-membrane vesicles (DMVs) that have been shown to be the site of CoV RNA synthesis. The replicase complex on the DMVs then mediates the replication of the positive-strand RNA genome to generate full-length and subgenomic negative-strand RNAs, and the subsequent production of positive-strand gRNAs and sgmRNAs. The sgmRNAs are translated to generate viral structural and accessory proteins, and virus particles assemble with positive-strand gRNA in the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) and bud into vesicles, with subsequent release from the cell. Depending on the virus strain, this replication can be robust and result in destruction of the host cell or a low-level, persistent infection that can be maintained in cultured cells or infected animals.

A hallmark of CoV transcription is the generation of a nested set of mRNAs, with each mRNA having the identical 'leader' sequence of approximately 65–90 nt at the 5′ end (**Figure 4(a)**). The leader sequence is encoded only once at the 5′ end of the gRNA. Each subgenomic mRNA (sgmRNA) has the identical leader sequence fused to the 5′ end of the body sequence. How are the leader-containing mRNAs generated during CoV transcription? Current evidence supports a model of discontinuous transcription, whereby the replicase complex switches templates during the synthesis of negative-strand RNA

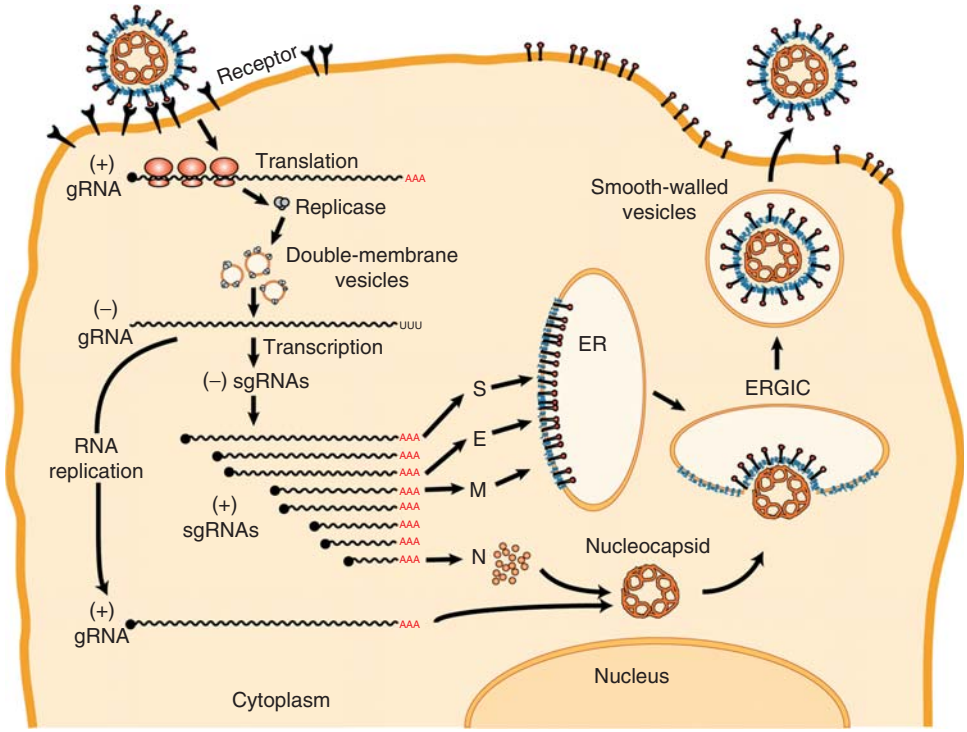


Figure 3 Replication cycle of CoVs. The spike glycoprotein on the virus particle interacts with host cell receptors to mediate fusion of the virus and host cell membranes and release of the positive-strand RNA genome into the cytoplasm. The 5′-proximal open reading frames (ORF1a and ORF1b) are translated from the gRNA to generate the replicase polyprotein. The replicase polyprotein is processed by viral proteases into 16 nonstructural proteins which assemble with membranes to generate double-membrane vesicles (DMVs) where RNA synthesis takes place. A nested set of 3′ co-terminal subgenomic (sg) RNAs is generated by a discontinuous transcription process. The sgRNAs are translated to generate the viral structural and accessory proteins. Viral gRNA is replicated and associates with nucleocapsid protein and viral structural proteins in the endoplasmic reticulum-Golgi intermediate compartment (ERGIC), where virus particles bud into vesicles before transport and release from the cell. Reprinted from Masters PS (2006) The molecular biology of coronaviruses. *Advances in Virus Research* 66: 193–292, with permission from Elsevier.

(**Figure 4(b)**). The key sequence element in this process is the transcriptional regulatory sequence (TRS). The TRS is a sequence of approximately 6–9 nt (5′-ACGAAC-3′ for SARS-CoV) which is found at the end of the leader sequence and at each intergenic region (the sites between the open reading frames encoding the viral structural and accessory proteins). Site-directed mutagenesis and deletion analysis has revealed the critical role of the TRS in mediating transcription of sgmRNAs. Deletion of any intergenic TRS results in loss of production of the corresponding sgmRNA. In addition, the CoV leader TRS and the intergenic TRS sequences must be identical for optimal production of the sgmRNAs. A three-step working model for template switching during negative-strand RNA synthesis has been proposed to describe the process for the generation of CoV leader-containing sgmRNAs (**Figure 4(b)**). In this process, the 5′ end and 3′ end of the gRNA form a complex with host cell factors and the viral replication complex. The 3′ end of the positive strand is used as the template for the initiation of transcription of negative-strand RNA. Negative-strand RNA synthesis continues up to the point of the TRS. At each TRS, the viral replicase may either read through the sequence to generate a longer template, or switch templates to copy the leader sequence. The template switch allows the generation of a leader-containing sgmRNA. In this model, alignment of the leader TRS, the newly synthesized negative-strand RNA, and the genomic TRS is critical for the template switching to occur. Disruption of the complex, or loss of base-pairing within the complex, will result in the loss of production of that sgmRNA. Further studies of the CoV replication complex may yield new insights into the role of the viral helicase and endoribonuclease in the generation of the leader-containing CoV RNAs.

Another hallmark of CoV replication is high-frequency RNA recombination. RNA recombination occurs when a partially synthesized viral RNA dissociates from one template and hybridizes to similar sequences present in a second template. Viral RNA synthesis continues and generates a progeny virus with sequences from two different parental genomes. This RNA recombination event is termed copy-choice recombination. Copy-choice RNA recombination can be demonstrated experimentally when two closely related CoV strains (such as MHV-JHM and

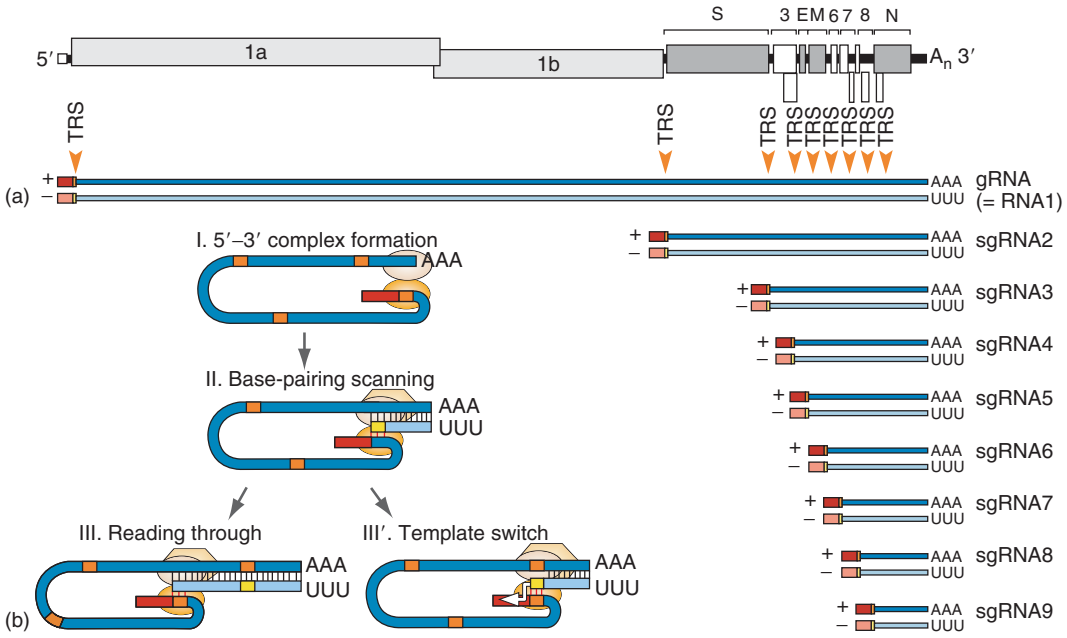


Figure 4 Model of SARS-CoV gRNA and sgRNAs, and a working model of discontinuous transcription. (a) Diagram of gRNA and the nested set of sgRNAs of SARS-CoV. The 5′ leader sequence, the transcriptional regulatory sequences (TRSs), and the positive- and negative-sense sgRNAs are indicated. (b) A working model of CoV discontinuous transcription. I. 5′–3′ complex formation. Binding of viral and cellular proteins to the 5′ and 3′ ends of the CoV gRNA is represented by ellipsoids. The leader sequence is indicated in red, the TRS sites are in orange. II. Base-pair scanning step. Minus-strand RNA (light blue) is synthesized from the positive-strand template by the viral transcription complex (hexagon). At the TRS site, base-pairing may occur between the template, the nascent negative-strand RNA, and the leader TRS sequence (dotted lines). III. The synthesis of negative-strand RNA can continue to make a longer sgRNA III, or a template switch can take place III′ to generate a leader-containing subgenomic negative-strand RNA, which could then serve as the template for leader-containing positive-strand sgRNAs. Modified from Enjuanes L, Almazán F, Sola I, and Zunia S (2006) Biochemical aspects of coronavirus replication: A virus–host interaction. *Annual Reviews in Microbiology* 60: 211–230.

MHV-A59) are used to coinfect cells. Recombinant viruses with cross-over sites throughout the genome can be isolated, although sequences within the spike glycoprotein may be a 'hot spot' for recombination due to the presence of RNA secondary structures that may promote dissociation and reassociation of RNA. It has been proposed that copy-choice recombination is also the mechanism by which many CoVs have acquired accessory genes, and it has been exploited experimentally for the deletion or insertion of specific sequences in CoV genomes to assess their role in virus replication and pathogenesis.

CoV Accessory Proteins

Sequence analysis of CoVs isolated from species ranging from birds to humans has revealed that all CoVs encode a core canonical set of genes, replicase (rep), spike (S), envelope (E), membrane (M), and nucleocapsid (N), and additional, so-called accessory genes (**Table 1**). The canonical genes are always found in the same order in the genome: rep-S-E-M-N. Reverse genetic studies (see below) have shown that this is the minimal set of genes required for efficient replication and assembly of infectious CoV particles. However, the genomes of all CoVs sequenced to date encode from one to eight additional ORFs, which code for accessory proteins. As the

Table 1 Coronavirus **canonical** and *accessory* proteins

Virus	*Proteins:* **canonical (rep-S-E-M-N)** *and accessory*
Group 1	
TGEV	**rep-S**-*3a,3b*-**E-M-N**-*7*
FIPV	**rep-S**-*3a*,*3b*,*3c*-**E-M-N**-*7a*,*7b*
HCoV-229E	**rep-S**-*4a,4b*-**E-M-N**
PEDV	**rep-S**-*3*-**E-M-N**
HCoV-NL63	**rep-S**-*3*-**E-M-N**
Group 2	
MHV	**rep**-*2a*, *HE*-**S**-*4-5a*,**E-M-N**,*7b*
BCoV	**rep**-*2a*, *HE*-**S**-*4a,4b-5*,**E-M-N**,*7b*
HCoV-OC43	**rep**-*2a*, *HE*-**S**-*5*,**E-M-N**,*7b*
HCoV-HKU1	**rep**-*HE*-**S**-*4*-**E-M-N**,*7b*
SARS-CoV	**rep-S**-*3a,3b*-**E-M**-*6-7a*,*7b-8a*,*8b*-**N**,*9b*
Bat-SARS-CoV	**rep-S**-*3*-**E-M**-*6-7a,7b-8*-**N**,*9b*
Group 3	
Avian IBV	**rep-S**-*3a,3b,3c*-**E-M**-*5a,5b*-**N**

name implies, these accessory proteins are not required for CoV replication in tissue culture cell lines, but they may play important roles in tropism and pathogenesis *in vivo*. How were these additional genes acquired? Current evidence indicates that these additional sequences may have been acquired by RNA recombination events between co-infecting viruses. For example, the hemagglutinin-esterase (HE) glycoprotein present in four different CoVs (MHV, BCoV, HCoV-OC43, and HCoV-HKU-1) was likely acquired by recombination of an ancestral CoV with the HE glycoprotein gene of influenza C. Interestingly, the expression of the HE gene has no effect on replication of the virus in cultured cell lines, but has been shown to enhance virulence in infected animals. Other CoV accessory genes may have been acquired through recombination with host cell mRNA or other viral mRNAs. The specific role of the accessory proteins in CoV replication and pathogenesis is under investigation. For SARS-CoV, accessory protein 6 has been implicated as an important factor in viral pathogenesis. Researchers have shown that mice infected with murine CoV expressing SARS-CoV protein 6 rapidly succumb to the infection, indicating that the protein 6 enhances virulence. In addition, recent studies suggest that SARS-CoV accessory proteins may play a role in blocking host cell innate immune responses, which may enhance viral replication and virulence. Other accessory proteins, such as SARS-CoV 3a and 7a, have been shown to be packaged into virus particles, where they may enhance infectivity or alter cell tropism. Future studies will be aimed at elucidating how CoV accessory proteins may modulate the virulence of CoV infection.

Manipulating CoV Genomes Using RNA Recombination and Reverse Genetics

Genetic manipulation of CoV sequences is challenging because of the large size (27–32 kbp) of the RNA genomes. However, two approaches have been developed to allow researchers to introduce mutations, deletions, and reporter genes into CoV genomes. These approaches are (1) targeted RNA recombination and (2) reverse genetics using infectious cDNA constructs of CoV. The first approach exploits high-frequency copy-choice recombination to introduce mutations of interest into the 3′ end of the CoV gRNA. In the first step of targeted RNA recombination, a cDNA clone encoding the region from the spike glycoprotein to the 3′ end of the RNA is generated. These sequences can be easily manipulated in the laboratory to introduce mutations or deletions, or for the insertion of reporter or accessory genes, into the plasmid DNA. Next, RNA is transcribed from the plasmid DNA and the RNA is transfected into cells coinfected with the CoV of interest. RNA recombination occurs between the replicating CoV and the transfected substrate RNA, and viruses with the 3′ end sequences derived from the transfected substrate RNA will be generated. The recombinant viruses are generated by high-frequency copy-choice recombination, but the challenge is to sort or select for the recombinant virus of interest from the background of wild-type virus. To facilitate selection of recombinant viruses, Masters and Rottier introduced the idea of host range-based selection. They devised a clever plan to use a mouse hepatitis virus (MHV) that encodes the spike glycoprotein from a feline CoV as the target for their recombination experiments. This feline-MHV, termed fMHV, will infect only feline cell lines. Substrate RNAs that encode the MHV spike and mutations of interest in the 3′ region of the genome can be transfected into feline cells infected with fMHV, and progeny virus can be collected from the supernatant and subsequently selected for the ability to infect murine cell lines. Recombinant CoVs that have incorporated the MHV spike gene sequence (and the downstream substrate RNA with mutations of interest) can be selected for growth on murine cells, thus allowing for the rapid isolation of the recombinant virus of interest. This host range-based selection step is now widely used by virologists to generate recombinant viruses with specific alterations in the 3′ end of the CoV genome.

The second approach for manipulating CoV sequences, generating infectious cDNA constructs of CoV, has been developed in several laboratories. Full-length CoV sequences have been cloned and expressed using bacterial artificial chromosomes (BACs), vaccinia virus vectors, and from an assembled set of cDNA clones representing the entire CoV genome. The generation of a full-length cDNA and subsequent generation of a full-length CoV gRNA allows for reverse genetic analysis of CoV sequences. Successful reverse genetics systems are now in place to study the replication and pathogenesis of SARS-CoV, MHV, HCoV-229e, and IBV. These reverse genetics systems have allowed researchers to introduce mutations into the replicase gene and identify sites that are critical for enzymatic activities of many replicase products such as the helicase, endoribonuclease, and the papain-like proteases. Reverse genetic approaches are also being used to investigate the role of the TRSs in controlling the synthesis of CoV mRNAs. Interestingly, the SARS-CoV genome can be 're-wired' using a novel, nonanonical TRS sequence, which must be present at both the ends of the leader sequence and at each intergenic junction. This 're-wired' SARS-CoV may be useful for generating a live-attenuated SARS-CoV vaccine. An important feature of this 're-wired' virus is that it would be nonviable if it recombined with wild-type virus, since the leader TRS and downstream TRS would no longer match in a recombinant virus. The development of reverse genetics systems for CoVs has opened

the door to investigate how replicase gene products function in the complex mechanism of CoV discontinuous transcription, and provides new opportunities to generate novel CoVs as potential live-attenuated or killed virus vaccines to reduce or prevent CoV infections in humans and animals.

Vaccines and Antiviral Drug Development

Because of the economic importance of CoV infection to livestock and domestic animals, a variety of live-attenuated and killed CoV vaccines have been tested in animals. Vaccines have been developed against IBV, TGEV, CCoV, and FIPV. However, these vaccines do not seem to provide complete protection from wild-type virus infection. In some cases, the wild-type CoV rapidly evolves to escape neutralization by vaccine-induced antibodies. In studies of vaccinated chickens, a live-attenuated IBV vaccine has been shown to undergo RNA recombination with wild-type virus to generate vaccine escape mutants. Killed virus vaccines may also be problematic for some CoV infections. Vaccination of cats with a killed FIPV vaccine has been shown to exacerbate disease when cats are challenged with wild-type virus. Therefore, extensive studies will be required to carefully evaluate candidate vaccines for SARS-CoV. A variety of approaches are currently under investigation for developing a SARS-CoV vaccine, including analysis of killed virus vaccines, live-attenuated virus vaccines, DNA immunization, and viral vector vaccines (such as modified vaccine virus Ankara, canarypox, alphavirus, and adenovirus vectors). The development of improved animal models for SARS will be essential for evaluating SARS-CoV candidate vaccines. Transgenic mice expressing human ACE-2 may be an appropriate small animal model. Initial studies suggest that Syrian hamsters and ferrets develop pneumonia and lung pathology similar to that seen in humans after infection with SARS-CoV, and therefore may be appropriate animal models for viral pathogenesis. CoV vaccine studies will benefit from an improved understanding of conserved viral epitopes that can be targeted for vaccine development.

The use of neutralizing monoclonal antibodies directed against the SARS-CoV spike glycoprotein is another approach that may provide protection from severe disease. The success in the development and use of humanized monoclonal antibodies against respiratory syncytial virus (family *Paramyxoviridae*) to protect infants from severe disease indicates that this approach is certainly worth investigating. Preliminary studies have indicated that patient convalescent serum and monoclonal antibodies directed against the SARS-CoV spike glycoprotein efficiently neutralize infectious virus. Further studies are essential to evaluate any concerns about potential antibody-mediated enhancement of disease and to determine if neutralization escape mutants arise rapidly after challenge with infectious virus. Studies evaluating monoclonal antibodies directed against a variety of structural proteins, and monoclonal antibodies directed against conserved sites in the spike glycoprotein will provide important information on the efficacy of passive immunity to protect against SARS.

Currently, there are no antiviral drugs approved for use against any human CoV infection. With the potential for the emergence or re-emergence of pathogenic CoV from animal reservoirs, there is considerable interest in identifying potential therapeutic targets and developing antiviral drugs that will block viral replication and reduce the severity of CoV infections in humans. Two promising targets for antiviral drug development are the SARS-CoV protease domains, the papain-like protease (PL^{pro}) and the 3C-like protease ($3CL^{pro}$, also termed the main protease, M^{pro}) (**Figure 5**). These two protease domains

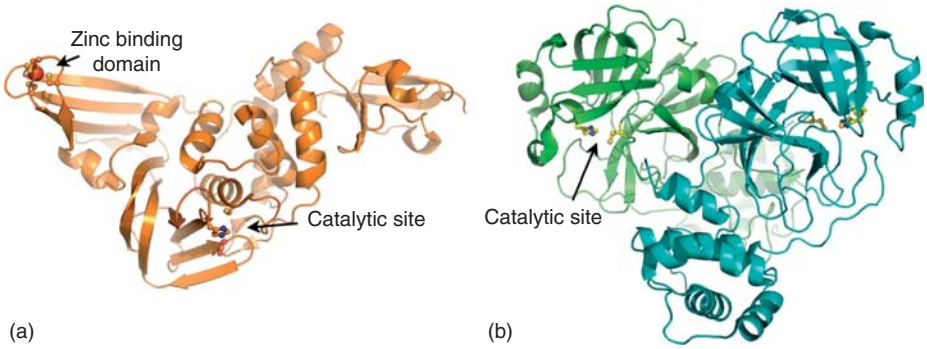


Figure 5 CoV proteases are targets for antiviral drug development; X-ray structures of the two SARS-CoV protease domains encoded in the replicase polyprotein. (a) The SARS-CoV papain-like protease (PL^{pro}) with catalytic triad cysteine, histidine, and aspartic acid residues, and zinc-binding domain indicated. (b) The 3C-like protease ($3CL^{pro}$, also termed main protease, M^{pro}) dimer with catalytic cysteine and histidine residues indicated.

are encoded within the replicase polyprotein gene and protease activity is required to generate the 16 replicase nonstructural proteins (nsp1–nsp16) that assemble to generate the viral replication complex. The crystal structure of the $3CL^{pro}$ was determined first from TGEV and then from SARS-CoV. Rational drug design, much of which was based on our knowledge of inhibitors directed against the rhinovirus 3C protease, has provided promising lead compounds for $3CL^{pro}$ antiviral drug development. Interestingly, these candidate antivirals have been shown to inhibit the replication of SARS-CoV and other group 2 CoVs such as MHV, and the less related group 1 CoV, HCoV-229e. This indicates that the active site of $3CL^{pro}$ is highly conserved among CoVs and that antiviral drugs developed against SARS-CoV $3CL^{pro}$ may also be useful for inhibiting the replication of more common human CoVs such as HCoV-229e, HCoV-OC43, HCoV-NL63, and HCoV-HKU1. Further studies are needed to determine if these inhibitors can be developed into clinically useful antiviral agents.

Analysis of SARS-CoV papain-like protease led to the surprising discovery that this protease is also a viral deubiquitinating (DUB) enzyme. The SARS-CoV PL^{pro} was shown to be required for processing the amino-terminal end of the replicase polyprotein and to recognize conserved cleavage site (-LXGG). The LXGG cleavage site is also the site recognized by cellular DUBs to remove polyubiquitin chains from proteins targeted for degradation by proteasomes. Analysis of the X-ray structure of the SARS-CoV PL^{pro} has revealed that it has structural similarity to known cellular DUBs. These studies suggest that CoV papain-like proteases have evolved to have both proteolytic processing and DUB activity. The DUB activity may be important in preventing ubiquitin-mediated degradation of viral proteins, or the DUB activity may be important in subverting host cell pathways to enhance viral replication. PL^{pro} inhibitors are now being developed using structural information and by performing high-throughput screening of small molecule libraries to identify lead compounds. Additional CoV replicase proteins, particularly the RNA-dependent RNA polymerase, helicase, and endoribonuclease, are also being targeted for antiviral drug development.

Future Perspectives

The development of targeted RNA recombination and reverse genetics systems for CoVs has provided new opportunities to address important questions concerning the mechanisms of CoV replication and virulence, and to design novel CoV vaccines. In the future, improved small animal models for testing vaccines and antivirals, and the availability of additional X-ray crystallographic structure information for rational drug design will be critical for further progress toward development of effective vaccines and antiviral drugs that can prevent or reduce diseases caused by CoVs.

See also: Coronaviruses: General Features; Nidovirales; Severe Acute Respiratory Syndrome (SARS); Torovirus.

Further Reading

Baker SC and Denison M (2007) Cell biology of nidovirus replication complexes. In: Perlman S, Gallagher T, and Snijder E (eds.) *The Nidoviruses*. Washington, DC: ASM Press.

Baric RS and Sims AC (2005) Development of mouse hepatitis virus and SARS-CoV infectious cDNA constructs. *Current Topics in Microbiology and Immunology* 287: 229–252.

Enjuanes L, Almazán F, Sola I, and Zuñiga S (2006) Biochemical aspects of coronavirus replication: A virus–host interaction. *Annual Reviews in Microbiology* 60: 211–230.

Lau YL and Peiris JS (2005) Pathogenesis of severe acute respiratory syndrome. *Current Opinion in Immunology* 17: 404–410.

Li W, Wong SK, Li F, *et al.* (2006) Animal origins of the severe acute respiratory syndrome coronavirus: Insights from ACE2-S-protein interactions. *Journal of Virology* 80: 4211–4219.

Masters PS (2006) The molecular biology of coronaviruses. *Advances in Virus Research* 66: 193–292.

Masters PS and Rottier PJM (2005) Coronavirus reverse genetics by targeted RNA recombination. *Current Topics in Microbiology and Immunology* 287: 133–160.

Perlman S and Dandekar AA (2005) Immunopathogenesis of coronavirus infections: Implications for SARS. *Nature Reviews Immunology* 5: 917–927.

Ratia K, Saikatendu K, Santarsiero BD, *et al.* (2006) Severe acute respiratory syndrome coronavirus papain-like protease: Structure of a viral deubiquitinating enzyme. *Proceedings of the National Academy of Sciences, USA* 103: 5717–5722.

Shi ST and Lai MMC (2005) Viral and cellular proteins involved in coronavirus replication. *Current Topics in Microbiology and Immunology* 287: 95–132.

Stadler K, Masignani V, Eickmann M, *et al.* (2003) SARS – Beginning to understand a new virus. *Nature Reviews Microbiology* 1: 209–218.

Thiel V and Siddell S (2005) Reverse genetics of coronaviruses using vaccinia virus vectors. *Current Topics in Microbiology and Immunology* 287: 199–228.

Wang L, Shi Z, Zhang S, Field H, Daszak P, and Eaton BT (2006) Review of bats and SARS. *Emerging Infectious Diseases* 12: 1834–1840.

Yang H, Xie W, Xue X, *et al.* (2005) Design of wide-spectrum inhibitors targeting coronavirus main proteases. *PLoS Biology* 3: 1742–1751.

Yount B, Roberts RS, Lindesmith L, and Baric RS (2006) Rewiring the severe acute respiratory syndrome coronavirus (SARS-CoV) transcription circuit: Engineering a recombinantion-Resistant genome. *Proceedings of the National Academy of Sciences, USA* 103: 12546–12551.

Relevant Websites

http://patric.vbi.vt.edu – Coronavirus bioinformatics resource, PATRIC (PathoSystems Resource Integration Center).

http://www.cdc.gov – Severe acute respiratory syndrome (SARS) resource, Centers for Disease Control and Prevention.

Cotton Leaf Curl Disease

S Mansoor, I Amin, and R W Briddon, National Institute for Biotechnology and Genetic Engineering, Faisalabad, Pakistan

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Host plant resistance Refers to the ability of a natural host species to control population of the parasite. This is the most desired way to limit damage caused by the causative agent.

Koch's postulates The criteria conceived by Robert Koch in 1890 to establish a causal relationship between a microbe and a disease. These conditions are that the pathogen must be found in all symptomatic hosts, be grown in pure culture, and should be able to cause the disease when reintroduced into a healthy host. The pathogen must then be reisolated from the experimentally infected host.

Pathogen-derived resistance An engineered type of resistance to pathogens created by expression of pathogen-derived genes, or sequences, that interfere with infection by the homologous and closely related pathogens.

Introduction

Cotton leaf curl disease (CLCuD) is a serious disorder of cotton and several other malvaceous hosts that is transmitted by the whitefly *Bemisia tabaci*. CLCuD is endemic across most of Pakistan and northwestern India. The disease has also been reported from Egypt, Sudan, Nigeria, Malawi, and South Africa. Affected plants exhibit very distinctive symptoms; they consist of vein swelling, upward or downward curling of the leaves, and the formation of enations on the main veins on the undersides of leaves. The enations frequently develop into cup-shaped, leaf-like structures (**Figure 1**). Conspicuously CLCuD-infected cotton plants are greener than noninfected plants due to the proliferation of chloroplast-containing tissues. Symptoms are highly variable between cotton varieties, and they also depend on the age of the plant at infection. Plants infected late in the season show only mild symptoms and do not suffer a significant yield reduction. Plants infected early are severely stunted, with tightly rolled leaves, and they usually yield no harvestable lint.

Cotton has been grown in tropical and subtropical regions of the world since prehistoric times and is increasingly being adapted to more temperate climates as well. The genus *Gossypium* encompasses *c.* 50 species, of which five are tetraploid and the remainder are diploid. Of these, four produce spinable fiber and are cultivated as cotton. These include two diploid species, *G. arboreum* and *G. herbaceum*, and two tetraploid species, *G. hirsutum* and *G. barbadense*. More than 90% of world cotton production is obtained from *G. hirsutum*. Diploid cotton species were grown throughout Asia and Africa before the introduction of tetraploid cotton from the New World. The diploid cotton species grown in the Old World are completely immune to CLCuD.

History of CLCuD

CLCuD in Africa

The first report of CLCuD was from Nigeria in 1912, where the disease was a sporadic but minor problem, with a second outbreak in this country occurring in 1924. In North Africa (mainly Egypt and Sudan), *G. barbadense* is the main cotton species cultivated. In these areas, CLCuD is endemic, although it is only sporadically a problem. This was not the case during the early part of the twentieth century when the disease caused major crop losses, particularly in Sudan. An epidemic in Sudan in 1927–28 stimulated interest, and it was during this time that the disease was shown to be transmitted by the whitefly, *B. tabaci*. Extensive work to understand the disease showed that it was also graft transmissible. Although the causative agent was not identified, a virus-like agent was suspected. The disease in this case was brought under control by imposing a cotton-free cultivation period and the introduction of virus-tolerant *G. barbadense* varieties. The disease continues to occur sporadically throughout the region but does not cause major losses.

CLCuD in Southern Asia

CLCuD was noted infrequently across the Indian subcontinent prior to the 1980s. In Pakistan, cotton production, the main foreign exchange earner for the country, suffered heavily from an epidemic of CLCuD which initiated in the vicinity of the city of Multan in the mid-1980s and spread to virtually all cotton-growing areas, as well as into western India. The epidemic has been attributed to the introduction of high-yielding, but also highly susceptible, cotton varieties such as S-12 and CIM70. The cotton



Figure 1 Typical symptoms of CLCuD-affected cotton (*G. hirsutum*) plants. Symptoms include upward curling of the leaves, vein swelling, vein darkening, and enations on the main veins on the undersides of leaves which frequently develop into cup-shaped, leaf-like structures (left). A severely infected plant showing a reduction in leaf size, downward leaf curling, leaf crumpling, and severe stunting (right).

species native to this region, *G. arboreum*, is immune to CLCuD, but unfortunately this does not produce the high-grade cotton lint desired by the processing industry. Nevertheless, *G. arboreum* continues to be grown on a small scale. Although CLCuD remained endemic, losses due to the disease were reduced in both India and Pakistan during the 1990s by the gradual replacement of susceptible varieties with locally developed, tolerant, and resistant cotton varieties. By the late 1990s, cotton production was again at record levels, exceeding the output achieved prior to the epidemic. Then, in 2001, there was a change in the prevalent virus population affecting cotton in Pakistan, as will be described later.

Etiology of CLCuD

The etiology of CLCuD from two regions (North Africa (Egypt and Sudan) and the Indian subcontinent) has been determined. In both regions, the disease is caused by begomovirus complexes consisting of monopartite begomoviruses (genus *Begomovirus*, family *Geminiviridae*) and a recently identified single-stranded DNA satellite termed DNA β. Invariably, begomovirus-DNA β infections of cotton are also associated with a third component, known as DNA-1. This is a satellite-like molecule that plays no essential part in the etiology of CLCuD.

Elucidation of the Etiology of CLCuD

During the early 1990s, the losses to the Pakistan and Indian cotton industries due to the newly emergent CLCuD initiated an urgent investigation into the etiology of the disease. A number of distinct begomoviruses were initially cloned and fully sequenced but infectivity of these was not shown to cotton; thus, Koch's postulates were not fulfilled. Later efforts to introduce one of these viruses, now known as cotton leaf curl Multan virus (CLCuMV), into cotton resulted in mild, atypical symptoms. This indicated that either CLCuMV was not involved in causing the disease or that some other essential component was required to do so.

The genomes of many begomoviruses consist of two components. Component DNA-A encodes all viral functions required for replication, control of gene expression, and encapsidation. The second, DNA-B, encodes two products involved in movement of the virus between and within plant cells. A small number of begomoviruses, mostly isolated from tomato, have monopartite genomes, appearing to be able to dispense with the functions encoded by DNA-B, their genomes consisting of only a homolog of the DNA-A component of bipartite viruses. For these viruses, the products of other genes are able to take over the functions of the DNA-B products in movement. One possible effect of the absence of DNA-B is that monopartite begomoviruses are limited to phloem-associated cells, whereas the majority of bipartite begomoviruses can spread to tissues outside the phloem. The monopartite begomoviruses include tomato yellow leaf curl virus, a major cause of losses to tomato crops globally.

The absence of a detectable DNA-B component associated with CLCuMV suggested that this was a monopartite virus. However, the inability to infect cotton and induce typical disease symptoms was troubling and mirrored the situation with several other begomoviruses at the time. Further analysis of plants identified an additional, novel, subviral component in CLCuD-affected plants. Similar components have been shown to be associated with a number of other monopartite begomoviruses and

this group of molecules has been named DNA-1. However, DNA-1 was found not to play a part in the etiology of CLCuD. Nevertheless, this finding caused a renewed analysis of subviral DNA molecules associated with the monopartite begomoviruses. The breakthrough finally came with the identification of a second subgenomic DNA associated with the monopartite begomovirus, ageratum yellow vein virus, and soon thereafter with CLCuMV, as well as numerous other viruses. This group of molecules has become known as DNA β. Inoculation of CLCuMV with CLCuD DNA β induced full CLCuD symptoms in cotton and established the etiology of the disease as well as fulfilled Koch's postulates.

Components of the CLCuD Complex

CLCuD-associated DNA-1 component

DNA-1 components are satellite-like molecules that are associated with most (but not all) begomovirus-DNA β complexes (**Figure 2**). Their function remains unclear, since they are not required for infectivity or symptom induction in host plants. They are small (~1380 nt), circular, single-stranded DNA molecules that contain a single gene which encodes a rolling-circle replication initiator protein (known for plant-infecting, single-stranded DNA viruses as the replication-associated protein (Rep)). These molecules are capable of autonomous replication in the cells of host plants; hence they are described as satellite-like since, by definition, satellites depend upon their helper virus for replication. However, these components require the helper begomovirus for movement in plants and for transmission between plants, presumably by *trans*-encapsidation in the helper virus' coat protein.

Surprisingly, the DNA-1 components are closely related to the Rep-encoding, satellite-like components of nanoviruses. Nanoviruses are a second family of plant-infecting, single-stranded DNA viruses which are transmitted by aphids. As well as the so-called master Rep (mRep) component, the component which encodes the Rep responsible for replicating all the *bona fide* virus components, some nanovirus infections are also associated with additional Rep-encoding components. These satellite-like molecules are distinct from the mRep in both sequence and in the organization of the genome (the positioning of the promoter being different from that of the mRep). The DNA-1 components associated with begomovirus complexes are believed to have evolved from the nanovirus satellite-like components; most probably by component exchange during co-infection.

The major difference between DNA-1 molecules and nanovirus satellite-like components is size. The components of nanoviruses are typically 1000–1100 nt in length, whereas DNA-1 components are on average 1380 nt – almost exactly half the size of the genomes of their helper begomoviruses. The majority of this size increase is due to the presence, in DNA-1, of ~200 nt region of sequence which is rich in adenine. The function of this A-rich sequence is unclear; it is not essential for the molecule, since DNA-1 components with the A-rich sequence deleted remain capable of replication and movement in plants in the presence of a begomovirus. It is likely that there is a very subtle selection mechanism for maintenance of these additional sequences, most likely for efficient encapsidation by the helper virus' coat protein.

The presence of a DNA-1 component with the CLCuD complex in North Africa has not been investigated.

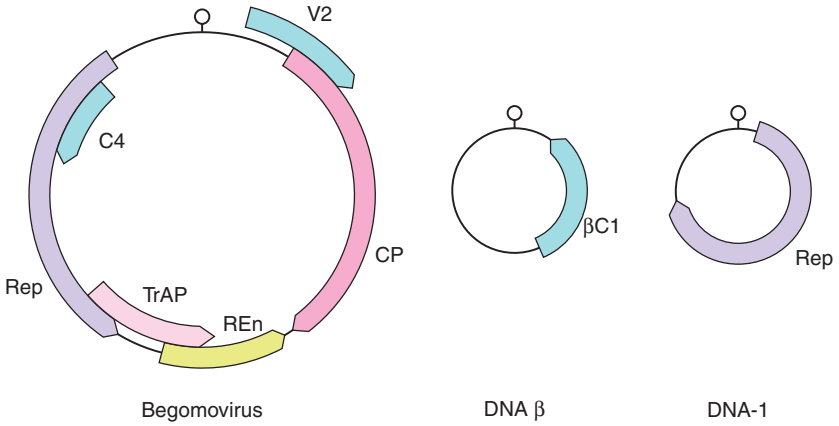


Figure 2 Components associated with CLCuD. Shown are the begomovirus genome and the associated DNA β and DNA-1 components. Only the begomovirus and DNA β component are required to cause the disease. The position and orientation of genes encoded on these are indicated as arrows and labeled as coat protein (CP), replication-associated protein (Rep), transcriptional-activator protein (TrAP), replication enhancer protein (REn), and DNA βC1 protein (βC1). The function of the begomovirus-encoded V2 and C4 genes remain unclear. The ubiquitous stem–loop structure containing the nonanucletide loop sequence TAATATTAC (which for geminiviruses is the origin of virion-strand, rolling-circle DNA replication) is indicated by the small circle for each component.

CLCuD-associated DNA β components

DNA β components are a recently identified class of single-stranded DNA satellites that are associated with monopartite begomoviruses (family *Geminiviridae*). DNA β depends on the begomovirus for its proliferation and is responsible for the accumulation of the helper virus to levels normally found in their original hosts in the field, suggesting its involvement in either replication, systemic movement, or the suppression of a host defense mechanism. They are typically half the length of their helper begomoviruses (~1350 nt) and share no sequence homology with their helper virus other than the presence of a potential stem–loop structure containing the ubiquitous nonanucleotide sequence TAATATTAC. DNA β components encode a single gene, the product of which (known as βC1) is a pathogenicity determinant (determines the symptoms of the infection), a suppressor of post-transcriptional gene silencing (overcoming host plant defenses), upregulate viral DNA levels *in planta*, and bind DNA. DNA β satellites depend upon their helper viruses for replication, movement in plants, and transmission between plants, presumably by *trans*-encapsidation in the helper virus' coat protein. DNA β components have been cloned from a diverse range of hosts and DNA sequence comparison shows them to be highly diverse. Despite their highly diverse nature, only a single type of DNA β is associated with CLCuD and is replicated by several distinct begomoviruses, both experimentally and in the field. Expression of βC1 of CLCuD in tobacco, either stably transformed or from a virus vector, induces virus-like symptoms, demonstrating that DNA β is the most important pathogenicity determinant of the disease. However, the DNA β associated with CLCuD from Sudan is distinct from the one associated with the disease on the Indian subcontinent.

Monopartite begomoviruses associated with CLCuD

On the Indian subcontinent, seven distinct begomovirus species (CLCuMV, cotton leaf curl Alabad virus (CLCuAV), cotton leaf curl Kokhran virus (CLCuKV), cotton leaf curl Rajasthan virus (CLCuRV), papaya leaf curl virus (PaLCuV), tomato leaf curl Bangalore virus (ToLCBV), and cotton leaf curl Burewala virus (CLCuBV)), either singly or as dual/multiple infections, have been shown to be involved in causing CLCuD (**Figure 3**). Some of these viruses have been shown experimentally to induce CLCuD in cotton when co-inoculated with CLCuD DNA β. Among these begomoviruses, only ToLCBV is infectious to tomato, in the absence of a DNA β, causing tomato leaf curl disease. An eighth virus, cotton leaf curl Bangalore virus (**Figure 3**), is associated with CLCuD in southern India (isolated from *G. barbadense*) and is thus unlikely to be part of the epidemic of CLCuD affecting the north of the country; its infectivity to cotton has not been shown experimentally. The viruses shown to be associated with CLCuD are not monophyletic (**Figure 3**), and thus are unlikely to have a common recent ancestor. This suggests that the viruses have been drawn into the disease by gaining an interaction with CLCuD DNA β, likely from other malvaceous hosts since a host range including cotton is a prerequisite for causing CLCuD.

The majority of begomoviruses associated with CLCuD show features both of monopartite as well as bipartite begomoviruses. These viruses replicate to high level in experimental hosts such as *Nicotiana benthamiana*, a situation that differs from bipartite begomoviruses which accumulate to only very low levels, or not at all, in the absence of DNA-β. Experimentally CLCuD DNA β can be replicated and maintained by other begomoviruses that are not found in cotton, including the bipartite begomovirus tomato leaf curl New Delhi virus (ToLCNDV; a virus that occurs widely in solanaceous crops across the Indian subcontinent). However, ToLCNDV has not been reported to infect cotton, suggesting that begomoviruses mobilized into cotton are also limited by their host range. The presence of so many viruses causing a single disease in cotton suggests that they were mobilized into this host after gaining an interaction with CLCuD DNA β, most probably following co-infection of a common host such as tomato. The presence of multiple begomoviruses may help the disease complex to adapt to other hosts or help in overcoming resistance. Nevertheless, there are other monopartite begomoviruses found on the Indian subcontinent that are not found in cotton, suggesting that some begomoviruses are better adapted to cotton. Experimentally some begomoviruses that are not found in the region (such as ageratum yellow vein virus originating from Southeast Asia and tomato leaf curl virus originating from Australia) are capable of supporting replication of CLCuD DNA β. The ability of CLCuD DNA β to interact with begomoviruses from other parts of the world indicates that CLCuD is a global threat to cotton cultivation. No study has been carried out to assess the ability of bipartite begomoviruses from the New World to support replication of CLCuD DNA β. No native monopartite begomoviruses occur in the New World, although TYLCV has recently been introduced and now affects crops in the Caribbean, southern North America, and Central America.

In North Africa, a single begomovirus, cotton leaf curl Gezira virus, has been shown to be associated with CLCuD. This virus is only distantly related to the Asian CLCuD-associated begomoviruses, being instead more closely related to other begomoviruses infecting plants of the Malvaceae originating from Africa and the Mediterranean (**Figure 3**). This is typical of the relationship of begomoviruses within an area (viruses being more related geographically than by the host from which they are

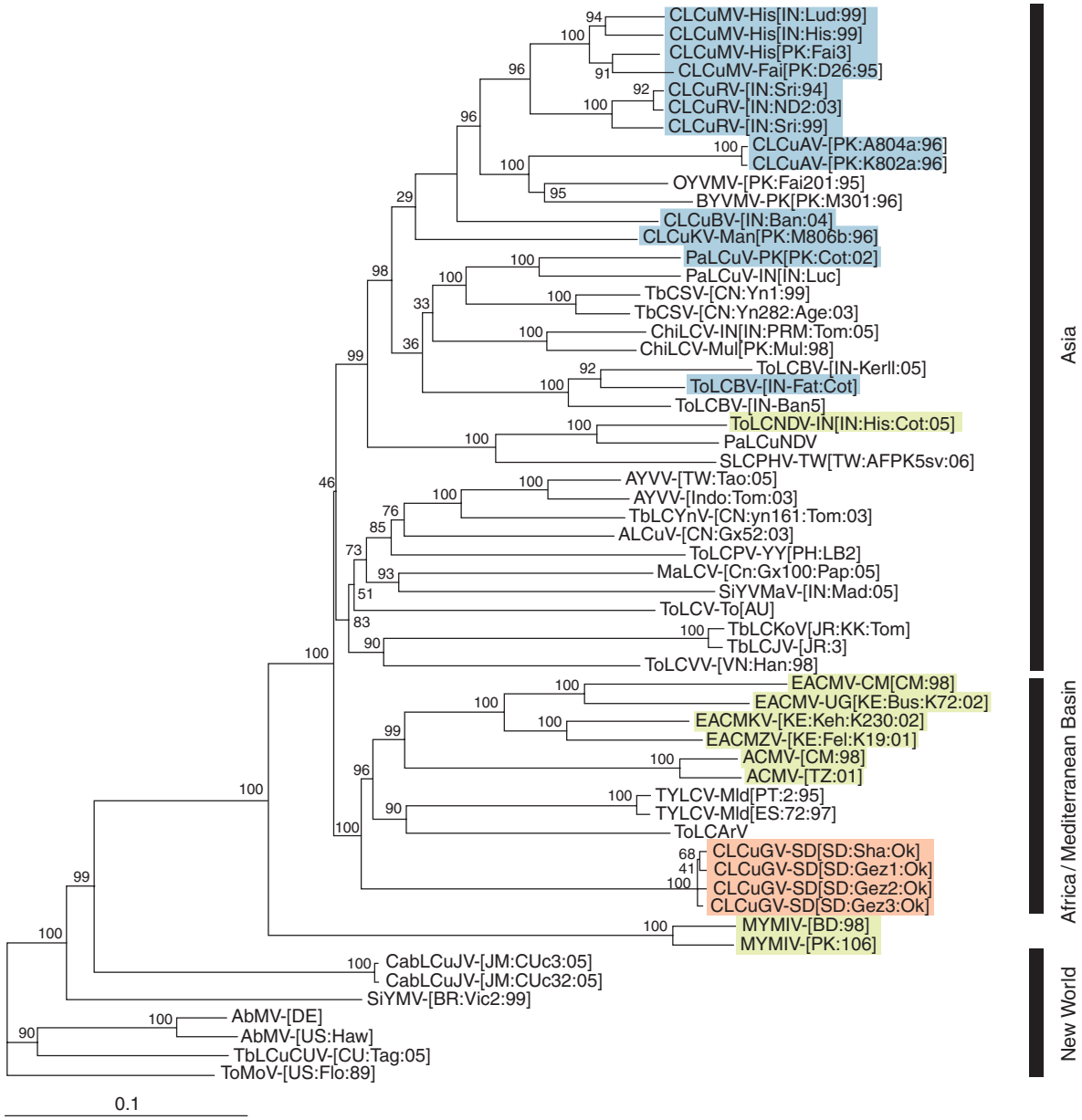


Figure 3 Phylogenetic dendrogram derived from an alignment of 58 selected full-length sequences of the genomes (or DNA A genomic components) of begomoviruses. Indicated on the right are the viruses originating from the New World, Africa/Mediterranean Basin, and Asia. Viruses associated with CLCuD on the Indian subcontinent are highlighted in blue whereas those associated with CLCuD in North Africa are highlighted in brown. Viruses known to be bipartite are highlighted in yellow. The viruses are labeled as abutilon mosaic virus (AbMV), African cassava mosaic virus (ACMV), ageratum leaf curl virus (ALCuV), ageratum yellow vein virus (AYVV), Bhendi yellow vein mosaic virus (BYVMV), cabbage leaf curl Jamaica virus (CabLCuJV), chilli leaf curl virus (ChiLCV), cotton leaf curl Bangalore virus (CLCuBV), cotton leaf curl Gezira virus (CLCuGV), cotton leaf curl Kokhran virus (CLCuKV), cotton leaf curl Multan virus (CLCuMV), cotton leaf curl Rajasthan virus (CLCuRV), cotton leaf curl Alabad virus (CLCuAV), East African cassava mosaic Kenya virus (EACMKV), East African cassava mosaic virus (EACMV), East African cassava mosaic Zanzibar virus (EACMZV), malvastrum leaf curl virus (MaLCV), okra yellow vein mosaic virus (OYVMV), mungbean yellow mosaic India virus (MYMIV), papaya leaf curl New Delhi virus (PaLCuNDV), papaya leaf curl virus (PaLCuV), sida yellow mosaic virus (SiYMV), sida yellow vein Madurai virus (SiYVMaV), squash leaf curl Philippines virus (SLCPHV), tobacco curly shoot virus (TbCSV), tobacco leaf curl Cuba virus (TbLCuCUV), tobacco leaf curl Japan virus (TbLCJV), tobacco leaf curl Kochi virus (TbLCKoV), tobacco leaf curl Yunnan virus (TbLCYnV), tomato leaf curl Bangalore virus (ToLCBV), tomato leaf curl New Delhi virus (ToLCNDV), tomato leaf curl Pune virus (ToLCPV), tomato leaf curl virus (ToLCV), tomato leaf curl Vietnam virus (ToLCVV), tomato mottle virus (ToMoV), tomato yellow leaf curl virus (TYLCV), and tomato leaf curl Arusha virus (ToLCArV). Strain and isolate designation follow established convention. Vertical lines are arbitrary; horizontal lines are proportional to calculated mutation distances. The numbers at nodes indicate percentage bootstrap values (1000 replicates). The tree was rooted on the sequence of ToMoV originating from North America.

isolated or the disease they cause) and indicates that the CLCuDs occurring in Asia and Africa likely have distinct evolutionary origins and are not, as some have claimed, the result of accidental introduction of the virus to Pakistan/India from North Africa.

Control of CLCuD

Natural Resistance to the CLCuD Complex

Resistance to the Asian CLCuD complex is available in the form of *G. arboreum*. This cotton species is immune to CLCuD (in fact, no geminiviruses are reported that infect it) and is grown commercially on a small scale. However, the cotton fiber it produces is not of a sufficient quality or quantity to make it a viable alternative to *G. hirsutum*. Introgression of resistance from *G. arboreum* into *G. hirsutum* is also technically not a simple task (*G. arboreum* being diploid whereas *G. hirsutum* is tetraploid), although some efforts in that direction are in progress.

Limited resistance/tolerance is available in *G. hirsutum* germplasm. The cultivars LRA 511 and CP15/2 were the basis of much of the conventional resistance used to control CLCuD in Pakistan/India during the late 1990s. Cultivation of these cultivars was successful in alleviating losses to CLCuD in endemic areas and during this period cotton production in Pakistan showed an upward trend. However, recently, leaf curl symptoms were noted on previously resistant cotton varieties, indicating a change in the prevalent CLCuD complex.

Breakdown of resistance to CLCuD

During 2001, in the vicinity of the town of Burewala (Punjab, Pakistan), previously resistant cotton varieties began to exhibit the symptoms typical of CLCuD infection. Since then there has been a resurgence of the disease with all previously resistant varieties and virtually all cotton-growing areas of Pakistan and neighboring India affected. Initial analysis of the resistance-breaking strain of CLCuD shows it to be a typical begomovirus-DNA β complex (although the presence of a DNA-1 component has not been confirmed). Only a single begomovirus, which has since been named cotton leaf curl Burewala virus (CLCuBV), a recombinant virus consisting of sequences derived from the previously occurring CLCuMV and CLCuKV, has been identified in all resistant varieties infected. Similarly, the DNA β associated with resistance breaking is recombinant. In this case, the molecules consist for the most part of sequences derived from the original CLCuD DNA β, but with a small fragment of the satellite conserved region (SCR), ~80 nt, derived from a DNA β first identified from tomato exhibiting tomato leaf curl disease symptoms. Although these components are infectious to experimental hosts, it has thus far not been possible to infect resistant cotton varieties with clones of CLCuBV and the recombinant DNA β; the precise molecular basis for resistance breaking thus remains unclear.

Engineered Resistance to the CLCuD Complex

With the absence of any diversity in the natural resistance to CLCuD in the *G. hirsutum* germplasm, efforts have been made to obtain genetically engineered resistance to the disease complex prevalent on the Indian subcontinent. The major challenge to obtaining transgenic CLCuD resistance is the diversity of viruses which cause the disease. It is essential to introduce a broad-spectrum resistance, which is effective against all the viruses present in the field, if the approach is to stand any chance of durability. The strategies under investigation all rely on post-transcriptional gene silencing (PTGS) or transcriptional gene silencing (TGS) – homology-dependent, RNA-mediated phenomena which stimulate the plant's own defenses to target the invading virus. Since the one 'target' present in all CLCuD-affected plants is CLCuD DNA β, initial studies attempted to induce PTGS/TGS against this molecule, with little success. More fruitful have been the studies that have targeted the Rep and AV2 genes, by antisense expression as either full-length (AV2) or truncated (Rep) coding sequences. Both these strategies are presently being assessed in cotton under field conditions. However, it remains to be seen whether the sequences being used provide a sufficiently broad-spectrum resistance to all the CLCuD-associated begomoviruses to be effective and durable under field conditions.

See also: Banana Bunchy Top Virus; Beta ssDNA Satellites; Nanoviruses; Plant Resistance to Viruses: Geminiviruses; Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Satellite Nucleic Acids and Viruses; Tomato Leaf Curl Viruses from India; Tomato Yellow Leaf Curl Virus; Virus Induced Gene Silencing (VIGS).

Further Reading

Asad S, Haris WAA, Bashir A, *et al.* (2003) Transgenic tobacco expressing geminiviral RNAs are resistant to the serious viral pathogen causing cotton leaf curl disease. *Archives of Virology* 148: 2341–2352.

Briddon RW (2003) Cotton leaf curl disease, a multicomponent begomovirus complex. *Molecular Plant Pathology* 4: 427–434.

Briddon RW, Bull SE, Amin I, *et al.* (2004) Diversity of DNA-1; A satellite-like molecule associated with monopartite begomovirus-DNA β complexes. *Virology* 324: 462–474.

Briddon RW and Markham PG (2000) Cotton leaf curl virus disease. *Virus Research* 71: 151–159.

Fauquet CM and Stanley J (2005) Revising the way we conceive and name viruses below the species level: A review of geminivirus taxonomy calls for new standardized isolate descriptors. *Archives of Virology* 150: 2151–2179.

Idris AM, Briddon RW, Bull SE, and Brown JK (2005) Cotton leaf curl Gezira virus-satellite DNAs represent a divergent, geographically isolated Nile Basin lineage: Predictive identification of a satDNA REP-binding motif. *Virus Research* 109: 19–32.

Mansoor S, Briddon RW, Bull SE, *et al.* (2003) Cotton leaf curl disease is associated with multiple monopartite begomoviruses supported by single DNA β. *Archives of Virology* 148: 1969–1986.

Mansoor S, Khan SH, Bashir A, *et al.* (1999) Identification of a novel circular single-stranded DNA associated with cotton leaf curl disease in Pakistan. *Virology* 259: 190–199.

Sanjaya VVS, Prasad V, Kirthi N, Maiya SP, Savithri HS, and Sita GL (2005) Development of cotton transgenics with antisense AV2 gene for resistance against cotton leaf curl virus (CLCuD) via *Agrobacterium tumefaciens*. *Plant Cell, Tissue and Organ Culture* 81: 55–63.

Cowpea Mosaic Virus

G P Lomonossoff, John Innes Centre, Norwich, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Cowpea mosaic virus (CPMV) is the type member of the genus *Comovirus* which includes 13 additional members in the family *Comoviridae*. CPMV was first isolated from an infected cowpea (*Vigna unguiculata*) plant in Nigeria in 1959. Subsequently, it has been found to occur in Nigeria, Kenya, Tanzania, Japan, Surinam, and Cuba. While its natural host is cowpea, it can infect other legumes, and *Nicotiana benthamiana* has proven to be an extremely valuable experimental host. In nature, CPMV is usually transmitted by leaf-feeding beetles, especially by members of the Chrysomelidae. CPMV has also been reported to be transmitted by thrips and grasshoppers. The beetle vectors can acquire the virus by feeding for as little as one minute and can retain and transmit the virus for a period of days or weeks. The virus does not, however, multiply in the insect vector. Experimentally, CPMV is mechanically transmissible.

In Nigeria, infection of cowpeas with CPMV causes a considerable reduction in leaf area, flower production, and yield. Infected plant cells show a number of characteristic cytological changes. These include the appearance of viral particles, a proliferation of cell membranes and vesicles in the cytoplasm, and a variety of modifications to plasmodesmata.

Physical Properties of Viral Particles

Viral particles can reach a yield of up to 2 g kg^{-1} of fresh cowpea tissue and can be readily purified by polyethylene glycol precipitation and differential centrifugation. The particles are very stable with a thermal inactivation point in plant sap of 65–75 °C and a longevity in sap of 3–5 days at room temperature. Once purified, the particles can be stored for prolonged periods at 4 °C. The ease with which virus particles can be propagated, purified, and stored has undoubtedly contributed to the early popularity of CPMV as an object of study.

CPMV preparations consist of nonenveloped isometric particles, 28 nm in diameter, which can be separated on sucrose density gradients into three components, designated top (T), middle (M), and bottom (B), with sedimentation coefficients of 58S, 95S, and 115S, respectively. The three components have identical protein compositions, containing 60 copies each of a large (L) and small (S) coat protein, with sizes 42 and 24 kDa, respectively, as calculated from the nucleotide sequence. The discovery, in 1971, that CPMV particles contained equimolar amounts of two different polypeptides suggested that the capsids had an architecture more similar to the animal picornaviruses than to other plant viruses of known structure. This provided an early clue as to the common origins of plant and animal viruses.

The difference in the sedimentation behavior of the three centrifugal components of CPMV lies in their RNA contents. Top components are devoid of RNA, while middle and bottom components each contain single molecules of positive-strand RNA of 3.5 and 6.0 kbp, respectively. The two RNA molecules were originally termed middle (M) and bottom (B) component RNA after the component from which they were isolated. However, more recently they have been referred to as RNA-2 and RNA-1, respectively. The three-component nature of CPMV preparations is summarized in **Figure 1**. The determination of the component structure of the virus, and particularly the relationship between this and infectivity, was important in establishing the principle that plant viruses frequently have divided genomes, the individual components of which are separately encapsidated.

Because of their differing RNA contents, the three components of CPMV also differ in density and can hence be separated by isopycnic centrifugation on cesium chloride gradients. While T and M components give single bands of densities of 1.30 and 1.41 g ml^{-1}, respectively,

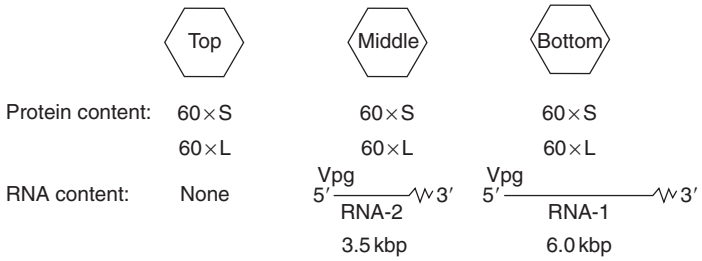


Figure 1 The three-component nature of CPMV indicating the protein and RNA content of each component.

B component gives two bands of 1.43 and 1.47 g ml^{-1}, the proportion of the denser band increasing under alkaline conditions. This increase in density results from an increase in capsid permeability, which allows the exchange of the polyamines present in B components (where they serve to neutralize the excess negative charges from RNA-1) for cesium ions.

CPMV preparations are not only centrifugally heterogeneous but can also be separated in two forms, fast and slow, electrophoretically. Both electrophoretic forms contain all three centrifugal components. The proportion of the two electrophoretic forms in a given virus preparation varies both with the time after infection at which the virus was isolated and the age of the preparation itself. Conversion of one form to the other is caused by loss of 24 amino acids from the C-terminus of the S protein.

Viral Structure

X-ray crystallographic studies on CPMV, as well as the related comoviruses bean pod mottle virus (BPMV) and red clover mottle virus (RCMV), have provided a detailed picture of the arrangement of the two viral coat proteins in the three-dimensional structure of the particle. Overall, the virions are icosahedral, with 12 axes of fivefold and 20 axes of threefold symmetry, and resemble a classic $T = 3$ particle. The two coat proteins taken together consist of three distinct β-barrel domains, two being derived from the L and one from the S protein. Thus, in common with the $T = 3$ viruses, each CPMV particle is made up of 180 β-barrel structures. The S protein, with its single domain, is found at the fivefold symmetry axes and therefore occupies a position analogous to that of the A-type subunits in $T = 3$ particles (**Figure 2**). The N- and C-termini domains of the L protein occur at the threefold axes and occupy the positions equivalent to those of the C- and B-type subunits of a $T = 3$ particle, respectively (**Figure 2**). This detailed analysis confirmed the earlier suggestion that CPMV particles are structurally homologous to those of picornaviruses, with the N- and C-terminal domains of the L protein being equivalent to viral protein VP2 and viral protein VP3, respectively, and the S protein being equivalent to viral protein VP1 (**Figure 2**). However, CPMV particles are structurally less complex than those of picornaviruses. The L and S subunits lack the extended N- and C-termini found in VP2, VP3, and VP1 of picornaviruses and there is no equivalent of VP4. Moreover, CPMV subunits lack the relatively large insertions between the strands of β-sheet, sequences that form the major antigenic determinants of picornaviruses. No RNA is visible in either the M or B components of CPMV, in contrast to the situation found with BPMV where segments of ordered RNA could be detected in middle components.

Genome Structure

Both M and B (but not T) components of a virus preparation are essential for infection of whole plants. As CPMV is a positive-strand RNA virus, a mixture of the genomic RNAs within the particles can also be used to initiate an infection. However, RNA-1 is capable of independent replication in individual plant cells but this leads to the establishment of gene silencing, rather than a productive infection, in the absence of RNA-2. Both genomic RNAs have a small basic protein (VPg) covalently linked to their 5′ termini and both are polyadenylated at their 3′ ends. The elucidation of the overall structure of the RNA segments once more underscored the similarity between CPMV and picornaviruses. However, unlike picornaviruses, the VPg is linked to the viral RNA via the β-hydroxyl group of its N-terminal serine residue rather than via a tyrosine. The VPg is not required for the viral RNAs to be infectious.

The complete nucleotide sequences of both genomic RNAs were reported in 1983, making CPMV one of the first RNA plant viruses to be completely sequenced. The length of the RNAs are 5889 and 3481 nucleotides, for RNA-1 and RNA-2, respectively, excluding the poly(A) tails and the full sequences appear in GenBank under accession numbers NC_003549 and NC_003550. The two genomic RNAs have no sequence homology apart from that at the 5′ and 3′ termini. Full-length infectious cDNA clones of both RNAs of CPMV have been

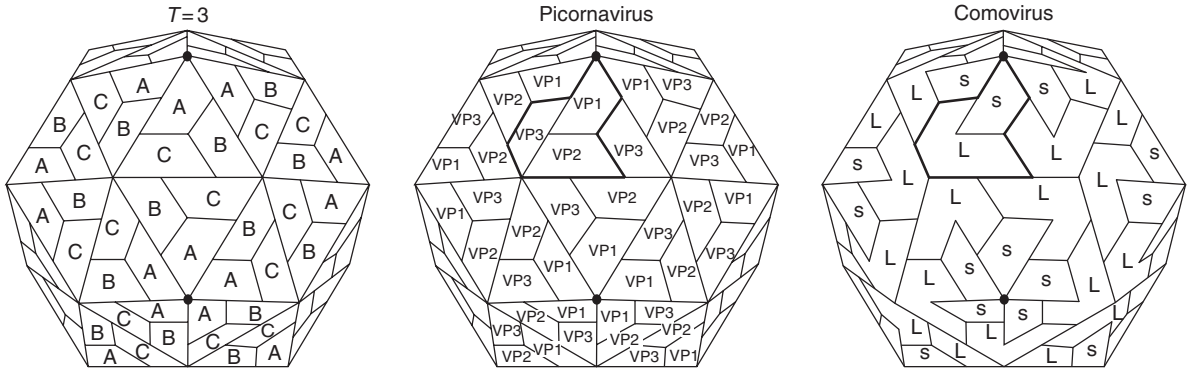


Figure 2 Arrangement of the coat protein subunits of comoviruses (including CPMV) compared with those of simple $T = 3$ viruses and picornaviruses. The asymmetric unit of $T = 3$ viruses contains three β-barrels contributed by three coat protein subunits with identical amino acid sequences (labeled A, B, and C). The asymmetric unit of a picornavirus also contains three β-barrels, but in this case each is contributed by a different coat protein (labeled viral protein VP1, viral protein VP2, and viral protein VP3). The comovirus capsid is similar to that of a picornavirus except that two of the β-barrels (corresponding to viral protein VP2 and viral protein VP3) are fused to give the L protein. Reproduced from Lomonossoff GP, Shanks M, *et al*. (1991) Comovirus capsid proteins: Synthesis, structure and evolutionary implications. *Proceedings of the Phytochemical Society of Europe* 32: 76.

constructed, allowing the genome to be manipulated. This was an important development since it allowed both reverse genetic experiments to be undertaken and for the virus to be used for biotechnological applications.

Expression of the Viral Genome

Both genomic RNAs contain a single long open reading frame, which occupies over 80% of the length of the RNA. A combination of *in vitro* translation and protoplast studies has unraveled the basic mechanism of gene expression of the virus. Both RNAs of CPMV are expressed through the synthesis and subsequent cleavage of large precursor polyproteins. This was the first example of a plant virus using this strategy for the expression of its genome, a strategy that was subsequently shown to be used by a number of other plant viruses. On RNA-1, initiation of translation occurs at the first AUG encountered on the sequence (at position 207) and results in the synthesis of a protein of approximately 200 kDa (the 200K protein). This initial product undergoes rapid cotranslational autoproteolysis to give proteins with apparent sizes of 32 and 170 kDa (the 32K and 170K proteins). The 170K protein undergoes further cleavages to give the range of virus-specific proteins shown in **Figure 3**. *In vitro* translation studies using mutant RNA-1 molecules have shown that all the cleavages occur most efficiently in *cis*. The 170K product can initially be cleaved at three different sites to give three different combinations of secondary cleavage products, 58K + 112K, 60K + 110K, and 84K + 87K. *In vitro*, and probably also *in vivo*, the 60K and 110K are stable and do not undergo further cleavage reactions. This is particularly curious in the case of the 110K protein as it contains both the 24K proteinase domain and a cleavage site. By contrast, the 112K and 84K proteins do undergo further cleavages. The end products of the cleavage pathway of the 170K protein are, from N- to C-terminus, the 58K protein, the VPg, the 24K proteinase, and the 87K protein.

Initiation of translation of RNA-2 occurs at two different positions on the RNA and results in the synthesis of two carboxy coterminal proteins, the 105K and 95K proteins (**Figure 3**). This double initiation phenomenon, which occurs as a result of 'leaky scanning', is found with the RNA-2 molecules of all comoviruses. In the case of CPMV, synthesis of the 105K protein is initiated from an AUG at position 161 while initiation from an AUG at position 512 directs the synthesis of the 95K protein. CPMV RNA-2 has an additional AUG (position 115) upstream of both these initiation sites but this feature is not conserved in the RNA-2 molecules of other comoviruses. Both RNA-2-encoded primary translation products are cleaved by the RNA-1-encoded proteolytic activity to give either the 58K or the 48K protein (depending on whether it is the 105K or 95K protein that is being processed) and the two viral coat proteins. Processing of the RNA-2-encoded polyproteins, at least at the site between the 48K and L coat protein, has been shown to require the presence of the 32K protein as well as the 24K proteinase.

Functions of the Viral Proteins

Functions have been ascribed to most of the regions of the polyproteins encoded by both RNA-1 and RNA-2 of CPMV. In most cases, however, it is not certain at what stage(s) in the cleavage pathway they manifest their activity.

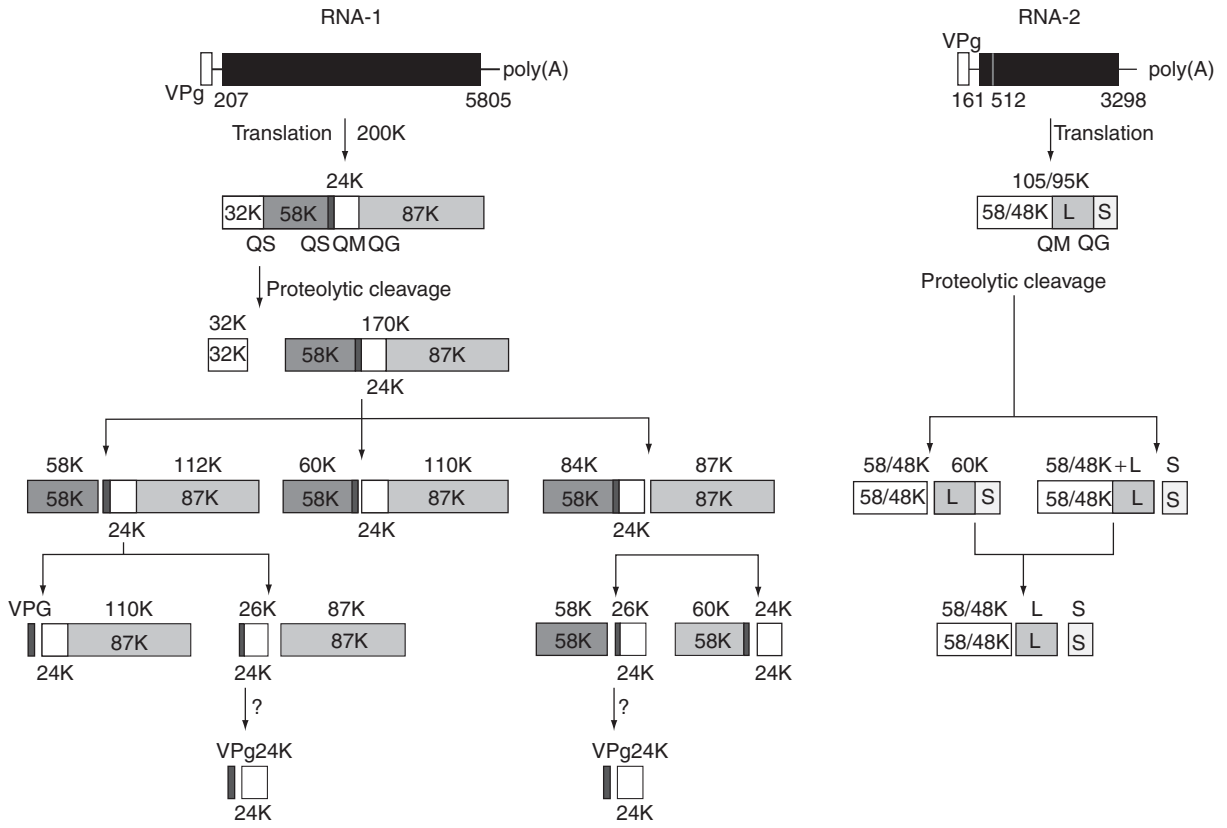


Figure 3 Expression of CPMV RNA-1 and RNA-2. Both RNAs contain a single long open reading frame, which is processed to yield a series of proteins. The positions of the initiation and termination codons and the dipeptides at the proteolytic processing sites are shown.

In the case of RNA-1, the 32K protein, which is rapidly cleaved from the N-terminus of the 200K primary translation product, is a cofactor which modulates the activity of the virus-encoded protease. As described earlier, the presence of the 32K protein is required for the cleavage of the RNA-2-encoded 105K and 95K proteins but is not essential for the cleavage of the RNA-1-encoded 170K protein. It does, however, seem to play a role in determining the rate at which cleavage of the 170K protein occurs. When mutant RNA-1 molecules carrying deletions in the region encoding the 32K protein are translated *in vitro*, the rate of processing of the 170K protein is greatly increased, indicating that the 32K protein acts as an inhibitor of processing. This inhibition may be achieved through the interaction of the 32K with the 58K domain of the 170K protein. The mechanism by which the 32K protein enables the 24K proteinase to cleave in *trans* is unclear.

The RNA-1-encoded 58K protein is associated with cell membranes and contains a nucleotide-binding motif. The 60K protein (**Figure 3**), containing the amino acid sequence of the 58K protein linked to VPg, is involved in rearrangements in the endoplasmic reticulum of CPMV-infected cells and acts in concert with the 32K protein. The 24K protein is the virus-encoded proteinase that carries out all the cleavages on both the RNA-1- and RNA-2-encoded polyproteins. Its proteolytic activity has been shown to be expressed in a number of the processing intermediates that contain its sequence. Indeed, it is not known whether the free form of the protein has any biological significance. Although the proteinase contains a cysteine at its active site, it is structurally related to serine proteases, such as trypsin, rather than cellular thiol proteases, such as papain. In this regard, it is similar to the 3C proteinases of picornaviruses. All comoviral cleavage sites identified so far have glutamine (Q) residue at the −1 position. The enzyme encoded by a given comovirus is specific for the polyproteins encoded by that virus and is unable to cleave the polyproteins from other comoviruses either in *cis* or in *trans*.

The 87K protein is believed to contain the virus-encoded RNA-dependent RNA polymerase (RdRp) activity since it contains the G-D-D sequence motif found in all such enzymes. It also has amino acid sequence

homology to the $3D^{Pol}$ polymerases encoded by picornaviruses. However, when replication complexes capable of elongating nascent RNA chains were isolated from CPMV-infected cowpea plants, they were found to contain the 110K protein (**Figure 3**), consisting of the sequence of 87K protein linked to the 24K proteinase, rather than the free 87K protein.

In the case of RNA-2, the 48K protein, derived from processing of the 95K protein, is involved in potentiating the spread of the virus from cell to cell. This protein is found in tubular structures that are formed in the plasmodesmata of infected cells. Tubules extending into the culture medium can also be seen in protoplasts either infected with CPMV or transiently expressing the 48K protein. Virus particles can be seen within these tubules when protoplasts are infected with CPMV but not when only 48K protein is expressed. At present, no definite role has been assigned to the 58K protein, which is produced by processing of the 105K protein. Mutants in which translation of the 105K protein is disrupted replicate poorly, if at all. In light of these observations, it has been suggested that the 105K protein may play a role in the replication of RNA-2. Apart from containing many hydrophobic and aromatic amino acids, the approximately 10 kDa of protein present in the 58K but absent from the 48K protein is not conserved between comoviruses. The viral coat proteins are required to enable capsids to be formed. As well as protecting the genomic RNAs, capsid formation is essential for the virus to be able to spread from cell to cell through modified plasmodesmata and long-distance movement also requires capsid formation. An additional function in suppressing gene silencing is also provided by the C-terminal region of the S protein.

Replication

CPMV replicates to high level in infected cells. Replication is believed to involve the initial transcription of the incoming positive-sense RNA into minus-strands followed by initiation and synthesis of new plus-strands from the recently formed minus-strands. It has been shown that the 5′ends of both the plus- and minus-strands are covalently linked to the VPg, suggesting that this protein has an essential role in the initiation of RNA synthesis. There also appears to be a tight linkage between the translation of the viral RNAs and their replication.

Replication of the viral RNAs has been shown to occur in the membraneous cytopathological structures, which are formed in the cytoplasm of cells during infection through the action of the RNA-1-encoded 32 and 60K proteins. Both CPMV-specific double-stranded replicative form (RF) RNA and an enzyme activity capable of completing nascent RNA strands can be isolated from such structures. Purified preparations of the enzyme activity contain the RNA-1-encoded 110K protein and two host-encoded proteins of 68 and 57 kDa. However, at present, no enzymatic activity capable of initiating RNA synthesis *in vitro* has been described.

Relationships with Other Viruses

Together with the genera *Nepovirus* and *Fabavirus*, the genus *Comovirus* belongs to the family *Comoviridae*. Within the family, the greatest affinity is between the genera *Comovirus* and *Fabavirus*. On a wider scale, consideration of genome structure and organization, translational strategy, and amino acid homologies between the virus-encoded proteins has led to grouping the family *Comoviridae* with the families *Potyviridae* and *Picornaviridae* as members of picorna-like superfamily of viruses. Members of this superfamily are all nonenveloped positive-strand RNA viruses with 3′ polydenylated genomic RNAs, which have a protein (VPg) covalently linked to their 5′ ends. All members of the supergroup have a similar mode of gene expression, which involves the synthesis of large precursor polyproteins and their subsequent cleavage by a virus-encoded proteinase. The members of the superfamily all contain similar gene order, membrane-bound protein-VPg-proteinase-polymerase (see **Figure 3**) and share significant amino acid sequence homology in the membrane-bound proteins, the proteinases, and polymerase coding regions. Comovirus capsids are also clearly structurally related to those of picornaviruses (**Figure 2**).

Use in Biotechnology

CPMV has been extensively used as a vector for the expression of foreign peptides and proteins in plants. To date, all vectors have involved modifications to RNA-2 (**Figure 4**). In the first instance, antigenic peptides (epitopes) were genetically fused to exposed loops on the surface of the viral capsids. The resulting chimeric virus

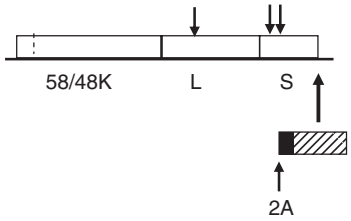


Figure 4 Structure of CPMV RNA-2-based vectors used to express heterologous peptides and proteins in plants. Positions where epitopes have been inserted into the sequence of the coat proteins are shown by black arrows. The position where foreign proteins (shown hatched) have been inserted into RNA-2 is indicated. The FMDV 2A sequence is shown as a black box at the N-terminus of the foreign protein.

particles (CVPs) could be propagated in plants and the modified virions purified. When injected into experimental animals, CVPs can elicit the production of antibodies against the inserted epitope and in a number of instances can confer protective immunity against the pathogen from which the epitope was derived. This was a significant breakthrough as it represented the first instance where protection against an animal pathogen was conferred by material produced from a plant virus-based vector.

In an alternative approach, the sequence encoding an entire heterologous polypeptide has been fused to the C-terminus of the RNA-2-encoded polyprotein via a 2A catalytic peptide derived from foot-and-mouth disease virus (FMDV). The inclusion of the 2A sequence promotes efficient release of the foreign polypeptide from the polyprotein (**Figure 4**). This system has been used to express antibody derivatives in cowpea plants and crude plant extracts containing the antibodies have been shown to be capable of passively immunizing newborn pigs against challenge with the porcine coronavirus, transmissible gastroenteritis virus.

Recent developments in the use of comoviruses in biotechnology include the creation of combined transgene/viral vector systems based on CPMV, and the use of CPMV particles in bionanotechnology.

See also: Nepovirus; Plant Virus Diseases: Economic Aspects; Poliomyelitis; Virus Particle Structure: Nonenveloped Viruses.

Further Reading

Cañizares MC, Lomonossoff GP, and Nicholson L (2005) Development of cowpea mosaic virus-based vectors for the production of vaccines in plants. *Expert Review of Vaccines* 4: 687–697.

Gergerich RC and Scott HA (1996) Comoviruses: Transmission, epidemiology, and control. In: Harrison BD and Murant AF (eds.) *The Plant Viruses 5: Polyhedral Virions and Bipartite RNA Genomes*, 77p. New York: Plenum.

Goldbach RW and Wellink J (1996) Comoviruses: Molecular biology and replication. In: Harrison BD and Murant AF (eds.) *The Plant Viruses 5: Polyhedral Virions and Bipartite RNA Genomes*, 35p. New York: Plenum.

Lin T and Johnson JE (2003) Structure of picorna-like plant viruses: Implications and applications. *Advances in Virus Research* 62: 167–239.

Lomonossoff GP, Shanks M, *et al.* (1991) Comovirus capsid proteins: Synthesis, structure and evolutionary implications. *Proceedings of the European Phytochemical Society* 32: 76.

Cowpox Virus

M Bennett, University of Liverpool, Liverpool, UK
G L Smith, Imperial College London, London, UK
D Baxby, University of Liverpool, Liverpool, UK

© 2008 Elsevier Ltd. All rights reserved.

History

The first published account of cowpox in man and cattle is probably that of Edward Jenner in his *Inquiry* published in 1798, although others, such as Benjamin Jesty, performed immunization of humans with cowpox material earlier. Jenner described the clinical signs of cowpox in both hosts, and how infection in man with *Variolae vaccinae* ('known by the name of the cowpox') provided protection against smallpox. At that time, smallpox was responsible for between 200 000 and 600 000 deaths each year in Europe and about 10% of all deaths in children. Jenner's discovery, despite the concern of some over the consequences of inoculating bovine material into man, soon led to the establishment of smallpox vaccination schemes around the world. However, not until Pasteur's work *c.* 100 years later was the principle of immunization used again. In fact, it was Pasteur who suggested that all such immunizations be called vaccines in honor of Jenner's work.

Although Jenner's first vaccines probably came indirectly from cattle, later vaccine material was often derived from horses, and the origin(s) of modern vaccinia virus (VACV) the smallpox vaccine, remain unknown. That cowpox virus (CPXV) and VACV are different was first published in 1939, since when further biological and genetic studies have confirmed that VACV represents a species in its own right, and is not simply a mutant of CPXV or a recombinant of variola virus (VARV) and CPVX.

Even Jenner seems to have had difficulty finding cowpox cases, and CPXV is not endemic in cattle. Rather, it is endemic in rodents, and cattle and man are merely accidental hosts. The domestic cat is the animal diagnosed most frequently with clinical cowpox in Europe.

Taxonomy and Classification

CPXV represents the species *Cowpox virus*, a member of the genus *Orthopoxvirus* in the family *Poxviridae*, and the international reference strain, Brighton Red, was isolated from farm workers in contact with infected cattle in 1937. CPXV can be differentiated from other orthopoxviruses

(OPVs) by a combination of biological tests, including the ability to produce hemorrhagic pocks on chorioallantoic membranes, the production of A-type inclusions (ATIs) in infected cells, and its ceiling temperature for growth (40 °C, the highest temperature at which the virus will replicate), by minor antigenic differences, by restriction enzyme digestion of the entire genome, particularly with *Hin*dIII, by sequencing of certain genes, and by polymerase chain reaction (PCR). There is considerably more variation between some CPXV isolates than between strains of other OPV species, such that some CPXV strains might be reclassified as separate species rather than strains of the same species. This variation is seen in biological properties (e.g., ceiling temperature of growth, and possibly virulence in different hosts) and genome (restriction enzyme fragment polymorphism, gene content, and nucleotide sequence). It is not yet known whether these differences reflect variation in geographic range or reservoir host.

Properties of the Virion

CPXV has a typical OPV morphology, and is indistinguishable from VACV by electron microscopy. Virions are brick shaped, approximately 300 nm × 200 nm × 200 nm in size, and are enveloped. There are two types of virion. The simpler form, termed intracellular mature virus (IMV), consists of a biconcave core, and within each concavity lies a lateral body. The whole is surrounded by a lipid membrane, and an outer layer of protein. The core contains DNA and proteins, many of which are virus-encoded enzymes. The second form is called extracellular enveloped virus (EEV), and these virions are surrounded by an additional lipid envelope that is fragile and is derived from either the *trans*-Golgi network or endosomes.

The life history of CPXV within the cell is very similar to that of VACV, except that intracellular nonenveloped, yet infectious, virions can become incorporated into large intracytoplasmic ATIs (**Figure 1**). It is thought that these inclusions help protect the virus after cell lysis, and so are important in survival in the environment and spread from animal to animal. In contrast, EEV is more important for spread within individual hosts.

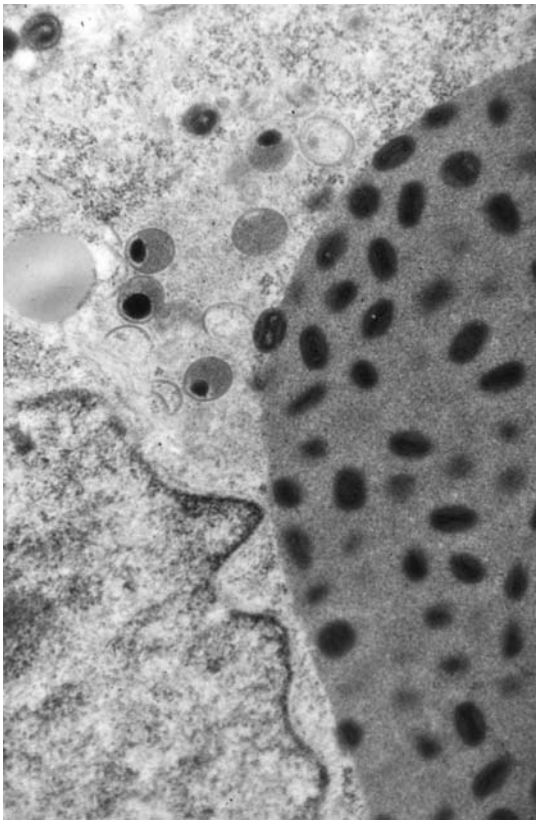


Figure 1 A CPXV-infected cell, showing intracytoplasmic virion synthesis and an ATI containing intracellular mature virions.

Properties of the Genome

The genome consists of linear, double-stranded DNA with covalently linked inverted repeats at the termini. The CPXV genome is the largest of all the OPV genomes and for strain Brighton Red is 224 501 bp. Restriction endonuclease mapping and nucleotide sequencing demonstrated that the middle portion of the CPXV genome (approximately 100 kbp) is highly conserved between OPVs, but more variation occurs toward either end. Digestion with *Hin*dIII usually differentiates CPXVs from other known OPVs, but isolates, particularly geographically distinct strains, do vary in profile, and digestion of genomic DNA with other enzymes often reveals much greater differences between strains. However, overall the genome is fundamentally very stable. The main exception to this is the deletion of 32–39 kbp of DNA from the right end of the genome and its replacement by DNA from the left end: these terminal transpositions are the cause of the 1% white pocks observed on infected chorioallantoic membranes. The size of the transposed fragment varies (5–50 kbp) but the net effect is a change in the length of the inverted terminal repeat (ITR), the duplication of some genes, and the loss of others.

The nucleotide sequences of two CPXV genomes, and of particular genes for a much greater number of isolates, have been determined. CPXV not only has the largest OPV genome, but it also encodes the greatest number of complete protein-coding open reading frames (ORFs) and has relatively few genes that are broken by mutation into fragments. Such broken ORFs are more common in other OPVs such as VARV, camelpox virus (CMLV), and taterapox virus (TAPV). These observations suggest that CPXV might be closest in its genetic complement to the

ancestral poxvirus from which OPVs have evolved. PCR-based diagnostic methods are now used to distinguish CPXV from other OPVs.

Properties of Proteins

The genome of CPXV Brighton Red is predicted to encode more than 200 proteins. Like other OPVs, the proteins encoded in the central part of the genome are mostly essential for virus replication, and in this region 89 genes have counterparts in all sequenced chordopoxviruses. Generally, these CPXV proteins are very closely related to their counterparts in VACV, VARV, and other OPVs. This is certainly true for the proteins that are present in the infectious virion, and the finding that the proteins on the surface of IMV and EEV are very closely related provides a plausible explanation why VACV and CPXV were effective vaccines against smallpox. In contrast, genes located toward the genome termini are nonessential for virus replication in cell culture and these include approximately half of all CPXV genes. Notably, CPXV encodes many genes that are not found in VACV strains.

Interest has focused on proteins concerned with immune modulation, and these are encoded mostly near the left and right ends of the genome. Experimentation has demonstrated that several of these genes (or their orthologs in VACV) are associated with virulence in accidental hosts, but their functions in natural rodent hosts may be more subtle. Possibly their role in natural infection, where clinical disease is not seen and presumably not relevant to transmission, is to moderate the host's immune response, ensuring efficient contact between individuals and therefore transmission.

Other proteins expressed by CPXV but not VACV have been studied, such as those affecting the ability to grow on Chinese hamster ovary (CHO) cells and produce ATIs. Growth of CPXV in CHO cells is dependent on production of a 77 kDa protein and the insertion of the gene encoding this protein into VACV enables that virus to replicate in CHO cells. Formation of ATIs involves the production of two late proteins. One, of 160 kDa, is the major component of the inclusion itself. The other is required for occlusion of virions into the ATI.

A red pock character is associated with the *crmA* gene, which encodes a 38 kDa protein that is one of the most abundant early gene products and a serine protease inhibitor that inhibits apoptosis and the cleavage of pro-interleukin (IL)-1β to IL-1β. This gene is lost in the frequent deletion mutants referred to above. The essential difference between the wild-type and mutant white pock is the greater hemorrhage in the former and the massive leukocyte infiltration in the latter. Although a protein with 93% amino acid sequence identity is encoded by VACV strain Western Reserve, pocks formed by this virus are white irrespective of whether or not the protein (B13) is expressed.

Physical Properties

Little work has been done directly on the physical properties of CPXV, which are usually assumed to be similar to those of VACV and ectromelia virus (ECTV). The outer lipid envelope of extracellular forms of CPXV is labile and readily lost by mechanical stress, but is not essential for virus infectivity because its removal releases an infectious IMV particle. The virus is generally very hardy, and can survive for months in dry scab material at room temperature, and indefinitely at −70 °C. It is inactivated by moist heat at 60 °C for 10 min, and by hypochlorites, phenolics, and detergents, but less effectively by alcohols. The significance of survival in the environment to the epidemiology of cowpox is unknown. The transmission dynamics of natural infections suggest that most transmission is direct. However, in populations of rodents on small islands, infection occurs as small epidemics followed by apparent extinction: whether these epidemics result from immigration of infected individuals, or survival of the virus in the environment with rare reintroduction into naive populations, has not been resolved.

Replication

The mechanisms of DNA replication and temporally regulated transcription and translation of CPXV mRNA are assumed to be very similar to those in VACV. Virus replication takes place in the cytoplasm, and virus assembly occurs in areas known as B-type inclusions. Like VACV, CPXV virions can leave the cell as IMV by lysis of the cell membrane, or as EEV by exocytosis following fusion between the outer membrane of intracellular enveloped virus (IEV) and the plasma membrane. CPXV differs from VACV in that its genome also encodes for large proteinaceous inclusions known as ATIs, into which virions are incorporated (**Figure 1**). These inclusions are released by cell lysis, and are thought to act as protective packets that aid survival of the virus outside of the animal and therefore increase the chance of spread to another host on fomites.

Geographic and Seasonal Distribution

CPXV has been isolated, or detected by PCR and sequencing, throughout much of Northern Europe and as far east as Kazakhstan. Although cases of human and feline cowpox may be seen at any time of year, infection is most common

in the late summer and autumn, which probably reflects increased infection in the reservoir hosts, which in turn reflects increased numbers of hosts at that time of year.

Host Range and Propagation

CPXV has a wide host range both *in vivo* and *in vitro*. It has been isolated from cattle, humans, domestic cats (perhaps the most common source of human infection), dogs, a horse, and a variety of zoo animals including cheetahs, ocelots, panthers, lynx, lions, pumas, jaguars, anteaters, elephants, rhinoceroses, and okapis. All of these are, however, accidental hosts, and the virus circulates mainly in wild rodents. The main reservoir hosts appear to be voles (*Clethrionomys* spp. and *Microtus* spp.) and wood mice (*Apodemus* spp.) throughout the virus' range. House mice (*Mus musculus*) and rats (*Rattus norvegicus*) are infected rarely and are probably accidental hosts (explaining the limited geographic range of the virus), although they may, like cats, act as liaison hosts and transmit the infection onward to man. Other rodents may also act as reservoirs toward the eastern range of the virus. CPXV has been isolated from wild susliks and gerbils (*Rhombomys* spp., *Citellus* spp., and *Meriones* spp.) in Turkmenia. Guinea pigs and rabbits have been infected experimentally with CPXV.

CPXV can be isolated and propagated on the chorioallantoic membrane of hens' eggs, but, unlike VACV, it does not grow in feather follicles of adult chickens. It can be propagated in a variety of cell cultures derived from human, simian, bovine, feline, murine, and rabbit tissues.

Genetics

CPXV produces red hemorrhagic pocks on the chorioallantoic membrane with about 1% white pock mutants. The virus that produces the white pocks breeds true and the change in pock character reflects deletion and transposition events near the termini of the genome. White pock mutants are better able to grow on arginine-deprived cells than is the parent virus. Other properties that are inherited independently, and vary between individual strains, include production of the hemagglutinin, incorporation of virions into ATIs, resistance to heat inactivation, ceiling temperature for growth, and virulence for newborn mice and chick embryos. OPVs undergo genetic recombination in dual-infected cells, for example producing hybrids of CPXV and VARV.

Evolution

The genetic relationships of CPXV and other OPVs have been studied by bioinformatics. Nucleotide sequencing demonstrated that the OPVs are very closely related as a whole (although the North American OPVs appear more distantly related), but each species can be readily differentiated by biological properties and genome structure. Bioinformatic analyses demonstrated that the two CPXV genomes sequenced are quite divergent and it was proposed they should be reclassified as separate species. As noted above, the presence of the greatest number of intact genes and fewest broken genes suggests that CPXV is closest to the ancestral OPV. With the exception of terminal transposition events (see above), CPXV isolates are genetically stable *in vitro* and *in vivo*. Modern isolates often have near-identical restriction enzyme profiles to isolates made many years ago, and the Brighton Red strain behaves the same now as when first isolated over 60 years ago. However, isolates of CPXV do differ in restriction enzyme pattern and minor biological characteristics, and generally greater differences occur between geographically distinct isolates. Furthermore, the greatest variation appears to be seen among isolates in central Europe, and this may reflect a central European origin for CPXV and divergence as individual strains spread out into different host reservoirs and different geographical areas.

CPXV isolates are all clearly different from other OPVs, such as VACV and VARV, but the genome sequence of horsepox virus (from Central Asia) shows that it is intermediate between VACV and CPXV and shares some properties of each virus.

Serologic Relationships and Variability

Within the genus *Orthopoxvirus*, there is extensive antigenic cross-reactivity in all serologic tests, although minor differences can allow differentiation of species using monoclonal antibodies. No significant serologic differences have been reported among CPXV strains.

Epidemiology

CPXV is rarely isolated from cattle, and serologic surveys show that cattle are not the reservoir host. Most human infection cannot be traced to contact with infected cattle, but about half of the recent human cases in Britain were traced to contact with an infected cat. The domestic cat, although the species in which clinical cowpox is most frequently diagnosed in Europe, is not the reservoir host of CPXV either. Although cat-to-cat transmission can occur, antibody to CPXV is uncommon in surveys of healthy cats. There is no evidence that CPXV can become endemic in any of the zoo animals that have been infected, but with increasing reliance on zoo populations for the survival of many species, these outbreaks may cause conservation problems.

Rather, CPXV is endemic in rodent populations. Transmission has been studied in most detail in several populations of voles (*Clethrionomys glareolus* and *Microtus agrestis*) and wood mice (*Apodemus sylvaticus*) in northern England, where it has been used as a model system for investigating the ecology of endemic infections in wildlife populations. Transmission dynamics appear linked to host population dynamics, with the highest incidence of infections occurring in the autumn when population sizes are at their greatest. This is reflected in the highest incidence of infection in cats and human beings also at this time of year. Although cross-species transmission may occur, empirical evidence combined with mathematical models suggests that most transmission occurs among members of the same rodent species, even where two rodent host species share the same habitat.

Transmission and Tissue Tropism

Both natural rodent hosts and laboratory rodents can be infected with CPXV by the oral and respiratory routes as well as skin inoculation: natural hosts can often be infected with less virus than is required to infect cell culture, as is the case with ECTV where the lethal dose for some strains of mice may be less than one plaque-forming unit. However, it is not known how the virus is transmitted in the wild, and transmission has not occurred among laboratory-housed rodent hosts. This may be because a particular behavior, not elicited in the laboratory, is involved in transmission. Transmission rates in the wild can be very high, and longitudinal studies of naturally infected populations suggest that much transmission, although not all, is frequency, rather than density, dependent, which might itself suggest an important role for particular behaviors in transmission.

Among the occasional hosts of CPXV, the most frequent route of infection appears to be through the skin, probably through a cut or abrasion. Domestic cats, however, can be infected experimentally by oronasal inoculation, and limited respiratory spread sometimes occurs in domestic or zoo cat colonies.

Virus replication in cattle and man is mainly limited to the epidermis at the site of entry, and possibly also to draining lymph nodes. In cats, virus can be isolated not only from skin lesions but also from lymphoid, lung, and turbinate tissue. Skin inoculation of cats is followed by virus replication both at the site of entry and in draining lymph nodes, which leads to the development of a viremia, and virus can be isolated from the white cell fraction of blood, from the spleen, and other lymphoid organs. After about 7–10 days, virus can be detected in the epidermis, leading to the development of secondary skin lesions. The viremia in cats appears to last 1–8 days, and no virus has been isolated from cats after the skin lesions have healed, which may take 5–6 weeks.

Oral, nasal, and skin inoculation of various rodents with CPXV also causes systemic infection. Virus has been isolated from lung, kidney, liver, and lymph nodes of susliks and gerbils, and detected by PCR in a similar range of tissues, and the cellular fraction of blood from naturally and experimentally infected British wild rodents. Laboratory infection and studies of naturally infected wild voles and wood mice suggest that they remain infected for around 4 weeks before clearing the infection.

Virulence

It is not known whether different strains of CPXV vary in virulence for most accidental hosts, including man. In a small-scale experiment, no differences in the ability to cause infection or a primary lesion in domestic cats could be detected between the Brighton Red strain and isolates from a cheetah and a domestic cat. Differences in virulence of different strains do exist for newborn laboratory mice and chick embryos, but these differences are not associated with the ability to infect various accidental hosts and it is not known whether they have any significance in the maintenance of different strains of CPXV in different reservoir tests.

Wild-type CPXV outgrows the white pock mutants and is more virulent for laboratory animals.

Clinical Features of Infection

In cattle, CPXV causes teat lesions, but little apparent systemic disease. Human infection is characterized usually by a single skin lesion, often on a hand or the face. Spread of skin lesions in man is usually the result of direct transmission, for example, from hand to face, but multiple lesions may also occur if there is a preexisting skin condition. Cowpox in man is often accompanied by systemic signs such as nausea, fever, and lymphadenopathy, and children are often hospitalized. Death is rare, and usually the result of an underlying condition, such as immunodeficiency, which increases the severity of disease.

CPXV infection in domestic cats is usually a more severe disease than in cattle or man. There is often a history of a single primary lesion, especially on the head or a forelimb, but by the time the cat is presented for veterinary attention widespread skin lesions have usually developed. The primary lesions vary enormously in character, and secondary bacterial infection is common. The widespread secondary lesions first appear as small erythematous macules, which develop into papules and ulcers over several days. These scab over, and the cat

usually recovers within 6–8 weeks. Cats may be slightly pyrexic in the early stages of the disease and some show signs of mild upper respiratory disease. More severe illness such as large nonhealing lesions or pneumonia usually, but not always, results from secondary bacterial infection or immunosuppression. In some zoo-kept cats, such as cheetahs, pneumonia is more common and is associated with a high mortality rate.

No obvious clinical signs are observed in either naturally or experimentally infected rodents (unless the experimental dose used is high). However, longitudinal studies of naturally infected wild voles and wood mice, at both the individual and population levels, as well as experimental infections, have shown an effect on rodent fecundity. Infected voles and mice delay reproduction compared to uninfected animals in the wild by perhaps a whole season.

Pathology and Histopathology

Skin lesions associated with cowpox in most accidental hosts are typical of those expected of an OPV infection, developing through papule, vesicle, pustule, ulcer, and healing stages, although macroscopic vesicles often ulcerate quickly because of abrasion or, in the case of domestic cats, because the epidermis is too thin in most areas to support a vesicle. Microscopic examination reveals hypertrophy and hyperplasia of infected cells, multilocular vesicle formation, large, intracytoplasmic eosinophilic inclusion bodies (ATIs) (**Figure 1**) in epithelial cells, and a vigorous polymorph infiltration of the dermis. Immunostaining demonstrates virus antigen in epithelial cells of the skin, hair follicles, and sebaceous glands, and in dermal macrophages.

Even in lungs and turbinates of cats from which large amounts of virus can be isolated, there are often no gross lesions, and microscopic lesions may be difficult to find. In cats showing clinical signs of pneumonia, there is often an interstitial pneumonia, and, again, eosinophilic inclusions can be seen in infected cells. The tonsils and lymph nodes of infected cats contain many large reactive follicles, and immunostaining may demonstrate antigen in macrophage-like cells. Some follicles have large necrotic centers, suggesting that virus replication is occurring here.

After 3 days of growth on the chorioallantoic membrane of 12 day chick embryos, CPXV causes hemorrhagic pocks approximately 2 mm in diameter, with a few (usually 1%) white pocks. Microscopically, the red pocks consist of ectodermal proliferation and hypertrophy with many cells containing ATIs, and extensive edema and hemorrhage into the mesoderm. The histopathology of white pocks is similar but consists of more inflammatory infiltration and no hemorrhage.

Immune Response

Relatively little is known about the immune response to CPXV in naturally infected hosts. Antibody can be detected by enzyme-linked immunosorbent assay (ELISA), immunofluorescence, virus neutralization (VN) (usually done with the IMV form of virus) with or without complement, complement-fixation, and hemagglutination-inhibition (HAI) tests. HAI antibody can be detected before VN antibody, and in cattle and cats begins to decline after about 6 months. HAI antibody is therefore more useful for the diagnosis of acute infections than VN antibody, and in epidemiological studies indicates more recent infection than VN antibody alone.

As in VACV and ECTV infections, cell-mediated immunity plays an important role in protection against CPXV disease, but less work has been published specifically on the cell-mediated immunity to CPXV. A possible delayed-type hypersensitivity response has been reported in cats.

Prevention and Control

Infection in man and domestic animals is relatively uncommon and so measures to prevent infection are generally not warranted. Vaccination against smallpox is no longer routine, and might not protect against the development of a skin lesion, but would reduce the severity of any systemic illness. VACV infection of cattle and man often causes lesions and disease similar to cowpox. VACV does not grow well in cats, and its efficacy as a vaccine (although not necessarily as a vaccine vector) in felids is uncertain, although it has been recommended for some zoo animals.

Although CPXV can cause quite severe disease in man, it does not appear to be very infectious. Human-to-human spread of cowpox has not been reported (in contrast to VACV). Many human cases of cowpox have been traced to contact with infected cats, but we know of no cases of cat-to-human transmission after cowpox was diagnosed in the cat. Simple hygiene – washing hands after handling the cat, keeping the cat or scab material away from cuts and the eyes – seems adequate to prevent transmission to man, although special measures might be taken for the very young, elderly, or immunosuppressed. Similarly, if an outbreak occurs in cattle, the main route of spread among the cows is through milking equipment, and simple hygiene should suffice to control spread.

Future Perspectives

CPXV is one of several OPVs that have wildlife reservoirs; others include monkeypox virus, raccoonpox virus,

and Californian volepox virus. Buffalopox virus is now regarded as a variant of VACV and may also have a wild animal reservoir in India. Occasionally, these and other OPVs may infect other, accidental, hosts, such as domestic animals: there are reports of uncharacterized OPVs being isolated from horses, for example, in Africa, North America, and Australia, and a recent report of raccoonpox virus infection in a cat in Canada. Study of the ecology of CPXV is therefore useful as a model for OPV maintenance in a wildlife reservoir host and the mechanisms of transmission from animals to man. It is also being studied in wild rodents as an ecological model of transmission of endemic infections, and the interactions between host and parasite dynamics.

See also: Adenoviruses: Pathogenesis; Mousepox and Rabbitpox Viruses; Smallpox and Monkeypox Viruses; Vaccinia Virus.

Further Reading

Baxby D, Bennett M, and Getty B (1994) Human cowpox; A review based on 54 cases, 1969–93. *British Journal of Dermatology* 131: 598–607.

Bennett M, Gaskell RM, and Baxby D (2005) Feline poxvirus infection. In: Greene C (ed.) *Infectious Diseases of the Dog and Cat,* 3rd edn., pp. 158–160. Philadelphia: WB Saunders.

Carslake D, Bennett M, Hazel S, Telfer S, and Begon M (2006) Inference of cowpox virus transmission rates between wild rodent host classes using space–time interaction. *Proceedings of the Royal Society Series B* 272: 775–782.

Essbauer S and Meyer H (2007) Genus *Orthopoxvirus*: Cowpox virus. In: Mercer AA, Schmidt A and Weber O (eds.) *Poxviruses*, pp. 75–88. Basel: Birkhäuser Verlag.

Fenner F, Wittek R, and Dumbell KR (1989) *The Orthopoxviruses.* London: Academic Press.

Gubser C, Hué S, Kellam P, and Smith GL (2004) Poxvirus genomes: A phylogenetic analysis. *Journal of General Virology* 85: 105–117.

Moss B (2001) *Poxviridae*: The viruses and their replication. In: Fields BN, Knipe DM, Howley PM, *et al.* (eds.) *Virology,* 4th edn., pp. 2849–2883. Philadelphia: Lippincott-Raven Publishers.

Pickup DJ, Ink BS, Hu W, Ray CA, and Joklik WK (1986) Hemorrhage in lesions caused by cowpox virus is induced by a viral protein that is related to plasma protein inhibitors of serine proteases. *Proceedings of the National Academy of Sciences, USA* 83: 7698–7702.

Shchelkunov SN, Safronov PF, Totmenin AV, *et al.* (1998) The genomic sequence analysis of the left and right species-specific terminal region of a cowpox virus strain reveals unique sequences and a cluster of intact ORFs for immunomodulatory and host range proteins. *Virology* 243: 432–460.

Telfer S, Bennett M, Bown K, *et al.* (2002) The effects of cowpox virus on survival in natural rodent populations: Increases and decreases. *Journal of Animal Ecology* 71: 558–568.

Telfer S, Bennett M, Bown K, *et al.* (2005) Infection with cowpox virus decreases female maturation rates in wild populations of woodland rodents. *Oikos* 109: 317–322.

Tulman ER, Delhon G, Afonso CL, *et al.* (2006) Genome of horsepox virus. *Journal of Virology* 80: 9244–9258.

Relevant Website

http://www.poxvirus.org – Poxvirus Bioinformatics Research Center.

Coxsackieviruses

M S Oberste and M A Pallansch, Centers for Disease Control and Prevention, Atlanta, GA, USA

Published by Elsevier Ltd.

Glossary

Chemosis Edema of the bulbar conjunctiva.
Conjunctival hyperemia Increased blood flow to the conjunctiva.
Cytopathic effect Degeneration of cultured cells due to virus infection and replication, often characteristic of a given virus.
Exanthem Illness characterized by skin eruption (rash).
Myalgia Nonspecific muscle pain or tenderness.
Nuclear pyknosis Condensation and reduction in size of the nucleus, often the result of a pathogenic process.
Pleurodynia Illness characterized by chest pain due to inflammation of the pleura, the membrane surrounding the lungs and chest cavity.
Protomer Basic unit of a virus capsid, containing one or more viral proteins.

History

The coxsackieviruses (genus *Enterovirus*, family *Picornaviridae*) were discovered in the late 1940s, as a result of intense efforts to develop better systems to propagate and study poliovirus. At the time, nonhuman primates were

the model system for poliovirus and poliomyelitis, as cell culture and inoculation of suckling mice were just being introduced. Investigators at the New York State Department of Health inoculated suckling mice with fecal suspensions from two suspected poliomyelitis cases. The mice became paralyzed, but the viruses were not polioviruses – the virus was later named 'coxsackievirus' (CV) for the patient's home town, Coxsackie, New York. Subsequent studies identified related viruses, some causing spastic paralysis rather than the flaccid paralysis observed with the initial isolates. Based on these differences in pathogenicity, the viruses were classified as group A coxsackieviruses (flaccid paralysis) or group B coxsackieviruses (spastic paralysis), with the individual virus types being numbered sequentially within each group (e.g., coxsackievirus A2 or coxsackievirus B5). Once cell culture became established as a standard laboratory technique, additional viruses were discovered that were able to replicate in culture but caused no disease in mice. Since many of these were derived from stools of healthy individuals and they were not yet known to cause disease in humans, they were termed enteric cytopathic human orphan viruses or 'echoviruses' – these were also named using consecutive numbers (e.g., echovirus 9). The early coxsackieviruses had been isolated from cases of 'nonparalytic poliomyelitis' (aseptic meningitis), showing that this disease was not necessarily caused by polioviruses. Other illnesses, including herpangina, rash, pleurodynia, and myocarditis, were also shown to be associated with coxsackievirus infection; later, the echoviruses were later shown to be associated with many of these same illnesses.

Taxonomy and Classification

As mentioned, the term 'coxsackievirus' was applied to enteroviruses that caused paralysis upon intracerebral inoculation in suckling mice, with the type of paralysis – flaccid versus spastic – being used to place strains in group A or group B. When it was discovered that antigenically related viruses could have varying degrees of pathogenicity in mice, the original naming convention was abandoned and since 1967 all new enteroviruses (whether coxsackievirus-like or echovirus-like) have been named 'enterovirus' followed by a sequential number, starting with enterovirus 68. Enteroviruses have traditionally been classified antigenically, using well-characterized, standardized antisera in a neutralization assay, either in cell culture or in suckling mice (for viruses that replicate poorly in culture). Using this approach, 30 coxsackievirus serotypes were defined, with 24 in group A and six in group B. Coxsackievirus A23 was later discovered to be antigenically identical to echovirus 9, so CVA23 was dropped as a distinct serotype.

It was recognized early on that there were many limitations to the antigenic typing approach. In addition to the labor-intensive nature of the neutralization assay and the requirement for extensive characterization of new antisera, different serotypes may cross-react in complex combinations and individual strains of a given type may react differently with different preparations of homotypic antisera. Once nucleotide sequences became available for a large number of serotypes, it became clear that the sequences of certain parts of the capsid-coding region could serve as a surrogate for antigenic type. In the late 1990s and early 2000s, several investigators developed typing systems based on polymerase chain reaction (PCR) amplification of a portion of the capsid region, followed by sequencing and analysis of the sequence to determine type. Eventually, VP1 was settled upon as the most reliable region for molecular virus typing. Sequence comparisons have shown that CVA15 is a strain of CVA11 and CVA18 is a strain of CVA13.

Sequence relationships are now recognized as a primary discriminating characteristic in enterovirus taxonomy. There are currently eight species in the genus *Enterovirus*: *human enterovirus* (HEV) A–D, *Poliovirus*, *Bovine enterovirus*, *Porcine enterovirus*, and *Simian enterovirus A*. There are current proposals to merge *Poliovirus* with HEV-C and to move the two human rhinovirus species from their own genus into *Enterovirus*. The coxsackieviruses are distributed among three species: HEV-A (CVA2–8, CVA10, CVA12, CVA14, and CVA16), HEV-B (CVA9, CVB1–6), and HEV-C (CVA1, CVA11, CVA13, CVA17, CVA19–22, and CVA24) (**Table 1**).

Host Range and Virus Propagation

Coxsackieviruses are primarily human pathogens but many can also infect certain nonhuman primate species and all serotypes are able to infect and cause disease in mice (by definition), though there is some variability among strains of any given type. Infection of nonhuman primates often fails to induce clinical disease, but CVA7

Table 1 Coxsackievirus taxonomy

Species	*Serotype*
Human enterovirus A[a]	CVA2–8, CVA10, CVA12, CVA14, CVA16
Human enterovirus B[b]	CVA9, CVB1–6
Human enterovirus C[c]	CVA1, CVA11, CVA13, CVA17, CVA19–22, CVA24

[a]Also includes EV71, EV76, EV89–92.
[b]Also includes all echoviruses, EV69, EV73–75, EV77–88, EV93, EV97–98, EV100–101.
[c]Also includes polioviruses, EV95–96, EV99, and EV102.

can induce polio-like paralysis in monkeys. Swine vesicular disease virus, an important livestock pathogen because symptoms of illness are similar to those of foot and mouth disease, is closely related genetically and antigenically to CVB5. Genetic and epidemiologic studies strongly suggest that human CVB5 was introduced into swine decades ago, with subsequent adaptation and diversification in the new host species.

The best specimens for virus isolation are stool specimens or rectal swabs, throat swabs or washings, and cerebrospinal fluid, in that order. Isolation from stool is most sensitive because virus is usually present at higher titer and it is present in stool longer than in any other specimen. Nonfecal specimens are most likely to yield virus isolates if they are obtained early in the acute phase of illness. For cases of acute hemorrhagic conjunctivitis, the best specimens are conjunctival swabs and tears. A number of different primary cell cultures and continuous cell lines, both generally of human or monkey origin, have been used for isolation and propagation of coxsackieviruses. These cell systems include primary monkey kidney cells; monkey kidney cell lines such as BGM, Vero, and LLC-MK2; and human cell lines such as HeLa, Hep2, KB, and RD. Virus infection and replication results in a characteristic cytopathic effect which is observed microscopically 1–7 days after inoculation. The cells become rounded and refractile, with nuclear pyknosis and cell degeneration. Ultimately, the cells are lysed and become detached from the surface of the culture vessel.

Attempts at virus isolation in cell culture may sometimes be unsuccessful, necessitating the inoculation of suckling mice. For example, CVA1, CVA19, and CVA22 are rarely, if ever, isolated in culture, but grow readily in suckling mice. If the virus titer is extremely low, blind passage in mice may be necessary; blind passage may also be needed to allow the virus to adapt to growth in mice. The two groups of coxsackieviruses can be distinguished by the distinct pathology that they cause in mice. With CVA infection, newborn mice develop flaccid paralysis and severe, extensive degeneration of skeletal muscle (sparing the tongue, heart, and CNS), and they may have renal lesions. Death usually occurs within a week. CVB infection proceeds more slowly and is characterized by spastic paralysis and tremors associated with encephalomyelitis, focal myositis, brown fat necrosis, myocarditis, hepatitis, and acinar cell pancreatitis.

Epidemiology, Geographic Distribution, and Seasonality

The coxsackieviruses are distributed worldwide, but there can be significant geographic and temporal variation in prevalence of individual serotypes. These differences are attributed to differences in climate and public hygiene, overall population immunity (e.g., an increase in the number of susceptible individuals in the years following an outbreak), and other factors. In the United States, for example, CVA9 is among the most commonly reported enteroviruses, but circulation tends to peak every 3–5 years. CVB2, CVB4, and CVB5 are also common, with cyclic peaks of activity, and CVB5 has been associated with large outbreaks, while CVB1 and CVB3 circulate at low, relatively constant levels, and CVB6 is rarely reported. Like the other enteroviruses, most coxsackievirus infections tend to occur during the warmer months in areas with a temperate climate, peaking in late summer or fall. In the tropics, peak activity generally correlates with the rainy season.

Coxsackieviruses are isolated in the highest titer and for the longest time in stool specimens but can also be isolated from respiratory secretions. Fecal-oral transmission and spread by contact with respiratory secretions (person-to-person, fomites, and possibly large particle aerosol) are the most important modes of transmission. The relative importance of the different mode probably varies with the virus and the environmental setting. Viruses that cause a vesicular exanthem are also presumably spread by direct or indirect contact with vesicular fluid, which contains infectious virus. Exceptions to the usual modes of enterovirus transmission are the agents of acute hemorrhagic conjunctivitis, coxsackievirus A24 variant (CA24v), and enterovirus 70 (a member of HEV-D). These two viruses are seldom isolated from respiratory or fecal specimens and are probably transmitted primarily by direct or indirect contact with eye secretions.

Virion Structure and Host Cell Receptors

Coxsackievirus virions, like those of other picornaviruses, are approximately 30 nm in diameter, with little discernable fine-scale structure (**Figure 1**). Sixty copies of each of the mature virion proteins, VP1–VP4, form an icosahedral virion particle with pseudo $T = 3$ symmetry. The proteins VP1–VP3 combine to form the virion protomer, with VP4 internal to the particle.

Virus infection is dependent on the presence of specific receptors. At least four distinct receptors are used by coxsackieviruses for entry into human cells. CVA9 uses integrin $\alpha_v\beta_3$, CVB1–6 use the 'coxsackievirus–adenovirus receptor' (CAR) and some may also use decay-accelerating factor (DAF), and some of the HEV-C coxsackieviruses use intracellular adhesion molecule 1 (ICAM-1). The receptor (s) used by most viruses in HEV-A, and by many of the viruses in HEV-C remain unknown.

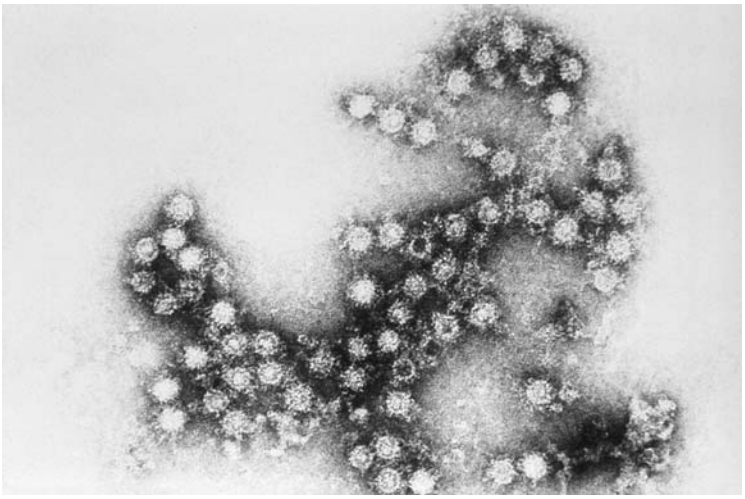


Figure 1 Electron micrograph of coxsackievirus B4 virions. Image courtesy of CDC Public Health Image Library.

Genetics, Genetic Diversity, and Evolution

Like the other enteroviruses, the coxsackievirus genome is a single-stranded, polyadenylated, positive-sense RNA of approximately 7.4 kbp, with a 22-amino-acid virus-encoded protein ($3B^{VPg}$) covalently linked to the 5′ end. Flanked by 5′- and 3′-nontranslated regions (NTRs), the single long open reading frame encodes a polyprotein of approximately 2200 amino acids that is processed during and following translation by viral proteases to yield the mature viral polypeptides (**Figure 2**). The P1 region encodes the capsid proteins 1A-1D (VP4, VP2, VP3, and VP1, respectively). The P2 and P3 regions encode proteins involved in polyprotein processing, RNA replication, and shutdown of host cell protein synthesis.

Coxsackieviruses, like other picornaviruses, evolve extremely rapidly, because the viral RNA-dependent RNA polymerase is error-prone and lacks a proofreading function (like other RNA virus replicases). Most of the nucleotide substitutions are translationally silent, because most substitutions that become fixed in a virus population are in the third ('wobble') position of codons. This phenomenon is likely due to intense selection against amino acid substitutions, especially in the proteins that are required for replication, protein processing, and shutdown of host cell synthesis. Most viable amino acid substitutions occur in surface loops of the external capsid proteins, VP1, VP2, and VP3, while residues that contribute to the proteins' beta-barrel structure tend to be highly conserved within a serotype and, to a large extent, within a species. Within a serotype, VP1 nucleotide sequence can vary by up to 25%, representing near-saturation of synonymous sites, as well as multiple nonsynonymous substitutions – the amino acid sequence can vary by as much as 15% with a type.

The different enterovirus species each form a distinct phylogenetic group throughout the coding region, as well as in the 3′-NTR. In the 5′-NTR, the human enteroviruses form only two clusters: cluster I contains HEV-C and HEV-D, while cluster II contains HEV-A and HEV-B. The nonhuman species each form distinct 5′-NTR clusters. The coxsackie B virus capsid sequences cluster together as a group, probably reflecting their shared, but unique, use of CAR as a receptor. In other parts of the coding region, the CVB are not phylogenetically coherent; rather, they interdigitate with other viruses in HEV-B, due to the high frequency of RNA recombination within any given enterovirus species.

Pathogenesis and Immunity

Much of the disease associated with coxsackievirus infection is thought to be a direct result of tissue-specific cell destruction, analogous to the cytopathic effect in cultured cells (**Figure 3**). For the most part, the detailed mechanisms of virus-induced disease have not been well-characterized. The primary site of infection is typically the respiratory or gastrointestinal epithelium, leading to viremia that may result in a secondary site of tissue infection. Such secondary spread of virus to the CNS can result in aseptic meningitis or, rarely, encephalitis or paralysis. Other tissue-specific infection can result in pleurodynia or myocarditis. Disseminated infection can lead to exanthems, nonspecific myalgias, or severe multiple-organ disease in neonates. Some disease manifestations, enteroviral exanthems and myocarditis, for example, are thought to result from the host immune response to the infection.

Enterovirus infection elicits a strong humoral immune response. Often the response is heterotypic; that is, infection with one serotype induces a broad immune response

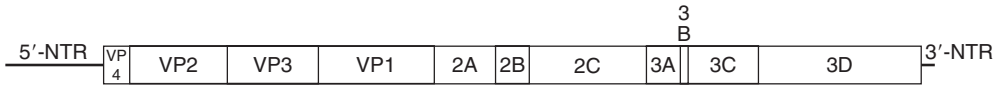


Figure 2 Coxsackievirus genome, indicating the locations of the mature viral proteins, 1A-1D (VP4, VP2, VP3, and VP1, respectively), 2A–2C, and 3A–3D, flanked by the 5′- and 3′-nontranslated regions (NTRs).

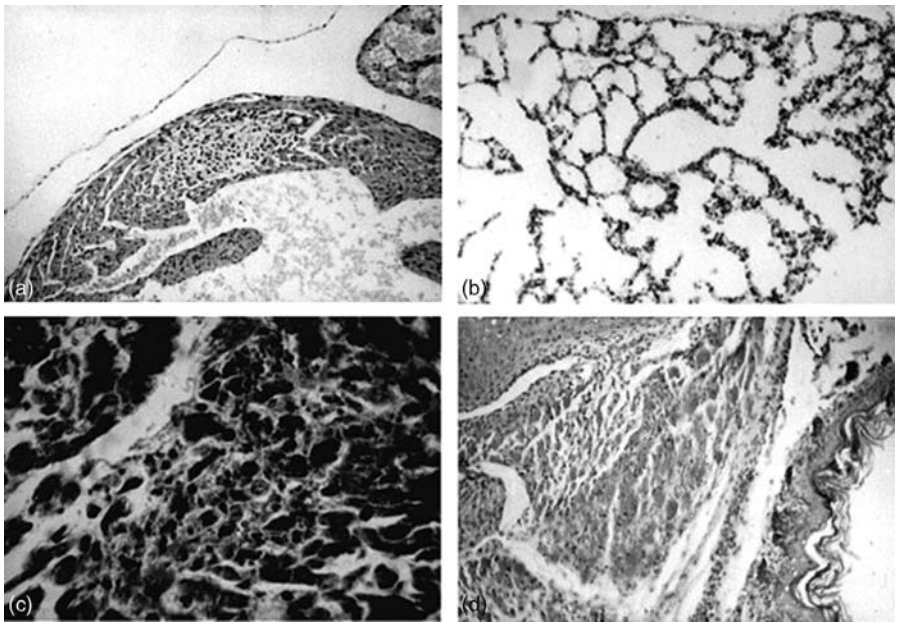


Figure 3 Tissue damage caused in baby mice by a freshly isolated strain of coxsackievirus B (a–c) or coxsackievirus A (d). (a) A large necrotic focus in the atrial myocardium (× 100); (b) steatitis in the interscapular brown fat pad (× 250); (c) a ventricular focus of necrotic cardiomyocytes (× 450); (d) intercostal muscles with diffuse hyalinization of myocytes and mild inflammatory infiltrate (×250). Reproduced from Pozzetto B and Gaudin OG (1999) Coxsackieviruses (*Picornaviridae*). In: Granoff A and Webster R (eds.) *Encyclopedia of Virology*, 2nd edn. San Diego: Academic Press, with permission from Elsevier.

that cross-reacts with several other serotypes. Young children develop a more homotypic response, whereas older children and adults tend to develop a more heterotypic response. This age difference in the specificity of the antibody response probably reflects exposure to a greater number of serotypes with increasing age. The heterotypic response may reflect the presence of epitopes that are shared among multiple serotypes, but the actual mechanism is unknown. The presence of antibody does not prevent infection or primary virus replication, but it is sufficient to protect from disease, probably by limiting spread of the virus to secondary sites such as the CNS. Infection also elicits a T-cell response which helps clear the virus, but the cell-mediated immune response is not required for protection from disease.

Clinical Manifestations

Coxsackievirus infections can result in a wide variety of disease syndromes (**Table 2**). Most infections are asymptomatic or result in only mild upper respiratory tract symptoms (common cold). Other mild enteroviral illness, such as fever, headache, malaise, and mild gastrointestinal symptoms, may also occur. Serious illness that brings the patient to the attention of a physician is much less frequent. Inapparent infections and prolonged excretion of virus, especially in stools, are common. These properties of enterovirus infection, and the fact that enterovirus infection is extremely common, make it difficult to establish a definitive link between infection and specific disease unless the virus can be isolated from a nonsterile site that is linked to the observed pathology (e.g., isolation from cerebrospinal fluid in the case of aseptic meningitis). Often, the association between infection and disease is based on studies of outbreaks in which a large number of persons with the same clinical signs and symptoms have evidence of infection with the same serotype. Such studies have clearly demonstrated that enterovirus infection can cause aseptic meningitis, pericarditis, pleurodynia, myocarditis, and encephalitis.

Table 2 Diseases commonly associated with coxsackievirus infection

Illness or syndrome	*Group A*	*Group B*
Acute hemorrhagic conjunctivitis	X[a]	
Aseptic meningitis	X	X
Encephalitis[b]	X	X
Exanthema	X	X
Hand, foot, and mouth disease	X[c]	
Hepatitis	X	X
Herpangina	X	
Infantile diarrhea	X	
Myocarditis/pericarditis		X
Paralysis[b]	X	X
Pleurodynia		X
Respiratory illness	X	X
Severe systemic infection in infants		X
Undifferentiated febrile illness	X	X

[a]CVA24 variant.
[b]Rare.
[c]Primarily CVA16 and EV71; also CVA6, CVA10.

Central Nervous System Disease

The most commonly recognized serious manifestation of enterovirus infection is CNS disease, usually aseptic meningitis, but sometimes, encephalitis or paralysis. Aseptic meningitis is the most common CNS infection in the United States, and enteroviruses are the main recognized cause of aseptic meningitis in both children and adults in developed countries. Other than poliovirus, echoviruses and group B coxsackieviruses are most commonly isolated from cases of enterovirus-associated CNS disease, but this may be largely an artifact of low-efficiency isolation of group A coxsackieviruses in culture.

Febrile Rash Illnesses

Herpangina is a common illness in school-age children, characterized by vesicular inflammation of the oral mucosa, including throat, tonsils, soft palate, and tongue. Herpangina has commonly been associated with CVA2–6, CVA8, and CVA10, as well as with some of the echoviruses. Hand, foot, and mouth disease (HFMD) is characterized by vesicular rash on the palms, soles, and oral mucosa, with frequent involvement of the limbs and trunk. CVA 16 and EV71 are the most common viruses isolated from HFMD cases, but CVA4, CVA6, and CVA10 are also frequent causes. Many of the coxsackieviruses (both groups A and B) may also be associated with undifferentiated rash, often in connection with other symptoms such as meningitis.

Prenatal, Perinatal, and Neonatal Infection

A number of enterovirus serotypes have been associated with severe illness in neonates, including generalized disseminated infection that can mimic some aspects of bacterial sepsis. CVB4 infection is associated with a higher risk for severe disease in infants less than 1 month old, but other CVB serotypes and other enteroviruses can also cause similarly severe disease in this age group. Neonatal disease can also include other typical enterovirus syndromes, such as meningitis, encephalitis, pneumonia, hepatitis, myocarditis, and pancreatitis. One systematic study in Nassau County, New York, estimated that one of every 2000 infants in that area was hospitalized during the first 3 months of life for CVB sepsis-like disease, with significant mortality. This probably underestimates the true rate, since virus isolation studies may be insensitive and other enteroviruses were not included in the estimate.

Although there are few studies that examine the relationship between enterovirus infection and adverse effects on the fetus, one study found serologic evidence of CNS infection with CVB in ventricular fluid from 4 of 28 newborns with congenital neural tube defects. The infants had neutralizing antibody to only one CVB serotype in the ventricular fluid, but to several in serum. The unique distribution of antibodies in the ventricular fluid compared to that in serum supports the purported association. The mothers had antibodies to the same serotype as well as some other CVB serotypes. No virus was isolated from infants or mothers. Two other studies have documented an association between enterovirus infection and miscarriages and stillbirths. Further studies are needed to assess the possibility of enterovirus infection of the fetus.

Acute Hemorrhagic Conjunctivitis

Coxsackievirus A24 variant and enterovirus 70 are associated with acute hemorrhagic conjunctivitis. This disease is different from other enteroviral illnesses, having occurred in global pandemics since its introduction around 1969. The incubation period for these agents is shorter than for other enteroviruses (24–72 h); systemic illness is much less common and conjunctival replication the rule. The disease is characterized by acute onset of lacrimation, severe pain, chemosis and periorbital edema, photophobia, conjunctival hyperemia, and mild-to-severe subconjunctival hemorrhages. The disease is usually bilateral. It is generally self-limiting, but may lead to secondary bacterial infection.

Other Acute Illnesses

Group B viruses have been associated with pleurodynia, also known as 'Bornholm disease' or 'epidemic myalgia'. Pleurodynia is characterized by sudden onset of chest pain, due to inflammation of the diaphragm, accompanied by general malaise, headache, fever, and sore throat. Enteroviruses, primarily the coxsackieviruses, are also

a common cause of respiratory illness. Most of these infections are relatively mild – even subclinical – and restricted to the upper respiratory tract (e.g., common cold, croup, and epiglottitis). Occasionally, the lower respiratory tract becomes infected, resulting in more serious illness, such as bronchiolitis or pneumonia, especially in very young children.

Cardiac Disease

Although the association between myocarditis and pericarditis and enterovirus infection is clearly established, it is not yet clear how often enterovirus infections are responsible for the disease syndromes. One study has shown that CVB IgM in a group of patients with acute myocarditis is significantly higher than in controls. Enterovirus RNA has also been detected in myocardial biopsy specimens from patients with myocarditis. These and other studies suggest, but do not clearly show, that CVB infection may be associated with a large fraction of cases of acute myocarditis. By contrast, different studies have failed to show conclusive evidence for the involvement of enterovirus infection in idiopathic dilated cardiomyopathies.

Diabetes

As with myocarditis, numerous studies have suggested a link between enterovirus infection and the onset of type I diabetes mellitus. In some cases, enteroviruses, such as CVB4, have been isolated from pancreas of fulminant diabetes cases, but most of the evidence is indirect, through serologic studies. Several mechanisms have been hypothesized to explain the possible link between infection and diabetes onset, including direct, lytic destruction of insulin-producing beta cells in the pancreas; molecular mimicry of self-antigens, resulting in induction of prediabetic autoimmunity that eventually leads to beta-cell destruction; and indirect damage mediated through activation of a nonspecific inflammatory response in the pancreas ('bystander effect'). A number of studies are attempting to address various steps in virus-induced diabetes pathogenesis, in human patients and in animal model systems.

Treatment and Prevention

Antiviral therapy is not presently available for enterovirus infections, so treatment is directed toward ameliorating symptoms. Drugs have been identified that exhibit antiviral activity against several enteroviruses in tissue culture and experimental animals, and some have been tested in human clinical trials; however, no enterovirus drugs are currently licensed in the United States or elsewhere. Interferon has been proposed for treatment of acute hemorrhagic conjunctivitis, but further studies are needed to evaluate its effectiveness. Chronic enterovirus infections in patients with agammaglobulinemia have been treated with γ-globulin, and in some cases this has controlled the infection.

No vaccines are available to prevent coxsackievirus infection or disease. General preventive measures include enteric precautions and good personal hygiene. Nosocomial infections are most serious in newborns and persons with compromised immune systems, but others may also be affected. Hospital staff can inadvertently carry the virus between patients or become infected themselves and spread the virus. The main strategy to prevent nosocomial infections is to manage patients with suspected enterovirus infection using enteric precautions. During outbreaks, patients and staff can be cohorted. In some cases, neonatal nurseries were closed to new admissions during newborn outbreaks to prevent further spread of the virus.

See also: Echoviruses; Enteroviruses: Human Enteroviruses Numbered 68 and Beyond; Enteroviruses of Animals; Evolution of Viruses; Human Eye Infections; Picornaviruses: Molecular Biology; Poliomyelitis; Quasispecies; Recombination; Rhinoviruses; Viral Receptors.

Further Reading

Bergelson JM, Cunningham JA, Droguett G, *et al.* (1997) Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science* 275(5304): 1320–1323.

He Y, Bowman VD, Mueller S, *et al.* (2000) Interaction of the poliovirus receptor with poliovirus. *Proceedings of the National Academy of Sciences, USA* 97(1): 79–84.

Huber S and Ramsingh AI (2004) Coxsackievirus-induced pancreatitis. *Viral Immunology* 17: 358–369.

Hyöty H and Taylor KW (2002) The role of viruses in human diabetes. *Diabetologia* 45: 1353–1361.

Hyypiä T and Stanway G (1993) Biology of coxsackie A viruses. *Advances in Virus Research* 42: 343–373.

Khetsuriani N, LaMonte A, Oberste MS, and Pallansch MA (2006) Enterovirus Surveillance – United States, 1970–2005. *Morbidity and Mortality Weekly Report Surveillance Summaries* 55(SS-8): 1–20.

Kim K-S, Hufnagel G, Chapman N, and Tracy S (2001) The group B coxsackieviruses and myocarditis. *Reviews in Medical Virology* 11: 355–368.

Muckelbauer JK, Kremer M, Minor I, *et al.* (1995) The structure of coxsackievirus B3 at 3.5 Å resolution. *Structure* 3: 653–667.

Pallansch MA and Oberste MS (2004) Coxsackievirus, echovirus, and other enteroviruses. In: Gorbach SL, Bartlett JG, and Blacklow NR (eds.) *Infectious Diseases,* 3rd edn, pp. 2047–2051. Philadelphia, PA: Lippincott Williams and Wilkins.

Pallansch MA and Roos R (2006) Enteroviruses: Polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology*, 5th edn Philadelphia, PA: Lippincott Williams and Wilkins.

Pozzetto B and Gaudin OG (1999) Coxsackieviruses (*Picornaviridae*). In: Granoff A and Webster R (eds.) *Encyclopedia of Virology,*, 857pp San Diego: Academic Press.

Rossmann MG, He Y, and Kuhn RJ (2002) Picornavirus–receptor interactions. *Trends in Microbiology* 10(7): 324–331.

Semler BL and Wimmer E (eds.) (2002) *Molecular Biology of Picornaviruses.* Washington, DC: ASM Press.
Stanway G, Brown F, Christian P, *et al.* (2005) Picornaviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 757–778. San Diego, CA: Elsevier Academic Press.
Tracy S, Oberste MS, and Drescher K (eds.) (2008) *Coxsackie B viruses. Current Topics in Microbiology and Immunology.* Berlin: Springer.

Crenarchaeal Viruses: Morphotypes and Genomes

D Prangishvili and T Basta, Institut Pasteur, Paris, France
R A Garrett, Copenhagen University, Copenhagen, Denmark

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Hyperthermophile An organism that grows optimally at temperature of 80 °C or higher.

Introduction

The susceptibility to viral infection is as common for the domain Archaea as it is for the other two domains of life, the Bacteria and the Eukarya. Initially, the knowledge about archaeal viruses was restricted to head–tail viruses infecting members of the kingdom Euryarchaeota. However, the isolation of viruses from the other archaeal kingdom, the Crenarchaeota, has demonstrated that head–tail phages constitute only a part of viral diversity associated with this domain.

The double-stranded (ds) DNA viruses of Crenarchaeota isolated by Wolfram Zillig and colleagues were so unusual in their morphotypes that for their classification four novel viral families had to be introduced: *Fuselloviridaae, Lipothrixviridae, Rudiviridae,* and *Guttaviridae.*

Further studies on viral diversity in geothermally heated hot habitats, where Crenarchaeota predominate demonstrated that unusual morphotypes are a general feature of crenarcheal viruses and resulted in isolation of more viruses with unique morphologies. They have been classified into three novel viral families the approval of which is pending at the ICTV: Globuloviridae, Ampullaviridae, and Bicaudaviridae.

In this article we summarize our present knowledge on viruses infecting hyperthermophilic crenarchaea (**Table 1**). Virion morphotypes and constituents are described and data regarding different stages of replication cycle presented. Sequences of dsDNA genomes of all viruses are discussed jointly, in accord with the idea that crenarchaeal viruses form a distinctive group, unrelated to any other viruses, with a small pool of shared genes and a unique origin, or more likely, multiple origins.

Morphotypes

The vast majority of the viruses replicated by Crenarchaeota have morphotypes that have not been observed among dsDNA viruses infecting Bacteria and Eukarya. In this section we describe different morphotypes of crenarchaeal viruses grouped according to their gross shape features.

Spindle-Shaped Viruses

Spindle-shaped virions, single or two-tailed, constitute a large fraction of the known archaeal viruses and are abundant in hot acidic springs where crenarchaea predominate.

Virions of the family *Fuselloviridae*, represented by *sulfolobus* spindle-shaped viruses (SSVs), are enveloped with a short tail attached to one of the two pointed ends and measure about (55–60) × (80–100) nm (**Figure 1**(**a**)). The tail carries fibers which serve for adsorption to the host membrane. The structure of the inner core of the enveloped virions which generates the unusual shape is unknown. The mature virions were observed intracellulary (**Figure 1**(**a**)) indicating that the budding process is not required for the coating by a lipid-containing membrane.

The virions of *Acidianus* two-tailed virus (ATV) are most unusual because they undergo extensive morphological changes outside the host cell (**Figures 1**(**c**)–**1**(**g**)). When released from the host cell, the particles are spindle-shaped with an overall length of 250–300 nm and a diameter of 110–120 nm (**Figure 1**(**c**)). Then, specifically at temperatures above 75 °C, a tail-like appendage protrudes from each of the pointed ends (**Figures 1**(**d**)–**1**(**g**)). The particles with fully developed tails are about 1 μm in length. This process is linked with a reduction of the volume of the virion by a factor of 2, concurrent with a slight expansion of its surface area.

The tails are tube-like and terminate with an anchor-like structure formed by two furled filaments (**Figure 1**(**g**)). This structure is employed for adsorption on the host cell surface. The tube contains a filament of an unknown nature,

Table 1 Crenarchaeal viruses

Morphotype	*Species*	*Virus family*	*Origin*	*Host*	*Lipids*	*Genome form and size (bp)*	*G+C content (%)*	*Genome integration*	*Accession no.*
Linear	AFV1	*Lipotrixviridae*	Y	*Acidianus*	+	linear, 20 869	37	−	AJ567472
	AFV2	*Lipotrixviridae*	P	*Acidianus*	−	linear, 31 787	36	−	AJ854042
	TTV1	*Lipotrixviridae*	I	*Thermoproteus tenax*	+	linear, 15 900	37	ND	X14855 (85%)[a]
	TTV2	*Lipotrixviridae*	I	*T. tenax*	ND	ND	ND	ND	ND
	TTV3	*Lipotrixviridae*	I	*T. tenax*	ND	ND	ND	ND	ND
	SIFV	*Lipotrixviridae*	I	*Sulfolobus*	+	linear, 40 852	33	−	AF440571 (97%)[a]
	SIRV1	*Rudiviridae*	I	*Sulfolobus*	−	linear, 32 308	25	−	AJ414696
	SIRV2	*Rudiviridae*	I	*Sulfolobus*	−	linear, 35 450	25	−	AJ344259
	ARV1	*Rudiviridae*	Y	*Acidianus*	−	linear, 24 655	39	−	AJ875026
Spindle-shaped	SSV1	*Fuselloviridae*	J	*Sulfolobus*	ND	ccc, 15 465	40	+	XO7234
	SSV2	*Fuselloviridae*	I	*Sulfolobus*	ND	ccc, 14 796	39	+	AY370762
	SS-K1	*Fuselloviridae*	R	*Sulfolobus*	ND	ccc, 17 385	39	+	AY423772
	SSVRH	*Fuselloviridae*	Y	*Sulfolobus*	ND	ccc, 16 473	39	+	AY388628
	ATV	"Bicaudaviridae"[b]	P	*Acidianus*	ND	ccc, 62 730	41	+	AJ888457
	STSV1	unclassified	C	*Sulfolobus*	+	ccc, 75 294	35	−	AJ783769
Spherical	PSV	"Globuloviridae"[b]	Y	*Pyrobaculum*	+	linear, 28 337	48	−	AJ635162
	TTSV1	"Globuloviridae"[b]	K	*Thermoproteus*	+	linear, 20 933	50	ND	AY722806
	STIV	*unclassified*	Y	*Sulfolobus*	ND	ccc, 17 663	36	ND	AY569307
Bottle-shaped	ABV	"Ampullaviridae"[b]	P	*Acidianus*	+	linear, 23 900[c]	48	ND	ND
Droplet-shaped	SDNV[d]	"Guttaviridae"	I	*Sulfolobus*	ND	circular, 20 000[c]	41	ND	ND

[a]Percentage of the determined sequence.
[b]Proposed families.
[c]Approximate value.
[d]The virus do not exist any more in laboratory collections.
J, Japan; I, Iceland; R, Kamchatka, Russia; Y, Yellowstone National Park, USA; P, Pozzuoli, Italy; K, Korea; C, China; ND, not determined.

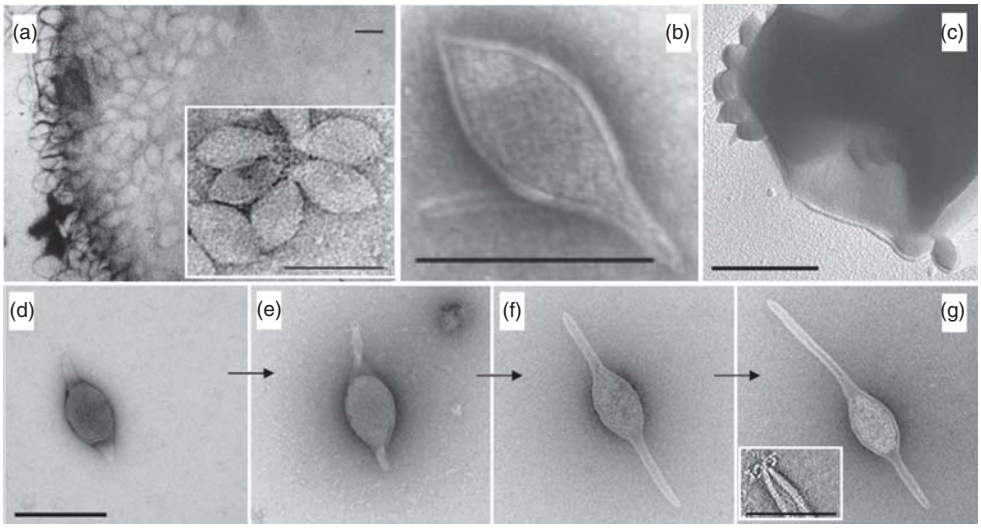


Figure 1 Electron micrographs of spindle-shaped viruses. (a) SSV1 and its extrusion from the host cell; (b) STSV1; (c–g) ATV at different stages of extracellular morphogenesis (in inserts, a horizontal slice through the 3D reconstruction of terminal structure by electron tomography); negative stain with 3% uranyl acetate. Scale: 100 nm (a); 200 nm (b, e–g); 500 nm (c).

which exhibits a structural periodicity. Protrusion of tails does not require the presence of host cells, an exogenous energy source or any cofactors. The only requirement is temperature in the range of host growth, above 75 °C.

The virus *Sulfolobus tengchongensis* spindle-shaped virus 1 (STSV1) is the largest of the known spindle-shaped viruses (107 × 230 nm) and has a single short tail (**Figure 1**(**b**)). The tail length varies in the range from 0 to 133 nm suggesting that the virus may also undergo an extracellular morphogenesis similar to ATV.

Rod-Shaped and Filamentous Viruses

The combination of rod or filamentous shape and dsDNA genome occurring among members of *Rudiviridae* and the *Lipothrixviridae* is unique in the viral world.

The three characterized rudiviruses *Sulfolobus* rod-shaped virus 1 (SIRV1), *Sulfolobus* rod-shaped virus 2 (SIRV2), and Acidianus rod-shaped virus 1 (ARV1) are nonenveloped stiff rods about 23 nm wide and 610–900 nm long (**Figure 2**(**a**)). The particle length is proportional to the size of the dsDNA genome (**Table 1**). The rods are formed by a tube-like superhelical nucleoprotein complex that consists of linear dsDNA and copies of a single DNA-binding protein. The tubes are closed at each end by plug-like structures about 50 nm in length. Each plug carries three tail fibers (**Figure 2**(**a**), inset) that are probably involved in adsorption to host cell membranes.

The lipothrixviruses include the highest number of known species among the crenarchaeal viral families. Virions are filamentous and mostly flexible, except for *Thermoproteus tenax* virus 1 (TTV1), and enveloped (**Figure 2**(**b**)).

The structures of the virion termini, as well as of the inner core are diverse, and owing to this diversity, as well as to presumed different replication strategies, four genera have been established in the family.

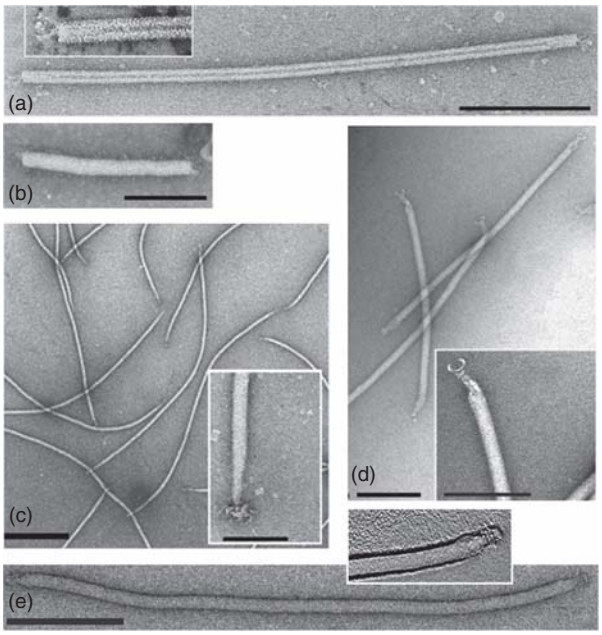


Figure 2 Electron micrographs of linear viruses. (a) SIRV1; (b) TTV1; (c) SIFV; (d) AFV1; (e) AFV2; terminal structures are shown in inserts. Negative stain with 3% uranyl acetate. Scale: 200 nm (a–e) (in insets, 100 nm).

The body of *Sulfolobus islandicus* filamentous virus (SIFV) particles tapers and ends in identical mop-like structures (**Figure 2**(**c**)), the *Acidianus* filamentous virus 1 (AFV1) has claw-like terminal structures (**Figure 2**(**d**)), and *Acidianus* filamentous virus 2 (AFV2) carries a complex collar with

two sets of filaments, resembling a bottle brush with a solid round cap at each end (**Figure 2**(**e**), inset). The virion termini of TTV1 have not been analyzed in detail.

The terminal structures of lipothrixviruses serve for the adsorption onto the host cell. Both termini of a given virus may be involved in this process. The mop-like terminal structures of SIFV have been shown to unfold like spiders legs prior to attachment to cell membranes. Most lipothrixviruses adsorb to host cell membrane with exception of AFV1 which uses the claw-like ends for attachment to host pili.

The architecture of virion cores differs between the lipothrixviruses although they all carry linear dsDNA genomes. The cores of AFV1 and TTV1 are helical, and the latter was shown to contain equimolar amounts of two DNA-binding proteins. The DNA of SIFV is wound around a zipper-like array of subunits of a single protein. Such a core arrangement is reminiscent of nucleosomes. In contrast, no regular structure was detected in the core of AFV2.

Spherical Viruses

The spherical viruses infecting hyperthermophilic Crenarchaeota encompass *Pyrobaculum* spherical virus, PSV, *Thermoproteus tenax* spherical virus 1, TTSV1, and *Sulfolobus* turreted icosahedral virus, STIV (**Figure 3**). This is the only morphotype of crenarchaeal viruses that is also observed in the domains Bacteria and Eukarya.

Virions of PSV are around 100 nm in diameter and they carry an envelope which encases tightly packed nucleoprotein in a superhelical conformation (**Figures 3**(**a**) and **3**(**b**)). Such core structure is unique for a DNA virus but resembles the core structure of single-stranded RNA viruses of the family *Paramyxoviridae*. The virion surface is covered by a variable number of spherical protrusions, about 15 nm in diameter, most likely involved in adsorption to the host cell.

The properties of the virion structure and genome of TTSV1 are very similar to those of PSV suggesting that these viruses belong to the same family.

The virions of the STIV are nonenveloped icosahedra with a diameter of about 74 nm (**Figure 3**(**c**)). The capsid most likely contains an internal lipid envelope. Single-particle reconstruction revealed a unique virus architecture including complex, turret-like appendages at the virion surface. The crystal structure of the major capsid protein of STIV is very similar to those of the bacterial tectivirus PRD1 and the eukaryal phycondnavirus PBCV-1, suggesting a common ancestry, despite the fact that the corresponding protein sequences show no significant similarity.

Bottle- and Droplet-Shaped Viruses

The extraordinary shapes of crenarchaeal viruses are exemplified by two viruses with most unusual virion structures, *Acidianus* bottle-shaped virus, ABV, and *Sulfolobus neozealandicus* droplet-shaped virus, SNDV (**Figure 4**).

The virions of ABV resemble in their shape a bottle with an overall length of 230 nm and a width varying from 75, at the broad end, to 4 nm, at the pointed end (**Figure 4**(**a**)). The broad end of the virion exhibits 20 (± 2) thin rigid filaments, which appear to be inserted into a disk, or ring, and are interconnected at their bases. The 9-nm-thick envelope encases cone-shaped core that is formed by a torroidally supercoiled nucleoprotein filament (**Figures 4**(**b**) and **4**(**c**)). The narrow end of virions is likely involved in cellular adsorption and in channeling of viral DNA into a host cell. The function of filaments at the opposite end remains unclear.

The SNDV virions are droplet-shaped, 110–185 nm long and 70–95 nm wide. The pointed end is densely covered by thin fibers (**Figure 4**(**d**)). The core is protected by a beehive-like structure, the surface of which appears to be built up of components stacked in a helical manner.

Virion Constituents

The protein content of particles of crenarchaeal viruses is often complex. The number of the major protein components present in the virions varies from two proteins for rudiviruses, fuselloviruses, and lipothrixviruses up to 11 proteins for the ATV. Moreover, in the majority

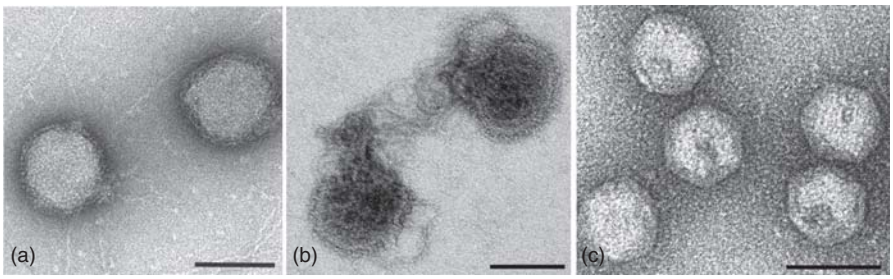


Figure 3 Electron micrographs of spherical viruses. (a) PSV; (b) partially disrupted virions of PSV releasing helical nucleoprotein filament; (c) STIV. Negative stain with 3% uranyl acetate. Scale = 100 nm.

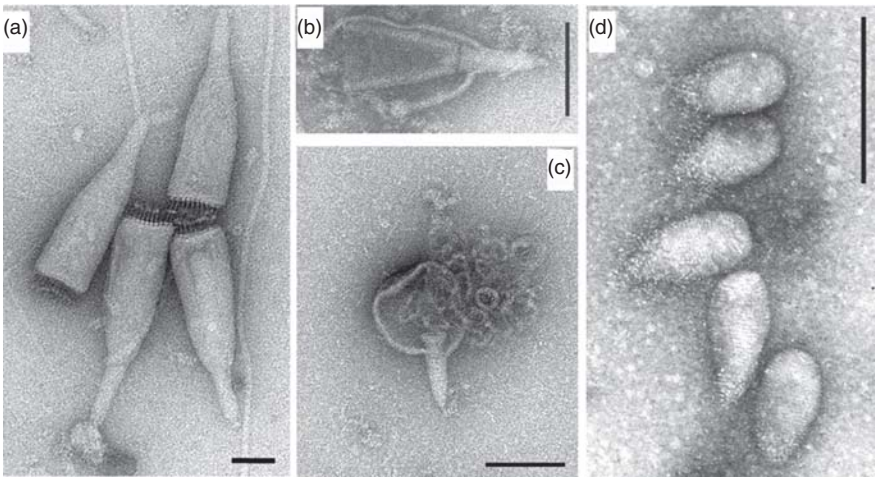


Figure 4 Electron micrographs of bottle-shaped and droplet-shaped viruses. (a) ABV; (b and c) partially disrupted ABV virions, revealing an inner cone-shaped core and its constituent nucleoprotein filament; (d) SNDV. Negative stain with 3% uranyl acetate. Scale: 50 nm (a); 100 nm (b, c); 200 nm (d).

of viruses a few minor protein components are also present. Several structural proteins have been identified (**Figure 5**).

Virion proteins have an important role in protecting the viral DNA from the harsh conditions of the host's habitat. For example, the single DNA-binding protein of nonenveloped rudiviruses can partially protect the virion structure during autoclaving at 120 °C for 10 min. The particles are completely disrupted only after a further 50 min.

Some of the structural proteins can form structures of higher order. This is exemplified by the 90 kDa protein from ATV that is rich in coiled-coil motifs and can generate long filaments. They may contribute to the protrusion of tail-like appendages during the extracellular development of ATV. The virions of PSV exhibit three major protein components the largest of which can build dimers and higher aggregates. This protein also contains two-thirds of hydrophobic amino acid residues, about 15% of which are aromatic, and no cysteine residues, properties which are likely to contribute to high thermostability.

Almost all crenarchaeal viruses are enveloped. The only exception are the members of the *Rudiviridae*. The envelopes of the PSV, and of all lipothrixviruses, except AFV2, have been shown to contain host lipids that have been modified probably by virus-encoded enzymes. The STIV particles have an internal host lipids-containing envelope. The presence of lipids in virions of other crenarchaeal viruses has not yet been reported.

Virus–Host Interactions

Little is known about the modes of cellular entry and the assembly mechanisms of the crenarchaeal viruses. Presumably, after attachment to the host cell surface either the whole virion is taken up or the viral DNA is injected through the host membrane. The latter mechanism could be employed by the virions of ABV that were shown to release a nucleoprotein complex through the pointed end (**Figure 4**(**c**)). Another mechanism is possible for the AFV1 which attaches with its claw-like terminal structures onto the host pili. The virions could be pulled into the cell by retraction of the pili.

Hosts of cultured crenarchaeal viruses are extreme acidophiles (*Sulfolobus*, *Acidianus*), or neutrophiles and obligate anaerobes (*Pyrobaculum*, *Thermoproteus*). Infectivity of the latter organisms with viruses is unaffected by exposure to oxygen. For all of the hosts which grow optimally at 80 °C or above, viral infection occurs most effectively at the optimal growth temperature of the host such that viruses can also be considered as hyperthermophiles.

Viruses of the Crenarchaeota are rather exceptional with respect to virus–host interactions because they generally do not kill their host during release of progeny virions. Instead, the viruses persist in host cells and are not segregated even after prolonged growth and several dilutions of infected cell cultures. This is the result of an equilibrium that is established between the viral genome replication and cell division. It is usually argued that such inoffensiveness of crenarchaeal viruses is beneficial, helping them to avoid direct, and possibly prolonged, exposure to the harsh environmental conditions of the host habitat.

The only two examples of crenarchaeal viruses that cause lysis of their hosts are TTV1 and ATV.

The ATV exhibits the characteristics of a true temperate virus. At the optimal temperature of host growth, 85 °C, infection with ATV results in integration of the viral genome into the host chromosome and lysogenization of the host cell. The lysogeny can be interrupted by

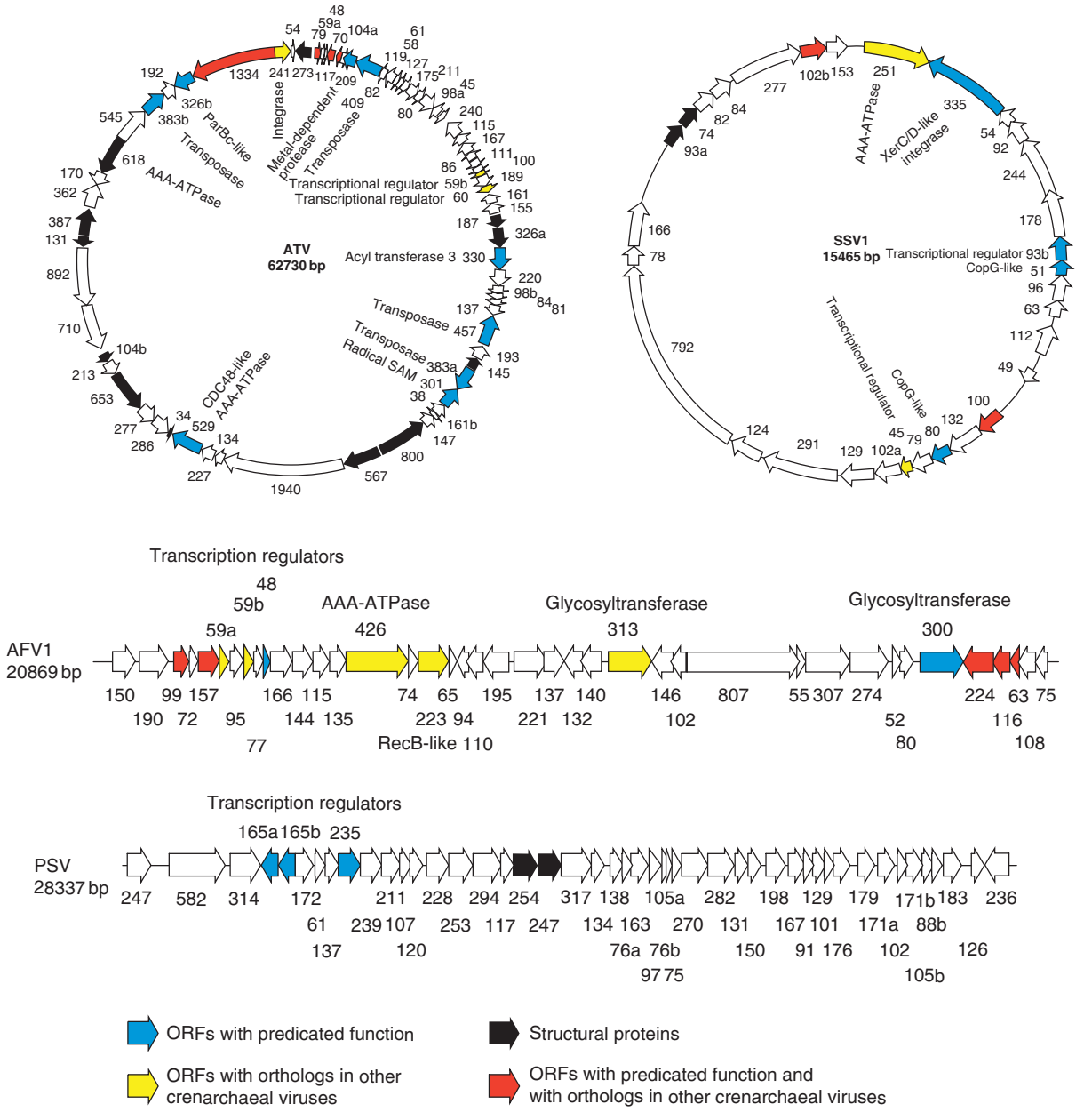


Figure 5 Schematic representation of the genome organizations found in four different crenarchaeal viruses. Arrows represent predicted viral ORFs and their size and orientation. Numbers assigned to the ORFs correspond to number of amino acids in the encoded proteins. The genomes are not in scale to each other. ORFs have been assigned functions according to sequence similarities with genes in public databases.

subjecting the host cell to stress conditions, for example, UV-irradiation, treatment with mytomycin C, freezing–thawing cycles, or host growth at suboptimal temperatures. Induction of virus replication eventually leads to cell lysis.

The integration of the ATV genome into the host chromosome is facilitated by a virus-encoded integrase. The genes encoding these enzymes have been detected also on circular genomes of the fuselloviruses and STSV1. The former insert generally within specific host tRNA genes and the integrated form of the latter has not been detected. The fuselloviruses are present in the infected host cell cultures in episomal and integrated form but it is not known whether the two forms exist in the same cell. The replication of the SSV1 can be induced by ultraviolet irradiation leading to temporary growth inhibition,

but not lysis, of host cells. Subsequently, virus proliferation decreases and the host cells assume their earlier growth rate.

The linear genomes of crenarchaeal viruses do not encode integrases and they have not been detected in chromosomes of their hosts. Moreover, their replication is not affected by UV-irradiation or other stress factors.

Genome Structure

The crenarchaeal viruses investigated so far all carry dsDNA genomes. The genomes of the fuselloviruses ATV and STSV1 are circular, 15–75 kbp in size (**Table 1**). Known rudiviruses, lipothrixviruses, and PSV contain linear genomes in the size range 25–45 kbp.

For the fuselloviruses and some lipothrixviruses, about half of each genome, including genes for viral structural proteins, is always highly conserved in gene content and order with the remainder of the genome being more variable.

Modifications have been detected in two of the viral genomes. The SNDV carries a *dam*-like N(6) methylation of adenine residues in GATC sequences while the DNA of the STSV1, which encodes three putative modifying enzymes, undergoes methylation of specific cytosine residues within the sequence CCGG. The latter modifications are probably absent from the host chromosmal DNA.

Most of the linear genomes carry inverted terminal repeats (ITRs) that can vary significantly in their size between viruses. The rudiviral genomes exhibit large ITRs (600–2000 bp), the PSV carries a 190 bp ITR, and the lipothrixvirus AFV1 has a very short 11 bp ITR.

Genomic termini of some crenarchaeal viruses are modified. In the rudiviruses SIRV1 and SIRV2 the two DNA strands are covalently linked at the ends, generating a 4 bp hairpin loop which can be degraded by *Bal*31 endonuclease. A similar structure may occur at the termini of the PSV genome.

Restriction fragments containing the terminal sequences of the TTV1 and AFV1 produce low molar yields on phenol extraction suggesting the presence of tightly bound proteins at the ends of genomic DNA.

Replication of Viral Genomes

The genome replication of crenarchaeal viruses has not been studied experimentally. Consequently, the knowledge about the replication mechanisms employed and participating enzymes is very scarce. Nevertheless, some clues about possible replication strategies of crenarchaeal viruses could be obtained from the sequences of the genomes and the genome arrangements.

Linear genomes of some lipothrixviruses exhibit an internal, repeat-rich, non-protein-coding region. The AFV2 carries a 1008 bp element bordered by an ITR that is rich in repeat sequences. Such regions could constitute internal origins of replication.

Even more remarkable are the genomic features of the STSV1. Its circular genome is highly asymmetric and divides into equal halves with respect to gene orientation. Between the two halves of the genome was located the 1.4 kbp long intergenic region with an unusually high AT content, including two sets of tandem repeats and two sets of inverted repeats. This region constitutes the putative origin of replication. Most likely, genome replication proceeds bidirectionally in the theta-mode from the proposed origin.

The genomic termini of lipotrixvirus AFV1 do not resemble any other virus. The two very short (11 bp) ITRs are preceded by about a 300 bp region consisting of many direct repeats of the pentanucleotide TTGTT, or close variants thereof. Such structures resemble telomeric ends of eukaryotic chromosomes and raise the possibility of a primitive telomeric mechanism operating in AFV1 genome replication.

Rudivirus SIRV1 replicates its genome via head-to-head and tail-to-tail linked intermediates. This suggested a self-priming mechanism of replication, similar to that proposed for large eukaryotic dsDNA viruses including poxviruses. Consistent with this proposal are the similarities in the structures of linear genomes of these archaeal and eukaryal viruses, including long ITRs and covalently closed termini. Moreover, each of these viruses encodes a Holliday junction resolvase which is likely to resolve the replicative intermediates into single-progeny genomes. The large ITRs of the rudiviruses carry highly conserved sequence located 100–150 bp from the genomic termini that could serve as signal for the initiation of replication.

Transcription of Viral Genes

The studies on transcription of the fusellovirus SSV1 resulted in identification of archaeal promoter sequences and were crucial for our seminal understanding of mechanisms of transcriptional regulation in archaea. SSV1 transcripts were mapped after UV induction of virus production in the lysogenes. Eight constitutive and one UV-inducible transcript were identified.

The first detailed analysis of transcription over the complete replication cycle was performed on rudiviruses SIRV1 and SIRV2. Already 30 min post infection all the ORFs, with one exception, are transcribed. Nevertheless, some ORFs have different transcription patterns during the replication cycle. One example is the ORF coding for coat protein that is initially transcribed as polycistronic

mRNA but its monocistronic transcript was detected in the later stage of virus infection. The rather simple and ordered transcriptional pattern of the rudiviruses fits with fairly unsophisticated virus–host relationship (stable carrier state) which probably does not require much transcriptional control.

SIRV promoters, like those of their hosts, carry a TATA-like box and adjacent to it a purine-rich region, corresponding to the transcription factor B binding site. In addition, virus-specific consensus element, the trinucleotide GTC is located immediately downstream from the TATA-like box. The GTC motif is also present in one-third of the promoter regions of another rudivirus, the ARV1.

Many viral transcripts for single genes and the first genes of SIRV operons are leaderless, and lack the consensus Shine–Dalgarno motif GGTG.

Genomics of Crenarchaeal Viruses

The most prominent feature of genomes of known crenarchaeal viruses is the extremely low number of gene products with homologs in the public sequence databases (**Figure 5**).

Genes with Confirmed Functions

Putative functions of only few genes (with homologs in public databases) have been confirmed experimentally. They encode the dUTPase and the Holliday junction resolvase of the rudiviruses SIRV1 and SIRV2 and the integrase/recombinase of the fusellovirus SSV1. In addition genes for structural proteins were identified.

Genes with Putative Functions Unique for Individual Viruses

A few genes of crenarchaeal viruses have homologs in archaea and/or bacteria. These include DNA-modification methylases of the STSV1, superfamily 2 helicases of the lipothrixvirus SIFV, ABC-class ATPase of the rudivirus ARV1, and thioldisulfide isomerase of the globulovirus TTSV1.

Genes with Putative Functions Shared by Different Viruses

Many crenarchaeal viruses share a repertoire of genes with putative functions including transcription regulators, ATPases, enzymes of DNA precursor metabolism, and RNA modification, and glycosylases.

The most common gene products in crenarchaeal viruses are small proteins containing ribbon–helix–helix (RHH) domain. These proteins are transcription regulators, common in archaea, and are nearly as abundant as classical helix–turn–helix (HTH) domains. The RHH proteins of crenarchaeal viruses are rather heterogeneous, and apparently have a complex history, probably involving multiple independent acquisitions as well as horizontal gene transfers.

Other putative transcription regulators encompass HTH-domains, looped-hinge helix domains, and Zn-fingers. Remarkable is the presence of C2H2 Zn-finger proteins with moderate similarity to a variety of eukaryotic Zn-fingers but no obvious homologs in prokaryotes.

Each of the crenarchaeal viruses encodes at least one of the P-loop ATPases, which are known to be the most abundant protein domain in prokaryotes and the great majority of viruses, and typically are involved in viral replication, transcription, or packaging. Other predicted enzymes with probable functions in DNA replication are the RecB-family nucleases in the *Lipothrixviridae*, *Rudiviridae*, and *Fuselloviridae*, the XerC/D-like integrase/recombinase in the Bicaudaviridae, and the STSV1. As for the Holliday junction resolvases of the *Rudiviridae*, these enzymes may be involved in intermediate resolution during viral genome replication.

The *Rudiviridae* and STSV1 encode a dUTPase and/or the flavin-dependent thymidylate synthase (ThyX) which apparently are involved in DNA precursor metabolism, a function widely represented in DNA viruses of Bacteria and Eukarya. Another function common for bacterial viruses, RNA modification, is also represented in the members of *Rudiviridae*: they encode predicted tRNA-ribosyltransferase and S-adenosylmethionine-dependent methyltransferase. The former enzyme is encoded also by STSV1 and the latter enzyme by the lipothrixvirus SIFV.

All known members of the *Rudiviridae* and *Lipothrixviridae* encode glycosyltransferases that may be involved in modification of virions proteins and/or the host cell wall during viral entry and/or release. In members of other viral families in this process maybe implicated such enzymes as a membrane-associated acyltransferase (in ATV) and nucleoside-diphosphate-sugar epimerase (in STSV1).

The gene pool shared by crenarchaeal viruses includes also two protein families with unknown functions. One of these, exemplified by the AFV1 protein 03 of 99 amino acids, is found exclusively in the *Rudiviridae* and *Lipothrixviridae*. Another family of small proteins has a single bacterial representative, the uncharacterized protein YddF of *Bacillus subtilis*, with all other members found in crenarchaeal viruses: in the *Rudiviridae, Lipothrixviridae, Fuselloviridae*, the ATV, and STIV.

Orthologous Genes

Results of identification of orthologous genes among the genes shared by crenarchaeal viruses are in astounding

accord with evolutionary relationships postulated on the basis of viral morphotypes. (Because of the uncertainty regarding the very existence of a unique common ancestor virus, orthologs were defined only conditionally, as genes that are more closely related to each other in a given set of viruses than they are to any homologs that may exist outside that set of genomes.)

A significant number of orthologs (18–44) are observed among viral species assigned to the same genus, which probably derived from relatively recent common ancestors. These are four members of the *Fuselloviridae*, three members of the *Rudiviridae*, and two members of the Globuloviridae.

A smaller number of orthologous genes (5–9), representing moderately related groups which probably have evolved from a single ancestral virus in a more distant past, were observed for members of different genera in the family *Lipothrixviridae*: the betalipothrixvirus SIFV, gammalipothrixvirus AFV1, and deltalipothrixvirus AFV2. A comparable number of orthologous genes were observed also between some members of different families, the *Rudiviridae* and *Lipothrixviridae*, suggesting a common ancestry for these two families.

In those cases when the viruses share only 1–4 orthologous genes, it remains unclear, whether a common ancestral virus ever existed. The existence of a common viral ancestor is even less likely, when no orthologs are detected among viruses, as it occurs for the two members of the Globuloviridae and the rest of the crenarchaeal viruses.

Conclusions and Future Prospects

Known dsDNA viruses infecting hyperthermophilic crenarchaea represent a remarkable collection of unique morphotypes, not encountered among dsDNA viruses of the Bacteria and Eukarya. It is now clear that crenarcheal viruses are not just an oddity but represent a general picture of viral diversity in hot environments. Therefore, the observed variety of viral morphotypes in hot environments is probably only a tip of an iceberg and the isolation of new viruses should remain an important part of future studies.

Analysis of the sequences of crenarchaeal viral genomes demonstrated that they form a distinct group, evolutionary unrelated to dsDNA viruses of the other two domains of life. Moreover, the majority of predicted genes of crenarchaeal viruses have no homologs in public sequence databases and only a few of the viral genes could be assigned functions. Consequently, this hinders our understanding of the fundamental processes that take place during the replication cycle of the crenarchaeal viruses. It is now time to initiate studies of the mechanisms of these processes, notably attachment, virion assembly and release, transcription, and genome replication. The instrumental discoveries made during studies of bacteriophages in the past argue that the studies of crenarchaeal viruses have potential to provide the archaeal research community with robust genetic tools.

The discovery of the unique group of crenarchaeal viruses provides a new perspective on events related to the origin of diverse groups of DNA viruses and their evolution. It is a challenge for future studies to reveal evolutionary aspects of involvement of sets of nonorthologous genes in establishment of similar lifestyles in the three unrelated groups of dsDNA viruses.

See also: Viruses Infecting Euryarchaea.

Further Reading

Häring M, Rachel R, Peng X, Garrett RA, and Prangishvili D (2005) Viral diversity in hot springs of Pozzuoli, Italy, and characterization of a unique archaeal virus, *Acidianus* bottle-shaped virus, from a new family, the *Ampullaviridae*. *Journal of Virology* 79(15): 9904–9911.

Häring M, Vesterdaard G, Rachel R, Chen L, Garrett R, and Prangishvili D (2005) Virology: Independent virus development outside a host. *Nature* 436(7054): 1101–1102.

Kessler A, Brinkman AB, van der Oost J, and Prangishvili D (2004) Transcription of the rod-shaped viruses SIRV1 and SIRV2 of the hyperthermophilic archaeon *Sulfolobus*. *Journal of Bacteriology* 186(22): 7745–7753.

Nadal M, Mirambeau G, Forterre P, Reiter WD, and Duget M (1986) Positively supercoiled DNA in a virus-like particle of an archaebacterium. *Nature* 321: 256–258.

Prangishvili D (2003) Evolutionary insights from studies on viruses of hyperthermophilic archaea. *Research in Microbiology* 154(4): 289–294.

Prangishvili D and Garrett RA (2005) Viruses of hyperthermophilic crenarchaea. *Trends in Microbiology* 13: 535–542.

Prangishvili D, Garrett RA, and Koonin EV (2006) Evolutionary genomics of archaeal viruses: Unique viral genomes in the third domain of life. *Virus Research* 117: 52–67.

Prangishvili D, Stedman K, and Zillig W (2001) Viruses of the extremely thermophilic archaeon *Sulfolobus*. *Trends in Microbiology* 9(1): 39–43.

Rachel R, Bettstetter M, Hedlund BP, *et al.* (2002) Remarkable morphological diversity of viruses and virus-like particles in hot terrestrial environments. *Archives of Virology* 147(12): 2419–2429.

Rice G, Stedman K, Snyder J, *et al.* (2001) Viruses from extreme thermal environments. *Proceedings of the National Academy of Sciences, USA* 98: 13341–13345.

Rice G, Tang L, Stedman K, *et al.* (2004) The structure of a thermophilic archaeal virus shows a double-stranded DNA viral capsid type that spans all domains of life. *Proceedings of the National Academy of Sciences, USA* 101: 7716–7720.

Wiedenheft B, Stedman K, Roberto F, *et al.* (2004) Comparative genomic analysis of hyperthermophilic archaeal *Fuselloviridae* viruses. *Journal of Virology* 78: 1954–1961.

Xiang X, Chen L, Huang X, Luo Y, She Q, and Huang L (2005) *Sulfolobus tengchongensis* spindle-shaped virus STSV1: Virus–host interactions and genomic features. *Journal of Virology* 79: 8677–8686.

Zillig W, Prangishvili D, Schleper C, *et al.* (1996) Viruses, plasmids and other generic elements of thermophilic and hyperthermophilic Archaea. *FEMS Microbiology Reviews* 18: 225–236.

Crimean–Congo Hemorrhagic Fever Virus and Other Nairoviruses

C A Whitehouse, United States Army Medical Research Institute of Infectious Diseases, Frederick, MD, USA

Published by Elsevier Ltd.

Glossary

Argasid ticks Any member of the family *Argasidae*, which are soft-shelled; commonly called soft ticks.
Ecchymosis The escape of blood into the tissues from ruptured blood vessels.
Ixodid ticks Any member of the family *Ixodidae*, which have a hard outer covering called scutum; commonly referred to as hard ticks.
Petechiae Minute reddish or purplish spots containing blood that appear in the skin or mucous membrane, especially in some infectious diseases.

Introduction

The genus *Nairovirus* within the family *Bunyaviridae* includes 34 predominantly tick-borne viruses. These viruses are enveloped, with a tripartite negative-sense, single-stranded RNA genome. Among the members of this genus, the most important pathogens are the Crimean–Congo hemorrhagic fever virus (CCHFV), which causes severe and often fatal hemorrhagic fever in humans, Crimean–Congo hemorrhagic fever (CCHF), and Nairobi sheep disease virus (NSDV), which circulates in Africa and Asia and causes acute hemorrhagic gastroenteritis in sheep and goats.

CCHF was first recognized during a large outbreak among soldiers and agricultural workers in the mid-1940s in the Crimean peninsula but the etiologic agent was not isolated at that time. In 1956 a virus named Congo virus was isolated from a febrile patient in the Belgian Congo (now the Democratic Republic of the Congo) and it was later recognized that Congo virus was the same as Crimean hemorrhagic fever virus, isolated in 1967 from a patient with this disease in (now) Uzbekistan. The disease now occurs sporadically throughout much of Africa, Asia, and Europe and results in an approximately 30% case–fatality rate. CCHF is characterized by a sudden onset of high fever, chills, severe headache, dizziness, and back and abdominal pains. Additional symptoms can include nausea, vomiting, diarrhea, neuropsychiatric disorders, and cardiovascular changes. In severe cases, hemorrhagic manifestations, ranging from petechiae to large areas of ecchymosis, develop. Ixodid ticks of numerous genera serve both as vector and reservoir for CCHFV. Ticks in the genus *Hyalomma* are particularly important in the ecology of this virus and exposure to these ticks represents a major risk factor for contracting disease. Other important risk factors include direct contact with blood and/or body fluids from infected patients or animals. The highly pathogenic nature of CCHFV has restricted research on the virus to biosafety level 4 (BSL-4) laboratories and has led to the fear that it might be used as a bioweapon.

NSDV, which is an important cause of veterinary disease, was originally isolated from sheep from Nairobi, Kenya in 1910. Dugbe virus was originally isolated from adult male *Amblyomma variegatum* ticks collected from cattle in Ibadan, Nigeria in 1964. Both Dugbe and Ganjam viruses (a variant of NSDV) have been isolated repeatedly from ticks removed from domestic animals and have both caused febrile illnesses in humans. Most other nairoviruses have been isolated from ixodid ticks or from argasid ticks, which are ectoparasites of seabirds and other birds, and their medical or veterinary significance is not known.

History

Crimean–Congo Hemorrhagic Fever Virus

A disease now considered to be CCHF was described by a physician in the twelfth century from the region that is presently Tadzhikistan. The description was of a hemorrhagic disease with the presence of blood in the urine, rectum, gums, vomitus, sputum, and abdominal cavity. The disease was said to be caused by a louse or tick, which normally parasitizes a blackbird. The arthropod described may well have been larvae of a species of *Hyalomma* ticks, which are frequently found on blackbirds. CCHF has also been recognized for centuries under at least three names by the indigenous people of southern Uzbekistan: *khungribta* (blood taking), *khunymuny* (nose bleeding), or *karakhalak* (black death). Various other names, including acute infectious capillarotoxicosis and Uzbekistan hemorrhagic fever, have been used for centuries in Central Asia to refer to CCHF.

In modern times, CCHF (then known as Crimean hemorrhagic fever (CHF)) was first described as a clinical entity in 1944–45 when about 200 Soviet military personnel and civilian farmers were infected during an epidemic in war-torn Crimea. Shortly thereafter, a viral etiology was suggested by reproducing a febrile syndrome in psychiatric patients who were inoculated with a filterable agent from the blood of CHF patients. Further evidence of a viral etiology and of a suspected tick-borne route of infection was demonstrated by inducing a mild form of

disease in healthy human volunteers after their inoculation with filtered suspensions of nymphal *H. marginatum* ticks. In 1967, the Russian virologist M. P. Chumakov and his colleagues at the Institute of Poliomyelitis and Viral Encephalitides in Moscow were the first to use newborn white mice for CHF virus isolation.

In 1956, Dr. Ghislaine Courtois at the Provincial Medical Laboratory in what was then Stanleyville, Belgian Congo, isolated a virus, referred to as Congo virus, from the blood of a 13-year-old boy who had fever, headache, nausea, vomiting, and generalized joint pains. Interestingly, a second isolation of the virus was made from the blood of Dr. Courtois, who subsequently became ill with symptoms similar to those of the boy. Subsequent work showed that Crimean hemorrhagic fever virus was antigenically indistinguishable from Congo virus and were in fact, the same virus, leading to the new name, Crimean–Congo hemorrhagic fever virus.

Other Important Members of the Genus *Nairovirus*

NSDV, an important cause of livestock disease, was originally isolated from sheep in Nairobi, Kenya in 1910. Dugbe virus was originally isolated from *A. variegatum* ticks from Ibadan, Nigeria in 1964. Ganjam virus (**Figure 1**), which is now considered to be a strain of NSDV, was originally isolated from *Haemaphysalis intermedia* ticks collected from goats in the Ganjam district of India in 1954. Most other nairoviruses were first isolated in the 1960s or 1970s from ticks parasitizing seabirds and other birds (**Table 1**).

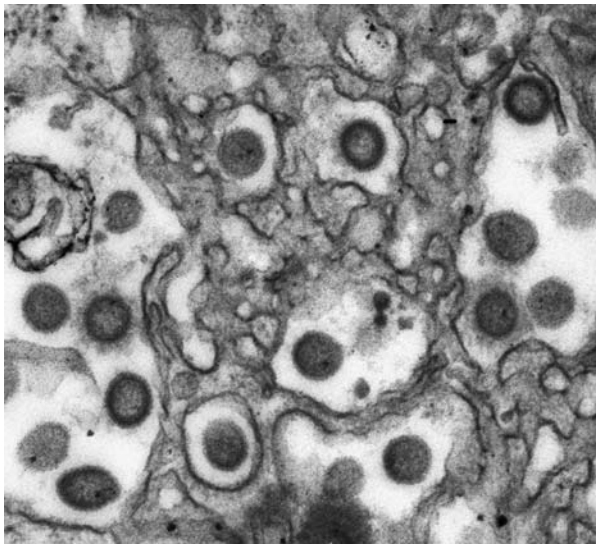


Figure 1 Electron micrograph of Ganjam virus. Photo courtesy of Frederick A. Murphy, Centers for Disease Control and Prevention, Atlanta, Georgia.

Taxonomy and Classification

According to the Eighth Report of the International Committee on the Taxonomy of Viruses, the species *Crimean–Congo hemorrhagic fever virus* is classified in the genus *Nairovirus*. There are seven recognized species in the genus, containing a total of 34 viruses, most of which are transmitted by either ixodid or argasid ticks (i.e., hard or soft ticks, respectively) (**Table 1**).

Geographical Distribution

The known distribution of CCHFV covers the greatest geographic range of any tick-borne virus and there are reports of viral isolation and/or disease from more than 30 countries in Africa, Asia, southeast Europe, and the Middle East. Evidence for its presence in France, Portugal, Egypt, and India is based only on limited serologic observations. Interestingly, after several decades of only serologic evidence of the existence of CCHFV in Turkey, a large outbreak of disease in that country began in 2002, is ongoing, and has resulted in more than 900 cases. This highlights the ability of CCHF to emerge in geographic regions that have previously been devoid of the disease.

Ecology and Epidemiology

As do other tick-borne zoonotic agents, CCHFV generally circulates in nature unnoticed in an enzootic tick-vertebrate-tick cycle. CCHFV has been isolated from numerous domestic and wild vertebrates, including cattle and goats, sheep, hares, hedgehogs, and even domestic dogs. Sera from wild mammals of several species have been shown to have antibodies to CCHFV and seroepidemiological studies have also detected antibodies to CCHFV in domestic cattle, horses, donkeys, sheep, goats, and pigs from various parts of Europe, Asia, and Africa. Interestingly, there has been only one report of antibody to CCHFV detected from a reptile, a tortoise from Tadzhikistan.

Although many domestic and wild vertebrates are infected with CCHFV, as evidenced by development of viremia and/or antibody response, birds, in general, appear to be refractory to infection with CCHFV. One interesting exception is ostriches, which become infected with CCHFV and have been the source of several cases of CCHF associated with slaughtering ostriches in South Africa.

The natural cycle of CCHFV includes transovarial (i.e., passed through the eggs) and trans-stadial (i.e., passed directly from immature ticks to subsequent life stages) transmission among ticks in a tick–vertebrate–tick cycle. CCHFV has been isolated from ticks in at least 31 species. Viral isolations have been made from ticks of two species

Table 1 List of currently accepted species and other viruses within the genus *Nairovirus*[a]

Virus	*Original source*	*Year of isolation*	*Abbreviation*
Crimean–Congo hemorrhagic fever virus			
Crimean–Congo hemorrhagic fever virus	Human	1967	CCHFV
Hazara virus	*Ixodes* sp.	1964	HAZV
Khasan virus	*Haemaphysalis* sp.	1971	KHAV
Dera Ghazi Khan virus			
Dera Ghazi Khan	*Hyalomma* sp.	1966	DGKV
Abu Hammad virus	*Argas hermanni*	1971	AHV
Abu Mina virus	*Argas streptopelia*	1963	AMV
Kao Shuan virus	*Argas* sp.	1970	KSV
Pathum Thani virus	*Argas* sp.	1970	PTHV
Pretoria virus	*Argas* sp.	1970	PREV
Dugbe virus			
Dugbe virus	*Hyalomma* sp.	1966	DUGV
Nairobi sheep disease virus	Sheep	1910	NSDV
Ganjam virus[b]	*Haemaphysalis* sp.	1954	GANV
Hughes virus			
Hughes virus	*Ornithodoros* sp.	1962	HUGV
Farallon virus	*Carios capensis*	1964	FARV
Fraser Point virus	ND[c]	ND	FPV
Great Saltee virus	*Ornithodoros* sp.	1976	GRSV
Puffin Island virus	*Ornithodoros maritimus*	1979	PIV
Punta Salinas virus	*Ornithodoros* sp.	1967	PSV
Raza virus	*Carios denmarki*	1962	RAZAV
Sapphire II virus	*Argas cooley*	ND	SAPV
Soldado virus	*Ornithodoros* sp.	1963	SOLV
Zirqa virus	*Ornithodoros* sp.	1969	ZIRV
Qalyub virus			
Qalyub virus	*Ornithodoros erraticus*	1952	QYBV
Bakel virus	ND	ND	BAKV
Bandia virus	*Mastomys* sp.	1965	BDAV
Omo virus	*Mastomys* sp.	1971	OMOV
Sakhalin virus			
Sakhalin virus	*Ixodes* sp.	1969	SAKV
Avalon virus	*Ixodes* sp.	1972	AVAV
Clo Mor virus	*Ixodes* sp.	1973	CMV
Kachemak Bay virus	*Ixodes signatus*	1974	KBV
Taggert virus	*Ixodes* sp.	1972	TAGV
Tillamook virus	*Ixodes uriae*	1970	TILLV
Thiafora virus			
Thiafora virus	*Crocidura* sp. (shew)	1971	TFAV
Erve virus	*Crocidura russula* (shew)	1982	ERVEV

[a]Species names are in italics. Under each virus species name is listed other viruses of undetermined taxonomic status.
[b]Considered to be an Asian variant of NSDV.
[c]ND, not determined.

in the family *Argasidae* and from ticks of seven genera of the family *Ixodidae*. However, the virus appears to be most efficiently transmitted by ticks of the genus *Hyalomma* (**Figure 2**) and, in general, the known occurrence of CCHFV in Europe, Asia, and Africa coincides with the geographic distribution of these ticks. Although *Hyalomma* spp. ticks are considered the most important in the epidemiology of CCHF, the virus has been isolated from ticks in other genera (i.e., *Rhipicephalus*, *Boophilus*, *Dermacentor*, and *Ixodes* spp.) as well, which may contribute to its wide geographical distribution.

The principal vector of NSDV is the ixodid tick *Rhipicephalus appendiculatus*, which occurs throughout East and Central Africa. Most other nairoviruses have been isolated from ticks parasitizing birds and their ecology has not been well studied.

Properties of the Virion and Genome

The morphology and structure of the CCHFV virion was first described in the early 1970s from the brains of infected newborn mice. It is now known that CCHFV and nairoviruses in general, are typical of other members of the family *Bunyaviridae* in terms of their basic structure, morphogenesis, replication cycle, and physicochemical



Figure 2 Dorsal view of a *Hyalomma marginatum marginatum* female tick. Photo courtesy of Dr. Zati Vatansever, Ankara University Faculty of Veterinary Medicine, Ankara, Turkey.

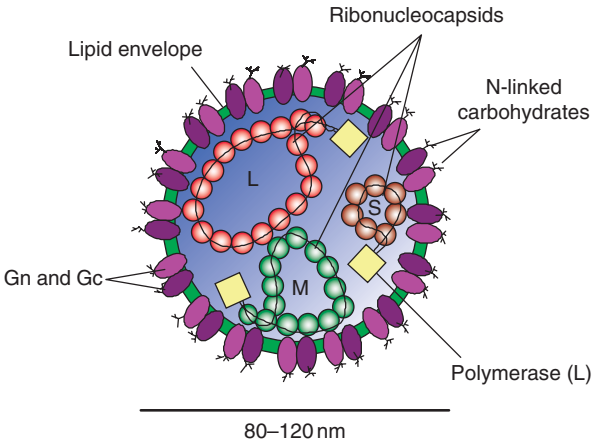


Figure 3 Schematic cross-section of a *Bunyaviridae* virion. The three RNA genome segments (S, M, and L) are complexed with nucleocapsid protein to form the ribonucleocapsids. The nucleocapsids and RNA-dependent RNA polymerase are packaged within a lipid envelope that contains the viral glycoproteins, Gn and Gc. Adapted from Schmaljohn CS and Hooper JW (2001) Bunyaviridae: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology*, 4th edn., pp. 1447–1472. Philadelphia: Lippincott Williams and Wilkins, with permission from Lippincott Williams and Wilkins.

properties. Virions are spherical, approximately 100 nm in diameter, and have a host cell-derived lipid bilayered envelope approximately 5–7 nm thick, through which protrude glycoprotein spikes 8–10 nm in length (**Figure 3**). Virions of members of the family *Bunyaviridae* contain three structural proteins: two envelope glycoproteins (Gn and Gc (previously termed G2 and G1)), named in accordance with their relative proximity to the amino or carboxy terminus of the M segment-encoded polyprotein, respectively, and a nucleocapsid protein (N), plus a large polypeptide (L), which is the viron-associated RNA-dependent RNA polymerase (**Figure 3**).

The genome is typical of those of other members of the family and is composed of three negative-strand RNA segments, S, M, and L, encoding the N nucleocapsid, Gn and Gc glycoproteins, and the L polymerase, respectively. The RNA segments are complexed with N to form individual S, M, and L nucleocapsids, which appear to be circular or loosely helical. The M segment of nairoviruses is 30–50% larger than the M segments of members of other genera in the *Bunyaviridae* family and has a potential coding capacity of up to 240 kDa of protein. At least one of each of the S, M, and L ribonucleocapsids must be contained in a virion for infectivity; however, equal numbers of nucleocapsids may not always be packaged in mature virions. Recent data show that the N protein is targeted to the perinuclear region of infected cells in the absence of native RNA segments and that this targeting is actin filament dependent. The first 8–13 nucleotide bases at the 3′-termini of all three RNA segments have a sequence (3′-AGAGUUUCU-) that is conserved within viruses of the genus, with a complementary consensus sequence at the 5′-termini. Base-pairing of the terminal nucleotides is predicted to form stable panhandle structures and noncovalently closed circular RNAs, which have been directly observed by electron microscopy of RNA extracted from the bunyavirus *Uukuniemi Virus*.

The principal stages of the replication process for viruses in the *Bunyaviridae* are similar to those of many other enveloped viruses. The viral glycoproteins are believed to be responsible for recognition of receptor sites on susceptible cells. Viruses that attach to receptors on susceptible cells are internalized by endocytosis, and replication occurs in the cytoplasm. Virions mature by budding through endoplasmic reticulum into cytoplasmic vesicles in the Golgi region, which are presumed to fuse with the plasma membrane to release virus.

Recently, a reverse genetics system was developed for CCHFV. The development of such a system was a major step forward in efforts to understand the biology of the virus. Developing an infectious clone for CCHFV will allow for more extensive studies of its biology and pathogenesis, and may ultimately lead to better therapeutic and prophylactic measures against CCHFV infections.

Phylogenetic Relationships

Many early studies, based on serological testing, suggested that there are very few significant differences among strains of CCHFV. Recent data based on nucleic acid sequence analyses have revealed extensive genetic diversity. For example, analysis of the S RNA segment reveals

the existence of seven distinct virus groups (i.e., genotypes) (**Figure 4**). These genotypes show a strong correlation with the geographical area of viral isolation. Furthermore, studies have discovered similar genotypes in distant geographical locations. Movement of CCHFV-infected livestock or uninfected livestock carrying infected ticks may explain some of the movement of viral genetic lineages within diverse regions. Other explanations include the movement of virus via migratory animals or birds that are either infected or are carrying virus-infected ticks. It is interesting that the Greek strain AP92 differed greatly from other European strains, and therefore is in a group by itself (**Figure 4**). AP92 strain was originally isolated in Greece from a *Rhipicephalus bursa* tick and has not yet been associated with disease in humans.

Sequence information on the L RNA segment has lagged behind those of the S and M RNA segments; nevertheless, there is evidence that the S and L RNA segments have the same evolutionary history. Thus, essentially identical S and L tree topologies are seen when analyzing all the available segment sequences. This, however, is not the case for the M RNA segment. This is generally taken for evidence of RNA segment reassortment. RNA viruses with segmented genomes have the capacity to reassort their genomic segments into new, genetically distinct viruses if the two or more viruses co-infect the same target cell.

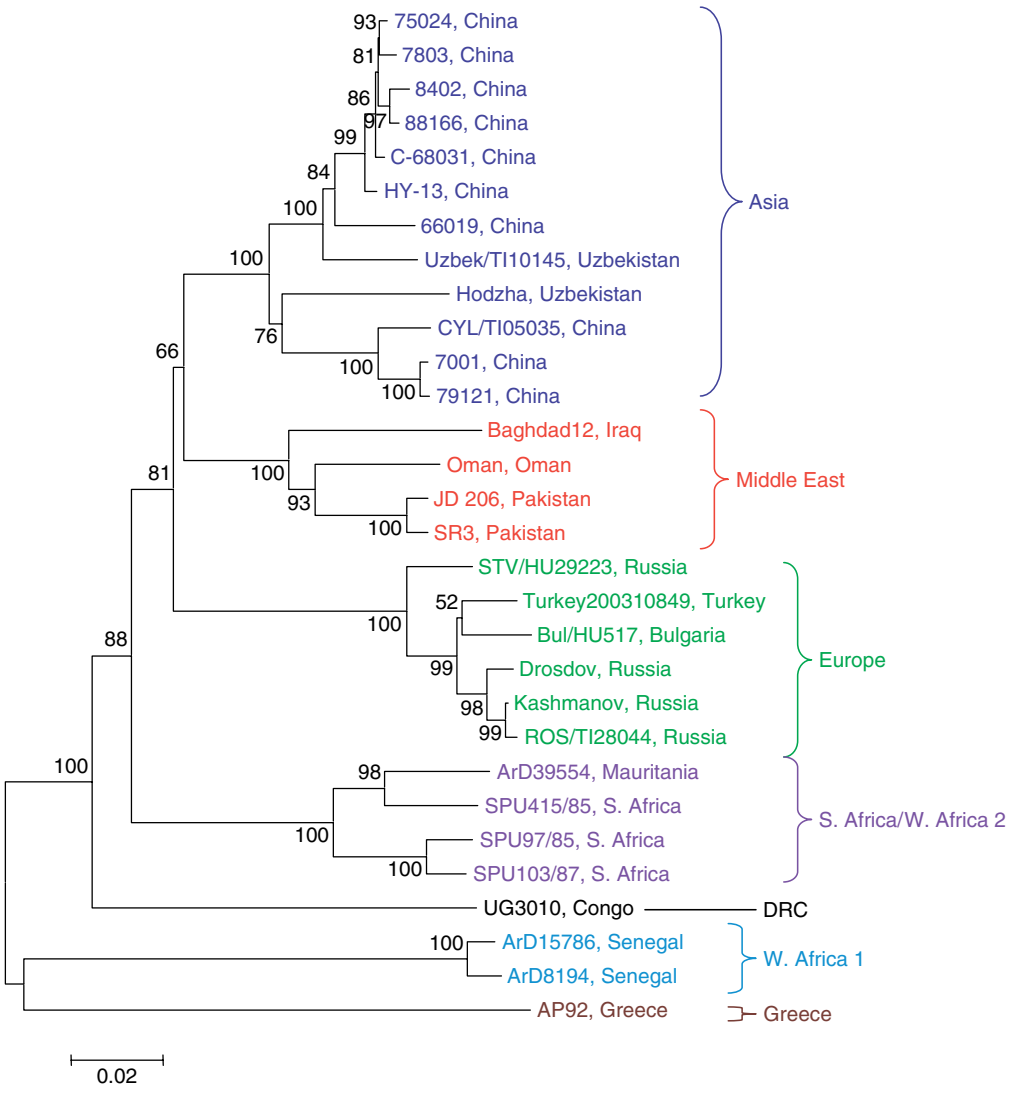


Figure 4 Phylogenetic relationships of the S segment RNA of CCHFV. Complete sequences of S segment RNA were aligned and analyzed by a neighbor-joining method with Kimura two-parameter distances by using MEGA software (version 3.1). The lengths of the horizontal branches are proportional to the number of nucleotide differences between taxa. Bootstrap values above 50%, obtained from 500 replicates of the analysis, are shown at the appropriate branch points. CCHFV strains are described as strain designation, country of origin. Scale = 2% divergence.



Figure 5 Ecchymosis seen on the arm of a CCHF patient. Photo courtesy of Dr. Miro Petrovec, University of Ljubljana, Slovenia.

Indeed, several examples of RNA segment reassortment have been documented among viruses of the family *Bunyaviridae*, including CCHFV. Furthermore, reassortment appears to be much more frequently observed among CCHFV M RNA segments than among S and L RNA segments.

Clinical Features

Except for newborn mice, humans appear to be the only host of CCHFV in which disease is manifested. In contrast to the inapparent infection in most other vertebrate hosts, human infection with CCHFV often results in severe hemorrhagic disease. The typical course of CCHF is noted by some authors as progressing through four distinct phases, that is, 'incubation', 'prehemorrhagic', 'hemorrhagic', and 'convalescence'; it is noteworthy that the duration and associated symptoms of these phases can vary greatly. In general, the incubation period after a tick bite can be as brief as 1–3 days, but can be much longer depending on several factors including route of exposure. After the incubation period, the prehemorrhagic period is characterized by sudden onset of fever, chills, severe headache, dizziness, photophobia, and back and abdominal pains. Additional symptoms such as nausea, vomiting, diarrhea, and an accompanying loss of appetite are common. Fever is often very high (39–41 °C) and can remain continually elevated for 5–12 days or may be biphasic. It is interesting that neuropsychiatric changes have been reported in some CCHF patients. These have included sharp changes in mood, with feelings of confusion and aggression and even some bouts of violent behavior. Cardiovascular changes can also be seen and include bradycardia and low blood pressure. In severe cases, 3–6 days after the onset of disease, hemorrhagic manifestations develop. These can range from petechiae to large areas of ecchymosis and often appear on the mucous membranes and skin, especially on the upper body and/or extremities (**Figure 5**). Bleeding in the form of melena, hematemesis, and epistaxis is also commonly seen by day 4 or 5 and can often be characterized by dark 'coffee grounds' vomitus and tarlike stools resulting from intestinal hemorrhages. Bleeding from other sites including the vagina, gingival bleeding, and, in the most severe cases, cerebral hemorrhage have been reported. About 50% of the patients develop hepatomegaly. Not surprisingly, poor prognosis is associated with cerebral hemorrhage and massive liver necrosis. Mortality rates for the various CCHF epidemics and outbreaks have varied greatly. The average mortality rate is often cited at 30–50%; however, rates as high as 72.7% and 80% have been reported from the United Arab Emirates and China, respectively. During the recent outbreak in Turkey, of the 500 cases reported to the Turkish Ministry of Health, 26 (5.2%) were fatal. Mortality rates of nosocomial infections are often much higher than those acquired naturally through tick bite. The exact reasons for this phenomenon are not known, but may simply relate to viral dose.

Pathogenesis

Because of the difficulties of working with CCHFV (e.g., the need for specialized containment laboratories) and the lack of an animal model of disease, the pathogenesis of CCHF is poorly understood. Capillary fragility is a common feature of CCHF, suggesting infection of the endothelium. This is surely where the alternative term 'capillary toxicosis', given to CCHF by the early Soviet workers, was derived. Localization of CCHFV in tissues by immunohistochemistry has shown that mononuclear phagocytes and endothelial cells are major sites of viral infection. Endothelial damage would account for the characteristic rash and contribute to hemostatic failure

by stimulating platelet aggregation and degranulation, with consequent activation of the intrinsic coagulation cascade. Thrombocytopenia appears to be a consistent feature of CCHF infection and platelet counts can often be extremely low beginning at the early stage of illness in fatal cases.

Some have argued that the characteristic endothelial damage seen in CCHF is not necessarily the result of direct infection of the endothelial cells by CCHFV. Evidence is mounting that for viral hemorrhagic fever, caused by the Ebola virus, much of the cellular damage and resulting coagulopathy results from multiple host-induced mechanisms. These include massive apoptosis of lymphocytes both intravascularly and in lymphoid organs; induction of pro-inflammatory cytokines, including tumor necrosis factor (TNF)-α; and the dysregulation of the coagulation cascade leading to disseminated intravascular coagulation (DIC). Indeed, many of these same features are seen in CCHF, including DIC, vascular dysfunction, and shock. Clearly, more work needs to be done in this area to completely understand the pathogenesis of CCHF.

Diagnosis

A diagnosis of CCHF should be considered when severe flu-like symptoms with a sudden onset are seen in patients with a history of tick bite, travel to an endemic area, and/or exposure to blood or other tissues of livestock or human patients. Early diagnosis is essential, both for the outcome of the patient and because of the potential for nosocomial infections. The differential diagnosis should include rickettsiosis (tick-borne typhus or African tick bite fever caused by *Rickettsia conorii* and *R. africae*, respectively), leptospirosis, and borreliosis (relapsing fever). Additionally, other infections, which present as hemorrhagic disease such as meningococcal infections, hantaviral hemorrhagic fever, malaria, yellow fever, dengue, Omsk hemorrhagic fever, and Kyasanur Forest disease should be considered. In Africa, Lassa fever and infection with the filoviruses, Ebola and Marburg, must also be included in the differential diagnosis.

The diagnosis of CCHF is confirmed by detecting viral nucleic acid by reverse transcription-polymerase chain reaction (RT-PCR), demonstration of viral antigen by enzyme-linked immunosorbent assay (ELISA), or isolation of the virus. Any attempts at isolating and culturing the virus should be performed only under BSL-4 containment. The traditional method and 'gold standard' for CCHFV isolation has been by intracranial or intraperitoneal inoculation of a sample into newborn mice. However, isolation in cell culture is far simpler and safer and provides a more rapid result. Virus can be isolated from blood and organ suspensions in a wide variety of susceptible cell lines including LLC-MK2, Vero, BHK-21, and SW-13 cells with maximal viral yields after 4–7 days of incubation. In some cases however, depending on the cell line and strain, the virus may induce little or no cytopathic effect (CPE). In these cases, the presence of virus can be identified by performing an immunofluorescence assay (IFA) with specific antibodies to CCHFV or by RT-PCR.

The neutralizing antibody response to CCHFV, as well as to other nairoviruses, is weak and difficult to demonstrate. Both IgG and IgM antibodies are detectable by IFA by about day 5 of illness and are present in the sera of survivors by day 9. The IgM antibody declines to undetectable levels by the fourth month after infection, and IgG titers may also begin to decline gradually at this time, but remain demonstrable for at least 5 years. An antibody response is rarely detectable in fatal cases and diagnosis is usually confirmed by isolating the virus from the serum or from liver biopsy specimens or by demonstrating the presence of CCHFV antigen by immunohistochemical techniques of paraffin-embedded liver sections or by IFA of liver impression smears.

Molecular-based diagnostic assays, such as RT-PCR, provide a useful complement to serodiagnosis and now often serve as the front-line tool both in the diagnosis of CCHF and in epidemiological studies of the disease.

Prevention and Control

Several groups of individuals are considered to be at risk of contracting CCHF – specifically, people from endemic areas who are liable to be fed upon by ticks, particularly *Hyalomma* spp. ticks. These include individuals who work outdoors, particularly those who work with large domestic animals. Exposures such as crushing infected ticks and butchering infected animals have also been a frequent source of CCHFV infection. Other groups who are at risk include those caring for CCHF patients. In fact, the risk of nosocomial infection in healthcare workers is well documented and can be extremely high, especially during the hemorrhagic period of disease.

Avoiding or minimizing exposure to the virus is the best means of preventing CCHF. Persons in high-risk occupations (i.e., slaughterhouse workers, veterinarians, sheep herders, etc.) should take every precaution to avoid exposure to virus-infected ticks or virus-contaminated animal blood or other tissues. For example, wearing gloves and limiting exposure of naked skin to fresh blood and other tissues of animals are effective practical control measures. Likewise, medical personnel who care for suspected CCHF patients should practice standard barrier-nursing techniques. Tick control may not be practical in many regions of the world where *Hyalomma*

ticks are most prevalent. However, acaricide treatment of livestock in CCHFV-endemic areas is effective in reducing the population of infected ticks. Applying commercially available insect repellents (i.e., meta-*N,N*-diethyl toluamide (DEET)) to exposed skin and using clothes impregnated with permethrin can provide some protection against tick bites. As for other tick-borne viruses, inspecting one's body and clothes for ticks, and their prompt removal can minimize the risk of infection. An inactivated vaccine, prepared from the brains of infected suckling mice, was used in Eastern Europe and the former Soviet Union in the past, but no vaccines are currently available.

Treatment

Treatment options for CCHF are limited and primarily consist of supportive and replacement therapies. Standard treatment consists of monitoring patients with replacement of red blood cells, platelets, and other coagulation factors. Immunotherapy has been attempted by passive transfer of CCHF survivor convalescent plasma, but the efficacy of this treatment is not clear. There is currently no specific antiviral therapy for CCHF approved for use in humans by the US Food and Drug Administration. An antiviral drug, ribavirin, is effective against CCHFV in culture and has shown the most promise in treating CCHF patients over the years. However, for the best patient outcome, treatment should be started early, ideally, before day 5 of illness.

Disclaimer

The views, opinions, and findings contained herein are those of the author and should not be construed as an official Department of the Army position, policy, or decision unless so designated by other documentation.

See also: Orthobunyaviruses; Rift Valley Fever and Other Phleboviruses.

Further Reading

Flick R and Whitehouse CA (2005) Crimean–Congo hemorrhagic fever virus. *Current Molecular Medicine* 5: 753–760.

Hoogstraal H (1979) The epidemiology of tick-borne Crimean–Congo hemorrhagic fever in Asia, Europe, and Africa. *Journal of Medical Entomology* 15: 307–417.

Linthicum KJ and Bailey CL (1994) Ecology of Crimean–Congo hemorrhagic fever. In: Sonenshine DE and Mather TN (eds.) *Ecological Dynamics of Tick-Borne Zoonoses*, pp. 392–437. New York: Oxford University Press.

Montgomery E (1917) On a tick-borne gastro-enteritis of sheep and goats occurring in British East Africa. *Journal of Comparative Pathology and Therapy* 30: 28–57.

Schmaljohn CS and Hooper JW (2001) *Bunyaviridae:* The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology*, 4th edn., pp. 1447–1472. Philadelphia: Lippincott Williams and Wilkins.

Swanepoel R (1995) Nairovirus infections. In: Porterfield JS (ed.) *Exotic Viral Infections*, pp. 285–293. London: Chapman and Hall.

Watts DM, Ksiazek TG, Linthicum KJ, and Hoogstraal H (1989) Crimean–Congo hemorrhagic fever. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. II, pp. 177–222. Boca Raton: CRC Press.

Whitehouse CA (2004) Crimean–Congo hemorrhagic fever. *Antiviral Research* 64: 145–160.

Cryo-Electron Microscopy

W Chiu and J T Chang, Baylor College of Medicine, Houston, TX, USA
F J Rixon, MRC Virology Unit, Glasgow, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

X-ray crystallography has yielded atomic models of icosahedral viruses of up to 700 Å diameter (e.g., bluetongue virus, reovirus, and bacteriophage PRD1). However, crystallography has not been successful with larger viruses, is not yet able to yield high-resolution details of nonicosahedral components in intact virus particles, and is of limited use in examining partially purified or low-concentration specimens or samples with mixtures of different conformational states. Many of these limitations can be overcome by using cryoelectron microscopy (cryoEM) and image reconstruction approaches. Furthermore, cryoEM has been useful not only to virologists for understanding virus assembly and virus–antibody and virus–receptor interactions, but also to electron microscopists for driving developments in electron imaging technology.

In the early 1970s, DeRosier, Crowther, and Klug introduced the method for icosahedral particle reconstruction, which marked the beginning of three-dimensional (3-D) electron microscopy for spherical viruses. Initially, these studies used stained and dried specimens. However, in the early 1980s, Dubochet and co-workers developed

vitrification methods that use rapid freezing of solutions to preserve the intact structures of biological macromolecules for electron imaging. They demonstrated the feasibility of reconstructing the 3-D structure of a virus particle in its native conformation without using stain or fixative.

Both the quality of the electron images and the resolving power of the single-particle reconstruction algorithms have been continuously improved in the last 20 years to the extent that it is now possible to routinely obtain virus structures at subnanometer resolutions (6–10 Å). Although not yet equal to X-ray crystallographic resolution, this does allow identification of secondary structure elements such as long α-helices and large β-sheets in protein subunits of virus particles.

All the subnanometer resolution cryoEM structures of viruses solved so far, have made use of the icosahedral properties of the particle. However, the assembly and infection processes in many viruses depend on structures which break the icosahedral symmetry of the particle. Details of the organization of these nonicosahedral components are absent from almost all crystal structures of virions due to the icosahedral averaging used in the data processing. Similarly, cryoEM reconstructions from single-particle images that are dependent on icosahedral averaging do not show the nonicosahedral components.

Recently, image reconstruction techniques have been introduced to compute a density map without imposing icosahedral symmetry. This approach has been successfully used to visualize all the structural components of bacteriophages T7, Epsilon15, and P22 at moderate resolution (17–20 Å).

Another imaging method called electron cryotomography (cryoET) has been used to reconstruct large enveloped viruses such as human immunodeficiency virus (HIV), vaccinia virus, and herpes simplex virus (HSV) at 100–60 Å resolution. Subsequent single-particle averaging of certain computationally extracted components has yielded their 3-D structures to 50–30 Å resolution. This approach is ideal for obtaining low-resolution information from virus particles containing both conformationally constant and variable components.

CryoEM of Single Virus Particles

CryoEM of single particles consists of four basic steps: cryo-specimen preparation, imaging in an electron cryomicrosope, image reconstruction, and structural interpretation. Because there are excellent reviews that describe the detailed procedures involved, in this article the focus is on the fundamental concepts of this methodology, including some practical considerations where appropriate.

Cryo-Specimen Preparation

Specimens in the electron microscope have to be examined in a high vacuum (10^{-6} torr) environment, which would dehydrate and thus destroy the native structure of any normal biological specimen. Fortunately, the rapid freezing methods developed by Dubochet and co-workers can preserve specimens embedded in a thin layer of vitreous ice. Using this procedure, the virus particles are maintained in their original solution state. Vitrification is the process by which water solidifies without undergoing crystallization and it requires extremely rapid cooling. This can be achieved by applying the sample to a microscope grid that has been overlaid with a thin film of holey carbon, blotting away excess sample, and plunging the very thin aqueous film into liquid ethane that has been cooled to liquid nitrogen temperature. An ideal situation is for the ice to be slightly thicker than the virus particles. In practice, there is always variation in ice thickness even across a single microscope grid. For any new specimen, a process of systematic trial and error will eventually identify freezing conditions that yield optimal ice thickness in at least some parts of a grid.

An important practical consideration in cryo-specimen preparation is the concentration of the sample, which ideally should be in the order of 10^{10} particles ml^{-1}. A subnanometer-resolution icosahedral reconstruction generally needs images of several thousands of particles in different orientations. Therefore, a high particle concentration will reduce the number of micrographs that have to be collected, which will, in turn, reduce the number of data sets to be processed. An added benefit is that having a large number of particles per micrograph facilitates the determination of the defocus value of each micrograph, which is necessary for subsequent data processing. However, such concentrations can be difficult to obtain with some virus samples, in which case the necessary data collection and processing can become very time consuming.

Low-Dose Single-Particle Imaging

Following sample preparation, the grids containing the ice-embedded virus particles can be stored in liquid nitrogen until placed into the electron microscope for imaging. Throughout all stages of the process, the frozen-hydrated specimens must be handled extremely carefully to avoid any ice contamination from moisture in the atmosphere. The specimen is kept at or below liquid nitrogen temperature during the electron microscopy session. Frozen-hydrated biological specimens are extremely sensitive to radiation and must be scrutinized using a very low electron dose. They are examined initially at low magnification (100–1000×) to find a suitable area on the grid and

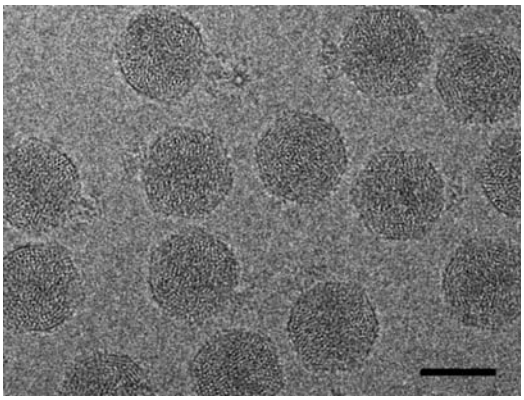


Figure 1 CryoEM imaging. Electron image of frozen-hydrated Epsilon15 phage taken at 300 kV displays the capsids as dark circles. The viral DNA inside is observed as a fingerprint pattern. Scale = 500 Å. Courtesy of Joanita Jakana, Baylor College of Medicine.

then images are recorded at a higher magnification (40 000–80 000×) from the chosen area (**Figure 1**).

The total electron dose that can be tolerated by a specimen depends only slightly on the specimen temperature and the accelerating electron voltage. For most practical purposes, a typical dose is about 15–25 electrons $Å^{-2}$ for single-particle imaging aimed at a resolution of better than 20 Å. A major variable in recording any electron micrograph is the defocus setting for the objective lens, which will vary according to the targeted resolution of the experiment and the type of microscope used. Higher defocus increases image contrast while smaller defocus yields higher-resolution information. Typically, the data for reconstruction are collected with defocus values between 1 and 3 μm in order to produce visible image contrast in the raw micrograph while preserving the higher-resolution features retrievable through image processing.

There are two ways to record an electron micrograph: on photographic film or with a charge-coupled device (CCD) camera. The advances in CCD camera technology now enable most medium-resolution data (down to 6–8 Å) to be recorded directly in digital format ready for subsequent steps of image processing. Given the rapid growth in CCD technology, it is likely that photographic film will eventually be replaced completely.

Icosahedral Reconstruction

Many viruses are composed of spherical protein shells arranged icosahedrally. **Figure 1** shows a representative cryoEM image of this type of particle. It is important that the particles are oriented randomly in the matrix of embedding ice because the task of image reconstruction involves combining particles from different

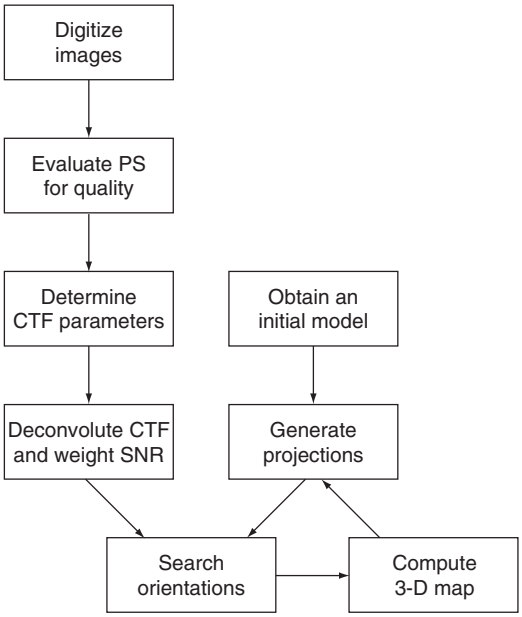


Figure 2 A 3-D image reconstruction scheme for icosahedral particles. The quality of digitized images is evaluated by analyzing their power spectrum (PS). Next, the contrast transfer function (CTF) parameters are determined for each image and used to deconvolute and weigh them according to their signal-to-noise ratio (SNR). The orientations of the deconvoluted images are determined by comparing them against projections from a 3-D map generated from a starting model. The oriented images are used to reconstruct a better 3-D map, which is subsequently used to generate better projections. The new projections are used to improve the accuracy of the search for the image orientations. The 3-D map improves after each iteration until it converges and the processing can stop.

orientations into a 3-D density map. The separate steps in image reconstruction are outlined in **Figure 2** and described briefly below. To simplify the technical aspects of the procedure, numerous image-processing software packages that can be used for icosahedral reconstruction have been developed.

Initially, each micrograph captured from a CCD camera or digitized from photographic film is evaluated for its image quality based on the power spectrum derived from the average of multiple Fourier transforms of computationally isolated particle images. This power spectrum contains information about the structure of the particles, the imaging conditions used, and the image quality. The ring pattern in the power spectrum (**Figure 3**(**a**)) reflects the imaging condition known as the contrast transfer function (CTF). It can vary in appearance according to factors such as defocus, astigmatism, specimen drift, and signal decay as a function of resolution, all of which are attributable to various instrumental factors. The signal-to-noise ratio (SNR) in each micrograph can be computed from the circularly averaged power spectrum. The SNR plot (**Figure 3**(**b**)) indicates the potential signal strength

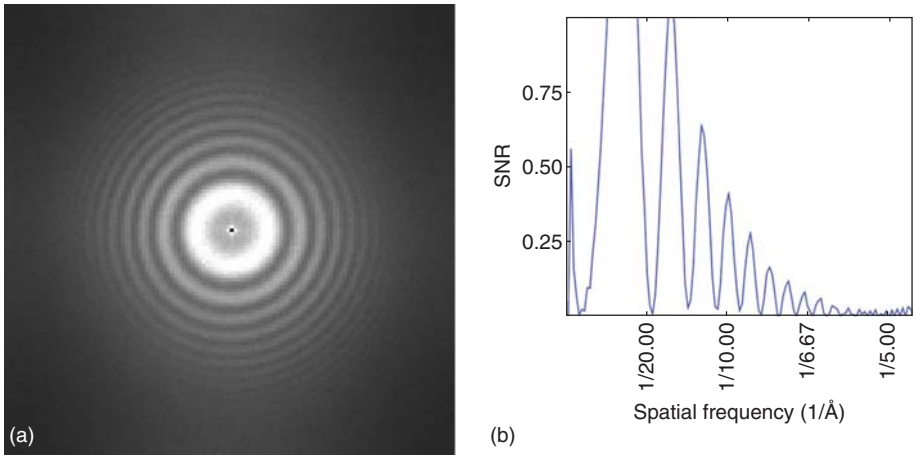


Figure 3 Evaluating image quality. (a) The power spectrum derived from averaging the Fourier transforms of particles boxed out from electron images such as those in **Figure 1**. (b) SNR plot of the boxed particle images as a function of spatial frequency (resolution) computed from the circularly averaged power spectrum. For a conservative measure of 10% SNR, the usable data extends beyond 8 Å resolution. Courtesy of Donghua Chen, Baylor College of Medicine.

at different resolutions and gives an indication of the ultimately achievable resolution in the reconstruction.

In order to obtain a correct structure, each raw particle image has to be deconvoluted by its CTF and be compensated for the signal decay at high resolution (**Figure 2**). The image deconvolution and signal-to-noise weighing are usually carried out for each raw particle image before the orientation of the particles is determined. Following orientation determination, the corrected particle images are merged to synthesize the 3-D map. Alternatively, the deconvolution can be carried out on the preliminary 3-D maps computed from each micrograph, with the deconvoluted maps being merged subsequently to produce the final map.

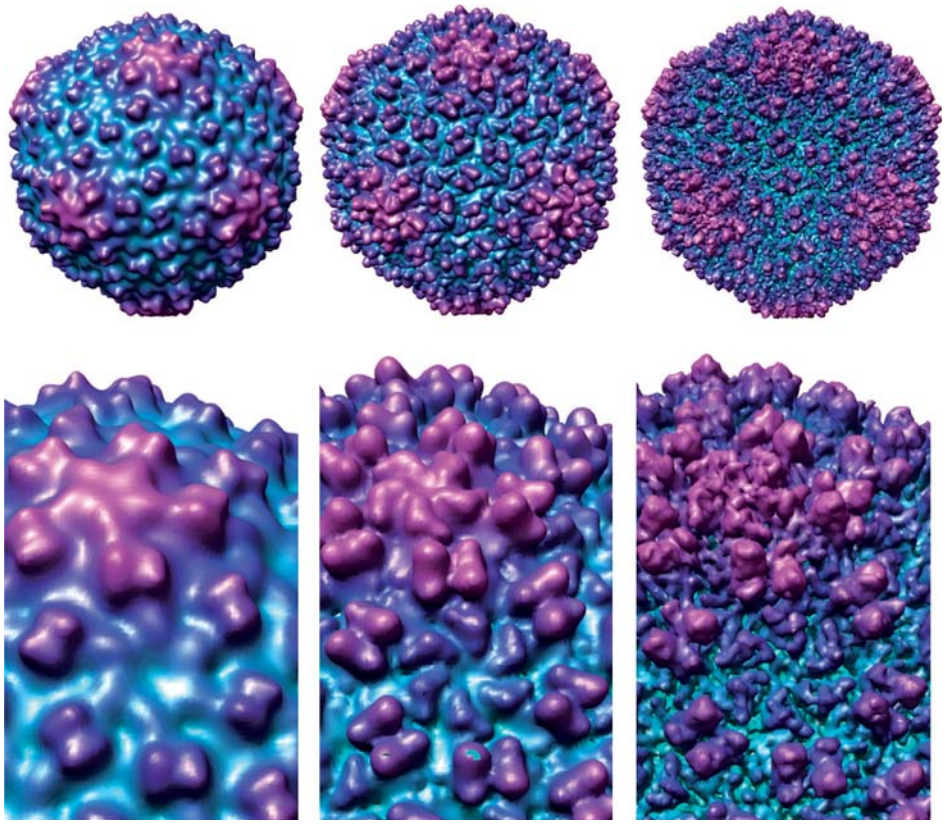
The most computationally demanding aspect of image processing is the estimation and refinement of the five spatial parameters (three for rotations around x-, y-, and z-axes, and two for center in x- and y-directions) for each particle image (**Figure 2**). There are a number of different algorithms and software packages that can be used to perform this critical task. Most of these programs require an initial model of the particle, from which they generate multiple projection images showing the particle model in different orientations. These projection images are then compared with each of the raw particle images. The projection image that gives the closest match is assumed to have the same orientation as the virus particle that produced the raw image. Each algorithm uses different criteria for comparing the reference and raw particle images to derive the spatial parameters. Throughout a series of iterative refinement steps, the model is continuously updated and its resolution is gradually increased (**Figure 2**).

During the steps of orientation and center determination, and 3-D reconstruction, the algorithms assume that the particle has icosahedral symmetry. This means that the particle is made up of 60 repeated (asymmetric) units, which are related to each other by five-, three-, and twofold symmetry operations. If the particle does not have the expected symmetry, the algorithm will not yield a consistent map and will not be able to derive its structure. On the other hand, it is possible that in some particles the icosahedral symmetry is preserved only up to a certain resolution, which would limit the ultimate resolution of the reconstruction. This is analogous to a protein crystal only diffracting up to some resolution limit beyond which the crystal structure cannot be resolved.

Interpreting CryoEM Single-Particle Density Maps

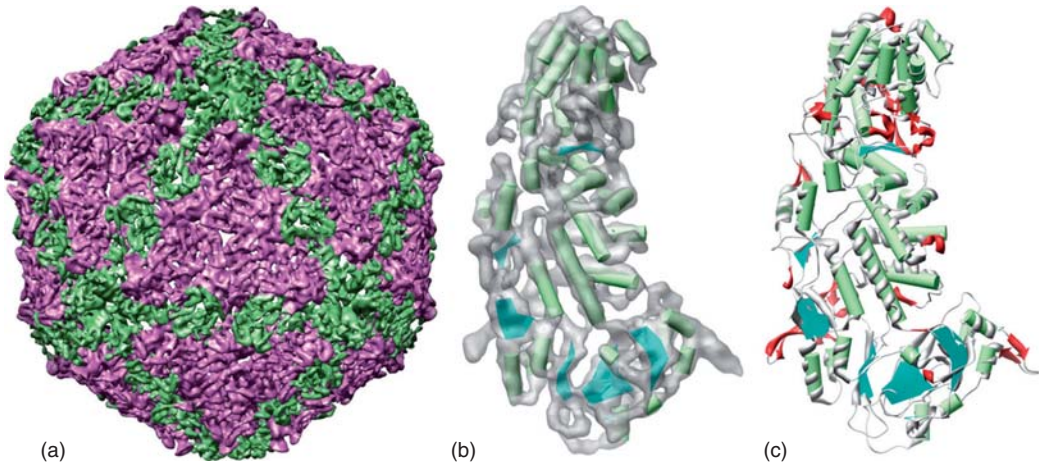
The challenge in interpreting a cryoEM density map varies according to its resolution. Maps of different resolutions reveal different structural features of the icosahedral shell proteins (**Figure 4**). A low-resolution map (down to 30 Å) can yield the size, shape, and organization (triangulation number) of the icosahedral particle. In fact, cryoEM is probably the most reliable technique for providing such information. In a moderate resolution map (20–10 Å), the subunit boundaries may begin to be resolved. The segmentation of the map into subunits can be done qualitatively by visual inspection of the structure in front of a graphics terminal or quantitatively by a software program. The accuracy of the segmentation is ultimately dictated by the resolution of the map.

When a cryoEM density map reaches subnanometer resolution, long α-helices and large β-sheets become visually recognizable and can be assessed quantitatively (**Figure 5**). In intermediate resolution (7–9 Å) density maps, it has been shown that it is possible to correctly



(a) (b) (c)

Figure 4 CryoEM maps of Epsilon15 phage at different resolutions. The surface-rendered images of the whole capsid (top row) and a close-up of the asymmetric unit (bottom) show that (a) at 40 Å resolution, the overall morphology and triangulation number can be distinguished; (b) at 20 Å resolution, it is possible to visualize the subunit separation in the hexons and pentons; and (c) at 9.5 Å resolution, α-helices are discernible as rod-like densities (EMD-1176). The display was colored radially.



(a) (b) (c)

Figure 5 CryoEM map of rice dwarf virus matches the crystal structure. (a) The inner capsid map segmented from a 6.8 Å resolution cryoEM reconstruction of the entire virus, with the two different conformations of the capsid protein P3A and P3B colored green and violet, respectively (EMD-1060). (b) Segmented inner capsid shell protein P3A with predicted secondary structure elements annotated as cylinders (α-helices) and surfaces (β-sheets). (c) Overlaying the crystal structure (ribbons, 1UF2) onto the secondary structure prediction shows a good match. (a) Reproduced from Zhou ZH, Baker ML, Jiang W, *et al*. (2001) Electron cryomicroscopy and bioinformatics suggest protein fold models for rice dwarf virus. *Nature Structural Biology* 8: 868–873. (c) Reproduced from Baker ML, Ju T, and Chiu W (2007) Identification of secondary structure elements in intermediate-resolution density maps. *Structure* 15: 7–19, with permission from Elsevier.

identify and annotate nearly all of the helices with $\geq$2.5 turns and sheets with $\geq$2 strands. For example, the match (**Figure 5**(**c**)) between the secondary structural elements determined from the 6.8 Å cryoEM map of the rice dwarf virus and the crystal structure that was obtained later demonstrated the reliability of the cryoEM reconstruction and of the structural feature identification.

Asymmetric Reconstruction of an Icosahedral Particle with a Unique Vertex

Though icosahedral reconstruction methods have been very successful in providing structural information on capsid shell proteins, they reveal nothing about any nonicosahedral components. Recently, however, several image-processing procedures have emerged for reconstructing spherical virus particles with tail structures without imposing symmetry. Roughly speaking, the reconstruction procedure uses an initial icosahedral reconstruction of the particle as a starting model for the asymmetric reconstruction. Because only one vertex of the virus particle has the additional mass of the tail, a crude model can be generated by computationally adding a cylinder of density at one of the fivefold vertices of the icosahedral reconstruction. This tailed model is projected in each of the 60 icosahedrally equivalent views and compared with the raw particle image to identify the view that produces the best match. Once the orientations of all the images have been determined, they are used to reconstruct the map without imposing any symmetry. This process of projection matching is iterated using a constantly updated reconstruction, until the cylindrical symmetry of the tail is broken, revealing its true structure. **Figure 6** shows the asymmetric reconstruction of Epsilon15 phage where, in addition to the capsid shell, multiple nonicosahedrally ordered molecular components and the internal DNA can be visualized.

Modeling Virus Particles with CryoEM Density Map Constraints

It is not uncommon for components of a virus particle to be crystallized and structurally determined while only a low- or medium-resolution cryoEM map of the entire virus particle is available. In these cases, a pseudo-atomic model of the virus particle can often be built by docking the crystal structures of the solved components into the cryoEM map. The docking can be done visually or quantitatively using a fitting program. Examples of this are provided by adenovirus and herpesvirus. In neither case has the intact capsid been crystallized, but inserting the crystal structures of the adenovirus hexon and penton base proteins, or a domain of HSV-1 major capsid protein

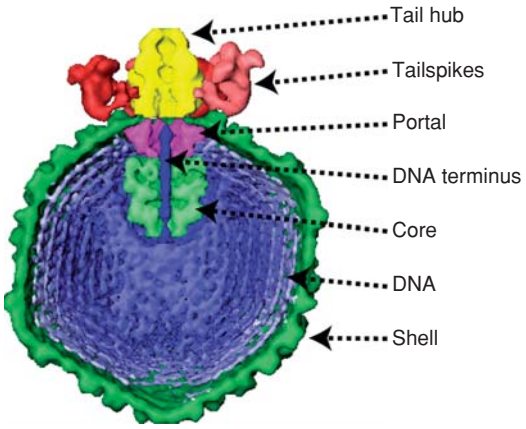


Figure 6 Asymmetric reconstruction of Epsilon15 phage. The map reveals all the structural components of the capsid shell, DNA, core, portal, tailspikes, and tail hub (EMD-1175). Reproduced from Jiang W, Chang J, Jakana J, Weigele P, King J, and Chiu W (2006) Structure of Epsilon15 phage reveals organization of genome and DNA packaging/injection apparatus. *Nature* 439(7076): 612–616.

into the respective cryoEM structures, has provided us with pseudo-atomic models that reveal important features of the capsids. An outstanding example of how cryoEM and crystallographic data can be merged to establish a pseudo-atomic model is provided by the T4 bacteriophage baseplate (**Figure 7**). Here, the crystal structures of six separate molecular components have been fitted into the reconstruction of the baseplate, leading to a proposal for the mechanism of DNA injection into the host cell.

Where there is no crystal structure available, it may still be possible to construct a set of structural models using either comparative modeling or *ab initio* modeling based on the amino acid sequence alone. In these cases, a cryoEM density map can be used as a template to select the most native-like model. A domain of the VP26 capsid protein of HSV, which has a molecular mass of ~10 kDa, has been modeled *ab initio* using the cryoEM structure of the HSV capsid as a selection constraint. Although the validity of this approach has not yet been confirmed by crystal data, it is encouraging that the model fitting the cryoEM data was consistent with published genetic and biochemical data for VP26.

Low-Dose Cryoelectron Tomographic Imaging of Virus Particles

Particles of some viruses have components that assume different conformations in different particles, making it impossible to generate a 3-D density map by averaging these particles. For this type of particle, the only option available is tomography. While the reconstruction

techniques described above work by combining views of multiple particles in different orientations, tomographic reconstruction combines multiple views of the same particle as its orientation is changed by rotating it between consecutive exposures using a tilting microscope holder. Therefore, this technique allows a low-resolution map to be generated by collecting a tilt series from any cryoEM specimen, and it has been used successfully with a range of viruses including HSV virions and capsids, vaccinia virus, and HIV. Because of the constraints on the number of images that can be acquired due to the effects of radiation damage, cryotomography yields only low-resolution data. Therefore, the strategy is to record multiple images of the specimens at low magnification and under low-dose condition. Typically, a magnification of 10 000–25 000× and a dose of 1–2 electrons $Å^{-2}$ per micrograph are used (**Figure 8**).

Once captured, the tilt series images are aligned and merged to generate a 3-D tomogram. Because the grid supporting the specimen obscures the electron beam, the range of tilts that can be used is limited to about ±70°. As a result, there is a missing wedge of data, which means that each tomogram is subject to distortion and must be interpreted cautiously.

Although tomographic reconstructions are generally of low resolution (100–60 Å), post-tomographic averaging of components with constant conformations can yield higher resolutions. For example, post-tomographic data sorting, mutual alignment, and averaging have been used to generate a model of the herpesvirus capsid with an averaged view of the single portal vertex (**Figure 9**(**a**)). Similarly, the spike of the simian immunodeficiency virus (SIV) particle has been aligned and averaged to produce a map that permitted docking of the crystal structures of the spike proteins (**Figure 9**(**b**)). In this type of approach, the final resolution is limited by a number of factors, including the accuracy of the alignment, the number of independent views of the components included in the

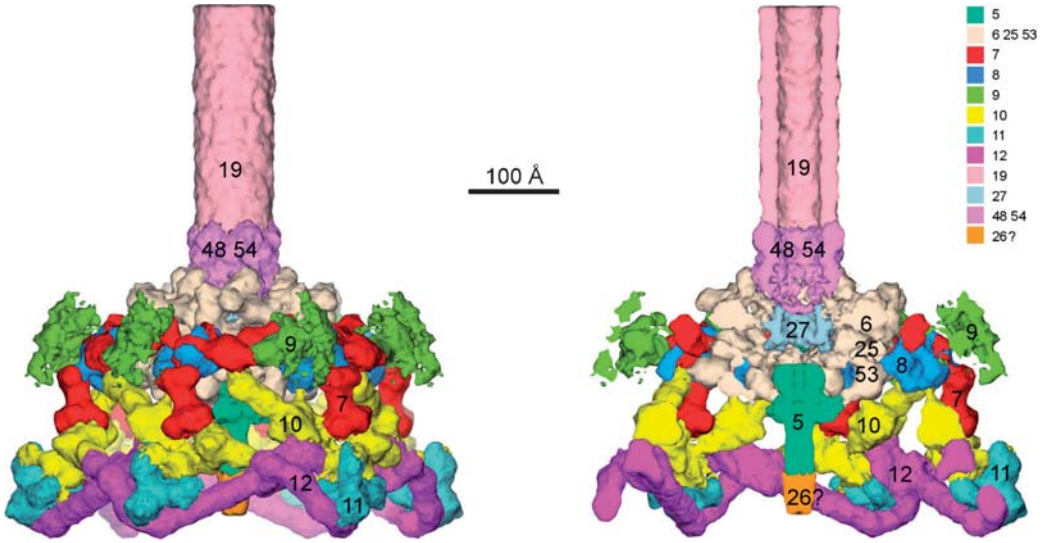


Figure 7 Pseudo-atomic models. Side and cut-away view of the T4 phage tube–baseplate complex. Six crystallized components of the T4 baseplate were docked into the cryoEM map, which permitted identification of the baseplate components (color-coded gene products with legend on right) (EMD-1086). Courtesy of M. G. Rossmann at Purdue University and reproduced with permission from Rossmann MG, Mesyanzhinov VV, Arisaka F, and Leiman PG (2004) The bacteriophage T4 DNA injection machine. *Current Opinion in Structural Biology* 14(2): 171–180.

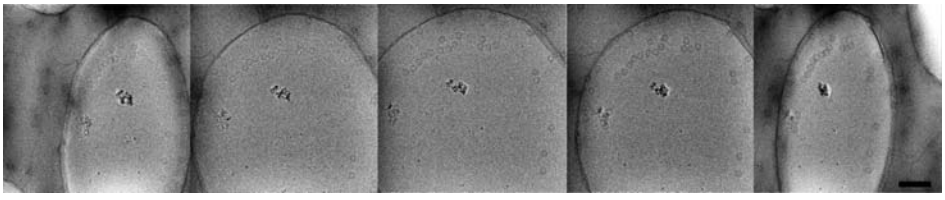


Figure 8 Tomography images. Selected CCD frames recorded at −60°, −30°, 0°, +30°, and +60° tilts (left to right) show herpesvirus capsids forming an arc in the top half of each image. Scale = 5000 Å.

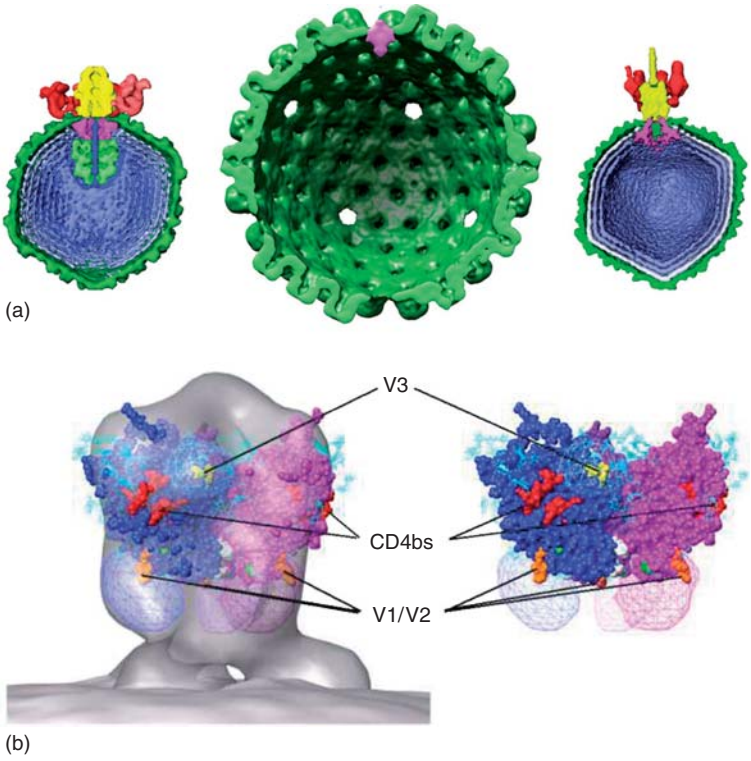


Figure 9 Increasing resolution by averaging subvolumes from tomographic reconstructions. (a) Structure of the herpes simplex virus-1 capsid (center) shown after removal of the pentons to reveal the unique portal vertex (purple). This structure was derived by averaging 13 subvolumes in different tomographic reconstructions (EMD-1308). The arrangement of the portal in the capsid floor is similar to that seen in the tailed double-stranded DNA (dsDNA) phages Epsilon15 (left, EMD-1175) and P22 (right, EMD-1222). (b) Averaging membrane spikes from tomographic reconstructions of SIV particles and then imposing threefold symmetry improves the resulting map, thereby allowing the crystal structure of the spike protein to be fitted. The V1/V2 (orange) and V3 (yellow) loops and CD4 binding sites (red) are labeled in this model. (a) Reproduced from Chang JT, Schmid MF, Rixon FJ, and Chiu W (2007) Electron cryotomography reveals the portal in the herpesvirus capsid. *Journal of Virology* 81(4): 2065–2068, with permission from American Society for Microbiology. (b) Courtesy of K. Taylor and K. H. Roux at Florida State University and reproduced with permission from Zhu P, Liu J, Bess J, *et al.* (2006) Distribution and three-dimensional structure of AIDS virus envelope spikes. *Nature* 441(7095): 847–852.

averaging, the symmetry of the components, the proper image deconvolution, the correct compensation for the missing wedge data, and the conformational uniformity of the components.

Biological Insights from CryoEM Maps

The extent of biological information that can be obtained from cryoEM reconstruction varies according to the resolution of the map as well as the design of the experiment. As shown in **Figures 4** and **5**, different structural features can be derived from the cryoEM maps at varying resolutions, ranging from shape, size, triangulation number, capsomere morphology and number, quaternary, tertiary, and secondary structural features of individual protein subunits. Here, a small and not exhaustive set of examples is provided to demonstrate the usefulness of cryoEM maps in the context of virology.

Conservation of Structural Motifs among Viruses

Questions regarding the origin of viruses and their distant evolutionary relationships are handicapped by the low sequence conservation generally found in these small and rapidly evolving entities. Therefore, structural comparison has been used as an alternative indicator of their relatedness. The best-known example of structural analysis revealing a link between supposedly unrelated viruses is that of bacteriophage PRD1 and human adenovirus. Although early cryoEM analysis had shown that the PRD1 capsid structure was very similar to that of adenovirus, it was not until the crystal structures of their respective major capsid proteins were compared that the evolutionary relationship between these viruses was firmly established.

However, in cases where crystal structures are not available, cryoEM maps determined at subnanometer

resolution can provide sufficient information on the pattern of secondary structure elements and thus of the protein folds. **Figure 10** shows a structural comparison of the capsid proteins of the eukaryote-infecting herpesvirus (*Herpesviridae*) and the prokaryote-infecting tailed DNA bacteriophages (*Caudovirales*). Although these proteins have no detectable sequence homology, it is clear from the similar spatial distributions of secondary structural elements that they share a characteristic fold. CryoEM-derived structural data have also revealed conserved features in the unique portal vertices of the two virus types, including a characteristic subunit arrangement and a similar location within the capsid shell (**Figure 9**(**a**)). These observations have led to the conclusion that these very distinct, extant viruses must have arisen from the same primordial progenitors.

Structural Polymorphism during Virus Maturation

Many virus particles undergo structural changes during their morphogenesis. For example, tailed bacteriophages typically form their capsid shell around an internal scaffold of proteins. This initial assembly product (the procapsid) then undergoes a maturation process during which the DNA is packaged, the internal scaffold is lost, and the capsid shell undergoes extensive structural reconfiguration. **Figure 11** demonstrates the structural changes in P22 bacteriophage as revealed from reconstructions of the procapsid and of the mature virion. Not only can changes in the overall size and shape of the capsid be observed but also correlative changes in the quaternary and tertiary structures of individual subunits can be

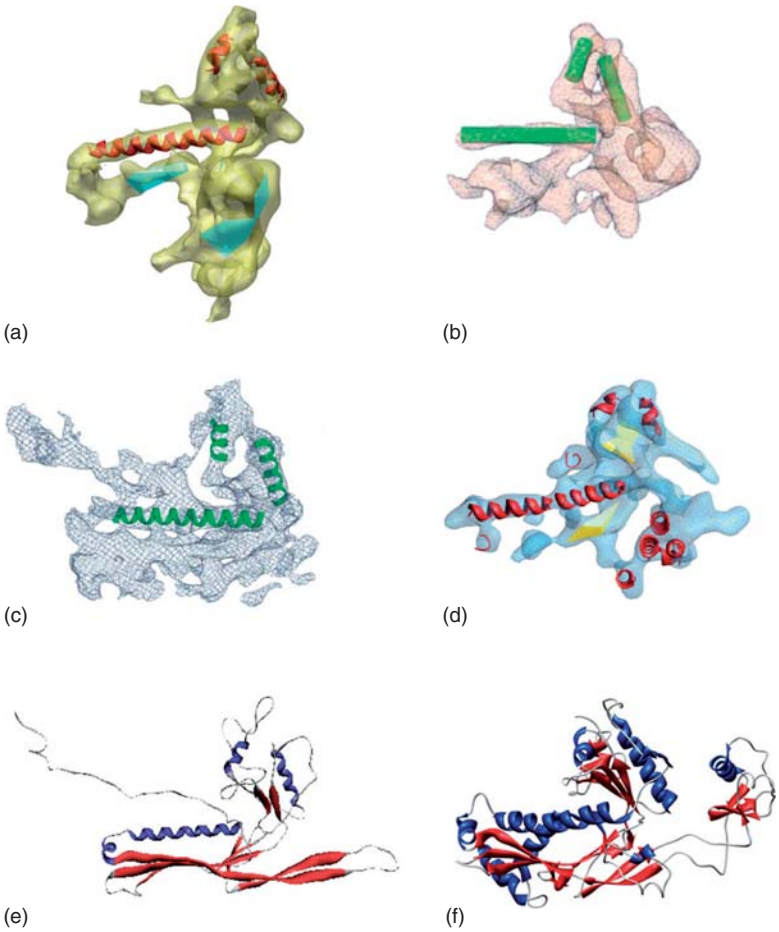


Figure 10 Evolutionary conservation of a structural motif in dsDNA virus particles. CryoEM structure of (a) Epsilon15 phage shell protein, with annotated helices and sheets (EMD-1176), (b) P22 phage coat protein gp5 (EMD-1101), (c) Phi29 phage head protein gp8 (EMD-1120), (d) HSV-1 major capsid protein VP5 floor domain, and crystal structures of (e) HK97 phage head protein gp5 (1OGH) and (f) T4 phage head protein gp24 (1YUE) all have similar structural signatures, which suggest a common ancestry even though they have little sequence similarity. Reproduced from Jiang W, Chang J, Jakana J, Weigele P, King J, and Chiu W (2006) Structure of Epsilon15 phage reveals organization of genome and DNA packaging/injection apparatus. *Nature* 439(7076): 612–616.

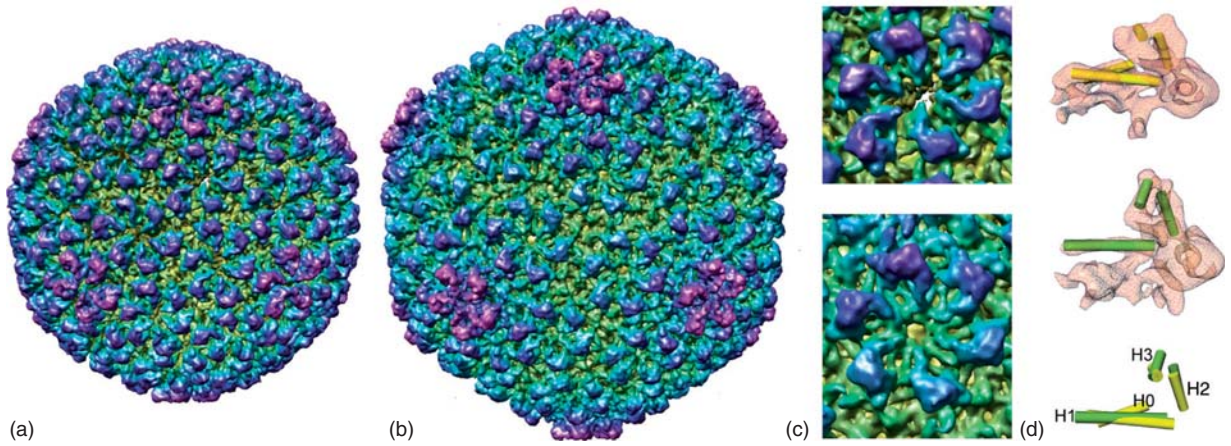


Figure 11 Structural changes during virus maturation. (a) The spherical procapsid of P22 is ~600 Å in diameter, and its hexons are elongated, with a hole in the center. (b) The angular mature capsid is ~700 Å in diameter, and its hexon subunits are arranged hexagonally with more lateral interactions, resulting in a smaller hole (EMD-1101). (c) A close-up view of the procapsid (top) and mature (bottom) hexon. (d) The P22 capsid protein gp5 rearranges when maturing from the procapsid (top, annotated secondary structures in yellow) to the capsid (middle, annotated secondary structures in green), resulting in changes to secondary, tertiary, and quaternary structure, as shown by overlapping the annotated secondary structures (bottom). (d) Reproduced from Jiang W, Li Z, Zhang Z, Baker ML, Prevelige PE, and Chiu W (2003) Coat protein fold and maturation transition of bacteriophage p22 seen at subnanometer resolutions. *Nature Structural Biology* 10:131–135.

noted. For instance, the organization of subunits in the hexon capsomere changes from a skewed shape to a six-fold symmetrical arrangement. In addition to the overall domain movements in the hexon subunit that bring about this change, the cryoEM structures also reveal more detailed changes such as the refolding of an N-terminal α-helix. Large-scale structural changes have also been visualized during maturation of the HSV capsid. In addition to comparing relatively stable forms of a particle, cryoEM can be used to follow rapid structural changes (e.g., by changing the pH of the buffer immediately before freezing) as observed in Semliki forest virus, Sindbis virus, and La Crosse virus.

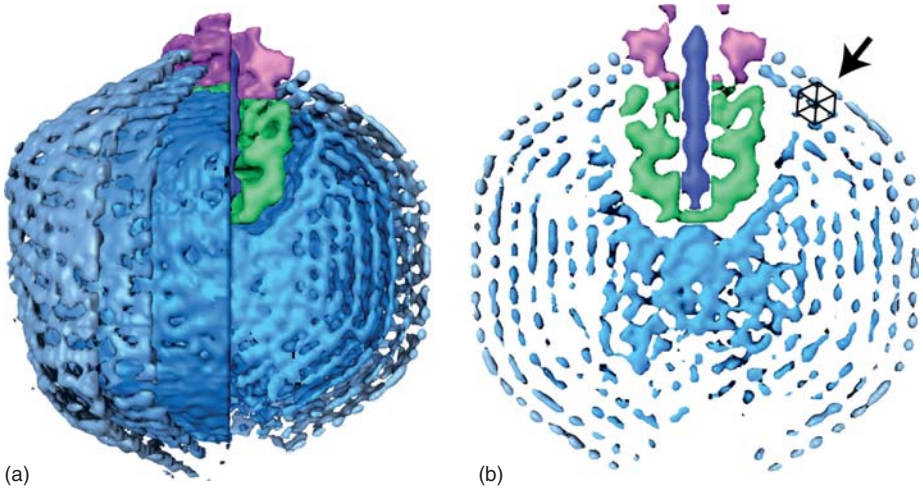
Viral Genome Organization

To date, most information on how viral nucleic acids are arranged within particles has come from cryoEM reconstructions. Low-resolution cryoEM reconstruction has revealed that small sections of the RNA genomes in some RNA viruses are bound icosahedrally to the capsid shell proteins, although the bulk of the genome was not resolved even in these cases. However, in general, viral genomes are not packaged icosahedrally within particles and so cannot be visualized by icosahedral reconstruction techniques.

Those double-stranded DNA viruses that package their genomes into preformed capsids pose a particularly interesting dilemma because this must be accomplished without organizing proteins (such as histones) to counter the electrostatic charge of the DNA and the organization must allow for efficient entry and ejection without forming knots or tangles. The arrangement of the nucleic acids in such capsids has been extensively studied and debated. Proposed models include the co-axial spool, spiral fold, liquid crystal, and folded toroid. CryoEM has led the way in shedding light on this question. Analysis of a bacteriophage T7 mutant that was found to orient preferentially in vitreous ice showed the capsids having either concentric rings (axial view) of DNA spaced about 25 Å apart or punctate arrays (side view) suggesting that the DNA was organized in concentric layers. Subsequently, it became possible to examine the DNA in more detail using asymmetric reconstruction algorithms. In Epsilon15 phage, for example, DNA strands are packed as concentric layers in the capsid cavity suggesting a co-axial spool arrangement (**Figure 12**(**a**)), and a slice normal to the DNA strands and parallel to the tail axis shows hexagonal features (**Figure 12**(**b**)). A DNA strand connecting each layer has not yet been observed, but this could be the result of errors in the alignment of the particle images during reconstruction, or due to heterogeneity in where the DNA winding commences. The goal of such studies is to trace the path of the entire nucleic acid molecule within the virion. Asymmetric reconstruction methods at higher resolution are well poised for these determinations.

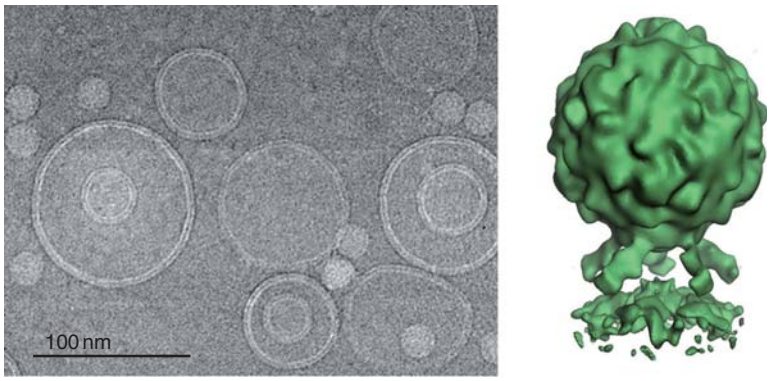
Virus–Antibody and Cell Receptor Complexes

There is increasing interest in using cryoEM to analyze aspects of virus biology involving interactions with other molecules such as receptors or antibodies. A good



(a) (b)

Figure 12 Viral genome organization in tailed dsDNA phages. (a) In Epsilon15 phage the dsDNA (blue) is arranged in concentric layers surrounding a protein core (green) and portal (purple) (EMD-1175). (b) A slice parallel to the tail axis and normal to the dsDNA strands shows hexagonal features (arrow over polygon).



(a) (b)

Figure 13 Virus–receptor interactions. (a) CryoEM image of poliovirus attached to liposomes with embedded receptors. (b) The reconstruction shows the icosahedral capsid binding to five receptors on the membrane, which orients the capsid so that a fivefold vertex is directed toward the membrane. Courtesy of J. M. Hogle at Harvard Medical School and reproduced with permission from Bubeck D, Filman DJ, and Hogle JM (2005) Cryo-electron microscopy reconstruction of a poliovirus–receptor-membrane complex. *Nature Structural and Molecular Biology* 12: 615–618.

example of the value of this approach is provided by work on virus–receptor interactions in the small icosahedral picornaviruses, notably poliovirus and the common cold virus (rhinovirus). In order to recognize and enter their target cells, viruses must usually bind to specific receptor molecules on the cell surface. When the crystal structure of the rhinovirus particle was first determined, it revealed the existence of a depression or 'canyon' around each fivefold axis of the particle, which was proposed as the binding site for the receptor. However, direct confirmation of this proposal was not obtained until icosahedral reconstruction was carried out on cryoEM images of virus particles bound to their receptors. Although virus receptors are components of the cell membrane, these initial binding studies were carried out using soluble fragments of receptor molecules.

Typical eukaryote cells are too large to freeze rapidly by normal methods making it difficult to study virus binding *in situ*. However, by embedding receptor molecules in artificial liposomes that were small enough to vitrify (**Figure 13**(**a**)), the interaction between the poliovirus particle and its receptor could be visualized in the context of a membrane. Comparison of the micrographs with projection images of an existing icosahedral reconstruction allowed the orientation of the virus particles bound to the liposome to be determined. Then, in a process roughly analogous to that used in the asymmetric reconstruction of tailed bacteriophage particles (described earlier), an

artificial density was introduced at the point on the membrane closest to the virus particle and the reconstruction was repeated without imposing icosahedral symmetry. The structure that resulted from this procedure showed the virus particle, receptor molecules, and adjacent parts of the liposome membrane (**Figure 13**(**b**)), thereby demonstrating that the particle adopts a consistent alignment with respect to the lipid membrane. This not only provided information on how the receptor molecules interact with the virus particle in the context of a membrane, but also revealed the presence of a perturbation to the membrane induced by virus binding. This perturbation might represent an early stage in the formation of the pore, which is necessary for release of the viral genome into the cell.

Concluding Remarks

CryoEM is an important technique that has been used to determine the structures of many viruses. In many cases, achieving subnanometer resolutions has revealed new insights into the structural organization and biological properties of viruses. CryoEM results have been complemented by docking crystal structures or by molecular modeling to provide additional information. Furthermore, conformational changes, structural transformations, and virus–receptor interactions have been observed. Even in cases where the sample is pleomorphic, electron cryotomography has revealed important information. Overall, the study of viruses has been enormously enriched by the use of cryoEM.

Acknowledgment

Research support has been provided by National Institutes of Health, National Science Foundation, and the Robert Welch Foundation.

See also: Electron Microscopy of Viruses; Virus Particle Structure: Nonenveloped Viruses; Virus Particle Structure: Principles.

Further Reading

Adrian M, Dubochet J, Lepault J, and McDowall AW (1984) Cryo-electron microscopy of viruses. *Nature* 308(5954): 32–36.

Baker ML, Ju T, and Chiu W (2007) Identification of secondary structure elements in intermediate-resolution density maps. *Structure* 15(1): 7–19.

Baker TS, Olson NH, and Fuller SD (1999) Adding the third dimension to virus life cycles: Three-dimensional reconstruction of icosahedral viruses from cryo-electron micrographs. *Microbiology and Molecular Biology Reviews* 63(4): 862–922.

Chang JT, Schmid MF, Rixon FJ, and Chiu W (2007) Electron cryotomography reveals the portal in the herpesvirus capsid. *Journal of Virology* 81(4): 2065–2068.

Crowther RA, Amos LA, Finch JT, DeRosier DJ, and Klug A (1970) Three dimensional reconstructions of spherical viruses by Fourier synthesis from electron micrographs. *Nature* 226(5244): 421–425.

Dubochet J, Adrian M, Chang JJ, *et al.* (1988) Cryo-electron microscopy of vitrified specimens. *Quarterly Reviews of Biophysics* 21(2): 129–228.

Jiang W, Chang J, Jakana J, Weigele P, King J, and Chiu W (2006) Structure of Epsilon15 phage reveals organization of genome and DNA packaging/injection apparatus. *Nature* 439(7076): 612–616.

Jiang W and Chiu W (2006) Electron cryomicroscopy of icosahedral virus particle. In: Kuo J (ed.) *Methods in Molecular Biology*, pp. 345–363. Totowa, NJ: The Humana Press.

Johnson JE and Rueckert R (1997) Packaging and release of the viral genome. In: Chiu W, Burnett RM, and Garcea RL (eds.) *Structural Biology of Viruses*, pp. 269–287. New York: Oxford University Press.

Lucic V, Forster F, and Baumeister W (2005) Structural studies by electron tomography: From cells to molecules. *Annual Review of Biochemistry* 74: 833–865.

Rossmann MG, He Y, and Kuhn RJ (2002) Picornavirus–receptor interactions. *Trends in Microbiology* 10(7): 324–331.

Rossmann MG, Mesyanzhinov VV, Arisaka F, and Leiman PG (2004) The bacteriophage T4 DNA injection machine. *Current Opinion in Structural Biology* 14(2): 171–180.

Smith TJ (2003) Structural studies on antibody–virus complexes. *Advances in Protein Chemistry* 64: 409–454.

Zhou ZH, Baker ML, Jiang W, *et al.* (2001) Electron cryomicroscopy and bioinformatics suggest protein fold models for rice dwarf virus. *Nature Structural Biology* 8: 868–873.

Zhou ZH, Dougherty M, Jakana J, He J, Rixon FJ, and Chiu W (2000) Seeing the herpesvirus capsid at 8.5 Å. *Science* 288(5467): 877–880.

Cucumber Mosaic Virus

F García-Arenal, Universidad Politécnica de Madrid, Madrid, Spain
P Palukaitis, Scottish Crop Research Institute, Dundee, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Chlorotic Yellowing or light green symptoms induced by virus infection affecting chlorophyll accumulation.

Cross-protection Inhibition of systemic virus accumulation and disease by prior inoculation of plants with a mild or symptomless strain of the same virus.

Filiformism Narrowing of the leaf blade, often leading to symptoms referred to as shoestring.
Pseudorecombination Reassortment of the genomic RNAs of two or more strains of a multipartite RNA virus to generate novel combinations of the full complement of genomic RNAs.
Satellite RNA A subviral RNA genome dependent on a helper virus for both replication and encapsidation.
Tonoplast The membrane surrounding the central vacuole of plant cells.

History

Cucumber mosaic virus (CMV) was first described as a disease of cucurbits in 1916 by Doolittle in Michigan and Jagger in New York. The virus can infect a large number of indicator plant species and has been isolated from over 500 naturally infected species. Cross-protection was used in the 1930s to discriminate isolates of CMV with differences in phenotypes or host range (strains). CMV was not purified reliably until the middle 1960s. Later serology and hybridization technology were used to detect and differentiate two major subgroups of CMV. The nucleotide sequence and the genome organization of one strain of each CMV subgroup were determined between 1984 and 1990, while biologically active cDNA clones of several CMV strains were developed in the early 1990s. The major functions of each of the five encoded proteins have been assigned, although each protein is also involved in other host–virus relationships.

Taxonomy and Classification

Isolates of CMV are heterogeneous in symptoms, host range, transmission, serology, physicochemical properties, and nucleotide sequence of the genomic RNAs. On the basis of different criteria (e.g., serological typing, peptide mapping of the coat protein, sequence similarity of their genomic RNA) CMV isolates can be classified into two major subgroups, now named subgroup I and subgroup II. The percentage identity in the nucleotide sequence between pairs of isolates belonging to each of these subgroups ranges from 69% to 77%, depending on the pair of isolates and the RNA segment compared, dissimilarity being highest for RNA2. Nucleotide sequence identity among isolates within a subgroup is above 88% for subgroup I and above 96% for subgroup II, indicating a higher heterogeneity of subgroup I. Analysis of the open reading frames (ORFs) and 5′ noncoding regions of RNA3 of subgroup I isolates shows a group of closely related isolates forming a monophyletic cluster, named subgroup IA; the rest of subgroup I isolates are included in the nonmonophyletic group IB. Analyses of RNA2 show that subgroups IA and IB constitute monophyletic groups, while analyses of RNA1 show no clear division into groups IA and IB. Hence, the different genomic segments have followed different evolutionary histories. Cross-protection occurs between strains from all subgroups. Isolates of subgroup I and II can be distinguished using monoclonal antibodies, and isolates from subgroup IA, IB, and II can be distinguished by reverse transcriptase polymerase chain reaction (RT-PCR). CMV isolates differ from isolates of the other two cucumovirus species, *Tomato aspermy virus* (TAV) and *Peanut stunt virus*, having only 50–67% nucleotide sequence identity, depending on the RNA and isolates being compared.

Geographic Distribution

CMV isolates have a worldwide distribution, having been reported from both temperate and tropical regions. Most reported isolates belong to subgroup I. Subgroup II isolates are found more frequently in cooler areas or seasons of temperate regions. This has been associated with lower temperature optima for *in planta* virus accumulation shown for the few isolates characterized for this property. Most isolates in subgroup IB have been reported from East Asia, which is presumed to be the origin of this subgroup. Subgroup IB isolates also have been reported from other areas, for example, the Mediterranean region, California, Brazil, and Australia. Those in the Mediterranean could have been introduced recently from East Asia.

Host Range and Propagation

The host range of the collective isolates of CMV is over 1300 species in more than 500 genera of over 100 families, with new hosts reported each year. Some recently described strains from new hosts have lost the ability to infect many of the typical hosts of CMV. This may be a general feature for adaptation to unusual hosts. CMV infects most of the major horticultural crops as well as many weed species; the latter act as reservoirs for the virus. Infection of various indicator plants was used to differentiate CMV from other viruses, since unlike most other viruses of cucurbitaceous or solanaceous hosts, CMV could infect representative species of both families. These include cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), and tobacco (*Nicotiana tabacum*), all systemic hosts of CMV, as well as cowpea (*Vigna unguiculata*) and *Chenopodium quinoa*, which limit CMV infection to the inoculated leaves, although there are legume strains that will

infect cowpea systemically. Most isolates of CMV are best propagated in squash (marrow) (*Cucurbita pepo*), tobacco, *N. clevelandii*, or *N. glutinosa.*

Virus Structure and Properties

CMV has icosahedral particles 29 nm in diameter, which sediment as a single component with an $S_{20,w}$ of 98.6– 104*c* (*c* being virus concentration in mg ml^{-1}). Particles are built of 180 capsid protein subunits arranged with $T = 3$ quasisymmetry, contain about 18% RNA, and have an extinction coefficient at 260 nm (1 mg ml^{-1}, 1 cm light path) of 5.0. RNA1 and RNA2 are encapsidated in different particles, whereas RNA3 and RNA4 are probably packaged together in the same particle, some particles may contain three molecules of RNA4. Thus, virus preparations contain at least three different types of particles, but with similar morphology and sedimentation properties. Virus particles also contain low levels of the RNA species designated RNA4A, RNA5, and RNA6. There is a limit to the size of encapsidated RNAs, those larger than RNA1 are not encapsidated *in vivo.* CMV particles are stabilized by RNA–protein interactions, and no empty particles are formed. Particles disrupt at high neutral chloride salt concentrations or at low sodium dodecyl sulfate concentrations; biologically active particles can be reassembled by lowering the salt concentration or removing the sodium dodecyl sulfate. Particles are stable at pH 9.0 and do not swell at pH 7.0, an important difference with bromoviral particles.

The structure of CMV particles has been resolved at 3.2 Å by X-ray crystallography. The $T = 3$ lattice of capsids is composed of 60 copies of three conformationally distinct subunits designated A, B, and C that form trimers with quasi-threefold symmetry. There are 20 hexameric capsomers of B and C subunits with quasi-sixfold symmetry and 12 pentameric capsomers formed by A subunits with fivefold symmetry. The exterior radius along the quasi-sixfold axes is 144 Å, the RNA is tightly packaged against the protein shell and leaves a hollow core of about 110 Å along the threefold axes. The protein subunit has a β-barrel structure, with the long axis of the β-barrel domain oriented roughly in a radial direction. The N-terminal 22 amino acids of the capsid protein are needed for particle assembly. This region is positively charged and, in the B and C subunits, forms amphipatic helices that run parallel to the quasi-sixfold axes. There is an external region of negative electrostatic potential that surrounds the fivefold and quasi-sixfold axes and locates above regions of positive potential which extend to cover nearly homogeneously the inner surface of capsids, where interaction with encapsidated RNA occurs. Electrostatic distributions in CMV particles explain the physicochemical conditions required for particle stability.

Genome Organization

The genome of CMV consists of five genes distributed over three, single-stranded, positive-sense, capped, genomic RNAs: RNA1 (3.3–3.4 kb) encodes the *c.* 111 kDa 1a protein. RNA2 (3.0 kb) encodes the 98 kDa 2a protein, as well as the 13–15 kDa 2b protein, which is translated from a 630–702 nt subgenomic RNA designated RNA 4A that is co-terminal with the 3′ end of RNA2. The ORF expressing the 2b protein overlaps with the ORF encoding the 2a protein, but in a +1 reading frame. RNA3 encodes the 30 kDa 3a protein, as well as the 25 kDa 3b protein, which is expressed from a 1010–1250 nt subgenomic RNA designated RNA4 that is co-terminal with the 3′ end of RNA3. The 224–338 nt 3′ nontranslated regions of all three genomic and both subgenomic RNAs are highly conserved, forming a tRNA-like structure as well as several pseudoknots. The 5′ nontranslated regions of RNA1 (95–98 nt) and RNA2 (78–97 nt) are more conserved in sequence with each other than with those of RNA3 (96–97 nt or 120–123 nt). CMV also produces an RNA5 of unknown function, which is co-terminal with the 3′ nontranslated regions of RNA1 and RNA2. RNA4A and RNA5 are only encapsidated by subgroup II strains. CMV particles also encapsidated a low level of tRNAs, which have been reported in the literature as CMV RNA6. Although rarely reported, some strains of CMV can also encapsidate defective RNAs derived from CMV RNA3.

Satellite RNAs

CMV can also support satellite RNAs varying in size from 333 to 405 nt. These satellite RNAs are dependent upon CMV as the helper virus for both their replication and encapsidation, but have sequence similarity to the CMV RNAs limited to no more than 6–8 contiguous nt. More than 100 satellite variants have been found associated with over 65 isolates of CMV from both of the CMV subgroups. These satellite RNAs usually reduce the accumulation of the helper viruses and on most hosts also reduce the virulence of CMV. However, this attenuation of disease is not due to competition between the helper virus and the satellite RNA for a limited amount of replicase or capsid protein. Some CMV satellite RNAs can also be replicated and packaged by strains of the cucumovirus TAV, although these satellite RNAs do not attenuate the symptoms induced by TAV. Certain satellite RNAs in some selected hosts can enhance the disease induced by CMV. In the case of tomato plants infected by CMV and certain satellites, this has led to systemic necrosis observed in the fields of several Mediterranean countries. This necrosis is actually caused by sequences of the complementary-sense satellite RNA produced in large quantities during satellite RNA replication.

Genetics

The CMV 1a and 2a proteins encode proteins that replicate the virus, but which also function in promoting virus movement in several host species. The 2b protein is an RNA-silencing suppressor protein that antagonizes the salicylic acid defense pathway and also influences virus movement in some hosts. The 3a protein is the major movement protein of the virus and is essential for both cell-to-cell as well as long-distance (systemic) movement. The 3b protein is the sole viral capsid protein and is also required for cell-to-cell and long-distance movement, although the ability to form virions is not a requirement for movement. All of the CMV-encoded proteins are RNA-binding proteins. Viral RNAs from different strains and subgroups can be exchanged to form novel viruses, allowing mapping of some phenotypes to specific RNAs. Higher-resolution mapping requires the use of biologically active cDNA clones for generating chimeras and site-directed mutants. By these approaches, the following functions have been delimited to specific RNAs, with some mapped to specific nucleotide changes: hypersensitive response in tobacco, rapid local and systemic movement in squash, seed transmission in legumes, temperature-sensitive replication in melon; and replication of satellite (all in RNA1); hypersensitive response versus systemic infection in cowpea, suppression of gene silencing, and host range and pathology responses (all in RNA2); limited movement between epidermal cells, systemic movement in cucurbits, virion assembly, hypersensitive response on *Nicotiana* sp., local and systemic infection in cucurbits or in maize, symptom responses, and aphid transmission (all in RNA3).

Replication

CMV replication takes place on the vacuole membrane (the tonoplast). Replication involves the 1a and 2a proteins of the virus and presumably several host proteins. The purified CMV replicase contains the 1a and 2a proteins, as well as a host protein of *c.* 50 kDa of unknown function. The 1a protein of CMV contains a putative N-terminal proximal methyltransferase domain believed to be involved in capping of the RNAs, as well as a putative C-terminal proximal helicase domain, presumed to be required for the unwinding of the viral RNAs during replication. The 1a protein has been found to be able to bind to several tonoplast intrinsic proteins, although what roles these have in virus replication has not been established. The N-terminal region of the 2a protein interacts with the C-terminal region of the 1a protein *in vivo* and *in vitro*. Phosphorylation of the 2a protein prevents interaction with the 1a protein. The C-terminal half of the 2a protein contains conserved domains found in RNA polymerases and therefore together with the 1a protein forms the core of the CMV replicase.

CMV replication is initiated by the binding of the tonoplast-associated replicase to the tRNA-like structure and various pseudoknots present in the 3′ nontranslated region of the positive-sense CMV RNAs. Minus-sense RNA is then synthesized from each of the genomic RNAs and the synthesized minus-sense RNA acts as a template for synthesis of new plus-stranded genomic RNAs. The minus-sense RNA2 and RNA3 also serve as the templates for the synthesis of the two plus-sense subgenomic RNAs (4 and 4A), through recognition of the subgenomic promoters present on the minus-sense RNAs. The subgenomic RNAs are not themselves replicated, but defective RNAs and satellite RNAs are replicated by the CMV replicase. Differences in the relative levels of accumulation of the various CMV genomic RNAs and satellite RNAs have been observed in different host species, which probably is due to host-specific differences in template copying.

Movement

CMV moves cell to cell via plasmodesmata between cells until it reaches the vasculature, when the virus moves systemically via the phloem. The viral RNAs move as a nucleoprotein complex between cells involving the 3a movement protein and some involvement by the capsid protein. This movement does not involve interactions with microtubules. No specific plant proteins have yet been identified as being involved in cell-to-cell movement. CMV appears to move between epidermal cells as well as from epidermal cells down to mesophyll cells toward vascular cells. The 2b protein also influences the path of virus movement, since without the 2b protein, the virus moves preferentially to and between mesophyll cells. The virus replicates in all of these cell types, but not in the sieve elements of the vasculature. Virion assembly may take place inside sieve elements from RNAs and capsid protein moving from neighboring vascular cells. Virion assembly is necessary in some, but not all, species for systemic infection. A 48 kDa phloem protein (PP1) from cucumber phloem exudates interacts with CMV particles *in vitro* and increases virus particle stability. How the virus moves from the vasculature back to mesophyll and epidermal cells is unknown. The virus moves from plant to plant either by transmission by the aphid vectors of CMV, or in some cases via seed transmission, at a low but variable frequency.

Pathology

The symptoms induced by CMV are not generally specific to CMV, but rather reflect sets of host responses to viral pathogens. Therefore, symptoms such as light

green–dark green mosaics, generalized chlorosis, stunting, leaf filiformism, and local chlorotic or necrotic lesions associated with various strains of CMV are not specific to CMV, but can also be elicited by other viruses in the same plant species. Some strains of CMV can induce a bright yellow chlorosis in some *Nicotiana* species. This can be due to either specific amino acid changes in the viral capsid protein, or the presence of a chlorosis-inducing satellite RNA. A white-leaf disease of tomato also was due to the effects of a particular satellite RNA, as well as systemic necrosis and lethal necrosis disorders seen in the field of several Mediterranean countries. Some pathogenic responses, such as local lesions versus systemic infection in cowpea have been mapped to two amino acid changes in RNA 2, while other pathogenic responses have been mapped to sequences present in more than one viral RNA molecule, and to different viral sequences for different strains, indicating that several interactions are involved in the elicitation of some symptom responses.

Cytopathology associated with infection by CMV includes viral inclusions within the cytoplasm and vacuoles, and as membrane-bound clusters in sieve elements. In some cases, angular inclusions corresponding to virus crystals can be seen in vacuoles by staining and light microscopy. CMV infection usually also leads to proliferation of cytoplasmic membranes, which originate from the plasma membrane, the endoplasmic reticulum, or the tonoplast. Effects on the nucleus or nucleolus (e.g., vacuolation), usually due to virion accumulation, have been observed with some strains of CMV. Similarly, some strains have caused effects on mitochondria or chloroplasts. Yellowing strains, in particular, show effects on chloroplasts development leading to smaller and rounded chloroplasts that have fewer grana and starch granules. These various effects appear to be host specific. How virus–plant interactions lead to cytopathic effects remains unknown; however, as the virus expands from the initial site of infection, rings or zones of responses occur, in which the expression patterns of numerous plant genes are altered in a spatial- and temporal-specific manner.

Transmission

Seed transmission of CMV has been reported in many plant species, with efficiencies varying from less than 1% up to 50%. Virus may be present in the embryo, endosperm, and seminal integuments, as well as in pollen. RNA1, and possibly protein 1a, affects the efficiency of seed transmission.

Horizontal transmission of CMV is vectored by aphids in a nonpersistent manner. Over 80 species of aphids have been reported to transmit CMV, *Aphis gossypii* and *Myzus persicae* being two efficient and most studied vectors. Transmission efficiency depends on several factors, particularly the specific combination of virus isolate and aphid species, and the accumulation of particles in the source leaf. Differences in transmissibility of various isolates by different aphid species are determined solely by the virus coat protein and amino acid determinants for transmission have been mapped. The amino acid positions that determine transmission are either exposed on the outer surface of capsids (e.g., amino acid position 129, on the β strand H-I loop) or lay in the inner surface (e.g., position 162). Hence, the effect could be through direct interaction with the aphid mouth parts or by affecting particle stability. Loss of transmissibility upon repeated mechanical passage seems to be rare, perhaps because of tradeoffs with particle stability, but particle stability is not always correlated with transmissibility. Transmission efficiency also depends on the host plant, as shown by the resistance to transmission on melon genotypes containing the *Vat* gene, which does not confer resistance to the aphid or the virus, but impairs aphid transmission.

Ecology and Epidemiology

CMV infects a wide range of food crops, ornamentals, and wild plant species. Economic losses in crops are highest in field-grown vegetables and ornamentals, pasture legumes, and banana. In recent times, CMV has caused severe epidemics in many crops, including necrosis of tomato in Italy, Spain, and Japan; mosaic and heart rot of banana worldwide; mosaic of melons in California and Spain; mosaic of pepper in Australia and California; and mosaic of lupins and other legumes in Australia and the USA. In crops in which seed transmission is effective (e.g., pasture or fodder legumes), the primary inoculum for epidemics may be the seedlings from infected seeds. In most vegetable and ornamental crops, seed transmission does not occur or is negligible, and the primary inoculum must come from outer sources as other crops or weeds, which should be near the crop as aphid transmission is nonpersistent. In the absence of crops during unfavorable seasons, infected perennial weeds or crops, and infected seeds from weeds act as reservoir inoculum. Seed transmission has been shown to be important in several weed species from different regions. In spite of its general broad host range, there is evidence of host adaptation or preference for some CMV strains, which might have important consequences for inoculum flows among host species. Also, the dynamics of virus infection may differ largely in weeds and crops within a region, indicating that the relevant reservoirs and inoculum sources for crops need not be the most frequently infected weeds. In banana, secondary spread of infection within the

crop is ineffective for most strains, and alternative hosts are both primary and secondary inoculum sources for epidemics.

Variation and Evolution

In agreement with the high mutation rates of RNA genomes, populations of CMV derived from biologically active cDNA clones were found to be genetically diverse. When the cDNA-derived population was passaged in different hosts, the amount of genetic diversity depended on the host species. Genetic diversity has been shown to be countered by genetic drift associated to population bottlenecks during systemic colonization of the host and, probably also, during host-to-host transmission. Sequence analyses have shown different evolutionary constraints for the different viral proteins, which show different evolutionary dynamics. A second source of genetic variation is the exchange of RNA sequences by recombination or by reassortment of genomic segments. Experimentally, recombination has been shown to occur between the 3′ nontranslated region of the genomic RNAs, with recombinants being up to 11% of the population. Recombinants in the 3′ nontranslated region may have an increased fitness in some hosts, as shown for isolates infecting alstroemeria. Recombination in the RNA3 also was frequent in mixed infection between CMV and TAV. Recombination between CMV strains or CMV and TAV strains seems to be facilitated by stem–loop structures in the RNA. In spite of abundant evidence for frequent recombination, analyses of the genetic structure of field populations of CMV show that recombinant RNAs are not frequent, and that selection operates against most recombinants. A second mechanism of genetic exchange is reassortment of genomic segments also called pseudorecombination. Reassortants exchanging any genomic segment have been obtained between different CMV strains, which multiply efficiently under experimental conditions. Natural reassortants also have been described, and reassortment may have played an important role in the evolution of CMV, as suggested by the different phylogenies obtained for each genomic RNA. In field population, reassortant isolates are rare and seem to be selected against, as is the case for recombinants. Evidence for selection against genotypes originating by genetic exchange suggests co-adaptation of the different viral genes that, when disrupted, results in a decreased fitness. Analyses of the population structure of CMV in Spain and California indicate a metapopulation structure, with local extinctions and recolonizations, which suggests that population bottlenecks occur, probably associated with unfavorable seasons for the host plants and/or the aphid vectors. Interestingly, this is not the case for the population structure of the satellite RNA. Analyses in Italy and Spain during epidemics of CMV plus satellite RNAs have shown that the satellite RNAs have an undifferentiated population. The different population structure for CMV and its satellite RNAs indicates that the satellite RNAs expanded as a molecular parasite on the CMV population, rather than satellite expansion was linked to a particular CMV isolate.

Control

Control of CMV can be achieved by planting of resistant crops, but resistance in many crop species is often not available to a broad range of CMV strains. The use of insecticides to control the aphid vectors of the virus has met with only limited success, since the virus is transmitted in a nonpersistant manner and thus before the aphids would have been killed by the insecticide, it would have transmitted the virus. Rather, insecticides are used to reduce aphid numbers and thus reduce the progressive spread of infections. Since many species of weeds act as reservoirs for the virus and many of these are asymptomatic hosts, it is important to remove these from the borders of fields to eliminate the source of infectious material that could then be spread by aphids. This also applies to removal of infected crop plants during the growing season. Others sources of resistance include the use of transgenic plants expressing either protein-mediated or RNA silencing-mediated resistance. Although most of these approaches have led to resistance to only members of one of the two major subgroups, the use of pyramiding of viral segments from different subgroups offers the promise of obtaining a broad spectrum resistance to CMV together with other viruses infecting the same crop species. Transgenic expression of satellite RNAs has also been used to confer resistance to CMV. This has met with success, but has raised concerns about using a virulent pathogen, as did the use of mild strains of CMV for cross-protection against severe strains.

See also: Brome Mosaic Virus; *Ilarvirus*.

Further Reading

Edwardson JR and Christie RG (eds.) (1991) Cucumoviruses. *CRC Handbook of Viruses Infecting Legumes*, pp. 293–319. Boca Raton, FL: CRC Press.

Kaper JM and Waterworth HE (1981) Cucumoviruses. In: Kurstak E (ed.) *Handbook of Plant Virus Infections and Comparative Diagnosis*, pp. 257–332. New York: Elsevier/North-Holland.

Palukaitis P and Garcia-Arenal F (2003) Cucumoviruses. *Advances in Virus Research* 62: 241–323.

Palukaitis P and García-Arenal F (2003) Cucumber mosaic virus. In: Antoniw J and Adams M (eds.) *Description of Plant Viruses. DPV400*, Rothamstead Research, UK: Association of Applied Biologists. http://www.dpvweb.net/dpv/showdpv.php?dpvno=400 (accessed September 2007).

Palukaitis P, Roossinck MJ, Dietzgen RG, and Francki RIB (1992) Cucumber mosaic virus. *Advances in Virus Research* 41: 281–348.

Cytokines and Chemokines

D E Griffin, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Chemokine Chemotactic cytokine that regulates trafficking of cells during immune responses.
Cytokine Soluble protein produced by a variety of cells that mediates cell-to-cell communication important for innate and adaptive immune responses.
Inflammasome A multiprotein complex of more than 700 kDa that is responsible for the activation of caspases 1 and 5, leading to the processing and secretion of the pro-inflammatory cytokines IL-1β and IL-18.
Lymphotoxin A cytokin secreted by activated T_H1 cells, fibroblasts, endothelial and epithelial cells.

Introduction

Cytokines are small soluble proteins produced by a wide variety of cells and are important mediators of cell-to-cell communication. Production of these biologically potent proteins is particularly associated with generation of the innate and acquired immune responses. Cytokines regulate both the initiation and maintenance of immune responses and some also induce production of cellular antiviral molecules. Chemokines (chemotactic cytokines) regulate trafficking of leukocytes during immune responses and infiltration of leukocytes into infected tissues. Both cytokines and chemokines share the properties of pleiotropy and redundancy. Each cytokine and chemokine has more than one function and most functions can be performed by more than one of these soluble factors.

Cytokines can act at a distance, but their effects are usually most potent in the areas in which they are produced. They mediate their effects by binding to small numbers of high-affinity receptors on responding cells. Cytokine receptors have a modular design consisting of two or more chains, each with a single transmembrane-spanning domain. Individual chains may participate in formation of the receptor for more than one cytokine. Chemokine receptors are single G-protein-coupled molecules with seven-transmembrane domains. The biologic function of many cytokines and chemokines is regulated by a short half-life for both the protein and the mRNA and by the production of circulating cytokine inhibitors such as soluble forms of the cytokine receptor or biologically inactive forms of the cytokine itself. A number of viruses, particularly in the herpesvirus and poxvirus families, encode inhibitory homologs of cytokines, chemokines, or their receptors, attesting to their importance for antiviral immunity.

Overview of Cytokine Networks

Cytokines associated with the innate immune response are those induced early after infection by virus replication *per se*. One of the most important of these early immunologically nonspecific responses are type 1 (α and β) interferons (IFNs) that are generally synthesized and released by infected cells. Some cells, for example, plasmacytoid dendritic cells, produce IFN rapidly after infection. IFN-α/β can induce an antiviral state in surrounding cells and can also influence the characteristics of the subsequent adaptive immune response. In addition, phagocytic cells, responsible for processing and presentation of viral antigens to initiate the antigen-specific immune response, produce a variety of cytokines, including IFN-α/β. These antigen-presenting cells, generally dendritic cells and monocyte/macrophages, may or may not be infected or produce infectious virus. Important cytokines and chemokines produced by antigen-presenting cells in the initial phases of the response to many viral infections are tumor necrosis factor (TNF), monocyte chemotatic proteins (MCP), and interleukin (IL)-1, IL-6, IL-12, and IL-18. These soluble factors promote the activation of T cells and recruitment of inflammatory cells into areas of virus replication. IL-12 stimulates natural killer (NK) cells to produce IFN-γ, promotes differentiation of $CD8^+$ cytotoxic T lymphocytes, and influences the early development and differentiation of CD4 T cells toward a delayed type hypersensitivity cellular immune response (type 1).

$CD4^+$ and $CD8^+$ T lymphocytes are the effectors of the specific cellular immune response and function primarily through synthesis of cytokines. T cells do not produce cytokines constitutively, but upregulate expression in response to stimulation through the T-cell receptor. $CD8^+$ T cells are stimulated by viral antigens presented in association with class I major histocompatibility (MHC) antigens. This type of stimulation is most likely to occur if the virus replicates in the antigen-presenting cell because antigens presented in association with MHC class I must be processed by proteasomes in the cytosol. $CD8^+$ T cells often have cytotoxic activity through production of granzymes and perforin or Fas ligand, but also produce substantial amounts of IFN-γ and lymphotoxin (LT). Viral antigens are presented to $CD4^+$ T cells as

peptides in association with class II MHC antigens. Processing of antigens for presentation in association with MHC class II occurs through an endosomal pathway; therefore, virus replication is not required. Activated $CD4^+$ T cells produce IL-2, a T-cell growth factor that supports proliferation of both $CD4^+$ and $CD8^+$ T cells.

With further development of the virus-specific immune response, $CD4^+$ T cells tend to differentiate into different types of T-helper (Th) cells. Type 1 $CD4^+$ T (Th1) cells develop under the influence of IFN-γ and produce primarily IFN-γ and TNF-β (LT-α) and are associated with classic delayed type hypersensitivity or cellular immune responses. Type 2 $CD4^+$ T cells develop under the influence of IL-4 and produce primarily IL-4, IL-5, and IL-13 and are associated with strong antibody responses to viral antigens. A third type of $CD4^+$ effector cells (Th17) develop under the influence of transforming growth factor (TGF)-β and produce IL-17 and IL-6 and are associated with inflammation and autoimmune disease. Cytokines produced by $CD4^+$ T cells are necessary for B-cell development and for the switch from B cells producing IgM to B cells producing more mature forms of immunoglobulin such as IgG, IgA, and IgE and for B cell maturation into long-lived antibody-secreting cells. Both $CD4^+$ and $CD8^+$ T-cell responses are associated with mononuclear infiltrates into areas of virus replication. Regulatory T cells control and dampen ongoing immune responses, in part, through production of IL-10 or TGF-β.

Brief Description of Cytokine Families

TNF

The TNF family has three members: TNF-α, TNF-β/LT-α, and LT-β. These cytokines have approximately 30% homology and interact with cellular receptors in the TNF receptor (TNFR) superfamily. Activated macrophages are the major source of TNF-α and T cells are the major source of LT. TNF-α is synthesized as a proprotein (26 kDa) which is membrane bound and cleaved by a specific multidomain cell-surface metalloprotease to yield a 17 kDa protein. The active form of the soluble protein is a cone-shaped trimer that can bind to either of two TNFRs (TNFR1, p55 or TNFR2, p75). Binding of TNF to TNFR1 induces nuclear factor kappa B (NF-κB) and can trigger apoptosis. Homotrimers of LT-α also bind to TNFR1 and TNFR2, but LT-β reacts with a separate member of the TNFR family, the LT-β receptor. TNF interacts with a wide variety of cells systemically and locally and induces pro-inflammatory cellular responses such as expression of adhesion molecules, release of cytokines, chemokines and procoagulatory substances, synthesis of acute phase proteins, and production of fever. TNF also has some antiviral activity and is an important early participant in the immune response to infection. In bacterial infections, TNF is one of the mediators of endotoxin-induced (septic) shock. TNF and LT also play an important role in development of lymphoid tissues because lymphoid architecture in mice with deletions of these genes is abnormal.

IFN-γ

IFN-γ is a glycosylated protein of 25 kDa that is produced by NK cells and by type 1 CD4 and CD8 T cells (immune IFN). Although IFN-γ induces antiviral activity, it is structurally unrelated to IFN-α/β and uses a distinct receptor. However, IFN-γ shares some components of the intracellular signaling pathways for type 1 IFN. The biologically active form of IFN-γ is a dimer. A primary role for IFN-γ is the activation of macrophages to increase phagocytosis, tumoricidal properties, and intracellular killing of pathogens, particularly bacteria and fungi. IFN-γ induces macrophage production of a variety of inflammatory mediators and reactive oxygen and nitrogen intermediates. IFN-γ increases expression of high-affinity immunoglobulin F_c receptors on phagocytes, which increases recognition of opsonized microorganisms by these cells. IFN-γ also increases expression of MHC antigens by macrophages and this facilitates antigen presentation to T cells. The IFN-γ cell-surface receptor is composed of two chains, IFN-γR1 (α) and IFN-γR2 (β). The α (R1) chain is sufficient for binding, but the β (R2) chain is required for signaling and receptor complex formation. T-cell responsiveness is regulated by receptor expression. Both Th1 and Th2 cells express IFN-γR1, but only Th2 cells express IFN-γR2. Therefore, Th1 cells produce, but do not respond to, IFN-γ while Th2 cells respond to, but not produce this cytokine. Macrophages express both receptor chains and are a primary target of IFN-γ activity.

IL-1

The IL-1 family has three members: IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1RA). IL-1α and IL-1β have only distantly related amino acid sequences, but similar structure, and recognize the same receptors. IL-1RA binds the IL-1 receptor without transducing a signal and blocks the activities of IL-1α and IL-1β. Activated monocytes and macrophages are the major source of IL-1, although many other cells can produce this cytokine. IL-1β is synthesized as a precursor protein of 31 kDa that is processed by the caspase interleukin-1 β-converting enzyme (caspase-1) within the 'inflammasome' to the active secreted 17 kDa form. IL-1 acts systemically, as well as locally, and can produce fever, sleep, and anorexia, frequent symptoms of viral infection. Hepatocytes are among the cells that produce IL-1RA as a part of the acute-phase response to inflammation and infection, presumably to control the effects of IL-1. There are two IL-1 receptors but only IL-1R1 transduces an activation

signal to responding cells. IL-1R2 appears to function as a decoy receptor that regulates IL-1 activity by binding the cytokine without transducing a signal.

IL-18, also known as IFN-γ-inducing factor, is an 18 kDa cytokine produced by macrophages that shares structural features with the IL-1 family of proteins. It is synthesized as a proprotein that is cleaved by caspase 1 to its biologically active form. IL-18 stimulates IFN-γ production by T cells, NK cytotoxicity, and T-cell proliferation.

IL-2

IL-2 is a unique 15 kDa cytokine produced primarily by activated type 1 $CD4^+$ T cells. IL-2 plays an important role in supporting proliferation of activated helper T cells, cytotoxic T cells, B cells, macrophages, and NK cells. For stimulated $CD4^+$ T cells, IL-2 acts in an autocrine fashion because Th1 cells can produce IL-2 and upregulate high-affinity IL-2 receptors as well. The high-affinity IL-2R consists of three chains – IL-2Rα, β, and γ – which are expressed on the surface of activated T cells. IL-2Rα is a unique protein, whereas IL-2Rβ and IL-2Rγ are members of the hematopoietic growth factor receptor superfamily. Members of this superfamily share a 210-amino-acid domain in the extracellular region of the molecule that contains a distinct cysteine and tryptophan motif at the N-terminus and a Trp-Ser-X-Trp-Ser motif at the C-terminus. The whole domain is folded into two barrel-like structures with the ligand-binding region between. In addition to the IL-2 receptor, this receptor superfamily includes components of receptors for cytokines IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, granulocyte macrophage-colony stimulating factor (GM-CSF), and granulocyte-colony stimulating factor (G-CSF). Signal transduction is through the IL-2Rβ chain. Intermediate-affinity IL-2R βγ receptors are expressed on monocytes and NK cells. IL-2Rγ (γ constant) also participates in the formation of the multichain IL-4, IL-7, IL-9, and IL-15 receptors. Defects in expression of this molecule lead to severe combined immunodeficiency in humans. Binding of IL-2 to the high-affinity receptor leads to T-cell proliferation.

IL-4

There are five members of the IL-4 family of cytokines: IL-3, IL-4, IL-5, IL-13, and GM-CSF. These cytokines have approximately 30% protein sequence homology, have similar α-helical structures, are linked in the same chromosomal region, and are produced by type 2 $CD4^+$ T cells. IL-3 and GM-CSF are two of the four major human myeloid growth factors that also include G-CSF and M-CSF. IL-3 is produced by activated T cells and binds to a heterodimeric receptor. The β subunit of the IL-3R is shared with the receptors for IL-5 and GM-CSF.

IL-4 and IL-13 have similar effects on B cells and monocytes. Both can serve as a costimulating factor for B-cell proliferation, drive activated B cells into immunoglobulin secretion, and, in human B cells, induce class switching to IgG4 and IgE. IL-4 is regarded as an anti-inflammatory cytokine because it antagonizes the effects of IFN-γ on macrophages and downregulates type 1 $CD4^+$ T-cell responses. IL-13 induces monocyte proliferation and monocytosis *in vivo*. IL-4 is important for promoting the differentiation of $CD4^+$ T cells to type 2 cytokine production. IL-4 and IL-13 are produced by type 2 $CD4^+$ T cells. IL-4 is also produced by a subset of $CD4^+$ T cells that are $NK1.1^+$ (NK T cells), by type 2 $CD8^+$ T cells and by mast cells, basophils, and eosinophils.

IL-5

IL-5, along with IL-3 and GM-CSF, acts as an eosinophil stimulating factor and its receptor shares a common β chain with these cytokines. The high-affinity IL-5R has a unique α chain. IL-5 induces proliferation and differentiation of eosinophil progenitors, whereas IL-3 and GM-CSF probably act at earlier stages of development. IL-5 is produced by type 2 $CD4^+$ and $CD8^+$ T cells and by NK cells. IL-5 also induces antigen-stimulated B cells to differentiate into immunoglobulin-secreting plasma cells and enhances secretion of IgA.

IL-6

IL-6 is a member of a family of cytokines and neuronal differentiation factors that includes leukemia inhibitory factor, oncostatin M, IL-11, and ciliary neurotrophic factor. The receptors for these cytokines share the transmembrane protein gp130 that transduces the cytokine signal. IL-6 is a functionally diverse cytokine produced by macrophages and monocytes early in the response to infection. It induces hepatic synthesis of acute phase proteins, supports proliferation of B cells, and has an important role in production of IgA.

IL-7

IL-7 is a 15 kDa protein that is produced primarily by epithelial cells and supports the growth and development of immature B and T cells. IL-7 is produced by bone marrow stromal cells, thymic epithelial cells, keratinocytes, and intestinal epithelial cells and supports lymphocyte development in these locations. In the thymus, IL-7 induces rearrangement of germline T-cell receptor genes by upregulating expression of the RAG-1 and RAG-2 recombinases. The α chain of the IL-7 receptor is a member of the hematopoietic growth factor family and associates with the IL-2Rγ chain to form a functional high-affinity IL-7R.

IL-10

IL-10 is an 18 kDa polypeptide that forms homodimers. It is predicted to be a member of the 4 α–helix bundle family of cytokines. It is produced by a wide variety of cells, including type 2 $CD4^+$ and $CD8^+$ cells, B cells, mast

cells, macrophages, and keratinocytes. Epstein–Barr virus encodes a viral homolog of IL-10 that is functionally active. The activities of IL-10 are diverse, but its principal function appears to be limitation of inflammatory responses. IL-10 inhibits cytokine production by Th1 cells, NK cells, and macrophages and has been associated with viral persistence. It suppresses the synthesis of TNF-α, IL-1α, IL-6, IL-8, GM-CSF, and G-CSF by macrophages while increasing the production of IL-1RA. IL-10 stimulates proliferation and differentiation of activated B cells and T cells and of mast cells and is important for differentiation of regulatory T cells. The functional IL-10R consists of two subunits, IL-10R1 and IL-10R2, both of which belong to the class II cytokine receptor family. Expression of both subunits is required for signal transduction.

IL-12

IL-12 is a structurally unique heterodimeric cytokine composed of two disulfide-linked 35 and 40 kDa polypeptides. The 40 kDa protein conveys receptor-binding activity, is shared with IL-23 and is constitutively produced by many cells. Only the heterodimer is biologically active, so regulation of synthesis of the 35 kDa chain controls IL-12 activity, while synthesis of the IL-23 19 kDa chain controls IL-23 activity. IL-12 is produced by macrophages and dendritic cells and stimulates the production of IFN-γ by NK and T cells. IL-12 is important for differentiation of $CD4^+$ T cells into Thl cells and IL-23 promotes differentiation into Th17 cells. At least two classes of the IL-12 receptor exist. The IL-12R β1 chain is expressed in both Th1 and Th2 cells while the β2 chain is expressed only in Th1 cells. The IL-23 receptor is composed of IL-12β1 and a unique IL-23R subunit. For IL-12 responsiveness and for T-cell differentiation, both β1 and β2 subunits are necessary so only potential Th1 cells respond to this cytokine. Both IL-12R β1 and β2 subunits are related to the gp130 group of cytokine receptors. Homodimers of p40 bind with high affinity to the IL-12R, transduce no signal and block the effect of the heterodimer.

IL-15

IL-15 is a novel cytokine functionally related to IL-2, but without significant IL-2 sequence homology. It binds to a receptor with the β and γ chains of the IL-2R, but has its own unique α chain. IL-15 induces T-cell proliferation, enhances NK cell function, is a potent T-lymphocyte chemoattractant, and stimulates production of IL-5. Unlike IL-2, it is produced primarily by monocyte/macrophages and endothelial cells.

IL-17

IL-17 (IL-17A) is the founding member of a family of six cytokines (IL-17A-F). IL-17 is a 155-amino acid glycoprotein that functions as a disulfide-linked 35 kDa homodimer. IL-17 is produced by a separate lineage of activated $CD4^+$ (Th17) and $CD8^+$ T cells that have been implicated in autoimmune disease.

Chemokines and Chemokine Families

The chemokine superfamily includes a large number of structurally related, basic, heparin-binding, small molecular weight (8–10 kDa) proteins that have potent chemotactic and immunoregulatory functions. Chemokines attract specific, but overlapping, populations of inflammatory cells, induce integrin activation, and can be divided into four families with different biologic activities based on the spacing of the first two of four conserved disulfide-forming cysteine residues in the amino-terminal part of the molecule. The largest groups are the CXC or α-chemokines and the CC or β-chemokines. In general, CXC chemokines are the primary attractants for neutrophils, whereas CC chemokines modulate responses of monocytes, macrophages, lymphocytes, basophils, and eosinophils. C chemokines have only one set of cysteines and are chemotactic for lymphocytes. The CX3C chemokine fractalkine is unusual in that it is a type 1 transmembrane protein with the chemokine domain on top of an extended mucin-like stalk. Herpesviruses and poxviruses encode viral chemokine binding proteins (vCKBPs) that are important regulators of the host immune response.

α-Chemokines

The first CXC chemokine to be identified was platelet factor 4 (PF4, CXCL4) that is stored in the granules of platelets that also contain neutrophil-activating protein (NAP-2, CXCL7) ready to be released at sites of tissue injury. The best-characterized α-chemokine is IL-8 (CXCL8) that can be rapidly induced in most cells and is also chemotactic for neutrophils. Other α-chemokines with chemotactic activity for neutrophils include GRO-α (CXCL1), GRO-β (CXCL2), GRO-γ (CXCL3)γ, epithelial cell-derived neutrophil activating protein (CXCL5), and granulocyte chemotactic protein-2 (CXCL6). Sequence identities between these various family members range from 24% to 46%. IP-10 (CXCL10) is a structurally similar, but functionally distinct, CXC chemokine that is an attractant for monocytes and T cells rather than granulocytes.

β-Chemokines

The CC chemokines have two large subgroups: the MCPs and the macrophage inflammatory proteins (MIPs). MCP-1 (CCL2), produced by endothelial cells in response to IL-1β and TNF-α, functions as a dimer, attracts and activates monocytes, NK cells, $CD4^+$, and $CD8^+$ T cells, promotes

Th2 responses and is a potent histamine-releasing factor for basophils. MCP-2 (CCL8) and MCP-3 (CCL7) are structurally and functionally related chemokines important in Th2 responses, whereas MIP1α (CCL3) and MIP1β (CCL4) are important in Th1 responses. Other CC chemokines commonly increased during viral infection are RANTES (CCL5) and I-309 (CCL1). Sequence identities between family members are 29–71%. These chemokines activate integrins, thereby inducing or increasing adhesion to endothelial cells or extracellular matrix proteins to promote development of an inflammatory response. Two novel, related β chemokines, MCP-4 (CCL13) and eotaxin (CCL11), are preferential chemoattractants for eosinophils. CCL11 is produced by epithelial and endothelial cells, often in concert with IL-5.

Chemokine Receptors

Chemokine receptors are seven-transmembrane, G-protein-coupled molecules that signal through the pertussis toxin-sensitive Gαi subfamily of hetereotrimeric G proteins. In general, the α chemokine receptors (CXCRs) are distinct from the β receptors (CCRs). Each receptor can bind multiple chemokines within its class and each chemokine can bind multiple receptors. Therefore, the specificity of cellular attraction to a site and subsequent activation is probably achieved by selective mixtures of chemokines and cellular receptors. These molecules serve as secondary receptors for entry of human immunodeficiency virus into T cells and monocytes.

CXCR5 and CCR7 and their ligands (CXCL13, CCL19, and CCL21) are major regulators of dendritic cell and lymphocyte trafficking to secondary lympoid tissue. Th1 cells more frequently express CXCR3, CXCR6, CCR2, CCR5, and CX3CR1 than Th2 cells which more frequently express CCR3, CCR4, and CCR8. Tissue-specific homing is chemokine dependent. For instance, T cells that home to skin preferentially express CCR4 and CCR10, whereas their ligands CCL2 and CCL27 are produced by dermal macrophages, dendritic cells, and keratinocytes. B cells producing IgG upregulate CXCR4 which promotes homing.

See also: Entomopoxviruses; Epidemiology of Human and Animal Viral Diseases; Epstein–Barr Virus: General Features; Epstein–Barr Virus: Molecular Biology; Immune Response to viruses: Antibody-Mediated Immunity; Immune Response to viruses: Cell-Mediated Immunity; Immunopathology.

Further Reading

Boehm U, Klamp T, Groot M, and Howard JC (1997) Cellular responses to interferon-gamma. *Annual Review of Immunology* 15: 747–795.
Brown MA and Hural J (1997) Functions of IL-4 and control of its expression. *Critical Reviews in Immunology* 17: 1–32.
Del Prete G (1998) The concept of type-1 and type-2 helper T cells and their cytokines in humans. *International Reviews of Immunology* 16: 427–455.
Dinarello CA (1996) Biologic basis for interleukin-1 in disease. *Blood* 87: 2095–2147.
Kolls JK and Linden A (2004) Interleukin-17 family members and inflammation. *Immunity* 21: 467–476.
Moore KW, de Waal Malefyt R, Coffman RL, and O'Garra A (2001) Interleukin-10 and the interleukin 10 receptor. *Annual Review of Immunology* 19: 683–765.
Murphy PM (2003) Chemokines. In: Paul WM (ed.) *Fundamental Immunology,* 5th edn., pp. 801–840. Philadelphia: Lippincott Williams and Wilkins.
Sugamura K, Asao H, Kondo M, *et al.* (1996) The interleukin-2 receptor gamma chain: Its role in the multiple cytokine receptor complexes and T cell development in XSCID. *Annual Review of Immunology* 14: 179–205.
Taga T and Kishimoto T (1997) Gp130 and the interleukin-6 family of cytokines. *Annual Review of Immunology* 15: 797–819.
Trinchieri G (1998) Immunobiology of interleukin-12. *Immunologic Research* 17: 269–278.
Weaver CT, Harrington LE, Mangan PR, Gavriell M, and Murphy KM (2006) Th17: An effector CD4 T cell lineage with regulatory T cell ties. *Immunity* 24: 677–688.

Cytomegaloviruses: Murine and Other Nonprimate Cytomegaloviruses

A J Redwood, L M Smith, and G R Shellam, The University of Western Australia, Crawley, WA, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cytomegalia From Greek *kytos*, cell, and *megas*, large. Refers to the cellular enlargement or swelling (cytomegalia) seen in cytomegalovirus-infected cells. This swelling is typically accompanied by the presence of intranuclear inclusion bodies, which are sometimes called 'owl eye' inclusion bodies because the dense staining inclusion body is surrounded by a cleared halo.

Classification of Cytomegaloviruses

Cytomegaloviruses (CMVs) are large, enveloped, double-stranded DNA viruses with an icosahedral capsid that belong to subfamily *Betaherpesvirinae* of the family *Herpesviridae*. There are three genera. Genus *Cytomegalovirus* contains human cytomegalovirus (HCMV; species *Human herpesvirus 5*) and a number of other primate CMVs. Genus *Muromegalovirus* contains murine cytomegalovirus (MCMV; *Murid herpesvirus 1*) and rat cytomegalovirus (RCMV; *Murid herpesvirus 2*). Genus *Roseolovirus* contains human herpesviruses 6 and 7 (HHV-6 and HHV-7; *Human herpesvirus 6* and *Human herpesvirus 7*). Other CMVs that have not yet been fully classified within the *Betaherpesvirinae* are guinea pig cytomegalovirus (GPCMV; *Caviid herpesvirus 2*), tree shrew herpesvirus (THV; *Tupaiid herpesvirus 1*), swine cytomegalovirus (SuHV-2; suid herpesvirus 2), European ground squirrel cytomegalovirus (ScHV-1; sciurid herpesvirus 1), and American ground squirrel cytomegalovirus (ScHV-2; sciurid herpesvirus 2).

The recognized nonprimate CMVs are described in **Table 1**. With the exception of MCMV, RCMV, and GPCMV, little is known about the life cycle and pathogenesis of nonprimate CMVs. Nevertheless, general characteristics include strict species specificity, an ability to induce cytomegalia in infected cells, cell-associated replication in cell culture, a slow replication cycle and the establishment of persistent and latent, lifelong infection in the natural host. Tropism for secretory glands, particularly the salivary gland, is also a common feature. Infection is generally asymptomatic unless the host is immunosuppressed or has an immature immune system. In such hosts, infection may result in morbidity or even mortality.

Table 1 Classification of known and putative nonprimate CMVs by the International Committee on Taxonomy of Viruses

Family	00.031	*Herpesviridae*
Subfamily	00.031.2	*Betaherpesvirinae*
Genus	00.031.2.02	*Muromegalovirus*
Examples	00.031.2.02.001	*Murid herpesvirus 1* (murine cytomegalovirus)
	00.031.2.02.002	*Murid herpesvirus 2* (rat cytomegalovirus)
Unassigned in the subfamily	00.031.2.00.004	*Caviid herpesvirus 2* (guinea pig cytomegalovirus)
	00.031.2.00.049	*Tupaiid herpesvirus 1* (tree shrew herpesvirus)
Unassigned in the family	00.031.0.00.062	Suid herpesvirus 2 (swine cytomegalovirus)
	00.031.0.00.063	Sciurid herpesvirus 1 (European ground squirrel cytomegalovirus)
	00.031.0.00.046	Sciurid herpesvirus 2 (American ground squirrel cytomegalovirus)

Murine Cytomegalovirus

Murid herpesvirus 1 is the type species of the genus *Muromegalovirus*. The term murine cytomegalovirus (MCMV) is more commonly used for the virus than murid herpesvirus 1. The natural host for MCMV is the house mouse, *Mus musculus domesticus*. Because of the strict species specificity of CMVs, MCMV is widely used as an animal model of HCMV infection. Consequently, more is known about MCMV than any other nonprimate CMV.

Virion Structure and Morphology

CMV virions are spherical, *c.* 230 nm in diameter, and comprise four morphologically distinct elements: the core, capsid, tegument, and envelope. The core encompasses the double-stranded DNA viral genome, which is packaged as a single linear molecule into the protein capsid.

The viral capsid is composed of 162 capsomers comprising hexons and pentons in a $T = 16$ icosohedral lattice structure. The capsids of CMVs are larger and incorporate a larger genome than other herpesviruses. Unlike other CMVs, MCMV preparations may contain a high proportion of multicapsid virions, which contain a number of capsids enclosed within a common membrane. The capsid of MCMV is composed of five proteins: the major capsid protein (MCP), the minor capsid protein, the minor capsid binding protein, the smallest capsid protein, and an assembly/protease. These are encoded by genes *M86*, *M85*, *M46*, *M48.2*, and *M80*, respectively.

The tegument of MCMV is a proteinaceous layer of material between the capsid and envelope, and resembles the matrix of other viruses. By electron microscopy the tegument is seen to have an ordered structure, particularly proximal to the capsid. The tegument proteins of MCMV have been defined by their homology to known HCMV tegument proteins. In HCMV there are at least 25 proteins associated with the tegument. Typically, tegument proteins are phosphorylated (and have the prefix pp). At least nine MCMV tegument proteins with homologs in HCMV have been detected in MCMV virions. These are the upper and lower matrix phosphoproteins (encoded by *M82* and *M83*), large tegument protein (*M48*), pp150 (*M32*), and the gene products from *M25*, *M47*, *M51*, *M94*, and *M99*. The role of most tegument proteins is unknown, but the function of some can be inferred from the function of HCMV homologs. For example, the upper matrix protein of HCMV, also known as pp71, is a transcriptional transactivator that regulates immediate early gene expression, possibly by

inhibiting the effects of the host cell transcriptional inhibitor hDaxx. Other tegument phosphoproteins are likely to play similar roles in the transcriptional regulation of viral genes.

The envelope of MCMV is composed predominantly of lipids obtained from the intracellular membranes of the host cell and contains a considerable number of virus-encoded glycoproteins. The envelope of CMVs is more pleiomorphic, and contains more glycoproteins, than envelopes of other herpesviruses. The major envelope glycoprotein, a product of the *M55* gene, is the highly conserved glycoprotein B (gB) and is a dominant B-cell antigen in CMV-infected animals and humans. It has been found in every mammalian herpesvirus and is one of the most highly conserved herpesvirus proteins. The glycoproteins of CMVs form three distinct complexes that mediate viral attachment and entry into host cells (**Table 2**).

Genome Architecture and Coding Potential

The MCMV genome comprises a single unique sequence with short terminal direct repeats and several short internal repeats. Unlike that of HCMV, the linear genome of MCMV does not have an isomeric structure because it lacks internal repeat sequences related to the terminal repeats. The nomenclature devised for MCMV genes numbers them left to right along the genome. MCMV genes with homologs in HCMV are assigned the uppercase prefix '*M*', while genes with no sequence identity with HCMV genes are identified by the lowercase prefix '*m*'.

The genome of the Smith strain of MCMV is 230 278 bp in size, and has a coding potential estimated to be between 170 and 204 open reading frames (ORFs), including newly defined splice variants of previously described ORFs. MCMV shares with other members of the herpesvirus family a number of evolutionarily conserved proteins, which are involved in processes such as DNA replication and virion maturation and structure, and are located within the central region of the genome. The two terminal regions of the genome contain genes that are unique to MCMV and include the *m02* and *m145* gene families, respectively (**Figure 1**). Many of the genes in these unique regions encode proteins that are involved in modulation of the host's immune response.

Replication

Viral replication is initiated when infectious virions enter a susceptible host cell (**Figure 2**). A series of receptors have been implicated in the binding, fusion, and entry of CMVs. Most information has come from studies of HCMV, in which infection is initiated by loose tethering of the virus via viral gCI and gCII complexes and heparan sulfate proteoglycans (HSPGs) on the cell surface. Tethering promotes a stronger binding reaction between the viral gCI complex and host cellular receptors. These receptors have not been fully defined, although for HCMV the epidermal growth factor receptor (EGFR) is thought to be one of the host receptors important in viral binding. This receptor binding promotes receptor co-localization with other host proteins, in particular integrins, into lipid rafts. The β1 and β3 chains of integrins have been implicated in the entry of MCMV into host cells. Integrin-binding domains have been localized to the N-terminus of gB in all betaherpesviruses and most gammaherpesviruses. Finally, receptor binding and/or co-localization triggers intracellular signaling and fusion between the host cell and viral membranes. Cellular signaling appears important for translocation of the capsids to the nucleus.

Table 2 Structural glycoproteins of MCMV

Glycoprotein (gene)	*Complex*	*Essential*	*Function*
gB (*M55*)	gCI	Yes	Binds to cellular receptors (e.g., EGFR) and triggers intracellular signaling. *May activate host TLR2. Major B-cell epitope*
gN (*M73*)	gCII	Yes	With gM binds HSPG
gM (*M100*)	gCII	Yes	With gN binds HSPG
gH (*M75*)	gCIII	Yes	As a component of the gCIII complex is involved in binding and membrane fusion. *May activate host TLR2*
gL (*M115*)	gCIII	Yes	Required for transport of gH to the gCIII complex. As a component of the gCIII complex is involved in binding and membrane fusion
gO (*m74*)	gCIII	No; deletion virus has small plaque size	Enhances cell-to-cell spread of MCMV. As a component of the gCIII complex is involved in binding and membrane fusion
gp24 (*m73.5*)		Unknown	Unknown

The data are a compilation of information from HCMV and MCMV. Text in italics denotes the host response to glycoproteins. In HCMV, gB forms the disulfide-linked homodimeric gCI complex. The gCII complex is a disulfide-linked heterodimer between gM (*UL100*) and gN (*UL73*). The HCMV glycoproteins gL, gH, and gO (encoded by the genes *UL115*, *UL75,* and *UL74,* respectively) form the noncovalently associated heterotrimeric complex gCIII. All three complexes are essential for viral replication. MCMV homologs are believed to serve a similar function, although gN has only been demonstrated in the virions of HCMV. Note that gp24 has only been demonstrated in MCMV. In HCMV, gB and gH appear to bind TLR2, but this has not been demonstrated for MCMV.

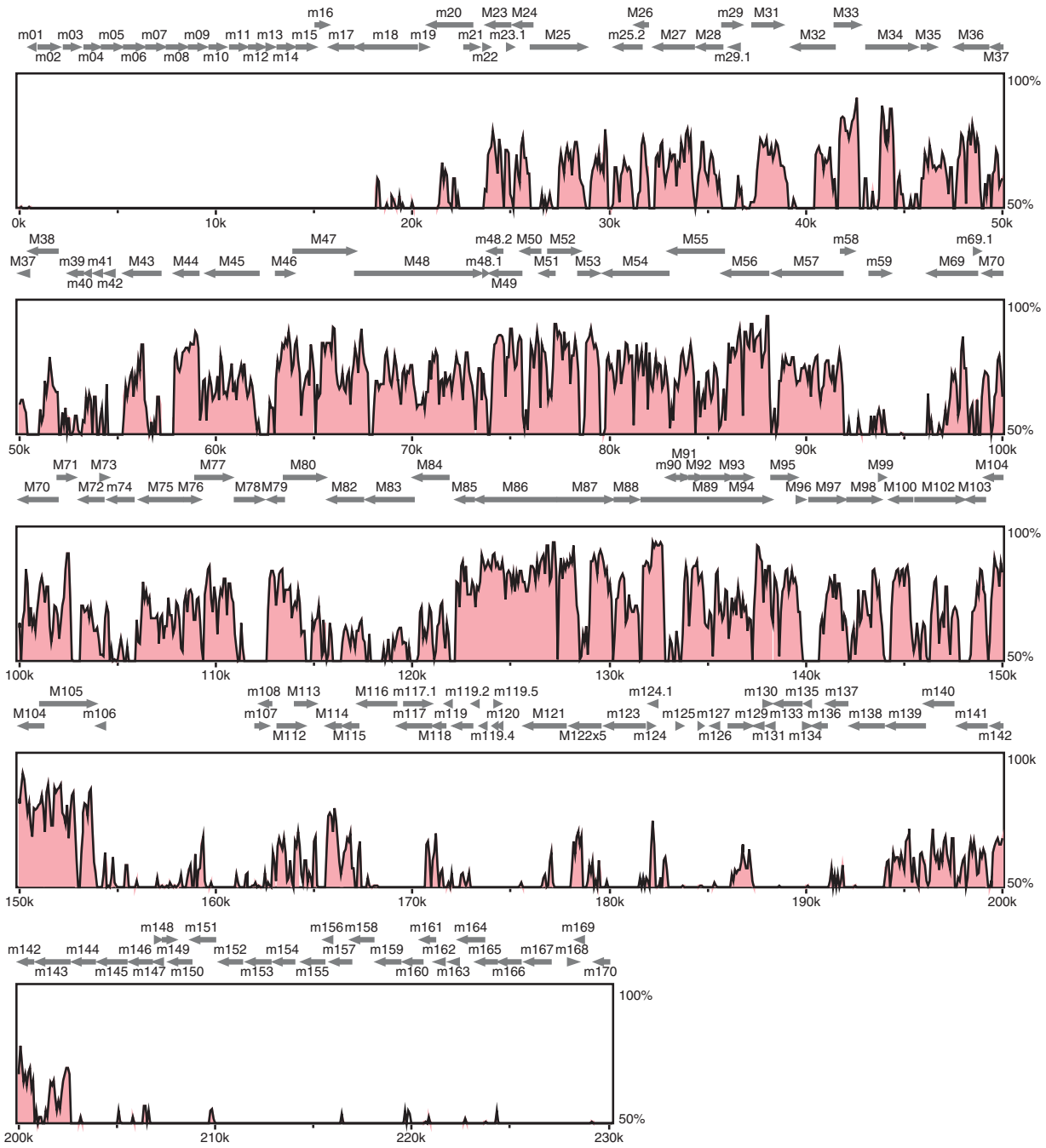


Figure 1 Moving window comparison of the sequence similarity (shown from 50% to 100%) of the MCMV and RCMV genomes. Selected MCMV genes are marked with an arrow above the plot. The central regions of the genome are highly conserved and represent the herpesvirus-conserved genes. The left and right terminal sequences contain species-specific genes and therefore show little or no sequence similarity. Image created using LAGAN and VISTA (http://genome.lbl.gov/vista/index.shtml).

The exact mechanism by which viral DNA enters the nucleus is unknown. However, it is believed that viral DNA migrates to nuclear domain 10 (ND10) complexes within the nucleus where viral gene transcription occurs. All betaherpesviruses have three gene sets, α, β, and γ, that are temporally regulated. These genes are expressed in the immediate early (IE), early (E), and late (L) phases of viral replication, respectively. The IE phase occurs immediately after viral DNA enters the nucleus and is controlled by the major IE promoter (MIEP) in MCMV. The MIEP controls expression of the transcriptional activator genes *ie1* (*m123*) and *ie3* (*M122*). While both the *ie1* and *ie3* genes are essential for replication, the former is required specifically at low multiplicities of

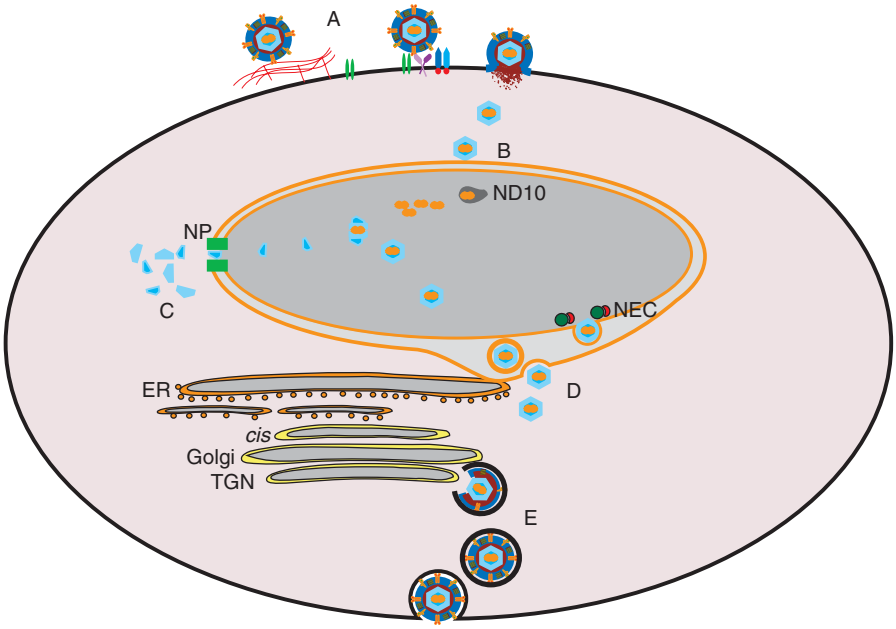


Figure 2 CMV life cycle. (A) Virus is loosely tethered to the host cell by binding to HSPG via either gB (gCI complex) or gM/gN (gCII complex). Virus is bound more tightly by association with cell receptors such as EGFR (HCMV). Binding causes receptor clustering and the interaction of other receptors such as host cell integrins. The interaction between host receptors and viral glycoproteins induces membrane fusion and the delivery of viral capsid and tegument proteins into the host cell cytoplasm. (B) Viral capsid is transported to the nucleus and, by a process that is not understood, the viral DNA enters the nucleus. Viral DNA is localized at ND10 complexes where viral gene expression is initiated. (C) Viral proteins, including capsid proteins, are produced in the cytoplasm. DNA encapsidation and capsid assembly occur in the nucleus. Capsid proteins either diffuse into the nucleus via nuclear pores or are transported due to the presence of nuclear localization motifs. Large capsid proteins, such as MCP, which lack these motifs, are transported to the nucleus in association with capsid proteins that possess them. (D) Viral capsids acquire a primary envelope as they migrate through the inner nuclear membrane (INM). Viral proteins p35 and p38 (*M50* and *M53*) form the nuclear egress complex (NEC) and serve to disassociate the INM. Transport of the capsid through the outer nuclear membrane results in de-envelopment. (E) The final viral envelope is derived from cytoplasmic organelles, possibly in the *trans*-Golgi network (TGN). Fully formed virions enter the secretory pathway and exit the cell by exocytosis.

infection. A third IE gene, *ie2*, not present in HCMV, is transcribed from a different promoter and in the opposite direction. The *ie2* gene is not essential for MCMV replication either *in vitro* or *in vivo*.

There is an absolute requirement for IE gene expression prior to E gene expression. Genes transcribed during the E phase of viral replication include those required for entry into the L phase of viral replication, and other genes, such as the immune evasion genes. L phase genes mostly encode structural proteins and are expressed after the start of viral DNA replication, which occurs in the nucleus approximately 16 h after infection. During DNA replication, the sequences at the genomic termini fuse via a 3′ nucleotide extension to form the intermediates for MCMV replication, which occurs by a rolling-circle mechanism. Maturation of the MCMV genome involves the processing and cleavage of newly synthesized concatemeric viral DNA into genome-length monomers prior to packaging into preformed nucleocapsids in the cell nucleus. Herpesvirus-conserved *pac1* and *pac2* DNA sequence motifs are required for cleavage and packaging of the MCMV genome.

The origin of DNA replication of MCMV is the *ori* Lyt region between *M57* and *M69* (**Figure 1**), which extends over 1.7 kbp and is extremely rich in repeat sequences that act as binding elements for various transcription factors. MCMV-encoded proteins required for origin-dependent replication include the DNA polymerase (*M54*), a polymerase accessory protein (*M44*), the single-stranded DNA-binding protein (*M57*), and a helicase-primase complex encoded by *M70*, *M102*, and *M105*. All of these proteins have been detected within purified MCMV virions.

The formation of capsids and the packaging of viral DNA occur in the nucleus of infected cells. Capsid proteins are produced in the cytoplasm and are transported back to the nucleus across the nuclear membrane. The transport of capsid proteins is a result of either their small size, which allows diffusion across the nuclear pore complex, or the presence of nuclear localization signals. Large capsid proteins such as the MCP, which do not contain nuclear localization signals, are transported in association with those that do. Viral DNA is packaged into complete capsids and transported to the cytoplasm via the nuclear membrane where the capsids acquire their primary

envelope as they bud through the inner nuclear membrane (INM) as shown in **Figure 2**. In MCMV infection, the virus penetrates the INM with the aid of *M50*/p35 and a partner gene, *M53*/p38. The proteins p35 and p38 form the nuclear egress complex (NEC) and recruit cellular kinases to the INM, specifically to the nuclear lamina. Recruitment of cellular kinases results in phosphorylation and degradation of the lamina, and facilitates egress of the virus from the nucleus.

The mechanism of egress of CMVs from infected cells is incompletely understood. Studies from other herpesviruses suggest that there are two phases of envelopment. A primary envelopment involving the host's INM precedes a final secondary envelopment from another host compartment, possibly the *trans*-Golgi network (TGN), where the virions also gain their tegument. The mature enveloped virions, now present in secretory vesicles, are transported to the plasma membrane, where they are released into the extracellular space by exocytosis (**Figure 2**).

Pathogenesis

During acute experimental intraperitoneal infection, MCMV replicates predominantly in the spleen and liver and to a lesser extent in the lungs. However, the virus persists in the lungs for longer than in the spleen or liver. Other organs infected during the acute phase include the adrenal glands, kidneys, heart, and ovaries. During the chronic or persistent phase, the virus replicates predominantly in the salivary gland. Intranasal inoculation, which may mimic natural infection, results in viral replication predominantly in the lungs and salivary gland. Histologically, MCMV-infected cells exhibit typical swelling or cytomegalia (from which the virus derives its name) with intranuclear inclusion bodies. In MCMV-infected mice, mononuclear cell infiltration may be observed in inflammatory responses in the heart, lung, adrenals, and other organs.

The seroprevalence of MCMV in free-living mice can be up to 100%, particularly when mouse population densities are high. Transmission of the virus is presumed to be via saliva, possibly as a result of biting and grooming. However, MCMV is found in urine and breast milk, as well as the reproductive tract of male and female mice. This suggests additional modes of transmission, such as from mother to pup during feeding and by sexual activity. Interestingly, infection with multiple strains of MCMV is common, suggesting that mice are either infected simultaneously with multiple MCMV strains or that the lack of sterilizing immunity allows sequential reinfection of mice.

A number of diseases induced by HCMV in humans have been modeled with MCMV in mice. These include myocarditis, hepatitis, adrenalitis, and interstitial pneumonitis. However, natural congenital infection has not been modeled in mice, since MCMV, unlike HCMV, does not cross the placenta in immunocompetent hosts. Mice have also been used to investigate host resistance to CMV. A number of factors affect the capacity of MCMV to cause disease in mice. These include the dose and route of inoculation of the virus as well as the age and genetic constitution of the host. Several genetically linked innate resistance mechanisms have been identified in mice, including *Cmv1*, *Cmv3*, and *Cmv4*. These loci control resistance to MCMV via innate immune responses. Other genetic resistance mechanisms have been demonstrated in New Zealand white mice (*Cmv2*) and in a mouse strain carrying an *N*-ethyl-*N*-nitrosourea-induced mutation of the protein Unc93b1 in which signaling via Toll-like-receptors (TLRs) 3, 7, and 9 is deficient. TLRs 3, 7, and 9 recognize single-stranded RNA, double-stranded RNA (dsRNA), and unmethylated DNA, respectively. Previous studies have highlighted the importance of TLRs 2, 3, and 9 in resistance to MCMV.

Latency

Persistent infection is a common feature of the betaherpesviruses. The infection may be either chronic, in which infectious virus is produced at very low levels for long periods in particular organs or tissues such as the salivary gland, or latent, in which infectious virus is no longer detected, although the viral genome is present in certain cells in the body. Reactivation from latency usually occurs during immunosuppression to yield infectious virus, which may induce disease and be transmitted to susceptible hosts.

Regulation of transcription is tightly controlled during latency. In MCMV, regulation of IE gene expression is an early checkpoint on the way from latency to recurrence. The IE1/3 transcriptional unit gives rise to IE1 and IE3 mRNAs by differential splicing, which is driven by the $P^{1/3}$ promoter with a strong upstream enhancer that serves as a molecular switch, connecting IE1/3 transcription to the cellular environment. External stimuli, such as the pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α), act as the first signal in the reactivation pathway, inducing transcription factors which activate the enhancer by binding to defined sequence motifs. IE3 is believed to be the major transactivator of E gene expression. MCMV latency is controlled after the initiation of IE1/3 transcription and this is the second checkpoint in the pathway of molecular reactivation. The full mechanism of reactivation has not been elucidated, but models postulate a multistep system of MCMV reactivation involving many checkpoints before the production of infectious virus.

Host Immune Responses and Viral Evasion Strategies

The host immune response to MCMV and the many countermeasures employed by MCMV reflect a dynamic

host and pathogen interaction and co-evolution. A range of host immune responses involving antibody, $CD4^+$ T cells, $CD8^+$ T cells, and natural killer (NK) cells help control MCMV infection, and are summarized in **Table 3**.

Innate intracellular defense mechanisms, such as interferon production (see below), apoptosis, and the dsRNA-dependent protein kinase R (PKR)-mediated shutdown of protein synthesis, are all targets of MCMV genes. There are three genes encoded by MCMV whose products inhibit apoptosis and facilitate tropism in specific cell types: apoptosis is inhibited in macrophages by the product of *M36*, in endothelial cells and fibroblasts by the product of *M45*, and in endothelial cells by the product of *m41*. An additional gene, *m38.5*, appears to be a homolog of the HCMV viral mitochondria-localized inhibitor of apoptosis (vMIA) encoded by *UL37*. Finally, the products of MCMV genes *m142* and *m143* form a dsRNA-binding complex that inhibits host PKR-mediated shutdown of protein synthesis.

NK cells are the primary host cell involved in the early innate response to MCMV. NK cells limit the severity, extent, and duration of acute infection and also affect the subsequent acquired immune response. MCMV infection can be lethal in their absence. NK cell control of MCMV infection is mediated by direct lysis of infected cells and by the production of cytokines. Direct NK cell killing is mediated predominantly by perforin, particularly in the spleen. Cytokine contol of infection is most evident in the liver and is predominantly mediated by interferon gamma (IFN-γ). MCMV encodes at least six genes that

Table 3 Immune evasion strategies of MCMV

		MCMV immune subversion		
Host control	*Effect*	*Gene*	*Function*	*Mechanism*
Innate immunity				
NK cells	Control of MCMV replication in acute infection	*m145*	Inhibits NK cell killing	Downregulates MULT-1, a ligand for the NK cell activating receptor, NKG2D, on infected cells
	Cytokine production enhances later immune responses such as T-cell activation and may affect DC/NK cell cross talk	*m152*	Inhibits NK cell killing	Downregulates RAE-1, a ligand for the NK cell activating receptor, NKG2D, on infected cells
		m155	Inhibits NK cell killing	Downregulates H60, a ligand for the NK cell activating receptor, NKG2D, on infected cells
		m144	Inhibits NK cell killing	MHC homolog, ligand unknown
		m138	Inhibits NK cell killing	Downregulates MULT-1 and H60 ligands for the NK cell activating receptor, NKG2D, on infected cells
		m157	Activates NK cells	Binds the NK cell activating receptor Ly49H (Cmv1)
Monocyte/ macrophages	Phagocytosis of infected cells and cytokine production	Nil	MCMV infects monocytes and macrophages, increasing IL-10 expression and reducing MHC class II expression	Infected monocytes disseminate virus to other organs such as the salivary gland. Differentiation in the tissues to macrophages allows for productive infection
Cytokines	Various effects on both innate and acquired immune responses	*M27*	Inhibits innate intracellular resistance to MCMV	Downregulates STAT-2, induces type I and type II IFN resistance
		M33	Migration of cells, including smooth muscle cells	Agonist independent GPCR, functional homolog of HCMV US28
		m129/131	Promotes inflammation	Chemokine homolog, macrophage chemoattractant. May aid dissemination of MCMV to the salivary gland within macrophages

Table 3 Continued

		MCMV immune subversion		
Host control	*Effect*	*Gene*	*Function*	*Mechanism*
Acquired immunity				
DCs	Priming of T cells Cytokine production Activation of NK cells	*m147.5* and other unidentified genes	Inhibition of T-cell priming Inhibits NK cell function Reduction in IL-2 and IL-12 production	Failure of DC maturation, possibly due to multiple MCMV genes affecting MHC expression, co-stimulatory molecule expression, and cytokine production. *m147.5* specifically downregulates CD86
CD8 T cells	Direct killing of infected cells	*m04*	Blocks CTL function	Binds MHC class I and remains associated on cell surface
	Control of reactivation from latency	*m06*	Blocks CTL function	Targets MHC class I to lysosome for degradation
		m152	Blocks CTL function	Retains MHC class I in ERGIC
CD4 T cells	Control of viral replication in the salivary gland	Nil		
Antibody	Reduces viral dissemination Reduces MCMV titer after reactivation Passive transfer protects mice from MCMV	*m138*	Fc receptor homolog (fcr-1). No known role in evasion of antibody responses	

MCMV has multiple mechanisms for evading host control. NK cells and $CD8^+$ CTLs are the primary effector cells in host control and this is reflected in the number of immune evasion strategies MCMV employs to evade these mechanisms. Dendritic cells (DCs) also play a major role in the acquired immune systems control of MCMV infection by priming MHC class I and II restricted responses and, in the innate response, by cytokine release and by activation of NK cells. MCMV causes functional paralysis of DC and inhibits the expression of MHC and co-stimulatory molecules on the surface of DCs. These effects presumably serve to reduce T-cell priming as well as the activation of NK cells and may alter the type of immune response generated by perturbing cytokine responses. Macrophages are a major host control mechanism, but MCMV subverts this role by infecting monocytes and using the cells as a means of dissemination to other organs such as the salivary gland. Once in the tissues, monocytes differentiate into macrophages and become permissive for MCMV production. MCMV-encoded chemokine (MCK-2) and chemokine receptor (*M33*) may serve to recruit inflammatory cells to the site of infection to aid viral dissemination.

affect NK cell responses (**Table 3**). The complexity of this interaction is demonstrated in **Figure 3**.

$CD8^+$ cytotoxic T lymphocytes (CTLs) are crucial for the resolution of acute MCMV infection in BALB/c mice. CTLs lyse MCMV-infected fibroblasts *in vitro* and are found in the spleen of infected mice within 3 days. Sensitized CTLs recognize both structural and nonstructural viral antigens. This has been best studied in BALB/c mice where almost 50% of CTLs are directed against the nonstructural pp89 (*IE1*) protein of the Smith strain of MCMV. Epitope mapping has identified the immunodominant H-$2L^d$-restricted pp89 peptide as the nonomer YPHFMPTNL. Other peptides recognized in BALB/c mice are encoded by genes *m04*, *M45*, *m164*, *M83*, and *M84*. Recent studies in C57BL/6 mice have also identified a broad range of CD8 T-cell epitopes.

MCMV encodes a number of genes that are believed to affect CTL-mediated control of infection. Some, such as *m04*/gp34, *m06*/gp48, and *m152*/gp40, affect major histocompatibility complex (MHC) class I molecule expression on the surface of infected cells and/or inhibit CTL recognition and killing (**Table 3** and **Figure 3**). Their role *in vivo* is yet to be fully explained, but MCMV mutants in which all three genes are deleted exhibit reduced growth in the salivary glands of mice. MCMV also inhibits T-cell priming by causing functional paralysis of dendritic cells (DCs). The MCMV genes responsible for this effect are largely unknown, although *m147.5* is known to downregulate the co-stimulatory molecule B7.2 on the surface of infected DCs.

Antibody and $CD4^+$ T cells play lesser roles in the resistance of mice to MCMV infection. Antibody reduces dissemination of the virus within the host and reduces viral loads after reactivation from latency. As yet, there are no known MCMV genes that target antibody function directly. The MCMV-encoded (*m138*) Fc receptor inhibits NK cell function but does not appear to play a role in inhibiting host-antibody-mediated protection. Similarly, no MCMV-encoded gene has been identified that directly targets host $CD4^+$ T-cell responses. This perhaps reflects the moderate role of host $CD4^+$ T cells in resistance to MCMV, although $CD4^+$ T cells are important in the control of MCMV replication in the salivary gland.

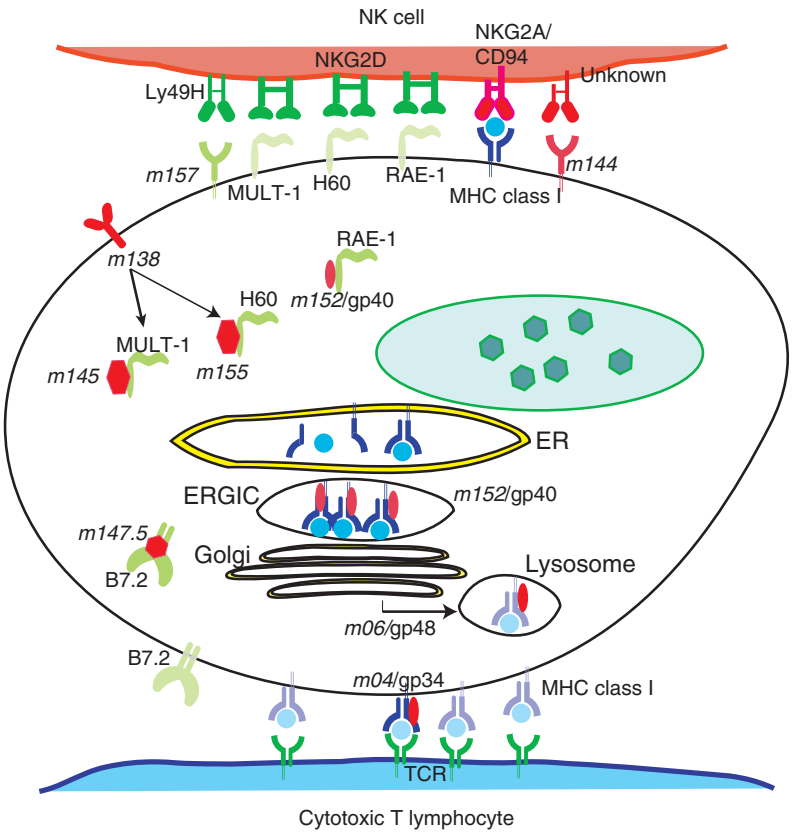


Figure 3 MCMV NK cell and CD8$^+$ CTL immune evasion strategies. MCMV has multiple methods of moderating host immune responses. Receptors or genes that inhibit immune responses are shown in red; those that activate immune responses are shown in green. The MHC class I molecules activate immune responses when ligated by host TCR (T-cell receptor) but inhibit responses when ligated by host NK cell NKG2A/CD94 heterodimer and are therefore shown in blue. When downregulated by an MCMV gene, host proteins are shown on the surface of the cell with reduced shading. MCMV targets NK cells by multiple mechanisms. MCMV downregulates host stress ligands that activate NK cells via the NK cell activating receptor NKG2D. MCMV genes *m138*, *m145*, *m152*, and *m155* affect the host proteins MULT-I and H60, MULT-1, H60, and RAE-1, respectively. These interactions have been depicted to occur in the cytoplasm because their actual locations are unknown. MCMV *m144* is an MHC homolog and inhibits NK cells by interaction with an unknown ligand, presumably an NK cell inhibitory receptor. Host CTL responses are avoided by interference with MHC class I expression. The gp48 (*m06*) protein targets MHC class I for degradation in the lysosome, gp40 (*m152*) retains MHC class I in the endoplasmic reticulum-Golgi intermediate compartment (ERGIC), and gp34 (*m04*) binds to MHC class I and inhibits CTL function (and possibly NK cell function). The gene product from *m147.5* downregulates the co-stimulatory molecule B7.2 and functions with other unidentified genes to inhibit DC function and reduce T-cell priming and NK cell function. Note, NKG2D is also found on T cells as a co-stimulatory receptor, but the role of *m138*, *m145*, *m152*, and *m155*, if any, on T-cell response to MCMV has not been explored.

Cytokine responses are also crucial to the resolution of MCMV infection in mice. This is exemplified by the increased sensitivity of interferon-αβ receptor 1 chain (IFNAR1) and IFN-γR1 knockout mice to MCMV. IFNAR1/IFN-γR1 double-knockout mice are exquisitely sensitive to MCMV infection. This increased sensitivity is probably due to the combined effects of defective cellular innate and acquired immune responses due to loss of IFN-γ (type II IFN) signaling as well as the loss of innate intracellular responses resulting from a loss of type I IFN signaling. As a countermeasure to host type I and II IFNs, MCMV induces a state of type I and II IFN resistance by *M27*-mediated downregulation of STAT2. In addition, infection is enhanced by an MCMV-encoded chemokine and chemokine receptor. *M33* encodes a G protein-coupled receptor (GPCR) homolog that is constitutively active in an agonist-independent manner and promotes dissemination of the virus to the salivary gland, possibly by recruitment of macrophages. The MCMV-encoded (*m129/131*) chemokine MCK-2 serves a similar function in that it increases inflammation, and deletion of *m129/131* reduces viral titers in the salivary gland (**Table 3**).

Rat Cytomegalovirus

CMVs have been identified in, and isolated from, several species of rat. The Maastricht strain of RCMV is the most

widely studied and was isolated from the brown rat, *Rattus norvegicus*. Another strain of RCMV, the English strain, was isolated from *R. norvegicus*; however, its classification as a CMV has been questioned. Recently, more putative strains of RCMV have been isolated from *R. norvegicus*, *Rattus rattus* (black rat), and *Rattus argentiventer* (rice field rat). This article focuses on the Maastricht strain of RCMV.

The Maastricht strain of RCMV was isolated from a wild brown rat in 1982, and has been maintained *in vitro* and *in vivo* in inbred laboratory strains of *R. norvegicus*. The genome has been fully sequenced and consists of a unique region bounded by terminal repeats. Its genome is 230 138 bp in length and is collinear with that of MCMV, encoding at least 170 ORFs, approximately two-thirds of which share significant sequence homology with genes found in MCMV (**Figure 1**). One RCMV gene, *r127*, appears to be unique among CMVs, encoding a homolog of the parvovirus *rep* gene. As with MCMV, RCMV encodes several genes (e.g., the GPCRs *R33* and *R78*, the MHC class I homolog *r144*, and the CC chemokine homologs *r129* and *r131*) which are homologs of cellular genes and which have therefore presumably been appropriated by the virus during evolution.

As is the case with other CMVs, RCMV is able to modulate and subvert host immune responses. RCMV downregulates the level of MHC class I molecules on the surface of infected cells. However, unlike MCMV, this effect is short-lived and does not result in proteolytic degradation of MHC class I molecules, rather in their delayed exit from the endoplasmic reticulum (ER). The RCMV gene *r131* is a pro-inflammatory CC-chemokine homolog, and appears to act similarly to the MCMV gene *m129/131* in that deletion of these genes results in decreased inflammation at the site of inoculation. This *r131*-mediated inflammation may recruit susceptible target cells to the site of infection, allowing virus dissemination throughout the body. *R33* has been shown to promote smooth muscle cell migration; however, it may play other roles in viral disease.

Pathogenesis

Following intraperitoneal inoculation of RCMV into rats, low levels of virus can be found in the visceral organs and bone marrow at 4 days post infection (p.i.). Virus can be detected in the salivary gland, in the striated duct cells, from 10 days p.i., with virus titers peaking at 28 days p.i., before gradually decreasing over several months. Interestingly, although at 6 months p.i. viral DNA can occasionally be detected in the visceral organs, infectious virus can only ever be isolated from the salivary gland at this time point. RCMV is capable of entering latency within the infected rat, although the major sites of latent infection are unknown. Virus can be reactivated from latency following immunological stress, such as immunosuppression or stimulation with allogeneic cells, resulting in virus replication.

As is the case with other CMVs, RCMV infection does not result in overt disease in immunocompetent hosts. However, in the immunocompromised animal, it may cause considerable morbidity and mortality. In immunocompetent rats, RCMV is capable of causing vascular injury, such as endothelial cell damage and leukocyte adherence to the aortic endothelium. RCMV infection may also lead to an influx of macrophages and lymphocytes and loosening of endothelial cells from the basement membrane. Inoculation of virus into immunocompromised animals, however, produces a different scale of pathology. Extensive cell necrosis results in organ damage, with multiple hemorrhages in lung, liver, spleen, and kidney. Virus also induces damage to the microvascular epithelium, vasculitis, and thrombotic occlusions.

The tendency of RCMV to induce vascular disease has made it a good model for studying the role of HCMV in these diseases in humans. Atherosclerosis is a chronic inflammatory disorder of large- and medium-sized arteries, and seroepidemiological and histopathological studies have implicated HCMV in the pathogenesis of this disease. RCMV induces vascular lesions with endothelial cell damage and leukocyte presence in the subendothelium, the presence of subendothelial foam cells, and morphological changes to the large blood vessels of infected rats. It has been suggested that RCMV induces smooth muscle cell migration, promotes leukocyte influx, and increases cellular expression of adhesion molecules, inflammatory cytokines, and chemokines, all components of the atherosclerotic process. Infection of rats with RCMV has also been shown to be a suitable model for arterial restenosis and transplant vascular sclerosis (chronic rejection). Both conditions have been linked to infection with HCMV in humans.

Guinea Pig Cytomegalovirus

Classic viral inclusions were first seen in the salivary gland of guinea pigs in the 1920s. However, GPCMV was not isolated until 1957. Since this time, almost all research conducted on GPCMV has used the Hartley strain of the virus.

The genome of GPCMV, while not fully sequenced, is approximately 230 kbp in length and is collinear with those of other CMVs, sharing genes with HCMV, MCMV, and RCMV within the central two-thirds of its genome but having GPCMV-specific genes near the termini. Several genes have been fully sequenced, particularly those with significant homology to potential HCMV vaccine targets. Again, as with other CMVs, gene expression is temporally regulated with IE, E, and L kinetics, although expression in the latter categories has been less well studied.

Following inoculation with GPCMV, viremia occurs for approximately 10 days, with infectious virus being detectable, and disease evident, in lungs, spleen, liver, kidney, thymus, pancreas, and brain for about another 3 weeks. Virus may be detected in the salivary gland for up to 10 weeks p.i.

GPCMV is unique among rodent CMVs in its ability to cross the placenta and infect the fetus. Hence, it is widely used as a model of congenital infection with HCMV. The guinea pig placenta consists of a single trophoblast layer separating maternal and fetal circulation, and is histologically similar to the human placenta. Infection during early pregnancy leads to pup resorption, while infection late in pregnancy generally leads to pup mortality. Following infection mid-gestation, GPCMV can be detected in placental tissue, even in the face of maternal anti-GPCMV antibodies. However, even in this case, only a proportion of pups attached to infected placentas are infected with GPCMV. These infected pups exhibit CMV disease in the brain, visceral organs, and inner ear, analogous to congenital infection with HCMV. GPCMV has been extensively used in studies of vaccination against congenital CMV infection. Vaccines against both gB and GP83 have been shown to protect against congenital GPCMV infection, although protection against pup mortality was dependent on maternal antibody titer.

Other Nonprimate Cytomegaloviruses

Swine cytomegalovirus is endemic in swine herds worldwide, and has also been shown to reactivate from pig-to-baboon xenotransplants. It causes rhinitis in young swine and is able to cross the placenta, resulting in generalized disease, runting, and fetal death. Initial sequence analysis of the DNA polymerase complex genes suggests that this virus is more closely related to HHV-6 than to CMVs. Tree shrew herpesvirus has been sequenced and has been found to resemble MCMV and other betaherpesviruses. A CMV has been isolated from deer mice (*Peromyscus maniculatas*) in North America, and has been characterized as a CMV based on physical and biological properties and genetic homology with several genes of other CMVs. CMVs have also been isolated from European and American ground squirrels, and designated as sciurid herpesvirus 1 and sciurid herpesvirus 2, respectively.

Agents resembling CMVs have also been described in hamsters, moles, voles, field mice, the Australian native rodent antechinus, cats, and dogs. These agents have not been characterized further and may not actually be CMVs.

See also: Cytomegaloviruses: Simian Cytomegaloviruses; Herpesviruses: General Features; Human Cytomegalovirus: General Features; Human Cytomegalovirus: Molecular Biology; Human Herpesviruses 6 and 7.

Further Reading

Mocarski ES, Jr. (2004) Immune escape and exploitation strategies of cytomegaloviruses: Impact on and imitation of the major histocompatibility system. *Cellular Microbiology* 6: 707–717.

Rawlinson WD, Farrell HE, and Barrell BG (1996) Analysis of the complete DNA sequence of murine cytomegalovirus. *Journal of Virology* 70: 8833–8849.

Reddehase MJ (ed.) (2006) *Cytomegaloviruses Molecular Biology and Immunology.* Wymondham, UK: Caister Academic Press.

Schleiss MR (2002) Animal models of congenital cytomegalovirus infection: An overview of progress in the characterization of guinea pig cytomegalovirus (GPCMV). *Journal of Clinical Virology* 25: S37–S49.

Shellam GR, Redwood AJ, Smith LM, and Gorman S (2007) Mouse cytomegalovirus and other herpesviruses. In: Fox JG, Barthold SW, Davisson MT, Newcomer CE, Quimby FW, and Smith AL (eds.) *The Mouse in Biomedical Research, Vol. 2: Diseases,* 2nd edn., pp. 1–48. Amsterdam: Academic Press.

Vink C, Beuken E, and Bruggeman CA (2000) Complete DNA sequence of the rat cytomegalovirus genome. *Journal of Virology* 74: 7656–7665.

Cytomegaloviruses: Simian Cytomegaloviruses

D J Alcendor and G S Hayward, Johns Hopkins School of Medicine, Baltimore, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Paralogs Genes that share homology through gene duplication and often have diverged functions or expression patterns.

Stealth virus An unproven chimeric virus that lacks or suppresses genes that trigger immune responses and is thereby able to go undetected by the immune system.

Introduction

In addition to the well-studied cytomegaloviruses (CMVs) of human (HCMV; salivary gland virus) and mouse (MCMV), related agents with typical CMV-like characteristics have been described in rats (RCMV), guinea pigs (GpCMV), pigs (PCMV), elephants (EEHV, elephant endotheliotropic herpesvirus), Old and New World primates (collectively known as simian CMVs and represented

by viruses such as RhCMV), and tree shrews (HVTupaia) (**Table 1**). GpCMV was originally recognized in 1920 and served as a model system for the biology and pathogenicity of HCMV disease for many years.

Most natural nonhuman primate populations studied harbor persistent or latent infections with host-specific simian CMVs. Isolates have been reported from almost all major primate groups, including gorilla, chimpanzee, bonobo, drill, baboon, rhesus and other macaques, African green monkey, spider monkey, owl monkey, capuchin, and marmoset. Infection is also common in captive breeding populations and, even when infection is usually inapparent in the absence of immunsuppression, virus is shed intermittently in urine and saliva. Indeed, simian CMVs are potential contaminants in primary cell cultures obtained from primate sources. Also, all simian CMVs can be adapted to grow in human fibroblasts. For research purposes, the main value of simian CMVs is for genome evolutionary comparisons and as animal models for HCMV disease, in which they are potentially superior to rodent CMVs. The genomes of primate CMVs differ among themselves and from HCMV to a surprisingly large degree, but nonetheless are more closely related to HCMV than are their equivalents in nonprimates.

Classification and Evolution

CMVs are members of the family *Herpesviridae* and belong to subfamily *Betaherpesvirinae* (**Table 1**). Formally, this subfamily is divided into three genera, namely *Cytomegalovirus* (HCMV-like viruses from primates), *Muromegalovirus* (MCMV-like viruses from rodents), and *Roseolovirus* (HHV-6-like viruses, so far only from great apes). There is

Table 1 Primate and other viruses classified in or potentially belonging to the subfamily *Betaherpesvirinae*

Genus	*Host group*	*Host species*	*Common abbreviation*	*Formal name*	*RefSeq accession*
Cytomegalovirus	Old World primate	Human	HCMV	*Human herpesvirus 5*	NC_001347; NC_006273
		African green monkey	AgmCMV	*Cercopithecine herpesvirus 5*	
		Rhesus macaque	RhCMV	*Cercopithecine herpesvirus 8*	NC_006150
		Chimpanzee	CzCMV	*Pongine herpesvirus 4*	NC_003521
Possible members		Bonobo	BoCMV		
		Baboon	BaCMV		
		Vervet	SA6	Cercopithecine herpesvirus 3	
		Vervet	SA15	Cercopithecine herpesvirus 4	
	New World primate	Marmoset	MaCMV	Callitrichine herpesvirus 2	
		Owl monkey	HVAotus type 1	Aotine herpesvirus 1	
		Owl monkey	HVAotus type 3	Aotine herpesvirus 3	
		Cebus	CeCMV		
		Capuchin	AL-5	Cebine herpesvirus 1	
		Capuchin	AP-18	Cebine herpesvirus 2	
Muromegalovirus	Rodent	Mouse	MCMV	*Murid herpesvirus 1*	NC_004065
		Rat	RCMV1	*Murid herpesvirus 2*	NC_002512
Possible member		Rat	RCMV2		
Roseolovirus	Old World Primate	Human	HHV-6A	*Human herpesvirus 6*	NC_001664
		Human	HHV-6B	*Human herpesvirus 6*	NC_000898
		Human	HHV-7	*Human herpesvirus 7*	NC_001716
Possible member		Chimpanzee	PaHV-6		
Proboscivirus (proposed)	Elephant	African elephant	EEHV	Elephantid herpesvirus 1	
Unassigned	Various	Guinea pig	GpCMV	*Caviid herpesvirus 2*	
		European ground squirrel	SqCMV1	Sciurid herpesvirus 1	
		American ground squirrel	SqCMV2	Sciurid herpesvirus 2	
		Pig	PCMV	Suid herpesvirus 2	
		Tree shrew	HVTupaia	*Tupaiid herpesvirus 2*	NC_002794

one genus pending, *Proboscivirus* (EEHV). Based on the relatively high G + C content of their genomes and their adaptability to grow in fibroblasts in cell culture, most traditionally recognized 'CMVs' are likely to be evolutionarily more similar to genera *Cytomegalovirus* or *Muromegalovirus* than to the (A + T)-rich genus *Roseolovirus*. The taxonomy of the *Betaherpesvirinae* will be shaped further with the continuing addition of members that are currently not fully classified , such as GpCMV, PCMV, and HVTupaia.

The genomes of nine betaherpesviruses are currently available, namely those of HCMV (strain AD169 in 1990, 229 354 bp; strain Merlin in 2004, 235 645 bp), CzCMV (in 2003, 241 087 bp), RhCMV (strain 68-1 in 2003, 221 459 bp; strain CMV 180.92 in 2006, 215 678 bp), MCMV (strain Smith, in 1996, 230 278 bp), RCMV (strain Maastricht in 2000, 229 896 bp), HHV6A (strain U1102 in 1995, 159 321 bp), HHV-6B (strain Z29 in 1999, 162 114 bp), HHV-7 (strain JI in, 1996, 144 861 bp; strain RK in, 1998, 153 080 bp), and HVTupaia (in 2001, 195 857 bp). The first complete betaherpesvirus genome sequenced was that of the AD169 isolate of HCMV. However, AD169 is a highly passaged laboratory strain that had suffered deletions and duplications during passaging in culture. Five more HCMV genomes have now been sequenced in the form of bacterial artificial chromosomes, and AD169 has been replaced by strains Merlin and FIX as the prototype HCMV genomes. Two strains of AgmCMV have now also been sequenced (GR2715 and Colburn), and work is in progress on EEHV as well as several other nonhuman primate CMV species.

All formally recognized and probable betaherpesviruses are listed in **Table 1**, with their common and official names and the RefSeq accession numbers of their complete sequences. Two very different muromegaloviruses (with somewhat different gene contents) are both known as RCMV, and are represented by the Maastricht (M) and English (E) strains as RCMV1 and RCMV2, respectively. CMV-like viruses have been associated with fatal hemorrhagic disease in young African and Asian elephants. Ongoing genetic analysis indicates that they are very distinct from members of the three recognized genera, and this has resulted in proposal of a new genus, *Proboscivirus*. An unusual herpesvirus isolate, referred to as 'stealth virus', was reported to have been recovered from a human patient with central nervous system (CNS) neuropathy, but genetic analysis has revealed that this virus is virtually identical to several characterized simian CMV isolates from African green and vervet monkeys (AgmCMV). Another AgmCMV isolate, 'Colburn', was also originally thought to have been of human origin, but it is unclear whether it is truly a human isolate rather than a contaminant from primary monkey cell cultures. Based on genome analyses, the bovine herpesvirus BHV-4 and two equine herpesviruses EHV-2 and EHV-5 were originally thought to be CMVs, but were reclassified into the genus *Rhadinovirus* in subfamily *Gammaherpesvirinae*.

Typically, betaherpesviruses have no more than 40 of their 110–165 genes in common with alpha- and gammaherpesviruses. Another 35 genes appear to be betaherpesvirus specific and are likely to be shared by all CMV and HHV-6-like viruses, whereas most of the remaining genes are unique to different virus genera or individual species. As judged by the presence of several large families of related genes in HCMV, AgmCMV, and RhCMV, it appears that the CMVs, which possess the largest of all mammalian herpesvirus genomes, underwent a rapid genomic expansion in the early stages of mammalian evolutionary radiation. In contrast, the roseoloviruses (HHV-6 and HHV-7) and HVTupaia can be considered to be mini-CMVs lacking most of the repeated gene families and the entire S segment of the genome. Unlike other herpesviruses, betaherpesviruses in general do not encode either thymidine kinase or the small subunit of ribonucleotide reductase, although EEHV has both of these. However, probably all betaherpesviruses encode a phosphotransferase (UL97) activity that is the target for the effective anti-CMV agent ganciclovir and its derivatives. Like all other known herpesviruses, primate CMVs encode the typical set of six core DNA replication proteins including DNA polymerase (POL), DNA primase (PRI), helicase (HEL), single-stranded DNA-binding protein (SSB), polymerase processivity factor (PPF), and primase accessory factor (PAF). In contrast to the CMVs and muromegaloviruses, the roseoloviruses and EEHV encode a homolog of the HSV UL9 origin-binding protein (OBP).

Virion Structure

Primate CMV virions are structurally similar to those of other members of the *Herpesviridae*, and are essentially indistinguishable from those of HCMV in the electron microscope. However, many of the virion proteins of each species display characteristic size variations by polyacrylamide gel electrophoresis (PAGE) analysis. Large nuclear and cytoplasmic inclusion bodies detectable in lytically infected cells by light microscopy are a hallmark of all betaherpesviruses.

Genome Structure

The genomes of all known CMV DNA molecules are *c.* 210–240 kbp in size, and most have a G + C content of around 58%, in contrast to the 145–166 kbp and 46% G + C content of HHV-6 and HHV-7. Structurally, two distinct CMV genome types can be discerned. The

genomes of New World primate CMVs (HVAotus types 1 and 3), HCMV, and CzCMV represent one type, having internal inverted repeats flanking two unique regions (the L segment consisting of unique region U_L flanked by inverted repeats TR_L/IR_L, and the S segment consisting of unique region U_S flanked by inverted repeats TR_S/IR_S), which generate four isomeric arrangements of the segments, similar to the pattern observed in herpes simplex virus. On the other hand, AgmCMV, RhCMV, HVTupaia, and several rodent CMVs (MCMV, RCMV, and GpCMV) have noninverting DNA molecules. HHV-6 and HHV-7 lack the entire 40 kbp S segment of HCMV and the L segment is bounded by large direct terminal repeats of 5–10 kbp. MCMV has a similarly sized gene block in place of the S segment, but the region has very little organizational resemblance or similarity to the S segment of HCMV. Large blocks of genes at the left and right ends of the L segment of each genome are also unique to each of the three genera for which complete sequence data are available, including the segments mapping to the right of and adjacent to the major immediate-early (MIE) genes in nonhuman primate simian CMV genomes, which differ dramatically from each other as well as from the equivalent regions in HCMV.

DNA and amino acid sequence analysis indicates that species from different mammalian hosts are diverged in accordance with their host phylogeny, with different genes having diverged at different rates. For example, in a region that is highly conserved between HCMV and AgmCMV, the major single-stranded DNA-binding proteins (UL57) have 72% overall amino acid sequence identity, whereas a group of three adjacent betaherpesvirus-specific glycoproteins mapping to the left of the MIE region (UL118, UL120, and UL121) vary from as much as 35% identity overall to as little as 15% in one region. Similarly, the betaherpesvirus-specific IE2 (UL122) immediate-early (IE) regulatory proteins of HCMV and AgmCMV have 58% amino acid sequence identity, but only over the C-terminal half of the protein, and both are equally diverged from RhCMV in this region. Amino acid sequence differences between homologous CMV genes from even the most closely related host species, such as HCMV and CzCMV, are frequently at least 20–30%.

Even the DNA molecules of individual isolates of a single primate CMV species are often distinguishable on the basis of multiple restriction fragment length polymorphisms stemming from overall intraspecies nucleotide variations of up to 3–4%. In fact, different isolates or strains of HCMV and simian CMVs display a number of significant genomic differences. First, so-called laboratory strains of HCMV (such as AD169 and Towne), AgmCMV (Colburn), and RhCMV (68-1) have all undergone deletions and rearrangements. They have also accumulated frameshift or truncation mutations in a number of genes that appear to be critical for preserving natural tropism for endothelial and macrophage cell types, but are evidently selected against during adaptation to fibroblast cells. These changes particularly affect a set of three small genes (UL128, UL130, and UL131A). Even the less passaged HCMV (strain Toledo) has undergone a large inversion that affects UL128.

A second type of strain sequence difference has been documented in HCMV for many years within a subset of 'variable' genes, which include gB (UL55), gN (UL73), gO (UL74), exon 3 of UL37 (UL37ex3), UL9, RL12, RL13, UL144, UL146, and UL147. In each case, even when analyzed directly by polymerase chain reaction (PCR) sequencing of clinical samples, the proteins from different genomes can display up to 30–60% divergence at the amino acid level, with collections of isolates examined falling into clusters of 3, 5, 8, or even 15 subtypes, depending on the locus. The subtype patterns in different 'variable' gene loci are unlinked, and this is indicative of high levels of mixed infections, recombination, and chimerism in HCMV samples. The origin and biological significance of subtype patterns are not well understood, but are most likely related to founder and bottleneck effects during the recent evolutionary spread and migration of humans. Isolates of CMV from nonhuman primate host species also display similar patterns of variability.

The sequenced prototype betaherpesviruses contain several interesting, and in some cases genus-specific, genes that are related to cellular genes and appear to be used primarily for evasion of immune responses. For example, HCMV encodes four viral G protein-coupled receptors (vGPCRs; UL33, UL78, US27, and US28), at least one of which (US28) functions as a broad-spectrum chemokine receptor that promotes migration of smooth muscle cells, and three glycoproteins (US3, US10, and US11) that together function to destabilize or inhibit cellular HLA-mediated responses. HHV-6 and MCMV each encode two GPCR proteins. HCMV and MCMV also each encode diverged homologs of HLA-I (at different locations in their genomes), and HCMV also possesses two separate anti-apoptotic proteins known as vMIA (UL37) and vICA (UL36). Spliced genes in MCMV and RCMV encode two functional β-chemokines, whereas HCMV encodes two α-chemokines (UL146 and UL147). HHV-6 (but not HHV-7) encodes an REP protein apparently captured from adeno-associated virus, and both HHV-6 and HHV-7, as well as RCMV but not MCMV, encode different OX-2-related proteins. RhCMV and AgmCMV, but not HCMV or CzCMV, encode a highly spliced COX2 gene, and HCMV, RhCMV, and AgmCMV encode a spliced vIL-10 gene, whereas CzCMV does not.

The total genome contents of the two 'great ape' CMVs (HCMV and CzCMV) differ by at most a half dozen out of 165 clearly defined genes. However, differences between HCMV and the 'Old World primate' CMVs (RhCMV and AgmCMV) are numerous. One

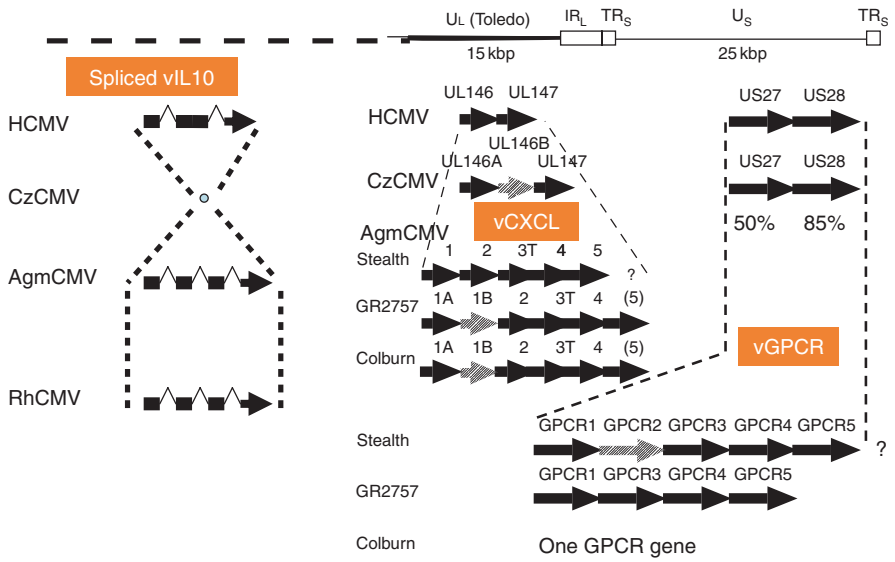


Figure 1 Comparison of the organization of the spliced vIL10 genes, the UL146 vCXCL α-chemokine ligand gene cluster, and the US28 vGPCR chemokine gene cluster in CMV genomes. Amino acid sequence identity between HCMV and CzCMV is indicated for the US27 and US28 proteins. Stealth, GR2757, and Colburn are distinct isolates of AgmCMV. The position of the additional (but inverted) 15 kbp segment in HCMV strain Toledo U_L compared to strain AD169 is indicated by the solid line. The spliced vIL10 gene is present in HCMV, AgmCMV, and RhCMV but absent from CzCMV. Another spliced gene (the COX2 gene) is present (at another location) in AgmCMV and RhCMV, but not in HCMV or CzCMV.

manifestation is the presence of either five or six tandemly repeated but highly diverged paralogs of the paired US27/US28 vGPCR genes of HCMV in multiple strains of RhCMV and AgmCMV, and the presence of either seven or eight tandemly repeated but highly diverged paralogs of the paired UL146/UL147 α-chemokine genes of HCMV in different strains of AgmCMV (**Figure 1**). Also, there are three, rather than two, of the α-chemokine genes in this cluster in CzCMV and at least three in RhCMV. Both the vGPCR and α-chemokine gene clusters appear to be undergoing highly dynamic evolution in Old World primate CMVs.

Epidemiology and Physical Properties

Features of host range, virus propagation, virus transmission, tissue tropism, pathogenicity, histopathology, and immune responses of primate CMVs, together with the physical properties and assembly pathways of the virions, and a number of biochemical properties of their structural proteins, closely resemble those of HCMV and MCMV. In general, long-term persistent asymptomatic infection is nearly universal among natural primate populations in the wild, with primary infections occurring in infants and juveniles. Even captive colony-borne animals are rarely free of these viruses, which can be shed intermittently in saliva and urine throughout the host's lifespan. Like all other herpesviruses, primate CMVs have enveloped icosahedral capsids and replicate in the nucleus. Infection of humans with simian CMVs via prolonged contact with seropositive monkeys or apes, or from vaccines grown in unscreened primary cell cultures from primate sources, is plausible, but has not been documented unambiguously. Laboratory-adapted strains such as AgmCMV Colburn grow vigorously in human fibroblast cell culture (**Figure 2**) and have been known to outgrow HCMV in cell culture. However, primary isolates grow slowly in both human and simian fibroblasts.

Replication Strategies

The lytic cycle pathway of gene expression for primate CMVs follows the typical herpesvirus cascade of IE mRNAs and proteins followed by activation of delayed-early (DE) then late (L) class genes, with synthesis of viral DNA. In cell culture, fully permissive host cell types for HCMV are restricted almost exclusively to diploid cells, including human fibroblasts, vascular endothelial cells (and the U373 astrocytoma cell line), smooth muscle cells, and differentiated macrophages. Fresh clinical HCMV isolates usually need to be adapted for efficient growth in fibroblasts by multiple rounds of passaging, which is accompanied by selection of inactivating point mutations (or deletions) in certain 'cell tropism' genes and a loss of ability to grow in endothelial cells or macrophages.

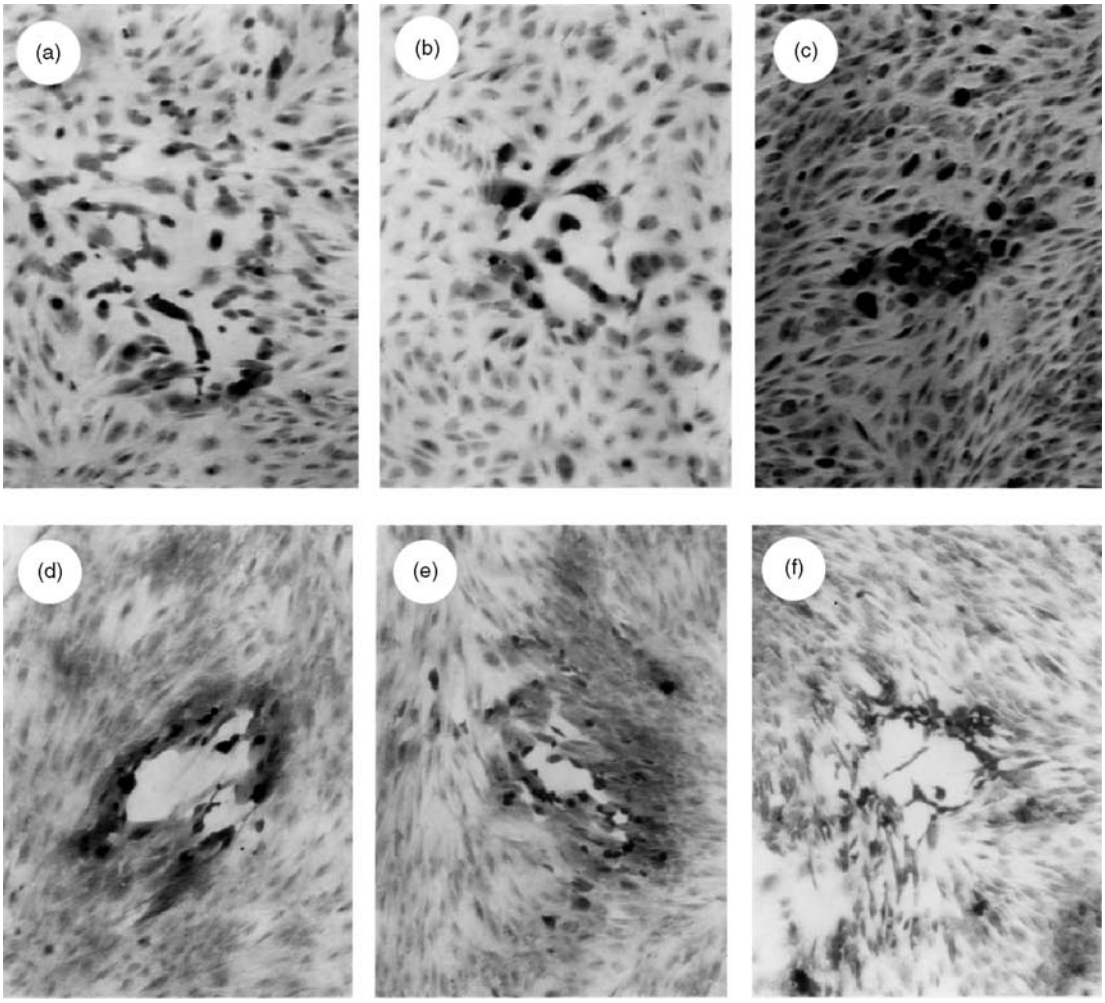


Figure 2 Infection of human diploid fibroblasts (HF cells) with human and other primate CMVs. HF cells at passage level 15 were infected and examined daily for cytopathic effects. All infections produced characteristic cytomegalic cells in culture. (a) HCMV (Towne strain) plaque at 7 days after infection; (b) AgmCMV plaque at 8 days after infection; (c) BaCMV plaque at 11 days after infection; (d) CzCMV plaque at 14 days after infection; (e) RhCMV plaque at 14 days after infection; (f) CeCMV plaque at 8 days after infection.

Laboratory strains of HCMV replicate their DNA efficiently by 72 h in human fibroblasts, but express only the MIE proteins and fail to synthesize any viral DNA in rodent or monkey fibroblasts. AgmCMV, RhCMV, BaCMV, and CzCMV replicate their DNA and form plaques in both human and simian fibroblasts (**Figure 2**). In contrast, MCMV carries out viral DNA synthesis in human, monkey, and rodent fibroblasts. Most transformed human cell types are nonpermissive for HCMV replication, with the block occurring between DNA entry and synthesis of MIE mRNA. However, some differences between HCMV and AgmCMV have been observed at this stage. For example, HCMV fails to synthesize MIE proteins in infected human (NTera) or mouse (F9) teratocarcinoma stem cells, in human 293 or NBE cells, or in mouse L-cells. In contrast, AgmCMV produces MIE proteins in infected NTera, 293, and NBE cells, but not in F9 or L-cells. After differentiation with retinoic acid (RA), NTera stem cells can become permissive for HCMV MIE expression and infectious virus production. Similarly, F9 cells treated with RA induce AgmCMV, but not HCMV, MIE expression. These biological differences among the different species of Old World primate CMVs appear to coincide with structural differences in the organization of *cis*-acting transcriptional control elements and adjacent accessory domains located both upstream of and within the large first intron of the MIE genes. Although a major site *in vivo* for quiescent inactive infection for HCMV and MCMV is believed to involve monocytes, many other sites of inapparent noncytopathic infection occur, but whether any of these can be defined as the true site of reactivatable latent state infection has not yet been resolved.

Control of Gene Expression

Similarly organized MIE transcription units, which encode regulatory proteins that trigger the lytic cycle, have been described for HCMV, CzCMV, AgmCMV, RhCMV, BaCMV, GpCMV, RCMV, MCMV, HHV-6, and HHV-7. In each case, these transcription units produce several multiply spliced mRNAs whose expression is controlled by powerful upstream *cis*-acting enhancer regions. The predominant viral mRNAs synthesized after infection of permissive cells in the presence of cycloheximide (an inhibitor of protein synthesis) are the MIE mRNAs, and these are also the only HCMV or AgmCMV mRNAs and proteins produced after infection of nonpermissive rodent fibroblasts. At least two types of phosphorylated nuclear regulatory proteins are encoded by the MIE transcription unit. These include the highly abundant, acidic IE1 (UL122) nuclear protein and the less abundant IE2 (UL123) DNA-binding transactivator/repressor protein. The IE2 DNA-binding transcription factor proteins are not only essential for stimulating transcription of downstream DE and L HCMV promoters, but they also specifically downregulate their own MIE promoters (negative autoregulation) and are probably also engaged in control of viral DNA replication, altering cell cycle function and blocking interferon and apoptotic responses. In particular, both the IE1 and IE2 proteins target to subnuclear domains known as PODs (protein oncogenic domains), which contain the PML proto-oncoprotein, and appear to modify these sites (also known as ND10 (nuclear domain 10)) for utilization by the viral genomes to initiate viral IE transcription and viral DNA synthesis.

In contrast to the rest of the viral genome, the IE1 and the IE2 coding regions of the MIE transcription units in HCMV, CzCMV, AgmCMV, MCMV, RCMV, HHV-6, and HHV-7 (and probably all other betaherpesviruses) are highly CpG-suppressed, which suggests that they are accessible to cellular methylation events at some stage of the viral life cycle during which all other viral genes are transcriptionally silent. The 490-amino-acid residue HCMV IE1 protein has only 15% amino acid sequence identity with AgmCMV or RhCMV IE1, and much less still is conserved between the primate and MCMV and RCMV versions, although all of the betaherpesvirus IE1 proteins (except for those of HHV-6 and HHV-7) have a highly acidic, Glu-rich C-terminus. In contrast, the 579-amino-acid residue IE2 proteins of HCMV, AgmCMV, RhCMV, MCMV, RCMV, and their much larger counterparts in HHV-6 and HHV-7, exhibit between 25% and 58% sequence identity over the C-terminal 270-residue conserved DNA-binding domain. As expected, the CzCMV IE1 and IE2 proteins are much more similar to the HCMV versions.

The upstream MIE promoter/enhancer regions, which serve to sense the intranuclear environment and control entry into or out of the lytic cycle, are large, complex, noncoding DNA sequence domains that consist of often multicopy, high-affinity binding sites for numerous constitutive or inducible cellular transcription factors. In HCMV, CzCMV, AgmCMV, RhCMV, BaCMV, GpCMV, and MCMV, these sites include response elements for cyclic AMP (CRE, PKA), phorbol esters (TPA, PKC), and RA, together with recognition motifs for SRF/ETS, CREB, SP-1, AP-1, and nuclear factor kappa B (NFκB). Even among the five Old World primate CMVs that have been examined in detail, the organization of these sites within the MIE enhancer regions differs significantly, and the number and pattern of the adjacent tandemly repeated 15 and 30 bp high-affinity NFI/YY1 motifs also differ greatly (**Figure 3**). Overall, the MIE control region in AgmCMV encompassing the BENT, NFI, ENH, and INTRON segments totals 2.3 kbp in size, much more than the approximately 1.1 kbp MIE control region in HCMV.

Other specific regulatory proteins common to HCMV and HHV-6, such as the UL36 and UL37 proteins, members of the US22 family, the UL82 and UL83 matrix phosphoproteins, and the UL84 replication-associated protein, are all conserved in primate CMVs. However, the second immediate early promoter (IES) and its novel, complex NFκB-containing enhancer is found in HCMV and CzCMV upstream from gene US3, and is not conserved in RhCMV or AgmCMV.

The CMV lytic cycle origin of DNA replication (Ori-Lyt) is located to the right of and adjacent to the single-stranded DNA-binding protein gene (UL57), near the center of the HCMV, CzCMV, AgmCMV, RhCMV, and MCMV genomes. It differs significantly from virus to virus in structural organization and apparently cannot be complemented by the protein replication machinery from another virus within this group. Although Ori-Lyt is located at the equivalent site in the genomes of HHV-6 and HHV-7 (and presumably also EEHV), it differs in structure and in its UL9-dependent mode of DNA replication from that in CMVs. It is still not known whether any betaherpesviruses utilize a latent cycle origin of DNA replication, such as that found among gammaherpesviruses.

Future Perspectives

Persistent, inapparent infection with CMVs is probably virtually ubiquitous in most individuals of all mammalian species. However, the biological and pathological properties of primate CMVs have attracted the interest of herpesvirologists and clinicians interested in HCMV disease in acquired immune deficiency syndrome (AIDS) and organ transplant patients, not so much because of the serious morbidity or economic consequences of these infections in their own hosts, but more often as models for latency and pathogenesis or immunological responses in HCMV. MCMV, RCMV, GpCMV, RhCMV, and

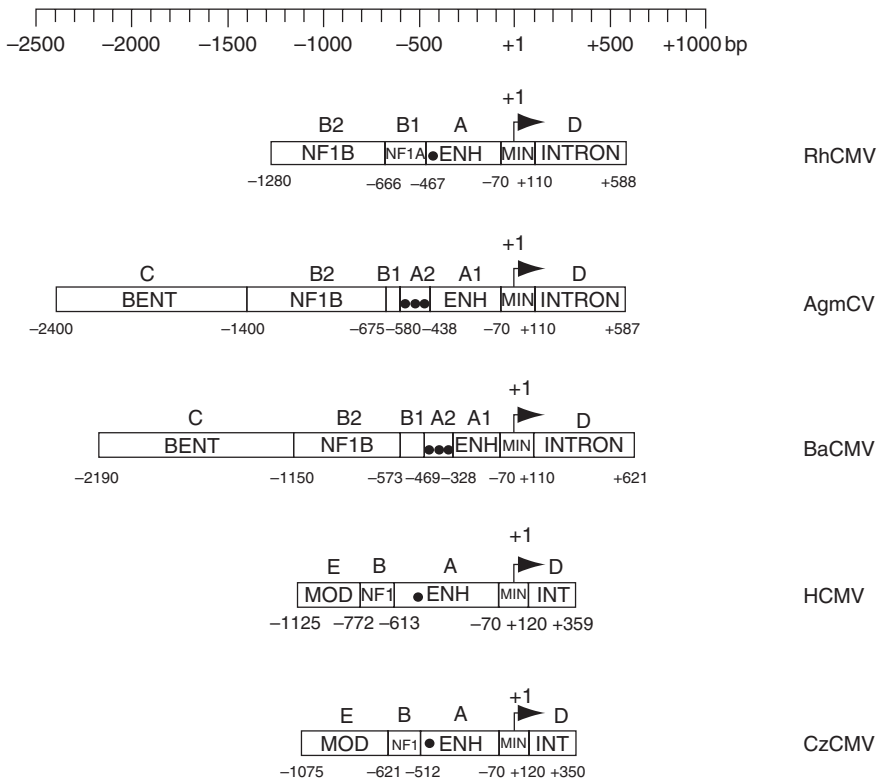


Figure 3 Upstream control regions in the MIE genes of HCMV and other primate CMVs. The diagram illustrates the respective size and organization of domains containing specific transcription factor-binding motif patterns. These include clusters of nuclear factor 1 (NF1) and YY1 sites, the distal enhancer containing multiple CRE, SRE, and NFκB sites (ENH), the proximal promoter and TATATAA box region (MIN) that includes the start site of transcription at +1 (arrowed), and the 5′ half of the large first intron (INTRON). Where appropriate, additional upstream AT-rich (BENT) and modulatory (MOD) regions are also included. Domain boundaries are designated as + or – nucleotide positions with reference to the start site of transcription and given in bp. The domains have also been designated A–E with subcategories 1 or 2 in the case of the NF1 and ENH block. Adapted from Chan YJ, Chiou CJ, Huang Q, and Hayward GS (1996) Synergistic interactions between overlapping binding sites for the serum response factor and ELK-1 proteins mediate both basel enhancement and phorbal ester responsiveness of primate cytomegalovirus major immediate-early promoters in monocyte and T-lymphocyte cell types. *Journal of Virology* 70: 8590–8605, with permission from American Society for Microbiology.

BaCMV have all been investigated as models for virus reactivation, and for control of acute and chronic disease by anti-CMV agents (such as ganciclovir, a nucleotide analog that specifically inhibits CMV DNA replication) in association with allografted organ transplants or immunosuppression. There is also interest in questions related to whether different CMVs can cross host species barriers with possible pathological consequences, which is of concern with BaCMV and PCMV for xenografted organ transplants. Primate CMVs also have comparative value in molecular genetics and biochemical analysis because of their similarities to and differences from HCMV or HHV-6 and HHV-7. Current basic scientific interest in genomic evolution (made possible by large-scale DNA sequencing), as well as the role of transcriptional gene regulation mechanisms in determining cell tropism and the switching between latent and lytic cycle virus–cell interaction pathways, and the many novel mechanisms used by betaherpesviruses for immune evasion, should lead to an expansion of these types of studies with nonhuman primate CMVs over the next several years.

See also: Cytomegaloviruses: Murine and Other Nonprimate Cytomegaloviruses; Herpesviruses: General Features; Human Cytomegalovirus: General Features; Human Cytomegalovirus: Molecular Biology; Human Herpesviruses 6 and 7; Simian Alphaherpesviruses; Simian Gammaherpesviruses.

Further Reading

Bahr U and Darai G (2001) Analysis and characterization of the complete genome of tupaia (tree shrew) herpesvirus. *Journal of Virology* 75: 4354–4370.

Bankier AT, Beck S, Bohni R, *et al.* (1991) The DNA sequence of the human cytomegalovirus genome. *DNA Sequence* 2: 1–12.

Chan YJ, Chiou CJ, Huang Q, and Hayward GS (1996) Synergistic interactions between overlapping binding sites for the serum response factor and ELK-1 proteins mediate both basal enhancement and phorbal ester responsiveness of primate cytomegalovirus major immediate-early promoters in monocyte and T-lymphocyte cell types. *Journal of Virology* 70: 8590–8605.

Chee MS, Bankier AT, Beck S, *et al.* (1990) Analysis of the protein-coding content of the sequence of human cytomegalovirus strain AD169. *Current Topics in Microbiology and Immunology* 154: 125–169.

Davison AJ, Dolan A, Akter P, *et al.* (2003) The human cytomegalovirus genome revisited: Comparison with the chimpanzee cytomegalovirus genome. *Journal of General Virology* 84: 17–28.

Dolan A, Cunningham C, Hector RD, *et al.* (2004) Genetic content of wild-type human cytomegalovirus. *Journal of General Virology* 85: 1301–1312.

Dominguez G, Dambaugh TR, Stamey FR, Dewhurst S, Inoue N, and Pellett PE (1999) Human herpesvirus 6B genome sequence: Coding content and comparison with human herpesvirus 6A. *Journal of Virology* 73: 8040–8052.

Gibson W (1983) Protein counterparts of human and simian cytomegaloviruses. *Virology* 128: 391–406.

Gompels UA, Nicholas J, Lawrence G, *et al.* (1995) The DNA sequence of human herpesvirus-6: Structure, coding content, and genome evolution. *Virology* 209: 29–51.

Hansen SG, Strelow LI, Franchi DC, Anders DG, and Wong SW (2003) Complete sequence and genomic analysis of rhesus cytomegalovirus. *Journal of Virology* 77: 6620–6636.

Kaplan AS (1973) *The Herpesviruses,* chs. 12 and 13. New York: Academic Press.

Megaw AG, Rapaport D, Avidor B, Frenkel N, and Davison AJ (1998) The DNA sequence of the RK strain of human herpesvirus 7. *Virology* 244: 119–132.

Nicholas J (1996) Determination and analysis of the complete nucleotide sequence of human herpesvirus 7. *Journal of Virology* 70: 5975–5989.

Rawlinson WD, Farrell HE, and Barrell BG (1996) Analysis of the complete DNA sequence of murine cytomegalovirus. *Journal of Virology* 70: 8833–8849.

Richman LK, Montali RJ, Garber RL, *et al.* (1999) Novel endotheliotropic herpesviruses fatal for Asian and African elephants. *Science* 283: 1171–1176.

Rivailler P, Kaur A, Johnson RP, and Wang F (2006) Genomic sequence of rhesus cytomegalovirus 180.92: Insights into the coding potential of rhesus cytomegalovirus. *Journal of Virology* 80: 4179–4182.

Staczek J (1990) Primate cytomegaloviruses. *Microbiological Reviews* 54: 247.

Vink C, Beuken E, and Bruggeman CA (2000) Complete DNA sequence of the rat cytomegalovirus genome. *Journal of Virology* 74: 7656–7665.

D

Defective-Interfering Viruses

L Roux, University of Geneva Medical School, Geneva, Switzerland

© 2008 Elsevier Ltd. All rights reserved.

This article is reproduced from the previous edition, volume 1, pp 371–375, © 1999, Elsevier Ltd.

History

In 1943, Henle and Henle reported the decreased infectivity for mice of influenza virus stocks obtained after a series of undiluted passages in embryonated chicken eggs. In the early 1950s, von Magnus showed that such undiluted passages generate incomplete virus particles capable of limiting the growth of infectious virus (hence exhibiting interference). This first characterization was soon followed by similar reports by Mims, on the one hand, and Cooper and Bellet, on the other, dealing respectively with Rift Valley fever virus and vesicular stomatitis virus (VSV). In the late 1950s, Cooper and Bellet went so far as to assign interference to sedimentable particles, but failed to identify them as antigenically related to VSV. From the mid-1960s on, the characterization of other positive- and negative-stranded RNA virus defective particles continued. In 1970, a review by A. Huang and D. Baltimore set the basic definition of defective interfering (DI) particles and emphasized their widespread occurrence. Since then, DI particles have been described for almost all the known DNA and RNA viruses, including plant and even fungal viruses.

Structure

DI particles have the same protein composition as their homologous nondefective 'parents', often called St. However, they differ from the St particles in the primary structure of their genome. As emphasized later, DI genomes lack part of the genetic information. They may or may not serve as coding sequences. However, they always conserve the *cis*-sequences needed for replication initiation (origins of replication), sometimes present in more than one copy, and sequences involved in encapsidation. Foreign sequences can also be inserted. DI particles can sometimes be separated from St particles on the basis of size, when the size of the particle closely corresponds to the size of the genome (for instance rhabdovirus), or on the basis of particle density differences (changes in nucleic acid to protein ratios). Often, however, only viral stocks enriched in DI particles are available owing to the size heterogeneity of the virus particles.

Generation of DI Genomes

DI DNAs very likely arise from various recombinational events not necessarily linked to genome replication, and which result in deletion, tandem duplication, insertion of host DNA, and polymerization of small monomer sequences. DI RNAs have been proposed to arise almost exclusively during genome replication by a mechanism of 'leaping polymerase' consisting of polymerase stop/fall-off or slippage/reinitiation events (**Figure 1**). In this model the replicase complex moves with the nascent RNA still attached to it. Depending on where reinitiation takes place, and on the number and the direction of the leaps, the resulting molecules can be of the copy-back type, with more or less intramolecular inverted complementary sequences (a), of the internal deletion type (b), and of the duplication type (c). Multisteps (b) or (c) and combinations of steps (b) and (c) can, moreover, lead to various mosaic types. Insertion of host RNA is also observed, especially in plant DI RNA. The frequency at which the polymerase leaps and resumes its synthesis is unknown. The probability for this exercise to be successful in producing a viable DI genome has been estimated for VSV to range in the order of 10^{-7}–10^{-8} per genome replication.

Defectiveness

The DI genomes contain interrupted or rearranged open reading frames. They partly or completely lack the full

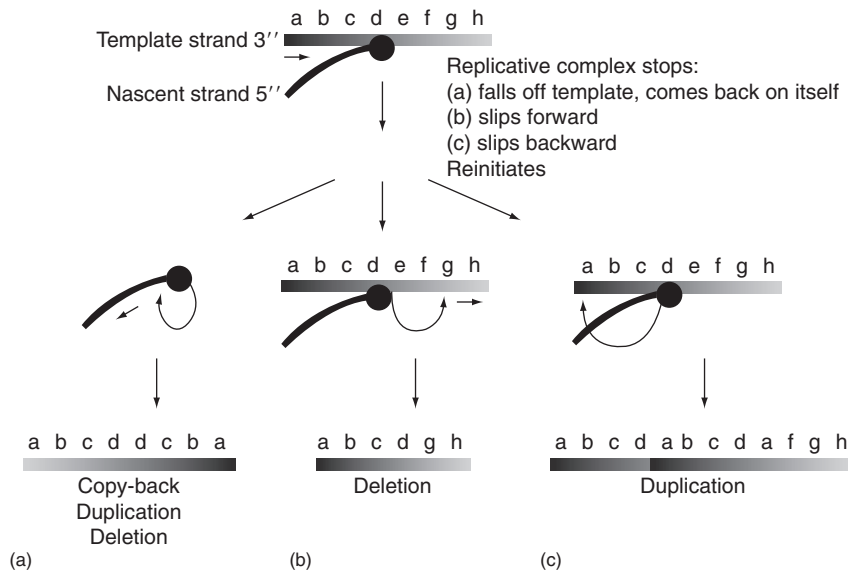


Figure 1 Defective RNA genome generation.

coding capacity of the viral genome. They are therefore defective, and depend for their replication and for their propagation (formation of virus particles) on the functions provided by the homologous standard virus (helper). Co-infection of cells with DI and standard particles is therefore essential for DI particle multiplication. Consequently, low-multiplicity infections, and particularly plaque purification, represent conditions which decrease, and potentially eliminate, DI particles from a viral preparation.

Interference

As stated earlier, the generation of a defective genome is likely to represent a rare event. This event would never be seen unless it was successfully amplified. During this amplification step the defective genome is preferentially replicated over the nondefective genome. This ability to replicate efficiently at the expense of the nondefective genome is called interference. The mechanisms of interference are not completely understood. They obviously change depending on the specificity of the viruses, and appear to be also affected by the host cell types. In general, interference involves an early step in genome replication, and can be pictured as a competition for limiting replication 'factors' (viral replicase, encapsidation proteins, host cell factors). Reiterated origins of replication or encapsidation sites on DI DNAs, presence of higher affinity sites for the replicase or for the encapsidation on both positive and negative polarity DI RNAs, and shorter length of the replicating units, higher availability for replication of molecules not involved in transcription, have been shown or postulated as taking part in the interference mechanism.

Defective Interfering versus Defective Viruses

Based on the outcome of experimental co-infections of defective with nondefective viruses, a distinction has been made between defective interfering or defective noninterfering particles, according to the ability of the defective viruses to selectively restrict nondefective virus replication. This distinction may not apply during the first events following the generation of a defective genome. As this is bound to be a rare event, an interference mechanism has to be invoked any time this defective genome is amplified to the point it can be detected, or become predominant.

Cyclic Variations of Defective Interference

The dependence of DI genome replication on functions provided by the nondefective genome on the one hand, and the interference exhibited by DI genome on the other hand, result in out-of-phase cyclic variations of both DI and St genome replication. As illustrated in **Figure 2**, efficient St genome replication must precede extensive DI genome replication. This in turn establishes conditions of high interference which results in inhibition of St genome replication. Decrease of helper function availability leads to DI genome replication dampening, and therefore to release from interference, allowing efficient St genome replication to resume. These cyclic variations have been observed in serial passages of St and DI viruses in cell culture, as well as in persistent infections. The periodicity of a complete cycle is generally a matter of days or of a few serial passages.

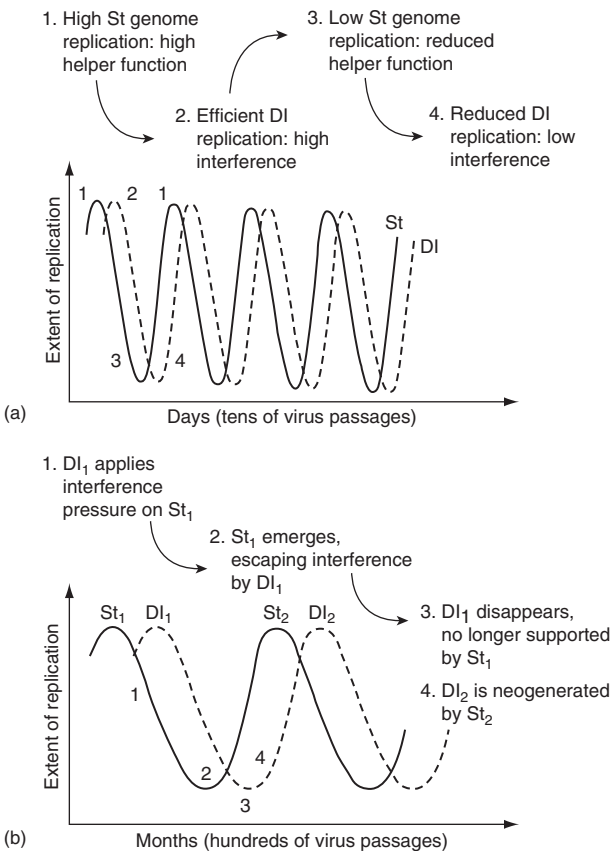


Figure 2 Cyclic variation of DI replication in (a) days, and (b) months.

Assay for DI Particles

DI particles can be detected by physical separation on velocity or density gradients when applicable (see the section titled 'Structure'). The presence of subgenomic nucleic acids in viral stocks or in infected cells (distinct from viral messengers) can also be diagnostic. The ability to decrease the infectivity of a viral stock or to protect infected cells from the lytic infectious virus (see the section titled, 'Biological effects') are used in various biological assays to estimate quantitatively and qualitatively the DI particle composition of a viral stock. These assays, although appropriate to characterize DI particle preparations, are generally not sensitive enough to exclude, when negative, the presence of DI particles in a viral preparation. The test that still remains the most dependable to assess presence or absence of DI particles consists of multiple independent serial undiluted passages. It is based on the observation that a viral stock contaminated with an undetectable amount of DI particles will, on subsequent independent serial passages, promote in each series amplification of the same contaminating DI particles. A DI particle-free stock, on the other hand, will yield in each series different DI particles or different sets of DI particles.

Biological Effects

DI particles have been shown to modulate the course of an infection. In cell culture, attenuation of the cytopathic effects is the most frequently described, and DI particles can promote cell survival and establishment of persistent infections. As far as negative-stranded RNA viruses are concerned, copy-back DI RNAs appear to prevent induction of apoptosis through a mechanism which has still to be unravelled, in which a certain category of small leader RNAs may participate. A possible role of the matrix M protein, the concentration of which is decreased to the point where viral assembly and budding at the cell surface is highly diminished in the presence of DI genomes, has also been considered. DI ability selectively allows emergence of St virus (St_2) which escapes interference (**Figure 2**(**b**)). St_2, resistant to interference, is selectively amplified over St_1, still sensitive to interference, and soon becomes predominant. It loses its ability to support DI_1, which therefore disappears. St_2 will generate its own DI_2, which in turn will favor emergence of a new St variant. Thus, DI viruses serve as mutational drivers favoring virus evolution, through cycles of high and low interference whose periodicity is this time measured in months or in hundreds of viral passages (compare **Figures 2**(**a**) and **2**(**b**)).

DI Particles in Experimental Animals

DI particles are generated and amplified in the whole animal, as well as in cell culture. They can change the symptoms of viral infection from rapid death to slow, progressing paralysis. They can sometimes fully protect the animals from an otherwise lethal infection. Interference is likely to be involved in this modulation of symptoms, but other phenomena like increased interferon induction and immune response modulation are reported.

DI Particles in Natural Infections

Involvement of DI particles in natural infections is still poorly documented. This is partly because the experimental results supporting the strong potential for infection modulation of DI particles have not been fully recognized, as detection of DI particles in natural infections is not straightforward. Unpredictable cyclic variations in DI replication, efficiency of DI replication

changing with the types of infected tissues, and potent interfering ability associated with poor DI particle replication are all factors which undoubtedly make DI particle detection difficult *in vivo*. Last but not least, virus isolation, which is used to characterize the infectious agent associated with a disease, often represents conditions (low multiplicity of infection) known to impair DI particle replication drastically.

Nevertheless, association of a chicken influenza virus strain, efficiently producing DI particles, with an epidemic of low morbidity and low mortality, and conversely, a high-mortality epidemic associated with a strain free of DI particles, have been reported. Murine and feline leukemia virus strains causing immune deficiency syndromes are shown to contain predominantly replication-defective viral genomes before onset and during the development of the disease. The pathogenicity of some bovine and swine pestiviruses has clearly been associated with presence of DI RNAs in the animals. For the bovine viral diarrhea virus (BVDV), a pestivirus of the same family as hepatitis C virus, the presence of a particular DI RNA can turn noncytopathic virus into a fatal infectious agent. In plants, at least three examples of DI RNAs are described to be involved in infection modulation. Interestingly, depending on the types of viruses, DI RNAs can either attenuate or exacerbate the symptoms.

DI RNAs are identified in stool and blood samples of humans suffering from hepatitis A virus, an infection known to be rather moderate and prolonged. DI particles are identified in measles virus-attenuated vaccine preparations which have been, and are being, widely and successfully used (raising the question of DI particle participation in vaccine attenuation). Measles viruses defective in viral assembly are currently found associated with human subacute sclerosing panencephalitis (SSPE). The brain cells of SSPE patients were, moreover, shown to harbor many species of measles virus copy-back DI RNAs. Direct amplification of a portion of the HIV *tat* gene from infected patients demonstrates that about a third of the sequences correspond to defective *tat* function. Moreover, human immunodeficiency virus (HIV)-1 sequences isolated from a cohort of six blood or blood product recipients infected with one donor all contained a similar deletion in the *nef* gene. Remarkably, all the patients harboring this deleted viral genome remained free of HIV-related diseases 12–16 years after infection, suggesting that this defective species of HIV genome is responsible for this decreased pathology. Epstein–Barr virus (EBV) replicative infections developing in human epithelial lesions involve a deleted rearranged form of EBV DNA (het DNA). This het DNA is associated, in experimental infections, with disruption of latency and persistent productive infections. Specific identification of viral hepatitis B (HBV) genomes containing an interrupted precore antigen (HBeAg) coding sequence in patients dying from fulminant hepatitis suggests that such defective genomes may be responsible for the exacerbation of the disease. This contrasts with more recent data reporting experimental evidence for the existence of DI-like viruses in HBV human chronic carriers; fluctuations between these naturally occurring core internal deletion variants and helper HBV in three chronic carriers were reminiscent of the cycling phenomenon in other DI viral systems.

Future Perspectives

Defective interfering particles are ubiquitous in the realm of animal and plant viruses. In experimental conditions they appear as necessary companions of their nondefective homologs. Capable of affecting the extent of viral growth, the course of viral infections, and serving as selective pressure to drive mutational changes, they can be seen as natural regulators of virus evolution. The demonstration of their participation in natural infections, and of their ability to affect the course of diseases, constitutes a challenge for the years to come. As pointed out by the few examples listed earlier, their direct detection in infected tissues will certainly be needed to assert their involvement in natural infections. The availability of sensitive detection techniques (like polymerase chain reactions), allowing direct observation of viral genomes without the possible distortion of virus isolation, bears great hope. More than giving increased insights into the physiopathology of viral infections, in times where the modifications of the viral genomes represent an imperative step in the generation of viral recombinant vaccines or of appropriate vectors for gene targeting, DI viral genomes represent natural versions of defective genomes that can serve as model tools for creation of more adapted vectors.

See also: Orthobunyaviruses; Orthomyxoviruses: Molecular Biology; Orthomyxoviruses: Structure of antigens.

Further Reading

Barrett ADT and Dimmock NJ (1986) Defective interfering viruses and infection of animals. *Current Topics in Microbiology and Immunology* 128: 55.

Perrault J (1981) Origin and replication of defective interfering particles. *Current Topics in Microbiology and Immunology* 93: 151.

Roux L, Simon AE, and Holland JJ (1991) Effects of defective interfering viruses on virus replication and pathogenesis. In: Shatkin A (ed.) *Advances in Virus Research,* vol. 40, 181pp. New York: Academic Press.

Dengue Viruses

D J Gubler, John A. Burns School of Medicine, Honolulu, HI, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arbovirus A virus transmitted to vertebrates by hematophagous (blood-feeding) arthropods.
Endemic A disease constantly present in a human population.
Extrinsic incubation The time between infection and becoming infectious.
Hyperendemicity The co-circulation of multiple dengue virus serotypes in the same population.
Vertical transmission Transmission of a virus from a female arthropod to her progeny.

History

Dengue fever is a very old disease; the earliest record of a dengue-like illness found to date is in a Chinese encyclopedia of disease symptoms and remedies, first published during the Chin Dynasty (AD 265–420) and formally edited in AD 610 (Tang Dynasty) and again in AD 992 during the Northern Sung Dynasty. There are reports of epidemics of dengue-like illnesses in the French West Indies in 1635 and in Panama in 1699. By the late 1700s, the disease had a worldwide distribution in the tropics, with epidemics of a clinically compatible disease occurring in 1779 in Batavia (Jakarta), Indonesia and Cairo, Egypt, and in 1780 in Philadelphia, Pennsylvania, USA. From the late 1700s to World War II, repeated epidemics of dengue-like illness occurred in most tropical and subtropical regions of the world at 10- to 30-year intervals. There is no documentation, however, that dengue viruses were responsible for all these epidemics because diagnosis was based only on clinical reports. Clinical descriptions of some early epidemics were compatible with chikungunya virus infection, which has a transmission cycle similar to that of the dengue viruses. It is likely that epidemic chikungunya did occur, but recent data show that the dengue viruses, not chikungunya virus, were responsible for the majority of epidemics in the past 50 years.

The virus etiology of dengue fever was not documented until 1943–44, when Japanese and American scientists simultaneously isolated the viruses from soldiers in the Pacific and Asian theaters during World War II. Albert Sabin isolated dengue viruses from soldiers who became ill in Calcutta (India), New Guinea, and Hawaii. The viruses from India, Hawaii, and one strain from New Guinea were antigenically similar, whereas three others from New Guinea were different. These viruses were called dengue 1 (DENV-1) and dengue 2 (DENV-2) and designated as prototype viruses (DENV-1, Hawaii, and DENV-2, New Guinea C). The Japanese virus, isolated by Susumu Hotta, was later shown to be DENV-1. Two more serotypes, called dengue 3 (DENV-3) and dengue 4 (DENV-4), were subsequently isolated by William McD. Hammon and his colleagues from children with hemorrhagic disease during an epidemic in Manila, Philippines, in 1956. Although thousands of dengue viruses have been isolated from different parts of the world since that time, all fit antigenically into the four serotype classification.

Many early workers suspected that dengue viruses were transmitted by mosquitoes, but actual transmission was first documented by H. Graham in 1903. In 1906, T. L. Bancroft demonstrated transmission by *Aedes aegypti*, later known to be the principal urban mosquito vector of dengue viruses. Subsequent studies in the Philippines, Indonesia, Japan, and the Pacific showed that *Aedes albopictus* and *Aedes polynesiensis* also were efficient secondary vectors for dengue viruses.

During and following World War II, *Ae. aegypti* greatly expanded its distribution in Asia, becoming the dominant day-biting mosquito in most Asian cities. Multiple dengue virus serotypes were also disseminated widely at that time. A dramatic increase in urbanization in the postwar years created ideal conditions for increased transmission of urban mosquito-borne diseases. These changes, plus an increased movement of people within and among countries of the region via airplane, resulted in increased movement of dengue viruses between population centers, increased frequency of epidemic activity, the development of hyperendemicity (co-circulation of multiple serotypes), and the emergence of epidemic dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS) in many countries of Southeast Asia during the 1960s. By 1975, DHF/DSS was a leading cause of hospitalization and death among children in the region. During the 1980s and 1990s, epidemic DHF/DSS continued to expand geographically in Asia. In the 1970s DHF/DSS moved into the Pacific Islands after an absence of 25 years. In the Americas, where *Ae. aegypti* had been eradicated from many countries as a result of efforts to control yellow fever, increased epidemic dengue fever closely followed the reinfestation of countries by this mosquito in the 1970s, 1980s, and 1990s.

With the development of hyperendemicity, DHF/DSS has emerged as a global public health problem in the past 25 years. In 2007, dengue fever is the most important

arbovirus disease of humans, with more than 2.5 billion people living in areas at risk for dengue in a belt around the tropics of the world (**Figure 1**). An estimated 100 million dengue infections and 500 thousand cases of DHF/DSS occur each year. The average case–fatality rate for DHF/DSS is 5%.

Taxonomy and Classification

Dengue viruses belong to the family *Flaviviridae,* genus *Flavivirus*. There are four serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. They belong to a larger, heterogeneous group of viruses called arboviruses. This is an ecological classification, one which implies that transmission between vertebrate hosts, including humans, is dependent on hematophagous arthropod vectors.

As are other flaviviruses, dengue viruses are comprised of a single-stranded RNA genome surrounded by an icosahedral nucleocapsid. The latter is covered by a lipid envelope, which is derived from the host cell membrane from which the virus buds. The complete virion is about 50 nm in diameter. The mature virion contains three structural proteins as follows: the nucleocapsid core protein (C), a membrane associated protein (M), and the envelope protein (E). Functional domains responsible for virus neutralization, hemagglutination, fusion, and interaction with virus receptors are associated with the E protein. Epitope mapping has demonstrated three to four major antigenic sites.

Antigenically, the four dengue viruses make up a unique complex within the genus *Flavivirus*. Although the four dengue serotypes are antigenically distinct, there is evidence that serologic subcomplexes may exist within the group. For example, a close genetic relationship has been demonstrated between DENV-1 and DENV-3 and between DENV-2 and DENV-4. The sizes of the genomic open reading frames of DENV-1, DENV-2, DENV-3, and DENV-4 are 3392 to 3396, 3391, 3390, and 3387 amino acids, respectively, the shortest among the mosquito-borne flaviviruses. An amino acid sequence positional homology of 63–68% is observed among the DENV serotypes compared to 44–51% between DENVs and other flaviviruses such as yellow fever and West Nile.

There are 53 flaviviruses recognized by the International Committee on Taxonomy of Viruses (this classification considers dengue 1 virus with four serotypes instead of four distinct viruses), including the genus prototype, yellow fever virus, and Japanese encephalitis, Murray Valley encephalitis, St. Louis encephalitis, West Nile, Zika and other viruses, all of which are transmitted by mosquitoes. Another group of flaviviruses are tick-borne and include tick-borne encephalitis, Omsk hemorrhagic fever, and Kyasanur Forest disease viruses. A small number of flaviviruses have no known arthropod vector, and three have been isolated only from insects.

Geographic and Seasonal Distribution

Dengue viruses have a worldwide distribution in the tropics (**Figure 1**). The viruses are endemic in most urban centers of the tropics, with transmission occurring year-round, and epidemics occurring every 3–6 years. It is well documented, however, that dengue viruses are maintained during interepidemic periods in most tropical areas and, although the risk of infection is lower than during epidemic periods, it is still substantial to unsuspecting visitors.

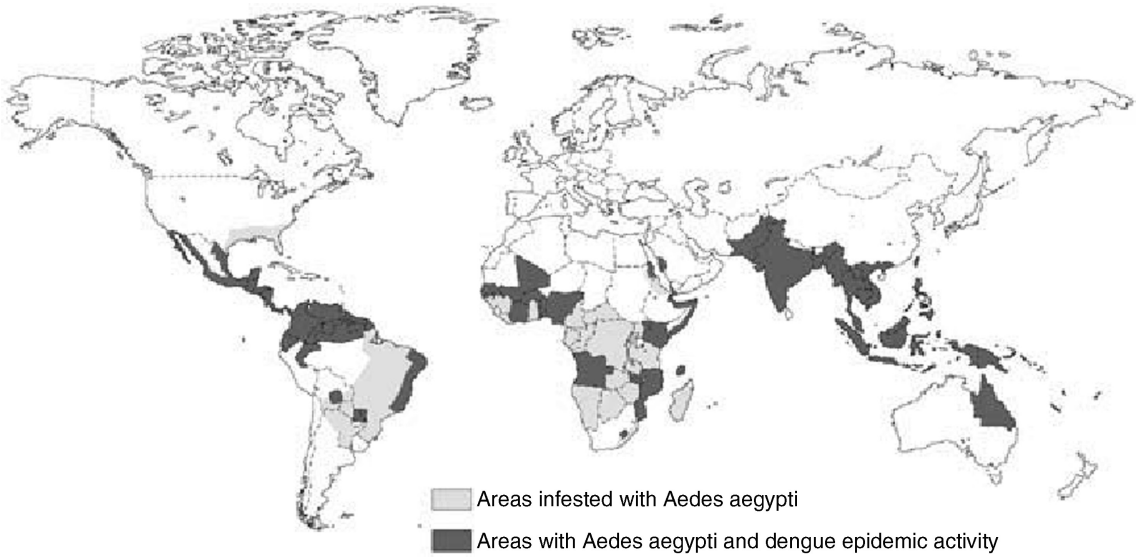


Figure 1 Global distribution of dengue fever and its principal epidemic mosquito vector, *Aedes aegypti, 2007*.

Peak transmission of dengue viruses usually is associated with periods of higher rainfall in most endemic countries. Factors influencing seasonal transmission patterns of dengue viruses are not well understood, but obviously include mosquito density, which may increase during the rainy season, especially in those areas where water level in larval habitats is dependent on rainfall. In areas where water in storage containers is not influenced by rainfall, however, other factors such as higher humidity and moderate ambient temperatures associated with the rainy season increase survival of infected mosquitoes, thus increasing the chances of secondary transmission to other persons. Virus strain and serotype, and herd immunity also influence transmission dynamics.

Host Range and Virus Propagation

There are only three known natural hosts for dengue viruses: *Aedes* mosquitoes, humans, and lower primates. Viremia in humans may last 2–12 days (average, 4–5 days) with titers ranging from undetectable to more than 10^8 mosquito infectious doses 50 (MID_{50}) ml^{-1}. Experimental evidence shows that several species of lower primates (chimpanzees, gibbons, and macaques) become infected and develop viremia titers high enough to infect mosquitoes, but do not develop illness. Viremia levels in lower primates are more transient, often lasting only 1–2 days if detectable, with titers seldom reaching 10^6 MID_{50} ml^{-1}.

Dengue viruses are known to cause clinical illness and disease only in humans. Baby mice, which are used for the isolation and assay of many other arboviruses, may show no signs of illness after intracerebral inoculation with most unpassaged strains of dengue viruses. Experimentally, however, some strains can be adapted to produce illness and death in baby mice. SCID, $Rag^{2-1-8c-1}$ and AG129 mice have been used for pathogenesis studies.

Mosquitoes only of species of the genus *Aedes* appear to be natural hosts for dengue viruses. Species of the subgenus *Stegomyia* are the most important vectors in terms of human transmission, and include *Ae. (S.) aegypti*, the principal urban vector worldwide, *Ae. (S.) albopictus* (Asia, the Pacific, Americas, Africa, and Europe), *Ae. (S.) scutellaris* spp. (Pacific), and *Ae. (S.) africanus,* and *Ae. (S.) luteocephalus* (Africa). It is uncertain what role *Ae. Albopictus* plays in transmission in areas where it has been recently introduced. Species of the subgenera *Finlaya* (Asia) and *Diceromyia* (Africa) appear to be important mosquito hosts involved in forest maintenance cycles of these viruses. Two other species, *Ochlerotatus* (=*Aedes*) *(Gymnometopa) mediovittatus* (Caribbean) and *Oc.* (=*Aedes*) *(Protomacleaya) triseriatus* (North America), have been shown to be excellent experimental hosts of dengue viruses.

Low passage or unpassaged dengue viruses can be propagated with consistent results only in laboratory-reared mosquitoes and in mosquito cell lines. Mosquito species most commonly used for *in vivo* propagation include *Ae. aegypti, Ae. Albopictus,* and *Toxorhynchites* spp., all of which can be reared with ease in the laboratory. Only three mosquito cell lines show high susceptibility to dengue viruses: C6/36 from *Ae.albopictus,* AP-61 from *Ae. Pseudoscutellaris,* and TRA-284 from *Tx. amboinensis*.

Dengue viruses can also be propagated in baby mice (see above) and in several vertebrate cell lines. These all have lower susceptibility to infection than do mosquito cells however, and dengue viruses must be adapted to each system by serial passage before consistent results can be obtained. Mammalian cell lines commonly used include LLC-MK2 and VERO (monkey kidney), BHK-21 (baby hamster kidney), FRhL (fetal rhesus lung), and PDK (primary dog kidney).

Genetics

Laboratory and epidemiologic studies have documented that genetic variants of dengue viruses occur in nature. DENV-3 isolated during epidemics in Puerto Rico in 1963 and 1977, and in Tahiti in 1965 and 1969, are antigenically and biologically very similar to each other, but very different from Asian strains of the same serotype. Similar antigenic differences were observed between Caribbean and Asian strains of DENV-4.

Oligonucleotide fingerprinting, restriction enzymes, primer extension sequencing, and nucleotide sequence comparison all have been used to study genetic variation among dengue viruses. In general, viruses circulating in the same geographic region during the same general time frame show genetic homogeneity, while differing from viruses of the same serotype from other regions. Because there is no good animal model for dengue however, it is not well understood how genetic variation influences phenotypic expression, in terms of clinical presentation or epidemic potential.

With increased transmission worldwide, dengue viruses have increased in diversity in recent years, most likely influencing both virulence and epidemic potential. This is supported by epidemiologic and virologic studies conducted in areas with sequential, but contrasting disease severity and transmission dynamics, such as Sri Lanka prior to and after the first DHF epidemic there in 1989. Other evidence includes the striking difference in replication characteristics between the South Pacific/American and the Southeast Asian subtypes of DENV-3 and DENV-2.

The number of genetic subtypes identified in each serotype varies with the method used, but more viruses have now been studied by partial nucleotide sequencing. Based on sequencing a 600 bp region of the envelope protein, which correlates well with sequencing the entire

envelope protein, there are two distinct genotypes of DENV-1, four of DENV-2, four of DENV-3, and three of DENV-4. Of considerable epidemiologic interest is that there are currently two genotypes of DENV-2 circulating in the American region. One, designated American, has been in the Americas since at least 1952, whereas the second, designated Southeast Asia, was isolated for the first time from dengue patients in Jamaica in 1982 shortly after the 1981 Cuban epidemic of DHF/DSS. Analysis by restriction enzymes and primer extension sequencing has shown that the Jamaican virus is nearly identical to strains of DENV-2 isolated in Vietnam in 1987, suggesting that the DENV-2 virus causing the Cuban epidemic was introduced from Vietnam. This conclusion is supported by the fact that many Cubans were working in Vietnam on various aid projects during the late 1970s and early 1980s. The Southeast Asia genotype has subsequently become the predominant and most wide-spread strain of DENV-2 virus in the American region.

Although there have been increasingly frequent reports of intraserotypic recombination events among the dengue viruses, the data suggest that this has not been important in their evolution. However, with increased occurrence of the cocirculation of multiple serotypes in an area (hyperendemicity), there have been increased reports of concurrent infections with two serotypes. This will increase the probability that interserotypic recombination might occur.

Evolution

The two principal theories of flavivirus evolution are: (1) that the tick-borne and mosquito-borne flaviviruses evolved from viruses with no known vector and (2) that the tick-borne and no known vector viruses evolved from a common ancestor and the mosquito-borne viruses arose separately. The origin of dengue viruses is unknown. Their natural history, however, suggests a long association with mosquitoes, possibly prior to becoming adapted to lower primates and humans. Biologically, dengue viruses are highly adapted to their mosquito hosts, being maintained by vertical transmission (from female mosquito to her offspring) in those species responsible for the forest maintenance cycle, with periodic amplification in lower primates (**Figure 2**). Such forest cycles have been documented in Southeast Asia and Africa. At some point in the past, probably with the clearing of the forests and development of human settlements in Asia, these viruses moved out of the jungle and into a rural environment where they were, and still are, transmitted to humans by peri-domestic mosquitoes such as *Ae. albopictus*. Migration of people ultimately moved the viruses into the cities of the tropics where they became 'urbanized' and transmitted by the highly domesticated, urban *Ae. aegypti* mosquito, which had been spread around the world via sailing ships and increased commerce.

Because of the rather slow rate of change (genetic drift) of the dengue virus genome, viruses isolated over long periods of time in the same geographic region still show striking homogenicity. The greatest genetic difference between dengue virus strains was observed between DENV-2 and DENV-3 isolated from forest mosquitoes in Africa and Asia, respectively, and viruses of the same serotype isolated from humans or mosquitoes in nearby urban areas. This would suggest that there is little gene flow between the forest and urban cycles. On the other hand, both laboratory and field evidence suggest that significant genetic changes that influence epidemic potential do occur in nature (see above).

Serologic Relationships and Variability

Dengue viruses share common morphology, genomic structure, and antigenic determinants with 52 other flaviviruses.

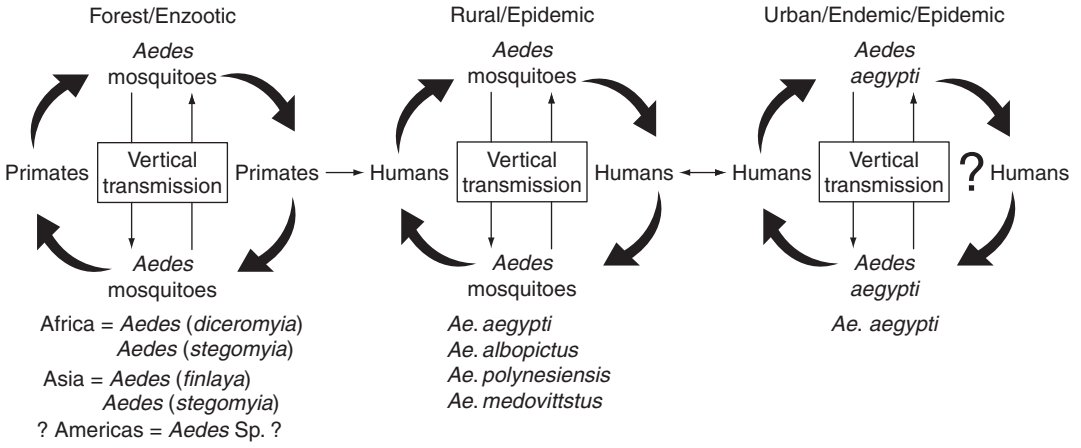


Figure 2 Natural transmission and maintenance cycles of dengue viruses.

Serologic tests most frequently used to determine antigenic relationships have included the hemagglutination-inhibition (HI), complement fixation (CF), and the plaque reduction neutralization (PRNT) tests. Because all flaviviruses share common antigenic determinants, identification of individual family members using these tests is difficult. The dengue viruses make up one antigenic complex within the family *Flaviviridae*. They share complex-specific antigenic determinants on both structural and nonstructural proteins. Serotypes within the dengue virus complex are most accurately and easily identified with an indirect immunofluorescent antibody (IFA) assay using serotype-specific monoclonal antibodies which react with epitopes on the structural protein. They can also be readily identified using polymerase chain reaction (PCR).

Both antigenic and biologic variation among dengue viruses have been documented. As noted above, DENV-3 viruses isolated in the Caribbean and the South Pacific in the 1960s were found to be antigenically distinct from the prototype and Asian strains of DENV-3 using PRNT. They were also biologically unique in that they did not grow as well in baby mice and mosquitoes as did Asian strains. DENV-4 viruses isolated in the Caribbean after the introduction of this serotype into that region in 1981 were antigenically distinct from DENV-4 viruses from Asia.

Field and epidemiologic evidence for natural strain variation among dengue viruses is more circumstantial. When DENV-2 was introduced into the South and Central Pacific islands in 1971 after an absence of more than 25 years, epidemics occurred on numerous islands. Marked variation was observed in disease severity, viremia levels, and epidemic potential in epidemics on the various islands. This variation was observed with both DENV-1 and DENV-2 in the Pacific and with DENV-3 in Indonesia. Some DENV strains appeared naturally attenuated, causing mild illness with low viremia levels of short duration, whereas others caused explosive epidemics with severe hemorrhagic disease and high viremia levels. Factors that could influence epidemic transmission and disease severity, other than differences in the virus strain, were ruled out as a cause of this variation. Recent studies in the American region have shown that variation among strains of DENV-2 and DENV-4 has influenced epidemic transmission.

Epidemiology

Dengue viruses occur in nature in three basic maintenance cycles (**Figure 2**). The primitive forest cycle involves canopy-dwelling mosquitoes and lower primates. A rural cycle, primarily in Asia and the Pacific, involves peridomestic mosquitoes (*Ae. albopictus* and *Ae. scutellaris* Spp.) and humans. The urban cycle, which is the most important epidemiologically and in regard to public health and economic impact, involves the highly domesticated *Ae. aegypti* mosquito and humans. The viruses are maintained in most large urban centers of the tropics, with epidemics occurring at periodic intervals of 3–6 years.

A combination of increased urbanization in the tropics, changing life styles, and lack of effective mosquito control has made most tropical cities highly permissive for transmission of dengue viruses by *Ae. aegypti*. Increased air travel by humans provides the ideal mechanism to transport dengue viruses between population centers. As a result, in the past 25 years there has been a dramatic global increase in the movement of dengue viruses within and between regions, resulting in increased epidemic activity, development of hyperendemicity, and the geographic spread and increased incidence of the severe and fatal form of disease, DHF/DSS. Once observed only in Southeast Asia, epidemic DHF/DSS has spread to west Asia, the Peoples Republic of China, the Pacific islands, and the Americas in the past 25 years.

Factors responsible for the emergence and spread of the severe form of disease, DHF/DSS, are not fully understood. The changing disease pattern described above provides support for both principal hypotheses regarding the pathogenesis of DHF/DSS, secondary infection, and virus virulence. Thus, increased movement of viruses between population centers results in increased transmission and the development of hyperendemicity, which then increases the probability of a secondary infection, of a virulent virus emerging via genetic change or being imported from another area.

Increased transmission of multiple dengue serotypes raises the iceberg further out of the water, and increases the probability that severe disease will occur, regardless of whether the underlying cause is due to increased virulence, immune enhancement, or, more likely, a combination of both (**Figure 3**).

Dengue is primarily an urban disease. Most major epidemics of DHF/DSS occur in tropical urban centers where

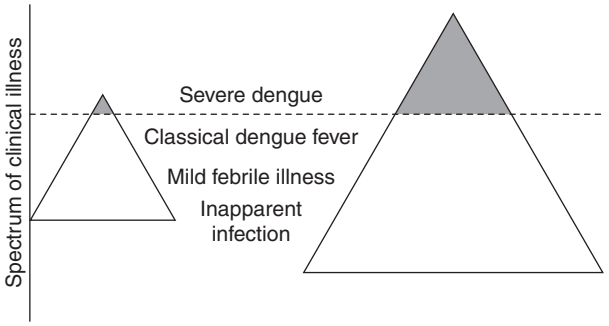


Figure 3 The iceberg concept of dengue/dengue hemorrhagic fever. The severe form of disease represents only the tip that protrudes from the water. As the incidence of infection increases, so too does the severe and fatal form of disease.

large and crowded human populations live in intimate contact with the principal mosquito vector, *Ae. aegypti*. This mosquito is a highly domesticated, day-biting species that lives and breeds in and around the home. High mosquito densities often occur in tropical cities because of water-storage practices and the accumulation of domestic trash. Primary larval habitats for *Ae. aegypti* include a variety of domestic water-storage containers such as clay jars and pots, metal drums, cement cisterns, and many other artificial containers found in the domestic environment that collect and hold rain water. The latter include, but are not limited to flower vases and pots, used automobile tires, buckets, bottles, cans, old machinery, etc.

Transmission and Tissue Tropism

Most dengue virus transmission is by the bite of an infective mosquito vector. Any of the four serotypes may cause high levels of viremia in humans ($\geq 10^8$ MID_{50} ml^{-1}) that lasts an average of 4–5 days (range, 2–12 days). If a competent mosquito vector takes a blood meal from a person during this viremic phase, virus is ingested with the blood meal and infects the cells of the mosquito mesenteron. After 8–12 days, depending on ambient temperature, the virus, and the mosquito, the virus will disseminate and infect other tissues, including the mosquito salivary glands. When the mosquito takes a subsequent blood meal, virus is injected into the person along with the salivary fluids. Dengue virus infection has no apparent effect on the mosquito, which is infected for life.

Ae. aegypti is a highly competent epidemic vector of dengue viruses. It lives in close association with humans because of its preference to lay eggs in artificial water-holding containers in the domestic environment, and to rest inside houses and feed on humans rather than other vertebrates. It has a nearly undetectable bite and is very restless in the sense that the slightest movement will make it interrupt feeding and fly away. It is not uncommon, therefore, to have a single mosquito bite several persons in the same room or general vicinity over a short period of time. If the mosquito is infective, all of the persons bitten may become infected.

In addition to transmitting the virus to humans or lower primates, the female mosquito may also transmit the virus vertically to her offspring through her eggs. Although the implications of vertical transmission are not fully understood, it is thought to be an important mechanism in the natural maintenance cycles of dengue viruses, especially in rural and forest settings.

The primary site of replication of dengue viruses after injection into humans by the feeding mosquito is believed to be dendritic cells. Other tissues from which these viruses have been isolated include phagocytic monocytes, liver, lungs, kidneys, lymph nodes, stomach, intestine, and brain, but it is not known to what extent the virus replicates in these tissues. Pathological changes similar to those observed in yellow fever, with focal central necrosis, have been observed in the liver of some patients who died of dengue virus infection. There is some evidence that the viruses also replicate in endothelial cells and possibly in bone marrow cells. Encephalopathy has been documented in dengue infection but whether dengue viruses cross the blood–brain barrier and replicate in the central nervous system is still open to question. Dengue viruses have been transmitted by blood transfusion and organ transplantation.

Pathogenicity

There is still some controversy about the pathogenesis of DHF/DSS. Evidence suggests that at least two pathogenetic mechanisms are associated with severe dengue infection. Classical DHF/DSS is characterized by a vascular leak syndrome which, if not corrected, may rapidly lead to hypovolemia, shock, and death. The underlying pathogenetic mechanism for this syndrome is thought to be an immune enhancement phenomenon whereby the infecting virus complexes with non-neutralizing dengue antibody, thus enhancing infection of mononuclear phagocytes. The latter produce vasoactive mediators, which are responsible for increased vascular permeability. Loss of plasma from the vascular compartment may range from mild and transient to severe and prolonged, the latter often resulting in irreversible shock and death. Although classical DSS is most commonly associated with secondary dengue infections, it has also been documented in primary infections, which suggest that subneutralizing levels of homologous antibody or other immune factors may also cause immune enhancement.

In vitro studies have shown that not all dengue viruses can be enhanced and that there are qualitative differences in the enhancing ability of antibody to dengue viruses. This raises the question as to whether dengue virus strains vary in their ability to stimulate production of enhancing antibody, whether this is associated with virulence, and, if so, how this relates to the immune enhancement hypothesis. Because an animal model is not available, no good data exist that demonstrate variation in virulence among dengue viruses. However, an accumulating body of both experimental and field data suggest the dengue viruses, like most other animal viruses, vary in their virulence and in their epidemic potential. When DENV-1 and DENV-2 viruses were introduced into the Pacific in the early 1970s after an absence of more than 25 years, some islands experienced explosive epidemics, with patients having high viremia levels and severe and fatal hemorrhagic disease, whereas other islands with similar ecology experienced only sporadic

or silent transmission, with low or undetectable viremias and mild illnesses. Virus strain variation was the only logical explanation for these differences. Recent laboratory evidence suggests that a major Cuban epidemic of DHF/DSS in 1981, the first of its kind in the American Region, was caused by a DENV-2 introduced from Vietnam, which was genetically distinct from the original American DENV-2. Data from this epidemic support both the immune enhancement and virus virulence hypotheses, which are not mutually exclusive. The most consistent feature associated with the emergence of DHF/DSS in an area is the development of hyperendemicity. This increases the probability of secondary infection, which is thought to be associated with DHF/DSS. However, hyperendemicity is also associated with increased transmission and movement of viruses between population centers, which increases the probability of genetic change and introduction of virus strains that have greater epidemic potential or virulence (**Figure 4**).

Patients infected with dengue viruses may also experience severe and uncontrolled bleeding, usually from the upper gastrointestinal (GI) tract. This severe hemorrhagic disease may be associated with multiple organ failure, and is more difficult to manage than classical DHF/DSS. The underlying pathogenetic mechanism for this type of bleeding is clearly different from that of the vascular leak syndrome, and involves disseminated intravascular coagulation and thrombocytopenia.

A third type of severe and fatal dengue infection, which may or may not involve overt hemorrhagic disease, is encephalopathy. Although many patients with this syndrome present clinically as viral encephalitis, conclusive evidence that dengue viruses infect the central nervous system has not yet been documented. Available data suggest that neurologic symptoms may be secondary to cerebral hemorrhage, edema, or other indirect effects of dengue virus infection.

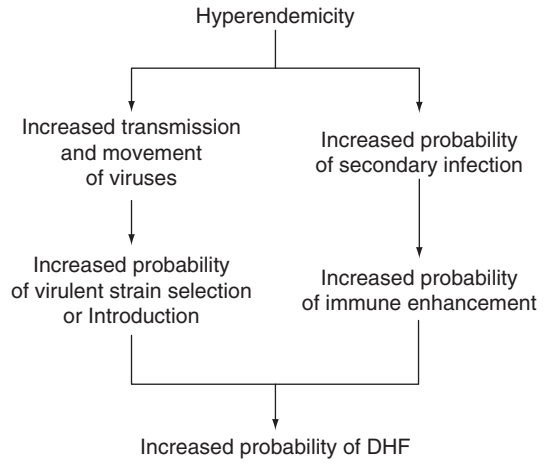


Figure 4 The cocirculation of multiple virus serotypes in a community (hyperendemicity) is the most important risk factor for the occurrence of dengue hemorrhagic fever and is compatible with the two principal hypotheses of pathogenesis, immune enhancement and virus strain variation.

Clinical Features of Infection

Dengue infection causes a spectrum of illness in humans ranging from clinically inapparent to severe and fatal hemorrhagic disease with the latter representing only the tip of the iceberg (**Figure 3**). The incubation period may be as short as 3 days and as long as 14 days, but most often is 4–7 days. The majority of patients present with mild, nonspecific febrile illness, or with classical dengue fever. The latter is generally observed in older children and adults, and is characterized by sudden onset of fever, frontal headache, retroocular pain, and myalgias. Rash, joint pains, nausea and vomiting, and lymphadenopathy are common. The acute illness, which lasts for 3–7 days, is usually benign and self-limiting, but it can be very debilitating, and convalescence may be prolonged for several weeks.

The hemorrhagic form of disease, DHF/DSS, is most commonly observed in children under the age of 15 years, but it also occurs in adults in areas of lower endemicity. It is characterized by acute onset of fever and a variety of nonspecific signs and symptoms that may last 2–7 days. During this stage of illness, DHF/DSS is difficult to distinguish from many other viral, bacterial, and protozoal infections. In children, upper respiratory symptoms caused by concurrent infection with other viruses or bacteria are not uncommon. The differential diagnosis should include other hemorrhagic fevers, hepatitis, leptospirosis, typhoid, malaria, measles, influenza, etc.

The critical stage in DHF/DSS occurs when the fever subsides to or below normal. At that time, the patient's condition may deteriorate rapidly with signs of circulatory failure, neurologic manifestations, shock and death if proper management is not implemented. Skin hemorrhages such as petechiae, easy bruising, bleeding at the sites of venepuncture, and purpura/ecchymoses are the most common hemorrhagic manifestations; GI hemorrhage may occur, usually after, but in some cases before, onset of shock.

The World Health Organization (WHO) has defined strict criteria for diagnosis of DHF/DSS, with four major clinical manifestations: high fever, hemorrhagic manifestations, hemoconcentration, and circulatory failure. WHO has classified DHF/DSS into four grades according to severity of illness: grades I and II represent the milder form of DHF and grades III and IV represent the more severe form, DSS (**Table 1**). Thrombocytopenia and hemoconcentration are constant features. However, there is some disagreement with the WHO case definition in that some patients may present with severe and uncontrollable upper GI bleeding with shock and death in the absence of

Table 1 World Health Organization classification of dengue hemorrhagic fever

Grade I	Fever accompanied by nonspecific constitutional symptoms, with a positive tourniquet test and/or scattered petechiae as the only hemorrhagic manifestation
Grade II	The same as grade I, but with spontaneous hemorrhagic manifestations
Grade III	Circulatory failure manifested by rapid, weak pulse, narrowing of pulse pressure (20 mmHg or less), or hypotension
Grade IV	Profound shock with undetectable pulse and blood pressure

From Anonymous (1997) *Dengue Hemorrhagic Fever: Diagnosis, Treatment and Control*. Geneva: World Health Organization.

hemoconcentration or other evidence of the vascular leak syndrome. These patients by the WHO criteria cannot be categorized as having DHF/DSS. In addition, hepatomegaly may not be a constant feature in all epidemics of DHF/DSS. The WHO is currently reevaluating the case definitions for dengue fever, DHF and DSS.

Dengue virus infection is associated with a variety of neurologic and psychiatric disorders, including headache, dizziness, hysteria, and depression. In addition, some patients present with clinical symptoms of viral encephalitis, but as noted above, there is no conclusive evidence that CNS infection occurs.

Treatment for DHF/DSS is symptomatic, and the prognosis of the disease depends on early recognition, initiation of corrective fluid replacement, and management of shock. Definitive diagnosis can only be made in the laboratory by serologic and/or virologic methods.

Pathology and Histopathology

The pathology of dengue virus infection is not well understood because systematic postmortem studies have not been done on patients representing all types of clinical presentations. The major pathophysiologic abnormality in classical DHF/DSS is an increase in vascular permeability which leads to leakage of plasma. Patients may have serous effusions in the pleural and abdominal cavities and a variable amount of hemorrhaging in most major organs. Studies have not revealed destructive inflammatory vascular lesions, but some swelling and occasional necrosis have been observed in endothelial cells.

Limited studies on patients with a fatal outcome have demonstrated focal necrosis of the hepatic cells, Councilman bodies, and hyaline necrosis of Kupffer cells in the liver. Changes in the kidney are suggestive of an immune complex type of glomerulonephritis. There is depression of bone marrow elements, which improve when the patient becomes afebrile. Biopsy studies of the skin rash have demonstrated perivascular edema with infiltration of lymphocytes and monocytes.

Immune Response

Persons infected with dengue viruses produce IgM and IgG antibodies, both of which appear 5–7 days after onset of illness in primary infections. The highest titers of IgM antibody are produced in primary dengue infections, but IgM antibody is also produced in secondary and tertiary infections. IgM antibody is transient and generally disappears 30–90 days after onset of illness in primary infections and after shorter periods in secondary and tertiary infections. IgG antibody, by contrast, persists for at least 60 years and probably for the life of the patient. In persons experiencing their first dengue or flavivirus infection, peak IgG titers are reached 14–21 days after onset of illness and seldom exceed 640–1280, although there are exceptions. In secondary infections, on the other hand, there is an immediate anamnestic IgG immune response to dengue complex-and/or flavivirus-specific antigenic determinants. In these patients, IgG antibody titers may exceed 20 480. Both IgM and IgG antibodies neutralize dengue viruses, and infection provides life-long immunity to that specific dengue virus serotype.

Both IgM and IgG antibodies to dengue viruses cross-react with other flavivirus antigens, including those of yellow fever, Japanese encephalitis, West Nile, and St. Louis encephalitis viruses. Cross-reactivity with viruses in the dengue complex is more extensive than with other flaviviruses, and makes interpretations of serologic results difficult. In patients with second and third flavivirus infections, original antigenic sin reactions are not uncommon. In geographic areas where several flaviviruses are endemic, therefore, definitive laboratory diagnosis can only be made by virus isolation or nucleic acid detection, and in patients with primary infection, by PRNT. Normally, a combination of laboratory (serologic and virologic), clinical, and epidemiologic data is used to make a diagnosis of dengue and other flavivirus infections.

Because IgG antibody persists for many years, its presence in a single serum sample is not diagnostic unless it occurs at high titer ($\geq$1280 by HI and PRNT, $\geq$256 by CF or $\geq$163 840 by IgG ELISA), which is considered presumptive evidence of a recent infection. Lower IgG titers simply indicate that the person has had a previous infection at some time in the past. Paired serum samples are required to confirm a current infection by demonstrating a fourfold or greater rise in IgG antibody. The presence of detectable IgM antibody in a singe serum sample is considered to be diagnostic for a recent infection because this isotype does not persist for long periods. The diagnosis is considered presumptive, and not confirmatory for a current infection, however, because IgM antibody may persist for 90 or more days.

Extensive work done in recent years has demonstrated that immunopathogenetic mechanisms play a major role in the pathophysiology of severe dengue infection. Generally, CD4+ and CD8+ T-cell responses are directed against multiple viral proteins. After primary infection, memory T cells proliferate in response to multiple dengue serotypes with both specific and cross-reactivities. After secondary infection, the T cell proliferation is of low-affinity and may be more cross-reactive to the first infecting virus serotype than the second, a phenomenon analogous to original antigenic sin seen in the antibody response.

Dengue virus infection results in the production of a number of chemokines, including IL-8, RANTES, MIP-1α, and MIP-β, which attract T cells and other inflammatory cells. Some, such as IL-8, are associated with plural effusions and may be important in inducing increased vascular permeability in patients with dengue infection.

There is accumulating evidence that cell-mediated immunity also plays a role in terminating dengue infections. Recent work suggests that dendritic cells (DCs) are likely the initial site of virus replication. Dengue virus infection stimulates DC maturation and activation, and production of TNF-α and INF-α. The DCs migrate to T-cell-rich lyphoid organs where T cells are activated, stimulating memory responses and releasing cytokines and chemokines. Circulating virus in the blood activates more T and B cells. During a second dengue infection, T-cell responses are likely to be dominated by a subset of specific memory T cells, which produce IFN-δ and CD40L, resulting in greater DC activation, T cell stimulatory capacity, IL-12 release, increased secretion of TNF-α and IFNδ, as well as potential dysregulation of cytokine responses. As viremia is cleared, the cascade of events initiated by an early type 1 cytokine response may contribute to the pathogenesis of DHF. The cross-reactive T cells that have low affinity to the second infecting virus serotype (see above) may be ineffective in clearing viremia, thus permitting a higher virus load and more severe disease.

Prevention and Control of Dengue

The options available for prevention and control of DF/DHF are limited. Although currently not available, considerable progress has been made in recent years in development of a vaccine (for DF/DHF). Effective vaccination to prevent DF/DHF will likely require a tetravalent, live attenuated vaccine. Promising candidate attenuated vaccine viruses have been developed and have been evaluated in phase I and II trials.

Progress has also been made on developing second-generation, recombinant dengue vaccines by using cDNA infectious clone technology. At least three candidate chimeric vaccines have been constructed by inserting the PrM and E genes into the backbone of yellow fever 17D vaccine virus, into an attenuated DENV-2 (PDK-53) backbone and into an attenuated DENV-4 backbone. The yellow fever 17D chimeric vaccine is further along and appears promising after phase I safety trials. The other two, plus a subunit candidate vaccine, are still in the preclinical phase.

The development of other new technology vaccines is in its infancy. Despite the promising progress, it will likely be 5–7 or more years before an effective, safe, economical dengue vaccine is commercially available. Also very promising has been the rapid progress in developing antiviral drugs that can be used in treatment of dengue infection, and perhaps, even in prevention and control programs.

Currently, the only way to prevent dengue infection is to control the mosquito vector that transmits the virus. Unfortunately, our ability to control *Ae. aegypti* is limited. For more than 25 years, the recommended method of control was the use of ultralow volume (ULV) application of insecticides to kill adult mosquitoes. Field trials in Puerto Rico, Jamaica, and Venezuela, however, showed that this method was not effective in significantly reducing natural mosquito populations for any length of time. This supports epidemiologic observations that ULV has little or no impact on epidemic transmission of dengue viruses.

The only truly effective method of controlling *Ae. aegypti* is larval control, that is to eliminate or control the larval habitats where the mosquitoes lay their eggs. Most important larval habitats are found in the domestic environment, where most transmission occurs. To have sustainability of prevention and control programs, some responsibility for mosquito control should be transferred from government to citizen homeowners. For long-term sustainability, mosquito control programs must be community-based and integrated. Persons living in *Ae. aegypti* infested communities have to be educated to accept responsibility for their own health destiny by helping government agencies control the vector mosquitoes, and thus prevent epidemic DF/DHF/DSS.

Countries with endemic dengue should develop active, laboratory-based surveillance systems that can provide some degree of epidemic prediction. Finally, prevention of excess mortality associated with DHF/DSS can be achieved by educating physicians in endemic areas on clinical diagnosis and management of DHF/DSS. As demonstrated in countries such as Thailand, early recognition and proper management are the key to keeping DHF/DSS case–fatality rates low.

Future

Continued population growth and urbanization of the tropics, changing lifestyles, increased air travel and lack of effective mosquito control have been the most important factors responsible for the dramatic increased incidence and geographic expansion of DHF/DSS in the past

25 years. DF/DHF/DSS has become a global public health problem in the tropics and it is anticipated that this trend will continue unless something is done to reverse it. More effective integrated prevention and control strategies must be developed and implemented worldwide in the tropics. Ultimately, development of an economical, safe, and effective vaccine holds the greatest promise for sustainable prevention and control.

See also: Arboviruses; Diagnostic Techniques: Serological and Molecular approaches; Flaviviruses: General Features.

Further Reading

Anonymous (1997) In: *Dengue Haemorrhagic Fever: Diagnosis, Treatment and Control*, 58 p. Geneva: World Health Organization.

Calisher CH, Shope RE, Brandt W, *et al.* (1989) Antigenic relationships among viruses of the genus Flavivirus (family Flaviviridae). *Journal of General Virology* 70: 37–43.

Gubler DJ (1989) Aedes aegypti and Aedes aegypti-borne disease control in the 1990's: Top down or bottom up. *American Journal of Tropical Medicine and Hygiene* 40: 571.

Gubler DJ (1998) Dengue and dengue hemorrhagic fever. *Clinical Microbiology Reviews* 11: 480–496.

Gubler DJ (2002) Epidemic dengue/dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. *Trends Microbiology* 10: 100–103.

Gubler DJ and Kuno G (eds.) (1997) *Dengue and Dengue Hemorrhagic Fever.* Wallingford, UK: CAB International.

Gubler DJ, Kuno G, and Markoff L (2007) Flaviviruses. In: Knipe D and Howley P (eds.) *Fields Virology,* 5th edn., pp. 1153–1252. Philadelphia: Lippincott Williams and Wilkins.

PAHO (1994) *Dengue and Dengue Hemorrhagic Fever in the Americas: Guidelines for Prevention and Control*, No. 548. Washington, DC: Publicaciones Cientificas.

Phuong CX, Nhan NT, Kneen R, *et al.* (2004) Clinical diagnosis and assessment of severity of confirmed dengue infections in Vietnamese children: Is the World Health Organization classification system helpful? *Americal Journal of Tropical Medicine Hygiene* 70: 172–179.

Pugachev KV, Guirakhoo F, and Monath TP (2005) New developments in flavivirus vaccines with special attention to yellow fever. *Current Opinion Infectious Diseases* 18: 387–394.

Rico-Hesse R (2003) Microevolution and virulence of dengue viruses. *Advances in Virusus Research* 59: 315–341.

Thomas SJ, Strickman D, and Vaughn DW (2003) Dengue epidemiology: Virus epidemiology, ecology, and emergence. *Advances in Virusus Research* 61: 235–289.

Thongeharoen P (ed.) (1993) *Monograph on Dengue/Dengue Hemorrhagic Fever*, No. 22. New Delhi: World Health Organization.

Diagnostic Techniques: Microarrays

K Fischer, A Urisman, and J DeRisi, University of California, San Francisco, San Francisco, CA, USA

© 2008 Published by Elsevier Ltd.

Motivation for New Diagnostic Methods

Numerous human diseases exist for which viral etiologies are suspected, yet specific causal agents are not known. Among these are up to 20% of cases of acute hepatic failure, up to 35% of cases of acute aseptic meningitis and acute encephalitis, up to 50% of cases of acute respiratory infections, and numerous other conditions. In addition, infectious agents may be involved in the pathogenesis of a number of chronic conditions, most notably disorders such as chronic inflammation, autoimmune and degenerative conditions, as well as some forms of cancer. While it is unlikely that viruses cause all of these diseases, identifying causative agents in even a modest number of disorders will have profound implications for understanding, diagnosis, and treatment of these conditions.

New approaches to viral diagnostics and discovery are needed to overcome the shortcomings of existing methods. These methods include viral culture, electron microscopy, serology, specific polymer chain reaction (PCR)-based methods, and techniques based on subtractive hybridization. While these methods have been critical for identifying many important human and nonhuman pathogens, each of these methods has intrinsic limitations. For example, many viruses are refractory to culture. Inspection by electron microscopy may prove difficult depending on the titer and morphological features of the virus. Serology- and PCR-based techniques are highly specific methods, but their specificity frequently renders them ineffective for the detection of variant or novel viral species. In the case of PCR, there exist dozens if not hundreds of variations on the method which may extend the scope of the assay, usually through multiplexing or by the use of degenerate primers, yet the number of possible targets that can be interrogated remains small relative to the number of known viral pathogens. Finally, subtractive hybridization techniques, while unbiased, are difficult to troubleshoot and essentially impossible to scale up for high throughput.

DNA Microarrays

While there exist many different forms of DNA microarrays, produced by both researchers and corporations,

they fundamentally share the same properties. All microarrays exist as some form of solid substrate, typically glass or silicon, to which is bound different species of nucleic acid. Each microarray may contain 10^5–10^6 different species of DNA, arranged in a grid. Fluorescently labeled nucleic acid derived from any biological sample may be interrogated by hybridization to the microarray. In this manner, the abundance of thousands of different nucleic acid species may be simultaneously measured. The most common application of microarray technology is the measurement of relative gene expression, often for entire genomes. While gene expression profiling has enjoyed tremendous success over the last decade, it has long been realized that the microarray format would be amendable to the detection of exogenously derived nucleic acids for purpose of identifying the presence of pathogens in a background of host material. The general concepts and methodologies are similar to those required for expression profiling; however, there are key differences to consider for the design of the array and the mechanics of sample processing. Furthermore, most implementations of virus-detection microarrays strive only to determine the presence of viral sequences, rather than attempting to quantify the amount of virus in a particular sample. In practical terms, this allows more aggressive amplification strategies to be used during sample preparation.

Microarray Design for Viral Detection

Algorithms to guide the process of sequence selection for expression microarrays are well developed, and many of the design principles apply directly to microarrays for viral detection. The parameters shared in common include physical properties of the oligonucleotide itself, such as the propensity of the sequence to form hairpins, melting temperature, and sequence complexity. Beyond this, the design considerations for viral detection and expression profiling differ substantially. First, the majority of microarrays intended for viral detection are designed to specifically detect the products of a multiplex PCR reaction using specific primers. In this role, the array simply sorts the product of amplification. The design specifications for these types of arrays are straightforward and are primarily guided by the choice of flanking PCR primers for virus amplification. This configuration of array does not exploit the full potential of the microarray for panviral detection. However, the design parameters for a generalized virus detection chip that does not rely on specific multiplex PCR primers must take into account additional factors.

While it is essential that probes for expression arrays are unique with respect to target sequence (to prevent cross hybridization from other mRNA species), the same does not necessarily apply to viral probes. In fact, to maximize the probability of detecting any viral species from a known family, it is often desirable to choose sequences that are the most conserved among a group of viruses. For example, while there exists a large range of sequence diversity among human rhinoviruses, sequences in the 5′ UTR are highly conserved, even among more distant picornaviruses. These sequences may thus serve as a type of universal 'hook' to capture both existing and new variants of these RNA viruses. In case of novel pathogens, such as the SARS coronavirus, the use of conserved sequences was a key determinant for successful detection by microarray. Clearly, unique species-specific or even genotype-specific oligonucleotides can further augment the discrimination of the microarray. A logical extension of this strategy takes into account features of viral taxonomy. Rather than choosing all conserved, or all unique sequences, one may attempt to cover, by design, each level of the taxonomic tree for each family. Thus, some sequences would be chosen to be species specific (terminal nodes on the tree), some would be genus specific, and so on. Various bioinformatic tools, including online databases, are currently available to assist in such design efforts. In all cases, it is critical to prescreen each choice for matches within the human genome to prevent inappropriate cross-hybridization to host material.

A more simple approach is to simply tile overlapping oligonucleotides spanning the entire genome of the viruses in question. This approach is appropriate for relatively small panels of viruses since each species will result in large numbers of sequences, depending on genome length. In general, this approach becomes impractical when extended beyond a few viral species.

After satisfying the basic design requirements, more sophisticated considerations may also contribute to choice of viral sequence for representation on a microarray. For example, to enhance detection of latent herpesviruses, it may be advantageous to overrepresent sequences specific for genes specific for latent phase expression, rather than those involved in lytic processes. In this case, it is assumed that the RNA rather than DNA will be analyzed, which highlights the importance of sample processing and amplification considerations.

Sample Processing and Amplification

The protocol by which nucleic acids are isolated from specimens, and the subsequent amplification of the material, if needed, is also important to consider, as this may also affect both microarray and experimental design. In general, isolation of total RNA is the preferred and more conservative route. While this may seem biased toward RNA viruses, all DNA viruses produce mRNA as part of their lifecycle, so this choice does not exclude them. However, if viral particles are collected, or host material is removed from the specimen by filtration or other

size-selection techniques, it may be advisable to isolate total nucleic acid (both RNA and DNA) to maximize sensitivity. The origin of the sample also bears on this issue. When processing a relatively acellular material, such as cerebral spinal fluid, a total nucleic acid extraction would be appropriate, whereas in the case of a solid organ, such as liver or brain, an RNA extraction would avoid the unnecessary complexity brought by co-purifying massive quantities of the host genomic DNA.

After nucleic acid has been isolated, an amplification step is typically employed to generate sufficient quantities for successful microarray hybridization. In certain situations, where large amounts of primary material are available, the yield of nucleic may be such that an amplification step may be bypassed altogether, but these situations are the exception. The choice of an amplification strategy is closely linked to the design of the array itself. Several virus-detection microarrays have been designed to serve as detectors for the products of multiplex PCR amplification strategies, but in these cases, the broad spectrum and unbiased nature of the microarray is not realized.

For panviral microarrays, where no assumptions are made as to the probable identity of the target, a general, randomized amplification strategy is required. Numerous random amplification strategies exist, but all begin with a priming step using a randomized oligo of various lengths, ranging from 6 to 15 bp. At this point, PCR adapters may be added, either by ligation, or through priming via a common sequence linked to the random primer. Alternatively, various RNA polymerase promoter sequences may be appended which then allow linear amplification. For all these methods, contamination with previously isolated material is a critical concern and good laboratory practices and appropriate controls, such as nontemplated amplification reactions, must be an integral part of the protocol.

The overall complexity of the sample, with respect to the viral species, is a critical factor for the success of random amplification strategies. As previously noted, the ideal samples are those that contain relatively low amounts of host cellular material and high titers of virus. For many biological specimens, it may be possible to reduce the complexity by filtration, centrifugation, or by pretreatment of the raw material with various nucleases. In the latter case, free host material such as genomic DNA and ribosomal RNA may be degraded, yet viral-packaged nucleic acid will escape destruction. Such 'preprocessing' can significantly enhance the signal to noise of the final microarray assay, although these steps add both cost and complexity to the overall protocol. Potentially, the use of novel microfluidic techniques for particle size discrimination may serve as a rapid and reproducible way to deterministically reduce complexity in biological samples.

Bioinformatics

Analysis of hybridization results depends greatly on the design of the experiment and of the DNA microarray in particular. Microarrays that are narrowly targeted, for the purposes of distinguishing between strains of a particular virus species, for example, human influenza or smallpox, are subjected to different analysis techniques than are multigenus, multifamily, or even panviral arrays. The utility of DNA microarrays in all these fields is demonstrable when investigators use appropriate analysis techniques.

In strain and species typing applications there are usually ample controls available to researchers. By use of control hybridizations, characteristic species or strain hybridization patterns can be classified manually. Often these patterns are the result of iterative microarray design where features with the desired specificity are spatially clustered on the chip. These patterns can be used as templates for visual inspection of the experimental arrays where different classes of microarray features are enumerated and the input sample is thereby placed into one of the known groups or unclassified as ambiguous. Machine-learning techniques, such as probabilistic neural networks, are also sometimes used in these studies, but to date have not been show to be more accurate than simple enumerative methods.

Broader explorations of viral populations, for example, panviral studies, must face the problem that the number of control samples available is small when compared to the number of distinct viral genomes that may be encountered. Many viral targets are extremely mutable, introducing one or more mutations per virus genome for every cycle of replication. Such diversity quickly outstrips the resources of any effort to perform exhaustive control hybridizations.

In panviral studies standard methods such as hierarchical clustering and several particular bioinformatic approaches can be employed. First the microarray should be designed to include conserved regions of the target viruses to minimize the chance that a divergent but related virus will escape detection. Second, estimates of the hybridization patterns expected from the possible targets for which sequence data is available can be generated using biophysical models of hybridization. Experimental hybridization patterns can then be compared to the model profiles using a correlation metric. If a virus present in a hybridization shares homology with one of the sequences used to estimate the hybridization profiles, this similarity will be reported as a significant correlation between the profile and the experimentally observed pattern. In the last method, the intensity history of the microarray's features can be compared to an experimental hybridization. Extraordinarily strong signal at a particular feature can indicate *bona fide* virus, especially when signal

at taxonomically related features is similarly elevated. During the design stage of the experiment, it is important to consider that in multifamily, metagenomic studies it is critical to have negative controls to characterize the background of microarray designed with broad specificity, while in studies with narrower focus a significant number of positive controls are needed for each class of virus being considered.

Real-World Applications: Research and Clinical

To date, only a few studies have examined the use of viral-detection microarrays using actual prospectively collected patient samples, as opposed to viruses cultured in the lab or previously characterized retrospective samples. As of the time of this writing, no large-scale study has been published comparing the use of virus microarrays (using a random amplification) to traditional laboratory medicine diagnostics, such as commercial DFA kits and PCR assays. Preliminary data and studies using modest numbers of clinical samples suggest that a microarray-based viral diagnosis outperforms conventional DFA assays, both in terms of sensitivity and specificity. In comparison to a PCR assay using specific primer pairs, microarray assays are likely to have comparable specificity, yet the sensitivity is anticipated to be somewhat lower, depending on amplification strategy. However, microarray-based assay coupled to a random amplification protocol provides much broader detection capabilities, often including essentially every known viral pathogen. When considering the potential of DNA microarrays for clinical diagnostics, it may be the case that the microarray will serve a complementary role to specific PCR assays, especially when the latter fails to yield positive results.

For clinical cases where conventional diagnostic assays have failed, the use of a panviral microarray assay may allow identification of new, or unanticipated pathogens. In a recent case report, Chiu and colleagues reported the diagnosis of a previously healthy 28-year-old woman suffering from a severe respiratory tract infection of unknown etiology. Extensive panels of diagnostics for bacteria, fungi, and viruses failed to reveal any positive results, and all viral cultures remained negative. DNA microarray analysis of RNA isolated from endotracheal aspirate revealed the presence of human parainfluenza virus-4, a virus that is not normally included on standard DFA panels or PCR tests. Viral sequence was recovered directly from the patient sample confirming the identity of the virus, and it was further shown that the patient seroconverted during the time of the illness. While it is generally believed that human parainfluenza virus-4 causes only mild, self-limiting infections, this data taken together suggests that the spectrum of disease may extend to respiratory failure in an otherwise healthy adult. This example demonstrates an attractive feature of the microarray assay, namely, the power to detect the unexpected.

In addition to the detection of previously known pathogens, DNA microarrays have also been effective for the detection of novel viral species. In the case of SARS, total nucleic acid from a supernatant from an infected vero cell culture revealed a coronavirus signature consisting of oligonucleotides originating from avian infectious bronchitis, human and bovine coronaviruses, and, interestingly, several astroviruses. At first glance, the hybridization signal from astrovirus-derived oligonucleotides would seem to be aberrant. In fact, this is expected, since several astroviruses and coronaviruses share conserved sequences at the 3′ end of their genomes. These particular sequences were represented on the microarray since the panviral design algorithm purposely selected conserved sequences within and among viral families.

The same principles applied to a separate study in which a novel xenotropic gamma retrovirus was detected in prostate tumor biopsies of men with a mutant variant of the *RNASEL* gene. Integration sites and full-length genomes were subsequently cloned and the virus was demonstrated to be replication competent, thus validating the microarray result. Again, the broad-spectrum nature of the DNA microarray was critical to the success of the project, since there were no preconceptions that such a virus might be a candidate, given that no xenotropic gamma retrovirus had been previously observed in a human subject.

Limitations

Several important limitations of using microarrays for viral detection and discovery should not be overlooked. The most important limitation of the approach is its reliance on known viral sequences. Although most of the novel viruses discovered in the last decade share homology with previously known viruses, viruses lacking even short regions of homology cannot be detected by any hybridization-based method. In the case of profoundly divergent viruses, more brute-force approaches, such as shotgun sequencing, are likely to be applicable. Other existing and likely surmountable limitations of the microarray-based methods are their cost, the need for specialized equipment, and access to computational resources. It is likely that these limitations will become less pronounced as streamlined versions of the technology become available through academic and commercial efforts. It may also be the case that the utility of DNA microarrays may be surpassed by next-generation, massively parallel

shotgun sequencing technologies, which would permit cheap, fast, and unbiased analysis of clinical samples.

Scientific and Public Health Implications

Currently, a substantial fraction of human disease, with a presumed viral cause, goes without a clinical diagnosis. This is especially true for common ailments, such as upper respiratory tract infections, where despite advances in PCR assays, the etiology of 30–60% of infections remains unidentified. Without considering the complexities of diagnostic regulatory approvals, an unbiased DNA microarray approach to the detection of viral pathogens should substantially increase the number of successful diagnoses, and as a consequence, may lead to improved therapeutics and supportive care. It should be noted that use of a virus microarray extends beyond the analysis of clinical samples. The wide scope of detection and the power to discover new pathogens has broad application to agriculture, veterinary medicine, ecology, and environmental genomics. While it is impossible to accurately predict the number of undiscovered viral pathogens remaining on this planet, tools such as virus-detection DNA microarrays permit rapid inroads into this fascinating and important aspect of virology.

See also: Diagnostic Techniques: Plant Viruses; Diagnostic Techniques: Serological and Molecular approaches.

Further Reading

Chiu CY, Rouskin S, Koshy A, *et al.* (2006) Microarray detection of human parainfluenzavirus 4 infection associated with respiratory failure in an immunocompetent adult. *Clinical Infectious Diseases* 43: e71–e76.

Lin B, Wang Z, Vora GJ, *et al.* (2006) Broad-spectrum respiratory tract pathogen identification using resequencing DNA microarrays. *Genome Research* 16: 527–535.

Lin FM, Huang HD, Chang YC, *et al.* (2006) Database to dynamically aid probe design for virus identification. *IEEE Transactions on Information Technology in Biomedicine* 10: 705–713.

Urisman A, Fischer KF, Chiu CY, *et al.* (2005) E-Predict: A computational strategy for species identification based on observed DNA microarray hybridization patterns. *Genome Biology* 6: R78.

Urisman A, Molinaro RJ, Fischer N, *et al.* (2006) Identification of a novel gammaretrovirus in prostate tumors of patients homozygous for R462Q RNASEL variant. *PLoS Pathogen* 2: e25.

Wang D, Coscoy L, Zylberberg M, *et al.* (2002) Microarray-based detection and genotyping of viral pathogens. *Proceedings of the National Academy of Sciences, USA* 99: 15687–15692.

Wang D, Urisman A, Liu YT, *et al.* (2003) Viral discovery and sequence recovery using DNA microarrays. *PLoS Biology* 1: e2.

Wang Z, Daum LT, Vora GJ, *et al.* (2006) Identifying influenza viruses with resequencing microarrays. *Emerging Infectious Diseases* 12: 638–646.

Diagnostic Techniques: Plant Viruses

R Koenig and D-E Lesemann, Biologische Bundesanstalt für Land- und Forstwirtschaft, Brunswick, Germany
G Adam, Universität Hamburg, Hamburg, Germany
S Winter, Deutsche Sammlung für Mikroorganismen und Zellkulturen, Brunswick, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cylindrical inclusions (CI) CI are induced in infected cells by all viruses belonging to the family *Potyviridae*. The term pinwheel inclusion is also widely used, because it describes the typical appearance of the CI in cross sections. CI are composed of one virus-encoded 66–75 kDa nonstructural protein which aggregates to monolayer sheets forming a complicated structure in which several curved sheets are attached to a central tubule. Details of the CI architecture are specific for the virus species and can serve as an additional identification feature of the virus.

Immunosorbent techniques (e.g., enzyme-linked immunosorbent assay, immunosorbent electron microscopy) Techniques in which a viral antigen is trapped on a solid matrix by means of specific antibodies that are bound to the matrix by adsorpiton.

Plant virus vectors Plant viruses can be transmitted specifically by various vector organisms, for example, aphids, mites, nematodes, or plasmodiophorid protozoans (*Olpidium* sp., *Polymyxa* sp.).

Viroplasm Cytoplasmic inclusions induced by members of the *Caulimoviridae*, *Rhabdoviridae, Reoviridae,* and *Bunyaviridae* are about the size of nuclei and are not bound by any membrane. The viroplasm consists of amorphous and/or fibrillar material and may or may not enclose immature or mature virus particles. It is generally assumed that viroplasms are the site of virus synthesis.

Introduction

Diagnostic techniques for plant viruses are indispensable for at least three major sets of applications:

1. the identification and classification of viruses associated with 'new' plant diseases (primary diagnosis);
2. the routine detection of known viruses, for example, in
   - indexing programs designed to provide healthy plant propagation material (seeds, tubers, grafting material, etc.);
   - breeding programs designed to select virus-resistant plants; and
   - epidemiological investigations designed to monitor the spread of viruses into new areas and in quarantine tests designed to prevent this spread;
3. investigations on the functional, pathogenic, and structural properties of a virus, for example, on
   - its spread within a plant or a vector;
   - changes produced in the ultrastructure of infected cells;
   - intracellular localization of the virions and of nonstructural viral proteins;
   - the time course of the expression of various parts of the viral genome; and
   - the surface structure of virus particles, for example, the accessibility or nonaccessibility to antibodies of various parts of coat protein in the virions.

The primary diagnosis usually requires the application of a number of different techniques, whereas for routine diagnosis only one well-adapted technique is employed. For studying additional properties of a virus, in order to understand its pathogenic effects, one or several techniques may be necessary. The most important diagnostic techniques for plant viruses are based on their morphological, serological, molecular, and pathogenic properties, quite often on a combination of them.

Techniques Used for the Identification and Classification of Viruses Associated with New Plant Diseases (Primary Diagnosis)

Inspection of Symptoms

The first step in attempts to identify the causal agent of a new plant disease is usually a careful inspection of the type of symptoms present in the natural host. **Figure 1** shows some typical virus symptoms. Mosaics of green and yellow (chlorotic) tissue areas (**Figure 1(a)**) or of green and brownish (necrotic) ones (**Figure 1(c)**) or irregular yellow spots (mottling) (**Figure 1(b)**) may be seen on leaves, flowers, or fruits. Flowers may show irregular discolorations (flower break) (**Figure 1(d)**). More or less severe growth reduction (stunting) and/or malformations of either the whole plants or of individual parts, such as flowers and tubers, are also often observed.

Adsorption Electron Microscopy

A quick means to detect the presence of viruses – preferentially in symptom-showing parts of a plant – is the electron microscopical examination of a crude sap extract. In adsorption electron microscopy (**Figure 2(a)**), the virus particles are adsorbed to support films on electron microscope grids and are negatively stained, for example with uranyl acetate. Rod-shaped, filamentous, or isometric particles are readily distinguished from normal plant constituents. More than 30 different morphological types of viruses (examples in **Figure 3**) have been recognized. Particle morphology may serve as a guide for further identification, for example, by means of serology or molecular analyses (see below). Unfortunately, many viruses with very different pathogenic and other properties may be morphologically indistinguishable. Another severe limitation of this test is its rather low sensitivity. Thus, viruses occurring in low concentrations may not be detected. These drawbacks may be overcome in the immunoelectron microscopical tests described in the following sections.

Immunosorbent Electron Microscopy

In immunosorbent electron microscopy (ISEM) (**Figures 2(b), 4(a),** and **4(b)**), the electron microscope grids carrying a support film are coated with antibodies specific for viruses having the same morphology as the ones found in the electron microscopical adsorption test or with antibodies to viruses being suspected to be present in the plant material under investigation. Over a thousand times more virus particles can be detected on such antibody-coated grids than on uncoated grids or on grids coated with antibodies to an unrelated virus. This ISEM test is a very versatile technique. Its sensitivity and the broadness of cross-reactivities detected can be modulated by the length of time during which the virus-containing fluids are in contact with the antibody-coated grids. When the incubation time is short (15 min or less), mainly the homologous and very closely related viruses are trapped. However, after incubation overnight, the efficiency of virus trapping increases and more distantly related viruses are also detected. Very small amounts of antisera are needed (1 µl of crude antiserum can support 200 ISEM tests) and the quality of the antisera need not necessarily be high, since antibodies to normal host constituents do not interfere with virus-specific reactions and low-titered antisera can also be used. Since ISEM tests are labor intensive – they are not suitable for the routine detection of viruses in large numbers of samples.



Figure 1 Typical symptoms induced by plant viruses: (a) mosaic symptoms on abutilon, (b) mottling symptoms on zucchini fruits, (c) chlorotic and necrotic stripe mosaic on barley leaves, (d) flower break on lilies.

Immunoelectron Microscopical Decoration Tests

In the immunoelectron microscopical decoration test (**Figures 2(c), 4(c),** and **4(d)**), the reaction between free antibodies and viruses which have been immobilized on electron microscope support films either by adsorption or serological binding can be visualized as antibody coating of particles ('decoration'). At low antiserum dilutions (e.g., 1:50), homologous antisera produce a dense coating. Reactions with heterologous antisera may be recognized by a much weaker coating efficiency. The closeness of serological relationships may be assessed by comparing the dilution end points (titers) of antisera with homologous and heterologous viruses. The more refined immunogold-labeling technique uses antibodies that are labeled with colloidal gold particles having diameters between 5 and 20 nm. The binding of small amounts of antibodies to individual epitopes, for example, at the extremities of rod-shaped or filamentous virus particles (**Figure 4(d)**), can be visualized by means of this technique.

The immunoelectron microscope decoration tests are the most reliable means for verifying reactions between

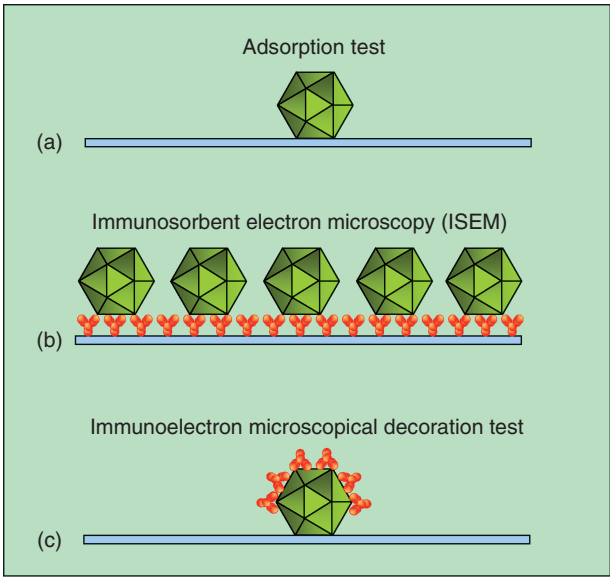


Figure 2 Schematic representation of various electron microscopical tests. For detailed explanations, see text. Adapted with permission from Koenig R and Lesemann D-E (2001) Plant virus identification. In: *Encyclopedia of Life Sciences*. Copyright 2001, © John Wiley & Sons Limited.

viruses and antibodies. Mixed infections by morphologically indistinguishable viruses are readily detected (**Figure 4(c)**). The presence of antibodies can be recognized even on a single virus particle. Thus, viruses occurring at low concentrations, as often happens in field samples, can nevertheless be analyzed directly. The detectability of viruses occurring at low concentration may be increased when the decoration test is preceded by an ISEM step. Antisera even with high host plant reactivities can be used successfully, because the specific antibody attachment to virus particles is directly visualized. The technique is not suited for detecting small antigenic differences between viruses and since it is labor intensive and cannot be automated, it is not suitable for routinely testing large numbers of samples.

Observations on Host Plant Reactions

The transmission of new viruses to experimental host plants is another helpful method to identify and characterize suspected 'new' viruses. Commonly used indicator and propagation hosts are *Chenopodium* species, for example, *C. quinoa* (**Figure 5**), and *Nicotiana* species, for example, *Nicotiana benthamiana*. These plant species are susceptible to a great number of plant viruses, but by far not to all of them. Host range studies may be helpful in finding a suitable host for propagating a new virus and for differentiating it from other similar viruses. In experimental hosts, viruses may reach higher concentrations than in their natural hosts and they may often be purified more easily from them, because natural hosts frequently contain tannins, glycosides, slimy substances, and other inhibitory compounds. Virus purification is necessary for the production of specific antisera which are needed for many diagnostic procedures, such as the above-mentioned immunoelectron microscopical techniques and many others, some of which will be described in the following sections.

Nucleotide Sequence Analyses

Nucleotide sequence analyses are the most reliable means to identify a virus either as a 'new' one or as a 'new' strain of a known virus. These analyses are more time-consuming than the above-described immunoelectron microscope tests. The latter can provide preliminary information concerning the putative genus or family membership of a new virus within 1–2 days. This preliminary information may serve as a valuable basis for further molecular studies, because it allows the design of broad specificity primers for cDNA synthesis and amplification of genome portions by means of the polymerase chain reaction (PCR). Such primers will be derived from highly conserved genome areas in the genera or families suggested by electron microscope studies.

Genome sequences of viruses with circular DNAs can be amplified directly by means of isothermic rolling-circle amplification (RCA) techniques using random primers and the DNA polymerase of *Bacillus subtilis* bacteriophage Phi29. This enzyme in addition to polymerase activity possesses strand-displacement activity, thus allowing circular DNA to be replicated to a nearly unlimited extent. Since random primers can be used, no previous sequence information is necessary. Virus variants or viruses in mixed infections can be recognized after cleavage with restriction endonucleases.

For RNA viruses, reverse transcription of the genome into cDNA is necessary. The PCR products obtained with broad specificity primers can often be sequenced directly without the need of time-consuming and costly cloning procedures. Several commercial companies offer this service. The sequences are compared with those of other viruses in the respective taxonomic group that can be obtained from gene banks, for example, 'Pubmed' or the 'Descriptions of Plant Viruses'. The latter as well as the 'ICTVdB Index of Viruses' offer additional information on previously described viruses.

If no information on the possible genus or family membership of a new virus is available from initial electron microscope studies, the double-stranded RNA (dsRNA) found as a replication intermediate in plants infected with RNA viruses may serve as a template for cDNA synthesis. Reverse transcription may be initiated by a 'universal' primer containing a sequence of about 20 defined nucleotides on its 5′ end and a 3′ tail consisting of six variable nucleotides that may bind randomly to various internal sites in the denatured dsRNA. The cDNAs

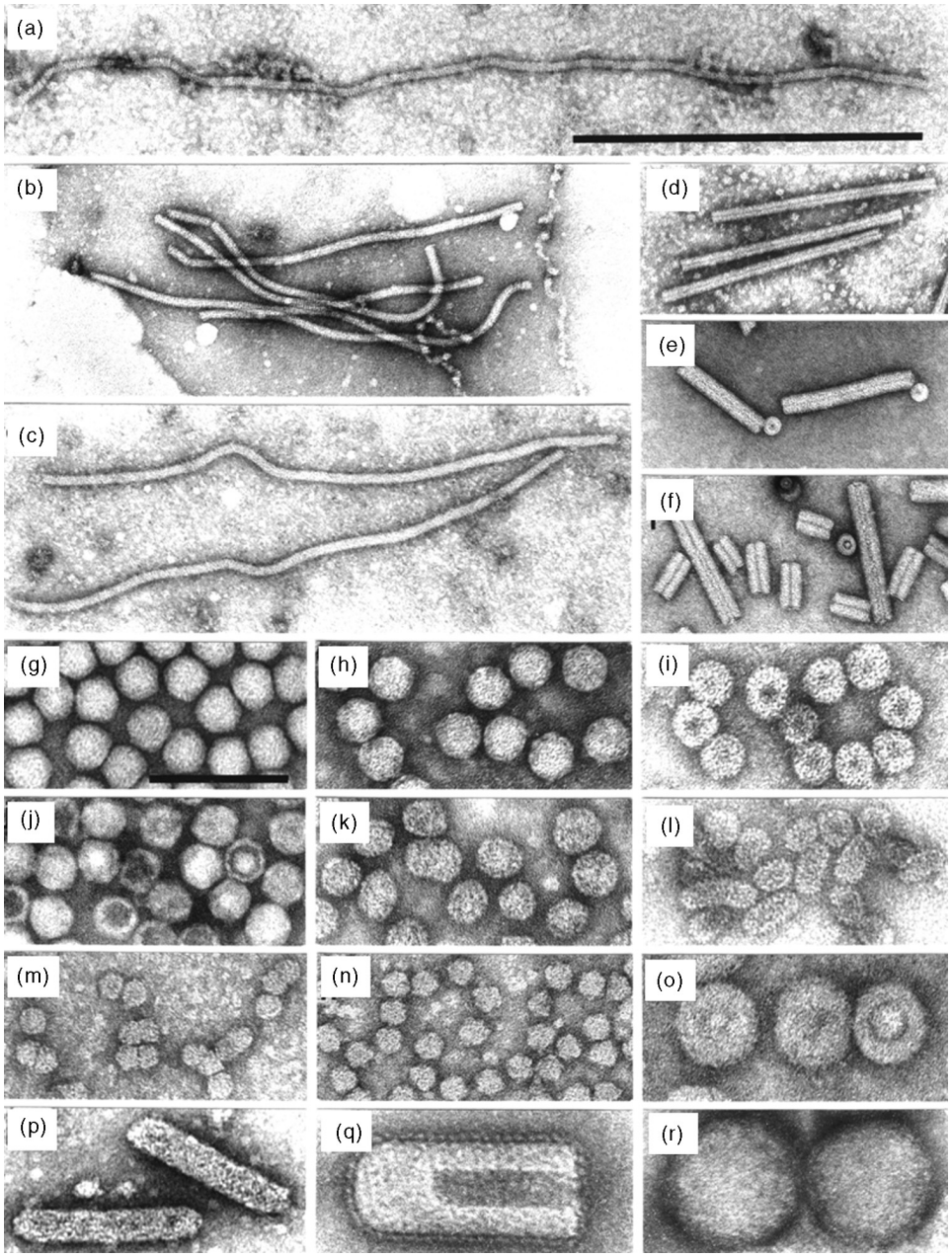


Figure 3 Various morphological types of plant viruses. Filamentous and rod-shaped particles: (a) beet yellows virus (genus *Closterovirus*), (b) potato virus X (genus *Potexvirus*), (c) potato virus Y (genus *Potyvirus*), (d) tobacco mosaic virus (genus *Tobamovirus*), (e): barley stripe mosaic virus (genus *Hordeivirus*), (f) tobacco rattle virus (genus *Tobravirus*). Isometric particles: (g) maize chlorotic mottle virus (genus *Machlomovirus*) , (h) barley yellow dwarf virus (genus *Luteovirus*), (i) cucumber mosaic virus (genus *Cucumovirus*), (j) arabis mosaic virus (genus *Nepovirus*). Irregular isometric to bacilliform particles: (k) apple mosaic virus (genus *Ilarvirus*), (l) alfalfa mosaic virus (genus *Alfamovirus*). Geminate or isometric 18 nm diameter particles: (m) tomato yellow leaf curl virus (genus *Begomovirus*), (n) faba bean necrotic yellows virus (genus *Nanovirus*). Isometric or bacilliform particles: (o) cauliflower mosaic virus (genus *Caulimovirus*), (p) cacao swollen shoot virus (genus *Badnavirus*) . Bullet-shaped or spherical particles: (q) Laelia red leaf spot-type virus (genus *Rhabdovirus*), (r) tomato spotted wilt virus (genus *Tospovirus*). The magnification bar shown in (a) equals 500 nm; the same magnification is also used in (b) to (f). The magnification bar shown in (g) equals 100 nm; the same magnification is also used in (h) to (r). Adapted with permission from Koenig R and Lesemann D-E (2001) Plant virus identification. In: *Encyclopedia of Life Sciences*. Copyright 2001, © John Wiley & Sons Limited.

obtained are rendered double-stranded and are amplified by means of PCR using a second primer that contains only the sequence of the 20 defined nucleotides forming the 5′ end of the 'universal' primer. The many different PCR products obtained will form a smear after electrophoresis in an agarose gel. The more slow-moving fraction of this smear is eluted from the gel. It contains the larger-sized PCR products that are cloned into a suitable

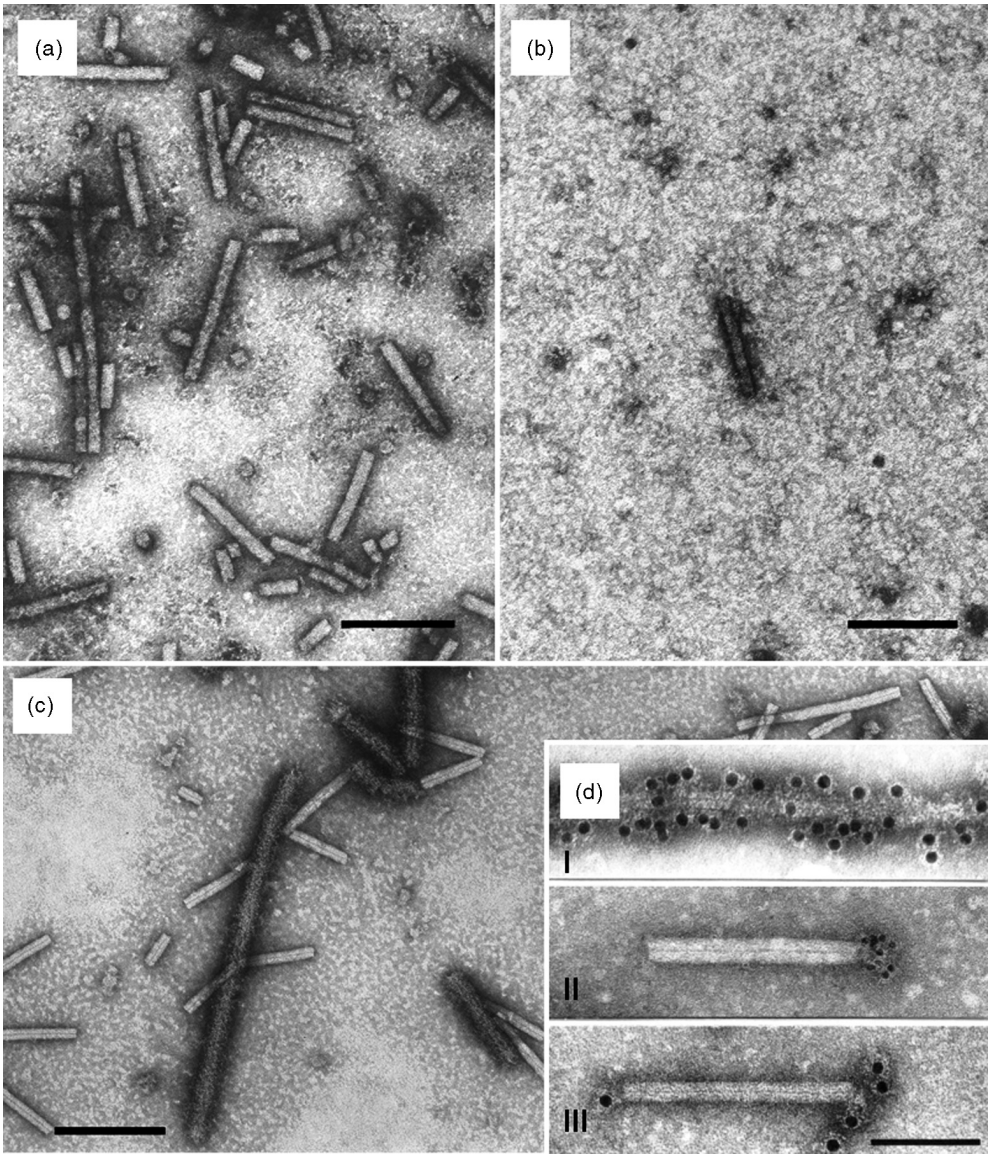


Figure 4 Immunoelectron microscopical analyses of virus-containing fluids: (a) Many virus particles are trapped within 15 min on a grid which had been coated with the homologous antiserum. (b) Only one particle is adsorbed nonspecifically from the same extract as in (a) on a grid coated with serum from a nonimmunized rabbit. (c) Detection of a mixed infection with two morphologically indistinguishable viruses: only those virus particles are decorated for which the antiserum is specific. (d) Binding of monoclonal antibodies to virus particles visualized by immunogold labeling (10 nm gold particles in the upper and lower parts and 5 nm gold particles in the middle part of the figure, respectively). In the upper part of the figure, the antibodies are bound along the entire surface of the particle, and in the middle and lower parts of the figure, on one or both extremities of the particles, respectively. Scale = 200 nm (a–c); 100 nm (d). Adapted with permission from Koenig R and Lesemann D-E (2001) Plant virus identification. In: *Encyclopedia of Life Sciences*. Copyright 2001, © John Wiley & Sons Limited.

vector. Nucleotide sequences are determined with clones containing differently sized inserts. Gaps between individual portions of a sequence may be bridged up by means of PCRs using suitably designed primers on each of these sequences. Complete sequences may be obtained by means of various RACE techniques (rapid amplification of cDNA ends). Since genome recombinations are frequently found with plant viruses, it is important to determine the full-length or almost-full-length nucleotide sequences for newly described viruses in order to allow comparisons to be made along the entire length of the genome.

Techniques That are Especially Useful for the Routine Detection of Plant Viruses

Most of the techniques that are presently used for the routine detection of plant viruses are either based on the use of enzyme-labeled antibodies or on the amplification of portions of the viral genome by means of the PCR or other nucleic acid amplification techniques (see below). Tests based on symptom formation on indicator plants to which the viruses are transmitted either mechanically or by grafting have been used extensively as diagnostic tools

in earlier times, but they are increasingly replaced by molecular or serological techniques which provide results within 1–2 days rather than after weeks or months. In addition, these latter techniques do not require extensive greenhouse or outdoor space, are more specific, do not require the propagation of unwanted pathogens, and – very importantly – can be readily automated.

Enzyme-Linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA), especially its double antibody sandwich form (DAS ELISA), is presently probably the most widely used technique for the routine detection of plant viruses. The principle of DAS ELISA is outlined in **Figure 6**. Virus particles present even in low concentrations in crude sap extracts of diseased plants are trapped by specific antibodies that have been immobilized on the surface of multiwell polystyrene plates.

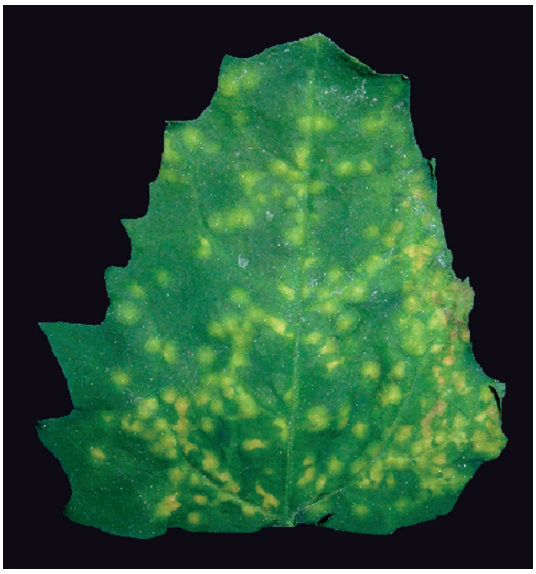


Figure 5 Chlorotic local lesions produced by a plant virus (beet necrotic yellow vein virus) on a leaf of the test plant *Chenopodium quinoa*.

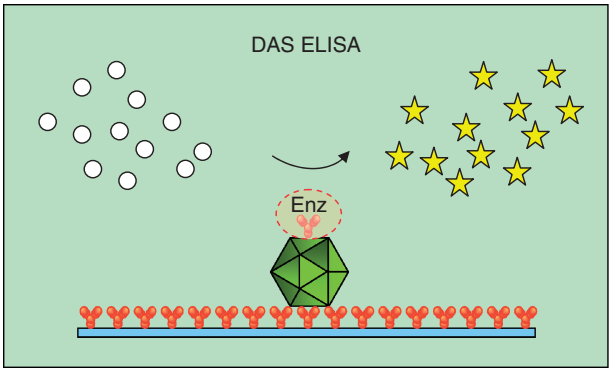


Figure 6 Schematic representation of the principle of DAS ELISA. For a detailed explanation, see text.

The trapped particles are detected by means of enzyme-labeled antibodies. The enzyme attached to these antibodies is able to convert an unstained substrate into a brightly colored reaction product. Alkaline phophatase is used most commonly for antibody labeling in plant virus work. One molecule of alkaline phosphatase attached to an antibody molecule can dephosphorylate large numbers of molecules of the colorless *p*-nitrophenylphosphate to yield the bright yellow *p*-nitrophenol. Both the initial trapping step and the dye-production step make the test more than a 1000-fold more sensitive than the previously used precipitin tests which relied on the detection of a visible precipitate formed as a result of the antigen/antibody reaction. Triple antibody sandwich ELISAs are used especially in work with monoclonal antibodies (MAbs), because the latter often lose their activity when labeled directly with an enzyme. The binding of mouse or rat MAbs is detected by means of enzyme-labeled antibodies from rabbits specific for mouse or rat immunoglobulins, respectively. This requires an additional assay step, but often results in increase in sensitivity of virus detection and a broadening of the range of serologically related viruses which can be detected.

Tissue Print Immunoblotting

In the tissue print immunoblotting assay (**Figure 7**), the surfaces of freshly cut virus-infected plant organs, for example, of leaves or roots, are firmly pressed on a nitrocellulose or nylon membrane. Viruses contained in the sap become adsorbed to this membrane and are detected by means of antibodies labeled with an enzyme that is able to convert a colorless soluble substrate into a colored insoluble product. For alkaline phospatase, 5′-bromo-4-chloro-3-indolylphosphate (BCIP) is commonly used as a substrate. Together with 4-nitrotetrazoliumchloride blue (NTB), its dephosphorylation products form an intense blue deposition of diformazan. The binding of unlabeled antibodies produced in a rabbit can be detected by means of enzyme-labeled antibodies produced against rabbit immunogloblins in a different animal species, such as goats. Small portions of a leaf are sufficient for the routine detection of a virus. The test also allows studies of the distribution of a virus in large organs, such as sugar beet tap roots.

PCR and Other Nucleic Acid Amplification Techniques

PCR techniques using specific primers have become very popular for the routine detection of plant viruses. They are in general more sensitive than serological techniques and hence can be used even for the detection of many viruses in woody plants, for example, in fruit trees, for which ELISA is usually not sensitive enough.

With RNA viruses, transcription of the RNA into cDNA prior to PCR is necessary (reverse transcription PCR, RT-PCR). Viral RNAs can be extracted from

infected tissues by means of various commercially available kits which often are based on the selective binding of RNAs to silica gel-containing membranes and microspin technology. Alternatively, virus particles can be trapped from plant sap by means of specific antibodies which have been immobilized, as in ELISA, on a polystyrene surface, for example, in an Eppendorf tube. In this immunocapture RT-PCR (IC-RT-PCR), the nucleic acid is released from the virus by heat treatment and reverse-transcribed (**Figure 8**). Fragments of the generated cDNA are amplified and converted to dsDNA by means of a heat-stable DNA polymerase in the presence of deoxynucleotide triphosphates (dNTPs) and specific upstream and downstream primers flanking the sequence. The formation of correctly sized PCR products is checked by means of electrophoresis in agarose gels which are stained with ethidium bromide.

As mentioned earlier, viruses with circular DNA can readily be detected by means of the isothermic RCA technique using the DNA polymerase of *B. subtilis* bacteriophage Phi29. RT-LAMP (loop-mediated isothermal amplification) might be a promising isothermic alternative to PCR that can be used for RNA viruses.

In multiplex PCRs, several viruses can be detected simultaneously using pairs of primers specific for different viruses. The primers are designed in a way that each virus in a mixture yields a differently sized PCR product. The sensitivity of conventional PCRs can be further increased by means of a second amplification step using nested primers that anneal to internal sequences in the first PCR product which may be present in a concentration too low for direct detection.

The technical simplicity of PCR has made it a routine procedure in many laboratories, including virus-diagnostic laboratories. However, despite the ease of the method, careful interpretation of the results and positive, internal, and negative controls are extremely important in PCR analyses. The reason is that samples may easily become contaminated during working procedures by minute amounts of cDNAs or PCR products that may be present, for example, in aerosols in laboratories. UV irradiation is a useful means to decontaminate working areas.

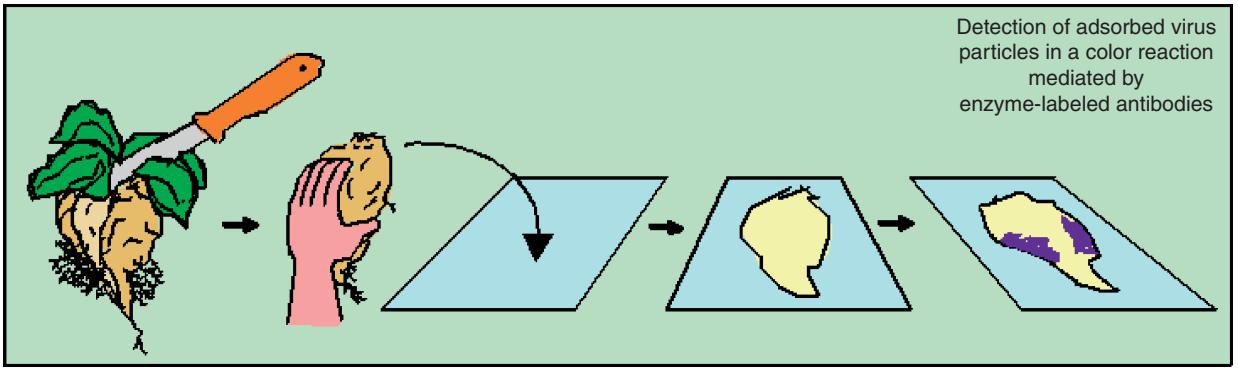


Figure 7 Schematic representation of the tissue print immunoblotting assay – for details, see text. Adapted with permission from Koenig R and Lesemann D-E (2001) Plant virus identification. In: *Encyclopedia of Life Sciences*. Copyright 2001, © John Wiley & Sons Limited.

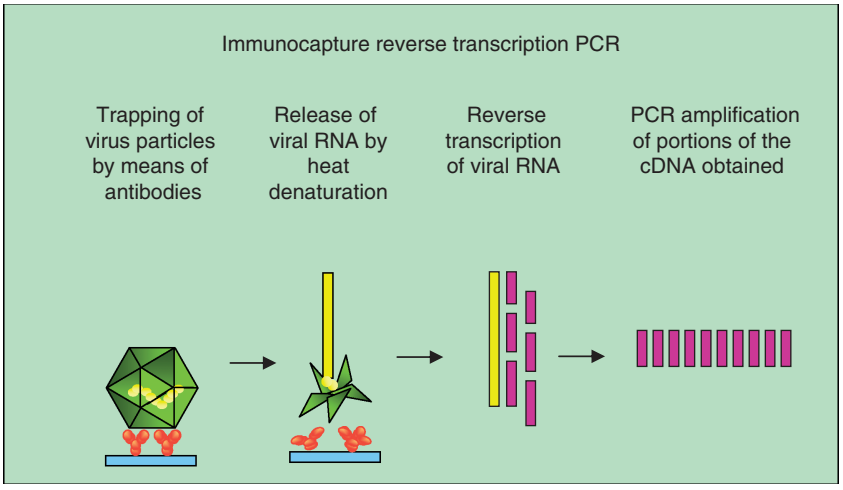


Figure 8 Schematic representation of the principle of IC-RT-PCR – for details, see text. Adapted with permission from Koenig R and Lesemann D-E (2001) Plant virus identification. In: *Encyclopedia of Life Sciences*. Copyright 2001, © John Wiley & Sons Limited.

Real-Time PCR

Real-time PCR combines the amplification and identification of target sequences in one complex reaction. Between the upstream and downstream amplification, primers most commonly a TaqMan probe is annealed specifically to the target sequence. This probe carries a fluorophore quencher dye attached to its 3′ end and a fluorpophore reporter dye attached to its 5′ end. During PCR, the polymerase replicates the template to which the probe is bound. Due to the 5′ exonuclease activity of the enzyme, the probe annealed to the target sequence is then cleaved and the reporter is thus released from the proximity of the quencher. This leads in each cycle to an increase in the fluorescence of the reporter dye that will be proportional to the number of amplicons that have been created. Because the increase in fluorescence signal is detected only if the target sequence is complementary to the probe, nonspecific amplification is not detected.

Real-time PCR is an attractive alternative to conventional PCR because of its rapidity, sensitivity, reproducibility, and the reduced risk of carryover contamination. In addition, it is cost effective when employed in high-throughput diagnostic work where it can be conveniently automated. As other PCR-based techniques, real-time PCR permits the differentiation of strains or pathotypes that differ in a few nucleotides only. With the possibility of quantification, real-time PCR is a highly versatile molecular technique for routine virus detection and identification.

Microarrays

The simultaneous identification of a number of viral nucleotide sequences, theoretically comprising all possible virus targets in a given sample, is possible by means of microarray analysis, a technique originally designed for gene expression studies with large numbers of nucleic acid sequences. With the availability of rapidly increasing viral sequence data, sequences conserved in all members of a genus or, alternatively, sequences highly specific for one or a few isolates can be defined and used in such hybridization analysis. PCR products or nowadays rather oligonucleotides less than 50 nt in length complementary to informative conserved or discriminatory sequences in virus genomes are printed on glass slides. Several hundred to a few thousand oligonucleotide spots which act as capture probes are compiled on a so-called DNA chip. The target sequences, for example, cDNA for the total RNA of a virus-infected plant, are labeled with fluorescent dyes, for example, cyanine 3/cyanine 5, during reverse transcription and are hybridized to the probe sequences on the slide. Their ability or failure to bind to the probes is subsequently analyzed using specific laser scanners and image analysis software.

Microarray analysis permits the parallel detection of a large number of pathogen sequences in one plant sample and can be useful, for example, in quarantine assays for identifying the 'array' of pathogen sequences present in one sample. The technique is commonly used for the detection and genotyping of human viruses, for example, HIV and hepatitis virus C. Its application in plant virology, however, is still at the level of proof of principle and its use for high-throughput testing is still limited by the high cost of chip production and target labeling. Also, preamplification steps may be necessary to enrich target sequences present in low concentrations.

Techniques That are Especially Useful for Studying Functional, Physical, and Structural Properties of Plant Viruses

Western Blotting

The correct sizes and the time course of the expression of structural and nonstructural proteins of a virus can be determined by means of Western blotting. In this technique, the proteins present in a plant extract are first separated by means of sodium dodecyl sulfate (SDS) polyacrylamide electrophoresis and are then transferred electrophoretically to a nitrocellulose or nylon membrane where the viral proteins are detected by means of enzyme-labeled antibodies as described above for tissue print immunoassay.

Epitope Mapping by Investigating the Binding of MAbs to Overlapping Synthetic Oligopeptides and Subsequent Immunoelectron Microscopical Studies

Epitopes, that is, binding sites of viral coat proteins recognized by antibodies, can be identified by studying the ability or inability of MAbs to bind to a series of overlapping synthetic oligopeptides corresponding to the amino acid sequence of the viral protein. The binding sites of those MAbs which have reacted with one or several of the oligopeptides are subsequently checked on the native virus particles by means of immunoelectron microscopical decoration tests using the sensitive immunogold-labeling technique (see above). By this means, information can be obtained on the accessibility or nonaccessibility of antibody-binding sites in the intact virus particles (**Figure 4(d)**).

Methods for Studying the Distribution of a Virus within a Plant or a Vector

The distribution of a virus inside a plant or a vector can be investigated macroscopically or by means of a light microscope using the above-mentioned tissue print

immunoassay or by means of antibodies labeled with fluorescent dyes. Alternatively, the coding sequences for the green fluorescent protein (GFP) or other fluorescent tags can be attached to the coding sequences for structural or nonstructural viral proteins, provided that infectious cDNA clones of a virus are available. Investigations on the movement of a tagged virus or its tagged nonstructural proteins, such as of movement proteins, between different cell compartments are best performed by means of confocal laser scan microscopy (CLSM), which produces blur-free high-resolution images of thick specimens at various depths as well as computer-generated three-dimensional (3-D) reconstructions.

Studies on the Ultrastructure of Infected Host Cells by Means of Conventional Electron Microscopy

Alterations of the fine structure in infected host cells are studied by means of conventional electron microscopy in ultrathin sections that are obtained from plant tissues after chemical fixation, dehydration, and embedding in resin-like polymers such as epoxides or methacrylates (Epon, Araldite, Spurr's resin, LR White, or LR Gold). Cryosectioning after rapid freezing procedures is used less frequently. Cytological alterations are easily detected in ultrathin sections from well-selected tissue samples. The preparation of the sections, however, is time consuming and requires special equipment and expertise. Also, not all plant viruses induce characteristic cellular alterations.

Cytological alterations appearing as unusual inclusions may be recognized in infected cells also by means of light microscopy, especially after appropriate staining. However, details of their fine structure and the complete spectrum of cellular alterations can be analyzed only in ultrathin sections. Tissues from plant organs showing macroscopically visible symptoms like chlorosis and necrosis may show pathological alterations of various cell organelles, for example, chloroplasts, mitochondria, peroxisomes, nuclei or membranes of the endoplasmic reticulum. These alterations may be indicative of the damaging physiological effects induced by the infection in incompletely adapted host plants, rather than of virus-specific effects on cytology. However, virus-specific alterations may also be recognized in infected cells. They are most clearly seen in cells of hosts which do not produce pronounced macroscopically visible symptoms (latent infections).

Characteristic inclusions may be formed by accumulated virus particles (**Figure 9**(**a**)), nonstructural viral proteins (e.g., in the typical cylindrical inclusions induced by potyviruses; **Figure 9**(**b**)), proliferated host membrane elements, or they may be composed of complex

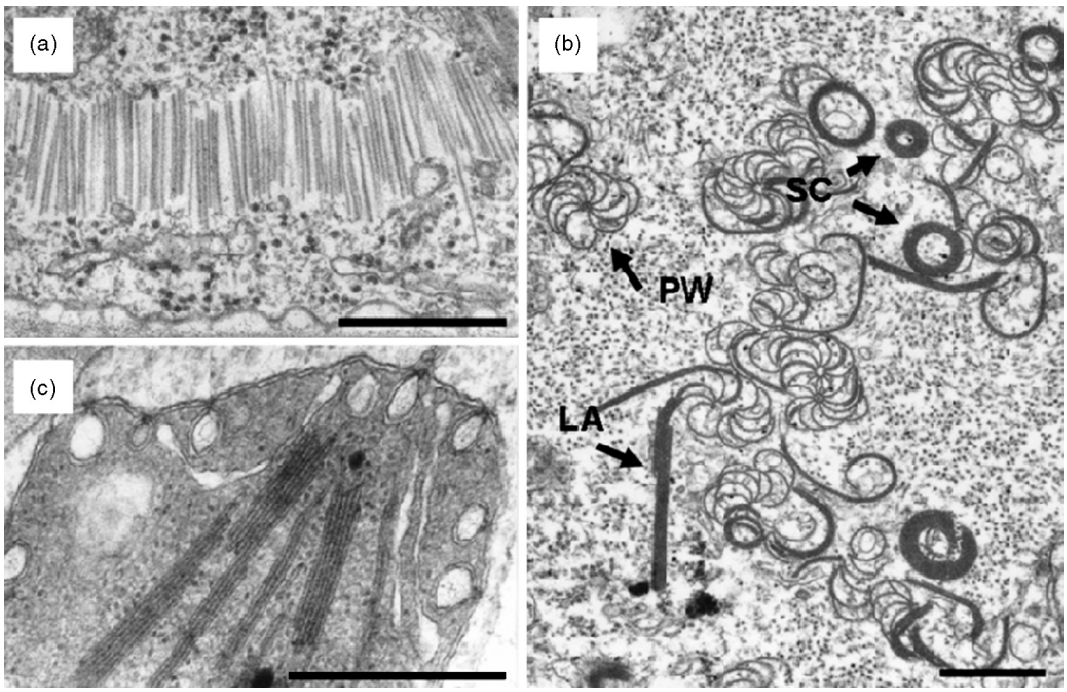


Figure 9 Electron-microscopically visible cytological alterations due to virus infections. (a) Plate-like cytoplasmic aggregate formed by the particles of a rod-shaped virus (cross section) – the highly regular *in vivo* arrangement with particle ends in register has been disturbed during fixation. Scale = 500 nm. (b) Ultrathin cross sections of pinwheels (PWs), scrolls (SCs), and laminated aggregates (LAs) formed by a nonstructural protein of a potyvirus. (c) Flask-shaped vesicles associated with the peripheral membrane of a chloroplast typical for tymovirus infections; the fibrillar content of the vesicles suggests the presence of dsRNA. Scale = 500 nm. Adapted with permission from Koenig R and Lesemann D-E (2001) Plant virus identification. In: *Encyclopedia of Life Sciences*. Copyright 2001, © John Wiley & Sons Limited.

aggregates of one or more of these components with host cell constituents. The particular structure and composition of such inclusions may be characteristic for a virus. Members of the Sindbis-like supergroup of positive-stranded single-strand RNA (ssRNA) viruses may induce virus-specific or genus-specific membrane-associated vesicles (**Figure 9(c)**), whereas members of the picorna virus-like supergroup may induce accumulations of free vesicles. Both types of vesicles contain dsRNA and are believed to be associated with viral RNA replication. Viruses with genomic nucleic acids other than positive-stranded ssRNA may induce viroplasm-like inclusions presumably involved in virus replication.

The fine structure of virus particle aggregates is usually determined by the morphology of the viruses (see **Figure 9(a)**) and is, thus, mostly specific for a virus genus. Occasionally, however, the particle arrangement may be specific for a certain virus strain. Inclusions composed of nonstructural viral proteins may exhibit very diverse and sometimes virus-specific shapes and fine structures (see **Figure 9(b)**). Proliferated membrane inclusions and membrane-associated vesicles are also species or genus specific (see **Figure 9(c)**).

Outlook: New and Neglected Techniques with Promise for Plant Virus Work

New technical developments that are promising for plant virus work include the use of liquid arrays and Padlock probes. A useful technique for RNA quantification that has been widely neglected by plant virologists is the RNase protection assay.

Liquid Arrays

Instead of applying antibodies to different viruses as an array to a planar surface, differently colored plastic beads are coated with different antibodies and serve as a basis for a miniaturized DAS ELISA in which the second antibody is labeled with a green fluorescent dye. In ELISA plates, mixtures of such beads carrying different antibodies allow the simultaneous testing of up to 100 different antigens. The assay is demultiplexed by means of a flow-through fluorescence reader that identifies by means of a laser beam on the basis of the color of a bead the antibody that has been used for coating the bead. Subsequently, a second laser beam shows how much fluorescent antibody has been bound to a specific type of bead because of the presence of the antigen in the testing material.

Padlock Probes

Padlock probes are DNA molecules whose 3′ and 5′ ends are complementary to adjacent parts of the target sequence (**Figure 10**). They become circularized in a ligation reaction when they fit the target sequence completely. In addition, they contain forward and backward primer-binding regions (P1 and P2) which can be used for amplification using unlabeled (**Figure 10(c)**) or labeled primers (**Figure 10(b)**). The probes also contain a ZipCode region which can be used either for hybridizing

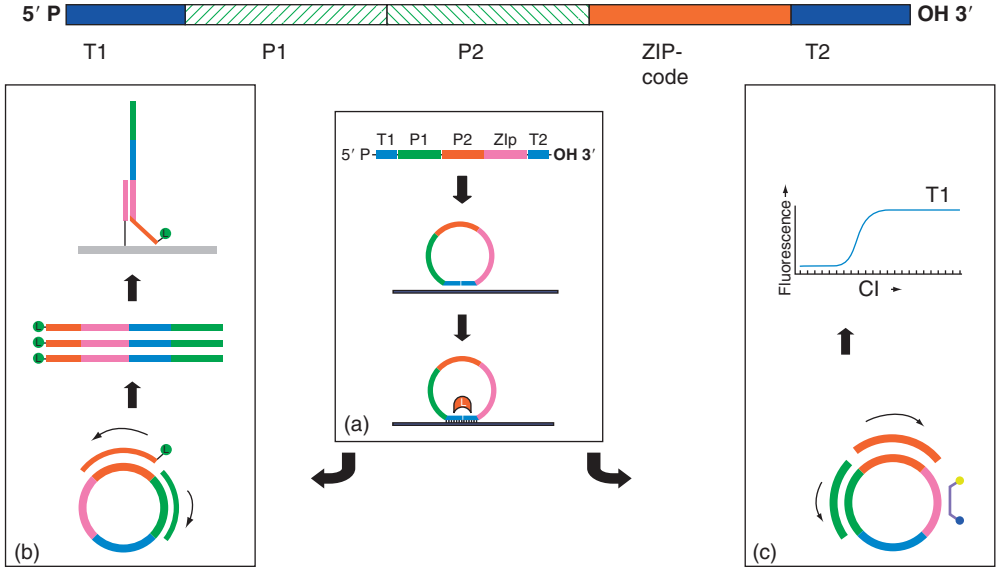


Figure 10 The Padlock probe, structure, and application for nucleic acid-based detection. The top drawing shows the construction of a Padlock probe. It consists of the two target-specific regions T1 and T2, the primer-binding regions P1 and P2, and the ZipCode, which serves as identification sequence. The probe is hybridized to the target and in case of a complete fit it is circularized (a). Subsequently the probe can serve either as a template for PCR amplification using fluorescent-labeled primers (b). In this case, the labeled amplicons are hybridized via the ZipCode to oligonucleotide arrays for differential detection (b). Alternatively, the circularized probe can be used for quantitative PCR with a TaqMan probe directed against the ZipCode (c). Courtesy of C. D. Schoen and P. J. M. Bonants, Plant Research International BV, Wageningen, The Netherlands.

the labeled Padlock amplicons to oligonucleotide arrays (**Figure 10(b)**) or as binding region for a TaqMan probe or a molecular beacon in quantitative real-time PCR (**Figure 10(c)**).

RNase Protection Assay

These tests offer a simple and cheap method to quantify RNA without PCR. They are especially useful when several RNAs have to be quantified simultaneously. Discrete-sized complementary RNAs are labeled, for example, by radioactive isotopes, and are hybridized in solution with the target RNAs to yield labeled dsRNAs. After RNase digestion of the unhybridized probe and target RNAs, the labeled dsRNAs are separated electrophoretically in a denaturing polyacrylamide gel and are visualized and quantified according to the label used.

See also: Diagnostic Techniques: Microarrays; Electron Microscopy of Viruses.

Further Reading

Abdullahi I, Koerbler M, Stachewicz H, and Winter S (2005) The 18S rDNA sequence of *Synchytrium endobioticum* and its utility in microarrays for the simultaneous detection of fungal and viral pathogens of potato. *Applied Microbiology and Biotechnology* 68: 368–375.

Baulcombe DC, Chapman SN, and Santa Cruz S (1995) Jellyfish green fluorescent protein as a reporter for virus infections. *Plant Journal* 7: 1045–1053.

Boonham N, Fisher T, and Mumford RA (2005) Investigating the specificity of real time PCR assays using synthetic oligonucleotides. *Journal of Virological Methods* 130: 30–35.

Boonham N, Walsh K, Smith P, *et al.* (2003) Detection of potato viruses using microarray technology: Towards a generic method for plant viral disease diagnosis. *Journal of Virological Methods* 108: 181–187.

Bystrika D, Lenz O, Mraz I, *et al.* (2005) Oligonucleotide-based microarray: A new improvement in microarray detection of plant viruses. *Journal of Virological Methods* 128: 176–182.

Commandeur U, Koenig R, Manteuffel R, *et al.* (1993) Location, size and complexity of epitopes on the coat protein of beet necrotic yellow vein virus studied by means of synthetic overlapping peptides. *Virology* 198: 282–287.

Francki RIB, Milne RG, and Hatta T (1985) *Atlas of Plant Viruses* Vols. I and II. Boca Raton, FL: CRC Press.

Froussard P (1992) A random-PCR method (rPCR) to construct whole cDNA library from low amounts of RNA. *Nucleic Acids Research* 20: 2900.

Haible D, Kober S, and Jeske H (2006) Rolling circle amplification revolutionizes diagnosis and genomics of geminiviruses. *Archives of Virology* 135: 9–16.

Koenig R and Lesemann D-E (2001) Plant virus identification. *Encyclopedia of Life Sciences.* London: Nature Publishing Group.

Rott ME and Jelkmann W (2001) Characterization and detection of several filamentous viruses of cherry: Adaptation of an alternative cloning method (DOP-PCR), and modification of an RNA extraction profile. *European Journal of Plant Pathology* 107: 411–420.

Tzanetakis IE, Keller KE, and Martin RR (2005) The use of reverse transcriptase for efficient first- and second-strand cDNA synthesis from single- and double-stranded RNA templates. *Journal of Virological Methods* 124: 73–77.

van Regenmortel MHV, Bishop DHL, Fauquet CM, Mayo MA, Maniloff J, and Calisher CH (1997) Guidelines to the demarcation of virus species. *Archives of Virology* 142: 1505–1518.

Varga A and James D (2006) Use of reverse transcription loop-mediated isothermal amplification for the detection of plum pox virus. *Journal of Virological Methods* 138: 184–190.

Webster CG, Wylie SJ, and Jones MGK (2004) Diagnosis of plant viral pathogens. *Current Science* 86: 1604–1607.

Relevant Websites

http://www.pri.wur.nl – Characterization identification and detection, Plant Research International – Research, Wageningen UR.
http://www.csl.gov.uk – Diagnostic Protocols, Special Interest, Central Science Laboratory, York.
https://www.invitrogen.com – D-LUX Assays, Invitrogen.
http://www.whatman.com – FTA Cards (Product of Whatman).
http://www.ncbi.nlm.nih.gov – ICTVdB Index of Viruses; PubMed, NCBI.
http://www.pcrstation.com – Inverse PCR, PCR Station.
http://www.brandeis.edu – LATE-PCR, Wangh Laboratory of Brandeis University.
http://www.luminexcorp.com/technology – Liquid Arrays Technology, Luminex.
http://www.ambion.com – Nuclease Protection Assays: The Basics, Ambion, Inc.
http://www.dpvweb.net – Plant Virus and Viroid Sequences (compiled 11 Jul. 2007), Descriptions of Plant Viruses.
http://pathmicro.med.sc.edu – Real Time PCR Tutorial (updated 7 Dec. 2006), Microbiology and Immunology On-line, University of South Carolina.
http://aptamer.icmb.utexas.edu – The Ellington Lab Aptamer Database.

Diagnostic Techniques: Serological and Molecular Approaches

R Vainionpää, University of Turku, Turku, Finland
P Leinikki, National Public Health Institute, Helsinki, Finland

© 2008 Elsevier Ltd. All rights reserved.

Glossary

EIA Enzyme immunoassays are methods used to estimate virus-specific IgG and IgM antibodies or virus antigens by enzyme-labeled conjugates.

PCR By the polymerase chain reaction (PCR) and with specific primers, DNA sequences can be multiplied.
RT-PCR For RNA, nucleic acid has to be transcribed with reverse transcriptase (RT) enzyme to complementary DNA prior to PCR.

Introduction

Specific virus diagnostics can be used to determine the etiology of acute viral infection or the reactivation of a latent infection. Two approaches can be used: demonstration of a specific antibody response or of the presence of the virus itself. Serological methods are used for measuring the antibody response while the presence of virus can be demonstrated by cultivation or demonstration of specific antigens or gene sequences. For the latter, molecular diagnostic methods have become more and more widely applied.

In this article, we briefly describe the principles of the most important serological methods and molecular applications that are used to provide information about the viral etiology of the clinical condition presumed to be caused by a viral infection.

The diagram of the course of acute virus infection (**Figure 1**) indicates the optimal methods for viral diagnosis. Following transmission, the virus starts to multiply and after an incubation period clinical symptoms appear with simultaneous shedding of infectious virus. Virus-specific antibodies appear somewhat later (from some days to weeks, called a window period). When the virus-specific antibody production reaches the level of detection, at first immunoglobulin M(IgM) antibodies and some days later immunoglobulin G(IgG) antibodies appear, and the amount of infectious virus starts to decrease. If this is the first encounter with this particular virus, that is, a primary immune response, IgG antibody levels can stay at a relatively low level, whereas in a later contact with the same antigen, that is, in secondary response, IgG levels increase rapidly and reach high levels while IgM response may not be detectable at all. Antibodies are usually investigated from serum samples taken at acute and convalescent phase of the infection. In selected cases other materials such as cerebrospinal fluid and other body fluids can also be analyzed.

The presence of infectious virus or viral structural components can be investigated directly from various clinical specimens either by virus isolation, nucleic acid detection assays, or antigen detection assays. In order to reach the best diagnosis for each patient, it is important to select the most suitable method using the right sample collected at the right time.

Principles of Serological Assays

During most primary infections IgM antibody levels peak at 7–10 days after the onset of illness and then start to decline, disappearing after some weeks or months. An IgM response is usually not detected in reactivated infections or reinfections. The production of IgG antibodies starts a few days after IgM response and these antibodies often persist throughout life.

Serological diagnosis is usually based on either the demonstration of the presence of specific IgM antibodies or a significant increase in the levels of specific IgG antibodies between two consecutive samples taken 7–10 days apart. The antigen for the test can be either viable or inactivated virus or some of its components prepared by virological or molecular methods. Isotype-specific markers or physical separation are used to demonstrate the isotype of the reacting antibody. In some cases, even IgG subclass specificities are determined although they have limited value in diagnostic work.

During the early phase of acute infection the specific avidity of IgG antibodies is usually low but it increases

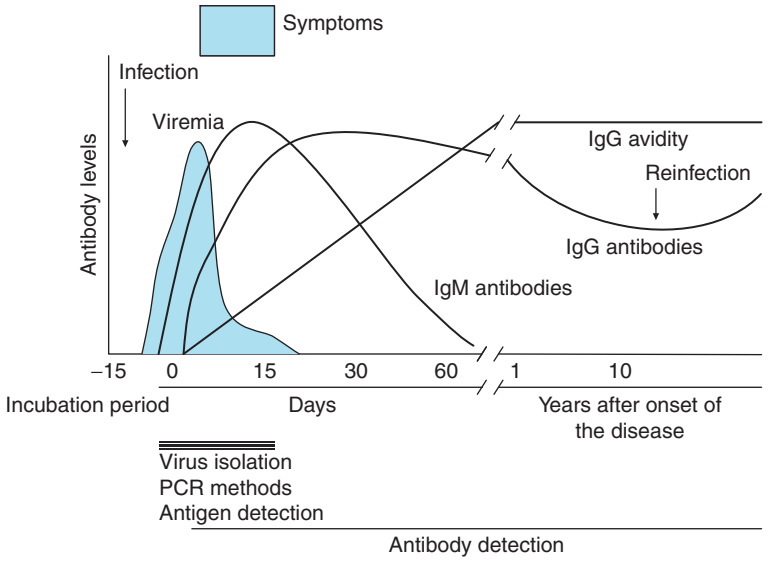


Figure 1 The course of virus infection. The shedding of infectious virus after incubation period and typical antibody response. Recommended diagnostic laboratory methods have been marked.

during the maturation of the response. Diagnostic applications of the measurement of the avidity of IgG antibodies against specific antigens have been developed to help distinguish serological responses due to acute infections from those of chronic or past infections.

Serological assays are useful for many purposes. In primary infections they often provide information about the etiology even after the acute stage when infectious virus or its components can no longer be demonstrated in the samples. They are widely used for screening of blood products for the risk of certain chronic infections, evaluation of the immune status, and need for prophylactic treatments in connection with certain organ transplantations. They are also widely used for epidemiological studies, determination of vaccine-induced immunity, and other similar public health purposes.

Serological assays have their limitations. In some infections the antibody response is not strong enough or the limited specificity of the antigens used in the assay does not allow unambiguous interpretation of the results. In infections of newborns the presence of maternal antibodies may render the demonstration of the response in the baby impossible. In immunocompromised patients the serological response is often too weak to allow the demonstration of specific responses. In these cases other virological methods should be considered.

Other clinical specimens than sera can be used for antibody assays. IgM and IgG antibody determinations from cerebrospinal fluid are used for diagnosis of virus infections in the central nervous system although new molecular methods are increasingly replacing them. Recently, increasing attention has been given to the use of noninvasive sample materials such as saliva or urine. They are becoming important for public health purposes but their value for diagnosing individual patients is still limited.

Principles of the Most Common Serological Tests

Neutralizing Antibody Assay

Antibodies that decrease the infectious capacity of the virus are called neutralizing antibodies. They are produced during acute infection and often persist during the entire lifetime. They are also useful as an indication of immunity. Both IgM and IgG antibodies participate in the neutralization.

In the assay, known amounts of infectious virus are mixed with the serum sample and incubated for a short period after which the residual infectivity is measured using cell cultures or test animals. This infectivity is then compared with the infectivity of the original virus and the neutralizing capacity is calculated from this result. Today, neutralizing antibody assays are often done by plaque reduction assays with better accuracy but with somewhat more complex technical requirements.

Neutralizing antibody assay is specific and sensitive, but time-consuming and laborious, and therefore it is not widely used in routine diagnostic services.

Hemagglutination Inhibition Test

Many viruses bind to hemagglutinin molecules found at the surface of red blood cells of various animal species and this can cause aggregation of red cells in suitable conditions. Prevention of this aggregation, called hemagglutination inhibition, by specific antiviral antibodies in the patient's serum has been widely used for diagnostic purposes. The test, known as hemagglutination inhibition test, has important diagnostic and public health applications in certain infections, most notably in influenza where antibodies measured by this test show additional specificity compared to other tests and therefore provide more detailed information about the immunity and past infections of individuals. However, for the diagnosis of individual patients, the assay is no longer widely used and is replaced by more modern immunoassays.

In the test, a virus preparation with a predetermined hemagglutinating capacity is mixed with the serum sample and after proper incubation the residual hemagglutination capacity is measured. Both IgM and IgG antibodies are able to inhibit hemagglutination.

Complement Fixation Test

The complement fixation test (CFT) is a classical laboratory diagnostic test, which is still used for determination of virus antibodies in patient sera or cerebrospinal fluid samples during an acute infection. The test mainly measures IgG antibodies.

The test is based on the capacity of complement, a group of heat-labile proteins present in the plasma of most warm-blooded animals to bind to antigen–antibody complexes. When the complexes are present on the surface of red blood cells, complement causes their lysis which can be visualized by a suitable experimental setup.

In the actual test, the complement in the patient's serum is first destroyed by heating; the serum is then mixed with appropriate viral antigen and after incubation; when the antigen–antibody complexes are formed, exogenous complement (usually from fresh guinea pig serum) is added. This complement then binds to the complexes and having been 'fixed', it is then no longer able to cause lysis of added indicator red cells. Usually, sheep red cells coated with antisheep red cell antibodies are used as indicator to measure the presence of any residual complement. The effect is measured by a suitable test protocol. Serial dilutions of the patient serum are used

and the highest dilution where the serum can still prevent complement activity in the indicator system is taken as the CFT titer of the sample. The tests are usually carried out on microtiter plates and the results are observed by eye.

CFT is still used for diagnosis of acute virus infection. It measures certain types of antibodies which occur only during the acute phase of the infection. Therefore, CFT is not suitable for investigation of immune status. The assay procedure is quite complex, because the test is dependent on several biological variables, which have to be standardized by pretesting. The method is less sensitive than many other immunoassays. In addition, the method is very labor intensive and is not amenable to automation. The use of CFT in virus diagnostics is increasingly replaced by modern immunoassays.

Immunoassays

In immunoassays, antibodies binding to specific immobilized antigens can directly be observed using bound antigens and proper indicators such as labeled anti-immunoglobulin antibodies. The antigens can be immobilized to plastic microtiter plates, glass slides, filter papers or any similar material. Different immunoassays are nowadays widely used to measure virus-specific IgM and IgG antibodies. The most recent formats of immunoassays make it possible to detect simultaneously both antigens and antibodies decreasing significantly the window period between infection and immune response. Numerous commercial kits with high specifity and sensitivity are available. Automation has made immunoassay techniques more rapid, accurate, and easier to perform.

In the basic format of solid-phase immunoassays, virus-infected cells, cell lysates, purified or semipurified, recombinant viral antigens or synthetic peptides are immobilized to a solid phase, usually plastic microtiter wells or glass slides. Patient's serum is incubated with the antigen and the bound antibody, after washing steps, is visualized using labeled anti-immunoglobulin antibodies ('conjugate') (**Figure 2(a)**). If the label used is an enzyme, the test is called enzyme immunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA) and the bound antibody is detected by an enzyme-dependent color reaction. If a fluorescent label is used, the method is called immunofluorescent test (IFT). The enzyme labels most commonly used are horseradish peroxidase (HRP) and alkaline phosphatase (AP). In HRP-EIA the color-forming system consists of ortho-phenyldiamine (OPD) as a chromogen and hydrogen peroxidase (H_2O_2) as a substrate. If the HRP-conjugate is bound to antibody–antigen complexes, the colorless chromogen becomes yellow and color intensity is measured with a photometer at a wavelength of 490–492 nm. The intensity of the color is proportional to the amount of bound conjugate and to the amount of specific antibodies in a patient serum sample. If the serum contains no specific antibodies, the conjugate is not bound and no color reaction occurs. By using either anti-IgG or anti-IgM conjugates it is possible to determine separately immunoglobulin subclasses.

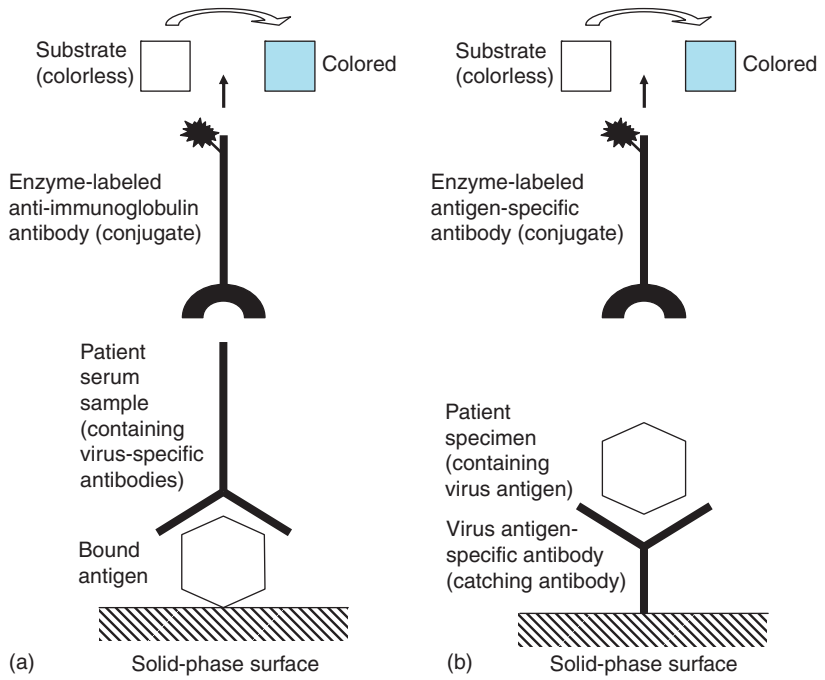


Figure 2 Enzyme immunoassay. (a) Detection of virus-specific antibodies. (b) Detection of virus antigens.

The specificity and sensitivity of these immunoassays are high. The sensitivity can be improved further, by using an additional incubation step where IgM antibodies are first enriched ('captured') in the sample by using anti-IgM immunoglobulin. Modifications to improve assay specificity by various methods of antigen handling and by using monoclonal antibodies or synthetic peptides have been developed.

Immunofluorescent tests were used in the past for measuring virus-specific antibodies, but are now replaced by EIA techniques. The principle of the method is similar to EIAs. In IFT infected cells are placed on a glass slide and bound antibodies are detected by fluorescein-labeled anti-immunoglobulin antibodies. The glass slides are examined under a fluorescence microscope. The method is specific and sensitive, but quite labor intensive and reading the test demands considerable experience.

Immunoblotting

In some infections (e.g., that caused by human immunodeficiency virus (HIV)), antibodies against certain components of the virus are more informative than other less-specific antibodies and they are detected by immunoblotting assays. Different virus antigens, prepared by gel diffusion or other techniques, are absorbed as discrete bands on a solid strip of cellulose or similar material and the strip is incubated with the patient's serum. Antibodies present in the serum bind to specific antigens and are detected using an HRP-conjugate and nitroblue tetrazolium as the precipitating color chromogen. The color reaction is observed and compared to positive and negative control samples assayed on separate strips.

Lateral-Flow and Latex Tests

A technique known as lateral-flow technology has also been used to identify antibodies or antigens. These tests involve application of serum or other samples directly on a strip of suitable material such as cellulose, where the antibodies are diffused laterally and eventually reach a site in the strip where appropriate antigen has been applied and chemically fixed. Specific antibodies become bound to the site while nonreacting antibodies diffuse out from the area. The presence of antibodies is visualized using labeled conjugates.

Although such tests are not quantitative, they are valuable for infections where the presence of specific antibodies is indicative, such as HIV infection. Performance of the test is often very simple and the result is available in a few minutes or a few hours, making such tests suitable for bed-side screening. In more advanced tests, several different antibodies can be detected by a single assay and the test conditions can be modified further so that antigens can also be detected. Many such tests have become commercially available in recent years.

For some applications, coated latex particles have replaced strips with fixed antigen as the solid phase. Binding of specific antibodies can be visualized with chromogenic or otherwise labeled indicator antibodies or a positive reaction can be detected by agglutination of the latex particles.

Point-of-Care Tests

Point-of-care tests (POC tests) are becoming increasingly common in clinical practice. Most of them are based on easy-to-use lateral-flow or latex particle technology and are able to give the result in a few minutes. POC tests are nowadays available for antibody screening of an increasing number of virus infections (HIV, hepatitis C virus (HCV), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr virus (EBV)). Some authorities still question the validity of POC tests for clinical use although there is considerable evidence that many of the commercially available kits give reliable results.

Detection of Viral Antigens

The presence of viral antigens in clinical specimens, such as nasopharyngeal aspirates, fecal specimens, vesicle fluids, tissue specimens, as well as serum samples can be demonstrated by antigen detection assays.

In immunofluorescence tests, cells from a clinical specimen are fixed on a glass slide and viral antigens present in the cells are detected by fluorescein-labeled virus-specific antibodies. More reliable results can be obtained using enzyme immunoassay or time-resolved fluoroimmunoassay (TR-FIA). Europium-labeled monoclonal antibodies can be used as a conjugate. Solubilized antigens in clinical specimens are first captured using specific monoclonal antibodies bound to a solid phase, and are then detected with enzyme- or europium-labeled virus-specific antibodies (**Figure 2(b)**).

Antigen detection methods are especially recommended in the case of virus reactivation, for example, for herpes simplex and varicella zoster virus diagnosis where the serological response can be very weak. Antigen detection assays are also widely used in respiratory tract infections like influenza and respiratory syncytial virus infections. A simple test for the demonstration of rotavirus and adenovirus antigens in children with gastroenteritis is also available.

Nucleic Acid Detection Assays

Direct demonstration of viral nucleic acids in clinical samples is an increasingly used technique for virus diagnosis. Using the polymerase chain reaction (PCR) with specific

primers, viral sequences can be rapidly multiplied and identified. These techniques are largely replacing classical virus isolation. They are rapid to perform and in many cases more sensitive than virus isolation or antigen detection methods making earlier diagnosis possible. They have proved particularly valuable for the diagnosis of viruses that cannot be cultivated such as papillomaviruses, parvoviruses, and hepatitis viruses. Semiquantitative and quantitative applications have been developed allowing monitoring of viral load during antiviral treatment. These tests cannot distinguish between viable and replication-incompetent virus, warranting caution in the interpretation of the results in certain cases. Also, sensitivity to cross-over contamination in the laboratory has caused some problems in clinical laboratory settings.

The specificity of these tests is based on the extent of pair-matching sequences between the viral nucleic acids and the primers. Extremely high sensitivity is typical for PCR methods; 1–10 copies of viral nucleic acid can be detected in a few hours. PCR methods are available for both RNA and DNA viruses. For RNA viruses viral nucleic acid has to be transcribed with reverse transcriptase (RT) enzyme to complementary DNA (RT-PCR).

Viral nucleic acid is extracted from the sample material and amplified in three successive steps. The double-stranded DNA is first heat-denaturated and separated into single strands. The specific target fragment of DNA strand is then amplified (**Figure 3**) by pairs of target-specific oligonucleotide primers, each of which hybridize to one strand of double-stranded DNA. The hybridized primers act as an origin for heat-stable polymerase enzyme and a complementary strand is synthesized via sequential addition of deoxyribonucleotides. After annealing of the primers, extension of the DNA fragment will start. These cycles are repeated 35–40 times, each cycle resulting in an exponentially increasing numbers of copies.

After the amplification is completed, the products can be detected by several methods. Agarose gel electrophoresis combined with ethidium bromide staining of the products is a classical method (**Figure 4**). The size

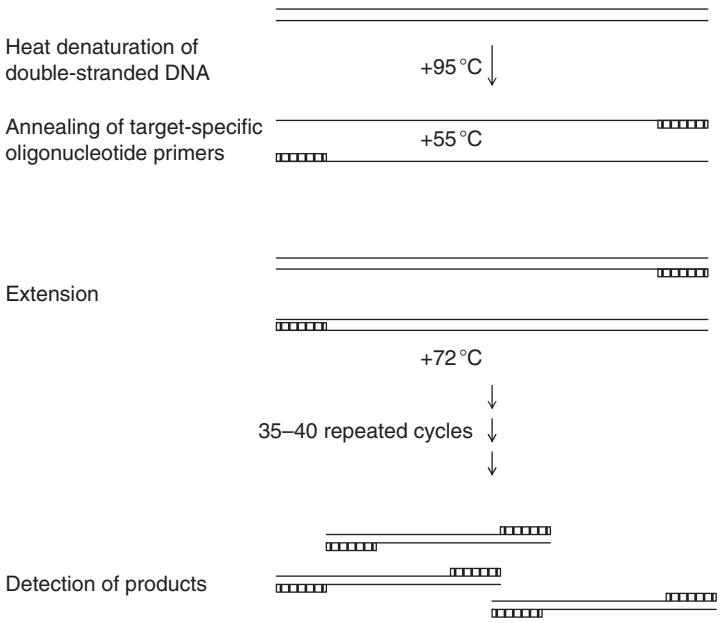


Figure 3 Polymerase chain reaction.

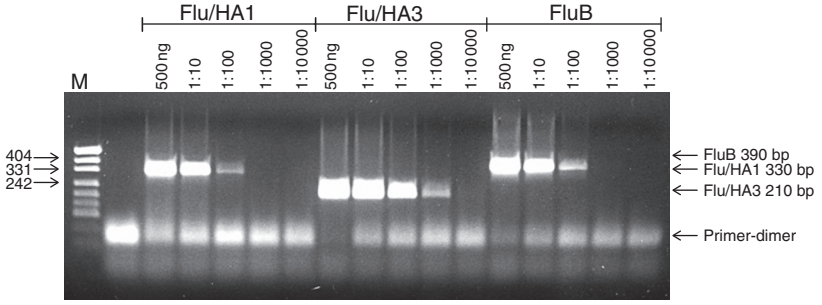


Figure 4 Detection of PCR products (amplicons) by an agarose gel electrophoresis after ethidium bromide staining.

of the amplified product is compared to control amplicons and other standards in the same gel. Various hybridization assays, based on labeled complementary oligonucleotides (probes), are also used to improve the sensitivity and specificity of the detection.

The amplified fragments can also be sequenced giving additional information about the virus. Comparison of the sequences with known virus sequences allows identification of species, strains, or subtypes that may be important for public health or medical purposes. Sequencing after RT-PCR is also the current method-of-choice for investigating the emergence of antiviral drug resistance among HIV-infected patients.

Real-time PCR instruments monitor accumulation of amplicons by measuring the fluorescence continuously in each cycle of the reaction. The earlier the amplification product becomes detectable over the background, the higher is the amount of virus in the sample (**Figure 5**). One application, based on the use of melting temperatures, allows simultaneous detection and analysis of several different nucleic acids. It also allows testing for more than one virus from the same sample (**Figure 6**).

The PCR assays are extremely sensitive and can therefore be influenced by inhibitors of the polymerase enzyme that are sometimes present in clinical samples. Internal controls can be included into reaction mixtures. Nucleases present in samples or in reagents can also cause false negative results by degrading viral nucleic acids. Furthermore, amplicons may also cause product carry-over and false positive results. Extremely high care has to be applied in handling the clinical specimens, the reagents, as well as the reaction products.

One of the great advantages of the PCR technology is its potential to detect new emerging viruses. By using primers from related viruses or so-called generic primers important information regarding the new virus can be obtained for further development of more specific tests. A good example is the severe acute respiratory syndrome (SARS) virus, for which specific diagnostic tests became available soon after the taxonomic position of the virus became known. The technology also allows safe handling and transport of virus samples, since extraction buffers added to the samples inactivate virus infectivity.

Future Perspectives

Driven by public health, scientific and commercial interests, new diagnostic tests for the laboratory diagnosis of viral infections are continuously being developed. The main area for development will probably be new molecular detection methods, where automation will provide rapid, well-standardized, and easy-to-use technology.

Use of multianalyte methods is becoming a practical reality and they might significantly change diagnostics of infectious diseases in future. They provide an opportunity to screen simultaneously for a wide range of viruses increasing the rapidity of the diagnostic procedure. A single microarray test (microchip) can contain thousands of virus-specific oligonucleotide probes spotted on a glass slide. Several kit applications for detecting viral nucleic acids and antigens or virus-specific antibodies already exist. Microarrays based on random PCR amplification can be used to detect a variety of viruses belonging to different families. Screening of some other infection markers can be also included in the same test format. Microarrays are not widely used for clinical purposes because of limited sensitivity and the difficulties of developing analytical instruments suitable for diagnostic laboratories. Another line of

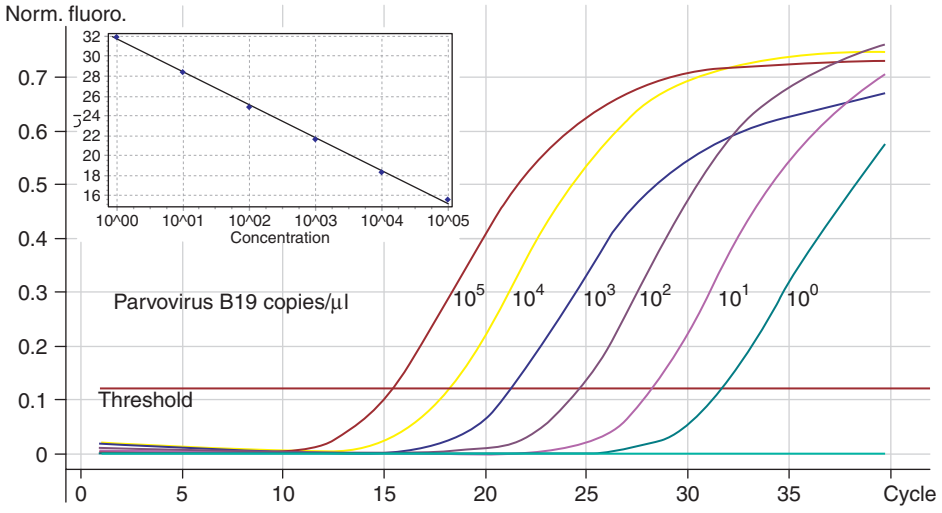
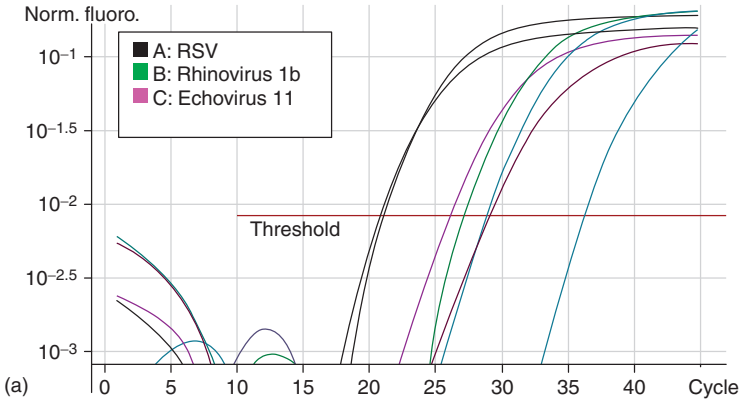
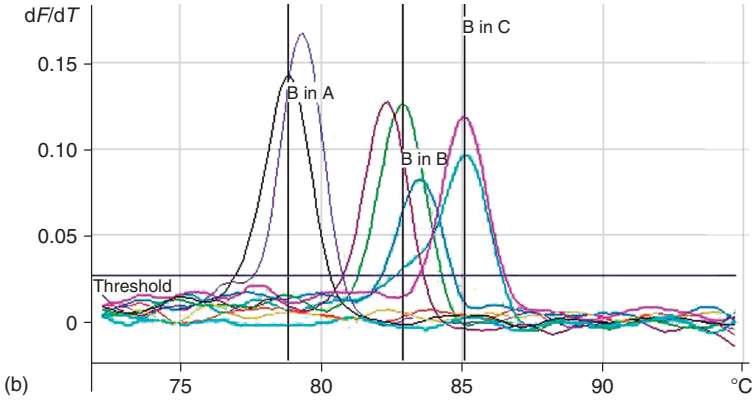


Figure 5 Quantitative real-time PCR with fluorescent-labeled probes for parvovirus B19.





	Sample	*Sample type*	C_t	T_m	*Virus*
	Patient 1	Unknown	21,10	79,35 (B in A)	RSV
	Patient 2	Unknown	28,99	85,15 (B in C)	Enterovirus
	Patient 3	Unknown	29,18	82,35 (B in B)	Rhinovirus
	Patient 4	Unknown	36,35	83,6 (B in B)	Rhinovirus
	Extraction aqua	NTC			
	Rhinovirus 1b	Positive control	27,26	82,9 (B in B)	Rhinovirus
	Echovirus 11	Positive control	26,22	85,1 (B in C)	Enterovirus
	RSV	Positive control	20,91	78,85 (B in A)	RSV
	RT aqua	NTC			
	PCR aqua	NTC			

(c)

Figure 6 RT-PCR with real-time detection (a) and with melting curve analysis (b) for the detection of respiratory syncytial virus (RSV), rhinovirus, and enterovirus in respiratory secretions (c). C_t is a threshold cycle number, T_m is a melting temperature, and NTC is a nontemplate control. Unpublished results by Waris M, Tevaluoto T, and Österback R.

development is the increasing number of POC tests, which may form an important part of future diagnostic testing of infectious diseases.

See also: Antigenicity and Immunogenicity of Viral Proteins; Diagnostic Techniques: Microarrays; Immune Response to viruses: Antibody-Mediated Immunity.

Further Reading

Halonen P, Meurman O, Lövgren T, *et al.* (1983) Detection of viral antigens by time-resolved fluoroimmunoassay. In: Cooper M, *et al.* (eds.) *Current Topics in Microbiology and Immunology,* vol. 104, pp. 133–146. Heidelberg: Springer.

Hedman K, Lappalainen M, Söderlund M, and Hedman L (1993) Avidity of IgG in serodiagnosis of infectious diseases. *Review of Medical Microbiology* 4: 123–129.

Hukkanen V and Vuorinen T (2002) Herpesviruses and enteroviruses in infections of the central nervous system: A study using time-resolved fluorometry PCR. *Journal of Clinical Virology* 25: S87–S94.
Jeffery K and Pillay D (2004) Diagnostic approaches. In: Zuckerman A *et al.* (eds.) *Principle and Practise of Clinical Virology*, pp. 1–21. Chichester, UK: Wiley.
Wang D, Coscoy L, Zylberberg M, *et al.* (2002) Microarray-based detection and genotyping of viral pathogens. *Proceedings of the National Academy of Sciences, USA* 99: 15687–15692.

Dicistroviruses

P D Christian, National Institute of Biological Standards and Control, South Mimms, UK
P D Scotti, Waiatarua, New Zealand

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cohort A group of similar individuals.
Dipteran A member of the insect order Diptera: true flies.
Hemipteran A member of the insect order Hemiptera: true bugs (including aphids).
Hymenopteran A member of the insect order Hymenoptera: wasps and bees.
Intergenic region Region between the two open reading frames in the dicistrovirus genome.
Lepidopteran A member of the insect order Lepidoptera: moths and butterflies.
Orthopteran A member of the insect order Orthoptera: crickets and grasshoppers.
Penaeid A shrimp from the family Penaeidae.
Picorna-like Viruses that are ostensibly like members of the *Picornaviridae*; but the term is generally used to refer to any small (*c.* 30 nm in diameter) icosahedral viruses with single-stranded RNA genomes.
Polyprotein A protein that is cleaved after synthesis to produce a number of smaller functional proteins.
Vertical transmission Transmission of virus directly from an infected mother to her offspring.
VPg A virally encoded protein covalently linked to the 5′ end of the viral genome.

Introduction

After the early 1960s, it became apparent that invertebrates, as well as vertebrates and plants, played host to a number of small (<40 nm in diameter) icosahedral viruses with RNA genomes. The initial descriptions of many of these viruses involved little more than their physical and biochemical characteristics such as diameter, density, and S-value. By the 1970s, many new invertebrate small RNA viruses were being isolated and described and even minimal characterization made it clear that new families of viruses were emerging from this assemblage of small RNA-containing viruses of invertebrates, along with apparent members of existing virus families. Two major families of viruses recognized in the late 1970s and early 1980s were the *Tetraviridae* and *Nodaviridae*. Any of the other viruses were simply considered to be 'invertebrate picornaviruses' or 'small RNA viruses of insects'.

The properties of many of the yet-unclassified viruses were found to be very similar to those of the mammalian picornaviruses. In particular, the size of the virions (*c.* 30 nm), the composition of the capsids (three major proteins of around 30 kDa), and single-stranded, positive-sense RNA genomes all suggested these were invertebrate picornaviruses and this was very much the prevalent feeling – until 1998. At this point, the first full genome sequence of one such invertebrate virus, drosophila C virus (DCV), was published and surprisingly revealed a genome organization strikingly different from the picornaviruses, and indeed quite different from any other viruses known at that time. During the next several years, the genomes of a number of insect small RNA-containing viruses were sequenced and it became clear that two organizational paradigms existed. The first group became the *Dicistroviridae* while the second has become the (currently) unassigned genus *Iflavirus*.

Taxonomy and Classification

The family *Dicistroviridae* currently comprises 12 species, most of them in the only genus recognized so far, *Cripavirus* (**Table 1**). There are a number of other potential candidates for the family but these have yet to be accepted as species by the International Committee on Taxonomy of Viruses. For the purposes of this article, we will limit our discussion to only those species shown in **Table 1**.

Biophysical Properties

Dicistroviruses appear roughly spherical under the electron microscope in negative stained preparations with particle diameters of approximately 30 nm and no envelope (**Figure 1**). The mature virions contain three major structural proteins, VP1, VP2, and VP3 of between 28 and 37 kDa, although taura syndrome virus (TSV) does appear to have one larger structural protein of 56 kDa. In many mature virion preparations, one or more minor structural components can be present which are larger than the major capsid proteins and are presumed to be the precursor(s) of structural proteins. In some viruses, a fourth smaller structural protein (VP4) – of between 4.5 and 9 kDa – is also present in the virion. A summary of the biophysical properties of dicistrovirus virions and the size and composition of the genome is given in **Table 2**.

The virions exhibit icosahedral, pseudo $T = 3$ symmetry and are composed of 60 protomers. Each of the protomers is composed of a single molecule of each of the structural proteins VP2, VP3, and VP1. The protomers are arranged so that the molecules of VP1 are set around the fivefold axes (**Figure 2**), with VP4 lying inside the virion below the molecules of VP1. Where the molecules of VP1 come together the surface of the virion shows a slightly raised crown – similar to that on the surface of poliovirus and other picornaviruses (**Figure 3**). In contrast to poliovirus, the surface of cricket paralysis virus (CrPV) does not show the characteristic deep canyon around the fivefold axes – which in the case of the former is where the receptor-binding site is known to be. The mature virions have a buoyant density in neutral CsCl of between 1.34 and 1.39 g cm^{-3} and sedimentation coefficients that range between 153S and 167S. For those viruses where physicochemical stability has been assessed, for example, CrPV, the virions are stable at pH 3.0 and are resistant to treatment with detergents and organic solvents such as ether and chloroform.

The virions contain a single molecule of linear, positive-sense, single-stranded RNA (ssRNA) of approximately 9000–10 000 nt in size. Structural studies have not revealed any ordered structure to the RNA within the virion. Terminal modifications to the RNA include a covalently linked protein at the 5′ end of the genome (referred to as the VPg) and a polyA tract at the 3′ end.

Organization of the Dicistrovirus Genome

The single-stranded genomes possess a 5′ untranslated region (5′ UTR) of 500–800 nt followed by two open reading frames (ORFs) of *c.* 5500 and 2600 nt. The ORFs are separated by an untranslated region of ~190 nt, commonly referred to as the intergenic region (IGR) (**Figure 4**).

Table 1 Members of the virus family *Dicistroviridae*. Isolate and vernacular names are shown in brackets. Accession number for whole genome sequences are also given. A recently suggested taxonomy that places ABPV, KBV, SINV-1, and TSV as unassigned species in the family is followed

Genus	*Species (isolate name)*	*Accession number*	*Abbreviation*
Cripavirus	*Cricket paralysis virus* [type species] (cricket paralysis virus)	[AUF218039]	CrPV
	Aphid lethal paralysis virus (aphid lethal paralysis virus)	[AUF536531]	ALPV
	Black queen-cell virus (black queen-cell virus)	[AUF183905]	BQCV
	Drosophila C virus (drosophila C virus)	[AUF014388]	DCV
	Himetobi P virus (himetobi P virus)	[AUB017037]	HiPV
	Plautia stali intestine virus (plautia stali intestine virus)	[AUB006531]	PSIV
	Rhopalosiphum padi virus (rhopalosiphum padi virus)	[AUF022937]	RhPV
	Triatoma virus (triatoma virus)	[AUF178440]	TrV
Unassigned species in the family	*Acute bee paralysis virus* (acute bee paralysis virus)	[AUF150629]	ABPV
	Kashmir bee virus (Kashmir bee virus)	[AUY452696]	KBV
	Solenopsis invicta virus-1 (solenopsis invicta virus-1)	[AUY634314]	SINV-1
	Taura syndrome virus (taura syndrome virus)	[AUF277675]	TSV

The virion proteins (VPs) have been shown, by direct sequence analysis, to be encoded by an ORF proximal to the 3′ end, while the more 5′ ORF encodes protein(s) that have sequence motifs lying in the order (5′–3′) Hel-Pro-Rep, a feature common among a large number of other positive-sense RNA viruses such as picornaviruses, comoviruses, sobemoviruses, caliciviruses, sequiviruses, and potyviruses.

Where present, the sequence coding for the small virion protein VP4 is in the ORF encoding the structural proteins, between the region coding for the capsid proteins VP2 and VP3 (as it is also in the iflaviruses). VP4 is cleaved from VP3 during the maturation of the virion. The VPg is encoded in the nonstructural protein-encoding region, and in most dicistroviruses there are multiple copies of the VPg coding sequence which show some degree of heterogeneity.

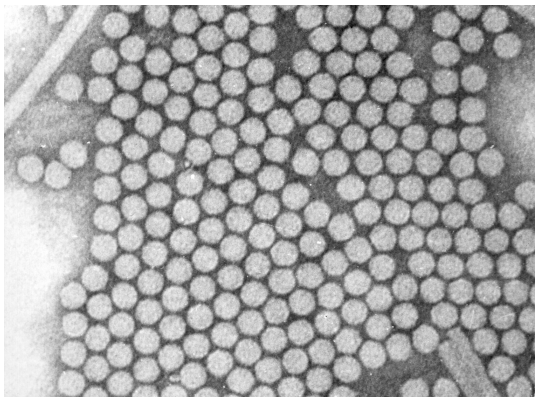


Figure 1 Negative stained electron micrograph of isometric particles CrPV showing rod-shaped particles of tobacco mosaic virus (diameter 18 nm) in the upper left- and lower right-hand corners. Electron micrograph supplied courtesy of Carl Reinganum.

Virus Replication and Genome Expression

The mechanism of virus entry into susceptible cells is unknown. Initiation of protein synthesis coincides with the shutdown or downregulation of host cell protein synthesis. During infection, a large number of precursor proteins are produced which are then cleaved to produce an array of smaller polypeptides. In the case of CrPV (one of the few dicistroviruses for which a permissive cell culture system exists), the structural proteins are produced in supramolar excess relative to the nonstructural proteins. Like many positive-sense RNA viruses, no subgenomic RNAs (sgRNAs) are produced during the infection cycle.

The absence of sgRNAs indicates that the translation of ORF2 could be initiated by an internal ribosome entry site (IRES) similar to the mechanism known from picornaviruses. Experimental studies did indeed demonstrate this and that both the 5′ UTR and IGR of several dicistroviruses including CrPV, plautia stali intestine virus (PSIV), and rhopalosiphum padi virus (RhPV) can act as IRES elements to direct initiation of translation in either *in vitro* translation systems or in cultured invertebrate cells.

However, perhaps the most unique feature of the replicative strategy of dicistroviruses is the fact that translation of the structural proteins from IGR–IRES element does not require the presence of a methionine codon. Computer modeling has shown that the IGRs of all dicistroviruses have predictable stem–loop structures. While there are two basic types among the dicistroviruses (**Figure 5**), these are fundamentally the same with six stem–loops – the most 3′ of which forms a pseudoknot with the codons involved in translation initiation. The initiation of translation is thought to be mediated by the codon which forms the part of the pseudoknot with stem loop VI, immediately upstream of the triplet which encodes the first amino acid of the mature VP2 (**Table 3**). In most cases, the initiation codon is CCU (Pro) while the first codon of

Table 2 Summary of some biophysical properties of dicistroviruses

Virus	*Molecular weight of major capsid proteins (kDa)*[a]	*Buoyant density in CsCl (g ml^{-1})*	*Particle diameter (nm)*
Acute bee paralysis virus	35, 33, 24, 9	1.34	30
Aphid lethal paralysis virus	34, 32, 31 (41)	1.34	27
Black queen-cell virus	34, 32, 29, 6	1.34	30
Cricket paralysis virus	35, 34, 30 (43)	1.37	27
Drosophila C virus	31, 30, 28, 9 (37)	1.34	27
Himetobi P virus	37, 33, 28	1.35	29
Kashmir bee virus	41, 37, 25, 6	1.37	30
Plautia stali intestine virus	33, 30, 26, 5	n.d.	30
Rhopalosiphum padi virus	31, 30, 28 (41)	1.37	27
Solenopsis invicta virus-1	n.d.	n.d.	31
Taura syndrome virus	55, 40, 24 (58)	1.34	31
Triatoma virus	39, 37, 33	1.39	30

[a]Minor virion components are shown in brackets. These are presumed to be precursors of VP4–VP3.

VP2 encodes an alanine residue (**Table 3**). It has been shown experimentally that the glutamine at the 5′ end of PSIV VP2 can be replaced with any other amino acid to produce a mature protein in an *in vitro* translation system.

Where cell culture systems are available, pulse-chase studies have shown that translation of dicistrovirus genomes results in the production of polyproteins that are then cleaved to produce the structural and nonstructural proteins. For the virion proteins of some dicistroviruses, the cleavage sites between proteins in the structural polypeptide have been experimentally determined and are shown in **Table 3**. These sites show some degree of conservation and point toward the involvement of cysteine proteases. While the viruses themselves encode cysteine protease-like peptides in the nonstructural region there is also some evidence, for CrPV at least, that host-cell-encoded proteases may also be involved in the processing of viral polypeptides.

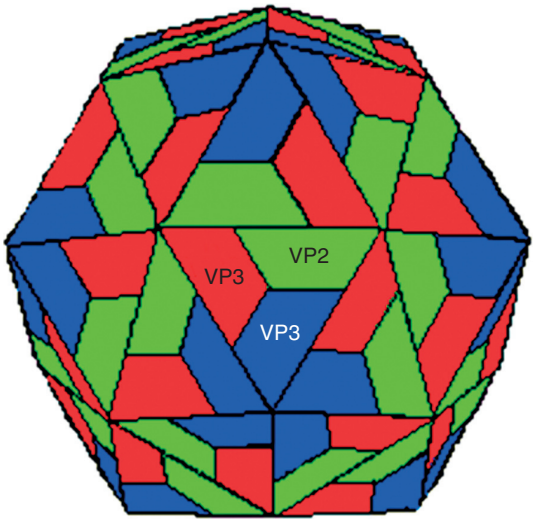


Figure 2 Diagram showing the surface packing of the coat proteins (VP1, VP2, VP3) of CrPV. Reproduced with permission from Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy*: *Eighth Report of the International Committee on Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press.

Host Range

To date, all members of the *Dicistroviridae* have been isolated from invertebrates, generally from a single species, or at most three closely related species. The hymenopteran viruses, acute bee paralysis virus (ABPV) and black queen-cell virus (BQCV), are known only from honeybees (*Apis mellifera*) but Kashmir bee virus (KBV) has also been isolated from the Asiatic hive bee *Apis dorsata*. It should also be noted that ABPV and KBV have been identified in the parasitic mite *Varroa destructor*, although there is no clear evidence that the virus is capable of replicating in this host. Solenopsis invicta virus-1 (SINV-1) is only known to infect the ant species, *Selonopsis invicta*.

Dicistroviruses found in homopterans and hemipterans (which includes aphids and true bugs, respectively) have slightly broader host ranges. RhPV has been isolated from laboratory and field populations of the aphids *Rhopalosiphum padi*, *R. maidis*, *R. rufiabdominalis*, *Schizaphis graminum*, *Diuraphis noxia*, and *Metapolophium dirrhodum*. Aphid lethal paralysis virus (ALPV) has only been isolated from aphids, but in this case from only three species: *R. padi*, *M. dirrhodum*, and *Sitobian avenae*. Himetobi P virus (HiPV) and PSIV are found in true bugs rather than aphids with HiPV having been isolated from the leafhoppers *Laodelphax striatellus*, *Sogatella furcifera*, and *Nilaparvata lugens*, and PSIV from the brown-winged green bug, *Plautia stali*. Triatoma virus is also a virus of hemipterans and has been found in the hematophagous triatomine bug, *Triatoma infestan*, which is also a vector of the protozoan agent that causes Chagas' disease in South America.

DCV is the only dicistrovirus with a host range restricted to dipterans and has been isolated from *Drosophila melanogaster* and the sibling species *D. simulans*. TSV is a virus of penaeid shrimps and has been isolated from a number of species including *Litopenaeus vannamei*, *L. stylirostris*, *Metapenaeus ensis*, and *Penaeus monodon*. The majority of the dicistroviruses have relatively restricted host ranges and, at most, have only been isolated from insects of a single order. CrPV is the striking exception. Originally isolated from the field crickets *Teleogryllus*

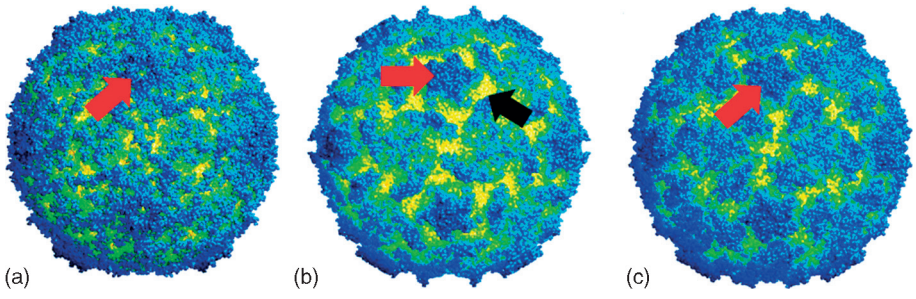


Figure 3 The surface structure of (a) CrPV, (b) poliovirus, and (c) rhinovirus 14. The red arrows show the raised crown around the fivefold axes and in poliovirus the black arrow indicates the deep canyon around this raised crown. Courtesy of John Tate.

oceanicus and *T. commodus*, CrPV has subsequently been isolated from a further 20 species belonging to five taxonomic families: *Orthoptera*, *Hymenoptera*, *Lepidoptera*, *Hemiptera*, and *Diptera*. Interestingly, while a number of other RNA-containing viruses are known from lepidopterans (moths and butterflies), that is, the iflaviruses and tetraviruses, as well as a number of uncharacterized viruses, CrPV is the only dicistrovirus isolated from lepidopterans – in fact from ten lepidopteran species.

All of the above records refer to the natural host range of the viruses. To a certain extent, studies on the experimental host range of dicistroviruses are limited and those that have been carried out have not substantially extended the known host ranges. Again the exceptions are CrPV and DCV. CrPV replicates in a number of established insect cell lines including those from *Drosophila*, the hemipteran *Agallia constricta*, and the lepidopterans *Pieris rapae*, *Plutella xylostella*, *Spodoptera ornithogalli*, and *Trichoplusia ni*. In addition to insect cell lines, CrPV has also been found to replicate readily in larvae of the greater waxmoth, *Galleria mellonella*. This is an easy insect to rear and maintain and virus yields can be very high. Apart from CrPV, the only other dicistrovirus shown to replicate in cultured cells is DCV, which multiplies in several *Drosophila* cell lines (some DCV isolates also replicate in the greater waxmoth). Reports that TSV can replicate in some mammalian cell lines have never been substantiated and may simply be attributable to the production of a cytopathic effect in the absence of virus replication.

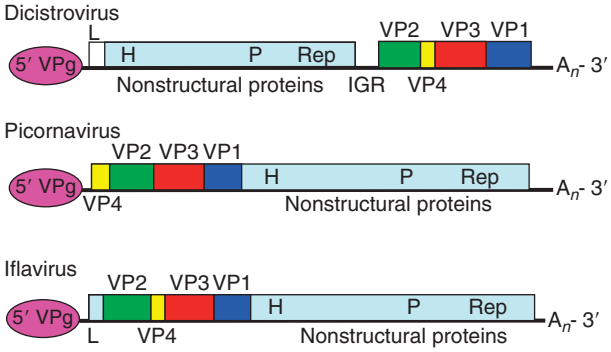


Figure 4 Diagrammatic representation of the genomic arrangement of the dicistroviruses, picornaviruses, and iflaviruses. The helicase (H), protease (P), and replicase (Rep) domains of the nonstructural proteins are indicated.

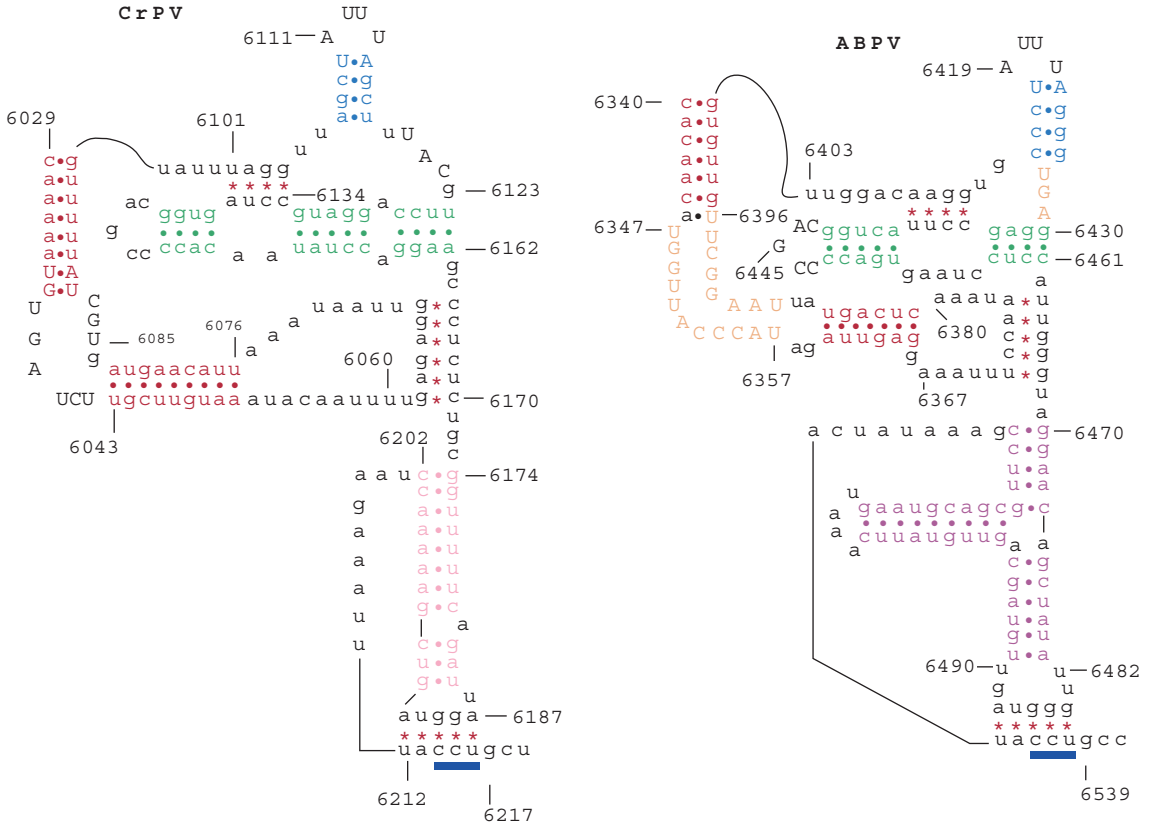


Figure 5 Predicted structure of the IGR–IRES elements of dicistroviruses. The structure represented by that of CrPV is shared by most dicistroviruses with the exception of ABPV, KBV, SINV-1, and TSV that have a structure similar to that represented by ABPV. The stem loop VI pseudoknot stuctures are shown in pink for CrPV and in purple for ABVP. The first translated codon is underlined in blue. Adapted with permission from Nobuhiko Nakashima.

Table 3 Some properties of the translation and processing of the structural polyprotein of the dicistroviruses. Only cleavage sites that have been empirically deduced from sequencing of the virion proteins are shown

Virus	*First residue*[a]	*Initiation codon*	*Cleavage VP2/VP4*	*Cleavage VP4/VP3*	*Cleavage VP3/VP1*
Cricket paralysis virus	A	CCU	IYAQ/AASE	LFGF/SKPT	n.d.
Acute bee paralysis virus	n.d.	CCU	VTMQ/INSK	IFGW/SKPR	ASMQ/INLA
Aphid lethal paralysis virus	A	CCU	n.d.	n.d.	n.d.
Black queen-cell virus	A	CCU	MLAQ/AGLK	LFGF/SKPL	MVAG/SNSG
Drosophila C virus	A	CCU	n.d.	MLGF/SKPT	IVAQ/VMGE
Himetobi P virus	A	CUA	AREQ/VNLN	APGF/KKPD	STAQ/EQAN
Kashmir bee virus	n.d.	CCU	n.d.	n.d.	n.d.
Plautia stali intestine virus	Q	CUU	LILQ/SGET	AFGF/SKPQ	LTLQ/SGDT
Rhopalosiphum padi virus	A	CCU	n.d.	THGW/SKPL	SIAQ/VGTD
Solenopsis invicta virus-1	n.d.	CCU	n.d.	n.d.	n.d.
Taura syndrome virus	A	CCU	n.d.	MFGF/SKDR	PSTH/AGLD
Triatoma virus	n.d.	CUC	n.d.	ALGF/SKPL	PIAQ/VGFA

[a]The first residue of the mature VP2.

Pathology and Transmission

The names of several of the dicistroviruses imply that virus infection can produce a noticeable disease symptom, that is, CrPV, ABPV, ALPV, and BQCV (potential queens die as propupae or pupae in hive cells in which the walls turn black). However, the majority of virus–host interactions produce no noticeable disease. Although BQCV kills some queens in their cells, most infected larvae, pupae, and workers appear to be completely unaffected.

Nevertheless, while disease symptoms may not be evident, in most of the cases studied, dicistrovirus infections reduce the life span of the infected individual. Or, more precisely, the presence of a dicistrovirus in a cohort (group of similar individuals) of insects coincides with a reduced life span relative to an uninfected cohort. This point is explicitly made since no studies have yet been undertaken where an invertebrate host has been nonlethally sampled for virus through its life span. In many other virus–host systems, for example, plants, mammals, or even fish and amphibians, it is possible to sample for virus without killing the host so the progress of infection can be measured at the level of the individual. With invertebrates, this has not been done and would generally be very difficult or impossible. The experimental approach has therefore been to introduce the virus into an uninfected cohort and compare the effects to a control cohort.

Despite these practical limitations, a number of studies have demonstrated the routes of virus transmission. BQCV has been detected in the eggs, larvae, and offspring of queens that were found to be infected – indicating that the virus is vertically transmitted. Similar findings have been made with ALPV which can be vertically transmitted in the aphid host, *R. padi*; this infection subsequently results in reduced longevity and fecundity. It has also been shown that ALPV RNA can be detected in the developing embryos inside infected females.

DCV is also vertically transmitted although virus is not present in the cytoplasm of the egg but is associated with the chorion on the egg surface. Infection presumably occurs when emerging larvae ingest the virus since they do not become infected if the chorion is removed. A similar phenomenon is found with CrPV and *T. oceanicus* where surface sterilization/dechorionation of eggs with dilute hypochlorite blocks the transmission of the virus to emerging nymphs.

Evidence of horizontal transmission is in many respects even more difficult to obtain than data on vertical transmission. However, experimental feeding studies indicate that dicistroviruses can be transmissed horizontally. In the case of triatoma virus (TrV) and its host *T. infestans*, infected insects excrete virus in their feces and since triatomine bugs are coprophagous and TrV is infectious *per os*, the virus can be readily spread. With DCV, it has been found that when uninfected males are placed with uninfected females, these males become infected (and vice versa). In fact, even if uninfected female flies are placed on media on which infected males have been allowed to feed for several hours, the females become infected. Such manipulations with other virus and invertebrate host systems are not as easy as those involving *Drosophila*, as it is difficult to obtain colonies free of viruses and the insects are not as easy to manipulate in the laboratory.

Evidence of vectors playing a role in dicistrovirus transmission is limited to honeybee viruses and their parasitic mite, *Varroa destructor*. It has been known for some time that the prevalence of a number of honeybee viruses increases in the presence of varroa mites. Initially, it was thought that the stress caused by varroa infestation induced the viruses to replicate. However, recently it has been shown that ABPV and KBV can be detected in mites implicating them more strongly in transmission of the viruses. In contrast, BQCV, although present at high

frequency in adult honeybees, has not been detected in mites, suggesting that they play little or no role in transmission.

Geographic and Strain Variation

Some dicistroviruses infect specific hosts with a wide geographic distribution or have been found to infect a number of species spread over a large geographic range. Several studies have looked at strain variation between geographic isolates of dicistroviruses using a variety of techniques. CrPV and DCV possess a range of biological and serological characteristics that show differentiation between geographical isolates. With CrPV, two major serogroups have been identified that separate Australian and New Zealand isolates from North American isolates. With DCV, isolates from different localities varied both in their pathogenicity and virus yield after injection into virus-free flies. Additional genetic studies on CrPV and DCV have utilized ribonuclease T1 fingerprinting and subsequently polymerase chain reaction restriction endonuclease analysis. Estimates of the maximum nucleotide divergence between isolates of CrPV and DCV were about 10%. In the case of CrPV, it was found that that the North American isolates were quite distinct from the antipodean isolates, a finding which reflects the serological data.

More recently, a number of molecular studies with the honeybee dicistroviruses and TSVs have been undertaken to determine the levels of genetic variation between isolates of these viruses. In most instances, slightly different regions of the virion protein coding regions (usually regions of VP3) have been used, which makes direct comparison between studies quite difficult. Nevertheless, ABPV isolates show levels of nucleotide identity between 90% and 100%, but isolates from the same geographic region are more closely related than isolates from more distant locations. One such study has revealed that viruses isolated from different central Europe regions were more similar to each other than to isolates from North America or from the UK. Similar patterns are also evident for BQCV and KBV with nucleotide identity within species at 90–100%. To put these values in perspective, the region used for the KBV studies is *c.* 75% identical to the same region from ABPV.

For TSV, the situation is slightly different, and it has been found that levels of nucleotide identity between isolates from North and Central America and Asia range from 95% to 100%. These lower levels of diversity may indicate that TSV has rapidly spread into many of the regions and hosts where it is now found – a hypothesis that is supported to some extent by the rapid emergence of the disease over the last two decades. There is also some evidence that there are serological differences between isolates.

Relationships within the Family

The taxonomy of the dicistroviruses currently recognizes only one genus, *Cripavirus*, into which most of the species are placed. However, comparisons of the coding sequences of the dicistroviruses reveal that they are only distantly related. For instance, the amino acid identity between the structural polyproteins of different species ranges from 19% to 66% while the amino acid similarity ranges from 37% to 80%. Using these data, the phenogram presented as **Figure 6** shows the pattern of relationships and reveals only two closely related pairs of species: CrPV and DCV (which also share a serological relationship) and ABPV and KBV. All other species are only distantly related. While the genus *Cripavirus* is currently proposed to include all but ABPV, KBV, SINV-1, and TSV, this genus will probably be divided and new genera established to accept the currently unassigned species listed above.

Similarity with Other Taxa

The dicistroviruses share various properties with a number of other positive-sense ssRNA virus genomes. Historically, these viruses have been collectively grouped as the picornavirus superfamily which has been considered to include the virus families *Comoviridae*, *Picornaviridae*, *Potyviridae*, *Sequiviridae*, *Caliciviridae*, and more recently the unassigned genera *Iflavirus*, *Sadwavirus*, and *Cheravirus* and the recently created family *Marnaviridae*. In all of the groups mentioned above, the gene order for the nonstructural proteins is the same, viz. Hel-Pro-Rep (as in **Figure 4**). When compared to other positive-sense viruses, these genes appear to be more closely related to each other

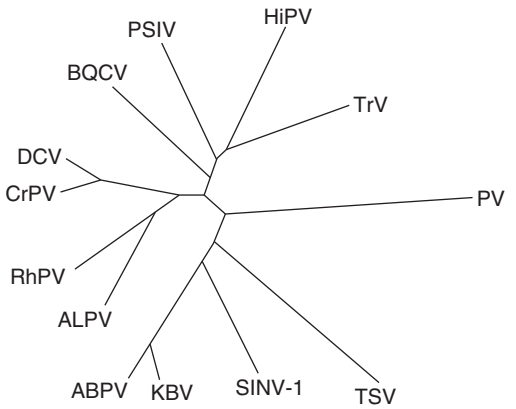


Figure 6 Phenogram showing the relationships among members of the family *Dicistroviridae,* constructed from the amino acid identity of the structural proteins encoded in ORF2. The phenogram was constructed using the neighbor-joining algorithm of the MEGA software. The sequence from Poliovirus type 3 [L23844] was used as an outgroup for the analysis. Branch lengths are drawn approximately to scale.

than to genes from other viruses, for example, coronaviruses, toroviruses, and tetraviruses. However, there is a subset of the above taxa which shares a larger set of properties: for example, isometric virus particles with pseudo $T = 3$ symmetry (formed of three units of eight-stranded β-barrel); the absence of sgRNAs during replication; and the presence of a 3–4 kDa VPg. On the basis of these properties, the grouping would exclude the *Caliciviridae* (sgRNAs, large VPg, and true $T = 3$ symmetry) and the *Potyviridae* (large VPg, rod-shaped particles with helical symmetry).

There are also a large number of other isolates of invertebrate picorna-like viruses that share some properties with dicistroviruses, for example, single-stranded postive-sense RNA genomes, isometric virions of around 30 nm in diameter, and up to three virion proteins of around 30 kDa. Further studies will undoubtedly reveal additional structural and organizational paradigms among the yet uncharacterized picorna-like viruses of invertebrates besides increasing the number of known dicistroviruses.

See also: Iflavirus; Insect Pest Control by Viruses; Picornaviruses: Molecular Biology; Taura Syndrome Virus.

Further Reading

Bailey L and Ball BV (1991) *Honey Bee Pathology,* 2nd edn. Sidcup: Harcourt Brace Jovanovich.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.* San Diego, CA: Elsevier Academic Press.

Miller LK and Ball LA (eds.) (1998) *The Insect Viruses.* New York: Plenum Press.

Disease Surveillance

N Noah, London School of Hygiene and Tropical Medicine, London, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Case–fatality rate Number of persons dying of an infection divided by total number of persons with the disease. Thus, CFR of 5% means that 5 of 100 persons with the infection died.

Epidemic and outbreak Most infectious disease epidemiologists do not distinguish between these terms and use them interchangeably. Generally, an outbreak is defined as a localized increase in cases, whereas an epidemic is more widespread, perhaps affecting a whole country.

GUM clinics The term GenitoUrinary Medicine clinics is often used for special clinics for sexually transmitted infections (STIs).

Incidence The rate of new infections (number by population) in a given time – for example, 5 cases of influenza per 1000 per year. Good for short-term infections.

Outbreak When an infection occurs at a frequency higher than expected for that time or place. It is basically an increased incidence which is usually unexpected. Two linked cases is also theoretically an outbreak.

Pandemic This term should be restricted to infections affecting many countries, though it does not have to be worldwide. SARS spared many countries and indeed some continents, but it was a pandemic. AIDS is undoubtedly a pandemic.

Prevalence The number of infections at any one time in a given population, expressed as a rate. Good for chronic infections and serological studies (e.g., prevalence of varicella antibody at age 15 in a given population is 95%).

Introduction

Surveillance is undoubtedly an essential – indeed critical – ingredient of any disease control program. It is used to monitor the impact of an infection, the effect of an intervention or health promotion strategy, health policy, planning, and delivery. Surveillance is the ongoing and systematic collection of routine data which are then analyzed, interpreted, and acted upon. It is essentially a practical process, which nevertheless can be useful in other ways. Its main purpose is to analyze time trends – but these can include not simply fluctuations in overall numbers, but also changes in age and sex distributions, geographical locations, and even possibly, in some of the more sophisticated established surveillance systems, at-risk groups (such as particular social, ethnic and

occupational groups). Surveillance is essential for evaluating the impact of an intervention, such as mass vaccination, on a population. It can be a fairly sensitive system for the detection of outbreaks earlier than they would have been recognized otherwise.

The word ongoing in the description of surveillance helps to distinguish it from a survey, which is usually finite, tends to focus more on one or more groups of persons, and involves a questionnaire. Nevertheless, surveillance does not have to continue forever – when it is no longer useful, it should be stopped.

To be systematic is another important ingredient of surveillance. If reporting centers are not consistent in what they report, nor regular, the data they send in will be uninterpretable, and probably useless. Defining what needs to be reported and agreeing on the criteria for making a reportable diagnosis are necessary to make sense of the data.

The collection of routine data is another important characteristic of surveillance, especially with surveillance of laboratory infections. Generally, testing of samples is done for diagnosis, not primarily for surveillance. As laboratory testing is expensive, making further use of the results by contributing to surveillance makes for more efficient use of information, and the contribution to surveillance itself can often justify further testing. Typing echoviruses or coxsackie viruses can seldom be justified on clinical grounds alone, but the surveillance of serotypes can provide valuable information on the epidemiology of these viruses, their clinical characteristics, seasonality, and age and sex distributions. Echovirus 4 for example tends to be rare, fairly localized even within a country, but, when it occurs, it has a high aseptic meningitis rate, and causes a short and sharp autumn outbreak. Echovirus 9 on the other hand is far more common, more widespread geographically when it causes an epidemic, more benign, with a macular rash, and with meningitis not an especially common manifestation.

Although surveillance is essentially a practical exercise, this article attempts to show that surveillance can also be useful in giving us clues about an infection, whether it be its natural history, etiology, severity, or outcome.

Collection of Data: Sources of Data in Surveillance of Viruses

Death Certification

In most developed and middle-income countries, deaths are certified primarily for legal reasons, but have proved to be an important data source to use for surveillance. Clearly, they tend to be useful mainly for serious infections, and may suffer from inaccuracies, but remain a useful basic data source. Diseases with a high mortality rate and a short duration of illness (the viral hemorrhagic fevers for example) will obviously be better represented by death certification, than those with a low mortality. HIV/AIDS, before the era of HAART, had a high mortality but long periods of symptomless and symptomatic infection, so that death certification data needed careful interpretation. Laboratory reporting systems can be another useful source of information on mortality from infection.

Notifications

Surveillance systems such as statutory notification tend to be based mainly on clinical features. These can be very useful for common diseases with distinctive clinical syndromes, such as measles and mumps. It is important however not to make a disease notifiable unless there is a good reason for it: 'good' reasons include a mass vaccination program (when surveillance is virtually mandatory), any other mass control program, serious diseases for which contact tracing, mass or close contact prophylaxis, or investigation into source, is necessary. Serious less common viral infections such as poliomyelitis are notifiable in most countries. This is because contact tracing and preventive measures can be taken. Broader clinical diagnoses, such as aseptic (viral) meningitis, may on the face of it be less useful to notify, as it is usually impossible at the bedside to distinguish such causes of it as, for example, the coxsackie B viruses, echoviruses, and mumps. Nevertheless, notification of aseptic meningitis can be useful because a rapid rise in notified cases may need to be investigated. Moreover, the timing of any epidemic may give a clue to etiology – mumps meningitis tends to increase in spring and is usually accompanied by a concurrent outbreak of clinical mumps, while enterovirus epidemics are more likely to occur in autumn.

Other Clinical Sources of Data

Specific general practitioner (GP) surveillance systems are useful to provide data of epidemiological value for infections that are not notifiable, such as the common cold or chickenpox (in UK). They are of course clinically based, but are nevertheless useful, and often surprisingly accurate, possibly because those GPs who subscribe to a surveillance system are motivated to do so. GP surveillance systems are often sentinel-based, that is, based on a sample of GPs in a country, region, or area. Thus, they are good for common infections which to make notifiable would possibly be wasteful, for example, chickenpox. Moreover each sentinel would normally provide complete reporting. In the English system, GPs provide data on the base populations of their practices, so that rates of infection can be provided as a routine, a feature that is almost unique among surveillance systems. GP surveillance systems also tend to be good for timeliness and completeness.

Laboratory Data

It is useful to think of laboratory data as being of qualitative rather than quantitative value as they add quality and detail to disease surveillance. Thus, in the example already used for aseptic meningitis, a precise diagnosis of mumps, echovirus, or coxsackie type is particularly useful in clustering and outbreaks. Indeed, as the enteroviruses exhibit a strong late summer/autumn seasonal pattern with each enterovirus returning to its baseline in winter, when numbers of a particular type continue to be reported at a level higher than the winter baseline, the return of this virus to cause another epidemic in the following summer can usually be safely predicted. Laboratory data are also essential in qualifying food poisoning and gastroenteritis. Separating viral from bacterial causes is a useful first step, as their management tends to be very different. Norovirus gastroenteritis can be food-borne, but also spreads very easily from person-to-person because an extremely small dose is necessary for infection to occur, and the virus being fairly resistant to the environment will survive for some time. Management therefore must concentrate on hygiene. With most bacterial causes of food poisoning, especially the salmonellas, management often depends on the removal of the offending food. Laboratory surveillance is particularly essential for unraveling the mass of respiratory viral infections that inflict humans – respiratory syncytial virus, adenoviruses, parainfluenza viruses, rhinoviruses, etc. Indeed, it is particularly useful for influenza – not only separating it from influenza-like illness, but also in identifying influenza A and B, and if A, the subtype and variant.

Surveillance of Outbreaks

Surveillance of outbreaks (as opposed to individual infections) can be revealing, and important to allow public health measures. The existence of the noroviruses was suspected in the UK many years before the organisms were identified.This was because some outbreaks which did not fit the characteristics of known infections but had characteristics of their own had occurred.

Hospital Admissions

These can be useful for certain more serious infections, such as hepatitis and encephalitis. They can be unwieldy, generally lack detail, and often are published a year or more after the events have occurred.

Serological Surveillance

Serological surveillance has become increasingly important, and is a useful tool in assessing the immunity of a population, though it can also be used to identify vulnerable individuals. The immunity of a population can be vaccine-induced, and serological surveillance is a valuable adjunct to the methods available to monitor a mass immunization program. Vulnerable age groups can be identified, and booster doses of the vaccine introduced. An example of the importance of serological surveillance in determining public health policy is included below (analysis by person).

Surveillance of Viruses in Nonhuman and Environmental Sources

To build a picture of an infection, animals, birds, and the environment have been placed under surveillance. Rabies in foxes and other wildlife, influenza in birds, pigs, and other animals, are examples of important and fairly successful surveillance systems.

Other Sources of Data

Records of sickness absence, absence from school, calls to an emergency room, if available, can provide speedy information that something has happened, but tend to be nonspecific. Surveillance of antiviral resistance will become important.

Surveillance of HIV/AIDS

The association of HIV/AIDS with stigma makes surveillance of this infection especially difficult. The uniqueness and seriousness of this infection warrants a separate section. It is an example of the importance of tailoring surveillance to a specific serious infection if it becomes necessary to do so.

In the UK and some other countries with data protection acts, HIV infection, as with other STIs, is not notifiable. Special confidential surveillance systems through clinicians and GUM clinics, as well as laboratories, are in place. These are especially important for assessing risk factors. Inclusion of risk factors is essential for targeted intervention – for example, the proportions and rates of new diagnoses attributed to men who have sex with men, heterosexual sex, mother-to-infant, blood transfusion, IVDUs, and other needlestick injury.

Laboratory reporting is essential. Death certification is useful, though it has been shown that men who have sex with men, and probably those with other risk factors, are under-represented. In the UK, matching reported cases with death certificates is very important, as it allows for detection of deaths due to AIDS (such as pneumonia) as well as deaths associated with AIDS, and which are seen now in HIV-infected individuals – these include liver and cardiovascular disease, overdoses, and malignancies.

A surveillance system, based on unlinked anonymous testing of samples of blood taken routinely from certain at-risk population or occupational groups has been shown to provide valuable information on HIV infection in these populations. Specific screening systems for blood donors, military recruits, commercial sex workers, and family planning/termination of pregnancy clinics are also useful if these populations are to be targeted. Behavioral surveillance should also be seriously considered, to assist in identifying future trends, healthcare planning, as well as for specific health promotion efforts.

Attributes of Surveillance Systems

Completeness

Incompleteness is an almost universal drawback of most notification systems. They should never be dismissed for this reason alone. Statutory notification systems can be essential for surveillance and control. For common infections completeness may not be worth striving for, because notifications (assuming consistency in reporting) will generally provide information on trends, as well as fairly accurate information on age, sex and seasonal distributions, and possibly on place. The effect of mass vaccination programs can also be monitored fairly closely with statutory notification systems, as with measles (**Figure 1**) and acute paralytic poliomyelitis (**Figure 2**) in the UK. When a mass vaccination or other universal control program reduces the incidence of an infection to low levels, completeness becomes much more essential. For serious infections also, such as SARS or Lassa fever, for which contact tracing or other control measure is necessary, completeness is essential.

In active surveillance, reporters make negative returns if they have had no cases during the reporting period, to ensure completeness. In some countries, enhanced surveillance has been used to assess more accurately the true incidence of an infection – regions or districts are chosen to report all cases of a particular infection or infections. It is a hybrid of active and sentinel surveillance.

Timeliness

Timeliness is important for infections for which urgent public health measures have to be undertaken, such as poliomyelitis and viral hemorrhagic fever, as well as any outbreak. In some instances, infections, not normally urgent, can become so as an elimination program progresses. In a country with elimination of measles as its goal, a case of indigenous or imported measles needs to be dealt with urgently, as it may lead to an outbreak if not controlled immediately. Laboratory data are often not timely, and hospital data generally even less so, but can make up in accuracy what they lose in timeliness.

Accuracy

Accuracy is clearly important, though some minor degrees of inaccuracy can be tolerated in some common infections. Clinical data are most liable to have some inaccuracies, though even laboratory data can be inaccurate. Case definitions and quality control systems can be useful to improve accuracy.

Representativeness

For surveillance to provide an accurate picture of the impact of a particular infection, representativeness is essential. It is perhaps the most important quality for any surveillance system. Having a wide coverage of reporting clinicians and laboratories, or a well-chosen sample of sentinel sites, is necessary for the data collected to be representative of an infection in a country. Sometimes it may be necessary to assess data from various sources, such as notifications/GPs (clinical), hospital, laboratory, and death certificates.

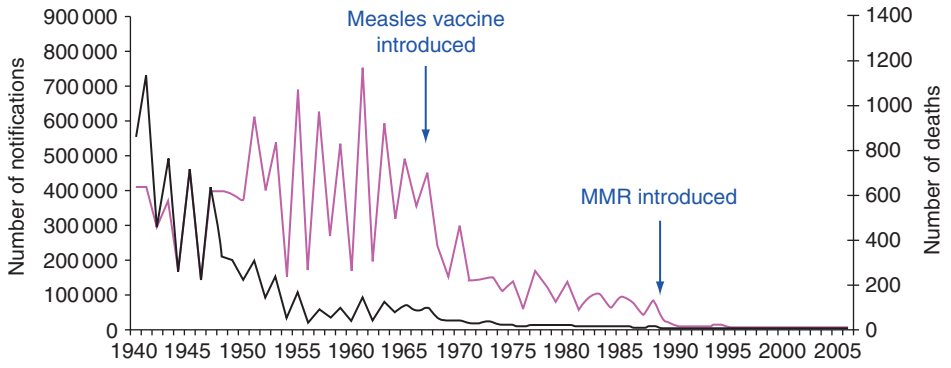


Figure 1 Measles notifications: cases and deaths, England and Wales 1940–2006. Reproduced from the Health Protection Agency (www.hpa.org.uk).

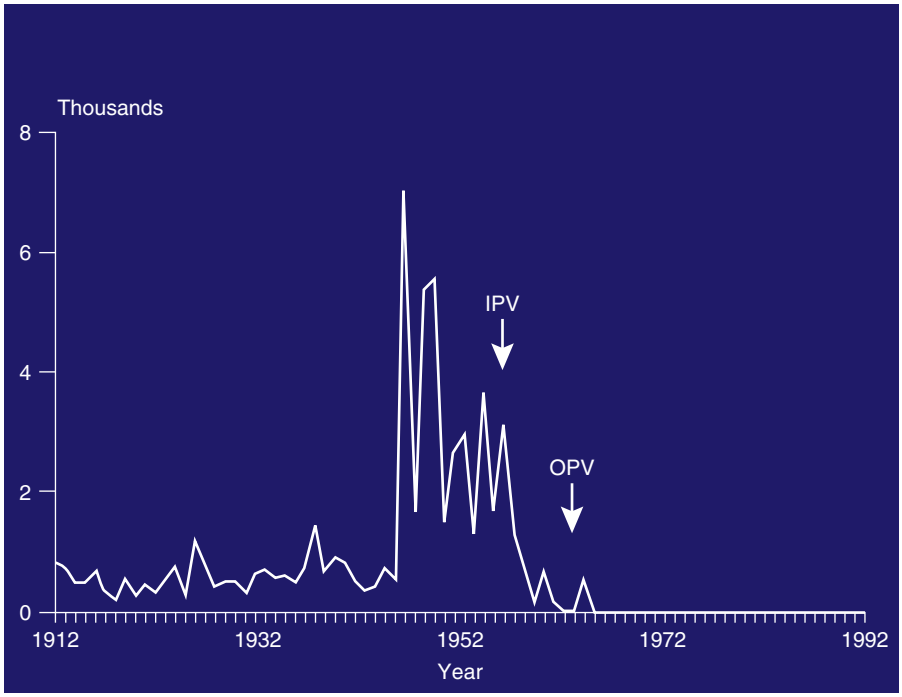


Figure 2 Notifications of acute poliomyelitis in England and Wales 1912–93. Reproduced from the Health Protection Agency (www.hpa.org.uk).

Consistency

Consistency is another crucial basic attribute of any surveillance system. Reporters must know what to report (case definition) and how often. Otherwise it will not be possible to interpret trends.

Analysis of Data

Time

The three basic analyses by time, place, and person should be routine. Computer programs have made analyses of data quicker but somewhat less flexible. There is no standard period for analysis by time. Depending on what the surveillance intends to show, yearly, quarterly, monthly, four-weekly, or weekly time intervals can be used. In surveillance, time intervals shorter than this are rarely used. Monthly intervals have the disadvantage of having unequal number of days in each month, and are difficult to use when reporting is weekly; for seasonal trends four-weekly periods are better but cannot be divided into quarterly periods. In viral surveillance, four-weekly rather than weekly intervals tend to be most useful in showing seasonal changes. There is more likely to be more variation ('noise') in weekly intervals, making for less smooth changes. For secular trends, quarterly or annual intervals are generally used. Analyzing by time can reveal regular changes in the periodicity of viruses, enabling some of them to be predicted.

A basic knowledge of seasonal and secular patterns makes it easier to detect changes that signify a possible epidemic, and to differentiate these from a random variation. It is important to remember when analyzing laboratory data that there is often an interval, which can be 2 weeks or more, between date of onset and date of reporting.

Person

Analysis by age and sex is another basic analysis in surveillance. It can identify those most affected, and vulnerable groups. Changes in age distributions may provide important clues about a changing viral infection, and the effect of mass interventions on the age distribution of an infection can be monitored. Changes in the age distribution of measles in 1994 in the UK signified that an epidemic in older children was imminent, and the vaccine schedule was changed to include an extra booster injection (MR) to children aged 5–16 years. This averted the outbreak and the booster dose became a permanent feature of the routine immunization schedule in the UK. Indeed the changes in age distribution following mass vaccination could be considered an epidemiological side effect of mass vaccination. Requests for occupational groups and travel histories should be selective. For poliomyelitis, SARS, dengue, and the viral hemorrhagic fevers, travel histories are required. Occupational group may be useful for norovirus, and hepatitis types A, B, or C. Specific risk factors may be worthwhile for HIV, hepatitis B and C.

Place

Analysis by place can pinpoint local outbreaks. Some echoviruses (e.g., echovirus type 4) can cause rare short local outbreaks, other types (e.g., types 9 and 11) are more common and more widespread. Food-borne outbreaks of hepatitis A are generally picked up locally through routine surveillance, but sometimes more extensive outbreaks caused by a more widely distributed foodstuff, including shellfish or frozen soft fruit, may be identified.

Interpretation

Collection and analysis are generally routine functions; skill is required in interpretation of the data. No statistic is perfect, and surveillance data, like all data, must be interpreted with caution. One must take into account the origins of the data – clinical, laboratory, or hospital. Not only must the reliability, or otherwise, of the data be evaluated, but what the data signify in the natural history of the infection must also be recognized.

Every viral infection has its stages and these must be recognized before surveillance data can be sensibly interpreted. At what stage are the data being collected important to understanding and interpretation? Using influenza or hepatitis A as examples, and a defined population (**Table 1**), only a proportion of persons in the defined population will be infected with the virus. They can only be comprehensively detected by screening, and serological surveillance will identify these persons, or assess population immunity (B1 in **Table 1**). In HIV/AIDS surveillance, unlinked anonymous testing of samples of blood taken routinely at say, an antenatal clinic, can give vital information on the prevalence of HIV infection, since in this infection, presence of antibody denotes infection, not immunity. A smaller proportion will be ill (B2), but only some of these will visit a doctor (B3). Surveillance systems based on GP consultation rates have now been recognized as an important addition to the spectrum of a disease, and many countries have excellent systems. Of those patients that do visit their family doctor, only some will be admitted to hospital (B4). Finally, only some will die (B5).

For laboratory data, the stages are slightly different (**Table 2**), but still important to understanding what the reported data mean. As before, only a proportion of persons will be infected (B1), a smaller proportion will be ill (B2), and a smaller proportion still visit their doctors (B3). Not all doctors will send specimens to a laboratory (B4), and only a proportion of these specimens (B5), depending on accuracy of the identification process, the method of transport, the fragility of the organism, and the swabbing or other sampling technique, will be positive. Finally, depending on the level of consistency of reporting, only some of these will be reported (B6). It is important to recognize these stages in interpreting surveillance data.

It is important moreover to recognize the biases that will inevitably occur between these stages. Collection of data in routine surveillance is not normally a scientific process as one has to rely on readily available data – data obtained mostly for other reasons, such as to make a definitive diagnosis. Only the most severe cases die, and death certification thus provides, at best, a limited view of any disease. Similarly, only certain types and severity of cases will be admitted to hospital (some admissions are for social reasons for example) or even visit their family doctor. Certain age, sex, and perhaps social or occupational groups are more likely to seek medical help, be investigated and be reported. In laboratory data, more severe cases, or children, are perhaps much more likely to be investigated in detail.

These shortcomings of surveillance data do not make them useless – but their strengths and limitations must be recognized.

Feedback

If interpretation is turning statistics into information, feedback is getting the information across to those that matter, and those that need to know, so that action – the objective of surveillance – can be taken. Without feedback, surveillance is pointless. Feedback is most likely to be informative if undertaken by those most closely involved in the surveillance cycle, and who understand the significance of the data they are receiving.

Feedback should be aimed at contributors and those in public health. Contributors will then be aware of which viruses are circulating and this will help them to know what to look for in their own tests (e.g., what echovirus or

Table 1 Stages of a viral infection

Clinical
A. Uninfected
B. Infected
1. Asymptomatic
2. Symptomatic unreported
3. Symptomatic, sees a doctor
4. Symptomatic, admitted to hospital
5. Symptomatic, dies/survives

Table 2 Stages in laboratory diagnosis

A. Uninfected
B. Infected
1. Asymptomatic
2. Symptomatic, unreported
3. Symptomatic, sees a doctor
4. Symptomatic, specimens submitted
5. Symptomatic, specimens positive
6. Symptomatic, specimens reported to surveillance system

adenovirus types are in circulation). Moreover, routine surveillance will undoubtedly uncover outbreaks of infection, which will need further investigation and control at local, national, and even international level. An interesting side effect of a flourishing microbiological/feedback surveillance system is that it often stimulates better quality control within reporting laboratories.

Regular feedback is helpful, not only to contributors, but also to those who can act for the public health. Generally, the periodicity of feedback should reflect the frequency of reporting – weekly feedback for weekly reports for example. Regular topic-based reviews are important.

Evaluation of Surveillance

A surveillance system is like a country's train system. Once the rail lines have been built, the goods that will be carried along those lines can be changed according to need. Similarly, in a surveillance network, once the lines of communication have been laid down, the data being reported can be changed according to what is most important at the time (though probably not too frequently). Nevertheless, surveillance systems should ideally be frequently evaluated for usefulness, as well as for accuracy, efficiency, and effectiveness.

They should also be sufficiently flexible, so that 'new' or emerging infections can be included in an emergency or when the need arises. The successful implementation of international surveillance for SARS was instrumental in controlling it. Emergency surveillance was also essential following the tsunami of 2004, and is also necessary for the successful management of other disasters following earthquakes, hurricanes, and floods.

Surveillance systems should be evaluated before they are set up, and again at regular intervals thereafter. Before implementing a surveillance system, is there an adequate public health and administrative infrastructure in place to take action? Are the data to be collected representative and sufficiently timely for the specific infection? Are they useful, and is action being taken on the information? If not, is the feedback inadequate?

Global and International Surveillance

The ease of modern travel, the distribution of goods (especially foodstuffs) across increasingly wide parts of the world, and the uncontrollable spread of birds and other wildlife across boundaries has made global and international surveillance essential for outbreak and infection control. Surveillance of influenza now requires the expertise of many professionals – epidemiologists, virologists, and vaccinologists, clinicians, statistical modelers, veterinarians, managers, and planners – in many different countries so that information can be exchanged, and attempts made on a global basis, to prevent the next pandemic. Only recently, a new variant of chikungunya virus jumped from Kenya, where it seems to have started, to islands in the Indian Ocean and hence to India. It has now, in 2007, even reached Italy. In Reunion alone, it affected 265 000 people, an astoundingly high incidence of 34%, and an estimated case-fatality rate of 1/1000. In India 1.3 million persons are thought to have been affected (so far, to February 2007).Two species of mosquito have been involved, *Aedes aegypti* and *A. albopictus*. Epidemics of dengue and West Nile virus have also spread widely recently. AIDS/HIV was destined to become a global problem almost from the time of its first discovery. On a more positive note, SARS was contained through the use of international surveillance; and surveillance was the backbone of the smallpox eradication program.

International surveillance can also be used for the detection of international outbreaks of food poisoning caused by the distribution of foodstuffs across a wide number of countries. An outbreak of hepatitis A in England was caused by frozen raspberries grown and frozen in another country and another outbreak of hepatitis A, this time in Czechoslovakia (before it became separate republics) was caused by strawberries used to make ice cream; the strawberries had been imported from another Eastern European country. There are now well-established trans-European surveillance systems for salmonella infections and legionnaires' disease.

The need for surveillance will never diminish or disappear. Surveillance systems will only improve, become increasingly sophisticated, and become increasingly relied upon and used. Control of infection will not be possible without it.

See also: Bovine Spongiform Encephalopathy; Central Nervous System Viral Diseases.

Further Reading

Charrel RM, de Lamballerie X, and Raoult D (2007) Chikungunya outbreaks – The globalization of vectorborne diseases. *New England Journal of Medicine* 356: 769–771.

Chin J (ed.) (2002) *Control of Communicable Diseases Manual,* 17th edn. Washington, DC: APHA.

Chorba TL (2001) Disease surveillance. In: Thomas JC and Weber DJ (eds.) *Epidemiologic Methods for the Study of Infectious Diseases*, ch. 7. Oxford, UK: Oxford University Press.

Communicable Disease Report(1994) National measles and rubella immunisation campaign. *Communicable Disease Report Weekly* 4(31): 146–150.

Heymann D and Rodier GR (1998) Global surveillance of communicable diseases. *Emerging Infectious Diseases* 4: 362–365.

Noah N (2006) *Controlling Communicable Disease*, chs. 1–4, pp. 14–19. Maidenhead, UK: Open University Press.

UNAIDS (2003) Introduction to second generation HIV surveillance. http://www.data.unaids.org/Publications/IRC-pub03/2nd_generation_en.ppt (accessed September 2007).
Thomas MEM, Noah ND, and Tillett HE (1974) Recurrent gastroenteritis in a preparatory school caused by *Shigella sonnei* and another agent. *Lancet* 1: 978–981.

Relevant Websites

http://www.cdc.gov – CDC (Centers for Disease Control and Prevention).
http://www.hpa.org.uk – Communicable Disease Report Weekly, Communicable Disease Report, Health Protection Agency (HPA).
http://www.hpa.org.uk – Enter-net(Gastrointestinal), International, Health Protection Agency.
http://www.who.int – Epidemiologic Surveillance, WHO.
http://www.who.int – Epidemiology, WHO.
http://www.eiss.org – European Influenza Surveillance Scheme (EISS).
http://www.eurosurveillance.org – Eurosurveillance is Freely Available Weekly and Monthly, European Centre for Disease Prevention and Control (ECDC).
http://www.hpa.org.uk – Health Protection Agency (HPA).
http://www.hpa.org.uk – HIV and Sexually Transmitted Infections, Health Protection Agency.
http://www.who.int – National Influenza Pandemic Plans, WHO.
http://www.hpa.org.uk – Response, Co-ordination and Specialist Support for Outbreaks and Incidents, Centre for Infections, HPA.
http://www.who.int – Surveillance of Noncommunicable Disease Risk Factors, WHO.
http://www.EWGLI.org – The European Working Group for Legionella Infections, EWGLI.

DNA Vaccines

S Babiuk, National Centre for Foreign Animal Disease, Winnipeg, MB, Canada
L A Babiuk, University of Alberta, Edmonton, AB, Canada

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Electroporation The perturbution of cell membranes by very weak electrical impulses to allow the uptake of plasmid DNA.
Th1 immune response The quality of the immune response represented by a more active cell-mediated immune response with the concomitant secretion of specific cytobines such as interferon gamma.
Th2 immune response The quality of the immune response represented by a larger humoral antibody response, with the secretion of specific cytobines such as interleukin-4.

Introduction

Although the era of vaccinology has begun over 200 years ago, some of the early advances continue to form the foundation of our most successful vaccines. For example, even though it is over 200 years since Jenner introduced the concept of vaccination, using a live virus vaccine to protect people from smallpox, the concept of vaccinating with live agents continues. Indeed, some of the most effective vaccines are live-attenuated or live-heterologous vaccines. These vaccines have been successful in eliminating smallpox, or dramatically reducing the economic impact of diseases such as measles, polio, and many animal viral infections such as rinderpest. The reason for this success is that as the agent replicates, it resembles a natural infection, thereby stimulating the appropriate immune responses, including humoral and cell-mediated immunity. Furthermore, the persistence of the infecting agent, over an extended period of time, also provides long-term memory. Approximately 100 years after Jenner, Pasteur introduced a new approach to vaccination by killing the infectious agent and introducing the killed agent into the body as a nonreplicating vaccine. Similar to the original live vaccines, killed vaccines continue to be used for many infectious agents and are an integral component of our current armamentarium for disease control. Indeed, in some cases, live vaccines, which, in many cases, are more effective than killed vaccines, can be used initially to reduce the level of circulating agent in the environment, followed by killed vaccines to 'mop up' the remaining agent. The best example of such an approach is the use of live polio virus vaccine to provide broad immunity and reduce the viral load in the environment or country of interest and then use killed polio virus vaccine as a final step in disease eradication. The reason for such a strategy is that live vaccines can revert to virulence and may have certain risks associated with their use, which are worth taking when the disease burden is high but less so when the disease burden is low.

A century after Pasteur, we embarked on a new era of vaccination, primarily driven by advances in molecular biology and biotechnology, as well as a much deeper understanding of the host immune responses and antigens of each pathogen that are involved in providing protection from infection. These advances are the underpinning of

the era of vaccination employing genetic engineering. This resulted in the development of vaccines against hepatitis B and human papillomavirus, which not only prevent infection but also reduce the development of tumors. These vaccines were genetically engineered to contain all of the critical epitopes involved in inducing protective immunity. However, they do not contain any nucleic acid, thereby making them relatively safe and they are considered to be killed vaccines. Unfortunately, they require adjuvants and generally do not induce the broadest range of immune responses often seen with live vaccines.

Since production and purification of genetically engineered subunit vaccines are generally very expensive, require strong adjuvants, and generally induce a skewed systemic Th2 response, the quest continues for the development of more effective vaccines resembling live vaccines. To combine the benefits of a subunit vaccine such as safety with the broad immune responses produced by live vaccines, the concept of introducing the gene encoding putative protective proteins in a plasmid into the host was proposed. Indeed, this was heralded as a 'third generation of vaccinology', genetic vaccination, or DNA vaccination. The major advantage of such an approach, especially for viral infections, is that the antigen is expressed endogenously and the antigens are, therefore, processed in a manner resembling a viral infection. As a result, the antigen is presented by both major histocompatibility complex (MHC) I and MHC II pathways, inducing a more balanced immune response. This article describes the progress made to date in the area of DNA vaccination, including a description of the DNA vaccines that are already licensed as well as some of the challenges faced by this technology. Furthermore, the opportunities to overcome these challenges will also be discussed.

Concept of DNA Vaccines

The major reason for continued interest in DNA-based vaccines is their simplicity in concept, ease of production, potential to develop a broad range of immune responses, as well as their perceived safety and ability to induce immunity in neonates in the absence or presence of maternal antibodies. With regards to simplicity, a DNA vaccine is comprised of a plasmid containing various regulatory elements to ensure efficient production of the plasmid in bacterial systems, such as an origin of replication and a selectable marker as well as an expression cassette containing the gene of interest under a eukaryotic promoter usually human cytomegalovirus for efficient expression of the gene inserted into mammalian cells. Since the general features of a plasmid are identical for all vaccines, the single platform makes DNA vaccines very attractive from the prospective of manufacturing. Thus, the only difference between different vaccines would be the gene insert. Thus, if a company establishes a process for manufacturing one plasmid-based vaccine, they can use the same process for production and purification of a variety of different vaccines.

Recent advances in our understanding of pathogenesis, comparative biology, molecular biology, bioinformatics, and immunology make identification of putative protective antigens to most pathogens relatively easy. Second, identifying and isolating the gene encoding a protective antigen, combined with gene sequencing to ensure the cloned gene contains the correct sequence and is correctly positioned in the plasmid, is relatively straightforward. Furthermore, the gene sequence can also be modified to optimize the codon biases for expression in the host cell of interest. Once the plasmid is constructed, the production of the plasmid is relatively simple, using well-established fermentation processes in *Escherichia coli*. Since *E. coli* grows to very high densities, the quantity of plasmid produced in this manner is very high. Combining fermentation with well-established downstream processing currently developed for plasmid purification makes this process very attractive to commercial companies. Indeed, processes for plasmid purification are considered easier to perform than protein purification used in subunit vaccine production. Currently, a number of companies have established the downstream processing steps for purifying high-quality plasmids suitable for DNA vaccine production. These processes can easily be transferred to developing countries to provide secure supplies of vaccine for the developing world, should that be necessary. Indeed, all of the processes including fermentation, plasmid purification, and quality control can occur in a time frame as short as 1 month. This is a significant advantage over current subunit vaccine protein production.

The process of inserting a gene into the plasmid is routine and once the process is developed for one vaccine, the same process can be used for inserting any gene of interest. The development of a new vaccine would not require additional skills, except knowledge of the gene of interest. As a result, extensive trials and procedures required to demonstrate the absence of reactogenic components often required for subunit or conventional vaccines is reduced to a minimum. This not only reduces the concerns for safety, but also reduces the time for reaching the market.

Safety Issues with DNA-Based Vaccines

DNA vaccines are considered to be safe since they can be highly purified to remove extraneous materials and are noninfectious. The infectious nature is a common concern with live-attenuated vaccines where reversion to virulence has occurred. Preliminary trials in animals and humans demonstrated low inherent toxicity and since

the plasmids are often administered in physiological saline, in liposome-based formulations, or various other carriers which have already been tested and used over extended periods in humans, these vaccines are considered relatively safe. Initially, there was concern regarding the potential of the introduced plasmids to integrate into somatic or germline cells. However, studies have suggested that the probability of this occurring is 3 orders of magnitude lower than spontaneous mutations. Finally, there was concern that introduction of large amounts of DNA might lead to the generation of anti-DNA antibodies. Fortunately, a number of studies designed to test this possibility have demonstrated that this is likely to be a rare event if it would occur at all. Thus, it is currently widely believed that DNA-based vaccines are relatively safe.

Attractiveness of DNA-Based Vaccines

One desirable feature of any vaccine is the ability to protect individuals against a variety of disease agents following single administration of a vaccine. Thus, multicomponent vaccines are gaining popularity, not only because of the breadth of protection they induce, but also because of improved compliance since individuals do not need to return for numerous vaccinations. One limitation of combining multiple conventional vaccines is the possibility of interference between various components in the vaccine. With DNA vaccines this is also a possibility, but it should be possible to identify which component is interfering in induction of immunity. For example, in the study of a vaccine containing nine different plasmids encoding nine different malarial antigens, reduced immune responses to various components were observed compared to responses induced by individual plasmids. Similarly, we observed interference between plasmids encoding the genes for bovine herpesvirus-1 gD, parainfluenza-3 HA, and influenza virus HA. We also showed that co-administration of BHV-1 gD reduced the immune responses to BHV-1 gB. Since in these last two instances, the gD gene was responsible for the interference, it will be necessary to re-engineer this gene to remove the interference. In contrast, the use of genes encoding the four different dengue serotypes did not result in any interference. Indeed, combining the four serotypes resulted in higher antibody levels against dengue-4 than if animals were immunized with a monovalent dengue-4 plasmid-based vaccine. Similarly, broadening of the immune response was seen with multiple DNA vaccine components of various HIV proteins. Thus, it appears that each plasmid combination may need to be investigated independently for maximal benefit.

In addition to combining plasmids encoding different genes of interest, it is also possible to introduce two different genes encoding two different proteins from either the same or different pathogens in a single plasmid, thus further reducing the cost of production since one vaccine would protect against two different agents. This can be achieved by generating a plasmid which contains two gene expression cassettes or by simply linking the genes from two pathogens and inserting them into the plasmid. In this case, the two genes would be expressed as a single protein from a single promoter. Since the conformational changes imposed by linking two proteins together may alter their immunogenicity and the stability of the plasmid may be compromised as a result of its size, a number of plasmids have been constructed with each gene driven by its own promoter or by insertion of an encephalomyocarditis virus (EMCV) internal ribosome entry site (IRES) to translate the second gene. In some of these cases, the level of expression of the second gene was not as effective as that of the first gene, whereas in other instances there was no reduction in gene expression of either gene. Using such manipulations, it should be possible to broaden the protection to induce immunity to a number of different diseases simultaneously.

Neonatal Immunization

Since many diseases occur during the first few weeks or months of life, it is critical to induce immunity from vaccines as early as possible. Unfortunately, most vaccines given to neonates are less effective than in adults. There are many reasons for this. In the case of live vaccines, the presence of maternally derived antibodies generally limit the degree of replication of the vaccine, resulting in poor immune responses due to reduced antigenic mass produced *in vivo*. In the case of killed vaccines where there is less interference by maternally derived antibodies, the immune response is generally skewed to a Th2 bias response, thought to be due to the immaturity of the immune system. Using DNA vaccines, it has been shown that not only was it possible to induce immunity in neonates, but immunity could even be induced *in utero*. Thus, sheep immunized *in utero* during the third trimester were born fully immune. More importantly, the animals developed long-term memory. Indeed, the induction of memory by DNA vaccines is a very attractive feature of these vaccines since immune memory and duration of immunity are desirable for many vaccines. Numerous other studies have also shown the induction of long-term memory of T cells and B cells following DNA vaccination. This has been shown in a variety of models including pigs, sheep, and cytomologous monkeys. Memory could be further increased by incorporating plasmids encoding for IL-12 or IL-15 with the DNA vaccine.

It is also interesting that DNA vaccines appear to be able to induce immune responses in the presence of high levels of antibody. Thus, the fact that DNA vaccines can

induce immunity in neonates in the presence or absence of maternal antibodies makes this approach to vaccination extremely attractive, especially for diseases such as herpes simplex virus-2, human immunodeficiency virus (HIV), hepatitis B, group B strep, and chlamydia which often infect children during birth or shortly thereafter.

Limitations and Clinical Applications of DNA Vaccines

Although there are over 1000 publications demonstrating the effective induction of immunity using DNA vaccines in mice which protect mice from subsequent infection, DNA vaccines have not been as successful in larger animals or humans. Possibly the greatest challenge to adopting DNA vaccination as a routine in large animals and humans is the poor efficiency of transfection leading to suboptimal induction of immunity. This has limited the introduction of vaccines into the market. However, recently two DNA vaccines were licensed for commercial use. The first was a vaccine to immunize fish against infectious hematopoietic necrosis virus, followed shortly after with one for West Nile virus in horses. Although there have been numerous clinical trials in humans, the greatest success has been when DNA vaccines were used to prime the individual, followed by booster with a recombinant or subunit vaccine. Indeed, the DNA prime, followed by protein boost is currently the method of choice for induction of the immune responses using DNA vaccines in humans. Thus, it is our contention that if better delivery mechanisms could be introduced to enhance the transfection efficiency in large animals and humans, DNA vaccines have the potential to become a critical component in our armamentarium against infectious diseases.

Vaccine Delivery

Once the plasmids are purified, they are introduced into the host (animals or humans) and the DNA is taken up by specific cells where the gene is expressed and protein is produced. Unfortunately, only a very small percentage of the DNA is internalized, which is one of the drawbacks of DNA vaccination. Even for the DNA molecules that are internalized, not all are transported to the nucleus where they express their encoded antigen. However, the few molecules that enter the nucleus are transcribed and translated to produce protein, making the animal cells a bioreactor. Thus, the vaccine antigen is produced in the animal. Even though the quantity of protein produced by the transfected cell is very low, the protein is presented to the immune system and induces a broad spectrum of immune responses including antibody- and MHC-I-restricted CTL responses. Clearly, the magnitude of the immune response as well as the quality of the immune response can be influenced by the route of delivery; intradermal delivery is generally better than intramuscular since the numbers of antigen-presenting cells are more numerous in the skin than in muscle. Indeed, muscle cells are not generally capable of presenting antigen to the immune system, although immunity occurs as a result of antigen release from myocytes leading to cross-priming. The quality of the immune response can also be modulated by the background of the plasmid. A number of groups have modified the plasmid background to incorporate sufficient and appropriate CpG motifs to stimulate the innate immune response. By stimulating the innate immune response, it is possible to recruit the appropriate cells to the site of antigen expression and create a cytokine microenvironment conducive to developing an adaptive immune response. To improve the cytokine microenvironment conducive to induction of immunity, a number of investigators have co-administered DNA vaccines with plasmids expressing cytokines, defensins, or other immune stimulator molecules to recruit dendritic cells to the vaccination site. Other constructs have contained both the antigen and the cytokine/chemokines within the single plasmid. In most cases, the immune response was enhanced by the co-administration of cytokines such as granulocyte-macrophage colony-stimulating factor (GMCSF) and interleukins IL-2, IL-12, and IL-15. In addition, the cytokine–chemokine combination plasmids could bias the immune response. For example, macrophage inflammatory protein-1 (MIP-1)β biased the immune response of Th2-like response, whereas MIP-2 and MIP-1α favored a Th1 like response. Currently, a number of studies are ongoing using IL-12 and IL-15 for enhancing immune response to HIV vaccines.

Since intracellular uptake of DNA is not a very efficient process, numerous investigators have employed a variety of methods to enhance DNA uptake into cells. One very efficient delivery system is encapsulation of DNA in liposomes or polylactide-L-glycolide (PLG) microparticles. DNA delivery by these microparticles serves two purposes. First, the encapsulated DNA is protected from degradation, and, second, the liposomes or microparticles facilitate uptake of the DNA into antigen-presenting cells. Indeed, it is even possible to deliver the microparticles orally to induce mucosal immunity as well as systemic immunity. Since encapsulation of DNA in PLG is thought to cause DNA damage due to the process of encapsulation, other investigators have attached the DNA to the surface of PLG microspheres. This process has resulted in excellent immune responses regardless of whether the PLG microparticles were given mucosally or delivered systemically, suggesting the microspheres play a crucial role in DNA uptake in the cells.

Improving uptake of DNA is thought to be critical for enhancement of immunity, and since the response is

better when more cells are transfected, significant efforts have been focused on enhancing DNA uptake by various physical methods. The earliest approach to enhancing cell uptake was the use of a gene gun. In this instance, the DNA is coated on gold particles and is propelled into the skin. Some of these particles directly penetrate cells, whereas others are taken up by antigen-presenting cells. Although this approach has been shown to be effective, the quantity of DNA that can be coated onto gold beads is generally low, thereby reducing the effectiveness of such an approach.

The limitation of the quantity of DNA that can be taken up by cells by a gene gun can be overcome by jet injection of DNA. Depending on the pressure used to deliver the DNA, the DNA can be deposited either in the epidermis, subcutaneously, or even intramuscularly. However, an even more efficient method to deliver DNA into cells is the use of electroporation. Various companies have developed devices to both inject and electroporate the DNA simultaneously. Electroporation does not only enhance DNA uptake, but it also causes localized inflammation and induces the various mediators of innate immunity, resulting in enhanced immune responses. These studies have demonstrated the importance of modifying the local environment at the site of injection since the mere enhancing of expression does not always increase the immune responses. These studies demonstrate that one requires all of the elements of the immune response to be present at the site, as well as the specific antigen for the immunity to be induced.

Future Prospects

DNA vaccines represent an exciting addition to an already impressive track record of vaccines, which have saved millions of lives and improved the quality of life of almost every individual on the planet. Furthermore, vaccines have added significant economic benefits to society. Unfortunately, individuals still suffer from many infections. The reasons for this are varied, including the cost of vaccines, politics, as well as distribution issues. One of the major challenges to distribution is the need for a cold chain. If this could be overcome, the vaccines could be distributed to even remote regions of the world. DNA vaccines offer an advantage in this regard. Furthermore, it should be possible to develop these vaccines more economically, ensuring that the poor in remote regions could be vaccinated. Unfortunately, for this dream to become a reality, it will be necessary to improve the transfection efficiency of DNA vaccines to ensure that sufficient antigen is produced to induce an immune response. Currently, improvements in DNA vaccines often have been marginal. What is required is a 10–50-fold improvement to make the vaccines economical in most veterinary species and for humans in the developing world. However, the fact that two different DNA vaccines have already been licensed provides hope that within the next decade we will see a number of new DNA-based vaccines licensed and shown to be effective in reducing both morbidity and mortality. Whether these vaccines will be comprised solely of DNA-based vaccines or will be comprised of DNA-based vaccines which will prime the immune response, followed by boosting with conventional or recombinant vaccines, remains to be determined. Indeed, we will probably see both types of vaccine configurations in the future.

See also: AIDS: Vaccine Development; Immune Response to viruses: Antibody-Mediated Immunity; Immune Response to viruses: Cell-Mediated Immunity; Vaccine Strategies.

Further Reading

Babiuk S, Babiuk LA, and van Drunen Littel-van den Hurk S (2006) DNA vaccination: A simple concept with challenges regarding implementation. *International Reviews of Immunology* 25: 51–81.

Gerdts V, Snider M, Brownlie R, Babiuk LA, and Griebel P (2002) Oral DNA vaccination *in utero* induces mucosal immunity and immune memory in the neonate. *Journal of Immunology* 168: 1877–1885.

O'Hagan DT, Singh M, and Ulmer JB (2004) Microparticles for delivery of DNA vaccines. *Immunological Reviews* 199: 191–200.

Ulmer JB, Wahren B, and Liu MA (2006) DNA vaccines: Recent technological and clinical advances. *Discovery Medicine* 6: 109–112.

van Drunen Littel-van den, Hurk S, Babiuk SL, and Babiuk LA (2004) Strategies for improved formulation and delivery of DNA vaccines to veterinary target species. *Immunological Reviews* 199: 113–125.

E

Ebolavirus

K S Brown and A Silaghi, University of Manitoba, Winnipeg, MB, Canada
H Feldmann, National Microbiology Laboratory, Public Health Agency of Canada, Winnipeg, MB, Canada

© 2008 Elsevier Ltd. All rights reserved.

History

Ebola virus (EBOV) first emerged as the causative agent of two major outbreaks of viral hemorrhagic fever (VHF) occurring almost simultaneously along the Ebola River in Democratic Republic of Congo (DRC, formerly Zaire) and Sudan in 1976 (see **Table 1**). Over 500 cases were reported, with case fatality rates (CFRs) of 88% and 53%, respectively. It was later recognized that these two outbreaks were caused by two distinct species of EBOV (*Zaire ebolavirus* and *Sudan ebolavirus*). In 1989, a novel virus, Reston ebolavirus (REBOV), was isolated from naturally infected cynomolgus macaques (*Macaca fascicularis*) imported from the Philippines into the United States. All shipments except one were traced to a single supplier in the Philippines; however, the actual origin of the virus and mode of contamination for the facility have never been ascertained. While pathogenic for naturally and experimentally infected monkeys, limited data indicate that REBOV may not be pathogenic for humans as animal caretakers were infected without producing clinical symptoms. In 1994, the first case of Ebola hemorrhagic fever (EHF) occurred in western Africa in the Tai Forest Reserve in Côte d'Ivoire (Ivory Coast). An ecologist was infected by performing a necropsy on a dead chimpanzee whose troop had lost several members to infection with Côte d'Ivoire ebolavirus (CIEBOV). A single seroconversion was later documented, suggesting another nonfatal human case in nearby Liberia. Zaire ebolavirus (ZEBOV) reemerged in Kikwit, DRC, in 1995, causing a large EHF outbreak with 81% CFR. Sudan ebolavirus (SEBOV) reemerged in 2000–01 in the Gulu District in northern Uganda. There were over 425 cases (53% CFR), making it the largest EHF epidemic documented so far. Starting in 1994, an endemic focus of ZEBOV activity became obvious in the northern boarder region of Gabon and the Republic of Congo (RC) with multiple small EHF outbreaks over the past decade. Most infections there have been associated with the hunting and handling of animal carcasses (mainly great apes). In addition, ZEBOV has virtually decimated the chimpanzee and gorilla populations in those areas. At least three laboratory exposures to EBOV have occurred; one in Russia (2004) was fatal.

Taxonomy and Classification

Filoviruses are classified in the order *Mononegavirales*, a large group of enveloped viruses containing nonsegmented, negative-sense (NNS) RNA genomes. The family *Filoviridae* is separated into two distinct genera, *Marburgvirus* and *Ebolavirus*. The genus *Ebolavirus* is subdivided into four species – *Zaire ebolavirus*, *Sudan ebolavirus*, *Côte d'Ivoire ebolavirus*, and *Reston ebolavirus*. Filoviruses are classified as maximum containment (biosafety level 4 (BSL-4)) agents as well as category A pathogens based on their generally high mortality rate, person-to-person transmission, potential aerosol infectivity, and absence of vaccines and chemotherapy.

Biological and Physical Properties of Virion

EBOV particles are pleomorphic, appearing as U-shaped, 6-shaped, circular forms, or as long filamentous, sometimes branched forms varying greatly in length (up to 14 000 nm), but have a uniform diameter of ~80 nm (see **Figures 1(a)** and **1(b)**). EBOV virions purified by ratezonal gradient centrifugation are bacilliform in outline and show an average length associated with peak infectivity of 970–1200 nm. Except for the differences in length, EBOVs seem to be very similar in morphology. Virions contain a helical ribonucleoprotein complex RNP or nucleocapsid roughly 50 nm in diameter bearing cross-striations with a periodicity of approximately 5 nm, and a dark, central axial space 20 nm in diameter running

Table 1 Ebola hemorrhagic fever (EHF) outbreaks from 1976 to 2005

Ebola species	*Year*	*Outbreak location (country)*	*Place of origin*	*Human cases (% case fatality rate)*
Zaire ebolavirus	1976	Yambuku (DRC)	DRC	318 (88)
	1977	Tandala (DRC)	DRC	1 (100)
	1994	Ogooue-Invindo province (Gabon)	Gabon	51 (60)
	1995	Kikwit (DRC)	DRC	315 (79)
	1996	Mayibout (Gabon)	Gabon	37 (57)
	1996	Booue (Gabon); Johannesburg (South Africa)	Gabon	61 (74)
	2001–02	Ogooue-Invindo province (Gabon); Cuvette region (RC)	Gabon?[a]	124 (79)
	2002–03	Cuvette region (RC); Ogooue-Invindo province (Gabon)	RC?[a]	143 (90)
	2003	Mboma and Mbandza (RC)	RC	35 (83)
	2005	Etoumbi and Mbomo in Cuvette region (RC)	RC	12 (75)
	2007	Kampungu (DRC)	DRC	Ongoing
Sudan ebolavirus	1976	Nzara, Maridi, Tembura, Juba (Sudan)	Sudan	284 (53)
	1979	Nzara, Yambio (Sudan)	Sudan	34 (65)
	2000–01	Gulu District in Mbarrara, Masindi (Uganda)	Uganda	425 (53)
	2004	Yambio Country (Sudan)	Sudan	17 (41)
Côte d'Ivoire ebolavirus	1994	Tai Forest (Ivory Coast)	Ivory Coast	1 (0)
	1995	Liberia (Liberia)	Liberia?[a]	1 (0)
Reston ebolavirus	1989	Reston, Virginia (also Pennsylvania and Texas) (USA)	Philippines[b]	4 (0)[c]
	1992	Siena (Italy)	Philippines	0[d]
	1996	Alice, Texas (USA)	Philippines	0[d]

[a]Place of origin unconfirmed.
[b]Reston virus has only been traced to a single monkey-breeding facility in the city of Calamba, the Philippines, which was depopulated in 1996 and is no longer in operation.
[c]Mortality in monkeys was estimated at 82%.
[d]Only monkeys were infected in these outbreaks; no reported human cases.
DRC, Democratic Republic of the Congo; RC, Republic of the Congo.

the length of the particle. The RNP complex is composed of the genomic RNA and the RNA-dependent RNA polymerase (L), nucleoprotein (NP), and virion proteins 35 and 30 (VP35 and VP30). A lipoprotein unit-membrane envelope derived from the host cell plasma membrane surrounds it. Spikes approximately 7–10 nm in length, spaced apart at ~10 nm intervals, are visible on the virion surface and are formed by the viral glycoprotein (GP). Virus particles have a molecular weight of approximately $3–6 \times 10^8$ Da and a density in potassium tartrate of 1.14 g cm^{-3}. Virus infectivity is quite stable at room temperature. Inactivation can be performed by ultraviolet (UV) light and γ-irradiation, 1% formalin, β-propiolactone, and brief exposure to phenolic disinfectants and lipid solvents, like deoxycholate and ether.

Properties of Genome

The EBOV genome consists of a molecule of linear, nonsegmented, negative-stranded RNA which is noninfectious, not polyadenylated, and complementary to viral-specific messenger RNA. The genome amounts to *c.* 1.1% of the total virion. EBOV genomes are ~19 kbp in length and fairly rich in adenosine and uridine residues. Genomes show a linear gene arrangement in the order 3′ leader–NP–VP35–VP40–GP–VP30–VP24–L–5′ trailer (see **Figure 1(b)**). All genes are flanked at their 3′ and 5′ ends by highly conserved transcriptional start (3′-CUnCnUn-UAAUU-5′) and termination signal sequences (3′-UAAUUCUUUUU-5), respectively, all of which contain the pentamer 3′-UAAUU-5′. Most genes are separated by intergenic sequences variable in length and nucleotide composition. A feature of all EBOV genomes is the fact that some intergenic regions overlap by the conserved pentamer (UAAUU) sequence. ZEBOV and SEBOV show three such overlaps within the intergenic sequences of VP35/VP40, GP/VP30, and VP24/L, whereas REBOV shows only two between VP35/VP40 and VP24/L. Extragenic leader and trailer sequences are present at the 3′ and 5′ genome ends. These sequences are complementary at their very extremities, showing the potential to form

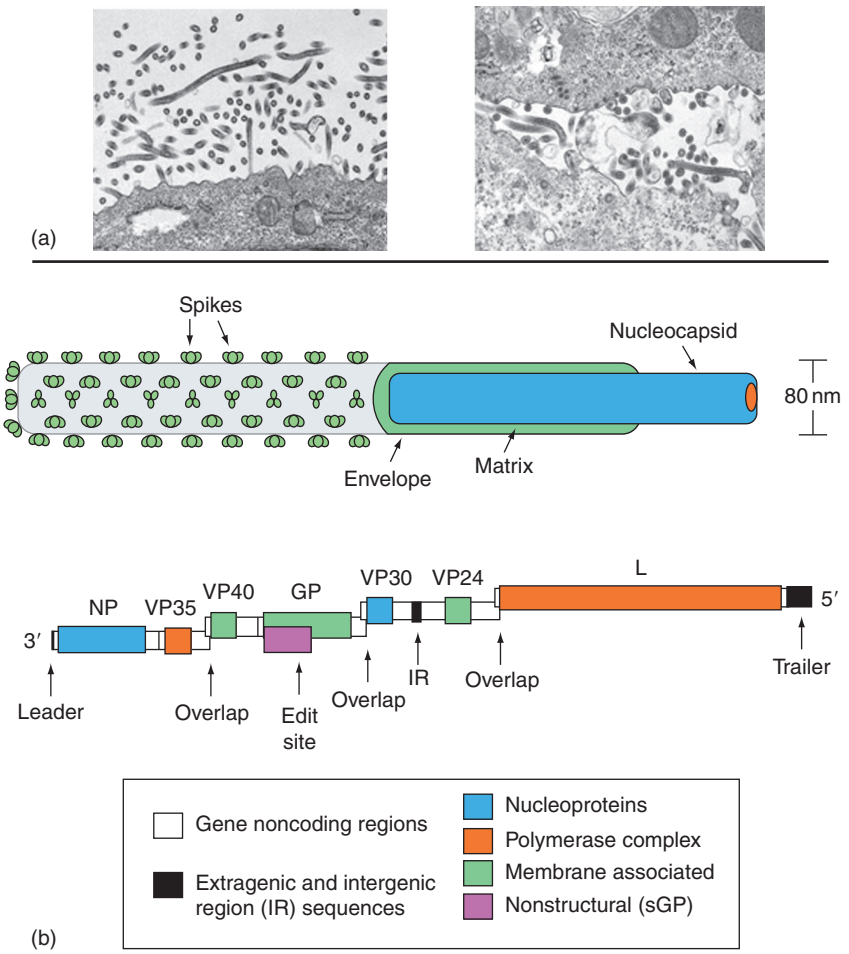


Figure 1 Particle morphology. (a) Transmission electron microscopy of Ebola virus particle. Both graphs show images of Vero E6 cells infected with Zaire ebolavirus. (b) Ebola virus particle structure and genome organization. The upper part provides a scheme of the virus particle separated into four components. The inner core consists of the nucleocapsid which is a structure formed by the single-stranded, negative-sense RNA genome associated with the two nucleoproteins (NP, VP30) and the polymerase complex (L and VP35). The matrix is built by the viral matrix protein VP40 and a minor component VP24. The envelope is derived from the infected cell during assembly/budding. The spikes consist of a homotrimer of the glycoprotein (GP).

stem–loop structures. Phylogenetic analyses on the basis of the GP gene of the different Ebola species show a 37–41% difference in their amino acid and nucleotide sequences. Analysis within one species shows remarkable genetic stability between strains (the variation in nucleotide sequences has been shown to be <7% and even <2% among distinct ZEBOV strains), unexpected for an RNA virus, but highly indicative that these viruses have reached a high degree of fitness to fill their respective niches.

Properties of Viral Proteins

Virions contain seven structural proteins with presumed identical functions for the different viruses (see **Table 2**). The electrophoretic mobility patterns of these proteins are characteristic for each species.

RNP Complex and Matrix Proteins

Four proteins are associated with the viral RNP complex: NP, L, VP30, and VP35 (see **Figure 1(b)**). These proteins are involved in the transcription and replication of the genome. NP and VP30 represent the major and minor nucleoproteins, respectively. They interact strongly with the genomic RNA molecule, and are both phosphoproteins. VP30 is also a zinc-binding protein that behaves as a transcriptional activator. The L and VP35 proteins form the polymerase complex. The L protein, like other L proteins of NNS RNA viruses, represents the RNA-dependent RNA polymerase. Motifs linked to RNA (template) binding, phosphodiester bonding (catalytic site), and ribonucleotide triphosphate binding have all been described. VP35 appears to behave in a mode similar to that of the phosphoproteins found in other NNS RNA viruses, acting as a cofactor that affects the mode of RNA synthesis (transcription vs

Table 2 Ebola virus proteins: functions and localization

Gene order[a]	*Ebola virus proteins*	*Protein function (localization)*
1	Nucleoprotein (NP)	Major nucleoprotein, RNA genome encapsidation (component of RNP complex)
2	Virion protein 35 (VP35)	Polymerase complex cofactor, type I interferon antagonist (component of RNP complex)
3	Virion protein 40 (VP40)	Matrix protein, virion assembly, and budding (membrane associated)
4 (primary)	Secreted glycoprotein (sGP) (nonstructural)	May have immunomodulatory role (secreted)
4 (secondary)	Glycoprotein (GP)	Receptor binding and membrane fusion (membrane associated)
5	Virion protein 30 (VP30)	Minor nucleoprotein, RNA binding, transcriptional activator (component of RNP complex)
6	Virion protein 24 (VP24)	Minor matrix protein, virion assembly, type I interferon antagonist (membrane associated)
7	Polymerase (L)	RNA-dependent RNA polymerase, enzymatic portion of polymerase complex (component of RNP complex)

[a]Gene order refers to 3′–5′ gene arrangement as shown in **Figure 1(b)**.

replication). VP35 is also known to be a type I interferon (IFN) antagonist; it blocks IFN-α/β expression by inhibiting IFN regulatory factor 3 (IRF-3). VP40 functions as the viral matrix protein and represents the most abundant protein in the virion. It plays a number of roles in viral infection related to assembly and budding of the viral particles. The production of VP40 by itself is sufficient to initiate the budding process for the production of virus-like particles (VLPs); however, the production of these particles is greatly enhanced by the addition of GP and NP. Recent studies have shown that EBOV hijacks the cellular protein machinery in order to mediate assembly and budding from the cellular membranes. These functions have been associated with overlapping late domain sequences (PTAP and PPEY) found in VP40. Late domains interact with cellular proteins such as Tsg101 and Nedd4 and affect a late step in the budding process. VP24 is thought to be a secondary (minor) matrix protein that, unlike VP40, is only incorporated into virions in small amounts. It has an affinity for the plasma membrane and perinuclear region of infected cells. The precise role of VP24 in replication is unclear; however, VP24 is a known type I IFN antagonist. Unlike VP35, VP24 blocks the translocation of phosphorylated STAT1 into the nucleus, subverting the antiviral response. More recently, VP24 has been associated with adaptation in rodent hosts.

Glycoproteins

The structural GP is a type I transmembrane protein inserted into the membrane as a trimer (see **Figure 1(b)**). It functions in viral entry, influences pathogenesis, and acts as the major viral antigen. The GP gene shows two open reading frames (ORFs) encoding for the precursors of GP (pre-GP) and a nonstructural secreted glycoprotein (pre-sGP), which is the primary product of this gene. Translation of pre-GP can only be achieved through mRNA editing, where one adenosine residue is added at a seven-uridine-stretch template sequence, resulting in a frameshift of the primary ORF. GP is cytotoxic when expressed at higher levels, leading to the hypothesis that mRNA editing may be evolutionarily related to the control of overexpression of this protein. The N-terminal $\sim$300 amino acids of the pre-GP are identical to those of pre-sGP, but the C termini of each protein are unique. pre-GP is translocated to the endoplasmic reticulum (ER) by an N-terminal signal sequence, and anchored by an extremely short membrane-spanning sequence at the C-terminus. The protein is glycosylated in the ER and Golgi apparatus with both N-linked and O-linked glycans. Most of the O-linked glycans are located in a mucin-like region (rich in theronine, serine, and proline residues) located in the middle of the protein. pre-GP is then cleaved by a subtilisin/kexin-like convertase such as furin, leading to the formation of disulfide-bonded $GP_{1,2}$. The smaller C-terminal cleavage fragment GP_2 contains the transmembrane region that anchors GP to the membrane. Interestingly, proteolytic cleavage of pre-GP is not required for infectivity or for virulence, indicating that the uncleaved precursor can mediate receptor binding and fusion. The production of pre-sGP differentiates EBOV from Marburg viruses, which do not express this soluble protein. pre-sGP is also translocated into the ER, modified in the secretory (e.g., oligomerization, glycosylation) pathway, and cleaved by furin near the C-terminus to release a short peptide termed delta peptide. No biological properties have been attributed to delta peptide. Recent matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis suggests that sGP forms a homodimer in a parallel orientation, held together by disulfide bonds between the most N-terminal and C-terminal cysteine residues. sGP circulates in the blood of acutely infected humans. Its exact function is still unknown; however, an interaction with the cellular and

humoral host immune responses has been postulated. In contrast to GP which mediates endothelial cell (EC) activation and decreases EC barrier functions, sGP seems to have an anti-inflammatory role.

Viral Life Cycle

The viral life cycle consists of several events: binding/entry, uncoating, transcription, translation, genome replication, and packaging/budding (see **Figure 2**). EBOVs have selective tropism primarily for monocytes, macrophages, and dendritic cells (DCs), although other cell types such as fibroblasts, hepatocytes, and ECs can be infected. In contrast, lymphocytes generally do not support EBOV replication, which is believed to be due to the lack of expression of the viral receptor(s). Although several molecules have been identified as possible viral receptors in various *in vitro* systems, it is not clear if any of the identified molecules are actually used *in vivo* or the degree of requirement for infection and disease. Folate receptor alpha was the first identified possible receptor but entry was also shown to occur in the absence of the molecule. C-type lectins such as DC-SIGN, DC-SIGNR, and hMGL were shown to be able to enhance binding but are not required for infection. Expression of members of the Tyro3 family converted the poorly susceptible Jurkat T cells into susceptible cells for particles pseudotyped with ZEBOV GP and enhanced ZEBOV infection. Receptor binding results in endocytosis into endosomes, possibly via clathrin-coated pits and caveolae although some studies suggest that they may not be necessary. Acidification of the endosomes is necessary for fusion of the viral and endosomal membranes, which is mediated by a region in GP_2. Proteolysis by cathepsins B and L in the acidic endosomes might be essential for infectivity.

Transcription and genome replication seems to follow the general principles for *Mononegavirales*. There seems to

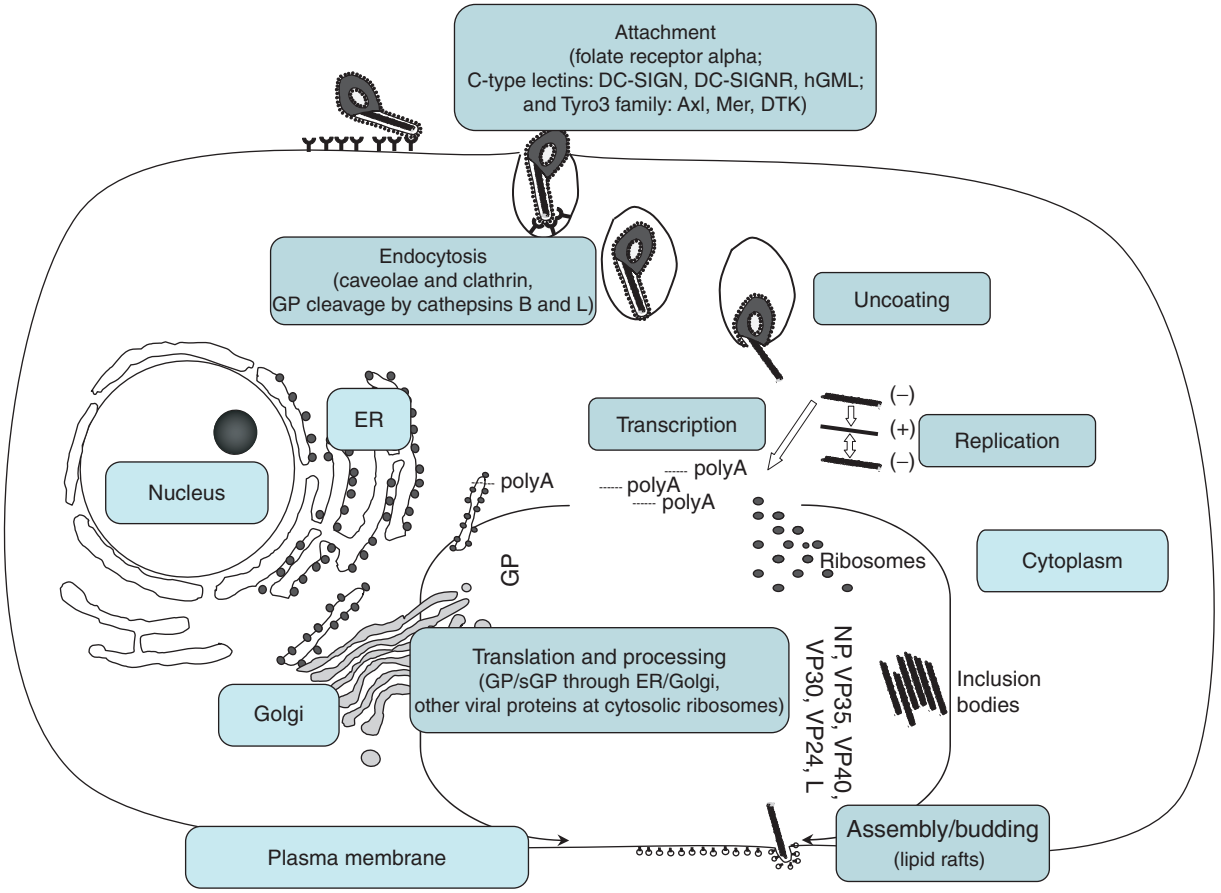


Figure 2 Ebola virus life cycle. The virus attaches to specific receptor(s) on the plasma membrane which leads to endocytosis via caveolae and clathrin-mediated pathways. The viral GP is cleaved in the endosome by cathepsins L and B. After uncoating, transcription and replication take place in the cytoplasm. All proteins except the GPs are translated on free ribosomes in the cytoplasm, while the glycoproteins are produced and modified in the ER and Golgi. Nucleocapsids formed in inclusion bodies interact with the matrix protein VP40 at the plasma membrane. Assembly and particle budding occurs at lipid rafts in the plasma membrane.

be a gradual decrease in mRNA levels from 3′ to 5′ end of the genome. The encapsidated viral genome serves as the template for transcription, which in the case of EBOV requires the L proteins, VP35 and VP30. mRNA transcripts are monocistronic, capped, polyadenylated, and contain long noncoding regions at their 3′ and/or 5′ ends. Stem–loop structures at the 5′ ends of mRNA transcripts may affect transcript stability, ribosome binding, and translation. The switch from transcription to replication seems to be triggered by an accumulation of viral proteins, especially NP, in the cytoplasm. Large amounts of NP are localized in inclusion bodies to which other viral proteins are recruited. These inclusion bodies may function as sites of RNP complex formation.

Membrane/lipid rafts have been identified as platforms for the assembly of virions; GP trimers conveyed to the surface membrane have an affinity for these rafts that is associated with palmitoylation of the membrane-spanning anchor sequence. RNP complexes interact with VP40, which is deposited at the plasma membrane via the late retrograde endosomal pathway. Abolition of the late domains in VP40 blocks particle formation, but only partially affects viral replication, suggesting that other domains or proteins must be involved. VP24 is also associated with the plasma membrane and enhances particle formation, but a specific role in particle maturation has not been identified. GP trimers seem to interact with VP40 and/or VP24 to finalize the budding process.

Host Range and Experimental Models

Natural Hosts and Geographic Distribution

EBOVs typically infect humans and nonhuman primates (NHPs); ZEBOV and SEBOV appear to be the main causes of lethal infections in humans, and thus are of primary public health concern. EBOVs appear to be indigenous to the tropical rain forest regions of central Africa (with the exception of REBOV), as indicated by the geographic locations of known outbreaks and seroepidemiological studies. The discovery of REBOV in the Philippines suggests the presence of a filovirus in Asia. Many species have been discussed as possible natural hosts; however, no nonhuman vertebrate hosts or arthropod vectors have yet been definitely identified. Epidemiological data have suggested monkeys as a potential reservoir of filoviruses; however, the high pathogenicity of EBOV for NHPs does not generally support such a concept. Similarities in biological properties to other viral hemorrhagic fever agents, such as ‘Old World’ arenaviruses, favor a chronic infection of an animal that regulates survival of the viruses in nature. More recently, viral RNA and virus-specific antibodies could be detected in African fruit bat species, suggesting that bats might be a reservoir for EBOV.

Experimental Models

Experimental hosts include monkeys (specifically rhesus and cynomolgus macaques), for which infection with ZEBOV is usually 100% lethal, guinea pigs (which show febrile responses 4–10 days after inoculation, but not uniform lethality), and newborn and immunocompromised mice when inoculated with wild-type virus. The resistance of the adult rodent models to EBOV infection has led to the production of guinea pig- and mouse-adapted ZEBOV strains (GPA-ZEBOV and MA-ZEBOV), produced through the serial passage of the virus through progressively older animals. These adapted strains are able to present uniform lethality in their respective hosts. MA-ZEBOV demonstrates reduced virulence in NHPs, whereas GPA-ZEBOV remains uniformly lethal in this model. Infected mice do not exhibit strong coagulation abnormalities (a hallmark of EBOV infection in humans and NHPs) and also slightly differ in other clinical symptoms. Mice, however, represent a good screening model for studies of antiviral and host immune responses. Infected guinea pigs do present coagulation defects that more closely resemble a human or an NHP infection. For growth in cell culture, primary monkey kidney cells and monkey kidney cell lines (e.g., Vero) are often used, but EBOV can replicate in many mammalian cell types including human ECs and monocytes/macrophages.

Clinical Features

Nonspecific, flu-like symptoms such as fever, chills, and malaise appear abruptly in infected individuals after an incubation period that ranges from 2 to 21 days, but on average lasts 4–10 days. Subsequently, multisystemic symptoms such as prostration, anorexia, vomiting, chest pain, and shortness of breath develop. Macropapular rash associated with varying degrees of erythema may also occur and is a valuable differential diagnostic feature. At the peak of the disease, vascular dysfunction signs appear ranging from petechiae, echymoses, and uncontrolled bleeding at venipuncture sites, to mucosal bleeding and diffuse coagulopathy. Massive blood loss is atypical, although it may happen in the gastrointestinal tract, and is not sufficient to lead to death. Fatal cases develop shock, multiorgan failure, and coma with death occurring between days 6 and 16. Survivors can have multiple sequelae such as hepatitis, myelitis, ocular disease, myalgia, asthenia, and psychosis. The mortality and severity of symptoms are viral species dependent, with ZEBOV causing 60–90% and SEBOV 50–60% lethality. Viremia in fatal cases can reach peak levels of 10^9 genomes ml^{-1}, while survivors have peak levels of about 10^7 genomes ml^{-1}. Viral antigen can be found systemically, although it is most abundant in the spleen and liver.

Diagnosis of EHF is based on the detection of virus-specific antibodies, virus particles, or particle components. The procedures are the same as for Marburg hemorrhagic fevers.

Pathogenicity

Most of the information available regarding pathogenicity is derived from ZEBOV infections in humans and animal models. Death seems to be the result of systemic shock due to vascular dysfunction, which is caused by a complex interaction of the immune system with vascular physiology. Three major processes – increase in vascular permeability, disseminated intravascular coagulation, and impaired protective immunological responses – are the main events that lead to shock and death. The first two processes are the product of a series of events starting with infection of primary target cells (monocytes/macrophages (Mø) and DCs) and later leading to activation and decrease of barrier function of ECs, while the third is still being actively investigated (see **Figure 3**).

Infected Mø are strongly activated, secreting pro-inflammatory molecules such as interleukin 6(IL-6), tumor necrosis factor alpha (TNF)-α, IL-1β, nitric oxide (NO), and spread the infection systemically. In contrast, infection of DCs results in impaired activation, with no upregulation of co-stimulatory molecules such as major histocompatibility complexes (MHCs) I and II, CD80, CD86, and CD40. Early interaction with primary target cells is independent of virus replication and current models suggest that GP in the repetitive context of a particle is required for activation, possibly by binding and cross-linking cellular receptors. Activation of ECs by mediators such TNF-α and NO is believed to be the main cause for the decrease in EC barrier function. TNF-α, NO, and pro-inflammatory cytokines increase vascular permeability and EC surface adhesion molecule expression, which are

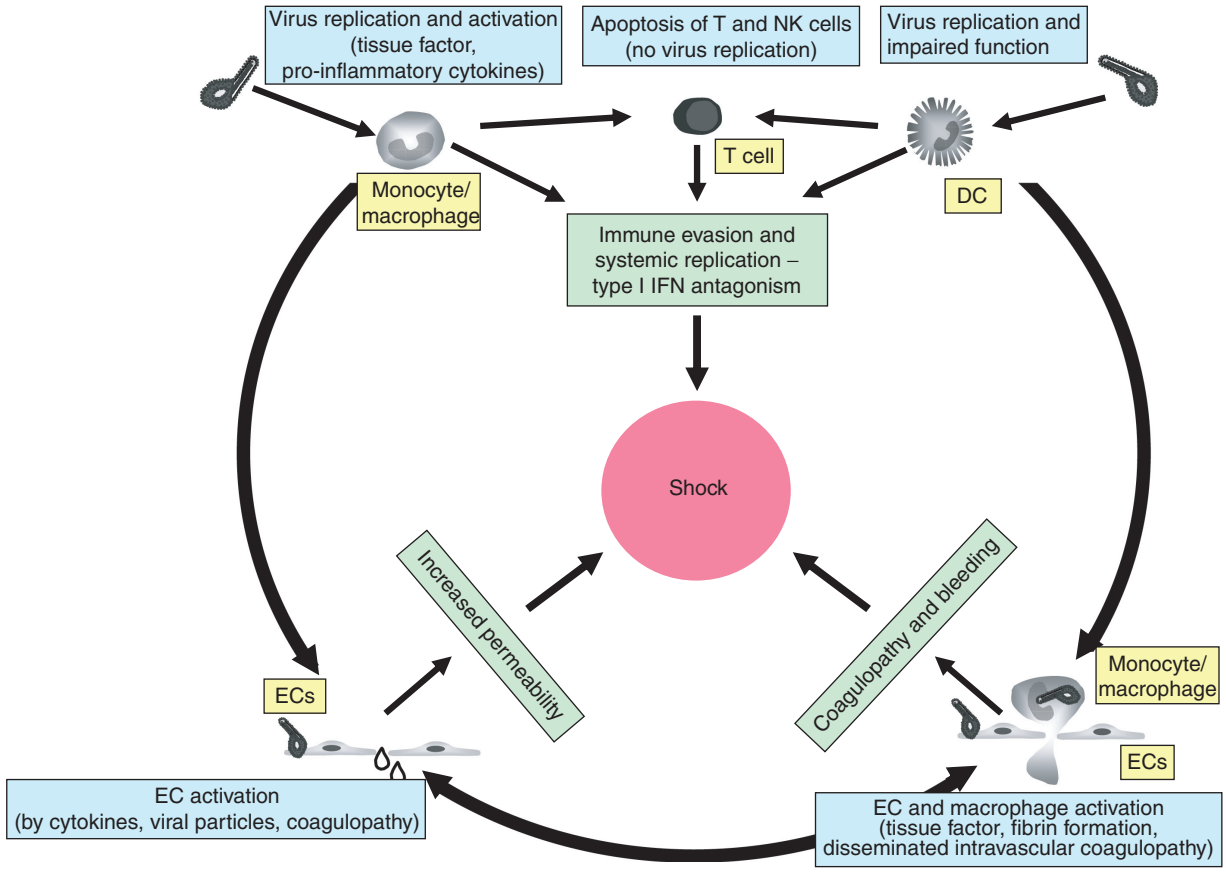


Figure 3 Ebola virus pathogenesis. Shock, the final event in severe/lethal cases, is caused by three processes, which influence each other: systemic viral replication and immune evasion, increase in vascular permeability, and coagulopathy. Infection of primary target cells such as monocytes/macrophages and DCs results in systemic spread of the virus and differential activation. Monocytes/macrophages are activated to produce pro-inflammatory cytokines and tissue factor (TF), while DC activation is impaired, leading to poor protective immune responses. Type I interferon responses are also inhibited by virus-encoded inhibitors (VP35 and VP24). Despite no infection of T- and natural killer (NK) cells, there is extensive apoptosis in those cell types. ECs are activated by pro-inflammatory cytokines and virus, which leads to increased permeability. TF expression induces coagulopathy, which is also able to increase inflammation.

necessary in extravasation of immune cells in inflamed tissue. Although these pro-inflammatory cytokines are an integral part of a normal, localized immune response by attracting and activating immune cells to the site of infection, in the context of a systemic and extensive response, they have a negative effect by inducing shock. ECs also serve as target cells and infection results in activation as indicated by upregulation of adhesion molecule expression followed by cytolysis. However, *in vivo* EC destruction can only be observed at the end stage of the disease.

Infection of Mø also results in the expression of tissue factor (TF), which can impair the anticoagulant–protein-C pathway by downmodulating thrombomodulin. TF is believed to be an important protein in the development of disseminated intravascular coagulopathy (DIC), as inhibition of TF delays death in NHPs and even protects 33% of the infected animals. TNF-α can also induce TF expression in ECs, which would further amplify coagulopathy and DIC. Coagulopathy is known to enhance inflammation, and this could lead to a vicious cycle where inflammation enhances coagulopathy, which in turn would amplify inflammation.

Rapid systemic viral replication is an integral part of the pathogenesis, as infection and even binding of particles results in not only Mø activation but also DC impairment. ZEBOV infection in humans and NHPs results in rapid massive viremia and high titers in various organs, especially spleen and liver. This correlates with an impaired innate and adaptive immune response. However, high viremia and lethality are only seen in guinea pigs and mice after host adaption or infection of various immune-deficient mice, suggesting that evasion of immune responses plays a pivotal role in pathogenesis. Mice lacking the IFN-α/β receptor or STAT1, the main signaling molecule in response to type I IFN, are extremely susceptible to ZEBOV. Treatment of NHPs with IFN-α did not protect the animals but delayed death, suggesting that type I IFN may be an important innate molecule in delaying viral replication. Interestingly, MA-ZEBOV is less sensitive to IFN-α treatment in murine macrophages compared to wild-type ZEBOV, and analysis of genomic mutations in GA- and MA-ZEBOV indicated that mutations in NP and VP24 are sufficient for a lethal phenotype. VP24 inhibits responses to interferon *in vitro*, but it is not clear yet if the mutations in the adapted strains are required for type I IFN evasion or other functions. ZEBOV is also able to inhibit IFN gene induction through VP35, and deletion of the region in VP35 involved in IFN antagonism results in a highly attenuated virus. Furthermore, comparative *in vitro* gene microarray analysis demonstrated a correlation between cytotoxicity and IFN antagonism, which was strongest with ZEBOV and weakest with REBOV.

Adaptive immune responses are also impaired during infection. As mentioned above, upregulation of co-stimulatory molecules is inhibited in DCs, which reduces activation of T cells. There is also a dramatic drop in number of T and natural killer (NK) cells despite the fact that lymphocytes do not support ZEBOV replication. It is believed that lymphopenia is caused by 'bystander' apoptosis, although the mechanism is not well understood. Activation of T cells could also be inhibited by treatment *in vitro* with a 17-amino-acid peptide present in GP_2, which has homology to a known immunosuppressive peptide in the retroviral Gag protein; yet there is no evidence that the complete ZEBOV GP has the same capabilities.

Treatment

The current treatment of EHF is strictly supportive, involving fluid and electrolyte replenishment and pain reduction. Due to the remote location of the outbreaks and limited resources available in the affected regions, treatment options have not been tested in patients. Several experimental treatment strategies have been successful in the rodent models, but failed in the NHP model, which is considered the most accurate in modeling human disease.

Therapeutic antibodies are still considered a short-term solution despite varying success in animal models and humans. Convalescent serum was used in a limited number of patients during the Kikwit 1995 ZEBOV outbreak but the success is a matter of dispute. Passive immunization with hyperimmune horse serum resulted in protection of hamadryl baboons, whereas it only delayed death in cynomolgus macaques. Monoclonal antibody treatment is successful in rodent models but has failed in preliminary NHP studies.

Currently the most feasible and promising approach relates to interference with coagulation using the recombinant nematode anticoagulant protein c2 (rNAPc2). Administration of the drug, which is already in clincial trials for other applications, as late as 24 h post infection resulted in 33% survival in the rhesus macaque model. Even more potent seems to be post-exposure treatment with a recombinant vesicular stomatitis virus (VSV) expressing the ZEBOV GP, which resulted in 50% protection when given 30 min post infection, but the mechanism of post-exposure protection is not yet understood. It is expected that approval of this attenuated replication-component vector will be difficult.

The recent advances in the understanding of EBOV pathogenesis and replication will open new avenues for intervention therapy. Novel antiviral strategies such as viral gene silencing through specific siRNA, cathepsin inhibition, and functional domain interference with small peptides showed promise in tissue culture and partially also in rodent models. Strategies targeting host responses are important alternative options and include anticytokine therapy and modulation of coagulation pathways. It should be noted that more classical approaches such as ribavirin treatment, which has been successfully used to treat other VHFs, are not indicated for EHF.

Vaccines

There are no approved vaccines against EBOV. Similar to therapeutic approaches, many initial vaccination strategies were successful in rodents but failed in NHPs. Nevertheless, there are several promising experimental strategies. Nonreplicating adenoviruses expressing the ZEBOV GP and NP were able to induce sterile immunity in cynomolgus macaques within 28 days or more post vaccination, either alone in a single shot approach or in combination with DNA priming and adenovirus boost. Although DNA priming is not necessary for protection of NHPs, it may be required to overcome the problem of preexisting immunity to human adenoviruses. DNA vaccination alone against EBOV is already in Phase I clinical trial, where it was shown to be safe and effective in inducing humoral and cellular immune responses.

Recombinant VSV expressing ZEBOV GP also induced sterile immunity when given 28 days prior to challenge of cynomolgus macaques, but was also able to protect 50% of rhesus macaques when administered 30 min post challenge. These results, together with the fact that prior immunity against the vector is extremely low, and the only target of neutralizing antibodies, VSV G protein, is removed from the vaccine vector, indicate that this platform may be successful in humans if licensing for the replication-competent vector can be achieved. Human parainfluenza virus-based vectors and VLPs are successful in protecting rodents, and preliminary results suggest that they even show efficacy in NHPs. Safety testing of vaccine vectors and the establishment of immune correlates is a priority for all these and future vaccine platforms.

Note: Investigation of an ongoing hemorrhagic fever outbreak in southwestern Uganda revealed what appears to be an additional distinct species of Ebola virus associated with this outbreak. Preliminary genome sequence analysis suggests the most closely related Ebola virus species would be the *Côte d'Ivoire ebolavirus*. Initial outbreak investigations suggest that infection with this newly discovered virus is associated with a lower case fatality than infections with Zaire ebolavirus (60–90%) or Sudan ebolavirus (50–60%) (T. G. Ksiazek, Centers for Disease Control and Prevention, Atlanta, GA, United States, personal communication).

Acknowledgments

The Public Health Agency of Canada (PHAC), Canadian Institutes of Health Research (CIHR), and CBRNE (Chemical, Biological, Radiological & Nuclear) Research and Technology Initiative (CRTI), Canada, supported work on filoviruses at the National Microbiology Laboratory of the Public Health Agency of Canada.

See also: Marburg Virus.

Further Reading

Feldmann H, Geisbert TW, Jahrling PB, *et al.* (2005) *Filoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 645–653. San Diego, CA: Elsevier Academic Press.

Feldmann H, Jones SM, Klenk HD, and Schnittler HJ (2003) Ebola virus: From discovery to vaccine. *Nature Reviews Immunology* 3: 677–685.

Feldmann H, Jones SM, Schnittler HJ, and Geisbert TW (2005) Therapy and prophylaxis of Ebola virus infections. *Current Opinion in Investigational Drugs* 6: 823–830.

Geisbert TW and Hensley LE (2004) Ebola virus: New insights into disease aetiopathology and possible therapeutic interventions. *Expert Reviews in Molecular Medicine* 6: 1–24.

Geisbert TW and Jahrling PB (2004) Exotic emerging viral diseases: Progress and challenges. *Nature Medicine* 10(supplement 12): S110–S121.

Hensley LE, Jones SM, Feldmann H, Jahrling PB, and Geisbert TW (2005) Ebola and Marburg viruses: Pathogenesis and development of countermeasures. *Current Molecular Medicine* 5: 761–772.

Hirsch M (ed.) (1999) *Journal of Infectious Diseases* 179(supplementum 1): S1–S288.

Jasenosky LD and Kawaoka Y (2004) Filovirus budding. *Virus Research* 106: 181–188.

Paragas J and Geibert TW (2006) Development of treatment strategies to combat Ebola and Marburg viruses. *Expert Reviews of Anti-Infective Therapy* 4: 67–76.

Pattyn SR (ed.) (1978) *Ebola Virus Hemorrhagic Fever.* Amsterdam: Elsevier/North-Holland Biomedical Press.

Reed DS and Mohamadzadeh M (2007) Status and challenges of filovirus vaccines. *Vaccine* 25: 1923–1934.

Sanchez A, Geisbert TW, and Feldmann H (2007) Marburg and Ebola viruses. In: Knipe DM, Howley PM Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1409–1448. Philadelphia: Kluwer/Lippincott Williams and Wilkins.

Walsh PD, Abernethy KA, Bermejo M, *et al.* (2003) Catastrophic ape decline in western equatorial Africa. *Nature* 422: 611–614.

Zaki SR and Goldsmith CS (1999) Pathologic features of filovirus infections in humans. *Current Topics in Microbiology and Immunology* 235: 97–116.

Echoviruses

T Hyypiä, University of Turku, Turku, Finland

© 2008 Elsevier Ltd. All rights reserved.

History and Classification

Echoviruses belong to the species *Human enterovirus B* (HEV-B), in the genus *Enterovirus* of the family *Picornaviridae*. Original classification of picornaviruses was based mainly on physicochemical properties and pathogenesis in experimental animals. At the beginning of the twentieth century, poliomyelitis was transmitted to

monkeys using a filterable agent from clinical patients and 40 years later, new related human picornaviruses (coxsackieviruses), that characteristically caused disease in newborn mice, were isolated. Based on the disease signs observed in mice they were classified into A and B subgroups. After introduction of tissue cultures for virus propagation, a number of new viruses were isolated that exhibited similar physicochemical characteristics (resistance to organic solvents and low pH) with polio- and coxsackieviruses, but grew exclusively in cell culture. Since the disease association of these viruses was at that time unclear, they were grouped among ECHO viruses, which stands for enteric (isolated mainly from stool samples), cytopathogenic (cytopathic effect observed in tissue culture), human (no disease in monkeys or in newborn mice), orphan (disease association not confirmed). There are 28 distinct echovirus serotypes (EV1–33); EV1 and EV8 represent the same serotype, serotype 10 was identified to be a reovirus, serotype 28 was reclassified as a rhinovirus, and serotypes 22 and 23 were reclassified as members of a separate picornavirus genus, *Parechoviridae* (**Table 1**). Polioviruses, coxsackieviruses, and echoviruses formed human enteroviruses. Later, new enterovirus serotypes were not classified into the subgroups but were given successive numbers in the order of their identification (enteroviruses 68–71).

When sequence analysis became available, it was possible to determine the genetic relatedness of individual virus serotypes and strains. It was shown that echoviruses form a genetically rather coherent cluster and they are closely related to certain other enteroviruses. Molecular criteria replaced the previous enterovirus subgroup division in classification and currently echoviruses belong to the HEV-B species together with coxsackie B virus 1–6, coxsackievirus A9, and enterovirus 69 serotypes. More recently, identification of new enterovirus types has been exclusively based on comparison of the VP1 gene sequences, which correlate well with the previous serotype division. Currently, there are approximately 100 distinct enterovirus types, 63 of which have been assigned to serotypes. Further studies of the new members of the genus will show how many of them share biological properties with the classical echovirus serotypes.

Table 1 Summary of the molecular and clinical characteristics of echoviruses

Family	*Picornaviridae*
Genus	*Enterovirus*
Species	*Human enterovirus B*
Serotypes	1–7, 9, 11–21, 24–27, 29–33
Virus capsid	Nonenveloped, icosahedral, contains 60 copies of each of the capsid proteins VP1–4
Genome	Single-stranded, infectious RNA, approximately 7400 nt
Viral proteins	Cap-independent translation utilizing the internal ribosome entry site; translated as a polyprotein (2200 amino acids); the capsid proteins are at the N-terminus and the C-terminal part of the polyprotein contains the nonstructural proteins (2A, 2B, 2C, 3A, 3B, 3C, and 3D)
Receptors	
Most echoviruses	Decay-accelerating factor (CD55)
Echovirus 1	$\alpha 2\beta 1$ integrin
Echovirus 9 (Barty)	αV integrins
Entry route	Endocytosis
Replication	Viral RNA synthesis takes place on cytoplasmic membranes
Clinical manifestations	Meningitis, encephalitis, generalized infections of newborns, myocarditis, rashes, respiratory infections, severe infections in immunocompromised patients
Diagnosis	RT-PCR, virus isolation, serology

Molecular Characteristics and Replication

Virus Particle

In general, echoviruses share the basic properties of other representatives of the enterovirus genus, where polioviruses are considered as the type members. Enteroviruses are composed of a protein capsid consisting of 60 copies of each of the four structural proteins (VP1–4) and enclosing a single-stranded infectious RNA genome, approximately 7400 nt in length. The diameter of the icosahedral virus particle is around 30 nm and the RNA genome represents about 30% of the molecular mass of the virion. VP1–3 are exposed on the surface of the virus particle and they all share the eight-stranded antiparallel β-barrel structure. These capsid proteins are responsible for the recognition of the cellular receptors and they contain the neutralizing B-cell epitopes, located in the variable loops connecting the β-strands and in the C-termini of the proteins. VP4 is located inside the virion and it contains a myristic acid, covalently linked to the N-terminus. Five VP1 molecules form a star-shaped structure around the fivefold symmetry axis that is surrounded by a surface depression (a canyon), while VP2 and VP3 molecules alternate around the threefold axis. The structural subunits are a protomer, composed of one copy of each of the capsid proteins and a pentamer including five protomers. The three-dimensional structure of at least two echovirus serotypes (EV1 and EV11) have been determined by X-ray crystallography and some additional serotypes have also been studied by cryoelectron microscopy. In general, the detailed architecture of these echoviruses closely resembles that of other enteroviruses. Structural, immunological, and other biological

reasons for the existence of almost 30 distinct echovirus serotypes in contrast to three polioviruses are currently not known.

The organization of the echovirus genome is analogous to other enteroviruses and the functions of the proteins have been mainly predicted on the basis of the findings reported for polioviruses. The capsid proteins are located at the N-terminal part (P1 region) of the polyprotein encoded by the viral RNA and the nonstructural proteins, necessary for replication, are at the C-terminal part (P2 and P3 regions). The 5′ noncoding region (NCR) contains around 750 nt, and it includes the structured elements important in translation (internal ribosome entry site, IRES) and genome replication. A small viral protein (3B, also known as VPg) is covalently linked to the 5′ terminus of the genome. The length of the 3′ NCR, also containing predicted secondary structures, is approximately 100 nt and it is followed by a poly(A) tract. The variation in the lengths of all echovirus genomes is in the range of 100 nt. The maximum difference between the 5′ NCR sequences is less than 25%; the capsid protein genes are rather variable (around 60–80% identity between the amino acid sequences of VP1), and less diversity is found at the nonstructural region (80–100% amino acid identity).

Replication Cycle

In most picornaviruses, the interaction with a specific cellular receptor takes place through structures located on the bottom of the canyon with the terminal domains of immunoglobulin (Ig) superfamily molecules. This interaction leads to conformational changes which give rise to further uncoating and release of the genome into the cytoplasm. Instead, many echoviruses attach to decay-accelerating factor (DAF, CD55) that belongs to a family of regulatory proteins protecting the cells from autologous complement-mediated lysis. DAF contains four short consensus repeat domains and is anchored to the surface of the plasma membrane. DAF-binding echoviruses interact mainly with the consensus repeat domain 3 but may also recognize binding sites in domains 2 or 4. Interaction of DAF with echoviruses does not lead to similar alterations in the conformation of the virus particle that are observed after interaction of the Ig superfamily member receptors. Structural studies have revealed that the binding site of DAF in echovirus 7 is located in the VP2 protein close to the twofold symmetry axis in contrast to the binding site of the Ig superfamily molecules in the canyon. DAF is also recognized by certain coxsackie A and B viruses but the binding mechanisms are different and the conformational changes in these viruses are induced by subsequent interaction with members of the Ig superfamily molecules. Therefore, it is thought that additional cellular factors are also needed for successful internalization and uncoating of the DAF-binding echoviruses. Candidate molecules include β2-microglobulin, complement control protein CD59, and heparan sulfate.

Sequence analysis of the mouse-pathogenic echovirus 9 Barty strain revealed that there was a 10-amino-acid-long insert to the C-terminal end of the VP1 protein when compared to the Hill strain of the same serotype which does not cause disease in mice. This insert contains an arginine-glycine-aspartic acid (RGD) motif, known to interact with αV integrins, and this sequence has also been found in clinical isolates. Interestingly, coxsackievirus A9, another mouse-pathogenic member of the HEV-B species, contains a functional RGD motif in a similar position and recognizes αV integrins.

Echovirus 1 is the only picornavirus that is known to interact with α2β1 integrin (a collagen receptor) on the cell surface. The virus binds to the I (inserted) domain in the α2 subunit which is also recognized by collagen but there are remarkable differences between these interactions. The binding site has been localized to the outer wall of the canyon. As in the case of DAF interaction with other echoviruses, binding of the α2I-domain to EV1 does not bring about the conformational changes needed for uncoating *in vitro*. Attachment of the virus to the integrin gives rise to clustering of the receptor molecules which is known to be able to induce signaling events which may play an important role in the subsequent internalization processes.

Entry of echoviruses has been studied in serotypes 1 and 11. The integrin interaction of EV1 is followed by endocytosis of the virus–receptor complex into cytoplasmic structures which are rich in caveolin-1. This internalization process is initiated in lipid rafts and, subsequently, the virus entry can occur either in caveolae or through a faster route into caveosomes, which are preexisting intracellular organelles. How the EV1 genome is released from the caveosomes to the cytoplasm, is currently not known. Echovirus 11 appears to use similar mechanisms during entry. In the plasma membrane, DAF is present in lipid rafts, which are cholesterol-rich domains, and it has been shown that cholesterol depletion inhibits DAF-mediated echovirus infection. The virus can also be copurified with components found in lipid rafts.

When released to the cytoplasm, the picornavirus genome is infectious and it can act directly as an mRNA encoding a large polyprotein that is subsequently processed to the mature viral polypeptides. Based on similarity echovirus nonstructural proteins with those of poliovirus, viral replication cycles are evidently very similar. The proteolytic cleavages take place as a cascade and some of the intermediate products (2BC, 3AB, and 3CD) exhibit also specific activities. 2A is a protease responsible for the cleavage between the VP1 and 2A proteins and, in addition, it causes shut-off of host-cell protein synthesis

by cleaving a cellular factor needed for cap-dependent translation. Proteases 3C and 3CD are responsible for other proteolytic processing events except the cleavage between VP4 and VP2 which may be autocatalytic.

In the infected cells, the viral nonstructural proteins associate with host-cell membrane compartments and modify these to establish a specialized complex for viral RNA synthesis. In these replication complexes, containing almost all the nonstructural proteins with multiple interactions with each other and viral RNA, the genome is copied through a complementary template to new genomic strands. 3D is an RNA-dependent RNA polymerase responsible for the synthesis of the positive and negative strands. Due to the deficient proof-reading activity of the viral RNA-dependent RNA polymerases, errors are frequently generated during the replication and in picornaviruses every new genome contains approximately one mutation. 3B (VPg), found at the 5′ termini of the RNA strands during replication but not during translation, plays a role in the priming of the RNA synthesis. The role of the 3AB protein is thought to be anchoring of VPg to the membranes for priming. The exact role of the 2B protein in the replication cycle is not known. It increases membrane permeability that may facilitate release of the mature viruses from the cells and it also interferes with cellular protein secretion. The 2C protein contains three motifs found in NTPases/helicases. Moreover, 2C and some other nonstructural proteins are involved in rearrangements of intracellular vesicles during formation of the replication complexes. In the assembly of infectious virions, the protomers form pentamers which can associate to form empty capsids. It is not clear whether the viral genome can be inserted into these particles or if they represent a reservoir of the structural units, finally assembling around viral RNA. The mature viruses are released as a result of the lysis of the cells.

Viral replication has dramatic effects in the host cell, including inhibition of cellular protein synthesis and secretion, interference with RNA synthesis and transport, and activation of other processes that together induce the cytopathic effect. Both apoptotic and anti-apoptotic effects, caused by enterovirus replication in cells, have been reported and also more detailed alterations can be detected. In EV1-infected cells, approximately 2% of the studied genes were induced whereas around 0.5% were downregulated. The activated genes included immediate-early response genes as well as genes involved in apoptotic pathways and cell growth regulation. Viral macromolecule synthesis was also observed to activate protein kinases known to control expression of several cellular genes. Further studies on the events during the infection may reveal cellular activities that are necessary for the infection or play a crucial role in the pathogenesis and they can become new potential targets of chemotherapy.

Clinical Manifestations

In general, the disease pattern caused by echoviruses is highly variable and most of the infections are evidently subclinical like those caused by other human enteroviruses. No specific disease condition can be directly associated with a certain echovirus and different serotypes cause identical clinical illnesses, while the same serotype can have a different clinical outcome in patients during the same epidemic.

Members of HEV-B species are among the most common causes of meningitis that may occur as sporadic cases as well as large epidemics. Typically, the symptoms and signs include fever, headache, nausea, stiffness of the neck, and may be associated with respiratory illness, rash, or myalgia. The manifestations may also exhibit signs of meningoencephalitis. Paralysis is a rare consequence of echovirus infection although some cases have been reported.

In neonates, a severe generalized infection, often clinically indistinguishable from bacterial sepsis, can include meningitis or meningoencephalitis, myocarditis, and hepatitis. A similar syndrome is caused by echoviruses and coxsackie B viruses. The transmission occurs transplacentally or soon after delivery from the mother but can also originate from other infected infants. Immunocompromised patients may develop serious chronic echovirus infections, like meningoencephalitis, occasionally with fatal outcome. These complications occur in individuals with B-cell deficiencies and gammaglobulin has been used for the prevention and treatment of the infections.

Echoviruses are associated with mild respiratory infections (e.g., common cold, bronchiolitis, and herpangina) that cannot be clinically distinguished from illnesses caused by other viral respiratory pathogens. Entroviruses cause maculopapular rashes with similar manifestations caused by other infections. Roseola-like skin manifestations can also occur during enterovirus infections and the 'Boston exanthem', caused by echovirus 16, was the first one of these infections recognized. Although echoviruses and other enteroviruses replicate in the gastrointestinal tract and nausea as well as diarrhea can be associated with the infection, they are not considered as major causative agents of acute viral gastroenteritis. Echoviruses have been isolated from individual cases of conjunctivitis but their role in the disease compared to enteroviruses causing epidemics (coxsackievirus A24 and enterovirus 70) is not important. Epidemics of uveitis, a severe eye infection, caused by echoviruses 11 and 19, have been described in Russia.

Myocarditis is often associated with coxsackie B viruses but other enteroviruses are evidently also responsible for this disease where specific diagnosis is usually difficult. Although neonatal myocarditis can be fatal, the manifestations of the disease in older age groups are often nonspecific, and the cardiac involvement is usually suspected

on the basis of ECG findings and severe symptoms are uncommon. There is also evidence that enteroviruses could have an etiological role in the development of dilated cardiomyopathy.

Members of the HEV-B species have also been associated with the development of type 1 diabetes. This is based largely on seroepidemiological studies but, for instance, echoviruses have also been isolated from prediabetic and diabetic individuals and enterovirus genomes have been detected in the blood of patients with recent onset of diabetes. However, the role of enteroviruses in the process leading to type 1 diabetes remains unknown today. Echoviruses and other enteroviruses have also been suspected to have a role in amyotrophic lateral sclerosis, a chronic progressive neurological disorder, but further studies are required to clarify this association. Involvement of viruses, including enteroviruses, in the chronic fatigue syndrome has also been suspected, but this disease is still poorly characterized and the pathogenesis is not understood. It is known that echoviruses can cause chronic infections in cell culture models and availability of new animal models will further clarify their pathogenesis in the future. Sensitive and specific molecular methods will also cast light in the occurrence of echoviruses in diseases where the etiology is presently unknown.

Based on the observations about the protective effects of the inactivated poliovirus vaccine and appearance of severe chronic enterovirus infections in agammaglobulinemic patients, it is thought that protective immunity in enterovirus infections is mainly antibody mediated. It seems that the response is largely serotype specific and systemic infections may result in life-long immunity. Cell-mediated immunity against enterovirus infections is still rather poorly understood but there is evidence that cross-reactive epitopes between virus serotypes may exist.

Laboratory Diagnosis

Echoviruses can be detected from the cerebrospinal fluid (CSF) and respiratory tract samples during the acute phase of infection, but they may be excreted in stool samples for weeks or even months after the infection. Identification of enteroviruses has been classically based on isolation in cell culture followed by neutralization typing using antiserum pools. Although the advantage of the methods is that it gives information about the serotypes, the problem is the time and labor required. In spite of accelerated protocols, based on immunological staining of the infected cells, the isolation method is of limited use when specific diagnosis is required at the acute phase of the illness.

Introduction of the reverse transcription-polymerase chain reaction (RT-PCR) methods for the molecular detection of human picornaviruses revolutionized rapid diagnosis of the enterovirus infections, in particular those affecting the central nervous system. By this means, it is possible to obtain the results in a few hours which has an important impact in the differential diagnosis and treatment of the illness. Currently used RT-PCR methods take advantage of the conserved regions in the 5′ noncoding region of the genome and do not therefore allow further typing of the detected enteroviruses. However, it has been shown that sequences of the VP1 gene correlate well with the current enterovirus serotypes which makes it possible to obtain more detailed information about the individual virus types. Often amplification of the VP1 sequences, utilizing less well-conserved primers, is not possible directly from the clinical material and requires a cell culture enrichment step. In the future, multiplex RT-PCR and microarray methods may provide us with new tools where the high sensitivity of the molecular methods can be combined with an informative collection of data about the pathogen.

There are no highly sensitive and specific standardized serological assays available for enteroviruses. Neutralization assays are serotype specific, but due to the large number of different viruses, the usage of this method is restricted to epidemiological studies. In-house methods, based on enzyme immonoassays or complement fixation tests, are widely used in diagnostic laboratories. The antigens are usually heat-treated to increase cross-reactivity and synthetic peptides containing conserved sequences are also in use. The presence of enterovirus-specific IgM antibodies or an increase in antibody titers between samples collected at the acute and convalescent phase of the illness are considered as a sign of acute infection.

Epidemiology

Transmission of echoviruses occurs through the respiratory and gastrointestinal routes and the epithelial tissue at these sites is the primary location of replication. This can be followed by viremia and infection of secondary target organs. It is possible that respiratory transmission may predominate in areas with improved hygiene. Several nosocomial outbreaks have been described in neonatal units. In addition to direct contact with infected individuals, the infection can be obtained from contaminated water and food. There is no evidence of animal reservoir for these viruses.

In many studies, echoviruses have been the most commonly isolated enteroviruses and there is an extensive number of reports of outbreaks from different geographical areas during several decades in the literature. Most frequently, the echoviruses have been isolated from children of less than 5 years of age that can be understood for immunological reasons and because of the increased risk of transmission. The occurrence of the infections in this

age group does not necessarily correlate with the appearance of disease, since, for instance, meningitis may be detected more frequently in older children. Approximately half of the isolations originate from infections of the central nervous system and isolation from respiratory and gastrointestinal infections are more rarely used. One of the reasons for this is most probably that in mild infections specific diagnostic procedures are more rare. EV4, EV6, EV7, EV9, EV11, and EV30 have been among the predominating serotypes.

Enterovirus infections occur typically during the late summer and early autumn months in temperate climates while elsewhere, the viruses often appear to circulate through the year. The infections are more common in males and in individuals with lower socioeconomic status. Due to the high number of serotypes and the population immunity, there are considerable differences between the epidemiological periods, and variation from small local outbreaks to widespread epidemics is seen. There is also extensive fluctuation in the occurrence of the serotypes during the years and, for instance, echovirus 30 that has been responsible for large meningitis epidemics worldwide may sometimes represent almost half of the isolations but be absent during another epidemic season.

The epidemiological studies during the twentieth century were based on virus isolation and subsequent typing which provided information about the circulating serotypes. Since the RT-PCR assays only can give information about the presence or absence of enteroviruses in clinical samples, this epidemiological surveillance is significantly changing its character. However, introduction of molecular epidemiological analysis, based on the comparison of genomic sequences, has given new information about the circulation and evolution of the virus strains. The approach has been used in studies of, for example, echovirus 30 and the investigations have shown that there has been one predominant genotype with several distinct lineages circulating in Europe and in Northern America since 1978. These strains are clearly different from those isolated some decades ago and some genetic lineages seem to have completely disappeared during this time.

It has become clear that recombination is a frequent phenomenon in the evolution of enteroviruses. It has been mostly found to occur among the members of the species and in particular in the genome region encoding nonstructural proteins. Since the virus isolates are identified primarily on the basis of the serotype, based on the capsid region sequences, this heterogeneity of the genomes has not previously been observed in the epidemiological studies. Therefore, future studies may give a new picture about the circulating and reemerging genetic lineages. Although it is clear that the nonstructural gene region is evolving rapidly, the rules for the appearance of genetically mosaic strains which become predominant by their superior fitness and other properties remain still unclear. The importance of these events in the generation of new pathogenic strains would be highly important to understand and molecular epidemiological approaches and studies on picornavirus evolution in general will hopefully be able to cast new light on these questions.

Future Perspectives

There are currently no vaccines available against other human picornaviruses except polioviruses and hepatitis A virus. The number of echovirus and other enterovirus serotypes makes vaccine development particularly challenging, since the protection from infection is primarily thought to be mediated by serotype-specific neutralizing antibodies. The most promising chemotherapeutic agent developed against clinical picornavirus infections so far has been pleconaril, a capsid-stabilizing small molecule. It has been successfully used in severe enterovirus infections but due to side effects observed in a treatment study against common cold it is not in general use. Other molecules, based on a similar principle, as well as inhibitors of the virus-specific proteases have also been developed, and they are likely to become tools for the treatment of enterovirus infections in the near future. Since echoviruses, with a few exceptions, do not replicate in experimental animals, testing of antiviral drugs is problematic. However, there are promising results showing that the infection can be successfully transmitted to transgenic mice expressing human receptors for an echovirus.

An interesting application in cancer therapy is the use of naturally occurring or modified viruses. For instance, adenovirus-based systems have been used for this purpose. There are also enteroviruses that have been reported to have such an effect. An example is EV1 shown to exhibit tropism for human ovarian cancer cells.

As illustrated by these few examples, increasing knowledge of echoviruses and their replication is likely to provide us with new tools for the treatment of these infections in the future. New disease associations may also be found by using the improved diagnostic methods. Development of an enterovirus vaccine is a particularly important and demanding goal. Understanding of the cell tropism and detailed multiplication mechanisms of these viruses in the body may also provide us with new tools for the development of tools against clinical illnesses.

Acknowledgments

The original studies carried out in our laboratory were supported by grants from the Academy of Finland and the Sigrid Juselius Foundation.

See also: Coxsackieviruses; Enteric Viruses; Enteroviruses: Human Enteroviruses Numbered 68 and Beyond; Enteroviruses of Animals.

Further Reading

Minor PD and Muir PA (2004) Enteroviruses. In: Zuckerman AJ, Banatwala JE, Pattison JR, Griffiths PD, and Schoub BD (eds.) *Principles and Practice of Clinical Virology*, pp. 467–489. New York: Wiley.

Pallansch MA and Roos RP (2001) Enteroviruses: Polioviruses, coxsackievirus, echoviruses, and newer enteroviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 723–775. Baltimore, MD: Lippincott Williams and Wilkins.

Racaniello VR (2001) *Picornaviridae*:The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 685–722. Lippincott Wiliams & Wilkins.

Rotbart H (2002) Enteroviruses. In: Richman DD, Whitley RJ, and Hayden FG (eds.) *Clinical Virology*, pp. 971–994. Washington, DC: ASM Press.

Semler B and Wimmer E (eds.) (2002) *Molecular Biology of Picornaviruses.* Washington, DC: ASM Press.

Ecology of Viruses Infecting Bacteria

S T Abedon, The Ohio State University, Mansfield, OH, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Adsorption Phage diffusion and attachment to a bacterium.

Burst Release of phage from a bacterium (usually upon lysis). Burst size is the number of phage released per bacterium.

Chronic release The continuous release of phage progeny from infected bacteria in a manner that does not involve bacterial death or infection loss.

Community The species (more than one) found within a given environment.

Ecosystem The sum of the biotic (living community) and abiotic (nonliving) components of a single environment, such as a lake or a field.

Epifluorescent microscopy Microscopic observation of light reemitted from specimen-bound dyes.

Extracellular search The diffusion-mediated movement of a phage virion from the point of release from an infected bacterium through the irreversible attachment of a phage to a susceptible bacterium. The extracellular search for new bacteria to infect may be thwarted by virion decay.

Flow cytometry Technique involving detection of suspended particles (such as bacteria) as they flow through a laser beam.

Latent period The time beginning with phage adsorption to a bacterium and ending with phage release from a bacterium.

Metagenomic sequencing The isolation and sequencing of genomic nucleic acid from whole communities including bacteriophage communities such as those found within a sample volume of seawater.

Population A geographically associated group of interbreeding, potentially interbreeding, or clonally related individuals. A localized grouping of individuals of a single species.

Pseudolysogeny A latent phage infection in which penetration of the phage genome into the bacterial cytoplasm has occurred but subsequent steps towards either productive infection or lysogeny are delayed. Pseudolysogeny is thought to occur primarily when phage infect bacteria that are in a significantly starved state.

Pulsed-field gel electrophoresis A technique for gel separation of large DNA molecules such as the whole genomes of bacteriophage.

Single-step growth A technique involving adsorption of phage to bacteria that is followed by a determination of the timing of phage-progeny release from the now-infected bacteria as well as the total number of phage released per bacterium. The word ‘single’ (or ‘one’) is used to indicate that the released phages are prevented from infecting (or adsorbing to) subsequent bacteria within the experimental vessel.

Spatial structure Environmental impediments to mixing, diffusion, and organism motility such that the present spatial position of an organism serves as a good predictor of future spatial position. In the microbiology laboratory spatial structure is often imposed upon cultures via the addition of agar to growth media.

Introduction

Bacteriophages are viruses of bacteria. An enormous number of bacteriophages are found the world over, on the order of 10^{30} or more. By way of comparison, 10^{30} is approximately the weight of the Sun, in pounds. This chapter presents an overview of the interaction between bacterial viruses and their environments: phage ecology. Phage ecology is a process- or mechanism-centered exploration of how bacteriophages exist in the wild and of their impact there.

Phage Existence within Environments

Phage populations have been documented in numerous environments. With the availability of modern molecular, microscopic, and flow-cytometric techniques, it is relatively easy to obtain direct counts or to molecularly characterize uncultured phage virions obtained from environmental samples. A variety of techniques may also be employed to study viral infection/production (aka, proliferation) within environments, though none of these methods are ideal. Estimations of lysogeny in environments are possible but similarly are not ideal.

Determination of phage numbers by viable (typically plaque; **Figure 1**) count originally dominated phage environmental endeavors. Direct microscopic counts, however, are generally much higher than viable counts, for example, by as much as 10 000-fold. For isolation of viable phage from environmental samples, selective enrichment techniques are often employed. Representative phage (more generally, virus) numbers by direct counts range from 1×10^4 to 2×10^7 per ml seawater. Assuming virion degradation between sampling and enumeration in the above estimations, these numbers are estimated to be as high as $(3–100) \times 10^6$ per ml. Sediment and terrestrial counts can be even higher than phage numbers in open water.

Especially using epifluorescence microscopy, direct counts are accomplished more easily than is either phenotypically characterizing environmental phage or demonstrating phage environmental impact on specific bacteria. Partially making up for these deficiencies, the various molecular approaches – including pulsed-field gel electrophoresis and metagenomic sequencing of phage environmental populations – allow characterization of phage environmental diversity, especially at the level of genotype. While electron microscopy of environmental samples does allow some phenotypic characterization, particularly of virion morphology, none of these approaches are substitutes for phenotypic analysis of individual phage in at least 'phage' pure cultures. In most cases such characterization is not possible because the majority of presumptive phage hosts cannot be cultured in the laboratory.

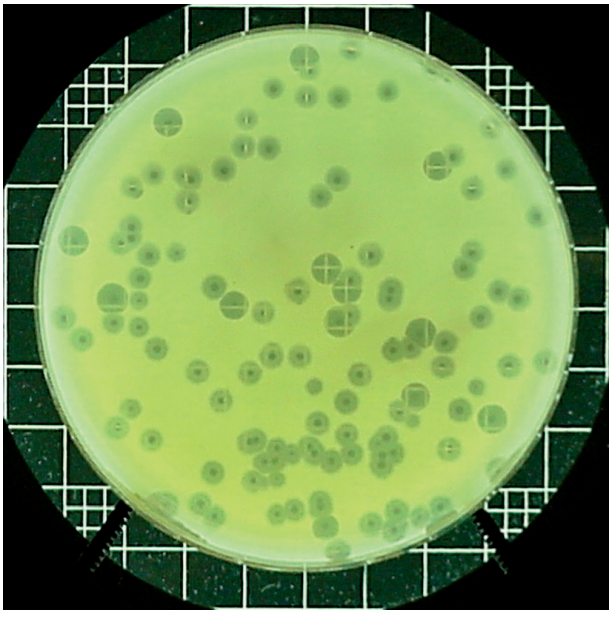


Figure 1 Bacteriophage plaques. Shown are two strains of phage RB69 as grown on the host bacterium *Escherichia coli* CR63. The larger plaques are made by a mutant, called sta5, which displays a significantly shorter latent period than the smaller-plaqued wild type. To accentuate the plaque-size differential, incubations were done at room temperature for 2 days.

Phage Ecology

The science of ecology may be differentiated into a number of more-or-less distinct subdivisions. These include organismal, population, community, ecosystem, and landscape ecologies. Also included, especially under the heading of organismal ecology, are physiological and evolutionary ecologies. Though important to an understanding of ecology in general, in most cases little effort is made within the phage ecology literature to distinguish among these various categories, plus there exist numerous overlaps among these categories. Indeed, phage biologists (including this author) often are trained first as microbiologists (or molecular geneticists, etc.) and only later or much less so as ecologists. As a consequence, phage publications relevant to our understanding of phage ecology typically will not describe their contents from a classically ecological perspective. A major goal of this entry, therefore, will be to expose readers to phage ecological thinking as organized within a more general ecological framework, presented in overview as follows.

Phage organismal ecology considers phage adaptations within environments, with emphasis on phage survival, reproduction, and dissemination. Phage physiological ecology considers the impact of environments on phenotypes, which for phage would be manifested either

through modifications in the physiology of bacterial hosts or in the environmental impact on virion properties. Phage evolutionary ecology considers the selective benefits of phage adaptations especially from an evolutionary perspective.

Phage population ecology considers phage intraspecific interactions, particularly in terms of how those interactions control phage population size, dispersion, and growth rates. Phage community ecology considers phage interactions with other species, especially with bacteria but also with other types of phages as well as with biotic components of the greater bacterial environments, for example, such as in terms of phage-encoded exotoxins which impact the eukaryotic hosts of bacteria. Phage ecosystem ecology considers phage impact on nutrient cycling and energy flow within ecosystems, which by necessity represents a much more abiotic emphasis than other approaches. Phage landscape ecology, which has been little considered at least explicitly within the phage literature, would consider the phage impact on interactions between distinct ecosystems, such as between a pond and a surrounding forest, a sewage treatment plant and downstream environments, or an animal (serving as an ecosystem itself) and the extra-animal environment. Note that these various means of looking at phage ecology build upon one another such that ecosystem ecology builds upon community ecology which builds upon population ecology which builds upon organismal ecology which, finally, is understood best in light of a strong molecular appreciation of phage biology.

Phage Organismal Ecology

Phage organismal ecology may be considered from the perspective of environmental impacts on phage 'growth' parameters, as well as the impact of these parameters on phage ecological success as measured in terms of reproduction, dissemination, and survival. 'Growth' parameters include things such as adsorption rates, infection timing, productive-infection versus lysogeny decisions, burst sizes, and virion decay rates. Since our understanding of phage growth parameters is derived from the laboratory characterization of phages, in this section the author describes this aspect of phage ecology employing a very much laboratory-centered emphasis.

The traditional means of studying most phage growth parameters are single-step growth experiments and phage adsorption determinations. We can fit these growth parameters into a generalized scheme of the phage life cycle, and then consider how variation in the assorted steps of this life cycle can impact phage ecological success. These steps, in order, include (**Figure 2**) (1) a diffusion-driven phage-virion extracellular search for bacteria to infect, (2) the phage adsorption process during which a relatively

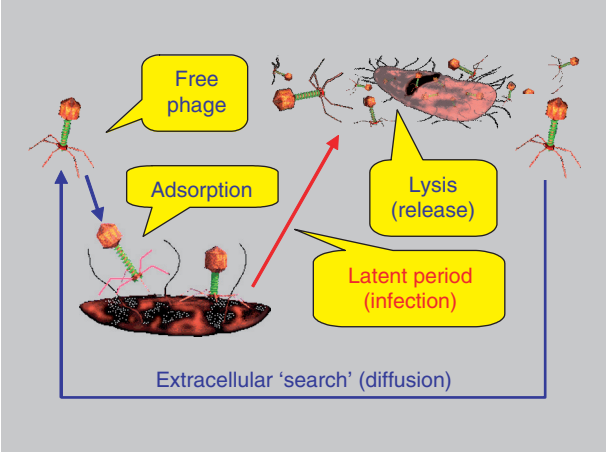


Figure 2 Lytic bacteriophage life history. Images © James A. Sullivan, used with permission.

inert phage-virion and adsorption-susceptible bacterium are together converted into a phage-infected bacterium, (3) the phage infection which can vary in its phenotypic expression from restricted to abortive to productive to pseudolysogenic (or 'carrier state') to lysogenic, and (4) some means of phage-virion release into the extracellular environment. The latter, depending on the phage, may or may not involve host-cell and infection death (i.e., via lysis versus chronic/continuous release, respectively).

Consideration of how variation in these assorted steps can impact phage ecological success is presented in the following sections. For example, we can expect rapid virion adsorption – particularly rapid phage attachment given encounter with a phage-susceptible bacterium – to be favored over less-rapid adsorption unless phenotypes conferring rapid adsorption interfere with phage survival or fecundity. For instance, T4-like phages may enhance their survival within extra-colonic environments by taking on a temporarily adsorption-incompetent but inactivation-resistant state. Another counter to the evolution of more-rapid phage adsorption/attachment may be phage growth within relatively spatially structured environments, if there exist tradeoffs between time spent disseminating versus time spent infecting bacteria. Note that these considerations of phage adsorption rates more generally reflect differences between phage properties 'as virions' versus 'as during bacterial infection', differences which are explored more fully in the following section.

Bacteria-Like versus Virion-Like Modes of Existence

The selective advantage associated with various phage infection and release strategies, similar to phage adsorption rates, will be dependent on environmental conditions, particularly in terms of bacteria numbers, bacteria

physiology, and phage decay rates. In general, we can assume that conditions that favor bacterial growth and survival over phage population growth and survival will tend to favor more bacteria-like modes of phage replication/survival over more phage/virion-like modes; more precisely, we can expect life history biases toward phage existence infecting a bacterium rather than existence as a free virion. Thus, low bacterial densities, bacterial physiologies that poorly support significant phage fecundity, or virion-specific environmental antagonists (including phage-restricting bacteria) will tend to favor extended phage latent periods, once commitment to adsorption has occurred. These infecting states may include pseudolysogeny, lysogeny, or productive but longer-lasting infections, including infections by phage that release their progeny chronically rather than lytically. Alternatively, we can assume that conditions that favor phage population growth will tend to favor more phage-like modes of replication, that is, ones that emphasize the virion state over the infected state. This more virion-emphasizing state can include shorter, productive phage infection – especially lytic infections that favor earlier virion release over higher infection fecundity, but also more-rapid chronic release at the expense of longer-term infection survival.

The advantage of displaying a more phage-like existence under these latter conditions may be viewed from two perspectives. The first is that such conditions should allow for rapid phage-population expansion. Less intuitively, that expansion comes at the expense of bacteria survival, and therefore any phage which persists within a more bacteria-like (extended latent period) mode of infection may be more susceptible to attack by unrelated phages under conditions which are favorable toward phage population expansion (see, e.g., 'Kill the winner', below). Also favoring the virion over the infecting state, a temperate phage can increase its target number and reduce its genomic target size via lysogen induction (i.e., by changing from a single bacterial lysogen to multiple copies of phage). Such induction can occur following bacterial exposure to DNA damaging agents. In considering phage population growth and survival, we thus can envisage phage adaptation within real ecosystems as involving especially a compromise between conflicting tendencies toward greater emphasis on existence as an infection versus greater emphasis toward existence as a virion.

Phage Population Ecology

Considerations of phage population ecology may be differentiated into two categories: (1) the impact of phage adaptations and environmental conditions on phage population growth within well-mixed, fluid environments (such as within broth in the laboratory) and (2) the impact of phage adaptations and environmental conditions on phage population growth within spatially structured environments (such as within soft-agar overlays in the laboratory). These categories will be considerd in turn, emphasizing phage population growth in broth-like environments. Like phage organismal ecology, much of our understanding of phage population ecology derives from laboratory characterization of phage, but also from exercises in modeling phage population growth. We can also distinguish phage population considerations into those operating at low phage densities versus those operating at high phage densities. In both cases density refers to a single phage population since inter-(as opposed to intra-) species interactions are considered under the province of phage community rather than phage population ecology. These latter considerations are much simpler to envisage during phage broth-culture growth.

The growth of phage in broth can be considered to occur over at least four distinct steps. These are as follows:

1. Phage entrance into an environment. This entrance can be physical, in terms of phage movement from one place to another, it can be physiological in terms of the induction of an already-present lysogen, or it can be genetical in terms of phage host-range mutation (or recombination event) such that a previously phage-insusceptible bacterial population suddenly becomes susceptible to sympatric phage.
2. A period of phage-population exponential growth that spans from the point of the initial phage infection of a susceptible bacterium within an environment through to the point of transition whereby most susceptible bacteria have become phage infected.
3. The transition to and then span over which most of the phage-susceptible bacteria within a population are phage infected.
4. A postinfection period during which phage numbers greatly outnumber those of bacteria.

In many cases, each of these steps is at best a transient phenomenon for a given combination of phage, bacterium, and environment. To keep things simple, during this initial discussion assume the least complex of environments: one that is homogeneous, well-mixed, closed (e.g., not a continuous culture), and of finite volume. That is, the equivalent of a shaken or stirred broth-filled flask. Also for the sake of simplicity, assume that bacteria do not replicate nor change physiologically, other than by phage infection, over the course of phage population growth.

In general terms, once a phage virion has been released from an infected bacterium, it has entered into a period of what is described as an extracellular search for new bacteria to infect. The duration of this search will be a function of the product of the density of phage susceptible bacteria within a given environment – which in turn is a function of the range of bacterial types that a given phage

may infect – and the phage adsorption constant, which will vary as a function of phage, bacterium, and environmental parameters. The likelihood that a given phage will successfully adsorb to and infect a bacterium is additionally a function of a phage's decay rate, with higher decay rates reducing the likelihood of successful virion infection. Decay can be a function of abiotic insults that result in virion capsid or nucleic acid damage, phage adsorption to abiotic materials, biotic insults including engulfment by eukaryotes as well as restriction (i.e., death or inactivation) mediated by phage-adsorbed bacteria, or even emigration out of an environment. To a phage entering a previously phage-free environment, the likelihood that phage population growth will occur therefore is a function of bacterial numbers, rates of phage adsorption per bacterium, and rates of phage decay.

If a successful adsorption occurs, the likelihood that phage population growth will continue beyond this first adsorption will additionally be a function of phage burst size, with each additional phage produced per infection essentially possessing an additional, nearly identical likelihood of successful bacterial adsorption. Extending this point, we can assume with either higher bacterial numbers, more-effective/rapid virion adsorption per bacterium, lower rates of decay, or higher initial phage numbers that there will be a greater likelihood that phage population growth will be initiated upon phage entrance into a new environment. The potential for a phage population to grow within a given environment can be described as its existence conditions.

Once phage population growth has begun, it seems unlikely that there would exist any intrinsic inhibitions on phage evolution toward faster population growth. This is not to say that there may not be blocks extrinsic to phage population growth, including various tradeoffs that could exist, such as potential conflicts between, on the one hand, the breadth of a phage's host range (more generally, niche) and, on the other hand, rates of phage population growth on any given host population (which would be a conflict between ecological generalization vs. specialization). However, both in terms of intraspecific and interspecific competition among phages, it is the phage that reaches a bacterium first that should have the greatest potential to 'claim' that bacterium for infection. Thus, a phage that grows its population fastest will have access to more bacteria sooner, and therefore enjoy among its progeny greater overall numbers of bacterial infections within a given environment. In general, we can expect faster overall population growth given greater phage burst sizes, shorter phage latent periods, or faster phage adsorption. Phage decay can also affect rates of phage population growth, and this occurs via reductions in effective phage burst size, where the qualifier 'effective' refers to the number of phage released from a given bacterial cell which go on to successfully infect a new bacterium.

Things change, within a given environment, as a phage population reaches the limits of its population growth. At this point the selective pressures on phage growth change from ones that are phage-density independent to ones which are phage-density dependent. These phage-density-dependent limits vary in their relevance, however, depending on bacterial densities along with phage fecundities. At one extreme, bacterial densities are so low, or phage fecundities (effective burst sizes) so small, that phage populations never reach densities of sufficient size to greatly affect bacterial populations. Under these conditions multiple phage adsorption to a given bacterium is of low likelihood and competition between phage for individual bacteria small. Selection here should be biased toward greater virion or infection durability, for example, rather than higher rates of phage population growth. We have this expectation because bacterial rarity presumably would put a premium on phage survival prior to bacterium encounter and also because phage potential to migrate to new bacteria-containing environments could be a function of longer-term phage survival, either as virions or as bacterial infections.

At the other extreme, bacterial densities as well as phage fecundities may be sufficiently high that a majority of phage-susceptible bacteria may become phage infected and therefore no longer available for further infection by the same phage type. Prior to this point we may expect selection to favor phage that display rapid population growth, even if such rapidity should come at the expense of effective burst size, either in terms of actual burst size or in terms of virion resistance to decay. Thus, at higher bacterial densities a phage could display faster population growth by shortening its latent period. Such shortening, however, is most easily accomplished at the expense of the overall duration of the period during which phage-progeny mature, resulting in a reduced phage burst size. Alternatively, one could envisage a loss of phage genes that otherwise could contribute to long-term virion survival should the presence of those genes come at the expense of effective phage burst size or further latent-period shortening. One possible example of such evolution could be a common loss of temperance among phage contaminating industrial dairy ferments.

Adaptation that results in declines in phage survival could be shortsighted within environments in which densities of new bacteria to infect are in rapid decline. Instead, we can envisage an advantage bestowed upon phage infections during 'final rounds' of infection – that is, at the point where most bacteria within a population have become phage infected – that would result from larger effective burst sizes. In this way more or more robust phages are produced to better assure phage survival until access to new bacterial populations occurs. Somewhat equivalently, a phage may accrue advantage by displaying a more bacteria-like lifestyle under conditions

where bacterial densities are in decline, such as via temperate-phage reduction to lysogeny rather than display of a lytic infection.

Many of these same effects take place during phage population growth within spatially structured environments, such as within bacterial lawns immobilized within soft agar, that is, as phage plaques (**Figure 1**) or as within soil. A difference, though, is that in addition to temporal differentiation between density- and density-dependent selection, there are also spatial differences. Thus, phage multiplicity is higher toward the center of plaques versus toward their periphery. In other words, at a plaque's leading edge, selection acting upon phage characteristics is more density independent than toward the center of plaques. Thus, we can expect selection for faster phage population growth to occur at a plaque's periphery, whereas, by contrast, selection at a plaque's center should be biased more toward a greater effective burst size.

Phage Community Ecology

Phage community ecology includes issues of phage predation on bacteria as well as the establishment of mutualistic-like relationships such as those between prophage and bacteria, forming lysogens. The phage community ecology literature is more closely aligned with the ecological literature, which is to say that a good deal of phage community ecology has been done by individuals trained first as ecologists who have then taken to phage as model systems rather than by individuals trained first by studying more organismal or more molecular aspects of phage biology. As such, phage community ecology has a much more theoretical basis than either phage organismal or population biologies, including the employment of simulations of phage–bacterial interactions, especially within continuous cultures (chemostats). Phage community ecology has a much more environmental emphasis than these other ecologies, as workers consider, especially, the phage impact on bacterial populations. Phage community ecology can also involve consideration of interactions between different phage species or grazing of phage by protists.

We can distill three general governing principles of phage community ecology, especially with regard to phage impact on bacteria: (1) The greater a phage population density then, all else held constant, the greater the phage impact on a bacterial population, and the faster that phage populations reach these higher densities, then the sooner their impact. (2) The greater the density of phage-sensitive bacteria, then, potential changes in bacterial physiology aside, the greater the population size that phage can grow to and the sooner they will grow to that population size. (3) Bacteria can hide in various ways from phage – numerically, genetically, physiologically, spatially – but doing so can come at a cost in terms of, for example, bacterial growth rates.

Real world applications of these concepts include that of 'Kill the winner' where it is expected that populations of same or similar bacterial types, that grow to higher densities, will tend to become more susceptible to phage-mediated population crashes. These crashes result solely from bacteria having reached these higher densities. By culling especially more successful bacterial populations, phage may serve as mediators of a frequency-dependent selection among bacterial communities that results in greater bacterial diversity than environments could otherwise sustain.

A second and related real-world consideration is that bacteria within especially eutrophic (i.e., high-nutrient) environments, where phage virions can be especially abundant, may display physiologies that represent trade-offs between rates of bacteria growth and phage-free survival. That is, bacteria may be subject to physiological and other burdens that come with deploying protective measures. Protective measures are potentially diverse and certainly include things such as restriction-modification systems. Additionally, bacteria may take on more-protective modes of existence such as within extracellular polysaccharides or biofilms.

Bacteria may also display reduced versatility because of loss (temporarily or permanently; fully or partially) of membrane proteins involved, for example, in nutrient transport, proteins which could otherwise serve as phage receptors. That is, the more proteins (or other molecules) a bacterium displays on its surface, the more phage types that bacterium may be susceptible to. Because many of these mechanisms of phage resistance can result in reduced bacterial fitness, we should expect that the strength of selection for phage resistance should be directly proportional to phage density within a given environment. Phage, in turn, can display adaptations that serve to overcome various bacterium-mediated mechanisms of phage resistance. Bacteria can also evolve to mitigate the costs associated with displaying phage resistance.

A third real-world consequence of phage impact on bacteria is as a consequence of phage-mediated transduction of bacterial genetic material. Phage do this by two basic mechanisms: generalized transduction in which bacterial DNA is incorporated into consequently defective phage virions (with essentially equal probability of incorporation across the bacterial chromosome) and specialized transduction, which traditionally involves the incorporation of the bacterial genomic DNA that flanks prophage as a consequence of imprecise prophage excision upon induction. A third means, essentially an extension of the concept of specialization transduction, is via phage 'morons', which involves the incorporation via illegitimate recombination of bacterial genes into various locations within temperate phage, which are then passed

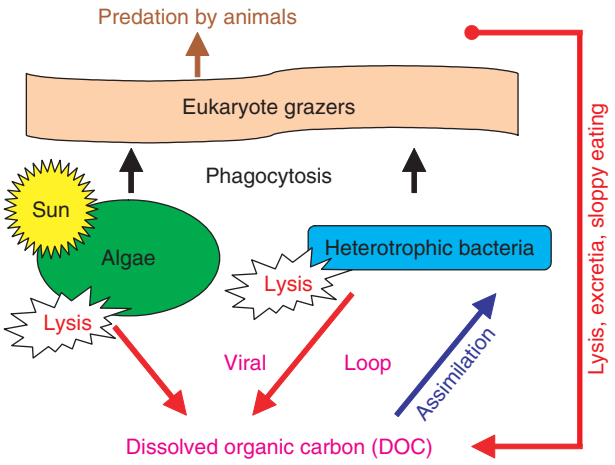


Figure 3 Microbial loop as short circuited by virus-(particularly phage-) induced lysis. The viral loop consists of generation of dissolved organic carbon via virus-induced lysis of especially heterotrophic bacteria and cyanobacteria (the latter here included under the heading, Algae), which is followed by assimilation of a fraction of that material into heterotrophic bacteria. The viral loop reduces the efficiencies by which photosynthetically fixed organic carbon is transferred to higher trophic levels, such as to aquatic animals, via the microbial loop.

on to bacteria via subsequent establishment of lysogeny. These genes, along with prophage as a whole, can impact bacterial phenotype including the fitness of lysogens, the latter at least some of the time for the better. These latter mechanisms of transduction serve as a means of transferring genetic material from bacterium to bacterium while also transferring genetic material from bacterium to phage.

Phage Ecosystem Ecology

Phage ecosystem ecology is more firmly rooted in considerations of phage environmental interactions than in the theory of phage community ecology. The primary emphasis of this field is on the impact of phage infection of prokaryotes on the movement of nutrients and energy from prokaryotes to higher trophic levels. That is, phage infection influences the acquisition of bacteria (including cyanobacteria) by bacteria-eating eukaryotes. The real-world significance of this emphasis derives from prokaryotes serving as the base of many, especially aquatic, ecosystems and the role that these bacteria play in carbon sequestration. That is, within aquatic environments most dissolved organic material becomes available to eukaryote grazers only once bacteria have assimilated it. With phage-induced bacterial lysis, however, a significant fraction, up to approximately 25% of the carbon entering these ecosystems photosynthetically, is converted to a dissolved organic state. This creates a 'viral loop' that shunts organic carbon away from what is known as the aquatic 'microbial loop', which otherwise moves carbon from prokaryotes to grazers (i.e., especially phagotrophic protists) to grazer-consuming animals (**Figure 3**). Another way of stating the significance of the phage impact on the survival of aquatic bacteria is that it is approximately equivalent to the impact of protist grazing. The impact of phage infection versus protist grazing, however, varies with habitat, with phage displaying a greater impact especially in more extreme, including low-oxygen environments.

See also: Epstein–Barr Virus: General Features; Virus Evolution: Bacterial Viruses.

Further Reading

Abedon ST (2006) Phage ecology. In: Calendar R and Abedon ST (eds.) *The Bacteriophages*, pp. 37–64. Oxford: Oxford University Press.
Abedon ST (2007) *Bacteriophage Ecology: Population Growth, Evolution, and Impact of Bacterial Viruses.* Cambridge, UK: Cambridge University Press.
Abedon ST and LeJeune JT (2005) Why bacteriophage encode exotoxins and other virulence factors. *Evolutionary Bioinformatics Online* 1: 97–110.
Abedon ST and Yin J (2008) Bacteriophage plaques: Theory and analysis. In: Clokie M and Kropinski A (eds.) *Bacteriophages: Methods and Protocols*, Totowa, NJ: Humana Press.
Ackermann H-W and DuBow MS (1987) *Viruses of Prokaryotes.* Boca Raton, FL: CRC Press.
Breitbart M, Rohwer F, and Abedon ST (2005) Phage ecology and bacterial pathogenesis. In: Waldor MK, Friedman DI, and Adhya SL (eds.) *Phages: Their Role in Bacterial Pathogenesis and Biotechnology*, pp. 66–91. Washington, DC: ASM Press.
Brüssow H and Kutter E (2005) Phage ecology. In: Kutter E and Sulakvelidze A (eds.) *Bacteriophages: Biology and Application*, pp. 129–164. Boca Raton, FL: CRC Press.
Carlson K (2005) Working with bacteriophages: Common techniques and methodological approaches. In: Kutter E and Sulakvelidze A (eds.) *Bacteriophages: Biology and Application*, pp. 437–494. Boca Raton, FL: CRC Press.
Chibani-Chennoufi S, Bruttin A, Dillmann ML, *et al.* (2004) Phage-host interaction: An ecological perspective. *Journal of Bacteriology* 186: 3677–3686.
Goyal SM, Gerba CP, and Bitton G (1987) *Phage Ecology.* Boca Raton, FL: CRC Press.
Lenski RE (1988) Dynamics of interactions between bacteria and virulent bacteriophage. *Advances in Microbial Ecology* 10: 1–44.
Levin BR and Lenski RE (1985) Bacteria and phage: A model system for the study of the ecology and co-evolution of hosts and parasites. In: Rollinson D and Anderson RM (eds.) *Ecology and Genetics of Host-Parasite Interactions*, pp. 227–242. London: Academic Press.
Paul JH and Kellogg CA (2000) Ecology of bacteriophages in nature. In: Hurst CJ (ed.) *Viral Ecology*, pp. 211–246. San Diego: Academic Press.
Weinbauer MG (2004) Ecology of Prokaryotic Viruses. *FEMS Microbiological Reviews* 28: 127–181.
Wommack KE and Colwell RR (2000) Virioplankton: Viruses in aquatic ecosystems. *Microbiology and Molecular Biology Reviews* 64: 69–114.

Relevant Website

http://www.cellsalive.com – Cells alive.

Electron Microscopy of Viruses

G Schoehn and R W H Ruigrok, CNRS, Grenoble, France

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Most viruses are too small to be observed with light microscopy and many are too large or too irregular to crystallize. Therefore, electron microscopy (EM) is the method of choice for the direct visualization of viruses and viral proteins or subviral particles. However, electrons do not travel far in air and therefore the inside of the microscope has to be under very high vacuum (10^{-5} to 10^{-8} torr). This implies that biological samples have to be dehydrated and/or fixed. It is important that the biological sample is stabilized (or fixed) so that its ultrastructure remains as close as possible to that in the biologically active material when exposed to the vacuum.

The penetrating power of electrons in biological material is also very limited, which means that the specimens must either be very thin or must be sliced into thin sections (50–100 nm) to allow electrons to pass through. This is not a real problem for the imaging of viruses because the sizes of viruses are usually in this range but if we want to study viruses interacting with cells during infection or replication, sectioning is necessary.

Contrast in the transmission electron microscope basically depends on the atomic number of the atoms in the specimen; the higher the atomic number, the more electrons are scattered and the larger the contrast. Biological molecules are composed of atoms of very low atomic number (carbon, hydrogen, nitrogen, phosphorus, and sulfur). Staining methods using heavy metal salts or image analysis have to be used to enhance the contrast in electron micrographs.

In order to make this article easier to read we will illustrate the various EM-preparation techniques with images of intact adenovirus particles, individual capsid proteins or protein complexes from adenoviruses. Adenoviruses are double-stranded DNA viruses with an icosahedral capsid with a pseudo T number of 25 and a total protein mass of around 125 MDa. The major capsid components are the hexon, a trimeric protein with a basal hexagonal shape, and the penton that is a noncovalent complex between the pentameric penton base and the trimeric fiber protein. Two hundred and forty hexons form the 20 facets of the icosahedron, whereas the pentons form and project from the 12 vertices. The fiber binds to host cell receptors with its C-terminal knob domain whereas protein loops that extend from the penton base and that contain an Arg-Gly-Asp (RGD) sequence are required to bind to a secondary cell receptor to trigger endocytosis of the virus. The capsid also contains minor components, proteins IIIa, VI, VIII and IX that glue the hexons and pentons together at specific positions in the capsid. For a schematic drawing of the virus, see **Figure 1(a)**.

Techniques for Single Particle Imaging

Before any sample preparation takes place, one should be informed about the nature of the sample: its molecular weight, some information on its physical size, and the chemical nature of the components (proteins, nucleic

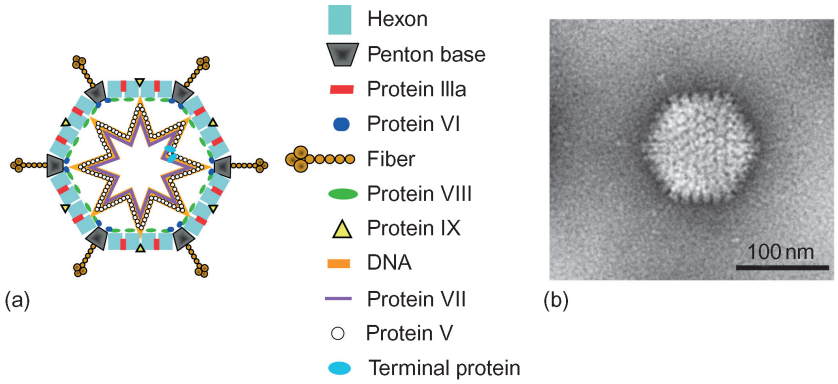


Figure 1 The adenovirus particle. (a) Schematic view of an adenovirus particle. A schematic view through a sliced adenovirus showing the different structural proteins of the virus. The adenovirus particle is an icosahedral particle with on each vertex a noncovalent complex between a trimeric fibre and a pentameric base. This complex is called the penton. (b) Electron micrograph of an adenovirus particle negatively stained with a 1% solution of ammonium molybdate pH 7.5. The particle is seen in the same orientation as in (a). The approximate molecular weight of an adenovirus is 150 MDa and the size of the particle is 1000 Å in diameter.

acids, lipid membranes). Further, it is important to verify by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) whether the protein(s) in the sample is (are) intact and not totally or partially degraded. Information on possible contamination of protein samples with nucleic acids can be obtained by measuring the optical density spectrum at 220–350 nm. Such a spectrum can also give information on whether a protein sample contains aggregated protein since a significant absorption at wavelengths above 300 nm is then observed. Such controls are important because the imaging techniques that are described here will always lead to a picture. However, one wants to know how the picture relates to the active biological sample.

Negative Staining

For negative staining the sample is placed on a support film, bathed in a solution of a heavy metal salt that is subsequently blotted, and the sample is then left to dry in air (**Figure 2**). The stain will dry around the molecules, and the area where the molecules happen to be will appear light and the space around it dark, that is, opaque for electrons. As support films one can use plastic films, commercially available or home-made, but these films tend to become hydrophobic after some time and need to be cleaned first with a plasma cleaner. Plastic films are mechanically resistant but are often rather thick and give a high background noise in the images. Thin carbon films give better results. These films are evaporated onto the surface of freshly cleaved mica by heating carbon at high temperatures under vacuum.

For sample preparation it is important to know the concentration of the sample. For small molecules like isolated capsomer proteins, a concentration between 0.05 and 0.1 mg ml^{-1} often gives the best results. When the concentration is too low the stain will not be spread out evenly on the support film and if the concentration is too high the molecules will be one on top of the other and the picture will be difficult to interpret. For intact virus particles such as adenoviruses and influenza viruses, a concentration of 1 mg ml^{-1} should be used. Using a drawn-out glass Pasteur pipette (the sample is taken up into the pipette by capillary force) 1–2 μl of the sample is touched at the carbon–mica interface of a small piece of precut carbon on mica (**Figure 2(a)**). The surface of the carbon on the outside is hydrophobic but at the interface the carbon is clean and hydrophilic and the sample is sucked in between the carbon and mica layers. Then the carbon film with the adsorbed sample is floated off in a pool of negative-stain (usually uranyl acetate (UA), ammonium molybdate, or sodium silicotungstate, SST) and a copper EM grid is placed on the top of the floating film. The grid plus carbon film is then picked up by placing a small piece of paper on the grid and, as the paper wets, it is lifted up taking grid and carbon film up from the pool of stain. In this way the edges of the carbon film that stick out over the rim of the copper grid do not fold back onto the bottom of the grid, creating carbon layers at both sides of the grid with an inclusion of stain. The paper-grid-carbon-sample sandwich is placed on filter paper to blot and air-dry for at least 5 min before insertion into the column of an electron microscope (**Figure 2(a)**).

Different heavy atom salts that are available commercially can be used for negative staining. However, chemical reactions between the salt and the specimen are possible. When the salt binds specifically to the sample molecules one may obtain positive staining rather than negative staining. With positive staining one does not obtain an image of a clear sample molecule on a dark background but a dark molecule on a clear background. It should also be taken into consideration that some salts for staining might have non-neutral pH values. Finally, stain molecules have defined sizes and the resolution of the image may be limited by the grain size of the stain molecules.

Uranyl acetate has a low pH (pH 4.4) and is known to cross-link and stabilize fragile macromolecular assemblies. This stain is especially useful for enveloped viruses since the binding of uranyl ions to the negatively charged lipid heads stabilizes the membranes. This stain does not stay close to the support film but will also stain the top of virus particles so that one obtains a superimposed image of the surface features that touch the support film plus the features on the opposite side of the virus particle. It is not such a good stain for thin objects that lie flat on the support film because the contrast is reduced by the stain lying on top of the object. In **Figure 2** the molecules in plate C (right) were stained with uranyl acetate. Note that uranyl acetate, because of its ability to chemically react with proteins, lipids, and nucleic acids, is known to cause positive staining as well as negative staining. One serious problem with this salt is that it is no longer available in some countries since even nonenriched uranium may be considered to be of military importance. Another problem is that it is slightly radioactive. Sodium silicotungstate is a chemically inert stain with a neutral pH. The stain stays very close to the support film and outlines only those features of a virus or protein that are in contact with the film and it is therefore often used to stain small proteins. The size of the stain molecule is rather large (i.e., 10 Å in diameter) and this feature may limit the resolution of image reconstructions. The stain is often sold as silicotungstic acid. The acid needs to be solubilized in water, neutralized with NaOH and the salt is precipitated with cold ethanol. In **Figure 2** plates (b), (c) (left), and (d) were stained with SST. This stain allows visualization (and sometimes determination of their oligomeric state) of proteins as small as 60 kDa (**Figure 2(d)**).

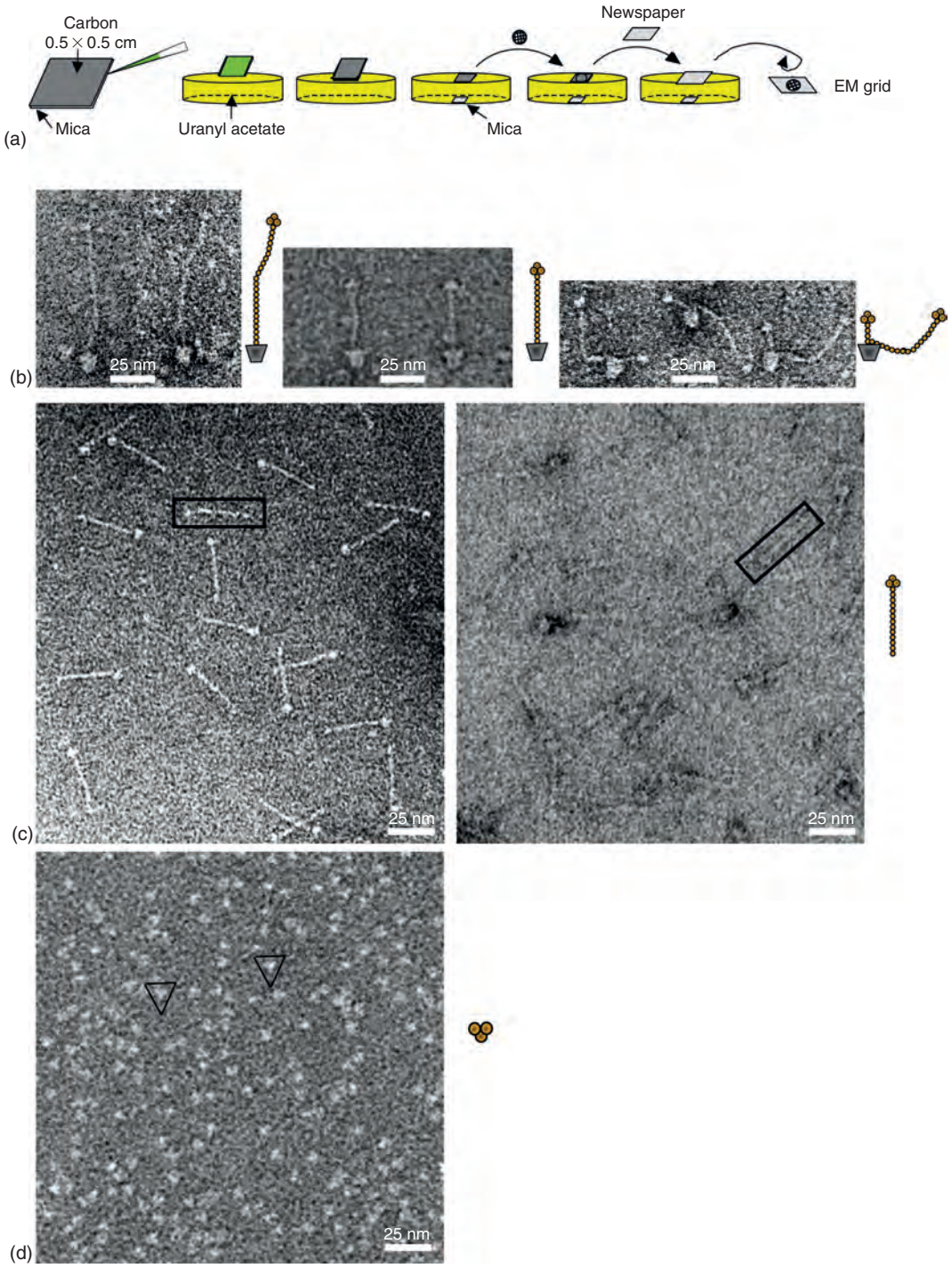


Figure 2 Negative staining technique and results. (a) The different steps of the negative staining method: the sample is adsorbed onto the carbon support film by touching a pipette with the sample to the mica and carbon interface. Then the sample/carbon is floated off the mica onto the surface of a solution of a heavy atom salt. An EM grid is placed on the surface of the carbon and then a piece of paper is used to pick up the grid with carbon film. The paper with grid, carbon, and sample is blotted on filter paper and the grid is then air-dried before observation in the microscope. (b) Electron micrographs of different adenovirus pentons negatively stained with sodium silicotungstate. From left to right one can see a bovine adenovirus 3 penton base (very long and bent fiber), a human adenovirus 41 penton with the long fiber, and an avian adenovirus penton (one base with two different fibers sticking out). The penton base is around 300 kDa is size, whereas the fiber is between 120 and 350 kDa. (c) Electron micrograph of an isolated adenovirus 2 fiber stained with sodium silicotungstate (left) and uranyl acetate (right). In each case, a single fiber is highlighted by a black rectangle. The molecular weight of one fiber is 190 kDa. (d) Electron micrograph of a recombinant canine adenovirus fiber-head expressed in *Escherichia coli*, stained with sodium silicotungstate. The molecular weight of the trimeric particle is around 60 kDa and it is sometimes possible to recognize the triangular shape of the particle (black triangles).

Phosphotungstic acid (PTA) can be used at pH values between 5 and 7. The staining characteristics are similar to those of SST although PTA often does not spread out well during air-drying and can remain in thick puddles. The stain in these puddles is so thick that protein structures cannot be visualized. This stain can be rather destabilizing for lipid membranes and enveloped viruses often show membrane blebs that are squeezed out of the regular lipid envelope during air-drying.

Ammonium molybdate has a neutral pH. During drying, the stain stays quite high on the support film. It is therefore especially useful for the staining of large particles (**Figure 1(b)**). This stain can be used for cryo-negative staining when very high stain concentrations (16%) are used. The preparation of the grids is as described below for regular cryo-microscopy. The advantage over regular cryo-microscopy is the much higher contrast. The disadvantage is that of all negative staining techniques: only the outside of the particles is visualized by negative staining without any information on the structures inside the virus particles.

Advantages and limitations

With the negative staining technique it is possible to visualize small proteins and sometimes determine their oligomeric state in a very rapid manner. The main limitation of this technique results from the air-drying step. If the sample is fragile, which is the case with enveloped viruses, some flattening can occur during drying and lipid blebs can be squeezed out. Finally, the molecular forces that act upon proteins, complexes, or viruses during the adsorption phase onto the support film can also induce conformational changes.

Cryo-Electron Microscopy

In order to overcome the adsorption step, stain artifacts and, most importantly, the drying step, Dr. Jacques Dubochet and collaborators developed a new sample preparation technique: the frozen-hydrated sample imaging or cryo-EM (**Figure 3**). For this technique the sample concentration must be much higher than that for negative staining because there is no direct support film that has a

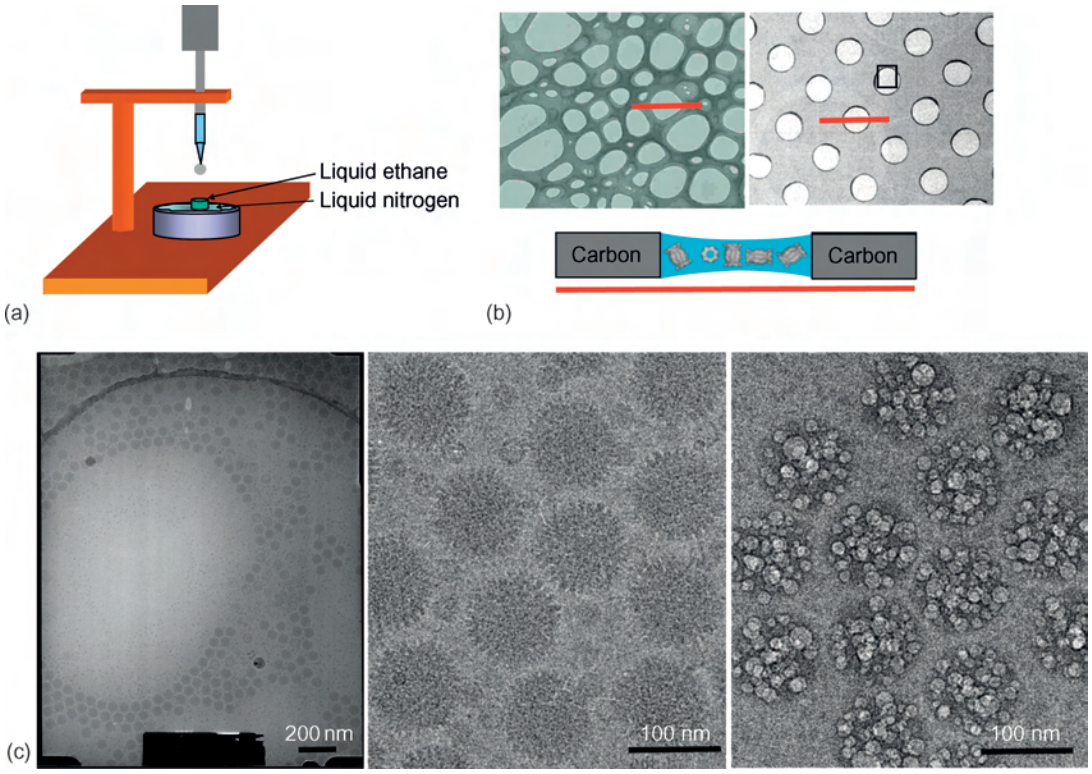


Figure 3 Cryo-electron microscopy (EM) technique and results. (a) Plunger used for rapid freezing of the sample. A grid covered with a holey carbon film is loaded with the sample just before blotting and plunging it very rapidly into liquid ethane cooled down by liquid nitrogen. (b) Examples of home-made holey carbon (left) and commercial quantifoil grid (right). The red bar represents a slice through a hole showing the sample in the frozen vitrified water (bottom). The rectangle on the quantifoil grid represents the portion of field indicated by the red bar above. (c) Cryo-EM images of adenovirus particles. The sample was prepared on a quantifoil film. The picture on the left part is an entire micrograph showing the repartition of the adenovirus particles according to the ice thickness (one part of the hole is totally empty due to the fact that the ice is too thin. The middle part shows a higher magnification of the cryo-EM micrograph. Details are visible on the virus particle; the smaller particles are adenovirus-associated viruses. On the right, the same adenovirus sample is shown after an 1 min exposure to the electron beam. Irradiation damage (bubbles on the picture) is clearly visible.

concentrating effect. The support film for the sample in solution is a carbon film with holes, either home-made (rather irregular, **Figure 3(b)**, left film) or commercially obtained (these films have very regular, evenly spaced holes, **Figure 3(b)**, right film). An aliquot of 2–4 μl of purified sample at a concentration of 0.5–1 mg ml^{-1} is pipetted onto a copper grid covered by the carbon film. Excess liquid is removed by brief blotting with a piece of blotting paper and the grid is then plunged into liquid ethane cooled by a liquid nitrogen bath (**Figure 3(a)**). The rate at which the temperature of the buffer in the holes is lowered is so high that there is no time for the formation of ice crystals; the water forms vitreous ice (also called amorphous ice). Because of the absence of ice crystals, the sample is not deformed but stays in its native state and the vitreous ice is translucent for electrons. The frozen grids have to be transferred into a cryo-holder and inserted into the microscope. The temperature of the sample has to stay very low (i.e., under −160 °C) in order to prevent the vitreous ice to transform into hexagonal or cubic ice. If this irreversible conversion occurs the structure of the sample will be destroyed. All cryo-EM work has to be done at low temperature and the microscope has to be equipped with cryo-EM tools (cryo-holder, anti contaminator blades around the specimen in the microscope in order to avoid the deposition of water and other vapors onto the sample). The ranges of salt concentration and pH that can be used are quite large and include physiological conditions.

Advantages and limitations

Because no heavy atoms are added to the sample, the contrast in the images is very low (**Figure 3(c)**) (the contrast is partly generated by the difference in density between the frozen water and the protein, i.e., 1 g/ml vs. 1.3 g ml^{-1}). This low contrast makes the use of image analysis indispensable. The combination of low contrast and noise also limits the minimum size of the protein/macromolecular assemblies that can be studied by cryo-EM to about 200 kDa. Radiation damage is also a big problem in cryo-EM. As soon as the sample is exposed to the electron beam its starts to be destroyed (**Figure 3(c)**), right). However, minimal dose systems have been developed to limit the electron dose that hits the sample by exposing it only at the nominal magnification during the photographic or charged-coupled device (CCD) camera exposure time. Basically, the electron dose has to be less than 10 e^{-}Å^{-2} in order to preserve the structure of the sample.

There have been a number of recent technological advances that have made the use of this technique easier. The blotting time of the grid is one of the important steps in sample freezing, but is also one of the less reproducible ones. The thickness of the ice in the hole has to be as close as possible to the diameter of the sample. Automated procedures for producing reproducible cryo-EM grids have been developed and commercial devices for obtaining reproducible ice thickness are now available. The introduction of support films with regularly spaced holes in parallel with automated freezing will make automated data acquisition possible.

Cryo-Electron Tomography

Electron tomography allows the study of the 3D organization of thin individual cell organelles and bacterial cells at nanometer resolutions without slicing them. This technique is also able to reconstruct the 3D structure of nonregular viruses. In contrast to all other EM reconstruction techniques, the samples are unique objects. For tomography, a tilt-series is taken from a single object (from −70° to +70°). CCD cameras are used for the recording of many images of the same sample without serious radiation damage. The second step is to align all the images coming from the tilt series in order to generate a 3D model of the object. Tomography holds the potential to routinely enable reconstructions that will allow the fitting of intermediate-resolution EM-structures or high-resolution X-ray-structures into the features of the tomography reconstruction.

Metal Shadowing

The angular shadowing of protein samples consists of evaporation under vacuum of a thin film of metal at an angle over a dried sample on support film (**Figure 4(a)**). The sample is mixed with glycerol and then deposited on the surface of a mica sheet. The mica is then introduced into an evaporator and dried. The sample is then tilted for evaporation. Different kinds of metal can be evaporated onto the surface of the sample (titanium, gold, platinum-paladium alloy). Subsequently a carbon film is evaporated onto the mica, which is then floated off on a water bath, much like the methods used for negative staining above, and the metal comes off the mica with the carbon. Different metals will lead to different grain sizes. Differences in sample height will lead to differences in metal deposit and, hence in contrast (see **Figure 4(b)**). This technique is the only technique that can determine the handedness of a helix and distinguish, for example, between icosahedral 7*d* or 7*l* surface lattices. Rotational shadowing is based on the same principle as angular shadowing but the sample is rotated during evaporation (a fixed angle of evaporation is kept). This technique is especially helpful for the visualization of small proteins or nucleic acids (**Figure 4(b)**). Note that the dimensions of shadowed proteins are difficult to interpret because the metal shadow will add significantly to the thickness of the protein.

Preparation of Cells or Very Large Particles for EM Observation

One of the most interesting aspects of virus studies is the analysis of virus infection, trafficking, and particle

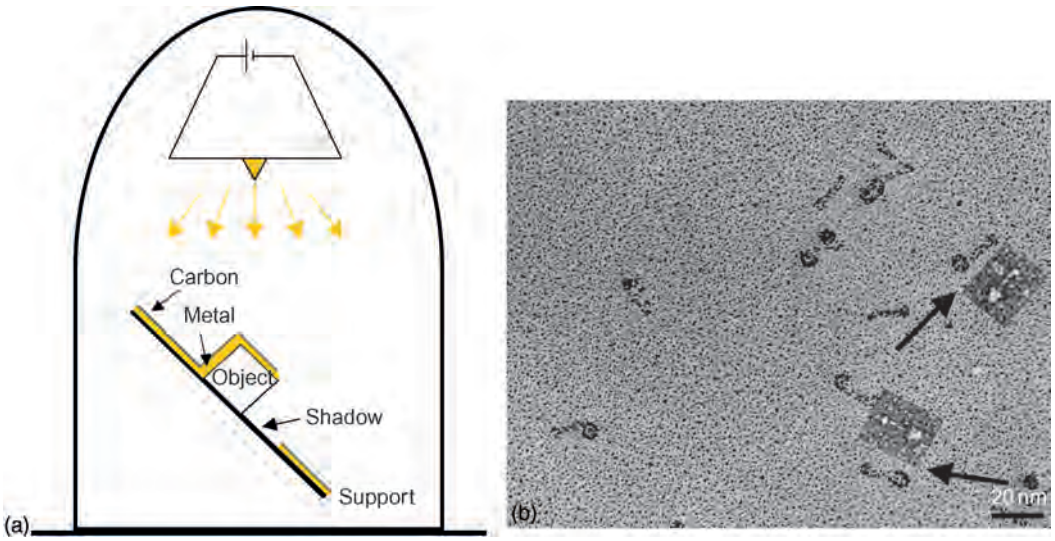


Figure 4 Principle of rotary shadowing. (a) Principle of shadowing. The shadowing process occurs under high vacuum. The specimen is in a tilted position. At the top, the metal to be evaporated is shown in yellow. It is heated until evaporation by a high-intensity current and will be deposited onto the sample. For rotating shadowing, the specimen is not fixed in a tilted position but rotated while keeping the same tilt angle. (b) Human adenovirus 3 penton base was rotatively shadowed using platinum. For comparison the same object negatively stained using sodium silicotungstate has been placed on the same figure (insets are arrowed).

formation in cells. Unfortunately, cells and tissues are generally too thick to be directly observed by EM and they first have to be sectioned.

Thin and Ultrathin Sectioning

Classical thin sectioning

The cells or tissues are first stabilized by chemical fixation (usually with aldehydes such as formaldehyde or gluteraldehyde) in order to immobilize the protein and nucleic acid components of the cell (**Figure 5**). Subsequently, fixation with osmium tetroxide will immobilize and stain the membranes of the cell. Note that all these fixatives are very toxic. Then the material is dehydrated in ethanol or acetone and embedded in plastic. Ultrathin sections (60 nm) cut with diamond knives using an ultramicrotome are floated on water, transferred to specimen support grids and examined in the transmission electron microscopy (TEM). Before observation, the sections are further contrasted with uranyl acetate and lead citrate. There are many different types of plastic resins. The hardest resins (epoxy) will best preserve the ultrastructure of the cells during sectioning but will not allow antibody labeling of sections because the antibodies have no or little access to their antigens. There exist soft or spongy resins (Lowicryl) that do permit antibody labeling but these resins are not hard enough to guarantee ultrastructure conservation.

Application: Antibody labeling

Successful immunocytochemistry is only possible when antibodies (or other affinity markers) are able to bind to their target ligands. For extracellular antigens this is not a problem, but for molecules inside cells the markers must be able to access the antigens. For EM it is important to use preparation methods that open the cells and allow introduction of antibodies without destroying the normal cellular organization. The antigens must also be preserved during the different sectioning steps.

The cryo-section technique is now one of the two most important techniques for subcellular immunocytochemistry. Specialized resin formulas have been developed specifically for use in immunocytochemistry. These include the Lowicryl resins, LR White and LR Gold, Unicryl, and MonoStep. They all have low viscosity and can be polymerized, at low temperatures, with ultraviolet light.

In practice, this method is used with electron opaque markers, which bind to the antibodies and show their location within the cell. In this way, subcellular antigens recognized by antibodies can be localized directly with TEM. Colloidal gold coupled to protein A (a protein from bacterial cell walls that binds to the Fc portion of antibodies) has been used extensively in recent years to localize antibodies on resin and frozen sections of biological materials (**Figure 5**). The ability to produce homogeneous populations of colloidal gold with different particle sizes enabled the use of these probes to co-localize different structures on the same section.

Cryo-sectioning

As for single particles, it is possible to freeze biological material fast enough to vitrify the water present inside the cells. Vitrification of water occurs when the freezing is so fast that ice crystals have no time to form. Vitrified biological material can be sectioned at low temperatures. Because no drying occurs and no salt has been added,

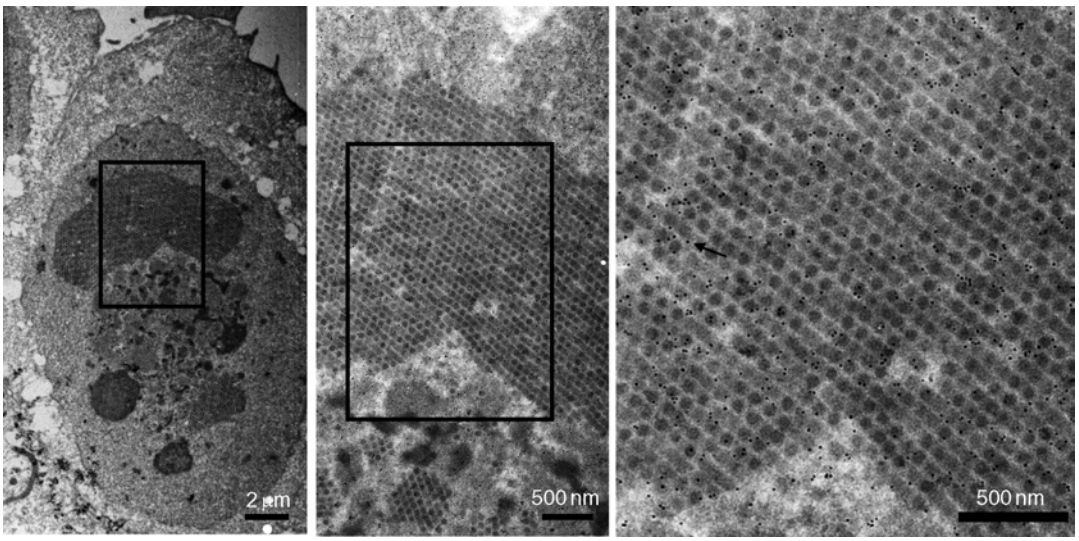


Figure 5 Thin sectioned, adenovirus infected cells. Going from left to right one can see an increase of magnification going from a general view of the infected nucleus to clearly recognizable particles. On the right, the infected cells have been labeled with anti-adenovirus-hexon (major capsid component) antibody coupled with colloidal gold (arrow). Cells were fixed with 2.5% glutaraldehyde in 100 mM HEPES buffer pH 7.4, post-fixed with 1% osmium tetraoxide and dehydrated with ethanol and then embedded in Epon prior to slicing. Grids were stained with saturated uranyl acetate in 50% ethanol and then with 1 M lead citrate.

cryo-sections better represent the native state of cells although the contrast in the sample is low. Presently, there are seven main rapid freezing methods possible:

1. Immersion freezing – the specimen is plunged into the cryogen.
2. Slam (or metal mirror) freezing – the specimen is impacted onto a polished metal (usually copper) surface cooled with liquid nitrogen or helium.
3. Cold block freezing – two cold, polished metal blocks attached to the jaws of a pair of pliers squeeze-freeze the specimen.
4. Spray freezing – a fine spray of sample in liquid suspension is shot into the cryogen (usually liquid propane).
5. Jet freezing – a jet of liquid cryogen is sprayed onto the specimen.
6. Excision freezing – a cold needle is plunged into the specimen, simultaneously freezing and dissecting the sample.
7. High-pressure freezing – freezing the specimen at very high pressure avoids the expansion of water that occurs during freezing.

CEMOVIS: Cryo-EM of vitreous sections

Dr. Jacques Dubochet in Lausanne has developed the cryo-EM of vitreous sections (CEMOVIS) technique. This technique which consists of cutting very thin sections of a vitrified sample requires good vitrification of the sample and good cutting. Progress in these two domains has been of crucial importance. The first one is high-pressure freezing, which made it possible to increase the thickness of a typical biological sample that can be vitrified from 10 to 100 µm. The second progress came from a better understanding of the cutting process which allowed optimization of the cutting. It is now possible to obtain reproducibly ribbons of homogeneous, 50 nm thick sections in which biological structures are remarkably preserved. It is also possible to observe for the first time, at a resolution approaching 2 nm, the ultrastructure of cells and tissues in their native state.

The next step using CEMOVIS is to combine it with electron tomography. Recording a large number of tilted images (typically 100) should allow the calculation of a 3D model of the specimen to a resolution approaching 4 nm. This is a real challenge for studying virus replication.

Image Analysis

As mentioned above, in tomography, about a hundred images of the same particle are combined in a computer in order to calculate a 3D reconstruction of a single particle. The resolution obtained is presently around 30 Å. However, computer analysis of images is also needed for cryo-EM images because the contrast in cryo-EM is too low. The images of thousands of particles can be combined to obtain a high-contrast model of the particle provided that all particles have the same composition and conformation. The resolution of models derived with this method can be as good as 7 Å for large protein complexes or intact virus particles. A large number of mathematical approaches are available to calculate such 3D reconstructions. Different approaches exist to calculate models of

nonsymmetrical complexes, for helical structures, and for icosahedral virus capsid structures. These techniques requires classical EM photographs to be digitized by scanning the negatives or the images have to be directly recorded in the microscope using a CCD camera.

Figure 6 shows 3D reconstructions of adenovirus type 5 particles from negatively stained particles and from cryo-EM images. The two models are identical but the cryo-model has a higher resolution (around 10 Å compared to 35 Å for the negative stain model). Because the contrast in the negatively stained images is high, such a model from negatively stained particles can be used as starting model for the cryo-reconstruction. The major difference between the two models is that in the model derived from the stained particles only the outside of the virus capsid is visualized, whereas for the cryo-model all proteins inside and outside of the capsid are visible. The cryo-EM model of the capsid was combined with the atomic resolution data derived from the crystal structure of the two main adenovirus capsomers, the trimeric hexon

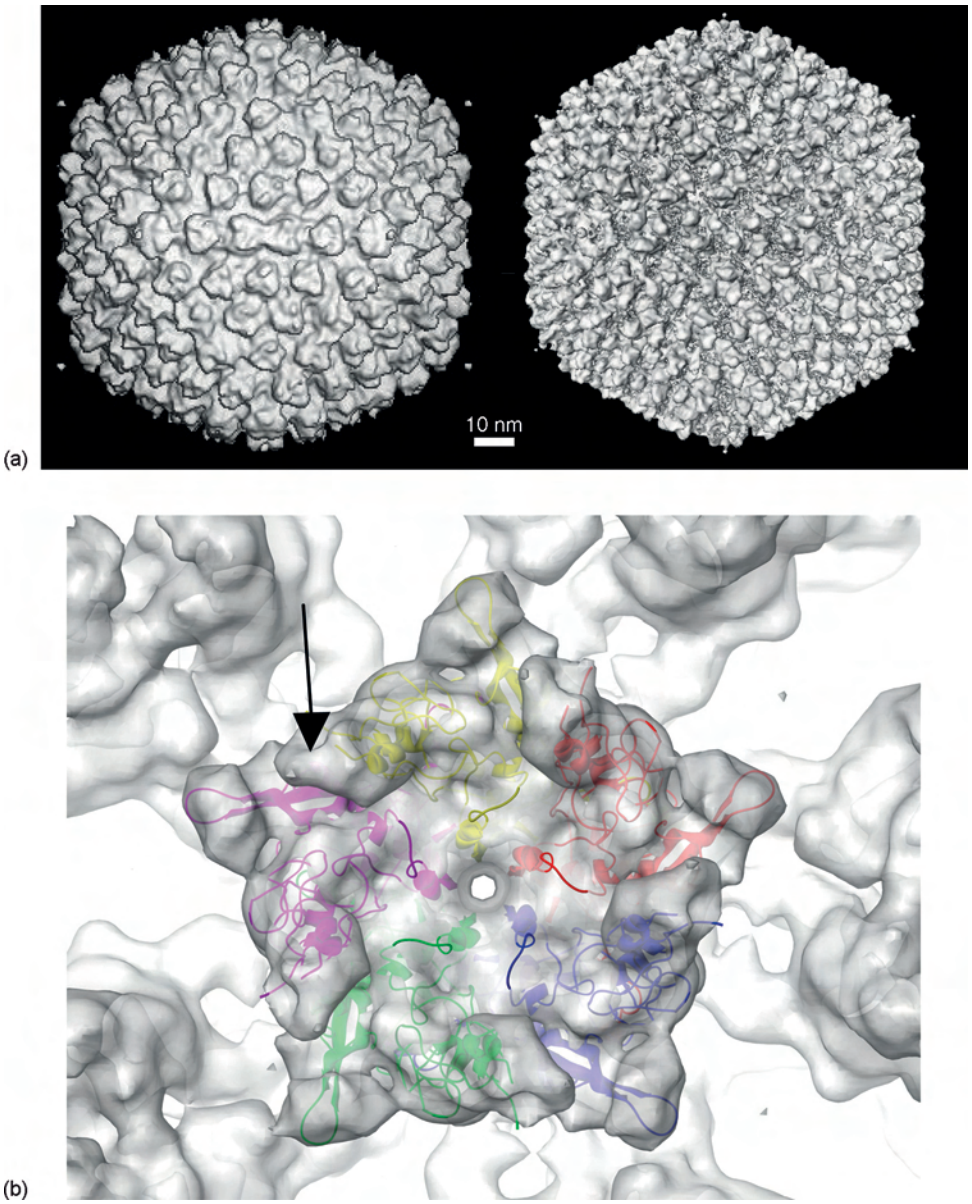


Figure 6 Image analysis and combination with other techniques (a) 3D reconstruction of adenovirus 5 particle after imaging in negative staining (left) and cryo-EM (right). Respectively 150 and 3000 individual negatively stained or cryo-EM images were combined to obtain 3D reconstructions at 35 Å (negative staining) and 10 Å (cryo-EM) resolution. (b) Combination of EM with X-ray crystallography. Fitting of the X-ray structure of the adenovirus penton base into the EM density. The arrow highlights a mobile part of the protein that is resolved in electron microscopy but not in X-ray crystallography.

and the pentameric penton base. By fitting the crystal structures into the EM density, a quasi-atomic model can be derived that describes the exact position of all capsomers and the interacting surfaces and protein loops between the capsomers. **Figure 6** shows details of the fit of the crystal structure of the penton base into a penton base from the EM model. A number of different algorithms are available to perform these fits, some of which take into account the possibility of flexibility between protein domains. The fit in **Figure 6** (arrow) shows density in the EM model that is not filled by density from the crystal structure. Some loops of the molecule are flexible and therefore invisible in the crystal structure, whereas the lower resolution of the EM model does allow imaging.

The Future

In our opinion, the future of EM in virology lies in an extended use of the combination of structural data as shown above. Fitting crystal structures of intact proteins or domains into protein complexes (viral complexes or complexes between viral and host proteins) will show how proteins interact in the infected cell. Although the EM models have a resolution of only around 10 Å, the information on the interface of the proteins in the complex is of near-atomic resolution. These techniques can be applied to regular viruses. For irregular viruses, such as influenza and paramyxoviruses, tomography will allow the construction of models with a resolution of around 40 Å that will show how the proteins in the virus particle interact. In particular, it will be interesting to see the packaging of the nucleocapsids in these viruses. At present we do not know how the DNA is packaged in adenovirus particles and it is possible that the icosahedral averaging techniques result in a loss of this information if the packing symmetry of the DNA is in fact not icosahedral. The same problem exists for capsid features that are not icosahedral such as unique capsid vertices that may be used for the packaging of DNA in adenoviruses and herpesviruses. Such problems may be overcome by using reconstruction techniques that are not based on icosahedral symmetry.

See also: Virus Particle Structure: Nonenveloped Viruses; Virus Particle Structure: Principles.

Further Reading

Al-Amoudi A, Dubochet J, and Studer D (2002) Amorphous solid water produced by cryosectioning of crystalline ice at 113 K. *Journal of Microscopy* 207: 146–153.

Al-Amoudi A, Chang JJ, Leforestier A, *et al.* (2004) Cryo-electron microscopy of vitreous sections. *EMBO Journal* 23: 3583–3588.

Chiu W, Baker ML, Jiang W, Dougherty M, and Schmid MF (2005) Electron cryomicroscopy of biological machines at subnanometer resolution. *Structure* 13: 363–372.

Dubochet J, Adrian M, Chang JJ, *et al.* (1988) Cryo-electron microscopy of vitrified specimens. *Quarterly Reviews of Biophysics* 21: 129–228.

Fabry CM, Rosa-Calatrava M, Conway JF, *et al.* (2005) A quasi-atomic model of human adenovirus type 5 capsid. *EMBO Journal* 24: 1645–1654.

Griffiths G, McDowall A, Back R, and Dubochet J (1984) On the preparation of cryosections for immunocytochemistry. *Journal of Ultrastructure Research* 89: 65–78.

Grunewald K and Cyrklaff M (2006) Structure of complex viruses and virus-infected cells by electron cryo tomography. *Current Opinion in Microbiology* 9(4): 437–442.

Harris JR (1997) *Royal Microscopy Society Handbook 35: Negative Staining and Cryoelectron Microscopy.* Oxford: Bios Scientific Publishers.

Sartori N, Richter K, and Dubochet J (1993) Vitrification depth can be increased more than 10-fold by high pressure freezing. *Journal of Microscopy* 172: 55–61.

Tokuyasu KT (1986) Application of cryoultramicrotomy to immunocytochemistry. *Journal of Microscopy* 143: 139–149.

Tokuyasu KT and Singer SJ (1976) Improved procedures for immunoferritin labeling of ultrathin frozen sections. *Journal of Cell Biology* 71(3): 894–906.

Wepf R, Amrein M, Burkli U, and Gross H (1991) Platinum/iridium/carbon: A high-resolution shadowing material for TEM, STM and SEM of biological macromolecular structures. *Journal of Microscopy* 163: 51–64.

Emerging and Reemerging Virus Diseases of Plants

G P Martelli and D Gallitelli, Università degli Studi and Istituto di Virologia Vegetale del CNR, Bari, Italy

© 2008 Elsevier Ltd. All rights reserved.

Introduction

A number of RNA plant viruses constitute new threats to economically relevant vegetable crops, grapevine, and citrus. These are listed among new disease-causing agents, and are therefore denoted 'emerging or re-emerging' being often new viruses or virus strains responsible for serious diseases. The emerging plant viruses and their known vectors are quickly expanding in new areas as a consequence of the increasing international trade and the

large numbers of people traveling in many countries. Once introduced, the fitness of these viruses to new agroclimatic conditions increases, leading to the development of severe epiphytotics.

Vegetable Viruses

Pepino Mosaic Virus

Geographical distribution

Pepino mosaic virus (PepMV) was originally described in Peru on pepino (*Solanum muricatum*) and found to infect tomato and related wild species symptomlessly, in experimental trials. Since 1999, PepMV outbreaks have been reported almost simultaneously in many European countries (Austria, Bulgaria, Finland, France, Germany, Hungary, Italy, Netherlands, Norway, Poland, Slovakia, Spain, Sweden, Switzerland, Ukraine, and UK) where it is considered an emerging pathogen of tomato glasshouses. PepMV was also found in Canada, USA (Arizona, California, Colorado, Florida, Oklahoma, Texas), Ecuador, and Chile. After discovering the PepMV occurrence in tomato grown in Europe and North America, a survey carried out in central and southern Peru and Ecuador demonstrated that the virus was present in Peruvian tomato crops as well as in the following wild *Lycopersicon* species: *L. chilense*, *L. chmilewskii*, *L. parviflorum*, *L. peruvianum*, and in *L. pimpinellifolium* in Ecuador.

Disease symptoms and yield losses

In pepino, the virus causes yellow mosaic in young leaves, whereas affected tomato plants show a wide array of symptoms. These include stunting of the whole plant, bubbling of the leaf surface, interveinal chlorosis, mosaic and green striations on stem and sepals. Foliar symptoms resemble hormonal herbicide damage, while lower leaves show necrotic lesions that resemble damage caused by water that dripped onto the plant. Fruits from early infected tomato plants may show blotchy ripening and gold marbling. Ripe fruits develop yellow speckles and spots that make them unmarketable. Observations by Dutch scientists indicate that symptoms are more readily seen during autumn and winter months, and are masked during warmer months. Yield losses caused by PepMV are probably below 5%, although surveys in glasshouse-grown tomatoes reported crop reductions up to 40%. The disease spreads very rapidly and crop losses may be significant if early action is not taken to eliminate infected sources. Although Peruvian virus strains seem to be little virulent, new strains of higher virulence could arise through recombination. Because of the adverse effects on quality and yield, PepMV is becoming one of the most important tomato pathogens in Europe.

Causal agent and classification

PepMV (genus *Potexvirus*, family *Flexiviridae*) has helically constructed semirigid filamentous particles with a modal length of 508 nm and a diameter of 11 nm. Sequencing of a number of strains has shown that virions contain a single molecule of linear, positive-sense, single-stranded RNA (ssRNA) 6410–6425 nt in size. The genome organization is typical of the genus *Potexvirus*, with five open reading frames (ORFs): ORF1, encoding a putative replicase of 164 kDa; ORFs 2–4, coding for the triple gene block proteins 1–3 (TGBp) of 26 kDa, 14 kDa, and 9 kDa, respectively; and ORF5, encoding the 25 kDa coat protein (CP). Phylogenetic analyses carried out on replicase, TGBp1, and CP amino acid sequences revealed that PepMV is closely related to narcissus mosaic virus (NMV), scallion virus X (SVX), cymbidium mosaic virus (CymMV), and potato aucuba mosaic virus (PAMV) (**Figure 1**). Tomato isolates of PepMV can be distinguished from the original isolate from pepino on the basis of symptomatology, host range, and sequence data. On the other hand, tomato isolates from Europe appear very similar to each other sharing nucleotide sequence identities higher than 99% and in the range of 95–96% with PepMV from *L. peruvianum* from Peru. Nucleotide sequence comparisons between US and the European tomato isolates show only 79–82% identity. Gene-for-gene comparison between sequenced isolates suggests that TGBp1 and TGBp3 are more suitable than either the replicase or CP gene products for discriminating virus isolates.

Epidemiology

PepMV is transmitted by contact and readily spread mechanically by contaminated hands, tools, shoes, clothing, and plant-to-plant contact. The virus is thought to remain viable in dry plant material for as long as 3 months. Seed transmission or surface seed contamination in tomato is suspected but not demonstrated. In the UK, the virus is frequently found in imported fruits. Infection of other solanaceous crops such as eggplant, tobacco, and potato has only been observed in experimental trials, while infection in pepper has not been demonstrated. Cucumber can be artificially infected but the virus does not appear to spread systemically in the plant.

Alternative hosts that may serve as virus reservoirs were studied in Spain, where native plants with virus-like symptoms growing in or around tomato fields were collected and analyzed for the presence of PepMV. As many as 18 weed species were found to be infected.

Because of the high similarity of tomato virus isolates, a common origin seems likely although where this origin is located is still unclear. The extent of PepMV distribution in Peru, together with the fact that many of the wild Peruvian *Lycopersicon* populations sampled were isolated and had not been manipulated by man, led to the conclusion that this virus has been present in the region for a

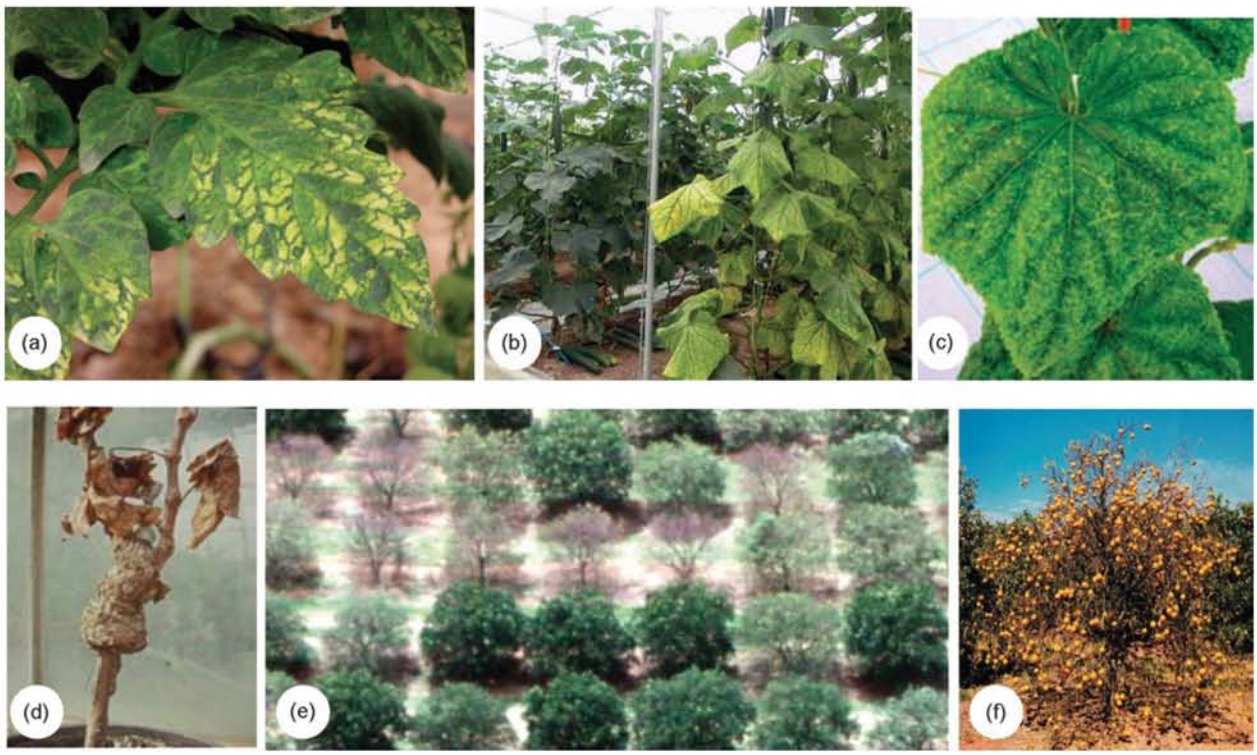


Figure 1 (a) Strong symptoms of PepMV on tomato; (b) symptom of CYSDV on melon; (c) severe vein yellowing on cucumber infected by CVYV; (d) swelling at the graft union and death of the scion of a vine grafted on Kober 5BB, infected by *Grapevine leafroll-associated virus 2;* (e) a citrus grove affected by sudden death disease; and (f) a citrus plant killed by sudden death disease. (a) Courtesy of Dr. E. Moriones. (b) Courtesy of Dr. E. Moriones. (c) Courtesy of Dr. I. M. Cuadrado. (e, f) Courtesy of Dr. J.Bovè.

long time and that other factors might be involved in its spread. *Myzus persicae* does not transmit PepMV. In southern Spain a faster spread of PepMV was observed in bumble-bee pollinated greenhouses than in those with no pollinator insects.

Control

Recommended control strategies for PepMV focus on sanitation. Use of certified seed lots, complete removal (including roots) of infected plants, limited access to affected rows, and sanitation of clothing and tools are all critical. The increasing concern caused by this new disease has led the European Commission to authorize member countries to take measures to prevent the spread of PepMV within the European Union.

Criniviruses

Criniviruses are whitefly-transmitted RNA viruses that cause plant diseases with increasingly important yield impact, consequent to the sudden explosion of whitefly populations in temperate regions in the last 10–20 years. All these viruses induce yellowing symptoms in their hosts, are generally phloem-limited, nonmechanically transmissible, and have large ssRNA genomes. Cucurbit yellow stunting disorder virus (CYSDV), tomato infectious chlorosis virus (TICV), and tomato chlorosis virus (ToCV) are important emerging criniviruses.

Cucurbit Yellow Stunting Disorder Virus

Geographical distribution

CYSDV was first observed in the southeastern coast of Spain, on melon and cucumber grown under plastic. The virus was also found in the Canary Islands, Egypt, France, Israel, Jordan, Lebanon, Mexico, Morocco, Portugal, Saudi Arabia, Syria, Texas, Turkey, and the United Arab Emirates.

Disease symptoms and yield losses

Protected crops of cucumber and melon show severe yellowing that starts as interveinal mottle of older leaves to develop into complete yellowing of the leaf lamina, except for the veins, followed by rolling, brittleness, and stunting. On the whole, symptoms are virtually indistinguishable from those caused by beet pseudo yellows virus (BPYV). Zucchini can also be infected but symptoms have not been described. Disease incidence in protected

crops can easily reach 100%. In Spain and many other countries, CYSDV is considered the prevailing virus of protected cucurbit crops.

Causal agent and classification

CYSDV (genus *Crinivirus*, family *Closteroviridae*) has a narrow host range limited to the family *Cucurbitaceae* and is confined to phloem tissues. Virions are flexuous filaments with lengths between 750 and 800 nm. Genome consists of two molecules of ssRNA of plus-sense polarity designated RNA-1 and -2. RNA-1 is 9123–9126 nt long and contains five ORFs with papain-like protease, methyltransferase, RNA helicase, and RNA-dependent RNA polymerase domains in the first two overlapping ORFs, a small 5 kDa hydrophobic protein, and two further downstream ORFs potentially encoding proteins 25 and 22 kDa in size, respectively. RNA-2 is 7976 nt long and contains the hallmark ORFs of the family *Closteroviridae*, encoding, in the order, a heat shock protein 70 (HSP70) homolog, a 59 kDa protein, the major (CP) and the minor (CPm) CP. In the 3′-terminal region, RNA-1 contains an ORF potentially encoding a protein of 25 kDa which has no homologs in any databases, and RNA-2 has an unusually long 59 noncoding region. Subgenomic RNAs were detected in CYSDV-infected plants, suggesting that they serve for the expression of internal ORFs. CYSDV can be divided into two divergent groups of isolates. One group is composed of isolates from Spain, Lebanon, Jordan, Turkey, and North America, the other of isolates from Saudi Arabia. Nucleotide identity between isolates of the same group is greater than 99%, whereas identity between groups is about 90%.

Epidemiology

Natural hosts of CYSDV are restricted to cucurbits: watermelon, melon, cucumber, and zucchini. *Cucurbita maxima* and *Lactuca sativa* are experimental host plants. The life cycle of CYSDV is dependent on its vector, the whitefly *Bemisia tabaci*, as viral outbreaks are associated with heavy infestations of whitefly biotypes A, B, and Q. Transmission of CYSDV by biotype B is more effcient than by biotype A, whereas biotype Q transmits as efficiently as biotype B. *Trialeurodes vaporariorum* was displaced *by B. tabaci* as the dominant whitefly along the southeastern coast of Spain when CYSDV took over BPYV as the agent of yellowing diseases of cucurbits. Acquisition periods of 18 h or more and inoculation periods of 24 h or more seem necessary for high transmission rates of CYSDV, which can persist for at least 9 days in the vector. The virus is not known to be seed-borne.

Control

Control of CYSDV consists in controlling *B. tabaci*, and on eliminating infection sources. Chemical control of *B. tabaci* has not been effective in preventing the spread and in reducing the incidence of the disease because the vector has a wide host range and quickly develops resistance to most of existing insecticides. Roguing infected plants and weeds that can act as hosts for the vector and removal of overwintering crops prior to the emergence of adult whiteflies may prove useful. This helps if applied over large areas and where there is no continuous cropping in glasshouses, which are the sites of whitefly survival and the source of virus spread throughout the year. Growing plants under physical barriers such as low-mesh tunnels may also have a positive effect. No resistant cultivars are currently available commercially but experimental evidence for delayed viral infection and decreased symptom severity in accessions of *Cucumis sativus* has recently been obtained.

Tomato Infectious Chlorosis Virus

Geographical distribution

TICV infections have been reported from California, France, Greece, Indonesia, Italy, Japan, North Carolina, Spain, and Taiwan. This virus may also be present in the Czech Republic but the record has not been confirmed.

Disease symptoms and yield losses

Symptomatic tomato plants in open field and greenhouses exhibit interveinal yellowing in older leaves, followed by generalized yellowing. Symptoms can be confused with nutritional disorders (i.e., magnesium deficiency), pesticide toxicity, or natural senescence as older leaves may also turn red. Necrosis and occasional upward rolling of the leaves have also been reported. On the whole, infected plants are less vigorous and with fruits that may show delayed ripening. There is no information on the effects of TICV on artichoke and lettuce crops or ornamental species known to be natural hosts. Disease incidence may vary from one or few plants to severe outbreaks, depending on the abundance of whitefly populations. In California and Greece, disease incidence between 80% and 100% was reported.

Causal agent and classification

The natural hosts of TICV (genus *Crinivirus*, family *Closterovidae*) include members of the families *Chenopodiaceae*, *Compositae*, *Ranuncolaceae*, and *Solanaceae* where the virus is confined to phloem tissue. Virions are flexuos filaments with lengths between 750 and 800 nm. Genome consists of two molecules of ssRNA of positive polarity, denoted RNA-1 and RNA-2, whose nucleotide sequence has partially been determined. Both RNAs contain the hallmark ORFs of the family *Closteroviridae.*

Epidemiology

TICV is spread by the whitefly *T. vaporariorum* but not by *B. tabaci* and it is not mechanically transmitted. Besides

tomato, natural infections have been detected in *Callistephus chinensis*, *Chenopodium album C. murale*, *Cynara cardunculus*, lettuce, *Physalis ixocarpa* (tomatillo), globe artichoke, *Nicotiana glauca*, *Petunia hybrida*, *Picris echioides*, *Ranunculus* sp., and *Zinnia elegans*.

Control

TICV has the potential to cause significant losses to tomato and other naturally susceptible crops if it becomes established. Control largely depends on the efficacy of treatments and strategies to limit populations of *T. vaporariorum*. Tomato seedlings for planting should come from disease-free stocks. The general management strategies outlined for CYSDV can also be adopted for TICV.

Tomato Chlorosis Virus

Geographical distribution

ToCV infections have been reported from the US (Colorado, Connecticut, Florida, Louisiana), Europe (France, Greece, Italy, Portugal, Spain), Morocco, Israel, Puerto Rico, South Africa, and Taiwan.

Disease symptoms and yield losses

Symptoms are very similar to those induced by TICV, that is, progressive yellowing of the whole plant. Apparently, there are no estimates of yield losses, although since its discovery, the virus represents a serious problem for tomato production in many parts of the world.

Causal agent and classification

ToCV (genus *Crinivirus*, family *Closteroviridae*) virions are flexuous filaments encapsidating two molecules of positive-sense ssRNA denoted RNA-1 and RNA-2, whose complete nucleotide sequence has been determined. RNA-1 consists of 8595 nt organized into four ORFs and encodes replication-associated proteins. RNA-2 is 8244 nt long and putatively encodes nine ORFs comprising in the order, the HSP70 homolog, a 59 kDa protein, CP, and CPm, which may be involved in determining the unique, broad vector transmissibility of the virus. Phylogenetically, ToCV is closely related to sweet potato chlorotic stunt virus (SPCSV) and CYSDV.

Epidemiology

ToCV is transmitted by *T. vaporariorum*, *B. tabaci* biotypes A and B, and *T. abutilonea*. Besides tomato, the natural host range includes pepper, *Datura stramonium*, *Physalis wrightii*, *Solanum nigrum*, and *Z. elegans*.

Control

ToCV has the potential to cause significant losses to tomato if it becomes established. Control depends on the efficacy of treatments and strategies to limit populations of its broad range of vectors. The management strategies outlined for CYSDV apply also for ToCV.

Cucumber Vein Yellowing Virus

Geographical distribution

Cucumber vein yellowing virus (CVYV) has been recorded form Greece, Israel, Jordan, Portugal, Spain, Sudan, and Turkey.

Disease symptoms and yield losses

In cucumber, CVYV causes pronounced vein clearing deformation of the leaves followed by a generalized chlorosis and necrosis of affected plants. Fruits show light to dark green mottling. Nonparthenocarpic cucumbers are symptomless carriers of CVYV while parthenocarpic cucumbers develop severe symptoms. Stunting was also observed in cucumber and melon and sudden death in protected melons in Portugal. In watermelon, symptoms are often light or not expressed, but splitting of the fruits has occasionally been observed. In zucchini, symptoms vary from chlorotic mottling to vein yellowing of the leaves, but symptomless infections have also been recorded. Symptoms severity may be increased by synergistic reactions with other viruses. Yield losses have not been quantified. CVYV could present a threat to cucurbits grown outdoors or under glasshouses, although its actual impact in the complex pathosystem affecting cucurbit crops in the Mediterranean and subtropical regions has not been determined.

Causal agent and classification

CVYV (genus *Ipomovirus*, family *Potyviridae*) has filamentous particles and is transmitted by *B. tabaci* but, unlike other whitefly-transmitted viruses, it can also be transmitted by mechanical inoculation. CVYV genome consists of a single molecule of plus-sense ssRNA of 9751 nt (ALM 23 isolate) containing most hallmarks of the genome of members of the family *Potyviridae*. The absence of a coding region for the helper component-proteinase seems to be a distinctive trait of CVYV. Two CVYV strains have been recognized from Israel and Jordan, that is, CVYV-Is and CVYV-Jor. The two strains induce similar vein-clearing symptoms in cucumber and melon, but CVYV-Jor infections in cucumber are more severe. CVYV is more closely related to *Sweet potato mild mottle virus* (SPMMV) than any other species in the family *Potyviridae*.

Epidemiology

CVYV naturally infects cucumber, melon, watermelon squash, and zucchini and several weed species are also natural hosts of the virus (e.g., *Ecballium elaterium*, *Convolvulus arvensis*, *Malva parviflora*, *Sonchus oleraceus*, *Sonchus asper*, and *Sonchus tenerrimus*). All experimental hosts belong to the

family *Cucurbitaceae* and include *Cucurbita moschata*, *Cucurbita foetidissima*, and *Citrullus colocynthis*. CVYV is semipersistent in its white-fly vector (*B. tabaci*) which retains the virus for less than 6 h. Therefore, individuals moving to nonhost plants may not remain viruliferous long enough to transmit the virus. *Aphis gossypii* and *M. persicae* are not vectors. Whether CVYV is seedborne has not been determined.

Control

CVYV management is mainly based on the use of virus-free stock plants as well as of *B. tabaci* populations as already outlined for CYSDV. Several detection methods based on molecular hybridization and polymerase chain reaction (PCR) have been developed and used for screening *Cucurbitaceae* germplasm that can show some degree of resistance to CVYV.

Graft Incompatibility in Grapevines

Geographical Distribution

Cases of graft union disorders have been documented from Europe ('Kober 5BB incompatibility', 'Syrah decline'), California, New Zealand, Italy, Australia, and Chile ('Young vine decline'), and again California ('Roostock stem lesions', 'Necrotic union', and 'Stem necrosis'), but are likely to occur also in other viticultural countries.

Disease Symptoms and Yield Losses

The increased use of grapevine clonal material is revealing unprecedented and widespread conditions of generalized decline that develop dramatically in certain scion–rootstock combinations. Newly planted vines grow weakly, shoots are short, leaves are small sized, with margins more or less extensively rolled downwards, and the vegetation is stunted. The canopy shows autumn colors off season so that leaves turn reddish in red-berried varieties or yellow in white-berried varieties much earlier than normal. A prominent swelling forms at the scion/rooststock junction ('Kober 5BB imcompatibility', 'Young vine decline') sometimes accompanied by necrosis at the graft union ('Necrotic union'), and variously extended necrotic lesions develop on the roostock stem ('Roostock stem lesion', 'Stem necrosis'). Severely affected vines decline and may die within 1 or 2 years. Syrah decline is a severe disease characterized by early reddening of the leaves and swellings with grooves and deep cracks at the graft union. Appearance of graft union disorders depends more on the rootstock than on the scion. For instance, European grape varieties grafted on tolerant rootstocks (e.g., Freedom, Harmony, Salt creek, 03916, 101-14) exhibit a green canopy and perform well, whereas varieties grafted on susceptible roostocks (e.g., Kober 5BB, 5C, 1103P, 3309) develop a discolored canopy, decline, and may die.

Causal Agents and Classification

An ordinary strain of grapevine leafroll-associated virus 2 (GLRaV-2) is consistently associated with Kober 5BB incompatibility in Europe, appears to be involved in California's young vine decline, and was detected in diseased Chilean and Argentinian grapes. GLRaV-2, a definitive member of the genus *Closterovirus* (family *Closteroviridae*), has flexuous filamentous particles *c.* 1600 nm long an RNA genome 15 528 nt in size made up of nine ORFs. A virus originally detected in cv. Redglobe in California called grapevine rootstock stem lesion-associated virus (GRSLaV) proved to be a molecular and biological variant of GLRaV-2 (GLRaV-2 RG). Other molecular variants of GLRaV-2 were reported from New Zealand, Chile, and Australia in association with young vine decline conditions. Based on the differential responses of a panel of 18 rootstocks, up to five different graft-transmissible agents inducing incompatibility were detected in California. Of these, only GLRaV-2 RG, the putative agent of roostock stem lesion, was identified. The agents of necrotic union and stem necrosis are unknown. Equally unkown is the agent of Syrah decline, although there is circumstantial evidence that grapevine rupestris stem pitting-associated virus (GRSPaV) may have a bearing on its aetiology. GRSPaV, a definitive member of the genus *Foveavirus* (family *Flexiviridae*), has filamentous particles *c.* 730 nm in length, a genome 8726 nt in size comprising five or six ORFs, and occurs in nature as a family of molecular variants.

Transmission

GLRaV-2 and GRSPaV have no known vectors, but GRSPaV is pollen- and seed-borne. Infected propagative material is the major means for dissemination of both viruses.

Control

Use of certified virus-free scionwood and rootstocks is recommended. Currently known graft incompatibility agents can be eliminated with reasonable efficiency by heat therapy, meristem tip culture, or a combination of the two. If scionwood is infected, the use of sensitive rootstocks is to be avoided.

Sudden Death of Citrus

Geographical Distribution

Citrus sudden death (CSD) has only been reported from the State of Saõ Paulo in Brazil, where it has already killed about 1 million trees. It has the potentiality for spreading to other Brazilian States and neighboring countries.

Disease Symptoms and Yield Losses

CSD is a destructive disease first observed in 1999 in Brazil on sweet orange and mandarin trees grafted on Rangpur lime (*Citrus limonia*) or Volkamer lemon (*Citrus volkameriana*). Outward symptoms of infected trees and modifications of the bark anatomy at the bud union resemble very much those elicited by citrus tristeza, the major difference being that CSD develops on trees grafted on tristeza-resistant rootstocks. Symptoms of CSD are characterized by a generalized discoloration of the leaves which are pale green initially, then turn yellowish and abscise. With time, defoliation becomes more intense, the trees do not push new vegetation, and the root system decays. A rapid decline and death of the plant ensues, due also to phloem degeneration in the graft union region. The phloem of the susceptible rootstocks Rangpur lime and Volkamer lemon shows a characteristic yellow strain. Until their sudden collapse, infected trees bear a normal crop.

Causal Agent and Classification

All trees affected by CSD host a strain of CTV and, with 99.7% association, a spherical virus denoted citrus sudden death-associated virus (CSDaV). This virus is a tentative member of the genus *Marafivirus* (family *Tymoviridae*), has isometric particles about 30 mn in diameter, a ssRNA genome 6805 nt in size with a high cytosine content (37.4%), encompassing two ORFs. ORF1 codes for a large polyprotein with a predicted M_r of 240 kDa which is processed to yield the replication-associated proteins (methyltransferase, helicase, and RNA-dependent RNA polymerase), a papain-like protease, and two CP subunits 21 and 22 kDa in size, respectively. One of the CP subunits is produced from the cleavage of the C-terminus of the polyprotein, the other is translated from a subgenomic RNA. ORF2 codes for a putative protein 16 kDa in size, with some relationship with a movement protein of viruses belonging to the sister genus *Maculavirus* (family *Tymoviridae*). It has not been ultimately established whether CTV or CSDaV are the causal agents of CSD, but their consistent association with the disease suggests that both may have a bearing on its etiology.

Epidemiology

CSD is transmitted by grafting and can be disseminated with propagation material. Natural spreading is by aphids (*Toxoptera citricida*) with a temporal and spatial pattern similar to that of citrus tristeza. CSDaV was detected in *T. citricida* that had fed on infected trees and was transmitted experimentally to healthy plants. Since marafiviruses are not aphid-borne, it was hypothesized that CSDaV may use CTV as a helper virus.

Control

Production and distribution of healthy scion material to be grafted onto tolerant rootstocks (Cleopatra mandarin, Swingle citrumelo) can help restraining the spread of CSD in newly established groves. Grafting the affected trees above the graft union with seedlings of tolerant roostocks allows their recovery.

See also: Pepino Mosaic Virus; Plant Virus Diseases: Economic Aspects; Plant Virus Diseases: Fruit Trees and Grapevine; Virus Databases.

Further Reading

Boubals D (2000) Le dépérissement de la Syrah. Compte-rendu de la réunion du Groupe de Travail National. *Progrés Agricole et Viticole* 117: 137–141.

Célix A, López-Sesé A, Almarza N, Gómez-Guillamón ML, and Rodríguez-Cerezo E (1996) Characterization of cucurbit yellow stunting disorder virus, a *Bemisia tabaci*-transmitted closterovirus. *Phytopathology* 86: 1370–1376.

Duffus JE, Liu HY, and Wisler GC (1996) Tomato infectious chlorosis virus – A new clostero-like virus transmitted by *Trialeurodes vaporariorum*. *European Journal of Plant Pathology* 102: 219–226.

Greif C, Garau R, Boscia D, *et al.* (1995) The relationship of grapevine leafroll-associated virus 2 with a graft incompatibility condition of grapevines. *Phytopathologia Mediterranea* 34: 167–173.

Lecoq H, Desbiez C, Delécolle B, Cohen S, and Mansour A (2000) Cytological and molecular evidence that the whitefly-transmitted cucumber vein yellowing virus is a tentative member of the family *Potyviridae*. *Journal of General Virology* 81: 2289–2293.

Legin R and Walter B (1986) Etude de phénomènes d'incompatibilité au greffage chez la vigne. *Progrés Agricole et Viticole* 103: 279–283.

Maccheroni W, Allegria MC, Greggio CC, *et al.* (2005) Identification and genomic characterization of a new virus (*Tymoviridae* family) associated with citrus sudden death disease. *Journal of Virology* 79: 3028–3037.

Meng B and Gonsalves D (2003) Rupestris stem pitting associated virus of grapevines: Genome structure, genetic diversity, detection, and phylogenetic relationship to other plant viruses. *Current Topics in Virology* 3: 125–135.

Roman MP, Cambra M, Juares J, *et al.* (2004) Sudden death of citrus in Brazil: A graft-transmissible bud union disease. *Plant Disease* 88: 453–467.

Rubio L, Soong J, Kao J, and Falk BW (1999) Geographic distribution and molecular variation of isolates of three whitefly-borne closteroviruses of cucurbits: Lettuce infectious virus, cucurbit yellow stunting disorder virus, and beet pseudo-yellows virus. *Phytopathology* 89: 707–711.

Soler S, Prohens J, Díez MJ, and Nuez F (2002) Natural occurrence of *Pepino mosaic virus* in *Lycopersicon* species in Central and Southern Peru. *Journal of Phytopathology* 150: 49–53.

Uyemoto JK, Rowhani A, Luvisi D, and Krag R (2001) New closterovirus in 'Redglobe' grape causes decline of grafted plants. *California Agriculture* 55(4): 28–31.

Van der Vlugt RAA, Cuperus C, Vink J, Stijger ICMM, Lesemann DE, Verhoeven JTJ, and Roenhorst JW (2002) Identification and characterization of Pepino mosaic potexvirus in tomato. *Bulletin OEPP/EPPO Bulletin* 32: 503–508.

Verhoeven JTJ, van der Vlugt RAA, and Roenhorst JW (2003) High similarity between tomato isolates of *Pepino mosaic virus* suggests a common origin. *European Journal of Plant Pathology* 109: 419–425.

Wisler GC, Duffus JE, Liu H-Y, and Li RH (1998) Ecology and epidemiology of whitefly-transmitted closteroviruses. *Plant Disease* 82: 270–279.

Emerging and Reemerging Virus Diseases of Vertebrates

B W J Mahy, Centers for Disease Control and Prevention, Atlanta, GA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Amplicon The product nucleic acid obtained from a polymerase chain reaction.
Bocavirus A genus of the family *Parvoviridae* containing bovine, canine, and human species.
Kaposi's sarcoma A skin tumor which frequently develops in young males infected with HIV.
Kawasaki disease A mucocutaneous lymph node syndrome that has features of a virus infection but so far no causative agent has been discovered. There are about 120 cases per 100 000 population in Japan, a sixfold higher incidence than in the USA.
picobirnavirus A virus containing a genome consisting of two segments of double-stranded RNA, 2.6 and 1.5 kb in length.
Sigmodontinae A subfamily of rodents in the family Muridae that contains over 500 species, confined to the American continent.
Vero cells A heteroploid cell line derived from the kidney of a normal African green monkey (*Cercopithecus aethiops*).

Introduction

It became apparent during the last two decades of the twentieth century that new infectious diseases were increasingly being recognized in the human and animal populations. This led to the establishment of a formal committee of the Institute of Medicine of the National Academy of Sciences, USA, who reported on their deliberations in 1992, in a report edited by Joshua Lederberg and Richard Shope. This was followed 10 years later by a second report, edited by Mark Smolinski, Margaret Hamburg, and Joshua Lederberg, which appeared in 2003. Among the factors they cited as contributing to emergence were microbial adaptation and change, human susceptibility to infection, climate and weather, changing ecosystems, economic development and land use, human demographics and behavior, technology and industry, international travel and commerce, breakdown of public health measures, poverty and social inequality, war and famine, lack of political will, and finally intent to harm.

Recognition of Emerging and Reemerging Virus Diseases

The advent of highly specific molecular techniques such as the polymerase chain reaction (PCR) in the early 1980s permitted the detection and grouping of viruses on the basis of genome nucleotide sequence analysis, and in several respects these techniques have replaced serological analyses for the characterization of viruses. Although it is still important to isolate viruses in cell culture for their complete characterization, it is now possible directly to detect viruses in diseased tissues by PCR, then, by sequencing the amplicon, to determine whether a new virus has emerged to cause the disease. In fact, many viruses which do not readily grow in cell culture can only be differentiated by sequence analysis. The papillomaviruses are an example. Their study was very difficult until the advent of sequence analysis, which now has revealed more than 100 types in humans, and many more in animals and birds. For differentiation, three virus genes (E6, E7, and L1) are sequenced, and if the combined sequence of these three genes differs by more than 10% from known papillomaviruses, the virus is considered to be a new type. Other viruses which have not been grown in cell culture include many caliciviruses, and the ubiquitous anelloviruses, such as torqueteno (TT) virus, which can be detected and sequenced in the blood of most humans and many other vertebrate species.

Hepatitis C virus was originally described as non-A, non-B hepatitis virus because of the severe disease it caused but the virus would not grow in cell culture, and eventually was detected in blood known to be infected with the virus by reverse transcription of the RNA present using random primers then expressing the resultant DNA in the bacteriophage lambda gt 11. Thousands of clones were screened using patient blood as a source of antibody before positive clones were detected, which then allowed the development of enzyme immunoassays that could detect the virus in blood and so were used to screen blood destined for transfusion, saving millions of lives worldwide. Once the complete genome of hepatitis C virus was sequenced, it became apparent that there are many different genotypes circulating in the world, with different pathogenic properties.

Nucleotide sequence analysis has also been extremely useful in tracing the origins of viruses. For example, when hantavirus pulmonary syndrome, caused by a bunyavirus

of rodents, Sin Nombre virus, was initially detected in 1993 in the Four Corners region of Western USA, it was found that rodents inside a house where people had been infected carried a virus identical in sequence to virus isolated from human cases. However, rodents caught at various distances from the house had increasingly variable genome RNA sequences, providing strong evidence that these rodents, deer mice (*Peromyscus maniculatus)*, were the source of the infection. Subsequently more than 30 other hantaviruses, some of which also cause hantavirus pulmonary syndrome in humans, were isolated from rodents throughout North and South America. Each new virus seems to be associated with a different genetic variant of rodent host, and all rodents that carry the virus belong to the subfamily Sigmodontinae, unique to the American continent.

A particularly powerful tool for the initial recognition of an emerging virus is the application of immunohistochemistry to diseased tissues. Provided a comprehensive collection of antibodies is available, the particular virus or related group of viruses can often be detected. For example, when Hendra virus first appeared in 1995 in Australia, causing the death of a horse trainer and 14 of his horses, antibody against the virus was sent to the Centers for Disease Control and Prevention (CDC). Then, in 1999, CDC was asked to investigate a newly emerged epidemic that had appeared in Malaysia, killing more the 100 people and causing disease in many pigs. Initially, it was suspected to be caused by a virus related to Japanese encephalitis virus, but a virus was isolated from the pigs that replicated in Vero cells, and reacted in an immunofluorescence test against the Hendra virus antiserum. This could subsequently be used on patient tissues to study the pathogenesis of the disease, and after comparison of the genome sequences of Hendra virus and Nipah virus they were found to be closely related and are now classified in the genus *Henipavirus* of the *Paramyxoviridae*.

Finally, molecular methods can be used to detect new, emerging viruses in the absence of disease in the host. In 2001, Allander and colleagues searched for RNA viruses in human respiratory secretions using random primer PCR, and discovered a hitherto unknown parvovirus with a sequence related to the bovine and canine parvoviruses, which are grouped together in the genus *Bocavirus*. The new virus was called human bocavirus, and many research groups worldwide have now confirmed the presence of the virus, particularly in pediatric samples, although it is still not certain how important this virus is in causing morbidity and mortality. Their method also amplified a human coronavirus from the respiratory samples, and when sequenced this turned out to be HKU1, a recently emerged coronavirus detected by scientists at Hong Kong University. It is possible that a systematic search of human samples using such molecular techniques might reveal more hitherto unknown human viruses.

Human Demographics and Behavior

In some cases, the emerging viruses themselves have contributed to other viruses emerging and reemerging in the population. This is especially true of human immunodeficiency virus (HIV), the cause of acquired immune deficiency syndrome (AIDS), which rapidly spread following its emergence in the early 1980s to infect more than 40 million people worldwide by the end of the twentieth century. Because of its severe effects on the immune system, the virus leads to numerous other infections in the HIV-infected population. For example, picobirnaviruses, that had been detected in fecal samples from chickens and rabbits, were difficult to detect in human fecal samples until a cohort of men with AIDS was examined, and in these humans picobirnavirus was detected for the first time. Some rare diseases have become common in persons with AIDS. For example, the human polyomavirus known as JC virus can cause the rare brain disorder known as progressive multifocal leukoencephalopathy (PML). Normally, the virus remains dormant in the kidney, but in HIV-infected individuals the HIV-encoded transactivator Tat acts as a transactivator of JCV leading to PML which progresses to death within 4 months after infection. Other important virus infections which emerge in AIDS patients are human herpesviruses (cytomegalovirus, herpes simplex viruses 1 and 2, varicella-zoster virus, and human herspesvirus 8, which causes Kaposi's sarcoma).

HIV is mainly spread through sexual activity between an infected and a noninfected person, and is most common in those who indulge in high-risk sexual behavior with multiple partners. It can also be transmitted by direct contact with infected blood, and is common in persons who indulge in intravenous drug use, particularly when needles, syringes, or equipment used to prepare drugs for injection are shared. It is therefore an example of a virus disease which is dependent on risky human behavior for its maintenance in the human population.

The ability of such new infections to spread in the population has been greatly enhanced by population growth and ease of movement as a result of rapid air travel. A dramatic recent example of this was the appearance of the coronavirus causing severe acute respiratory syndrome (SARS) in late 2002 which spread by air travel from a single infected Chinese physician who infected 12 persons in a Hong Kong hotel. These infected persons then traveled by air and spread the infection to more than 8000 individuals worldwide, 10% of whom died. The virus then apparently receded from the human population in July 2003. Only recently was it discovered that the SARS coronavirus has a natural reservoir in Chinese horseshoe bats (*Rhinolophus sinicus*). Some other species such as Himalayan palm civets and raccoon dogs from which the virus has been isolated may serve as amplification hosts.

Following the recognition of the human coronavirus SARS, research on coronaviruses intensified, and this led to the discovery in 2004 of two previously unrecognized human viruses, one found by Hong Kong University, called HKU1 virus, and another reported almost simultaneously from the Netherlands, called NL63, and from Yale University, called New Haven coronavirus. The latter viruses probably represent two isolates of the same virus species. They are clearly associated with lower respiratory tract infection in children, but initially it was claimed that New Haven coronavirus was also associated with Kawasaki disease in children. This intriguing claim was rapidly investigated and refuted by several different groups in Japan, Taiwan, and elsewhere, and the cause of Kawasaki disease, which has features resembling a virus infection, remains unknown.

Zoonotic Diseases

A majority of recent emerging virus diseases have been zoonoses (i.e., diseases transmitted from animals to humans under natural conditions). Some of the more important of these include HIV-1, which was transmitted to humans from chimpanzees in Central Africa around 1931, and HIV-2, transmitted from sooty mangabeys to humans in West Africa around 1940.

Other important recent examples are the viruses of the genus *Henipavirus*. Hendra virus was first recognized through a disease outbreak in some horse stables in Hendra, Queensland, Australia, when 14 horses and their trainer died from pulmonary disease with hemorrhagic manifestations in 1994. The reservoir of the virus was found to be in large fruit-eating bats (*Pteropus* spp.), and one year later a horse farmer 600 miles away in Mackay, Queensland, died of encephalitis from the same virus. Then, in 1999, a related virus was discovered in Malaysia following a major outbreak of respiratory disease in pigs and neurological disease in humans in their close contact. More than 100 humans died, and in a successful effort to control the disease 1.1 million pigs were slaughtered. The causative virus was isolated from a fatal human case that had lived in Nipah River Village, and so was named Nipah virus. Hendra and Nipah viruses are clearly members of the family *Paramyxoviridae*, but have been placed in a separate genus as their RNA genome is about 19 kb in length, larger than that of any other paramyxovirus.

Nipah virus, like Hendra virus, was found to have a reservoir in *Pteropus* bats, and has since been identified in fatal human disease outbreaks in India in 2003 and Bangladesh in 2004.

Other new viruses which apparently have a reservoir in fruit bats include Menangle virus, a new paramyxovirus which emerged in a commercial piggery near Sydney, Australia, to cause stillbirths and abortion in pigs. Menangle virus also caused disease in two workers in the piggery. A new virus related to Menangle virus emerged during an investigation of urine samples from *Pteropid* bats collected on Tioman island, off the coast of Malaysia, in 2001, and was named Tioman virus. During the same investigation, a new orthoreovirus was isolated from *Pteropus hypomelanus* in 1999 and called Pulau virus, and more recently a related orthoreovirus called Melaka virus was isolated from a human case of acute respiratory disease in Melaka, Malaysia. Serological studies of sera collected from human volunteers on Tioman island showed that 13% had antibodies against both Pulau and Malaka viruses.

Another important group of zoonotic diseases are rodent-borne, and caused by members of the genus *Hantavirus* of the family *Bunyaviridae*. These viruses first emerged during the Korean War of 1950–52, when thousands of UN troops developed a mysterious disease with fever, headache, hemorrhage, and renal failure with a fatality rate of 5–10%. It was more than a quarter of a century before the causative virus was isolated from field mice in Korea, and named Hantaan virus, the cause of hemorrhagic fever with renal syndrome (HFRS) in humans.

Then, in 1993, a new hantavirus emerged in the Four Corners region of Southwestern USA as the cause of a severe acute respiratory disease syndrome, with a fatality rate close to 40%, and named Sin Nombre virus. This virus was shown to be transmitted to humans by inhalation of virus present in the urine, feces, or saliva of deer mice (*Peromyscus maniculatus*). It seems likely that this disease had existed for many years, and was only recognized in 1993 because of a clustering of human cases as a result of a regional upsurge in the rodent population resulting from climatic conditions causing increased availability of rodent food. Fortunately, in most of these infections, humans appear to be a dead-end host, and transmission between humans does not occur except with the Andes virus in South America.

Rodent-borne viruses of the family *Arenaviridae* also cause a number of serious zoonotic diseases in humans. The 'Old World' arenaviruses such as Lassa fever virus have been known for some time, but still cause thousands of fatal hemorrhagic fever cases every year in West Africa. However, 'New World' arenaviruses such as Junin virus causing Argentinian hemorrhagic fever and Machupo virus causing Bolivian hemorrhagic fever have long been recognized in South America. Recently, new arenaviruses have emerged, probably as a result of deforestation, which results in rodents seeking shelter in human habitation, and brings them into closer contact with people. These viruses include Guanarito virus that causes Venezuelan hemorrhagic fever with 36% mortality rate from confirmed cases, and Sabia virus isolated in 1990 that causes Brazilian hemorrhagic fever with a high fatality rate, including two laboratory acquired cases.

Rabies is a zoonotic disease of great antiquity that has mainly been associated with carnivores, such as dogs. The virus is excreted in the saliva of infected animals, and following infection it moves through the nervous system to attack the brain, causing aggressive behavior which results in the animal biting humans and animals with which it comes into contact and thereby spreading the virus infection. Fortunately, due to early work by Louis Pasteur, a vaccine was developed that protects humans or other animals from infection, and can also be given immediately post exposure, and the domestic dog population in the developed world is vaccinated and does not pose a risk to humans. However, in some developing countries, it is not uncommon for a rabid dog to bite and infect more than 25 people before it can be put down, and worldwide there are still some 30 000 human rabies deaths per year. Using molecular sequencing techniques, it is now possible to distinguish the genotypes of rabies viruses associated with different species of host, as the virus has become adapted through frequent transmission between members of the same host species. In the USA, there are six recognized terrestrial animal genotypes, in raccoons in eastern states, skunks in north-central states, skunks in south-central states, coyotes in southern Texas, red foxes in Alaska, gray foxes in Arizona, and several genotypes associated with particular species of bat. In fact, most fatal cases of human rabies in the USA can now be traced to bats, which are often not detected when the person is bitten; so rabies is not suspected and vaccination is not undertaken until the disease has taken hold.

Ecological Factors Favoring Virus Emergence

Many important virus diseases are spread by arthropods, and exposure to new arthropods and the viruses they carry is critical to the emergence of new virus diseases. Dengue hemorrhagic fever is caused by dengue virus which is transmitted mainly by the Asian mosquito (*Aedes albopictus*), and dengue fever is one of the most rapidly emerging diseases in tropical regions of the world. There are four serotypes of dengue virus, and it seems that consecutive infections with two antigenic types can lead to the more serious disease of dengue hemorrhagic fever with shock syndrome, which, if untreated, can result in up to 50% mortality. Unfortunately, through the importation of vehicle tires containing water from Korea, the Asian mosquito was introduced into the USA, and is now present in several regions of the Southern states. It can act as a vector not only for dengue virus, but also for California encephalitis virus.

In Europe, the emergence of two important animal diseases has occurred through the movement of arthropod vectors into the Iberian Peninsula. African horse sickness virus causes a disease that can be fatal to horses, mules, and donkeys, and is transmitted by nocturnal biting flies of the genus *Culicoides*. These were introduced inadvertently into Spain, and the disease is now endemic around Madrid and regions to the south. African swine fever virus is transmitted by ticks of the genus *Ornithodorus* and it causes a fatal disease resembling classical swine fever in domestic pigs. It first emerged in Portugal and Spain in 1957, France in 1964, Italy in 1967, and Cuba in 1971. Through slaughter of infected animals, the disease was eradicated from Europe, except Sardinia, by 1995.

The most recent dramatic example of the movement of a virus vector is provided by West Nile virus, a flavivirus first isolated in Uganda in 1937. This virus uses birds as a reservoir host, and is transmitted from birds to humans and other vertebrates by mosquitoes. In 1999, cases of encephalitis in New York were found to have been caused by a strain of West Nile virus that was phylogenetically similar to a virus isolated from geese in Israel.

At the same time, many birds, especially corvids, began dying in New York State. Since the introduction in 1999, West Nile virus has become well established throughout the USA and moved north into Canada and south into the Caribbean and into Mexico. It is not known how the virus moved from Israel to the USA, but the most reasonable explanation is that it was carried in an infected mosquito or possibly an infected bird in the hold of an aircraft. Transmission by an infected human seems less likely since the titer of virus in human blood is usually too low for efficient mosquito transmission.

It is clear, nevertheless, that once it arrived in North America, West Nile virus found an extremely favorable environment with abundant avian and arthropod hosts that facilitated its spread throughout the American continent.

Prospects

The emergence of new viruses is likely to continue as viruses evolve and find new ecological niches in the human and animal population. It is noticeable that most newly recognized viruses have been RNA viruses, perhaps since RNA evolves at a faster rate than DNA, for which host cells have developed efficient proofreading enzymes. It will be important in the future to detect new viruses before they can emerge to cause disease in the population. The SARS epidemic provides an excellent example. Before the epidemic, only two human coronaviruses were known, human coronaviruses 229E and OC43. Despite the fact that serious coronavirus diseases were well known in other vertebrates, such as feline infectious peritonitis and avian infectious bronchitis virus, it was not until the SARS epidemic that research on human coronaviruses led to the discovery of three new human coronaviruses – SARS, HKU1, and NL63/New Haven.

There are other genera of viruses that cause serious disease in animals but have not been adequately investigated in humans. An example is the genus *Arterivirus*, which has members causing serious disease in horses and pigs, but has not been reported at all in humans. This could be a worthwhile area for future investigation.

Another critical factor in the future control of emerging viruses is better vector control. When mosquito control was conducted using DDT, dengue fever virus was virtually eliminated from the Americas in the 1970s, but environmental concerns led to the widespread banning of the use of DDT, so that since the 1980s there has been a considerable expansion of dengue fever in South America, with the appearance of dengue hemorrhagic fever there for the first time. There is a real need to improve mosquito control measures to control this disease. Although there are prospects for a dengue virus vaccine, this is so far not available.

Finally, one of the most important viruses that continue to emerge in different antigenic forms is influenza virus. The main reservoir of influenza viruses is in birds, and over the past century several pandemics of influenza have emerged, the most serious of which was in 1918. Pandemic strains usually arise by a process of antigenic shift, where one of the genes encoding the hemagglutinin and/or the neuraminidase of influenza virus is replaced by one from birds. New pandemics occurred in 1918 (H1N1 subtype), 1957 (H2N2 subtype), and 1968 (H3N2 subtype). Since 1968, there have been no new pandemics, but it is widely expected that another will occur. At the time of writing, there is worldwide concern that a highly pathogenic avian influenza virus (H5N1 subtype), which has caused some human infections and deaths in persons in close contact with infected birds, might mutate or recombine to generate a virus which would be highly transmissible in the human population. Plans are being developed in many countries and by the WHO to try to prepare for such an event by generating possible vaccines against such a virus and stockpiling antiviral drugs.

See also: Emerging and Reemerging Virus Diseases of Plants; Epidemiology of Human and Animal Viral Diseases.

Further Reading

Choo QL, Kuo G, Weiner A, *et al.* (1992) Identification of the major, parenteral non-A, non-B hepatitis agent (hepatitis C virus) using a recombinant cDNA approach. *Seminars on Liver Diseases* 12: 279–288.

Chua KB, Bellini WJ, Rota PA, *et al.* (2000) Nipah virus: A recently emergent deadly paramyxovirus. *Science* 288: 1432–1435.

De Villiers EM, Whitley C, and Gunst K (2005) Identification of new papillomavirus types. *Methods in Molecular Medicine* 119: 1–13.

Gratz NJ (2004) Critical review of the vector status of *Aedes albopictus*. *Medical and Veterinary Entomology* 18: 215–227.

Hayes EB and Gubler DJ (2006) West Nile virus: Epidemiology and clinical features of an emerging epidemic in the United States. *Annual Review of Medicine* 57: 181–194.

Hsu VP, Hossain MJ, Parashar UD, *et al.* (2004) Nipah virus encephalitis reemergence, Bangladesh. *Emerging Infectious Diseases* 10: 2082–2087.

Kahn JS (2006) The widening scope of coronaviruses. *Current Opinion in Pediatrics* 18: 42–47.

Korber B, Muldoon M, Theiler J, *et al.* (2000) Timing the ancestor of the HIV-1 pandemic strains. *Science* 288: 1789–1796.

Ksiazek TG, Erdman D, Goldsmith CS, *et al.* (2003) A novel coronavirus associated with severe acute respiratory syndrome. *New England Journal of Medicine* 348: 1953–1966.

Mahy BWJ and Brown CC (2000) Emerging zoonoses: Crossing the species barrier. *Revue Scientifique Et Technique Office International Des Epizooties* 19: 33–40.

Mahy BWJ and Murphy FA (2005) The emergence and re-emergence of viral diseases. In: Mahy BWJ and ter Meulen V (eds.) *Topley & Wilson's Microbiology & Microbial Infections,* 10th edn., pp. 1646–1689. London: Hodder-Arnold.

Mushahwar IK, Erker JC, Muerhoff AS, *et al.* (1999) Molecular and biophysical characterization of TT virus: Evidence for a new virus family infecting humans. *Proceedings of the National Academy of Sciences, USA* 96: 3177–3182.

Nichol ST, Spiropolou CF, Morzunov S, *et al.* (1993) Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science* 262: 914–917.

Smolinski MS, Hamburg MA, and Lederberg J (eds.) (2003) *Microbial Threats to Health, Emergence, Detection and Response,* 367pp. 367pp. Washington, DC: The National Academies Press.

Stephenson I (2005) Are we ready for pandemic influenza H5N1? *Expert Review of Vaccines* 4: 151–155.

Emerging Geminiviruses

C M Fauquet and M S Nawaz-ul-Rehman, Danforth Plant Science Center, St. Louis, MO, USA

© 2008 Published by Elsevier Ltd.

Glossary

Geminivirus A class of plant viruses with a genome composed of single-stranded DNA encapsidated in geminate particles.

Pandemic An epidemic at the regional or worldwide level.

Satellite An infectious molecule that depends on a helper virus for crucial functions.

Introduction

Geminiviruses, which are circular single-stranded DNA (ssDNA) plant viruses, are divided into four different genera (*Curtovirus*, *Mastrevirus*, *Topocuvirus*, and *Begomovirus*). All geminiviruses are transmitted by insects; the most devastating begomoviruses are transmitted by whiteflies (*Bemisia tabaci*). In the last 20 years, we have witnessed in many parts of the world the emergence of newly discovered or previously recorded geminivirus diseases. These emergences are attributable to several factors, including an explosion of whitefly populations in many parts of the world, an increase in human activities, and a large development of global trade introducing new plant hosts, vectors, and viruses in different ecosystems. As there are too many geminivirus emerging diseases to be discussed here, we will restrict our description to the three most important diseases caused by begomoviruses that will illustrate the impact of the various factors influencing geminivirus emergences in the world.

The Tomato Yellow Leaf Curl Disease

Geographical Distribution

The tomato yellow leaf curl virus (TYLCV) was originally reported/isolated from Israel and was described in the 1960s, mostly infecting tomatoes and causing very severe symptoms and huge losses. In Spain and Italy, tomato production increased considerably in the 1980s, and closely related viruses were identified in these countries which were first mistakenly identified as new strains of TYLCVs, although the Israeli virus was subsequently identified in these countries. Furthermore, recombinants between local viruses and TYLCV were found and are now prevalent in several regions of both countries. TYLCV was later exported to several countries around the Mediterranean region and worldwide. In 1994, TYLCV was identified in the Caribbean islands and in 1997 was identified for the first time in the USA. Since then, the virus has been exported worldwide (**Figure 1**) and is present in the following countries: USA (California, Georgia, New Mexico, Arizona), Mexico, Puerto Rico, Dominican Republic, Cuba, Tunisia, Morocco, Egypt, Spain, Italy, France, Greece, Turkey, Sudan, India, China, Japan, and Australia.

Symptoms and Yield Losses

The symptoms induced by TYLCV are typically a leaf curling of the leaves with different levels of yellowing (**Figure 2**). However in a single field of infected tomato plants a variety of symptoms can be observed from green to purple leaf curling, thickening of the veins and the stems, and various levels of stunting. In some instances, the leaf surface is reduced a minimum, with large veins and thick lamina. When plants are infected at an early stage, the plant fail to produce fruits and will stay stunted. Losses are in general very high, and depending on the earliness of the infection and on the level of resistance of the variety can reach 100%. TYLCV is considered a major threat for farmers growing tomatoes, both for commercial or subsistence purposes.

Causal Agent with Classification

TYLCV belongs to the genus *Begomovirus*, in the family *Geminiviridae*. Begomoviruses are either monopartite and bipartite, meaning that their genome is composed of a single or two molecules of circular ssDNA and TYLCV is a monopartite virus. This feature does not explain the prevalence of TYLCV in the world, as there are many monopartite geminiviruses in the Old World that do not show the characteristics of a new virus emergence. Regarding the genome organization, TYLCV is a typical monopartite begomovirus coding for six open reading frames (ORFs). A total of five strains of TYLCV, called TYLCV-Iran, -Gezira, -mild, -Oman, and -Israel, have been identified, but it is mostly the Israeli strain that has, so far, spread all over the world.

Epidemiology

Begomoviruses are not seed transmitted, nor mechanically transmitted (with a few exception in lab conditions); they are only transmitted by the whitefly *Bemisia tabaci*. In the 1960s, TYLCV was found in local weeds in Israel, serving as virus reservoir, when tomatoes were planted in the field. In the 1980s in Spain, the year-round cultivation of tomatoes under plastic screen houses promoted the spread of the disease, but beans were also found as a host of the virus. Over the years, it was found that the privileged mode of spreading of the disease in the world was via international trade of tomato plantlets, but it was demonstrated that the green tissue attached to the fruits (sepals, stems) was a very efficient virus reservoir for local whiteflies. In several instances (Dominican Republic and Florida), it was observed that TYLCV became the prevalent virus, displacing the local geminiviruses previously infecting tomatoes. This could be due to the fact that TYLCV is very aggressive and could become prevalent because of a better fitness to the host. In addition to the trade impact, the explosion of the B-type population of whiteflies can explain the worldwide spread of TYLCV. This particular B-type population of whiteflies is very ubiquitous and infects more than 1000 different plant hosts, providing the insect with enormous resources for winter survival and local adaptation.

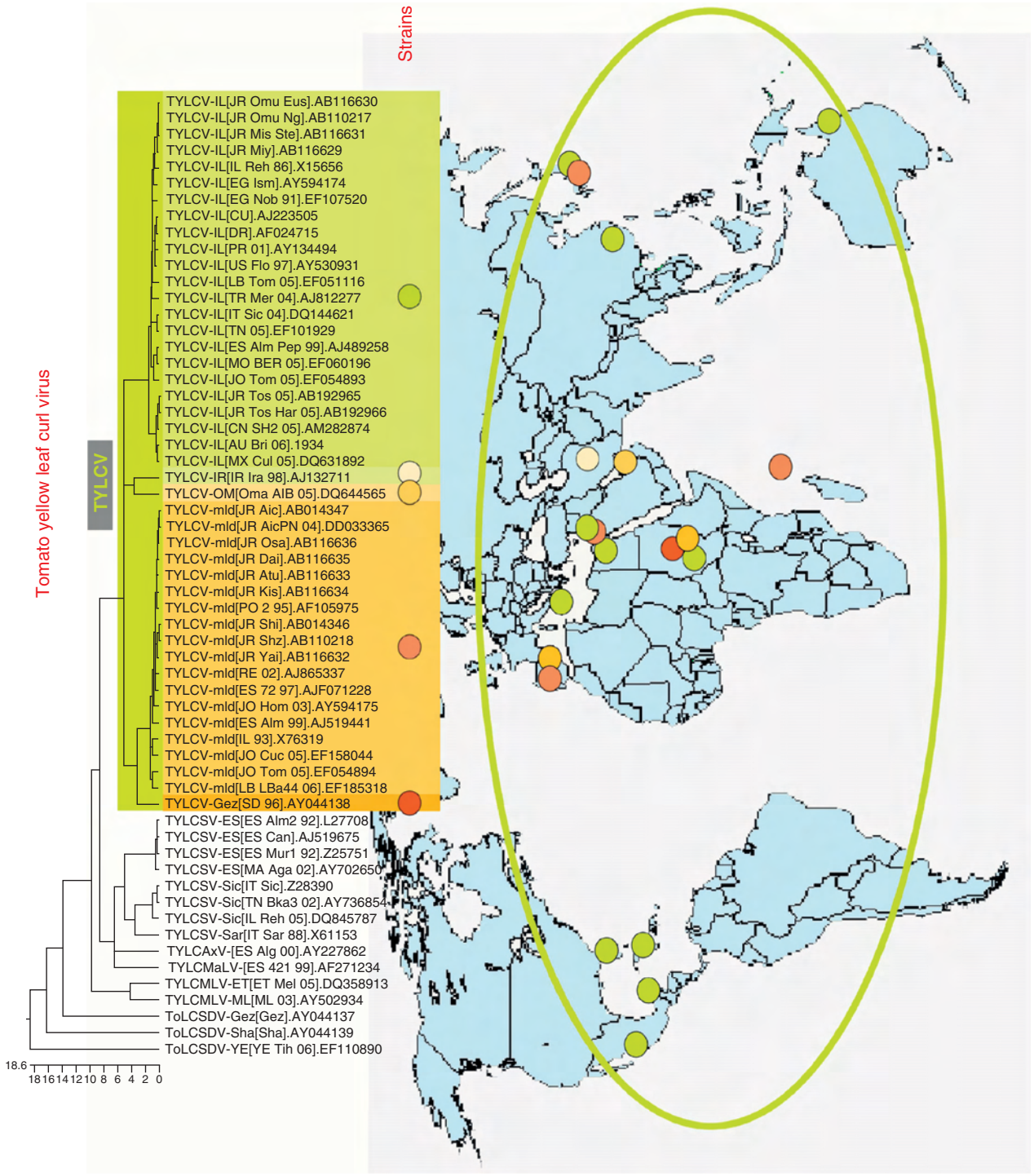


Figure 1 World map on which each of the 44 members representing five begomovirus strains of the TYLCV species have been indicated. The abbreviated name and accession numbers of the isolates are listed in phylogenetic tree in the upper part of the diagram. The phylogenetic tree has been built using their complete A component sequence. The Clustal V algorithm of the program MegAlign from DNAStar has been used and distances in percentage difference are indicated on the left. The tree shows a partition in six major clusters, one for each of the six designated viruses, TYLCV, tomato yellow leaf curl Sardinia virus (TYLCSV), tomato yellow leaf curl Axarquia virus (TYLCAxV), tomato yellow leaf curl Malaga virus (TYLCMalV), tomato yellow leaf curl Mali virus (TYLCMLV), and tomato leaf curl Sudan virus (ToLCSDV). These six species constitute the so-called TYLCV cluster of the Old World begomoviruses. The individual viruses composing the TYLCV cluster are positioned on the world map, as filled circles of various colors representing their pertaining to one of the six specific TYLCV strains, as indicated in the colored boxes at the bottom of the tree. On the world map, the individuals pertaining to the TYLCV species are shown with filled circles.

Control

Geminiviruses are in general very difficult to control. The most efficient way is the use of virus-resistant varieties when they are available, acceptable, and affordable. The cost of such varieties is limiting their use to industrial tomato plantations and therefore most of the tomato fields in poor farmers fields are extremely susceptible. Another limiting factor is the taste and shape of virus-resistant tomatoes that do not match local requirements and encourage the use of susceptible varieties. Controlling the insect, by chemical means, is practically impossible and very expensive. Agricultural practices, although efficient in certain conditions, have been of little help to control TYLCV in a practical manner.



Figure 2 Typical symptoms of TYLCV on tomato in a field in Jordan. Courtesy of Mohammed Abhary.

The African Cassava Pandemic

Geographical Distribution

The cassava geminivirus pandemic in Africa started in the early 1990s and is still going on today. It started in Uganda in 1994, invaded several countries in East Africa around Lake Victoria, before crossing the mountains in Rwanda-Burundi, to infect the entire Congo Basin and invade up to Gabon and the south of Cameroon (**Figure 3**). Cassava plants were already infected with African cassava mosaic

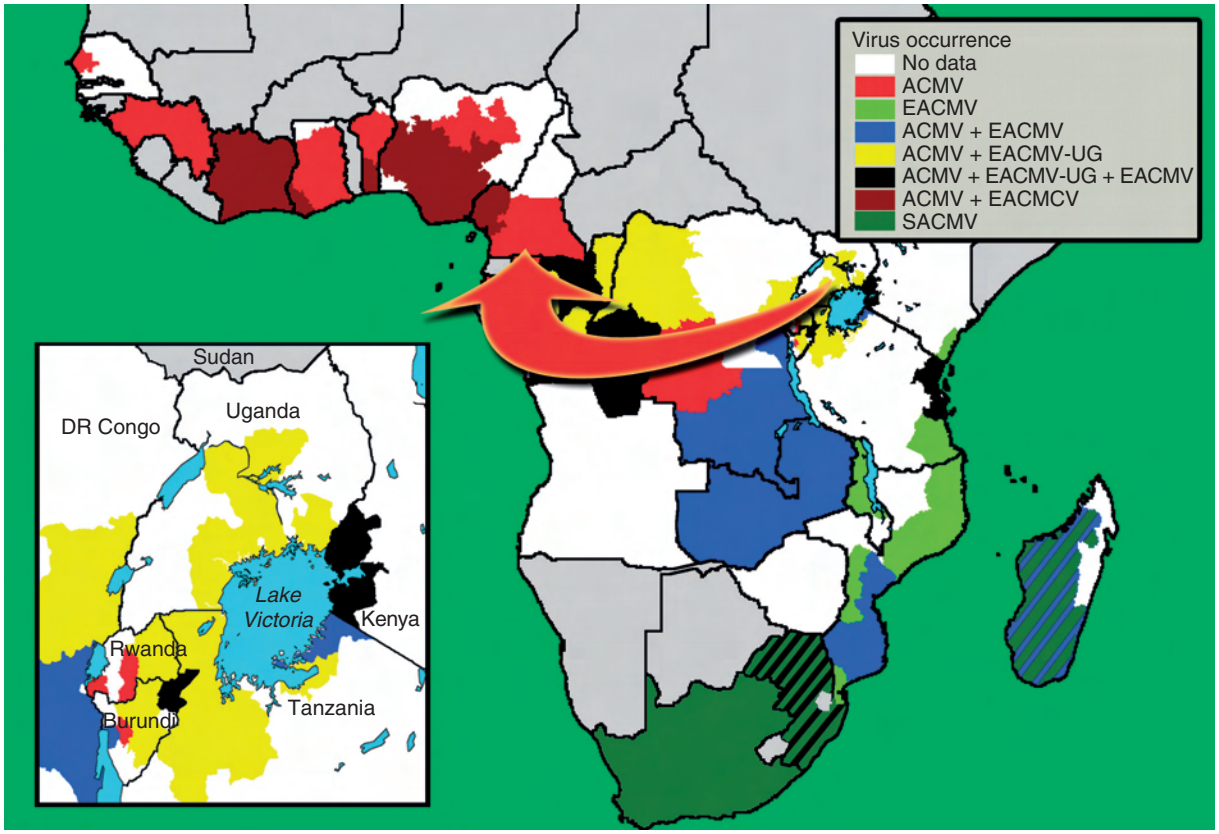


Figure 3 Distribution of cassava mosaic geminiviruses in Africa obtained from CMD surveys in Africa, 1998–2004. ACMV, African cassava mosaic virus; EACMV, East African cassava mosaic virus; EACMV-UG, East African cassava mosaic virus – Uganda; EACMCV, East African cassava mosaic Cameroon virus; SACMV, South African cassava mosaic virus.

virus (ACMV) and other viruses, but became superinfected with an Ugandan strain of East African cassava mosaic virus (EACMV-UG), which displaced other viruses.

Symptoms and Yield Losses

Symptoms due to the EACMV-UG are extreme reduction of the surface leaf, yellowing of the lamina, and downcurling of the leaves. The entire infected plants look yellow compared to healthy plants, with reduced size and extremely limited foliage. Furthermore, in many instances, plants are infected with both EACMV-UG and ACMV, and these two viruses synergize to cause a very dramatic syndrome, called 'candlestick syndrome'. In this instance, the stem is thick and turns purple, the leaves are even more reduced in size, with very thick veins and almost no lamina (**Figure 4**(**d**)). Cassava is normally propagated using the stem, but if the plants have been co-infected with ACMV and EACMV-UG, the stems cannot be used anymore and cassava cannot be propagated to the next season. This caused dramatic cassava shortages in Uganda between 1994 and 2000, estimated to losses amounting to 6 million tons per year, and famine and death for thousands of people.

Causal Agent with Classification

ACMV and EACMV-UG are both members of the genus *Begomovirus*, in the family *Geminiviridae*, similarly to TYLCV above. These viruses however are bipartite viruses, with a second DNA molecule coding for two movement proteins. Furthermore, EACMV-UG is a recombinant between ACMV and EACMV, with a fragment of 550 nt from the core part of the coat protein of ACMV integrated into the EACMV genome. Although there is no direct proof that this recombinant has any specific advantage over the nonrecombinant EACMV, it is a fact that the EACMV-UG strain is present in all the plants that show severe symptoms and is strictly correlated with the development of the pandemic across the African continent. In addition, these two viruses do synergize to cause a dramatic 'candlestick' symptom, preventing the cuttings from these infected plants to grow for the next generation. The explanation for this phenomenon resides in the fact that each virus codes for a very strong and differential gene silencing suppressor, and when both suppressors are present, the infected plant collapses from the resulting dual virus infection. This is the first time that such synergism between two geminiviruses has been found.

Epidemiology

ACMV and EACMV-UG are spread by the whitefly *Bemisia tabaci* and through the cassava cuttings, as there is no virus-clean material propagation system in place in Africa. The explosion of the cassava mosaic pandemic in Africa is currently explained by a conjunction of several mechanisms. (1) The synergism between the two viruses certainly played a key role, as it translates into a huge increase in viral DNA in the tip of the plants by 30–100 times, thereby enhancing the whitefly transmission capacity. (2) The recombinant fragment could provide an advantage to EACMV-UG, as it has been shown that the whitefly transmission epitopes are coded by this fragment and that there has been co-adaptation as far as the rate of transmission is concerned, between the local whitefly and viruses. (3) Finally, there has been a tremendous increase in whitefly populations adapted to cassava in Uganda, where the epidemic started. The conjunction of these three elements is believed to be at the triggering and maintenance of the cassava mosaic disease pandemic in Africa.

Control

Similar to the TYLCV, the only available method of control of the cassava mosaic disease is the use of virus-resistant cassava cultivars. Fortunately, such plants were made available to farmers in Uganda by the end of the 1990s, and the use of these plants allowed to restore the cassava production in Uganda. This however did not stop

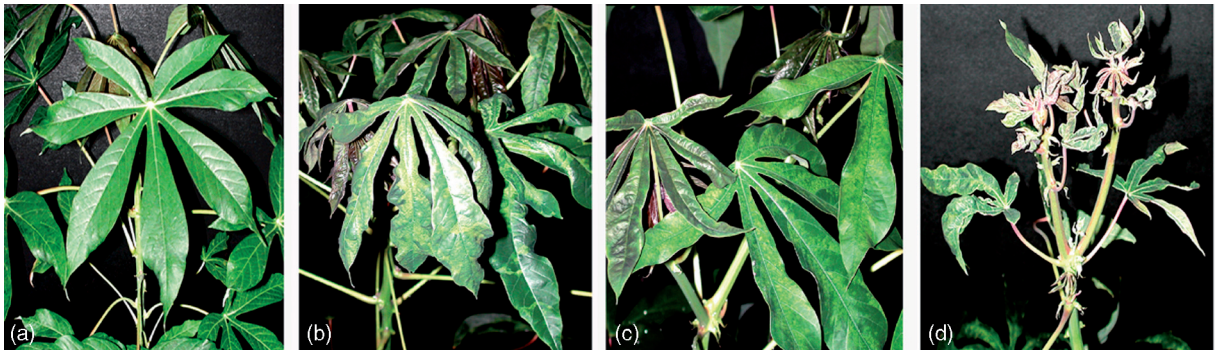


Figure 4 Typical symptoms of cassava infected with ACMV (b) and EACMCV from Cameroon (c), and synergism between the two viruses expressing the 'candlestick' symptoms (d), compared to the control healthy cassava leaves (a).

the pandemic that is still going on in West Africa. Furthermore, many of the farmers in Uganda, and elsewhere, are now going back to their preferred cassava plant material that is not virus resistant, but highly appreciated for their organoleptic and processing qualities. Consequently, more virus-resistant cassava cultivars, acceptable to farmers, are needed.

The Cotton Leaf Curl Disease in the Indian Subcontinent

Geographical Distribution

The leaf curl disease of cotton (*Gossypium hirsutum* L.) was first observed in Multan, Pakistan, in 1967. The disease reappeared in 1987 in its epidemic form in most of the cotton-growing areas of Pakistan. In 1992, the disease was observed throughout the central and southern parts of Punjab in Pakistan. During 1997–98, the disease was established in the border areas of India (Rajasthan, Haryana, and Punjab), joining the southern Punjab of Pakistan. The most devastating virus known as cotton leaf curl Multan virus (CLCuMV) is attributed to huge losses throughout the Indian subcontinent. The estimated direction of movement for the disease is from the center of Pakistan to northern and southern districts of India. Nowadays, the disease can be observed in all primary or secondary cotton-growing areas of the Indian subcontinent (**Figure 5**).

Symptoms and Losses

Cotton leaf curl disease (CLCuD) is not a seed-borne disease, but is only transmitted through whiteflies. After 2–3 weeks of virus inoculation by the insects, vein thickening on young leaves can be observed. Later, upward or downward curling of leaves starts and a cup-shaped leaf enation appears at the underside of the leave (**Figure 6**). The infected plants remain stunted throughout their life cycle and may lose 100% of the total yield. Disease spread can be observed with the wind direction. During the last two decades, the disease caused heavy cotton yield losses which reached up to US$ 5 billion in Pakistan in 1992–97. Recently, the introduction of relatively resistant varieties has selected a more aggressive virus in 2002 in central Pakistan, called cotton leaf curl Burawalia virus (CLCuBuV), and this strain is now prevailing in all cotton-growing areas of Pakistan.

Causal Agent and Classification of Cotton Leaf Curl Disease

The begomoviruses associated with CLCuD are monopartite begomoviruses with an associated ssDNA satellite molecule known as DNA-β (beta satellite).

According to sequence analysis of the available sequences in gene bank, there are viruses belonging to seven different species involved in the etiology of CLCuD named as, CLCuMV, cotton leaf curl Kokhran virus (CLCuKV), cotton leaf curl Rajasthan virus (CLCuRV), cotton leaf curl Allahabad virus (CLCuAV), cotton leaf curl Burawalia virus (CLCuBuV), cotton leaf curl Bangalore virus (CLCuBV), and papaya leaf curl virus (PaLCuV). Cotton leaf curl Bangalore virus is only present in the Bangalore district of southern India, while viruses belonging to the other six species are widespread throughout the cotton-growing areas of the north of the Indian subcontinent.

The area of maximum diversity for CLCuD is in central parts of Pakistan, hosting viruses belonging to five species of cotton leaf curl viruses (CLCuVs) and one of PaLCuV.

CLCuD is in reality caused by a complex of viruses associated with virus satellites. A monopartite geminivirus, belonging to different species of viruses, is typically associated with a beta satellite (DNA-β) or a DNA-1 satellite. If DNA-β's have only been found associated with geminiviruses, DNA-1 satellites have originally been found associated to nanoviruses. Both satellite genomes are encapsidated by the geminivirus capsid protein, and thereby both are transmitted by whiteflies. Full-length clones of several CLCuVs DNA-A were unable to reproduce the symptoms in cotton and other different hosts such as tobacco. Infectious clones of DNA-1 were also not capable of inducing symptom development of CLCuVs in the inoculated plants.

With the discovery of DNA-β, it was proved that both DNA-A and DNA-β are necessary to induce typical symptoms of CLCuD, while DNA-1 is not necessary to induce the symptoms and is seldom found in association with the disease complex. At present, the origin of DNA-β is unknown but it can be transreplicated by diverse geminiviruses associated with the CLCuD complex. The DNA-β is only ±1300 nt long and codes only for one ORF called βC1. The gene βC1 codes for a strong suppressor of gene silencing, thereby providing a tremendous advantage to the cognate helper virus. DNA-1 satellite codes for a Rep protein that allows this satellite to be replicated on its own, but the satellite depends on the helper virus for all the other functions, such as gene-silencing suppression, movement, and transmission. DNA-β is dependent on DNA-A for replication, movement, and transmission. The genome organization of DNA-A is typical of any other begomovirus and has no similarity with its associated satellite molecules. Both DNA-β and DNA-1 are approximately half the size of DNA-A, that is, 1350 nt.

Epidemiology

Cultivated cotton or upland cotton (*G. hirsutum*) was introduced into the Indian subcontinent from southern

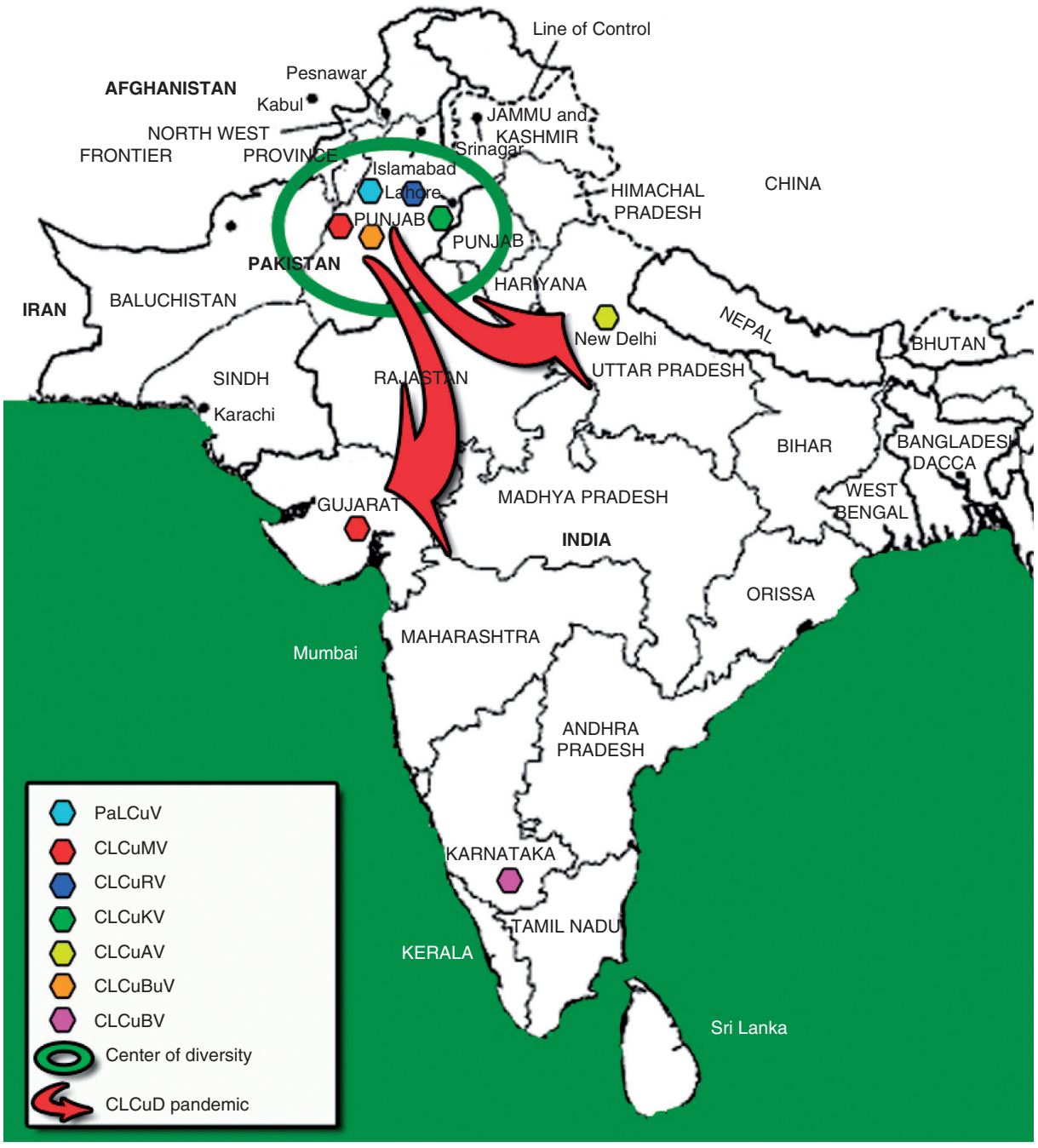


Figure 5 Localization of cotton leaf curl geminiviruses in the Indian subcontinent. PaLCuV, papaya leaf curl virus; CLCuMV, cotton leaf curl Multan virus; CLCuRV, cotton leaf curl Rajasthan virus; CLCuKV, cotton leaf curl Kokhran virus; CLCuAV, cotton leaf curl Allahabad virus; CLCuBuV, cotton leaf curl Burawalia virus; CLCuBV, cotton leaf curl Bangalore virus.

Mexico during the nineteenth century. In its native place, cotton is not associated with virus diseases as in the Indian subcontinent. CLCuD complex most probably evolved from some wild species of cotton growing over a long period of time or from other weed hosts present before the cultivation of upland cotton in this part of the world. In 1991, a new variety of cotton (S12) was introduced from Texas, because of its excellent fiber qualities; unfortunately, this new variety was hypersensitive to CLCuD and the disease propagated very quickly after this introduction. The CLCuD threat was greatly increased due to intensive cotton cultivation, monocropping pattern of agriculture, and overlapping seasons of other crop plants. CLCuVs can infect a number of diverse hosts in the



Figure 6 Downward leaf curl symptoms of cotton induced by the CLCuMV in association with the cotton leaf curl Multan beta satellite.

families Malvaceae and Solanaceae. The virus infection was not highly noticed at the early beginning of the epidemic, when perhaps it could have been possible to eradicate the disease. The disease is transmitted through whitefly in a persistent manner. The disease spread is directly correlated with the spread of the whitefly population, along the prevailing wind direction.

Control

Controlling the whitefly vector population is an important aspect to control CLCuD. Insecticides used to control whitefly play a vital role in the control of disease, but still cannot completely eliminate the disease. Over the years, breeders have selected several varieties of cotton with various levels of resistance, but either this level of resistance was unsatisfactory or has been broken by the appearance of new strains of the virus, such as the CLCuBuV from Burawalia. Recently RNA interference (RNAi) has been used and shown to be efficient, in model plants, to control the disease. However, there is still a need to do more work to control the disease in crop plants. In order to create a broad-spectrum resistance to control the disease complex, there is a need of integrated efforts of conventional and genetic engineering approaches.

Conclusion

The three examples of geminivirus emergence presented here provide examples of the importance of different factors that favor the emergence of geminiviruses under very different conditions and environments. In the case of TYLCV, it is a relatively simple case with a single monopartite geminivirus that is transported, through human-based international trade, to various places in the world, and where this very effective virus overcomes existing local viruses and prevails. Although we do not know the molecular and biological basis for the prevalence of TYLCV, it is clear that this virus has a better fitness in tomato and other hosts, compared to local geminiviruses, whether they be bipartite New World geminiviruses or monopartite Old World geminiviruses.

In the case of cassava mosaic geminiviruses, the situation is more complex as it involves at least two different geminiviruses, a new whitefly population, better adapted to cassava, as well as human participation to move the viruses, and probably the whiteflies, in new environments over chains of mountains, where the disease can again explode. It is therefore the apparent conjunction of various biological and human-based activities that promoted the pandemic in Africa. On the biological side, at least three elements can be related to the emergence: (1) the encountering of two geminiviruses with differential and combinatorial gene silencing suppressors that promoted synergism; (2) the occurrence of an apparent successful recombination between these two viruses; and (3) the adaptation of a new population of whiteflies to cassava. The pandemic has been able to travel eastward more than 3500 km in 12 years, and this would not have happened without human intervention. It remains to be seen if the pandemic will continue in West Africa or if it will fade away in this new ecological zone.

The CLCuD case is even a more complex situation where human intervention by introducing new cotton varieties, highly susceptible to local unknown viruses and satellites, as well as the absence of human intervention to stop the disease, triggered the CLCuD pandemic in the Indian subcontinent, costing billions of dollars to poor farmers and impacting Pakistan economy to about 30% for years. On the virological side, this pandemic revealed a new concept, that is, the crucial role of satellites like the DNA-β for inducing symptoms and finally for allowing the whole pandemic. It is the association of a single DNA-β to a variety of helper geminiviruses that is causing the disease and not a single monopartite geminivirus. It is now obvious that recombination of both DNA-β and its geminivirus helpers is an important evolutionary mechanism to create new diversity to overcome virus resistance, revealing the power of geminiviruses and their satellites to adapt to new ecological situations.

The common factor among these three examples is that human intervention, or lack of intervention, is providing viruses and their satellites opportunities to promote

new disease emergences in combination with ecological changes, which are also linked to human impact. These dramatic pandemics, costing billions of dollars in losses and thousands of lives, offer however opportunities to the scientists to better understand the biological causes of these emerging diseases. Taking advantage of these global scale 'experiments', it may be possible to identify the relevant biological factors that could in future allow such diseases to be controlled.

See also: African Cassava Mosaic Disease; Beta ssDNA Satellites; Cotton Leaf Curl Disease; Nanoviruses; Plant Resistance to Viruses: Geminiviruses; Tomato Leaf Curl Viruses from India.

Further Reading

Briddon RW (2003) Cotton leaf curl disease, a multicomponent begomovirus complex. *Molecular Plant Pathology* 4: 427–434.
Czosnek H (ed.) (2007) *Tomato Yellow Leaf Curl Virus Disease.* New York: Springer.
Legg JP and Fauquet CM (2004) Cassava mosaic geminiviruses in Africa. *Plant Molecular Biology* 56: 585–599.
Mansoor S, Briddon RW, Zafar Y, and Stanley J (2003) Geminivirus disease complexes: An emerging threat. *Trends in Plant Science* 8: 128–134.
Polston JE and Anderson PK (1997) Emergence of whitefly transmitted geminiviruses in tomato in the Western Hemisphere. *Plant Disease* 81: 1358–1369.
Rojas MR, Hagen C, Lucas WJ, and Gilbertson RL (2005) Exploiting chinks in the plant's armor: Evolution and emergence of geminiviruses. *Annual Review of Phytopathology* 43: 361–394.

Endogenous Retroviruses

W E Johnson, New England Primate Research Center, Southborough, MA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Metazoan A multicellular animal.
Monophyletic A taxonomic grouping comprised exclusively of an ancestor and its descendants.
Phylogenetic tree A diagram of the evolutionary relationships between entities descended from a common ancestor.
Proto-oncogene A gene whose normal function is to control cell growth or differentiation, but which can be converted into a cancer-causing gene (oncogene) by mutation or disregulation.

Introduction

At the heart of the retrovirus replication cycle is a stage in which the viral genome, having been converted from single-stranded RNA into double-stranded DNA by the viral reverse transcriptase, is inserted at random into a chromosome of the infected cell. The resulting DNA 'provirus' directs expression and assembly of progeny virions. The integrated provirus itself is contiguous with the rest of the host-cell chromosome, just like any other stretch of genomic DNA sequence. Consequently, if a cell harboring a provirus continues to divide and multiply, the provirus will be inherited by daughter cells during subsequent rounds of cell division like any other cellular gene. If retroviral integration occurs in a cell belonging to germline tissue of a host organism (e.g., in an oocyte or in an embryonic cell destined to develop into germline tissue), the resulting provirus constitutes an insertional mutation, and the new allele can potentially be passed on to the next generation like any other chromosomal locus. As a new allele, the proviral locus will be subject to the processes of random genetic drift and selection and, as a result, many such events may quickly be lost. But some will spread in the population and, given enough time, may eventually become fixed. In fact, this scenario has played out many millions of times over the course of metazoan evolution, such that the genomes of almost all animal species contain numerous sequences resembling proviruses. These elements are referred to as endogenous retroviruses (ERVs). While exogenous retroviruses spread from individual to individual by infection (horizontal transmission), endogenous retroviruses, as components of the germline, are inherited in the same fashion as other genes. The two modes are not mutually exclusive, and virions expressed from ERVs can give rise to spreading infections within the host and may also be transmitted to other individuals.

Structure

The typical retroviral provirus consists of *gag*, *pro*, *pol*, and *env* open reading frames, which encode the viral structural and nonstructural proteins, bookended by two identical regulatory regions called long terminal repeats (LTRs).

No chromosomal DNA is lost as a consequence of integration; however, a short stretch of host-cell DNA (typically around 4–6 bp) is duplicated, resulting in direct repeats (DRs) of cellular sequence flanking the provirus. Important *cis*-acting sequences for replication are located within the LTRs, and primer recognition sequences for reverse transcription are located at the junctions between the 5′ LTR and *gag* (first-strand synthesis), and between *env* and the 3′ LTR (second-strand synthesis). Retroviruses use a cellular transfer RNA molecule (tRNA) as the primer for the first step in reverse transcription; thus, the primer binding site (PBS) is complementary to the 3′ end of a cellular tRNA. The second primer is derived from a purine-rich stretch of the viral RNA itself (polypurine tract or PPT). The generic structure of a provirus is therefore:

DR-5′LTR-PBS-gag-pro-pol-env-PPT-3′LTR-DR

In general, ERVs can be recognized as genomic loci conforming to this basic arrangement, even though in many cases significant portions of the original provirus may be missing (**Figure 1**). There are no viral or cellular mechanisms for precise deletion of integrated proviruses, but ERV sequences can be lost or rearranged, in whole or in part, by the same general processes affecting all chromosomal DNA. For example, homologous recombination can occur between the two LTRs of an ERV, resulting in deletion of the intervening proviral sequences and leaving behind a single LTR (or solo-LTR). Solo-LTRs are abundant in animal genomes, and in many cases may significantly outnumber intact proviruses.

Discovery and Distribution

Even prior to the discovery of reverse transcriptase in 1970 (and confirmation that retroviruses replicate through a proviral intermediate), there was some phenotypic evidence for the existence of ERV loci. Then beginning in the early 1970s, the widespread existence of ERVs was quickly revealed using genomic DNA samples and nucleic acid hybridization techniques. Novel ERV sequences were cloned initially using probes derived from existing retroviruses, by accident during the cloning and characterization of unrelated genes, and later by using a variety of polymerase chain reaction (PCR)-based approaches. By far the richest vein of new ERV sequences has come from mining the human genome databases and subsequent whole-genome sequences of other organisms.

The distribution of ERV among modern species speaks of their age. ERV families or specific ERV loci are often shared among the genomes of related species, indicating that the integration events occurred in the genomes of ancestors shared by those species. For example, a large number of ERVs are located in identical positions in humans and closely related primates, such as chimpanzees, bonobos, and gorillas. Thus, these loci must have originated in a common ancestor of these species. This also provides a lower bound to the estimated time of integration based on the approximate time of divergence of these lineages. In the case of an ERV locus shared exclusively by humans and chimpanzees, for example, the provirus will be no less than about 5 million years old, whereas a locus shared by humans, apes, and Old World monkeys will be greater than 25 million years old.

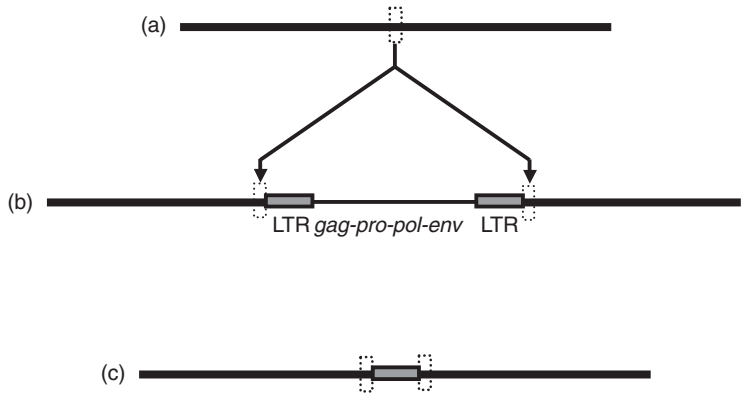


Figure 1 Structure of an ERV. Thick black line represents a segment of a host-cell chromosome. Thin line (labeled *gag-pro-pol-env*) and gray boxes (LTR) represent integrated viral sequences. Small boxes (dashed line) indicate short segment of chromosomal DNA duplicated (arrowheads) at the time of proviral integration. (a) Chromosomal segment prior to integration of a provirus. (b) Same chromosomal locus, but containing an integrated provirus. No chromosomal DNA is lost due to integration, and a short segment (~4–6 bp) is duplicated. When this structure occurs in the germline, it constitutes an ERV. At the time of integration, the LTRs of the ERV are identical, but these sequences will thereafter diverge due to the accumulation of random mutations; thus, the degree of divergence between the LTRs of an ancient ERV are a reflection of the age of the provirus. (c) Same locus, after recombination between the two LTRs, has resulted in loss of the intervening proviral sequence. Only a single copy of the LTR sequence remains, along with the flanking repeats.

In general, the wider spread an ERV-sequence family is among extant species, the more ancient that family is. More modern ERVs are found among a smaller subset of species or are even confined to a single species. (This is a generalization because viruses have the potential for horizontal, cross-species transmission; ERV sequences derived from a modern retrovirus could appear in the genomes of more than one lineage.) Modern ERVs are also more likely to be intact (and capable of expressing virus), and are often closely related to known exogenous retroviruses.

Origins

There are at least four possible scenarios to explain the presence of multicopy ERV families in the germline: (1) repeated, *de novo* infection of germline tissue by an exogenous retrovirus; (2) expression of an existing endogenous provirus with subsequent reinfections of germline tissue; (3) intracellular retro-transposition from an existing ERV; and (4) duplication of genomic DNA fragments already containing an integrated ERV (however, even in this case, the original ERV sequence must have arisen via one of the first three mechanisms). Although there is experimental proof-in-principle for all four processes, it is generally believed that scenarios involving extracellular replication and infection (i.e., the first two mechanisms) predominate in nature.

Whatever the mechanism, presence in the germline requires that integration, takes place either in cells of germline tissue or, because the provirus is maintained during cell division, in a cell lineage destined to develop into germline tissue. Once integrated, the selective forces that work on the proviral sequences will depend on a number of factors, including the state of the provirus at the time of integration. For example, did the viral genome suffer attenuating or debilitating mutations during reverse transcription? Was it intact and capable of expression at the time of provirus formation? Did integration occur in junk DNA, close to a gene, or in a gene? Was the provirus silenced after integration (e.g., by methylation), and in what developmental stages and in which tissues was the provirus expressed? In other words, whether or not a newly formed provirus will persist as an endogenous retrovirus is determined in part by its immediate effect on the survival of the infected cell and its ultimate effect on reproductive fitness of the host organism. ERVs that are strongly counterselected due to pathogenic or detrimental effects on the host are likely to be lost from the gene pool.

Although the bulk of integrations may occur in junk DNA, experimental evidence suggests that integration may occur preferentially into transcriptionally active areas, which increases the likelihood of insertion in and near genes. Insertional inactivation of critical genes can be immediately deleterious to the host, or may only be lethal in the homozygous state (in the latter case, an ERV-containing locus could persist for many generations as a minor allele). Integration can also lead to activation or aberrant regulation of genes near the site of integration. For example, activation of proto-oncogenes in the vicinity of integrated proviruses has been detected many times in experimental animal models of retroviral pathogenesis; it is possible that similar scenarios could also occur during formation of an ERV. Expression of the ERV can potentially be a source of pathogenic virus or can contribute to pathogenecity by recombination with other retroviruses.

ERV sequences may also have beneficial consequences for the host. Laboratory studies with both chickens and inbred mice have identified several ERV-derived genes that now function to reduce or prevent exogenous retroviral infection. ERV sequences also have the potential to provide new cellular functions during the course of evolution. The human Syncytin protein may be a striking example of the latter; this protein is in fact the envelope protein of an ancient endogenous provirus that appears to have been co-opted during evolution to function in human placental morphogenesis.

Stochastic factors will also influence the persistence of an ERV locus in the genome. Proviruses in the germline are subject to the same processes that affect all genomic sequences, including substitutions, deletions, insertions, recombination, gene conversion, random assortment, and genetic drift. Even in the absence of selective pressures, a newly formed ERV represents a minor allele and stands a good chance of being lost by chance. In the absence of positive selection, random accumulation of mutations over time will eventually degrade the viral open reading frames and the *cis*-acting elements required for expression. Thus, the interplay between random processes, as well as the nature and extent of selective forces acting on the ERV, will affect the way in which these sequences evolve during their residence in the germline.

Taxonomy and Classification

The term 'endogenous retrovirus' does not refer to a biological entity distinct from other retroviruses, but simply describes any DNA provirus, retroviral in origin, that has found its way into an organismal germline. This is true regardless of whether the provirus is still capable of expressing infectious virions. In fact, even highly degraded proviruses containing large deletions, insertions, and substitutions are often referred to as ERVs, as long as they are still clearly derived from a retrovirus.

Phylogenetic trees incorporating multiple genera of retroviruses are typically constructed using amino acid alignments corresponding to conserved domains in the reverse transcriptase gene. When representatives of multiple ERV families are included in such analyses, they do

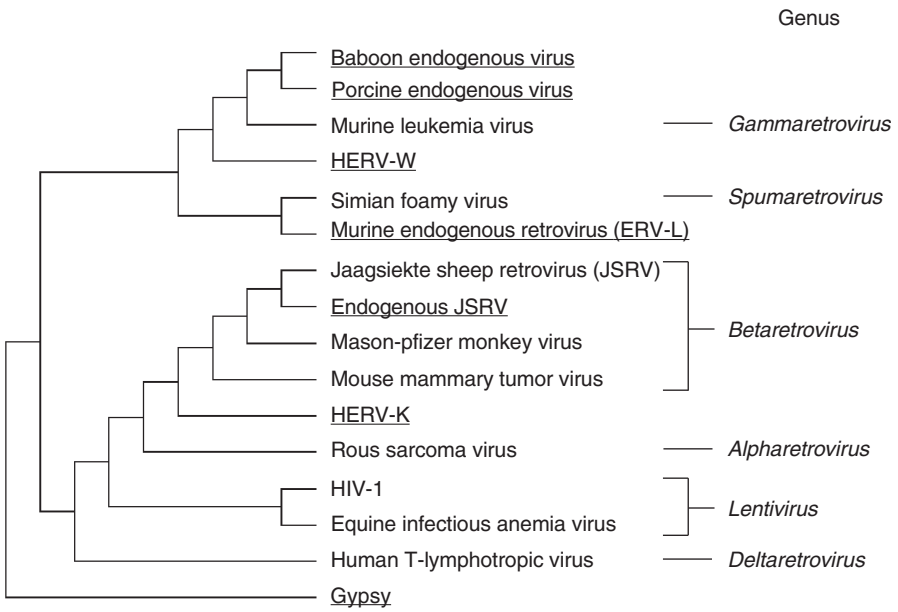


Figure 2 Phylogenetic tree based on amino acid alignment of reverse transcriptase from a variety of retroviruses. ERVs are underlined. Viruses representing genera in the family *Retroviridae* are indicated. Gypsy is an endogenous retroviral element from the invertebrate species *Drosophila melanogaster*. Tree was generated by maximum parsimony and represents one of five equally parsimonious trees. Note that ERV sequences are interleaved with exogenous sequences and do not represent a separate lineage.

not form a monophyletic cluster, but rather are scattered within and between the various genera of exogenous retroviruses (**Figure 2**). Moreover, modern ERVs may have very closely related, exogenous counterparts. Thus, ERVs do not constitute a separate taxonomic division from exogenous retroviruses (this refers to ERVs as a whole; any individual ERV locus or family may still represent a novel (and possibly extinct) retroviral genus).

Although there are exceptions, the vast majority of ERVs (particularly the ancient ERVs) is not closely related to known exogenous retroviruses, is no longer capable of expressing virus, and has no other associated biological or phenotypic properties to facilitate classification. The interleaving of ancient ERV sequences with extant retroviruses in phylogenetic trees, along with similarities to modern viruses in provirus organization, indicates that ancient ERVs are not ancestral stages in the evolutionary pathway leading to retroviruses (i.e., they are not protoretroviruses), but rather represent fully evolved (albeit extinct) sibling species to modern retroviruses.

There is as yet no officially recognized nomenclature for endogenous retroviruses, and a variety of naming conventions exist. In general, these represent myriad naming practices coined by various investigators at the time each novel ERV was discovered. For ERVs originally identified in the human genome, the practice has been adopted of using the acronym HERV (for human endogenous retrovirus) followed by a single letter that specifies the most likely tRNA primer (based on complementarity to the putative PBS sequence). Thus, HERV-K refers to endogenous proviruses with a PBS sequence complementary to a lysine tRNA. This convention has made the HERV literature much more accessible, but in practice it still leads to some ambiguity, because: (1) otherwise unrelated retroviruses may share the same or similar tRNAs; (2) many ERV loci may not contain a recognizable PBS element; and (3) in some cases, the PBS will be sufficiently degraded by substitutions that unambiguous prediction of tRNA specificity is not possible. Despite the name, the vast majority of HERV loci is not unique to humans but is shared with other, closely related primates (having first appeared in a common ancestor); thus, HERV loci should not be thought of as human specific, but rather as human orthologs of loci found in other primate species. Because some HERV families have hundreds or thousands of members, when a specific HERV locus is being described in reference to the chromosomal location, database accession number or cosmid clone is sometimes used for clarity (e.g., HERV-K6p22 refers to a provirus of the HERV-K family found at band 22 on the short arm of chromosome 6). However, this practice is recent and many of the original clone names are still in use.

Conclusion

Given the sheer number and ubiquity of ERVs among extant species, it is likely that retroviruses have made major contributions to both the content and structure of animal genomes for hundreds of millions of years. Retroviruses can give rise to new DNA content via

proviral integration as well as by retro-transposition or transduction of nonviral sequences. Because many ERVs constitute multilocus families of closely related sequences, they can mediate intergenic recombination events leading to large-scale deletions, duplications, interchromosomal translocations, and inversions of chromosomal sequence. All of these processes are also mutagenic, and therefore provide sources of variation upon which selective forces may operate. ERVs can also recombine with exogenous retroviruses during infection, providing additional sources of viral variation and giving rise to retroviruses with altered or novel properties. In modern times, the advent of xenotransplantation (using appropriate animals as organ donors for human transplantation) has raised concerns that ERVs expressed in the donor organs could introduce new retroviruses into the human population. Finally, because of the vast archive of retroviral sequences present in animal genomes in the form of ERVs, the deep evolutionary past of the *Retroviridae* is amenable to scientific exploration in a way that other viral families are not.

See also: Bovine and Feline Immunodeficiency Viruses; Equine Infectious Anemia Virus; Evolution of Viruses; Feline Leukemia and Sarcoma Viruses; Fish Retroviruses; Foamy Viruses; Host Resistance to Retroviruses; Human Immunodeficiency Viruses: Molecular Biology; Human T-Cell Leukemia Viruses: General Features; Jaagsiekte Sheep Retrovirus; Mouse Mammary Tumor Virus; Organ Transplantation, Risks; Origin of Viruses; Reticuloendotheliosis Viruses; Retrotransposons of Vertebrates; Retroviral Oncogenes; Retroviruses: General Features; Retroviruses of Birds; Retroviruses of Insects; Simian Immunodeficiency Virus: Natural Infection; Simian Retrovirus D; Visna-Maedi Viruses.

Further Reading

Best S, Le Tissier PR, and Stoye JP (1997) Endogenous retroviruses and the evolution of resistance to retroviral infection. *Trends in Microbiology* 5: 313–318.

Boeke JD and Stoye JP (1997) Retrotransposons, endogenous retroviruses, and the evolution of retroelements. In: Coffin JM, Hughes SH, and Varmus H (eds.) *Retroviruses*, pp. 343–435. New York: Cold Spring Harbor Laboratory Press.

Gifford R and Tristem M (2003) The evolution, distribution and diversity of endogenous retroviruses. *Virus Genes* 26: 291–315.

Jern P, Sperber GO, and Blomberg J (2005) Use of endogenous retroviral sequences (ERVs) and structural markers for retroviral phylogenetic inference and taxonomy. *Retrovirology* 2: 50.

Lower R, Lower J, and Kurth R (1996) The viruses in all of us: Characteristics and biological significance of human endogenous retrovirus sequences. *Proceedings of the National Academy of Sciences, USA* 93: 5177–5184.

Mager DL and Freeman JD (1995) HERV-H endogenous retroviruses: Presence in the New World branch but amplification in the Old World primate lineage. *Virology* 213: 395–404.

Mi S, Lee X, Li X, *et al.* (2000) Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* 403: 785–789.

Sverdlov ED (2000) Retroviruses and primate evolution. *BioEssays* 22: 161–171.

Turner G, Barbulescu M, Su M, Jensen-Seaman MI, Kidd KK, and Lenz J (2001) Insertional polymorphisms of full-length endogenous retroviruses in humans. *Current Biology* 11: 1531–1535.

Weiss RA (2006) The discovery of endogenous retroviruses. *Retrovirology* 3: 67.

Endornavirus

T Fukuhara and H Moriyama, Tokyo University of Agriculture and Technology, Fuchu, Japan

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cytoplasmic male sterility (CMS) Male sterility caused by a cytoplasmic factor.
Isogenic line Genetically identical pure-breeding group of individuals.
Mycovirus A virus that infects fungal species.
Replicon A genetic unit of replication.

Introduction

Double-stranded RNAs (dsRNAs) have frequently been identified in various healthy plants, from algae through to higher plants. These dsRNAs are not transcribed from the host genome DNAs, and they have important properties in common that differ from those of conventional viruses: (1) most of these dsRNAs have no obvious effect on the phenotype of their host plants; (2) they are present at a constant low concentration in the host plant; (3) they are efficiently transmitted to the next generation via seeds, but their horizontal transfer (infection) to other plants has never been proved. The size of these dsRNAs varies from 1.5 to 20 kbp. Smaller dsRNAs (~2.0 kbp) are often found with virus-like particles and some of these dsRNAs have already been classified as viruses in the genera *Alphacryptovirus* and *Betacryptovirus* of the family *Partitiviridae*. Partitiviruses have two unrelated linear dsRNA segments, each about 2.0 kbp in size. Larger

dsRNA segments (more than 10 kbp) are unlikely to be associated with distinct virus-like particles, because no distinct virus-like particles have been detected in preparations obtained with various purification procedures. Thus, these large dsRNAs were previously referred to as RNA plasmids, enigmatic dsRNAs, or endogenous dsRNAs.

Large dsRNAs (Endornaviruses) in Plants

In 1966, a cytoplasmic male sterility (CMS) trait, termed '447', in broad bean (*Vicia faba*) was described, and in 1976, electron microscopy observations revealed that the cytoplasm of 447 male-sterile plants contained cytoplasmic spherical bodies (CSBs) that resembled vesicles of about 70 nm in diameter. These CSBs were found in all tissues of the 447 plants, and contained a high-molecular-weight dsRNA. An absolute correlation between the CMS trait and the presence of the CSB containing the dsRNA has been established.

In the 1990s, large dsRNAs of about 14 kbp in length were found in many strains of cultivated rice (*Oryza sativa* L.) in Japan (temperate *japonica* rice) and the Philippines (tropical *japonica* rice), but not in the *indica* subspecies of *O. sativa*. A similar dsRNA was also detected in one strain (W-1714) of wild rice (*O. rufipogon*), which is considered an ancestor of *O. sativa*. dsRNA-carrying plants and dsRNA-free plants were found for the Nipponbare cultivar of temperate *japonica* rice, but these two isogenic lines were indistinguishable in their phenotypes. Even rice breeders and farmers could not distinguish these two lines; in fact, dsRNA-carrying and dsRNA-free rice plants were found in the same rice paddy field at our university farm. If the dsRNA-carrying line had a lower harvest yield than the dsRNA-free line, rice breeders and/or farmers would have recognized this phenotype and discarded the lower-yielding line. Given that this did not occur, these two lines can be considered identical with respect to all phenotypes, including harvest yield. Electron microscopy observations revealed that all dsRNA molecules from rice plants are linear. Differential centrifugation and sucrose density-gradient centrifugation revealed that the dsRNA in rice is localized in the cytoplasm.

Large dsRNAs of about 14 kbp in length have also been found in other crops, but only three of these dsRNAs, from the 447 male-sterile strain of broad bean (*V. faba*), the Nipponbare cultivar of cultivated rice (*O. sativa*), and the W-1714 strain of wild rice (*O. rufipogon*), have been sequenced completely. All three dsRNAs encode a single long open reading frame (ORF), in which conserved motifs for RNA-dependent RNA polymerase (RdRp) and RNA helicase (Hel) are found (**Figure 1**). The large dsRNAs are RNA replicons that can

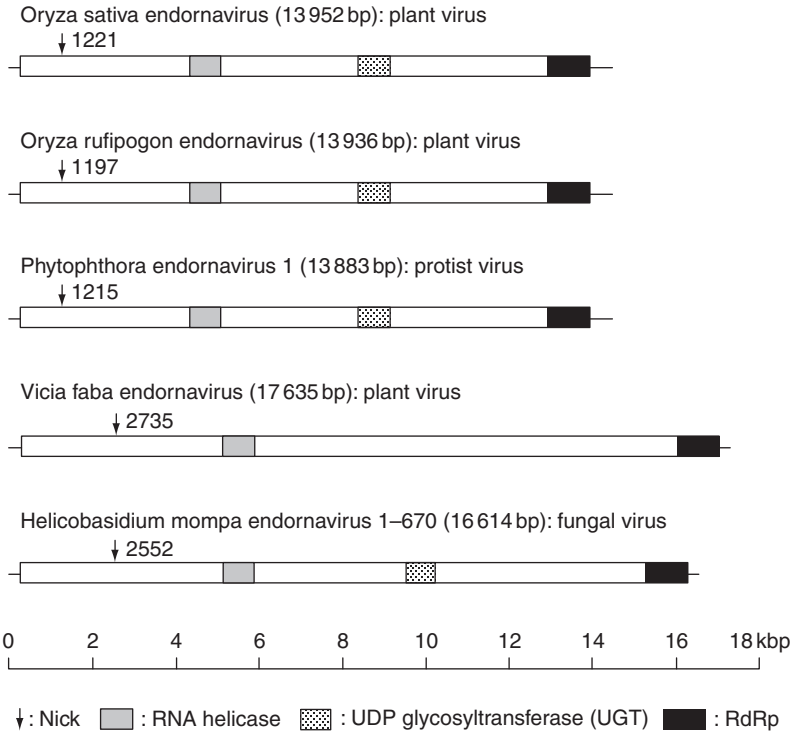


Figure 1 Comparison of the genome organizations of five endornaviruses: oryza sativa endornavirus (OsEV), oryza rufipogon endornavirus (OrEV), phytophthora endornavirus 1 (PEV1), vicia faba endornavirus (VfEV), and helicobasidium mompa endornavirus 1–670 (HmEV1–670).

replicate independently of their host genome by using their own RdRps, as will be discussed later. Phylogenetic analyses of the amino acid (aa) sequences of the RdRp and Hel motifs indicate that the three large dsRNAs that have been sequenced share a common ancestor with the alpha-like supergroup of positive-sense, single-stranded RNA (ssRNA) viruses, which includes many plant viruses, such as those of the genera *Tobamovirus* and *Cucumovirus*. Based on this information, in its eighth report, the International Committee on Taxonomy of Viruses (ICTV) accepted that these large plasmid-like dsRNA replicons are members of a new virus genus, *Endornavirus*. The dsRNAs found in cultivated rice, wild rice, and broad bean have been named oryza sativa endornavirus (OsEV), oryza rufipogon endornavirus (OrEV), and vicia faba endornavirus (VfEV), respectively (**Figure 1**).

Nonplant Endornaviruses

dsRNAs are found not only in plants, but also frequently in a variety of fungi; in fact, they have been recorded in all major fungal taxonomic groups, including various plant pathogens. The genomes of most mycoviruses consist of dsRNAs, and these fungal dsRNA viruses have so far been classified into five families: *Reoviridae, Totiviridae, Partitiviridae, Chrysoviridae,* and *Hypoviridae*. Members of only one of these families, the *Hypoviridae*, contain a single large dsRNA genome about 10–12 kbp in length, in contrast to members of the other families that contain one or multiple small dsRNA genomes about 2–6 kbp in length. However, many unclassified dsRNAs are still found in various fungi, some of which are larger than 10 kbp in length.

The AG3 isolate of the fungus *Rhizoctonia solani* is the major cause of rhizoctonia disease in potato, and contains two large dsRNAs, L1 (25 kbp) and L2 (23 kbp), in some strains of the fungus. The nucleotide sequence of the L2 dsRNA has been partially determined, and was found to be similar to the RdRp regions of the ORFs encoded by plant endornaviruses.

The violet root rot fungus, *Helicobasidium mompa*, occurs on various plants, and contains various-sized dsRNAs. A large dsRNA (L1 dsRNA) in the V670 strain of *H. mompa* has been identified as a hypovirulence factor, and has been sequenced completely. It encodes a single long ORF (5373 aa), in which conserved RdRp and Hel motifs are found (**Figure 1**). A BLAST search using the aa sequence of this ORF revealed significant sequence similarities to the dsRNAs of members of the genus *Endornavirus*. These results indicated that the large dsRNA in *H. mompa* belongs to the genus *Endornavirus*, and it was thus named helicobasidium mompa endornavirus 1–670 (HmEV1–670).

Plant pathogens of the genus *Phytophthora* have many of the same biological properties as fungi. However, on the basis of sequence similarities they have been classified together with diatoms and brown algae in a protist group known as the stramenopiles or chromista. The complete nucleotide sequence of the 13.9 kbp dsRNA isolated from a *Phytophthora* isolate from Douglas fir has also been published recently. The dsRNA encodes a single long ORF (4548 aa), whose genome organization is very similar to that of known endornaviruses (**Figure 1**). Phylogenetic analyses support the classification of this dsRNA (named *Phytophthora endornavirus* 1 (PEV1)) as a member of the genus *Endornavirus*.

Therefore, entire nucleotide sequences have been published for a total of five endornaviruses, three from plants (OsEV, OrEV, and VfEV), one from a fungus (HmEV1-670) and one from a protist (PEV1). Despite the diversity of their hosts, a comparison of the genome organizations of the five endornaviruses indicates that they share a unique structure (**Figure 1**): all five endornaviruses encode a single unusually long ORF, in which conserved motifs for RdRp and Hel are found in the same regions, and they contain a site-specific nick in the 5′ region of the coding strand.

Tentative Members of the Genus *Endornavirus*

In the 1990s, dsRNAs of about 14 kbp in size were found in many cultivars of barley (*Hordeum vulgare*), kidney bean (*Phaseolus vulgaris*), and bell pepper (*Capsicum annuum*). Barley and bell pepper plants hosting the dsRNAs are available commercially, so these plants must be healthy and common (not special) cultivars. These dsRNAs have been reported to be efficiently transmitted to the next generation. Therefore, these large dsRNAs with plasmid-like properties may be members of the genus *Endornavirus*. Unfortunately, only limited sequence information for these dsRNAs is available (except for an approximately 630-nt sequence of the dsRNA from kidney bean), so it is currently unclear whether they do in fact belong to the genus *Endornavirus*.

Partial sequences of the large dsRNAs from kidney bean (*P. vulgaris,* cv. Black Turtle) and bottle gourd (*Lagenaria siceraria*) have been reported recently, and a single ORF was found in dsRNAs from both species. Homologous sequence searches for these ORFs indicate that both ORFs contain sequences similar to the entire RdRp regions of endornaviruses, and that, in turn, the RdRp domains of endornaviruses are similar to the pfam00978 (RNA_dep_RNApol2) domain in the Conserved Domain Database. RdRps of ssRNA viruses of the alpha-like superfamily are classified as the pfam00978 domain. Molecular phylogenetic analyses using the aa sequences encoded by the RdRp regions (about 470 aa residues) of the kidney bean and bottle gourd dsRNAs as well as of the five endornaviruses and the 10 most diverse

members of pfam00978 were carried out. The resulting phylogenetic tree shows that the kidney bean and bottle gourd dsRNAs form a monophyletic group with the known endornaviruses, a grouping that was supported by a high bootstrap probability (100%; **Figure 2**(**a**)), indicating that these two dsRNAs are members of the genus *Endornavirus*.

Furthermore, partial sequences of dsRNAs from barley (*H. vulgare*, cv. Kashima), melon (*Cucumis melo*), Malabar spinach (*Basella alba*), seagrass (*Zostera marina*), and strain V1369 of the violet root rot fungus (*H. mompa*) have also been recently reported. The sequences are short, but commonly contain core RdRp regions of about 80 aa residues. Phylogenetic analyses were carried out for these five dsRNA sequences, seven known endornaviruses (including kidney bean and bottle gourd dsRNAs), and 10 ssRNA viruses. Although the analyzed RdRp regions of the dsRNAs and viruses were short (~80 aa), the phylogenetic tree was essentially the same as the tree in **Figure 2**(**a**), in which the five dsRNAs mentioned above formed a monophyletic group with the seven endornaviruses (**Figure 2**(**b**)). These results suggest that these five dsRNAs from four plants and one fungus are members of the genus *Endornavirus*. Therefore, there is a growing list of new dsRNA viruses that belong or very probably belong to the genus *Endornavirus*, distributed over the plant, protist, and fungal kingdoms (**Table 1**).

Proteins Encoded by Endornaviruses

All five endornaviruses that have been completely sequenced encode a single unusually long ORF, from 4500 to 5500 aa residues in length, which is one of the unique molecular features of endornaviruses (**Figure 1**). Conserved motifs encoding RdRp and Hel are found in the same regions of these long ORFs. The genes encoding RdRp and Hel are structurally similar to those encoded by the ssRNA genome of members of the alpha-like supergroup of RNA viruses. Thus, the endornavirus RdRp and Hel probably function in the replication of the dsRNA genome, just as their homologs encoded by ssRNA viruses function in the replication of the ssRNA genome. Indeed, viral RdRp activity has been detected in plants containing OsEV or VfEV (to be discussed later).

The enzyme uridine diphosphate (UDP) glycosyltransferase (UGT) is commonly found in eukaryotes. This enzyme catalyzes the addition of the glycosyl

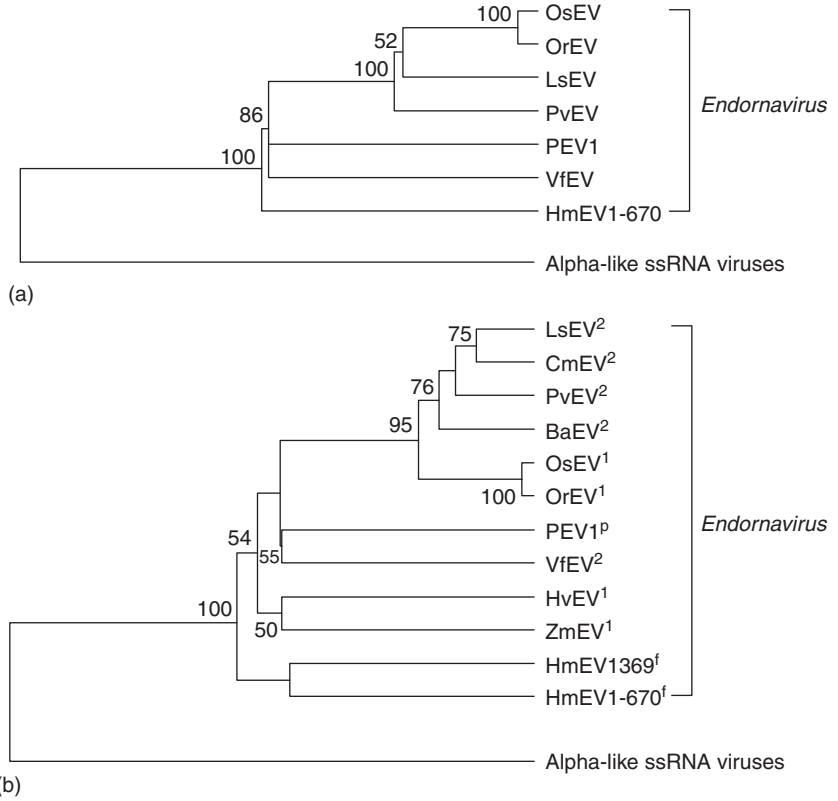


Figure 2 Phylogenetic positions of endornaviruses. (a) About 470 aa of the RdRp regions of seven endornaviruses and 10 alpha-like ssRNA viruses and (b) about 80 aa of the RdRp regions of 12 endornaviruses and 10 alpha-like ssRNA viruses were analyzed by using the ClustalX and MEGA2 (Molecular Evolutionary Genetics Analysis) programs, and the resulting neighbor-joining (NJ) trees are shown. A bootstrap test was performed with 100 resamplings. The hosts of endornaviruses are monocotyledonous plants (1), dicotyledonous plants (2), fungi (f), and a protist (p).

Table 1 Members in the genus *Endornavirus*

	Host	*Virus*	*Abbreviation*
Plant	Rice	Oryza sativa endornavirus	OsEV
	Wild rice	Oryza rufipogon endornavirus	OrEV
	Broad bean	Vicia faba endornavirus	VfEV
	Kidney bean	Phaseolus vulgaris endornavirus	PvEV
	Bottle gourd	Lagenaria siceraria endornavirus[a]	LaEV
	Barley	Hordeum vulgare endornavirus[a]	HvEV
	Malabar spinach	Basella alba endornavirus[a]	BaEV
	Melon	Cucumis melo endornavirus[a]	CmEV
	Seagrass	Zostera marina endornavirus[a]	ZmEV
Fungus	Violet root rot fungus	Helicobasidium mompa endornavirus 1–670	HmEV1–670
	Violet root rot fungus	Helicobasidium mompa endornavirus 1369[a]	HmEV1369
Protist	Phytophthora	Phytophthora endornavirus 1	PEV1

[a]Tentative member.

group from a UDP-sugar to a small hydrophobic molecule. UGTs have been identified in dsDNA viruses, including baculoviruses and nucleopolyhedroviruses from insects. In insect cells, the viral ecdysteroid UGT inactivates the molting hormones (ecdysteroids) of the host insect by sugar conjugation. Although a UGT gene has never been found in the genomes of the alpha-like supergroup of ssRNA viruses, it is found in strains of two hypoviruses (fungal dsRNA viruses), cryphonectria hypovirus 3 and 4 (CHV3 and CHV4). Amino acid sequences significantly similar to that of UGT are found in four of the five endornaviruses that have been sequenced in their entirety (**Figure 1**; they are not found in VfEV). Because the locations of the UGT motifs between the Hel and RdRp domains are conserved among the four endornaviruses, a common ancestor of the endornaviruses was likely to have also had a putative UGT gene. VfEV might have lost this gene during course of evolution, or a putative UGT gene encoded by VfEV might vary so much from other putative UGT genes that it was not identified as such. The functions of the putative UGT proteins in endornaviruses are unknown.

Because the single ORF of endornaviruses is very large (**Figure 1**), it very likely encodes several other proteins in addition to those already discussed. The ORF must encode a polyprotein, so it must also encode one or more proteinases to cleave the putative polyprotein into functional units (including RdRp, Hel, and UGT). A similar situation occurs in the potyviral genome, which contains a single large ORF encoding a polyprotein of more than 3000 aa residues; this also has proteinase activities which cleave the polyprotein into functional units. Furthermore, endornaviruses may encode an RNA-silencing suppressor, given that many other viruses contain this protein for protecting themselves against the host defense (RNA silencing) system.

Site-Specific Nick in the Coding Strand

All five endornaviruses that have been sequenced completely contain a site-specific nick in the 5′ region of the coding strand, which divides not only the coding strand but also the single long ORF (**Figure 1**). The biological implications of the nick remain unknown. However, it must affect at least two important steps in the life cycle of the endornavirus, because the divided coding strand can no longer be used as either a template for noncoding strand synthesis or an mRNA for translation of the putative polyprotein. The molecular mechanism that generates the nick is also unknown: an unknown endonuclease encoded by either the host genome or the endornavirus itself might cleave the coding strand site specifically. These two molecular features, the single long ORF and the site-specific nick, are unique, and have never been found in other known RNA viruses.

Inheritance (Vertical Transmission)

The transmission of endornaviruses relies essentially on cell division, and endornaviruses may survive in cooperation with their host cells. No horizontal spread has been observed in the field, and no potential vectors have been identified. OsEV-carrying plants and OsEV-free plants are found coexisting in the *japonica* rice cultivar Nipponbare, and these two isogenic lines cannot be distinguished on the basis of their appearance. Crossing experiments with OsEV-carrying and OsEV-free plants have indicated that the efficiency of OsEV transmission via pollen is more than 98%, and that of transmission via ova is 100%. Although OsEV is localized in the cytoplasm, it is transmissible to progeny plants via pollen as well as ova. Analyses of the F2 progeny plants of cv. Nipponbare indicated that the absence of OsEV is not associated with any particular gene(s) in the OsEV-free plants. The observed inheritance of OsEV is very different from that of other cytoplasmic genetic elements (e.g., chloroplasts and mitochondria), which are usually inherited only via egg cells. Thus, the propagation of OsEV, and probably other plant endornaviruses, may be entirely dependent

on seed-mediated transmission. The high efficiency of transmission of OsEV via both pollen and ova is likely to be responsible for the wide distribution of OsEV in cultivars of *japonica* rice.

OsEV has been found in many cultivars of *japonica* rice (*O. sativa*) but not in any cultivars of *indica* rice (a subspecies of *O. sativa*). *Japonica* and *indica* are distinguishable on the basis of their phenotype (e.g., grain shape) and genotype (e.g., the distribution of transposons in their genomes). To determine the reason why OsEV is not found in *indica* rice cultivars, reciprocal crosses between an OsEV-carrier *japonica* variety (cv. Nipponbare) and an OsEV-free *indica* variety (cv. IR 26 or Kasalath) were performed. When cvs. IR 26 and Nipponbare were used, efficient transmission of OsEV via ova (93%) and pollen (89%) was observed. However, when the Kasalath and Nipponbare cultivars were used, OsEV transmission efficiency to F1 progeny was 68% via ova and 20% via pollen, and transmission to F2 progeny plants also followed a complicated, non-Mendelian inheritance pattern. These results suggest that OsEV is unstable in *indica* rice plants, which may lack one or more genes that are involved in the maintenance (replication) of OsEV.

Because OsEV is efficiently transmitted to progeny plants via pollen, reciprocal crosses between cultivated rice (*O. sativa*, cv. Nipponbare) and wild rice (*O. rufipogon*, W-1714) were performed to introduce two evolutionarily related endornaviruses (OsEV and OrEV) into F1 progeny plants. Unusual cytoplasmic inheritance of these endornaviruses was observed in F1 hybrids: the evolutionarily related endornaviruses were incompatible, and in some F1 plants the resident OsEV of an egg cell from cultivated rice was excluded by the incoming OrEV from a pollen cell from wild rice. The two coexisting endornaviruses in some F1 hybrids segregated away from each other in the F2 plants. However, the total number of the two coexisting endornaviruses in the F1 hybrid cells remained constant relative to the number of endornaviruses in the parent cells (approximately 100 copies/cell). The stringent regulation of endornavirus copy number may be responsible for their unusual inheritance pattern. This phenomenon is similar to the plasmid incompatibility that occurs with two evolutionarily related DNA plasmids in *Escherichia coli*.

Copy Number Regulation of OsEV

OsEV is found in every tissue as well as at every developmental stage in its host plants. Regardless of the host rice cultivar, the relative concentration of OsEV in comparable cell types is approximately constant. A comparison of the relative amounts of OsEV and rice DNA (430 Mb per haploid genome) showed that there are approximately 100 copies of OsEV per cell. No significant difference in OsEV concentration was found between seedlings, roots, mature leaves, and stems. Crossing experiments indicated that a mechanism for stringent regulation of endornavirus copy number may exist in host rice cells.

A 14-kb dsRNA has also been found in another ecospecies of cultivated rice, the tropical *japonica* rice cultivars grown in the Philippines, which are also classified as *O. sativa*. A partial nucleotide sequence from the RdRp region of the dsRNA from cv. Gendjah Gempel (tropical *japonica* rice) has been determined, revealing that this sequence has already diverged from that of the dsRNA from cv. Nipponbare (temperate *japonica* rice from Japan). The dsRNAs from cv. Nipponbare (temperate *japonica* rice) and cv. Gendjah Gempel (tropical *japonica* rice) are hereafter referred to as OsEV-N and OsEV-G, respectively. Although the three dsRNAs (OsEV-N, OsEV-G, and OrEV) in the genus *Oryza* have already diverged from one another at the nucleotide sequence level, the copy number of each dsRNA genome in their respective host cells is almost identical (100 copies per cell).

In contrast, the number of endornavirus copies in cultured cells was found to be about 10 times that in the cells of seedlings; a similarly high level of OsEV has been observed in each culture established from an independent temperate *japonica* seed (cv. Nipponbare). Although no changes in the copy number of OsEV were found in suspension cultures over the course of 3 years, the copy number returned to the original low level in rice plants regenerated from the cultured cells. An approximately tenfold increase of OsEV-G and OrEV copy numbers also occurred in suspension cultures of cv. Gendjah Gempel (tropical *japonica* rice) and W-1714 (wild rice) seeds, respectively. Thus, the copy numbers of OsEV-N, OsEV-G, and OrEV are dependent on the physiological conditions of the host plant, and may be maintained by a similar regulatory system in each rice genotype.

The amount of OsEV-N, OsEV-G, and OrEV also increases about 50-fold in the pollen grains of the host plants relative to the rest of the plant. There is no evidence for the horizontal transmission of these endornaviruses, so their propagation seems to depend on steady replication before every host cell division (not only mitosis but also meiosis) and efficient transmission to the next generation via egg and pollen. An unregulated increase in the amount of endornaviruses in cells could result in disease or death of the host plant, as seen with conventional viruses. Conversely, a decrease in the number of endornaviruses could cause the virus to disappear from a host plant (germ cells). The mating of host plants must be an opportunity for endornavirus propagation. An increase in the endornavirus copy number only in pollen grains must be a strategy to ensure their transmission to host progeny, despite their cytoplasmic localization. Unlike almost all other viruses, endornaviruses are not able to transmit horizontally, but they are capable of vertical transmission, which the majority of viruses cannot do.

Replication (RdRp Activity)

In 1990, the RdRp activity of endornaviruses was reported from a study of dsRNA in the 447 male sterile strain of broad bean (VfEV). Purified cytoplasmic membranous vesicles containing the dsRNA (VfEV) had RdRp activity, which required Mg^{2+} ions and the four nucleotide triphosphates, and was unaffected by inhibitors of cellular DNA-dependent RNA polymerases (e.g., alpha-amanitin and actinomycin D). Treatment of the vesicles with a nonionic detergent released the VfEV dsRNA together with the RdRp, which retained specific activity for synthesis of VfEV dsRNA.

RdRp activity can also be detected in the crude microsomal fraction of cultured rice cells that contain OsEV. Like VfEV RdRp activity, this RdRp activity was also highest in the presence of all four nucleotide triphosphates and Mg^{2+} ions, and was resistant to inhibitors of DNA-dependent RNA polymerases. This RdRp activity increased approximately 2.5-fold in the presence of 0.5% deoxycholate. Treatment of the purified microsomal fraction with proteinase K plus deoxycholate suggested that the RdRp enzyme complex, with its own 14-kb RNA template, is located in vesicles. So far, RdRp activity has been found only in two endornavirus-containing plants, broad bean containing VfEV and rice containing OsEV. Therefore, the available data concerning endornavirus replication are limited. There is a need to identify and characterize the endornavirus-and/or host-encoded proteins that are involved in endornavirus replication.

Molecular Evolution of Endornaviruses

Endornaviruses have been found in two ecospecies of cultivated rice (temperate *japonica* rice cultivars in Japan and tropical *japonica* rice cultivars in the Philippines), both of which are classified as *O. sativa*. They have also been found in one strain (W-1714) of wild rice (*O. rufipogon*, an ancestor of *O. sativa*). A comparison of the nucleotide and deduced amino acid sequences of the core regions of the RdRp domains of these three endornaviruses (OsEV-N, OsEV-G, and OrEV) indicates that the three endornaviruses are evolutionarily related and that OsEV-N is evolutionarily closer to OsEV-G than to OrEV. The relationships between these endornaviruses are consistent with the evolutionary relationships between the host plants. Therefore, the endornaviruses found in the genus *Oryza* are likely to have been transmitted vertically through seeds at least since the time when *O. sativa* and *O. rufipogon* diverged from each other several thousand years ago. These endornaviruses have probably evolved independently within their own host plants, which have always generated self-pollinated progeny.

Similar evolutionary divergence of endornaviruses within host plants has been reported for the bottle gourd (*L. siceraria*) endornaviruses. Large dsRNAs (*Lagenaria siceraria endornavirus*) are found in several cultivars of bottle gourd, including three Japanese cultivars and two Western cultivars. Northern blot analysis of these endornaviruses from five cultivars indicated that these endornaviruses have evolutionarily diverged from each other within their host plants. Therefore, because of their plasmid-like properties, namely vertical transmission only, endornaviruses in rice and bottle gourd are thought to have evolved within their host plants.

However, as shown in **Figure 2**(**b**), the four endornaviruses in monocotyledonous plants (OsEV, OrEV, HvEV, and ZmEV) do not form a monophyletic group in the endornavirus phylogenetic tree. Similarly, the kidney bean endornavirus (PvEV) and the broad bean endornavirus (VfEV) do not cluster together, although their hosts both belong to the family Fabaceae Therefore, the phylogenetic relationships among these endornaviruses are not consistent with the phylogenetic relationships of their host plants. There are three possible explanations for these unexpected relationships between endornaviruses and their hosts. (1) Endornaviruses have speciated independently of their hosts. Several types of endornaviruses may have diverged before monocotyledonous and dicotyledonous plants diverged, and the families Poaceae (rice and barley) and Fabaceae (kidney and broad beans) now carry at least two independent lineages of endornaviruses. (2) The ancestors of endornaviruses were horizontally transmissible. (3) Some endornaviruses are horizontally transmissible, but horizontal transmissions occur only very rarely. The last two explanations may conceivably be possible, because the rare transmission events could have occurred during a sufficiently long time, over millions of years.

dsRNAs of various sizes are frequently found in plants, fungi, protozoans, and insects; in fact, dsRNAs (or dsRNA viruses) with plasmid-like properties can be considered to be widely distributed throughout all four eukaryotic kingdoms. Plasmid-like dsRNA replicons including endornaviruses and ssRNA viruses could have evolved from a common RNA replicon ancestor, in the process developing two different propagation strategies: ssRNA viruses became independent of the host cell (e.g., developed rapid replication of its genome RNA(s) and extracellular stability by encapsulation within a capsid protein), and dsRNA replicons developed a highly symbiotic relationship with the host cell (e.g., developed stringent copy-number regulation and efficient vertical transmission). dsRNA replicons have probably coevolved with their host cells to be symbiotic. Indeed, efficient vertical transmission and constant copy number are likely necessary to ensure the stability of the symbiotic relationship.

See also: Alphacryptovirus and *Betacryptovirus*; Hypoviruses; Totiviruses.

Further Reading

Brown GG and Finnegan PM (1989) RNA plasmids. *International Review of Cytology* 117: 1–56.

Fukuhara T (1999) Double-stranded RNA in rice. *Journal of Plant Research* 112: 131–138.

Gibbs MJ, Pfeiffer P, and Fukuhara T (2004) Genus *Endornavirus*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 603–605. San Diego, CA: Elsevier Academic Press.

Tavantzis SM (ed.) (2001) *dsRNA Genetic Elements: Concepts and Applications in Agriculture, Forestry, and Medicine.* Boca Raton, FL: CRC Press.

Enteric Viruses

R F Bishop and C D Kirkwood, Murdoch Childrens Research Institute Royal Children's Hospital, Melbourne, VIC, Australia

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The intestinal tract, lined by replicating epithelial cells, bathed in nutrient fluids and maintained at optimal temperature provides an ideal milieu for growth of many viruses. 'Enteric viruses' represent a wide spectrum of viral genera that invade and replicate in the mucosa of the intestinal tract, and that can be grouped as follows:

- viruses causing localized inflammation at any level of the intestinal tract, predominantly in small intestinal mucosa, resulting in acute gastroenteritis, for example, rotaviruses, caliciviruses, adenoviruses, astroviruses;
- viruses that multiply at any level of the intestinal tract, causing few enteric symptoms prior to producing clinical disease at a distant site, for example, measles virus, reoviruses (in mice) , enteroviruses (including polioviruses, coxsackieviruses, enteroviruses, hepatitis A and E); and
- viruses that spread to the intestinal tract during the later stages of systemic disease, generally in an immunocompromised host, for example, human immunodeficiency virus (HIV), cytomegalovirus.

This article focuses upon the first category of viruses that cause enteric disease associated with primary replication in the intestinal tract.

Viruses Associated with Acute Gastroenteritis

Acute gastroenteritis is one of the most common health problems worldwide. More than 700 million cases are estimated to occur annually in children less than 5 years of age, resulting in few deaths in developed countries, but more than 2 million deaths in developing countries.

Worldwide, a diverse group of viral, bacterial, and parasitic pathogens cause acute enteric symptoms including nausea, vomiting, abdominal pain, fever, and acute diarrhea. Infections with viral agents, unlike those with bacterial or parasitic pathogens, cannot be treated with antibiotics, and many cannot be prevented by improvements in quality of drinking water, food, or sanitation.

Until the early 1970s most viral agents causing gastroenteritis in humans were largely unknown. Studies using electron microscopy of intestinal contents resulted in the discovery of numerous viral enteropathogens now classified as caliciviruses, rotaviruses, astroviruses, or 'enteric' adenoviruses. Caliciviruses are now recognized as the most important cause worldwide of outbreaks of viral gastroenteritis in humans of all age groups. Rotaviruses are the single most important cause of life-threatening diarrhea in children <5 years old. Astroviruses and adenoviruses also cause severe diarrhea in children. **Table 1** lists the characteristics of the major viruses associated with acute gastroenteritis. Other viruses linked to gastroenteritis in humans include coronaviruses, toroviruses, picornaviruses, and picobirnaviruses. Understanding many features of these 'enteric viruses' has been based on parallel studies of related viruses infecting animals.

Caliciviruses

History

The family *Caliciviridae* contain small RNA viruses that cause enteric disease in a wide variety of hosts including cattle, pigs, rabbits, and humans. Infections in other hosts, for example, sea lions, cats, and primates, appear to cause predominantly systemic and respiratory symptoms. Caliciviruses are small nonenveloped viruses of 27–35 nm diameter (**Figure 1**) with a genome comprising

Table 1 Characteristics of major enteric viruses causing acute gastroenteritis in humans

Characteristic Family	*Norovirus/ Sapovirus Caliciviridae*	*Rotavirus Reoviridae*	*Adenovirus Adenoviridae*	*Astrovirus Astroviridae*
	Divided into genogroups, each with distinct genetic clusters	Six groups (A–F) Group A-multiple serotypes based on outer capsid proteins.	Six subgenera, more than 50 serotypes Enteric serotypes (Ad40–41)	Eight serotypes
Virion size (nm)	28–35	70	80	28
Capsid organization	Two structural proteins (orf2–56–62 kDa, orf3–22 kDa)	Two outer capsid proteins (VP7–38 kDa, VP4–88 kDa) Two inner protein layers (VP6–41 kDa, VP2–88 kDa)	Capsomer – composed of three proteins: hexon, penton, and fiber.	Precursor cleaved into several proteins (e.g., 20 kDa, 29 kDa and 31 kDa)
Nucleic acid	ssRNA (plus sense)	dsRNA	dsDNA	ssRNA (plus sense)
Genome organization	Three open reading frames (1, 2, and 3)	11 segments which encode specific protein.	Linear chromosome with multiple transcription/ translation units	2 open reading frames (1a, 1b, and 2)

ss, single-stranded; ds, double-stranded; kDa, kilodalton.

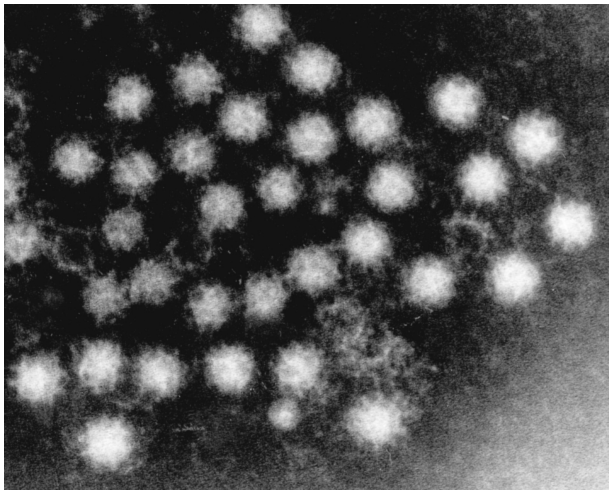


Figure 1 Electron micrograph of negatively stained calicivirus particles (NoV) in fecal extract.

a single-stranded positive-sense polyadenylated RNA genome of 7400–7700 nucleotides (nt). Typical calicivirus particles show cup-like hollows (calices) on the virus surface. Some caliciviruses have an indistinct appearance described as 'feathery'.

The 'Norwalk agent' (now classified as a calicivirus) was identified in 1972 by Kapikian and colleagues, using immune-electron microscopy (IEM) to search for the causative agent of an 1968 outbreak of gastroenteritis in humans in Norwalk, Ohio, USA. Many related viruses have been implicated as causes of gastroenteritis, and given names identifying the geographical location of the outbreak (e.g., Marin County, Snow Mountain, Hawaii, Sapporo).

Classification

Classification of caliciviruses was hampered for many years by the inability to culture these viruses, and study their genetic and protein structure. Caliciviruses causing enteric infections (in humans and other animals) are classified as belonging to the family *Caliciviridae*, which is divided into four genera. The genus *Norovirus* (NoV), and genus *Sapovirus* (SaV) cause human and animal infections. Other genera infect rabbits (*Lagovirus*) or sealions, cats, and primates (*Vesivirus*). The genome organization and reading frame usage differs between the four genera. For noroviruses, the genome contains three open reading frames (ORFs). The largest (ORF1) encodes a polyprotein which undergoes proteolytic cleavage to produce an NTPase, a 3c-like protease, and an RNA-dependent RNA polymerase (RdRp). ORF2 encodes the major capsid protein and ORF3 a putative minor capsid protein.

Noroviruses are subdivided into five genogroups (GI–GV). Genogroups GI, GII, and GIV infect humans, GIII infects cattle and GV infects mice. GI and GII contain at least 10 and 20 distinct genetic clusters, respectively. Sapoviruses are divided into genogroups (GI–GV), of which GI, GII, GIV, and GV infect humans. GI and GII are further divided into four and three genetic clusters.

The inability to culture human caliciviruses delayed the introduction of diagnostic tests, resulting in an under-appreciation of the significance of these agents for many years. Currently, over 20 different reverse transcription-polymerase chain reaction (RT-PCR) assays targeting regions on the RdRp gene and the capsid gene have been described and utilized in epidemiological studies. This large genetic diversity of human caliciviruses makes routine detection difficult.

Geographic and Seasonal Distribution

Human NoV are the leading causes of 'nonbacterial gastroenteritis' outbreaks in all age groups worldwide. Outbreaks frequently occur in communities such as

nursing homes, hospitals, schools, and cruise ships. No consistent seasonal variation has been observed. Infection involves transmission via person-to-person contact or ingestion of contaminated food and water.

Epidemiological studies have identified caliciviruses in 60–95% of outbreaks in many countries. Estimates of disease burden in USA suggest that caliciviruses are responsible annually for 23 million illnesses and 50 000 hospitalizations. Strains of NoV GII cluster 4 (GII-4) have been the most common type identified worldwide in the past 5 years (2001–2005) in both adults and children. Prevalence rates of calicivirus (predominantly NoV) in young children admitted to hospital with acute gastroenteritis in many countries range from 3.5% to 20% annually. Strains of SaV, while playing a minor role overall, are more generally associated with childhood gastroenteritis than with disease in older children and adults. Caliciviruses may be important enteric pathogens in patients with hereditary or acquired immunodeficiency.

Genetics

The genera *Norovirus* and *Sapovirus* are genetically diverse, and multiple strains co-circulate in human populations. Individual dominant strains emerge every 2–5 years, and often have a global impact, such as the GII-4 strains identified in USA, Europe, Japan, and Australia in 1995/1996 and again in 2004. NoV recombinant strains, with polymerase and capsid genes derived from different ancestral clusters, have been identified in Thailand and Australia. Repeated attempts to adapt NoV and SaV to growth in cell culture have failed. Diagnostic techniques and analysis of antigenic variation rely predominantly on molecular biological techniques. Cloning and expression of the major viral capsid protein (VP1) in baculovirus expression systems has led to the formation of virus-like particles (VLPs) morphologically similar to native virus and their incorporation into enzyme immunoassay (EIA) assays.

Pathogenesis

Pathogenesis of NoV and SaV infection is poorly understood as a result of the long-standing inability to adapt these viruses to cell culture, and the absence of a small animal model. Symptomatic enteritis in human volunteers infected with 'Norwalk agent' showed changes in jejunal biopsies (mucosal inflammation, absorptive cell abnormalities, villus shortening, and crypt hypertrophy) that persisted for at least 4 days after remission of clinical symptoms and reverted to normal after 2 weeks. No identifiable viral particles were detected by electron microscopy in any affected intestinal tissue. The recent demonstration that human noroviruses can infect and replicate in a three-dimensional cell culture model of human intestinal epithelium, should improve our understanding of the pathogenesis, and antigenic diversity of this important group of enteric viruses. These studies will also be enhanced by discovery of a norovirus that infects mice, and that replicates after transfection of cultured kidney cells.

Immune Responses, Prevention and Control

Mechanisms of immunity to NoV are unclear. Infection results in formation of IgG and IgM serum antibody that are broadly reactive within, but not between, genogroups. The role of these antibodies in immune protection is unknown. Infected individuals can develop short-term immunity to homologous viruses but the molecular diversity of NoV circulating in communities makes it difficult to predict whether long-term immunity can develop. It is unclear why a proportion of exposed individuals remain uninfected during outbreaks. Recent studies suggest that histo-blood group antigens and the secretor status may be genetic susceptibility markers for infection. At present the major control strategies for prevention of human calicivirus infection rely on prevention of contamination of food and water supplies.

Rotaviruses

History

Virus particles, later classified in the genus *Rotavirus*, were first described in 1963 by Adams and Kraft as a cause of epidemic diarrhea in infant mice (EDIM). Similar particles (NCDV) were recognized in 1969 by Mebus and colleagues as a cause of severe diarrhea in newborn calves in Nebraska, USA. Neither virus was considered relevant as a causative agent of severe diarrhea in young children until 1973 when Bishop, Davidson, Holmes, and Ruck described a 'new virus' (later shown to be antigenically related to EDIM and NCDV) in duodenal biopsies and diarrheal feces from young children admitted to hospital in Melbourne, Australia with severe acute diarrhea. Named because of their wheel-like appearance in negatively stained extracts examined by electron microscopy (rota = Latin for wheel) rotaviruses have since become established as causes of severe acute diarrhea in the young of many mammalian and avian species worldwide. Rotavirus enteritis affects all children regardless of socioeconomic status, and results in over 600 000 deaths annually in young children in developing countries.

Classification

Rotaviruses are nonenveloped icosahedral viruses of 70 nm (**Figure 2**) diameter that belong to the genus *Rotavirus*

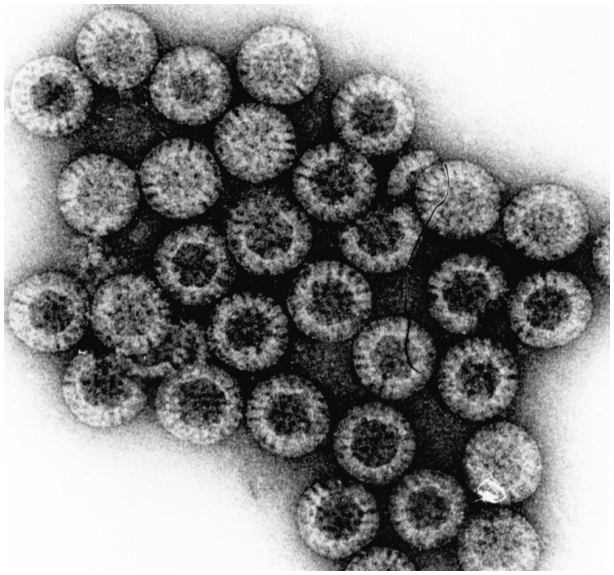


Figure 2 Electron micrograph of negatively stained triple-shelled rotavirus particles in fecal extract.

within the family *Reoviridae*. The double-stranded RNA genome is contained within a triple-layer of viral proteins (VPs) comprising a core (VP1, VP2, VP3), an inner capsid (VP6), and an outer capsid (VP4, VP7). Rotaviruses are classified into groups A to G based on serology of the VP6 protein. The majority of human and mammalian infections, due to group A viruses, are further classified into serotypes by antigenic differences on VP7 (G-serotypes) and into genotypes by genetic differences on VP4 (P-genotypes). To date there are at least 11 of 15 G-serotypes and 15 of 26 P-genotypes identified in humans. Groups B and C have been identified infrequently in humans. Groups D to G have been identified only in nonhuman mammalian or avian species.

Geographic and Seasonal Distribution

Five rotavirus serotypes G1P[8], G3P[8], G4P[8], G2P[4], and G9P[8] have been the most common serotypes causing severe human disease globally during the past 30 years. G1P [8] strains have been consistently present worldwide. Yearly winter epidemics of rotavirus disease are regularly observed in countries with temperate climates, whereas rotavirus disease is prevalent year-round in tropical climates lacking defined winter seasons.

Host Range

Group A rotaviruses infect humans and other mammals repeatedly throughout life. Most primary rotavirus infections in animals occur during the neonatal period. Most primary rotavirus infections in humans occur during the first 24 months of life. Children worldwide will experience at least one rotavirus infection by 5 years of age.

In general, group A rotaviruses are species specific. However, rotavirus strains with gene segments of feline, bovine, or porcine origin have been isolated from children, suggesting the occurrence of cross-species infection in nature. Cross-species infections can be established experimentally, and comprise one of the strategies for human vaccine development.

Genetics

The rotavirus genome consists of 11 segments of double-stranded RNA that can be separated by polyacrylamide gel electrophoresis, allowing epidemiological studies mapping the genetic diversity of strains within and between serotypes. Each gene segment encodes a separate protein, with the exception of gene segment 11 which encodes two proteins. Reassortment of genes between human strains and human and animal strains occurs *in vivo* and *in vitro*.

Pathogenesis

Rotaviruses are transmitted from person to person by the fecal–oral route, or via aerosols. Rotaviruses replicate in the cytoplasm of mature nonreplicating enterocytes lining the upper portions of the small intestinal villi, eventually causing cytolysis. Profuse watery diarrhea results from a combination of mechanisms including malabsorption secondary to loss of enterocytes responsible for absorption and digestion, activation of the enteric nervous system, and stimulation of intestinal secretion by the rotavirus nonstructural protein NSP4. Rotavirus antigenemia and viremia occur during the acute phase of severe primary rotavirus disease. As a result, complete rotavirus particles have been found in liver, lung, spleen, pancreas, thymus, and kidneys of experimental animals. It is not clear if the virus is replicating at these sites.

Clinical Features

Clinical symptoms in children are strongly influenced by age, with severe often life-threatening diarrhea occurring after primary infection in young children, and in aged people in nursing homes. Excretion of rotavirus particles in detectable numbers (by EIA, RT/PCR) continues for 5–10 days, and occasionally up to 50 days. Excretion can continue for months in immunodeficient children and animals. Reinfections occur throughout life, and are usually asymptomatic or associated with mild symptoms. Symptoms of primary infection require medical attention in 1:5 children, result in hospitalization in 1:65 children and death in 1:293 (almost all in young children in developing countries). Treatment is based upon replacement

of fluid and electrolyte loss, usually achieved by oral administration of fluids containing glucose and electrolytes. Occasionally, delayed repair of the small intestinal mucosa is associated with disaccharide or monosaccharide malabsorption leading to malnutrition.

Immune Response/Prevention and Control

Primary rotavirus infection protects against severe symptomatic disease on reinfection, and is associated with humoral and cellular immune responses to individual rotavirus proteins. Neutralizing antibody to VP4 and VP7 outer capsid proteins contribute to protection, possibly by interfering with viral replication and limiting the extent of intestinal damage. The role of immune responses to other proteins is uncertain. Virus-specific cytotoxic T cells are not essential for protection.

The importance of rotavirus disease worldwide and its contribution to childhood mortality in developing countries has resulted in strong initiatives, supported by the World Health Organization, to develop live oral rotavirus vaccines to be administered to infants before 3 months of age. Two contrasting vaccines, a single attenuated G1P[8] human rotavirus and a pentavalent human–bovine G1-G4,P[8] reassortant vaccine, have been proved to be safe and effective in preventing severe rotavirus disease. Both have the potential to radically change global childhood mortality and morbidity.

Adenovirus

History and Classification

Adenoviruses were first detected in 1953 in cultured fragments of tonsillar and adenoidal tissue from children. They are nonenveloped icosahedral viruses approximately 80 nm in diameter (**Figure 3**). The genome is composed of double-stranded DNA. The family *Adenoviridae* comprises three genera: *Mastadenovirus* (mammalian) classified into subgenera A–F representing more than 50 serotypes, *Aviadenovirus* (birds), and a newly recognized genus *Atadenovirus* identified in sheep and reptiles. Most are readily cultivatable. Adenovirus infections occur worldwide in many mammalian species, are species specific, usually associated with disease in the respiratory, urinary, and ocular systems, and are frequently shed in feces in the absence of any gastrointestinal symptoms.

In 1975, Flewett and colleagues in Birmingham, UK, noticed the presence of large numbers of adenovirus particles in negatively stained extracts of diarrheal stools examined by EM. These proved difficult to culture, were designated 'enteric' adenoviruses (EAd), and are now classified as serotypes EAd40 and EAd41 within subgenus group F. Cultivation of EAd remains difficult. The most reliable growth has been achieved in human embryonic kidney cells (293 cells) immortalized by transfection with regions of Ad5.

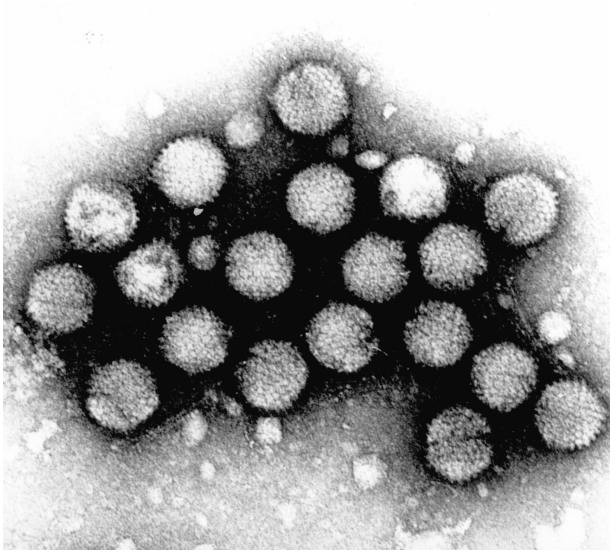


Figure 3 Electron micrograph of negatively stained 'enteric' adenovirus particles (showing characteristic hexagonal shape) in fecal extract. Courtesy of professor M. Studdert.

Geographic and Seasonal Distribution

EAd40 and EAd41 occur worldwide, causing severe acute enteritis in 5–15% of hospitalized young children. Outbreaks occur at unpredictable intervals year-round with no seasonal prevalence. Nosocomial epidemics occur in day-care nurseries and in hospital wards for children and adults. Group A adenoviruses (serotypes 12, 18, 31) have also been implicated in epidemics, usually in older age groups.

Genetics/Evolution

The adenovirus virion is composed of at least 10 different structural polypeptides and contains a linear 33–45 kbp DNA. Virus capsomers are arranged as hexons, the corners of which have antenna-like (fiber) projections presumed involved in cell attachment. The DNA genomes of groups A–F are genetically diverse, and differences can be illustrated by analysis using genome restriction endonucleases. The heterogenous genome in groups A–F makes recombination between subgenera unlikely, with exception of groups A and F which show a close evolutionary relationship.

Diagnoses of EAd infection rely on EIA that detects the hexon antigen common to groups A–F, followed by determination of restriction enzyme patterns and/or reactions in EIA incorporating neutralizing, monoclonal antibodies specific for Ed40 and 41.

Pathogenesis

EAd replicate within the epithelial cells of the small intestine. Group A adenoviruses have also been grown from mesenteric lymph nodes and appendices. The mechanisms causing diarrhea are not clear, but destruction of infected epithelial cells has a role.

Clinical Features

Adenovirus diarrhea is more common in infants <12 months old than in older children, and can be protracted with a mean duration of 12 days. Adenovirus diarrhea occurs in immunocompromised patients. Nonseasonal epidemics of EAd diarrhea occur in hospital wards, orphanages, and day-care nurseries. Occasional fatal cases have been reported in children. Evidence from animal models (with non-EAd) suggests that viremia occurs, and can lead to infection of other tissues. The natural history of disease and development of immunity is unknown.

Astroviruses

History and Classification

Astroviruses were first described in the UK in 1975 by Madeley and Cosgrove studying an outbreak of diarrhea in newborn babies in an obstetric hospital nursery. Astroviruses are small, round nonenveloped plus-stranded RNA viruses 28–30 nm diameter (**Figure 4**) occasionally exhibiting virions with a superficial star shape. They are members of the genus *Astrovirus* in the family *Astroviridae*. They have been detected in humans (children and adults) and a range of mammalian (sheep, cattle, pigs, dogs, cats, and mice) and avian (turkeys, ducks) species, usually associated with diarrhea. There are currently eight serotypes of human astrovirus, designated HAstV 1–8, based on reactivity with polyclonal antisera. HAstV 1 is most common worldwide.

Prevalence rates as a cause of diarrhea vary from 2% to 16% (hospital-based studies), and 5% to 17% (community-based studies). Most astrovirus infections have been recorded during colder months in temperate climates and year-round in tropical countries. A longitudinal study in Mayan children in a poor community in Mexico found a high prevalence (61%) of astrovirus infection in a birth cohort of 271 children followed for 3 years. Infection occurred primarily in infants <12 months old, and showed a high rate of asymptomatic infection and prolonged shedding (2–17 weeks) in many infants. Astrovirus infection has also been associated with persistent diarrhea (lasting for 14 days or more) in children in Bangladesh. Astroviruses are widespread in developed countries, causing outbreaks in day-care centers, hospitals, and nursing homes for the elderly. They are an important cause of enteritis in immunocompromised patients.

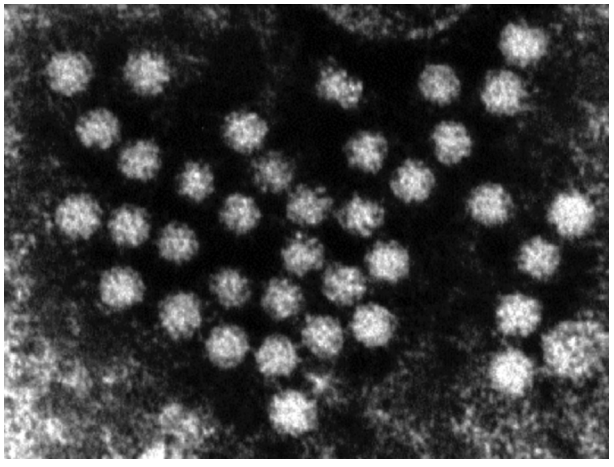


Figure 4 Electron micrograph of negatively stained astrovirus particles (showing star-shape) in fecal extract.

Genetics and Evolution

The HAst genome is a polyadenylated plus-stranded RNA molecule of approximately 7 kbp. The genome contains two open reading frames (ORFs), ORF1a and -1b, code for nonstructural proteins and ORF2 encodes for the capsid protein. Genetic diversity in all serotypes exists, but no association has been shown between serotypes and ability to cause severe gastroenteritis. Astrovirus diagnostic assays include commercially available EIA kits, electron microscopy and RT-PCR detection and genotyping of diarrheal feces.

Pathogenesis

Acute astrovirus infection induces a mild watery diarrhea in young children that lasts for 2–3 days and may be associated with vomiting, fever, and anorexia. The lack of a small animal model has hampered studies of the mechanism of astrovirus-induced diarrhea. Experimental models of astrovirus enteritis in turkeys and in gnotobiotic lambs show mild histopathological changes in the intestine (despite high mortality from severe osmotic diarrhea) together with viremia. Experimental astrovirus infection in calves is asymptomatic, with viral replication apparently targeted to M cells. It is possible that none of these animal models illuminate pathogenesis of HAstV infection in humans. Cultivation of HAstV was initially difficult, but can now regularly be achieved using a human colon cancer derived epithelial cell line (CaCo2 cells).

Coronaviruses/Toroviruses

Members of the genera *Coronavirus* and *Torovirus* are enveloped plus-strand single-stranded RNA viruses belonging to the family *Coronaviridae*. Electron microscopy shows them to be pleomorphic fringed particles 100–140 nm at maximum dimension (**Figure 5**). Coronaviruses and toroviruses can be distinguished by differences in peplomer structure and reaction in IEM using specific antisera.

Coronaviruses and toroviruses cause diarrhea, respiratory, and/or hepatic disease in many animal species, including cattle, mice, swine, cats, and dogs. In general, most of these viruses are species specific and disease is most severe in infant animals. Transmission is fecal–oral and due to virus lability may require close contact. Coronaviruses and toroviruses have been implicated in human diarrheal disease but there is still no consensus about their importance. Similar particles have been seen frequently in children without diarrhea, particularly in children in developing countries. Morphological similarities between these viruses and fragments of intestinal brush border make diagnosis difficult. Several studies have implicated coronaviruses as causative agents of necrotizing enterocolitis outbreaks in newborn babies.

Toroviruses were first described as a cause of diarrhea by Woode and colleagues in 1979 when Breda virus was identified in a severe outbreak of neonatal calf diarrhea in USA. Toroviruses are now also known to infect horses (Berne virus) and swine. Human infections were first described by Flewett *et al.* in Birmingham UK in 1984, but have rarely been reported since then.

The pathogenesis of diarrhea has been studied in animal models using infection with coronavirus (TGE) in piglets, and with Breda viruses in calves. Both replicate in epithelial cells of small intestine and descending colon causing diarrhea 24–72 h later. Breda virus also replicates in crypt cells.

Many animal coronaviruses can be propagated in cell culture. Isolation of human enteric coronaviruses is difficult and serological studies can be confounded by antibody resulting from repeated respiratory coronavirus infection. Enteric infection can be confirmed in feces by detection of viral RNA by RT-PCR, or IEM using antibodies to the viral envelope glycoproteins.

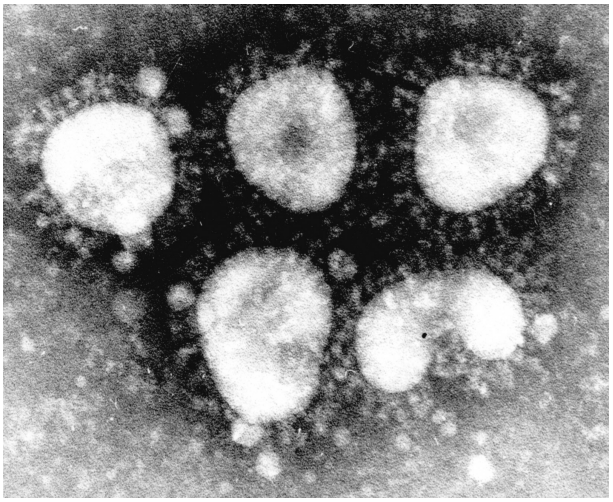


Figure 5 Electron micrograph showing large fringed pleomorphic coronavirus particles (with adherent antibody) in fecal extract.

Picornaviruses

Picornaviruses are 24–30 nm featureless spherical particles containing single-stranded positive-sense RNA. They have been found in diarrheal feces from humans but their etiological role is often not clear. The first clear evidence implicating a picornavirus as an enteric pathogen identified Aichi virus, as a cause of oyster-associated epidemics of gastroenteritis in Japan in 1989. Aichi viruses are now classified as a new genus *Kobuvirus* within the family *Picornaviridae* (kobu = Japanese for knob). Isolation of Aichi virus in Vero cells has permitted development of EIA and RT-PCR assays based on nucleotide sequence data. Serological assays show seroconversion resulting from infection and a prevalence rate for antibody of 7.2% in Japanese children aged 7 months to 4 years and rising to 80% in adults by age 35.

The new genus *Parechovirus* within the family *Picornavirideae* contains at least one serotype (previously echovirus 22) that has been implicated as an enteric pathogen in humans.

Parvoviruses

Parvoviruses are small 22–26 nm single-stranded DNA viruses comprising a genus *Parvovirus* in the family *Parvoviridae*. Some animal parvoviruses have been clearly linked to enteritis including bovine, feline, mink, and canine strains. Canine parvovirus infection emerged after 1977. This lethal neonatal enteric infection, accompanied by viremia and widespread systemic infection, shows a pathogenesis distinct from most enteropathogenic viruses. The virus infects and destroys crypt epithelial cells resulting in flat mucosa with fused and stunted villi. Damage has been likened to that caused by radiation.

Other small viruses, resembling parvoviruses, have been seen by EM in diarrheal feces in humans. Evidence linking them to causation of disease is not convincing. They have often been present as dual infections with known enteric pathogens. In addition, their resemblance to some phages makes diagnosis uncertain.

Picobirnaviruses

These are a group of currently unclassified small viruses detected in the feces of humans and animals without diarrhea. Picobirnaviruses are 35–41 nm particles with a bi- or tri-segmented dsRNA genome and have been detected in Europe, South America, and Australia. They are found significantly more often in patients with HIV-related diarrhea than those without diarrhea. Their role in gastroenteritis in healthy individuals remains unknown.

See also: Astroviruses; Birnaviruses; Enteroviruses: Human Enteroviruses Numbered 68 and Beyond.

Further Reading

Atmar RL and Estes MK (2001) Diagnosis of noncultivable gastroenteritis viruses, the human caliciviruses. *Clinical Microbiology Reviews* 14: 15–37.

Bresee JS, Widdowson M-A, Monroe SS, and Glass RI (2002) Foodborne viral gastroenteritis: Challenges and opportunities. *Clinical Infectious Diseases* 35: 748–753.

Chadwick D and Goode JA (2001) *Gastroenteritis Viruses. Novartis Foundation Symposium*, p. 238. New York: Wiley.

Chiba S, Estes MK, Nakata S and Calisher CH (eds.) (1996) *Viral gastroenteritis. Archives of Virology* (supplement 12): 119–128.

Duckmanton L, Luan B, Devenish J, Tellier R, and Petric M (1997) Characterisation of torovirus from human faecal specimens. *Virology* 239: 158–168.

Franco MA, Angel J, and Greenberg HB (2006) Immunity and correlates of protection for rotavirus vaccines. *Vaccine* 24: 2718–2731.

Glass RI (2006) New hope for defeating rotavirus. *Scientific American* 294: 33–39.

Hall GA (1987) Comparative pathology of infection by novel diarrhoea viruses. In: Bock G and Whelan J (eds.) *Novel Diarrhoea Viruses. Ciba Foundation Symposium,* vol. 128, pp. 192–217. Chichester: Wiley.

Hansman GS, Oka T, Katayami K, and Takeda N (2007) Human Sapovirus: Genetic diversity, recombination and classification. *Reviews in Medical Virology* 17: 133–141.

Mendez-Toss M, Griffin DD, Calva J, *et al.* (2004) Prevalence and genetic diversity of human astroviruses in Mexican children with symptomatic and asymptomatic infections. *Journal of Clinical Microbiology* 42: 151–157.

Ramig RF (1997) Genetics of the rotaviruses. *Annual Reviews of Microbiology* 51: 225–255.

Ramig RF (2004) Pathogenesis of intestinal and systemic rotavirus infection. *Journal of Virology* 78: 10213–10220.

Schwab KJ, Estes MK, and Atmar RL (2000) Norwalk and other human caliciviruses: Molecular characterization, epidemiology and pathogenesis. In: Cary JW, Linz JE and Bhatnagar D (eds.) *Microbial Foodborne Diseases: Mechanisms of Pathogenesis and Toxin Synthesis*, pp. 469–493. Lancaster, USA: Technomic Publishing Company.

Straub TM, Honer zu Bentrup K, Orosz-Coghlan P, *et al.* (2007) *In vitro* cell culture infectivity assay for human noroviruses. *Emerging Infectious Diseases* 13: 396–403.

Tiemessen CT and Kidd AH (1995) The subgroup F adenoviruses. *Journal of General Virology* 76: 481–497.

Yamashita T, Sugiyama M, Tsuzuki H, *et al.* (2000) Application of a reverse transcription-PCR for identification and differentiation of Aichi virus, a new member of the picornavirus family associated with gastroenteritis in humans. *Journal of Clinical Microbiology* 38: 2955–2961.

Enteroviruses of Animals

L E Hughes and M D Ryan, University of St. Andrews, St. Andrews, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

In the 1950s, the use of monkey kidney cell cultures for the growth of poliovirus revealed the presence of simian viruses and further study showed some of these to have properties consistent with enteroviruses. In parallel, investigations of viruses infecting domestic animals also revealed the presence of enteroviruses (and enterovirus-like particles) in pigs and cattle. Enteroviruses have since been isolated from African buffalo, water buffalo, sheep, goat, deer, and impala and many have been shown to be related to bovine enterovirus isolates.

In the past, the classification of enteroviruses was primarily based upon the physico-chemical properties of virions, growth in tissue-cultured cell lines, and by serotyping. In practice, this can be difficult with some isolates being poorly recognized by the reference antisera, or, the occurrence of (misleading) cross-reactivities between serotypes. The expansion of the sequence database together with more sensitive cloning/sequencing techniques have facilitated the elucidation of the genome structures of many picornaviruses. Such analyses have replaced other techniques in the classification of picornaviruses, and this article discusses characteristics which are important for the classification of animal enteroviruses.

Animal Enteroviruses

Bovine Enteroviruses

Bovine enteroviruses (BEVs) are endemic in cattle in many regions of the world with infection typically

asymptomatic and apparently healthy animals acting as carriers. There has, however, been some association of BEVs with diarrhoea and abortion. Studies in Spain have shown that BEV is widespread and variants co-circulate in cattle around the country, with the virus less prevalent in cattle from extensive farms (69%) than in cattle from intensive farms (94%). Analysis of samples collected from a farm in the USA indicated that the virus was present in the spring in 2–4-month-old calves but that the infection had probably been cleared by summer.

Recently, it has been shown that infectious virus particles are present in water from animal watering tanks, pastures, and streams/rivers in regions where BEVs are endemic, demonstrating the ability of these viruses to survive in aqueous environments. Interest in these viruses has widened over recent years with proposals that they can be utilized as markers for fecal contamination of the environment. BEVs have also been proposed as surrogate viruses for evaluating FMDV contamination of farms and the evaluation of extraction/detection techniques.

Porcine Enteroviruses

Historically, the more economically significant diseases of pigs caused by the porcine enteroviruses (PEVs) included neurological disorders (Teschen/Talfan disease), fertility disorders, dermal lesions, pneumonia, and diarrhoea. With the major reappraisal of the taxonomy of these viruses, these diseases are now associated with the teschoviruses (see below).

Swine Vesicular Disease Virus

Swine vesicular disease virus (SVDV) causes a highly contagious disease of pigs that spreads rapidly through contact with infected pigs and a contaminated environment. The disease is variable from mild or subclinical infections to lesions on the snout and feet that are indistinguishable from those caused by FMDV. For this reason, routine surveillance of SVDV is maintained in European countries.

SVDV was first described in Italy in 1966 and since then, numerous outbreaks have occurred throughout Europe and Asia. SVDV was largely eradicated from Europe during the 1970s and 1980s, but a new strain, possibly originating in the Far East, entered Europe during 1992. This strain spread to the Netherlands, Belgium, Portugal, Spain, and Italy. In 2003–04, clinical outbreaks were only reported in Portugal (leading to the slaughter of 2168 pigs) while subclinical infections are continuously detected in southern Italy. A long-term study of pigs infected with a recent Italian isolate showed the vRNA and virus could be detected long after the initial infection, and provided good preliminary evidence that this virus can establish persistent infections.

Simian Enteroviruses

Many simian viruses – including enteroviruses – were isolated from a range of nonhuman primate tissue-cultured cells used in biomedical research and vaccine development and from primates used for biomedical research. Twenty enterovirus serotypes were defined, with cross-reactivity in some cases. All these isolates were, however, distinct in that they did not show cross-reactivity with the (then) known human enteroviruses. Very little is known about the pathogenesis of these viruses. Simian enterovirus A (A-2 plaque virus) was isolated from a human, but caused viremia in tamarin monkeys 1–2 weeks post inoculation. Virus could not be detected in fecal samples.

'Animal' versus 'Human' Enteroviruses

Viruses of the genus *Enterovirus* of the family *Picornaviridae* primarily cause infections of the gastrointestinal tract, where large numbers of progeny virions are produced and shed in the feces. The virus particles are stable in a wide range of pH, temperature, and salinity conditions and may remain infective in the environment for long periods.

A number of viruses formerly thought to be enteroviruses are now known to be members of other genera. Presently, those animal viruses assigned to the enteroviruses include SVDV, BEV-1 and -2, and PEV-9 and -10.

Currently five human and three animal species comprise the genus *Enterovirus*. The separation of the genus into human and animal species is not as clear as in the past. Nucleotide sequence and serological data strongly suggest that some viruses have passed from man to domestic animals, or vice versa. For many years, SVDV was known to have a close relationship with human coxsackievirus B5 (CBV-5) and, indeed, this animal enterovirus is now classified in the species *Human enterovirus B* (HEV-B). The antigenic and molecular relationships between the two viruses suggest that CBV-5 crossed from humans to pigs – probably in the early 1950s – and has since adapted to the new host.

Another human enterovirus, type 70 (EV70; spp. *Human enterovirus D*) first appeared in humans in 1969 causing widespread outbreaks of acute hemorrhagic conjunctivitis. In a small proportion of cases (1:10 000–1:17 000), this generally benign ocular infection was accompanied by a disease with a presentation very similar to poliomyelitis. Interestingly, studies on animal sera collected prior to the human outbreaks showed the presence of anti-EV70 antibodies in cattle, sheep, swine, chickens, goats, dogs, and wild monkeys. EV70 disappeared from the human population in the 1980s, but the data suggest that this human disease was the result of a zoonosis.

Recent sequence analyses of simian enteroviruses has shown that some of these viruses are closely related to

viruses within the species *Human enterovirus A*, while others are proposed as new members of *Human enterovirus B*. It is not surprising, therefore, that the present 'human' enterovirus classification scheme contains several animal viruses.

Genome Structure and Classification

The classification of picornaviruses has been in a state of flux for a number of years. Originally classification was based on serological, pathogenic and biophysical properties, but now genome structure is paramount. In some instances, such analyses confirmed previous classifications, while in others reclassification occurred – either by simply renaming, regrouping, or, more fundamentally, by the formation of new genera.

Entero- and Rhinovirus Genome Structures

Briefly, a viral protein (VPg), is covalently linked to the 5′ end of the genome and followed by a nontranslated region, the 5′ NTR (**Figure 1(a)**). A single, long, open reading frame (ORF) encodes a polyprotein of ~2200 aa. The N-terminal domain of the polyprotein (P1) comprises the four capsid proteins, while the replicative proteins comprise the central domain (P2) and C-terminal domain (P3) of the polyprotein. The full-length translation product predicted by the single ORF is not observed within infected cells due to rapid, intramolecular, proteolysis by the two virus-encoded proteinases, $2A^{pro}$ and $3C^{pro}$ (**Figure 1(a)**). The P1, P2, and P3 'primary' cleavage products subsequently undergo proteolytic processing to yield the individual virus proteins. After the stop codon, there is a short 3′ NTR and a poly(A) tail (**Figure 1(a)**).

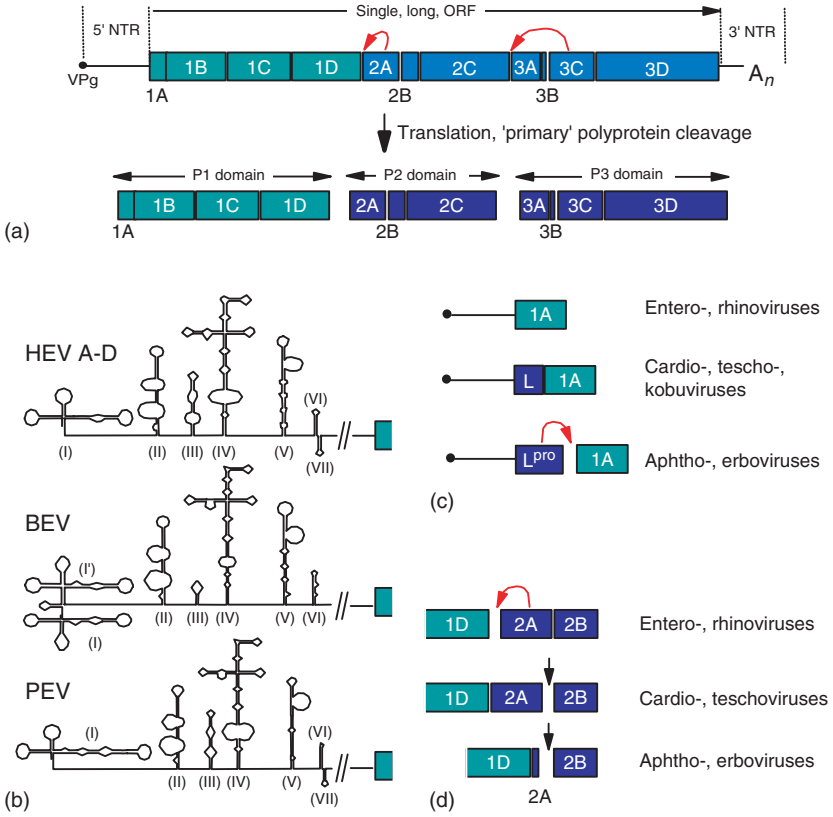


Figure 1 Enterovirus genome organization. The oligopeptide VPg (3B) is covalently attached to the 5′ terminus of vRNA. The long 5′ nontranslated region (5′ NTR) precedes the single, long, ORF (boxed area). The polyprotein comprises three domains: the capsid proteins precursor (P1; green boxed areas) and the nonstructural replication protein domains P2 and P3 (blue boxed areas). The 3′ portion of the genome comprises a short 3′ NTR and a poly(A) tail. The 'primary' (cotranslational) cleavages of the polyprotein mediated by the 2A and 3C proteinases are shown (red curved arrows) (a). The type 1 IRES RNA secondary structural features together with the individual domain features (I–VII) are shown for *Human enteroviruses A–D* (HEV A–D), bovine enterovirus (BEV), and porcine enterovirus (PEV) (b). In the case of enteroviruses, the N-terminal portion of the polyprotein is the capsid protein 1A (VP4), whereas in cardio- and teschoviruses, for example, the polyprotein starts with an L protein. Aphtho- and erbovirus polyproteins commence with an L proteinase (L^{pro}) which cleaves at its own C-terminus (c). Entero- and rhinovirus 2A proteins are proteinases ($2A^{pro}$) which cleave the polyprotein to form their own N-termini, whereas the 2A proteins of other genera (cardio-, tescho-, aphtho-, and erboviruses) mediate a cotranslational ribosome 'skipping' effect (producing an apparent prolyprotein cleavage), but these 2A proteins are not proteinases (d).

A few, but major, differences between the genomes of enteroviruses and other genera are now of prime importance in the classification of viruses within the *Picornaviridae*. Specifically, the RNA secondary structural features of the 5′ NTR (**Figure 1(b)**, the presence/absence of an L protein at the N-terminus of the polyprotein (**Figure 1(c)**), and the nature of the 2A region of the polyprotein (**Figure 1(d)**) are now taken to be the discriminative features. It has been shown, however, that comparison of VP1 (1D) sequences alone is sufficient to assign isolates to enterovirus species. Note that the high-level of recombination within noncoding regions and regions encoding nonstructural proteins (see below) means care should be taken when comparing sequences from these regions for taxonomic purposes.

The 5′ nontranslated region

The 5′ terminal ~100 nt of picornavirus vRNA folds into a cloverleaf-shaped structure (domain I; **Figure 1(b)**). A large proportion of the remainder of the 5′ NTR forms a complex secondary structure comprising multiple domains which functions as an internal ribosome entry site (IRES). This region confers a cap-independent mode of initiation of translation of the viral RNA: the ^{7me}G cap structure associated with eukaryotic mRNAs is replaced in picornaviruses by the oligopeptide 3B (or VPg) covalently linked to the 5′ terminus of vRNA. While the 5′ NTR has the same functions across the *Picornaviridae*, there are differences in the overall structure and this may be used to aid the classification of viruses. The entero- and rhinoviruses have a 'type 1' IRES (**Figure 1(b)**), while many of the other picornaviruses have a type 2 IRES.

The leader protein

A leader protein (L) is present at the N-terminus of the polyprotein in the aphtho-, erbo-, cardio-, tescho-, and kobuviruses (**Figure 1(c)**). In the cardioviruses, L has been shown to interact with Ran-GTPase disrupting nucleocytoplasmic trafficking of cellular proteins. In the aphtho- and erboviruses, however, L is a papain-like proteinase (L^{pro}). This proteinase cleaves at its own C-terminus and is involved in shut-off of host cell protein synthesis via cleavage of eIF4G. Expression of the erbovirus L protein in cells did not, however, lead to efficient inhibition of cap-dependent translation. In the entero- and rhinoviruses, no L protein is present: the N-terminus of the polyprotein is the capsid protein, VP4 (1A).

The 2A protein

Picornavirus 2A proteins are diverse in sequence and function (**Figure 1(d)**). The 2A proteins of entero- and rhinoviruses are very similar, and are structurally related to the small subclass of serine proteinases – although the active site nucleophile is not serine, but cysteine. They catalyze an intramolecular proteolytic cleavage at their own N-termini (*in cis*), separating the structural protein polyprotein domain (P1) from the nonstructural proteins (P2, P3; **Figure 1(a)**). Importantly, this virus-encoded proteinase also cleaves a host-cell translation factor (like L^{pro}), the initiation factor eIF4G, which brings about the 'shut-off' of host-cell (cap-dependent) mRNA translation.

The 2A regions of all other picornaviruses are quite different (**Figure 1(d)**). In the case of the aphtho-, cardio-, erbo-, and teschoviruses, the 2A protein is not a proteinase. This type of 2A may be identified by a C-terminal –NPG– motif. In these cases, the N-terminal residue of protein 2B is, invariably, proline. An apparent 'cleavage' of the nascent polyprotein occurs between the glycine and proline residues at the 2A/2B junction (-NPG $\Downarrow$ P-). This processing event is not, however, proteolytic but rather mediated by a translational effect termed ribosome 'skipping'.

Genome Structure of BEV

The genome organizations of several BEV isolates have been reported. BEV 5′ NTRs range from 812 to 822 nt and are unusual in that the 5′ cloverleaf-like secondary structure is duplicated (domains I and I′; **Figure 1(b)**). Furthermore, the putative domains III and VI of the IRES region differ in size and shape from other enteroviruses. The ORF varies from 6498 nt in BEV-2 to 6525 nt in BEV-1. The 3′ NTRs of BEV are 71–75 nt and although the predicted folding pattern (two stem–loop structures – a potential pseudoknot-like element) resembles other enteroviruses, no nucleotide sequence similarity exists.

Originally classified into several serotypes, only two serotypes (BEV-1 and BEV-2) are now recognized within the species *Bovine enterovirus*. A recent study has suggested, however, that the taxonomy of these viruses be revised. Sequence comparisons provided data that the bovine enteroviruses form two major clusters characterized by structural features and levels of sequence identity. These clusters have been designated BEV-A and BEV-B and appear to correlate with the two serotypes BEV-1 and BEV-2. It is also suggested that these clusters represent species, rather than serotypes. Further subgrouping within the clusters is proposed with BEV-A comprising two geno-/serotypes and BEV-B comprising three geno-/serotypes.

Genome Structure of PEV

Sequence analyses of what were formerly regarded as PEVs have shown that all but three now form the genus *Teschovirus*. Based upon the presence of an L protein and the nature of the 2A proteins, it is further proposed that one of these three, PEV-8 (together with some simian enteroviruses; see below) forms a new genus – *Sapelovirus*. The two remaining 'true' enteroviruses are PEVs 9 and 10. The 5′ NTR of PEV-9 is 809 nt (814 nt for PEV-10).

Both 5′ NTRs are predicted to adopt a secondary structure similar to that of the human enteroviruses. However, the 5′ cloverleaf has a unique insertion that enlarges domain I (**Figure 1(b)**). Interestingly, this domain has been previously shown to bind the 3CD proteinase in poliovirus and rhinovirus 14, forming a ribonucleoprotein complex required for initiation of positive-strand RNA synthesis. The long ORFs encode a polyprotein (2168 aa – PEV9; 2171 aa – PEV10) with a very similar organization to other enteroviruses (**Figure 1(a)**). The 3′ NTRs of both viruses are similar in sequence and length (72 nt – PEV9; 71 nt – PEV10), and predicted to form two stem–loop structures.

Genome Structure of SVDV

Complete genome sequences of the virulent J1′73 strain of SVDV and the avirulent H3′76 strain have been determined. Furthermore, partial sequences (5′ NTR, capsid protein VP1, protein 3BC) have been determined from a large number of isolates for epidemiological studies. The genome structure is very similar to that of the other enteroviruses (**Figure 1(a)**). Although only a single serotype, SVDV shows high genetic variability. The IRES is very similar to the human enterovirus type 1 IRES (**Figure 1(b)**; spp. HEV-B), although variability between isolates was observed in the 'spacer' region extending from the 3′ end of the IRES to the initiation codon. Indeed, several isolates had blocks of sequence (6–125 nt) deleted in this region.

A key determinant of virulence was mapped to a single residue within the 2A proteinase. Residue 20 of the avirulent H3′76 strain is isoleucine, the virulent J1′73 strain, arginine (this site is adjacent to His21, a component of the catalytic triad of $2A^{pro}$). Pigs were infected with viruses rescued from an infectious copy of the virulent strain bearing mutations at this single site. Each mutant tested showed much reduced virulence correlating with the different efficiencies of the two forms of the proteinase to promote translation of the vRNA.

Genome Structure of Simian Enteroviruses

Formerly, the 20 simian picornaviruses were provisionally classified as enteroviruses. Analysis of partial (5′ NTR, VP1, and 3D) sequences showed that the simian viruses SV2, -16, -18, -42, -44, -45, and -49 are not enteroviruses, but comprise a new genus *Sapelovirus*. The complete genome sequence of SV2 confirmed that this virus was not an enterovirus since it does not possess a type 1 IRES and is thought, like PEV-8, to possess an L protein. The sequence of the 2A protein was quite different to other picornaviruses, although the presence of the conserved motif –GxCG– (also present in PEV-8) suggests that SV2/PEV8 2A proteins may be proteinases. If these viruses do possess an L protein and a 2A proteinase, then they represent an interesting link between the enteroviruses and other genera.

Analyses of the 5′ NTR and VP1 sequences showed that A13, SV19, -26, -35, -43, -46 were members of *Human enterovirus A*, SA5 a member of *Human enterovirus B*, and SV6, N125 plus N203 were related, but apparently form a new enterovirus species most closely related to *Human enterovirus A*. The complete genome sequence of simian enterovirus A (A-2 plaque virus) showed an organization characteristic of enteroviruses (**Figure 1(a)**), but along with SV4 and SV28 forms another new enterovirus species, this time most closely related to *Human enterovirus B*.

Virus Replication

Most of the receptors identified for picornaviruses belong to the immunoglobulin superfamily or the integrin receptor family. However, the receptors for the animal enteroviruses remain to be determined. Internalization occurs via endocytosis, and as the endosome undergoes acidification, changes in the virion structure lead to the release of the vRNA into the cytoplasm. The vRNA acts as an mRNA; the first step of replication is, therefore, translation of the single, long ORF. The autocatalytic processing by the virus-encoded proteinases $2A^{pro}$ and $3C^{pro}$ produces the three 'primary' processing products P1, P2, and P3 (**Figure 1(a)**) – the full-length (predicted) translation product is not observed. Subsequently further, 'secondary', proteolytic processing of these precursors occurs mediated by $3C^{pro}$. In the case of poliovirus, it has been demonstrated that the P1 capsid protein precursor is processed not by $3C^{pro}$, but by $3CD^{pro}$ – also a proteinase. The function of the vRNA soon switches from that of an mRNA, to that as a template for –ve strand synthesis. The –ve-strand RNA then serves as a template for the synthesis of +ve-strand RNA. A large excess (~80-fold) of +ve-, over –ve-, sense RNA is observed. Although the RNA-dependent RNA polymerase ($3D^{pol}$) is an enzyme, overall the replication of vRNA is not 'enzymic' as such, since protein 3B is used to prime RNA synthesis and is covalently linked to the 5′ terminus of both +ve- and –ve-sense RNA.

A characteristic feature of picornavirus replication is the disappearance of the Golgi apparatus concomitant with the appearance of virus-induced vesicles. It is upon these structures that RNA replication occurs; proteins 2C and 3A are key players in this remodeling of the endomembrane system. An increase in the intracellular level of Ca^{2+} is also observed; protein 2B has been shown to function as a 'viroporin' releasing calcium ions stored in the endoplasmic reticulum.

Recombination occurs with high frequency, reported to be in the region of 10%. The polymerase complex, together with the nascent strand, may 'switch' template

and complete the synthesis of the vRNA from another parent. Analyses of recombinants have shown that viable progeny arise largely from template switching within noncoding regions, or regions encoding nonstructural proteins.

The process by which vRNA is encapsidated is not clear. A feature of this process is the 'maturation' cleavage of VP0 (1AB) into VP4 and VP2 (1B) – again poorly understood. Replication is rapid with cell death and release of progeny virions occurring within ~9 h for BEV and SVDV for most cell types.

Virion Structure and Properties

Particles have a buoyant density of 1.30–1.34 g ml^{-1} in cesium chloride gradients, are resistant to ether, and stable throughout a wide range of pH (3–10). The ability to tolerate low pH is seen as an adaptation to survive passage through the acidic conditions of the stomach. Under the electron microscope, particles are seen as icosahedrons, 25–30 nm in diameter (**Figure 2(a)**). Capsids are nonenveloped, comprising 60 copies of each of the capsid proteins VP4, VP2, VP3 (1C), and VP1. Capsid proteins are arranged into protomers and 60 of these structural units form an icosahedron (symmetry: pseudo $T = 3$; **Figure 2(b)**).

The atomic structures of both BEV and SVDV capsids have been determined. The folding pattern of BEV polypeptides VP1–3 is similar resulting in an eight-stranded antiparallel β-sheet structure. The VP4 protein is much smaller than the other capsid proteins, and lies across the inner surface of the capsid. The N-terminus of VP4 is close to the icosahedral fivefold axes of symmetry and the C-terminus close to the threefold axes. Furthermore, in all picornaviruses, the N-terminal residue of VP4 is covalently bonded to a myristic acid group giving the capsid five symmetry-related myristoyl moieties around the inner surface of the capsid, a channel running from the inner to outer surface at this point. It has been proposed that the myristic groups attached to the VP4 proteins may insert into the host membrane aiding entry.

A radial depth-cued image of the BEV particle is shown in **Figure 2(c)**. The topologies of the BEV major capsid proteins and the overall architecture of the virion are similar to those of related picornaviruses. Some differences were observed, however; the external loops are relatively truncated giving a comparatively 'smooth' appearance. A 'canyon' receptor binding region (running around the fivefold axes of symmetry) is observed in the structures of polio- and rhinoviruses. In BEV, this depression is partially filled by a five-residue extension of the G-H loop of VP3. The extended VP3 loop of BEV has been implicated in receptor binding, although the cell-surface receptor has not been identified. BEV is usually cultured in hamster kidney (BHK-21) cells but is known to be readily adaptable to grow in human cervical carcinoma (HeLa) cells to equivalent titer and can cause cytopathic effect in an extensive range of cell types *in vitro*. The BEV receptor is, therefore, thought to be a ubiquitous cell-surface glycoprotein. The crystal structure also revealed that the virus maintains a hydrophobic pocket within VP1, occupied by a specific 'pocket factor' which appears to be myristic acid. The pocket factor is thought to stabilize the capsid and it has been proposed that a kinetic equilibrium exists between occupied and unoccupied pocket states, with occupation inhibiting 'uncoating' of the vRNA. In purified BEV preparations, a small proportion of the precursor protein VP0 is detected and it is suggested that in a few protomers this precursor remains uncleaved. This maturation cleavage is completely absent in the genera *Parechovirus* and *Kobuvirus* – these viruses possessing only three structural proteins.

The crystal structures of two SVDV isolates, UK/27/72 and SPA/2/93, have been reported. These two structures are in agreement and are similar to those of other enteroviruses, with SVDV being most similar to coxsackievirus B3 (CBV-3). The major capsid proteins (VP1–3)

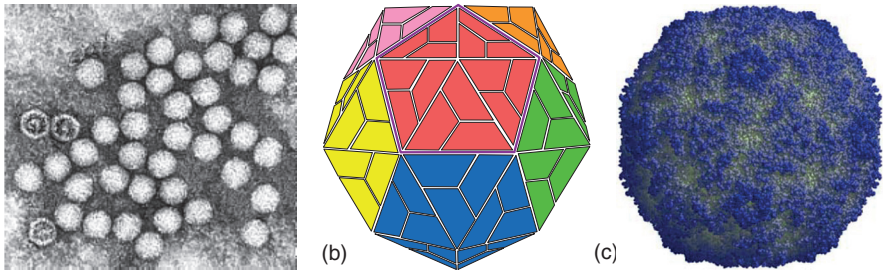


Figure 2 The structure of enterovirus particles. (a) A negatively stained electron microscopic image of enterovirus shows unenveloped particles ~25 nm in diameter. (b) The icosahedral structure of the particle is shown. Five copies each of capsid proteins 1A–D form a pentamer, twelve of which form the complete particle. (c) A radially depth-cued image produced from the X-ray crystallographic data of the BEV particle showing surface projections (dark blue) – particularly notable at the fivefold axis of symmetry. The particle is smoother than the surface of other enteroviruses. Rasmol image courtesy of Dr. J.-Y. Sgro, University of Wisconsin, USA.

possess the conserved β-sheet structure with some notable differences. VP1, making up most of the outer surface area, is often the most variable, and while the β-sheet is conserved, the connecting loops vary in length between SVDV and CBV-3. Five copies of VP1 associate to form each of the fivefold vertices, and in SVDV it has been noted that an arginine residue from each of the five VP1s form a crown at the apex of the fivefold axes of symmetry. The hydrophobic pocket formed by VP1 is also present in SVDV and electron density clearly showed that this accommodates a fatty acid pocket factor. The hydrophobic pocket situated at the base of the canyon extends from a pore on the surface inward, until it is blocked by VP1 loops. In CBV-3, the pocket factor has been identified as palmitic acid. The dimensions of the pocket are similar in CBV-3 and SVDV, but in the case of SVDV the electron density suggests that the pocket factor is a longer molecule – similar to sphingosine.

The VP2 and VP3 proteins alternate around the threefold axes of symmetry. The VP2 of SVDV possesses a puff region composed of two sequential loops between sheets E and F, with the second loop being more exposed on the surface. This is the least conserved area between SVDV and CBV-3. Furthermore, in poliovirus, this area has been implicated in the binding of the virus to its cell-surface receptor. The C-terminus of the VP3 protein is external and forms a major surface protrusion termed the 'knob'; this structure is present in SVDV and CBV-3. VP3 is also important in the stability of the pentamer with fivefold neighboring VP3 proteins forming a β-cylinder.

VP4 is situated on the inner surface of the capsid and is the most conserved of the capsid proteins. The central region of this protein was found to be disordered with little secondary structure. VP4 begins close to the fivefold axes and snakes toward the nearest threefold axes, with the C-terminus of VP4 lying close to the N-terminus of VP2. The N-terminal glycine of VP4 is covalently attached to myristic acid and the myristoyl groups of adjacent VP4s group around the fivefold axes, under the VP3 β-cylinder. In general, the surface structure of SVDV is very similar to that of coxsackieviruses.

Rather than a continuous, circular, canyon in SVDV, there are five distinct depressions. The C-terminus of VP3, the first loop of the VP2 puff, and residues of the C-terminal VP1 loop form a ridge between these depressions. Further depressions are present on the twofold axes with the enclosing four walls composed of symmetrically related pairs of VP2 and VP3. SVDV, the six CBV serotypes (CBV-1–6), and many adenoviruses share a common receptor on human cells. The coxsackieadenovirus receptor (CAR) has two immunoglobulin-like extracellular domains, a transmembrane domain and a cytoplasmic domain. Cryoelectron microscopy has provided evidence that CAR binds into the canyon of CBV-3, mostly interacting with VP1 but with contributions from VP2 and VP3. CBV-5 (thought to have given rise to SVDV in pigs) uses CAR as a primary receptor and decay-accelerating factor (DAF; CD55) as a co-receptor. Recent isolates of SVDV have been shown to have lost the ability to bind human DAF but have not developed the ability to bind pig DAF, suggesting that SVDV may have adapted to use another co-receptor which is tailored to the new host.

Concluding Remarks

Understandably, in terms of research effort, for many years the animal enteroviruses have been the poor cousins of their human 'relatives'. The greatly expanded sequence database has led to a wholescale reappraisal of the taxonomy of these viruses. It is clear that within this group close relationships exist between animal and human viruses and that viruses have passed from man to animals, and vice versa. The high mutation rate in combination with high frequency of recombination means these viruses can rapidly adapt to new host species. Animal enteroviruses are, therefore, worthy of study not only for their intrinsic interest and economic impact but, through enzoonosis, they pose an ever-present threat to human health.

See also: Coxsackieviruses; Enteroviruses: Human Enteroviruses Numbered 68 and Beyond.

Further Reading

Hughes AL (2004) Phylogeny of the *Picornaviridae* and differential evolutionary divergence of picornavirus proteins. *Infections, Genetics and Evolution* 4: 143–152.

Hyypia T, Hovi T, Knowles NJ, and Stanway G (1997) Classification of enteroviruses based on molecular and biological properties. *Journal of General Virology* 78: 1–11.

Krumbholz A, Dauber M, Henke A, *et al.* (2002) Sequencing of porcine enterovirus groups II and III reveals unique features of both virus groups. *Journal of Virology* 76: 5813–5821.

Oberste MS, Maher K, Flemister MR, *et al.* (2000) Comparison of classic and molecular approaches for the identification of untypeable enteroviruses. *Journal of Clinical Microbiology* 38: 1170–1174.

Oberste MS, Maher K, and Pallansch MA (2002) Molecular phylogeny and proposed classification of the simian picornaviruses. *Journal of Virology* 76: 1244–1251.

Oberste MS, Maher K, and Pallansch MA (2003) Genomic evidence that simian virus 2 and six other simian picornaviruses represent a new genus in *Picornaviridae*. *Virology* 314: 283–293.

Pöyry T, Kinnunen L, Hovi T, and Hyypiä T (1999) Relationships between simian and human enteroviruses. *Journal of General Virology* 80: 635–638.

Zang G, Haydon DT, Knowles NJ, and McCauley JW (1999) Molecular evolution of swine vesicular disease virus. *Journal of General Virology* 80: 639–651.

Zell R, Dauber M, Krumbholz A, *et al.* (2001) Porcine teschoviruses comprise at least eleven distinct serotypes: Molecular and evolutionary aspects. *Journal of Virology* 75: 1620–1631.

Zell R, Krumbholz A, Dauber M, Hoey E, and Wutzler P (2006) Molecular-based reclassification of the bovine enteroviruses. *Journal of General Virology* 87: 375–385.

Enteroviruses: Human Enteroviruses Numbered 68 and Beyond

T Hovi, S Blomqvist, C Savolainen-Kopra, and M Roivainen, National Public Health Institute (KTL), Helsinki, Finland

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Acute flaccid paralysis (AFP) A descriptive diagnosis used in the context of poliomyelitis eradication initiative (PEI), coordinated by the World Health Organization (WHO). A case of AFP is a patient with acute onset paralytic symptoms without known etiology. Within the PEI, an AFP patient is suspected of having poliomyelitis and should be tested for poliovirus excretion.

Acute hemorrhagic conjunctivitis (AHC) A rapidly developing severe inflammatory disease of the skin covering the eyeballs, including bleeding of the inflamed skin.

Hand-foot-and-mouth disease (HFMD) An acute febrile disease of children with flat or raised red spots, which may later develop to vesicles, in the mouth as well as on palms and soles of feet.

Phylogenetic typing Determination of genetic type of an unknown strain by sequence and phylogenetic analysis of partial genome sequence.

Prime strains Virus strains showing one-way cross-reactivity with prototype strains of a serotype and thus reflecting within-serotype antigenic variation. Antisera against a prototype strain do not neutralize a prime virus strain but an antiserum against a prime strain will neutralize the prototype strain.

Introduction

Historical Aspects

Original taxonomic subgrouping of human enterovirus (EV) isolates was based on host species range and pathogenicity in humans or experimental animals. Accordingly, the strains were classified as polioviruses (PVs), coxsackie viruses of subgroup A (CVA), or B (CVB), or echoviruses (E). Each subgroup was further divided in three or more serotypes according to results of cross-neutralization assays with hyperimmune antisera raised against individual strains. Toward the end of 1960s, after initial characterization of 3 PV serotypes, 24 CVA serotypes, 6 CVB serotypes, and 34 E serotypes, it was realized that antigenic relationships did not always correlate with other phenotypic features used in the taxonomic subgrouping. Subsequently, new serotypes were no more distributed in the established subgroups but instead given a running number from 68 onward. Within a few years, five new serotypes, EV68 through EV72, were identified. However, EV72 turned out to be very different from typical EVs and was later reclassified as hepatitis A virus in a genus of its own (*Hepatovirus*) in the family *Picornaviridae*.

Development of molecular techniques in virus strain characterization, notably phylogenetic analysis of (partial) genomic sequences, enabled novel approaches in virus taxonomy and also revived taxonomical interest in EV strains in the late 1990s. The official taxonomic subclassification of the genus *Enterovirus* now includes four phylogenetically distinct EV species with strains infecting humans, *Human enterovirus (HEV) A–D* (**Table 1**).

General Features of Human Enteroviruses

Human enteroviruses (HEVs) naturally share many basic biological properties with other picornaviruses, but may also show features typical of the species, serotype, or even strain in question. Although many of the features often referred to as typical of EVs have in reality been examined only for poliovirus type 1/Mahoney, we assume here that in principle they are also likely to be true for enterovirus types 68 and beyond. Exceptions to the 'rules', if known, will be mentioned in the subsequent serotype-specific articles.

EVs are typically acid stabile, nonenveloped, icosahedral particles composed of 60 structural subunits each comprising one copy of each of the four structural proteins, capsid protein 1–4 (VP1–VP4) surrounding the about 7500 nucleotide (nt) long RNA genome. Steps of the replication cycle follow the common pattern of viruses with messenger-sense single-stranded RNA genome.

Humans are the sole natural hosts of HEVs. Transmission of EV readily takes place through close direct contacts, for example, within a family, but also through indirect contacts such as by touching by fingers objects or surfaces contaminated with feces or respiratory excreta. Out of the large number of human enterovirus serotypes, only polioviruses can be prevented today by vaccines, developed already half a century ago.

Enterovirus 68 (HEV-D)

History

Human enterovirus 68 (EV68) was originally isolated from four children with pneumonia and bronchiolitis in

Table 1 Distribution of enterovirus (sero)types in the old and new taxonomic subclasses

	Classical subgroup of enteroviruses/sero(type) number				
Species	*Poliovirus*	*Coxsackie-virus A*	*Coxsackie-virus B*	*Echovirus*	*New enterovirus*
Human enterovirus A ($N = 18$)		CVA2–CVA8, CVA10 CVA12, CVA14, CVA16			EV71, EV76[a] EV89[a]–EV92[a]
Human enterovirus B ($N = 57$)		CVA9	CVB1–CVB6	E1–E7, E9 E11–E21 E24–E27 E29–E33	EV69, EV73[a]–EV75[a] EV77[a]–EV88[a] EV93[a], EV97[a], EV–98[a] EV100[a], EV101[a]
Human enterovirus C ($N = 16$)	PV1–PV3	CVA1, CVA11, CVA13 CVA17 CVA19–CVA22 CVA24			EV95[a], EV96[a] EV99[a], EV102[a]
Human enterovirus D ($N = 3$)					EV68, EV70, EV94[a]

[a]Novel types identified by the VP1 coding sequence and registered by the Picornavirus Study Group of ICTV.
PV, poliovirus; CVA, coxsackievirus subgroup A; CVB, coxsackievirus subgroup B; E, echovirus; EV, enterovirus. Explanation for lacking numbers: CVA15 and CVA18 have been reclassified as strains of CVA11 and CVA13, respectively; CVA23 was found to be same as echovirus 9; echovirus 8 is a strain of E1; echovirus 10 has been reclassified as reovirus type 1, echovirus 28 as rhinovirus type 1A; echovirus types 22 and 23 now belong to another genus, *Parechovirus*; the initially identified echovirus 34 is a strain of E33, enterovirus 72 was classifies as hepatitis A virus in the genus *Hepatovirus*.

California in 1962. One of these simultaneous isolates, recovered from an oropharyngeal swab of a 10-month-old female, was defined as the prototype strain Fermon. Since then isolations of EV68 have been rare. Human rhinovirus 87 (prototype strain Corn) was recently shown to both antigenically and genetically represent the same enterovirus serotype as EV68. Neutralization assays with antisera raised against EV68 Fermon and HRV87 Corn demonstrated one-way cross-reactivity between these virus strains indicating that strain Fermon could be considered a prime strain of the Corn.

Epidemiology

Most of the relatively few known EV68 isolates have been obtained from respiratory specimens, but occasional isolations from stool and sewage specimens have also been made. The transmission of EV68 is more plausible by the respiratory than by the fecal–oral route. EV68 may affect persons of different ages, although it has been most often detected in young children. Despite the infrequent isolation from clinical specimens, EV68 is probably a highly common virus since antibodies against EV68 are frequently found among Finnish people.

Cellular Interactions

Isolates of EV68 have been obtained from a variety of human and monkey cell lines. Besides, at least some of the currently circulating EV68 strains are able to induce cytopathic effect (CPE) in nonprimate mouse L cells. The host cell surface molecules involved in the initiation of EV68 infection are not known, but recent studies indicate that both the decay-accelerating factor (DAF) and a yet unknown sialic acid moiety may be required. Receptor specificity of HRV87 Corn was studied among other human rhinoviruses. The requirement for the presence of sialic acids on the HeLa cells for attachment of HRV87 distinguished it from other HRVs, the receptor of which was either LDL receptor or ICAM-1. On the other hand, pretreatment of HeLa Ohio cells with a monoclonal antibody to the SCR-3 epitope of DAF inhibited both the HRV87 and EV68 induced CPE.

Clinical Presentation and Pathogenesis

EV68 has a clear association with respiratory diseases. Most clinical isolates of EV68 have been obtained from respiratory specimens of patients with respiratory tract infection. Studies on strains EV68 Fermon, HRV87 Corn, and some recent clinical EV68 isolates have shown that these viruses share important biological features with human rhinoviruses, the most prominent causative agents of common colds. Unlike typical EVs, strains of EV68 lose their infectivity in acidic environment and besides, grow more effectively at $+33\,^{\circ}C$ than at $+37\,^{\circ}C$. Clinical illness or symptoms described upon isolation of EV68 included pneumonia, bronchiolitis, upper respiratory tract infection, wheezing, asthma, emesis, respiratory distress, crackles, cough, and asthma exacerbation.

Enterovirus 69 (HEV-B)

Enterovirus 69, strain Toluca, was isolated in 1959 from a rectal swab of a healthy Mexican child. Later detections of EV69 among enterovirus isolates have remained very rare but the virus has been found in different parts of the

world, mainly in patients with nontypical respiratory disease. Respiratory excreta and stool specimens may yield the virus demonstrating typical enteroviral CPE in monkey kidney and human tumor cell lines. Further details of the replication cycle have not been elucidated. Some antigenic cross-reactivity with echovirus 6 has been reported but according to phylogenetic analysis of the entire genome, EV69 is a typical member of the species *HEV-B* with its own distinct branch in a tree constructed on VP1 sequences and variable, region-dependent clustering with other HEV-B serotypes as regards the nonstructural protein coding sequences.

Enterovirus 70 (HEV-D)

History

An emergence of a new type of viral conjunctivitis was observed in Acera, Ghana, in June 1969. The disease, locally first called 'Apollo 11 disease' because of the coincidental landing of Apollo 11 on the moon, was named as acute hemorrhagic conjunctivitis (AHC) and it spread rapidly over Africa and Asia. The causative agent was isolated in Japan, Singapore, and Morocco in 1971 and assigned as enterovirus 70 (EV70).

Phylogenetic and epidemiological analyses have confirmed that EV70 emerged from one focal place in August 1967 ± 15 months, which is about 2 years before the first recognition of the AHC pandemic. It has been speculated that EV70 may have originated from an animal or insect picornavirus. This hypothesis is supported by the wide *in vitro* host range of EV70 and detection of EV70 neutralizing antibodies from animal sera collected from Ghana and Senegal already around the estimated time of emergence of EV70.

Epidemiology

EV70 has caused two pandemics and several epidemics of AHC. The first pandemic began in Ghana and spread over Africa and Asia during 1969–1971. The largest number of cases occurred in crowded coastal areas of tropical countries. At the same time, outbreaks were also observed in some European countries. The second pandemic started in southern India in 1980 and spread extensively during 1980–1982, now also including the Western Hemisphere. The epidemic EV70 strains from Asia and America in 1980 and 1981 were shown to be genetically closely related and to differ significantly from the strains recovered during the first pandemic. Since the two large pandemics, which caused about 100 million cases of AHC, smaller outbreaks have been described at least in Singapore, China, Brazil, and Cuba in 1980s and in American Samoa, Israel, Japan, and India in 1990s.

EV70 is highly contagious and spreads fast under crowded and unhygienic conditions. The virus may be transferred by fomites as well as by direct inoculation by contaminated fingers. High levels of relative humidity prolong the survival of EV70. Transmission of EV70 occurs usually at school or home, but may also occur, for example, in eye clinics. During the epidemic, the highest number of cases is typically among school-aged children. Women in child-bearing age have been reported to be affected more frequently than men in the same age.

EV70 induces immune response, which provides only short-lived protection. Consequently, multiple epidemics have been seen in a given geographical region even within a 5-year period. Although EV70 typically causes clear-cut epidemics, the virus may continue endemic circulation in the affected population after the main outbreak. Sporadic cases of AHC or neurological diseases may occur over a period of some years.

Cellular Interactions and Pathogenesis

In humans, EV70 exhibits a restricted tropism to conjunctival and corneal cells, but like several other HEVs, it also has a propensity for infecting the central nervous system (CNS). *In vitro*, EV70 has the ability to infect a wide spectrum of mammalian cell lines, which is untypical for most HEVs.

EV70 attaches to the short consensus repeat domain 1 of DAF, on HeLa cells. However, additional sialylated binding factors on the cell surface are needed for productive infection. The DAF is also used as a receptor by several other EVs, but the requirement for the cell surface sialic acid is unique among HEVs. It has been speculated that this recognition of sialic acid attached to underlying glycans by a particular glycosidic linkage might contribute to the exceptional *in vitro* host range and tissue tropism of EV70.

Clinical Presentation and Pathology

EV70 is, together with a variant of CVA24, the leading causative agent of AHC. The typical site for EV70 entry is conjunctiva, where the virus may be transferred by fomites as well as by direct inoculation by contaminated fingers. The incubation period is about 24 h. The disease is usually localized to the eye and characteristically produces subconjunctival hemorrhage, which ranges from discrete petechiae to large patches of hemorrhage covering the bulbar conjunctiva. Symptoms include severe eye pain, photophobia, and blurred vision. Less specific symptoms like headache, nasal discharge, and sore throat may also occur. The cornea may be transiently involved in the form of epithelial keratitis. Asymptomatic EV70 infections are uncommon. Recovery is usually complete within less than 10 days with no significant ocular sequelae.

During the two EV70 pandemics, neurological manifestations were reported in about 1 out of 10 000 AHC patients. Male adults were more likely to be affected than women. The neurological symptoms resembling poliomyelitis developed as late as several weeks after the onset of AHC. Significant antibody titers against EV70 were demonstrated in the serum and more relevantly in the cerebrospinal fluid (CSF) of the patients.

Diagnosis

AHC caused by EV70 or other viruses are clinically indistinguishable. EV70 is most frequently isolated from conjunctival and oropharyngeal specimens, occasionally also from feces. Although several different cell lines may support EV70 replication, isolation of EV70 in cell culture is considered insensitive and today, EV70 infection is typically diagnosed by serological means or by reverse transcriptase-polymerase chain reaction (RT-PCR) methods. Demonstration of EV70 specific IgM antibodies in sera indicates a recent EV70 infection. The titers of IgM antibodies against EV70 begin to drop by the fifth week after onset of illness. In addition, neutralizing antibodies can be measured from sera, tears, or CSF. EV70 specific RNA by RT-PCR has been successfully detected from eyewash specimens, eye swabs, conjunctival swabs, and tears. Sequence analysis of (partial) VP1 is an useful tool for determination the origin of the outbreak.

Prospects for Prevention, Control, and Treatment

Currently no practical treatment method or effective vaccine is available for AHC caused by EV70. Preliminary studies aiming at development of the vaccine have been conducted with immunogenicity of the candidate materials tested in rabbits. Immunizations with UV-inactivated EV70 or bacterially produced EV70 VP1 protein or its nonoverlapping N and C terminal fragments were reported to elicit a classical humoral immune response including EV70-neutralizing antibodies in rabbit sera.

Disinfection and control of EV70 has been studied in experimental eye clinic conditions. When placed in eye drop solution and kept at room temperature, the infectivity of EV70 is sustained for more than 20 days. The most convenient and efficient disinfection procedures against EV70 is either drying the instruments and keeping them in a moisture-free environment or heating at 90 °C for 5 s.

Enterovirus 71 (HEV-A)

History

EV71 was initially isolated in 1969 from a case of fatal encephalitis in California. Since then it has caused several outbreaks in different parts of the world including Australia, Bulgaria, Hong Kong, Hungary, Japan, and Sweden. Clinical symptoms of infection have been various, sometimes associated with CNS involvement. In recent years Southeast Asia has suffered from large epidemics of EV71, manifested, for example, in Taiwan 1998 with 405 patients hospitalized and 78 fatalities.

Epidemiology and Genetic Characteristics

EV71 appears worldwide with both endemic and epidemic circulation. Most infections occur in children under 5 years. Infections are transmitted by feco–oral route, but respiratory droplet transmission is also possible. Epidemics occur year around with peaks in late summer and autumn, occasionally in 3-year cycles.

EV71 is a member of HEV-A species with a close genetic and antigenic relationship to CV-A16. Nucleotide and amino acid homologies are 77% and 89%, respectively, between these viruses. At least four distinct genogroups A, B, C, D, with subgroups B1–4 (5), C1–4, have been identified. The EV71 prototype strain, BrCr, is the sole member of genogroup A. Strains belonging to genogroups B and C have mainly caused the epidemics in the Asia-Pacific region. Large sets of strains worldwide have been subjected to molecular epidemiological and sequence analysis, but neurovirulence has not been connected to a single genotype and its genetic determinants have remained elusive.

Cellular Interactions and Pathogenesis

The receptor molecules involved in EV71 are not known. In the laboratory, EV71 strains replicating in Vero and RD or other primate cell lines produce CPE typical of EVs. Like other EVs, EV71 interferes with the cellular apoptosis program by inhibiting the activation during early steps of infection and then enhancing apoptosis in the later stages of the infection. It is not known if apoptosis has a significant role in the generation of symptoms *in vivo*. Immune response after infection is considered to be typical of enterovirus infection, involving both humoral and cellular responses with the humoral immunity likely to provide protection against reinfections. Animal models using either mice or monkeys have been developed for *in vivo* studies of EV infection. Alpha interferon seems to be an important component of the innate immunity limiting spread of the virus in the body. In an oral-infection model in newborn mice, the virus was found to spread from the intestines to several tissues including the spinal cord, eventually causing brain-stem encephalitis. The virus also spread to unprotected littermates and the dams. The model thus has several features similar to the human EV71 infection and is likely to be useful in future.

Clinical Presentation and Pathology

Enterovirus 71 is one of the two major HEV-A serotypes causing the relatively common hand-foot-and-mouth disease (HFMD) in children, the other being CVA16. In the western world, CVA16 has been dominating but in Southeast Asia, EV71 has caused severe outbreaks since the late 1990s with sometimes transmission of CVA16 coinciding. The usually self-limiting HFMD starts with fever followed by appearance of flat or raised red spots in the tongue, gums, and inside of the cheeks, as well as on palms, soles of feet, and occasionally buttocks. The spots may develop to blisters and in the mouth further turn to ulcers. The rash does not usually itch. Neurological complications, aseptic meningitis, encephalitis, and poliomyelitis-like polyneuritis are relatively common in EV71 but rare in CVA16 infections. In the large Southeast Asian epidemics during the last decade, meningo-encephalitis has occasionally been an alarmingly common complication with significant associated mortality especially in young children. Neurological disease may occasionally occur without recognizable rash. Pulmonary edema may contribute to the death and is considered to be secondary to dysregulation of blood circulation due to virus-induced damage in the brain stem resulting in dysregulation of glucose homeostasis, rather than putative infection of pulmonary tissues.

Diagnosis

EV71 infection has traditionally been confirmed in cell culture with virus isolation and serotyping using samples from throat swab or vesicular fluid or postmortem samples from the brainstem or spinal cord. Serological diagnosis with a high single titer or in a minority of cases a fourfold rise in antibody has also been used. New methods for rapid serotype-specific identification to replace the traditional tedious ones have been developed prompted by recent outbreaks. These include RT-PCR, real-time RT-PCR hybridization probe assay, DNA microchip array, and IgM-capture enzyme-linked immunosorbent assay (ELISA). Phylogenetic analysis of VP1 and VP4 capsid protein sequences have been used in tracing genetic origin of the strains.

Prospects for Prevention, Control, and Treatment

Severe outbreaks of EV71 in Southeast Asia have triggered studies aiming at development of vaccine and/or antiviral chemotherapy, but neither vaccine nor specific chemotherapy is available in the clinic. Initial studies in experimental animals have reported immunogenicity of Vero-cell-grown inactivated virions, baculovirus-expressed empty VP0-VP3-VP1 procapsids, and even transgenic tomatoes expressing the VP1 protein, but no clinical studies have been published by mid-2006. Spreading of the virus via both respiratory and feco–oral routes restricts the efficacy of attempts to control spreading of the virus in human populations. Pregnant women have been advised to avoid contacts with children suffering from HFMD although no data have been accumulated to suggest that maternal EV71 infection would be more dangerous to the fetus than infections in general. *In vitro* and animal model studies suggest that alpha interferon and lactoferrin may limit EV71 replication. In cell culture, siRNAs targeted to different genomic regions of EV71 were found to have antiviral activity.

The Newly Discovered HEV Types

History

Nontypable enterovirus isolates have been detected as long as enterovirus isolations have been carried out. Between 1970 and 2000, they were more or less ignored, and their existence perhaps interpreted by assuming that they might represent prime strains of known serotypes, rare serotypes not represented in the antiserum pools used, or mixtures of two EVs too difficult to sort out in the neutralization assays. By the end of 1990s, it became evident that phylogenetic analysis of VP1 coding sequences of conventionally serotyped enterovirus isolates results in defined clusters showing complete agreement with the phenotypic serotype. Subsequently, several groups have used this approach to identify nontypable isolates in historical collections of enterovirus strains, or among new isolates. The Picornavirus Study Group of ICTV has by mid-2006 listed almost 30 new types and the running number of the next new serotype is beyond 100. It is interesting that new enterovirus types have been described in all four HEV species and that most of them are in the species *HEV-B,* already traditionally the largest HEV species (**Table 1**).

For all the newly discovered enterovirus types, detection of perhaps only one or at the best, a handful of independent isolates have been reported. For most strains, only the source of origin and the VP1 sequence is known. Therefore, it is not possible to describe in further detail more than a few examples of these novel types.

New Types Forming a Subcluster within the Species HEV-A

Four of the new HEV-A serotypes have been studied in some detail. All but one of the altogether 19 isolates were derived from Bangladeshi children with acute flaccid paralysis (AFP) and collected in 1999–2002. The remaining isolate was from an adult gastroenteritis patient in France from 1991. It is not clear if these infections had any role in the generation of the recorded symptoms. Sequences of the VP1 coding region formed four serotype-like clusters

close to but separated as a group from the classical HEV-A cluster as well as from the simian enterovirus isolates tentatively classified in the HEV-A species. Eleven strains, including the French one, were given type number 76 while two, four, and two strains segregated in the clusters labeled EV-89, EV-90, and EV-91, respectively. Sequences of the entire capsid coding region confirmed this clustering and monophyletic origin of each new type as well as that of the new subcluster within HEV-A. The observed monophyletic segregation of the new types from the classical HEV-A serotypes extended to the nonstructural protein coding part of the genomes. Hence, evidence for recombination between these new HEV-A types and the classical ones (or the simian ones) was not obtained. Analysis of larger numbers of independent sequences may be required before final conclusion about the matter but it is possible that this subcluster has diverged from the other HEV-A strains far enough so that functional combatibility of genomic parts joined in putative recombination events is too poor to produce viable progeny with sufficient fitness for enrichment and widespread transmission.

Selected New Types of the Species HEV-B

Epidemiological background and some genomic features of HEV types 73–75 and 77–78 have been published. While parts of these strains are derived from poliovirus surveillance, either from patients with AFP or their contacts, a significant proportion is from patients suffering from a variety of clinical entities typical of classical HEV-B infection, ranging from acute respiratory infection to aseptic meningitis. In phylogenetic analysis, these new types do not form a subcluster within HEV-B but are rather distributed in different parts of the multipronged tree of HEV-B.

HEV-94, A Proposed New Type in the Species HEV-D

During environmental polio surveillance in Egypt four nonpoliovirus strains were isolated from sewage specimens in L20 B cells, a recombinant murine cell line expressing human poliovirus receptor. The VP1 sequences of these isolates clustered together and showed less than 70% nucleotide and less than 80% amino acid sequence similarity to the closest established enterovirus serotype, EV70. This suggests that the isolates form a new enterovirus type, EV94, within the species *HEV-D*. In parallel with this finding AFP surveillance in the Democratic Republic of Congo revealed two nonserotypable virus strains whose VP1 sequences clustered very close to those of the above Egyptian isolates suggesting that they belong to the same serotype. Altogether, VP1 sequences of all these six EV94 isolates formed a monophyletic group within the species *HEV-D*. Enterovirus 94 cannot be considered as a new virus type although just recently discovered, since, according to the results of seroprevalence studies carried out in Finland, neutralizing antibodies to this virus strain were common already two decades ago.

Comparison of the whole genome sequence of the prototype strain of EV94 with those of EV68 and EV70 revealed no evidence for recombination between the prototype strains of these three HEV-D serotypes. In further characterization, the prototype strain turned out to be acid resistant suggesting that this virus strain, unlike EV68 and some strains of EV70, is a typical enteric pathogen capable of surviving the acidic stomach during the passage to the presumed intestinal replication sites.

The natural host range and the cellular receptor for EV94 are not yet known. Although two strains of EV94 were isolated from stool specimens of patients with AFP, it is not yet known whether these virus strains could be causative agents of neurological symptoms.

Future Prospects

It is likely that the number of human enterovirus (sero) types will still increase as more and more previously nontypable isolates will be characterized genetically. While genetic and serological identification of the HEV serotype usually is in good agreement, there are exceptions to this rule. Some serotypes contain strains showing wide genetic divergence in the VP1 coding region and sometimes strains of genetically relatively homogenous clusters show strong antigenic variation. On the other hand, it is unlikely that anybody would take the trouble and serotype all the new genetically identified types in the classical way using monotypic antisera. Serotyping of isolates is a tedious and expensive procedure and may in the future remain as a research tool only. For identification of clinical isolates, genetic tools are more rapid and useful, for example, in tracking the transmission routes.

See also: Coxsackieviruses; Enteroviruses of Animals; Epidemiology of Human and Animal Viral Diseases; Evolution of Viruses; Human Eye Infections; Picornaviruses: Molecular Biology; Poliomyelitis; Quasispecies; Rhinoviruses.

Further Reading

McMinn PC (2002) An overview of the evolution of enterovirus 71 and its clinical and public health significance. *FEMS Microbiology Reviews* 26: 91–107.

Nokhbeh MR, Hazra S, Alexander DA, *et al.* (2005) Enterovirus 70 binds to different glycoconjugates containing α-2, 3-linked sialic acid on different cell lines. *Journal of Virology* 79: 7087–7094.

Oberste MS, Maher K, Michele SM, Belliot G, Uddin M, and Pallansch MA (2005) Enteroviruses 76, 89, 90 and 91 represent a novel group within the species *Human enterovirus A*. *Journal of General Virology* 86: 445–451.

Oberste MS, Nix WA, Maher K, and Pallansch MA (2003) Improved molecular identification of enteroviruses by RT-PCR and amplicon sequencing. *Journal of Clinical Virology* 26: 375–377.

Pallansch MA and Roos RP (2001) Enteroviruses: Polioviruses, coxsackieviruses, echoviruses and newer enteroviruses. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 4th edn., pp. 723–775. Philadelphia, PA: Lippincott Williams and Wilkins.

Racaniello VR (2001) *Picornaviridae*: The viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 839–893. Philadelphia, PA: Lippincott Williams and Wilkins.

Smura T, Junttila N, Blomqvist S, *et al.* (2007) Enterovirus 94, a proposed new serotype in human enterovirus species D. *Journal of General Virology* 88: 849–858.

Relavant Website

http://www.picornaviridae.com – Picornavirus (refer to Enterovirus).

Entomopoxviruses

M N Becker and R W Moyer, University of Florida, Gainesville, FL, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The entomopoxviruses (EVs) comprise the *Entomopoxvirinae*, one of the two subfamilies of the family *Poxviridae*. EVs infect insect hosts and were first described in 1963. These viruses are found in a number of insect species but are particularly well characterized within the butterflies and moths. The other subfamily of poxviruses is the *Chordopoxvirinae*, which contains the well-known variola virus, the causative agent for smallpox, and vaccinia virus (VV), the vaccine strain for smallpox. The EVs possess a number of the same features as the chordopoxviruses (CVs); however, there are striking differences between the two subfamilies. The similarities include general virion morphology, a double-stranded DNA genome, and a cytoplasmic life cycle. The differences include the host range of the viruses, the presence of occluded virus in EVs, the composition and organization of the DNA genome, differences in gene regulation, and the optimal temperature for growth, which is 27 °C for EVs versus 37 °C for CVs. Although a large number of EVs have been identified, very little is known about most of them with the exception of the *Amsacta moorei* EV (AMEV), which is amenable to growth in culture.

Phylogeny

Within the subfamily *Chordopoxvirinae* are eight genera. The causative agent of smallpox (variola virus) and the virus used for vaccination against smallpox (VV) are members of the genus *Orthopoxvirus*. Other CVs that cause human disease are cowpox virus and monkeypox virus within this same genus and molluscum contagiosum virus in the genus *Molluscipoxvirus*.

Within the subfamily *Entomopoxvirinae* are three genera, recently renamed by the International Committee on Taxonomy of Viruses: *Alphaentomopoxvirus*, *Betaentomopoxvirus*, and *Gammaentomopoxvirus*. The genera are defined predominantly by the host range of the viruses and by the morphology of the virus particle. The genus *Alphaentomopoxvirus* contains members that infect beetles (coleopterans), and the type species is *Melolontha melolontha entomopoxvirus*. Little is known about the molecular biology of the members of this genus. The genus *Betaentomopoxvirus* contains the best-studied members of the EVs. Betaentomopoxviruses infect either butterflies and moths (Lepidoptera) or grasshoppers and locusts (Orthoptera). The type species is *Amsacta moorei entomopoxvirus*. The host of AMEV is the red hairy caterpillar from India, a member of the tiger moths. Members of the genus *Gammaentomopoxvirus* infect flies, mosquitoes, or midges (Dipterans), and the type species is *Chironomus luridus entomopoxvirus*. There are also a number of unclassified EVs. Included in this group is the well-studied virus from the grasshopper, *Melanoplus sanguinipes* (MSEV). Formerly classified as a betaentomopoxvirus owing to its host range, this virus has been reclassified based on genomic sequencing data and comparison to the genomic sequence of AMEV. Evolutionary trees based on the DNA polymerase sequence indicate not only that the EVs are distinct and evolutionarily separated from the CVs, but that AMEV and MSEV are also highly divergent from each other. There are other unclassified EVs that infect Hymenoptera (bees and wasps). The best studied of these is *Diachasmimorpha* EV, whose host is the parasitic wasp, *Diachasmimorpha longicaudata*.

Virion Structure

The general virion structure is similar to that of the orthopoxviruses in that it consists of an inner core of electron-dense material surrounded by one or two lateral bodies (**Figure 1**). Within the core are the double-stranded DNA genome and a number of proteins required for the cytoplasmic life cycle of the virus. The entire virion is enveloped by a membrane. **Table 1** indicates the differences in virion size and shape of the lateral bodies of virions from different genera.

One of the unique features of EVs in comparison to CVs is that in nature they exist in an occluded form (**Figure 1**). A variable number of virions are embedded within a paracrystalline protein matrix consisting almost exclusively of a single protein, spheroidin. This is a large protein of 100–115 kDa that is abundant at late times during infection. Spheroidins from different viruses infecting hosts of the same insect order have greater than 80% identity to each other; however, spheroidins from viruses that infect hosts from different orders, such as coleopterans versus lepidopterans, have little similarity. The baculoviruses are another family of double-stranded DNA insect viruses that also produce occluded virus. In baculoviruses, it is the polyhedrin protein that forms the occlusion bodies (OBs). Despite a similar function, spheroidin has little similarity to the baculovirus polyhedrin protein. A large number of cysteines are present with the spheroidin protein, leading to the hypothesis that the OB structure is held together by disulfide bonds.

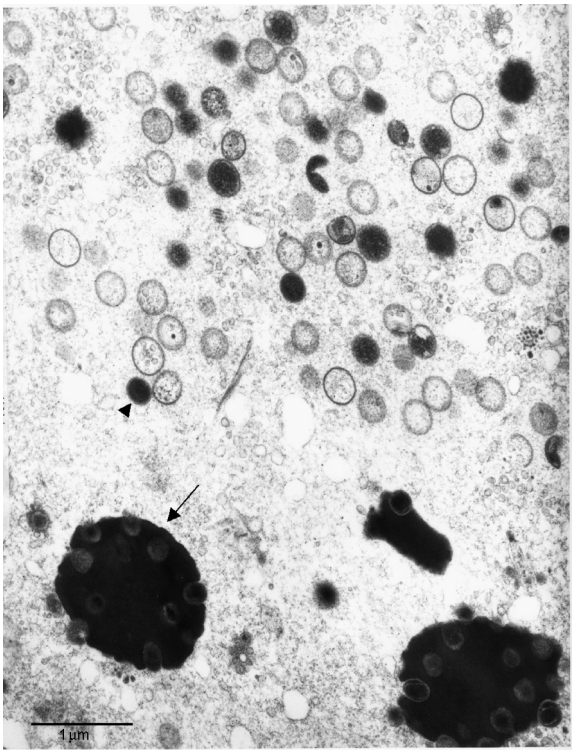


Figure 1 AMEV-infected *Lymantria dispar* cell (cell line Ld652). Different stages of virion development are seen, including mature virions indicated by an arrowhead and OBs indicated by an arrow.

The function of the OBs is to provide stability for the virus in the environment until ingested by another caterpillar. OBs contain a variable number of virion particles and only mature virions are occluded. OBs are alkaline sensitive, consistent with the high pH of the insect gut. Spheroidin is not required for virulence and the gene is nonessential in tissue culture. Nonoccluded virus is poorly infectious in caterpillars when ingested, but quite virulent when injected. OBs can be induced to form in the absence of virions by the expression of spheroidin protein in cell culture. An alkaline protease is associated with some preparations of EV OBs, and may be useful for dissolution of the OB in the insect gut. AMEV OBs are associated with an alkaline protease when isolated from insects, but not when the virus is grown in culture.

Pathology

EVs infect primarily the larvae of insects rather than the adult insects. There are reports of infections of adult insects within laboratory settings, however. Most EVs have a restricted host range, although MSEV does infect several grasshopper species. Infection occurs primarily through an oral route with the ingestion of OBs. Once in the gut, the OBs are dissolved by the alkaline environment. The released virions attach to the midgut epithelium and then appear to fuse with the cell membrane. The virus then infects the fat body of the insect and this becomes the primary site of replication for the virus.

Table 1 Properties of EV virions

	Alphaentomopoxvirus	*Betaentomopoxvirus*	*Gammaentomopoxvirus*
Size	450 × 250 nm	350 × 250 nm	320 × 230 × 110 nm
Core	Unilateral concave core	Cylindrical	Biconcave
Lateral body	Single	Sleeve shaped	Two
Virion shape	Ovoid	Ovoid	Brick

EVs are reported to infect the hemocytes and AMEV does infect primary hemocytes in culture. Some researchers argue that the presence of virus in the hemocytes *in vivo* represents phagocytosis rather than infection. As the disease progresses within the insect, the hemolymph frequently turns white with OBs, and the larva itself can take on a whitish or white spotted appearance. The fat body disintegrates and the virus is disseminated throughout the body.

Infected larvae are lethargic and uncoordinated and exhibit a decrease in feeding. The duration of the instar phase is increased before progression to the next instar and few infected larvae continue through pupation. In some EV infections, this is thought to be due to the increased levels of juvenile hormone. There are reports of regurgitation and defecation of AMEV particles by infected *Estigmene acrea* larvae. The time to death of an infected insect is dependent both on the dose of virus received and the larval instar stage. Younger larvae appear to be more susceptible to lower doses of virus and die more quickly than older larvae. The lowest reported LD_{50} is 2.4 spheroids. *Melanoplus sanguinipes* larvae succumbing to an MSEV infection within less than 12 days are reported to lack OBs, although infections of greater than 14 days do possess OBs. The shorter time to death is the result of larger doses of virus.

Fusolin/Spindles

In addition to spheroidin, some, but not all, members of the alphaentomopoxviruses and many of the lepidopteran members of the betaentomopoxviruses produce another protein termed fusolin that forms crystalline spindles. Among the viruses known to produce spindles are *Melolontha melolontha* EV, *Heliothis armigera* EV, *Choristoneura fumiferana* EV, *Choristoneura biennis* EV, *Pseudaletia separata* EV, and *Anomala cuprea* EV (ACEV). Spindles do not contain virions, though spindles are occasionally found within the OBs of these viruses. Similar to spheroidin, fusolin is highly expressed at late times during infection and is a smaller protein of 38 kDa.

Spindles purified from EV infections enhance the infectivity of a number of baculoviruses. One proposed mechanism of this enhancement is through dissolution of the noncellular peritrophic membrane found within the insect gut. An alternate hypothesis is that the spindles increase the amount of baculovirus-to-cell fusion. Spindles from ACEV have also been demonstrated to enhance the infectivity of ACEV. Clearly, spindles are not required for pathogenicity as evidenced by their absence from most members of the gammaentomopoxviruses and the orthopteran betaentomopoxviruses. It should be noted that although AMEV is a lepidopteran virus it does not produce spindles or encode a gene for fusolin.

Genome Organization

Genome sizes of the EVs are larger than that of the best-studied CV, VV. The estimated sizes for EV genomes range from 200 to 390 kbp. Two EV genomes have been sequenced, those of AMEV and MSEV. The genome size of AMEV is 232 kbp and of MSEV is 236 kbp. Both genomes are highly A + T-rich, with an A + T content for each genome of approximately 82%, which is in line with DNA melting experiments for EV genomes. In comparison, VV has a genome that is 67% A + T. Both the MSEV and AMEV genomes contain inverted terminal repeats at the ends of the genome.

The organization of the AMEV and MSEV genomes is surprisingly unlike that of the orthopoxviruses. Within the orthopoxviruses there is a core of conserved genes flanked by variable genes involved in pathogenesis and host range. Although many of the same genes are present in EVs, the collinear core is not conserved in either MSEV or AMEV. In fact, it is the lack of conservation of genome organization between AMEV and MSEV, both originally classified as betaentomopoxviruses, that resulted in MSEV being removed from this genus and listed as an unclassified virus.

The available sequence data from the EVs has been included in recent bioinformatics analysis of all available poxvirus sequences. From this analysis, 49 gene families were identified that are conserved across all species of poxviruses. Homologs of the VV G3L gene have recently been identified in AMEV and MSEV, bringing the total of conserved gene families to 50. These 50 genes presumably comprise the minimal complement of genes required for poxvirus function. These conserved gene families include proteins necessary for DNA replication and transcription, polyadenylation of mRNA, and the major structural proteins found within the virion core. An analysis of CVs alone increases the number of conserved genes from 50 to 90. Clearly, the inclusion of the EVs in this type of analysis refines the minimal core of proteins required by a poxvirus.

Replication

Like other poxviruses, EVs complete their life cycle entirely within the cytoplasm of the cell. A number of enzymes required for DNA synthesis and for temporal regulation of RNA expression are encoded by EVs. These include DNA polymerase, RNA polymerase, poly(A) polymerase, topoisomerase, and a number of transcription factors. Replication within the insect host occurs primarily within the fat body. Many EVs then proceed to infect the hemocytes and other tissues. In cell culture, DNA replication occurs between 6 and 12 h post infection (hpi). At the optimum temperature of 27 °C, the life

cycle takes approximately 18–24 h. By 9 hpi, host-cell protein synthesis is shut off. The sites of DNA replication and virion assembly are known as viroplasms or viral factories. Although many virions are packaged into OBs, some are not. Those virions that are not occluded proceed to bud through the cell membrane into the hemocoel of the insect or the cell culture medium *in vitro*. These nonoccluded virus particles are infectious and are thought to be responsible for virus spread with the organism.

RNA production occurs in a temporal fashion with early gene expression occurring from 0 to 6 hpi. Included in the early gene products are transcription factors required for intermediate and late gene production. No intermediate genes have been studied in the EVs, but homologs of several VV intermediate genes are present within both the AMEV and MSEV genomes. Late gene expression appears to follow DNA replication and begins at 9 hpi. The DNA regulatory elements that are present in VV to control this temporal gene expression are present in both AMEV and MSEV, although the functionality of these elements during an EV infection has not been explored. Among the conserved regulatory elements are the TTTTTNT termination sequence at the end of early genes, and the consensus late promoter TAAATG. Similar to the CVs, there is no RNA splicing of EV transcripts.

A unique discovery within the late genes of AMEV is the presence of discrete late transcripts. The late transcripts of VV lack specific 3′ termini, resulting in polydisperse 3′ ends. The rare exceptions to this are several genes that have been identified as having discrete 3′ RNA termini that are formed via cleavage of polydisperse transcripts. A number of major late transcripts of AMEV are discrete in length, unlike what is observed in VV. These include the transcripts for the structural proteins p4a and p4b, the superoxide dismutase, and RAP94, a component of the early RNA polymerase. The spheroidin transcript is also discrete.

Molecular Biology

Since the sequencing of two EV genomes, a number of interesting features of the EVs have come to light. One of these is the apparent absence of some members of the RNA polymerase complex. There are eight components of the RNA polymerase complex in VV and these are conserved among all sequenced CVs. Three of these proteins have no obvious homologs in AMEV and MSEV. Whether these subunits are not required in an insect cell environment or whether their roles are fulfilled by other nonhomologous proteins is not yet clear. Several transcription factor homologs are also missing from the EVs. Together, this might indicate that the EVs regulate transcription in a different fashion from their orthopoxvirus relatives.

In vitro transcription catalyzed by permeabilized AMEV virions requires different conditions from those needed for transcription from VV virions. Unlike VV reactions, which use low levels of detergent and reducing agent, AMEV transcription requires higher levels of reducing agent and the requirement for detergent is less stringent, as transcription can occur in the absence of detergent but not in the absence of reducing agent. AMEV reactions are optimal when an ATP-generating system is present. Such a system is not required for VV virion-mediated transcription. This indicates that the structure of the AMEV virion is different in its response to both reducing agents and detergents. Our attempts to isolate AMEV cores with methodology optimized for the preparation of VV cores has also indicated that key AMEV core proteins partition differently from their VV counterparts.

The transcripts of AMEV are polyadenylated at the 3′ termini, but the composition of the poly(A) polymerase complex appears to be different from that of VV. In VV, poly(A) polymerase is a heterodimer. AMEV has two homologs of the small VV subunit in addition to one homolog of the large subunit. This raises the question of whether both small subunits are involved in polyadenylation or whether there might be tissue or temporal specificity conferred on the complex by the different subunits. It is not clear how common two small poly(A) polymerase subunits are among EVs, since the only other fully sequenced EV (MSEV) only has one small subunit. Spheroidin and other transcripts from EVs have been shown to contain a nonencoded 5′ poly(A) head similar to that reported for a number of VV transcripts.

Protein gel analysis of ^{35}S-labeled virions indicates that AMEV contains 36–37 structural proteins and MSEV contains 39–45. The proteolytic processing of the large structural proteins that is detected in VV infections has not been found in EV infections. The enzymes thought to be responsible for this proteolytic cleavage in VV are the products of the I7L and G1L genes. These genes are conserved in AMEV and MSEV, indicating that protein processing may occur even though it is as yet undetected.

Both AMEV and MSEV encode an NAD^+-dependent DNA ligase. In contrast, VV encodes an ATP-dependent DNA ligase. The AMEV DNA ligase is capable of joining singly nicked DNA fragments. These are the first examples of an NAD^+-dependent DNA ligase outside of the eubacteria. The recently sequenced crocodile poxvirus has also been reported to encode an NAD^+-dependent DNA ligase.

Poxviruses are known for the wide variety of proteins that they encode to evade the host immunomodulatory response. VV and other orthopoxviruses encode serine protease inhibitors as well as chemokine binding molecules and decoy receptor molecules. Due to the host range of the

EVs, they encode other types of defense molecules. Among these are the *inhibitor of apoptosis* (*iap*) genes. The *iap* of AMEV has been well characterized and functionally inhibits apoptosis. A related AMEV gene that functions to inhibit apoptosis is a homolog of the baculovirus pan-caspase inhibitor, p35.

Another novel protein expressed by AMEV is a Cu–Zn superoxide dismutase (SOD). Although a number of the orthopoxviruses encode genes with homology to this class of SODs, neither the VV or myxoma virus proteins are functional in that capacity, although they are present within the virion. The SOD expressed by AMEV is functional as an SOD but is not essential for virus growth in culture. The deletion of the *sod* gene from AMEV appears to have no effect on the growth of the virus in gypsy moth larvae.

Summary

It is clear that this large subfamily of the family *Poxviridae* provides a wealth of possible information about the basic mechanisms of the poxvirus lifecycle. There appear to be a number of interesting variations on the molecular details which define this overall family of viruses. There are clear similarities to the vertebrate poxviruses in virion morphology, double-stranded DNA genome, cytoplasmic life cycle, and RNA expression. Yet the differences between the CVs and EVs are significant and represent an area of research that has not been fully explored. The data that have been obtained from genomic sequencing has been essential to identifying some of the different proteins that are present in the EVs, as well as identifying potentially missing homologs of VV proteins. It is important to note that there are large differences at the DNA level between the two sequenced EVs, indicating that there is probably a wide variety of unique features within the EVs as a group. As more sequence information becomes available, the diversity of this family of viruses may become more evident.

See also: Apoptosis and Virus Infection; Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Poxviruses; Vaccinia Virus.

Further Reading

Afonso CL, Tulman ER, Lu Z, *et al.* (1999) The genome of *Melanoplus sanguinipes* entomopoxvirus. *Journal of Virology* 73: 533–552.

Arif BM and Kurstak E (1991) The entomopoxviruses. In: Kurstak E (ed.) *Viruses of Invertebrates* pp. 179–195. New York: Marcel Dekker.

Bawden AL, Glassberg KJ, Diggans J, *et al.* (2000) Complete genomic sequence of the *Amsacta moorei* entomopoxvirus: Analysis and comparison with other poxviruses. *Virology* 274: 120–139.

Becker MN, Greenleaf WB, Ostrov DA, and Moyer RW (2004) *Amsacta moorei* entomopoxvirus expresses an active superoxide dismutase. *Journal of Virology* 78: 10265–10275.

Becker MN and Moyer RW (2007) Subfamily *Entomopoxvirinae*. In: Mercer A, Schmidt A, and Weber O (eds.) *Poxviruses*, pp. 251–269. Basel: Birkhäuser.

Gubser C, Hue S, Kellam P, and Smith GL (2004) Poxvirus genomes: A phylogenetic analysis. *Journal of General Virology* 85: 105–117.

Li QJ, Liston P, and Moyer RW (2005) Functional analysis of the *inhibitor of apoptosis* (*iap*) gene carried by the entomopoxvirus of *Amsacta moorei*. *Journal of Virology* 79: 2335–2345.

Li QJ, Liston P, Schokman N, Ho JM, and Moyer RW (2005) *Amsacta moorei* entomopoxvirus inhibitor of apoptosis suppresses cell death by binding grim and hid. *Journal of Virology* 79: 3684–3691.

Miller LK and Ball LA (1998) *The Insect Viruses.* New York: Plenum.

Moss B (2001) *Poxviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology*, 4th edn., pp. 2849–2883. Philadelphia: Lippincott Williams & Wilkins.

Upton C, Slack S, Hunter AL, Ehlers A, and Roper RL (2003) Poxvirus orthologous clusters: Toward defining the minimum essential poxvirus genome. *Journal of Virology* 77: 7590–7600.

Winter J, Hall RL, and Moyer RW (1995) The effect of inhibitors on the growth of the entomopoxvirus from *Amsacta moorei* in *Lymantria dispar* (gypsy moth) cells. *Virology* 211: 462–473.

Epidemiology of Human and Animal Viral Diseases

F A Murphy, University of Texas Medical Branch, Galveston, TX, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Airborne transmission Transmission via large droplets and via very small droplet nuclei (aerosols) emitted from infected persons during coughing or sneezing or from environmental sources.

Case-control study An epidemiological study in which the risk factors of humans or animals with a disease are compared with those without the disease.

Cohort study Attempt to identify the cause of a disease by comparing exposed and nonexposed (control) populations in a prospective epidemiological study.

Common vehicle transmission Pertains to fecal contamination of food and water supplies.

Direct contact transmission Involves actual physical contact between an infected subject and a susceptible subject (e.g., kissing, biting, coitus).
Epidemic Major increase in disease incidence affecting either a large number of humans or animals or spreading over a large area.
Epidemiology The study of the determinants, dynamics, and distribution of diseases in populations.
Fomite An inanimate object that may be contaminated with virus and become the vehicle for transmission.
Herd immunity The immune status of a population that affects viral transmission rates. Often used in describing the elimination of a virus from a population when there are too few susceptible hosts remaining to sustain a transmission chain.
Horizontal transmission The transfer of infectious virus from one human or animal to another by any means other than vertical transmission.
Iatrogenic transmission Transmission via health care procedures, materials, and workers (e.g., physicians, nurses, dentists, veterinarians).
Incidence rate (or attack rate) A measure of the occurrence of infection or disease in a population over time – it refers to the proportion of a population contracting a particular disease during a specified period.
Mathematical model (epidemiological) A means to convey quantitative information about a host-virus interaction, such as an epidemic or an emerging disease episode, by the construction of a set of predictive mathematical algorithms.
Nosocomial transmission Pertains to infections acquired while a patient, human or animal, is in hospital.
Prevalence rate The ratio, at a particular point in time, of the number of cases currently present in the population divided by the number of subjects in the population at risk; it is a snapshot of the occurrence of infection or disease at a given time.
Species jumping (or host range extension) Referring to a virus that derives from an ancient reservoir life cycle in animals, but has subsequently established a new life cycle in humans or a different animal species and no longer uses, the original animal reservoir.
Transmission The process by which a pathogen is shed from one host and infects the next.
Vector-borne transmission Involves the bites of arthropod vectors (e.g., mosquitoes, ticks, sandflies).
Vertical or transplacental transmission Occurs from mother to fetus prior to or during parturition, either across the placenta, when the fetus passes through the birth canal, or via colostrum and milk.
Vertical transmission Transmission of virus from parent to progeny through the genome, sperm, or ovum or extracellularly (e.g., through colostrum or across the placenta).
Zoonosis Disease which is naturally transmitted to humans from an ongoing reservoir life cycle in animals or arthropods, without the permanent establishment of a new life cycle in humans.

Introduction

Viral disease epidemiology is the study of the determinants, dynamics, and distribution of viral diseases in populations. The risk of infection or disease in a population is determined by characteristics of the virus, the host, and the host population, as well as behavioral, environmental, and ecological factors that affect virus transmission from one host to another. Epidemiology attempts to meld these factors into a unified whole. The depiction of the interaction of factors favoring the emergence of a viral disease (**Figure 1**), called 'the convergence model', is taken from the US Institute of Medicine study, Microbial Threats to Health, Emergence, Detection and Response (National Academy Press, 2003). At the center is a box representing the convergence of factors leading to 'the black box', reflecting the reality that many unknown interactions are important virologically and epidemiologically.

The foundations of epidemiology predate the microbiological and virological sciences, starting with Hippocrates, the Greek physician and father of medicine, who in the fourth century BC made important epidemiologic observations on infectious diseases. John Snow is called the father of modern epidemiology because he developed excellent quantitative methods while studying the source of a cholera outbreak at the Broad Street pump in London in 1849. Snow was followed by William Farr, who in the 1870s advanced the use of vital statistics and clarified many of the principles of risk assessment and retrospective and prospective studies. Their vision is reflected in the fast-changing science of epidemiology which is now supported by advanced computer technology, sophisticated statistical methods, and very sensitive and specific diagnostic systems.

Assessment of Disease Occurrence and Outcome

By introducing quantitative measurements of disease trends, epidemiology has come to have a major role in improving our understanding of the overall nature of

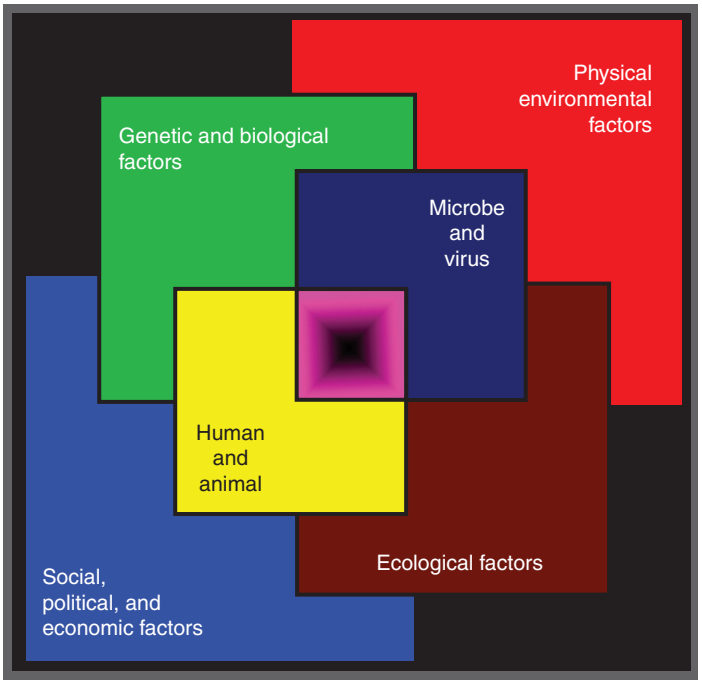


Figure 1 The Convergence Model. Factors contributing to the emergence of viral diseases. At the center is a box representing the convergence of factors leading to the emergence of a viral disease. The black center represents unknown interactions, 'the black box'. Reproduced from: Smolinski MS, Hamburgh MA, and Lederburg J (eds.) (2003) *Microbial Threats to Health, Emergence, Detection and Response*, **Figure 1** p. 3. Washington, DC: National Academies Press. Reprinted with permission from the National Academy Press, copyright (2003), National Academy of Sciences.

disease and in alerting and directing disease control activities. Epidemiology is also effective in (1) clarifying the role of particular viruses and viral variants as the cause of disease, (2) clarifying the interaction of viruses with environmental determinants of disease, (3) determining factors affecting host susceptibility, (4) unraveling modes of transmission, and (5) field testing of vaccines and antiviral drugs.

The comparison of disease experience between populations is expressed in the form of 'rates'. The terms 'incidence rate' and 'prevalence rate' are used to describe quantitatively the frequency of occurrence of infection or disease in populations. 'Incidence rate' (also called attack rate) is defined as the ratio of new cases occurring in a population to the size of the population during a specified period of time. Prevalence rate is the ratio of the total number of cases occurring in a population to the size of the population during a specified period of time. 'Seroprevalence rate' relates to the occurrence of antibody to a particular virus in a population. Because viral antibodies, especially neutralizing antibodies, often last a lifetime, seroprevalence rates usually represent cumulative experience with the virus. The term 'case–fatality rate' is used to indicate the percentage of subjects with a particular disease that die from the disease. All these rates may be affected by various attributes that distinguish one individual from another: age, sex, genetic constitution, immune status, pregnancy, nutritional status, and various behavioral and medical care and patient management parameters. The most widely applicable attribute is age, which may encompass immune status as well as various physiological variables.

A viral disease is characterized as 'endemic' when there are multiple or continuous chains of transmission resulting in continuous occurrence of disease in a population over a period of time. 'Epidemics' are peaks in disease incidence that exceed the endemic baseline or expected rate of disease. The size of the peak required to constitute an epidemic is arbitrary and is related to the background endemic rate and the anxiety that the disease arouses (e.g., a few cases of rabies is regarded as an epidemic, whereas a few cases of influenza is not). A 'pandemic' is a worldwide epidemic.

Epidemiologic Studies

A proper description of an outbreak of disease or an epidemic must include the parameters of 'person (or subjects in the case of animals), place, and time'. Such descriptive information is a necessary first step in describing the occurrence, distribution, course, threat, and anticipated action response to the initial recognition of a cluster of cases of disease. Much of the initial

investigation called for rests in common sense, observational acuity, and an insightful 'index of suspicion'. Much of the initial investigation has been termed 'shoe-leather epidemiology'. The trigger for such initial investigation is most often an astute clinician (physician or veterinarian) or an astute pathologist.

Case-Control Studies and Cohort Studies

There are two basic analytic techniques used to investigate relationships between cause and effect and to evaluate risk factors of disease. These are the 'case-control study' and the 'cohort study'. In the case-control study, investigation starts after the disease has occurred – it is a retrospective study, going back in time to determine causative events. Although this kind of study does not require the creation of new data or records, it does require careful selection of the control group, matched to the test group so as to avoid bias. The retrospective case-control study lends itself to quick analysis and is relatively inexpensive to carry out. In the cohort study, the prospective study, investigation entails the gathering of new data to identify cause–effect relationships. This kind of study is expensive and does not lend itself to quick analysis as groups must be followed until disease is observed. However, when cohort studies are successful, proof of cause–effect relationship is often incontrovertible.

Molecular Epidemiologic Studies

The term 'molecular epidemiology' is used to denote the use of any of a large number of molecular biological methods in support of epidemiologic investigations. For example, with herpesviruses, restriction endonuclease mapping has provided a means of identification of unique viral genotypes – in an epidemiologic study recognized as the first based upon viral molecular characterization, the source of herpes simplex virus 1 causing disease in a hospital newborn nursery was traced to one persistently infected nurse rather than any of several other possible shedders. With rotaviruses and bluetongue viruses, polyacrylamide gel electrophoresis of the segmented viral RNA has been used epidemiologically, for example, to unravel outbreaks involving multiple viral variants. Panels of monoclonal antibodies have been used to distinguish virus variants for epidemiologic purposes; they have been particularly useful in elucidating host-range and geographic variants of rabies virus. Today, partial sequencing has become the most commonly used molecular epidemiologic methodology; partial sequencing of poliovirus isolates recovered from patients indicates whether they are wild type (even local or introduced wild type), attenuated vaccine type, or a vaccine type that has reacquired neurovirulence during human passage. Partial sequencing of foot-and-mouth disease viruses can offer the same kind of geographic information of virus movement as has proved so useful in polio control and eradication programs, but because of political sensitivities in some countries a robust international reference laboratory system has not been established that could provide the same kind of practical disease control information as has been the case with polio. Thus, with many human and animal viruses molecular epidemiologic studies are flourishing, but more such studies should lead to international reference laboratory systems to guide prevention and control actions. Such studies are developing rapidly today to deal with the threat of a human pandemic of avian influenza, but there are many more viral diseases, especially animal diseases, in need of this kind of development.

Etiologic Studies and the Proof of Causation

One of the landmarks in the history of infectious diseases was the development of the Henle-Koch postulates which established the evidence required to prove a causal relationship between a particular infectious agent and a particular disease. These simple postulates were originally drawn up for bacteria, but were revised in 1937 by Rivers and again in 1982 by Evans in attempts to accommodate the special problem of proving disease causation by viruses (**Table 1**). In many cases, virologists have had to rely on indirect evidence, 'guilt by association', with associations based on epidemiologic data and patterns of serologic positivity in populations. Today, many aspects of epidemiologic investigation play roles, especially in trying to distinguish an etiological, rather than coincidental or opportunistic relationship between a virus and a given disease.

For example, early in the investigation of human acquired immunodeficiency syndrome (AIDS), before its etiology was established, many kinds of viruses were being isolated from patients and many candidate etiologic agents were being advanced. Prediction that the etiologic agent would turn out to be a member of the family *Retroviridae* was based upon years of veterinary research on animal retroviruses and animal retroviral diseases. This prediction was based upon recognition of common biologic and pathogenetic characteristics of AIDS and animal retroviral diseases. This prediction guided many of the early experiments to find the etiologic agent of AIDS; later, after human immunodeficiency virus (HIV1) was discovered, its morphological similarity to equine infectious anemia virus, a prototypic member of the genus *Lentivirus*, family *Retroviridae*, was the key to unraveling confusion over the fact that the human virus killed host lymphocytes rather than transforming them as typical oncogenic retroviruses would do. Ever since, this essence of comparative medicine has been guiding HIV/AIDS research in many areas, including drug design,

Table 1 Criteria for disease causation: a unified concept appropriate for viruses as causative agents of disease, based on the Henle–Koch postulates, and modified by A. S. Evans

1. Prevalence of the disease is significantly higher in subjects exposed to the putative virus than in those not so exposed.
2. Incidence of the disease is significantly higher in subjects exposed to the putative virus than in those not so exposed (prospective studies).
3. Evidence of exposure to the putative virus is present more commonly in subjects with the disease than in those without the disease.
4. Temporally, the onset of disease follows exposure to the putative virus, always following an incubation period.
5. A regular pattern of clinical signs follows exposure to the putative virus, presenting a graded response, often from mild to severe.
6. A measurable host-immune response, such as an antibody response and/or a cell-mediated response, follows exposure to the putative virus. In those individuals lacking prior experience, the response appears regularly, and in those individuals with prior experience, the response is anamnestic.
7. Experimental reproduction of the disease follows deliberate exposure of animals to the putative virus, but nonexposed control animals remain disease free. Deliberate exposure may be in the laboratory or in the field, as with sentinel animals.
8. Elimination of the putative virus and/or its vector decreases the incidence of the disease.
9. Prevention or modification of infection, via immunization or drugs, decreases the incidence of the disease.
10. ''The whole thing should make biologic and epidemiologic-sense.''

diagnostics, and vaccine development. HIV/AIDS epidemiologic research has often been intertwined with research on the several simian immunodeficiency viruses (SIVs).

Seroepidemiologic Studies

Seroepidemiology is useful in public health and animal health investigations and in research to determine the prevalence or incidence of particular infections, to evaluate control and immunization programs, and to assess past history when a 'new' virus is discovered. When paired serum specimens are obtained from individuals several weeks apart, the initial appearance of antibody in the second specimen or a rise in antibody titer indicates recent infection. Similarly, the presence of specific immunoglobulin M (IgM) antibody in single serum samples, indicating recent infection, may be used in seroepidemiologic studies. Correlation of serologic tests with clinical observations makes it possible to determine the ratio of clinical to subclinical infections.

Sentinel Studies

Because of advanced diagnostic/serologic methods, sentinel studies can yield many valuable data in timely fashion about impending disease risks. For example, sentinel chicken flocks are set out for the early detection of the presence of arboviruses such as West Nile virus in the United States. These flocks are bled and tested weekly for the presence of virus or antiviral antibody; they provide an early warning of the levels of virus amplification that occur before epidemics.

Vaccine Trials

The immunogenicity, potency, safety, and efficacy of vaccines are first studied in laboratory animals, followed by small-scale closed trials, and finally in large-scale open trials. Such studies employ epidemiologic methods, rather like those of the cohort (prospective) study. In most cases, there is no alternative way to evaluate new vaccines, and the design of trials has now been developed so that they yield maximum information with minimum risk and acceptable cost.

Virus Transmission among Individuals

Viruses survive in nature only if they are able to be transmitted from one host to another, whether of the same or another species. Transmission cycles require virus entry into the body, replication, and shedding with subsequent spread to another host.

Virus Entry

Portals of virus entry into the body include the skin, respiratory tract, intestinal tract, oropharynx, urogenital tract, and conjunctiva. In some cases, viruses use a particular portal of entry because of particular environmental or host-behavior factors and in other cases because of specific viral ligands and host-cell receptors. In many cases, disruption of normal host-defense mechanisms leads to entry that might otherwise be thwarted; for example, papillomaviruses may enter the deep layers of the skin via abrasions, acid-labile coronaviruses may enter the intestine protected by the buffering capacity of milk, and influenza viruses may enter the lower respiratory tract because a drug has dampened cilial action of the respiratory epithelium.

Virus Shedding

The exit of virus from an infected host is just as important as entry in maintaining its transmission cycle. All portals used by viruses to gain entry are used for exit. The

important elements in virus shedding are virus yield (from the standpoint of the virus, the more shedding the better) and timeliness of yield (again, the earlier the shedding the better). Viruses that cause persistent infections often employ remarkable means to avoid host inflammatory and immune responses so as to continue shedding. For example, the epidemiologically important shedding of herpes simplex viruses 1 and 2 that perpetuates the viruses in populations requires recrudescence of persistent ganglionic infection, centrifugal viral genomic transit to peripheral nerve endings, and productive infection of mucosal epithelium, all in the face of established host immunity.

Modes of Virus Transmission

Virus transmission may be 'horizontal' or 'vertical'. The vast majority of transmission is horizontal, that is, between individuals within the population at risk. Modes of horizontal transmission of viruses can be characterized as direct contact, indirect contact, common vehicle, airborne, vector-borne, iatrogenic, and nosocomial. Vertical or transplacental transmission occurs between the mother and her fetus or newborn. Some viruses are transmitted in nature via several modes, others exclusively via one mode (see **Table 2**).

'Direct contact transmission' involves actual physical contact between an infected subject and a susceptible subject (e.g., kissing, Epstein–Barr virus, the cause of mononucleosis, biting (e.g., rabies); coitus (sexually transmitted viral diseases)). Indirect contact transmission occurs via 'fomites', such as shared eating utensils, improperly sterilized surgical equipment, or improperly sterilized non-disposable syringes and needles.

'Common vehicle transmission' pertains to fecal contamination of food and water supplies (e.g., norovirus diarrhea). Common vehicle transmission commonly results in epidemic disease.

'Airborne transmission' typically results in respiratory infections (and less typically in intestinal infections), but these infections may also be transmitted by direct and

Table 2 Examples of human and animal virus transmission patterns

Infectious agent/disease	*Mode of transmission*	*Portal of entry*
Influenza virus/influenza	Contact/direct/indirect via droplets and droplet nuclei	Respiratory tract
Rhinoviruses/common cold	Contact/direct/indirect via droplets and droplet nuclei and fomites	Respiratory tract
Rubella virus/congenital rubella	Contact/direct/indirect via droplets and droplet nuclei	Respiratory tract
	Vertical/congenital	Transplacental
Rotaviruses/diarrhea	Contact/direct/indirect via fomites	Intestinal tract (oral)
Poliovirus/poliomyelitis	Contact/direct/indirect via fomites	Intestinal tract (oral)
Norovirus/diarrhea	Common vehicle/fecal contamination of water	Intestinal tract (oral)
Hepatitis A virus/hepatitis	Common vehicle/fecal contamination of food	Intestinal tract (oral)
Variant Creutzfeldt–Jakob disease prion/prion disease (spongiform encephalopathy)	Common vehicle/bovine spongiform encephalopathy prion contamination of beef or beef products	Intestinal tract (oral)
Herpes simplex virus/genital herpes	Contact/direct (sexual)	Genital tract
Human immunodeficiency virus 1/acquired immunodeficiency syndrome (AIDS)	Contact/direct (sexual), contact/direct (blood) Vertical/congenital	Genital tract, bloodstream, transplacental, at birth and via breast feeding
Rabies virus/rabies	Zoonotic/contact/direct (saliva)	Skin (bite wound)
Russian spring summer encephalitis virus/encephalitis	Zoonotic/arthropod-borne	Skin (tick bite)
Dengue viruses/dengue	Zoonotic/arthropod-borne	Skin (mosquito bite)
Sin Nombre and related viruses	Zoonotic/contact/direct (rodent urine, saliva and feces)	Respiratory tract
Lassa virus	Zoonotic/contact/direct (rodent urine, saliva and feces)	Respiratory tract and intestinal tract (oral)
Ebola and Marburg viruses	Zoonotic/reservoir host unknown; secondary cases contact/direct/nosocomial and iatrogenic	Index cases unknown, likely respiratory tract or skin and mucous membranes; secondary cases, contact and iatrogenic (injection)
Leukemia viruses/leukemias (proven only in animals)	Vertical/germ-line	Transmitted as genetic trait

indirect contact. Airborne transmission occurs via large droplets and via very small droplet nuclei (aerosols) emitted from infected persons during coughing or sneezing (e.g., influenza) or from environmental sources. Large droplets (>10 μm in diameter) settle quickly, but droplet nuclei evaporate forming dry particles (<5 μm in diameter) which remain suspended in the air for extended periods. Droplets may travel only a meter or so while droplet nuclei may travel over much longer distances.

'Vector-borne transmission' involves the bites of arthropod vectors (e.g., mosquitoes, ticks, and sandflies).

'Iatrogenic transmission' involves health care procedures, materials, and workers (e.g., physicians, nurses, dentists, and veterinarians).

'Nosocomial transmission' pertains to infections acquired while a patient, human or animal, is in hospital.

'Vertical or transplacental transmission' occurs from mother to fetus prior to or during parturition. Certain retroviruses are vertically transmitted in animals via the integration of viral DNA directly into the DNA of the germline of the fertilized egg. Other viruses are transmitted to the fetus across the placenta; yet others are transmitted when the fetus passes through the birth canal. Another vertical transmission route is via colostrum and milk. Vertical transmission of a virus may or may not be associated with 'congenital disease' (i.e., disease that is present at birth) which may be lethal (and the cause of abortion or stillbirth) or the cause of congenital abnormalities. The herpesviruses, especially cytomegaloviruses, and rubella virus cause important congenital diseases in humans, and pestiviruses, such as bovine viral diarrhea virus, in animals.

Common Patterns of Virus Transmission

Enteric infections are most often transmitted by direct contact and by fomites in a 'fecal–oral cycle' that may include fecal contamination of food and water supplies; diarrheic feces may also splash to give rise to aerosols (droplets and droplet nuclei). Respiratory infections are most often transmitted by the airborne route or by indirect contact via fomites in a 'respiratory cycle', that is, virus is shed in respiratory secretions and enters its next host through the nares during inhalation. The respiratory cycle is responsible for the most explosive patterns of epidemic disease in humans and all domestic animal species.

Perpetuation of Viruses in Nature

Perpetuation of a virus in nature depends upon the maintenance of serial infections, that is, a chain of transmission; the occurrence of disease is neither required nor necessarily advantageous.

Influence of the Clinical Status of the Host

Infection without recognizable disease is called 'subclinical' or 'clinically inapparent'. Overall, subclinical infections are much more common than those that result in disease. Their relative frequency accounts for the difficulty of tracing chains of transmission, even with the help of laboratory diagnostics. Although clinical cases may be somewhat more productive sources of virus than subclinical infections, because the latter do not restrict the movement of the infected host, they can be most important as sources of viral dissemination. In most acute infections, whether clinically apparent or not, virus is shed in highest titers during the late stages of the incubation period, before the influence of the host-immune response takes effect. Persistent infections, whether or not they are associated with episodes of clinical disease, also play an important role in the perpetuation of many viruses in nature. For example, prolonged virus shedding can reintroduce virus into a population of susceptibles all of which have been born since the last clinically apparent episode of infection. This is important in the survival of rubella virus in some isolated populations. Sometimes the persistence of infection, the production of disease, and the transmission of virus are dissociated; for example, togavirus and arenavirus infections may have little adverse effect on their reservoir hosts (arthropods, birds, and rodents), but transmission may be very efficient. On the other hand, the persistence of infection in the central nervous system, as with measles virus in subacute sclerosing panencephalitis (SSPE), is of no epidemiological significance, since no infectious virus is shed from this site.

Influence of Virulence of the Virus

The virulence of the infecting virus may directly affect the probability of its transmission. The classic example of this is rabbit myxomatosis. In Australia, mosquito-borne transmission of myxoma virus was found to be most effective when infected rabbits maintained highly infectious skin lesions for several days before death. Highly virulent strains of the virus were found to kill rabbits so quickly that transmission did not occur, and naturally attenuated strains were found to produce minimal lesions that healed quickly and did not permit transmission. Virus strains at either extreme of this virulence spectrum were found not to survive in nature, but virus strains of intermediate virulence have circulated for many years.

Influence of Host Population Immunity

With most viruses, endemic or epidemic transmission leads to a level of immunity in the host population that affects or even interrupts further transmission. The 'herd immunity' effect is countered in some cases by viral

antigenic variation. For example, influenza viruses undergo genetic variations ('shift' and 'drift') such that persons immune to previously circulating virus strains are susceptible to new strains. Assessing these genetic changes is the main objective of laboratory-based surveillance programs, which in turn are the basis for decisions on the formulation of each year's influenza vaccine.

Influence of Population Size

It is self-evident that the long-term survival of a virus requires that it be continuously transmitted from one host to another. In general, for rapidly and efficiently transmitted viruses such as many respiratory viruses, local survival of the virus requires that the susceptible host population be very large. A virus may disappear from a population because it exhausts its potential supply of susceptible hosts as they acquire immunity to reinfection with the same virus. Depending on duration of immunity and the pattern of virus shedding, the 'critical population size' varies considerably with different viruses and with different host species. The most precise data on the importance of population size in acute nonpersistent infections come from studies of measles. Persistence of measles virus in a population depends upon a continuous supply of susceptible children. Analyses of the incidence of measles in large cities and in island communities have shown that a population of about half a million persons is needed to ensure a large enough annual input of new susceptible hosts, by birth or immigration, to maintain measles virus in the population. Because infection depends on respiratory transmission, the duration of epidemics of measles is correlated inversely with population density. If a population is dispersed over a large area, the rate of spread is reduced and the epidemic may last longer, so that the number of susceptible persons needed to maintain transmission chains is reduced. On the other hand, in such a situation a break in the transmission chain is much more likely. When a large proportion of the population is initially susceptible, the intensity of the epidemic builds up very quickly and attack rates are almost 100% ('virgin-soil epidemic'). On the other hand, when measles vaccination programs are implemented properly the virus disappears completely from the regional population.

Influence of Zoonotic Transmission Cycles

Because most viruses are host-restricted, most viral infections are maintained in nature within populations of the same or related species. However, there are a number of viruses that may have multiple hosts and spread naturally between several different species of vertebrate host, for example, rabies and eastern equine encephalitis viruses. The term 'zoonosis' is used to describe multiple-host infections that are transmissible from animals to man. The zoonoses, whether involving domestic or wild animals or arthropods, usually represent important problems only under conditions where humans are engaged in activities involving close contact with animals or exposure to arthropods.

Influence of Arthropod Transmission Cycles

Many viral zoonoses are caused by arboviruses. Arboviruses have two classes of hosts, vertebrate and invertebrate. Over 500 arboviruses are known, of which about 100 cause disease in humans and 40 in domestic animals; some of these are transmitted by ticks, some by mosquitoes, and yet others by phlebotomine flies (sandflies) or *Culicoides* spp. (midges). Arthropod transmission may be 'mechanical', where the arthropod acts as a 'flying pin', or more commonly, 'biological', involving replication of the virus in the arthropod vector. The arthropod vector acquires virus by feeding on the blood of a viremic person or animal. Replication of the ingested virus, initially in the arthropod's gut, and its spread to the salivary glands takes several days; the interval varies with different viruses and is influenced by ambient temperature. Virions in the salivary secretions of the vector are injected into human or animal hosts during subsequent blood meals. Most arboviruses have localized natural habitats in which specific receptive arthropod and vertebrate hosts are involved in the viral life cycle. Vertebrate reservoir hosts are usually wild mammals or birds; humans are rarely involved in primary transmission cycles, although the exceptions to this generalization are important (e.g., Venezuelan equine encephalitis, yellow fever, and dengue viruses). Humans are in most cases infected incidentally, for example, by the geographic extension of a reservoir vertebrate host and/or a vector arthropod. Ecological changes produced by human activities disturb natural arbovirus life cycles and have been incriminated in the geographic spread or increased prevalence of arbovirus diseases.

Mathematical Modeling

From the time of William Farr, who studied epidemic disease problems in the 1870s, mathematicians have been interested in 'epidemic curves' and secular trends in the incidence of infectious diseases. With the development of computer-based mathematical modeling techniques, there has been a resurgence of interest in the population dynamics of infectious diseases. There has also been a resurgence in controversies surrounding the use of models; critics say 'for every model there is an equal and opposite model'. So, the proof of the value of models lies in their practical application, and in recent years there have been more and more successes. For example, when for counterterrorism reasons universal

smallpox vaccination was being considered, models that showed that vaccine could be used effectively after rapid detection of a terrorism incident led to a decision to stockpile, but not widely use vaccine. As another example, when a foot-and-mouth disease epidemic raged in the United Kingdom in 2001, a model showed that only the most vigorous stamping-out campaign could get ahead of the movement of the virus across the country. The model, seeming eminently logical now, importantly provided the kind of veracity and political will needed to accelerate the stamping-out campaign.

Models may be used to determine (1) patterns of disease transmission, (2) critical population sizes to support the continuous transmission of viruses with short and long incubation periods, (3) the dynamics of endemicity of viruses that become persistent in their hosts, and (4) the variables in age-dependent viral pathogenicity. Computer modeling also provides useful insights into the effectiveness of disease control programs. Much attention has been given to modeling the future of the AIDS epidemic in the United States and the rest of the world. Such models usually start with historical data on the introduction of the etiologic virus, HIV1, proceed to the present stage of the epidemic where the disease has become well established in many countries and in fewer countries subject to prevention and treatment strategies, and then proceed to project its course into the future. During the first 10 years of the AIDS epidemic in the United States, African countries, and then in Asian countries, most models underestimated developing trends; more recently models have become more accurately predictive – but in many places more and more sobering.

Implications for Disease Prevention

Knowledge of the epidemiology and modes of transmission of infectious diseases is critical to the development and implementation of prevention and control strategies. Data on incidence, prevalence, and mortality contribute directly to the establishment of priorities for prevention and control programs while knowledge of viral characteristics and modes of transmission are used in deciding prevention strategies focusing on vaccine development and delivery, environmental improvements, enhancement of nutritional status, improvement in personal hygiene, and behavioral changes.

See also: Disease Surveillance; Viral Pathogenesis; Zoonoses.

Further Reading

Evans AS and Kaslow RA (eds.) (1997) *Viral Infections of Humans. Epidemiology and Control,* 4th edn. New York: Plenum Medical Book Company.

Heymann DL (ed.) (2005) *Control of Communicable Diseases Manual,* 18th edn. Washington, DC: American Public Health Association Press.

Mandell GL, Bennett JE, and Dolin R (eds.) (2000) *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases,* 5th edn. New York: Churchill Livingstone.

Monto A (2005) The epidemiology of viral infections. In: Mahy BWJ and ter Meulen V (eds.) *Virology: Topley and Wilson's Microbiology and Microbial Infections,* vol. 1. London and Washington, DC: Hodder Arnold and ASM Press.

Murphy FA, Gibbs EPJ, Horzinek MC, and Studdert MJ (1999) *Veterinary Virology,* 3rd edn. New York: Academic Press.

Nathanson N (2006) Epidemiology. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, and Martin MA (eds.) *Fields Virology,* 5th edn. Philadelphia: Lippincott Williams and Wilkins.

Nathanson N and Murphy FA (1997) Evolution of viral diseases. In: Nathanson N, Ahmed R, Griffin DE, *et al.* (eds.) *Viral Pathogenesis*, Philadelphia: Lippincott-Raven Press.

Nowak MA and May R (2001) *Virus Dynamics: Mathematical Principles of Immunology and Virology.* Oxford, UK: Oxford University Press.

Smolinski MS, Hamburg MA, and Lederberg J (2002) *Emerging Microbial Threats to Health in the 21st Century.* Institute of Medicine/ National Academy of Sciences. Washington, DC: National Academy Press.

Smalinski MS, Hamburgh MA, and Lederburgh J (eds.) (2003) *Microbial Threads to Health, Emergence, Detection and Response*, 367pp. Washington: National Academies press.

Thrusfield MV and Bertola G (2005) *Veterinary Epidemiology,* 3rd edn. London: Blackwell Publishing.

Epstein–Barr Virus: General Features

L S Young, University of Birmingham, Birmingham, UK

© 2008 Elsevier Ltd. All rights reserved.

Taxonomy and Genome Structure

Epstein–Barr virus (EBV; species *Human herpesvirus 4*) is a member of the genus *Lymphocryptovirus*, which belongs to the lymphotropic subfamily *Gammaherpesvirinae* of the family *Herpesviridae.* EBV is closely related to the lymphocryptoviruses (LCVs) present in Old World nonhuman primates, including EBV-like viruses of chimpanzees and rhesus monkeys. These viruses share homologous sequences and genetic organization, and infect the B lymphocytes of their host species, resulting in the establishment of latent infection *in vivo* and transformation

in vitro. A transforming, EBV-related virus has also been isolated from spontaneous B-cell lymphomas of common marmosets and is thus the first EBV-like virus to be identified in New World primates. The genome of this marmoset LCV revealed considerable divergence from the genomes of EBV and Old World primate EBV-related viruses, suggesting that this virus represents a more primitive predecessor of the LCVs infecting higher-order primates.

The EBV genome is composed of linear, double-stranded DNA, approximately 172 kbp in length. EBV has a series of 0.5 kbp terminal direct repeats (TRs) and internal repeat sequences (IRs) that divide the genome into short and long, largely unique sequence domains. EBV was the first herpesvirus to have its genome completely cloned and sequenced. Since the EBV genome was sequenced from an EBV DNA *Bam*HI fragment cloned library, open reading frames (ORFs), genes, and sites for transcription or RNA processing are frequently referenced to specific *Bam*HI fragments, from A–Z, in descending order of fragment size. The virus has the coding potential for around 80 proteins, not all of which have been identified or characterized.

History

In 1958, Denis Burkitt described a lymphoma that represented the most common tumor affecting children in certain parts of East Africa. The geographical distribution of this malignancy suggested that the development of Burkitt lymphoma (BL) might be due to an infectious agent possibly related to malaria. In 1964, the successful establishment of cell lines from explants of BL enabled Tony Epstein and Yvonne Barr to identify herpesvirus-type particles by electron microscopy within a subpopulation of tumor cells *in vitro* (**Figure 1**). Werner and Gertrude Henle subsequently demonstrated that BL-derived cell lines expressed antigens that were recognized not only by sera from patients with BL but also by sera from patients with infectious mononucleosis (IM). Similar seroepidemiological studies also suggested a link between infection with this virus (now called Epstein– Barr virus after its discoverers) and undifferentiated nasopharyngeal carcinoma (NPC), leading to the subsequent direct demonstration of EBV DNA in the tumor cells of NPC. The ability of EBV to immortalize B lymphocytes efficiently *in vitro* and to induce tumors in nonhuman primates established this virus as a putative oncogenic agent in humans. Over the last 20 years EBV has been implicated in a variety of other lymphoid and epithelial malignancies (**Table 1**).

Geographic and Seasonal Distribution

EBV is ubiquitous, being found as a widespread and largely asymptomatic infection in all human communities.

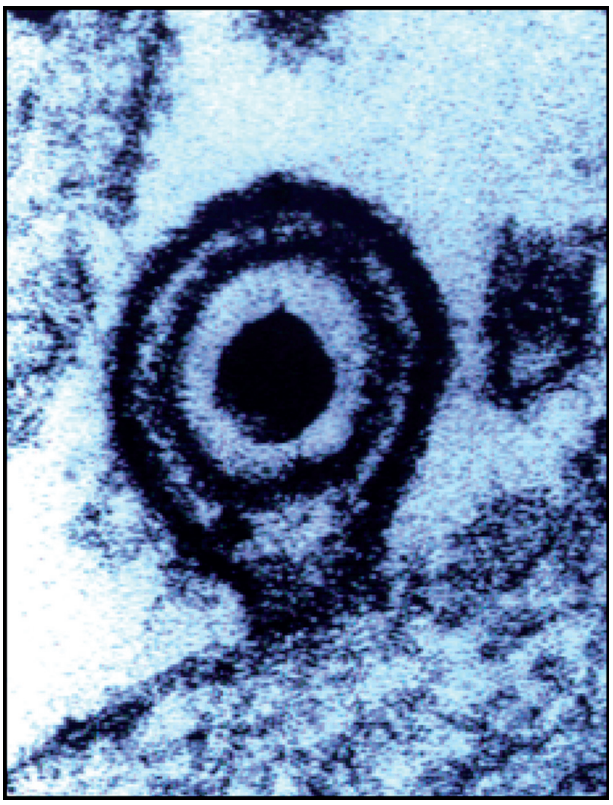


Figure 1 Electron micrograph of the Epstein–Barr virus virion.

Table 1 EBV-associated tumors

Tumor	*Subtype*	*EBV genome +ve (%)*
Burkitt's lymphoma	Endemic	100
	Sporadic	10–20
	AIDS	30–40
Nasopharyngeal carcinoma	Undifferentiated	100
	SCC	10–40
Hodgkin's disease	Mixed cellularity	>80
	Nodular sclerosing	30–40
	Lymphocyte predominant	<10
T-cell lymphoma	Nasal	100
	Others	10–40
Immunoblastic lymphoma	Transplant	100
	AIDS	>90

Primary infection often occurs early in life, particularly in tropical areas and in persons of lower socioeconomic class. Thus, in tropical Africa and New Guinea primary EBV infection is common in the first year of life whereas in Western communities infection is continually acquired throughout childhood and early adulthood. Although the EBV-associated malignancies BL and NPC exhibit an unusual geographic distribution, this appears not to be due to differences in EBV infection but due to additional cofactors.

Host Range and Virus Propagation

Humans are the natural host for EBV infection. EBV has a unique ability to transform resting B cells into permanent, latently infected, lymphoblastoid cell lines (LCLs) in which every cell carries multiple copies of circular, extra chromosomal, viral DNA (episomes) and produces a number of latent proteins, including six EBV-encoded nuclear antigens (EBNAs 1, 2, 3A, 3B, 3C, and -LP) and three latent membrane proteins (LMPs 1, 2A, and 2B); this form of infection is referred to as latency III. When peripheral blood lymphocytes from healthy EBV seropositives are placed in culture, the few EBV-infected B lymphocytes that are present regularly give rise to spontaneous outgrowth of EBV-transformed LCLs, provided that immune T lymphocytes are either removed or inhibited by the addition of cyclosporin A to the culture. LCLs can also be generated by direct infection of resting B lymphocytes with EBV derived from the throat washings of seropositive individuals or from producer B-cell lines. LCLs have provided an invaluable, albeit incomplete, model of the lymphomagenic potential of EBV.

Certain nonhuman primates, particularly the cotton-top tamarin, have been used as experimental hosts for EBV, but virus infection in these animals is associated with the induction of lymphomas. LCLs generated by EBV transformation of cotton-top tamarin B lymphocytes *in vitro* spontaneously produce large amounts of virus compared with their human counterparts and as such have been used as a source of EBV; for example, the prototype strain of EBV is B95.8, which is produced from a tamarin LCL originally immortalized with EBV from a patient with IM. However, the lack of a fully permissive system for propagating EBV *in vitro* has hampered our understanding of virus replication and prevented the generation of EBV mutants. Over recent years a number of systems have been developed for the generation of recombinant forms of EBV which rely on the manipulation of the entire virus genome within bacteria followed by rescue and propagation in human cells.

Genetics and Strain Variation

EBV isolates from different regions of the world or from patients with different virus-associated diseases are remarkably similar when their genomes are compared by restriction fragment length polymorphism analysis. However, variations in repeat regions of the EBV genome are observed among different EBV isolates. Analysis of the EBV genome in a number of BL-cell lines revealed gross deletions, some of which account for biological differences. For example, P3HR-1 virus, which is nontransforming, has a deletion of the EBNA2-encoding gene. Strain variation over the EBNA2-encoding (*Bam*HI WYH) region of the EBV genome permits all virus isolates to be classified as either 'type 1' (EBV-1, B95.8-like) or 'type 2' (EBV-2, Jijoye-like). This genomic variation results in the production of two antigenically distinct forms of the EBNA2 protein that share only 50% amino acid sequence homology. Similar allelic polymorphisms (with 50–80% sequence homology depending on the locus) related to the EBV type occur in a subset of latent genes, namely those encoding EBNA-LP, EBNA3A, EBNA3B, and EBNA3C. These differences have functional consequences as EBV-2 isolates are less efficient in *in vitro* B-lymphocyte transformation assays compared with EBV-1 isolates. A combination of virus isolation and seroepidemiological studies suggest that type 1 virus isolates are predominant (but not exclusively so) in many Western countries, whereas both types are widespread in equatorial Africa, New Guinea, and perhaps certain other regions.

In addition to this broad distinction between EBV types 1 and 2, there is also minor heterogeneity within each virus type. Individual strains have been identified on the basis of differences compared with B95.8, ranging from single-base mutations to extensive deletions. While infection with multiple strains of EBV was originally thought to be confined to immunologically compromised patients, more recent studies demonstrate that normal healthy seropositives can be infected with multiple EBV isolates and that their relative abundance and presence appears to vary over time. Coinfection of the host with multiple virus strains could have evolutionary benefit to EBV, enabling the generation of diversity by genetic recombination. Such intertypic recombination has been demonstrated in human immunodeficiency virus (HIV)-infected patients and appears to arise via recombination of multiple EBV strains during the intense EBV replication that occurs as a consequence of immunosuppression.

The possible contribution of EBV strain variation to virus-associated tumors remains contentious. Many studies have failed to establish an epidemiological association between EBV strains and disease and suggest that the specific EBV gene polymorphisms detected in virus-associated tumors occur with similar frequencies in EBV isolates from healthy virus carriers from the same geographic region. However, this does not exclude the possibility that variation in specific EBV genes is responsible for the distinct geographic distribution of virus-associated malignancies. In this regard, an LMP1 variant containing a 10-amino-acid deletion (residues 343–352) was originally identified in Chinese NPC biopsies and has oncogenic and other functional properties distinct from those of the B95.8 LMP1 gene. It is therefore likely that variation in LMP1 and other EBV genes can contribute to the risk of developing virus-associated tumors, but more biological studies using well-defined EBV variants are required.

Evolution

EBV, like other herpesviruses, has probably evolved with humans. The relatedness of the LCVs at both the genomic and protein levels does not correlate with the evolutionary relatedness of their Old World primate hosts. This implies that the selective pressures governing the evolution of these viruses are different from those responsible for the evolution of primate species. It has been suggested that the specific tissue tropism of LCVs may have constrained their evolutionary divergence. A comparison of marmoset LCV with the LCVs present in Old World primates provides some insight into LCV evolution approximately 35 million years before the appearance of EBV. The acquisition of accessory genes by Old World LCVs supports the contention that these viruses have coevolved with their hosts possibly as a means of maintaining their biological properties.

The evolutionary relationship between the type 1 and type 2 strains of EBV remains obscure. They may have evolved from a common progenitor virus or through recombination of the ancestral LCVs infecting Old World primates. The pronounced (but not exclusive) segregation of EBV-2 isolates within equatorial regions suggests that environmental factors (including immunological competence) may have influenced EBV evolution and may still be responsible for the effective competition between EBV-2 isolates and the ubiquitous EBV-1 family.

EBV Primary Infection and Persistence

EBV infects the majority of the world's adult population and, following primary infection, the individual remains a lifelong carrier of the virus (**Figure 2**). In underdeveloped countries, primary infection with EBV usually occurs during the first several months to few years of life and is often asymptomatic. However, in developed populations, primary infection is more frequently delayed until adolescence or adulthood, in many cases producing the characteristic clinical features of IM. EBV is orally transmitted, and infectious virus can be detected in oropharyngeal secretions from IM patients, from immunosuppressed patients, and at lower levels from healthy EBV seropositive individuals. These observations, together with the fact that EBV-transformed LCLs *in vitro* tend to be poor producers of the virus and B lymphocytes permissive of viral replication have not been demonstrated *in vivo*, suggest that EBV replicates and is shed at epithelial sites in the oropharynx and/or salivary glands. This is supported by the demonstration of replicating EBV in the differentiated epithelial cell layers of oral 'hairy' leukoplakia, a benign lesion of the tongue found in immunocompromised patients. However, it is also likely that EBV-infected B cells are reactivated within the local mucosal environment and that this contributes to virus shedding at oropharyngeal sites (**Figure 2**).

The inability to detect EBV routinely in normal epithelial cells and the demonstration that EBV can be completely eradicated by irradiation in bone marrow transplant recipients suggests that B lymphocytes are the main site of EBV persistence (**Figure 2(b)**). This is supported by the B lymphotropism of EBV which is mediated by the binding of the major viral envelope glycoprotein gp350 to the CR2 receptor on the surface of B cells. Virion penetration of the B-cell membrane requires further interactions between the EBV glycoprotein gp42 (which forms a ternary complex with gH and gL viral glycoproteins) and human leukocyte antigen (HLA) class II molecules. It appears that the presence or absence of HLA class II in virus-producing cells influences the tropism of EBV for B cells or epithelial cells by affecting the availability of gp42. Other CR2-independent pathways may be responsible for EBV infection of epithelial cells, including secretory component-mediated immunoglobulin (Ig) A transport, integrin interactions with polarized epithelium, and direct cell-to-cell contact, but these are relatively inefficient and of unknown relevance to EBV infection *in vivo*.

The B-cell tropism of EBV and its ability to establish a latent infection in these cells both *in vitro* and *in vivo* further supports the contention that B lymphocytes are the reservoir of lifelong persistent infection (**Figure 2(b)**). Detailed examination of EBV infection *in vivo* has shown that the virus persists in the $IgD^{-}CD27^{+}$ memory B-cell subset and that these cells have downregulated the expression of most, if not all, viral genes. The precise route of entry of EBV-infected B cells into the memory compartment remains a subject of much debate. This reservoir of infected cells is stably maintained thereafter, apparently subject to the same physiologic controls as the general mucosa-associated memory B-cell pool. EBV persistence within this B-cell population brings with it the possibility of fortuitous antigen-driven recruitment of infected cells into germinal centers (GCs), leading to progeny that either re-enter the circulating memory pool or differentiate to plasma cells that may migrate to mucosal sites. The different forms of latency that are manifest in virus-associated malignancies (discussed below) may represent latency programs that have evolved to accommodate such changes in host cell physiology (**Figure 2**). Thus GC transit appears to activate a latency program where only the genome maintenance protein EBNA1 is expressed (latency 0), while exit from GCs is possibly linked to the transient expression of the EBV-encoded latent membrane proteins, LMP1 and LMP2 (latency II). The ability of these proteins to mimic the key signals required for B cells to undergo a GC reaction, namely T-cell help via the CD40 pathway (constitutively provided by LMP1) and activation of the cognate B-cell receptor (augmented by LMP2A), supports this strategy whereby EBV exploits the physiological process of B-cell differentiation to access and maintain persistent

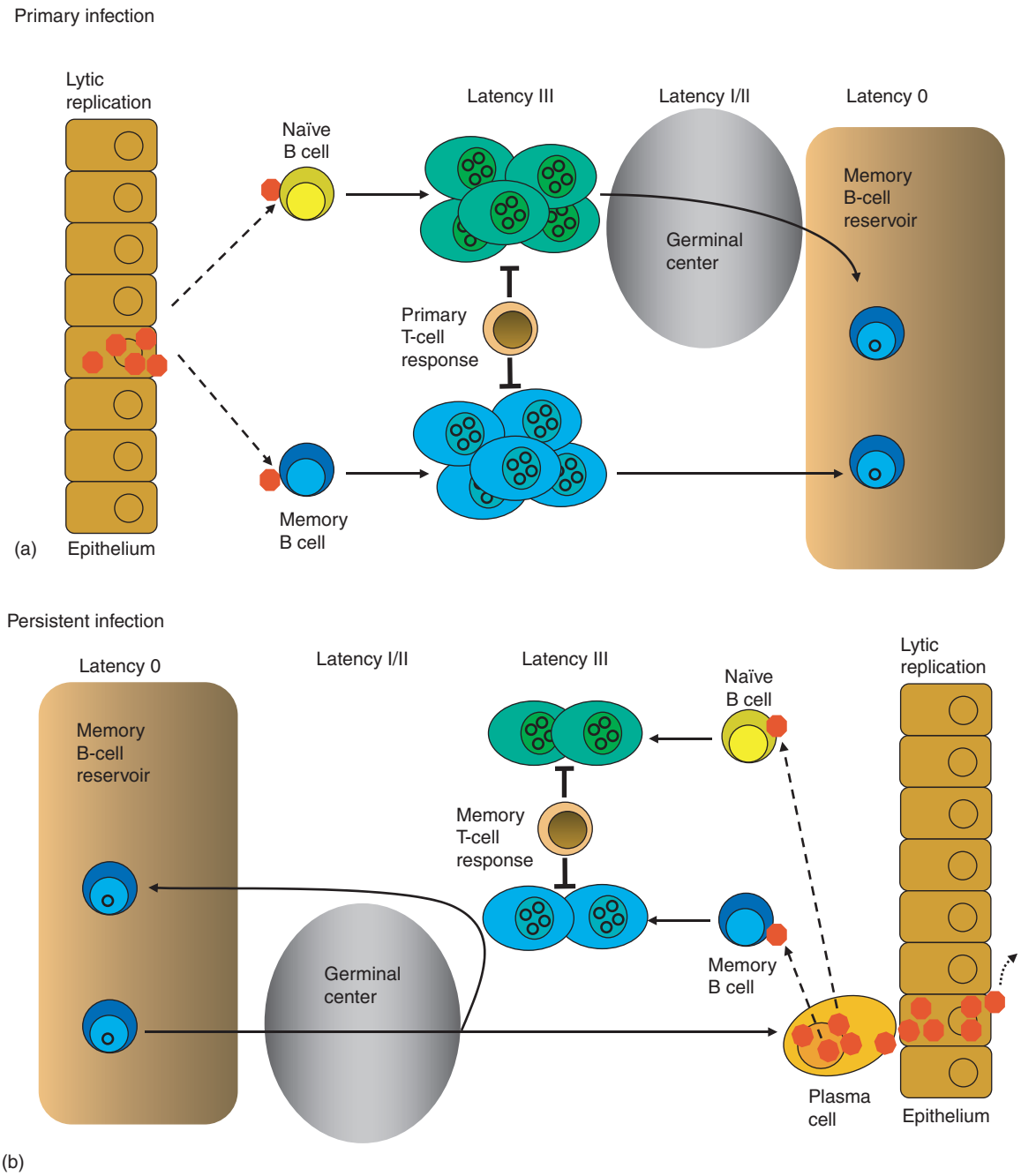


Figure 2 EBV primary infection and persistence. Diagram showing putative *in vivo* interactions between EBV and host cells. (a) Primary infection. EBV replicates in epithelial cells and spreads to lymphoid tissues as a latent growth-transforming (latency III) infection of B cells. Many infected B cells are removed by the emerging EBV-specific T-cell response. Some infected cells escape by downregulating EBV latent genes to establish a stable pool of resting virus-positive memory B cells. (b) Persistent infection. EBV-infected memory B cells become subject to the physiological controls governing memory B-cell migration and differentiation. Occasional recruitment into germinal center (GC) reactions resulting in activation of different EBV latency programs and re-entry into the memory cell reservoir or plasma cell differentiation with activation of virus lytic cycle. Infectious virions then initiate foci of EBV replication in epithelial cells and also new growth-transforming infection of naïve and/or memory B cells.

infection in the memory B-cell pool. The possible commitment of these cells to plasmacytoid differentiation may trigger entry into lytic virus replication, thereby providing a source of low-level virus shedding into the oropharynx. There may also be circumstances in which infected cells in the reservoir can reactivate back to proliferative infections similar to those of an EBV-transformed LCL.

Immune Response

EBV elicits both humoral and cell-mediated immune responses in infected hosts. Primary infection with EBV is associated with the rapid appearance of antibodies to replicative antigens such as viral capsid antigen (VCA), early antigen (EA), and membrane antigen (MA; gp350/220) with a later serological response to EBNA proteins. In IM, these responses are exaggerated and are accompanied by autoantibodies such as rheumatoid factor as well as a heterophile antibody response directed against antigens on the surface of sheep erythrocytes. These autoantibodies are the result of EBV-induced polyclonal B-cell activation. In the chronic asymptomatic virus carrier, antibodies to VCA, MA, and EBNAs are found, the titers of which remain remarkably stable. Of these antibodies, those against MA are particularly important as they have virus-neutralizing ability and can also mediate antibody-dependent cellular cytotoxicity. As discussed below, the levels of these EBV-specific antibodies are elevated in different EBV-associated diseases.

As with other persistent viruses, cell-mediated immunity plays an important role in controlling EBV infection. Primary EBV infection elicits a robust cellular immune response and the lymphocytosis observed in IM is a consequence of the hyperexpansion of cytotoxic CD8+ T cells with reactivities against both latent and lytic viral antigens. These reactivities are subsequently maintained at high level (up to 5% of the total circulating pool) in the CD8+ memory T-cell pool. An EBV-specific CD4+ T-cell response also contributes toward the control of EBV infection and, along with the CD8+ response, appears to be important in preventing the unlimited proliferation of EBV-infected B cells. Thus, impairment of the T-cell response, either by immunosuppressive drug therapy or by HIV infection, is responsible for the development of polyclonal lymphoproliferations that can progress to frank monoclonal non-Hodgkin's lymphomas (see below). These lesions can be controlled by adoptive therapy with EBV-specific T cells. The growth and survival of BL, NPC, and Hodgkin's lymphoma (HL) in immunocompetent individuals implies that the tumor cells can evade EBV-specific T-cell surveillance. This may be achieved by restricting EBV latent gene expression to those viral proteins not efficiently recognized by the T-cell responses (i.e., away from expression of the highly immunogenic EBNA3 family) and/or by downregulation of target cell molecules required for immune recognition such as major histocompatability complex (MHC) class I and the antigen-processing machinery.

EBV-Associated Diseases

Infectious Mononucleosis

Primary infection with EBV in childhood is usually asymptomatic but when delayed until adolescence or early adulthood can manifest clinically as IM, a self-limiting lymphoproliferative disease associated with the hyperexpansion of cytotoxic $CD8^+$ T cells that are reactive to both lytic and latent cycle viral antigens. These reactivities are subsequently maintained in the $CD8^+$ T-cell memory pool at high levels (up to 5% of circulating $CD8^+$ T cells), even in individuals with no history of IM. The incidence of IM is low in developing countries where asymptomatic primary infection predominantly occurs in childhood. In certain poorly defined situations, IM-like symptoms can persist, resulting in chronic active EBV infection associated with elevated antibody titers to virus lytic antigens but low titers to the EBNAs.

Lymphomas in Immunosuppressed Individuals

Patients with primary immunodeficiency diseases such as X-linked lymphoproliferative syndrome (XLP) and Wiscott–Aldrich syndrome are at increased risk of developing EBV-associated lymphomas. Because these tumors are extremely rare, little is known about the precise contribution of EBV and the associated pattern of viral gene expression in these lymphomas. Mortality from XLP is high with around 50% of patients developing fatal IM after primary infection with EBV and an additional 30% of patients developing malignant lymphomas. The defect responsible for XLP is mutation of an adaptor molecule, SAP, which mediates signaling in a wide range of immune cells and is involved, via its interaction with the SLAM family of receptors, in both innate and adaptive immune reactions.

Allograft recipients receiving immunosuppressive therapy and patients with acquired immune deficiency syndrome (AIDS) are also at increased risk for development of EBV-associated post-transplant lymphoproliferative disease (PTLD) and lymphomas. These lesions range from polyclonal EBV-driven lymphoproliferations, much like that observed *in vitro* in virus-transformed LCLs (latency III, **Figure 3**), to aggressive monoclonal non-Hodgkin's B-cell lymphomas (NHLs) in which additional cellular genetic changes (e.g., mutation of p53) are present. The incidence of both PTLD and B-cell lymphomas in allograft recipients varies with the type of organ transplanted and with the type of immunosuppressive regimen used. Allogeneic bone marrow or solid organ transplantation into EBV seronegative children is a particular risk factor for the development of these lesions. A proportion of these lesions in post-transplant patients resolve in response to a reduction in immunosuppression or to targeted therapies such as anti-CD20 monoclonal antibody or adoptive EBV-specific T cells.

The incidence of NHL in AIDS patients is increased approximately 60-fold compared to the normal population. Around 60% of these tumors are large B-cell lymphomas like those found in allograft recipients, 20% are primary brain lymphomas, and 20% are of the BL type. EBV

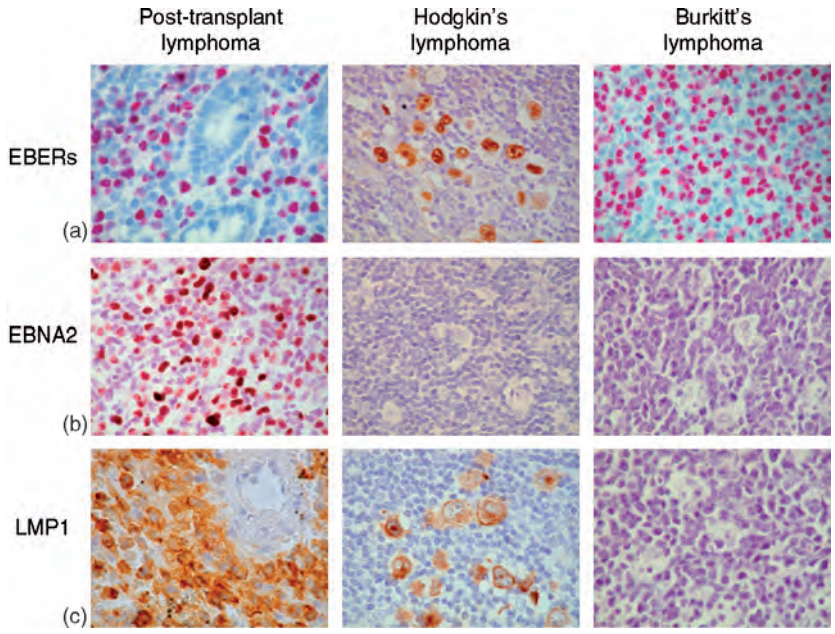


Figure 3 EBV-associated lymphomas display different patterns of virus latent gene expression. While the post-transplant lymphomas associated with EBV infection express a pattern of latent gene expression similar to that observed in virus-transformed B cells *in vitro* (latency III), Hodgkin's lymphoma (HL; Latency II) and Burkitt's lymphoma (BL; Latency I) express more restricted forms of EBV latency. (a) Upper panels show *in situ* hybridization for the abundant nonpolyadenylated EBER transcripts, which are expressed in all forms of EBV latent infection. (b) The middle panels depict immunohistochemical staining for the EBNA2 protein, which is only expressed in post-transplant lymphoma and not either HL or BL. (c) The bottom panels show immunohistochemical staining for the LMP1 protein, which is expressed in post-transplant lymphoma and HL but not in BL.

infection is present in approximately 50% of AIDS-related NHL, nearly all the primary brain and Hodgkin's lymphomas, and around 40% of the BL tumors.

Burkitt's Lymphoma

The endemic form of BL, which is found in areas of equatorial Africa and New Guinea, represents the most common childhood cancer (peak age 7–9 years) in these regions with an incidence of up to 10 cases per 100 000 people per year. This high incidence of BL is associated with holoendemic malaria, thus accounting for the climatic variation in tumor incidence first recognized by Denis Burkitt. More than 95% of these endemic BL tumors are EBV-positive compared with 20% of the low incidence, sporadic form of BL which occurs worldwide. In areas of intermediate BL incidence, such as Algeria and Malaysia, the increased number of cases correlates with an increased proportion of EBV-positive tumors. The pattern of EBV gene expression in BL is generally restricted to EBNA1 and the nonpolyadenylated EBER transcripts (latency I), although broader virus gene expression involving the EBNA3 proteins has been observed in a subset of tumors (**Figure 3**).

A consistent feature of BL tumors, irrespective of geographical location or EBV status, is chromosome translocations involving the long arm of chromosome 8 (8q24) in the region of the c-myc proto-oncogene and either chromosome 14 in the region of the Ig heavy-chain gene or, less frequently, chromosomes 2 or 22 in the region of the Ig light-chain genes. The cell surface phenotype of BL cells and the presence of ongoing Ig gene mutation supports the GC origin of this tumor.

Seroepidemiological studies have demonstrated elevated antibody titers to EBV VCA and EA in BL patients compared to children without the tumor. These elevated antibody titers have been found to precede the development of BL and can therefore be used to screen 'at risk' individuals. Most early cases of BL can be successfully treated using chemotherapeutic regimens that include cyclophosphamide. However, access to drugs and advanced tumor presentation have limited the efficacy of such therapies.

Hodgkin's Lymphoma

Epidemiological studies originally suggested a possible role for EBV in the etiology of HL. Thus, elevated antibody titers to EBV antigens have been detected in HL patients and these are present before the diagnosis of the disease. Furthermore, there is an increased risk of HL following IM. EBV has been demonstrated in around 40% of HL cases with both viral nucleic acid and virus-encoded latent proteins (EBNA1, LMP1, LMP2 – latency

II) localized to the malignant component of HL, the so-called Hodgkin's and Reed–Sternberg (HRS) cells (**Figure 3**). These malignant B cells tend to carry nonproductive Ig genes consistent with their GC origin and implicating EBV infection in the rescue of these crippled cells from their usual apoptotic fate. The association of HL with EBV is age-related; pediatric and older adult cases are usually EBV-associated whereas HL in young adults is less frequently virus-positive. The proportion of EBV-positive HL in developing countries is high, consistent with a greater incidence of HL in children and more frequent prevalence of the mixed cellularity histiotype; the histological subtype of HL in which EBV infection is most frequently detected. Although the incidence of HL is relatively low (1–3 per 100 000 per year), this tumor is not geographically restricted, making its association with EBV significant in world health terms.

Virus-Associated T-Cell and NK-Cell Lymphomas

EBV-positive monoclonal lymphomas of either CD4+ or CD8+ T-cell origin are more frequently found in Southeast Asian populations, arising as a consequence of either virus-associated haemophagocytic syndrome (VAHS) or in the setting of chronic active EBV infection. A more aggressive extranodal tumor predominantly expressing the CD56 natural killer (NK) cell marker is also EBV positive and manifests as an erosive lesion (lethal midline granuloma) of the nasal cavity.

Nasopharyngeal Carcinoma

The association of EBV with undifferentiated NPC (WHO type III) was first suggested by serological evidence and then confirmed by the demonstration of EBV DNA in NPC biopsy material. NPC is particularly common in areas of China and southeast Asia, reaching a peak incidence of 20–30 cases per 100 000 per year. Incidence rates are particularly high in Cantonese males, highlighting an important genetic predisposition as well as a role for environmental cofactors such as dietary components (e.g., salted fish). NPC tumors are characterized by a prominent lymphoid stroma, and the interaction between these activated lymphocytes and adjacent carcinoma cells appears to be crucial for the continued growth of the malignant component. EBV latent gene expression in NPC is restricted to EBNA1, the LMP2A/B proteins, the EBER transcripts, and the *Bam*HI-A rightward transcripts (BARTs), with around 20% of tumors also expressing the oncogenic LMP1 protein (**Figure 4** – latency II). The presence of monoclonal EBV episomes in NPC indicates that virus infection preceded the clonal expansion of the malignant cell population. More detailed analysis of the genetic changes in NPC suggests that some of these (particularly deletions in chromosomes 3p and 9p) occur early before EBV infection. Extensive serological screening for EBV-specific antibody titers in high-incidence areas, in particular IgA antibodies to VCA and EAs, have proved useful in diagnosis and in monitoring the effectiveness of therapy. More recent studies have demonstrated that the quantitative analysis of tumor-derived EBV DNA in the blood of patients with NPC using real-time polymerase chain reaction (PCR) is of both diagnostic and prognostic utility. EBV infection is also associated with the more differentiated forms of NPC (WHO types I and II) particularly in those geographical regions with a high incidence of the type III tumor. EBV-positive carcinomas, which morphologically resemble NPC (undifferentiated carcinomas of nasopharyngeal type), have been described at other anatomical sites (e.g., thymus, tonsil, lungs, skin) but the extent of the association of these tumors with EBV is geographically variable.

Gastric Carcinoma and Other Epithelial Tumors

EBV infection is also present in around 10% of typical gastric adenocarcinomas, accounting for up to 75 000 cases per year. These tumors resemble NPC in carrying monoclonal EBV genomes, having a restricted pattern of EBV gene expression (EBERs, EBNA1, LMP2A, BARTs, and BARF1) and in the appearance of virus infection as a relatively late event in the carcinogenic process. The geographical variation in the association of EBV with gastric adenocarcinomas probably reflects ethnic and genetic differences. EBV-positive tumors have distinct phenotypic and clinical characteristics compared with EBV-negative tumors.

A number of other more common carcinomas such as breast cancer and liver cancer have been reported to be infected with EBV. Difficulties in confirming these associations have raised concerns about the use of PCR analysis to detect EBV infection and about the specificity of certain monoclonal antibody reagents. It is possible, however, that a small proportion of tumor cells can be infected with EBV, perhaps sustaining a low-level replicative infection, and that this might contribute to the growth of the carcinoma.

Prevention and Control of EBV Infection

The significant burden of EBV-associated tumors worldwide has prompted the development of novel therapeutic strategies that either specifically target viral proteins or exploit the presence of the virus in malignant cells. Pharmacological approaches include the use of agents to induce the expression EBV lytic cycle antigens, including virus-encoded kinases (EBV thymidine kinase and

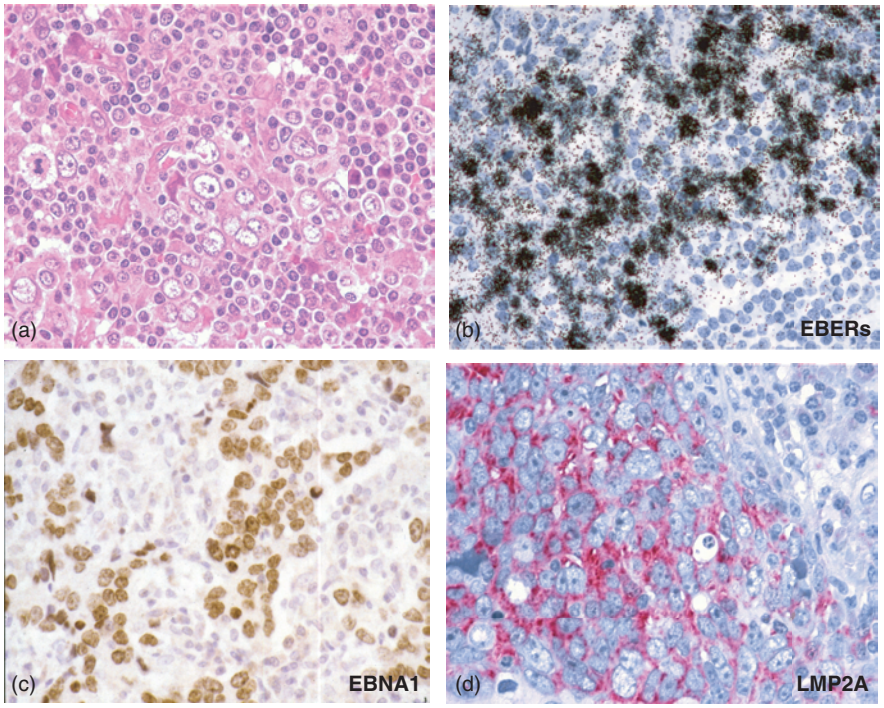


Figure 4 EBV gene expression in nasopharyngeal carcinoma (NPC). (a) shows a hematoxylin and eosin stain of a NPC tumor showing the intimate association between the undifferentiated carcinoma cells (large nuclei) and the activated lymphoid stroma (small intensely staining nuclei). (b–d) *in situ* hybridization for the EBER transcripts and immunohistochemical staining for EBNA1 and LMP2A.

BGLF4, a protein kinase) that will then phosphorylate the nucleoside analog gancyclovir to produce its active cytotoxic form. Demethylating agents such as 5′ azacytidine are able to de-repress lytic, as well as potentially immunogenic, latent genes and are currently in early-stage clinical trials in patients with NPC, HL, and AIDS-associated lymphoma. Hydroxyurea is a chemotherapeutic agent that can induce loss of EBV episomes and has shown some clinical efficacy in patients with EBV-positive AIDS-related central nervous system lymphoma. Other approaches are based on the more specific targeting of individual EBV proteins using either single-chain antibodies, siRNA, or dominant-negative molecules. The use of adoptive EBV-specific T-cell therapy for the treatment of existing PTLD and in the prophylactic setting has been extremely successful. This approach is also showing signs of clinical efficacy in the treatment of NPC and HL but in this setting the use of T cells enriched for reactivities to subdominant targets (e.g., LMP2A and EBNA1) is important. More direct vaccine approaches to treat patients with EBV-associated tumors or to prevent disease development are being examined including (1) whole EBV latent antigens delivered by a virus vector or in autologous dendritic cells; (2) peptide or polytope vaccination; and (3) prophylactic vaccination against MA (gp350/220) to generate a neutralizing antibody response.

Future Perspectives

EBV was discovered over 40 years ago and its DNA was fully sequenced in 1984. Much work has contributed to the unequivocal identification of EBV as oncogenic in humans. In more recent years, the nature of the interaction of EBV with the immune host has become clearer, illustrating the complex mechanisms that the virus exploits to persist in the memory B-cell pool. The fine detail of this interaction with normal B cells is still in its infancy, and a major question remains over the replicative lifecycle of EBV *in vivo*, particularly with regard to the relative role of B cells versus epithelial cells in this process. The development of more efficient *in vitro* systems for studying EBV infection and replication in different cell types will help to unravel the complex interplay between the virus and the cell. The use of EBV recombinants continues to shed light on the role of latent genes in the transformation process, on the requirements for the efficient production of progeny virus, and on the role of membrane glycoproteins in the infection process. Understanding the host cell–virus interaction will be dependent on the generation of appropriate *in vitro* models, particularly systems which allow a more detailed understanding of the tumor microenvironment and the role of the local cytokine milieu. There are many interesting aspects of EBV that remain to be understood, including the role of virus-encoded

microRNAs and the possible contribution of lytic cycle antigens to virus persistence and oncogenesis. The challenge will be to exploit these new mechanistic insights both to gain a better understanding of EBV infection *in vivo* and to develop novel therapies for treating virus-associated disease.

See also: Epstein–Barr Virus: Molecular Biology; Herpesviruses: Discovery; Herpesviruses: General Features; Herpesviruses: Latency; Kaposi's Sarcoma-Associated Herpesvirus: General Features; Kaposi's Sarcoma-Associated Herpesvirus: Molecular Biology.

Further Reading

Kieff E and Rickinson AB (2001) Epstein–Barr virus and its replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 2511–2573. Philadelphia: Lippincott Williams and Wilkins.

Kuppers R (2005) Mechanisms of B cell lymphoma pathogenesis. *Nature Reviews Cancer* 5: 251–262.

Kutok JL and Wang F (2006) Spectrum of Epstein–Barr virus-associated diseases. *Annual Review of Pathology: Mechanisms of Disease* 1: 375–404.

Lo KW and Huang DP (2002) Genetic and epigenetic changes in nasopharyngeal carcinoma. *Seminars in Cancer Biology* 12: 451–462.

Rickinson AB and Kieff E (2001) Epstein–Barr virus. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 2575–2627. Philadelphia: Lippincott Williams and Wilkins.

Robertson ES (ed.) (2005) *Epstein–Barr Virus.* Norfolk, England: Caister Academic Press.

Tao Q, Young LS, Woodman CB, and Murray PG (2006) Epstein–Barr virus and its associated cancers – Genetics, epigenetics, pathobiology and novel therapeutics. *Frontiers in Bioscience* 11: 2672–2713.

Thorley-Lawson DA and Gross A (2004) Persistence of the Epstein–Barr virus and the origins of associated lymphomas. *New England Journal of Medicine* 350: 1328–1337.

Young LS and Rickinson AB (2004) Epstein–Barr virus: 40 years on. *Nature Reviews Cancer* 4: 757–768.

Epstein–Barr Virus: Molecular Biology

J T Sample and C E Sample, The Pennsylvania State University College of Medicine, Hershey, PA, USA

© 2008 Elsevier Ltd. All rights reserved.

Properties of the Virion

The morphology of the virion of Epstein–Barr virus (EBV; species *Human herpesvirus 4*, genus *Lymphocryptovirus*, family *Herpesviridae*) is typical of all herpesviruses, and comprises four major components. At the core is the viral genome, which is encased within an icosadeltahedral nucleocapsid that consists of 162 capsomeres and is approximately 100 nm in diameter. The tegument represents the amorphous proteinaceous region between the nucleocapsid and membrane envelope, the latter of which the virus acquires as the nucleocapsid buds through cellular membranes. Analysis of virion protein content by mass spectrometry has identified 6 viral capsid proteins, 12 glycoproteins, and as many as 17 tegument proteins. The tegument also contains several host proteins, for example, actin and Hsp70. The most abundant viral glycoprotein is gp350, which binds to the major cellular receptor for EBV attachment on B cells, CD21.

Properties of the Genome

Organization and Coding Potential

The EBV genome is a double-stranded (ds) DNA molecule of approximately 172 kbp. In its linear configuration, as within the virion, it is bounded on both ends by a variable number of 538-bp direct terminal repeats (TRs). The genome is separated into short (12 kbp) and long (134 kbp) predominately unique sequence domains by 7–10 copies of a 3-kbp repeat, IR1. Moreover, the long unique domain is divided into four shorter domains (U2–U5) by repetitive elements containing short direct repeats (IR2–IR4) (**Figure 1**). Each of the noted internal repeats contributes coding information to a viral protein. Based on identification of potential translational open reading frames (ORFs), the existence of approximately 75 genes was initially predicted from the genomic nucleotide sequence of the B95–8 isolate of EBV. Further, a 12-kbp deletion from the B95–8 genome removes three ORFs present in wild-type isolates, and sequence analyses of EBV cDNAs have identified several additional genes that were not apparent from the genomic sequence due to the highly spliced nature of their mRNAs. Thus, currently there are an estimated 80–85 EBV proteins, though not all have been confirmed. In addition to these, EBV expresses two classes of noncoding RNAs: EBV-encoded small RNA (*EBER*) 1 and 2, which are highly structured RNAs of 167 and 172 nucleotides, respectively, and two clusters of micro-RNAs (miRNAs) derived from two different loci.

The majority of EBV genes contribute to the production of progeny virus, and their expression is limited to

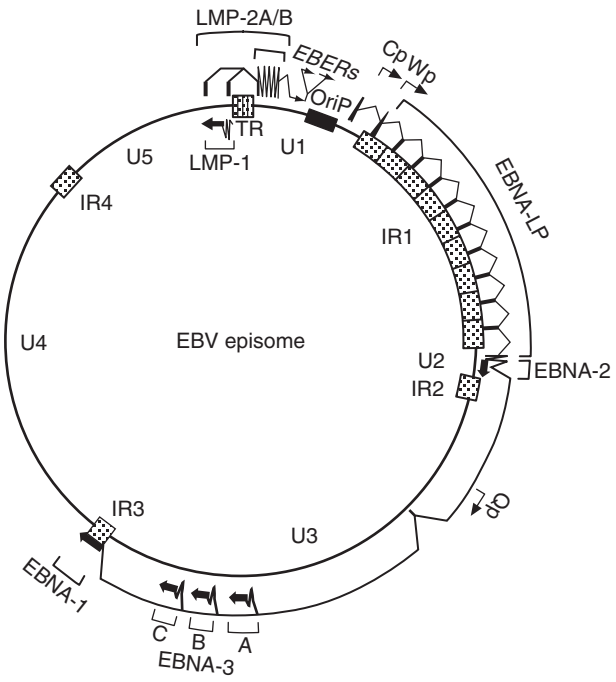


Figure 1 Structure and latency-associated transcription of the EBV genome. The episomal form of the genome that is maintained in latently-infected cells is shown; repeat elements (TR, IR1–IR4), unique sequence domains (U1–U5), and the latent-infection origin of DNA replication *oriP* are indicated, as are the latency-associated mRNAs and *EBERs*. The common EBNA-gene promoters Cp and Wp, and the EBNA-1 exclusive promoter Qp are depicted by bent arrows. Exons that contain ORFs are bracketed. The *BARTs* (not shown; see **Figure 2**) originate in U4 and terminate in U5.

the virus replicative or lytic cycle of infection. Up to an additional 12 genes, plus the *EBERs* and miRNAs, are expressed during the latent (nonproductive) programs of EBV infection. Few lytic-cycle mRNAs are spliced, and those that are in this class are relatively simple, generally containing only a single splice that removes a relatively short intron of no more than a few hundred base pairs. A discussion of the many lytic-cycle-specific genes is beyond the scope of this article, but their organization within the EBV genome and mRNA structures may be followed up via the 'Further reading' section. By contrast, all of the latency-associated gene mRNAs are spliced, and most splicing is extensive and occurs over 8–90 kbp. The nine known latency-associated proteins of EBV are grouped into two classes: the latent membrane proteins (LMP-1, LMP-2A, and LMP-2B) and the EBV-encoded nuclear antigens (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, and EBNA-LP). A transcriptional map of the EBV genome displaying the structures of the mRNAs encoding these proteins is presented in **Figure 1**. Notably, expression of the EBNA proteins is from a common transcription unit that spans 90 kbp of the viral genome, the primary transcripts from which are alternatively spliced to yield individual EBNA mRNAs. Moreover, the common highly spliced 5′ portion of the EBNA mRNAs contains an ORF composed primarily of a two-exon repeat, derived from each of the 7–10 IR1 elements, that encodes EBNA-LP (leader protein). A similar level of complexity is displayed by the LMP-2A and LMP-2B genes, each of which has a unique transcriptional promoter and 5′ exon, but share exons 2–9. A translational start codon is present in the unique exon of the LMP-2A mRNA, whereas the start codon for the LMP-2B ORF is within the common second exon, in frame with the LMP-2A ORF; consequently, LMP-2A differs from LMP-2B by the presence of 119 amino acid residues at its N-terminus. Further, because transcription of the LMP-2 genes traverses the fused TRs, they can only be expressed from the circular, latency-associated form of the EBV genome. The remaining latency-associated gene encodes for a family of alternatively processed and polyadenylated RNAs that co-terminate in the *Bam*HI-A restriction fragment of the EBV genome – hence their designation *Bam*HI-A rightward transcripts, or *BARTs* (**Figure 2**). These appear to arise from a common promoter, and contain several short ORFs. However, while there is ample circumstantial evidence to support protein expression from several of these ORFs, this has yet to be demonstrated with certainty in the context of EBV infection.

Genetic Diversity

There are two distinct genetic strains or subtypes of EBV, EBV-1 and EBV-2 (alternatively subtypes A and B), which were initially defined by specific sequence differences in their respective EBNA-2 ORFs. This was subsequently extended to the EBNA-3 ORFs (EBNA-3A, EBNA-3B, and EBNA-3C), which appear to have arisen through gene duplications from a common progenitor, and the *EBERs*. The respective degree of amino acid sequence identity between the EBNA-3 proteins of EBV-1 and EBV-2 ranges from 72% to 88%. The complete nucleotide sequence has recently been determined for the prototypic EBV-2 isolate, AG876, and no additional genetic loci were identified that would segregate with the type 2 genotype. Thus, the defining differences between EBV-1 and EBV-2 are limited to a subset of genes associated exclusively with the latent form of EBV infection. However, the biological significance of this divergence is currently unclear. *In vitro*, EBV-2 has an apparently lower transformation efficiency than EBV-1 isolates, such as B95–8. This is perhaps not surprising given that EBNA-2, EBNA-3A, and EBNA-3C are essential for B-cell immortalization. However, the molecular basis for this is unclear, as there is no strong evidence to indicate that these EBV-2 proteins differ significantly in function from their EBV-1 counterparts. The observation that EBV-2 isolates are more

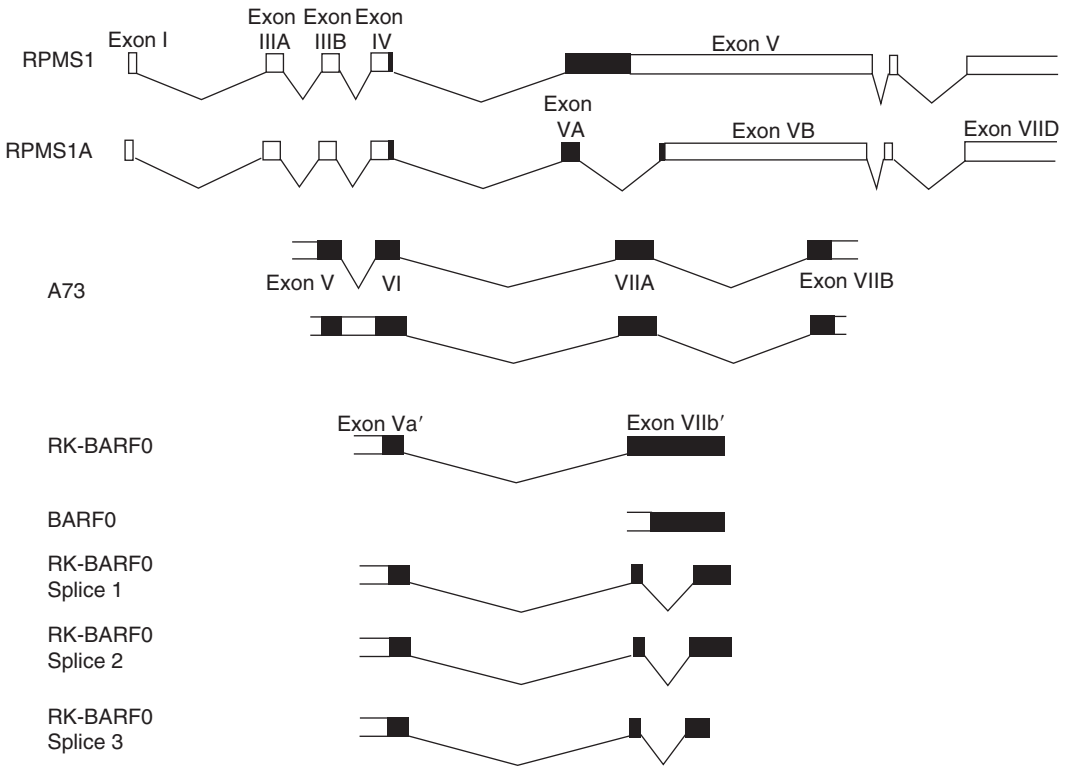


Figure 2 Structure of the latency-associated *BART* RNAs of EBV. Shown are portions of the 5′ and 3′ co-terminal *BARTs* that may encode protein. Potential ORFs within these highly spliced RNAs are shaded in black, and are designated RPMS1, A73, RK-BARF0 and BARF0; shorter versions are also indicated that arise through alternative splicing, but maintain the same translational reading frame. Transcription of the *BART* gene originates from a promoter upstream of exon I, and extends through the 3′-most exon (VII) that contains the ORF BARF0. Note that the three groups of *BARTs* are not shown as aligned relative to each other.

restricted geographically, notably to areas in which EBV-positive Burkitt lymphoma (BL) is endemic, initially raised the possibility of a link between this subtype and pathogenic potential. However, EBV-1 isolates also circulate in areas where EBV-2 is present, and the relative association of either subtype with BL more likely reflects its respective prevalence within the local population.

In addition to genetic differences that define EBV subtype, a number of sequence polymorphisms at various loci have been noted relative to the reference strain B95–8, and the LMP-1 ORF in numerous EBV isolates has a 30-bp deletion in the region encoding the C-terminal domain of this oncoprotein. Although EBV isolates with the LMP-1 deletion appear to be relatively widespread, the nondeleted form generally represents the more common strains circulating within any given population. Significant but non-type-specific differences have also been detected in the EBNA-1 ORF. Although clinical significance has at times been ascribed to some of these genetic variations, such reports are largely anecdotal. Another notable genetic anomaly within the EBV genome is a 6.8–7.4-kbp deletion that removes a portion of the EBNA-LP ORF and the entire EBNA-2 and BHLF1 ORFs (the latter encodes a protein of unknown function expressed during the lytic cycle of infection). Recent work has demonstrated that perhaps as many as 15% of BL tumors carry EBV with this deletion. Interestingly, these tumor cells often also carry the wild-type EBV genome, though it appears to be transcriptionally silent, such that EBV gene expression originates only from the deleted genome. This deletion likely reflects a selection against cells that express EBNA-2, the expression of which is normally repressed through epigenetic mechanisms within BL cells and during normal establishment of long-term latency within the B-cell reservoir of EBV (see below). Thus, deletion of the EBNA-2 locus may represent an alternative, albeit accidental, pathway in lymphomagenesis leading to BL.

Molecular Biology of Infection

Current knowledge of the EBV life cycle at the molecular level has come almost exclusively from *in vitro* studies, largely by necessity due to the lack of a representative and tractable animal model of EBV infection. These have primarily employed either lymphoblastoid cell lines (LCLs) generated by EBV immortalization of primary

human B lymphocytes *in vitro*, or lines established from BL tumors. Regardless of their origin, these B-cell lines characteristically restrict EBV infection to its latent phase, through which *in vivo* the virus is able to persist within its host and potentially contribute to malignancy. Currently, there is a considerably better understanding of EBV latency than of the viral replicative cycle, at least from the perspective of viral protein functions. This is primarily the consequence of the more intense effort over the past four decades to define the oncogenic nature of EBV, which is derived from its latency-associated gene functions, and the lack of an experimental cell system that efficiently supports EBV replication *in vitro*. Nonetheless, virus replication is a fundamentally important aspect of the EBV life cycle, as it is essential for maintenance of the virus in the human population.

Virus Replication

Although some latently infected B-cell lines can be induced to replicate EBV in response to chemical agents such as phorbol esters and inhibitors of histone deacetylases, or by crosslinking of cell-surface immunoglobulin, the efficiency of induction is generally poor in that 20–30% of the treated cells, at best, will actually produce virus. Further, these do not necessarily represent physiological pathways of reactivation. While epithelial cells are thought to be significant sites of EBV replication *in vivo*, epithelial cell lines are difficult to infect. This may reflect alternative pathways of cell entry, such as cell-to-cell spread (e.g., through contact with productively infected B cells) or IgA-mediated entry into epithelial cells via the receptor for the dimeric form of IgA that is bound to EBV virions. Further, other factors, such as the differentiation state of the cell, influence whether infected epithelial cells will support virus replication or establishment of a latent infection.

Despite these drawbacks, the general process of EBV replication is reasonably well understood owing to parallels that can be drawn to the replication of other herpesviruses, notably the herpes simplex viruses that replicate well in cell culture and for which an extensive body of knowledge exists. This is aided by comparative sequence analyses of the mammalian herpesvirus genomes, which have revealed blocks of conserved genes that are expressed during the respective viral replication cycles. Thus, while EBV also possesses a subset of unique lytic-cycle genes that contribute to its distinct biology and ability to occupy a specific niche within the host, its replication is believed to occur through a basic process common to all herpesviruses. This occurs through a highly ordered cascade of viral gene expression that is initiated by the immediate-early class of genes (those that do not require *de novo* protein synthesis for transcription), followed by expression of the early and then the late genes. In general, immediate-early and early proteins regulate or contribute to viral gene expression and DNA replication, whereas late proteins are either structural components of the virion or are required for functions such as assembly and egress of the virus particle. A discussion of the unique aspects of EBV replication as they contribute to the EBV life cycle in the host is presented below.

Latency

Although EBV can exist as a latent infection in epithelial cells within the host, B lymphocytes are the major reservoir of latent EBV and the source of new virus (following reactivation) that is ultimately required to populate EBV-naïve hosts. Primary B cells can be readily infected by EBV *in vitro*, leading to direct establishment of a latent infection that promotes cell immortalization through the actions of the latency-associated EBV genes, leading to continuous growth and proliferation in culture. Shortly after infection, the TR elements of the EBV genome fuse to generate a closed circular DNA molecule or episome (**Figure 1**), a variable but relatively stable number of which (10–100) is ultimately established and maintained within the nuclei of latently infected B cells. Replication of the EBV episome in latently infected cells is mediated by the cellular DNA replication machinery, occurs once per cell division cycle, and is initiated within an origin of DNA replication, *oriP*, located in the short unique domain of the viral genome (**Figure 1**). Two functions of a single EBV protein, EBNA-1, are required for genome maintenance during latency. First, through binding to the dyad symmetry (DS) element of *oriP* that contains four EBNA-1-binding sites, EBNA-1 directs assembly of host replication proteins to initiate DNA synthesis. Second, through binding to the family of repeats (FR) element of *oriP* (20 EBNA-1-binding sites), EBNA-1 tethers the EBV genome to host chromosomes through its interaction with a cellular chromosome-associated protein, ensuring that EBV episomes are evenly partitioned upon cell division. By contrast, genome replication during the lytic cycle does not utilize *oriP*, but two origins of DNA replication (*oriLyt*) that function independent of EBNA-1 and lie 100 kbp apart within the long unique sequence domain. Further, replication of viral DNA during the lytic cycle is mediated predominantly by virally encoded DNA polymerase and accessory proteins.

Latency-Associated Gene Expression and Function

Immediately upon infection of a resting B cell, the EBNA promoter Wp is activated by cellular transcription factors, resulting initially in the expression of EBNA-LP and

EBNA-2, the ORFs for which are the first encountered within the multicistronic EBNA transcription unit. Shortly afterwards, there is an EBNA-2-mediated switch in transcription to the common EBNA promoter Cp, approximately 3 kbp upstream of Wp (**Figure 1**). In reporter gene assays, Cp can be regulated, either positively or negatively, by virtually all members of the EBNA family (see below). Thus, the switch to Cp-mediated EBNA expression is thought to afford the virus a means by which to finely regulate EBNA levels and ultimately to regulate LMP-1, LMP-2A, and LMP-2B, whose promoters are also controlled by members of the EBNA family. By approximately 24 h after infection, expression of the full complement of EBV latency-associated genes is evident, including the noncoding RNAs *EBER-1* and *EBER-2* (it is currently unclear at what point the *BARTs* and the newly discovered miRNAs are expressed). In response to expression of the latency-associated genes, the B cell is driven into the cell cycle, and thereafter is able to proliferate indefinitely as an LCL. *In vivo*, this expression of the full repertoire of latency-associated genes, known as the growth or Latency III program, serves to rapidly establish a pool of infected B cells. However, as discussed later, there is a reprogramming of EBV gene expression *in vivo* that substantially restricts viral gene expression to enable infected cells to persist in the face of the cellular immune response to EBV proteins.

EBNA-1

EBNA-1 is the only member of the EBNA family that binds to DNA in a sequence-specific manner, a property critical to its functional interaction with *oriP*. EBNA-1 also has a strong but nonspecific binding affinity for RNA, though the reason for this is unclear. As the genome maintenance protein, EBNA-1 is essential for the establishment and maintenance of latent infection, and thus the pathogenic potential of EBV. This is underscored by the fact that EBNA-1 is the only viral protein known to be expressed in all EBV-associated tumors. EBNA-1 also contributes to regulation of its own expression and that of other EBNA proteins. It does this by activating transcription from the common EBNA promoter Cp (via binding to the FR element of *oriP*) and negatively regulating its own expression from an EBNA-1 exclusive promoter, Qp (see below), which is approximately 43 kbp downstream of *oriP* and contains two EBNA-1-binding sites. The mechanisms by which EBNA-1 functions in these regards are undefined, and although it is possible that it may likewise regulate cellular gene expression, thus far there is a lack of conclusive evidence to support this. Whether EBNA-1 also contributes directly to tumorigenic potential has been examined in mice transgenic for EBNA-1. However, while the results of one group suggest that EBNA-1 can promote B-cell lymphomagenesis in this model, another group found, using independently derived transgenic lines, that EBNA-1 had no demonstrable oncogenic properties. Therefore, to date the principal functions of EBNA-1 appear limited to its roles in genome maintenance and the regulation of EBV gene expression.

EBNA-LP and EBNA-2

After EBNA-1, EBNA-2 has been one of the most extensively studied EBV latency proteins, largely due to the fact that it was the first viral protein shown to be essential for B-cell immortalization by EBV. EBNA-2's function has been studied extensively in EBV-negative B-cell lines, where it increases expression of numerous cellular genes, for example, the B-cell activation marker CD23, CD21 (the B-cell receptor for EBV attachment), the oncogenes *c-fgr* and *c-Myc*, and cyclin D2. This list has further grown with the advent of gene array technology. EBNA-2 is also a crucial transactivator of transcription from the EBV LMP-1, LMP-2A, and LMP-2B promoters, and the EBNA promoter Cp. EBNA-2 does not bind directly to DNA; instead, it is tethered to target promoters via interactions with cellular transcription factors such as CSL (alternatively known as RBP-Jκ or CBF-1), PU.1 and AUF1, allowing increased transcription through recruitment of basal transcription factors by EBNA-2's strong activation domain.

One protein targeting EBNA-2 to DNA is the ubiquitous cellular transcription factor CSL, which normally inhibits transcription by recruiting a co-repressor complex to the promoter. Through its interaction with CSL, EBNA-2 displaces this repressor complex, instead recruiting co-activators. Binding sites for CSL are found in all of the viral promoters responsive to EBNA-2 mentioned above and are key for their regulation by EBNA-2. CSL normally participates in the Notch signaling pathway. Upon ligand-mediated activation of Notch, its intracellular domain is released by proteolysis, enters the nucleus, and binds to CSL, displacing the co-repressor complex and recruiting transcriptional coactivators. Thus, EBNA-2 essentially acts as a constitutively activated Notch protein, though EBNA-2 and Notch likely possess unique functions as suggested by the observation that activated Notch can only partially rescue mutant EBV lacking an EBNA-2 gene in a B-cell immortalization assay. Although the full complement of genes targeted by CSL is unknown, Notch and EBNA-2 do appear to activate expression of many of the same EBV and cellular genes. An additional function of EBNA-2 and Notch is the ability to protect against apoptosis mediated by the nuclear hormone receptor family member Nur77 through a direct association. The fact that Notch is activated in several cancers is consistent with the belief that EBNA-2's interaction with CSL is an essential component of EBV-mediated immortalization.

A second cellular factor targeting EBNA-2 to DNA, specifically to the LMP-1/2B bidirectional promoter (**Figure 1**), is PU.1, and also the highly related protein Spi-B. Both are members of the Ets family of transcription factors, which recognize the same DNA sequence element. Whereas expression of PU.1 is restricted to and plays an important role in the development of B lymphocytes and myeloid cells, less is known about Spi-B, whose expression is limited to B and T lymphocytes. The Ets family of transcription factors, which are clearly important for B-cell development and differentiation, regulate a wide variety of cellular genes. The expression of at least some of these genes, therefore, is likely to be regulated by EBNA-2 and important in EBV-mediated control of B-cell growth and differentiation.

The activity of EBNA-2 is regulated by a number of the other EBNA proteins. Transcription mediated through CSL is repressed by the EBNA-3 proteins, which, by also binding to CSL, prevent its association with EBNA-2 and DNA, thus inhibiting activation of transcription. Relatively little is known of the function(s) of EBNA-LP other than its role as a coactivator of EBNA-2-mediated transcription. Although EBNA-LP is critical for the efficient immortalization of B lymphocytes by EBV, it is not absolutely essential. In primary B lymphocytes stimulated with the EBV gp350, expression of both EBNA-2 and EBNA-LP results in increased expression of cyclin D2. EBNA-LP also appears to specifically coactivate the viral LMP-1/2B bidirectional promoter and Cp, rather than all EBNA-2 target promoters. EBNA-LP augments transcription in a manner requiring only the activation domain of EBNA-2, suggesting that EBNA-LP may act as an adaptor between EBNA-2's activation domain and the basal transcription machinery, though only a weak direct interaction is observed between full-length EBNA-2 and EBNA-LP. Although EBNA-LP has been reported to associate with several cellular proteins, its association with Sp100, which displaces both Sp100 and heterochromatin protein 1 from PML nuclear bodies, is likely to play the most direct role in coactivation along with heat shock protein 72, also an EBNA-LP partner.

The EBNA-3 family

The EBNA-3 family (EBNA-3A, EBNA-3B, and EBNA-3C) apparently arose by gene duplication of a single progenitor, though little notable sequence homology now exists between the proteins. EBNA-3A and EBNA-3C are required for B-cell immortalization by EBV, whereas EBNA-3B is dispensable. One common function of the EBNA-3 proteins is their ability to bind CSL through a conserved domain in their respective amino termini, lending further support to the belief that these proteins are related. As mentioned in the previous section, interaction of CSL with the EBNA-3 proteins inhibits EBNA-2/CSL-mediated transcription in reporter gene assays, though this may not occur in EBV-infected cells, in which the EBNA-3 proteins are expressed at much lower levels. The fact that all three proteins bind to CSL suggests that this interaction is important, and studies with recombinant virus lacking EBNA-3 CSL-interacting domains have demonstrated that at least association of EBNA-3A with CSL plays an essential role in immortalization of B lymphocytes. Both EBNA-3A and EBNA-3C possess transactivation domains within their C termini and bind to a variety of cellular transcription factors, indicating that they likely function themselves as transcriptional activators. Indeed, EBNA-3C can maintain the levels of LMP-1 that otherwise fall in the G1 phase of the cell cycle, and reporter gene assays have demonstrated that this effect is mediated through the PU.1 site in the LMP-1 promoter. EBNA-3C can bind to PU.1 and Spi-B proteins *in vitro*, and results of chromatin immunoprecipitation assays have revealed that EBNA-3C is indeed present on the LMP-1/2B bidirectional promoter. The EBNA-3 proteins are therefore likely to influence gene expression in both CSL-dependent and -independent mechanisms.

Co-transformation assays employing rat embryo fibroblasts have demonstrated that both EBNA-3A and EBNA-3C can immortalize these cells in collaboration with an activated *ras* gene. This function is dependent on a site that mediates interaction with the cellular transcriptional repressor CtBP. The EBNA-3 proteins are implicated in preventing cell-cycle arrest that normally occurs in response to genotoxic agents, presumably by inactivating cell-cycle checkpoint controls. Thus, the EBNA-3 proteins are also likely to contribute to EBV-induced cell growth and proliferation during the Latency III program in a manner independent of their ability to regulate viral and cellular gene expression.

LMP-1

LMP-1 is unique among the EBV latency-associated proteins in that it alone induces the classic phenotypic changes associated with transformation when expressed in rodent fibroblasts. Expression of LMP-1 is essential to maintain proliferation of EBV-infected LCLs, and transgenic mice that express LMP-1 in their B cells develop lymphomas. However, LMP-1 alone is not sufficient to sustain B-cell proliferation, underscoring the importance of the contributions of the other EBV proteins expressed during the Latency III program.

LMP-1 is comprised of a small cytoplasm-orientated domain at its N-terminus, followed by six transmembrane domains and a larger cytoplasmic domain at the C-terminus. Two regions of the C-terminal domain, transformation effector sites (TES)-1 and -2, are essential for efficient B-cell immortalization by EBV. Through these domains, LMP-1 associates directly with intracellular signaling proteins of the tumor necrosis factor receptor

(TNFR) family. LMP-1 aggregates within lipid rafts of the plasma membrane, resulting in ligand-independent, constitutive activation of these signaling proteins, a critical outcome of which is activation of the transcription factor NFκB. JNK, p38/MAPK, and STAT proteins are also activated by LMP-1, though activation of STAT has been shown not to be important for LMP-1's effects in B lymphocytes. Through these pathways, LMP-1 affects the expression of cellular genes such as adhesion molecules associated with antigen activation of B lymphocytes, anti-apoptotic proteins such as Bcl-2, Bfl-1, and A20, as well as various cytokines. Additional genes activated in the presence of LMP-1 continue to be identified. The membrane proximal TES-1 site interacts with TNFR-associated factors or TRAFs, mediating a low-level activation of NFκB that drives the initial proliferation of B lymphocytes following EBV infection. TES-2, located at the very C-terminus of LMP-1, mediates the majority of NFκB activation through binding to the TNFR-associated death domain protein TRADD, and is required for the continued growth of EBV-infected B lymphocytes in culture. The TNFR family comprises members associated with the promotion of cell growth (CD40) as well as cell death (FAS receptor), and LMP-1 mimics many of the functions of CD40, partially rescuing the defects in CD40-null mice. Thus, LMP-1 plays a direct role in the immortalization associated with EBV infection.

Within epithelial cells, LMP-1 is able to activate expression of the epidermal growth factor receptor through TRAF signaling, resulting in inhibition of terminal differentiation. This pathway is likely to mediate the development of epithelial hyperplasia observed in LMP-1 transgenic mice, and suggests a significant role for LMP-1 in the more restricted Latency II program maintained in nasopharyngeal carcinoma (NPC).

LMP-2A and LMP-2B

LMP-2A and LMP-2B are integral plasma membrane proteins comprised almost entirely of 12 transmembrane domains, with both their N and C termini oriented toward the cell cytoplasm. The unique 5′ exon of LMP-2A encodes a 119-amino acid residue N-terminal domain not present in LMP-2B, which contains only the transmembrane domains and the common 27-residue C-terminal tail. It is through its N-terminal domain that LMP-2A, aggregated in lipid rafts within the plasma membrane, recruits the protein tyrosine kinase Lyn and subsequently Syk to activate a pro-survival signaling cascade that includes a number of signaling proteins in the B-cell receptor (BCR) pathway, for example, Akt/protein B kinase, phosphatidylinositol-3 kinase (PI3K), phospholipase-Cγ2 (PLCγ2), and mitogen-activated protein kinase (MAPK). Consequently, LMP-2A mimics a constitutively active BCR that provides a submitogenic survival signal important for long-term persistence of latently infected B cells. The level of signaling by LMP-2A appears to be tightly regulated through its association with Nedd4 ubiquitin ligases, which control levels of LMP-2A and its associated proteins through ubiquitin-mediated protein degradation. Also, LMP-2B, which is unable to interact with Lyn and Syk, is believed to regulate LMP-2A negatively by interfering with juxtapositioning of the N-terminal domains of LMP-2A (required for trans-phosphorylation of tyrosine motifs) within lipid rafts. LMP-2A also limits the reactivation of EBV within LCLs that can be induced by crosslinking of surface immunoglobulin, that is, the BCR. Inhibition of BCR signaling appears to occur as a result of LMP-2A-dependent reduction of Lyn available for BCR signaling. However, it is unclear whether this is a significant means of inhibiting EBV reactivation *in vivo*. LMP-2A is not essential for immortalization of B lymphocytes *in vitro*, probably because its pro-survival functions are superseded by those of LMP-1.

With the exception of the Latency I program in BL, LMP-2A is probably expressed in all latent EBV infections. Further, LMP-2A expression in NPC and EBV-positive cases of Hodgkin lymphoma implies a role for it in these human cancers. Indeed, despite being nonessential for B-cell immortalization, LMP-2A is tumorigenic when expressed in immortalized epithelial cell lines, and the pro-survival function of LMP-2A is believed to rescue the Reed–Sternberg cells of Hodgkin lymphoma – derived from germinal center (GC) B cells – from apoptosis. Transformation of epithelial cells by LMP-2A is thought to occur through stabilization of β-catenin via activation of the PI3K/Akt pathway. Inhibition of TGF-β-induced apoptosis by LMP-2A may further contribute to its tumorigenic potential within epithelial cells. LMP-2A is also able to promote migration of epithelial cells *in vitro* through upregulation of integrin-α-6 expression, suggesting that it plays an ancillary role in tumorigenesis as well by contributing to metastasis.

BARTs

The *BARTs* are expressed in all forms of EBV latency *in vitro*, and are detectable by PCR in the peripheral blood of healthy EBV carriers. While they are not essential to immortalization of B cells by EBV *in vitro*, their universal expression suggests that they play a critical role(s) in EBV biology. These are highly spliced transcripts that contain poly(A) tails on their common 3′ termini, and several putative ORFs have been identified within various members of the *BART* family, suggesting that they indeed function as mRNAs (**Figure 2**). Though proteins encoded by these ORFs can be expressed from eukaryotic expression vectors in cells following transfection, definitive identification of virally encoded proteins within infected cells has yet to be reported. There are four *BART* ORFs for which there is at least indirect evidence that they encode proteins: BARF0, RK-BARF0 (slightly longer than BARF0 due to an RNA

splicing event), RPMS1, and A73 (**Figure 2**). While serum from NPC patients does not recognize endogenous protein by immunoblotting, it will detect bacterially produced BARF0 polypeptide. Three *BART*-encoded polypeptides have been shown in yeast two-hybrid screens to interact with cellular proteins. RPMS1 and RK-BARF0 interact with components of the Notch signaling pathway: RPMS1 with CSL and its associated co-repressor CIR, and RK-BARF0 with Notch4. A73 interacts with the cytoplasmic receptor for activated protein kinase 1 (RACK1), though the functional significance of this is unclear. The interaction of RPMS1 with CSL and CIR is believed to repress promoters with CSL-binding sites (e.g., the LMP-1 promoter) by stabilizing the CSL–CIR repressor complex on the DNA. Interestingly, though different from the mechanism of EBNA-3 repression of CSL-regulated transcription, the outcome is similar. Interaction of RK-BARF0 with Notch4 results in translocation of the unprocessed form of Notch4 directly to the nucleus, which is believed to then activate expression of LMP-1. Thus, one set of functions for RPMS1 and RK-BARF0 may be analogous to those of the EBNA-3 and EBNA-2 proteins, respectively, in the regulation of LMP-1, but during the Latency II program in which these EBNA proteins are absent.

EBERs

EBER-1 and *EBER-2* are small, highly structured noncoding RNAs whose genes lie adjacent to one another immediately upstream of *oriP* (**Figure 1**) and are transcribed by cellular RNA polymerase III (Pol III). They are expressed in all forms of EBV latency and are restricted to the cell nucleus, where they are present at approximately 10^7 copies per cell (levels of *EBER-1* are approximately tenfold higher than *EBER-2*). While *EBER* expression is not essential for immortalization of B lymphocytes by EBV *in vitro*, like EBNA-LP the *EBERs* enhance the efficiency of this process. The most interesting property of the *EBERs* is their ability to enhance the tumorigenic potential of some EBV-negative BL cell lines, suggesting that they may play a role in tumor maintenance. How the *EBERs* promote tumorigenic potential is unresolved. There are numerous reports that the *EBERs* can interact with and influence the actions of proteins within the type 1 (antiviral) interferon response pathway that bind to dsRNA, namely the dsRNA-activated protein kinase PKR and RIG-I. However, as the *EBERs* are present in ribonucleoprotein complexes and unlikely to be available for interaction with these predominately cytoplasmic proteins, these interpretations of *EBER* effects *in vitro* or under experimental conditions that take the *EBERs* out of their normal intracellular context must be viewed with caution.

The *EBERs* are present in RNA–protein complexes that include La/SS-B (*EBER-1* and *EBER-2*) and the ribosomal protein L22 (predominately *EBER-1*). La is critical for the synthesis and protection from exonucleolytic digestion of small cellular transcripts generated by Pol III (e.g., tRNAs) that have a UUU-OH moiety at their 3′ termini, which is bound by La. La also retains some Pol III transcripts within the nucleus via this interaction, as it probably does the *EBERs*. As Pol III transcripts themselves, La binding to the *EBERs* probably represents a normal physiological interaction. *EBER* interaction with L22 results in retention of approximately 50% of the cellular pool of L22 within the nucleoplasm. The functional consequence of this is unknown, but presumably it influences L22 function. In bacterial ribosomes, L22 lies within the exit tunnel and regulates the passage of nascent polypeptide by interaction with a motif within the secretion monitor protein, secM. L22 may perform an analogous function in mammalian cells that is affected by *EBER-1*, though it is possible that *EBER-1* interaction with L22 influences a nonribosomal function of L22 (as yet undescribed), or imparts upon it a gain of function.

miRNAs

Two clusters of EBV miRNAs have recently been identified that are derived from two loci within the viral genome: the BHRF1 locus encoding for a pro-survival Bcl-2 homolog, and a portion of the *BART* locus. Analysis of miRNA expression in various EBV-infected cells has revealed that the *BART* miRNAs are highly expressed in latently infected epithelial cells (Latency II program), but at much lower levels in B-cell lines. By contrast, the BHRF1 miRNAs are present in high levels within B cells maintaining the Latency III program, but are very low or undetectable during the restricted latency programs in B (Latency I) and epithelial (Latency II) cell lines. Three of the *BART* and one of the BHRF1 miRNAs are induced to higher levels upon induction of the lytic cycle. The identity of the genes post-transcriptionally silenced by these miRNAs (both viral and cellular) and the contribution of virus-mediated RNA interference to EBV biology and pathogenic potential are currently unclear, but are currently the focus of an intense research effort.

Infection in the Host

For two decades after the discovery of the immortalizing effect of EBV on B lymphocytes *in vitro*, latency within LCLs was widely accepted as the model of EBV persistence within the host (although the extent of EBV gene expression in LCLs was not appreciated at the time). Increasing evidence indicated, however, that such cells are highly susceptible to killing by $CD8^+$ cytotoxic T lymphocytes (CTLs), and subsequently that this cellular immune response is directed at epitopes derived from the latency-associated proteins of EBV, particularly members of the EBNA-3 family. Thus, it was unlikely that EBV could persist within an immunocompetent host solely in

cells with the characteristics of an LCL. The subsequent observation that some BL cell lines are resistant to CTL killing, particularly at low passage in culture, was largely explained by the soon-to-follow discovery that expression of known latency-associated proteins in these cells was restricted to EBNA-1. EBNA-1, moreover, is not a good inducer of the CTL response. This is due in part to poor presentation of EBNA-1 peptides with HLA class I antigen on the infected cell surface as a consequence of self-inhibition of proteolytic processing of EBNA-1 by its substantial Gly-Ala repeat domain. Thus, B cells that limit EBV expression to EBNA-1 are not particularly vulnerable to the host's immune surveillance.

Restricted patterns of EBV gene expression were later described for other EBV-associated tumors, for example, NPC and Hodgkin lymphoma, though cells in these tumors express the LMP proteins in addition to EBNA-1. This led to the classification scheme of latency-associated gene expression patterns or programs: Latency I exemplified by BL cells (EBNA-1 only); Latency II, in which the LMP proteins are expressed in addition to EBNA-1; and Latency III or the growth program, in which expression of the full complement of latency-associated genes is supported. The *EBERs* and *BARTs* are also expressed in each of these programs, whereas the miRNAs are differentially expressed, as discussed above. Importantly, the realization that tumor cells can support alternative programs of latency-gene expression provided the basis for studies that have led to the current understanding of EBV persistence within the host. With the aid of reverse transcription-PCR and cell-isolation procedures, it has been possible to evaluate EBV gene expression in phenotypic subsets of B cells, and even approaching the single-cell level. Analyses of EBV gene expression in B cells from peripheral blood and tonsils have provided a reasonably good, though certainly not complete, picture of the pathway(s) taken by EBV to establish itself permanently within the B-cell pool of its host.

Establishment of Persistent Infection

The major cellular reservoir of EBV within the peripheral blood are resting memory B cells ($IgD^-/CD20^+$) that express the LMP-2 proteins, and probably the *EBERs* and *BARTs* (referred to as the Latency program). Periodic activation of cell division results in EBNA-1 expression via Qp (suppressed in resting cells by the retinoblastoma protein) to prevent loss of the viral genome, thereby maintaining EBV within this reservoir. The virus is believed to enter the memory cell pool not directly, but by either driving or participating in a GC reaction, the normally antigen-driven process that gives rise to the long-lived memory B cells and the plasma cells that produce high-avidity antibody. This is supported by the presence of EBV within BL cells, which are derived from GC B cells and are thought to acquire the virus early in the oncogenic process, quite possibly during the primary establishment of EBV latency. The pathway by which EBV is believed to gain entry into the memory B-cell pool is illustrated in **Figure 3**, support for which has largely come from PCR-based analyses of EBV gene expression in tonsil B cells following surgical resection associated with either acute infectious mononucleosis or tonsillitis of unknown etiology. The basic premise of this model is that EBV infects a naïve resting B cell (IgD^+), driving rapid proliferation and expansion of infected cells, much as it does *in vitro* through its Latency III program. Gradually, however, there is an epigenetic down-regulation of EBV gene expression (as in BL cells) that both enables infected B cells to evade the developing CTL response and is compatible with long-term persistence in a resting B cell.

Conclusive evidence that EBV enters the memory cell pool by participating in a GC reaction, however, has been elusive. EBV-positive B cells appear infrequently within the GCs themselves, and those that do are not undergoing somatic hypermutation of *Ig V* genes, arguing against EBV driving or participating in a classic GC reaction. These cells harbor somatically mutated *V* gene rearrangements, suggesting that EBV may gain entry into GC B cells by direct infection, but that this pool of infected cells expands without further hypermutation. However, it is unlikely that EBV directly inhibits somatic hypermutation, as BL cell lines can continue to undergo this process *in vitro* despite infection with EBV or, in EBV-negative BL cells, stable expression of the individual latency genes. An important caveat to these observations is that, as a consequence of the pathology that prompted tonsillectomy, EBV infection in these B cells may not represent entirely normal events in the establishment of long-term latent infection.

The Role of Virus Replication

It is unclear whether establishment of a persistent latent infection in B cells requires an initial round of productive infection in epithelial cells within the oropharynx, or results instead from direct infection of B cells, most likely within Waldeyer's ring of the oropharyngeal lymphoid tissue. Extended treatment of EBV-positive individuals with acyclovir, an inhibitor of herpesvirus replication, does not reduce the level of latently infected cells in the peripheral blood, suggesting that intermittent reactivation of EBV is not essential for persistence. By contrast, reactivation of EBV from latency is necessary to pass the virus onto naïve hosts to maintain the virus in the human population. This is believed to occur sporadically from latently infected memory B cells that begin to replicate virus in response to their terminal differentiation into plasma cells. Virus produced from B cells has a slightly higher tropism for epithelial cells, whereas virus produced in epithelial cells is considerably more infectious for B cells. This is due to the relative

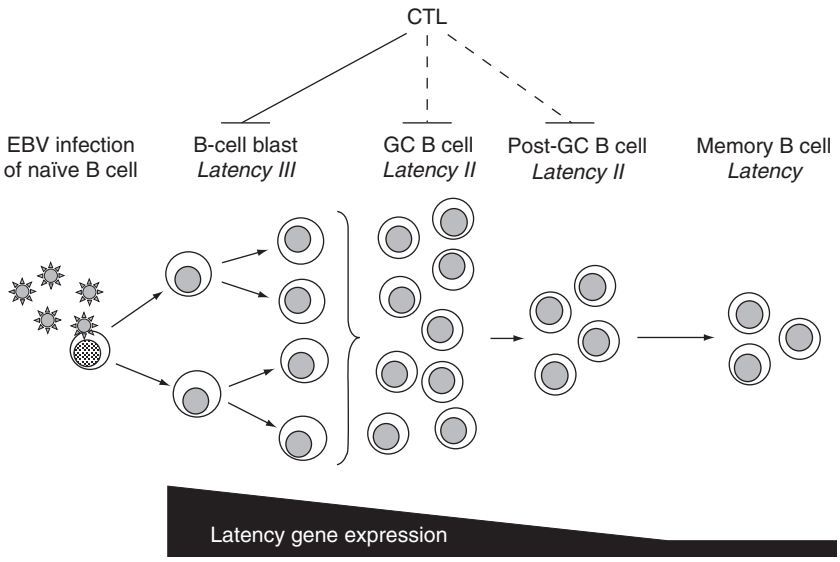


Figure 3 Establishment of persistent EBV latent infection. Shown is the currently favored model for the establishment of latency within memory B cells, the major long-term reservoir of EBV. As latently infected B cells progress from an initial stage of EBV-driven cell proliferation through a germinal center (GC) reaction, ultimately entering the memory B-cell pool, there is a restriction of latency-associated EBV genome expression. This occurs primarily through epigenetic silencing of the common EBNA promoters, Cp and Wp. The different latency-gene expression programs at the various stages are indicated in italics. The Latency program within memory B cells is similar to Latency I in BL cells, except for the expression of LMP-2A, and EBNA-1 expression is repressed; periodic cell division results in reactivation of EBNA-1 expression from the cell-cycle-regulated promoter Qp, as in Latency I. Solid to dashed lines indicate reduced potential for killing by cytotoxic T lymphocytes (CTLs) directed at epitopes derived from EBV proteins expressed in the Latency III and II programs.

amount of two EBV glycoprotein complexes on the virion envelope: gH/gL and gH/gL/gp42. B cells are preferentially infected by virions rich in gH/gL/gp42, whereas the presence of gp42 (prevalent on virus produced in epithelial cells) blocks interaction of gH/gL with its putative receptor on epithelial cells, which do not express the B-cell receptor for virus attachment, CD21, that is bound by gp350. B-cell-derived virus has lower levels of gp42 due to its interaction with HLA class II antigen (lacking in epithelial cells), which targets it for degradation. Recent evidence indicates that EBV present in saliva is programmed preferentially to infect B cells (high in gp42), suggesting that virus reactivated from B cells goes through an amplification step in tonsillar epithelium before it is secreted to be passed onto naïve hosts.

Activation of EBV replication is initiated by expression of the viral immediate early protein Zta – a protein related to the cellular AP-1 family of transcription factors – that binds to specific DNA motifs in a number of EBV early-gene promoters, initiating the cascade of lytic-cycle gene expression. This is done in collaboration with a second EBV transactivator, Rta, itself activated by Zta. Zta and Rta also participate in replication of EBV DNA during the lytic cycle through interaction with their response elements within *oriLyt* domains. An interesting property of Zta is its preference for binding DNA that has been methylated. Because the EBV genome is highly methylated within latently infected B cells *in vivo*, the propensity of Zta for methylated DNA gives further credence to the model in which new virus originates from reactivation within the memory B-cell reservoir of EBV.

To aid replication of virus in the host, EBV encodes a number of proteins that, while not directly involved in virion production, influence the intra- or extracellular milieu to enhance virus production. For example, the EBV BHRF1 ORF encodes a homolog of the cellular anti-apoptotic protein Bcl-2, and presumably enhances virus production by countering induction of programmed cell death in response to infection. Similarly, Zta and Rta, in addition to their direct contributions to EBV gene expression and DNA replication, interact with or regulate the expression of a number of cellular proteins, examples of which are p53, NFκB, pRB, p21, TGF-β and the retinoic acid receptor. These properties of Zta and Rta likely provide the optimal cellular environment for virus replication. EBV also expresses several proteins during the lytic cycle that are capable of modulating the host immune response. These include homologs of interleukin 10 (vIL-10), a negative regulator of the immune response, and a secreted form of the receptor for colony stimulating factor 1 (CSF-1) able to bind and neutralize CSF-1. Further, gp42, in addition to aiding in infection of

B cells, is able to inhibit HLA class II-mediated antigen presentation, and Zta is able to inhibit type 2 interferon signaling by downregulating expression of the γ interferon receptor.

See also: Epstein–Barr Virus: General Features; Herpesviruses: Discovery; Herpesviruses: General Features; Herpesviruses: Latency; Simian Gammaherpesviruses; Tumor Viruses: Human.

Further Reading

Cai X, Schäfer A, Lu S, *et al.* (2006) Epstein–Barr virus microRNAs are evolutionarily conserved and differentially expressed. *PLoS Pathogens* 2: 236–246.

Farrell PJ (2005) Epstein–Barr virus genome. In: Robertson ES (ed.) *Epstein–Barr Virus*, pp. 263–288. Norfolk, England: Caister Academic Press.

Kieff E and Rickinson AB (2006) Epstein–Barr virus and its replication. In: Fields BN, Knipe DM, Howley PM, *et al.* (eds.) *Fields Virology,* 5th edn., vol. 2, pp. 2603–2654. Philadelphia: Lippincott Williams and Wilkins.

Rickinson AB and Kieff E (2006) Epstein–Barr virus. In: Fields BN, Knipe DM, Howley PM, *et al.* (eds.) *Fields Virology,* 5th edn., vol. 2, pp. 2655–2700. Philadelphia: Lippincott Williams and Wilkins.

Robertson ES (ed.) (2005) *Epstein–Barr Virus.* Norfolk, England: Caister Academic Press.

Tselis A and Jenson HB (eds.) (2006) *Epstein–Barr Virus.* New York, NY: Taylor and Francis Group, LLC.

Equine Infectious Anemia Virus

J K Craigo and R C Montelaro, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Equine infectious anemia (EIA), colloquially known as swamp fever, has been documented in numerous diverse geographical areas and is currently considered a worldwide disease that occurs only in members of the family Equidae. EIA was first identified as an infectious disease of horses by veterinarians in France in 1843. In 1904, the infectious organism that caused EIA was identified as a 'filterable agent', making EIA one of the first animal diseases to be assigned a viral etiology.

Despite this early identification of the equine infectious anemia virus (EIAV), the characterization of this virus was extremely slow because of the difficulties experienced in the isolation and propagation of the virus in cell culture. Thus, the major focus on the control of EIA has been the development of regulatory policies that involve the identification and elimination of EIAV-infected horses. More recently, advances in animal vaccine strategies and the demand for animal models for AIDS vaccine development have provided renewed impetus to the development of an EIAV vaccine to prevent virus infection. EIAV also offers an important model for the role of antigenic variation in a persistent retrovirus infection.

Classification

EIAV is classified as a member of the genus *Lentivirus* based on criteria of virion morphology, serological properties, and genomic sequence homologies. There has been no formal further subdivision of EIAV isolates into subtypes.

Properties of the Virion

The EIAV particle has the general morphology of a lentivirus, including an oblong core enclosed in a viral envelope with surface projections (**Figure 1**). The oblong core observed in EIAV is characteristic of lentiviruses, in contrast to the icosahedral cores found in most oncoviruses. This distinctive structural feature was the initial indication that HIV-1 was related to EIAV and a member of the lentivirus rather than the oncovirus subfamily of retroviruses. The virus particles appear roughly spherical in the electron microscope (**Figure 2**), although there are various degrees of polymorphism depending on the sample preparation. The overall diameter of the virion is approximately 100 nm. The surface projections extend about 7 nm and appear to be distributed on the viral surface in a symmetrical pattern.

Properties of the Genome

The EIAV genome consists of a dimer of single-stranded, positive-sense RNA. The genomic organization of EIAV is characteristic of a complex retrovirus, but is the simplest and smallest of characterized human and animal

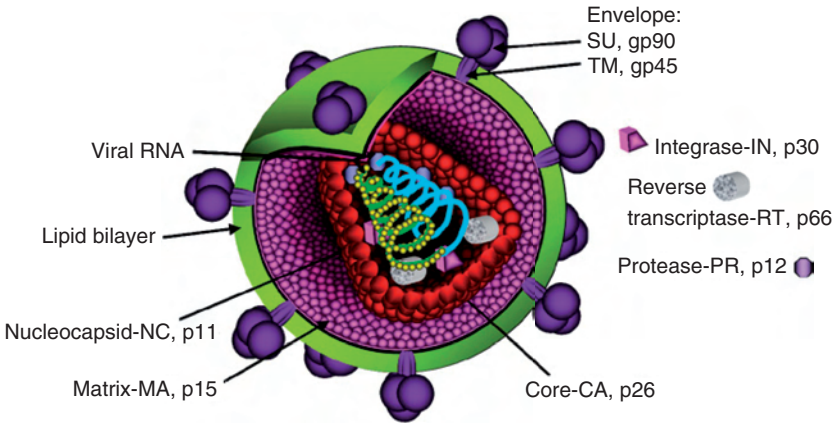


Figure 1 Virion morphology and protein organization of EIAV.

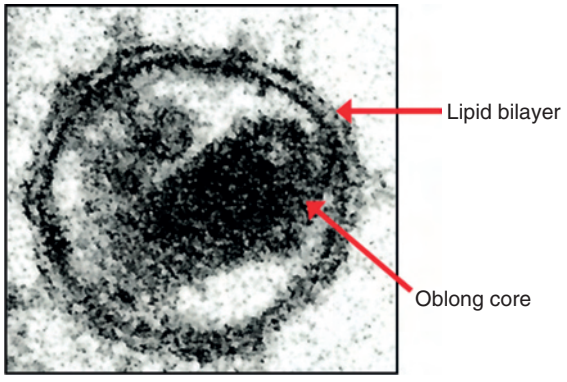


Figure 2 Transmission electron micrograph of an EIAV particle.

lentiviruses (**Figure 3**). The viral RNA contains about 8200 bp and contains three major genes (*gag, pol,* and *env*) encoding viral structural proteins and three minor genes (*tat, S2,* and *rev*) that encode nonstructural proteins that regulate various aspects of virus replication. The order of the EIAV genome is 5′-R-U5-*gag-pol-env*-U3-R-3′. The *tat* and *S2* genes are encoded as distinct alternate reading frames within the *pol–env* intergenic region, while the *rev* gene is encoded by alternate reading frames contained at the beginning of the *env* gene sequences and following the 3′ end of the *env* gene. The relatively small size of the EIAV genome compared to other lentiviruses is primarily due to the much smaller size of the *pol–env* intergenic region.

The EIAV *gag* gene encodes the four viral core proteins in the order of 5′-p15-p26-p11-p9–3′, while the *env* gene encodes the two envelope glycoproteins of the virus in the order of 5′-gp90-gp45–3′. The *pol* gene encodes a complex of enzymes with an organization of 5′-protease-reverse transcriptase-RNase H-dUTPase-integrase-3′. As with other lentiviruses, the EIAV *tat* and *rev* genes encode important regulatory proteins that either transactivate virus transcription (Tat) or control viral transcription patterns (Rev) after infection of host cells. The function of the 8 kDa protein encoded by the *S2* gene remains to be defined, although mutation studies of this gene indicate that it is not essential for *in vitro* viral replication and results in a single $\log_{10}$ reduction in viral replication in equines. The terminal LTR sequences of EIAV contain the usual complex of transcriptional regulatory domains distinctive of lentiviruses.

Properties of the Viral Proteins

The proteins encoded by the *gag* and *env* genes of EIAV constitute the major structural proteins of the virus (cf. **Figure 1**). The gp90 protein is a highly glycosylated, hydrophilic surface (SU) protein that forms the outermost knobs of the envelope projections, while the gp45 is a sparsely glycosylated, hydrophobic transmembrane (TM) protein that forms the membrane-spanning spike of the envelope projection. There are approximately 300 copies each of the envelope glycoproteins per virion, and the surface projections are composed of trimers of gp90 and gp45. The final component of the EIAV envelope structure is the fatty acylated p15 that forms a continuous matrix (MA) immediately beneath the lipid bilayer of the virus particle. The virion core shell or capsid (CA), composed predominantly of p26 molecules, encloses a helical ribonucleoprotein complex containing the basic nucleoprotein (NP), p11, and various polymerases (RT, IN, and DU) in close association with the viral RNA genome. The location of the final core protein, p9, is not certain, but it has been proposed as a linker protein between the core shell and envelope matrix. Recent experiments have indicated that the EIAV p9 protein mediates late stages of viral budding.

The *gag*-encoded proteins of the virus are present in molar amounts that are at least tenfold greater than the envelope glycoproteins, in the range of 3000–5000 copies

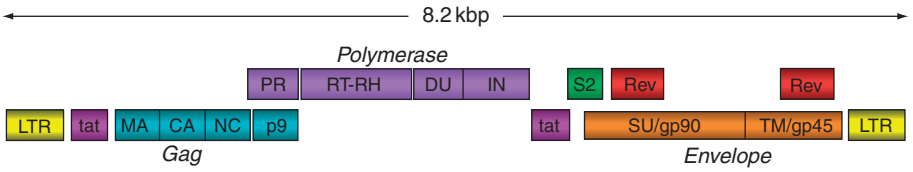


Figure 3 Organization of the EIAV genome indicating viral genes (italic print) and the respective proteins encoded by these genes (block print).

per virus particle. In contrast, the *pol*-encoded enzymes appear to be present in the virus at levels of about 10 molecules per virion.

Replication Strategy

The EIAV replication cycle is characteristic of retroviruses in general and lentiviruses in particular (summarized in **Figure 4**). Viral recognition, attachment, and penetration of target cells is believed to be mediated by specific interaction of the viral envelope glycoproteins and cellular receptor proteins contained in the plasma membrane. Until recently, there were no recognized receptors for EIAV. Recently, a cellular receptor for EIAV, designated ELR1, has been identified as a member of the tumor necrosis family of receptors (TNFRs). Once inside the target cell cytoplasm, the virus reverse transcriptase copies the single-stranded RNA genome into double-stranded DNA provirus that is then transported to the nucleus of the cell. There is no evidence for translation of the incoming EIAV genome at this stage of infection. Once inside of the cell nucleus, the EIAV integrase mediates apparently random but limited incorporation of the provirus DNA into cellular chromosomes, although some extrachromosomal viral DNA is always present in productively infected cells. There is typically a total of about 10 copies of proviral DNA per infected cell. Cytopathic infections by EIAV have been correlated with higher ratios of integrated to unintegrated proviral DNA.

Transcription of the proviral DNA by cellular polymerases produces a complex pattern of viral messenger RNA species whose relative proportions may differ depending on the virus strain and target cell. In all instances, the predominant EIAV transcripts are an 8.2 kbp transcript representing the full-length genomic RNA and translated to produce the *gag* and *pol* gene products, and a 3.5 kbp mRNA that is a singly spliced transcript translated to produce the viral envelope proteins. Lentivirus-infected cells usually contain in addition to these major viral transcripts, a heterogeneous population of small multiply spliced RNA that are used to produce the various regulatory proteins. In the case of EIAV, however, infected cells reveal only minute quantities of these small, multiply spliced RNA species. The relatively low abundance of small transcripts may in part reflect the relative genetic simplicity of EIAV and the

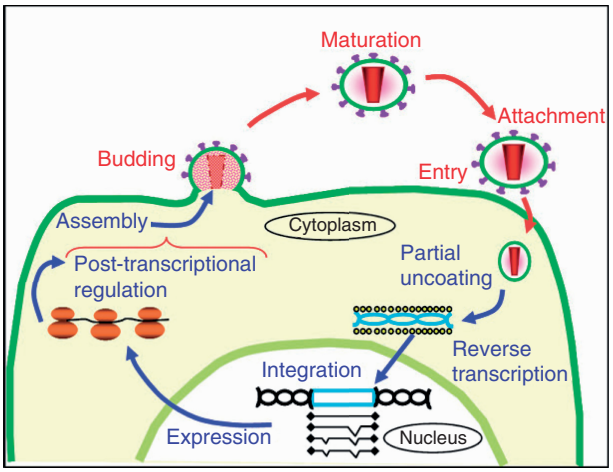


Figure 4 Schematic representation of the EIAV life cycle.

lack of extensive splicing to ensure production of all of the minor viral genes. On the other hand, it is intriguing that the 3.5 kbp transcript is in fact a tricistronic messenger that can produce by *in vitro* translation the viral Tat and Rev proteins in addition to the more abundant envelope glycoproteins. The use of a tricistronic messenger may represent a novel mechanism of maximizing genetic efficiency in EIAV replication.

The viral Gag and Env proteins are initially produced as polyproteins that are cleaved into the mature virion components by a combination of viral and cellular proteases. The only modification documented in the core proteins is a fatty acylation at the N terminus of the matrix protein, p15. The envelope proteins are modified by N linked glycosylation. Although the gp90 and gp45 polypeptides contain about 400 amino acid residues, the gp90 contains an average of 17 potential N linked glycosylation sites, while the gp45 contains only about five potential glycosylation sites. By comparison to HIV-1 gp120, it is assumed that all potential glycosylation sites are occupied by complex oligosaccharides.

The precise mechanisms of EIAV assembly have not been completely dissected, although it is assumed that it follows the general model for retrovirus assembly. It appears to be a highly concerted process that is mediated by interactions between the viral Gag polyproteins and host cofactors. Accordingly, the viral envelope

glycoproteins are initially inserted into the plasma membrane to create distinct sites of virus assembly at which the Gag polyprotein is accumulated beneath the membrane lipid. Subsequently, Gag proteins recruit host cellular components that also regulate the endocytic pathway for assembly and budding. To date, early (AP-2) and late endocytic proteins (TSG101) as well as the actin cytoskeleton have been associated with EIAV virion production. The intermediate viral proteins are next cleaved into the mature virion proteins as the particle buds from the cell surface and is released to produce progeny virions.

Epidemiology, Geographic and Seasonal Distribution

EIAV has been diagnosed in many areas of the world and is considered a worldwide disease of horses. Although localized outbreaks of disease can occur, the incidence of EIAV-infected horses is the highest in tropical and subtropical climates, presumably due to the longer warm seasons and more abundant populations of insect vectors that may transmit EIAV among horses. During the past 20 years, the EIAV infection rate reported by the USDA has dropped from about 4% to less than 0.2%. However, these testing results do not reflect the general horse population as less than 10% of the horses in the United States are tested for EIA, usually because of requirements for transportation across state lines or for participation in organized shows or races. General surveys of unregulated herds in the Southeast United States demonstrate infection rates of up to 15%. EIAV infections are especially prevalent in Central and South America, where limited surveys of Latin American countries have demonstrated infection rates in unregulated herds frequently approach 50% indicating that EIAV infection is epidemic in these areas. Although the probability of EIAV infection by insect vectors is greatest during seasons that are warm, infections can occur throughout the year via mechanical transfers of blood by hypodermic needles and other veterinary instruments. Sexual transmission of EIAV has not been demonstrated to date.

Host-Range and Virus Propagation

EIAV appears to infect only members of the family Equidae. There is no evidence to support the concept of natural or experimental infections of humans or of other mammalian species. EIAV infection of horses results in high levels of virus replication, persistent infection, and clinical disease. EIAV infection of donkeys produces only limited virus replication, presumably a persistent infection, but no signs of clinical disease.

Field strains of EIAV can only be propagated *in vitro* in cultures of equine monocyte or macrophage cells, where virus infection typically produces a cytopathic effect within several days. Large-scale production of EIAV is limited to cell culture-adapted strains of virus that can be grown in primary cultures of equine dermal cells or fetal equine kidney cells and in a limited selection of nonequine continuous cell lines, including canine fetal thymus (Cf2th) cells and the Fea and FEF feline cell lines. The cell-adapted strains of EIAV are noncytopathic to these permissive cell lines. Field strains of EIAV retain their pathogenic properties when propagated in leukocyte cultures, but usually become avirulent when adapted to other types of cell cultures. Cell-adapted strains of EIAV that retain their virulence have been produced by back passage of avirulent cell-adapted strains in ponies or horses. There is no evidence for infection of cultured human cells by EIAV.

EIAV production in cell cultures is most easily detected by the presence of viral antigens or reverse transcriptase activity in culture media.

Genetics

Like other retroviruses, EIAV replication is mediated by a virion reverse transcriptase (RT) that copies the viral RNA genome into proviral DNA that is found in the nucleus of infected cells randomly integrated into the cellular chromosome and as extrachromosomal molecules. There are typically only about 10 copies of EIAV DNA per infected cell. EIAV replication in horses is characterized by relatively rapid and diverse genomic mutations that produce an apparently wide variety of variant virus strains. Analyses of sequential antigenic variants of EIAV from experimentally infected horses suggest that the rate of mutation in the envelope gene of the virus is greater than 10^{-2} base substitutions per site per year. The fidelity of DNA synthesis by purified EIAV RT has been measured *in vitro*, and an average error rate of 1/700 bp has been estimated. This value is similar to the *in vitro* error rate calculated for human immunodeficiency virus (1/700 bp), but is significantly higher than the rate observed for oncovirus RT such as avian myeloblastosis virus (1/3000 bp). The error-prone nature of EIAV RT produces significant biological diversity that is important in EIAV persistence and pathogenesis. As demonstrated for HIV and SIV, recombination between variant EIAV genomes in infected cells may also contribute to genetic diversification during persistent infection.

Evolution

Phylogenetic analyses based on the nucleotide sequences of various retroviruses indicate that EIAV is most closely related to the ungulate lentiviruses (visna-maedi virus,

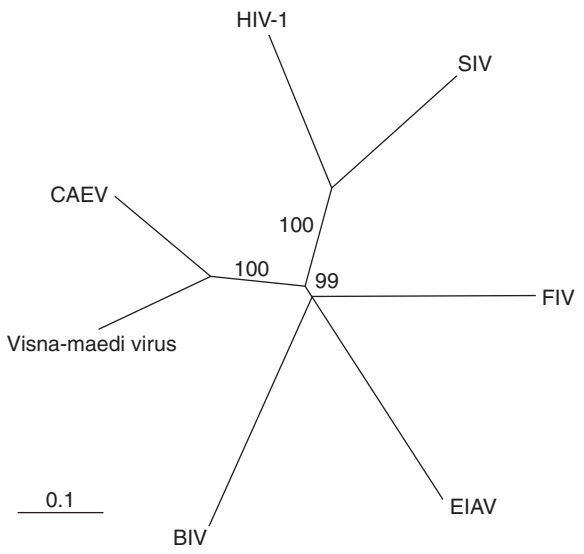


Figure 5 Phylogenetic tree of the complete genomes of identified lentiviruses.

caprine arthritis-encephalitis virus, and bovine immunodeficiency virus) and equally divergent from the human and simian immunodeficiency viruses (**Figure 5**).

Studies of EIAV evolution through analyses of sequence variation during persistent infection in experimentally infected equids have clearly identified dynamic changes in envelope sequences that alter viral antigenic properties, evidently as a result of immune selection. Variation of the envelope gene has therefore served as a distinct marker for analysis of viral population evolution. Detailed molecular characterization of envelope variation during sequential disease cycles in experimentally infected ponies revealed the presence of distinct EIAV envelope variants with each wave of viremia. Examination of inapparent stage viral populations from the plasma of ponies indicated that evolution of the viral quasispecies is continuous, even with relatively low levels of detectable virus replication in the periphery or tissues. These results suggest that even in the absence of detectable plasma virus, viral populations, most likely in tissue reservoirs, continue to replicate and evolve, seeding the plasma with new viral quasispecies.

Serologic Relationships and Variability

Field and laboratory isolates of EIAV display a remarkable variability in antigenic properties. The core proteins of the virus contain conserved antigenic determinants that are the basis of current serological diagnostic assays. The viral glycoproteins also contain a limited number of conserved antigenic determinants, but predominantly present an array of variable antigenic sites that can be distinguished by their reactivity with monoclonal antibodies or by neutralization with polyclonal immune serum from infected horses. The range of variation observed among EIAV isolates has precluded any classification of virus strains on the basis of serological properties.

Immune serum from EIAV-infected horses is reactive with the respective major core protein of most animal and human lentiviruses, but not with any of the major core proteins of oncoviruses. Immune serum taken from other species infected with a lentivirus generally do not reveal a cross-reactivity with EIAV or with other lentiviruses. This one-way serological reactivity suggests that horses infected with EIAV uniquely recognize a conserved lentivirus-specific, antigenic determinant.

Transmission and Tissue Tropism

Blood from persistently infected horses is the most important source of EIAV for transmission; *in utero* transmissions of EIAV from mare to foal are evidently very rare. This blood transfer can be affected by man or blood-feeding vectors. EIAV has been shown to remain infectious on hypodermic needles for up to 96 h, emphasizing the potential for transmission via routine animal husbandry or veterinary medical practices. However, the mechanical transmission of EIAV by arthropods, especially horseflies, is generally accepted as the major natural means of transmission in the field. Transmission of EIAV by a single horsefly carrying only approximately 10 nl of virus-infected blood has been documented under experimental conditions.

The target cell during persistent EIAV infections appears to be exclusively cells of the monocyte/macrophage lineage; there is no evidence for infection of lymphocytes as observed with some other lentiviruses. The virus burden in infected horses is predominantly in tissue macrophage found in liver, kidney, and spleen with much lower levels of virus found in lymph nodes, bone marrow, or in circulating monocytes. Thus, the relatively high levels of viremia (10^{4-6} $TCID_{50}$) observed during episodes of chronic EIA evidently result primarily from the production and release of virus from infected tissue macrophage, rather than an extensive infection of blood monocytes.

Pathogenicity

Field isolates and laboratory strains of EIAV differ markedly in their pathogenicity, ranging from avirulent to lethal strains of the virus. Little is known about the viral determinants or host factors that influence the course of virus replication and pathogenesis. In other lentiviruses, differences in viral pathogenesis have been mapped to specific changes in viral envelope genes or to changes in gene regulatory sequences in the viral genome. It is likely that variation in EIAV pathogenicity will follow a similar pattern.

Clinical Features of Infection

The clinical response of horses following artificial inoculation or natural exposure to EIAV is variable and depends in part on host resistance factors, viral virulence factors, and environmental factors (e.g., weather and work load). In general, EIAV infections can be apparent with distinctive clinical symptoms or inapparent without any clinical signs of EIA. The clinical disease is typically described as acute, chronic, or asymptomatic (**Figure 6**).

Acute EIA is most often associated with the first exposure to the virus, with fever and hemorrhages evident from 7 to 30 days after exposure. Acute disease is thought to be associated with massive virus replication in and destruction of infected macrophages. Horses in the initial phase of acute EIA will be seronegative, because the immune system has had insufficient time to respond to the viral antigens. During the peak of the febrile response in acute EIA, viremia of greater than 10^6 horse-infectious doses per ml of whole blood is often observed. The initial acute phase of EIA infection may not be seen by the veterinarian unless there is an epizootic of the infection in a group of horses. Even then, the horses must be under close supervision before the initial fever and anorexia are detected. Neither anemia nor edema is seen at this stage of disease.

The more classic clinical signs of EIA such as loss of weight, anemia, diarrhea, and edema are seen later during recurring cycles of the illness, which appear at irregular intervals ranging from a couple of weeks to several months. The frequency and severity of clinical episodes in horses with chronic EIA usually decline with time, about 90% occurring within 1 year of infection. Horses with chronic EIA are seropositive and have variable viremia levels which are the highest during the periodic febrile episodes. Although a small percentage of chronic EIA cases may result in death, the predominant clinical course of infection is a cessation of detectable clinical symptoms by the end of the first year post infection and the establishment of a lifelong inapparent carrier stage. However, animals can experience a recrudescence of viremia and disease due to stress or immune suppression from a contemporous bacterial or viral infection.

The highest percentage of EIAV-infected horses in the field are in fact inapparent carriers. These horses have no clinical illness associated with the viral infection, and viremia is usually undetectable. However, these inapparent carriers maintain high levels of EIAV-specific antibodies, suggesting a chronic low level of virus replication. Inapparent carriers can be shown to harbor infectious EIAV by transfusions of whole blood (200 ml) to recipient horses that become seropositive to EIAV within 3–6 weeks post inoculation. In addition, treatment of certain inapparent carriers with immunosuppressive drugs or exposure to extreme stress can cause the recrudescence of chronic EIA, even in some horses that have been free of clinical symptoms for years.

Pathology and Histopathology

Gross and histopathological lesions in EIAV-infected horses are variable and closely associated with levels of viral replication. In the acute stage of EIA, gross pathological lesions consist of swelling of the parenchymatous organs, and hemorrhages can be observed in most tissues.

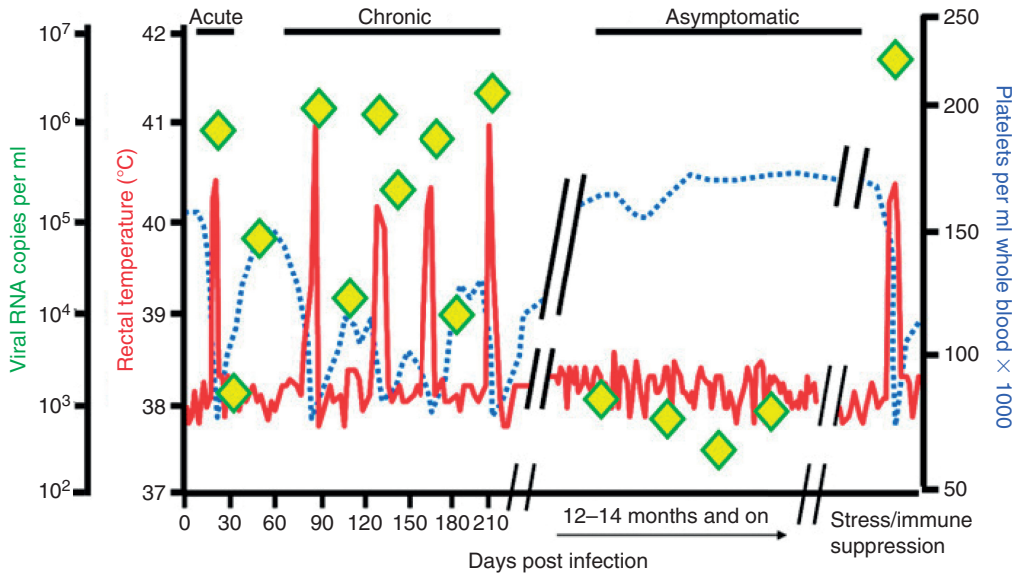


Figure 6 Clinical profile associated with EIAV infection of horses indicating the characteristic stages of EIA. Febrile episodes are defined as rectal temperatures above 39 °C (103 °F), and thrombocytopenia is defined as platelet levels below 105 000 μl^{-1} of blood.

The most pronounced histopathological lesions are hepatic and lymphoid necrosis in association with large numbers of activated macrophage and Kupffer cells. Hepatic necrosis is most severe near the central vein evidently resulting from degenerative changes in the parenchymal cells. Lesions in the spleen are characterized by degenerative erythrocytes, and small focal hemorrhages are found in the splenic capsule and adjacent tissue. Lymphocytic infiltrations can be observed in several organs including liver, spleen, lymph node, kidney, heart, and lung. The majority of these lesions are thought to be the combined effect of immune-mediated lysis of virus-infected cells and an immune complex-mediated inflammatory response.

The pathological changes in the chronic form of EIA include a developing immunological control of virus replication. Gross pathological lesions include splenomegaly, lymphadenopathy, and hepatomegaly. Microscopic changes are characterized by infiltration of lymphoid cells in almost all organs and tissues. Anemia has long been considered the hallmark of EIA. The two major causes of anemia, hemolysis and bone marrow depression, are closely associated with replicating virus. Hemolysis is immunologically mediated. Erythrocytes are coated with the viral surface glycoprotein which in the presence of specific antibodies and bound C3 induces erythrophagocytosis and complement-mediated hemolysis. Bone marrow suppression is less well characterized but appears to be associated with iron deficiency. Thrombocytopenia is frequently the earliest pathology observed during chronic EIA and can precede the detection of virus-specific antibodies. The mechanism for the marked reduction in blood platelets is unknown, but has recently been associated with cytokine dysregulation.

Immune Responses

Horses infected with EIAV typically become seropositive in standard serological assays within 21 days post infection. The humoral immune responses are predominantly against the viral envelope glycoproteins, gp90 and gp45, and the major core protein, p26. All currently approved diagnostic assays for EIAV infection are based on the detection of antibody to the major core antigen, although the antibody response to the envelope glycoproteins is at least tenfold greater than the antibody titer measured against the p26 protein. Significantly lower levels of antibody can be detected against virtually all of the other structural and nonstructural proteins encoded by EIAV. Interestingly, EIAV-specific antibody levels remain relatively constant throughout the course of chronic EIA and even in the unapparent stage of infection.

The immune responses generated during chronic EIA initially mediate significant pathogenesis in the presence of sufficient levels of EIAV antigenimia, but progressively evolve to establish a strict immunologic control over virus replication. Thus, the EIAV system is unique among lentiviruses in that the host immune responses routinely accomplish an effective control of aggressive virus replication and recurring clinical disease to maintain an indefinite inapparent stage of infection. The immune correlates of this protection remain to be defined. However, it has been shown that the neutralizing capacity of serum antibodies elicited to EIAV during the chronic stage of disease progressively increases, indicating an evolution of immune responses to the sequential generation of antigenic variants of virus. In addition, recent studies have demonstrated a lengthy and complex evolution of antibody and cellular immune responses to experimental EIAV infections of horses demonstrating a dynamic maturation process that apparently correlates with the development of protective immunity. The specific humoral or cellular immune correlates of protection have not yet been identified. The ability of the horse immune responses to overcome the array of persistence and escape mechanisms employed by EIAV suggests that a successful vaccine is feasible.

Prevention and Control

The transmission of EIAV infection has been controlled by improving animal husbandry techniques to prevent the spread of infected blood, by reducing the horsefly population in the vicinity of herds, and primarily by identifying and segregating or sacrificing horses that are seropositive for the virus. In the United States, the most common diagnostic assays are an agar gel immunodiffusion test, the Coggins assay, which was developed in the early 1970s and enzyme-linked immunoassay that was approved by the USDA as an EIA diagnostic assay in the 1980s and 1990s. Both diagnostic assay procedures are based on the detection of serum antibodies to the major viral core protein, p26. The diagnostic enzyme-linked immunosorbent assay (ELISA) assay has been engineered to produce a sensitivity that is equivalent to the previously established Coggins test. More sensitive diagnostic assays based on the detection of antibodies to the EIAV envelope glycoproteins have been developed, but have not yet been approved for commercial use. The most sensitive and specific assay for detecting EIAV infection is horse inoculation tests with 200 ml of whole blood from the horse being tested. The horse inoculation test is used only in rare cases where the standard serological assays may give ambiguous results.

There is currently no effective vaccine for the prevention of EIAV infection and disease. The primary challenge in developing an effective EIAV vaccine is overcoming the antigenic diversity intrinsic to this virus. An important

practical requirement in the development of any EIAV vaccine is compatibility with established regulatory policies and diagnostic assays. The ability of EIAV-infected horses to routinely establish immunologic control over virus replication and disease suggests that an effective vaccine can indeed be developed, if the critical natural immune correlates of protection can be elicited by a candidate vaccine. An attenuated live EIAV vaccine with a reported protection efficacy of about 70% has been used in China since the early 1980s, but the effectiveness of this vaccine remains to be confirmed outside of that country. Evaluation of other candidate EIAV vaccines (live-attenuated, inactivated whole virus, subunit vaccines, synthetic peptides, etc.) under experimental conditions has revealed a spectrum of vaccine efficacy that ranges from 'sterile protection' (prevention of infection upon inoculation with EIAV) to severe elevation of EIAV replication, and exacerbation of disease. These results indicate that immune responses to EIAV are a double-edged sword that can either mediate protection or yield vaccine enhancement. Vaccine enhancement has previously been reported for other viral infections (dengue virus, respiratory syncitial virus, feline infectious peritonitis virus) and is of special concern with macrophage-tropic viruses. Similar examples of vaccine protection and enhancement have been reported in studies of experimental vaccines for other lentiviruses, including feline immunodeficiency virus, caprine arthritis-encephalitis virus, and visna-maedi virus. These observations in several diverse animal lentivirus systems suggest that the potential for immune enhancement may be a general property of lentiviruses, including HIV-1. Current efforts in the production of a commercial EIAV vaccine are focused on the development of a vaccine that can achieve sufficient maturation of immune responses to provide protection from virus infection, but allow the serological differentiation between vaccinated and infected horses. In this regard, DNA vaccine strategies appear to be well suited to accomplish these criteria for a commercial EIAV vaccine.

Future Research

EIAV provides a dynamic system for examining the interaction between virus populations and host immune responses that are evolving in response to each other. In addition, EIAV offers a remarkable model for studying the delicate balance between immune responses to a persistent virus infection that result in disease and those that have beneficial results. A characterization of the nature of protective and enhancing immune responses can provide important information about the mechanisms of lentivirus disease and the type of immune responses to be elicited or avoided by a vaccine. The results of these studies in the EIAV system should be applicable to other lentiviruses, including HIV-1.

See also: Human Immunodeficiency Viruses: Antiretroviral agents; Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Origin; Human Immunodeficiency Viruses: Pathogenesis; Immune Response to viruses: Antibody-Mediated Immunity; Vaccine Strategies; Viral Pathogenesis.

Further Reading

Cook RF, Issel CJ, and Montelaro RC (1996) Equine infectious anemia virus. In: Studdert R (ed.) *Viral Diseases of Equines*, pp. 295–323. Amsterdam: Elsevier.

Cordes TA and Issel CJ (1996) Equine infectious anemia: A status report on its control. USDA Animal and Plant Health Inspection Service Publication No. APHIS 91–55–032.

Montelaro RC, Ball JM, and Rushlow KE (1992) Equine retroviruses. In: Levy J (ed.) *The Retroviridae,* vol. 2, pp. 257–360. New York: Plenum.

Montelaro RC and Bolognesi DP (1995) Vaccines against retroviruses. In: Levy J (ed.) *The Retroviridae,* vol. 2. New York: Plenum.

Sellon DC, Fuller FJ, and McGuire TC (1994) The immunopathogenesis of equine infectious anemia virus. *Virus Research* 32: 111.

Evolution of Viruses

L P Villarreal, University of California, Irvine, Irvine, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Dendrogram A schematic line drawing, often tree-like, that represents evolutionary relationships between species.

Error catastrophe A threshold at which a high error rate of genome replication can no longer maintain the integrity of essential genetic information.

ERV An endogenous retroviral-like genetic element, containing a long terminal repeat

that is found in the genomes of many organisms.

Fitness landscape A hypothetical representation of a three-dimensional surface that represents relative fitness of genetic variants.

Muller's ratchet A decrease in fitness resulting from a repetition of a genetically restricted founder population.

Quasispecies A population of viral genomes that are the product of error-prone replication usually envisioned as a swarm or cloud of related genomes.

Red Queen hypothesis An evolutionary concept in which an organism must evolve at high rates in order to maintain its competitive advantage.

Reticulated evolution A pattern of evolution in which elements are derived from different ancestors represented as a tree with cross-connections between distinct branches.

Sequence space A multidimensional representation of all possible sequences for a given genome.

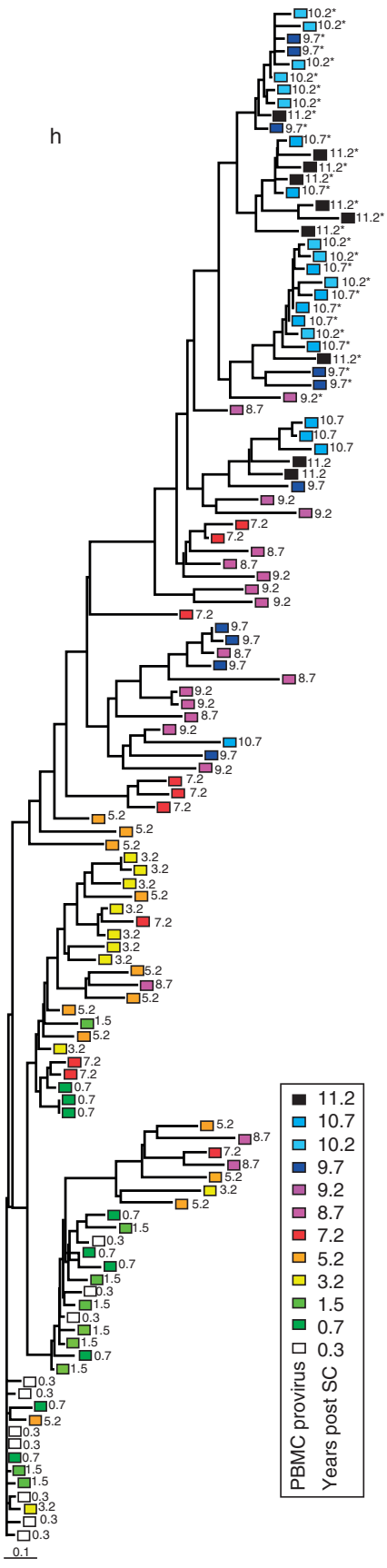
Introduction

The initial study of virus evolution sought to explain how virus variation affects viral and host survival and to understand viral disease. However, we now realize that virus evolution is a basic issue, impacting all life in some way. In general, the principles of virus evolution are very much the same Darwinian principles of evolution for all life, involving genetic variation, natural selection, and survival of favorable types. However, virus evolution also entails features such as high error rates, quasispecies populations, and genetic exchange across vast reticulated gene pools that extend the traditional concepts of evolution. Evolution simply means a noncyclic change in the genetic characteristics of a virus; and viruses are the most rapidly evolving genetic agents for all biological entities. Principles of virus evolution provide an integrating framework for understanding the diversity of viruses and the relationships with host as well as providing explanations for the emergence of new viral disease. Most emerging viral diseases are due to species jumps from persistently infected hosts that have long-term virus–host evolutionary histories (called natural reservoirs). Since early human populations (i.e., small bands of hunter gatherers) could not have supported the great human viral plagues of civilization (e.g., smallpox virus/variola and measles), these viruses must also have originated from species jumps that adapted to humans. In recent history, the emergence of HIV demonstrates that virus evolution continues to impact human populations. Early observation established that viruses often show significant variation in virulence. Such variation was used as an early, yet risky, form of vaccination (i.e., variolation against smallpox). The variation that occurred with passage into alternate tissue and host was also used to make various vaccines (rabies in 1880s) or attenuate viral virulence, such as live yellow fever vaccine. But variation also allows some viruses, such as influenza A, to escape neutralization by vaccination. However, variation in viral disease or virulence does not provide a quantitative basis to study evolution.

The study of virus variation and evolution is an applied science that allows the observation of evolutionary change in real time. For example, human individuals (or populations) infected with either human immunodeficiency virus 1 (HIV-1) or hepatitis C virus (HCV) show progressive or geographical evolutionary adaptation associated with the emergence of specific viral clades that affect disease therapy and progression (such as resistance to antiviral drugs). **Figure 1** shows HIV variation in an individual human patient whereas **Figure 2** shows HCV variation in the human population. Virus evolution is also important for the commercial growth of various organisms, such as the dairy industry (lactose fermenting bacteria), the brewing industry, agriculture, aquaculture, and farming. In all these applied cases major losses can result from virus adaptation to the cultivated species, often from viruses of wild species. Some organisms appear much less prone to viral adaptations (e.g., nematodes, ferns, sharks). Virus evolution can also be applied to technological innovation, as in phage display. This is a process in which a terminal surface protein of some filamentous bacterial virus can be genetically engineered for novel surface protein expression. By generating diversity *in vitro* (with up to 10^{15} types), and applying the principles of evolution (random variation) to biochemical selection (such as binding to a chemical substrate), a reiterative amplification can find solutions to problems in biochemistry, such as surface interactions or catalytic activity.

Virus Evolution as a Basic Science

Virus variation is a global issue. In the last decade it has been established that viruses are the most numerous biological entities on the planet. The oceans and soil harbor vast numbers of viral-like particles (VLPs), mostly resembling the tailed DNA viruses of bacteria. In addition, some of these environmental viruses are unexpectedly large and complex, such as the phycodnaviruses of algae or mimivirus of amoeba, a 1.2 Mb DNA virus that can encode nearly 1000 genes. Thus viruses represent a vast and diverse source of novel genes. However, the evolutionary dynamics of this population and its effect on hosts is not well understood. It is likely that this virus gene pool also affects host evolution since prophage colonization is



known for all prokaryotic genomes. Thus, this vast pool of viruses connects directly to prokaryotes and the 'tree of life'. The study of virus evolution has become an extension of all evolution.

Distinctions from Host Evolution

For the most part, virus evolution conforms to the same Darwinian principles as host evolution, involving variation and natural selection. However, viruses have multiple origins, and are thus polyphyletic. There are six major categories of viruses (+RNA, –RNA, dsRNA, retro, small DNA, large DNA) that have no common genes and hence have no common ancestor. However, these categories all have conserved hallmark genes (i.e., capsid proteins, Rd RNA pol, RT, primase, helicase, and RCR initiation proteins) which are all monophyletic and which may trace their origins to primordial host/viral gene pools. Many DNA viruses, for example, have replication strategies and polymerases that are clearly distinct from that of their host, which depends on such hallmark genes. Thus, virus evolution appears ancient but inextricably linked to its host. Also, in contrast to host evolution, viral quasispecies show a population-based adaptability that extends the selection of the fittest to include populations of otherwise unfit genomes (described below). Viruses can clearly cross the usual host-species barriers so that viral evolution can be reticulated in vast genes pools. For example, bacterial DNA viruses and +RNA viruses can show high rates of recombination across viruses that infect numerous host species. Accordingly, tailed DNA phage of bacteria appear to represent one single vast gene pool. Viruses can also violate concepts of death and extinction, reassembling genomes from parts and/or repairing lethal damage by multiplicity reactivation. In addition, damaged (defective) viruses can also affect virus and host evolution. Such defectives are found in many types of viruses and can also be found in many host genomes (as defective prophage or defective endogenous retroviruses). Such defective viruses can clearly affect host survival. In all these characteristics, viruses extend the Darwinian principles of host evolution.

Figure 1 HIV population analysis from an infected individual. Shown is a neighbor-joining phylogram derived from maximum likelihood distance between all sequences. Sequences are represented by a square for PBMC sequences or a triangle for plasma sequences. The arbitrary color gradient corresponds to the time of sampling. Adapted from Shankarappa R, Margolick JB, Gange SJ, *et al*. (1999) Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus Type 1 infection. *Journal of Virology* 73: 10489–10502, with permission from the American Society for Microbiology.

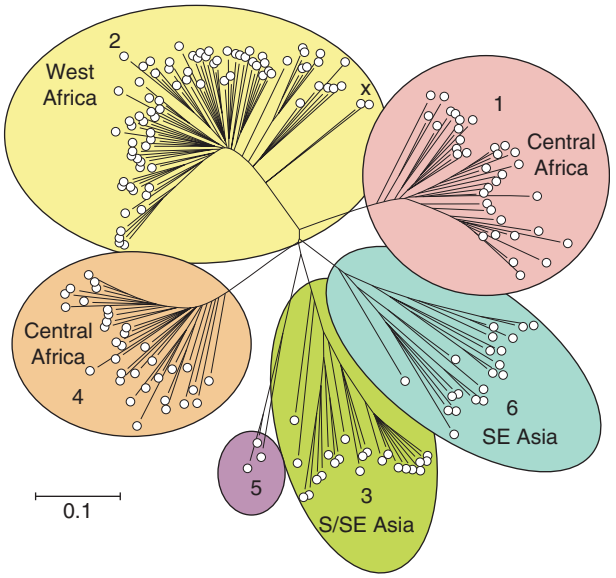


Figure 2 Unrooted phylogenetic analysis of HCV nucleotide sequences from globally distributed human isolates. Adapted from Simmonds P (2001) Reconstructing the origins of human hepatitis viruses. *Philosophical Transactions of the Royal Society of London* 356: 1013–1026, with permission from Royal Society Publishing.

A History of Virus Evolution

The coherent study of virus evolution awaited the development of sequence technology to measure mutations and genetic variation in viral populations. Concepts of natural selection, fitness, and propagation of favorable variation had long been established in the evolutionary biology literature prior to the growth of virology. Thus, mathematical models, such as Fisher population genetics, concerned with gene frequency in a (sexually exchanging) species population, had been well developed and seemed directly applicable to virus evolution, which resemble that of host genes. Here, viral fitness was typically expressed as relative replication rates (replicative fitness) but sometimes host virulence and disease were also used. However, as presented below, a comprehensive definition of viral fitness remains problematic. The first quantitative measurements of the rate of virus mutation was done in the 1940s with bacterial phage. The mutation rates were expressed as a set of ordinary differential equations that were subsequently used to develop the quasispecies equations as applied to error-prone RNA genomes (see below). However, the species definition of a virus poses a problem for evolutionary thinking and challenges how we define kinship in viruses. Unlike the sex-defined host, a virus species is currently defined as a polythetic class: a mosaic of related parts of which not all elements are shared (such as host range, genome relatedness, antigenic properties). No specific defining characteristic or gene exists for a virus species and sexual exchange need not be included. This is an inherently fuzzy definition, like defining a 'heap', which although clear, cannot be specified by its number of parts. The ensuing molecular characterization of many virus populations supports this species definition. The challenge then is to understand viral evolutionary patterns working with such fuzzy definitions. Yet, conserved patterns of virus evolution are still seen, some of which suggest viruses are indeed an ancient lineage, possibly extending into the primordial RNA world.

Error-Prone Replication and Quasispecies

In the 1970s, Manfred Eigen and also Peter Shuster developed a fundamental theoretical model of virus evolution. A set of ordinary differential equations was published that described what was called 'quasispecies'. Starting from measurements of phage mutation rates, they considered the consequences of high error rates as expected from RNA replication (an error-prone noncorrecting replication process). The resulting population shared many properties and was called quasispecies, a society (or community) of individuals that are the error products of replication. The name 'quasispecies' thus describes a chemically diverse set of molecules and was not intended to refer to a biological species (i.e., genetic exchanging). However, as discussed above, the fuzzy definition of virus species and quasispecies overlaps somewhat, which has been a source of confusion. Several premises were used to develop this theory: (1) the individual products ignore one another and interact only as individuals; (2) the system is not at equilibrium and resources are not limiting. Based on relative replication, the growth of favorable types is described which provides a mathematical definition of replicative fitness. The original equations represent an idealized generalized system of infinite population size and are not directly applicable to the real world, although they provide valuable insights into real world systems. The equations do not address variable mortality (longevity), interference, exclusion, competition, complementation, and persistence, or how such issues affect nonreplicative fitness definitions. The issue of mortality and fitness is interesting from the perspective of viruses. For example, an interfering defective virus can be considered dead, but can clearly interfere with and drive the extinction wild-type template replication in quasispecies. In some cases, the quasispecies equations appear to be mathematically equivalent to classical Wright and Fisher population genetics equations as applied by Kimura and Maroyama to asexual haploid populations at the mutation-selection balance. However, these two approaches begin with distinct perspectives, and it was the assumption of high error in the quasispecies equations that had a major impact on experimentation and our current understanding of virus evolution. This has

also led to some counter intuitive conclusions, such as the concept of selection of 'the fittest' compared to the consensus character of the master template. Quasispecies from a virus with high error rates (such as HIV-1) might be composed of all mutant progeny RNAs such that the consensus template (the mean, the fittest, or the master template) may not actually exist. With classical population genetics, an asexual clonal population should fix the clonal sequence. With quasispecies, this is not observed. The first laboratory measurements of viral quasispecies were made using Qβ RNA polymerase *in vitro*. Error estimates ranged from 10^{-3} to 10^{-4} substitutions per site per year (an error rate applicable to most RNA viruses). With Qβ, the replication of many nonviable mutants generated a genetic spectra that had a characteristic makeup. For example, separate DNA clones of Qβ were initially distinct from each other but quickly generated the same RNA quasispecies as before cloning. Additional lab measurements have shown that quasispecies can have significant adaptive fitness (above the cloned master template) and display memory; that is, they can retain information of prior selections in a minority of the population. Complementation, interference, competition suppression, and extinction have all been measured in various quasispecies, thus indicating a collective form of evolution and violating an original premise of genome independence in these equations. In addition, the sequence diversity in a quasispecies is now seen as a source of adaptive potential, not simply error (see below). Despite these results, the concept of quasispecies has still been highly useful, and not simply as a theoretical development. For example, the live poliovirus vaccine is clearly a heterogeneous quasispecies. Within this population exists a minority of neurovirulent variants that are suppressed by the majority avirulent virus. A main point of the quasispecies concept is that it provides an understanding of high adaptability from a population that has many, even lethal, mutations. It is interesting that the proposed early RNA world would have also been a collective quasispecies world.

Error Catastrophe, Sequence Space

Quasispecies theory also predicts a situation known as error catastrophe, defined as an error rate threshold at which information is lost and the system decays. If error rates are too high, or the information content (genome length) too extensive, the system will be unable to maintain its information integrity. This predicts a basic limit on the size of RNA genomes, consistent with the observation that the largest RNA genomes are only about 27–32 kbp (coronaviruses). There is a possible therapeutic use of error catastrophe: drugs (possibly Ribavarin and 5-fluorouracil) that increase the error rates of RNA polymerase can potentially push a virus beyond its error threshold and induce a catastrophe. Quasispecies is an inherently fuzzy and dynamic population that has no sharp boundaries or specific members and has been metaphorically referred to as swarms and clouds. Here, cloud is a metaphor for the population landscape that exists in high-dimensional hyperspace and cannot be readily visualized. The concept of sequence space has been used to represent topography of the distribution of all mutants. Kinship relationships between mutants can be measured by Hamming distance; the minimal steps needed to specify the difference between two mutants. In spite of high error and adaptation rates (and sometimes high recombination rates), RNA viruses are not able to explore all potential sequence space. Selection significantly limits the quasispecies, since the potential sequence space is hyperastronomical even for a moderately sized virus. For example, an RNA virus with 10 000 nt would correspond to 10^{6000} possible sequences, well beyond what could be explored by even the potentially vast number of viruses over the lifetime of the world. In addition, there are clearly mechanistic constraints that prevent many possible sequences, such as necessary domains of ± strand RNA folds, physical association with ribonucleotide proteins, virion packaging and assembly – all in addition to usual selection for gene fitness (function) that all severely limit possible adaptations. This creates a multipeak 'fitness' landscape in hyperspace (see **Figure 3**). Assuming fitness itself can have a single definition (i.e., replicative fitness, not subjected to variable and stochastic competition), we can visualize this space as many steep valleys and ledges (in this case with 10 000 dimensions). Normally we think that adaptation by natural selection is the force to explore and move through fitness landscape. But as the deep valleys are often lethal, they cannot be explored via natural selection. Here we see the major adaptive power of the quasispecies

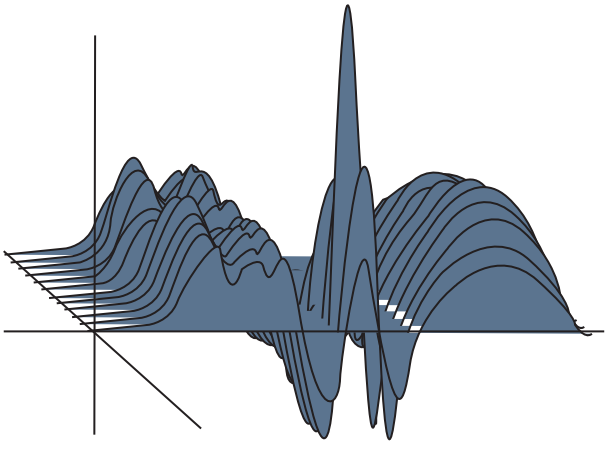


Figure 3 Hypothetical fitness landscape for an RNA virus. Assuming one definition for a nonrelativistic fitness (such as replicative fitness), the coordinates indicate relative fitness. Those below the *y*-axis are interfering or lethal variants.

collective. Since random, even lethal, errors and drift are inherent in a quasispecies, lethal valleys can be readily crossed by such variable genomes, allowing the master genome to adapt by natural selection to a new fitness peak. Thus, error-prone replication and the generation of mutant clouds allows for much better exploration of sequence space and eventual adaptability.

When viruses are transmitted to new hosts, they can experience a genetic bottleneck since a relatively small number of viral genomes could be involved (aka low multiplicity passage). If this process is serially repeated, a phenomenon known as 'Muller's Ratchet' can result in lost competitiveness as the essentially clonal RNA virus accumulates deleterious mutations (sometimes measured as pfu/plaque). However, in lab studies, virus extinction from serial passage does not occur, presumably due to plaque selection for a restored phenotype. Even a single plaque is in reality a small population (due to nonideal particle/pfu ratios and ID_{50}). However, lost competitiveness with other viruses is seen with clonal laboratory passage. However, if a quasispecies population is passed, this generally results in increased competitive fitness. Such passage can produce a seemingly never-ending better version of the virus that outcompetes all prior versions of the same virus (although virion yields and absolute replication are not necessarily improved). This has been likened to the Red Queen hypothesis in that the viruses are evolving at high rates, simply to maintain their competitive position, so as not to be displaced as the dominant viral type. Virus–virus competition is thus a crucial selection.

Never-Ending Adaptation

A real world example of the potentially never-ending virus adaptation is shown in **Figure 4**. The HA and NA genes of human influenza A virus have been monitored for several decades. As shown, the prevalent master template of the virus circulating in the human population has been continually changing, due to immune selection and stochastic viral immigration, necessitating yearly vaccine changes (also shown). Although such a population dynamic has been stably maintained in the human population, all prior versions have essentially become extinct, as they do not reappear.

Not all RNA virus populations show this dynamic of a continual change or even the diversity expected from quasispecies. Even in influenza virus A, avian isolates from natural host (waterfowl) can be genetically stable. Some RNA (and retro) viruses with high error rates can nevertheless maintain stable populations in specific hosts. For example, measles virus shows much less antigenic drift in human infections compared to influenza virus A. Hepatitis G virus (a human prevalent and distant relative of HCV) shows little variation in even isolated human populations. The filoviruses (Ebola virus and Marburg virus) have shown no genetic variation in Zaire isolates from 20 years apart. Hendra virus, isolated from Australian fruit bats, and Nipah virus from Malaysian fruit bats also show little genetic diversity. Arenaviruses and hantavirus are also genetically stable in their natural rodent host. The reasons for such population stability have not been well evaluated. In some cases (measles), purifying selection would seem likely. In other cases, persistence and low replication rates seem to apply. For example, simian foamy virus (SFV) and human T-lymphotropic virus II (HTLV-II) generate only about 10^{-8} substitutions per site per year, probably due to low replication rates.

Virus–Host Congruence and RNA Stability

There are now many examples of species-specific RNA virus/host coevolution, indicating very slow rates of virus evolution. Since error rates must be similar, this appears to be at odds with the quasispecies theory. For example, Hantavirus (genus *Bunyavirus*) coevolution with its rodent host, suggests a 20 million year association. Arenaviruses (ssRNA bisegmented ambisense) also coevolve in Old/New World rodents. These viruses are of special interest with regard to emergence as they represent the source of five hemorrhagic human fevers (such as Lassa virus). In all these examples, however, it appears that the virus causes a persistent unapparent infection in its natural host and that human disease is due to species jump.

Tools

Although viral genomes were the first to be sequenced, the initial focus was simply to identify similarity between viral genes, not to evaluate distant evolutionary relationships. The most popular tool for finding similarity is BLAST (Basic Local Alignment Search Tool) from the National Centers for Biotechnology Information (NCBI), which calculates similarity between query sequences and infers a probability based on a matrix database. Various versions of BLAST are the most used tool in bioinformatics to trace evolution. Although BLAST will identify similar genes, it is also necessary to compile and evaluate the similarities in sets of the related sequences. Multiple sequence alignment software, such as ClustalW, is used for this purpose. Phylogenetic relationships are then inferred from tree-building software. This software includes maximum parsimony, neighbor-joining, and maximum likelihood methods. The statistical significance of the tree (relative to all possible trees) can then be evaluated by algorithms such as bootstrap. More recently, Bayesian analyses, such as Bali-Phy, which implements a Markov Chain Monte Carlo (MCMC) method and calculates joint posterior

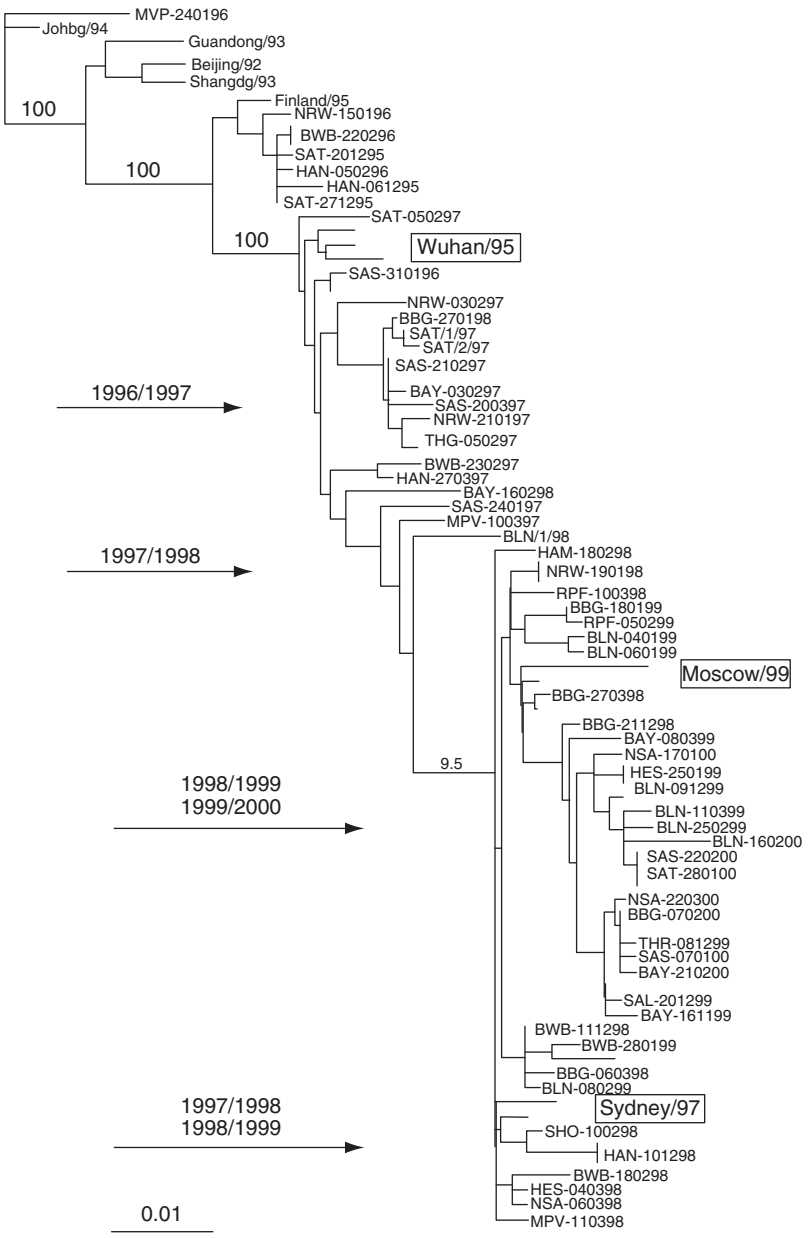


Figure 4 Phylogenetic tree of yearly influenza A/H3N2 viruses variants based on the hemaglutinin gene. Locations of specific vaccine strains are boxed. Reproduced from Schweiger B, Zadow I, and Heckler R (2002) Antigenic drift and variability of influenza viruses. *Medical Microbiology and Immunology* 191: 133–138, with permission from Springer-Verlag.

probabilities of phylogeny and alignment have become popular. This software has the added potential of using sliding windows and evaluating multiple trees, such as in virus–host coevolution. These methods, however, use the single master consensus sequence as its query and do not evaluate quasispecies, collective-based populations. Also, the clearly reticulated or hybrid character of some virus evolution is problematic. Also, distant evolutionary relationships are no longer preserved in the same sequences. Here, conservation of structural motifs, assembly patterns, gene order, and replication strategy are used to identify distant kinship.

Patterns of RNA Virus Evolution

The sometimes extreme variation of RNA virus sequence has led some to propose that most family lineages appear to be only about 10 000 years old, which is clearly at odds with much older estimates. The +RNA viruses in particular show a remarkable diversity of genomes and replicator mechanisms. These families also show much evidence of recombination and a tendency to cross host barriers. About 38 families of +RNA viruses with up to four segments are known. There are four distinct classes of replicase recognized in viruses that also share a common

genetic plan. These RNA viruses have three helicase superfamilies, two protease superfamilies, and two jelly roll capsid domains. For the most part, capsid and RdRpol sequences are congruent except for members of the families *Luteoviridae* and *Tetraviridae*, which appear to have undergone recombination between these two gene lineages. The smallest +RNA virus is a member of the bacterial virus family *Leviviridae*, which has only four genes. This simple virus appears to represent the ancestral +RNA virus. Curiously, no RNA virus has yet been found to infect archaebacteria. The largest +RNA viruses belong to the genus *Coronavirus* (27–32 kbp). The most recently described +RNA viruses are members of the family *Marnaviridae* infecting bats and marine organisms sea life, which appear to be basal to evolution of picornaviruses. However, natural populations of some +RNA viruses can be stable. For example, dengue virus (*Flaviviridae*) shows low rates of amino acid substitution (e.g., nonsynonymous to synonymous ratios). Since it is an acute arbovirus infection with high error rates, strong selective constraints likely account for this stability involving multiple (systemic) tissues and vector transmission.

Negative-strand RNA viruses have distinct patterns of evolution, which is traced via their polymerase genes. Gene order tends to be highly conserved. The unsegmented viruses, such as rhabdoviruses, lyssaviruses, and paramyxoviruses, do not undergo significant recombination so their variation tends to be by point mutations and deletions. Although high error rates, variation, and quasispecies generation can be seen in laboratory settings, natural isolates, such as lyssaviruses and measles virus, tend to be relatively homogeneous. For example, lyssaviruses show a slow rate of evolution (5×10^{-5}/site/year). Lyssavirus persistent infections in natural host might contribute to this stability. However, measles virus is a strictly human-specific acute infection so its stability is likely due to purifying selection.

Patterns of DNA Virus Evolution: Tailed Phage

Large DNA viruses of bacteria, archaea, and eukaryotes appear to be evolutionarily linked. Although little sequence conservation can be identified between the T4 phage of bacteria, the halophage of archaea, the members of the family *Phycodnaviridae* infecting algae, and the herpes viruses of vertebrate eukaryotes, all show similarities in their gene programs, DNA polymerase types, capsid structures, and capsid assembly, consistent with a common ancestor. For example, both the Enterobacteria phage T4 (T4) and herpes simplex virus 1 (HSV-1) have $T = 1$ capsid symmetry with 60 copies of capsid protein. The bacterial DNA viruses would appear to represent the ancestor of all these viruses, but the origins of these phages now appear lost in the primordial gene pool. These DNA viruses can have large genomes that could not be sustained by error-prone replication. Thus, many DNA viruses do have error-correcting DNA replication, with error rates that approach or equal those of their host cells (10^{-8}). Giant bacterial phage genomes (*Bacillus megaterium* phage G, of about 600 genes), and algal phycodnaviruses have now been characterized. Even larger DNA viruses of amoeba (acanthamoeba polyphaga mimivirus) coding for more than 1000 genes are known to be abundant in some water habitats.

The tailed phage of bacteria have been called the dark matter of genetics, due to their numerical dominance ($\sim 10^{31}$ *en toto*). This corresponds to about 10^{24} productive infections per second on a global scale. Most host-restricted phage lineages clearly conserve sets of core proteins (especially capsid genes), but others (the broader T-even phages) do not conserve any hallmark genes. Hallmark genes, when present, are usually recognized by conserved domains within proteins, such as replication and structural proteins. Replicator strategy and gene order are also frequently conserved. Phage also tends to conserve genes that are active against other phage (i.e., DNA modification, lambda RexA, T4 rII). With the sequencing of numerous phage genomes, however, a large number of novel genes have been identified. Currently, 350 full genomes of tailed phage and 400 prophage from bacterial genomes have been sequenced. In general, large DNA viruses are tenfold overrepresented in small single-domain genes (~100 aa). Comparative genomics, especially of lactobacterial phages, suggest that most phage genomes evolve as mosaics, with sharp boundaries between genes as well as at protein domains within genes (see **Figure 5**). Recombination between lytic, temperate, and cryptic prophages appears to account for this gene and subgene domain variation. Some specific phages have mechanisms to generate specific gene diversity (such as bordetella phage using RT for surface receptor diversity), but most diversity is the product of recombination. Two broad patterns of phage variation have been observed corresponding to host-unassociated lytic and host-associated (congruent) temperate phage. In most bacterial genomes (ECOR *Escherichia coli* collection, cyanobacteria, *B. subtilis*), patterns of prophage colonization account for significant genetic distinctions between closely related host strains. The general picture for tailed phage of bacteria is that they are not the products of reduction of host genomes.

Large Eukaryotic DNA Viruses

As noted, evolutionary links between tailed phage and large DNA viruses of eukaryotes are apparent. The phycodnaviruses of unicellular green algae clearly have many phage-like characteristics, including the presence of

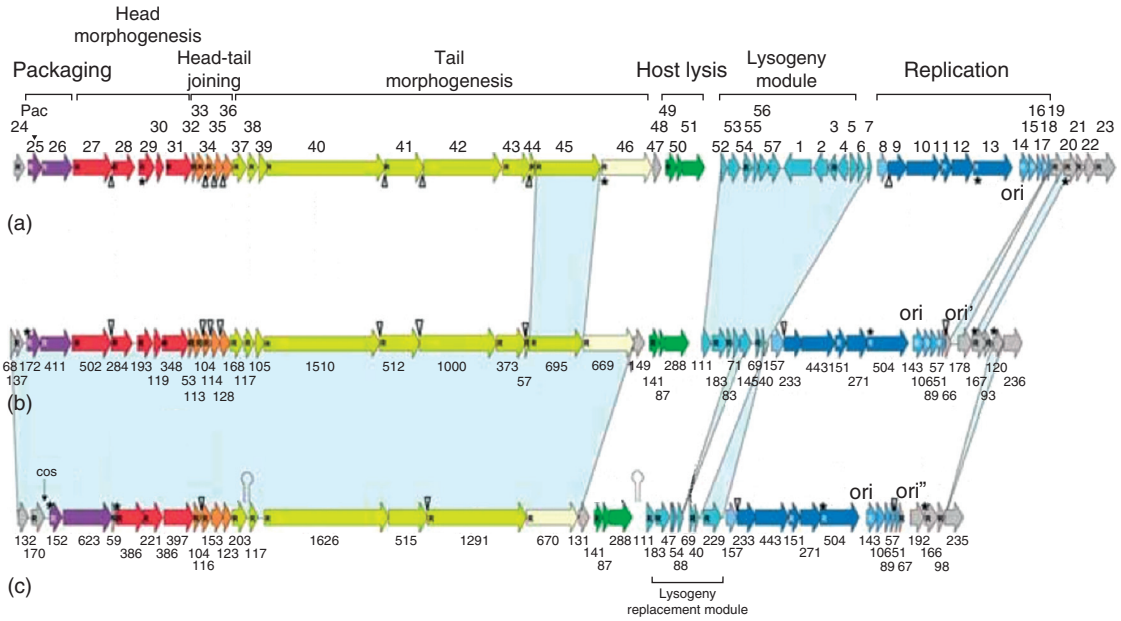


Figure 5 Genome comparison of temperate *S. thermophilus* phage 01205, virulent *S. thermophilus* Sfi11, and the virulent *S. thermophilus* Sfi19. Probable gene functions are indicated and genomes have been divided into functional units. Genes belonging to the same module are indicated with the same color. Areas of shading indicate regions of major difference. From Desiere F, Lucchini S, Canchaya C, Ventura M, and Brüssow H (2002) Comparative genomics of phages and prophages in lactic acid bacteria. *Antonie van Leeuwenhoek* 82: 73–91.

restriction/modification enzymes, homing endonucleases, the injection of viral DNA, and the external localization of the viral capsid. They also have many characteristics of eukaryotic DNA viruses, such as a clearly herpes virus-related DNA polymerase, PCNA proteins, nonintegrating DNA, and numerous signal-transduction proteins. Thus, phycodnaviruses show hybrid characteristics of prokaryotic and eukaryotic viruses.

The evolutionary pattern of the large DNA viruses of eukaryotes is generally best traced by comparing their respective DNA-dependent DNA polymerases (DdDp). These exist in distinct classes that are typically specific for each viral lineage and are usually the most highly conserved of the set of core genes within a viral lineage. However, some viruses, such as the white spot syndrome virus (WSSV) infecting shrimps, have almost no genes in common with other DNA viruses. Generally, the specific set of core genes is clade specific. The first fully sequenced viral genome tree was that of the baculoviruses (see **Figure 6**). The overall pattern of evolution shows the conservation of the core set in which most clades can be differentiated from one another mainly by acquisition of several novel viral genes (although some lineage-specific gene loss is also apparent). In another example, coccolithoviruses differ from related phycodnaviruses by the acquisition of 100 kbp gene set, including six subunits of DdDp core genes. Similar patterns of divergence can be seen with the herpesvirus family members. In addition, most herpesvirus clades also show coevolution with their host. However, the poxviruses (orthopoxviruses), show a different overall evolutionary pattern and are not congruent with host. The more ancestral orthopoxvirus members, such as cowpox virus and mousepox virus, have greater gene numbers that appear to have been lost in the human-specific and virulent smallpox virus. Avipoxviruses have even greater gene diversity but the entomopoxviruses are the most complex and diverse of all. The complexity and brick shape of the poxviruses originally inspired the view that these viruses might evolve from bacterial cells following the reduction of complexity. However, DNA sequencing makes it clear that viral core genes have no bacterial analogs. In some instances, viral lineages have clearly fused with other viral and host lineages. For example, the baculovirus Autographa californica MNP virus (AcMNPV) has acquired a gypsy-like retrovirus (e.g., TED), an endogenous retrovirus associated with moth development. The polydnaviruses (circular DNA viruses) are fused into their host genomes (as endogenous DNA viruses) of some parasitoid wasps, essential for survival of the wasp larvae.

Small DNA Viruses

The small, double-stranded, circular DNA viruses (*Papillomaviridae* and *Polyomaviridae*) show evolutionary patterns that are highly host linked. Virus and host evolution are mostly congruent, and virus evolution tends to be slow.

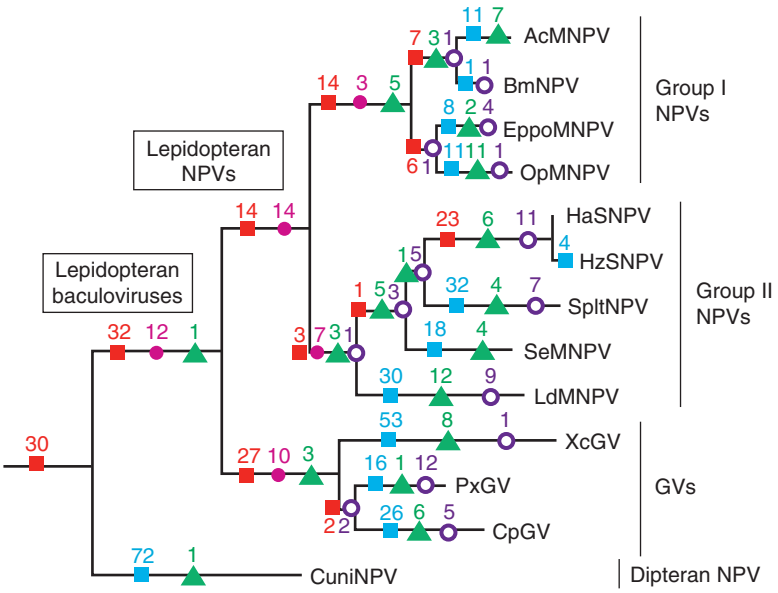


Figure 6 Gene content map of 13 complete sequences of baculoviruses, including the genus *Granulovirus*. The tree shows the most parsimonious hypothesis of changes in gene content during baculovirus evolution. Colors and shapes indicate gene conservation, acquisition, and loss. Reproduced from Herniou EA, Olszewski JA, Cory JS, O'Reilly DR (2003) The genome sequence and evolution of baculoviruses. *Annual Review of Entomology* 48: 211–234, with permission from Annual Reviews.

For example, approximately 100 human papillomaviruses show congruent evolution with human (and primate) host. This seems to be due to both a highly species- and tissue-specific virus replication, as well as a tendency to establish persistent infections. However, the rolling circular replicon (RCR) viruses, such as parvoviruses, can have distinct evolution patterns. Both mouse minute virus (MMV) and canine parvoviruses can have quasispecies-like populations, which can show evolutionary rates at 10^{-4} substitutions per site per year. Such rates are at the lower end of those seen with RNA viruses. In bacteria, RCR viruses and RCR plasmids appear to represent a common gene pool. Other poorly characterized small eukaryotic DNA viruses, such as human torque teno virus, are asymptomatic but show high variation during persistence for unknown reasons.

Endogenous and Autonomous Retroviruses

Retroviruses present a special problem in understanding patterns of eukaryotic virus evolution. Like prophage of bacteria, retroviruses both stably colonize their host as endogenous or genomic retroviruses (ERVs) that are often defective, but may also sometimes emerge from their host (especially rodents) to produce autonomous virus. In addition, retroviruses are polyphyletic and prone to generating quasispecies due to high error rates as well as high rates of recombination. The most common conserved retrovirus genome elements are domains within the long terminal repeats (LTRs), RT, integrase, protease, gag protein, and env protein. Of these, env are the most often altered or deleted in host genomes. In addition, tRNA primer sites (such a lys [K] tRNA) are also often conserved and used for classification (i.e., human endogenous retrovirus, HERV-K family). However, each of these retroviral elements can potentially have distinct patterns of evolution and conservation, generating distinct dendrograms. Vertebrates, especially mammals, seem to host many retroviral elements within their genomes. Their autonomous retroviruses have a tendency to infect cells of the immune system. Murine leukemia virus (MLV) is the best-studied simple autonomous retrovirus, but many endogenous MLV relatives also exist. Retroviruses are present in genomes of early eukaryotes but significantly expanded in vertebrates. Gypsy-like retroviruses (aka chromoviruses, defined via RT and gag similarity) are often found conserved as full-length elements including env genes in most lower eukaryote genomes (e.g., *Caenorhabditis elegans*), but were mostly lost from tetrapods. Some lower eukaryotes clearly prevent colonization by ERVs, such as *Neurospora* fungi (via the RIP exclusion system). Many endogenous retroviruses are congruent with host evolution, whereas other ERVs are recently acquired and highly host specific. In terms of gene diversity, the retroviral *env* are the most diverse. There are five RT-based families recognized such as *Retroviridae, Hepadnaviridae, Caulimovoridae, Pseudoviridae,* and *Metaviridae*, the latter three being especially prevalent as genomic elements in flowering plants (especially Gypsy). Yet not all retroviruses seem able to colonize host germ line. For example, lentiviruses (such as simian

immunodeficiency virus (SIV) and HIV) show no examples of endogenization compared to the simpler MLV-related viruses that can be both autonomous (i.e., MLV, Gibbon ape leukemia virus) and endogenous (i.e., *Mus dunni* ERV, koala ERV). The converse can also be true, since no autonomous versions of HERV K, for example, are known.

Early views proposed that retroviruses evolved from nonviral retroposons (LTR RT elements, non-LTR LINE-like elements). These non-LTR elements, have distinct nonretroviral mechanistic features and core protein domains, but retain some virus-like domains of RT; thus, they appeared to predate retroviruses. However, we now know that gypsy-like retroviruses were present in the earliest eukaryotes. In addition, some LTR-containing elements, such as Gypsy, had initially been considered ancestral to retroviruses because all copies seemed to be defective. However, it is now established that complete gypsy retroviruses are conserved as ERVs in some yeast and *Drosophila* strains. Thus, although endogenous and exogenous retroviruses appear to evolve from each other, there is no evidence that exogenous retroviruses have emerged from non-LTR LINE-like elements.

The congruence between ERVs and host eukaryote evolution is sometimes striking. For example, all mammals have acquired their own peculiar versions of ERVs (and LINES). Recently, it has become clear that the placental mammals have conserved several families of ERV-derived *env* genes that provide an essential function for placental tissue (ERV W-syncytin 1, ERV FRD syncytin 2, enJSRV). Clearly, retrovirus evolution is highly intertwined with that of their hosts.

Never-Ending Emergence

A remaining concern of virus evolution is to understand the emergence of new viral pathogens. The unpredictable and stochastic nature of such virulent adaptations makes predictions difficult, as the link between virulence and evolution is vague. For example, the genetic changes that made the SARS virus (persisting in bats) into an acute human pathogen are still not predictable. Viral fitness and selection, and how they change from persistent states with acute species jumps, are not yet defined. However, some variables contribute to the likelihood of viral emergence, such as virus ecology. The population density and dynamics of the new host and the ecological interactions-between new and stable viral host are often crucial. In addition, virus–virus interactions can be important, allowing for recombination and/or reassortments or lowering immunological selective barriers via immunosuppression. The emergence of HIV-1 from different, persistent SIVs of African monkeys through chimpanzees into a new human disease, for example, includes the same issues. Also, the potential emergence of pandemic human influenza from avian (Anatiformes) sources, such as H5N1, remains a great concern. Thus, virus evolution will continue to interest us as we seek to predict, control, or eradicate viral agents of disease.

See also: Antigenic Variation; Coronaviruses: Molecular Biology; Emerging and Reemerging Virus Diseases of Plants; Emerging and Reemerging Virus Diseases of Vertebrates; Emerging Geminiviruses; Origin of Viruses; Phylogeny of Viruses; Picornaviruses: Molecular Biology; Quasispecies; Retrotransposons of Vertebrates; Virus Databases; Virus Evolution: Bacterial Viruses; Virus Species.

Further Reading

Desiere F, Lucchini S, Canchaya C, Ventura M, and Brussow H (2002) Comparative genomics of phages and prophages in lactic acid bacteria. *Antonie Van Leeuwenhoek* 82(1–4): 73–91.

Domingo E (2006) *Current Topics in Microbiology and Immunology, Vol. 299: Quasispecies: Concept and Implications for Virology.* Berlin: Springer.

Herniou EA, Olszewski JA, Cory JS, and O'Reilly DR (2003) The genome sequence and evolution of baculoviruses. *Annual Review of Entomology* 48: 211–234.

Hurst CJ (2000) *Viral Ecology.* San Diego: Academic Press.

Schweiger B, Zadow I, and Heckler R (2002) Antigenic drift and variability of influenza viruses. *Medical Microbiology and Immunology* 191: 133–138.

Shankarappa R, Margolick JB, Gange SJ, *et al.* (1999) Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection. *Journal of Virology* 73: 10489–10502.

Simmonds P (2001) Reconstructing the origins of human hepatitis viruses. *Philosophical Transactions of the Royal Society of London* 356: 1013–1026.

Roossinck MJ (2005) Symbiosis versus competition in plant virus evolution. *Nature Reviews Microbiology* 3(12): 917–924.

Villarreal LP (2005) *Viruses and the Evolution of Life.* Washington, DC: ASM Press.

Zanotto PM, Gibbs MJ, Gould EA, and Holmes EC (1996) A reevaluation of the higher taxonomy of viruses based on RNA polymerases. *Journal of Virology* 70(9): 6083–6096.

F

Feline Leukemia and Sarcoma Viruses

J C Neil, University of Glasgow, Glasgow, UK

© 2008 Elsevier Ltd. All rights reserved.

History

The description of murine leukemia viruses by Moloney and others stimulated an intensive search for similar viruses in other species. William Jarrett made the perceptive observation that lymphomas (lymphosarcomas) in cats often occurred at particularly high incidence in certain households, and in 1964 he showed that typical type C retroviruses could be demonstrated in the tumor cells by electron microscopy. He went on to show that these feline leukemia viruses (FeLVs) could be transmitted to cats where they induced lymphosarcomas and a range of degenerative diseases, including anemias and thymic atrophy.

Following these studies, Snyder and Theilen isolated a retrovirus from a feline fibrosarcoma that rapidly reproduced this tumor on inoculation into experimental cats. It is now recognized that feline sarcoma viruses (FeSVs) arise by recombination between FeLV and cellular proto-oncogenes and that, in contrast to FeLV, these viruses are not transmitted from cat to cat.

In clinical veterinary medicine, FeLV remains one of the most important viruses affecting the cat, despite advances in control of this infection. As a naturally occurring disease in an outbred host, FeLV has served as a paradigm for the natural history and molecular pathogenesis of the γ-retrovirus subfamily. It also played a foundational role in cancer genetics as a transducing agent which led to the discovery of novel cellular transforming genes.

Taxonomy and Classification

FeLVs are RNA viruses and belong to the family *Retroviridae*. They are further classified in the genus *Gammaretroviruses*.

Virion and Genome Structure

The virion particles are around 100 nm in diameter and consist of an outer membrane derived from the host cell surrounding a spherical core particle. The core encapsidates the viral genome which, as in other members of this viral family, is present as two linear single-stranded RNA molecules linked as a dimer. The virion RNA is positive-stranded and resembles cellular RNA having a 5′ cap and a 3′ poly(A) tract. As deduced from sequencing of proviral forms, the FeLV genome is around 8 kb long with a 67 base terminal redundancy and the gene order gag-pol-env.

The particles have surface spikes composed of multimers of the two env-coded proteins, the gp70 surface glycoprotein (SU), and the p15E transmembrane anchor protein (TM). Inside the envelope are the structural *gag* gene products which form a spherical core particle composed of p27, the major capsid protein (CA), with an outer layer formed by the p15 matrix protein (MA). Another gag product, the p10 nucleocapsid (NC), is associated with the virion RNA. Other minor virion proteins encoded by the *pol* gene comprise the protease (PR), reverse transcriptase (RT), and integrase (IN) enzymes.

Replication

Virus replication proceeds, following binding to specific host cell-surface receptors, internalization, and uncoating. The virion RNA is converted to a double-stranded DNA form by the virion RT which uses a proline tRNA primer and carries a ribonuclease H function that degrades the virion RNA. After nuclear translocation, viral integration is catalyzed by the IN protein and involves the creation of a staggered cut in cellular DNA with a consequent 4 bp duplication of host DNA at the insertion site.

As illustrated in **Figure 1**, the proviral form is flanked by long terminal repeats (LTRs) of 480–560 bp. These are generated during reverse transcription by duplication of unique sequences at the 3′ (U3) and 5′ (U5) ends of the RNA genome. The LTRs contain promoters and enhancers that drive transcription of viral RNA and also processing signals for cleavage and polyadenylation of the

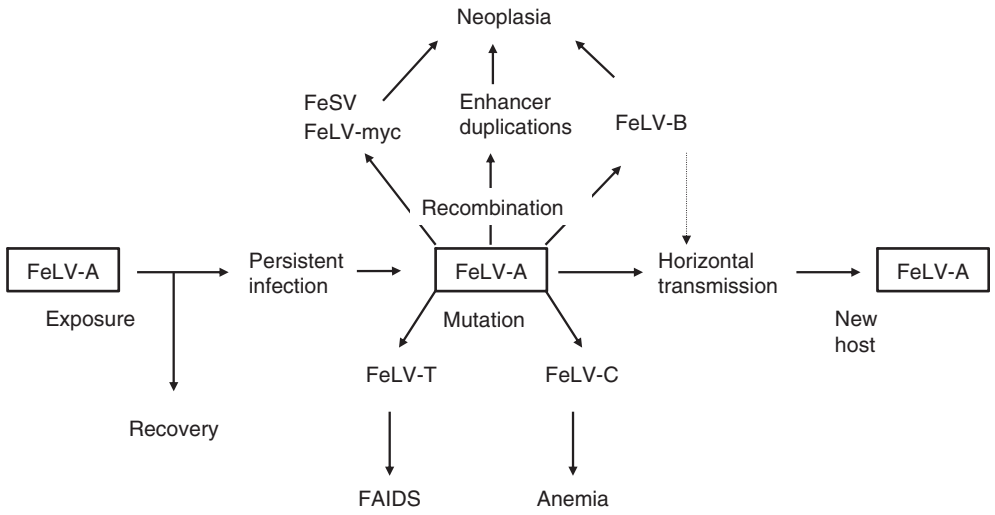


Figure 1 Life cycle of FeLV in its host, the domestic cat. FeLV-A is the most readily transmitted form and is found in 100% of isolates. In cats which become persistently viremic, this prototypic virus may evolve by recombination or point mutations to generate pathogenic variants that can lead to the rapid demise of the infected host animal. Multiple variants can arise in a single host. Few of these variants show any capacity for horizontal transmission to new hosts with the exception of FeLV-B. In this way, FeLV inflicts a substantial disease burden without significant reducing host numbers.

RNA transcripts. The 5′ LTR functions to initiate transcription while the 3′ LTR acts primarily as an RNA processing signal.

The virion RNA can function as a messenger RNA for Gag and Pol products, while a spliced subgenomic mRNA of around 3 kb encodes the Env products. Most full-length RNA translation products terminate at the 3′ end of *gag* to produce the Pr65gag precursor, while a small percentage read through into *pol* by misreading of an UAG termination codon, generating the Pr180Gag-pol precursor. During and after the budding process, the virion aspartyl PR catalyzes the cleavage of both precursor proteins to their mature forms. The envelope gene products are synthesized as a Pr80env precursor and processed by cellular PRs to the mature, disulfide-linked gp70 and p15E envelope proteins. The *gag* gene is also abundantly expressed in an alternative, glycosylated form via an upstream AUG codon. This product is expressed on the cell surface and shed after cleavage by cellular PRs. It is dispensable for *in vitro* virus replication but is highly conserved and may play a role *in vivo*.

Assembly of virus particles occurs at the cell surface by extrusion of cores which form at the budding site, concomitantly acquiring a host cell-derived outer membrane with virus-coded surface spikes. Virus replication and release is often noncytopathic.

Geographic Distribution

FeLV occurs worldwide in domestic cat populations although prevalence varies significantly and has declined in pet cats in areas where active control measures have been instigated. FeLV has also been isolated from the European wild cat (*Felis sylvestris*). Endogenous retroviral sequences closely matched to FeLV are found in the same species and in related small felids such as the sand cat (*F. margarita*) and the jungle cat (*F. chaus*). Although not a direct source of disease, these endogenous sequences can participate in recombination with exogenous FeLV to generate variant viruses with altered host range.

Epidemiology

The outcomes of FeLV infection fall into several categories, which vary in likelihood according to the age and immune status of the exposed host. The majority of cats undergo a transient infection lasting up to 3 months, during which they are viremic and shed virus. They then develop neutralizing antibody and concomitantly clear infectious virus and a little later, virus antigen from the blood stream. Some cats appear to clear virus infection successfully, while others may harbor latent virus in the bone marrow for some years. A recent study based on analysis of virus loads by polymerase chain reaction (PCR) suggests that animals that control viremia may be further subdivided into abortive, regressive, or latent infection. Even where latent infection persists, reactivation is not a frequent event and the vast majority of these cats do not develop an FeLV-related disease. However, at this stage virus can be reactivated by immunosuppression. The final group (persistent or progressive infection) remains actively infected, shedding virus from epithelial surfaces and displaying high titer plasma viremia. Such cats may remain apparently healthy for 2–3 years before succumbing to an FeLV-related disease.

The proportion of cases falling into these groups differs between multicat households and those households containing one or two free-ranging cats. In the former case, the introduction of an FeLV carrier results in repeated exposure of susceptible cats, often at a young age so that up to 30–40% become persistently viremic and at risk of disease. Approximately 50% of free-ranging urban and suburban cats have serological evidence of exposure to FeLV but only 1–5% of these cats are actively infected and the disease incidence is correspondingly lower.

Dual infection with FeLV and feline immunodeficiency virus (FIV) occurs and is associated with rapid disease onset, particularly if cats with preexisting FeLV infection encounter FIV. Rapid death of dually infected animals may reduce the apparent overlap of these agents in the field, but the populations at risk of infection also differ. FIV infection rates increase directly with age while persistent FeLV infection has a peak incidence in young cats.

FeLV Subgroups and Host Range

FeLV isolates were initially classified as subgroup A, B, C according to their viral interference properties in feline fibroblast cells *in vitro*. Viruses of a given subgroup prevent superinfection (interfere) with other viruses of the same subgroup (**Table 1**). This property is based on the use of three different host cell-surface receptors by FeLV-A, B, and C, and the blockade of these receptors in persistently infected cells by viral Env glycoproteins. The lymphotropic variant FeLV-T has more complex entry requirements, and does not replicate well in fibroblast cells. It appears that this isolate requires the FeLV-A receptor and an auxiliary mechanism in which a truncated *env* gene product encoded by endogenous FeLV sequences (FeLIX) is used as a co-receptor through binding to the FeLV-B receptor. Primary receptors for subgroups A, B, and C have been identified and shown to be transmembrane transporter molecules which the virus has subverted to gain entry to the host cell.

Natural isolates contain either subgroup A alone, or mixtures of subgroups A + B, A + C, or A + B + C. FeLV isolates of subgroup A are generally restricted to growth in feline cells, whereas subgroups B and C have a greatly expanded host range, infecting cat, human, mink, and canine cells. FeLV infection is generally noncytopathic and persistent and the virus is commonly propagated in long-term cultures of embryo-derived fibroblasts. Some strains such as FeLV-T or FeLV-C are cytopathic or induce apoptosis in lymphoid cells *in vitro*, reflecting their *in vivo* pathogenic properties.

Clinical Features and Pathology

Of those cats which become persistently viremic following FeLV exposure, over 80% die within 3.5 years. Most young cats infected with FeLV die from degenerative diseases rather than from tumors. Profound immunosuppression associated with thymic atrophy is a common finding in kittens. Other diseases seen in FeLV-infected cats include enteritis, immune complex glomerulonephritis, pancytopenia, and hemolytic anemia. Erythroid hypoplasia, an acute disease involving failure of red cell development past the burst-forming unit (BFU) stage, is specifically associated with FeLV subgroup C.

The most common neoplasm induced by FeLV is lymphosarcoma of T-cell origin, usually restricted to the thymus or sometimes occurring as a multicentric tumor in lymph nodes. The tumors often develop between 1 and 3 years after infection and the first signs may be chronic wasting and anemia. At presentation, the normal architecture of the lymphoid organ has usually been destroyed by a monomorphic infiltrate of lymphoblastic cells. The thymic tumors frequently display a rearrangement of the T-cell antigen receptor β-chain gene and may also express the co-receptor molecules CD4+ and/or CD8+. FeLV is also commonly associated with myeloid leukemias and a myelodysplastic syndrome-like disease, as well as with other forms of hematopoietic malignancy. Multicentric fibrosarcoma is a rare sequel to FeLV infection but these tumors are

Table 1 Properties of feline leukemia virus subgroups

Subgroup	*Origin*	*Receptor*	*Function*	*Pathogenesis*
A	Exogenous	feTHTR1?	Thiamine transport?	Minimally pathogenic to acute immunosuppression
B	Recombination FeLV-A × endogenous FeLV	Pit-1 (Pit-2)	Phosphate transport	More common in leukemic cats Some isolate-specific diseases, e.g., FeLV-GM1 myeloid leukemia
C	Mutation of FeLV-A (Env vrA)	FLVCR	Heme export	Erythroid hypoplasia
T	Mutation of FeLV-A (outside RBD)	? FeLV-A receptor +FeLIX, Pit-1		Acute immunosuppression

often associated with the *de novo* generation of a FeSV. FeLV is also found in association with 35% of alimentary tumors, primarily of B cell origin, but the virus is not always clonally integrated in these tumors and the role of the virus is, therefore, unclear. FIV can also increase the frequency of malignant diseases in cats, and some rare cases arise on a background of dual infection.

Envelope Gene Variation and Pathogenicity

The common infectious form of FeLV is FeLV-A which is a remarkably highly conserved virus as shown by sequence analysis of several strains and serotypic analysis of a much larger number. FeLV variants frequently arise from FeLV-A by mutation and recombination, and such variants are often implicated in the acute diseases which develop in persistently infected cats. The variant viruses generated from FeLV-A are generally dependent on the continued presence of the prototype for their propagation *in vivo*. However, as the variants are less efficiently transmitted and are in some cases rapidly fatal, they tend to die out with the host while the prototypic FeLV-A continues to colonize new hosts (**Figure 1**).

The most commonly isolated FeLV recombinants are subgroup B viruses (**Table 1**). These are derived by recombination between FeLV-A and endogenous FeLV-related proviruses which are found in the genome of the domestic cat and related small feline species. Although the endogenous FeLV-related proviruses all appear to be replication defective, their envelope genes can be rescued by the recombination process leading to the generation of FeLV-B viruses. FeLV-B can infect cells refractory to, or already containing, FeLV-A by virtue of their distinctive receptor specificity.

The anemia-inducing FeLV-C isolates are rarer and appear to be derived from FeLV-A by mutation of a single variable domain (VRA) of the *env* gene. The acute disease properties of these variants appear to be due to compromised viability of erythroid progenitor cells due to downregulation of the FeLV-C receptor, a vital heme exporter.

Minor *env* mutations also appear to give rise to the acutely immunosuppressive FeLV-FAIDS variants. The prevalence of acutely immunosuppressive viruses in nature is unknown, but immunosuppressive disease is a common manifestation of FeLV infection.

The relationship of subgroup variation to oncogenesis is complex. FeLV-B recombinants are more common in tumor bearing than in infected asymptomatic cats. This higher frequency might reflect merely longer-standing infection, but some FeLV-B-containing isolates have an altered spectrum of neoplastic disease. For example, FeLV-GM1, which contains a replication-defective FeLV-B component, induces mainly myeloid leukemia.

FeLV Oncogenesis: Virus Evolution and Mutagenesis of Cellular Oncogenes

Two modes of virus-induced host gene mutation have been described in FeLV-associated cancers. The first is 'transduction', where recombination leads to the generation of an acutely oncogenic variant in which viral gene sequences are replaced by a host-derived insert. Such viruses are replication defective and are found in nature in association with a replication-component FeLV helper virus.

Multicentric fibrosarcomas of young cats are relatively rare, but are generally FeLV positive and frequently involve a novel sarcoma virus. Similarly, transduction of c-*myc* has been observed in up to 20% of naturally occurring thymic lymphosarcomas in FeLV positive cats. In all, nine different host cell genes have been shown to be transduced by FeLV (**Table 2a**). The transducing viruses induce tumors with short latency in cats and in the case of FeSVs may transform cells in tissue culture.

Alternatively, host genes can be affected by proviral 'insertional mutagenesis' (*cis*-activation). Four known oncogenes and an uncharacterized novel integration locus have been identified as common tumor-specific insertion

Table 2a FeLV gene transduction in neoplasia

Gene	*Normal function of host gene product*	*Associated tumor*	*Examples*[a]
abl	Plasmamembrane protein kinase	Fibrosarcoma	FeSV-HZ2
fes	Plasmamembrane protein kinase	Fibrosarcoma	FeSV-GA,-HZ1,-ST
fgr	Plasmamembrane protein kinase	Fibrosarcoma	FeLV-GR,-TP1
fms	Receptor protein kinase (CSF-1 receptor)	Fibrosarcoma	FeSV-SM,-HZ5
kit	Receptor protein kinase (SCF receptor)	Fibrosarcoma	FeSV-HZ4
myc	Transcription factor	T-cell lymphoma	FeLV (T3, T17, FTT)
Notch2	Transmembrane receptor	T-cell lymphoma	(Inoculum FeLV-61E)[a]
sis	Growth factor (B chain PDGF)	Fibrosarcoma	FeSV-PI
tcr	T-cell antigen receptor (β-chain)	T-cell lymphoma	FeLV-T17

[a]Isolated from naturally occurring tumors apart from the indicated exception.

Table 2b Insertional mutagenesis and FeLV oncogenesis

Common integration site	*Gene function*	*Tumor*
fit-1	Transcription factor (c-*myb*)	T-cell lymphoma
flvi-1	Unknown	non-T, non-B lymphoma
flvi-2 (bmi-1)	Transcription factor	T-cell lymphoma
c-myc	Transcription factor	T-cell lymphoma
pim-1	Protein kinase	T-cell lymphoma

sites for FeLV in thymic lymphosarcomas (**Table 2b**). In this respect FeLV oncogenesis appears remarkably similar to that of the murine γ-retroviruses, and most studies of this process have been conducted recently in the laboratory mouse which has the advantages of complete genome sequence and the opportunity to manipulate the germline.

Changes within the LTR are also a feature of tumor-associated FeLV. In thymic lymphosarcomas, sequence duplications of the core enhancer domain are frequently found, and have been shown to arise *de novo* from infection with molecularly cloned FeLV isolate lacking such features. By analogy with the murine oncoretroviruses, the duplications are likely to increase the oncogenicity of the virus and reduce the latent period for tumor formation, possibly by increasing the potency of viral enhancer activity on nearby cellular promoters. There is evidence that these adaptive changes to the LTR operate tissue-specifically and proviruses carrying different duplications of LTR regions 5′ and 3′ to the core-enhancer region have been identified in myeloid leukemias and non-T, non-B splenic lymphomas, respectively. Chimeric murine retroviruses carrying the FeLV enhancer region have been generated, confirming that the tissue specificity is carried by this structure.

Immune Response

Unlike infection with the lentiviruses HIV and FIV, FeLV infection of cats may lead to recovery. There is evidence that both virus-specific cytotoxic T cells (CTLs) and neutralizing antibodies play a role in resistance as either can be used to prevent or limit infection by passive transfer. In natural infection, CTLs to virus structural protein epitopes can be detected as early as a week after infection and may be the primary means of control of infection as neutralizing antbodies are not detected until around 6 weeks post infection.

In the early literature on FeLV a distinction was made between antiviral immunity and antitumor immunity. Cats with antitumor (FOCMA) antibody were thought to be protected from tumor development. This antibody response is now believed to be directed to endogenous FeLV *env* proteins and its role in modulating tumor development is uncertain.

Transmission

Cats persistently infected with FeLV shed virus in their saliva, urine, and feces but, as the virus is fragile, close contact is required for transmission. The most frequent routes involve saliva and transplacental spread. Kittens infected *in utero* become persistently infected, but the consequences of infection in older cats depend on a number of factors. There is an age-related resistance to infection such that cats up to 12 weeks of age are highly susceptible, but above 16 weeks they are difficult to infect either naturally or experimentally.

FeLV subgroup A is always found in field isolates and about half also contain FeLV-B, whereas FeLV-C is present in only 1–2% of isolates. Although FeLV-B can arise *de novo* by recombination, it may also be transmitted between cats. This occurrence is dependent on pseudotype formation in which the genome of the B virus becomes enclosed in an envelope containing glycoproteins of the A subgroup.

Prevention and Control

Successful control measures can be adopted in multicat households by removing or isolating persistently infected animals. Productively infected cats are detected by virus isolation from plasma or more usually by enzyme-linked immunosorbent assay (ELISA) for virus antigen in the blood. A few cats remain persistently antigenemic but nonviremic. These cats do not usually transmit the virus unless they are shedding virus in the milk or saliva. Assays are conducted twice, 3 months apart to exclude cats that are transiently viremic.

Numerous vaccine strategies have been shown to offer protection against FeLV in laboratory conditions (**Table 3**) and FeLV was the first retrovirus for which commercial vaccines were developed. Vaccines in current use include whole inactivated virus preparations, subunit vaccines from recombinant viral Env protein expressed in bacterial cells, and a canarypox recombinant virus expressing Gag and Env. These vaccines offer a measure of protection against experimental challenge and are under evaluation for longer-term efficacy in the field. These vaccines do not generate sterilizing immunity but appear to prime the immune system to favor clearance of virus infection instead of persistent viremia.

Table 3 FeLV vaccines

Vaccine	*Protection*	*Commercial use*
Live attenuated FeLV	Yes	No (safety concerns)
Inactivated whole virus	Yes	Yes
Subunit vaccines		
SU from *E. coli*	Yes	Yes
ISCOM-Env (native)	Yes	No
Lymphoma cell extract	Yes (poor in some studies)	Withdrawn
Live vector vaccine		
Vaccinia-Env	No	No
Canarypox-Env-Gag	Yes	Yes
Feline herpesvirus-Env	Partial	No

Future Perspectives

There is continuing interest in control of FeLV infection due to its importance in veterinary medicine. In the future we can look forward to improvements in vaccine efficacy and further dissection of the host responses that confer protection. While the focus of attention of cancer genetics has moved on to more easily manipulated models, FeLV remains as a useful touchstone for our understanding of retroviral pathogenesis in an outbred, naturally infected host. Also, with the impetus of FIV as a model for human AIDS, the generation of reagents to probe the feline immune system offers new opportunities for comparative study of FeLV.

See also: Bovine and Feline Immunodeficiency Viruses.

Further Reading

Flynn JN, Dunham SP, Watson V, and Jarrett O (2002) Longitudinal analysis of feline leukemia virus-specific cytotoxic T lymphocytes: Correlation with recovery from infection. *Journal of Virology* 76: 2306–2315.

Hanlon L, Barr NI, Blyth K, *et al.* (2003) Long-range effects of retroviral activation on c-myb over-expression may be obscured by silencing during tumor growth *in vitro*. *Journal of Virology* 77: 1059–1068.

Mendoza R, Anderson MM, and Overbaugh J (2006) A putative thiamine transport protein is a receptor for feline leukemia virus subgroup A. *Journal of Virology* 80: 3378–3385.

Miyazawa T (2002) Infections of feline leukemia virus and feline immunodeficiency virus. *Frontiers in Bioscience* 7: D504–D518.

Roca AL, Nash WG, Menninger JC, Murphy WJ, and O'Brien SJ (2005) Insertional polymorphisms of endogenous feline leukemia viruses. *Journal of Virology* 79: 3979–3986.

Sparkes AH (2003) Feline leukaemia virus and vaccination. *Journal of Feline Medicine and Surgery* 5: 97–100.

Torres AN, Mathiason CK, and Hoover EA (2005) Re-examination of feline leukemia virus: Host relationships using real-time PCR. *Virology* 332: 272–283.

Tsatsanis C, Fulton R, Nishigaki K, *et al.* (1994) Genetic determinants of feline leukemia virus-induced lymphoid tumors: Patterns of proviral insertion and gene rearrangement. *Journal of Virology* 68: 8294–8303.

Filamentous ssDNA Bacterial Viruses

S A Overman and G J Thomas Jr., University of Missouri – Kansas City, Kansas City, MO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cloning vector The DNA molecule of a virus, plasmid, or cell into which a foreign DNA fragment can be integrated without loss of self-replicating activity. The vector introduces the foreign DNA fragment into an appropriate host cell for autonomous replication, usually in large quantity.

Phage library An ensemble of up to about 10^{10} phage clones, each harboring a different foreign coding sequence in-frame with either the N- or C-terminal region of a capsid protein gene. The clone thus allows display of a different 'guest' peptide on the virion surface.

Raman spectroscopy The branch of optical spectroscopy (named after its 1928 founder, C. V. Raman) concerned with measurements of the intensities of light scattered inelastically by molecules that have been excited by monochromatic radiation. The resulting spectrum, usually a plot of scattering intensity (in arbitrary units) versus energy (in wave number or cm^{-1} units), reflects the transfer of discrete energy quanta from the impingent photons to vibrational energy states of the molecules. The Raman spectrum, which is determined by both intramolecular bonding arrangements (covalency and conformation) and intermolecular interactions, provides a sensitive

signature of molecular structure and local environment.

Trans-envelope network A multiprotein complex that is located in the envelope of a bacterial cell and brings the inner and outer membranes in close proximity to one another.

Ultraviolet resonance Raman spectroscopy (**UVRR**) A type of Raman spectroscopy (see above) in which the wavelength of the exciting monochromatic radiation is in the ultraviolet region (i.e., wavelength $\lambda < 400$ nm, or wave number $\sigma > 25\,000$ cm^{-1}), so as to achieve resonance with electronic absorption processes of the molecules. In UVRR spectra, only the vibrational states of the chromophore are represented.

Introduction

The filamentous ssDNA viruses (genus *Inovirus*) are members of a genus of morphologically similar virions that infect different bacteria via molecular recognition of a host-specific pilin. The most well studied of these phages are the closely related M13, fd, and f1 virions, which infect *Escherichia coli* displaying a conjugative F-pilus. The genome sequences of these F-specific phages are sufficiently similar that they are collectively called Ff phage. Other filamentous phages that have been studied include IKe, which infects *E. coli* displaying a conjugative N-pilus, Pf1, which infects *Pseudomonas aeruginosa* strain PAK by binding to the bacterial type IV pilus, Pf3, which infects *P. aeruginosa* strain PAO by binding not to the inherent type IV pilus but to the conjugative RP4 pilus, and PH75, which infects *Thermus thermophilus*. These filamentous phages are nonlytic and nonlysogenic. On the other hand, the filamentous ssDNA bacteriophage CTXφ, which infects *Vibrio cholera* by recognition of a toxin-co-regulated pilus (TCP), is lysogenic.

Genetic, biochemical, and biophysical methods have been used to study the Ff bacteriophage life cycle, which is unique in its use of the bacterial cell envelope for virus assembly. Features of the nonlytic Ff assembly pathway are (1) the prolific production of phage particles, (2) the extrusion of progeny virions through the cell envelope, and (3) the requirement of stable transmembrane (TM) domains in virally encoded proteins that participate in the assembly process. Also noteworthy in Ff morphogenesis is that genomes of variable size can be packaged without deleterious consequences. For example, although the single-stranded DNA (ssDNA) genome of wild-type Ff (~6400 nt) is sheathed by 2750 copies of the major capsid protein, viable variants have been isolated containing as many as 12 000 nt and corresponding modifications in the filament length and number of subunits in the sheath. Biophysical studies have shown that the Ff phage particles are highly thermostable and highly flexible. Polymorphism of the viral capsid is also revealed by electron cryomicroscopy.

The life cycle of the filamentous ssDNA bacteriophage enables its use as a model for membrane-associated nucleoprotein assembly and as a valuable tool for molecular cloning, phage display, and pharmacotherapy. The filamentous ssDNA bacteriophage also serves as a vehicle for orientation of small molecules in solution spectroscopic (nuclear magnetic resonance, NMR) applications and as a model for nanowire self-assembly. Details of the molecular structure and life cycle of the filamentous ssDNA bacteriophage are considered in this article.

Taxonomy and Classification

The filamentous ssDNA bacterial viruses, which are visualized in electron micrographs as thin cylindrical filaments about 7 nm in diameter and ranging from 700 to 2000 nm in length, belong to the genus *Inovirus* of the family *Inoviridae*. They are distinguished from rod-shaped members of the genus *Plectrovirus* of the same family (typically 85–280 × 10–15 nm dimensions) by their greater contour length, smaller diameter, and lower flexural rigidity. The many species of the genus *Inovirus* so far identified have been categorized into four broad groups on the basis of the types of bacteria infected. The species encompassed by these four groups are listed in **Table 1**, in accordance with the classification of the International Committee on Taxonomy of Viruses (ICTV). The best characterized of the filamentous viruses with respect to both biological and structural properties is the coliphage M13, which serves as the prototype of the species *Enterobacteria phage M13*. This species also includes the closely related phages f1, fd, AE2, dA, Ec9, HR, and ZJ/2.

Physical Properties

Table 2 lists selected physical properties of several well-studied filamentous ssDNA bacterial viruses. Although each of the viruses included in **Table 2** exhibits a characteristic filamentous shape, significant differences occur in their contour lengths and in the mass ratio of capsid protein to DNA. Accordingly, differences are also anticipated in the packing arrangement of capsid subunits with respect to the encapsidated genome. The overall length of the viral filament is dictated by both the genome size and the number of subunits required to electrostatically balance the negatively charged DNA phosphates, which is accomplished by the distribution of basic side chains (Lys and Arg) near the subunit C-terminus. On the basis of fiber X-ray diffraction results, two distinct symmetry classes (I and II) have been

Table 1 Species of the genus *Inovirus*[a]

Host type	*Species*
Enterobacteriaceae	*Enterobacteria phage M13*,[b] *C-2, If1, IKe, I2–2, PR64FS, SF, tf-1, X,* and *X-2*
Spirillaceae	*Vibrio phage 493, fs1, fs2, CTX, v6, Vf1, Vf33,* and *VSK*
Pseudomonadaceae	*Pseudomonas phage Pf1, Pf2,* and *Pf3*
Xanthomonadaceae	*Xanthomonas phage Cf16, Cf1c, Cf1t, Cf1tv, Lf, Xf, Xfo,* and *Xfv*

[a]The species are categorized by host type.
[b]Enterobacteria phages fd, f1, and several others have been classified as a type of *Enterobacteria phage M13*.

Table 2 Physical properties of filamentous ssDNA bacteriophages

Property	*M13*	*Pf1*	*Pf3*	*Xf*	*PH75*[a]
Virion length (nm)	880	1900	680	980	910
Percent mass protein	87	93	85	85	84
Capsid subunits per virion	2750	7370	2500	3500	2700
Residues per subunit	50	46	44	44	46
Nucleotides per genome	6407	7390	5830	7420	6760
Nucleotides per subunit	2.3	1.0	2.3	2.1	2.5
λ_{max} (nm)	269	270	264	263	267
Extinction coefficient ($cm^2\,mg^{-1}$)	3.84	2.07	4.53	3.52	3.77
Symmetry class[b]	I	II	II	II	II

[a]The thermophilic filamentous ssDNA bacteriophage, PH75, which infects *Thermus thermophilus*, has not yet been assigned to a species, genus, or family by the International Committee on Taxonomy of Viruses.
[b]Class I phages have capsid subunits arranged with C_5S_2 symmetry (fivefold rotational symmetry and approximately twofold screw axes) and class II phages have capsid subunits arranged with $C_1S_{5.4}$ symmetry (no rotational symmetry and a single-start superhelical array of approximately 5.4 subunits per turn).

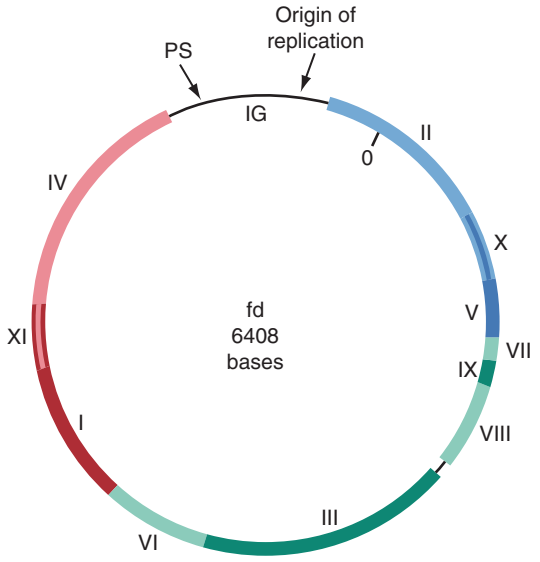


Figure 1 The genome organization of the Ff (fd) bacteriophage. All genes (I–XI) are labeled and color-coded by function of the gene product (blue, DNA synthesis; green, capsid; red, virus assembly). The single-stranded DNA genome of fd contains 6408 nt, which are numbered clockwise from the unique *Hin*dII site located in gene II. The intergenic region (IG) is located between genes II and IV and includes the packaging signal (PS) and the origin of replication. Adapted from Petrenko VA and Smith GP (2005) Vectors and modes of display. In: Sidhu S (ed.) *Phage Display in Biotechnology and Drug Discovery*, pp. 63–110. Boca Raton, FL: CRC Press.

defined to categorize subunit/DNA packing arrangements: subunits of the class I phages (e.g., M13, f1, and fd) are arranged with approximate C_5S_2 symmetry, while class II phages (e.g., Pf1, Pf3, and PH75) exhibit $C_1S_{5.4}$ symmetry. Further details of virion structure are discussed in the section titled 'Composition and structure'.

Genome Organization

The genomes of many filamentous viruses, including M13, f1, fd, IKe, Pf1, Pf3, PH75, and CTX, have been sequenced. Comparative analyses indicate that at least those of M13, f1, fd, IKe, and PH75 are similarly organized, as shown in **Figure 1** for fd.

Of the 11 virally encoded Ff genes, two (X and XI) overlap and are in-frame with larger genes (II and I, respectively). The Ff genome contains only one significant noncoding region (intergenic region, IG) consisting of about 500 nt. The IG contains the origin of replication, as well as a functionally and structurally distinct region called the packaging signal (PS), which is capable of forming a hairpin of 78 nt. During the phage life cycle, recognition of the genomic PS by virally encoded proteins initiates assembly of the viral particle. The viral genes are expressed from the origin of replication in a counterclockwise direction and are organized on the genome in functional groups, such that genes II, V, and X are grouped together and express proteins that facilitate DNA replication; genes III, VI, VII, VIII, and IX are grouped together and express structural proteins found in the mature infectious virion; and genes I, IV, and XI are grouped together and express proteins that direct virus assembly. The CTX genome lacks gene IV but contains genes that encode proteins specific to the CTX life cycle.

Composition and Structure

The typical filamentous virion consists of a covalently closed, ssDNA genome (5–10 kbp) sheathed by several thousand copies of the major capsid protein (pVIII, ~5 kDa) plus a few copies of minor proteins at the filament ends (**Figure 2**). The numbers of pVIII subunits and DNA nucleotides in several well-studied filamentous phages are given in **Table 2**. The pVIII subunits of the *Enterobacteria phage M13* species members (including phages M13, f1, and fd) have been studied extensively and can be discussed collectively. (Note that the amino acid sequence of the pVIII subunits in fd and f1 are identical and differ from that of M13 by a single amino acid change, namely Asp12 of fd (and f1) is replaced by Asn12 in M13; see **Table 3**.) It is evident from **Table 3** that the pVIII sequence in many filamentous viruses comprises three distinct components, an acidic N-terminal region, a hydrophobic central region, and a basic C-terminal region, suggesting that these fulfill distinct functions in phage morphogenesis.

Here, we consider in further detail the composition and structure of phage fd, which has served as the focus of many detailed and comprehensive biochemical and biophysical studies. The pVIII subunit is generally modeled as a gently curved α-helix that is tilted at a small average angle ($16 \pm 4^{\circ}$) relative to the filament axis. The N-terminus of pVIII is exposed on the exterior of the capsid and the C-terminus lines the core, where it is presumed capable of contacting phosphates of the packaged ssDNA. Fiber X-ray diffraction studies indicate that pVIII subunits of the capsid lattice are arranged in a right-handed slew with near-fivefold rotational symmetry and twofold screw symmetry (**Figure 2**). Five copies of each of four minor proteins form the ends of the fd filament. The initially assembled end ('head') contains minor proteins pVII (3.6 kDa) and pIX (3.6 kDa), while the ultimately assembled end ('tail') contains minor proteins pIII (42.6 kDa) and pVI (12.3 kDa). The packaged genome is oriented with its IG region (PS hairpin) at the head, gene III region near the tail, and the intervening non-base-paired antiparallel strands of the DNA loop spanning the length of the filament core (**Figure 2**). Phage display and genetic studies suggest further that the minor proteins pVII and pVI at the head and tail, respectively,

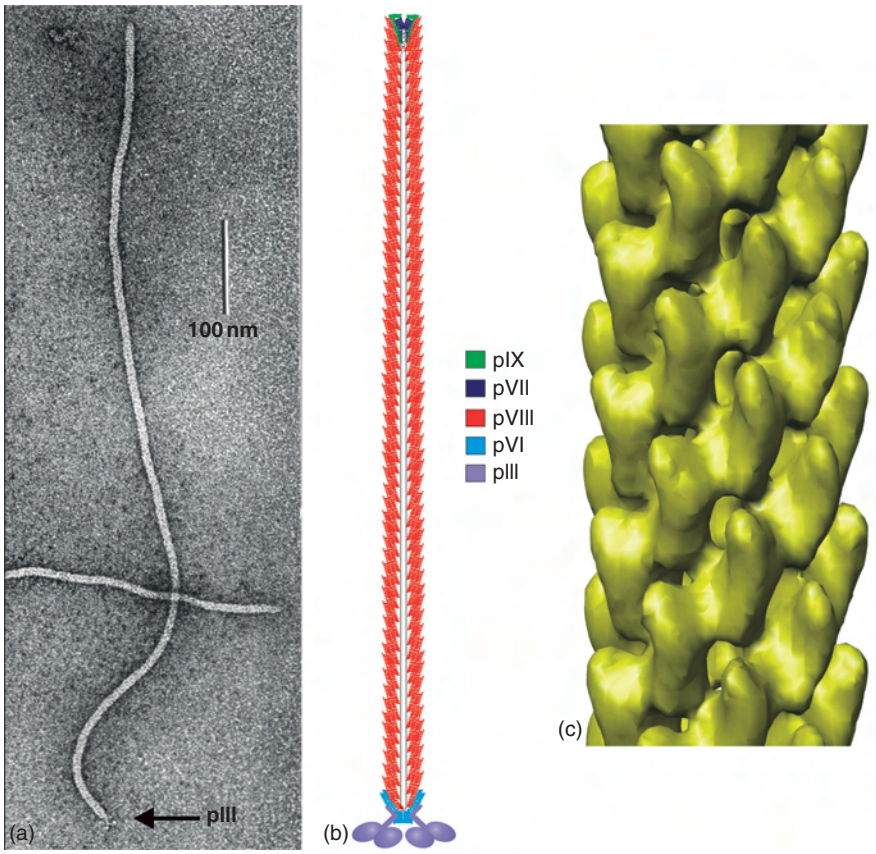


Figure 2 The fd filamentous ssDNA bacteriophage. (a) A negatively stained electron micrograph showing one complete virion (top to bottom) oriented with the pIII tail (arrow) at the bottom. The flexibility of the virion, which is apparent, is consistent with the experimentally determined equilibrium persistence length of 1.0 μm. (b) Cartoon of fd (not to scale) showing locations of the minor coat proteins. (c) Reconstruction of a 24 nm segment of fd at ~8 Å resolution from images obtained by electron cryomicroscopy. (a) Courtesy of Carla W. Gray, University of Texas, Dallas. (c) Courtesy of E. H. Egelman, University of Virginia.

Table 3 pVIII sequences for filamentous ssDNA bacteriophages

Phage	*pVIII sequence*[a]
M13	[1]AEGDDPAKAAFNSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS[50]
Pf1	[1]GVIDTSAVESAITDGQGDMKAIGGYIVGALVILAVAGLIYSMLRKA[46]
Pf3	[1]MQSVITDVTGQLTAVQADITTIGGAIIVLAAVVLGIRWIKAQFF[44]
Xf	[1]SGVGDGVDVVSAIEGAAGPIAAIGGAVLTVMVGIKVYKWVRRAM[44]
PH75	[1]MDFNPSEVASQVTNYIQAIAAAGVGVLALAIGLSAAWKYAKRFLKG[46]

[a]The hydrophobic segment of each pVIII sequence is underlined.

form contacts with the neighboring pVIII subunits, while pIX and pIII decorate the surfaces of the filament tips. Each pIII subunit is known to contain a globular N-terminal domain that is visible in electron micrographs (**Figure 2**).

In the mature fd virion, pVIII is predominantly α-helical and the C-terminal end of the α-helix (residues 40–50) consists of 'basic' and nonpolar side chains along opposite faces. Although direct experimental evidence is lacking, model-building exercises have revealed that it is possible to position the positively charged side chains of this amphipathic helix proximal to the surface of the capsid core, where interaction with packaged DNA would be facilitated. The net positive charge of the C-terminal region apparently functions to neutralize the negatively charged DNA phosphates. This is supported by the fact that mutating Lys 48 of pVIII to an uncharged side chain results in the assembly of an appropriately elongated (∼35%) fd virion; that is, additional pVIII subunits are required to package the genomic complement. The opposite nonpolar (hydrophobic) face of the C-terminal helix provides a suitable surface for intersubunit contacts.

The N-terminal region of pVIII (residues 1–20) also forms an amphipathic helix, in this case with 'acidic' and nonpolar faces. The former is exposed on the capsid surface, while the latter is presumably engaged in inter-subunit contacts. In aqueous environments of low or moderate ionic strength, electron micrographs reveal extensive intervirion clustering, which may be mediated by the acidic (and/or polar) residues of the capsid surface. In some micrographs, liquid-crystalline phase formation is evident. Although high ionic strength conditions tend to disfavor such intervirion clustering, the bundles once formed are not readily dissociated.

The highly hydrophobic central region of the pVIII α-helix (residues 21–39) appears to serve as the pillar of capsid stability. This α-helical segment also appears to function as a distinct domain in the assembly process, as is discussed in the next section. Studies based upon site-directed and random mutations of residues throughout the central α-helix region have contributed significantly to an understanding of inter-subunit contacts in both the mature virion and its assembly precursors. As implied above, hydrophobic contacts between subunits involve not only the central region of the α-helix but also the nonpolar faces of the N- and C-terminal segments. The periodicity of small nonpolar side chains (Gly, Ala) along specific faces of the α-helix facilitates close packing of subunits. For example, small side chains appear to be required at pVIII positions 25, 34, 35, and 38 for fd capsid stability. A similar requirement applies to the capsid subunits of other filamentous bacteriophages.

Genetic and biophysical studies of proteins show that the pentapeptide motif Gly/Ala-X-X-X-Gly/Ala (where X is any residue) increases the thermostability of packed α-helices. Of the sequences listed in **Table 3**, all contain at least one Gly/Ala-X-X-X-Gly/Ala motif within the central hydrophobic region (residues 21–39). Interestingly, the corresponding segment of the pVIII subunit of the thermophilic *T. thermophilus* phage PH75 contains four Gly/Ala-X-X-X-Gly/Ala motifs among 11 small side chains (residues Ala and Gly). Conversely, the mesophile infecting fd phage contains two Gly/Ala-X-X-X-Gly/Ala motifs among six small side chains of the 19-peptide region. In summary, hydrophobic interactions lead to close packing of pVIII helices, which is likely the principal source of capsid stability.

The multifunctional minor capsid protein pIII, which directs host infection, progeny virus assembly termination, and virion tail stabilization, has been the subject of several structural studies. The 406-amino-acid protein comprises three domains, namely two closely associated and globular N-terminal domains (N1 and N2, consisting of residues 1–67 and 88–218, respectively) that protrude from the capsid, and a stalk-like C-terminal domain (residues 253–406) that is anchored to the capsid. The domains are linked by flexible, glycine-rich sequences. The crystal structure of the N1–N2 fragment indicates a largely β-stranded structure.

The conformation of the packaged genome is not known for fd or any other filamentous phage. Because DNA typically represents a very small percentage of the virion mass (6–16%, **Table 2**), most biophysical probes do not yield definitive structural information regarding the packaged genome. Methods that selectively probe the ssDNA bases, such as ultraviolet resonance Raman (UVRR) spectroscopy, reveal strong hypochromic effects in the fd genome, which implies close contact between the purine and pyrimidine bases. Conversely, UVRR studies of Pf1 suggest unstacked bases in its packaged genome.

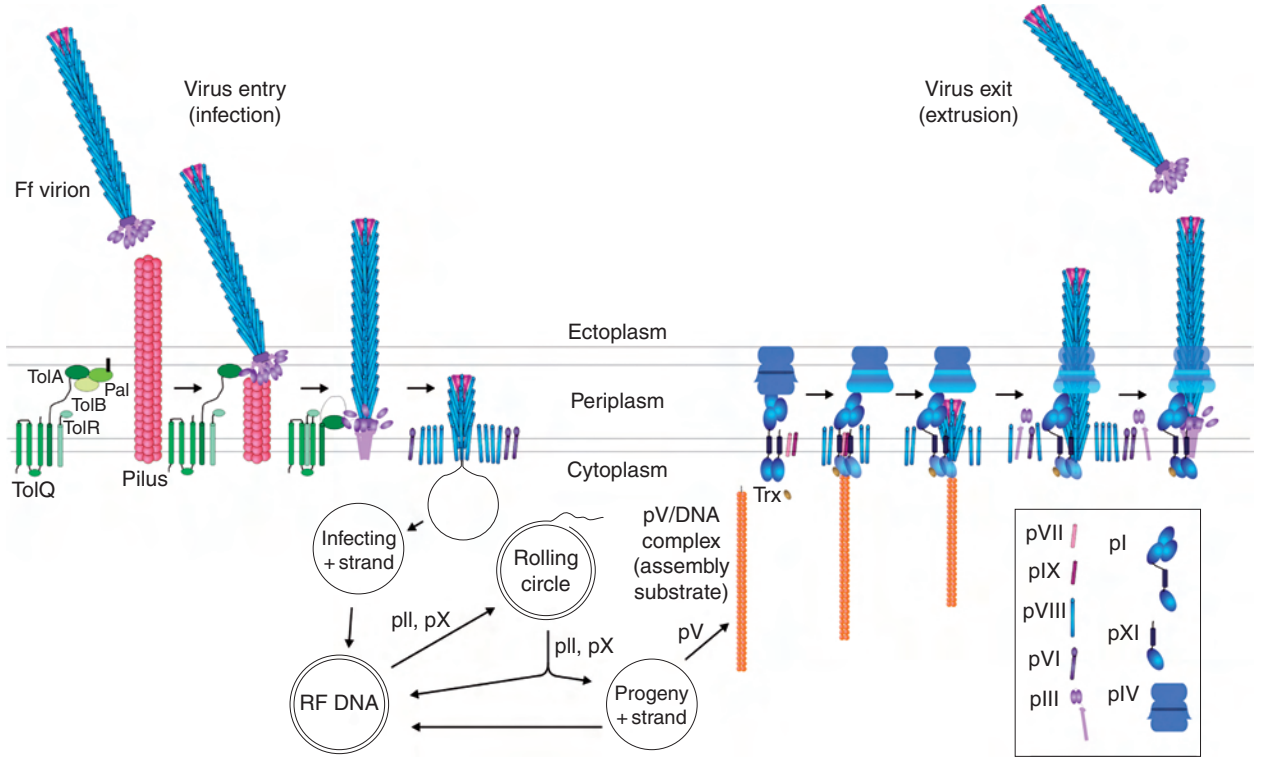


Figure 3 Schematic representation of the Ff life cycle. In the periplasmic space, the arrows show progressive stages of disassembly (left) and assembly (right) as discussed in the text. Viral and bacterial components are not drawn to scale (e.g., the actual dimensions of the Ff virion are 6.5 × 880 nm). Pilin biogenesis proteins are not shown.

Raman spectroscopy also indicates very different local deoxynucleoside conformations in the packaged ssDNA molecules of phages fd and Pf1.

Life Cycle

Infection

The mechanism of host cell infection by a filamentous bacteriophage has been studied in detail only for the bacteriophage Ff, which recognizes the conjugative F pilus of *E. coli* cells harboring the F plasmid. The process involves a trans-envelope network of host proteins – the Tol–Pal system – that is located in the *E. coli* cell envelope and serves to maintain the stability of the outer membrane. The Tol–Pal system includes the cytoplasmic membrane proteins TolA, TolR, and TolQ, which are anchored to the inner membrane, and the proteins TolB and Pal, which are associated with the outer membrane. TolA consists of an N-terminal TM domain, a periplasmic spanning α-helix, and a C-terminal globular domain also located in the periplasmic space. These domains are linked by glycine-rich segments. Like TolA, TolR has TM and periplasmic domains, whereas TolQ lacks a periplasmic domain and is located predominantly in the cytoplasm. TolA, TolR, and TolQ are associated with one another via their TM domains and all are necessary for viral infectivity. TolB is a periplasmic protein and Pal is a lipoprotein that is anchored to the outer membrane. Interaction of TolA with both TolB and Pal connects the outer and inner membranes of the *E. coli* cell. In the initial step of infection (**Figure 3**), the N2 domain of pIII binds the F-pilus, inducing a conformational change in N1–N2 that exposes a site of the N1 domain specific for TolA binding. The conformational change in N1–N2 allows a fast *cis*–*trans*-isomerization of the peptide bond between Gln212 and Pro213. When the peptide bond is in the more stable *trans*-form, the TolA binding site is exposed. Isomerization to the initial *cis*-form is a slow reaction, the rate of which is determined by the sequence of amino acids flanking Pro213. The rapid isomerization of the Gln212–Pro213 bond to the *trans*-form is a molecular switch controlled by the slower reverse reaction, which acts as a molecular timer. During the time that the N1 TolA binding site is exposed, the F-pilus is believed to retract through a secretin channel in the outer membrane of the host cell, by depolymerization of pilin, thus translocating pIII into the periplasm. The N1 domain of pIII binds the periplasmic C-terminal domain of TolA, the second phage receptor, forming a bridge between the adsorbed phage particle and the bacterial inner membrane. Subsequently, the C-terminal domain of pIII directs continuation of the infection process. Genetic studies suggest that the TolA–N1 interaction causes a conformational

change in the pIII C-terminal domain, which 'unlocks' the capsid. Once unlocked, a TM region of the pIII C-terminal domain likely interacts with the inner membrane to initiate dissociation of the capsid proteins, pVIII, pVII, and pIX, into the inner membrane with concomitant release of the viral genome into the cytoplasm. The capsid proteins of the infecting virion are retained in the membrane for use in the assembly of progeny virions.

DNA and Protein Synthesis

Upon entry into the cytoplasm, the ssDNA viral genome is converted into a replicative double-stranded form (RF) by the activity of host-encoded polymerases (RNA Pol and DNA Pol III) and a host-encoded single-stranded DNA-binding protein (SSB). To initiate this event, Pol synthesizes a short RNA primer beginning at nucleotide 5753. Pol III extends this primer the length of the viral strand, and Pol I and ligase close the complementary strand to produce a circular, dsDNA molecule. Gyrase supercoils the circle, thus resulting in the RF DNA, which serves as the template for transcription of viral genes.

Synthesis of ssDNA viral molecules requires the virally encoded protein pII, which specifically nicks the viral strand ((+)-strand) of RF DNA. The resultant 3′ hydroxyl group is used by the host DNA synthesizing apparatus (Pol III, SSB, and Rep helicase) to generate a new (+)-strand by the rolling-circle mode of replication, thus displacing the parent (+)-strand, which is subsequently circularized by pII. The newly synthesized RF DNA is closed and supercoiled for additional rounds of replication. The displaced and circularized (+)-strand can serve as either a substrate for additional RF DNA formation (template for (−)-strand synthesis) or a virus genome (encapsidation). At early stages of infection, the former path is more likely; at later stages, as the concentrations of virally encoded proteins become large, encapsidation is favored.

The (−)-strand serves as the template for transcription of viral genes, beginning at gene II. The presence of two strong terminators, one of which is rho-independent between genes VIII and III and the other rho-dependent in the IG, divides the genome into regions that are relatively frequently (genes II–VIII) and infrequently (gene III–IG) transcribed. The former has three strong promoters that lead to three primary transcripts. Post-transcriptional modification of these primary transcripts results in a set of six smaller and more stable mRNA molecules, all of which encode pVIII and a subset (four) of which encode pV. Multiple transcripts facilitate synthesis of the large amounts of pV and pVIII required to coat the prolifically produced (+)-strands (up to 200–300 progenies per cell). The frequently transcribed region also includes genes for the minor capsid proteins pVII and pIX. However, the translational initiation sites for synthesis of these proteins are weak. Accordingly, pVII and pIX are not produced in abundance. Proteins pII and pX, which likewise are encoded in the frequently transcribed region but not required in abundance, are regulated at the translational level via pV binding to the mRNA transcripts. This inhibition is evident at high cellular concentrations of pV, when amounts of the protein exceed the threshold required to sequester (+)-strands targeted for viral assembly.

Transcription of the genome and translation of the mRNA transcripts produces 11 viral proteins, of which pII, pX, and pV are required for (+)-strand synthesis, pIII, pVI, pVII, pVIII, and pIX are incorporated into the capsid, and pI, pIV, and pXI facilitate membrane-associated assembly. pII (409 residues) is a strand-specific endonuclease required for (+)-strand and RF syntheses; pX (111 residues) is synthesized from an AUG site within and in-frame with the C-terminal coding region of pII; pV (87 residues) forms a stable homodimer, the crystal structure of which has been solved to 1.8 Å and gives insight into the mechanism of binding of antiparallel ssDNA strands.

Each of the five capsid proteins of Ff exhibits a sequence consistent with an α-helical TM domain. All reside in the cytoplasmic membrane prior to assembly into the virion. Nascent pVIII contains a 23-residue signal sequence, which is removed from the N-terminus by a periplasmic signal peptidase, yielding the 50-residue subunit of the mature capsid. The N-terminal segment (1–19) of the mature subunit resides on the periplasmic face of the inner membrane, the middle 20 or so residues span the membrane, and the C-terminal segment (40–50) is situated in the cytoplasm. Although Sec proteins of *E. coli* ordinarily facilitate membrane insertion and translocation, these processes for pVIII are Sec-independent and mediated instead by the host-encoded protein YidC. Maturation of the pIII precursor, which contains an 18-residue N-terminal signal, is also accomplished following membrane translocation. A C-terminal sequence of 23 hydrophobic residues anchors pIII to the inner membrane, while most of the protein is located in the periplasmic space. The three remaining minor capsid proteins, pVI (112 residues), pVII (33 residues), and pIX (32 residues), are membrane-inserted without the aid of a signal sequence.

The three morphogenetic proteins of Ff are also located in the bacterial cell envelope. pI (348 residues) contains an internal TM region and spans the inner membrane with residues 1–253 in the cytoplasm and 273–348 in the periplasm. pXI (108 residues), which is synthesized from within the pI coding region, shares the sequence of the pI C-terminal segment. Accordingly, pXI is anchored to the membrane by a short N-terminal TM region, while most of the protein resides in the periplasm. The only viral protein located in the outer membrane is pIV. The 426-residue precursor is translocated into the periplasm where a 21-residue signal sequence is removed. The resulting 405-residue protein is integrated into the

outer membrane as an oligomer of 14 subunits, each with its N-terminus in the periplasm. A cryoelectron microscopy-based image reconstruction of detergent solubilized pIV at 22 Å resolution reveals a barrel-like cylindrical complex of three domains – an N-terminal ring (N-ring) of inner diameter 6.0 nm, a middle ring (M-ring) blocked by protein density, and a C-terminal ring (C-ring) of inner diameter 8.8 nm. In a recent model of the pIV multimer, it has been proposed that the C-ring is embedded in the outer membrane and the M- and N-rings are located in the periplasmic space, where a virus assembly-induced conformational change would allow opening of the gated M-ring and widening of the narrow N-ring.

Virus Assembly

Virus assembly occurs in the bacterial envelope at sites where the inner and outer membranes are associated by a trans-envelope network of proteins comprising the virally encoded pI, pIV, and pXI. This network is not transient and exists even in the absence of virus assembly. In addition to pI, pIV, and pXI, virus assembly is also dependent on host-encoded thioredoxin, ATP hydrolysis and the proton motive force across the cytoplasmic membrane. Thioredoxin must be in the reduced state; however, redox capability of the protein is unnecessary. pI likely catalyzes ATP hydrolysis, since its cytoplasmic domain contains an ATP-binding Walker motif, which is necessary for phage assembly.

The substrate for assembly is the pV-coated viral genome, in which the PS is exposed at one end. The PS likely forms an initiation complex with the cytoplasmic domains of pI, pVII, and pIX. pV is stripped from the genome concurrent with association of the positively charged C-terminal pVIII domain to the ssDNA molecule. The viral genome is translocated across the inner membrane as pVIII subunits assemble to it. A proposed hinge region between the TM and N-terminal amphipathic domains of pVIII may facilitate the transition of the capsid subunit from the inner membrane to the viral coat, although direct experimental evidence is lacking. Concurrent with assembly of the capsid, the progeny virion exits the bacterial cell through the pIV channel. The periplasmic N-terminal and middle domains of the pIV channel must undergo large conformational changes to allow passage of the 7 nm virus particle. The narrow N-terminal ring of the channel (6 nm i.d.) must expand and the gated middle ring must open. Since pI binds to both the initiation complex and the pIV channel, the initiation of virus assembly may be coupled to pIV conformational change by way of pI, which would avoid unintended channel opening. Additionally, if energy is necessary to widen the pIV channel or open its gate, pI may provide this energy via ATP hydrolysis.

Elongation of the progeny phage continues until the end of the viral genome is reached, at which time pVI and pIII associate with the tip of the viral capsid to terminate assembly and release the virion into the extracellular milieu. Without pVI and pIII, virus assembly continues by packaging additional genomes, one after the other, until the resultant polyphage is removed from the cell by mechanical shearing. Genetic studies have shown that separate regions of the C-terminal domain of pIII are important for termination of virus assembly, release of the viral filament from the host cell, and stabilization of the virion particle. An 83-residue C-terminal segment (residues 324–406) allows incorporation of pVI, but not release of the progeny phage. Ten additional residues (313–322) within the 313–406 segment confer the ability to release progeny phage from the bacterial cell envelope. A pIII C-terminal fragment of at least 121 residues is needed to release stable filamentous ssDNA bacteriophage progeny from an *E. coli* cell.

Biotechnology

Several aspects of the unique life cycle and morphology of the Ff bacteriophage have been exploited for biotechnological applications. (1) The size of the packaged genome is variable, such that it can easily tolerate an insertion of up to 6 kbp, which is compensated by the additional assembly of the proportionate number of pVIII subunits, as noted earlier. (2) The orientations of pVIII within the capsid lattice and pIII at the virion tail allow fusion of non-native peptides and proteins to exposed sites of the viral particle without significant disruption of phage viability or capsid stability. (3) The mature virion is very stable to changes in pH, temperature, and ionic strength. (4) Phage preparations yield high titers resulting in efficient and inexpensive large-scale phage production. (5) The phages are distinct from animal and plant viruses, and are generally not toxic to mammalian cells.

The filamentous ssDNA bacteriophage M13 has been used as a cloning vector for decades. The foreign gene to be cloned is usually inserted between functional sequences of the IG region of the genome. The virus assembly process allows up to 12 kbp of DNA to be packaged without adverse effects on phage viability. Inserts greater than 6 kbp, which result in a genome larger than 12 kbp, are possible with compensatory mutations elsewhere in the genome. One advantage of cloning with M13 is that both single-stranded and double-stranded products can be isolated from progeny phage and intracellular RF DNA, respectively.

Most biotechnological applications of M13 are byproducts of the revolutionary exploitation of the phage as a platform from which peptides (or proteins) can be displayed. To produce a phage that displays a foreign peptide, the coding sequence is inserted in-frame with that of a capsid protein, usually pVIII or pIII, so that the fused

peptide is exposed on the surface of the virion. Minor capsid proteins pVI, pVII, and pIX have also been successfully used for phage display, although to a lesser extent than pIII and pVIII. The display of the peptide must not interfere with capsid protein function. In the case of pVIII tethering, the peptide must be compatible with translocation of the capsid subunit across the inner membrane and with capsid assembly. Accordingly, peptides are usually fused to the pVIII N-terminus. For pIII, the peptide must not occlude the pilin binding site. Interference of the displayed peptide with the life cycle and/or stability of the phage can often be overcome by using a helper phage to facilitate production of hybrid virions containing both wild-type and fused capsid proteins, thus minimizing the adverse effect of the display. When sequences from a DNA library are inserted into the genome for display, a phage library containing up to 10^{10} different displayed peptides is produced.

The applications of phage display for biotechnological progress range from identification of molecular recognition elements between interacting proteins and ligands to targeted drug delivery for treatment of diseased mammalian cells. Biomedical applications include (1) antibody selection using a phage-displayed epitope library to facilitate the design of vaccines with optimal binding affinity for target epitopes; (2) immunopharmacotherapy for cocaine addiction, in which cocaine-sequestering antibodies displayed on a filamentous ssDNA bacteriophage are delivered to the central nervous system intranasally; (3) *in vivo* visualization of β-amyloid plaques by delivery of phage-displayed anti-β-amyloid antibodies to the brain with subsequent staining with fluorescent-labeled anti-phage antibody; and (4) cell-targeted gene and drug delivery, in which the phage is essentially a therapeutic nanocourier. Filamentous ssDNA bacteriophage display systems have also been successfully applied for nonmedical biotechnological purposes, for example, in the synthesis and assembly of nanowires for lithium ion battery electrodes. In this case, the tetrapeptide Glu-Glu-Glu-Glu is fused to the N-terminus of pVIII and treated to bind cobalt oxide forming a Co_3O_4 nanowire. These Co_3O_4 viral nanowires electrostatically self-assemble to form a nanostructured monolayer, which is used as the negative electrode in the construction of a Li-ion battery.

See also: Icosahedral Tailed dsDNA Bacterial Viruses.

Further Reading

Bennett NJ and Rakonjac J (2006) Unlocking of the filamentous bacteriophage virion during infection is mediated by the C domain of pIII. *Journal of Molecular Biology* 356: 266–273.

Davis BM and Waldor MK (2005) Filamentous phages linked to virulence of *Vibrio cholera*. *Current Opinion in Microbiology* 6: 35–42.

Eckert B, Martin A, Balbach J, and Schmid FX (2005) Prolyl isomerization as a molecular timer in phage infection. *Nature Structural and Molecular Biology* 12: 619–623.

Frenkel D and Solomon B (2002) Filamentous phage as vector-mediated antibody delivery to the brain. *Proceedings of the National Academy of Sciences, USA* 99: 5675–5679.

Nam KT, Kim D-W, Yoo PJ, *et al.* (2006) Virus-enabled synthesis and assembly of nanowires for lithium ion battery electrodes. *Science* 312: 885–888.

Opalka N, Beckmann R, Boisset N, *et al.* (2003) Structure of the filamentous phage pIV multimer by cryo-electron microscopy. *Journal of Molecular Biology* 325: 461–470.

Petrenko VA and Smith GP (2005) Vectors and modes of display. In: Sidhu S (ed.) *Phage Display in Biotechnology and Drug Discovery*. pp. 63–110. Boca Raton, FL: CRC Press.

Samuelson JC, Chen M, Jiang F, *et al.* (2000) YidC mediates membrane protein insertion in bacteria. *Nature* 406: 637–641.

Tsuboi M and Thomas GJ, Jr. (2007) Polarized Raman and polarized infrared spectroscopy of proteins and protein assemblies. In: Uversky VN and Permyakov EA (eds.) *Protein Structures: Methods in Protein Structure and Stability Analysis*, ch. 3.4. Hauppage, NY: Nova Science Publishers.

Wang YA, Yu X, Overman SA, *et al.* (2006) The structure of the filamentous bacteriophage. *Journal of Molecular Biology* 361: 209–215.

Webster RE (1999) Filamentous phages. In: Granoff A and Webster RG (eds.) *Encyclopedia of Virology,* 2nd edn., pp. 547–552. London: Academic Press.

Filoviruses

G Olinger, USAMRIID, Fort Detrick, MD, USA
T W Geisbert, National Emerging Infectious Diseases Laboratories, Boston, MA, USA
L E Hensley, USAMRIID, Fort Detrick, MD, USA

© 2008 Published by Elsevier Ltd.

Glossary

D-dimers Fibrinolysis is mediated by plasmin, which degrades fibrin clots into D-dimers. Clinically, D-dimers can be used to diagnose DIC.

Disseminated intravascular coagulation (DIC) An acquired syndrome characterized by inappropriate accelerated systemic activation of coagulation with fibrin deposition in the microvasculature and consumption of procoagulants and platelets.

Fibrin degradation products (FDPs) The protein fragments produced after digestion of fibrinogen or fibrin by plasmin. Typically, a hallmark of disseminated intravascular coagulation. Also, referred to as fibrin-split products.
RNAi The silencing of the expression of a selected gene by a homologous portion of double-stranded RNA. Also, referred to as RNA interference.
Tissue factor (TF) A procoagulant protein responsible for activation of the extrinsic coagulation pathways.

Introduction

In 1967, the first filovirus outbreak occurred in Germany and Yugoslavia among laboratory workers handling African green monkeys and/or tissues from contaminated monkeys imported from Uganda. The causative agent was identified as a new virus called Marburg virus (MARV). MARV re-emerged in 1975 in Johannesburg, South Africa, when a man who had recently returned from Zimbabwe became ill. The infection was spread to the man's traveling companion and a nurse at the hospital. While the man died of the disease, the other two cases recovered from the illness after receiving supportive care. The following year in 1976, a mysterious outbreak swept through several remote villages in Africa. The disease was rapidly fatal (318 cases; mortality rate 88%). Examination of samples revealed a previously unknown virus similar to that of the MARV. This virus was named Ebola after a local river in central Africa and became the second member of the family *Filoviridae*. Since 1976, there have been a number of outbreaks of both types of filoviruses. Isolation and characterization of the causative agents of the Ebola outbreaks resulted in the identification of a number of related viruses. Today *Filoviridae* forms a separate family within the order *Mononegavirales* and can be divided into two genera: *Marburgvirus* and *Ebolavirus*. The latter is composed of four distinct species with varying degrees of lethality in man, *Zaire ebolavirus* (~90% lethal), *Sudan ebolavirus* (~50 %), *Reston ebolavirus* (unknown lethality), and *Ivory Coast ebolavirus* (unknown lethality). In contrast, the MARV genus is composed of a single species, *Lake Victoria marburgvirus*. However, there have been a number of isolates of MARV identified with varying degrees of morbidity and mortality. The case–fatality (CF) rate from the most recent isolate, MARV-Angola, has been reported to be ~90%. Although there has been a tremendous amount of work performed on filoviruses, the source of these outbreaks remains undetermined and there remains no approved vaccine or therapeutic intervention.

Background

Ebola Virus History

Ebola virus (EBOV) was first discovered during near-simultaneous outbreaks in the former Zaire and Sudan. These outbreaks were due to serologically distinct viral species that would be designated ZEBOV and SEBOV, respectively. The CF was ~88% in the initial ZEBOV outbreak and ~53% in the SEBOV outbreak. The source of these outbreaks was never determined. These outbreaks were followed by several smaller outbreaks in the late 1970s. After an almost 20-year absence, a new EBOV outbreak was reported in 1989 in a colony of cynomolgus monkeys (*Macaca fascicularis*), imported from the Philippines to a holding facility in Reston (VA, USA). Although the Reston species appeared to be highly lethal in nonhuman primates (NHPs), no disease was reported in any of the documented human exposures. Although there have been a number of subsequent outbreaks of REBOV in NHP facilities both in and outside of the US, to date there have been no cases of human infections reported. Investigations traced the source of all REBOV outbreaks to a single export facility in the Philippines (Laguna Province); however, the original source and mechanism of entry into this facility remain unknown. In 1994, EBOV reemerged in Africa with the identification of a fourth species, ICEBOV, as well as the detection of ZEBOV for the first time in Gabon. The fourth species of EBOV, ICEBOV, was identified in Côte d'Ivoire following the exposure of a researcher during a necropsy to determine the cause of death of local chimpanzee populations. The source for the infection of the chimpanzees was undetermined. The outbreak in Gabon occurred in the Ogooue-Ivindo Province; a total of 51 cases and 31 deaths were documented (CF = 61%) in this outbreak.

The largest outbreak of EBOV occurred in 1995 in Kikwit, Democratic Republic of the Congo (DRC) (formerly Zaire). A total of 310 cases with 250 deaths (CF = 81%) were reported. Approximately one-fourth of all cases were reported among healthcare workers. This outbreak was quickly followed by two additional outbreaks that occurred in DRC and Gabon. Only one of the three primary cases identified could be linked to contact with a dead chimpanzee. A total of 31 cases with 21 deaths (CF = 68%) were documented. The second outbreak occurred as a series of unrelated cases in hunters during July and August 1996. Reminiscent of previous outbreaks (Gabon and Côte d'Ivoire), there were reports of several dead great apes (gorillas and chimpanzees) in the same area. A total of 60 cases with 45 deaths (CF = 75%) were reported. Interestingly, sequence analysis of the isolates from 1994 and 1996 differed by less than 0.1% in the glycoprotein and polymerase genes.

Ongoing, sporadic outbreaks of EBOV continue to be reported in central Africa. Most often, these outbreaks

have been associated with infection of the great ape population. It is believed that these outbreaks are to blame for the widespread loss of these animals. It has been reported that the population of Western lowland gorillas has dropped by 50% and as much as 90% in some areas. Conservationists have stated that it will take decades for the populations of these animals to recover.

Marburg Virus History

MARV was first identified in 1967 during simultaneous outbreaks in Germany and Yugoslavia among laboratory workers handling African green monkeys and/or tissues from contaminated monkeys imported from Uganda. Secondary cases were reported among healthcare workers and family members. All secondary cases had direct contact with a primary case. A total of 32 cases and 7 deaths (CF = 21%) occurred during this outbreak. Over the next several decades MARV was associated with multiple sporadic and isolated cases among travelers and residents in southeast Africa.

From 1998 to 2000 a number of MARV cases were reported among young male workers at a gold mine in Durba, DRC. Cases were subsequently detected in the neighboring village. Although a few cases were detected among family members, secondary transmission appeared to be rare. Subsequent virological investigation revealed that these cases were due to multiple introductions of different strains from an as-of-yet unidentified source. In total there were 154 cases with 128 deaths (CF = 83%) reported.

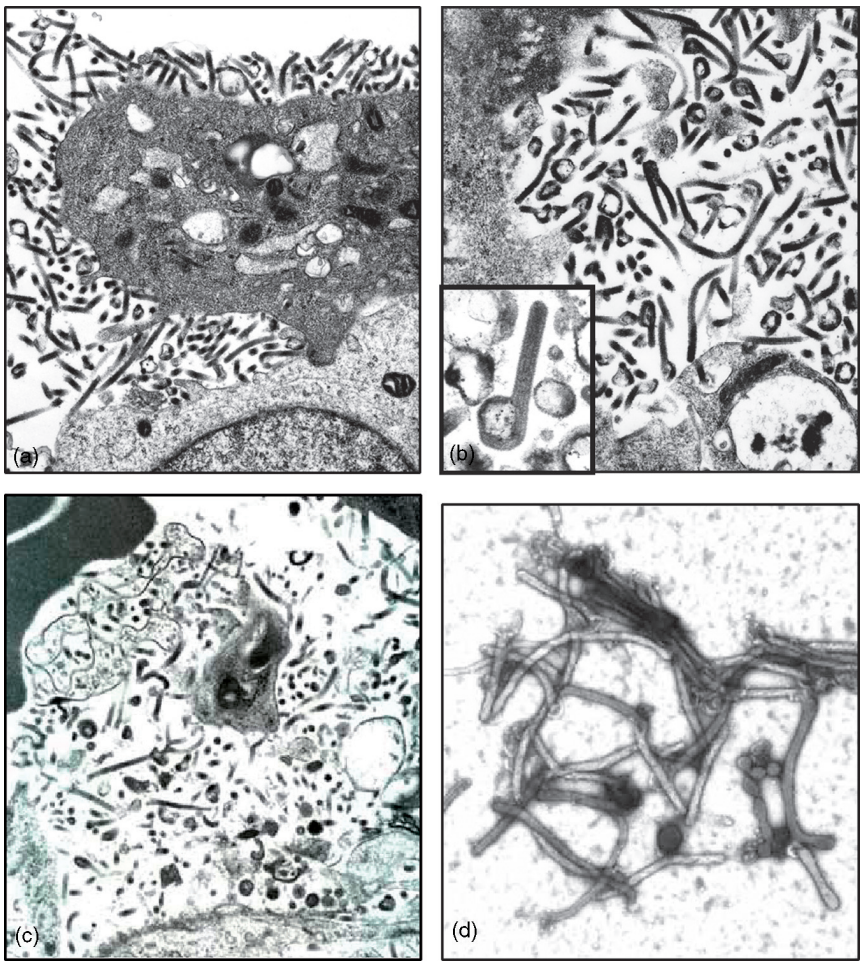
The largest and most deadly outbreak of MARV occurred in 2004–05 in the Uige Province of Angola. A total of 252 cases and 227 deaths (CF = 90%) were reported. Unlike previous outbreaks, a large number of the victims were children under the age of five. Virological analyses suggest that this isolate is very close to the strain from the original cases in 1967 in Europe. The reason for the increased virulence of the Angola isolate remains unclear.

Transmission

Both EBOV and MARV are transmitted by direct contact with the blood, secretions, organs, or other bodily fluids from infected persons, or infected animals. Many of the initial cases in outbreaks can be traced to the handling of infected animals, particularly chimpanzees and gorillas. Amplification has most often been associated with hospitals, where the virus is spread through nosocomial routes, or through burial ceremonies, where it is not uncommon for mourners to have direct contact with the deceased. While the modes of transmission have been well documented, the true source of these outbreaks and the reservoir for these viruses remain unclear. Although NHPs have been a source of infection for humans, the highly virulent nature of filoviruses in these animals suggests that they are not the reservoir. Different hypotheses have been developed to try to explain the origin of EBOV outbreaks. The presence of bats or contact with bat guano is often a common theme during outbreaks of both MARV and EBOV. Laboratory observation has shown that fruit and insectivorous bats experimentally infected with ZEBOV can support viral replication without apparent signs of illness. In a recent survey of small animals in Africa near areas of human or great ape EBOV outbreaks, asymptomatic infection of three species of fruit bats, *Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*, was reported. The exact ecology of EBOV and MARV remains to be determined and may include multiple natural reservoirs.

Virion Structure and Composition

Filoviruses are enveloped, nonsegmented, negative-strand RNA viruses. The virus envelope is derived from the lipid membrane envelope from the host cellular plasma membrane and therefore the lipid and protein composition reflect that of the infected cell. Filovirus virions have a characteristic thread-like, filamentous morphology (**Figure 1**). Pleomorphic virus structures ranging from straight tube-like structures, branched structures, and curved filaments shaped like a 6 (shepherd's crook), a U, or a circle. The diameter of filovirus virions are typically 80 nm, while the length can vary between 130 and 14 000 nm. The average length of a virus particle ranges from about 800 to 1000 nm with MARV being closer to 800 nm and EBOV closer to 1000 nm. Structurally, the virus particle is composed of seven viral proteins. The glycoprotein (GP), a type I transmembrane protein complex composed of two proteins, a ~140 kDa GP_1 and ~26 kDa GP_2. Normally, the two GP subunits are disulfide linked; however, when GP_1 is not disulfide-linked with GP_2, the GP_1 can be released in a soluble form from infected cells. Glycosylation, both N- and O-linked, provides approximately 50% of the GP mass. Complex cellular processing including glycosylation and proteolytic cleavage of the GP_1–GP_2 precursor protein leads to the generation of the GP_1–GP_2 heterotrimeric spikes in the virus envelope. GP_1 allows for receptor binding while GP_2 contains the fusion domain necessary for viral entry into the host cell. Surface projections of GP are dispersed evenly over the entire surface at 10 nm intervals. Functionally, the virus GP allows for attachment, receptor-mediated endocytosis, fusion with endocytotic vesicles, and release of the virion core into the host cell cytoplasm. To date, no specific host cell receptor has been identified. A variety of putative receptors and cofactors have been identified including the asialoglycoprotein receptor, folate receptor-α, and C-type lectins. Given the wide cell tropism of the virus, or the receptors are likely either constitutively expressed proteins, that involve multiple proteins with conserved binding domains, or



involve cofactors that facilitate virus entry. Viral particles may gain entry into the cell by co-opting common phagocytic pathways leading to a nonspecific entry mechanism.

The remaining six viral proteins are found within the virion, underlying the host-derived membrane. The viral protein 40 (VP40) functions as a matrix protein, and is essential for efficient viral assembly and budding. VP24 has been demonstrated to participate in the spontaneous formation of the nucleoprotein complex. Similar to VP40, studies indicate that VP24 behaves as a minor matrix protein. The nucleoprotein (NP) and VP30, VP35, polymerase (L) proteins, and the viral genomic RNA form the ribonucleoprotein complex. The nucleocapsids form a symmetrically helical filamentous structure underlying the viral envelope and the core structure has a striated appearance similar to that of rhabdoviruses. The L protein, the largest protein, functions as the virus-associated RNA-dependent RNA polymerase. VP30 functions as an EBOV-specific heterologous transcription activation factor that recognizes a secondary stem–loop structure during transcription. Similar to other transactivator factor proteins, VP30 exists in both phosphorylated and unphosphorylated forms within virions. In addition to their function as structural proteins, VP35 and VP24 have been shown to act as interferon antagonists.

Genome Organization and Expression

The linear genome is approximately 19 000 nt in length and consists of a single-stranded, negative-sense RNA (**Figure 2**). The genomic nucleic acid is not infectious. The genome contains highly conserved, transcription initiation and termination sites. Genomes contain intergenic sequence regions that vary in length, nucleotide sequences, and contain regions of gene overlap. While EBOV and MARV have a substantial degree of structural similarity in their respective genome organization, limited nucleotide sequence, homology is observed. Similar to the majority of other mononegaviruses, filovirus replication and transcription occurs in the cytoplasm of the infected

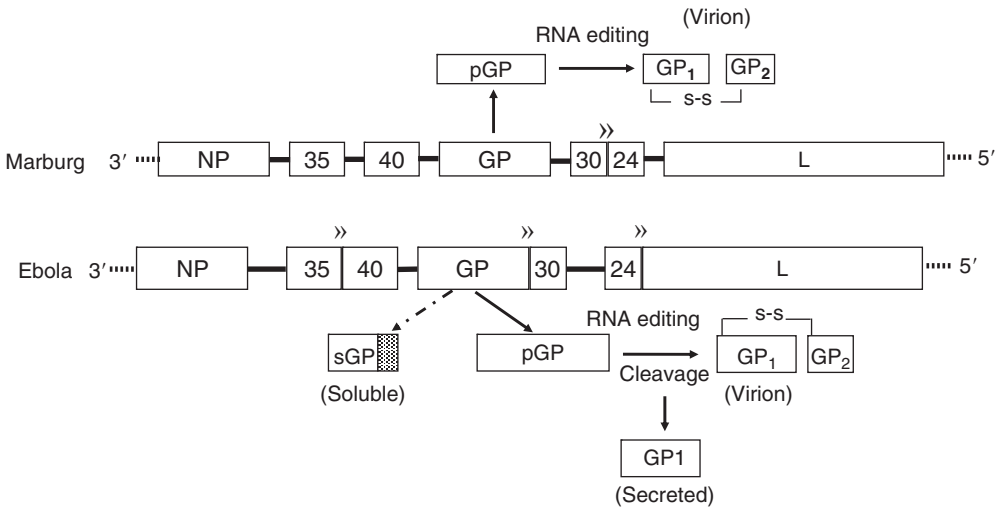


Figure 2 Filovirus genome organization and generation of glycoproteins. Linear, negative-sense, single-stranded, RNA monopartite genome, ~19 000 nt. Relative positions of the encoding regions for viral proteins depicted as boxes; NP, nucleoprotein; 35, VP35; 40, VP40; GP, glycoprotein; 30, VP30; 24, VP24; L, viral polymerase. Leader and trailer and intergenic regions (nontranscribed regions) shown as lines. Overlapping regions indicated by ≫ symbol. For Ebola, glycoprotein gene generates a soluble GP (sGP) and precursor GP (pGP). pGPs for Ebola and Marburg are cleaved by host-derived enzymes to generate a GP_1 and GP_2 subunit that are disulfide linked.

cell. The viral-encoded RNA-dependent RNA polymerase generates full-length positive-sense antigenomes that serve as templates for the synthesis of the progeny negative-sense genomes. Filoviral subgenomic mRNAs are monocistronic and polyadenylated. A single genome contains seven genes that are arranged linearly. Monocistronic subgenomic mRNAs complementary to the viral RNA lead to the generation of seven filovirus proteins found within the virion. For both MARV and EBOV, each gene encodes a single polypeptide, except for the GP. The structural glycoprotein GP_1 and GP_2 are generated as a single precursor polypeptide that is cleaved by host cell pro-protein convertases to generate the two subunits of the final protein. EBOV is different from MARV in that an additional viral protein is generated; a nonstructural, soluble form of GP, referred to as secreted GP (sGP). sGP is translated from an mRNA that has undergone RNA editing. Interestingly, the majority of GP mRNA encodes for the sGP which has been detected in cell culture and serum from EBOV-infected patients. Both soluble GP_1 and sGP have been implicated in the pathogenesis of EBOV by acting as decoy proteins that sequester neutralizing antibodies during infection; however, the exact role of the soluble forms of GP remains to be elucidated.

Pathogenesis

Clinical Features/Presentation

The clinical manifestations of EBOV and MARV are often compared to that of severe sepsis or septic shock. Following a 2–21 day incubation period, cases often present with a variety of nonspecific symptoms including high fever, chills, malaise, and myalgia. As the disease progresses, there is evidence of multisystemic involvement, and manifestations include prostration, anorexia, vomiting, nausea, abdominal pain, diarrhea, shortness of breath, hypotension, edema, confusion, maculopapular rash, and eventually coma. Patients normally progress rapidly with death occurring 6–9 days after the onset of symptoms. The development of petechiae, ecchymoses, mucosal hemorrhages, and uncontrolled bleeding at venipuncture sites are indicative of development of abnormalities in coagulation and fibrinolysis. Massive loss of blood is atypical and, when present, is largely restricted to the gastrointestinal tract. Fulminant infection typically evolves to shock, convulsions, and, in most cases, diffuse coagulopathy.

Animal Models

While only limited information is available for pathophysiology of human cases of EBOV or MARV, a lot has been learned from studies employing experimentally infected NHPs. Although the majority of studies in humans and NHPs to date have focused on ZEBOV, limited studies suggest that MARV may behave in a similar manner. EBOV and MARV are most often characterized by three common factors: development of lymphocyte apoptosis, aberrant production of pro-inflammatory cytokines, and the development of coagulation abnormalities. Sequential analysis of samples collected from ZEBOV-infected NHP shows early and prominent infection of monocyte/macrophages (MO/Φ) and dendritic cells (DCs). The importance of this selective targeting has been well documented.

Infection of the MO/Φ not only facilitates distribution of virus to the spleen and lymph nodes but also triggers a cascade of events including the upregulation and production of the procoagulant protein tissue factor (TF) as well as a number of pro-inflammatory cytokines/chemokines and oxygen free radicals. *In vitro* studies of human MO/Φ have confirmed these observations. Similar to septic shock caused by bacterial pathogens, disease triggered by EBOV or MARV infection is thought to involve inappropriate or maladaptive host responses. It is likely that these responses are more important to the development of the observed pathology than any structural damage directly induced by viral replication. Infection of DC likely facilitates disease progression by hindering the ability of the host to orchestrate an effective immune response to clear the virus.

Although lymphocytes do not appear to be productively infected by filoviruses, the importance of their role in the development of the disease pathology cannot be understated. The loss of lymphocytes through apoptosis has been well documented. Flow cytometry demonstrated a striking loss of circulating CD8+ T cells and natural killer (NK) cells prior to or corresponding with the onset of symptoms. The exact mechanism(s) responsible for triggering this widespread loss of lymphocytes remains unclear. RNA analysis of peripheral blood mononuclear cells (PBMCs) collected from NHPs has demonstrated the upregulation of a number of pro-apoptotic genes and anti-apoptotic genes.

Infection of MO/Φ also appears to be critical in the triggering and propagation of the coagulation abnormalities observed in filovirus infections. Recently, it was demonstrated that infection of MO/Φ *in vitro* or *in vivo* resulted in the upregulation of TF. Interestingly, this expression was observed only in infected cells, suggesting a direct correlation with the virus. Analysis of sera from infected animals revealed the presence of microparticles or small membrane vesicles with procoagulant and pro-inflammatory properties. Analysis of these microparticles showed that many of these expressed TF and may therefore have been derived from filovirus-infected MO/Φ.

The contribution of the endothelium as well as the infection of endothelium remains controversial. *In vitro* studies have demonstrated that endothelial cells are easily infected by either MARV or EBOV. However, analysis of tissues collected from human cases has produced conflicting results. Analysis of sequential tissues from NHP suggests that endothelial cells are not targeted until the later stages of disease. Electron microscopy examination of tissues showed activation of endothelial cells but no widespread damage to the vasculature. Analyses of clinical chemistries support this observation, with a drop in small molecular weight proteins but not total proteins. The cause for this vascular leakage is unclear and is likely multifactorial. Fibrin degradation products (FDPs), thrombin, reactive oxygen species, pro-inflammatory cytokines, as well as activation of the complement and kinin systems can all contribute to increased vascular permeability. Rapid increases in levels of D-dimers were observed in sera of EBOV-infected NHP with initial increases being detected before the onset of clinical symptoms. While the contributions of the complement and kinin systems in the development of EBOV and MARV pathogenesis are yet to be evaluated, the production of pro-inflammatory cytokines and oxygen free radicals has been well documented. In EBOV-infected NHP, IFN-α, interleukin-6 (IL-6), monocyte chemoattractant protein 1 (MCP-1), macrophage inflammatory protein (MIP)-1α, and MIP-1β were detected in sera at the early to mid-stages of disease. IFN-β, IFN-γ, IL-18, and TNF-α were also detected in sera but not until the later stages of disease.

In recent years, the importance of the interaction between coagulation and inflammation as a response to severe infection has become increasingly appreciated. In addition to driving upregulation of procoagulant proteins, inflammatory mediator, may inhibit fibrinolytic activity, and downregulate natural anticoagulant pathways, in particular, the protein C anticoagulant pathway. Uncontrolled activation of coagulation systems may lead to disseminated intravascular coagulation (DIC). DIC is a syndrome with both bleeding and thrombotic abnormalities characterized in part by the presence of histologically visible microthrombi in the microvasculature. These microthrombi may hamper tissue perfusion and thereby contribute to multiple organ dysfunction and high mortality rates. Before acute DIC can become apparent, there must be a sufficient stimulus to deplete or overwhelm the natural anticoagulant systems. During activation of the clotting system, the host regulates the process through the production and activation of a variety of inhibitors of the clotting system. In this process, however, the inhibitors are consumed, and if the rate of consumption exceeds the rate synthesized by liver parenchymal cells, plasma levels of inhibitors will decline. Analysis of tissue and sera from ZEBO-infected NHPs shows widespread fibrin deposition and marked decreases in plasma levels of protein C. The drops in protein C correlate with increase in D-dimers and disease progression.

Host Responses

The low sporadic disease incidence and remote locations of most outbreaks have led to limited careful study of the host responses to virus infection. Perturbations of the innate and acquired immune responses are consistent with fatal disease outcome. In a few studies patients that survived Ebola infections were reported to have generated a virus-specific immunoglobulin M (IgM) response and to have transient expression of pro-inflammatory cytokines. In sharp contrast, fatal EBOV cases have dysregulated cytokine expression levels, coagulation abnormalities, fibrin deposition, DIC, and evidence of cell death and the lack of an effective adaptive immune response. Through direct and

indirect mechanisms, and pathways that are not clearly understood, the fatal cases are associated with the lack of B- or T-lymphocyte responses. Altered innate responses, both cellular and soluble, combined with early infection of MO/Φ and DC, likely impair the host's ability to initiate an appropriate adaptive immune response to clear filovirus-infected cells. Simultaneously, virus-induced or inappropriate host defenses lead to dysregulated innate immune responses. Filovirus proteins VP35 and VP24 interfere with and modulate these key immune pathways by inhibiting cellular antiviral defenses. Ultimately, these events result in immune dysregulation and uncontrolled explosive virus replication.

Animal models have offered some insight into protective immune responses. In EBOV infection in mice, early interferon responses are vital to controlling virus replication. In concert, EBOV-specific antibody and cellular responses help eliminate virus infection in this animal model. Protective filovirus vaccines have been shown to induce antibody and/or cellular responses that offer protection in rodents and NHPs. Therefore, an appropriate innate and adaptive immune response can offer immunity to both MARV and EBOV.

Treatment and Prevention

Currently, there are no approved therapies or vaccines for the treatment and/or prevention of EBOV and MARV. Control and treatment are limited to containment and basic supportive care. Suspected cases should be isolated from other patients and strict-barrier-nursing techniques implemented. Contact tracing and follow-up of people who may have been exposed through close contact with cases are essential. If not already implemented proper disinfection and handling of materials from infected patients must be initiated. Education of the community as to the presentation of the disease, the nature by which it is transmitted, and the sources by which patients are likely to come into contact with the virus are critical to preventing and containing future outbreaks.

Although there are no approved vaccines there has been considerable progress in the identification and successful testing of candidate vaccines in animal models. These platforms can be separated into several categories: replication-deficient platforms, replication-competent platforms, and virus-like particles (VLPs). The replication-deficient platforms are viral vectors such as adenovirus or Venezuelan equine encephalitis virus (VEEV) expressing filoviral proteins. The first successful strategy to protect NHP from EBOV employed a replication-deficient adenovirus expressing EBOV GP and NP. In this strategy, a DNA prime of GP and NP genes was followed by an adenovirus-expressing GP boost. Subsequent studies demonstrated protection using a single adenovirus GP vaccination 28 days before EBOV challenge. Questions have been raised about the durability of the replication-deficient strategies as well as the ability of these strategies to overcome any pre-existing immunity to the vaccine platform. Efficacy has also recently been demonstrated using a live-attenuated (replication-competent) vesicular stomatitis virus (VSV) expressing the GP of EBOV. Additional work demonstrated that by simply swapping out the EBOV GP for either the MARV or Lassa fever virus GP, this platform could provide full protection against lethal MARV or Lassa fever virus challenge, respectively. The VSV platform has the additional benefit in that it has been demonstrated to provide 100% protection against MARV when administered 30 min after MARV exposure and ~50% protection for EBOV when rapidly administered in an EBOV post-exposure setting. While it is likely that the replication-competent platforms may provide increased durability there is some concern about the safety of administration of any live vaccine. Therefore, additional studies are needed to fully evaluate the safety profile of this platform. Finally, filovirus-like particles generated by the co-expression of viral membrane proteins (GP and VP40) are currently being evaluated in NHPs. This strategy may be beneficial in that it will likely overcome the safety concern associated with the use of live-attenuated platforms and/or the issues of preexisting immunity. Consistent with other protein vaccines, there are concerns about the durability of the immunity, adjuvant choice, as well as the necessary dosing regimen to achieve long-lasting immunity.

In addition to the wealth of candidate vaccine platforms developed and tested over the last few years, there have also been a number of candidate therapeutics that been evaluated in animal models. These strategies have met with varying degrees of success and can be divided into two basic categories, those that directly target the virus and those that target the host and/or the disease manifestations in the host. Recently, investigators reported success using either antisense oligonucleotides or RNA interference (RNAi) corresponding to sequences in the viral genomic or mRNA *in vivo*. Additional studies will be needed to evaluate whether the antisense technology can provide any therapeutic value when used after exposure and whether the success of the RNAi strategies in guinea pig models will translate into the more stringent NHP models. Reduction in morbidity and mortality has also been observed using therapeutics targeting the clinical manifestations in the NHP models. Treatment of ZEBOV-infected NHP with a recombinant protein, recombinant nematode anticoagulant protein c2 (rNAPc2) that blocks the TF-mediated activation of the extrinsic pathway of blood coagulation, resulted in ~33% survival as well a substantial increase in the mean time to death. Interestingly, substantial reductions in plasma levels of IL-6, MCP-1, and viral load were noted in rNAPc2-treated animals. It is unknown if treatment with other anticoagulant strategies that block the

intrinsic and/or common pathways may also offer comparable and/or improved therapeutic benefit.

Finally, it is important to mention therapeutic strategies that are yet to provide any survival benefit when tested in NHP models. Although type I IFNs have shown promise in the mouse model of EBOV infection, testing of IFN-α2 failed to protect NHPs against EBOV or MARV. In addition, *S*-adenosylhomocysteine, which had been hypothesized to protect mice against EBOV, at least in part through induction of a type I IFN response, also failed to protect NHP. Currently, alternative IFN therapies and formulations are under evaluation. It is unclear if these approaches will have any improved efficacy in NHPs. In addition, heparin, convalescent serum, and equine anti-EBOV immunoglobulin have been used to treat infections in humans and/or NHPs, but were inconsistent and appeared to be of little therapeutic value. While passive antibody treatment has been successful in mice, the benefit of antibody immunotherapy remains controversial. Although results in rodent models have been encouraging, initial studies in NHPs have not been as successful. Additional studies are needed to fully evaluate the utility of this therapeutic avenue.

Acknowledgment

Work on filoviruses at USAMRIID was supported by the Medical Chemical/Biological Defense Research Program, US Army Medical Research, and Material Command.

See also: Ebolavirus.

Further Reading

Bosio CM, Moore BD, Warfield KL, *et al.* (2004) Ebola and Marburg virus-like particles activate human myeloid dendritic cells. *Virology* 326: 280–287.

Feldmann H, Geisbert TW, Jahrling PB, *et al.* (2004) *Filoviridae*. In: Fauquet C, Mayo MA, Maniloff J, Desselberger U and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses,*, pp 645–653. San Diego, CA: Elsevier Academic Press.

Feldmann H, Volchkov VE, Volchkova VA, and Kenk HD (1999) The glycoproteins of Marburg and Ebola virus and their potential roles in pathogenesis. *Archives of Virology Supplementum* 15: 159–169.

Geisbert TW, Young HA, Jahrling PB, Davis KJ, Kagan E, and Hensley LE (2003) Mechanisms underlying coagulation abnormalities in Ebola hemorrhagic fever: Overexpression of tissue factor in primate monocytes/macrophages is a key event. *Journal of Infectious Diseases* 1(188): 1618–1629.

Grolla A, Lucht A, Dick D, Strong JE, and Feldmann H (2005) Laboratory diagnosis of Ebola and Marburg hemorrhagic fever. *Bulletin de la Société de Pathologie Exotique* 98(3): 205–209.

Harlieb B and Weissenhorn W (2006) Filovirus assembly and budding. *Virology* 344: 64–70.

Hensley LE, Jones SM, Feldmann H, Jahrling PB, and Geisbert TW (2005) Ebola and Marburg viruses: Pathogenesis and development of countermeasures. *Current Molecular Medicine* 5: 761–772.

Ignatyev GM (1999) Immune response to filovirus infections. *Current Topics in Microbiology and Immunology* 235: 205–217.

Jones SM, Feldmann H, Stroher U, *et al.* (2005) Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nature Medicine* 11: 786–790.

Mohamadzadeh M, Chen L, Olinger GG, Pratt WD, and Schmaljohn AL (2006) Filoviruses and the balance of innate, adaptive, and inflammatory responses. *Viral Immunology* 19: 602–612.

Paragas J and Geisbert TW (2006) Development of treatment strategies to combat Ebola and Marburg viruses. *Expert Review Anti-Infective Therapy* 4: 67–76.

Peterson AT, Bauer JT, and Mills JN (2004) Ecologic and geographic distribution of filovirus disease. *Emerging Infectious Diseases* 10: 40–47.

Reed DS, Hensley LE, Geisbert JB, Jahrling PB, and Geisbert TW (2004) Depletion of peripheral blood T lymphocytes and NK cells during the course of Ebola hemorrhagic fever in cynomolgus macaques. *Viral Immunology* 17: 390–400.

Schnittler HJ and Feldmann H (1999) Molecular pathogenesis of filovirus infections: Role of macrophages and endothelial cells. *Current Topics in Microbiology and Immunology* 235: 175–204.

Theriault S, Groseth A, Artsob H, and Feldmann H (2005) The role of reverse genetics systems in determining filovirus pathogenicity. *Archives of Virology Supplementum* 19: 157–177.

Fish and Amphibian Herpesviruses

A J Davison, MRC Virology Unit, Glasgow, UK

© 2008 Elsevier Ltd. All rights reserved.

This article is a revision of the previous edition articles by Andrew J Davison, volume 1, pp 553–557, and Allan Granoff, volume 1, pp 51–53, © 1999, Elsevier Ltd.

Glossary

Fingerling Young fish between the fry and adult stages.

Fry Newly hatched or very young fish.

Tegument The layer of proteins situated between the capsid and the envelope in herpesvirus particles; equivalent to the matrix in other viruses.

Classification and History

Most currently recognized fish herpesviruses infect species that are farmed or harvested from the wild for human consumption. The conditions used in aquaculture may enhance the disease potential of these pathogens and increase their likelihood of detection. It is probable, therefore, that many more fish herpesviruses await discovery. A similar prospect applies to amphibian herpesviruses, since the two known examples originated from a single host species. Herpesviruses of fish and amphibians, like those of higher vertebrates, are usually highly species specific in the natural setting. This indicates that they have evolved in close association with their hosts over long periods of time. Many of these viruses cause mortality only in young fish, and some are also agents of epidermal hyperplasia or neoplasia. Some have been studied to such a limited extent that causal links with the disease whose occurrence led to their identification have not yet been established adequately.

Several fish and amphibian herpesviruses have been classified as members of the family *Herpesviridae*. These include viruses that infect white sturgeon, *Acipenser transmontanus* (acipenserid herpesviruses 1 and 2), Japanese eel, *Anguilla japonica* (anguillid herpesvirus 1), carp, *Cyprinus carpio* (cyprinid herpesvirus 1), goldfish, *Carassius auratus* (cyprinid herpesvirus 2), northern pike, *Esox lucius* (esocid herpesvirus 1), rainbow trout, *Oncorhynchus mykiss* (salmonid herpesvirus 1), channel catfish, *Ictalurus punctatus* (ictalurid herpesvirus 1), Pacific salmon, *Oncorhynchus* species (salmonid herpesvirus 2), walleye, *Stizostedion vitreum* (percid herpesvirus 1), and turbot, *Psetta maxima* (pleuronectid herpesvirus 1). Only one of these viruses (ictalurid herpesvirus 1) has been classified further into a species (*Ictalurid herpesvirus 1*) and a genus (*Ictalurivirus*). Candidate herpesviruses have also been reported in black bullhead (*Ictalurus melas*), Japanese flounder (*Paralichthys olivaceus*), pilchard (*Sardinops sagax*), and redstriped rockfish (*Sebastes proriger*). The viruses representing the classified amphibian herpesviruses (ranid herpesviruses 1 and 2) originated from the leopard frog, *Rana pipiens*. The tenuous genetic relationships between herpesviruses of fish and amphibians and those of higher vertebrates have prompted the proposal that the former group be separated into a new family (*Alloherpesviridae*) and grouped with other herpesviruses into an order (*Herpesvirales*). Elucidation of the genetic relationships among these viruses, upon which detailed subclassification will depend, is at an early stage.

This article focuses on three principal viruses, one infecting an amphibian and two infecting fish. These are Lucké tumor herpesvirus (LTHV; ranid herpesvirus 1), channel catfish virus (CCV; ictalurid herpesvirus 1), and koi herpesvirus (KHV; proposed cyprinid herpesvirus 3). Comments are made on other viruses where appropriate.

In 1934, Lucké reported that intranuclear acidophilic inclusion bodies typical of certain virus infections were present in renal adenocarcinomas of the leopard frog. He concluded 4 years later that the tumor was caused by a virus. Almost two decades elapsed before herpesvirus particles were observed in frog kidney tumor cells by electron microscopy (**Figure 1**), and another decade followed before a causative relationship was established between LTHV and the tumor. The complete genome sequence of the McKinnell strain of LTHV was published in 2006 along with that of the other amphibian herpesvirus, the Rafferty strain of frog virus 4 (FV4; ranid herpesvirus 2).

In 1968, Fijan reported the first isolation of CCV from young channel catfish, in which it causes an acute disease of high mortality. The first detailed characterization of the virus was published 3 years later, and showed that capsids with the typical herpesvirus morphology are assembled in infected cell nuclei. Three-dimensional reconstructions

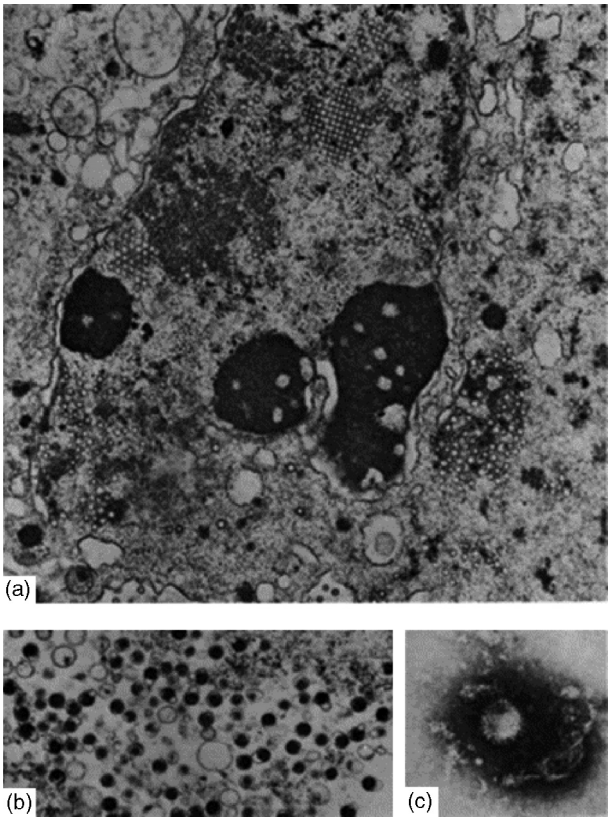


Figure 1 (a) Thin section of an inclusion-bearing Lucké tumor cell with typical herpesvirus particles in various stages of development in the nucleus. Magnification ×19 400. (b) Enveloped extracellular virions. Magnification ×24 000. (c) Negatively stained nonenveloped particles showing typical herpesvirus morphology. Magnification ×110 000. Reproduced from Granoff A (1972) Lucké tumor-associated viruses – a review. In: Biggs PM, de-The G, and Payne LN (eds.) *Oncogenesis and Herpesviruses*, pp. 171–182. Lyons: International Agency for Research on Cancer, with permission from WHO.

derived by cryoelectron microscopy have shown that, apart from minor dimensional differences, the CCV capsid is strikingly similar to that of herpes simplex virus type 1 (HSV-1) (**Figure 2**). The complete genome sequence of the Auburn strain of CCV was published in 1992.

In 1998, a herpesvirus-like agent was identified as the cause of high mortality among carp, including expensive ornamental fish known as koi. The first outbreaks of this new disease may have occurred as early as 1996. In initial studies, this agent (KHV) was also known as carp interstitial nephritis and gill necrosis virus. Consideration of developmental, morphological, and, most recently, genetic attributes have led to the virus being proposed formally as a member of the proposed family *Alloherpesviridae*. The complete genome sequences of three strains of KHV (from Japan, USA, and Israel) were published in 2007.

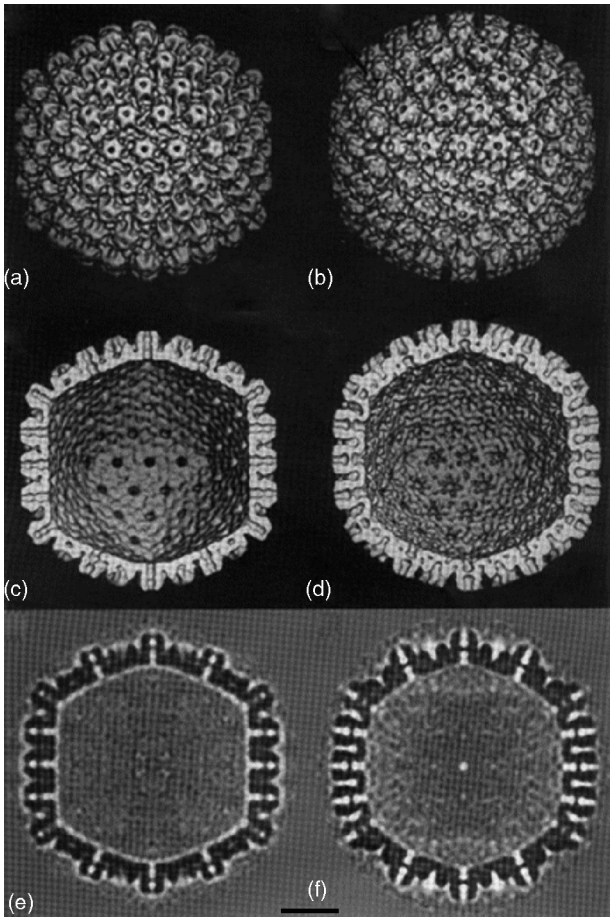


Figure 2 Comparison of the three-dimensional capsid structures of CCV (a, c, e) and HSV-1 (b, d, f). The capsids are viewed along a twofold symmetry axis. Outer surfaces (a and b); inner surfaces (c and d); central thin sections (e and f). The distribution of capsomers (a and b) is distinctive for the triangulation number, $T = 16$, with lines of three hexons connecting pentons along each edge of the icosahedral surface lattice. Scale = 25 nm. Reproduced from Booy FP, Trus BL, Davison AJ, and Steven AC (1996) The capsid architecture of channel catfish virus, an evolutionarily distant herpesvirus, is largely conserved in the absence of discernible sequence homology with herpes simplex virus. *Virology* 215: 134–141, with permission from Elsevier.

Geographic and Seasonal Distribution

The Lucké tumor occurs in north-central and northeastern parts of the USA and southern Canada. In the 1960s, the frequency of affected frogs reached 9% in some collections. However, leopard frog populations have since declined drastically, and the tumor has also become very rare. Production of LTHV within tumors is temperature dependent and therefore seasonal. Tumor cells contain acidophilic intranuclear inclusions and virus particles at temperatures below 11.5 °C but not at higher temperatures, and transition between these states can be induced by temperature shift. The mechanisms by which temperature regulates virus growth have not been elucidated, and their operation *in vivo* and *in vitro* (explanted tumors) indicates that the immune system is not a primary factor. Despite the absence of virus particles at higher temperatures, there is some evidence that tumors express virus-specific proteins.

The occurrence of CCV disease has paralleled the intensification of channel catfish farming in the southern USA and other warmer temperate regions. The lack of reported CCV isolations from wild channel catfish indicates that factors such as dense stocking and poor environmental conditions may predispose farmed fish stocks to outbreaks of disease. A key factor is water temperature, since epizootics occur in the summer months and the incubation period has been shown to be considerably shorter at higher temperatures up to 30 °C.

KHV has spread to many countries worldwide, presumably as a result of intensive aquaculture and unregulated transport of live fish in the absence of diagnostic assessment. Water temperature is an important seasonal factor influencing disease, with losses tending to occur during periods at 18–25 °C (spring and autumn). In one study, experimental infections of koi resulted in a cumulative mortality of 95% at 23 °C, with marginally fewer deaths occurring more rapidly at 28 °C and more slowly at 18 °C. Mortalities did not occur at 13 °C, but shifting virus-exposed fish from 13 to 23 °C resulted in rapid onset of mortality.

Pathology, Clinical Features, and Pathogenesis

Little information is available on the natural transmission of LTHV and the factors that influence the occurrence of tumors. It is possible that virus is transmitted by contact with urine or by infection of oocytes in tumor-bearing frogs. Although FV4 infects the embryos and larvae of *R. pipiens*, surviving animals do not develop tumors. Examinations of

the gene complements of the two viruses have not provided a ready explanation of this biological difference.

CCV causes an acute hemorrhagic disease in juvenile channel catfish. It is readily transmitted from fish to fish, probably entering through the gills. In artificial settings, it can also be transmitted by injection or orally by ingestion of contaminated food. After experimental infection, CCV can be isolated from the kidneys and then from other organs, in some of which impressively high titers of virus may be attained. The primary route for virus shedding is probably via the urine.

CCV can be remarkably virulent in susceptible populations of channel catfish. Under optimal conditions the incubation period can be as short as 3 days, and mortality can rise rapidly to 100%. Signs of distress may be accompanied by convulsive swimming, including a 'head-up' posture, and lethargy and death follow. Externally, affected fish may display protruding eyes, a distended abdomen and hemorrhages, largely on the ventral surface. Internally, viscera may be enlarged and hemorrhagic, but certain organs, such as kidneys, liver, and spleen, may be pale. The digestive tract is empty of food, instead containing a mucoid secretion, and a fluid accumulation is present in the peritoneal cavity. Histopathological examination reveals widespread and profound changes. Initially, the kidneys show edema, hemorrhage, and necrosis, and then these features develop in the liver and digestive tract. Electron microscopic studies reveal virus particles in affected organs.

Given the virulence of CCV in young catfish, it is reasonable to suppose that the virus might persist in an inapparent or latent form in adult fish, as do higher vertebrate herpesviruses in their hosts. There is evidence in support of this. Viral mRNA and antigens have been detected in adult fish and, in more recent studies, virus was recovered by co-cultivation of tissues from wintering adult fish and virus DNA was detected by polymerase chain reaction (PCR) in various tissues of surviving fish many months after infection. There is evidence that the CCV genome may exist in circular or concatemeric form in surviving fish. However, the site of latency has not been identified. Reactivated CCV from adult fish could be transmitted horizontally, and there is circumstantial evidence that vertical transmission may also occur.

KHV is highly contagious, has an incubation period of 2–3 days, and exhibits mortality rates of 80–100%. Clinical signs include increased mucus production, pale patches on the skin and gills, labored breathing, and swelling and then necrosis of the gill filaments. Pathological lesions are evident primarily in the gills but may be present in the kidney, skin, liver, spleen, brain, and gastrointestinal tract. Gill disease is characterized by hyperplasia and then fusion of secondary lamellae, inflammation of the gill rakers, and subsequent necrosis. Kidney disease, if present, is characterized by a peritubular and interstitial nephritis. Virus particles are present in affected organs. The routes of infection and shedding are not known, though the gills and skin are obvious candidates, and cohabitation is an effective means of transmitting the disease. Depending on water temperature, virus may survive for periods of days to weeks. Immunohistochemistry and PCR analyses have demonstrated large amounts of the virus in the gills, skin, kidneys, and spleen with smaller amounts in liver, brain, and intestinal tract, mirroring the pathological involvement of these organs. Given its identity as a herpesvirus, it is likely that KHV has a latent aspect to its life cycle. There is some evidence for this, in that temperature-dependent reactivation of KHV has been shown to occur experimentally in surviving carp several months after initial exposure.

Two other carp herpesviruses have been characterized. From limited DNA sequence data, both appear to be closely related to, though distinct from, KHV. Carp pox herpesvirus (cyprinid herpesvirus 1) has been implicated as the cause of a localized epithelial hyperplasia, which manifests itself as benign smooth nodules on the skin and may be transferred by applying material from the lesions to the abraded skin of other fish or following bath exposures of young carp or koi to virus from cell culture. The agent is difficult to grow successfully in cell culture, and thus is isolated infrequently from clinical cases. Hematopoietic necrosis herpesvirus of goldfish (cyprinid herpesvirus 2) was first observed in Japan in 1992, and since then severe outbreaks of the associated disease have occurred in several countries. The virus affects goldfish and not carp, and the disease shares some pathological manifestations similar to that caused in carp by KHV, although the hematopoietic tissue in the kidney rather than the gills is the principal virus target. Similar to cyprinid herpesvirus 1, the goldfish virus is difficult to isolate, although it has been successfully cultured on occasion, with the experimental demonstration that upon bath exposure goldfish will succumb to typical signs of the disease and death.

Two salmonid herpesviruses have been shown to cause virulent disease either naturally or experimentally. Herpesvirus salmonis (salmonid herpesvirus 1; SalHV-1) was isolated on several occasions from a rainbow trout hatchery in the state of Washington. Oncorhynchus masou virus (salmonid herpesvirus 2; SalHV-2) was isolated from Japanese land-locked salmon, and has the property of causing epithelial tumors in survivors of experimental infection. Two additional herpesviruses, the NeVTA and Yamame tumor viruses, have been isolated from *Oncorhynchus* species. They appear to be related to each other, but it is not known whether they represent distinct salmonid herpesviruses or whether they are isolates of SalHV-2 or, as seems less likely, SalHV-1.

Diseases ostensibly due to several other fish herpesviruses have been described. Walleye herpesvirus is

associated with epidermal hyperplasia, but causality has not been demonstrated. Similarly, the role of turbot herpesvirus has not been proven in episodes of substantial mortality among farmed fry.

Growth Properties

The Lucké tumor occurs naturally in *R. pipiens*. Tumors can be induced readily in *R. pipiens* embryos or larvae inoculated with LTHV-containing extracts of Lucké tumors or virus purified by density gradient centrifugation. The ascites frequently produced by tumor-bearing frogs contains virus that can induce tumors when inoculated into embryos. Tumors have also been induced experimentally in other species of the host genus. Despite attempts employing cell lines from a wide range of hosts, it has not proved possible to culture LTHV *in vitro* from homogenates of Lucké tumors or the urine of tumor-bearing frogs. Therefore, it has not been possible to investigate the replication cycle. In contrast, FV4 can be grown in cell culture, and produces cytopathic effects typical of herpesviruses within 10–21 days after infection of *R. pipiens* embryo or adult kidney cells at 25 °C. These include cell rounding and vacuolization, enlargement of nuclei and development of intranuclear inclusions, and formation of syncytia.

CCV causes acute infection only in young channel catfish up to about 6 months old, the degree of mortality depending on the strain of fish. Certain other closely related species, such as the blue catfish (*Ictalurus furcatus*), may be infected experimentally by injection, but other species are refractory even by this route. Host cell requirements are a little less stringent in cell culture, but virus growth still only occurs in ictalurid and clariid fish cell lines. Among commonly used cell lines BB (*Ictalurus nebulosus* or brown bullhead) and CCO (channel catfish ovary), the latter is more susceptible. Optimal virus yield is obtained at 25–30 °C, and about 50% of progeny virus is released into the culture medium. Virus infection causes the formation of syncytia, particularly at lower multiplicities of infection, followed eventually by disaggregation and lysis. Under optimal conditions, peak virus yield may be attained only 12 h after infection. Thus, CCV is one of the fastest growing herpesviruses *in vitro*.

Experimental determinations have shown that about 50 CCV proteins are expressed or induced in infected cell culture. As with other herpesviruses, expression appears to be coordinately regulated. The major immediate early protein, which is likely to regulate the expression of other virus genes, has an apparent molecular mass of 117 000 Da. The capsid contains an abundant constituent (the major capsid protein) with a molecular mass of 128 000 Da and at least two smaller proteins. The major capsid protein of higher vertebrate herpesviruses is invariably larger, having a molecular mass of about 150 000 Da. Different isolates of CCV may be differentiated by restriction endonuclease digestion of their DNAs, but are recognizably similar. CCV DNA replicates in the nuclei of infected cells via head-to-tail concatemers made up of a unit comprising the unique region linked to a single copy of the direct repeat.

KHV disease is restricted to koi and common carp, and other fish tested have been shown to be resistant even after extensive exposure to infected carp at permissive temperatures. A cell line derived from koi fin has been used to culture KHV, with optimal growth occurring at 15–25 °C and no growth at 10 or 30 °C. The highest virus yields are generated 7 days after infection at 20 °C, with most infectivity released into the culture medium. KHV strains are highly similar to each other as assessed by restriction endonuclease and sequence analyses. A total of 31 virion proteins have been detected. Studies of KHV gene expression are in their infancy.

SalHV-1 causes disease when injected into young rainbow trout maintained at 6–9 °C, but not in other salmonid species. A similar temperature optimum is characteristic of growth in the rainbow trout cell line RTG-2. SalHV-2 has a slightly wider host range, causing virulent disease in the young of several members of the genus *Oncorhynchus* and the rainbow trout. It has a higher temperature optimum for growth in RTG-2 cells than SalHV-1 (15 °C).

Genetics and Evolution

The complete genome sequences of two fish and two amphibian herpesviruses have been determined: those of CCV, KHV, LTHV, and FV4. Partial data are available for other viruses in this group. **Table 1** shows some of the basic characteristics of the sequenced genomes. All have a type A genome structure, consisting of a unique region (U) flanked by a direct repeat at the termini (TR). However, this structure is not universal among fish and amphibian herpesviruses, as the SalHV-1 genome has a type D structure. All of the sequenced genomes contain a number of families of genes that are related (usually distantly) to each other and have presumably arisen by gene duplication. KHV has the largest genome thus far reported for any herpesvirus.

Figure 3 shows the predicted gene layout in CCV, which has the smallest sequenced genome among the fish and amphibian herpesviruses. Analysis of the sequence indicates the presence of 62 genes in U (two spliced) and 14 genes in TR. Several gene functions have been predicted from computer-aided comparisons of predicted CCV proteins with proteins from other organisms. Ten genes belong to four gene families: two encoding related sets of protein kinases (PK1 with ORF14, ORF15, and ORF16; PK2 with ORF73 and ORF74), one encoding proteins similar to a

Table 1 Characteristics of sequenced fish and amphibian herpesvirus genomes

Virus	*Genome size*[a] *(bp)*	*G + C composition (mol.%)*	*Genes*[b]	*Gene families*
CCV	134 226 [18 556]	56.2	76	4
KHV	295 146 [22 469]	59.2	156	5
LTHV	220 859 [636]	54.6	132	15
FV4	231 801 [912]	52.8	147	15

[a]The total size of the DNA sequence is given, with the size of TR in square brackets.
[b]Genes that are duplicated by virtue of their presence in the terminal repeat are counted only once.

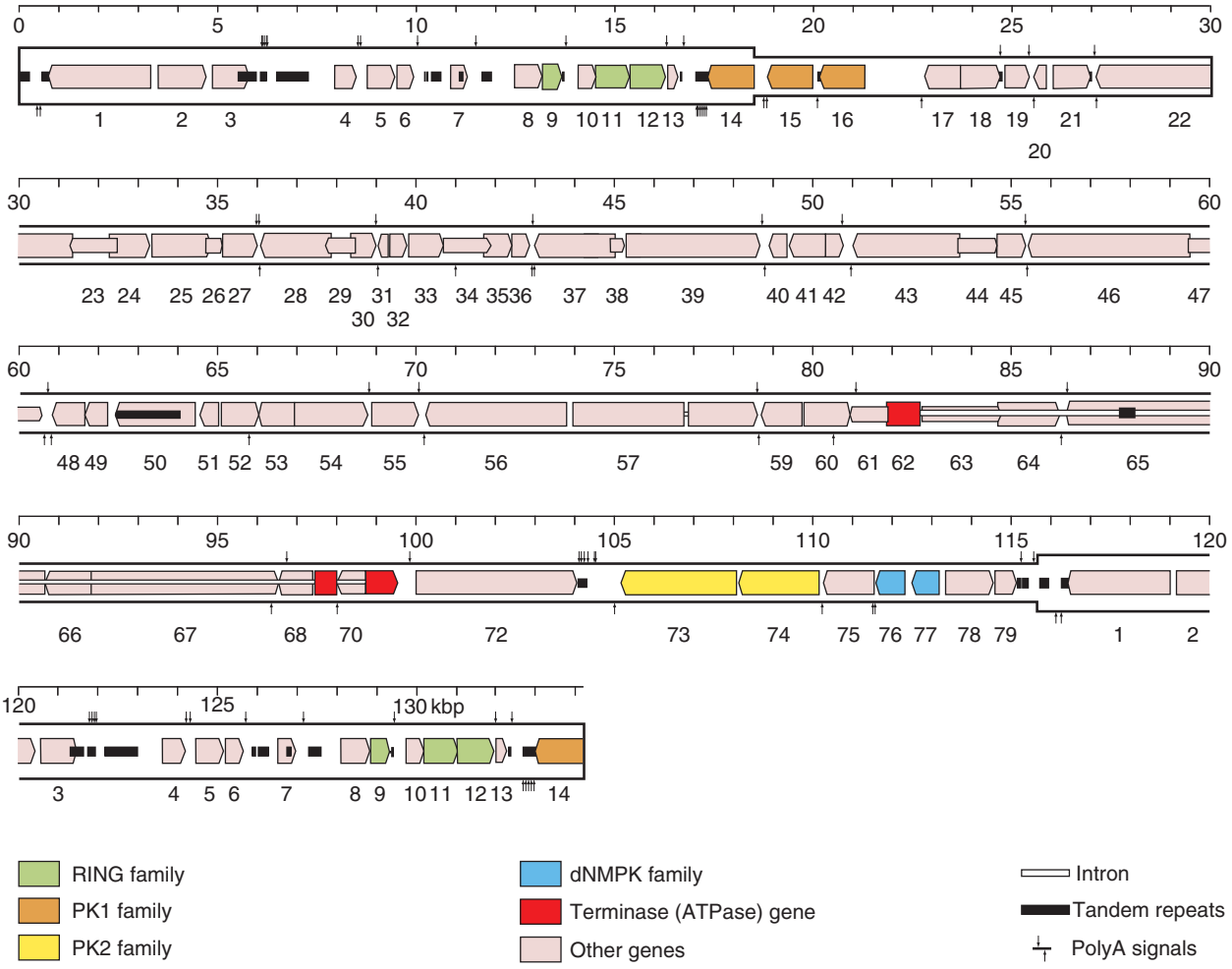


Figure 3 Arrangement of predicted protein-coding open reading frames (ORFs) in the CCV genome. ORFs are colored and numbered with the 'ORF' prefix omitted. The thinner portion of the genome denotes the unique region (U) and the thicker portions the terminal repeats (TR).

bacteriophage deoxynucleoside monophosphate kinase (dNMPK; ORF76 and ORF77), and one encoding proteins containing a potential zinc-binding domain (RING; ORF9, ORF11, and ORF12). Other genes encode a potential zinc-binding protein (ORF78), a protein containing a myosin-like domain (ORF22), a helicase (ORF25), a DNA polymerase (ORF57, composed of two exons), a deoxyuridine triphosphatase (ORF49), a thymidine kinase (ORF5), a subtilisin-like proprotein convertase (ORF47), the ATPase subunit of the putative terminase (ORF62, composed of three exons), a putative primase (ORF63), a protein containing an ovarian tumor (OTU)-like cysteine protease domain (ORF65), an envelope glycoprotein (ORF46), and seven other membrane-associated proteins (ORF6, ORF7, ORF8, ORF10, ORF19, ORF51, and ORF59). The principal constituent proteins of CCV virions have been identified by proteomic analysis. The capsid includes the major capsid protein (ORF39), a potential scaffold protein

(ORF28), and two other proteins that possibly constitute the intercapsomeric triplex (ORF27 and ORF53). Several virus proteins in the tegument have been identified, in addition to actin. The major envelope protein is specified by ORF59.

LTHV, FV4, and KHV have substantially more genes than CCV, and many of these are members of gene families or genes apparently captured from the cell or other viruses. For example, the frog herpesviruses, unlike the fish herpesviruses, encode a DNA (cytosine-5-)-methyltransferase and, presumably as a result, have extensively methylated genomes. Overall, the two frog herpesvirus genomes are the most closely related of the four, sharing 40 genes (when genes with marginal similarity are excluded). CCV shares 19 genes with the frog herpesviruses and only 15 with KHV. The modest number of conserved genes indicates that the evolutionary distance among lower vertebrate herpesviruses is substantially greater than that among higher vertebrate herpesviruses.

Where functions may be predicted for the conserved genes, they are central to herpesvirus replication, being involved in capsid morphogenesis, nucleotide metabolism, DNA replication, and DNA packaging. The arrangement of homologous genes in LTHV and FV4 is collinear, except for one block of genes that is in a different position and orientation in the two genomes. In contrast, homologous genes in the frog herpesviruses are situated in several rearranged blocks in the more distantly related fish herpesviruses. This parallels the situation among members of different subfamilies of higher vertebrate herpesviruses. As with higher vertebrate herpesviruses, homologous genes tend to be located centrally in the genomes.

The salmonid viruses SalHV-1 and SalHV-2 have been shown to be distinct viruses on the base of serological comparisons, DNA hybridization studies, and limited DNA sequencing. The SalHV-1 genome is approximately 174 kbp in size and has homologues of at least 18 CCV genes in several rearranged blocks. Sequence data indicate that these two viruses are related to each other and, more distantly, to CCV.

No proteins encoded by fish and amphibian herpesviruses are detectably related in their primary sequences to proteins that are specific to higher vertebrate herpesviruses (e.g., those that make up the capsid, of which the structure is conserved). This observation suggests that the two groups diverged from their common ancestor a long time ago. The best (and perhaps only) sequence-based evidence for a common evolutionary origin of the two groups depends on conservation of the putative ATPase subunit of the terminase, which is encoded by a spliced gene containing three exons (**Figure 3**). This protein is thought to be involved in packaging replicated DNA into the capsid and is also distantly conserved in T4-like bacteriophages, thus contributing to the notion that herpesviruses may share an ancient origin with bacteriophages.

Immune Response

Understanding of the immune responses to amphibian and fish herpesviruses is not well developed, and data obtained under relatively normal conditions of infection are limited largely to CCV. Adult channel catfish produce peak neutralization titers on average 8 or 9 weeks after primary immunization with CCV, and a further moderate increase in titer of short duration is apparent after boosting. The importance of virus growth in eliciting the immune response is indicated by the observation that heat-inactivated virus is poorly immunogenic. Serum neutralization indexes have been used to document a link between outbreaks of CCV disease and potential carriers of the virus. These studies, though not extensive, have given some indication that seroconverted adult fish are able to transmit CCV.

Control and Prevention

The devastating economic losses occasioned by certain of the fish herpesviruses prompt the need for intervention protocols. Avoidance and containment of outbreaks presently involve the use of ostensibly virus-free or partially resistant breeding stock, as clean environmental conditions as practicable, destruction of affected populations, and disinfection of ponds. The use of temperature variation regimes to aid the survival of infected fish has also been mooted. It is clear from the KHV situation, where individual fish may be very valuable, that effective diagnosis and monitoring of stock movements should be considered in order to avoid the spread of highly virulent diseases.

CCV is sensitive to nucleoside analogs that inhibit the growth of higher vertebrate herpesviruses. In general, these compounds are phosphorylated by the virus, but not the cellular, thymidine kinase. Further steps in phosphorylation probably involve cellular enzymes. The triphosphate form then inhibits DNA replication by direct interaction with the DNA polymerase or by incorporation into nascent DNA. These compounds include the widely used antiherpetic drug acyclovir. Phosphonoacetate, which inhibits the DNA polymerase by mimicking pyrophosphate, is also inhibitory. Against considerations of cost and administration, however, it is doubtful whether antiviral therapy will ever be generally practicable for the treatment of fish herpesvirus diseases.

The poor immunogenicity of inactivated CCV and KHV preparations suggests that the use of live, attenuated viruses is likely to be more effective in vaccination strategies. However, given the potential for latent vaccine virus to revert to wild type, thorough characterization of vaccine candidates and development of assays to differentiate vaccine from wild-type virus are essential (for a precedent

in higher vertebrate herpesviruses, consider pseudorabies virus). The use of avirulent strains of virus in which virulence genes are replaced by detection markers may lead in the long term to safe, efficacious vaccines.

CCV attenuated by passage in a clariid fish cell line was able to provide substantial protection against the lethal effect of infection by virulent virus, particularly when the initial immunization was boosted. This virus exhibits several genomic differences from wild type, most notably a substantial deletion in ORF50, which potentially encodes a secreted glycoprotein. In this context, deletion of ORF5, which encodes thymidine kinase, has been shown to cause attenuation of virulence in channel catfish fingerlings. Attenuated versions of KHV produced by serial passage in koi fin cell culture and subjecting clones to further mutation *in vitro* did not induce lethal disease and protected immunized fish against challenge. This protection correlated with antibody production.

DNA vaccines also hold promise in combating fish herpesvirus diseases, particularly in their avoidance of utilizing intact virus. From seven CCV genes tested, ORF59 (encoding the major envelope glycoprotein) and ORF6 (encoding a predicted membrane protein) produced significant resistance to challenge, with combined vaccination even more effective.

Acknowledgment

The author is grateful to Ronald Hedrick for comments on the pathological features of KHV in light of his unpublished data.

See also: Herpesviruses: General Features; Pseudorabies Virus.

Further Reading

Aoki T, Hirono I, Kurokawa K, *et al.* (2007) Genome sequences of three koi herpesvirus isolates representing the expanding distribution of an emerging disease threatening koi and common carp worldwide. *Journal of Virology* 81: 5058–5065.

Booy FP, Trus BL, Davison AJ, and Steven AC (1996) The capsid architecture of channel catfish virus, an evolutionarily distant herpesvirus, is largely conserved in the absence of discernible sequence homology with herpes simplex virus. *Virology* 215: 134–141.

Davison AJ (1992) Channel catfish virus: A new type of herpesvirus. *Virology* 186: 9–14.

Davison AJ, Cunningham C, Sauerbier W, and McKinnell RG (2006) Genome sequences of two frog herpesviruses. *Journal of General Virology* 87: 3509–3514.

Gilad O, Yun S, Adkison MA, *et al.* (2003) Molecular comparison of isolates of an emerging fish pathogen, koi herpesvirus, and the effect of water temperature on mortality of experimentally infected koi. *Journal of General Virology* 84: 2661–2667.

Granoff A (1972) Lucké tumor-associated viruses – a review. In: Biggs PM, de-The G and Payne LN (eds.) *Oncogenesis and Herpesviruses*, pp. 171–182. Lyons: International Agency for Research on Cancer.

Granoff A (1983) Amphibian herpesviruses. In: Roizman B (ed.) *The Herpesviruses,* vol. 2, pp. 367–384. New York: Plenum.

Ilouze M, Dishon A, and Kotler M (2006) Characterization of a novel virus causing a lethal disease in carp and koi. *Microbiology and Molecular Biology Reviews* 70: 147–156.

McKinnell RG, Lust JM, Sauerbier W, *et al.* (1993) Genomic plasticity of the Lucké renal carcinoma: A review. *International Journal of Developmental Biology* 37: 213–219.

Naegele RF, Granoff A, and Darlington RW (1974) The presence of Lucké herpesvirus genome in induced tadpole tumors and its oncogenicity: Koch–Henle postulates fulfilled. *Proceedings of the National Academy of Sciences, USA* 71: 830–834.

Pikarsky E, Ronen A, Abramowitz J, *et al.* (2004) Pathogenesis of acute viral disease induced in fish by carp interstitial nephritis and gill necrosis virus. *Journal of Virology* 78: 9544–9551.

Plumb JA (1989) Channel catfish virus. In: Ahne W and Kurstak E (eds.) *Viruses of Lower Vertebrates*, pp. 198–216. Berlin: Springer.

Wolf K (1983) Biology and properties of fish and reptilian herpesviruses. In: Roizman B (ed.) *The Herpesviruses,* vol. 2, pp. 319–366. New York: Plenum.

Fish Retroviruses

T A Paul, R N Casey, P R Bowser, and J W Casey, Cornell University, Ithaca, NY, USA
J Rovnak and S L Quackenbush, Colorado State University, Fort Collins, CO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cytopathic effect Morphologic changes in cells resulting from virus replication.
Dysplasia An abnormality in the appearance of cells and indicative of a preneoplastic change.
Hyperplasia An increase in the number of cells of an organ or tissue.
Leiomyosarcoma Neoplasia of smooth muscle cells.
Malpighian cells Cells of the deepest layer of the epidermis.
Neoplasia An abnormal growth of cells.

Oncogene Cellular or viral gene whose products are capable of inducing a neoplastic phenotype.
Proto-oncogene A normal cellular gene which when inappropriately expressed or mutated becomes an oncogene.

Introduction

Retroviruses have been documented in a wide range of vertebrate species including lower vertebrates such as frogs, snakes, sharks, fish, birds, and turtles. To date, however, the majority of these reported sequences represent only partial fragments of the retrovirus. In recent years, the complete genomic sequence of exogenous fish retroviruses from Atlantic salmon (*Salmo salar*), walleye (*Sander vitreus*), and snakehead (*Ophicephalus striatus*), and an endogenous retrovirus of zebrafish (*Danio rerio*) have been determined. Analysis of these viral genomes indicates a high degree of diversity among the fish retroviruses as well as several unique features compared to mammalian and avian retroviruses.

The etiological relationship between retroviruses and cancer is well established in mammalian and avian systems. Simple retroviruses can promote cell proliferation by inducing the ectopic expression of captured cellular oncogenes (viral transduction for acutely transforming viruses) in which an oncogene recombines into the viral genome, or by integration near or within the coding sequence of cellular proto-oncogenes (nonacute transforming viruses). Transcription factors encoded by some complex retroviruses, like human T-cell leukemia virus-1 (HTLV-1), also have nonacute oncogenic potential by deregulating cellular pathways controlling cell proliferation.

Like their mammalian and avian counterparts, many fish neoplastic/proliferative diseases are suspected of having a retroviral etiology. Retroviruses are reported to be associated with 13 spontaneous proliferative diseases of fish based on the observation of retrovirus-like particles and, in some cases, reverse transcriptase activity in neoplastic lesions. Seven of these diseases with putative viral etiologies display a seasonal cycle, that is, they develop and regress annually. Retrovirus-associated tumors of the skin have received the most attention, since they are most visible and easily sampled. Skin tumors with suspected viral etiologies have been found in white sucker (*Castostomus commersoni*), walleye, yellow perch (*Perca flavescens*), and European smelt (*Osmerus eperlanus*). Retroviruses have also been linked to lymphoma in northern pike (*Esox lucius*), leukemia in chinook salmon (*Oncorhynchus tshawytscha*), and leiomyosarcoma of the swimbladder in Atlantic salmon. One noteworthy piscine retrovirus, the snakehead fish retrovirus (SnRV), has not been associated with tumor induction. In most of these systems, the etiologic relationship of retroviral infections and neoplasms rests on circumstantial evidence such as observations of viral particles by electron microscopy and the presence of reverse transcriptase activity. However, sequencing of fish retroviruses has facilitated use of molecular-based diagnostic reagents and, in some cases, shed light on potential modes of pathogenesis. In particular, the walleye retroviruses, walleye dermal sarcoma virus (WDSV) and walleye epidermal hyperplasia virus type 1 (WEHV-1) and type 2 (WEHV-2), express a subset of accessory genes exclusively during oncogenesis, specifically implicating the encoded proteins in the process of tumorigenesis.

Retroviruses and their Life Cycle

Retrovirus particles are composed of viral structural and enzymatic proteins and two copies of the positive-sense single-stranded RNA genome. The proteins are surrounded by a lipid bilayer, acquired from the host cell, in which viral envelope glycoproteins are embedded. Retroviral infection is initiated when the surface glycoprotein binds to its cognate receptor on the surface of a susceptible cell. Upon fusion of the viral and cellular membranes, the viral core enters the cytoplasm where the virally encoded enzyme, reverse transcriptase, copies the RNA genome into DNA. The viral integrase protein binds reverse transcribed DNA and mediates its integration into the genomic DNA of the host. In rare cases, retroviral infection of host germ-line cells and subsequent integration into the chromosomes can establish retroviral sequences as heritable genetic elements, known as endogenous retroviruses.

Based on the complexity of the viral genome, retroviruses can be divided into two broad categories: simple and complex. Simple retroviruses, including members of the *Gammaretroviruses* and *Alpharetroviruses*, contain three genes: *gag*, *pol*, and *env*. Expression of a full-length unspliced messenger RNA (mRNA) and a spliced mRNA serve as the templates for translation of the retroviral structural and enzymatic proteins. These proteins are first translated as polyproteins from *gag* and *pol*, respectively, and are processed by a virally encoded protease. The viral envelope glycoprotein is encoded from a singly spliced viral transcript containing the *env* gene.

Complex retroviruses are distinguished from simple retroviruses on the basis of their additional coding capacity and pattern of viral gene expression. This category of retroviruses includes *Lentiviruses* such as human immunodeficiency virus type 1 (HIV-1), *Spumaviruses*, and the *Deltaretroviruses*, human T-cell leukemia virus (HTLV-1), and bovine leukemia virus (BLV). Complex retroviruses produce multiply spliced transcripts that encode two classes

of accessory/regulatory proteins. One class includes transcriptional regulatory proteins such as HTLV-1 Tax and HIV-1 Tat, which act *in trans* to directly regulate the activity of the viral promoter. A second class, including HTLV-1 Rex and HIV-1 Rev, act post transcriptionally, to facilitate transport of unspliced and singly spliced viral transcripts to the cytoplasm. The combined action of these proteins divides the replication cycle into two temporal phases: an early, regulatory phase, and a later, structural phase. During the regulatory phase, low levels of fully spliced transcripts encoding *trans*-activators increase levels of transcription leading to the accumulation of post-transcriptional regulatory proteins. These proteins allow production of unspliced and singly spliced mRNAs that encode the viral structural proteins.

Both simple and complex retroviruses have been identified in fish species. Retroviruses isolated from walleye and snakehead fish resemble complex retroviruses based upon their genome structure and transcriptional profile, while simple retroviruses have been identified in Atlantic salmon and zebrafish.

Skin Tumors in Walleye and Their Associated Retroviruses

Walleye dermal sarcoma (WDS) and walleye discrete epidermal hyperplasia (WEH) are neoplastic and hyperplastic skin lesions in walleye that have been etiologically associated with infection by three distinct retroviruses (**Figure 1**). WDS and WEH were first reported in 1969 on fish from Oneida Lake in New York. Since then, WDS and WEH have been reported on walleyes throughout North America. These diseases have a seasonal cycle; tumor incidence is highest in the late fall and early spring months at frequencies of 27% and 5% for WDS and WEH, respectively. Lower tumor prevalence in summer months is associated with regression on individual adult fish.

WDS appears as a cutaneous mesenchymal neoplasm arising multicentrically within the superficial dermis and overlaid with epidermis. Fall tumors appear highly vascularized due to a network of capillaries, while spring tumors are frequently white with ulcerated surfaces. Invasive tumors are rarely observed in feral fish. WEH lesions are benign mucoid-like plaques with distinct boundaries. They can range from 2 to 3 mm in diameter to large lesions with irregular borders that may be as large as 50 mm across. Histologically, this disease appears as an epidermal proliferation consisting primarily of Malpighian cells.

Experimental transmission of WDS and WEH has been achieved with cell-free filtrates. Interestingly, only cell-free filtrates prepared from the spring, regressing WDS, not fall, developing WDS, are able to transmit disease. Infection of walleye fingerlings less than 9 weeks of age results in the frequent development of invasive tumors.

Electron microscopic observation of retrovirus particles and cell-free transmission of both diseases suggest a retroviral etiology for WDS and WEH. WDSV was isolated from WDS tissue, and two independent retroviruses, WEHV1, WEHV2, were isolated from WEH lesions. Similar hyperplastic lesions on yellow perch in Oneida Lake are associated with two new retroviruses, perch epidermal hyperplasia virus types 1 and 2 (PEHV1, PEHV2).

Isolation and Sequencing of Retroviruses from WDS and WEH Lesions

WDSV, WEHV1, and WEHV2 have been molecularly cloned and sequenced (**Figure 2**). The genome structures of WDSV, WEHV1, and WEHV2 indicate large and complex viral genomes (12.7, 12.9, and 13.1 kbp, respectively).

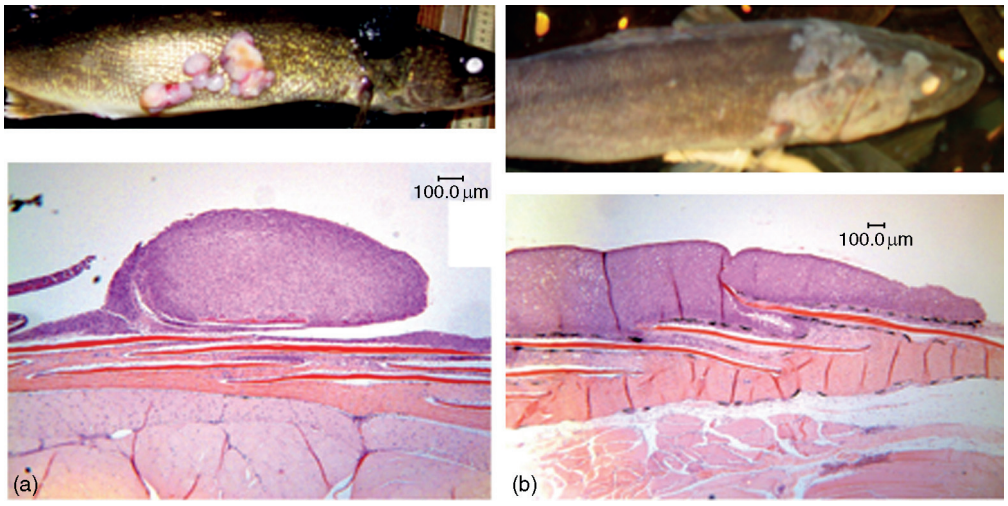


Figure 1 (a) Gross and histological images of walleye dermal sarcoma and (b) walleye epidermal hyperplasia.

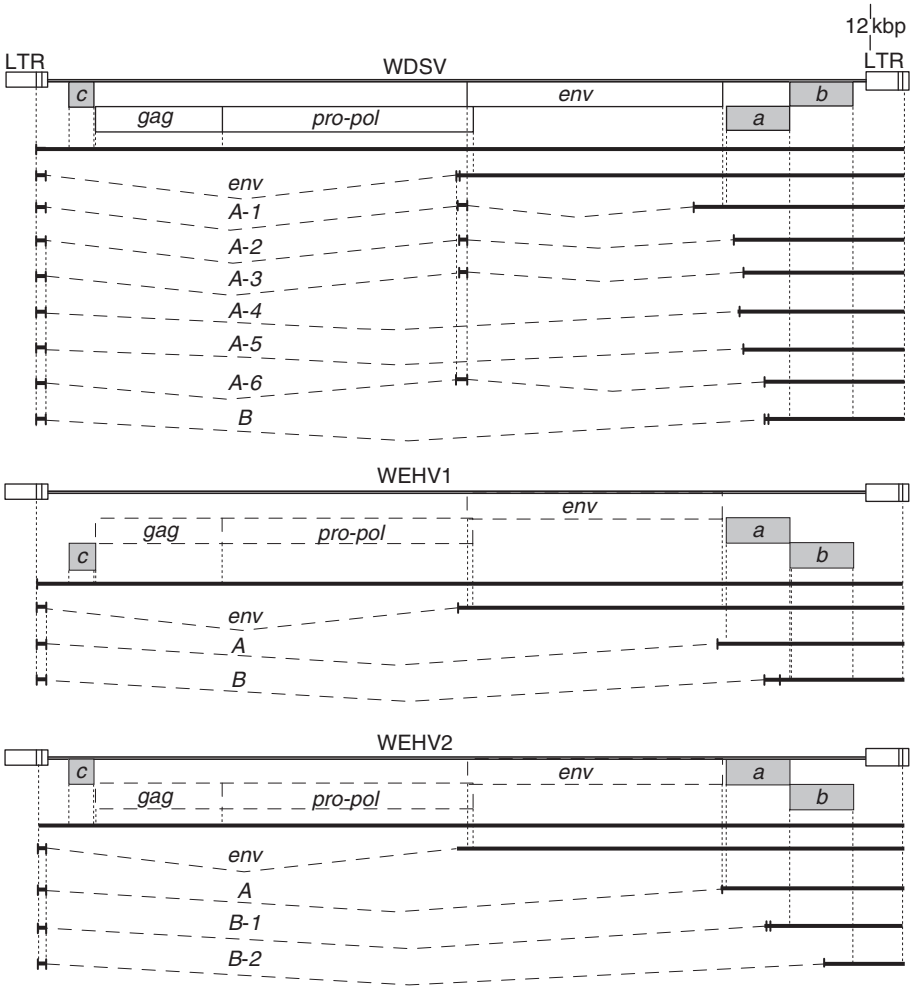


Figure 2 Genomic and transcriptional maps of WDSV, WEHV1, and WEHV2. Genomic organization of viral DNA with retroviral genes and accessory genes *orfa*, *orfb,* and *orfc* is depicted under each virus heading. The envelope-spliced transcript (*env*) and additional spliced transcripts (*A* and *B*), which are capable of expressing accessory genes, are illustrated. The vertical dashed lines indicate the boundaries of the exons, introns, and open reading frames.

All three viruses have intact open reading frames (ORFs) capable of encoding the structural and enzymatic genes *gag*, *pol*, and *env*. *gag* (viral capsid) and *pol* (reverse transcriptase) are in the same reading frame and synthesized as a polyprotein through a termination suppression mechanism. *pro* (viral protease), responsible for cleaving the polyprotein, is located in the same reading frame as *pol*. A unique feature of all three viruses is the presence of additional orfs that have tentatively been called *orfa*, *orfb*, and *orfc*. The *orfa* and *orfb* genes are located between *env* and the 3′ long terminal repeat (LTR), while *orfc* is located between the 5′ LTR and the start of *gag*. Database searches with OrfA protein amino acid sequences suggest limited homology with D-type cellular cyclins within the cyclin-box motif, and are referred to as rv-cyclins. WEHV1 rv-cyclin has a 20% amino acid identity and 35% similarity with human cyclin D-3, whereas WEHV2 and WDSV rv-cyclin are most similar to human cyclin D-1 (22%/35% and 19%/29% amino acid identity/similarity). Similarities with known walleye and other piscine cyclins are not significantly greater. The WEHV cyclin homologs are 37% identical within the cyclin box motif and 21–28% identical with the WDSV cyclin. Sequence homologies between rv-cyclin and OrfB in the walleye retroviruses suggest they arose by a gene duplication event.

Roles of the WDSV Accessory Proteins in Pathogenesis

Transcriptional mapping by RT-PCR and Northern blot analyses of developing and regressing WDS and WEH have demonstrated temporal gene expression profiles and complex splicing patterns analogous to those seen in the complex retroviruses of mammals. In developing WDS tumors, only low levels of multiply spliced subgenomic transcripts are detected. These transcripts predominantly contain the coding sequences for rv-cyclin and OrfB. Regressing spring tumors contain high levels of full-length and subgenomic

transcripts as well as unintegrated viral DNA and transmissible virus. OrfC is likely encoded by the full-length transcript coincident with tumor regression.

A detailed transcriptional analysis of WDSV showed an alternative splicing pattern of the *orfa* transcript (**Figure 2**). In developing tumors, the *orfa* transcript contains the coding sequence for a full-length rv-cyclin protein that localizes to the nucleus of mammalian and walleye cells. Alternatively spliced forms of this transcript encode amino-terminal truncated forms of the rv-cyclin protein, which localize in the cytoplasm of cells. The different forms of rv-cyclin may play functionally different roles in developing and regressing tumors. A single, spliced transcript encodes the OrfB protein, which is predominantly localized in the cytoplasm in tumor explant cells, but is capable of shuttling into and out of the nucleus when expressed in piscine and mammalian cells.

The OrfC protein from WDSV has been shown to localize to mitochondria and to disrupt mitochondrial function, which results in apoptosis. Only the full-length viral transcript found in regressing tumors is capable of encoding OrfC. Apoptotic cells are present in regressing, but not developing, WDS tumor sections. Therefore, the OrfC protein may be responsible for a direct viral mechanism of tumor regression.

WDSV *orfa* and *orfb* are the only virus transcripts found in developing tumors, suggesting direct roles for rv-cyclin and OrfB in the process of tumorigenesis, and their oncogenic potential has been demonstrated by several experimental approaches. WDSV *orfa* supported growth of a yeast (*Saccharomyces cerevisiae*) strain conditionally deficient for the synthesis of the G1 to S cyclins that are necessary for cell cycle progression. WEHV *orfa* did not support yeast growth in this model. Transgenic expression of WDSV *orfa* in mice from a skin-specific promoter caused a moderate to severe squamous epithelial hyperplasia and dysplasia dependant on skin injury. This suggests that rv-cyclin is not oncogenic as a result of a 'single-hit' mechanism, but rather, that secondary genetic or epigenetic events (possibly wound repair) are necessary for tumor development and progression. A similar phenotype has been described in transgenic mice expressing *v-jun* oncogene from the H-2K^k major histocompatibility complex (MHC) class I antigen gene promoter. When expressed in mammalian or piscine cell culture, WDSV rv-cyclin localized in the nucleus and was found by co-immunoprecipitation to be associated with cyclin dependent kinase 8 and cyclin C, general transcription initiation factors, and RNA polymerase II transcription complexes. In piscine cells, rv-cyclin inhibited transcription from the WDSV promoter independent of *cis*-acting DNA sequences. However, rv-cyclin can activate other viral promoters in fish cells, and can activate the WDSV promoter in select mammalian cells; thus rv-cyclin activation and inhibition of transcription are dependent on both the promoter and the cell type. WDSV rv-cyclin contains a defined transcription activation domain in the carboxy end of the protein, and the isolated activation domain is capable of interacting with co-activators of transcription. WEHV rv-cyclins do not have a corresponding carboxy-region activation domain. WDSV rv-cyclin may exhibit oncogenic potential by differential regulation of host gene expression such as proto-oncogenes or tumor suppressors.

Less is known about the capabilities of the OrfB protein, but initial studies indicate its direct association with the regulation of signal transduction pathways. It is concentrated, in tumor explant cells, at focal adhesions, and along actin stress fibers. Established OrfB-expressing lines are resistant to the chemical induction of apoptosis, suggesting a role in oncogenesis.

The origins of the accessory genes of complex retroviruses are unclear. This includes the origins of the WDSV accessory genes encoding rv-cyclin, OrfB, and OrfC. In the case of rv-cyclin and OrfB, their extreme divergence from host cyclin sequences indicates that any transduction event was ancient and led, ultimately, to an exclusive, complex viral species. The OrfC protein, like the accessory proteins of other complex retroviruses, has no clear homology to host proteins.

Control of the Seasonal Cycle of Disease

An interesting aspect of WDS is the control of the seasonal switch in viral gene expression and associated tumor regression. Potential modulators of viral gene expression include host immunity and endocrine activity (accompanying spawning) and environmental factors such as water temperature, physical trauma, and sunlight. A number of *cis*-acting elements important for transcription activation have been identified in the WDSV promoter. There is differential binding of proteins from developing and regressing tumor nuclear extracts to a 15 bp repeat region in the WDSV promoter. This element may be critical to the induction of high levels of virus expression. The WDSV rv-cyclin protein negatively regulates the WDSV promoter in tissue culture cells. Presumably, it is advantageous for the virus to have lower levels of gene expression during tumor development to avoid immune surveillance as well as the cytopathic effects associated with virus production. While rv-cyclin may function in the repression of virus expression during tumor growth, the host, environmental, or viral signals that switch on full virus expression are yet to be determined.

Snakehead Retrovirus

The SnRV was isolated from a productively infected cell line derived from a Southeast Asian striped snakehead

fish. Cell culture supernatant from these infected cells demonstrated high levels of RT activity and the presence of type C-like retrovirus particles. Additionally, supernatant from the infected cell line induced cytopathic effects in cultures of a bluegill cell line (BF-2).

Molecular approaches were utilized to identify and sequence the SnRV from infected cells. The large 11.2 kbp genome of SnRV contains intact coding regions for *gag*, *pol*, and *env* (**Figure 3**). An arginine tRNA primer binding site used for reverse transcription initiation distinguishes SnRV from other retroviruses. The structure and transcriptional profile of SnRV suggests a complex expression pattern capable of encoding an ORF located between *env* and the 3′ LTR and two very small ORFs termed ORF1 and ORF2. Within the leader sequence of SnRV resides a start codon located just upstream of the major splice donor site that could potentially encode a 14 amino acid peptide (LP). Expression of Env from a singly spliced transcript is predicted to utilize the LP initiation codon and fuses this 14 amino acid peptide in frame to downstream *env* sequences. A transcript with four exons and two initiator codons would encode ORF1 and ORF2 proteins. The 3′ ORF would be expressed from a transcript containing three exons and would encode a protein of 24 kDa. Finally, the fourth spliced transcript may encode 3′ ORF or possibly a LP-Env cytoplasmic domain (CD) fusion protein. The 3′ ORF contains an N terminal acidic domain, cysteine residues, and a basic region, motifs commonly found in transcriptional activators. These small ORFs have no significant homology to any known proteins in the databases, and their role in the viral life cycle is unknown. No endogenous copies of SnRV have been identified in the snakehead genome and an uninfected cell line has been established.

Although the presence of SnRV in wild populations has not been thoroughly examined, the virus has been independently isolated from two separate snakehead cell lines derived from whole fry tissue and from caudal peduncle tissue from juvenile fish. In all cases, fish from which these cell lines were derived appeared healthy.

Salmon Swimbladder Sarcoma-Associated Retrovirus

An outbreak of neoplastic disease of the swimbladder of Atlantic salmon was first reported at a Scottish commercial marine fish farm in 1975. Affected salmon were sluggish and in poor condition. A viral etiology was suspected, and electron microscopic evaluation of tumors revealed the presence of budding viral particles with retroviral morphology. A second outbreak occurred between 1995 and 1997 in brood stock salmon held at the North Attleboro National Fish Hatchery in Massachusetts. The fish exhibited skin discoloration and hemorrhages on the fins and body and showed a general debilitation and lack of vigor. In May of 1997, significant mortality was noted at the facility such that 35% of the population was affected by late spring of 1998. All of the affected fish displayed swollen abdomens due to multinodular masses on internal and external surfaces of the swimbladder. In several cases, these multinodular masses occupied the entire swimbladder (**Figure 4**). Histologic examination revealed that the tumors were composed of well-differentiated fibroblastic cells that were arranged in interlacing bundles, which were classified as leiomyosarcomas.

Isolation of a Retrovirus from Salmon Swimbladder Sarcoma

An exogenous retrovirus, termed Atlantic salmon swimbladder sarcoma virus (SSSV), was initially identified in tumors by degenerate RT-PCR of tumor RNA, and the entire viral sequence completed by DNA sequencing of

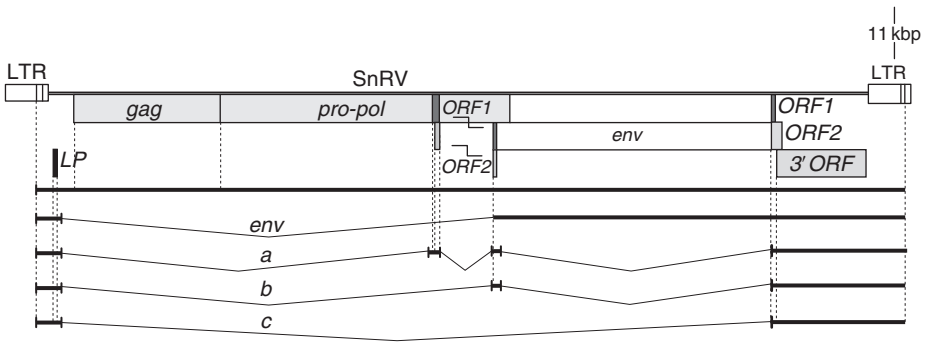


Figure 3 Genomic and transcriptional map of SnRV. Predicted coding regions are shown as open or shaded boxes. The envelope-spliced transcript (*env*) and additional spliced transcripts (*a*, *b*, *c*), which are capable of expressing accessory genes, are illustrated. LP indicates the location of a predicted leader peptide. The vertical dashed lines indicate the boundaries of the exons, introns, and open reading frames.

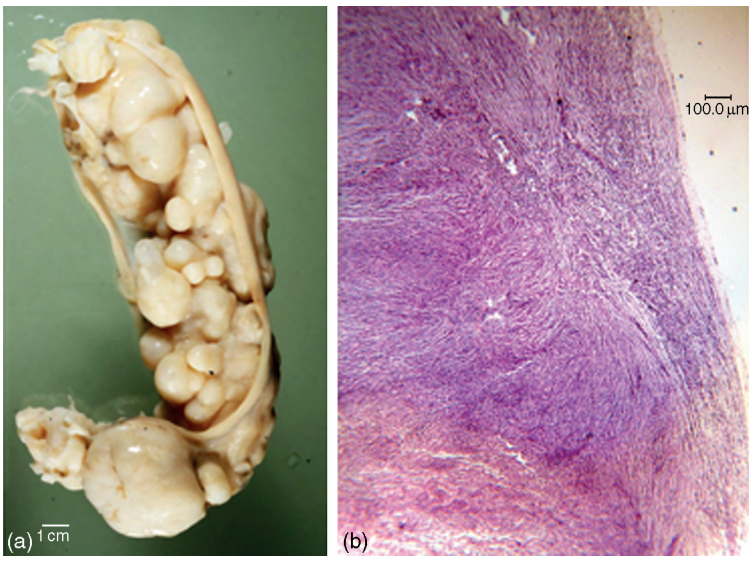


Figure 4 (a) Gross and (b) histological images of Atlantic salmon swimbladder sarcoma.

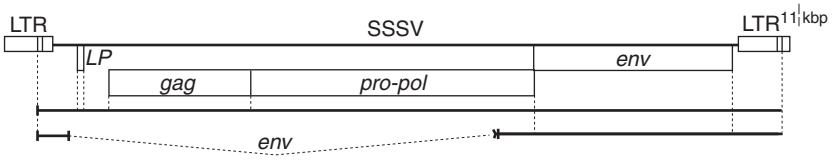


Figure 5 Genomic and transcriptional map of SSSV. Predicted coding regions are shown as open boxes. The envelope-spliced transcript (*env*) is illustrated. LP indicates the location of a predicted leader peptide. The vertical dashed lines indicate the boundaries of the exons, introns, and open reading frame.

proviral DNA. In contrast to the complex walleye retroviruses, SSSV is a simple retrovirus. The viral genome contains ORFs capable of expressing *gag*, *pol*, and *env* (**Figure 5**). Additionally, a short 25 amino acid leader peptide of unknown function is located upstream of the Gag-Pol polyprotein ORF. SSSV differs from other simple retroviruses by not having related endogenous sequences in the host genome, and SSSV is the only retrovirus to use a methionine-tRNA as a plus-strand primer. Additionally, sequences in *pol* display homology to central polypurine tract regions identified in complex retroviruses. Central polypurine tracts facilitate the formation of a single-stranded DNA region called a central DNA flap as a result of reverse transcription priming from this internal site. In HIV-1, this feature plays a major role in complex retroviral replication and allows efficient infection of nondividing cells. It is intriguing that SSSV displays a high proviral copy number (greater than 30 copies per cell) with a polyclonal integration pattern in swimbladder tumors. SSSV must be capable of initiating multiple rounds of infection within the same cell. The specific mechanisms leading to the high copy number and its implications in the pathogenesis of disease are of significant interest for future research.

Sequence Comparisons of SSSV with Other Retroviruses

A comparison of the sequence of SSSV viral proteins with other retroviruses indicates large regions of homology with mammalian type C and type D viruses in Pol and Env. Additionally, a 179 amino acid region in the C terminus of Gag and a 1064 amino acid region of Pro–Pol displays 23% and 33% identity to the related WDSV proteins, respectively. SSSV has striking homology to the sequence of the zebrafish endogenous retrovirus (ZFERV). BLAST analysis of the Gag, Pol, and Env ORFs of SSSV reveals a 25% identity with ZFERV over 533 amino acids within Gag, a 40% identity over 533 amino acids of Pol, and a 39% identity over 429 amino acids of Env.

Prevalence, Seasonality, and Transmission of SSSV

A PCR diagnostic assay was developed using sequences within the *pol* gene of SSSV. Prevalence at the North Attleboro fish facility has been found to be 52% and 5% of a natural stock of Pleasant River salmon were found to harbor SSSV.

Like other retroviral diseases of fish, observations suggest that salmon swimbladder sarcoma is seasonal. First, of the 34% fish mortality at the North Attleboro facility between 1997 and 1998, 57% occurred in June. Second, in the following year, the surviving salmon had an SSSV incidence (measured by PCR) that cycled with a peak in late summer to early winter and then diminished in late winter to early spring. The period of highest SSSV incidence correlated with salmon spawning runs in late fall. Interestingly, WDSV prevalence, which peaks during late spring, also correlates with the time of spawning. This suggests that endocrine changes during spawning runs may be a critical factor in the observed seasonal variation in disease incidence.

Pathogenesis of SSSV

The existence of SSSV sequences in association with an outbreak of the swimbladder sarcoma suggests a role for the retrovirus in pathogenesis of the disease. As a simple retrovirus with no transduced cellular oncogenes, one possible mechanism of tumorigenesis is the insertional activation of a cellular proto-oncogene. The high copy number of proviruses in tumors has made the analysis of insertional activation events difficult, but multiple insertions increase the likelihood of such a mechanism. It is also feasible that SSSV may express an oncogenic viral gene product. This mechanism of oncogenesis has been proposed for the Jaagsiekte sheep retrovirus (JSRV) in the induction of ovine pulmonary adenocarcinoma and in Friend spleen focus-forming virus (SFFV)-associated erythroid hyperplasia in mice. Additionally, the presence of common integration sites within JSRV-associated tumors suggests that insertional mutagenesis may act in concert with the envelope in tumor development. Thus, a multifactorial mechanism for the development of salmon swimbladder sarcoma must also be considered.

In addition to sarcoma, a number of other distinct pathologies are associated with SSSV infection. These diseases are frequently debilitating and are, therefore, of significance to the aquaculture industry. Atlantic salmon at the North Attleboro hatchery, during the SSSV outbreak, presented with multifocal hemorrhages, sloughing of the epidermis, lethargy, wasting, and failure to mature sexually in addition to swimbladder tumors.

Zebrafish Endogenous Retrovirus

Endogenous retroviruses have been identified in almost all vertebrate genomes, and most are defective due to mutations and deletions. An endogenous retrovirus, ZFERY with a genome of 11.2 kbp has been identified in the Tubingen stock of zebrafish, and contains intact coding regions for *gag*, *pol*, and *env*. The *gag* and *pol* genes are in the same reading frame. While the majority of endogenous retroviruses are transcriptionally silent because of mutation or methylation, ZFERV remains transcriptionally active. In addition to genomic transcripts that encode Gag and Pol proteins, an unusual multiply spliced *env* transcript is produced. Expression appears highest in the larval and adult zebrafish thymus, and no expression was detected in 2-day old embryos, suggesting that ZFERV expression may be tied to thymic development.

Phylogeny of Fish Retroviruses

Retroviruses have been classified into seven genera based largely on highly conserved amino acid sequences in the retroviral reverse transcriptase gene. While the majority of the viral sequences employed in this classification represent mammalian and avian retroviruses, a new genus termed *Epsilonretroviruses*, representing the fish retroviruses WDSV, WEHV-1, WEHV-2, has been added to the most recent classification. As more retroviral sequences from lower vertebrates have been identified, it has become apparent that this classification scheme may be inadequate to represent the apparent diversity.

Based on the unique characteristics of SnRV, including its genomic organization, tRNA primer, and complex transcriptional profile, the virus is yet to be definitively placed in the current classification. The large size of the genome, genetic organization, and presence of additional ORFs suggest that SnRV is closest to the spumaviruses and walleye retroviruses, but its limited sequence homology suggests SnRV is divergent from these groups.

Phylogenetic analysis indicates that, while the walleye retroviruses cluster in a group representing the *Epsilonretroviruses*, SSSV and ZFERV appear to represent a new branch of piscine retroviruses between the walleye retroviruses and the *Gammaretroviruses*, a genera that includes the murine leukemia virus (MLV)-related retroviruses (**Figure 6**). The SnRV appears quite divergent from the other fish retroviruses by its placement in a distinct branch near the *Spumaviruses*. This would suggest that SnRV is quite divergent from the genus *Epsilonretrovirus* and may represent yet another group of retroviruses. Interestingly, a more encompassing phylogenetic analysis using all known retroviral sequences from lower vertebrates, including partial endogenous retroviral *pol* fragments from Stickleback (*Gasterosteus aculeatus*), brook trout (*Salvelinus fontinalis*), Brown trout (*Salmo trutta*), freshwater whiting (*Corogonus lavaretus*), and puffer fish (*Fugu rubripes*), indicates that the majority of the fish viruses, excluding SnRV, cluster together with MLV-related viruses in a group

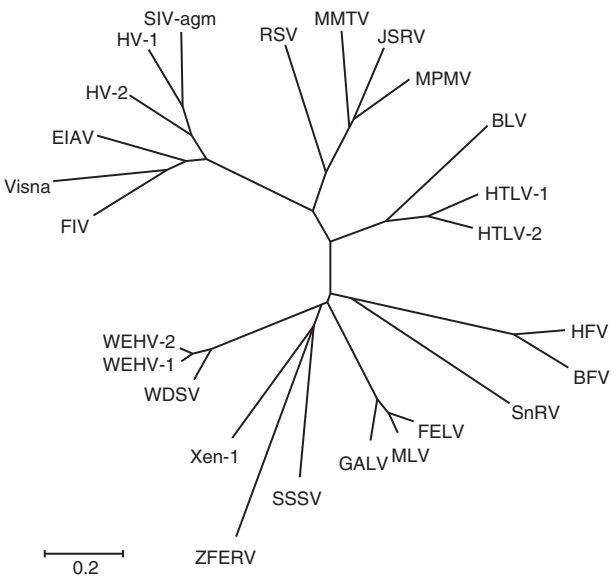


Figure 6 Unrooted phylogenetic tree of representative retroviruses based on an amino acid alignment of seven conserved domains in reverse transcriptase. Retroviruses are designated as follows: MPMV (Mason–Pfizer monkey virus), JSRV (Jaagsiekte sheep retrovirus), MMTV (mouse mammary tumor virus), RSV (Rous sarcoma virus), EIAV (equine infectious anemia virus), FIV (feline immunodeficiency virus), Visna (visna virus), HIV-2 (human immunodeficiency virus-2), HIV-1 (human immunodeficiency virus-1), SIV-agm (simian immunodeficiency virus-agm), HTLV-1 (human T-cell leukemia virus type 1), HTLV-2 (human T-cell leukemia virus 2), BLV (bovine leukemia virus), SSSV (salmon swimbladder sarcoma virus), SnRV (snakehead retrovirus), BFV (bovine foamy virus), HFV (human foamy virus), FeLV (feline leukemia virus), MLV (murine leukemia virus), GALV (gibbon ape leukemia virus), WDSV (walleye dermal sarcoma virus), WEHV-1 (walleye epidermal hyperplasia virus type 1), WEHV-2 (walleye epidermal hyperplasia virus type 2), ZFERV (zebrafish endogenous retrovirus), Xen-1 (Xenopus endogenous retrovirus-1).

separate from most non-MLV related mammalian retroviruses. This raises the possibility that some retroviral groups maybe restricted to particular vertebrate classes. However, it is evident from the diversity among the fish retroviruses, that there is a high degree of heterogeneity within this group.

Acknowledgment

This research was supported in part by USDA grants 99-35204-7485 and 02-35204-12777 to J.W.C., National Oceanic and Atmospheric Administration award no. NA86RG0056 to the Research Foundation of State University of New York for New York Sea Grant to P.R.B., American Cancer Society grant RPG-00313–01-MBC to S.L.Q., and National Institutes of Health grant CA095056 to S.L.Q. T.A.P. was supported by National Institutes of Health training grant 5T32CA09682.

See also: Equine Infectious Anemia Virus; Feline Leukemia and Sarcoma Viruses; Foamy Viruses; Human Immunodeficiency Viruses: Antiretroviral agents; Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Origin; Human Immunodeficiency Viruses: Pathogenesis; Human T-Cell Leukemia Viruses: General Features; Human T-Cell Leukemia Viruses: Human Disease; Mouse Mammary Tumor Virus; Reticuloendotheliosis Viruses; Retroviruses of Birds; Simian Retrovirus D; Visna-Maedi Viruses.

Further Reading

Bowser PR, Wolfe MJ, Forney JL, and Wooster GA (1988) Seasonal prevalence of skin tumors from walleye (*Stizostedion vitreum*) from Oneida Lake, New York. *Journal of Wildlife Diseases* 24: 292–298.

Coffin JM, Hughes SH, and Varmus HE (eds.) (1997) *Retroviruses,* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Hart D, Frerichs GN, Rambaut A, and Onions DE (1996) Complete nucleotide sequence and transcriptional analysis of the snakehead fish retrovirus. *Journal of Virology* 70(6): 3606–3616.

Hernious E, Martin J, Miller K, Cook J, Wilkinson M, and Tristem M (1998) Retroviral diversity and distribution in vertebrates. *Journal of Virology* 72(7): 5955–5966.

Holzschu DL, Fodor SK, Quackenbush SL, *et al.* (1995) Nucleotide sequence and protein analysis of a complex piscine retrovirus, walleye dermal sarcoma virus. *Journal of Virology* 69(9): 5320–5331.

Lairmore MD, Stanley JR, Weber SA, and Holzschu DL (2000) Squamous epithelial proliferation induced by walleye dermal sarcoma retrovirus cyclin in transgenic mice. *Proceedings of the National Academy of Sciences, USA* 97(11): 6114–6119.

LaPierre LA, Casey JW, and Holzschu DL (1998) Walleye retroviruses associated with skin tumors and hyperplasias encode cyclin D homologs. *Journal of Virology* 72: 8765–8771.

LaPierre LA, Holzschu DL, Bowser PR, and Casey JW (1999) Sequence and transcriptional analyses of the fish retroviruses walleye epidermal hyperplasia virus types 1 and 2: Evidence for a gene duplication. *Journal of Virology* 73(11): 9393–9403.

Paul TA, Quackenbush SL, Sutton C, Casey RN, Bowser PR, and Casey JW (2006) Identification and characterization of an exogenous retrovirus from Atlantic salmon swimbladder sarcomas. *Journal of Virology* 80(6): 2941–2948.

Poulet FM, Bowser PR, and Casey JW (1994) Retroviruses of fish, reptiles, and molluscs. In: Levy JA (ed.) *The Retroviridae,* vol. 3, pp. 1–38. New York: Plenum.

Quackenbush SL, Holzschu DL, Bowser PR, and Casey JW (1997) Transcriptional analysis of walleye dermal sarcoma virus (WDSV). *Virology* 237: 107–112.

Rovnak J, Hronek BW, Ryan SO, Cai S, and Quackenbush SL (2005) An activation domain within the walleye dermal sarcoma virus retroviral cyclin protein is essential for inhibition of the viral promoter. *Virology* 342(2): 240–251.

Rovnak J and Quackenbush SL (2002) Walleye dermal sarcoma virus cyclin interacts with components of the mediator complex and the RNA polymerase II holoenzyme. *Journal of Virology* 76: 8031–8039.

Shen CH and Steiner LA (2004) Genome structure and thymic expression of an endogenous retrovirus in zebrafish. *Journal of Virology* 78(2): 899–911.

Fish Rhabdoviruses

G Kurath and J Winton, Western Fisheries Research Center, Seattle, WA, USA

Published by Elsevier Ltd.

Glossary

Cytopathic effect (CPE) Damage caused in cultured cells due to insult such as infection with virus.
Genogroup Grouping of virus isolates within a species based on genetic data, such as nucleotide sequences and phylogenetic analyses.
Novirhabdovirus A member of the taxonomic genus *Novirhabdovirus*, within the family *Rhabdoviridae*. This genus contains many important fish rhabdoviruses.
Nucleocapsid For a rhabdovirus, the inner structure of the virion comprised of the viral genomic RNA wrapped in nucleocapsid (N) protein with associated phosphoprotein (P) and polymerase (L) proteins.
Spill-over and spill-back events Transmission of pathogens, and consequently disease, between wild and cultured populations of animals (in this case, fish).
Vesiculovirus A member of the taxonomic genus *Vesiculovirus*, within the family *Rhabdoviridae*. This genus currently contains only mammalian rhabdoviruses but several fish rhabdoviruses are very closely related to this genus.

Introduction

Some of the most significant viral pathogens of fish are members of the family *Rhabdoviridae*. The viruses in this large and important group cause losses in populations of wild fish as well as among fish reared in aquaculture. While many of the best known fish rhabdoviruses produce acute disease and high mortality, others have been isolated from chronic or asymptomatic infections. Fish rhabdoviruses often have a wide host and geographic range, and infect aquatic animals in both freshwater and seawater.

The diseases caused by fish rhabdoviruses are generally characterized as acute, hemorrhagic septicemias affecting multiple organs. Death is usually due to organ failure and subsequent loss of osmoregulation. In addition to petechial hemorrhages, gross signs include accumulation of ascites, darkening, and exopthalmia. Internally, necrosis of multiple organs is evident upon histological examination. Mortality is frequently highest in younger fish and can be explosive in aquaculture settings, resulting in losses approaching 100% in some cases. Fish that survive infection typically develop protective immunity. Transmission of fish rhabdoviruses occurs both horizontally between fish, and vertically through egg-associated transmission from an infected adult to its progeny. The role of long-term carriers and reservoirs is an area requiring further research.

Understanding of fish rhabdovirus infections improved significantly following the establishment of a variety of fish cell lines, beginning in the 1960s. Infection of cultured cell lines produces typical cytopathic effects (CPEs) consisting of cell rounding and necrosis following the release of mature virions by budding. The rate of the appearance of CPE is related to the incubation temperature, which is typically set to mirror the temperature optimum for the fish host (e.g., 10–25 °C). Highly reproducible laboratory challenge models have been developed for several important fish rhabdoviruses and their principal hosts. These tools have been used extensively to further our understanding of host–pathogen relationships and the role of environmental factors such as temperature on the virus replication cycle and the disease process.

The rhabdoviruses recovered from fish and described in the literature comprise a diverse collection of isolates (**Table 1**). Those studied in detail have been assigned to one of two quite different groups: isolates that are members, or likely members, of the established genus, *Novirhabdovirus*, and those fish rhabdovirus isolates that are most similar to members of the genus *Vesiculovirus* (**Figure 1**). Formal species names have been assigned to only four fish rhabdoviruses, all within the genus *Novirhabdovirus*, while several others are recognized as tentative members of a genus. There are also several partially characterized or unusual isolates that have been reported as rhabdoviruses based largely on their characteristic bullet-shaped morphology in electron micrographs. Upon further study, some of the fish rhabdoviruses listed in **Table 1** may be shown to be the same virus species.

Novirhabdoviruses of Fish

The genus *Novirhabdovirus* was established in 1998, and includes four official species as shown in **Table 1**. Infectious hematopoietic necrosis virus (IHNV) and viral hemorrhagic septicemia virus (VHSV) are economically significant viruses that cause epidemics in salmonid fish. IHNV is endemic in salmonids of northwestern North America and has become established in Europe and Asia through historical aquaculture-related activities. VHSV

Table 1 Fish rhabdoviruses from the published literature and their status in the *Eighth Report of the International Committee on Taxonomy of Viruses* (ICTV). Virus names shown as indented are isolates described in the literature that are either now considered to be indistinguishable from a recognized species (indicated in parentheses), or those that appear to be similar to the virus directly above but require further characterization

Named virus	*Abbreviation*	*Status in 8th ICTV*
I. Species and tentative species in the genus *Novirhabdovirus*		
Infectious hematopoietic necrosis virus	IHNV	Type species of the genus
Oregon sockeye virus (IHNV)	OSV	
Sacramento River Chinook virus (IHNV)	SRCV	
Viral hemorrhagic septicemia virus	VHSV	Formal species in genus
Egtved virus (VHSV)		
Brown trout rhabdovirus (VHSV)		
Cod ulcus syndrome rhabdovirus (VHSV)		
Carpione brown trout rhabdovirus (VHSV)	583	
Hirame rhabdovirus	HIRRV	Formal species in genus
Snakehead virus	SHRV	Formal species in genus
Eel virus B12	EEV-B12	Tentative species in genus
Eel virus C26	EEV-C26	Tentative species in genus
II. Vesiculovirus-like viruses from fishes		
Spring viremia of carp virus	SVCV	Tentative species in genus
Swim bladder inflammation virus (SVCV)	SBI	
Pike fry rhabdovirus	PFRV	Tentative species in genus
Grass carp rhabdovirus	GCV	None
Tench rhabdovirus		None
Eel virus American	EVA	Tentative species in genus
Eel virus European X	EVEX	None
Eel virus C30, B44 and D13	C30, B44, D13	None
Rhabdovirus anguilla	None	
Ulcerative disease rhabdovirus	UDRV	Tentative species in genus
Perch rhabdovirus		None
Pike perch rhabdovirus		None
Pike rhabdovirus	DK5533	None
Grayling rhabdovirus		None
European lake trout rhabdovirus	903/87	None
Swedish sea trout rhabdovirus	SSTV	None
Starry flounder virus	SFRV	None
III. Incompletely characterized fish rhabdoviruses		
Rio Grande cichlid virus		Unassigned animal rhabdovirus
Siniperca chuatsi rhabdovirus	SCRV	None
Scophthalmus maximus rhabdovirus	SMRV	None

was originally known as a major pathogen of trout in Western Europe, but in recent years it has also been revealed as a relatively ubiquitous pathogen of numerous species of marine fish in both the Pacific and Atlantic Oceans. Hirame rhabdovirus (HIRRV) and snakehead rhabdovirus (SHRV) are important pathogens of cultured fish in Asia. Hirame rhabdovirus has been isolated from several species of wild and cultured marine fish suffering from a hemorrhagic septicemia. In Japan and Korea, HIRRV causes economically significant disease, especially in cultured Japanese flounder (hirame). Snakehead rhabdovirus has been isolated from both wild and cultured snakehead in Southeast Asia where it causes a severe ulcerative disease. These four viruses have all been well studied, and each one has been developed as an important research model for studies of various aspects of fish rhabdovirus molecular biology. In addition to these formal species in the genus, there are two tentative members that have been isolated from eel, but these and several other fish rhabdoviruses reported in the literature are less well characterized.

The virions of novirhabdoviruses have the typical bullet-shaped morphology of rhabdoviruses and are *c.* 150–190 nm in length and 65–75 nm in diameter (**Figure 2(a)**). Virions are comprised of five viral proteins. The single glycoprotein (G) on the surface of enveloped virus particles has been shown to be a major antigenic protein that is, by itself, capable of eliciting a protective immune response. The matrix protein (M) is an inner component of the virions that binds both the cytoplasmic domain of the G protein and the viral nucleocapsid and, for IHNV, has been shown to downregulate host transcription. The major structural component of the nucleocapsid is the viral nucleocapsid (N) protein that associates with

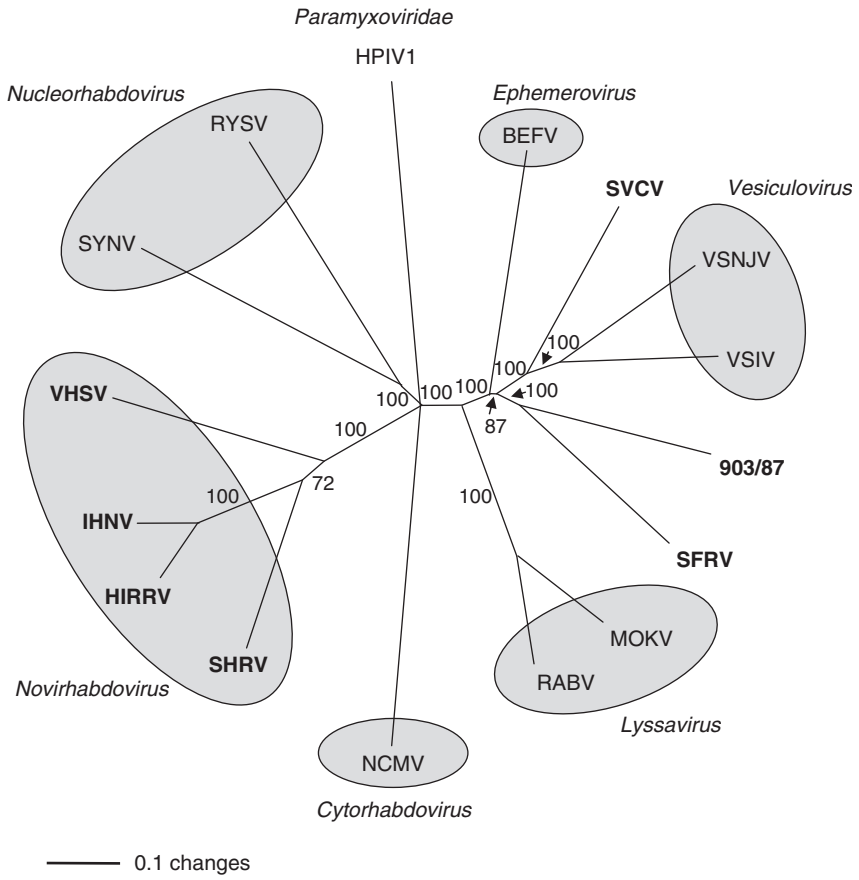


Figure 1 Phylogenetic tree showing the relationship of fish rhabdoviruses (in bold) with other representative members of the family *Rhabdoviridae*. Partial polymerase protein sequences comprising ~460 amino acids at the N-terminus of the L protein were used in a neighbor-joining distance program. Two groups of fish rhabdoviruses are shown either as members of the genus *Novirhabdovirus* or as vesiculovirus-like isolates. Abbreviations used are: BEFV, bovine ephemeral fever virus; SVCV, spring viremia of carp virus; VSNJV, vesicular stomatitis New Jersey virus; VSIV, vesicular stomatitis Indiana virus; 903/87, European lake trout rhabdovirus; SFRV, starry flounder rhabdovirus; MOKV, Mokola virus; RABV, rabies virus; NCMV, northern cereal mosaic virus; SHRV, snakehead virus; HIRRV, hirame rhabdovirus; IHNV, infectious hematopoietic necrosis virus; VHSV, viral hemorrhagic septicemia virus; SYNV, sonchus yellow net virus; RYSV, rice yellow stunt virus. The paramyxovirus human parainfluenza virus 1 (HPIV-1) was used as the outgroup. Phylogenetic analysis used 1000 bootstrapped data sets, and nodes with bootstrap values less than 70% were collapsed to polytomies.

full-length viral genomic RNA and facilitates its interaction with the viral polymerase. The large polymerase protein (L) is a complex protein that carries out most of the enzymatic functions of genome replication and transcription. The phosphoprotein (P) is an essential cofactor that is required for polymerase function. These five virion proteins are found in all known rhabdoviruses and, to date, it appears that their roles are conserved across genera. In addition to these familiar rhabdovirus proteins, the hallmark of the novirhabdoviruses is the presence of a sixth protein, the nonvirion (NV) protein, which has not yet been identified in any rhabdovirus of another genus. The NV protein is not found in virus particles, but it is synthesized, often in only trace amounts, within infected cells. The presence of an apparently functional NV gene is conserved in all novirhabdovirus isolates examined to date, but the role of this protein is not well defined. Through the use of reverse genetics, it has been shown that the NV gene and its encoded protein product are not essential for replication in cultured cells or in fish, but in some cases it enhances replication and pathogenicity.

Fish rhabdovirus replication occurs in the cytoplasm of infected cells by processes common to all rhabdoviruses. During the replication cycle the viral RNA-dependent RNA polymerase (L) transcribes individual mRNAs for each viral gene, and also copies the RNA genome to produce progeny genomes. Viral mRNAs are translated into viral proteins that traffic to the outer cell membrane where assembly with viral genomic RNA occurs and budding through the cell membrane releases new enveloped virus particles. All novirhabdovirus genomes are single-stranded minus-sense RNA molecules *c.* 11 000 nt in length. The genome organization is conserved across the genus, and is shown for the type species *Infections hematopoietic necrosis*

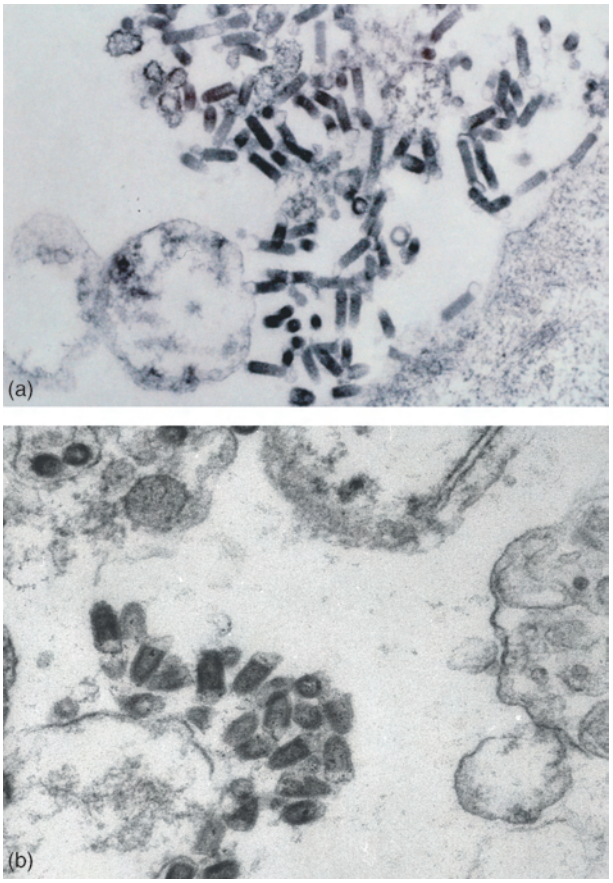


Figure 2 Electron micrograph showing a representative member of each of the two groups of fish rhabdoviruses. (a) *Viral hemorrhagic septicemia virus*, a species in the genus, *Novirhabdovirus*. (b) Starry flounder rhabdovirus, a vesiculovirus-like rhabdovirus of fish. Scale = 200 nm (a, b).

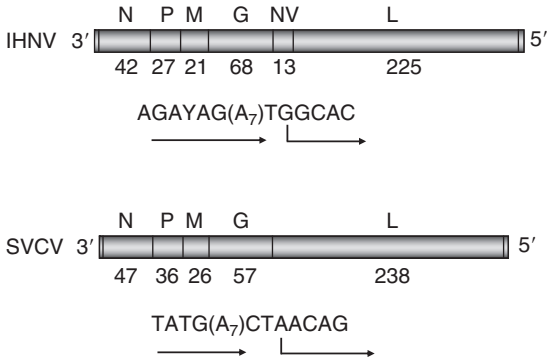


Figure 3 Genome organization of representatives of the two major subgroups of fish rhabdoviruses. IHNV represents the genus *Novirhabdovirus* and spring viremia of carp virus (SVCV) represents the vesiculovirus-like rhabdoviruses of fish. Both have negative-sense single-stranded RNA genomes of *c.* 11 000 nt containing nucleocapsid (N), phosphoprotein (P), matrix (M), glycoprotein (G), and polymerase (L) genes as shown. Species in the genus *Novirhabdovirus* have an additional gene encoding an NV protein. Approximate sizes (in kDa) of the encoded proteins are shown below each gene. Conserved gene junction sequences for each virus are shown in positive-sense (mRNA) orientation below the genome with arrows indicating putative transcription stop (ending with seven A's) and start signals.

virus in **Figure 3**. The six viral genes occur in the order 3′-N–P–M–G–NV–L-5′. This is identical to the genome organization of members of the genera *Vesiculovirus* and *Lyssavirus*, with the exception of the additional NV gene at the G–L junction. The genome termini have complementary leader and trailer regions of approximately 50–60 and 40–115 nt, respectively. The untranscribed, intergenic regions are single nucleotides, typically A or T, at all gene junctions except for the G–NV junction, which is slightly more variable. Putative transcription start and stop signals for each gene, shown in **Figure 3**, are highly conserved among individual genes. They are also conserved among novirhabdovirus species and differ from the conserved regulatory sequences of rhabdoviruses in other genera.

Genetic diversity within novirhabdovirus species has been well characterized for IHNV and VHSV. Genetic typing of hundreds of IHNV field isolates from North America, as well as from Europe, Russia, and Asia, has shown surprisingly low diversity, with a maximum of 9% nucleotide sequence diversity in a relatively variable region of the G gene. Despite this overall low diversity, genetic typing clearly resolves IHNV isolates into three major genogroups that correlate with their geographic origin in North America. Similar typing of extensive global collections of VHSV isolates has revealed much greater diversity, with up to 16% nucleotide diversity in full-length G gene sequences, and four phylogenetic subgroups that also correlate with geographic range. For both the IHNV and VHSV subphylogenies, additional studies with partial nucleocapsid (N) gene sequences have confirmed the genogroups defined by partial G gene analyses. Diversity between species of novirhabdoviruses can be illustrated with full G protein amino acid sequences. Within the genus, IHNV and HIRRV are most closely related with 74% identity (83% similarity) in their G proteins. Within the entire genus, the G proteins of the four member species are more divergent, with as little as 37% identity (54% similarity). Comparisons outside the genus reveal 13–23% identity (27–48% similarity) between the G proteins of novirhabdoviruses and the fish vesiculovirus-like isolates or members of other rhabdovirus genera.

Vesiculovirus-Like Viruses of Fish

At present, no fish rhabdoviruses are accepted as formal species within the genus *Vesiculovirus*, but many rhabdoviruses isolated from fish are clearly closely related to members of this genus, and distinct from the members of

the genus *Novirhabdovirus*. Some vesiculovirus-like fish rhabdoviruses are considered tentative species in the genus *Vesiculovirus* (**Table 1**). The best studied vesiculo-like fish rhabdovirus is spring viremia of carp virus (SVCV), which has a long history of causing severe epidemics among cultured carp in Europe. SVCV is well characterized at both the biological and molecular levels, and it is considered the representative of the vesiculovirus-like fish rhabdoviruses. Full-length genome sequences are available for several strains of SVCV, and numerous other isolates are partially sequenced, facilitating phylogenetic analyses that confirm its close relationship to the mammalian vesiculoviruses. Many isolates of a similar virus, pike fry rhabdovirus, have also been described, and several other fish rhabdoviruses have been isolated from various cold- and warm-water hosts including various species of eel, snakehead, trout, and perch. By serological assays and phylogenetic analyses, at least some of these isolates appear to be distinct vesiculo-like viral species. Phylogenetic trees of partial G or L gene sequences indicate that these isolates form an emerging cluster of tentative species around the mammalian genus *Vesiculovirus*, as illustrated in **Figure 1**. Future work by the ICTV will likely establish some formal species among the vesiculo-like fish rhabdoviruses and clarify whether they should be accepted as members of the genus *Vesiculovirus*.

Virions of the fish vesiculo-like rhabdoviruses are typically shorter and wider than novirhabdoviruses, with bullet-shaped particles measuring *c.* 80–150 nm in length and 60–90 nm in diameter (**Figure 2(b)**). Particles are composed of five viral proteins that correspond to the five major virion proteins found in all rhabdoviruses. The virus replication cycle roles of the N, P, M, G, and L proteins are as described above for novirhabdoviruses. Among fish rhabdoviruses, the distinguishing feature of the fish vesiculo-like viruses is the absence of an NV gene at the G–L junction, indicating that, whatever the role of the NV protein in novirhabdoviruses, it is not essential for rhabdovirus replication in fish *per se*.

The negative-sense RNA genome of SVCV is just over 11 000 nt in length, and it has genes encoding the five major viral proteins in the same order found in all rhabdovirus genomes (**Figure 3**). The upstream untranslated region of each gene is a conserved 10 nt sequence and the downstream untranslated regions are more variable. The 3′ and 5′ termini of the genome have complementary leader and trailer regions of *c.* 20–60 and 12–20 nt, respectively. The untranscribed, intergenic regions between genes are dinucleotides, which, for SVCV, are all CT with the exception of the G–L junction that has the tetranucleotide CTAT. Putative transcriptional start and stop signals, shown in **Figure 3**, are highly conserved among individual genes and among SVCV isolates. These regulatory signals at the SVCV gene junctions are nearly identical to those of mammalian virus members of the genus *Vesiculovirus*, and they differ from the regulatory sequences conserved among members of other rhabdovirus genera, including novirhabdoviruses. As an example of the levels of sequence similarity, the SVCV G protein has 31–33% amino acid sequence identity (52–53% similarity) with the G proteins of members of the genus *Vesiculovirus*, but only 19–24% identity (40–47% similarity) with G proteins of 11 rhabdoviruses from all other genera including *Novirhabdovirus*. Among several viruses reported as isolates of SVCV, partial G gene nucleotide sequences have 83–100% identity, and phylogenetic analyses resolved four genogroups that correlated with geographic origin in Eurasia. Among SVCV and other tentative species of fish vesiculo-like rhabdoviruses, partial G or L gene nucleotide sequences typically have 70–80% identity.

Fish Rhabdovirus Detection and Control

Because the diseases caused by fish rhabdoviruses are important to aquaculture, diagnostic methods for the detection and identification of the most significant pathogens are well established. Many cultured cell lines derived from finfish support the replication of a broad range of fish rhabdoviruses and are used routinely in cell culture-based assays to detect the presence of infections or to quantify infectious virus. Serological methods using polyclonal or monoclonal antibodies are used for both diagnostics and research, and molecular techniques such as DNA probes, polymerase chain reaction (PCR) assays, and quantitative polymerase chain reaction (qPCR) assays have been developed for confirmatory diagnosis, identification, and quantification of the most significant rhabdoviruses affecting fish. In many regions of North America, Europe, and Asia, the majority of all cultured fish stocks are surveyed on a regular basis for viral pathogens by fish health professionals.

Understanding the potential impacts of fish rhabdovirus infections on the health of native species and fish reared in aquaculture has led to increased attention to biosecurity and in measures to prevent the spread of aquatic animal diseases. During the mid-1900s, the inadvertent spread of several fish rhabdoviruses by movement of infected eggs or juvenile fish was responsible for several intercontinental introductions of pathogens with serious economic impacts. National and international standards now require health inspections for certain fish species prior to international or interstate transport. Several fish rhabdoviruses are on the list of pathogens for which inspections are required. The global trade in aquaculture species is reasonably well regulated. However, the huge global trade in ornamental fish is essentially unregulated and is a documented source of transboundary virus movement.

Considerable attention has also been given to improving methods for the control or prevention of aquatic animal diseases. These include sound management practices such as improved sanitation, the use of pathogen-free well water sources for early life-stage rearing, the installation of water treatment technologies for facilities using large volumes of water from open sources, and more frequent fish health inspections that make use of improved technologies. In aquaculture settings, the ability to break the cycle of vertical transmission between generations by the disinfection of eggs with an iodine solution has been an important control approach for fish rhabdoviruses.

Research on vaccines to protect fish from rhabdovirus infections has been conducted for more than 30 years. Initially, this work focused on traditional approaches including inactivated viruses or live-modified vaccines. Later, recombinant DNA technology produced experimental vaccines using bacterial or baculovirus expression systems as well as peptide immunogens. While some of these traditional and molecular vaccines were reported to be highly efficacious in both laboratory and field trials, prohibitive development and licensing costs, inconsistent efficacy, or safety concerns have prevented their use on a commercial scale. More recently, DNA vaccines have been engineered for protecting fish against the novirhabdoviruses, IHNV, VHSV, and HIRRV. These vaccines have been studied extensively and show exceptionally high efficacy against severe viral challenges under a wide range of conditions. An IHNV DNA vaccine was licensed for use in Canada in 2005. With regulatory concerns and vaccine delivery methods currently being addressed, it is possible that these will be among the first DNA vaccines to be used commercially for preventative veterinary care.

Current Aspects

The number of reported fish rhabdoviruses continues to grow as a result of the growth of aquaculture, the increase in global trade, the development of improved diagnostic methods, and the expansion of surveillance activities. The increase in nucleotide sequence information available for many fish rhabdoviruses has not only improved our understanding of the genetic diversity among members of the group, but also complicated our ability to form neat taxonomic categories. Upon further analysis, the creation of several new virus species and, possibly, genera may be needed to accommodate the range of fish rhabdoviruses, particularly the unassigned or less well characterized members of the family.

Due to their economic importance, the rhabdoviruses of finfish are among the best studied of all aquatic animal viruses, providing models for their function, movement, and persistence in the environment. There is an increasing understanding that rhabdoviruses are important pathogens of wild or free-ranging fish in both freshwater and marine ecosystems. Extensive oceanographic surveys have revealed marine fish as important reservoirs for virus infections of fish in freshwater and marine aquaculture. In addition to causing natural mortality that may limit population size, anthropogenic stressors can act synergistically to amplify the effects of disease on the overall health and survival of aquatic species. Field studies of the relatively unique disease interface between cultured and wild aquatic host populations are providing insight into virus spill-over and spill-back events, and anthropogenic impacts on fish health in altered environments.

Fish rhabdoviruses are also serving as useful components of model systems to study vertebrate virus disease, epidemiology, and immunology. The availability of a variety of established fish cell lines, the creation of new-generation reagents and tools, and the well-established laboratory challenge models that can use statistically robust numbers of trout, catfish, or zebrafish make these model systems particularly attractive and powerful. Recent work includes the use of DNA vaccines against fish rhabdoviruses in mechanistic studies to determine how DNA vaccines elicit strong innate and adaptive immune responses in vertebrate hosts. Reverse genetics systems for both IHNV and SHRV have been developed and used to investigate the roles of individual viral genes and proteins. These studies have already shown that the G genes of different fish rhabdovirus species can be interchanged, and that the NV gene is not essential for virus replication. In the area of immunology, quantitative real-time PCR assays and microarray analyses have been used to profile the fish immune gene response following either viral infection or DNA vaccination. Future work will include the use of these models to address questions such as the genetics of host disease resistance, viral host specificity and virulence, viral fitness and competition, and host immune selection as a driver of virus evolution.

See also: DNA Vaccines; Fish Viruses; Vesicular Stomatitis Virus.

Further Reading

Ahne W, Bjorklund HV, Essbauer S, Fijan N, Kurath G, and Winton JR (2002) Spring viremia of carp (SVC). *Diseases of Aquatic Organisms* 52: 261–272.

Betts AM, Stone DM, Way K, *et al.* (2003) Emerging vesiculo-type virus infections of freshwater fish in Europe. *Diseases of Aquatic Organisms* 57: 201–212.

Bjorklund HV, Higman KH, and Kurath G (1996) The glycoprotein genes and gene junctions of the fish rhabdoviruses spring viremia of carp virus and hirame rhabdovirus: Analysis of relationships with other rhabdoviruses. *Virus Research* 42: 65–80.

Bootland LM and Leong JC (1999) Infectious hematopoietic necrosis virus. In: Woo PTK and Bruno DW (eds.) *Fish Diseases and Disorders, Vol. 3: Viral, Bacterial and Fungal Infections*, pp 57–121. New York: CABI.

Bremont M (2005) Reverse genetics on fish rhabdoviruses: Tools to study the pathogenesis of fish rhabdoviruses. *Current Topics in Microbiology and Immunology* 292: 119–141.
Einer-Jensen K, Ahrens P, Forsberg R, and Lorenzen N (2004) Evolution of the fish rhabdovirus viral haemorrhagic septicaemia virus. *Journal of General Virology* 85: 1167–1179.
Hoffmann B, Beer M, Schutze H, and Mettenleiter TC (2005) Fish rhabdoviruses: Molecular epidemiology and evolution. *Current Topics in Microbiology and Immunology* 292: 81–117.
Kim D-H, Oh H-K, Eou J-I, *et al.* (2005) Complete nucleotide sequence of the hirame rhabdovirus, a pathogen of marine fish. *Virus Research* 107: 1–9.
Kurath G (2005) Overview of recent DNA vaccine development for fish. In: Midtlyng PJ (ed.) *Fish Vaccinology, Developments in Biologicals,* vol. 121, pp. 201–213. Basel: Karger.
Kurath G, Garver KA, Troyer RM, Emmenegger EJ, Einer-Jensen K, and Anderson ED (2003) Phylogeography of infectious haematopoietic necrosis virus in North America. *Journal of General Virology* 84: 803–814.
Smail DA (1999) Viral haemorrhagic septicaemia. In: Woo PTK and Bruno DW (eds.) *Fish Diseases and Disorders, Vol. 3: Viral, Bacterial and Fungal Infections*, pp 123–147. New York: CABI.
Snow M, Bain N, Black J, *et al.* (2004) Genetic population structure of marine viral haemorrhagic septicaemia virus (VHSV). *Diseases of Aquatic Organisms* 61: 11–21.
Stone DM, Ahne W, Sheppard AM, *et al.* (2002) Nucleotide sequence analysis of the glycoprotein gene of putative spring viraemia of carp viruses and pike fry rhabdovirus isolates reveals four distinct piscine vesiculovirus genogroups. *Diseases of Aquatic Organisms* 53: 203–210.
Tordo N, Benmansour A, Calisher C, *et al.* (2004) Family *Rhabdoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses,* pp. 623–644. San Diego, CA: Elsevier Academic Press.
Wolf K (1988) *Fish Viruses and Fish Viral Diseases.* Ithaca, NY: Cornell University Press.

Fish Viruses

J C Leong, University of Hawaii at Manoa, Honolulu, HI, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Anadromous Migrating from the sea to freshwater to spawn.
Cardiomyopathy A weakening of the heart muscle or a change in heart muscle structure.
Salmonid Belonging to, or characteristic of the family Salmonidae which includes the salmon trout and whitefish.
Swimbladder An air-filled sac near the spinal cord in many fishes that helps maintain buoyancy.

Introduction

Viruses that infect fish and cause disease are represented in 14 of the families listed for vertebrate viruses by the International Committee on the Taxonomy of Viruses (27 May 2005). The fish viruses containing DNA genomes are listed in the families *Iridoviridae*, *Adenoviridae*, and *Herpesvirdae* and those with RNA genomes are listed in the families *Picornaviridae*, *Birnaviridae*, *Reoviridae*, *Rhabdoviridae*, *Orthomyxoviridae*, *Paramyxoviridae*, *Caliciviridae*, *Togaviridae*, *Nodaviridae*, *Retroviridae*, and *Coronaviridae*. As more fish species are brought under culture, there will be additions to this list, and possibly new viruses will be assigned to families not previously characterized in vertebrates.

Viral diseases have had a tremendous economic impact on both wild and farm-reared fish. A herpesvirus outbreak in 1998 and early 1999 reduced the pilchard (*Sardinops sagax neophilchardus*) fishery in southern Australia by two-thirds. In 2001, infectious salmon anemia virus (ISAV) was discovered in cultured Atlantic salmon in Cobscook Bay, Maine. The discovery of this orthomyxovirus virus forced farmers to destroy about 2.6 million fish in an effort to contain the spread of the disease. The cost to the Maine salmon industry (valued at more than $100 million) was about $24 million (USDA Animal and Plant Health Inspections Service (APHIS) estimates). More recently, the rhabdovirus viral hemorrhagic septicemia virus (VHSV), a very serious pathogen of marine and freshwater fish in Europe, was detected in the Great Lakes of North America in 2005. On 24 October 2006, APHIS issued an emergency order that blocked the live export of 37 fish species from any of the eight Great Lakes states. The order caused strong protests from fish farmers who make their living with live bait shipments and fish-stocking programs that sustain the Great Lakes' $4.5 billion fishing industry. Since vaccines and/or therapeutics for fish viruses are not readily available, containing the spread of these viruses by restricting movement and destruction of the affected population has been the only effective control strategy. Thus, the World Animal Health Organization (OIE) lists nine reportable diseases of fish (**Table 1**), seven of which are viral diseases.

Known and characterized fish viruses numbered only 16 in 1981, with an additional 11 observed by electron microscopy. Now, there are over 125 described viruses of fish and countless reports of electron microscopic observations of

Table 1 Diseases of fish listed by the OIE, 2006

Epizootic hematopoietic necrosis	Iridovirus
Infectious hematopoietic necrosis	Novirhabdovirus
Spring viremia of carp	Vesiculovirus
Viral hemorrhagic septicemia	Novirhabdovirus
Infectious salmon anemia	Orthomyxovirus
Epizootic ulcerative syndrome	Fungal infection
Gyrodactyloses	Flat worm fluke
Red sea bream iridovirus diseases	Iridovirus
Koi herpesvirus disease	Herpesvirus

viruses in wild-caught and cultured fish. The dramatic increase in reports of new fish viruses correlates with growth of the aquaculture industry that has increased production more than fivefold since 1985 to now represent more than 30% of global fishery production.

RNA Viruses of Fish

Rhabdoviridae

Fish rhabdoviruses are considered among the most serious viral pathogens of aquacultured fish, affecting predominantly salmon and trout. The first fish rhabdovirus was described in 1938 by Schaperclaus in European rainbow trout and, 6 years later, the Sacramento River Chinook virus was reported by Rucker. Since then, these viruses have been isolated, grown in tissue culture cells, and the genomes have been cloned and sequenced. The salmon virus is now called *Infectious hematopoietic necrosis virus* (IHNV) which is the type species for a new genus *Novirhabdovirus* in the *Rhabdoviridae*. Other fish rhabdovirus species assigned to this genus include *Viral hemorrhagic septicemia virus* (VHSV), *Hirame rhabdovirus* (HIRRV), and *Snakehead rhabdovirus* (SHRV). All of these viruses have negative-sense ssRNA genomes with a physical map ordered from the 3′-end as follows: leader-N-P-M-G-NV-L, where N is the nucleoprotein gene, P is the phosphoprotein gene, M is the matrix protein gene, G is the glycoprotein gene, NV is the nonvirion protein gene, and L is the virion RNA polymerase gene. The presence of the NV gene distinguishes the rhabdoviruses in the genus *Novirhabdovirus*, 'novi' standing for nonvirion.

Reverse genetic analysis of the IHNV, SHRV, and VHSV NV genes has, to date, provided mixed results. For IHNV and VHSV, deletion of the NV gene ameliorated virus-induced cytopathic effect (CPE) in tissue culture cells and reduced pathogenicity in fish. However, deletion of the NV gene for SHRV did not affect virus production or virus-induced CPE in tissue culture cells, or reduce pathogenicity in live fish challenges.

IHNV is a virus of salmonid fish and outbreaks of this virus in rainbow trout (*Oncorhynchus mykiss*), sockeye salmon (*O. nerka*), Chinook salmon (*O. tshawytscha*), Atlantic salmon (Salmo salar), and masou salmon (*O. masou*) have been economically devastating to the fish farmers. This virus prefers colder temperatures with optimal growth at 8–15 °C. VHSV is also a serious pathogen of salmonid fish, but its host range is broader and it has been shown to kill Pacific herring (*Clupea pallasi*), Pacific cod (*Gadus macrocephalus*), whitefish *(Coregonus* sp.), European sea bass (*Dicentrarchus labrax*), and turbot (*Scophthalmus maximus*). This broad host range has caused a great deal of concern to authorities in the USA since VHSV was first reported there in 2006 and found to affect freshwater drum (*Aplodinotus grunniens*), round goby (*Neogobius melanostomus*), smallmouth bass (*Micropterus dolomieu*), bluegill (*Lepomis machrochirus mystacalis*), crappie (*Pomoxis nigromaculatus*), gizzard shard (*Dorsoma cepadianum*), and other species occurring in the Great Lakes. HIRRV affects hirame, the Japanese flounder (*Paralychthys olivaceus*), which is a highly prized food fish in Japan. Its host range includes ayu (*Pleuroglossus altivelis*) as well as salmonid fish. SHRV was isolated from snakehead fish (*Channa striatus*) suffering from epizootic ulcerative syndrome (EUS) in Thailand. It is not considered the etiologic agent of EUS which is caused by a fungal pathogen. However, zebrafish (*Brachydanio rerio*), exposed to tissue culture grown SHRV develop, will develop petecchial hemorrhages and die.

Spring viremia of carp virus (SVCV), unlike the fish rhabdoviruses described above, does not contain an intervening gene between its glycoprotein and L genes. Analysis of the SVCV genome sequence indicates that it clusters with the members of the genus *Vesiculovirus* that includes vesicular stomatitis virus. SVCV was first identified as the etiologic agent of an acute hemorrhagic disease in common carp (*Cyprinus carpio*) in Europe in 1972. The disease has since been found in Asia, the Middle East, and most recently in South and North America. Outbreaks in the USA have raised concerns that indigenous fish species in the minnow family (*Cyprinidae*), some of which are endangered species, may be susceptible to SVCV. Other virus isolates that are closely related in nucleotide sequence to SVCV are the pike fry rhabdoviruses (PFRV) from a variety of freshwater fish in Europe including pike (*Esox lucius*), common bream (*Abramis brama*), roach (*Rutilus rutilus*), eel virus Europe X (EVEX), eel virus American (EVA); ulcerative disease rhabdovirus (UDRV); the grass carp rhabdovirus isolated from grass carp (*Ctenopharyngdon idella*); trout rhabdovirus 903/87 (TRV 903/87); and sea trout rhabdovirus 28/97 (STRV-28/97).

Phylogenetic analyses comparing aligned data for the N and G genes of members of the family *Rhabdoviridae* have confirmed the classification of the six genera: *Lyssavirus*, *Vesiculovirus*, *Ephemerovirus*, *Cytorhabdovirus*, *Nucleorhabdovirus*, and *Novirhabdovirus*. To date, fish rhabdoviruses are restricted to the genera *Novirhabdovirus* and *Vesiculovirus*. Analyses using the viral P gene sequences indicate that the aquatic vesiculoviruses form a separate cluster from the arthropod-borne vertebrate vesiculoviruses.

Paramyxoviridae

The first description of a paramyxovirus-like virus in fish was reported in 1985. During a routine health assessment of Chinook salmon juveniles in Oregon, tissue culture cells inoculated with a cell-free homogenate of organ tissue exhibited syncytia formation. Electron micrographs of the infected cell line showed enveloped, pleomorphic virus particles with a diameter of approximately of 125–250 nm and a single helical nucleocapsid with a diameter of 18 nm and a length of 100 nm. No disease syndrome was observed in trout and salmon fingerlings in subsequent infectivity trials with the tissue cultured virus. A second fish paramyxovirus that caused epidermal necrosis in juvenile black sea bream (*Acanthopargrus schlegeli*) was identified in Japan by electron microscopy. This virus was never cultured *in vitro*. The most recent description of a fish paramyxovirus was from Atlantic salmon post-smolts suffering from inflammatory gill disease in Norway. The genome of this virus has been cloned and partial sequences from the viral L protein have been used to determine the phylogenetic placement of this virus among the *Paramyxoviridae*. The Atlantic salmon paramyxovirus (ASPV) clustered with the subfamily *Paramyxovirinae*, in the genus *Respirovirus* that includes human parainfluenza virus and Sendai virus.

Orthomyxoviridae

Infectious salmon anemia virus (ISAV) is the only fish orthomyxovirus that has been fully described to date. There are eight RNA segments in the ISAV genome. Segment 1 encodes PB2, a component of the virion RNA polymerase; segment 2 encodes PB1; segment 3, the nucleocapsid protein NP; segment 4, the RNA polymerase PA; segment 5, acetylcholinesterase P3 or fusion protein; segment 6, hemagglutinin; segment 7, protein P4 and P5; and segment 8, proteins P6 and P7. The proteins P4 and P5 may be the ISAV counterparts to the membrane proteins M1 and M2 of influenza A virus; proteins P6 and P7 may be related to the nonstructural proteins NS1 and NEP of influenza A virus. The ISAV hemagglutinin does agglutinate fish red blood cells or mammalian red blood cells.

A comparative sequence analysis of the PB1 gene of ISAV and other members of the *Orthomyxoviridae* led to its assignment as the type species of a new genus *Isavirus*. More recent comparative analyses of the fusion protein gene (segment 5) and the hemagglutinin gene (segment 7) indicate that ISAV isolates can be divided into two subtypes, a North American subtype and a European subtype.

ISAV causes a highly lethal disease with affected farmed Atlantic salmon displaying severe anemia, leucopenia, ascetic fluids, hemorrhagic liver necrosis, and petecchiae of the viscera. The virus also causes disease in sea trout (*Salmo trutta*), rainbow trout, and Atlantic herring.

Picornaviridae

The first reported observation of picorna-like viruses in fish was made in 1988 from rainbow smelt (*Osmerus mordax*) in New Brunswick, Canada. Since then, picornaviruses have been isolated from barramundi, turbot, sea bass, grass carp, blue gill, grouper (*Epinephelus tauvina*), Japanese parrotfish (*Oplegnathus fasciatus*), and salmonid fish. In most of these descriptions, the presumptive characterization of the etiologic agent as a picornavirus was based on growth in tissue culture cells and the observation of crystalline arrays in the cytoplasm of small virus particles with a size and morphology consistent with picornaviruses. Analysis of RNA extracted from purified blue gill virus has indicated that it is single-stranded RNA virus. Sequence characterization of the viral genomes has not been carried out and there is some suggestion that at least some of these viruses might actually be betanodaviruses. In many cases, diseased fish infected with these viruses contain picorna-like virus particles in the brain and medulla and the victims display corkscrew-like swimming and eventually die.

Nodaviridae

Members of the *Nodaviridae* that infect fish belong to the genus *Betanodavirus* for which the type species is *Striped jack nervous necrosis virus* (SJNNV). These viruses are nonenveloped with icosahedral symmetry and virion diameters of approximately 30 nm. The viral genome consists of two molecules of positive-sense ssRNA. RNA1, the largest RNA genome segment encodes the viral polymerase. RNA2 encodes the virion capsid protein. A third RNA, transcribed from the 3′ terminal region of RNA1, encodes a 75 amino acid protein that bears little similarity with the B2 and B1 proteins encoded by a similar RNA3 in the alphanodaviruses. Despite this, the SJNNV B2 protein RNA has RNA silencing-suppression activity, as does the B2 protein of insect-infecting alphanodaviruses.

The betanodaviruses are the causative agents of viral nervous necrosis or viral encephalopathy and retinopathy in a variety of cultured marine fish. The disease affects young fish and produces a necrosis and vaculoation in the brain, spinal cord, and retina in most cases. It has been reported in striped jack (*Pseudocaranx dentex*), grouper (*Epinephelus* spp.), red drum (*Sciaenops ocellatus*), guppy (*Poicelia reticulate*), barfin flounder (*Verasper moseri*), red sea bream, tiger puffer (*Takifugu rubripes*), Japanese flounder, Atlantic halibut (*Hippoglossus hippoglossus*), amberjack (*Seriola dumerili*), sea bass, and barramundi. The recent detection of betanodaviruses in apparently healthy aquarium fish and invertebrates has raised concerns that the disease could be spread by trade in aquarium fish, particularly from Southeast Asia. Comparative sequence analyses of the coat protein genes for 25 isolates suggest that there are

four genotypic variants: tiger puffer nervous necrosis virus (TPNNV), striped jack nervous necrosis virus (SJNNV), barfin flounder nervous necrosis virus (BFNNV), and red-spotted grouper nervous necrosis virus (RGNNV).

Nidovirales

The family *Coronaviridae* comprises two genera, *Coronavirus* and *Torovirus*, and is classified with the families *Arteriviridae* and *Roniviridae* in the order *Nidovirales*. Members of the *Coronaviridae* share the common feature of pleomorphic, enveloped virions with diameters of 126–160 nm and prominent surface projections. The nucleocapsid is helical and contains a single molecule of linear, positive-sense ssRNA. Coronavirus-like particles have been isolated from a common carp from Japan showing petecchial hemorrhages on the skin and abdomen. A similar virus has also been isolated from moribund colored carp (*Cyprinus carpio*) with ulcerative dermal lesions. The investigators were able to grow the virus in epithelioma papulosum cyprini (EPC) cells and produce the same disease in carp injected with the tissue culture grown virus. Further characterization of these virus isolates was never carried out and there was no confirmation that they are, indeed, coronaviruses.

Recently, a novel virus with morphological features resembling those found in rhabdo-, corona-, and baculoviruses has been detected during the routine diagnostic screening of white bream (*Blicca bjoerkna*) in Germany. Ultrastructural studies indicated that the cell-free virions contain of a rod-shaped nucleocapsid similar to that seen in baculoviruses. Virions are bacilliform-shaped structures somewhat reminiscent of plant rhabdoviruses with an envelope containing coronavirus-like spikes. Sequence analysis has indicated that the 26.6 kbp white bream virus (WBV) contains five open reading frames, ORF1a, -1b, -2, -3, and -4, which are produced from a 'nested' set of 3′-coterminal mRNAs. The largest mRNA is of genome length. ORF1a and ORF1b form the viral replicase gene. ORF1a encodes several membrane domains, a putative ADP-ribose 1′-phospatase, and a chymotrypsin-like serine protease. ORF1b encodes the putative polymerase, helicase, ribose methyltransferase, exoribonuclease, and endoribonuclease activities. These characteristics are consistent with classification of WBV in the order *Nidovirales*. Phylogenetic analyses of the helicase and polymerase core domains indicate that WBV is more closely related to toroviruses than to coronaviruses and it has been suggested that a new nidovirus genus *Bafinivirus* be established (from bacilliform fish nidoviruses).

Togaviridae

The family *Togaviridae* comprises the genera *Alphavirus* and *Rubivirus* among the vertebrate viruses. These viruses have spherical virions, 70 nm in diameter, with a lipid envelope containing glycoprotein peplomers and a ssRNA genome which is capped at the 5′-end and polyadenylated at the 3′-end. Salmonid alphaviruses (SAVs) cause mortality in salmon and trout in Europe (Norway, France, UK, and Ireland). At least three subtypes of SAV exist: Salmon pancreas disease virus (SPDV/SAV-1) in Atlantic salmon; sleeping disease virus (SDV/SAV-2) in rainbow trout; and Norwegian salmonid alphavirus (NSAV/SAV-3). An early study on the evolutionary relationships of the alphaviruses has indicated that SAVs represent a separate and distant group in the genus *Alphavirus*.

Pancreas disease, due to SPDV (SAV-1) infection, was first described in Scotland in Atlantic salmon. It occurs during the first year at sea following transfer of young fish from freshwater tanks. The fish become anorexic and exhibit sluggish swimming activity with mortality rates reaching 10–50%. Histological examination of the affected fish has shown pancreatic acinar necrosis, and cardiac and skeletal myopathy. In rainbow trout, SDV (SAV-2) infection is characterized by the unusual behavior that fish lie on their side at the bottom on the tank. The lesion responsible for this behavior is red and white muscle degeneration. The histological lesions are similar to those observed in SPDV infection with progressive pancreatic necrosis and atrophy, mulifocal cardiomyopathy and muscle degeneration.

Caliciviridae

San Miguel sea lion virus (SMSV) is classified in the species *Vesicular exanthema of swine virus* in the genus *Vesivirus* in the family *Caliciviridae*. Investigators have found that the serotype 7 strain of the virus (SMSV-7), isolated from the opaleye fish (*Girella nigrigans*), can produce vesicular exanthema in swine. Thus, it is a virus that can jump from fish to mammals. The same serotype has also been reported in elephant seals and a sea lion trematode. Tissue culture grown serotype 5 SMSV injected into opaleye replicated to high titer 15 °C, producing $10^{7.6}$ $TCID_{50}$ per gram of spleen. There is no apparent disease in opaleye caused by this virus.

SMSV virions are nonenveloped with icosahedral symmetry and are 27–40 nm in diameter. The genome consists of a 7.5–8.0 kbp linear, positive-sense ssRNA that contains a covalently linked protein (VPg) attached to its 5′-end. The 3′-end of the genome is polyadenylated. The nonstructural polypeptides are encoded as a polyprotein in the 5′-end of the genomic RNA, while the single structural protein is encoded in the 3′-end. The identity of nonstructural polypeptides 2C (RNA helicase), 3C (cysteine protease), and 3D (RNA-dependent RNA polymerase) has been suggested by similarity to highly conserved amino acid motifs in the nonstructural proteins of the picornavirus superfamily. Phylogenetic analysis of the capsid protein region of caliciviruses including the

Sapporo-like human caliciviruses indicate that the genus *Vesivirus* includes SMVL-1, SMSV-4, SMSV-13, SMSV-15, SMSV-17, three feline caliciviruses, and the primate calicivirus Pan-1. These viruses are distant from the human caliciviruses and the rabbit caliciviruses.

Retroviridae

The family *Retroviridae* consists of two subfamilies, the *Orthoretrovirinae*, containing six genera, and the *Spumaretrovirinae*, containing only one genus. The piscine retroviruses constitute the genus *Epsilonretrovirus*, a genus established within the *Orthoretrovirinae* to include the piscine retroviruses: walleye dermal sarcoma virus (WDSV), walleye epidermal hyperplasia virus type 1 (WEHV-1), walleye epidermal hyperplasia virus type 2 (WEHV-2), and snakehead retrovirus (SnRV). The genomes of all of these viruses have been sequenced. There are also numerous reports of C-type (retrovirus-like) particles of about 110–150 nm in epidermal papillomas of European smelt (*Osmerus eperlanus*) and in cells cultured from neurofibromas of damselfish (*Pomacentrus partitus*). A retrovirus has also been suggested as the etiological agent of plasmacytoid leukemia in Chinook salmon.

The first report of a retrovirus-like agent in fish was made in 1976 in lymphosarcoma of northern pike and muskellunge (*Esox masquinongy*). The lymphosarcoma lesions contained a reverse transcriptase-like DNA polymerase with a temperature optimum of 20 °C. The first molecular evidence for a piscine retrovirus was reported in 1992 for a type C retrovirus from dermal sarcomas that form on the surface of adult walleye. These tumors are formed on the surface of adult walleye (*Stizeostedion vitreum*) in the fall and regress in the spring. The genome of the virus (13.2 kbp) was larger than all other known retroviruses at the time. Sequence analysis indicated that WDSV contained three additional open reading frames: ORF C at the 5′ terminal end; and ORF A and ORF B at the 3′ terminal end. ORF A encodes a D-cyclin homolog (retroviral cyclin) that locates in the nucleus of tumor cells in interchromatic granule clusters. ORF C encodes a cytoplasmic protein that targets the mitochondria and is associated with apoptosis. It is expressed in regressing tumors when full-length viral RNA is synthesized. The function of the protein encoded in ORF B, which is distantly related to ORF A, remains unknown. The WDSV protease cleavage sites have been identified to contain glutamine in the P2 position. The WDSV reverse transcriptase is rapidly inactivated at temperatures greater than 15 °C; a finding that is consistent with adaptation to growth in a coldwater fish species.

Two additional retroviruses have been cloned from epidermal hyperplasias on walleye. The genome sequences indicate that they are distinctly different from each other (77% identity) and from WDSV (64% identity). Walleye epidermal hyperplasia viruses 1 and 2 (WEHV-1 and -2) have genome organizations similar to WDSV. Each of the walleye retroviruses produces lesions when a cell-free filtrate from homogenized tumors is injected in naïve walleye juveniles.

Complete nucleotide sequence and transcriptional analyses of snakehead fish retrovirus have also been reported. The proviral genome is arranged in a typical 5′-LTR-gag-pol-env-LTR-3′ retrovirus organization. There are three additional ORFs: ORF1 encoding a 52 aa protein (5.7 kDa); ORF2 encoding a 94 aa protein (11 kDa); and ORF3 encoding a 205 aa protein (24 kDa). BLAST searches for possible homologs of these proteins have not produced any meaningful matches and their functions remain unknown. The SnRV genome differs from the retroviruses of walleye in that it has no ORF between the Unique region in the 5′ LTR (U5) and the gag region. The pathogenicity of SnRV has also not been determined.

In 2006, a novel piscine retrovirus was identified in association with an outbreak of leiomyosarcoma in the swimbladders of Atlantic salmon. The swimbladder sarcoma virus (SSSV) provirus is 10.9 kbp in length with a simple *gag*, *pro-pol*, *env* gene arrangement similar to that of murine leukemia viruses. Phylogenetic analysis of pol sequences suggests that SSV is most closely related to the sequenced zebrafish endogenous retrovirus (ZFERV) and that these viruses represent a new group of piscine retroviruses.

Reoviridae

Reoviruses that infect aquatic animals are grouped in the genus *Aquareovirus* in the family *Reoviridae* and are characterized by a nonenveloped double capsid shell, 11 segments of double-stranded RNA and seven structural proteins. John Plumb isolated the first finfish reovirus, golden shiner virus, GSRV, from golden shiner (*Notemigonus crysoleucas*) in 1979. Since then, several reovirus-like agents have been reported in piscine, molluscan, and crustacean hosts. Each has 11 segments of dsRNA and grow at temperatures that reflect their host range. The aquareoviruses have been divided by RNA–RNA hybridization kinetics into six groups (A–F) and several tentative species (**Table 2**). The type species of the genus *Aquareovirus* is *Aquareovirus A* which includes striped bass (*Morone saxatilis*) reovirus (SBRV). Like other reoviruses, the aquareoviruses are ether resistant and resistant to acid to pH3.

Most aquareovirus isolates are nonpathogenic or of low virulence in their host species. Grass carp virus (GCV; species *Aquareovirus C*) is the exception and appears to be the most pathogenic aquareovirus. GCV was isolated from grass carp in the People's Republic of China, causing severe hemorrhagic disease and affecting about 85% of infected fingerling and yearling populations.

Table 2 Species and tentative species of aquareoviruses

Aquareovirus A
Angelfish reovirus AFRV
Atlantic salmon reovirus HBR
Atlantic salmon reovirus ASV
Atlantic salmon reovirus TSV
Chinook salmon reovirus DRC
Chum salmon reovirus CSV
Threadfin reovirus
Herring reovirus HRV
Masou salmon reovirus MSV
Smelt reovirus
Striped bass reovirus
Aquareovirus B
Chinook salmon reovirus B
Chinook salmon reovirus LBS
Chinook salmon reovirus YRC
Chinook salmon reovirus ICR
Coho salmon reovirus CSR
Coho salmon reovirus ELC
Coho salmon reovirus SCS
Aquareovirus C
Golden shiner reovirus[a]
Grass carp reovirus
Aquareovirus D
Channel catfish reovirus
Aquareovirus E
Turbot reovirus
Aquareovirus F
Chum salmon reovirus PSR
Coho salmon reovirus SSR
Tentative species of Aquareoviruses
Chub reovirus
Landlocked salmon reovirus
Tench reovirus

[a]Grass carp reovirus and Golden shiner reovirus are variants of the same virus. Table 2 taken from *ICTVdB-The Universal Virus Database*, version 4. http://www.ncbi.nlm.nih.gov/ICTVdb/ICTVdB.

Full-length and partial genome sequences for several members of the genus *Aquareovirus* have been reported. The complete sequence is available for several isolates of GCV, golden shiner reovirus (species *Aquareovirus C*), chum salmon (*O. keta*) reovirus (species *Aquareovirus A*), golden ide (*Leuciscus idus melanotus*) reovirus (tentative species), and striped bass reovirus (species *Aquareovirus A*). Segment 6 of the guppy reovirus has been determined and threadfin (*Eleutheronema tetradactylus*) reovirus (untyped) segments 10, 6, and 11 are available. Segment 1 encodes a putative guanylyl/methyl transferase; segment 2 encodes the RNA-dependent RNA polymerase; segment 3 encodes a dsRNA binding protein with NTPase and helicase activity; segment 4, a nonstructural protein; segment 5, a NTPase core protein; segment 6, the outer capsid protein; segment 7, a nonstructural protein; segment 8, a core protein; segment 9, a nonstructural protein; segment 10, the external capsid protein; and segment 11, a nonstructural protein. Phylogenetic comparisons of the available sequences support the current taxonomic classification of the aquareoviruses and orthoreoviruses in two different genera, a distinction that was made originally on their genome segment number and specific econiches.

Birnaviridae

Members of the *Birnaviridae* have single-shelled non-enveloped capsids and genomes comprising two segments of double-stranded RNA. There are three genera in this family: *Aquabirnavirus*, *Avibirnavirus*, and *Entomobirnavirus*. The names of each genus denote the host specificity. The larger genome segment A encodes a polyprotein containing the virion capsid protein VP2, an autocatalytic protease NS, and an internal capsid protein VP3 in the physical order 5′-VP2-NS-VP3–3′ in the positive sense. There is an additional 17 kDa protein encoded in a second reading frame at the 5′-end of RNA segment A and it has been shown to be a novel anti-apoptosis gene of the Bcl-2 family. Segment B encodes the virus RNA-dependent RNA polymerase. There is no evidence of 5′-capping of any of the viral mRNAs.

The type species of the genus *Aquabirnavirus* is *Infectious pancreatic necrosis virus* (IPNV). Infectious pancreatic necrosis is a highly contagious viral disease of salmonid fish. The disease most characteristically occurs in fry of rainbow trout, brook trout (*Salvelinus fontinalis*), brown trout (*S. trutta*), Atlantic salmon, and several species of Pacific salmon. In salmonid fish, the virus causes an acute gastroenteritis and destruction of the pancreas. The signs of the disease are typically darkening, a pronounced distended abdomen, and a spiral swimming motion. The virus has also been associated with disease in Japanese eels (*Anguilla japonica*) in which it causes nephritis, menhaden (*Brevoortia tryrranus*) in which it causes a 'spinning disease', and in yellowtail fingerlings (*Seriola quinqueradiata*). A birnavirus has been associated with hematopoietic necrosis, causing high mortalities in turbot with renal necrosis, and birnaviruses have been isolated from clams exhibiting darkened gills and gill necrosis. A nontypical apoptosis has been observed in cultured cells infected by IPNV.

Transmission of the virus can occur via the feces of piscivorous birds. Fish that survive an IPNV outbreak become IPNV carriers and continue to shed the virus for life. Most IPNV isolates are antigenically related and belong to one large serogroup A. There is only one virus in serogroup B, a clam Tellina virus. More recent studies using comparisons of the deduced VP2 amino acid sequence have identified six genogroups. Genogroup 1 (equivalent to serotype A1) comprises four subgroups: genotypes 1, 2, 3, and 4. With increased culture of marine species of fish, there have been increasing reports of mortalities in yellow tail and amberjack in Japan from marine aquabirnaviruses.

Several vaccines have been developed for IPNV, including bacterially produced capsid protein and a DNA vaccine. The protein vaccine has been moderately effective in reducing the lethal effects of IPNV infection in Atlantic salmon in Norway. However, control methods still reply on quarantine and certification of eggs/fry as disease free.

DNA Viruses of Fish

Iridoviridae

In the family *Iridoviridae*, the genera *Iridovirus*, *Lymphocystivirus*, *Ranavirus*, and *Megalocystivirus* contain all of the known iridoviruses that infect fish. Their common features are icosahedral virions, 120–350 nm in diameter, that may acquire an envelope, and a viral genome consisting of one molecule of linear dsDNA of 100–303 kbp. Lymphocystis disease was one of the first fish diseases to be described due, in large part, to the characteristic giant cells observed in the connective tissue and benign nodules in the skin of plaice (*Pleuronectes platessa*) and flounder (*Platichthys flesus*). The causative agent, lymphocystis disease virus (LCDV), has been detected in more than 140 species of freshwater, estuarine, and marine fishes.

Six iridoviruses genomes have been completely sequenced, including those of the *Lymphocystis disease virus 1* (LCDV-1, genus *Lymphocystivirus*), Chilo iridescent virus (CIV, species *Invertebrate iridescent virus 6*, genus *Iridovirus*), Tiger frog virus (TFV, species *Frog 3 virus*, genus *Ranavirus*), *Infectious spleen and kidney necrosis virus* (ISKNV, genus *Megalocystivirus*), *Abystoma tigrinum virus* (ATV, genus *Ranavirus*), and Singapore grouper iridovirus (SGIV, tentative species, genus *Ranavirus*). Comparisons of the different iridovirus genomes have revealed that many genes have been conserved during evolution and, among closely related species, the gene order is well preserved. The number of genes (ORFs) in viruses of this family range from 93 (ATV) to 468 (CIV). There are 195 ORFs in LCDV-1 and 120 ORFs in GIV.

Other iridovirus diseases of fish include epizootic hematopoietic necrosis which is caused by viruses in the species *Epizootic hematopoietic necrosis virus* (EHNV, genus *Ranavirus*) in perch and rainbow trout, European sheatfish virus (ESV, genus *Ranavirus*) in sheatfish (*Silurus glanis*), and European catfish virus (ECV, genus *Ranavirus*) in catfish. ESV and ECV are classified as the same species, *European catfish virus*. *Santee–Cooper ranavirus* (SCRV) is the species name given to three iridoviruses: largemouth bass iridovirus (LMBV), doctor fish virus (DFV-16), and guppy virus (GV-6). The white sturgeon iridovirus group (WSIV) is comprised of a group of unassigned viruses that infect sturgeon in North America and Russian sturgeon (*Acipenser guldenstadi*) in Europe. Red seabream iridoviruses (RSIV) (genus *Megalocystivirus*) causes mortality in cultured juvenile red sea bream in Japan. It has also been observed in grouper in Thailand. Two goldfish iridovirus-like viruses (goldfish virus 1 and 2, GFV-1 and -2) have been isolated from swimbladder tissue culture of healthy goldfish. Electron microscopic observations of iridoviruses in the cytoplasm of erythrocytes (viral erythrocytic necrosis, VEN) have been observed in many marine and anadromous bony fish.

Herpesviridae

Herpesviruses have been isolated from channel catfish (*Ictalurus punctatus*), common and koi carp (*C. carpio*), common goldfish (*Carassius auratus*), eel (*Anguilla* spp.), rainbow trout, masou salmon, lake trout (*S. namaycush*), sturgeon, walleye, and Japanese flounder. Channel catfish virus is the only fish herpesvirus assigned to the genus, *Ictalurivirus*, and this genus is not assigned to any of the three subfamilies (*Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae*) of the family *Herpesviridae*. The other fish herpesviruses, cyprinid herpesviruses 1 and 2 (CyHV-1 and CyHV-2), koi herpesvirus (CyHV-3), salmonid herpesvirus 1 and 2 (SalHV-1 and -2), eel herpesvirus (Anguilla herpesvirus, AngHV-1), and the acipenserid or white sturgeon herpesviruses remain as unassigned members of the family *Herpesviridae*. Electron micrographic evidence of herpesviruses has been found in sharks, eels, pike, flounder, perch, angelfish, grouper, and other fish.

The genomes of fish herpesviruses range in size from 134 to 295 kbp and the physical organization of the genome varies sufficiently to suggest that they have evolved separately from the herpesviruses of birds and mammals. The genome of *Ictalurid herpesvirus 1* (IcHV-1) or Channel catfish virus has a unique long (UL) region flanked by a substantial direct repeat that is similar to the betaherpesviruses of the genus *Roseolovirus*. The SalHV-1 genome is more similar in organization to the alphaherpesviruses of the genus *Varicellovirus* with a unique short (US) region flanked by a unique long (UL) region which is not flanked by a repeat. Phylogenetic comparisons of the individual genes including the DNA polymerase gene, the major capsid protein gene, the intercapsomeric triplex protein gene, and the DNA helicase gene indicate that the three cyprinid viruses are closely related and are distinct from IcHV-1.

These viruses are serious pathogens in their respective hosts. IcHV-1 outbreaks among juvenile catfish result in mortality and fish that survive the infection become carriers. The cyprinid herpes viruses produce a systemic disease with lesions in hematopoietic tissue in goldfish and papillomas on the caudal regions in koi carp. SalHV-2 was isolated from the ovarian fluid of masou salmon and it induces syncytia formation and lysis of

infected cells. Epithelial papillomas are induced in young masou salmon injected with tissue culture-grown virus. The acipenserid herpesviruses cause serious losses in hatchery-reared young of white sturgeon (*Acipenser transmontanus*).

Adenoviridae

Adenovirus particles have been observed in lesions in a number of fish species and have been isolated from white sturgeon, dabs (*Limanda limanda*), cod, and Japanese red sea bream. The white sturgeon adenovirus has been isolated in tissue culture and its hexon protein and protease gene sequences are available. Based on an alignment of partial DNA polymerase gene sequences of the sturgeon adenovirus and 24 other adenovirus types, it is clear that the fish adenovirus is distantly related to the other adenoviruses, and might constitute a fifth genus of the *Adenoviridae*.

Adenovirus infection in cod produces an epidermal hyperplasia. In California, white sturgeon adenovirus affects young fish in hatcheries. Infection is characterized by epithelial hyperplasia and enlarged cell nuclei. A lympholeukemia has been observed in red sea bream and papillomas have been observed in dabs infected with adenoviruses.

See also: Fish and Amphibian Herpesviruses; Fish Rhabdoviruses; Fish Retroviruses; Aquareoviruses; Infectious Pancreatic Necrosis Virus; Infectious Salmon Anemia Virus.

Further Reading

Attoui H, Fang Q, Jaafar FM, *et al.* (2002) Common evolutionary origin of aquareoviruses and orthoreoviruses revealed by genome characterization of Golden shiner reovirus, Grass carp reovirus, Striped bass reovirus, and Golden ide reovirus (genus *Aquareovirus*, family *Reoviridae*). *Journal of General Virology* 83: 1941–1951.

Essbauer S and Ahne W (2001) Viruses of lower vertebrates. *Journal of Veterinary Medicine, Series B* 48: 403–475.

Hoffmann B, Beer M, Schutz H, and Mettenleiter TC (2005) Fish rhabdoviruses: Molecular epidemiology and evolution. *Current Topics in Microbiology and Immunology* 292: 81–117.

Lewis TD and Leong JC (2004) Viruses of fish. In: Leung KY (ed.) *Current Trends in the Study of Bacterial and Viral Fish and Shrimp Diseases. Molecular Aspects of Fish & Marine Biology,* vol. 3, 39–81.

Paul TA, Quackenbush SL, Sutton C, Casey R, Bowser P, and Casey JW (2006) Identification and characterization of an exogenous retrovirus from Atlantic salmon swimbladder sarcomas. *Journal of Virology* 80: 2941–2948.

Phelan PE, Pressley ME, Witten PE, Mellon MT, Blake S, and Kim CH (2005) Characterization of Snakehead rhabdovirus infection in zebrafish (*Danio rerio*). *Journal of Virology* 79: 1842–1852.

Schutze H, Ulferts R, Schelle B, *et al.* (2006) Characterization of White bream virus reveals a novel genetic cluster of Nidoviruses. *Journal of Virology* 80: 11598–11609.

Skall HF, Olesen NJ, and Mellergaard S (2005) Viral haemorrhagic septicaemia virus in marine fish and its implications for fish farming – A review. *Journal of Fish Diseases* 58: 509–529.

Troyer RM and Kurath G (2003) Molecular epidemiology of infectious hematopoietic necrosis virus reveals complex virus traffic and evolution within southern Idaho aquaculture. *Diseases of Aquatic Organisms* 55: 175–185.

Wolf K (1988) *Fish Viruses and Fish Viral Diseases.* Ithaca, NY: Cornell University Press.

Flaviviruses of Veterinary Importance

R Swanepoel, National Institute for Communicable Diseases, Sandringham, South Africa
F J Burt, University of the Free State, Bloemfontein, South Africa

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Argasid Soft-skinned tick, member of the family *Argasidae*.
Arthropod Any member of the phylum Arthropoda, including insects and arachnids (spiders, scorpions, ticks, and mites).
Culicoid midge Blood-sucking midge of the genus *Culicoides*.
Instar Stage in the life cycle of arthropods, for example, egg, larva, nymph, and adult in mosquitoes; egg, larva, nymph, and adult in ticks.
Ixodid Tick with a hardened shell or scutum.
Microhabitat The environmental niche in which an organism is found.
Phlebotomine fly Blood-sucking sandfly of the genus *Phlebotomous*.
Phylogenetics The study of the genetic relatedness of organisms.
Transovarial transmission of virus Passage of virus infection through the eggs of arthropods to the succeeding generation, thus ensuring perpetuation of the virus.
Vectors Blood-sucking arthropods (mosquitoes, midges, sandflies, and ticks) which transmit viruses from one vertebrate to another.
Virion A complete virus particle with its protein coat and core of DNA or RNA nucleic acid.

Introduction

Approximately 94 viruses are currently assigned to three genera (*Flavivirus, Pestivirus*, and *Hepacivirus*) within the family *Flaviviridae* (L. *flavus* = yellow, from the type species *Yellow fever virus*, which causes jaundice); the number of viruses in the family changes with periodic discovery of new members or revision of the taxonomic status of existing viruses. Diseases of major medical and veterinary importance caused by hepaciviruses and pestiviruses are discussed elsewhere in this encyclopedia, while this article summarizes aspects pertaining only to the veterinary importance of members of the genus *Flavivirus*.

Viruses of the *Flaviviridae* have spherical virions 40–60 nm in diameter, with a tightly applied lipid envelope incorporating envelope (E) protein spikes which are glycosylated in pestiviruses and flaviviruses. The genomes consist of a single, linear molecule of positive-sense, single-stranded RNA (ssRNA), ranging in size from approximately 9.5 kbp in hepaciviruses, to 10.7 kbp in flaviviruses, and 12.5 kbp in pestiviruses. Replication takes place in the cytoplasm, with morphogenesis, transport, and maturation of virions occurring in cell membrane vesicles. A single open reading frame (ORF) in the genome is translated directly into a polyprotein which is cleaved into structural proteins, the RNA-associated internal capsid or core (C) protein, envelope proteins (E1, E2), and a membrane (M) protein, plus seven nonstructural proteins which exhibit viral polymerase and other enzymatic activities. The E proteins mediate binding of virus to host cells, are responsible for the hemagglutinating property exhibited by most flaviviruses, and constitute the major antigenic determinants of protective immunity. Viruses of the three genera are not closely related, with less than 30% nucleotide sequence homology and an absence of antigenic cross-reactivity, but they share genome organization and replication strategies. Viruses of the genera *Hepacivirus* and *Pestivirus* are most closely related structurally, and have in common a lack of arthropod vectors and the tendency to cause persistent infection in their vertebrate hosts.

The 82 recognized or tentative members of the genus *Flavivirus* include two inherent viruses of mosquitoes, cell fusion agent virus (CFAV) and Kamiti River virus (KRV), which have no known medical or veterinary significance and which may be assigned to a new genus, plus Tamana bat virus from Trinidad, which may also be assigned to a new genus. Most recently, there has been molecular identification of a tick-associated agent, Ngoye virus from Senegal, which has not as yet been successfully cultured in a laboratory host system, has no known medical or veterinary significance, and is only distantly related to existing flaviviruses. The remaining flaviviruses are antigenically related to each other and were originally assigned to serogroups on the basis of close antigenic affinities. Recent classification of the viruses based on phylogenetic analyses shows remarkable concordance with antigenic classification, and with the biological properties of the viruses, such as vertebrate host and arthropod vector associations.

About 21% of the members of the genus have been isolated from mammals only and have no known vectors (NKV viruses), a further 51% are usually transmitted by mosquitoes, and the remaining 28% appear to be transmitted principally by ticks, although definitive demonstration of transmission by arthropod vectors has been reported in a proportion of instances only. Depending on which genes are subjected to phylogenetic analysis, different conclusions may be reached with regard to the evolution of the flaviviruses, but from limited analyses of whole genome sequences it appears that NKV viruses diverged from a putative ancestor before mosquito-borne viruses, and that tick transmission is a recently acquired trait within the genus. Many flaviviruses were discovered in the course of surveys conducted on vertebrate and arthropod populations, rather than in the investigation of disease syndromes, and consequently many of the viruses have no known medical or veterinary significance. From the genetic distance which exists between the recently discovered Ngoye virus and other members of the genus, and between existing mosquito-borne viruses, it has been extrapolated that several thousand flaviviruses remain to be discovered.

Various mechanisms are postulated to ensure the persistence of arboviruses through prolonged periods of vector inactivity during winters and dry seasons, including the migration of infected vertebrate hosts, hibernation of infected adult vectors, and passage of virus through the eggs of vectors to infect the succeeding generation, so-called transovarial transmission of infection, which generally occurs with low frequency. Unlike mosquitoes, ticks feed on vertebrates in different stages (instars) of their life cycles, as larvae, nymphs, and adults, so that even in the absence of transovarial transmission of infection the perpetuation of virus can be ensured by the long intervals which intervene between the feeding of successive instars on different hosts.

Viruses transmitted by mosquitoes often cause sporadic infections which may pass unrecognized in endemic areas, but they are capable of causing explosive outbreaks when exceptionally heavy rainfall creates favorable breeding conditions for the vectors, or human manipulation of the environment, such as the building of dams and irrigation schemes, or the implementation of intensive livestock production systems, results in the juxtaposition of susceptible human or farm animal populations with vectors. Tick-borne virus infections tend to occur at endemic level within areas of vector distribution, and larger outbreaks are less immediately linked to climatic events which may favor population explosions of the wild hosts of immature ticks, but human intervention, such as changes in livestock

farming systems, can also precipitate epidemics. A few arthropod-borne flaviviruses are named for the disease syndromes with which they are associated, but most are named for the geographic location from which the initial isolate was obtained.

Mosquito-Borne Flaviviruses Causing Livestock or Wildlife Diseases

Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) was first isolated in 1935 in Japan as a cause of encephalitis in humans. Severe outbreaks of human disease occur at intervals in India, China, Korea, and Japan, and lesser outbreaks or sporadic cases are recorded in Nepal, Bangladesh, Sri Lanka, Laos, Cambodia, Vietnam, the Far East provinces of the Russian Federation, Thailand, Malaysia, Singapore, Philippines, Indonesia, Papua New Guinea, Taiwan, Guam, Saipan, and Myanmar. There are four genotypes of the virus, but they are all cross-protective. The virus is transmitted by *Culex tritaeniorhyncus* and other culicine mosquitoes and circulates between the mosquitoes, water birds, particularly egrets, herons and ducks, and domestic pigs. Overt outbreaks of disease are associated with rainfall and the irrigation of rice fields. The virus infects a wide range of vertebrates, including donkeys, cattle, and dogs, but most animals do not manifest disease. There is fetal wastage and abortion in sows. Humans and horses are incidental hosts which develop encephalitis, but play no significant role in the circulation of the virus. Tens of thousands of human infections occur each year, and while less than 1% of infected persons develop encephalitis the death rate is high in such patients. Case fatality rates may exceed 50% in horses with encephalitis. The virus is not known to cause disease in wild vertebrates, but bats can circulate virus following experimental infection, and low prevalences of antibody to JEV have been found in reptiles and amphibians.

St. Louis Encephalitis Virus

St. Louis encephalitis virus (SLEV) was isolated in 1933 from human brain as the causative agent of a large outbreak of encephalitis in St. Louis, Missouri, and was subsequently found to be transmitted by culicine mosquitoes, which are capable of breeding in drainage water in urban settings. The virus is widely distributed in North, Central, and South America, but the disease is seen mainly in the USA where it was considered to be the most important arbovirus pathogen prior to the introduction of West Nile virus (WNV) in 1999. There is some regional variation in the mosquitoes involved, but the main vectors are members of the *Cx. pipiens* complex, plus *Cx. tarsalis* and *Cx. nigripalpus*. Human infection with SLEV is usually asymptomatic but a low proportion of patients develop encephalitis with an approximately 10% case fatality rate. Outbreaks of varying magnitude occur at irregular intervals throughout the USA, but there are regional differences in occurrence of the infection depending on host and breeding-site preferences of the mosquito species concerned. Asymptomatic infection occurs in domestic animals such as horses, cattle, goats, sheep, pigs, cats, dogs and poultry, as well as in wild birds and small mammals including raccoons, opossums, rodents, and bats, but only birds are thought to play a significant role in the circulation of the virus. SLEV was isolated from the brain of a gray fox with encephalitis, and forest mammals including sloths are thought to be involved in transmission cycles in South America.

West Nile Virus

Following the intial isolation of WNV from a febrile patient in Uganda in 1937, sporadic cases and outbreaks of benign febrile disease were recorded in humans in Africa, the Near East and Asia, with the largest outbreaks occurring in Israel in 1950–54 and 1957, and in South Africa in 1974. It was found that WNV is widely endemic in southern Africa in localities where the vectors, ornithophilic (bird-feeding) culicine mosquitoes, and avian hosts of the virus are present, such as at dam sites with reed beds and heronries. Outbreaks of human infection, which occur when high rainfall or hot weather favors mosquito breeding, are associated with high seroconversion rates in wild birds, and 13 species of birds in South Africa were found to support replication of the virus without developing disease. It was deduced that the widespread dispersal of the virus was associated with migrating birds. Cattle and sheep appeared to undergo inapparent infection and could also theoretically serve as hosts for infection of mosquitoes. Subsequently, limited experimental evidence was obtained to indicate that WNV is capable of causing abortion in pregnant sheep.

The occurrence of meningoencephalitis was first observed in elderly WNV patients in Israel in 1957, and subsequently also in young children in India. The occurrence of horse encephalitis was recognized in Egypt in 1962, and in 1962–66 outbreaks of meningoencephalitis were observed in both humans and horses in France. There was a marked increase in the frequency and severity of outbreaks of human disease during the 1990s, often involving horses as well, and including epidemics in Algeria, Romania, Morocco, Tunisia, Italy, Russia, France, and Israel. Moreover, the outbreaks in Romania and Israel were characterized by concurrent mortality in birds. The vectors of WNV included *Cx. univitattus* and *Cx. antennatus* in Africa and Europe, members of the *Cx. pipiens* complex in Europe and Israel, and members of the *Cx. vishnui* complex plus *Cx. fatigans* in Asia.

The presence of WNV in the Western Hemisphere was first recognized in New York in 1999, and over the next few years it spread rapidly throughout the country and beyond into Canada and South America. Vectors in North America included members of the *Cx. pipiens* complex, plus *Cx. restuans*, *Cx. tarsalis*, and *Cx. nigripalpus*. The virus circulating in the USA was found to be genetically most closely related to a WNV isolate associated with goose mortality in Israel in 1998, suggesting that the virus was imported into America from the Near East, either in an infected bird, mosquito, human, or other animal, although the exact mechanism of the introduction will probably remain unknown.

In North America, WNV infection was confirmed in more than 300 species of birds, with death rates approaching 100% in corvids (crows, magpies, and jays), and with robins constituting important hosts for the infection of the mosquito vectors. It is notable that the virus spared African bird species in the New York zoo where deaths of local species were first observed. Infection was also recorded in 30 species of mammals, reptiles, and amphibians. Approximately 10% of infected horses manifested clinical illness, with a high proportion of these developing fatal encephalitis. Vaccine for horses became available in 2005. Although fatal infections were recorded in dogs and a wolf, infection rates were low in cats and dogs. Fatal disease was observed in farmed alligators in the USA and Mexico, with infection resulting from the feeding of infected horse meat, and also from transmission between alligators in crowded conditions. Serological evidence of infection was also found in small wild animals, such as raccoons, opossums, and squirrels, but the pathogenicity of the virus for these hosts is uncertain. Serological evidence of infection was obtained in farmed Nile crocodiles in Israel in the absence of disease. The Kunjin strain of WNV transmitted by *Cx. annulirostris* mosquitoes in Australia generally causes febrile illness in humans, seldom encephalitis, and was confirmed to be associated with encephalitis in horses on one occasion only.

The increase in the frequency of neurologic infections, and the occurrence of human, horse, and bird fatalities in the Northern Hemisphere, raised the question of whether there had been a change in the pathogenicity of WNV. Phylogenetic analyses revealed that WN isolates fell into two lineages. Lineage 1 included isolates from Africa north of the equator, Europe, Asia, and North America, with Kunjin virus from Australia constituting a subtype within this lineage. Lineage 2 consisted solely of viruses from Africa and Madagascar. The findings were interpreted to support the emergence of exalted virulence in lineage 1, with lineage 2 isolates considered to be associated with endemic infection of low virulence in Africa. However, WNV belonging to lineage 2 has recently been isolated from a goshawk with fatal encephalitis in Hungary, and three additional lineages of the virus have been described from the Czech Republic, Georgia, and India. Moreover, despite the apparently low level of virus activity observed in southern Africa in recent years, WNV was isolated from the brain of a suspected rabid dog in Botswana (initially described as having an infection with Wesselsbron virus), and in South Africa from humans and a foal with encephalitis, plus an ostrich chick in a commercial flock where extensive mortalities occurred. Experiments in mice confirmed that neuroinvasive WNV strains occur within both lineage 1 and 2. Thus, it is possible that the virulence of WNV in southern Africa may previously have been underestimated, while the perceived enhanced virulence of the virus in recent epidemics in the Northern Hemisphere may partly be due to the emergence or re-emergence of existing strains of WNV in geographic locations with immunologically naive populations, high medical alertness, and active surveillance programs.

Israel Turkey Meningoencephalomyelitis Virus

A disease caused by Israel turkey meningoencephalomyelitis virus (ITV) infection in turkeys was first observed in Israel in 1958 and the causative virus isolated in 1959. ITV was later found to be transmitted by culicine mosquitoes, but was also isolated from culicoid midges, and phlebotomine sandflies were shown to be capable of transmitting the virus. In 1978 the virus was isolated as a cause of disease of turkeys in South Africa. The virus has not been found in any other country, and disease has only been reported in turkeys. The disease generally occurs seasonally, only in birds older than 10 weeks, and it is characterized by progressive paresis and paralysis, with morbidity and mortality rates ranging from 15% to 80%. Turkey breeder hens exhibit a severe drop in egg production. Vaccines are available.

Sitiawan Virus

Sitiawan virus (SITV) was recently isolated as a cause of deaths of chick embryos and broiler chickens in Malaysia. It is closely related to the mosquito-borne Tembusu virus, which has no known medical or veterinary significance.

Wesselsbron virus

Wesselsbron virus (WESSV) was identified in 1955 in South Africa as a cause of deaths of newborn lambs in a flock where a modified live vaccine against the mosquito-borne Rift Valley fever virus (RVFV) (not a flavivirus) had been administered 2 weeks previously. There was concern that the RVFV vaccine may have caused the deaths, but it was concluded that the new virus, which also proved to be transmitted by *Aedes* species mosquitoes of the subgenera *Ochlerotatus* and *Neomelanoconion*, occurred in outbreaks together with RVFV and caused similar disease in sheep,

cattle, and goats, namely, deaths of young animals and abortion in pregnant animals. Consequently, an attenuated WESSV vaccine was developed by serial intracerebral passage of virus in infant mice, and this was marketed for use together with RVFV vaccine on nonpregnant animals. During extended outbreaks of RVF in South Africa and Namibia in 1974–76 some 13.9 million doses of the live WESSV vaccine and 22 million doses of live RVFV were used indiscriminately on livestock, with resultant widespread occurrence of abortions and congenital malformations in sheep and cattle. It was confirmed experimentally that both of the vaccines were abortigenic and teratogenic. However, it subsequently became clear that WESSV is more widely endemic in southern Africa than RVFV, and although sporadic isolations of virus have been made from livestock in South Africa and Zimbabwe, mainly in association with disease of newborn lambs and calves, the virus has not been incriminated as the cause of large outbreaks of disease in farm animals, nor has a veterinary problem been described elsewhere. The vaccine, which confers lifelong immunity, continues to be marketed for use in nonpregnant sheep and goats.

Altogether, WESSV has been isolated from livestock and a gerbil in southern Africa, a camel in Nigeria, mosquitoes in South Africa, Zimbabwe, Uganda, Kenya, the Central African Republic (CAR), Cameroon, Ivory Coast, Senegal, Guinea and Thailand, a tick in CAR, naturally infected humans in South Africa, Senegal, and CAR, and from human laboratory infections in South Africa, Uganda, Nigeria, Senegal, CAR, and the USA. In addition, antibody has been found in humans and/or livestock in Malawi, Zambia, Botswana, Namibia, Angola, Mozambique, and Madagascar. The principal vectors of the virus appear to be aedine mosquitoes, and although the virus has been isolated from a tick, there is no evidence that ticks play a significant role in transmission of the virus. The few human infections recorded in South Africa were acquired from contact with infected animal tissues or mosquito bite, while human infections reported in Senegal and CAR seem to be consistently associated with mosquito bite. The virus generally causes benign febrile illness in humans, but a patient who acquired laboratory infection from the splashing of virus into an eye developed encephalitis from which he recovered without sequelae.

It cannot be excluded that WESSV or various other flaviviruses could emerge as important pathogens under particular circumstances, as occurred with WNV in North America in 1999. In this connection it should be noted that Bagaza virus has been isolated from aborted cattle fetuses on three occasions in South Africa, that WNV and Banzi virus were shown to cause abortion in experimentally infected pregnant sheep, and that Usutu virus, initially isolated from mosquitoes, a rodent and birds in the context of surveys in Africa, emerged as a cause of bird deaths in Austria in 2001.

Yellow Fever Virus

Descriptions of disease compatible with yellow fever date back to 1492. However, many medical conditions were confused in early records, and the name yellow fever was first applied to a fatal disease of humans in the Caribbean region in 1750. Shortly thereafter, the presence of the disease was recognized in South America and West Africa. Over the next 150 years the virus, introduced by infected people on ships, caused epidemics in many port cities in North America and Europe. From 1900 onwards the Walter Reed commission from the USA investigated yellow fever in Cuba and, using American military volunteers, demonstrated that the disease could be transmitted from person-to-person by *Ae. aegypti* mosquitoes, and that the infection was caused by a filterable agent present in the blood of patients (filtration was an early method used to distinguish viruses from bacteria). It was not until 1927 that yellow fever virus (YFV) was isolated in a laboratory host system, rhesus monkeys. Meanwhile, the identification of the *Ae. aegypti* vectors, which breed in small volumes of water, had facilitated the implementation of mosquito control methods which virtually eliminated circulation of YFV in urban settings. Initially, there was speculation as to whether YFV had originated in Africa or South America, but it was concluded that the virus and *Ae. aegypti* mosquitoes had been translocated from Africa in ships centuries ago. The occurrence of outbreaks of the disease on ships was well documented.

Resurgence of urban yellow fever in South America led to the discovery of jungle or sylvatic circulation of the virus in 1932. In South America, YFV circulates in the forest canopy between monkeys and *Haemagogus* species mosquitoes. Persons who live near or enter forests acquire infection and introduce it into urban environments where epidemics are generated by the circulation of YFV between humans and *Ae. aegypti* mosquitoes. In Africa, mosquitoes such as *Ae. africanus* transmit YFV between primates at forest canopy level, while mosquitoes which feed at intermediate levels, including *Ae. bromeliae* and members of the *Ae. furcifer-taylori* group, serve as link hosts to spread infection to humans which in turn serve to initiate urban transmission cycles involving *Ae. aegypti* mosquitoes. South American primates including tamarins, marmosets and howling, squirrel, owl, and spider monkeys undergo severe infection with variable mortality, while African monkeys and apes generally undergo viremic infection without overt illness. This was interpreted to indicate that there had been a longer period of co-evolution of YFV with African primates, but African YFV isolates were found to be of reduced virulence for neotropical monkeys. Asian monkeys are susceptible to severe and fatal YFV infection. Birds, reptiles, and amphibia are resistant to infection, and although several mammals including hedgehogs are capable of developing

viremia, it appears that they have limited exposure to known vectors.

Effective vaccines were developed in the 1930s and used extensively in conjunction with mosquito control to reduce the occurrence of the human disease. However, the existence of sylvatic transmission cycles precludes eradication of the virus, and since 1980 there has been a marked resurgence of the disease in Africa with outbreaks being recorded in countries extending from Senegal in the north, to Angola in the south, and eastwards to Sudan, Ethiopia, and Kenya. No recent urban outbreaks have been recorded in South America, but fairly extensive outbreaks involving sylvatic vectors have occurred in Peru and Brazil.

Many of the remaining mosquito-borne flaviviruses are believed to circulate in birds and although a few, including Rocio, Spondweni, and Zika, have been associated with human disease on occasion, they have no known veterinary significance. It should be noted, however, that many of the flaviviruses which are pathogenic for humans produce symptomatic infection in a minority of people, and it can be surmised that infections which produce similar low morbidity in farm animals are likely to pass unrecognized.

Tick-Borne Flaviviruses

It has emerged from recent phylogenetic analyses that the tick-borne flaviviruses comprise three groups: a seabird group which includes three viruses with no known medical or veterinary significance, a Kadam virus group containing a single virus known only to have been isolated from ticks removed from cattle in Uganda and a camel in Saudi Arabia, but which represents a distinct evolutionary lineage, and the so-called mammalian tick-borne group of viruses. Within the mammalian group Gadgets Gully, Royal Farm, and Karshi viruses and are more distantly related to others and are known only to have been isolated from ticks, while the remaining viruses, which were formerly clustered together in a tick-borne encephalitis antigenic complex, include important pathogens of humans and livestock. However, genetic differences between some of these latter viruses are smaller than those which exist between other flaviviruses, and hence they have been tentatively designated as types and subtypes of a proposed tick-borne encephalitis virus (TBEV) species.

Powassan Virus and Deer Tick Virus

Powassan virus (POWV) was first isolated as a cause of fatal encephalitis in a child in Ontario, Canada, in 1958. The virus was subsequently found in western Canada, and northeast, north-central and western USA. The principal vector was found to be the tick *Ixodes cookei*, and the main host for the tick and virus is believed to be the woodchuck or groundhog (*Marmota monax*). Other *Ixodes* species and squirrels have also been implicated in the circulation of the virus, and there have been isolations from other ticks including *Dermacentor andersoni*. Twelve human cases of encephalitis with four fatalities have been reported in North America. Antibody has been found in a range of animals, and virus was isolated from two dead foxes, although it was not clear that the virus had caused their deaths. The virus was experimentally shown to be capable of causing encephalitis in horses. Reportedly, POWV was isolated in 1978 from the blood of a person who had been bitten by a tick in the Maritime Province (Primorsky) in the Far East of the former Soviet Union (currently the Russian Federation), and later the virus was isolated from *Ixodes persulcatus* and other ticks and mosquitoes, and antibodies were found in birds in Primorsky. In 1996, deer tick virus (DTV) was isolated from deer ticks, *Ixodes scapularis*, in the eastern USA and found to be closely related to POWV. The white-footed mouse (*Peromyscus leucopus*) is thought to be the main host for the tick and virus, although other mammals, particularly white-tailed deer, are important hosts for the tick. As yet, no disease associations have been made for DTV. Although POWV and DTV are closely related, the results of recent phylogenetic studies confirm that the viruses circulate independently. However, the studies did not include POWV isolates from the western USA or the Russian Federation.

Kyasanur Forest Disease Virus

Kyasanur forest disease virus (KFDV) was discovered in 1957 as a cause of fatal febrile disease of humans in Karnataka State, India. The vectors are ticks of the genus *Haemaphysalis*, particularly *H. spinigera*, and two species of *Ixodes* ticks, but the virus has also been isolated from the argasid (soft-skinned) tick *Ornithodoros crossi*. Rodents are suggested to be the main hosts of the virus. About 500 human cases of the disease are recorded each year in Karanataka State, with a mortality rate of 5–10%. Infection occurs mainly in people who enter forests to gather wood, and the disease is seasonal with tick activity. Fatal disease occurs in langur and macaque monkeys in the forests, but no disease manifestations have been recorded in domestic animals. The virus has been isolated from rodents, shrews, and bats.

Omsk Hemorrhagic Fever Virus

Omsk hemorrhagic fever virus (OHFV) was isolated in 1947 from the blood of a patient with hemorrhagic disease in western Siberia in the former Soviet Union. The disease was first observed in 1941, and large numbers of cases were recorded in 1945–49. Since that time only sporadic cases have been recognized in the Omsk, Novosibirsk,

Kurgan, and Tjumen regions of western Siberia. The disease affects mostly muskrat trappers and their families. Muskrats, or musquash (*Ondatra zibethicus*), are large rodents which are trapped for their fur. They are indigenous to North America and were introduced into Siberia in 1925. No equivalent virus or disease has been found in North America. The principal vector of the virus in Siberia was found to be the tick *Dermacentor reticulatus* (syn. *D. pictus*) but the virus was also isolated from *D. marginatus* and other ticks and mites. Muskrats and local voles, some of which undergo benign infection, serve as reservoir hosts for the infection of ticks. Muskrats live in swamps, and virus which is present in the excretions of infected muskrats, or leaches into water from dead muskrats, can infect other muskrats or humans. OHFV was also found in mosquitoes and antibody in water birds, but these do not appear to play a significant role in circulation of the virus. Humans acquire infection mainly from contact with infected muskrat tissues, but also from infected water, or from tick bite.

Tick-Borne Encephalitis Virus

A human encephalitis syndrome was recognized in the far eastern provinces of the former Soviet Union at least as far back as the late nineteenth century, and has been known under a variety of names including Russian spring–summer encephalitis and Far Eastern tick-borne encephalitis. The causative agent, now named tick-borne encephalitis virus-Far Eastern subtype (TBEV-FE), was isolated from human patients in 1937 and later from its *Ixodes persulcatus* tick vectors. The virus occurs in the Primorsky, Khabarovsk, Krasnoyarsk, Altai, Tomsk, Omsk, Kemerovo, Western Siberia, Ural and Priural regions of the Russian Federation, China, and eastern Europe. After World War II the existence of a similar disease was recognized in several countries in Central Europe and adjacent parts of the Soviet Union, and a second virus, tick-borne encephalitis virus-European subtype (TBEV-Eu), was isolated from humans and *Ix. ricinus* ticks. Slight antigenic differences were shown to occur between the two viruses, and TBEV-Eu had human case fatality rates of 0.5–2.0%, in contrast to 5–20% for TBEV-FE. It has since been established that TBEV-Eu occurs in Albania, Austria, Belarus, Bornholm Island of Denmark, Bosnia, Bulgaria, China, Croatia, Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Kazakhstan, Latvia, Lithuania, Norway, Poland, Romania, Russia, Slovakia, Slovenia, Sweden, Switzerland, Turkey, and Ukraine, although the distribution tends to be focal, dependent on suitable tick habitat. The *Ix. persulcatus* tick vector of TBEV-FE is distributed across nontropical Asia from Japan to eastern Europe, where there is overlap with the distribution of the *Ix. ricinus* vector of TBEV-Eu which extends across western Europe to the British Isles and Ireland. Other tick species play a minor role in transmission of the TBEV viruses. Phylogenetic analyses in the 1990s revealed the existence of a third subtype of virus, tick-borne encephalitis virus-Siberian subtype (TBEV-Sib), which is transmitted by *Ix. persulcatus* in the Irkutsk region of Siberia and has similar virulence for humans as does TBEV-FE. It was deduced that the TBEVs evolved in a cline in a westerly direction across the Eurasian continent over the past few thousand years. However, TBEV-FE and TBEV-Sib infections have recently been detected in Europe.

All three subtypes of TBEVs are believed to circulate between ticks and small vertebrates such as field mice and voles which undergo benign viremic infection. Antibody is also found in larger vertebrates such as squirrels, deer, badgers, and farm animals which develop low-grade viremia and are believed not to constitute a significant source of virus for infection of ticks. About 5000–10 000 cases of human disease are recorded each year, mainly in the Russian Federation, with the lowest incidence occurring in Austria where there is widespread use of vaccine. Humans acquire TBEV infection from tick bite, or from the ingestion of unpasteurized infected milk of sheep, goats, and cows. Less than 5% of humans develop symptomatic infection, but high death rates occur in patients who develop nervous disease. There may be neurological sequelae in those who recover, and a few patients develop chronic infections. A fatal hemorrhagic syndrome of humans has been described in the Novosibirsk region of Siberia in association with TBEV-FE infection. Recently, fatal TBEV-Eu infection was reported in captive monkeys in Germany which were inadvertently exposed to ticks.

Louping Ill Virus

Descriptions of a sheep disease conforming to louping ill (Scottish dialect *louping* = leaping) date back more than two centuries in Scotland, but it was not until 1929 that the causative virus was isolated, and shortly thereafter shown to be transmitted by the tick *Ix. ricinus*. Other species of ticks can be infected but do not appear to play a significant role in the transmission of the virus. Louping ill virus (LIV) occurs in hill sheep farming areas of Britain and Ireland where there is suitable microhabitat for the ticks. Phylogenetic evidence suggests that the virus was probably originally introduced into Ireland from western Europe as a result of the livestock trade, and successively evolved as Louping ill virus-Ireland (LIV-Ir), and Louping ill virus-British (LIV-Brit) as it spreads through Wales, England, Scotland, and into Norway.

LIV causes fatal encephalomyelitis in a proportion of sheep depending on age and predisposing factors, including concurrent infection with agents such as the tick-borne parasite *Ehrlichia phagocytophila*. In areas with high challenge

rates the disease is seen most frequently in lambs once they have lost their maternal immunity, but in areas with low tick burdens primary infection and disease may be seen in sheep of all ages. Infection is less frequently associated with disease in cattle, horses, pigs, farmed red deer, dogs, red grouse, and humans. Infection also occurs in roe deer, mountain hares, feral goats, voles, shrews, and field mice. The only recognized disease problem in wild vertebrates exists in red grouse (*Lagopus lagopus scotticus*), which have a commercial hunting value, and it has been demonstrated that the chicks can acquire infection from ingesting ticks. Sheep and red grouse are the only hosts which develop sufficiently intense viremia for the infection of ticks. However, it has been shown experimentally that the so-called phenomenon of nonviremic transmission of infection between ticks feeding on the same host, facilitated by factors present in tick saliva, occurs with LIV-infected and noninfected ticks co-feeding on mountain hares, which are important hosts of *Ix. ricinus* ticks. The same phenomenon has been demonstrated with TBEV-Eu, and this implies that there should be reassessment of the role of vertebrate hosts in the circulation of tick-borne viruses.

Spanish sheep encephalitis virus, Turkish sheep encephalitis virus, and Greek goat encephalitis virus are recently recognized subtypes of TBEV analogous to LIV that have been associated with encephalitis in sheep and goats in Spain, Greece, Bulgaria, and Turkey, but their distribution and epidemiology are as yet incompletely understood.

Negishi Virus

A virus given the name Negishi virus (NEGV) was isolated in 1948 from a cerebrospinal fluid sample from a child near Tokyo with suspected Japanese encephalitis, and was found to be antigenically related to the tick-borne encephalitis group of flaviviruses. There were no further isolations of NEGV in Japan and recently the virus was found to be genetically closely related to LIV. However, in 1993 a virus was isolated from a patient with encephalitis in Hokkaido, the northern island of Japan where *Ix. persulcatus* ticks occur, and subsequently the virus was also obtained from ticks and blood samples from dogs in Hokkaido. The new isolates proved to be phylogenetically most closely related to TBEV-FE.

See also: Flaviviruses: General Features; Tick-Borne Encephalitis Viruses; West Nile Virus; Yellow Fever Virus.

Further Reading

Billoir F, de Chesse R, Tolue H, de Micco P, Gould EA, and de Lamballerie X (2000) Phylogeny of the genus *Flavivirus* using complete coding sequences of arthropod-borne viruses and viruses with no known vector. *Journal of General Virology* 81: 781–790.

Cook S, Bennett SN, Holmes EC, de Chesse R, Moureau G, and de Lamballerie X (2006) Isolation of a new strain of the *Flavivirus* cell fusing agent virus in a natural mosquito population from Puerto Rico. *Journal of General Virology* 87: 735–748.

Cook S and Holmes EC (2006) A multigene analysis of the phylogenetic relationships among the *Flaviviruses* (family: *Flaviviridae*) and the evolution of vector transmission. *Archives of Virology* 151: 309–325.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.* San Diego, CA: Elsevier Academic Press.

Grard G, Lemasson JJ, Sylla M, *et al.* (2006) Ngoye virus: A novel evolutionary lineage within the genus *Flavivirus*. *Journal of General Virology* 87: 3273–3277.

Grard G, Moureau G, Charrel RN, *et al.* (2006) Genetic characterization of tick-borne *Flaviviruses:* New insights into evolution, pathogenetic determinants and taxonomy. *Virology* 361: 80–92.

Kono Y, Tsukamoto K, and Hamid MABD (2001) Encephalitis and retarded growth of chicks caused by Sitiawan virus, a new isolate belonging to the genus *Flavivirus*. *American Journal of Tropical Medicine and Hygiene* 63: 94–101.

Pybus OG, Rambaut A, Holmes EC, and Harvey PH (2002) New inferences from tree shape: Numbers of missing taxa and population growth rates. *Systematic Biology* 51: 881–888.

Flaviviruses: General Features

T J Chambers, Saint Louis University School of Medicine, St. Louis, MO, USA

© 2008 Published by Elsevier Ltd.

Glossary

Cirrhosis Disease of the liver, with fibrosis and distortion of the cellular architecture.
Enzootic A disease or maintenance transmission cycle which occurs in a continuous manner among nonhuman animals within a certain geographic zone.
Epizootic Above average activity or occurrence of disease or pathogenic agent among nonhuman animals.
Sylvatic Located in forested regions.
Thrombocytopenia A reduction in the normal quantity of circulating blood platelets.
Zoonosis A disease of animals communicable to man.

General Features

The family *Flaviviridae* includes three genera, *Flavivirus, Pestivirus,* and *Hepacivirus,* that together comprise approximately 80 known viruses (**Table 1**), many of which cause diseases in human or animal hosts. Viruses in these genera are antigenically distinct from viruses in other genera and overall sequence homology is approximately 20%. The flaviviruses constitute the largest genera within the family, including arthropod-borne viruses that cause encephalitis and hemorrhagic fever. Pestiviruses exhibit a host range that includes pigs and ruminants. Hepaciviruses cause chronic liver disease in humans. Flaviviruses were originally classified as the 'group A' members of the family *Togaviridae*, distinguished serologically from 'group A' arboviruses (e.g., equine encephalitis viruses) but later assigned as constituting a unique family of viruses. Although vector associations together with antigenic properties previously served to classify the *Flaviviridae*, modern taxonomy based on nucleotide sequencing has been used to establish their phylogenetic and evolutionary relationships.

Members of the *Flaviviridae* are enveloped viruses which share a common genome organization and replication strategy. Their virions are small, spherical particles containing the capsid–genome complex and membrane-associated envelope proteins, which confer antigenic properties. The genomes are small, single-stranded, plus-sense RNA molecules ranging in size from 9.6 to 12.3 kbp. Viral proteins are encoded within a single long open reading frame that contains the structural proteins, followed by nonstructural proteins (protease(s), RNA helicase, RNA-dependent RNA polymerase (RdRp), other enzymatic activities involved in RNA replication, and several small hydrophobic membrane proteins).

Flaviviruses and pestiviruses exhibit generally broad cellular host range *in vitro*, whereas growth of hepaciviruses is very restricted. Entry of viruses into host cells occurs by receptor-mediated endocytosis, requiring a low pH-dependent fusion activity of the major envelope proteins. Cellular receptors involved in the entry process have been difficult to definitively identify. Viral replication occurs in association with intracellular membranes via a negative-strand RNA intermediate. Characteristic ultrastructural changes are induced in the membranes. Mature viruses are generated by budding through host intracellular membranes followed by release at the cell surface. Further details of these processes and unique features of each genus are described below.

Table 1 Taxonomy of viruses of the family *Flaviviridae*

- Genus *Flavivirus* (12 complexes[a]; 1 tentative species/subspecies)
  - Tick-borne viruses
    - Mammalian tick-borne virus complex (10 members)
    - Seabird tick-borne virus complex (3 members)
  - Mosquito-borne viruses
    - *Aroa virus* complex (4 members)
    - *Dengue virus* complex (5 members)
    - *Japanese encephalitis virus* complex (10 members)
    - *Kokobera virus* complex (2 members)
    - *Ntaya virus* complex (6 members)
    - *Spondweni virus* complex (2 members)
    - *Yellow fever virus* complex (10 members)
  - Viruses with no known arthropod vector
    - *Entebbe bat virus* complex (3 members)
    - *Modoc virus* complex (6 members)
    - *Rio Bravo* complex (7 members)
  - Tentative members of the genus
    - Alkhurma virus (1 member)
- Genus *Pestivirus* (4 species plus 1 tentative species)
  - *Border disease virus* (2 serotypes)
  - *Bovine viral diarrhea virus-1* (4 serotypes)
  - *Bovine viral diarrhea virus-2* (2 serotypes)
  - *Classical swine fever virus* (*Hog cholera virus*), (4 serotypes)
  - Tentative species in the genus
    - Pestivirus of giraffe
- Genus *Hepacivirus* (1 species)
  - *Hepatitis C virus* (6 genotypes)
  - Unassigned viruses in the family
    - Tamana bat virus
    - Cell-fusing agent
    - GB virus A
    - GBV A-like agents
    - GB virus B
    - GB virus C/Hepatitis G virus

[a]Complex refers to closely related viruses, representing genetic and antigenic clusters, and viral species in the complexes are enumerated in parentheses, or otherwise indicated. All others represent virus strains or serotypes.

Reproduced in revised form from Calisher CH and Gould EA (2003) Taxonomy of the virus family Flaviviridae. *Advances in Virus Research* 59: 1–19, with permission from Elsevier.

Flaviviruses

History and Classification

The genus includes >70 viruses, approximately 40 of which are mosquito-borne, 16 tick-borne, and 18 without a known vector. The prototypic flavivirus, yellow fever virus (from the Latin *flavus* for 'yellow'), is recognized as a human disease agent for at least the past 300 years. In the early twentieth century, the virus was isolated and characterized as an arthropod-transmitted agent, and a live-attenuated vaccine (yellow fever strain 17D) was developed. Other flaviruses causing a range of human diseases were identified within the nineteenth and twentieth centuries. The most important of these are Japanese encephalitis (JEV), West Nile virus (WNV), the tick-borne encephalitis (TBEV), and the dengue (DENV) viruses, which cause outbreaks of human disease on a large scale. Many other flaviviruses not listed in **Table 1** are causes of acute fever, meningoencephalitis, or hemorrhagic fever in humans on a regional basis throughout the world.

Based on comparative genomic sequence analysis, it is hypothesized that flaviviruses evolved as separate

tick-borne, mosquito-borne, and no-known-arthropod vector lineages, which diverged at an early point after the origin of the genus *Flavivirus* from a common ancestor whose identity remains unknown. This ancestral virus is assumed to have originated in central Africa as long ago as 10 000 years. There has been a general agreement between the phylogenetic clustering of flaviviruses and classification on the basis of serological relationships. Viruses such as the cell-fusing agent (CFA) and Tamana bat virus display homology that is distant enough to suggest a possible link among the three genera, but exact relationships remain unclear. According to this scheme, tick-borne viruses diverge first, followed by the mosquito-borne viruses. Yellow fever virus is representative of so-called Old World mosquito-borne (species *Aedes*) viruses. Additional clades, such as the dengue viruses and those of the JEV antigenic complex, vectored primarily by *Culex* mosquitoes, are believed to have diverged subsequently.

Epidemiology and Clinical Disease

The epidemiologic situation for flaviviruses involves both the appearance of new viruses, with several described in recent years, as well the emergence and re-emergence of established viruses. A number of contributing factors, such as human activities (urbanization, transportation, and land-use practices) and also natural phenomena (bird migration, host–vector relationships, and climate change), drive this process. Flaviviruses exhibit both global and regional distribution based on these various factors. Dengue has become the most significant cause of viral hemorrhagic fever globally, with a dramatic increase in prevalence of dengue involving up to 2.5 billion persons at risk and an annual infection rate of 50 million cases. JEV is regarded as the most important cause of epidemic viral encephalitis worldwide, causing up to 50 000 cases annually, and has spread from Southeast Asia to the south and west in recent years. West Nile virus, originally recognized as a cause of febrile illness primarily in Africa, the Middle East, and India, caused outbreaks of severe encephalitis in Eastern Europe and the former Soviet Union in the 1990s prior to its due appearance in the US in 1999. Since 1999, it has become distributed across North America and parts of Central and South America. In North America, it has caused annual outbreaks of encephalitic disease involving thousands of cases. Yellow fever is currently restricted to tropical Africa and South America, where it regularly causes endemic and periodic epidemic disease involving thousands of cases on a regular basis. The true incidence is believed to be underreported and may be as high as 200 000 cases per year, mainly in Africa.

Most flaviviruses are zoonotic viruses maintained in enzootic transmission cycles which depend on birds, rodents, or nonhuman primates as reservoirs, with humans usually dead-end hosts. However, dengue viruses are exceptions, due to their adaptation to humans and maintenance in urban-based areas in transmission cycles that do not require animal reservoirs as they seem to exist in sylvatic cycles in Africa and Asia.

Structure and Replication

The structure and replication of the flaviviruses have served for many years as a model for the entire family, since the first report of the genomic sequence for yellow fever virus in 1985. The use of molecular clones, together with the ease of propagating most of these viruses in cell lines *in vitro*, and the advent of modern expression systems for production and purification of viral proteins, have facilitated progress for understanding the molecular biology of the family. A number of different molecules in different cell types have been characterized as candidate receptors, including glycosaminoglycans. The best characterized receptor interaction is between dengue virus and dendritic cell (DC)-specific intercellular adhesion molecule 3 (ICAM-3)-grabbing nonintegrin (DC-SIGN), present on human dendritic cells. Fc and complement receptors can also mediate entry of the dengue viruses and several other viruses through the process of antibody-dependent enhancement occurring in the presence of subneutralizing antibody levels.

The virions contain a lipid envelope, have a buoyant density of 1.19–1.3 g ml^{-1}, and are sensitive to inactivation by heat, acid pH, organic solvents, and detergents. Currently the virion structure is best understood for dengue viruses and TBEVs. Dengue viruses, as reconstructed from cryoelectron microscopy (cryo-EM) images at 24 Å resolution, exist as a smooth particle comprised of dimeric units of the envelope (E) protein arranged in a head-to-tail fashion on its surface with icosahedral symmetry, encasing a nucleocapsid composed of capsid protein and genomic RNA (**Figure 1**). These genomes are on the order of 11 kbp (42 S sedimentation) and differ from other flavivirus genera in having a 5′ cap. The 5′ untranslated region (UTR) encodes a unique secondary RNA structure. The 3′ UTR contains conserved sequences and secondary structures, including a unique terminal stem–loop structure required for viral RNA replication (**Figure 2**). Such structures are found among both mosquito and tick-borne viruses despite differences in nucleotide sequence, arguing for conserved mechanisms relating to viral replication.

The flavivirus polyprotein undergoes co- and post-translational processing to produce the mature viral proteins. Structural proteins (capsid, prM (precursor to M), and E (envelope)) are generated by cleavage by host signalase in association with membranes of the endoplasmic reticulum, whereas nonstructural proteins (NS1, -2A, -2B, -3, -4A, -4B, and -5) are generated primarily by cleavage reactions mediated by the viral NS3 serine protease together with its NS2B cofactor. NS1 is a soluble,

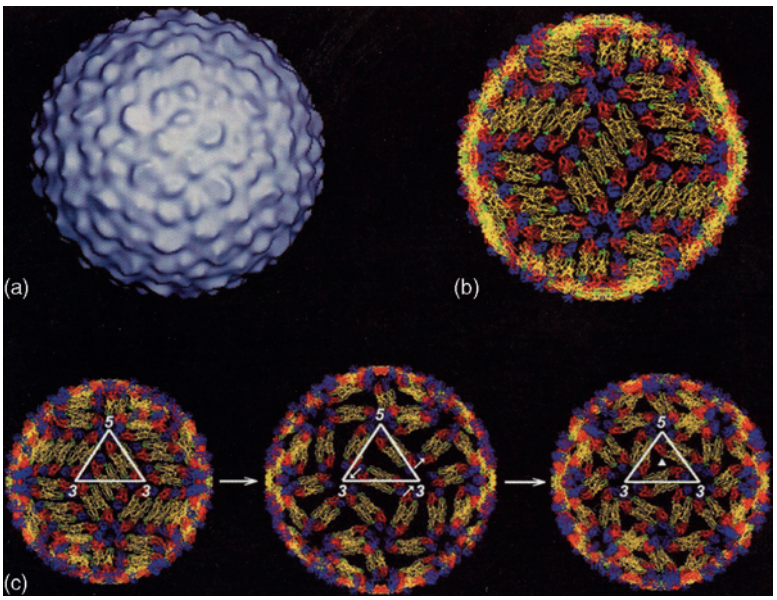


Figure 1 Structure of the dengue-2 virion as determined by cryo-EM. (a) An image reconstruction model of the virion in which the smooth surface is shown. (b) E protein dimers are displayed in the 'herringbone' arrangement, as fitted into the electron density map. (c) Acid-catalyzed rearrangement of the E protein dimers into the fusion-active state. Triangle represents the icosahedral asymmetric unit with three- and fivefold symmetry axes shown. The small arrows indicate the rotation of the E protein according to the proposed model. Reproduced from Lindenbach BD and Rice CM (2003) Molecular biology of the flaviviruses. *Advances in Virus Research* 59: 23–61, with permission from Elsevier, and adapted from Kuhn RJ, Zhang W, Rossmann MG, *et al.* (2002) Structure of dengue virus: Implications for flavivirus organization, maturation, and fusion. *Cell* 8: 717–725.

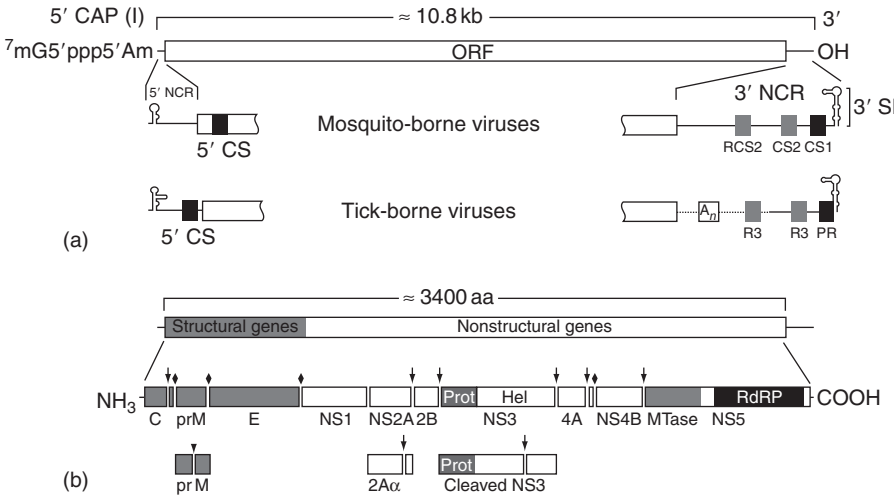


Figure 2 Organization and expression of the flavivirus genome. (a) RNA elements of the 5′ and 3′ noncoding regions (NCRs) flanking the open reading frame (ORF) for mosquito- and tick-borne viruses are indicated. The 5′ CAP structure and 5′ and 3′ stem–loop (SL) structure are shown. Conserved sequence elements for mosquito- and tick-borne viruses are denoted by CS, RCS, and CS, R, PR, respectively. A_n indicates a polyadenylate sequence contained within the variable region (dotted line) of some tick-borne isolates. (b) Expression and processing of the polyprotein. Cleavage events mediated by host signalase and furin-like enzyme are depicted by solid diamonds and triangle, respectively. Question mark indicates processing of NS1-2A by an unknown host enzyme. Downward arrows indicate cleaveage by the viral NS3 protease (PROT). HEL, helicase; MTase, methyltransferase. Reproduced from Lindenbach BD and Rice CM (2003) Molecular biology of the flaviviruses. *Advances in Virus Research* 59: 23–61, with permission from Elsevier.

cell surface, and intracellular protein which is involved in viral RNA synthesis, together with NS3 (protease, helicase, and NTPase activities), NS5 (methyltransferase and RdRP activities), and the hydrophobic proteins of the NS2 and NS4 regions. Viral replication occurs in association with intracellular membranes that are reorganized into packets of vesicles, convoluted membranes, and paracrystalline arrays. The capsid protein is responsible for

formation of the viral nucleocapsid. During subsequent virus maturation, prM acts as a molecular chaperone for the E protein, and undergoes a furin-like cleavage to form the fusion-competent heterodimer prM–E complex. The E protein is the major virion surface protein that harbors receptor binding and fusion activity. Structural characterization of the TBEV E protein has revealed that this protein resembles the E1 protein of the alphaviruses, both of which are designated as 'class II' viral fusion proteins, distinguished from the class I proteins typified by the influenza hemagglutin by major differences in overall fold, and a flat elongated orientation on the virion surface rather than an extended spike.

Pathogenesis

Flaviviruses are readily propagated in a variety of cell lines in culture, including cells of mammalian origin, in which cytopathic effects and plaque formation are common, as well as in mosquito-derived cells. Many of these infected cell types exhibit cell fusion and syncytia formation and are capable of supporting persistent infection. *In vivo*, flaviviruses cause syndromes in humans ranging from febrile illness to acute meningoencephalitis and hemorrhagic fever. Flaviviruses are inherently neurotropic viruses, possibly reflecting the propensity of viruses of the early phylogenetic branches to propagate in the central nervous system of arthropod vectors, and in turn, in vertebrate hosts, in which disease manifestations occur. Arthropod vectors undergo chronic infections that serve to deliver virus into enzootic and zoonotic transmission cycles that lead to infections of humans and animals. Whereas many of the encephalitic flaviviruses which typically depend on avian species as reservoirs and for amplification cycles, dengue and yellow fever viruses have adapted to primate hosts, and cause hemorrhagic fever as a primary disease manifestation. Nonhuman primates have been used for characterization of yellow fever and dengue viral infections and for evaluation of experimental vaccines for dengue viruses. Most flaviruses are pathogenic for laboratory rodents, particularly mice, which have been used as models for experimental studies of acute flavivirus encephalitis.

Flavivirus resistance is a phenomenon observed in certain inbred strains of mice, in which markedly reduced susceptibility to encephalitic strains, such as of WNV, have been observed. The genetic basis of this resistance has been mapped to a single autosomal dominant locus which encodes the Oas1B gene. It is not known yet if there is an analogous resistance mechanism that operates in humans to explain variability in susceptibility to these viruses. A recent study has also suggested a link between CCR5D32 (a common mutant allele of the chemokine and human immunodeficiency virus (HIV) receptor CCR5) and fatal WNV infection.

Flaviviruses are transmitted via feeding by chronically infected arthropod vectors. Following local replication at the site of inoculation and in regional lymph nodes, virus disseminates by viremia, with distribution to target organs in the periphery, including primarily visceral organs in the case of hemorrhagic fever viruses, and meninges, brain, and spinal cord in the case of encephalitic agents. Skin Langerhans (dendritic) cells are implicated as sites of initial virus replication as well as early spread of virus to lymphatic tissues. Monocytes and macrophages are also major targets of infection by dengue viruses. Flaviviruses also interact with many other cell types *in vivo* and elicit changes in cell surface molecules that are believed to influence pathogenesis of encephalitis and hemorrhagic fevers. Upregulation of surface molecules such as major histocompatibility complex (MHC) I and ICAM-1 has been observed.

Pathogenesis of both hemorrhagic fever due to yellow fever virus and acute encephalitis due to JEV complex and TBEV complex members are generally associated with cytopathic effects of the virus in target organs, although many viruses have been shown to induce apoptotic cell death as well. Flavivirus infections of the central nervous system, as typified by members of JEV complex or TBEV complex viruses, range from mild meningitis to severe destructive encephalitis, in which neurons of the brain and spinal cord are targeted. Mortality rates are as high as 40% and permanent neurologic effects may occur in survivors. Persistent and congenital infections have been described for some of the encephalitic viruses. Primary infection with dengue viruses either is generally characterized by flu-like illness or is asymptomatic. However, some secondary infections with heterologous serotypes result in a distinct form of hemorrhagic disease. This involves an immunopathogenic process of antibody-dependent enhancement of infection mediated by heterologous antibodies, in concert with activation of cross-reactive memory T-cells, cytokine release, and diffuse capillary leakage. This is referred to as dengue hemorrhagic fever and may be accompanied by a shock syndrome. Yellow fever causes a unique form of acute hemorrhagic fever with characteristic cytopathology in the liver and kidneys, and is fatal in up to 50% of severe cases.

Diagnosis and Prevention

Because flaviviruses may cause many different disease syndromes, disease manifestations are variable. Although diagnosis is often suspected on the basis of clinical findings, case history, and geographical distribution, a range of laboratory tests are available for determining infection with specific viruses, including virus isolation, serologic techniques (enzyme immunoassay, hemagglutination inhibition, immunofluorescence, complement fixation, dot blot immunoassays, and plaque neutralization), antigen

detection, and reverse transcriptase/polymerase chain reaction (RT/PCR) amplification. Detection of immunoglobulin M (IgM) by immunoassay is a common diagnostic test for flaviviruses, but in some cases persistence of high IgM levels from previous infections with other flaviviruses can complicate the diagnosis of recent infections. Detection of IgM antibody in cerebrospinal fluid is of use in cases of flavivirus encephalitis. RT/PCR of serum is a sensitive and rapid technique that has increasing application for flaviviruses, but must be carried out during the viremic stage, which can differ from one flavivirus infection to another. For instance, RT/PCR for dengue viruses loses sensitivity after the first week of infection due to clearance of viremia.

Outside of mosquito control measures, vaccination is the only available means of preventing flavivirus infections in humans. The live-attenuated yellow fever 17D vaccine has been used since its original development in 1936 for prevention of yellow fever and is one of the safest and most effective vaccines ever developed for human use. Inactivated vaccines are available for prevention of diseases caused by JEV and TBEV. Development of vaccines for dengue viruses and WNVs have been pursued because of the increasing public health burdens associated with these agents, but are still in investigational stages. At this time, there are no antiviral agents or other therapies licensed for treatment of flavivirus infections.

Pestiviruses

History and Classification

The genus *Pestivirus* includes four accepted species that are important causes of veterinary disease in farm animals which are of great economic consequence worldwide: *Classical swine fever virus*, *Bovine viral diarrhea virus 1* and *2*, and *Border disease virus*. The host range of pestiviruses is restricted to 'artiodactyles' (cloven-hoofed ruminants and pigs). Classical swine fever virus (CSFV) infects members of the Suidae as the natural hosts. Bovine viral diarrhea virus (BVDV) is a pathogen primarily of cattle. Classical swine fever was characterized (e.g., 'hog cholera') in the 1800s, but the viral cause not established until *c.* 1904. The diseases caused by BVDV in cattle were described in the 1940s and 1950s. Border disease virus (BDV) was discovered in 1959 in the border region of England and Wales, as a congenital viral infection affecting sheep and goats. Pestiviruses exhibit great genetic diversity, and characterization of new isolates, including those from wildlife species, about which understanding is limited, will have future impact on the classification of viruses of this genus.

Prior to use of genomic sequencing, pestiviruses were differentiated by host species of origin, and antigenic cross-reactivity prevented assignments to different serogroups, even though differences among groups could be demonstrated with monoclonal antibodies. Comparison of genomic sequences later revealed the existence of two genotypes of bovine viral diarrhea virus (BVDV-1 and BVDV-2), which became significant after it was recognized during the 1980s that BVDV-2 strains could cause a severe acute hemorrhagic syndrome, with some of the first isolations of BVDV-2 arising during BVDV1 vaccine breaks. The two genotypes are distinguishable antigenically, but not to a level which allows classification into different serotypes. Subtypes (BVDV-1a, -1b, -2a, -2b) also exist. BVDVs also display pronounced heterogeneity in phenotype (e.g., biotype and virulence type). Biotypes (cytopathic vs. noncytopathic) are defined on the basis of cytopathic effects in cultured epithelial cells. However, virulence phenotype *in vivo* correlates with the noncytopathic rather than the cytopathic biotype.

The authentic form of BDV is predominantly an ovine pathogen, although pigs are also affected. Three major genotypes have recently been identified within the BDV species, referred to as BDV-1, BDV-2, and BDV-3.

Epidemiology and Clinical Disease

BVDVs cause infections throughout the world. In the US, both BVDV-1a and BVDV-1b isolates are common, with BVDV-1b more prevalent. The prevalence of BVDV-2 appears to be greater in North America than elsewhere, and is relatively rare in Europe and Asia. However, a number of isolates have been made in South America. BVDV-2a isolates also are more predominant than BVDV-2b, which are rare. BVDV is endemic in many cattle populations, with maximum rates of persistent infection sometimes reaching 1–2%, and seropositivity as high as 65%. BVDVs also cross species barriers among animal hosts, with or without evidence of disease.

Ruminant pestiviruses frequently cause persistent infections in cattle and sheep, which serve as reservoirs for maintenance of virus, resulting in transmission from asymptomatic viremic animals to naive animals upon introduction into new herds; transmission by acutely infected animals can also occur. Contact with waste products as well as with contaminated animal feed and farm equipment may also spread infection. Animals acquire pestiviruses through the nasal and oropharyngeal tracts. Direct contact is the most efficient means of transmission. Other transmission routes may apply, as in the case of closed, nonpasturing herds which sometimes develop BVDV infections. A number of factors including the density of cattle populations and regional practices of cattle trading affect the introduction of BVDV into naive herds.

Manifestations of illnesses caused by BVDV are diverse, ranging from mild transient infections to severe acute disease involving the enteric, respiratory, and hematologic systems. Postnatally acquired infections may cause fever, diarrhea, and cough associated with virus shedding, but

seroconversion and clearance of infection leads to lifelong immunity. Persistent infections (PIs) occur after virus crosses the placenta in nonimmune animals to cause intrauterine infection early in fetal life. PI animals continuously excrete large amounts of virus and remain BVDV-antibody-negative due to immune tolerance. These animals may develop mucosal disease (MD), a fatal disease with profuse bloody diarrhea due to extensive ulceration of the gastrointestinal tract.

CSFV is the cause of a highly contagious and serious disease of pigs, and is of worldwide importance. Classical swine fever (CSF) is endemic in large portions of Asia, and has been reported in parts of Central and South America. CSF has been progressively eradicated from Western Europe, but periodic reintroduction has occurred. Eradication efforts have also generally led to control of CSF in North America and Australia. CSF can also be spread through pig products, with swill-feeding of pigs a particularly efficient means of transmission, in many regions now outlawed. Spread by semen of infected boars can also occur. CSF is an acute illness with significant mortality, but has become less common now than milder chronic forms of CSF and persistent infections following intrauterine transmission. The latter can result in stillbirth or yield piglets which develop late-onset disease.

BDV is an economically important cause of congenital infection in sheep and goats with a probable worldwide distribution; prevalence in sheep may be up to 50%. Vertical transmission results principally in abortion and stillbirth. Surviving animals are small and weak, and exhibit tremor and hairy fleece ('hairy shaker syndrome'). Infections are spread by these persistently infected animals, and can cause acute postnatal infections. Border disease can also be caused by bovine viral diarrhea disease types.

Structure and Replication

Pestivirus virions are 40–60 nm in size, contain three glycoproteins in the virion envelope complexed as disulfide-linked homo- and heterodimers, and have a buoyant density of 1.134 g ml^{-1} in sucrose. They are sensitive to heat, organic solvents, and detergents, but in contrast to flaviviruses, BVDV and CSFV are highly stable in acidic environments, although virus entry is through a low-pH endosome compartment. CD46 has been identified as a receptor for BVDV; however, glycosaminoglycans, such as heparan sulfate, may act as a receptor for cell culture-adapted BVDV and CSFV. It is believed that pestiviruses invade host cells after binding by envelope glycoproteins E^{rns} and E2. Low-density-lipoprotein receptor has also been proposed as a BVDV receptor.

The pestivirus genome is *c.* 12.3 kbp, with variant genomes of larger and smaller size generated by recombination events (**Figure 3**). Pestivirus genomes lack a 5′ cap structure, and translation is mediated by a 5′ internal ribosome entry site (IRES) element that has similarities to the hepatitis C virus (HCV) IRES. The 3′ UTR contains a short polyC tract, as well as variable and conserved regions, the latter of which encodes hairpin structures. The open reading frame codes for 4000 amino acids, and is processed into at least 12 mature proteins: N^{pro}, C, E^{rns}, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B. N^{pro} is a unique N-terminal leader autoprotease, which is followed by the four structural proteins C, E^{rns}, E1, and E2. E^{rns}, which is not found in other viruses of the family, is a heavily glycosylated protein that is secreted from cells and exhibits a ribonuclease activity and a lymphocytotoxic activity that is involved in pathogenesis. E1 and E2 are integral membrane proteins that form the heterodimeric complex which mediates virus entry. Antibodies

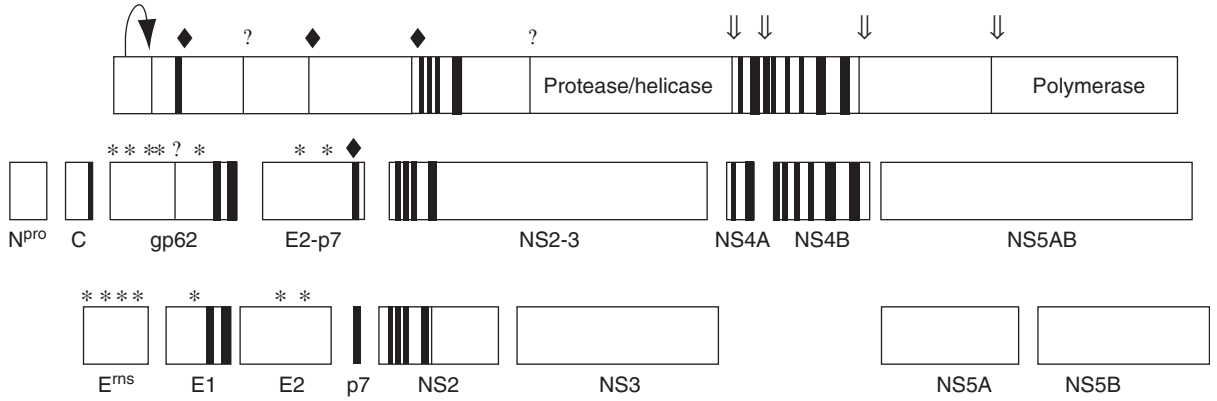


Figure 3 Pestivirus genome organization and processing, based on the cytopathic NADL strain of BVDV. Open boxes indicate mature proteins, with shaded regions representing stretches of hydrophobic amino acids. Diamonds and question marks indicate proteolytic processing by signalase and an unknown host enzyme, respectively. Downward arrows mark cleavages by the viral serine protease. The autoproteolytic cleavage by N^{pro} is shown as a curved arrow. Asterisks indicate proteins which undergo glycosylation. Reproduced from Lindenbach BD and Rice CM (2001) *Flaviviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., vol. 1. Baltimore, MD: Lippincott Williams and Wilkins, with permission.

directed against E^{rns} and E2 confer protective immunity. The p7 protein serves as a membrane leader sequence for NS2. NS2, together with the N-terminus of NS3, is an autoprotease with specificity for the NS2–NS3 junction. This cleavage is necessary for RNA replication and pathogenesis of BVDV disease, but, paradoxically, the uncleaved NS2–NS3 precursor is required for virion formation. NS3 also has multiple functions, including protease (together with the NS4A cofactor), helicase, and nucleoside triphosphatase activities. By analogy with the hepaciviruses, NS4B is involved in cellular membrane reorganization and scaffolding for the replicase complex, which includes NS5B (RdRp). NS5A is a large, hydrophilic phosphorylated zinc metalloprotein analogous to the hepacivirus NS5A, and likely to function in the regulation of viral RNA synthesis.

Pathogenesis

Growth of pestiviruses in cell culture generates virus yields that are not robust, due to intracellular sequestration of the viral progeny and association with serum components that also cause difficulties with virus purification. Whereas susceptible cell culture systems are mainly derived from cells from the natural host species, lack of cytopathogenicity (e.g., 'noncp' biotypes) is common, in contrast to the cytopathic variants ('cp' biotypes), which arise spontaneously during infections in animal hosts.

In vivo, pestiviruses cause acute and persistent infections. BVDV causes a complex pathogenesis involving broad tissue tropism, and a propensity for high cytopathogenicity through mechanisms that remain poorly defined. There is evidence that cytopathic strains cause cell death by activating apoptosis through a double-stranded RNA (dsRNA)-dependent pathway, as well as by mechanisms related to oxidative stress. This process may underlie the development of MD associated with superinfection by a cytopathic strain.

Infection of cows by noncytopathic BVDV during the first 4 months of pregnancy can lead to birth of persistently infected calves immunologically tolerant to BVDV. Subsequently, the calves develop MD after superinfection by cp BVDV. Cytopathic strains associated with such cases are generated by mutations occurring in the genomes of noncytopathic strains, and require the production of NS3 which is cleaved from its NS2-3 precursor. This phenomenon is often a consequence of recombination events which introduce sequences allowing cleavage by host ubiquitin C-terminal hydrolase, or in other cases, by the N^{pro} autoprotease itself. Point mutations can also result in cytopathic variants. Production of NS3 is believed to be the basis for both BVDV cytopathogenicity in cell culture and pathogenesis of MD in immunologically tolerant PI calves.

Following inoculation of the mucosal surface, BVDV gains access to and replicates in lymphatic tissue, in many cases associated with leukopenia. Viremia has been experimentally demonstrated within 2–4 days. In severe cases, as in MD supervening in persistently infected calves, there is disseminated infection, mucosal ulceration, and hemorrhagic disease associated with platelet deficiency and dysfunction. Persistent viral infection is typically found in lymphoid tissue of the intestine, circulating mononuclear cells, and neurons in the central nervous system.

CSF is a disseminated infection that in its most severe form involves extensive thrombosis, endothelial damage, and severe thrombocytopenia resulting in hemorrhagic disease, necrosis, and multiorgan dysfunction. Viral infection of lymphoid tissue causes depletion of lymphocytes and germinal follicles and induces an immunosuppressive state with delayed appearance of CSF-specific antibodies. Superinfection with other pathogens can complicate the disease course.

BDV typically causes a less inflammatory disease, although a variant type of disease with necrosis of central nervous system has been described. Persistent infection of newborn lambs commonly involves widespread lymphoid infection and teratogenic lesions in multiple organs, with endocrine and neurologic abnormalities.

Diagnosis, Control, and Prevention

In the context of control programs for BVDV, diagnostic assays are employed for both surveillance at the herd and population levels, and also at the level of individual animals for identification of persistently infected animals that require elimination. Tests to determine immunity (antibody in serum or milk) as well as presence of virus are commonly done. Detection of antibodies by virus neutralization test or by antibody ELISAs are both used for diagnosis. Presence of virus can be demonstrated by virus isolation, antigen detection, or RT-PCR. Use of these assays depends on a variety of considerations, including age of animals to be tested, the epidemiological situation, and vaccination history.

Laboratory confirmation of CSFV infection has traditionally depended on virus isolation and detection of antigen in organ tissue, although more rapid techniques, such as antigen ELISA and RT-PCR, have been developed. Early diagnosis of infection based on antibody detection is limited by the slow appearance of virus-specific antibodies and lack of specificity of some antibody ELISA tests.

Vaccines have been developed against both CSFV and BVDV. However, control programs based on detection and eradication of persistently infected animals, as well as on quarantining and maintaining closed herds, have been employed to reduce the economic losses caused by these viruses. National eradication campaigns conducted in Scandinavia in the 1990s were particularly successful, facilitated by development of ELISA for detection of

BVDV antibodies. Due to the high effectiveness of these programs, the threat of reintroduction of virus into uninfected herds became apparent, and vaccination was recommended in some regions as a prophylactic measure against a resurgence of infections. Although some questions remain about the duration of protection and its efficacy in preventing fetal infection, newer vaccine regimens combined with comprehensive BVDV control programs are probably the best approach to lowering the incidence of PI in calves and reducing the problem of BVDV outbreaks. Commercial BVDV vaccines include either inactivated or modified live vaccines. Although there are some data to indicate that both antibody and cellular immune mechanisms are important for protection against BVDV infection, more studies are needed to define the protective mechanisms associated with these vaccines.

Live-attenuated vaccines for CSF have been available since the 1960s, but are not universally employed because of interference with traditional serologic methods to demonstrate absence of disease. Stamping-out policies are therefore employed as control efforts. Research into the use of marker vaccines, which do not exhibit interference in detection of infection, is ongoing, together with modeling studies to assess the impact of vaccine and eradication policies on control of disease.

Hepaciviruses

History and Classification

After serologic screening tests for the detection of hepatitis B and hepatitis A viruses were introduced in the 1970s to eliminate these viruses from human blood supplies, the role of HCVs in causing up to 90% of transfusion-associated hepatitis was recognized. The term hepatitis C virus (HCV) was coined in 1989 after identification of an RNA genome of this virus from cDNA libraries of human serum containing the infectious agent. In humans, infection with HCV commonly evolves into a chronic persistent hepatitis which can lead to progressive hepatic cirrhosis and hepatocellular carcinoma. Lack of a suitable cell culture system for propagating HCV had until only recently been a major impediment to progress in understanding the details of its replication. In addition, studies of HCV pathogenesis have been hampered by the lack of animal model systems, and progress has depended on use of clinical data from human cases, or from the use of a chimpanzee model.

Hepaciviruses display great genetic heterogeneity on a worldwide basis and are classified into six genotypes based on nucleotide sequence similarity, and further classified into multiple subtypes (identified by lowercase letters: e.g., 1a, 1b, 2a) as well as quasispecies for a given viral isolate. These divisions correspond to sequence divergence rates of *c.* 30–35%, 20–25%, and 1–10%, respectively. This level of genetic diversity has implications for the epidemiology of HCV transmission, clinical manifestations of liver disease caused by HCV, response to treatment, and development of a prophylactic vaccine.

Epidemiology and Clinical Disease

It is estimated that there are at least 170 million chronically infected HCV subjects worldwide, with prevalence varying from 0.1% to as high as 18% (Egypt). In areas where HCV has been eliminated from blood supplies by donor screening, use of illicit injectable drugs remains the most important mode of transmission. Various other forms of exposure, including occupational and sexual exposures, are involved in some cases of transmission. HCV is a primary cause of cirrhosis, a significant cause of hepatocellular carcinoma, and in the United States is the leading reason for liver transplantation. There is great variability in the spectrum of disease. Acceleration of disease is observed in persons who are older at the time of infection, and in the setting of continuous alcohol exposure, and co-infection with HIV or hepatitis B virus (HBV). Individuals who sustain infection at a younger age tend to have slower progression.

Genotypes 1, 2, and 3 are found worldwide, but with regional differences with respect to distribution. Genotypes 1a and 1b are predominant in the United States and Europe, 2a and 2b are relatively common in North America and Europe, and type 3a common in the United States and Europe among intravenous drug abusers. Genotype 4 is notable in the Middle East/North Africa region, whereas 5 is found in South Africa, and 6 in Hong Kong. Additional genotypes (e.g., 7–11) have been identified but have been proposed as variants of genotype 6. Genotypes are useful in establishing genetic markers of HCV in epidemiologic investigations and for determining modes of transmission. Differences in terms of disease potential and treatment response among genotypes also have been described, such as the relative resistance of genotype 1 to interferon alpha (IFN-α) therapy. This genotype has been associated with more aggressive liver disease as well, but it is unclear if this results from a longer duration of infection among people infected with this genotype. Genotypic differences may affect usefulness of diagnostic immunoassays for HCV antibody which are based on various portions of the HCV genome.

Structure and Replication

In contrast to those of flaviviruses and pestiviruses, hepacivirus virions have been difficult to visualize, and exhibit larger size and lower buoyant density (1.03–1.1 $g\,ml^{-1}$) as a result of association with immunoglobulins and low-density lipoproteins. HCV genomes contain a 9.6 kb

RNA which differs in some structural features from flaviviruses and pestiviruses (**Figure 4**). The 5′ UTR is the most highly conserved region of the genome and contains an IRES which mediates cap-independent translation of viral RNA. The structure of the IRES has been resolved, including studies of complexes with the ribosomal 40S subunit and the human translation factor eIF3. These efforts may facilitate the design of small molecules capable of inhibiting this critical viral factor. Because of its high sequence conservation, the 5′ UTR has also been used for development of sensitive detection assays for HCV infection. The 3′ UTR exhibits a tripartite structure, containing several unique elements, including a short variable region, a poly(U/UC) tract, and a highly conserved RNA sequence (the X-tail). A unique RNA structure, the *cis*-acting replication element (CRE) within the NS5 region, has also been identified.

The HCV polyprotein (*c.* 3000 amino acids) is processed by cellular (signalase) and viral proteases into mature viral proteins, consisting of the core, E1 and E2, the hydrophobic p7 peptide, and six nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B). Unique properties of several of these proteins have been characterized. The RNA-binding core protein has been associated with a range of effects on host cells, including transactivation, but the relevance to HCV pathogenesis *in vivo* is unknown. Alternative forms of core, produced by frameshifting, have been described. The E1 and E2 proteins form the heterodimeric complex of the virion that serves receptor binding, membrane fusion, and entry. These proteins exhibit the highest mutation rate at both the nucleotide and amino acid level. E2 in particular contains a hypervariable region (HVR-1) which undergoes an extremely high mutation rate and, because antibody epitopes are found within this region, it is believed that the mutations provide a mechanism for HCV to escape antibody responses involved in controlling virus infection. The small membrane-associated p7 protein forms an amantadine-sensitive ion channel which may function in the secretory pathway during virus maturation.

HCV encodes two known proteases involved in polyprotein processing, the NS2-3 autoprotease, and the NS3 serine protease, that with its NS4A cofactor mediates cleavage of the remainder of the nonstructural region. The structure of the NS3-4A protease has been solved, promoting interest in a new class of antiviral agents that target its protease activity. NS3 can also cleave adaptor proteins (Toll-like receptor-3 adaptor TRIF (TICAM-1)

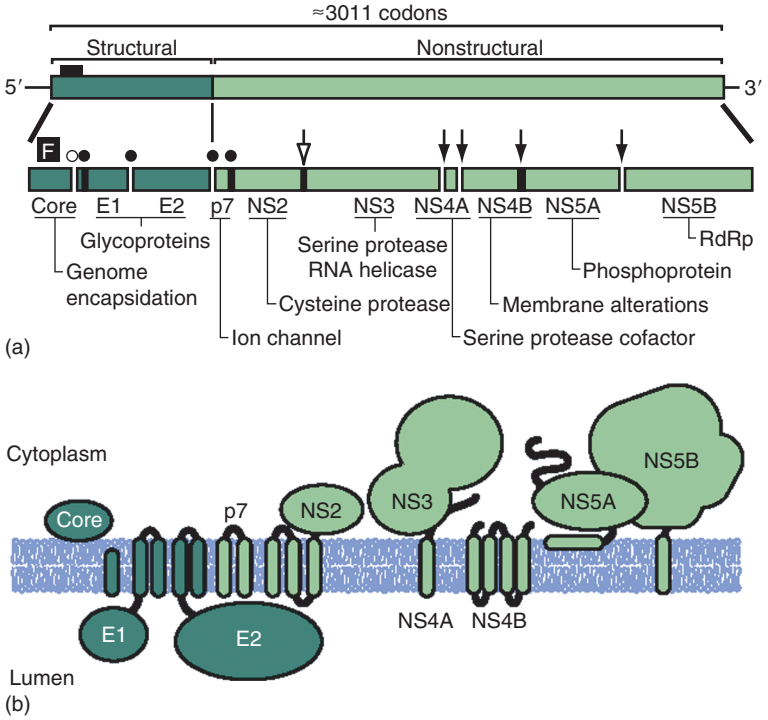


Figure 4 HCV genome organization and processing. (a) The HCV genome and polyprotein encoding structural and nonstructural genes and known functions. Closed and open circles indicate signal peptidase cleavage sites and signal peptide peptidase cleavage sites, respectively. F represents an alternative open reading frame protein produced by ribosomal frameshifting within the core region. Open and closed arrows indicate cleavage by the NS2 autoprotease and the NS3/4A serine protease, respectively. (b) Proposed topology of the HCV proteins with respect to the intracellular membrane. Reprinted by permission from Macmillan Publishers Ltd; *Nature* (Lindenbach BD and Rice CM (2005) Unravelling hepatitis C virus replication from genome to function. *Nature* 436(7053): 933–938), Copyright (2005).

and Cardiff (IPS-1, VISA, or MAVS)) required for activation of interferon regulatory factor 3, indicating a role for NS3 in downregulating the dsRNA signaling pathway that leads to induction of interferon beta and other genes involved in antiviral defense.

The NS4B protein is a mediator of the reorganization of intracellular membranes that together with other viral and host proteins generates the 'membranous web' scaffold for formation of RNA replication complexes. NS5A is a multidomain protein containing an RNA-binding activity. Differential phosphorylation of NS5A appears to have a role in regulation of HCV RNA synthesis, as a molecular switch for critical steps in the transition from RNA synthesis to virus packaging. NS5A also interacts with interferon-induced dsRNA-activated protein kinase PKR. Mutations in the domain of NS5A which affect this interaction (interferon sensitivity-determining region (ISDR) have been reported to influence the response of HCV to interferon alpha. The NS5B protein contains the RdRp activity that forms the core of the replication complex, and together with other NS3, NS5A, and cellular factors, synthesizes the genome-length plus- and minus-strand RNAs involved in the replication of the virus. NTPase and helicase activities participating in this process are encoded in the NS3 protein. Cellular proteins that have been implicated in function of the replication complex by interacting with NS5A and NS5B include VAP-A and -B (vesicle-associated membrane protein-associated proteins A and B), geranylgeranylated protein FBL-2, and cyclophilin B. These proteins appear to affect the assembly and function of the replication through such interactions such as membrane localization and RNA binding.

Recent progress in understanding HCV genome replication has been achieved through the use of HCV replicon systems. Initially established for genotype 1b, these were subgenomic constructs that encoded only the 5′ and 3′ structures and the nonstructural proteins required for viral RNA replication. Studies with additional iterations of replicons, including those for other genotypes, as well as full-length versions that exhibit efficient replicative functions *in vitro*, are excellent tools for studying the virus life cycle, in the absence of robust propagation of infectious HCV viruses in cultured cells. Crystal structures of several HCV proteins harboring key enzymatic functions, including NS3 protease, helicase, NS5A, and NS5B are known and are being applied to the development of HCV-specific antiviral agents.

The exact cell entry process for HCV has not been definitively characterized. It is believed that CD81, a tetraspanin family protein widely found on cell substrates, is involved in HCV entry, but requirements for other cell surface molecules on hepatocytes probably exist. Other proposed receptors include the LDL receptor, the class B type I scavenger receptor, mannose-binding lectins DC-SIGN and L-SIGN, heparan sulfate proteoglycans, and the asialoglycoprotein receptor. Further studies are needed to demonstrate the role of such molecules in HCV infections, and will be facilitated with new model systems such as retrovirus pseudoparticles that express HCV glycoproteins and exhibit low-pH-dependent infectivity for hepatic cell lines.

Pathogenesis and Clinical Disease

HCV causes acute and chronic forms of viral hepatitis. Acute hepatitis resembles that caused by other agents of viral hepatitis, but overt symptoms are not as frequent, despite biochemical evidence of liver disease in most subjects and appearance of HCV RNA in the serum. HCV causes persistent infection in most infected individuals, who then have chronic hepatitis throughout their lives in the absence of treatment and who remain at risk for cirrhosis and hepatocellular carcinoma. The infection is often diagnosed by screening of asymptomatic blood donors, or by the appearance of either chronic fatigue or features otherwise typical of viral hepatitis. Such individuals have persistent viremia and evidence of chronic inflammatory disease on liver biopsy.

Currently HCV is believed to cause persistent infection because primary and secondary immune responses are inadequate in breadth and magnitude to control the infection. Underlying mechanisms include the inherent difficulty in optimizing an immune response within the liver, and HCV-specific factors which suppress the host immune response, including effects of the core protein on host gene expression, inhibition of the interferon-induced protein kinase PKR by E2 and NS5A proteins, and NS3 inhibition of the dsRNA signaling pathway. The high genetic variability of HCV results in viral variants that may contribute to virus persistence through escape from antibody and from cytotoxic lymphocyte responses directed at specific epitopes.

The mechanism of liver injury associated with persistent HCV infection is believed to be immune-mediated and initiated by virus-specific T-lymphocytes which infiltrate the liver. Such cells are predominantly of a T_H-1 phenotype and produce interferon gamma and tumor necrosis factor alpha (TNF-α). Because these cytokines have been implicated in noncytolytic clearance of other viral agents in the liver, other mechanisms of hepatocyte injury and death associated with HCV have been proposed, including Fas-Fas ligand-mediated apoptosis, perforin-dependent killing by cytolytic T-cells, or TNF-α itself. HCV is not highly cytopathic for hepatocytes; however, fat deposits and eosinophilic inclusions are often seen in liver cells. In the absence of virus clearance, repeated episodes of hepatocellular injury and regeneration trigger development of hepatic fibrosis, cirrhosis, and eventual hepatocellular carcinoma. Additional factors that promote this process include coexistent infection with

other agents of viral hepatitis, alcohol intake, and iron overload. Extrahepatic manifestations can also occur in association with HCV infection, typically manifesting as skin and renal disease caused by a systemic vasculitis involving immune complexes with viral proteins.

Diagnosis and Treatment

Diagnosis of chronic hepatitis C infection is established by screening enzyme immunoassay to detect circulating antibodies to HCV proteins, followed by confirmatory determination of HCV RNA in serum, using qualitative and quantitative tests based on target (RT-PCR and transcription-mediated amplification) and signal amplification (branched DNA (bDNA)) techniques. Quantitation of serum HCV RNA helps to identify patients likely to benefit from treatment and undergo virus clearance as defined by a sustained viral response (SVR). Determination of the HCV genotype guides treatment decisions, as genotypes 1 and 4–6 are less amenable to treatment than are genotypes 2 or 3. Therapeutic trials have shown that combinations of interferons and ribavirin are more effective than is interferon alone and pegylated (long-acting) interferons have yielded improved SVR rates. An SVR is less common in patients with genotype 1 infections, high pretreatment HCV RNA levels, or advanced stages of fibrosis. Liver biopsy is used to establish the pre-treatment stage of liver disease and to facilitate decisions concerning the need for therapy. Advances in the future diagnosis and management of HCV will require close monitoring of the transmission of this infection, as well as extending treatment to populations not usually evaluated in clinical trials, and the development of new therapies, including antiviral agents targeting the viral protease and RNA polymerase.

GB Viruses

GB viruses are unassigned members of the family that include GB-A and -B, which are marmoset viruses, and GB-C, which was detected in the serum of human chronic hepatitis cases. GB-C and hepatitis G were discovered coincidentally and currently given the taxonomic assignment GBV-C/HGV. Currently there are five genotypes of this virus.

GBV-C/HGV is related to HCV, sharing 29% amino acid homology, and similarities in genome organization and structure, including a 5′ IRES, and a 3000 amino acid polyprotein but there are notable differences. GBV-C/HGV contains a capsid protein in the nucleocapsid; but this protein has not been identified. The E2 protein lacks both the extensive glycosylation and the hypervariable region found in the HCV E2 protein, which may in part explain the less frequent occurrence of persistent infection by GBV-C/HGV.

The 3′ UTR also differs from that of HCV. GBV-C/HGV is not hepatotropic, but rather replicates in mononuclear cells and bone marrow. Infection of humans is common, but no disease association has yet been identified. Instead, a beneficial effect of GBV-C/HGV infection on the outcome of HIV infection has been observed, presumably as a result of altered cellular immune responses caused by persistent infection.

See also: Classical Swine Fever Virus; Flaviviruses of Veterinary Importance; Tick-Borne Encephalitis Viruses; Yellow Fever Virus.

Further Reading

Chambers TJ and Monath TP (eds.) (2003) *The Flaviviruses: Advances in Virus Research,* vols. 59–61. San Diego, CA: Academic Press.

Chang KM (2003) Immunopathogenesis of hepatitis C virus infection. *Clinics in Liver Disease* 7: 89–105.

Cocquerel L, Voisset C, and Dubuisson J (2006) Hepatitis C virus entry: Potential receptors and their biological functions. *Journal of General Virology* 87: 1075–1084.

Calisher CH and Gould EA (2003) Taxonomy of the virus family Flaviviridae. *Advances in Virus Research* 59: 1–19, with permission from Elsevier.

Gubler DJ and Kuno G (eds.) (1997) *Dengue and Dengue Hemorrhagic Fever.* London: CABI.

Heinz FX, Collett MS, Purcell RH, *et al.* (2000) Family *Flaviviridae*. In: Van Regenmortel MHV, Fauquet CM Bishop DHL, *et al.* (eds.) *Virus Taxonomy: Seventh International Committee for the Taxonomy of Viruses*, pp. 859–878. San Diego, CA: Academic Press.

Houe H (1999) Epidemiological features and economical importance of bovine virus diarrhoea virus (BVDV) infections. *Veterinary Microbiology* 64: 89–107.

Kovács F, Magyar T, Rinehart C, *et al.* (2003) The live attenuated bovine viral diarrhea virus components of a multi-valent vaccine confer protection against fetal infection. *Veterinary Microbiology* 96: 117–131.

Kuhn RJ, Zhang W, Rossmann MG, *et al.* (2002) Structure of dengue virus: Implications for flavivirus organization, maturation, and fusion. *Cell* 8: 717–725.

Lindenbach BD, Evans MJ, Syder AJ, *et al.* (2005) Complete replication of hepatitis C virus in cell culture. *Science* 309: 623–626.

Lindenbach BD and Rice CM (2001) *Flaviviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., vol. 1. Baltimore, MD: Lippincott Williams and Wilkins.

Lindenbach BD and Rice CM (2003) Molecular biology of the flaviviruses. *Advances in Virus Research* 59: 23–61.

Lindenbach BD and Rice CM (2005) Unravelling hepatitis C virus replication from genome to function. *Nature* 436(7053): 933–938), Copyright (2005).

Radostits OM, Gay CC, Blood DC, and Hinchcliff KW (eds.) (2000) Bovine virus diarrhoea, mucosal disease, bovine pestivirus complex. In: *Veterinary Medicine, A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats, Horses,* 9th edn., pp. 1085–1105. New York: WB Saunders.

Ridpath JF (2003) BVDV genotypes and biotypes: Practical implications for diagnosis and control. *Biologicals* 2: 127–133.

Tassaneetrithep B, Burgess TH, Granelli-Piperno A, *et al.* (2003) DC-SIGN (CD209) mediates dengue virus infection of human dendritic cells. *Journal of Experimental Medicine* 197: 823–829.

Tautz N, Meyers G, and Thiel HJ (1998) Pathogenesis of mucosal disease, a deadly disease of cattle caused by a pestivirus. *Clinical and Diagnostic Virology* 10: 121–127.

Flexiviruses

M J Adams, Rothamsted Research, Harpenden, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Triple gene block (TGB) protein A set of three proteins in overlapping reading frames that function together in facilitating cell-to-cell movement of plant viruses.

Introduction

The creation of the family *Flexiviridae* was approved by the International Committee on the Taxonomy of Viruses (ICTV) in 2004 to include a number of existing genera and some unassigned species of plant viruses. The family is so named because its members have flexuous, filamentous virions, and there are a number of characteristics that distinguish it from the families *Closteroviridae* and *Potyviridae*, the two other families of plant viruses that have virions of similar morphology (**Table 1**).

Main Characteristics

The chief characteristics shared by all members of the family are considered in this section.

Virion Morphology

Virions are flexuous filaments, 12–13 nm in diameter, and usually 500–1000 nm in length, depending on the genus. They are constructed from helically arranged coat protein subunits in a primary helix with a pitch of about 3.4 nm and nine to ten subunits per turn. Each particle contains a single molecule of the full-length genomic RNA. Smaller 3′ terminal subgenomic RNAs may also be encapsidated in some species, sometimes in shorter virions. In negative contrast electron microscopy the virions usually appear to be cross-banded. A typical electron micrograph is shown in **Figure 1** (for red clover vein mosaic virus, genus *Carlavirus*) but in the genera *Trichovirus* and *Vitivirus*, the virions appear to be rather more flexuous and hence resemble more those of the family *Closteroviridae*.

Genome

All members have a monopartite, positive-sense, single-stranded RNA (ssRNA) genome in the size range 5.5–9.5 kbp, depending on the species. This forms about 5–6% of the virion by weight. The RNA is capped at the 5′ terminus and there is a 3′ polyA tail.

Replication and Translation

In all members, there is a short 5′ nontranslated region followed by a single large alpha-like replication protein (145–250 kDa). This contains (in this order) conserved methyltransferase, helicase, and RNA-dependent RNA polymerase (RdRp) motifs and appears to be the only protein required for replication, which is believed to take place in the cytoplasm. Translation of the smaller open reading frames (ORFs) downstream occurs from one or more 3′ terminal subgenomic mRNAs. In some species, there is a 'leaky scanning' mechanism that ensures that two or three different proteins are translated from the same mRNA.

Genomic Organization

Genomic organization varies between genera but there are up to six ORFs ordered from 5′ to 3′:

1. a large alpha-like replication protein (see above);
2. one or more cell-to-cell movement proteins (MPs): in some genera there is a single MP of the '30K' superfamily

Table 1 Characteristics of the families of filamentous plant viruses[a]

Family	*Genome size (kbp)*	*RNAs*[b]	*ORFs*	*Translation strategy*[c]	*5′ end VPg*[d]	*3′-polyA*
Closteroviridae[e]	15–20	1, 2, or 3	10+	Subgenomic	No	No
Flexiviridae	5.5–9.5	1	2–6	Subgenomic	No	Yes
Potyviridae	9–12	1 or 2	1 or 2	Polyprotein	Yes	Yes

[a]All these have ssRNA genomes that encode in the virion (positive) sense.
[b]Numbers of genomic RNA components.
[c]Proteins are translated either from a set of nested subgenomic mRNAs or are cleaved by virus-encoded proteases from a polyprotein precursor.
[d]Presence or absence of a genome-linked protein covalently bound to the 5′ terminus of the RNA.
[e]Members of the family *Closteroviridae* have distinctive, tailed virions. The tail is constructed from a second, minor, coat protein.

(distantly related to the 30 kDa MP of tobacco mosaic virus (genus *Tobamovirus*)) while in the others there is a set of three overlapping proteins known as a 'triple gene block' (TGB);

3. a single capsid (coat) protein (CP) (21–45 kDa);
4. a final ORF (in some viruses only), which is thought to have RNA-binding properties and is probably a suppressor of gene silencing; it partially overlaps the 3′ end of the CP gene in some species.

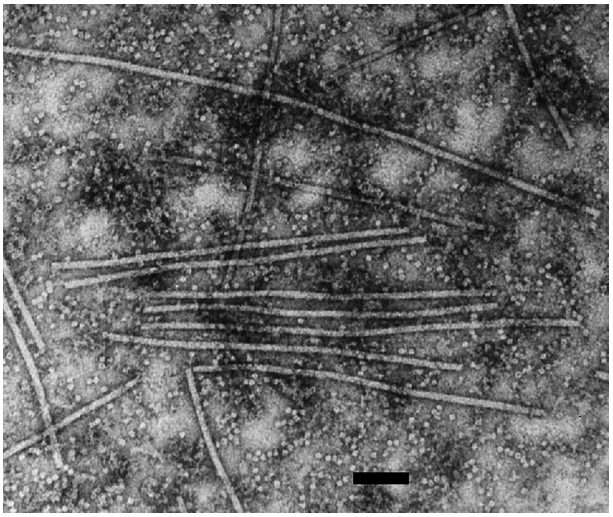


Figure 1 Electron micrograph of virions of red clover vein mosaic virus (genus *Carlavirus*), negatively stained. Scale = 100 nm.

Biological Properties

Biologically, the viruses are fairly diverse. They have been reported from a wide range of herbaceous and woody mono- and dicotyledonous plant species but the host range of individual members is usually limited. Natural infections by members of the genera *Capillovirus*, *Citrivirus*, *Foveavirus, Mandarivirus*, *Trichovirus*, and *Vitivirus* are mostly or exclusively of woody hosts. Many of the viruses have relatively mild effects upon their host. All species can be transmitted by mechanical inoculation, often readily. Many of the viruses have no known invertebrate or fungal vectors but allexiviruses and some trichoviruses are thought to be mite borne, most carlaviruses are transmitted naturally by aphids in a nonpersistent manner, and a range of vectors have been reported for different vitiviruses. Aggregates of virus particles accumulate in the cytoplasm but there are generally no distinctive cytopathic structures.

Component Genera and Species

Some properties of the nine genera included in the family and of six species that are not assigned to any genus are summarized in **Table 2**. These are mostly distinguished by the number of ORFs, the type of MP encoded, and the sizes of their virions and replication protein genes. In some genera, there are additional ORFs not present in other members of the family. These distinctions based on

Table 2 Genera and unassigned species included within the family *Flexiviridae*

Genus and unassigned species[a]	*Virion length (nm)*	*ORFs*	*MP type*[b]	*Rep (kDa)*	*CP (kDa)*
Potexvirus (28)	470–580	5	TGB	146–191	22–27
Mandarivirus (1)	650	6	TGB	187	36
Allexivirus (8)	~800	6	TGB	175–194	26–28
Carlavirus (35)	610–700	6	TGB	226–238	31–40
Foveavirus (3)	800+	5	TGB	244–247	28–45
Capillovirus (3)	640–700	2 or 3	30K	214–241	25–27
Vitivirus (4)	725–785	5	30K	195–196	18–23
Trichovirus (4)	640–760	3 or 4	30K	216–217	21–22
Citrivirus (1)[c]	960	3	30K	227	41
Banana mild mosaic virus (virus: BanMMV)	580	5	TGB	205	27
Banana virus X (virus: BanVX)	?	5	TGB	nd[d]	24
Cherry green ring mottle virus (virus: CGRMV)	1000+	5	TGB	230	30
Cherry necrotic rusty mottle virus (virus: CNRMV)	1000+	5	TGB	232	30
Potato virus T (virus: PVT)	640	3	30K	nd	24
Sugarcane striate mosaic-associated virus (virus: SCSMaV)	950	5	TGB	222	23
Possible species					
Botrytis virus X (BotV-X)	720	5	na[e]	158	44

[a]Numbers of species recognized within each genus are shown in parentheses (2006 data).
[b]Either a set of three overlapping proteins (TGB) or a single protein of the '30K' superfamily (phylogenetically related to the 30 kDa MP of *Tobacco mosaic virus*, genus *Tobamovirus*).
[c]Genus proposed in 2005 for the species *Citrus leaf blotch virus* (virus: CLBV).
[d]Not determined (only partial sequence data available).
[e]Not applicable (no MP encoded).

genome organization are also supported by phylogenetic analyses (see below). Genome organization is summarized in **Figure 2**.

A recently described mycovirus, botrytis virus X, shares many of the characteristics of the family and its CP and replication protein are phylogenetically related to members of the family *Flexiviridae*, in particular to members of the genera *Potexvirus* and *Allexivirus*. It is likely that a new genus will be proposed to accommodate this species in due course.

Phylogenetic Relationships

The relationships detected between the genera within the family and those between members of the family *Flexiviridae* and other viruses are dependent on the genome region used for analysis. This probably indicates that the viruses have arisen from reassortment of entire genes (or blocks of genes). These relationships are summarized below.

Replication Protein

A dendrogram based on the nucleotide sequence of the replication proteins (**Figure 3**) supports the taxonomic division of the family *Flexiviridae* into genera and falls into two major parts. One part contains the genera *Allexivirus*, *Mandarivirus*, and *Potexvirus* together with botrytis virus X. These proteins are the smaller ones in the family (145–195 kDa) and the plant-infecting members all have a TGB. The second part of the tree contains those viruses with larger proteins (195–250 kDa). It includes all those with a '30K'-like MP (*Capillovirus*, *Citrivirus*, *Trichovirus*,

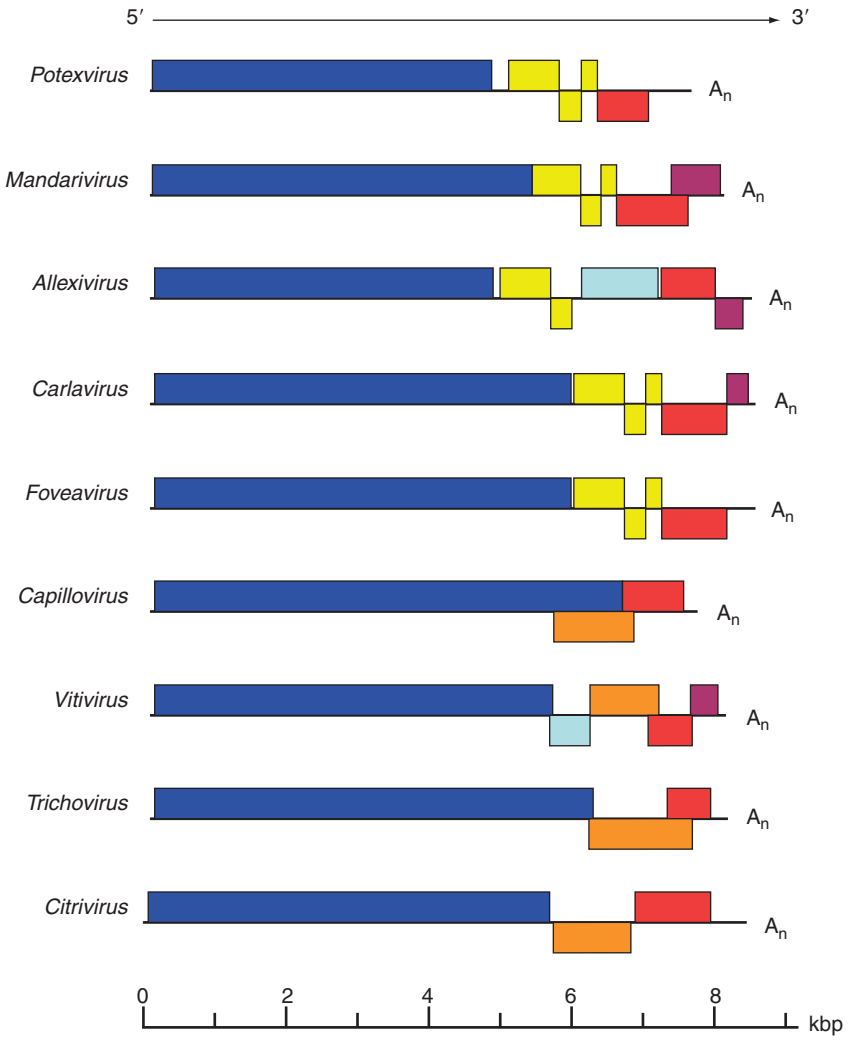


Figure 2 Diagram showing genome organization for the component genera of the family *Flexiviridae*. Blocks represent predicted ORFs. Replication proteins are shown in dark blue, '30K-like' MPs in orange, TGB proteins in yellow, CPs in red, and RNA-binding proteins in purple. ORFs of unknown function are shown in light blue.

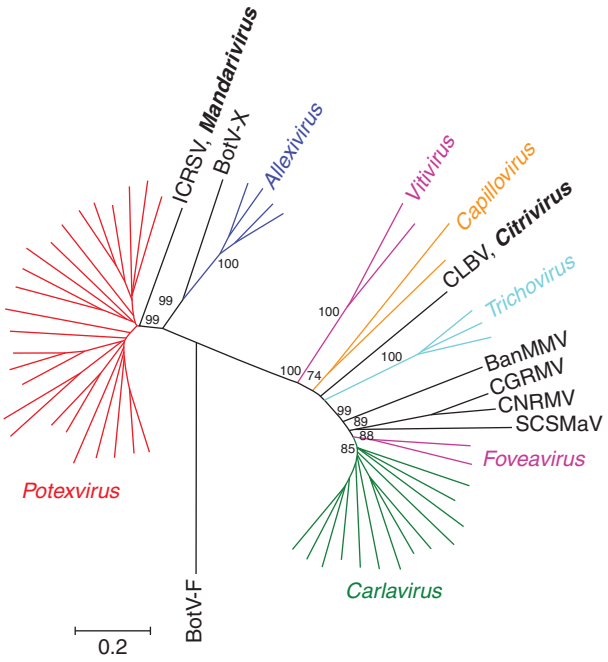


Figure 3 Phylogenetic tree of the replication proteins of all sequenced members of the family *Flexiviridae* with botrytis virus X and botrytis virus F. For abbreviations, see **Table 2**. The neighbor-joining tree is based on the codon-aligned nucleotide sequences. Values on the major branches show the percentage of trees in which this grouping occurred after bootstrapping the data (10 000 replicates). The scale bar shows the number of substitutions per base.

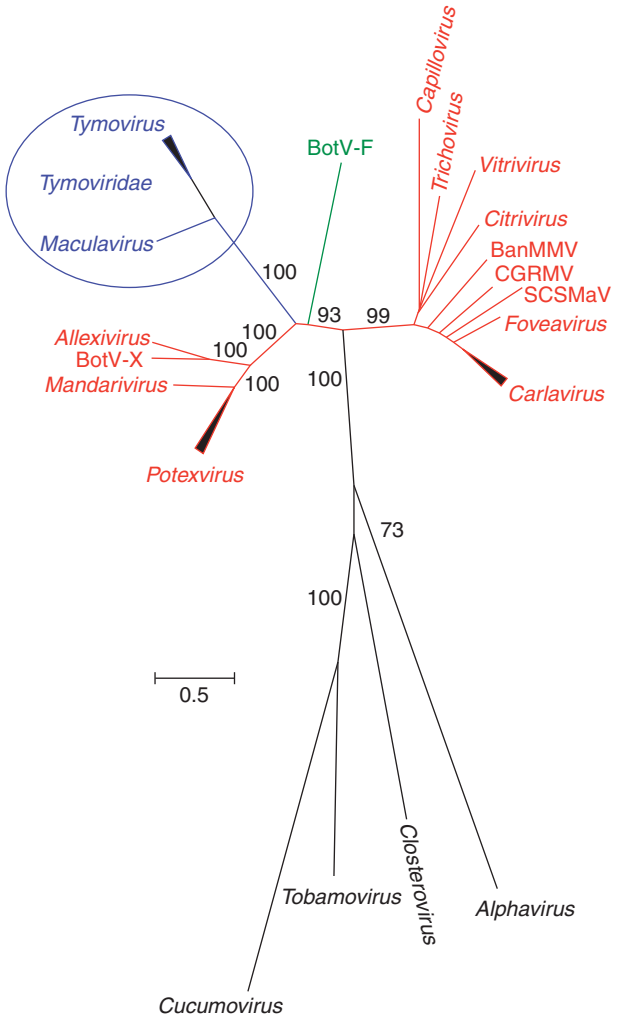


Figure 4 Phylogenetic tree of the replication proteins of selected members of the families *Flexiviridae* (in red) and *Tymoviridae* (in blue) with other related viruses. For abbreviations, see **Table 2**. The neighbor-joining tree is based on the aligned peptide sequences. Values on the major branches show the percentage of trees in which this grouping occurred after bootstrapping the data (500 replicates). The scale bar shows the number of substitutions per base.

and *Vitivirus*), together with the TGB-containing species that make up the genera *Carlavirus* and *Foveavirus* and some unassigned viruses that have foveavirus-like genome organization.

Most studies of 'longer distance' relationships between viruses have been based upon the motifs within the replication protein as these are relatively conserved within ssRNA viruses. Such studies consistently place the family *Flexiviridae* within a larger group ('alpha-like' or 'Sindbis-like' with 'type III' RdRp) that includes the family *Tymoviridae* (plant-infecting viruses that have isometric particles) and, more distantly, the families *Togaviridae*, *Bromoviridae*, *Closteroviridae,* and the rod-shaped plant viruses including the genus *Tobamovirus.* Phylogenetic analysis of the entire replication protein shows that this protein in members of the *Tymoviridae* is related to that found in the genera *Allexivirus, Mandarivirus*, and *Potexvirus* but distinct from that found in the remaining members of the family *Flexiviridae* (**Figure 4**). This might suggest that the *Tymoviridae* and *Flexiviridae* could be placed within the same family or that the current family *Flexiviridae* could be divided into two separate families. However, the relationships between other genes (see below) and differences in virion morphology and cytopathology suggest that the current classification makes better biological sense. The replication protein of the mycovirus botrytis virus F also appears to belong to the *Flexiviridae–Tymoviridae* group but falls outside any of the major groups and is probably best left unclassified at present.

Movement Protein

'30K'-like MPs occur in the genera *Capillovirus*, *Citrivirus*, *Trichovirus,* and *Vitivirus* and also in the unassigned potato virus T. The '30K' superfamily proteins are largely recognized by structural similarities rather than by primary sequence comparisons. Nonetheless, phylogenetic analysis shows that those from the family *Flexiviridae* are related to one another (although *Vitivirus* is more distant) and that

there are some similarities to the corresponding proteins in the rod-shaped plant virus genera *Furovirus*, *Tobravirus*, and *Tobamovirus* (**Figure 5**).

TGB Proteins

Within the family, the TGB proteins (for those genera that have them) are related in much the same way as their CPs. A TGB module is also found in members of the genera *Benyvirus*, *Hordeivirus*, *Pecluvirus*, and *Pomovirus*. However, the first and third proteins of the TGB of the rod-shaped viruses are considerably larger than the corresponding proteins in the family *Flexiviridae* and clearly form a separate grouping in phylogenetic analyses (**Figure 6**). In some species, notably all members of the genus *Allexivirus*, a sequence similar to that coding for the third TGB protein can be recognized but there is no standard AUG start codon and it is not known whether the protein is expressed.

Coat Protein

A phylogenetic tree of the CP sequences shows that most viruses with a '30K'-like MP in the genera *Capillovirus*, *Trichovirus*, and *Vitivirus* are only distantly related to those in the remainder of the family but that citrus leaf blotch virus, the single member of the genus *Citrivirus*, groups with a well-defined group containing all the TGB-containing members (**Figure 7**).

It is difficult to detect any significant relationship between the CPs of members of the *Flexiviridae* and those of other viruses beyond the modest similarity that occurs between the CPs of all viruses with filamentous particles.

RNA-Binding Protein

Putative RNA-binding proteins are found in the genera *Allexivirus*, *Carlavirus*, *Mandarivirus*, and *Vitivirus*. The proteins are clearly related and share a strictly conserved core amino acid sequence GxSxxAxR/KRRAx$_5$Cx$_{1-2}$Cx$_{5-13}$C. In phylogenetic analyses, the proteins from each genus cluster separately. The proteins probably act as silencing suppressors and are related to those of the closteroviruses (p23 of citrus tristeza virus and homologs in other species) (**Figure 8**).

General Comments

The limitations of phylogenetic analysis are demonstrated by the complex relationships within and outside the family

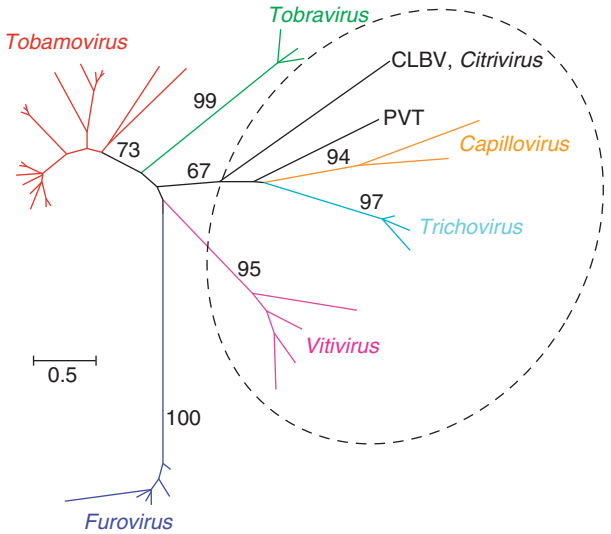


Figure 5 Phylogenetic tree of the '30K-like' MPs of the family *Flexiviridae* (in dotted oval) and the corresponding proteins of rod-shaped plant viruses. For abbreviations, see **Table 2**. The neighbor-joining tree is based on the aligned peptide sequences. Values on the major branches show the percentage of trees in which this grouping occurred after bootstrapping the data (500 replicates). The scale bar shows the number of substitutions per base.

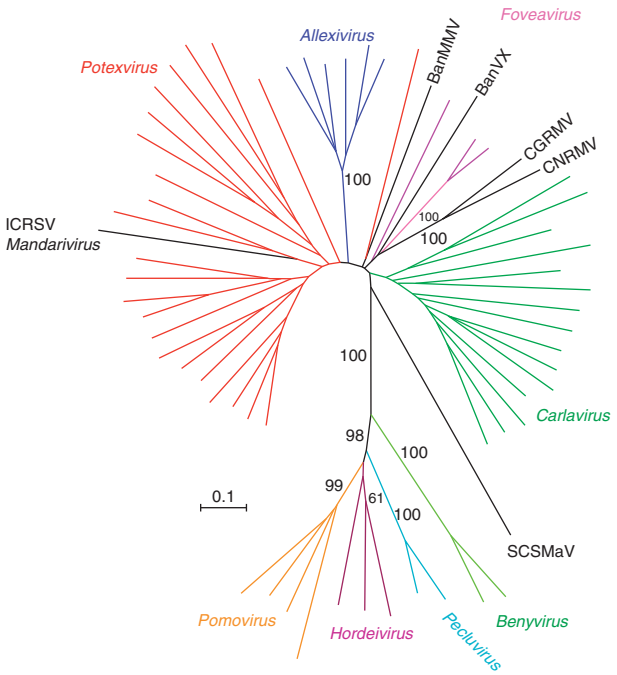


Figure 6 Phylogenetic tree of the TGB proteins of the family *Flexiviridae* and related rod-shaped plant viruses. For abbreviations, see **Table 2**. The neighbor-joining tree is based on the codon-aligned nucleotide sequences of all three proteins. Values on the major branches show the percentage of trees in which this grouping occurred after bootstrapping the data (10 000 replicates). The scale bar shows the number of substitutions per base.

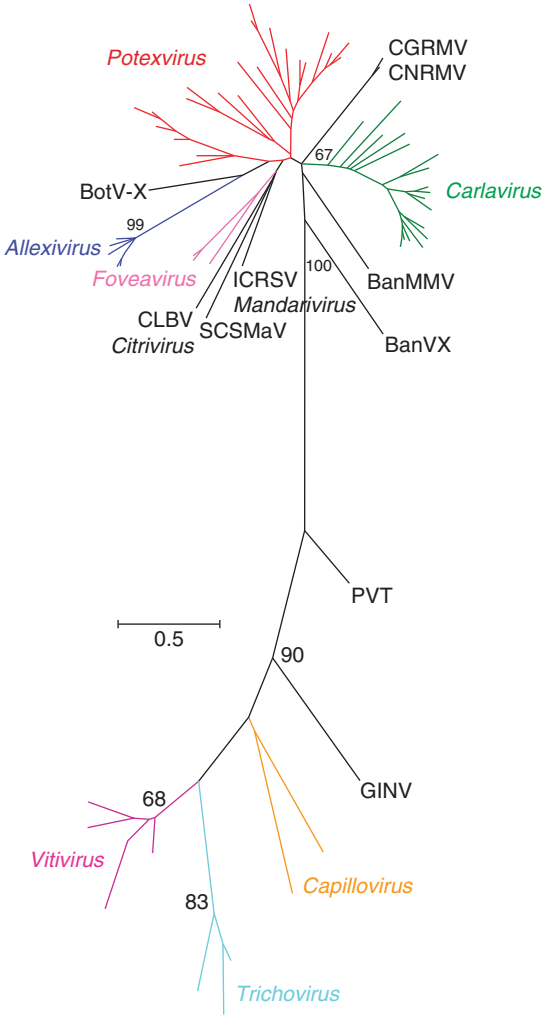


Figure 7 Phylogenetic tree of the CPs of all sequenced members of the family *Flexiviridae*. For abbreviations, see **Table 2**. The neighbor-joining tree is based on the aligned peptide sequences. Values on the major branches show the percentage of trees in which this grouping occurred after bootstrapping the data (500 replicates). The scale bar shows the number of substitutions per base.

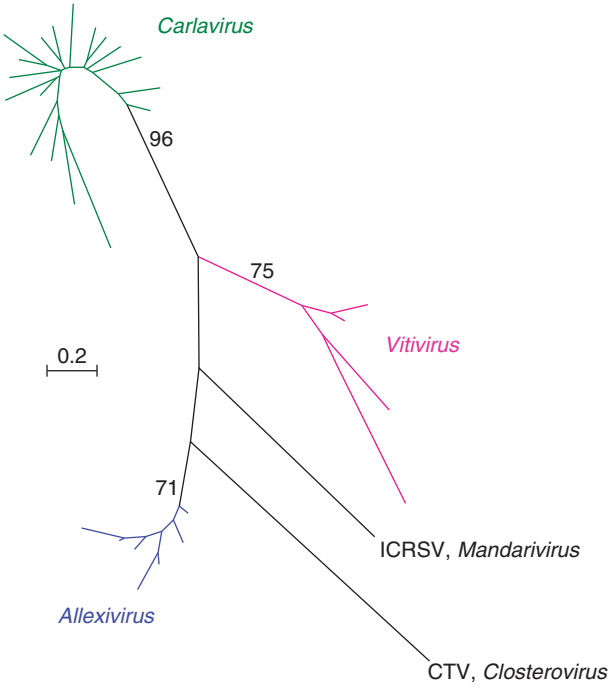


Figure 8 Phylogenetic tree of the nucleic acid-binding proteins of members of the family *Flexiviridae* and citrus tristeza virus (CTV, genus *Closterovirus*). The neighbor-joining tree is based on the aligned peptide sequences. Values on the major branches show the percentage of trees in which this grouping occurred after bootstrapping the data (500 replicates). The scale bar shows the number of substitutions per base.

that are revealed when different genes are used in the analysis (**Table 3**). In particular, there are big differences in the relative positions of the genera *Carlavirus*, *Citrivirus*, and *Foveavirus* to the rest of the family. The single virus in the genus *Mandarivirus* consistently groups with the genus *Potexvirus*, but it has a larger CP and an RNA-binding protein related to those of the genera *Allexivirus* and *Carlavirus*. Outside the family, the replication proteins are related to those found in the *Tymoviridae* and to botrytis virus F. By contrast, the movement protein(s), whether TGB or '30K'-like, are clearly related to those of the rod-shaped viruses, which are much more distantly related in their replication proteins.

Table 3 Summary of the main phylogenetic relationships within the family *Flexiviridae*

Genus	*Rep*[a]	*MP*[b]	*CP*[c]	*RNABP*[d]
Potexvirus	1	TGB	1	No
Mandarivirus	1	TGB	1	Yes
Allexivirus	1	TGB	1	Yes
(Botrytis virus X)	1	na[e]	1	No
Carlavirus	2	TGB	1	Yes
Foveavirus+[f]	2	TGB	1	No
Capillovirus	2	30K	2	No
Vitivirus	2	30K	2	Yes
Trichovirus	2	30K	2	No
Citrivirus	2	30K	1	No

[a]Type of replication protein.
[b]Either a set of three overlapping proteins (TGB) or a single protein of the '30K' superfamily.
[c]Type of coat protein.
[d]Presence of RNA-binding protein.
[e]Not applicable (no protein encoded).
[f]Including the unassigned members of the family: *Banana mild mosaic virus, Banana virus X, Cherry green ring mottle virus, Cherry necrotic rusty mottle virus,* and *Sugarcane striate mosaic-associated virus*.

See also: Allexivirus; *Capillovirus, Foveavirus, Trichovirus, Vitivirus*; *Carlavirus*; *Carmovirus*; Citrus Tristeza Virus; Phylogeny of Viruses; *Potexvirus*; Potyviruses; *Tobamovirus*; Tymoviruses.

Further Reading

Adams MJ, Antoniw JF, Bar-Joseph M, *et al.* (2004) The new plant virus family *Flexiviridae* and assessment of molecular criteria for species demarcation. *Archives of Virology* 149: 1045–1060.

Howitt RLJ, Beever RE, Pearson MN, and Forster RLS (2001) Genome characterization of Botrytis virus F, a flexuous rod-shaped mycovirus resembling plant 'potex-like' viruses. *Journal of General Virology* 82: 67–78.

Howitt RLJ, Beever RE, Pearson MN, and Forster RLS (2006) Genome characterization of a flexuous rod-shaped mycovirus, Botrytis virus X, reveals high amino acid identity to genes from plant 'potex-like' viruses. *Archives of Virology* 151: 563–579.

Koonin EV and Dolja VV (1993) Evolution and taxonomy of positive-strand RNA viruses: Implication of comparative analysis of amino acid sequences. *Critical Reviews in Biochemistry and Molecular Biology* 28: 375–430.

Martelli G, Adams MJ, Kreuze JF, and Dolja VV (2007) Family *Flexiviridae*: A case study in virion and genome plasticity. *Annual Review of Phytopathology* 45: 73–100.

Melcher U (2000) The '30K' superfamily of viral movement proteins. *Journal of General Virology* 81: 257–266.

Morozov SY and Solovyev AG (2003) Triple gene block: Modular design of a multifunctional machine for plant virus movement. *Journal of General Virology* 84: 1351–1366.

Wong S-M, Lee K-C, Yu H-H, and Leong W-F (1998) Phylogenetic analysis of triple gene block viruses based on the TGB 1 homolog gene indicate a convergent evolution. *Virus Genes* 16: 295–302.

Foamy Viruses

M L Linial, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Provirus A viral DNA that is incorporated into the genetic material of a host cell.
Syncytia Structures in a monolayer cell culture formed by fusion of multiple cells that form a large cell with multiple nuclei.
Zoonotic infection An infection of animals that is transmitted to humans.

Introduction

Foamy viruses (FVs, also known as spumaretroviruses or spumaviruses) have been known for over 50 years as the cause of cytopathic effects in cell cultures derived from monkey tissues. The original name, spumaviruses, is derived from the Latin word *spuma* for the foamy appearance of infected tissue culture cells. A foamy virus was isolated from a human-derived tissue culture in 1971 and called HFV for human foamy virus. The cell culture that yielded virus was obtained from a Kenyan patient with a nasopharyngeal carcinoma. At the time, this isolate was thought to be the first human retrovirus. Isolation of HFV generated a considerable amount of interest because of its possible link with human cancer. However, subsequent work over the past decade has definitively shown that the HFV isolate is a chimpanzee virus derived from the species of chimpanzee present in East Africa. This has led to the suggestion that the Kenyan patient had acquired FV through a zoonotic infection. This chimpanzee isolate is now designated PFV for prototype foamy virus. Unless otherwise indicated, the information in this article refers specifically to PFV.

Epidemiology

FVs have been isolated from a number of hosts (**Table 1**), including all nonhuman primates (NHPs) that have been examined, as well as cats, cows, and horses. It is interesting that all species naturally infected with lentiviruses are also infected with harbor foamy viruses, although the reverse is not true. FV infection of natural hosts has not been linked to any pathologies. The widespread presence of FV suggests that it is part of the natural host microbial flora. In a small number of cases examined, infection of other species such as mice or rabbits has also failed to yield any manifestation of disease.

A large number of publications have suggested links between FVs and a variety of human diseases. However, ultimately none of these have been verified. Currently, there is no evidence of widespread infection of humans with FV. However, human infections are well documented. All the known cases can be traced to interactions with NHP. Veterinarians, primate center workers, bush meat hunters, pet owners, and people associated with monkey parks and monkey temples in Asia have been found to be infected. The frequency of infection in these high risk

groups is about 2–3% of those examined. Humans have been shown to be infected by the presence of specific antibodies, and in some cases by sequencing provirus from peripheral blood cells. In the few cases examined, human-to-human transmission has not been observed. Recent studies have shown that primate FVs can be transmitted to monkeys via blood transfusions from naturally infected animals. Whether or not this would also be true for infected human blood is not known. Limited studies have been made to determine whether humans can be infected by feline FVs from domestic cats. To date, there is no evidence for human infection by these agents.

Taxonomy and Classification

FVs are clearly retroviruses based on their genomic organization and general life cycle. However, they differ in fundamental ways from other retroviruses. These differences have led to their reclassification into a subfamily of *Retroviridae*. All other groups of retroviruses such as human immunodeficiency virus (HIV) are now called orthoretroviruses, or true retroviruses, while foamy viruses are spumaretroviruses (**Table 2**). Many aspects of their replication place FV closer to the hepadnaviruses such as human hepatitis B virus (HBV) than to orthoretroviruses.

Table 1 Foamy virus isolates

Host species	*Virus isolate*
Feline	FFV
Bovine	BFV
Equine	EFV
Rhesus macaque	SFVmac
African green monkey	SFVagm
Squirrel monkey	SFVsqu
Galago	SFVgal
Chimpanzee	SFVcpz
Spider monkey	SFVspm
Capuchin monkey	SFVcap
Baboon	SFVbab
Orangutan	SFVora
Gorilla	SFVgor
Marmoset	SFVmar
Human (not a natural host)	PFV (chimpanzee origin)

Viral Infections

Primate FV have been shown to have a very broad host range in tissue culture, with almost all vertebrate cell types being susceptible. In tissue culture, many cell types are lytically infected with virus. Such infected cells fuse to form large multinucleated cells, or syncytia. These syncytia rapidly die. Infected cells become filled with large numbers of vesicles, giving the cell cultures a foamy appearance (hence the name). An example of an FV-infected tissue culture is shown in **Figure 1**, using a hamster-derived indicator cell line in which infected cells stain blue. The arrows indicate multinucleate syncytia. However, some cell types in culture, including some human hematopoietic cell lines, are not killed after infection. Cell lines such as human Jurkat T cells are infected, evidenced by the presence of full-length viral DNA in the chromosomes. Few or no virions are produced from Jurkat cells, and there is no apparent effect on the health of the cells. These cell types are latently infected, and the infection persists indefinitely. The inability of virus to replicate in such persistent cultures may be caused by a transcriptional block. It appears that viral replication in tissue culture cells is invariably associated with cell death. However, it is not known whether or not this is true *in vivo*.

FV infections of organisms are lifelong in all species examined, including humans. There are no documented examples of viral clearance. Antibodies to viral proteins are present at all times after initial infection. Virus is believed to be transmitted between individuals through saliva. NHPs acquire the virus through biting or grooming. Young animals are not infected, and appear to be resistant to virus infection because of acquisition of maternal antibodies. Once such antibodies wane, serum conversion is rapid, and by 3 years of age, most animals are infected. In some populations of NHPs, infection reaches nearly 100% in adult animals. In naturally infected NHPs, viral DNA sequences can be found in most tissues, at very low levels. However, viral replication has only been documented in oral mucosal tissues, and virus is present in saliva. It is likely that FVs are latent in most tissues, and viral replication is confined to a small subset of cells in the oral mucosa. Such infected cells could secreted into saliva and be responsible for viral transmission.

Table 2 Classification of retroviruses (family *Retroviridae*)

Subfamily	*Genus*	*Type species*
Orthoretrovirinae (Orthoretrovirus)	*Alpharetrovirus*	*Avian leukosis virus*
	Betaretrovirus	*Mouse mammary tumor virus*
	Gammaretrovirus	*Murine leukemia virus*
	Deltaretrovirus	*Bovine leukemia virus*
	Epsilonretrovirus	*Walleye dermal sarcoma virus*
	Lentivirus	*Human immunodeficiency virus*
Spumaretrovirinae (Spumavirus)	*Spumavirus*	*Simian foamy virus*

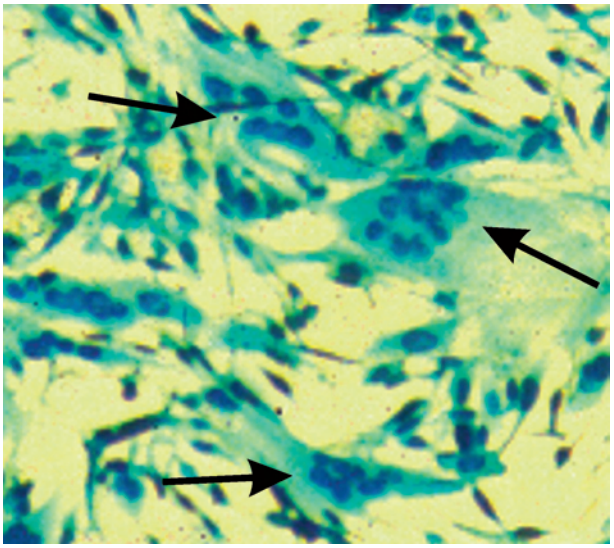


Figure 1 Baby hamster kidney indicator cells infected with PFV. These cells contain an indicator gene that turns the cell nuclei blue after staining when the viral transactivator Tas is present. The arrows point to multinucleate syncytia formed by PFV infection.

Virion Properties

FVs resemble other retroviruses in that they are fairly pleotropic in morphology, with large extracellular spikes. One distinct difference is that the FV core is electron lucent rather than condensed into a spherical core as in murine leukemia virus or into a cone-shaped core as in HIV (**Figure 2**(**a**), red arrow). The morphology of mature, infectious FV resembles that of immature gammaretroviruses. Foamy virions have a density of about 1.16 gm ml^{-1}, as do orthoretroviruses. The infectious particles are surrounded by long spikes, composed of the viral glycoproteins encoded by the *env* gene (**Figure 2**(**a**), purple dotted arrow). Like most retroviruses, FV bud from cellular membranes (**Figure 2**(**b**), black arrow). Most often budding is from intracellular membranes, and the majority of virions remain cell associated. A cartoon of an FV particle is shown in **Figure 2**(**c**). Virions are composed of two large structural proteins (Gag, blue ovals), surrounded by glycoprotein spikes (green). Particles contain the viral enzymes, including reverse transcriptase, encoded in the Pol protein (red). Like orthoretroviruses, FVs package RNA, but unlike

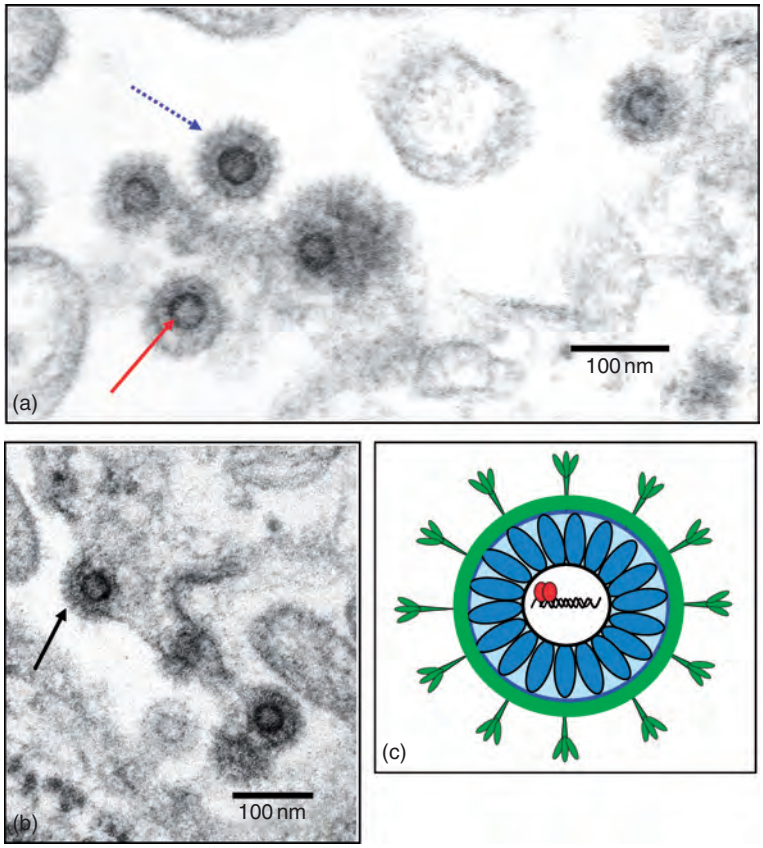


Figure 2 Morphology of foamy virus particles. (a, b) Negatively stained electron micrographs of tissue culture cells infected with PFV. (a) Intracellular particles; (b) budding particle; (c) depiction of a mature particle. The viral Gag proteins are shown in blue, the Env proteins in green, and the Pol proteins in red. The DNA genome is indicated by the black helix.

orthoretroviruses, the infectious genome of FVs is double-stranded DNA (dsDNA) (depicted by black lines), implying that reverse transcription occurs during assembly and/or budding (see below). Because of the mechanism of Pol synthesis, there are many fewer Pol molecules per foamy virion compared to the orthoretroviruses.

Genome and Protein Organization and Expression

Foamy viruses are complex retroviruses. Their genomes are greater than 12 kb (**Figure 3**(**a**)), placing them among the largest retroviruses. They encode the three genes common to all retroviruses (*gag*, *pol*, and *env*), as well as additional genes (*tas* and *tas*/*bel2* encoding Bet), whose gene products are not virion associated (**Figure 3**(**b**)). The Gag, Pol, and Env gene products are all polyproteins that are cleaved by proteases to smaller mature products. The Gag and Pol precursor proteins are cleaved by the viral protease (PR) encoded within the Pol protein (cleavage sites are indicated by the yellow arrows), whereas the Env precursor protein is cleaved by a cellular furin-like protease (indicated by the white arrows). The Gag protein is synthesized from an RNA that is indistinguishable from the viral genome. The Pol and Env proteins are synthesized from spliced mRNAs that utilize the same 5′ splice site (5′ss) but different 3′ splice sites (**Figure 3**(**c**)). Synthesis of Env from a spliced mRNA is common to all retroviruses. In contrast, synthesis of Pol from a spliced RNA is unique to FV. Orthoretroviruses synthesize Pol as a Gag–Pol read-through protein from genomic RNA. Since the orthoretrovirus precursor Pol protein has Gag determinants, it is co-assembled with Pol into virions. Lack of Gag determinants on FV Pol (**Figure 3**(**d**)) indicates that Pol encapsidation into virions occurs by a different mechanism. Tas and Bet are synthesized from a second promoter (IP) and Bet is made from a spliced mRNA joining part of *tas* to *bel2* (**Figure 3**(**c**)).

There are several noticeable differences in organization of the FV Gag, Pol, and Env proteins compared to orthoretroviruses (**Figure 3**(**b**)). The FV Gag is only cleaved once by PR at a site near the C-terminus and there are no mature cleavage products analogous to the matrix, capsid, or nucleocapsid proteins of orthoretroviruses.

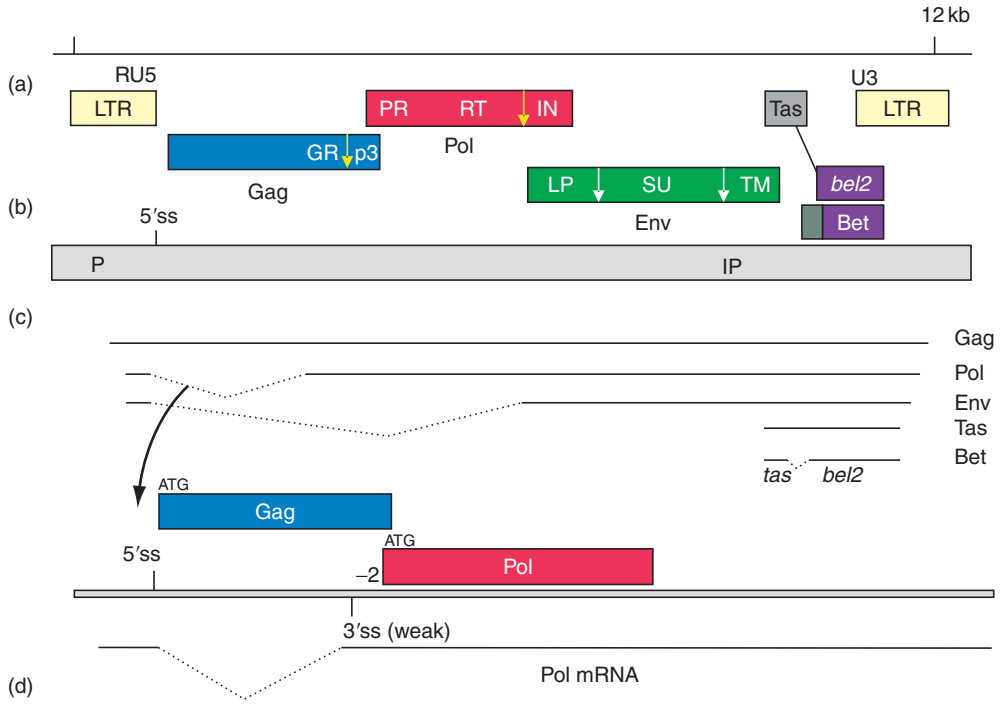


Figure 3 Viral genome. (a, b) The size of the PFV proviral genome and all of the gene products. The long terminal repeats (LTRs) contain the regulatory elements created by reverse transcription of the viral genomic RNA, duplicating RU5 and U3. The yellow arrows show sites of cleavage by the viral protease, the white arrows show sites of cleavage by a cellular protease. The Gag gene encodes the structural proteins. GR indicates three glycine-arginine-rich regions. One cleavage event occurs releasing a 3 kDa peptide (p3). The Pol gene contains the three enzymatic domains, protease (PR), reverse transcriptase (RT), and integrase (IN). The Env gene contains three domains, leader peptide (LP) unique to foamy viruses, surface (SU), and TM (transmembrane). Tas is the viral transactivator protein, and Bet is made from two exons, Tas and *bel2*. The function of Bet is not completely understood. (c) The location of the two viral promoters; The canonical retroviral promoter (P) in the LTR and the unique internal promoter (IP) encoded within the *env* gene. 5′ss indicates the major splice site. Indicated below are the major mRNAs. (d) The unique spliced mRNA that gives rise to the Pol protein. ATG indicates the start sites for translation. Adapted from Linial ML (1999) Foamy viruses are unconventional retroviruses. *Journal of Virology* 73: 1747–1755, with permission from American Society for Microbiology.

FV Gag lacks the hallmarks of other retroviral Gag proteins such as the cysteine–histidine boxes and the major homology region (MHR). The only distinct feature of FV Gag is the presence of three glycine-arginine-rich (GR) boxes near the C-terminus of the protein. These are involved in assembly and/or RNA binding. Most retroviral Gag proteins have a post-translational modification that adds a myristylation signal at the N-terminus. However, this does not occur in foamy viruses. The Pol protein encodes the four enzymatic activities associated with all retroviruses: PR, reverse transcriptase (RT) with its associated RNase H activity (RH), and integrase (IN). However, only one cleavage occurs in Pol, leading to a PR–RT (RH) fusion protein and an IN protein. The Env protein is composed of three subunits. In addition to the usual surface (SU) and transmembrane (TM) proteins, there is an N-terminal leader peptide (LP). The LP is equivalent to the signal peptide of other retroviruses that is cleaved and discarded. However, in FV, the LP is incorporated into particles with the same stoichiometry as SU and TM. In orthoretroviruses, the signal peptide is not incorporated into particles.

FVs also encode two proteins which are not found in virions, Tas and Bet. They are translated from mRNAs transcribed from the internal promoter located within the *env* coding region (IP; **Figure 3(c)**). Tas is a transcriptional transactivator, which binds to sequences in both the long terminal repeat (LTR) promoter and the IP, although the sites have different sequences. The IP has a higher affinity for Tas, and is activated first after infection. When additional Tas is synthesized from the IP, the level becomes high enough to allow transcription from the LTR and production of the virion proteins Gag, Pol, and Env. A block at the transcriptional level, preventing sufficient Tas to be synthesized to allow LTR transcription, is thought to occur in latent infections. Some latently infected cells can be activated by protein kinase C pathway activators, leading to viral production and ultimately to cell death. The second nonstructural protein, Bet, is an approximately 60 kDa protein with no obvious functional domains. Although all foamy virus Bet proteins have some similarities, there are no other proteins encoded by any organism with homology. Although the function of Bet is unknown, there are many tantalizing findings. Bet is both nuclear and cytoplasmic, and is also released from cells. It can be taken up by uninfected cells and there is some evidence that it can prevent infection. Although primate foamy virus Bet has not been found to be required for viral infectivity, it does appear to be required for replication of feline FV in some tissue culture cells. The role of Bet in infections of animals has not been assessed. Overexpression of Bet appears to have some effect on activation of the LTR promoter by Tas. Most recently, a number of groups have shown that Bet can inhibit the antiretroviral effects of the host protein APOBEC3, which is also an inhibitor of HIV and other retroviruses. However, the exact function(s) of Bet in viral replication *in vitro* and *in vivo* remains to be determined.

Viral Life Cycle

An overview of the FV life cycle is depicted in **Figure 4**. The steps in viral replication that differ in some aspects from those of orthoretroviruses are shown in red text. Some aspects of the viral life cycle are similar to that of the Hepadnaviruses such as hepatitis B virus (HBV), as indicated below.

1. *Viral entry.* The receptor(s) have not been identified for any foamy viruses. Identification is made difficult by the lack of cells that are resistant which could be used for selection. FV entry has been shown to use a pH-dependent endocytic pathway that is resistant to chloroquine, showing

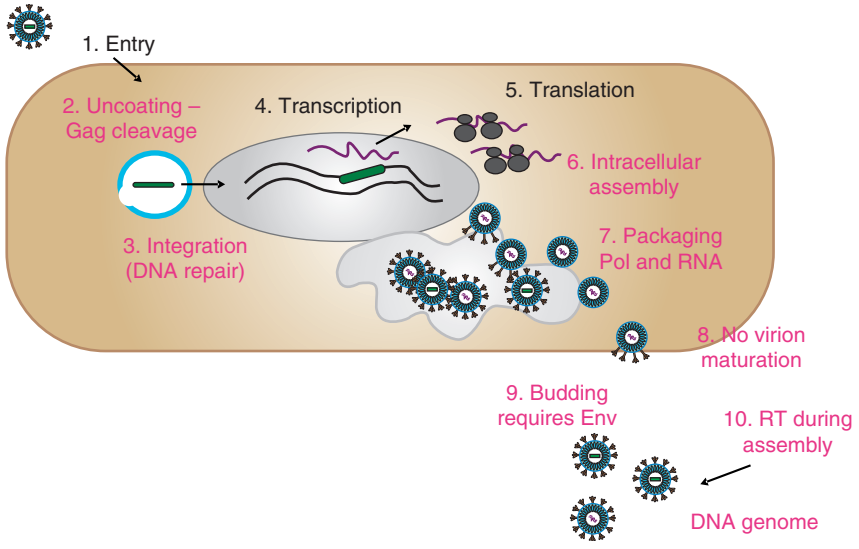


Figure 4 Viral life cycle. The major events in FV replication are indicated, with the steps that differ from orthoretroviruses indicated in red. For details, see text.

that the details of entry differ from that of the well-studied vesicular stomatitis virus.

2. *Uncoating.* FV Gag proteins are only cleaved once upon assembly/release by the viral protease PR. However, during viral entry, at least one additional PR cleavage occurs, which is required for infectivity. Presumably, this cleavage is important for uncoating of the viral capsid to release viral nucleic acid. Incoming particles traffic to the cellular microtubule organizing center (MTOC).

3. *Integration.* Although virions contain both RNA and DNA, the infectious genome is double-stranded linear DNA, reverse transcription having occurred late in the replication cycle (step 10, below). FVs share the property of packaging RNA but having DNA genomes with HBV. FV integration appears to occur in a manner similar to orthoretroviruses, although some repair of the dsDNA may occur at this step. The viral integrase (IN) is required for productive infection.

4 and 5. *Transcription and translation.* These occur as in other retroviruses using cellular machinery. As noted above, FV transcription from the two promoters requires the viral DNA binding protein Tas. Unlike other complex retroviruses in the genera *Deltaretrovirus* and *Lentivirus*, no viral proteins have been shown to be involved in RNA splicing and/or export of unspliced RNA from the nucleus. A region in the LTR which is present in both *gag* and subgenomic RNAs is necessary for efficient translation of Gag and Pol proteins.

6–8. *FV assembly and Pol and RNA packaging.* FV assembly occurs intracellularly, rather than at the plasma membrane, in a manner similar to that of Mason–Pfizer monkey virus (MPMV), a type D simian retrovirus. Like MPMV, FV Gag contains a cytoplasmic targeting and retention signal (CTRS) that is required for viral assembly. Newly synthesized Gag traffics to the MTOC which appears to be involved at some stage of viral assembly. RNA is incorporated into particles through a specific region of the RNA that interacts with one or more of the arginine-rich GR boxes. The RNA sequence is not exclusively located near the 5′ of the RNA where orthoretroviral packaging sequences (psi) are located. The FV psi sequence is more complex, and appears to include additional sequences encoded within the *pol* gene. The FV Pol protein is synthesized from a spliced mRNA and lacks Gag sequences. Thus, encapsidation is not through Gag assembly domains. Instead, viral RNA is required for Pol packaging. The Pol encapsidation sequences, like psi, are complex and involve both the U5 region of the LTR as well as sequences at the 3′ end of the *pol* gene. Protein–protein interactions of Gag and Pol may be required for Pol encapsidation. Because of the requirement for RNA sequences for binding, fewer Pol particles are packaged than in orthoretroviruses. As described above, cleavage of Gag into smaller subunits does not occur. This probably is responsible for the morphology of infectious virions that resemble immature retroviruses lacking a condensed core.

9. *Budding.* Orthoretroviral particles can bud from cells in the absence of the envelope glycoproteins, although the presence of Env can increase the efficiency of their release. In contrast, FVs absolutely require Env proteins to bud from cells, reminiscent of HBV. The component of FV Env that is required for particle egress has been mapped to the N-terminus of the LP protein. In the absence of LP intracellular particles accumulate. In general, FV budding is quite inefficient with a vast majority of infectivity remaining cell associated. It is likely that transmission from infected to naive animals occurs via cell-associated virus, rather than free virus.

10. *Reverse transcription.* Unlike orthoretroviruses but similar to HBVs, reverse transcription of the FV RNA genome occurs during assembly. The mechanism of reverse transcription appears to be identical to that of orthoretroviruses, using a $tRNA_{lys1,2}$ as a primer. Budded virions contain full-length dsDNA that it is required for infectivity. DNA extracted from virions has been shown to be infectious when fused into cells. The RT is unique in that it also contains the protease domain. FV RT is highly active and processive *in vitro.*

Viral Genetics and Evolution

FVs are the most ancient of retroviruses, and of all RNA viruses. It is estimated that they have been present in NHP populations for over 60 million years and in Old World primates for over 30 million years. They appear to have cospeciated with their hosts. The lack of significant homologies to orthoretroviruses in their Gag proteins, suggest that they may have evolved separately from orthoretroviruses. FV viral genomes are very stable and there is much less sequence diversity than seen with other retroviruses, either within or between species. The viral RT appears to be as error prone as that of other retroviruses, so the low sequence diversity may reflect the lower total viral load per animal, as replication is confined to the oral mucosa. It is assumed that FVs undergo recombination and reassortment as seen in the other genera, but this has not been studied.

Viral Vectors

Orthoretroviruses, including murine leukemia virus and HIV, have been developed into vectors for gene therapy applications. The large genome size, the broad host range, and especially the nonpathogenic nature of FV, have made them excellent candidates for gene therapy vector development. While simian and feline FVs have been developed as vectors, most work has been concentrated on PFV. Vector development has been the impetus for much of the present information about the replication

and infection by FVs. FVs are rather stable. They cannot complete replication in nondividing tissue culture cells. If nondividing cells are infected, FVs can apparently persist in the quiescent cells until the cells become activated. At that time, viral infection proceeds and the FV genome integrates into the host chromosome. Viral integrations into normal human cells do not occur preferentially within genes although there is a significant preference for GC-rich regions of the genome.

Thus far, it has not been possible to create stable packaging cell lines which contain all of the viral gene products. Instead, as in the case of HIV, cells are transfected with three vectors encoding separately the *gag*, *pol*, or *env* genes, and a fourth vector encoding the gene of interest embedded in an RNA containing the RNA and Pol packaging sequences. FV cannot be pseudotyped with vesicular stomatis virus glycoproteins (VSV G), so the cognate FV Env protein is used. This has an equally broad host range as VSV G. The vectors do not use the viral promoters, so the Tas gene product is not required. Resulting replication-defective FV vectors have been successfully used in both mice and dogs to repopulate all hematopoeitic cell lineages with the gene encoded by the vectors. This indicates that the vectors can integrate into early hematopoietic stem cells. Work proceeds on using FV vectors to repair genetic defects, and also to interfere with the replication of pathogens, such as HIV. Thus far, no human trials with FV vectors have been reported.

Future Perspectives

There are currently two major foci of current foamy virus research. The first is the continued development and refinement of vectors for a variety of applications. The published results thus far have been encouraging and human trials are probably not far away. The second area is surveillance of human populations at risk for FV infection. The progenitors of HIV are apparently not pathogenic in their natural hosts, and the human pathogen arose by recombination between two monkey viruses with a chimpanzee intermediate host. There are obvious parallels with FVs, where humans are increasingly in contact with NHP harboring a variety of FV. Future studies need to determine whether or not humans shed virus in saliva, and if human-to-human transmission can be documented. But many other interesting questions remain to be determined. It is not known how FVs have adapted so perfectly to their hosts, while maintaining the ability to be highly cytopathic, at least *in vitro*. The intricate control mechanisms could reveal new strategies for dealing with other cytopathic retroviruses such as HIV. There are many unanswered questions about the unique FV replication pathway. How is Pol packaged into virions? Why is FV RT activated during assembly whereas HIV RT is not activated until after infection of new cells? What is the FV receptor? Where do the interactions between Gag and Env occur and how do they allow viruses to bud from cells? We have just begun to scratch the surface in unraveling the replication of these fascinating retroviruses.

See also: Assembly of Viruses: Enveloped Particles; Feline Leukemia and Sarcoma Viruses; Gene Therapy: Use of Viruses as Vectors; Hepatitis B Virus: General Features; Human Immunodeficiency Viruses: Molecular Biology; Simian Retrovirus D; Vesicular Stomatitis Virus; Viral Pathogenesis.

Further Reading

Lecellier C-H and Saïb A (2000) Foamy viruses: Between retroviruses and pararetroviruses. *Virology* 271: 1–8.

Linial ML (1999) Foamy viruses are unconventional retroviruses. *Journal of Virology* 73: 1747–1755.

Linial ML (2007) Foamy viruses. In: Knipe DM and Howley PM (eds.) *Fields Virology*, 5th edn., ch. 60, pp. 2245–2262. Philadelphia: Lippincott Williams and Wilkins.

Meiering CD and Linial ML (2001) Historical perspective of foamy virus epidemiology and infection. *Clinical Microbiology Reviews* 14: 165–176.

Murray SM and Linial ML (2006) Foamy virus infections in primates. Review. *Journal of Medical Primatology* 35: 225–235.

Trobridge G, Vassilopoulos G, Josephson N, and Russell DW (2002) Gene transfer with foamy virus vectors. *Methods in Enzymology* 346: 628–648.

Foot and Mouth Disease Viruses

D J Rowlands, University of Leeds, Leeds, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Serotype Animals recovered from infection with a virus belonging to a given serotype are resistant to subsequent infection with viruses belonging to that serotype but are still susceptible to infection with viruses belonging to any of the other serotypes.

History

The earliest account that clearly describes foot-and-mouth disease (FMD) was made by Fracastorius in 1546 and it is still today one of the most important diseases of domestic livestock.

It was the first animal disease demonstrated to be caused by a filterable agent in 1897 by Loeffler and Frosch, who also demonstrated the presence of neutralizing antibody in serum. It is also the first virus for which serotype differences were recognized. Further milestones were the demonstration by Waldemann and Pope in 1921 that guinea pigs could be infected and by Skinner in 1951 that it caused a lethal infection in suckling mice. Subsequently, cultivation of the virus in tissue culture cells has enabled studies of viral structure and replication and also the large-scale production of vaccines.

Taxonomy and Classification

Foot-and-mouth disease viruses (FMDVs) are a species within the genus *Aphthovirus* of the family *Picornaviridae*. The nature and organization of the genome, mode of replication, and structure of the virion are, in general, similar to other viruses in the family. The subdivision of the *Picornaviridae* into four genera, *Enterovirus*, *Rhinovirus*, *Cardiovirus*, and *Aphthovirus*, was originally based on physicochemical properties such as susceptibility to acid inactivation, buoyant density of CsCl solution, and the nucleotide composition. Analyses of evolutionary relationships by nucleotide sequence comparisons have largely endorsed the original classifications; however, the family has now expanded to include nine genera. Of special note is the recent inclusion of equine rhinitis A virus (ERAV) in the genus *Aphthovirus*. Properties which distinguish the FMDVs are: (1) extreme sensitivity to acid inactivation (<pH 6.8 in low ionic strength buffer); (2) high buoyant density in CsCl (1.43–1.50 g cm^{-3}); (3) possession of a poly(C) tract in the 5′ untranslated region (UTR) of the RNA (a property shared with some cardioviruses); (4) three separately encoded VPg proteins; (5) the use of two alternative in-frame protein translation initiation sites; and (6) a leader protease protein located in the N-terminal region of the polyprotein.

Properties of the Virion

As for other picornaviruses, FMDV particles are nonenveloped icosahedrons comprising 60 copies each of four structural proteins, VP1–VP4, encapsidating a single copy of the single-stranded positive-sense genomic RNA. The crystallographic structure of the virus (**Figure 1**) has shown that the three larger proteins (VP1–VP3) have the eight-stranded antiparallel β-barrel folding motif seen in other picornaviruses and some plant viruses. VP4 is disposed on the inner surface of the particle. In common with other picornaviruses, VP1 molecules are located around the axes of fivefold symmetry whereas VP2 and VP3 alternate around the two- and threefold symmetry axes. Heat or acid degradation of the particles results in dissociation into pentameric subunits, consisting of five copies each of VP1–VP3, with release of the RNA and VP4.

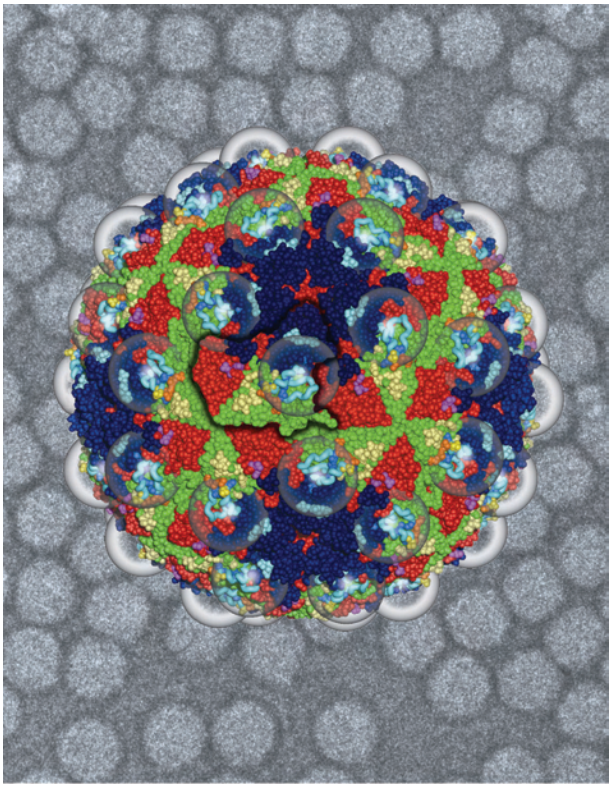


Figure 1 CPK rendition of reduced FMDV, serotype O superimposed on a cryoelectron micrograph of particles. Color codes: VP1, blue; VP2, green; VP3, red (VP4 is internal and not visible in these views). The VP1 G–H loop is shown as a worm in cyan with the Arg-Gly-Asp residues in orange CPK. Antigenic residues are color-coded according to their classification into sites: sites 1 and 5 (mid-blue), site 2 (pale yellow), site 3 (light blue), site 4 (magenta). The potential occupancy of the mobile VP1 G–H loop is modeled by a transparent sphere centered at the midpoint between the two ends of the loop. A protomeric subunit is outlined in black. Courtesy of E. Fry and D. Stuart.

Several features of the structure are unique to FMDV. The protein shell is generally thinner and the external surface is smoother than in other picornaviruses. This is a result of the smaller sizes of VP1–VP3 (VP1 213, VP2 218, VP3 220 amino acids for serotype O1 virus) compared to other picornaviruses. The truncations are in the loop regions linking the core elements of the β barrels, which in other picornaviruses form prominent features at the outer surface. The deep grooves or pits encircling the fivefold axes of many picornaviruses are not present in FMDV and the position equivalent to these invaginations

is occupied by the C-terminal portion of VP1. An important exception to the generally smooth contours of the surface of FMDV is provided by the G–H loop of VP1 (**Figure 1**). This large loop extends from about residue 130 to 160 of which residues around 135–158 are too disordered to be visible in electron density maps. In serotype O1 viruses, the disorder of the VP1 G–H loop is induced by a disulfide bond between the cystine residues VP2 130 and VP1 134. Under reducing conditions this bond is broken and the G–H loop collapses onto the surface of the virus in an ordered configuration. This feature includes an immunodominant antigenic site to which a high proportion of virus-neutralizing antibodies are directed. Also, synthetic peptides representing sequences from this region are immunogenic and can induce protective immunity. The sequence of this region is variable both in composition and length between different viruses with the exception of a highly conserved receptor binding motif which includes the triplet, Arg, Gly, Asp (see the section titled 'Cell attachment and entry').

Properties of the Genome

The genome consists of a single molecule of single-stranded positive-sense RNA, which is infectious. The order of the gene products on the genome is basically similar to other picornaviruses but there are some unique features (**Figure 2**). The genomic RNA terminates at the 5′ untranslated end with a small protein, VPg, linked by a phosphodiester bond through a Tyr. There is a variable length of poly(A) tract at the 3′ end. Uniquely, the FMDV genome encodes three distinct VPgs, which are used with equal efficiency. The reasons for this gene triplication are unknown. In fact, virus derived from an infectious cDNA clone from which one or two of the VPg copies had been deleted is still infectious, although RNA synthesis is reduced. The 5′ UTR is exceptionally long (*c.* 1300 nt), even by picornavirus standards (**Figure 3**). There is an uninterrupted poly(C) tract of 100–200 residues, depending on the virus isolate, located *c.* 400 nt from the 5′ end. The function of the poly(C) tract is unknown and it is not present in ERAV. There is evidence from FMDV and mengovirus (a cardiovirus) that the length of poly(C) is related to pathogenicity.

The sequence between the 5′ end and the poly(C) tract has a high degree of secondary structure and is predicted to fold into an almost complete hairpin. Its function is unknown but by analogy to the highly structured 5′ sequence of poliovirus RNA it is likely to be involved in the control of RNA replication. To the 3′ side of the poly(C) tract there are a variable number of repeat domains that are predicted to fold as pseudoknot structures. The 3′ 435 nucleotides of the 5′ UTR fold into a series of stem/loop structures similar to those present in the equivalent region of cardiovirus 5′ UTRs and function as an internal ribosome entry site (IRES) to initiate protein synthesis. A stem/loop structure (*cre* – *cis*-active replication element), located between the pseudoknots and the IRES, functions as a template for the uridylation of VPg, which is an essential step in the initiation of viral RNA synthesis.

Protein Products

The protein-coding region is a continuous open reading frame of 6999 or 6915 nt for FMDV serotype A[10],

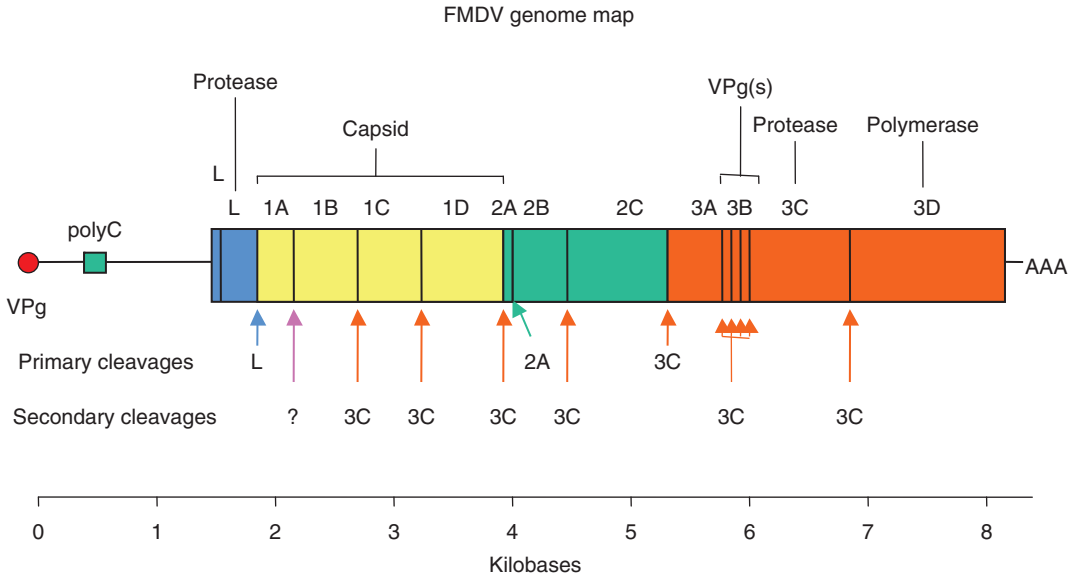


Figure 2 Genome map of FMDV. Boxed region is the polyprotein translation product with processing sites arrowed.

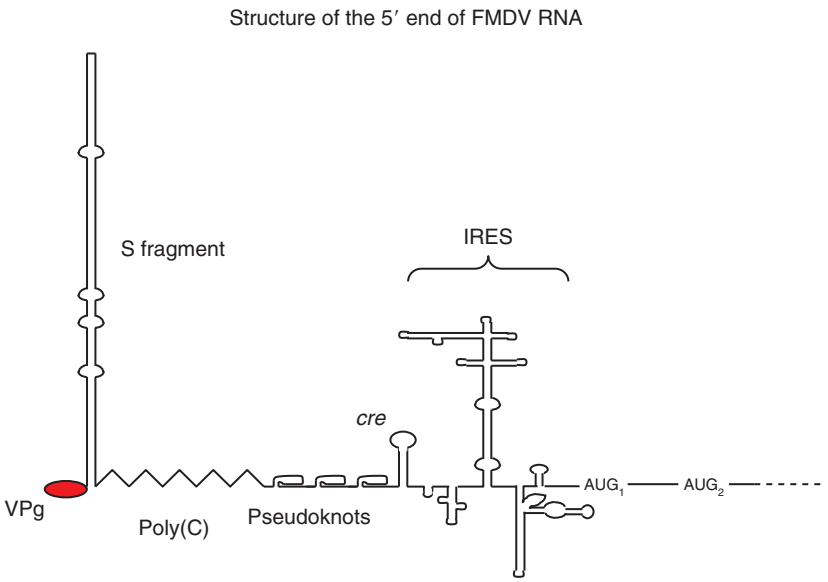


Figure 3 Secondary structure prediction for the 5′ UTR of FMDV RNA with major functional motifs individually labeled.

depending on which of two functional in-frame initiation codons is used. The order of the gene products is shown in **Figure 2**.

Leader protein. The leader protein(s), Lab and Lb, which precede the structural proteins, have a proteolytic function which cleaves at the L–P1 junction and also affects the cell translation machinery (see the section titled 'Translation'). Lb can perform all of the known functions of L and the significance of Lab is unknown, although presence of two polyprotein initiation sites is completely conserved. The L proteins are not essential for virus viability since virus derived from an infectious clone with deleted L domain can replicate. However, it has an attenuated phenotype and has been proposed as a candidate live vaccine.

P1 region. The P1 region consists of the structural proteins 1A, 1B, 1C, and 1D, which are equivalent to VP4, VP2, VP3, and VP1, respectively.

P2 region. The 2A protein is vestigial in size compared to other picornaviruses, being only 18 amino acids long, but it enables the nascent separation of the polyprotein at the 2A–2B junction. 2C has a nucleoside triphosphate binding motif and it appears to have a role in RNA replication since amino acid changes in this protein can relieve the inhibition of RNA synthesis seen in the presence of guanidine. The precursor protein 2BC influences membrane trafficking in infected cells, a role performed by 3A in poliovirus.

P3 region. The role of 3A in FMDV is unclear but it does not seem to function in a similar manner to the 3A protein of poliovirus, which serves as a membrane-bound donor of VPg during viral RNA synthesis.

3B or VPg occurs as three tandem copies of 23, 24, and 24 amino acids. Although differing in sequence, each is rich in Pro, Arg, and Lys residues and has a single Tyr at position 3. Each of the individual VPg molecules is highly conserved between the A, O, and C serotype viruses. The VPg molecules are post-translationally modified to function as primers in RNA synthesis (see next section). All encapsidated RNA molecules terminate with a VPg molecule and each of the three forms is found in equal abundance. Actively translating viral RNA lacks VPg, and cell extracts contain an enzyme that cleaves the phosphodiester bond to produce RNA terminating with a 5′-monophosphate. The observation that all virion-associated RNA terminates in VPg suggests that it may have a role in the selection of molecules for encapsidation. Mutagenesis experiments with an infectious cDNA clone have provided some support for this conclusion.

$3C^{pro}$ is the protease responsible for the majority of the processing cleavages. In common with other picornaviruses, sequence analysis suggests that the catalytic site of $3C^{pro}$ is related to trypsin, a serine protease, but with the replacement of the nucleophilic serine residue with cysteine.

$3D^{pol}$ is the RNA-dependent RNA polymerase responsible for RNA replication and VPg uridylation (see next section). The crystal structure has been solved in the apo form and in complex with primer and template.

The 3CD precursor has a distinct function as a catalyst for the $3D^{pol}$-mediated uridylation of VPg.

The molecular structures of virus particles, L^{pro}, $3C^{pro}$, $3D^{pro}$, and precursor 3CD have all been determined by X-ray crystallography.

RNA Replication

RNA of infecting virus functions as a template for the synthesis of a negative-sense complementary strand, which

serves, in turn, as template for the synthesis of positive-sense strands, identical to the original infecting molecule. Positive strands are synthesized in a complex structure (replicative intermediate (RI)) consisting of a single negative-strand template and several (around six) nascent positive strands. RNA synthesis is asymmetrical in favor of positive strands. A proportion of the negative-strand templates occur as full-length double-stranded hybrids (replicative form (RF)) and appear to take no further part in RNA synthesis. RF molecules accumulate in the cell during viral replication. Each RNA replication event is initiated by priming with a uridylated VPg molecule.

A single molecule of viral RNA is sufficient to initiate infection, which implies that it can function sequentially as a template for translation, to produce the polymerase enzyme(s), and as a template for RNA replication. Single-stranded viral RNA is infectious in the presence of inhibitors of host cell DNA-dependent RNA polymerase. Double-stranded viral RNA is also infectious but not in the presence of inhibitors of the cellular polymerases.

Translation

FMDV RNA is efficiently translated in a cell-free system (rabbit reticulocyte lysate) to produce protein products similar to those found in infected cells. The 435 nucleotides upstream of the first AUG initiation codon are folded into a complex structure similar to that of the equivalent region of cardiovirus RNAs. This sequence acts as an IRES, allowing initiation of translation in the absence of the host cell cap-binding complex. The rate of total protein synthesis in virus-infected cells does not change until its decline toward the end of the growth cycle, when cytopathic effects (CPEs) are apparent. There is, however, a marked change in the profile of proteins produced. When viral replication is maximal, virtually no host cell proteins are produced. This 'swap-over' of translation from host to viral products is similar to the situation in cardiovirus-infected cells and differs from the kinetics of translation following infection of cells with entero- or rhinoviruses. In the latter, infection results in a rapid shutdown of host cell protein translation, which is followed later by a resumption of protein synthesis due to the increasing production of viral proteins. The shutdown induced by entero- and rhinoviruses is largely, if not entirely, due to a virus-induced cleavage of a host protein, p220 or eIF4G, an important component of the cap-binding complex required for the initiation of translation of host mRNAs. In these viruses, eIF4G cleavage is indirectly induced by $2A^{pro}$ protease. Cardioviruses do not induce eIF4G cleavage and appear to simply outcompete host mRNAs for utilization of the translation machinery. Although the kinetics of protein translation in FMDV-infected cells resemble those of cardiovirus-infected cells, eIF4G is cleaved. In contrast to entero- and rhinoviruses, FMDV cleavage of eIF4G is not induced by 2A but by L. $3C^{pro}$ can also cleave eIF4G but at a different site from L^{pro} and later in the infection cycle. Inhibition of host cell protein synthesis is likely to be of advantage to the virus both by removing competition for access to the translation machinery and also by inhibiting the expression of innate immunity response genes in infected cells.

Post-Translation Processing

The polyprotein translation product of FMDV RNA is proteolytically processed by three of four virus-encoded enzyme activities (**Figure 2**). Three of the cleavages occur nascently on the growing polypeptide chain. The first separates L from P1, the structural protein precursor, and is carried out by leader protein. The cleavage is within a Lys-Gly dipeptide and probably occurs normally in *cis* but can also occur in *trans*. L–P1 cleavage is the only processing step which is inhibited by the tripeptide D-Val-Phe-Lys-CH_2Cl. Both Lb and Lab are proteolytically active.

The second primary cleavage occurs at the junction of 2A and 2B and is catalyzed by the 18-amino-acid 2A sequence. There is good evidence that the mechanism of 'cleavage' at the 2A/2B junction involves a novel process of interrupted translation, referred to as ribosomal skipping, rather than proteolytic cleavage.

The third primary cleavage is between 2C and 3A and is catalyzed by $3C^{pro}$ protease. This protease is responsible for all other processing cleavages, apart from that which generates VP2 (1B) and VP4 (1A) from the precursor VP0 (1AB). Many cleavages catalyzed by $3C^{pro}$ protease occur at Glu-Gly junctions but other dipeptides are recognized and $3C^{pro}$ of FMDV is the most promiscuous of the picornavirus proteases. The cleavage event to produce VP2 and VP4 from the precursor, VP0, occurs in the final stages of virus maturation. The mechanism of this cleavage is not known.

Virus Assembly and Release

A variety of assembly intermediates containing equimolar amounts of VP1, VP2, and VP0 are detected in infected cells. These correspond to monomer and pentamer subunits of the icosahedral capsid and 75S empty particles, which lack viral RNA but possess antigenic properties similar to mature viral particles. Following pulse-labeling experiments, empty particles can be 'chased' into viral particles, but it has not been shown that they are on the direct morphogenetic pathway. All encapsidated RNA

terminates with VPg, suggesting that this plays a role in selection. Altered VPg molecules within an infectious cDNA clone are less efficiently encapsidated.

Paracrystalline arrays of virus are visible in infected cells and are released by lysis of the cell. There is evidence that some viral particles are secreted prior to cell disruption.

Cell Attachment and Entry

The large mobile G–H loop of VP1 located at the surface of FMDV particles mediates their binding to susceptible cells. The sequence of the loop contains a highly conserved motif, Arg, Gly, Asp, which is the hallmark of ligands for a number of heterodimeric cell surface molecules called integrins. Synthetic peptides including this sequence can compete with virus for cell attachment and treatment of the virus with proteolytic enzymes such as trypsin, which cleave within the G–H loop, also prevents virus binding. Although the virus can bind to a number of integrins there is good evidence that $\alpha_v\beta_6$ is the preferred receptor *in vivo*.

Following attachment the virus is internalized by endocytosis and release of the RNA is triggered by acidification in early endosomes. Reduction of the pH below ~6.8 seems to be all that is required to initiate the infection process and the mechanism by which the genome is delivered to the cytoplasm is unknown. Many tissue-culture-adapted strains of the virus have evolved *in vitro* to use heparin sulfate at cell surface as an alternative receptor.

Geographic Distribution

FMD occurs widely and is endemic in many countries, especially in tropical regions (**Figures 4** and **5**). North America, Australia, New Zealand, and Japan are free of the disease and maintain this status by rigorous application of import controls and quarantine. Mass vaccination campaigns have virtually eliminated the virus from some areas, for example, Europe, but have been less effective in others, largely due to logistical problems of vaccine distribution and the techniques of animal husbandry employed. The global distribution of the serotypes is shown in **Figures 4** and **5**.

Host Range and Viral Propagation

The virus typically infects cloven-hoofed species with domestic cattle being the most susceptible. Domestic pigs are also important hosts and are particularly effective in propagating the disease, since they secrete large quantities of virus in the form of aerosols. In sheep and goats, the clinical manifestations of infection are usually less

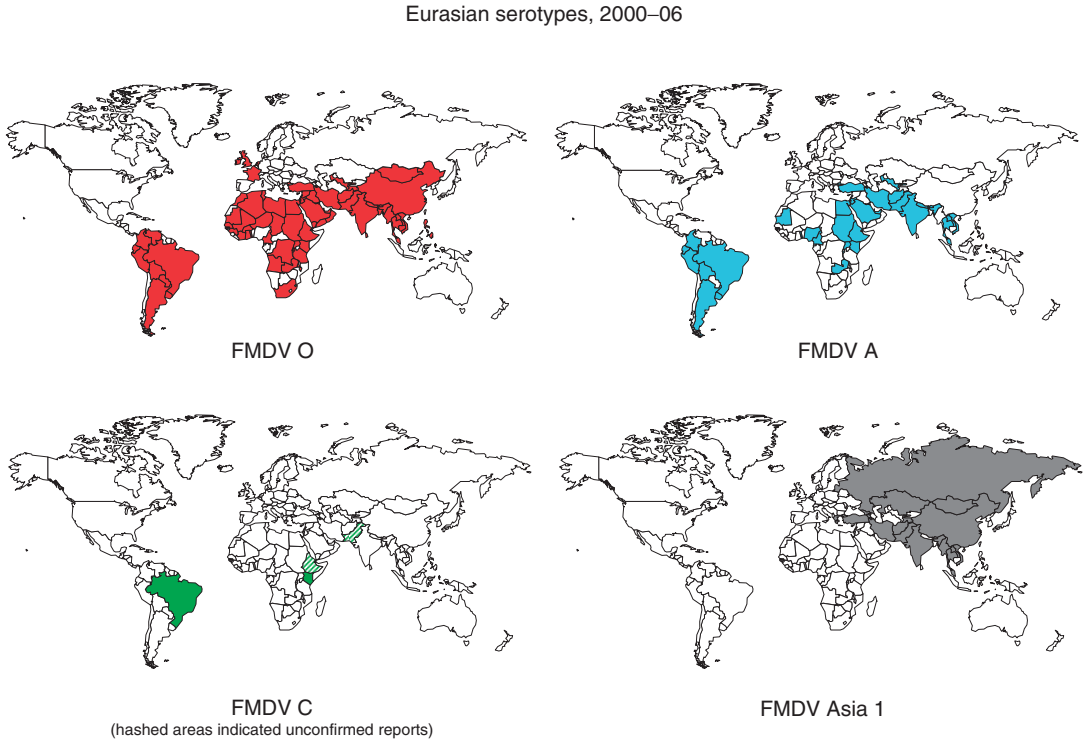


Figure 4 Global distribution of FMDV serotypes O, A, C, and Asia 1 between 2000 and 2006. Courtesy of N. Knowles.

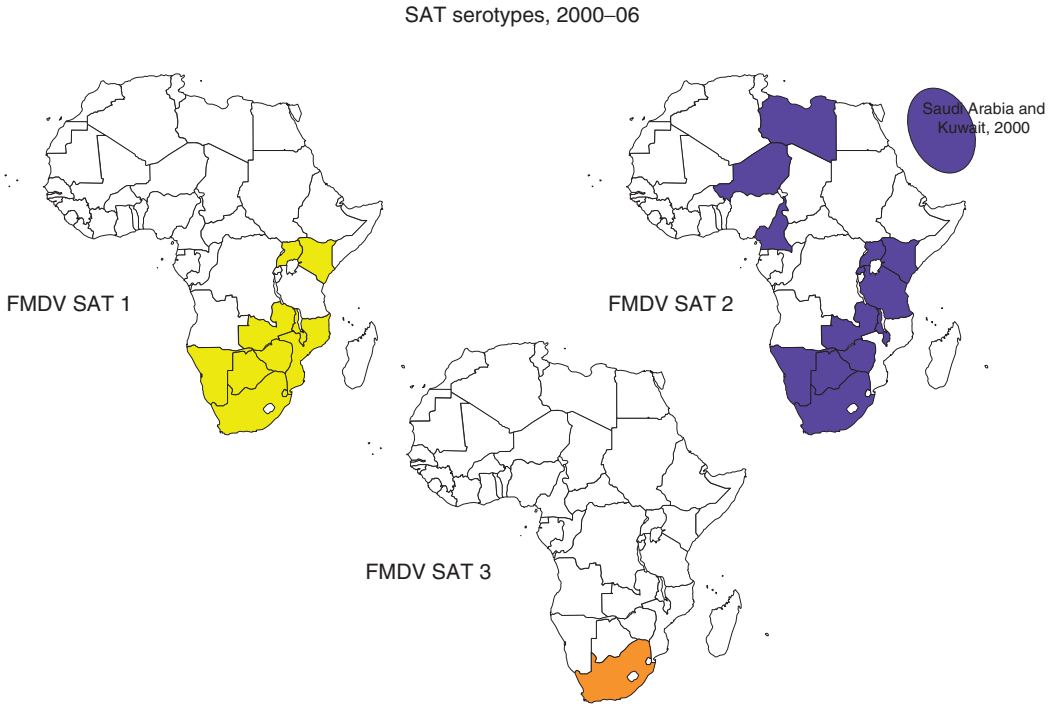


Figure 5 Distribution of FMDV serotypes SAT 1, SAT 2, and SAT 3 between 2000 and 2006. Courtesy of N. Knowles.

severe than those seen in cattle and pigs. Natural infection of Indian elephants and of camels has been reported. Many wild species of deer and antelope are susceptible to infection, and in African Cape buffalo infection is asymptomatic. Persistent infection with prolonged shedding of virus for months or years has been reported in wild and domestic species. A wide range of animals, including Australian marsupials and birds, have been infected under laboratory conditions and, very rarely, infection of human has been demonstrated.

The most important small animals for laboratory investigations are the guinea pig and the suckling mouse. In the former, injection of virus intradermally into plantar pads results in the formation of vesicular lesions both at the site of injection and in the mouth and the remaining feet, and so resembles the lesion distribution in naturally infected susceptible species. Intraperitoneal infection of suckling mice results in rapid death. The viruses can be propagated in primary cells and cell lines of bovine or porcine origin. Cells derived from the BHK-21 line are most widely used for research or vaccine production purposes. The virus can be titrated by plaque assay in cultured cell monolayers or by cytopathic end point dilution assay in microtiter plates.

Genetics and Evolution

In common with other RNA viruses, the mutation rate is extremely high and virus populations exist as quasispecies in which each individual genome is likely to differ from every other. Antigenic sites on the viral particle are tolerant of sequence variation, and antigenic diversity is a significant property of the virus.

In addition to evolution by the accumulation of point mutation, genomic recombination occurs at a high rate *in vitro*. The frequency and genomic location of recombinatorial events mirror the genetic relatedness of the parental viruses.

Serological Relationships and Variability

Seven serotypes of FMDV are recognized, the distinction of serotypes being that an animal convalescent from infection by virus of one serotype is fully susceptible to viruses of any of the remaining six. In addition to the major serotype differences, there is considerable antigenic variation between viruses within serotypes. The serological relationships between FMDV isolates are paralleled by genetic relationships as evidenced by RNA sequence analyses (**Figure 6**).

For epidemiological studies and vaccine strain selection, the serological relationships between field virus isolates or laboratory strains are expressed as *r* values, that is, the ratio of the neutralizing titers of immune sera against heterologous and homologous viruses. The serological relationships between virus isolates are frequently nonreciprocal, showing that closely related viruses may induce broadly cross-reactive or narrowly specific immune responses.

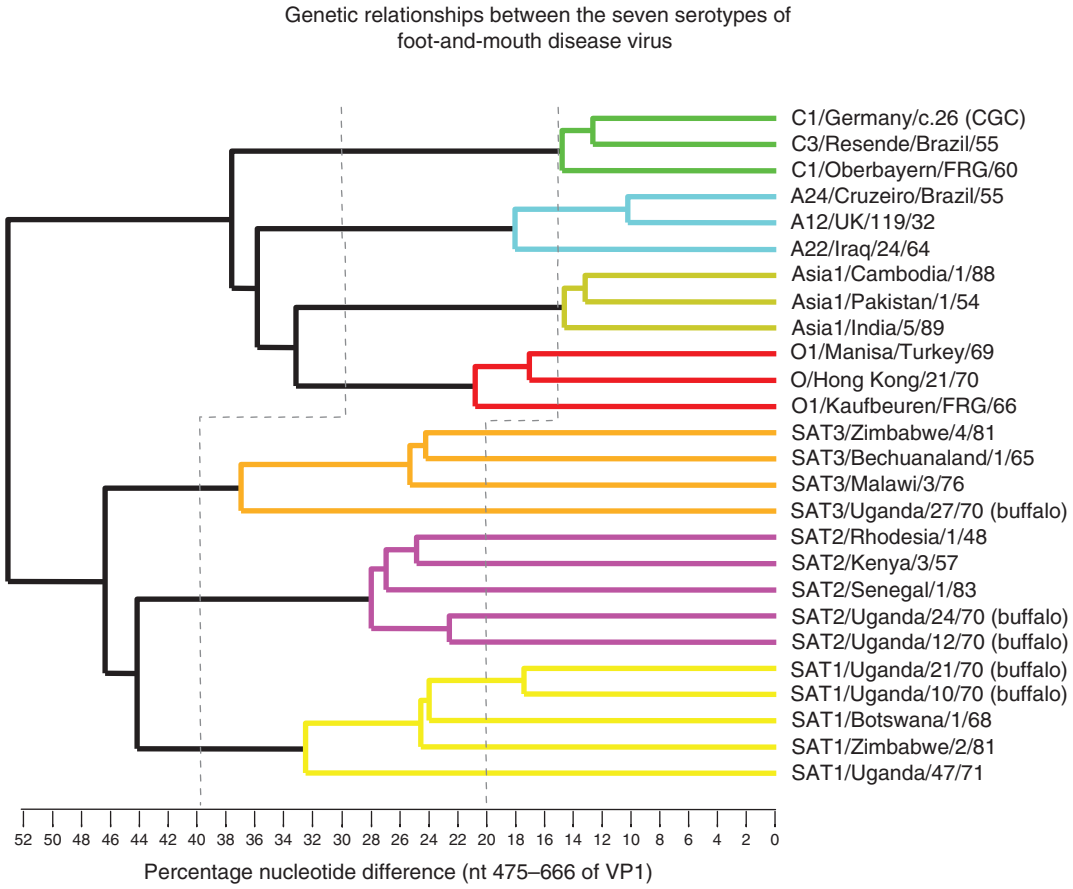


Figure 6 Genetic relatedness of FMDV strains and serotypes based on sequences within the VP1 gene. Courtesy of N. Knowles.

Complement fixation assay and enzyme-linked immunosorbent assay (ELISA) using polyclonal sera or monoclonal antibodies are also used for epidemiological studies.

Epidemiology

In regions endemic for FMD, the virus is most likely maintained in persistently infected animals. It has been shown experimentally that infected bovines can secrete virus for long periods after the initial episode of disease. In some areas the wild animal population may act as a reservoir for infection (e.g., Cape buffalo in Africa). In nonendemic areas, infection may be introduced from a variety of sources such as the importation of infected livestock, contaminated animal products such as carcasses containing bone (in contrast to meat, the postmortem acidification of bone marrow and other nonmuscular tissues is insufficient to inactivate the virus), or contaminated materials. More locally, transmission of infection is by direct transport of contaminated animals or materials or by wind-borne carriage of infectious aerosols. It is also suspected that the virus can be passively transmitted by migrating birds.

Transmission and Tissue Tropism

The principal route of infection appears to be via aerosol impinging on the pharynx and respiratory tract. Aerosols may be produced locally during feeding on contaminated foodstuff or may be transmitted over considerable distances under appropriate meteorological conditions. Pigs secrete particularly high levels of virus-contaminated aerosols. In addition to mucosal secretion, high levels of virus are found in milk. Pasture may be contaminated with virus from urine and feces.

Vesicular lesions appear in the mouth on the tongue, gums, and cheeks and later on interdigital mucosa and coronary bands of the feet. Virus can be isolated from many tissues in the body. The onset of clinical disease is usually very rapid and lesions can develop as early as 1–2 days after infection, depending on the virus strain and level of exposure.

Pathogenicity

The pathogenicity of FMDV varies according to the virus strain, host species, and age. The factors that govern the

virulence of FMDVs are poorly understood and as with most viruses are probably multifactoral. Domestic cattle are usually the most susceptible species and morbidity is usually *c.* 100% in nonvaccinated animals. Wild bovines, such as African Cape buffalo, may produce no clinical manifestations. Although the disease is rarely fatal in adult domestic animals (<5%), significant mortality may occur in young animals (*c.* 50%).

Clinical Features of Infection

Infection typically produces a rapidly progressing febrile illness and the development of often massive vesicular lesions in the mouth and on the feet. The lesions rupture with considerable loss of epithelial tissue (**Figure 7**). The resulting discomfort discourages feeding until the lesions heal by the infiltration of fibrous tissue. The severity of the disease results in long-term loss of productivity in terms of meat and milk yield, and lameness may be a serious consequence for draught animals. Abortion and chronic subfertility are also common. Other organs infected include mammary glands, pancreas, and heart.

Immune Response

Infection elicits a vigorous humoral antibody response and, after recovery, immunity to reinfection with viruses of the same serotype is prolonged. The role of cytolytic T cells in recovery is unclear.

Four antigenic sites recognized by antibodies capable of neutralizing the virus have been described. One of these is an immunodominant linear sequence comprising the G–H loop of VP1, and synthetic peptides representing this tract are effective in inducing high levels of virus-neutralizing antibody and can protect animals from infection.



Figure 7 Severe symptoms of FMD showing extensive sloughing of linguinal epithelium. Crown copyright. Reproduced from ''Foot and mouth disease: Ageing of lesions'' (Defra, 2005) by permission of the Controller of HMSO.

Antibody responses to several of the nonstructural proteins are induced during infection and these are being developed as markers to distinguish vaccinated from infected animals.

Prevention and Control

In disease-free regions, natural protection afforded by geographical barriers is rigorously reinforced by strict controls on the importation of susceptible animals and potentially contaminated materials. Where outbreaks occur sporadically, due to occasional introduction from external sources, embargoes on animal movement and slaughter of infected herds have been successful in maintaining a disease-free national herd. In endemic areas, control is by mass vaccination. Ring or barrier vaccination is also used to limit the spread of infection.

Dramatic demonstrations of the vulnerability of naive livestock to large-scale epidemics of FMD were provided by the outbreaks of serotype O virus in the UK and of serotype A virus in South America in 2001. The outbreak in the UK resulted in the slaughter of over 10 million animals (most of which were uninfected) to control spread of the disease and the cost to the country was several billions of pounds. In South America, the outbreak was controlled by the resumption of blanket vaccination of all livestock.

The first vaccines against FMD were produced by formalin inactivation of lymph drawn from lesions on the tongues of infected cattle. This source of immunizing virus was replaced by the Frenkel method of culture in fragments of epithelium stripped from the tongues of slaughtered cattle. Most vaccine in use today is produced by growing the virus in suspensions of BHK-21 cells in fermentation vessels of up to 10 000 l capacity. Approximately 2×10^9 monovalent doses of vaccine are administered annually. Aziridines have largely replaced formalin as the inactivant, since the inactivation kinetics of the latter are nonlinear and residual live virus in vaccines has occasionally been the source of outbreaks of disease. The serotype and strain composition of vaccines have to be tailored for local requirements. Inactivated virus is usually adjuvanted by adsorption onto aluminum hydroxide gel, and saponin may also be added to enhance potency. In pigs, such vaccines elicit only immunoglobulin M responses, and for this species vaccines are formulated with oil adjuvants. The use of oil-adjuvanted vaccines are now being extended to cattle.

Solid protection requires high levels of neutralizing antibodies, and, to achieve this with inactivated vaccines, immunization is repeated two to three times a year. The development of live-attenuated vaccines has largely been abandoned mainly due to the complexity of antigenic diversity and the fear of reversion to a virulent phenotype.

Future Perspectives

There is scope for developments in FMD vaccines to improve stability, cross-protective efficacy, and duration of immunity. Peptide vaccines, recombinant viral capsids, and rationally designed nonreverting attenuated viruses are potential routes by which these goals may be achieved. The production of non-biodegradable peptides and the creation of an attenuated L protein deletion mutant may be important developments. Since the massive outbreaks in the UK and in South America in 2001, there is increased interest in the development of drugs to halt the rapid spread of infection. Improved rapid diagnostic methods are being explored as is the ability to accurately distinguish infected from vaccinated animals.

As to the molecular properties of the virus, both the determination of the crystallographic structure of the particle and several of the nonstructural proteins and the cloning and manipulation of full-length infectious cDNA molecules are important steps toward understanding the unique features of the virus.

See also: Antigenic Variation; Antigenicity and Immunogenicity of Viral Proteins; Cardioviruses; Evolution of Viruses; Immune Response to viruses: Antibody-Mediated Immunity; Picornaviruses: Molecular Biology; Replication of Viruses; Viral Receptors; Virus Particle Structure: Nonenveloped Viruses.

Further Reading

Barteling SJ and Vreeswijk J (1991) Developments in foot and mouth disease vaccines. *Vaccine* 9: 75–88.
Brooksby JB (1982) Portraits of viruses: Foot and mouth disease virus. *Intervirology* 18: 1–23.
Brown F (1989) The development of chemically synthesised vaccines. *Advances in Veterinary Science and Comparative Medicine* 33: 173.
Defra (2005) Foot and Mouth Disease – Ageing of Lesions. http://www.defra.gov.uk/footandmouth/pdf/ageing-lesions.pdf (accessed January 2008).
Fry E, Logan D, Fox G, Rowlands D, Brown F, and Stuart D (1990) Architecture and topography of an aphthovirus. *Seminars in Virology* 1: 439–451.
Mahy BWJ (ed.) (2005) *Foot-and-Mouth Disease Virus.* Heidelberg: Springer.
Rowlands DJ (ed.) (2003) Special issue: Foot-and-mouth disease virus. *Virus Research* 91: 1–161.
Sobrino F and Domingo E (eds.) (2004) *Foot-and-Mouth Disease – Current Perspectives.* Norfolk, UK: Horizon Bioscience.

Fowlpox Virus and Other Avipoxviruses

M A Skinner, Imperial College London, London, UK

© 2008 Elsevier Ltd. All rights reserved.

Taxonomy

Fowlpox virus, the prototype of the *Avipoxvirus* genus, is the best-studied species. There are currently nine other recognized species (*Canarypox virus*, *Juncopox virus*, *Mynahpox virus, Pigeonpox virus*, *Psittacinepox virus, Quailpox virus, Sparrowpox virus, Starlingpox virus,* and *Turkeypox virus*) and three tentative species (Crowpox virus, Peacockpox virus, and Penguinpox virus). Avipoxvirus infections have been observed in more than 230 of the known 9000 species of birds, spanning 23 orders, yet little is known about the genome diversity, host range, and host specificity of the causative agents.

History

With its characteristic lesions, ubiquitous distribution among domesticated poultry, and large virion size, fowlpox virus (FWPV) was one of the earliest recognized viruses. It also played important roles in the development of modern virological techniques during the middle decades of the twentieth century. Fowlpox was one of the first diseases of livestock and poultry for which effective vaccines were developed, as early as the late 1920s. These vaccines led to effective control of the disease and its virtual eradication from commercial poultry production in temperate regions. Fowlpox remains enzootic in most tropical and subtropical regions where poultry is produced. It represents, after Newcastle disease, the second largest virus infection of backyard poultry in Africa and is thus of considerable socioeconomic importance.

Virion Structure

Like other poxviruses, the brick-shaped avipoxviruses are large enough (at $330\,\text{nm} \times 280\,\text{nm} \times 200\,\text{nm}$) to be resolved by light microscopy. Indeed, the particles were originally observed following staining with basic dyes

(such as basic fuchsin in Gimenez stain) and were termed 'elementary particles' or 'Borrel bodies'. Overall, the structure of the avipoxvirus virion is assumed to be similar to that of the much better studied vaccinia virus (VACV). Likewise, the replication cycle of the avipoxviruses is assumed to be essentially similar to that elucidated for the mammalian poxviruses. However, there are differences between avipoxviruses and the commonly studied mammalian poxviruses in the complement of structural genes and in morphogenesis of extracellular enveloped virions (described below), both indicative of significant structural differences at the molecular level. As a consequence, it is not clear which proteins are present in the envelope of avipoxvirus extracellular enveloped virus (EEV).

Genome Size and Organization

The avipoxviruses appear to have some of the largest known genomes of viruses of vertebrates, more than 300 kbp for canarypox virus (CNPV), encoding about 300 proteins. By way of comparison, this is 100 times the size of the smallest animal virus genomes and half the size of the genome of the smallest free-living bacterium. Among mammalian poxviruses, the central two-thirds of the genome generally encode conserved structural proteins and enzymes. In contrast, the terminal regions are more variable and divergent, encoding genus and species-specific, nonessential proteins, which are frequently involved in virus–host interactions affecting host–range, pathogenesis, and virulence. This general distribution holds in the avipoxviruses but, relative to the mammalian poxviruses, several large genome rearrangements (translocations, inversions, and transversions), which could have occurred in either avian or mammalian lineages (or both), have resulted in transfer of likely nonessential genes into more central locations.

Gene Complement

Although avipoxviruses appear to encode equivalents of most of the internal structural proteins of the VACV core, they lack some important proteins found on the surface of the intracellular mature virus (IMV) of VACV (such as D8 and A27), on the surface of the EEV (such as A33, A56, and B5), as well as on the surface of intracellular enveloped viruses, or IEV (such as A36).

Avipoxviruses also encode proteins not found in mammalian poxviruses, such as homologs of the host PC-1 nucleotide phosphodiesterase, DNaseII (DLAD), alpha-SNAP, and the lipid-pathway enzyme involved in Stargardt's macular dystrophy (ELOVL4). Such proteins appear to be nonessential for replication in tissue culture and are presumed to be nonstructural. Avipoxviruses also encode multiple members of several gene familes, notably the 31 ankyrin-repeat proteins encoded by FWPV (51 by CNPV) and the six copies of the massive B22 ortholog (2000 amino acid residues) found only as a single copy in some mammalian poxviruses, such as variola virus (VARV), cowpox virus (CPXV), and molluscum contagiosum virus (MOCV). The latter gene family therefore accounts for about 35 kbp of the additional sequence found in the avipoxvirus genome.

Antigens

Three major immunodominant antigens of FWPV that are recognized by murine monoclonal antibodies have been identified. They correspond to immunodominant antigens recognized by hyperimmune serum from FWPV-infected chickens and to equivalent immunodominant antigens of mammalian poxviruses. They are the 30/35 kDa IMV surface protein fpv140 (corresponding to VACV H3), the 39 kDa virion core protein fpv168 (corresponding to VACV A4), and the 63 kDa 'virion occlusion' protein fpv191 (corresponding to the p4c protein retained in very few VACV strains). Neutralizing antisera or monoclonal antibodies have not been reported.

Survival Factors

A-Type Inclusion Protein

The avipoxviruses express a number of proteins that might be considered as factors to potentiate their survival in the face of various environmental stresses. These include the A-type inclusion (ATI) protein, which forms large cytoplasmic inclusions. These inclusions can contain large assemblies of embedded virions (analogous to spheroidin inclusions in the entomopoxviruses), which would probably be better protected from dessication in desquamated dermis. It is likely that entry of avipoxviruses into the ATI requires the presence of fpv191 (p4c, virion occlusion protein) on the surface of the IMV, assuming the mechanism is as elucidated for CPXV.

Photolyase

Avipoxviruses encode photolyases (e.g., fpv158), which are capable of repairing ultraviolet (UV)-induced pyrimidine dimers in DNA in a light-dependent manner. Such lesions might be induced in viral genomic DNA when the virion is exposed to the environment in desquamated epithelium, or while the virion is near the surface of a skin lesion.

Glutathione Peroxidase

Like MOCV, avipoxviruses encode a glutathione peroxidase. Such proteins might be able to protect the virus

from environmental oxidative stress. However, the FWPV protein (fpv064) is probably not an ortholog of the MOCV protein (MC066), which is most closely related to cellular glutathione peroxidase. Instead it is probably a paralog, most closely related to cellular phospholipid hydroperoxide glutathione peroxidase. The avipoxvirus proteins might protect infected cells from the oxidative burst of immune cells or affect cell signaling pathways.

Immunomodulators

Unlike most mammalian poxviruses, but in common with MOCV, no obvious candidates for proteins interfering with the host type I interferon response have been identified. Thus the avipoxviruses lack orthologs of the VACV double-stranded RNA-binding protein (E3) and the eIF2 mimic (K3). No soluble homologs of type I interferon receptors (equivalent to VACV B18) have yet been identified in the avipoxviruses, although a soluble binding protein (e.g., fpv016) for type II interferon has been identified biochemically. Conversely, avipoxviruses also encode proteins not generally found in mammalian poxviruses, such as homologs of transforming growth factor β (e.g., fpv080), which has also been found in deerpox virus, and IL10 (e.g., CNPV018), also found in orf parapoxvirus.

No obvious soluble chemokine-binding proteins encoded by avipoxviruses have been identified, in contrast to the mammalian poxviruses. However, proteins resembling cellular serpentine chemokine receptors of the 7-transmembrane spanning G-protein-coupled receptor family are encoded by avipoxviruses (e.g., fpv021, fpv027, and fpv208), as are single copies by some mammalian poxviruses (capripoxviruses, suipoxviruses, yatapoxviruses, and deerpox virus). Like MOCV, avipoxviruses encode putative IL18-like proteins (e.g., fpv214) and chemokine-like proteins (e.g., fpv060, fpv061, fpv116, and fpv121).

Features of Avipoxviruses Shared with Molluscum Contagiosum Virus

The mammalian poxvirus to which the avipoxviruses are most closely related phylogenetically is MOCV. The avipoxviruses also share a number of other features with MOCV. For example, they lack orthologs of the VACV proteins E3 and K3 involved in evading the type I interferon response. Whereas avipoxviruses encode a family of proteins (e.g., fpv097, pfv098, fpv099, fpv107, fpv122, and fpv123) with homology to the large VARV protein B22, MOCV (like VARV and CPXV) encodes only a single protein (MC035). In CPXV, as in VARV, the gene encoding the B22 ortholog (CPXV219) is located in the variable region at the right terminus of the genome. The gene encoding the MOCV ortholog MC035 is, however, more central, located between orthologs of VACV E4L and E6R. This is syntenic with the location of FWPV genes encoding B22 orthologs fpv097, fpv098, and fpv099, also found between orthologs of VACV E4L and E6R.

Like the avipoxviruses, MOCV lacks orthologs of VACV IMV surface proteins A27 and D8 as well as EEV proteins A56 and B5 (though, unlike the avipoxviruses, MOCV does encode orthologs of VACV EEV protein A33 and IEV protein A36).

Virus Replication

Host Range Restriction

Avipoxviruses are incapable of productive replication in mammalian cell lines, including those (such as BHK-21) permissive or semipermissive for the host-range-restricted MVA variant of VACV, and those defective in the type I interferon pathway, such as Vero cells. Otherwise, the replicative cycle is largely similar to that elucidated for VACV, although considerably slower (taking about 24 h rather than 12 h). Genome transcription and replication mechanisms are essentially the same, with relatively well conserved polymerases and other enzymes. VACV promoters function well in cells infected with avipoxviruses and are frequently used to drive foreign gene expression in recombinant avipoxviruses, and the inverse appears to be true. Virus-induced shut-off of host gene expression, clearly observed with VACV, is far less dramatic with FWPV.

Genetic Reactivation

The long-recognized phenomenon of 'genetic reactivation' illustrates that many of the essential replicative components of poxviruses are interchangeable. Thus the proteins of a UV-inactivated poxvirus can rescue the genome of a heat-inactivated poxvirus, even when the two viruses are from different genera. This is well illustrated by the modern practise of rescuing naked (and often genetically manipulated) genomic DNA of a mammalian poxvirus by infection of transfected cells with FWPV. The rescued mammalian poxvirus can be recovered and amplified by passage through mammalian cells, which eliminates the rescuing FWPV due to its host range restriction.

Virion Morphogenesis

The most obvious difference between the replication cycles of avipoxviruses and that of VACV is in the formation of EEV. VACV EEV formation predominantly involves wrapping of IMV with a double membrane derived from the *trans*-Golgi network (to form IEV) followed by subsequent exocytosis to form cell-associated enveloped virus (CEV) and EEV. Avipoxviruses, however, form EEV by a more conventional pathway in

which IMV buds through the cell membrane to acquire an envelope. This difference, observed by electron microscopy (**Figure 1**), is consistent with the absence from the avipoxvirus genome of genes involved in wrapping and exocytosis in VACV.

Lipid Metabolism

There have been reports that FWPV has a profound effect on host cell lipid metabolism. It is tempting to speculate that this might be attributable to some of the unusual genes carried by FWPV, notably fpv048 encoding a protein with unusually high sequence similarity to the ELOVL4 host protein, a fatty acid elongation factor implicated in macular degeneration.

Virus Propagation

Avipoxviruses replicate only in avian cells; replication in mammalian cells is abortive and no infectious progeny are produced. Some vaccine strains of FWPV, such as the Cyanamid Webster FPV-M vaccine, even display a preference for chick embryo skin cells (CESs) over chick embryo fibroblast cells (CEFs). FWPV FP9 has been effectively adapted to CEFs but it displays a distinct preference for primary as opposed to secondary CEFs. Plaques on CEFs are not lytic, but the cytopathic effects manifest as changes in cell morphology, resulting in areas of altered refractive index with the plaques best viewed by dark field illumination. FWPV fails to plaque and replicates poorly in the recently derived chicken fibroblast cell line, DF-1. Replication is similarly poor in the chemically transformed cell line OU-2. It can be plaqued and replicated quite efficiently in quail cell lines, such as QT-35, but the presence in these cells of viable endogenous Marek's disease virus (a herpesvirus) means that their use for preparation of vaccines is not advisable. Of these possible cell substrates for avipoxvirus propagation, currently only CEFs and CESs are licensed for use in the production of human vaccines.

Publications reporting the replication of uncharacterized avipoxviruses of unknown origin in embryonic bovine tracheal cells or in BHK-21 are atypical and, until corroborated, should be viewed with caution.

Molecular Phylogenetics

The taxonomy and classification of avipoxviruses are important to the study of epidemiology and hence to

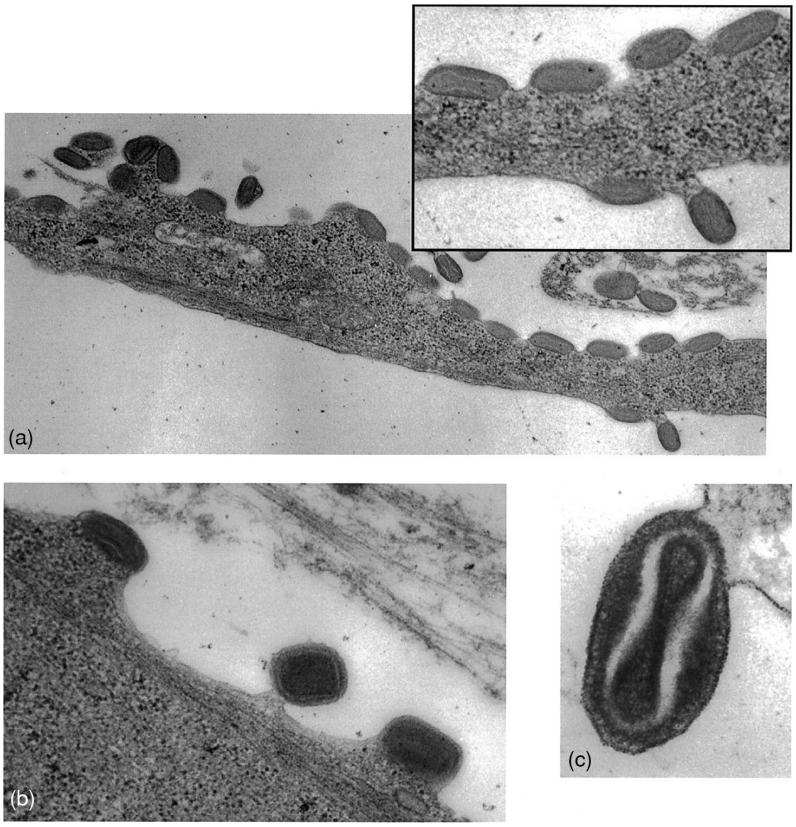


Figure 1 FWPV IMV particles budding through the membranes of infected chick embryo fibroblasts. Magnifications: (a) ×27 000 (inset ×51 000); (b) ×60 000; (c) ×170 000. Reproduced from Boulanger D, Smith T, and Skinner MA (2000) Morphogenesis and release of fowlpox virus. *Journal of General Virology* 81: 675–687, with permission from the Society for General Microbiology.

the future ability to control the diseases. Recent molecular studies confirm earlier impressions that avipoxviruses can jump species to cause disease. Thus isolation of a virus from a turkey, for instance, is not sufficient to demonstrate that turkeypox virus is the cause and, more importantly, that the outbreak can be controlled by a turkeypox virus vaccine.

Avipoxviruses can be highly diverged at the molecular level. For instance, the relatively highly conserved FWPV and CNPV P4b proteins (encoded by genes fpv167 and cnpv240, respectively) share only 64.2% amino acid sequence identity. This level of divergence is comparable to that seen between different genera of mammalian poxviruses (see **Figure 2**). In fact, recent molecular studies have demonstrated that avipoxviruses fall into three major diverged groups: (1) the FWPV-like viruses, (2) the CNPV-like viruses, and (3) the psittacinepox viruses (**Figure 2**). As their name suggests, the psittacinepox viruses have so far only been isolated from psittacines. In contrast, viruses of the FWPV-like and CNPV-like groups appear either inherently capable of infecting a wider range of birds or have evolved and adapted to be able to infect different birds. FWPV-like viruses have been isolated from turkeys, pigeons, ospreys, albatrosses, doves, and falcons. Viruses closely related to, and even barely distinguishable from, CNPV can cause disease in great tits, sparrows, stone curlews, and houbaras. CNPV-like viruses have been isolated from pigeons and starlings. A report in 2006 from Virginia in the USA indicated that one particular CNPV-like virus caused disease in a wide range of birds (robins, crows, herons, finches, doves, hawks, gnatcatchers, mockingbirds, and cardinals). It was not known whether the virus that caused the epornitic had a wider geographic distribution throughout the USA.

It is apparent that pigeonpox can be caused by two distinct types of virus, either FWPV-like or CNPV-like. The FWPV-like virus is very closely related to viruses from turkeys and osprey, and a little less closely related to those from an albatross, a falcon, and a dove. The CNPV-like virus is closely related to one from a starling. Infection of avian species outside the normal host range of the particular avipoxvirus can result in altered pathogenesis compared to that observed in the normal host. For instance, a virus which was isolated from an Andean condor (*Vultur gryphus*), in which it had caused an aggressive, diphtheritic form of the disease, produced only mild lesions in inoculated chickens. Conversely, viruses causing mild poxvirus lesions in wild birds, such as those found in 50% of short-toed larks (*Calandrella rufescens*) and 28% of Bertholet's pipits (*Anthus berthelotti*) in the Canary Islands, might cause more severe disease in naive populations of other avian species. It is, for instance, suspected that canaries might not be the natural reservoir for CNPV, which causes them a devastating disease.

Epizootiology

Evidence of poxvirus infection has been observed in many avian species but little is known about the natural host range of the causative agents, due to the lack of robust techniques for the identification and differentiation of isolates. Initially, the viruses were distinguished by their ability to cause disease in a range of test species, such

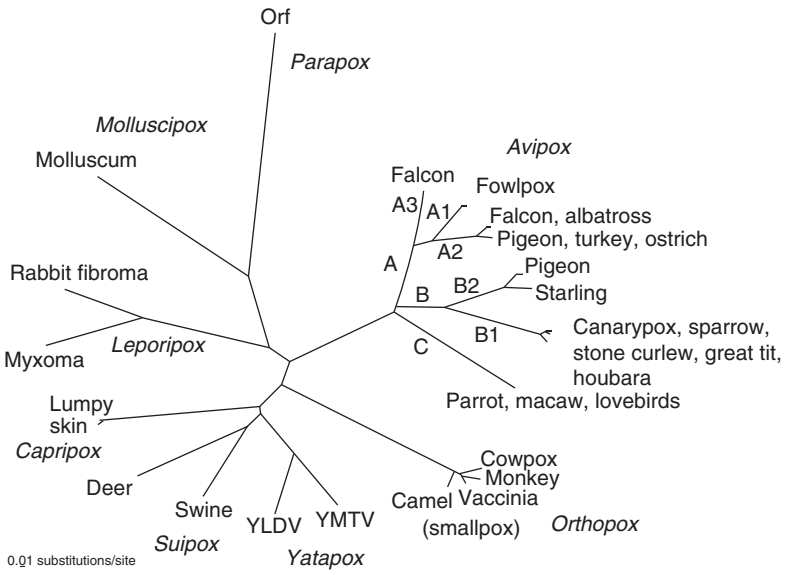


Figure 2 A phylogram showing phylogenetic relationships and evolutionary distances of avipoxvirus clades and mammalian poxvirus genera based on neighbor-joining analysis (supported by bootstrapping) of P4b orthologs.

as chickens, pigeons, turkeys, and quail. Such infectivity studies probably overestimate the degree of similarity between the viruses, as many isolates seem able to cause disease in the test species, possibly due to the inoculation of high doses. *In vivo* analysis of antigenic cross-reactivity allowed the viruses to be grouped or distinguished by their ability or inability to induce protective immunity against each other. Thus, psittacinepox virus offered no protection against FWPV or pigeonpox virus, or vice versa, nor did quailpox virus. Similarly, mynahpox virus showed no cross-protection with fowl, pigeon, psittacine, quail, or turkey poxviruses, and a condor poxvirus induced no protection against FWPV. *In vitro* serological techniques were applied to only a small proportion of the observed isolates because initial propagation in chick embryos or chick embryo fibroblast cells often failed. Possibly as a consequence of the limited characterization of isolates, there are only the ten recognized species of avipoxviruses.

Reintroduction programs appear to be particularly susceptible to problems caused by poxvirus infection. For instance, houbara bustard reintroduced into the Middle East have proved susceptible to uncharacterized avipoxvirus infections from unknown sources. Similar programs, such as that for the closely related great bustard on Salisbury Plain, Wiltshire, in the UK may face similar threats. There are also concerns that avipoxviruses might threaten marginal species such as some species of birds in the Hawaiian Islands and especially the kakapo, the flightless, nocturnal New Zealand owl parrot.

Transmission

Poxviruses produce two types of virion particles that are both infectious: IMV released into the environment only by desquamation of skin lesions and EEV released from the cell to spread to secondary sites of infection. In the wild, the primary route of infection is mechanical transmission of blood-borne EEV by biting insects (mosquitoes and midges). Lesions are therefore characteristically restricted to the featherless areas around the eyes and nares, on the comb, wattle, lower legs, and feet. Fowlpox remains a problem when control of biting insects is difficult but is generally not a problem for intensive production systems in the temperate areas of Northern Europe and the northern USA, even in the absence of vaccination. Problems with epizootics have emerged with free-range flocks, presumably because they are more exposed to biting insects, as well as to pecking by infected birds. In subtropical and tropical climates, fowlpox poses a significant problem requiring vaccination of commercial flocks.

FWPV can also transmit via inhalation or ingestion of dust (or dander, representing virus-infected cells shed from cutaneous lesions), or aerosols, leading to the 'diphtheritic form' of the disease, with lesions on the mucous membranes of the mouth, pharynx, larynx, and sometimes trachea. More likely to occur at high population density, this form of the disease is associated with commercial poultry flocks or collections of captive or domesticated birds (including quarantine facilities). However, such lesions may also form as a consequence of secondary viremia following primary cutaneous lesions, and viral genetic factors may play a role. Avipoxvirus infections of passerine birds (e.g., canaries and finches) can cause significantly higher rates of mortality than those of chickens or turkeys.

Diagnosis

Clinical Signs

FWPV and many other avipoxviruses primarily cause disease in mature birds. The cutaneous form of avipox, including fowlpox in chickens and turkeys, spread mechanically by biting insects or pecking, is normally relatively mild (**Figures 3** and **4**) but the diphtheritic form (**Figure 5**) causes higher mortality by occlusion of the oropharynx. A third form of poxvirus disease causes a pneumonia-like illness in canaries, with high mortality.

Histology

Smears of material scraped from cutaneous lesions or postmortem sections from diphtheritic lesions (or airway epithelium in the case of pneumonia) can be stained with hematoxylin/eosin, or Gimenez stain. The presence of eosinophilic inclusions (A-type inclusions, Bollinger bodies) in the cytoplasm is diagnostic of poxviruses. Basophilic (or B-type) inclusion bodies may also be seen in the cytoplasm (these represent the sites of virus replication, the so-called 'viral factories'), as may Borrel bodies (the virions themselves).

Virus Isolation and Detection

Virus may be passaged and amplified *in vivo* through specific pathogen-free chickens via wing web scarification or through embryonated eggs via chorio-allantoic membrane infection. The method of choice for virus propagation, however, is *in vitro* culture on CEFs for amplification through liquid culture or for plaque purification under semisolid overlay. Not all avipoxvirus isolates will form plaques, or even propagate, on CEFs. Primary cultures of other poultry species (duck, turkey, or quail) may also be used.

Serology

Serological methods such as enzyme-linked immunosorbent assays (ELISAs), available as commercial flock

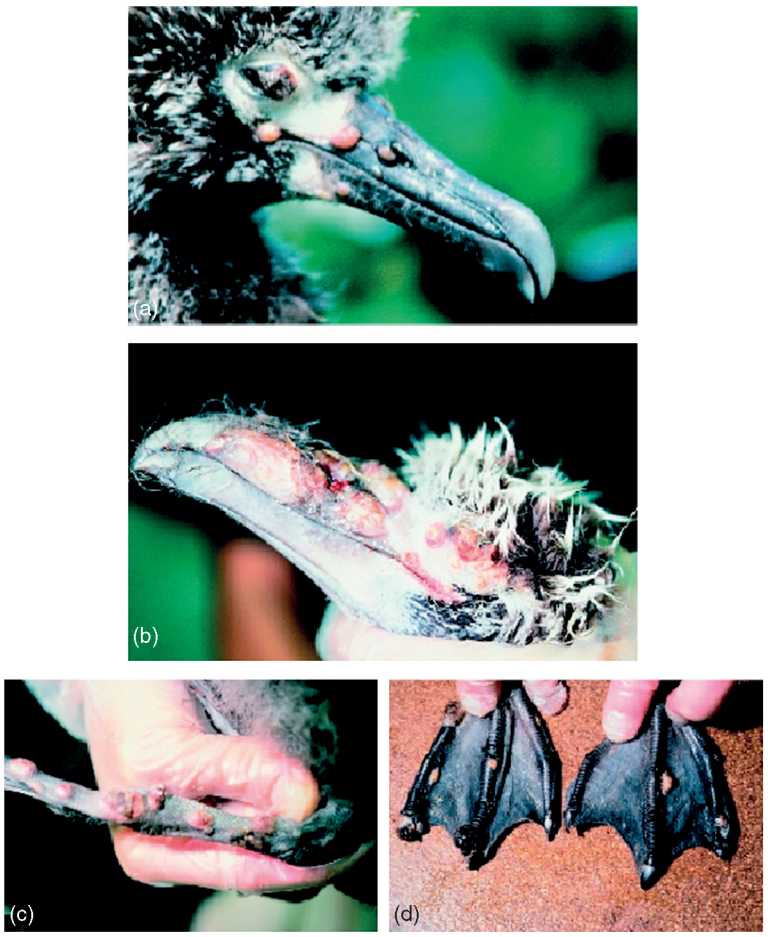


Figure 3 Typical avipoxvirus lesions seen on the head and feet of a Laysan albatross. Reproduced from Hansen W (1999) Avian pox. In: Friend M and Franson JC (eds.) *Field Manual of Wildlife Diseases, General Field Procedures and Diseases of Birds*, pp. 163–169, with permission from USGS.

monitoring kits, can be used to provide evidence for ongoing or prior infection in the flock. Virus neutralization tests are more specific but are technically more demanding and the responsible antigenic epitopes have not been identified.

Monoclonal Antibodies

Monoclonal antibodies against the three major immunodominant structural antigens of FWPV have been isolated and characterized, as described above. None appears to be neutralizing but all can be used in ELISAs and western blotting.

Restriction Enzyme Digestion and Southern Blotting

Before extensive genome sequencing, Southern blotting was the only available molecular diagnostic method, involving examination of restriction enzyme digest profiles. Without the use of radioisotopes, the method was relatively insensitive.

PCR-Based Analysis

With the advent of DNA sequence data for the avipoxviruses, polymerase chain reaction (PCR) amplification became the molecular diagnostic method of choice to identify avipoxvirus infections. This has been based routinely on the sequence of the gene encoding the P4b protein (the A3 ortholog encoded by gene fpv167), with primers for a 578 bp fragment. However, all avipoxviruses produce fragments of the same length, so the avipoxvirus could only be identified either by sequencing the PCR product or by restriction enzyme fragment polymorphism profiling.

A second PCR locus (the H3 locus), which works with most (but not all) avipoxviruses, has the advantage that viruses of the two major groups (CNPV-like and FWPV-like) can be discriminated purely on the basis of the size of the PCR product (**Figure 6**).

The genome sequences of two different FWPV strains, one extensively culture-passaged and attenuated, and of a pathogenic CNPV have been determined. They confirmed



Figure 4 Extensive avipoxvirus lesions seen on the head of a bald eagle. Reproduced from Hansen W (1999) Avian pox. In: Friend M and Franson JC (eds.) *Field Manual of Wildlife Diseases, General Field Procedures and Diseases of Birds*, pp. 163–169, with permission from USGS.

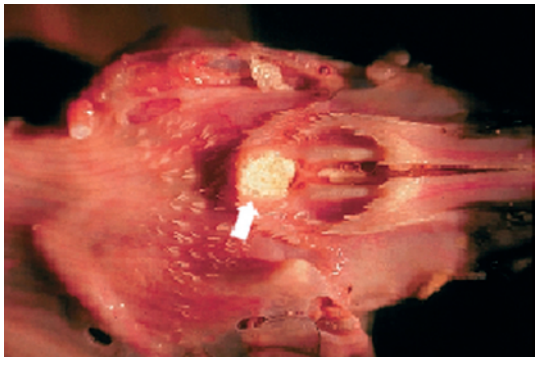


Figure 5 Diphtheritic lesion in a Laysan albatross. Reproduced from Hansen W (1999) Avian pox. In: Friend M and Franson JC (eds.) *Field Manual of Wildlife Diseases, General Field Procedures and Diseases of Birds*, pp. 163–169, with permission from USGS.

the divergence and differences between the avipoxviruses and the mammalian poxviruses typified by VACV. In fact, phylogenetic analysis showed that avipoxviruses are most closely related to MOCV of humans, a relationship confirmed by other specific aspects of their molecular biology (see **Figure 2**). Despite having sequence information for the prototypic members of the two major groups of avipoxviruses, it has still proved extremely difficult to identify conserved primer sequences that work in PCR reactions for all other members of the genus, further illustrating the extent of sequence divergence and diversity within this group of viruses.

Intraspecies and Interstrain Differentiation

Despite their large genome size, the high level of conservation within species of avipoxviruses makes it difficult to differentiate between different strains or isolates or between vaccines and pathogenic strains. Although numerous loci within FWPV have been surveyed, only one locus (the H9 locus), has been reported to allow clear differentiation. It appears to be an unstable region, with deletions of different lengths found in various FWPV strains, isolates, and vaccines. Three commercial fowlpox vaccines were found to have the same parental sequence found in two clinical isolates, while two other different commercial vaccines shared an identical deletion at the H9 locus (see **Figure 7**).

Control and Prevention

Vaccination

Since its introduction in the 1920s, vaccination against fowlpox using live FWPV or pigeonpox virus (now known to be closely related antigenically to FWPV) has become commonplace and extensive, such that the disease has been nearly eradicated from developed countries in temperate zones. Most currently available commercial vaccines originate from early isolates, though a minority were isolated in the mid-1960s.

Vaccination may be undertaken from 10 days or 4 weeks of age, depending on the residual pathogenicity of the vaccine, which is often related to whether the vaccine

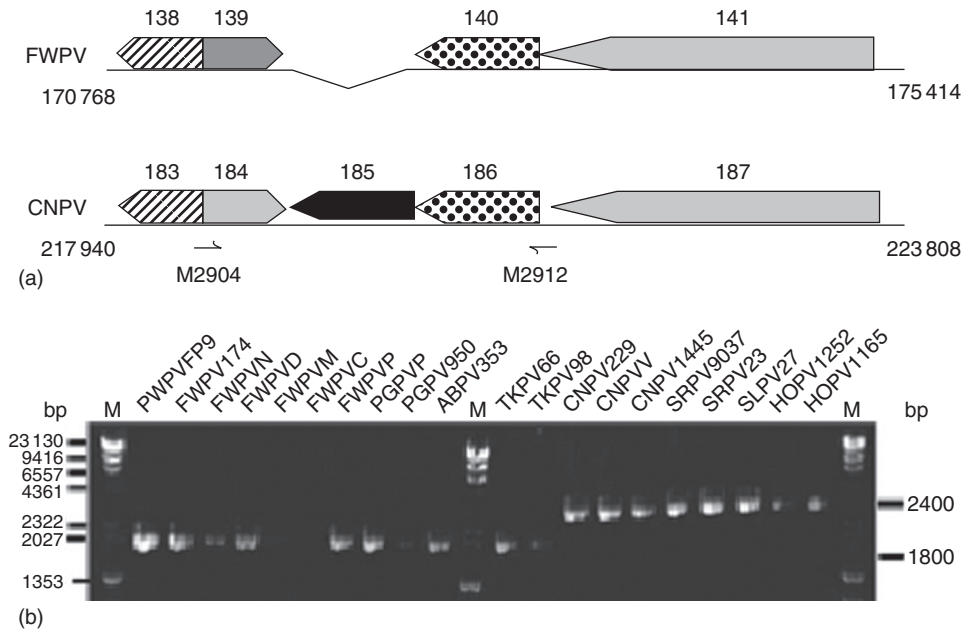


Figure 6 PCR-based differentiation of FWPV- and CNPV-like viruses. (a) Arrangement of FWPV genes fpv138 to fpv141 at the H3 locus aligned with their CNPV orthologs. The positions of primers used for PCR analysis are indicated. (b) The PCR analysis shows the length difference in the amplified DNA fragment obtained from FWPV-like viruses (1800 bp) and from CNPV-like viruses (2400 bp). Reproduced from Jarmin S, Manvell R, Gough RE, Laidlaw SM, and Skinner MA (2006) Avipoxvirus phylogenetics: Identification of a PCR length polymorphism that discriminates between the major clades. *Journal of General Virology* 87: 2191–2201, with permission from the Society for General Microbiology.

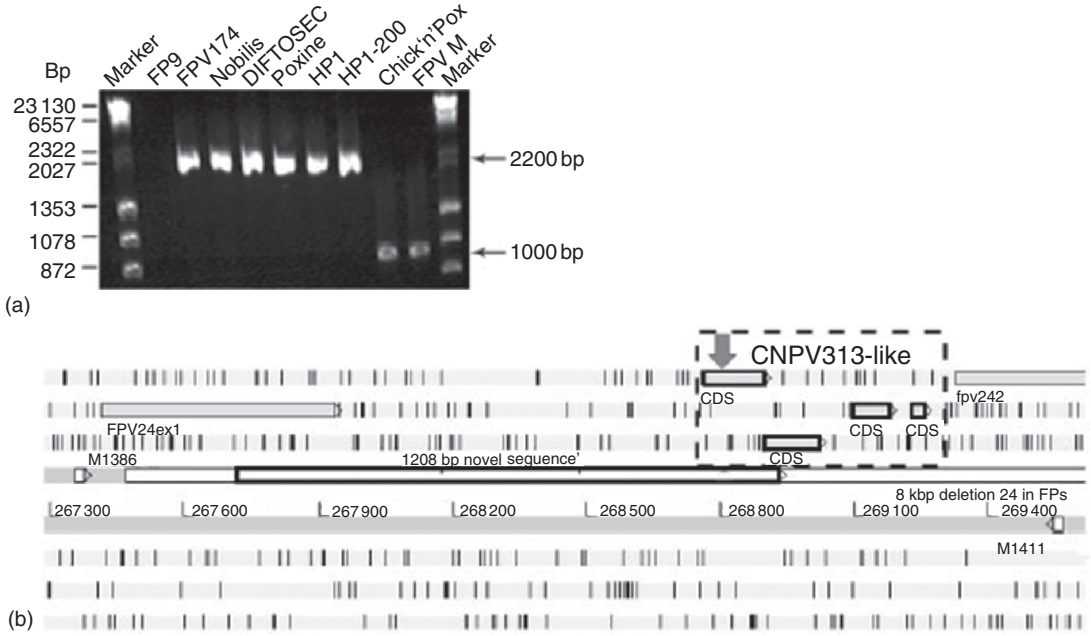


Figure 7 PCR analysis of the H9 locus of FWPV. (a) The 2200 bp product for field viruses (FPV174, HP1, and HP1-200) and three vaccines (Nobilis, DIFTOSEC, and Poxine) and identically deleted 1000 bp products for two other vaccines (Chick'n'Pox and FPV M). No product was seen for an extensively passaged and attenuated virus FP9. (b) Organization (to scale) of the H9 locus (FWPV genes fpv241 to fpv242), including the 1200 bp product, which is deleted from the vaccines, shown for FWPV HP1. The positions of primers used for the PCR are shown. Reproduced from Jarmin SA, Manvell R, Gough RE, Laidlaw SM, and Skinner MA (2006) Retention of 1.2 kbp of 'novel' genomic sequence in two European field isolates and some vaccine strains of fowlpox virus extends open reading frame fpv241, *Journal of General Virology* 87: 3545–3549 with permission from the Society for General Microbiology.

is propagated in cell culture or in embryonated eggs. Inoculation is into the wing web using a bifurcated needle or by scarification of the thigh. The formation of a small lesion at the site of vaccination 6–8 days later indicates a good take, with immunity developing in 8–15 days. Though desirable, alternative routes for vaccination (aerosol, intranasal, or via drinking water) have not generally proved successful.

Treatment

There are no demonstrated effective treatments for avipoxvirus infections other than palliative treatment and to prevent or treat secondary bacterial infections.

Variant FWPVs

Antigenic Variants

Outbreaks of fowlpox have been reported within flocks previously vaccinated with FWPV or pigeonpox virus vaccines. If the vaccination had been effective, which is often not the case, antigenic variation might be suspected, though serotype specificity would be novel among poxviruses. Little is known about protective epitopes for humoral or cellular immune responses in FWPV and there has only been preliminary characterization of the 'variant' isolates.

Tumorigenic Variants

FWPV has also been associated with cases of dermal squamous cell carcinoma in poultry in Brazil. It is not clear whether FWPV is a causative agent and, if so, whether a variant is involved.

The Role of Integrated Reticuloendotheliosis Virus Sequences

FPV-S, a commercial FWPV vaccine known to be contaminated with reticuloendotheliosis virus (REV), was found to carry a near full-length, infectious progenome of REV integrated into the poxvirus genome. The provirus has subsequently been found in most, if not all, pathogenic field isolates of FWPV, but the majority of vaccine strains of FWPV carry only noninfectious, long terminal repeat (LTR) sequences of REV. The provirus and the LTR sequences have only ever been found at the same single locus, although differences exist between the REV LTR sequences retained in different viruses. It appears likely that a single, ancestral event inserted the provirus into the FWPV genome between genes fpv201 and fpv203, in contrast to the multiple REV insertions that have been observed in Marek's disease virus. It is not known whether REV can reintegrate at the site of a retained LTR or whether this would regenerate a pathogenic virus. There are no REV sequences in the completely sequenced genome of CNPV, but they have been detected in a commercial CNPV vaccine as well as in some commercial pigeonpox virus vaccines. Full-length REV sequences have been detected in an isolate from a turkey.

Recombinant Avipoxvirus Vaccines

Recombinant FWPV as a Vaccine Vector in Poultry

Shortly after the development of methods for isolating recombinant VACV in the early 1980s, FWPV was developed as an equivalent recombinant vector for use in poultry. Several commercial vaccine and laboratory attenuated strains were used as vectors against a number of important poultry pathogens, especially avian influenza virus, Newcastle disease virus, infectious bronchitis virus, avian hemorrhagic enteritis virus, Marek's disease virus, turkey rhinotracheitis virus, REV, and infectious bursal disease virus, as well as *Mycoplasma gallisepticum*. Commercial recombinant FWPV vaccines against Newcastle disease virus and avian influenza virus have been licensed for commercial use in the USA. Those against avian influenza have also been licensed for use in Mexico; indeed, between 1997 and 2003, approximately 459 million doses of a recombinant fowlpox-H5 vaccine were used in Mexico as part of a program to control H5N2. The same recombinant and similar viruses developed in China are being used in Southeast Asia to counter the highly virulent avian influenza H5N1 strain.

Recombinant Avipoxviruses as Vaccine Vectors in Mammals

It was demonstrated in the late 1980s that recombinant avipoxviruses (initially FWPV and then CNPV) carrying antigens from mammalian viral pathogens could enter mammalian cells and express the foreign antigens, even though the recombinants could not replicate and spread to neighboring cells. Moreover, vaccination of mammals with the recombinants could elicit immune responses, which could be protective against viral challenge. This discovery, which was surprising because of the low level of antigen expressed from the single-round, abortive infections, offered the prospect of extremely safe recombinant vaccines. Several recombinant avipoxvirus vaccines, mainly based on CNPV, have been developed primarily for veterinary use. However, the more recent focus on T-cell-mediated immunity has seen more use of FWPV-based

vectors. Many clinical trials have been conducted, are underway, or are planned, against viral diseases such as acquired immune deficiency syndrome (AIDS), parasitic diseases including malaria, and various cancers. In many of these trials, the recombinant avipoxviruses are used in combined (so-called 'prime-boost') regimes with the same antigens expressed by different vectors, such as DNA plasmids, the host-restricted MVA variant of VACV, or adenoviruses. These regimes allow immune responses to be boosted by multiple vaccinations without eliciting excessive responses against vector-specific proteins.

Recombinant avipoxvirus vaccines are yet to be licensed for agricultural use in Europe, though recombinant CNPV vaccines against equine influenza and feline leukemia have received European Medicines Agency (EMEA) approval. Both are licensed for use in the USA, besides similar recombinants against canine distemper and West Nile virus (for use in horses).

See also: Cowpox Virus; Entomopoxviruses; Herpesviruses of Birds; Leporipoviruses and Suipoxviruses; Molluscum Contagiosum Virus; Mousepox and Rabbitpox Viruses; Parapoxviruses.

Further Reading

Boulanger D, Smith T, and Skinner MA (2000) Morphogenesis and release of fowlpox virus. *Journal of General Virology* 81: 675–687.

Hansen W (1999) Avian pox. In: Friend M and Franson JC (eds.) *Field Manual of Wildlife Diseases, General Field Procedures and Diseases of Birds*, pp. 163–169. Madison, WI: US Geological Survey.

OIE (World Organisation for Animal Health) (2004) Page on Fowlpox diagnostic tests and vaccines from Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (5th edn.) http://www.oie.int/eng/normes/mmanual/A_00113.htm (updated 23 July 2004).

Jarmin S, Manvell R, Gough RE, Laidlaw SM, and Skinner MA (2006) Avipoxvirus phylogenetics: Identification of a PCR length polymorphism that discriminates between the major clades. *Journal of General Virology* 87: 2191–2201.

Jarmin SA, Manvell R, Gough RE, Laidlaw SM, and Skinner MA (2006) Retention of 1.2 kbp of 'novel' genomic sequence in two European field isolates and some vaccine strains of fowlpox virus extends open reading frame fpv241. *Journal of General Virology* 87: 3545–3549.

Skinner MA, Laidlaw SM, Eldaghayes I, Kaiser P, and Cottingham MG (2005) Fowlpox virus as a recombinant vaccine vector for use in mammals and poultry. *Expert Review of Vaccines* 4: 63–76.

Skinner MA (2007) *Poxviridae*. In: Pattison M, McMullin P, Bradbury J, and Alexander D (eds.) *Poultry Diseases*, 6th edn. New York: Elsevier.

Tripathy DK and Reed WM (2003) Pox. In: Saif YM, Barnes HJ, Fadly A, Glisson JR, McDougald LR and Swayne DE (eds.) *Diseases of Poultry,* 11th edn., pp. 253–265. Ames, IA: Iowa State University Press.

Fungal Viruses

S A Ghabrial, University of Kentucky, Lexington, KY, USA
N Suzuki, Okayama University, Okayama, Japan

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Hyphal anastomosis The union of a hypha with another resulting in cytoplasmic exchange.
Hypovirulence Attenuated fungal virulence mediated by virus infection, mitochondrial defects, or mutations in the fungal genome.
Mycoviruses Viruses that infect and multiply in fungi.
Vegetative incompatibility A genetically controlled self/nonself recognition system in fungi that determines the ability to undergo hyphal anastomosis.

Introduction

Relative to plant virology, animal virology, or bacterial virology, fungal virology is new. In 1948, an economically important disease of the cultivated mushroom, *Agaricus bisporus*, characterized by malformed fruiting bodies and serious yield loss, was first reported in a mushroom house owned by the La France Brothers of Pennsylvania. The disease was called 'La France disease', and similar diseases were reported shortly thereafter from Europe, Japan, and Australia. Different designations, such as 'X-disease', 'watery stripe', 'brown disease', and 'die-back' were given to basically the same disease as the La France. The significance of the 1948 report lies in the fact that it led to the discovery of fungal viruses. In 1962 Hollings noted the presence of at least three types of virus particles in diseased mushroom sporophores. This was the first report of virus particles in association with a fungus and is regarded as the dawn of mycovirology. The subsequent discovery that dsRNA of mycoviral origin was responsible for interferon-inducing activities of cultural filtrates of several species of *Penicillium* spp. greatly stimulated the search for fungal viruses.

Fungi, like other living organisms, can be infected by a number of viruses, and mycoviruses are found in all the major groups of fungi. Although mycoviruses are widely

prevalent, only those infecting a limited number of fungal host species have been studied, for example, the yeast *Saccharomyces cerevisiae*, edible mushroom, and phytopathogenic fungi. Given the predicted vast number of fungal species (approximately 10 000 known species and many more unknown species), it is expected that a greater number of unrecognized mycoviruses occur in nature. Support for this idea comes from recent extensive searches of field fungal isolates that showed relatively high frequencies of virus infection, for example, approximately 65%, 20%, and 2–28% of *Helicobasidium mompa*, *Rosellinia necatrix,* and *Cryphonectria parasitica* isolates were found to be infected, respectively.

Biological Properties

Host Range

The natural host range of mycoviruses is likely to be restricted to the same or closely related vegetative compatibility groups that allow lateral transmission. Until recently, there were no known experimental host ranges for fungal viruses because of lack of suitable infectivity assays. Experimental host ranges for some mycoviruses, however, were recently demonstrated and shown to extend to different vegetative compatibility groups and even to different genera. For example, the prototype mycoreovirus (mycoreovirus 1) can replicate and induce phenotypic alterations in different vegetative compatibility groups of the chestnut blight fungus, *Cryphonectria parasitica*, similar to those exhibited by the original virus-containing strain. Furthermore, CHV1-EP713, the type member of the family *Hypoviridae*, can replicate in and confer hypovirulence onto members of several other fungal genera, for example, *Endothia gyrosa* and *Valsa ceratosperma*, in addition to its natural host, *C. parasitica.*

Symptom Expression

Although the majority of the viruses that infect phytopathogenic fungi have been reported to be avirulent, phenotypic consequences of infections with mycoviruses can vary from symptomless to severely debilitating, and from hypovirulence to hypervirulence. Mycoviruses that attenuate the virulence of plant pathogenic fungi provide excellent model systems for basic studies on development of novel biological control measures and for dissecting the mechanisms underlying fungal pathogenesis. In general, infections due to mycoviruses are both symptomless and persistent. Latency benefits the host for survival, and persistence helps the virus in the absence of extracellular modes of transmission. To ensure their retention, some mycoviruses have evolved to bestow selective advantage to their host (e.g., the killer phenotypes in yeasts and smuts). Because of their ability to secrete killer toxins, yeast killer strains have been utilized by the brewing industry to provide protection against contamination with adventitious sensitive strains. The genes encoding the smut killer toxins have been used for the development of novel transgenic approaches to control the corn smut.

Macroscopic symptoms caused by fungal viruses are a consequence of alterations in complex physiological processes that involve interactions between host and virus factors. Several virally encoded proteins are identified as symptom determinants including the papain-like protease, p29, of some hypoviruses. This protein acts to repress host pigmentation and conidiation regardless of whether it is expressed from host chromosomes or the homologous virus genome. Another interesting example is the proteins encoded by the totivirus *Helminthosporium victoriae* 190S virus. Co-expression of the capsid and RNA-dependent RNA polymerase (RdRp) proteins results in empty capsid production and phenotypic changes similar to those induced in virus-infected isolates, suggesting that viral replication is not required for symptom development. Host factors involved in symptom expression are not well studied except for host genes involved in the killer phenomenon in yeast infected with the totivirus L-A and associated satellite dsRNAs. Transcriptome analysis was performed for only limited virus/natural host combinations. Of the 2200 *C. parasitica* genes, 13.4% are either upregulated or downregulated upon infection with the prototype severe strain of the hypovirus CHV1, while only 7.5% are altered in their transcription levels by infection with a mild strain of CHV1. One-half of the genes responsive to the infection with the latter virus are commonly altered in transcription by the severe strain, which generally causes greater magnitude of transcriptional changes.

Transmission

Mycoviruses lack an extracellular phase to their life cycles. They are transmitted intracellularly during cell division, sporogenesis, and cell fusion. Lateral (horizontal) transmission usually occurs only between individuals within the same species, which belong to the same or closely related vegetative compatibility groups. Vegetative compatibility is governed genetically. Mycoviruses may be eliminated during sexual spore formation. Although the totiviruses and narnaviruses that infect the yeasts are effectively transmitted via ascospores, the mycoviruses infecting the ascomycetous filamentous fungi are eliminated during ascospore formation. Whereas the ssRNA and dsRNA mushroom viruses are transmitted efficiently via basidiospores, the virus-containing strains of the basidiomycete *H. mompa* are cured during the sexual sporulation processes. Therefore, whether a mycovirus is transmitted through sexual spores depends on the host/virus

combination involved. Whereas mycovirus transmission through asexual spores occurs frequently, its rate varies greatly depending on the combination of viral and host strains. It was recently shown that the papain-like protease p29, encoded by CHV1, might play a role in virus transmission since it enhances the transmission of the homologous virus (CHV1) as well as heterologous viruses (mycoreoviruses) in *C. parasitica* conidia.

While rare in nature, interspecies transmission has been reported between members within the same genus including *Cryphonectria*, *Sclerotinia*, and *Ophiostoma* that share the same habitats. It remains unknown whether interspecies barrier is overcome by physical contacts or by vectors. Experimental evidence for vector transmission of fungal viruses, however, is lacking.

Mixed Infections

Mixed infections with two or more unrelated viruses and accumulation of defective dsRNA and/or satellite dsRNA are common features of mycovirus infections. Examples include the sphaeropsis sapinea totiviruses SsRV1 and SsRV2, saccharomyces cerevisiae totiviruses ScV-L-A and ScV-LB/C, and penicillium stoloniferum partitiviruses PsV-S and PsV-F.

There are a number of known examples of mixed infections with plant or animal viruses where one virus either interferes with or enhances the replication of the other one. As a consequence, reduction or increase in symptom severity may arise. Recent extensive searches for fungal viruses confirmed relatively high mixed infection rates in some phytopathogenic fungi like *C. parasitica*, *R. necatrix*, and *H. mompa*. In contrast to mixed virus infections in plants or animals, possible interactions between co-infecting mycoviruses in single hosts is little studied because of the limitation in experimental manipulation of viruses and hosts. Recent studies suggest synergistic interactions between a hypovirus (phylogenetically related to potyviruses and viruses belonging to the picorna-like superfamily) and a mycoreovirus through a hypovirally encoded protein. In this case, one-way synergism is observed in that only hypovirus infection transactivates the replication of a mycoreovirus, which has a replication strategy distinct from that of hypoviruses.

Fungal Virus Taxonomy

A list of the virus families and genera into which mycoviruses are classified is included in **Table 1**. Members of some fungal virus families, for example, the families *Narnaviridae*, *Chrysoviridae*, and *Hypoviridae*, infect only fungi, while members in other families, for example, the families *Metaviridae*, *Pseudoviridae*, *Reoviridae*, *Totiviridae,* and *Partitiviridae*, infect fungi, protozoa, plants, or animals. Except for the rhizidiomyces virus with dsDNA genome (the only member in the genus *Rhizidiovirus*), almost all other mycoviruses have RNA genomes and many have dsRNA genomes. Viruses with (–)-strand RNA or ssDNA genomes have yet to be found in fungi.

dsRNA Mycoviruses

Mycoviruses with dsRNA genomes represent the majority of the fungal viruses so far reported. With the exception of the mycoreviruses (genus *Mycoreovirus,* family *Reoviridae*), which have spherical double-shelled particles 80 nm in diameter, the dsRNA mycoviruses are typically isometric particles 25–50 nm in diameter. They are classified, based on the number of genome segments, into three families, *Totiviridae*, *Partitiviridae,* and *Chrysoviridae*. Viruses in the family *Totiviridae* have nonsegmented dsRNA genomes coding for a CP and an RdRp. At present, three genera have been placed in this family: *Totivirus*, *Giardiavirus*, and *Leishmaniavirus*. Viruses in the genus *Totivirus* infect fungi, whereas those belonging to the latter two genera infect parasitic protozoa. At least two distinct RdRp expression strategies have been reported for totiviruses: (1) those that express their RdRp as a fusion protein (CP-RdRp or Gag-Pol) by ribosomal frameshifting, such as the yeast L-A and the viruses that infect parasitic protozoa and (2) those that synthesize RdRp as a separate nonfused protein by an internal initiation mechanism (e.g., a coupled termination–reinitiation mechanism), as proposed for Hv190SV and others that infect filamentous fungi. Phylogenetic analysis of CP or RdRp sequences of totiviruses reflects these differences, and separate phylogenetic clusters can be generated. Hv190SV and other totiviruses that infect filamentous fungi are closer to each other than to viruses infecting yeast, smut fungi, and protozoa. The fact that independent alignments of CP and RdRp sequences give similar phylogenetic relationships supports the conclusion that totiviruses infecting filamentous fungi should reside in a genus of their own. The genus *Victorivirus* has been proposed to include the totiviruses that infect filamentous fungi with Hv190SV, as the type species (**Table 1**). The genomes of partitiviruses and chrysoviruses consist of two and four segments, respectively.

The unclassified dsRNA mycovirus agaricus bisporus virus 1 (AbV1), also designated La France isometric virus, causes a serious disease of cultivated mushroom (named La France disease). AbV1 is of special interest because of its historical and economic importance. The AbV1 virions, isolated from diseased fruit bodies and mycelia, are isometric 36 nm in diameter and co-purify with nine dsRNA segments (referred to as disease-associated dsRNAs). The size of dsRNA segments varies from 3.6 to 0.78 kbp, three of which are believed to be satellites. It is not clear at present whether the nine dsRNA segments are encapsidated individually, in various combinations, or all nine segments are packaged in single particles. Based on

Table 1 List of viral families and genera into which fungal viruses are classified

Family/genus/virus species[a]	*Virus abbreviation*	*No. of segments*	*Accession number*
Double-stranded DNA genome			
Unassigned			
Rhizidiovirus			
Rhizidiomyces virus	RhiV	1	
RNA reverse transcribing genome			
Pseudoviridae			
Pseudovirus			
Saccharomyces cerevisiae Ty1 virus	SceTy1V	1	M18706
Hemivirus			
*Saccharomyces paradoxus Ty5 virus**	SceTy5V	1	U19263
Metaviridae			
Metavirus			
*Saccharomyces cerevisiae Ty3 virus**	SceTy3V	1	M34549
Double-stranded RNA genome			
Reoviridae			
Mycoreovirus			
Cryphonectria parasitica mycoreovirus 1 (9B21)	MyRv1–9B21	11	AY277888; AY277889; AY277890; AB179636; AB179637; AB179638; AB179639; AB179640; AB179641; AB179642; AB179643
Totiviridae			
Totivirus			
Saccharomyces cerevisiae virus L-A (L1)	Sc V-L-A	1	J04692; X13426
Victorivirus[b]			
Helminthosporium victoriae 190SV	Hv190SV	1	U41345
Partitiviridae			
Partitivirus			
Atkinsonella hypoxylon virus	AhV	2	L39125; L39126; L39127
Chrysoviridae			
Chrysovirus			
Penicillium chrysogenum virus	PcV	4	AF296439; AF296440; AF296441; AF296442
Single-stranded (+) RNA genome			
Narnaviridae			
Narnavirus			
Saccharomyces 20S narnavirus	ScNV-20S	1	M63893
Mitovirus			
Cryphonectria mitovirus 1	CMV1	1	L31849
Barnaviridae			
Barnavirus			
Mushroom bacilliform virus	MBV	1	
Hypoviridae[c]			
Hypovirus			
Cryphonectria hypovirus 1-EP713	CHV-1/EP713	1	M57938
Endornavirus[c]			
*Phytophthora endornavirus 1**	PEV1	1	AJ877914
Unassigned			
Botrytis virus F	BVF	1	AF238884
Sclerotinia sclerotiorum debilitation-associated RNA virus	SsDRV	1	AY147260
Diaporthe RNA virus	DRV	1	AF142094
Sclerophthora macrospora virus A	SmV A	2	AB083060; AB083061
Sclerophthola macrospora virus B	SmV B	1	AB012756

[a]The type species of the specified genus are listed. Otherwise, a fungal virus member of the genus is listed and marked with an asterisk.
[b]Proposed new genus in the family *Totiviridae*.
[c]Grouped with dsRNA viruses in the Eighth Report of ICTV.

the size of the particles, cesium sulfate gradient profile, and results of dsRNA and protein analyses of the gradient fractions, it is highly unlikely that all dsRNAs are packaged together in single particles. More realistically, AbV1 represents a multiparticle system in which the various particle classes have similar densities. Interestingly, phylogenetic analysis of the conserved motifs of AbV1 RdRp, encoded by dsRNA segment 1, and other

dsRNA mycoviruses showed that AbV1 is closely related to the multipartite chrysoviruses.

ssRNA Viruses

There are a number of mycoviruses with apparent ssRNA genomes that do not code for capsid proteins and exist more or less predominantly as dsRNA 'replicative' forms in their hosts. Because of lack of true virions, these viruses were easier to isolate and study as their dsRNA forms, and some were grouped with dsRNA viruses (e.g., family *Hypoviridae*). However, there is ample evidence at present that many of these viruses replicate and express their genomes like (+)-strand RNA viruses and that the lineage of their RdRp and helicase genes are within the lineages of (+)-strand RNA viruses. The simplest types of these viruses include members of the genera *Narnavirus* and *Mitovirus* (family *Narnaviridae*), whose RNA genomes code only for RdRp and the viruses exist as RNA/RdRp nucleoprotein complexes. The corresponding dsRNAs can be isolated from infected tissues, usually in lesser molar amounts than the genomic ssRNA. Phylogenetic analysis of RdRps of members of the family *Narnaviridae* along with those of other fungal viruses and related taxa indicate a distant relationship between members of the family *Narnaviridae* and bacteriophages belonging to the family *Leviviridae*.

Lack of true virions is characteristic of two other groups of classified viruses, those belonging to the family *Hypoviridae* and the genus *Endornavirus*. Although these viruses were grouped with dsRNA viruses in the latest ICTV Report, their genome organization and expression strategies are indicative of (+)-strand RNA viruses. Viruses in the family *Hypoviridae* are phylogenetically related to the (+)-strand RNA viruses in the family *Potyviridae* (picorna-like virus supergroup). Comparisons of hypovirus-conserved motifs of RdRp, helicase, and protease with those of members of the family *Potyviridae* suggest that viruses in the genus *Bymovirus* are the closest relatives to hypoviruses. Phytophthora endornavirus (PEV1) is the only nonplant virus in the genus *Endornavirus* of plant viruses. Although *Phytophthora* species and other members of the class Oomycetes have many biological properties in common with fungi, they are currently classified, based on sequence similarities, in a protist group known as the Stramenopiles. Endornaviruses are believed to have evolved from an alpha-like virus that has lost its capsid gene. This is consistent with the recent finding that RdRps of PEV1 and other endornaviruses cluster with those of families and genera in the alpha-like virus superfamily of (+)-strand RNA viruses.

The mushroom bacilliform virus (MBV; genus *Barnavirus*, family *Barnaviridae*) is the only mycovirus known to have bacilliform virions. MBV has a (+)-strand RNA genome that contains seven open reading frames (ORFs), three of which encode a putative chymotrypsin-like serine protease, a putative RdRp, and a CP. The polypeptides encoded by the remaining four ORFs have no homology to known proteins. Amino-acid sequence comparisons of the putative protease and RdRp suggest that MBV is evolutionarily related to sobemoviruses and poleroviruses. Although double infections of cultivated mushrooms with MBV and ABV1 are of common occurrence, the role of MBV in the ensuing dieback disease of cultivated mushroom remains unknown.

Unassigned ssRNA Viruses

It is worth noting that a relatively large number of mycoviruses remain unassigned including some well-characterized ones like botrytis virus F (BVF), sclerotinia sclerotiorum debilitation-associated RNA virus (SsDRV), diaporthe RNA virus (DRV), and sclerophthora macrospora viruses A and B (SmVA and SmV B). BVF has flexuous rod-shaped particles comparable in size and morphology to ssRNA plant 'potex-like' viruses. Amino-acid sequence identities of the conserved helicase and RdRp regions and the coat protein genes are greatest to those of potex-like viruses. The main difference between BVF and these plant viruses is the lack of a movement protein. Although particle morphology along with amino-acid sequence similarities of both the replicase and coat protein genes support the classification of BVF in the family *Flexiviridae*, it is obvious that the mycovirus BVF is distinct enough to belong to a new genus in this family. It is proposed that a new genus tentatively designated '*Mycoflexivirus*' is created to include BVF (**Table 1**). The genome of SsDRV contains a single ORF encoding a protein with significant sequence similarity to the replicases of the 'alphavirus-like' supergroup of (+)-strand RNA viruses. The SsDRV-encoded putative replicase protein contains the conserved methyl transferase, helicase, and RdRp domains characteristic of the replicases of potex-like plant viruses (flexiviruses) and BVF. Although phylogenetic analysis of the conserved RdRp motifs verified that SsDRV is closely related to BVF and to the allexiviruses in the family *Flexiviridae*, SsDRV is distinct enough from these viruses, mainly based on the lack of coat protein and movement protein, to justify the creation of yet another genus in the family *Flexiviridae*.

DRV is another naked RNA mycovirus that is associated with hypovirulence of its fungal host. It has two large ORFs present in the same reading frame, which are most likely translated by readthrough of a UAG stop codon in the central part of the genome. The longest possible translation product has a predicted molecular mass of about 125 kDa, which shows significant homology to the nonstructural proteins of carmoviruses of the (+)-strand RNA virus family *Tombusviridae*. Interestingly, transcripts derived from full-length cDNA clones were

infectious when inoculated to spheroplasts and the transfected isolates exhibited phenotypic traits similar to the naturally infected isolate.

Sclerophthora macrospora virus A (SmV A) found in *S. macrospora,* the pathogenic fungus responsible for downy mildew of gramineous plants, is a small icosahedral virus containing three segments of (+) ssRNAs (RNAs 1, 2, and 3). Whereas RNA 1 contains the RdRp motifs, RNA 2 codes for a capsid protein. RNA 3 is a satellite RNA. Whereas the deduced amino acid sequence of RdRp shows some similarity to RdRps of members of the family *Nodaviridae*, the amino acid sequence of the viral CP shows similarity to those of members in the family *Tombusviridae.* The capsid of SmV A is composed of two capsid proteins, CP 1 and CP 2, both encoded in ORF2. CP 2 is apparently derived from CP 1 via proteolytic cleavage at the N-terminus. The genome organization of SmV A is distinct from those of other known fungal RNA viruses, and suggests that SmV A should be classified into a new genus of mycoviruses.

Sclerophthora macrospora virus B (SmV B), which is also found in *S. macrospora,* has small icosahedral, monopartite virions containing a (+) ssRNA genome. The viral genome has two large ORFs: ORF1 encodes a putative polyprotein containing the motifs of chymotrypsin-related serine protease, and ORF2 encodes a capsid protein. The genome arrangement of SmV B is similar to those belonging to the genera S*obemovirus, Barnavirus,* and *Polerovirus.* The putative domains for the serine protease, VPg, RdRp, and the CP are located in this order from the 5′ terminus to the 3′ terminus. SmV B, however, is distinctive since its genome has only two ORFs. The genome organization of the barnavirus MBV, on the other hand, resembles that of poleroviruses. These results suggest that SmV B, like SmV A, should also be classified into a new genus of mycoviruses.

The mycoviruses belonging to the families *Pseudoviridae*, *Metaviridae*, *Reoviridae* (genus *Mycoreovirus*), *Totiviridae*, *Partitiviridae*, *Chrysoviridae*, *Narnaviridae,* and *Barnaviridae* are discussed in more detail elsewhere in this encyclopaedia.

Replication and Gene Expression Strategy

Replication cycles of fungal viruses are not well studied except for a few cases including the *Saccharomyces cerevisiae* L-A virus. For dsRNA fungal viruses including members of the *Totiviridae*, *Partitiviridae*, *Reoviridae*, and *Chrysoviridae*, virus particles or subviral particles, containing RdRp, are believed to play pivotal roles in RNA transcription and replication. Replication of naked RNA mycoviruses represented by members of the family *Hypoviridae* may occur in infection-specific, lipid-membranous vesicles presumed to contain viral RdRp and RNA helicase. These vesicles are able to synthesize *in vitro* both plus and minus RNA at a ratio of 1:8. The narnavirus RNA, encoding only a single protein (RdRp), is associated with RdRp rather than being encapsidated. These RNA/RdRp complexes seem to play a key role in RNA replication.

Fungal viruses, like many RNA viruses of plants and animals, employ noncanonical translational strategies for expressing their genomes. These include −1 (*Totiviridae*) and +1 frameshifting (*Totiviridae*, *Pseudoviridae*, *Metaviridae*), termination-coupled initiation (*Totiviridae*, *Hypoviridae*), and IRES-mediated initiation (*Totiviridae*). Furthermore, a readthrough of a termination codon strategy is proposed for translation of the 3′ proximal ORF of DRV. A noncanonical mechanism may also be required for efficient translation of mRNA of narnaviruses, which lack poly(A) tails. The (CAA) n repeats found at the 5′ UTR of chrysoviruses are implicated in translation augmentation, as observed for the 5′ UTR sequence of tobacco mosaic virus. Translation of the viral genes that are regulated by these mechanisms is considered critical for virus viability.

Recent Technical Advances in Fungal Virology

Fungal virology has been thwarted by many constraints on manipulation of fungal viruses. Many, if not all, plant and animal viruses can be inoculated into individuals/tissue cultures of plant and animal hosts. Some assays with those hosts allow quantitative detection of biologically active viruses. As for mycoviruses, it is rather rare to be able to inoculate into host fungi because of experimental limitations, which often makes the etiology of mycoviruses difficult to establish. Fungal cells have rigid cell walls and are usually difficult to digest for preparation of cell-wall-free protoplasts ready for transformation or transfection. Even if protoplasts are made, their maintenance like animal cell culturing is not possible. Furthermore, fungal hosts usually have self/nonself recognition systems operating at inter- and intraspecies levels. Intraspecies barriers are based on vegetative incompatibility/compatibility that is governed genetically. This is often regarded as one of the host defense barriers that inhibits virus transfer between individuals. To overcome these barriers, a few methods are available. The prototypic hypovirus cryphonectria parasitica hypovirus 1 (CHV1) is the first for which a reverse genetics is established. Infection with different CHV1 strains can be launched either from cDNA integrated into host chromosomes or *in vitro*-synthesized RNA viral cDNA. It is noteworthy that via bombardment of mycelia, not protoplasts, the infectious CHV1 cDNA clone can be integrated into chromosomes of fungi other than the natural host *C. parasitica.* cDNA-based transfection systems are now available for three other species of RNA viruses: *Diaporthe RNA virus*, *Saccharomyces cerevisiae 20S narnavirus,* and *Saccharomyces cerevisiae 23S narnavirus.*

Mycoviruses in general lack infectivity as purified virions. However, all members of the new genus *Mycoreovirus* including mycoreovirus 1 (MyRV1), MyRV2, and MyRV3 were found to be infectious as purified particles when applied to fungal protoplasts. It is of interest in this regard that treatment of purified virions with trypsin or chymotrypsin was not required for infectivity. Protoplast fusion provides an alternative approach to introduce mycoviruses into vegetatively incompatible fungal strains that are incapable of hyphal anastomosis. Intra- and interspecies virus transfer via protoplast fusion has been reported in *Aspergillus* spp. This method is particularly useful for viruses for which infectious particles or cDNA-derived RNA are unavailable. Another recent revelation is that monokaryotic strains are able to serve as an intermediate virus transmitter between different mycelial incompatibility groups within the same species of *R. necatrix*.

To complete Koch's postulates, virus curing is as important as virus inoculation, because the virus must be back inoculated into an isogenic, virus-free strain with the same genetic background as the original virus-infected strain. Virus-free isolates may be obtained from germlings of asexual spores if virus transmission through spores is less than 100%. Alternatively, virus-free strains may be isolated by hyphal tip culturing, as in the case for *H. mompa* and *R. necatrix*. This technique is applicable to fungi infected with a virus that is transmitted to 100% of the asexual spores or for fungi that produce little or no spores.

Future Perspectives

Yeast as a (Model) Host to Study Viral Replication

S. cerevisiae has provided an excellent system to investigate virus assembly and replication of the dsRNA totivirus L-A and the ssRNA narnaviruses that infect yeast. With the robust yeast genetics, a number of host factors involved in totivirus replication were identified, many of which are related to translation events. Furthermore, the yeast provides an 'artificial' viral host model system to explore host genes affecting viral replication on a genomewide basis. Genetic screens of a collection of 4500–4800 single-gene deletion yeast strains (Yeast Knockout strain collection) have been successfully conducted for identifying host factors involved in replication and recombination with two different plant ssRNA viruses: brome mosaic virus and tomato bushy stunt virus (TBSV). Each screen led to the identification of approximately 100 host genes (approximately 1.8% of the entire yeast genes) that affect virus replication. Interestingly, the replication of the two viruses in yeast is affected by a different set of genes. A similar approach was employed to identify genes affecting the recombination of TBSV. This type of use of the yeast system can be expanded to a (+)-strand RNA animal virus and a (−)-strand RNA vertebrate virus. It may be surprising that the yeast has yet to be used for viruses infecting filamentous fungi. The yeast should be able to serve as a model host for mycoviruses other than those that naturally infect yeast.

Host Defense against Fungal Viruses

Host defense responses against viruses have not been explored intensively. RNA silencing is regarded as one of the host defense strategies of eukaryotes to molecular parasites including viruses, and operates in a number of fungi including important model and phytopathogenic fungi. However, no direct evidence is shown for RNA silencing that target mycoviral RNA. Recently, the hypovirus p29 was shown to be a suppressor of RNA silencing targeting a transgene that functions in both plant and fungal cells. This suggests that RNA silencing may function as an antiviral mechanism in fungal cells. The observation that p29 enhances the replication of a heterologous virus supports this idea. There are genetic elements involved in RNA silencing, for example, that are conserved widely from fungi to vertebrates. Functional roles of these factors in RNA silencing as antiviral reactions will need to be elucidated. Unraveling the mechanism by which the hypovirus p29, or other mycovirus-encoded RNA silencing suppressors, may block the RNA silencing pathway will be an interesting challenge.

Role of Mycoviruses in Plant–Fungal Mutualistic Associations

The question of whether mycoviruses are involved in the mutualistic interactions between endophytic fungi and their host plants is of considerable interest because of the attractive beneficial features of these associations and because of the common occurrences of fungal viruses in all major groups of fungi. This question was recently addressed in an intriguing report that presented evidence for a dsRNA mycovirus being involved in the mutualstic interaction between a fungal endophyte (*Curvularia protuberata*) and a tropical panic grass. This association allows both organisms to grow at high soil temperatures. The virus in question, which was designated curvularia thermal tolerance virus (CThTV), has unusual genome organization with an unknown genome expression strategy. CThTV has apparently a bipartite genome (RNA 1 and RNA 2), but no evidence that these RNAs are packaged in the 27-nm isometric particles isolated from the fungal host. Although many questions pertinent to the fungal endophyte, CThTV, and the veracity of the evidence for viral etiology remain unanswered, this report will undoubtedly stimulate the search for mycoviruses in other mutulastic fungal endophytes. In this regard, it is noteworthy that the well-characterized mutualistic endophyte, *Epichloë festucae*, was found to harbor a totivirus, but no phenotypes were associated with virus infection.

Mycovirus as Biocontrol Agents and as Tools for Fundamental Studies

The hypovirulence phenotype in the chestnut blight fungus (*Cryphonectria parasitica*) is an excellent and well-documented example for a mycoviral-induced phenotype that is currently being exploited for biological control. The debilitating disease of *Helminthosporium victoriae*, the causal agent of Victoria blight of oats, and the disease phenotype of the Dutch elm disease fungus *Ophiostoma novo-ulmi* are examples of pathogenic effects of dsRNA fungal viruses. An understanding of the molecular basis of disease in these fungal-virus systems would provide excellent opportunities for development of novel biocontol strategies of plant pathogenic fungi. Mycoviruses also continue to serve as versatile tools to study the virulence of host fungi, as recognized in studies with the hypovirus/*Cryphonectria parasitica* system, in which substantial advances in our understanding of the molecular basis of hypovirulence have been made.

See also: Barnaviruses; Hypovirulence; Hypoviruses; Metaviruses; Mycoreoviruses; Narnaviruses; Partitiviruses of Fungi; Partitiviruses: General Features; Pseudoviruses; Ustilago Maydis Viruses; Viral Killer Toxins; Yeast L-A Virus.

Further Reading

Buck KW (1986) Fungal virology-an overview. In: Buck KW (ed.) *Fungal Virology*, pp. 1–84. Boca Raton, FL: CRC Press.

Buck KW (1998) Molecular variability of viruses of fungi. In: Bridge PD, Couteaudier Y and Clarkson JM (eds.) *Molecular Variability of Fungal Pathogens*, pp. 53–72. Wallingford: CAB International.

Ghabrial SA (1994) New developments in fungal virology. *Advances in Virus Research* 43: 303–388.

Ghabrial SA (1998) Origin, adaptation and evolutionary pathways of fungal viruses. *Virus Genes* 16: 119–131.

Ghabrial SA (2001) Fungal viruses. In: Maloy O and Murray T (eds.) *Encyclopedia of Plant Pathology*, vol. 1, pp. 478–483. New York: Wiley.

Goodin MM, Schlagnhaufer B, and Romaine CP (1992) Encapsidation of the La France disease-specific double stranded RNAs in 36 nm isometric viruslike particles. *Phytopathology* 82: 285–290.

Hillman BI and Suzuki N (2004) Viruses in the chestnut blight fungus. *Advances in Virus Research* 63: 423–472.

Howitt RLJ, Beever RE, Pearson MN, and Forster RLS (2001) Genome characterization of Botrytis virus F, a flexuous rod-shaped mycovirus resembling plant 'potex-like' viruses. *Journal of General Virology* 82: 67–78.

Márquez LM, Redman RS, Rodriguez RJ, and Roossinck MJ (2007) A virus in a fungus in a plant: Three-way symbiosis required for thermal tolerance. *Science* 315: 513–515.

McCabe PM, Pfeiffer P, and Van Alfen NK (1999) The influence of dsRNA viruses on the biology of plant pathogenic fungi. *Trends in Microbiology* 7: 377–381.

Nuss DE (2005) Hypovirulence: Mycoviruses at the fungal–plant interface. *Nature Reviews Microbiology* 3: 632–642.

Sun L-Y, Nuss DL, and Suzuki N (2006) Synergism between a mycoreovirus and a hypovirus mediated by the papain-like protease p29 of the prototypic hypovirus CHV1-EP713. *Journal of General Virology* 87: 3703–3714.

Tavantzis S (ed.) (2001) *Fungal dsRNA Elements: Concepts and Application in Agriculture, Forestry and Medicine.* Boca Raton, FL: CRC Press.

Van der Lende TR, Duitman EH, Gunnewijk MGW, Yu L, and Wessels JGH (1996) Functional analysis of dsRNAs (L1, L3, L5, and M2) associated with isometric 34-nm virions of *Agaricus bisporus* (White Button Mushroom). *Virology* 217: 88–96.

Wickner RB (1996) Double-stranded RNA viruses of *Saccharomyces cerevisiae*. *Microbiological Reviews* 60: 250–265.

Furovirus

R Koenig, Institut für Pflanzenvirologie, Mikrobiologie und biologische Sicherheit, Brunswick, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Furovirus Siglum derived from fungus-transmitted rod-shaped virus.

History

Soil-borne wheat mosaic virus (SBWMV) is the type species of the genus *Furovirus*. Originally it was classified as a possible member of the tobamovirus group, because its rod-shaped particles resemble those of the tobamoviruses. In 1991, the fungus-transmitted rod-shaped viruses which all have several genome segments were separated from the tobamoviruses with a monopartite genome to form a new group, named furovirus group, with SBWMV as the type member. Molecular studies performed in the following years revealed that the genome organization of many of these furoviruses greatly differed from that of SBWMV. Eventually four new genera were created, that is, the genus *Furovirus* with SBWMV as the type species, the genus *Benyvirus* with *Beet necrotic yellow vein virus*, the genus *Pomovirus* with *Potato mop-top virus*, and the genus *Pecluvirus* with *Peanut clump virus* as type species, respectively. The genus *Furovirus* presently comprises five species, that is, SBWMV, *Soil-borne cereal mosaic virus* (SBCMV), *Chinese wheat mosaic virus* (CWMV), *Oat golden stripe virus* (OGSV), and *Sorghum*

chlorotic spot virus (SrCSV). SBCMV was simultaneously and independently described by two different working groups who had suggested the names European wheat mosaic virus (EWMV) and soil-borne rye mosaic virus (SBRMV), respectively. These names are no longer in use.

Host Ranges, Diseases, and Geographic Distribution

SBWMV has been known since the early 1920s. It causes mosaic, stunting, and severe losses of yield in winter wheat in the USA where it is widely distributed in the central parts. As with other soil-borne diseases the symptoms often occur in patches in the fields. SBWMV may also naturally infect barley. A deviating strain of SBWMV which differs from the type strain considerably in its nucleic acid sequences, but not in the proteins translated from them has been observed to be rapidly spreading in upper New York State since 1998. Diseases on wheat with symptoms similar to those described for SBWMV are caused by SBCMV in Europe, CWMV in China and by the distantly related Japanese strain (jap) of SBWMV in Japan. A virus closely related to SBWMV jap has recently been isolated from barley in France. SBWMV jap has also been obtained from barley in Japan. SBCMV is now widely distributed in Europe where it infects mainly wheat in Italy, France, and England, but mainly rye in Germany, Denmark, and Poland. SBWMV has been detected on a single field in Germany for the first time in 2002 with apparently no tendency to spread. OGSV has been detected in oats at various sites in Britain, France, and the USA (North Carolina). In oats it induces conspicuous chlorotic striping of leaves, but it fails to infect wheat. SBWMV, SBCMV, CWMV, and OGSV may be transmitted mechanically (sometimes only with difficulty) to some *Chenopodium* and *Nicotiana* species. SrCSV was isolated in 1986 from a single sorghum line from a breeder's plot in Kansas/USA. It is readily mechanically transmissible to maize where it produces a bright yellow mosaic and elongated ringspot symptoms several weeks after inoculation. Local infections are produced on mechanically inoculated *Chenopodium quinoa*, C. *amaranticolor*, and *N. clevelandii*. Attempts to transmit the virus back to *Sorghum bicolor* or to winter wheat either mechanically or by growing plants in soil in which infected sorghum was growing were unsuccessful.

Plants infected naturally by furoviruses are often also infected by bymoviruses, that is, wheat spindle streak mosaic virus in Europe and North America, wheat yellow mosaic virus in East Asia or oat mosaic virus in Europe and the USA. The symptoms caused by these bymoviruses are very similar to those caused by the furoviruses, and both the furoviruses and the bymoviruses are transmitted by *Polymyxa graminis* (see below).

Transmission in Nature and Long-Distance Movement in Infected Plants

Furoviruses, with the possible exception of SrCSV, are soil-borne and transmitted by the zoospores of *Polymyxa graminis,* a ubiquitous plamodiophorid protozoan formerly considered to belong to the fungi. *Polymyxa*-transmitted viruses (or their RNAs?) are taken up by the plasmodia of the vector in infected root cells, but there is no evidence that they multiply in the vector. The multinucleate plasmodia which are separated from the host cytoplasm by distinct cell walls may either develop into zoosporangia from which secondary viruliferous zoospores are released within a few days. Alternatively, the plasmodia may form cystosori which act as resting spores and may survive in the soil for many years even under extreme conditions. They may be distributed on agricultural equipment, by irrigation or even by wind blow. Upon germination they release primary zoospores transmitting the virus. Zoospores inject their contents into the cytoplasm of root cells where new plasmodia are formed. Zoospores treated with antisera to SBWMV or resting spores treated with 0.1 N NaOH or HCl retained their ability to transmit virus into plants indicating that the infectious viral material was carried inside. Immunolabeling and *in situ* RNA hybridization studies have revealed the presence of SBWMV movement protein and RNA but not of SBWMV coat protein in the resting spores of *P. graminis*. This might suggest that the vector does not transmit SBWMV in form of its particles but rather as a ribonucleoprotein complex possibly formed by the movement protein and the viral RNAs. The furovirus movement protein belongs to the '30 K' superfamily of movement proteins which are known to mediate cell-to-cell and vascular transport of viruses by binding viral nucleic acids and carrying them through plasmodesmata and through the vasculature. There is also strong evidence that two transmembrane regions found in the coat protein readthrough proteins of furoviruses are involved in the transmission process.

Immunogold-labeling studies have suggested that SBWMV uses the xylem in order to move from infected roots to the leaves. It may enter primary xylem elements before cell death occurs and then move upward in the plant after the xylem has matured into hollow vessels. There is also evidence for lateral movement between adjacent xylem vessels.

Control

Polymyxa-transmitted plant viruses may survive in soil for decades in the long-living resting spores of the vector. The diseases caused by these soil-borne viruses are, therefore, much more difficult to control than those caused, for

instance, by insect-transmitted viruses. Chemical control, for example, by soil treatment with methyl bromide, is neither efficient nor acceptable for economic and ecological reasons. Growing resistant or tolerant varieties currently represent the only practical and environmentally friendly means to lower the impact of these diseases on yield. Immunity to the wheat-infecting furoviruses has not been found. The tolerant varieties which have been developed contain high virus levels in the root system. The partial resistance in some varieties may be overcome when the plants are grown at temperatures >23 °C. Cereal genotypes with resistance to *P. graminis* have so far not been identified.

Particle Properties

Furovirus particles are nonenveloped hollow rods which have a helical symmetry (**Figure 1**). The diameter of the particles is *c.* 20 nm and in leaves of freshly infected field grown plants in the spring the predominant lengths are *c.* 280–300 and 140–160 nm. Due to internal deletions in the coat protein readthrough protein genes shorter particles arise later in season in naturally infected plants and in laboratory isolates which may outcompete the 140–160 nm particles. The single coat protein species of furoviruses has a molecular mass of *c.* 20 kDa.

Antigenic Properties

Serological relationships exist between all five furoviruses. Monoclonal antibodies (MAb) prepared in several laboratories were either species or even isolate specific or allowed broad-range detection of several or all furoviruses except for SrCSV that was not included in such studies. MAbs to SBWMV have also been used to determine the accessibility of stretches of the coat protein amino acid chain on the virus particles. The C-terminus of the coat protein is apparently exposed along the length of the particles and is readily removed by treatment with trypsin.

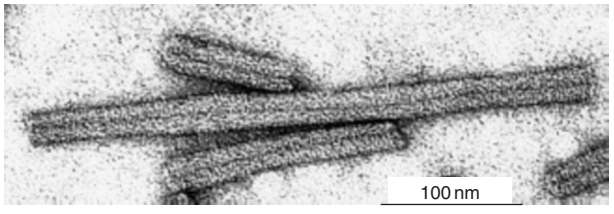


Figure 1 Particles of *Soil borne wheat mosaic virus* in a purified preparation negatively stained with uranyl acetate. Courtesy of Dr. D.-E. Lesemann and Dr. J. Engelmann, BBA Braunschweig.

Nucleic Acid Properties and Differentiation of Furoviruses

Furovirus genomes consist of two molecules of linear positive-sense ssRNAs. Their complete or almost complete nucleotide sequences have been determined for all five furoviruses described so far. They are 5′ capped and terminate in a functional tRNA-like structure with an anticodon for valine. This tRNA-like structure is preceded in the 3′ untranslated region by an upstream hairpin and an upstream pseudoknot domain (UPD) with two to seven possible pseudoknots. The genome organization is more or less identical for all furoviruses although there are considerable differences in nucleotide sequences (**Figure 2**). The percentages of sequence identities range from *c.* 60% to 75% for RNA1 and from *c.* 50% to 80% for RNA2 of different furovirus species. With strains of CWMV and SBCMV percentages of sequence identities are >94% for RNA1 and >85% for RNA2. Classification on the basis of sequence dissimilarities would suggest that SBWMV jap might be considered to be a separate species rather than a strain of SBWMV. However, the fact that in reassortment experiments a mixture of RNA1 of SBWMV jap and RNA2 of the type strain of SBWMV yielded an infectious progeny and the observation that the biological properties of all wheat-infecting furoviruses are very similar has been taken by some researchers as evidence that the type and the Japanese strains of SBWMV as well as CWMV and SBCMV should all be regarded as distantly related strains of the same species. As with viruses in other genera the decision whether related viruses should be considered as different strains of one virus or as separate species is not always easy, because these categories are men-made and many border-line cases exist in nature.

Organization of the Genome and Properties of the Encoded Proteins

Furoviral RNA1 codes for two N-terminally overlapping, presumably replication-associated proteins and for a *c.* 37 kDa movement protein (**Figure 3**). The shorter of the two replication-associated proteins contains methyltransferase and helicase motifs and the longer one, in addition, the RNA-dependent RNA polymerase motifs (**Figure 3**). The green fluorescent protein (GFP)-labeled 37 kDa protein of SBWMV, as opposed to its GFP-labeled coat protein, was shown to move from cell to cell in leaves of wheat but not in those of tobacco (a nonhost) and, similar to other viral movement proteins, to accumulate in the cell wall of both SBWMV-infected wheat leaves and transgenic wheat plants expressing the 37 kDa protein.

Furoviral RNA2 codes for the *c.* 20 kDa coat protein, a *c.* 84 kDa coat protein readthrough protein and a *c.* 19 kDa

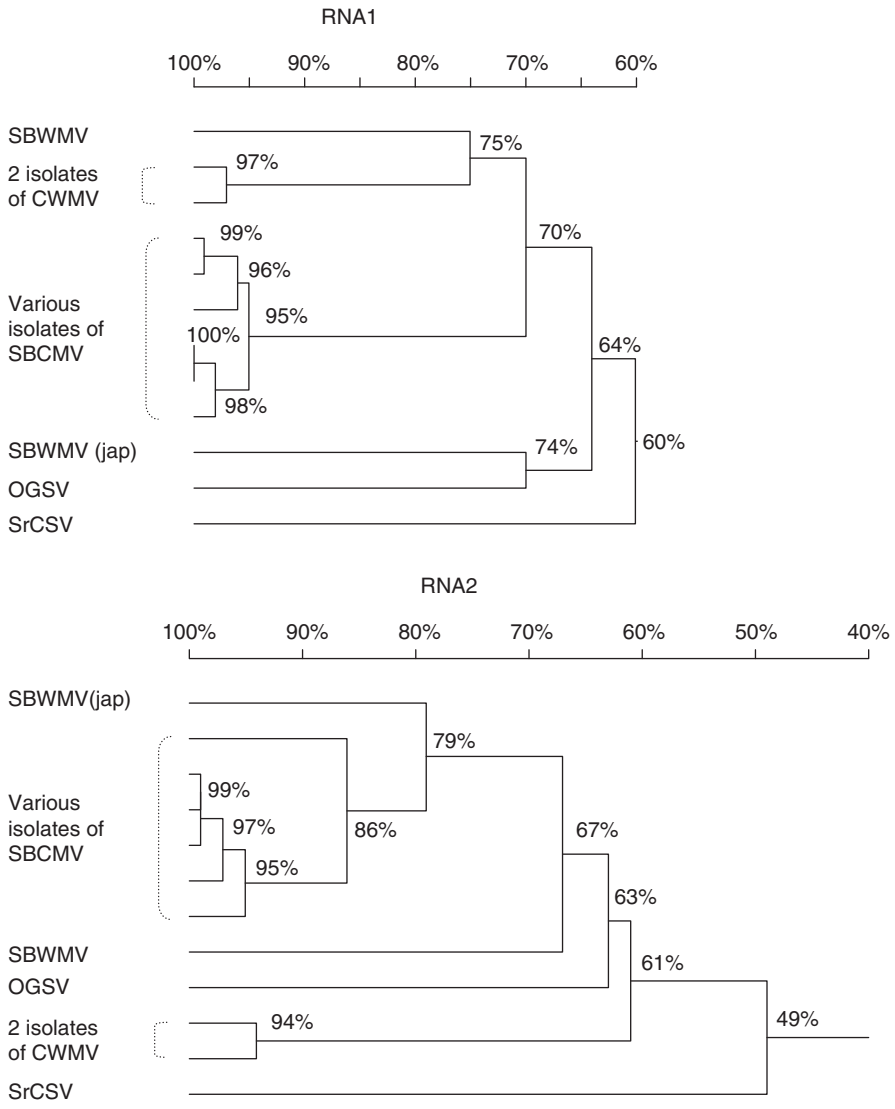


Figure 2 Percentages of nucleotide sequence identities among furoviral RNA1 and furoviral RNA2, respectively.

cysteine-rich protein. A 24 kDa protein found in *in vitro* translation experiments and also *in planta* has been shown to be coat protein with a 40-amino-acid N-terminal extension initiated at a conserved CUG codon 120 nt upstream of the AUG initiation codon for the coat protein gene. Neither the coat protein with this N-terminal extension nor the coat protein readthrough protein are required for SBWMV virion formation and systemic infection. SBWMV with full-sized RNA2 is found in naturally infected wheat in winter or early spring. Prolonged cultivation of field-infected plants, virus propagation by repeated mechanical inoculations or growth at elevated temperatures result in spontaneous deletions in the coat protein readthrough domain which may vary in size from 519 to 1030 nt. Computer analyses have revealed the presence of two complementary transmembrane domains (TM1 and TM2) in the coat protein readthrough proteins of furoviruses and also in those of the likewise *Polymyxa*-transmitted benyviruses and pomoviruses and in the P2 proteins of the *Polymyxa*-transmitted bymoviruses. The TM helices are apparently tightly packaged with ridge/groove arrangements between the two helices and strong electrostatic associations. It has been suggested that they facilitate the movement of the virus across the membrane surrounding the plasmodia in the plant cells. Nontransmissible deletion mutants lack the second transmembrane region partially or completely. The cysteine-rich 19 kDa protein of SBWMV is a suppressor of post-transcriptional gene silencing.

The products of the two 3′ proximal ORFs on RNA1 and RNA2, that is, the movement protein and the cysteine-rich protein, have not been found in *in vitro* translation experiments. It is assumed that they are expressed from subgenomic RNAs.

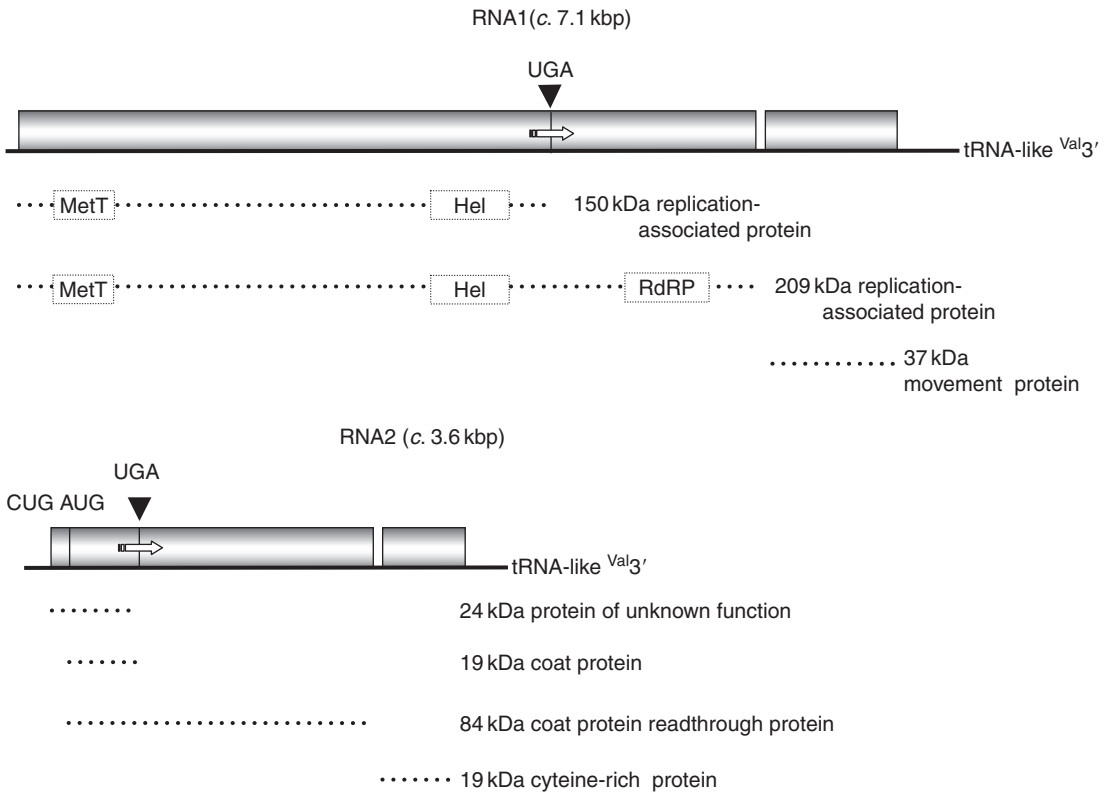


Figure 3 Organization of furoviral genomes (illustrated for SBWMV). The arrows in the open reading frames (boxed) indicate a readthrough translation due to a leaky stop codon.

Diagnosis

Symptoms caused by furoviruses may differ depending on environmental conditions, such as moisture and temperature. Nutrient deficiencies, winter injury or other viruses (especially bymoviruses), may produce symptoms that are easily confused with those caused by wheat-infecting furoviruses. Serological tests such as the enzyme-linked immunosorbent assay (ELISA), tissue print immunoassay, and immunoelectron microscopy as well as polymerase chain reaction (PCR) techniques allow a reliable detection. Real-time reverse transcriptase-PCR (RT-PCR) and PCR assays based on TaqMan chemistry have been developed for the detection and quantitation not only of SBCMV but also of its vector *P. graminis*. Real-time assays were found to be a 1000 times more sensitive than ELISA for the quantitation of SBCMV, and a 100 times more sensitive than conventional PCR for the quantitation of *P. graminis*.

Similarities and Dissimilarities with Other Taxa

The morphology of furoviruses resembles that of other rod-shaped viruses, that is, of benyviruses, pecluviruses, pomoviruses, hordeiviruses, tobraviruses, and tobamoviruses. The CPs of all these viruses have a number of conserved residues, for example, RF and FE in their central and C-terminal parts, respectively, which are presumably involved in the formation of salt bridges. Furoviruses, like pecluviruses and tobraviruses but unlike the other aforementioned viruses, have bipartite genomes. They differ from pecluviruses by having their movement function encoded on a single ORF rather than a triple gene block. They also differ from pecluviruses as well as from tobraviruses by having a coat protein readthrough protein gene. The gene for their cysteine-rich protein is located on RNA2, whereas with pecluviruses and tobraviruses it is located on RNA1. The cysteine-rich proteins of furoviruses, pecluviruses, and tobraviruses act as suppressors of post-transcriptional gene silencing and are phylogenetically interrelated, whereas those of beny- and pomoviruses are surprisingly unrelated to them. Furoviruses have two N-terminally overlapping replication-associated proteins (**Figure 2**) which in their amino acid sequences and structure are related to those of pomoviruses, pecluviruses, tobraviruses, and tobamoviruses. The 37 kDa movement protein of the furoviruses which belongs to the '30 K' superfamily of movement proteins relates them to the dianthoviruses and the tobamoviruses, whereas the valine-accepting tRNA-like structures on the 3′ ends of their RNAs relate them to the tymoviruses.

In other properties, such as particle morphology and mode of transmission, dianthoviruses and tymoviruses are very different from furoviruses. Furoviruses like tobamoviruses have an upper pseudoknot domain in the 3′ untranslated regions of their genomic RNAs.

See also: Benyvirus; Cereal Viruses: Wheat and Barley; *Hordeivirus*; *Pecluvirus*; *Pomovirus*; Potyviruses; Vector Transmission of Plant Viruses.

Further Reading

Adams MJ, Antoniw JF, and Mullins JG (2001) Plant virus transmission by plasmodiophorid fungi is associated with distinctive transmembrane regions of virus-encoded proteins. *Archives of Virology* 146: 1139–1153.

An H, Melcher U, Doss P, *et al.* (2003) Evidence that the 37 kDa protein of soil-borne wheat mosaic virus is a virus movement protein. *Journal of General Virology* 84: 3153–3163.

Diao A, Chen J, Gitton F, *et al.* (1999) Sequences of European wheat mosaic virus and oat golden stripe virus and genome analysis of the genus furovirus. *Virology* 261: 331–339.

Driskel BA, Doss P, Littlefield LJ, Walker NR, and Verchot-Lubicz J (2004) Soilborne wheat mosaic virus movement protein and RNA and wheat spindle streak mosaic virus coat protein accumulate inside resting spores of their vector, *Polymyxa graminis*. *Molecular Plant–Microbe Interactions* 17: 39–48.

Goodwin JB and Dreher TW (1998) Transfer RNA mimicry in a new group of positive-strand RNA plant viruses, the furoviruses: Differential aminoacylation between the RNA components of one genome. *Virology* 246: 170–178.

Hariri D and Meyer M (2007) A new furovirus infecting barley in France closely related to the Japanese soil-borne wheat mosaic virus. *European Journal of Plant Pathology* 118: 1–10.

Kanyuka K, Ward E, and Adams MJ (2003) *Polymyxa graminis* and the cereal viruses it transmits: A research challenge. *Molecular Plant Pathology* 4: 393–406.

Koenig R, Bergstrom GC, Gray SM, and Loss S (2002) A New York isolate of *Soil-borne wheat mosaic virus* differs considerably from the Nebraska type strain in the nucleotide sequences of various coding regions but not in the deduced amino acid sequences. *Archives of Virology* 147: 617–625.

Koenig R, Pleij CW, and Huth W (1999) Molecular characterization of a new furovirus mainly infecting rye. *Archives of Virology* 144: 2125–2140.

Miyanishi M, Roh SH, Yamamiya A, Ohsato S, and Shirako Y (2002) Reassortment between genetically distinct Japanese and US strains of *Soil-borne wheat mosaic virus*: RNA 1 from a Japanese strain and RNA 2 from a US strain make a pseudorecombinant virus. *Archives of Virology* 147: 1141–1153.

Ratti C, Budge G, Ward L, *et al.* (2004) Detection and relative quantitation of soil-borne cereal mosaic virus (SBCMV) and *Polymyxa graminis* in winter wheat using real-time PCR (TaqMan). *Journal of Virological Methods* 122: 95–103.

Shirako Y, Suzuki N, and French RC (2000) Similarity and divergence among viruses in the genus *Furovirus*. *Virology* 270: 201–207.

Te J, Melcher U, Howard A, and Verchot-Lubicz J (2005) Soil-borne wheat mosaic virus (SBWMV) 19K protein belongs to a class of cysteine rich proteins that suppress RNA silencing. *Virology Journal* 2: 18.

Torrance L and Koenig R (2005) Furovirus. In: Fauquet CM, Mayo M, Maniloff J, Desselberger U and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp 1027–1032. San Diego, CA: Elsevier Academic Press.

Verchot J, Driskel BA, Zhu Y, Hunger RM, and Littlefield LJ (2001) Evidence that soil-borne wheat mosaic virus moves long distance through the xylem in wheat. *Protoplasma* 218: 57–66.

Yamamiya A and Shirako Y (2000) Construction of full-length cDNA clones to Soil-borne wheat mosaic virus RNA 1 and RNA 2, from which infectious RNAs are transcribed *in vitro*: Virion formation and systemic infection without expression of the N-terminal and C-terminal extensions to the capsid protein. *Virology* 277: 66–75.

Fuselloviruses of Archaea

K M Stedman, Portland State University, Portland, OR, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Acidophile An organism whose optimal growth is at acidic pH, often 2 or below.
Archaea One of the three domains of life as defined by Carl Woese, completely microbial and separate from bacteria.
Archaeon Singular of Archaea or Archaebacteria.
Cryptic plasmid Plasmid with no known function.
Cryptic protein Protein with no known function.
Extreme thermophile An organism whose optimal growth temperature is above 80 °C.
Fusiform Having a spindle shape.
Thermoacidophile An organism that is thermophilic and acidophilic.
Thermophile An organism whose optimal growth temperature is above 50 °C.
Tyrosine recombinase One of a family of DNA recombining enzymes with an active site tyrosine.

Introduction

Fuselloviruses are unique spindle-shaped viruses with a short tail at one end that have so far only been observed in archaeal viruses or extreme environments dominated by

Archaea (**Figure 1**). The first fusellovirus to be found and characterized was SSV1, a virus-like particle of *Sulfolobus shibatae*, an extremely thermoacidophilic archaeon (optimal growth at 80 °C and pH 3). Fusellovirus genomes are relatively small double-stranded circular DNA molecules from 15 to 17 kbp. Only one gene, the viral integrase, shows clear similarity to genes outside the fuselloviruses. Their genomes persist in host cells both as episomes and integrated into the host genome. The genome is positively supercoiled when packaged in virus particles. Virus production can be induced by UV-irradiation. Virion production is usually constitutive at a low level and does not impede host growth or lyse the host cells. Viruses appear to be produced by budding at the cellular membrane.

Fuselloviruses have been found throughout the world and five complete genomes have been reported (**Table 1**). Fusellovirus-like DNA sequences have been found in many more locations by culture-independent techniques. Intriguing virus-plasmid hybrids have been discovered along with the SSV1-like viruses. SSV1, or portions thereof, has been used to make the first widely used vectors for genetic manipulation in thermophilic Archaea. Biochemical characterization has focused on the viral integrase with recent results showing that the integrase is similar to eukaryotic recombinases. Interestingly, the integrase gene and integration do not appear to be absolutely necessary for virus function. Nonetheless, the presence of the integrase gene appears to give such viruses a competitive advantage. Two structures of SSV1 encoded proteins have been solved to high resolution, but elucidation of the structure of the entire virion and many of the proteins remains elusive.

A number of archaeal viruses have been described as having a similar morphology to the SSV viruses. However, the spindle-shaped viruses of methanogens that were originally grouped with the fuselloviruses are very pleiomorphic and probably do not belong in this family. Additionally, the haloviruses His1 and His2 have very different genomes and replication and have been placed in the new genus *Salterprovirus*. Other spindle-shaped viruses of Archaea are much larger than the fuselloviruses.

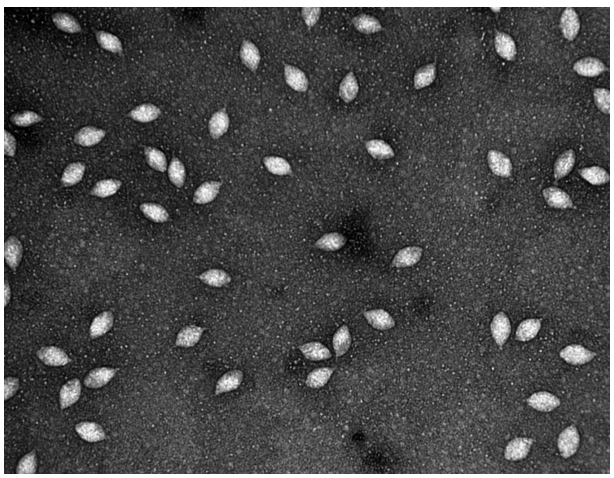


Figure 1 SSV1 image. Typical transmission electron micrograph of negatively stained SSV1 virions. Virus particles are 60 nm across and 90 nm long.

Table 1 Sequenced fuselloviruses

Virus	*Genome size (bp)*	*Location of isolation*
SSV1	15 495	Kyushu, Japan
SSV2	14 794	Reykjanes, Iceland
SSV3	15 230	Krisovik, Iceland
SSV-RH	16 473	Yellowstone, USA
SSV-K1	17 385	Kamchatka, Russia
pSSVx[a]	5 705	Reykjanes, Iceland

[a]Satellite.

History

The virus SSV1 was originally detected as a UV-inducible plasmid in a *Sulfolobus* isolate from Beppu Onsen in Kyushu, Japan by Wolfram Zillig and his co-workers in the early 1980s. Production of a 60×90 nm spindle-shaped virus-like particle was shown soon thereafter. The term 'virus-like particle' was used because infection of an otherwise uninfected strain could not initially be shown. It appears to be very difficult, if not impossible, to cure a *Sulfolobus* strain of a fusellovirus once infected. This may be due to the integration of the virus genome into a tRNA gene in the host genome by the virus integrase gene. SSV1 integrates into the CCG arginyl tRNA gene, whereas other fuselloviruses integrate into other tRNA genes. SSV1 became a model for the understanding of transcription in Archeaea. It was in the study of SSV1 genes and their promoters that Wolf-Dieter Reiter noticed that they resembled eukaryotic promoters with their canonical TATA-boxes. Together with previous work from the Zillig laboratory that had shown that the DNA-dependent RNA polymerases in Archaea were similar to eukaryotic DNA-dependent RNA polymerases, there was a strong evidence that the archaeal transcription machinery was eukaryote-like. The complete 15 495 bp genome sequence of SSV1 was determined in 1990. Genome analysis showed that other than the previously characterized virus coat protein genes, VP1 and VP3, an apparent DNA-binding protein, VP2, and the viral integrase (see below), none of the other 34 open reading frames (ORFs) showed any similarity to proteins in the known databases (**Figure 2**).

A major breakthrough in the study of SSV1 was made when Christa Schleper showed that SSV1 could infect *Sulfolobus solfataricus*, an uninfected *Sulfolobus* isolated from Pisciarelli, Italy, showing conclusively that SSV1

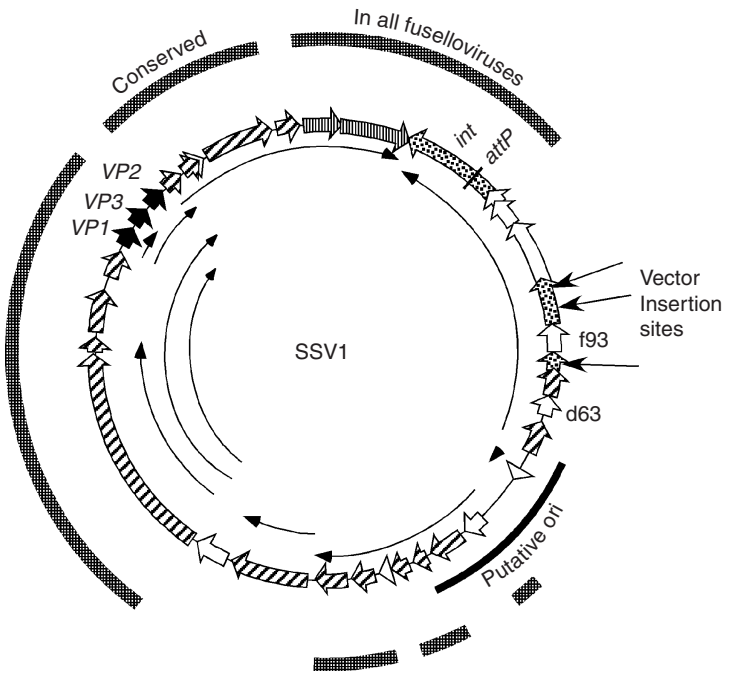


Figure 2 The SSV1 genome with its ORFs is shown, together with mapped transcripts. The attachment site in the viral integrase gene is shown as '*attP*'. Known viral genes are labeled. *VP1*, *VP3*, and *VP2* are virus structural genes. The location of the ORFs whose products have been crystallized, f93 and d63, are labeled. The putative origin of replication is labeled as 'Putative ori'. Insertion points for full-length shuttle vectors are shown with arrows outside the viral genome. Dotted ORFs have been shown to be not essential for virus function. Diagonally striped ORFs appear to be important for virus function. Vertically striped ORFs are conserved in pSSVx. ORFs conserved in all fusellovirus genomes are indicated by a stippled curve, outside of the ORF map.

was a virus. She was also able to transform *Sulfolobus* for the first time using this DNA. With this technique, plaque tests were established, the virus could be characterized, and large quantities could be purified. *Sulfolobus solfataricus* is one of the best studied of the thermophilic Archaea and a complete genome sequence and many other data are available. The development of the plaque test also allowed screening for fuselloviruses (and other viruses) in samples from throughout the world.

Fuselloviruses

Isolates

Using a plaque test and related spot-on-lawn halo techniques, first Wolfram Zillig's group and then others isolated fuselloviruses from Iceland, the USA, and Russia, in addition to SSV1 from Japan. The Zillig group found that approximately 8% of isolates from habitats with $T > 70\,^{\circ}\mathrm{C}$ and $\mathrm{pH}^{+} < 4$ contained SSVs that could be detected by their ability to infect *S. solfataricus*. First characterized was SSV2, from Iceland, followed by SSV-RH from Yellowstone National Park in the USA and SSV-K1 from the Kamchatka peninsula in Russia. All of these viruses had the typical spindle-shape and size of fuselloviruses and all contained *c.* 15 kbp genomes (**Table 1**). Those tested appeared to integrate into the host genome and were also inducible with UV-irradiation. However, the level of induction varied greatly between isolates as did the tRNA gene used as the site of virus integration into the host genome.

The complete genome sequences of all of these new fuselloviruses were determined and it was found that only about 50% of the genome is conserved (**Figure 2**). In some cases there is no sequence similarity whatsoever between parts of the virus genomes. The overall nucleotide identity is only about 55%, but the level of amino acid identity varies from undetectable to about 80%. The genomes have similar organization, with the exception of a large insertion and inversion in the SSV-K1 genome. Strangely, the putative DNA-binding protein, VP2, is missing in all genomes other than SSV1. The question then arose whether these genomic differences were due to geographical isolation of the viruses or to large amounts of local heterogeneity. To address this question the complete genome of a new SSV from Iceland, SSV3, was determined and compared to SSV2. It was found to have about 70% overall nucleotide identity to SSV2 and most of the ORFs were well conserved between the two viruses. However, there were a number of ORFs that were not conserved in SSV2, the other Icelandic virus, but were found in fuselloviruses from other parts of the world.

Culture-Independent Studies

The availability of multiple genome sequences for the fuselloviruses allowed the design of oligonucleotide probes that could be used to amplify conserved parts of fusellovirus genomes using the polymerase chain reaction. This technique was used on DNA samples collected directly from the environment, pioneered by Mark Young and his group. They showed that in a single spring that was known to harbor both *Sulfolobus* and fuselloviruses, the composition and relative abundance of fusellovirus sequences changed on at least a monthly basis. They also showed that a great deal of diversity was present in a single spring, much more diversity than seen in their hosts. Analysis of the sequence data over time indicated that there was a great deal of migration between springs and possibly a very large reservoir of fuselloviruses worldwide. Similar data from Lassen Volcanic National Park in the USA indicate this to be the case. These data contrast with the conservation seen between the whole SSV2 and SSV3 genomes from Iceland mentioned above. This conundrum remains to be resolved.

Plasmid Virus Hybrids

During the investigation of SSV2 from Iceland, a small virus-like particle was observed. This virus-like particle corresponded to a small plasmid, then named pSSVx, that was present in the same culture as SSV2 and appeared to be dependent on it or another complete fusellovirus for propagation. The genome sequence of pSSVx indicated that it was a fusion of a plasmid from the pRN family of cryptic *Sulfolobus* plasmids and two ORFs from a fusellovirus. It is not clear if activity of these ORFs is required for the plasmid to be packaged or if a *cis*-acting DNA sequence is required. At least one more of these virus-plasmid pairs has been reported in viruses from Iceland. This plasmid not only provides insight into virus and plasmid function but also is the basis for new genetic tools for *Sulfolobus*.

Fusellovirus-Based Vectors

One of the major reasons for sequencing the *S. solfataricus* genome and for isolation of viruses and plasmids of thermophilic Archaea was to be able to establish a genetic system for the analysis of these organisms. Many of the first and most successful steps have been taken using SSV1 or other fuselloviruses as the basis for plasmids that replicate in *Sulfolobus* and allow recombinant DNA to be transformed into *Sulfolobus*. A number of repeated sequences near the UV-inducible promoter, Tind, together with divergent promoters, led to the proposition that this region was the origin of replication (**Figure 2**). Pieces of the SSV1 genome containing this region have been incorporated into vectors that appear to replicate in *S. solfataricus* after transformation. However, these vectors have not been widely used. More successful vectors use the whole SSV1 genome with an insertion of an *Escherichia coli* plasmid in a region that was shown not to be critical for virus function to create infectious clones (**Figure 2**). Vectors based on this technique are now in their third generation. They have been successful in complementing mutants, overexpressing homologous and heterologous genes, and for preliminary gene-expression studies.

Fusellovirus Integrases

The only gene in fusellovirus genomes to show clear similarity to other genes is the virus integrase gene. It shows distant but clear similarity to the large family of site-specific tyrosine recombinases. Fusellovirus integrases are, however, unlike most other integrases in that the viral attachment site is within the integrase gene. Thus, integration disrupts the viral integrase gene. The attachment site in all fusellovirus integrases is in the N-terminal domain of the protein, and it is assumed, but not proved, that the remainder of the protein is not active, even though it contains all of the conserved catalytic amino acids responsible for recombination. The host attachment sites are in tRNA genes and proviral insertion preserves most of the tRNA gene so that it should remain functional. Intriguingly, all of the fuselloviruses sequenced to date integrate or are predicted to integrate into different tRNA genes. No additional host or virus genes appear to be required for either integration or excision. No excision of a provirus has been observed to date. Extensive *in vitro* studies with the SSV1 integrase indicate that the integrase itself is necessary and sufficient for both integrative or deletion reactions. Elegant molecular genetic studies have shown that the active site of the integrase is shared between two monomers and performs '*trans*-cleavage', similar to eukaryotic recombinases, but not bacterial ones.

Very recently, it was shown that the virus integrase and integration are not necessary for virus function. This was somewhat surprising due to the conservation of the integrase gene in all fusellovirus genomes sequenced to date. Additionally, a number of partial integrase genes, apparently made by past virus integration events, are present in not only the *S. solfataricus* genome but also in many other extremely thermophilic Archaea where they are thought to be highly involved in horizontal gene transfer.

Viruses lacking the integrase gene appeared not to integrate, but were stably maintained in laboratory cultures and under a number of stress conditions. However in

head-to-head competition experiments between viruses containing and lacking the virus integrase gene respectively, the construct without the integrase was rapidly out-competed.

Structures

A structural genomics program to elucidate all of the structures of the products of all of the ORFs in the SSV1 genome has produced two high-resolution structures to date. The first, the product of ORF f93, is clearly a winged helix-domain containing protein, almost undoubtedly involved in DNA binding. However, its role in virus function is not clear. This ORF is also not conserved in most of the other SSV genomes. A high-resolution structure for the ORF product d63 has been solved. It is a very simple four-helix bundle which may be involved in protein–protein interactions but again its function is not clear. Recently developed genetic tools may help in the elucidation of these functions.

Other 'Fusiform' Viruses

The viruses of extremely halophilic Archaea, His1 and His2, have strikingly similar morphology to the *Sulfolobus* fuselloviruses, but their genomes are linear and their replication is protein primed. Therefore, they have been recently assigned to the new floating genus *Salterprovirus*. The virus-like particle reported from *Methanococcus voltae* strain A3 at first glance appears to be very similar to the *Sulfolobus* fuselloviruses, but it has a much larger genome and many different shapes in transmission electron microscopy. A virus-like particle with a similar shape to the *Sulfolobus* fuselloviruses, PAV1, has been isolated from a deep-sea *Pyrococcus abysii* strain. This virus has not yet been fully characterized and its infectivity has not yet been shown. Its genome has no sequence similarity to the *Sulfolobus* SSVs.

A number of other viruses with a spindle shape with or without projections at one or both ends have been isolated from a number of thermoacidophilic Archaea. Most of them, however, have either been assigned to other virus families or have not yet been classified. Generally, their genomes are very different both in size and sequence from the known fuselloviruses and often their virions have very different sizes.

See also: Crenarchaeal Viruses: Morphotypes and Genomes; History of Virology: Bacteriophages; Satellite Nucleic Acids and Viruses; Taxonomy, Classification and Nomenclature of Viruses; Viruses Infecting Euryarchaea.

Further Reading

Albers SV, Jonuscheit M, Dinkelaker S, *et al.* (2006) Production of recombinant and tagged proteins in the hyperthermophilic archaeon *Sulfolobus solfataricus*. *Applied and Environmental Microbiology* 72(1): 102–111.

Arnold HP, She Q, Phan H, *et al.* (1999) The genetic element pSSVx of the extremely thermophilic crenarchaeon *Sulfolobus* is a hybrid between a plasmid and a virus. *Molecular Microbiology* 34(2): 217–226.

Prangishvili D, Forterre P, and Garrett RA (2006) Viruses of the Archaea: A unifying view. *Nature Reviews Microbiology* 4(11): 837–848.

Schleper C, Kubo K, and Zillig W (1992) The particle SSV1 from the extremely thermophilic archaeon *Sulfolobus* is a virus: Demonstration of infectivity and of transfection with viral DNA. *Proceedings of the National Academy of Sciences, USA* 89(16): 7645–7649.

Stedman KM (2005) Fuselloviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy Eighth Report of the International Committee on Taxonomy of Viruses*, pp 107–110. San Diego, CA: Elsevier Academic Press.

Stedman KM, Clore A, and Combet-Blanc Y (2006) Biogeographical diversity of archaeal viruses. In: Logan NA, Pappin-Scott HM, and Oynston PCF (eds.) *SGM Symposium 66: Prokaryotic Diversity: Mechanisms and Significance*, pp. 131–144. Cambridge: Cambridge University Press.

Stedman KM, Prangishvili D, and Zillig W (2005) Viruses of Archaea. In: Calendar R (ed.) *The Bacteriophages,* 2nd edn., pp. 499–516. New York: Oxford University Press.

Stedman KM, She Q, Phan H, *et al.* (2003) Relationships between fuselloviruses infecting the extremely thermophilic archaeon *Sulfolobus*: SSV1 and SSV2. *Research in Microbiology* 154(4): 295–302.

Wiedenheft B, Stedman KM, Roberto F, *et al.* (2004) Comparative genomic analysis of hyperthermophilic archaeal *Fuselloviridae* viruses. *Journal of Virology* 78(4): 1954–1961.

G

Gene Therapy: Use of Viruses as Vectors

K I Berns and T R Flotte, University of Florida College of Medicine, Gainesville, FL, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Biodistribution Tissues infected by a viral vector.
Episomal form Existence of the transgene as an extrachromosomal element.
Gene therapy Introduction of exogenous genes into cells to correct genetic or physiologic defects.
Nonenveloped virus A virus particle in which the viral coat is not surrounded by a lipid membrane.
Nonhomologous recombination Recombination between two unrelated DNA sequences.
Persistent infection An infection in which the viral genome is retained for extended periods, often for life, in host cells.
Replication competent A vector which can self-replicate and potentially spread from cell to cell.
Transgene A gene carried by a vector into a target cell.
Vector The means by which is a transgene is introduced into a target cell.

Introduction

Gene therapy represents the ultimate application of the evolution of molecular genetics from the elucidation of the structure of the double helix to patient treatment. There are only two requirements: the gene or DNA to be used to correct a genetic defect and a vehicle to introduce the DNA into the patient's cells. With the advent of cloning and recombinant DNA technology and the determination of the sequence of the human genome, a large number of genes are available for the therapy of genetic defects. Yet the clinical application of gene therapy remains in its infancy, albeit it has probably moved beyond the neonatal period. The major reason for the lack of more extensive progress has been the challenge of developing effective vectors. Successful vectors must be able to get the transgene (the new gene to be introduced) to the appropriate cell, transport the transgene into the cell, and then into the nucleus. Once present in the nucleus, a successful vector will express the transgene at the desired level and most often for an extended period of time. A major concern is toxicity, which may reflect either a host reaction to the vector itself, a commonly observed problem for early-generation vectors, or to the transgene, if it is perceived as nonself, which could occur in the case of a person with a null mutation. A further consideration is the ability of any DNA molecule introduced into the nucleus to potentially integrate into the host cell genome, most often by nonhomologous recombination. Nonhomologous recombination, which can occur at many sites in the genome, has the potential of disrupting normal gene expression by either altering the gene product or the regulation of the expression of the gene. Thus, important cellular functions may be lost which can impair the normal biology of the cell and in some cases lead to oncogenic transformation. Because of these considerations or hurdles, development of safe, effective vectors has proved to be much more challenging than initially appreciated.

Introduction of the transgene into patients can be done either *in vivo* or *ex vivo*. A variety of routes have been employed for administration *in vivo*; intramuscular, intravenous, via the airway, intraocular (either subretinal or into the vitreous humor), etc. The alternative approach is to remove the target cells from the body, introduce the transgene, and return the cells to the body. This method has been used primarily with the bone marrow. A second consideration is whether the vector will preferentially persist as an extrachromosomal element or will integrate into the host genome, either randomly or at a specific location. If the target consists of cells which divide rarely, if at all, then persistence as an extrachromosomal element may be an advantage. However, if the cell is a stem or progenitor cell which will undergo a sizable number of divisions, the vector is likely to be diluted out if it is not integrated into the genome.

A number of types of vectors have been tried, ranging from purified DNA administered either IV or via a gene gun, to liposomes, to vectors derived from viruses. The latter have been a favorite because viruses have evolved in nature to function by delivering and expressing genetic material within cells. All DNA viruses which replicate in the nucleus (including retroviruses) cause persistent infections which usually last for the life of the host. However, most of the viruses we know about also cause disease and thus the challenge is to engineer the virus so that it can function as a vector without causing disease. Interestingly, two of the viruses to be discussed below are not known to cause human disease. In any event, what has become clear is that to develop safe and effective vectors requires a detailed knowledge of the fundamental biology of the viruses to be used.

Despite the various challenges to be surmounted, gene therapy has developed to the point where there have been numerous clinical trials in which various levels of toxicity have been observed. In the most paradoxical example 12 children in France with severe combined immunodeficiency disease were cured by gene therapy; however, two of the children developed leukemia caused by the vector (happily, they have been successfully treated for this problem, as well). Clearly, much developmental work remains to be done, yet the promise is great. Several of the types of viral vectors which have been developed are described in the sections below.

There are several general considerations in the design of viral vectors. One is the host range or tissue targeting of the vector. A broad host range means that the vector can potentially be used to target many organs; however, if target specificity is desirable, a broad host range is not desirable. In other cases the desired target normally may not be infected by the vector. Thus, in many instances there have been attempts to modify the original host range. This has been achieved naturally by the use of different serotypes with differing host ranges, by modifying the genes encoding the capsid so that new epitopes are present on the surface of the virion, or, in some cases, by chemical linking of ligands to surface proteins. Another approach to altering host range is by creation of pseudotypes, enveloped viruses in which the envelope glycoprotein of a second virus is substituted for the normal constituent, for example, vesicular stomatitis glycoprotein has been substituted for a retrovirus envelope protein. Vector host range may also be affected by the alteration of the regulation of gene expression, so that it can occur or not in specific tissues.

Retrovirus Vectors

Retroviruses have long been considered to be good candidates to be developed as vectors for gene therapy. The viral capsid is surrounded by a lipoprotein envelope and contains two identical copies of a linear, single stranded RNA of plus polarity, in addition to at least two enzymes, reverse transcriptase and an integrase. As a consequence the viral life cycle passes through a double stranded DNA intermediate which integrates at a variety of sites in the cellular genome through the action of the virion integrase. Three different types of retroviruses have been used as vectors; the oncogenic retroviruses, lentiviruses, and spumaviruses. The oncogenic retroviruses have a wide host range (which can be extended by pseudotyping, in which the envelope proteins can be replaced by glycoproteins from other viruses), but can only integrate their genome in dividing cells after the nuclear membrane is removed before mitosis occurs. This property has limited their utility on the one hand; on the other, it has some desirable features because it limits the potential for horizontal spread since most cells in the body do not divide. Since the oncogenic retroviruses cause cancer, it is important that vector preparations not contain replication competent retrovirus (RCR). The viral genome contains three genes, *gag*, *pol*, and *env*. All of these are deleted from vector constructs; the only original sequences that are retained are the long terminal repeats (ltr), the packaging signal, and an ori. Vector production requires the normal gene products and the challenge is to make the probability of recombination to produce RCR very small so that quality checks can be passed. Since the viral genome integrates at a variety of sites, another potential concern is insertional mutagenesis in which either an essential gene is disrupted or a normally silent gene under tight regulation gains the ability to be expressed constitutively. The former seems relatively remote since most genes are present in at least two copies in a diploid genome. However, the latter could be potentially a one-hit phenomenon and lead to cancer. Indeed, this appears to have happened in a clinical trial, which otherwise was one of the great success stories of gene therapy. Twelve French infants with an X-linked form of severe combined immunodeficiency were treated with a vector derived from Moloney murine leukemia virus; nine of the children were 'cured' of this otherwise lethal disease. However, two children developed leukemia; in both cases the disease was clonal and the cells in each case contained the vector inserted next to the LMO gene. The conclusion was that the inserted vector had turned on gene expression, leading to the disease. Fortunately, it has been possible to successfully treat the children for this additional problem. Interestingly, the French government decided to continue the trial since the disease was otherwise lethal; that is, the benefit outweighed the proven risk.

Lentivirus vectors are derived from human immunodeficiency virus (HIV) and, thus, there is the real and psychological challenge of using a vector based upon a dangerous human pathogen. In addition to the three genes contained in the genomes of all retroviruses, the

lentivirus genome contains six additional genes which are the products of splicing and function in a variety of regulatory roles. Again the approach has been to generate vectors from which all the viral genes have been deleted in producer cells where the helper genes required have a minimal amount of sequence homology so that there is very little chance of recombination to generate RCR. Lentivirus vectors are easier to produce in higher titers than the oncogenic retrovirus vectors and have the great potential advantage that they can successfully infect both dividing and nondividing cells and integrate the genome in both cases to establish persistence. Whether the psychological question can be successfully overcome remains to be seen. An alternative possibility is the development of spumavirus vectors. These have many of the same desirable features as lentivirus vectors without the problem of being derived from a serious pathogen. Spumavirus is a common human infection but has never been convincingly associated with human disease. They have been used successfully in a variety of animal models and indeed seem promising.

Adenovirus Vectors

Certain DNA viruses offer the potential to transduce both dividing and nondividing cells, with a much lower risk of integration-related carcinogenesis. Recombinant adenovirus (rAd) and recombinant adeno-associated virus (rAAV) have both been used in clinical gene therapy trials, as have recombinant herpes simplex virus (rHSV). Adenoviruses are nonenveloped, double stranded DNA viruses with a 36 kb genome that contains both early genes (encoding regulatory proteins expressed prior to DNA replication during a lytic life cycle) and late genes (encoding structural proteins expressed after DNA replication). Adenoviruses are present in over 60 serotypes in humans, and they commonly cause acute, self-limited infections of the respiratory and gastrointestinal tracts of humans. rAd vectors based on the group C adenoviruses, Ad2 and Ad5, were initially developed by deleting portions of the early genes E1a and E3 and inserting a therapeutic gene of interest. These first-generation rAd vectors are relatively easy to propagate and mediate robust short-term expression in a wide range of cell types, *in vitro* and *in vivo*. rAd vectors also trigger innate and adaptive immune responses in the host under most circumstances, which can limit both the duration of expression and the safety of *in vivo* gene therapy. An acute inflammatory response to a first-generation rAd vector resulted in the death of one patient with partial ornithine transcarbamylase (OTC) deficiency in a well-publicized incident. Nonetheless, clinical trials of rAd vectors continue in cancer patients and as recombinant vaccines.

Recognition of the immunogenicity of rAd vectors has led to the generation of later-generation versions of the vector, in which more viral coding sequences have been deleted. Second-generation vectors were produced by deletion of other early genes such as E2a and E4, in addition to the E1a and E3 deletions described above. The second-generation vectors mediate less expression of viral proteins, especially the late proteins, and trigger less adaptive immunity than first-generation vectors. The latest version of the rAd technology, known as the high-capacity or helper-dependent adenoviral (HD-Ad) vector, has a greater payload for therapeutic genes and expression elements, a lower risk of adaptive immune responses, and a longer duration of effect. HD-Ad vectors are currently beginning early-stage clinical trials.

Adeno-Associated Virus Vectors

Adeno-associated viruses (AAVs) are nonpathogenic, and include among others, human and primate parvoviruses, with a nonenveloped icosahedral capsid and a 4.7 kbp single stranded DNA genome. The genome contains genes required for replication (*rep*) and for the capsid components (*cap*). The use of internal promoters and alternate splicing allows for the production of a total of four Rep proteins (Rep78, Rep68, Rep52, and Rep40) and three capsid proteins (VP1, VP2, and VP3). These two genes are flanked by two palindromic inverted terminal repeats (ITRs), which contain all of the *cis*-acting elements required for replication and packing of the AAV genome.

AAVs are not adenoviruses, but were originally discovered as contaminants of adenovirus cultures. Wild-type AAV requires a helper virus (usually an adenovirus or herpesvirus) for efficient replication. In the absence of helper virus co-infection, AAV enters into the latent stage of its life cycle, either by maintaining itself as a stable episome or by integrating its DNA into the host genome, often into a specific site on human chromosome 19, the AAVS1 site. This site-specific integration is dependent upon the AAV Rep protein, which is deleted from rAAV vectors. Thus, the vectors have been found to persist primarily as episomal form, with a low frequency of non-site-specific integration. rAAV vectors are also capable of transducing nondividing cells and have been shown to persist long term *in vivo* in nondividing cells in animal models. One of the major limitations of the rAAV system is the small packaging capacity of the virion, with a payload of approximately 4.5 kb of exogenous DNA. This may be overcome in certain instances by taking advantage of the propensity of rAAV vector genomes to form heteromultimers, thus allowing for *trans*-splicing between two different vector genomes within a single target cell.

rAAV vectors have been used in clinical trials in cystic fibrosis, hemophilia B, limb-girdle muscular dystrophy, Parkinson's disease, Batten's disease, and alpha-1 antitrypsin (AAT) deficiency. Results of these early phase trials

confirm the fact that rAAV is safe over a wide dose range when administered to the airways, muscle, and central nervous system. Delivery to the liver in patients with hemophilia B resulted in transient elevations of liver enzymes that appeared to be associated with cell-mediated immune responses to rAAV capsid proteins retained in hepatocytes. While these immune responses may have limited the duration of therapeutic effect of the vector, no clinically important toxicity was observed.

All of the previous clinical trials experienced with rAAV have been using the type strain, AAV serotype 2. In recent years, over 100 new serotypes and genomic variants of AAV have been identified. Many other serotypes have been developed as rAAV vectors and some have substantially higher efficiency for transduction of specific tissues than rAAV2. One trial of an alternative serotype has just been initiated in AAT deficient patients with AAV serotype 1 capsids. The preclinical rAAV1 experience demonstrates that switching to a different AAV serotype capsid can still retain the basic property of long-term persistence of vector DNA, yet capsid–receptor interactions may lead to a distinct pattern of vector biodistribution to distant organs and to increased innate immunity as the distinct capsids interact with pattern recognition receptor proteins. Altered biodistribution could be advantageous if it leads to increased transduction of a target cell population. However, it could also lead to an increased risk of inadvertent germ line transmission of vector DNA, a phenomenon that is generally to be avoided. The clinical utility of alternative rAAV serotype vectors may become clearer in the near future as more clinical trials with these vectors are undertaken.

Both rAd and rAAV vector capsids have also been genetically engineered to create receptor-targeted versions of their respective vectors. Essentially, this approach involves insertion of specific peptides that may serve as ligands for specific cell-surface receptors. These 'designer' vectors have the potential for highly efficient and specific cell targeting. One key to the use of targeting technology is the identification of sites within the capsid which can be modified without a loss of the integrity of the capsid. Another important factor is the size of the peptide insert that may be tolerated within the insertion site. In the case of rAAV, one of the three capsid proteins, VP2, can either be completely deleted from the vector without loss of infectivity (and with a modest increase in packaging capacity), or can be modified at its N-terminus to include very large inserts, including single-chain antibodies. This form of rationally designed vector appears to hold much promise for future vector development, as does another variant of rAAV capsid alteration, an *in vitro* evolution, or DNA shuffling approach. In these types of approaches libraries of rAAV capsid variants are developed by mutagenesis and/or recombination of domains between distinct AAV serotypes. Serial passage on relatively nonpermissive cell types allows for positive selection for novel AAV variants capable of transducing those cell types, thus further expanding the repertoire of AAV capsids available as vehicles for therapeutic gene transfer.

Herpesvirus Vectors

Human herpesviruses are large double stranded DNA viruses with a complex structure. An envelope is separated from the nucleocapsid by the tegument which is composed of a large number of proteins which subserve various functions during viral infection, including turning off cell protein synthesis and initiating viral transcription. The herpes simplex virus (HSV) genome is composed of two parts, a long unique sequence and a short unique sequence, both bounded by terminal repeats. There are three classes of cellular genes: (1) immediate early, which control gene expression; (2) early, involved in DNA replication; and (3) late, which encode structural proteins. HSV preferentially infects cells of the central nervous system; after initial entry through epithelial cells of the skin or mucosa the virions travel retrograde through axons to cell bodies, particularly in sensory ganglia, where the virus most commonly establishes a latent infection. The viral genome is maintained as an extrachromosomal circle. Because the neurons which are persistently infected do not divide, the latent state is maintained unless a variety of environmental stimuli which can stress the cell activate viral replication. HSV vectors of two types have been developed and most often are intended to be lytic and cidal for tumors of the brain. The first type of vector has had several viral genes which are normally associated with virulence removed (immediate early genes and the gene for the tegument protein which shuts off cell protein synthesis) and replaced with genes specifically toxic to tumor cells. The second type is termed an amplicon, which consists of a cassette containing the transgene with its regulatory sequences, the HSV origin of replication and the packaging signal. The former type has a transgene capacity of almost 30 kbp while the latter can accommodate almost the full 160 kbp in the full genome. Since the latter expresses no viral genes, toxicity associated with wild-type virus and an inflammatory immune response can be avoided (unless caused by the transgene). HSV vectors of the first type have been used in several clinical trials and in phase I trials have been found to be nontoxic and to indicate the possibility of beneficial effects on the target tumors, although the number of patients in completed trials has been too small for definitive results.

Clinical Trials

The future of clinical gene therapy may lie with the appropriate matching of vector properties with the properties

of the cell target and the desired timing of transgene expression. Situations in which transient, high-level expression is needed may be best addressed with rAd or liposomal vectors. First-generation rAd vectors may be particularly well suited for recombinant vaccine approaches since the innate responses to Ad may serve as an adjuvant for adaptive immune responses to the transgene product. Longer-term expression in nondividing cells might be better addressed with lentivirus or rAAV vectors. Finally, long-term expression in rapidly dividing cells might be best addressed with onco-retrovirus or lentivirus vectors since episomal rAAV vector genomes may be lost in such cells. The choice of vector and route of administration is often dictated by the disease process itself. While many applications of gene therapy have been for recessive genetic disorders, which usually require long-term gene expression within a particular organ, others have aimed to induce immune responses to malignant tumors, to stimulate a new vascular supply to ischemic limbs or segments of myocardium, to augment wound healing, or to relieve inflammatory diseases, such as rheumatoid arthritis. Each of these examples would require a different route of administration, as well as different timing and level of gene expression in order to achieve the desired therapeutic effect.

As more gene therapy vectors are developed into clinical products for specific diseases, a regulatory pathway to the clinic has emerged in USA and in other industrialized countries. The paradigms established for biological agents by the US Food and Drug Administration (FDA) provide the model for such regulatory approval. The pathway begins with proof-of-principle studies in which an appropriate vector is chosen to transfer the therapeutic gene into a cell culture or animal model. These studies generally address the efficiency and duration of gene expression, the biological effects of the transgene product, and functional correction of the defect. The gene transfer approach used in the proof-of-principle study generally mimics the ultimate clinical use of the vector, in terms of whether the vector is injected directly *in vivo* into a target organ, or is used for *ex vivo* transduction of a cell population that is amenable to reimplantation.

If the proof-of-principle studies demonstrate a potentially therapeutic effect, additional preclinical safety testing is usually undertaken in animals. These studies often are done in a larger number of animals, so that important potential toxicities can be understood and their frequency predicted. In some cases, both small animal (usually rodent) models and larger animal (occasionally nonhuman primate) models will be used. Formal safety studies should cover a fairly broad dose range and should include evaluations at both early and late time points. Safety studies in gene therapy have special considerations, including studies of the biodistribution of vector genomes to distant organs, particularly to germ cells within the gonads, studies of the immune responses to vector components and transgene products, and possibly a survey for vector-related tumorigenesis. The complete toxicology package should address potential toxicity of the vector over a specific dose range, and thus guide both the dosing and the safety studies within the early-phase clinical trials.

Traditionally, clinical trials have included phase I safety studies, phase II studies that focus on safety and biological effects, and phase III studies to prove efficacy and document lower frequency toxicities. In recent years, there has been a growing emphasis on phase IV studies, which occur after licensure of a drug or biological product for distribution and sale. In addition, there is a new concept of phase 0 studies in gene transfer, which are used as an experimental context to learn about properties of a given vector or vector class, without necessarily being part of the regulatory process for a specific gene therapy product. Once again, clinical gene therapy studies have certain special considerations, including sampling of semen (as an indicator of potential for inadvertent germ line transmission in males), studies of the immune responses to the vector and transgene product, and long-term follow-up of gene therapy study participants. The latter is designed to monitor the long-term effects of vector-mediated mutagenesis, that might result in secondary malignant changes in target cells at a later time. There are also special regulatory processes for gene therapy trials, particularly those that receive federal funding in USA. These include the involvement of the Office of Biotechnology Activities at the National Institutes of Health, and its Recombinant DNA Advisory Committee, as well as the use of Data and Safety Monitoring Boards by sponsoring NIH institutes for ongoing monitoring of clinical trials.

Given the fairly broad range of available vectors and the rapidly developing systems for clinical testing of vector candidates, it seems likely that a growing number of gene therapy agents will reach clinical application in the coming years. However, the long-term impact of gene therapy will likely take much longer to be fully realized. It remains to be seen just how broad that impact will be.

See also: Plant Virus Vectors (Gene Expression Systems); Viral Suppressors of Gene Silencing; Virus Induced Gene Silencing (VIGS).

Further Reading

Baum C, Schambach A, Bohne J, and Galla M (2006) Retrovirus vectors: Toward the plentivirus? *Molecular Therapy* 13: 1050–1063.

Epstein AL, Marconi P, Argnani R, and Manservigi R (2005) HSV-1-derived recombinant and amplicon vectors for gene transfer and gene therapy. *Current Gene Therapy* 5: 445–458.

Ghosh SS, Gopinath P, and Ramesh A (2006) Adenoviral vectors: A promising tool for gene therapy. *Applied Biochemistry and Biotechnology* 133: 9–29.

Hibbitt OC and Wade-Martins R (2006) Delivery of large genomic DNA inserts >100 kb using HSV-1 amplicons. *Current Gene Therapy* 6: 325–336.
Loewen N and Poeschla EM (2005) Lentiviral vectors. *Advances in Biochemical Engineering Biotechnology* 99: 169–191.
Mergia A and Heinkelein M (2003) Foamy virus vectors. *Current Topics in Microbiology and Immunology* 277: 131–159.
Morris KV and Rossi JJ (2006) Lentiviral-mediated delivery of siRNAs for antiviral therapy. *Gene Therapy* 13: 553–558.
Schambach A, Galla M, Modlich U, *et al.* (2006) Lentiviral vectors pseudotyped with murine ecotropic envelope: Increased biosafety and convenience in preclinical research. *Experimental Hematology* 34: 588–592.
Shen Y and Nemunaitis J (2006) Herpes simplex virus 1 (HSV-1) for cancer treatment. *Cancer Gene Therapy* 13: 975–992.
Wu Z, Asokan A, and Samulski RJ (2006) Adeno-associated virus serotypes: Vector toolkit for human gene therapy. *Molecular Therapy* 14: 316–327.

Genome Packaging in Bacterial Viruses

P Jardine, University of Minnesota, Minneapolis, MN, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Chromosome Encapsidated nucleic acid polymer that is the genomic component of the phage virion.
Concatamer A long nucleic acid polymer made up of tandemly linked genomes.
Packaging ATPase Enzyme complex that binds to, or is part of, the procapsid and translocates the chromosome from the outside to the inside of the capsid.
Procapsid The preformed, precursor capsid into which the genome is packaged.
Prohead See procapsid.
Virion The mature, infectious phage particle.

Introduction

The parasitic phase of the virus life cycle begins with the delivery of a viral genome into the host cell. Therefore, the bringing together of the virus chromosome with the viral capsid can be considered the culmination of the process of virus assembly that yields the infectious particles that will continue the next round of host-dependent replication. Bacteriophages have evolved complex mechanisms by which they ensure that this process is both effective and efficient.

As in other virus types, the encapsidation of the bacteriophage chromosome occurs by one of two distinct pathways. The first is the co-assembly of the components of the proteinaceous capsid with the chromosome at the terminus of viral replication. By this mechanism, capsid components assemble around the phage chromosome via the principles of self-directed assembly, with large classes of single-stranded DNA (ssDNA) and single-stranded RNA (ssRNA) phages being general examples. The ssDNA filamentous phages of the family *Inoviridae*, such as M13, achieve co-assembly of chromosome and capsid via an extrusion process by which ssDNA chromosome, coated with binding protein, is translocated through the cell membrane. The chromosome picks up external protein components of the capsid at membrane associated assembly sites. This extrusion/co-assembly is ATP dependent. In contrast, ssRNA phages of the family *Leviviridae*, such as MS2, present a co-assembly of an icosahedral capsid around a highly structured ssRNA chromosome.

In the second general pathway of genome encapsidation, the focus of this article, capsid assembly and genome replication remain separated until they converge in an event during which the phage chromosome is packaged into a preformed capsid using a process distinct from those that drive co-assembly. Unlike co-assembly, genome packaging is often mediated by mechanisms that energetically drive the chromosome from the outside to the inside of the capsid. In many phage systems, this event requires the assembly of specialized molecular machinery and the input of energy to push the nucleic acid chromosome into a confined space against a concentration gradient that result in the compaction of the nucleic acid polymer by several orders of magnitude.

There are three types of genome packaging that represent three large groups of phages and are distinguished by the type of nucleic acid substrate that is packaged. These consist of (1) the double-stranded DNA (dsDNA) phages of the order *Caudovirales*, which includes the families *Podo-*, *Sipho-*, and *Myoviridae*, and others groups including the family *Tectiviridae*; (2) the ssDNA phages of the family *Microviridae*; and (3) the double-stranded RNA (dsRNA) phages of the family *Cystoviridae*. All three of these groups of phages have evolved complex and highly efficient strategies to select and translocate viral chromosomes from the cytosol of the host cell into a preformed capsid.

General Considerations

One of the most crucial aspects of genome packaging in bacteriophage, as in other viruses, is the appropriate selection of virus chromosomal nucleic acid polymer from the milieu of nucleic acid inside the host cell. This selectivity is achieved by the binding and recognition of precursor viral chromosomes by components of the genome packaging apparatus and targeting them to the preassembled precursor capsid. This targeting event can be coupled to other events such as genome replication, as in the family *Microviridae*, be part of the chromosomal maturation process, as in the dsDNA phages, or precede the final replicative stages of the chromosome as found in the family *Cystoviridae*. Nomenclature can be diverse throughout the literature, with precursor capsids being referred to as procapsids, proheads, or preheads, depending on the system. This article refers to the precursor capsid targeted for packaging as the prohead or procapsid as is the convention for each system.

Additionally, distinction must be made between the bacteriophage genome and the packaged chromosome that becomes part of the infectious progeny virion. In some cases, these two terms are synonymous, as in the family *Microviridae* and the dsDNA phages lambda, ø29, and PRD1, since the chromosome is limited to a single copy of the genome. Exceptions arise for two reasons: (1) the genome is segmented into multiple chromosomes, as in the family *Cystoviridae*; and (2) the chromosome is terminally redundant as a result of a headful packaging mechanism using a concatamer as a substrate, as in caudoviruses such as the T-phages, P22, SPP1, etc. A singular exception is the Mu-like bacteriophage, in which the chromosome is comprised of a complete copy of the phage genome flanked by host-derived DNA sequences, a result of excision of the phage DNA from the host chromosome.

Finally, the inclusion of the packaging motor complex into the mature virion is variable, depending on the phage. In general, the enzyme complex responsible for chromosome translocation detaches from the packaged capsid in the dsDNA phages and is replaced by tail organelles. In the family *Microviridae*, the packaging complex is part of the DNA replication complex that is similarly not retained on the capsid surface. In contrast, the packaging complex of the family *Cystoviridae* remains attached to the capsid and is part of the mature virion. A summary of the key components and processes involved in packaging is presented in **Table 1**.

dsDNA Packaging

Since dsDNA phages are the most prevalent biological pathogens in the biosphere, the dominant type of genome packaging is the dsDNA packaging seen in the families *Podo-*, *Sipho-*, *Tecti-*, and *Myoviridae* (**Figure 1**). As with other packaging motifs, dsDNA phages pre-assemble an empty, immature capsid that docks with the phage chromosome for packaging. Universally, DNA packaging in dsDNA phages involves three viral components: (1) the preassembled prohead or procapsid, (2) the matured DNA chromosome, and (3) the ATP-dependent DNA-translocating enzymes.

The dsDNA phage chromosome is packaged into an immature, icosahedral prohead. The architecture of the capsid in these dsDNA phages is different from other icosahedral phages. At a unique fivefold vertex of the prohead, a portal structure is embedded, replacing one of the 12 pentamers of the icosahedron. This portal is a

Table 1 Packaging facts

Phage family	*Genome type*	*Point of entry into procapsid*	*Enzymes involved*	*Nucleic acid polymer translocated*	*Completion events*
Sipho-, Podo-, Myo-, Tectiviridae	dsDNA	Unique fivefold vertex via portal	Large and small packaging enzyme complex	Concatameric dsDNA (lambda, P22, SPP1, T-phages) Unit-length genome inserted into host chromosome (Mu) Unit-length chromosome with terminal proteins (phi29, PRD1)	Cleavage of concatamer, motor detachment, and tailing Cleavage of chromosome from host genome, motor detachment, and tailing Motor detachment and tailing
Microviridae	ssDNA	Twofold axis	Replication complex, J protein	ssDNA	
Cystoviridae	dsRNA	Fivefold vertex	Translocating NTPase complex	ssRNA	Replication of ssRNA to dsRNA

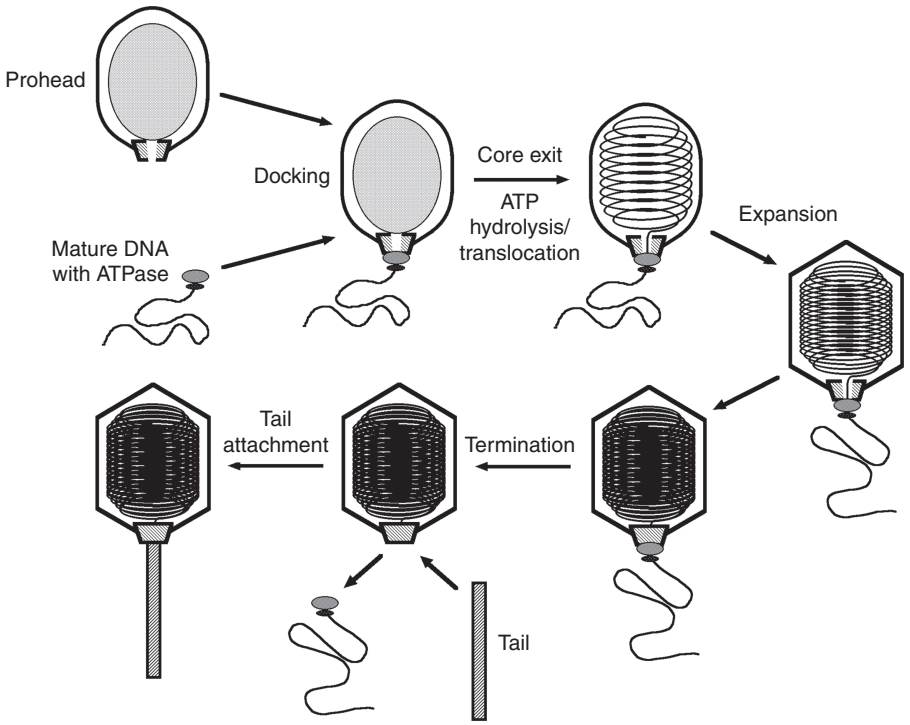


Figure 1 Schematic of generalized dsDNA phage assembly. A prohead interacts with the packaging ATPase holoenzyme–DNA complex via its head–tail connector. ATP hydrolysis powers translocation of the mature DNA, and at some point the scaffold core is ejected, either whole or following proteolysis. After an amount of DNA enters the head the shell capsomeres rearrange, making the head more angular and, in most phages, increasing the head volume. DNA translocation continues until a full complement of DNA enters the head, determined by either the unit length of the DNA, sequence recognition of the DNA length, or a headful mechanism. The ATPase–DNA complex detaches from the connector and is replaced by neck, tail, and/or tail fiber components, yielding a mature, infectious virion. Reproduced from Calendar R (ed.) (2006) *The Bacteriophages*. New York: Oxford University Press, with permission from Oxford University Press.

12-fold homo-oligomer of the phage-encoded portal protein forming a channel through the capsid wall. In phages with prolate heads, the portal vertex is always at one end of the long axis of the prohead. In most cases, it is believed that the portal is responsible for initiation of capsid polymerization in conjunction with an associated scaffold structure. The portal provides the binding site for the rest of the DNA packaging machinery and is the channel through which the DNA passes into, and ultimately out of, the capsid. After packaging, it is the axis around which the neck and tail components assemble, hence the designation connector for this structure in some systems.

The dsDNA phage prohead lattice is in an immature form prior to genome packaging. The immature prohead lattice is often physically less stable than in the mature virion. In some cases, accessory domains of the major capsid protein required for lattice assembly are proteolytically cleaved prior to packaging. In most phages, the conformational switch of the capsid lattice to the mature form occurs during packaging. This rearrangement of the capsid lattice is accompanied by an increase in the sixfold symmetry of the capsid hexons, and an overall increase, or expansion, of the head shell volume and consequent thinning of the shell thickness. Expansion ranges from the unperceivable, as in ø29, to the dramatic, as in HK97 where the capsid volume doubles.

Considerable diversity in the structural organization and maturation pathway of the DNA chromosome exists in these phages. DNA maturation converges at the point of chromosome translocation in that all dsDNA phages studied employ a common mechanism for genome translocation. The differences in DNA chromosome structure in the dsDNA phages arise as a result of differing strategies that have evolved to replicate phage dsDNA inside the cell. The central challenge, not faced by RNA phages, is the inability to initiate DNA-dependent DNA replication from a linear dsDNA without loss of information from the 5′ ends of the replicated DNA polymer. This 'riddle-of-the-ends' is solved by dsDNA phages by using one of four strategies: (1) replication of a circular form of the genome (P1); (2) producing multiple, tandemly linked copies of the genome, termed concatamers, in a single replicating molecule such that complete chromosomes can be produced by cleavage (P22, T-phages, SPP1); (3) replication of the genome as part of integrated segment imbedded in the host cell DNA genome (Mu); and (4) initiate replication of linear DNA using a priming mechanism dependent on a covalently linked terminal

protein that does not lead to degeneracy of the chromosome (ø29, PRD1). The first three replication strategies require a DNA cleavage reaction as part of the packaging initiation process, since only linear dsDNA of a certain size can be packaged. Bacteriophage lambda combines the first two replication strategies in that early replication is by theta replication of its circular genome that switches to the lambda rolling circle mechanism to produce long, multigenome concatamers which serve as the packaging substrate. The fourth replication mechanism above does not require a cleavage event since the DNA substrate is present in the cell in unit length form.

The DNA substrate is recruited from the cytosol by the enzymatic components of the packaging apparatus. In general, there are two proteins involved, one large and one small, that form a holoenzyme complex. Their stochiometry, relative to each other and to the procapsid, is still unclear, with reports from various systems ranging from four complexes per motor up to six. The 'small' subunit is a DNA binding and recognition protein, while the 'large' component is an ATPase. These proteins assemble on the DNA substrate, with the small subunit recognizing a specific sequence in the DNA termed the pac site. This process has been particularly well described in phage lambda (**Figure 2**). It is believed that initial DNA-binding step is mediated by other factors of cellular origin (integration host factor (IHF) in the case of lambda). (In phages with terminal proteins at the DNA ends, the terminal protein is analogous to the small subunit.) The specificity of this recognition ensures that only phage chromosomes will be packaged into the receptive phage prohead. In the case of phages with either circular- or concatameric-replicated DNA, the large subunit of these enzymes cut the DNA to produce a free end. This endonuclease activity is also responsible for production of a second endonuclease cut of the DNA at the end of packaging in phages that package segments of concatameric DNA, thus the designation 'terminase' for this enzyme ensemble in many phages (P22, lambda, T-even, T-odd, SPP1, Mu, etc). The designation terminase is not relevant to phages with unit length chromosomes such as ø29 and PRD1 since no cleavage is required; thus, these enzymes are more accurately termed packaging ATPases.

Once formed, the packaging ATPase–DNA complex docks with the prohead via the portal complex. The initial events that mediate the insertion of the DNA into the portal are unclear. Once initiated, DNA translocation occurs via an ATP hydrolysis-mediated process. The energy requirement for this process is clear when one considers the forces against which this molecular machine is working. First, entropy dictates that the concentration of DNA outside and inside the prohead should be equal. Thus, entropy works against dsDNA packaging throughout the process. Second, dsDNA is a relatively stiff polymer, with a persistence length (the minimum curvature of relaxed dsDNA) on the same scale as the capsid into which the DNA is being packaged in most phages. Therefore, energy is required to bend the DNA within the confines of the prohead. Finally, DNA has a net negative surface charge owing to the phosphate backbone of the polymer. As the DNA becomes compacted into the prohead, DNA strands are forced into close contact and electrostatic repulsion builds. Once again, energy must be invested into the DNA to achieve this end state. The end result is that the compacted DNA is pressurized inside the capsid to several atmospheres, requiring that the packaging motor complex must exert a translocating force on the order of 100 pN, making these molecular motors some of the strongest described in any biological systems. It is not clear how the DNA is organized to accommodate the degree of packing density, which approaches that of crystalline DNA. It is clear that the DNA must be

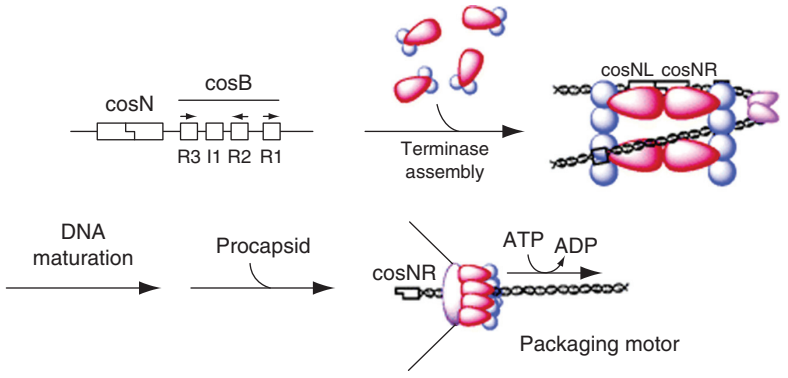


Figure 2 Model for terminase assembly at the cos site. The terminase protomer is a heterotrimer composed of one gpA subunit (red lobes) tightly associated with a gpNu1 dimer (blue spheres). Four protomers assemble at cos, resulting in bending of the duplex into a 'packasome' complex. Duplex bending by a gpNu1 dimer (purple lobes) bound to the R3 and R2 elements is central to the assembly of the packaging machinery. DNA maturation includes duplex nicking and separation of the DNA strands. Reproduced from Ortega M and Catalano CE (2006) Bacteriophage lamda gpNu1 and *E. coli* proteins cooperatively bind and bend viral DNA: Implications for the assembly of a genome packaging motor. *Biochemistry* 45: 5180–5189, with permission from ACS Publications.

pressed together in some form of hexagonal array that follows the contour of the capsid (**Figure 3**), which is the most energy-efficient conformation. Several models, including solenoids, liquid crystals, and folded toroids, have been suggested, but to date no structural study has been able to clearly resolve the structure of the packaged chromosome.

Packaging is terminated either by simply reaching the end of the unit-length chromosome (ø29, PRD1), or by a second DNA cleavage event necessitated by the translocation of a concatameric packaging substrate. In the latter, this cleavage event relies on the signaling from the packaged DNA density inside the capsid to the translocating complex on the outside of the capsid. In some cases, the near filling of the capsid permits the recognition and cleavage of the next approaching pac sequence, as with the lambda cos sequence, such that all progeny have identical genomes. In other instances, the second cleavage event is strictly 'headful', meaning the restriction of the DNA being packaged is sequence independent and relies on the detection of packaged DNA density inside the head. In this case, the capsid volume has evolved to exceed the capacity required to package a chromosome the length of the genome; thus the packaged DNAs are terminally redundant. This terminal redundancy is crucial to the progeny in that it will buffer the genome against loss during the next replicative infection. How this signal is transmitted from the portal to the packaging enzymes is unclear.

As described above, prohead assembly often involves the assembly of a precursor capsid that is structurally distinct from the mature capsid. In many cases, the prohead is yet to undergo ejection of the core-scaffold components that mediate head shell polymerization into the size and shape required by the virus. Similarly, final conformational rearrangements that stabilize the capsid shell allow the binding of accessory stabilizing proteins to the head surface, or release of the packaging motor and assembly of neck and tail components required to make

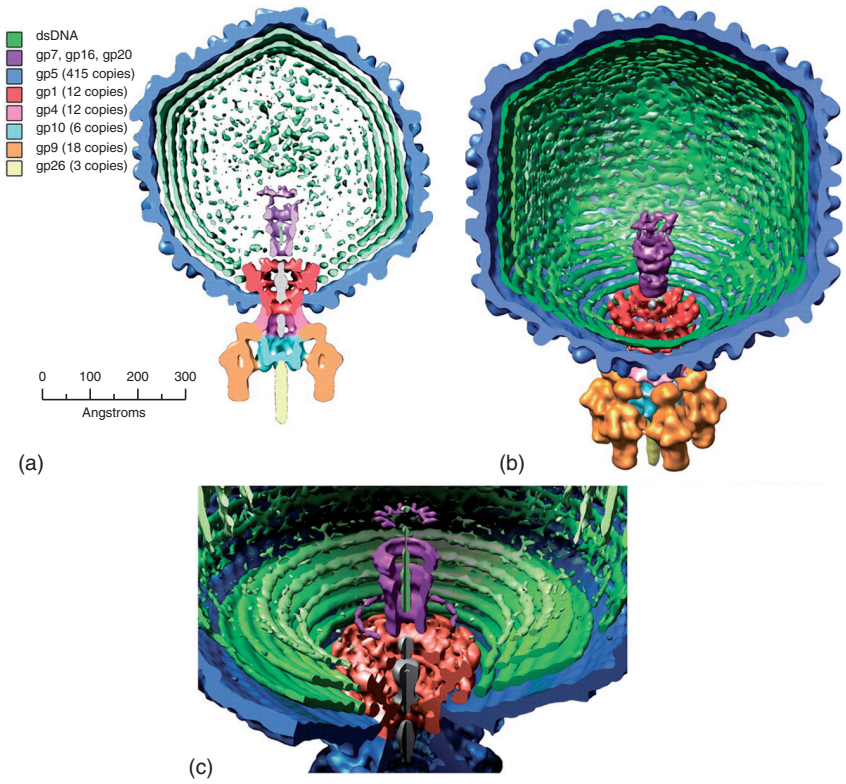


Figure 3 The interior features of the P22 virion. (a) The locations, deduced from many previous molecular biological studies, of the assembled gene products within a cutaway view of the reconstructed density of the P22 virion. Gene products 1, 4, 9, 10, and 26 make up the tail machine. Layers of dsDNA (green) are clearly visible as concentric shells within the capsid; they break into distinct rings of density near the portal vertex. Density (green) in the center of the channel formed by the ejection proteins (purple) could be the end of the P22 chromosome; however, density on this axis within the portal protein ring (red) does not appear to be consistent with DNA. (b) A cutaway view of the internal portion of the asymmetrically reconstructed particle contoured at 3, showing the 12-fold symmetry of the portal (red), the putative ejection proteins (purple), and individual strands of dsDNA (green). (c) Close-up view of the packaged interior upon 12-fold averaging along the tail tube axis. Although the E-proteins (purple) themselves in reality may or may not exhibit 12-fold symmetry, this view demonstrates the channel-like nature of the structure they form in the virion, as well as the dsDNA (green) that may be seated within their channel. Three concentric shells of spooled DNA are clearly visible. Reproduced from Lander GC, Tang L, Casjens SR, *et al*. (2006) The structure of an infections P22 vision shows the signal for headful DNA packaging. *Science* 312(5781): 1791–1795, with permission from American Association for the Advancement of Science.

the completed virion infectious. The end result of the separation of these final events in assembly permits the specific targeting of immature proheads by the DNA packaging machinery and completion of assembly (i.e., packaging motor detachment and neck/tail assembly) only after packaging has been completed. Upon completion of packaging, the ATPase complex detaches from the packaged head and neck and tail components assemble. This generally involves the assembly of several neck proteins, followed by the assembly of the tail in completed form (e.g., T4, lambda) or by the stepwise addition of tail components to the packaged head (P22, ø29).

The mechanism of DNA translocations in these motors remains unresolved, owing mostly to the complex nature of the portal motor complex. The role of the ATPases is clear in that they bind and hydrolyze ATP, transferring the energy of this reaction to the physical movement of the DNA substrate. It is widely held that the ATPases therefore have an active role in translocation, and function in a manner similar to the helicases, as is clear in the dsRNA phage packaging complex. What is unclear is whether the portal protein actively participates in this process. Regardless of a direct role in translocation, the portal protein ring does appear to play a role in signaling the end of translocation, as illustrated by mutants in the portal proteins of SPP1 and P22 phages which package altered chromosome lengths. This suggests the head-full sensor for these phages lies in the portal protein, and that the signal for the cleavage of the DNA upon completion of packaging is transmitted to the ATPase by the portal ring.

ssDNA Packaging

The archetype ssDNA phage assembly system is øX174 due to its extensive genetic and structural characterization over recent decades. Unlike the dsDNA phages, genome packaging in ssDNA phages is coupled to genome replication. The substrate genome for replication is a closed, circular dsDNA that is copied using a complex of host cell enzymes. This replicating complex switches to rolling circle replication upon interaction with viral proteins A and C, a host helicase (the *rep* protein), and the procapsid. As the positive polarity DNA is extruded from the rolling circle, it is transferred into the procapsid through a depression in the twofold axis of the preformed icosahedral shell. Unlike dsDNA phages, this point of entry is not structurally unique like a portal, but the replication/packaging complex binding confers exclusion to the other twofold axis on the capsid surface. Packaging in øX174 is dependent on an ssDNA binding protein J, which enters the capsid during packaging, and binds the DNA via charge interaction. J protein also mediates interaction of the packaged DNA with the inner surface of the capsid. Changes in capsid size and stability also occur during packaging, as with the dsDNA phages. In contrast to the dsDNA phages, phages of the family *Microviridae* do not require the high chromosome density inside the capsid that requires extensive input of energy for packaging. Thus, the energy of translocation harnessed from coupled DNA replication and DNA binding is sufficient in generating the required packaging force to efficiently produce infectious progeny.

ssRNA Packaging

A large class of dsRNA phages, the family *Cystoviridae*, translocate their genome into preformed capsids. These phages are analogous in their replication and structure to the eukaryotic reoviruses, and are best characterized in the ø6 group. Unlike the dsDNA phages, the cystoviruses translocate a single-stranded nucleic acid polymer into the procapsid. This circumstance is the product of the replication strategy of the virus, during which positive-strand RNA synthesized during infection serves as both template for translation and substrate for genome packaging. Conversion of the ssRNA to dsRNA occurs inside the capsid after translocation at the end of the assembly pathway. Also, unlike the dsDNA or ssDNA phages, the dsRNA genome is segmented into three chromosomes of different lengths (S, M, and L for small, medium, and large, respectively). This requires the translocation apparatus to not only select chromosomal nucleic acid polymer as in the dsDNA phages, but to select and process multiple ssRNAs in sequence (designated s, m, and l in the ssRNA form prior to conversion to S, M, and L) to allow for fidelity in the assembly of the mature virion.

The translocating NTPase of this class of viruses, the P4 enzyme, has been extensively characterized by biochemical and structural analysis. (Unlike the ATPases in the dsDNA phages, the packaging enzymes in the family *Cystoviridae* are not limited to ATP, but can use other NTPs depending on the phage.) A hexameric complex of this enzyme is assembled at each fivefold vertex of the precursor procapsid. The mechanism by which only one of the 12 vertices is selected to translocate a bound ssRNA substrate is unclear. The complex is believed to function in a manner analogous to helicases, translocating ssRNA through the hexamer and into the capsid. The P4 prohead complex recognizes stem loop structures at the 5′ end of the ssRNAs to be packaged, presumably through binding sites on the capsid surface adjacent to the ATPase. The order of binding, and thus packaging, is controlled by the alteration of the capsid binding site that occurs as RNA is packaged. Thus, a naive procapsid binds the s fragment specifically, packages it, and in doing so undergoes a conformational change that alters the procapsid RNA binding domain such that it now binds the m fragment,

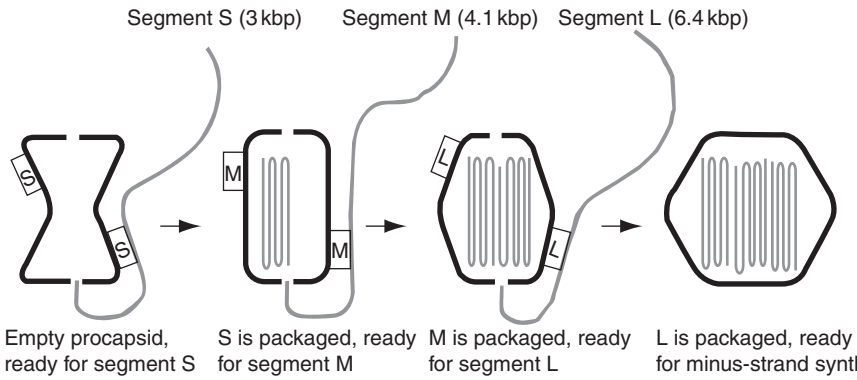


Figure 4 The packaging model of ø6. The empty procapsid shows only binding sites for S. After a full-size S is packaged, the S sites disappear and M sites appear. After a full-size M is packaged, the M sites disappear and L sites appear. After a full-size L is packaged, minus-strand synthesis commences. Reproduced from Calendar R (ed.) (2006) *The Bacteriophages*. New York: Oxford University Press, with permission from Oxford University Press.

which is then packaged, followed by the l fragment (**Figure 4**). The determinate of these conformational changes in the capsid to allow recognition of the variant 5′ ends of the chromosomes is the length of packaged RNA, rather than sequence.

Concluding Remarks

The phenomenon of genome packaging remains one of the most intensively studied processes of virion assembly, bringing together diverse but complementary fields of biochemistry, molecular genetics, structural biology, and biophysics. Since many of the phage studied have evolved mechanism analogous to eukaryotic viruses, efforts to resolve the mechanisms involved in genome packaging in phage promise to be reflected in their eukaryotic counterparts. Since genome translocation has no direct link to most cellular processes, inhibition of genome packaging has been long held to serve as a future target for antiviral therapy once the mechanisms of chromosome and capsid maturation and chromosome translocation are resolved. Regardless of this practical consideration, the efficiency, complexity, and power of this process will remain a target of investigation for its own sake.

See also: Filamentous ssDNA Bacterial Viruses; Icosahedral ssRNA Bacterial Viruses; Virus Particle Structure: Nonenveloped Viruses.

Further Reading

Calendar R (ed.) (2006) *The Bacteriophages.* New York: Oxford University Press.

Lander GC, Tang L, Casjens SR, *et al.* (2006) The structure of an infections P22 virion shows the signal for headful DNA packaging. *Science* 312(5781): 1791–1795.

Ortega M and and Catalano CE (2006) Bacteriophage lamda gpNu1 and *E. coli* IHF proteins cooperatively bind and bend viral DNA: Implications for the assembly of a genome packaging motor. *Biochemistry* 45: 5180–5189.

Giardiaviruses

A L Wang and C C Wang, University of California, San Francisco, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

History

In 1986, a 6.3 kbp linear double-stranded (ds) RNA molecule was observed in nucleic acid extracts of *Giardia lamblia* Portland I trophozoites. *Giardia lamblia* is an anaerobic parasitic flagellate that inhabits the upper gastrointestinal tracts of humans as well as many other mammals. When infecting humans, the parasitic protozoan causes giardiasis, an acute diarrhea that often progresses to chronic, carrier-stage for adults and severe malnutrition for children. Further examination of this 6.3 kbp dsRNA isolated from the extract of *G. lamblia* revealed it to be the genome of a small isometric virus, hence named giardiavirus (GLV), that specifically infects this protozoan.

It is interesting to note that although GLV was first detected in an isolate of *G. lamblia* Portland I (P1) obtained

from Dr. D. G. Lindmark of Cleveland State University, the same P1 isolate from the American Type Culture Center is virus free. It therefore remains a mystery as to how the Cleveland P1 became exposed to the virus. All the information included here has been derived from studies of the virus originally isolated from this Cleveland P1 strain.

Taxonomy and Classification

GLV belongs to the family *Totiviridae*, genus *Giardiavirus*, of RNA viruses. This family is characterized by the non-segmented dsRNA genome and simple virion structure. Members of *Totiviridae* include the yeast dsRNA virus (ScV-L). Many of the viruses recently discovered from protozoa, such as the trichomonas vaginalis virus (TVV), leishmania RNA virus (LRV), and eimeria stiedae virus (ESV), also belong to this family.

Host Range and Geographic Distribution

Purified GLV readily infects many virus-free isolates of *G. lamblia* trophozoites, but not any other parasitic protozoa tested, including *Tritrichomonas foetus, Trichomonas vaginalis, Trypanosoma brucei brucei, Entamoeba histolytica*, and *Eimeria stiedae*. The virus has also been shown not to infect two transformed human intestinal cell lines. It is therefore believed that giardiavirus has a rather narrow host range. It probably infects only *G. lamblia* in nature. On the other hand, the cellular host for this virus, *G. lamblia*, parasitizes many mammals other than humans. Viruses that are identical in shape and size of genomic dsRNA and share dsRNA sequence homology with one another have been detected from many *G. lamblia* strains and isolates obtained from humans, guinea pigs, cats, beavers, llamas, and sheep. The human isolates were collected from Belgium, Poland, England, Israel, Ecuador, Puerto Rico, and various states in the USA. Since *G. lamblia* is found in almost all parts of the world, affecting developing as well as developed countries, it is expected that GLV follows its host and is distributed worldwide.

Physical and Biochemical Characteristics

Under the electron microscope, GLV appears as an icosahedron of 36 nm diameter, consisting of an electron-dense core encapsidated in a shell 5–6 nm thick (**Figure 1**). In addition to the 6.3 kbp linear dsRNA genome, the virion contains a major polypeptide of 100 kDa (p100) which is most likely the viral capsid. Purified virions are good antigens in mice. Polyclonal antibodies raised against whole virions react with p100 in Western blots and

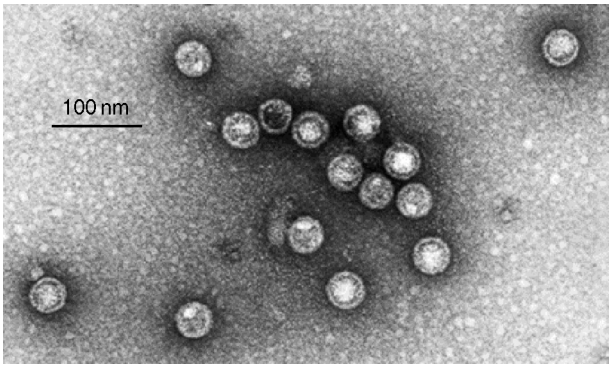


Figure 1 Giardiavirus.

can effectively block viral infection in the *in vitro* culture of *G. lamblia* trophozoites. The same antiserum also reacts positively with a minor component of the virion, a polypeptide of 190 kDa, which is believed to be the viral RNA-dependent RNA polymerase (RDRP). Indeed, GLV-encoded RDRP activity has been detected in the infected cell extract as well as the purified GLV fractions.

Antisera raised against synthetic dsRNA cross-react only with the viral dsRNA and are not protective against viral infection of *G. lamblia* trophozoites *in vitro*. Giardiavirus does not contain any lipids nor is the p100 polypeptide glycosylated.

The entire nucleotide sequence of the linear GLV dsRNA genome has been determined. It consists of 6277 bp and is flush at either end. As the dsRNA can be readily radiolabeled by [^{32}P]pCp and RNA ligase, this molecule must have a free 3′-OH group at one or both of its 3′ termini. The exact structure at the 5′ termini of the dsRNA has not yet been elucidated. It is known that the dsRNA molecule cannot be phosphorylated by T4 polynucleotide kinase, and that the denatured dsRNA molecule can be circularized with T4 RNA ligase. Therefore, the 5′ termini of the GLV genomic dsRNA are probably phosphorylated. Additionally, no covalently linked protein is found at these termini such as in the case of poliovirus.

Organization and Molecular Biology of the GLV dsRNA Genome

The 6277 nt GLV dsRNA genome contains only two large open reading frames (ORFs), both on the same strand of RNA (**Figure 2**). The first ORF (nts 368–3015) encodes the precursor polypeptide for the GLV capsid protein. Thirty-two amino acid residues from the N-terminus of this precursor protein are apparently removed by a cellular cysteine protease before the processed capsid protein is assembled into the virion.

The second ORF (nts 2806–5976) is –1 relative to ORF 1, and the two ORFs overlap by 220 nts. Amino acid

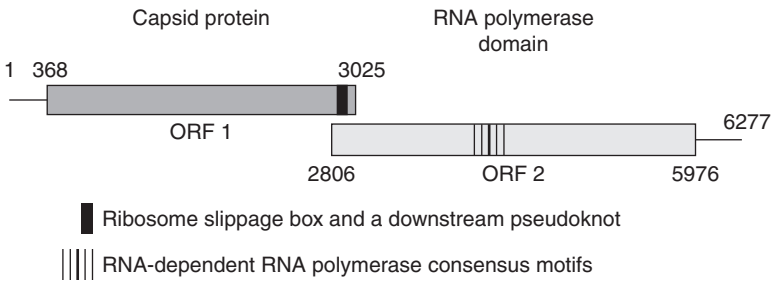


Figure 2 Organization of the giardiavirus dsRNA genome.

sequence motifs conserved for all RDRPs have all been found in ORF 2. It is now known that the 190 kDa GLV RNA polymerase is synthesized as a fusion protein of ORFs 1 and 2 at a level that is 2–5% of p100. Apparently, ribosomes carrying the growing nascent polypeptide chain are stalled when they encounter the pseudoknot structure residing within the 220 nt overlap of these two ORFs. At a 2–5% frequency, the ribosomes slip back one reading frame and proceed to translate ORF 2 as the C-terminus of a fusion protein. The ability of this GLV overlap fragment to induce −1 ribosomal frameshifting has been demonstrated in a reporter system in yeast.

Flanking the two ORFs, there are 367 and 301 nt untranslated regions respectively on the 5′ and 3′ sides. These two regions contain sequence elements that are critical for initiation of transcription and replication of GLV RNA. For example, deletion of a single nucleotide from the 5′ terminus of the (+)-strand GLV RNA totally abolishes transcription of GLV mRNA. Similarly, deletion or alteration of sequences in these two regions drastically reduces the level of progeny viral RNA.

In recent years, GLV has been successfully used as a vector for the introduction of foreign genes into *G. lamblia*. When a portion of the GLV genome is replaced with the firefly luciferase gene in a cDNA construct downstream from a T7 promoter, chimeric RNA can be synthesized *in vitro* using T7 polymerase. *Giardia* cells that are infected with wild-type GLV and electroporated with the chimeric RNA show luciferase activity of a millionfold above background. The chimeric RNA is replicated as dsRNA and packaged into recombinant virions that are shed into the culture supernatant. These recombinant viruses, together with wild-type GLV, can in turn infect naive *Giardia* trophozoites to produce luciferase activity. The list of foreign genes tested in this system include genes encoding neomycin phosphotransferase, hygromycin phosphotransferase, and green fluorescence protein. All can be delivered and expressed at high levels. Inclusion of a fragment, nts 368–631 in the coding region of p100, as the N-terminus of a fusion protein with the foreign gene product has been shown to enhance translation level by 5000-fold. This region therefore may contain elements that can promote interaction between GLV mRNA and ribosomes of *G. lamblia*.

Giardiavirus mRNA is uncapped and not adenylated. Translation is initiated at an internal ribosomal entry site (IRES) in the mRNA consisting of a 253 nt segment in the 5′ untranslated region (5′ UTR) and a 264 nt stretch in the ORF immediately downstream from the 5′ UTR. The initiation codon is localized at the center of an unstructured 31 nt segment flanked by complex secondary structures in the 5′ UTR and the ORF of the IRES. The precise position of the initiation codon is critical for translation initiation. It suggests a direct recruitment of the 40 S small ribosomal subunit to the initiation codon without ribosomal scanning, which is known to be absent from the translation machinery in *Giardia*.

Infection and Replication

GLV infects susceptible isolates of *G. lamblia* very efficiently. It has been estimated that demonstrable infection can be achieved at a multiplicity of infection as low as 10 GLV particles per cell. The exact mode of viral entry into the cell has not been elucidated. However, a number of virus-free *G. lamblia* strains have been found resistant to GLV infection. When trophozoites of these strains are electroporated with the single-stranded (ss) GLV RNA (see below), they become infected by GLV and can support and complete the cycle of replication, producing progeny virions that are fully infective to sensitive strains. It is therefore probable that certain cellular component(s) such as viral receptors are involved in the initial interaction between GLV and susceptible strains.

Using purified GLV to infect virus-free *G. lamblia* WB strain, it was shown by *in situ* hybridization that dsRNA replication was first detected in the cytoplasm. Toward the late stage of infection, viral dsRNA was found to spread into the twin nuclei of the infected flagellate. Transmission electron microscopic examination of thin sections of infected cells at stationary phase also reveals paracrystals of virus-like particles in the nuclei (**Figure 3**). It has been estimated that an infected cell may harbor as many as 10^5 GLV particles without lysis. Meanwhile, mature and infectious GLV particles begin to appear in the supernatant of *Giardia* culture medium 42 h after infection. It is not known whether a specific cellular

process is involved in the release of giardiavirus into the culture supernatant, although cell lysis has not been observed as a consequence of viral infection.

The growth rate of newly infected cells decreases with increasing multiplicity of infection. As the ratio of the infecting virus to cell increases, the percentage of the nonadhering (nondividing) trophozoites also increases. However, in established infected cell lines or at moderate multiplicity of infection (<1000), the infected cell assumes normal appearance and maintains the same growth rate as its uninfected counterpart. Furthermore, GLV infection persists indefinitely throughout repeated subculturings.

In addition to the 6.3 kbp genomic dsRNA, an ssRNA (SS) of identical length that is homologous to only one of the two strands of GLV dsRNA is also found in the GLV-infected cellular extract. Studies of the infection time course showed that, in contrast to GLV dsRNA which increases steadily from 23 to 141 h after infection, SS becomes detectable at about the same time, peaks at 42–50 h after infection, then gradually declines. Electroporation of gel-purified SS into the uninfected WB cells resulted in the recovery of dsRNA from the cell extract and infectious GLV particles in culture supernatant, demonstrating that SS is the viral messenger RNA as well as the full-length replicative form of the viral genome. Recent nucleotide sequence analysis of GLV cDNA clones also verifies that it is the SS strand that encodes the large ORFs. There is no subgenomic viral RNA detected inside the infected cell.

The RNA products synthesized *in vitro* by the virion-associated RDRP are homologous to SS and complementary to the negative strand. Transcription of viral message therefore must proceed conservatively by utilizing the dsRNA as its template.

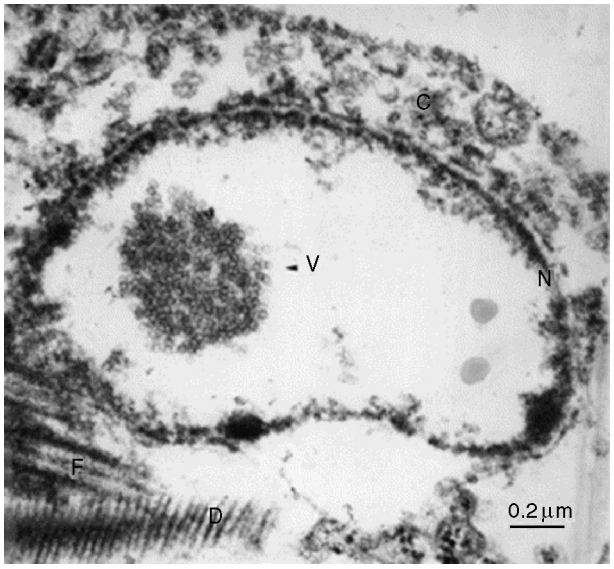


Figure 3 Thin section of a giardiavirus-infected cell. C, cytoplasm; N, nuclear envelope; F, flagella; D, ventral disk; V, aggregates of virus particles.

Evolution

The extent to which a person is affected by giardiasis varies widely. The outcome of the severity of parasitic infection depends on many variables derived not only from the parasite but also from the human host. Attempts at correlating the presence or absence of GLV with the severity of giardiasis have been inconclusive, and we still do not know the role GLV might play in the delicate balance of the host–parasite interaction, if any.

Some of the characteristics of GLV are listed with those of other dsRNA viruses of *Totiviridae* in **Table 1**. When compared with ScV-L, the best-known virus of this group, GLV and ScV are similar in many ways: (1) single molecules of genomic dsRNAs of similar sizes; (2) single major capsid polypeptides of similar sizes; (3) virion-associated RDRP with similar amino acid sequence motifs deduced from the genomic sequence, (4) ability to synthesize viral messages from intact virions *in vitro*; and (5) use of ribosomal frameshifting for the synthesis of viral polymerase. However, the two viruses do not cross-infect and the two dsRNAs do not cross-hybridize in Northern blots. GLV dsRNA has also been shown not to cross-hybridize with dsRNAs of TVV or LRV. Comparison of the nucleotide sequence from GLV

Table 1 Physical properties of some small dsRNA viruses

Virus	*Shape*	*Diameter (nm)*	*Density (gm/ml^{-1})*	*dsRNA (μm)*	*dsRNA (kbp)*	*Capsid protein (kDa)*
GLV	Isometric	33	1.368	1.50	6.277	100
TVV	Isometric	33	1.468	1.50	4.3–4.8	85
LRV	Isometric	32			5.3	
ESV	Isometric	35		1.63	6.5	
BBV	Isometric	38	1.358		5.5	
ScV-L	Isometric	33–41	1.368	1.31	4.3–4.8	88
UmV-P1	Isometric	41–43	1.418		6.3	73

GLV, giardia lamblia virus; TVV, trichomonas vaginalis virus; LRV, leishmania RNA virus; ESV, eimeria stiedae virus; BBV, babesia bovis virus; ScV-L, saccharomyces cerevisiae L; UmV-P1, ustilago maydis virus.

cDNAs with that of ScV-L, LRV or TVV indicates that, despite the similar organization of the viral genomes, GLV share very little overall sequence identity with any of these viruses whose genomic sequences have been completely determined. GLV is therefore not closely related to any of these viruses evolutionarily.

See also: Leishmaniaviruses; Totiviruses; Ustilago Maydis Viruses; Yeast L-A Virus.

Further Reading

Garlapati S and Wang CC (2005) Structural elements in the 5′-untranslated region of giardiavirus transcript essential for internal ribosome entry site-mediated translation initiation. *Eukaryotic Cell* 4 (no. 4): 742–754.

Wang AL and Wang CC (1991) Viruses of the protozoa. *Annual Review of Microbiology* 45: 251.

Wang AL and Wang CC (1995) The double-stranded RNA genome of giardiavirus In. In: Boothroyd JC (ed.) *Molecular Approaches to Parasitology*, 179pp. New York: Wiley.

H

Hantaviruses

A Vaheri, University of Helsinki, Helsinki, Finland

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Coevolution Evolution of a virus together with its rodent/insectivore host.
Hantavirus A virus in the genus *Hantavirus*.
Reassortant A virus having genome segments of two different viruses.
Robovirus A virus transmitted from rodents to other vertebrates without arthropods.
Sympatric Occupying the same or overlapping geographic areas without interbreeding.

Historical Introduction

Hantavirus infections are not new to humankind. The first description of a hemorrhagic fever with renal syndrome (HFRS)-like disease can be found in a Chinese medical account written in about AD 960 and the earliest definite description of HFRS comes from Far East Russian clinical records dating back to 1913. During World Wars I and II, HFRS became an important military problem; for example, 'field nephritis' in Flanders during World War I may well have been caused by a hantavirus. In Manchuria in the mid-1930s, 12 000 Japanese soldiers caught the disease and military researchers were investigating the cause of the disease, sometimes using prisoners of war in infection experiments. Finnish and German soldiers encountered an HFRS-like epidemic in Finnish Lapland in 1943–44. During the Korean conflict in 1950–53, the disease again gained much attention when about 3000 United Nations troops contracted it – since then known as Korean hemorrhagic fever – with a 5–10% case–fatality rate. The milder form of HFRS, nephropathia epidemica (NE), common in Fennoscandia (Scandinavia and Finland), was first described by Swedish authors in 1934. However, the infecting agent of HFRS remained unknown until 1976, when the Korean researcher Ho Wang Lee discovered that cryostat-sectioned lungs of striped field mice (*Apodemus agrarius*), trapped near the Hantaan river, contained virus-specific antigen reactive with HFRS-patient sera. By a similar approach, the causative agent of NE was demonstrated in 1980 in bank voles, *Clethryonomys glareolus*, trapped in Puumala, Finland. Another agent, this one from urban rats in Seoul, Korea, was also found to cause HFRS. In the early 1990s, a further distinct European human-pathogenic hantavirus was isolated from the yellow-necked mouse, *Apodemus flavicollis*, near the village of Dobrava in Slovenia, and a few years later, the related, less pathogenic, Saaremaa virus from striped field mice on Saaremaa Island, Estonia. Thottapalayam virus from an insectivore (*Suncus murinus*, a shrew) in India, Prospect Hill virus from a meadow vole (Microtus *pennsylvanicus*) in USA, and Thailand virus from a bandicoot rat (*Bandicota indica*) already had been isolated in the early 1970s to the 1980s – they all apparently are nonpathogenic for humans.

Human disease had not been known to be caused by a hantavirus in the Americas until a cluster of acute respiratory distress syndrome cases with a high (60%) case–fatality rate in the Four Corners area of the American Southwest (where Arizona, Utah, Colorado, and New Mexico are contiguous) was recognized in May 1993. Subsequent studies led to the discovery of Sin Nombre virus and other hantaviruses that cause hantavirus pulmonary syndrome (HPS), all transmitted from sigmodontine rodents indigenous to the New World. An increasing number of pathogenic hantaviruses have been reported in South America, including Andes virus, which can be transmitted person to person, and which have high case–fatality rates. While only about 2000 HPS cases have been reported so far, approximately 150 000 HFRS cases are estimated to occur worldwide annually.

Virology

Hantaviruses are enveloped viruses with a tripartite negative-stranded RNA genome and belong to the genus

Hantavirus of the large virus family *Bunyaviridae*. The 6.4 kb L (large) genome segment encodes the ~250 kDa RNA polymerase, the ~3.6 kb M (medium) segment, the two glycoproteins 68–76 kDa Gn and 52–58 kDa Gc (formerly known as G1 and G2), and the ~1.7 kb S (small) segment the 50–54 kDa nucleocapsid protein (N). In addition, the S segment of hantaviruses carried by arvicolid and sigmodontine rodents has another overlapping (+1) open reading frame (ORF) named NSs, which has been shown to inhibit the interferon response. Viral messenger RNAs of the members of the *Bunyaviridae* are not polyadenylated and are truncated relative to the genomic RNAs at the 3′ termini. Messenger RNAs have 5′-methylated caps and 10–18 nontemplated nucleotides derived from host cell mRNAs. The termini of all three segments are highly conserved and complementary to each other, a feature that has assisted in cloning and discovery of new hantaviruses.

Unlike most other bunyaviruses, hantaviruses are not arthropod borne (arboviruses), but are rodent borne (roboviruses). Each hantavirus is primarily carried by a distinct rodent/insectivore species, although several host switches seem to have occurred during the tens of millions of years of their co-evolution with their carrier hosts. We now know that the genetic diversity of hantaviruses is generated by (1) genetic drift (accumulation of point mutations and insertions/deletions) leading to mixtures of closely related genetic variants, quasispecies; (2) genetic shift (reassortment of genome fragments within a given virus genotype/species); and (3) according to recent findings, also by homologous recombination, a mechanism not previously observed for negative-strand RNA viruses.

Ecology and Epidemiology

Hantavirus infections are prime examples of emerging and reemerging infections. As are most of these infections, hantaviral diseases are zoonoses. With the exception of the South American Andes virus, hantavirus infections are thought to be transmitted to humans primarily from aerosols of rodent excreta (feces, urine, saliva). Only some hantaviruses cause disease in humans. In Asia, Hantaan and Amur viruses, carried by *Apodemus* spp. mice, cause severe HFRS, and Seoul virus, carried by rats, a milder disease. In Europe, there are three major hantaviral pathogens: Puumala virus carried by bank voles causes NE; Dobrava virus, carried by yellow-necked mice, causes severe HFRS; and the genetically and antigenically closely related Saaremaa virus carried by striped field mice causes mild NE-like disease. There also are reports that Seoul virus, carried by rats (*Rattus norvegicus* and *Rattus rattus*), causes HFRS of moderate severity both in Europe and North America. In addition, European common voles (*Microtus arvalis* and *Microtus rossiaemeridionalis*) carry Tula hantavirus, which can asymptomatically infect humans. Topografov hantavirus isolated from Siberian lemmings (*Lemmus sibiricus*) has not been detected in North European lemmings (*Lemmus lemmus*), although it can replicate in them. Infections of hantaviruses in rodents are asymptomatic and, often, persistent.

Hantavirus infections are quite common in Europe. Puumala virus occurs in Northern Europe, European Russia, and parts of central-western Europe. Dobrava virus is found mainly in the Balkans and the neighboring Central European areas. Saaremaa virus has been detected in Eastern and Central Europe, and in Slovenia the two viruses Dobrava and Saaremaa occur sympatrically. Apart from infections of laboratory rats, Seoul virus has been detected in wild rats in France and in several city harbors. It is apparent that many parts of Europe, such as Britain, Poland, and Byelorussia, and most of Africa, remain completely or relatively unstudied with regard to hantaviruses. This suggests either that HFRS is rare or nonexistent in these regions or is not generally recognized and is not diagnosed by local biomedical communities.

In Northern Europe, HFRS as well as the carrier rodents exhibit peaks in 3–4 year cycles, while in Central Europe the HFRS incidence follows the fluctuations of 'mast years', that is, the abundance of beech and oak seeds for the hantavirus-carrying rodents. In Central Europe, HFRS peaks in the summer whereas in Northern Europe most cases occur in late autumn and early winter, from November to January. Risk factors for acquiring hantavirus infections and HFRS include professions such as forestry, farming, and military, or activities such as camping, and the use of summer cottages. Cigarette smokers and males are more likely to be infected than are females. In the Americas, the increased precipitation during El Niño/Southern Oscillation in South and North America has been suggested as the main reason for the peaks in rodent population densities and for the consequent increased number of HPS cases.

Sigmodontine-borne hantaviruses circulating in North America (**Table 1**) form three phylogenetically distinct groups: those associated with *Peromyscus* spp. and *Reithrodontomys* spp. rodents, and the third carried by *Sigmodon* spp. and *Oryzomys* spp. rats. The first group carries both human pathogens (Sin Nombre virus, New York virus) and viruses not thus far associated with human disease (e.g., Blue River virus, Limestone Canyon virus). *Reithrodontomys*-borne viruses have not been shown to cause human disease. Hantaviruses discovered in South America are associated with several tribes of Sigmodontinae, mostly Oryzomyini-associated viruses, several of which are important human pathogens, including Andes, Lechiguanas, and Laguna Negra viruses. Results of phylogenetic analyses of South American hantaviruses suggest that several host-switching events have occurred during coevolution with their

Table 1 Hantavirus types

Virus	*Host*	*Distribution (origin)*	*Disease*
Murid-borne viruses	Mice and rats		
Hantaan (HTNV)[a]	Striped field mouse (*Apodemus agrarius*)	Asia (Korea)	HFRS
Da Bie Shan	Chinese white-bellied rat (*Niviventer confucianus)*	Asia (China)	NR
Seoul (SEOV)	Rat (*Rattus rattus, R. norvegicus*)	Worldwide (Korea)	HFRS
Dobrava (DOBV)	Yellow-necked mouse (*A. flavicollis*)	Europe (Slovenia)	HFRS
Saaremaa (SAAV)	Striped field mouse; western (*A. agrarius)*	Europe (Estonia)	HFRS
Thailand (THAIV)	Bandicoot rat (*Bandicota indica*)	Thailand (Thailand)	NR
Amur (AMRV)	Korean field mouse (*A. peninsulae*)	Asia (Far East Russia, China)	HFRS
Arvicolid-borne viruses	Voles and lemmings		
Puumala (PUUV)	Bank vole (*Clethrionomys glareolus*)	Europe (Finland)	HFRS
Hokkaido (HOKV)	Red bank vole (*C. rufocanus*)	Asia (Japan)	NR
Tula (TULV)	European common vole (*Microtus arvalis*)	Europe (Russia)	NR
Prospect Hill (PHV)	Meadow vole (*M. pennsylvanicus*)	North America (USA)	NR
Bloodland Lake (BLLV)	Prairie vole (*M. ochrogaster*)	North America (USA)	NR
Isla Vista (ISLAV)	Californian vole (*M. californicus*)	North America (USA)	NR
Khabarovsk (KHAV)	Reed vole (*M. fortis*)	Asia (Far East Russia)	NR
Topografov (TOPV)	Lemming (*Lemmus sibiricus*)	Siberia (Russia)	NR
Vladivostok (VLAV)	Reed vole (*M. fortis*)	Asia (Far East Russia)	NR
Sigmodontine borne	New World sigmodontine rodents		
Sin Nombre (SNV)	Deer mouse (*Peromyscus maniculatus*)	North America (USA)	HPS
Monongahela (MGLV)	Deer mouse (*P. maniculatus nubiterrae*)	North America (USA)	HPS
New York (NYV)	White-footed mouse (*P. leucopus*)	North America (USA)	HPS
Blue River (BRV)	White-footed mouse (*P. leucopus*)	North America (USA)	NR
Limestone Canyon (LCV)	Brush mouse (*P. boylii)*	North America (USA)	NR
Bayou (BAYV)	Rice rat (*Oryzomys palustris*)	North America (USA)	HPS
Black Creek Canal (BCCV)	Hispid cotton rat (*Sigmodon hispidus*)	North America (USA)	HPS
Muleshoe (MULV)	Hispid cotton rat (*S. hispidus*)	North America (USA)	NR
Andes (ANDV)	Long-tailed pygmy rice rat (*Oligoryzomys longicaudatus*)	South America (Argentina)	HPS
Lechiguanas (LECV)	Rice rat (*O. flavescence*)	South America (Argentina)	HPS
Oran (ORNV)	*O. longicaudatus*	South America (Argentina)	NR
Bermejo (BERV)	*O. chacoensis*	South America (Argentina)	NR
HU39694	Unknown	South America (Argentina)	HPS
Choclo (CHOV)	Pygmy rice rat (*O. fulvescens*)	Central America (Panama)	HPS
Calabazo	*Zygodontomys brevicauda*	Central America (Panama)	NR
Laguna Negra (LANV)	Vesper mouse (*Calomys laucha*)	South America (Paraguay)	HPS
Rio Mamore (RIOMV)	Small-eared pygmy rice rat (*O. microtis*)	South America (Bolivia)	NR
Caño Delgadito (CADV)	Cane mouse (*S. alstoni*)	South America (Venezuela)	NR
El Moro Canyon (ELMCV)	Western harvest mouse (*Reithrodontomys megalotis*)	North America (USA)	NR
Rio Segundo (RIOSV)	Mexican harvest mouse (*R. mexicanus*)	Central America (Costa Rica)	NR
Maciel (MACV)	Dark field mouse (*Necromys benefactus*)	South America (Argentina)	NR
Pergamino (PERV)	Grass field mouse (*Akodon azarae*)	South America (Argentina)	NR
Juquitiba	Unknown	South America (Brazil)	HPS
Araraquara	Unknown	South America (Brazil)	HPS
Castelo dos Sonhos	Unknown	South America (Brazil)	HPS
Insectivore-borne	Insectivores		
Thottapalayam (TPMV)	Asian house shrew (*Suncus murinus*)	Asia (India)	NR

Distinct hantavirus species listed in the 8th ICTV Report are shown in bold.
HFRS, hemorrhagic fever with renal syndrome; HPS, hantavirus pulmonary syndrome; NR, disease not recorded.
[a]Type species.

rodent hosts. The phylogenetic split between murine and sigmodontine rodents presumably dates to a divergence that occurred between subfamilies 30 million years ago, when the precursors of the sigmodontine rodents crossed the Bering Strait into the Americas. Notably, sigmodontine rodents are found only in the Americas and thus it is unlikely that HPS would occur in Eurasia, unless imported.

Clinical Picture and Pathogenesis

Amur, Dobrava, Hantaan, Seoul, Puumala, and Saaremaa viruses all cause HFRS but the infections differ considerably in severity. All are characterized by acute-onset, fever, headache, abdominal pains, backache, temporary renal insufficiency (first oliguria, proteinuria,

and increase in serum creatinine, and then polyuria), and thrombocytopenia, but the extent of hemorrhages (hematuria, petechiae, internal hemorrhages), requirement for dialysis treatment, hypotension, and case–fatality rates are much higher in HFRS caused by Amur, Dobrava, or Hantaan viruses than in NE caused by Puumala or Saaremaa viruses. About a third of NE patients experience temporary visual disturbances (myopia), which is a very characteristic if not pathognomonic sign of the disease. Notably, the clinical consequences of all of the hantaviral pathogens in humans vary from none to fatal. Severe NE is associated with a certain haplotype, HLA-B8, DR3, DQ2 alleles, severe HPS with HLA-B35, and mild NE with HLA-B27. Yet, although Puumala virus infection is generally associated with mild HFRS, NE may have significant long-term consequences. A 5-year followup study demonstrated that 20% of NE patients had a somewhat increased systolic blood pressure and proteinuria. This is important, since the infection is so common in many areas of Europe. In addition, in some patients, Puumala virus infection may infect the pituitary gland and lead to mortality or at least to hypophyseal insufficiency requiring hormone-replacement therapy.

The pathogeneses of HFRS and HPS are poorly understood. However, it is known that β3 integrins can mediate the entry of pathogenic hantaviruses and that hantaviruses can regulate apoptosis. Also, there is evidence that increased capillary permeability is an essential component in the pathogenesis of both HFRS and HPS, although different target tissues, kidneys and lungs, respectively, are affected in the two diseases. HFRS and HPS patients show activation of tumor necrosis factor α (TNF-α) in the plasma and tissues and high levels of urinary secretion of the pro-inflammatory cytokine interleukin 6 (IL-6) are seen in NE. Studies with a monkey model mimicking human Puumala virus infection and HPS-like disease in Andes virus-infected Syrian hamsters may assist in elucidating the mechanism of pathogenesis.

HPS is characterized by pulmonary edema but death often results from cardiac failure; thus the term hantavirus cardiopulmonary syndrome (HCPS) has been proposed for the disease. HFRS and HPS, although primarily targeted at kidneys and lungs, respectively, share a number of clinical features, such as capillary leakage, TNF-α, and thrombocytopenia; notably, hemorrhages and alterations in renal function occur also in HPS and pulmonary involvement is not rare in HFRS.

Of the four structural proteins, both in humoral and cellular immunity, the nucleocapsid protein appears to be the principal immunogen. Cytotoxic T-lymphocyte responses are seen in both HFRS and HPS and may be important for both protective immunity and pathogenesis in hantavirus infections.

Diagnostics and Prevention

The diagnosis of acute hantavirus infection is primarily based on serology. Both immunofluorescence tests and enzyme immunoassays are widely used for detection of specific IgM or low-avidity IgG antibodies, characteristics of acute infection. In addition, immunochromatographic 5 min IgM-antibody tests have been developed. Hantaviruses show extensive serological cross-reactivity, especially within each of the three virus subgroups (murid, arvicolid, and sigmodontine borne; **Table 1**), but for accurate typing, neutralization tests are needed. For example, in Paraguay, a considerable seroprevalence of hantavirus antibodies, as high as 40%, is noted in people without a history of HPS. Average seroprevalences in Finland and Sweden suggest that only 10–25% of Puumala virus infections are diagnosed; thus, most infections either are subclinical or are mild or atypical and remain undiagnosed.

Viral RNA usually can be detected in the blood of HPS patients using polymerase chain reaction with samples collected during the first week of illness, which is useful because it also identifies the infecting virus genotype. The same is true for severe forms of HFRS but in the case of Puumala virus infections, viral RNA cannot be regularly detected in the blood or urine of NE patients.

Vaccines against hantaviral infections have been used for years in China and Korea, but not in Europe or the Americas. No specific therapy is used in Europe, but both ribavirin and interferon-α have been administered in trials in China. A major problem is that at the time the patients are hospitalized, the rate of virus replication is declining, so that reduction of virus replication no longer is necessary for the patient. Thus, prevention of hantaviral infections continues to rely on reduced contact with excreta from infected rodents.

See also: Bunyaviruses: General Features; Human Eye Infections; Zoonoses.

Further Reading

Brummer-Korvenkontio M, Vapalahti O, Henttonen H, Koskela P, Kuusisto P, and Vaheri A (1999) Epidemiological study of nephropathia epidemica in Finland 1989–96. *Scandinavian Journal of Infectious Diseases* 31: 427–435.

Calisher CH (ed.) (1990) *Hemorrhagic Fever with Renal Syndrome, Tick and Mosquito-Borne Viruses: Archives of Virology, Supplementum 1,* pp. 1–347. Vienna: Springer.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses, Bunyaviridae, Hantavirus*, pp. 704–707. San Diego, CA: Elsevirer Academic Press.

Kallio-Kokko H, Uzcategui N, Vapalahti O, and Vaheri A (2005) Viral zoonoses in Europe. *FEMS Microbiology Reviews* 29: 1051–1077.

Kaukinen P, Vaheri A, and Plyusnin A (2005) Hantavirus nucleocapsid protein: A multifunctional molecule with both housekeeping and ambassadorial duties. *Archives of Virology* 150: 1693–1713.

Khaiboullina SF, Morzunov SP, and St. Jeor SC (2005) Hantaviruses: Molecular biology, evolution and pathogenesis. *Current Molecular Medicine* 5: 773–790.
Kukkonen SKJ, Vaheri A, and Plyusnin A (2005) L protein, the RNA-dependent RNA polymerase of hantaviruses. *Archives of Virology* 150: 533–555.
Maes P, Clement J, Gavrilovskaya I, and Van Ranst M (2004) Hantaviruses: Immunology, treatment, and prevention. *Viral Immunology* 17: 481–497.
Muranyi W, Bahr U, Zeier M, and van der Woude FJ (2005) Hantavirus infection. *Journal of the American Society of Nephrology* 16: 3669–3679.
Peters CJ and Khan AS (2002) Hantavirus pulmonary syndrome: The new American hemorrhagic fever. *Clinical Infectious Diseases* 34: 1224–1230.
Pini N (2004) Hantavirus pulmonary syndrome in Latin America. *Current Opinion in Infectious Diseases* 17: 427–431.
Plyusnin A (2002) Genetics of hantaviruses: Implications to taxonomy (review). *Archives of Virology* 147: 665–682.
Schmaljohn CS and Nichol ST (eds.) (2001) Hantaviruses. *Current Topics in Microbiology and Immunology* 256: 1–191.
Terajima M, Vapalahti O, Van Epps HL, Vaheri A, and Ennis FA (2004) Immune response to Puumala virus infection and the pathogenesis of nephropathia epidemica. *Microbes and Infection* 6: 238–245.
Vapalahti O, Mustonen J, Lundkvist Å, Henttonen H, Plyusnin A, and Vaheri A (2003) Hantavirus infections in Europe. *Lancet Infectious Diseases* 3: 653–661.

Henipaviruses

B T Eaton and L-F Wang, Australian Animal Health Laboratory, Geelong, VIC, Australia

© 2008 Elsevier Ltd. All rights reserved.

History

The first henipavirus was isolated in 1994 following an outbreak of acute respiratory disease in a stable in Brisbane, Australia. In less than 3 weeks, 21 of 30 horses became infected and 14 either died or were euthanized. Two people who worked at the stable also became infected and one died. The virus responsible was a previously undescribed paramyxovirus, subsequently called Hendra virus (HeV) after the suburb where the outbreak occurred. In the subsequent decade, HeV was identified as the cause of equine deaths at four separate locations in Queensland, spilling over on two occasions to cause single human infections, one of which was fatal. In the latter instance, the patient succumbed to encephalitis over a year after infection during the necropsy of HeV-infected horses. Initial serological evidence suggested that fruit bats (flying foxes) in the genus *Pteropus* were reservoir hosts and in 1996, HeV was isolated from several Australian flying fox species.

The second member of the genus, Nipah virus (NiV), emerged in 1998–99 in Perak State, Peninsular Malaysia, as the cause of an outbreak of respiratory and encephalitic disease of low morbidity and mortality in pigs. The virus spread to humans causing febrile encephalitis among pig farmers and those who had direct contact with pigs. The epidemic moved south to the intensive pig-farming areas of Negeri Sembilan in December 1998 and was only brought under control by the imposition of movement restrictions and the culling of over 1 million pigs. Two hundred and sixty-five human cases of encephalitis were documented with 105 deaths. A cluster of 11 cases with one death occurred among abattoir workers in Singapore. NiV was subsequently isolated from the urine of Malaysian flying foxes in 2002. NiV re-emerged in Bangladesh and an adjoining area of India (West Bengal) in 2001, and since then outbreaks of encephalitis caused by the virus have re-occurred in Bangladesh almost every year, with case–fatality rates approaching 75%.

Taxonomy and Classification

The full-length genome sequence of HeV revealed it to be a member of the family *Paramyxoviridae*, subfamily *Paramyxovirinae*, but one with unique genetic features that precluded its classification in the genera *Morbillivirus, Respirovirus*, or *Rubulavirus*, the three genera established in the subfamily at that time. Complete genome sequencing of NiV revealed a high degree of similarity to HeV and, in 2002, the genus *Henipavirus* was created to accommodate these novel paramyxoviruses. The genus *Henipavirus* is now one of five genera in the subfamily *Paramyxovirinae* (**Figure 1**).

Several molecular features distinguish henipaviruses from other paramyxoviruses. First, the genome length of NiV and HeV at 18 246 and 18 234 nt, respectively, is approximately 15% larger than most other paramyxoviruses. It is not the extra length *per se* that is unique to henipaviruses. The unclassified paramyxoviruses J virus and Beilong virus have genomes over 19 000 nt in length. The novel henipavirus genomic feature is the length of the noncoding regions at the 3′ end of five of the six genes, which in most cases are 3–13 times longer than their *Paramyxovirinae* counterparts. The function of these regions is unknown. A second unique feature of henipaviruses

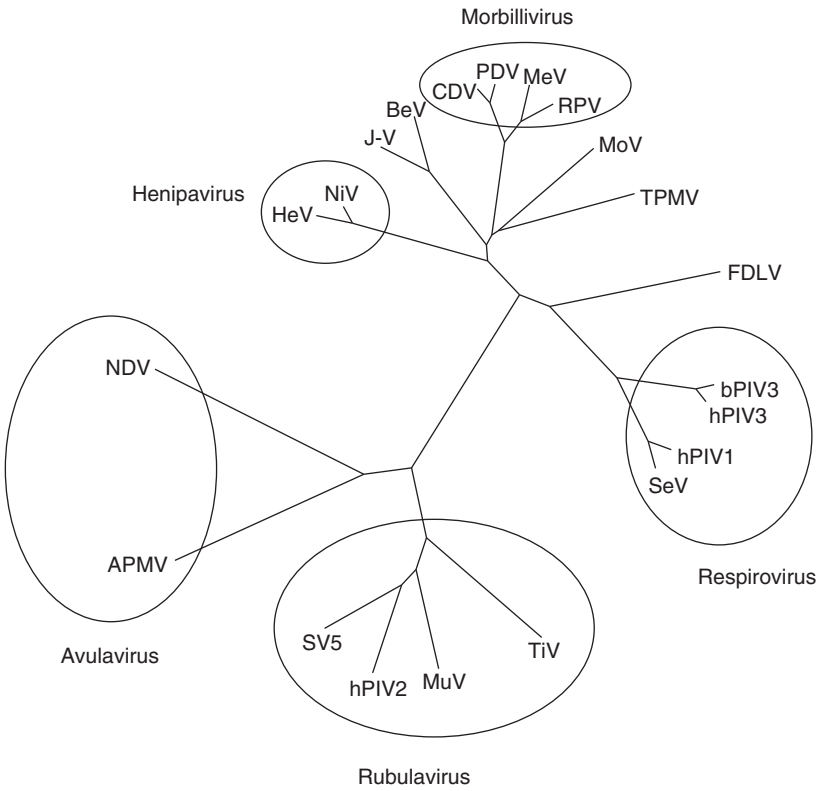


Figure 1 Classification of henipaviruses. The number of genera in the subfamily *Paramyxovirinae* was raised in 2002 from three (*Respirovirus*, *Morbillivirus*, and *Rubulavirus*) to five by the addition of two new genera, *Avulavirus* and *Henipavirus*. The phylogenetic tree shown here is based on an alignment of the deduced amino acid sequence of the L gene of selected family members using the neigbor-joining method. Viruses are grouped according to genus and abbreviated as follows. *Avulavirus* genus: APMV (avian paramyxovirus), NDV (Newcastle disease virus); *Respirovirus* genus: SeV (Sendai virus), hPIV1 (human parainfluenzavirus 1), hPIV3 (human parainfluenzavirus 3), bPIV3 (bovine parainfluenzavirus 3); *Morbillivirus* genus: MeV (measles virus), CDV (canine distemper virus), RPV (rinderpest virus), PDV (phocine distemper virus); *Henipavirus* genus: HeV (Hendra virus), NiV (Nipah virus); *Rubulavirus* genus: hPIV2 (human parainfluenzavirus 2), MuV (mumps virus), SV5 (simian parainfluenzavirus 5), TiV (Tioman virus); and unclassified viruses: MoV (Mossman virus), BeV (Beilong virus), FDLV (Fer-de-lance virus), TPMV (Tupaia paramyxovirus), and J-V (J virus).

commensurate with their status in a separate genus within the family *Paramyxoviridae* is the presence of 3′ and 5′ genomic terminal sequences 12 nt in length, that differ from all other paramyxoviruses and contain the genus-specific promoter elements for replication and transcription.

Molecular Biology

Although the genomes of henipaviruses, respiroviruses, and morbilliviruses are organized in an identical manner and, with the exception of the P protein, virus-encoded proteins in each genus are similar in size, analyses have revealed striking differences between some henipavirus proteins and their respirovirus and morbillivirus counterparts.

The attachment glycoproteins of paramyxoviruses are designated as hemagglutin-neuraminidase (HN), hemagglutinin (H), or glycoprotein (G) on the basis of their capacity to agglutinate red blood cells and remove sialic acid from carbohydrate moieties. The HN-positive status of respiroviruses, avulaviruses, and the vast majority of rubulaviruses reflects their use of sialic acids as cell surface receptors. In contrast, morbilliviruses and henipaviruses bind to cells using sialic acid-independent mechanisms. SLAM (signaling lymphocyte-activation molecule) is regarded as a universal morbillivirus receptor and ephrin B2 has been identified as a functional receptor for both HeV and NiV. Ephrin B2 is a member of a family of cell surface glycoprotein ligands that bind to surface-expressed tyrosine kinases known as Eph receptors. Binding of ephrins to Eph receptors leads to bidirectional signaling and cell-to-cell communication. The widespread distribution of ephrin B2 among mammals may help explain the extensive host range of henipaviruses (see below). Like the fusion protein of all other paramyxoviruses, the henipavirus F protein is activated by proteolytic cleavage of a precursor F_0 protein. Henipaviruses invariably generate systemic infections and most paramyxoviruses which do so synthesize F proteins

which are cleaved at a multibasic cleavage site by furin, a cellular protease located in the *trans*-Golgi network. Surprisingly, the cleavage site of both HeV and NiV is a single basic amino acid, arginine and lysine, respectively. What is more, that basic residue and the amino acids in the vicinity of it are not required for proteolytic cleavage. A second unique feature of henipavirus F proteins is the presence of an endocytosis consensus motif YXXϕ (where Y is tyrosine, X is any amino acid, and ϕ is an amino acid with a bulky hydrophobic group) in the cytoplasmic tail of the protein. Endocytosis is required for F protein cleavage activation, an observation consistent with the fact that cathepsin L, a lysosomal cysteine protease that lacks a distinctive cleavage recognition site, is responsible for cleavage of the HeV F protein.

In paramyxoviruses, mechanisms to inhibit the antiviral effects of interferon are encoded by the P gene, which generates multiple proteins (P, V, W, and C in henipaviruses) by means of internal translation initiation sites, overlapping reading frames, and a transcription process in which nontemplated G nucleotides are inserted at a conserved editing site, resulting in a reading frameshift during translation. Insertions of one and two G residues generate transcripts which encode the V and W proteins, respectively. Whereas the henipavirus V protein resembles that of other paramyxoviruses in having a conserved cysteine-rich C-terminal domain, the henipavirus W protein is unusual because the C-terminal domain is significantly longer than that of most *Paramyxovirinae*, where translation of the encoding P gene transcript terminates soon after the editing site.

Host Range and Viral Propagation

HeV and NiV replicate in a variety of mammalian cell lines. The rate of replication, the size of syncytia generated, and the location of nuclei in syncytia vary depending on cell type and virus species. Infected Vero cell monolayers yield titers in excess of 10^8 infectious virions per milliliter. The broad host range of henipaviruses has been corroborated by experiments in which cells infected with vaccinia virus recombinants and expressing henipavirus cell attachment (G) and fusion (F) proteins on the surface fuse with cells from a gamut of vertebrate sources. Susceptibility to virus infection and virus glycoprotein-mediated fusion *in vitro* are reflected in the wide range of mammalian hosts sensitive to virus infection *in vivo*. The host range of henipaviruses is considered uncommon for paramyxoviruses, which usually adopt a narrow host range. In addition to bats, NiV has been shown to infect animals in six other mammalian orders. Although the widespread distribution of ephrin B2 in vertebrates provides an explanation for the diverse host range of henipaviruses, there are mammalian species such as mice which express functional ephrin B2, shown to bind henipaviruses, but remain resistant to infection with HeV and NiV.

Serologic Relationships

NiV was initially described as Hendra-like on the basis of its reactivity with anti-HeV antibodies, a similarity corroborated by complete genome sequencing. No serological relationship between henipaviruses and other paramyxoviruses has been confirmed. The close serological relationship of HeV and NiV makes it difficult to identify antibodies to each virus and determine the virus species circulating in specific geographical areas. A four- to eightfold difference between homologous and heterologous neutralization titers is the basis upon which antibody specificity has been determined.

Four neutralizing epitopes have been mapped on the globular head of the HeV G protein. Two discontinuous epitopes are located on the base of the head and two on the top, in locations resembling those identified as neutralizing sites in other paramyxoviruses. The amino acid homology between HeV and NiV is relatively high at one of the discontinuous epitopes but decreases significantly at the other three sites.

Geographical Distribution

HeV has been isolated from horses, humans, and flying foxes in Australia and NiV from humans, pigs, and flying foxes in Malaysia, Bangladesh, and Cambodia. NiV has also been isolated from fruit partially eaten by flying foxes in Malaysia, and NiV RNA has been detected in human patients and bats in India and Thailand, respectively. Comparative neutralization studies using Australian bat sera confirm that HeV populates the southern domain of the henipavirus distribution. Conversely, Malaysian and Indonesian sera preferentially neutralize NiV, rather than HeV. Equivalent neutralization titers to both HeV and NiV suggest that a related virus may be circulating in the Cambodian bat population. The geographic distribution of *Pteropus* species which range from the east coast of Africa, through the Indian subcontinent and Southeast Asia, north to Okinawa and south to Australia, suggests that henipaviruses may also be found in flying fox populations in geographically more diverse locations.

Evolution and Genetics

Genetically diverse populations of NiV circulate in Southeast Asia. In Malaysia, three closely related lineages of NiV have been identified. A number of identical isolates from humans and pigs during the major outbreak in

the pig-farming communities in central Malaysia in 1999 constitute the first lineage. A 1999 isolate from the initial focus of infection in northern Malaysia is the sole representative of a second lineage and NiV isolated from the urine of flying foxes on Tioman Island off the east coast of peninsular Malaysia constitutes the third lineage, one that is genetically closer to viruses isolated during the major outbreak. It has been suggested that there may have been two bat-to-pig spillover events in Malaysia, only one of which caused significant transmission to humans. However, the role played by virus evolution in pigs, particularly during the period following introduction into the pig population in 1998 and prior to the major outbreak in 1999, remains uncertain.

Unlike the Malaysian isolates which displayed an overall sequence divergence of less than 1%, regardless of source, NiV isolates from human patients in Bangladesh in 2004 differed from the Malaysian isolates by approximately 7% in the protein-coding regions and up to 25% in the non-coding regions. The heterogeneity of Bangladesh isolates suggested multiple spillovers of NiV from flying foxes into the human population. A further evolutionary lineage situated between the NiV-Malaysia isolates and NiV-Bangladesh isolates appears to exist in Cambodia.

The paucity of HeV isolates has restricted speculation on the evolution of this henipavirus in Australia. Partial sequencing suggests that viruses isolated from equine and human sources during the initial outbreak in Brisbane in 1994 and from flying foxes two years later were identical.

Epidemiology and Transmission

Human henipavirus infections in Australia and Malaysia occurred as a result of transmission of the viruses from flying foxes via horses and pigs, respectively. In contrast, NiV may have been directly transmitted to humans in Bangladesh, where it is believed that date palm juice contaminated with bat secretions constituted a potential transmission route. The mode of transmission from bats to spillover hosts in Australia and Malaysia is not known. It has been suggested that horses and pigs may have been infected by HeV and NiV through masticated fruit pulp spat out by flying foxes or by flying fox urine which contaminated pastures or pig sties. Alternatively, the source of virus may have been infected fetal tissues or fluids, a suggestion based on the fact that HeV outbreaks occurred during the birthing period of some species of flying fox, the isolation of HeV from a pregnant flying fox and its fetus, and the transplacental transmission of HeV in experimental infections.

HeV has been transmitted from horse to man on four occasions. The source of the virus may have been the saliva of infected horses, the nasal discharge commonly found at the terminal stages of the disease, or a wide range of infected tissues made accessible by necropsy. The paucity of virus in the bronchi or bronchioles of infected horses suggests transmission of HeV to either man or horse by aerosol is highly unlikely and experimental infection has confirmed the poor transmissibility of HeV in horses.

Risk factors for human infection by NiV in Malaysia were contact with pigs or fresh pig products and because the virus was present in a wide range of organs, the greatest likelihood arose for those in direct contact with sick or dying pigs on farms during farrowing or slaughtering or in abattoirs. NiV is readily observed in the respiratory epithelium of naturally and experimentally infected pigs, a feature suggesting that the virus probably spread to humans and within the pig population by aerosol or by direct contact with oropharyngeal or nasal secretions. Although NiV was present in the urine and respiratory secretions of patients, human-to-human transmission in Malaysia was extremely rare. In contrast, in Bangladesh, epidemiologic evidence indicates spread of the virus from person to person.

Tissue Tropism

Henipaviruses cause systemic infections displaying a predilection for vascular endothelial cells. The identification of ephrin B2 as a henipavirus receptor not only provides an explanation for the diverse host range of these viruses, but also helps to explain the observed systemic distribution of viral antigen particularly in arterial endothelial cells and smooth muscle. Ephrin B2 is found in arteries, arterioles, and capillaries in multiple tissues and organs but is absent from venous components of the vasculature. It is also found in arterial smooth muscle. Ephrins play critical roles in axonal guidance during vertebrate embryonic development and ephrin B2 is expressed on neurons. The availability of ephrin B2 or other receptors, the requirement for co-receptors, or the differing capacities of cells to support the production of infectious virus may determine whether henipaviruses act as respiratory or neurological pathogens.

Pathogenicity

The outcome of henipavirus infections can range from high mortality, as seen with HeV in horses and NiV in humans, through low mortality and morbidity, best exemplified by NiV in pigs, to asymptomatic as observed in flying foxes. The highly virulent nature of henipaviruses in humans and the lack of therapeutic modalities have led to the classification of HeV and NiV as Biosafety Level 4 (BSL4) pathogens. Experimental infections reveal that

henipaviruses are infectious following either oronasal or parenteral administration. Figures for the minimum lethal dose of henipaviruses are known only for NiV in golden hamsters (*Mesocricetus auratus*) where the LD_{50} was 270 plaque-forming units (pfu) and 47 000 pfu following intraperitoneal and intranasal administration, respectively.

Clinical Features of Infection

Henipaviruses display either predominantly respiratory or neurological tropisms depending on the host. In natural infections of horses and young pigs with HeV and NiV, respectively, and in experimental infections of cats with either virus, respiratory symptoms predominate. Neurological symptoms were also observed in a proportion of HeV-infected horses. Experimental infection of horses and cats is usually fatal, with death or euthanasia occurring 5–10 days post infection. Following natural infection of horses, however, the observations during the initial outbreak indicated that some animals displayed respiratory symptoms but survived and others responded to infection asymptomatically. HeV-induced respiratory disease in horses may be accompanied by facial swelling and ataxia and *in extremis*, a copious frothy nasal discharge. Natural infection of pigs with NiV is usually asymptomatic, an outcome also observed after experimental administration of NiV by the ocular and oronasal route. When symptoms are present, they vary according to the age of the pig. Young animals present primarily with respiratory symptoms. Older animals display increased salivation and nasal discharge and on occasion develop neurological signs such as trembling, muscle spasms, and an uncoordinated gait. In Malaysia, pigs displaying symptoms of NiV infection, such as nasal discharge and rapid and labored respiration, had a harsh and nonproductive cough, which gave rise to the name 'barking-pig-disease'.

In contrast, NiV infection in humans is usually associated with severe acute encephalitis and although a proportion of cases presented with respiratory disease, particularly in Bangladesh, the majority displayed fever, headache, drowsiness, dizziness, myalgia, vomiting, and a reduced level of consciousness. Clinical signs such as the absence of reflexes and the irregular twitching of muscles or parts of muscles and an abnormal doll's eye reflex are indicative of brainstem and upper cervical spinal cord dysfunction. In the Malaysian outbreak, 105 of 256 patients died, a mortality rate of 41%. However, this figure reduces to *c.* 30% when individuals who experienced either a mild or asymptomatic infection are taken into account. In Bangladesh, 66 of 90 patients died in outbreaks in 2001, 2003, and 2004, giving a combined case–fatality rate of approximately 70%. Whereas HeV or NiV infection of cats presents a model of respiratory infection, the clinical and pathological features observed in NiV-infected hamsters resemble those found in human cases of encephalitis.

Both HeV and NiV can cause prolonged infection in humans before manifesting as causes of severe neurological disease. In Malaysia, NiV persisted in a proportion of patients (*c.* 10%) who either recovered from encephalitis or who had experienced an asymptomatic infection. Such cases of relapsed and late-onset encephalitis respectively presented from months to years after the initial infection. Only four cases of human HeV infection have been recorded. Two displayed influenza-like symptoms and one died. A third, fatal case of encephalitis occurred over a year after a self-limiting episode of meningitis, attributed to HeV infection.

Pathology and Histopathology

The respiratory disease caused by HeV in horses is characterized by pulmonary edema and congestion. Viral antigen is found in endothelial cells in a range of organs including lungs, lymph nodes, kidneys, spleen, bladder, and meninges, and virus can be recovered from a number of internal organs, including lung, and from saliva and urine. Young pigs infected with NiV present primarily with respiratory symptoms caused by tracheitis and bronchial and interstitial pneumonia. After experimental infection by the ocular and oronasal routes, virus replication occurs in the oropharnyx and spreads to the respiratory tract and lymphoid tissues before appearing in the trigeminal ganglion and neural tissue. Viral antigen is found, particularly in clinically affected animals, in both lungs and meninges and virus is recovered from a range of tissues including tonsil, nasal, and throat swabs and lung. Detection of NiV in the urine of infected pigs is uncommon.

The primary site of replication and dynamics of henipavirus spread in humans are unknown but the distribution and time of appearance of lesions throughout the vasculature and in the brain in NiV encephalitis suggest that hematogenous spread delivers the virus from primary to secondary sites of replication in widely dispersed vascular endothelial cells. Inflammation of blood vessels, particularly small arteries, arterioles, and capillaries, occurs in most organs but is prominent in brain, lung, heart, and kidney. The interval between maximum vasculitis in the brain and parenchymal infection in NiV encephalitis suggests that infection of neurons occurs as a result of vascular damage and that neurological impairment may be due, not only to the effects of ischemia and infarction, but also viral infection of neurons. Although NiV antigen is found infrequently in epithelial cells of the bronchi and kidney, replication in such locations may play a role in virus dissemination. Recent outbreaks of NiV in Bangladesh strongly suggest human to human transmission.

No disease syndromes associated with henipaviruses have been documented in wild flying fox populations. Nor does HeV cause clinical disease in experimentally infected flying foxes and only a proportion of infected bats respond with sporadic vasculitis in the lung, spleen, meninges, kidney, and gastrointestinal tract. The mode of henipavirus transmission between flying foxes is unknown but the presence of virus in the placenta and transmission without apparent harm to the fetus in experimentally infected bats suggests that horizontal transmission is feasible.

Immune Response

Adaptive

In susceptible nonpteropid species, henipaviruses elicit strong humoral immune responses. No studies have been made of cellular responses to henipavirus infection. Antibodies to P, N, and M proteins are evident in Western blots and anti-F and anti-G antibodies are readily detected by enzyme-linked immunosorbent assay (ELISA), using both traditional and bead-based formats, and by immune precipitation. In contrast to the consistent antibody response in susceptible, nonvolant species, in flying foxes there appears to be little direct correlation between detectable virus replication and the appearance of antiviral antibody. Antibody production is irregular and of uncertain longevity.

Anti-NiV antibodies were present in a majority of patients with clinical NiV encephalitis and IgM antibodies occurred more frequently than IgG antibodies in both serum and cerebrospinal fluid (CSF). The appearance of specific IgM antibodies in serum preceded their appearance in the CSF, a sequence consistent with viremia preceding central nervous system infection.

Innate

Henipaviruses inhibit both interferon (IFN) induction and IFN signaling. In the induction phase, the detection of viral double-stranded (ds)RNA by both RNA helicase enzymes and Toll-like receptor 3 (TLR3) leads to the transcription of IFN. Henipaviruses inhibit this process in a number of ways. The V protein, like that of other paramyxoviruses, blocks the activity of the helicase sensor in the cytoplasm but does not inhibit the TLR3 pathway. In contrast, in a process unique to paramyxoviruses and by virtue of the nuclear localization signal in its C-terminal domain, the W protein inhibits IFN induction by blocking a late nuclear step in the activation process which is shared by both helicase and TLR3 pathways.

In the IFN signaling pathway, IFN binds to cell surface receptors in a paracrine manner and initiates a sequence of reactions that leads to activation of members of a family of proteins called signal transducers and activators of transcription (STAT). STAT proteins activate transcription of hundreds of genes some of whose products inhibit virus replication. In a strategy unique to paramyxoviruses, henipaviruses inhibit IFN signaling by sequestering STAT proteins in high molecular weight complexes. The V and P proteins bind STAT proteins in the cytoplasm, whereas the W protein co-localizes with STAT in the nucleus. The extra length of the henipavirus P gene compared with that of other *Paramyxovirinae* results in the encoded P, V, and W proteins having an N-terminal extension of *c.* 100 amino acids compared with their morbillivirus and respirovirus counterparts. This domain appears to provide henipaviruses with a multifaceted anti-IFN signaling activity.

Prevention and Control

Therapeutic options for treatment of infections caused by henipaviruses are limited and currently use of ribavirin appears to be the only recourse. The drug inhibits replication of HeV in cells in culture, and in an open-label study of 194 patients during the NiV outbreak in Malaysia, its use resulted in a 35% reduction in mortality. Duration of ventilation and total hospital stay were both significantly shorter in those receiving ribavirin. Human monoclonal antibodies to the henipavirus G protein also appear to have therapeutic potential. Antibodies which react with the soluble G protein of HeV have been generated from naive recombinant human antibody libraries and they neutralize both HeV and NiV *in vitro*.

A number of promising strategies to develop henipavirus vaccines are being explored. NiV F and G glycoproteins expressed from vaccinia virus elicit neutralizing antibodies in both mice and hamsters and in the latter both anti-F and anti-G antibodies protected from a lethal NiV challenge, although they did not prevent virus replication. Murine anti-F and anti-G antisera also inhibited membrane fusion mediated by F and G glycoproteins expressed in cell culture. More importantly, because of the attractiveness of a subunit compared with a recombinant vaccine, purified soluble HeV and NiV G proteins elicit potent neutralizing antibody responses in rabbits. Antibodies neutralized both HeV and NiV in cell culture and displayed a slightly higher titer against the homologous virus.

A further therapeutic option relies on the fact that henipavirus F_1 glycoproteins have α-helical heptad repeat (HR) domains proximal to the fusion peptide and transmembrane domain at the N- and C-terminal of the protein, respectively. HR domains form a trimer-of-hairpins structure during the fusion of virus and cell membranes. Peptides corresponding to the N- and C-terminal HR domains of HeV and NiV form trimer-of-hairpins structures *in vitro*. Addition of exogenous peptide from either HR domain blocks formation of the trimer-of-hairpins, inhibits cell

fusion mediated by vaccinia virus-expressed F and G proteins, and prevents virus infection of cells in culture.

Future Perspectives

The presence of HeV or NiV in flying foxes throughout Australia, Southeast Asia, and part of the Indian subcontinent suggests that the distribution of henipaviruses may parallel that of pteropid species, which range from Madagascar to the South Pacific. Although the factors responsible for the emergence of henipaviruses have not been clearly elucidated, the destruction of native habitats, a process which is unlikely to abate, forces flying foxes into contact with man as they seek food in areas frequented by humans. Although documented outbreaks of disease caused by henipaviruses are relatively infrequent, lack of knowledge of the modes of transmission between fruit bats and from fruit bats to spillover hosts, the high infectivity of the viruses for certain species, their broad species tropism, and their zoonotic potential will ensure that further work on henipaviruses remains a priority.

See also: Measles Virus; Mumps Virus; Paramyxoviruses of Animals; Rinderpest and Distemper Viruses.

Further Reading

Eaton BT, Broder CC, Middleton D, and Wang LF (2006) Hendra and Nipah viruses: Different and dangerous. *Nature Reviews Microbiology* 4: 23–35.

Eaton BT, Mackenzie JS, and Wang LF (2006) Henipaviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 5th edn., pp. 1587–1600. Philadelphia: Lippincott Williams and Wilkins.

Field HF, Mackenzie JS, and Daszak P (2007) Henipaviruses: Emerging paramyxoviruses associated with fruit bats. *Current Topics in Microbiology and Immunology* 315: 133–160.

Hyatt AD, Zaki SR, Goldsmith CS, Wise TG, and Hengstberger SG (2001) Ultrastructure of Hendra virus and Nipah virus. *Microbes and Infection* 3: 297–306.

Lamb RA and Parks GD (2006) *Paramyxoviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 5th edn., pp. 1449–1496. Philadelphia: Lippincott Williams and Wilkins.

Wong KT, Grosjean I, Brisson C, *et al.* (2003) A golden hamster model for human acute Nipah virus infection. *American Journal of Pathology* 163: 2127–2137.

Wong KT, Shieh WJ, Zaki SR, and Tan CT (2002) Nipah virus infection, an emerging paramyxoviral zoonosis. *Springer Seminars in Immunopathology* 24: 215–228.

Hepadnaviruses of Birds

A R Jilbert, Institute of Medical and Veterinary Science, Adelaide, SA, Australia
W S Mason, Fox Chase Cancer Center, Philadelphia, PA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The avian hepadnaviruses belong to the genus *Avihepadnavirus* in the family *Hepadnaviridae*. Within this genus, duck hepatitis B virus (DHBV) and heron hepatitis B virus (HHBV) are assigned to the species *Duck hepatitis B virus* and *Heron hepatitis B* virus, respectively. These avian viruses are related phylogenetically through similarities in genome sequence and organization of open reading frames (ORFs) to human hepatitis B virus (HBV) and other hepadnaviruses that infect mammals (genus *Orthohepadnavirus*). The major site of hepadnavirus infection and replication is the hepatocyte, the predominant cell type of the liver, constituting about 60–70% of liver cell mass. There are $\sim 5 \times 10^{10}$ hepatocytes in the liver of an adult duck.

Hepadnaviruses contain a relaxed circular double-stranded DNA (rcDNA) genome which is converted in the nucleus to covalently closed circular DNA (cccDNA). cccDNA acts as the virus transcriptional template and is used by host RNA polymerase II to produce a greater than genome length, 'pregenomic' RNA, referred to as the pregenome, and a number of other mRNA species. Hepadnaviruses have a unique method of replication that involves reverse transcription of pregenomic RNA into DNA. Virus replication and release are generally considered to be noncytopathic and disease activity is attributed to the host immune response to infected hepatocytes.

The avian hepadnaviruses are naturally transmitted *in ovo* via vertical transmission of virus from an infected female duck to the egg, with virus replication occurring in the yolk sac and liver of the developing embryo. Ducks infected *in ovo* develop widespread and persistent DHBV infection of the liver but have minimal or no liver disease as they are immune tolerant to the virus. Although experimental infection of newly hatched ducks also leads to widespread and persistent DHBV infection of the liver, DHBV-infected ducks, unlike HBV-infected humans, do not develop severe liver disease, cirrhosis, or liver cancer. However, they provide a reproducible experimental system for studying virus kinetics and immune clearance, and much of what we know of the hepadnavirus replication

strategy has been discovered from studies of DHBV *in vitro* and *in vivo*. DHBV-infected ducks are also used to evaluate new antiviral therapies and vaccines with the ultimate aim of applying the same strategies to the treatment of HBV infections in humans.

Virion and Particle Structure

Like the mammalian hepadnaviruses, avian hepadnaviruses are enveloped viruses possessing an icosahedral nucleocapsid and ~3000 nt rcDNA genome (**Figure 1**). DHBV, the prototypic avian hepadnavirus, has a diameter of 40–45 nm, and different isolates contain genomes ranging in length from 3021 to 3027 nt. In infected ducks, mature enveloped DHBV virions are released from hepatocytes directly into the bloodstream. Virus titers in chronically infected ducks are up to 1×10^{10} virions per ml of serum.

The envelope of DHBV is composed of a lipid bilayer containing multiple copies of two transmembrane virus proteins, Pre-S/S and S (36 and 17 kDa, respectively). These two proteins are synthesized from the same virus ORF, with initiation of Pre-S/S occurring upstream of S. The two proteins share a common carboxy terminus. An additional 28 kDa protein antigenically related to Pre-S/S and S has been detected in DHBV-infected liver but it is unclear whether this is a degradation product of Pre-S/S or is translated from an AUG codon mapping between the start sites of Pre-S/S and S. As for HBV, the envelope proteins not only participate in virion formation but also self-assemble into noninfectious virus-like DHBV surface antigen particles (DHBsAg particles). This process occurs in the endoplasmic reticulum and results in the release from infected hepatocytes of a 500- to 1000-fold excess of DHBsAg particles over infectious virions. DHBsAg particles do not contain a virus nucleocapsid and lack virus nucleic acids. DHBsAg particles are pleomorphic and spherical, with a diameter of ~35–60 nm, and the larger particles are almost indistinguishable from DHBV virions by electron microscopy (EM). In contrast, during HBV infection, filamentous forms of surface antigen-containing particles (HBsAg) are produced as well as 22 nm spherical HBsAg particles, both of which are readily distinguished from those of HBV by EM.

The nucleocapsid of DHBV is icosahedral, as observed by cryoelectron microscopy, and is comprised of dimers of 30 kDa nonglycosylated DHBV core antigen (DHBcAg). In mature virions, the rcDNA genome and a virus-encoded DNA polymerase are contained in the virus nucleocapsid (**Figure 1**), which also contains cellular chaperones.

The DHBV polymerase is a 90 kDa molecule that functions as both an RNA-dependent DNA polymerase and a DNA-dependent DNA polymerase. The polymerase also has an RNase H activity that digests the RNA pregenome during virus replication. The protein sequence of the DHBV polymerase has four separate domains listed from the N terminal end, that include a terminal protein (TP), 'spacer', reverse transcriptase (RT), and RNase H domain. Each of these domains has one or more key roles during virus replication, as described below.

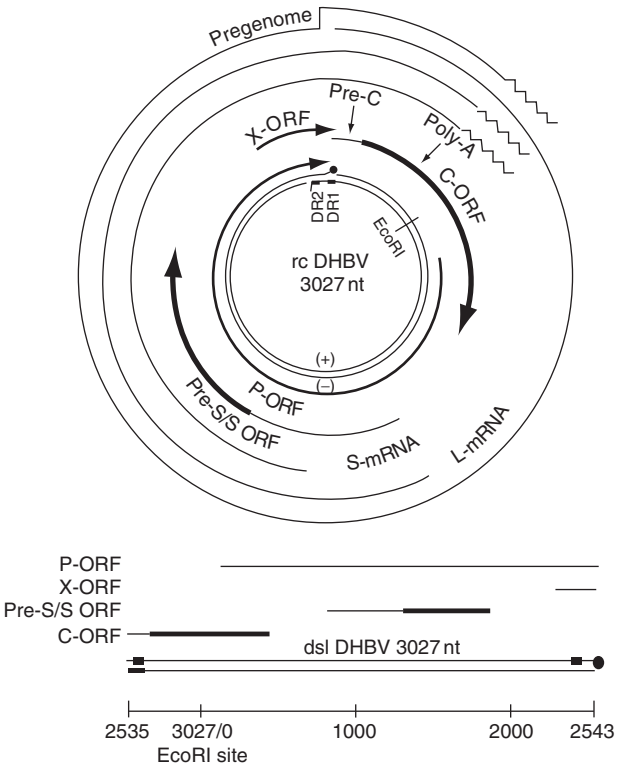


Figure 1 Organization of the DHBV genome. The rcDNA genome is shown at the top center, with the virus polymerase covalently attached to the 5′ end of the negative strand and an 18 nt RNA bound to the 5′ end of the positive strand. The DHBV genome contains two direct repeat (DR) sequences (DR1 and DR2). The locations of the virus ORFs, DR1 and DR2 and maps of the virus mRNAs are also shown. None of the major mRNAs is spliced. Thus, each has its own promoter. However, they share a common polyadenylation (poly-A) signal in the virus C-ORF. The pregenome RNA is terminally redundant and serves as the mRNA for both the C-ORF and, less frequently, the downstream P (polymerase) ORF. The pregenome lacks the AUG of the Pre-C/C-ORF, which instead is translated from an mRNA a few nt longer at the 5′ end than the pregenome (not shown). The L and S mRNAs encode the Pre-S/S and S envelope proteins, respectively. The more rarely synthesized double-stranded linear (dsl) virus genome is shown at the bottom. The common nucleotide numbering system is also displayed, proceeding in a clockwise direction from a conserved EcoRI restriction site.

Also released from hepatocytes and circulating in the blood of infected ducks is the so called DHBV e antigen (DHBeAg), a proteolytically processed form of the 35 kDa Pre-C/C protein. Different forms of DHBeAg range in size from a nonglycosylated 27 kDa to 30–33 kDa glycosylated forms. DHBeAg is thought to play an important

role in either the initiation or maintenance of persistent infection by delaying or diverting the host immune response to DHBcAg. Both DHBsAg and DHBcAg are highly immunogenic. Anti-DHBc antibodies can be detected by ELISA in the serum of ducks during acute and persistent DHBV infections. Anti-DHBs antibodies are produced during the resolution phase of acute DHBV infection and are generally not detected in ducks with persistent infection except in complexes with circulating DHBsAg. Anti-DHBs antibodies bind virus particles and are able to block DHBV infection of cells. For this reason, ducks that have recovered from acute DHBV infection are immune to challenge with DHBV.

Genome Organization

As noted above, all hepadnaviruses have a similar genome organization, despite the low DNA sequence identity between the mammalian and avian hepadnaviruses (40%). The negative strand of the DHBV genome contains S-ORF, C-ORF, and P-ORF encoding the surface or envelope proteins, core and e antigen proteins, and polymerase protein, respectively (**Figure 1**). The DHBV genome was originally thought to contain only these three ORFs and to lack an ORF encoding a protein analogous to the X protein of the mammalian hepadnaviruses. It was subsequently discovered that avian hepadnaviruses, including HHBV and DHBV, have a fourth ORF, the X-ORF, which directs synthesis of a candidate X protein that is translated using an unconventional start codon. The significance of this observation is still unclear. Using woodchuck hepatitis virus (WHV), a close relative of HBV, the X gene was shown to be essential for successful WHV infections *in vivo*. In contrast, knockout of the X gene of DHBV did not alter the time course of DHBV infection. As with wild-type DHBV, the X gene knockout of DHBV spread rapidly through the liver of newly hatched ducks and caused persistent DHBV infection.

The positive strand of the DHBV genome does not contain any functional ORFs. All virus mRNA species are produced from the negative strand of the virus genome.

The difference in size between the genomes of the avian (e.g., DHBV with a genome of 3027 nt) and the mammalian hepadnaviruses (e.g., HBV with a genome of 3200–3300 nt), is due in part to deletion of a 150 nt stretch in S-ORF. This region in the HBV genome encodes the so-called 'a' determinant, a highly immunodominant region to which most of the neutralizing anti-HBV antibodies are directed. This region in HBV is present on the small form of HBsAg, the S protein. The absence of this region in DHBV results in a lack of immunodominance, and both DHBV Pre-S/S and S domains have been shown to induce high titer, neutralizing, anti-DHBs antibodies.

Replication Cycle

Hepatocytes, the major parenchymal cell of the liver, are the primary site of DHBV infection. The cell specificity of infection is determined by the presence of specific receptors required for virus binding and entry into hepatocytes. In early studies, DHBsAg particles, which appear to have the same envelope structure as DHBV virions, were shown to bind cell surface receptors on primary duck hepatocytes in a species-specific manner. Receptor binding occurs via the Pre-S/S region of DHBV and is followed by cell entry via receptor-mediated endocytosis. Recent studies have determined that the cellular protein carboxypeptidase D (180 kDa) binds DHBsAg particles and DHBV with high affinity and is found on both internal and external membranes of the cell. However, whereas DHBV has a narrow host range, carboxypeptidase D is expressed on many cell types that are not susceptible to DHBV infection. In addition, despite the observed binding of DHBsAg to carboxypeptidase D, transfection of cells with carboxypeptidase D cDNA does not confer susceptibility to DHBV, suggesting that other co-receptors or mechanisms may be operating. An additional DHBV-binding protein, glycine decarboxylase (120 kDa) has been identified. Its cellular expression is restricted to the liver, kidney, and pancreas. Interestingly, DHBV infection and replication has been detected in a few percent of cells in both the kidney and pancreas (see below and **Figure 5**). Thus, these proteins are potential components of a DHBV receptor complex and probably have a role in determining DHBV organ tropism. However, definite proof that these are the receptors that lead to DHBV binding, receptor-mediated endocytosis, and infection is still lacking.

Whatever the receptor, the immediate sequel to virus uptake is transport of the rcDNA genome to the nucleus to form cccDNA (**Figure 2**). The negative strand of the rcDNA DHBV genome is nicked, with a 9 nt terminal redundancy and with the virus polymerase molecule covalently attached to its 5′ end. Covalent attachment of the virus polymerase occurs during priming of reverse transcription through a tyrosine located in the TP domain of the polymerase. Similarly, the positive strand of the DHBV genome has an 18 nt RNA primer attached to its 5′ end (**Figure 1**). In addition, the positive strand is incomplete, with a minimum gap of 12 nt. Thus, following transport to the nucleus, the covalently linked polymerase protein, the RNA primer, and the terminal redundancy in the negative strand are removed, the positive strand is completed, and ligation of the ends of each strand takes place. As noted above and discussed in detail below, ~10% of circulating DHBV virions contain double-stranded linear (dsl) genomes that result from a defect that occurs during synthesis of positive-strand DNA. Following infection these dsl genomes can also enter the nucleus to form cccDNA, which in this case is formed

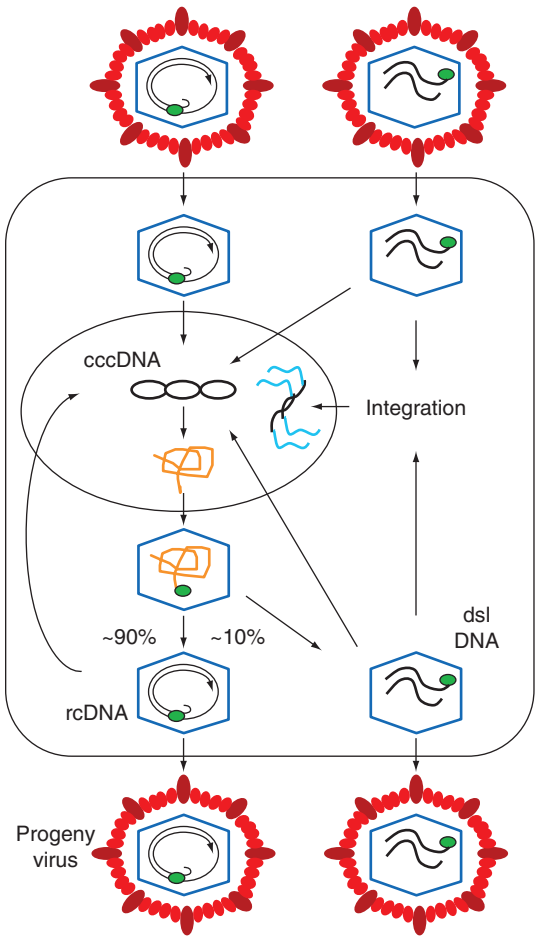


Figure 2 DHBV infection of hepatocytes. Virions, shown at the top, may have either an rcDNA or, more rarely, a dsl DNA genome. Upon infection virus nucleocapsids are uncoated and move to the nucleus. Virus DNA is released from nucleocapsids and probably enters the nucleus at nuclear pores. Both rcDNA and dsl DNA give rise to cccDNA, the virus transcriptional template. cccDNA is used by host-cell RNA polymerase II to produce pregenomic RNA that is reverse transcribed to rcDNA or dsl DNA. dsl DNA also has a propensity to integrate into host-cell DNA (see text). Early in infection, cccDNA copy number increases by intracellular amplification from rcDNA and dsl DNA made in the cytoplasm via reverse transcription. Later, this pathway is shut down and nucleocapsids containing rcDNA and dsl DNA bud into the endoplasmic reticulum and are released as progeny virus.

by illegitimate recombination between the ends of the dsl DNA. This recombination event typically involves some loss of sequences, and cccDNA formed from dsl genomes is generally defective.

DHBV cccDNA molecules exist within the nucleus of each infected hepatocyte as a population of virus minichromosomes that bind up to 20 nucleosomes per 3000 nt molecule. cccDNA does not undergo semiconservative replication. New copies are therefore formed from rcDNA and dsl DNA synthesized in the cytoplasm of infected hepatocytes. Once formed, cccDNA is transcribed by host RNA polymerase II to produce pregenomic and other virus mRNAs (**Figures 1** and **2**), followed by protein production, assembly of nucleocapsids from the viral core protein and packaging of pregenomic RNA. Each virus mRNA is produced from its own promoter and virus RNA molecules including the pregenome have a 5′ cap and are polyadenylated at a common site (nt 2778–2783) on the DHBV genome numbered according to the sequence of the Australian strain of DHBV (GenBank AJ006350) where the unique EcoRI site is nucleotide 1.

Packaging of pregenomic RNA is facilitated by the presence of an encapsidation signal, epsilon (ε), located at the 5′ end (nt 2566–2622) and at the 3′ end of the pregenome, within the terminal redundancy (**Figure 3(a)**). Pregenome packaging is dependent upon binding of the virus polymerase to the 5′ ε sequence. In fact, the pregenome is able to serve as the mRNA for both core and polymerase proteins (**Figure 1**). However, once polymerase is translated, it may bind to its own message and block its further translation. Once packaging into nucleocapsids has occurred, virus DNA synthesis takes place via reverse transcription of the pregenomic RNA, with production of replicative intermediate DNA (RI DNA) to produce rcDNA and dsl DNA genomes in a ~10:1 ratio (**Figures 3(f)** and **3(h)**).

DNA synthesis begins with reverse transcription of 4 nt (5′ UUAC 3′) in the bulge in the stem–loop structure of ε, leading to synthesis of 4 nt of DNA (5′ GTAA 3′) (**Figure 3(b)**). As noted above, a tyrosine residue in the TP domain of the polymerase serves as the primer of reverse transcription. Following synthesis by reverse transcription of the first 4 nt, the complex is translocated to DR1, in particular the 3′ copy of DR1, DR1* (**Figure 3(c)**).

DR1 is a 12 nt sequence located 6 nt from the 5′ end of the pregenome at nt 2541–2552. It is therefore also present in the terminal redundancy of the pregenome. The same sequence known as DR2 is located about 50 nt upstream of the terminal redundancy at nt 2483–2494. Once transferred to DR1*, where the 4 nt can base pair due to sequence homology, reverse transcription reinitiates and continues to the 5′ end of the pregenome, to produce a full-length negative strand with a 9 nt terminal redundancy (**Figures 3(d)** and **3(e)**). Most of the pregenome is degraded during negative-strand elongation by the RNase H activity of the virus polymerase. However, the 5′ ~18 nt of the pregenome, including the cap and all of the 5′ copy of DR1, escapes RNase H degradation and serves as the positive DNA strand primer. Positive-strand synthesis leading to rcDNA formation initiates near the 5′ end of the negative strand. The primer is first translocated from its original location at the 3′ end of the template to DR2 where it can hybridize due to the sequence identity of DR1 and DR2 (**Figure 3(e)**). Following positive-strand elongation to the 5′ end of the negative strand, circularization occurs to facilitate continuation

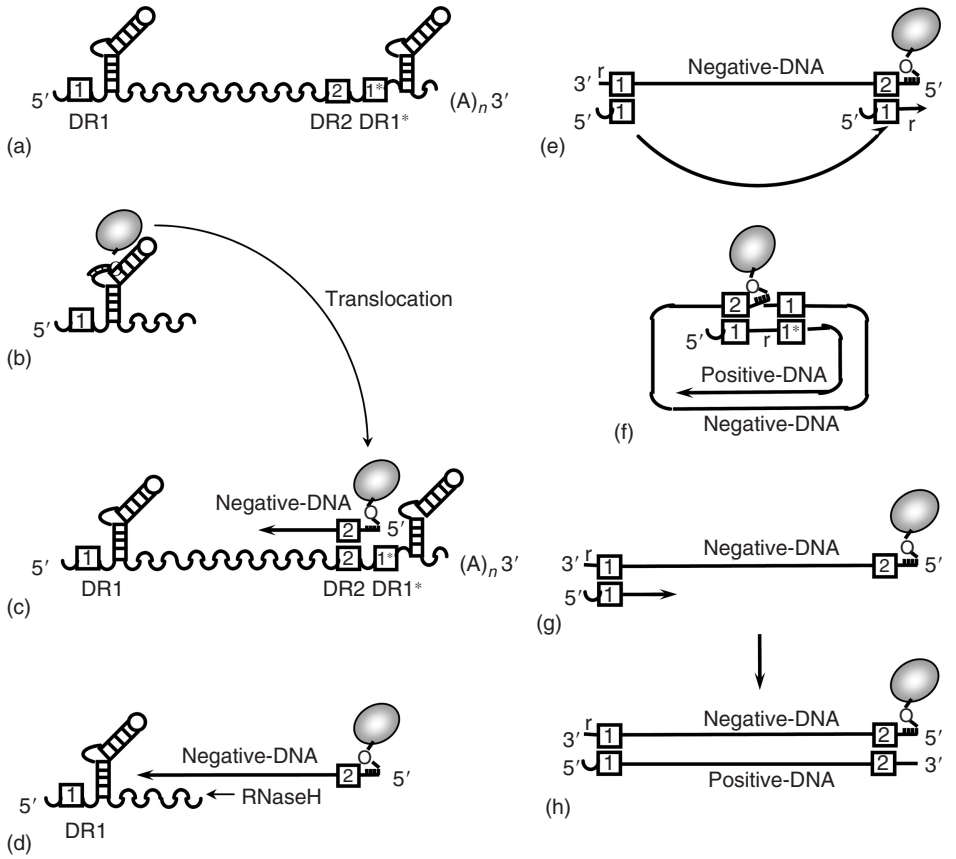


Figure 3 Mechanism of DHBV DNA synthesis. Virus DNA synthesis begins with reverse transcription of 4 nt (5′ UUAC3′) of the bulge in the stem–loop structure, ε, and leads to synthesis of the first 4 nt (5′ GTAA3′) of negative-strand DNA, as shown in (b). Polymerase with the nascent transcript then translocates to the right-hand copy of DR1, DR1*, and reverse transcription of the negative strand and degradation of the pregenomic RNA template by RNase H proceeds as shown in (c) and (d). Following completion of the negative strand, the 5′ 18 nt of the pregenome, including the cap, are typically translocated to DR2 and positive-strand DNA synthesis then initiates from this RNA primer. In this case, rcDNA formation is of necessity an early step in positive-strand synthesis ((e) and (f)). About 10% of the time, positive-strand synthesis initiates without translocation of the primer (g), leading to the formation of dsl DNA (h). The details of DNA synthesis are described in greater detail in the text.

of positive-strand synthesis to produce mature rcDNA (**Figure 3**(**f**)). Circularization is facilitated by the 9 nt terminal redundancy on the negative-strand template.

dsl DNA genomes reflect a failure of positive-strand primer translocation from DR1 to DR2, resulting in a phenomenon known as *in situ* priming to produce a DNA that is collinear with pregenomic RNA up to DR1* (**Figure 3**(**h**)). Interestingly, both the polymerase, the protein primer of negative-strand synthesis and the RNA primer of positive-strand synthesis remain associated with virus DNA throughout virion assembly. Biochemical evidence suggests that each nucleocapsid contains only a single copy of polymerase protein, implying that a single protein is simultaneously the primer of DNA synthesis and the polymerase for both RNA- and DNA-dependent DNA synthesis.

Newly made rcDNA and dsl DNA are enveloped by budding into the endoplasmic reticulum and exported as progeny virus via the Golgi apparatus, or are transported to the nucleus to make additional copies of cccDNA, typically found at 10–30 copies per hepatocyte. Nuclear transport is negatively regulated by the virus envelope proteins, which direct nucleocapsids into the pathway of virus assembly. This negative regulation is essential because excessive accumulation of cccDNA will kill the host cell. Strong negative regulation may also be important because of the stability of cccDNA, and new synthesis of cccDNA may only be necessary to restore cccDNA levels in the progeny following division of infected hepatocytes. Negative regulation of cccDNA synthesis also appears to occur during HBV infection, but it is still not known whether the viral envelope proteins have an essential role in this process.

dsl DNA also integrates randomly into chromosomal DNA and has been detected in ~0.01–0.1% of hepatocytes during acute infections by both DHBV and the mammalian hepadnaviruses. Higher levels of integrated virus DNA accumulate during chronic infections. This

probably reflects ongoing import of rcDNA and dsl DNA into the nucleus to restore cccDNA copy number as infected hepatocytes divide to replace those killed by the host immune response, thereby providing new dsl DNA genomes for integration. Integrated forms of hepadnavirus DNA are usually unable to act as templates for transcription of pregenomic RNA since the promoter for this virus RNA is located at the 3′ end of the integrant, not the 5′ end, and integrated DNA is therefore not able to direct virus replication (see **Figure 1**). In addition, virus sequences may be lost during the integration process, particularly from the ends of the dsl molecule.

Since each integrated virus–cell DNA junction is unique, and will be present only once in the liver unless the infected hepatocyte divides, integration sites can be used to identify and track the fate of individual hepatocyte lineages. In particular, assays for integrated DNA were used to show that hepatocytes present in the liver following recovery from an acute WHV infection were derived from previously infected hepatocytes, indicating the existence of mechanisms for removal of cccDNA from infected hepatocytes.

Phylogenetic Information

Assigned species within the genus *Avihepadnavirus* include DHBV isolated from Pekin ducks (*Anas domesticus*) and HHBV from grey herons (*Ardea cinerea*). Many DHBV isolates have been found in domesticated ducks and, in the wild, in the mallard, the species from which most domesticated ducks are derived. Viruses less closely related to DHBV have been isolated from geese and other duck species and include the Ross's goose hepatitis B virus (RGHBV) from Ross's geese (*Anser rossii*), Mandarin duck hepatitis B virus (MDHBV) from Mandarin ducks (*Aix galericulata*), and the snow goose hepatitis B virus (SGHBV) from snow geese (*Anser caerulescens*). The stork hepatitis B virus (STHBV) has been isolated from white storks (*Ciconia ciconia*), with additional viruses isolated from demoiselle (*Anthropoides virgo*) and grey crowned cranes (*Balearica regulorum*) (**Figure 4**). By genome sequencing, HHBV and STHBV are the most distant from DHBV (**Figure 4**). HHBV was assigned as a species based both on genome divergence and a host range difference from DHBV. Current information does not provide clear evidence for designation of additional new species among the isolates shown in **Figure 4**.

Thus, the avihepadnaviruses are currently classified phylogenetically into 'Chinese' and 'Western Country' isolates as well as four highly distinct lineages that include SGHBV, RGHBV, plus MDHBV, STHBV, and HHBV. Sequence divergence within the 'Chinese' and 'Western Country' DHBV strains is 5.99% and 3.35%, respectively, while divergence between the strains is 9.8%. SGHBV diverges from DHBV by 11–13%, RGHBV and MDHBV by 17–19% and STHBV and HHBV by 22–24%. Additional closely related viruses have been isolated from the puna teal (*Anas puna*), ashy-headed sheldgoose (*Chloephaga poliocephala*), Orinoco sheldgoose (*Neochen jubata*), and Chiloe wigeon (*Anas sibilatrix*) (**Figure 4**).

Pathogenesis and Control of Diseases

In persistently DHBV-infected ducks, the natural route of virus transmission is from the bloodstream to the egg. Virus replication then occurs in the yolk sac and developing liver and pancreas of the embryo (**Figures 5(a)–5(c)**) and results in congenital DHBV infection. Congenitally DHBV-infected ducks remain persistently infected for life, with infection in >95% of hepatocytes (**Figure 5(e)**). Bile duct cells are also infected with DHBV and express high levels of DHBcAg and DHBsAg (**Figure 5(e)**). Virus replication also occurs in acinar and islet cells in the pancreas (**Figure 5(f)**), and in glomeruli (**Figure 5(d)**) and tubular epithelial cells in the kidney. Cells located in the germinal centers of the spleen contain nonreplicating DHBV DNA, which is thought to be associated with follicular dendritic cells. In ducks with congenital infection, levels of DHBV DNA and DHBsAg in the bloodstream gradually decrease over 800 days and anticore antibodies can be detected from ~90 days post hatch, consistent with a high degree of immune tolerance.

Persistent DHBV infection also results following experimental inoculation of newly hatched ducks, and inoculation of infected serum containing the equivalent of one virion is sufficient to initiate DHBV infection. Infection of newly hatched ducks with DHBV has allowed detailed studies of the kinetics and tissue specificity of DHBV infection. Inoculation of 2- to 4-week-old ducks with DHBV results in either acute or persistent infection depending on the age of the duck at the time of inoculation and the dose of DHBV administered. Ducks from this age group that develop persistent infection have histological changes in the liver with mild mononuclear cell infiltration of portal tracts but no evidence of lobular hepatitis or extensive liver damage. Experimental inoculation of 4-month-old ducks with high doses of DHBV leads to either persistent infections with mild to marked liver disease or to transient infections that are rapidly cleared.

Since hepadnavirus infection is noncytopathic, the liver damage seen during DHBV infection has been attributed to the immune response directed against infected hepatocytes. Although cirrhosis and primary hepatocellular carcinoma (HCC) are not reported to occur in DHBV infection, the clinical and serological events, hepatocyte specificity, and ability of the host to rapidly resolve an infection involving the entire hepatocyte population are similar for DHBV, WHV, and HBV.

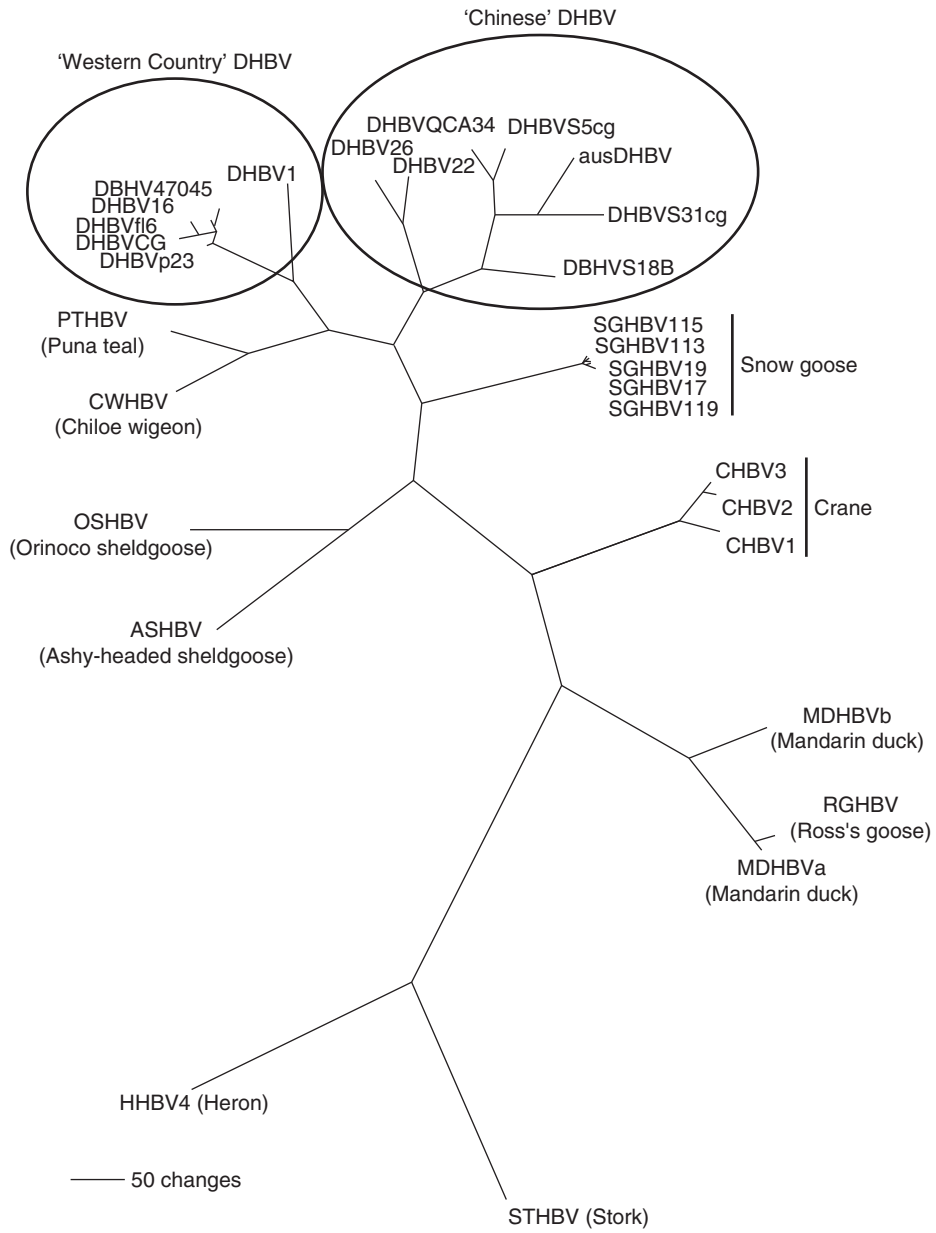


Figure 4 Phylogenetic relationships of avian hepadnaviruses. A dendrogram file was constructed using Clustal X and full-length virus sequences and displayed using Treeview. The following sequences were included: 'Chinese' DHBV isolates, accession numbers M32990, M32991, X60213, AJ006350, M21953, X58568, X58569; 'Western Country' DHBV isolates, K01834, M60677, X12798, X74623, AF047045, X58567; snow goose hepatitis B virus isolates: AF110996, AF110997, AF110998, AF110999, AF11000; Ross's goose hepatitis B virus: M95589; grey heron hepatitis B virus: M22056; stork hepatitis B virus: AJ251937; crane hepatitis B virus isolates: AJ441111, AJ441112, AJ441113; Mandarin duck hepatitis B virus isolates: AY494848, AY494849; Chiloe wigeon hepatitis B virus: AY494850; puna teal hepatitis B virus: AY494851; Orinoco sheldgoose hepatitis B virus: AY494852; and ashy-headed sheldgoose hepatitis B virus: AY49485.

Humans infected with HBV, especially when less than a year of age, often develop a healthy carrier state which can last for several decades without clinically evident liver disease. Similarly, persistent infection with avian hepadnaviruses, resulting either from *in ovo* infection or experimental inoculation after hatching, generally results in only mild hepatitis similar to the 'healthy' carrier state in humans. The failure to detect liver disease, cirrhosis and HCC in persistently DHBV-infected ducks may to be linked to the timing and mode of transmission of these viruses, since they are usually transmitted vertically by *in ovo* transmission resulting in congenital infection with immune tolerance and an absence of, or only mild, liver disease. The inability of DHBV-infected ducks to progress to HCC may also be affected by their limited life span in captivity, which is generally less than 5 years.

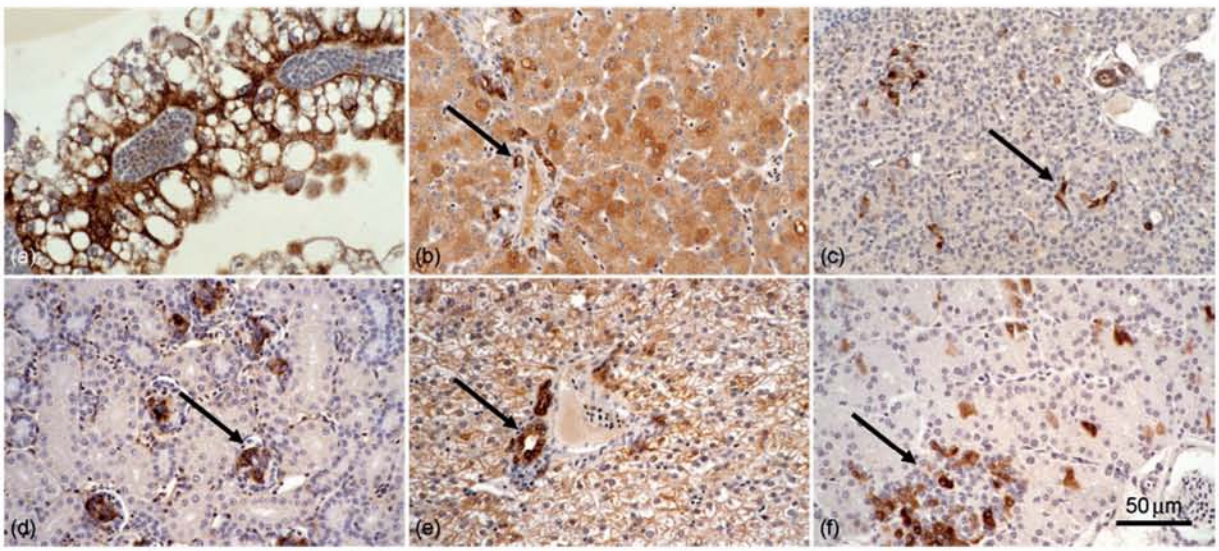


Figure 5 Hepatic and extrahepatic infection with DHBV of a 14-day-fertilized duck embryo (a–c) and a 2-week-old congenitally infected duck (d–f). Immunoperoxidase staining of DHBV core antigen in yolk sac (a), liver (b, e), pancreas (c, f), and kidney (d). DHBV is transmitted *in ovo* from the bloodstream of the persistently DHBV-infected female duck to the egg, resulting in DHBV replication in yolk sac cells (a), hepatocytes and bile duct cells (arrow) in the liver (b), and pancreatic acinar cells (c). DHBV infection persists in congenitally DHBV-infected ducks in glomerular cells in the kidney ((d); arrow), in >95% of hepatocytes and in bile duct cells of the liver ((e); arrow shows high levels of DHBV core antigen detection in bile duct cells), and in pancreatic acinar and islets cells ((f); arrow indicates a group of DHBV core antigen-positive pancreatic beta islet cells; alpha islets also positive (not shown)). Tissues were fixed in ethanol:acetic acid, immunostained with polyclonal anti-DHBV core antibodies and counterstained with hematoxylin.

HCC was detected in ducks in a Chinese province but in those areas aflatoxin exposure may have been common and the key contributory factor in the development of the liver cancers.

Conclusion

Although the avian hepadnaviruses share only 40% nucleotide sequence identity with HBV and the other mammalian hepadnaviruses, they are nearly identical in genome organization and replication strategy. Indeed, the molecular details of hepadnavirus replication were initially worked out with DHBV and later extended to HBV, and the similarities were found to be striking. The biology of infection of HBV and DHBV is also strikingly similar, with hepatocytes the primary target of infection and immune-mediated cell death the primary cause of liver disease in infected hosts.

The only major difference between the two viruses is how they are able to persist in their respective host populations. DHBV appears to be maintained primarily by vertical transmission to the developing embryo, whereas the primary route for maintenance of HBV in the human population is by horizontal transmission during the first year of life from mother to child, or among young children. Both routes lead to chronic infection, but it is generally believed that the degree of immune tolerance to the virus is much greater following vertical than horizontal transmission. This probably explains the greater disease activity in chronically infected humans than ducks. This difference is also seen in the woodchuck model. Chronic WHV infection results, as in HBV in humans, primarily from horizontal transmission, leading to a much more rapid disease progression than found in ducks.

HBV was discovered in 1967, WHV in 1978, and ground squirrel hepatitis B and DHBV viruses shortly thereafter. Among these, DHBV was the easiest to work with because of the ready availability of hosts from commercial sources. Thus, in the initial absense of a cell culture system, much of the early work on hepadnavirus replication focused on DHBV. As a result, DHBV provided much of the information on how the hepadnaviruses replicate as well as detailed early information on the biology of infection and virus spread, including possible identification of the cell surface receptor for the virus. Studies of DHBV have also provided important information on how cccDNA is made and on its high degree of stability in nondividing cells. In addition, the DHBV model has been used to demonstrate the reproducible and rapid kinetics of the spread of DHBV infection *in vivo* and to determine the high specific infectivity of DHBV, where one virus particle has been shown to be infectious in neonatal ducks.

Because of the reproducible nature of the kinetics of infection and the predictable outcomes of infection using defined doses of DHBV, the model is especially useful for the evaluation of new antiviral therapies and vaccine strategies for HBV infection in humans. Similarities in the replication strategy and polymerase enzymes of the avian

and human hepadnaviruses allow evaluation of new antiviral drugs in *in vivo* models of HBV infection, including the development of antiviral drug-resistant mutants and competition between wild-type and mutant DHBV strains.

See also: Hepadnaviruses: General Features; Hepatitis B Virus: General Features; Hepatitis B Virus: Molecular Biology.

Further Reading

Foster WK, Miller DS, Scougall CA, Kotlarski I, Colonno RJ, and Jilbert AR (2005) The effect of antiviral treatment with Entecavir on age- and dose-related outcomes of duck hepatitis B virus infection. *Journal of Virology* 79: 5819–5832.

Funk A, Mhamdi M, Will H, and Sirma H (2007) Avian hepatitis B viruses: Molecular and cellular biology, phylogenesis, and host tropism. *World Journal of Gastroenterology* 13: 91–103.

Gong SS, Jensen AD, Chang CJ, and Rogler CE (1999) Double-stranded linear duck hepatitis B virus (DHBV) stably integrates at a higher frequency than wild-type DHBV in LMH chicken hepatoma cells. *Journal of Virology* 73: 1492–1502.

Guo H, Mason WS, Aldrich CE, *et al.* (2005) Identification and characterization of avihepadnaviruses isolated from exotic anseriformes maintained in captivity. *Journal of Virology* 79: 2729–2742.

Jilbert A and Locarnini S (2005) *Avihepadnaviridae*. In: Thomas H, Lemon S, and Zuckerman A (eds.) *Viral Hepatitis,* 3rd edn., pp. 193–209. Adelaide: Blackwell Publishing.

Jilbert AR, Freiman JS, Gowans EJ, Holmes M, Cossart YE, and Burrell CJ (1987) Duck hepatitis B virus DNA in liver, spleen, and pancreas: Analysis by *in situ* and Southern blot hybridization. *Virology* 158: 330–338.

Jilbert AR and Kotlarski I (2000) Immune responses to duck hepatitis B virus infection. *Developmental and Comparative Immunology* 24: 285–302.

Jilbert AR, Miller DS, Scougall CA, Turnbull H, and Burrell CJ (1996) Kinetics of duck hepatitis B virus infection following low dose virus inoculation: One virus DNA genome is infectious in neonatal ducks. *Virology* 226: 338–345.

Jilbert AR, Wu TT, England JM, *et al.* (1992) Rapid resolution of duck hepatitis B virus infections occurs after massive hepatocellular involvement. *Journal of Virology* 66: 1377–1388.

Meier P, Scougall CA, Will H, Burrell CJ, and Jilbert AR (2003) A duck hepatitis B virus strain with a knockout mutation in the putative X ORF shows similar infectivity and *in vivo* growth characteristics to wild-type virus. *Virology* 317: 291–298.

Miller DS, Bertram EM, Scougall CA, Kotlarski I, and Jilbert AR (2004) Studying host immune responses against duck hepatitis B virus infection. *Methods in Molecular Medicine* 96: 3–25.

Summers J, Smith PM, and Horwich AL (1990) Hepadnavirus envelope proteins regulate covalently closed circular DNA amplification. *Journal of Virology* 64: 2819–2824.

Yang W and Summers J (1995) Illegitimate replication of linear hepadnavirus DNA through nonhomologous recombination. *Journal of Virology* 69: 4029–4036.

Yang W and Summers J (1999) Integration of hepadnavirus DNA in infected liver: Evidence for a linear precursor. *Journal of Virology* 73: 9710–9717.

Zhang YY, Zhang BH, Theele D, Litwin S, Toll E, and Summers J (2003) Single-cell analysis of covalently closed circular DNA copy numbers in a hepadnavirus-infected liver. *Proceedings of the National Academy of Sciences, USA* 100: 12372–12377.

Hepadnaviruses: General Features

T J Harrison, University College London, London, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The infectious nature of viral hepatitis has been recognized throughout human history, including by documentation of epidemics of jaundice (campaign jaundice) associated with warfare. Parenteral transmission of hepatitis (serum hepatitis) was recognized from the end of the nineteenth century following transmission by routes such as tattooing, the reuse of syringes and needles, and contamination of vaccines by 'stabilization' with human 'lymph' or serum. The viral etiology of infectious and serum hepatitis was recognized in studies involving transmission to volunteers, during and after World War II. In addition, these studies established different incubation times for infectious (short incubation) and serum (long incubation) hepatitis. The terms hepatitis A and hepatitis B were coined and became accepted for infectious (or epidemic) and serum hepatitis, respectively.

Progress with hepatitis B began with the serendipitous discovery of the envelope protein of the virus, hepatitis B surface antigen (HBsAg), originally termed Australia antigen. This was discovered in 1965 by Blumberg, who precipitated an antigen–antibody complex by immunodiffusion of sera from a multiply transfused patient and an Australian aboriginal, during a study of blood and leukocyte antigens. The protein was later recognized to be associated with transmission of hepatitis and could be detected in the sera of a proportion of patients with viral hepatitis. Electron microscopic studies in the late 1960s led to the discovery of particles of around 20 nm diameter that are now known to be composed of membrane-embedded HBsAg, secreted from the hepatocytes as non-infectious, subviral particles. In 1970, Dane visualized larger, 42 nm particles with electron dense cores. Originally termed 'Dane particles', these are the hepatitis B virions and were shown later to contain a small circular

DNA genome that was partially single-stranded and associated with a DNA polymerase activity that could render the molecule fully double-stranded.

The development of tests for HBsAg, and for antibodies to hepatitis A virus, led to the recognition of transmission of hepatitis, termed non-A, non-B hepatitis (NANBH), potentially by other viruses. Post-transfusion (or parenterally transmitted), PT-NANBH is caused principally by hepatitis C virus and enterically transmitted, ET-NANBH, by hepatitis E virus. The genomes of these viruses were cloned in 1989. Earlier, in 1977, Rizzetto had described a novel antigen–antibody system, termed delta/anti-delta, in patients with hepatitis B. The delta antigen is now known to be the nucleocapsid protein of hepatitis D virus (HDV), a defective virus that requires HBsAg to form its envelope.

The Hepadnaviruses

Hepatitis B virus (HBV) is now recognized as the prototype of a family of viruses that infect mammals and birds. The *Hepadnaviridae* (named for *hepa*totropic *DNA* viruses) are divided into mammalian (*Orthohepadnavirus*) and avian (*Avihepadnavirus*) genera.

In the 1960s, it was noted by Snyder that eastern woodchucks (*Marmota monax*) in a colony in the Philadelphia Zoo were highly susceptible to the development of chronic hepatitis and hepatocellular carcinoma, and he proposed a viral etiology. The sera of animals with chronic hepatitis proved to contain a virus with similar biochemical properties to HBV and this was named woodchuck hepatitis virus (WHV). A search for similar viruses in relatives of the woodchuck turned up a closely related virus, ground squirrel hepatitis virus (GSHV) in the Beechey ground squirrel (*Spermophilus beecheyi*; *Spermophilus* and *Marmota* are genera of the family *Sciuridae*). Other related viruses are endemic in Richardson ground squirrels (*Spermophilus richardsonii*) in Canada (Richardson ground squirrel hepatitis virus, RGSHV) and arctic ground squirrels (*Spermophilus parryi kennicotti*) in Alaska (arctic squirrel hepatitis virus, ASHV).

Reports of hepatocellular carcinoma in farmed ducks in China led to the discovery of duck hepatitis B virus (DHBV; the 'B' is included to avoid confusion with the picornavirus, duck hepatitis virus, and is retained in the names of the other avian hepadnaviruses) in flocks of Pekin ducks (*Anas domesticus*) in China and the USA. In the USA, up to 10% of ducks in some commercial flocks are persistently infected. The discovery of DHBV was followed by the identification of related viruses in grey herons (*Ardea cinerea*) in Germany (heron hepatitis B virus, HHBV), in Ross's geese (*Anser rossii*; Ross's goose HBV, RGHBV) and snow geese (*Anser caerulescens*; snow goose HBV, SGHBV), in white storks (*Ciconia ciconia*; STHBV) and in grey crowned cranes (*Balearica regulorum*; CHBV). Recently, other hepadnaviruses have been isolated from a variety of exotic ducks and geese, including teal, widgeon, and sheldgeese. However, because these birds were kept in captivity, it is not clear that these novel viruses are native to the species from which they were first isolated. Indeed, RGHBV and SGHBV also were isolated from captive birds and are quite distinct viruses (**Figure 1**), despite the fact that the two avian species mix and may interbreed in the wild.

Biology of the Viruses

As noted above, hepadnavirus particles consist of a DNA-containing nucleocapsid enveloped with the surface proteins embedded in a lipid bilayer derived from the internal membranes of the host cell. Excess surface proteins are secreted as subviral particles, which may serve to subvert the immune response to the surface proteins. The surface open reading frame (ORF) in the mammalian viruses contains three, in-frame initiation codons leading to the synthesis of large (L, pre-S1+pre-S2+S), middle (M, preS2+S), and small (or major, S) surface proteins (**Figure 2(a)**). The pre-S1 region contains an endoplasmic reticulum-retention signal involved in virion assembly; its incorporation into subviral particles leads to the formation of tubular, rather than spherical, forms. The avian viruses have only large (pre-S+S) and small (S) surface proteins, the middle protein being absent (variants of HBV with small deletions that abrogate synthesis of the middle protein also seem to be viable). For both classes of virus, the ligand that interacts with the major cellular receptor seems to reside within the domain unique to the large surface protein and it is this interaction that seems to be responsible for the high degree of species specificity of this virus family. The primary receptor for DHBV is carboxypeptidase D but the receptors and co-receptors of the mammalian viruses remain to be identified.

Within the nucleocapsid, the genome (around 3.2 kbp for HBV, 3.3 kbp for the rodent viruses, and 3.0 kbp for the avian viruses) is composed of two linear strands of DNA held in a circular configuration by base-pairing of a short region where the 5′ ends overlap. As noted above, one strand (the plus strand) of the HBV genome is incomplete, usually being approximately 60–80% full length. On the other hand, the genome of DHBV tends to be almost completely double-stranded, while it has been reported that SGHBV produces a significant proportion of virions containing single-stranded DNA.

With its small size, the HBV genome was one of the first viral genomes to be cloned and sequenced completely.

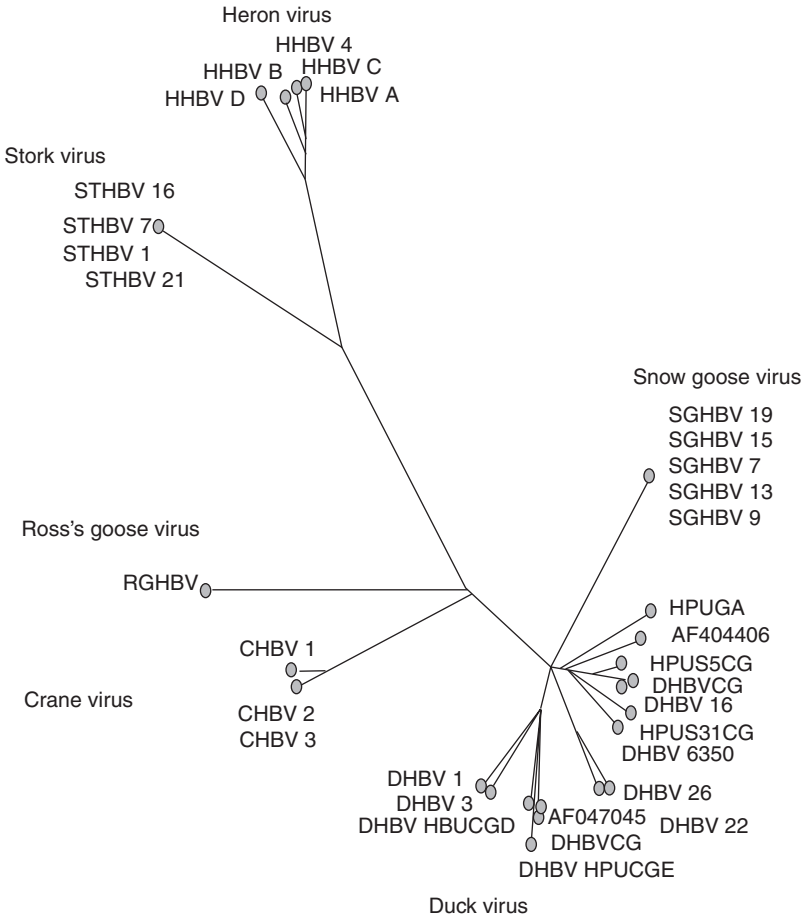


Figure 1 An unrooted phylogenetic tree showing the genetic relatedness between hepadnaviruses isolated from ducks, geese, herons, storks, and cranes. Reproduced from Prassolov A, Hohenberg H, Kalinina T, *et al.* (2003) New hepatitis B virus of cranes that has an unexpected broad host range. *Journal of Virology* 77: 1964–1976, with permission from American Society for Microbiology.

Four overlapping ORFs are conserved among the mammalian viruses (**Figure 2**(**a**)). These encode the surface and nucleocapsid proteins, the polymerase, and a small protein called x (HBxAg) for its unknown function. HBxAg is now known to be a transactivator of transcription and is presumed to act by upregulating the activity of the viral promoters and may be important in 'kick-starting' a new infection. The X ORF is absent from the genomes of the avian viruses (**Figure 2**(**b**)). However, the avian viruses do encode an ORF that resembles X but lacks an initiation codon; it is not know whether this is expressed during infection.

The hepadnaviruses replicate by reverse transcription of a pregenomic RNA and have been termed 'pararetroviruses'. The replication of HBV, many features of which were elucidated using as a model DHBV in its natural host, is discussed elsewhere in this encyclopedia. Briefly, the viruses are believed to enter the host cell via endosomes with delivery of the genomes to the nucleus, where they are converted to a covalently closed circular (ccc) form that is the template for transcription. The pregenomic RNA, which is also the mRNA for the nucleocapsid protein and polymerase, has a stem–loop structure near to the 5′ end. This is recognized by the polymerase, which also acts as a protein primer, and DNA synthesis begins, also signaling encapsidation. Reverse transcription and second-strand synthesis take place in immature cores in the cytoplasm; completion of the cores being followed by envelopment and exocytosis. Early in infection, some nascent cores cycle back to the nucleus to build up a pool of ccc DNA templates.

Genetic Variation and Epidemiology

HBV originally was typed serologically on the basis of the reactivity of HBsAg. All types share a common determinant known as *a*; this has been mapped to a hydrophilic region, roughly between amino acid residues (aa) 111 and 156 of the (226 aa) small surface protein, S. This highly

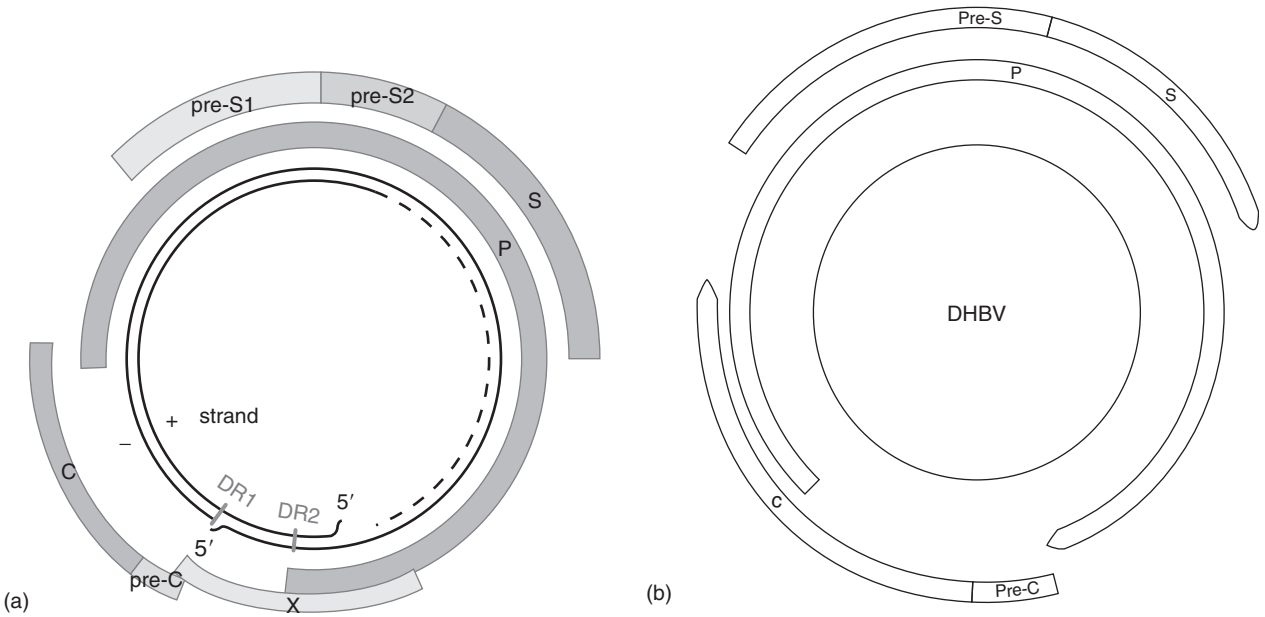


Figure 2 (a) Organization of the HBV genome. The inner circles depict the complete minus strand and incomplete plus strand and the positions of the direct repeats (DR) are indicated. The blocks surrounding the genome show the locations of the four overlapping ORFs; C and S contain two and three in-frame initiation codons, respectively. (b) Organization of the DHBV genome. A simplified view illustrating the single pre-S region and lack of an X ORF. (a) Reproduced from Kidd-Ljunggren K, Miyakawa Y, and Kidd AH, *et al.* (2002) Genetic variability in hepatitis B viruses. *Journal of General Virology* 83: 1267–1280, with permission from Society for General Microbiology.

conformational region, comprising a number of overlapping epitopes, is the main target of the humoral response, and antibodies synthesized during convalescence or in response to the hepatitis B vaccine are protective. Two pairs of mutually exclusive subdeterminants, *d* or *y* and *w* or *r*, correlate with variation (in both cases between lysine and arginine) at aa 122 and aa 160, respectively. Thus, four principal subtypes are recognized (*adw*, *adr*, *ayw*, and (rarely) *ayr*) and these show varying geographical distribution.

The error-prone nature of HBV DNA replication, via an RNA intermediate and without proofreading, is balanced by constraints on variation imposed by overlapping ORFs and the various *cis*-acting elements all being embedded in ORFs. Nonetheless, individual isolates of HBV vary by up to 10–14% of nucleotide positions and genotypes have been defined on the basis of >8% nucleotide sequence divergence. Currently, eight genotypes (A–H) are recognized and most of these have been divided further into subgenotypes. Genotype A is found in northern Europe and North America, B and C in east and Southeast Asia, D in a wide area though southern Europe and North Africa to India, E in western Africa, and F in South and Central America. Arguably, too few of the most recently described genotypes have been isolated to establish an epidemiological pattern, but G has been found in the USA and Europe, and H in the USA and Central America.

HBV has also been isolated from a variety of nonhuman primates. The first such complete sequence was derived from HBV from a captive chimpanzee and, at the time, it was not possible to determine whether this represented a divergent human isolate (i.e., the result of an unintentional human-to-chimpanzee transmission) or a genuine chimpanzee virus. Some years later, a related virus was isolated from a white-handed gibbon (*Hybolates lar*) and complete sequences now are available from a considerable number of chimpanzees, gorillas, orangutans, and gibbons. The most divergent of the primate hepadnaviruses sequenced at the time of writing is from the woolly monkey (*Lagothrix lagotricha*), a New World monkey. Woolly monkey HBV (WMHBV) shows around 20% nucleotide sequence divergence from HBV.

Figure 3 shows a dendrogram of the eight human HBV genotypes along with the primate viruses and rooted using WMHBV as an outgroup. Fewer genome sequences are available for the rodent hepadnaviruses. Isolates of WHV vary in fewer than 4% of nucleotide positions and differ from GSHV and ASHV in around 15% (no sequence is available for RGSHV). **Figure 4** shows a dendrogram of the primate and rodent hepadnavirus sequences, rooted using DHBV as an outgroup.

As noted above, the avian hepadnaviruses show somewhat less-restricted host specificity than those infecting mammals. **Figure 1** shows a phylogenetic tree of viruses isolated from ducks, geese, herons, storks, and cranes.

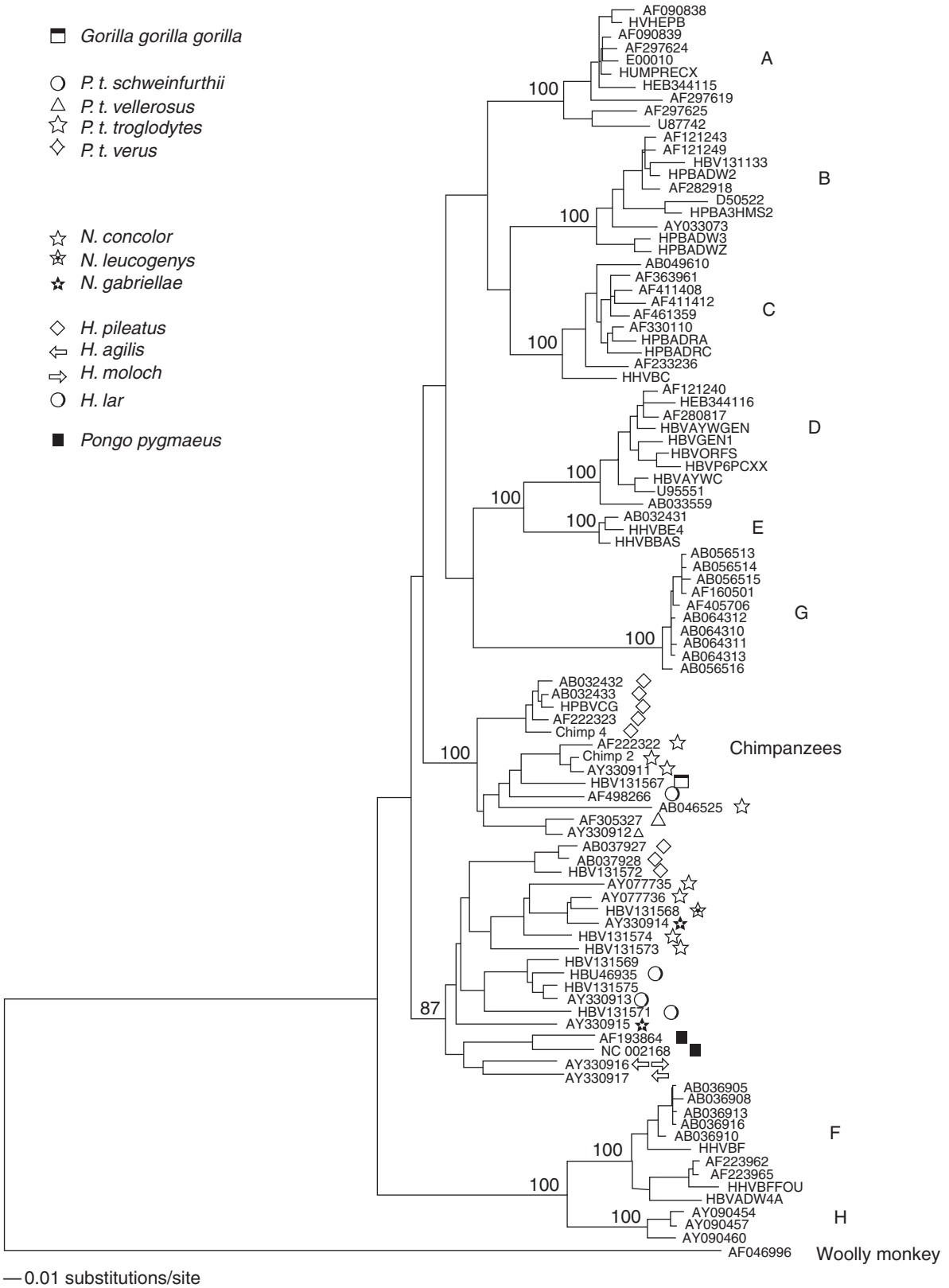


Figure 3 Dendrogram showing the relatedness of the eight genotypes of HBV and hepatitis B viruses isolated from nonhuman primates. The tree is rooted using the woolly monkey HBV sequence as an outgroup. Reproduced from Starkman SE, MacDonald DM, Lewis JCM, Holmes EC, and Simmonds P (2003) Geographic and species association of hepatitis B virus genotypes in non-human primates. *Virology* 314: 381–393, with permission from Elsevier.

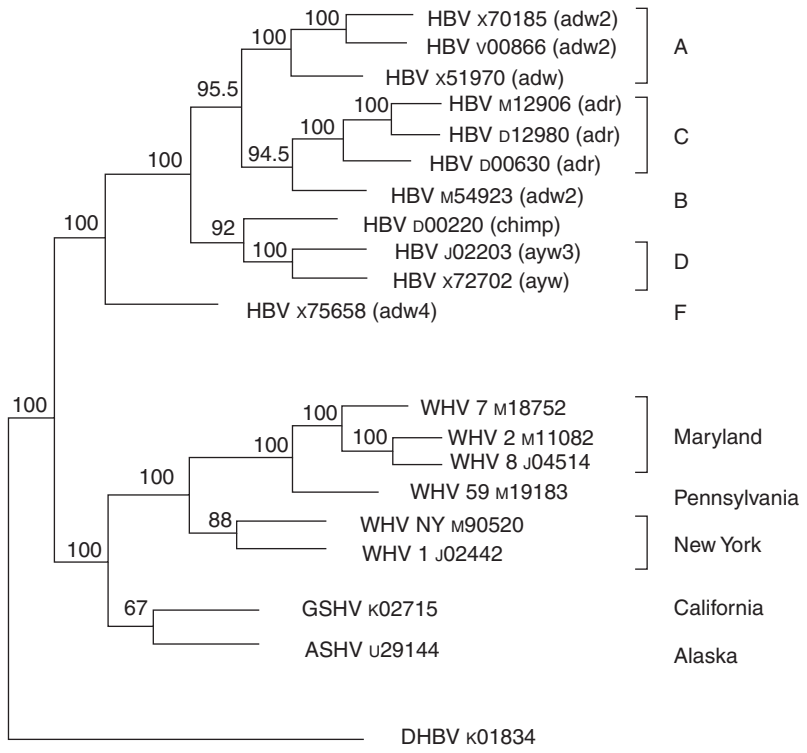


Figure 4 Dendrogram showing the relatedness of HBV and the rodent hepadnaviruses, rooted using DHBV as an outgroup. Reproduced from Testut P, Renard CA, Terradillos O, *et al.* (1996) A new hepadnavirus endemic in arctic ground squirrels in Alaska. *Journal of Virology* 70: 4210–4219, with permission from American Society for Microbiology.

Some of these viruses may originate from species of birds other than those from which they were first isolated, and it seems quite possible that many other varieties of avian hepadnaviruses remain to be described.

Clinical Features and Pathology

The clinical features of acute viral hepatitis in humans are nonspecific and are not dependent on the etiology of the infection; they include fatigue, anorexia, myalgia, and malaise. Jaundice may be evident in the more severe cases, but often the infections may be anicteric (without jaundice) or even asymptomatic. In hepatitis B, these clinical features are evidence of a robust immune response to the virus and a sign that the infection will be cleared by the immune system. In a minority of cases, less than 5% of immune competent adults, asymptomatic infections persist in individuals who do not mount a vigorous immune response. Such persistent infections, originally termed the chronic carrier state, are defined formally by the persistence of HBsAg in serum for more than 6 months.

When a persistently infected woman gives birth, she will almost invariably pass on the virus to her infant. HBV normally does not cross the placenta and transmission is believed to occur during or immediately after the birth process. Infants who are infected perinatally have a very high probability (>90%) of becoming persistently infected – they are extremely immune tolerant of the virus and the infection may persist even for life. A soluble protein, HBeAg, which is related to the nucleocapsid protein, is secreted from the infected hepatocytes and may cross the placenta and induce tolerance in the foetus. A protein equivalent to HBeAg is made by all of the hepadnaviruses, including the avian viruses.

HBV infections of children up to the age of 5 also are much more likely to progress to chronicity than those in adults. Thus, in regions of the world where HBV is highly endemic, and more than 8% of individuals are persistently infected, the virus is maintained in the population by mother-to-infant transmission and horizontal transmission from persistently infected young children to their peers. These regions include sub-Saharan Africa, China, and Southeast Asia (**Figure 5**). Areas of intermediate endemicity (2–7% of individuals persistently infected) include North Africa, Eastern Europe, and northern Asia. In areas of low endemicity, such as North America and Western Europe, important routes of transmission include sexual contact and parenteral exposure.

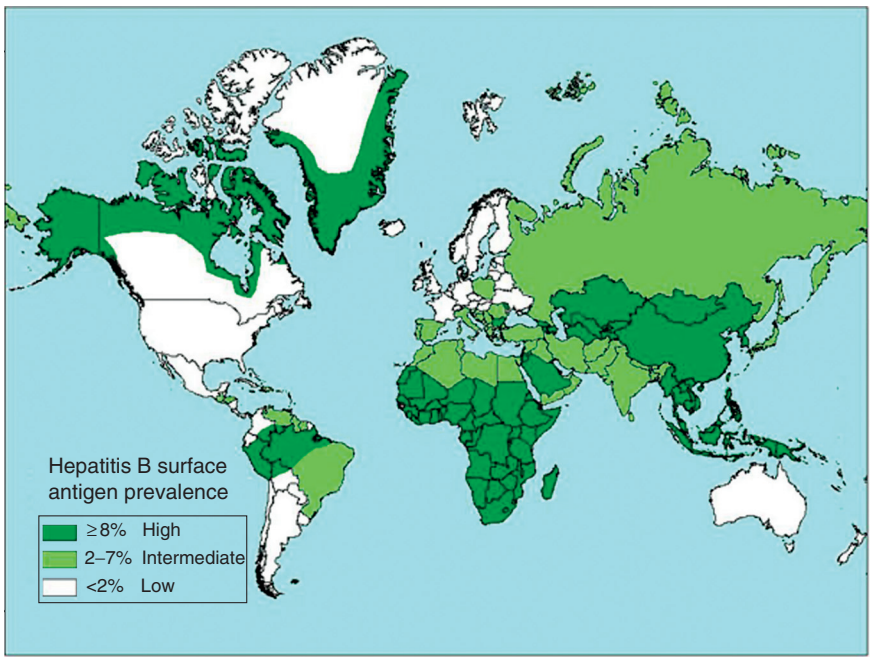


Figure 5 Global prevalence of persistent HBV infection (HBsAg in serum). Reproduced from http://www.cdc.gov/ncidod/diseases/hepatitis/slideset/hep_b/slide_9.htm, with permission from Central Food Technological Research Institute.

HBV seems not to be cytopathic and, in immune tolerant individuals, massive amounts of virus may be produced over long periods of time with little damage to the liver. However, such replication leads to the accumulation of hepatocytes with integrated (partial) copies of the viral genome and these seem to be at high risk of becoming neoplastic. Integration is not part of the HBV life cycle and seems to be a dead-end for the virus, but may be detected in almost all HBV-associated primary liver tumors.

In persistent infections, tolerance may break down over time, particularly with a cell-mediated immune response to the virus. Liver damage is attributable especially to the lysis of infected hepatocytes by cytotoxic T cells and peptides derived from the core (C) ORF seem to be a major target. In the long term, chronic active hepatitis may lead to the development of fibrosis, cirrhosis, and ultimately, end-stage liver failure. Cirrhosis also is a high risk for the development of primary liver cancer (hepatocellular carcinoma, HCC). However, almost all HCCs arising in HBV-infected livers contain chromosomally integrated HBV DNA and, in contrast to hepatitis C, in hepatitis B HCC may develop in the absence of cirrhosis and even on a background of almost normal liver histology.

The rodent hepadnaviruses can also become persistent in their natural hosts and chronic hepatitis, cirrhosis, and HCC may ensue. On the other hand, DHBV infection of ducks results in very little pathology, the infection may be passed vertically through the egg, and hatchlings are extremely immune tolerant of the virus. The avian and rodent systems have been studied experimentally. DHBV in its natural host has been especially useful. As noted above, the DHBV system had an important role in the elucidation of the mode of replication of the viral DNA. It has also been valuable for testing nucleoside and nucleotide analogs for potential therapeutic use. Despite their closer relatedness to humans, woodchucks are not best suited to experimentation; they are large, hibernate in the winter, and wild-caught animals have many parasites. Nevertheless, these animals have been used in various studies, including investigations of the actions of antiviral agents, the immune response in chronic infection, and the development of HCC.

Prevention and Treatment of Infection

HBV infection can be prevented by immunization, intramuscular administration of HBsAg leading to a protective anti-HBs response. The first vaccine (so-called plasma-derived vaccine) was produced by purifying HBsAg from the plasma of hepatitis B carriers. Second-generation vaccines contain HBsAg produced from yeast or (less commonly) mammalian cells by recombinant DNA technology. The World Health Organization recommends universal immunization of infants and this is now carried out in many countries. The key is to break the chain of transmission from infected mothers to their infants, and

giving the vaccine within 24 h of birth protects 70–90% of such babies. Protective efficacy may be increased by giving passive protection with hepatitis B immune globulin (HBIG) at a contralateral site. In Taiwan, one of the first countries with a high prevalence of HBsAg to introduce universal immunization of infants, that prevalence has been reduced from more than 10% to less than 1% in the immunized cohort. Furthermore, although HCC is very rare in children, the incidence of childhood HCC has been reduced and all indications are that universal immunization against hepatitis B will, in time, lead to a marked decrease the incidence of HCC worldwide.

Prior to the introduction of nucleoside and nucleotide analogs, persistent HBV infection was treated most often by the administration of interferon. Up to 30% of individuals so treated cleared the virus. Interferon seemed to work by upregulating the cytotoxic T-cell response to the virus, rather than by a direct antiviral action. Responses were notably poor in patients from China and the Far East, presumably because these individuals had been infected early in life and were very immune tolerant.

As noted above, HBV replicates via reverse transcription of an RNA pregenome, and some of the nucleoside and nucleotide analogs introduced for the treatment of human immunodeficiency virus (HIV) infection are also active against HBV. The first to be licensed for such use was lamivudine (3TC); monotherapy reduces the viral load considerably but resistance develops in around 15% of treated patients per year. Resistance parallels that seen in HIV, with mutations affecting the 'YMDD' motif in the active site of the polymerase. Resistance to adefovir dipivoxil arises less frequently and the drug is active against lamivudine-resistant HBV. Other drugs also have been licensed but regimens of combined therapy are less well developed than those used for HIV. It should be noted also that, while such treatments reduce the viral load considerably, they rarely result in complete clearance of the infection.

Hepatitis D Virus

HDV merits a brief mention because of its particular association with HBV. As noted above, a novel antigen, termed delta, was discovered in patients with hepatitis B and this turned out to be the nucleocapsid protein of HDV. The HDV genome is a single-stranded circle of RNA that resembles the viroids and virusoids of plants; it is believed to be replicated by the host RNA polymerase II, with cleavage and rejoining of the circle mediated by a ribozyme activity. Unlike the viroids and virusoids, the RNA contains (in the antigenomic sense) an ORF, encoding the delta antigen. HDV requires HBsAg for envelopment and exocytosis, and for subsequent binding and entry to the target hepatocyte. HDV has been transmitted experimentally to WHV-infected woodchucks and is there enveloped by WHsAg.

HDV was first discovered in Italy and is found in the Mediterranean area, reportedly with a declining prevalence, and also in the Far East and South America. The virus may be acquired as a coinfection with HBV or by super-infection of someone already HBsAg-positive. In both cases, disease may be more severe than with HBV alone, and chronic delta hepatitis may progress to cirrhosis more frequently, and more rapidly, than chronic hepatitis B. The hepatitis B vaccine also protects against coinfection but there is no licensed vaccine to protect hepatitis B carriers against HDV infection.

See also: Hepatitis B Virus: General Features; Hepatitis B Virus: Molecular Biology; Hepadnaviruses of Birds.

Further Reading

Cougot D, Neuveut C, and Buendia MA (2005) HBV induced carcinogenesis. *Journal of Clinical Virology* 34(supplement 1): S75–S78.

Harrison TJ (2006) Hepatitis B virus: Molecular virology and common mutants. *Seminars in Liver Disease* 26: 87–96.

Kidd-Ljunggren K, Miyakawa Y, Kidd AH, *et al.* (2002) Genetic variability in hepatitis B viruses. *Journal of General Virology* 83: 1267–1280.

Lavanchy D (2005) Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. *Journal of Clinical Virology* 34(supplement 1): S1–S3.

McMahon BJ (2005) Epidemiology and natural history of hepatitis B. *Seminars in Liver Disease* 25(supplement 1): 3–8.

Norder H, Courouce A-M, Coursaget P, *et al.* (2004) Genetic diversity of hepatitis B virus strains derived worldwide: Genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 47: 289–309.

Prassolov A, Hohenberg H, Kalinina T, *et al.* (2003) New hepatitis B virus of cranes that has an unexpected broad host range. *Journal of Virology* 77: 1964–1976.

Schultz U, Grgacic E, and Nassal M (2004) Duck hepatitis B virus: An invaluable model system for HBV infection. *Advances in Virus Research* 63: 1–70.

Starkman SE, MacDonald DM, Lewis JCM, Holmes EC, and Simmonds P (2003) Geographic and species association of hepatitis B virus genotypes in non-human primates. *Virology* 314: 381–393.

Taylor JM (2006) Hepatitis delta virus. *Virology* 344: 71–76.

Tennant BC, Toshkov IA, Peek SF, *et al.* (2004) Hepatocellular carcinoma in the woodchuck mode of hepatitis B virus infection. *Gastroenterology* 127: S283–S293.

Testut P, Renard CA, Terradillos O, *et al.* (1996) A new hepadnavirus endemic in arctic ground squirrels in Alaska. *Journal of Virology* 70: 4210–4219.

Zoulim F (2006) Antiviral therapy of chronic hepatitis B. *Antiviral Research* 71: 206–215.

Hepatitis B virus, Division of Viral Hepatitis, Centers for Disease Control and Prevention. http://www.cdc.gov/ncidod/diseases/hepatitis/slideset/hep_b/slide_9.htm (accessed August 2007).

Hepatitis A Virus

A Dotzauer, University of Bremen, Bremen, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Antibody prevalence The percentage of a population with antibodies against a certain disease at a given time.
Aplastic anemia Disease characterized by a decrease in blood cells resulting from underproduction due to bone marrow failure; also called hypoplastic anemia.
Incidence The rate of occurrence of new cases of a particular disease in a population in a certain period of time.
Polyprotein processing Cascade of proteolytic cleavage events resulting in release of mature proteins from the polyprotein.
Serine-like protease Proteolytic enzyme characterized by a catalytic triad similar to that in serine-type proteases with Ser, His, and Asp.

History

Jaundice has been known as an epidemic disease for centuries, but the earliest outbreaks of what was almost certainly hepatitis A were documented by Rayger, occurring in Preßburg, now Slovakia, in 1674 and 1697. Besides sporadic occurrence of the disease, characterized by a slow increase and slow decrease of the number of infected persons in the course of months (spread by person-to-person contact), with an overall small number of infections, larger vehement epidemics (spread by contaminated water and food) were reported in later times. The first pandemic wave occurred in the 1860s and the first considerable record of the disease was registered during the American Civil War.

In the second half of the nineteenth century, the disease became known as 'icterus catarrhalis' (catarrhal jaundice; inflammation of the biliary tract was supposed) or 'icterus epidemicus' (epidemic jaundice) as well as campaign jaundice, as epidemics are common in military medical history.

At the turn of the nineteenth to the twentieth century, the disease was recognized as infectious and transmissible by person-to-person contact. The terms 'hepatitis epidemica' (epidemic hepatitis) and 'hepatitis infectiosa' (infectious hepatitis) were introduced as synonyms for catarrhal jaundice.

Detailed epidemiologic recordings have been conducted since the beginning of the twentieth century. Two large pandemic waves were observed during the twentieth century, the first one originating during World War I and reaching its summit between 1918 and 1922, and the second one originating in the early 1930s reaching its widest distribution with the beginning of World War II.

During World War II, hepatitis had been demonstrated to be caused by at least two separate filterable agents, and the resulting diseases were called hepatitis A (infectious hepatitis) and B (serum hepatitis), and the etiological agents hepatitis A virus (HAV) and hepatitis B virus (HBV), respectively.

After World War II, epidemiologic studies in human volunteers showed that hepatitis A is spread by the fecal–oral route, and provided information on the duration of viremia and shedding of virus in feces. In the late 1960s and early 1970s, it was shown that marmoset monkeys and chimpanzees could serve as animal models for human hepatitis A, and replication of HAV in cell cultures was established between 1979 and 1981.

The etiologic agent was identified through immune electron microscopy by Feinstone *et al.* in 1973.

The molecular cloning of the HAV genome in the 1980s revealed that the genomic organization of HAV is similar to that of picornaviruses, and the first infectious cDNA clone of HAV was reported by Cohen *et al.* in 1987.

The disease manifestations by hepatocellular destruction could be attributed to an immunopathogenic mechanism in the late 1980s by Vallbracht *et al.*

An inactivated vaccine has been available since 1992.

Taxonomy

HAV is the only member of the genus *Hepatovirus* within the family *Picornaviridae.*

All human HAV strains known belong to only one serological group, but phylogenetic analysis of the VP1–2A junction region and the VP1 coding region, respectively, revealed that several distinct HAV genotypes (seven in the case of VP1–2A junction analysis, five in the case of VP1 region analysis), which include several subgenotypes, can be distinguished by the degree in their genetic heterogeneity. These genotypes correlate with the geographic origin of the virus isolates. Genotype I is the most common type worldwide, particularly genotype IA. The cell culture-adapted viruses most commonly used are variants of the Australian strain HM175 (genotype IB) and the German strain GBM (genotype IA).

Morphology and Physicochemical Properties

The infectious spherical virion is a nonenveloped particle with a diameter of *c.* 27 nm. The icosahedral capsid, which embodies the viral RNA genome, contains 60 copies of each of the three major proteins, VP1 (also known as 1D), VP2 (1B), and VP3 (1C) (see **Table 1**). It is not known whether the small protein VP4 (1A) is integrated into the capsid. The mature HAV virion seems to have a different structure than other picornaviruses, as a usually prominent feature of picornaviruses and one which represents the viral attachment site to cellular receptors, the canyon surrounding the fivefold symmetry axes, is obviously missing.

The HAV particle has a buoyant density of *c.* 1.33 g cm^{-3} in CsCl and a sedimentation coefficient of *c.* 160S. Empty capsids found in feces have a sedimentation constant of about 70S. HAV is extremely resistant to acid (pH 1.0 for 2 h at room temperature) and thermal inactivation (60 °C for 1 h).

Genome Organization and Expression, Replication, Morphogenesis

The linear, single-stranded, positive-sense RNA genome of HAV is ~7500 nt in length and is not capped but covalently linked by a tyrosine-O^4-phosphodiester bond to the 2.5 kDa viral protein 3B (also known as VPg; see **Table 1**). It encompasses a structurally complex 5′ nontranslated region (NTR) of ~740 bases, followed by a single open reading frame encoding a polyprotein (*c.* 250 kDa; ~2230 amino acids) and a 3′ NTR of ~60 bases which terminates with a poly(A) tail of about 60 nt. In the polyprotein, the capsid proteins and those with functions during virion assembly represent the N-terminal third (VP4, VP2, VP3, VP1, also known as P1 region, and 2A) (see **Table 1** and **Figure 1**) with the remainder of the polyprotein comprising a series of proteins required for RNA replication (2B and 2C followed by 3A to 3D) (see **Table 1** and **Figure 1**).

The viral polyprotein is translated directly from the messenger-sense genomic RNA, which is released into the cellular cytoplasm after uncoating of the virion. An internal ribosomal entry site (IRES) located within the 5′ NTR (see **Table 1**) is involved in the cap-independent initiation of protein synthesis. The IRES of HAV differs from that of other picornaviruses and forms its own group (type III picornavirus IRES). Translation from the HAV IRES requires all of the initiation proteins, including eIF4E and intact eIF4G, and infection with HAV does not result in cleavage of the translation initiation factor eIF4G to block cap-dependent host protein synthesis, as featured by other picornaviruses. The HAV IRES directed initiation of translation, which depends on the entire 5′ NTR, is enhanced by sequences of the 5′ terminal coding region and by the cellular protein poly(A) binding protein (PABP), which mediates circularization of the template RNA and seems to be necessary for HAV protein translation. Involvement of the host cells poly(C) binding protein 2 (PCBP2), polypyrimidine tract binding protein (PTB), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is described, but the functional significance of these interactions is uncertain.

Proteolytic processing of the primary polyprotein (see **Figure 1**) occurs simultaneously with translation and is largely carried out by the viral 3C protease, which is a serine-like protease in which cysteine replaces the nucleophilic serine in the catalytic triad of the active center and for which, in contrast to other picornaviruses, no additional cellular substrate has been described so far.

Synthesis of viral RNA by the viral 3D polymerase follows the accumulation of the nonstructural proteins spanning 2B to 3D, which induce the assembly of a macromolecular replication complex on membranes that are recruited from the endoplasmatic reticulum. The proteins 2B, 2C, and 3A may be involved in the structural rearrangements of the intracellular membranes. RNA transcription is most likely protein-primed, with the uridylylated VPg protein 3B representing the primer for the negative-sense RNA replication intermediate and the subsequent positve-sense genomic RNA synthesis. A participation of the 5′ terminal NTR structures in the switch from translation to replication on the same viral RNA is suggested.

HAV morphogenesis is poorly understood. The primary polyprotein cleavage event occurs at the 2A/2B junction mediated by the 3C protease resulting in the structural precursor P1–2A. The steps then resulting in particle formation are not entirely clear, but the following model is suggested (see **Figure 1**). The P1–2A structural precursors assemble to a pentameric structure and are further cleaved by the 3C protease to generate the precursors VP4–VP2 (also known as VP0) and VP1–2A, as well as VP3 resulting in the structural building block $(VP0, VP3, VP1–2A)_5$. The 2A C-terminal extension of VP1 is a critical structural intermediate in virion morphogenesis, maybe clamping the pentamer at the fivefold symmetry axis. After assembly of 12 such pentamers with the genomic RNA to provirions ($12 \times (VP0, VP3, VP1–2A)_5$-RNA), two subsequent maturation cleavage events occur. First, the cleavage at the VP1/2A junction leading to the removal of 2A seems to result from the action of an unknown host protease and, second, VP0 is cleaved into VP4 and VP2 by an unknown mechanism. 2A has never been identified in infected cells, which may indicate an extracellular cleavage event for the VP1–2A processing, and the role of VP4 in virion morphogenesis is mysterious. While the HAV polyprotein appears to

Table 1 HAV regulatory genomic regions and HAV proteins; all numbering refers to HAV strain HM175 (accession no. NC_001489)

Regions/proteins	*Nucleotide position*	*Length in amino acids*	*Proposed or known function*	*Remarks*
5′ nontranslated region (5′ NTR)	1–734		5′ stem–loop region (nucleotides 1–94): required for RNA replication	Mechanisms regulating switch from translation to replication are not clear
			Polypyrimidine tract pY1 (nucleotides 99–138): unknown function	
			Internal ribosomal entry site (IRES) (nucleotides 152–734): directs cap-independent initiation of translation	Picornaviral type III IRES
3′ nontranslated region (3′ NTR)	7416–7478		Involved in regulation of RNA replication	
			Extended by 40–80 adenylates, which are structurally involved in translation (closed-loop model)	
1A	735–803	23	VP4	Not myristoylated
			Morphogenesis	So far not identified in mature virions
1B	804–1469	222	Capsid protein VP2	During morphogenesis, precursor VP4–VP2 (VP0), presumably cleaved autocatalytically
1C	1470–2207	246	Capsid protein VP3	
1D	2208–3023/ 3026/ 3029	272 273 274	Capsid protein VP1	Heterogeneous C-terminus, presumably depending on HAV strain and host cell
				During morphogenesis, precursor VP1–2A, presumably cleaved by cellular protease
2A	3024/3027/ 3030–3242	71/72/73	Morphogenesis	So far not identified in infected cells
2B	3243–3995	251	Structural rearrangements of intracellular membranes for replication	Peripheral association with membranes
				Influence on membrane permeability
				Mutations accompany adaptation to growth in cell culture
2C	3996–5000	335	Structural rearrangements of intracellular membranes for replication	Integral association with membranes
				Mutations accompany adaptation to growth in cell culture
3A	5001–5222	74	As 3AB VPg precursor (pre-VPg)	Interacts with membranes, may anchor pre-VPg to cellular membranes through a central region of 21 hydrophobic amino acids
3B	5223–5291	23	VPg (virus protein genomic)	Tyr^{3} attached to 5′ terminus of genomic RNA
			Supposed protein primer for RNA replication	
3C	5292–5948	219	Sole protease	Serine-like protease, with replacement of Ser by Cys in catalytic triad: Cys^{172}, His^{44}, Asp^{84}
3D	5949–7415	489	RNA-dependent RNA polymerase	

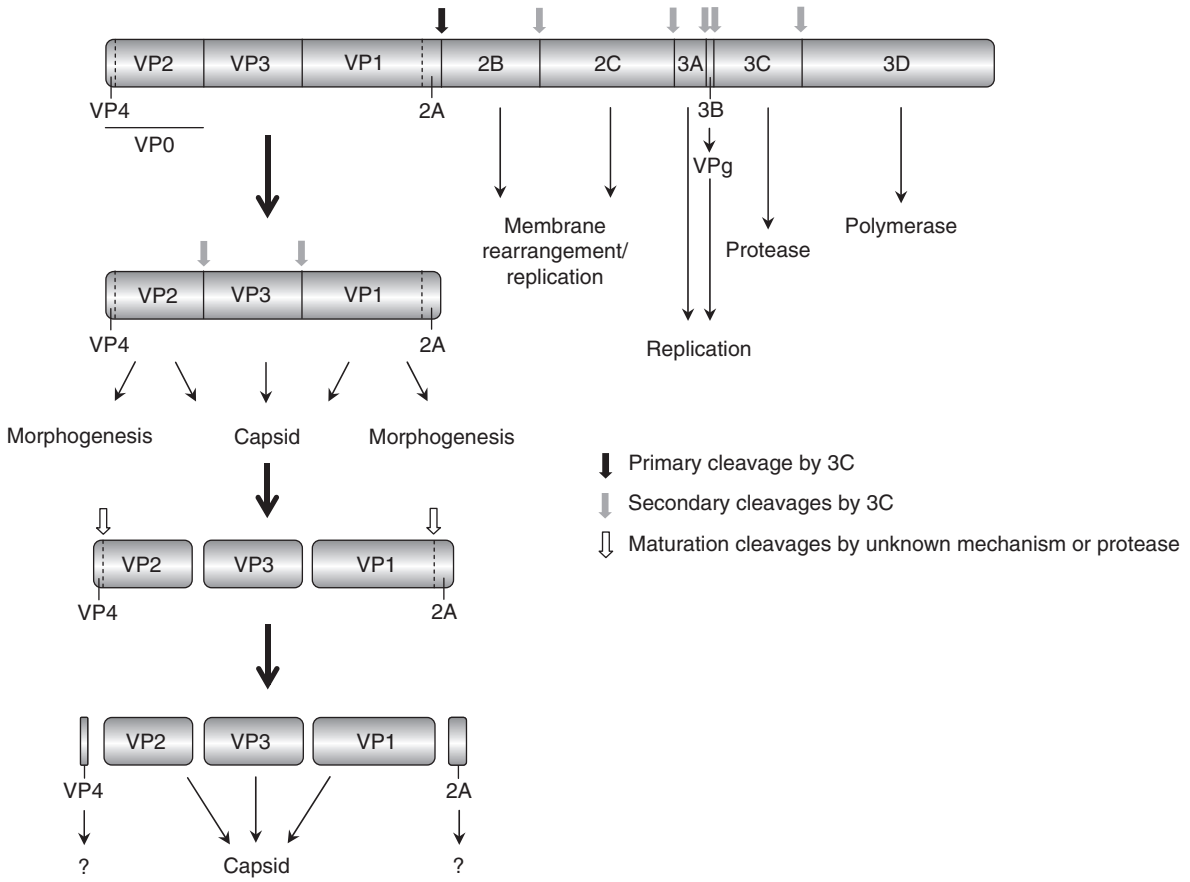


Figure 1 HAV polyprotein processing and known or supposed functions of the viral proteins during morphogenesis and replication. The polyprotein that results from IRES-directed translation is cleaved by the viral protease 3C to release certain precursor proteins with biologically relevant functions as well as the mature nonstructural proteins. Primary cleavage results in release of the structural precursor VP4–VP2–VP3–VP1–2A, which associate to assembly intermediates (pentamers), which are stabilized by 3C cleavage of the VP3 junctions (VP4–VP2 (VP0), VP3, VP1–2A)$_5$. After assembly of the intermediate protein building blocks and the viral genome to provirions, maturation cleavages occur through an unknown proteolytic activity to release VP4 from VP2 and by a so far unknown cellular protease to release 2A from VP1, resulting in the infective virion with 60 copies of each of the main structural proteins VP1, VP2, and VP3.

possess the short VP4 segment at its N-terminus, this putative VP4 moiety has never been identified in virions. Moreover, the HAV-VP4 sequence does not contain a myristoylation signal, which is important for the morphogenesis of other picornaviruses.

Host Range, Transmission, and Tissue Tropism

Under natural conditions, only humans and certain species of nonhuman primates seem to be susceptible to HAV. These primates, which are also used as animal models, include chimpanzees, marmosets, and owl monkeys.

HAV is transmitted via the fecal–oral route. As the virus is excreted in feces, it is typically acquired by ingestion of feces-contaminated food or water. Direct person-to-person spread occurs under poor hygienic conditions.

The site of replication is the liver. The events that occur during the passage of HAV across the intestinal epithelium into blood, in which the virus reaches the liver, are not clearly understood. Although HAV antigen and the T-cell immunoglobulin mucin 1 (TIM1; function so far unknown) protein, which has been identified as a cell surface protein binding HAV (HAV_{cr1}), could be detected in different organs, such as kidney, spleen, and gastrointestinal tract, no extrahepatic sites of HAV replication have been clearly identified. Furthermore, it was demonstrated that infection of polarized intestinal cells does not result in penetration of the epithelium. A functional cellular receptor, whose selective expression in the liver is assumed, could not be identified so far. Some studies suggest that the hepatotropism of HAV may be supported by immunoglobulin A (IgA)-virus complexes (HAV/IgA), as HAV/IgA uptake via the hepatocellular asialoglycoprotein receptor (ASGPR) results in infection of hepatocytes (IgA-carrier hypothesis).

The virus progeny produced in the liver is then released back into the intestinal tract via bile.

Cell Culture and Growth Characteristics

HAV can infect a variety of primate and nonprimate cell lines, including nonhepatic cells, *in vitro*. The virus exhibits a protracted replication cycle and normally establishes a noncytolytic, persistent infection with low virus yields, and there is no evidence that HAV notably interferes with the macromolecular synthesis of its host cell. After infection of cultivated cells with wild-type virus, a minimum of 8 weeks elapses before HAV can be isolated. Although a more rapid replication and higher virus titers are obtained after serial virus passages in cultivated cells resulting in cell culture-adapted viruses, even the replication of these virus variants is not detectable within the first few days after infection. Adaptation of HAV to growth in cultivated cells seems to be achieved by varying sets of multiple interacting mutations, with adaptive mutations within the IRES enhancing viral translation in a cell-type-specific fashion, and mutations clustering in the 2B and 2C proteins (see **Table 1**) increasing replication regardless of the cell line used.

The virus apparently downregulates its own replication and this may, for example by supporting the ability of HAV to inhibit innate cellular antiviral defense mechanisms, be important for the establishment of persistent infections. A large proportion of the virus progeny remain cell associated, but extensive release of HAV from the cells also occurs, caused by an unknown mechanism.

Several cytopathogenic variants of HAV have been isolated which induce apoptosis resulting in cell death. These variants are highly cell culture adapted and characterized by a rapid replication phenotype and high virus yields. In these variants, both the downregulation of viral replication and the ability to inhibit the innate defense mechanisms are less effective. The molecular mechanisms resulting in apoptosis are not known.

Clinical Features and Pathology

Infections with HAV may produce a wide spectrum of manifestations ranging from silent infections, over icteric courses to fatal fulminant hepatitis. The acute icteric course of infection varies between common, over prolonged to relapsing hepatitis A. Persistent infections or chronic disease have not been described.

The clinical presentation of the disease depends on the age of infection. The likelihood of having symptoms and the severity of the disease increases with the age of the patient. Inapparent infections (asymptomatic or at least anicteric) are normally observed in very young children, under the age of 2. However, clinically obvious disease can occur even in infancy (aged 2 weeks to 8 months) and may be characterized by prolonged courses. In children under 5 years of age *c.* 3%, in children 5–15 years old *c.* 30%, and in individuals over the age of 18 years as many as 70% may develop a clinically apparent disease.

Common Course of Infection

The incubation period ranges from 2 to 6 weeks with a mean duration of 4 weeks. The prodromal (preicteric) period of normally 4–6 days (which may vary from 1 day to more than 2 weeks) is characterized by nonspecific symptoms, like anorexia, nausea with vomiting, malaise, abdominal pain, loss of appetite, accelerated pulse, rash, headache, and fever (38–39 °C) as well as by gastrointestinal symptoms, normally in form of obstipation, but diarrhea is also observed.

The prodromal symptoms disappear with the onset of jaundice, which is seldom abrupt (in 15% of the cases no obvious prodrome is observed before appearance of jaundice). The icteric phase, which ranges from 2 to >22 days (mean duration 3 weeks), is marked by jaundice, which may start with scleral icterus, dark beer-colored urine (conjugated bilirubinuria), clay-colored stool, and clearly decelerated pulse.

The reconvalescence period ranges from 3 to 6 weeks, but fatigue, dullness, right upper quadrant tenderness, and fast exhaustion may remain for 2–4 months. In almost all cases the liver is enlarged.

The clinical symptoms are accompanied by several biochemical parameters (see **Figure 2**). Elevation of aminotransferase levels in serum (alanine aminotransferse (ALT) and to a lesser degree aspartate aminotransferase (AST)), which reflect hepatocellular damage with release of the liver enzymes into the circulation, roughly correlate with the severity of the disease. Elevation of serum alkaline phosphatase activity and in the serum bilirubin level relate to intrahepatic cholestasis.

At the onset of symptoms, seroconversion to anti-HAV occurs.

Large amounts of HAV, which are produced in the liver and released into the gastrointestinal tract via bile, already occur in the feces during the late incubation period when no clinical symptoms are observable and are shed for approximately three weeks until a few days after the onset of elevated levels of liver enzymes in the serum. Fecal shedding of HAV reaches its maximum just before the onset of hepatocellular injury.

Viremia occurs a few days before and during the early acute stage of the clinical and biochemical hepatitis, in which it roughly parallels the shedding of virus in feces, but at a lower magnitude.

More sensitive methods (especially nucleic acid amplification technologies) demonstrated that low levels of viral RNA may be present in feces and blood for many weeks.

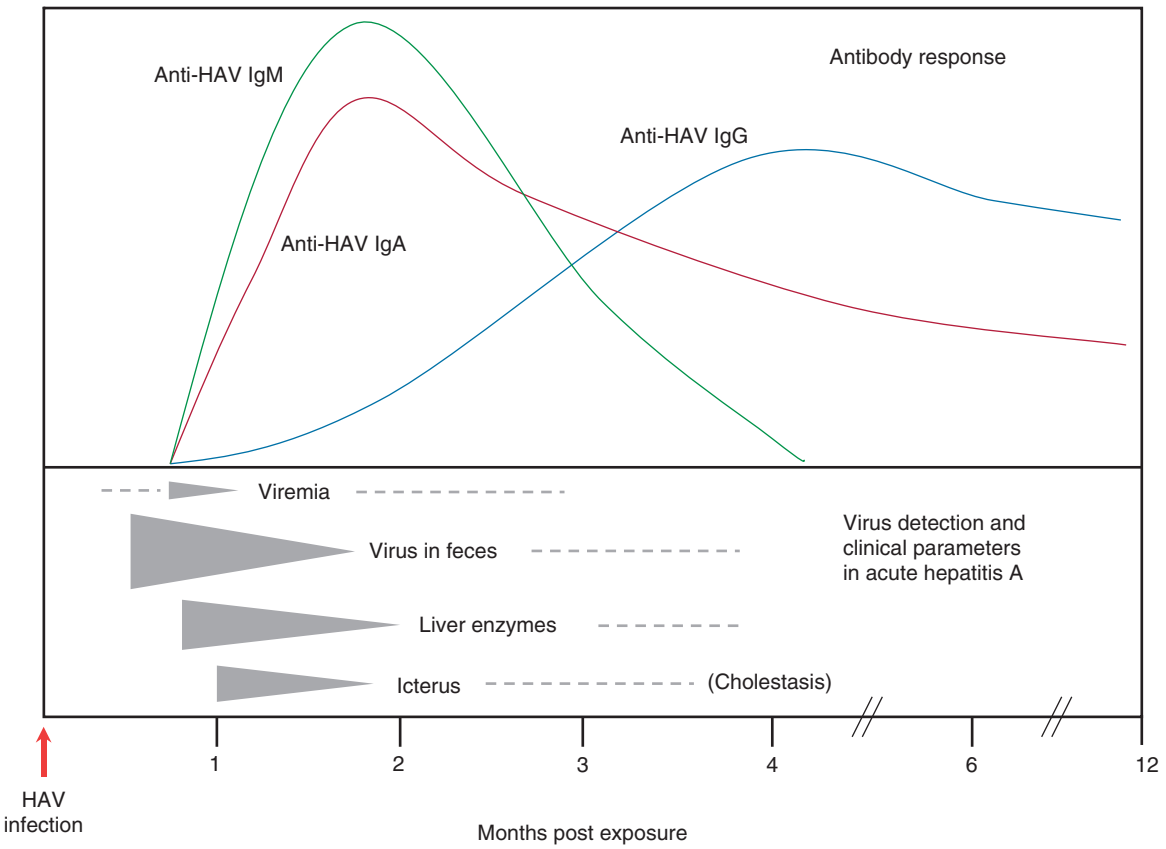


Figure 2 Course of clinically relevant events in acute hepatitis A. This figure schematically shows the mean duration and intensity of certain parameters. The dotted lines indicate that the duration and intensity of the events may vary.

Prolonged Hepatitis A

In 8.5–15% of the cases, jaundice lasts for up to 17 weeks. The biochemical abnormalities are resolved by 5 months. Occasionally, prolonged courses are accompanied by high serum bilirubin levels persisting for months (cholestatic hepatitis A). The cholestatic form is marked by extensive itching of the skin.

Relapsing Hepatitis A

After initial improvement in symptoms and liver test values (serum aminotransferase levels), one or more relapses of the disease (mostly biphasic) are described for up to 20% of the patients. These relapses occur between 30 and 90 days after the primary episode, when high titers of neutralizing antibodies are already present. The severity of symptoms, the biochemical abnormalities, and the immunoglobulin M (IgM) response are essentially the same as observed during the initial phase, with a tendency to greater cholestasis. The pathogenesis of relapsing hepatitis is not understood and basically two hypotheses are suggested: that the disease is a manifestation of a persistent viral infection, or, alternatively, that the relapse may represent a manifestation of an enterohepatic cycling of HAV, which may be supported by anti-HAV IgA (IgA-carrier hypothesis). But none of these hypotheses is currently supported by *in vivo* data.

Fulminant Hepatitis A

In rare cases, acute hepatitis A results in a fatal deterioration in liver function with massive destruction of liver cells. Surprisingly, no vigorous inflammatory response is observed. The fatality rate is below 1.5% of all hospitalized icteric cases. This course of the disease is accompanied with fever over 40 °C. This outcome is more frequent in adults, especially in patients over 50 years of age, than in children, and the risk is increased in patients with underlying chronic liver disease.

Extrahepatic Manifestations

Extrahepatic manifestations of the disease are rare and the etiology is uncertain. Besides frequently observed transient suppression of hematopoiesis, rare cases of aplastic anemia (pancytopenia) with a lethality rate of over 90% are described, and it was demonstrated that HAV infects monocytes and inhibits their further differentiation. In some patients, interstitial nephritis was observed. In connection

with the finding that the HAV-binding receptor HAV_{cr1} is identical with the T-cell immunoglobulin mucin 1 (TIM1), a protein suggested to play a role in asthma susceptibility, and by statistical evaluation of medical records, it was suggested that HAV exposure may leave a protective effect on the development of asthma and allergic diseases.

Pathology

The pathology of the liver in acute hepatitis A has been studied in humans and several animal models. The pathological lesions, which are caused by an immunopathogenic mechanism, are characterized by hepatocellular necrosis, which is most prominent in periportal regions, accompanied with large inflammatory infiltrates of mononuclear cells.

Innate and Adaptive Immune Response

Innate Immune Response

HAV, which does not interfere with the replication of other viruses, prevents the synthesis of beta-interferon (IFN-β), but is not resistant to alpha- and beta-interferon (IFN-α/β) exogenously added to persistently infected cells. It could be demonstrated that HAV does inhibit dsRNA-induced transcription of IFN-β by blocking effectively interferon regulatory factor 3 (IRF-3) activation due to an interaction of HAV with the mitochondrial antiviral signaling protein MAVS (also known as IPS-1, VISA, or Cardif), which is a component of the retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated gene 5 (MDA-5) signaling pathway. Signaling through the Toll-like receptor 3 (TLR-3) pathway may also be partially impaired.

HAV also has the ability to prevent apoptosis induced by accumulating dsRNA, but the underlying mechanism is not clear.

Gamma interferon (IFN-γ) produced by HAV-specific HLA-dependent cytotoxic T lymphocytes (CTLs) may contribute to the elimination of HAV infections by inducing an antiviral state in the later course of the infection.

Adaptive Immune Response

Neutralizing anti-HAV IgM antibodies are present in almost all patients at the onset of the symptoms (see **Figure 2**). These antibodies disappear in the course of 3 months, but in the case of prolonged courses IgM can be detected up to 1 year after onset of icterus. Anti-HAV IgA antibodies are also detectable at the onset of the symptoms (see **Figure 2**). This response reaches its peak titer 50 days post infection and may last for >5 years. The majority of the IgA remains as serum IgA in circulation and is not secreted into the intestinal tract as secretory IgA by the polymeric immunoglobulin receptor (pIgR) pathway. But a significant fraction of this serum IgA is released into the gastrointestinal lumen via bile by liver functions under participation of the hepatocellular IgA-specific asialoglycoprotein receptor (ASGPR). The role of IgA antibodies in the protection against HAV infections appears to be limited, and studies suggest that HAV-specific IgA may serve as a carrier molecule for a liver-directed transport of the virus, supporting the hepatotropic infection by uptake of HAV/IgA immunocomplexes via the ASGPR (IgA-carrier hypothesis). Neutralizing anti-HAV immunoglobulin G (IgG) antibodies are also detectable 3 weeks post infection for the first time, but this response develops slowly, reaching its peak titer 4 months post infection (see **Figure 2**). Anti-HAV IgG persists lifelong, although the titer may fall to undetectable levels after several decades. Neutralizing antibodies, which are effective in eliminating the virus from the blood, do recognize a conformational epitope clustered into a major, immunodominant antigenic site involving residues contributed by VP3 and VP1.

HAV-specific, HLA-restricted cytotoxic $CD8^+$ T lymphocytes (CTLs) have been detected within the liver during acute HAV infection and play prominent roles both in eliminating the virus and in causing liver injury (immunopathogenesis). Gamma interferon (IFN-γ), released by these CTLs, may stimulate HLA class I expression on hepatocytes and in the following promote upregulation of the normally low level display of antigen on liver cells. Specific T-cell epitopes have not been identified so far.

Diagnosis

Since the clinical presentation of hepatitis A cannot be distinguished from hepatitis due to the other hepatitis viruses, serologic tests or nucleic acid amplification techniques are necessary for a virus-specific diagnosis.

The routine diagnosis of acute hepatitis A is made by detection of anti-HAV IgM in the serum of patients (see **Figure 2**). A further option is the detection of virus in the feces.

In order to improve the safety of blood and blood products, blood screening with HAV-specific polymerase chain reaction (PCR) is performed, which reduces the window period of up to 3 weeks post infection during which HAV infection fails to be diagnosed by serologic assays.

Epidemiology

HAV occurs worldwide and accounts for over 1.5 million clinical cases reported annually. The seroprevalence pattern ranges from high endemicity, such as in Africa, South Asia, and Latin America, where infection normally occurs in childhood, over intermediate endemicity, such as in Eastern Europe and the northern parts of Asia, to low endemicity, such as in Western Europe and North

America, where the majority of the population remains susceptible to HAV infection. However, the epidemiology pattern is complex and continuously changing, with considerable heterogeneity among different countries. In general, the anti-HAV antibody prevalence inversely correlates with the quality of the hygienic standards, and the incidence declines in many populations through improvements in public sanitation and living conditions. These improvements result in an increase of the pool of susceptible adults, with a shift in the age of infection to older age groups, in which a more severe disease is observed, leading to an increased morbidity.

A minor seasonal distribution of HAV infections is observed, with a peak occurring during fall and winter, mentioned in almost all earlier and contemporary reports, nowadays possibly as a result of exposure during summer vacation spent in endemic countries.

At special risk for acquisition of hepatitis A are international travelers from areas of low endemicity to endemic areas, employees of child-care centers and sewage plants, gully workers, injecting drug users, homosexually active men, and persons with an increased risk of developing a fulminant disease, such as persons with chronic hepatitis C virus (HCV) infections.

The high physical stability of HAV provides a good opportunity for common-source transmission. Community-wide outbreaks are reported in association with infections of food handlers, and linked to contaminated food and drink, or uncooked clams from contaminated water. Hepatitis A is most commonly acquired by sharing the household with an infected person.

Prevention and Control

There is no specific treatment for hepatitis A. As almost all HAV infections are spread by the fecal–oral route, good personal hygiene, high-quality standards for public water supplies, and proper disposal of sanitary waste are important measures to reduce virus transmission.

Until the availability of an active prophylaxis, the disease could be prevented for up to 5 months with a certainty of 80–90% by passive immunization with pooled IgG of at least 100 IU anti-HAV. IgG is still used for postexposure prophylaxis. If administered within 2 weeks after exposure, either development of the disease is prevented or the severity of the disease as well as virus shedding is reduced.

Since 1992, inactivated vaccines for active immunization have been available. These vaccines contain purified, formalin-inactivated virions produced in cell culture, which are absorbed to an aluminum hydroxide adjuvant. They are highly immunogenic and protect against both infection and disease caused by all strains of HAV with 100% efficacy for at least 10 years, which is consistent with the finding that all human HAV strains belong to one single serotype.

Candidate live, attenuated HAV vaccines have been developed using virus adapted to growth in cell culture, but were poorly immunogenic. Nonetheless, such a vaccine has been widely used in China and appears to be capable of inducing protective levels of antibody.

See also: Hepatitis B Virus: General Features; Hepatitis C Virus; Hepatitis E Virus; Innate Immunity: Introduction.

Further Reading

Bell BP (2002) Global epidemiology of hepatitis A: Implications for control strategies. In: Margolis HS, Alter MJ, Liang JT, and Dienstag JL (eds.) *Viral Hepatitis and Liver Disease*, pp. 9–14. Atlanta: International Medical Press.

Cuthbert JA (2001) Hepatitis A: Old and new. *Clinical Microbiology Reviews* 14: 38–58.

Gerety RJ (ed.) (1984) *Hepatitis A.* London: Academic Press.

Gust ID and Feinstone SM (1988) *Hepatitis A.* Boca Raton, FL: CRC Press.

Hepatitis B Virus: General Features

P Karayiannis and H C Thomas, Imperial College London, London, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Icterus Jaundice, or yellowing of the skin and particularly the whites of the eyes, due to failure to excrete bilirubin, a bile pigment.

History

It was not until the mid-1960s that hepatitis A (HAV) and B (HBV) viruses were recognized as the causative agents for infectious and serum hepatitis, respectively. These studies, performed by Krugman and colleagues at the

Willowbrook State School for mentally handicapped children, were preceded a few years earlier by the description of the Australia antigen in the sera of patients with leukemia. The connection between Australia antigen, or hepatitis B surface antigen (HBsAg) as it is now known, and HBV became apparent in later studies performed by the teams of Prince and Blumberg. These studies set the groundwork for the subsequent serological tests for the diagnosis of HBV and allowed detailed investigations into the epidemiological and virological aspects of infection.

Electron microscopic studies in 1970 by Dane and Almeida and their colleagues led to the visualization of the infectious virion or Dane particle, and the nucleocapsid core, respectively. This was followed in the early 1970s by the characterization of the virus genome, the virion-associated proteins, and the detailed definition of the serological profiles in acute and chronic HBV infection, performed primarily by Robinson's group at Stanford. The connection between the virus and the development of hepatocellular carcinoma (HCC) followed soon after, but the absence of a cell culture system for propagation of the virus impeded the study of its molecular biology. This changed in the early 1980s with the development of genetic engineering techniques that allowed the cloning of the viral genome, the study of its protein funtions, and the unravelling of the fascinating mechanism of its replication strategy. The polymerase chain reaction (PCR) allowed the speedy amplification and sequencing of virus isolates that led to the identification of quasispecies, virus mutants, and genotypes by bioinformatic approaches. In 1987, Carman and colleagues described the molecular basis of HBe antigen negative viremia (precore stop mutation – see below) and described the first, and most common, vaccine escape variant (arginine 145) in vaccinated children born to HBV-infected mothers (see below).

The introduction of a plasma-derived vaccine, following its extensive evaluation by Smuzness and colleagues in chimpanzees, constitutes another historical landmark in HBV research. This was soon followed by the production of a recombinant vaccine, which has effectively reduced the prevalence of the infection in many countries of the world where the virus was endemic. Almost concurrently, interferons were used for the first time in the treatment of chronic hepatitis B. These remain, with the subsequently introduced nucleos(t)ide analogs, the main treatment options in order to prevent progression of chronic liver disease to cirrhosis and HCC.

Taxonomy and Classification

Hepatitis B is the prototype virus of the family *Hepadnaviridae*, a name that signifies the hepatotropism and DNA nature of the genome of its members. There are two genera within the family. The genus *Orthohepadnavirus* contains members that infect mammals, and, other than HBV, includes hepadnaviruses that infect rodents such as woodchucks (woodchuck hepatitis virus, WHV) and squirrels (70% nucleotide identity). In recent years, HBV-like isolates have also been obtained from primates such as chimpanzees, gibbons, gorillas, orangutans, and woolly monkeys. These are more closely related to HBV and may in fact represent progenitors of the human viruses (**Figure 1**). The *Avihepadnavirus* genus on the other hand contains members that infect birds such as ducks (duck hepatitis B virus, DHBV), herons, storks, and geese. Over the years, the woodchuck and duck animal models, as well as chimpanzees, which are susceptible to infection with human HBV isolates, have proved invaluable in the study of the replication of these viruses, the natural history of

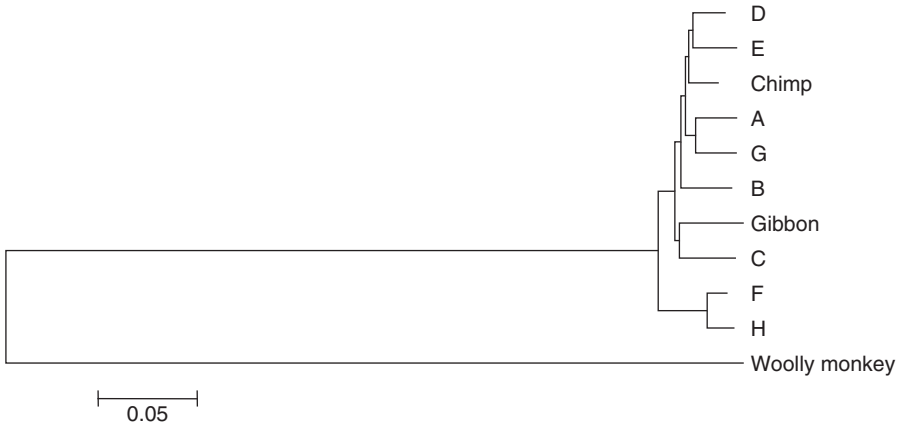


Figure 1 Phylogenetic tree based on the nucleotide sequences from the HBsAg region of all known human HBV genotypes and isolates from a chimp, gibbon, and woolly monkey. The tree was constructed using the Mega 2 software and rooted to the woolly monkey sequence.

infection, and the testing for efficacy of vaccines and antiviral drugs.

Distribution and Epidemiology

Conservative estimates place the number of persons chronically infected with HBV at over 350 million worldwide. The prevalence of HBV infection varies by geographical region, so that in northwestern Europe, North America, and Australia it is 0.1–2%. In the Mediterranean region, Eastern Europe, Middle East, Indian subcontinent, and Central and South America, it is 3–7%, while in Africa and the Far East it is 10–20%. This geographical distribution of HBV infection is mirrored by the incidence of HCC in the same regions. In areas of high endemicity, the virus is transmitted perinatally from carrier mothers to their infants, or horizontally from infected siblings and other children in early childhood. In areas of intermediate endemicity apart from perinatal transmission, household and sexual contact, as well as percutaneous exposure, are likely routes of infection. Finally, in Western countries, transmission is nowadays through sexual contact or intravenous drug use.

Virion Structure and Genome Organization

The infectious virion or Dane particle measures about 42 nm in diameter and consists of an outer envelope containing hepatitis B surface proteins (HBsAg) in a lipid bilayer (**Figure 2**). This in turn encloses the nucleocapsid core of the virus, within which lies the viral genome and a copy of its polymerase. Apart from virions, liver hepatocytes release into the circulation subviral particles devoid of nucleic acid and consisting entirely of HBsAg. These are the 22 nm spheres and the filamentous forms of similar diameter, which outnumber the virions by 100–10 000-fold.

The viral genome is a relaxed circular, partially double-stranded DNA molecule of 3.2 kbp in length, and contains four wholly or partially overlapping open reading frames (ORFs) (**Figure 3**). In addition, all regulatory elements such as enhancers, promoters, and encapsidation and replication signals lie within these ORFs. The Pre-S/S ORF encodes the three envelope glycoproteins, which are known as the large (L), middle (M), and small (S) HBsAgs, produced by differential initiation of translation at each of three in-frame initiation codons. The proteins are therefore co-terminal and the sequence of the more abundant small protein is shared by the other two. The M protein has an additional 55 amino acid residues at its N-terminus encoded by the Pre-S2 region, while the L protein includes in addition another 125 residues from the Pre-S1 region. The Pre-S1 protein is thought to contain the region responsible for the virus interaction with the hepatocyte receptor. All three proteins are glycosylated, while the L and S proteins may also be present in an unglycosylated form in particles.

The precore/core ORF contains two in-frame initiation codons and therefore yields two translation products. Initiation of translation from the first results in synthesis of the precore polypeptide, which forms the precursor of the soluble hepatitis B e antigen (HBeAg). This protein contains a signal peptide at its N-terminus that anchors the protein in the endoplasmic reticulum membrane. Cleavage by signal peptidase in the lumen is followed by further processing of the C-terminus. The resulting protein is the HBeAg, a nonstructural protein and marker of active virus replication. Moreover, the protein is thought to have a tolerogenic effect on the immune response to the virus. The nucleocapsid or core protein is synthesized following initiation of translation at the second initiation codon. The HBeAg and core proteins are translated from two separate transcripts known as the precore mRNA and the pregenomic RNA (pgRNA), respectively. The

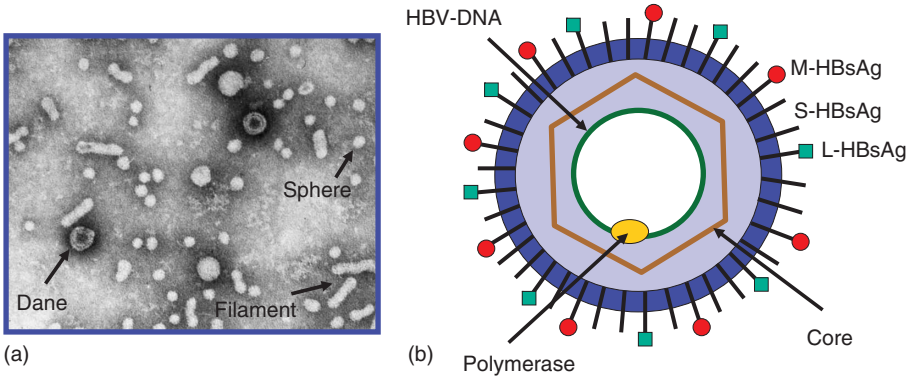


Figure 2 Structure of the hepatitis B virion. (a) Electron migrograph of HBV purified from plasma showing the infectious Dane particle and the spherical and filamentous subviral particles. (b) Cartoon of the virion and its components.

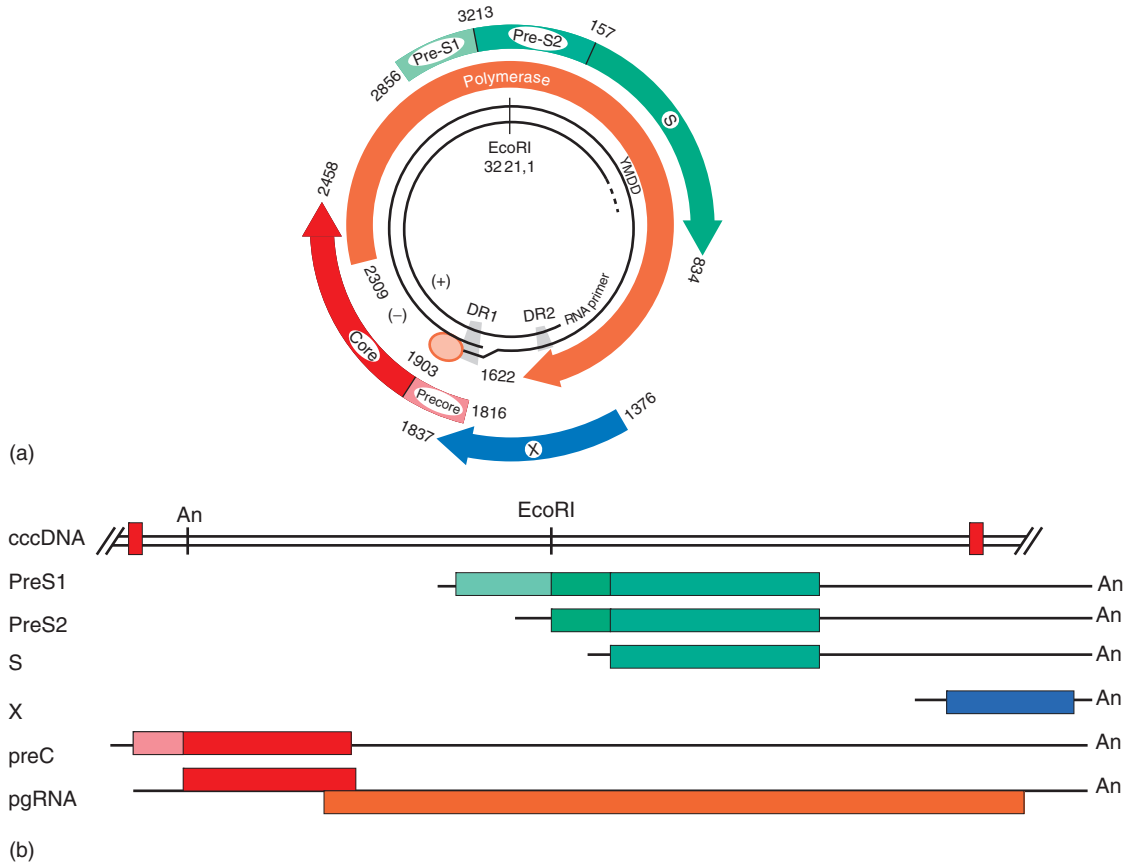


Figure 3 Genome organization of the virus (a) and the transcripts encoding the various viral proteins (b), synthesized from cccDNA. All of them terminate at the common polyadenylation site. Reproduced from Hunt CM, Mc Gill JM, Allen MI, *et al.* (2000) Clinical relevance of hepatitis B viral mutations. *Hepatology* 31: 1037–1044. American Association for Liver Diseases. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

latter also encodes the polymerase ORF of the virus, which covers almost all of the genome. The polymerase has multifunctional enzymatic actions as described below. Finally, the fourth ORF encodes for the X protein, which modulates host cell signal transduction and acts as a gene transactivator under experimental conditions.

Replication Strategy

The life cycle of the virus begins with its attachment to the appropriate hepatocyte receptor, which still remains unknown. In contrast, the region between residues 21 and 47 of Pre-S1 has long been known to be involved in virus binding to the hepatocyte membrane (**Figure 4**). The virion is internalized and uncoated in the cytosol, whence the genome translocates to the nucleus, where it is converted into a double-stranded covalently closed circular DNA (cccDNA) molecule, following completion of the shorter positive (+)-strand and repair of the nick in the negative (–)-DNA strand. The cccDNA constitutes the template for viral transcript synthesis by the host RNA polymerase II. All the transcripts terminate at a common polyadenylation signal situated within the proximal end of the core ORF, and their synthesis is controlled by individual promoters and the two enhancer elements, Enh 1 and 2.

The pgRNA is longer than genome length (3.5 kbp) and, apart from encoding the core and polymerase proteins, also forms the template for (–)-DNA strand synthesis (**Figure 5**). The polymerase has three functional domains, each one in turn involved in DNA priming (terminal protein), reverse transcription (rt), and pgRNA degradation (RNase H). There is also a spacer region of unknown function between the terminal protein and the rt domain. Once synthesized, the polymerase engages epsilon (ε), a secondary RNA structure at the 5′ end of the pgRNA, triggering encapsidation of the complex by the core protein. The subsequent steps in virus nucleic acid synthesis then take place within the nucleocapsid. Host cell factors including chaperones from the heat shock protein family are thought to be instrumental in aiding encapsidation, stabilization, and activation of the polymerase.

As pgRNA is longer than genome length, its terminal sequence duplicates the elements contained in its 5′ end

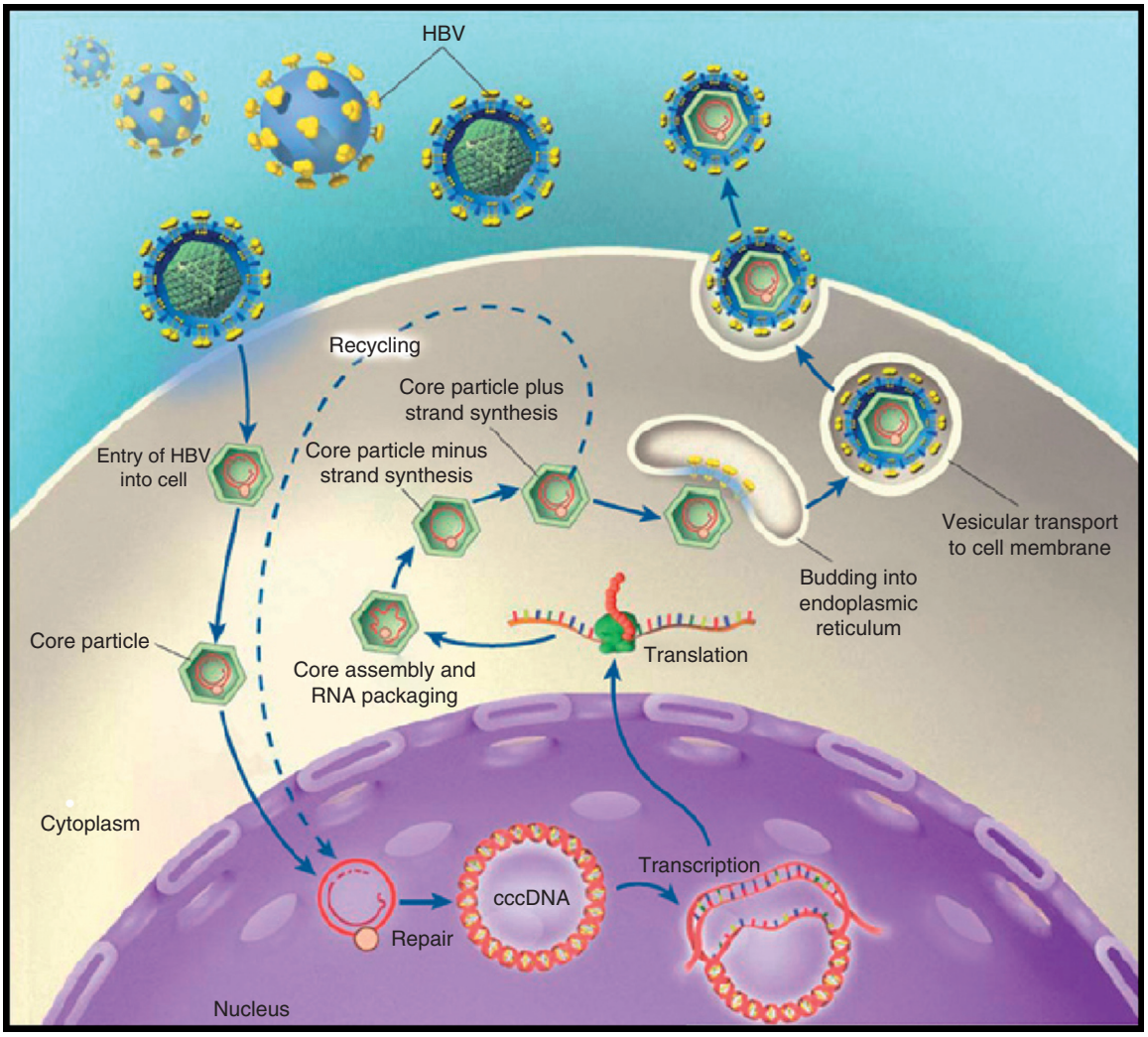


Figure 4 Diagrammatic representation of the life cycle of the virus. Reproduced from Ganem D and Prince AM (2004) Hepatitis B virus infection – Natural history and clinical consequences. *New England Journal of Medicine* 350: 1118–1129, with permission from Massachusetts Medical Society.

and includes the direct repeat 1 (DR1) and ε (**Figure 5**). The bulge of the ε structure serves as a template for the synthesis of a 3–4-nt-long DNA primer, which is covalently attached to the polymerase through a phosphodiester linkage between dGTP and the hydroxyl group of a tyrosine residue in the terminal protein (96). This event involves the ε structure at the 5′ end of the pgRNA, and is then followed by the translocation of the polymerase–primer complex to the 3′ end, where it hybridizes with the DR1 region with which it shares similarity. How this translocation occurs remains unknown. As the complex proceeds toward the 5′ end of the pgRNA, the (−)-DNA strand is synthesized by reverse transcription and the RNA template is concurrently degraded by the RNase H activity of the polymerase, except for the final 18 or so ribonucleotides. A second translocation event then occurs during which the ribonucleotide primer hybridizes with the DR1 region at the 5′ end of the newly synthesized (−)-DNA strand. A template exchange occurs that allows the (+)-DNA strand synthesis to proceed along the 5′ end of the complete (−)-DNA strand, effectively circularizing the genome. (−)- and (+)-DNA strand synthesis occurs within the nucleocapsid as already mentioned, and this is facilitated through pores allowing entry of nucleotides. Once the maturing nucleocapsid is enveloped by budding through the endoplasmic reticulum membrane, the nucleotide pool within the capsid cannot be replenished, hence the incomplete nature of the (+)-DNA strand.

Subtypes

The S protein sequence shared by all three envelope proteins contains the major immunogenic epitope of the virus referred to as the '*a*' determinant. This epitope, shared by all isolates of the virus, is recognized by

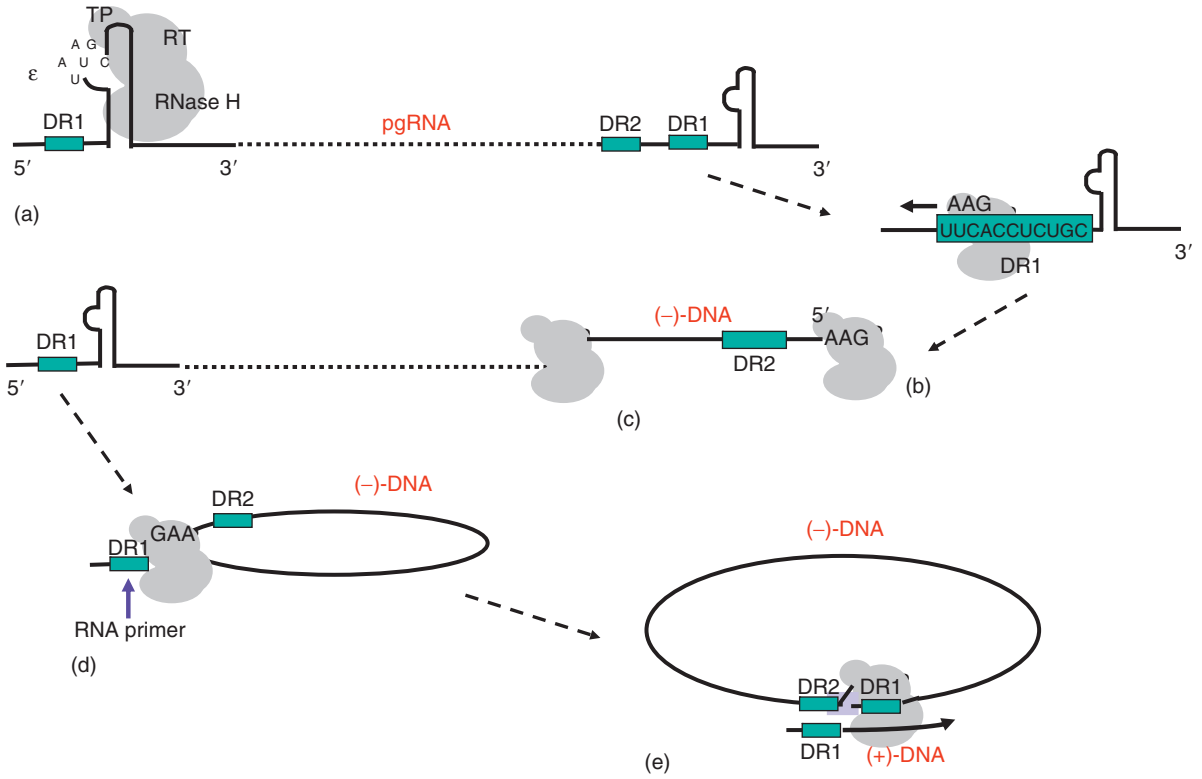


Figure 5 Replication strategy of the virus. (a) Primer synthesis; (b) translocation and binding to DR1; (c) synthesis of the (–)-DNA strand; (d) RNA primer fragment preserved from the degradation of the pgRNA; (e) (+)-DNA strand synthesis. Reproduced from Karayiannis P (2003) Hepatitis B virus: Old, new and future approaches to antiviral treatment. *Jouranl of Antimicrobial Chemotherapy*. 51: 761–785, with permission from Oxford University Press.

neutralizing antibodies and is conformational in nature, probably encompassing residues 110–160. In addition, there are subtypic specificities originally detected by antibodies. The presence of lysine (K) or arginine (R) at position 122 confers *d* or *y* specificity, respectively. Similarly, specificities *w* and *r* are conferred by the presence of K or R at position 160. Moreover, the *w* subdeterminant can be further divided into *w*1–*w*4 specificities. There are nine subtypes of the virus (**Table 1**) depending on the presence of other subtype-determining residues elsewhere in the '*a*' determinant region.

Genotypes

Nucleotide sequencing studies soon established that the virus could be divided into genotypes based on sequence divergence of >8% (**Table 1**). There are currently eight genotypes, designated A–H, with characteristic geographical distribution. Genotypes A and D occur frequently in Africa, Europe, and India, while genotypes B and C are prevalent in Asia. Genotype E is restricted to West Africa, and genotype F is found in Central and South America. The distribution of genotypes G and H is less clear, but these have been described from isolates in central America and Southern Europe. These genotypes can be further subdivided into a total of 21 subgenotypes; A1–3, B1–5, C1–5, D1–4, and F1–4. Subgenotypes of B and C differ in their geographical distribution, with B1 dominating in Japan and B2 in China and Vietnam, while B3 and B4 are confined to Indonesia and Vietnam, respectively. Subgenotype C1 is common in Japan, Korea, and China, C2 in China, Southeast Asia, and Bangladesh, C3 in Oceania, and C4 in Australian aborigines. Recombinants between genotypes A and D, as well as between B and C, have also been described.

Likely differences between genotypes in relation to pathogenesis and response to antiviral treatment are beginning to emerge. Genotype C is more frequently associated than B with abnormal liver function tests, lower rates of seroconversion to anti-HBe, higher levels of serum HBV-DNA, cirrhosis, and HCC. Moreover, there is a better sustained response to interferon treatment in patients with genotype B than those with C, and in patients with genotype A than those with D. Genotype A infection appears to be associated with biochemical remission and clearance of HBV-DNA more frequently than genotype D, and has a higher rate of HBsAg clearance compared with genotype D.

Table 1 Genotype determining amino acid variation over the '*a*' determinant region of HBsAg, and relationship between genotypes and subtypes

Genotype	*Subtype*	*Amino acid sequence of 'a' determinant (positions 122–160)*																																				
	Adw	K	T	C	T	T	P	A	Q	G	N	S	M	F	P	S	C	C	C	T	K	P	T	D	G	N	C	T	C	I	P	I	P	S	S	W	A	F	A	K
A	adw2/ayw1																																							
	a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B	adw1/ayw1	*a*	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C	ayr/adrq+/adrq-/adr	*a*	-	-	-	I	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	R
		-	-	-		I	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	V	R
D	ayw2/ayw3/ayw4	R	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-
		R	-	-	M	-	T	-	-	-	T	-	-	-	-	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-
E	ayw4	R	-	-	-	-	L	-	-	-	T	-	-	-	-	-	-	-	-	S	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-
F	adw4q-/adw2/ayw4	*a*	-	-	-	-	L	-	-	-	T	-	-	-	-	-	-	-	-	S	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	L	G	-
G	adw2	-	-	-	-	-	-	-	-	-	-	-	-	Y	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H	adw4	-	-	-	-	-	T	V	-	-	T	-	-	Y	-	-	-	-	-	-	-	-	S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	G	-

[a]R or K.

In contrast to the differences observed in response to interferon therapy, treatment with nucleos(t)ide analogs does not show differential responses between genotypes.

Variants

The HBV genome is not as invariant as originally thought. As HBV replicates through an RNA intermediate that is reverse-transcribed, this step in the replication cycle of the virus is prone to errors made by the viral reverse transcriptase. It is estimated that the HBV genome evolves at a rate of $1.4–3.2 \times 10^{-5}$ nucleotide substitutions/site/year. The virus therefore circulates in serum as a population of very closely related genetic variants, referred to as a quasispecies. Although a lot of these variants would have mutations that would be deleterious to the virus, as a result of constraints imposed by the overlapping ORFs, some would be advantageous, either offering a replication advantage or facilitating immune escape. These are discussed below in the clinical settings in which they have been described.

Markers of Infection

Serological diagnosis of HBV infection relies on the detection of HBsAg in serum, and its persistence for longer than 6 months indicates progression to chronic infection. Appearance of anti-HBs (antibody to HBsAg) indicates recovery from infection, or acquired immunity after preventive vaccination. Detection of HBeAg denotes active viral replication, as does the detection of serum HBV-DNA by qualitative or quantitative PCR tests. Seroconversion to anti-HBe occurs after recovery from acute infection, and less often during the chronic phase, either spontaneously or after therapeutic intervention. In the latter case, this leads to a quiescent phase of disease that can be long term, but does not necessarily mean that virus replication ceases completely (see below). Detection of antibody to core antigen (anti-HBc) of IgM class at high level is a marker of acute infection, whereas total anti-HBc (primarily IgG) is detectable during both acute and chronic infection.

Natural History of the Disease

Exposure to HBV may result in asymptomatic, acute icteric, or, in some instances, fulminant hepatitis (0.1–0.5%). Approximately 5% of adults and 95% of perinatally infected young children become persistently infected. The outcome depends on the age of the patient and genetic factors determining the efficiency of the host immune response. Genetic factors influencing outcome (in more than one study) include polymorphisms of the MHC class II glycoproteins, which influence presentation of viral peptides during induction of the cellular immune response, and mannin-binding lectins, which bind to mannose-terminated carbohydrate residues such as those present on the C-terminus of the Pre-S2 region of the middle envelope protein facilitating phagocytosis. The risk of chronicity in children decreases with increasing age. A small proportion of carriers each year may become HBsAg negative (0.05–2%, depending on age of infection), thus leading to resolution of the hepatitis.

Acute HBV Infection

The incubation period following exposure is 3–6 months. In the week before icterus appears, some patients develop a serum sickness-like syndrome including arthralgia, fever, and urticaria. The clinical picture varies from asymptomatic anicteric infection to protracted icterus and, in some patients (<1%), liver failure (fulminant hepatitis). The acute infection is self-limiting and most patients recover within 1–2 months after the onset of icterus.

Chronic HBV Infection

This is defined as persistent viremia of more than 6 months duration and accompanied by hepatic inflammation. The latter is based on histological examination of liver biopsy material that is followed by assigning of scores for necroinflammatory activity (out of 18) and stage of fibrosis (out of 6), which are used to decide whether a patient needs therapy.

Course of Chronic Infection

Chronic HBV infection is quite variable and is typically characterized by four phases. These phases are the immune tolerant, the immune clearance, the nonreplicative (immune-controlled low-level infection), and the reactivation phase that may be seen in some patients, particularly in Southern Europe and the Far East (**Figure 6**). During the immune-tolerant phase, the patient is HBsAg- and HBeAg-positive with high levels of HBV-DNA, but with near-normal or minimally elevated alanine aminotransferase (ALT) levels. Children infected at birth or soon after are more likely to go through this phase, which may last for 2–3 decades. During the immune clearance phase more commonly seen in those infected in adult life, HBeAg and HBV-DNA levels decrease, ALT levels increase, and necroinflammatory changes are seen in liver biopsies. Loss of HBeAg may be accompanied by an ALT flare, culminating in seroconversion to anti-HBe and entry to the nonreplicative phase when the infection is under

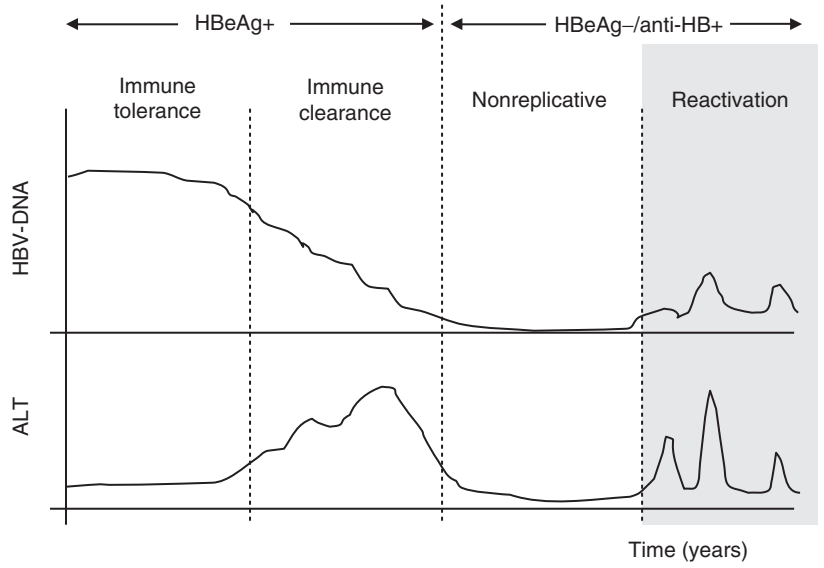


Figure 6 Diagram of the natural history of chronic HBV infection showing the immune tolerant, immune clearance, nonreplicative, and reactivation phases. Reproduced from Karayiannis P, Carman WF, and Thomas HC (2005) Molecular variations in the core promoter, precore and core regions of hepatitis B virus, and their clinical significance. In: Thomas HC, Lemon S, and Zuckerman AJ (eds.) *Viral Hepatitis* 3rd edn., pp. 242–262. London: Blackwell, with permission from Blackwell Publishing.

immune control. Viral DNA integration into the host genome may take place during chronic infection and persist during the nonreplicative (low replicative) phase. During this phase, plasma HBV-DNA may or may not be detectable, while ALT levels return to normal. This serological profile characterizes the 'inactive carrier state', which is maintained thereafter. Some patients, however, for reasons that still remain unknown, show disease reactivation accompanied by ALT rises and return of viremia. Such patients may exhibit fluctuations in ALT levels with occasional severe exacerbations. Continued necroinflammatory activity may lead to fibrosis, and faster development of cirrhosis and HCC.

Mutant Viruses and Chronic Infection

Anti-HBe-positive patients in the reactivated phase of the disease are also referred to as the HBeAg-negative viremic group. Genomic analyses has revealed that such patients carry natural mutants of the virus that have either reduced levels (core promoter variants) or complete abrogation of HBeAg (precore variants) production. These variants are selected at the time of, or soon after, seroconversion, and become dominant during the reactivation phase. The most common precore mutation is the G1896A substitution, which creates a premature stop codon in the precursor protein from which HBeAg is elaborated. This mutation affects the stem of the ε encapsidation signal, but leads to stronger base pairing with the A1896 change in genotypes with a T at position 1858 of the precore region, such as B, C, D, and E. The double mutation affecting the core promoter region (A1762T, G1764A) is thought to result in decreased transcription of the precore mRNA, with a knockon effect on HBeAg production, while pgRNA production remains the same or is even upregulated. It is now apparent that additional mutations in this region may contribute to this phenotype.

Vaccination

Prophylactic vaccination offers the only means of interrupting the transmission of the virus. Vaccines currently used consist of recombinantly expressed HBsAg in yeast such as *Saccharomyces cerevisiae*. In adults, the vaccine is administered intramuscularly into the deltoid at 0, 1, and 6 months. In countries of high and medium seroprevalence, universal vaccination programmes have been instituted, and HBV vaccination is recommended for infants born to carrier mothers within 12 h of birth, given together with hepatitis B immune globulin (HBIg). The response to the vaccine is determined by measuring anti-HB levels 1–4 months after the last dose of the vaccine, and the minimum protection level is set at 10 mIU ml^{-1}.

Development of anti-HBs following vaccination has been recorded in 90–95% of healthy individuals, with lower response rates in hemodialysis and hemophiliac patients (70%). Recent studies on the duration of antibodies have shown maintenance of levels above the 10 mIU ml^{-1} cutoff for 12 years, in up to 80% of individuals immunized at a young age. Booster immunizations therefore may not be required for at least 10 years after

vaccination, and some countries are reconsidering the necessity for this.

The beneficial effects of HBV vaccination are becoming increasingly apparent, particularly in reducing new infections. There has been a dramatic drop in HBV prevalence in populations where the disease was endemic. In Taiwan, 15 years after the start of the vaccination programme, the prevalence of HBsAg in children under 15 years of age has decreased from 9.8% in 1984 to 0.9% in 1999. Similarly, the incidence of HCC has been on the decline from 0.7 per 100 000 children between 1981 and 1986, to 0.57 and 0.36 in 1986–90 and 1990–94, respectively.

Vaccine Escape Mutants

In spite of vaccination and the presence of a satisfactory antibody level, it has been observed that in some instances breakthrough infections occur, the commonest of which involves a mutant with a G145R substitution in the '*a*' determinant region of HBsAg. This and additional mutations in this region have been shown to result in altered antigenicity, accompanied by failure of HBsAg recognition by neutralizing antibody. Such mutant viruses have also been described in the liver transplantation setting, where use of HBIg or monoclonal anti-HBs is recommended in an attempt to prevent infection of the new liver graft.

Treatment

The agents currently available for the treatment of chronic HBV infection are divided into two main groups: the immunomodulators, which include interferon-alpha (IFN-α), and nucleos(t)ide analogs such as lamivudine (3TC), adefovir dipivoxil (Hepsera), entecavir (Baraclude), and telbivudine (Tyzeka or Sebivo), which are currently approved for this purpose. The immunomodulators act by promoting cytotoxic T-cell activity for lysis of infected hepatocytes and by stimulating cytokine production for control of viral replication. Nucleos(t)ide analogs on the other hand are chain terminators acting at the stage of DNA synthesis.

HBeAg-Positive Patients

Treatment with pegylated IFN-α-2, the current standard treatment administered once a week for up to 1 year, achieves seroconversion to anti-HBe in 32% of patients, compared to 18%, 12%, 21%, and 23% with lamivudine, adefovir, entecavir, and telbivudine, respectively. The latter drugs are taken orally daily, in contrast to the weekly intramuscular injections of pegylated interferon. These responses are sustainable in over 95% of patients.

HBeAg-Negative Patients

Treatment of these patients with pegylated interferon for a year results in virologic remission (HBV-DNA $<20\,000$ IU ml^{-1}; equivalent to 105 copies ml^{-1}) in about 44% of them, followed by ALT normalization. This response appears durable in around 20%. Similarly, treatment with nucleos(t)ide analogs for a year leads to HBV-DNA becoming undetectable in between 65% and 90% of treated patients. However, on stopping therapy, only a small minority have a sustained response at the end of 24 weeks of follow up.

Protracted Treatment

To manage such relapses following initial interferon treatment, nucleos(t)ide therapy should be started and continued long term in both HBeAg-positive and -negative patients not achieving a sustained response after a trial of pegylated interferon for 6–12 months. In HBeAg-positive patients, prolonged treatment with lamivudine, for example, leads to increased seroconversion rates from 17% in year 1 to 27% and 40% for years 2 and 3, respectively. Besides, prolonged treatment leads to normalization of ALT levels and an obvious improvement in the histological findings of liver biopsy material. Unfortunately, in many cases, there are breakthrough infections which are attributed to the development of resistance as described below. In such cases, virological breakthrough is soon accompanied by biochemical (ALT rise) and histological relapse. The latter can be avoided by switching to a different nucleos(t)ide analogue that has no cross-resistance with the previous one. Monitoring at three monthly intervals for viral resistance, using molecular assays, is essential.

Resistance

Lamivudine resistance develops in about 24% of patients at year 1 rising to >70% by year 5. Adefovir resistance on the other hand is delayed, being 0% at year 1, 3% at year 2, and rising to 28% by year 4. Entecavir resistance has only been seen so far in patients with lamivudine resistant strains. Longer term follow-up with this nucleoside analogue is ongoing. Nevertheless, it appears that this analog has a high genetic resistance barrier while lamivudine has a low one. Resistance to telbivudine is already a problem after a year of treatment but it appears initially to be more potent than lamivudine.

Molecular Basis of Drug Resistance

The rt domain of the HBV polymerase contains six subdomains (A–F) that are spatially separated, but closely associated with, the normal function of the protein. The characteristic YMDD (tyrosine-methionine-aspartate-aspartate) motif of the catalytic site is located within

subdomain C. Subdomains A, C, and D are most likely involved with dNTP binding and catalysis, whereas subdomains B and E interact with the pgRNA template and primer. Amino acid substitutions that confer resistance to lamivudine predominantly affect the YMDD motif, so that the methionine (M) at position 204 is changed either to valine (YVDD, rtM204V) or isoleucine (YIDD, rtM204I). The former mutation is almost always associated with a second one in subdomain B, involving a substitution of leucine with methionine at position 180 (rtL180M). Adefovir resistance is conferred by mutations rtN236T in subdomain D and rtA181V in subdomain B. In the small number of entecavir-resistant cases detected so far, in addition to the lamivudine-resistant substitutions additional ones that include rtI169T, rtT184G, rtS202I, and rtM250V have been identified.

See also: Hepatitis A Virus; Hepadnaviruses of Birds; Hepadnaviruses: General Features; Hepatitis B Virus: Molecular Biology.

Further Reading

Carman WF, Jazayeri M, Basune A, Thomas HC, and Karayiannis P (2005) Hepatitis B surface antigen (HBsAg) variants. In: Thomas HC, Lemon S, and Zuckerman AJ (eds.) *Viral Hepatitis,* 3rd edn., pp. 225–241. London: Blackwell.

Ganem D and Prince AM (2004) Hepatitis B virus infection – Natural history and clinical consequences. *New England Journal of Medicine* 350: 1118–1129.

Hadziyannis SJ and Papatheodoridis GV (2006) Hepatitis B e antigen-negative chronic hepatitis B: Natural history and treatment. *Seminars in Liver Disease* 26: 130–141.

Hunt CM, Mc Gill JM, Allen MI, *et al.* (2000) Clinical relevance of hepatitis B viral mutations. *Hepatology* 31: 1037–1044.

Karayiannis P (2003) Hepatitis B virus: Old, new and future approaches to antiviral treatment. *Journal of Antimicrobial Chemotherapy* 51: 761–785.

Karayiannis P, Carman WF, and Thomas HC (2005) Molecular variations in the core promoter, precore and core regions of hepatitis B virus, and their clinical significance. In: Thomas HC, Lemon S, and Zuckerman AJ (eds.) *Viral Hepatitis,* 3rd edn., pp. 242–262. London: Blackwell.

Thomas HC (2006) Hepatitis B and D. *Medicine* 35: 39–42.

Zoulim F (2006) Antiviral therapy of chronic hepatitis B. *Antiviral Research* 71: 206–215.

Hepatitis B Virus: Molecular Biology

T J Harrison, University College London, London, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Pseudogene Nonfunctional DNA sequence that is very similar to that of a known gene.
Tolerogen A substance that produces immunological tolerance.

Introduction

Hepatitis B virus (HBV) is the prototype of the family *Hepadnaviridae*, a group of viruses that infect mammals (primates and rodents) and birds. These viruses are distantly related to the retroviruses and, although their genomes are DNA, they are replicated by the reverse transcription of an RNA pregenome.

Structure and Replication of HBV

The 42 nm hepatitis B virion is composed of the DNA genome packaged together with a copy of the virus-encoded polymerase in an icosahedral nucleocapsid made up of dimers of the hepatitis B core antigen, HBcAg. In turn, the nucleocapsid is covered by an envelope composed of a lipid bilayer derived from internal cellular membranes and embedded with the hepatitis B surface protein, HBsAg. The open reading frame (ORF) encoding HBsAg has three in-frame initiation codons (**Figure 1**) which are used for the translation of the large (L, pre-S1 + pre-S2 + S), middle (M, pre-S2 + S), and small (S or major) surface proteins. All three proteins, which share the same C-terminus, are found in the virions.

The early events in infection of the hepatocyte are not well understood, but begin with the virion binding to an unidentified receptor (and, most likely, co-receptors) on the plasma membrane. The first contact seems to involve a domain located near to the N-terminus of L (and not present in M or S), and other interactions involving S also seem to be important. Virus entry likely is via the endosomal route and results in the delivery of the genome, with or without the nucleocapsid, to the nucleus.

The 3.2 kbp genome is composed of two linear strands of DNA held in a circular configuration by base pairing of a short region ('cohesive end region') where the 5′ ends overlap (**Figure 1**). The plus strand is incomplete so that the circle is partially single-stranded. After delivery to the

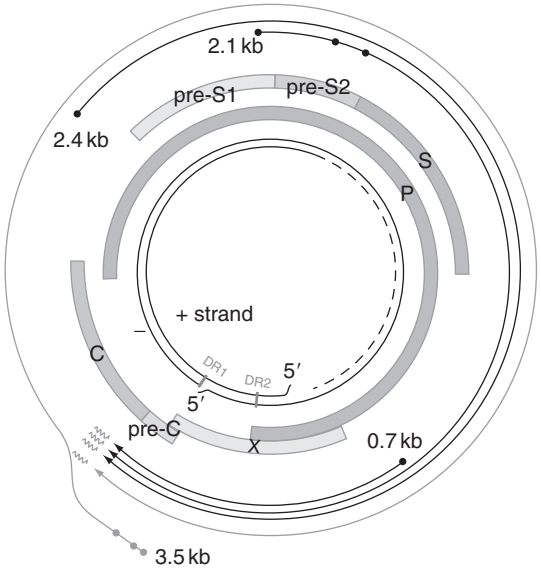


Figure 1 Organization of the HBV genome. The inner circles depict the complete minus strand and incomplete plus strand, and the positions of the direct repeats (DRs) are indicated. The blocks surrounding the genome show the locations of the four overlapping ORFs; C and S contain two and three in-frame initiation codons, respectively. The outer arrows show the viral transcripts, solid circles indicate the positions of the 5′ ends, and the arrowheads indicate the common polyadenylation site. Reproduced from Kidd-Ljunggren K, Miyakawa Y, and Kidd AH (2002) Genetic variability in hepatitis B viruses. *Journal of General Virology* 83: 1267–1280, with permission from Society for General Microbiology.

nucleus, the plus strand is completed (probably by a host enzyme), the primers that remain attached to the 5′ ends of both strands are removed, and the ends are ligated with the introduction of superhelical turns to yield covalently closed circular (ccc)-DNA. This cccDNA associates with histones and other host proteins and resides in the nucleus as a 'minichromosome', the template for transcription of the viral RNAs.

The genome encodes four overlapping ORFs, specifying the core and surface proteins, the polymerase, and a small protein known as HBx (**Figure 1**). All of the *cis*-acting elements, including two enhancers, four promoters, and the single polyadenylation signal, are embedded within the ORFs. Two families of RNAs (2.1 and 3.5 kb in size) are sufficiently abundant to be detectable by northern blotting of RNA from HBV-infected human liver. The 2.1 kb family is translated from a promoter (surface promoter 2, SP2) in the pre-S1 region and encodes M and S. The 3.5 kb RNAs, transcribed from the core promoter, include the precore RNA, which is translated to yield hepatitis B e-antigen (HBeAg), described in detail below, and the pregenomic RNA, which also encodes HBcAg and the polymerase. Surface promoter 1 (SP1), upstream of the surface ORF, transcribes a less-abundant 2.4 kb RNA that encodes L, and the X promoter transcribes an RNA of ~0.7 kb, HBx. All of the RNAs are 3′ co-terminal, being polyadenylated in response to a polyA signal in the core (or C) ORF (**Figure 1**).

The pregenomic RNA is the 3.5 kb transcript that is reverse-transcribed to the HBV genome and also acts as the mRNA for HBcAg and the polymerase. HBcAg forms dimers and has arginine-rich motifs at the C-terminal end that are believed to interact with the viral nucleic acid in the nucleocapsid. Less frequently, the downstream ORF is translated to yield the polymerase, probably following a ribosomal translocation that bypasses the core initiation codon. There is a stem–loop structure, termed epsilon (ε), near to the 5′ end of the pregenomic RNA (**Figure 2**(**a**)), and the polymerase binds to a bulge on the stem, priming first strand synthesis and signaling packaging of the pregenome and polymerase into 'precores', as the dimers of HBcAg self-assemble around the RNA–protein complex. The primer is the N-terminal domain of the polymerase, in which a tyrosine residue is covalently linked to guanosine, and this is extended by a further three residues on the bulge. The extended primer then translocates to a complementary sequence near to the 3′ end of the RNA (**Figure 2**(**b**)).

The direct repeats (DR1 and DR2) are motifs of 11 nt that occur twice in the HBV genome (**Figure 1**). Located at the positions of the 5′ ends of the two strands, they play an important role in template switches during viral DNA synthesis. The translocated primer binds within DR1, and minus-strand synthesis commences with concomitant degradation of the template by an RNaseH activity in the C-terminal domain of the polymerase (**Figure 2**(**c**)). Completion of minus-strand synthesis leaves a capped oligoribonucleotide (which was the 5′ end of the pregenome) containing the DR1 sequence (**Figure 2**(**d**)). This translocates to the copy of DR2 near to the 5′ end of the minus strand and primes plus-strand synthesis, which then proceeds to the 5′ end of the minus strand (**Figures 2**(**e**) and **2**(**f**)). The short (~8 nt) terminal redundancy of the minus strand allows another template switch and circularization of the genome. Completion of the nucleocapsid during plus-strand synthesis starves the polymerase of the substrate, leaving the characteristic partially single-stranded structure that typifies the HBV genome (**Figures 2**(**g**) and **2**(**h**)).

Mature cores then bud through cellular membranes containing HBsAg (S, M, and L) and are exocytosed. A vast excess of subviral particles, which lack nucleocapsids and are noninfectious, are also secreted from the hepatocyte and are presumed to overwhelm the immune response of the host. These comprise 22 nm spheres, composed of S and M, and tubular forms of the same diameter, composed of S, M, and L (**Figure 3**). An endoplasmic-retention signal within the pre-S1 domain of L is involved in the formation of the virions and tubular subviral particles.

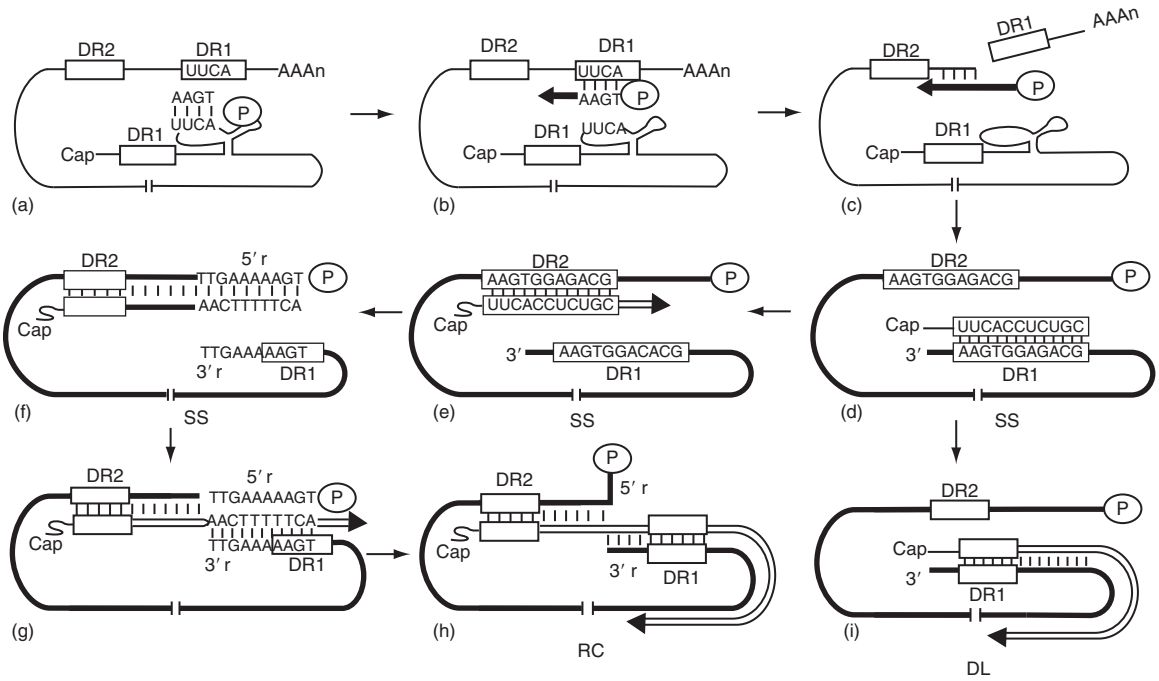


Figure 2 Replication of the HBV genome. See text for details. Reproduced from Liu N, Ji L, Maguire ML, and Loeb DD (2004) *cis*-Acting sequences that contribute to the synthesis of relaxed-circular DNA of human hepatitis B virus. *Journal of Virology* 78: 642–649, with permission from American Society for Microbiology.

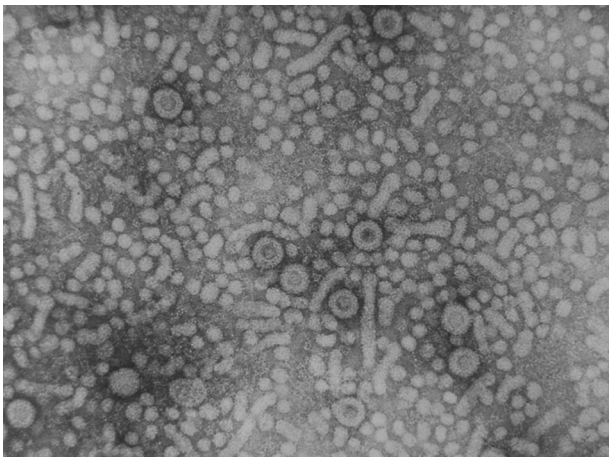


Figure 3 Electron micrograph of particles from the plasma of an infectious carrier. The 42 nm virions (Dane particles) are outnumbered by 22 nm spherical and tubular noninfectious, subviral particles.

The Surface Protein, HBsAg

The 226-amino-acid (aa) residue S protein is quite hydrophobic and structural models suggest that it contains four membrane-spanning regions (**Figure 4**(**a**)). The major hydrophilic loop is exposed on the surface of viral particles and is the main target of the immune response to HBsAg. This domain is rich in cysteine residues, which are believed to be linked by disulfide bridges, forming a complex structure that includes the *a* determinant and *d*/*y* and *w*/*r* subdeterminants. Antibody (anti-HBs) responses to this domain give immunity to infection in convalescence or following immunization with hepatitis B vaccine. Approximately half of the S residues are N-glycosylated at aa 146 (in the *a* determinant) so that the protein exists in two forms, p24 and gp27.

The N-terminus of S is exposed on the surfaces of the virions and subviral particles and, in M, is extended by 55 aa (the pre-S2 region). The pre-S2 region is glycosylated in M to give two proteins, gp33 and gp36, depending upon the glycosylation of asparagine 146 in the S domain. M is much less abundant than S in the virion and subviral particles. The SP2 promoter lacks a 'TATA box' and the 5′ ends of the 2.1 kb RNAs are heterogeneous, most being located downstream of the pre-S2 initiation codon (**Figure 1**). Furthermore, that codon is in a poor 'Kozak' context for recognition by the ribosome; consequently, much more S than M is produced.

The large surface protein, L, has a further extension of the N-terminus by the pre-S1 region. Again, there are two forms, p39 and gp42, depending on glycosylation at aa 146 of S (the pre-S2 region is not glycosylated in L). The glycine at aa 2 of the pre-S1 region is myristylated and embedded in the membrane. L has a dual topology: upon expression, the pre-S loop may translocate into the endoplasmic reticulum (ER) lumen, so that it is exposed on the exterior of the particle and presents the

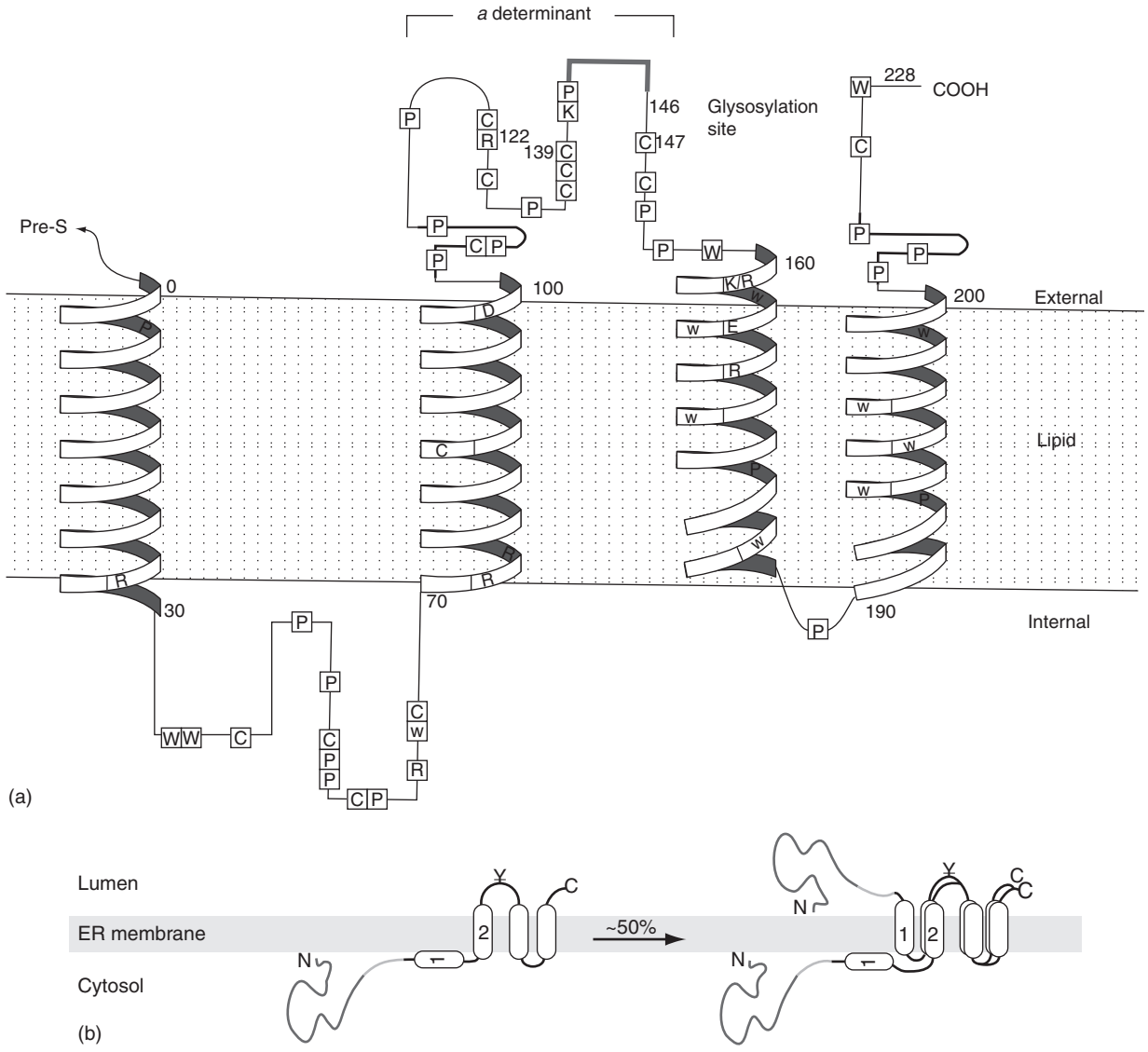


Figure 4 (a) Predicted structure of the major surface protein, S. Note the abundance of cysteine residues in the major hydrophilic region. (b) Dual topology of the large surface protein, L. (a) Reproduced from Stirk HJ, Thornton JM, and Howard CR (1992) A topological model for hepatitis B surface antigen. *Intervirology* 33: 148–158, with permission from Karger Publishers. (b) Reproduced from Lambert C, Mann S, and Prange R (2004) Assessment of determinants affecting the dual topology of hepadnaviral large envelope proteins. *Journal of General Virology* 85: 1221–1225, with permission from Society for General Microbiology.

receptor-binding domain. However, in around 50% of the L molecules, the loop does not translocate but remains in the cytosol and is believed to interact with the nucleocapsid during budding and maintain this contact in the virion (**Figure 4**(**b**)).

The Hepatitis B Vaccines

The hepatitis B vaccines contain HBsAg and are given via an intramuscular injection to stimulate a protective anti-HBs response. The first-generation (so-called 'plasma derived') vaccine was produced by purifying HBsAg from donated blood. Despite some worries that blood-borne viruses (particularly human immunodeficiency virus (HIV)) potentially might contaminate particular batches, this method of vaccine production is safe provided that manufacturing protocols, including steps that inactivate potential infectivity, are adhered to strictly. Even today, plasma-derived vaccines constitute the majority of doses given worldwide. Currently, most doses of hepatitis B vaccine given in the West are produced by expression of HBsAg in yeast (*Saccharomyces cerevisiae*), and several vaccines based on expression in mammalian cells also have been licensed.

Most individuals produce a protective immune response with the standard, three-dose regimen of immunization,

but there is a problem of nonresponsiveness in up to 5% of recipients, particularly the immune-suppressed and older individuals, and especially males. The vaccines contain only S and there has been interest in whether vaccines containing pre-S epitopes may circumvent this problem of nonresponsiveness. Up to 50% of nonresponders may sero-convert following immunization with a vaccine containing pre-S epitopes and the nonresponsiveness of many of the remainder seems to be genetically based.

Most countries worldwide have now introduced universal immunization of infants. Although the vaccine may be incorporated into the Expanded Programme of Immunization, it is essential that it is given as soon as possible after birth to the infants of infectious mothers, in order to break the chain of mother-to-infant transmission (see below). Immunization within 12 h of birth protects around 70% of the infants of carrier mothers and up to 90% success may be achieved when passive immunization with hepatitis B immune globulin (HBIG) also is given, at a contralateral site, to neutralize any maternal virus that reaches the newborn infant. Rarely, however, the antibody may select variants with mutations that alter the amino acid sequence of the *a* determinant. Such 'antibody escape' variants may also be selected in liver-transplant recipients given HBIG in an attempt to prevent HBV infection of the graft and, indeed, in the natural course of infection, with seroconversion to anti-HBs. The most common mutations affect the highly conserved codons 144 and 145 (for aspartic acid and glycine, respectively) of S. Fortunately, there is no evidence that these 'antibody escape' variants can be transmitted to individuals with vaccine-induced immunity.

Hepatitis B e-Antigen, HBeAg

This antigen circulates as a soluble protein in the plasma of some persistently infected individuals and is recognized as a marker of infectivity. The synthesis of HBeAg is nonessential for virus replication and its function is thought to be as a tolerogen; it may be especially important in perinatal transmission. HBV does not cross the placenta, but HBeAg is believed to do so and to circulate in the fetus. The infants of viremic mothers almost invariably become infected at or around the time of birth. At least 90% of such infants become persistently infected and often are extremely immune-tolerant of the virus, with very high viral loads that may persist for decades or even for life. When they are females, they infect their children in turn; thus, HBeAg seems to be important in maintaining HBV in the human population through the generations.

As noted above, the pregenomic RNA is translated from the second initiation codon in the core ORF to produce the nucleocapsid protein. The precore RNA has its cap site further upstream, so that it contains the precore initiation codon and the entire core ORF is translated (**Figure 5**). There are an additional 29 aa, encoded by the precore region, at the N-terminus of the precursor to HBeAg (p25) and these include a signal sequence that targets the protein to the ER. Here, the cellular signal peptidase cleaves this sequence and the protein is secreted with further proteolysis, removing the arginine-rich domain at the C-terminus.

Seroconversion from HBeAg positivity to anti-HBe, with clearance of HBV replication, is often a feature of recovery from persistent infection. However, a significant proportion of patients with circulating anti-HBe remain viremic, infected with HBV variants that do not synthesize, or synthesize reduced quantities of, HBeAg. The most common mutation affects codon 28 in the precore region, changing it from a tryptophan to a termination codon and preventing the synthesis of p25. Because this mutation affects base-pairing in ε, it is constrained in some genotypes and subgenotypes of HBV. Other, less common 'precore mutations' introduce termination codons elsewhere in the precore region, destroy the precore initiation codon, or modify the signal peptidase cleavage site.

Another class of mutations found in HBV from anti-HBe-positive individuals affects the core promoter. These mutations decrease the transcription of the precore RNA (and may increase the transcription of the pregenomic RNA), resulting in reduced synthesis of HBeAg. Core promoter mutations may be associated with a particularly high risk of developing hepatocellular carcinoma (HCC), especially in genotype C.

Treatment of Chronic Hepatitis B

Prior to the introduction of specific antiviral therapeutics, persistent HBV infection was treated with interferon, with mixed success. In some studies, up to one-third of treated individuals cleared the virus, but the treatment was rather less successful in certain populations, particularly individuals from the Far East. Interferon seems to work by modulating the immune response of the host, rather than a direct antiviral effect, and was found to be most successful in individuals who had evidence of hepatitis or, in other words, already were mounting a cellular immune response to the virus.

Because the HBV genome is replicated via an RNA intermediate, treatment with nucleoside and nucleotide analogs is an option. The predicted amino acid sequence of the HBV polymerase is similar to those of retroviruses and a motif, Tyr-Met-Asp-Asp (YMDD), in the active site is highly conserved. The HBV polymerase has not been crystallized but its structure is inferred from those of other RNA-dependent DNA polymerases, particularly that of HIV type 1 (HIV-1). Several conserved regions,

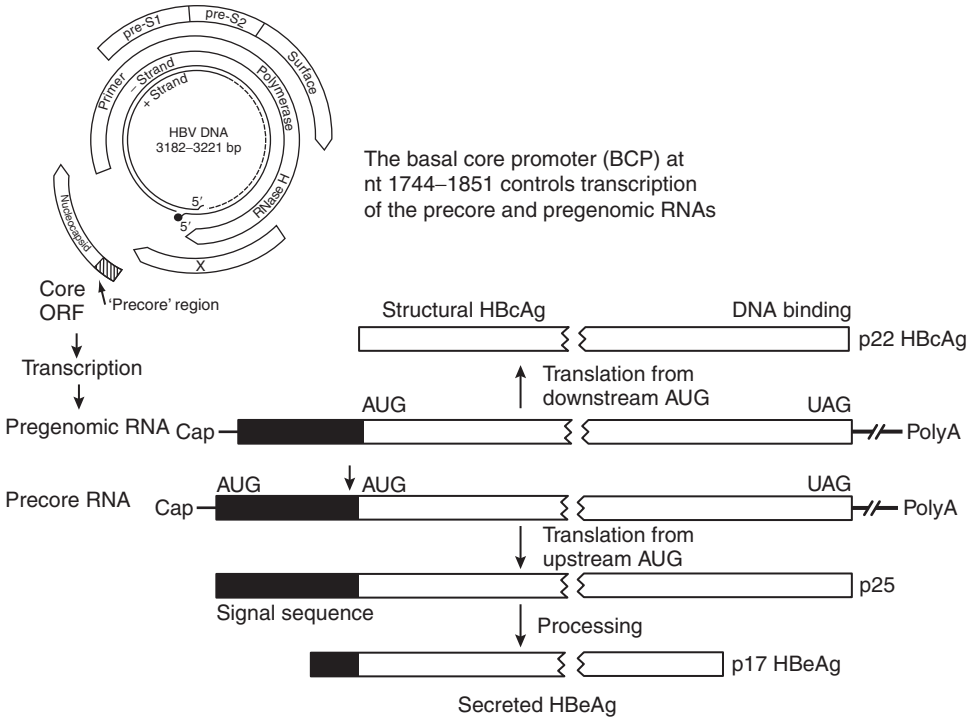


Figure 5 Expression of the HBV core ORF. The pregenomic RNA is translated from the second initiation codon in the ORF to yield HBcAg. The precore RNA is translated from the upstream initiation codon to p25, which is processed to HBeAg. The arrow indicates the position of the most common precore mutation, changing a tryptophan to a termination codon. Reproduced from Harrison TJ (2006) Hepatitis B virus: Molecular virology and common mutants. *Seminars in Liver Disease* 26: 87–96, with permission from Thieme International (Stuttgart).

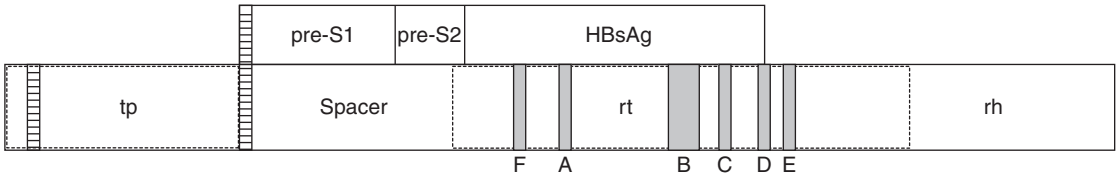


Figure 6 Conserved domains in the HBV reverse transcriptase. The polymerase and overlapping surface ORFs are shown. tp, terminal protein (primase); rt, reverse transcriptase; rh, RNaseH. Reproduced from Stuyver L, Locarnini S, Lok A, *et al*. (2001) Nomenclature for antiviral-resistant human hepatitis B virus mutations in the polymerase region. *Hepatology* 33: 751–757, with permission from the American Association for the Study of Liver Diseases.

designated A–F, are recognized within the reverse transcriptase domain and the YMDD motif is located within conserved region C (**Figure 6**).

Despite the similarity between the HIV and HBV polymerases, many of the nucleoside analogs first used against HIV, such as zidovudine, were found not to be effective against HBV. Lamivudine, a deoxycytidine analog which acts as a chain terminator during reverse transcription of the pregenome and can result in a 4–5 $\log_{10}$ suppression of viral load, was the first nucleoside analog to be used successfully and licensed for therapy of hepatitis B. However, prolonged monotherapy with lamivudine results in the emergence of resistant virus in approximately 40% of patients after 2 years of therapy (and 65% after 5 years). Resistance to lamivudine is associated with point mutations which result in substitution of the methionine residue in the YMDD motif by either valine or isoleucine, changes which parallel lamivudine resistance in HIV-1. The valine (but usually not the isoleucine) substitution is associated with a second, leucine-to-methionine substitution in the upstream, conserved region B and this seems to compensate partially for the adverse effect of the former on replication efficiency.

Another nucleoside analog, adefovir dipivoxil, inhibits the replication not only of wild-type HBV but also of lamivudine-resistant mutants. Resistance to adefovir emerges at a slower rate than to lamivudine, reaching around 22% after 2 years for patients initially treated

with lamivudine, and perhaps less frequently for nucleoside-naive individuals. Adefovir resistance is associated particularly with substitutions in conserved regions D or B and these mutants are susceptible to lamivudine. The absence of substitutions in conserved region C is consistent with the concept that adefovir inhibits the priming of reverse transcription, rather than chain elongation, and suggests that combination therapy with lamivudine and adefovir may inhibit the emergence of resistant mutants.

In vitro, the deoxyguanosine analog entecavir inhibits priming of reverse transcription as well as chain elongation of both the minus and plus strands. The drug is active against lamivudine- and adefovir-resistant HBV. Resistance to entecavir has been described in patients who had been treated previously with lamivudine and in whom lamivudine-resistant mutants had already been selected, but resistance seems to arise in otherwise treatment-naive patients at a rate of less than 1% per year. Other analogs, including telbivudine, which is similar in structure to lamivudine but seems to have better efficacy, have also been licensed recently for use in hepatitis B but the design of regimens for combination therapy lags way behind that for HIV.

HBV and HCC

When tests for HBsAg were first developed, it became clear that regions of the world with a high prevalence of chronic hepatitis B and a high annual incidence of HCC were coincident and that, in those regions, most individuals with HCC were HBsAg-positive. In Taiwan, a large prospective study confirmed the increased risk of HCC for carriers of HBsAg, and there is now evidence that the hepatitis B vaccine will reduce considerably the risk of developing the tumor. Although the importance of hepatitis C virus as a cause of HCC has increased considerably over recent decades, HBV remains a critical and preventable cause of one of the most common cancers of humans.

Cloning of the HBV genome enabled the use of virus-specific DNA sequences as probes in Southern hybridization assays, leading to evidence of chromosomal integration of viral sequences in at least 80% of HBV-associated HCCs. The chromosomal sites of integration of viral DNA seem to be random. Nevertheless, the tumors are clonal with respect to the integrated HBV DNA (often with multiple copies or partial copies of the genome), suggesting they arise from a single cell and that integration is the first step in the oncogenic process. In fact, integrated HBV DNA often can be detected by Southern hybridization of DNA from liver biopsies of infected patients who do not have HCC, suggesting that clonal expansion of hepatocytes containing integrated HBV DNA may be common in persistent infection.

Although the chromosomal sites of integration are random, there are hot spots in the viral genome, particularly around the direct repeats, for recombination with human DNA. A double-stranded linear form of HBV DNA, an aberrant product (and not a replicative intermediate) generated when the plus-strand primer fails to translocate and primes *in situ* (**Figure 2(i)**), may be that which recombines with the host DNA. Integration seems to be of no value to the virus, it is not a part of the replication cycle, and the integrants often are rearranged and never have sufficient redundancy to act as templates for the transcription of the 3.5 kb pregenome. On the other hand, the surface ORF often is expressed from integrants and many HBV-associated HCCs secrete HBsAg.

Despite considerable study, there is no consensus regarding the mode of HBV oncogenesis. Interest has focused on the product of the X ORF; this acts as a transactivator of transcription and seems to be important in 'kick-starting' the infection by stimulating transcription from the viral promoters. The protein seems to have pleiotropic effects in the cell, including stimulating various signal pathways and perhaps also preventing apoptosis. Transgenic mice with liver-specific expression of HBx develop HCC. Truncated middle surface proteins, produced following transcription of incomplete surface ORFs in integrated HBV DNA, also may act as transactivators, perhaps by locating in the plasma membrane and interacting with the receptors that initiate various signaling cascades. Some mice with the entire HBV genome as transgenes show accumulation of the large surface protein (L) in the ER of the hepatocytes and the resultant ER stress also seems to lead to the development of HCC.

Researchers have also investigated the hypothesis that HBV acts as an insertional mutagen, perhaps by inactivating a tumor suppressor gene or causing the inappropriate expression of a normally quiescent gene. Early studies, based on the analysis of genomic libraries of tumor DNA, identified insertions in or next to human genes, such as for cyclin A and the retinoic acid receptor β gene. However, these were isolated instances and no common targets were identified. More recently, amplification of virus–host junctions using polymerase chain reaction (PCR)-based techniques has identified further examples, particularly the human telomerase reverse transcriptase (hTERT), which seems to be a target of integration in several tumors. It is worth noting that, while the initial sites of HBV DNA integration are random, the integrants in tumors are a selected subset that may be implicated in the oncogenic process.

Woodchuck hepatitis virus (WHV) also causes HCC in its natural host. In contrast to HBV, in woodchuck tumors WHV DNA often is integrated within or adjacent to myc family oncogenes (c-myc or N-myc). In fact, woodchucks possess an additional copy of N-myc, a retrotransposed pseudogene that lacks introns. Insertion of the

WHV enhancer 5′ or 3′ of the myc-coding region activates transcription from the myc promoter.

See also: Hepadnaviruses: General Features; Hepadnaviruses of Birds; Hepatitis B Virus: General Features.

Further Reading

Bertoletti A and Gehring AJ (2006) The immune response during hepatitis B virus infection. *Journal of General Virology* 87: 1439–1449.

Bouchard MJ and Schneider RJ (2004) The enigmatic X gene of hepatitis B virus. *Journal of Virology* 78: 12725–12734.

Harrison TJ (2006) Hepatitis B virus: Molecular virology and common mutants. *Seminars in Liver Disease* 26: 87–96.

Kidd-Ljunggren K, Miyakawa Y, and Kidd AH (2004) Genetic variability in hepatitis B viruses. *Journal of General Virology* 83: 1267–1280.

Kremsdorf D, Soussan P, Paterlini-Brechot P, and Brechot C (2006) Hepatitis B virus-related hepatocellular carcinoma: Paradigms for viral-related human carcinogenesis. *Oncogene* 25: 3823–3833.

Lambert C, Mann S, and Prange R (2004) Assessment of determinants affecting the dual topology of hepadnaviral large envelope proteins. *Journal of General Virology* 85: 1221–1225.

Liu N, Ji L, Maguire ML, and Loeb DD (2004) *cis*-Acting sequences that contribute to the synthesis of relaxed-circular DNA of human hepatitis B virus. *Journal of Virology* 78: 642–649.

Norder H, Courouce A-M, Coursaget P, *et al.* (2004) Genetic diversity of hepatitis B virus strains derived worldwide: Genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 47: 289–309.

Stirk HJ, Thornton JM, and Howard CR (1992) A topological model for hepatitis B surface antigen. *Intervirology* 33: 148–158.

Stuyver L, Locarnini S, Lok A, *et al.* (2001) Nomenclature for antiviral-resistant human hepatitis B virus mutations in the polymerase region. *Hepatology* 33: 751–757.

Zoulim F (2006) Antiviral therapy of chronic hepatitis B. *Antiviral Research* 71: 206–215.

Hepatitis C Virus

R Bartenschlager and S Bühler, University of Heidelberg, Heidelberg, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Pseudotypes Retroviral vector particles bearing heterologous glycoproteins on their surfaces.
Replicon DNA or RNA molecule capable of self-replication in a cell or in an adequate *in vitro* system (e.g., cell lysate).
Sustained virological response (**SVR**) Continuous absence of HCV RNA from the serum starting 6 months after cessation of antiviral therapy.

Introduction

In the 1970s, when blood tests for the detection of hepatitis A virus (HAV) and hepatitis B virus (HBV) became available, many blood samples responsible for post-transfusion hepatitis were negative when tested for these two viruses. Therefore, this third form of transfusion-associated hepatitis was named non-A, non-B hepatitis. However, in 1989, Choo and co-workers discovered hepatitis C virus (HCV) as the causative agent of parenterally transmitted non-A, non-B hepatitis. Initially, they isolated a viral complementary DNA clone from the serum of a chimpanzee experimentally infected with non-A, non-B hepatitis and used it to establish a screening test for antibodies in patients infected with this agent. By using this initial cDNA clone as a hybridization probe, Choo and colleagues then isolated a near full-length viral genome that was readily classified as a close relative of the animal pathogenic pestiviruses based on similarities of genome organization and virion properties.

Taxonomy and Geographical Distribution

HCV has been classified as the only member of the genus *Hepacivirus* and grouped together with the genera *Pestivirus*, *Flavivirus*, and tentatively the GB-viruses, in the family *Flaviviridae*. According to phylogenetic analyses, HCV is more closely related to the pestiviruses than to the flaviviruses.

Based on genomic heterogeneity, six major genotypes, having more than 30% nucleotide sequence divergence, and more than 70 subtypes differing from each other by 10–30% at the nucleotide sequence level, have been defined. Subtypes are designated by lowercase letters following the number of the genotype (e.g., genotype 1 subtype b = 1b). While genotype 1 and 2 viruses are prevalent almost worldwide, HCV genotypes 3–6 are to a large extent restricted to distinct geographical regions, including the Indian subcontinent and Southeast Asia (genotype 3), Africa and Middle East (genotype 4), South Africa (genotype 5), and Southeast Asia (genotype 6). Individual genotypes have not been ascribed to particular disease manifestations, except for a higher prevalence of

steatosis with patients infected with genotype 3 viruses, but genotypes are important predictors of therapy outcome (see below).

Transmission

HCV is mainly transmitted by parenteral exposure to blood and blood products. The development of effective screening tests for blood and blood products and the implementation of viral disinfection procedures have almost excluded this route of transmission in countries where these measures are in place. Thus, the major remaining risk factor for acquiring HCV infection in developed countries is the use of contaminated needles in injection drug use. In some countries, HCV infection has been spread primarily by the use of inadequately sterilized medical instruments. In contrast to HBV infection, sexual transmission and maternal–infant spread of HCV are much less frequent.

Clinical Manifestation

Acute HCV infections are usually asymptomatic or, in about 30% of cases, associated with nonspecific symptoms such as abdominal pain, fatigue, weakness, poor appetite, and nausea. During an incubation period of 15–75 days, HCV RNA becomes detectable in serum by reverse transcription-polymerase chain reaction (RT-PCR), and virus titers usually peak at 10^5–10^7 genomes/ml between weeks 6 and 10, irrespective of disease outcome (**Figure 1(a)**). Two to four weeks after onset of viremia serum alanine aminotransferase (ALT) levels begin to rise, indicative of hepatocellular injury. Due to these nonspecific signs and symptoms, acute infection often remains unrecognized. The duration of viremia in acute hepatitis C is unpredictable and can vary from 2 to more than 4 months. Some patients even become HCV RNA negative during early convalescence but later on viremia rebounds. Overall 50–80% of HCV infections lead to a chronic carrier state (**Figure 1(b)**). About 30% of these chronically infected persons progress to liver cirrhosis 10–30 years after primary infection and hepatocellular carcinoma occurs in up to 2.5% of these patients. In contrast to chronic hepatitis B, in case of persistent HCV infection a hepatocellular carcinoma only develops on the basis of prior cirrhosis.

Pathology and Histopathology

Hepatocytes are the primary target of HCV. Therefore, the histological alterations of chronic hepatitis C are hepatocellular injury, portal and parenchymal inflammation, and necrosis. The injury of the hepatocyte is thought to be induced primarily by the immune reaction rather than by viral cytopathogenicity. Liver damage is typically spotty and focal with accompanying chronic inflammatory cells, macrophages, and, eventually, variable degrees of fibrosis. The progression rate of hepatic fibrosis is the major determinant for the outcome of chronic hepatitis C in terms of developing cirrhosis and hepatocellular carcinoma. Unfortunately, there are only a few histological markers that are more often associated with hepatitis C than with other causes of hepatitis, such as steatosis. Although the liver is the primary target, persistent HCV infection is often associated with extrahepatic symptoms, such as renal complications, lymphoma, and diabetes. A high proportion of patients with chronic hepatitis C develop cryoglobulinemia, which may account for some of these extrahepatic manifestations.

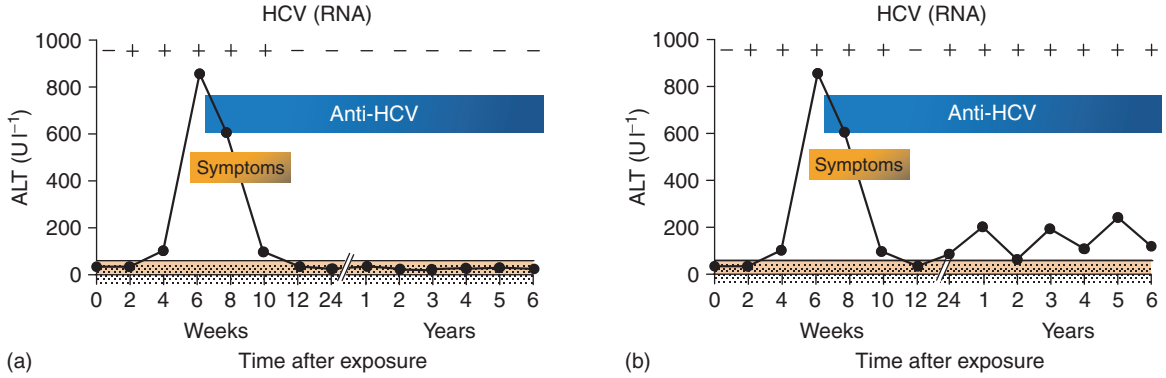


Figure 1 Course of acute and chronic HCV infection. (a) In acute HCV infection, viral RNA is typically detectable between weeks 2 and 10 after virus exposure. ALT levels rise between weeks 4 and 10 peaking around week 6. This is also the time when the first symptoms manifest. HCV-specific antibodies arise late in infection. In self-limiting infection HCV is cleared as measured by RNA levels and ALT returns to normal levels. (b) In chronic hepatitis C the early phase is similar to acute infection but later on the virus persists. In the chronic phase, ALT levels fluctuate as does viremia.

Genome Organization

The genome of HCV is a 9.6 kb single-stranded RNA molecule of positive polarity (**Figure 2(a)**). It is flanked by two highly structured nontranslated regions (NTRs). The 5′ NTR contains an internal ribosome entry site (IRES) (**Figure 2(b)**), mediating cap-independent translation of a polyprotein of *c.* 3000 amino acids in length. The 3′ NTR is composed of a 40-nt-long variable region, a polypyrimidine tract (heterogeneous length), and the 98-nt-long highly conserved 3′ terminal X-tail (**Figure 2(b)**). Both the 5′ and the 3′ NTRs contain *cis*-acting RNA elements (CREs) that are required for viral replication. Within the 3′ terminal part of the NS5B gene, three additional CREs are localized (5BSL3.1–5BSL3.3), wherein 5BSL3.2 is absolutely required for viral RNA replication by forming a long-distance RNA–RNA interaction with the middle loop in the X-tail.

The organization of the HCV polyprotein and the functions of the individual gene products are depicted in **Figure 2(a)**. The structural proteins, core and the envelope proteins 1 (E1) and 2 (E2), are located within the N-terminal part of the polyprotein preceding the p7 protein which appears to be an ion channel and therefore was grouped into the viroporin protein family. The nonstructural (NS) proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B are encoded in the remainder. The NS2 protein together with the N-terminal protease domain of NS3 is responsible for the autocatalytic cleavage of the NS2–NS3 junction (**Figure 2(a)**). It is a dimeric cysteine protease with a composite active site. The same NS3 protein domain carries a serine-type protease that after association with its cofactor NS4A cleaves the residual junctions between the NS proteins. Moreover, the same protease also cleaves two cellular signaling molecules involved in the induction of the innate immune response (see below). Two additional enzymatic

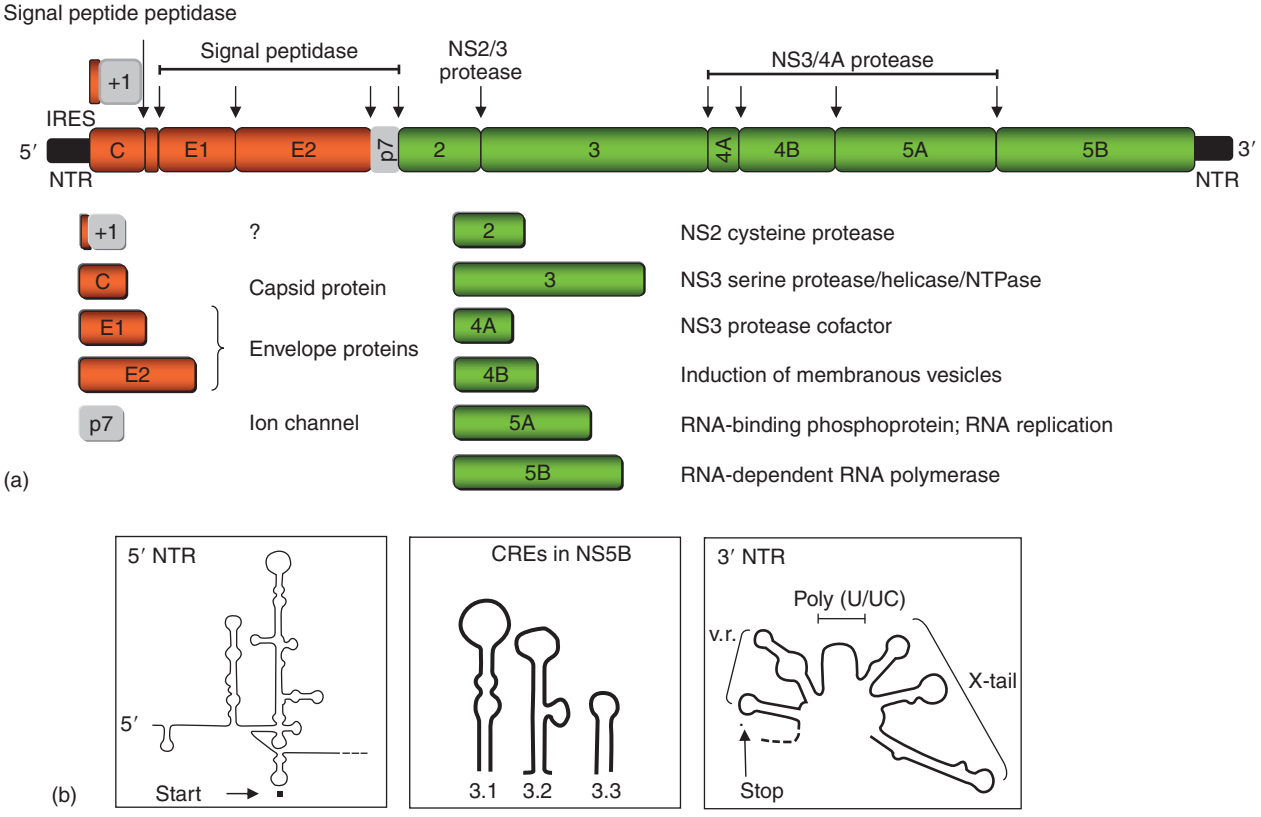


Figure 2 Illustration of the HCV genome organization, gene products, and *cis*-acting RNA elements (CREs). (a) HCV genome organization, gene products, and their functions in the replication cycle. The HCV coding region is shown as bar with arrows above indicating the positions of polyprotein cleavage and involved proteases. The structural region in the N-terminal third of the polyprotein is drawn in orange, and the region encoding the nonstructural proteins is drawn in green. The core+1 protein(s) and the p7 protein are drawn in gray. The HCV coding region is flanked by two nontranslated regions (5′ and 3′ NTR), indicated in dark gray. The 5′ NTR contains an internal ribosome entry site (IRES). Functions of the individual gene products are given in the lower panel. (b) Schematic representation of CREs. The 5′ NTR (left panel) contains the IRES as well as structures important for viral RNA replication. Three CREs are located in the 3′ terminal part of the NS5B coding region (middle panel). The loop region of stem–loop 5BSL3.2 forms a long-distance RNA–RNA interaction essential for RNA replication with the loop region of the middle stem–loop in the X-tail. The right panel displays the organization of the 3′ NTR with variable region (v.r.), poly(U/UC) tract, and X-tail. Positions of start and stop codons are indicated with dots.

activities (RNA helicase and nucleoside triphosphatase) reside in the C-terminal two-thirds of NS3. Alterations of intracellular membranes, in particular membranous vesicles, are mainly induced by the 27 kDa integral membrane protein NS4B. These vesicles accumulate in the perinuclear region, are called the membranous web, and are the site of viral RNA replication. So far, the role of the phosphorylated zinc metalloprotein NS5A in the viral replication cycle is unclear. NS5A is composed of an N-terminal amphipathic α-helix serving as a membrane anchor and three largely cytosolic domains. The X-ray crystal structure of RNA-binding domain I was resolved and shown to form homodimers resulting in a basic groove. NS5A phosphorylation is mediated by cellular kinases, in particular the α isoform of the protein kinase CKI. The NS5A phosphorylation state appears to affect replication efficiency indicating that NS5A is an important replication factor. Furthermore, NS5A may also contribute to interferon-alpha (IFN-α) resistance that is often observed with genotype 1 and 4 viruses. The 68 kDa protein NS5B is the RNA-dependent RNA polymerase (RdRp). It is a membrane-associated enzyme with a structural organization similar to that of other polymerases with palm, finger, and thumb subdomains. However, it differs from most other RdRps by having a fully encircled active site, which is due to tight interactions between the finger and thumb subdomains.

In addition to the polyprotein, a heterogeneous group of HCV proteins is expressed either by ribosomal frameshifting into the +1 ORF or by internal translation initiation. The resulting proteins, collectively designated core +1, are not essential for replication and virus production in cell culture, and their role *in vivo*, if any, remains to be elucidated.

HCV Replication Cycle

HCV infection starts by binding the envelope glycoprotein E1/E2 complex on the surface of the virus particle to its cognate receptor(s) presumably leading to clathrin-mediated endocytosis and a subsequent fusion step from within an acidic endosomal compartment (**Figure 3**). Cellular factors implicated in virus binding and entry are glycosaminoglycans, scavenger receptor class B type 1 (SR-B1), CD81, and low-density lipoprotein (LDL) receptor. However, for most of these factors, the precise role is not well understood and one or several additional factors may be required for productive entry. Upon release of the RNA genome, the polyprotein is expressed by IRES-dependent translation occurring at the rough endoplasmic reticulum (rER) where host cell signal peptidases, signal peptide peptidases, and viral proteases catalyze polyprotein cleavage.

During or after cleavage, the membrane-associated replication complex, which catalyzes the RNA amplification via negative-strand RNA intermediates, is formed (**Figure 3**). These membrane-associated complexes are composed of viral RNA, viral proteins, and most likely host cell factors. Newly synthesized positive-strand RNAs either are used for translation or serve as templates for further RNA synthesis or interact with the core protein to form the viral nucleocapsid. The E-proteins are retained at rER membranes indicating that viral envelopes are generated by budding into the lumen of this organelle. Progeny particles are thought to be exported by the secretory pathway and after fusion of the transport vesicle with the plasma membrane, virions are released. However, most of these steps are poorly understood and several assumptions were made that are based on studies with heterologous expression systems and analogies to closely related viruses.

Virion Properties

HCV particles are enveloped and spherical and have a diameter of 55–60 nm as determined by filtration and electron microscopy (**Figure 4**). By analogy to other flaviviruses, HCV particles are composed of at least the genomic RNA, the core protein, and the two envelope proteins E1 and E2 which are embedded into the lipid envelope. The core protein forms the internal viral capsid (presumably 30–35 nm in diameter) that shelters the single-stranded RNA genome (**Figure 4**). The S_{20W} is approximately 200S and infectious HCV virions isolated from the plasma sample of infected patients and chimpanzees have low buoyant densities in the range of 1.05–1.10 g ml^{-1}.

Tissue Tropism and Host Range

Hepatocytes are considered to be the natural target cells for HCV. Viral RNA was also detected in peripheral blood mononuclear cells (PBMCs) and bone marrow cells but it is unclear whether productive infection occurs in these cells. In cell culture, HCV RNA replication was demonstrated in non-liver cells like human T- and B-cell lines or embryonic kidney cells (293). Furthermore, certain mouse cell lines can support replication of HCV replicons demonstrating that the viral replication machinery is also functional in a murine host cell environment.

Experimental Systems

Animal Models

Possibilities to propagate HCV *in vivo* are rare. For many years HCV could be propagated only in chimpanzees after experimental inoculation with virus containing samples from patients or synthetic *in vitro* transcripts derived from cloned infectious genomes. More recently, transgenic mice

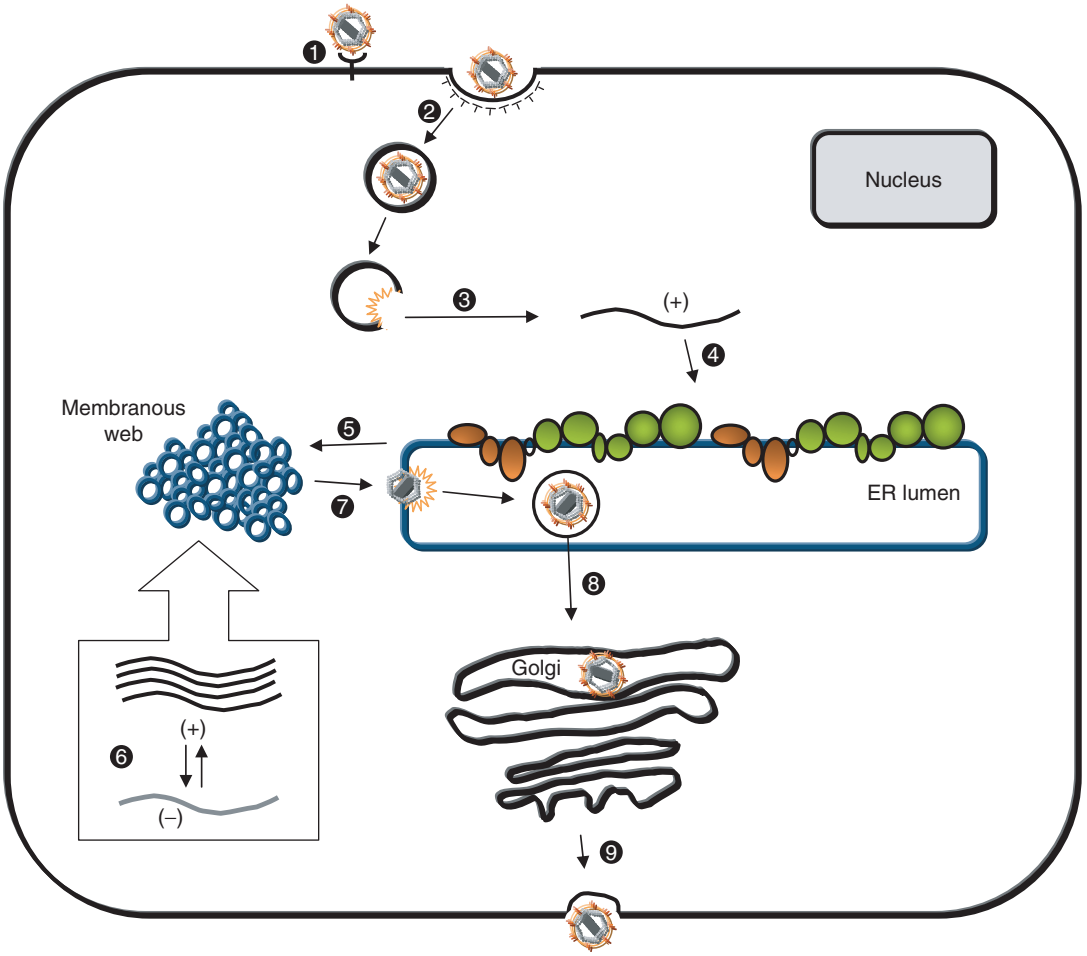


Figure 3 HCV replication cycle. (1) HCV virion binds to one or several receptors on the surface of the cell. Glycosaminoglycans, SR-B1, CD 81, and eventually LDL receptor are required for or contribute to virus binding and entry. (2) The virus particle supposedly enters the cell via clathrin-mediated endocytosis. (3) After a low-pH-mediated fusion step from within an acidic endosome and uncoating, the HCV genome is liberated into the cytoplasm of the host cell. (4) The viral RNA genome is translated at the rough endoplasmic reticulum (rER). (5) The membranous web presumably originating from ER membranes is formed. (6) It is the site of viral RNA amplification which occurs via negative-strand RNA intermediates. Newly synthesized positive-strand RNA is either used for translation or replication or the RNA is packaged into nascent capsids (7). This may occur at the ER where the E-proteins are retained. It is assumed that virions are generated by budding into the ER lumen. (8) Progeny virions are thought to be exported by the secretory pathway and, after fusion of the transport vesicle with the plasma membrane, virions are released (9).

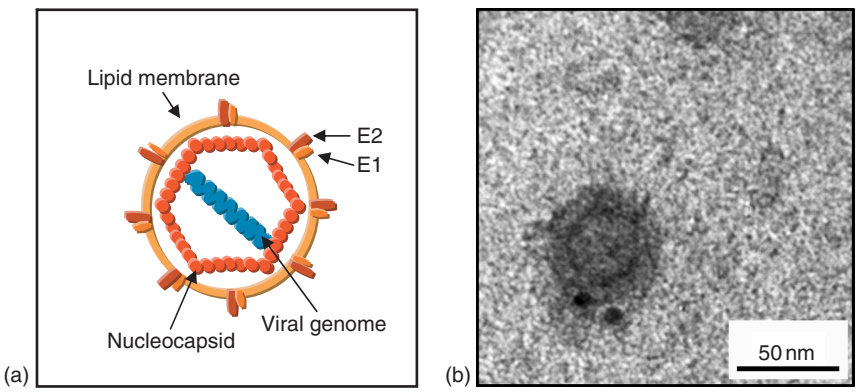


Figure 4 Composition of HCV particles. (a) Schematic of the HCV virion. (b) Electron micrograph of HCV virion produced in cell culture. The spherical particle has an outer diameter of about 60 nm and an inner core of about 30 nm. E2 was detected by immunoelectron microscopy using an E2-specific antibody and a secondary antibody conjugated to 10 nm gold particles.

xenografted with primary human hepatocytes were found to be susceptible to HCV infection. Virus replication occurs in the transplanted human tissue but, given the technical challenge, this mouse model is not widely available. Alternative models to study HCV *in vivo* are the closely related GB-viruses, especially GBV-B that replicates in tamarins (*Sanguis* sp.), causing an acute self-limiting infection of the liver or, under certain experimental conditions, a persistent infection.

Cell Culture Systems

Development of efficient cell culture systems for HCV propagation was difficult and was not successful until more than 10 years after the discovery of the virus. The first breakthrough was the development of subgenomic replicons composed of the NTRs, a selectable marker (*neo*), and a heterologous IRES directing translation of the HCV replicase (NS3–NS5B) (**Figure 5**(**a**)). When transfected into a human hepatocarcinoma cell line (Huh-7) and subjected to selection (e.g., with G418 in case of replicons containing the *neo* gene), stable cell lines were established that carry autonomously replicating HCV replicons. These viral RNAs replicate to very high levels and are maintained persistently when the cells are passaged under conditions of continuous selective pressure (e.g., G418). Owing to its high efficiency, this replicon system was of enormous value for studying HCV replication and HCV–host interaction, and for the development of antivirals targeting any of the viral replicase components (e.g., NS5B RdRp or the NS3 protease).

Since the first description of this system in 1999 numerous improvements have been made. These include the identification of replication-enhancing mutations (so-called adaptive mutations), different replicon formats allowing short-term replication analyses, and high-throughput screening assays. Thus far, replicons from different HCV isolates and two genotypes (1 and 2) are available, as are various cell lines, including two of murine origin.

Studies of the early steps of the HCV replication cycle are often performed using HCV pseudoparticles (HCVpp) (**Figure 5**(**b**)). These are retroviral capsids harboring a retroviral vector RNA into which a reporter gene has been inserted and which are surrounded by a lipid envelope carrying mature HCV E1/E2 glycoprotein complexes. This HCVpp system is an important tool to analyze the infection process and to measure HCV neutralization.

Robust production of infectious HCV particles in cell culture finally became possible in 2005 (**Figure 5**(**c**)). Key to this achievement is a particular HCV isolate designated JFH-1 (abbreviation for Japanese fulminant hepatitis) that was cloned from the serum of a patient with fulminant hepatitis C. When the JFH-1 genome is introduced into Huh-7 cells, virus particles are released that are infectious for naive Huh-7 cells, chimpanzees, and mice with human liver xenografts. Efficiency of this virus system has been increased by the construction of chimeric JFH-1 genomes, cell-culture-adapted virus variants, and by using highly permissive Huh-7 cell clones for HCV replication and entry. Very recently, a highly cell-culture-adapted variant of a genotype 1a HCV isolate has been

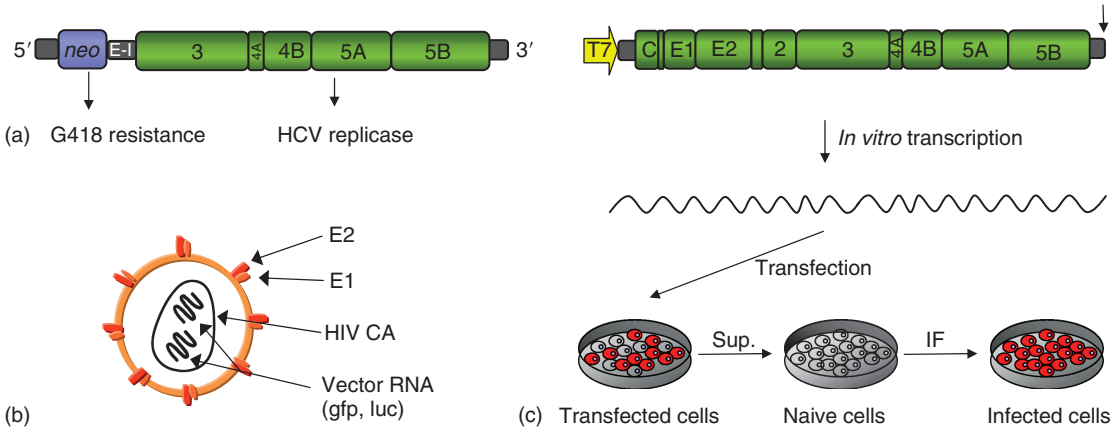


Figure 5 Cell-based systems for HCV. (a) Schematic illustration of the structure of a subgenomic HCV replicon carrying the selectable marker *neo* that confers G418 resistance. Translation of *neo* is directed by the 5′ NTR of HCV whereas translation of the replicase (NS3 to NS5B) is directed by the encephalomyocarditis virus (EMCV)-IRES. (b) Schematic of an HCV pseudoparticle (HCVpp). The retroviral vector RNA carries a reporter gene encoding, for example, for the green fluorescent protein (gfp) or the luciferase (luc). Two copies of vector RNA are packaged into the viral capsids surrounded by a lipid envelope carrying functional E1/E2 complexes. (c) Principle of the HCV infection system. Viral genomes are synthesized by *in vitro* transcription using a plasmid encoding a DNA copy of the viral genome. The T7 promoter at the 5′ end and a ribozyme or a restriction site (arrow) at the 3′ end are used to obtain run off transcripts with authentic termini. *In vitro* transcripts are transfected into Huh-7 cells and virus containing supernatant is transferred onto naive Huh-7 cells. Infection of these cells is detected, for example, by immunofluorescence (IF).

constructed that also supports virus production in Huh-7 cells, but virus titers are extremely low.

HCV–Host Interaction

Replication Factors

Several cellular proteins appear to contribute to HCV replication. For example, the ubiquitously expressed human vesicle-associated membrane protein-associated protein A (VAP-A) and its isoform VAP-B were identified as interaction partners of NS5A and NS5B. NS5A hyperphosphorylation seems to disrupt the VAP-A association and thereby negatively regulates HCV RNA replication. It is thought that VAP-A directs HCV nonstructural proteins to cholesterol-rich, detergent-resistant membranes, which are the presumed sites of HCV RNA replication.

Another host cell factor interacting with NS5A is FBL-2 belonging to the family of proteins that contain an F box and multiple leucine-rich repeats. FBL-2 is modified by geranylgeranylation which is important for NS5A interaction. FBL-2 appears to be required for HCV RNA replication. Likewise, Cyclophilin B (CyPB), a cellular protein interacting with the NS5B RdRp, seems to contribute to replication of genotype 1 isolates by promoting RNA-binding capacity of NS5B. CyPs are a family of peptidyl-prolyl *cis–trans*-isomerases which catalyze the *cis–trans*-interconversion of peptide bonds N-terminal of proline residues, facilitating changes in protein conformation. Cyclosporine or derivatives thereof potently block HCV RNA replication and it is assumed that this is due in part to sequestration of CyPB, which binds to cyclosporine with high affinity.

Also the host cell lipid metabolism plays a fundamental role in the HCV replication cycle. Treatment of replicon cells with lovastatin, an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase, or with an inhibitor of protein geranylgeranyl transferase I induced the disintegration of the HCV replication complex. Fatty acids can either stimulate or inhibit HCV replication, depending on their degree of saturation. Saturated and monounsaturated fatty acids stimulate viral replication, whereas polyunsaturated fatty acids impair replication.

Innate Immunity

Both in cell culture and in the majority of patients treated with IFN-α, a rapid and efficient block of HCV replication occurs. This result is somewhat surprising given the high rate of persistence of HCV infections (50–80%) and the finding that, in infected liver, type 1 IFN-induced genes are activated. In several studies it was concluded that HCV proteins, such as core, interfere with the various steps of the IFN-α/β-induced signaling and that some HCV proteins appear to block individual IFN-α/β-induced effectors. One prominent example is NS5A, assumed to block activity of the double-strand RNA-activated protein kinase PKR by binding to PKR via a particular NS5A region. This region overlaps with the so-called interferon-sensitivity-determining region in NS5A, assumed to correlate with outcome of antiviral therapy. However, this original assumption is still contradictory and it is still unclear whether HCV indeed interferes with one or several type 1 IFN-induced effector molecule(s). It also remains to be clarified by which mechanism interferons block HCV RNA replication.

Much less controversial are the mechanisms by which HCV interferes with the induction of innate antiviral defense. Several studies have shown that the NS3 protease proteolytically cleaves two signal-transducing molecules: TRIF, linking the activation of Toll-like receptor 3 to kinase complexes responsible for the phosphorylation of interferon response factor-3 (IRF-3) and CARDIF (also called MAVS, ips-1, VISA) relaying the activation of retinoic-acid-inducible gene 1 (RIG-1) also to IRF-3 phosphorylation. As a result, IRF-3-dependent genes are not expressed including IFN-β and IRF-7 and cells remain sensitive to virus infection. Although linking the block of IFN-β expression to persistence is attractive, it is unclear if and to what extent that is the case. On one hand blocking IRF-3 activation would not affect the antiviral program induced by type 1 IFN (e.g., produced by activated dendritic cells or administered during therapy), whereas on the other hand this block may affect the secretion of cytokines required for the development of a vigorous adaptive immune response and its attenuation may facilitate persistence.

Adaptive Immunity

The role of HCV-specific antibodies in controlling viral infection is not clear. They appear to be dispensable for viral clearance and do not protect from reinfection, neither in experimentally infected chimpanzees nor in humans after multiple exposure to HCV. However, there is evidence that the presence of HCV-specific antibodies at least partially attenuates infection. For instance, antibodies neutralizing HCV virions of different genotypes have been detected in sera of chronic hepatitis C patients but the frequency of these antibodies appears to be low. Control of acute HCV infection is primarily achieved by a rigorous and multispecific T-cell response. Thus, successful antiviral response generally encompasses multiple major histocompatibility complex (MHC) class-I and class-II restricted T-cell epitopes and a profound expansion of $CD8^+$ and $CD4^+$ T cells. In contrast, persistent infections are characterized by oligoclonal T-cell responses and a low frequency of HCV-specific T cells. The underlying reasons for the weak response

in the majority of patients are not clear but several possibilities have been suggested: (1) an impaired antigen presentation that might be due to interference of HCV with dendritic cell function; (2) $CD4^+$ T-cell failure due to deletion or anergy; (3) mutational escape in important T- (and B-)cell epitopes; and (4) functional impairment of HCV-specific $CD8^+$ T cells. How T-cell impairment is brought about is unclear but one attractive possibility is that the defect induced in innate immunity results in a defect in $CD4^+$ T-cell help. In fact, HCV-induced loss of T-cell help appears to be the key event of immune evasion.

Diagnosis

Routine screening tests for detecting HCV infections are based on serological assays measuring HCV-specific antibodies (most often by enzyme-linked immunosorbent assay (ELISA)) and nucleic acid-based tests to determine viral RNA. Current ELISA assays have a specificity of >99% and they are positive in 99% or more of immunocompetent patients in whom viral RNA is detectable. Given the higher sensitivity, the diagnostic window can be reduced by using nucleic acid-based tests. Qualitative RNA detection assays have been implemented in many blood banks in European Union countries and in the US. The risk to acquire transfusion-associated hepatitis C in such countries has been reduced to <1/million blood donations. Determination of HCV genotypes, which is an important parameter for current antiviral therapy, is based on analyzing viral RNA either by hybridization or direct sequence analysis or by using genotype-specific primers for PCR.

Treatment

Current antiviral therapy is based on the combination of a polyethylene glycol conjugated form of IFN-α with ribavirin resulting in an overall sustained virological response (SVR) of about 60%. However, the success rate depends very much on the genotype of the infecting virus. While up to 85% of genotype 2- and 3-infected patients develop SVR, only about 45% of patients infected with genotype 1 viruses do so. In addition, this therapy has numerous side effects, such as flu-like symptoms, including increased body temperature, headache and muscle pain; neuropsychiatric alterations and hemolytic anemia are also severe side effects of this combination therapy. Thus, therapy often has to be discontinued and many patients are not eligible for this treatment. Numerous efforts are therefore undertaken to develop selective drugs targeting viral functions without causing side effects. The first promising candidates are currently in clinical trials and have shown potent antiviral efficacy. Most advanced are inhibitors of the NS3 protease and the NS5B RdRp. However, monotherapy with these compounds leads to rapid selection for therapy-resistant HCV variants. Future therapy of HCV infection most likely will be based on a combination therapy, which may include a selective drug and IFN.

See also: Hepatitis A Virus; Hepatitis B Virus: General Features.

Further Reading

Appel N, Schaller T, Penin F, *et al.* (2006) From structure to function: New insights into hepatitis C virus RNA replication. *Journal of Biological Chemistry* 281: 9833–9836.

Bartenschlager R (2006) Hepatitis C virus molecular clones: From cDNA to infectious virus particles in cell culture. *Current Opinion in Microbiology* 9: 416–422.

Bartenschlager R, Frese M, and Pietschmann T (2004) Novel insights into hepatitis C virus replication and persistence. *Advances in Virus Research* 63: 71–180.

Bartosch B and Cosset FL (2006) Cell entry of hepatitis C virus. *Virology* 348: 1–12.

Blight KJ, Kolykhalov AA, and Rice CM (2000) Efficient initiation of HCV RNA replication in cell culture. *Science* 290: 1972–1974.

Bowen DG and Walker CM (2005) Adaptive immune responses in acute and chronic hepatitis C virus infection. *Nature* 436: 946–952.

Choo QL, Kuo G, Weiner AJ, *et al.* (1989) Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 244: 359–362.

Foy E, Li C, Sumpter R, *et al.* (2003) Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. *Science* 300: 1145–1148.

Gale M Jr. and Foy M (2005) Evasion of intracellular host defence by hepatitis C virus. *Nature* 436: 939–945.

Lindenbach BD and Rice CM (2005) Unravelling hepatitis C virus replication from genome to function. *Nature* 436: 933–938.

Lindenbach BD, Evans MJ, Syder AJ, *et al.* (2005) Complete replication of hepatitis C virus in cell culture. *Science* 309: 623–626.

Lohmann V, Körner F, Koch J, *et al.* (1999) Replication of subgenomic hepatitis C virus RNAs in a hepatome cell line. *Science* 285: 110–113.

Manns MP, Wedemeyer M, and Cornberg M (2006) Treating viral hepatitis C: Efficacy, side effects, and complications. *Gut* 55: 1350–1359.

Wakita T, Pietschmann T, Kato T, *et al.* (2005) Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nature Medicine* 11: 791–796.

Zhong J, Gastaminza P, Cheng G, *et al.* (2005) Robust hepatitis C virus infection *in vitro*. *Proceedings of the National Academy of Sciences, USA* 102: 9294–9299.

Hepatitis Delta Virus

J M Taylor, Fox Chase Cancer Center, Philadelphia, PA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Antigenome For hepatitis delta virus (HDV), the antigenome refers to an exact complement of the genome. It also is a single-stranded circular RNA.
Editing In recent years it has become clear that many RNAs undergo nucleotide sequence changes relative to the nucleic acid templates from which they are derived. There are many different forms of this process, collectively known as editing. It can occur during or after the process of RNA transcription. HDV RNA undergoes a specific form of post-transcriptional editing in which certain adenosines are deaminated to inosine.
Genome For a virus the genome is that nucleic acid species present within virus particles. For HDV, the genome is a single-stranded circular RNA.
Ribozyme When it was realized that certain RNA molecules could have enzymatic activities similar to certain proteins, such RNAs were defined as ribozymes. Both the genomic and antigenomic RNAs of HDV contain regions that undergo specific self-cleavage, and are thus defined as ribozymes.
Rolling-circle replication For agents that have a circular genome, whether it be of DNA or RNA, there is the possibility that replication can initiate at one or more locations, leading to the synthesis of species longer than the original circle. Such multimers may then undergo processing (e.g., by cleavage and ligation) to form new unit-length products. Such a mechanism, which is referred to as rolling-circle replication, applies to certain agents and has been implicated for the replication of HDV.
Viroid Among the infectious agents of plants there are small circular single-stranded RNAs that seem to replicate without the aid of a helper virus or the synthesis of any encoded protein. These agents do not fulfill the definition of a virus and so have been named viroids.

Classification

Hepatitis delta virus or hepatitis D virus (HDV) was discovered in patients with a more severe form of human hepatitis B virus (HBV) infection. HDV is a subviral satellite of HBV. HDV is often called a virus but strictly speaking it does not satisfy the definition of a virus and should be called subviral; HDV infection and assembly of new virus particles depends upon the envelope protein provided by the natural helper virus, HBV. HDV is also called a satellite of HBV because there is no nucleotide homology between the genomes of HBV and HDV.

No other infectious agents of animals resemble HDV. There are agents in plants that share several important characteristics of RNA genome structure and replication. These plant agents include the viroids and certain satellite RNAs and satellite viruses. Nevertheless, there are also enough major differences between HDV and these plant agents that the International Committee on Taxonomy of Viruses has agreed to assign HDV as a separate genus, *Deltavirus*, with only itself as a member.

Structure

HBV, the natural helper virus of HDV, encodes three envelope proteins. These are also used for the assembly of HDV, but in a different way.

Infectious HBV particles are roughly spherical with a diameter of about 42 nm. Inside these is an icosahedral nucleocapsid of about 27 nm diameter. HBV assembly is inefficient in that infectious particles are found in serum in the presence of a 1000–1 000 000-fold excess of empty particles. These empty particles consist of 25 nm spheres and filaments with a 22 nm diameter and a heterogenous length.

Infectious HDV particles contain a ribonucleoprotein composed of the HDV RNA genome in a complex with more than 70 copies of the delta antigen, the only protein encoded by HDV. It is considered from electron microscopy and filtration studies that infectious HDV particles are spherical and at least 38 nm in diameter. However, such studies are not as clear-cut as for HBV. First, they do not show which particles actually contain RNA. Second, they do distinguish which of the RNA-containing particles are infectious. What seems clear is that the same HBV envelope protein domains are needed for HDV and HBV infectivity. That is, it is likely that for infection HDV uses the same as yet unidentified receptor as HBV.

The internal ribonucleoprotein complex of the HDV RNA genome and delta proteins has been demonstrated but the actual structure has not been clarified yet.

Replication

The RNA genome (**Figure 1**) is a single-stranded RNA of about 1700 nt in length. It is unique for many reasons

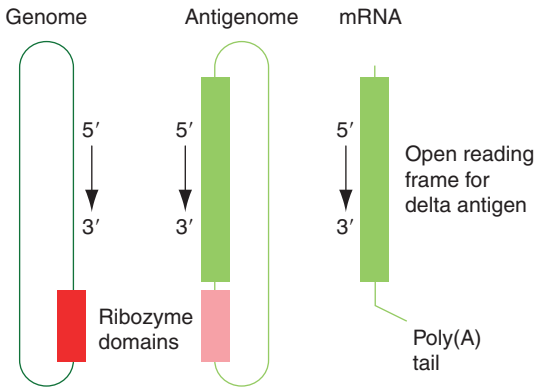


Figure 1 A representation of the three RNAs detected during the replication of HDV. The first is the 1679 nt single-stranded circular RNA found within virions, and thus defined as the genome. The second is an exact complement of the genome, defined as the antigenome, and it along with the genome is found in infected cells. A third RNA, the least abundant, is cytoplasmic, 5′-capped and 3′-polyadenylated, and is the mRNA for the translation of the delta protein. The genome and antigenomic circles are drawn as elongated in order to represent their ability to form extensive intramolecular base pairing and fold into an unbranched rod-like structure. Note that both the genome and the antigenome each contain a domain that will act as a self-cleaving ribozyme.

including its very small size and its circular conformation. The replication takes place by a mechanism that is fundamentally different from the reverse transcription pathway used by the helper virus HBV. HDV replicates with RNA rather than DNA intermediates. Inside infected cells we find not only the circular RNA genome but also an exact complement, the antigenome, which is also circular (**Figure 1**). A third RNA is also complementary to the genome. It is about 800 nt in length, 5′-capped and 3′-polyadenylated, and acts as the mRNA for the translation of the delta protein (**Figure 1**).

HDV genome replication seems to take place in the nucleus and involves redirection of the host RNA polymerase II. Because the genomic RNA is circular, it is possible for transcription to make multimeric RNAs from this template, in what is called a rolling-circle mechanism. Both the genomic and antigenomic RNAs contain a ribozyme that cleaves the RNA at a unique location. A detailed structure for one of these ribozymes has been reported. Ribozyme cleavage allows the processing of the multimeric RNAs into unit-length species, that are then somehow ligated to makes circles. The RNases of animal cells are mainly exonucleases and so circular RNAs are much more stable than the corresponding linear species. In infected liver tissue, the circular genomic RNAs accumulate to around 300 000 copies per average cell.

The mRNA of an infectious HDV needs to be translated into what is known as the small delta protein. This 195 amino acid protein is highly basic. It can dimerize using a region near the N-terminus, through what is known as an antiparallel coiled-coil domain. It can also bind with specificity to HDV RNAs, and localize to the nucleus. For a combination of these and maybe additional reasons, the small delta protein is essential for genome replication. However, during this replication, some of the antigenomic RNAs are post-transcriptionally modified at a specific location, by a form of RNA-editing. The editing involves a double-stranded RNA-specific adenosine deaminase, now known as ADAR. The editing occurs in the middle of the amber termination codon for the open reading frame of the small delta protein. This leads to the translation of a protein that is 19 amino acids longer. This 214-amino-acid long delta protein is a dominant negative inhibitor of genome replication. However, it also has a positive role. Most of it becomes farnesylated at a cysteine located four amino acids from the new C-terminus. This modified protein is essential for the late phase of HDV replication, the assembly of new virus particles, using the envelope proteins of the helper virus, HBV.

Epidemiology

HDV infections are transmitted via infected blood or blood products. There are two main classifications of HDV infection. A co-infection is one in which the individual receives both HBV and HDV at the same time. A superinfection is one in which the individual has a prior infection with HBV, usually a chronic infection, and is then infected with HDV. Such superinfections have a much greater chance of being more extensive and also a greater chance of going on to chronicity.

It was not realized until 1980 that HDV was an infectious agent. Convenient tests for HDV were soon developed and epidemiological information gathered. The initial test was for antibody directed against the delta antigen and then tests were developed for the antigen itself. Now we have much more sensitive tests for the RNA, but such tests are not routinely used.

HDV was first studied among populations in southern Italy. Of course it is only found in situations where HBV is also present, but the converse is not always true. There are geographic populations in which HBV infections are more likely to be associated with HDV. Data indicate that the fraction of HBV infections that are also HDV-associated can range from $<1\%$ to $>10\%$. The high levels have been reported for areas such as the Amazon basin in South America and southern regions of the former Soviet Union. Also, within a geographic population there may be individuals, such as intravenous drug users who share needles, where the fraction is very high, $>70\%$.

Levels of HDV infection have been greatly reduced in recent years. This is in part due to changes in the behavior of susceptible individuals, especially the intravenous drug users, and also due to screening of blood supplies.

In addition, the introduction of widespread immunization against HBV, which simultaneously protects against HDV, will ultimately reduce both the number of chronic HBV carriers and HDV infections.

Clinical Features

The replication of HBV is not directly cytopathic. The liver damage arises because of the involvement of the host immune response to these infections. The same is probably also true for HDV. In addition, there are experimental situations where HDV infections can be directly cytopathic.

In certain geographic areas, such as the Amazon basin, HDV infections can have a very high risk of producing a fulminant hepatitis, sometimes with death in less than 1 week.

Control

Immunization against the natural helper virus, HBV, confers protection against HDV. However, for nonimmunized individuals and especially for those already chronically infected with HBV, the risks remain high.

For an individual infected with HDV, there is no good therapy that directly targets the HDV infection. In one study, treatment with high doses of interferon was associated with some cures. Of course the interferon therapy also attacks the HBV infection.

Recently, antiviral therapies based upon inhibitors of the HBV reverse transcriptase are being directed against HBV infections. One might expect that these therapies would indirectly inhibit cycles of HDV replication, but such has not been the case.

See also: Hepatitis B Virus: General Features; Hepatitis B Virus: Molecular Biology.

Further Reading

Casey JL (ed.) (2006) *Current Topics in Microbiology and Immunology, Vol. 307: Hepatitis Delta Virus.* Berlin: Springer.
Farci P, Roskams T, Chessa L, *et al.* (2004) Long-term benefit of interferon alpha therapy on chronic hepatitis D: Regression of advanced hepatic fibrosis. *Gastroenterology* 126: 1740–1749.
Handa H and Yamaguchi Y (eds.) (2006) *Hepatitis Delta Virus.* Georgetown, TX: Landes Bioscience.
Taylor JM (2006) Hepatitis delta virus. *Virology* 344: 71–76.

Hepatitis E Virus

X J Meng, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Animal reservoir A large animal population serving as a source of virus supply for the transmission of virus(es) to other animals including humans.
Enzootic An epidemiology term describing multiple continuous disease presence in an animal population in a defined geographic region and time period.
Zoonosis Infectious diseases transmissible under natural conditions from vertebrate animals to humans.

Introduction

The initial evidence for the existence of a new form of enterically transmitted viral hepatitis came from serological studies of waterborne epidemics of hepatitis in India in 1980, as cases thought to be hepatitis A were tested negative for antibodies to the hepatitis A virus (HAV). To conform to the standard nomenclature of viral hepatitis, this new form of viral hepatitis was named hepatitis E. In 1983, viral hepatitis E was successfully transmitted to a human volunteer via fecal–oral route with a stool sample collected from a non-A, non-B hepatitis patient, and virus-like particles were visualized by electron microscope in the stool of the infected volunteer. Subsequently, the agent was also successfully transmitted to nonhuman primates. However, the identity of the virus was not known until 1990 when the genomic sequence of the new virus, named hepatitis E virus (HEV), was determined. The recent identification and characterization of animal strains of HEV, swine hepatitis E virus (swine HEV) from pigs in 1997 and avian hepatitis E virus (avian HEV) from chickens in 2001, have broadened the host ranges and diversity of the virus, and also provided two unique homologous animal model systems to study HEV replication and pathogenesis.

Convincing evidence indicates that hepatitis E is a zoonotic disease, and pigs and perhaps other animal species are reservoirs for HEV.

Properties and Structure of Virions

HEV is a symmetrical, icosahedral, spherical virus particle of approximately 32–34 nm in diameter without an envelope (**Figure 1**). The capsid protein encoded by the open reading frame (ORF) 2 gene of HEV is the only known structural protein on the virion. The N-terminal truncated capsid protein, which contains amino acid residues 112–660, can self-assemble into empty virus-like particles when expressed in baculovirus. The buoyant density of HEV virions is reportedly 1.35–1.40 g cm^{-3} in CsCl, and 1.29 g cm^{-3} in potassium tartrate and glycerol. The virion sedimentation coefficient is 183 S. HEV virion is sensitive to low-temperature storage and iodinated disinfectants but is reportedly stable when exposed to trifluorotrichloroethane. HEV virion is more heat labile than is HAV, another enterically transmitted hepatitis virus. HAV was only 50% inactivated at 60 °C for 1 h but was almost totally inactivated at 66 °C. In contrast, HEV was about 50% inactivated at 56 °C and almost totally inactivated (96%) at 60 °C. The fecal–oral route of transmission indicates that HEV is resistant to inactivation by acidic and mild alkaline conditions in the intestinal tract.

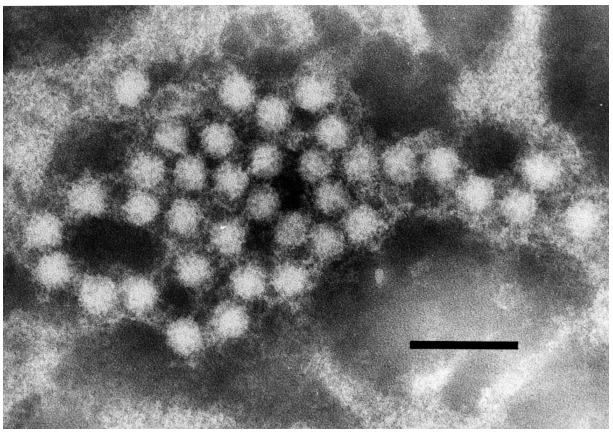


Figure 1 Electron micrograph of 30–35 nm diameter particles of an avian strain of the HEV. The virus particles were detected from a bile sample of a chicken with hepatitis–splenomegaly syndrome. Scale = 100 nm. Reproduced from Haqshenas G, Shivaprasad HL, Woolcock PR, Read DH, and Meng XJ (2001) Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis–splenomegaly syndrome in the United States. *Journal of General Virology* 82: 2449–2462, with permission from Society for General Microbiology.

Taxonomy and Classification

HEV was initially classified in the family *Caliciviridae* on the basis of its superficial similarity in morphology and genomic organization to caliciviruses. However, subsequent studies revealed that the HEV genome does not share significant sequence homology with caliciviruses, and that the codon usage and genomic organization of HEV are also different from that of caliciviruses. Therefore, in the *Eighth Report of the International Committee on Taxonomy of Viruses* (ICTV), HEV was declassified from the family *Caliciviridae*, and was placed in the sole genus *Hepevirus*. The proposed family name *Hepeviridae* has yet to be officially approved by ICTV.

The species in the genus *Hepevirus* includes the four recognized major genotypes of HEV (**Figure 2**): genotype 1 (primarily Burmese-like Asian strains), genotype 2 (a single Mexican strain), genotype 3 (strains from rare endemic cases in the United States, Japan, and Europe, and swine strains from pigs in industrialized countries), and genotype 4 (variant strains from sporadic cases in Asia, and swine strains from pigs in both developing and industrialized countries). A tentative new species in the genus *Hepevirus* is the recently discovered avian HEV from chickens (**Figure 1**). The nucleotide sequence identity differs by 20–30% between genotypes, and by *c.* 40–50% between avian HEV and mammalian HEVs. Despite the extensive nucleotide sequence variations, however, it appears that there exists a single serotype of HEV.

Genome Organization and Gene Expression

The genome of HEV, which possesses a cap structure at its 5′ end, is a single-stranded, positive-sense RNA molecule of approximately 7.2 kb in length. The viral genome consists of a short 5′ noncoding region (NCR) of approximately 26 bp, three open reading frames (ORFs 1–3), and a 3′ NCR. ORF3 overlaps ORF2, but neither ORF2 nor ORF3 overlaps with ORF1 (**Figure 3**). The ORF1 at the 5′ end encodes viral nonstructural proteins that are involved in viral replication and protein processing.

Functional motifs, characteristic of methyltransferases, papain-like cystein proteases, helicases, and RNA-dependent RNA polymerases (**Figure 3**), were identified in ORF1 based upon sequence analyses and analogy with other single-strand positive-sense RNA viruses. ORF2, located at the 3′ end, encodes the major capsid protein that contains a typical signal peptide sequence, and three potential glycosylation sites. The capsid protein contains the most immunogenic epitope, induces neutralizing antibodies against HEV, and is the target for HEV vaccine development. ORF3 encodes a small

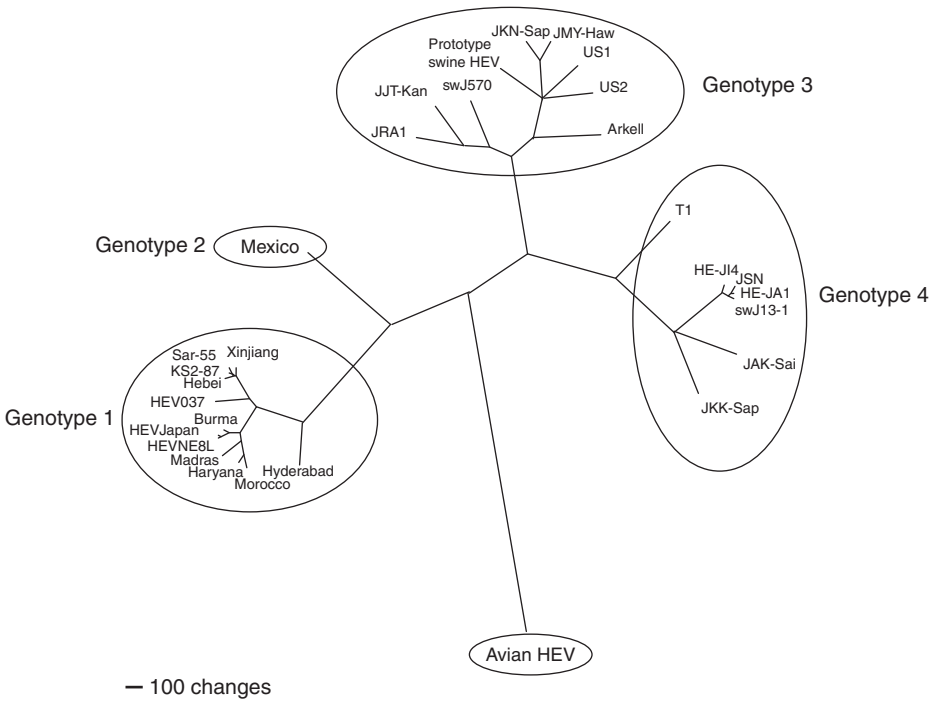


Figure 2 A phylogenetic tree based on the complete genomic sequences of available human, swine, and avian HEV strains. The tree was constructed with the aid of the PAUP program by using heuristic search with 1000 replicates. A scale bar, indicating the number of character state changes, is proportional to the genetic distance. The four recognized major genotypes of HEV are indicated. The avian HEV in a branch distinct from mammalian HEV is tentatively classified as a separate species. Reproduced from Huang FF, Sun ZF, Emerson SU, *et al.* (2004) Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. *Journal of General Virology* 85: 1609–1618, with permission from Society for General Microbiology.

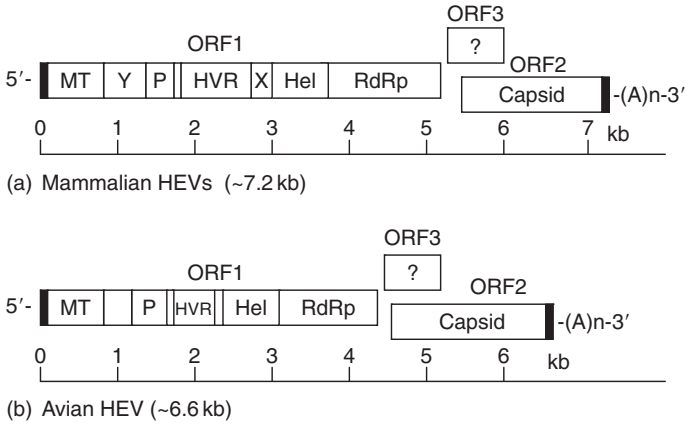


Figure 3 Schematic diagram of the genomic organization of HEV: a short 5′ (solid box at the 5′ end), a 3′ NCR (solid box at the 3′ end), and three ORFs. ORF2 and ORF3 overlap each other but neither overlaps ORF1. ORF1 encodes nonstructural proteins including putative functional domains, ORF2 encodes putative capsid protein, and ORF3 encodes a small protein with unknown function. MT, methytransferase; X, 'X' domain; Y, 'Y' domain; P, a proline-rich domain that may provide flexibility; HVR, a hypervariable region; Hel, helicase; RdRp, RNA-dependent RNA polymerase.

cytoskeleton-associated phosphoprotein. The N-terminus of ORF3 has a cysteine-rich region, binds to HEV RNA, and enters into a complex with the capsid protein. The C-terminal region of the ORF3 protein is a multifunctional domain, and may be involved in HEV virion morphogenesis and viral pathogenesis. Antibodies to ORF3 protein have been detected in some animals (rhesus monkeys and chickens) experimentally infected

with HEV; however, it remains unknown if ORF3 is a structural protein in the virion.

The transcription and translation mechanisms of HEV genes are still poorly understood, largely due to the lack of an efficient cell culture system for the propagation of HEV. By using an *in vitro* HEV replicon system, a bicistronic subgenomic mRNA was identified and found to encode both ORF2 and ORF3 proteins. Subgenomic mRNAs were also detected in infected liver tissues. The ORF3 gene contains a *cis*-reactive element and encodes a protein that is essential for virus infectivity *in vivo*. However, recent studies showed that the expression of ORF3 protein is not required for virus replication, virion assembly, or infection of hepatoma cells *in vitro*. The translation and post-translational processes of the ORF1 polyprotein remain largely unknown. When expressed in baculovirus, the nonstructural ORF1 polyprotein was found to process into smaller proteins that correlate with predicted functional domains in ORF1. However, no processing of the ORF1 polyprotein was detected when it was expressed in bacterial or mammalian expression systems.

Epidemiology

HEV is transmitted primarily through the fecal–oral route, and contaminated water or water supplies are the main sources of infection. Hepatitis E, generally affecting young adults, is an important public health disease in many developing countries of the world where sanitation conditions are poor. Rare epidemics are associated with fecal contamination of water, and are generally restricted in developing countries of Asia and Africa, and in Mexico. Most cases of acute hepatitis E occurred as endemic or sporadic forms worldwide in both industrialized and developing countries. The genotypes 1 and 2 HEV strains are associated with epidemics, whereas genotypes 3 and 4 strains cause sporadic cases of hepatitis E. A unique feature of HEV infections is the high mortality rate, up to 25%, in infected pregnant women, although the mechanism of fulminant hepatitis during pregnancy is still not known.

Seroprevalence of HEV antibodies is age dependent, with the highest rate occurring in young adults of 15–40 years of age. In endemic countries, the seroprevalence rate, generally ranging from 3% to 27%, is much lower than expected, although higher seroprevalence rates have been reported in some endemic countries such as Egypt. Surprisingly, a significant proportion of normal blood donors in the United States (4–36%) and other industrialized countries were tested seropositive for immunoglobulin G (IgG) anti-HEV antibodies, suggesting prior infections with HEV (or an antigenically related agent). The source of seropositivity in individuals from industrialized countries is not clear but a zoonotic origin via direct contact with infected animals or consumption of raw or undercooked animal meats has been suggested.

Swine HEV

The identification and characterization of swine HEV from pigs in the United States lend credence to the zoonosis concept for HEV. Since the initial report in 1997, swine HEV has now been identified from pigs in more than a dozen countries. Thus far, the viruses identified from pigs worldwide belong to either genotype 3 or 4, which both cause sporadic cases of acute hepatitis E. Recently, a genotype-1-like swine HEV was reportedly identified from a pig in Cambodia but independent confirmation of this finding is still lacking. Genetic and phylogenetic analyses of the complete genomic sequences of swine HEV revealed that swine HEV is very closely related, or identical in some cases, to the genotype 3 and 4 strains of human HEV (**Figure 2**). Seroepidemiological studies demonstrated that swine HEV is ubiquitous in pigs in the Midwestern United States, and that about 80–100% of the pigs in commercial farms in the United States were infected. Similar findings were also reported in more than a dozen other developing and industrialized countries, indicating that swine HEV infection in pigs is common worldwide. The ubiquitous nature of swine HEV infection in pigs provides a source of virus supply for zoonotic infection. Swine HEV infection generally occurs in pigs of 2–4 months of age. The infected pigs remain clinically normal, although microscopic evidence of hepatitis has been observed.

Avian HEV

Avian HEV was first isolated and characterized in 2001 from bile samples of chickens with hepatitis–splenomegaly (HS) syndrome in the United States, although a big liver and spleen disease virus (BLSV) with 62% nucleotide sequence identity to HEV (based on a 523 bp genomic region) was reported in 1999 from chickens in Australia. HS syndrome is an emerging disease of layer and broiler breeder chickens in North America characterized by increased mortality and decreased egg production. Dead birds have red fluid or clotted blood in their abdomens, and enlarged livers and spleens. Avian HEV shared *c.* 80% nucleotide sequence identity with the Australian BLSV, suggesting that big liver and spleen disease (BLS) in Australia and HS syndrome in North America are caused by variant strains of the same virus.

Avian HEV is morphologically, genetically, and antigenically related to human HEV. The complete genomic sequence of avian HEV is about 6.6 kb in length (**Figure 3**), which is about 600 bp shorter than that of human and

swine HEVs. Although avian HEV shares only *c.* 50–60% nucleotide sequence identity to mammalian HEVs, the genomic organization, functional motifs, and common antigenic epitopes are conserved between avian and mammalian HEVs (**Figure 3**). Phylogenetic analyses indicated that avian HEV is distinct from mammalian HEV (**Figure 2**) and may represent either a fifth genotype of HEV or a separate species within the genus *Hepevirus*. Like swine HEV, avian HEV infection in chickens is widespread in the United States: *c.* 71% of chicken flocks and 30% of chickens are positive for IgG antibodies to avian HEV. Like human and swine HEVs, avian HEV antibody prevalence is also age dependent: *c.* 17% of chickens younger than 18 weeks are seropositive, as compared to *c.* 36% of adult chickens. Avian HEV isolates recovered from chickens with HS syndrome in the United States displayed 78–100% nucleotide sequence identities to each other, and 56–61% identities to known strains of human and swine HEVs.

Host Range and Cross-Species Infection

Swine HEV has been shown to cross species barriers and infect both rhesus monkey and chimpanzee. Infection of nonhuman primates, the surrogates of man, with swine HEV demonstrated the possibility of human infection with swine HEV. Conversely, genotype 3 human HEV has been shown to infect pigs under experimental conditions. However, attempts to experimentally infect pigs with genotypes 1 and 2 strains of human HEV were unsuccessful. Cross-species infection of HEV has also been reported in other animal species. Lambs were reportedly infected with human HEV isolates. Similarly, Wistar rats were reportedly infected with a human stool suspension containing infectious HEV, although others failed to confirm the rat transmission results. Cross-species infection by avian HEV has also been demonstrated, as avian HEV recovered from a chicken with HS syndrome was able to successfully infect turkeys. However, an attempt to experimentally infect two rhesus monkeys with avian HEV was unsuccessful. Thus, it appears that, unlike swine HEV, avian HEV may not readily infect humans.

There is potentially a wide host range of HEV infection. In addition to swine and chickens, antibodies to HEV have been reportedly detected in several other animal species including rodents, dogs, cats, sheep, goats, cattle, and nonhuman primates, suggesting that these animals have been exposed to HEV (or a related agent) and thus might serve as reservoirs. However, the source of seropositivity in these animal species, with the exception of pigs and chickens, could not be definitively identified since virus was either not recovered from these species or the recovered virus could not be sequenced to confirm its identity. A significant proportion of wild rats caught in different geographic regions of the United States (ranging from 44% to 90%) and other countries tested positive for IgG anti-HEV antibodies. Rodents are frequently found in both urban and rural environments and, thus, could potentially play an important role in HEV transmission. The existence of a wide host range of animal species positive for HEV antibodies further supports the zoonosis concept for HEV.

Pathogenesis and Tissue Tropism

The pathogenesis of HEV is largely unknown. It is believed that HEV enters the host through the fecal–oral route. However, the primary site of HEV replication is not known. In primates and pigs experimentally infected with human and swine HEVs, virus replication in the liver has been demonstrated. It is believed that, after replication in liver, HEV is released to the gallbladder from hepatocytes and then is excreted in feces. In pigs experimentally infected with human and swine HEVs, in addition to the liver, HEV replication was also identified in extrahepatic tissues including small intestine, colon, hepatic, and mesenteric lymph nodes. Although the clinical and pathological significance of these extrahepatic sites of virus replication is not known, it is believed that HEV may first replicate in the gastrointestinal tract after oral ingestion of the virus, and subsequently spreads to the target organ liver via viremia. It has been well documented that pregnancy increases the severity and mortality of the disease. The overall mortality rate caused by HEV in infected pregnant women can reach up to 25%, although, under experimental conditions, fulminant hepatitis E could not be reproduced in infected pregnant rhesus monkeys or infected pregnant sows. Therefore, the mechanism of fulminant hepatitis E in infected pregnant women remains unknown.

Zoonotic Risk

Hepatitis E is now considered a zoonotic disease, and pigs (and possibly other animal species) act as reservoirs. It has been demonstrated that pig handlers such as pig farmers and swine veterinarians in both developing and industrialized countries are at increased risk of HEV infection. For example, swine veterinarians in the United States are 1.51 times (swine HEV antigen, $p = 0.03$) more likely to be positive for HEV antibodies than age- and geography-matched normal blood donors. Also, it has been reported that HEV antibody prevalence in field workers from the Iowa Department of Natural Resources is significantly higher than that in blood donors, suggesting that human populations with occupational exposure to wild animals also have a higher risk of HEV infection. Transmissions of

hepatitis E from a pet cat and a pet pig to human owners were also reported.

As a fecal–orally transmitted disease, waterborne epidemics are the characteristic of hepatitis E outbreaks in humans. Large amounts of viruses are excreted in feces of infected pigs and other animals. Therefore, swine and other animal manure and feces could be a source of contamination of irrigation water or coastal waters with concomitant contamination of produce or shellfish. Strains of HEV of both human and swine origin have been detected in sewage water. Consumption of contaminated shellfish has been implicated in sporadic cases of acute hepatitis E. Sporadic and cluster cases of acute hepatitis E have also been epidemiologically and genetically linked to the consumption of raw or undercooked pig livers. Approximately 2% of the pig livers sold in local grocery stores in Japan and 11% in the United States were tested positive for swine HEV RNA. The contaminating virus in commercial pig livers sold in the grocery stores of the United States remains fully infectious. Most importantly, the virus sequences recovered from pig livers in grocery stores are closely related, or identical in a few cases, to the viruses recovered from human hepatitis E patients in Japan. Recently, a cluster of four cases of acute hepatitis E were definitively linked to the consumption of raw deer meat in two families in Japan. The viral sequence recovered from the leftover frozen deer meat was 99.7–100% identical to the nucleotide sequence of viruses recovered from the four human patients. Family members who ate none or little deer meats were not infected. Taken together, these data provide convincing evidence of zoonotic HEV transmission either via direct contact with animals or animal wastes or via consumption of infected animal meats. Recently, numerous novel strains of HEV genetically closely related to swine HEV have been identified from patients with acute hepatitis E in many countries, suggesting that these novel strains of human HEV may be of swine origin.

Transmission and Natural History

The primary transmission route for HEV is fecal–oral. Feces from infected humans and other animals contain large amounts of viruses and are likely the main source of virus for transmission. However, under experimental conditions, reproduction of HEV infection in nonhuman primates and pigs via the oral route of inoculation has proven to be difficult, even though the animals can be readily infected by HEV via the intravenous route of inoculation. Chickens were successfully infected via the oral route of inoculation with avian HEV. Other route(s) of transmission cannot be ruled out.

The natural history of HEV is not known. The identification of HEV strains from swine and chickens and their demonstrated ability of cross-species infection and the existence of a wide host range of seropositive animals has further complicated the understanding of HEV natural history. Since human-to-human transmission is uncommon for HEV, therefore a speculative scenario is that HEV is constantly circulating among different animal species, and that each species is infected by a unique strain that is enzootic in respective animal species. Some species, but not all, carry HEV strains that are genetically very similar to human HEV and are transmissible to humans. Therefore, HEV can infect humans through direct contact with infected domestic or farm animals or with feces-contaminated water or via consumption of infected raw or undercooked animal meats. If this happens in a developing country, it may result in an endemic or epidemic due to the poor sanitation conditions. However, if this happens in an industrialized country with good sanitation measures, it will occur only as a sporadic case. It will now be important to determine whether there are basic differences in host range or pathogenicity between epidemic or endemic strains in humans and enzootic strains that are mainly circulating in animal species.

Immunity

The immune response to HEV infection, characterized by a transient appearance of immunoglobulin M (IgM) HEV antibodies followed by long-lasting IgG antibodies, appears late during the period of viremia and virus shedding in stool. The HEV capsid protein is immunogenic and induces protective immunity. The capsid proteins between mammalian and avian HEV strains share common antigenic epitopes, and all HEV strains identified thus far appear to belong to a single serotype. Cross-challenge experiments in primates have demonstrated cross-protection following infection with different genotypes of human HEV strains. The cell-mediated immune response against HEV infection is largely unknown.

Prevention and Control

A vaccine against HEV is not yet available. The experimental recombinant HEV vaccines appear to be very promising; however, their efficacies against the emerging strains of HEV including animal strains with zoonotic potential need to be thoroughly evaluated. In the absence of a vaccine, important preventive measures include the practice of good hygiene, and avoiding drinking water of unknown purity or consuming raw or undercooked pig livers. The demonstrated ability of cross-species infection by swine HEV raises a public health concern, especially for high-risk groups such as pig handlers. An effective

measure to prevent potential zoonotic transmission for pig and other animal handlers is to wash hands thoroughly after handling infected animals.

See also: Hepatitis A Virus.

Further Reading

Emerson SU, Anderson D, Arankalle A, *et al.* (2004) Hepevirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 851–855. San Diego, CA: Elsevier Academic Press.

Emerson SU and Purcell RH (2003) Hepatitis E virus. *Reviews in Medical Virology* 13: 145–154.

Haqshenas G, Shivaprasad HL, Woolcock PR, Read DH, and Meng XJ (2001) Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis–splenomegaly syndrome in the United States. *Journal of General Virology* 82: 2449–2462.

Huang FF, Sun ZF, Emerson SU, *et al.* (2004) Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. *Journal of General Virology* 85: 1609–1618.

Meng XJ (2000) Novel strains of hepatitis E virus identified from humans and other animal species: Is hepatitis E a zoonosis? *Journal of Hepatology* 33: 842–845 (editorial).

Meng XJ (2003) Swine hepatitis E virus: Cross-species infection and risk in xenotransplantation. *Current Topics in Microbiology and Immunology* 278: 185–216.

Meng XJ (2005) Hepatitis E as a zoonosis. In: Thomas H, Zuckermann A, and Lemon S (eds.) *Viral Hepatitis,* 3rd edn., pp. 611–623. Oxford: Blackwell.

Meng XJ and Halbur PG (2005) Swine hepatitis E virus. In: Straw BE, Zimmerman J, D' Allaire S, and Taylor DJ (eds.) *Diseases of Swine,* 9th edn., pp. 537–545. Ames, IA: Blackwell/Iowa State University Press.

Meng XJ, Purcell RH, Halbur PG, *et al.* (1997) A novel virus in swine is closely related to the human hepatitis E virus. *Proceedings of the National Academy of Science, USA* 94: 9860–9865.

Meng XJ, Shivaprasad HL, and Payne C (in press) Hepatitis E virus infections. In: Saif M (ed.) *Diseases of Poultry*, 12th edn., ch. 14. Ames, IA: Blackwell/Iowa State University Press.

Purcell RH and Emerson SU (2001) Hepatitis E virus. In: Knipe D, Howley P, Griffin D, *et al.* (eds.) *Fields Virology,* 4th edn., pp. 3051–3061. Philadelphia: Lippincott Williams and Wilkins.

Herpes Simplex Viruses: General Features

L Aurelian, University of Maryland School of Medicine, Baltimore, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

History

The word 'herpes' as applied to spreading, ulcerative skin manifestations is traced to Hippocrates. In 1736, Jean Astruc classified the condition as sexually transmitted. Its infectious nature was demonstrated in 1921, when lesion material was serially transmitted in rabbits. In 1929 Goodpasture concluded that a latent state is established in ganglionic neurons, and in the 1930s it became evident that the manifestation is gingivostomatitis in seronegative subjects and recurrent 'fever blisters' in those who are seropositive. We now know that two distinct viruses preferentially cause facial or genital lesions. A critical advance has been the development of antiviral chemotherapy.

Classification

Herpes simplex virus serotypes 1 and 2 (HSV-1 and HSV-2) are members of the family *Herpesviridae.* They belong to the subfamily *Alphaherpesvirinae* and the genus *Simplexvirus.* Features of the *Alphaherpesvirinae* include a variable host range, relatively short reproductive cycle, rapid spread in culture, efficient destruction of infected cells, and latency establishment in sensory ganglia.

Structure

Virus particles are 150–200 nm in diameter and consist of four components: core, capsid, tegument, and envelope (**Figure 1(b)**). The core contains the 152 kbp linear, double-stranded DNA genome. It has two covalently linked components consisting of unique sequences (U_L and U_S) bracketed by inverted repeats (**Figure 1(a)**). Open reading frames (ORFs) are located within the unique and repeat sequences, the latter in two copies per genome. Some genes (e.g., R1 (large subunit of ribonucleotide reductase) and $\gamma_1$34.5) contain regions with cellular homology, and were presumably captured during evolution.

The capsid is a 100–110 nm protein shell with $T = 16$ icosahedral symmetry (**Figure 1(c)**). The faces and edges of the icosahedron are formed by 150 hexons. The vertices are made by pentons. The triplexes are present in the threefold positions between hexons and between hexons and pentons. One of the 12 vertices is believed to be the portal complex through which the genome enters and leaves the capsid, replacing one of the pentons. Over 30 proteins are in the mature particle, eight of which form the capsids. VP5 forms the bulk of the pentons and hexons. VP26 occupies the outer surfaces of the hexons. VP19C and VP23 form the triplexes, which stabilize the

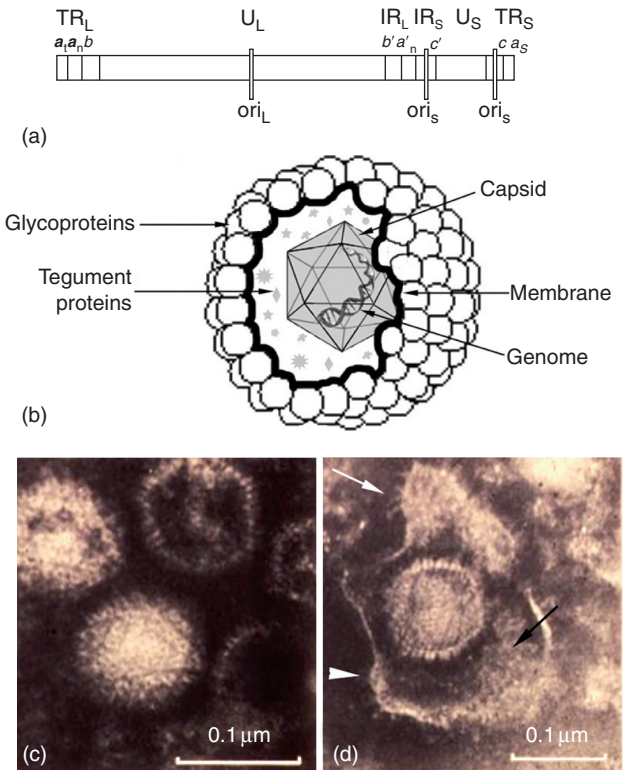


Figure 1 Structure. (a) The HSV genome has two covalently linked components consisting of unique sequences (U_L and U_S) bracketed by inverted repeats (IR_L/TR_L and IR_S/TR_S) and contains three origins of replication (one Ori_L and two Ori_S). (b) The virus particle consists of four components: core, capsid, tegument, and envelope. (c) Negatively stained virus capsids. Capsomers are $9.5 \times 12.5\,nm^2$ (longitudinal section) and evidence a 4 nm in diameter channel that runs from the surface along the long axis. (d) Enveloped virus particle. The capsid is surrounded by an asymmetrical fibrous-like tegument (black arrow). The envelope (arrowhead) is decorated with spikes projecting from its surface that consist of glycoproteins (white arrow).

capsid shell through interactions with adjacent VP5 molecules. The tegument surrounds the capsid. It is asymmetrically distributed and may appear fibrous on negative staining. Tegument proteins are involved in initiating virus replication (e.g., the transactivator VP16) and inhibiting host cell translation (e.g., the virion host shutoff (vhs) protein). The envelope is the outer covering, which is acquired from cellular membranes. It is decorated with spikes that contain viral glycoproteins (**Figure 1(d)**).

Host Range

Humans are the natural host, but HSV also infects experimental animals and embryonated chicken eggs. The animal species, virus type, route of inoculation, and state of immune competence affect the outcome of infection. In the mouse, intracerebral, footpad, intranasal, and intraperitoneal inoculations are used as models of human fatal neurological or visceral disease. Eye infection is used as a model of encephalitic and latent disease. Certain mouse strains (viz., C57BL/6) exhibit natural resistance, while newborn mice are particularly sensitive. Skin infection was described in mice, rabbits, and guinea pigs, the latter providing the best approximation of human recurrent disease. A wide variety of cell lines support HSV growth. Human primary and secondary cultures are generally more sensitive than established nonhuman cell lines and are used for virus isolation.

Virus Replication

The replicative cycle is represented schematically in **Figure 2**.

1. *Entry.* Virus glycoproteins gC and/or gB bind heparan sulfate chains on the cell surface, followed by fusion of the viral envelope with the cell membrane. Fusion requires viral glycoproteins gB, gD, gH, and gL and one cellular entry receptor that binds gD. Receptors include the herpesvirus entry mediator (HVEM), a member of the tumor necrosis factor receptor superfamily, and nectins 1 and 2, which are members of the immunoglobulin superfamily. The same receptor may also initiate endocytosis, in a cell-type specific context. A cellular entry receptor that binds gB was postulated.

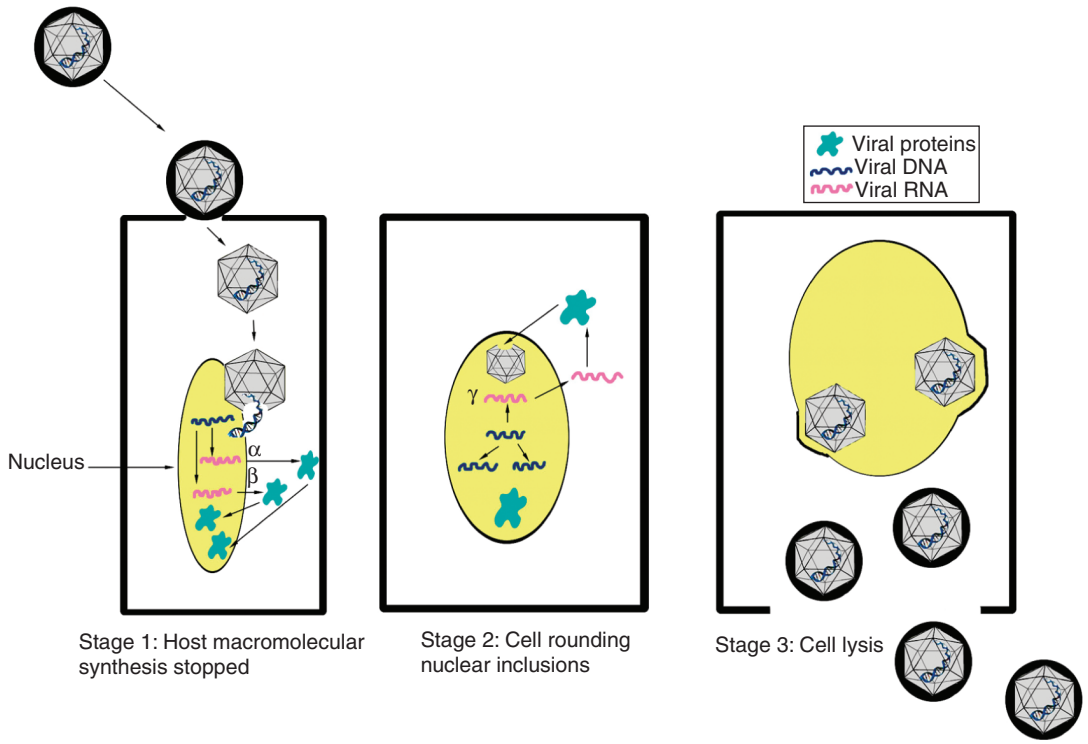


Figure 2 Replicative cycle. Schematic representation includes temporally regulated transcription of IE (α), E (β), and L (γ) genes and the stages of cellular alterations.

2. *Transcription.* Entering capsids dock at the nuclear pore complex and release their genomes. The viral DNA traverses the nuclear pore channel as a condensed, densely packed rod-like structure. In the nucleus, the HSV-1 genomes associate with promyelocytic leukemia protein nuclear bodies (or nuclear domain 10 (ND10)), giving rise to virus replication compartments. The genome encodes up to 84 proteins, and 38 HSV-1 ORFs are essential for virus replication in cell culture. HSV transcripts can have common initiation signals but different termination sites, different initiation sites but co-terminal ends, or differ in their initiation and termination sites but have partially collinear sequences. At least three gene pairs are known to occupy both DNA strands at complementary positions, generating antisense transcripts, the expression of which is mutually exclusive. Only a few HSV mRNAs are spliced (e.g., ICP0).

Three classes of genes are coordinately transcribed and temporally regulated during lytic infection: immediate early (IE), early (E), and late (L) (also known as α, β, and γ, respectively). IE (α) genes (e.g., ICP4, ICP27, ICP0, and ICP22) are transcribed first and play crucial roles in the regulation of productive infection. ICP4 is required for transcription of the E (β) and L (γ) genes. Early after infection, ICP27 co-localizes with ICP4 and has transcriptional and post-transcriptional regulatory activities. Late in infection, it functions in the processing and efficient export of viral mRNAs. ICP0 is not essential, but it is a major transactivator of HSV gene expression and stimulates virus growth, particularly in cells infected at a low multiplicity of infection (moi). ICP22 and its in-frame N-terminal truncation US1.5 are required for virus replication in most cell types. E gene products include enzymes (e.g., thymidine kinase (TK) and DNA polymerase (Pol)), which are required for virus DNA replication. Their expression requires functional IE genes. An exception is R1, which is regulated with IE kinetics. HSV-2 R1 responds to cellular AP-1 transcription factors. L gene expression requires viral DNA synthesis.

The promoters of the IE and R1 genes contain reiterated core enhancer elements (TAATGARAT) that are targeted by VP16 in conjunction with the cellular protein Oct-1 and the transcriptional co-activator HCF-1 in order to mediate transcription. The presence of VP16 is associated with the recruitment of transcription factors (e.g., TFIIB and TFIIH) and the TATA-binding protein (TBP), and assembly of an RNA polymerase II preinitiation complex. Oct-1 is critical for virus replication at low moi.

3. *DNA replication.* Upon reaching the nucleus, the viral genome circularizes and serves as template for DNA replication. DNA replication initiates in an origin-independent manner and continues through an origin-dependent process, perhaps by a rolling-circle and/or homologous recombination-driven mechanism. Homologous recombination may help maintain the integrity of the viral genome through repair of mutated/damaged DNA, and is likely to

contribute to the intratypic molecular diversity of the viral genome. The majority of the replicative intermediates are long concatemers that appear to be generated through sequence replacement or insertion. They contain genomic units with U_L and U_S segments in different orientations that give rise to four possible isomers in equimolar ratios. The biologic relevance of isomerization is unclear. Viral proteins required for DNA replication in cell culture are UL30/UL42, a highly processive heterodimeric DNA polymerase; UL5/UL8/UL52, a heterotrimeric helicase–primase complex; UL29, a single-stranded DNA-binding protein; and UL9, an origin-binding protein that is only required early post infection (pi). Host recombination–repair proteins are also recruited to viral DNA replication sites.

The HSV genome contains three origins of replication with high-affinity binding sites for UL9: a single copy of Ori_L located in the middle of the U_L segment and two copies of Ori_S located in the repeats flanking the U_S segment (**Figure 1**(**a**)). Mutants lacking Ori_L or both copies of Ori_S are replication competent, indicating that Ori_L and Ori_S can compensate for each other. However, differences in their respective functional efficiencies have been reported. Ori_L may be involved in HSV-1 morbidity and mortality and latency reactivation in mice.

4. *Assembly and egress.* Assembly occurs in the nucleus (**Figure 3**(**a**)). The viral genome is inserted into a spherical intermediate capsid (procapsid) by a molecular motor made up of viral proteins UL6 together with UL15 and UL28, which are transiently associated with the capsids, recognize packaging signals on DNA, and cleave replicated concatameric DNA into monomeric units. During packaging, the protein scaffold within the procapsid is cleaved and removed, and the procapsid undergoes morphological changes that result in a more robust and angularized structure. Proteins UL17 and UL25 stabilize capsid-DNA structures. DNA-containing capsids exit the nucleus by budding through the inner and outer nuclear membranes (**Figure 3**(**b**)). This involves the viral protein complex UL31/UL34, which recruits protein kinase (PK) C to the nuclear membrane, thereby causing lamin B phosphorylation and lamina permeabilization.

The mechanism of egress of the alphaherpesviruses has been the subject of a long-standing controversy. According to one model, primary envelopment occurs by budding through the inner nuclear membrane and is followed by transport of primary virions within vesicles from the outer nuclear membrane through the endoplasmic reticulum and secretory Golgi pathway to the cell surface. To account for the lack of identity between primary and mature virions, the second model proposes that enveloped capsids undergo de-envelopment at the outer nuclear membrane and secondary re-envelopment at the *trans*-Golgi network, where they acquire their mature envelope by budding into a cytoplasmic organelle that transports them to the plasma membrane (**Figure 3**(**b**)). Release is by

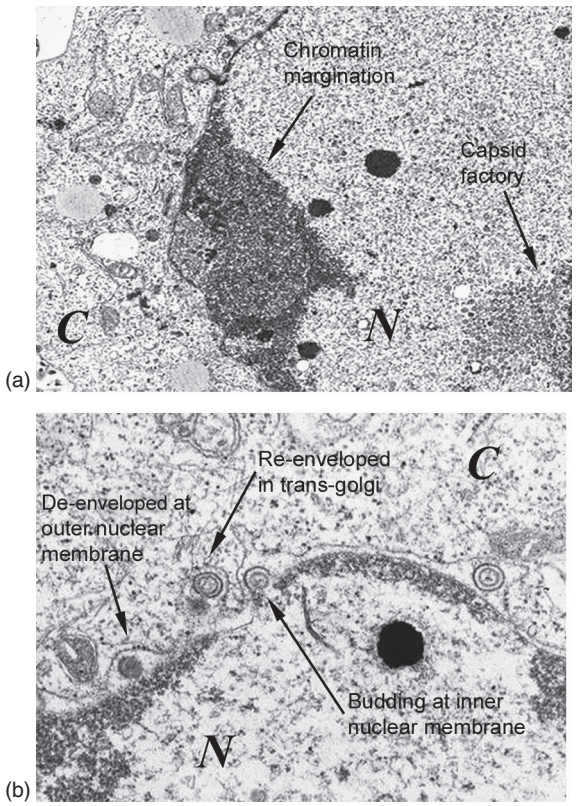


Figure 3 Virus replication. Thin sections of HSV-2-infected cells (12 h after infection) stained with uranyl acetate and lead citrate show nuclear (N) and cytoplasmic (C) compartments. (a) Intranuclear capsids are in different stages of assembly and the nucleus shows chromatin margination. (b) Virion assembly documents budding through the inner nuclear membrane, de-envelopment at the outer nuclear membrane, and re-envelopment in the *trans*-Golgi network. Magnification ×55 700.

exocytosis. During latency reactivation, HSV-1 envelopment likely occurs in neuronal varicosities and growth cones, where the primary site is the *trans*-Golgi network, and traffic/egress is along microtubules. In epithelial cells and neurons, newly assembled virions are directed to specialized cell surface domains (viz., epithelial cell junctions and neuronal synapses) for rapid entry into adjacent connected cells (cell-to-cell spread). Glycoproteins gE/gI bind ligands or receptors at these sites, promoting spread.

5. *Host cell effects.* The IE proteins inhibit cellular DNA synthesis and cause cell cycle arrest. Onset of E gene expression coincides with irreversible shutoff of host cell macromolecular synthesis, which is primarily mediated by the tegument phosphoprotein vhs released into the cytoplasm of the infected cells. Vhs degrades cellular mRNA through an endoribonuclease activity regulated, at least in part, through complexing with VP16. Virus replication is associated with cell rounding, chromatin margination, and the formation of nuclear inclusions (**Figures 2** and **3**).

Serologic Relationships

The HSV-1 and HSV-2 genomes have been sequenced. The genomes share a similar ORF organization, and overall sequence identity in coding region is 83%. Regions of low identity encode glycoprotein gG (used in HSV type-specific serology) and the R1 N-terminal domain, which has PK activity in HSV-2 but not HSV-1. Other biologic differences between HSV-1 and HSV-2 include virus yields in rabbit kidney cells, pock size on the chorioallantoic membrane of embryonated eggs, thermal stability, sensitivity to 5-iododeoxyuridine and interferon (IFN), and neoplastic potential.

Genetics

Epidemiologically unrelated isolates of the same HSV serotype are not identical (intratypic polymorphism). Differences result from base substitutions that may add or eliminate a restriction endonuclease cleavage site or substitute an amino acid, occasional deletions, or variable numbers of repeated sequences at various genome sites. The restriction endonuclease cleavage patterns of a given strain are relatively stable even after many years of serial passage *in vitro*, and may be related to neurovirulence.

Epidemiology and Associated Risks

HSV infects 80% of the adult population globally. Primary HSV-1 infection usually occurs by 5 years of age and is generally asymptomatic. It is estimated that 50 million adults have oral herpes in the USA. A recent cross-sectional survey of the general population in eight European countries revealed intercountry and intracountry differences in age-standardized HSV-1 seroprevalence (52–84%), but a significant proportion of recently screened adolescents in the USA and Europe were HSV-1 seronegative.

The current age-adjusted prevalence of HSV-2 in the USA is 20–25%. Individuals with genital HSV in the USA range between 40 and 60 million (one in five adolescents and adults) and new cases occur at a rate of *c.* 0.5–1 million per year. These estimates may be low, because the disease is not reported and many individuals do not seek medical attention, are misdiagnosed, or shed virus while asymptomatic. Factors that influence acquisition of HSV-2 include gender (women greater than men), race (blacks more than whites), marital status (divorced versus single or married), place of residence (city greater than suburb), and number of sexual partners (higher number has increased risk). Female prostitutes and homosexual men have the highest seroprevalence (75–98%). Women are about 45% more likely than men to be infected with HSV-2. The probability of infection is less than 10% for US women having one partner. It increases to 40%, 62%, and >80% as the number of partners increases to 2–10, 11–50, and >50, respectively. For heterosexual men, these risks are 0, 20%, 35%, and 70% for each of the risk groups, respectively. For homosexual men, the risks are greater than 60% and 90% for those with 11–50 and >50 partners, respectively. HSV-2 infection was also associated with a threefold increased risk of human immunodeficiency virus (HIV) acquisition by meta-analysis calculation of age- and sexual behavior-adjusted relative risks. Recently, HSV-1 became the most common cause of symptomatic first episode anogenital HSV among young people. Among US college students, the proportion of newly diagnosed genital HSV-1 infections increased from 31% in 1993 to 78% in 2001 ($P < 0.001$). HSV-1 was more common in females than males and in persons aged 16–21 than >22.

The major risk to the fetus is maternal primary or initial genital infection, the rate of which is 0.5–10% per year. The incidence of HSV-2 shedding at delivery is 0.01–0.6%, irrespective of past history and time of gestation. Most women whose children are infected (60–80%) are asymptomatic at the time of delivery, and they have neither a past history of genital HSV nor a sexual partner reporting genital infection. The incidence of cervical shedding in asymptomatic pregnant women averages 3%. Clinical diagnosis of genital HSV infection during pregnancy is a significant independent predictor of perinatal HIV transmission. HSV suppression during pregnancy may be a strategy to reduce perinatal HIV transmission.

HSV-induced encephalitis (HSE) is the most common viral encephalitis with an estimated incidence of 2.3 cases per million population per year. The age distribution is biphasic with one peak at 5–30 years of age and a second peak at >50 years of age. HSV-1 accounts for 95% of adult cases and the majority are due to reactivation from latency. HSV-2 does not generally cause encephalitis in adults, but the virus can cause aseptic meningitis, predominantly with primary infection. HSV-2 was isolated from the cerebrospinal fluid in 0.5–3% of patients with aseptic meningitis.

Transmission and Tissue Tropism

HSV is transmitted during close personal contact. The risk of transmission is directly related to virus load. Virus shedding in the saliva was reported in 20% of children 7–24 months old, 18% of those 3–14 years old, 2.7% in those >15 years old, and 2–9% of asymptomatic adults. HSV-1 was isolated from the mouth for 7–10 days after primary infection. Oral secretions from 36–45% of patients examined at prodrome (24–48 h before clinical recurrence)

were HSV-1 positive by polymerase chain reaction (PCR). Genital HSV-1 results from self-inoculation or from oral sexual practices.

Sexual contact is the primary route of HSV-2 transmission. Asymptomatic shedding was reported for the majority of patients. Half of the time it occurs more than 7 days before or after a symptomatic recurrence, and it is believed to be the major cause of transmission. However, cervical lesions are generally unnoticed, and they may be confused with asymptomatic shedding. Most studies of asymptomatic patients used PCR, the sensitivity of which in these subjects is at least four- to fivefold higher than that of virus isolation. When virus titers were measured, they were 100–1000 times higher in clinical lesions than in secretions from asymptomatic subjects, suggesting that transmission due to contact with active lesions is likely to be more efficacious. In discordant couples, 50% of seronegative partners remained seronegative for 16 months of contact, but use of condoms or daily suppressive therapy may be an option to reduce the risk of transmission. Intrapartum transmission accounts for 75–80% of neonatal HSV cases. Approximately 30 babies were identified in the world literature with symptomatic congenital HSV disease. This frequently resulted from ascending infections in women who have had prolonged rupture of membranes before delivery.

In children and young adults, HSE usually results from primary infection by virus entering the central nervous system (CNS) through neurotropic spread by way of the olfactory bulb. The hematogenous route of infection is common in immunosuppressed patients and neonates. Murine studies suggest that gender influences hematogenous HSV-1 infection in the CNS, and apolipoprotein E (ApoE) is involved in HSV-1 colonization of the brain from the blood. HSV infects the skin, eyes, liver, lungs, adrenal glands, pancreas, small and large intestine, CNS, and bone marrow. The trigeminal, vagal, and sacral dorsal root ganglia are the major sites of latent HSV infection.

Pathogenicity

The HSV-1 portal of entry is generally the oropharyngeal mucosa. Following replication at the site of infection, the trigeminal ganglia are invariably colonized (latency). Initial HSV-2 replication is at genital sites with colonization of the sacral ganglia. Animal studies suggest that sex hormones modulate susceptibility to HSV-2, with estradiol, but not progesterone, delaying vaginal pathology. Virus growth in the CNS (after intracerebral inoculation of mice), and its invasion after peripheral inoculation (neurovirulence) were ascribed to several genes, notably TK and $\gamma_1$34.5. The two serotypes have distinct pathogenicity in neurons. HSV-1 induces apoptosis through ICP0-mediated activation of the JNK/c-Jun pathway, and apoptosis is a component of HSE. HSV-2 does not trigger apoptosis in CNS neurons or cause encephalitis in human adults. This is likely due to the PK activity of the HSV-2 R1 protein (also known as ICP10), which is poorly conserved in HSV-1 and overrides apoptotic cascades through activation of survival pathways (viz., Ras/Raf-1/MEK/ERK and PI3-K/Akt) (**Figure 4**).

An unusual aspect of HSV pathogenicity is the infection of stem cells, both epithelial (**Figure 5**(**a**)) and CD34+ bone marrow derived. HSV-2 infection of human cervical cells causes atypia (**Figure 5**(**b**)) and the virus also infects spermatozoa (**Figure 5**(**c**)). Potential risks associated with these infection patterns require better elucidation, particularly as related to stem cells, an area of current research interest.

Clinical Features of Infection

HSV-1 and HSV-2 exhibit similar clinical manifestations. They infect neonates, children, and adults, and produce a wide spectrum of diseases including mucous membrane and skin lesions, ocular, visceral, and CNS disease. The incubation period is 1–26 days (median 6–8 days). Age, gender, host genetic factors, immune competence, associated illnesses, and virulence of the infecting virus strain influence severity.

Most (70–90%) childhood HSV-1 infections are asymptomatic. In children 1–3 years of age, the major manifestion is gingivostomatitis, a serious infection of the gums, tongue, mouth, lip, facial area, and pharynx, often accompanied by high fever, malaise, myalgias, swollen gums, irritability, inability to eat, and cervical lymphadenopathy (**Figure 6**(**d**)). Later in life, the major HSV-1 clinical manifestation is an upper respiratory tract infection, generally pharyngitis, and a mononucleosis-like syndrome. Reactivated HSV-1 is associated with mucosal ulcerations or lesions at the mucocutaneous junction of the lip presenting as small vesicles that last 4–7 days (known as herpes labialis, cold sores, or fever blisters). Other HSV-1 skin diseases include primary herpes dermatitis (a generalized vesicular eruption), eczema herpeticum (usually a manifestation of a primary infection in which the skin is the portal of entry) (**Figure 6**(**e**)), and traumatic herpes (resulting from traumatic skin breaks due to burns or abrasions). Herpetic whitlow is an occupational hazard (dentists, hospital personnel, wrestlers) resulting from infection of broken skin (often on fingers). Herpes folliculitis is a rare HSV-1 manifestation that often presents with lymphocytic folliculitis devoid of epithelial changes characteristic of virus infection. Acute HSV-1 rhinitis is a primary infection of the nose

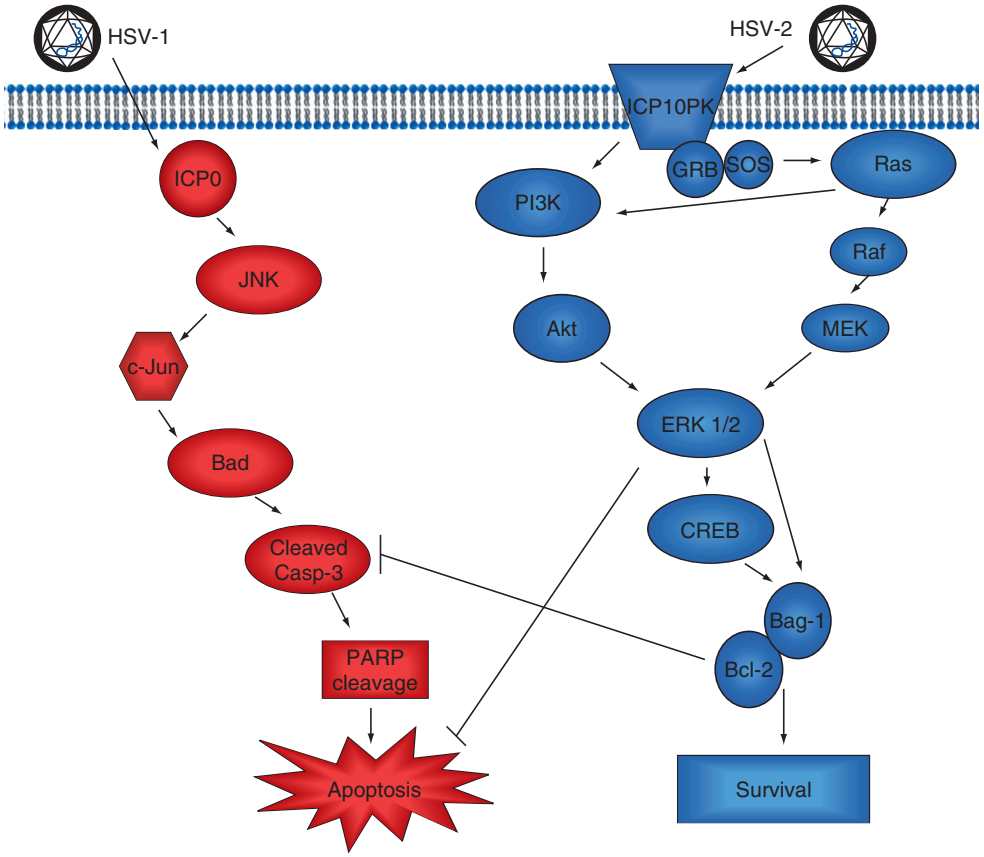


Figure 4 Distinct HSV-1 and HSV-2 pathogenicity. Schematic representation of signaling pathways activated by neuronal cell infection with HSV-1 or HSV-2. HSV-1 activates the pro-apoptotic JNK/c-Jun pathway and triggers apoptosis, likely mediated by the ICP0 protein. HSV-2 activates the MEK/ERK and PI3K/Akt survival pathways and blocks apoptosis, mediated by the PK function of the R1 protein (also known as ICP10).

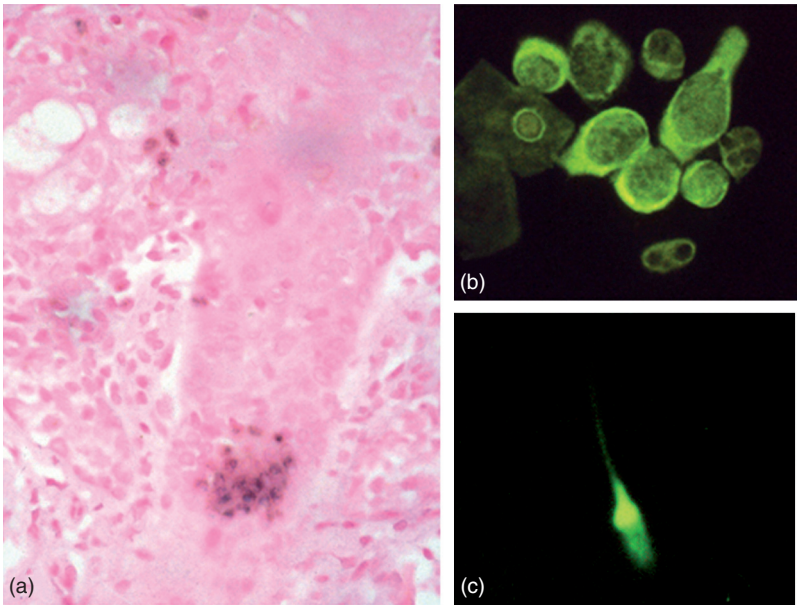


Figure 5 Pathogenicity aspects. Immunohistochemistry and immunofluorescent staining with HSV antibody reveals HSV infection of human epidermal (follicular) stem cells (a), cervical cells (resulting in mild to moderate atypia) (b), and a sperm cell (c).

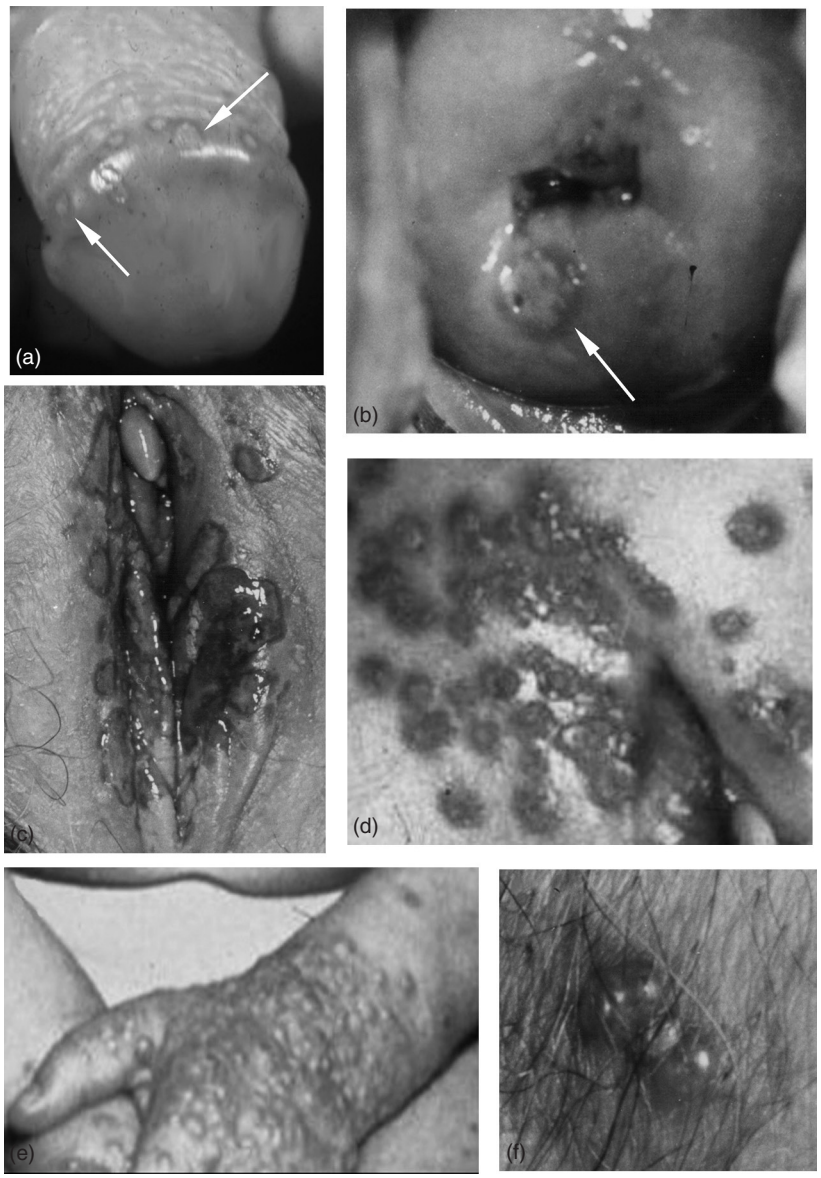


Figure 6 Clinical manifestations. HSV-2 induced lesions of penis (a), cervix (b), and vulva (c, f). Clinical HSV-1 manifestations include gingivostomatitis (d) and eczema herpeticum (e). Arrows in (a) and (b) identify vesicular lesions.

recognized by the appearance of tiny vesicles in the nostrils usually associated with fever and enlarged cervical lymph nodes. HSV-1 infections of the eye can lead to stromal keratitis, one of the leading causes of blindness in the Western world. This disease has a virus-specific T-cell (CD4+) component. Chorioretinitis is a manifestation of disseminated HSV infection that may occur in neonates or in patients with AIDS. HSV-1 accounts for nearly all cases of HSE in adults (and 10–20% of all viral encephalitis cases). Both serotypes can cause encephalitis in infants. With treatment, HSE mortality rates are 15% in newborns and 20% in others. Survivors have neurological sequelae involving impairments in memory, cognition, and personality. Untreated, mortality rates are 60–80%. Genital HSV-1 infections may be less severe and prone to recur than HSV-2 infections.

In females, HSV-2 infection is manifested by vesicles on the mucous membranes of the labia and the vagina (**Figure 6**(**f**)). Severe forms result in ulcers that cover the entire area surrounding the vulva (**Figure 6**(**c**)). Symptoms of primary infection include itching, pain, and lymphadenopathy. Cervical involvement is common, although it generally passes unnoticed (**Figure 6**(**b**)) and the patient is often mistaken for having asymptomatic shedding. Common sites of primary HSV-2 infection in males are the shaft of the penis, the prepuce, and the glans

penis (**Figure 6(a)**). Urethritis is the main local expression accompanied by a watery discharge often resulting in dysuria. Symptomatic urethritis is rare in recurrent disease, but virus can often be cultured from the urethra. The infection is more severe in females than in males and systemic symptoms are more common. They often accompany the appearance of primary lesions and include fever, headache, photophobia, malaise, and generalized myalgias. Dysuria, urinary retention, urgency and frequency, pain, and discharge are also seen. Systemic symptoms are generally not seen in recurrent disease. A prodrome often signals a recurrence. It is characterized by a tingling sensation that may precede the lesions by a few hours to 2 days, could be accompanied by radiating radicular pain, and is related to loss of sodium conductance in neurons that support replication of reactivated virus. Anal and rectal infections are seen primarily in homosexuals, as a result of anal intercourse.

HSV-1- and HSV-2-associated erythema multiforme (HAEM) is a recurrent skin disease that follows reactivation of latent virus and occurs at distal sites. HAEM lesional skin contains HSV DNA fragments (most often comprising the viral Pol gene) that are delivered by migrating infected CD34+ stem cells. Lesion development is initiated by virus gene expression and contains virus-specific and autoimmune components. The virus-specific component consists of a restricted population of Th1 cells (primarily, CD4+ Vβ2) that produce IFN-γ. The autoimmune component is in response to antigens released by lysed/apoptotic virus-infected keratinocytes. A genetic predisposition is implicated.

Immunocompromised adults can develop a severe generalized disease that is occasionally responsible for herpetic hepatitis. HSV pneumonitis accounts for 6–8% of cases of interstitial pneumonia in recipients of bone marrow transplants. Mortality due to HSV pneumonia in immunosuppressed patients is >80%. Generalized HSV with involvement of adrenal glands, pancreas, small and large intestine, and bone marrow was reported in the immunocompromised patient. HSV has also been isolated from 40% of patients with acute respiratory distress syndrome. HSV-1 DNA was recently found by PCR in gastrointestinal sensory neurons and in the geniculate ganglion and adjacent areas, respectively, implicating HSV in recurrent gastrointestinal disorders and Bell's palsy. HSV-1 seropositivity was also associated with myocardial infarction and coronary heart disease in older adults, and with Alzheimer's disease (AD). AD is believed to result from frequent episodes of mild encephalitis and neuronal apoptosis resulting from virus reactivation in individuals homozygous or heterozygous for ApoE4. Virus DNA was detected in the brain from elderly people in some, but not other, studies. Uncommon complications of HSV infection include monoarticular arthritis, adrenal necrosis, idiopathic thrombocytopenia, and glomerulonephritis.

Latency

Following replication in the skin, sensory or autonomic nerve endings are infected by cell-to-cell spread (retrograde transport). Virions, or more likely capsids, are thought to be transported intraxonally to the nerve cell bodies in the ganglia (primarily trigeminal and sacral for HSV-1 and HSV-2, respectively), where viral DNA is maintained as an episome that is largely transcriptionally silent. Human trigeminal ganglia contain approximately 20 viral genome copies per latently infected neuron. Virus isolates obtained at various times from the same patients have different restriction endonuclease patterns, indicating that ganglia can be colonized by multiple HSV strains. Certain stimuli cause virus reactivation with concomitant reverse axonal transport of virus progeny to a peripheral site at or near the portal of entry (anterograde transport) (**Figure 7(a)**). Reactivating stimuli include fever, axonal injury, exposure to ultraviolet irradiation (sunlight), emotional stress, and possibly hormonal irregularities. Development of clinically apparent recurrent lesions is probably influenced by the amount of virus, virus–cell interactions, and the rapidity/efficacy of immune-mediated clearance. In the guinea pig model, latent virus reactivates spontaneously. In other animal models, virus is reactivated by neurectomy, ganglionic trauma, electrical stimulation, epinephrine iontophoresis, cadmium treatment, or ganglia explantation and organ culture.

The mechanism of latency establishment and reactivation is still unclear. One possibility is that they are controlled by cellular factors required for virus replication, such as HCF, which translocates to the nucleus at reactivation. However, the general consensus is that viral genes control latency, although their identity is still controversial. The only HSV transcript abundantly expressed during latency is the 2.0 kbp intron derived from the 8.3 kbp primary latency-associated transcript (LAT). Because LAT overlaps the 3′ end of ICP0, it was assumed to contribute to latency establishment by acting as antisense to ICP0. An alternative hypothesis was that ICP0 splicing and expression are suppressed by the ORF P protein (apparently encoded by a minor LAT). However, both spliced and intron-containing ICP0 transcripts were found in latently infected ganglia, and LAT or ORF P did not increase their levels. Others concluded that LAT promotes efficient latency reactivation. However, mutants lacking LAT were shown to reactivate as well as the wild-type virus. One group has reported the expression of a LAT protein encoded by an ORF within the 2.0 kbp intron, which acts somewhat like ICP0 in that it enhances lytic genes and promotes interaction with cellular transcription factors. Most studies attribute the role of LAT in latency reactivation to its anti-apoptotic activity, which ensures a large pool of latently infected neurons that contribute to efficient virus reactivation. It is thought that LAT

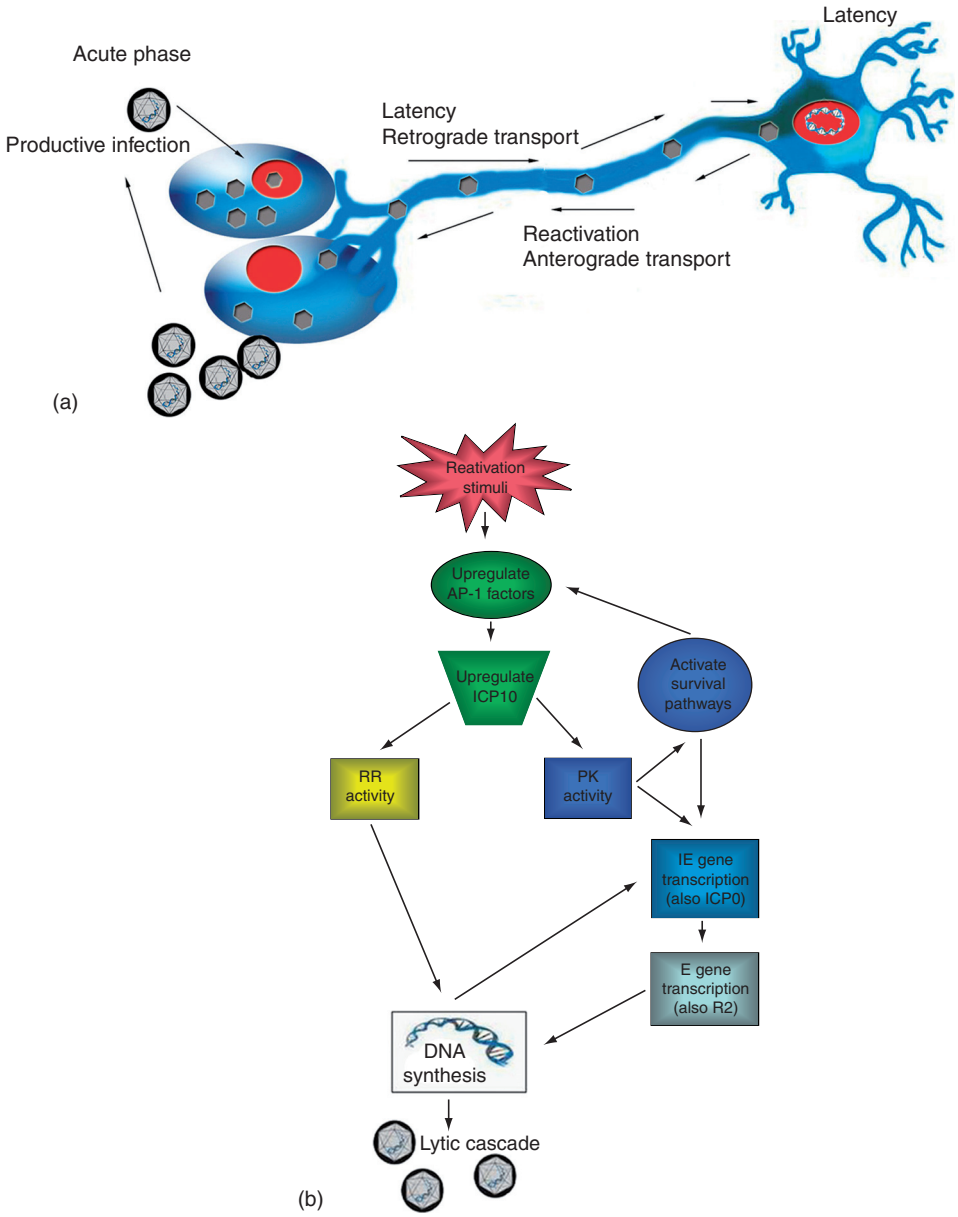


Figure 7 Schematic representation of latency establishment and reactivation. (a) Following replication in the skin, capsids are axonally transported to nerve cell bodies where viral DNA is maintained as a circularized episome. Reactivating stimuli cause reverse axonal transport of virus progeny. (b) Model for involvement of R1 (ICP10) in HSV-2 latency reactivation. Reactivating stimuli upregulate AP-1 factors, thereby causing ICP10 overload. ICP10 supplies the PK activity, which is required for IE gene transcription and a feedback amplification loop through activation of the Ras survival pathway. It also supplies the RR activity that is required for DNA synthesis. The outcome is initiation of the lytic cascade.

counteracts apoptosis induced by corticosteroids, which are upregulated by reactivation-inducing stress stimuli. However, controversy remains. While LAT enhances reactivation in the rabbit eye model, it does not seem to be required in small animal models. LAT anti-apoptotic activity was not studied in neurons, although apoptosis is cell-type specific. LAT anti-apoptotic activity may be unique to certain HSV-1 strains. Apoptosis was also associated with LAT downregulation by stress-induced cAMP early repressors (ICER). The presence of STAT1 response elements in the LAT promoter suggests that cytokines may initiate or contribute to LAT-mediated virus reactivation.

The HSV-2 LAT fails to substitute for its HSV-1 counterpart in promoting latency reactivation, and it does not modulate HSV-2 latency reactivation or its establishment. An HSV-2 mutant deleted in the PK domain of R1 (ICP10) was shown to be significantly impaired in latency establishment/reactivation, and virus reactivation (by ganglia

co-cultivation) was inhibited by an ICP10 antisense oligonucleotide, indicating that ICP10 might be involved in HSV-2 latency. In this model, latency-reactivating stimuli upregulate AP-1 transcription factors, thereby inducing expression of ICP10, which is the only virus protein the basal expression of which is regulated by AP-1. The PK function of ICP10 induces IE gene expression and, thereby, the entire viral transcriptional cascade. It also activates the Ras/MEK/ERK pathway that blocks apoptosis, ensuring a large pool of surviving neurons that contribute to efficient virus reactivation, and provides a feedback amplification loop for ICP10 upregulation The ribonucleotide reductase activity of ICP10 contributes to viral DNA synthesis. The outcome is virus replication (**Figure 7(b)**).

HSV Infection during Pregnancy and Neonatal Disease

Localized genital infection is the most common form during pregnancy, but rare visceral involvement with approximately 50% mortality was reported. Fetal deaths occurred in 50% of cases, although mortality did not correlate with the death of the mother. Maternal primary infection prior to 20 weeks of gestation was associated with spontaneous abortion in 25% of infected women. Fetal infection is generally due to virus shed at the time of delivery. Skin lesions are the most commonly recognized features, but at least 70% of untreated cases have disseminated disease. Neonatal infection is decreased by surgical abdominal delivery when membranes are ruptured for less than 4 h. For women with a past history of genital infection, HSV culture can establish whether virus is shed at the time of delivery.

Pathology and Diagnosis

Histopathologic changes are a combination of virus-induced alterations (cell ballooning, condensed nuclear chromatin, and nuclear degeneration) and associated inflammatory responses. Virus-induced changes are generally within the parabasal and intermediate cells of the epithelium. Multinucleated giant cells are also formed and a clear (vesicular) fluid containing large quantities of virus, cell debris, and inflammatory cells appears between the epidermis and the dermal layer. An intense inflammatory response is seen in dermal structures, particularly in primary infection, and is accompanied by an influx of mononuclear cells, as host defenses are mounted. With healing, the vesicular fluid becomes pustular and, then, scabs. Scarring is uncommon. Similar histopathologic findings are seen in mucous membranes. Perivascular cuffing and areas of hemorrhagic necrosis are also seen in the area of infection, particularly in organs other than skin. In the brain oligodendrocytic involvement, gliosis, and astrocytosis are common. Virus culture is the gold standard for diagnosis, but results require approximately 2 weeks. Accordingly, in severe infections, treatment initiation should not be delayed. The increased sensitivity and rapidity of PCR are desirable for diagnosis of severe infections.

Host Immune Responses

An early nonspecific containment phase (innate immunity) and a later HSV-specific effector phase (adaptive immunity) contribute to protection. Natural killer (NK) cells and rapid production of IFN type I provide a threshold of resistance to HSV-1 infection and were associated with the natural resistance of certain mouse strains. In mice, IFNα/β inhibit the onset of IE gene expression, limit virus spread into the nervous system, and activate NK cells. Dendritic cells (DCs) are required for activation of NK cells and CD4+ and CD8+ T cells in response to HSV-1. Conventional CD11c+CD8α+ DCs are the principal antigen-presenting cells during acute HSV-1 infection. The plasmocytoid CD11c+B220+ DCs secrete large amounts of type I IFN *in vitro* after exposure to HSV-1 and help lymph node DCs to induce HSV-specific cytotoxic T cells (CTLs) optimally during a primary immune response. Various reports have incriminated or refuted the association of HLA with HSV infections.

HSV antibody is first seen on days 5–10 pi and persists indefinitely. Antibody titers are higher in subjects with a history of recurrent disease, reflecting virus reactivation. Antibodies to structural proteins are followed by those directed against gD, gB, ICP4, gE, gG, and gC. IgM and IgG antibodies can be demonstrated at different times pi. In children, antibodies to ICP4 may be predictive of long-term neurologic outcome. Infected newborns produce IgM antibodies (particularly against gD) within the first 3 weeks pi. Transplacentally acquired antibodies do not protect the newborn.

T cells play the major role in recovery from HSV infection. Their development is delayed in newborns (2–4 weeks), presumably explaining the increased sensitivity to disease progression in this age group. Effector mechanisms include: (1) delayed-type hypersensitivity (DTH), an early (days 5–10 pi) HSV-type common response that remains inducible for at least 2 years after infection; (2) effector lymphokines, such as IFN-γ, interleukin 2 (IL-2), and other soluble factors; and (3) CTL. CTL clones are specific for cell surface glycoproteins, primarily gD or gE, and intracellular IE proteins.

Naturally acquired HSV immunity reduces the severity of the disease, but it does not prevent latency establishment, recurrent disease, or reinfection. HSV has developed various mechanisms of immune evasion, notably inhibition of antigen presentation by the MHC class I

complex through binding of the cellular protein that transports antigenic peptides into the endoplasmic reticulum (TAP) by the viral IE protein ICP47 (**Figure 8**). Other immune-evasion mechanisms include resistance to complement attack (mediated by gC), prevention of antibody binding (mediated by gE/gI), and blocking of CD8+ CTL activity through US3-mediated inhibition of caspase activation. HSV-1 also blocks the IFN response at multiple sites, including decreased Jak1 kinase and Stat2, which are associated with IFN signaling. However, the exact role of immune evasion in recurrent disease is unclear. It is believed that reactivated virus circumvents immune inhibition by replicating before immunity is alerted, or by shifting the immunological balance toward tolerance. For example, induction of Th2 cytokines (e.g., IL-10) could downregulate the Th1 antiviral response by inhibiting DC maturation/function. Generation of downregulatory factors, such as prostaglandin E2 (PGE2) and TGFβ, DC infection, and/or induction of T regulatory cells (Tregs) could also be involved. Our longitudinal studies of HSV patients, done approximately 20 years ago, implicated Tregs in the immune downregulation that enables recurrent disease development. Tregs have made a comeback recently and were again implicated in recurrent disease, now in the mouse model.

Neoplastic Transformation

Seroepidemiologic studies have associated HSV-2 infection with an increased risk of human squamous cervical cancer. Current opinion is that HSV-2 is only a co-carcinogen. Notwithstanding, a wealth of experimental evidence indicates that HSV-2 is a tumor virus. It causes neoplastic transformation of immortalized cells (including those of human origin) and causes tumors in animals, both under conditions that interfere with virus replication. Transformation is mediated by R1 (ICP10) PK through activation of the Ras/MEK/ERK pathway, a function that is conserved because it is required for latency reactivation. The HSV-1 R1 gene does not have oncogenic potential, but HSV-1 causes mutagenesis and gene amplification. In humans, HSV-2 can cause severe hyperproliferative lesions that may have an increased risk for neoplastic conversion. We reported the case of a patient with common variable immunodeficiency who presented with hyperproliferative lesions (pseudo-epitheliomatous hyperplasia) caused by an acyclovir-resistant HSV-2 mutant through overexpression of ICP10PK (**Figure 9**).

Therapy and Prevention

A recent critical advance has been the development of antiviral chemotherapy. Agents currently used are acyclovir, valaciclovir, and famciclovir, which have superior oral bioavailability (**Table 1**). Episodic oral therapy speeds healing and resolution of primary and recurrent HSV lesions, but subsequent recurrence rates are not affected. Long-term daily suppressive therapy may reduce the frequency of reactivation in patients with frequent recurrent episodes, but it does not eliminate ganglionic latency and reactivation occurs after therapy is discontinued. Its effect on asymptomatic virus shedding is still unclear. Daily oral administration for 6–12 months appears to be safe, but a potential concern of long-term treatment is that nonreplicating HSV-2 is oncogenic. Intravenous acyclovir is the agent of choice for treatment of neonatal HSV and HSE. These drugs are phosphorylated by the HSV TK at a 10^6-fold faster rate than the cellular enzyme. After additional phosphorylation by cellular enzymes, the triphosphate (active form) blocks viral DNA synthesis by acting as an inhibitor and substrate of viral DNA polymerase, becoming incorporated into the growing DNA chain and causing termination of chain growth. HSV DNA polymerase has a much higher affinity for the

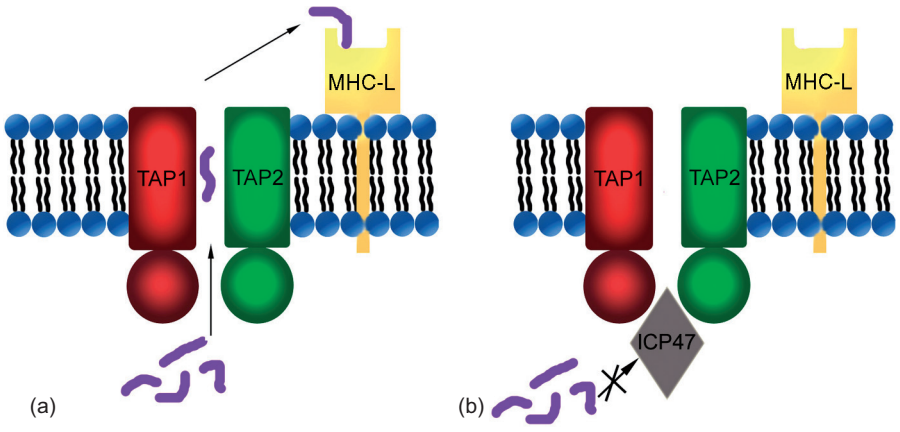


Figure 8 Immune evasion. (a) TAP-mediated transport of antigenic peptides for presentation by MHC class I. (b) ICP47 binds TAP, thereby inhibiting antigen presentation.

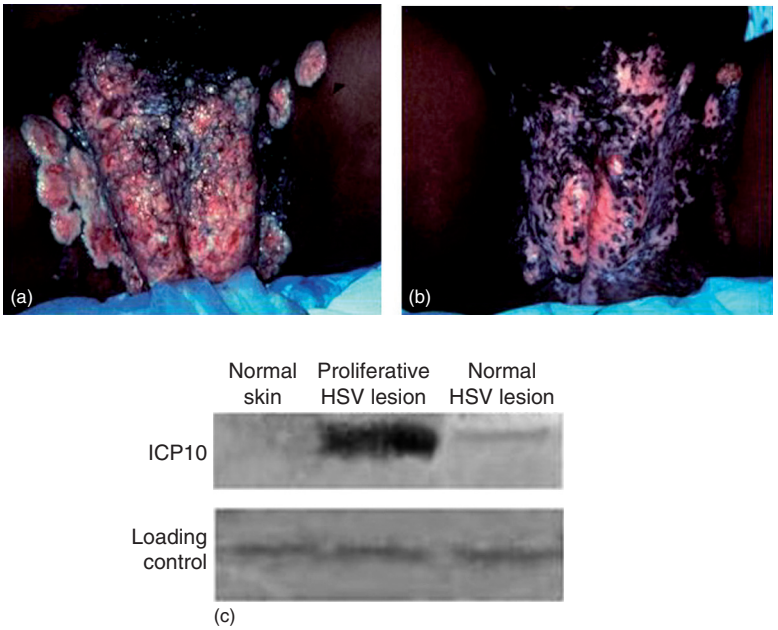


Figure 9 HSV-2 causes human hyperproliferative lesions. Hyperproliferative lesion caused by acyclovir-resistant HSV-2 (a) was treated with intravenous foscarnet (b). The lesion was associated with increased expression of the proliferation-inducing ICP10 protein (c). (a) Reprinted from *Journal of the American Academy of Dermatology*, 37, Beasley KL, Cooley GE, Kao GF, Lowitt MH, Burnett JW, and Aurelian L, Herpes simplex vegetans: Atypical genital herpes infection in a patient with common variable immunodeficiency, 860–863, Copyright (1997), with permission from The American Academy of Dermatology, Inc.

Table 1 CDC treatment recommendations for genital HSV

		Recurrent disease	
	First clinical episode (mg for 7–10 days)	*Episodic (mg × days)*	*Suppressive (mg, daily)*
Acyclovir	400 tid 200 5×/day	400 tid (5 d) 800 bid (5 d) 200 5×/day (5 d)	400 bid
Valaciclovir	1000 bid	500 bid (3–5 d) 1000 qd (5 d)	500 qd 1000 qd
Famciclovir	250 tid	125 bid (5 d)	250 bid up to 1 year

drug than the cellular enzyme. As DNA synthesis is required for the drugs to function, they cannot destroy the virus during latency. Acyclovir resistance emerges with moderate frequency and it crosses to valaciclovir and famciclovir.

Foscarnet and cidofovir are used to treat infections caused by acyclovir-resistant virus. Cidofovir undergoes two stages of phosphorylation, via monophosphate kinase and pyruvate kinase, to form an active metabolite, cidofovir diphosphate (CDVpp), that bears structural similarity to nucleotides and acts as a competitive inhibitor and an alternative substrate for DNA polymerase. Nephrotoxicity is a potential complication with parenteral use of cidofovir, and it should be administered with oral probenecid after prehydration to minimize adverse effects. A topical cidofovir formulation is currently under investigation. Acyclovir-resistant HSV can become sensitive after treatment with cidofovir, suggesting that alternating acyclovir and cidofovir therapies may provide a strategy for managing the emergence of alternating acyclovir-resistant and -sensitive infections.

It is becoming increasingly evident that prevention of HSV infection through vaccination may be an unrealistic goal. A more modest goal has been to reduce the clinical symptoms of primary infection. In two Phase III trials, an HSV-2 gD fragment formulated with a mixture of alum and 3-deacylated monophosphoryl lipid A (3-dMPL) was shown to reduce clinical symptoms of primary HSV-2 infection (approximately 70% efficacy) in women that were HSV-1 and HSV-2 seronegative. Males mounted similar immune responses, but they were not protected and the activity of the vaccine was attributed to the adjuvant used in its composition. Continued research is focusing on HSV-1 recombinants engineered to express immunostimulatory

molecules, such as IL-12, which bridges innate and adaptive immunity.

Another vaccination goal has been to prevent/reduce the frequency of recurrent disease episodes in infected patients (therapeutic vaccine). One such vaccine is an HSV-2 mutant rendered replication incompetent and Th1 polarizing through deletion of ICP10PK. This vaccine caused a 75–90% reduction in the number of guinea pigs with spontaneous recurrent disease and reduced the frequency and duration of recurrent episodes experienced by the rare animals that were not absolutely protected. In Phase I/II clinical trials, the vaccine was tolerated well and recurrences were completely eliminated in 44% of the vaccinated patients at 1 year of follow up. This compares to 13% without recurrences in the placebo group ($p = 0.024$). There was also a significant reduction in the number of episodes relative to the previous year ($p < 0.001$) and in the number of recurrent episodes in the vaccinated relative to the placebo group ($p = 0.04$). Efficacy was gender independent, and was seen in both HSV-1 and HSV-2 seropositive subjects. Virus-specific CD8+ CTL and CD4+ Th1 cells mediated protection.

HSV Vectors and Oncolytic Viruses for Gene Therapy

One vector type, known as an amplicon, consists of a plasmid engineered to contain an HSV-1 origin of replication and packaging site along with a bacterial origin of replication in which a selected therapeutic gene is introduced. Amplicons are packaged by co-transfection with a defective HSV-1 helper virus, giving rise to a mixed population of virus particles that consist of the packaged amplicon and the helper virus. Helper-free amplicons were also developed. Another vector type consists of HSV-1 mutants rendered avirulent through deletion of relevant genes. Therapeutic genes delivered with these vectors limit neuronal death (e.g., Bcl-2), restore energy metabolism (e.g., glucose transporter), or promote neuronal survival (e.g., growth factors). HSV vectors were also developed to deliver short hairpin RNA to inhibit various transcripts (e.g., Aβ precursor). Drawbacks include residual toxicity, loss of immune-evasion mechanisms required for repeat treatment, and the loss of function by surviving neurons. HSV-2, which does not cause encephalitis or apoptosis in the CNS, may be a better candidate for the development of vectors for gene therapy of neurologic disorders. ΔRR, a recently developed growth-compromised and avirulent HSV-2 mutant (even after intracerebral inoculation), delivers the viral anti-apoptotic protein ICP10PK and has been shown to have protective activity in various paradigms of neuronal cell death *in vitro* and in animal models. The surviving neurons retain functional (synaptic) activity.

Oncolytic viruses are promising tools for cancer gene therapy. HSV-1 mutants G207 and MGH1 have double deletions in both copies of the neurovirulence gene $\gamma_1 34.5$ and an insertional mutation in the R1 gene. They are avirulent upon intracerebral inoculation, and were shown to kill CNS tumor cells (e.g., glioma), decrease tumor growth, and prolong survival in tumor-bearing nude mice. Tumor specificity appears to be related to increased MEK activity in tumor as compared to normal cells. Function requires intact T-cell activity. Additional HSV-1-based oncolytic viruses were constructed to express IL-4, GM-CSF, or the CD40 ligand. Others use the glioma-specific nestin promoter to drive $\gamma_1 34.5$ (to increase tumor specificity), or lack ICP47 (to reduce immune evasion). An HSV-2-based oncolytic virus, which is deleted in ICP10PK, was recently shown to eradicate tumors in >80% of breast cancer mouse xenografts. However, mutations may arise in untargeted regions of the viral genome, and their clinical significance is still unclear. Another cancer gene therapy approach (known as suicide) is based on the ability of HSV TK to preferentially activate ganciclovir (GCV), thereby killing tumor cells that contain the TK gene (delivered with an HSV-1, retrovirus, or adenovirus vector) as well as surrounding tumor cells (bystander effect).

Future

Studies of the molecular pathogenesis of HSV neuronal and stem cell infections and the role and modulation of HSV immunity in disease pathogenicity (notably recurrent disease) and protection remain principal goals for future research efforts. Prophylactic and therapeutic vaccines are badly needed. Further research must also focus on the mechanism of latency establishment and virus reactivation, and overcome problems of drug resistance. Risks associated with reinfection, particularly by drug-resistant virus strains, need elucidation. Significant progress may be achieved by developing novel oncolytic viruses, and further research is needed to elucidate the potential of HSV as a vector for gene therapy of neurodegenerative diseases.

See also: Herpes Simplex Viruses: Molecular Biology; Herpesviruses: General Features; Herpesviruses: Latency.

Further Reading

Aurelian L (2004) Herpes simplex virus type 2 (HSV-2) vaccines: New ground for optimism? *Clinical and Vaccine Immunology* 11: 437–445.

Aurelian L (2005) HSV-induced apoptosis in herpes encephalitis. *Current Topics in Microbiology and Immunology* 289: 79–112.

Aurelian L, Ono F, and Burnett J (2003) Herpes simplex virus (HSV)-associated erythema multiforme (HAEM): A viral disease with an autoimmune component. *Dermatology Online Journal* 9: 1.
Beasley KL, Cooley GE, Kao GF, Lowitt MH, Burnett JW, and Aurelian L (1997) Herpes simplex vegetans: A typical genital herpes infection in a patient with common variable immunodeficiency. *Journal of the American Academy of Dermatology* 37: 860–863.
Burton EA, Fink DJ, and Glorioso JC (2005) Replication-defective genomic HSV gene therapy vectors: Design, production and CNS applications. *Current Opinion in Molecular Therapy* 7: 326–336.
Carr DJ and Tomanek L (2006) Herpes simplex virus and the chemokines that mediate the inflammation. *Current Topics in Microbiology and Immunology* 303: 47–65.
Kimberlin DW and Whitley RJ (2005) Neonatal herpes: What have we learned. *Seminars in Pediatric Infectious Diseases* 16: 7–16.
Leone P (2005) Reducing the risk of transmitting genital herpes: Advances in understanding and therapy. *Current Medical Research and Opinion* 21: 1577–1582.
Novak N and Peng WM (2005) Dancing with the enemy: The interplay of herpes simplex virus with dendritic cells. *Clinical and Experimental Immunology* 142: 405–410.
Ono F, Sharma BK, Smith CC, Burnett JW, and Aurelian L (2005) CD34+ cells in the peripheral blood transport herpes simplex virus (HSV) DNA fragments to the skin of patients with erythema multiforme (HAEM). *Journal of Investigative Dermatology* 124: 1215–1224.
Shen Y and Nemunaitis J (2006) Herpes simplex virus 1 (HSV-1) for cancer treatment. *Cancer Gene Therapy* 13: 975–992.
Smith CC (2005) The herpes simplex virus type 2 protein ICP10PK: A master of versatility. *Frontiers in Bioscience* 10: 22820–22831.

Herpes Simplex Viruses: Molecular Biology

E K Wagner[†] **and R M Sandri-Goldin,** University of California, Irvine, Irvine, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

The Virion

The Capsid

Like all herpesviruses, herpes simplex virus (HSV) type 1 (HSV-1) and the closely related HSV type 2 (HSV-2) have enveloped, spherical virions. The genome of HSV-1 is densely packaged in a liquid-crystalline, phage-like manner within a 100 nm icosahedral capsid. The capsid comprises 162 capsomeres of 150 hexons and 12 pentons. Hexons contain six molecules of the 155 kDa major capsid protein (VP5 or UL19) with six copies of the vertex protein VP26 (UL35) at the tips. Pentons contain five copies of VP5. Hexons are coordinated in threefold symmetry with a triplex structure made up of two other proteins: one copy of VP19C (UL38) and two copies of VP23 (UL18) per triplex. Small amounts of other viral proteins are also associated with the capsid; these include VP24 (derived from UL26), a maturational protease, and the UL6 gene product. The capsid portal formed from the UL6 gene product is now thought to occupy one of the penton positions, which leaves the capsid with 11 rather than 12 VP5-containing pentons.

The Tegument

The capsid is surrounded by a layer consisting of approximately 20 tegument or matrix proteins. These include the α-*trans* inducing factor (αTIF, VP16, or UL48), and a virion-associated host shutoff function (vhs or UL41).

The Envelope

The trilaminar viral lipid envelope forms the outer surface of the virion. This envelope has a diameter of 170–200 nm, although exact dimensions vary depending upon the method of visualization. The envelope is derived from the host cell nuclear membrane and contains at least 10 virally encoded glycoproteins. One glycoprotein (gG–US4) has sufficient differences in amino acid sequence to serve as a type-specific immune reagent to differentiate HSV-1 and HSV-2. At least four (gL–UL1, gH–UL22, gB–UL26, and gD–US6) are involved in virus penetration into the host cell. The sequence of gB is highly conserved among a broad range of herpesviruses. Another glycoprotein (gC–UL44) facilitates the initial attachment of the virion to glycosaminoglycans (GAGs) on the cell surface. Two others (US7–gI and US8–gE) function together as a heterodimer that binds the Fc region of immunoglobulin G (IgG) and influences cell-to-cell spread of virus.

The Genome

The complete genomic sequences of prototype strains of HSV-1 and HSV-2 are available in standard databases. Homology between HSV-1 strains is >99% for most regions of the genome while overall homology between HSV-1 and HSV-2 approximates 85% within translational reading frames and is significantly less outside them. The HSV-1 genome is shown schematically in **Figure 1**. It is a linear, double-stranded (ds) DNA duplex, 152 000 bp in length, with a base composition of 68% G+C. The genome circularizes upon infection. Because

[†] Deceased

the genome circularizes, the genetic/transcription map is conveniently shown as a circle. The genome contains at least 77 open reading frames (ORFs) translated into funtional proteins. Therefore, the map is complex.

The organization of the genome can be represented as $a_n b U_L b' a'_m c' U_S c a$. The genome is made up of five important regions. These are:

1. The ends of the linear molecules, with the left end comprised of multiple repeats of the 'a' sequence (a_n), which are important in circularization of the viral DNA upon infection and in packaging the DNA in the virion. Variable numbers of the 'a' sequence in inverted form (a'_m) are present internally in the genome.
2. The 9000 bp long inverted repeat (R_L – 'b' region). This region encodes an important immediate early regulatory protein (ICP0), and most of the 'gene' for the latency-associated transcript (LAT), including the promoter. It also encodes the ORF for the ICP34.5 gene, which is important in neurovirulence, and two partially overlapping ORFs antisense to that of ICP34.5, namely ORF-O and ORF-P. Two other short translational reading frames 5′ of the LAT cap site, termed ORF-X and ORF-Y, are also present, but have unknown functions.
3. The long unique region (U_L), which is 108 000 bp long, and encodes at least 58 distinct proteins. It contains an origin of DNA replication (ori_L) and genes encoding DNA replication enzymes, including the DNA polymerase. A number of capsid proteins and glycoproteins are encoded in U_L as well as many other proteins.
4. The 6600 bp short inverted repeats (R_S – 'c' region) encode the very important immediate early transcriptional activator, ICP4. R_S also encodes another origin of DNA replication (ori_S) and the promoters for two other immediate early genes.
5. The 13 000 bp short unique region (U_S) contains 13 ORFs, a number of which encode glycoproteins important in viral host range and response to host defense.

The arrangement of the inverted repeat elements allows the unique regions of the viral genome to invert relative to each other into four 'isomers': prototype (P), inversion of U_L relative to U_S (I_L), inversion of U_S relative to U_L (I_S), and inversion of both U_L and U_S (I_{LS}). Inversion is facilitated by the 'a' sequence elements noted above.

Viral Replication

Entry

Initial association of HSV with a host cell is mediated by the association with the envelope glycoproteins gB (UL27) and gC (UL44) and glycosamminoglycans (GAGs), such as heparin sulfate, on the cell surface. Virus internalization involves the mediation of four essential glycoproteins, gB (UL27), gH (UL22), gD (US6), and gL (UL1). In many cells, this is mediated by interaction of gD with a cellular surface protein named the herpesvirus entry mediator (HVEM). HVEM is a member of the TFN/NGF family of proteins. Other cellular surface proteins may also function in a similar capacity since some cells lacking this receptor still allow efficient viral entry. Following entry, HSV exists as a de-enveloped virion in the cytoplasm. This particle must then be transported to the nucleus by a process that requires the cell microtubule machinery, and then the viral DNA released into the nucleus (uncoating). The details of these processes are poorly understood.

Transcription and DNA Replication

Once in the nucleus, the viral DNA becomes available for transcription by host RNA polymerase II into mRNAs. The viral genome is accompanied into the nucleus by the VP16 or αTIF protein (UL48), which functions in enhancing immediate early viral transcription via cellular transcription factors. Gene expression occurs in three phases – immediate early or α (the first phase), early or β (which requires the immediate early protein ICP4), and late or γ (which requires the immediate early proteins ICP4 and ICP27 and viral DNA replication for maximal expression). DNA replication has a significant influence on viral gene expression. Early expression is reduced following the start of DNA replication, while late genes begin to be expressed at high levels. Immunofluorescence studies show that DNA replication occurs at discrete sites or replication compartments in the nucleus. Many of the early proteins are involved in viral DNA replication. Some of these early proteins, such as the two-subunit DNA polymerase and the three-subunit helicase–primase, participate directly in DNA synthesis, while others, such as thymidine kinase and the two-subunit ribonucleotide reductase, are involved indirectly by increasing the pools of deoxynucleoside triphosphates.

RNA Export, Translation, and Post-Translational Processing

HSV mRNAs are capped at the 5′ end and processed to form the 3′ end by polyadenylation, but the majority of viral transcripts do not undergo splicing. HSV transcripts are exported to the cytoplasm using the cellular TAP/NXF1 mRNA export pathway, and this process is facilitated by the HSV immediate early protein ICP27, which binds to viral RNAs as well as to the TAP/NXF1 receptor. HSV mRNA is translated on polyribosome structures using the host translational machinery.

Extensive proteolytic processing of HSV proteins does not occur, but the maturation of the capsid absolutely

requires the activity of the maturational protease encoded by the UL26 gene product. Many viral proteins are extensively phosphorylated, including the immediate early regulatory proteins ICP4, ICP0, and ICP27, as well as a number of structural proteins. Other modifications such as arginine methylation of ICP27 have also been described. The precise role of such post-translational modifications upon the function of these proteins has not been well characterized.

Assembly and Release

Following DNA replication in the nucleus, the completed viral genomes are packaged into preformed capsids as the scaffolding proteins (the products of UL26.5 and the 3′ portion of UL26) are displaced. This assembly process entails several proteins that comprise the DNA encapsidation/cleavage machinery. The packaging process also involves the 'a' sequence at the other end of the genome-sized DNA fragment, leading to cleavage of the growing DNA chain and resulting in encapsidation of a full-length genome. Assembly is accompanied by a major change in capsid structure (maturation), which entails the action of the UL26 viral protease. This is followed by a very complicated process of egress. While there is controversy on how this process occurs, in the currently favored model, the nucleocapsid first buds through the inner nuclear membrane acquiring an envelope. The virion then enters the cytoplasm by fusing with the outer nuclear membrane, thereby losing its initial envelope. Subsequently, it becomes re-enveloped by budding into intracellular membranous compartments and proceeds through the cytoplasm. Whatever the exact mechanism, infectious virus can be recovered from cells many hours before cellular disintegration and release of virions into the extracellular medium. Virus spread by cell to cell contact and/or cell fusion is probably an important feature of the pathogenesis of infection in the host and can be readily observed with infections in cultured cells.

Viral Transcripts and Proteins

Each viral protein is expressed from its own independently regulated transcript. The viral transcription map is included in **Figure 1**. Independent starts of overlapping transcripts, (rare) splicing, and temporally differentiated polyadenylation site utilization result in the number of independent transcripts expressed exceeding 100.

General Properties of Transcripts and Promoters

Generally, the ORFs of HSV-1 are expressed as unique, intronless mRNA molecules that are controlled by temporal class-specific promoters. Nested, partially overlapping transcripts, each encoding a unique ORF but utilizing a shared polyadenylation site, are common (e.g., transcripts encoding UL24, UL25, and UL26 and those encoding US5, US6, and US7). Some complementary reading frames also exist, such as the mRNAs encoding ORF-P and ORF-O with that encoding ICP34.5 and transcripts encoding reading frames complementary to UL43.

Most HSV promoters are recognizable as eukaryotic RNA polymerase II promoters with obvious 'TATA' box homologies 20–30 nucleotides (nt) 5′ of the mRNA cap sites. The *cis*-acting elements mediating transcription of immediate early (α) transcripts include upstream enhancer elements at sites distal to and extending to several hundred nucleotides upstream of the cap sites, as well as several transcription factor binding sites located within the region from −120 to −50. The enhancers contain multiple copies of a 'TAATGARAT' sequence, which interacts with cellular transcription factors of the POU family (notably, Oct-1). Enhancement of transcription occurs through the action of virion-associated tegument protein VP16 or αTIF (UL48), which is a powerful transcriptional activator. The *cis*-acting elements mediating transcription of early (β) promoters include several transcription factor binding sites and the TATA box at −30, but the 'TAATGARAT' enhancer elements are not present. Leaky-late (βγ) and late transcript (γ) promoters contain critical sequence elements mediating full levels of transcription at or near the cap site, and at least some contain transcription factor binding sites in the proximal part of the leader sequence downstream of the start site of transcription. HSV-1 promoters representing the temporal classes of productive infection are shown in **Figure 2**.

Nomenclature of Proteins

The proteins encoded by HSV-1, their functions (if known), and the locations of their coding regions on the genome are given in **Table 1**.

Currently accepted protein nomenclature is a mix of historic and systematic systems. Proteins encoded in the repeats and a number of other proteins of well-established function are referred to by their historic or functional names. Proteins encoded by ORFs in the U_L and U_S regions are often referred to by the location of the ORF. Both nomenclatures are used in **Table 1**.

Functional Classification of Proteins

Regulatory proteins

The cascade of viral gene expression begins with the expression of immediate early or α proteins. There are five immediate early genes and their expression is highly activated by the action of VP16 or αTIF (UL48), which interacts with cellular transcription factors, Oct1 and

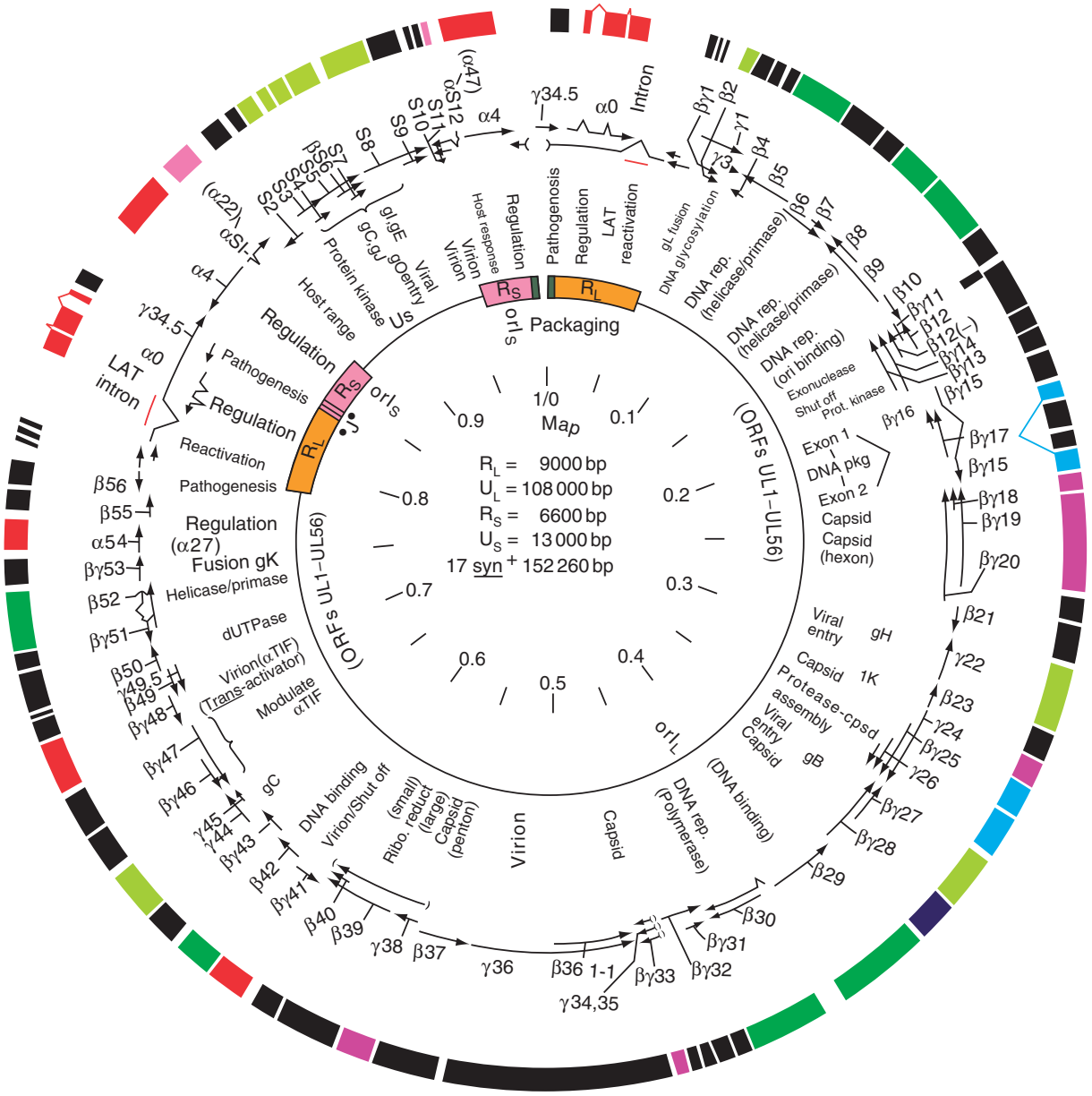


Figure 1 The genetic and transcriptional map of HSV-1 (strain 17*syn*$^+$). The map is shown as a circle to indicate the fact that the linear genome circularizes upon infection. ORFs predicted from sequence data are named numerically according to their position on the viral genome, and transcripts expressing all or part of them are indicated with the arrowheads showing the transcription termination/polyadenylation sites. Known kinetic classes of transcripts are indicated; the LATs in the R_L regions are the only transcripts expressed during latent infection. Also shown are the location of the origins of replication and the 'a' sequences at the genome ends which are involved in encapsidation. In addition, and where known, the functions of individual genes are shown. In the literature, gene names sometimes contain a subscript (e.g., U_L19 rather than UL19).

HCF. The resulting complex binds to the 'TAATGARAT' sequences upstream of immediate early gene promoters. Three of the five immediate early genes, ICP4, ICP0, and ICP27, have roles in regulating viral gene expression at the level of transcription. ICP4 is a transcriptional activator that is required for the transcription of early and late viral mRNAs. Multiple phosphorylated forms of the protein have been identified in the infected cell. ICP4 apparently stabilizes the formation of the transcription initiation complexes at the TATA box of viral promoters.

The essential ICP27 protein is multifunctional, acting at both the transcriptional and post-transcriptional levels to regulate viral and cellular gene expression. Different functional domains of ICP27 are required for its activities, which include recruiting cellular RNA polymerase II to sites of viral DNA, inhibiting cellular splicing, promoting

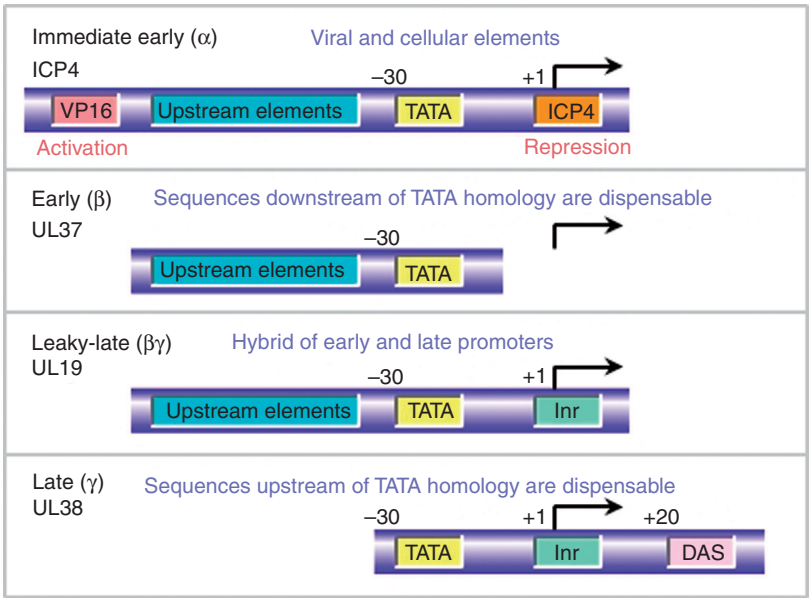


Figure 2 The architecture of HSV promoters. Features of the promoters controlling representative members of the kinetic classes of viral transcripts expressed during productive infection are shown. Inr, initiator element; DAS, downstream activator sequence.

export of viral intronless mRNAs, and enhancing translation of viral transcripts. ICP27 interacts with myriad cellular and viral proteins, and it binds to viral RNAs. ICP27 is phosphorylated and methylated on some arginine residues, and these modifications presumably help regulate its various activities.

ICP0 is also a multifunctional protein that is, first and foremost, a transcriptional activator. ICP0 appears to play a central role in regulating whether HSV infection goes into the lytic phase or the virus becomes latent. ICP0 is a member of the family of E3 ubiquitin ligase enzymes that have a so-called RING finger zinc-binding domain. This domain confers on ICP0 the ability to induce the proteasome-dependent degradation of a number of cellular proteins. This process results in multiple consequences, including the disruption of cellular nuclear substructures known as ND10 or PML nuclear bodies. ICP0 interacts with or forms complexes with a large number of proteins. It also undergoes extensive post-translational modification, including phosphorylation by viral and cellular kinases, and ubiquitination. The phosphorylation status of ICP0 alters as infection progresses, suggesting that the change in ICP0 localization from nuclear to predominantly cytoplasmic at later times of infection may in part be regulated by phosphorylation.

DNA replication proteins

Seven early proteins are required for the formation of the viral DNA replication complex: The DNA polymerase (UL30) functions in a complex with UL42, a processivity factor. The ori binding protein (UL9) binds to the critical core sequence 'GTTCGCAC' in ori_S and may function as a dimer. Equimolar amounts of proteins UL5, UL8, and UL52 make up the helicase–primase complex; and ICP8 (UL29), the major single-stranded DNA-binding protein functions in a manner analogous to phage T_4 gene 32 protein in keeping the replication fork open.

A number of other early proteins are involved in genome replication by altering the pool of deoxyribonucleoside precursors and for repair and proofreading functions. These include thymidine kinase (UL23), the large and small subunits of ribonucleotide reductase (UL39 and UL40), and deoxyuridine triphosphatase (UL50). An enzyme with a potential repair function is uracil-DNA glycosylase (UL2).

Structural proteins

More than 30 HSV late proteins are associated with mature virions. These include five capsid proteins, of which VP5 is the major capsid protein. A large number of tegument proteins and 10 or more glycoproteins are also found in mature virions.

Capsid assembly can be readily accomplished both *in vitro* and in insect cells with proteins expressed from a panel of recombinant baculoviruses expressing the four principal capsid proteins (UL19–VP5, UL38–VP19C, UL18–VP23, and UL35–VP26), the scaffolding protein (UL26.5), and the maturational protease (UL26). Other proteins have been implicated genetically in the capsid assembly process, but are dispensable in minimal *in vitro* systems.

Packaging of the viral DNA into the mature capsid requires a large number of viral proteins, including UL6, UL15, UL17, UL25, UL28, UL32, and UL33. The protein encoded by UL15, in conjunction with that encoded by UL28, may function as a terminase, cleaving

Table 1 Genetic functions encoded by HSV-1

Location	*Name*	*Function*
R_L	'a'	*cis* genome cleavage, packaging signal
	ICP34.5	RL1, neurovirulence, inhibits cellular apoptosis
	ORF-O	Modulates ICP0, ICP22 – inhibits splicing
	ORF-P	Modulates ICP4
	ICP0	RL2 (α0, IE1) immediate early *trans*-activator, E3 ubiquitin ligase, disrupts nuclear ND10 structures
	LAT-intron	Stable accumulation in nuclei of latently infected neurons
	LAT	*c*. 600 nt in 5′ region facilitates reactivation
	ORF-X	Unknown
	ORF-Y	Unknown
U_L	UL1	gL, essential glycoprotein that functions as a heterodimer with gH – involved in viral entry
	UL2	Uracil DNA glycosylase, DNA repair
	UL3	Nuclear protein
	UL4	Nuclear protein
	UL5	Part of helicase–primase complex, essential for DNA replication
	UL6	Capsid portal protein, capsid maturation, DNA packaging in capsid
	UL7	Unknown, dispensable in cell culture
	UL8	Part of helicase–primase complex, essential for DNA replication
	UL9	Ori-binding protein, essential for DNA replication
	UL10	gM, glycoprotein of unknown function
	UL11	Tegument protein, capsid egress, and envelopment
	UL12	Alkaline exonuclease, DNA packaging (?), capsid egress
	UL12.5	C-terminal 2/3 of UL12, expressed by separate mRNA – specific function unknown
	UL13	VP18.8, tegument-associated protein kinase
	UL14	Unknown
	UL15	DNA packaging, spliced mRNA, exons flank UL16 and UL17
	UL16	Tegument protein
	UL17	Cleavage and packaging of DNA
	UL18	VP23, capsid protein, triplex
	UL19	VP5, major capsid protein, hexon
	UL20	Membrane-associated, virion egress
	UL21	Tegument protein, auxiliary virion maturation function (?)
	UL22	gH, glycoprotein involved in viral entry as heterodimer with gL
	UL23	TK, thymidine kinase
	UL24	Nuclear protein
	UL25	Tegument protein, capsid maturation, DNA packaging
	UL26	VP24 (N-terminal half), protease, minor scaffolding protein (C-terminal half)
	UL26.5	Scaffolding protein
	UL27	gB, glycoprotein required for virus entry, mediates binding through interaction with GAGs in plasma membrane
	UL28	ICP18.5, capsid maturation, DNA packaging
	UL29	ICP8, single-stranded DNA-binding protein, essential for DNA replication
	ori_L	Origin of DNA replication
	UL30	DNA polymerase
	UL31	Nuclear lamina protein involved in nuclear egress
	UL32	Capsid maturation, DNA packaging
	UL33	Capsid maturation, DNA packaging
	UL34	Nuclear membrane protein involved in nuclear egress
	UL35	VP26, capsid protein
	UL36	VP1/2, tegument protein
	UL37	Tegument phosphoprotein
	UL38	VP19C, capsid protein, triplex
	UL39	Large subunit of ribonucleotide reductase
	UL40	Small subunit of ribonucleotide reductase
	UL41	vhs, virion host shutoff protein, degrades mRNA
	UL42	Polymerase accessory protein, essential for DNA replication
	UL43	Multiple membrane spanning protein
	UL43.5	Antisense to UL43
	UL44	gC, glycoprotein involved in initial stages of virion-cell association with GAGs, complement binding protein
	UL45	Membrane associated
	UL46	VP11/12, tegument associated

Continued

Table 1 Continued

Location	*Name*	*Function*
	UL47	VP13/14, tegument associated
	UL48	αTIF, VP16, virion-associated transcriptional activator, enhances immediate early transcription
	UL49	VP22, tegument protein
	UL49.5	Membrane protein associated with gM, glycosylated in other herpesviruses (gN) but not in HSV-1
	UL50	dUTPase, nucleotide pool metabolism
	UL51	Unknown
	UL52	Part of helicase–primase complex, essential for DNA replication
	UL53	gK, glycoprotein involved in virion egress
	UL54	ICP27, immediate early regulatory protein, multifunctional regulator, inhibits splicing, mediates viral RNA export, enhances translation initiation
	UL55	Unknown
	UL56	Tegument protein, affects pathogenesis
R_L		See R_L above
	R_L/R_S junction	Joint region, site of inversions of long and short segments, contains 'a' sequences
R_S	LAT-poly(A) site	Polyadenylation site for the primary latency associated transcript and the transcripts encoding ORF-O and ORF-P
	ICP4	RS1, essential immediate early transactivator, required for early and late gene expression
	ori_S	Origin of DNA replication
U_S	ICP22	US1, nonessential immediate early protein, affects host range
	US2	Unknown
	US3	Tegument-associated protein kinase, phosphorylates UL34 and US9
	US4	gG, glycoprotein of unknown function
	US5	gJ, glycoprotein of unknown function
	US6	gD, glycoprotein involved in virus infectivity and entry, binds HVEM
	US7	gI, glycoprotein, which as heterodimer with gE binds IgG-Fc and influences cell-to-cell spread of virus
	US8	gE, glycoprotein, which as heterodimer with gI binds IgG-Fc and influences cell-to-cell spread of virus
	US8.5	Nucleolar protein
	US9	Tegument-associated phosphoprotein (type II membrane protein)
	US10	Tegument-associated protein
	US11	Tegument-associated phosphoprotein, RNA binding, post-transcriptional regulation
	US12	ICP47, immediate early protein, inhibits major histocompatibility complex type 1 antigen presentation in human and private cells

genome-sized fragments from the growing concatemeric replication complex. The UL15–UL28 complex also appears to mediate the initial entry of viral DNA into the capsid, that is, it serves a 'docking' function. The alkaline exonuclease gene (UL12) also plays a role in DNA packaging.

Proteins involved in pathogenesis and cytopathology

After the onset of viral DNA synthesis, a large fraction of the viral genome is represented in abundant amounts of complementary mRNA that can form stable dsRNAs, which serve to activate protein kinase R (PKR). Activated PKR phosphorylates the α subunit of the translation initiation factor 2 (eIF-2α), which shuts off protein synthesis. To combat this response, HSV encodes a protein named $\gamma_1$34.5 (ICP34.5), which binds phosphatase 1α and redirects it to dephosphorylate eIF-2α, thereby preventing the shutoff of protein synthesis by activated PKR. One of the immediate early proteins, ICP47, appears to have a role in modulating host response to infection by specifically interfering with the presentation of viral antigens on the surface of infected cells by major histocompatibility complex class I (MHC-I), precluding immune surveillance by $CD8^+$ T lymphocytes at the very onset of infection.

A number of the envelope glycoproteins found in the intact virion appear to have a significant role in controlling host response. The complex of glycoproteins gE (US9) and gI (US8) functions as an IgG Fc receptor blocking the presentation of this region of the antibody molecule and precluding activation of the complement cascade. In addition, gC (UL44) binds several components of the complement complex, and viruses with mutations in gD (US6) display altered pathogenesis in mice.

Other viral gene products also have a role in cytopathology and pathogenesis. While dispensable for productive infection in some cells, the immediate early protein ICP22 is required for HSV replication in others – perhaps by mediating the expression of a set of late transcripts. Viruses lacking thymidine kinase (UL23) have significantly altered pathogenic patterns in experimental animals while certain mutations in the DNA polymerase gene (UL30) have altered patterns of neurovirulence.

Viral mutants defective in the virion host-shutoff protein (vhs; UL41) also have altered virulence in mice.

Latent Infection

HSV-1 latency is characterized by the persistence of viral genomes as episomes in the nuclei of sensory neurons. During latency, lytic functions are repressed and only one region of the genome is transcribed abundantly: the region encoding the LATs. The LAT domain is transcriptionally complex, and while the predominant species that accumulates during latency is a 2.0 kb stable intron, other RNA species are transcribed from this region of the genome, including a number of lytic or acute-phase transcripts. The LAT promoter (**Figure 3**) has not been characterized in quite as much detail as those promoters controlling productive cycle transcripts. It appears to have a number of regulatory elements important in neuronal expression over extended periods of time.

Latent infection with HSV can be viewed as having three separable phases: establishment, maintenance, and reactivation. In the establishment phase, the virus must enter a sensory neuron, and, following entry, there must be a profound restriction of viral gene expression so that the cytopathic results of productive infection do not occur. Thus, productive cycle genes are quiescent transcriptionally and functionally and only LAT is expressed. The maintenance of the HSV genome in latently infected neurons requires no viral gene expression apart from the LATs. Latent HSV genomes are harbored within the nucleus of a nondividing sensory neuron and do not need to replicate. HSV DNA is maintained as a nucleosomal, circular episome in latent infections and low levels of genome replication might occur or be necessary for the establishment or maintenance of a latent infection from which virus can be efficiently reactivated.

Reactivation of HSV results in the appearance of infectious virus at the site of entry in the host. Expression of only a 350 bp region of LAT near its 5′ end is both necessary and sufficient to facilitate reactivation in several animal models. Whatever the specific mechanism for this facilitation, it does not involve expression of a protein; rather, the active region appears to function by a *cis*-acting mechanism, the details of which are currently obscure. The process of reactivation is triggered by stress as well as other signals that may transiently lead to increased transcriptional activity in the harboring neuron, which may achieve a threshold that leads to lytic infection. The latency and reactivation cycle is shown in **Figure 4**.

Latent Phase Transcripts

Latently infected neurons represent 1–10% of the total neurons in the ganglia. In some neurons, an 8.5 kb polyadenylated primary LAT is expressed, which extends through the joint region (across the 'a' sequences) to a polyadenylation signal next to that of the ICP4 gene. The primary LAT is extensively processed, forming a stable 2 kb poly(A-) intron in lariat form, which accumulates in the latently infected neuronal nucleus. The mechanism by which LATs contribute to the establishment of and reactivation from latency has not been elucidated; in part, this is because LAT mutants behave somewhat differently in the different animal models used to study HSV latency. Because LAT does not become translated into protein, the RNA is believed to be the regulatory factor. It has been proposed that LAT may function as an antisense RNA or a small interfering RNA (siRNA). Future studies will reveal whether these possibilities are involved.

Future Trends

HSV possesses a number of features that will make it a continuing research model in the future. Among the most obvious are: (1) the neurotropism of the virus, along with the ability to generate recombinants containing foreign

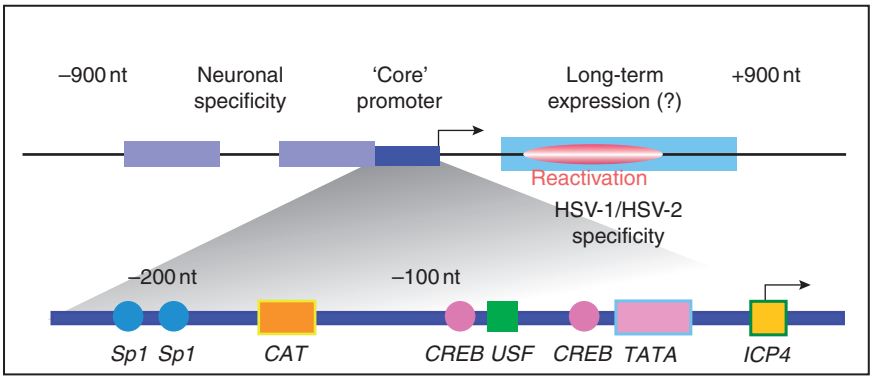


Figure 3 The architecture of the HSV LAT promoter. The transcriptional initiation site (arrow) and binding sites for transcriptional factors are shown in the expansion of the 'core' promoter.

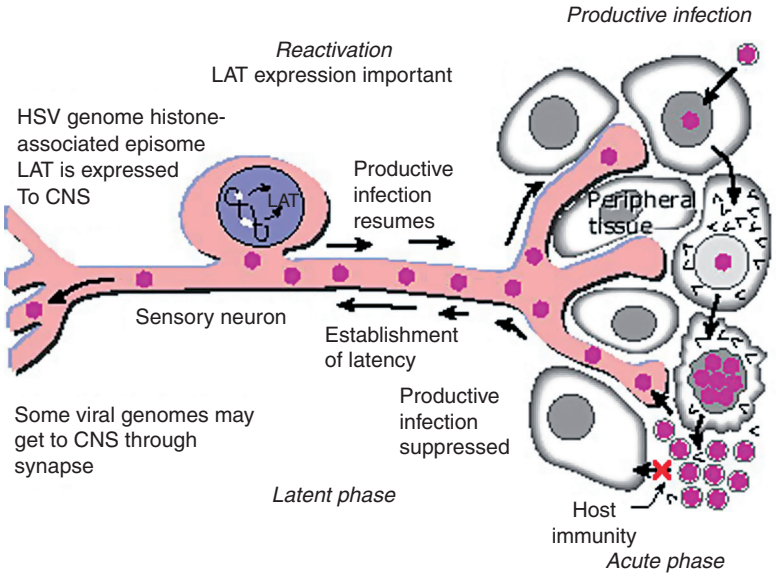


Figure 4 Schematic representation of HSV latent infection and reactivation. CNS, central nervous system.

genes, recommends HSV as a vector for introducing specific genes into neural tissue; (2) the fact that replication of HSV is restricted in terminally differentiated neurons suggests its potential as a therapeutic agent against neural tumors, and several studies have suggested real promise; (3) the abundance of viral genes dedicated to the efficient replication of viral DNA in the productive cycle provides a number of potential targets for antiviral chemotherapy; and (4) the number of multifunctional viral proteins that interact with many different cellular proteins makes HSV a useful model for detailed analysis of specific aspects of eukaryotic gene expression and cellular functions.

See also: Herpes Simplex Viruses: General Features; Herpesviruses: General Features; Herpesviruses: Latency.

Further Reading

Advani SJ, Weichselbaum RR, Whitley RJ, and Roizman B (2002) Friendly fire: Redirecting herpes simplex virus-1 for therapeutic applications. *Clinical Microbiology and Infection* 89: 551–563.

Bloom DC (2004) HSV LAT and neuronal survival. *International Reviews of Immunology* 23: 187–198.

Campadelli-Fiume G, Cocchi F, Menotti L, and Lopez M (2000) The novel receptors that mediate the entry of herpes simplex viruses and animal alphaherpesviruses into cells. *Reviews in Medical Virology* 10: 305–319.

Enquist LW, Husak PJ, Banfield BW, and Smith GA (1998) Infection and spread of alphaherpesviruses in the nervous system. *Advances in Virus Research* 51: 237–347.

Roizman B and Knipe DM (2001) Herpes simplex viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology* 4th edn., pp. 2399–2459. Philadelphia: Lippincott Williams and Wilkins.

Taylor TJ, Brockman MA, McNamee EE, and Knipe DM (2002) Herpes simplex virus. *Frontiers in Bioscience* 7: 752–764.

Wagner EK and Bloom DC (1997) The experimental investigation of herpes simplex virus latency. *Clinical Microbiology Reviews* 10: 419–443.

Herpesviruses of Birds

S Trapp and N Osterrieder, Cornell University, Ithaca, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

A large number of herpesviruses are found in avian host species of several different orders. Similar to their mammalian counterparts, they generally have a relatively narrow host range *in vitro* and *in vivo*. At present, only the gallid herpesviruses (GaHV-1, GaHV-2, and GaHV-3), meleagrid herpesvirus 1 (MeHV-1; also referred to as herpesvirus of turkeys, HVT), and psittacid herpesvirus 1 (PsHV-1) have been classified taxonomically by the International Committee on Taxonomy of Viruses (ICTV) as members of the *Alphaherpesvirinae* subfamily. Genomic sequences have been reported for all of these alphaherpesviruses and deposited in the GenBank sequence

database. Avian beta- or gammaherpesviruses have not been identified.

Taxonomically unclassified herpesviruses have been discovered in a wide variety of avian hosts; including the bald eagle (acciptrid herpesvirus 1, AcHV-1), duck (anatid herpesvirus 1, AnHV-1), black stork (ciconiid herpesvirus 1, CiHV-1), pigeon (columbid herpesvirus 1, CoHV-1), falcon (falconid herpesvirus 1, FaHV-1), crane (gruid herpesvirus 1, GrHV-1), bobwhite quail (perdicid herpesvirus 1, PdHV-1), cormorant (phalacrocoracid herpesvirus 1, PhHV-1), black-footed penguin (sphenicid herpesvirus 1, SpHV-1), and owl (strigid herpesvirus 1, StHV-1). Of these viruses, only AnHV-1 causes a known disease, duck virus enteritis (DVE), which accounts for significant economic losses. DVE, also referred to as duck plague, is a highly contagious acute disease of ducks, geese, and swans, and is characterized by hemorrhagic lesions in the blood vessels, gastrointestinal mucosa, and lymphoid tissues. The clinical symptoms of DVE are unspecific, and often mortality is the first observation in infected waterfowl flocks. Morbidity and mortality in domestic ducks may range from 5% to 100%.

Gallid herpesvirus 1 (GaHV-1), frequently referred to as infectious laryngotracheitis virus (ILTV), belongs to the type species of the genus *Iltovirus* and is the causative agent of a highly contagious, acute respiratory tract disease of domestic fowl termed infectious laryngotracheitis (ILT). ILT causes considerable economic losses to the poultry industry worldwide owing to mortality and decreased egg production associated with the disease. Epizootic forms of ILT are characterized clinically by severe respiratory symptoms such as dyspnea and hemorrhagic expectorations, and are generally associated with high mortality (5–70%). Enzootic infections, predominantly those occurring in poultry flocks of developed countries, are associated with mild symptoms (nasal discharge, conjunctivitis, and decreased egg production) or subclinical symptoms. Modified-live vaccines against ILTV infections are available, but their use is commonly restricted to areas where ILT is enzootic. However, most of the current modified-live virus vaccines have residual virulence, and several ILT outbreaks have been attributed to reversions of the ILTV vaccine strains to a virulent phenotype.

ILTV is the only virus allocated to the genus *Iltovirus*. However, recent genomic information indicates clearly an affiliation of PsHV-1 with this genus. PsHV-1 causes Pacheco's disease, a generally fatal disease of psittacine birds characterized by acute onset and massive necrotizing lesions of the crop, intestines, pancreas, and liver. Parrot species from multiple geographic regions are highly susceptible to Pacheco's disease. However, captive parrots such as macaws, Amazon parrots, and cockatoos account for the majority of clinical cases of Pacheco's disease; hence, the disease is of great concern to pet traders and exotic bird breeders worldwide. An association of subclinical PsHV-1 infections with mucosal papillomatosis in neotropical (Central and South American) parrots has been reported, but causality has not yet been demonstrated. Recently, genomic DNA of a novel psittacine alphaherpesvirus has been amplified from mucosal and cutaneous papillomas of African grey parrots. This virus is related to, but phylogenetically distinct from, PsHV-1, and has been designated psittacid herpesvirus 2 (PsHV-2).

Gallid herpesvirus 2 (GaHV-2) or Marek's disease virus (MDV) is the etiologic agent of Marek's disease (MD), an economically important, neoplastic, and neuropathic disease of chickens. MDV is highly infectious and routinely causes >90% morbidity and mortality in susceptible, unvaccinated animal populations. MD manifests as various clinical syndromes of varying severity. Of these, T-cell lymphoproliferative syndromes, including fowl paralysis (classic MD) and fatal MD lymphoma (acute MD), are most frequently associated with the disease. However, infections of chickens with the most recent clade of MDV strains are characterized by massive inflammatory brain lesions that are clinically manifested as transient paralysis and/or peracute death. Owing to its biological features, namely its lymphotropism and oncogenic potential, MDV was long thought to be related to Epstein–Barr virus, a lymphomagenic gammaherpesvirus. However, based on its genomic organization, MDV was reclassified as an alphaherpesvirus; thus, MDV is genetically more closely related to herpes simplex virus 1 (HSV-1) and varicella-zoster virus (VZV) (**Figure 1**). MDV belongs to the type species of the genus *Mardivirus*, into which two other closely related but distinct species have been grouped, represented by gallid herpesvirus 3 (GaHV-3) and MeHV-1 (HVT). Although the three viruses are closely related, only MDV causes MD while GaHV-3 and HVT are nonpathogenic. Therefore, the old nomenclature referring to GaHV-3 and HVT as MDV (serotypes) 2 and 3 is misleading and should be avoided.

Control of MDV-caused syndromes by vaccination was started in the late 1960s using an attenuated MDV strain (HPRS-16att) or the nonpathogenic HVT. Because MDV field strains are evidently evolving toward greater virulence in the face of vaccination, combination vaccines consisting of HVT and GaHV-3 or an attenuated MDV strain (CVI988-Rispens) are currently used to immunize chickens against the latest clade of MDV strains. Based on their ability to cause breaks of vaccinal immunity, MDV strains are subdivided into four different pathotypes: mildly virulent (m), virulent (v), very virulent (vv), and very virulent plus (vv+).

History

ILT was first described in 1925 as tracheolaryngitis, and shortly thereafter its viral etiology was demonstrated. The term infectious laryngotracheitis was adopted in 1931

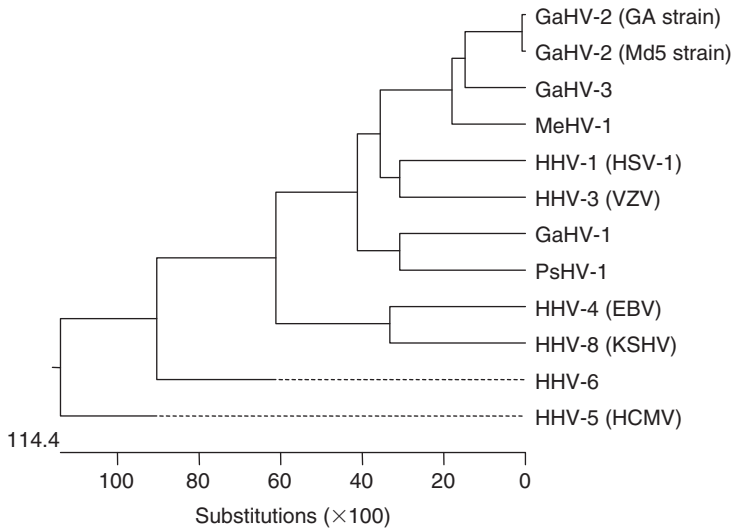


Figure 1 Genetic relationship of avian herpesviruses. A ClustalV-based comparison of the amino acid sequences of the DNA polymerase proteins of the viruses is shown. Human herpesviruses 5 (human cytomegalovirus) and 6, representatives of the *Betaherpesvirinae*, were chosen as outgroups. It is evident that the two representatives of the *Iltovirus* genus, GaHV-1 (ILTV) and PsHV-1 are closely related, as are the three members of the genus *Mardivirus*, GaHV-2 (MDV), GaHV-3, and MeHV-1 (HVT). These results also indicate independent evolution of *Alphaherpesvirinae* (GaHV-1 and GaHV-2) within one bird species (*Gallus gallus*). EBV, Epstein–Barr virus; HCMV, human cytomegalovirus; HHV, human herpesvirus; HSV-1, herpes simplex virus type 1; KSHV, Kaposi's sarcoma-associated herpesvirus; VZV, varicella-zoster virus.

by the Special Committee on Poultry Diseases of the American Veterinary Medical Association. ILT was the first major viral avian disease against which an effective vaccine was developed, being achieved in the 1930s by Hudson and Beaudette at Cornell University. Pacheco's disease was first recognized by Pacheco and Bier in the late 1920s in Brazil, but was not seen again until the 1970s, when outbreaks of the disease occurred in wild-caught neotropical parrots and a psittacine herpesvirus was identified as the etiologic agent.

The first account of MD dates from 1907, when József Marek (after whom the disease was named in 1960), a preeminent clinician and pathologist at the Royal Hungarian Veterinary School in Budapest, reported a generalized neuritis interstitialis or polyneuritis in four adult cockerels, which were affected by paralysis of the limbs and wings. Marek's detailed histological examination revealed that the sciatic nerve and areas of the spinal cord of the affected birds were infiltrated by mononuclear cells, an observation that is still made today after infection with most MDV strains. In the late 1920s, Pappenheimer and colleagues at Rockefeller University recognized the lymphoproliferative character of MD, and proposed that polyneuritis and visceral lymphoma were manifestations of the same disease. The identification of a highly cell-associated herpesvirus as the etiological agent of MD in the late 1960s by Churchill and colleagues led to the development of vaccines that achieved unparalleled success in preventing the disease, and provided the first example of immune prophylaxis against a cancer induced by an oncogenic virus.

Host Range and Virus Propagation

Like most avian herpesviruses, ILTV exhibits a very narrow host range. The chicken is the primary natural host, but pheasants and peafowl have also been reported to be susceptible. Turkeys have been shown to be susceptible to infection under experimental conditions, but other avian species are probably refractory. Embryonated chicken eggs are also susceptible, and can be used to propagate the virus. Chorioallantoic membrane (CAM) plaques resulting from necrosis and proliferative tissue reactions can be observed as early as 2 days post inoculation (pi). The virus can also be propagated in a variety of primary avian cell cultures, including chicken embryo liver cells (CELCs), chicken embryo kidney cells (CEKCs), and chicken kidney cells (CKCs). In addition, a permanent chicken cell line (LMH) derived from a chemically induced liver tumor was shown to permit replication of cell culture-adapted ILTV. Viral cytopathology *in vitro*, characterized by the formation of syncytia and nuclear inclusion bodies, can be observed as early as 4–6 h pi.

PsHV-1 infects a broad spectrum of psittacine species. In addition, it has been isolated from a superb starling, and some evidence suggests that other passerine species, such as the common cardinal, zebra finch, and canary, are also susceptible to PsHV-1 infections. Embryonated chicken eggs and primary chicken embryo cells (CECs) derived from 10- to 12-day-old chicken embryos are used for virus propagation *in vitro*.

MDV infections and MD-like symptoms have been observed in quail, turkeys, and pheasants, but the chicken

is considered the natural host of MDV. Primary chicken cells (CKCs and CECs) or duck embryo fibroblasts (DEFs) are used routinely to propagate the virus *in vitro*. However, for primary isolation and propagation of low-passage MDV isolates, the use of CKC or DEF is recommended to retain wild-type properties of the virus. Permanent avian cell lines such as OU2, DF-1, and QM7 allow only low level or abortive MDV replication. On permissive cells, MDV-induced plaques ($\leq$1 mm in diameter) develop very slowly (in 5–14 days pi) on primary isolation and 3–7 days pi after cell culture adaptation. In addition, 1-day-old chicks can be used for primary isolation and propagation of MDV *in vivo*.

Morphology

All herpesvirus particles share a complex and characteristic multilayered structure in which the nucleocapsid containing the viral DNA is separated from the envelope by a proteinaceous layer called the tegument. While both MDV and ILTV capsids have a diameter of approximately 125 nm, the incorporation of highly variable amounts of tegument into ILTV virions results in diameters of enveloped ILTV particles that vary between 195 and 350 nm. In contrast, enveloped MDV particles have a diameter of 150–160 nm. The morphologies of GaHV-3 and HVT closely resemble that of MDV, but, in thin sections, HVT capsids commonly show a characteristic cross-shaped appearance. The morphology of PsHV-1 has not been studied in detail, but typical herpesvirus particles have been visualized.

Genetics

The genomes of herpesviruses are linear, double-stranded DNA molecules that range in size from approximately 120 to 300 kbp. A total of six different genome organizations, referred to as classes A through F, are distinguished in the *Herpesviridae*, and only class D and E genomes are found in the *Alphaherpesvirinae*. ILTV and PsHV-1 have class D genomes and the three members of the *Mardivirus* genus (MDV, GaHV-3, and HVT) have class E genomes. Class D genomes consist of two unique sequence regions (unique-long, U_L; unique-short, U_S) with inverted repeats (internal repeat, IR; terminal repeat, TR) flanking U_S. Class E genomes consist of U_L and U_S, each bracketed by inverted internal (IR_L, IR_S) and terminal repeats (TR_L, TR_S).

The ILTV genome sequence, as assembled from 14 overlapping genome fragments from different virus strains, is approximately 147 kbp in length and has a G+C content of 48.2%. The PsHV-1 genome is 163 kbp in length and has a nucleotide composition of 61% G+C. ILTV and PsHV-1 contain 76 and 73 protein-coding open reading frames (ORFs), respectively, and the majority of these ORFs exhibit significant homologies to genes of the prototypic alphaherpesvirus, HSV-1. However, ILTV and PsHV-1 exhibit several unusual structural features, for example, a large internal inversion of the region within U_L containing genes UL22 to UL44 (a suffix is sometimes used in gene name, e.g., U_L 22). Also, U_L in both viruses contains a unique cluster of five ORFs (designated A to E) that do not share significant sequence similarity with known viral or cellular genes. The function of these ORFs is unknown, but they have been shown to be dispensable for ILTV replication *in vitro*. Another striking feature is the localization of the UL47 gene, which encodes a tegument protein and is usually localized in the U_L region of alphaherpesvirus genomes. UL47 is absent from the corresponding position in the ILTV and PsHV-1 genomes, but both viruses are still predicted to encode a homolog of UL47 (ILTV: UL47; PsHV-1: sORF1) in U_S.

One major structural difference between the closely related viruses ILTV and PsHV-1 can be found in the inverted repeat regions of the genomes. The ILTV repeats are considerably (18.5%) shorter than those of PsHV-1, and each harbors three genes: ICP4, US10, and sORF4/3. In contrast, ICP4 is the only gene located in the PsHV-1 repeat, and the US10 and sORF4/3 genes are located in U_S. Moreover, PsHV-1 U_L contains a homolog of UL16, an ORF that is conserved throughout the herpesvirus subfamilies but is absent from the ILTV genome, having presumably been lost during evolution. Conversely, ILTV U_L contains the UL48 gene and ILTV-specific UL0 gene, neither of which is found in the PsHV-1 genome.

The complete genomic sequences of representatives of all three species in the genus *Mardivirus* have been determined. The gene contents and linear arrangements of the genomes of MDV, GaHV-3, and HVT are similar in general, but vary considerably with regard to G+C content and size. Whereas the ~180 kbp MDV genome has a G+C content of 44.1%, the ~165 kbp GaHV-3 genome has a nucleotide composition of 53.6% G+C; the ~160 kbp HVT genome has a G+C content in between these two values (47.2%). A total of 103 (MDV), 102 (GaHV-3), and 99 (HVT) genes have been clearly identified in the mardivirus genomes. The majority of these genes are homologous to genes encoded by other alphaherpesviruses, but genus- and species-specific genes are also present.

Most of the differences between the three mardiviruses concern the TR_L and IR_L regions. In pathogenic MDV, they harbor a unique cluster of three species-specific genes, which are designated vTR, vIL-8, and meq. vTR, which encodes a viral homolog of telomerase RNA (vTR), exhibits 88% sequence identity to chicken telomerase RNA, and was presumably pirated from the chicken genome. The vIL-8 ORF encodes a CXC chemokine (viral interleukin-8, vIL-8) of 18–20 kDa in size, which functions as a chemoattractant for chicken mononuclear cells. The meq

gene encodes the 339-residue oncoprotein Meq (MDV EcoRI Q), a transcriptional regulator that is characterized by an N-terminal bZIP domain and a proline-rich C-terminal transactivation domain.

In addition, a genus-specific ORF (pp38), which is located in the junctions between the U_L and the adjacent repeat regions of all three mardivirus genomes, encodes a pp38 phosphoprotein of variable size dependent on the virus and the phosphorylation status of the protein. Another mardivirus-specific ORF, vLIP, is located in U_L in close proximity to pp38, and encodes a 120 kDa N-glycosylated protein, a portion of which shows significant similarity to the α/β hydrolase fold of pancreatic lipases and was thus termed viral lipase (vLIP). The HVT-specific vN-13 ORF encodes a 19 kDa protein (vN-13) that exhibits 63.7% amino acid sequence identity to cellular N-13, an anti-apoptotic member of the Bcl-2 family. While Bcl-2-like sequences have been identified in several gammaherpesviruses, HVT is the only alphaherpesvirus that encodes a Bcl-2 homolog.

One striking feature of all three mardiviruses is the presence of heterogenic telomeric repeat sequences at the genome termini. Interestingly, similar telomeric repeat sequences have been identified in the genomes of two betaherpesviruses, human herpesviruses 6 and 7 (HHV-6 and HHV-7), and one gammaherpesvirus, equid herpesvirus 2 (EHV-2). The structural or functional significance of these telomeric repeats is unknown, but they might be responsible for genome integration and maintenance of virus genomes in latently infected (tumor) cells.

Pathobiology

Pathogenesis, Clinical Features, and Pathology of Iltovirus Infections

ILTV is readily transmitted from infected to susceptible chickens, and virus shedding and spread mainly occur via the respiratory and ocular routes. Early cytolytic replication of ILTV in the epithelia of the upper respiratory tract results in syncytia formation and subsequent desquamation. Following the acute phase of infection, which lasts for approximately 6–8 days, ILTV establishes latency in the central nervous system (CNS), in particular in trigeminal ganglia. No clear evidence exists for a viremic phase in the course of lytic infection, latency, or reactivation. Sporadic reactivations from the latent state are usually asymptomatic, but generally lead to productive replication in the upper respiratory tract and virus shedding, which can result in infection of susceptible contact animals. The severity of clinical symptoms of ILT depends on the virulence of a particular ILTV strain or isolate, and mortality rates range from 0% to 70%. Severe epizootic forms of ILT are characterized clinically by marked dyspnea and hemorrhagic expectorations. Clinical signs of the milder, enzootic forms include nasal discharge, conjunctivitis, sinusitis, gasping, and decreased egg production. The incubation period of ILT ranges from 6 to 12 days. Pathomorphological alterations may be found in the conjunctiva and throughout the respiratory tract, but are most consistently detected in the larynx and trachea. Typical gross lesions include mucoid to hemorrhagic tracheitis, conjunctivitis, infraorbital sinusitis, and necrotizing bronchitis. However, in the case of the mild enzootic forms of ILT, conjunctivitis and sinusitis are often the only detectable gross lesions. Microscopic lesions include epithelial syncytia, desquamation, and submucosal edema. Eosinophilic intranuclear inclusion bodies in epithelial cells are detectable from day 1 to 5 pi, but disappear as desquamation of infected epithelial cells progresses.

Similar to ILTV, PsHV-1 is shed from the upper respiratory tract but also in the feces of infected parrots. Transmission of the virus occurs by direct contact between infected and susceptible animals or indirectly by contact with contaminated fomites or environmental contamination. The target sites for primary lytic viral replication and the establishment of latency are unknown. The systemic character of Pacheco's disease strongly argues for a viremic phase of infection during which the virus disseminates into multiple organ sites. Anorexia, depression, diarrhea, nasal discharge, and ataxia are among the most common clinical signs of the disease, but a variety of symptoms may be observed, largely depending on which organ system is affected. However, the majority of diseased animals die peracutely, that is, before the onset of clinical symptoms. Latently infected parrots that survive the disease play an important epidemiological role as asymptomatic virus carriers and shedders. The incubation time of Pacheco's disease ranges from 3 to 14 days. Gross pathological lesions are unspecific and may be found in a multitude of organ systems, including the respiratory and gastrointestinal tract, liver, and CNS. Microscopically, Pacheco's disease is characterized by a necrotizing hepatitis with minimal associated inflammation and the presence of intranuclear inclusion bodies (Cowdry type A).

Pathogenesis, Clinical Features, and Pathology of MD

MDV shed from the skin and associated with feathers and dander is highly infectious and can persist for extended periods in the environment. Transmission of the virus occurs by inhalation, either through direct contact or through virus present in dust and dander. In the current model of MDV pathogenesis, phagocytic cells in the lower respiratory tract become infected either directly or after an initial round of replication in epithelial cells. Within 24 h of uptake, the virus is detectable in the primary and secondary lymphoid organs such as thymus, bursa of Fabricius, and spleen. Following primary productive replication, which takes place in B-lymphocytes, the virus

infects activated $CD4^+$ lymphyocytes, and, rarely, $CD4^-CD8^-$ T-cells or $CD8^+$ T-cells. Infected $CD4^+$ T-cells serve as the target for the establishment of MDV latency and are also the means of virus dissemination within an infected animal. Besides epithelial layers in visceral organs, MDV enters the feather follicle epithelium, where cell-free infectious virus is assembled and released into the environment.

In latently infected T-cells, viral DNA is commonly integrated into the cellular genome. MDV is one of the few herpesviruses that achieve genome maintenance during the quiescent stage of infection through this mechanism, the details of which are entirely enigmatic. It is well known that latency is a prerequisite for oncogenic transformation by MDV, but only small subsets of latently infected T-cells become ultimately transformed and proliferate to generate tumors. Depending on the virulence of the virus strain and the susceptibility of the chicken population, mortality rates range from 0% to >90%. Clinical and pathological signs of MD vary according to the specific syndrome. The leading symptom of the classical form of the disease (fowl paralysis) is flaccid or spastic paralysis of the limbs and/or wings caused by lymphoproliferative peripheral nerve lesions. Chickens affected by MD lymphoma (acute MD) generally exhibit unspecific symptoms such as weight loss, anorexia, depression, and ruffled plumage, but (transient) paralysis may also be seen. Unlike the classic form of paralysis, transient paralysis is caused by inflammatory lesions of the CNS and peripheral nervous system. Most v and vv MDV strains induce MD lymphoma and/or (transient) paralysis in susceptible chicken lines. Recent outbreaks of MD caused by vv+ MDV strains are characterized clinically by transient paralysis, a massive rash affecting mainly the extremities, and/or peracute death.

Enlarged peripheral nerves and lymphomatous lesions are the most frequently observed gross pathological findings in MD. Lymphomatous lesions can develop as early as 14 days after infection and generally manifest as diffuse infiltrations and/or solid lymphomas, which affect a variety of organs, including the viscera (heart, liver, spleen, kidney, gonads, adrenal gland, etc.), skeletal muscle, and skin. Two main peripheral nerve pathologies are described: neoplastic proliferation that sometimes involves secondary demyelination (type A) and primary inflammatory cell-mediated demyelination (type B). MD lymphomas are cytologically complex and essentially comprised of lymphocytes and macrophages. MD tumors mainly consist of T-cells, but only a minority of these are transformed, the majority representing immune T-cells that try to contain the neoplasm.

Tumors induced by avian retroviruses represent a differential diagnosis for MDV-induced tumors, but onset of disease is delayed and nerve lesions are generally absent. Histologically, intranuclear inclusions are always absent from retrovirus-infected cells. A hallmark of MDV-transformed cells is expression of the viral oncoprotein Meq, and upregulation of the Hodgkin's antigen, CD30, has also been reported.

Prevention and Control

Control of Iltovirus Infections

ILTV-infected chickens produce peak virus-neutralizing antibody titers around 21 days post infection or vaccination. ILTV-neutralizing antibodies decline over the following months, but remain detectable for years. Although the detection of ILTV-specific antibodies by virus neutralization test is an important means of serological diagnosis, neutralizing antibody titers do not reflect the immune status of infected chickens or vaccinees, as immunity against ILT largely rests on local and cell-mediated immune response in the upper respiratory tract. Maternal antibodies to ILTV are transmitted to the chicken embryo via the egg yolk, but do not confer passive immunity against ILT or interfere with vaccination. For many years, modified-live virus (MLV) vaccines have been used to immunize chickens against ILT. They are administered via the intraocular route and for rapid mass vaccination via the drinking water or aerosolization. Immunization of chickens with attenuated ILTV vaccine strains results in latently infected carrier animals and spread of vaccine virus to nonvaccinates. However, spread to nonvaccinates results in consecutive bird-to-bird passages during which the vaccine virus may revert to a virulent phenotype. Therefore, the use of ILTV MLV vaccines has commonly been restricted to areas where infectious laryngotracheitis is enzootic. Experimental inactivated whole-virus vaccines and subunit preparations containing affinity-purified immunogenic ILTV glycoproteins were tested successfully as an immune prophylactic alternative to MLV vaccines. However, due to high costs of production and individual administration, the practical use of these vaccines in the field is debatable. Thus, the generation of MLV vaccines based on genetically engineered ILTV strains appears to be a more promising approach to the development of safe and efficacious ILTV vaccination protocols for the future.

Immune prophylaxis against Pacheco's disease is available in the form of an inactivated vaccine that is administered subcutaneously or intramuscularly. However, the vaccine is only protective against certain PsHV-1 serotypes, and its administration has been associated with adverse side effects, including granuloma formation and paralysis. Treatment with antiherpetic nucleoside analogues such as acyclovir can be used for pro- and metaphylactic measures in aviaries affected by Pacheco's disease. Strict quarantine protocols together with diagnostic screenings and isolation of birds that have been exposed to PsHV-1 are the most effective control measures for parrot breeding facilities and pet stores.

Control of MD

MD is controlled worldwide by vaccination of 18-day-old embryos *in ovo* or 1-day-old chickens by subcutaneous/intramuscular injection into the neck. Vaccine practices, however, vary between countries and, for example, broilers usually remain unvaccinated in Europe whereas all chickens are vaccinated in the USA. These different vaccine regimens exist largely because of differences in the production practices of broilers, which live longer in the Americas to produce carcasses with higher body mass as preferred by the consumer. Prior to the introduction of vaccines against MD in the late 1960s and early 1970s, the disease had an economically devastating impact on the poultry industry, and mortality rates reached as high as 30–60%. All MD vaccines used today are MLV preparations, and some of them have been used for almost 40 years. The first vaccines were based on HVT isolate FC126 (used mainly in the USA) and on HPRS16att (Europe), a formerly virulent strain that was rendered nonpathogenic by serial passage in cultured cells. Shortly after the introduction of vaccination in Europe, a naturally avirulent MDV strain, CVI988, was isolated, which has formed the basis of the MDV-based vaccines that are currently in use worldwide.

It became evident early on that MD vaccination is able to reduce and delay tumor development, but does not induce sterile immunity, which leads to a situation where constant evolutionary pressure is on field viruses to adapt to, and ultimately escape, vaccination. Consistent with this hypothesis, new MDV strains have been isolated that are able to break vaccine protection provided by HVT. The appearance of new, more virulent strains led to the introduction of so-called bivalent vaccines consisting of HVT and GaHV-3 strain SB-1. The latter was isolated from chickens, is completely avirulent, and is closely related genetically and antigenically to HVT and MDV. The bivalent vaccine was able to protect against more virulent (vv) viruses, but in the early 1990s even more virulent (vv+) MDV strains began to emerge. Most recently, repeated vaccinations using various combinations of vaccines (HVT + GaHV-3, HVT + MDV, MDV alone) are employed mainly in the USA to keep MD in check.

With the threat of new, even more virulent strains breaking through the current vaccination protocols, the development of novel vaccines is a huge challenge to the scientific community. Next to the efficacy of the vaccine preparations, the production process of vaccines in primary chicken embryo cultures is a major problem. The cost of producing these vaccines is high for several reasons, most notably the maintenance of pathogen-free chicken flocks, and approaches to find alternatives have been undertaken. The availability of infectious clones of various MDV strains and of HVT holds promise for a novel generation of rationally designed and efficacious vaccines.

See also: Herpesviruses of Horses; Herpesviruses: General Features; Retroviruses of Birds; Human Herpesviruses 6 and 7.

Further Reading

Davison F and Nair V (eds.) (2004) *Marek's Disease: An Evolving Problem.* London: Academic Press.

Guy JS and Bagust TJ (2003) Laryngotracheitis. In: Saif YM, Barnes HJ, Fadly A, Glisson JR, McDougald LR, and Swayne DE (eds.) *Diseases of Poultry,* 11th edn., ch. 5, pp. 121–134. Ames, IA: Iowa State.

Kaleta EF (1990) Herpesviruses of free-living and pet birds. In: Purchase HG, Arp LH, Domermuth CH, and Pearson JE (eds.) *A Laboratory Manual for the Isolation and Identification of Avian Pathogens*, pp. 97–102. Athens, GA: American Association of Avian Pathologists.

Osterrieder N, Kamil JP, Schumacher D, Tischer BK, and Trapp S (2006) Marek's disease virus: From miasma to model. *Nature Reviews Microbiology* 4: 283–294.

Panigraphy B and Grumbles LC (1984) Pacheco's disease in psittacine birds. *Avian Diseases* 28: 808–812.

Thureen DR and Keeler CL, Jr. (2006) Psittacid herpesvirus 1 and infectious laryngotracheitis virus: Comparative genome sequence analysis of two avian alphaherpesviruses. *Journal of Virology* 80: 7863–7872.

Herpesviruses of Horses

D J O'Callaghan, Louisiana State University Health Sciences Center, Shreveport, LA, USA
N Osterrieder, Cornell University, Ithaca, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

History

The first disease attributed to an equine herpesvirus (EHV), the agent that is now referred to as equid herpesvirus 1 (EHV-1, equine abortion virus; equine rhinopneumonitis virus), was documented at the University of Kentucky Agricultural Experiment Station in Lexington, KY. EHV-1 was first shown to be associated with spontaneous abortions in pregnant mares in 1932. In 1941, equine abortions were found to be associated with

mild respiratory disease with clinical signs similar to those associated with equine influenza virus infections. EHV-1 was also shown to be the etiological agent of epizootic respiratory disease in young horses. Based on these investigations, the disease was termed viral rhinopneumonitis, and the agent was called equine rhinopneumonitis virus. In the 1980s, however, the two disease manifestations, viral abortion and viral rhinopneumonitis, were shown to be caused by two closely related but clearly distinct viruses now classified as EHV-1 (equine abortion virus) and EHV-4 (equine rhinopneumonitis virus).

Equid herpesvirus 2 (EHV-2) was first isolated from horses in 1963. The cytopathology caused by this virus closely resembled that of cytomegalovirus infections, which were first described in 1921 in humans. EHV-2 is a ubiquitous, slow-growing virus that infects horses at a very young age (<2 years) and establishes a lifelong chronic infection such that the horse becomes a continuous shedder of the virus. To date, no major disease has been attributed to EHV-2. However, an association of EHV-2 with chronic throat infections (the 'lumpy bumpies') or recurrent eye disease has been established by some investigators, although the causal relationships have not been proved unequivocally. Also, EHV-2 may be a cofactor in EHV-1 and/or EHV-4 infections in that it is able to modulate EHV-1 and EHV-4 replication by immunosuppression, causing general malaise, or by modulation of EHV-1 gene expression through EHV-2-specific transcriptional transactivators.

Equid herpesvirus 3 (EHV-3; equine coital exanthema virus, ECE virus) was first isolated independently in 1968 in Canada, Australia, and the USA. EHV-3 is the etiological agent of equine coital exanthema, a generally mild genital infection of mares and stallions that is transmitted venereally and has been largely eradicated.

Equid herpesvirus 4 (EHV-4) is associated mainly with respiratory disease and has also been associated occasionally with equine abortions. On the other hand, EHV-1 is associated primarily with equine abortions, but frequently causes respiratory disease in young animals, highly fatal neurological disease, fulminating neonatal pneumonitis, and, very rarely, an exanthematous condition involving the external genitalia of the mare.

Equid herpesvirus 5 (EHV-5) is closely related to EHV-2 and no information on possible disease(s) caused by this virus is currently available.

Equid herpesvirus 9 (EHV-9), previously referred to as gazelle herpesvirus 1, was described very recently. It has been shown to be closely related to EHV-1 (and asinine herpesvirus 1) and exhibits a broad host range *in vivo*. While clinical signs in horses seem to be relatively mild, EHV-9 can cause lethal encephalitides in other animals, such as gazelles and goats.

Taxonomy and Classification

Eight of the nine EHVs have been classified as species in the family *Herpesviridae*. The species names are *Equid herpesvirus 1* through *Equid herpesvirus 9*, with the exception of *Equid herpesvirus 6*, which is not yet in existence as EHV-6 has not yet been fully assigned to a species. EHV-6, EHV-7, and EHV-8 are donkey herpesviruses that are also referred to as asinine herpesviruses 1, 2, and 3, respectively, and will not be discussed in detail in this article. The morphology of all six members is typical of the herpesviruses in that they are enveloped, contain an icosadeltahedral capsid, and have a proteinaceous coat, the so-called tegument, which surrounds the nucleocapsid. EHVs are composed of six distinct species: (1) EHV-1 is the major equine pathogen causing fetal abortions, respiratory illness, and neurological disease; (2) EHV-2 (and perhaps EHV-5) establish mainly asymptomatic, long-term persistent infections; (3) EHV-3 is the causative agent of mild progenital exanthema; (4) EHV-4 is a major respiratory pathogen that differs significantly from EHV-1 at the DNA level and is associated occasionally with equine abortions; and (5) EHV-9 can cause mostly subclinical encephalitides in horses, while infections of other hosts are often lethal. In the latter respect, EHV-9 possesses biological properties very akin to an alphaherpesvirus of pigs, pseudorabies virus (PRV). EHV-1, EHV-3, EHV-4, and EHV-9 are members of subfamily *Alphaherpesvirinae*, genus *Varicellovirus*. EHV-2 and EHV-5 are members of subfamily *Gammaherpesvirinae*, genus *Rhadinovirus*.

Geographic and Seasonal Distribution

EHV-1 and EHV-4 are distributed worldwide, and infections can occur year-round. Over the past 30 years in the USA, EHV-1 'abortion storms' have occurred in many areas. Major outbreaks have also been reported in Australia and England. Due to the nature of the disease manifestation, viral abortions caused by EHV-1 exhibit a seasonal cumulation in the spring on broodmare farms, whereas EHV-1 and EHV-4 infections of the upper respiratory tract (rhinopneumonitis), as well as the neurological form of EHV-1 disease, are observed mainly on race tracks and after crowding of large numbers of animals.

EHV-2 has also been isolated in many countries, including England, Switzerland, Germany, the USA, and South Africa. The existence of the closely related EHV-5 was described for England and Australia. More thorough studies, however, have led to the assumption that both EHV-2 and EHV-5 are distributed worldwide.

To date, EHV-3 has been isolated in five countries: Germany, USA, Australia, Canada, and England. EHV-9 so far has only been described in captive gazelles in Japan, although zoo animals in other parts of the world have been shown to harbor antibodies against the agent.

Host Range and Virus Propagation

Although the horse is the natural host of the EHVs, a variety of animals and tissue culture systems can be used to propagate the viruses. Regarding the major equine pathogen, EHV-1, experimental animals include Syrian hamsters and baby hamsters, chick embryos, baby mice and adult mice, and kittens. Primary tissue culture systems used to propagate EHV-1 include cells from a variety of equine tissues such as fetal lung, dermis, spleen, and kidney, as well as cells from domestic cats, dogs, hamsters, rabbits, mice, sheep, and swine. In the laboratory, permanent tissue culture systems commonly used to cultivate EHV-1 include primate HeLa, Vero, and CV-1 cells, rabbit kidney (RK) cells, mouse L–M cells, and equine NBL-6 and Edmin337 cells.

The host range for EHV-2, EHV-3, EHV-4, and EHV-5 is more restrictive than that for EHV-1. Except for RK cells and primary cat cells, EHV-2 and EHV-5 growth appears to be restricted to cells of equine origin. The host range for EHV-3 and EHV-4 is limited to cells of equine origin, although Vero cell culture-adapted EHV-4 strains have been described. EHV-9 is a notable exception of the rule of narrow host range of EHVs *in vivo*. As mentioned above, this virus was first isolated from an outbreak of fatal encephalitis in zoo gazelles and was later characterized in detail and shown to be most closely related to EHV-1. EHV-9 has a wide host range *in vivo* and *in vitro*.

Genetics

All six EHVs contain a linear, double-stranded DNA genome ranging between 140 and 184 kbp. The reported sizes of the genome are: EHV-1, 150 kbp; EHV-2, 184 kbp; EHV-3, 144 kbp; EHV-4, 145 kbp; and EHV-5, 179 kbp. The size of the EHV-9 genome has not yet been determined, but is likely to be close to that of EHV-1. The genomes of EHV-1, EHV-3, EHV-4, and probably EHV-9 exist in two isomeric forms, since the short region (S) can invert relative to the fixed orientation of the unique long region (U_L). The S region is composed of a central segment of unique sequences (U_S) bracketed by a pair of inverted sequences (IRs). In the case of the Kentucky A (KyA) tissue culture-adapted strain of EHV-1, each IR is 12.8 kbp. In contrast, the genomes of EHV-2 and EHV-5 exist as one isomer and are comprised of a large (149 kbp) central segment of unique sequences that is bracketed by a pair of direct repeat sequences. Each of the terminal direct repeat segments is 18 kbp, and the total genome size is 179–184 kbp. Other characteristics of the genomes are shown in **Table 1**.

There are varying degrees of homology at the DNA level among the six EHVs. The sequences shared by EHV-1, EHV-3, EHV-4, and EHV-9 appear to be arranged collinearly and are dispersed throughout the genome. EHV-1 and EHV-4 exhibit 55–84% identity at the DNA level and are antigenically very closely related, and antibodies can cross-neutralize. Levels of identity between EHV-1 and EHV-9 are even higher than those between EHV-1 and EHV-4. EHV-1 and EHV-2 show negligible identity, as do EHV-2 and EHV-3. EHV-1 and EHV-3 exhibit approximately 10% identity at the DNA level. Lastly, EHV-2 and EHV-5 show approximately 60% identity at both the DNA and protein levels.

The cloning of the genomes of a number of EHV-1 strains, among them the attenuated KyA and pathogenic RacL11 strains, as bacterial artificial chromosomes (BACs) has been achieved. These clones are infectious and provide a basis for rapid and efficient mutagenesis of the EHV-1 genome in prokaryotic cells. A variety of recombinant viruses that lack nonessential genes or portions of an essential gene have been generated and used in experiments to elucidate the functions of these genes in virus replication and/or pathogenesis.

Genome Structure

The entire genomes of EHV-1 strains, Ab4 and V592, EHV-4 strain NS80567, and EHV-2 strain 86/67 have been sequenced. The EHV-1 strain Ab4 genome is 150 224 bp in size, while that of EHV-4 is 145 597 bp. Both genomes contain 76 open reading frames (ORFs) potentially encoding proteins, with 4 duplicated in

Table 1 Properties of equine herpesvirus genomes

Member	*Subfamily*	*Isomers*	*S value*	*G+C content*	*Clinical manifestations*
EHV-1	Alpha	2	49–55	56%	Abortion, respiratory infection, paralysis
EHV-2	Gamma	1	61.8	57%	Chronic throat infection; perhaps recurrent eye infection
EHV-3	Alpha	2	55.4	66%	Equine coital exanthema
EHV-4	Alpha	2	ND[a]	50%	Respiratory infections
EHV-5	Gamma	1	ND	ND	Not known; possibly pneumonia
EHV-9	Alpha	2	ND	ND	Mild infections in horses; encephalitis in other animals (e.g., gazelles and goats)

[a]ND, not determined.

repeated regions in EHV-1 and -3 in EHV-4, giving a total of 80 ORFs in EHV-1 and -79 in EHV-4. The 63 ORFs in the U_L region are arranged colinearly between EHV-1 and EHV-4 and with those of herpes simplex virus (HSV) and varicella-zoster virus (VZV). Several genes mapping within IR and U_S of the S region differ in arrangement from those of other alphaherpesviruses. In addition, EHV-1 and EHV-4 contain a limited number of unique genes that are present in neither HSV-1 nor VZV and which might represent the viruses' gene repertoire involved in determining host specificity.

Each identical IR of the S region of the KyA tissue culture-adapted strain of EHV-1 is composed of 12 777 nucleotides. Six genes and the origin of DNA replication (ORI) have been mapped to IR: (1) the IR1 gene (gene 64) is an immediate-early (IE) gene encoding a spliced 6.0 kbp mRNA and a major phosphoprotein (1487 amino acid residues) with an apparent molecular mass of 203 kDa; (2) the IR2 gene is an early gene that is embedded within the IR1 gene, and its 4.4 kbp mRNA encodes a 130 kDa polypeptide (1165 amino acid residues) – the protein product of the IR2 gene represents a truncated form of the IE polypeptide; (3) the IR3 gene is a delayed-early gene encoding a 0.9 kbp mRNA that overlaps the IE promoter region on the opposite DNA strand; (4) the EHV-1 ORI maps downstream of IR3 and exhibits 60% identity to the corresponding ORI of HSV-1 and HSV-2; (5) the IR4 gene (gene 65) is a homolog of the ICP22 gene of HSV-1 and is differentially regulated to encode a 1.4 kbp early mRNA and a 1.7 kbp late mRNA; (6) the IR5 gene (gene 66) is a homolog of the US10 gene of HSV-1; and (7) the EHV-1 unique IR6 gene (gene 67) is a 'very early' gene encoding a 1.2 kbp mRNA and a 31/33 kDa phosphoprotein that has been shown to be a capsid constituent and a major determinant of virulence in some EHV-1 strains.

Additional EHV-1 genes in the S region map in the U_S segment and include homologs of the HSV-1 US2 gene, the protein kinase gene, the US9 gene, and genes that encode the glycoproteins (g) gG, gD, gI, and gE. In addition, a unique gene (EUS4 or gene 71) was mapped in U_S. This gene encodes a highly O-glycosylated protein referred to as gp2. Some size variations of gp2 were documented for EHV-1 strains KyA, Ab4, RacL11, and an EUS4 gp2 null mutant was apathogenic in a murine model of EHV-1 infection. Replacement of the truncated EUS4 gene of the apathogenic KyA strain with the EUS4 gene of the pathogenic RacL11 strain, which encodes the full size gp2 of 791 amino acid residues, resulted in a 'transfer' of pathogenic properties, indicating that gp2 is a major determinant of virulence. While gD is essential for virus replication in cultured cells, gI and gE are not, but do play a role in virulence. Restoration of the gI and gE sequences to a gI/gE deletion mutant or to vaccine strain KyA, which has an attenuated phenotype, restores virulence in the equine and murine models of infection.

Functional analyses of genes encoded in U_L have also been performed. Among the genes investigated are those encoding thymidine kinase, gB, gC, and gM, which show strong homology to their HSV-1 and VZV counterparts. Functional homology between HSV-1 and EHV-1 genes has been demonstrated for gM and gB.

The genomic sequences of EHV-2 and EHV-4 have been determined, but intensive research on gene functions has not yet been performed. Nothing is known about EHV-3 or EHV-9 gene functions.

Replication

The receptor for EHV-1 entry has not been identified, but recent studies showed that the virus utilizes a unique entry receptor, as it efficiently entered and replicated in cells that lack the entry receptors HveA, HveB, and HveC used for entry by other alphaherpesviruses, such as HSV-1, HSV-2, and PRV. As with other alphaherpesviruses, EHV-1 entry is mediated by gD.

The genes of the tissue culture-adapted KyA strain of EHV-1 are regulated at the transcriptional and translational levels in a temporal fashion, and three kinetic classes of genes designated IE, early, and late have been described (see **Table 2**). The sole IE gene (IR1) maps in both IR segments and gives rise to a spliced 6.0 kbp

Table 2 Regulatory genes of EHV-1

Gene	*Temporal class*	*Gene product*	*Function in replication*
IE (gene 64)	Immediate early	1487 aa[a]	Transactivates early genes and activates some late genes
IR4 (gene 65)	Early	293 aa	Enhances IE protein DNA-binding and binds TBP
UL5 (gene 5)	Early	470 aa	Binds the IE protein, TFIIB, and TBP
EICP0 (gene 63)	Early	419 aa	Promiscuous transactivator, which antagonizes IE protein function; binds IE protein, TFIIB, TBP
IR2	Early	1165 aa	Dominant negative regulator, which blocks IE protein binding to promoters
IR3	Early	0.9 kb RNA	Antisense to IE mRNA; possible precursor of microRNA
ETIF (gene 12)	Late	479 aa	Transactivates the IE promoter; essential for virus egress

[a]aa, amino acid residues in the primary translation product.

mRNA. Multiple IE polypeptide species have been observed, and the major IE protein (IE1, 203 kDa) is a nuclear-localized phosphoprotein that is capable of transactivating other viral genes and autoregulating its own transcription. The transactivation domain (residues 3–89), the DNA-binding domain (residues 422–597), and the nuclear localization domain (PPAPKRRV; residues 963–970) of the IE protein have been mapped. Recent studies have revealed that the IE protein harbors domains for binding general transcription factors, such as TFIIB and TATA-binding protein (TBP), and thus serves to promote the formation of pre-initiation complexes that mediate viral transcription.

Following IE polypeptide synthesis, approximately 45 early transcripts can be detected. Four of the early proteins serve as regulatory proteins and are designated IR4 (EICP22), UL5 (EICP27), EICP0 (UL63), and IR2. The IR4 protein interacts physically with the IE protein and serves to enhance the DNA binding of the IE protein to its target sequence (ATCGT) present within the promoters of EHV-1 genes characterized to date. The IR4 protein also binds to TBP and is present at viral early promoters in association with the IE protein and TBP. These interactions explain the synergistic effect on the transactivation of viral genes mediated by the IE and IR4 proteins. The EHV-1 UL5 protein exhibits limited identity to HSV-1 ICP27, and is essential for virus replication in cell culture. It acts synergistically with either the IE protein or the EICP0 early regulatory protein to activate expression of both early and late viral gene expression. This early regulatory protein also interacts physically with both the IE protein and TBP, and serves to enhance formation of transcriptional complexes on viral promoters. The third early regulatory protein is EICP0, which is a powerful and promiscuous transactivator that can independently activate expression of viral genes of all three temporal classes. Ironically, EICP0 cannot activate its own promoter, possibly due to a 28 bp negative regulatory element that maps at nucleotides (nt) −204 to −177 within the EICP0 promoter. The EICP0 protein binds to the IE protein and to cellular TFIIB and TBP, but is not a DNA-binding protein. The EICP0 and IE proteins have an antagonistic relationship that may result from their physical interaction in the nucleus and/or from competition for binding to TFIIB and TBP. Both viral proteins bind to the same domain in TFIIB. Deletion of the EICP0 gene greatly impairs virus replication and severely retards late gene expression, suggesting that this regulatory protein is important in the switch from early to late transcription. The fourth early regulatory protein is the IR2 protein, which is a truncated form of the IE protein lacking its essential transactivation and serine-rich domains. The IR2 protein serves a negative regulatory role as it downregulates viral gene expression by acting as a dominant negative protein that blocks IE protein binding to viral promoters and/or by squelching the limited supply of TFIIB and TBP. In addition to these four early auxiliary regulatory proteins, the EHV-1 unique IR3 gene contributes a regulatory role as it encodes a small transcript that is antisense to a portion of the IE transcript. In transient transfection assays, the IR3 transcript downregulates IE gene expression and is only minimally expressed as a protein. Initial studies suggest that the IR3 transcript is processed to a microRNA, and this indicates that the IR3 gene may use novel mechanisms to downregulate IE gene expression at late times of infection.

Early gene expression is followed by viral DNA replication and the production of approximately 29 late transcripts has been detected. Although these transcripts have been positioned on the viral genome, only a small number of protein products have been identified and characterized (see above).

Viral DNA replication initiates at approximately 4 h post infection and requires the virus-encoded DNA polymerase. DNA replication is thought to occur by the rolling circle mechanism whereby long concatemers of the viral genome are generated, cleaved, and then packaged into the maturing virions. The UL15 homolog of HSV-1, one of the two spliced EHV-1 genes known to date, appears to be essentially involved in the generation of unit length genomes and their packaging into mature capsids. Sequences at the L terminus of the EHV-1 genome are composed of direct repeats (DR1 = 18 bp and DR4 = 16 bp) as well as unique sequences (Uc = 60 bp), while sequences at the terminus of the S region contain a 54 bp region designated Ub. Thus, the sequence arrangement at the concatameric junction following replication of the EHV-1 genome is Ub-DR1-Uc-DR4, which represents a functional cleavage/packaging signal, similar, but not identical, to that of HSV-1.

The start of viral DNA synthesis initiates late gene expression and the synthesis of the late regulatory protein EHV transinducing factor (ETIF), a counterpart to the alpha-transinducing factor of HSV-1. This 60 kDa protein is multifunctional and plays at least three roles in EHV-1 replication. First, ETIF is present in several molecular sizes in the tegument and contributes to overall virion structure. Second, after virus entry and uncoating, ETIF serves to transactivate the IE promoter by binding to cellular factors that mediate its association with the TAATGARATT sequence at nt−630 to −620 in the IE promoter. This transactivation function to initiate viral gene programming is important but not essential, as EHV-1 DNA is infectious and virus progeny are produced in cells transfected with plasmids carrying the EHV-1 genome. The activation of a viral promoter by ETIF is specific for the IE promoter as ETIF has not been shown

to transactivate any early or late promoter tested to date. Recent experiments with an ETIF-deleted virus and an ETIF-complementing cell line revealed a third function for ETIF, and one that is essential for virus replication. Ultrastructural studies of cells infected with ETIF-deleted virus showed a marked defect in secondary envelopment of viral nucleocapsids at cytoplasmic membranes, such that few enveloped virions are produced. Thus, this transactivator protein also plays a key role in secondary envelopment.

Three different EHV-1 capsid species have been identified and probably correspond to the forms found in HSV-1, which are designated type A, B, and C capsids. The EHV-1 capsid species were designated: (1) L capsids, which appear to be empty; (2) I capsids which possess an electron-lucent, immature core structure in the shape of a cross; and (3) H capsids, which contain an electron-dense, mature core. All three capsids appear at approximately 6 h post infection. I capsids are believed to be a major precursor in the formation of mature capsids. The major capsid protein has an apparent size of 148 kDa, and other structural proteins have also been identified. As reported for other herpesviruses, EHV-1 maturation occurs by interaction of mature nucleocapsids with the inner portion of the nuclear membrane resulting in the formation of enveloped particles. As noted above, recent studies with ETIF-deleted EHV-1 support the model of the sequential envelopment/de-envelopment/re-envelopment pathway for egress of EHV-1 from the cell.

Infectious EHV-1 particles contain an envelope whose protein component is composed mainly of glycoproteins. To date, 12 EHV-1 glycoproteins have been identified and an association of gB, gC, gD, gG, gH, gL, gM, gp2, and the tegument protein VP13/14 with purified virions has been demonstrated. The glycoproteins have been shown in other alphaherpesviruses to be involved in virus binding, virus penetration, and cell-to-cell spread of infection. In the case of EHV-1, these functions were confirmed for gB, gD, gM, and gp2, and the latter two proteins were shown to be nonessential for virus growth. In contrast, gB- or gD-deleted EHV-1 mutants are unable to grow in cell culture. The defect in replication of gB- and gD-negative viruses is caused by an entry defect and the inability to spread from infected to uninfected cells. Detailed functional analyses for the other glycoproteins have not been performed, but are now facilitated by the use of engineered virus mutants produced by targeted gene deletion or disruption using infectious DNA clones of the virus. Even less is known for proteins that make up the third component of the mature virion, the tegument. Only one protein that is related to an HSV-1 tegument protein has been analyzed in detail. As discussed above, recent studies have shown that ETIF is essential for virus maturation and egress.

Defective Interfering Particles and Persistent Infection

EHV-1, EHV-2, and EHV-5 have been shown to mediate persistent infection. In the case of EHV-1, defective interfering particles (DIPs) have been shown to initiate and maintain this outcome. EHV-1 DIPs have been generated *in vivo* in the Syrian hamster model, and therefore may be relevant during EHV-1 infection of the natural host. DIPs are replication defective and require standard EHV-1 as a helper. The overwhelming majority of EHV-1 DNA sequences are absent from DIPs. The packaged DIP DNA molecule is a concatamer of EHV-1 sequences ranging in size from 5.9 to 7.3 kbp, repeated head to tail until it is approximately the size of the standard viral genome. DNA sequencing has revealed that sequences from three regions of the EHV-1 genome are conserved in DIPs: (1) the L terminus, including genes UL3, UL4 and the 3′ portion of UL5; (2) the junction between U_L and the internal IR; and (3) the central portion of IR, including ORI and the 5′ portion of gene IR4. The UL3 and UL4 genes in DIP genomes are 100% identical to those of infectious virus, but their functions in virus replication remain to be elucidated. The DIP genome also contains a perfectly conserved cleavage/packaging signal. The sequences at the L terminus and IR are joined by a homologous recombination event mediated by a conserved 8 bp sequence present at both the L terminus and within the IR4 gene to generate a unique ORF present only in DIPs. This ORF is expressed as a 31 kDa 'hybrid protein' comprising the N-terminal 196 amino acid residues of the IR4 protein (the homolog of HSV-1 ICP22) linked in frame to the C-terminal 68 amino acids of the UL5 protein (the homolog of HSV-1 ICP27). Unique to EHV-1 persistently infected cells (not detected in EHV-1 cytolytic infection) is a 2.2 kbp transcript that maps to the U_L/IR junction and is antisense to the IE mRNA. Interestingly, this transcript exhibits significant homology to the latency associated transcripts of HSV-1, which appear to be associated with HSV-1 reactivation rather than establishment of latency.

Lastly, in EHV-1 persistently infected cells, transcription of certain viral genes appears delayed compared with cytolytically infected cells. Recent findings reveal that expression of the 31 kDa IR4/UL5 hybrid protein downregulates expression of specific EHV-1 promoters. Moreover, altered forms of the EHV-1 IE polypeptides have been observed only in persistently infected cells. Taken together, these studies indicate that altered or aberrant viral regulatory mechanisms may be involved in establishing or maintaining persistent infection. Ongoing studies with recombinant forms of the DIP genome indicate that the hybrid gene is not essential for DIP replication, but is important in the ability of EHV-1 DIPs to establish persistent infection.

Evolution

The six EHVs are biologically distinct. Initial DNA sequence analyses have revealed that genes identified to date are collinearly arranged in the genomes of EHV-1, EHV-3, and EHV-4, all of which possess a two-isomer genomic structure. Evolutionary relationships have become more apparent now that EHV-1, EHV-2, and EHV-4 have been sequenced and data on EHV-5 sequences and genomic organization are available. It is clear that EHV-1, EHV-4, and EHV-9 are closely related and may have arisen from the same ancestor. The same is true for EHV-2 and EHV-5. However, it is not possible to determine precise details of the EHV ancestor since additional sequence data (especially on EHV-3) are not available.

Serological Relationship and Variability

The six EHVs share certain antigens, but are antigenically distinct. EHV-1, EHV-4, and EHV-9 are closely related antigenically, such that cross-neutralizing antibodies are generated. Also, EHV-2 and EHV-5 are closely related. Almost no data are available on the relationship of EHV-3 to other members of the EHVs. However, all of the EHVs are believed to share complement-fixing antigens.

Epidemiology

Rhinopneumonitis caused by EHV-1 and EHV-4 is spread by direct or indirect contact (ingestion and inhalation). The viruses are most commonly shed in nasal droplets for up to 3 weeks after initial infection and are present in large amounts in aborted fetuses and the placenta.

EHV-1 infection can also result in spontaneous abortions in pregnant mares. Horses are most susceptible to EHV-1 infection between the eighth and eleventh months of pregnancy. The peak incidence is in the ninth and tenth months, at which time approximately 70% of abortions occur.

EHV-2 and EHV-5 have been isolated from the respiratory tract, kidneys, spleen, testicles, genital tract, and rectum. Once infected, the horse is a lifelong carrier and excreter of the virus. The exact modes of spread of EHV-2 and EHV-5 are unknown.

EHV-3 causes a mild coital exanthema that is spread by genital contact and – rarely – the respiratory route. An EHV-3 infection is usually cleared after 14 days and is not associated with equine abortions.

Transmission and Tissue Tropism

EHV-1, EHV-4, and EHV-9 are spread mainly by nasal discharge. EHV-2 and EHV-5 establish a chronic infection and may be spread by the respiratory route. EHV-3 is spread by genital contact. EHV-1 has a wide host range *in vitro* as described above, while EHV-4 is more limited. EHV-2, EHV-3, and EHV-5 are restricted mainly to cells of equine origin.

Pathogenicity

EHV-1 and EHV-4 cause rhinopneumonitis, which is often transient and mild but can be complicated by secondary bacterial infections and become more severe and long lasting. EHV-1 is also associated with spontaneous abortions as well as neurological disease. Although EHV-1 and EHV-4 respiratory infections are clinically indistinguishable, their pathogenesis is quite different. EHV-1 infection results in a systemic viremia that can lead to abortion and/or neurological disease. Alternatively, EHV-4 infection usually remains restricted to tissues of the upper respiratory tract. EHV-2 and also EHV-5 infections are acquired horizontally early in life usually by inhalation. EHV-2 and EHV-5 establish a chronic lifelong infection in peripheral blood mononuclear cells. EHV-3 causes an acute coital exanthema in both the mare and the stallion, and infection remains localized to the genitalia.

Clinical Features of Infection

EHV-1 and EHV-4 cause outbreaks of upper respiratory disease ('common cold') in young horses (mainly EHV-4 in adult horses) with no previous exposure to the viruses. Infection is characterized by a short incubation time (<1 day) followed by fever (39–41 °C), which can last between 1 and 4 days, sometimes with a second spike approximately 1 week after the primary pyrexia, and animals suffer from serous nasal discharge and congestion of the nasal mucosa and conjuctiva. Less frequently, one can detect a transitory period of anorexia, enlargement of the submandibular lymph nodes, and edematous swelling of the lower parts of the body and the extremities. An initial leukopenia is followed by leukocytosis before the temperature falls. Recovery is usually uneventful and occurs within 1 week. Older horses show few or no clinical signs, although increased sensitivity is seen in stressed horses. Death is not uncommon from natural acquired infection resulting in neurological disease.

Equine abortions induced by EHV-1 usually occur late in gestation (8–11 months). The foals are usually born dead, but, if alive, often succumb to pneumonia within the first few days. Clinical signs of neurological disease caused by EHV-1 are highly variable and include head pressing, ataxia, and paralysis with complete recumbence.

Both EHV-1 and EHV-4 have been isolated from the central nervous system (CNS) of infected horses, but EHV-1 is by far the leading cause of EHV-induced neurological disease. The virus strains do not invade the extravascular nervous tissue and are not neurotropic *per se*. Rather, the virus spreads from the respiratory tract to the CNS via infected leukocytes and infects endothelial cells of the blood vessels supplying the spinal cord. While the neurological form of the disease is usually lethal, some horses have fully recovered from it with no permanent neurological sequelae.

The roles of EHV-2 and EHV-5 in clinical disease are virtually unknown. However, the viruses have been associated occasionally with chronic throat infections and have been isolated from the respiratory tract of horses with respiratory disease.

An incubation period as long as 10 days can be observed following natural infection with EHV-3. The initial lesions are small (1–2 mm), raised, reddened papules. The lesions then progress rapidly to the pustular form, and there is a general reddening of the vaginal mucosa in the mare. The number of lesions increases in the first few days, and by day six, many of the lesions form ulcers up to 20 mm in diameter and 5 mm deep. Lesions can also be seen on the vulva, perineal skin, as well as on the penis and prepuce of the stallion. The disease is usually mild, with temperature, pulse, appetite, and respiration remaining close to normal. The severity of the disease can be increased by secondary bacterial infections; however, uneventful cases are usually cleared within 2 weeks. Lastly, EHV-3 is not abortigenic and does not lead to infertility.

Infections with EHV-9 lead to encephalitis in horses, but, more severely, in other species as well. Among the susceptible hosts are gazelles and goats, and also carnivores such as dogs and cats.

Pathology and Histopathology

Respiratory disease caused by EHV-1 and EHV-4 results in inflammation, congestion, and sometimes necrosis of the tissues of the upper respiratory tract. Extensive swelling of the nasal mucosa may occur and, in later stages, the lungs may become involved. One can find typical herpesvirus inclusion bodies in the nuclei of the respiratory epithelium. The respiratory infection can become more serious if followed by a secondary bacterial infection, which may lead to bacterial pneumonia.

Fetuses that are aborted as a consequence of EHV-1 infection present with widespread hemorrhages and edema, as well as a yellowish discoloration of the fetal conjunctiva and splenomegaly – if virus is transmitted from mother to animal. However, abortions without infection of the fetus also occur, and the pathogenesis in the uterine vasculature largely resembles that of the vasculitis observed in the neurological form of the disease. The classic histopathology includes the presence of eosinophilic intranuclear inclusion bodies in various organs of the aborted fetuses. Gross pathological and histopathological alterations are sometimes not as obvious, and only sensitive methods (virus isolation or PCR) are able to confirm the EHV-1 abortion. Classically, features associated with EHV-1-induced abortions differ in those fetuses aborted during the first 6 months of gestation as compared with those aborted after 6 months. Those before 6 months present with widespread cell necrosis and inclusion bodies in the liver and lung. Those after 6 months exhibit jaundice, subcutaneous edema, excessive pleural fluid, pulmonary edema, splenomegaly, and necrosis of the liver.

In experimental EHV-1 infections, a severe hepatitis is observed following intraperitoneal infection of the Syrian hamster, and a model of respiratory infection is also available in the hamster. Intranasal infection of mice with EHV-1 results in respiratory disease and subsequent cell-associated viremia, thus serving as a model that somewhat mimics the disease in equines. The murine model of EHV-1 infection is widely used in virulence and immunogenicity studies. Recent studies with a library of EHV-1 mutants reveal the importance of full-size gp2 in respiratory disease. DNA-array analyses of lung tissues confirmed earlier findings from RNase protection assays and showed that the massive influx of inflammatory cells into the lung is preceded by 2–13-fold increases in >30 inflammatory genes. Expression of these genes increased as early as 8–12 h post infection, and they encode major cytokines and chemokines, including interleukin-1β (IL-1β), IL-6, tumor necrosis factor alpha, macrophage inflammatory protein 1α (MIP-1α), MIP-1β, and MIP-2. Future investigations should give insight into specific domains of gp2 that activate this constellation of inflammatory genes and the signal transduction pathways that are involved.

EHV-2 (and also EHV-5) infection becomes widespread throughout the body, and the viruses have been isolated from a variety of tissues. The infected animal becomes a lifelong carrier, and the viruses remain highly cell associated.

Tissues affected following an infection with EHV-3 include the vaginal and vestibular mucosa, penis, prepuce, and the skin of the perineal region. One of the characteristics of an infection with EHV-3 is the sloughing of the surface epithelial cells. On occasion, the skin of the lips and mucus membranes of the respiratory tract may become involved, but the exanthema is usually mild.

Immune Response

Neutralizing antibodies are detected in the serum soon after an EHV-1 or EHV-4 infection. The antibodies can first be

detected from a week following infection and are most abundant after several weeks. However, the immunity is short lived, in that horses can be re-infected and exhibit respiratory symptoms just 3 months after the initial infection. Multiple exposures to either EHV-1 or EHV-4 will result in the development of neutralizing, cross-reactive antibodies. However, cell-mediated immune responses are likely to be primarily responsible for induction of a sustainable immunity.

Immunity to EHV-2, EHV-3, and EHV-5 is poorly understood. However, virtually all horses have antibodies to EHV-2 and EHV-5, confirming the general apathogenicity of these viruses.

Prevention and Control

A number of vaccine approaches are followed to combat EHV-1 infections; among them are modified live vaccines (e.g., Rhinomune and Prevaccinol), inactivated vaccines (e.g., Pneumabort K and Prestige), inactivated combination vaccines, which – among others – also contain EHV-4 (e.g., Innovator, Resequin, and Duvaxyn1,4), and subunit vaccines also covering both viruses (e.g., Cavalon IR). Unfortunately, many EHV vaccines cause undesirable side effects in the form of massive local reactions while not affording acceptable levels of protection, especially when inactivated combination vaccines are considered. All vaccines are given repeatedly to pregnant mares usually in the third, fifth, seventh, and ninth months of pregnancy, since, to ensure protection, a good level of population immunity is imperative. To protect against viral rhinopneumonitis outbreaks, the vaccine is usually given to all horses every 3–6 months. There is considerable ongoing discussion as to proper vaccination against the neurological disease, which has become more prevalent during past years. Recent studies seem to suggest that modified live virus vaccines are superior, especially to multivalent inactivated vaccines, with respect to duration of fever, virus excretion from the nasal mucosa, and development of neurological symptoms. These studies, however, need to be corroborated by further comparative studies and/or larger numbers of animals in experimental groups.

Clinical management often involves the use of antibiotics to prevent severe bacterial complications following the viral rhinopneumonitis. Control of EHV-1 and EHV-4 infections involves isolation and quarantine of infected horses (for at least 3 weeks) and sound hygiene for prevention of viral infection, since the viruses are highly contagious. Quarantine procedures are required more often with EHV-1 infections, since EHV-1 can lead to more serious diseases of the CNS. Since many of the EHV vaccines provide unacceptable levels of protection, the first step in the prevention and control of EHV-1 and EHV-4 infections involves specific management practices and adequate day-to-day care of the animals. The viruses can be spread easily in contaminated feed and water. In addition, minimizing stress and close contact of large groups of horses can prevent the spread of disease.

Future Perspectives

Considerable progress in unraveling the nucleotide sequences of EHV-1, EHV-2, EHV-4, and EHV-5 has been made during recent years, and EHV-1, EHV-2, and EHV-4 have been entirely sequenced. With this information in hand, it will be possible to pursue studies on gene expression and on those proteins that are involved in virulence of EHV. These studies will in turn open the possibility for a rational design of anti-EHV vaccines, especially against the most important pathogens, EHV-1 and EHV-4. These goals may be achieved by the use of viral deletion mutants that carry targeted gene deletions, which is greatly facilitated by the advent of a number of infectious DNA clones during the past years. Using novel molecular approaches, a better understanding of EHV biology will be possible and in the future will open new perspectives in the understanding of diseases caused by EHVs.

See also: Herpesviruses: General Features; Herpes Simplex Viruses: General Features; Herpes Simplex Viruses: Molecular Biology; Pseudorabies Virus; Varicella-Zoster Virus: General Features; Varicella-Zoster Virus: Molecular Biology.

Further Reading

Albrecht RA, Kim SK, and O'Callaghan DJ (2005) The EICP27 protein of equine herpesvirus 1 is recruited to viral promoters by its interaction with the immediate-early protein. *Virology* 333: 74–87.

Buczynski KA, Kim SK, and O'Callaghan DJ (2005) Initial characterization of 17 viruses harboring mutant forms of the immediate early gene of equine herpesvirus 1. *Virus Genes* 31: 229–239.

Goodman LB, Wagner B, Flaminio MJBF, *et al.* (2006) Comparison of the efficacy of inactivated combination and modified-live virus vaccines against challenge infection with neuropathogenic equine herpesvirus type 1 (EHV-1). *Vaccine* 24: 3636–3645.

Kim SK, Albrecht RA, and O'Callaghan DJ (2004) A negative regulatory element (bp –204 to –177) of the EICP0 promoter of equine herpesvirus 1 abrogates the EICP0 protein's *trans*-activation of its own promoter. *Journal of Virology* 78: 11696–11706.

Pagamjay O, Sakata T, Matsumura T, Yamaguchi T, and Fukushi H (2005) Natural recombinant between equine herpesvirus 1 and 4 in the ICP4 gene. *Microbiology and Immunology* 49: 167–179.

Paillot R, Ellis SS, Daly JM, *et al.* (2006) Characterisation of CTL and IFN-gamma synthesis in ponies following vaccination with a NYVAC-based construct coding for EHV-1 immediate early gene, followed by challenge infection. *Vaccine* 24: 1490–1500.

Rudolph J, O'Callaghan DJ, and Osterrieder N (2002) Cloning of the genomes of equine herpesvirus type 1 (EHV-1) strains KyA and RacL11 as bacterial artificial chromosomes (BAC). *Journal of*

Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health 49: 31–36.
Smith PM, Kahan SM, Rorex CB, von Einem J, Osterrieder N, and O'Callaghan DJ (2005) Expression of the full length form of gp2 of equine herpesvirus 1 (EHV-1) completely restores respiratory virulence to the attenuated EHV-1 strain KyA in CBA mice. *Journal of Virology* 79: 5105–5115.
Soboll G, Whalley JM, Koen MT, *et al.* (2003) Identification of equine herpesvirus-1 antigens recognized by cytotoxic T lymphocytes. *Journal of General Virology* 84: 2625–2634.
Telford EAR, Studdert MJ, Agius CT, Watson MS, Aird HC, and Davison AJ (1993) Equine herpesviruses 2 and 5 are γ-herpesviruses. *Virology* 195: 492–499.
Telford EAR, Watson MS, Aird HC, Perry J, and Davison AJ (1995) The DNA sequence of equine herpesvirus 2. *Journal of Molecular Biology* 249: 520–528.
Telford EAR, Watson MS, McBride K, and Davison AJ (1992) The DNA sequence of equine herpesvirus-1. *Virology* 189: 304–316.

Herpesviruses: Discovery

B Ehlers, Robert Koch-Institut, Berlin, Germany

© 2008 Elsevier Ltd. All rights reserved.

History of Herpesvirus Discovery

Diseases caused by herpesviruses have been known since ancient times, but it was not until the beginning of the twentieth century that the viruses causing these diseases were detected. Human alphaherpesviruses (members of subfamily *Alphaherpesvirinae*) such as herpes simplex virus (HSV) and varicella-zoster virus (VZV) were the first to be propagated in cell culture and visualized by electron microscopy. Later, betaherpesviruses (members of subfamily *Betaherpesvirinae*) such as human cytomegalovirus (HCMV) and gammaherpesviruses (members of subfamily *Gammaherpesvirinae*) such as Epstein–Barr virus (EBV) were discovered and studied in detail. EBV was the first herpesvirus whose genome was cloned in bacteria and completely sequenced. In the middle of the last decade of the twentieth century, the complete genome sequences of roughly 40 herpesvirus species had accumulated in public databases. These human and animal herpesviruses had previously all been cultured, and their physical, morphological, and antigenic characteristics had been studied. This was a cumbersome process and usually carried out only with viruses causing important diseases.

For the primary characterization of uncultured viruses, alternative, more-straightforward approaches were needed. Immunological cross-reaction and nucleic acid hybridization were the first tools used for the detection of unknown viruses closely related to known species. Later, nonspecific amplification methods were developed that detected nucleic acids of unknown DNA and RNA viruses directly in clinical samples. Expression libraries of genomic DNA or cDNA were screened immunologically with sera from patients infected with the putative etiologic agent. Then subtractive approaches were developed to isolate sequences in one DNA sample that were absent from another, and one of these, representational difference analysis, led to the discovery of human herpesvirus 8 (HHV-8; also known as Kaposi's sarcoma-associated herpesvirus). Although very powerful, these nonspecific amplification methods are most appropriate for the detection of agents in defined samples from diseased individuals where a microbiologic etiology is strongly indicated. They are too laborious for broad screening of large sample numbers.

In 1988, Mack and Sninsky were the first to describe a polymerase chain reaction (PCR)-based method for the identification of uncharacterized viruses related to known virus groups, using degenerate primers (a pool of all the possible combinations of sequences that could code for a given amino acid sequence). Later, and following the same principles, a PCR method for the universal detection of herpesvirus genomes was published by VanDevanter and co-workers. Despite its restriction to herpesviruses, this method was a hallmark because it had the potential to detect virtually all vertebrate herpesviruses by a straightforward approach. Since then, nearly all novel herpesviruses have been discovered with the help of this method (in its original version or with modifications), and many were found in individuals lacking a recognized disease history.

Panherpes PCR with Degenerate Primers

The method is based on the fact that herpesvirus genomes contain highly conserved genes, which are present in all because they code for proteins essential for viral growth. One of the most conserved is the herpesvirus DNA polymerase (DPOL) gene. Investigators aligned the DPOL genes of all herpesviruses for which sequence data were available, in order to identify short blocks of greatest amino acid identity that encode domains essential for DPOL function. This procedure allowed the design of

five degenerate primers that were used in a nested PCR – three primers in the first round and two primers in the second round (**Figure 1**). The region amplified in between the primers displayed a lower degree of conservation. This allowed investigators to assess (1) whether the detected herpesvirus was known or novel, and (2) to which herpesvirus subfamily the novel species could be assigned. In some cases, even a tentative assignment to a virus genus was possible.

The primers are degenerate in their 3′ part and nondegenerate in their 5′ part. As an example, the sense primer (TGV) of the second round is shown in **Figure 2**. The degenerate 3′ part enables it to bind universally to the DPOL gene of higher vertebrate herpesviruses in the first PCR cycle. The nondegeneracy of the 5′ part ensures amplification efficiency in subsequent cycles. The product amplified in the second round has a length of ~210–235 bp, depending on the virus. Only HCMV and chimpanzee cytomegalovirus, as well as some herpesviruses of insectivoral shrews, give rise to significantly larger amplification products of >300 bp (**Figure 3**).

Several variations of the original method were subsequently published, using (1) deoxyinosine-substituted primers, (2) a nondegenerate consensus sequence as 5′-part of the primer (also called consensus-degenerate hybrid oligonucleotide primers (CODEHOPs)), or (3) other conserved primer binding sites in the DPOL gene. Also, targeting of other highly conserved herpesvirus genes such as the glycoprotein B (gB) gene or the terminase gene was attempted. Degenerate consensus primers were also used to amplify a larger number of conserved genes ($n = 8$) of a novel porcine herpesvirus (porcine lymphotropic herpesvirus 3, PLHV-3). These sequence 'islands' were then connected with the help of long-distance PCR to yield a contiguous sequence of ~100 kbp (accession AY170316). This is the longest part of a herpesvirus genome of unknown sequence ever determined directly from primary virus-positive organ material solely through PCR (i.e., without virus cultivation).

How Many Herpesviruses Have Been Discovered?

Since the publication of these methods, the number of novel herpesviruses discovered has risen very fast. Prior to writing this article, more than 200 potential herpesvirus species had been detected, belonging to more than 20 mammalian orders (**Table 1**). These are many more than currently accepted by the International Committee on Taxonomy of Viruses (ICTV), which lists some 120 herpesvirus species (VIIIth Report). Sequences of more than 160 herpesviruses are available under the taxonomy browser of the NCBI, most detected by DPOL consensus primers. In addition, sequences indicating the existence of more than 100 additional herpesvirus species, not yet available in public databases, await publication. By combining all the data currently available, a total of approximately 300 detected

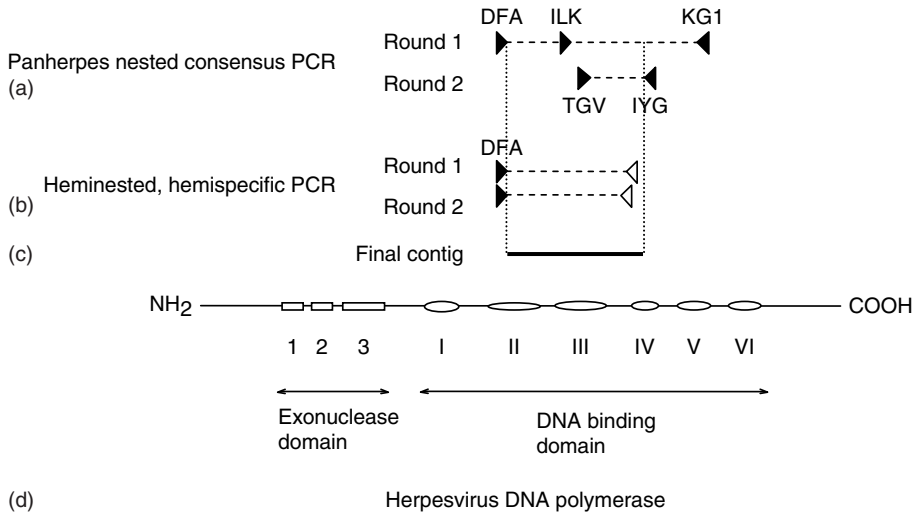


Figure 1 Panherpes consensus PCR with degenerate primers. Schematic diagram of the PCR strategy. (a) Initially, panherpes-nested PCR with degenerate and deoxyinosine-substituted primers (black triangles) is performed for amplification of novel herpesvirus DPOL sequences. In the first round, the sense primers DFA and ILK are combined with the antisense primer KG1. In the second round, the sense primer TGV and the antisense primer IYG are used. (b) Subsequent heminested PCR with the degenerate sense primer DFA and two virus-specific antisense primers (open triangles). The exact position of the virus-specific antisense primers is slightly different for each individual DPOL sequence but is always located between primers TGV and IYG. (c) The final contig is represented by the solid line (450–480 bp, without primer-binding sites). (d) The DPOL gene is shown schematically. Highly conserved, functionally important regions are represented by boxes and ovals, respectively, and numbered.

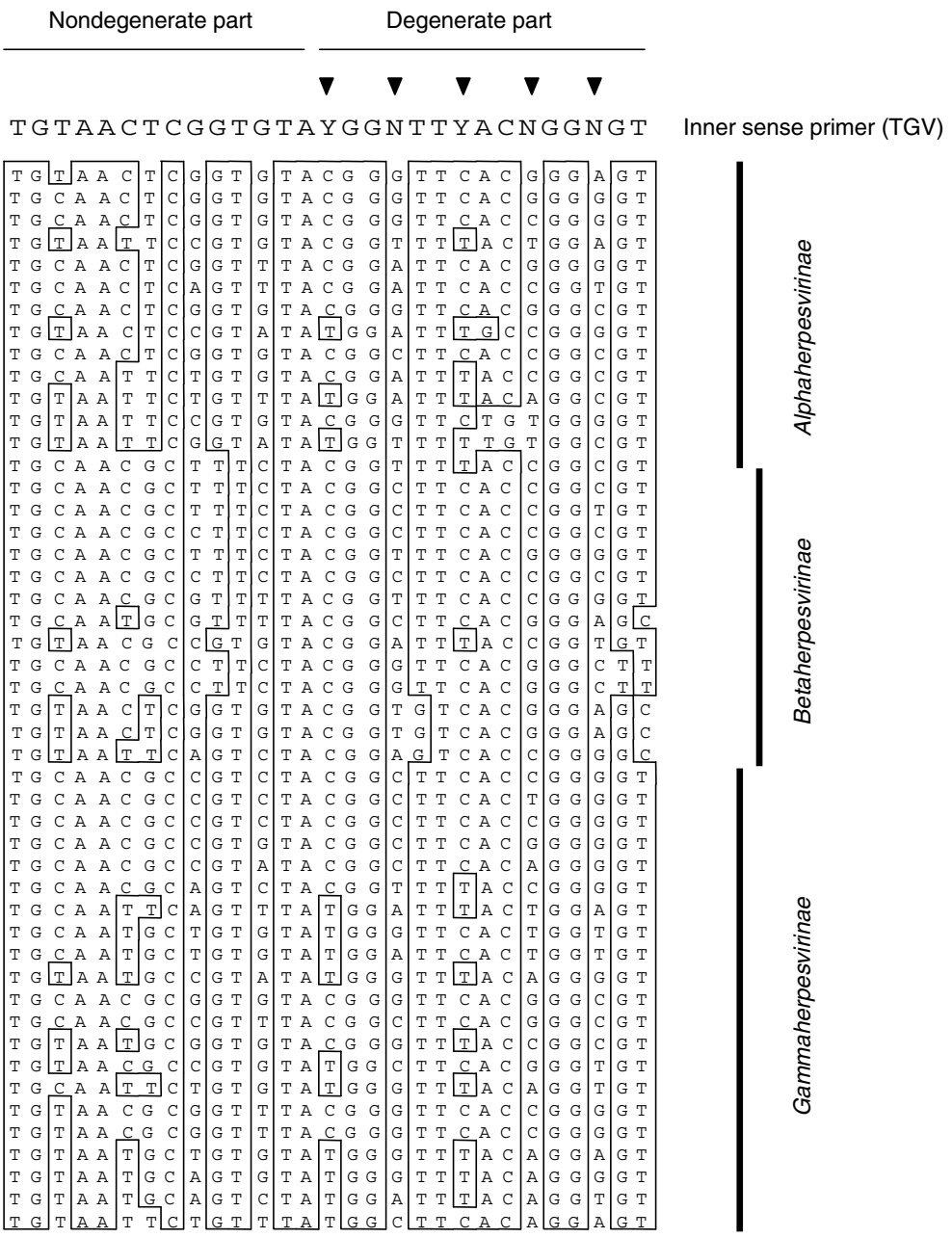


Figure 2 Primer-binding site of the inner sense primer of the panherpes consensus PCR. An alignment is shown of the binding regions of the inner sense primer (TGV) in alpha-, beta-, and gammaherpesviruses. Above, the TGV primer is shown, originally designed by VanDevanter and co-workers. In its degenerate part, the primer does not match perfectly the sequences of some alpha- and betaherpesviruses. Nonetheless, the DPOL sequences of these viruses are amplifiable by panherpes consensus PCR.

herpesvirus species can be presumed, the vast majority infecting a broad spectrum of mammalian hosts such as primates, elephants, ungulates, rodents, dolphins, carnivores, insectivores, manatees, and bats. Herpesviruses have also been found by consensus primer PCR in non-mammalian hosts such as reptiles, birds, and fishes (**Table 1**). Even a mollusk (Pacific oyster) has been shown to host a herpesvirus. The fact that herpesviruses are not yet known for many animal hosts is most probably due to lack of investigation.

Most of the novel viruses from which newly detected sequences originate have never been isolated or propagated in tissue culture, and a large number probably never will. On the other hand, some of the herpesviruses that were cultured in the premolecular era and classified formally have never been characterized genetically and are

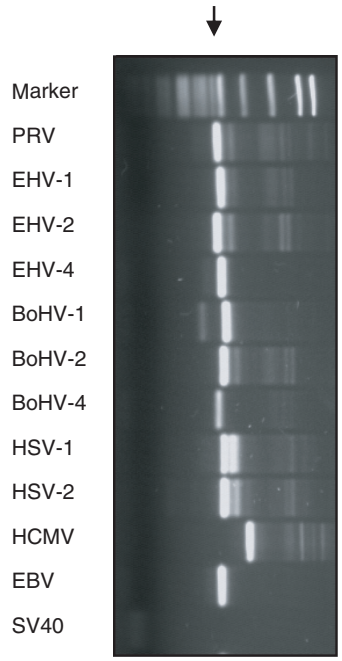


Figure 3 Amplified fragments obtained by panherpes consensus PCR. Partial DPOL sequences were amplified from various herpesviruses: porcine (PRV), equine (EHV-1, EHV-2, and EHV-4), bovine (BoHV-1, BoHV-2, and BoHV-4), and human (HSV-1, HSV-2, HCMV, and EBV). The arrow marks the amplified fragments, most of which have a length of ~230 bp. Note the considerably larger band (310 bp) obtained by amplification of HCMV. DNA of the polyomavirus SV40 was used as a negative control.

no longer available. Therefore, some of the new PCR sequences may represent rediscovery of a herpesvirus whose existence was reported many years earlier.

The mammalian herpesviruses may all be assigned to one of the subfamilies *Alpha-*, *Beta-*, and *Gammaherpesvirinae*, and many mammals host one or more herpesviruses from each subfamily. Humans, for example, are infected by three alpha-, three beta-, and two gammaherpesviruses (HSV-1, HSV-2, VZV, HCMV, human herpesviruses 6 and 7, EBV, and HHV-8, respectively). Other host species apparently lack herpesviruses of a certain subfamily (**Table 1**). For example, in dogs, birds, and reptiles, only alphaherpesviruses have been found, but no beta- or gammaherpesviruses. In Equidae and Bovidae, alpha- and gammaherpesviruses have been found, but no betaherpesviruses. These 'blind spots' may be due to evolutionary developments; that is, some herpesvirus species may have existed but died out later, or have never developed in certain host lineages. On the other hand, certain herpesvirus species may be low in prevalence, replicate to low titers and reside in narrow ecological niches with few or no pathological consequences, which would make their detection difficult. In addition, panherpes consensus PCR may be inefficient in detecting certain sequences because of insufficient primer binding. The latter could be due to the fact that the DPOL consensus primers were originally designed on the basis of a limited set of complete DPOL genes. This selection of genes may have been too small to ensure that the primers do not encounter mismatches in their binding sites within certain herpesvirus genomes. For example, betaherpesviruses are more heterogeneous in their DPOL sequences than alpha- or gammaherpesviruses, and some currently available sequences reveal mismatches with the consensus primers. Therefore, the sensitivity of the PCR may be suboptimal for some betaherpesviruses. Inclusion of novel DPOL sequences as a basis for redesign of current panherpes PCR systems may improve their universality and enable the discovery of additional herpesviruses that otherwise might remain undetected.

It is a common phenomenon that hosts are infected with more than one herpesvirus. The detection of all herpesviruses present in such samples is a technical challenge. Panherpes consensus PCR usually favors the amplification of one viral sequence and concurrently neglects others. This is a result of different binding efficiencies of the degenerate primers to each viral genome and the genome copy number of each virus in the sample. One solution for this problem is to design degenerate primers with a more limited spectrum of detection. These can be derived from less-conserved sites in the DPOL gene or from other, less well-conserved genes. For example, the gB gene allows the derivation of subfamily-specific or genus-specific degenerate primers only. Thus, samples which are positive for gammaherpesviruses in the panherpes DPOL PCR can be additionally screened with betaherpesvirus-specific gB primers. Such detection strategies often reveal the presence of several herpesviruses in a given sample, and this has resulted in the discovery of previously unknown herpesvirus sequences in primates and perissodactyls (odd-toed ungulates such as horse, zebra, and donkey).

Tentative Classification of Novel Herpesvirus Sequences

According to ICTV, "related herpesviruses are classified as distinct species if (a) their nucleotide sequences differ in a readily assayable and distinctive manner across the entire genome and (b) they occupy different ecological niches by virtue of their distinct epidemiology and pathogenesis or their distinct natural hosts." These requirements are only very rudimentarily fulfilled after determination of a short partial DPOL sequence from a primary sample. However, it is possible to make an initial attempt tentatively to classify the new virus entity.

It is a general rule (with some important exceptions such as herpes B virus and pseudorabies virus (PRV)) that

Table 1 Herpesviruses amplified through consensus PCR and their hosts

Host class	*Host order*	*Host species (examples)*	*Number of viruses in the host taxonomic order*	*Alpha-herpesvirinae*	*Beta-herpesvirinae*	*Gamma-herpesvirinae*
Mammalia						
	Primates	Humans and other Old World primates; New World primates	~100	x	x	x
	Scandentia	Tree shrew	3		x	x
	Proboscidea	Asian and African elephant	4		x	x
	Carnivora	Lion, hyena, cat, dog, badger, sea lion, seal	7	x		x
	Cetacea	Dolphins, whales	>10	x		x
	Rodentia	Mice, rats, guinea pig	>40		x	x
	Perissodactyla	Horse, donkey, zebra, tapir, pygmy hippopotamus	>10	x		x
	Artiodactyla	Cattle, rhinoceros, sheep, goat, and other ruminants; pig, babirusa, warthog	>35	x	x	x
	Insectivora	Shrews	3		x	
	Sirenia	Florida manatee	1			x
	Chiroptera	Bats	>5		x	x
Reptilia						
	Crocodilia	Alligator	1			
	Squamata	Monitor, chuckwalla, plated lizard	>5	x		
	Testudines	Tortoises and turtles	>15	x		
Aves						
	Galliformes	Chicken, turkey	4	x		
	Columbiformes	Pigeon	1	x		
	Psittaciformes	Parrot	2	x		
	Falconiformes	Vulture, falcon	2	x		
	Passeriformes	Finch	1	x		
Actinopterygii						
	Cypriniformes	Koi	3			
	Siluriformes	Channel catfish	1			
	Acipenseriformes	Sturgeon	3			
	Anguilliformes	Japanese eel	1			
	Perciformes	Tilapia larvae	1			

herpesviruses are specific for their hosts. Thus, related, albeit distinct, herpesvirus sequences from different hosts can be assigned as originating from distinct herpesvirus species. In line with this, analysis of the novel sequence is primarily performed by pairwise and multiple alignment with sequences of known and classified viruses.

When a more detailed view is desired, phylogenetic tree construction is performed. The most reliable phylogenetic trees are constructed with the proteins encoded by sets of conserved herpesvirus genes. This requires the determination of the complete genome sequence of the novel species in question (or at least a considerable part of it). For noncultured herpesviruses and with the amplification techniques presently at hand, this is either impossible or at least very time consuming. As a compromise, the short DPOL sequence, amplified with panherpes consensus PCR, is extended by many investigators in an upstream direction. The final contiguous sequence extends from the outer degenerate sense primer to the inner degenerate antisense primer (450 to >700 bp; the length depends on the virus) (**Figure 1**).

This DPOL region is sufficient for a first attempt to construct a phylogenetic tree in order to (1) identify the closest relatives among the herpesviruses already sequenced, and (2) classify tentatively the virus from which the novel sequence originated. As an example, a phylogenetic tree of all HHV-8-related gammaherpesviruses, for which sequences >450 bp are available, is shown in **Figure 4**. The tree contains 63 distinct sequences and reveals that a large group of Old World primate rhadinoviruses (RHVs) exists, and that this can be divided into two subgroups. One subgroup contains a human RHV (HHV-8), but a human member of the other subgroup has not been identified. Furthermore,

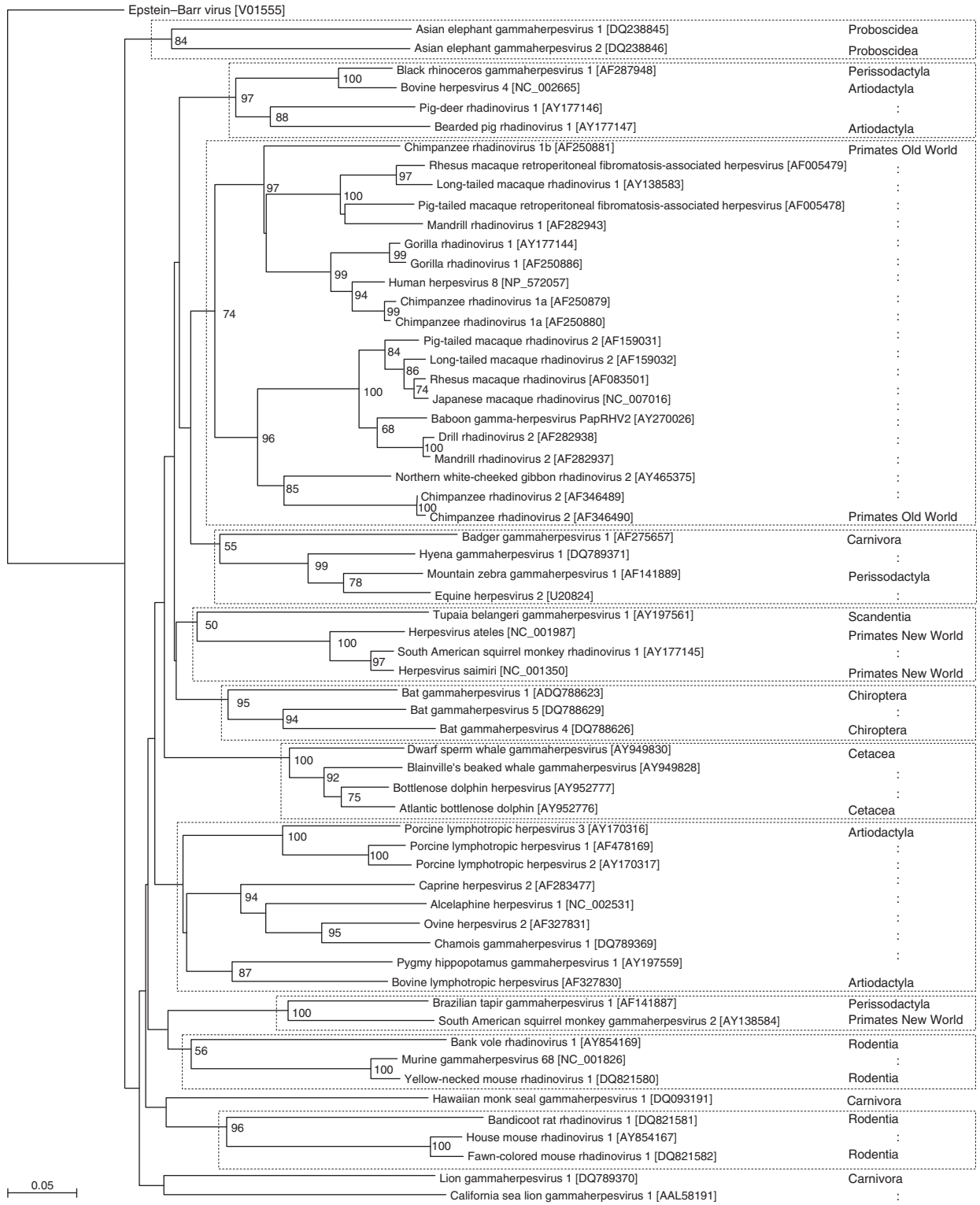


Figure 4 Phylogenetic analysis of well-known and recently discovered RHVs. A phylogenetic tree was constructed with DPOL sequences of 150–160 amino acid residues. They were aligned using the ClustalW module of MacVector™, and gaps were removed. The resulting alignment was analyzed using the neighbor-joining tree-construction module. A rooted phylogram is shown, with EBV as the outgroup. Bootstrap values of >50% are indicated at the nodes of the tree. All sequences are available in GenBank, and the accession numbers are given following the virus names. Host families are indicated.

two distinct RHV groups from artiodactyls (even-toed ungulates, such as cattle, sheep, goat, and pig) and rodents are present. Two mixed groups of carnivore/perissodactyl RHVs are seen, the latter also containing a New World primate RHV. Finally, distinct groups of cetacean (dolphin and whale) and chiropteran (bat) RHVs exist, and a small group of New World primate RHVs clusters loosely with a tree shrew RHV (host: *Tupaia belangeri*) (**Figure 4**).

In phylogenetic trees constructed using short conserved sequences, the deeper nodes close to the center of the tree are often not supported by statistical significance (e.g., see **Figure 4**). In addition, when a great number of herpesviruses from closely related hosts are very similar on the DPOL sequence level, tree resolution is insufficient. A good example is the huge number of EBV-like lymphocryptoviruses (LCVs) in Old World (African and Asian) primates. A satisfactory solution is the consensus amplification of a part of the gB gene, which is located immediately upstream of the DPOL gene in beta- and gammaherpesviruses. Subsequent long-distance PCR and sequencing of all amplification products results in a contiguous sequence of 3–4 kbp, covering large parts of the gB and DPOL genes – a 'bigenic approach' (**Figure 5**). This region codes for approximately 1100 amino acid residues and generally gives rise to considerably improved resolution in phylogenetic trees. This is exemplified by a tree in which 20 EBV-like LCVs of Old World and New World primates are included (**Figure 6**). In this tree, the New World LCVs are clearly separated from two groups of Old World LCVs. In the first group of Old World LCVs, EBV clusters with

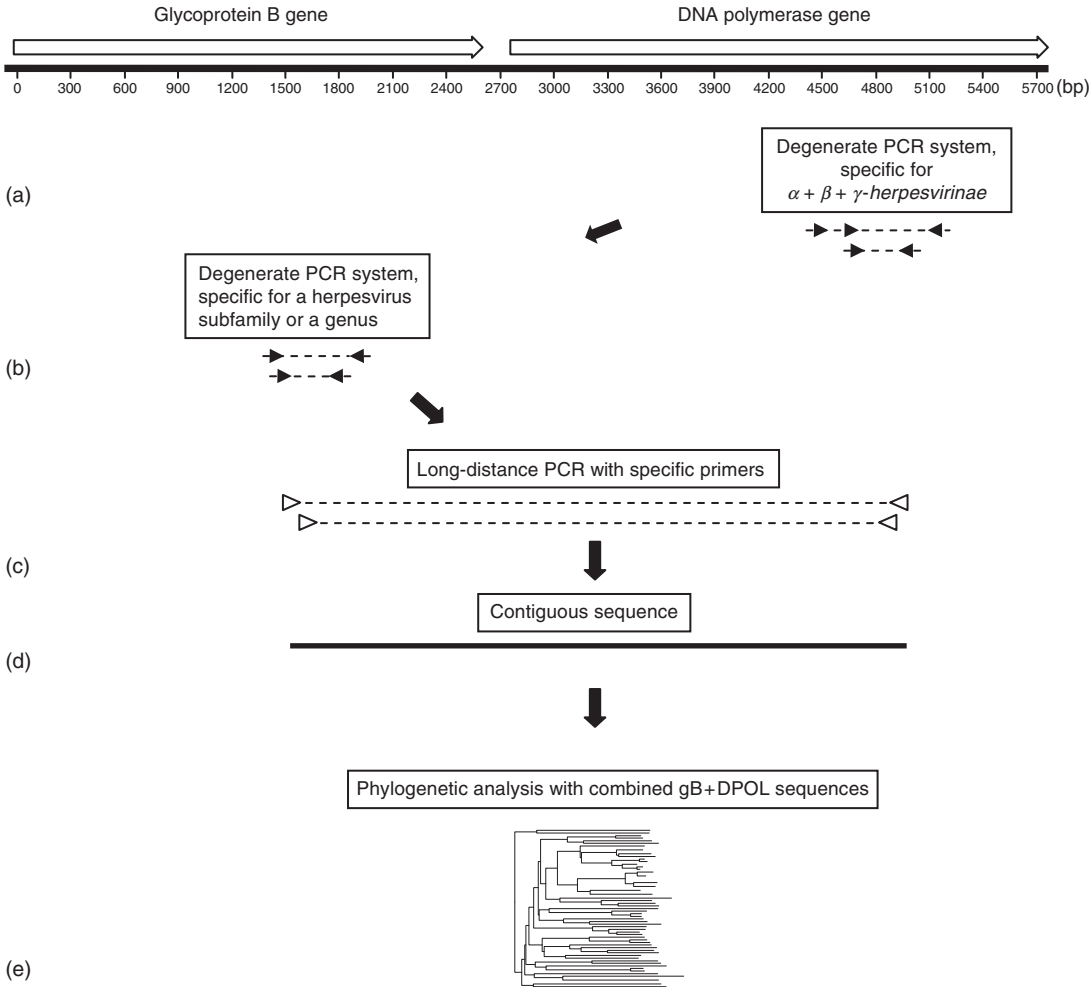


Figure 5 Bigenic amplification of beta- and gammaherpesviruses. Schematic diagram of the analytical strategy. (a) Initially, panherpes-nested PCR with degenerate and deoxyinosine-substituted primers (black triangles) is performed for amplification of the herpesvirus DPOL sequence. (b) According to the sequence of the amplification product and a tentative assignment of the novel DPOL sequence to a herpesvirus subfamily or genus, a degenerate PCR strategy for amplification of the gB gene is chosen, suited to the novel virus. (c) After both gB and DPOL sequences are determined, long-distance PCR is performed with specific primers binding to gB (sense) and DPOL (antisense). (d) A final contiguous sequence of >3 kbp is obtained (solid line) and (e) used for phylogenetic analysis.

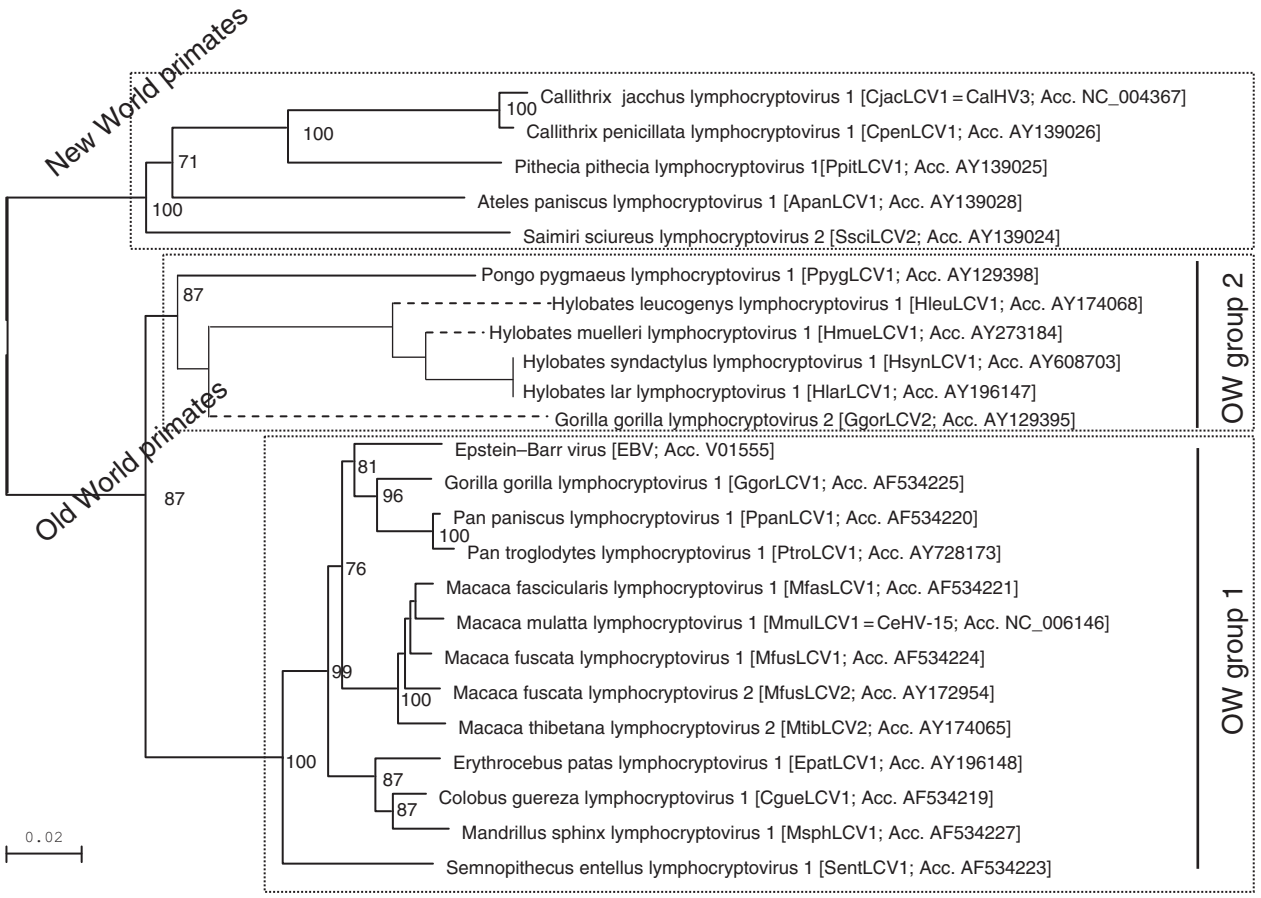


Figure 6 Phylogenetic analysis of well-known and recently discovered LCVs. 400 amino acid residues of gB and 700 residues of DPOL were concatenated for each LCV and analyzed as described in **Figure 2**. For most of the gibbon (genus *Hylobates*) LCVs and the second gorilla LCV (GgorLCV2), the analyzed gB to DPOL region was not available. Therefore, the branches of these viruses were taken from a tree that was constructed on the basis of short DPOL sequences (<160 residues), and are shown by broken lines. OW, Old World.

high probability with LCVs of great apes (chimpanzee, bonobo, and gorilla), as expected from co-evolution of the herpesviruses with their hosts. This subgroup is well separated from a subgroup of macaque LCVs and a subgroup consisting of other cercopithecine LCVs and a colobine LCV. The second group of great ape LCVs contains several distinct gibbon (genus *Hylobates*) LCVs, a second gorilla LCV, and an orangutan (*Pongo pygmaeus*) LCV (**Figure 6**). It can be speculated that this second group should also contain a second human LCV, but this virus, if it exists, remains to be discovered.

The 'bigenic approach' yields more sequence data and improves considerably the reliability of phylogenetic analyses. It has so far been applied to more than 30 novel herpesviruses from nine different orders of vertebrate hosts. It has the potential to become the gold standard for initial genetic characterization of novel beta- and gammaherpesviruses. However, it cannot be applied directly to alphaherpesviruses, in which the gB and DPOL genes are ordered differently, being separated by several kilobase pairs of sequence spanning two genes and, in some cases, the origin of DNA replication.

Pathogenic Impact of Novel Herpesviruses

The herpesviruses that were first discovered and studied in detail were those infecting either humans or animals of economical importance (pigs, PRV; cattle, bovine rhinopneumonitis virus (BoHV-1); horses, equine abortion virus (EHV-1); chickens, Marek's disease virus). Most of these viruses are alphaherpesviruses with overt primary pathogenicity. However, beta- and gammaherpesviruses such as HCMV or EBV often cause primary infections with mild or subclinical pathogenicity. Therefore, it is not surprising that for many of the more recently discovered herpesviruses a disease association is unknown. This is

especially the case for all sequences discovered through screening of healthy individuals, and even the detection of herpesviruses in autopsy specimens of animals suffering from lethal disease can be incidental. Therefore, further investigations are generally needed to confirm etiological involvement. Despite these difficulties, for some recently discovered animal herpesviruses, an association with a specific disease is probable.

One of the more exotic examples is the endotheliotropic elephant herpesvirus, which is a serious threat to the endangered Asian elephant. This virus was first described in 1999, and was found in benign skin lesions of African elephants. In several Asian elephants of American and European zoological gardens, this virus was found to cause a severe endotheliotropic infection with multiorgan failure and death within days or even hours after onset of symptoms. It is thought that the virus is well adapted to the African elephant but can cross the species barrier where both elephant species are housed together. Sequence analysis of the complete gB and DPOL genes (GenBank accession AF322977) placed the virus in the subfamily *Betaherpesvirinae*, and further classification into a new genus (*Proboscivirus*) has been proposed.

Crossing of a species barrier is also an attribute of several ruminant gammaherpesviruses. Ovine herpesvirus 2 (OvHV-2) and alcelaphine herpesvirus 1 (AlHV-1), two gammaherpesviruses infecting sheep and wildebeest, respectively, without clinical symptoms, are known also to infect cattle (OvHV-2 and AlHV-1) and pigs (OvHV-2). In these hosts, a lymphoproliferative disease with high mortality is caused, called malignant catarrhal fever (MCF). More recently, a number of novel gammaherpesviruses were discovered in several wildlife ruminants with an apparently similar pathogenic potential (e.g., GenBank accessions AY237360–AY237366 and AY212111–AY212114).

A lymphoproliferative disease of high mortality has been observed in pigs after pig-to-pig blood stem cell transplantation combined with immunosuppressive drug treatment. The disease was apparently associated with a novel porcine lymphotropic gammaherpesvirus (PLHV-1; GenBank accession AF478169), and resembled the post-transplantation lymphoproliferative disease (PTLD) which is associated with EBV after human-to-human allotransplantation. In pig-to-human xenotransplantation, PLHV-1 is discussed as a risk factor. Xenotransplantation is under investigation because it is seen as a solution for the shortage of human organs for allotransplantation.

Gammaherpesviruses are also closely associated with abnormal proliferation and cancer in primates. Several primate LCVs are known that are very similar to EBV in genome organization and gene content. Some have already been studied in detail and found to be associated with lymphoproliferative diseases that resemble those caused by EBV. Therefore, these LCV infections may be viewed as potential models for EBV infection. For the LCVs more recently detected by panherpes PCR methods (examples are in **Figure 6** and GenBank accessions AY608702–AY608714, AY28171–AY28177, and AF534219–AF534229), such associations remain to be elucidated.

A large number of primate RHVs have been discovered. The first to be described and one of the best studied is herpesvirus saimiri (HVS), which naturally infects a New World primate, the squirrel monkey (*Saimiri sciureus*), without producing symptoms. Upon experimental infection of other monkey species, it causes fatal acute T-cell lymphoma. In Old World primates, several RHVs have since been discovered that are genetically very similar to HHV-8. One, rhesus monkey rhadinovirus (RRV), has been completely sequenced by two research groups and found to induce hyperplastic lymphoproliferative disease in rhesus macaques upon experimental co-infection with simian immunodeficiency virus. This disease resembles a variant of multicentric Castleman's disease, which is a rare HHV-8-associated disease of the human lymphatic system that involves hyperproliferation of certain cytokine-producing B-cells. RRV infection of rhesus monkeys was therefore proposed as a model for certain aspects of HHV-8 pathogenicity in humans. Retroperitoneal fibromatosis in rhesus and pig-tailed macaques is associated with another group of macaque RHVs. Panherpes consensus PCR revealed that they are even more closely related to HHV-8 at the sequence level (**Figure 4**). In recent years, both groups of primate RHVs have been populated with many additional viruses, in particular those infecting great apes (chimpanzee, gorilla, and gibbon), and also other Old World monkeys (e.g., mandrill and baboon) and a New World monkey (squirrel monkey) (**Figure 4**). For the majority of these viruses, a disease association remains to be described.

The first alphaherpesvirus from great apes was isolated very recently from oral and pharyngeal ulcers of chimpanzees. It is a close relative of HSV-2. Serological evidence suggests that gorillas may host a similar alphaherpesvirus.

Fibropapillomatosis of marine turtles is an emerging neoplastic disease associated with infection by a novel virus, fibropapilloma-associated turtle herpesvirus (FPTHV). The DPOL gene of this virus has been amplified through panherpes consensus PCR and originates from an alphaherpesvirus. Later, several turtle species were reported to come down with the disease. These findings underline the fact that tumorigenicity is not an exclusive property of gammaherpesviruses.

In the world of reptiles, novel herpesviruses have also been reported to be associated with proliferative stomatitis in green tree monitors (*Varanus prasinus*) and plated lizards (*Gerrhosaurus major* and *Gerrhosaurus nigrolineatus*).

Several marine mammals of the family Cetaceae (dolphins and whales) have been described as suffering from lesions on oral and genital mucosa as well as from systemic, fatal disease. These were attributed to herpesviruses, and their assignment as alpha- and gammaherpesviruses, respectively, was reported recently on the basis of consensus PCR and sequence analysis.

Among fish, mass mortality in common carp (*Cyprinus carpio*) and colored carp or koi (*Cyprinus carpio koi*) has been observed during the last decade and was reported to be caused by a herpesvirus that differs substantially in sequence and gene content from mammalian herpesviruses but is related to a number of other fish herpesviruses from the families Cyprinidae (carps and minnows), Acipenseridae (ray-finned fish, including sturgeons), and Ictaluridae (catfish).

Finally, sporadic mortality of the Pacific oyster (*Crassostrea gigas*) is associated with a novel herpesvirus, ostreid herpesvirus 1. This is the only known herpesvirus that has an invertebrate host, and is the sole representative of a third major class of herpesvirus in addition to the two classes infecting vertebrates (mammals, birds, and reptiles, and bony fish and amphibians).

Outlook

For the vast majority of potential animal hosts, herpesviruses are completely unknown. This holds true in particular for mammalian families that are populated with a huge number of species, such as those encompassing rodents or bats. Also, many nonmammalian hosts remain to be investigated. The number of putative novel herpesvirus species is growing exponentially, most being characterized exclusively from limited sequence information. The novel sequences obtained can be used to refine the available panherpes consensus PCR methods in order to improve their general detection potential. Recent developments in diagnosing known and novel pathogens by microarray technology may evolve into an additional powerful tool for herpesvirus discovery. Thus, the goal of obtaining a much more extensive list of herpesviruses can be approached, and exciting questions tackled, such as the possible existence of as yet unidentified human herpesviruses.

See also: Epstein–Barr Virus: General Features; Fish and Amphibian Herpesviruses; Herpes Simplex Viruses: General Features; Herpesviruses: General Features; Human Cytomegalovirus: General Features; Kaposi's Sarcoma-Associated Herpesvirus: General Features; Varicella-Zoster Virus: General Features.

Further Reading

Chmielewicz B, Goltz M, Franz T, *et al.* (2003) A novel porcine gammaherpesvirus. *Virology* 308: 317–329.

Damania B and Desrosiers RC (2001) Simian homologues of human herpesvirus 8. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 356: 535–543.

Davison AJ (2002) Evolution of the herpesviruses. *Veterinary Microbiology* 86: 69–88.

Davison AJ, Eberle R, Hayward GS, *et al.* (2005) Family *Herpesviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 193–212. San Diego, CA: Elsevier Academic Press.

Ehlers B, Borchers K, Grund C, Frölich K, Ludwig H, and Buhk H-J (1999) Detection of new DNA polymerase genes of known and potentially novel herpesviruses by PCR with degenerate and deoxyinosine-substituted primers. *Virus Genes* 18: 211–220.

Ehlers B, Ochs A, Leendertz F, Goltz M, Boesch C, and Mätz-Rensing K (2003) Novel simian homologues of Epstein–Barr virus. *Journal of Virology* 77: 10695–10699.

Huang CA, Fuchimoto Y, Gleit ZL, *et al.* (2001) Posttransplantation lymphoproliferative disease in miniature swine after allogenic hematopoietic cell transplantation: Similarity to human PTLD and association with a porcine gammaherpesvirus. *Blood* 97: 1467–1473.

Kellam P (1998) Molecular identification of novel viruses. *Trends in Microbiology* 6: 160–165.

Lacoste V, Mauclere P, Dubreuil G, Lewis J, Georges-Courbot MC, and Gessain A (2000) KSHV-like herpesviruses in chimps and gorillas. *Nature* 407: 151–152.

Li H, Gailbreath K, Flach EJ, *et al.* (2005) A novel subgroup of rhadinoviruses in ruminants. *Journal of General Virology* 86: 3021–3026.

Luebcke E, Dubovi E, Black D, Ohsawa K, and Eberle R (2006) Isolation and characterization of a chimpanzee alphaherpesvirus. *Journal of General Virology* 87: 11–19.

Mack DH and Sninsky JJ (1988) A sensitive method for the identification of uncharacterized viruses related to known virus groups: Hepadnavirus model system. *Proceedings of the National Academy of Sciences, USA* 85: 6977–6981.

McGeoch DJ, Dolan A, and Ralph AC (2000) Towards a comprehensive phylogeny for mammalian and avian herpesviruses. *Journal of Virology* 74: 10401–10406.

Rose TM (2005) CODEHOP-mediated PCR – A powerful technique for the identification and characterization of viral genomes. *Virology Journal* 2: 20.

VanDevanter DR, Warrener P, Bennett L, *et al.* (1996) Detection and analysis of diverse herpesviral species by consensus primer PCR. *Journal of Clinical Microbiology* 34: 1666–1671.

Wang D, Coscoy L, Zylberberg M, *et al.* (2002) Microarray-based detection and genotyping of viral pathogens. *Proceedings of the National Academy of Sciences, USA* 99: 15687–15692.

Relevant Websites

http://blocks.fhcrc.org – CODEHOP to Design PCR Primers from Blocks, Fred Hutchinson Cancer Research Center.

http://www.ncbi.nlm.nih.gov – National Center for Biotechnology Information (NCBI) Home Page.

Herpesviruses: General Features

A J Davison, MRC Virology Unit, Glasgow, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The family *Herpesviridae* consists of a substantial number of animal viruses that have large, double-stranded DNA genomes and share a defining virion structure. Herpesviruses have been discovered in vertebrates from fish to humans, and one has been found in invertebrates (bivalves). They exhibit a wide range of pathogenic properties, ranging from inapparent in many instances to life-threatening (including cancer) in some, and have the ability to establish lifelong latent infections that can reactivate periodically. This chapter introduces the fundamental molecular properties of the herpesviruses: structure, replication, classification, genome, genes, and evolution.

Virion Structure

Herpesvirus particles (virions) are spherical and have an approximate diameter of 200 nm. They have a unique morphology consisting of four basic components (**Figure 1**).

Core

The core is occupied by the virus genome, which is a large, linear, double-stranded DNA molecule packaged at high density.

Capsid

The capsid is an icosahedron of diameter 125–130 nm. It is fashioned from 161 protein capsomeres, which are contributed by 150 hexons and 11 pentons, plus the portal in the 12th pentonal position. The hexons and pentons contain six and five copies each, respectively, of the major capsid protein, each copy of which in the hexons is decorated by a small, external protein. The portal consists of 12 copies of the portal protein and forms the vertex through which DNA enters and leaves the capsid. The capsomeres in the capsid shell are joined together via complexes known as triplexes, which contain two copies of one protein and one copy of another.

The immature capsid shell is constructed in the cell nucleus around a scaffold that consists of approximately 1200 copies of the scaffold protein plus approximately 150 copies of an N-terminally extended form of this protein that contains a protease domain in the additional region. The scaffold is replaced by the virus genome during DNA packaging in a process that involves proteolytic cleavage and loss of all the scaffold protein fragments except for the protease domain.

Tegument

The capsid is embedded in the tegument, and is often not located centrally therein. The tegument contains many virus protein species and is poorly defined structurally except for the region close to the capsid, where a degree of icosahedral symmetry exists.

Envelope

The tegument is wrapped in the lipid envelope, which contains several virus membrane glycoproteins as well as some cellular proteins.

Replication Cycle

The lytic replication cycle has been studied in detail for relatively few herpesviruses, most information having come from herpes simplex virus type 1 (HSV-1). As a consequence, there is a substantial degree of generalization – and even controversy – about applying conclusions from one virus across the whole family. This is especially pertinent to aspects of virus growth involving functions that are not genetically conserved. The mechanisms involved in establishment of, and reactivation from, latency are in this class, with different groups of herpesviruses having evolved different ways of ensuring that the virus stays with the host for life. Herpesviruses possess elaborate means of modulating the host responses to infection, and the genetic functions involved are largely not conserved across the family.

In broad outline, the phases of the lytic replication cycle are as follows. Details on the lytic and latent replication cycles (and pathogenic properties) of specific viruses are available in the relevant articles.

Entry

The virion envelope fuses to the cell membrane via the interactions of cellular receptors with envelope glycoproteins, and the capsid and tegument enter the cytoplasm. Some – perhaps many – of the incoming tegument proteins have specific roles in modulating the replication cycle, for example, in inhibiting host macromolecular synthesis and initiating virus transcription. Some tegument proteins also reach the nucleus, perhaps independently of the capsid.

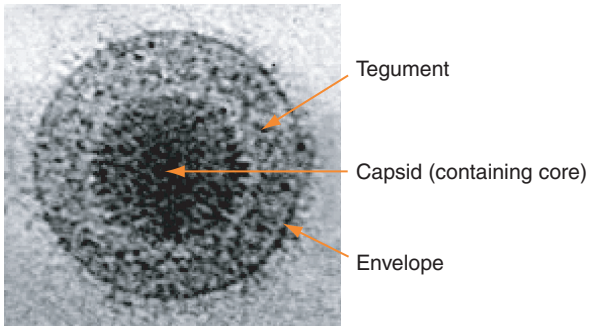


Figure 1 Structure of the herpesvirion exemplified by a cryoelectron microscope image of HSV-1. Adapted from Rixon FJ (1993) Structure and assembly of herpesviruses. *Seminars in Virology* 4: 135–144, with permission from Elsevier.

Expression

Capsids are transferred via microtubules to nuclear pores, through which the virus genome enters the nucleus. The genome is transcribed into mRNA by host RNA polymerase II in three major, regulated phases of transcription. Transcription of immediate early genes, unlike that of later phases, is not dependent on protein synthesis. Some of the immediate early proteins regulate the expression of early and late genes. Functions involved in DNA replication or nucleotide metabolism, and a subset of structural proteins, are in the former class, and many virion proteins are in the latter class.7

Some polyadenylated RNAs, or introns derived therefrom, appear to function via pathways not requiring translation into proteins. In some herpesviruses, small, noncoding RNAs are transcribed by RNA polymerase III. The functions of these RNAs are largely unknown.

Replication

In the nucleus, DNA replication initiates at one or more specific sequences (origins of replication) to generate head-to-tail concatemeric genomes, probably by a rolling circle mechanism. Unit-length genomes are cleaved from the concatemers and packaged into preformed capsids.

Maturation

Capsids acquire some tegument proteins in the nucleus and gain a temporary envelope by budding through the inner nuclear membrane. This envelope is lost upon transit across the outer nuclear membrane into the cytoplasm. Further tegument proteins are added in the cytoplasm, and the tegumented capsids gain their final envelope by budding into a post-Golgi compartment.

Exit

Mature virions are released from the cell by reverse endocytosis.

Classification

The task of classifying viruses, including herpesviruses, commenced in 1966 and is undertaken by the International Committee on Taxonomy of Viruses (ICTV). Various criteria have been utilized, as enabled by developments in knowledge and technology. The current taxonomy of herpesviruses, as laid out in the *Eighth Report of the ICTV* (2005), is summarized in **Table 1**. The family *Herpesviridae* is divided into three subfamilies populated by herpesviruses of higher vertebrates (mammals and birds): *Alphaherpesvirinae* (containing four genera: *Simplexvirus*, *Varicellovirus*, *Mardivirus*, and *Iltovirus*), *Betaherpesvirinae* (containing three genera: *Cytomegalovirus*, *Muromegalovirus*, and *Roseolovirus*), and *Gammaherpesvirinae* (containing two genera: *Lymphocryptovirus* and *Rhadinovirus*). The family also contains a genus (*Ictalurivirus*) that is not linked to any subfamily and has a single fish herpesvirus as a member.

In proposals under consideration by the ICTV (**Table 1**), an order (*Herpesvirales*) is introduced as the highest taxon. The family *Herpesviridae* is redefined as restricted to viruses of higher vertebrates (which in this context will eventually include reptiles as well as mammals and birds). Within this redefined family, an additional genus (*Proboscivirus*) is added to the *Betaherpesvirinae*, and two new genera (*Macavirus* and *Percavirus*) are split from the *Rhadinovirus* genus in the *Gammaherpesvirinae*. Two new families are also created: *Alloherpesviridae* to accommodate viruses of lower vertebrates (amphibians and fish), and *Malacoherpesviridae* for the single herpesvirus of invertebrates (bivalves).

The formal names of herpesvirus species follow a taxonomic designation derived from the natural host followed by the word 'herpesvirus' and a number (e.g., *Human herpesvirus 4* – the fourth of the eight species whose members infect humans). However, many herpesviruses are better known by common names (e.g., Epstein–Barr virus rather than human herpesvirus 4), and in this respect are characterized by a dual nomenclature. In addition, vernacular terms are often used to imply members of the family (e.g., herpesvirus), subfamily (e.g., gammaherpesvirus), or genus (e.g., rhadinovirus).

Herpesviruses are defined as separate species if their nucleotide sequences differ in a readily assayable and distinctive manner across the entire genome and if they occupy different ecological niches by virtue of their distinct epidemiology and pathogenesis or their distinct natural hosts. This definition implies that several criteria are employed in identifying a new species, and their application is to some extent arbitrary. The criteria are outlined below.

Morphological Criteria

Assignment of a virus to the family *Herpesviridae* depends primarily upon virion morphology, as described above.

Table 1 Present and proposed herpesvirus classification schemes. Species other than the type species of each genus are not included

Taxon level	*Current taxon*	*Proposed taxon*	*Common name of virus*
Order		*Herpesvirales*	
Family	*Herpesviridae*	*Herpesviridae*	
Subfamily	*Alphaherpesvirinae*	*Alphaherpesvirinae*	
Genus	*Simplexvirus*	*Simplexvirus*	
Type species	*Human herpesvirus 1*	*Human herpesvirus 1*	Herpes simplex virus type 1
Genus	*Varicellovirus*	*Varicellovirus*	
Type species	*Human herpesvirus 3*	*Human herpesvirus 3*	Varicella-zoster virus
Genus	*Mardivirus*	*Mardivirus*	
Type species	*Gallid herpesvirus 2*	*Gallid herpesvirus 2*	Marek's disease virus type 1
Genus	*Iltovirus*	*Iltovirus*	
Type species	*Gallid herpesvirus 1*	*Gallid herpesvirus 1*	Infectious laryngotracheitis virus
Subfamily	*Betaherpesvirinae*	*Betaherpesvirinae*	
Genus	*Cytomegalovirus*	*Cytomegalovirus*	
Type species	*Human herpesvirus 5*	*Human herpesvirus 5*	Human cytomegalovirus
Genus	*Muromegalovirus*	*Muromegalovirus*	
Type species	*Murid herpesvirus 1*	*Murid herpesvirus 1*	Murine cytomegalovirus
Genus	*Roseolovirus*	*Roseolovirus*	
Type species	*Human herpesvirus 6*	*Human herpesvirus 6*	Human herpesvirus 6
Genus		*Proboscivirus*	
Type species		*Elephantid herpesvirus 1*	Endotheliotropic elephant herpesvirus
Subfamily	*Gammaherpesvirinae*	*Gammaherpesvirinae*	
Genus	*Lymphocryptovirus*	*Lymphocryptovirus*	
Type species	*Human herpesvirus 4*	*Human herpesvirus 4*	Epstein–Barr virus
Genus	*Rhadinovirus*	*Rhadinovirus*	
Type species	*Saimiriine herpesvirus 2*	*Saimiriine herpesvirus 2*	Herpesvirus saimiri
Genus		*Macavirus*	
Type species		*Alcelaphine herpesvirus 1*	Wildebeest-associated malignant catarrhal fever virus
Genus		*Percavirus*	
Type species		*Equid herpesvirus 2*	Equine herpesvirus 2
Family		*Alloherpesviridae*	
Genus	*Ictalurivirus*	*Ictalurivirus*	
Type species	*Ictalurid herpesvirus 1*	*Ictalurid herpesvirus 1*	Channel catfish virus
Family		*Malacoherpesviridae*	
Genus		*Ostreavirus*	
Type species		*Ostreid herpesvirus 1*	Oyster herpesvirus

Biological Criteria

The three subfamilies *Alphaherpesvirinae*, *Betaherpesvirinae*, and *Gammaherpesvirinae* were defined initially on the basis of biological criteria, in the application of which a degree of generality is implicit: host range in cell culture, length of replication cycle, cytopathology, and characteristics of latency. Alphaherpesviruses exhibit a variable host range, have a short replication cycle, spread quickly with efficient destruction of infected cells, and establish latent infections primarily in sensory ganglia. Betaherpesviruses have a narrow host range and a long replication cycle with slow spread and cell enlargement. Viruses may become latent in secretory glands and lymphoreticular cells. Gammaherpesviruses are associated with lymphoproliferative diseases, and latent virus is present in lymphoid tissue. They infect B- or T-lymphocyte cells *in vitro*, in which infection is frequently arrested so that infectious progeny are not produced. However, some gammaherpesviruses are able to cause lytic infections in fibroblastoid or epithelial cell lines.

Serological Criteria

Closely related viruses may be detected using antisera or specific antibodies.

Genomic Criteria

The general layout of a genome in terms of unique and repeat regions (see below) is limited as a criterion since, although particular structures are found more commonly in certain subfamilies or genera, similar structures have evidently evolved more than once. The DNA sequences of herpesviruses are sufficiently diverged to limit extensive nucleic acid similarity (detectable by hybridization) to closely related viruses.

Sequence Criteria

Phylogenetic analysis based on sequence comparisons between conserved genes has become the predominant

criterion in classification of herpesviruses at all taxonomic levels. **Figure 2** shows one example of a phylogenetic tree supporting the current classification scheme. In recent years, many herpesviruses have been detected in animal tissues solely by polymerase chain reaction (PCR) of short sequences. Given the multifactorial nature of the herpesvirus species definition (see above), the absence of other information renders formal classification problematic in these cases.

Genome Organization

The herpesvirus genomes studied to date range in size from approximately 124 kbp (simian varicella virus) to 295 kbp (koi herpesvirus) and in nucleotide composition from 32% to 74% G+C. Alphaherpesviruses are not deficient in the 5′ CG dinucleotide, betaherpesviruses are deficient in very limited regions, and gammaherpesviruses are deficient throughout their genomes. 5′ CG deficiency is thought to be a consequence of spontaneous deamination of methylated cytosine residues in DNA over evolutionary time to produce 5′ TG. There is evidence that methylation of certain regions of the Epstein–Barr virus genome resident in peripheral blood lymphocyte cells may be involved in perpetuating infection. Extensive methylation of the 5′ CG dinucleotide has been found in the virion DNA of two frog herpesviruses, both of which encode the enzyme DNA (cytosine-5-)-methyltransferase.

Herpesvirus genomes differ in the arrangements of direct and inverted repeat regions with respect to unique

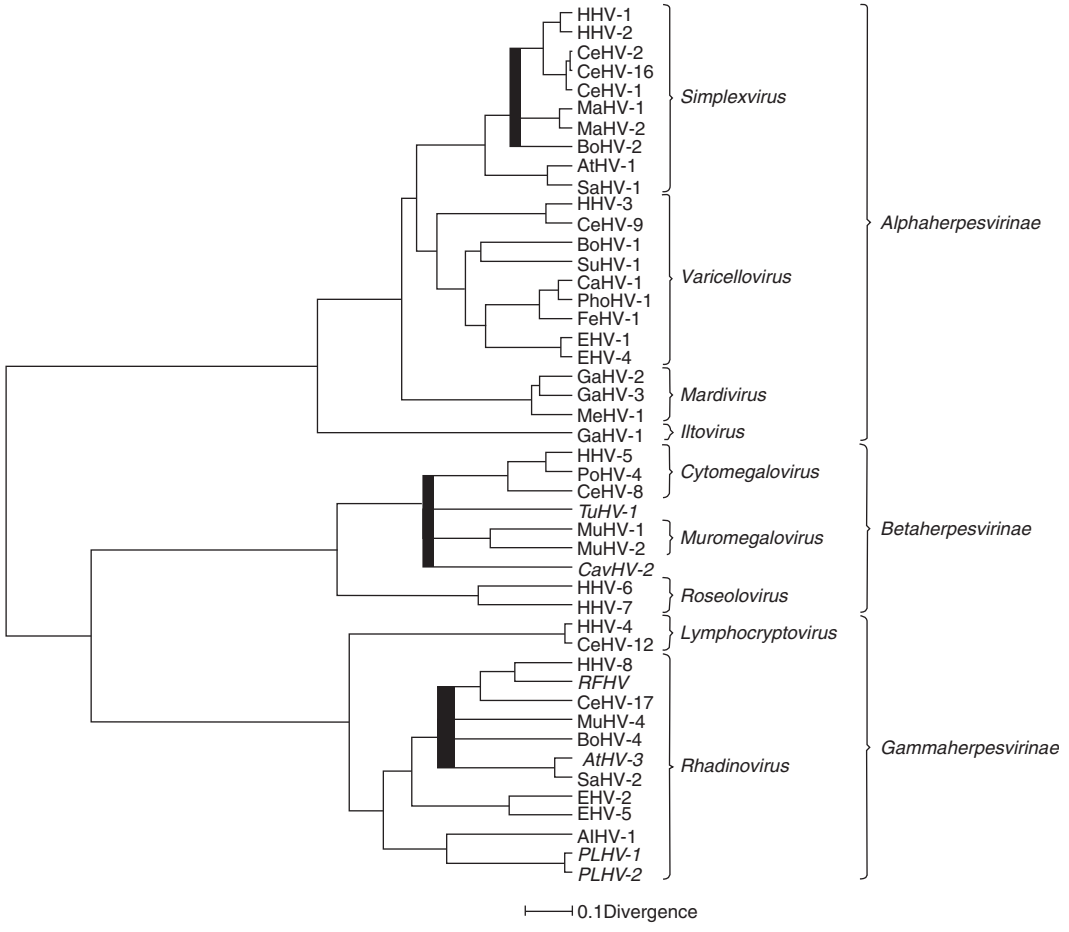


Figure 2 Phylogenetic tree for herpesviruses. The scope is limited to the three subfamilies that comprise the revised family *Herpesviridae*; see **Table 1**. The tree is a composite derived from sequence alignments of up to eight sets of core proteins, analyzed using the maximum-likelihood method with a molecular clock imposed. Thick lines denote regions of uncertain branching. Prefixes denote host species: H, human; Ce, cercopithecine; Ma, macropodid; Bo, bovine; At, ateline; Sa, saimiriine; Su, suid; Ca, canid; Pho, phocid; Fe, felid; E, equid; Ga, gallid; Me, meleagrid; Po, pongine; Tu, tupaiid; Mu, murid; Cav, caviid; and Al, alcelaphine. Other virus name abbreviations are: RFHV, retroperitoneal fibromatosis herpesvirus of macaques; and PLHV-1 and PLHV-2, porcine lymphotropic herpesviruses 1 and 2. Viruses that are not yet incorporated into genera are in italics. Genera and subfamilies are on the right. Adapted from McGeoch DJ, Dolan A, and Ralph AC (2000) Toward a comprehensive phylogeny for mammalian and avian herpesviruses. *Journal of Virology* 74: 10401–10406, with permission from American Society for Microbiology.

(nonrepetitive) regions. **Figure 3** illustrates the five types of structure that have been confirmed adequately. The type A structure (e.g., human herpesvirus 6) consists of a unique region (U) flanked by a direct terminal repeat (TR) at the genome ends. Type B genomes (e.g., human herpesvirus 8, also known as Kaposi's sarcoma-associated herpesvirus) contain variable numbers of a TR at each end of the genome. The type C structure (e.g., Epstein–Barr virus) has in addition a variable number of copies of an internal direct repeat (IR), which is unrelated to TR, and the presence of IR splits U into two unique regions (U_L and U_S). The type D structure (e.g., pseudorabies virus) also has two unique regions (U_L and U_S), with U_S flanked by an inverted repeat (TR_S/IR_S). In some type D genomes (e.g., varicella-zoster virus), U_L is flanked by a very small, unrelated inverted repeat (TR_L/IR_L). By virtue of recombination between inverted repeats, the two orientations of U_S are present in equimolar amounts in virion DNA populations, and U_L is present completely or predominantly in a single orientation. In the type E structure (e.g., HSV-1), TR_L/IR_L is larger and the two orientations of U_L and U_S are each present in equimolar amounts in virion DNA populations, giving rise to four genome isomers. A direct repeat (the *a* sequence) is also present at the termini, and an inverted copy (*a*′) is present internally.

In addition to large-scale repeats, regions consisting of tandem repeats of short sequences are found at various locations in many herpesvirus genomes. The elements that make these up may be identical to each other or merely similar, giving rise to simple or complex repeats, and their presence in variable numbers causes genome size heterogeneity.

Gene Content

To date, the genome sequences of herpesviruses belonging to 42 species have been published, with more than one strain determined for several of these species. Details are available via the genomic biology Web pages at the National Center for Biotechnology Information. In addition, a large amount of data is available for portions of other herpesvirus genomes. Analyses of these sequences provide detailed views of genetic content and phylogeny.

Herpesvirus genomes consist mostly of protein-coding sequences. Gene complements range from about 70 to 170 and are arranged about equally between the two DNA strands. They are not generally located in functionally related groups. Overlap between protein-coding regions in different reading frames on the same or opposing strands is infrequent and, where it does occur, is not extensive. In instances where extensive or complete overlap of two protein-coding regions has been mooted, evidence that both encode functional proteins is invariably unconvincing.

Most herpesvirus genes are transcribed from their own promoters and thus specify mRNAs with unique 5′ ends. Groups of genes are frequently arranged tandemly on the same DNA strand and share a common polyadenylation site, with mRNAs sharing the same 3′ end. Most genes are not spliced, particularly among the alphaherpesviruses. RNA polymerase II transcripts that do not encode proteins but may have other functions, and micro-RNAs, have been identified in representatives of all three subfamilies, and RNA polymerase III transcripts in certain gammaherpesviruses. The functions of such transcripts are largely unknown. Herpesvirus genomes also contain

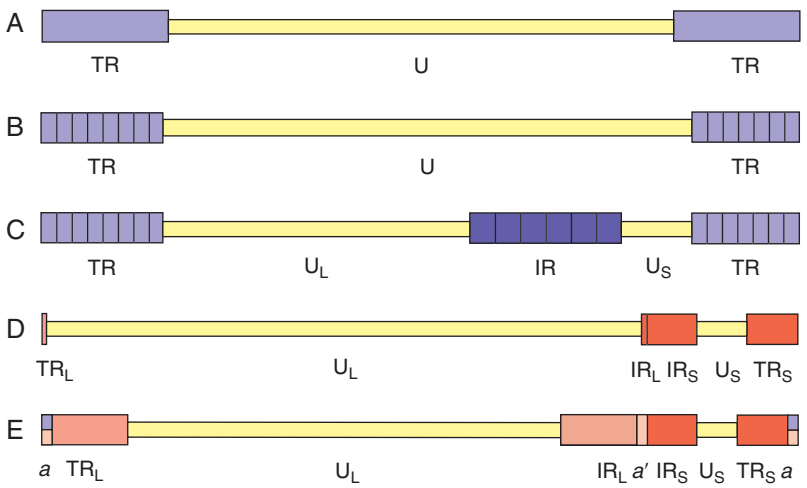


Figure 3 Types of herpesvirus genome structure. The disposition of unique regions (yellow), direct repeats (blue), and inverted repeats (red) are shown (not to scale). Within a structure, unique regions are unrelated to each other, and only the repeats depicted in the same color and shade are identical. Among the structures, repeats represented in the same color and shade are not necessarily related to each other. In the type E structure, an inverted copy of a direct repeat (*a*) at the genome termini is present internally (*a*′).

cis-acting signals in addition to those involved in transcriptional control, such as origins of DNA replication.

Sequence comparisons among members of the revised family *Herpesviridae* indicate that a set of 43 core genes has been inherited from the ancestor of the three subfamilies. This number of core genes is an approximation, since its derivation assumes that the *Alphaherpesvirinae* was the first subfamily to diverge (as indicated by molecular phylogeny; see **Figure 2**). Moreover, it is based not simply on primary sequence conservation but, in some cases, on positional and functional similarities. Three of the core genes derived from the ancestor have been lost subsequently from certain lineages. The core genes are generally arranged collinearly among members of the same subfamily, but in members of different subfamilies blocks of genes have been rearranged and sometimes inverted.

The most strongly represented functional categories among the core genes are involved in central aspects of lytic replication. Particularly prominent are genes encoding capsid proteins (six genes) or proteins involved in replication (seven genes) or processing and packaging of DNA (eight genes). Also featured are genes with roles in nucleotide metabolism or DNA repair (five genes), and, to a lesser extent, genes encoding tegument (seven genes) or envelope proteins (five genes). Genes with roles in niche-specific aspects of the life cycle, such as modulation of the host response and regulation of latency, do not generally belong to the core set. This feature illustrates the high degree of evolutionary flexibility that herpesviruses have exhibited in colonizing particular ecological niches. The fact that over half (in some cases well over half) of the genes in a herpesvirus genome may be removed individually without eliminating viral growth in cell culture, when all presumably have a role *in vivo*, testifies to the extensive interactions that the virus has with its host during natural growth and transmission.

The comments above refer to the revised family *Herpesviridae*, where most information is available. Similar points may be made about the proposed family *Alloherpesviridae*, though the number of core genes is considerably fewer (probably not more than 20), presumably due to the greater antiquity of the common ancestor.

Herpesvirus Evolution

The processes that have occurred during evolution of the three subfamilies of the *Herpesviridae* from their common ancestor are the same as in other organisms, and include the gradual effects of nucleotide substitution, deletion, and insertion to modify existing genes or produce genes *de novo*, and the recombinational effects of gene capture, duplication, and rearrangement. These processes are also evident in the proposed family *Alloherpesviridae*.

Herpesviruses are usually restricted to a single species in the natural setting, and severe symptoms of infection are often limited in the natural host to young or immunosuppressed individuals. This indicates that a substantial degree of co-adaptation and co-evolution have taken place over the long term between herpesviruses and their hosts. Although some exceptions suggesting ancient interspecies transfer have been registered, phylogenetic studies provide strong support for co-evolution as a general historic characteristic, in some instances supporting the idea that the viruses have speciated along with their hosts. This correspondence has allowed a timescale for herpesvirus evolution to be proposed on the basis of accepted dates for host evolution. For example, the most recent analyses suggest divergence dates of 400 million years (before present) for the three subfamilies, 120 million years for the genera *Simplexvirus* and *Varicellovirus* of the *Alphaherpesvirinae*, and 8 million years for HSV-1 and HSV-2. These dates are approximate, especially the further back they go, and vulnerable to the vicissitudes of estimated host dates and developments in analytical approaches.

The three lineages populated by the revised family *Herpesviridae* and the two proposed families *Alloherpesviridae* and *Malacoherpesviridae* share similarities in capsid structure and replication that indicate evolution from a common ancestor, the forerunner of all herpesviruses. However, in comparison with the situation within the families, detectable amino acid sequence similarity among proposed families is very limited, with evidence for a common ancestor focused on a single gene (encoding a subunit of the terminase complex responsible for DNA packaging). These features imply that the divergence events that gave rise to the three lineages are very ancient. Moreover, structural considerations focused on the capsid continue to stimulate the notion that herpesviruses might share an even earlier ancestor with T4-like bacteriophages.

Note: The proposals for new taxa mentioned in this article have been accepted by the ICTV.

See also: Herpesviruses: Discovery.

Further Reading

Davison AJ (2002) Evolution of the herpesviruses. *Veterinary Microbiology* 86: 69–88.

Davison AJ, Eberle R, Hayward GS, *et al.* (2005) Family *Herpesviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 193–212. San Diego, CA: Elsevier Academic Press.

Grunewald K, Desai P, Winkler DC, *et al.* (2003) Three-dimensional structure of herpes simplex virus from cryo-electron tomography. *Science* 302: 1396–1398.

Kieff E and Rickinson AB (2001) Epstein–Barr virus and its replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 2511–2573. Philadelphia, PA: Lippincott Williams and Wilkins.

McGeoch DJ, Dolan A, and Ralph AC (2000) Toward a comprehensive phylogeny for mammalian and avian herpesviruses. *Journal of Virology* 74: 10401–10406.
McGeoch DJ and Gatherer D (2005) Integrating reptilian herpesviruses into the family *Herpesviridae*. *Journal of Virology* 79: 725–731.
McGeoch DJ, Rixon FJ, and Davison AJ (2006) Topics in herpesvirus genomics and evolution. *Virus Research* 117: 90–104.
Mocarski ES and Tan Courcelle C (2001) Cytomegaloviruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 2629–2673. Philadelphia, PA: Lippincott Williams and Wilkins.
Moore PS and Chang Y (2001) Kaposi's sarcoma-associated herpesvirus. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 2803–2833. Philadelphia: Lippincott Williams and Wilkins.
Rixon FJ (1993) Structure and assembly of herpesviruses. *Seminars in Virology* 4: 135–144.
Roizman B and Pellett PE (2001) Herpes simplex viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 2399–2459. Philadelphia, PA: Lippincott Williams and Wilkins.
Zhou ZH, Dougherty M, Jakana J, *et al.* (2000) Seeing the herpesvirus capsid at 8.5 Å. *Science* 288: 877–880.

Herpesviruses: Latency

C M Preston, Medical Research Council Virology Unit, Glasgow, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ganglion A small organ containing the cell bodies, including nuclei, of neurons.
Iontophoresis Introduction through the skin or cornea by applying an electrical charge.

Introduction

Members of the family *Herpesviridae* exhibit the ability to remain latent in tissues of the host following primary infection. Latent virus is retained for the lifetime of the host, and can be reactivated to cause recurrent disease. Latency is a crucial property for the survival of herpesviruses, overcoming the requirement for rapid reinfection of new individuals in order to spread within a population. As described below, the characteristics of latency show variations when the different herpesvirus subfamilies are considered. Operationally, latency is defined as the presence of the viral genome without detectable virus production, coupled with the potential for resumption of virus replication in response to reactivation signals. For descriptive purposes, latency is characterized by three phases: establishment, maintenance, and reactivation.

Early thoughts on latency focused on two basic ideas, named the static and dynamic models. In the static model, the viral genome is considered to be nonreplicating due to a failure to undergo the normal program of gene expression, and reactivation is viewed as a change in intracellular conditions such that replication resumes. Dynamic models of latency envisage a slow persistent production of virus that is normally controlled by the host immune system and does not cause overt disease until a reactivation stimulus diminishes host defenses. There is currently no consensus that either of these models is correct; instead, research into latency continually reveals greater complexity in the interaction of virus with host. It is now thought that interference with virus replication occurs at many levels, ranging from repression of gene expression to control by immunological defenses. The requirement for long-term retention of the viral genome, coupled with an ability to spread within a population, demands that a complex and intimate relationship exists between virus and host. This relationship can be considered at two levels, namely the individual cell and the organism. At the cellular level, there must be mechanisms for sequestering the viral genome in a host cell that is potentially permissive for replication, and for retaining the ability to resume replication in response to appropriate stimuli. In terms of the organism, the virus must evade detection by the immune system during latency and must overcome host defenses when reactivating.

Latency will be considered in terms of the three subfamilies of the family *Herpesviridae*, focusing on human viruses since these are understood in the greatest detail.

Alphaherpesvirinae

The prototype of the subfamily *Alphaherpesvirinae*, herpes simplex virus (HSV) type 1 (HSV-1), is the most intensively studied in terms of latency. In humans, its natural host, primary exposure usually occurs during infancy and is characterized by a mild infection of the oropharynx that is frequently unnoticed. After the initial infection has resolved, a proportion of individuals experience periodic reactivation in response to stressful stimuli, such as excessive exposure to sunlight, resulting in the appearance of 'cold sore' lesions around the lip and less frequently on other areas of the face. HSV type 2 (HSV-2) is more

frequently associated with genital herpes, although nowadays genital HSV-1 is detected with increasing frequency. Genital herpes and facial cold sores are treated by application of acyclovir, but this agent is only effective in preventing lesions once reactivation has occurred. There is currently no antiviral that eradicates latent virus or prevents reactivation.

HSV establishes latency in sensory neurons that innervate the site of initial infection. The viral genome is retained in neurons of the relevant ganglia for the lifetime of the host, and upon reactivation virus is released into tissues served by neurons extending from the ganglion. During initial infection, replicating HSV enters nerve termini and virus particles are transported along axons in a retrograde manner to the ganglia. Upon reactivation, virus moves in an anterograde direction from the ganglion to the surface, where replication causes disease.

Animal models have been developed for the study of HSV latency, and although each has limitations, they have been important in linking molecular investigations with *in vivo* studies. Latency is established efficiently after infection of mice with HSV-1, but reactivation is difficult to achieve *in vivo*. The most common method of reactivating latent virus from mice is to explant ganglia, a process in which ganglia are removed by dissection and cultured in the laboratory. This invariably results in the appearance of infectious virus within a few days but its physiological relevance is debatable. Subjecting mice to transient hyperthermia reliably reactivates virus replication in ganglia *in vivo*, albeit at low efficiency. The best animal for the study of reactivation is the rabbit, since virus is spontaneously released in tear films after ocular infection and release can be stimulated by iontophoresis of epinephrine. Genital HSV-2 infection can be reproduced, to some extent, by intravaginal inoculation of guinea pigs.

In considering establishment of latency, a crucial question concerns the way in which the normally inexorable progression to lytic replication and cell death is interrupted. Transcripts and proteins characteristic of lytic infection cannot be detected easily during latency, indicating a global repression of gene expression. Furthermore, studies with HSV mutants have failed to identify a gene product that is dispensable for lytic replication but required for latency, thus the prevailing hypothesis is that establishment of latency results by default when viral gene expression is somehow arrested in neurons. It is currently thought that the block occurs early in the virus life cycle, and the most likely point for intervention by the cell is at the immediate early (IE) stage. A favored hypothesis contends that IE transcription is compromised in sensory neurons, possibly due to inefficient transport of the virion transactivator protein VP16 (virion protein number 16), which activates IE transcription, to the neuronal nucleus, or to lack of the cellular transcription factors Oct-1 (a cellular protein that binds sequences with the consensus ATGCAAAT) or host cell factor (HCF, a large cell protein), with which VP16 is able to form a complex. Alternatively, sensory neurons may contain proteins that act as competitive inhibitors of Oct-1 and/or HCF, blocking their interactions with VP16. The possibility of arrest at the IE stage is supported by experiments with tissue culture cells. HSV-1 mutants that are severely impaired for IE gene expression can be retained in cells for extended periods in a nontranscribed 'quiescent' state that apparently mimics the transcriptional silence characteristic of latency.

Despite the overall absence of viral lytic gene products, latently infected neurons can be readily identified by the presence of a 2 kbp viral RNA known as the latency-associated transcript (LAT). Thousands of molecules of this RNA are found in the nucleus, enabling its detection by *in situ* hybridization or RNA blots. The observed species is an unusually stable intron spliced from a larger precursor that is present in neurons at much lower levels. Structurally, LAT is a lariat, that is, a splicing intermediate of a type typically cleaved and rapidly degraded. The LAT lariat has an unusual branch point sequence that is not recognized by cellular debranching enzymes. A smaller 1.5 kbp RNA, derived by further splicing, is found only in neurons. Intriguingly, LAT is complementary to sequences encoding the C-terminal portion of the IE protein ICP0 (infected cell protein number 0), an important activator of gene expression, leading to the suggestion that LAT blocks ICP0 production by an antisense mechanism. At present, there is no evidence for protein products encoded by HSV-1 LAT or the longer precursor in latently infected neurons, although there is evidence that the bovine herpesvirus 1 latency-related transcript does encode one or more proteins.

The significance of LAT for latency is controversial. Viral mutants that are unable to produce LAT nonetheless establish latency and can be reactivated; thus, LAT does not have an essential role in any of the animal models currently available. In detail, however, LAT mutants are deficient in reactivation in a number of contexts, suggesting a modulatory role for the transcripts. In some animal models, the absence of LAT appears to reduce the efficiency of reactivation directly, whereas in others LAT mutants exhibit a defect in establishment, thereby indirectly reducing reactivation due to the smaller pool of latent genomes. It is relevant that LAT mutants cause greater destruction of neurons during the initial stages of infection. This may be because one of LAT's normal roles is to exert antisense inhibition of ICP0 expression, thereby restricting productive infection, but currently the favored interpretation is that LAT has anti-apoptotic properties that reduce neuronal death after infection. Therefore, LAT could be important for improving establishment of latency by preventing loss of infected neurons, but equally a direct effect on reactivation is possible by keeping reactivating neurons healthy for long periods.

During maintenance of latency, which can last for many decades in humans, it is thought that cellular factors contribute to the stable repression of transcription. The genome is believed to be organized into a chromatin-like structure, and indeed histones carrying post-translational modifications characteristic of inactive cellular chromatin are associated with latent HSV-1 genomes. Interestingly, the LAT region, which escapes repression, is associated with histones normally found on actively transcribed genes. Therefore, cellular mechanisms for global control of transcription may operate on latent HSV DNA.

The latent viral genome exists as a circular episome, structurally distinct from the linear molecule found in the virion. This suggests that the gene expression program is arrested at an early stage, since circularization occurs shortly after virus entry into the cell as a prelude to replication. Alternatively, circular molecules may be remnants from replication during productive infection. It is possible that circularization is important for the assembly of the latent genome into a chromatin structure. There is no evidence for integration of HSV DNA into the host genome during latency.

During latency in humans or experimental animals, only a fraction of neurons in the ganglion (typically 0.1–5% but in some cases up to 30%) harbor latent HSV-1, and neurons themselves constitute only about 5% of the cells in the ganglion. However, viral genomes can be detected by Southern hybridization of total ganglion DNA, demonstrating that neurons harbor many copies of HSV DNA. Analysis of single cells by polymerase chain reaction (PCR) confirms this conclusion and further reveals a wide disparity in the viral copy number per neuron. Most neurons harbor fewer than 100 viral genomes, but a minority can contain thousands.

Although it is clear that most latent genomes do not express lytic gene products at detectable levels, sensitive analysis of mouse ganglia by reverse transcriptase-PCR demonstrates the presence of viral transcripts from loci outside the LAT region. The transcripts are present in low amounts; they may signify infrequent viral lytic gene expression, but they may simply represent a small amount of background transcription of the latent genome. In an extensive analysis of mouse ganglia, a very small number of neurons (fewer than one per mouse) were undergoing an apparently productive infection, again showing that silencing of the genome is not absolute. Therefore, a low level of viral lytic gene expression may occur during latency.

Immunological studies support the view that virus lytic gene expression may occasionally occur during latency. Leukocytes, predominantly CD8+ T cells, can be found in ganglia from humans or latently infected experimental animals, often in intimate association with neurons. In mice, the infiltrating T cells are HSV-1 specific, with the majority recognizing a single immunodominant epitope on glycoprotein B. Furthermore, latently infected ganglia contain cytokines such as RANTES (an attractant for T cells), γ-interferon, and tumor necrosis factor (both produced by T cells). The continued presence of CD8+ T cells and derived cytokines is indicative of persistent stimulation of the immune system during latency. The favored interpretation of the data is that T cells respond to and eliminate a low level of virus that is produced from infected neurons.

The exact nature of the stimuli that provoke reactivation is unclear, and at present the general term 'stress' is used. The stress can be delivered at the periphery, directly to the neuron, or systemically. In experimental animals, explantation of ganglia efficiently reactivates latent HSV, but virus is only released from a small proportion of infected neurons. Procedures that provoke reactivation *in vivo*, such as hyperthermic treatment of mice, are even less efficient. The low efficiency imposes constraints on studying the molecular basis of reactivation, since it is not possible to identify the small number of reactivation-competent genomes among the total latent population. The normal program of HSV gene expression probably does not operate during reactivation because VP16 is absent during latency. It is suspected that stress causes alterations to the transcription factor profile of neurons, resulting in changes at the ICP0 promoter and synthesis of ICP0 protein which then derepresses the entire viral genome. An alternative view of reactivation is that stresses impair immunological mechanisms that normally prevent a low level of reactivating virus from causing disease. The impairment may occur in the ganglion, at the periphery, or both, with the outcome that virus is able to replicate. The application of PCR has revealed that low-level asymptomatic shedding of HSV in humans is more common than previously thought, supporting the view that local immunity prevents reactivated virus from causing disease.

A summary of HSV latency is shown in **Figure 1**.

Varicella-zoster virus (VZV) causes varicella (chickenpox) as primary infection and zoster (shingles) upon reactivation. VZV is latent in neurons within ganglia throughout the body because the primary infection is widespread rather than localized as in the case of HSV. Studies on VZV latency have proceeded slowly, relying on analysis of human tissue in the absence of a satisfactory animal model to reproduce latency. Although rats can be infected with VZV and harbor genomes in ganglia, virus cannot be reactivated. In humans, latent VZV genomes appear to be retained as circular episomes. The pattern of gene expression during latency, however, differs from that found with HSV. Transcripts representing four lytic genes (ORF21, ORF29, ORF62, and ORF63) have been detected in human ganglia, and there is evidence that protein products, particularly that of ORF63, are also present in infected human and rat neurons. Thus, despite many similarities, there may be significant differences in the details of VZV and HSV latency.

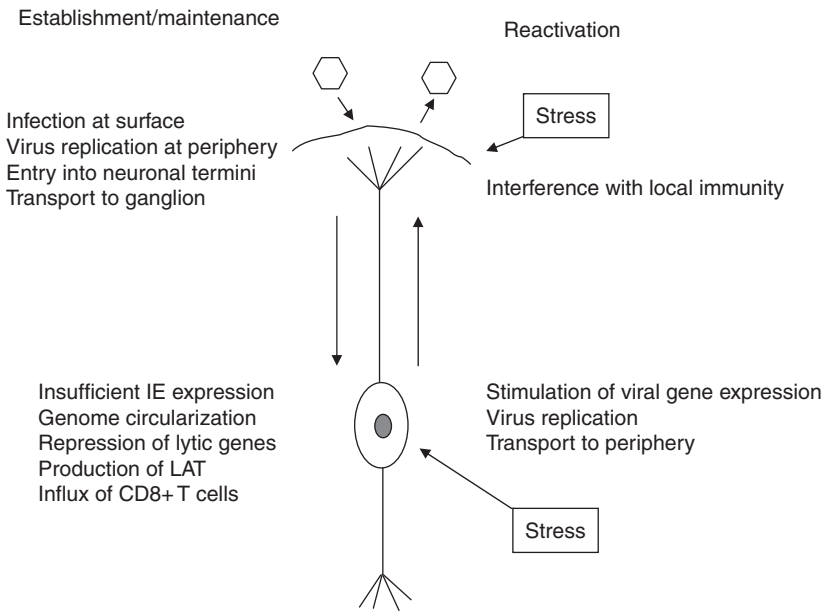


Figure 1 Schematic representation of HSV latency and reactivation.

Betaherpesvirinae

Most studies on latency of the subfamily *Betaherpesvirinae* are concerned with the cytomegaloviruses, particularly human cytomegalovirus (HCMV). In adults, initial infection with HCMV is normally asymptomatic unless it occurs *in utero*, but in immunocompromised individuals it may be serious. Reactivation of latent HCMV can cause rejection of organ transplants, due to replication of virus originating from either the recipient or the donated organ. In addition, AIDS patients can suffer a widespread HCMV infection that has serious consequences. HCMV can be transferred by blood transfusion, suggesting that circulating lymphocytes harbor latent virus, and sensitive PCR analysis, together with cell sorting, has detected the HCMV genome in a small proportion (1 in 10^4 cells) of peripheral blood monocytes. In addition, the HCMV genome can be detected in precursors of monocytes, the CD34+ myeloid progenitors in bone marrow, suggesting that these cells may be the primary reservoir of latent virus.

In view of the low proportion of myeloid cells containing latent HCMV, and the absence of animal models for this virus, molecular studies on HCMV latency have relied heavily on the use of cell culture models. The myelomonocytic line THP-1 is nonpermissive for HCMV replication, but becomes permissive upon differentiation by chemical treatment *in vitro*. Similarly, the teratocarcinoma line NT-2 is nonpermissive until induced to differentiate. The block in undifferentiated cells is due to failure of the major IE transcription unit to be expressed, resulting in arrest of the entire productive infection program. Plasmid-based transfection assays have indicated that specific sequence elements within the major IE promoter (MIEP) are targets for repression. Whereas the entire MIEP is inactive in undifferentiated cells but active in differentiated cells, a truncated MIEP, containing only the 300 base pairs proximal to the mRNA start site, is active in both situations. Mutational analysis has identified critical DNA elements in the MIEP at positions upstream of −300 that contain binding sites for two cellular proteins known to act as repressors. These proteins, known as Yin-Yang 1 (YY1) and Ets-2 repressor factor (ERF), mediate repression of the MIEP in transfection assays, and, furthermore, their levels decline after differentiation by chemical treatment. The implication is that differentiation changes the levels of repressors and hence permits IE gene expression in THP-1 and NT-2 cells.

Chromatin structure at the MIEP is a further important factor in determining permissiveness in cell culture models of HCMV latency. In undifferentiated cells, the MIEP is associated with methylated histones and heterochromatin protein 1 (HP1), both markers of inactive chromatin, whereas in differentiated cells the relative amount of HP1 is reduced and acetylation of histones, a correlate of active chromatin, is increased. It is suspected that YY1 and ERF exert their repressive effects by recruiting histone deacetylases (HDACs) to the MIEP, thereby promoting the formation of inactive chromatin, and this idea is supported by the fact that undifferentiated cells become permissive for IE transcription when treated with chemical inhibitors of HDACs.

The findings from the analysis of cell culture models have received support from studies on monocytes isolated from seropositive individuals and on CD34+ myeloid precursors from human bone marrow. Monocytes do not support HCMV replication, but virus is produced upon differentiation to macrophages in the laboratory. HCMV

can be reactivated *ex vivo* by inducing CD34+ cells from seropositive individuals to differentiate to a mature dendritic cell phenotype. Furthermore, differentiation of CD34+ precursors to dendritic cells reproduces the switch from inactive to active chromatin structure at the MIEP. Thus it is thought that HCMV remains latent in CD34+ myeloid precursors and that differentiation to macrophages or dendritic cells leads to intracellular changes that activate the MIEP and ultimately result in virus replication. Although the precise signals that provoke reactivation *in vivo* are not known, one reported means of inducing virus replication is by allogeneic stimulation of blood monocytes, achieved by mixing cells from histo-incompatible individuals. This may reproduce events that occur during transplantation.

The latent HCMV genome is thought to exist as an episome, but it is not known how it is retained in the host. It may be replicated during cell division, by cellular factors or with the participation of viral proteins, or alternatively a low level of persistent virus production may continually seed new latently infected cells. There have been reports of HCMV-specific transcripts produced during latency, but there is little consensus between different laboratories and thus the possibility that viral gene products control latency remains an open question.

At the level of the whole organism, reactivation of HCMV generally causes disease only when the immune system is compromised. A high proportion (>1%) of circulating CD8+ T cells is specific for HCMV, suggesting frequent stimulation of the immune system, presumably through virus reactivation. Indeed, differentiation of monocytes occurs constantly and if such an event frequently reactivates HCMV, immune clearance must be crucial to prevent disease. The potential significance of the immune response in limiting HCMV infection is best demonstrated in studies with murine cytomegalovirus (MCMV). In its natural host, the mouse, latent MCMV is found in numerous tissues, in contrast to the situation with HCMV. During MCMV latency, CB8+ T cells specific for a major IE protein are present, suggesting that immune control eliminates cells containing reactivating virus at an early stage of the gene expression program. Damage to the immune system can result in widespread infection, and although the simplest interpretation of this finding is that immune surveillance is critical for maintaining latency, it must be remembered that immunosuppression also represents a significant stress that may itself activate the latent genome.

HCMV latency is summarized in **Figure 2**.

Gammaherpesvirinae

Most latency studies with the subfamily *Gammaherpesvirinae* have focused on Epstein–Barr virus (EBV), a member of the genus *Lymphocryptovirus*. This virus is usually transmitted by exchange of saliva, with cells of lymphoepithelial structures such as the tonsils initially infected. Infection is generally asymptomatic but infectious mononucleosis, a self-limiting lymphoproliferative disease, can result if EBV is first acquired during adolescence or later. A defining feature of EBV is its ability to activate resting cultured B cells into unchecked proliferation with retention of the viral genome but without virus production, and indeed the distinction between latency and transformation is often blurred in discussion of this virus. It is clear, however, that EBV is latent in B cells, and that the virus utilizes many aspects of normal B cell development to establish and control latency. EBV, in contrast to HSV and HCMV, possesses a number of genes that are specific to the nonreplicating state and indeed are crucial for coordinating the establishment of latency. These encode the EBV nuclear antigens (EBNAs) 1, 2, 3A, 3B, 3C, and LP, and the latent membrane proteins (LMPs) 1, 2A, and 2B. Other loci specifying small RNAs, known as EBERs, and Bam A rightward transcripts are active during B cell transformation *in vitro*, although their significance for latency is unclear at present. During establishment of

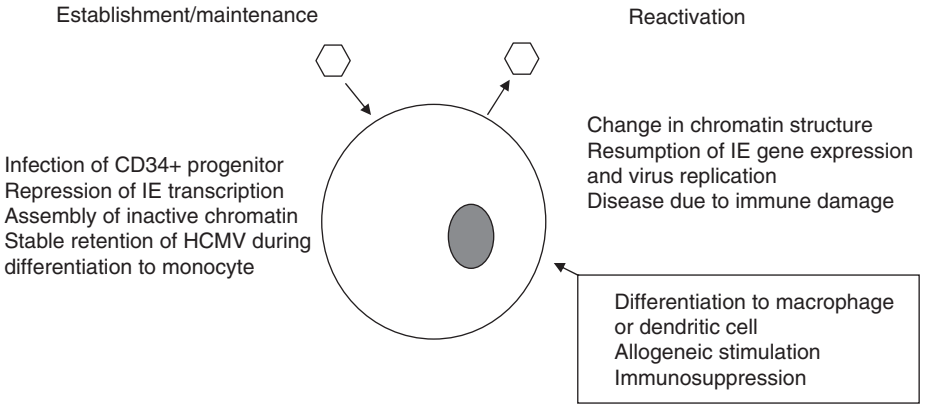


Figure 2 Stages in latency and reactivation of HCMV.

latency, the EBNAs and LMPs participate in a complex interaction with the host cell that results in utilization of the normal B cell development, to the advantage of the virus.

After penetration of the mucosal layers of the tonsils, virus replicates and naive B cells are infected and activated in a manner similar to that observed in culture. At this stage, no lytic gene transcription occurs but all of the EBNAs and LMPs are expressed in an interaction known as the growth program, or latency III. The combined action of the viral proteins gives the resting cell the characteristics of an activated B-lymphoblast, mimicking the first stage that occurs naturally after antigen recognition. Activated B cells are transported to the lymph node follicles, in which they replicate to form germinal centers. Again, following the normal pattern of B cell development after antigen stimulation, the infected cells undergo differentiation into memory B cells. At some stage during this process, the gene expression pattern changes to the default program, or latency II, in which only EBNA1, LMP1, and LMP2A are produced. Protein EBNA1 is crucial for maintaining the EBV genome in dividing cells, through stimulating its replication and by tethering it to cellular chromosomes. LMP1 and LMP2A provide survival signals that prevent the activated B cell from undergoing apoptosis. In the final step, the infected memory B cells are released from the germinal centers to become circulating memory B cells, and during this process gene expression changes to the latency program, or latency I, in which no viral gene products can be detected. During latency, the EBV genome is maintained as a circular episome. To retain the virus within the host, EBNA1 is produced during cell division, enabling the viral genome to replicate and persist in the memory compartment. Interestingly, each of the identified interactions with B cells is reflected in EBV-associated human tumors: viral gene expression in Burkitt's lymphoma (BL) cells resembles the latency program (but with EBNA1 continually expressed), Hodgkin's lymphoma has the default program, and nasopharyngeal carcinoma exhibits the growth program, albeit in epithelial-derived cells.

Reactivation of latent EBV occurs when a memory B cell differentiates into an antibody-secreting plasma cell, an event triggered by signaling from the B cell antigen receptor (BCR). The plasma cells migrate to the mucosal epithelium and virus is released into the saliva, possibly after replication in epithelial cells. The molecular mechanism of lytic cycle activation, and hence virus replication, has largely been studied by the use of BL cell lines, which express only EBNA1 and thus resemble the latent state in B cells. Cross-linking the BCR with immunoglobulin, possibly mimicking the natural differentiation signals provided by T cells, can activate EBV in certain BL lines. This process switches on expression of an IE protein, BZLF1, a transcription factor that triggers further viral gene expression and ultimately productive replication. The signal transduction pathways that respond to cross-linking the BCR result in activation of two transcription factors, MEF2D and ATF, which in turn induces histone acetylation and relief of repression by remodeling the chromatin structure. The BZLF1 protein activates its own promoter, providing a positive feedback loop that ensures rapid commitment to productive replication. In some BL cell lines, BZLF1 expression, and hence EBV replication, is activated in response to alternative agents, such as phorbol esters, but the relevance of these treatments to natural stimuli is unclear.

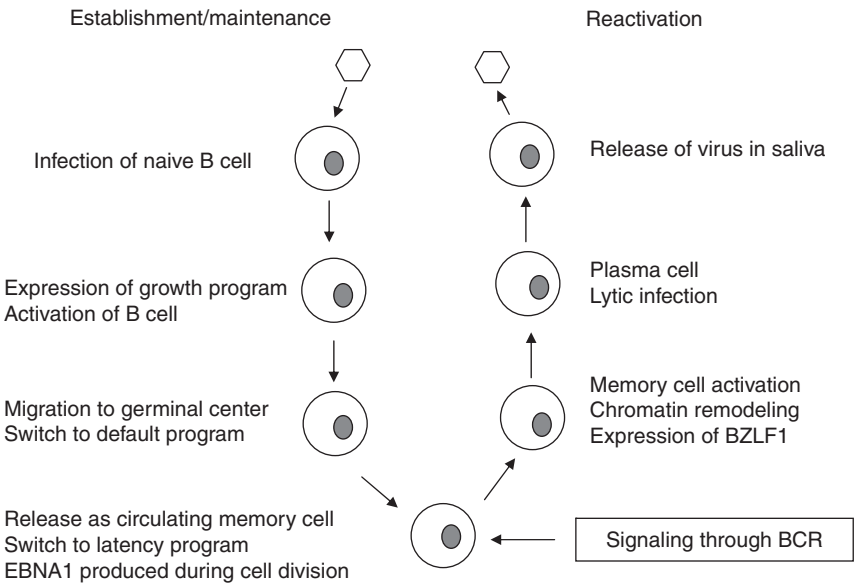


Figure 3 Establishment, maintenance, and reactivation of EBV latency in B cells.

The interaction between EBV and host B cells is summarized in **Figure 3**.

Members of the genus *Rhadinovirus* of the *Gammaherpesvirinae* have been studied in less detail than EBV. Murine herpesvirus 68 establishes latency in a number of cell types, including B cells, in mice. Kaposi's sarcoma-associated herpesvirus (KSHV) is also found in a variety of cell types, again including B cells. The natural pathways of latency and reactivation for these viruses are currently under investigation.

Common Themes

Although the examples described here deal with different viruses and host cells, some common themes emerge from the latent interactions. In establishment, specific features of the natural host cell are critical in preventing productive virus replication. Blocking IE protein production, an effective mechanism for inhibiting virus replication while minimizing cytopathology and immune recognition, appears to be common to establishment of latency. During maintenance, genomes are retained as circular episomes and viral gene expression is minimized to hide the virus from the immune system. Histones associated with the latent genome exhibit modifications characteristic of inactive chromatin. If repression of replication is not complete, low-level virus production may be neutralized by the immune system. Reactivation probably involves the virus utilizing normal cellular responses to a stimulus, but the stimulus may also damage the immune system and thereby cause disease.

See also: Bovine Herpesviruses; Human Cytomegalovirus: Molecular Biology; Epstein–Barr Virus: Molecular Biology; Herpes Simplex Viruses: Molecular Biology; Varicella-Zoster Virus: Molecular Biology; Herpesviruses: General Features; Kaposi's Sarcoma-Associated Herpesvirus: Molecular Biology.

Further Reading

Amon W and Farrell PJ (2004) Reactivation of Epstein–Barr virus from latency. *Reviews in Medical Virology* 15: 149–156.
Efstathiou S and Preston CM (2005) Towards an understanding of the molecular basis of herpes simplex virus latency. *Virus Research* 111: 108–119.
Jones C (2003) Herpes simplex virus type 1 and bovine herpesvirus 1 latency. *Critical Microbiology Reviews* 16: 79–95.
Kent JR, Kang W, Miller CG, and Fraser NW (2003) Herpes simplex virus latency-associated gene function. *Journal of Neurovirology* 9: 285–290.
Khanna KM, Lepisto AJ, Decman V, and Hendricks RL (2004) Immune control of herpes simplex virus during latency. *Current Opinion in Immunology* 16: 463–469.
Sinclair J and Sissons P (2006) Latency and reactivation of human cytomegalovirus. *Journal of General Virology* 87: 1763–1779.
Streblow DN and Nelson JA (2003) Models of HCMV latency and reactivation. *Trends in Microbiology* 11: 293–295.
Thorley-Lawson DA (2001) Epstein–Barr virus: Exploiting the immune system. *Nature Reviews* 1: 75–82.
Thorley-Lawson DA (2005) EBV the prototypical human tumor virus – just how bad is it? *Molecular Mechanisms in Allergy and Clinical Immunology* 116: 251–261.
Wagner EK and Bloom DC (1997) Experimental investigation of herpes simplex virus latency. *Clinical Microbiology Reviews* 10: 419–443.

History of Virology: Bacteriophages

H-W Ackermann, Laval University, Quebec, QC, Canada

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Bacteriophage 'Eater of bacteria' (Greek, phagein).
Eclipse Invisible phase during phage replication.
Lysogenic (1) Bacterium harboring a prophage. (2) Bacteriophage produced by such a bacterium.
Lysogeny Carriage of a prophage.
Metagenomics Sequencing of total genomic material from an environmental sample.
One-step growth Single cycle of phage multiplication.
Phage typing Testing bacteria with a set of phages of different host ranges.
Prophage Latent bacteriophage genome in a lysogenic bacterium.
T-even Phage of the T-series with an even number (T2, T4, T6).

Introduction

Phages or bacteriophages, also referred to as bacterial viruses, include eubacterial and archaeal viruses and are now more appropriately called 'viruses of prokaryotes'. Phages are ubiquitous in nature and may be the most

numerous viruses on this planet. They have a long evolutionary history, are considerably diverse, and include some highly evolved and complex viruses. Approximately 5500 phages have been examined in the electron microscope and they constitute the largest of all virus groups. The present system of phage taxonomy comprises 13 officially recognized families.

Following the discovery of phages, their study entered a symbiosis with the nascent science of molecular biology and then expanded to cover a multitude of subjects. The knowledge on phages grew by accretion and represents the efforts of thousands of scientists. For example, it took over 50 years and hundreds of publications to establish the genome map and sequence of coliphage T4. Phage literature is voluminous and comprises over 11 400 publications for the years 1915–65 and, to the author's reckoning, about 45 000 for 1965 to today. The volume of phage research is evident in the fact that there are two books each for single viruses, λ and T4, and that, by mid-2006, the journal *Virology* has published over 2000 phage articles since its start in 1955. The history of phages may be divided into three, partly overlapping periods. Because of the volume of phage research and the many scientists involved, the development of phage research is largely presented in tabular form. Any mention of individual scientists and facts is selective.

The Early Period: 1915–40

Discovery

Several early microbiologists had observed bacterial lysis. The British bacteriologist Ernest H. Hankin reported in 1896 that the waters of the Jumna and Ganges rivers in India killed *Vibrio cholerae* bacteria. The responsible agent passed bacteriological filters and was destroyed by boiling. In 1898, the Russian microbiologist Nikolai Gamaleya reported the lysis of *Bacillus anthracis* bacteria by a transmissible 'ferment'. It is probable that these scientists would have discovered bacterial viruses, if suitable techniques had been available.

Frederick William Twort (1877–1950), a British pathologist and superintendent of the Brown Institution in London, tried to propagate vaccinia virus on bacteriological media. He observed that 'micrococcus' colonies, in fact staphylococci, often became glassy and died. The agent of this transformation was infectious, passed porcelain filters, and was destroyed by heating. Twort proposed several explanations: an amoeba, an ultramicroscopic virus, a living protoplasm, or an enzyme with the power of growth. Subsequently, Twort joined the army and participated in the British Salonika campaign. Back at the Brown Institution, he published five more articles on phages and spent the rest of his career trying to propagate animal viruses on inert media.

Félix Hubert d'Herelle (1873–1949), born in Montreal, went to France as a boy and returned to Canada, styled himself a 'chemist', studied medicine without obtaining a degree, and finally went to Guatemala as a bacteriologist with minimal knowledge of this science. Later in Yucatan, he isolated the agent of a locust disease, the *Coccobacillus acridiorum* (now *Enterobacter aerogenes)*, was sent to the Pasteur Institute of Paris, returned to Mexico and France, and went to fight locusts in Argentine, Turkey, and Tunisia. He noticed the appearance of holes ('plaques') in coccobacillus cultures. In 1915, again in Paris, he investigated an epidemic of bacillary dysentery in military recruits. He noted that his *Shigella* cultures were destroyed by a filterable, plaque-forming agent, whose appearance coincided with convalescence of the recruits. His discovery was published in 1917. D'Herelle understood immediately that he had found a new category of viruses and he used phages for the treatment of bacterial infections. D'Herelle then went to Vietnam, Holland, Egypt, India (Assam), the USA, the Soviet Union (Tbilisi), and finally to Paris. He coined the term 'bacteriophage', devised the plaque test, and stated that there was only one kind of phage, with many races, the *Bacteriophagum intestinale*. He promoted and practiced phage therapy in many countries and founded several phage institutes. The Tbilisi Institute, which he co-founded with G. Eliava in 1934, still exists.

Twort and D'Herelle could not have been more different personalities. Twort was a medical doctor and a self-effacing person; he traveled little, and showed minimal interest in his discovery. He published only five more articles on phages. D'Herelle was self-educated, peripathetic, worldly, and combative. He devoted the rest of his career to phages and wrote five books and nearly 110 articles on them. The almost simultaneous discovery of bacteriophages by two different scientists is a remarkable coincidence.

Phage Research: General

The years 1920–40 were characterized by research on the nature of phages and lysogeny, phage therapy, and the detection of phages in a wide variety of bacteria and a wide range of habitats (**Table 1**). D'Herelle and his followers asserted that bacteriophages were viruses. A major argument was that they formed plaques. Others, observing that some bacteria produced lytic agents that could be transmitted from one culture to another, held that 'bacteriophages' were generated spontaneously and were endogenous in nature. The famous Belgian immunologist, J. Bordet, proposed that phages were enzymes produced by 'lysogenic' bacteria. He stated bluntly: "The invisible virus of d'Herelle does not exist." When Bordet discovered Twort's paper from 1915, a nasty controversy on the nature of phages and the priority of phage discovery ensued.

Table 1 Phage discovery: phages of selected host organisms

Year	*Investigators*	*Host species or group*
1915	Twort	*Staphylococcus* sp.
1917	D'Herelle	*Shigella dysenteriae*
1918	D'Herelle	*Salmonella typhi*
1921	D'Herelle	*Bacillus subtilis, Corynebacterium diphtheriae, Escherichia coli, Pasteurella multocida, Vibrio cholerae, Yersinia pestis*
1934	Cowles	Clostridia
1937	Whitehead and Hunter	Lactic acid bacteria
1947	Reilly and coll.	Streptomycetes
1963	Safferman and Morris	Cyanobacteria
1970	Gourlay	Mycoplasmas
1974	Torsvik and Dundas	Halophilic archaea
1983	Janekovic and coll.	Hyperthermophilic archaea

Phages Become Viruses

The viral nature of phages was demonstrated by physicochemical studies. W. J. Elford and C. H. Andrewes (1932) showed that phages could differ in size. M. F. Burnet and colleagues (1933) demonstrated that phages differed in antigenicity and resistance to inactivation by heat, urea, and citrate. M. Schlesinger (1934) found that phages were made of DNA and protein. The publication of phage electron micrographs (1940) settled the matter of phage nature and made the enzyme theory untenable. It was now clear that phages were particulate and morphologically diverse. In retrospect, however, both d'Herelle and his opponents were right: phages are viruses with a dual nature.

Phage Therapy

Phage therapy was practiced by D'Herelle himself (1919) soon after phage discovery. He treated human dysenteria, chicken cholera, bovine hemorrhagic fever, bubonic plague, cholera, and a variety of staphylococcal and streptococcal infections. His phage laboratory in Paris was devoted to phage therapy. Many people embarked on phage therapy. Spectacular successes and dismal failures ensued. The failures had many reasons: use of crude, uncontrolled, inactivated, or endotoxin-containing lysates, narrow host specificity of some phages; absence of bacteriological controls; and plain charlatanism. The literature on phage therapy peaked in 1930 and totaled 560 publications for 1920–40. At this time, antibiotics were introduced and interest in phage therapy quickly waned.

Phage Typing and Ecology

Host range tests had shown that bacteria, even of the same species, usually differed in phage sensitivity. Some strains were lysed and some were not. By testing bacteria with a battery of phages, they could be subdivided into 'phage types', which greatly facilitated the tracing of bacterial infections to their sources and gave epidemiology an enormous boost. The first phage typing scheme, introduced in 1938, was for *Salmonella typhi* bacteria, which were subdivided by means of a series of adapted derivatives of phage ViII. For the first time, viruses were found to have a practical use. Phage typing developed rapidly in the following decades.

New phages were sought and found everywhere. It appeared that most bacterial pathogens had phages (**Table 1**) and that these viruses were omnipresent in water and soil, on plants, in food, on the skin of humans and animals, and in their body cavities and excreta. It also was discovered that bacteriophages caused faulty fermentations in the dairy industry. A few bacteriophages isolated during this early period are still available today: a streptococcal and a T4-like phage, two microviruses, and, possibly, the original phage isolated by Twort himself (**Table 2**, **Figure 1**).

The early period ended with the rise of dictatorships and World War II, first in the Soviet Union and later in Europe. Scientists were silenced, persecuted, forced into exile, or outright killed (G. Eliava was shot in 1937 by Stalin's secret police and Eugène and Elisabeth Wollman disappeared in 1943 in a Nazi concentration camp). Phages now had their martyrs. D'Herelle himself, a Canadian citizen, was interned by the Vichy Government and prevented from pursuing phage therapy. Phage research was obliterated in much of Europe.

The Intermediate Period: 1939–62

Phages as Tools and Products of Molecular Biology

Phage research survived and attained new heights in the USA. It helped to start molecular biology and provided crucial insights into vertebrate and plant virology. In turn, phage research benefited enormously from both disciplines. The next two decades became a legendary period of phage research, as a small number of researchers produced results of fundamental scientific importance (**Tables 3** and **4**).

In 1939, E. L. Ellis devised the 'one-step growth' experiment to measure the kinetics of phage infection and to establish the viral nature of bacteriophages. He was joined by Max Delbrück (1906–81), a German immigrant and physicist. Delbrück proposed to concentrate phage research on the seven coliphages T1–T7, collected and studied by F. Demerec and U. Fano in 1945 and all easily propagated. Fortunately, these phages were both diverse and partially related, thus allowing useful comparisons. Electron microscopy showed that the T phages (T for type) belonged to four morphotypes: T1, T3–T7,

T2–T4–T6, and T5 (**Figure 2**). Morphotypes correlated with growth parameters and antigenic properties. The T phages became the raw material for fundamental studies which, although limited to tailed phages of the T series, provided a general understanding of the phage life cycle. It became clear that:

1. Phages have different morphologies.
2. Phages contain DNA and transmit it from parent to progeny. The DNA is the carrier of heredity and genetic information. Phage coats consist of proteins.
3. Phages adsorb to bacteria by their tails.
4. Phage DNA enters the bacteria while the protein coat remains outside.
5. Phage synthesis occurs in stages, one of which is the 'eclipse'. Neither phages nor particulate precursors can be detected during the eclipse period. Novel phages are liberated ready-made in a single event ('burst').

Lysogeny

The problem of lysogeny, a leftover from the previous period, was solved by the French bacteriologist André Lwoff (1902–94) from the Pasteur Institute of Paris. The Wollmans had already postulated the dual nature of some phages: they were infectious outside the bacterium and noninfectious inside. Starting in 1949, Lwoff isolated single cells of a phage-carrying strain of *Bacillus megaterium* with a micromanipulator and transferred them into microdrops of fresh medium. After 19 successive generations, there was still no lysis. When lysis occurred, it was sudden and phages were plentiful in the microdrops. Lysis and phage production were inducible, especially by ultraviolet (UV) light. It was concluded that lysogenic bacteria perpetuate latent phages as 'prophages' in a repressed state. They do not secrete phages. Spontaneously or after induction, the prophage enters a vegetative phase, new phages are produced, and the bacterium dies. A major review article published in 1953 clarified the nature of lysogeny. The prophage became the origin of the 'provirus' concept in vertebrate virology.

In 1951, Esther Lederberg isolated the lysogenic coliphage λ. Small and easily manipulated, it was to have an extraordinary future and became perhaps the best studied of all viruses. As the prototypic lysogenic phage, it was the subject of countless publications on the nature of

Table 2 Phage discovery: individual phages and phage groups

Year	*Investigators*	*Phage*	*Family*[a]	*Host genus*[b]
1915	Twort	Twort?	M	*Staphylococcus*
1926	Clark and Clark	C1	P	*Streptococcus*
1933	Burnet and McKie	C16	M	Enterics
		S13	*Microviridae*	Enterics
1935	Sertic and Boulgakov	ϕX174	*Microviridae*	Enterics
1945	Demerec and Fano	T1 to T7	M, P, S	Enterics
1951	E. Lederberg	λ	S	Enterics
	G. Bertani	P1 and P2	M	Enterics
	Zinder and J. Lederberg	P22	P	Enterics
1960	Loeb	f1	*Inoviridae*	Enterics
1961	Loeb and Zinder	f2	*Leviviridae*	Enterics
	Takahashi	PBS1	M	Bacillus
1963	Taylor	Mu	M	Enterics
1964	Okubo	SPO1	M	Bacillus
1965	Reilly	ϕ29	P	Bacillus
1967	Schito	N4	P	Enterics
1968	Espejo and Canelo	PM2	*Corticoviridae*	*Pseudoalteromonas*
	Riva and coll.	SPP1	S	*Bacillus*
1970	Gourlay	L1 (MVL1)	*Plectrovirus*	*Acholeplasma*
1971	Gourlay	L2 (MVL2)	*Plasmaviridae*	*Acholeplasma*
1973	Six and Klug	P4	M	Enterics
	Vidaver and coll.	ϕ6	*Cystoviridae*	*Pseudomonas*
1974	Olsen and coll.	PRD1	*Tectiviridae*	*Enterics*
1982	Martin and coll.	SSV1	*Fuselloviridae*	*Sulfolobus*
1983	Janekovic and coll.	TTV1	*Lipothrixviridae*	*Thermoproteus*
1994	Zillig and coll.	SIRV1	*Rudiviridae*	*Sulfolobus*
2002	Rachel and coll.	ATV	*Bicaudaviridae*[c]	*Acidianus*
2004	Häring and coll.	PSV	*Globuloviridae*[c]	*Pyrobaculum*
2005	Häring and coll.	ABV	*Ampullaviridae*[c]	*Acidianus*

[a]Tailed phages: M, *Myoviridae*; P, *Podoviridae*; S, *Siphoviridae*.
[b]Enterobacteria are considered as a single host 'genus' because of frequent cross-reactions.
[c]Archaeal viruses awaiting formal classification.

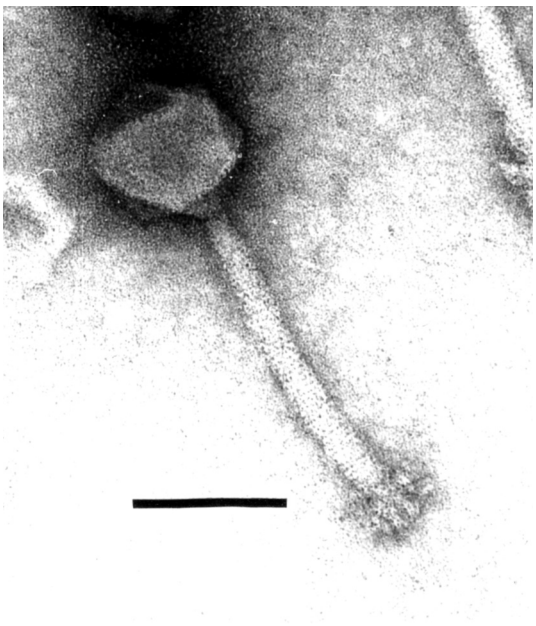


Figure 1 Phage Twort, uranyl acetate. Scale = 100 nm. The phage was reportedly deposited in 1948 by Twort himself in the collection of the Pasteur Institute of Paris.

lysogeny, genetic regulation, and transduction. It was shown that defective prophages could carry specific bacterial genes and insert them into bacteria (specific transduction). Certain phages such as P22 could transfer any bacterial genes and thus mediate 'general transduction'. This led to an understanding of phage conversion. It appeared that the converting phages were lysogenic and that their prophages converted bacteria by coding for novel properties, such as the production of antigens. As early as 1951, V. J. Freeman reported that the production of diphtheria toxin depended on the presence of prophages. The study of lysogeny culminated in 1962 when A. Campbell proposed his model of prophage insertion by a crossover process.

Varied and Interesting

In 1959, Brenner and Horne revolutionized electron microscopy by introducing negative staining with phosphotungstic acid. This produced pictures of unprecedented quality. They showed that T4 tails were contractile and that tail contraction was part of the T4 infective process. The same year, single-stranded (ss) DNA was discovered in the minute phage ϕX174 *(Microviridae)*. Filamentous phages *(Inoviridae)* and phages with ssRNA *(Leviviridae)* were isolated in 1960 and 1961, respectively. True marine phages and phages of cyanobacteria, then called 'blue-green algae', were also found. This offered some hope to control 'algal' blooms.

While phage therapy disappeared, phage typing became a major epidemiological tool (**Table 5**). In addition to the *S. typhi* typing scheme, international typing sets for *S. paratyphi B* and *Staphylococcus aureus* were devised and are still in use. Typing schemes for many medically important bacteria (e.g., corynebacteria, various *E. coli* and *Salmonella* serotypes, shigellae, *Pseudomonas aeruginosa*, and *Vibrio cholerae*) appeared. A literature survey by H Raettig lists no less than 2000 articles on phage typing, mostly in the years 1940–65. Phage research was considerably promoted by M H Adams' book *Bacteriophages*, which summarized phage research until 1957 and introduced the agar-double-layer method of phage propagation

Diversification and the Ongoing History of Bacteriophages: 1965–Today

Phage research now expanded explosively and divided into many, partly overlapping fields. No particular time periods are discernible; all fields developed gradually and are still expanding. As judged from literature listings, the present number of phage publications is 800–900 articles or monographs per year. The following is a selection of research activities.

Basic Phage Research

1. The present period of phage research started in 1965 (**Table 3**) after D. E. Bradley published a seminal, still cited paper on phage morphology (republished in 1967). Bradley classified phages into six basic types defined by morphology and nature of nucleic acid. Phages were divided into tailed phages (with contractile, long and noncontractile, or short tails), isometric phages with ssDNA or ssRNA, and filamentous phages. This became the basis of present-day phage classification.
2. Novel phages were isolated at a rate of about 100 per year. A first phage survey, published in 1967, listed 111 phages examined by electron microscopy, 102 of which were tailed and 9 isometric or filamentous. Most infected enterobacteria. Presently, the number of phages with known morphology exceeds 5500 and the number of host genera has grown to 155. It includes mycoplasmas, cyanobacteria, and archaea.
3. Replication and assembly of most phage families were studied. A highlight was the description of the assembly pathway of phage T4 (1967), obtained by a combination of electron microscopy and genetics.
4. Many phage genomes were sequenced. The first was that of ssRNA phage MS2 (*Leviviridae*), the first viral genome ever to be sequenced. This was followed by the sequences of various small viruses of the families *Microviridae*, *Inoviridae*, and *Leviviridae*. A landmark was the sequencing of the complete λ genome (1982), followed by that of coliphage T7. The complete

Table 3 Basic phage research

Year	*Investigators*	*Phages*	*Event or observation*
(a) The rise of molecular biology			
1939	Ellis and Delbrück	Coliphage	One-step growth experiment
1940	Pfankuch and Kausche; Ruska	Coliphage	Phages seen in the electron microscope
1945	Demerec and Fano	T1–T7	Differentiation by host range
1942	Luria and Anderson	T1, T2	Two different morphologies
1942–53	Anderson and coll.	T1–T7	Morphological studies
1946	Cohen and Anderson	T2	Contains 37% of DNA
1947	Luria	T1–T7	Multiplicity reactivation
1948	Putnam and Cohen	T6	Transfer of DNA phosphorus from parent to progeny phage
1949	Hershey and Rothman	T2	Genetic recombination between phage mutants
1950	Anderson	T4	DNA is located in the head and separated from protein by osmotic shock
	Lwoff and Gutmann		Clarification of the nature of lysogeny
1951	Cohen	T-even	Phage synthesis in stages: protein first, DNA later
	Herriott	T2	Empty phages consist of protein
1951	Doermann	T3, T-even	Eclipse period in phage multiplication
1952	Anderson	T2, T4, T5	Phages adsorb tail first
	Cohen	T-even	DNA contains 5-hydroxymethylcytosine
	Hershey and Chase	T2	DNA carries genetic information
	Zinder and J. Lederberg	P22	General transduction
1953	Lwoff		Review paper on lysogeny
1955	Lennox	P1	General transduction
1959	Adams		Summary of phage research
	Brenner and Horne		Introduction of negative staining
1960	Doty and coll.		DNA–DNA hybridization
1962	Campbell	λ	Model of prophage insertion
1963	Taylor	Mu	Acts as a transposon
(b) After 1965			
1965, 1967	Bradley	General	Phage classification
1967	Eisenstark	General	First phage count
	Wood and Edgar	T4	Assembly pathway completed
	Ikeda and Tomizawa	P1	Prophage is a plasmid
	Ptashne	λ	Isolation of repressor
1968	Signer	λ	Prophage may be a plasmid
1969	Six and Klug	P2	Depends on helper phage P2
1973	Arber	P1	Restriction endonucleases identified
1992	Young	General	Clarification of phage lysis
2000	Wikoff and coll.	HK97	Capsid structure
2005	Fokine and coll., Morais and coll.	T4, HK97, ϕ29	Common ancestry of tailed phages
(c) Phage-coded bacterial toxins			
1951	Freeman	β	Diphtheria toxin
1964	Zabriskie	A25	Erythrogenic toxin of streprococci
1970	Inoue and Iida	CEβ	Botulinus toxin C
1971	Uchida	β	Diphtheria toxin gene is part of phage
1984	O'Brien and coll.	H-19J, 933W	Shiga-like toxins of *E. coli*
1996	Waldor and Mekalanos	CTXϕ	Cholera toxin

sequence of the T4 genome was established in 1994. This was the beginning of large-scale phage genome sequencing (**Table 3**(**b**)) and of the novel science of 'genomics', devoted to computer-assisted comparisons of phage and other genomes. By 2006, the genomes of representatives of all phage families and of many individual tailed phages had been sequenced.

5. Bacteriophages appeared as major factors of bacterial virulence, coding for bacterial exotoxins (e.g., botulinus and Shiga toxins), antigens, and other virulence factors. A momentous discovery made in 1996 was that filamentous phages in their double-stranded replication form were able to convert nontoxigenic cholera strains to toxigenic forms. Virulence genes were found to be present in converting phages and integrated prophages. Sequencing of bacterial genomes showed that prophages with virulence factors were generally organized into 'pathogenicity islands'.

Table 4 Basic research: Milestones in genome sequencing

Year	*Investigators*	*Phages*	*Family*[a]	*Host*
1976	Fiers and coll.	MS2	*Leviviridae*	Enterics
1978	Sanger and coll.	ϕX174	*Microviridae*	Enterics
1980	Van Wezenbeek and coll.	M13	*Inoviridae*	Enterics
1981	Mekler	Qβ	*Leviviridae*	Enterics
1982	Sanger and coll.	λ	S	Enterics
1983	Dunn and Studier	T7	P	Enterics
1986	Vlcek and Paces	ϕ29[b]	P	*Bacillus*
1988	Mindich	ϕ6[b]	Cystoviridae	*Pseudomonas*
1994	Kutter and coll.	T4[b]	M	Enterics
1996	Grimaud	Mu	M	Enterics
1998	Christie and coll.	P2[b]	M	Enterics
1999	Mannisto and coll.	PM2	Corticoviridae	*Pseudoalteromonas*
2000	Vanderbyl and Kropinski	P22[b]	P	Enterics
2002	Mesyanzhinov and coll.	ϕKZ	M	*Pseudomonas*
2004	Lobocka and coll.	P1[b]	M	Enterics

[a]Tailed phages: M, *Myoviridae*; P, *Podoviridae*; S, *Siphoviridae*.
[b]Sequence completed.

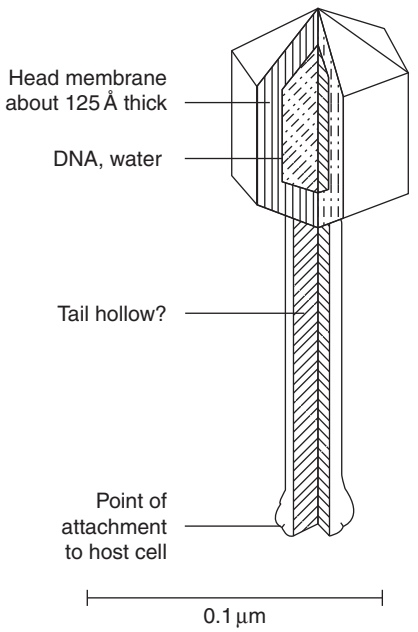


Figure 2 Drawing of phage T2 by T. F. Anderson (1952). Reproduced from Anderson TF, Rappaport C, and Muscatine NA (1953) On the structure and osmotic properties of phage particles. *Annales de l'Institut Pasteur* 84: 5–15, with permission from Elsevier.

6. Cryoelectron microcopy and three-dimensional image reconstruction revealed the capsid structure of various isometric phages (*Microviridae*, *Leviviridae*, *Tectiviridae*) and of the tail and base plate structure of phage T4. It was also shown that the capsids of adenoviruses and tectiviruses and of representatives of all three tailed phage families (T4, HK97, ϕ29) were structurally related. This indicated phylogenetic relationships between adeno- and tectiviruses and provided the long-sought evidence that tailed phages are a monophyletic evolutionary group.

Applied Research

See **Table 5** for a listing of research activities, arranged chronologically.

1. In the dairy industry, phages of lactococci and *Streptococcus thermophilus* were shown to cause major economic losses by destroying starter cultures and causing faulty fermentations. The phages involved were studied in detail by electron microscopy and genome sequencing.
2. Phages were investigated for use as indicators of fecal pollution. Phages with ssRNA (*Leviviridae*), somatic coliphages, and tailed phages of *Bacteroides fragilis* were proposed as indicators, but their usefulness was not conclusively proven. On the other hand, phages of many types proved useful as tracers of groundwater movements.
3. Phages were harnessed for the detection of bacteria, particularly by the construction of 'reporter' phages carrying luminescent (luciferase) genes, which are expressed when these phages infect a bacterium.
4. In biotechnology, phages are used as cloning and sequencing vectors, notably the filamentous phage M13 *(Inoviridae)*, phages λ and P1, and hybrids of phages and plasmids (named 'cosmids' or 'phagemids'). Phage products such as T4 ligase and T7 promoters are used in cloning reactions and phage lysozymes are used to free useful intracellular bacterial enzymes.
5. The technique of 'phage display', introduced in 1985, mainly uses filamentous phages (M13, fd). Foreign peptide genes are fused to phage coat proteins. This

Table 5 Applied and environmental phage research

Year	*Investigators*	*Phages*	*Event or observation*
(a) Phage therapy			
1919	D'Herelle		Treats human dysentery and chicken cholera
1921	Bruynoghe and Maisin		Treat human furunculosis
1925	D'Herelle		Treats plague in Egypt
1927–28	D'Herelle		Treats cholera in Assam
	Moore		Phages for control of plant disease
1938	Fisk		Treats septicemia in mice
1981–87	Slopek and coll.		Phage therapy in Poland
1982–87	Smith and Huggins		Treat diarrhea in calves, lambs, piglets
2001	Nelson and coll.		Phage lytic enzymes for elimination of streptococci in mice
(b) Diagnostics, industry, and biotechnology			
1936	Whitehead and Cox		Faulty fermentations in the dairy industry
1938	Craigie and Yen	VIII	Phage typing of *Salmonella typhi*
1942	Fisk		Phage typing of *Staphylococcus aureus*
1954	Cherry and coll.	O1	Identification of salmonellae
1955	Brown and Cherry	γ	Identification of *Bacillus anthracis*
1973	Lobban and Kaiser	T4	Ligase for cloning
	Studier	T7	Promoter for cloning
1977	Blattner and coll.	λ	Charon vectors for cloning
1977–83	Messing and coll., others	M13, fd	Cloning and sequencing vectors
1985	Smith	M13	Phage display
1987	Ulitzur and Kuhn	λ	Luciferase reporter phages
1990	Sternberg	P1	Cloning of large DNA fragments
(c) Phages and the environment			
1920	Dumas		Phages in soil
1923	Gerretsen and coll.		Phages in rhizobia
	Van der Hoeden		Phages in meat
1924	Zdansky		Phages in sewage
1955	Spencer		Indigenous marine phage
1966	Coetzee		Phages as pollution indicators
1974	Martin and Thomas		Phages as groundwater tracers
1987	Goyal and coll.		Book on phage ecology
1989	Bergh and coll.		Large numbers of phages in seawater
2002	Brüssow and Hendrix		Global phage population estimated at 10^{31}

powerful technique led to the development of antibody technology and has many medical and pharmaceutical applications.

6. Phage therapy reappeared. In France, it had always been practiced on a small scale. In the mid-1980s, a team in Wroclaw, Poland, conducted phage treatments of various human diseases (mainly pyogenic infections). After the fall of communism, the West learned with surprise that phage therapy had been practiced in the Soviet Union since the 1940s and that the institute founded in Tbilisi par d'Herelle and Eliava produced phages for the Soviet Union with up to 1200 employees engaged in production. Phage therapy is still practiced in Georgia.

Phage Ecology

1. Marine phages were investigated worldwide, including Arctic and Antarctic waters, largely by electron microscopy and the novel epifluorescence technique. Marine phages were found in vast numbers and appeared as part of a food web of phages, bacteria, and flagellates. Many marine phages were cyanophages and, all those investigated so far have tailed particles. Sequencing of uncultured virus DNA ('metagenomics') detected known entities such as T7-like phages and showed that only 25% of sequences matched known genes. Seawater appears as an immense phage reservoir (**Table 5**).
2. Phages were sought and found in extreme habitats such as volcanic hot springs, alkaline lakes, or hypersaline lagoons. This led to the discovery of several families of archaeal viruses (**Table 2**).

See also: Assembly of Viruses: Nonenveloped Particles; History of Virology: Vertebrate Viruses; Icosahedral dsDNA Bacterial Viruses with an Internal Membrane; Icosahedral Enveloped dsRNA Bacterial Viruses; Icosahedral ssDNA Bacterial Viruses; Icosahedral ssRNA Bacterial Viruses; Icosahedral Tailed dsDNA Bacterial Viruses; Inoviruses; Transcriptional Regulation in Bacteriophage.

Further Reading

Ackermann H-W (2006) 5500 bacteriophages examined in the electron microscope. *Archives of Virology* 152: 227–243.

Ackermann H-W and DuBow MS (1987) *Viruses of Prokaryotes, Vol. I. General Properties of Bacteriophages.* Boca Raton, FL: CRC Press.

Adams MH (1959) *Bacteriophages.* New York: Interscience Publishers.

Anderson TF, Rappaport C, and Muscatine NA (1953) On the structure and Ormotic properties of phage particles. *Annales de l'Institut Pasteur* 84: 5–15

Cairns J, Stent GS, and Watson JD (eds.) (1992) *Phage and the Origins of Molecular Biology.* (expanded edn.) Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Calendar R (2006) *The Bacteriophages,* 2nd edn., New York: Oxford University Press.

Goyal SM, Gerba CP, and Bitton G (eds.) (1987) *Phage Ecology.* New York: Wiley.

Kutter E and Sulakvelidze A (eds.) (2004) *Bacteriophages: Biology and Applications.* Boca Raton, FL: CRC Press.

Marks T and Sharp R (2000) Bacteriophages and biotechnology: A review. *Journal of Chemical Technology and Biotechnology* 75: 6–17.

Raettig H (1958) *Bakteriophagie 1917 Bis 1956. Vol. I. Einführung, Sachregister, Stichwortverzeichnis. Vol. II. Autorenregister.* Stuttgart: Gustav Fischer.

Raettig H (1967) *Bakteriophagie 1957–1965. Vol. I. Einführung, Sachregister, Stichwort-Index. Introduction, Index of Subjects, Reference Word Index. Vol. II. Bibliography.* Stuttgart: Gustav Fischer.

Summers WC (1999) *Felix d'Herelle and the Origins of Molecular Biology.* New Haven, CT: Yale University Press.

Tidona CA, Darai G, and Büchen-Osmond C (2001) *The Springer Index of Viruses.* Berlin: Springer.

Twort A (1993) *In Focus, Out of Step: A Biography of Frederick William Twort F.R.S. 1877–1950.* Stroud, UK: A. Sutton.

History of Virology: Plant Viruses

R Hull, John Innes Centre, Norwich, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

History shows that the study of virus infections of plants has led the overall subject of virology in the development of several major concepts including that of the entity of viruses themselves. To obtain a historical perspective of plant virology, five major (overlapping) ages can be recognized.

Prehistory

The earliest known written record describing what was almost certainly a plant virus disease is a poem in Japanese written by the Empress Koken in AD 752 and translated by T. Inouye as follows:

> In this village
> It looks as if frosting continuously
> For, the plant I saw
> In the field of summer
> The color of the leaves were yellowing

The plant, identified as *Eupatorium lindleyanum*, has been found to be susceptible to tomato yellow leafcurl virus, which causes a yellowing disease.

In Western Europe in the period from about 1600 to 1660, many paintings and drawings were made of tulips that demonstrate flower symptoms of virus disease. These are recorded in the Herbals of the time and in the still-life paintings of artists such as Johannes Bosschaert in 1610. During this period, blooms featuring such striped patterns were prized as special varieties leading to the phenomenon of 'tulipomania'. The trade in infected tulip bulbs resulted in hyperinflation with bulbs exchanging hands for large amounts of money or goods (**Table 1**).

In describing an experiment to demonstrate that sap flows in plants, Lawrence reported in 1714 the unintentional transmission of a virus disease of jasmine by grafting. The following quotation from Blair in 1719 describes the procedure and demonstrates that even in this proto-scientific stage, experimenters were already indulging in arguments about priorities of discovery.

> The inoculating of a strip'd Bud into a plain stock and the consequence that the Stripe or Variegation shall be seen in a few years after, all over the shrub above and below the graft, is a full demonstration of this Circulation of the Sap. This was first observed by Mr. Wats at Kensington, about 18 years ago: Mr. Fairchild performed it 9 years ago; Mr. Bradly says he observ'd it several years since; though Mr. Lawrence would insinuate as if he had first discovered it.

Recognition of Viral Entity

In the latter part of the nineteenth century, the idea that infectious disease was caused by microbes was well established, and filters were available that would not allow the passage of known bacterial pathogens. Mayer in 1886 showed that a disease of tobacco (*Mosaikkrankheit*; now

Table 1 Tulipomania: the goods exchanged for one bulb of Viceroy tulip

4 t of wheat	4 barrels of beer
8 t of rye	2 barrels of butter
4 fat oxen	1000 lb cheese
8 fat pigs	1 bed with accessories
12 fat sheep	1 full dress suit
2 hogsheads of wine	1 silver goblet

known to be caused by tobacco mosaic virus; TMV) could be transmitted to healthy plants by inoculation with extracts from diseased plants; Iwanowski demonstrated in 1892 that sap from such tobacco plants was still infective after it had been passed through a bacteria-proof filter candle. This work did not attract much attention until it was repeated by Beijerinck who in 1898 described the infectious agent as *contagium vivum fluidum* (Latin for contagious living fluid) to distinguish it from contagious corpuscular agents. Beijerinck's discovery is considered to be the birth of virology. In 1904 Baur showed that an infectious variegation of *Abutilon* could be transmitted by grafting, but not by mechanical inoculation. Beijerinck and Baur used the term 'virus' in describing the causative agents of these diseases, to contrast them with bacteria; this term had been used as more or less synonymous with bacteria by earlier workers. As more diseases of this sort were discovered, the unknown causative agents came to be called 'filterable viruses'.

Between 1900 and 1935, many plant diseases thought to be caused by filterable viruses were described, but considerable confusion arose because adequate methods for distinguishing one virus from another had not yet been developed.

The original criterion of a virus was an infectious entity that could pass through a filter with a pore size small enough to hold back all known cellular agents of disease. However, diseases were soon found that had virus-like symptoms not associated with any pathogen visible in the light microscope, but that could not be transmitted by mechanical inoculation. With such diseases, the criterion of filterability could not be applied. Their infectious nature was established by graft transmission and sometimes by insect vectors. Thus, certain diseases of the yellows and witches'-broom type, such as aster yellows, came to be attributed to viruses on quite inadequate grounds. Many such diseases are now known to be caused by phytoplasma or spiroplasma, and a few by bacteria or rickettsia.

The Biological Age

During most of the period between 1900 and 1935, attention was focused on the description of diseases using the macroscopic symptoms and cytological abnormalities as revealed by light microscopy, host ranges, and methods of transmission which were the only techniques available. The influence of various physical and chemical agents on virus infectivity was investigated, but methods for the assay of infective material were primitive. Holmes showed that the local lesions produced in some hosts following mechanical inoculation could be used for the rapid quantitative assay of infective virus. This technique enabled properties of viruses to be studied much more readily and paved the way for the isolation and purification of viruses a few years later. Until about 1930, there was serious confusion by most workers regarding the diseases produced by viruses and the viruses themselves. This was not surprising, since virtually nothing was known about the viruses except that they were very small. In 1931 Smith made an important contribution that helped to clarify this situation. Working with virus diseases in potato, he realized the necessity of using plant indicators, plant species other than potato, which would react differently to different viruses present in potatoes. Using several different and novel biological methods to separate the viruses, he was able to show that many potato virus diseases were caused by a combination of two viruses with different properties, which he named virus X (potato virus X, PVX) and virus Y (potato virus Y, PVY). As PVX was not transmitted by the aphid *Myzus persicae*, whereas PVY was, PVY could be separated from PVX. He obtained PVX free of PVY by needle inoculation of the mixture to *Datura stramonium* which does not support PVY. Furthermore, Smith observed that PVX from different sources fluctuated markedly in the severity of symptoms it produced in various hosts leading to the concept of strains.

An important practical step forward was the recognition that some viruses could be transmitted from plant to plant by insects. Fukushi recorded the fact that in 1883 a Japanese rice grower transmitted what is now known to be rice dwarf virus, RDV) by the leafhopper *Recelia dorsalis*. However, this work was not published in any available form and so had little influence. In 1922 Kunkel first reported the transmission of a virus by a planthopper; within a decade, many insects were reported to be virus vectors leading to the recognition of specific virus–vector interactions.

Since Fukushi first showed in 1940 that RDV could be passed through the egg of a leafhopper vector for many generations, there has been great interest in the possibility that some viruses may be able to replicate in both plants and insects. It is now well established that plant viruses in the families *Rhabdoviridae* and *Reoviridae* and the genera *Tenuivirus*, *Tospovirus*, and *Marafivirus* multiply in insects as well as in plants.

The Biochemical/Biophysical Age

Beale's recognition in 1928 that plants infected with TMV contained a specific antigen opened the age in which the

biochemical nature of viruses was elucidated. In the 1930s Gratia showed that plants infected with different viruses contained different specific antigens and Chester demonstrated that different strains of TMV and PVX could be distinguished serologically.

The high concentration at which certain viruses occur in infected plants and their relative stability turned out to be of crucial importance in the first isolation and chemical characterization of viruses, because methods for extracting and purifying proteins were not highly developed. In the early 1930s, various attempts were made to isolate and purify plant viruses using methods similar to those that had just been developed for purifying enzymes. Following detailed chemical studies suggesting that the infectious agent of TMV might be a protein, Stanley announced in 1935 the isolation of this virus in an apparently crystalline state. At first Stanley considered that the virus was a globulin containing no phosphorus but in 1936 Bawden *et al.* described the isolation from TMV-infected plants of a liquid crystalline nucleoprotein containing nucleic acid of the pentose type. They showed that the particles were rod-shaped, thus confirming the earlier suggestion of Takahashi and Rawlins based on the observation that solutions containing TMV showed anisotropy of flow. Electron microscopy and X-ray crystallography were the major techniques used in early work to explore virus structure, and the importance of these methods has continued to the present day. Bernal and Fankuchen applying X-ray analysis to purified preparations of TMV obtained accurate estimates of the width of the rods. The isolation of other rod-shaped viruses, and spherical viruses that formed crystals, soon followed. All were shown to consist of protein and pentose nucleic acid.

Early electron micrographs confirmed that TMV was rod-shaped and provided approximate dimensions, but they were not particularly revealing because of the lack of contrast between the virus particles and the supporting membrane. The application of shadow-casting with heavy metals greatly increased the usefulness of the method for determining the overall size and shape of virus particles but not structural detail. With the development of high-resolution microscopes and of negative staining in the 1950s electron microscopy became an important tool for studying virus substructure. From a comparative study of the physicochemical properties of the virus nucleoprotein and the empty viral protein shell found in TYMV preparations, Markham concluded in 1951 that the RNA of the virus must be held inside a shell of protein, a view that has since been amply confirmed for this and other viruses by X-ray crystallography. Crick and Watson suggested that the protein coats of small viruses are made up of numerous identical subunits arrayed either as helical rods or as a spherical shell with cubic symmetry. Subsequent X-ray crystallographic and chemical work has confirmed this view. Caspar and Klug formulated a general theory that delimited the possible numbers and arrangements of the protein subunits forming the shells of the smaller isodiametric viruses.

Until about 1948, most attention was focused on the protein part of the viruses. Quantitatively, the protein made up the larger part of virus preparations. Enzymes that carried out important functions in cells were known to be proteins, and knowledge of pentose nucleic acids was rudimentary. No function was known for them in cells, and they generally were thought to be small molecules primarily because it was not recognized that RNA is very susceptible to hydrolysis by acid, by alkali, and by enzymes that commonly contaminate virus preparations. In 1949 Markham and Smith isolated turnip yellow mosaic virus (TYMV) and showed that purified preparations contained two classes of particles, one an infectious nucleoprotein with about 35% of RNA, and the other an apparently identical protein particle that contained no RNA and that was not infectious. This result clearly indicated that the RNA of the virus was important for biological activity. Analytical studies showed that the RNAs of different viruses have characteristically different base compositions while those of related viruses are similar. About this time, it came to be realized that viral RNAs might be considerably larger than had been thought. A synthetic analog of the normal base guanine, 8-azaguanine, when supplied to infected plants was incorporated into the RNA of TMV and TYMV, replacing some of the guanine. The fact that virus preparations containing the analog were less infectious than normal virus gave further experimental support to the idea that viral RNAs were important for infectivity. However, it was the classic experiments in the mid-1950s of Gierer and Schramm, and Fraenkel-Conrat and Williams that demonstrated the infectivity of naked TMV RNA and the protective role of the protein coat. These discoveries ushered in the era of modern plant virology.

In the early 1950s Brakke developed density gradient centrifugation as a method for purifying viruses. Together with a better understanding of the chemical factors affecting the stability of viruses in extracts, this procedure has allowed the isolation and characterization of many viruses. The use of sucrose density gradient fractionation enabled Lister to discover the bipartite nature of the tobacco rattle virus genome. Since that time, density gradient and polyacrylamide gel fractionation techniques have allowed many viruses with multipartite genomes to be characterized. Their discovery, in turn, opened up the possibility of carrying out genetic reassortment experiments with plant viruses leading to the allocation of functions to many of the viral genes. Density gradient fractionation of purified preparations of some other viruses revealed noninfectious nucleoprotein particles containing subgenomic RNAs. Other viruses have been found to have associated with them satellite viruses or satellite RNAs that depend on the 'helper' virus for some function required during replication.

Further developments in the 1970s included improved techniques related to X-ray crystallographic analysis and a growing knowledge of the amino acid sequences of the coat proteins allowed the three-dimensional structure of the protein shells of several plant viruses to be determined in molecular detail.

For some decades, the study of plant virus replication had lagged far behind that of bacterial and vertebrate viruses mainly because there was no plant system in which all the cells could be infected simultaneously to provide the basis for synchronous 'one-step growth' experiments. However, following the initial experiments of Cocking in 1966, Takebe and colleagues developed protoplast systems for the study of plant virus replication. Although these systems had significant limitations, they greatly increased our understanding of the processes involved in plant virus replication.

Another important technical development has been the use of *in vitro* protein-synthesizing systems such as that from wheat germ, in which many plant viral RNAs act as efficient messengers. Their use allowed the mapping of plant viral genomes by biochemical means to begin.

The Molecular Biology Age

The molecular age opened in 1960 with the determination of the full sequence of 158 amino acids in the coat protein of TMV. The sequence of many naturally occurring strains and artificially induced mutants was also determined at about the same time. This work made an important contribution to establishing the universal nature of the genetic code and to our comprehension of the chemical basis of mutation.

Our understanding of the genome organization and functioning of viruses has come from the development of procedures whereby the complete nucleotide sequence of viruses with RNA genomes can be determined. In 1982 the genomes of both the first plant RNA virus (TMV) and DNA virus cauliflower mosaic virus (CaMV) were sequenced. Since then, the genomes of representatives of all the plant virus genera have been sequenced and there are many sequences of virus species.

The late 1980s and 1990s was a period when molecular biological techniques were applied to a wide range of aspects of plant virology. These included the ability to prepare *in vitro* infectious transcripts of RNA viruses derived from cloned viral cDNA allowing techniques such as site-directed mutagenesis to be applied to the study of genome function and reverse genetics being used to elucidate the functions of viral genes and control sequences. These approaches, together with others such as yeast systems for identifying interacting molecules, the expression of viral genes in transgenic plants, and labeling viral genomes in such a manner that their sites of function within the cell are known, were revealing the complexities of the interactions between viruses and their hosts. Nucleotide sequence information has had, and continues to have, a profound effect on our understanding of many aspects of plant virology, including (1) the location, number, and size of the genes in a viral genome; (2) the amino acid sequence of the known or putative gene products; (3) the molecular mechanisms whereby the gene products are transcribed; (4) the putative functions of a gene product, which can frequently be inferred from amino-acid-sequence similarities to products of known function encoded by other viruses; (5) the control and recognition sequences in the genome that modulate expression of viral genes and genome replication; (6) the understanding of the structure and replication of viroids and of the satellite RNAs found associated with some viruses; (7) the molecular basis for variability and evolution in viruses, including the recognition that recombination is a widespread phenomenon among RNA viruses and that viruses can acquire host nucleotide sequences as well as genes from other viruses; and (8) the beginning of a taxonomy for viruses that is based on evolutionary relationships. On the host side, advances in plant genome sequencing are identifying plant genes that confer resistance to viruses.

During the 1980s, major advances were made on improved methods of diagnosis for virus diseases, centering on serological procedures and on methods based on nucleic acid hybridization. Since the work of Clark and Adams reported in 1977, the enzyme-linked immunosorbent assay (ELISA) technique has been developed with many variants for the sensitive assay and detection of plant viruses. Monoclonal antibodies against TMV lead to a very rapid growth in their use for many kinds of plant virus research and for diagnostic purposes. The late 1970s and the 1980s also saw the start of application of the powerful portfolio of molecular biological techniques to developing other approaches to virus diagnosis, to a great increase in our understanding of the organization and strategy of viral genomes, and to the development of techniques that promise novel methods for the control of some viral diseases. The use of nucleic acid hybridization procedures for sensitive assays of large numbers of samples and the polymerase chain reaction, also dependent on detailed knowledge of genome sequences, are being increasingly used in virus diagnosis. Most recently DNA chips are being developed for both virus diagnostics and studying virus infection.

In the early 1980s, it seemed possible that some plant viruses, when suitably modified by the techniques of gene manipulation, might make useful vectors for the introduction of foreign genes into plants. Some plant viruses have been found to contain regulatory sequences that can be very useful in other gene vector systems, notably the widely used CaMV 35S promoter. Another practical application of molecular techniques to plant viruses has

been the modification of viral genomes so that products of interest to industry and medicine can be produced in plants. This is being done either by the introduction of genes into the viral genome or by modification of the coat protein sequence to enable epitopes to be presented on the virus.

Early attempts (early to mid-1900s) to control virus diseases in the field were often ineffective. They were mainly limited to attempts at general crop hygiene, roguing of obviously infected plants, and searching for genetically resistant lines. Developments since this period have improved the possibilities for control of some virus diseases. Heat treatments and meristem tip culture methods have been applied to an increasing range of vegetatively propagated plants to provide a nucleus of virus-free material that then can be multiplied under conditions that minimize reinfection. Such developments frequently have involved the introduction of certification schemes. Systemic insecticides, sometimes applied in pelleted form at the time of planting, provide significant protection against some viruses transmitted in a persistent manner by aphid vectors. It has become increasingly apparent that effective control of virus disease in a particular crop in a given area usually requires an integrated and continuing program involving more than one kind of control measure.

However, such integrated programs are not yet in widespread use. Cross-protection (or mild-strain protection) is a phenomenon in which infection of a plant with a mild strain of a virus prevents or delays infection with a severe strain. The phenomenon has been used with varying success for the control of certain virus diseases, but the method has various difficulties and dangers. In 1986 Powell-Abel and co-workers considered that some of these problems might be overcome by the application of the concept of pathogen-derived resistance of Sandford and Johnston. Using recombinant DNA technology, they showed that transgenic tobacco plants expressing the TMV coat-protein gene either escaped infection following inoculation or showed a substantial delay in the development of systemic disease. These transgenic plants expressed TMV coat-protein mRNA as a nuclear event. Seedlings from self-fertilized transformed plants that expressed the coat protein showed delayed symptom development when inoculated with TMV. Thus, a new approach to the control of virus diseases emerged. However, this approach revealed some unexpected results which led to the recognition that plants have a defense system against 'foreign' RNA. This defense system, initially termed post-translational gene silencing and now called RNA silencing or RNA interference (RNAi) was first recognized in plants and had, and is still having, a great impact on molecular approaches as diverse as disease control and understanding gene functions. Among the tools that have arisen from understanding this new phenomenon is virus-induced gene silencing which is being used to determine the functions of genes in plants and animals by turning them off.

However, the RNAi defense system in plants which targets double-stranded RNA, an intermediate RNA virus replication, raised the question of how RNA viruses replicated in plants. Studies on gene functions revealed that many plant viruses contain so-called 'virulence' genes. Many of these have been shown to suppress host RNA silencing, thus overcoming the defense system. Suppression of gene silencing is widespread among plant viruses and examples are being found in animal viruses. Thus, as noted at the beginning of this article, plant virology is still providing insights onto phenomena applicable to virology in general.

See also: Diagnostic Techniques: Plant Viruses; Nature of Viruses; Plant Virus Diseases: Economic Aspects; Tobacco Mosaic Virus; Vector Transmission of Plant Viruses; Vaccine Production in Plants; Viral Suppressors of Gene Silencing; Virus Induced Gene Silencing (VIGS).

Further Reading

Baulcombe DC (1999) Viruses and gene silencing in plants. *Archives of Virology* 15(supplement): 189–201.

Caspar DLD and Klug A (1962) Physical principles in the construction of regular viruses. *Cold Spring Harbor Symposium on Quantitative Biology* 27: 1–24.

Clark MF and Adams AN (1977) Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34: 475–483.

Fischer R and Emans N (2000) Molecular farming of pharmaceutical proteins. *Transgenic Research* 9: 279–299.

Hull R (2001) *Matthews' Plant Virology.* San Diego: Academic Press.

Li F and Ding SW (2006) Virus counterdefense: Diverse strategies for evading the RNA silencing mechanism. *Annual Review of Microbiology* 60: 507–531.

Lindbo JA and Dougherty WG (2005) Plant pathology and RNAi: A brief history. *Annual Review of Phytopathology* 43: 191–204.

Pavord A (1999) *The Tulip.* London: Bloomsbury.

Van der Want JPH and Dijkstra J (2006) A history of plant virology. *Archives of Virology* 151: 1467–1498.

History of Virology: Vertebrate Viruses

F Fenner, Australian National University, Canberra, ACT, Australia

© 2008 Elsevier Ltd. All rights reserved.

Introduction

As in other branches of experimental science, the development of our knowledge of viruses has depended on the techniques available. Initially, viruses were studied by pathologists interested in the causes of the infectious diseases of man and his domesticated animals and plants and these concerns remain the main force advancing the subject. The idea that viruses might be used to probe fundamental problems of biology arose in the early 1940s, with the development of the knowledge of bacterial viruses. These studies helped establish the new field of molecular virology in the period 1950–70, and this has revolutionized the study of viruses and led to an explosion of knowledge about them.

The Word Virus

Since antiquity the term virus had been synonymous with poison, but during the late nineteenth century it became a synonym for microbe (Pasteur's word for an infectious agent). It did not acquire its present connotation until the 1890s, after the bacterial or fungal causes of many infectious diseases had been discovered, using the agar plate, effective staining methods, and efficient microscopes. It then became apparent that there were a number of infectious diseases of animals and plants from which no bacterium or fungus could be isolated or visualized with the microscope. After the introduction in 1884 of the Chamberland filter, which held back bacteria, Loeffler and Frosch demonstrated that the cause of foot-and-mouth disease was a filterable (or ultramicroscopic) virus. The first compendium of all then known viruses was edited by T. M. Rivers of the Rockefeller Institute and published in 1928. Entitled *Filterable Viruses,* this emphasized that viruses required living cells for their multiplication. In the 1930s chemical studies of the particles of tobacco mosaic virus and of bacteriophages showed that they differed from all cells in that at their simplest they consisted of protein and nucleic acid, which was either DNA or RNA. Gradually, the adjectives filterable and ultramicroscopic were dropped and the word viruses developed its present connotation.

Early Investigations

Foot-and-Mouth Disease Virus

In 1898 F. J. Loeffler and P. Frosch described the filterability of an animal virus for the first time, noting that "the filtered material contained a dissolved poison of extraordinary power or that the as yet undiscovered agents of an infectious disease were so small that they were able to pass through the pores of a filter definitely capable of retaining the smallest known bacteria." Although the causative agent of foot-and-mouth disease passed through a Chamberland-type filter, it did not go through a Kitasato filter which had a finer grain. This led to the conclusion that the causative virus, which was multiplying in the host, was a corpuscular particle. Loeffler and Frosch gave filtration a new emphasis by focussing attention on what passed-through the filter rather than what was retained and established an experimental methodology which was widely adopted in the early twentieth century in research on viral diseases.

Yellow Fever Virus

Following the acceptance of the notion of filterable infectious agents, pathologists investigated diseases from which no bacteria could be isolated and several were soon shown to be caused by viruses. One of the most fruitful investigations, in terms of new concepts, was the work of the United States Army Yellow Fever Commission headed by Walter Reed in 1900–01. Using human volunteers, they demonstrated that yellow fever was caused by a filterable virus which was transmitted by mosquitoes and that the principal vector was *Aedes aegypti.* They also showed that infected persons were infectious for mosquitoes only during the first few days of the disease and that mosquitoes were not infectious until 7–10 days after imbibing infectious blood, thus defining the extrinsic incubation period and establishing essentially all of the basic principles of the epidemiology of what came to be called arboviruses (arthropod-borne viruses).

Physical Studies of Viruses

Further advances in understanding the nature of viruses depended on physical and chemical studies. As early as 1907 H. Bechhold in Germany developed filters of graded permeabilities and a method of determining their pore size. Subsequently, W. J. Elford, in London, used such membranes for determining the size of animal virus particles with remarkable accuracy.

In Germany, on the eve of World War II, H. Ruska and his colleagues had produced electron microscopic photographs of the particles of tobacco mosaic virus,

bacteriophages, and poxviruses. Technical improvements in instrumentation after the war, and the introduction of negative staining for studying the structure of viruses by Cambridge scientists H. E. Huxley in 1957 and S. Brenner and R. W. Horne in 1959 resulted in photographs of the particles of virtually every known kind of virus. These demonstrated the variety of their size, shape, and structure, and the presence of common features such as the icosahedral symmetry of many viruses of animals.

Following a perceptive paper on the structure of small virus particles by F. H. C. Crick and J. D. Watson in 1956, in 1962 D. L. D Caspar of Boston and A. Klug of Cambridge, England, produced a general theory of the structure of regular virus particles of helical and icosahedral symmetry. Structure of the virus particle became one of the three major criteria in the system of classification of viruses that was introduced in 1966.

The Chemical Composition of Viruses

If the ultramicroscopic particles found in virus-infected hosts were the pathogens, what did these particles consist of? Following observations on plant and bacterial viruses in 1935, in 1940 C. L. Hoagland and his colleagues at the Rockefeller Institute found that vaccinia virus particles contained DNA but no RNA. Thus evidence was accumulating that viruses differed from bacteria not only in size and their inability to grow in lifeless media, but in that they contained only one kind of nucleic acid, which could be either DNA or RNA.

The development of restriction-endonuclease digestion of DNA, based on studies of phage restriction by W. Arber (Nobel Prize, 1978) of Basel in the 1960s, and then elaborated by biochemist H. O. Smith of Baltimore in 1970, has simplified the mapping of the genomes of DNA viruses, a study initiated by D. Nathans (Nobel Prize, 1978), also of Baltimore, using Simian virus 40. The development of the polymerase chain reaction in 1985 by K. B. Mullis (Nobel Prize, 1993) revolutionized sequencing methods for both DNA and RNA, leading to the availability of the complete genomic sequences of most viruses and the ability to make diagnoses using minute amounts of material.

Investigations of bacterial viruses were motivated by scientific curiosity, but animal virology was developed by pathologists studying the large number of diseases of humans and livestock caused by viruses, and it has retained this practical bias. Over the first two decades of the twentieth century, testing of filtrates of material from a number of infected humans and animals confirmed that they were caused by viruses. Among the most important was the demonstration in 1911 by P. Rous (Nobel Prize, 1966), at the Rockefeller Institute, that a sarcoma of fowls could be transmitted by a bacteria-free filtrate.

The Cultivation of Animal Viruses

The first systematic use of small animals for virus research was Pasteur's use of rabbits, which he inoculated intracerebrally with rabies virus in 1881. It was not until 1930 that mice were used for virus research, with intracerebral inoculations of rabies and yellow fever viruses. By using graded dilutions and large numbers of mice, quantitative animal virology had begun. The next important step, initiated by E. W. Goodpasture in 1931–32, was the use of chick embryos for growing poxviruses. This was followed a few years later by the demonstration by F. M. Burnet that many viruses could be titrated by counting the pocks that they produced on the chorioallantoic membrane, whereas others grew well in the allantoic and/or amniotic cavities of developing chick embryos.

Tissue cultures had first been used for cultivating vaccinia virus in 1928, but it was the discovery by J. F. Enders, T. H. Weller, and F. C. Robbins of Harvard University (Nobel Prize, 1954) in 1949 that poliovirus would grow in non-neural cells that gave a tremendous stimulus to the use of cultured cells in virology. Over the next few years their use led to the cultivation of medically important viruses such as those causing measles (by J. F. Enders and T. C. Peebles in 1954) and rubella (by T. H. Weller and F. C. Neva in 1962). Even more dramatic was the isolation of a wide variety of new viruses, belonging to many different families. Also the different cytopathic effects produced by different viruses in monolayer cell cultures were found to be diagnostic.

The next great advance, which greatly increased the accuracy of quantitative animal virology, occurred in 1952, when the plaque assay method for counting phages was adapted to animal virology by R. Dulbecco (Nobel Prize, 1975), using a monolayer of chick embryo cells in a petridish. In 1958 H. Temin and H. Rubin applied Dulbecco's method to Rous sarcoma virus, initiating quantitative studies of tumor viruses. Biochemical studies of animal virus replication were simplified by using continuous cell lines and by growing the cells in suspension.

Biochemistry

During the 1950s virus particles were thought to be 'inert' packages of nucleic acid and proteins, although in 1942 G. K. Hirst of the Rockefeller Institute had shown that particles of influenza virus contained an enzyme, later identified by A. Gottschalk of Melbourne as a neuraminidase.

Further advances in viral biochemistry depended on methods of purification of virus particles, especially the technique of density gradient centrifugation. In 1967 J. Kates and B. R. McAuslan demonstrated the presence of a DNA-dependent RNA polymerase in purified vaccinia

virus virions, a discovery that was followed the next year by the demonstration of a double-stranded (ds) RNA-dependent RNA polymerase in reovirus virions and then of single-stranded (ss) RNA-dependent RNA polymerases in virions of paramyxoviruses and rhabdoviruses. In 1970 came the revolutionary discovery by D. Baltimore of Boston (Nobel Prize, 1975) and H. Temin of Wisconsin (Nobel Prize, 1975), independently, of the RNA-dependent DNA polymerase, or reverse transcriptase, of Rous sarcoma virus. Many other kinds of enzymes were later identified in the larger viruses; for example, no less than 16 enzymes have now been identified in vaccinia virus virions.

Just as investigations with bacterial viruses were of critical importance in the development of molecular biology, studies with animal viruses have led to the discovery of several processes that have proved to be important in the molecular biology of eukaryotic cells, although many of these discoveries are too recent for serious historical appraisal. Thus, in 1968 M. Jacobson and D. Baltimore of Boston showed that the genomic RNA of poliovirus was translated as a very large protein, which was then cleaved by a protease that was subsequently found in the viral replicase gene. RNA splicing was discovered in 1977, independently by P. A. Sharp (Nobel Prize, 1993) and L. T. Chow and their respective colleagues during studies of adenovirus replication. Work with the same virus by Rekosh and colleagues in London in 1977 resulted in the definition of viral and cell factors for initiation of new DNA strands using a novel protein priming mechanism. Capping of mRNA by m^7G and its role in translation was discovered in 1974, during work with reoviruses by A. J. Shatkin. Other processes first observed with animal viruses and now known to be important in eukaryotic cells were the existence of 3′ poly A tracts on mRNAs, the pathway for synthesis of cell surface proteins, and the role of enhancer elements in transcription.

Analysis of viral nucleic acids progressed in parallel with that of the viral proteins. Animal viruses were found with genomes of ssRNA, either as a single molecule, two identical molecules (diploid retroviruses), or segmented; dsRNA; ssDNA, dsDNA, and partially dsDNA. The so-called unconventional viruses or prions of scrapie, kuru, and Creutzfeld–Jacob disease, which have been extensively investigated since 1957 by D. C. Gajdusek of the US National Institutes of Health (Nobel Prize, 1976) and S. B. Prusiner of the University of California at San Francisco (Nobel Prize, 1997), appear to be infectious proteins. Soon after the discovery that the isolated nucleic acid of tobacco mosaic virus was infectious (see below), it was shown that the genomic RNAs of viruses belonging to several families of animal viruses were infectious; namely those with single, positive-sense RNA molecules. Then in 1973 came the discovery of recombinant DNA by P. Berg of Stanford University and his colleagues, using the animal virus SV40 and bacteriophage l.

Structure of the Virion

Using more sophisticated methods of electron microscopy, many animal virus virions have been shown to be isometric icosahedral structures, or roughly spherical protein complexes surrounded by a lipid-containing shell, the envelope, which contains a number of virus-coded glycoprotein spikes. X-ray crystallography of crystals of purified isometric viruses has revealed the molecular structure of their icosahedra; similar revealing detail has been obtained for the neuraminidase and hemagglutinin spikes of influenza virus.

Tumor Virology

After the discovery of Rous sarcoma virus in 1911, there was a long interval before the second virus to cause tumors, rabbit papilloma virus, was discovered by R. E. Shope in 1933. However, after that viruses were found to cause various neoplasms in mice, and in 1962 J. J. Trentin of Yale University showed that a human adenovirus would produce malignant tumors in hamsters. Since then, direct proof has been obtained that several DNA viruses (but not adenoviruses) and certain retroviruses can cause tumors in humans. Molecular biological studies have shown that oncogenicity is largely caused by proteins they produce that are encoded by viral oncogenes, a concept introduced by R. J. Huebner and G. Todaro of the US National Institutes of Health in 1969 and corrected, refined, and greatly expanded since the mid-1970s by J. M. Bishop, H. Varmus (Nobel Prize, 1989), and R. A. Weinberg of Boston.

Only one group of RNA viruses, the retroviruses, which replicate through an integrated DNA provirus, cause neoplasms, whereas viruses of five groups of DNA viruses are tumorigenic. Study of these oncogenic viruses has shed a great deal of light on the mechanisms of carcinogenesis. Oncogenic DNA viruses contain oncogenes as an essential part of their genome, which when integrated into the host cell DNA may promote cell transformation, whereas the oncogenes of retroviruses are derived from proto-oncogenes of the cell.

Impact on Immunology

Immunology arose as a branch of microbiology and several discoveries with animal viruses were important in the development of important concepts in immunology. It was the discovery in the 1930s by E. Traub in Tübingen of persistent infection of mice with lymphocytic choriomeningitis virus that led to the development by F. M. Burnet (Nobel Prize, 1960) of the concept of immunological tolerance in 1949. It was work with the same

virus that led to the discovery of MHC restriction by P. C. Doherty and R. Zinkernagel in Canberra (Nobel Prize, 1996) in 1974.

Vaccines and Disease Control

From the time of Pasteur's use of rabies vaccine in 1885, a major concern of medical and veterinary virologists has been the development of vaccines. Highlights in this process have been the development of the 17D strain of yellow fever virus by M. Theiler (Nobel Prize, 1951) of the Rockefeller Foundation laboratories in 1937, the introduction of influenza vaccine in 1942, based on Burnet's 1941 discovery that the virus would grow in the allantois, the licensing in 1954 of inactivated poliovaccine developed by J. Salk and in 1961 of the live poliovaccine introduced by A. B. Sabin, both based on the cultivation of the virus in 1949 by Enders, and development of the first genetically engineered human vaccine with the licensing of yeast-grown hepatitis B vaccine in 1986. Finally, 1977 saw the last case of natural smallpox in the world and thus the first example of the global eradication of a major human infectious disease, the result of a 10-year campaign conducted by the World Health Organization with a vaccine directly derived from Jenner's vaccine, first used in 1796.

Recognition of HIV-AIDS

In 1981 a new disease was described in the male homosexual populations of New York, San Francisco, and Los Angeles. It destroyed the immune system, and the disease was called the acquired immune deficiency syndrome (AIDS) and the causal virus, human immunodeficiency virus (HIV), a retrovirus, was first isolated by L. Montagnier, of the Pasteur Institute in Paris, in 1983. A sexually transmitted disease, it has now spread all over the world, with about 40 million cases worldwide, and without very expensive chemotherapy is almost always fatal. It is now clear that it arose from an inapparent but persistent infection of chimpanzees in Africa. In spite of enormous efforts over the past 20 years, it has so far been impossible to devise an effective vaccine.

Arthropod-Borne Viruses of Vertebrates

Arthropods, mainly insects and ticks, were early shown to be important as vectors of virus diseases of vertebrates (the arboviruses) and of plants. Sometimes, as in myxomatosis, carriage was found to be mechanical, but in most cases the virus was found to multiply in the vector as well as the vertebrate concerned. The development of methods of growing insect cells in culture by T. D. C. Grace in Canberra in 1962 opened the way for the molecular biological investigation of the replication of insect viruses and arboviruses in invertebrate cells.

Taxonomy and Nomenclature

After earlier tentative efforts with particular groups of viruses, viral nomenclature took off in 1948, with the production of a system of latinized nomenclature for all viruses by the plant virologist F. O. Holmes. This stimulated others, in particular C. H. Andrewes of London and A. Lwoff of Paris, to actions which resulted in the setting up of an International Committee on Nomenclature (later Taxonomy) of Viruses in 1966. Adopting the kind of viral nucleic acid, the strategy of replication, and the morphology of the virion as its primary criteria, this committee has now achieved acceptance of its decisions by the great majority of virologists. One interesting feature of its activities is that its rules avoid the controversies about priorities that plague taxonomists working with fungi, plants, and animals.

The Future

The future will see an explosive expansion of understanding and knowedge of viruses of vertebrates, with the application of techniques of genetic engineering, nucleic acid sequencing, the polymerase chain reaction, and the use of monoclonal antibodies. All these discoveries are addressed in the appropriate sections of this encyclopedia. Molecular biology, which was initially conceived during studies of bacterial viruses, is now being used to study all the viruses of vertebrates and the pathogenesis and epidemiology of animal viral diseases. The expansion in knowledge of these subjects in the next decade can be expected to exceed that of the previous century.

See also: History of Virology: Plant Viruses; History of Virology: Bacteriophages; Nature of Viruses.

Further Reading

Bos L (1995) The embryonic beginning of virology: Unbiased thinking and dogmatic stagnation. *Archives of Virology* 140: 613.

Cairns J, Stent GS, and Watson JD (eds.) (1962) *Phage and the Origins of Molecular Biology.* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory of Quantitative Biology.

Fenner F and Gibbs AJ (eds.) (1988) *Portraits of Viruses. A History of Virology.* Basel: Karger.

Horzinek MC (1995) The beginnings of animal virology in Germany. *Archives of Virology* 140: 1157.

Hughes SS (1977) *The Virus. A History of the Concept.* London: Heinemann Educational Books.

Joklik WK (1996) Famous institutions in virology. The Department of Microbiology, Australian National University and the Laboratory of Cell Biology, National Institute of Allergy and Infectious Diseases. *Archives of Virology* 141: 969.
Matthews REF (ed.) (1983) *A Critical Appraisal of Viral Taxonomy.* Boca Raton, FL: CRC Press.
Porterfield JS (1995) Famous institutions in virology. The National Institute of Medical Research, Mill Hill. *Archives of Virology* 141: 969.
van Helvoort T (1994) History of virus research in the twentieth century: The problem of conceptual continuity. *History of Science* 32: 185.
Waterson AP and Wilkinson L (1978) *An Introduction to the History of Virology.* Cambridge: Cambridge University Press.

Hordeivirus

J N Bragg, H-S Lim, and A O Jackson, University of California, Berkeley, Berkeley, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Hordeiviruses represent a unique genus of serologically related viruses with rigid rods that are composed of 96% protein and 4% RNA. Although members of the hordeiviruses have similar particle structure, they collectively infect both monocots and dicots and exhibit considerable biological diversity. The type member of the group, barley stripe mosaic virus (BSMV), has been known to cause serious worldwide disease problems in cultivated barley for more than 75 years, but during this time has been isolated only infrequently from wheat and wild oats. Because of the yield losses caused in barley, a number of strains of the virus have been isolated and their biological properties evaluated. The survival of BSMV in nature depends solely on direct plant-to-plant contact and seed transmission; consequently, virus spread can be prevented by planting virus-free seed. The advent of sensitive virus detection methods has permitted seed to be screened prior to planting, thus virus-free seed production has resulted in eradication of the virus in most areas of North America and Europe.

In addition to *Barley stripe mosaic virus*, three additional species, *Poa semilatent virus* (PSLV), *Lychnis ringspot virus* (LRSV), and *Anthoxanthum latent blanching virus* (ALBV) have been included in the genus *Hordeivirus* in the *Eighth Report of the International Committee on Taxonomy of Viruses.* This assignment is based on serological relatedness of the coat proteins, genome organization, and sequence relatedness of BSMV, LRSV, and PSLV. The coat proteins of BSMV, PSLV, and LRSV are the same size, but have different mobilities on polyacrylamide gels and are distantly related serologically. Serology and analyses of the coat protein sequences indicate that BSMV and PSLV are more closely related to each other than to LRSV. Serological studies also show that ALBV is closely related to BSMV, and may be a strain of BSMV. However, ALBV has not been investigated by hybridization or sequence analysis to determine whether it is a strain of BSMV or is a distinct virus.

PSLV has been recovered on two occasions from native grasses in Canada from widely separated locations, so the virus may be relatively abundant among members of the Poaceae in Western Canada. LRSV was first isolated from *Lychnis divaricata* seeds imported into the USA, and an additional LRSV strain, Mentha, has been isolated in Hungary. ABLV has been described only in Britain and little is known about its biological or biochemical properties. As is the case with BSMV, LRSV is highly seed transmitted, but seed transmission has not been reported for PSLV or ABLV. There are no known biological vectors for the four viruses, but all reach high concentrations in infected plants and are easily transmitted from plant to plant by mechanical contact.

In addition to their limited range of natural hosts, the experimental hosts of hordeiviruses have been expanded substantially by mechanical inoculation. BSMV has been shown to infect several monocots and a few dicots. Local lesions on *Chenopodium* species have been used for several genetic studies of BSMV disease phenotype, and systemic infections in *Nicotiana benthamiana* have provided a basis for analyses of movement functions and RNA silencing. PSLV also infects numerous species of the Poaceae and several dicots, while LRSV has been shown to infect several experimental dicot hosts, including members of the families. Caryophyllaceae and Labiatae. Reverse genetic systems for BSMV, PSLV, and LRSV have resulted in considerable information about biological properties of the viruses and the molecular biology of infection processes.

Genome Structure and Expression

Hordeivirus genomes are composed of three RNAs that have been designated α, β, and γ based on hybridization patterns of the RNAs. The sizes of the BSMV α, β, and γ genomic (g) RNAs vary between strains, with the ND18 strain sizes being 3.8, 3.2, and 2.8 kb, respectively. RNAs α and β are similar in size among strains of BSMV, but the sizes and complexity of the γ RNAs vary considerably.

The type strain gRNAγ has a 366 nucleotide (nt) sequence duplication at the 5′ end of the genome and comigrates with RNAβ in agarose gels. The Argentina mild strain contains mixtures of three gRNAγ species of 3.2, 2.8, and 2.6 kb. The 3.2 kb RNA has a duplicated sequence similar to that of the type strain, and the 2.6 kb RNA is defective as the result of a deletion in the 3′ terminus of the γa gene. The PSLV and LRSV α, β, and γ gRNAs are similar in size (3.9, 3.6, and 3.2 kb, and 3.7, 3.1, and 2.6 kb, respectively) and sequence to those of BSMV. Strain-specific comparisons are not available for PSLV and LRSV.

Each of the hordeivirus RNAs has a 7-methylguanosine cap at the 5′ terminus, and among the gRNAs within a species, the 5′ untranslated regions (UTRs) that are necessary for replication have very little sequence similarity. Obvious sequence similarity is not evident among the 5′ UTRs of the α and γ RNAs of BSMV, PSLV, and LRSV, but the RNAβ components of the three viruses have considerable sequence similarity. However, the 5′ UTR of LRSV RNAβ is 65 nt longer than that of BSMV or PSLV due to a 5′ terminal extension that is identical to 53 nt at the 5′ end of LRSV RNAα. Interestingly, a 70 nt 5′ UTR recombination between the α and γ RNAs of the BSMV CV17 strain has also been described. These results and the sequence variability exhibited by the Argentina mild strain of BSMV indicate that recombination has played an important role in evolution of the hordeiviruses.

The hordeiviruses gRNAs also contain a conserved 3′ terminal nontranslated sequence that is required for replication. The 3′ termini are variable in length between the different viruses, ranging from 148 nt in LRSV to 330 nt in PSLV. The sequences form tRNA-like structures that in BSMV and PSLV are capable of binding tyrosine *in vitro*. Sequence comparisons indicate that the structures are very similar except that the 3′ UTRs upstream of the conserved tRNA regions of the PSLV and BSMV RNAs have a large stem loop element and an array of possible pseudoknot stem loop regions that make the 3′ UTR longer than that of LRSV. An internal poly(A) sequence of varying length is located directly after the stop codon of the 3′ proximal gene of each RNA, and resides immediately upstream of the conserved 3′ tRNA termini in the BSMV and LRSV genomes. The poly(A) sequence is absent in the PSLV genome.

Hordeivirus gRNAs (**Figure 1**, BSMV) encode seven proteins. The replicase protein subunits are encoded by

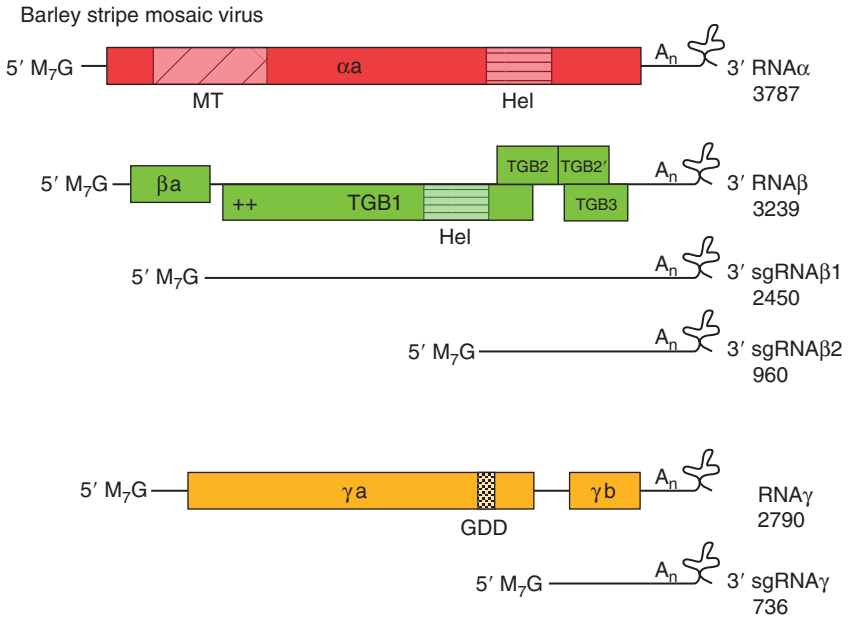


Figure 1 The tripartite genome of BSMV and sgRNAs used for expression of the genes encoded by the β and γ RNAs. Each RNA contains a 5′ cap structure (M_7G), and each 3′ proximal open reading frame (ORF) terminates with a UAA codon initiating an internal polyadenylate sequence (A_n) that precedes the 238 nt tRNA-like terminus. The three genomic RNAs (gRNAs), with ORFs represented by rectangular blocks, are designated α, β, and γ. The αa protein, which is the methyltransferase subunit of the RNA-dependent RNA polymerase (RdRp), is translated directly from RNAα. The αa protein is required for replication and contains methyltransferase (MT) and helicase (Hel) motifs that identify it as the 'helicase subunit' of the viral replicase complex. RNAβ encodes four major proteins. The coat protein, βa, is translated directly from the gRNAβ. The overlapping triple gene block (TGB) proteins, TGB1, TGB2, and TGB3, are each required for virus movement and are expressed from two subgenomic RNAs, sgRNAβ1 and sgRNAβ2, that are illustrated below gRNAβ. TGB1 contains two positively charged regions toward the N-terminus of the protein and an Hel domain, while TGB2 and TGB3 are small hydrophobic transmembrane proteins. RNAγ is bicistronic, and the 5′ ORF encodes the γa protein, which is the GDD-containing polymerase subunit of the RdRp. The cysteine-rich γb protein is involved in pathogenesis.

the α and γ gRNAs. The α RNAs encode a single protein (αa) that contains methyltransferase and helicase domains that are highly conserved between Sindbis-like viruses. The bicistronic γRNAs encode the polymerase (γa) sub unit, which contains the conserved GDD motif found in RNA-dependent RNA polymerase (RdRp) proteins of RNA viruses, plus a 3′ encoded cysteine-rich protein (γb).

RNAβ encodes the coat protein in the first open reading frame (ORF), and is followed by a series of overlapping ORFs termed the 'triple gene block' (TGB) that are also present in allexi-, beny-, carla-, fovea-, peclu-, pomo-, and potexviruses. The first ORF of the TGB encodes the TGB1 protein (formerly designated βb). This protein contains a conserved helicase domain similar to the helicase domain present in the αa protein. The remaining two TGB ORFs encode the small hydrophobic proteins, TGB2 and TGB3 (formerly βd and βc, respectively).

The αa, βa (coat protein), and γa proteins encoded by RNAα, RNAβ, and RNAγ, respectively, are translated directly from the gRNAs (**Figure 1**). Expression of the TGB proteins is mediated by two subgenomic (sg) RNAs, designated sgRNAβ1 and sgRNAβ2. TGB1 is translated from sgRNAβ1, and the other overlapping proteins, TGB2 and 3, are expressed from sgRNAβ2. The γb protein is translated from sgRNAγ. Each of the encoded proteins is expressed at different levels and times during the replication cycle, and the three sgRNAs have similar variances in their abundance during replication. High-level constitutive expression of sgRNAγ occurs throughout the replication cycle, but expression of sgRNAβ1 and sgRNAβ2 is temporal. In addition, sgRNAβ1 is considerably more abundant than sgRNAβ2. The three sgRNA promoters have little obvious sequence relatedness; however, the individual BSMV, LRSV, and PSLV sgRNA promoters share a number of blocks of conserved sequence. Pairwise combinations indicate that the BSMV and PSLV promoters have the highest conservation, whereas the LRSV promoters have undergone more divergence. These results and the protein comparisons described below support the existing evidence for a common origin of the hordeiviruses and buttress biological evidence suggesting that BSMV and PSLV are more closely related to each other than to LRSV. Additional comparisons of the TGB promoter sequences of several other viruses possessing TGB movement proteins have revealed no strong correlations in sequence or secondary structure between these putative promoter sequences and those of BSMV.

Replication of BSMV gRNAs requires the 5′ and 3′ terminal regions, but additional internal *cis*-elements are required for replication of each of the RNAs. RNAα replication is *cis*-preferential, with replication appearing to be coupled to translation of a functional αa protein. Each of the proteins encoded by RNAβ is dispensable for replication; however, the 117 nt intergenic region separating the βa (coat protein) ORF and the TGB1 ORF has a *cis*-acting function required for replication. In contrast, RNAγ replication is not dependent upon the 42 nt intergenic region separating γa and γb, but essential *cis*-acting elements are present in the first 500 nt of the γa gene.

Function and Relatedness of the Hordeivirus Proteins

Replicase Proteins (αa and γa)

BSMV gRNAs expressing αa and γa are able to replicate in protoplasts. The αa and γa proteins are essential subunits of the RdRp and are members of the tobamo-lineage of supergroup III RdRps. A histidine-tagged γa protein recovered from infected barley fractionates with the αa protein. The recovered complex exhibits BSMV RNA-specific polymerase activity and does not contain detectable levels of other BSMV-encoded proteins. Therefore, the αa and γa proteins constitute the helicase and polymerase subunits of the RdRp complex.

The αa protein contains amino-terminal methyltransferase and carboxy-terminal NTPase/helicase domains. The methyltransferase domain likely functions in capping of the viral RNAs and the DEAD box helicase domain consists of at least six amino acid motifs that are conserved among the RdRps of different groups of RNA viruses.

The γa RdRp subunit contains a GDD polymerase motif toward its C-terminus that is characteristic of the supergroup III polymerases, and this domain falls into the 'tobamo-lineage' among the plant viruses. Phylogenetic comparisons of hordeivirus polymerase subunits reveal more than 75% conservation within the genus and lower levels of conservation extend into members of the genera *Pecluvirus*, *Furovirus*, and *Tobravirus* (>65%), and *Tobamovirus* (>30%). Sequence comparisons of the NTPase, helicase, and GDD motifs have revealed significant conservation among the polymerase subunits of Sindbis-like viruses (supergroup III). Among the plant viruses within this group, the conserved domains of BSMV and PSLV are more closely related than those of LRSV, and these sequences also share close relatedness with viruses in the genera *Furovirus* and *Pomovirus*. Lower levels of sequence relatedness are observed when comparisons are extended to the genera *Tobravirus* and *Tobamovirus* and members of the family *Bromoviridae*.

Coat Protein (βa)

The coat protein (CP) is translated directly from gRNAβ and is the most abundant of the viral proteins in infected plants. The CPs of all hordeiviruses are approximately 22 kDa (~200 amino acids) in size, but exhibit different electrophoretic mobilities. The CP interacts with the genomic RNAs to form rod-shaped particles that appear to contain 3 nt per protein subunit, with approximately

24 protein subunits per turn of the helix. In agreement with their serological relatedness, the BSMV and PSLV coat protein sequences are more similar to each other than to the CP of LRSV. Although the CP shares several conserved motifs with other rod-shaped viruses, the hordeivirus CPs are closely related to that of members of the fungal-transmitted virus species *Peanut clump virus* and *Indian peanut clump virus* but show more limited similarity to SBWMV.

Studies of BSMV and PSLV show that the CP is not essential for infectivity of two systemic hosts (barley and *N. benthamiania*), or hosts that form local lesions (*Chenopodium* species). Moreover, infections elicited by CP-deficient BSMV mutants appear to be more aggressive, and the phenotype is more severe and protracted in barley than derivatives expressing the CP. Plants infected with CP deletion mutants remain stunted and appear to lack the recovery phase of infection that is usually observed in barley inoculated with BSMV. These results suggest that the CP is a multifunctional protein that may affect replication and gene expression through a feedback mechanism rather than acting to suppress host RNA silencing.

TGB Movement Proteins

The hordeiviruses encode Class I TGB proteins that differ in several features from the Class II TGB proteins of PVX and other viruses of the family *Flexiviridae*. The coordinated actions of each of the three TGB proteins are required for cell-to-cell and systemic movement. The proteins are expressed transiently and simultaneously during the early stages of infection and decline in abundance at later stages of infection.

TGB1

The TGB1 protein, encoded by the 5′ proximal gene of the TGB, is expressed from sgRNAβ1 and accumulates to high levels early in infection. Among the hordeivirus species, the TGB1 proteins range from 50 to 63 kDa in size. These proteins can be distinguished from the ~25 kDa Class II TGB proteins of the flexiviruses by having substantially larger N-terminal domains preceding the helicase domain. The N-terminal half of the hordeivirus TGB1 proteins contains two positively charged regions rich in lysine and arginine residues, while the C-terminal half of the protein contains an NTPase/helicase domain with seven conserved motifs (I, IA, II, III, IV, V, and VI) that are characteristic of superfamily I helicases of alpha-like viruses. The TGB1 protein has RNA helicase activity *in vitro* that is dependent on Mg^{2+} and ATP and can unwind RNA duplexes in both 5′- to -3′ and 3′- to -5′ directions. The TGB1 protein also binds ATP and dATP, and exhibits ATPase activity that maps to the helicase-containing, C-terminal half of the protein.

The TGB1 protein has RNA-binding activity at high ionic strength and exhibits a higher binding affinity for double-stranded (ds) RNAs than single-stranded (ss) RNAs. RNA sequence specificity has not been detected in binding studies, and TGB1 has little detectable affinity for DNA. The TGB1 proteins of both BSMV and PSLV contain multiple RNA-binding regions, and in PSLV, the N-terminal half of TGB1 containing the positively charged regions is able to bind RNA under high-salt conditions, whereas the C-terminal half containing the helicase motif binds RNA only under low-salt conditions.

A BSMV TGB1/RNA ribonucleoprotein (RNP) complex has been purified from infected barley, demonstrating that TGB1 also binds to RNA *in vivo*. The complex contains positive-sense α, β, and γ RNAs, but additional BSMV-encoded proteins are not present in detectable amounts. However, the TGB1 protein participates in both homologous interactions and in associations with TGB3. The TGB1 protein is membrane associated in infected cells, and visualization of a GFP:TGB1 fusion protein reveals paired foci on opposite sides of the cell wall, suggesting localization at plasmodesmata (PD). When TGB1 is transiently expressed in the absence of TGB2 or TGB3, the TGB1 protein is localized in cytoplasmic membranes. Due to its RNA-binding activities and presence in the RNP complex recovered from infected plants, the TGB1 protein appears to function in formation of viral RNA complexes that are transported to adjacent cells during interactions with TGB2 and 3.

TGB2 protein

Among the hordeivirus TGB proteins, the TGB2 protein contains the highest amount of sequence similarity, and the degree of sequence similarity extends to the class I TGB2 proteins encoded by PVX and other members of the family *Flexiviridae*. The TGB2 sequence contains two hydrophobic stretches and a conserved hydrophilic region that separates the hydrophobic residues. The hydrophobic regions are predicted to integrate into membranes with a U-shaped topology that directs the termini of the proteins to the cytoplasmic side of the membrane, and the strong conserved central portion of the protein is exposed to the ER lumen.

The TGB2 protein is associated with cellular membranes, and associates with the cortical ER and motile vesicles resembling Golgi stacks. In the presence of RNAα and RNAγ, a small portion of BSMV TGB2 fluorescent fusion proteins also associates with chlorophyll-containing vesicles that appear to be chloroplasts or derived from chloroplasts. From a functional perspective, a GFP:TGB2 fusion protein moves through several cells during transient expressions lacking TGB3, suggesting that TGB2 can modify the size exclusion limits of PD.

TGB3 protein

The TGB3 protein is encoded by the 3′ proximal ORF of sgRNAβ2 and is translated by leaky scanning of ribosomes past the TGB2 AUG, which is in a poor context for translation initiation. This mechanism normally produces about a 10:1 ratio of TGB2 to TGB3 during *in vitro* translation. The TGB3 proteins are the most poorly conserved of the TGB proteins, and the class 1 and 2 proteins are thought to have a polyphyletic origin, in contrast to the TGB2 proteins that are thought to have originated from a common ancestor.

The N-terminus of the approximately 17 kDa TGB3 protein from each of the hordeivirus species contains a stretch of conserved amino acids that includes one histidine and three cysteine residues. The TGB3 protein has two hydrophobic domains separated by a hydrophilic region that contains a conserved tetrapeptide (QDLN) sequence. The transmembrane regions are predicted to direct the termini of TGB3 toward the ER lumen. This topology is quite distinct from that of the class II TGB3 proteins, which have only a single membrane-spanning domain that results in location of the C-terminus on the cytoplasmic side of the ER.

When expressed alone, TGB3 localizes to paired peripheral bodies at the cell wall (CW) that appear to lie on opposite sides of the PD channels connecting adjacent cells. The targeting of PSLV TGB3 to the CW requires the central hydrophilic and the C-terminal transmembrane regions, whereas the BSMV TGB3 protein requires the five C-terminal residues, LSSKR, for PD targeting. Furthermore, TGB3 redirects the targeting of TGB1 and 2 to paired peripheral bodies at the CW, and protein interaction studies demonstrate that TGB3 binds with both TGB1 and TGB2.

Pathogenesis Protein (γb)

The 3′ proximal ORF of the hordeivirus γRNAs encodes a cysteine-rich γb protein that ranges in size from 16 to 20 kDa. The hordeivirus γb proteins share some structural similarities, but have little direct amino acid sequence similarity with the cysteine-rich proteins of tobra- and carlaviruses. Studies of the BSMV ND18 strain demonstrate that γb is not strictly required for infectivity in plants, but that it affects pathogenicity, viral RNA accumulation, and expression of genes encoded by RNAβ. Extensive mutational analyses of γb have revealed a number of distinct phenotypes in barley and *Chenopodium* that can ameliorate or exacerbate disease symptoms.

The majority of the cysteine residues in γb are concentrated in the N-terminal half of the protein. These residues are clustered in two zinc-finger-like motifs, designated C1 and C2. A basic motif (BM) rich in lysine and arginine residues is located between the cysteine-rich regions. The BSMV γb protein has been expressed and purified from *Escherichia coli* and shown to bind ssRNAs and zinc *in vitro*. The basic motif mediates RNA-binding activity, and the C1, BM, and C2 regions each show independent zinc-binding activity. A coiled-coil structure near the C-terminus of the γb protein mediates self-interactions that affect pathogenicity.

The PSLV γb protein is targeted to peroxisomes via a C-terminal SKL signal sequence, but deletion of the tripeptide does not affect infection phenotype. This sequence is also found in LRSV and in the BSMV type and China strain γb proteins, but is absent in the ND18 and CV17 strains. The BSMV ND18 γb:GFP fusion protein fluorescence is dispersed throughout the cytoplasm, and subcellular fractionation of infected barley tissue confirms that the majority of the protein is located in the soluble fraction. However, in the presence of the α and γ RNAs, a small portion of the γb protein is associated with vesicles surrounding the nucleus that contain chlorophyll. These associations with chloroplast membranes suggest that the γb protein may have a previously unrecognized role in virus replication or that it may protect viral replication factories against host RNA defense mechanisms.

Several lines of evidence indicate that a major function of γb is to serve as an inhibitor of host defense mechanisms that target RNA replication. A recombinant BSMV derivative that lacks γb expression compromises systemic virus infection and the γb function can be complemented by expression of other known silencing suppressor proteins. Similarly, expression of the γb protein is able to suppress silencing in heterologous virus-induced silencing systems. An agrobacterium-mediated transient silencing assay also provides strong support for a role of γb in suppression of RNA silencing. In this assay, γb interferes with silencing of GFP fluorescence that was induced by expression of a dsRNA. The BSMV γb coiled-coil region is required for silencing suppression in this system, suggesting that homologous interactions of γb are required for suppression activity. These results thus provide a persuasive argument that a major function of γb is to counter host innate RNA interference defenses.

Cytopathology and Replication

Microscopy studies of BSMV-infected barley indicate that most of the visible changes in leaf appearance can be attributed to the disruption of organelles, most notably chloroplasts, in the rapidly expanding cells of the growing leaf. The morphology and shape of chloroplasts are altered drastically during infection as grana become disorganized, and numerous small vesicles appear between the inner and outer membranes of the chloroplast envelope. Reduced amounts of chloroplast ribosomal RNA and decreased rates of synthesis of chloroplast proteins are also noted as BSMV RdRp activity increases. Some

evidence also indicates that chloroplasts or chloroplast membrane vesicles may become reoriented around the nuclei of infected leaf cells. Additional membranes often proliferate and form large globular inclusions of unknown function. Late in infection, virions accumulate to high levels in the cytoplasm in disordered arrays, but a small proportion of rod-shaped particles presumed to be of viral origin accumulate in the nuclei. Biochemical studies undertaken to extend the ultrastructural studies show that BSMV infection inhibits chlorophyll accumulation, and suggest that alterations in photosystem II may result in decreased light absorbance. Reductions in lipids and galactolipids are also correlated with infection and proplastid degradation.

Natural plant-to-plant transmission of hordeiviruses involves mechanical entry of virions into cells. A model for replication posits that virions are disassembled by ribosomes and that RdRp subunits are translated from the α and γ gRNAs (**Figure 2**). Indirect evidence indicates that the polymerase subunits assemble with host components and establish sites of replication on invaginated membrane vesicles originating from chloroplasts or proplastids. Based on dsRNA serological assays, replicating RNAs also appear to be associated with the vesicles. The requirement for translation of a functional αa protein for replication of RNAα raises the hypothesis that the protein may function to recruit RNAγ to sites of replication and initiate vesicle formation. During the replication cycle, the replicase initiates at the 3′ termini of each of the gRNAs during synthesis of dsRNAs, and also initiates sgRNA transcription at internal promoters on the minus strands of RNAβ and RNAγb. Recent results indicate that in the presence of RNAs α and γ, small amounts of TGB2 and the γb protein are targeted to abnormal chloroplast-like vesicles in barley and *N. benthamiana*. These results suggest that the γb protein and the TGB proteins may be recruited to the chloroplast vesicles to interfere with host defenses and to interact with viral RNAs destined for cell-to-cell movement. Serological studies also suggest that small amounts of CP are present in chloroplast-associated vesicles, so it is possible that the CP has regulatory functions related to replication. These findings thus fit a model whereby replication is initiated in vesicles associated with proplastids that mature into chloroplasts.

During the initial stages of replication, the TGB1 movement protein probably functions to unwind and encapsidate positive-sense gRNAs to form nucleoprotein complexes in the vesicles. Biochemical experiments have shown that the resulting TGB1 nucleoprotein complexes can be recovered from infected plants and that TGB1 associates with TGB2 and TGB3. These interactions are thought to mediate transit of the nucleoproteins through the cortical ER to the lumen of the PD. An attractive model postulates that during transit to the PD, the nucleocapsid is associated with vesicles containing RdRp and that these vesicles are targeted by actin to the periphery of the cell. Although cytochalasin D fails to noticeably affect the membrane localization of TGB1, recent work shows that the diameter of actin cables increases significantly in infected cells and in cells expressing TGB2 and TGB3. Transient expression studies with PSLV and BSMV also show that TGB3 forms opposing pairs of punctate foci across PD, so it is plausible that TGB3 interacts with TGB2 to target TGB1 nucleoprotein movement complexes, and possibly replication complexes, through the PD.

In contrast to viruses such as PVX, which contain class II TGB proteins, the coat protein of hordeiviruses is dispensable for cell-to-cell and vascular movement and, therefore, nucleocapsid complexes must have a primary role in movement. During the early phases of cell-to-cell spread, GFP:TGB1 forms brightly fluorescent concentric rings at the advancing infection front and greatly reduced fluorescence behind the front. These results suggest that the TGB proteins are rapidly downregulated after movement, as is the case with TMV where the 30 kDa protein appears to be targeted to the host 26S proteosome for degradation.

Pathogenesis

Natural mutants in both the noncoding and coding regions of BSMV RNAs have been shown to have strain-specific effects on pathogenesis. For example, the ND18 strain is unable to infect oats, whereas the CV42 strain is pathogenic in this host. ND18 virulence in oats can be engineered by altering a single amino acid in the αa protein, so this phenotype may result from relatively simple associations of αa with a host protein. Natural variation among BSMV strains has also facilitated identification of factors affecting seed transmission. Primary determinants influencing the efficiency of seed transmission reside in RNAγ sequences in the 5′ UTR and in the γb gene. Therefore, it appears that RNAγ sequences that are crucial to seed transmission influence replication and have effects that counter host RNA interference mechanisms. In a fourth case of strain-specific virulence, ND18 is able to systemically infect *N. benthamiana*, while the type strain is able to replicate and move in the inoculated leaf but is unable to invade systemically. This aberrant phenotype appears to be caused by the presence of a short ORF in the 5′ UTR that reduces γa translation. Infections with the ND18 or type strains also result in different local lesion phenotypes on *C. amaranticolor*. These differences are complex, but are primarily due to the presence of an amino-terminal 372-nucleotide duplication in RNAγ of the type strain that downregulates expression of γa, and to differences in amino acids of the γb proteins.

Advances in understanding pathogenesis have also been obtained by induction of mutations by reverse

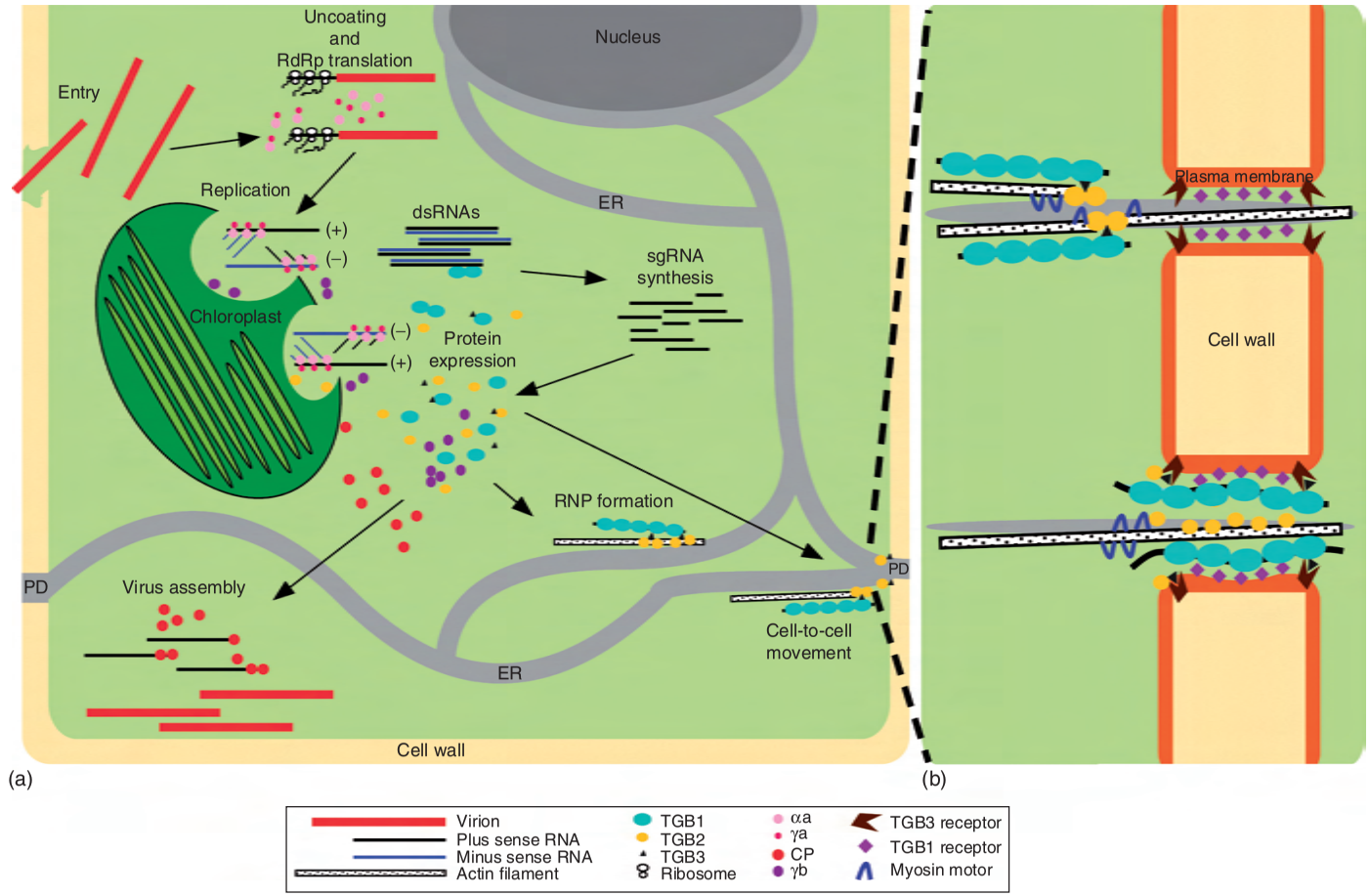


Figure 2 Model of the hordeivirus infection cycle and movement mechanisms. (a) Cycle of replication and formation of movement complexes. Virus particles mechanically enter the cell through breaks in the plant cell wall. Entry is followed quickly by uncoating of the gRNAs in the cytoplasm and translation of the αa and γa proteins to form the RdRp. The RdRp associates with host factors and recruits positive-sense gRNAs to chloroplast membranes where genome replication occurs on invaginated membrane vesicles. Production of positive- and negative-sense gRNAs results in the formation of dsRNA replicative intermedite species. The RdRp also initates transcription at internal promoters located on the negative-strand RNAs to generate sgRNAs that function as messengers for the translation of proteins encoded by the 3′ ORFs of the gRNAs. Small amounts of the TGB2 and γb proteins are also targeted to abnormal chloroplast-like vesicles where they may function in movement and protection against host defenses. The TGB1 protein binds to positive-sense gRNA to create hordeivirus ribonucleoprotein (RNP) movement complexes that associate with the TGB2 and 3 proteins for transport to the plasmodesmata (PD) to facilitate virus cell-to-cell spread. The CP, which is translated directly from gRNAβ, binds positive-sense gRNAs to form progeny virions. (b) Expanded view of events occurring during entry into PD. RNP movement complexes containing TGB1 and positive-sense gRNA associate with the TGB2 and TGB3 integral membrane proteins for transport along actin filaments that are associated with the ER. The TGB3 protein targets the complex to PD and is presumed to interact with host receptor proteins in the PD desmotuble. TGB2 mediates RNP entry into PD, possibly through remodeling of actin filaments within the desmotubule. Host proteins within the PD are predicted to interact with TGB1 to convey RNP complexes to the adjacent cell. The TGB2 and 3 proteins may move through PD along with the RNP, or be recycled by the endocytic pathway to facilitate the movement of additional RNP complexes.

genetics. The early results showed that γb is not strictly required for infectivity in plants, but that it has important pathogenicity effects. For example, null mutations in γb inhibit infections of the BSMV type strain in barley, but the ND18 strain is able to establish systemic infections albeit with an altered symptom phenotype. However, the failure of the type strain to establish systemic infections in the absence of γb can be reversed by mutations that increase the translation of the γa replicase or that remove an amino-terminal extension of the γa protein that may reduce the function of the protein. Individual site-specific mutations within the cysteine-rich region of γb can also result in a variety of phenotypes ranging from striking white regions to necrotic streaks on systemically infected leaves of barley. In addition, plants infected with site-specific γb mutants have secondary effects that culminate in greatly reduced abundance of RNAβ, the CP, and the TGB1 protein. Although the CP is not required for infectivity in barley, *N. benthamiania*, or *Chenopodium* species, infections elicited by CP-deficient BSMV mutants appear to be more aggressive, because recovery is protracted and the symptoms are more severe than in the presence of the CP. These results suggest that in addition to being essential for formation of virions, the coat protein may have a critical role in downregulating kinetics of replication late in infection. Overall, these genetic analyses suggest that complex interactions may affect virulence and disease phenotype, and that the levels of replication may have important effects on movement, host range, and seed transmission.

Natural genes that condition resistance to the ND18, type, and other BSMV strains have been identified in several barley cultivars. The recessive nature of these genes indicates that resistance is due to the absence of a function required for virus replication rather than an active response to infection. The fact that related strains of BSMV can overcome resistance suggests that resistance may result from mutation of a factor required for replication rather than the complete absence of a host factor. This hypothesis is supported by evidence that virus strains targeted by resistance genes are unable to replicate in protoplasts from resistant varieties, whereas the protoplasts fully support the replication of strains able to overcome the resistance genes. Accordingly, single amino acids or short stretches of amino acids in the αa and γa proteins appear to have been targeted by host resistance responses. As more resistance genes are identified, it will be interesting to see whether dominant genes target other functions such as the CP and movement proteins to elicit dominant protective mechanisms.

Hordeivirus Applications to Cereal Genomic Analyses

Virus-induced gene silencing (VIGS) has provided useful approaches to improve our understanding of the functional genomics of dicots, and several virus vectors have been developed for this purpose. Recently, BSMV has been developed into an effective VIGS vector by modifying sequences downstream of the γb gene to enable expression of untranslatable foreign inserts. This strategy was first applied to barley by targeting phytoene desaturase (PDS) for VIGS. Subsequently, applications of VIGS to downregulate genes known to be involved in disease resistance have also enabled identification of genes required for powdery mildew resistance in barley. Extension of the VIGS system to hexaploid wheat has also been used to effectively downregulate PDS, subunit H of the magnesium–protoporphyrin chelatase complex, and the β7 subunit of the 20S proteosome complex. An additional study has identified genes required for rust resistance in wheat. These studies indicate that BSMV provides a powerful and robust system for gene silencing that may have numerous applications for genetic analyses of a large number of cereal crops.

Future Perspectives

The broad host range of hordeiviruses and the information accumulated about virus genes involved in replication, movement, and responses to host resistance afford a rich resource for obtaining insight into the mechanics of infection. Although considerable effort has recently focused on the requirements and interactions of hordeivirus-encoded proteins, very little is known about how virus and host proteins interact to facilitate replication and movement. New genomics findings arising from host sequencing projects, development of advanced tools for analysis of host genes and cell biology, and the ability to modulate expression of individual genes are accelerating the pace of research findings and enabling dissection of individual steps in many of these processes. Of particular importance is characterization of host genes regulated during infection and the effects of these genes on pathogenesis. An understanding of events leading to the establishment of replication factories will provide important information on hordeivirus replication and the interplay between chloroplasts and cytoplasmic elements during the early stages of infection. Additional cell biological analyses of the TGB-encoded proteins and their interactions with host proteins are required to hone our understanding of the cell-to-cell and vascular movement mechanisms that permit systemic virus infections. In addition to enhancing the understanding of how viruses invade vegetative tissue, seed, and pollen, these studies may also provide insights into global aspects of subcellular transport, chloroplast, and PD functions in plants.

See also: Cereal Viruses: Wheat and Barley; Virus Induced Gene Silencing (VIGS).

Further Reading

Boevink P and Oparka K (2005) Virus–host interactions during movement processes. *Plant Physiology* 138: 1815–1821.

Bragg JN, Solovyev AG, Morozov SYu, Atabekov JG, and Jackson AO (2005) Genus *Hordeivirus*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1015–1026. San Diego,CA: Elsevier Academic Press.

Carroll TW (1986) Hordeiviruses: Biology and pathology. In: Van Regenmortel MHV and Fraenkel-Conrat H (eds.) *The Plant Viruses, The Rod-Shaped Plant Viruses,* vol. 2, pp. 373–395. New York: Plenum.

Edwards MC, Bragg JN, and Jackson AO (2005) Natural resistance mechanisms to viruses in barley. In: Loebenstein G and Carr JP (eds.) *Natural Resistance Mechanisms of Plants to Viruses*, pp 465–501. Dordrecht, The Netherlands: Springer.

Holzberg S, Brosio P, Gross C, and Pogue GP (2002) Barley stripe mosaic virus-induced gene silencing in a monocot plant. *Plant Journal* 30: 315–327.

Jackson AO and Lane LC (1981) Hordeiviruses. In: Kurstak E (ed.) *Handbook of Plant Virus Infections and Comparative Diagnosis*, pp. 565–625. Amsterdam: Academic Press.

Jackson AO, Petty ITD, Jones RW, Edwards MC, and French R (1991) Analysis of barley stripe mosaic virus pathogenicity. *Seminars in Virology* 2: 107–119.

Lawrence DM and Jackson AO (1998) Hordeiviruses. In: Foster G and Taylor S (eds.) *Plant Virology Protocols: From Virus Isolation to Transgenic Resistance,* vol. 81, pp. 99–106. Totowa, NJ: Humana Press.

Morozov SYu and Solovyev AG (2003) Triple gene block: Modular design of a multifunctional machine for plant virus movement. *Journal of General Virology* 84: 1351–1366.

Host Resistance to Retroviruses

T Hatziioannou and P D Bieniasz, Aaron Diamond AIDS Research Center, The Rockefeller University, New York, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

APOBECs A family of cytidine deaminases, the prototypic member is a component of apolipoprotein B editing complex.
Restriction factor A cellular protein whose major role is to inhibit retrovirus replication.
Vif Virion infectivity factor encoded by many lentiviruses which induces degradation of some APOBEC proteins.
Vpu Viral protein U, a small transmembrane protein encoded by HIV-1 and its close relatives.
Xenotropic Able to infect cells only from other species.

Introduction

The evolution of organisms and the viruses that colonize them is, obviously, closely linked. Often, pathogenic viruses impose a negative selection pressure on a host that results in the survival of a subpopulation in which infection is resisted, attenuated, or tolerated. In the case of retroviruses, the evolutionary association of virus and host can be exceptionally close, because of the unusual degree to which retroviral infection persists. Retroviral infection is normally lifelong, in part due to the propensity of retroviral genomes to irreversibly integrate into host DNA. Sometimes the targets of retroviral infection include germline cells, and infection of these cells, which generate so-called endogenous proviruses, enables persistence, not only for the host's own lifetime, but also that of its progeny. This persistence and inextricable association of host and virus may allow retroviruses to impose a uniquely sustained selection pressure and may explain why hosts have evolved unique and specific ways for their cells to resist colonization by retroviruses.

In principle, hosts could evolve resistance to the negative consequences of retroviral infection through changes in components of their innate and adaptive immune systems, but it has also become clear that host factors which directly inhibit the ability of retroviruses to replicate in host cells evolve under pressure of retroviral infection. Host resistance to retroviral infection can be acquired in many forms and be manifested at various stages of the retroviral life cycle (**Figure 1**). Of particular note are a collection of autonomously and constitutively active intracellular proteins, termed restriction factors, whose major function appears to be to provide intrinsic immunity to retroviral infection. As such, they are uniquely capable of actively preventing retrovirus replication at the very earliest stages of host colonization. Naturally, some retroviruses have adapted to cellular host resistance in its various forms. Since exogenous retroviruses evolve much more rapidly than their hosts, it seems obvious that they would do so. Nevertheless, some of the obstacles that cells have placed in the path of retroviral replication are not easily escaped by simple mutation, and in some cases retroviruses have learned

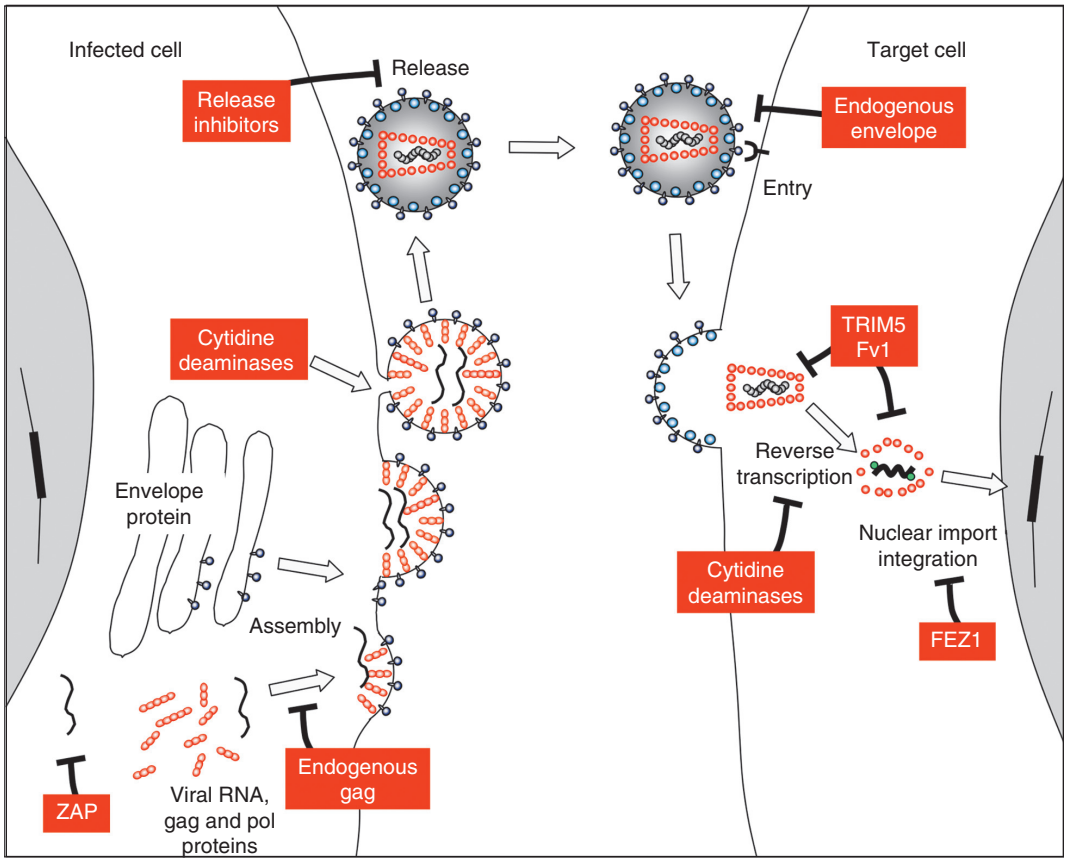


Figure 1 Overview of the retrovirus life cycle and activities encoded by cells that are known to inhibit retrovirus replication.

how to avoid or ablate host restriction factors in interesting and unique ways.

Host Cell Resistance Defined Early in the Retrovirus Life Cycle

Resistance to Retrovirus Entry

Imagine a cell attempting to thwart an infection event by a retrovirus. Optimal strategies would involve aborting the viral life cycle before integration into the genomic DNA and avoiding permanent residence of the retrovirus in the cell and all of its progeny. This could be achieved in numerous ways, an obvious approach being removal or alteration of the receptors that are exploited by retroviruses to enable entry. Several occurrences of this phenomenon are known. A striking example is the occurrence of endogenous murine leukemia viruses (MLVs) that are termed xenotropic because they are unable to replicate in their own hosts. Xenotropism generally occurs as a consequence of mutations that are acquired in the gene encoding the relevant receptor, presumably after colonization of the germline by the xenotropic MLV. Another important example of a receptor mutation that confers resistance to infection is present in humans. In this case a defective allele of the CCR5 co-receptor (*CCR5Δ32*) for HIV-1 exists at high frequency in certain human populations, and individuals that are homozygous for this mutation are resistant to infection by many strains of HIV-1. Although it is quite unlikely that HIV-1 itself is the source of the original evolutionary pressure that led to the dissemination of the *CCR5Δ32* allele, widespread HIV-1 infection in humans would clearly sustain or even increase the prevalence of the *CCR5Δ32* allele in populations where it is present. Other polymorphisms in the human *CCR5* gene also appear to have more modest effects on the acquisition and consequences of HIV-1 infection.

While receptor inactivation could clearly confer resistance to infection by certain retroviruses, some species have acquired or amplified specific genes that actively inhibit retroviral infection at the level of receptor–envelope interaction. Endogenous retroviruses themselves have proved to be a good source of such inhibitors and a few endogenous retroviral genes can efficiently control infection and inhibit the progression of disease caused by exogenous retroviruses. Certain of these preserved endogenous retroviral gene products, for example, Fv4 and Rmcf in mice, are viral envelope glycoproteins that bind and block the receptors shared by exogenous viruses, and constitute especially effective and specific entry

inhibitors. The natural cellular ligands of viral receptors can also provide resistance to infection. For example, the chemokine ligands of CCR5 act as competitive inhibitors of HIV-1 envelope binding and one of the genes encoding a CCR5 ligand (*CCL3-L1*) exists in variable copy numbers in humans. Individuals with higher copy numbers tend to be less likely to acquire HIV-1 infection, and once infected seem less susceptible to its effects. While it is not yet certain that these effects are not immunologically mediated, it seems most likely that the *CCL3-L1* gene product acts as a direct inhibitor of HIV-1 infection and replication in humans.

Post-Entry Resistance Factors Targeting Incoming Retroviruses

In addition to providing a source of receptor-blocking viral envelope proteins, endogenous retroviruses have provided additional factors that restrict retroviral replication. A particularly intriguing restriction factor of endogenous retroviral origin is Friend virus susceptibility factor 1 (Fv1), a protein with about 60% homology to human and mouse endogenous retroviral Gag proteins. *Fv1* was first characterized as a dominant, heritable trait in laboratory mice that conferred resistance to infection by particular MLV strains. The two principal alleles of Fv1 are functionally distinguished by the spectrum of MLV strains to which they confer resistance: The $Fv1^n$ allele renders cells permissive to infection by N-tropic MLV but restricts B-tropic MLV infection, whereas $Fv1^b$ renders cells permissive to B-tropic MLV but resistant to N-tropic MLV. Heterozygous animals that carry both *Fv1* alleles are resistant to both N- and B-tropic MLV strains, while certain MLV strains are not susceptible to restriction by either *Fv1* allele and are known as NB-tropic. Additional *Fv1* alleles that induce partly overlapping patterns of sensitivity/resistance exist, as do inactive variants. While Fv1 restriction is not absolute, it can be very substantial at low multiplicities of infection and Fv1 restriction can have extremely dramatic effects on MLV pathogenesis.

An important feature of Fv1-mediated resistance is that it is saturable and infection by any given virus particle is greatly facilitated by the presence of large numbers of additional restricted virus particles, even if they are inactivated. Most interestingly, the Fv1-imposed block in MLV infection occurs after reverse transcription has been completed but before integration of the provirus in the host genome. The viral determinant governing Fv1 sensitivity is the capsid protein and a single-amino-acid change from arginine to glutamate at position 110 is sufficient to convert an N-tropic MLV strain to B-tropism. Other residues in proximal positions can also affect Fv1 sensitivity and together these data suggest that Fv1 is an inhibitor that inactivates incoming MLV capsids in the cytoplasm of target cells.

Several non-murine mammalian cells, including those from humans, African green monkeys (AGMs), and cows, restrict infection by N-tropic, but permit infection by B-tropic, MLV. Remarkably, this occurs despite the absence of an Fv1-like gene in species other than mice. The characteristics of restriction of N-MLV in these cells are similar to those mediated by Fv1 in mouse cells, that is, restriction can be saturated and amino acid 110 of capsid determines restriction sensitivity. However, in most cases of nonmurine mammalian cells, capsid-specific blocks to infection appear to occur before reverse transcription is completed.

In addition, lentiviruses are also subject to a similar type of post-entry restriction in primate cells. Again, dominant saturable factors are responsible and the viral determinant governing restriction is the capsid protein. Cells from different primate species often exhibit distinct retrovirus restriction specificities. For example, human cells restrict infection by equine infectious anaemia virus (EIAV) but not primate lentiviruses. Conversely, AGM cells restrict multiple primate lentiviruses and EIAV but not simian immunodeficiency viruses (SIVs) naturally found in AGMs. Remarkably, in many cases, restriction of one retrovirus can be abolished by saturation with another even if the two viruses are not closely related, provided that both viruses are restricted in the target cell. For example, in AGM cells, which restrict N-tropic MLV as well as HIV-1, HIV-2, SIVmac, and EIAV, saturation with lentivirus particles can completely abrogate restriction of N-tropic MLV infection. These findings demonstrate the divergence in specificity of mammalian restriction factors but also predict the presence of a single restriction factor capable of recognizing retroviruses whose capsids share little sequence homology.

Indeed, recent work shows that the gene that is largely responsible for the retrovirus restriction properties of primate cells is *TRIM5* and that variation in its sequence can account for most of the variation in species-specific retrovirus restriction properties. Unlike Fv1, TRIM5 has no homology with any known retroviral sequences. Rather, *TRIM5* is one member of a family of dozens of cellular genes that share a similar architecture. Indeed, *TRIM5* itself exists as one of a small cluster of closely related *TRIM* genes on human chromosome 11. Each *TRIM* gene encodes a protein with an amino-terminal tripartite motif, comprised of a RING domain, one or two additional zinc-binding or 'B-box' domains, and a coiled-coil domain (**Figure 2**). The TRIM domain can be linked to one of a number of C-terminal domains, and a single *TRIM* gene can encode several variant proteins with different C-terminal domains as a result of alternative splicing. The α-spliced variant of *TRIM5* gene is responsible for retrovirus restriction and encodes a C-terminal SPRY domain, which is related to domains found in other proteins of diverse organization and function, as well as in

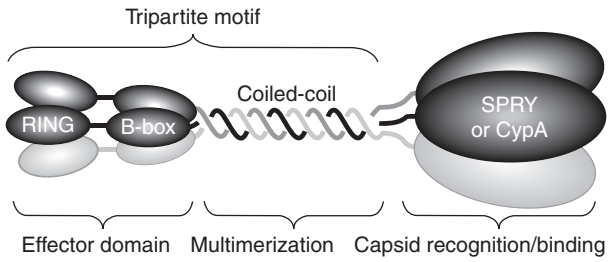


Figure 2 Domain and functional organization of the TRIM5 class of retrovirus restriction factors that block viral infection by targeting incoming capsids. Three major domains that are required for restriction are the N-terminal effector domain, of unknown function, a cenrtal coiled-coil that mediates trimerization, and a C-terminal domain that is responsible for capsid recognition.

several other members of the TRIM protein family. Functional dissection of the TRIM5α protein has revealed that the amino-terminal RING and the B-box sequences comprise a so-called effector domain that is required for restriction but not for recognition of incoming capsids. Conversely, the central coiled-coil is necessary for TRIM5 trimerization while the C-terminal SPRY domain is the principal specificity determinant that governs which retroviruses are inhibited by a given TRIM5α variant. The fact that TRIM5α exists as a trimer suggests a mode of capsid recognition that involves threefold symmetry, since the viral target of TRIM5 consists primarily of a hexameric array of capsid molecules. Moreover, residues on the outer surface of the hexameric capsid lattice of both HIV-1 and MLV have been shown to be important determinants of cell tropism and, in some cases, sensitivity to TRIM5α and Fv1.

The sequences of *TRIM5* genes in various primate species reveal rapid evolution and an excess of nonsynonymous mutations, specifically in the SPRY domain that determines restriction specifically. This finding is consistent with the notion that TRIM5 has been placed under evolutionary pressure by a variable selective force, most likely viral infections. Remarkably, in one particular New World monkey species, namely owl monkeys, a retrotransposition event has resulted in the almost precise replacement of the C-terminal SPRY domain with a cyclophilinA (CypA) domain. The resulting protein, termed TRIMCyp, inhibits HIV-1 and certain other retroviruses whose capsids bind to CypA.

The ability of certain retroviral capsid proteins, particularly that of HIV-1, to bind CypA can influence their sensitivity to TRIM5α as well as TRIMCyp, and there is a complex relationship between CypA:capsid interactions and sensitivity to restriction in various cell types. CypA binds to a flexible loop that is exposed on the surface of the viral capsid and overlaps with viral determinants that govern sensitivity to TRIM5α. The CypA:capsid interaction can be inhibited by drugs such as cyclosporine A (CsA), and treatment of certain human cells with CsA inhibits replication of HIV-1 while lentiviruses whose capsids do not bind CypA are unaffected. While CsA does not appear to render HIV-1 highly sensitive to human TRIM5α, mutations in the CypA-binding site do confer partial sensitivity to it and allow HIV-1 capsids to saturate human TRIM5α. These findings suggest that HIV-1 capsid may have specifically adapted to bind CypA in order to avoid inhibition by restriction factors, like TRIM5α, in humans.

However, the situation is more complex than these findings would suggest. The requirement for CypA for replication in human cells is HIV-1 strain and host cell-type dependent. Indeed, there are several examples of viral strains whose capsid binds CypA but in which disruption of the interaction has no detectable effect on virus replication. This phenotype is conferred by both naturally occurring and *in vitro* selected mutations that occur within the CypA binding loop but do not affect CypA binding. Remarkably, some of these mutations can confer CsA dependence in other human cell types. These differences among human cell types are not due to variation in TRIM5α sequence and suggest the existence of additional capsid-based restriction factors in human cells, whose activity is actually facilitated by the capsid:CypA interaction. Moreover, it appears that HIV-1 restriction by TRIM5α in Old World monkeys is facilitated by CypA:CA interactions.

Generally, the events immediately following entry of a retrovirus into the target cell are not well understood, complicating efforts to determine molecular details of the mechanism by which Fv1 and TRIM5α prevent retroviral infection. Although the hexameric capsid lattice forms the outer shell of the subviral complex that is delivered into the cell, biochemical studies suggest that the capsid shell may be discarded, in a process known as uncoating, shortly after entry. Therefore, there may be a relatively short time frame in which restriction factors such as TRIM5α and Fv1 can initiate events that inhibit infection. Experimental evidence is partly consistent with this notion, and irreversible TRIM5-mediated restriction events can indeed be initiated very rapidly, within minutes of virus entry into the target cell cytoplasm. TRIM5 can interact specifically with capsid proteins of restricted viruses *in vitro*, and direct binding of TRIM5 to capsids is almost certainly the initiating event in restriction. Ensuing events may involve accelerated capsid uncoating and/or degradation. While restriction by TRIM5 is often manifested as apparent inhibition of reverse transcription, this is not a necessary part of restriction since some TRIM5 variants do not affect viral DNA accumulation, nor does Fv1. The known involvement of RING domains in ubiquitin/sumoylation reactions is suggestive of potential effector mechanisms but neither of these pathways

appears to be required for restriction activity, although proteasome inhibition can restore DNA synthesis.

Other Cellular Inhibitors of Incoming Retroviruses

In addition to the intensively studied TRIM5 and Fv1 proteins, cell-type-dependent effects on the early post-entry event in retroviral replication suggest the existence of additional factors that may inhibit incoming retroviruses. For example, certain HIV-1 and HIV-2 strains appear restricted in particular human cells in a manner that depends on both the envelope and capsid. This finding suggests that the route of retroviral entry may affect the sensitivity to unknown capsid-specific restriction factors, although TRIM5 and Fv1 restriction is clearly independent of entry route. Moreover, studies of chemically mutagenized cell lines have revealed that overexpression of fasciculation and elongation protein zeta-1 can be detrimental to retroviral infection. Whether these additional apparent inhibitors of retroviral infection are physiologically relevant and the mechanisms by which they work has not yet been established. However, it is entirely possible that there remain more, and perhaps many, undiscovered inhibitors of incoming retroviruses.

Host Resistance to Infectious Virus Generation

Cells have also evolved alternative antiretroviral activities that act after retroviral DNA has integrated into the host genome. Several inhibitors that act at the level of viral genome accumulation, or affect particle assembly, release, or infectivity are known. While such activities cannot preserve the uninfected state of the cell that expresses them, these intrinsic immune functions can attenuate or prevent the spread of infection to subsequent generations of target cells.

Cellular Cytidine Deaminases as Retrovirus Resistance Factors

One such defense strategy relies on a group of genes, exemplified by *APOBEC3G*, that act primarily by deaminating cytidines in nascent retroviral DNA. *APOBEC3G* is one of a group of genes that exhibit cytidine deamination activity, and other members of this family (e.g., *APOBEC1* and *AID*) are involved in editing of specific cellular mRNAs and genomic loci in order to change their coding potential. APOBEC3G is incorporated into retroviral particles and deaminates nascent viral DNA during reverse transcription in subsequently infected target cells by virtue of its single-stranded DNA-specific deaminase activity (**Figure 3**). Cytidine deamination results in the generation of uracil-containing viral DNA, which likely becomes targeted by DNA repair/degradation enzymes. However, if the viral DNA escapes degradation then uracil is replicated to adenosine during second strand synthesis, resulting in viral DNA with characteristic G-to-A hypermutation. Generally, the burden of G-to-A mutations is high, rendering the proviral DNA incapable of encoding replication-competent progeny.

APOBEC3G is promiscuous with respect to its antiretroviral effect, because it is packaged into diverse retrovirus particles. The mechanism by which this is achieved involves a substantial nonspecific component, whereby APOBEC3G binds in an apparently sequence-independent manner to the RNA that is packaged into virions. Thus, APOBEC3G exploits an essential property of retroviruses to infiltrate particles and therefore makes it difficult for retroviruses to evolve mutations that avoid packaging APOBEC3G into virions. Perhaps because of this difficulty, primate lentiviruses have arrived at an unusual and highly effective solution to the problem imposed on them by APOBEC3G. Indeed, all primate lentiviruses encode an accessory gene, *vif,* whose only function appears to be to counteract APOBEC3G. Vif proteins bind simultaneously to APOBEC3G and to a ubiquitin ligase complex containing Cul5, elongin B, and elongin C to induce ubiquitination of APOBEC3G. Predictably, this leads to proteasome-dependent APOBEC3G degradation, effectively depletes APOBEC3G from the cell, and abolishes incorporation into virion particles. Interactions between APOBEC3G and Vif proteins form the basis of additional species tropism restrictions in primate lentiviruses. Although HIV-1 Vif can efficiently bind to and induce the degradation of human APOBEC3G, it is inactive against its rhesus macaque or AGM counterparts. In contrast, rhesus macaque and AGM APOBEC3G can be recognized by Vif proteins from SIVs found in macaques and AGMs. A single-amino-acid difference between human and monkey APOBEC3G proteins (K128D) is responsible for differential sensitivity to Vif proteins and imparts a second barrier to cross-species transmission for at least some primate lentiviruses. Foamy viruses that are also common in primates have also acquired a gene, Bet, whose function is to exclude APOBEC3 proteins from virion particles. Moreover, other retroviruses appear to avoid the APOBEC3 proteins without encoding accessory genes to exclude it from particles. However, in these cases the molecular mechanism of particle exclusion is not clearly defined.

Like *TRIM5*, the locus containing *APOBEC3G* has been under evolutionary pressure during mammalian speciation, most likely as a consequence of viral infections. Indeed, while mice have only one *APOBEC3* gene, humans have no less than seven that have arisen as a result of *APOBEC3* gene duplication and recombination events.

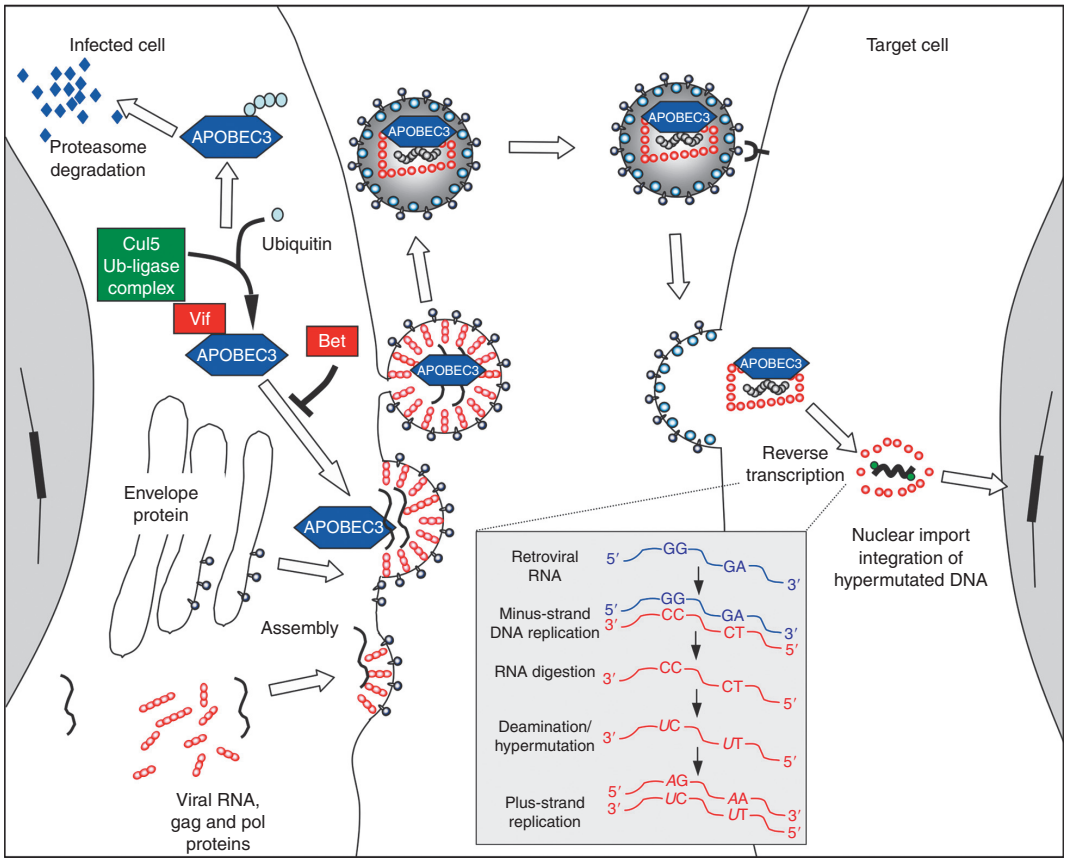


Figure 3 Major mode of action and alternative fates of APOBEC3 proteins that become incorporated into retrovirus particles and catalyzed cytidine deamination during infection of target cells. Some APOBEC3 proteins can be recruited by lentiviral Vif proteins to a ubiquitin ligase complex and degraded in a proteasome-dependent manner. Alternatively, foamy virus Bet proteins prevent incorporation of APOBEC3 into virus particles by unknown mechanisms.

Most of the *APOBEC3* genes, like TRIM5, exhibit excess nonsynonymous polymorphisms in primates, again suggesting evolutionary pressure. Several members of the *APOBEC3* gene cluster exhibit varying levels of activity against numerous retroviruses and retroelements, and some are targeted for degradation by primate lentiviral Vif proteins. In some cases, the APOBEC3 proteins are distinguishable by their substrate preference. For example, APOBEC3G-mutated retroviral DNA carries G-to-A changes primarily in the context of GG dinucleotides, while its close relative, APOBEC3F, preferentially targets GA dinucleotides. In the context of HIV-1-infected humans, APOBEC3G and its close relative APOBEC3F appear to be the major contributors to cytidine deamination-based host defense. Indeed, viral DNA carrying G-to-A hypermutation can be found quite easily in some patients, and the context in which the G-to-A changes occur suggests that both APOBEC3G and APOBEC3F are responsible.

While APOBEC3G and its relatives can clearly inhibit retrovirus replication by inducing catastrophic hypermutation, it may be that lentiviruses have turned its cytidine deamination activity to their advantage and are evolved to only partially resist APOBEC3 proteins. Less dramatic changes in viral DNA sequence may result from partial destruction of APOBEC3G by Vif, and infiltration of viral particles by a small number of APOBEC3 molecules. Indeed, Vif proteins with a range of potencies can be found in HIV-1-infected patients. Primate lentiviruses are capable of extremely rapid sequence divergence, and this is probably required to maintain their characteristic high levels of chronic replication in the presence of strong adaptive immune responses. Certainly, the A-rich nature of their genomes suggests that cytidine deaminases have played a role in influencing the rate and direction of sequence divergence in primate lentiviruses.

While cytidine deamination is thought to be the major mechanism by which APOBEC3 proteins inhibit retrovirus replication, some studies suggest that this class of proteins may also inhibit retrovirus and retroelement replication through mechanisms that do not involve hypermutation. In addition, it may be that inhibition does not require APOBEC packing into retroviral particles and that its presence in the cytoplasm of particular

target cells is sufficient to inhibit the early post-entry steps of the retrovirus life cycle. However, these additional apparent activities of APOBEC proteins are poorly understood at present.

Other Host Resistance Factors That Act during Virus Generation

The genomes of retroviruses appear to be the target of a further host defense activity that likely recognizes newly synthesized RNA. Zinc-finger antiviral protein (ZAP) inhibits the cytoplasmic accumulation of the genomes of some retroviruses and, more impressively, alphaviruses. Its mechanism of action may be related to that of the tristetraprolin-like proteins that it resembles, which induce the degradation of cytokine mRNAs by binding to AU-rich sequences in their 3′ untranslated regions. By doing so, ZAP may recruit viral RNAs to a collection of ribonucleolytic activities referred to as the exosome. ZAP obviously must distinguish between viral and cellular mRNAs but the molecular basis for this distinction is currently unknown.

It is also possible for cells to interfere with the assembly of retrovirus particles. There exists at least one example where defective Gag proteins encoded by an endogenous retrovirus, namely Jaagsiekte sheep retrovirus, can form heteromultimers with and interfere with the assembly of the Gag protein of its exogenous cousin. Yet another intrinsic immune mechanism targets retrovirus particle release. In some human cells, retrovirus particles are released very inefficiently, unless a protein that is expressed by a subset of primate lentiviruses, termed Vpu, is also present. Vpu is not required for the efficient release of retroviruses from some cell lines, but fusion of cells that do or do not require the presence of Vpu for efficient release results in heterokaryons that exhibit the Vpu-dependent virus release phenotype. While many retroviruses do not encode a Vpu protein, some envelope proteins appear to exhibit a similar activity. These findings suggest the existence of a cellular inhibitor of retrovirus release that is counteracted by Vpu or envelope proteins. Because Vpu can facilitate the release of a number of divergent retroviruses, the presumed release inhibitor must target particle release in a rather nonspecific way. A current model that explains many of the effects of Vpu invokes the existence of a release inhibitor that endows cells and/or nascent virions with an adhesive property. Virion–cell adhesion would result in the retention of viral particles on the cell surface, from where they would be subsequently endocytosed. Consistent with this model, retroviral particles generated by cells in which release is inefficient in the absence of Vpu accumulate at the cell surface and later within intracellular endosomes.

Perspectives

Historically, the ability or inability of retroviruses to colonize a particular species or cell type was thought to be defined almost entirely by its ability to parasitize host functions present therein that are necessary to complete its life cycle. However, recent findings have shown that evolution has equipped cells with an often robust and multicomponent defense against retroviruses that seek to colonize them. These activities constitute an equally important determinant of retroviral cell tropism. Studies of restriction factors are in their infancy, but these new insights have given a deeper understanding of the evolutionary relationships between retroviruses and their hosts and what governs the ability of retroviruses to engage in zoonosis. Moreover, the discovery of antiretroviral activities within cells that target pathogenic retroviruses may provide new opportunities for therapeutic intervention.

See also: Equine Infectious Anemia Virus; Foamy Viruses; Human Immunodeficiency Viruses: Pathogenesis; Jaagsiekte Sheep Retrovirus; Simian Immunodeficiency Virus: General Features.

Further Reading

Best S, Le Tissier P, Towers G, and Stoye JP (1996) Positional cloning of the mouse retrovirus restriction gene Fv1. *Nature* 382: 826–829.

Bieniasz PD (2004) Intrinsic immunity: A front-line defense against viral attack. *Nature Immunology* 5: 1109–1115.

Goff SP (2004) Genetic control of retrovirus susceptibility in mammalian cells. *Annual Review of Genetics* 38: 61–85.

Harris RS, Bishop KN, Sheehy AM, *et al.* (2003) DNA deamination mediates innate immunity to retroviral infection. *Cell* 113: 803–809.

Hatziioannou T, Perez-Caballero D, Yang A, Cowan S, and Bieniasz PD (2004) Retrovirus resistance factors Ref1 and Lv1 are species-specific variants of TRIM5{alpha}. *Proceedings of the National Academy of Sciences, USA* 101: 10774–10779.

Sayah DM, Sokolskaja E, Berthoux L, and Luban J (2004) Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. *Nature* 430: 569–573.

Sheehy AM, Gaddis NC, Choi JD, and Malim MH (2002) Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 418: 646–650.

Stremlau M, Owens CM, Perron MJ, Kiessling M, Autissier P, and Sodroski J (2004) The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* 427: 848–853.

Towers G, Bock M, Martin S, Takeuchi Y, Stoye JP, and Danos O (2000) A conserved mechanism of retrovirus restriction in mammals. *Proceedings of the National Academy of Sciences, USA* 97: 12295–12299.

Human Cytomegalovirus: General Features

E S Mocarski Jr., Emory University School of Medicine, Emory, GA, USA
R F Pass, University of Alabama School of Medicine, Birmingham, AL, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Cytomegaloviruses, also known as salivary gland viruses, are widely distributed, species-specific herpesviruses, with an evolutionarily related representative identified in most mammalian hosts when cells of the same species have been used for isolation. Human cytomegalovirus (HCMV), formally called human herpesvirus 5 (HHV-5), infects a majority of the world population by adulthood, but causes acute disease in only a small proportion of immunocompetent individuals. Developing areas of the world typically exhibit widespread transmission early in life, whereas more developed areas show a broader range of patterns. Individuals may escape infection early in life and remain susceptible during the childbearing years. Once rubella was controlled by vaccination, transplacental transmission of HCMV emerged as the major infectious cause of congenital hearing loss preventable by vaccination. HCMV is an opportunistic pathogen associated with disease in immunocompromised hosts, predominating in the settings of genetic or acquired immunodeficiency, allograft tissue and organ transplantation, and pregnancy. Disease pathogenesis requires active viral replication and focuses on different target tissues and organs in different clinical settings, particularly in circumstances where the ability to mount a cellular immune response has been compromised. Transmission of this virus in the general population depends on direct contact with infected bodily secretions.

History and Classification

HCMV is designated formally in the species *Human herpesvirus 5* and belongs to genus *Cytomegalovirus* in subfamily *Betaherpesvirinae* of the family *Herpesviridae*. Human herpesvirus 6 (HHV-6; often considered as two variants, HHV-6A and HHV-6B) and human herpesvirus 7 (HHV-7) belong to the species *Human herpesvirus 6* and *Human herpesvirus 7*, respectively, and represent genus *Roseolovirus* in the same subfamily. All betaherpesviruses replicate slowly in cell culture, remain cell-associated, and exhibit species specificity.

Starting in the early 1930s, the most severe form of HCMV-associated congenital disease, cytomegalic inclusion disease (CID), was recognized by an 'owl's eye' cytopathology in salivary gland, liver, lung, kidney, pancreas, and thyroid autopsy materials from infants. By the early 1950s, CID diagnosis was based on the presence of inclusion-bearing cells in urine, and viral etiology was established by isolation on cultured human fibroblasts. By the early 1970s, the host species specificity of viral replication, widespread distribution of HCMV-like agents in mammals, and tissue and cell type distribution in the diseased host were well recognized. Most importantly, the relationship between transplacental transmission and neurological damage in newborns was established and the social cost of the major sequela, progressive sensorineural hearing loss, placed a high priority on control of this infection through vaccination, which has not been realized to this day. Although primary infection results in the most severe disease with congenital infection, less frequent transmission following recurrent maternal infection nevertheless remains important in the overall epidemiology of disease. Placental infection appears to be considerably more frequent than transplacental transmission, which overall is on the order of 0.5–1% of live births in the USA or Europe. Transplacental transmission is less frequent during recurrent infection (<1% of newborns) than during primary infection (~30–40% of newborns). Young children are a major reservoir of this virus, often responsible for transmitting primary infection in pregnant women.

The second prominent setting of HCMV disease is in the immunocompromised host, and this has driven initiatives for therapeutic intervention. HCMV is associated with various diseases in which T-lymphocyte immune surveillance is compromised. Infection of immunocompetent individuals, ranging from immediately postpartum through adulthood, does not generally lead to significant illness other than occasional (heterophile negative) mononucleosis. A remarkably strong and broad T-cell response, particularly the cytotoxic T-cell response, contributes to lifelong suppression of active virus replication. Immunosuppressive therapy necessary to prevent T-cell-mediated allograft rejection reduces HCMV immunosurveillance, allowing the virus to replicate to levels that lead to various diseases. The incidence of HCMV pneumonia in allogeneic hematopoietic cell transplantation (HCT) and the widespread incidence of HCMV retinitis in acquired immune deficiency syndrome (AIDS) contributed to the development of the antiviral drugs ganciclovir, foscarnet, and cidofovir, and to the later development of the orally administered drug, valganciclovir, as well as ongoing investigation of maribavir. HCMV plays a suspected role in chronic vascular diseases, and this is best illustrated by cardiac allograft vascular disease incidence in heart transplantation and the contribution of this virus to incidence of bacterial and fungal infections in allogeneic HCT settings.

Geographic and Seasonal Distribution

As a ubiquitous virus, HCMV does not show a particular seasonal distribution, although initial acquisition varies with living circumstances. In general, prevalence is more widespread in younger individuals in developing countries than in developed countries, and, within developed countries, it is more widespread in urban than in suburban groups, and low-socioeconomic groups than in high-socioeconomic groups. In a US-based survey, overall HCMV prevalence was approximately 60%, with a higher prevalence in females (64%) than males (54%) and in non-Hispanic blacks (76%) and Mexican Americans (82%) than in non-Hispanic whites (51%). There is a doubling in the prevalence of HCMV infection between childhood and old age, which rises to include 75–85% of the population regardless of demographic considerations. In addition to age and socioeconomic level, early acquisition of HCMV is observed in immigrants and is associated with fewer years of education, large family size, and residence in the southern states.

Host Range and Virus Propagation

Cytomegaloviruses are most readily propagated in fibroblasts cultured from the origin host species, although efficient replication of HCMV in human fibroblasts is facilitated by mutations in the viral genome that reduce the ability to replicate in other host-cell types. Studies on autopsy materials from immunocompromised individuals with acute HCMV disease have shown evidence of virus replication in epithelial, endothelial, macrophage, and dendritic cells. HCMV antigens are also readily detected in nonpermissive peripheral blood (PB) neutrophils of diseased individuals, probably as a result of phagocytosis, and this has become the basis of an important diagnostic assay (antigenemia) specific for a major viral structural antigen (pp65). Fresh clinical isolates replicate efficiently on primary or secondary cultures of epithelial, endothelial, macrophage, and dendritic cells, but this tropism is lost when virus is propagated in fibroblasts due to mutations that accumulate in the viral genome. Extensively passaged laboratory strains acquire a variety of large and small deletions that remove or disrupt tropism genes as well as other genes that are dispensable for replication in cultured fibroblasts. Variants of common laboratory strains, all used under a single name (e.g., AD169, Towne), have arisen via independent propagation since their isolation in different laboratories around the world. Although cellular receptors for HCMV are important determinants of entry into host cells, species specificity and human cell tropism is not determined solely by the distribution of receptors for this virus; nonpermissive fibroblasts from other species generally allow viral attachment and penetration, but are blocked at an early postentry step. An exception appears to be HCMV isolated and adapted to fibroblasts, during which variants arise that lack the ability to enter primary human endothelial cells, epithelial cells, and myeloid cells due to acquisition of mutations.

Genetics and Replication

Betaherpesviruses have linear DNA genomes with direct terminal repeats that contain the *cis*-acting signals for cleavage and packaging of progeny viral DNA during replication. Based on a subset of protein-coding genes conserved across mammalian members of the *Herpesviridae* (the core genes), all herpesviruses likely follow common pathways of DNA replication, DNA encapsidation, and virion maturation. Otherwise, betaherpesvirus genomes exhibit some divergent characteristics. Primate cytomegaloviruses undergo genome rearrangement similar to some alphaherpesviruses. HCMV packages any one of four isomers and has a genome organization that includes a unique long (U_L) component surrounded by a set of inverted repeats (*ab–b′a′*, or TRL–IRL) and a unique short component (U_S) also surrounded by a second set of inverted repeats (*a′c′–ca*, or TRS–IRS) (**Figure 1**). HHV-6 and HHV-7 do not rearrange, and have genomes with a large terminal repeat. Murine or rat CMV genomes also do not rearrange and have terminal repeats that are the smallest (<50 bp) of any characterized herpesvirus.

Genome sequence analysis has revealed evolutionary relationships and codified the classification of herpesviruses. Betaherpesvirus genomes are collinear and exhibit more extensive genetic similarity with each other than with other herpesviruses, where the 40 core genes are arranged in different clusters. Betaherpesviruses encode approximately 170 protein-coding gene homologs, 40 of which are common to alphaherpesviruses and 46 of which are common to gammaherpesviruses. **Figure 1** depicts these gene sets along with an estimate of the overall genomic coding capacity.

The HCMV virion has typical herpesvirus structure, although somewhat larger than alphaherpesviruses or gammaherpesviruses, at 200–300 nm diameter. The 125 nm icosahedral nucleocapsid is composed of five herpesvirus core proteins: the major capsid protein (MCP) comprising the hexons and pentons, the minor capsid protein (mCP or TRI2) together with the minor capsid protein-binding protein (mCP-BP or TRI1) comprising the intercapsomeric triplexes, the small capsid protein (SCP) that decorates the hexon tips, and the portal protein (PORT) that comprises one penton position and is used for encapsidation of viral DNA. The nucleocapsid encloses a single linear molecule of DNA as well as origin of lytic DNA replication (*ori*Lyt)-associated RNA. The nucleocapsid is embedded in a tegument (or matrix) containing at least 27

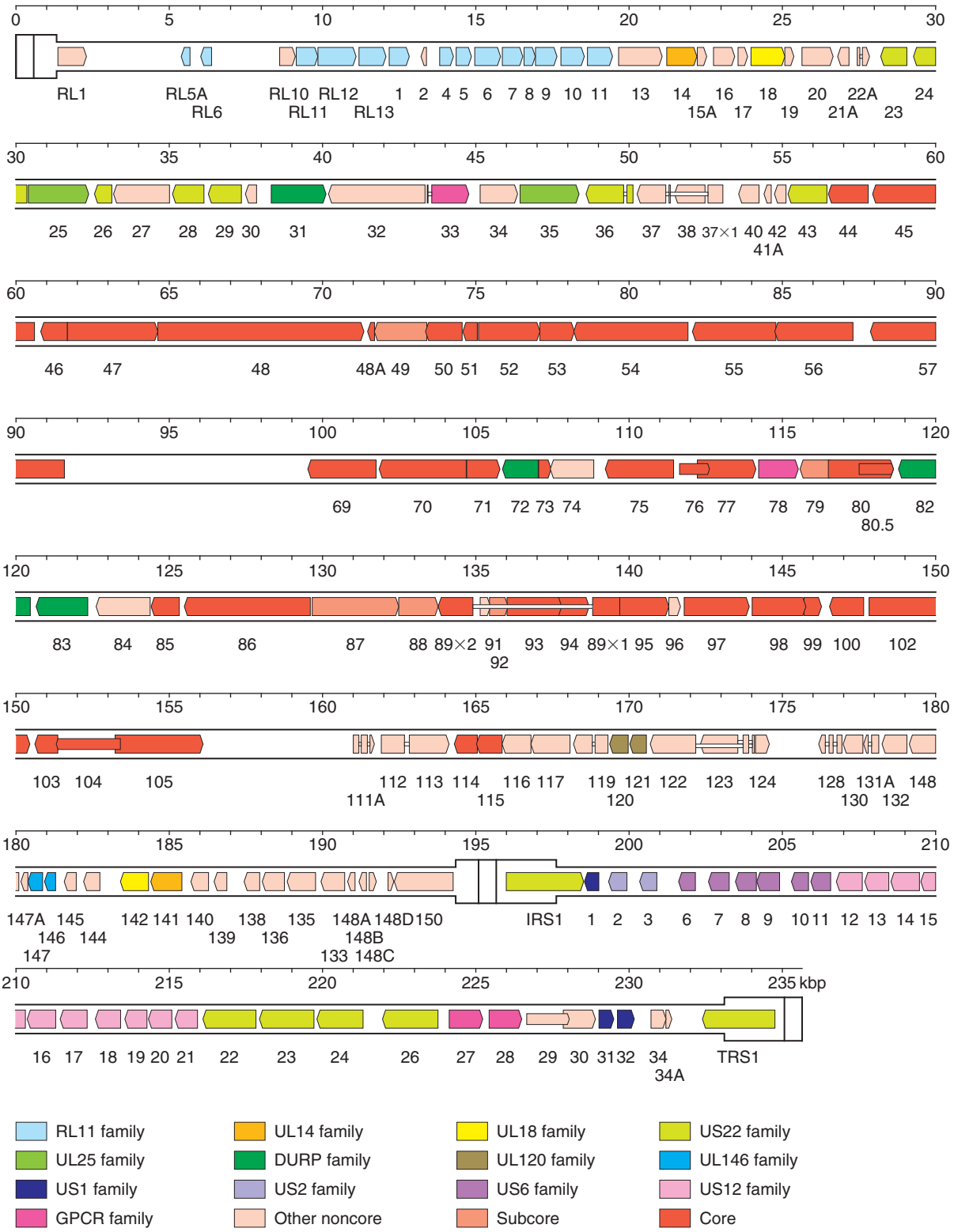


Figure 1 Genetic organization and content of wild-type human HCMV, based on low passage strain Merlin. The inverted repeats TRL/IRL (*ab–b′a′*) and TRS/IRS (*a′c′–ca*) are shown in a thicker format than U_L and U_S. Protein-coding regions are indicated by arrows and gene names are listed below. Introns are shown as narrow bars. Genes corresponding to those in TR_L/IR_L and TR_S/IR_S of strain AD169 are given their full nomenclature, but the UL and US prefixes have been omitted from UL1–UL150 and US1–US34A. Herpesvirus core genes (inherited from the ancestor of the alpha-, beta-, and gammaherpesviruses), subcore genes (inherited from the ancestor of beta- and gammaherpesviruses), other unique genes (homologs present only in betaherpesviruses), and members of families of related genes are color-coded as depicted in the key. Adapted from Dolan A, Cunningham C, Hector RD, *et al*. (2004) Genetic content of wild type human cytomegalovirus. *Journal of General Virology* 85: 1301–1312, with permission from Society for General Microbiology.

relatively abundant virus-encoded phosphoproteins as well as many more proteins and RNAs that are present in small amounts. About a dozen herpesvirus core as well as a dozen betaherpesvirus-specific structural proteins are considered tegument components. These appear to be involved in the earliest steps of infection and uncoating and release of the viral genome into the nucleus, as well as at late stages of replication for encapsidation of progeny viral DNA, capsid egress from nuclei, and, importantly, final envelopment and egress from the cytoplasm. Tegument proteins also modulate events in the host cell to block intrinsic resistance mechanisms that impede viral replication. The tegument is surrounded by a lipid bilayer envelope derived from cellular endoplasmic reticulum (ER)–Golgi intermediate compartment (ERGIC) membranes and into which some 20 or more virus-encoded glycoproteins are inserted. Unlike other herpesviruses that encode subgroup-specific envelope glycoproteins central to entry, the most prominent HCMV envelope glycoproteins are herpesvirus core gene products. These are known by common nomenclature as glycoprotein (g)B, gH:gL, and gM:gN, and all play essential replication functions. None of the numerous additional glycoproteins encoded by HCMV are essential for replication in cultured fibroblasts. Overall, HCMV is the most structurally complex herpesvirus, reflecting the large number of gene products encoded by this virus. During infection in cell culture, HCMV produces an abundance of noninfectious particles, including dense bodies that lack a nucleocapsid as well as other defective, capsid-containing particles. These particles constitute at least 99% of virion preparations, producing particle-to-PFU ratios of 100 or greater.

Complete or largely complete genome sequences have been determined for a number of laboratory and low-passage HCMV strains. All HCMV strains exhibit an average of >95% DNA sequence identity, although large deletion mutations are present in many highly propagated strains. Wild-type HCMV is estimated to carry a minimum of 166 protein-coding genes, the great majority of which are conserved in the closest relative of HCMV, chimpanzee CMV. A liberal upper estimate of 252 ORFs has been suggested. In addition to protein-coding genes and potential miRNA-like elements, the HCMV genome has three types of resident signals that act to control gene expression, DNA replication, and DNA packaging during replication. The genes on the viral genome are controlled by promoter-regulatory signals that are recognized by host-cell RNA polymerase II and influenced by viral regulatory proteins, and are expressed in a temporal cascade that initiates following penetration of the host cell.

Entry into cells depends upon envelope glycoproteins and release of virion DNA into the nucleus depends upon tegument proteins. Entry initiates with a series of distinct steps involving (1) binding to specific cell surface receptors, (2) fusion of the virion envelope with the cellular membrane to release nucleocapsids into the cytoplasm, (3) nucleocapsid association with cytoskeletal elements and translocation toward the nucleus, (4) nucleocapsid interaction with nuclear pores, and (5) release of the viral genome into the nucleus (**Figure 2**). Expression of the immediate early (IE) class of viral genes follows entry and is influenced by virion tegument proteins (virion transactivators). IE genes include viral functions that regulate gene expression by activating transcription and suppressing host repression systems, cell death, and major histocompatibility complex class I gene expression. As in all herpesviruses, different temporal classes of genes are interspersed across the HCMV genome. The IE genes are not clustered and map to diverse locations in the U_L, U_S, and IR_S/TR_S regions of the viral genome. Expression of the next set of genes, the delayed early (DE) genes, depends on the expression of regulatory IE gene products. DE genes control the initiation of viral DNA replication and a wide range of host-cell modulatory functions. Viral DNA synthesis initiates on the viral genome at oriLyt and relies on six core virus-encoded DNA synthesis proteins well as additional functions that facilitate this process. Expression of late (L) genes occurs last and includes a majority of virion structural proteins, a great many of which are herpesvirus core functions. DE and L gene classes also include as many as 100 immunomodulatory functions that impact the host cell and the immune response of the host, only a fraction of which have been studied (**Table 1**).

The replication cycle of HCMV is slow, requiring 48–72 h to begin the release of progeny, with a switch from E to L phase being delayed until 24–36 h post infection (hpi) and maximum levels of virus release starting at 72 –96 hpi. The basic features of HCMV capsid formation and maturation are likely to be common to all herpesviruses. Encapsidation of viral DNA is under the control of a set of viral proteins that package and cleave viral DNA molecules into preformed capsids. Seven herpesvirus core encapsidation proteins likely act as a complex to process a concatemeric DNA template via cleavage/packaging (*pac*) sites located within the terminal *a* sequences, and following the insertion of genome-length viral DNA, cleave to produce a filled nucleocapsid. The nucleocapsid follows a complex two-stage envelopment and egress process that starts in the nucleus and leads to virion release by exocytosis at the plasma membrane. This process depends upon many core herpesvirus functions and starts with primary envelopment at the inner nuclear membrane followed by a de-envelopment event at the outer nuclear membrane, a process that releases the nucleocapsid into the cytoplasm (**Figure 2**). Secondary envelopment occurs in the cytoplasm at ERGIC membranes with the resulting vesicles transporting mature virions to the cell surface using the cellular exocytic pathway. Dense bodies form in the cytoplasm,

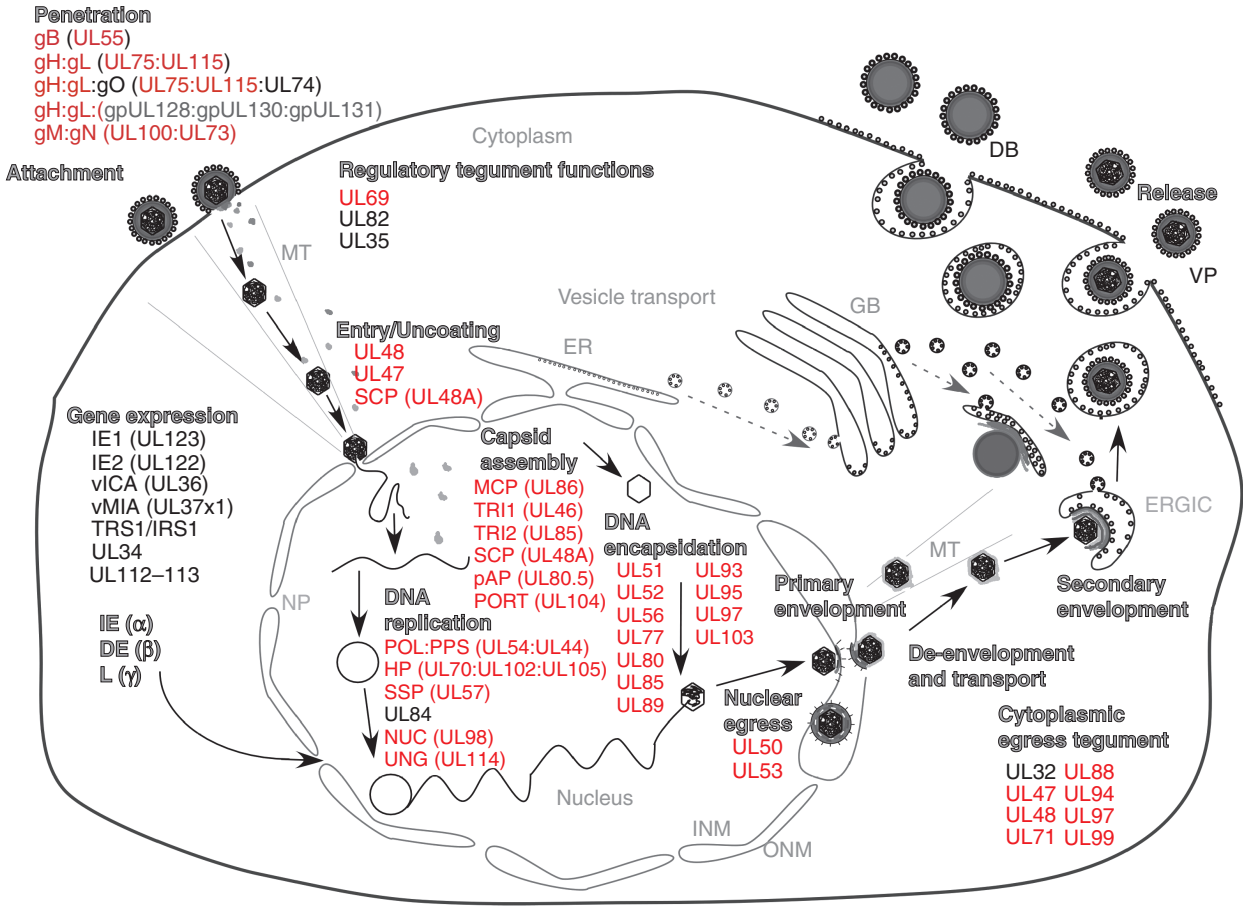


Figure 2 Summary of the HCMV replication pathway with focus on herpesvirus core (red) and betaherpesvirus-conserved (black) gene products playing known or predicted roles in replication. Major steps in productive replication are indicated in large gray outlined font, and black arrows indicate the procession of steps with individual functions identified by abbreviated names. The entry pathway shown employs direct fusion at the plasma membrane (attachment and penetration) although an endocytic pathway may also be important in certain cell types. Entry requires gB, gH:gL, possibly with gO, or gpUL128-gpUL130-gpUL131, and gM:gN.Virion tegument functions (UL69, UL82, UL35) are involved in regulation. UL47, UL48, and SCP are predicted to mediate transport on microtubules, docking at nuclear pores, and release of virion DNA from the capsid into the nucleus. Transcriptional regulation of viral and host-cell gene expression is mediated by IE genes (IE1, IE2) or DE genes (UL34, UL35, UL112-UL113). Cell death suppression is mediated by IE gene products vICA and vMIA. DNA replication depends on several core proteins (POL:PPS, HP, SSB, NUC, and UNG) as well as one betaherpesvirus-specific function (UL84). Capsid assembly uses core functions MCP, TRI1:TRI2, SCP, PORT, and pAP. Preformed capsids likely translocate to sites of DNA replication where several core proteins (UL51, UL56, UL77, UL80.5, UL85, UL89, UL93, UL95, UL97, UL103 gene products) are likely to be involved in encapsidation of viral DNA. Nuclear egress is likely to be controlled by UL50 and UL53 gene products, which are core proteins. Cytoplasmic egress (black arrows) and secondary (final) envelopment are controlled by core functions (UL47, UL48, UL71, UL88, UL94, UL97, UL99 gene products) as well as one betaherpesvirus-specific protein (UL32 gene product). Nucleocapsids are likely transported on microtubules and virion envelope glycoproteins follow vesicle transport to sites of final envelopment in the cytoplasm. Golgi body (GB), microtubules (MTs), and ER and ERGIC, as well as both virus particle (VP) and dense body (DB) final release steps are identified in the cytoplasm. Nuclear pores (NPs), inner nuclear membrane (INM), and outer nuclear membrane (ONM) are identified in the nucleus. The cellular vesicle transport pathway from the ER to GB is also designated (dashed gray arrow).

following only the cytoplasmic steps (**Figure 2**). Once maturation starts, infected fibroblasts continue to produce virus at peak levels for several days, depending on the viral strain and constellation of cell-death suppressors encoded by the strain. Based on systematic mutagenesis, about 80 viral genes play important roles in replication, with roughly 45 being essential for replication in fibroblasts.

Evolution

HCMV has a 236 kbp genome and the capacity to encode in excess of 166 gene products, including several gene families (**Figure 1**). This is considerably more than HHV-6 and HHV-7, whose smaller genomes (145–162 kbp) encode an estimated 84 or 85 gene products. Cytomegaloviruses from a wide variety of host species conserve the same 70

Table 1 HCMV gene characteristics and functions.

HCMV[a,b]	*HCMV gene family/gene name*[c]	*Function/comments/abbreviation*[d]
RL1		
RL5A	RL11 fam	
RL6	RL11 fam	
RL10	Virion env-gp	
RL11	RL11 fam; IgG Fc-binding glycoprotein	Modulation of antibody activity
RL12	RL11 fam; put mem-gp	
RL13	RL11 fam; put mem-gp	
UL1	RL11 fam	
UL2	Put mem	
UL4	RL11 fam; env-gp	
UL5	RL11 fam; virion envelope protein?	
UL6	RL11 fam; put mem-gp	
UL7	RL11 fam; put mem-gp	
UL8	RL11 fam; put mem-gp	
UL9	RL11 fam; put mem-gp	Temperance
UL10	RL11 fam; put mem-gp	Temperance
UL11	RL11 fam; mem-gp	
UL13	Put sec	
UL14	UL14 fam; put mem-gp	
UL15A	Put mem	
UL16	Mem-gp	Inhibits NK cell cytotoxicity via NKG2D ligands MICA and ULBPs; temperance
UL17		
UL18	UL18 fam; put mem-gp; MHC class I homolog	LIR-1 ligand
UL19		
UL20	T-cell receptor γ chain homolog	
UL21A[¥]		
UL22A	Virion env, sec-gp	CC chemokine-binding protein (also called UL21.5); possibly temperance
UL23	*US22 fam; teg*	*Temperance*
UL24	*US22 fam; teg*	
UL25	*UL25 fam: teg*	*Temperance*
UL26[¥]	*US22 fam; teg*	*Activator of major IE promoter; regulates teg phosphorylation*
UL27		*Maribavir resistance*
UL28[¥]	*US22 fam*	
UL29[¶]	*US22 fam*	*Temperance*
UL30[¥]		
UL31[¶]	*dUTPase-related protein (DURP) fam*	
UL32[†]	*Major teg-pp (pp150); highly immunogenic*	*Binds to capsids, cytoplasmic envelopment of virions*
UL33x1/ UL33x2	*GPCR fam, virion env*	*Constitutive signaling*
UL34[†]		*Represses US3 transcription*
UL35[¶]	*UL25 fam; teg-pp*	*Interacts with UL82 protein; regulates virion transactivation and assembly*
UL36x1/ UL36x2	*US22 fam; IE, teg; inhibitor of caspase-8-induced apoptosis*	*Cell death suppression (vICA)*
UL37x1[¶]	IE; mitochondrial inhibitor of apoptosis	Cell death suppression (vMIA)
UL37	*IE glycoprotein*	*Gene regulation, vMIA domain at amino terminus*
UL38[¶]	*Mem-gp*	*Cell death suppression*
UL40	Mem-gp	Signal peptide binds HLA-E to inhibit NK cell cytotoxicity via CD94:NKG2A
UL41A	Virion env-gp	Also called UL41.5
UL42	Put mem	
UL43	*US22 fam; teg*	
UL44[†]	*(core) DNA polymerase processivity subunit*	*Increases DNA pol product length (PPS)*
UL45	*(core) Teg*	*Large subunit ribonucleotide reductase homolog (enzymatically inactive); virion protein (RR1)*
UL46[†]	*(core) Capsid*	*Component of capsid triplexes (minor capsid binding protein; TRI1)*
UL47[¶]	*(core) Teg*	*Intracellular capsid transport; binds to UL48 protein? (LTPbp)*

Continued

Table 1 Continued

HCMV[a,b]	*HCMV gene family/gene name*[c]	*Function/comments/abbreviation*[d]
UL48†	*(core) Largest teg*	*Intracellular capsid transport? (LTP)*
UL48A†	*(core) Smallest capsid*	*Located on tips of hexons in capsids; capsid transport? (SCP) (also called UL48.5)*
UL49†		
UL50†	*(core) Inner nuclear mem*	*Nuclear egress of capsids; virion protein? (NEMP)*
UL51†	*(core) Terminase component*	*DNA packaging (TER3)*
UL52†	*(core)*	*Capsid transport in nucleus*
UL53†	*(core) Teg*	*Nuclear matrix protein; capsid egress; nuclear egress lamina protein*
UL54†	*(core) DNA polymerase*	*DNA polymerase catalytic subunit (POL)*
UL55†	*(core) Virion env-gp B (gB)*	*Homomultimers; heparan-binding; role in entry and signaling*
UL56†	*(core) Terminase component*	*Binds to DNA packaging motif, exhibits nuclease activity (TER2)*
UL57†	*(core)*	*Single-stranded DNA-binding protein (SSB)*
ori Lyt†		*DNA replication origin for productive infection (cis-acting); positional conservation, sequence divergence in betaherpesviruses.*
UL69¥	*(core) Multiple regulatory protein; teg*	*Tegument protein; contributes to cell cycle block; exhibits nucleocytoplasmic shuttling; promotes nuclear export of unspliced mRNA (MRP)*
UL70†	*(core) DNA helicase primase subunit*	*Unwinding DNA, primase homology (HP2)*
UL71†	*(core) Teg*	*Cytoplasmic egress*
UL72	*(core) DURP fam*	*Deoxyuridine triphosphatase homolog (enzymatically inactive) (dUTPase)*
UL73†	*(core) Virion env-gp N (gN)*	*Complexes with gM; entry*
UL74¶	*Virion env-gp O (gO)*	*Complexes with gH:gL*
UL75†	*(core) Virion env-gp H (gH)*	*Associates with gL; complexes with gO or UL128-UL130-UL131; entry role*
UL76†	*(core) Virion-associated regulatory*	
UL77†	*(core) Portal capping*	*DNA packaging (PCP)*
UL78	*GPCR fam; put chemokine receptor, env-gp*	
UL79†		
UL80†	*(core)*	*Precursor of maturational protease (PR; N terminus) and capsid assembly (scaffold) protein (AP; C terminus)*
UL80.5†	*(core)*	*Precursor of capsid assembly (scaffold) protein (pAP)*
UL82¥	*DURP fam; teg-pp (pp71; upper matrix)*	*Virion transactivator; ND10 localized; degrades Daxx*
UL83	DURP fam; major teg-pp (pp65; lower matrix)	Suppresses interferon response
UL84†	*DURP fam*	*Role in organizing DNA replication; exhibits nucleocytoplasmic shuttling; binds IE2*
UL85†	*(core) Capsid*	*Component of capsid triplexes (minor capsid protein; TRI2)*
UL86†	*(core) Major capsid*	*Component of hexons and pentons (MCP)*
UL87†		
UL88¶	*(core) Teg*	*Cytoplasmic egress*
UL89x1†/ *UL89x2*	*(core) Terminase component*	*ATPase subunit (TER1)*
UL91†		
UL92†		
UL93†	*(core) Teg*	*Capsid transport*
UL94†	*(core) Teg*	*Binds single-stranded DNA; cytoplasmic egress*
UL95†	*(core)*	*Encapsidation chaperone protein*
UL96†	*Teg*	
UL97¶	*(core) Viral serine-threonine protein kinase; teg*	*Phosphorylates ganciclovir; inhibited by maribavir; roles in DNA synthesis, DNA packaging and nuclear egress; mimics cdc2/CDK1 (VPK)*
UL98†	*(core) Deoxyribonuclease*	*(NUC)*
UL99†	*(core) Myristylated teg-pp pp28*	*Cytoplasmic egress tegument protein*
UL100†	*(core) Virion env-gp M (gM)*	*Complexes with gN*
UL102†	*(core) DNA helicase primase subunit*	*Unwinding DNA (HP3)*
UL103¶	*(core) Teg*	*Nuclear egress*
UL104†	*(core)*	*Portal protein; DNA encapsidation (PORT)*
UL105†	*(core) DNA helicase primase subunit*	*Unwinding DNA; helicase homology (HP1)*
UL111A	CMV interleukin 10	(cmvIL-10) and latency-associated (LA) vIL-10

Continued

Table 1 Continued

HCMV[a,b]	*HCMV gene family/gene name*[c]	*Function/comments/abbreviation*[d]
UL112[¥]/ *UL113*[¥]		*Transcriptional activation, orchestration of DNA replication*
UL114[¶]	*(core) Uracil-DNA glycosylase*	*Roles in excision of uracil from DNA and temporal regulation of DNA replication (UNG)*
UL115[†]	*(core) Virion env-gp L (gL)*	*Associates with gH; complexes with gO or UL128-UL130-UL131; entry*
UL116	Put mem-gp	
UL117[¥]		
UL119	IgG Fc-binding glycoprotein; virion *env-gp*	Mem related to OX-2; modulation of antibody activity
UL120	UL120 fam; put mem-gp	
UL121	UL120 fam; put mem-gp	
UL122[†]	*IE2 transactivator*	*Interacts with transcriptional machinery; repression via specific DNA-binding activity; cell cycle modulation (IE2)*
UL123[¥]	*Major immediate early 1 co-transactivator*	*Enhances activation by IE2; indirect effect on transcription machinery; binds HDACs; disrupts ND10 (IE1)*
UL124	*Mem-gp, latent protein*	
UL128	Put sec	Endothelial and epithelial cell tropism, env-gp complexes with gH:gL
UL130	Put sec	Endothelial and epithelial cell tropism, envelope gp complexes with gH:gL
UL131A	Put sec	Endothelial and epithelial cell tropism; envelope gp complexes with gH:gL
UL132[¶]	Virion env-gp	Temperance
UL148	Put mem-gp	
UL147A	Put mem	
UL147	UL146 fam; put sec-gp;	Put CXC chemokine
UL146	UL146 fam; sec-gp	hCXCR2-specific CXC chemokine
UL145		
UL144	Mem-gp; TNF receptor homolog	Regulates lymphocyte activation via BTLA
UL142	UL18 fam; put mem-gp; MHC class I homolog	Inhibits NK cytotoxicity
UL141	UL14 fam; mem-gp	Inhibits NK cell cytotoxicity by downregulating CD155 (CD226 ligand)
UL140	Put mem	Inhibits NK cytotoxicity
UL139	Put mem-gp	
UL138	Put mem	
UL136	Put mem	
UL135	Put sec	
UL133	Put mem	
UL148A	Put mem	
UL148B	Put mem	
UL148C	Put mem	
UL148D	Put mem	
UL150	Put sec	
IRS1	*US22 fam; IE protein; teg*	*Transcriptional activator, blocks PKR-mediated shutoff of translation*
US1	US1 fam	
US2	US2 fam; mem-gp	Degradation of MHC class I and possibly MHC class II
US3	US2 fam; IE gene; mem-gp	Inhibits processing and transport of MHC class I and possibly MHC class II
US6	US6 fam; put mem-gp	Inhibits TAP-mediated ER peptide transport
US7	US6 fam; mem-gp	
US8	US6 fam; mem-gp	Binds to MHC class I
US9	US6 fam; mem-gp	Cell-to-cell spread
US10	US6 fam; mem-gp	Delays trafficking of MHC class I
US11	US6 fam; mem-gp	Selective degradation of MHC class I
US12	US12 fam; put 7TM, GPCR?	
US13[¶]	US12 fam; put 7TM, GPCR?	
US14	US12 fam; put 7TM, GPCR?	
US15	US12 fam; put 7TM, GPCR?	
US16	US12 fam; put 7TM, GPCR?	Temperance
US17	US12 fam; put 7TM, GPCR?	Nuclear, fragmented
US18	US12 fam; put 7TM, GPCR?	
US19	US12 fam; put 7TM, GPCR?	Temperance
US20	US12 fam; put 7TM, GPCR?	
US21	US12 fam; put 7TM, GPCR?	

Continued

Table 1 Continued

HCMV[a,b]	*HCMV gene family/gene name*[c]	*Function/comments/abbreviation*[d]
US22	*US22 fam; teg*	*Released from cells*
US23[¶]	US22 fam; teg	
US24	US22 fam; teg	
US26[¥]	*US22 fam*	
US27	GPCR fam; virion env-gp	
US28	GPCR fam; env-gp	CC and CX3C chemokine receptor
US29	Put mem-gp, necessary in RPE cells	
US30	Put mem-gp	Temperance
US31	US1 fam	
US32	US1 fam	
US34	Put sec	
US34A	Put mem-gp	
TRS1[¶]	*US22 fam; IE protein; teg pp*	*Transcriptional activator; blocks PKR-mediated shutoff of translation*

[a]Commonly annotated ORFs of HCMV (see **Figure 1**). Based on the replication properties of HCMV mutant viruses, most genes are dispensable in cell culture (normal type). Viruses with mutations that influence replication efficiency exhibit one of three broad growth deficient phenotypes: failure to replicate (†), very poor replication (¥), or slight replication defect (¶) when assayed in human fibroblasts. Mutations of putative HCMV ORFs UL60 and UL61 are interpreted as disrupting *ori*Lyt rather than any protein-coding ORF. Certain mutant virus phenotypes vary depending on viral strain and strain variant tested.

[b]Betaherpesvirus-common genes are in italics, and include 40 herpesvirus core functions, labeled as 'core'. Gene family, characteristics, and functional information are provided for HCMV and a blank indicates the lack of information.

[c]Abbreviations: fam, family; pp, phosphoprotein; teg, tegument; gp, glycoprotein; mem-gp, membrane gp; env-gp, envelope gp, TM-gp, transmembrane gp; sec, secreted; put, putative; GPCR, G-protein-coupled receptor; 7TM, seven transmembrane-spanning; IE, immediate early.

[d]Functional information is speculative in some cases.

betaherpesvirus-common genes, as do HHV-6 and HHV-7. Betaherpesviruses exhibit the greatest divergence observed in the herpesviruses, with even the most closely related primate cytomegaloviruses exhibiting differences in gene content and different CMV strains containing a subset of strikingly variable genes. Human roseoloviruses and HCMVs lack detectable DNA sequence homology. The deduced protein-coding capacity of cytomegaloviruses is also highly diverged, although a set of betaherpesvirus-conserved proteins emerges from bioinformatic analysis of potential protein-coding regions and greater levels of conservation are observed in cytomegaloviruses of closely related host species. The diversity of genes observed in biologically related viruses may be a product of immune clearance pressure through co-evolution with particular hosts. Each betaherpesvirus appears to have co-evolved with its host species and exhibits little genetic evidence of cross-species transmission.

Pathogenesis and Latency

HCMV transmission is via contact with virus-positive body fluids and requires direct contact with infectious material, as is the case with most human herpesviruses. HCMV is shed for prolonged periods of time in urine, saliva, tears, semen, and cervical secretions, as well as in breast milk. HCMV transmission rates are highest where contact with body fluids occurs, such that HCMV is an important sexually transmitted disease as well as an infection that is transmitted among children in day-care centers and from young children to parents and other care providers. Thus, two distinct types of exposure are associated with horizontal transmission of HCMV: sexual activity among adults and direct contact with urine or saliva from young children. Children in day-care centers, who frequently transmit virus to each other through casual contact, continue to shed virus in saliva and urine for years without symptoms. Day-care workers have markedly increased rates of HCMV infection as do parents of young children who attend day-care centers, where transmission rates of 10% or greater per year have been reported. Hygiene is thought to play a role in transmission, and regular hand washing has been recommended to reduce transmission. Although shedding of HCMV is common among hospitalized individuals, medical-care professionals who are known to use good hygiene practices do not exhibit any increased risk of HCMV infection. Horizontal transmission may also occur through blood transfusion, blood and marrow (hematopoietic cell) allograft transplantation, and in the clinically important setting of solid organ transplantation from HCMV seropositive donors to naive recipients.

HCMV is transmitted vertically during pregnancy, as well as during the birthing process or from mother's milk. Transplacental transmission is a characteristic of HCMV and possibly roseoloviruses, and is not observed with alphaherpesviruses or gammaherpesviruses. Primary

infection has been reported to occur in 1–4% of pregnant women, and is associated with a significant risk for transplacental transmission. Between 20% and 40% of newborns delivered by women with primary infection are HCMV infected, compared to between 1% and 2% of newborns delivered by women with past infection. Primary infection is associated with more severe forms of congenital disease, although disease occurrence has been well documented in both settings. Transmission at birth as well as through mother's milk helps maintain HCMV infection in the population, with 25–50% of infants nursed by seropositive mothers acquiring HCMV by 1 year of age. Importantly, there are no known disease sequelae associated with intrapartum or postpartum acquisition of HCMV in healthy term newborns.

Although serotypes of HCMV have not been useful for epidemiology, various molecular genetic approaches that assess viral genome sequence variation have been used to differentiate viral strains in circulation. Comparison of polymerase chain reaction (PCR)-amplified segments of specific, highly variable genomic regions and protein-coding genes has been most useful, notably the noncoding genomic L–S junction (*a* sequence) as well as genes UL144, UL146, UL73, and UL74. To date, no particular viral strain has been unambiguously linked to any clinical disease.

HCMV appears to reside latent in progenitor cells of the myeloid lineage and to reactivate when these progenitors are stimulated by pro-inflammatory cytokines. HCMV is persistently detected in saliva approximately 3–5% of the time. Based on the distribution and levels of viral DNA, natural latency occurs in bone marrow-derived myelomonocytic cells that give rise to macrophages and dendritic cells, with approximately 0.01–0.001% of cells infected and each cell carrying two to ten genome copies of HCMV DNA. Little is known about sporadic reactivation in immunocompetent individuals, which leads to shedding in saliva and other bodily fluids. However, reactivation in allogeneic transplant settings appears to follow the elaboration of pro-inflammatory cytokines that are a component of tissue rejection. Patients with compromised immunity to HCMV amplify virus to levels that become a clinical threat. HCMV reactivation also occurs in settings without allogeneic stimulation. Pregnancy appears to allow reactivation at levels that are not a threat to women but may be a threat to a developing fetus. Severely immunocompromised patients, such as terminal AIDS patients, appear to support sufficiently high levels of HCMV replication for disease to occur.

Clinical Features and Pathology

HCMV infection in the immunocompetent host is typically clinically silent. A small percentage of mononucleosis is due to HCMV, which is typically less severe than that caused by Epstein–Barr virus. Severe or systemic HCMV disease affecting one or more organs only occurs in immunocompromised hosts and has been known to unveil underlying immunodeficiency in individuals with a previously unrecognized immune deficit.

Congenital disease is the hallmark of HCMV pathogenesis and follows transplacental transmission to the fetus any time during pregnancy, and is a particular risk for woman with primary infection. Congenital disease remains the most important medical and public health concern for HCMV, and is difficult to identify because primary infection is typically asymptomatic. A significantly lower rate of congenital disease follows recurrent compared to primary maternal infection during pregnancy, providing the key evidence that universal vaccination would benefit the population. In addition, primary infection at an earlier gestational age is most likely to manifest as disease in the newborn. HCMV disease recognized at birth often reflects neuronal damage (hearing loss, retinitis, optic neuritis, microcephaly, or encephalopathy) as well as damage to the reticuloendothelial system (hepatomegaly, splenomegaly, petechiae, or jaundice). Prematurity and poor intrauterine growth are common among newborns with symptomatic congenital HCMV infection. Although the majority of infected newborns are asymptomatic at birth, some still develop sequelae, notably sensorineural hearing loss, over the first few years of life. Most newborns with congenital HCMV infection, even those severely affected at birth, survive; congenital HCMV infection takes its toll by affecting hearing, eyesight, and mental capacity.

Very low birth weight premature infants who acquire HCMV infection during birth or postpartum (from human milk or transfusion) may develop systemic disease that is distinct from conventional congenital disease. Risk factors include very low birth weight, exposure to multiple units of blood and birth to a seronegative mother (lack of passive immunity). Transfusion-associated HCMV disease in newborns has been controlled by the use of seronegative (or leukocyte-depleted) blood products. Whether or not transmission of HCMV from a mother's milk to a very low birth weight premature newborn should be prevented remains an area of controversy.

HCMV has become one of the most common opportunistic pathogens as allograft transplantation has grown and as the AIDS epidemic has unfolded. Solid organ and hematopoietic cell allograft recipients all receive strong post-transplant immunosuppressive therapy and AIDS patients progress to a T-lymphocyte deficit that provides an opportunity for HCMV to replicate to high levels and cause disease. Active infection arises from primary infection of naive individuals, reinfection with additional strains of virus, and reactivation of latent virus. This aspect of disease pathogenesis is complicated because HCMV

shedding and viremia are common in the face of impaired cellular immunity even when HCMV disease is absent. Clinically silent HCMV infection may predominate even in the face of antiviral prophylaxis, and may only progress to disease as T-cell-mediated immune surveillance becomes severely compromised, such as occurs in AIDS patients with very low CD4 T-cell counts.

Clinical manifestations of disease in solid organ transplant patients include systemic, febrile illness (CMV syndrome, with malaise, arthralgia and rash; neutropenia, thrombocytopenia, and elevated liver enzymes), as well as a direct or indirect impact on specific organs. This disease risk has been reduced but not eliminated by preemptive therapy or prophylaxis with antiviral drugs. Clinical detection of infection using assays for viral antigens or nucleic acids without the need for virus culture successfully reduced the incidence of acute HCMV disease in the first few weeks following transplantation. Preemptive therapy has been beneficial in other high-risk patients such as allogeneic blood and marrow (hematopoietic cell) transplantation where late-onset disease and indirect effects have become more prominent. The detection of HCMV antigens (usually pp65 antigenemia) or nucleic acids (usually PCR detection of viral DNA in plasma or leukocytes) in blood is used to implicate this virus in disease. Solid organ transplant recipients may acquire HCMV pneumonitis, or gastrointestinal lesions, hepatitis, retinitis, pancreatitis, myocarditis, and, in rare circumstances, encephalitis or peripheral neuropathy, whereas hematopoietic cell transplant recipients suffer HCMV pneumonitis or gastrointestinal disease. Establishing an etiologic role for HCMV requires detection of virus in affected tissue or bronchoalveolar lavage or quantitation of HCMV in blood or plasma. The principal indirect effects of HCMV, particularly during solid organ transplantation, include allograft rejection, vascular disease complications, and increased risk of opportunistic fungal and bacterial infections. Primary infection is most likely to lead to disease when a naive recipient receives an organ from an HCMV seropositive donor, although the particular organ and immunosuppression regimen also influence outcome.

Although antiviral prophylaxis has proved to be very effective in preventing HCMV disease in solid organ transplant recipients, late-onset HCMV disease may follow prophylaxis in as many as 5% of patients. Prophylactic or preemptive antiviral treatment in hematopoietic cell transplant recipients reduces the incidence of HCMV disease during the first 3–4 months to around 5%; however, late-onset disease, usually more than 100 days after transplant, remains a problem. In all clinical transplant settings, HCMV has been associated with chronic disease consequences that prophylaxis and preemptive therapy have not completely alleviated. In the nontransplanted individual, HCMV has been a suspected cofactor in atherosclerotic vascular disease and in autoimmune vasculitis.

Highly active antiretroviral therapy (HAART) reduces incidence of HCMV retinitis and gastrointestinal disease in AIDS patients due to reconstitution or preservation of cellular immune function, although HCMV remains a significant risk to human immunodeficiency virus (HIV)-positive individuals when CD4 T-cell counts drop to low levels.

Four antiviral agents are currently approved in the USA for treatment of HCMV disease, ganciclovir, valganciclovir, cidofovir, and foscarnet, with intravenous ganciclovir and orally administered prodrug valganciclovir being first choices in most settings. These two are also common choices for prophylaxis and preemptive therapy. All of the anti-HCMV drugs have potentially significant toxicity and are limited to use in patients with disabling or life-threatening disease risk. Thus, additional drugs are under development. Prolonged use of any investigational or licensed drug may select for resistant virus, but drug-resistant mutants have not been observed to circulate in the population.

Immune Response, Prevention, and Control

Prevention of HCMV infections during pregnancy to reduce congenital disease risk is an important public health goal, and universal vaccination during childhood is believed to be the appropriate route to achieve this control. Attempts to develop live, attenuated, killed, and subunit vaccines over the past 30 years have not yet resulted in success. The Centers for Disease Control and Prevention (CDC) currently recommends that parents and caregivers of young children be informed of how HCMV is transmitted and of hygienic measures that reduce transmission, particularly to women who care for young children and may become pregnant.

Natural HCMV infection is highly immunogenic, resulting in broad humoral antibody and cellular T-lymphocyte responses that persist for life. The parameters of CD4 and CD8 T-lymphocyte responses vary with age and other characteristics, but the continued high levels of response in a majority of infected individuals suggests that virus antigen provides a continuous immunogenic stimulus throughout life. In addition to the adaptive immune response, innate cellular clearance by natural killer (NK) cells appears to be important in the initial stages of infection.

HCMV encodes a very large array of gene products that deflect intrinsic host-cell antiviral responses mediated at the transcriptional level through activation of the interferon pathways or cell death. In addition, this virus encodes a wide range of functions aimed at disarming the innate NK cell response as well as the effectiveness of adaptive humoral and T-cell responses. These

functions provide an impression that this virus survives in tight balance with host immune clearance mechanisms, and are summarized in **Table 1**. In addition, accumulating evidence from animal models of HCMV pathogenesis implicates pro-inflammatory viral gene products in creating the appropriate environment to allow efficient growth and dissemination to important tissue sites to assure transmission to new hosts.

Future Perspectives

Although there have been tremendous gains in knowledge of HCMV molecular biology and pathogenesis, this virus continues to be a very important cause of disease especially for immunocompromised hosts and for the fetus. To date, antiviral chemotherapy has been only partially successful in controlling HCMV infection in transplant and other immunocompromised patients and there remains a need for vaccination to reduce the incidence of congenital HCMV infection. An important goal for future research will be to translate the large and growing body of basic knowledge of HCMV biology into improved treatments and effective vaccines.

See also: Cytomegaloviruses: Murine and Other Non-primate Cytomegaloviruses; Cytomegaloviruses: Simian Cytomegaloviruses; Herpesviruses: General Features; Human Cytomegalovirus: Molecular Biology; Human Herpesviruses 6 and 7.

Further Reading

Davison AJ and Bhella D (2007) Comparative betaherpesvirus genome and virion structure. In: Arvin AM, Mocarski ES, Moore P, *et al.* (eds.) *Human Herpesviruses: Biology, Therapy and Immunoprophylaxis*, pp 177–203. Cambridge: Cambridge University Press.

Dolan A, Cunningham C, Hector RD, *et al*. (2004) Genetic content of wild type human cytomegalovirus. *Journal of General Virology* 85: 1301–1312.

Mocarski ES, Jr. (2007) Betaherpesvirus-common genes and their functions. In: Arvin AM, Mocarski ES, Moore P, *et al.* (eds.) *Human Herpesviruses: Biology, Therapy and Immunoprophylaxis*, pp 202–228. Cambridge: Cambridge University Press.

Mocarski ES Jr., Shenk T, and Pass RF (2006) Cytomegalovirus. In: Knipe DM, Howley PW, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 2701–2772. Philadelphia, PA: Lippincott Williams and Wilkins.

Human Cytomegalovirus: Molecular Biology

W Gibson, Johns Hopkins University School of Medicine, Baltimore, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

This chapter provides a general description of the molecular biology of human cytomegalovirus (HCMV) and highlights a number of specific features concerning the virus and its replication cycle. It is necessary to point out that most studies on the molecular biology of HCMV have utilized highly passaged, genetically deficient laboratory strains grown in human fibroblast cells. Although the findings are anticipated to apply in large part to wild-type strains grown in fibroblast and other cell types, and therefore to be relevant to the clinical situation, there are probably important differences to be discovered in the future.

Properties of the Virion

Typical of the herpesvirus group, the virion of HCMV (species *Human herpesvirus 5*, subfamily *Betaherpesvirinae*, family *Herpesviridae*) is approximately 230 nm in diameter and is composed of a DNA-containing capsid, surrounded by a less structured tegument layer, and bounded by a trilaminate membrane envelope. The capsid is isosahedral, has a diameter of approximately 110 nm, and is made up of four principal protein species that are organized into 162 capsomeres (150 hexamers plus 12 pentamers) and 320 triplexes located between the capsomeres. By analogy with herpes simplex virus type 1 (HSV-1), one of the pentamer positions is occupied by a portal complex (a dodecamer of the portal protein) through which DNA enters and leaves the capsid. The capsomeres are approximately 20 nm in length and 15 nm in diameter, have a channel about 3 nm in diameter that is open at the exterior end, possess favored cleavage planes along the longitudinal axis, and have short, spicule-like protrusions, probably representing the triplexes, extending out symmetrically and resulting in a pinwheel appearance of the capsomere viewed end on. The tegument region is approximately 50 nm thick and includes seven relatively abundant virus-encoded protein species, five of which are phosphorylated. The virion envelope is estimated to be 10 nm thick and contains at least 10 abundant protein species. Both the tegument and envelope contain a substantial number of

less abundant virus-encoded and host-cell proteins. In addition, the virion has been reported to contain phospholipids (phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol) and polyamines (spermidine and spermine). The properties of the virion DNA and proteins are described below.

Properties of the Genome

The genome of HCMV is composed of a linear, double-stranded DNA molecule with a size of 236 kbp in wild type virus. Thus, it is over 50% larger than that of HSV-1, and the largest among the human herpesviruses. It is a class E genome, like that of HSV-1, consisting of a long unique (U_L) and a short unique (U_S) sequence, both of which are flanked by much shorter inverted repeat sequences that enable U_L and U_S to invert relative to each other and give rise to the four structural isomers of the genome found in virions. The origin of DNA replication (*ori*-Lyt) has been localized to a 3–4 kbp region near the center of U_L. Like *ori*-L, one of three duplicated origin sequences in the HSV-1 genome, the HCMV *ori*-Lyt region is located adjacent to the promoter for the early, single-stranded DNA-binding protein (encoded by gene UL57). HCMV *ori*-Lyt includes a 2.4 kbp sequence that has no homology with other described virus DNA replication origins and contains direct and inverted repeat sequences. This region contains consensus cyclic AMP (cAMP) response elements and other transcription factor-binding sites, and 23 copies of the sequence AAAACACCGT that are conserved near the homologous *ori*-Lyt of simian cytomegalovirus (SCMV).

The complete nucleotide sequence of the high passage strain AD169 was the first to be determined for HCMV, with a size of 229345 bp. It has a G + C content of 57.2 mol%, and was assessed as containing 189 protein-coding open reading frames (ORFs), some of which are spliced and some of which are duplicated in the inverted repeats. The low passage strain Toledo was found to contain an additional ~15 kbp at the right end of U_L containing 19 additional ORFs. Analysis of the complete 235645 bp sequence of the low passage strain Merlin and a reevaluation of the genetic content of strain AD169 have indicated that wild-type HCMV contains ~165 protein-coding genes. The wild-type genome contains 13 families of related genes, many with members clustered into blocks and encoding predicted or recognized glycoproteins (e.g., the RL11, US6, and US27 families). Taken together, these gene families represent ~40% of the total gene number and in part account for the comparatively large size of the HCMV genome. It is also worth noting that the HCMV genome contains homologs of several cellular genes, including surface receptors such as class I major histocompatability (MHC) antigens, G protein-coupled receptors (GPCRs), and a tumor necrosis factor receptor.

Properties of the Proteins

HCMV proteins share the general pattern of expression characteristic of the herpesvirus group. Immediate early (IE or α), early (E or β), and late (L or γ) proteins are synthesized sequentially from corresponding mRNAs whose transcription is regulated in a temporal cascade. IE proteins are required to regulate transcription from their own promoters and those of subsequently expressed genes. E proteins include many of the enzymes and regulatory factors needed to carry out the synthesis of progeny DNA and proteins. L proteins include most of the virion structural proteins. Members of all three classes have been described for HCMV, though only a small number of the proteins encoded by HCMV have been identified. Those that have been reported include both nonvirion and virion species and their properties are briefly described below.

Nonvirion Proteins

Many of the recognized nonvirion proteins are made at early times after infection and localize to the nucleus. The first of these to appear is the major IE protein (IE1, encoded by UL123), a 72 kDa phosphoprotein that, together with products of a second IE gene, IE2 (UL122), can transactivate HCMV E promoters. Both IE1 and IE2 are expressed from spliced transcripts generated using the major IE promoter. In addition, the IE2 gene gives rise to a family of proteins ranging in size from 23 to 86 kDa that are generated by differential splicing and translational start sites. IE2 proteins can repress the major IE promoter by acting on a sequence immediately upstream of the transcription initiation site. Other IE proteins are encoded by genes IRS1, TRS1, US3, and UL36–38, and can act synergistically with IE1 and the US3 protein to regulate both cellular and virus gene transcription.

The E proteins include the 140 kDa virus DNA polymerase (encoded by UL54), a 140 kDa nuclear single-stranded DNA-binding protein (DB140; UL57), and a set of closely related nuclear phosphoproteins (UL112). Like its homologs in other herpesviruses, the DNA polymerase has an associated 3′-specific nuclease activity. Like its homolog (UL29) in HSV-1, UL57 is located adjacent to *ori*-Lyt, and its product DB140 localizes to discrete foci within the nuclei of infected cells and accumulates in the cytoplasm under conditions of inhibited virus DNA synthesis. UL112 encodes a set of four E, nuclear,

DNA-binding phosphoproteins (34, 43, 50, and 84 kDa) that appear to be produced by alternate splicing, are related to each other by their common N-terminal sequence, and are differentially phosphorylated. Expression of these proteins appears also to be regulated at both the transcriptional and post-transcriptional levels. The functions of these proteins are not known, and they may have a role in DNA replication or gene regulation.

Another nonvirion nuclear DNA-binding phosphoprotein is distinguished by its high abundance and kinetics of synthesis. The ~52 kDa species, DB52, is the product of gene UL44. On the basis of its reduced but not eliminated synthesis under conditions of inhibited virus DNA replication, DB52 is classified as a delayed-early (DE or γ_1) protein. Expression of this gene is under different promoter control at early and late times of infection, and there is evidence that its expression is also post-transcriptionally regulated. DB52 and its SCMV homolog bind to DNA *in vitro* and *in vivo* and associate with the virus DNA polymerase to enhance its activity, analogous to the HSV-1 UL42 protein. DB52, like the HSV-1 UL42 protein, has the structure of a homotrimeric sliding clamp similar to that of the cellular processivity factor, proliferating cell nuclear antigen. Three other proteins that function in virus DNA replication or processing are a phosphotransferase (encoded by UL97), and the ATPase (encoded by UL89) and its partner subunit (encoded by UL56) that constitute an apparent homolog of the bacteriophage DNA cleavage/packaging complex, terminase.

Several other nonvirion HCMV proteins have recently been shown to interfere with the MHC class I antigen presentation system (i.e. the US3, US6, and US11 proteins). These proteins are thought to help the virus-infected cell escape recognition and destruction by immune surveillance, and provide a mechanism for virus persistence in the immune-competent host. It is viewed as likely that wild-type HCMV encodes many proteins involved in modulating the host response to infection.

Virion Proteins

Estimates of the number of protein species in HCMV virions depend on the techniques used. Classical approaches utilizing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) have detected a total of 30–35 protein species. The characteristics of the most abundant of these proteins, including size, charge, carbohydrate and phosphate content, and deduced locations in the particle, are summarized in **Table 1** and described below. Virions have also been reported to contain several enzymatic activities (including protein kinase, DNA polymerase, DNase, and topoisomerase II), and several host proteins (including PP1 and PP2A phosphatases and a 45 kDa cellular actin-related protein), although it is not known whether these are specific associations. A larger number of proteins have been detected by a mass spectrometric analysis of virions, amounting to species encoded by 59 recognized HCMV genes and over 70 host proteins. The sensitivity of this technique is sufficiently high to raise questions about whether all these proteins are specifically incorporated into virions, and whether they are present in all virions.

The capsid shell is composed of four abundant protein species referred to as the major capsid protein (MCP), the minor capsid protein (mCP), the mCP-binding protein (mCP-BP), and the smallest capsid protein (SCP). Formation of the capsid shell is coordinated by precursors of an internally located maturational protease (assemblin) and assembly protein (pAP), which serve a scaffolding role and are present in nascent intranuclear capsids (e.g., B capsids) and in noninfectious enveloped particles (see below), but not in virions. These internal scaffolding proteins are the only abundant capsid proteins known to be post-translationally modified (**Table 1**).

The bulk of the protein mass of the virion tegument is contributed by its seven principal protein species. The largest of these, called the high molecular weight protein (HMWP), is the homolog of HSV-1 VP1/2 (encoded by HSV-1 UL36), and is an active ubiquitin-specific cysteine protease. HMWP forms heterooligomers with the HMWP-binding protein (HMWP-BP) encoded by the adjacent gene. Neither HMWP nor HMWP-BP is detectably phosphorylated or glycosylated. The basic phosphoprotein (BPP or pp150) and the upper matrix (UM or pp71) and lower matrix (LM or pp65) proteins account for the greatest protein mass of the tegument constituents and are highly phosphorylated, both *in vivo* and *in vitro*, by the virion-associated protein kinase. BPP is distinguished among the virion proteins by having the highest density of O-linked *N*-acetylglucosamine residues, which are attached at a single site or tight cluster of sites. UM is a transacting inducer of IE gene transcription that may be functionally analogous to the α-*trans*-inducing factor of HSV-1. LM appears to be an important component in cellular immunity and there are reports that it has an associated protein kinase activity. The '24K protein' (pp28) is also phosphorylated and, like its HSV-1 homolog UL11, forms oligomers (with the protein encoded by UL94). HMWP and BPP are thought to make direct contact with the capsid.

The virion envelope contains at least 10 protein species. The high degree of size and charge heterogeneity among this group has complicated their analysis. The most abundant species are distributed among three glycoprotein complexes (gCI, gCII, and gCIII), each of which is disulfide linked. The complex gCI consists of three forms

Table 1 Principle proteins of the HCMV virion

Descriptive name	*Predicted M_r*[a]	*Observed M_r*[b]	*Modification*[c]	*Charge*[d]	*Gene*[e]
Capsid					
Major capsid protein (MCP)	153.9	153		N	UL86
Portal protein	78.5			B	UL104
Precursor assembly protein (pAP)[f]	38.2	50	P, C	B	UL80.5
Minor capsid protein (mCP)	34.6	34		B	UL85
mCP-binding protein (mCP-BP)	33.0	35		N	UL46
Protease assemblin (A, A_N, A_C)[g]	28.0	28		A	UL80a
Smallest capsid protein (SCP)	8.5	8		B	UL48A
Tegument					
High mol. wt. protein (HMWP)	253.2	212		N	UL48
Basic phosphoprotein (BPP; pp150)	112.7	149	G, P	B	UL32
HMWP-binding protein (HMWP-BP)	110.1	115		N	UL47
80 K		80	P		
Upper matrix protein (UM; pp71)	61.9	74	P	N	UL82
Lower matrix protein (LM; pp65)	62.9	69	P	B	UL83
24 K (pp28)	28.0	24	P	B	UL99
Envelope					
Glycoprotein B (gB)	102.0	102	G, P, C	N	UL55
N-terminal portion (gB_N)	52.6	130	G	A	UL55
C-terminal portion (gB_C)	49.5	62	G, P	A	UL55
Glycoprotein H (gH)	84.4	84	G	N	UL75
Glycoprotein M (gM)	42.9	43			UL100
Glycoprotein N (gN)	14.5	57	G	A	UL73
Glycoprotein O (gO)	54.2	125	G		UL74
GPCR27	42.0	42	G	B	US27
GPCR33	46.3	44	G	B	UL33

[a]Molecular masses (kDa).
[b]Molecular masses (kDa) given as determined by SDS-PAGE.
[c]G, glycosylation; P, phosphorylation; C, proteolytic cleavage.
[d]Relative net charges were determined by 2-D PAGE (charge/size separation) with respect to the major capsid protein, which is approximately neutral in such separations. N, neutral; A, acidic; B, basic.
[e]Gene locations are shown in figure 1 of the article on Human Cytomegalovirus: General Features.
[f]Present in immature capsids and noninfectious enveloped particles but absent from virions.
[g]Assemblin (A) is derived from a 74 kDa precursor (encoded by UL80a), and is autoproteolytically converted to amino (A_N) and carboxyl (A_C) portions.

of glycoprotein B (gB). HCMV gB is synthesized as a 130–160 kDa, cotranslationally N-glycosylated precursor that undergoes at least four further modifications: (1) its 17–19 predicted N-linked oligosaccharides are converted from nontrimmed to trimmed high-mannose forms, and then a portion is further modified to complex structures; (2) O-linked carbohydrates are added; (3) the C-terminal region of the molecule is phosphorylated; and (4) the protein is cleaved between Arg460 and Ser461 to yield an intramolecularly disulfide-linked heterodimer composed of a highly glycosylated 115–130 kDa N-terminal fragment (gB_N), and a less extensively modified 52–62 kDa C fragment (gB_C). This gB_N–gB_C heterodimer can form intramolecular disulfide crosslinks, yielding a complex with the composition $(gB_N–gB_C)_2$.

Early studies of the complex gCII identified proteins with M_rs of 50–52 and >200 kDa representing forms of the integral membrane protein glycoprotein M (gM). These proteins have similar peptide maps indicating that they are closely related, and the 52 kDa component is comparatively highly O-glycosylated and sialylated. Subsequently, the 50–60 kDa glycoprotein N (gN, UL73) was recognized as a second component of gCII.

The complex gcIII was initially characterized as composed of glycoprotein H (gH) and glycoprotein L (gL; not listed in **Table 1**). HCMV gH is an 86 kDa species that is less extensively N-glycosylated than gB and its oligosaccharides are not processed beyond the high-mannose structure. It has been proposed to mediate virus–host membrane fusion during the initial steps of virus infection, and its intracellular trafficking associates with gL. A third component, the highly sialylated, O-glycosylated virion glycoprotein O (gO), was later shown to be specified by UL74. Recently, using strains of HCMV that more closely reflect wild-type virus, an alternative complex consisting of gH, gL, and two small proteins encoded by UL128 and UL130 has been implicated in tropism for nonfibroblast cell types.

Physical Properties

Infectivity of HCMV is eliminated by exposure to 20% ether for 2 h, by heating at 65 °C for 30 min, by exposure to ultraviolet light (4000 erg s^{-1} cm^{-2}; 400 μJ s^{-1} cm^{-2}) for 4 min, by acidic pH (<5), and by treatment with low concentrations of either ionic or nonionic detergents. Infectivity is also reduced following sonication, pelleting by ultracentrifugation, and repeated cycles of freezing and thawing.

Replication

General features of the HCMV replication cycle are typical of the herpesvirus group. Following virus adsorption and entry into the cell, the DNA is transported to the nucleus where it is replicated and packaged into preformed capsids. Maturation of the nucleocapsid involves acquisition of the tegument constituents followed by envelopment, which has been observed at both nuclear and cytoplasmic membranes.

DNA Replication

HCMV DNA is replicated in the nucleus. Parental DNA appears to circularize soon after entering the cell, but maximal rates of progeny DNA synthesis do not occur until approximately 3 days after infection. The 'endless' concatemeric nature of progeny DNA is consistent with a rolling circle mechanism of DNA replication. However, the finding that virus DNA synthesis proceeds bidirectionally from *ori*-Lyt under conditions inhibition of replication by ganciclovir ([9-[2-hydroxy-1-(hydroxymethyl)-ethoxy]methyl]guanine) is not easily reconciled with this model, and perhaps indicates an intermediate stage of replication based on a theta mechanism. Unit-length genomic DNA is released from the concatemers by cleavage at a specific site located in the *a* repeat elements, 30–35 bp from a herpesvirus group-common cleavage/packaging region referred to as the herpes pac homology sequence.

Characterization of Transcription

The pattern and control of HCMV gene expression shares many features with that of HSV-1. As during translation, transcription of the HCMV genome is temporally regulated into three major kinetic classes. The first class of mRNAs made are transcribed from input DNA and are referred to as IE (or α) RNAs. Their expression requires no preceding virus protein or DNA synthesis, and they are operationally defined as those RNAs that can be made in the presence of inhibitors of protein synthesis. The most abundant IE RNAs are transcribed from the major IE promoter. These transcripts are related to each other by alternate splicing patterns and encode IE1 and multiple forms of IE2. The promoter region for this transcription unit is one of the strongest known, presumably due to its high content of consensus binding sites for host-cell transcription factors such as nuclear factor 1, CAAT-binding protein, and SP1, and the large number of repeat sequences that contain cyclic AMP response elements and that may interact with additional DNA-binding proteins. Transcription from this region is enhanced by UM, whose function appears to be analogous to that of the HSV α-*trans*-induction factor. Lower abundance IE RNAs arise from genes IRS1, TRS1, US3, and UL36–38.

Expression of E (or β) RNAs requires the preceding synthesis of IE proteins, but does not require virus DNA synthesis. Thus, E RNAs are made in the presence of inhibitors of virus DNA synthesis, but not in the presence of inhibitors of protein synthesis. Both IE and E genes appear to be transcribed by host-cell RNA polymerase II. Expression of L (or γ) RNAs requires the preceding synthesis of both IE and E virus proteins, as well as synthesis of virus DNA. The possibility that post-transcriptional events may be involved in controlling HCMV RNA processing and translation is suggested by the presence of some E RNAs that are not translated until late times of infection.

Characterization of Translation

HCMV RNAs are translated by the host-cell protein-synthesizing system. Virus-specific proteins that may augment the host system (e.g., initiation or elongation factors) have not yet been identified. Each virus protein species appears to be encoded by a unique mRNA. Cells infected with HCMV do not show the generalized early shut-off of cell protein synthesis that occurs in HSV-1-infected cells. There are, nevertheless, very early changes in the metabolism of HCMV-infected cells that include stimulated transcription of some host genes (e.g., heat shock protein 70, ornithine decarboxylase, thymidine kinase, creatine kinase, and cyclooxygenase 2), decreased transcription of other host genes (e.g., fibronectin), and changes similar to those induced by the GPCR signaling pathway (e.g., decreased intracellular Ca^{2+} stores and increased levels of intracellular cAMP).

Post-Translational Modifications

Modifications of HCMV proteins include phosphorylation, glycosylation, and proteolytic cleavage. Phosphorylation has been detected on IE (e.g., IE1 and IE2), E

(e.g., UL112 protein) and L (e.g., DE nonvirion UL44 protein; late virion tegument (UL32, UL82, and UL83) proteins), and capsid (AP) proteins, all of which localize to the infected cell nucleus. At least some of these phosphorylations are likely to be catalyzed by reported virus-coded protein kinases. Glycosylation typifies many of the virion envelope glycoproteins and includes both N-linked (high-mannose and complex structures) and O-linked oligosaccharides. The only nonenvelope virion protein that has been demonstrated to be glycosylated is BPP, which has O-linked *N*-acetylglucosamine. Glycosylation of HCMV proteins is most likely carried out by host cell systems. The third modification that has been described for HCMV proteins is proteolytic cleavage. There are two maturational endoproteolytic cleavages, in addition to glycoprotein signal sequence cleavages. The first of these cuts between Arg460 and Ser461 in envelope gB and is catalyzed by a calcium-dependent cellular, furin-like proteinase. The other is a cleavage between residues Ala308 and Ser309 of the capsid assembly protein precursor (pAP). This latter cleavage is catalyzed by the genetically related, virus-encoded proteinase, assemblin (A), and is essential for the production of infectious virus.

Cytopathology

The mechanisms of cell recognition, binding, and penetration by HCMV are not yet understood, but there are at least five envelope proteins that may be involved: (1) a 'disintegrin-like' domain in virion envelope gB interacts with cellular integrins; (2) β_2-microglobulin is present on virions and may interact with cell-surface MHC proteins; (3) virion envelope gH interacts with a 90 kDa cell protein that may also be involved in binding or penetration; and (4) a highly O-glycosylated envelope glycoprotein complex (gcII) is able to bind heparin, and this interaction could promote early virus–cell interaction as proposed for cellular heparin and HSV-1. Different entry mechanisms may operate for different cell types.

Assembly of the capsid and DNA replication and packaging take place in the nucleus. As with HSV-1, some tegument constituents are presumed to be added to the maturing nucleocapsid in the nucleus, with envelopment and de-envelopment occurring as the tegument-coated capsid transits the two leaflets of the nuclear membrane into the cytoplasm. Further addition of tegument proteins is then thought to precede secondary envelopment at vesicle membranes in the infected cell cytoplasm.

The cytopathic effects of HCMV are distinctive. Early after infection, a transient cell rounding occurs, followed by overall enlargement (cytomegalia) and by the appearance of basophilic intranuclear inclusions, which gradually enlarge to fill and distort the nucleus into an elongated or kidney-shaped form late in infection. These intranuclear inclusions contain virus DNA, proteins, and capsids, and are thought to be nucleocapsid assembly sites. Late in infection, the cytoplasm contains numerous enveloped and nonenveloped capsids, large (>500 nm 'black holes') and smaller (250–500 nm 'dense bodies') electron-dense aggregates, and a large eosinophilic spherical region adjacent to the nucleus that exhibits strong F_C binding.

Release of HCMV from the cell results in three kinds of enveloped particles being freed into the growth medium of cultured fibroblasts: (1) virions; and two aberrant particles referred to as (2) noninfectious enveloped particles, which closely resemble virions in structure and composition but have no DNA and contain the capsid assembly protein that is absent from virions; and (3) dense bodies, which are solid, 250–600 nm spherical aggregates of LM that contain no DNA and are surrounded by an envelope layer.

Acknowledgment

I am grateful to Andrew Davison for his advice and kind assistance in updating the previous edition of this article.

See also: Herpesviruses: General Features; Human Cytomegalovirus: General Features.

Further Reading

Brignole EJ and Gibson W (2007) Enzymatic activities of human cytomegalovirus maturational protease assemblin and its precursor (pUL80a) are comparable: Maximal activity of pPR requires self-interaction through its scaffolding domain. *Journal of Virology* 81: 4091–4103.

Chee MS, Bankier AT, Beck S, *et al.* (1990) Analysis of the protein coding content of the sequence of human cytomegalovirus strain AD169. *Current Topics in Microbiology and Immunology* 154: 125–169.

Dolan A, Cunningham C, Hector RD, *et al.* (2004) Genetic content of wild type human cytomegalovirus. *Journal of General Virology* 85: 1301–1312.

Mocarski ES and Tan Courcelle C (2001) Cytomegaloviruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 2629–2673. Philadelphia: Lippincott Williams and Wilkins.

Varnum SM, Streblow DN, Monroe ME, *et al.* (2004) Identification of proteins in human cytomegalovirus (HCMV) particles: The HCMV proteome. *Journal of Virology* 78: 10960–10966.

Human Eye Infections

J Chodosh, A V Chintakuntlawar, and C M Robinson, University of Oklahoma Health Sciences Center, Oklahoma City, OK, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Blepharoconjunctivitis Inflammation of the eyelid and conjunctiva.
Dacryoadenitis Inflammation of the lacrimal gland.
Iridocyclitis Inflammation of the iris and ciliary body.
Meibomian gland Modified sebaceous glands lining the eyelid margin that provide the lipid layer of the preocular tear film.
Retinochoroiditis Inflammation of the retina and choroid (uveal) layers of the eye.

Introduction

The eye contains diverse tissues intricately linked to subserve visual function (**Figure 1**). The ocular adnexae – periorbita, eyelids and lashes, lacrimal and meibomian glands – produce, spread, and drain the preocular tear film, physically protect the sensitive ocular mucosa, and cushion the globe. The redundant conjunctiva with its low-viscosity tear film allows rapid multidirectional eye movements. Lymphoid tissues within the conjunctiva and lacrimal glands furnish acquired immune defense. The cornea and its tear film fashion the major refractive surface of the eye. The sclera forms the wall of the globe and scaffolds the intraocular tissues. The eye's lens provides additional refractive power and filters ultraviolet light. The iris diaphragm dynamically regulates the amount of light incident upon the retina, and together with the choroid and optic nerve head provides immune effector cells to the interior of the eye. The retina transduces light energy into neural signals; retinal function is requisite for vision. The vascular choroid nourishes the outer layers of the retina. The anterior (aqueous) and posterior (vitreous) humors provide internal pressure sufficient for maintenance of normal anatomic relationships, nourish the interior ocular tissues, provide immunosuppressive factors necessary to the maintenance of immune deviation, and during infection act as conduits for the distribution of inflammatory cells derived from the iris, ciliary body, and optic nerve head.

Eye infection by viruses most often follows direct contact with virus externally, either from infected secretions in the birth canal (herpes simplex virus, human papillomavirus), on fomites (adenovirus), or airborne particles (rhinovirus), or is acquired during viremia (human cytomegalovirus, measles virus). Other mechanisms of ocular viral infection include extension from contiguous adnexal disease (herpes simplex virus), spread from the upper respiratory tract via the nasolacrimal duct (rhinovirus), and transplacental passage of infectious virus (rubella virus). Rarely, ocular infection may disseminate elsewhere (enterovirus 70).

Acute viral infection produces stereotypic changes in ocular target tissues. Infection of the eyelid skin induces the formation of vesicles and ulcers. Viral infection of the conjunctiva results in vasodilatation, serous discharge, hyperplasia of conjunctival lymphoid follicles, and enlargement of the corresponding draining lymph nodes. Severe conjunctival infection can cause permanent scarring of the globe to the eyelids and turning in of the eyelashes against the eye. Viral infection of the corneal epithelium induces punctate epithelial cytopathic effect evident biomicroscopically as isolated swollen epithelial cells (punctate epithelial keratitis) and loss of individual epithelial cells (punctate epithelial erosions). When extensive, the punctate erosions may coalesce to form confluent epithelial ulcers with dendritic, dendritiform, or geographic morphology. With herpetic infection, corneal anesthesia can ensue, and in the absence of epitheliotropic neural growth factors, corneal epithelial integrity is impaired. Reduced corneal clarity and progressive sterile ulceration may result. Corneal stromal infection induces white blood cell recruitment; subsequent corneal scarring, vascularization, and lipid deposition may permanently reduce vision. Intraocular infection manifests in inflammatory cell deposits on the posterior surface of the cornea and on the vitreous scaffold, and in free-floating leukocytes and biomicroscopically visible protein spillage into the normally cell-free and protein-poor aqueous humor. Iridocorneal and iridolenticular adhesions may develop and lead to glaucoma and cataract. Retinal infection concludes with necrosis and lost function. Viral encephalitis and meningitis can result in cranial nerve inflammation and secondary dysfunction of vision and extraocular motility.

Classical viral pathogenic mechanisms of latency, reactivation, and carcinogenesis all can be demonstrated in the eye. Herpes simplex virus causes recurrent lytic epithelial keratitis when viral reactivation within sensory ganglia of the first division of the fifth cranial nerve gives rise to virus in the preocular tear film. Necrotizing herpes stromal keratitis follows viral reactivation within the cornea stroma. Intraepithelial neoplasia and invasive squamous cell carcinoma of the conjunctiva and cornea

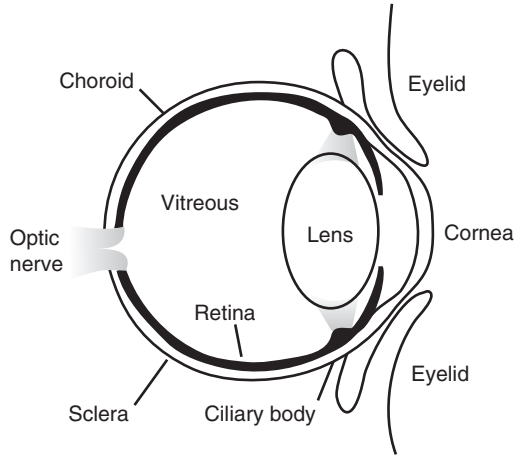


Figure 1 Cross section of the human eye.

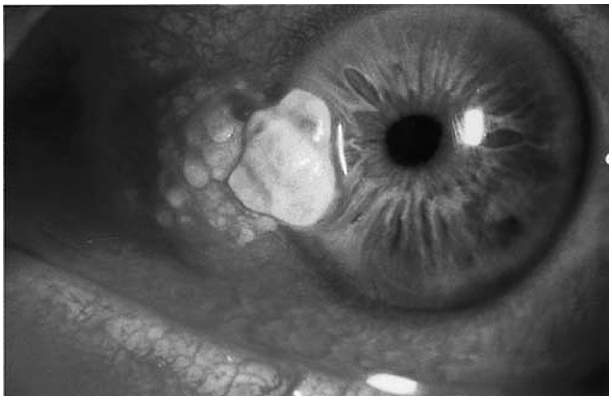


Figure 2 Squamous cell carcinoma of the corneal limbus is associated with infection by human papilloma virus types 16 and 18.

(**Figure 2**) have been associated with human papilloma virus types 16 and 18. When infected with oncogenic human papillomaviruses, corneal limbal stem cells can provide a persistent source of dysplastic ocular surface epithelium. Molecular mimicry has also been demonstrated as an immunopathogenic mechanism in ocular disease. Systemic infection with hepatitis C virus is associated with autoimmunity against a corneal stromal antigen and peripheral ulcerative keratitis. In a murine model of herpes simplex infection, non-necrotizing stromal keratitis accompanies T-cell reactivity against a corneal protein antigenically similar to a herpes simplex coat protein.

Ocular Immunology of Relevance to Viral Infection

Tissue diversity within the eye and adnexa compel varied means of innate immune defense. The eye's external surfaces (conjunctiva and cornea) encounter viruses by both airborne and contact routes. The eyelids, an intermittent barrier, periodically wipe the eye's surface free of debris and spread and drain the preocular tear film. The ability of the tear film to nonspecifically impede primary infection by viruses is unknown, although such mechanisms are well established for bacterial pathogens. An inhibitory effect of goblet cell-derived and intrinsic mucins and meibomian gland-derived lipids on viral adsorption to the ocular surface is speculative. Early in infection, aqueous tears from the main and accessory lacrimal glands furnish proinflammatory cytokines, and the conjunctival blood vessels provide both soluble and cellular components of innate immunity. After viral infection is established, aqueous tears carry lacrimal gland-derived monospecific secretory immunoglobulin A.

The constitutive defense armaments of the cornea and conjunctiva differ. The normal cornea is considered an immune-privileged site due to the high success rate of corneal transplantation; it lacks blood vessels, lymphatics, resident lymphoid cells, and Langerhans cells, expresses Fas ligand on its surface epithelium, and demonstrates reduced delayed hypersensitivity responses. Because corneal inflammation and subsequent scar reduce vision, corneal function is best served by its reduced immunologic responsiveness, also known as immune deviation. Necrotizing inflammation presupposes infection beneath the surface epithelium, and follows chemokine synthesis by infected corneal stromal fibroblasts. In contrast to the cornea, the conjunctiva is well endowed with blood and lymphatic channels, lymphoid cells, and Langerhans cells, and demonstrates classical delayed hypersensitivity responses. The immunology of the interior eye is less well established, but immune deviation appears to extend beyond the cornea to the aqueous and vitreous humors and to the central retina.

Ocular Disease Caused by RNA Viruses

Conjunctivitis is probably the most common viral ocular syndrome, and typically accompanies upper respiratory infections due to RNA viruses (**Table 1**). Rhinovirus, influenzavirus, respiratory syncytial virus, and parainfluenzavirus conjunctivitis typically are mild and self-limited, and most patients do not seek medical attention. More serious are the keratitis, uveitis, and retinitis caused by some RNA viruses. For example, influenzavirus infection of the respiratory tract, usually associated with a mild and short-lived conjunctivitis, less commonly causes inflammation in the lacrimal gland, cornea, iris, retina, optic and other cranial nerves.

Like influenzavirus, other RNA viruses can infect virtually every ocular tissue. For instance, rubella virus when acquired *in utero* may have devastating consequences for the eye. Characteristic features include

Table 1 Ocular targets of human RNA viruses

Virus	*Family*	*Subfamily/genus*	*Nuc. acid*	*Env.*	*Ocular target*
Rift Valley fever virus	*Bunyaviridae*	*Phlebovirus*	ss (−)	+	Conjunctiva Retina
Human coronavirus	*Coronaviridae*	*Coronavirus*	ss (+)	+	Conjunctiva
Dengue virus	*Flaviviridae*	*Flavivirus*	ss (+)	+	Conjunctiva
Hepatitis C virus	*Flaviviridae*	*Hepatitis C virus*	ss (+)	+	Cornea Lacrimal Glands Retina
West Nile virus	*Flaviviridae*	*Flavivirus*	ss (+)	+	Retina Uvea Optic nerve Cranial nerves
Yellow fever virus	*Flaviviridae*	*Flavivirus*	ss (+)	+	Conjunctiva
Influenzavirus	*Orthomyxoviridae*	*Influenzavirus* (A, B, C)	ss (−)	+	Lacrimal gland Conjunctiva Episclera Cornea Uvea Retina Optic nerve Cranial nerves
Measles (rubeola) virus	*Paramyxoviridae*	*Morbillivirus*	ss (−)	+	Conjunctiva Cornea Uvea Retina Optic nerve Cranial nerves
Mumps virus	*Paramyxoviridae*	*Paramyxovirus*	ss (−)	+	Lacrimal gland Conjunctiva Sclera Cornea Trabecular meshwork Uvea Optic nerve Cranial nerves
Newcastle disease virus	*Paramyxoviridae*	*Paramyxovirus*	ss (−)	+	Conjunctiva Cornea
Parainfluenza virus(es)	*Paramyxoviridae*	*Paramyxovirus*	ss (−)	+	Conjunctiva
Respiratory syncitial virus	*Paramyxoviridae*	*Pneumovirus*	ss (−)	+	Conjunctiva
Enterovirus(es): (includes poliovirus, coxsackievirus, echovirus, enterovirus)	*Picornaviridae*	*Enterovirus*	ss (+)	−	Conjunctiva Cornea Cranial nerves
Rhinovirus	*Picornaviridae*	*Rhinovirus*	ss (+)	−	Conjunctiva
Colorado tick fever virus	*Reoviridae*	*Coltivirus*	ds (+/−)	−	(?: reported to cause photophobia, retro-ocular pain)
Human T-cell lymphotropic virus-1	*Retroviridae*	*Deltaretrovirus*	ss (+)	+	Cornea Uvea
Human immunodeficiency virus	*Retroviridae*	*Lentivirus*	ss (+)	+	Lacrimal gland Retina
Rabies virus	*Rhabdoviridae*	*Lyssavirus*	ss (−)	+	(Transmission via corneal button)
Rubella virus	*Togaviridae*	*Rubivirus*	ss (+)	+	Cornea Uvea Lens Trabecular meshwork Retina Globe

+, Enveloped; −, nonenveloped; ss, single stranded; ds, double stranded; (+), positive-sense RNA genome; (−), negative-sense RNA genome.

Table 2 Ocular targets of human DNA viruses

Virus	*Family*	*Subfamily/genus*	*Nuc. acid*	*Env.*	*Ocular target*
Adenovirus	*Adenoviridae*	*Mastadenovirus*	ds	−	Conjunctiva Cornea
Herpes simplex virus, type 1 (HHV1)	*Herpesviridae*	*Alphaherpesvirinae/Simplexvirus*	ds	+	Eyelid Conjunctiva Cornea Trabecular meshwork Uvea Retina
Herpes simplex virus, type 2 (HHV2)	*Herpesviridae*	*Alphaherpesvirinae/Simplexvirus*	ds	+	Eyelid Conjunctiva Cornea Trabecular meshwork Uvea Retina
Varicella zoster virus (HHV3)	*Herpesviridae*	*Alphaherpesvirinae/Varicellovirus*	ds	+	Eyelid Conjunctiva Cornea Trabecular meshwork Uvea Retina Optic nerve
Epstein–Barr virus (HHV4)	*Herpesviridae*	*Gammaherpesvirinae/ Lymphocryptovirus*	ds	+	Lacrimal gland Conjunctiva Cornea Uvea Retina Optic nerve
Human cytomegalovirus (HHV5)	*Herpesviridae*	*Betaherpesvirinae/Cytomegalovirus*	ds	+	Retina Optic nerve
Human herpes virus 6 (HHV6)	*Herpesviridae*	*Betaherpesvirinae/Roseolovirus*	ds	+	Retina
Human herpes virus 8 (HHV8)	*Herpesviridae*	*Gammaherpesvirinae*	ds	+	Conjunctiva (Kaposi sarcoma)
Human papillomavirus	*Papovaviridae*	*Papillomavirus*	ds	−	Eyelid Conjunctiva Cornea
Molluscum contagiosum virus	*Poxviridae*	*Molluscipoxvirus*	ds	+	Eyelid Conjunctiva Cornea
Orf virus	*Poxviridae*	*Parapoxvirus*	ds	+	Eyelid
Smallpox (variola) virus	*Poxviridae*	*Orthopoxvirus*	ds	+	Eyelid Conjunctiva Cornea Uvea Optic nerve
Vaccinia virus	*Poxviridae*	*Orthopoxvirus*	ds	+	Eyelid Conjunctiva Cornea

ds, Double stranded; +, enveloped; −, nonenveloped; HHV, human herpes virus.

microphthalmos, corneal haze, cataracts, iris hypoplasia, iridocyclitis, glaucoma, and 'salt-and-pepper' pigmentary retinopathy. Rubella virus can be cultured from the lens of infected neonates at the time of cataract extraction. Congenital ocular abnormalities due to rubella, like those in other organ systems, are much worse when maternal infection ensues earliest in pregnancy.

In contrast to rubella virus, measles (rubeola) virus infection *in utero* rarely causes significant ocular disease. The classic triad of postnatally acquired measles – cough, coryza, and follicular conjunctivitis – can be accompanied by Koplik spots on the conjunctiva and a mild epithelial keratitis. Less common are optic neuritis, retinal vascular occlusion, and pigmentary retinopathy. Measles

keratopathy, a major source of blindness in the nonindustrialized world, typically presents as corneal ulceration in a malnourished child. A rare and fatal complication of measles virus infection, subacute sclerosing panencephalitis (SSPE), occurs in about 1 per 100 000 cases, and often years after clinically apparent measles. Along with devastating central nervous system damage, ocular abnormalities occur commonly in SSPE, including central retinal (macular) hyperpigmentation and inflammation, optic nerve atrophy, peripheral retinitis, and ocular motility disorders. Cortical blindness can occur in the absence of ocular involvement.

The most common ocular complication of mumps virus infection is dacryoadenitis, and this may occur concurrently with parotid gland involvement. Aseptic meningitis, associated oculomotor palsy, and optic neuritis also occurs. Follicular conjunctivitis, epithelial and stromal keratitis, iritis, trabeculitis, and scleritis have all been reported within the first 2 weeks after onset of parotitis.

Acute hemorrhagic conjunctivitis (AHC), caused predominantly by enterovirus type 70 and coxsackievirus A24 variant, but also by adenovirus type 11, is one of the most dramatic ocular viral syndromes. Sudden onset of follicular conjunctivitis associated with multiple petechial conjunctival hemorrhages characterizes AHC. The hemorrhages may become confluent and appear post-traumatic. In approximately 1 out of every 10 000 cases due to enterovirus type 70, a polio-like paralysis can ensue. Neurologic deficits are permanent in up to one-third of the affected individuals.

Human immunodeficiency virus (HIV) is the etiologic agent of the acquired immune deficiency syndrome (AIDS). Although HIV can be cultured from the retinas of individuals with AIDS, and has been shown to be present in the donated corneas of deceased AIDS patients, a direct relationship between local viral infection and ocular disease remains to be established. One example is the dry eye so common in AIDS patients. It is not known whether primary HIV infection of the lacrimal gland, immune deficit-induced potentiation of another virus such as Epstein–Barr virus within the lacrimal gland, or a putative HIV-induced neuro-immune-endocrine defect can account for AIDS-related dry eye. However, the severe immunosuppression of AIDS results in a host of other ocular diseases (discussed below).

Ocular Disease Caused by DNA Viruses

DNA viruses (**Table 2**) are responsible for most significant ocular viral infections in the industrialized world. Even the protean ocular manifestations of the HIV, an RNA virus, result largely from reduced immunity to DNA viruses.

Adenovirus is probably the most common DNA virus to cause eye disease. Three common ocular syndromes have been identified. Simple follicular conjunctivitis occurs with infection by many adenovirus types and may be subclinical. Pharyngoconjunctival fever typically follows infection with adenovirus types 3, 4, and 7. As the name implies, patients have pharyngitis, conjunctivitis, and fever, and may be misdiagnosed as having influenza. Epidemic keratoconjunctivitis, most often caused by adenovirus types 8, 19, and 37, is a highly contagious syndrome with significant morbidity. The conjunctivitis can be severe (**Figure 3**); associated inflammatory conjunctival membranes can permanently scar the eyelids to the globe. Corneal involvement begins as a punctate epithelial keratitis and may proceed to a large central epithelial ulcer. Stromal keratitis presents about 2 weeks after the conjunctivitis as multifocal subepithelial corneal infiltrates, and causes both foreign body sensation and reduced vision. The stromal infiltrates may resolve spontaneously, but can become chronic, require long-term treatment with corticosteroids, and cause persistent visual morbidity. A fourth ocular syndrome occasionally associated with adenovirus infection, AHC (discussed above), may be caused by adenovirus type 11. Interestingly, adenovirus type 11 also causes acute hemorrhagic cystitis. Follicular conjunctivitis (clinically indistinguishable from adenovirus conjunctivitis) can also be caused by Newcastle disease virus, an RNA virus that gives rise to fatal epidemics in poultry and infects the birds' human handlers.

The human herpes viruses are preeminent among DNA viruses in eye disease with at least seven of the eight known human herpes viruses associated with ocular disorders. Herpes simplex virus type 1 (HSV-1) is the most common herpes virus to cause eye disease, and herpes simplex keratitis is the most common cause of infectious blindness in the industrialized world. HSV-1 causes self-limited and relatively benign infections of the

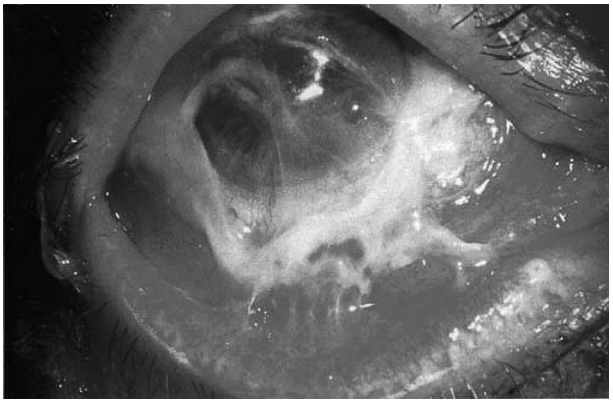


Figure 3 Epidemic keratoconjunctivitis. Infection with adenovirus serotype 19 has resulted in severe ocular surface inflammation.

eyelids, the conjunctiva, and the corneal epithelium, but infections of the corneal stroma, uvea, and retina may result in chronic or recurrent blinding stromal keratitis, uveitis, and retinal necrosis, respectively. Elevation of intraocular pressure due to involvement of the trabecular meshwork is not uncommon and may help to differentiate herpetic uveitis from noninfectious causes. Postnatally acquired HSV-2 ocular infection, less common than HSV-1, causes disease similar in most respects to HSV-1. Neonatal herpes simplex infection, acquired during transit through the birth canal and usually due to HSV-2, commonly causes vesicular blepharitis and conjunctivitis, but can also cause permanent visual loss due to keratitis, chorioretinitis, optic neuritis, and encephalitis of the visual cortex.

Varicella zoster virus, the etiologic agent of chickenpox and shingles, rarely causes keratouveitis with primary infection (chickenpox). However, vision-threatening keratitis, uveitis, and, less commonly, retinal necrosis are complications of varicella zoster virus reactivation in the distribution of the fifth cranial nerve (zoster ophthalmicus). Lid ulceration with frank tissue loss or lid malposition leads to corneal exposure and ulceration. Optic neuritis and cranial nerve paresis can accompany onset of the zoster rash. Sectoral iris atrophy is pathognomonic for zoster ophthalmicus. Postinfectious corneal anesthesia and secondary sterile corneal ulceration may follow herpes simplex types 1 and 2, but are most severe in zoster ophthalmicus. Chronic scleritis, keratitis, uveitis, and glaucoma may ultimately limit the visual acuity.

Acute systemic infection with Epstein–Barr virus may cause conjunctivitis and epithelial keratitis. Stromal keratitis occurs but is difficult to differentiate clinically from herpes simplex keratitis, and the true incidence of Epstein–Barr viral keratitis is unknown. Reports of uveitis and retinochoroiditis are unconfirmed. Delayed-onset optic neuritis following infectious mononucleosis is not uncommon.

Human cytomegalovirus (CMV) typically causes infectious retinitis (**Figure 4**) in immunocompromised patients with $CD4^+$ T-cell counts of less than $50\,\text{cells}\,\text{ml}^{-1}$. Although not the most common ocular complication of AIDS, CMV retinitis is the most common cause of blindness in AIDS patients. CMV retinitis in AIDS patients can be controlled but not cured. In contrast, congenital CMV infection in an otherwise normal fetus results in various degrees of retinochoroiditis, but is not progressive postnatally.

Human papillomavirus (HPV) causes a range of conjunctival tumors ranging from venereally acquired benign papillomas (HPV types 6 and 11) to invasive squamous cell carcinoma (**Figure 2**) (HPV types 16 and 18). Venereal papillomas are clinically similar to those of the larynx and anogenital tract. Ocular surface squamous neoplasia (conjunctival intraepithelial neoplasia and invasive squamous cell carcinoma) are most similar to dysplastic intraepithelial and invasive squamous lesions of the uterine cervix. Papillomatous eyelid neoplasms due to HPV also occur, and can be benign or malignant.

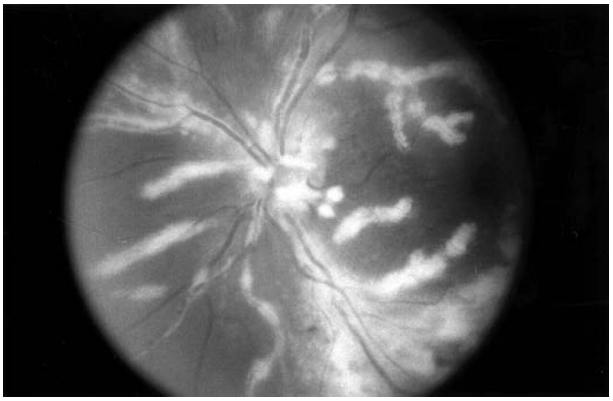


Figure 4 Cytomegalovirus retinitis. Discrete areas of perivascular necrosis and hemorrhage are typical.

Molluscum contagiosum virus is a poxvirus that may infect the eyelid skin or less commonly the conjunctiva. Skin lesions typically appear as elevated nodules with umbilicated centers, and may be multiple and quite large in HIV-infected patients. Molluscum lesions of the eyelid are fairly common in children, and can be associated with a follicular conjunctivitis that resolves with incisional or excisional biopsy of the lid lesion.

Prior to eradication, smallpox virus infection was associated with pustular blepharoconjunctivitis, secondary lid scarring, and stromal keratitis. In nonindustrialized nations, secondary bacterial infection of smallpox keratitis was a major source of blindness. Vaccination against smallpox virus with vaccinia virus was occasionally complicated by inadvertent autoinoculation of vaccinia into the eye, with potential for a severe blepharoconjunctivitis, keratitis, and globe perforation.

Ocular Complications of AIDS

Tay-Kearney and Jabs (1996) classified the ocular complications of HIV infection into five broad categories: (1) HIV retinopathy, (2) opportunistic ocular infections, (3) ocular adnexal neoplasms, (4) neuro-ophthalmic lesions, and (5) drug-induced manifestations.

HIV retinopathy is seen in over half of AIDS patients; cotton wool patches, or multifocal infarcts of the retinal nerve fiber layer, are the most common ocular sign of AIDS. Intraretinal hemorrhages occur less often. HIV can

be cultured from the retina of AIDS patients, but a direct relationship between retinal infection and AIDS retinopathy has not been established.

Some ocular infections, including CMV retinitis (**Figure 4**), *Pneumocystis carinii*, fungal, and mycobacterial choroiditis, and microsporidial keratoconjunctivitis are seen almost exclusively in AIDS. CMV retinitis is a major cause of morbidity in AIDS patients. Other infections, such as toxoplasmosis retinochoroiditis, ocular syphilis, herpes zoster ophthalmicus, and molluscum contagiosum of the eyelids are seen in immunocompetent as well as immunosuppressed individuals, but may be more severe and leave more profound deficits in HIV-infected patients. Herpes zoster ophthalmicus in young patients may be the first clinical clue to HIV infection. Acute retinal necrosis due to herpes simplex virus types 1 or 2, or varicella zoster virus, occurs more commonly in HIV-infected than in otherwise normal patients and can result in unilateral or bilateral blindness despite antiviral therapy.

Kaposi sarcoma of the eyelids or conjunctiva, associated with human herpes virus 8 infection, is exceedingly uncommon in immunocompetent individuals, but is probably the most common adnexal tumor in AIDS patients. Non-Hodgkin's lymphomas of the orbit, although rare overall, occur more frequently in AIDS patients than in the general population. Recently, squamous cell carcinoma of the ocular surface (conjunctiva and cornea) has been suggested as a marker for AIDS, but whether HIV infection potentiates HPV-induced carcinogenesis in the eye remains speculative.

Neuro-ophthalmic lesions in AIDS may occur directly due to HIV infection of the central nervous system, but most commonly are caused by cryptococcal meningitis or other opportunistic infections. Retinitis and uveitis due to anti-HIV medications can be confused with opportunistic intraocular infections.

Conclusion

Diverse ocular tissues act in concert to create vision. All of the tissues and structures within the eye are susceptible to viral infection, with consequences ranging from mild discomfort to severe pain and blindness, and almost all known human viruses cause ocular disease. Often, the same virus can infect widely disparate tissues within an eye. Classical viral pathogenic mechanisms are readily demonstrated in the eye, but the fine functions of ocular tissues within the visual axis (cornea, anterior chamber, lens, vitreous, and macula) compel altered immune responsiveness. The eye is uniquely affected by viral infection and provides an exceptional model for studies of viral pathogenesis and immunity.

Acknowledgments

This work is supported by US Public Health Service grants, R01 EY13124 and P30 EY12190, and a Physician-Scientist Merit Award (to J. Chodosh) from Research to Prevent Blindness, New York, NY.

See also: Equine Infectious Anemia Virus; Feline Leukemia and Sarcoma Viruses.

Further Reading

Biswas PS and Rouse BT (2005) Early events in HSV keratitis – setting the stage for a blinding disease. *Microbes and Infection* 7: 799–810.

Bonfioli AA and Eller AW (2005) Acute retinal necrosis. *Seminars in Ophthalmology* 20: 155–160.

Brandt CR (2005) The role of viral and host genes in corneal infection with herpes simplex virus type 1. *Experimental Eye Research* 80: 607–621.

Chodosh J and Stroop WG (1998) Introduction to viruses in ocular disease. In: Tasman W and Jaeger EA (eds.) *Duane's Foundations of Clinical Ophthalmology*, pp. 1–10. Philadelphia: Lippincott-Raven.

Cunningham ET, Jr., and Margolis TP (1998) Ocular manifestations of HIV infection. *New England Journal of Medicine* 339: 236–244.

Darrell RW (ed.) (1985) *Viral Diseases of the Eye.* Philadelphia: Lea & Febiger.

Garg S and Jampol LM (2005) Systemic and intraocular manifestations of West Nile virus infection. *Survey of Ophthalmology* 50: 3–13.

Goldberg DE, Smithen LM, Angelilli A,, and Freeman WR (2005) HIV-associated retinopathy in the HAART era. *Retina* 25: 633–649.

Green LK and Pavan-Langston D (2006) Herpes simplex ocular inflammatory disease. *International Ophthalmology Clinics* 46: 27–37.

Liesegang TJ (2004) Herpes zoster virus infection. *Current Opinion in Ophthalmology* 15: 531–536.

Natarajan K, Shepard LA,, and Chodosh J (2002) The use of DNA array technology in studies of ocular viral pathogenesis. *DNA and Cell Biology* 21: 483–490.

Pepose JS, Holland GN and Wilhelmus KR (eds.) (1995) *Ocular Infection & Immunity.* New York: Mosby.

Streilein JW (1996) Ocular immune privilege and the Faustian dilemma. *Investigative Ophthalmology & Visual Science* 37: 1940–1950.

Tay-Kearney ML and Jabs DA (1996) Ophthalmic complications of HIV infection. *Medical Clinics of North America* 80: 1471–1492.

Human Herpesviruses 6 and 7

U A Gompels, University of London, London, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The roseoloviruses human herpesviruses 6 and 7 (HHV-6 and HHV-7) infect almost all babies to give an 'infant fever', sometimes with rash (then termed 'exanthema subitum' or 'roseola infantum'), and persist throughout the host's lifetime. Infections with these viruses are generally regarded as benign and self-limiting, although severe complications have been recorded, with occasional associated fatalities, during some primary as well as secondary, reactivated infections in immunosuppressed (human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) or transplantation) patients. These viruses infect and can remain latent in leukocyte stem cells and neuronal cell types, with lytic replication in CD4+ T-lymphocytes. Immunomodulatory gene products can aid persistence and contribute to pathogenesis. There are two sides to this. On the one hand, studies on such a widespread, well-adapted virus allow a greater understanding of human immunity to infection and the possible development of new immunotherapies. On the other hand, recent associations of HHV-6 (in particular) with neurological disease, including 'status epilepticus', encephalitis, and multiple sclerosis (MS), have led to reevaluations of the pathogenic potential of the roseoloviruses. To date, there are no licensed vaccines or antiviral treatments available.

History and Classification

The first isolates of HHV-6 were characterized in 1986–88 in the USA (strains GS and Z29) and the UK (strain U1102), followed by Japan (strain HST). Using reagents derived from these laboratory strains, it was found that there are two strain groups, termed variants, HHV-6A (strains GS and U1102) and HHV-6B (strains Z29 and HST). Subsequently, HHV-6B was shown to be more prevalent in these countries, and most of the available data concern this variant. In comparison, HHV-6A appears to be an emergent infection. Complete genome sequences were derived from plasmid clones for HHV-6A (strain U1102; 159 kbp; accession X83413) and HHV-6B (strain Z29; 162 kbp; accession AF157706) in 1995 and 1999, respectively. These laboratory strains have subsequently been denoted as the prototype reference strains. They were isolated from viruses reactivated from adult immunosuppressed HIV/AIDS patients from African countries: HHV-6A strain U1102 from Uganda and HHV-6B strain Z29 from the Democratic Republic of Congo. Other laboratory strains include HHV-6A strain GS, the first report of HHV-6 infection, which was isolated from an adult HIV/AIDS patient in the USA; HHV-6A strain AJ, from an adult HIV/AIDS patient in the UK; and HHV-6B strain HST, from a pediatric patient with 'exanthema subitum' in Japan. HHV-6B strain HST is the only isolate from primary childhood infection to have been sequenced completely (accession AB021506), and partial genome sequences are available for HHV-6A strains GS and AJ.

HHV-7 was isolated in 1990 from an immunocompetent blood donor. Infections with this virus appear less severe, although the virus is as widespread as HHV-6 and persists at higher levels in the blood (possibly lacking 'true latency'), with frequent secretions in saliva as with HHV-6. HHV-7 can reactivate latent HHV-6 infections. Both viruses appear to give rise to 'exanthema subitum', although HHV-6 infection is earlier and predominates. The genomes of two strains of HHV-7 have also been sequenced: JI (145 kbp; U43400) and RK (153 kbp; AF037218).

The species *Human herpesvirus 6* and *Human herpesvirus 7* are classified in genus *Roseolovirus*, subfamily *Betaherpesvirinae*, family *Herpesviridae*, and are related to genus *Cytomegalovirus* in the same subfamily. The human virus that is most closely related to HHV-6 and HHV-7 is therefore human cytomegalovirus (HCMV). The DNA sequences of the HHV-6A and HHV-6B variants are very closely related overall (>95%), with most variation located in repetitive sequences at the ends of the genomes. Hypervariable loci are also present in other regions of the genome mostly near the ends, and include genes encoding the viral chemokine vCCL (U83), immediate early transcriptional regulators (U86 and U90), glycoprotein gQ (U100), and, at the center of the genome, glycoprotein gO (U47). These regions of substantial variation presumably contribute to the different biological properties of the variants. Comparisons of the genomes of HHV-6B strains Z29 and HST show less variation (1–2%).

Geographical Distribution

Serological studies show that HHV-6 and HHV-7 are widespread throughout the world, with over 95% of adults infected. However, the geographical distribution of HHV-6A and HHV-6B is difficult to establish. There is currently no serological assay that can distinguish between the variants, since the genes encoding immunodominant proteins

belong largely to the well-conserved group. The variable genes, which are diagnostically attractive, do not encode consistently immunogenic proteins. Thus, current estimates of the distribution of HHV-6A and HHV-6B have been derived using conventional polymerase chain reaction (PCR) of virus DNA or reverse transcription-polymerase chain reaction (RT-PCR) of virus RNA from tissue, blood, saliva, or cerebrospinal fluid (CSF) samples, with the use of additional techniques such as quantitative PCR, restriction endonuclease digestion, and sequencing of variable genes. In analyses of specific patient groups in North America (USA), Europe (UK), and East Asia (Japan), HHV-6B causes most (>97%) of the primary pediatric infections that give rise to HHV-6-associated infant fever with or without the 'exanthema subitum' rash. HHV-6A is detected, but as a minor component in mother–child pairs. In studies of adult bone marrow transplantation (BMT) and solid organ transplantation patients in the USA and the UK, the distribution of the variants seems similar to that in childhood infections, with primarily HHV-6B identified. In contrast, HHV-6A and HHV-6B were shown to be equally prevalent in hospitalized febrile infants in southern Africa (Zambia). Thus, there appear to be geographical differences in the distribution of HHV-6A and HHV-6B.

Biological differences may also be driven by certain variable genes, thus compounding any differences in geographic distribution. For example, in comparison to HHV-6B, HHV-6A infections are more neurotropic and more prevalent in CSF samples and congenital infections, as well as in early infections of infants less than 3 months old. In analyses of adult lung samples in the USA, both HHV-6A and HHV-6B were detected with equal prevalence. Some analyses have detected HHV-6A DNA in sera but not in peripheral blood mononuclear cells (PBMCs). Thus, the biological compartmentalization of the variants *in vivo* may differ, contributing an additional dimension to the geographical distribution of the variants.

Cellular Tropism, Laboratory Culture, and Latency

HHV-6 and HHV-7 are T-lymphotropic and neurotropic viruses. They have both been adapted to grow in CD4+ T-leukemic cell lines: for example, J-JHAN (Jurkat), HSB2, and Molt-3 cells for HHV-6; and SupT-1 cells for HHV-6 and HHV-7. However, higher titers of both HHV-6 and HHV-7 are obtained in activated cord blood lymphocytes or mononuclear cells (CBLs or CBMCs) or in peripheral blood lymphocytes (PBLs) or PBMCs. Screening of these cells is required prior to use, as infection with laboratory strains can result in reactivation of resident latent virus from adult blood and occasionally from cord blood.

CD4+ T-lymphocytes are permissive for replication and virus production, as shown *in vitro* and *in vivo* during viremia from acute infection. Infection results in cell death by necrotic lysis, with associated apoptosis in bystander cells. Lytic replication results in a characteristic cytopathic effect of ballooning cells (cytomegalia) and fused, multinucleated cells (syncytia). Studies *in vitro* show that CD4+, CD8+, and γδ T-lymphocytes can be infected, particularly by HHV-6A, which may have a wider tropism. HHV-7 binds to CD4 on T-lymphocytes, contributing to cellular tropism. HHV-6 binds to CD46, a ubiquitous receptor, and there may also be a specific co-receptor.

Similar to HCMV, and a feature of the betaherpesviruses in general, latency occurs within myeloid subsets or bone marrow progenitor cells. There is also evidence, particularly for HHV-6A, for replication in glial cells, including oligodendrocytes, astrocytes and a microglial cell type with similarities to monocytic/macrophage cells, which may also be a site of persistence in the central nervous system (CNS). HHV-7 circulates at higher levels than HHV-6, and can reactivate HHV-6. Both HHV-6 and HHV-7 can be shed asymptomatically in saliva, and salivary glands may be a site of persistence.

Recent studies indicate that 0.2% or 0.8% of blood donors in Japan or the UK, respectively, exhibit germline integration of HHV-6 genomes (either HHV-6A or HHV-6B). How this relates to latency or pathogenesis is being evaluated, but it can lead to high levels of virus DNA being detected in the blood (in cells and in sera due to cell breakage) in the absence of virus production, although a limited number of genes may be expressed. Germline integration can confound diagnoses by quantitative PCR, since in these individuals high levels of DNA do not correlate with viremia or symptoms. However, gene expression from the integrated genomes may contribute to chronic disease or immunity to HHV-6 infection.

Genome Organization

The roseolovirus genomes consist of a large unique region (U) flanked by a terminal direct repeat (DR).

The variation in size among the genomes is primarily due to differences in the size of DR. DR is itself bounded by smaller repeats (t) that are related to human telomeric repeats, and there is some suggestion that this arrangement may mediate a latent chromosome-like state or facilitate the rare integrations into the human genome that are observed (see above). At the ends of DR are 'pac' sequences for cleavage and packaging of unit-length genomes from replicative head-to-tail concatamers. Other repetitive sequences with known functions include the origin of lytic DNA replication (ORI) in the center of the genome. This combines features of ORIs in

betaherpesviruses (e.g., HCMV) and alphaherpesviruses (e.g., herpes simplex virus (HSV)). HHV-6 ORI is relatively large, as in HCMV, and contains binding sites for an origin-binding protein (OBP), as in HSV. HHV-6 and HSV each encode an OBP, whereas HCMV lacks a homolog to this gene.

The protein-coding regions predicted from the genome sequences (see **Figure 1**) are available in the sequence database accessions. The prototypic HHV-6 genome layout from HHV-6A strain U1102 is shown in **Figure 1**, with main differences between HHV-6 and HHV-7 as marked and cited below. Approximately 30% of the genome is

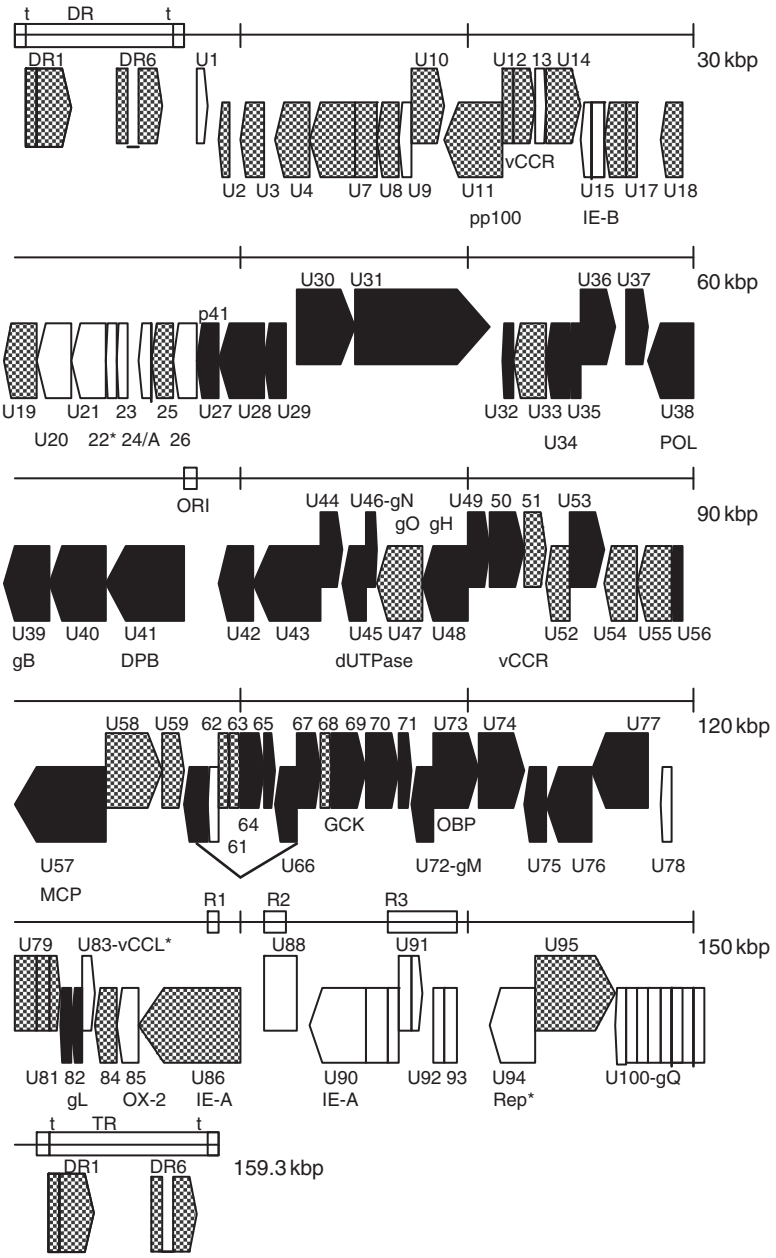


Figure 1 HHV-6 genome organization and locations of conserved genes. Protein-coding regions whose amino acid sequences are conserved among herpesviruses are indicated in black, those conserved only among betaherpesviruses are indicated as patterned, and those conserved in the roseoloviruses are indicated in white. HHV-6-specific genes are U22, U83, and U94 as asterisked, with additional possibly specific genes being U1, U9, U61, and U78. The U prefix is omitted from some gene names to enhance clarity. Repetitive sequences are marked: DR, terminal direct repeat; t, telomeric repeat; ORI, origin of lytic DNA replication; and R1, R2, and R3, locally repetitive sequences. Ancillary information is provided for certain genes mentioned in the text: p101, the major immunodominant tegument phosphoprotein; U94 Rep, the parvovirus Rep homolog and gene/replication regulatory latency gene; IE-A and IE-B, immediate early regulatory genes; POL, DNA polymerase; gB, gH, gL, gO, gM, gN, and gQ, envelope glycoproteins; vCCR, viral chemokine receptors; GCK, ganciclovir kinase; and chemokine vCCL.

taken up with genes encoding proteins that are conserved among mammalian and avian herpesviruses. These proteins dictate common features of replication, and include glycoproteins mediating virus entry by cell fusion, enzymes and accessory factors in the DNA replicase complex, and proteins for making the characteristic herpesvirus icosahedral nucleocapsid and packaging the genome into it. Proteins that are specific to betaherpesviruses are generally transcription factors controlling the gene expression cascade, functions for modulating the specificity of glycoproteins mediating virus entry by cell fusion, or proteins for modifying DNA virus replication. Functions specific to roseoloviruses that affect gene control and, in particular, immunomodulation presumably mediates latency and persistence.

Replication Cycle

Infection is initiated by virus binding to the cell, followed by penetration via virus-mediated cell fusion. As with other herpesviruses, HHV-6 and HHV-7 are first loaded onto the cell via proteoglycan interactions involving heparin or heparan sulfate. The herpesvirus-conserved gB and roseolovirus-specific gQ are involved at this stage. This is followed by membrane fusion mediated by the herpesvirus-conserved gH/gL complex. In HHV-6 and HHV-7, the gH/gL dimer also forms multimers, one with betaherpesvirus-specific gO, and the other with gQ (in two forms, gQ1 and gQ2). So far, no receptor has been identified for the gH/gL/gO complex, but the gH/gL/gQ1/gQ2 complex binds to the ubiquitous cellular receptor CD46 (which is also the measles virus co-receptor). The HHV-6A gH/gL/gQ1/gQ2 complex binds with much higher affinity than that of HHV-6B. HHV-7 additionally binds to the CD4 molecule (like HIV), but the virus attachment protein has not been identified.

Following transport of the nucleocapsid to the nucleus and release of the genome, expression of immediate early genes initiates the transcription cascade. The two spliced IE-A genes (U90 and U86) are positional counterparts of the well-characterized HCMV transcription factors, IE1 and IE2. U90 is particularly variable in sequence between HHV-6A, HHV-6B, and HHV-7. Like the corresponding region in HCMV, this major immediate early gene region is subject to CpG suppression, which is indicative of gene control by DNA methylation. This appears to be a common strategy for betaherpesviruses, which are latent in myeloid cells.

DNA replication is initiated at ORI utilizing a conserved replicase complex modified by the U55 protein, which corresponds to HCMV replication protein UL84, and also by the HHV-6-specific replication modulator, the U94 Rep protein. U94 Rep appears to be a latency-specific factor, and its absence from HHV-7 possibly contributes to the 'leaky latency' observed for this virus. The pac sites at the ends of the genome mediate cleavage of concatamers into unit-length genomes, which are then packaged into preformed nucleocapsids composed of herpesvirus-conserved capsid proteins. The virus buds through the nuclear membrane utilizing herpesvirus-conserved functions, and probably exits via the de-envelopment–re-envelopment pathway involving the *trans*-Golgi network (TGN), as has been shown for alphaherpesviruses.

Immunomodulation

HHV-6 and HHV-7 can have both direct and indirect modulatory effects on the immune system to favor persistence in immune cells. Direct effects include lysis of infected CD4+ lymphocytes during virus replication. In addition, apoptosis of bystander cells has been shown for both HHV-6- and HHV-7-infected cells. Indirect effects include modulation of the receptors on immune cells, thus affecting their function or specificity in immune activation or signaling. HHV-6 downregulates CD3 expression. Whereas HHV-7 downregulates CD4 expression, HHV-6 upregulates it. A protein encoded by both HHV-6 and HHV-7 (U21) downregulates major histocompatibility complex class I expression, which can lead to immune evasion of the antiviral TH1 cytotoxic T-cell response. A number of cytokines are dysregulated by HHV-6 infection, with IL-2 downregulated and IL-10, IL-12, TNFα, and IL-1 upregulated.

Like other primarily blood-borne herpesviruses, roseoloviruses engage the chemokine inflammatory system either to effect immune evasion or enhance virus dissemination. Homologs of chemokine receptors (vCCRs) are encoded by genes U12 and U51, and mimic properties of the human receptors in mediating immune cell traffic either constitutively, in homing to the lymph node, or inducibly, in the inflammatory response to infection. The HHV-6 vCCRs are betachemokine receptors that bind novel combinations of betachemokine ligands, including those with specificity for monocytic/macrophage cell types. Signaling by the U51 vCCR is affected by binding of different ligands and by G-protein levels in the cell. Early in infection when the U51 vCCR is produced, signaling leads to downregulation of the human CCR1-, CCR3-, and CCR5-specific chemokine Rantes/CCL5. In contrast, the HHV-7 vCCRs appear to mimic the constitutive homing chemokine receptors. HHV-6 infection can also mimic the constitutive receptor CCR7, which could allow homing of infected cells to sites of ligand secretion in the lymph node.

The HHV-6 U83 gene encodes a chemokine, vCCL. This gene is hypervariable between HHV-6A and HHV-6B but absent from HHV-7, and thus is a candidate for contributing to differences in virulence. HHV-6A vCCL has a high potency for human CCR1, CCR4,

CCR5, CCR6, and CCR8, which are present on mature monocytic/macrophage and T-cell subsets (TH2, skin homing) and immature dendritic cells. HHV-6B vCCL has a low potency for human CCR2 present on monocytes. These activities could aid immune evasion and virus dissemination, thus contributing to differences in tropism between the variants. They may also aid persistence via the chemoattraction of cell types for efficient antigen presentation and immune control.

In vivo reactivation of HHV-6 in BMT patients can lead to inhibition of outgrowth of cellular lineages. This is supported by *in vitro* data. HHV-6 infection is associated with monocyte dysfunction and suppression of macrophage maturation from bone marrow cultures. Similarly, HHV-6 infection can result in suppression of differentiation and colony formation from hematopoietic progenitor cells. For HHV-7, infection results in inhibition of megakaryocyte survival or differentiation.

Pathogenesis and Disease Associations

Infant Fever with or without Rash

HHV-6 and HHV-7 infect up to 95% of infants. Both HHV-6A and HHV-6B cause primary infant fever, and most infections occur following a drop in maternal immunity after 6 months of age. In the USA, HHV-6B accounts for 97% and HHV-6A for 3% of these infant infections. However, further studies in the USA and the UK show that in less common, earlier infections acquired either congenitally, during delivery, or heritably from germline integration, HHV-6A infections are more frequent, with a prevalence similar to that of HHV-6B. In southern Africa, infant febrile infections detected by PCR analysis of whole blood also indicate equal prevalence. Studies on acquisition of HHV-6B show that infection is symptomatic, with a fever of longer duration than other infections being the most common symptom (3–4 days, 39.4–39.7 °C in various studies). This is a frequent cause of referrals to physicians or hospital. HHV-7 is acquired later, in early childhood, with similar symptoms. As HHV-7 can reactivate HHV-6, the infections can be difficult to distinguish.

The main route of transmission appears to be spread by secretion of reactivated or persistent virus in the saliva from parents or siblings. Congenital infections with HHV-6, but not HHV-7, occur in 1% of births, a prevalence similar to that of HCMV, although HHV-7 has been detected in the placenta. Infections appear as asymptomatic compared to HCMV, although there is one report of neonatal HHV-6B infection associated with neurological disease, seizures, and mental retardation.

A minority of infants have other symptoms or complications, which may be mild or severe. Up to 10% have a skin rash similar to that of measles or rubella, which can confound diagnoses of those infections. This macular or papular rash spreads on face or trunk or both, and has previously been called 'exanthema subitum', 'roseola infantum', or sixth disease. The mean age for acquiring HHV-6 with rash is 7 months. Although this is primarily a benign, self-limiting disease, severe and occasionally life-threatening complications can develop, including lymphadenopathy, diarrhea, myocarditis, myelosuppression, and neurological disease. Indeed, HHV-6 has been reported to account for a third of childhood febrile seizures and direct or indirect mechanisms (via fever) are being evaluated. In the USA, where childhood infections by HHV-6B predominate, links with 'status epilepticus' have been suggested, HHV-6-associated childhood encephalitis cases can occasionally be fatal. The complications previously associated solely with HHV-6 rash are also found in HHV-6 infant fever without rash, and thus up to 90% of symptomatic HHV-6 infections may be undiagnosed or unrecognized.

Immunocompromised People

Solid organ transplantation

Owing to efficient and widespread early childhood transmission of HHV-6 and HHV-7, infections in the adult are primarily reactivations of latent virus, particularly in immunocompromised people such as transplantation patients. Where monitored, co-infections with HCMV present with more severe pathology ('CMV disease'), with a subgroup of delayed graft rejections in liver or kidney transplantation patients. HHV-6-linked encephalitis has occasionally been recorded in these patients, and also in cardiac and heart/lung transplantation patients. Immune suppression using monoclonal antibody to CD3 enhances HHV-6 reactivation and CMV disease; this treatment can also enhance HHV-6 replication *in vitro*. Where symptomatic correlates have been made, HCMV has a greater influence than HHV-6, and HHV-6 has a greater effect than HHV-7. Anecdotal correlates of HHV-6 infection in this context include fever, rash, pneumonitis, encephalitis, hepatitis, and bone marrow suppression. However, it is rare that HCMV reactivates in an HHV-6- or HHV-7-negative patient, as HHV-6 usually reactivates first, then HHV-7 concurrent with HCMV. Thus CMV disease is usually contingent on roseolovirus reactivation. An immunomodulatory role has been presented for HHV-6 reactivations that correlate with increased opportunistic infections, including those associated with HCMV and fungi. Occasional primary infections in the adult have been observed in rare HHV-6- or HHV-7-naive patients who become infected via the donor organ and experience more severe symptoms.

Bone marrow and hematopoietic stem cell transplantation

HHV-6 reactivation is associated with disease in BMT and hematopoietic or cord blood stem cell transplantation (SCT). HHV-6B predominates in Europe and the USA,

where typing has been done, reflecting the prevalence of this variant in primary infections of children there. Half of the patients have HHV-6 reactivations 2–4 weeks post-transplantation, with both direct and indirect immunomodulatory effects. Direct effects of reactivation can lead to HHV-6-associated encephalitis and reduced outgrowth of progenitor lineages, called 'bone marrow suppression' or stem cell inhibition. Indirect immunomodulatory effects leading to HCMV reactivation have also been recorded. The presence of virus DNA in the blood has been demonstrated by quantitative PCR, prior to encephalitis or CNS disease developing by 2–3 weeks post-transplantation. The highest levels were shown for cord blood SCT. Retrospective studies have shown that both HHV-6 reactivation and the use of monoclonal antibody to CD3 are independent risk factors for the development of encephalitis. Amnesia, delirium, or 'confusion' following HHV-6 reactivation have been reported, with estimates of 40% mortality for HHV-6-linked encephalitis in SCT patients. Further investigations are required, and should include possible complications in future stem cell therapy; prospective studies are underway to assess neurological involvement in BMT and SCT.

HIV/AIDS

Like HIV, both HHV-6 and HHV-7 can infect and kill or remain latent in CD4+ T-lymphocytes as well as monocytic/macrophage cells. In addition to direct effects via reactivation of HHV-6 and HHV-7, there may also be indirect interactions between these viruses. As the CD4 count in blood decreases with AIDS progression, so does detection of HHV-6 and HHV-7. However, in studies of disseminated reactivated infections, increased levels of HHV-6 DNA in various organs correlate with higher levels of HIV, suggesting interactions during AIDS progression. HHV-6 reactivations have been identified with HIV in lymph nodes, and have been associated with the early phases of lymphadenopathy syndrome. Individual case studies have recorded HHV-6A-associated fatalities in HIV/AIDS patients from pneumonitis and encephalitis. Furthermore, HHV-6-associated retinitis (on its own or in association with HCMV) has been identified in HIV/AIDS patients. However, the use of antiretroviral therapy in some countries has restored immune control of opportunistic infections such as HCMV, and probably HHV-6 and HHV-7.

Studies *in vitro* and in animal models point to possible mechanisms of interaction between HIV and HHV-6. HHV-6A has been shown to reactivate HIV from latency in monocytes *in vitro*. Ongoing studies of HHV-6A co-infection with simian immunodeficiency virus (SIV) in monkey models show enhanced disease. HHV-7 binds to CD4 and can compete with HIV *in vitro* for interaction with this receptor, and so inhibit infection. HHV-6A can induce CD4, thus enhancing infection with HIV in different cell types *in vitro*, whereas HHV-6A chemokine U83A can bind to CCR5, the HIV co-receptor, and inhibit infection. However, the HHV-6A U51 vCCR can bind, sequester, and downregulate the CCR5 ligand and the HIV-1 inhibitor, Rantes/CCL5, and thus enhance HIV-1 infection. Expression of the other HIV-1 co-receptor, CXCR4, is downregulated by HHV-6 infection and may inhibit progression. Thus, complex interactions between HIV-1 and HHV-6 may either inhibit or enhance HIV-1, depending on virus strain, specific gene expression, cell type, and stage of the lytic/latent life cycle.

Neuroinflammatory Disease and Persistence in the Brain

HHV-6 and HHV-7 have been identified as commensals in the brain, with latent or persistent infection in the CNS, and it is a major challenge to distinguish these silent infections from any locally activated ones associated with neurological disease. This problem is further complicated by the rare occurrence of germline integration of HHV-6A or HHV-6B as noted above. In studies of postmortem brain samples, HHV-6 has been identified more frequently than HHV-7 (*c.* 40% compared to 5%). Where genotyped, 75% of the HHV-6 was HHV-6B, reflecting the greater prevalence of this variant in childhood infections in the populations studied (China and the UK).

In the case of encephalitis, most data concern HHV-6, where active infections have been detected by a combination of identifying virus DNA in the CSF and sera and, where biopsies are available, by *in situ* hybridization in order to detect expression of lytic genes in various temporal classes. RT-PCR of virus RNA in blood may also identify specific viremia, but may often miss relevant localized reactivations in the CNS. Encephalitis associated with HHV-6A and HHV-6B has been identified as a rare complication during primary infection in childhood, and in adults. As described above, SCT and BMT patients are at most risk, since frequent HHV-6 infection/reactivation is linked with life-threatening HHV-6-associated encephalitis; further prospective studies are required.

Links with other neurological conditions have been described, including that of HHV-6B with 'status epilepticus' noted above, and prospective studies are underway. Evidence for active lesions associated with mesial temporal lobe specific regions of the brain, particularly the hippocampus, has been presented and evaluated for links with mesial temporal lobe epilepsy. Interestingly, this region of the brain is also associated with memory, and virus reactivation here may correlate with observations of amnestic periods linked with HHV-6 reactivations in some SCT and BMT patients. Neurological symptoms associated particularly with HHV-6A reactivations have also been linked with subsets of chronic fatigue syndrome patients (and in some cases termed HHV-6A encephalopathy), but the data are conflicting and prompt further studies.

Multiple sclerosis

Roseoloviruses have also been linked with a subset of MS patients, in particular the active HHV-6A infections in a subset of the relapsing remitting form of the disease, and in some studies they have been correlated with exacerbations. These HHV-6A infections occur in countries where the most prevalent childhood infections would be HHV-6B, although there are also some data for multiple reactivations along with HHV-6B or HHV-7. Thus, HHV-6A infections linked with MS could represent rare primary infections occurring during adulthood with this more neurotropic variant. Alternatively, an underlying condition may permit infection with multiple strains.

There is some evidence supporting mechanisms that link HHV-6 with MS. In one study, T-cells from MS patients had lower frequencies of activity against the immunodominant HHV-6 p101 (or pp100) protein (U11), suggesting defects in virus clearance. Furthermore, *in situ* hybridization and immunostaining of biopsy material from MS lesions showed the presence of HHV-6 DNA in oligodendrocytes, lymphocytes, and microglia, plus antigen expression in astrocytes and microglia. Studies on an oligodendrocyte cell line showed some productive infection with HHV-6A but abortive infection with HHV-6B; similar results were found using progenitor-derived astrocytes. *In vitro* studies on glial precursor cell infection by HHV-6 suggest that early virus proteins act in cell cycle arrest, thus inhibiting differentiation to the myelin-producing oligodendrocytes essential for repair of demyelination. In a study conducted in the USA, β-interferon treatment of MS patients resulted in lower amounts of detectable HHV-6 in sera. β-interferon also inhibited growth of HHV-6 cell culture. It has been suggested that tests with antiviral agents such as ganciclovir (GCV) will be required to examine the role of HHV-6 in MS. This will require careful selection of patients and use of appropriate controls, particularly if HHV-6 contributes only to a subsection of this disease.

Immune Response, Diagnosis, and Control

Protective Immunity

In immunocompetent people, HHV-6 and HHV-7 infections give rise to lifelong protective immunity. However, subsequent infections with multiple strains, either HHV-6A or HHV-6B, have been recorded even during early primary infection, with milder or no symptoms. Prior infection with HHV-6 does not prevent infection with HHV-7, but it may provide some cross-protective immunity.

Neutralizing antibodies can be generated after primary infection. Targets include gB as well as the gH/gL/gQ complex. As with other infections, not all antibodies are neutralizing, and thus high serological titers do not always correspond to protection against infection.

Cellular immunity also develops in response to these infections. It is usually HHV-6- or HHV-7-specific, but sometimes cross-reactive. HHV-6- or HHV-7-specific CD4+ T- and NK-cell clones have been isolated, as well HHV-6 antigen-specific clones to p101. Thus, efficient cellular immunity develops after infection, and immunomodulation of lymphoid and myeloid cells may contribute to a possible strategy for effective persistence.

Control

Compounds with activity specifically against HHV-6 are not available and require development. However, HHV-6 and HHV-7 share with HCMV genes encoding proteins involved in replication, and these serve as targets for certain antiviral compounds. Thus, many drugs that are active against HCMV and other herpesviruses also show some activity against HHV-6 and HHV-7. These include GCV, foscarnet, and cidofovir. Acyclovir, which is used to treat alphaherpesvirus, and sometimes gammaherpesvirus, infections, is not effective *in vitro* against HHV-6 or HHV-7, as these viruses do not have the thymidine kinase gene required for activity. The oral prodrug form of GCV (valganciclovir, vGCV) has some anecdotal efficacy during virus reactivations in BMT patients, but is not as efficacious as it is with HCMV, and some HHV-6 or HHV-7 antigenemia can still be observed after vGCV prophylaxis. However, as for HCMV, HHV-6 GCV-escape mutants have been observed *in vivo* in transplantation patients, indicating that there is some efficacy against virus replication. Mutations occur in gene U69, which encodes the homolog of the HCMV GCV kinase (GCK), or in gene U38, which encodes the DNA polymerase (POL). *In vitro*, the HHV-6 GCK is tenfold less active than that of HCMV, which perhaps explains the differences in response between these two betaherpesviruses. Prospective clinical trials are required to demonstrate clinical efficacy *in vivo*, and are underway with vGCV for herpesvirus-associated CNS disease.

Acknowledgments

The author is grateful for support from the Biotechnology and Biological Sciences Research Council (BBSRC, UK) and the Bill and Melinda Gates Foundation.

See also: Central Nervous System Viral Diseases; Cytomegaloviruses: Murine and Other Nonprimate Cytomegaloviruses; Cytokines and Chemokines; Defective-Interfering Viruses; Emerging and Reemerging Virus Diseases of Plants; Herpes Simplex Viruses: General Features; Herpesviruses: General Features; Human

Cytomegalovirus: General Features; Human Cytomegalovirus: Molecular Biology; Human Eye Infections; Organ Transplantation, Risks; Viral Receptors.

Further Reading

DeBolle L, Michel D, Mertens T, *et al.* (2002) Role of the human herpesvirus 6 U69-encoded kinase in the phosphorylation of ganciclovir. *Molecular Pharmacology* 62: 714–721.

Dewin DR, Catusse J, and Gompels UA (2006) Identification and characterization of U83A viral chemokine, a broad and potent beta-chemokine agonist for human CCRs with unique selectivity and inhibition by spliced isoform. *Journal of Immunology* 176: 544–556.

Dietrich J, Blumberg BM, Roshal M, *et al.* (2004) Infection with an endemic human herpesvirus disrupts critical glial precursor cell properties. *Journal of Neuroscience* 24: 4875–4883.

Dominguez G, Dambaugh TR, Stamey FR, Dewhurst S, Inoue N, and Pellett PE (1999) Human herpesvirus 6B genome sequence: Coding content and comparison with human herpesvirus 6A. *Journal of Virology* 73: 8040–8052.

Fotheringham J, Akhyani N, Vortmeyer A, *et al.* (2007) Detection of active human herpesvirus-6 infection in the brain: Correlation with polymerase chain reaction detection in cerebrospinal fluid. *Journal of Infectious Diseases* 195: 450–454.

Gompels UA (2004) Roseoloviruses: Human herpesviruses 6 and 7. In: Zuckerman AJ, Banatvala JE, Pattison JR, Griffiths PD and Schoub BD (eds.) *Principles and Practice of Clinical Virology,* 5th edn., pp. 147–168. Chichester, UK: Wiley.

Gompels UA and Kasolo FC (2006) HHV-6 genome: Similar and different. In: Krueger G and Ablashi D (eds.) *Perspectives in Medical Virology, Vol. 12: Human Herpesvirus-6,* 2nd edn., pp. 23–46. Elsevier: New York.

Gompels UA, Nicholas J, Lawrence G, *et al.* (1995) The DNA sequence of human herpesvirus-6: Structure, coding content, and genome evolution. *Virology* 209: 29–51.

Goodman AD, Mock DJ, Powers JM, Baker JV, and Blumberg BM (2003) Human herpesvirus 6 genome and antigen in acute multiple sclerosis lesions. *Journal of Infectious Diseases* 187: 1365–1376.

Hall CB, Caserta MT, Schnabel KC, *et al.* (2006) Characteristics and acquisition of human herpesvirus (HHV)-7 infections in relation to infection with HHV-6. *Journal of Infectious Diseases* 193: 1063–1069.

Hall CB, Long CE, Schnabel KC, *et al.* (1994) Human herpesvirus-6 infection in children: A prospective study of complications and reactivation. *New England Journal of Medicine* 331: 432–438.

Isegawa Y, Mukai T, Nakano K, *et al.* (1999) Comparison of the complete DNA sequences of human herpesvirus 6 variants A and B. *Journal of Virology* 73: 8053–8063.

Ward KN, Leong HN, Thiruchelvam AD, Atkinson CE, and Clark DA (2007) Human herpesvirus 6 DNA levels in cerebrospinal fluid due to primary infection differ from those due to chromosomal viral integration and have implications for diagnosis of encephalitis. *Journal of Clinical Microbiology* 45: 1298–1304.

Zerr DM, Corey L, Kim HW, Huang ML, Nguy L, and Boeckh M (2005) Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. *Clinical Infectious Diseases* 40: 932–940.

Zerr DM, Meier AS, Selke SS, *et al.* (2005) A population-based study of primary human herpesvirus 6 infection. *New England Journal of Medicine* 352: 768–776.

Human Immunodeficiency Viruses: Antiretroviral Agents

A W Neuman and D C Liotta, Emory University, Atlanta, GA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Host cell A cell that has been infected by a virus.
Provirus A virus that has integrated itself into the DNA of a host cell.
Viral load A measure of the severity of a viral infection which is reported in nucleic acid copies per milliliter of blood.
Viral tropism The specificity of a virus for a particular host tissue.
Virion A single virus particle.

Introduction

In 2006, there were an estimated 39.5 million people worldwide living with human immunodeficiency virus (HIV). An estimated 4.3 million new infections arose in 2006, with 65% of those in sub-Saharan Africa. Acquired immune deficiency virus (AIDS)-related infections accounted for about 2.9 million deaths in 2006 and over 25 million deaths since the syndrome was first recognized in 1981. AIDS is now recognized as one of the most destructive pandemics in recorded history, and with no effective vaccine or cure, treatment of the disease relies on antiretroviral therapy.

The life cycle of HIV begins with the attachment of the virion to the target cell via CD4 binding followed by binding to co-receptor, CCR5 or CXCR4. The viral lipid membrane then fuses to the host cell membrane, allowing the viral core, encapsidating HIV RNA and enzymes, to enter the host cell cytoplasm. HIV RNA subsequently undergoes reverse transcription into DNA, a process mediated by reverse transcriptase. The viral DNA enters the host cell nucleus and is integrated into the cellular DNA by the enzyme integrase. At this point, the provirus may remain latent for up to several years. When the host cell receives a signal to become active, the proviral DNA is transcribed into mRNA. The mRNA is

transported out of the nucleus and is translated into polyproteins. The polyproteins assemble to form an immature virus particle, which then buds out from the host cell. The enzyme protease cleaves these polyproteins into functional proteins, creating a mature virus.

Currently, 25 antiretroviral drugs are available, targeting three steps of the HIV life cycle: a fusion inhibitor, reverse transcriptase (RT) inhibitors (including two classes, nucleoside reverse transcriptase inhibitors (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs)), and protease inhibitors (PIs). Additional agents are being developed to target other steps in the viral life cycle. See **Table 1** for a list of antiretroviral drugs in development.

Most HIV patients are treated with highly active antiretroviral therapy (HAART), usually a combination of three antiretroviral agents. This treatment paradigm can often reduce a patient's viral load to below detectable levels for prolonged periods. The use of HAART over the past decade has led to significant declines in HIV-associated morbidity and mortality.

Entry Inhibitors

HIV entry into target cells is mediated by the envelope protein (Env), which is comprised of two glycoprotein subunits, the surface protein gp120 and transmembrane subunit gp41. Three distinct steps are required for the entry of HIV into host cells. First, the virus attaches to the cell surface via CD4 binding, changing the conformation of Env and exposing a co-receptor binding site on gp120, allowing the viral glycoprotein to bind to one of the co-receptors, CCR5 or CXCR4. The three gp41 glycoproteins in the trimeric envelope complex subsequently form a 6-helix bundle from interaction of the heptad repeat (HR-) 1 and HR2 domains present in the ectodomain of each subunit. The 6-helix bundle brings the viral and cellular membranes into close apposition and mediates their fusion. These three steps – attachment, co-receptor binding, and fusion – can be targeted by attachment inhibitors (including CD4 binding inhibitors), co-receptor binding inhibitors, and fusion inhibitors, respectively.

HIV entry inhibitors have recently exploded as potential therapeutic agents. The main reason for the great interest in this field is that entry inhibitors offer the opportunity to prevent infection of new target cells.

The CD4 receptor was identified in 1984 as the primary cellular receptor for HIV. Because gp120 is responsible for binding to the CD4 receptor on the surface of the host cell, the CD4–gp120 interaction is an attractive target for HIV therapy.

Several years after the discovery of CD4, it was determined that CD4 alone is insufficient to permit HIV infection. In 1996, CCR5 and CXCR4 were identified as the major co-receptors. It was determined that R5 tropic viruses, those which bind to CCR5, are involved in HIV transmission based on the discovery that CCR5Δ32 homozygotes are highly resistant to HIV infection. These individuals, mostly of northern European origin, have a 32 bp deletion in both copies of their CCR5 gene, which confers a recessive phenotype that is associated with resistance to HIV-1 infection and antibody production.

The CXCR4 and CCR5 co-receptors are appealing targets; however, their implementation will require viral phenotyping for co-receptor tropism. Patients infected with an X4 virus, for example, would need to be treated with a CXCR4-specific inhibitor.

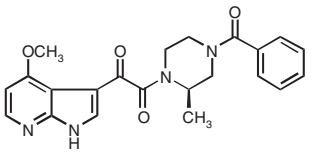
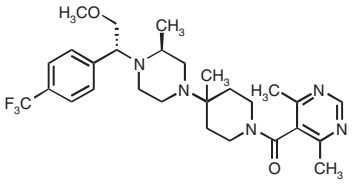
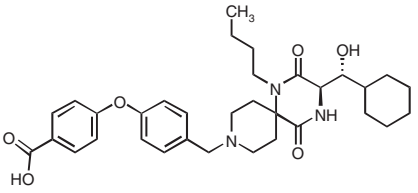
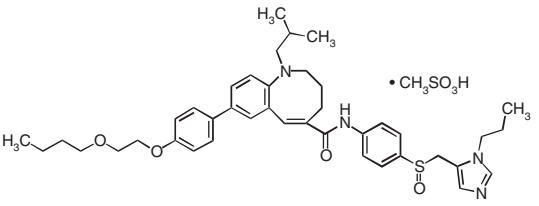
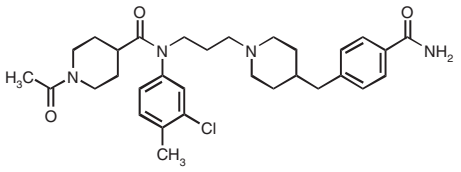
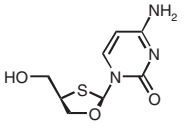
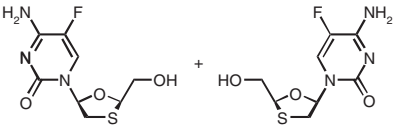
Maraviroc is a small molecule which became the first-in-class CCR5 antagonist and the first oral entry inhibitor with its FDA approval in August 2007 (**Table 2**). Maraviroc monotherapy including twice-daily doses of 300 mg led to a viral load reduction of 1.6 $\log_{10}$ copies ml^{-1}. In phase III studies, twice as many patients taking maraviroc along with optimized combination therapy as those taking a placebo with combination therapy had an undetectable viral load (less than 40 copies ml^{-1}) after 48 weeks.

Several other attachment and co-receptor binding inhibitors are currently in development, and biological data have been reported for the drugs in more advanced stages. TNX-355 is a monoclonal antibody which inhibits the interaction between gp120 and CD4. Intravenous infusion of 10 mg kg^{-1} led to a viral load reduction of 1.33 $\log_{10}$ copies ml^{-1}. Vicriviroc and INCB9471 are small-molecule antagonists of the CCR5 receptor. Twice-daily dosing of 50 mg of vicriviroc resulted in a viral load reduction of 1.62 $\log_{10}$ copies ml^{-1}. INCB9471 has recently been reported to show a 1.9 $\log_{10}$ copies ml^{-1} viral load reduction with 14 days of monotherapy. Additionally, the viral load continued to drop for several days after discontinuation of therapy.

The field of fusion inhibitors is led by enfuvirtide, a biomimetic 36-amino acid peptide that was approved for use in March 2003 (**Table 2**). Enfuvirtide is based on the sequence of the HR2 region of gp41. When the drug binds to HR1, HR2 is unable to bind and fusion cannot occur.

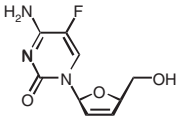
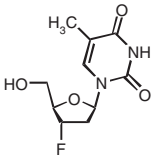
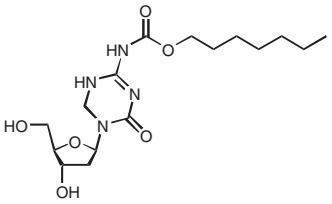
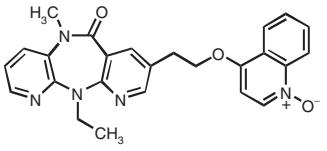
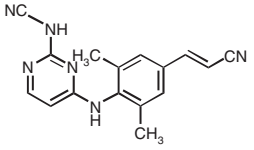
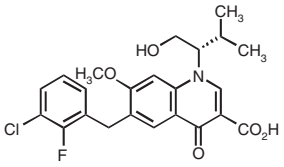
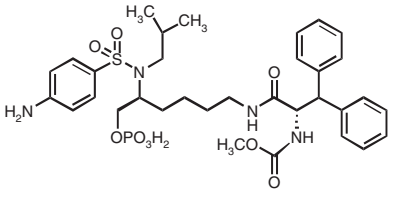
Enfuvirtide was shown to block virus-mediated cell-to-cell fusion with an IC_{90} of 1.5 ng ml^{-1}. A 15-day monotherapy study, with patients receiving 100 mg twice daily, resulted in an average plasma viral load reduction of 1.9 $\log_{10}$ copies ml^{-1}. The main disadvantage of enfuvirtide is its dosing regimen consisting of twice-daily subcutaneous injections. Furthermore, due to the complexity of its 106-step chemical synthesis, enfuvirtide is the most expensive antiretroviral drug on the market. Because of these obstacles, enfuvirtide is generally prescribed to patients for whom other antiretroviral agents have failed.

Table 1 HIV antiretroviral agents in clinical trials

Class	*Name*	*Structure*	*Phase of development*
Entry inhibitor (gp120-CD4)	BMS-378806		Phase I
Entry inhibitor (gp120-CD4)	TNX-355	Monoclonal antibody	Phase II
Entry inhibitor (CCR5)	Vicriviroc (SCH-D)		Phase II
Entry inhibitor (CCR5)	INCB9471	Structure not reported	Phase II
Entry inhibitor (CCR5)	PRO140	Monoclonal antibody	Phase I
Entry inhibitor (CCR5)	HGS004	Monoclonal antibody	Phase I
Entry inhibitor (CCR5)	AK602		Phase I
Entry inhibitor (CCR5)	TAK-652		Phase I
Entry inhibitor (CCR5)	TAK-220		Phase I
Fusion inhibitor	Suc-HSA	Succinylated human serum albumin	Phase I
Nucleoside reverse transcriptase inhibitor (NRTI)	Apricitabine (AVX754)		Phase II completed
NRTI	Racivir ±-FTC		Phase II completed

Continued

Table 1 Continued

Class	*Name*	*Structure*	*Phase of development*
NRTI	Elvucitabine		Phase II
NRTI	Alovudine		Phase II
NRTI	KP1461		Phase I
Non-nucleoside reverse transcriptase inhibitor (NNRTI)	BILR 355 BS		Phase II
NNRTI	Rilpivirine (TMC278)		Phase II
Integrase inhibitor	GS-9137		Phase II
Integrase inhibitor	GSK-364735	Structure not reported	Phase I
Protease inhibitor (PI)	PPL-100		Phase I

Continued

Table 1 Continued

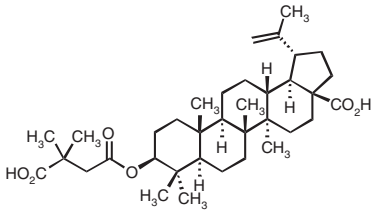
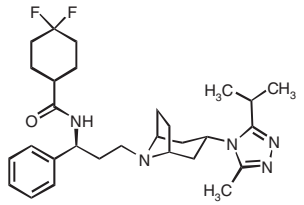
Class	*Name*	*Structure*	*Phase of development*
Maturation inhibitor	Bevirimat (PA-457)		Phase II

Table 2 FDA-approved HIV entry inhibitors

Name and structure	*Dosing schedule*[a]	*Major toxicity*[a]	*Key mutations conferring resistance*[b]
Acetyl-YTSLIHSLIEESQNQQEKN EQELLELDKWASLWNWF-amide Enfuvirtide (T-20, Fuzeon)	One subcutaneous injection of 90 mg b.i.d. into the upper arm, thigh, or abdomen	Injection site reactions (itching swelling, redness, pain or tenderness, hardened skin or bumps)	gp41 single point mutations between positions 36 and 45 gp41 double and triple point mutations between positions 36 and 45 gp41 mutations outside of positions 36–45 co-receptor tropism
Maraviroc (Selzentry) 	One 300 mg tablet b.i.d.	Liver toxicity, cough, pyrexia, upper respiratory tract infections, rash, musculoskeletal symptoms, abdominal pain, dizziness	Changes in the V3 loop, including A316T, and A319A/S in the CC1/85 strain and a deletion in RU570[c]

[a]The Body: The Complete HIV/AIDS Resource. http://www.thebody.com (accessed October 2007).
[b]Stanford University HIV Drug resistance Database. http://hivdb.stanford.edu (accessed October 2007).
[c]Westby M, Smith-Burchnell C, Mori J, *et al.* (2007) Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry. *Journal of Virology* 81: 2359–2371.

Reverse Transcriptase Inhibitors

Since the identification in 1983 of HIV as the etiological agent of AIDS, HIV RT has been one of the major targets for the development of antiretroviral drugs. HIV RT is a heterodimeric, RNA-dependent DNA polymerase (RdDp) that contains two subunits: the p66 catalytic unit and the p51 structural unit. Two classes of antiretroviral agents inhibit RT. These include NRTIs, which compete with normal 2′-deoxynucleoside triphosphates, and NNRTIs, which are allosteric, noncompetitive inhibitors of RT.

NRTIs are synthetic analogs of natural nucleosides that are incorporated into a nascent viral DNA chain. Because the NRTIs lack a 3′-hydroxyl group, incoming nucleotides are unable to form new phosphodiester linkages, therefore halting DNA replication (chain termination). NRTIs are administered as prodrugs, which must be phosphorylated to the active triphosphate before being incorporated into the DNA by HIV RT. Since these compounds enter the cell via passive diffusion, the prodrugs must be sufficiently lipophilic to penetrate the cell membrane.

Because NRTIs inhibit DNA polymerase, they can also affect normal cells. The drugs show much higher affinity for HIV reverse transcriptase compared to most human DNA polymerases; however, mitochondrial DNA polymerase is significantly inhibited by NRTIs. Various side effects of NRTIs can be attributed to mitochondrial dysfunction, including polyneuropathy, myopathy, cardiomyopathy, pancreatitis, bone-marrow suppression, and lactic acidosis.

Zidovudine, commonly known as AZT, was the first HIV drug approved by the FDA. AZT is a thymidine analog with an azido group replacing the 3′-hydroxyl group.

The azido group not only prevents binding of subsequent nucleosides to the DNA chain but also increases the lipophilicity of AZT, allowing it to easily cross the cell membrane.

Since the approval of AZT in 1987, six other NRTIs have come into the market: didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), abacavir (ABC), and emtricitabine (FTC). Zalcitabine is no longer manufactured as of February 2006. Although each of the NRTIs works by competitive inhibition of RT, they have significantly different dosing, toxicity, and resistance profiles (**Table 3**).

Several more NRTIs are in clinical trials. Apricitabine demonstrated a 1.65 $\log_{10}$ copies ml^{-1} viral load reduction after 10 days of 800 mg b.i.d. monotherapy. Other compounds in early clinical trials have not released efficacy data.

Nucleotide analogs, another type of RT inhibitors, are also available. While several nucleotide analogs are approved to treat various viruses, only tenofovir is approved for the treatment of HIV. Tenofovir is an acyclic nucleoside phosphonate analog that displays increased efficacy owing to its ability to bypass the nucleoside kinase step of activation. Tenofovir and other nucleoside phosphonate analogs also show activity against a broad range of DNA viruses and retroviruses (**Table 3**).

NNRTIs, on the other hand, are noncompetitive inhibitors that bind to a hydrophobic pocket near the polymerase active site. NNRTIs inhibit HIV-1 RT by locking the active catalytic site in an inactive conformation. Despite their structural diversity, most NNRTIs assume a similar butterfly-like structure consisting of two hydrophobic wings connected to a central polar body. The wing portions of the molecules contain significant π-electron systems and can act as π-electron donors to aromatic side chains of RT residues around the NNRTI binding pocket. Although NNRTIs bind at a common site on RT, they differ with regard to the exact amino acids of the binding site with which they interact. Therefore, NNRTIs show differences in their pharmacology and pharmacokinetic profiles, interactions with other drugs, and safety and toxicity profiles.

Four NNRTIs are currently licensed for clinical use: nevirapine, delavirdine, efavirenz, and etravirine. Nevirapine, a dipyridodiazepinone derivative, has been shown to reduce perinatal HIV transmission by 47% compared to treatment with AZT. However, the compound can cause life-threatening liver toxicity. Therefore, it is generally restricted to patients at lower risk for liver failure. Delavirdine is a bisheteroarylpiperazine derivative; it is not recommended as part of a first-line antiretroviral therapy program due to its inconvenient dosing regimen and lower efficacy compared to other NNRTIs. Efavirenz, a dihydrobenzoxazinone derivative, is the most commonly prescribed NNRTI due to its high efficacy, once-daily dosing, and relatively lower toxicity. Etravirine is second-generation NNRTI, a highly flexible diarylpyrimidine derivative with a higher genetic barrier to resistance than its precursors. It is the first NNRTI to demonstrate significant antiviral potency in patients with resistance to other NNRTIs.

Several other NNRTIs are currently in clinical trials and promise higher potency while eliciting fewer drug-resistant HIV strains. BILR 355 BS is a second-generation dipyridodiazepinone derivative that shows antiviral activity against a wide range of recombinant NNRTI-resistant viruses. Rilpivirine, like etravirine, is a diarylpyrimidine derivative. It shows a median viral load reduction of 1.20 $\log_{10}$ copies ml^{-1} regardless of dose.

Integrase Inhibitors

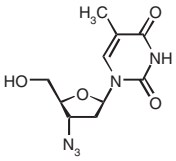
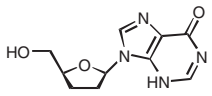
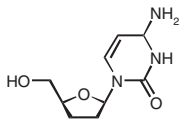
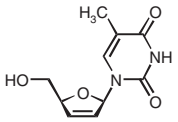
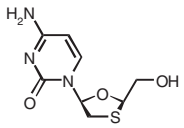
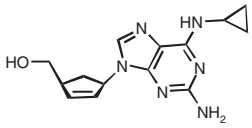
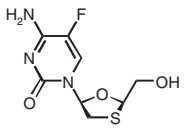
Following reverse transcription, HIV integrase mediates the integration of proviral cDNA by catalyzing two reactions. First, integrase mediates the endonucleolytic hydrolysis of the 3′ ends of the viral DNA. This 3′-processing step generates reactive nucleophilic hydroxyl groups. Integrase remains bound to the viral cDNA as a multimeric complex that bridges both ends of the viral DNA with intracellular particles called preintegration complexes (PICs). In the strand-transfer reaction, integrase mediates the nucleophilic attack of the viral 3′-hydroxyl cDNA across the major groove of the host DNA, resulting in a 5 bp stagger between the integrated viral and host DNA strands. The integration process is completed by DNA gap repair including a series of DNA polymerization, dinucleotide excision, and ligation reactions.

While integrase is essential for retroviral replication, there is no host-cell analog. Because integrase inhibitors do not interfere with normal cellular processes, they are an attractive target for antiretroviral agents. Integrase inhibitors were discovered as early as 1992, but not until 2007 was the first drug approved.

Raltegravir is a first-in-class integrase inhibitor that gained FDA approval in October 2007 (**Table 4**). Because it is the first integrase inhibitor on the market, raltegravir is efficacious even in patients with multidrug-resistant HIV. In phase III trials, raltegravir was found to reduce the HIV viral load to undetectable levels in nearly two-thirds of highly treatment-experienced patients after 16–24 weeks of combination therapy. While the initial approval is only for treatment-experienced patients, raltegravir may also prove to be an option for treatment-naive patients.

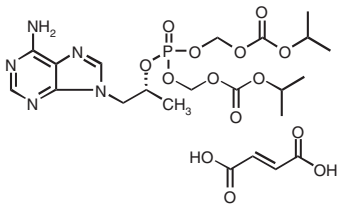
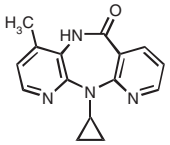
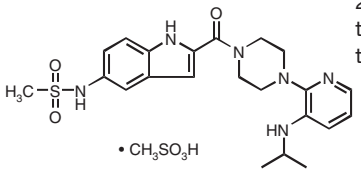
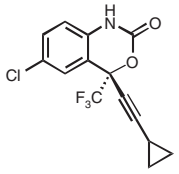
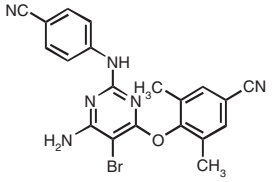
Two drugs in clinical trials, MK-0518 and GS-9137, and GSK364735 are shown in **Table 1**. GS-9137 is a dihydroquinoline carboxylic acid derivative which also acts by inhibition of strand transfer. GS-9137 displays a serum-free IC_{50} of 0.2 nM and has activity against viral strains resistant to NRTIs, NNRTIs, and protease inhibitors.

Table 3 FDA-approved reverse transcriptase inhibitors

Name and structure	*Dosing schedule*[b]	*Major toxicity*[b,c]	*Key mutations conferring resistance*[d]	*Impact of monotherapy*[e]
Zidovudine (AZT, Retrovir) 	300 mg tablet b.i.d.	Pancreatitis; lactic acidosis; hepatomegaly; anemia; myopathy; neutropenia	Thymidine analog mutations (TAMs): M41L, D67N/G, K70R, L210W, T215F/Y, K219E/Q/N T215 revertants: T215C/D/E/S/I/V T69 insertion mutations Q151M complex usually in combination with V75I, F77L, F116Y	1.0 $\log_{10}$ copies ml^{-1} decrease in viral load
Didanosine (ddI, Videx) 	200 mg tablet b.i.d. or 250 mg powder b.i.d.	Pancreatitis; lactic acidosis; hepatomegaly; peripheral neuropathy; optic neuritis	L74V/I K65R TAMs: M41L, D67N/G, L210W, T215F/Y, K219E/Q/N M184V/I	0.8 $\log_{10}$ copies ml^{-1} decrease in viral load
Zalcitabine (ddC, Hivid[a]) 	0.75 mg tablet t.i.d.	Pancreatitis; lactic acidosis; hepatomegaly; peripheral neuropathy; mouth and throat ulcers; neutropenia; stomatitis	T69A[f], L74V[g], K65R[h]	Less effective than either ddI or AZT
Stavudine (d4T, Zerit) 	40 mg capsule b.i.d.	Pancreatitis; lactic acidosis; hepatomegaly; peripheral neuropathy; lipodystrophy; hyperlipidemia; rapidly progressive ascending neuromuscular weakness	TAMs: M41L, D67N/G, K70R, L210W, T215F/Y, K219E/Q/N T215 revertants: T215C/D/E/S/I/V T69 insertion mutations Q151M complex usually in combination with V75I, F77L, F116Y	0.8 $\log_{10}$ copies ml^{-1} decrease in viral load
Lamivudine (3TC, Epivir) 	300 mg tablet daily or 150 mg tablet b.i.d.	Lactic acidosis; hepatomegaly	M184V/I K65R Q151M complex usually in combination with V75I, F77L, F116Y TAMs: M41L, D67N/G, L210W, T215F/Y, K219E/Q/N	Limited monotherapy data available
Abacavir (ABC, Ziagen) 	300 mg capsule b.i.d.	Hypersensitivity reaction which can be fatal	K65R L74V/I TAMs: M41L, D67N/G, L210W, T215F/Y, K219E/Q/N T69 insertion mutations	1.8 $\log_{10}$ copies ml^{-1} decrease in viral load
Emtricitabine (FTC, Emtriva) 	200 mg tablet q.d.	Lactic acidosis; hepatomegaly; hyperpigmentation	M184V/I K65R Q151M complex usually in combination with V75I, F77L, F116Y TAMs: M41L, D67N/G, L210W, T215F/Y	2.0 $\log_{10}$ copies ml^{-1} decrease in viral load

Continued

Table 3 Continued

Name and structure	*Dosing schedule*[b]	*Major toxicity*[b,c]	*Key mutations conferring resistance*[d]	*Impact of monotherapy*[e]
Tenofovir (Viread) 	300 mg capsule q.d.	Lactic acidosis; hepatomegaly; liver and kidney damage; reduction of bone mineral density	K61R TAMs: M41L, L210W, T215Y T215 revertants: T215C/D/E/S/I/V T69 insertion mutations	1.2 $\log_{10}$ copies ml^{-1} decrease in viral load
Nevirapine (Viramune) 	200 mg capsule q.d. for first two weeks, then 200 mg b.i.d.	Skin rash; liver damage	K103N/S Y181C/I G190A/S/E Y188L/H/C	1.5 $\log_{10}$ copies ml^{-1} decrease in viral load[i]
Delavirdine (Rescriptor) 	400 mg (two 200 mg tablets) t.i.d.	Skin rash; proteinuria; lipodystrophy	K103N/S Y181C/I P236L G190A/S/E	1.0 $\log_{10}$ copies ml^{-1} decrease in viral load[j]
Efavirenz (Sustiva, Stocrin) 	600 mg capsule q.d.	Skin rash; depression and other psychiatric symptoms; high triglyceride levels	K103N/S (± L100I, K101P, P225H, K238T/N) G190S/A/E Y188L/H/C Y191C/I	Limited monotherapy data available
Etravirine (Intelence) 	200 mg (two 100 mg tablets) b.i.d.	Moderate to severe skin reactions	Three or more of the 13 specific NNRTI mutations: V901I, A98G, L100I, K101E/P, V106I, V179D/F, Y181C/I/V, G190A/S[k]	2.4 $\log_{10}$ copies ml^{-1}decrease in viral load[k]

[a]Zalcitabine is no longer manufactured as of February 2006.

[b]The Body: The Complete HIV/AIDS Resource. http://www.thebody.com (accessed October 2007).

[c]Painter GR, Almond MR, Mao S, and Liotta DC (2004) Biochemical and mechanistic basis for the activity of nucleoside analogue inhibitors of HIV reverse transcriptase. *Current Topics in Medicinal Chemistry* 4: 1035–1044.

[d]Stanford University HIV Drug Resistance Database. http://hivdb.stanford.edu (accessed October 2007).

[e]Sharma PL, Nurpeisov V, Hernandez-Santiago B, Beltran T, and Schinazi RF (2004) Nucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. *Current Topics in Medicinal Chemistry* 4: 895–919.

[f]Fitzgibbon JE, Howell RM, Haberzettl CA, Sperber SJ, Gocke DJ, and Dubin DT (1992) Human immunodeficiency virus type 1 *pol* gene mutations which cause decreased susceptibility to 2′,3′-dideoxycytidine. *Antimicrobial Agents and Chemotherapy* 36: 153–157.

[g]St. Clair MH, Martin JL, Tudor-Williams G, *et al.* (1991) Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase. *Science* 253: 1557–1559.

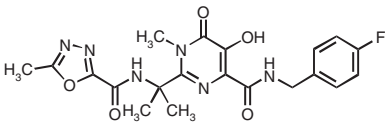
[h]Gu Z, Gao Q, Fang H, *et al.* (1994) Identification of a mutation at codon 65 in the IKKK motif of reverse transcriptase that encodes human immunodeficiency virus resistance to 2′,3′-dideoxycytidine and 2′,3′-dideoxy-3′-thiacytidine. *Antimicrobial Agents and Chemotherapy* 38: 275–281.

[i]Lange JMA (2003) Efficacy and durability of nevirapine in antiretroviral drug naïve patients. *Journal of Acquired Immune Deficiency Syndromes* 34: S40–S52.

[j]Para MF, Meehan P, Holden-Wiltse JH, *et al.* (1999) ACTG 260: a randomized, phase I–II, dose-ranging trial of the anti-human immunodeficiency virus activity of delavirdine monotherapy. *Antimicrobial Agents and Chemotherapy* 43: 1373–1378.

[k]Madruga JV, Cahn P, Grinsztejn B, *et al.* (2007) Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-1: 24-Week results from a randomized, double-blind, placebo-controlled trial. *Lancet* 370: 29–38.

Table 4 FDA-approved integrase inhibitors

Name and structure	*Dosing schedule*[a]	*Major toxicity*[a]	*Key mutations conferring resistance*[b]
Raltegravir (Isentress) 	One 400 mg tablet b.i.d.	Elevated levels of creatine phosphokinase (CPK) in muscles; diarrhea; nausea; headache	N155H, Q148K/R/H

[a]The Body: The Complete HIV/AIDS Resource. http://www.thebody.com (accessed January 2008).
[b]Markowitz M, Nguyen B-Y, Gotuzzo E, *et al.* (2007) Rapid and durable antiretroviral effect of the HIV-1 integrase inhibitor raltegravir as part of combination therapy in treatment-naïve patients with HIV-1 infection. *Journal of Acquired Immune Deficiency Syndromes* 46: 125–133.

A 10-day study in treatment-naive and treatment-experienced patients resulted in mean HIV RNA decreases of 1.91 $\log_{10}$ copies ml^{-1} in patients receiving 400 or 800 mg monotherapy, or 50 mg boosted with 100 mg of ritonavir.

Protease Inhibitors

HIV protease is an aspartyl proteinase responsible for cleaving the Gag and Gag-Pol polyproteins in a late stage of the viral life cycle. Because there is no corresponding aspartyl protease that cleaves the Gag polyprotein in mammalian cells, HIV protease has been a popular target for antiretroviral drug development.

HIV PIs are peptidomimetic products that prevent cleavage of Gag and Gag-Pol protein precursors, preventing virions from maturing and becoming infectious. PIs bind to the protease site with high affinity due to their structural similarity to the tetrahedral intermediate formed during hydrolytic cleavage of a peptide bond in the natural substrate. First-generation PIs have a hydroxyethylene core which acts as a nonhydrolyzable transition state isostere. These compounds bind in the protease active site in a manner that mimics the transition state formed during peptide cleavage.

Although many peptidomimetic inhibitors of HIV protease have been reported since the first one in 1990, researchers have faced challenges regarding the physicochemical and pharmacokinetic properties of PIs. Many PIs exhibit poor bioavailability, a short plasma half-life, poor aqueous solubility, and high protein binding, and often require frequent dosing or high pill burden in order to achieve the necessary drug concentration. The newer PIs are starting to resolve these problems. The currently available PIs are shown in **Table 5**.

Saquinavir was the first HIV PI studied clinically; it was approved by the FDA in 1995. It was discovered as part of a strategy to find transition-state mimetics of the Phe-Pro peptide bond. The original formulation exhibited potent *in vitro* viral activity but very poor bioavailability in humans (3–5%). In 1997, the FDA approved a soft gelatin capsule formation that provided higher plasma levels and bioavailability.

Ritonavir was discovered as a result of efforts to design inhibitors based on the C_2-symmetric structure of HIV protease. It shows better bioavailability than saquinavir and also inhibits cytochrome P450-3A4, a liver enzyme that normally metabolizes PIs. Therefore, ritonavir is administered in combination with other PIs in order to enhance their pharmacokinetic profiles.

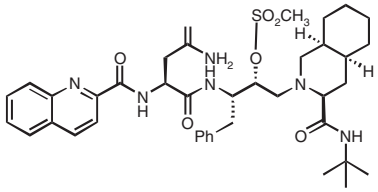
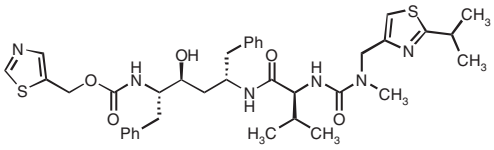
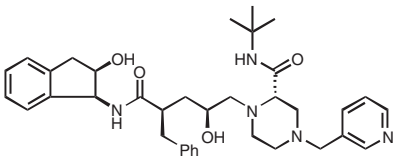
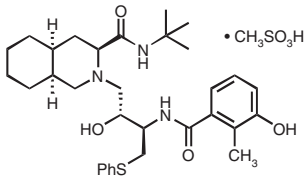
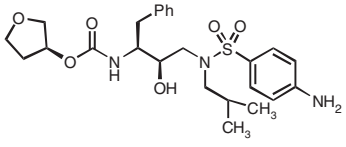
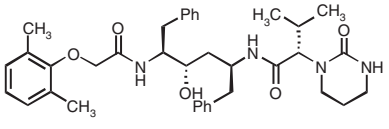
Indinavir resulted from a research program of mechanism-based drug design. The lead compounds preceding discovery of indinavir were modified to create smaller, less peptide-like structures with high water solubility and bioavailability. Nelfinavir was also discovered by rational design, which sought to maximize potency while improving on the pharmacokinetics of its predecessors.

An analysis of the molecular weight distribution of marketed drugs revealed that most drugs with acceptable pharmacokinetic profiles have molecular weights under 600 Da. Amprenavir therefore emerged from a program seeking to maintain high potency while reducing inhibitor size. This compound has a sulfonylated secondary amino hydroxyethyl core, with one of the sulfonyl oxygens playing a key structural role in binding to the enzyme. While amprenavir was the first FDA-approved PI for twice-daily dosing, the 1200 mg dose required by its poor aqueous solubility was very inconvenient, and amprenavir was later superseded by its prodrug fosamprenavir.

Lopinavir is a second-generation PI inhibitor based on the structure of ritonavir. It is potent against ritonavir-resistant virus and tenfold more potent than ritonavir in the presence of human serum. Because lopinavir is rapidly metabolized, it must be co-dosed with ritonavir in order to achieve good oral bioavailability.

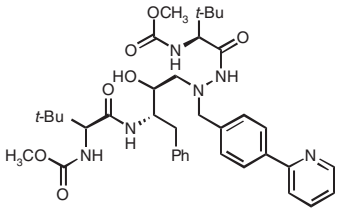
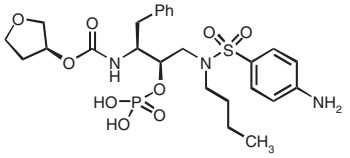
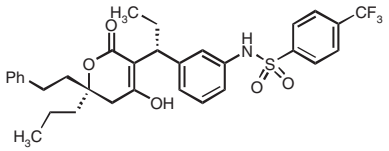
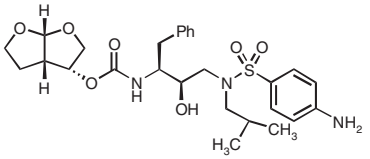
Atazanavir, an azapeptide PI inhibitor, is the first PI approved for once-daily dosing. It also appears to have a reduced effect on cholesterol and triglyceride levels as compared to other PIs.

Table 5 FDA-approved protease inhibitors

Name and structure	*Dosing schedule*[a]	*Major toxicity*[a]	*Key mutations conferring resistance*[b]	*Impact of monotherapy*[c]
Saquinavir (Invirase) 	Two 500 mg tablets b.i.d., boosted with ritonavir	Lipodistrophy; diabetes; increased bleeding in hemophiliacs; hyperlipidemia	G48V L90M ± G73S/C/T II84V I54V/T/L/M/A	0.7 $\log_{10}$ copies ml^{-1} decrease in viral load
Ritonavir (Norvir) 	Generally used as a boosting agent for other PIs, 100–400 mg q.d. or b.i.d. Maximum approved dose is 600 mg b.i.d.	Lipodystrophy; diabetes; increased bleeding in hemophiliacs; pancreatitis; paresthesias; hyperlipidemia; hepatitis	V82A/T/F/S I84V V32I I54V/T/L/M[d]	Limited monotherapy data available
Indinavir (Crixivan) 	400 mg capsule or 800 mg capsule b.i.d., boosted with ritonavir	Lipodystrophy; diabetes; increased bleeding in hemophiliacs; liver toxicity; kidney stones; jaundice; hyperlipidemia	V82A/T/F/S M46I/LI54V/T/M/L/A L90M	1.2 $\log_{10}$ copies ml^{-1} decrease in viral load[e]
Nelfinavir (Viracept) 	Two 600 mg tablets b.i.d.	Lipodystrophy; diabetes; increased bleeding in hemophiliacs; hyperlipidemia	D30N±N88D/S L90M±M46I/L I84V V82A/T/F/S	1.5 $\log_{10}$ copies ml^{-1} decrease in viral load
Amprenavir (Agenerase) 	24 50 mg capsules (1200 mg total) b.i.d.	Lipodystrophy; diabetes; increased bleeding in hemophiliacs; skin rash; oral paresthesias; hyperlipidemia		2.0 $\log_{10}$ copies ml^{-1} decrease in viral load[f]
Lopinavir (co-formulation with ritonavir is Kaletra) 	Administered with ritonavir in a single tablet. Two 200/50 mg (lopinavir/ritonavir) tablets b.i.d. or four 200/50 mg q.d.	Lipodystrophy; diabetes; increased bleeding in hemophiliacs; liver damage; hyperlipidemia	V82A/T/F/S I54V/L/M/A/T/SM46I/L I50V	1.9 $\log_{10}$ copies ml^{-1} decrease in viral load[g]

Continued

Table 5 Continued

Name and structure	*Dosing schedule*[a]	*Major toxicity*[a]	*Key mutations conferring resistance*[b]	*Impact of monotherapy*[c]
Atazanavir (Reyataz) 	300 mg capsule or two 200 mg capsules q.d., boosted with ritonavir	Lipodystrophy; diabetes; increased bleeding in hemophiliacs; jaundice	I50L N88S/D V82A/T/F/S I84V	1.6 $\log_{10}$ copies ml^{-1} decrease in viral load[h]
Fosamprenavir (Lexiva) 	Two 700 mg tablets q.d. or one 700 mg tablet b.i.d., boosted with ritonavir	Increased levels of cholesterol and triglycerides; lipodystrophy; diabetes; increased bleeding in hemophiliacs; skin rash	I50V I84V I54M/L/V/ T/A M46I/L	2.4 $\log_{10}$ copies ml^{-1} decrease in viral load[i]
Tipranavir (Aptivus) 	Two 250 mg capsules b.i.d., boosted with ritonavir	Lipodystrophy; diabetes; increased bleeding in hemophiliacs; liver damage; cerebral hemorrhage; hyperlipidemia; skin rash	V82A/T/F/S/ M/L I84V/ A/C L90M M46I/L/V	1.6 $\log_{10}$ copies ml^{-1} decrease in viral load[j]
Darunavir (Prezista) 	Two 300 mg tablets b.i.d., boosted with ritonavir	Lipodystrophy; diabetes; increased bleeding in hemophiliacs; skin rash; hyperlipidemia; hyperglycemia	I50V V82A/T/ F/S/M I8V/ A/C I47V/A	1.4 $\log_{10}$ copies ml^{-1} decrease in viral load[k]

[a]The Body: The Complete HIV/AIDS Resource. http://www.thebody.com (accessed October 2007).
[b]Stanford University HIV Drug Resistance Database. http://hivdb.stanford.edu (accessed October 2007).
[c]Eron JJ, Jr. (2000) HIV-1 protease inhibitors. *Clinical Infectious Diseases* 30(supplement 2): S160–S170.
[d]De Mendoza C and Soriano V (2004) Resistance to HIV protease inhibitors: Mechanisms and clinical consequences. *Current Drug Metabolism* 5: 321–328.
[e]Gulick RM, Mellors JW, Havlir D, *et al.* (1997) Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *New England Journal of Medicine* 337: 734–739.
[f]Adkins JC and Faulds D (1998) Amprenavir. *Drugs* 55: 837–842.
[g]Oldfield V and Plosker GL (2006) Lopinavir/ritonavir: A review of its use in the management of HIV infection. *Drugs* 66: 1275–1299.
[h]Barreiro P, Rendón A, Rodríguez-Nóvoa S, and Soriano V (2005) Atazanavir: The advent of a new generation of more convenient protease inhibitors. *HIV clinical trials* 6: 50–61.
[i]Chapman TM, Plosker GL, and Perry CM (2004) Fosamprenavir: A review of its use in the management of antiretroviral therapy-naïve patients with HIV infection. *Drugs* 64: 2101–2124.
[j]Dong BJ and Cocohoba JM (2006) Tipranavir: A protease inhibitor for HIV salvage therapy. *Annals of Pharmacotherapy* 40: 1311–1321.
[k]Arastéh K, Clumeck N, Pozniak A, *et al.* (2005) TMC114/ritonavir substitution for protease inhibitor(s) in a non-suppressive antiretroviral regimen: A 14-day proof-of-principle trial. *AIDS* 19: 943–947.

Because of the poor aqueous solubility of amprenavir, a more suitable analog was sought. Fosamprenavir, a phosphate ester prodrug of amprenavir, has excellent aqueous solubility due to the presence of the phosphate salt. Fosamprenavir is rapidly and extensively converted to amprenavir after oral administration and therefore maintains its positive pharmacokinetic profile.

Tipranavir, a nonpeptidic PI, shows excellent antiviral activity. However, its undesirable toxicity profile, including reports of hepatitis and hepatic failure, generally relegate this drug to salvage therapy for patients with resistance to other PIs.

Darunavir, the most recently approved PI, resulted from a drug discovery program aimed at replacing peptide segments of PIs with nonpeptidic isosteres – in particular, a bis-tetrahydrofuran system. The oxygen atoms in this portion of the molecule appear to form hydrogen bonds with aspartate residues in HIV protease. Because of these interactions, darunavir shows activity against a broad range of drug-resistant HIV strains.

Maturation Inhibitors

Recently, a new class of antiretroviral agents has been identified which blocks HIV-1 replication at a late step in the virus life cycle. PIs prevent the essential proteolytic processing of the Gag and Gag–Pol polyproteins, which leads to the structural maturation of the virus particle and activation of viral enzymes. Maturation inhibitors, on the other hand, act directly on the Gag protein by disrupting the conversion of the HIV capsid precursor p25 to the mature capsid protein p24. This results in the production of immature viral particles that have lost infectivity.

Bevirimat, also known as PA-457, is currently the only maturation inhibitor in clinical trials (**Table 1**). Bevirimat is a betulinic acid derivative, which exhibits a mean IC_{50} of 10.3 nM in assays using patient-derived WT virus isolates. The compound also retains a similar level of activity against a panel of virus isolates resistant to the three classes of drugs targeting the viral RT and PR enzymes. With an average 50% cytotoxicity value of 25 μM, the therapeutic index for bevirimat is *c.* 2500.

Combination Therapy

Antiretroviral agents are rarely used alone in treatment. Not only does monotherapy demonstrate inferior antiviral activity to combination therapy, but it also results in a rapid development of resistance. The most common antiretroviral regimens in treatment-naive patients generally contain two NRTIs along with one NNRTI or a single or ritonavir-boosted PI. Preferred regimens are shown below; patients who do not tolerate these combinations may try one of the alternative regimens. While these combinations are recommended by the Office of AIDS Research Advisory Council, each patient is encouraged to seek the optimal combination for his or her situation.

- Preferred
  - Atripla: efavirenz, tenofovir, emtricitabine
  - Atazanavir + ritonavir, tenofovir, emtricitabine
  - Fosamprenavir + ritonavir, tenofovir, emtricitabine
  - Lopinavir/ritonavir, tenofovir, emtricitabine
  - Efavirenz, zidovudine, lamivudine
  - Atazanavir + ritonavir, zidovudine, lamivudine
  - Fosamprenavir + ritonavir, zidovudine, lamivudine
  - Lopinavir/ritonavir, zidovudine, lamivudine

- Alternative
  - Nevirapine, abacavir, lamivudine
  - Atazanavir (unboosted), abacavir, lamivudine
  - Fosamprenavir (unboosted), abacavir, lamivudine
  - Fosamprenavir + ritonavir, abacavir, lamivudine
  - Lopinavir/ritonavir, abacavir, lamivudine
  - Nevirapine, didanosine, lamivudine
  - Atazanavir (unboosted), didanosine, lamivudine
  - Fosamprenavir (unboosted), didanosine, lamivudine
  - Fosamprenavir + ritonavir, didanosine, lamivudine
  - Lopinavir/ritonavir, didanosine, lamivudine

Conclusion

Significant progress in antiretroviral therapy has been made since AZT first hit the market in 1987. With 22 antiretroviral agents in four classes, patients have more choices than ever. Many of these new drugs also improve quality of life with more convenient dosing schedules and greatly improved tolerability.

Despite the important success of HAART, the evolution of many drug-resistant HIV strains has diminished the efficacy of antiretroviral therapy, and an ever-increasing number of patients have progressed to salvage therapy. An estimated 13% of adults receiving care in the USA exhibit resistance to all three drug classes, and 76% of patients show resistance to one or more drugs. Because so many individuals currently living with HIV infection are highly treatment experienced, there is a strong need for newer and more effective antiretroviral therapies.

See also: Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Origin; Human Immunodeficiency Viruses: Pathogenesis.

Further Reading

Adkins JC and Faulds D (1998) Amprenavir. *Drugs* 55: 837–842.

Arastéh K, Clumeck N, Pozniak A, *et al.* (2005) TMC114/ritonavir substitution for protease inhibitor(s) in a non-suppressive antiretroviral regimen: A 14-day proof-of-principle trial. *AIDS* 19: 943–947.

Barreiro P, Rendón A, Rodríguez-Nóvoa S, and Soriano V (2005) Atazanavir: The advent of a new generation of more convenient protease inhibitors. *HIV Clinical Trials* 6: 50–61.
Castagna A, Biswas P, Beretta A, and Lazzarin A (2005) The appealing story of HIV entry inhibitors: From discovery of biological mechanisms to drug development. *Drugs* 65: 879–904.
Chapman TM, Plosker GL, and Perry CM (2004) Fosamprenavir: A review of its use in the management of antiretroviral therapy-naïve patients with HIV infection. *Drugs* 64: 2101–2124.
Chrusciel RA and Strohbach JW (2004) Non-peptidic HIV protease inhibitors. *Current Topics in Medicinal Chemistry* 4: 1097–1114.
De Clercq E (2004) Antiviral drugs in current clinical use. *Journal of Clinical Virology* 30: 115–133.
De Clercq E (2004) Non-nucleoside reverse transcriptase inhibitors (NNRTIs): Past, present, and future. *Chemistry and Biodiversity* 1: 44–64.
De Mendoza C and Soriano V (2004) Resistance to HIV protease inhibitors: Mechanisms and clinical consequences. *Current Drug Metabolism* 5: 321–328.
Dong BJ and Cocohoba JM (2006) Tipranavir: A protease inhibitor for HIV salvage therapy. *Annals of Pharmacotherapy* 40: 1311–1321.
Eron JJ, Jr. (2000) HIV-1 protease inhibitors. *Clinical Infectious Diseases* 30(supplement 2): S160–S170.
Fitzgibbon JE, Howell RM, Haberzettl CA, Sperber SJ, Gocke DJ, and Dubin DT (1992) Human immunodeficiency virus type 1 *pol* gene mutations which cause decreased susceptibility to 2′,3′-dideoxycytidine. *Antimicrobial Agents and Chemotherapy* 36: 153–157.
Gu Z, Gao Q, Fang H, *et al.* (1994) Identification of a mutation at codon 65 in the IKKK motif of reverse transcriptase that encodes human immunodeficiency virus resistance to 2′,3′-dideoxycytidine and 2′,3′-dideoxy-3′-thiacytidine. *Antimicrobial Agents and Chemotherapy* 38: 275–281.
Gulick RM, Mellors JW, Havlir D, *et al.* (1997) Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *New England Journal of Medicine* 337: 734–739.
Lange JMA (2003) Efficacy and durability of nevirapine in antiretroviral drug naïve patients. *Journal of Acquired Immune Deficiency Syndromes* 34: S40–S52.
Madruga JV, Cahn P, Grinsztejn B, *et al.* (2007) Efficacy and safety of TMC125 (etravirine) in treatment-experienced HIV-1-infected patients in DUET-1: 24-Week results from a randomized, double-blind, placebo-controlled trial. *Lancet* 370: 29–38.
Markowitz M, Nguyen B-Y, Gotuzzo E, *et al.* (2007) Rapid and durable antiretroviral effect of the HIV-1 integrase inhibitor raltegravir as part of combination therapy in treatment-naïve patients with HIV-1 infection. *Journal of Acquired Immune Deficiency Syndromes* 46: 125–133.
Meadows DC and Gervay-Hague J (2006) *Current developments in HIV chemotherapy*. *ChemMedChem* 1: 16–29.
Office of AIDS Research Advisory Council (2006) Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents.
Oldfield V and Plosker GL (2006) Lopinavir/ritonavir: A review of its use in the management of HIV infection. *Drugs* 66: 1275–1299.
Painter GR, Almond MR, Mao S, and Liotta DC (2004) Biochemical and mechanistic basis for the activity of nucleoside analogue inhibitors of HIV reverse transcriptase. *Current Topics in Medicinal Chemistry* 4: 1035–1044.
Para MF, Meehan P, Holden-Wiltse JH, *et al.* (1999) ACTG 260: A randomized, phase I–II, dose-ranging trial of the anti-human immunodeficiency virus activity of delavirdine monotherapy. *Antimicrobial Agents and Chemotherapy* 43: 1373–1378.
Pereira CF and Paridaen JTML (2004) Anti-HIV drug development – an overview. *Current Pharmaceutical Design* 10: 4005–4037.
Piacenti FJ (2006) An update and review of antiretroviral therapy. *Pharmacotherapy* 26: 1111–1133.
Pommier Y, Johnson AA, and Marchand C (2005) Integrase inhibitors to treat HIV/AIDS. *Nature Reviews Drug Discovery* 4: 236–248.
Randolph JT and DeGoey DA (2004) Peptidomimetic inhibitors of HIV protease. *Current Topics in Medicinal Chemistry* 4: 1079–1095.
Sharma PL, Nurpeisov V, Hernandez-Santiago B, Beltran T, and Schinzai RF (2004) Nucleoside inhibitors of human immunodeficiency virus type 1 reverse transcriptase. *Current Topics in Medicinal Chemistry* 4: 895–919.
St. Clair MH, Martin JL, Tudor-Williams G, *et al.* (1991) Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase. *Science* 253: 1557–1559.
Temesgen Z, Warnke D, and Kasten MJ (2006) Current status of antiretroviral therapy. *Expert Opinion in Pharmacotherapy* 7: 1541–1554.
Westby M, Smith-Burchnell C, Mori J, *et al.* (2007) Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry. *Journal of Virology* 81: 2359–2371.

Relevant Websites

http://hivdb.stanford.edu – Stanford University HIV Drug Resistance Database.
http://www.thebody.com – The Body: The Complete HIV/AIDS Resource.

Human Immunodeficiency Viruses: Molecular Biology

J Votteler and U Schubert, Klinikum der Universität Erlangen-Nürnberg, Erlangen, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Pandemic An outbreak of an infectious disease that spreads across countries, continents, or even worldwide.
Zoonosis Transmission of a pathogen from one species to another (e.g., humans).

Introduction

Since the beginning of the 1980s, approximately 40 million people have been infected with the human immunodeficiency virus (HIV), the causative agent of the acquired immune deficiency syndrome (AIDS). To date, this multisystemic, deadly, and so far incurable disease has caused more than 20 million deaths. AIDS was first

described in 1981 in a group of homosexual men suffering from severe opportunistic infections. Two years later, a retrovirus was isolated from lymphocytes of AIDS patients and was later termed HIV. The two types, HIV-1 and -2, together with the simian immunodeficiency viruses (SIVs) found in nonhuman primates, delineate the genus of primate lentiviruses. Viruses of the genus *Lentivirus* preferentially replicate in lymphocytes and differentiated macrophages and often cause long-lasting and mostly incurable chronic diseases. In contrast to other retroviruses that require the breakdown of the nuclear membrane during mitosis for integration of the viral genome into host cell chromosomes, lentiviruses are uniquely capable of infecting nondividing cells, preferentially terminally differentiated macrophages and resting T cells.

Despite anti-retroviral therapy becoming more effective, infections with HIV remain one of the most devastating pandemics that humanity has ever faced. Unfortunately and despite enormous efforts, there is only limited hope that an effective vaccine will be developed in the near future and there are no other mechanisms available to stimulate the natural immunity against HIV. Another major drawback is the fact that the virus continues to persist even during prolonged therapy in latently infected host cells, classified as the viral reservoirs *in vivo*. Current anti-retroviral treatment is based on drugs that target either the viral enzymes, protease (PR) and reverse transcriptase (RT), or the envelope (Env) protein-mediated entry of the virus into the target cell.

Although introduction of highly active anti-retroviral therapy (HAART) in the mid-1990s, which involves the combination of different classes of both, PR and RT inhibitors, has led to a significant reduction in morbidity and mortality, an eradication of the virus from HIV-1-infected individuals has never been achieved. In addition, these antiviral drugs can induce severe adverse effects, particularly when administered in combination and over prolonged medication periods. A drawback to these treatments is that with the high mutation rate of HIV and replication dynamic, drug-resistant mutants are evolving. Cellular genes have much lower mutation rates, and a potential solution to this problem is to target cellular factors, enzymes, or complex mechanisms that are essential for replication of HIV in the host cells.

Genomic Organization and Replication of HIV

The *Retroviridae* belong to the huge group of eukaryotic retro-transposable elements. Retroviruses are distinguished from other viruses by their ability to reverse-transcribe their RNA genomes into DNA intermediates by using the enzyme RT. The provirus DNA genome is then integrated into the host cell chromosomes by action of the viral enzyme integrase (IN). All retroviruses contain at least three major genes encoding the main virion structural components that are each synthesized as three polyproteins that produce either the inner virion interior (Gag, group specific antigen), the viral enzymes (Pol, polymerase), or the glycoproteins of the virion Env. The genomic organization of HIV-1 is outlined in **Figure 1**. In addition to virus structural proteins and enzymes, some retroviruses code for small proteins with regulatory and auxiliary functions. In the case of HIV-1, these proteins comprise the two essential regulatory elements: Tat, which activates transcription, and Rev, which modulates viral RNA transport. In contrast, Nef, Vpr, Vif, and Vpu are nonessential for replication in certain tissue culture cells and are generally referred as accessory proteins (**Figure 1**).

According to the nomenclature defined by the International Committee on Taxonomy of Viruses (ICTV) convention, members of the *Orthoretrovirinae* contain RNA inside the virus particle and are released from the plasma membrane. However, the assembly of orthoretroviruses follows two different strategies: alpha-, gamma-, delta-, and lentiviruses (ICTV nomenclature) assemble on the inner leaflet of the plasma membrane and are released as immature virions. In contrast, betaretroviruses and spumaviruses first assemble in the cytoplasm to form immature particles that are then transported to the plasma (betaretroviruses) or internal (spumaviruses) membrane where virus budding occurs.

As with other retroviruses, the HIV replication cycle begins with virus attachment and penetration through the plasma membrane. The schematic replication cycle of HIV-1 is depicted in **Figure 2**. HIV binds to different cell receptors, among them the differentiation antigen, CD4, acting as primary receptor, as well as different specific chemokine receptors that act as co-receptors after binding to CD4. The first retroviral receptor ever identified was the CD4 T-cell receptor that was established in 1984 as the primary receptor for HIV-1 and -2 and SIV. However, the CD4 molecule alone is not sufficient to allow Env-mediated membrane fusion and virus entry, as certain primary isolates of HIV-1 preferentially replicate in T-cell lines (T-cell-line tropic, 'TCL'-tropic), while others establish productive replication only in macrophage cultures ('M'-tropic). Thus, a second class of HIV receptors was predicted and finally identified as a member of the G protein-coupled seven transmembrane domain receptor superfamily acting as so-called 'co-receptors' during HIV entry. Co-receptors, in general, function as cellular receptors for α- and β-chemokines. Two types of co-receptors arbitrate the disparity in cell-type tropism: the α-chemokine receptor CxCR4 that is typically present in T-cell lines, and the β-chemokine receptor CCR5 that is present on macrophages. It was shown that the genetic heterogeneity in co-receptor alleles determines the vulnerability to HIV infection and disease

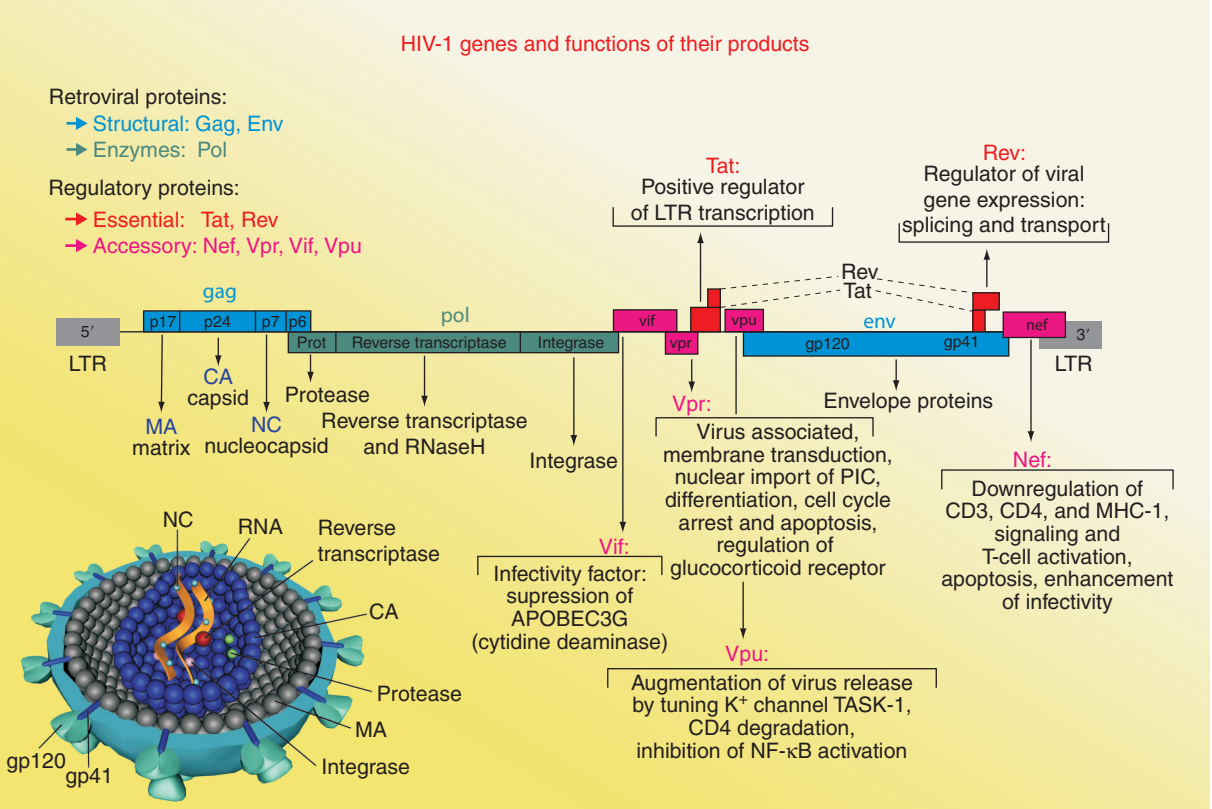


Figure 1 Genomic organization of HIV. This figure illustrates the complex organization of the HIV-1 genome comprising the standard retroviral elements for virus structure, *gag* and *env* (blue), the enzymes *pol* (green), and the six additional genes. These additional genes are either essential as *tat* and *rev*, or accessory as *vpr*, *vpu*, *vif*, and *nef*. The schematic model of the structure of the mature HIV-1 virus particle is given underneath.

progression. A well-characterized example is the identification of a truncated form of CCR5 (termed the 'CCR5/d32' mutation) that in its homozygous form almost completely protects individuals from HIV infection.

Virus entry starts with the so-called 'uncoating' of the virus which is the fusion between the virus and host cell membrane followed by the release of the viral core into the cytoplasm. Following this process, the viral RNA genome is transcribed by the reverse transcription complex (RTC) into double-stranded DNA, which, as part of the preintegration complex (PIC), is transported to the nucleus where the proviral genome is integrated into chromosomes. While there is evidence that the capsid (CA) molecules of the incoming virus are degraded via the ubiquitin proteasome system (UPS), the three virus proteins IN, matrix (MA), and accessory Vpr molecules together with the provirus genome form the PIC. Although the PIC components comprise various nuclear localization signals (NLSs), it is assumed that additional cellular and viral factors, including a central DNA flap that is formed during reverse transcription, facilitate the import of the PIC into the nucleus. It is worth noting that virus entry involves a number of consecutive and highly organized multistep events that might explain why approximately only 1 out of a 1000 mature HIV-1 particles are capable of establishing a productive infection.

After reverse transcription of the viral genome and nuclear import of the PIC, the proviral DNA is integrated into chromosomal DNA. Activation of HIV-1 long terminal repeat (LTR) promoter-driven retroviral gene expression, followed by export, transport, and splicing of the viral mRNA, the so-called late steps of the HIV-1 replication cycle are initiated at this point. These steps involve membrane targeting of Gag and Gag–Pol polyproteins, assembly, as well as budding and maturation of progeny virions. Upon activation of integrated provirus, viral mRNAs are processed and transported into the cytosol for translation of newly synthesized structural proteins that assemble at the plasma membrane into budding particles. The Gag polyprotein in HIV-1 and HIV-2 consists of different functional domains that mediate the recognition and binding of viral RNA, the membrane targeting of Gag and Gag–Pol polyproteins, virion assembly, and efficient particle release from the plasma membrane as the final step of virus budding. In general, the Gag polyprotein constitutes viral components that are both sufficient

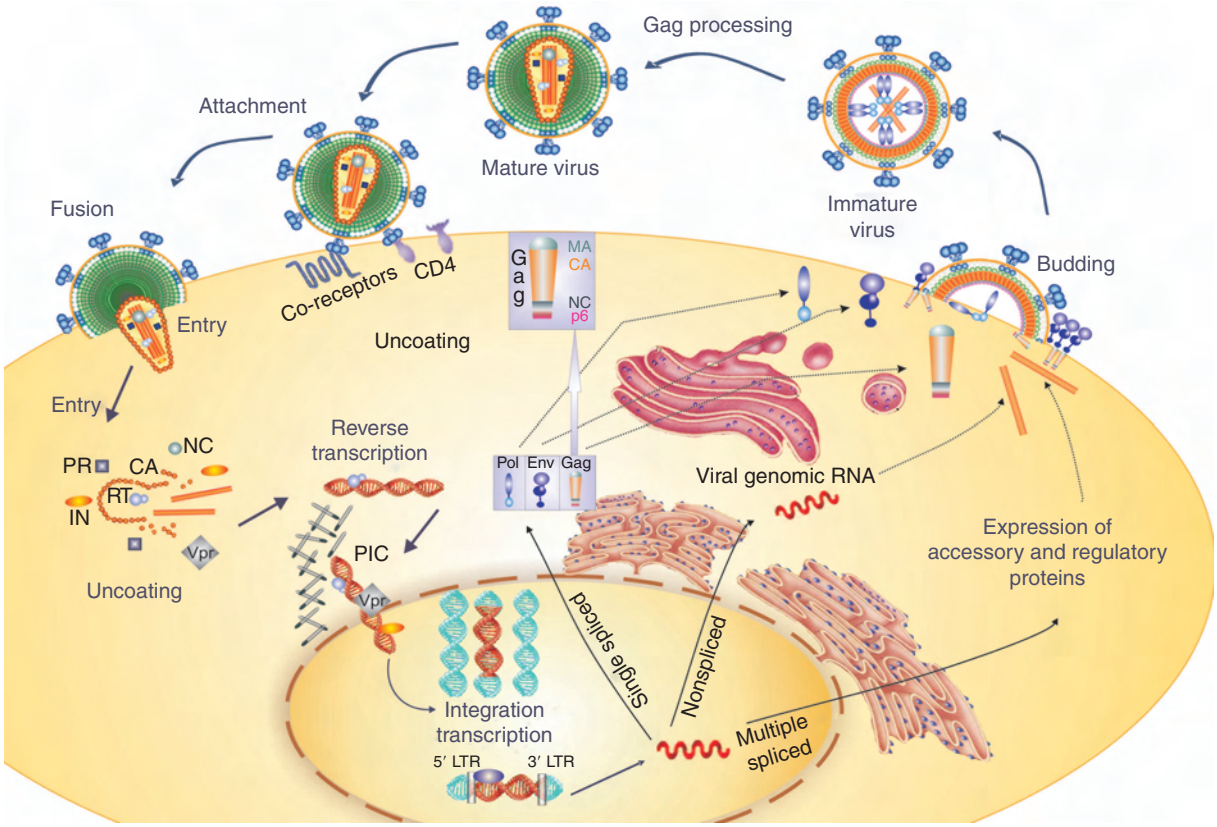


Figure 2 Replication cycle of HIV. Schematic overview of the different stages of the HIV-1 replication cycle.

and strictly required for virus particle assembly and budding, although further viral components such as the genomic RNA, the envelope, and the viral enzymes are required for production of infectious virions. The processing of the HIV-1 Gag polyprotein Pr55 by the viral PR generates the MA, CA, nucleocapsid (NC), as well as p6 proteins and the two spacer peptides, p2 and p1. The HIV-1 *pol*-encoded enzymes, PR, RT, and IN, are proteolytically released from a large polyprotein precursor, Pr160, the expression of which results from a rare frameshift event occurring during translation of Pr55. Like the Gag proteins, the Env glycoproteins are also synthesized from a polyprotein precursor protein. The resulting surface (SU) gp120 and transmembrane (TM) gp41 glycoproteins are produced by the cellular *protease* during trafficking of gp160 to the cell membrane. While gp120 contains the domains that mediate virus binding to CD4 and co-receptors, the gp41 anchors the trimetric complexes of TM/SU in the virus membrane and includes the determinants that regulate fusion between cellular and virus membranes during virus entry. The N-terminus of the extracellular ectodomain of gp41 harbors the so-called 'fusion peptide' that consists of a hydrophobic domain in concert with two helical motifs and regulates membrane fusion by formation of a six-helix bundle. The highly polymorphic SU protein gp120 is organized into five conserved domains (C1 to C5), and five highly variable domains that in most of the known SU sequences are concentrated near loop structures that are stabilized by disulfide bond formation.

In general, Gag proteins of different retroviruses exhibit a certain structural and functional similarity: MA mediates the plasma membrane targeting of the Gag polyprotein and lines the inner leaflet of the lipid bilayer of the mature virus particle, CA regulates assembly of Gag and forms the core shell of the infectious virus, and NC regulates packaging and condensation of the viral genome. In addition to these canonical mature retrovirus proteins, other Gag domains have been described, such as the HIV-1 p6 region that directs the incorporation of the regulatory protein Vpr into budding virions and governs efficient virus budding.

HIV particles bud from the plasma membrane as immature noninfectious viruses consisting predominantly of uncleaved Gag polyproteins. After virus release and in concert with PR activation which is autocatalytically released from the Gag–Pol polyprotein, processing of Gag and Gag–Pol polyproteins into its mature proteins and condensation of the inner core structure occurs that ultimately results in the formation of a mature

infectious virus. Besides PR and Env, at least two other viral factors are known to promote efficient virus release: the HIV-1-specific accessory protein Vpu and the p6 domain. While Vpu supports virus release by an ion channel activity, p6 contains at least two distinct late assembly (L) domains that are required for efficient separation of assembled virions from the cell surface by a yet-undefined mechanism that somehow involves the cellular multivesicular body (MVB) as well as the UPS.

Role of Cellular Factors in HIV Replication

From entry to release and maturation into infectious progeny virions, each individual step in the HIV replication cycle exploits cellular pathways. As for most other intracellular parasites, the replication of HIV-1 depends on the interaction with specific host cell factors, and some of these proteins are specifically incorporated into progeny virions. Conversely, replication of HIV-1 is blocked in cells of the infected host by the action of restriction factors that function as barriers to retroviral replication. These factors are part of the so-called 'innate immune system' for which several mechanisms are known to interfere with replication at different stages of the viral life cycle. HIV-1, however, has evolved strategies to undermine these antiviral responses and, as a consequence, successfully propagates in the specific host environment.

Well-characterized examples for host–virus protein interaction are the virus receptors (CD4 and chemokine receptors) which enable virus entry into specific host cells, the role of the chromatin-remodeling system, and the HMG I family proteins for proviral DNA integration, as well as the requirement of different factors of the endosomal protein trafficking and ubiquitination systems for virus release.

An important case of host–virus interaction is the role of the UPS in virus budding. Recent work has provided intriguing insight into the mechanism of how virus budding exploits the cellular machinery that is normally involved in vacuolar lysosomal protein sorting and MVB-biogenesis. In the case of HIV-1, the recruitment of these cellular factors to the virus assembly site is facilitated by the interaction between the primary L-domain of p6 with at least one important host factor, the tumor susceptibility gene product 101 (Tsg101), an E2-type ubiquitin ligase-like protein. The second L-domain at the C-terminus of p6 mediates the binding of Gag to AIP1/ALIX, a class E vacuolar protein sorting factor that also interacts with Tsg101. AIP1/ALIX also binds to late-acting components of the endosomal sorting complexes required for transport (ESCRTs) and is necessary for the formation of MVB at endosomal membranes. Further, in more recent studies, VPS37B was identified as a new component of the ESCRT that binds to Tsg101. There has been accumulating evidence that HIV-1 recruits the components of the MVB system to the budding machinery which follows two separate and cell-type-specific pathways: in T cells HIV-1 buds primarily from the cell surface, while in monocytes/macrophages the virus buds into vacuoles of the MVB system.

An example for the ability of HIV-1 to escape host cell restriction is the recently discovered relationship between the HIV-1 accessory protein Vif (virus infectivity factor), and APOBEC3G (apolipoprotein B mRNA editing enzyme catalytic polypeptide-like 3G). APOBEC3G is a member of the cellular cytidine deaminase DNA/RNA-editing enzyme family that has the unique capability of hypermutating retroviruses, including HIV-1, with terminal consequences. However, the antiviral effect of APOBEC3G does not correlate with the frequency of mutation induced by APOBEC3G, suggesting that cytidine deaminase activity is not the only underlying mechanism of the antiviral activity of APOBEC. Nevertheless, the resulting innate block in virus replication is counteracted by Vif, which in the virus producer cell binds to APOBEC3G, induces its polyubiquitination by an SCF-like E3 ubiquitin ligase (Cullin5-ElonginB+C), and finally initiates proteasomal degradation of APOBEC3G. It is assumed that by this mechanism Vif is precluding the presence of APOBEC3G in progeny virions.

A second family of proteins with anti-retroviral activity are proteins of the tripartite interaction motif (TRIM) family, exemplified by TRIM5α. TRIM proteins contain a series of three motifs comprising a RING-finger (really interesting new gene), a B-box, and a coiled-coil (CC) domain. Only TRIM5α contains an additional SPRY domain which mediates interaction with the viral CA and is mainly responsible for the species-specific restriction activity. It has been shown that TRIM5α blocks incoming retroviruses at an early step of virus replication, occurring sometime after virus entry and before reverse transcription is initiated, and this antiviral activity of TRIM5α is clearly CA dependent. The RING-finger acts as an E3-type ubiquitin ligase, suggesting that ubiquitination of incoming CA molecules leading to proteasomal degradation might be responsible for the antiviral activity. However, the molecular mechanisms employed by TRIM5α to restrict retroviral infection are still poorly understood and a matter of intensive debate.

HIV Regulator Proteins

While retroviruses share the same fundamental replication cycle and have the same basic genomic organization (e.g., the canonical *gag*, *pol*, and *env* genes), they vary in the content of additional small regulatory genes. These

proteins, except for Rev and Tat, dispensable for HIV-1 replication in certain cell lines *in vitro*, contribute enormously to pathogenesis and spread of HIV-1 *in vivo*.

The *trans*-Activator of Transcription (Tat) of HIV-1

It is now generally accepted that the HIV *trans*-activator (Tat) plays an important role in the pathogenesis of AIDS. Although originally described as an activator of the HIV-1 LTR promoter, Tat was later shown to regulate reverse transcription, to affect the expression of various cellular and viral genes, and to be released from infected cells. This so-called extracellular Tat, which acts as a cell membrane transducing peptide in the sense of a so-called 'trojan molecule', can affect neighboring cells, that are both uninfected and infected target cells. Indeed, there is accumulating evidence that Tat in its extracellular form plays a major role in AIDS-associated diseases like Karposi's sarcoma and HIV-associated dementia.

Tat can be expressed in two forms, as the 72-amino-acid one-exon Tat and as the 86–101-amino-acid (depending on the HIV-1 isolate) two-exon Tat expressed primarily early during infection. The 14–15 kDa Tat binds to an RNA stem–loop structure forming the Tat-responsive element (TAR) at the 5′ LTR region. It activates transcriptional elongation by stimulating the protein kinase TAK (Tat-associated kinase) resulting in hyperphosphorylation of the RNA polymerase II. In general, Tat stimulates the production of full-length HIV transcripts and is, therefore, essential for HIV replication.

The Regulator of Expression of Virion Proteins (Rev)

The compact organization of the HIV-1 genome and expression of all structural and regulatory proteins from a single promoter in the 5′-LTR requires a complex splicing regime of the primary transcript. The fully spliced mRNAs encoding Tat, Rev, and Nef are readily transported from the nucleus to the cytoplasm and, in consequence, these proteins are synthesized early in infection. However, the nuclear export of unspliced or single spliced mRNAs encoding structural proteins Gag and Env, the viral enzymes, as well as the accessory proteins Vif, Vpr, and Vpu, all require the activity of Rev. Hence, these proteins are expressed later during viral infection as the Rev concentration in the infected cell increases.

The Rev protein binds to the viral mRNAs via an arginine-rich RNA-binding motif that additionally serves as NLS required for the transport of Rev from the cytosol to the nucleus. Rev recognizes a stem–loop structure in the viral transcripts known as the Rev response element (RRE) that is located in *env*. After binding to RRE, Rev forms multimeric complexes of up to 12 monomers that are exported from the nucleus by interaction with nuclear export factors. These factors are recruited by a leucine-rich nuclear export signal (NES) located in the C-terminal region of Rev and promote the shuttling of the Rev–RNA complexes to the cytosol. Hence, Rev is required for the synthesis of viral proteins and is therefore essential for HIV replication.

The Lentivirus Protein R (Vpr)

Vpr is a virion-associated, nucleocytoplasmatic shuttling regulatory protein that is encoded by (and conserved among) primate lentiviruses, HIV-1, HIV-2, and the SIVs. Although dispensable for growth of HIV-1 in activated and dividing T cells, Vpr appears to play an important role in virus replication *in vivo*, since deletion of *vpr* and the related *vpx* genes in SIV severely compromises the pathogenic properties in experimentally infected rhesus macaques. Furthermore, HIV-2 and SIV also encode an additional Vpr-related protein, Vpx, that is believed to function synergistically with Vpr. The Vpr of HIV-1 is reported to exhibit numerous biological activities, including nuclear localization (based on the presence of at least two NLS), ion channel formation, transcriptional activation of HIV-1 and heterologous promoters, co-activation of the glucocorticoid receptor, regulation of cell differentiation, induction of apoptosis, cell cycle arrest, and transduction through cell membranes. Although significant amounts of Vpr (approximately 0.15-fold molar ratio to viral core proteins) are packaged into budding HIV-1 particles in a process dependent on Vpr's interaction with the C-terminal p6 domain of the Gag, the biological role(s) of virion-associated Vpr still remains to be fully elucidated.

The highly conserved 96-amino-acid Vpr has received considerable attention, and a number of biological functions have been attributed to its presence in various cellular and extracellular compartments. The most intensively investigated biological functions of Vpr are those affecting the translocation of the PIC of the incoming virus from the cytoplasm to the nucleus, and the arrest in the G_2 phase of the cell cycle. The nuclear targeting function of Vpr has been associated with productive infection of terminally differentiated macrophages by mediating integration of the proviral DNA into the host genome. It is assumed that Vpr causes dynamic disruptions in the nuclear envelope architecture that enables transport of the PIC across the nuclear membrane. Regarding the second function of Vpr, which leads to G_2 cell cycle arrest in HIV-1-infected T cells, it was suggested that this activity provides an intracellular milieu favorable for viral gene expression. Numerous cellular binding partners of

Vpr have been identified, and for some of these a specific role in G_2 arrest has been proposed. Nevertheless, the precise molecular mechanism underlying the Vpr-induced G_2 arrest remains unclear. A potential explanation for the obvious paradigm that Vpr prevents proliferation of infected T cells by arresting them in the G_2 phase was provided by the observation that viral gene expression is optimal in the G_2 phase and that Vpr can increase virus production by delaying cells at this stage of the cell cycle. Interestingly, there are sufficient amounts of Vpr in incoming virus particles to induce G_2 cell cycle arrest even prior to the initiation of *de novo* synthesis of viral proteins. The secondary structures in Vpr emerging from several analyses indicate the presence of an α-helix–turn–α-helix motif at the N-terminus and an extended amphipathic helical region at the C-terminus which might play a key role in self-association and the interaction of Vpr with heterologous proteins, such as p6, NC, Tat of the virus and the adenine nucleotide translocator of the mitochondrial pore.

Other studies suggest that the prolonged G_2 arrest induced by Vpr ultimately leads to apoptosis of the infected cell. Conversely, early anti-apoptotic effects of Vpr have been described which are superseded by its pro-apoptotic effects. These pro-apoptotic effects of Vpr may result from either effects on the integrity of the nuclear envelope or direct mitochondrial membrane permeabilization, perhaps involving Vpr-mediated formation of ion channels in biological membranes.

The HIV-1-Specific Virus Protein U (Vpu)

Vpu is exclusively encoded by HIV-1, with one exception – the HIV-1 related isolate SIV_{cpz} that encodes a Vpu-like protein similar in length and predicted structure to the Vpu from HIV-1 isolates. Vpu of HIV-1 represents an integral membrane phosphoprotein with various biological functions: first, in the endoplasmic reticulum (ER), Vpu induces degradation of CD4 in a process involving the ubiquitin proteasome pathway and casein kinase 2 (Ck-2) phosphorylation of its cytoplasmic tail (Vpu_{CYTO}). Second, Vpu augments virus release from a post-ER compartment by a cation-selective ion channel activity mediated by its transmembrane anchor (Vpu_{TM}). It was shown previously that Vpu can regulate cationic current when inserted into planar lipid bilayers and in *Xenopus* oocytes. Recent results indicate that the virus release function of Vpu_{TM} involves the mutually destructive interaction between Vpu and the K^+ channel TASK-1. Thus, the virus release function of Vpu might be mediated by tuning the activity of host cell ion channels at the cell membrane.

Structurally, Vpu is a class I oligomeric membrane-bound phosphoprotein composed of an amphipathic sequence of 81 amino acids comprising a hydrophobic N-terminal membrane anchor proximal to a polar C-terminal cytoplasmic domain. The latter contains a highly conserved region with the CK-2 phosphorylation sites that regulate Vpu's function in the ER. The current model of the structure and the orientation in the membrane of the N-terminal hydrophobic Vpu_{TM} provides evidence for water-filled five-helix bundles. However, more work is required to understand Vpu in its functional forms *in vivo* where it exists as the phosphoprotein in mulitprotein complexes involving CD4 and β-TrCP, a component of the SkpI, Cullin, F-box protein (SCF^{TrCP}) E3 ubiquitin ligase complex that regulates the ubiquitination and degradation of cellular proteins by the proteasome.

The Viral Infectivity Factor Vif

The 23 kDa phosphoprotein Vif represents a viral infectivity factor that is highly conserved among primate lentiviruses. Vif regulates virus infectivity in a cell-type-dependent fashion. Permissive cells like Hela, Cos, 293T, SupT1, CEM-SS, and Jurkat cells support HIV replication independent of Vif. In contrast, primary lymphocytes, macrophages, and certain cell lines like H9 cells require Vif for production of fully infectious viruses.

Until recently, the mechanism of how Vif supports formation of infectious virions remained mostly enigmatic. Several findings now support the intriguing hypothesis that at least one main function of Vif is to neutralize cellular cytidine deaminase APOBEC3G, which, in the absence of Vif, is encapsidated into progeny virions. It has been a long-known fact that Vif can act *in trans* when it is expressed in the virus-producer cell, but not in the target cell, indicating that Vif must be situated at the virus-assembly site. This model of a post-entry function for Vif is further supported by the observation that Vifs from diverse lentiviruses function in a species-specific mode. Several hypotheses have been put forward to explain the mechanism of Vif function: the activity of Vif might regulate reverse transcription and proviral DNA synthesis, Vif can support the formation of stable virus cores, or Vif might bind to viral RNA in order to support post-entry steps of virus replication. The discovery of the APOBEC3G–Vif interaction provides at least some explanation for these previous observations.

The Multifunctional Nef Protein

Originally, the 27 kDa N-terminal myristoylated membrane-associated phosphoprotein Nef was described as a 'negative factor' that suppresses the HIV LTR promoter activity. As one of the most intensively investigated HIV-1 accessory proteins, Nef has been shown to fulfill multiple functions during the viral replication cycle. In general, it is now widely accepted that inconsistent with its originally 'negative' nomenclature, Nef plays an important stimulatory role in the replication and pathogenesis

of HIV-1. The broad spectrum of Nef-associated activities so far described can be summarized in distinct classes: (1) modulation of cell activation and apoptosis; (2) change in intracellular trafficking of cellular proteins, particularly of cell receptor proteins like CD4 and major histocompatibility complex I (MHC-I); and (3) increase of virus infectivity.

Well characterized and one of the earliest observations in the course of studying the biology of Nef is the Nef-induced downregulation of cell surface CD4. This mechanism occurs on a post-translational level by augmentation of receptor internalization, where Nef binds to the adapter protein complex in clathrin-coated pits, followed by lysosomal degradation. Another influence on immune receptors is the Nef-mediated block in the transport of MHC-I antigen complexes to the cell surface, leading to the disturbance in the recognition of HIV-1 infected cells by cytotoxic T_{CD8}^{+} lymphocytes (CTLs). More recent studies also described downregulation of the co-stimulatory protein CD28 induced by Nef. It appears that the mechanisms by which Nef blocks cell surface expression of CD4, MHC-I, and CD28 are independent and can be genetically separated.

Nef is expressed relatively early in the HIV replication cycle, and there are reports that Nef is even incorporated into budding virions where it can be cleaved by the viral protease. Nef is present during almost all steps of the viral life cycle, which might explain the multiple functions deployed by this accessory protein. Major attention has been focused toward the Nef-mediated modulation of the T-cell signal transduction and activation pathways. Several lines of evidence supported the hypothesis that, by affecting T-cell receptors, Nef may support several aspects of the virus replication cycle, including regulation of apoptosis and immune evasion. Earlier studies in transgenic mice indicated Nef-dependent activation in T-cell signaling. Later studies indicate that Nef imitates T-cell receptor signaling motifs. The Nef-induced T-cell activation requires the tyrosine kinase Zap70 and the ζ-chain of the T-cell receptor. Further, Nef was shown to activate T cells by binding to the Nef-associated kinase, also known as the p21-activated kinase 2 (PAK2). In addition to the activation of T cells, Nef also induces cell death, since it was shown that expression of the death receptor Fas ligand (FasL) is activated by Nef, and that this upregulation might increase killing of lymphocytes attacking HIV infected cells.

A remarkable turn to our understanding of Nef function was offered recently when it was demonstrated that Nef proteins from the great majority of primate lentiviruses, including HIV-2, efficiently downregulate the T-cell receptor CD3 (TCD3), thereby suppressing activation and apoptosis in T cells. In contrast, *nef* alleles from HIV-1 and its closest related SIV isolates do not encode this activity. Hyperactivation of the immune system as caused by T-cell activation is now generally accepted as an important clinical marker of AIDS progression. Hence, downregulation of TCD3 and the resulting avoidance preceding T-cell activation yields viral persistence without damaging the host immune system and disease progression to AIDS. Thus, in contrast to HIV-1, SIV-derived Nef proteins are better described as persistence factors than as pathogenesis factors.

HIV Subtypes

Analysis of viral sequences allows HIV-1 to be classified into three distinctive groups, representing the HIV-1 lineages main (M), outlier (O), and non-M-non-O (N). It is assumed that each group resulted from an independent zoonotic transfer from chimpanzees, which were infected with SIV_{CPZ} into humans. In addition, eight HIV-2 lineages arose from separate zoonotic transfers from sooty mangabeys infected with SIV_{SM}. Most intriguingly, of these 11 zoonotic events that transferred primate lentiviruses from nonhuman primates to humans, only those resulting in the HIV-1 M-group finally led to the current global AIDS pandemic. Most of group O and group N strains originate from West Africa, especially Cameroon. However, the propagation of these strains was relatively restricted. HIV-2, predominantly A and B groups, is also for the most part endemic in West Africa, particularly in Côte d'Ivoire. In comparison to HIV-1, HIV-2 led to a relatively small number of infections in humans. It is conceivable that more transmissions from nonhuman primates to humans occurred, which, however, were not able to spread efficiently in the human population and therefore have never been detected. At least one reason for the low frequency of successful zoonotic events is that transmissions of primate lentiviruses across the species barrier can be controlled by the existence of species-specific restriction factors (e.g., TRIM5α, as detailed above) that act as barriers against retroviral transfers.

See also: Human Immunodeficiency Viruses: Origin; Human Immunodeficiency Viruses: Pathogenesis; Retroviruses: General Features; Simian Immunodeficiency Virus: Animal Models of Disease; Simian Immunodeficiency Virus: General Features; Simian Immunodeficiency Virus: Natural Infection.

Further Reading

Anderson JL and Hope TJ (2004) HIV accessory proteins and surviving the host cell. *Current HIV/AIDS Reports* 1(1): 47–53.

Coffin JM, Hughes SH, and Varmus HE (1997) *Retroviruses.* New York: CSHL Press.

Flint SJ, Enquist LW, Krung RM, Racaniello VR, and Skalka AM (2000) *Principles of Virology.* Washington, DC: ASM Press.

Freed EO (2002) Viral late domains. *Journal of Virology* 76(10): 4679–4687.
Freed EO and Mouland AJ (2006) The cell biology of HIV-1 and other retroviruses. *Retrovirology* 3: 3–77.
Goff SP (2004) Genetic control of retrovirus susceptibility in mammalian cells. *Annual Reviews of Genetics* 38: 61–85.
Klinger PP and Schubert U (2005) The ubiquitin–proteasome system in HIV replication: Potential targets for antiretroviral therapy. *Expert Review of Anti-Infective Therapy* 3(1): 61–79.
Li L, Li HS, Pauza CD, Bukrinsky M, and Zhao RY (2005) Roles of HIV-1 auxiliary proteins in viral pathogenesis and host–pathogen interactions. *Cell Research* 15(11–12): 923–934.
Morita E and Sundquist WI (2004) Retrovirus budding. *Annual Reviews of Cell and Developmental Biology* 20: 395–425.
Schubert U and McClure M (2005) Human immunodeficiency virus. In: Mahy BWJ and Ter Meulen V (eds.) *Virology,* 10th edn., pp. 1322–1346. London: Topley and Wilson.

Human Immunodeficiency Viruses: Origin

F van Heuverswyn and M Peeters, University of Montpellier 1, Montpellier, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Circulating recombinant forms These forms represent recombinant HIV-1 genomes that have infected three of more persons who are not epidemiologically related.
Endemic A classification of an infectious disease that is maintained in the population without the need for external inputs.
Epidemic A classification of a disease that appears as new cases in a given human population, during a given period, at a rate that substantially exceeds what is expected, based on recent experience.
Neighbor-joining method This clustering method constructs trees by sequentially finding of pairs of operational taxonomic units (OTUs) or neighbors that minimize the total branch length at each stage of clustering OTUs starting with a starlike tree.
Pandemic An epidemic that spreads through human populations across a large region (for e.g., a continent), or even worldwide.
Phylogenetic tree A phylogenetic tree, also called evolutionary tree, is a graphical diagram, showing the evolutionary relationships among various biological species of other entities that are believed to have a common ancestor, comparable to a pedigree showing which genes or organisms are most closely related. In a phylogenetic tree, each node with descendants represents the most common ancestor of the descendants, and the edge lengths in most trees correspond to time estimates. External nodes are often called operational taxonomic units (OTUs), a generic term that can represent many types of comparable taxa. Internal nodes may be called hypothetical taxonomic units (HTUs) to emphasize that they are the hypohetical progenitors of OTUs.
Prevalence The prevalence of a disease in a statistical population is defined as the total number of cases of the disease in the population at a given time, or the number of cases in the population, divided by the number of individuals in the population.

Introduction

Infectious diseases have been an ever-present threat to mankind. A number of important pandemics and epidemics arose with the domestication of animals, such as influenza and tuberculosis. Whereas the cause of some of the historic pandemics, such as the bubonic plague (the Black Death) that killed at least 75 million people, have been successfully eradicated, many others still cause high mortality especially in developing countries. Emerging infectious diseases continue to represent a major threat to global health. As such, HIV/AIDS is one of the most important diseases to have emerged in the past century. When on 5 June 1981, a report was published, describing five young gay men infected with *Pneumocystis carinii* pneumonia (PCP), no one could have imagined that 25 years later, more than 40 million people all over the world would be infected with the human immunodeficiency virus (HIV), the cause of the acquired immunodeficiency syndrome (AIDS). With more than 25 million deaths, HIV/AIDS continues to be one of the most serious public health threats facing humankind in the twenty-first century.

It is important therefore to identify where HIV came from, whether a natural host reservoir exists and how it was introduced into the human population. Today it is well established that human immunodeficiency viruses HIV-1 and HIV-2 are the result of several cross-species transmissions from nonhuman primates to humans.

West-Central African chimpanzees (*Pan troglodytes troglodytes*) are now recognized as the natural reservoir of the simian immunodeficiency viruses (SIVcpz*Ptt*), that are the ancestors of HIV-1. Similarly, HIV-2, which has remained largely restricted to west Africa, is the result of cross-species transmissions of SIVsmm from sooty mangabeys (*Cercocebus atys*).

Although it is clear now that HIV has a zoonotic origin, it remained for a long time less certain where, when, and how often these viruses entered the human population. In this article, we will describe in more detail the latest findings on the origin of HIV, more specifically of the three groups of HIV-1 (M, N, and O) and HIV-2.

Taxonomy, Classification, and Genomic Structure

Taxonomy

Human and *Simian immunodeficiency viruses* (HIV and SIV) belong to the genus *Lentivirus* of the family *Retroviridae*, characterized by their structure and replication mode. These viruses have two RNA genomes and rely on the reverse transcriptase (RT) enzyme to transcribe their genome from RNA into a DNA copy, which can then be integrated as a DNA provirus into the genomic DNA of the host cell. This replication cycle is common for all members of the family *Retroviridae*. As the name suggests, the genus *Lentivirus* consists of slow viruses, with a long incubation period. Five serogroups are recognized, each reflecting the vertebrate hosts with which they are associated (primates, sheep and goats, horses, cats, and cattle). A feature of the primate lentiviruses, HIV and SIV, is the use of a CD4 protein receptor and the absence of a dUTPase enzyme.

Classification

Classification of simian immunodeficiency viruses (SIVs)

SIVs isolated from different primate species are designated by a three-letter code, indicating their species of origin (e.g., SIVrcm from red-capped mangabey). When different subspecies of the same species are infected, the name of the subspecies is added to the virus designation, for example, SIVcpz*Ptt* and SIVcpz*Pts* to differentiate between the two subspecies of chimpanzees *P. t. troglodytes* and *P. t. schweinfurthii,* respectively. For chimpanzee viruses, the known or suspected country of origin is often included; for example, SIVcpzCAM and SIVcpzGAB are isolates from Cameroon and Gabon, respectively.

Currently, serological evidence of SIV infection has been shown for 39 different primate species and SIV infection has been confirmed by sequence analysis in 32 (see **Table 1**). Overall, complete SIV genome sequences are available for 19 species. Importantly, 30 species of the 69 recognized Old World monkey and ape species in sub-Saharan Africa have not been tested yet or only very few have been tested. Knowing that the vast majority (90%) of the primate species tested are SIV infected, many of the remaining species would be expected to harbor additional SIV infections. Only Old World primates are infected with SIVs, and only those from the African continent; no SIVs have been identified in Asian primate species. It is important also to note that none of the African primates naturally infected with SIV develop disease.

Classification of human immunodeficiency viruses (HIVs)

AIDS can be caused by two related lentiviruses; human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2). On the basis of phylogenetic analyses of numerous isolates obtained from diverse geographic origins, HIV-1 is classified into three groups, M, N, and O. Group M (for Major) represents the vast majority of HIV-1 strains found worldwide and is responsible for the pandemic; group O (for Outlier) and N (non-M–non-O) remain restricted to West-Central Africa. Group M can be further subdivided into nine subtypes (A–D, F-H, J, K), circulating recombinant forms (CRFs, CRF01–CRF32), and unique recombinants. The geographic distribution of the different HIV-1 M variants is very heterogeneous and differs even from country to country. Compared to HIV-1, only a limited number of HIV-2 strains have been genetically characterized and eight groups (A–H) have been reported.

Genomic Structure

All primate lentiviruses have a common genomic structure, consisting of the long terminal repeats (LTRs), flanking both ends of the genome, three structural genes, *gag, pol,* and *env* and five accessory genes, *vif*, *vpr*, *tat*, *rev,* and *nef*. Some primate lentiviruses carry an additional accessory gene, *vpx* or *vpu,* in the region between *pol* and *env.* Based on this genomic organization, we can distinguish between three patterns (**Figure 1**): (1) SIVagm, SIVsyk, SIVmnd1, SIVlho, SIVsun, SIVcol, SIVtal, and SIVdeb display the basic structure with three major and five accessory genes; (2) SIVcpz, SIVgsn, SIVmus, SIVden, SIVmon, and also HIV-1 harbor an additional accessory gene, *vpu*; HIV-1 and SIVcpz differ from the other members of this group by the fact that *env* and *nef* genes are not overlapping; (3) SIVsmm, SIVrcm, SIVmnd2, SIVdrl, and SIVmac, and HIV-2 harbor a supplemental accessory gene, *vpx*. For the remaining SIVs, SIVolc, SIVwrc, SIVasc, SIVbkm, SIVery, SIVblu, SIVpre, SIVagi, and SIVgor, full-length sequences are not yet available.

Table 1 Serological and/or molecular evidence for SIV infection in the African nonhuman primates

Genus	*Species*	*Common name*	*SIV*	*Geographic distribution*
Pan	*troglodytes*	Common chimpanzee	SIVcpz	West to East: Senegal to Tanzania
Gorilla	*gorilla*	Western gorilla	SIVgor[a]	Central: Cameroon, Gabon, Congo, Central Africa Republic
Colobus	*guereza*	Mantled guereza	SIVcol	Central: Nigeria to Ethiopia/Tanzania
Piliocolobus	*badius*	Western red colobus	SIVwrc[a]	West: Senegal to Ghana
Procolobus	*verus*	Olive colobus	SIVolc[a]	West: Sierra-Leone to Ghana
Lophocebus	*albigena*	Gray-cheeked managabey	?	Central: Nigeria to Uganda/Burundi
	aterrimus	Black crested mangabey	SIVbkm[a]	Central: Democratic Republic of Congo (DRC)
Papio	*anubis*	Olive baboon	?	West to East: Mali to Ethiopia
	cynocephalus	Yellow baboon	SIVagm-Ver[a]	Central: Angola to Tanzania
	ursinus	Chacma baboon	SIVagm-Ver[a]	South: southern Angola to Zambia
Cercocebus	*atys*	Sooty mangabey	SIVsmm	West: Senegal to Ghana
	torquatus	Red-capped mangabey	SIVrcm	West Central: Nigeria, Cameroon, Gabon
	agilis	Agile mangabey	SIVagi[a]	Central: northeast Gabon to northeast Congo
Mandrillus	*sphinx*	Mandrill	SIVmnd-1, SIVmnd-2	West Central: Cameroon (south of Sanaga) to Gabon, Congo
	leucophaeus	Drill	SIVdrl	West Central: southeast Nigeria to Cameroon (north of Sanaga)
Allenopithecus	*nigroviridis*	Allen's swamp monkey	?	Central: Congo
Miopithecus	*talapoin*	Angolan talapoin	SIVtal[a]	West Central: East coast of Angola into DRC
	ogouensis	Gabon talapoin	SIVtal	West Central: Cameroon (south of Sanaga)-Gabon
Erythrocebus	*patas*	Patas monkey	SIVagm-sab[a]	West to East: Senegal to Ethiopia, Tanzania
Chlorocebus	*sabaeus*	Green monkey	SIVagm-Sab	West: Senegal to Volta river in Burkina Faso
	aethiops	Grivet	SIVagm-Gri	East: Sudan, Erithrea, Ethiopia
	tantalus	Tantalus monkey	SIVagm-Tan	Central: Ghana to Uganda
	pygerythrus	Vervet monkey	SIVagm-Ver	South: South Africa to Somalia and Angola
Cercopithecus	*diana*	Diana monkey	?	West: Sierra-Leone to Ivory Coast
	nictitans	Greater spot-nosed monkey	SIVgsn	Central: forest blocks from West Africa to DRC
	mitis	Blue monkey	SIVblu[a]	East Central: East Congo to Rift-valley
	albogularis	Sykes's monkey	SIVsyk	East: Somalia to Eastern Cape
	mona	Mona monkey	SIVmon	West: Niger delta to Cameroon (north of Sanaga)
	campbelli	Campbell's mona	?	West: Gambia to Liberia
	pogonias	Crested mona	?	West Central: Cross River in Nigeria to Congo (east)
	denti	Dent's mona	SIVden	Central: south of Congo River
	cephus	Mustached guenon	SIVmus	West Central: Cameroon (south of Sanaga) to east of Congo River
	erythrotis	Red-eared monkey	SIVery[a]	West Central: Cross River in Nigeria to Sanaga in Cameroon, Bioko
	ascanius	Red-tailed monkey	SIVasc[a]	Central: South-East Congo to West Tanzania
	lhoest	l'Hoest monkey	SIVlho	Central: eastern Congo–Zaire to western Uganda
	solatus	Sun-tailed monkey	SIVsun	West Central: tropical forest of Gabon
	preussi	*Preuss's monkey*	SIVpre[a]	West Central: Cross river in Nigeria to Sanaga in Cameroon, Bioko
	hamlyni	Owl-faced monkey	?	Central: eastern DRC to Ruanda
	neglectus	de Brazza's monkey	SIVdeb	Central: Angola, Cameroon, Gabon to Uganda, western Kenya

[a]only partial sequences are available, ? only serological evidence for SIV infection.

Evolutionary History

HIV-2 and SIVsmm from Sooty Mangabeys

Shortly after the identification of HIV-1 as the cause of AIDS in 1983, the first SIV, SIVmac, was isolated from rhesus macaques (*Macaca mulatta*) at the New England Regional Primate Research Center (NERPRC). Retrospective research revealed that the newly identified SIV was introduced to the NERPRC by rhesus monkeys, previously housed at the California National Primate Research Center (CNPRC), where they survived an earlier (late 1960s) disease outbreak, characterized by immune suppression and opportunistic infections. A decade after the first outbreak, the story has been repeated in

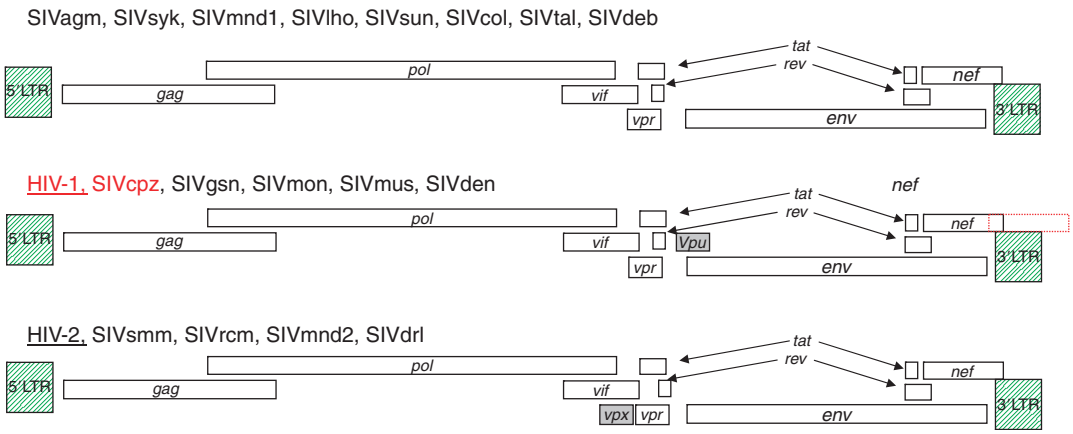


Figure 1 Genomic organization of the primate lentiviruses.

stump-nailed macaques (*Macaca arctoides*) in the same settings and 15 years later a lentivirus, called SIVstm, was isolated from frozen tissue from one of these monkeys. In both cases, the infected rhesus macaques had been in contact with healthy, but retrospectively shown SIVsmm seropositive sooty mangabeys at the CNPRC. The close phylogenetic relationship between SIVmac, SIVstm, and SIVsmm identified mangabeys as the plausible source of SIV in macaques. Since SIVmac induced a disease in rhesus macaques with remarkable similarity to human AIDS, a simian origin of HIV was soon suspected. The discovery in 1986 of HIV-2, the agent of AIDS in West Africa, and the remarkable high relatedness of HIV-2 with SIVsmm, naturally infecting sooty mangabeys in West Africa, reinforced this hypothesis.

In addition, the similarities in viral genome organization (the presence of *vpx*), the geographic coincidence of the natural range of sooty mangabeys and the epicenter of the HIV-2 epidemic in West Africa, as well as the fact that sooty mangabeys are frequently hunted for food or kept as pets, allowed the identification of SIVsmm from sooty mangabeys as the simian source of HIV-2.

HIV-1 and SIVcpz from Chimpanzees

The first SIVcpz strains, SIVcpzGAB1 and SIVcpzGAB2, were isolated from chimpanzees (*P. troglodytes*) in Gabon more than 15 years ago; of 50 wild-caught chimpanzees initially tested for SIVcpz infection, two (GAB1 and GAB2) harbored HIV-1 cross-reactive antibodies. Analysis of the SIVcpzGAB1 genome revealed the same genomic organization as HIV-1, including an accessory gene, named *vpu*, so far only identified in HIV-1. Furthermore, phylogenetic analysis indicated that SIVcpzGAB1 was more closely related to HIV-1 than to any other SIV. A few years later, a third positive chimpanzee, confiscated upon illegal importation into Belgium from the Democratic Republic of Congo (DRC, ex-Zaire), was identified among 43 other wild-caught chimpanzees. A virus, SIVcpzANT, was isolated and characterized, but this virus showed an unexpected high degree of divergence from the others. A fourth SIVcpz strain (SIVcpzUS) was obtained from a chimpanzee (Marilyn) housed in an American primate center and it was shown that SIVcpzUS was closely related to the SIVcpz strains from Gabon. Subspecies identification of the chimpanzee hosts revealed that the SIVcpzANT strain was isolated from a member of the *P.t. schweinfurtii* subspecies, whereas the other chimpanzees belonged to the *P.t. troglodytes* subspecies. These findings suggested two distinct SIVcpz lineages according to the host species: SIVcpz*Ptt*, from the West-Central African chimpanzees, and SIVcpz*Pts*, from eastern chimpanzees. HIV-1 strains are classified into three highly divergent clades, groups M, N, and O, each of which was more closely related to SIVcpz from *P. t. troglodytes* than to SIVcpz from *P. t. schweinfurthii*. These data pointed to the West-Central African subspecies, *P. t. troglodytes* as the natural reservoir of the ancestors of HIV-1.

Until recently, only a handful of complete or partial SIVcpz genomes have been derived. In addition to the four previously mentioned viruses, three other strains, SIVcpzCAM3, SIVcpzCAM5, and SIVcpzCAM13, have been identified in Cameroon and all clustered with the previously identified SIVcpz*Ptt* strains consistent with their species of origin. The discovery of HIV-1 group N in a Cameroonian patient in 1998 showed that HIV-1 N is closely related to SIVcpz*Ptt* in the envelope region of the viral genome, suggesting an ancient recombination event. This finding demonstrated the co-circulation of SIVcpz and HIV-1 N viruses in the same geographic area and provided additional evidence that the western part of Central Africa is the likely site of origin of HIV-1.

However, the number of identified SIV strains in chimpanzees was low, compared to that of other naturally occurring SIV infections. The major problem in studying SIVcpz infection in chimpanzees is their endangered status and the fact that they live in isolated forest regions. All

previously studied chimpanzees were among wild-caught but young and captive animals. They were initially captured as infants, mainly as a by-product of the bushmeat trade. Since the age of maturation of chimpanzees is around 9 for males and 10 for females, these infections were most probably the result of vertical transmission and do not reflect true prevalences among wild-living adult animals. Apparently, the frequency of vertical transmission is low among naturally infected primates, which can explain the low prevalence rates initially observed.

The recent development of noninvasive methods to detect and characterize SIVcpz in fecal and urine samples from wild ape populations boosted the search for new SIVcpz strains in the vast tropical forests of Central Africa. The first report of a full-length SIVcpz sequence obtained from a fecal sample, SIVcpzTAN1, was from a wild chimpanzee from the *P. t. schweinfurtii* subspecies in Gombe National Park, Tanzania. Subsequently, additional cases of SIVcpz*Pts* infections were documented in Tanzania (SIVcpzTAN2 to SIVcpzTAN5) and around Kisangani, northeastern DRC (SIVcpzDRC1). All the new SIVcpz*Pts* viruses formed a separate lineage with the initially described SIVcpzANT strain and suggest that the SIVcpz*Pts* strains are not at the origin of HIV-1 (**Figure 2**). A recent study in wild chimpanzee communities in southern Cameroon documented a prevalence of SIVcpz infection ranging from 4% to 35%, and identified 16 new SIVcpz*Ptt* strains. All of these newly identified viruses were found to fall within the radiation of SIVcpz*Ptt* strains from captive *P. t. troglodytes* apes, which also includes HIV-1 groups M and N, but not group O or SIVcpz*Pts* (**Figure 2**). The new SIVcpz*Ptt* viruses are characterized by high genetic diversity and SIVcpz*Ptt* strains were identified which are much more closely related to HIV-1 groups M and N than were any previously identified SIVcpz strains. Interestingly, the new SIVcpz*Ptt* viruses exhibited a significant geographic clustering and made it possible to trace the origins of present-day human AIDS viruses to geographically isolated chimpanzee communities in southern Cameroon.

HIV-1 and SIVgor from Gorillas

SIV infection has recently been described in western gorillas (*Gorilla gorilla*) in Cameroon. Surprisingly, the newly characterized gorilla viruses, termed SIVgor, formed a monophyletic group within the HIV-1/SIVcpz*Ptt* radiation, but in contrast to SIVcpz*Ptt*, they were most closely related to the HIV-1 group O lineage (**Figure 2**). However, the phylogenetic relationships between SIVcpz, SIVgor, and HIV-1 indicate that chimpanzees represent the original reservoir of SIVs now found in chimpanzees, gorillas, and humans. Given their herbivorous diet and peaceful coexistence with other primate species, especially chimpanzees, it remains a mystery by what route gorillas acquired SIVgor.

The data on SIV in wild chimpanzee and gorilla populations showed that distinct chimpanzee communities in southern Cameroon transmitted divergent SIVcpz to humans giving rise to HIV-1 groups M and N; and that chimpanzees transmitted HIV-1 group O-like viruses either to gorillas and humans independently, or first to gorillas which then transmitted the virus to humans. Additional studies are needed to determine if the other African great ape species, the eastern gorillas (*Gorilla berengei*) and the bonobo (*Pan paniscus*) harbor any SIV.

Cross-Species Transmission

Where?

HIV-1

Based on mitochondrial DNA sequences, common chimpanzees (*P. troglodytes*) are classified into four subspecies: *P. t. verus* in West Africa; *P. t. vellerosus*, restricted to a geographical area between the Cross River in Nigeria and the Sanaga River in Cameroon; *P. t. troglodytes* in southern Cameroon, Gabon, Equatorial Guinea, and the Republic of Congo; and *P. t. schweinfurtii* in the Democratic Republic of Congo, Uganda, Rwanda, and Tanzania. No evidence of SIV infection is found in *P. t. verus*, despite testing of more than 2000 chimpanzees, mostly captive animals exported to zoos or research centers in the US. Wild *P. t. vellerosus* apes have not been found to harbor SIVcpz, but only about 100 samples have been screened. The single reported SIV infection of *P. t. vellerosus*, SIVCAM4, was most probably the result of a cage transmission from a *P. t. troglodytes* ape, infected with SIVcpzCAM3. Since the three groups of HIV-1 (M, N, and O) all fall within the HIV-1/SIVcpz*Ptt*/SIVgor lineage, the cross-species transmissions giving rise to HIV-1 most likely occurred in western equatorial Africa. Furthermore, no human counterpart is found for SIVcpz*Pts* from *P. t. schweinfurtii*, which undermines the idea of a human-induced origin of HIV-1 by oral polio vaccine (OPV) programs in East-Central Africa in the late 1950s. It has been suggested that tissues derived from SIVcpz-infected chimpanzees, captured in the northeastern part of DRC were used for the polio vaccine production. However, this geographical region is situated in the middle of the *P. t. schweinfurtii* range and the characterization of a partial SIV genome (SIVcpzDRC1) from a wild chimpanzee in this region proved once more the inconsistency of the OPV theory (**Figure 2**).

The recent studies in wild chimpanzee communities in Cameroon, not only confirm the West-Central African origin of HIV-1, but also indicate that HIV-1 group M and N arose from geographically distinct chimpanzee populations. Phylogenetic analysis showed that all SIVcpz strains collected in southeast Cameroon formed a cluster with HIV-1 group M, whereas SIVcpz isolates from chimpanzee communities of a well-defined region in

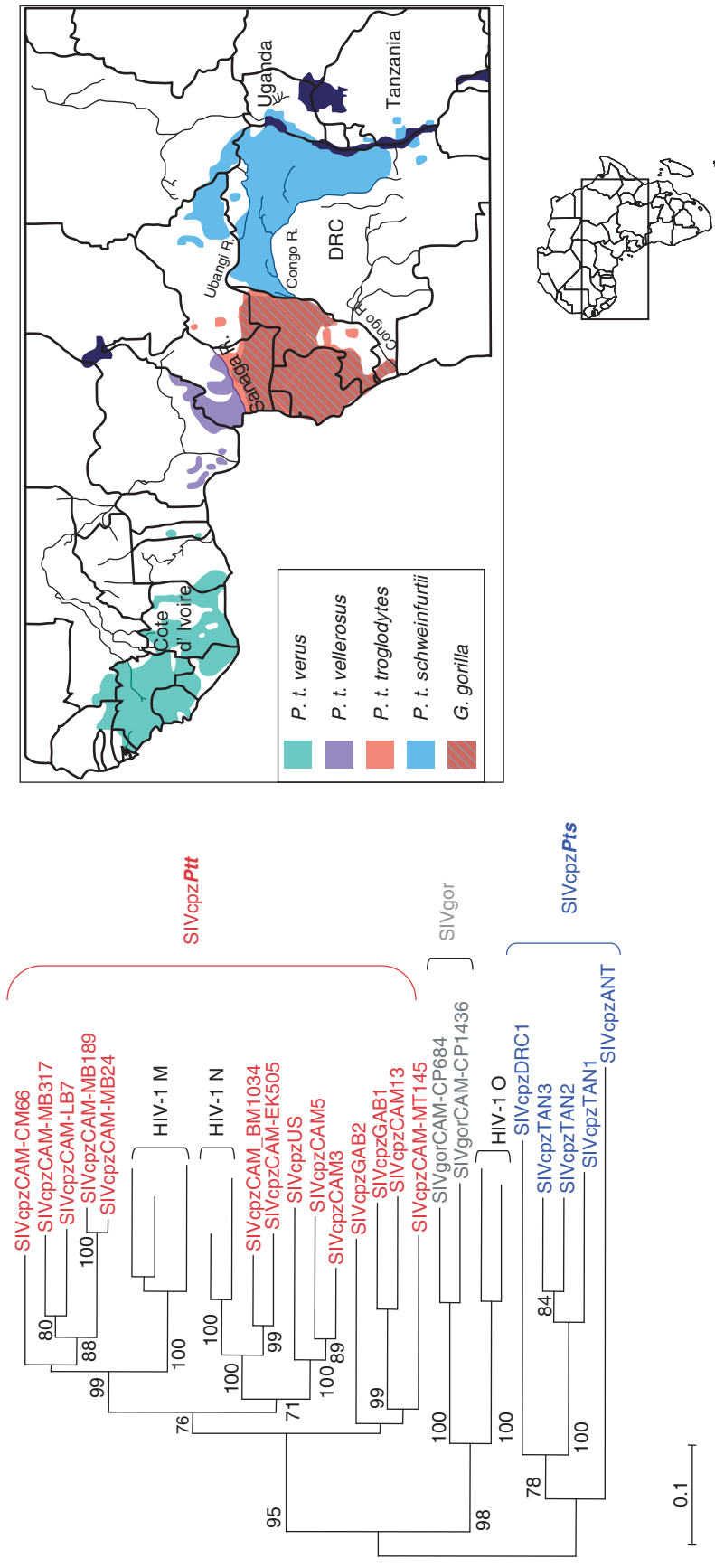


Figure 2 Natural range of chimpanzee subspecies and phylogeny of the HIV-1/SIVcpz/SIVgor lineage. The ranges of the four recognized chimpanzee subspecies are color-coded. The SIVcpz*Ptt* and SIVcpz*Pts* sequences in the phylogenetic tree are colored in red and blue, respectively, in accordance to the chimpanzee subspecies, illustrating that HIV-1 is more closely related to SIVcpz from West-Central African chimpanzees. The natural range for western gorillas (*Gorilla gorilla*) overlaps with the *P. t. troglodytes* range in West-Central Africa; SIVgor sequences are indicated in gray. The country of origin where SIVcpz strains were identified is indicated with a three-letter code: CAM for Cameroon, GAB for Gabon, TAN for Tanzania, DRC for the Democratic Republic of Congo, US for an animal in a primate center in the USA, and ANT for a captive animal in Antwerp, Belgium. This tree was derived by neighbor-joining analysis of partial *env*/*nef* nucleotide sequences. Horizontal branch lengths are drawn to scale.

south-central Cameroon clustered with the nonpandemic HIV-1 group N. It is also interesting to note that there is an uneven dissemination of SIV infection among chimpanzee populations, with the absence of SIV infection in some of them and with major geographical elements, like rivers, that can serve as important barriers.

As discussed above, HIV-1 group N resulted from an ancestral recombination event between divergent lineages. The discovery of such a recombinant virus in a geographically isolated chimpanzee community in southern Cameroon shows that HIV-1 N was already a recombinant in its natural hosts prior to its transmission to humans.

The origin of the third group of HIV-1, group O, remained uncertain until the recent identification of HIV-1 group O-like viruses in two different wild-living gorilla populations, in southern Cameroon (SIVgor). So all three HIV-1 groups seem to have their seeds in West-Central Africa. While HIV-1 group O infections remained restricted to West-Central Africa (Cameroon, Nigeria, Gabon, Equatorial Guinea) and HIV-1 N to Cameroon only, HIV-1 group M strains have spread across Africa and all the other continents.

HIV-2

A close phylogenetic relatedness is also observed between SIVsmm from sooty mangabeys (*Cercocebus atys*) and HIV-2 in West Africa. Sooty mangabeys are indigenous to West Africa, from Senegal to Ivory Coast, coinciding with the endemic center of HIV-2 (**Figure 3**). Eight groups (A–H) of HIV-2 have been described so far, but only subtypes A and B are largely represented in the HIV-2 epidemic, with subtype A in the western part of West Africa (Senegal, Guinea-Bissau) and subtype B being predominant in Ivory Coast. The other subtypes have been documented in one or few individuals only. Except for groups G and H, groups C, D, E, and F were isolated in rural areas in Sierra Leone and Liberia and these viruses are more closely related to the SIVsmm strains obtained from sooty mangabeys found in the same area than to any other HIV-2 strains. This suggests that the

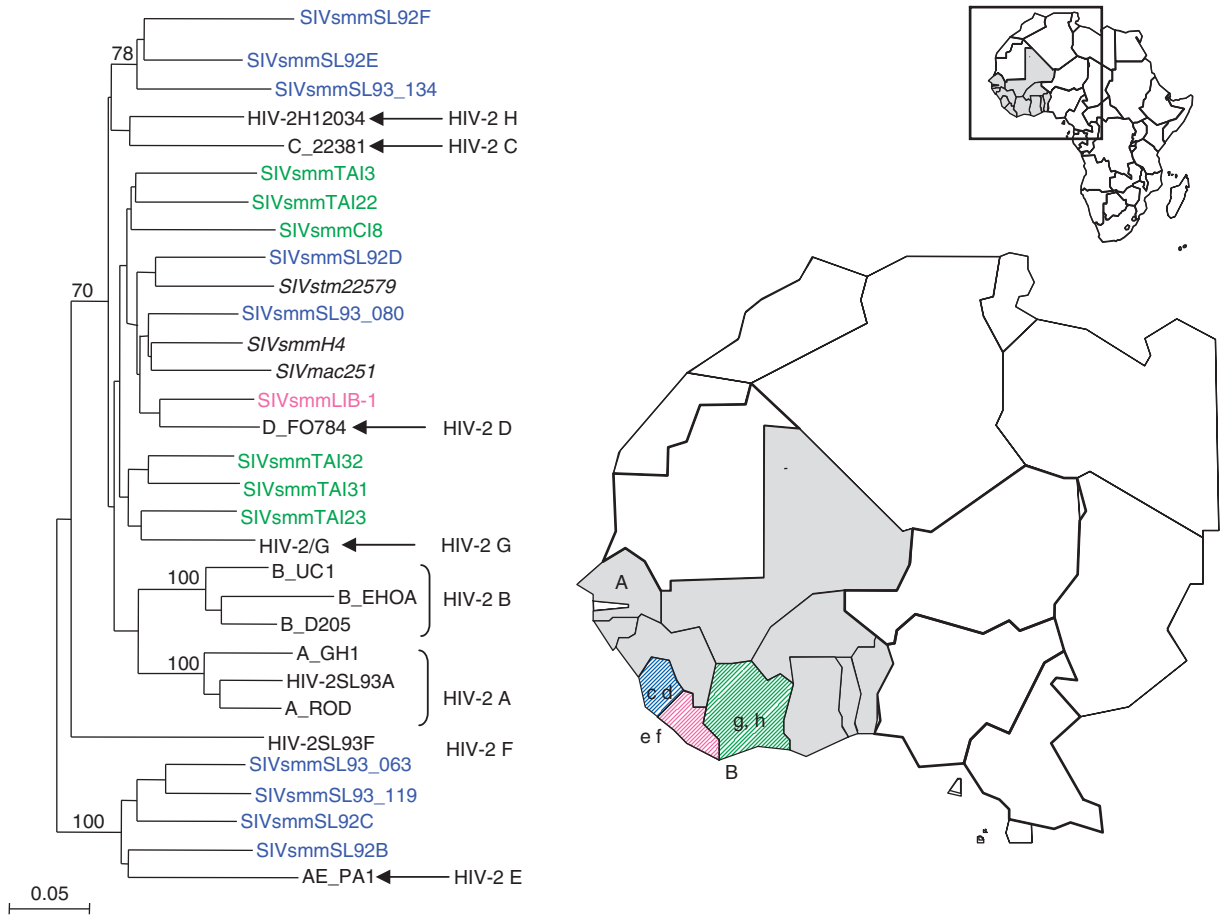


Figure 3 Geographic distribution of the different HIV-2 groups and phylogeny of the HIV-2/SIVsmm lineage. Countries where HIV-2 is endemic are colored in grey and overlap with the range of sooty mangabeys (*Cercocebus atys*) in West Africa. SIVsmm strains obtained from mangabeys in different regions are colored: green for Ivory Coast, blue for Sierra Leone, and pink for Liberia. SIVsmm strains isolated from mangabeys or macaques in US zoo's or primate centers are indicated in italic. This tree was derived by neighbor-joining analysis of partial *gag* nucleotide sequences from HIV-2 and SIVsmm sequences from West Africa. Horizontal branch lengths are drawn to scale.

different groups of HIV-2 must be the result of multiple independent cross-species transmissions of SIVsmm into the human population. Importantly, HIV-2 prevalence remains low and is even decreasing, since HIV-1 M is now predominating also in West Africa.

When?

It is clear now that each group of HIV-1 (M, N, and O) and HIV-2 (A–H) resulted from an independent cross-species transmission, followed by different viral evolution rates and possibly by one or more recombination events. HIV-1 group M can be further subdivided into subtypes, circulating recombinant forms and many unique recombinants, which had a heterogeneous geographical spread. The highest genetic diversity, in number of co-circulating subtypes and intrasubtype diversity has been observed in Central Africa, more precisely the western part of DRC, suggesting this region being the epicenter of HIV-1 M. Moreover, the earliest known HIV-1 virus, ZR59, isolated from an individual in Léopoldville (now Kinshasa) in 1959. The virus has been characterized as a member of HIV-1 M, subtype D. So, the common ancestor of this group should be dated prior to 1959. Molecular clock analyses estimated the date of the most recent common ancestor of HIV-1 group M around 1930 with a confidence interval of 1915–1941. A similar time frame is estimated for the origin of the HIV-1 group O radiation: 1920 with a range from 1931 to 1940. The oldest HIV-1 group O sample has been documented in a Norwegian sailor in 1964 infected in Cameroon (Douala), both groups show an exponential increase of the number of HIV infections during the twentieth century. However, the growth rate of HIV-1 group O ($r = 0.08$ [0.05–0.12]) is slower than the rate estimated for HIV-1 group M in Central Africa ($r = 0.17$). This is not surprising, considering the much lower prevalence of group O in Cameroon. Since the first identification of HIV-1 group N in 1998, about 10 group N infections have been described and all were from Cameroonian patients. The intra-group genetic diversity is significant lower for group N than for group M or O, which suggests a more recent introduction of the HIV-1 N lineage into the human population.

Similar analyses traced the origin of the pandemic HIV-2 groups A and B to be around 1940 with a confidence interval of ± 16 years, and around 1945 with a confidence interval of 1931–59, respectively.

How?

Although the precise conditions and circumstances of the SIVcpz and SIVsmm transmissions remain unknown, human exposure to blood or other secretions of infected primates, through hunting and butchering of primate bushmeat, represents the most plausible source for human infection. Also bites and other injuries caused by primates kept as pets can increase the probability of viral transmission. Direct evidence of human infection with other SIVs is not yet reported, but that retroviruses can jump from primates to humans has been documented for simian foamy viruses (SFV) and simian T-cell leukemia viruses (STLVs). For example, SFV infection has been detected in rural Central Africa among individuals who hunt monkeys and apes. The infection with SFV in an Asian man, who regularly visited an ancient temple, was most probably the result of a bite from a macaque. An analysis of the man's blood indicated the presence of an SFV strain similar to a strain from macaques living around the temple. So far, SFV has not been shown to cause disease in humans, but the long-term effects of SFV in humans are not yet known. These effects are well documented for SIV and its human counterpart HIV; the HIV-1 group M epidemic clearly illustrates the devastating results of a single cross-species transmission.

Other SIV Cross-Species Transmissions, Risk for Novel HIV?

A recent study on primate bushmeat in Cameroon revealed that about 10% is SIV infected and illustrates an ongoing exposure to a plethora of different SIVs. Today, 69 different nonhuman primate species are identified in Africa and for 32 species (47%) SIV infection could already be proved by genomic amplification of the virus. This means that, in addition to sooty mangabeys, chimpanzees, and possibly gorillas, at least 29 other primate species harbor SIV's, which poses a potential risk for transmission to humans, especially for those in direct contact with infected blood and tissues. Therefore, efforts to reduce exposure to SIV-infected primates should be a primary concern. However, the opposite is occuring; the bushmeat trade has increased significantly during the last few decades, especially through the development of the logging industry. As a consequence, roads are now penetrating the formerly isolated forest areas and create a free passage for transport of wood, bushmeat, people, and subsequently different pathogens. The surrounding villages change from modest, little communities to real trade centers, with up to several thousand inhabitants. In addition, human migration and social and economic networks support this industry. It is very likely that the estimated prevalence of SIV infection of wild monkey species is underestimated for three reasons: first, only half of the recognized species have been tested; second, for some species only a few monkeys were tested; and third, and the most important reason, the sensitivity of the available diagnostic tools, initially developed for HIV detection, may be inaccurate for the detection of divergent SIVs. The increasing magnitude of human exposure to SIVs, combined with socioeconomic changes, which

favor the dissemination of a plausible SIV transmission into the human population, could be the basis of novel zoonoses that lead in turn to novel HIV epidemics.

It is important to note that there is more needed than transmission of a virus to initiate an epidemic. After the virus has crossed the species barrier, adaptation to the new host is necessary to be able to spread efficiently within a population. The pathogenic potential of the virus and host genetic differences between individuals, as well as between species, determine susceptibility or resistance to further disease progression. In addition, environmental, social, and demographic factors play a major role in the further spread of new viruses.

Evolution of SIVs in their Natural Primate Hosts: The Origin of SIVcpz

With the increasing number of full-length SIV sequences from different primate species it becomes clear that cross-species transmissions and subsequent recombinations have occurred frequently among primates lentiviruses. Cross-species transmissions among co-habiting species in the wild has been observed between African green monkeys and patas monkeys in West Africa; between African green monkeys and baboons in southern Africa; and between different *Cercopithecus* species in Central Africa, that is, greater spot-nosed guenons (*C. nictitans*) and moustached (*C. cephus*) monkeys in Cameroon.

One of the most striking examples of cross-species transmission, followed by recombination, is SIVcpz in chimpanzees. Chimpanzees are known to hunt other primates for food, such as red-capped mangabeys (*Cercocebus torquatus*), greater spot-nosed guenons (*Cercopithecus nictitans*), and colobus monkeys (*Colobinae*). The isolation and characterization of the SIV genomes from the former monkey species revealed an unexpected high level of similarity between some parts of the SIVcpz genome and SIVrcm and SIVgsn. The 5′ region of SIVcpz (*gag*, *pol*, *vif,* and *vpr*) is most similar to SIVrcm, except for the accessory gene *vpx*, which is characteristic for the SIVrcm lineage, but absent in the HIV-1 and SIVcpz strains. The 3′ region of SIVcpz (*vpu*, *env,* and *nef*) is closely related to SIVgsn. Furthermore, SIVgsn is the first reported monkey virus to encode a *vpu* gene, the accessory gene characteristic of the HIV-1/SIVcpz lineage. Most probably, the recombination of these monkey viruses occurred within chimpanzees and gave rise to the common ancestor of today's SIVcpz lineages, which in turn were subsequently transmitted to humans. The cross-species transmission of this recombinant virus, or its progenitors, happened some time after the split of *P. t. verus* and *P. t. vellerosus* from the other subspecies, but possibly before the divergence between *P. t. troglodytes* and *P. t. schweinfurtii.*

The fact that gorillas are infected with an SIV from the SIVcpz lineage, represents a mystery, since only peaceful encounters have been documented among these sympatric apes. The evolutionary history of primate lentiviruses is complex and likely involved a series of consecutive interspecies transmissions, the timelines and directions of which remain to be deciphered.

Conclusion

We now have a clear picture of the origin of HIV and the seeds of the AIDS pandemic. SIVcpz, the progenitor of HIV-1, resulted from a recombination among ancestors of SIV lineages presently infecting red-capped mangabeys and *Cercopithecus* monkeys in West-Central Africa. HIV-1 groups M and O resulted from independent cross-species transmissions early in the twentieth century. The SIVcpz*Ptt* strains that gave rise to HIV-1 group M belonged to a viral lineage that persists today in *P. t. troglodytes* apes in south Cameroon. Most likely this virus was transmitted locally, but made its way to Kinshasa where the group M pandemic was spawned. HIV-1 group N, which has been identified in only a small number of AIDS patients from Cameroon, derived from a second SIVcpz*Ptt* lineage in south-central Cameroon and remained geographically more restricted. HIV-1 group O-related viruses are present in a second African great ape species, the western gorilla (*Gorilla gorilla*), but chimpanzees were the original reservoir of SIVgor.

Similarly, only HIV-2 group A and B play a major role in the HIV-2 epidemic, and most other groups (C–H) represent unique sequences found in a single patient. A possible explanation could be that some viruses were not able to adapt to the new host or that the environment was not suitable for epidemic spread.

Viral adaptation to the new host is one of the requirements for the generation of an epidemic, but also the interaction of sociocultural factors, such as deforestation, urbanization, and human migration, have been crucial in the emergence of the HIV-1 pandemic. While the origin of the HIV-1 and HIV-2 viruses has become clearer, important questions concerning pathogenicity and epidemic spread of certain SIV variants needs to be further elucidated.

See also: AIDS: Disease Manifestation; AIDS: Global Epidemiology; AIDS: Vaccine Development; Human Immunodeficiency Viruses: Antiretroviral agents; Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Pathogenesis; Simian Immunodeficiency Virus: Animal Models of Disease; Simian Immunodeficiency Virus: General Features; Simian Immunodeficiency Virus: Natural Infection.

Further Reading

Aghokeng AF and Peeters M (2005) Simian immunodeficiency viruses (SIVs) in Africa. *Journal of Neurovirology* 11(supplement 1): 27–32.

Hahn BH, Shaw GM, De Cock KM, and Sharp PM (2000) AIDS as a zoonosis: Scientific and public health implications. *Science* 287: 607–614.

Keele BF, Van Heuverswyn F, Li Y, *et al.* (2006) Chimpanzee: Reservoirs of pandemic and nonpandemic HIV-1. *Science* 313: 523–526.

Korber B, Muldoon M, Theiler J, *et al.* (2000) Timing the ancestor of the HIV-1 pandemic strains. *Science* 288: 1789–1796.

Lemey P, Pybus OG, Rambaut A, *et al.* (2004) The molecular population genetics of HIV-1 group O. *Genetics* 167: 1059–1068.

Santiago ML, Range F, Keele BF, *et al.* (2005) Simian immunodeficiency virus infection in free-ranging sooty mangabeys (*Cercocebus atys atys*) from the Tai Forest, Cote d'Ivoire: Implications for the origin of epidemic human immunodeficiency virus type 2. *Journal of Virology* 79: 12515–12527.

Sharp PM, Shaw GM, and Hahn BH (2005) Simian immunodeficiency virus infection of chimpanzees. *Journal of Virology* 79: 3891–3902.

VandeWoude S and Apetrei C (2006) Going wild: Lessons from naturally occurring T-lymphotropic lentiviruses. *Clinical Microbiological Reviews* 19: 728–762.

Van Heuverswyn F, Li Y, Neel C, *et al.* (2006) Human immunodeficiency viruses: SIV infection in wild gorillas. *Nature* 44: 164.

Worobey M, Santiago ML, Keele BF, *et al.* (2004) Origin of AIDS: Contaminated polio vaccine theory refuted. *Nature* 428: 820.

Human Immunodeficiency Viruses: Pathogenesis

N R Klatt, A Chahroudi, and G Silvestri, University of Pennsylvania School of Medicine, Philadelphia, PA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Apoptosis Programmed cell death.

Bystander cell death The death of HIV-uninfected cells.

CCR5 A G-protein-coupled, seven transmembrane spanning receptor for the chemokines RANTES (CCL5), MIP-1α (CCL3), and MIP-1β (CCL4) that is primarily expressed on T lymphocytes, dendritic cells, macrophages, and microglial cells.

CD4+ T lymphocytes (also called T-helper cells) Cells bearing CD4, a co-receptor of the T-cell receptor (TCR) complex, that recognize peptide antigens bound to MHC class II molecules and are important for both humoral and cellular immunity.

Cytotoxic T lymphocytes (CTLs) Cells that are capable of killing other cells. Most CTLs express CD8, a co-receptor of the TCR complex, and recognize antigenic peptides from cytosolic pathogens, particularly viruses, that are bound to MHC class I molecules.

Generalized immune activation The activation of all lymphocyte subsets in HIV infection, leading to increased cell death.

Mucosal-associated lymphoid tissue (MALT) The system of lymphoid cells found in the epithelia and lamina propria of the body's mucosal sites, including the gastrointestinal tract, lungs, eyes, nose, and the female reproductive tract.

Neutralizing antibodies Antibodies that can limit the infectivity of a pathogen or the toxic effects of a toxin by binding to the receptor-binding site on the pathogen/toxin and thus block entry into the target cell.

Simian immunodeficiency virus (SIV) Like HIV it is a single-stranded, positive-sense, enveloped RNA virus that is classified as a member of the genus *Lentivirus* of the family *Retroviridae*. The virus infecting chimpanzees (SIV_{cpz}) is the origin of HIV-1 and the virus infecting sooty mangabeys (SIV_{smm}) is the origin of HIV-2.

Introduction

The AIDS pandemic, with an estimated 40 million individuals infected with the causative agent, human immunodeficiency virus (HIV), is without question one of the key medical challenges of modern times. Twenty-four years after the first identification of HIV, the situation in the fields of AIDS research, prevention, and treatment reflects several major advances as well as a number of areas where the progress has been slow or nonexistent (**Table 1**). While the #1 challenge (low rate of treatment in developing countries, particularly sub-Saharan Africa) is, in essence, a reflection of the sociopolitical climate arising from a lack of stability, development, and health-care infrastructure in these countries, challenges #2 and #3 (absence of a preventative vaccine or a cure for HIV infection and AIDS) are a direct consequence of our incomplete understanding of the pathogenesis of HIV infection. Here we summarize the key advances that have improved our understanding of the mechanisms underlying the immunodeficiency that follows HIV infection.

While it is clear and universally accepted that HIV is the etiologic agent of AIDS and that the main

Table 1 Major achievements and remaining challenges in AIDS research

Major achievements in AIDS research:
1. Discovery and characterization of the etiologic agent.
2. Development of lab tests to monitor and prevent the infection via blood and blood products.
3. Definition of the origin of the epidemics.
4. Development of a large array of potent anti-HIV drugs, with consequent major reduction in mortality and MTCT in Western countries.

Major remaining challenges:
1. Abysmally low rate of treatment in developing countries.
2. Absence of a vaccine or a long-lasting microbicide.
3. Absence of a treatment that can eradicate infection.
4. Incomplete understanding of the pathogenesis of infection.

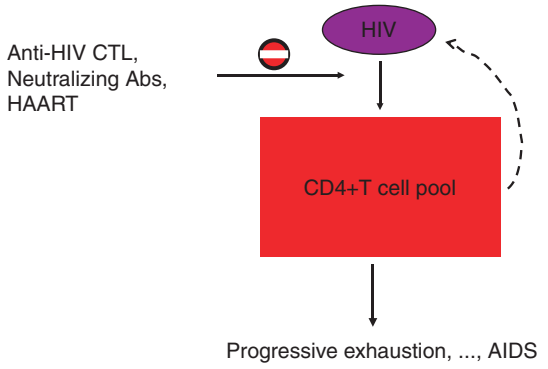


Figure 1 The classical or virus-centric model of AIDS pathogenesis.

pathophysiologic assault during HIV infection is the development of a state of chronic, progressive, and ultimately fatal immunodeficiency, several questions remain regarding the exact sequence and cause of the pathogenic events that define the progression from HIV infection to the development of AIDS. In particular, the precise mechanisms that underlie the progressive and generalized CD4+ T-lymphocyte depletion that represents the most striking and consistent laboratory finding in HIV-infected individuals as well as the best predictor of progression to AIDS are still unclear. While it is well established that HIV infects and kills CD4+ T lymphocytes, it is not known to what extent this direct effect of the virus contributes to the overall *in vivo* loss of these cells when measured against a series of indirect mechanisms of CD4+ T-lymphocyte depletion that are related to the state of generalized immune activation associated with HIV infection. Understanding the mechanisms responsible for the HIV-associated CD4+ T-lymphocyte depletion is a matter of utmost importance, as it is likely that greater knowledge of the immunopathogenesis of AIDS will pave the way for the implementation of novel therapeutic strategies.

Direct Cytopathic Effect of HIV on Infected CD4+ T Lymphocytes

A series of crucial discoveries made between 1983 (when HIV was first identified and proposed as the etiologic agent of AIDS) and 1996 (when highly active antiretroviral therapy, HAART, became available) resulted in a model for the pathogenesis of HIV infection that explains a large number of experimental and clinical findings (**Figure 1**). This model is based primarily on the following observations: (1) after transmission of HIV through either the sexual or intravenous route, the virus infects (using the CD4 molecule as well as one of the chemokine receptors CCR5 or CXCR4 as entry receptors) and then kills a subset of memory/activated CD4+ T lymphocytes; (2) the level of virus replication and the severity of CD4+ T-lymphocyte depletion are the key markers of disease progression from the initial asymptomatic phase of infection (lasting 2–15 years) to the phase characterized by constitutional symptoms and, eventually, the onset of opportunistic infections and cancer; (3) if HIV replication is controlled following the administration of antiviral drugs or spontaneously (in a small subset of HIV-infected individuals referred to as long-term nonprogressors, LTNP), the immunological damage induced by HIV is limited and survival improves dramatically. Collectively, these findings suggested that the pathogenesis of AIDS is mainly related to the direct, virus-mediated killing of CD4+ T lymphocytes that results in a slow but continuous erosion of the pool of these cells (**Figure 1**). This virus-centric model of AIDS pathogenesis was strengthened by the observations that HIV infection is associated with a high rate of virus turnover and a short lifespan of virus-infected cells. These studies were interpreted to mean that, in infected individuals, HIV kills large numbers of CD4+ T lymphocytes at a very fast pace, to the point that the compensatory drive to reconstitute the pool fails to maintain sufficient CD4+ T-lymphocyte numbers. Ultimately, this progressive depletion of CD4+ T lymphocytes results in the permanent loss of a key function of the human immune system, which in turn manifests itself as an increased susceptibility to opportunistic infections and neoplasms. It is important to note that, according to this model, the increased level of CD4+ T-lymphocyte proliferation that is consistently observed in HIV-infected patients is interpreted to be a homeostatic mechanism aimed at compensating for the loss of CD4+ T lymphocytes induced directly by HIV.

Further refinement of this model comes from studies defining the co-receptors used by HIV, in addition to CD4, to infect human cells. The main HIV co-receptor is the chemokine receptor CCR5 (CD195) that is used by the non-syncytium-inducing (NSI), macrophage-tropic

viruses that are preferentially transmitted from person-to-person and represent the majority of strains found in infected patients. CCR5 is expressed in a relatively small fraction (approximately 10–15%) of circulating and lymph node CD4+ T lymphocytes that mainly display a memory phenotype (i.e., CD45RO+), but is present on a much larger fraction of mucosal-associated memory CD4+ T lymphocytes. This latter finding is particularly important as it may explain why CD4+ T lymphocytes may be more severely depleted in mucosal tissues than in peripheral blood and lymph nodes (see below). The second important HIV co-receptor is CXCR4 (CD184), that is used by syncytium-inducing (SI), T-lymphocyte tropic viruses that are found in a minority of HIV-infected individuals, usually during advanced stages of disease. Interestingly, CXCR4 is expressed primarily by naïve CD4+ T lymphocytes, thus potentially accounting for the relative preservation of naïve CD4+ T lymphocytes during the early stages of HIV infection. These data are important not only for their elucidation of potential targets for therapeutic interventions aimed at blocking HIV entry (e.g., CCR5 inhibitors), but also because they help to explain the finding that not all subsets of CD4+ T lymphocytes are equally sensitive to HIV infection, such that depletion of memory/activated CD4+ T lymphocytes is associated with infection with CCR5-tropic viruses, and depletion of naïve CD4+ T lymphocytes is associated with CXCR4-tropic viruses.

Indirect Mechanisms of Immunopathogenesis during HIV Infection

The virus-centric model of HIV/AIDS pathogenesis outlined in the previous section has the unquestionable advantage of being fairly straightforward while still encompassing several important observations. More recently, however, this model, at least in its simplest formulation, has been challenged by a series of new insights into the experimental data. First, the long period of time between virus transmission and the development of AIDS in which the level of CD4+ T lymphocytes appears to be in a quasi-steady state is not compatible with the kinetics of a typical viral infection that should rapidly and exponentially consume all available target cells. The original assumption was that the long duration of the disease process is due to the regenerative capacity of the CD4+ T-lymphocyte compartment that is able to at least partially (or temporarily) compensate for the virus-induced killing of these cells. However, it is now believed that it would be too striking of a coincidence that in the vast majority of patients the rate of killing by HIV happens to be just slightly faster than the rate of production of new CD4+ T lymphocytes (and never slower, in which patients would remain healthy, nor significantly faster, in which patients would die within days to weeks). It was then shown that the fraction of infected CD4+ T lymphocytes was remarkably low (i.e., 0.01–1%) at any given time during the course of infection, thus raising the question of why the immune system cannot simply ramp up the production of new CD4+ T lymphocytes to compensate for the relatively limited losses induced by direct viral infection and killing. The further observations that, in fact, the majority of CD4+ T lymphocytes that die *in vivo* in HIV-infected patients appear to be uninfected (termed bystander cell death) and that, when rates of cell turnover are measured *in vivo* in HIV-infected patients, both CD4+ and CD8+ T lymphocytes show similarly elevated rates of death, together support a pathogenic model, whereby nonviral (or indirect) factors are primarily responsible for HIV disease progression. It is important to note that the increased rate of death of uninfected CD4+ T lymphocytes clearly indicates that direct killing by HIV cannot be the only mechanism involved in the depletion of CD4+ T lymphocytes seen in HIV-infected patients.

Taken together, the above-mentioned observations suggest that the pathogenesis of the HIV-associated CD4+ T lymphocyte depletion is a complex phenomenon and that the host response to the virus must play a key role. In particular, it has been proposed that the presence of a chronic generalized immune activation that involves all lymphocyte subsets is an important driving force in the loss of CD4+ T lymphocytes that is associated with progression to AIDS. This concept of HIV infection having a general effect on the host immune system is confirmed by the fact that a large number of functional abnormalities involving virtually all other immune cell types are associated with HIV disease progression. Importantly, several clinical studies have shown that, in HIV-infected patients, disease progression correlates better with markers of immune activation than with viral load. In this perspective, the observation of the short *in vivo* lifespan of HIV-infected CD4+ T lymphocytes has been re-interpreted as the result of their heightened state of activation that dooms them to die (via activation-induced apoptosis) rather than as a consequence of direct infection. As such, the impact of HIV infection on the lifespan of infected CD4+ T lymphocytes may be less dramatic than was originally proposed and it has now been hypothesized that HIV, in essence, jumps from one activated cell (that is destined to die of apoptosis) to another without changing the fate of these infected cells. It could thus be conceived that the number of available activated CD4+ T lymphocytes dictates, independently, both the level of virus replication and the overall rate of CD4+ T lymphocyte death, and that these two factors are correlated with one another only because they reflect the prevailing level of immune activation.

Further key support for the immune activation hypothesis derives from studies of the nonpathogenic

simian immunodeficiency virus (SIV) infections of natural hosts, such as sooty mangabeys (*Cercocebus atys*, SMs), African green monkeys (AGMs), mandrills, and numerous other nonhuman primate species. These natural SIV hosts are the origin of the human HIV epidemics through multiple episodes of cross-species transmission, with HIV-1 originating from the chimpanzee (*Pan troglodytes*) SIV_{cpz}, and HIV-2 originating from the SM SIV_{smm}. Naturally SIV-infected SMs are of interest also because SIV_{smm} is the source of the various rhesus macaque (*Macaca mulatta*)-adapted SIV_{mac} viruses (e.g., SIV_{mac239}, SIV_{mac251}) that are commonly used in laboratory studies of AIDS pathogenesis and vaccines. In natural SIV hosts, chronic high levels of virus replication and *in vivo* killing of CD4+ T lymphocytes are not associated with any signs of immunodeficiency in the setting of typically low levels of both immune activation and cellular immune responses to the virus. This phenotype suggests that natural SIV hosts have evolved to be disease-resistant without the acquisition of any special ability to control virus replication (via either immune or other mechanisms), but rather by an adaptation that attenuates the immune response to this retroviral infection (**Table 2**).

Interestingly, some recent findings may help to harmonize the classical (i.e., virus-centric) and immune activation models in that they support a more comprehensive model whereby the pathogenesis of HIV/AIDS involves both direct and indirect mechanisms (**Figure 2**). There are now thought to be two sequentially distinct phases in infection that involve very different pathogenic mechanisms. The first phase (acute infection) is characterized by a rapid, massive, directly HIV-mediated loss of memory CD4+ T lymphocytes that takes place mainly in mucosal tissues and may leave a profound scar on the overall function of the immune system. It is still unclear what determines the cessation of this brief early phase, although likely explanations include the consumption of available target cells and the generation of HIV-specific immune responses. The second phase (chronic infection) lasts typically for several years, and is characterized, in essence, by the immune system's struggle to both recover from the early injury (via activation of mechanisms of immune homeostasis) and control virus replication (via activation of humoral and cellular immune responses). Unfortunately, this latter endeavor proves almost always to be insurmountable as HIV is a pathogen of extraordinary genetic and structural plasticity allowing it to develop a series of highly effective strategies for immune evasion. The struggle of the second phase is characterized by the continuous generation of strong, though ultimately ineffective, immune responses to HIV, and by the attempt to maintain T-lymphocyte homeostasis in the face of massive T-lymphocyte loss due to both the direct effect of the HIV and bystander cell death. Sadly, this epic battle seems to end up inducing nothing but the very state of chronic immune activation that, as mentioned above, ultimately causes more damage than repair to the host immune system.

Table 2 Immunologic and virologic features of natural, nonpathogenic SIV infections vs. pathogenic HIV infection

	Natural SIVs	*HIV*
Chronic virus replication	Yes	Yes
Lack of immune control	Yes	Yes
Short lifespan of infected CD4+ T cells	Yes	Yes
Depletion of MALT CD4+ T cells	Yes	Yes
Systemic loss of CD4+ T cells	Rare	Yes
Generalized immune activation	No	Yes
Expression of CCR5 on CD4+ T cells	Low	High

Determinants of HIV Transmission and Early Replication

A clearer understanding of the host and viral factors determining the likelihood of virus transmission upon exposure and/or the course of infection after transmission will likely provide useful information for the design of new strategies to prevent HIV infection and AIDS. However, while some host factors regulating the risk of infection and disease progression have been identified, predicting the natural course of the infection remains very difficult at the level of the individual patient. Among the host genetic factors that have been shown to induce resistance to HIV infection, a predominant role has been ascribed to the 32 bp deletion in the CCR5 gene seen in 1% of Caucasian individuals. This deletion results in a frameshift mutation that causes the CCR5 protein to be completely absent from the surface of all cells. Thus, individuals homozygous for the Δ32 allele are resistant to transmission of CCR5-tropic viral strains (the overwhelming majority of viruses involved in horizontal sexual transmission). Other genes whose polymorphisms have been associated with some effect on HIV disease progression include SDF-1, CX3CR-1, CCL3, and many others. The clinical consequence of these discoveries is that small molecule antagonists or a monoclonal antibody directed against CCR5 are increasingly recognized as promising strategies to prevent HIV transmission.

The search for host factors associated with more effective immune responses to HIV (and, in fact, the identification of any type of correlate of immune protection) has proved much more frustrating. As of early 2007, it is still unclear whether and to what extent the immune response to HIV is capable of inducing absolute (i.e., by preventing infection) or relative (i.e., by lowering virus replication) resistance to virus transmission and early dissemination. While stronger and/or broader T-lymphocyte responses to the virus have been associated with better course of disease, it is still unclear whether this apparently more

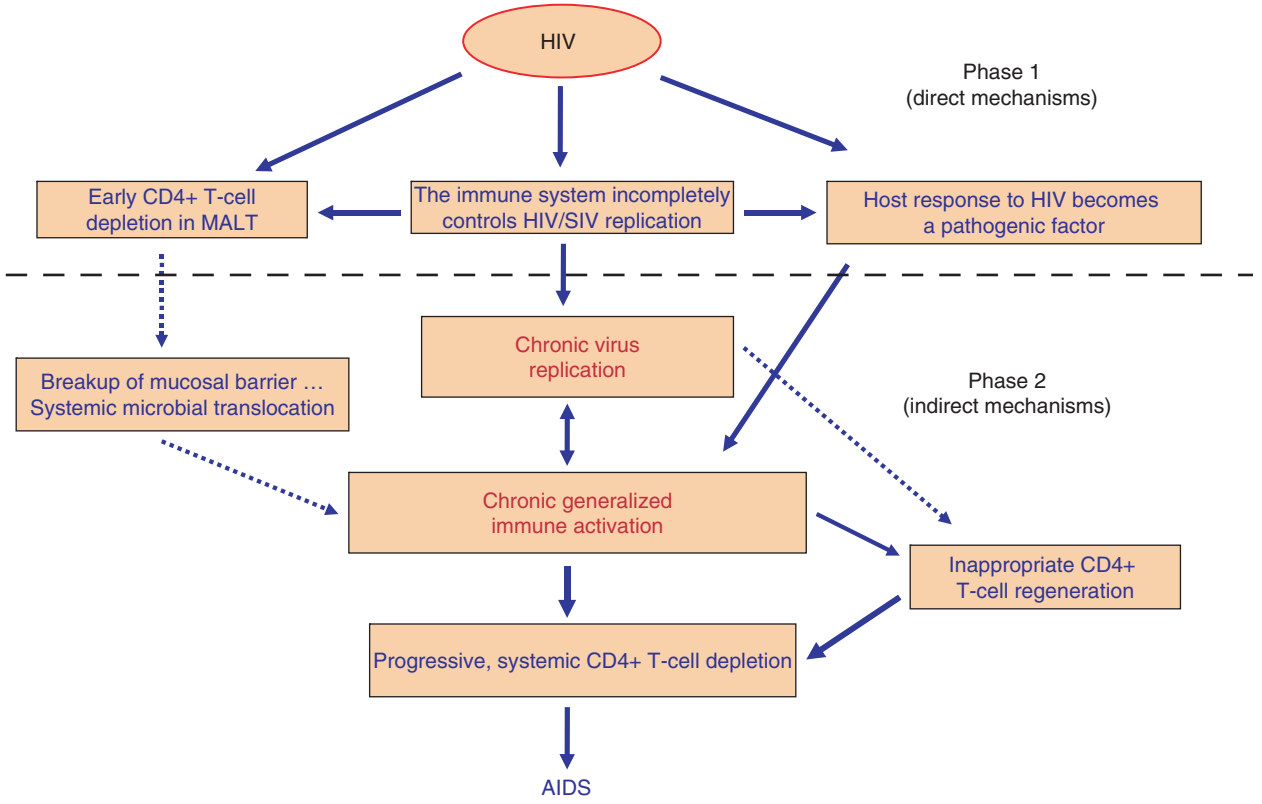


Figure 2 The biphasic model of AIDS pathogenesis. This model summarizes the key immunopathogenic events in acute and chronic infection that lead to progression to AIDS in HIV-infected individuals, taking into account both direct (virus-mediated) and indirect (immune activation-mediated) mechanisms.

effective immune response is cause or consequence of a more benign course of infection. Furthermore, the observation that natural SIV hosts, who are resistant to AIDS, show a low-level T-lymphocyte response to the virus and, more generally, an attenuated immune system activation clearly indicates that a strong host antiviral immune response is not necessary to lessen disease. While more studies are needed to fully elucidate the mechanisms underlying the lack of disease progression in natural SIV hosts, one clear message thus far is that co-evolution of SIV and its natural hosts have resulted in a happy equilibrium whereby the host immune function is preserved without any significant immune-mediated control of virus replication (**Table 2**).

Some recent advances in understanding what factors influence HIV-1 transmission come from recent studies of heterosexual transmission in Africa. One such study showed that the viruses that establish new infections encode an envelope surface glycoprotein (gp120) with a compact structure and reduced glycosylation that appears to be significantly more sensitive to antibody neutralization. To this end, it should be noted that the vast majority of gp120s isolated from patients with chronic HIV infection are resistant to neutralization by autologous sera. The implication of these findings for HIV vaccine development is significant, as they reveal a relative vulnerability of the HIV-Env glycoprotein in the very early phase of HIV infection. Also interesting is the finding that, in a cohort of discordant heterosexual partners (i.e., one HIV-infected and one HIV-uninfected) in Zambia, the sharing of HLA-B alleles is associated with a significant enhancement in the rate of transmission. This observation suggests that the foreign HLA molecules on virus-infected cells or the virus itself (acquired from the cell membrane) trigger protection against infection with a mechanism analogous to allogeneic transplant rejection; alternatively, transmission of viruses that have already escaped the cytotoxic T-lymphocyte (CTL) response may make infection more efficient in individuals with shared HLA alleles. While these reports shed some light on what type of adaptive immune response may control the spread of HIV infection, it is obvious that the virus appears to rapidly win the battle with the host immune system in the overwhelming majority of infected individuals.

When HIV first enters the body (most commonly via mucosal transmission but in certain instances through the bloodstream), the type and magnitude of the subsequent antiviral immune response is largely determined by immature dendritic cells (DCs), a cell type that is a component of the innate defense against mucosal penetration and

widespread dissemination of many pathogens. Importantly, the role of DCs in the early events of HIV infection is complex and not always beneficial (**Figure 3**). On the one hand, DCs take up HIV-1 via C-type lectin binding receptors and migrate to the draining lymph node where they prime HIV-specific immune responses by presenting HIV antigens to CD4+ T lymphocytes. On the other hand, DCs may also contribute to early HIV dissemination throughout the body, due to their migratory potential and the fact that the DC–CD4+ T lymphocyte contact often results in productive infection of CD4+ T lymphocytes. While manipulation of the DC–T lymphocyte interaction may conceivably improve the host immune response to HIV, our limited understanding of the molecular mechanisms responsible for this interaction hampers the design of simple interventions aimed at blocking the early dissemination of HIV infection.

The Acute/Early Phase of HIV Infection

The acute phase of HIV infection is characterized by a peak of virus replication that occurs coincident with a transient decline of circulating CD4+ T lymphocytes and the presence of a flu-like clinical syndrome. This acute infection lasts for approximately 2–3 weeks and ends at approximately the time of antibody seroconversion. Some recent elegant studies have highlighted the role of the mucosal-associated lymphoid tissue (MALT) in acute HIV infection. The MALT houses the majority of CD4+ T lymphocytes in the body, a large fraction of which express CCR5 and have an activated memory phenotype. A key feature of the early phases of HIV and SIV infections is the massive infection and depletion of MALT-associated CD4+CCR5+ memory T lymphocytes (**Figure 2**). HIV replication in activated CD4+CCR5+ memory T lymphocytes proceeds relatively unchecked for 2–3 weeks after transmission, resulting in large-scale depletion of these cells. During early pathogenic SIV infection of Asian macaques (i.e., non-natural hosts), up to 60% of memory CD4+ T lymphocytes in the intestinal lamina propria contain SIV-RNA at the peak of infection (day 10) and the majority of these cells are eliminated by day 14. Importantly, though, the best predictor of rapid disease progression may not be the extent of the early CD4+ T lymphocyte depletion from mucosal sites, but rather the ability to reconstitute the pool of memory CD4+ T lymphocytes in the MALT. MALT-associated memory CD4+ T lymphocytes are an integral component of the mucosal immunological barrier against invading pathogens,

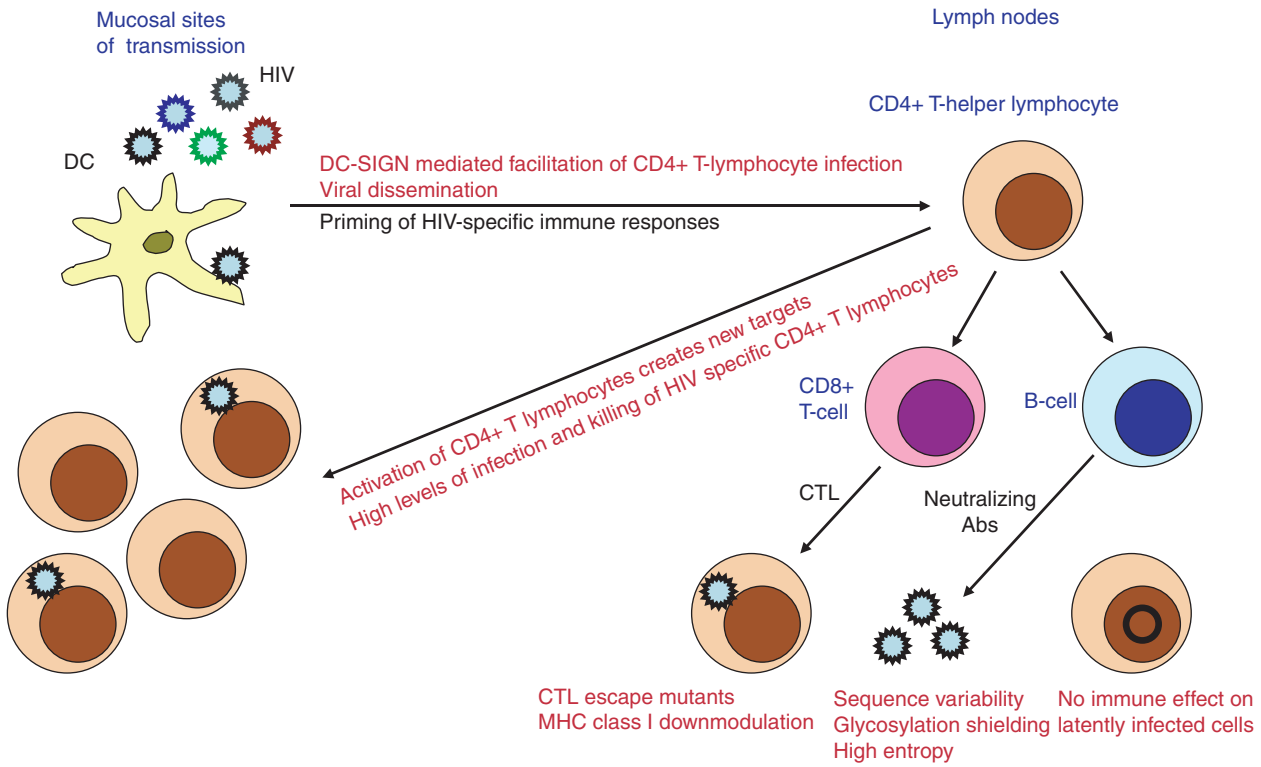


Figure 3 HIV transmission and generation of HIV-specific immune responses. HIV entry at mucosal sites is followed by capture by DCs that subsequently migrate to lymph nodes to present HIV antigens to CD4+ T-helper lymphocytes. In this process, DCs may also facilitate the infection of activated CD4+ T lymphocytes. CD4+ T lymphocytes help CD8+ T lymphocytes and B cells in mounting cellular and humoral anti-HIV immune responses. Legend in black indicates events of the HIV-specific immune response; legend in red indicates events leading to HIV immune evasion and dissemination.

and it is likely that their massive (and potentially irreversible) depletion may significantly affect the proper function of the mucosal barrier. This setting then allows for the emergence of numerous subclinical infections, thus inducing further microenvironment destruction and contributing to a state of chronic immune system activation (**Figure 2**). It should be noted, however, that to date the specific consequences of the early depletion of MALT-associated memory CD4+ T lymphocytes remain unknown, and it is still unclear why the opportunistic infections that are typical of full-blown AIDS do not appear until peripheral blood CD4+ T lymphocyte counts decline. Particularly puzzling is the observation that a rapid, severe, and persistent depletion of mucosal CD4+ T lymphocytes also occurs during nonpathogenic SIV infection of natural hosts such as SMs and AGMs (**Table 2**). However, in the context of the typically low levels of systemic and mucosal immune activation found in these species, no signs of mucosal immune dysfunction are observed, further emphasizing the potential protective role of an attenuated immune response during retroviral infection. Alternatively, it is possible that the early and rapid depletion of mucosal CD4+ T lymphocytes observed in pathogenic HIV/SIV infections simply reflects the systemic virus dissemination that is necessary to establish a chronically productive infection.

Generation of HIV-Specific Immune Responses and the Acute-to-Chronic Phase Transition

As mentioned above, the determinants of the relatively abrupt cessation of the early phase of HIV infection and the onset of the long chronic phase that occur at 2–3 weeks post infection are still poorly understood. However, several investigators have put forward the hypothesis that HIV-specific CTL responses as well as humoral responses directed against the virus envelope protein (i.e., neutralizing antibodies) play a central role in reducing the level of virus replication from its peak to set-point level. The evidence in favor of a significant role of CD8+ CTL-mediated responses reducing HIV replication include: (1) the temporal association between decline of viremia and emergence of cellular immune responses during primary HIV/SIV infection; (2) the increase in virus replication after experimental depletion of CD8+ T lymphocytes during pathogenic SIV infection of rhesus macaques; and (3) the observation that CTL escape mutations present in viruses transmitted between individuals with different HLA haplotypes are rapidly replaced by wild-type sequences. While CD8+ CTL-mediated responses play a significant role early on in protecting from unchecked HIV-1 replication and disease progression, their impact is ultimately limited by the extreme genetic variability of HIV that favors the emergence, during both acute and chronic infection, of viral escape mutants (**Figure 3**). The fact that HIV preferentially infects and kills HIV-specific memory CD4+ T lymphocytes (that are more likely to be activated, **Figure 3**) over memory T lymphocytes specific for other pathogens further complicates the situation, and results in a functional deficit in the anti-HIV response as compared to that directed against other pathogens. What must now be elucidated is whether preexisting, AIDS vaccine-induced HIV-specific CTLs will be more effective in controlling virus replication than those generated during natural infection. HIV-specific autologous neutralizing antibodies also exert potent and continuous selective pressure on the viral quasispecies. Unfortunately, HIV is able to escape antibody-mediated neutralization as efficiently as it does CTL control. A series of recent studies have clarified some of the mechanisms of HIV evasion from the humoral immune response (**Figure 3**). First, the envelope glycoprotein (Env) is highly variable, particularly in specific regions that form the surface of the virus. Second, the outer surface of gp120 is covered with a highly dynamic carbohydrate shield, in which glycans shift through point mutations to mask adjacent and distant epitopes. Third, conserved structural domains of Env are either sterically hindered or only transiently exposed. Fourth, receptor binding of Env is characterized by a high level of intrinsic entropy that makes it a moving target for antibody neutralization. The failure of both HIV-specific CTLs and neutralizing antibodies to completely suppress HIV replication during the transition between acute-to-chronic infection likely sets the stage for the following pathogenic events that are characterized by continuous, generalized immune activation in the face of chronic virus replication (**Figure 2**).

The Chronic Phase of Infection

Chronic HIV infection is a process lasting for several years that is characterized by a very slow decline of CD4+ T lymphocytes in peripheral blood, a small fraction of infected CD4+ T lymphocytes, and increased death rates of both CD4+ and CD8+ T lymphocytes. This last finding is related to the state of chronic, generalized immune activation that is now recognized as a major driving force behind CD4+ T lymphocyte depletion and a strong predictor of disease progression. The causes of the HIV-associated generalized immune activation are complex, and involve activation of T lymphocytes by specific antigens (e.g., HIV and a plethora of opportunistic pathogens), as well as bystander activation of T lymphocytes due to the presence of high levels of lymphotropic cytokines and other as yet poorly defined factors. But how does this increased immune activation induce CD4+ T lymphocyte depletion? First, CD4+ T lymphocyte activation provides new targets for HIV

replication (that occurs preferentially in activated CD4+ T lymphocytes, **Figure 3**), thus creating an environment conducive to further virus-mediated damage to the immune system. Second, the increased T-lymphocyte turnover driven by this chronic immune activation (and, to some extent, by the increased compensatory homeostatic proliferation of T lymphocytes) results in chronic consumption of the pools of naïve and resting-memory cells, with a resultant relative expansion of short-lived effector CD4+ T lymphocytes. Third, the chronic activation and proliferation of T lymphocyte results in perturbations of cell-cycle control and an increased propensity to undergo activation-induced apoptosis. It is also possible that the HIV-associated immune activation is, at least in part, responsible for the changes in the lymph node architecture and thymic function that likely limit the proper reconstitution of the CD4+ T lymphocyte pool. It is hypothesized that depletion is unique to CD4+ T lymphocytes (and involves CD8+ T lymphocytes only in the very late stages of HIV disease) not only because these cells are targeted by HIV, but also because they are more sensitive to the deleterious effects of immune activation and/or are less efficiently regenerated.

Evidently, the assertion that chronic T-lymphocyte activation is a key pathogenic mechanism during HIV infection is in an apparent conflict with data suggesting that cellular immune responses to HIV/SIV infections may limit primary infection and/or disease progression. While T-lymphocyte responses to HIV likely play a protective role early on, T-lymphocyte immunity virtually always fails to control HIV infection due to the emergence of viral escape mutants. Once HIV-specific cellular immune responses are unable to control the virus, other mechanisms may determine the net effect that this ongoing T-lymphocyte activation has on the course of disease.

Is it possible that there is a direct connection between the early damage that HIV causes to the MALT-based pool of memory CD4+ T lymphocytes and the generation of the HIV-associated generalized immune activation? It is known that MALT-associated memory CD4+ T lymphocytes are an integral component of the mucosal immunological barrier that protects the host from both invading pathogens as well as commensal enteric bacteria that may also be recognized as pathogens if they cross the intestinal epithelium. It is likely that a massive depletion of these cells may affect the proper function of this mucosal barrier and thus may open the door to a series of subclinical infections (i.e., micro-invasion of the intestinal mucosa) that will, in turn, induce further micro-environment destruction and contribute to the generation of a state of chronic immune system activation (**Figure 2**). While this model delineates an interesting mechanistic link between direct and indirect (i.e., virus vs. immune activation-mediated) mechanisms of HIV-associated CD4+ T-lymphocyte depletion, the specific consequences of the early depletion of MALT-associated memory CD4+ T lymphocytes are in fact unknown, and it remains unclear why the opportunistic infections that are typical of full-blown AIDS do not manifest until peripheral blood CD4+ T-lymphocyte counts decline.

Implications for HIV Therapy and Vaccine Development

As described above, AIDS pathogenesis likely recognizes two distinct phases, a brief acute phase dominated by the direct killing of large numbers of memory CD4+ T lymphocytes followed by a long chronic phase characterized by a slow attrition of the CD4+ T lymphocyte pool that is related mainly to the prevailing level of immune activation (**Figure 2**). However, many questions remain to be answered. First, a precise quantification of the relative contribution of the direct and indirect mechanisms of AIDS pathogenesis is still lacking. Second, we do not have a clear explanation as to why HIV appears to be so uniquely powerful in inducing a chronic state of immune activation (as opposed to other chronic viral infections), and why the HIV-induced immune activation is so disruptive of the immune system homeostasis. The fact that generalized immune activation follows HIV infection of humans, which is a very recent disease, but does not follow infections that date back many thousands or even millions of years (such as HCV infection of humans or SIV infection of natural hosts), would suggest that attenuation of the immune response to a chronically replicating virus is the result of an advantageous evolutionary adaptation of the host immune system. Unfortunately, the mechanisms underlying the ability of natural hosts for SIV infection to avoid the chronic immune activation seen in HIV-infected humans are still poorly understood.

This biphasic model of AIDS pathogenesis provides a few important implications for HIV therapy and vaccine design. In terms of therapy, an important observation is that a complete recovery of the mucosal CD4+ T-lymphocyte system is unlikely with the currently available antiretroviral drugs even in the presence of prolonged viral suppression. As such, additional immune-based interventions, in particular using CD4+ T-lymphocyte tropic cytokines (e.g., IL-2, IL-7) as well as factors promoting the CD4+ T-lymphocyte repopulation of the MALT, may be useful to improve the immune system recovery from the severe and persistent damages inflicted during the early phase of the infection. In addition, given the well-recognized pathogenic role of the generalized immune activation during the chronic infection, it is conceivable that, in many HIV-infected patients, immune-based interventions aimed at reducing immune activation, preserving proper cell-cycle control, and preventing excessive levels of bystander cell death may restore the overall CD4+ T-lymphocyte homeostasis.

Among these potentially interesting new interventions are those that interfere directly with the signaling pathways involved in establishing the generalized immune activation or cell-cycle dysregulation (i.e., toll-like receptors, cytokine receptors, nuclear factor kappa B (NF-κB), cell cycle-dependent kinases) and those that improve the regeneration and/or the differentiation of T lymphocytes (i.e., cytokines and growth factors). Finally, a clearer understanding of the reason(s) why SIV infection is nonpathogenic in natural hosts (and, in particular, how virus replication can proceed in the absence of immune system damage and chronic immune activation) will likely also reveal further immune-based therapies to delay progression of disease.

Advances in the understanding of the early events of HIV transmission, with special emphasis on the immunogenic vulnerabilities of Env and the correlates of immune protection at the mucosal level, may lead to the development of vaccine and microbicide strategies that can limit the acute HIV-induced damage. In terms of vaccine development, the main implication of the accumulated pathogenesis research is that there may be only a short window of opportunity to control this rapidly disseminating virus that produces such extensive damage to the host immune system. This premise is especially disheartening for CTL-based vaccines that, in order to be effective, will have to induce a persistently high level of mucosa-associated, long-lived, HIV-specific memory CD8+ T lymphocytes that can rapidly proliferate and differentiate into effectors in the event of viral infection. Recent findings are more encouraging for a vaccine that aims to induce a high titer of broadly neutralizing antibodies, particularly if one considers that structural bottlenecks seem to be required for the virus to be efficiently transmitted to a new host in certain settings. In addition, it should be noted that even a vaccine that is only partially effective in containing the early viral dissemination (and resultant immune system damage) may end up having a significant impact on shaping the course of disease.

In summary, there have been many advances in the field of HIV immunopathogenesis over the past 24 years, but there is much that remains to be elucidated, particularly at the mechanistic level. It is critical that, with the help of new and highly sophisticated tools of analyses, we endeavor to more fully understand the dynamic interaction between HIV and the host immune system in order to eradicate this disease.

See also: AIDS: Disease Manifestation; AIDS: Global Epidemiology; AIDS: Vaccine Development; Human Immunodeficiency Viruses: Antiretroviral agents; Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Origin; Simian Immunodeficiency Virus: Animal Models of Disease; Simian Immunodeficiency Virus: General Features; Simian Immunodeficiency Virus: Natural Infection.

Further Reading

Gordon S, Pandrea I, Dunham R, Apetrei C, and Silvestri G (2005) The call of the wild: What can be learned from studies of SIV infection of natural hosts? In: Leitner T, Foley B, Hahn B, *et al.* (eds.) *HIV Sequence Compendium 2005*, pp. 2–29. Los Alamos, NC: Theoretical Biology and Biophysics Group.

Grossman Z, Meier-Schellersheim M, Paul WE, and Picker LJ (2006) Pathogenesis of HIV infection: What the virus spares is as important as what it destroys. *Nature Medicine* 12(3): 289–295.

Guatelli JC, Siliciano RF, Kuritzkes DR, and Richman DD (2002) Human immunodeficiency virus. In: Richman DD, Whitley RJ, and Hayden FG (eds.) *Clinical Virology,* 2nd edn., pp. 685–730. Washington: ASM Press.

Lederman MM, Penn-Nicholson A, Cho M, and Mosier D (2006) Biology of CCR5 and its role in HIV infection and treatment. *JAMA* 296(7): 815–826.

Human Respiratory Syncytial Virus

P L Collins, National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA

Published by Elsevier Ltd.

Glossary

Fractalkine The sole known CX3C cytokine. It is present as a membrane-bound form that is upregulated by inflammation and promotes adhesion of monocytes and lymphocytes and as a secreted form that promotes chemotaxis of monocytes and lymphyocytes.

Furin A subtilisin-like cellular endoprotease present in the exocytic pathway with the consensus cleavage sequence Arg-X-Arg/Lys-Arg↓.

Glycosaminoglycan Long, unbranched, highly negatively charged polysaccharides that consist of repeating disaccharide subunits and are located on the cell surface or in the extracellular matrix.

Humanized antibody Substitution, by recombinant DNA techniques, of the antigen binding sequences of a nonhuman antibody molecule into the backbone of a human antibody molecule.
Mucin A family of large, heavily glycosylated, secreted or transmembrane proteins that form a protective barrier on the respiratory, gastrointestinal, and reproductive tracts.
Polylactosaminoglycan Carbohydrate of unknown function formed in the Golgi apparatus by the addition of a variable number of repeating units of lactosamine (galactose-β-1,4-N-acetylglucosamine-β-1,3) to an N-linked core oligosaccharide.
Rhinorrhea Runny nose.
Ribavirin A nucleoside analog (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) that has antiviral activity against a number of RNA viruses.
Syncytium A multinucleated cell, formed in the case of human respiratory syncytial virus by fusion of the plasma membrane of an infected cell with those of its neighbors.
Tachykinin A family of biologically active peptides (prototype, substance P) that can be potent vasodilators and can induce smooth muscle contraction directly or indirectly.

History

Human respiratory syncytial virus (HRSV) was first isolated in 1956 from a laboratory chimpanzee with upper respiratory tract disease. Shortly thereafter, an apparently identical virus was recovered from two children ill with pneumonia or croup and was identified as a human virus. HRSV quickly became recognized as the leading viral agent of pediatric respiratory tract disease worldwide. It has also gained recognition as a significant cause of morbidity and mortality in the elderly and severely immunocompromised individuals. Its inefficient growth in cell culture and propensity to easily lose infectivity due to physical instability impede research. HRSV lacks an approved vaccine or clinically effective antiviral therapy, although, as described below, passive immunoprophylaxis is available for infants at high risk for serious HRSV disease.

Classification

HRSV is a member of family *Paramyxoviridae* of order *Mononegavirales*, the nonsegmented negative-strand RNA viruses. *Paramyxoviridae* has two subfamilies: (1) subfamily *Paramyxovirinae* contains five genera whose members include Sendai virus, the human parainfluenza viruses (HPIVs), and mumps and measles viruses among others; and (2) subfamily *Pneumovirinae* contains genus *Metapneumovirus*, which includes avian and human metapneumoviruses, and genus *Pneumovirus*, which includes HRSV, bovine respiratory syncytial virus (BRSV), ovine respiratory syncytial virus (RSV), and pneumonia virus of mice (PVM). **Figure 1**(**a**) compares gene maps of the two genera of *Pneumovirinae* with those of a representative genus (*Respirovirus*) of *Paramyxovirinae*. **Figure 1**(**b**) illustrates the relatedness among members of the two genera of *Pneumovirinae* based on the nucleotide sequence of the N gene. HRSV is most closely related to BRSV. One might speculate that HRSV originally emerged in the past from infection of humans with BRSV-like virus.

Virion Structure and Viral Proteins

HRSV virions consist of spherical particles of 80–350 nm in diameter and filamentous particles that are 60–200 nm in diameter and up to 10 μm in length (**Figure 2**). Filaments appear to be the predominant form produced in cell culture. The virion contains a helical nucleocapsid (diameter 12–15 nm compared to 18 nm for *Paramyxovirinae*) packaged in a lipoprotein envelope acquired from the host cell plasma membrane during budding. The outer surface of the envelope contains a fringe of surface projections or spikes of 11–12 nm. HRSV lacks a hemagglutinin or neuraminidase.

HRSV encodes 11 proteins. Four are associated with the nucleocapsid: the major nucleocapsid protein N (56 kDa), the nucleocapsid phosphoprotein P (33 kDa), the transcription processivity factor M2-1 (22 kDa), and the large L protein (250 kDa). The N protein binds tightly along the entire length of genomic RNA as well as its positive-sense replicative intermediate that is called the antigenome. L is the major polymerase subunit containing the catalytic domains. P is thought to associate with free N and L to maintain them in soluble form for association with the nucleocapsid and probably also participates as a cofactor in RNA synthesis. N, P, and L are the viral proteins that are necessary and sufficient to direct RNA replication. They also direct transcription, but this is poorly processive unless the M2-1 protein is also present.

There are three transmembrane viral glycoproteins that are expressed on the surface of infected cells and are packaged in the virion: the large G glycoprotein (90 kDa), the fusion F glycoprotein (70 kDa), and the small hydrophobic SH protein (7.5–60 kDa, depending on the amount of added carbohydrate). These assemble separately into homo-oligomers that constitute the surface spikes: F assembles into trimers, G assembles into trimers or tetramers, and SH has been detected as a pentamer.

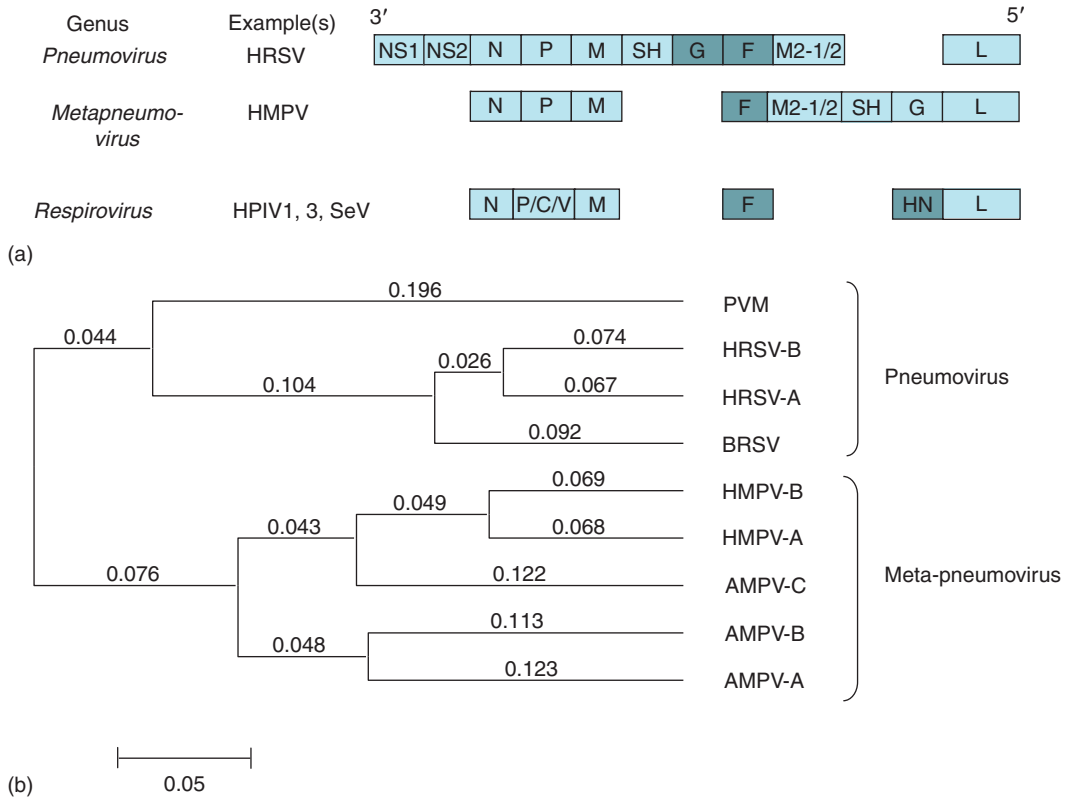


Figure 1 Comparison of human respiratory syncytial virus (HRSV) with other selected members of family *Paramyxoviridae*. (a) Alignment of the gene maps of HRSV, human metapneumovirus (HMPV), human parainfluenza virus (HPIV) types 1 and 3, and Sendai virus (SeV). (b) Relationships among the members of the two genera of subfamily *Pneumovirinae* based on the nucleotide sequence of the N gene. The scale bar represents approximately 5% nucleotide difference between pairs.

The G glycoprotein mediates viral attachment. It is a type II glycoprotein: there is a signal/anchor sequence near the N-terminus, with the C-terminal two-thirds of the molecule oriented extracellularly. G is heavily glycosylated, with several N-linked carbohydrate side chains and an estimated 24–25 O-linked side chains. The unglycosylated protein has an M_r of 32 500 compared to 90 000 for the mature protein. Most of the ectodomain is thought to have an extended, heavily glycosylated, mucin-like structure. In the middle of the ectodomain there is a predicted disulfide-linked tight turn (cystine noose) in the secondary structure. The cystine noose is overlapped on its N-terminal end by a 13-amino-acid conserved sequence of unknown significance and on its C-terminal end by a C–X3–C motif that it is embedded in a region of limited sequence relatedness with the CX3C chemokine fractalkine. A synthetic peptide containing this sequence has fractalkine-like chemotactic activity *in vitro*. The role of this apparent chemokine mimicry in the biology of HRSV is unclear. The tight turn, the conserved domain, and the fractalkine domain can be deleted from recombinant HRSV without significantly reducing its replication efficiency in cell culture or in the respiratory tract of mice. Thus, the region of G that is necessary for attachment has not been identified.

The F glycoprotein mediates cell-surface membrane fusion, which is responsible for delivering the viral nucleocapsid to the cytoplasm. In addition, F expressed at the cell surface mediates fusion of infected cells with their neighbors, resulting in the formation of syncytia. F is a type I glycoprotein, with an N-terminal cleaved signal anchor and a C-proximal membrane anchor. It has four or five N-linked carbohydrate side chains. F is synthesized as a precursor, F_0, which is cleaved intracellularly at two sites (amino acids 109/110 and 136/137) by a furin-like cellular protease. This yields the following fragments, in N- to C-terminal order: F_2 (109 amino acids), p27 (27 amino acids), and F_1 (438 amino acids). F_2 and F_1 remain linked by a disulfide bond and represent the active form of F. In BRSV, the p27 fragment has tachykinin activity and may play a role in promoting inflammation, but p27 of HRSV does not resemble a tachykinin.

The function of the SH glycoprotein is not known. Molecular modeling suggests that it might be an ion-channel-forming protein, although the significance of this to HRSV biology is unclear. SH is anchored in the

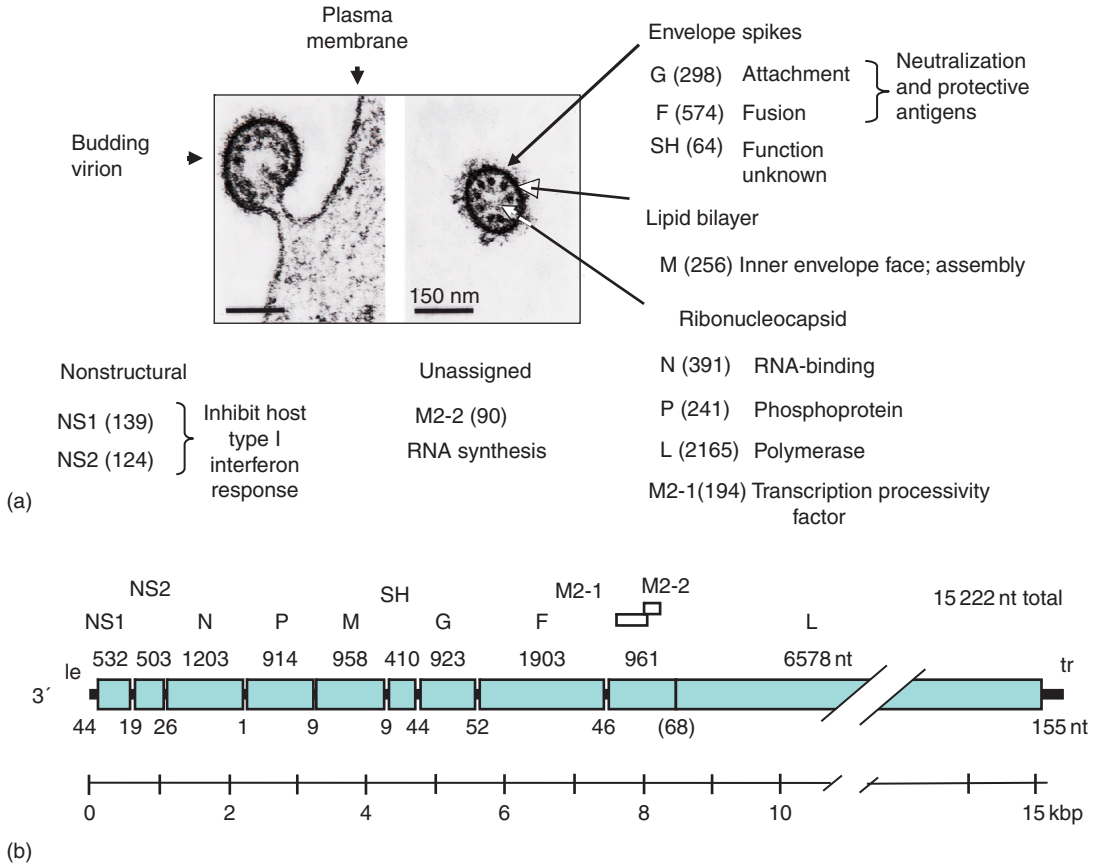


Figure 2 HRSV virion, proteins, and genome. (a) The electron photomicrographs illustrate an RSV virion budding from the plasma membrane of an infected cell (left) and a free virion (right). The viral proteins are indicated, with the amino acid length of the unmodified protein in parentheses. (b) The RNA genome is illustrated 3′ to 5′. Each large rectangle indicates a gene that is transcribed into a separate mRNA, with the gene nucleotide length shown above. The overlapping open reading frames of the M2 gene are indicated. The nucleotide lengths of the extragenic leader (le), intergenic, and trailer (tr) regions are shown underneath. The overlap between the M2 and L genes is indicated by its nucleotide length in parentheses. Nucleotide and amino acid lengths are for strain A2.

membrane by a centrally located signal/anchor sequence, with the N-terminus oriented intracellularly and the C-terminus extracellularly. A portion of SH present intracellularly and in virions is unglycosylated (M_r 7500), a portion has a single N-linked side chain (M_r 13 000–15 000), and a portion has the further modification of polylactosaminoglycan added to the N-linked sugar (M_r 21 000–60 000 or more). This multiplicity of forms is conserved in *Pneumovirinae* but its significance is unknown.

Remarkably, the G and SH genes can be deleted individually or in combination from viable HRSV. Deletion of SH does not reduce the efficiency of replication in cell culture and has only a modest attenuating effect on replication in mice, chimpanzees, or humans (the last observation is based on trials of experimental live vaccines). Deletion of G was attenuating in HEp-2 cells but not Vero cells, and was highly attenuating in mice and humans. Since SH and G are dispensable, at least in cell culture, it is thought that F can function as an alternative attachment protein in that setting.

The nonglycosylated M protein is thought to be located on the inner surface of the envelope and to have a central role in organizing the envelope and directing packaging of the nucleocapsid.

The M2-2 protein is expressed at a low level and its status as structural or nonstructural is unknown. Recombinant HRSV from which the M2-2 coding sequence was deleted replicates more slowly than wild-type HRSV in cell culture and is attenuated *in vivo*. This deletion virus exhibits an increase in transcription and a decrease in RNA replication, suggesting that M2-2 normally is involved in downregulating transcription and upregulating RNA replication.

NS1 and NS2 are small proteins and do not appear to be packaged significantly in the virion. Both proteins inhibit the host interferon response. NS1, and to a lesser extent NS2, inhibit the induction of interferon α/β by blocking activation of interferon regulatory factor 3. In addition, NS2, and to a lesser extent NS1, inhibit interferon α/β-induced signaling through the Janus

kinases/ signal transducers and activators of transcription (JAK/STAT) pathway, thus inhibiting the amplification of the interferon response, the upregulation of interferon-stimulated genes, and the development of the antiviral state. This appears to involve enhanced degradation of STAT2. Deletion of NS1 and NS2 individually or in combination yields viable viruses that replicate almost as efficiently as wild-type HRSV in cells that do not make interferon, but which are attenuated in interferon-competent cultured cells and *in vivo*.

Genome Organization, Transcription, and Replication

The HRSV genome is a single negative-sense strand of RNA that ranges in length from 15 191 to 15 226 nt for the strains that have been sequenced to date (**Figure 2**(**b**)). Its organization, expression, and replication follow the general pattern of *Mononegavirales*. The genome is neither capped nor polyadenylated. It is tightly encapsidated with N protein both in the virion and intracellularly. Based on the prototype A2 strain, the genomic RNA has a 44 nt 3′-extragenic leader at the 3′ end. This is followed in order by the NS1, NS2, N, P, M, SH, G, F, M2, and L genes. The L gene is followed by a 155 nt extragenic trailer region that comprises the 5′ end. The first nine genes are separated by intergenic regions of 1–52 nt. The last gene, L, initiates within the downstream nontranslated region of the M2 gene, and thus these two genes overlap by 68 nt.

In transcription, the viral polymerase initiates at a single promoter at the 3′ end of the genome and copies the genes by a sequential stop–start mechanism that yields subgenomic mRNAs. The upstream and downstream boundaries of each gene contain transcription signals: the upstream end consists of a conserved 10 nt gene-start motif that directs transcriptional initiation, and the downstream end consists of a moderately conserved 12–13 nt gene-end motif that directs termination and polyadenylation. The intergenic regions appear to lack any conserved motifs and seem to be nonspecific spacers. Between genes, the majority of polymerase remains template-bound and scans for the next gene. However, some polymerase dissociates from the template during transcription, yielding a polar gradient such that upstream genes are expressed more efficiently than downstream ones.

The HRSV mRNAs contain a virally synthesized 5′ cap [$m^7G(5')ppp(5')Gp$] and a 3′ polyadenylate tail. The latter is produced by reinterative copying by the viral polymerase on a tract of 4–7 U residues at the downstream end of each gene-end signal. Each mRNA encodes a single major viral protein except for the M2 mRNA, which contains two open reading frames (ORFs) that overlap by approximately 32 nt and encode two distinct proteins, M2-1 and M2-2 (**Figure 2**(**b**)). Expression of the downstream M2-2 ORF appears to be coupled to translation of the upstream M2-1 ORF and presumably involves a ribosomal stop–restart mechanism.

In RNA replication, the viral polymerase initiates in the same promoter region and ignores the gene-start and gene-end signals to make a full-length positive-sense copy called the antigenome. The antigenome is neither capped nor polyadenylated. It is tightly encapsidated with N protein and serves as the template for the synthesis of progeny genomes.

The first 11 nt at the 3′ end of the viral genome are essential for both transcription and RNA replication. The two processes involve overlapping sets of residues. For replication, nucleotides 16–32 are required in addition for encapsidation and the synthesis of complete antigenome. For transcription, nucleotides 36–43 are required in addition for full activity. The first 24 nt at the 3′ end of the antigenome have 81% sequence identity with the 3′ end of the genome, indicative of a conserved promoter element.

Viral Infection in Cell Culture and Experimental Animals

HRSV can infect and produce virus, with varying degrees of efficiency, in a surprisingly wide array of cell lines from various tissues and hosts. Replication is most efficient in epithelial cells, in particular the human HEp-2 line. HRSV that has been propagated *in vitro* generally retains its virulence.

Efficient infection by HRSV of established cell lines *in vitro* involves binding to cellular glycosaminoglycans, in particular heparan sulfate and chondroitin sulfate. Both G and F can mediate this binding. It is not known whether this represents attachment in total or whether it is an initial interaction that is followed by a second, higher-affinity step that remains to be identified. It is also not known how closely this mirrors the attachment process *in vivo*.

All events in HRSV infection occur in the cytoplasm. Viral mRNAs and proteins can be detected intracellularly by 4–6 h post infection. The accumulation of mRNAs has been reported to plateau by 14–18 h. This apparent shut-off of transcription might be due to accumulation of the M2-2 regulatory factor, since it does not appear to occur when the M2-2 coding sequence has been deleted. Apart from this, there is no apparent temporal regulation of gene expression. The release of progeny virus begins by 10–12 h, reaches a peak after 24 h, and continues until the cells deteriorate by 30–48 h. Although most of the infected cells in the culture are killed, persistent infections *in vitro* can readily be established.

In an *in vitro* model of human airway epithelium, HRSV infection was strictly limited to ciliated cells of the apical surface. Remarkably, HRSV infection caused

little gross cytopathic effect despite ongoing infection over several weeks. In particular, infection did not result in the formation of syncytia. The expression of the F protein was polarized to the apical surface, which might have prevented contact with neighboring cells and might explain the lack of syncytia. Thus, HRSV is not inherently a highly cytopathic virus in the absence of syncytia formation, and much of the damage to infected cells that occurs *in vivo* might be a consequence of immune attack rather than direct viral effects. HRSV infection causes a modest reduction in cellular DNA, RNA, and protein synthesis. Apoptosis does not occur until late in infection. The virus buds at the plasma membrane. In polarized cells, this occurs at the apical surface.

HRSV does not replicate to high titer *in vitro*. Most of the virus remains cell associated and is released by freeze–thawing or sonication. HRSV can rapidly lose infectivity during unfrozen storage or freeze–thawing. Stability can be improved by adjusting the harvested culture supernatants to pH 7.5 and 0.1 M magnesium sulfate, or by including 15% w/v sucrose. The yield of virus is relatively low: 10 PFU per cell is typical. Most of the infectious particles produced *in vitro* were trapped by a 0.45 μm filter, suggesting that the infectious unit is in the form of large filaments or aggregates. The instability and size heterogeneity of the virus produced *in vitro* makes purification and concentration difficult.

The most permissive hosts for HRSV are the human and the chimpanzee, and these are the only hosts in which respiratory tract disease can reliably be observed. HRSV can also replicate in the respiratory tract of several species of monkey as well as in hamsters, guinea pigs, infant ferrets, cotton rats, and mice. However, infection in these animals is only semipermissive, particularly in the case of rodents. In the BALB/c mouse, the most commonly used experimental model, intranasal infection with 10^6 PFU yields only 10^6 PFU in lungs harvested at the peak of virus replication 4 days post infection.

Genetics

Like RNA viruses in general, HRSV has a high misincorporation rate (approximately one substitution in 10^4 nt), providing the potential for diversity. RNA recombination involving exchange between viruses appears to be exceedingly rare. Defective interfering particles presumably occur but have not been described molecularly.

Negative-sense RNA is not directly infectious alone. However, as for *Mononegavirales* in general, complete infectious recombinant virus can be produced entirely from cloned cDNAs (reverse genetics). This involves transfecting cells with plasmids that express a complete antigenomic RNA and the proteins of the nucleocapsid, which in the case of HRSV are the N, P, M2-1, and L proteins. These components assemble (inefficiently) into a nucleocapsid that initiates a productive infection. This method can be used to introduce desired changes into infectious virus via the cDNA intermediate.

Another system that has been very useful for basic studies involves helper-dependent mini-replicons. These are short, internally truncated versions of genomic or antigenomic RNA in which the viral genes have been replaced by one or more reporter genes under the control of HRSV transcription signals. When complemented by the appropriate mix of plasmid-encoded HRSV proteins, a mini-replicon can be encapsidated, transcribed, replicated, and packaged into virus-like particles. The small size and simplicity of a mini-replicon makes it ideal for detailed structure–function studies.

Antigens and Antigenic Subgroups

The F and G proteins are the only known HRSV neutralization antigens. They have markedly different antigenic properties. Many of the available F-specific monoclonal antibodies (MAb's) efficiently neutralize HRSV *in vitro*, whereas most of those for G neutralize weakly or not at all. However, polyclonal antibodies specific to G neutralize infectivity efficiently. The high content of O-linked carbohydrate in the G protein may be a factor in its unusual antigenic properties, and indeed the sugars have been shown to be important, directly or indirectly, in the binding of many, but not all, MAb's. The sugar side chains might mask the viral polypeptide. In addition, microheterogeneity might exist in the placement and composition of the side chains, which might alter or mask epitopes. In seropositive humans, most of the HRSV proteins have been shown to stimulate HRSV-specific memory CD8+ T lymphocytes.

HRSV has a single serotype. However, isolates can be segregated into two antigenic subgroups A and B. These exhibit a fourfold difference in cross-neutralization *in vitro* by postinfection sera. Epitopes in F tend to be conserved whereas those for G are not, such that antigenic relatedness between the two subgroups is greater than 50% for the F protein compared to only 5% or less for G. At the amino acid level, F is 89% identical between subgroups while G is the most divergent of the proteins and is only 53% identical, with most of the diversity occurring in the ectodomain. Other proteins range from 76% (SH) to 96% identical (N). Thus, the two antigenic subgroups have substantial differences throughout the genome and represent two divergent lines of evolution, as opposed to differing at only a few antigenic sites.

In a 2 year window following infection, there was a 64% reduction in the incidence of infection by the same subgroup versus a 16% reduction against the heterologous subgroup. In epidemics, typically, there is an alternating

pattern with a 1–2 year interval with regard to the predominant subgroup. These observations indicate that antigenic dimorphism contributes to the ability of HRSV to reinfect, although its effect is modest rather than absolute.

Viral isolates obtained over successive years or decades exhibit some evidence of progressive amino acid changes and some changes in reactivity detectable with MAb's. However, all isolates retained a high level of antigenic relatedness and thus HRSV did not exhibit significant antigenic drift during this time frame.

Epidemiology and Clinical Factors

HRSV is the most important cause of serious viral respiratory tract disease in the pediatric population worldwide. In many areas it outranks all other microbial pathogens as a cause of bronchiolitis and pneumonia in infants less than 1 year of age. HRSV is conservatively estimated to cause 64 million infections and 160 000 deaths annually in the pediatric population. In the United States, HRSV is estimated to account for 73 400–126 300 hospitalizations annually for bronchiolitis and pneumonia in children of under 1 year of age, and is responsible for 90–1900 pediatric deaths annually. Hospitalization rates in developed countries are approximately one in 100–200 infections.

In a 13 year study of infants and children in the United States, HRSV was detected in 43%, 25%, 11%, and 10% of those hospitalized for bronchiolitis, pneumonia, bronchitis, and croup, respectively, and in aggregate accounted for 23% of pediatric hospitalizations for respiratory tract disease as a conservative estimate. Middle ear infection is a common complication. As another complication, approximately 40–50% of infants hospitalized with HRSV bronchiolitis have subsequent episodes of wheezing during childhood. HRSV infection also can exacerbate asthma.

Infection and reinfection with HRSV are frequent during the first years of life. Sixty to seventy percent of infants and children are infected by HRSV during the first year of life. The peak of serious disease is approximately 1–2 months of age (**Figure 3**). By age 2, 90% of children have been infected once with HRSV and 50% have been infected twice. The greatest incidence of serious disease occurs between 6 weeks and 6 months of age. Maternal antibodies account for the relative sparing of newborns. The major risk factors for serious HRSV disease in the pediatric population include chronic lung disease, congenital heart disease, prematurity, and young age (<6 months). While underlying disease is an important risk factor, the majority of infants hospitalized for HRSV disease were previously healthy.

Reinfection of older children and adults is common. For example, hospital staff members on pediatric wards

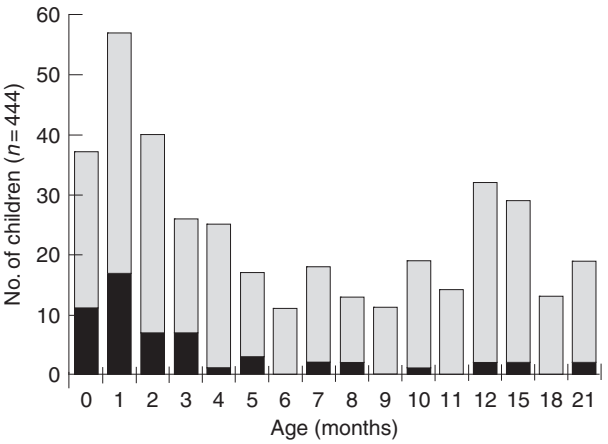


Figure 3 Age at the time of hospitalization for RSV disease at the Johns Hopkins Hospital during 1993–96. The filled bars indicate more severe disease. Adapted from Karron RA, Singleton RJ, Bulkow L, *et al*. (1999). Severe respiratory syncytial virus disease in Alaska native children. *Journal of Infectious Diseases* 180: 41–49.

have an infection rate of 25–50% during HRSV epidemics. Healthy individuals usually suffer only a common cold syndrome. However, HRSV is also an important cause of morbidity and mortality in the elderly, with an impact that is estimated to approach that of nonpandemic influenza virus. HRSV has been estimated to cause 17 000 deaths annually in the elderly in the United States. HRSV infection is also associated with a high mortality rate in children and adults with immunodeficiency or immunosuppression, particularly hematopoietic cell transplant recipients.

HRSV is highly contagious and is readily spread in day-care settings, to family members, in nursing homes, and in the hospital. Spread involves close contact and inoculation of conjunctival or mucosal surfaces by large droplets or by contaminated hands or objects. In experimental infections, HRSV had an incubation period of 4–5 days. Virus typically is shed for 7–12 days coincident with clinical disease, although sometimes shedding is longer and can continue after recovery.

Most primary infections are symptomatic, with upper respiratory tract disease and sometimes a fever. Twenty-five to forty percent of primary infections progress to the lower respiratory tract with the primary manifestations of serious disease being bronchiolitis or pneumonia. There is profuse rhinorrhea, coughing, intermittent fever, expiratory wheezes, and, frequently, middle-ear disease. Seriously ill infants have increased coughing and wheezing, rapid respiration, and hypoxemia, requiring the administration of humidified oxygen.

HRSV causes yearly epidemics that are centered in the winter months in temperate climates (**Figure 4**) or in the rainy season in the tropics, although there can also be local variations to this pattern.

The most definitive diagnosis is by virus isolation in cell culture. Rapid diagnosis can be made by (1) detection of viral antigen using an antigen-capture enzyme-linked immunosorbent assay (ELISA) or by an immunofluorescence analysis of exfoliated cells with HRSV-specific antibody or (2) detection of nucleic acid by reverse transcriptase polymerase chain reaction (RT-PCR). The sensitivity of detection can be over 90%.

Pathogenesis and Immunity

HRSV replicates primarily in the epithelial cells that line the lumen of the respiratory tract. It can also infect macrophages and dendritic cells and modify their functioning. Immunohistochemistry of airway tissues from infected individuals suggests a patchy distribution of infection, with only superficial cells expressing viral antigen. There is destruction of the epithelium and an influx of mononuclear cells, lymphocytes, plasma cells, and macrophages. Tissues become edematous and the secretion of mucus is excessive. Mucus, inflammatory cells, and debris from dead infected cells accumulate in the airways and can cause obstruction, which is a particular problem in infants due to the small diameter of their airways. With pneumonia, the alveolar spaces may fill with fluid and the interalveolar walls may thicken due to mononuclear cell infiltration.

HRSV is an acute infection that typically is completely cleared by host immunity, although sometimes shedding can persist for weeks. Studies in experimental animals and in the clinic indicate that virus-neutralizing secretory IgA antibodies, virus-neutralizing serum antibodies, and cytotoxic CD8+ T lymphocytes participate in resolving infection. These same effectors also confer protection against reinfection. Protection conferred by cytotoxic T lymphocytes appears to diminish within several weeks or months and thus is more important in the short term. Virus-neutralizing secretory IgA antibodies are particularly effective in restricting infection; this response is short-lived following primary infection but can increase in duration following reinfection. Virus-neutralizing serum antibodies provide long-lasting protection. However, they gain access to the respiratory lumen by the inefficient process of transudation, and thus protection is not efficient, particularly in the upper respiratory tract.

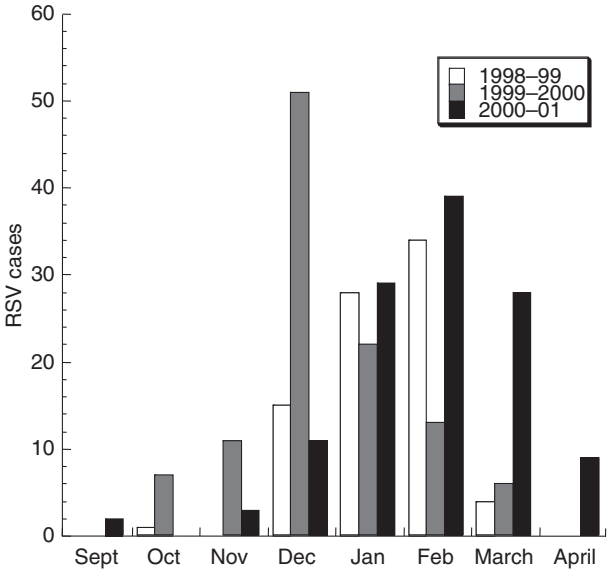


Figure 4 Variation in the timing of the RSV epidemic during three successive years at the Johns Hopkins Hospital. Unpublished data kindly provided by Karron RA, Johns Hopkins School of Hygiene and Public Health and School of Medicine.

Perhaps the most important determinant of HRSV pathogenesis is its ability to infect young infants early in life despite the presence of maternal serum antibodies (**Figure 3**). Why HRSV seems more able than most other respiratory pathogens to evade maternal antibodies and infect very early in life is not understood. The fact that the virus can infect in early infancy when disease is less easily supported and airways are small and more easily obstructed probably accounts for much of its impact.

The immune response during the first months of life is reduced due to immunologic immaturity as well as the immunosuppressive effects of maternal antibodies on humoral responses. This probably contributes to difficulty in controlling the virus and facilitates reinfection early in life. The Th2-biased nature of immune responses in early infancy has also been speculated to contribute to HRSV disease and reduced immune responses. In addition, the tropism of HRSV to the superficial epithelium might reduce its exposure to immune effectors and might also provide reduced immune stimulation.

There is some evidence that HRSV might have suppressive effects on cell-mediated immunity, although the significance and magnitude of these effects are unclear. The cystine noose of the G protein has been shown to inhibit innate immune responses in human monocytes. The inefficiency of virus-neutralizing serum antibodies in controlling HRSV presumably is also a factor in its ability to reinfect throughout life.

Treatment and Immunoprophylaxis

Treatment of severe disease is largely supportive, including mechanical ventilation in the most severe cases. Ribavirin has potent antiviral activity in cell culture and experimental animals, but it has not been clearly beneficial clinically and is not routinely used in most hospitals. Anti-inflammatory therapies have not been beneficial to date

but are still being investigated. Animal studies suggest a benefit of combining antiviral and anti-inflammatory therapies. Bronchodilators are commonly used but have, at most, modest benefits.

Passive immunoprophylaxis is available for infants who are at high risk for serious HRSV disease. This involves monthly intramuscular injections during the HRSV season of an HRSV-neutralizing MAb called palivizumab (Synagis), developed by MedImmune, Inc. This is a humanized version of a mouse MAb specific to the F protein.

A vaccine is not available. A major obstacle to developing a pediatric vaccine is that immunization ideally should start during the first weeks of life. As already noted, immune responses during the first few months of life are reduced due to immunologic immaturity and the immunosuppressive effects of maternal antibodies.

A vaccine consisting of formalin-inactivated, concentrated HRSV (FI-RSV) was evaluated in the 1960s. FI-RSV was found to be poorly protective and, unexpectedly, primed for immune-mediated enhanced HRSV disease that occurred on subsequent natural infection. In subsequent studies, FI-RSV was shown to induce antibodies that bound efficiently to viral antigen but did not efficiently neutralize infectivity. Antigen–antibody complexes might contribute to enhanced disease. In addition, compared to natural infection, FI-RSV caused a disproportionate stimulation of the Th2 subset of CD4+ T lymphocytes, whose role in enhanced disease was confirmed by depletion studies in experimental animals. Disease enhancement appears to be specific to killed or protein subunit HRSV vaccines and might occur because these nonreplicating vaccines do not efficiently stimulate natural killer cells and CD8+ T lymphocytes, which in turn play an important role in regulating the T-helper-cell response via secreted interferon γ. Disease enhancement appears to be an issue only for immunization early in life, and thus protein subunit vaccines appear to be safe in HRSV-experienced individuals.

Because of the phenomenon of vaccine-related disease enhancement, pediatric vaccine development is focused on the development of live-attenuated HRSV strains for intranasal administration. A number of candidate live vaccines developed by reverse genetics are presently in clinical trials. There is also a need for an HRSV vaccine for the elderly. Subunit vaccines are presently being developed for that age group.

See also: Human Respiratory Viruses; Paramyxoviruses.

Further Reading

Collins PL and Crowe JE, Jr. (2006) Respiratory syncytial virus and metapneumovirus. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 5th edn., pp. 1601–1646. Philadelphia, PA: Lippincott Williams and Wilkins.

Collins PL and Murphy BR (2005) New generation live vaccines against human respiratory syncytial virus designed by reverse genetics. *Proceedings of the American Thoracic Society* 2: 166–173.

Crowe JE, Jr. and Williams JV (2003) Immunology of viral respiratory tract infection in infancy. *Paediatric Respiratory Reviews* 4: 112–119.

Hall CB (2001) Respiratory syncytial virus and parainfluenza virus. *New England Journal of Medicine* 344: 1917–1928.

Hussel T, Baldwin CJ, O'Garra A, and Oppenshaw PJ (1997) T cells control Th2-driven pathology during pulmonary respiratory syncytial virus infection. *European Journal of Immunology* 27: 3341–3349.

Karron RA, Singleton RJ, Bulkow L, *et al*. (1999). Severe respiratory syncytial virus disease in Alaska native children. *Journal of Infectious Diseases* 180: 41–49.

McGivern DR, Collins PL, and Fearns R (2005) Identification of internal sequences in the 3 leader region of human respiratory syncytial virus that enhance transcription and confer replication processivity. *Journal of Virology* 79: 2449–2460.

Melero JA, Garcia-Barreno B, Martinez I, Pringle CR, and Cane PA (1997) Antigenic structure, evolution, and immunobiology of human respiratory syncytial virus attachment (G) protein. *Journal of General Virology* 78: 2411–2418.

Murphy BR, Sotnikov AV, Lawrence LA, Banks SM, and Prince GA (1990) Enhanced pulmonary histopathology is observed in cotton rats immunized with formalin-inactivated respiratory syncytial virus (RSV) or purified F glycoprotein and challenged with RSV 3–6 months after immunization. *Vaccine* 8: 497–502.

Polack FP, Irusta PM, Hoffman SJ, *et al.* (2005) The cysteine-rich region of the respiratory syncytial virus attachment protein inhibits the innate immune response elicited by the virus and endotoxin. *Proceedings of the National Academy of Sciences, USA* 102: 8996–9001.

Singleton RJ, Bulkow L, *et al*. (1999). Severe respiratory syncytial virus disease in Alaska native children. *Journal of Infectious Diseases* 180: 41–49.

Spann KM, Collins PL, and Teng MN (2003) Genetic recombination during co-infection of two mutants of human respiratory syncytial virus. *Journal of Virology* 77: 11201–11211.

Teng MN and Collins PL (2002) The central conserved cystine noose of the attachment G protein of human respiratory syncytial virus is not required for efficient viral infection *in vitro* or *in vivo*. *Journal of Virology* 76: 6164–6171.

Tripp RA, Jones LP, Haynes LM, *et al.* (2001) Cytokine mimicry by respiratory syncytial virus G glycoprotein. *Nature Immunology* 2: 732–738.

Wright PF, Karron RA, Madi SA, *et al.* (2006) The interferon antagonist NS2 protein of respiratory syncytial virus is an important virulence determinant in humans. *Journal of Infectious Diseases* 193: 573–581.

Young J (2002) Development of a potent respiratory syncytial virus-specific monoclonal antibody for the prevention of serious lower respiratory tract disease in infants. *Respiratory Medicine* 96 (supplement B): S31–S35.

Zhang L, Peeples ME, Boucher RC, *et al.* (2002) Respiratory syncytial virus infection of human airway epithelial cells is polarized, specific to ciliated cells, and without obvious cytopathology. *Journal of Virology* 76: 5654–5666.

Human Respiratory Viruses

J E Crowe Jr., Vanderbilt University Medical Center, Nashville, TN, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Bronchiolitis A disease condition characterized by trapping of air in the lungs with difficulty expiring (i.e., wheezing), caused by inflammation or infection of the bronchioles, the smallest and highest-resistance airways.
Croup A disease condition characterized by a difficulty in inspiration, associated with a barky cough, caused by inflammation or infection of the larynx, trachea, and bronchi.
Lower respiratory tract The anatomical region below the vocal cords, including the trachea, bronchi, bronchioles, and lung.
Pneumonia Infection of the alveolar space of the lungs.

Introduction

Respiratory virus infections of humans are the most common and frequent infections of man. Hundreds of different viruses can be considered respiratory viruses. Viruses that enter through the respiratory tract include viruses that replicate and cause disease that is restricted to the respiratory epithelium, and other viruses that enter through the mucosa but also spread by viremia causing systemic disease. An example of the latter is measles virus. SARS coronavirus is another. In general, viruses that do not cause viremia are capable of reinfecting the same host multiple times throughout life. In contrast, infections with systemic viruses induce lifelong immunity. Probably, the high rate of reinfection of mucosally restricted viruses reflects the difficulty and metabolic cost of maintaining a high level of immunity at the vast surface area of the mucosa. Virus-specific IgA levels are maintained at high levels generally only for several weeks or months after infection.

The Human Respiratory Tract

The anatomy and the cell types of the respiratory tract dictate to a large degree the type of disease observed during respiratory virus infection. The demarcation between the upper and lower respiratory tracts is the vocal cords. The structures of the upper respiratory tract, which are all interconnected, include the nasopharynx, the larynx, the Eustachian tube and middle ear space, and the sinuses. Significant collections of lymphoid tissue reside in the upper respiratory tract, the tonsils and the adenoids. The lower respiratory tract structures include the trachea, bronchi, bronchioles, alveoli, and lung tissue. The cell types that line the respiratory tract are complex, and exhibit different susceptibilities to virus infection. The predominant cell types are ciliated and nonciliated epithelial cells, goblet cells, and Clara cells. Smooth muscle cells are prominent features of the airways down to the level of the bronchioles, and the lung possesses type I and II pneumocytes.

Disease Syndromes

The disease syndromes that are associated with respiratory viruses generally follow the anatomy of the respiratory tract. Different viruses appear to have tropisms for different cells or regions of the respiratory tract; therefore, there are particular associations of viruses with clinical syndromes. The clinical diagnoses for infections with disease manifestations in the respiratory tract are rhinitis and the common cold, sinusitis, otitis media, conjunctivitis, pharyngitis, laryngitis, tracheitis, acute bronchitis, bronchiolitis, pneumonia, and exacerbations of reactive airway disease or asthma. Clinical syndromes with more systemic illness due to respiratory viruses include the influenza syndrome, measles, severe acute respiratory syndrome (SARS), and hantavirus pulmonary syndrome (HPS).

Viruses That Cause Respiratory Illness in Immunocompetent Humans

The principal causes of acute viral respiratory infections in children became apparent through large epidemiologic studies conducted soon after cell culture techniques became available. The landmark studies of association of viruses with clinical syndromes were performed in the 1960s and 1970s. Recent studies have increased our understanding of the causes of viral respiratory infection in infants, especially because of the advent of molecular tests such as the polymerase chain reaction (PCR), which is more sensitive than cell culture. Respiratory syncytial virus (RSV), parainfluenza viruses (PIVs), adenoviruses, and influenza viruses were identified initially as the most common causes of serious lower respiratory tract disease in infants and children. More recently, human metapneumovirus (hMPV) was identified as a major cause of serious illness. In the last 10 years, a number of additional viruses have been associated with respiratory illness, as discussed

below. However, still, infectious agents are not identified in 30–50% of clinical illnesses in large surveillance studies, even using sensitive diagnostic techniques such as viral culture on multiple cell lines, antigen detection assays, and RT-PCR based methods. It is not known if these illnesses are due to identified pathogens that are simply not detected due to low titers of virus in patient samples or if there are novel agents that are yet to be identified.

Immunocompromised Hosts

Reactivation of latent viruses, such as the herpesviruses HSV and CMV, and adenoviruses occurs in immunocompromised humans, particularly subjects with late-stage HIV infection, those with organ transplantation, and patients with leukopenia and neutropenia caused by chemotherapy. CMV is the most frequently recovered virus from diagnostic procedures such as bronchoalveolar lavage in immunosuppressed patients with pneumonia. These patients also suffer more frequent and more severe disease including mortality with common respiratory viruses, including RSV, hMPV, PIV, influenza viruses, rhinoviruses, and adenoviruses. Nosocomial transmission including large unit outbreaks is not uncommon, and can result in high frequency of transmission.

Specific Viral Causes of Respiratory Disease

Picornaviridae

A wide variety of picornaviruses cause respiratory disease, including rhinoviruses, the enteroviruses A to D including coxsackieviruses A/B, echoviruses, non-polio enteroviruses, and parechoviruses 1–3. Enterovirus infections occur most commonly in the summer months in temperate areas, which differs from the season of many of the other most common respiratory viruses such as paramyxoviruses and influenza virus. Rhinovirus infections occur year-round.

Rhinoviruses

Rhinovirus is a genus of the family *Picornaviridae* of viruses. Rhinoviruses are the most common viral infective agents in humans, and a causative agent of the common cold. There are over 105 serologic virus types that cause cold symptoms, and rhinoviruses are responsible for approximately half of all cases of the common cold. Rhinoviruses have single-stranded positive-sense RNA genomes. The viral particles are icosahedral in structure, and they are nonenveloped. Rhinovirus-induced common colds may be complicated in children by otitis media and in adults by sinusitis. Most adults, in fact, have radiographic evidence of sinusitis during the common cold, which resolves without therapy. Therefore the primary disease is probably best termed rhinosinusitis. Rhinovirus infection is associated with exacerbations of reactive airway disease in children and asthma in adults. It is not clear whether rhinovirus is restricted to the upper respiratory tract and induces inflammatory responses that affect the lower respiratory tract indirectly, or whether the viruses spread to the lower respiratory tract. In the past, it was thought that these viruses did not often replicate or cause disease in the lower respiratory tract. However, recent studies discern strong epidemiological associations of RVs with wheezing and asthma exacerbations, including episodes severe enough to require hospitalization. Likely, rhinoviruses can infect the lower airways to some degree, inducing a local inflammatory response. Another possibility is that significant local infection of the upper respiratory tract might induce regional elaboration of mediators that causes lower airways disease. Association of rhinovirus infection with lower respiratory tract illness is difficult to study because cell diagnosis by cell culture is not sensitive. RT-PCR diagnostic tests are difficult to interpret because they are often positive for prolonged periods of time and even asymptomatic individuals may have a positive test. Comprehensive serologies to confirm infection are difficult because of the large number of serotypes. Nevertheless, most experts believe rhinoviruses are a common cause of lower respiratory tract illness.

Coxsackieviruses

These viruses cause oral lesions and often are associated in children with a disease syndrome termed 'hand-foot-and-mouth disease'. The pharyngitis associated with this infection often is marked by the very characteristic findings of herpangina, a clinical syndrome of ulcers or small vesicles on the palate and often involving the tonsillar fossa associated with the symptoms of fever, difficulty swallowing, and throat pain. Outbreaks commonly occur in young children, in the summer.

Enteroviruses

Non-polio enteroviruses are common and distributed worldwide. Although infection often is asymptomatic, these viruses cause outbreaks of clinical respiratory disease, sometimes with fatal consequences. Studies have associated particular types with clinical syndromes, as enterovirus 68 with wheezing and enterovirus 71 with pneumonia.

Echoviruses

The term 'echo' in the name of the virus is an acronym for enteric cytopathic human orphan, although this may be an archaic notion since most echoviruses are associated with human diseases, most commonly in children. There are at least 33 echovirus serotypes. Echoviruses can be isolated from many children with upper respiratory tract

infections during the summer months. Echovirus 11 has been associated with laryngotracheitis or croup. Epidemiology studies also have associated echoviruses with epidemic pleurodynia, an acute illness characterized by sharp chest pain and fever.

Parechoviruses

These viruses have been assigned a new genus of the family *Picornaviridae* because of distinctive laboratory-based molecular properties. The most common member of the genus *Parechovirus*, human parechovirus 1 (formerly echovirus 22) is a frequent human pathogen. The genus also includes the closely related virus, human parechovirus 2 (formerly echovirus 23). Human parechoviruses usually cause mild respiratory or gastrointestinal illness. Most infections occur in young children. There is a high seroprevalence for parechoviruses 1 and 2 in adults, and a few clear descriptions of neonatal cases of severe disease.

Paramyxoviridae

Respiratory syncytial virus

RSV is a single-stranded negative-sense nonsegmented RNA genome virus of the family *Paramyxoviridae*, genus *Pneumovirus*. It is one of the most infectious viruses of humans and infects infants at a very young age, often in the first weeks or months of life. It is the most common viral cause of severe lower respiratory tract illness in children and one of the most important causes of hospitalization in infants and children throughout the world. There is one serotype, but circulating viruses exhibit an antigenic dimorphism such that there are two antigenic subgroups designated A and B. Reciprocal cross-neutralization studies using human sera showed that the antigenic groups are about 25% related. Reinfection is common and can be caused by viruses of the same subgroup. Yearly, epidemics of disease often peak between January and March in temperate regions. RSV infection causes mild upper respiratory tract infection in most infants and young children, but results in hospitalization in 0.5–1% of infants. Most children have been infected by the age of 2 years. There is an association of RSV infection early in life and subsequent asthma, although a causal relationship is controversial. Most hospitalized infants are otherwise healthy, but some groups are considered high risk for severe disease such as premature infants especially those with chronic lung disease and infants born with congenital heart disease. Immunocompromised patients of any age are at risk of severe disease.

Human parainfluenza viruses

These viruses constitute a group of four distinct serotypes (types 1–4) of single-stranded RNA viruses belonging to the family *Paramyxoviridae*. When considered as a group, they are the second most common cause of lower respiratory tract infection in young children. PIV3 is the most common cause of severe disease. Repeated infection throughout life is common. First infections are more commonly associated with lower respiratory tract disease, especially croup, while subsequent infections typically are limited to the upper respiratory tract. PIVs are detected using cell culture with hemadsorption or immunofluorescent microscopy, and RT-PCR.

Human metapneumovirus

In 2001, investigators in the Netherlands described a new human respiratory virus, hMPV. Evidence of near universal seroconversion was found in the general population by 5 years of age, suggesting ubiquitous infection in early childhood. This virus, a member of the genus *Pneumovirus* with RSV, differs from RSV in that it lacks the NS1 and NS2 nonstructural genes that counteract host interferons and it possesses a slightly different gene order. Studies of the role of hMPV in pediatric lower respiratory tracts infection (LRI) in otherwise healthy children in the United States, using a prospectively collected 25-year database and sample archive representing about 2000 children, revealed that nearly 12% of LRI in children was associated with a positive hMPV test. This and similar studies suggested that the virus is one of the major respiratory pathogens of early childhood. The clinical features of hMPV LRI were similar to those of other paramyxoviruses, most often resulting in cough, coryza, and a syndrome of bronchiolitis or croup. Interestingly, hMPV seemed to be clinically intermediate between RSV and PIV in that it tended to cause bronchiolitis with similar frequency to RSV but more frequently than PIV, while causing croup less often than the latter. Studies in subjects with conditions predisposing to increased risk of respiratory illness suggest that hMPV plays a significant role in exacerbations of asthma in children and adults, LRI in immunocompromised subjects, and in the frail and elderly.

Measles virus

Measles virus, a paramyxovirus of the genus *Morbillivirus* causes infection with systemic disease, also known as rubeola. The virus is spread both by direct contact/fomite transmission and by aerosol transmission, and therefore is one of the most highly contagious infections of man. The classical symptoms of measles include 3 or more days of fever that is often quite high and a clinical constellation of symptoms termed 'the three Cs': cough, coryza, and conjunctivitis. A characteristic disseminated maculopapular rash appears soon after onset of fever. Transient mucosal lesions in the mouth of a characteristic appearance (Koplik's spots) are considered diagnostic when identified by an experienced clinician. The virus causes a number

of systemic effects and can be complicated by severe pneumonia, especially when primary infection occurs in an unvaccinated adult or immunocompromised person of any age. Mortality in developing countries is high, especially when infection occurs in the setting of malnutrition.

Hendra and Nipah viruses

These emerging pathogens that are grouped in their own new genus *Henipaviruses* may not be respiratory pathogens in a conventional sense, but they are paramyxoviruses that probably infect humans by the respiratory route. Nipah virus is a newly recognized zoonotic virus, named after the location in Malaysia where it was first identified in 1999. It has caused disease in humans with contact with infectious animals. Hendra virus (formerly called equine morbillivirus) is another closely related zoonotic paramyxovirus that was first isolated in Australia in 1994. The viruses have caused only a few localized outbreaks, but their wide host range and ability to cause high mortality raise concerns for the future. The natural host of these viruses is thought to be a certain species of fruit bats present in Australia and the Pacific. Pigs may be an intermediate host for transmission to humans in Nipah infection, and horses in the case of Hendra. Although the mode of transmission from animals to humans is not defined, it is likely that inoculation of infected materials onto the respiratory tract plays a role. The clinical presentation usually appears to be an influenza-like syndrome, progressing to encephalitis, may include respiratory illness, and causes death in about half of identified cases.

Orthomyxoviridae

Influenza viruses

Influenza is a single-stranded segmented negative-sense RNA genome virus of the family *Orthomyxoviridae*. There are three types of influenza viruses: influenza virus A, influenza virus B, and influenza virus C. Influenza A and C infect multiple species, while influenza B infects humans almost exclusively. The type A viruses are the most virulent human pathogens among the three influenza types, and cause the most severe disease. The influenza A virus can be subdivided into different subtypes based on the antibody response to these viruses. The subtypes that have been confirmed in humans in seasonal influenza, ordered by the number of known human pandemic deaths, are: H1N1 which caused the 1918 pandemic, and H2N2 which caused the 1957 pandemic of avian influenza that originated in China, H3N2 which caused the pandemic of 1968. Currently, H3N2, H1N1, and B viruses cause annual seasonal epidemics. In addition, H5N1 virus infection of humans occurred during an epizootic of H5N1 influenza in Hong Kong's poultry population in 1997. The disease affected animals of many species and exhibited a high rate of mortality in humans. The virus is spreading throughout Asia, carried by wild birds. Human-to-human transmission does not occur efficiently at this time; however, there is widespread current concern about the potential for an H5N1 pandemic if the virus acquired transmissibility among humans. The H7N7 avian virus also has unusual zoonotic potential. In 2003 this virus caused an outbreak in humans in the Netherlands associated with an outbreak in commercial poultry on several farms. One death occurred and 89 people were confirmed to have H7N7 influenza virus infection. H1N2 virus appears to endemic in pigs and humans. H9N2, H7N2, H7N3, and H10N7 human infections have been reported. Influenza B virus is almost exclusively a human pathogen, and is less common than influenza A. It mutates less rapidly than influenza A, and there is only one influenza B subtype. In humans, common symptoms of influenza infection and syndrome are fever, sore throat, myalgias, headache, cough, and fatigue. In more serious cases, influenza causes pneumonia, which can be fatal, particularly in young children and the elderly. Influenza pneumonia has an unusually high rate of complication by bacterial superinfection with staphylococcal and streptococcal bacterial pneumonia occurring in as many as 10% of cases in some clinical series.

Adenoviridae

Viruses of the family *Adenoviridae* infect both humans and animals. Adenoviruses were first isolated in human lymphoid tissues from surgically removed adenoids, hence the name of the virus. In fact, some serotypes establish persistent asymptomatic infections in tonsil and adenoid tissues, and virus shedding can occur for months or years. These double-stranded DNA viruses are less than 100 nm in size, and have nonenveloped icosahedral morphology. The large dsDNA genome is linear and nonsegmented. There are six major human adenovirus species (designated A through F) that can be placed into 51 immunologically distinct serotypes. Human respiratory tract infections are mainly caused by the B and C species. Adenovirus infections can occur throughout the year. Sporadic outbreaks occur with many of the serotypes, while others appear to be endemic in particular locations. Respiratory illnesses include mild disease such as the common cold and lower respiratory tract illness, including croup, bronchiolitis, and pneumonia. Conjunctivitis is associated with infection by species B and D. There is a particular constellation of symptoms called 'pharyngoconjunctival fever' which is very frequently associated with acute adenovirus infection. In contrast, gastroenteritis has been associated most frequently with the serotype 40 and 41 virus of species F. Immunocompromised subjects are highly susceptible to severe disease during infection with respiratory adenoviruses. The syndrome of acute

respiratory disease (ARD), especially common during stressful or crowded living conditions, was first recognized among military recruits during World War II and continues to be a problem for the military following suspension of vaccination. ARD is most often associated with adenovirus types 4 and 7.

Coronaviridae

Members of the genus *Coronavirus* also contribute to respiratory illness including severe disease. There are dozens of coronaviruses that affect animals. Until recently, only two representative strains of human coronaviruses were known to cause disease, human coronavirus 229E (HCoV-229E) and HCoV-OC43. A recent outbreak of SARS-associated coronavirus (SARS-CoV) showed that animal coronaviruses have the potential to cross species to humans with devastating effects. There has been one major epidemic to date, between November 2002 and July 2003, with over 8000 cases of the disease, and mortality rates approaching 10%. SARS-CoV causes a systemic illness with a respiratory route of entry. SARS is a unique form of viral pneumonia. In contrast to most other viral pneumonias, upper respiratory symptoms are usually absent in SARS, although cough and dyspnea occur in most patients. Typically, patients present with a nonspecific illness manifesting fever, myalgia, malaise, and chills or rigors; watery diarrhea may occur as well. Recently, investigators reported the identification of a fourth human coronavirus, HCoV-NL63, a new group 1 coronavirus. Evidence is emerging that HCoV-NL63 is a common respiratory pathogen of humans, causing both upper and lower respiratory tract illness. Human coronavirus (HCoV) HKU1 was first described in January 2005 following detection in a patient with pneumonia. Several cases of respiratory illness have been associated with the virus, but the infrequent identification suggests to date that this putative group 2 coronavirus causes a low incidence of illness.

Herpesviridae

Several herpes viruses cause upper respiratory infections, especially infection of the oral cavity. Herpes simplex pharyngitis is associated with characteristic clinical findings, such as acute ulcerative stomatitis and ulcerative pharyngitis. Herpes simplex virus 1 (HSV-1) and herpes simplex virus 2 (HSV-2), also called human herpesvirus 1 (HHV-1) and human herpesvirus 2 (HHV-2), respectively, cause oral lesions, although over 90% of oral infections are caused by HSV-1. Primary oral disease can be severe, especially in young children, who sometimes are admitted for rehydration therapy due to poor oral intake. A significant proportion of individuals suffer recurrences of symptomatic disease consisting of vesicles on the lips. Epstein–Barr virus (EBV) mononucleosis syndrome is often marked by acute or subacute exudative pharyngitis; in some cases, the swelling of the tonsils in EBV pharyngitis is so severe that airway occlusion appears imminent. Most of the viruses of the family *Herpesviridae* can cause severe disease in immunocompromised patients (especially hematopoietic stem cell transplant patients), including cytomegalovirus (CMV), EBV, varicella-zoster virus, herpesvirus 6, herpesvirus 7, and herpesvirus 8.

Parvoviridae

Human bocavirus

A new virus was identified recently in respiratory samples from children with lower respiratory tract disease in Sweden. Sequence analysis of the viral genome revealed that the virus is highly related to canine minute virus and bovine parvovirus and is a member of the genus *Bocavirus*, subfamily *Parvovirinae*, family *Parvoviridae*. This virus was tentatively named human bocavirus (HBoV). HBoV has been identified as the sole agent in a limited number of respiratory samples from children hospitalized with respiratory tract disease. It remains to be seen whether the virus is causative of or merely associated with disease in these preliminary studies.

Bunyaviridae

Hantavirus

Over 400 cases of HPS have been reported in the United States. The disease was first recognized during an outbreak in 1993. About a third of recognized cases end in death. The Four Corners area outbreak is well known; however, cases now have been reported in 30 states. Patients with HPS usually present with a febrile illness beginning with symptoms of a flu-like illness. Physical examination is not specific, often only with findings of fever, and increased heart and respiratory rates. In addition to the respiratory symptoms, abdominal pain and fever are common. Diagnosis is often delayed until a severe illness occurs requiring mechanical ventilation.

Reoviridae

Rotavirus

Rotaviruses are dsRNA enteric viruses that are the most common cause of severe viral infectious gastroenteritis in children. Clinical series suggest that some children with gastroenteritis suffer upper respiratory symptoms during the prodrome of disease manifestation, and virus can be recovered from respiratory secretions. Some reports suggest

that rotavirus infection is associated with lower respiratory tract illness, although this association is unclear.

Reovirus

These dsRNA viruses (named using an acronym for respiratory enteric orphan virus) are not clearly associated with respiratory disease, but seroconversion rate is high in the first few years of life, and they probably cause minor or subclinical illness.

Retroviridae

Human immunodeficiency virus

Pharyngitis occurs with primary HIV infection and may be associated with mucosal erosions and lymphadenopathy.

Papovaviridae

Polyomaviruses

Polyomaviruses are small dsDNA genome nonenveloped icosahedral viruses that may be oncogenic. There are two polyomaviruses known to infect humans, JC and BK viruses. Eighty percent or more of adult US subjects are seropositive for these viruses. JC virus can infect the respiratory system, kidneys, or brain. BK virus infection causes a mild respiratory infection or pneumonia and can involve the kidneys of immunosuppressed transplant patients.

Co-Infections

Given the overlap in the winter season of these viruses in temperate areas, it is not surprising that co-infections with two or more viruses occur. In general, when careful studies using cell culture techniques were used for virus isolation, more than one virus was isolated from respiratory secretions of otherwise healthy subjects with acute respiratory illness in about 5–10% of cases in adults and 10–15% in children. There is little evidence that more severe disease occurs during co-infections, although there is insufficient evidence on this point to be definitive. The incidence of two molecular diagnostic tests being positive (generally RT-PCR, for these RNA viruses) is expected to be higher than that of culture, because molecular tests can remain positive for an extended period of time after virus shedding has ended.

Transmission

Respiratory viruses generally have two main modes of transmission, large particle aerosols of respiratory droplets transmitted directly from person-to-person by coughing or sneezing, or by fomites. Fomite transmission occurs indirectly when infected respiratory droplets are deposited on hands or on inanimate objects and surfaces with subsequent transfer of secretions to a susceptible subject's nose or conjunctiva. Most respiratory viruses, unlike measles virus or varicella zoster virus, do not spread by small particle aerosols across rooms or down halls. Therefore, contact and droplet precautions are sufficient to prevent transmission in most settings; handwashing is critical in healthcare settings during the winter season.

Antiviral Drugs for Respiratory Viruses

Ribavirin is a nucleoside antimetabolite pro-drug that is activated by kinases in the cell, resulting in a 5′ triphosphate nucleotide form that inhibits RNA replication. The drug was licensed in an aerosol form in the US in 1986 for treatment of children with severe RSV lower respiratory tract infection. The efficacy of aerosolized ribavirin therapy remains uncertain despite a number of clinical trials. Most centers use it infrequently, if ever, in otherwise healthy infants with severe RSV disease. Intravenous ribavirin has been used for adenovirus, hantavirus, measles virus, PIV, and influenza virus infections, although a good risk/benefit profile has not been established clearly for any of these uses.

A humanized mouse monoclonal antibody directed to the F protein of RSV, 'palivizumab', is licensed for prevention of RSV hospitalization in high-risk infants. It is efficacious in half or more of high-risk subjects. A more potent second-generation antibody is being studied in clinical trials. Experimental treatment of both immunocompetent and immunocompromised RSV-infected subjects has been reported but the efficacy of this approach is not established.

There are four licensed drugs in the US for treatment or prophylaxis of influenza. 'Amantadine' and 'rimantadine' are two of the drugs that interfere with the ion channel activity caused by the viral M2 protein of influenza A viruses, which is needed for viral particle uncoating following endocytosis. The other two drugs, 'oseltamivir' and 'zanamivir', are neuraminidase inhibitors that act on both influenza A and B viruses by serving as transition state analogs of the viral neuraminidase that is needed to release newly budded virion progeny from the surface of infected cells. The cell surface normally is coated heavily with the viral receptor sialic acid. Resistance to the ion channel inhibitors arises rapidly during prophylaxis or treatment, and in 2006 resistance levels became so common in circulating viruses that the CDC no longer recommends use of these drugs.

'Interferon-α' has been shown to protect against rhinovirus infections when used intranasally. This biological drug causes some side effects, such as nasal bleeding, and resistance to the drug developed during experimental use, so the molecule is no longer being developed for this

purpose. 'Pleconaril' has been tested for treatment of rhinovirus infection, as it is an oral drug with good bioavailability for treating infections caused by picornaviruses. This drug acts by binding to a hydrophobic pocket in the VP1 protein and stabilizing the protein capsid, preventing release of viral RNA into the cell. The drug reduced mucus secretions and other symptoms and is being further examined.

'Acyclovir' and related compounds are guanine analog antiviral drugs used in treatment of herpes virus infections. HSV stomatitis in immunocompromised patients is treated with 'famciclovir', or 'valacyclovir', and immunocompetent subjects with severe oral disease compromising oral intake are sometimes treated. These compounds have also been used prophylactically to prevent recurrences of outbreaks, with mixed results. Intravenous acyclovir is effective in HSV or varicella zoster virus pneumonia in immunocompromised subjects. 'Ganciclovir' with human immunoglobulin may reduce the mortality associated with CMV pneumonia in hematopoietic stem cell transplant recipients and has been used as monotherapy in other patient groups.

'Cidofivir' is a nucleotide analog with activity against a large number of viruses, including adenoviruses. Intravenous cidofovir has been effective in the management of severe adenoviral infection in immunocompromised patients but may cause serious nephrotoxicity.

Vaccines

There are licensed vaccines for influenza viruses. In the US, both a trivalent (H3N2, H1N1, and B) inactivated intramuscular vaccine and a live attenuated trivalent vaccine for intranasal administration is available. The efficacy of these vaccines is good when the vaccine strains chosen are highly related antigenically to the epidemic strain. Antigenic drift caused by point mutations in the HA and NA molecules leads to antigenic divergence, requiring new vaccines to be made each year. The influenza genome is segmented, which allows reassortment of two viruses to occur during co-infection, which sometimes leads to a major antigenic shift resulting in a pandemic. Pandemics occur every 20–30 years on average. There is current concern about the potential for an H5N1 pandemic, and experimental vaccines are being tested for this virus. To date, H5N1 vaccines have been poorly immunogenic compared to comparable seasonal influenza vaccines. Vaccines were developed for adenovirus serotypes 4 and 7, and these were approved for preventing epidemic respiratory illness among military recruits. Essentially, these were unmodified viruses given by the enteric route in capsules, instead of the respiratory route, which is the natural route of infection leading to disease. Inoculation by the altered route resulted in an immunizing asymptomatic infection. All US military recruits were vaccinated against adenovirus from 1971 to 1999 with near complete prevention of the disease in this population, but the sole manufacturer of the vaccine halted production in 1996 and supplies ran out 3 years later. Since 1999, adenovirus infection has reemerged as a significant problem in the military with approximately 10% of all recruits suffering illness due to adenovirus infection during basic training; some deaths have occurred. Live attenuated vaccine candidates are under development and being tested in phase I and II clinical trials for RSV and the PIVs. Mutant strains with reduced pathogenicity were isolated in the laboratory, tested, and sequenced. Now, vaccine candidates are being optimized by combining mutations from separate biologically derived viruses into single strains using recombinant techniques for generating RNA viruses from cDNA copies, a process called reverse genetics. Subunit vaccines have been developed for RSV, but there are safety concerns about their use in young infants because formalin inactivated vaccine induced a more severe disease response to infection in the 1960s. There are no vaccines against rhinoviruses as there is little or no cross-protection between serotypes, and it is not feasible to develop a vaccine for over 100 serotypes. Efforts to develop coronavirus vaccines are in the preclinical stage.

Summary

Viruses are the leading causes of acute lower respiratory tract infection in infancy. RSV is the most common pathogen, with hMPV, PIV-3, influenza viruses, and rhinoviruses accounting for the majority of the remainder of acute viral respiratory infections. Humans generally do not develop lifelong immunity to reinfection with these viruses; rather, specific immunity protects against severe and lower respiratory tract disease.

See also: Human Respiratory Syncytial Virus.

Further Reading

Booth CM, Matukas LM, Tomlinson GA, *et al.* (2003) Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. *JAMA* 289: 2801–2809.

Booth CM, Matukas LM, Tomlinson GA, *et al.* (2003) Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area – Erratum. *JAMA* 290: 334.

Collins PL and Crowe JE Jr. (2006) Respiratory syncytial virus and metapneumovirus. In: Knipe DM and Howley PM (eds.) *Fields Virology*, 5th edn., pp. 1601–1646. Philadelphia: Lippincott, Williams and Wilkins.

Fisher RG, Gruber WC, Edwards KM, *et al.* (1997) Twenty years of outpatient respiratory syncytial virus infection: A framework for vaccine efficacy trials. *Pediatrics* 99: E7.

Glezen WP, Frank AL, Taber LH, and Kasel JA (1984) Parainfluenza virus type 3: Seasonality and risk of infection and reinfection in young children. *Journal Infectious Diseases* 150: 851–857.

Glezen WP, Paredes A, Allison JE, Taber LH, and Frank AL (1981) Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. *Journal of Pediatrics* 98: 708–715.
Heymann PW, Carper HT, Murphy DD, *et al.* (2004) Viral infections in relation to age, atrophy, and season of admission among children hospitalized for wheezing. *Journal of Allergy and Clinical Immunology* 114: 239–247.
Karron RA and Collins PL (2006) Parainfluenza viruses. In: Knipe DM and Howley PM (eds.) *Fields Virology*, 5th edn., pp. 1497–1526. Philadelphia, PA: Lippincott Williams and Wilkins.
Martinez FD (2002) What have we learned from the Tucson Children's Respiratory Study? *Paediatric Respiratory Reviews* 3: 193–197.
Parrott RH, Kim HW, Arrobio JO, *et al.* (1973) Epidemiology of respiratory syncytial virus infection in Washington, DC. Part II. Infection and disease with respect to age, immunologic status, race, and sex. *American Journal of Epidemiology* 98: 289–300.
Subbarao K, Klimov A, Katz J, *et al.* (1998) Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science* 279: 393–396.
Williams JV, Harris PA, Tollefson SJ, *et al.* (2004) Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *New England Journal of Medicine* 350: 443–450.
Winther B, Hayden FG, and Hendley JO (2006) Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: Association with symptomatic illness and effect of season. *Journal of Medical Virology* 78: 644–650.
Wright PF, Neumann G, and Kawaoka Y (2006) Orthomyxoviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology*, 5th edn., pp. 1691–1740. Philadelphia, PA: Lippincott Williams and Wilkins.

Human T-Cell Leukemia Viruses: General Features

M Yoshida, University of Tokyo, Chiba, Japan

© 2008 Elsevier Ltd. All rights reserved.

Glossary

pX region HTLV sequence between the env and 3′ LTR, encoding Tax, Rex and other small regulatory proteins.
Rex Trans-modulator of viral RNA splicing and transport.
Tax Pleiotropic regulator activating viral and cellular replication interacting with cellular transcription factors, tumor suppressor proteins, and cell cycle checkpoints.

Introduction

Human T-cell leukemia virus 1 (HTLV-1) is the first established tumorigenic retrovirus of humans; exogenous to humans this virus is classified as the species *Human T-cell leukemia virus*, in *Deltaretroviridae*, within the family *Retroviridae*. HTLV-1 infection is associated with leukemia and neural disease, adult T-cell leukemia (ATL) and HTLV-1-associated myelopathy/tropical spastic parapasis (HAM/TSP), respectively. The genomic structure of the virus with genes for nonstructural proteins established a distinct viral genus that includes *Bovine leukemia virus*. HTLV-1 has no oncogene, but nevertheless transforms T cells rather efficiently and is identified as the etiologic agent of ATL. HTLV-1 has unique regulatory proteins, Tax and Rex, and Tax has been identified as a critical molecule not only in regulation of viral replication but also in induction of ATL.

History and Classification

After long and enormous efforts to identify a retrovirus in human tumors, HTLV was described in T-cell lines as a convincing human retrovirus. The first report of the virus (HTLV) was from a patient with Mycosis (MF) in the US, and another (adult T-cell leukemia virus (ATLV)) was from a patient with ATL in Japan. Subsequently, the MF case was characterized as ATL and the two isolates were established to be the same following a comparison of their genomes.

A prototypical retroviral genome contains the *gag*, *pol*, and *env* genes encoding the virion proteins including core proteins, reverse transcriptase, and surface glycoprotein, respectively. Acute leukemia viruses generally have an oncogene acquired from cellular genes that substitutes a part of the *gag*, *pol*, and *env* sequences. In contrast to these genomes, HTLV has additional genes in an extra pX region between *env* and the 3′ LTR (LTR – long terminal repeat). This unique genomic structure classified HTLV as a member of a distinct genus of the *Retroviridae*, which includes HTLV-1, and-2, bovine leukemia virus (BLV), and simian T-cell leukemia viruses (STLV-1, -2, and -3). HTLV-2 was isolated from a patient with hairy T-cell leukemia and its genome similarity to the type 1 is about 60%.

STLVs have been isolated from various species of Old World nonhuman primates, including the Japanese macaque, African green monkey, pig-tailed macaque, gorilla, and chimpanzee. Their genomes share 90–95% homology.

BLV infects and replicates in B cells of cows and sheep and induces B-cell lymphoma.

Geographic Distribution

Geographic Clustering

HTLV-1 carriers are defined by virus-specific antibodies. Nationwide surveys in Japan revealed the following: almost all ATL and HAM/TSP patients are infected with HTLV-1, 5–15% of adults in southwestern Japan are infected with HTLV-1 but this percentage is less in other areas of Japan. Worldwide, virus carriers are localized in the Caribbean islands and South America, Central Africa, and Papua New Guinea and the Solomon Islands. The prevalence of asymptomatic, seropositive adults varies significantly from district to district and even from village to village within these endemic areas. The unique clustering of virus infection in such remote areas is considered to reflect its mode of transmission and familial close contact such as sexual relations and breastfeeding. The presence of ATL and HAM/TSP is overlapping and clustered with HTLV-1 infection. Although some patients with ATL and healthy HTLV-1-infected carriers are found in nonendemic areas, they have frequently moved from an endemic area.

HTLV-2 is endemic in South America and is also detected in populations infected with HIV. STLVs are distributed in Old World monkeys in various areas, but not in New World monkeys. Although viruses were isolated from most species of Old World monkeys, some colonies are found to be virus-free even in the same geographic area.

Age-Dependent Infection

The frequency of HTLV-1 carriers increases with age after 20, reaching a maximum between 40 and 60 years of age. The prevalence is slightly (1.6 times) higher in females than in males, which is attributed to sexual transmission.

Familial aggregation

Familial aggregation of the HTLV-1 infection is apparent. If a mother is HTLV-1 positive, her children are frequently positive. This aggregation is due to viral transmission from husband to wife and mother to her children.

Host Range and Transmission

Receptor, Infection, and Transformation

The receptor for HTLV-1 infection is a glucose transporter, Glut-1, which is ubiquitously expressed on most cell types. Glut-1 is required for viral binding and infection, but it alone is not sufficient to understand preferential infection of CD4+ T cells *in vivo*. Cell-free viral particles of HTLV-1 released from established cell lines show extremely low infectivity *in vitro*, and co-cultivation with virus-producing cells is generally used to establish infection. A variety of cell types are infected including human T and B lymphocytes, fibroblasts, and epithelial cells, as well as cells from monkeys, rats, rabbits, and hamsters, but curiously not cells from mice. These cells infected by co-cultivation contain multiple integrated proviral copies of complete and defective genomes. Only T cells of the CD4+ phenotype are immortalized upon infection *in vitro*.

Despite the broad cell type host range *in vitro*, the cells infected *in vivo* are T cells. HTLV-1 infects both T cells with CD4+ and CD8+ phenotypes, although preferentially those of the CD4+ phenotype, but ATL cells are exclusively CD4+ T cells. Inoculation of infected cells can establish infection in rats, rabbits, and mice providing animal models for studies of the virus.

Natural Transmission

HTLV-1 is transmitted through (1) nursing of infants by infected mothers, (2) sexual contact, and (3) blood transfusion.

Mother to child. About 30% of the children born to seropositive mothers were seropositive. Infected T cells in breast milk are a source of transmissible virus. This mode of transmission was initially suggested by epidemiological studies and then direct evidence was obtained through cessation of breastfeeding by seropositive mothers that drastically reduced the seropositive rates in their children.

Sexual transmission. Wives with seropositive husbands are usually seropositive. Conversely, the husbands of seropositive wives show the same frequency of seropositivity as those in the region under study, indicating transmission occurs from husband to wife but not vice versa. Infected T cells in semen are thought to mediate transmission. This pathway may explain the higher (1.6 times) prevalence in females than in males, and is in sharp contrast to HIV infection. Infection in adulthood is not thought to be linked to subsequent ATL.

Blood transfusion. Transfusion of fresh and total blood transmits HTLV-1 into 60–70% of the recipients. The transfer of infected cells is critical for transmission. This mode of transmission does not lead to ATL, but does to HAM/TSP.

Zoonotic infections with retroviruses endemic in most Old World primates are described in people living in central African forests who have had contact with blood and body fluids of wild nonhuman primates. Such interspecies infection may explain the unique similarities between HTLVs and STLVs.

Genetics and Replication

Genome

The virion is spherical, enveloped with a membrane similar to that of the host cell and contains RNA genomes, core proteins, and reverse transcriptase, and envelope

glycoproteins on the surface (**Figure 1**). The RNA genome is copied into a 'proviral' DNA upon infection and analysis of the retroviral genome is generally performed with the integrated proviral genome.

The integrated HTLV-1 proviral genome is 9032 bp long and its organization is LTR-*gag*-*pol*-*env*-pX-LTR. The pX sequence contains multiple overlapping genes encoding for the regulatory factors for the viral replication, but none of them has any homology to cellular genes. The major regulatory factors are $p40^{tax}$ and $p27^{rex}$. Alternative splicing of the pX sequence also expresses various proteins such as p12(I), p10(I), p11(V).

Antisense viral transcripts from the pX region code for HBZ proteins (HTLV-1 bZIP with a leucine zipper motif), which represent additional regulatory proteins, and also function at the RNA level.

Replication with Feedback Regulation

Reverse transcription and integration

Upon infection, the viral RNA genome is reverse-transcribed into complementary DNA (cDNA) by viral reverse transcriptase in the virion and further copied into double-stranded DNA, defined as 'proviral DNA'. The proviral DNA is integrated into the host cell DNA establishing the viral infection.

Transcription and splicing

RNA transcription from the 5′ LTR to 3′ LTR generates the viral genomic RNA, and this step represents a potential regulatory stage of the viral replication cycle. HTLV genomic RNA serves as an mRNA for Gag and Pol proteins, but has to be spliced to express other viral proteins: into a 4.2 kbp (singly spliced) env mRNA for Env expression, and into a 2.1 kbp (doubly spliced) mRNA for the regulatory proteins, Tax and Rex (**Figure 1**). Various alternative splicing events also take place to express alternative proteins.

Genomic transcription depends on cellular factors that respond to 21 bp enhancers in the HTLV LTR, but initial transcriptional activity is weak. The low levels of viral transcripts are fully spliced into Tax/Rex mRNA. The Tax thus produced *trans*-activates transcription further enhancing viral transcription to produce more Tax/Rex mRNA. This *trans*-activation is mediated by Tax binding to a transcriptional factor, cAMP-responsive element binding protein (CREB) that responds to the 21 bp enhancers in the

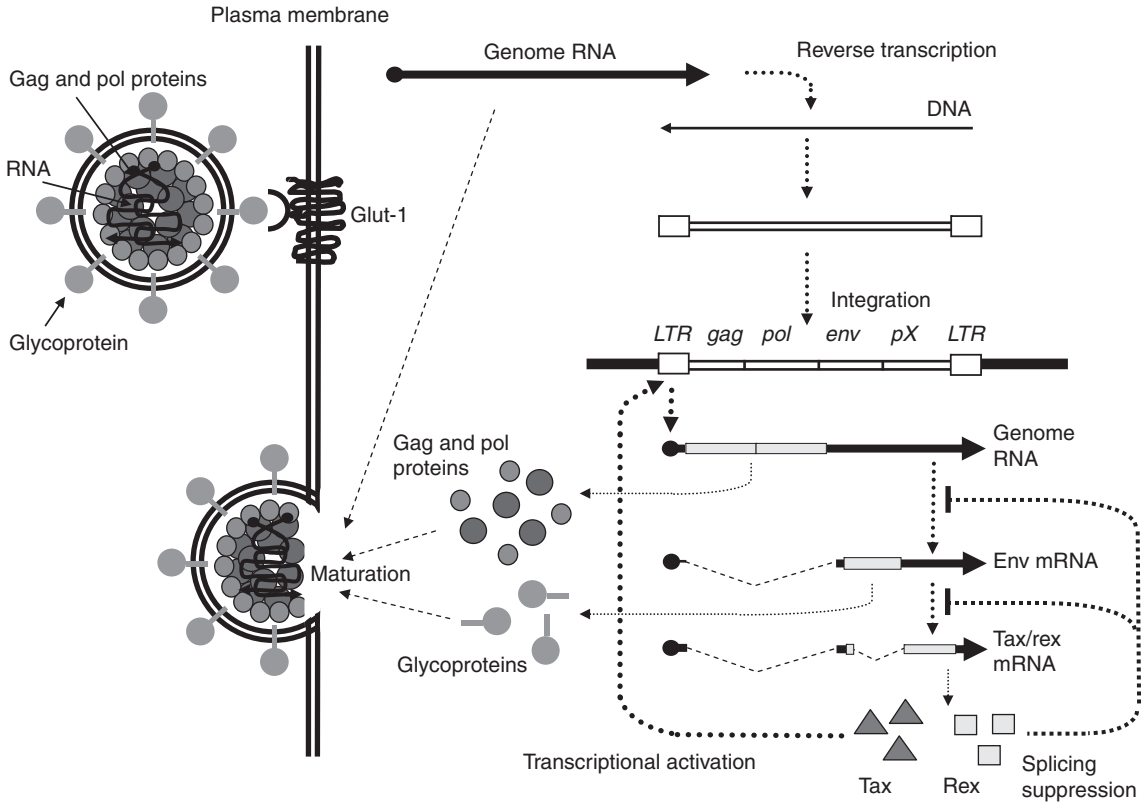


Figure 1 Replication cycle of HTLV. HTLV-1 binds to glucose transporter (Glut-1) and the genomic RNA is transcribed into complementary DNA by reverse transcriptase. The circularized proviral DNA is finally integrated into host chromosomal DNA establishing infection. The integrated provirus is transcribed into genomic RNA and expression of the viral proteins is regulated by Tax and Rex at transcriptional and splicing level making the expression transient. Genomic RNA and virion proteins mature at specific sites under plasma membrane and bud as the matured particles.

LTR. To express the virion proteins, Gag, Pol, and Env, the accumulated Rex protein specifically suppresses splicing of the viral RNA, and thus upregulates expression of unspliced genomic RNA and singly spliced *env* mRNA. In return, this Rex regulation reduces the level of spliced Tax/Rex mRNA, resulting in a lower level of Tax and ultimately reducing viral transcription. The combination of Tax and Rex functions exerts a feedback control on viral expression, making viral expression transient and resulting in the escape of infected cells from host immune surveillance. This feedback mechanism is unique to HTLV among oncogenic retroviruses and explains why HTLV is so repressed in expression and replication.

HTLV replication has also been reported to be regulated by small proteins such as p12(I), p10(I), p11(V), that are expressed by alternative splicing of the pX sequence. The regulatory mechanisms are not well understood but seem to be important for *in vivo* viral replication.

Maturation and budding

Genomic and *env* RNAs are efficiently accumulated by Rex and thus virion proteins, Gag, Pol, and Env, are transiently expressed. These virion proteins and genomic RNA come together and assemble at specific sites under the plasma membrane, finally budding by being enveloped with cellular membrane. The molecular mechanisms of these processes are not well characterized.

Variability and Evolution

The retroviral genome is rather labile in general since the reverse transcription process has no proofreading mechanism. In sharp contrast to HIV for example, however, the HTLV genome is highly stable and conserved among isolates from Japan, the Caribbean, and Africa. The viral isolates from Papua New Guinea may vary somewhat more but are still 90–95% homologous. This stable property is a reflection of an HTLV survival strategy that replicates the viral genome through infected cell replication rather than virus replication.

It has been suggested that HTLV and STLV are transmitted across species. Each of the isolates from various monkeys (STLV-1, -2, and -3) and humans (HTLV-1 and -2) are highly homologous and their variability is sometimes higher within the species than among species. Such unusual genomic conservation under different conditions is also well explained by a viral survival strategy through proliferation of infected cells.

Pathogenicity

Adult T-Cell Leukemia

Clinical features

Symptoms of ATL are variable and frequently complicated by skin lesions, enlargement of lymph node, liver and/or spleen, and infiltration of leukemic cells into various organs. Patients usually have antibodies to HTLV-1 proteins, show an increased level of serum lactate dehydrogenase (LDH), and most suffer from hypercalcemia.

The onset of ATL is observed in individuals between 20 and 70 years of age, the highest frequency being observed in people in their 40s and 50s. The male/female ratio of ATL incidence is 1.4/1.

Smoldering, chronic, acute forms of ATL and a lymphoma type have been recognized. Patients with smoldering ATL have a few or several percent of morphologically abnormal T cells in their peripheral blood, but do not show signs of severe illness for a long period. Patients with chronic ATL have rather high levels of HTLV-1-infected leukemic cells, but can maintain stable phenotypes for a certain period of time. Acute ATL is aggressive and resistant to any treatment; consequently, most patients die within one-half year of its onset.

Leukemic cells are exclusively CD4+ T cells with usually a highly lobulated nucleus. Leukemic cells generally have one complete integrated copy of an HTLV-1 provirus in their genome. Less frequently two copies are found, sometimes with defective forms. The site of integration is clonal in a given ATL patient, but different among patients. Leukemic cells carry aberrant chromosomes, frequently with multiple abnormalities, and express a high level of IL-2Ralpha, PTHrP, IL-1alpha, but no common abnormality has been described.

Etiology of ATL

An etiologic linkage of HTLV-1 with ATL is apparent from the observations: (1) ATL and HTLV-1 show identical geographic distribution, (2) almost all ATL patients are infected with HTLV-1, (3) leukemic cells are all infected with HTLV-1 but a vast majority of normal T cells are not, (4) leukemic cells are clonally integrated with an HTLV-1 provirus indicating their origin from a single infected cell, and (5) infection by HTLV-1 can immortalize T cells *in vitro* and the phenotypes of immortalized T cells are similar to those of leukemic cells.

There are approximately 1 million carriers of HTLV-1 in Japan. About 2–5% of all carriers of HTLV-1 are thought to develop ATL during their lifetime. While the vast majority of cases of ATL are associated with HTLV-1 infection, a form of ATL 'unrelated to HTLV-1 infection' has been described. The etiologic factor of these cases has not been identified.

Molecular mechanism of leukemogenesis

ATL cells have clonally integrated HTLV-1 proviruses; however, no common site for integration was observed among ATL patients. In this respect, HTLV-1 differs from animal chronic leukemia viruses, wherein integration was commonly adjacent to a proto-oncogene for its activation. Thus, the Tax protein has been focused on as a critical transforming protein.

Tax protein

Tax is able to transform fibroblasts and immortalize CD4+ T cells *in vitro*, and can induce tumors in transgenic mice. Tax exerts pleiotropic effects including (1) transcriptional activation of specific genes, (2) transcriptional repression of some other specific genes, (3) functional inactivation of tumor suppressor proteins, and (4) attenuation of cell-cycle checkpoints (**Table 1**). Cooperation of these pleiotropic functions is thought to contribute to ATL induction.

Transcriptional activation. Tax activates HTLV-1 genome transcription, which in turn is regulated by an enhancer binding protein, cyclic AMP-responsive element binding (CREB) protein. CREB has to be phosphorylated for active transcription, while Tax binds to CREB and activates it without a phosphorylation signal leading to a constitutively active protein. Similarly, Tax binds to other enhancer binding proteins such as nuclear factor kappa B (NF-κB) and serum responsive factor (SRF) and activates many specific cellular genes including IL-2Ralpha, IL-6, c-fos, and Bcl-x. NF-κB is alternatively activated by Tax through activation of IκB kinase (IKK) which disrupts inactive IκB–NF-κB complexes. The genes finally activated are linked to enhancement of cell proliferation or suppression of apoptosis.

Transcriptional repression. Tax also binds to transcription factors, CBP and P300, which interact with various enhancer binding proteins. The binding of Tax to CBP or p300 interferes with their interaction with the corresponding enhancer binding protein unless Tax is able to bind to enhancer binding protein, and consequently inhibits formation of a transcriptional initiation complex. The targets include p53-dependent transcription, DNA polymerase beta, p18ink4, Bax, and many others. The genes eventually repressed are linked to downregulation of p53-dependent stress responses, DNA repair and apoptosis.

Functional inactivation of tumor suppressor proteins. Tax directly binds to or modifies some tumor suppressor proteins and inactivates their negative regulation of the cell cycle. RB, APC, and p53 are targets. (a) Tax binds to and inactivates p16ink4 and p15ink4, which normally inhibit CDK4 and maintain an active RB pathway; consequently, Tax activates CDK4 which results in inactivation of RB and promotes cells to move from G1 arrest into S phase. Tax is also reported to bind directly to CDK4 to activate kinase activity. (b) Tax also binds to another tumor suppressor protein, hDlg, through its PDZ domain and inactivates its growth-retarding signal through APC and β-catenin. An abnormality in this pathway has been shown to play a critical role in colorectal tumors. (c) Tax inactivates p53 through phosphorylation and complex formation of p53–p300. Inactivation of the p53 pathway implies suppression of DNA repair resulting in the frequent fixation of mutations.

Attenuation of cell-cycle checkpoints. Check and review mechanisms for genomic processes before entering the next phase of the cell cycle is important to avoid genetic fixation of mutations. Tax interacts with hMad-1 and Chek-1 and attenuates the S- and G2-checkpoint functions. Tax also binds to Ran and Ran-binding protein and induces centrosome fragmentation and aneuploidy. These properties would explain a higher mutation rate in Tax-positive cells and may be the basis for why ATL cells have highly frequent choromosomal abnormalities.

Collectively, it has been proposed that these pleiotropic effects of Tax cooperate for abnormal cell proliferation. Significance of the Tax roles are twofold: (1) enhancing proviral replication and (2) promoting cells into leukemogenesis. Infected T cells are abnormally stimulated to proliferate through transcriptional activation/repression and inactivation of tumor suppressor proteins.

Table 1 Typical examples of pleiotropic Tax effects on cellular activities

Category/primary target	*Targeted gene or process*	*Targeted cellular activity*
Transcriptional activation		
CREB–CBP	HTLV-1 provirus	Viral replication/activation
	Unidentified	Transformation/activation
NF-κB-p300 and IKK-IκB	IL-2Ralpha, IL-6	Proliferation/activation
	Bcl-X	Apoptosis/suppression
SRF-CBP	c-Fos	Transformation/activation
Transcriptional repression		
p300–p53	p53-dependent transcription	Stress response/Suppression
	p18ink4	Proliferation/activation
CBP	DNA polymerase beta	DNA repair/suppression
	Bax	Apoptosis/suppression
Tumor suppressor protein		
p16ink4-CDK4	RB signaling	Cell cycle/promotion
p15ink4-CDK4		
hDLG-APC	APC signaling	Proliferation/activation
Kinase-p53	p53 signaling	Stress response/suppression
Checkpoint		
hMad-1	G2 checkpoint	Fidelity/suppression
Check-1	S, G2 checkpoint	Fidelity/suppression

Concomitantly, attenuation of cell-cycle checkpoints, reduction of DNA repair, and apoptosis would result in more mutations and their fixation, eventually leading to the leukemogenesis. In this respect, the pleiotropic effects of Tax may be equivalent to a multistep process for tumor formation.

Paradox in the leukemogenic mechanism

Tax plays a central role in the induction of ATL. Even its functional similarity to transforming proteins of DNA tumor viruses has been pointed out. However, it remains to be answered: (1) Why HTLV-1 transformation is selective for CD4+ T cells? and (2) Why ATL cells *in vivo* maintain tumor phenotypes in the absence of Tax? Tax/Rex mRNA is expressed only in a small percentage of tumor cells even using highly sensitive RT-PCR assays, yet tumor phenotypes are maintained in all ATL cells. The simplest answer for the latter question may be that Tax was critical for induction of tumors, but after establishment of tumors Tax is no longer required. If this is the case, what is the mechanism to maintain the tumor phenotypes?

An antisense gene, HTLV basic leucine zipper (HBZ) protein, may account for the paradox. This gene is transcribed from the pX region and codes for a DNA-binding protein with a bZIP domain. HBZ is able to moderately enhance T-cell proliferation *in vitro* and counteracts Tax *trans*-activation through dimer formation with other bZIP proteins. The antisense transcripts also operate at the RNA level. An exciting aspect of HBZ is that the antisense transcript is expressed in almost all ATL cases tested so far. It is therefore proposed to play a role after Tax *in vivo*. Tax is a potent antigenic protein; thus, its downregulation may make sense for tumor cell to escape from immune responses.

HAM/TSP and other diseases

Clinical features

HTLV-1 also induces a slowly progressive myelopathy known in tropical zones as tropical spastic paraparesis (TSP) and, in endemic areas of Japan, as HTLV-1-associated myelopathy (HAM). The unique phenotypes of HAM/TSP are chronic, symmetrical, bilateral involvement of the pyramidal tracts, at mainly the thoracic level of the spinal cord, and include progressive spastic paresis with spastic bladder and minimal sensory deficits. HTLV-1-infected T cells infiltrate into the spinal fluid and cord.

Most patients with HAM/TSP have much higher titers of HTLV-1 antibodies than those of asymptomatic carriers or ATL patients. This might be associated with particular types of human leukocyte antigens (HLAs). Despite their strong immunological responses to HTLV-1 infection, most HAM/TSP patients have larger populations of infected cells than do HTLV-1 carriers.

Etiology and other features

In endemic Japanese, all HAM/TSP patients are infected with HTLV-1. After screening for seropositive blood, the incidence of HAM/TSP has greatly decreased, clearly indicating that HTLV-1 is an etiologic agent of HAM/TSP. In contrast, TSP patients in the tropical areas are not always infected with HTLV-1, but the etiology in these cases is unknown. Indirect immunological reaction has been proposed as a pathogenic mechanism, but further studies are required.

The two HTLV-1-associated diseases, HAM/TSP and ATL, are mutually exclusive. The reason for this phenomenon is not well understood, but the route of primary viral infection may affect the pathogenic course: mucosal exposure to HTLV-1 for ATL, while primary infection of peripheral blood for HAM/TSP.

HTLV-1 infection is also proposed to be associated with some other diseases including uveitis, chronic lung disease, monoclonal gammopathy, and rheumatoid arthritis, but further systematic studies are required.

Prevention and Control of Infection

Transfusion of seropositive blood transmits HTLV-1 to two-thirds of the recipients. With the introduction of HTLV-1 screening systems in blood banks, viral transmission through transfusion has been greatly reduced. Application of these systems to populations in all endemic areas is critical to prevent HTLV-1 infection.

The major, natural route of viral transmission is from mother to child via infected T cells in breast milk. In Nagasaki City, Japan, pregnant women are tested for HTLV-1 antibodies by consent and those who are seropositive are recommended to avoid breastfeeding. A trial of this approach resulted in a drastic reduction in the incidence of seropositive children, from about 30% to just a few percent. The early success of this trial provides direct evidence for viral transmission through milk and suggests the possibility of eliminating ATL in the next few generations. Unfortunately, not all of children of seropositive mothers who did not breastfeed remained seronegative.

Future

Studies on HTLV-1 infection linked to ATL and HAM/TSP have progressed, but several very basic questions are still not answered: Why does only a small fraction of the infected population develop ATL or HAM/TSP? Why are ATL and HAM/TSP mutually exclusive? Why are only CD4+ T cells transformed into leukemic cells? What is required for ATL induction in addition to Tax? On the other hand, HTLV-1 transmission is now preventable in certain countries. However, it may not be

feasible everywhere in the world. For example, cessation of breastfeeding might result in more serious problems in children in certain environments. So, original questions should be asked: Is a vaccine possible for complete eradication of the HTLV-1? Is vaccination possible for preventing disease development after infection?

Modern technologies developed in conjunction with genome research are now becoming available and make it possible to revisit these questions utilizing new appraoches.

See also: Human Immunodeficiency Viruses: Origin; Human Immunodeficiency Viruses: Pathogenesis; Human T-Cell Leukemia Viruses: Human Disease.

Further Reading

Gallo RC (2002) Human retroviruses after 20 years: A perspective from the past and prospects for their future control. *Immunological Reviews* 185: 236–265.

Matsuoka M and Jeang KT (2005) Human T-cell leukemia virus type I at age 25: A progress report. *Cancer Research* 65: 4467–4470.

Yoshida M (2001) Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annual Review of Immunology* 19: 475–496.

Yoshida M (2005) Discovery of HTLV-1, the first human retrovirus, its unique regulatory mechanisms and insights into pathogenesis. *Oncogene* 24: 5931–5937.

Human T-Cell Leukemia Viruses: Human Disease

R Mahieux and A Gessain, Pasteur Institute, CNRS URA 3015, Paris, France

© 2008 Elsevier Ltd. All rights reserved.

Introduction

In 1980, Dr. Gallo's laboratory (National Institutes of Health, USA) reported the isolation of HTLV-1, the first oncoretrovirus to be discovered in humans. HTLV-1 was present in the peripheral blood cells obtained from an Afro-American patient suffering from a lymphoproliferative disease, originally considered as a cutaneous T-cell lymphoma, with a leukemic phase. The virus was thus named human T-cell leukemia/lymphoma virus (HTLV). Later, it was recognized that this cutaneous lymphoma was in fact an adult T-cell leukemia/lymphoma (ATLL), a severe T-cell lymphoproliferation, originally described in Japan in 1977 by Takatsuki. The epidemiological characteristics of ATLL in Japan suggested a strong environmental factor, which prompted researchers to characterize the tumor cells and to search for an oncogenic virus. In 1981, a virus was isolated in Japan and termed adult T-cell leukemia/lymphoma virus (ATLV). Japanese and American scientists rapidly demonstrated that both isolates represented the same virus, and agreed to name it HTLV-1. In parallel, the causal association between ATLL and HTLV-1 was established. In 1983, the authors initiated a series of studies in the French West Indies in order to investigate the epidemiological and clinical impact of HTLV-1 in this area. This led them to demonstrate the etiological association between this virus and a chronic neuromyelopathy originally named tropical spastic paraparesis (TSP) that is endemic in the Caribbean. A similar neurological entity was then uncovered in Japan and labeled as HTLV-1-associated myelopathy. These two diseases were further shown to be identical and this myelopathy is now referred to as HAM/TSP. HTLV-1 infection has also been associated with other clinical conditions including uveitis, infective dermatitis, and myositis (**Table 1**).

HTLV-1, which is not a ubiquitous virus, is present throughout the world, with clusters of high endemicity located often nearby areas where the virus is nearly absent. These highly endemic areas are the southwestern part of the Japanese archipelago; the Caribbean area and its surrounding regions; foci in South America including Colombia, French Guyana, parts of Brazil; some areas of intertropical Africa (such as South Gabon); and of the middle East (such as the Mashad region in Iran); and isolated clusters in Melanesia. The origin of this puzzling geographical or rather ethnic repartition is not well understood but is probably linked to a founder effect in certain ethnic groups, followed by the persistence of a high viral transmission rate due to favorable local environmental and cultural situations. Interestingly, in all the highly endemic areas, and despite different socioeconomic and cultural environments, HTLV-1 seroprevalence increases gradually with age, especially among women. This might be either due to an accumulation of sexual exposures with age, or due to a cohort effect.

The worldwide infected population is estimated around 15–20 million. Two to ten percent of infected persons will develop an HTLV-1-associated disease (ATLL, HAM/TSP, uveitis, infective dermatitis, etc.) during their life (**Table 1**). Three modes of transmission have been demonstrated for HTLV-1: (1) Mother to child transmission, which is mainly linked to prolonged breastfeeding after 6 months of age. Ten to twenty-five percent of the

Table 1 Diseases associated with HTLV-I infection

Adult disease	*Association*
Adult T-cell leukemia/lymphoma	++++
Tropical spastic paraparesis/HTLV-I-associated myelopathy	++++
Intermediate uveitis	+++
Infective dermatitis	+++
Myositis (polymyositis and SIBM)	+++
HTLV-I-associated arthritis	++
Pulmonary infiltrative pneumonitis	++
Invasive cervical cancer	+
Small cell carcinoma of lung	+
Sjögren disease	+
Childhood	*Association*
Infective dermatitis	++++
Tropical spastic paraparesis/HTLV-I-associated myelopathy (very rare)	++++
Adult T-cell leukemia/lymphoma (very rare)	++++
Persistent lymphadenopathy	++

The strength of association is based on epidemiological studies as well as molecular data, animal models, and intervention trials. ++++, proven association; +++, probable association; ++, likely association; +, possible association.
SIBM: sporadic inclusion body myositis.

breast-fed children born from HTLV-1 infected mothers will become persistently infected. (2) Sexual transmission, which mainly occurs from male to female, and is thought to be responsible for the increased seroprevalence with age in women. (3) Transmission with contaminated blood products (containing infected lymphocytes), which is responsible for an acquired HTLV-1 infection among 15–60% of the blood recipients.

From a molecular point of view, HTLV-1 possesses remarkable genetic stability, an unusual feature for a retrovirus. Viral amplification via clonal expansion of infected cells, rather than by reverse transcription, could explain this striking genetic stability, which can be used as a molecular tool to follow the migrations of infected populations in the recent or distant past and thus to gain new insights into the origin, evolution, and modes of dissemination of such retroviruses and their hosts. The few nucleotide substitutions observed among virus strains are indeed specific to the geographic origin of the patients rather than the pathology. Four major geographic subtypes (genotypes) have been reported. The origin of most of these geographic HTLV-1 subtypes appears to be linked to episodes of interspecies transmission between STLV-1-infected monkeys and humans, followed by variable period of evolution in the human host.

Adult T-Cell Leukemia/Lymphoma

Epidemiological Aspects

After the initial discovery and characterization of ATLL in Japan, the disease was reported in the USA and in Caribbean immigrants living in the United Kingdom. ATLL cases have now been reported in all HTLV-1 endemic areas including intertropical Africa, South and Central America, Iran, and Melanesia. Sporadic cases were also described in areas of low HTLV-1 endemicity, often in immigrant patients originating from regions where HTLV-1 is endemic. In Japan, the ATLL incidence rate shows a steep increase with age, the mean age of disease onset being 57 years old and the sex ratio (male/female) being 1.4. In Japan, the annual incidence of ATLL cases is approximately 700, while the number of HTLV-1 carriers reaches 1.2 million. This gives an estimated yearly incidence of ATLL of 0.6–1.5 for 1000 HTLV-1 carriers older than 40. Lastly, the additive life-time risk of developing ATLL was estimated to be 1–5 % among Japanese HTLV-1 carriers. Studies performed in Brazil, Gabon, and French Guyana concluded that ATLL prevalence is usually underestimated until a specific disease research is performed. This is mostly due to the severity of the disease, its rapid evolution, and to confusion between ATLL and similar diseases, such as Sézary's syndrome, Mycosis fungoides, or other T cell non-Hodgkin lymphomas. In addition, laboratory tests such as Western blot and molecular investigations are not easily available in most tropical countries.

Diagnosis Criteria and Classification

Because of the extensive diversity in the clinical presentation and evolution of the disease, Japanese clinicians and researchers have defined stringent diagnosis criteria for ATLL as well as a classification into four major subtypes (**Table 2**). These diagnostic criteria are the following: (1) Histologically and/or cytologically proven lymphoid malignancy with expression of specific T-cell surface antigens (mostly CD2+, CD3+, and CD4+). (2) Abnormal T lymphocytes (flower cells and small/mature T lymphocytes with incised or lobulated nuclei) present in the peripheral blood, except in the ATLL lymphoma type. (3) Antibodies against HTLV-1 antigens present in the patient's serum at diagnosis.

The four major subtypes, as defined by the Japanese lymphoma study group, are the smoldering, the chronic, the lymphoma, and the leukemic/acute form. Both chronic and smoldering types can progress toward the acute or lymphoma forms. This classification was proved to be useful to discriminate ATLL from other type of leukemias/lymphomas. Patients who develop the smoldering form and about 30% of those suffering of the chronic ATLL have a fairly 'good prognosis' as compared to those with a leukemic or lymphoma type whose survival median does not exceed 6–9 months (**Table 2**). Finally, criteria for determining the diagnosis of ATLL during epidemiological studies have also been proposed (**Table 3**).

Table 2 Diagnostic criteria for clinical subtype of HTLV-I associated ATLL

	Smouldering	*Chronic*	*Lymphoma*	*Acute*
Anti-HTLV-I antibody	+	+	+	+
Lymphocyte ($\times 10^3/\mu l$)	< 4	≥ 4[a]	< 4	*
Abnormal T lymphocytes	$\geq 5\%$[c]	+[b]	<1%	+[b]
Flower cells of T cell marker	#	#	No	+
LDH	$\leq$1.5N	$\leq$2N	*	*
Corrected Ca (mEq/liter)	< 5.5	<5.5	*	*
Histology-proven lymphadenopathy	No	*	+	*
Tumor lesion				
Skin and/or lung	*[c]	*	*	*
Lymph node	No	*	Yes	*
Liver	No	*	*	*
Spleen	No	*	*	*
Central nervous system	No	No	*	*
Bone	No	No	*	*
Ascites	No	No	*	*
Pleural effusion	No	No	*	*
Gastrointestinal tract	No	No	*	*

[a]Accompanied by T lymphocytosis ($3.5 \times 10^3/\mu l$ or more).
[b]If abnormal T lymphocytes are less than 5% in peripheral blood, histology-proven tumor lesion is required.
[c]Histology-proven skin and/or pulmonary lesion(s) is required if abnormal T lymphocytes are less than 5% in peripheral blood.
*no essential qualification except terms required for other subtype(s).
N: normal upper limit.
#: typical flower cells seen occasionally.
Adapted from Shimoyama M and The Lymphoma Study Group (1991) Diagnostic critreria and classification of clinical subtypes of adult T-cell leukemia-lymphoma. *British Journal of Haematology* 79: 428–437.

Clinical, Cytological, and Immuno-Virological Features

The predominant clinical findings of ATLL at onset are lymphadenopathy, hepatomegaly, splenomegaly, specific skin lesions, and hypercalcemia (**Table 4**). The symptoms may include abdominal pain, diarrhea, pleural effusion, ascites, and cough. White blood count ranges from normal up to a very high number of abnormal peripheral blood lymphocytes, especially in acute and chronic ATLL patients, while anemia, neutropenia, or thrombocytopenia are occasionally observed. ATLL cells differ in size and characteristics, depending on the subtype. As an example, at the typical acute/leukemic stage, most abnormal cells exhibit multilobulated nuclei and are named 'flower cells' (**Figure 1**). At the terminal stage, cells often significantly vary in size. They display a cytoplasmic basophilia and a marked nuclear lobulation. Chronic ATLL cells are generally small and of uniform shape with minor nuclear abnormalities such as indentation or convolutions. Smoldering ATLL cells are often relatively large with a bi- or trifoliate nucleus.

Most ATLL cells are mature T cells of helper/inducer phenotype (CD2+, CD3+, CD4+, CD7–, CD8–) and express activation markers (CD25+, HLA DP+, DQ+, DR+). CD4– and CD8– ATLL cells are uncommon, but a decrease of the CD3/T cell receptor expression is frequent. Changes in cell activation marker expression (CD4+CD8– to CD4+CD8+) have been observed in some patients during the course of the disease. Lymph-node histology analysis frequently demonstrates infiltration by medium and large T cells with irregular nuclei effacing the nodal architecture. This is consistent with the diagnosis of pleiomorphic large T-cell lymphoma. However, there is no specific histological pattern for ATLL.

Cytogenetic abnormalities are not specific for ATLL, but are frequently reported in acute and lymphoma patients. They include chromosome 14 translocations (14q 32, 14q 11) and 6q deletions. Trisomy 3, 7, and 21 as well as X chromosome monosomy or the loss of the Y chromosome were also reported. Mutations in the p53 encoding tumor-suppressor gene are detected in 20–30% of all patients, mostly those in advanced stages, suggesting the involvement of p53 mutation in a late phase of leukemogenesis, or as a consequence of the cellular transformation.

Detecting (by Southern blot) the clonal integration of HTLV-1 provirus(es) in the tumor cells represents the gold-standard for establishing that a patient suffers from ATLL (**Figure 2**). In most cases, only one copy of the provirus is integrated in the tumor cells. Nevertheless, two or more proviruses can infrequently be detected (5–20% of all ATLL cases). It is also worth noting that 5–20% of ATLL patients carry defective HTLV-1 proviruses. The 3′pX region is commonly conserved, while deletions occur frequently in *gag*, *pol*, and/or *env* open reading frames. Inverse-PCR, a technique that is more sensitive

Table 3 Registry criteria for definition of ATLL

Definition of ATLL	
Clinical/routine laboratory criteria	
Hypercalcemia	1 point
Skin lesions[a]	1 point
Leukemic phase[b]	1 point
Research laboratory criteria	
T-cell lymphoma or leukemia	2 points
HTLV-I antibody	2 points
TAC-positive tumor cells	1 point
HTLV-I-positive tumors[c]	2 points
ATLL classification	
Classical	≥7 points
Probable	5 or 6 points
Possible	3 or 4 points
Inconsistent with ATLL	<3 points
Exclusion criteria	
B-cell positivity, nodular or follicular lymphoma, lymphoblastic lymphoma, small lymphocytic lymphoma	

[a]Lymphomatous cells documented morphologically.
[b]More than 2% abnormal lymphocytes.
[c]Determined by PCR or Southern blot analysis of the DNA of tumoral cells and indicating a monoclonal integration of HTLV-1 provirus(es).
Adapted from Levine PH, Cleghorn F, Manns A, *et al.* (1994) Adult T-cell leukemia/lymphoma: A working print-score classification for epidemiological studies. *International Journal of Cancer* 59: 491–493.

Table 4 Main clinical features of ATLL

	Japan[a]	*Caribbean*[b]
Age at onset	58 years (range 27–82)	47 years
Sex ratio male/female	1.4	0.6
Lymphadenopathy	60%	70%
Hepatomegaly	26%	27%
Splenomegaly	22%	31%
Specific skin lesion	39%	41%
Hypercalcemia	32%	51%

[a]Based on a series of 187 Japanese patients and
[b]from a series of 57 patients, with 46 of Carribbean origin , seen in the United Kingdom.

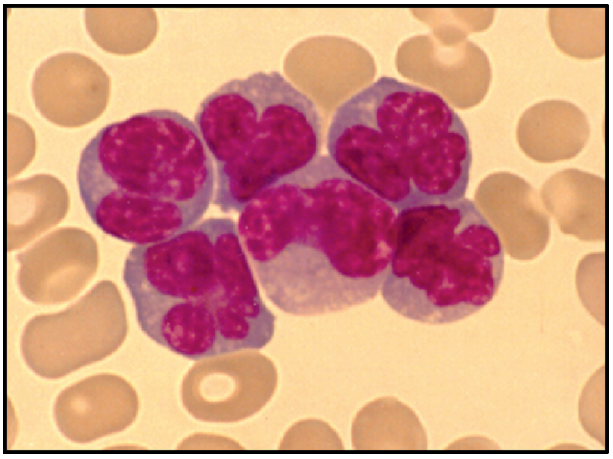


Figure 1 Peripheral blood smear from a Caribbean ATLL leukemic patient showing a cluster of atypical lymphoid cells with multilobulated nuclei (May-Grunwald-Giemsa staining).

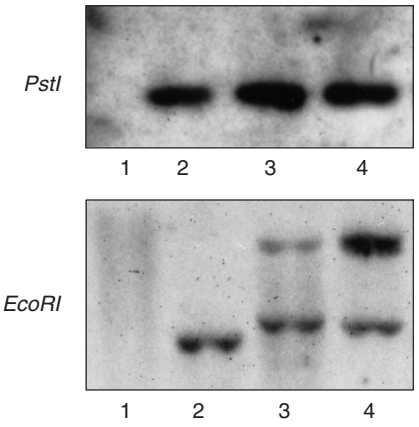


Figure 2 HTLV-1proviral integration as detected by Southern blot analysis. High molecular weight DNA was extracted from (1) negative control peripheral blood mononuclear cells and (2, 3, 4) from peripheral leukemic cells obtained from three different HTLV-1 patients diagnosed with ATLL. Because *EcoRI* restriction sites are absent from HTLV-1 provirus sequence, the observation of two bands (lane 3 and 4) demonstrates the clonal integration of two proviruses in the DNA of the leukemic cells.

than Southern blot is currently used for diagnosis of the monoclonal integration of HTLV-1 provirus(es) in the tumoral cells. Using this technique, oligoclonal or polyclonal proliferation of HTLV-1 infected T cell can also be detected in the peripheral blood lymphocytes of most HAM/TSP patients as well as in healthy HTLV-1 seropositive carriers. This also allows the precise identification of integration sites, the identification of each infected clone, and the tracing of the kinetics of the infected cells *in vivo*. The detection of persistent HTLV-1 infected monoclonal cell population could therefore be used as a method for monitoring individuals at risk for developing ATLL. Importantly, monoclonal expansion of HTLV-1 infected cells in carriers is directly associated with the onset of ATLL.

Specific Features and Complications

Hypercalcemia is very common among ATLL patients. It is detected in 20–30 % of the patients at admission and in more than 70 % of them during the entire clinical course, with or without lytic bone lesions. *In vitro*, ATLL cells induce the differentiation of hematopoietic precursor cells to osteoclasts through the expression of the RANKL (receptor activator nuclear factor kB) ligand on their surface, in cooperation with elevated M-CSF (mononuclear

phagocyte colony-stimulating factor) serum levels. This accelerates bone resorption and ultimately causes hypercalcemia. The degree of hypercalcemia might also be linked to the expression of the parathyroid hormone-related protein (PTHrP), since PTHrP also induces RANK ligand expression on osteoblasts.

Skin infiltration of a clonal population of HTLV-1 tumor cells represents a frequent ATLL clinical feature. It is present in 20–40% of all ATLL patients and in more than 50% of smoldering ATLL patients. Various cutaneous lesions have been described including papules, nodules, erythroderma, plaques, tumors, and ulcerative lesions. ATLL cells densely infiltrate both dermis and epidermis forming Pautrier's micro-abscesses. When skin lesions dominate the clinical picture, the disease is referred to as 'cutaneous ATLL' (**Figure 3**). In such cases, establishing the difference between these lesions and other cutaneous T-cell lymphomas (CTCLs) is complicated. A possible role of CCR4 (CC chemokine receptor 4) in skin invasion by ATLL cells has been suggested. Other factors such as expression of cutaneous lymphocyte antigen (CLA) by leukemic cells and inflammatory responses in injured skin that would lead to upregulation of thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC) are also likely to contribute to skin involvement of ATLL.

ATLL is also known to frequently invade the gastrointestinal tract, even though the exact incidence has not been determined yet. CCR9 is known to be involved in T-cell homing to the gastrointestinal tract. A recent study demonstrated that CCR9 is expressed by HTLV-1 T cells and ATLL cells expressing Tax and suggested that it may play a role in the gastrointestinal involvement of ATLL.

The frequency of opportunistic infections is quite high in ATLL patients, indicating that T-cell-mediated immunity is severely impaired in such patients. Infestation by *Strongyloides stercoralis* (*S.s*) is frequent among HTLV-1 seropositive carriers and *S.s* infected individuals are often infected by HTLV-1 in highly endemic areas. Numerous studies showed the presence of *S.S* in acute or lymphoma ATLL, suggesting that infection by *S.S* might play a significant role as a candidate cofactor for HTLV-1 induced leukemogenesis.

Pulmonary complications are also frequent in ATLL patients. These are either leukemic infiltrate or opportunistic infections. Central nervous system localization rarely occurs in ATLL (10%).

ATLL Pathogenesis

The development of an ATLL has been clearly associated with an early acquired HTLV-1 infection. The risk of being infected for a child born from an HTLV-1 infected mother is positively correlated with prolonged (>6 months) breastfeeding. After a limited number of replication cycles using its reverse transcriptase during primary infection, HTLV-1 replicates and increases its copy number through the proliferation of infected cells, thus using the cellular DNA polymerase which possesses a proofreading activity. Such a model could explain, in part, the very high genetic stability of HTLV-1. The clonal proliferation of the HTLV-1-infected CD4+ lymphocytes is likely to be linked to the pleiotropic effects of the viral Tax protein. *In vitro*, Tax expression induces expression of several cellular cytokines such as IL-2, IL-15, but also alters the cell-cycle control machinery, inhibits apoptosis, and promotes genetic instability. As seen above, the fact that oligo/monoclonal-infected cell populations are present in the PBMCs of HTLV-1 carriers or of HAM/TSP patients suggests that neither

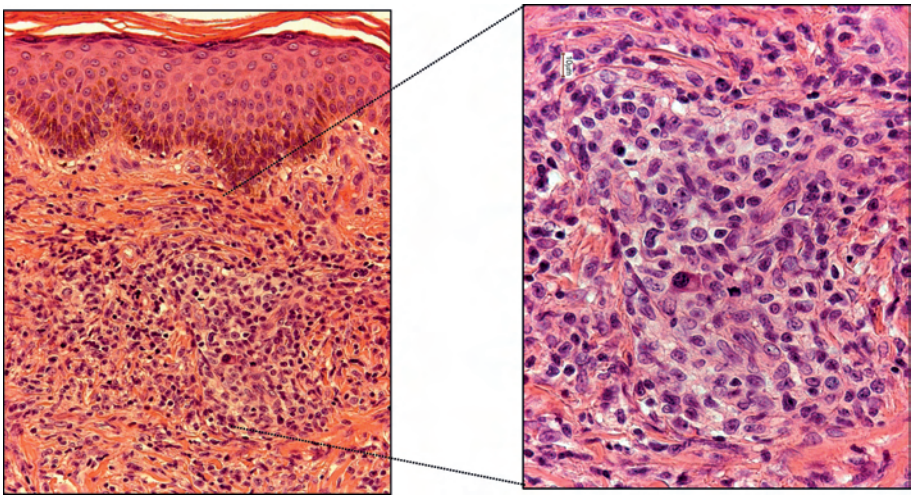


Figure 3 Histological analysis of skin invasion in an HTLV-1 patient from West Africa with a cutaneous ATLL showing an infiltration of the dermis by tumoral pleomorphic cells. These tumor cells are mature activated lymphoid T cells (CD2+, CD3+, CD4+, CD8–, and CD25+). Kindly provided by Dr. Michel Huerre, Institut Pasteur, Paris.

the monoclonal integration of the virus nor the CD4+ cell proliferation *per se* is sufficient to cause the disease. *In vivo*, the level of Tax expression is still debated. Several observations paradoxically suggested that HTLV-1 was transcriptionally silent *in vivo*, but it was shown lately that a high proportion (10%–80%) of naturally infected CD4+ peripheral blood mononuclear cells isolated from HAM/TSP patients or from HTLV-1 asymptomatic carriers are capable of expressing Tax *ex vivo*. It was also demonstrated that autologous $CD8^+$ T cells rapidly kill CD4+ cells that express Tax *in vitro* through a perforin-dependent mechanism. Altogether, these results suggest that virus-specific cytotoxic T lymphocytes (CTLs) participate in a highly efficient immune surveillance mechanism that tirelessly eradicates Tax-expressing HTLV-1-infected CD4+ T cells *in vivo*. Such CTL control of the HTLV-1-infected cell proliferation may also result in the prevention of ATLL. The role of the genetic background, especially HLA haplotypes, in such control could be crucial. A final step toward ATLL leukemogenesis consists in the accumulation of alterations in the host genome due to the pleiotropic effects of Tax (**Figure 4**).

Therapeutic Aspects of ATLL

The survival rate of ATLL patients, especially those who develop the acute leukemic or lymphoma forms, remains poor, and ATLL remains therefore one of the most severe lymphoproliferations. Treatment of ATLL patients using conventional chemotherapy (CHOP) has been and remains in most cases the standard first-line therapy. It has very limited benefit, since HTLV-1 cells are resistant to most apoptosis-inducing agents. A historical Japanese survey based on 818 ATLL cases reported a median survival time of 9 months, with a survival rate of only 27% and 10% at 2–4 years respectively. Survival is different when subtypes are considered into account, with a 4-year survival rate of 66% for the smoldering type, of 27% for the chronic type and of 5–6% for the lymphoma and acute types. Spontaneous regressions are observed in very few cases. The main prognosis factors associated with a poor response and a poor survival rate in ATLL patients are: a high LDH (lactate dehydrogenase) value, high leukemic counts (both of them reflecting a high tumor burden), hypercalcemia, and a poor clinical performance. Furthermore, the main obstacles to an efficient response to treatment are infectious complications (*pneumocystis carinii*, *cryptococcus meningitis*, disseminated herpes zoster), hypercalcemia, and liver or kidney dysfunction.

Various strategies other than CHOP have been used during clinical trials. This includes combination chemotherapy (CHOP plus methotrexate, CHOP in combination with etoposide, vindesine, ranimustine, mitoxantrone, or adriamycin), granulocyte macrophage-colony-stimulating factor (GM-CSF), supported combination chemotherapy, and other chemotherapy treatments that are highly toxic for bone marrow. Zidovudine (AZT) has been shown to inhibit HTLV-1 transmission *in vitro*. Several studies with AZT and interferon-alpha (IFN-α) have been conducted, both in the USA and in Europe (France and UK). They all demonstrated that response and survival in patients is more efficient when the drugs are used as first-line therapy. Until recently, the mechanism of action of AZT was a matter of debate. A recent study convincingly demonstrated that AZT can act as an inhibitor of the telomerase functions. More importantly, it was also shown that the p53 status

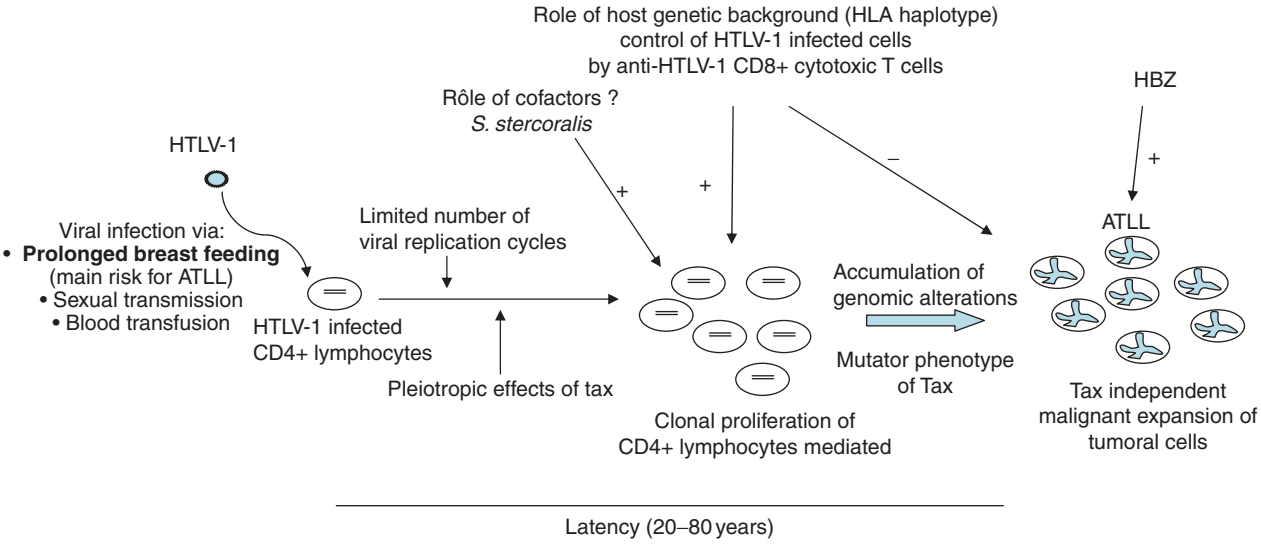


Figure 4 Pathogenesis of ATLL. Natural course from HTLV-1 primary infection by prolonged breast-feeding to the onset of the malignant proliferation of ATLL cells. HBZ: HTLV-1 bZIP factor. Adapted from Mahieux R and Gessain A (2003) HTLV-1 and associated adult T-cell leukemia/lymphoma. *Reviews in Clinical and Experimental Hematology* 7: 336–362.

(wild-type or mutated) is predictive of the response to AZT. Nuclear factor kappa B (NF-κB) inhibitor (Bay 11–7082) was found to be efficient for preventing the tumor growth in NOD-SCID (non-obese diabetic-severe combined immunodeficiency/gamma (null)) animals previously inoculated with HTLV-1 infected cells. Proteasome inhibitor PS-341 (Bortezomib) alone or combined with anti-CD25 (anti-Tac) therapy was partially successful in treating NOD/SCID animals or Tax transgenic mice. Allogenic bone marrow transplantation as well as allogenic hematopoietic stem cells transplantation have been performed on a limited number of patients. The estimated overall survival seems to compare favorably with historical data on chemotherapy, but definitive assessments are difficult to establish.

Tropical Spastic Paraparesis/HTLV-1 Associated Myelopathy

Spastic paraparesis without evidence of spinal cord compression has been described from various tropical and intertropical areas such as the Caribbean region, South and Central Africa, and India. Apart from cassava-related myelopathy and/or malnutrition in several African regions, lathyrism in India, and some infectious processes, the etiology of numerous spastic meylopathies remained elusive until 1985. The demonstration by our laboratory of the association between HTLV-1 and a form of tropical spastic paraparesis (TSP) of Unknown etiology, frequent in Martinique (French West Indies), led to the evaluation of the role of this oncoretrovirus, in this disease. Such an association was rapidly confirmed in Colombia and Jamaica. A year later, the association of HTLV-1 with a chronic spastic myelopathy of unknown etiology was documented in southern Japan, and this clinical entity was named HTLV-1 associated myelopathy (HAM). Soon after, it was recognized that HTLV-1-associated TSP and HAM were the same disease, the hybrid term HAM/TSP was adopted and WHO diagnostic criteria were established.

Hundreds of HAM/TSP patients have now been described in several HTLV-1 endemic areas, including Japan, several of the Caribbean islands (especially Jamaica, Trinidad, Martinique, Haiti), numerous countries of South and Central America (Brazil, Peru, Colombia), of Central and South Africa, as well as in Iran. Sporadic cases of HAM/TSP have also been described in Melanesia, in West African countries as well as in immigrants from high HTLV-1 endemic areas living in Europe and the USA. In Japan, the life-time risk among HTLV-1 carriers is estimated to be less than 2%, that is, lower than ATLL. HAM/TSP mainly occurs in adults, with a mean age at onset of 40–50 years, but some rare cases of HAM/TSP in children have been reported. In Japan, in contrast to ATLL, HAM/TSP is more common in women than in men at all ages, with a sex ratio (M/F) of around 1:2,3.

Diagnostic Criteria

Initially, HAM/TSP diagnostic criteria included: (1) chronic spastic paraparesis, which usually slowly progresses, with signs of bilateral pyramidal tract lesions manifested by increased knee reflexes, ankle clonus, and extensor plantar responses; (2) minor sensory signs of involvement of the posterior columns and spinothalamic tract; (3) a history of insidious onset with gait disturbance without an episode of complete remission; (4) no evidence of spinal cord compression or swelling on magnetic resonance imaging, myelography, or computed/tomographic scan; and (5) presence of specific anti-HTLV-1 antibodies in the serum and the cerebrospinal fluid (CSF).

In more than 90% of the cases, the neurological features of HAM/TSP involve: spasticity and/or hyperreflexia of the lower extremities, urinary bladder disturbance, lower extremity muscle weakness, and in around 50% of the cases, sensory disturbances with low back pain. Impotence is also frequent. Central functions and cranial nerves are usually spared. The evolution is generally chronic and progressive, and after 10 years of evolution, roughly 50% of the patients are wheel-chaired. The incubation period (between primary infection which occurs mainly in adults) to onset of the myelopathy signs ranges usually from years to decades, but HAM/TSP also developed within 3.3 years in 50% of the post-transfusion-associated cases.

Biologically, besides high level of antibodies directed against HTLV-1 antigens both in blood and CSF, ATLL-like cells can sometimes be detected on the blood smear and in the CSF. Furthermore, a high HTLV-1 proviral load is frequently observed in the peripheral blood lymphocytes from HAM/TSP patients. A mild to moderate increase of proteins may be present in the CSF. However, intrathecal production of specific antibody provides additional data to support the diagnosis of HAM/TSP and also contributes to eliminate other differential diagnoses. Multiple spotty high intensities in deep and subcortical areas on T2-weighted images are the most frequent findings in brain magnetic resonance imaging. A mild atrophy of the thoracic spinal cord can also be observed in few cases. From a pathological point of view, this neurological syndrome is characterized by a chronic inflammation with perivascular lymphocytic cuffing and mild parenchymal lymphocytic infiltrates (**Figure 5**(**a**)). The cells are mostly CD4+ in early disease and mostly CD8+ in latter disease. Pyramidal tract damage with myelin and axonal loss, mainly in the lower thoracic spinal cord are observed.

HAM/TSP can be associated with other HTLV-1-associated symptoms such as uveitis, myositis, pulmonary alveolitis, and arthritis. The coincidence of ATLL and HAM/TSP has been rarely reported.

Recently, a large group of neurologists established a proposal for a modification of the diagnostic criteria of

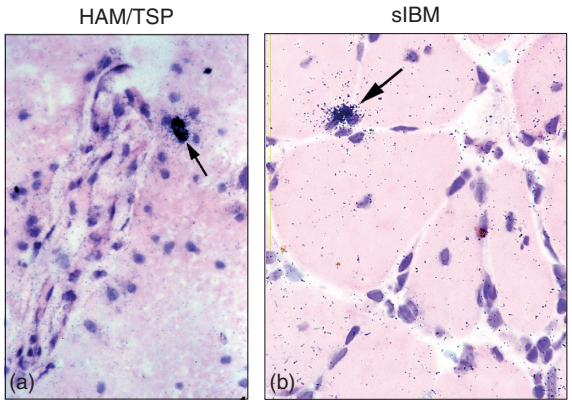


Figure 5 Detection of HTLV-1 *mRNA* expression by *in situ* hybridization with a ^{33}P *Tax* antisense riboprobe, on (a) frozen section from thoracic cord of a HAM/TSP patient, and (b) from a muscle section of a patient with an HTLV-1-associated sporadic inclusion body myositis (sIBM). Adapted from Ozden S, Seilhean D, Gessain A, Hauw J-J, and Gout O (2002) Severe demyelinating myelopathy with low human t cell lymphotropic virus type 1 expression after transfusion in an immunosuppressed patient. *Clinical Infectious Diseases* 34: 855–860 and Ozden S, Cochet M, Mikol J, *et al*. (2004) Direct evidence for a chronic CD8+-T-cell-mediated immune reaction to tax within the muscle of a human T-cell leukemia/lymphoma virus type 1-infected patient with sporadic inclusion body myositis. *Journal of Virology* 78: 10320–10327.

HAM/TSP. This was due to the fact that in some patients, especially those with early disease, HAM/TSP can be suspected but the complete WHO criteria are not met. These authors suggest that a HAM/TSP definite case corresponds to a nonremitting progressive spastic paraparesis with sufficiently impaired gait to be perceived by the patient. Sensory symptoms or signs may or may not be present. When present, they remain subtle and without a clear-cut sensory level. Urinary and anal sphincter signs or symptoms may or may not be present. Biologically, presence of HTLV-1 antibodies in serum and CSF confirmed by Western blot and/or a positive polymerase chain reaction (PCR) for HTLV-1 in blood and/or CSF should be demonstrated. Lastly, exclusion of other disorders that can resemble HAM/TSP is necessary.

HAM/TSP Pathogenesis

The pathogenesis of HAM/TSP is still poorly understood and viral and host factors as the proviral load and the immune response are considered to play a major role in disease progression. It remains unknown if the myelin and axonal loss, observed in HAM/TSP lesions, is a primary or secondary process and if it results from a direct viral effect or from an immune mediated process, as suggested by the marked lymphocytic infiltrates. Viral infection as demonstrated by *in situ* hybridization against *tax* mRNA has been clearly identified in some infiltrates by CD4+ lymphocytes by several teams, while the presence of HTLV-1 in neurons or oligodendrocytes remains controversial but unlikely.

At least three mechanisms have been proposed to explain the role of HTLV-1 in HAM/TSP development. The neurological damage is, in the first model, a direct consequence of the destruction by an antiviral attack mediated by cytotoxic T lymphocytes. These CTLs, frequent in HAM/TSP patients, both in the peripheral blood and CSF, are directed against HTLV-1 antigens, especially some immuno-dominant Tax epitopes expressed by HTLV-1-infected cells. In a second model, an autoimmune response could be due to either a chronic peripheral activation of autoreactive T cells by HTLV-1 infection, with infiltration of T lymphocytes into the CNS, or to a molecular mimicry, which could impair the immunological tolerance to the myelin antigens. A third possibility would be the bystander damage hypothesis. In this model, activated CD4+ T cells infected by HTLV-1, activated microglia, and CD8+ T cells could release into the spinal cord some myelinotoxic cytokines such as TNF-α or could directly damage the glial cells.

Therapeutic aspects of HAM/TSP

The long-term prognosis of HAM/TSP remains severe with a chronic evolution of a progressive disabling disorder without remission. Furthermore, its secondary complications may eventually lead to death, in some cases, after several years of evolution. Many small studies and cohort data have been performed using, among other drugs, corticosteroid therapy, danazol, vitamin C, interferon alpha, zidovudine, with very limited success. Recently, a randomized trial with zidovudine plus lamivudine has also been performed. Globally, a few short-term benefits have been observed, especially in early disease, but no treatment has been successful in chronic advanced disease. Failure to detect any clinical improvement is believed to be due to irreversible central nervous system damage in such patients. New controlled studies of both antiviral and anti-inflammatory agents are urgently required.

HAM/TSP is probably not the only neurological disorder associated with HTLV-1 infection. Indeed, an amyotrophic lateral sclerosis-like syndrome, as well as some peripheral neuropathies, and mild cognitive deficits have been described in some patients with HTLV-1 infection. It remains however difficult to prove a true association between such diseases and HTLV-1 infection, especially in high HTLV-1 endemic areas.

Infective Dermatitis

Infective dermatitis (ID) is a rare dermatological condition that was originally described in Jamaican children by Sweet in 1966. Subsequently, in 1990, La Grenade *et al.*

linked ID to HTLV-1 infection. A large series of patients with infective dermatitis have been described in Jamaica and more recently in Brazil. However sporadic cases of this clinical entity have also been reported in many other HTLV-1 endemic areas including Trinidad, Colombia, French West-Indies and French Guyana, Japan (where the disease seems very rare), and recently in Senegal (West Africa). In the great majority of cases, ID occurs in children from low socioeconomic backgrounds.

Infective dermatitis is a unique clinical entity characterized by a chronic and severe exudative dermatitis involving mainly the scalp, external ear and retroauricular areas, eyelid margins, paranasal skin, neck, axillae, and groin (**Figure 6**). Other symptoms include a chronic watery nasal discharge without other signs of rhinitis and/or crusting of anterior nares. A generalized fine papular rash is common in most of the severe cases. Clinically, the two differential diagnoses are atopic dermatitis and seborrheic dermatitis. In infective dermatitis, positive cultures for *Staphylococcus aureus* and/or beta-haemolytic streptococci are frequent from the anterior nares and skin samples. The evolution is typically chronic with relapse and several flares of superinfected lesions. Infective dermatitis responds well to antibiotic treatment, especially co-trimoxazole. However, relapse is very common if antibiotics are withdrawn. Infective dermatitis occurs mainly in young children (range 1–12 years), with an average age of onset of 2–6 years depending on the studies. Around 60% of the cases occur in girls. Anemia is frequent with a raised erythrocyte sedimentation rate and a hyper-immunoglobulinemia (IgD and IgE). The CD4 count, as well as the CD4/CD8 ratio, are elevated. Presence of rare ATLL-like cells is common in the peripheral blood. Pathological examination revealed an inflammatory lymphocytic infiltrate within the skin lesions. Epidemiological studies with long-term follow-up of ID patients have indicated that such disease may be associated with the later development of ATLL or of HAM/TSP. In a recent series from Brazil, neurological examination diagnosed six HAM/TSP cases among 20 children and adolescents with ID.



Figure 6 A 3-year-old boy from West Africa with infective dermatitis lesions: erosive dermatitis of the eyebrows, abscess of the scalp, and nasal discharge. Adapted from Mahe A, Meertens L, Ly F, *et al.* (2004) Human T-cell leukemia/lymphoma virus type 1-associated infective dermatitis in Africa: A report of five cases from Senegal. *British Journal of Dermatology* 150: 958–965.

Mothers of children with ID are nearly always infected by HTLV-1. Furthermore, they have breastfed their child for a long period of time (mean 20 months). Thus, nearly all ID children were infected by their mothers. This implies that reduction of HTLV-1 transmission from mother to child would be likely to prevent the occurrence of such disease as well as of ATLL. Such preventive medicine, currently performed in several areas of high HTLV-1 endemicity (Japan, West Indies), is based on HTLV-1 screening programs during pregnancy followed by adequate counseling that is adapted to the socioeconomic situation of each infected mother. The pathogenesis of ID is unknown but environmental, as well as host genetic factors are very likely involved in its occurrence.

Myositis

Myositis is an inflammatory myopathy that is represented by a heterogeneous group of muscle disorders. It is characterized by an acquired muscle weakness and inflammatory infiltrates of the muscle tissues. Depending on the clinical and histological features, myositis can be classified into dermatomyositis, polymyositis, and inclusion-body myositis. In 1989, Morgan *et al.* reported for the first time that 11 out of 13 Jamaican patients with idiopathic adult polymyositis were seropositive for HTLV-1. Later on, several epidemiological studies conducted in Jamaica, Martinique, and Japan, and based on larger series of patients, also indicated a higher prevalence of HTLV-1 antibodies in polymyositis patients as compared to that of the general population. Such data suggested a possible link between HTLV-1 and polymyositis. Lately, several sporadic cases of polymyositis, occurring in HTLV-1 infected patients of various origins, have been reported.

HTLV-1-associated polymyositis is a chronic disease that occurs mainly in adults 30–50 years of age. Patients complain mainly of musculo-skeletal symptoms including myalgia, joint and back pain, and proximal muscle weakness with muscle atrophia and falls. The four limbs can be affected. A patient's clinical pattern typically displays an elevation of muscle enzymes, especially creatine phosphokinase (CPK), and significant electromyographic abnormalities. Histological findings indicate an inflammatory myopathy with an increased variation in the fiber size and a mononuclear infiltrate located both between and within muscle cells, which appear often degenerate and necrotic. Muscle regeneration may also be present. This infiltration

is mainly due to T lymphocytes (both CD4+ and CD8+), but macrophages are also present. Expression of HTLV-1 *mRNAs* as well as of the viral proteins can be sporadically detected within the infiltrating T lymphocytes but not within muscle fibers. T-cell clones that are specifically directed against HTLV-1 Tax epitopes proliferate within the muscle lesions and may thus contribute to the pathogenesis. Patients respond poorly to corticosteroid therapy in most cases, and there is usually no sustained effect. Of note, HTLV-1 associated polymyositis is frequently associated with HAM/TSP.

A few cases of sporadic inclusion-myositis (sIBM) have also been reported in HTLV-1-infected patients. Such a disorder is a distinct form of chronic inflammatory myopathy for which a viral etiology has often been suspected. The lesions are characterized by vacuolated fibers that contain paired helical filaments similar to those of Alzheimer's diseases (**Figure 5(b)**). Immunohistochemical staining for ubiquitin may help to differentiate sIBM from polymyositis. sIBM can be associated with HAM/TSP.

Uveitis

Uveitis is an intraocular sight-threatening inflammatory disorder that can be caused by various infections, including tuberculosis, syphilis, toxoplasmosis, and cytomegalovirus. Uveitis, which does not have an infectious etiology, can occur in Bechet's diseases, Vogt–Koyanagi–Harada disease, and sarcoidosis. In roughly 40%, no cause can be identified and such disease is classified as idiopathic or unexplained uveitis. In the early 1990s, epidemiological studies demonstrated a higher prevalence of such idiopathic uveitis in HTLV-1 endemic areas from southern Japan than in areas where HTLV-1 has a lower prevalence. At the same time, some cases of uveitis were also observed in HAM/TSP patients. A large seroepidemiological study led by Mochizuki *et al.*, finally demonstrated without ambiguity that HTLV-1 infection was associated with idiopathic uveitis.

This clinico-virological entity is more frequent in females (around 60%) and the average onset age of the disease is 45 years, with few cases occurring in children. The major symptoms at initial presentation include sudden onset of occular floaters, foggy, and/or blurred vision. Unilateral disease is more common than bilateral (60% vs. 40%). The ocular signs consist mainly of iritis, vitreous opacities, and retinal vasculitis. Retinal exudates and hemorrhages are less frequent. Intermediate uveitis is more frequent than anterior or posterior lesions. Interestingly, co-morbid conditions include Graves disease and HAM/TSP. HTLV-1 uveitis responds well to topical and/or systemic corticosteroids and the intra-occular inflammation is markedly improved after steroid therapy. The visual prognosis is good in most cases. However, the inflammation tends to recur in about half of the cases. A specific opthalmological and systemic evaluation is necessary to eliminate other causes of uveitis in a given HTLV-1 infected individual. HTLV-1 antibodies are present in the aqueous humor at a level similar to that found in the plasma. HTLV-1-infected lymphocytes can be detected in the anterior chamber of the eye. Cytokines such as IL-6 and TNF-α produced by infiltrating lymphocytes are considered to play a major role in HTLV-1-associated uveitis pathogenesis. Infra-clinical uveitis can be detected through systematic examination in some HTLV-1 infected persons without any ocular symptoms.

Other Ocular Lesions Associated with HTLV-1 Infection

Kerato-conjonctivitis/sicca as well as interstitial chronic keratitis, are observed in HTLV-1 infected individuals, especially in HAM/TSP patients. The latter keratitis is also frequently associated with uveitis.

See also: Human T-Cell Leukemia Viruses: General Features.

Further Reading

Araujo AQ and Silva MT (2006) The HTLV-1 neurological complex. *Lancet Neurology* 5: 1068–1076.

Bazarbachi A, Ghez D, Lepelletier Y, *et al.* (2004) New therapeutic approaches for adult T-cell leukaemia. *Lancet Oncology* 5: 664–672.

Cleghorn F and Manns A (1994) Adult T-Cell leukemia/lymphoma: A working point-score classification for epidemiological studies. *International Journal of Cancer* 59: 491–493.

De Castro-Costa CM, Araujo AQ, Barreto MM, *et al.* (2006) Proposal for diagnostic criteria of tropical spastic paraparesis/HTLV-I-associated myelopathy (HAM/TSP). *AIDS Research and Human Retroviruses* 22: 931–935.

Grassmann R, Aboud M, and Jeang KT (2005) Molecular mechanisms of cellular transformation by HTLV-1 Tax. *Oncogene* 24: 5976–5985.

La Grenade L (1996) HTLV-I-associated infective dermatitis: Past, present, and future. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* 13(supplement 1): S46–S49.

La Grenade L, Manns A, Fletcher V, *et al.* (1998) Clinical, pathologic, and immunologic features of human T-lymphotrophic virus type I-associated infective dermatitis in children. *Archives of Dermatology* 134: 439–444.

Leon-Monzon M, Illa I, and Dalakas MC (1994) Polymyositis in patients infected with human T-cell leukemia virus type I: The role of the virus in the cause of the disease. *Annals of Neurology* 36: 643–649.

Levine PH, Cleghorn F, Manns A, *et al.* (1994) Adult T-cell leukemi/lymphoma: A working pring-score classification for epidemiological studies. *International Journal of Cancer* 59: 491–493.

Mahe A, Meertens L, Ly F, *et al.* (2004) Human T-cell leukaemia/lymphoma virus type 1-associated infective dermatitis in Africa: A report of five cases from Senegal. *British Journal of Dermatology* 150: 958–965.

Mahieux R and Gessain A (2003) A. HTLV-1 and associated adult T-cell leukemia/lymphoma. *Reviews in Clinical and Experimental Hematology* 7: 336–362.

Manns A, Hisada M, and La Grenade L (1999) Human T-lymphotropic virus type I infection. *Lancet* 353: 1951–1958.

Matsuoka M (2005) Human T-cell leukemia virus type I (HTLV-I) infection and the onset of adult T-cell leukemia (ATL). *Retrovirology* 2: 27.
Mochizuki M, Ono A, Ikeda E, *et al.* (1996) HTLV-I uveitis. *Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology* 13(supplement 1): S50–S56.
Ozden S, Cochet M, Mikol J, *et al.* (2004) Direct evidence for a chronic CD8+-T-cell-mediated immune reaction to tax within the muscle of a human T-cell leukemia/lymphoma virus type 1-infected patient with sporadic inclusion body myositis. *Journal of Virology* 78: 10320–10327.
Ozden S, Mouly V, Prevost MC, Gessain A, Butler-Browne G, and Ceccaldi PE (2005) Muscle wasting induced by HTLV-1 tax-1 protein: *An in vitro* and *in vivo* study. *American Journal of Pathology* 167: 1609–1619.
Ozden S, Seilhean D, Gessain A, Hauw J-J, and Gout O (2002) Severe demyelinating myelopathy with low human t cell lymphotropic virus type 1 expression after transfusion in an immunosuppressed patient. *Clinical Infectious Diseases* 34: 855–860.
Pinheiro SR, Martins-Filho OA, Ribas JG, *et al.* (2006) Immunologic markers, uveitis, and keratoconjunctivitis sicca associated with human T-cell lymphotropic virus type 1. *American Journal of Ophthalmology* 142: 811–815.
Proietti FA, Carneiro-Proietti AB, Catalan-Soares BC, and Murphy EL (2005) Global epidemiology of HTLV-I infection and associated diseases. *Oncogene* 24: 6058–6068.
Shimoyama M and The Lymphoma Study Group (1991) Diagnostic criteria and classification of clinical subtypes of adult T-cell leukemia-lymphoma. *British Journal of Haematology* 79: 428–437.
Taylor GP and Matsuoka M (2005) Natural history of adult T-cell leukemia/lymphoma and approaches to therapy. *Oncogene* 24: 6047–6057.

Hypovirulence

N K Van Alfen and P Kazmierczak, University of California, Davis, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ascomycete A division of fungi whose members produce spores in a saclike structure called the ascus.
Canker A dead section of bark on the branches or main trunks of trees.
Hyphae Vegetative growth structures of the fungus.
Hypovirulence A reduction of virulence.

Fungal Viruses

Hypovirulence is a phenomenon that can occur in virus-infected fungi. Although fungal viruses are now known to be common and widespread within all groups of fungi, their discovery was fairly recent. It was not until the early 1960s that viruses were found in fungi, the first discovery being of viral particles that were isolated from the edible mushroom *Agaricus bisporus*. Fungal viruses became more widely known after it was discovered that strains of *Penicillium* spp., which were strong inducers of interferon, were found to be infected with a double-stranded RNA (dsRNA) virus.

Most descriptions of fungal viruses suggest that they are not normally associated with clearly defined symptoms. The lack of known infectivity cycles of fungal viruses is a major barrier to the study of these viruses since it limits the ability to demonstrate Koch's postulates. Transmission of fungal viruses is known to naturally occur only during reproduction by the formation of spores and other such structures and by cytoplasmic fusion between hyphae of different strains of the same fungus. In this respect, transmission of fungal viruses is more similar to the transmission of plasmids than it is to the transmission of other viruses, that is, the infectious agent never leaves the cytoplasmic environment. With a few exceptions, the genomic material of known fungal viruses is dsRNA, and in viruses where sequences are known, they contain a virus-associated RNA-dependent RNA polymerase (RdRp). Because fungal virus genomes are clearly viral in origin and affinity, the apparent lack of a viral-like infectivity cycle has led researchers to postulate an ancient origin for fungal viruses.

The most extensively studied fungal viruses cause symptoms that can generally be classified into three general groups of symptoms: (1) killer phenotype, (2) hypovirulence, and (3) debilitation. The killer systems consist of a helper totivirus and associated satellite dsRNAs, which encode a protein toxin that is secreted and is lethal to strains of the same fungus lacking the virus. The best studied examples of killer systems are those found in the common baker's yeast, *Saccharomyces cerevisiae*. Viruses that reduce the virulence of plant pathogenic fungi either directly, or by debilitation, are the subject of this article.

Hypovirulence

A reduction of the virulence of a plant pathogenic fungus is known as hypovirulence. Generally, anything that reduces the ability of a pathogenic fungus to grow normally

will concomitantly reduce its virulence. So, by this general definition, viruses that debilitate a pathogen cause hypovirulence. It is useful, however, to distinguish this type of debilitating hypovirulence from a symptom of virus infection that is more directly associated with reducing virulence expression and/or other developmental processes of a fungus without significant effects on fungal growth. Virus-caused hypovirulence of both types has been described in diverse groups of plant pathogenic fungi. These mycoviruses that have hypovirulence as an associated symptom may have either ssRNA or dsRNA genomes and are found in the virus families *Chrysoviridae*, *Hypoviridae*, *Narnaviridae*, *Partitiviridae*, *Reoviridae*, and *Totiviridae*.

Perturbation of Virulence/Development

Other than the viruses associated with the killer phenotype of yeast, the most extensively studied fungal virus is cryphonectria hypovirus 1 (CHV1) that infects the ascomycete *Cryphonectria parasitica*. This fungus is the causal agent of chestnut blight and is the prime example of a virus that is able to cause the hypovirulence symptom without associated debilitation. Chestnut blight was first reported in North America during the summer of 1904 in the New York City Zoological Park. It was probably imported on nursery stock of Chinese or Japanese chestnuts, *Castanea mollissima* and *Castanea crenata*, respectively. The disease rapidly spread to the American chestnut, *Castanea dentata*, growing in surrounding woodlands and forests. Within 50 years, the disease spread throughout the range of the highly susceptible native tree. Few American chestnuts now survive in their natural range because of this disease. Prior to the blight, the American chestnut was the most common tree of the Eastern deciduous forest and was found on over 200 million acres across eastern North America. This is one of the most devastating diseases of recorded history.

Chestnut blight was also accidentally introduced into Europe, but during its spread in Italy it was noticed that chestnut trees in some orchards were recovering from the disease, that is, the cankers caused by the fungus stopped spreading. When the bark was peeled back, the fungal growth was found to be confined to the uppermost layers of the bark with no penetration occurring into the living tissues of the tree. The isolates of the fungus recovered from these cankers were of low virulence in assays, so were called hypovirulent by the French plant pathologist, J. Grente. Using genetically marked virulent and hypovirulent strains of the fungus, Van Alfen and colleagues demonstrated that a cytoplasmic element found in the hypovirulent strain was responsible for the symptoms and could be transferred cytoplasmically to the virulent strain, converting it into a hypovirulent strain. This cytoplasmic element was later found to be a fungal virus that is a member of the type species of the family *Hypoviridae*. **Figure 1** shows the phenotypic differences between virulent and hypovirulent isolates of *C. parasitica* on both the chestnut tree and in culture.

C. parasitica is also host to viruses classified in several other families of viruses: these include the *Reoviridae*, *Narnaviridae*, and *Chrysoviridae*. A hypovirulent phenotype of *C. parasitica* has also been described in strains infected by the virus NB631, a mitochondrial virus classified in the family *Narnaviridae*. This virus reduces virulence and growth of the fungus somewhat, but sporulation is normal. Two reoviruses of *C. parasitica*, C18 and 9B21 have been isolated. The best characterized of the two, 9B21, reduces virulence of the fungus significantly, but sporulation and pigmentation are not significantly affected. A very



Figure 1 Phenotypic changes in *C. parasitica* caused by the hypovirus CHV1. The photograph on the right is a tree that is infected with a virulent strain of the fungus. The photograph below shows this strain in culture. In addition to producing orange/brown pigments, virulent strains produce numerous conidia in asexual fruiting bodies, both in culture and on trees. The tree on the left is infected with a fungus containing CHV1. Strains of the fungus infected with this virus typically produce superficial healing cankers that do not kill the tree. Virus-infected strains of the fungus, as shown growing in culture, are not pigmented; they remain white and produce very few asexual conidia in culture. Canker photograph courtesy of Linda Haugen, University of Georgia, http://www.forestryimages.org. Culture photograph reproduced from McCabe PM, Pfeiffer PL, and Van Alfen NK (1999) The influence of dsRNA viruses on the biology of plant pathogenic fungi. *Trends in Microbiology* 7: 377–381, with permission from Elsevier.

unusual feature of 9B21 is that purified particles of the virus are able to infect fungal protoplasts.

Hypovirulence symptoms caused by viruses have been reported in many plant pathogenic fungi. Generally these viruses remain uncharacterized and in most cases the virulence of the fungus is reduced in association with a general debilitation of the infected fungus.

Structure and Classification of CHV1

Once it was demonstrated that a cytoplasmic element caused transmissible hypovirulence of the chestnut blight fungus there was a search for a fungal virus as the potential cause. This search culminated in the report of dsRNA associated with hypovirulent strains of the fungus. Further studies of the dsRNA showed that it was associated with fungal vesicles and that no detectable capsid was associated with this dsRNA, although RdRp activity was associated with the vesicles. The dsRNA of this 12.7 kb virus was cloned, sequenced, and an infectious clone of the virus was transformed into *C. parasitica*, confirming that this virus is responsible for transmissible hypovirulence. Based on the symptoms caused by the virus it has formally been named CHV1. This virus is the type member of the family *Hypoviridae* and was the first virus family described whose members have no capsid. Comparing the genome of CHV1 with other known viruses suggested that it is most similar to plant potyviruses and other picorna-like viruses.

Further study of the nature of the RdRp associated with fungal vesicles supported the hypothesis that the genome of this virus is derived from the replicative form of an ssRNA virus that lost its capsid during evolution. The coding strand of CHV1 contains two open reading frames: ORF A encodes a polyprotein that is processed into two polypeptides, p29 and p40 and ORF B which encodes a polyprotein from which only a single 48 kDa polypeptide p48 has thus far been shown to be autocatalytically processed. The sequences contained within ORF B indicate that it also contains RNA polymerase and helicase domains. **Figure 2** shows a schematic diagram of the genomic organization and expression strategy of CHV1.

Transmission of CHV1

There is no evidence that a typical infection cycle exists in the relationship of CHV1 with its fungal host. In order for infection of new hosts to occur the virus is transmitted through cytoplasmic exchange processes of the fungus. This cytoplasmic exchange occurs during fusion of hyphae (vegetative growth structures of the fungus) that happens frequently in fungal colonies and can occur between related strains of the fungus. In fungi, this hyphal fusion is controlled by vegetative incompatibility (*vic*) genes of the fusing strains; fusions most easily occur when alleles of the *vic* genes are the same in the two fungi. In *C. parasitica*, there are six known *vic* genes that control hyphal anastomosis. The more differences that occur in the *vic* loci, the less likely that transfer of CHV1 will occur. This vegetative incompatibility reaction is a defense in *C. parasitica* and other fungi against the spread of viruses. CHV1 spreads within a population with greater success if that population has few differences in alleles at the *vic* loci. Since strains of the fungus that contain CHV1 are incapable of sexual mating, infection by the virus suppresses allelic recombination at the *vic* loci and, as a result, may increase the effectiveness of virus spread within a population over time.

Very little is known about movement of the virus within the host fungus. However, the rate of movement of dsRNA within the fungus has been measured, and is rapid. The average rate of movement of the dsRNA within a fungal colony was found to be approximately 16 mm d^{-1}. This is 3–4 times faster than the colony growth rate during the same time period. Transmission electron microscope studies on the ultrastructure of hyphal anastomosis in *C. parasitica* reported that the dsRNA containing vesicles move from hypovirulent to virulent strains within 4–6 h after anastomosis. The incompatibility reaction controlled by the *vic* genes is an imperfect defense, since virus transfer can occur despite allelic differences in *vic* genes and virus transfer has been reported between different species of *C. parasitica*. There is clearly much left to be learned about virus movement within and between fungi.

The only other means by which the virus is known to spread is through asexual spores of the fungus. Although the

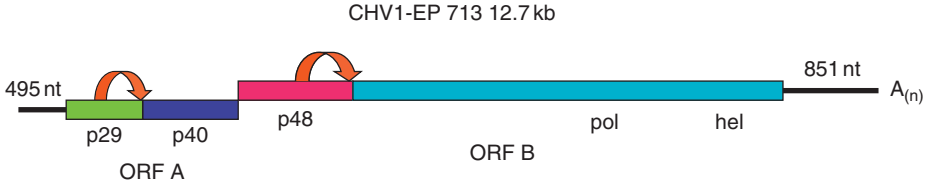


Figure 2 The genomic organization and expression strategy of CHV1–EP713, the prototypic strain of CHV1. Redrawn from Hillman BI and Suzuki N (2004) Viruses of the chestnut blight fungus, *Cryphonectria parasitica*. In: Maramorosch K and Shatkin AJ (eds.) *Advances in Virus Research*, vol. 63, pp. 423–472. San Diego, CA: Academic Press.

virus suppresses developmental processes of the fungus, it does not completely eliminate asexual sporulation. Many fewer asexual spores are produced by CHV1-infected strains of the fungus, but those asexual spores that are produced may contain the virus.

Hypovirulence as Developmental Perturbation

Hypovirulence caused by CHV1 is of interest not only because it provides a means of biological control for an important plant disease, but also because it offers the potential to understand critical control point(s) for development in a filamentous fungal pathogen. The perturbation of development, without fungal growth being affected, suggests that the virus interferes with development in specific ways that leave normal vegetative growth functions undisturbed. Understanding how the virus acts to perturb development in this fungus may provide insights of value on how to reduce the economic impact of filamentous fungi.

The symptoms of CHV1 infection of *C. parasitica* are a reduction of asexual sporulation, reduced pigmentation, female sterility, and hypovirulence (**Figure 1**). Careful growth measurements show little or no adverse effects of viral infection on fungal vegetative growth in culture. While there have been some claims that CHV1 reduces vegetative growth, these growth effects are likely an artifact of prolonged growth of the fungus in culture. We have found similar reduced growth rates in culture if some strains of the fungus, particularly EP713, are kept in culture too long. Such slow growth mutations are common in cultured fungi, and in our experience, are independent of virus infection. To prevent the accumulation of such growth mutations in prolonged culture, we routinely single-spore EP155 to select for a normal growth strain, and then reintroduce CHV1 by anastomosis to recreate EP713 with normal vegetative growth characteristics. Routine single-spore isolation is necessary to prevent the slower growth mutant strains from developing in culture.

Ultrastructural examination of virus-infected strains of the fungus show no significant cytopathology in CHV1-containing cells. The only differences observed between infected and uninfected cells are unique vesicles that are described as being located in association with unique Golgi in areas devoid of ribosomes and other cellular components. Recent cytological fractionation studies have isolated these small vesicles that accumulate in CHV1-infected strains and have shown that they are associated with the *trans*-Golgi network. These vesicles contain the virus and its replication-associated enzymes. **Figure 3** shows the accumulation of these vesicles in the CHV1-infected strain, EP802.

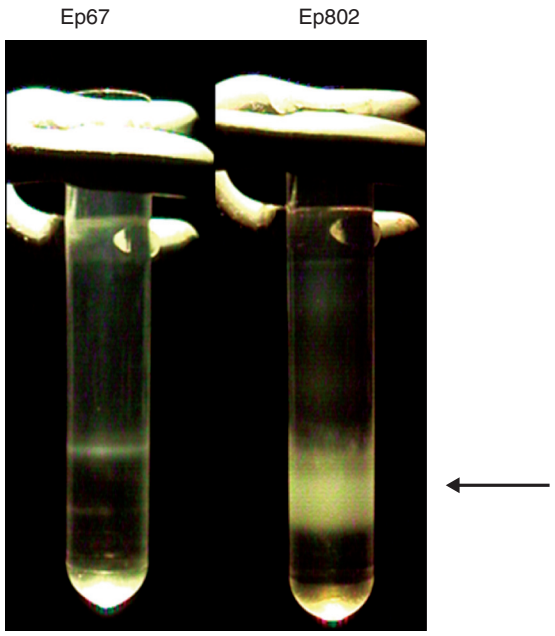


Figure 3 Subcellular fractionation on a Ficoll/^2H_2O gradient showing vesicle accumulation in CHV1-infected strain EP802 on the right and the isogenic virus free strain EP67 on the left. Gradient fractions were analyzed by Western and kex2 activity to show peak kex2 activity, AP-1μ, dsRNA, and viral helicase all cofractionate within this band. The endoprotease kex2 and the subunit for the AP-1 adaptor protein are markers for the *trans*-Golgi network.

Two general experimental approaches have been used to understand the molecular basis of how CHV1 causes symptoms in *C. parasitica*: (1) seek evidence that the virus perturbs normal fungal signal transduction pathways, and (2) identify commonalities in genes and proteins differentially expressed between normal and virus-infected strains of the fungus. A significant body of literature has been generated regarding how internal signal transduction pathways are affected in virus-infected strains, but, to date, there is no evidence of direct virus effects on signal transduction. As expected, signal transduction differs temporally and quantitatively between virus-infected and noninfected strains of the fungus. The most reasonable explanation of the published observations is that perturbation of signal transduction is the consequence of virus downregulation of development, rather than the cause. There is in fact no evidence that the symptoms are caused by direct virus perturbation of signal transduction and no testable hypothesis has emerged from these studies as to how the virus may be affecting signal transduction to cause the observed symptoms.

The approach that sought commonalities between differentially expressed proteins and genes associated with virus infection is leading to an understanding of how CHV1 may be causing symptoms. Given that the symptom of virus infection is a lack of development in infected strains,

significant differences in expression between the two strains were expected and observed. Early two-dimensional gel and differential hybridization studies identified a number of protein and gene expression differences between the infected and uninfected strains. A relatively small number of these genes/proteins that were highly expressed in uninfected but not in infected strains were characterized to seek commonalities between them.

Three of the genes highly expressed in uninfected strains, and not expressed in CHV1-infected strains, encoded sex pheromones. Two of the genes contain an 83bp ORF that encodes an identical 23-amino-acid peptide. This small peptide is similar to fungal lipopeptide sex pheromones: a C-terminal CAAX box with an asparagine residue 8–11 amino acids upstream of the box. CAAX is a prenylation signal, and farnesyl groups have been detected on the pheromones in other fungi. It was found that these peptides were expressed only in mating type 2 of uninfected strains of the fungus. Based on this similarity and loss of sexual mating upon deletion of the genes, the genes were named *mf2–1* and *mf2–2*. Deletion of *mf2–2* also resulted in a significant reduction in production of asexual fruiting bodies in culture and thus reduced numbers of asexual spores. Transcription run-on studies confirmed that these genes were transcriptionally downregulated in CHV1-infected cells.

Because *mf2–1* and *mf2–2* were so similar in structure to yeast pheromones, investigations were carried out to isolate the pheromone precursor gene of the opposite mating type. The gene isolated was shown to be similar in structure to the *S. cerevisiae* α-factor pheromone. There is a single copy of this gene expressed only by mating type 1 strains; it was named *mf1–1*. When the gene was deleted from *C. parasitica*, conidia or mycelial fragments normally used as spermatia in mating were sterile. Expression of this gene is significantly downregulated in CHV1-infected strains.

Differential protein expression studies identified two secreted proteins that are highly expressed by uninfected strains, but are downregulated in virus-infected cells. One of these is an extracellular laccase and the other an extracellular structural protein that was named cryparin. This later protein is a hydrophobin, a class of cell-surface proteins widely found in fungi and lichens. All hydrophobins are located on hyphal surfaces and confer hydrophobic properties to the surfaces, but they appear to have evolved different functions in various fungi. In *C. parasitica*, cryparin accumulates in fruiting bodies and is necessary for the eruption of the fungal fruiting bodies through the bark of the host tree. **Figure 4** shows the results of the deletion of cryparin.

The role of the laccase enzyme downregulated in CHV1-infected strains is not known. Knockout mutants did not exhibit any detectable phenotype, but did reveal the existence of multiple laccase enzymes expressed by the fungus. Laccases have been implicated in a number of

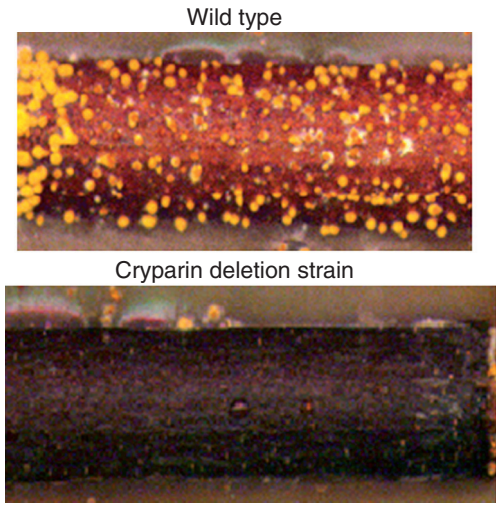


Figure 4 Stromal pustule (asexual fruiting bodies) eruption on chestnut wood. Sterile stem pieces of chestnut wood were inoculated with EP67 (upper panel) and the cryparin deletion strain (lower panel). Adapted from Kazmierczak P, Kim DH, Turina M, and Van Alfen NK (2005) A hydrophobin of the chestnut blight fungus, Cryphonectria parasitica, is required for stromal pustule eruption. *Eukaryotic Cell* 4: 931–936 with permission from American Society for Microbiology.

roles related to fungal development and virulence including degradation of lignin, formation of fruiting bodies, and pigment production.

The isolation and characterization of a number of the gene products downregulated in virus-infected cells was done to determine if there were any commonalities among them that could point to how the virus acts to cause symptoms. All of the gene products characterized are secreted into the extracellular environment and three of the proteins have sequences that suggest they are post-translationally processed by the same secretion pathway. Each protein is processed by an endoprotease after the signal peptide is cleaved, and the recognition signals suggest that they are processed by the same enzyme. **Figure 5** shows the preproproteins of Mf1–1, the fungal mating pheromone of mating type 1 strains, cryparin, and laccase. These three proteins have a signal peptide that presumably is cleaved early in the secretion process, followed by a propeptide region that terminates with a recognition sequence for processing by a kex2-like serine protease. These processing signals suggest that these three proteins are secreted via a kex2-like protein processing and secretion pathway. Kex2 processing is a post-Golgi function and the kex2 enzyme is a standard marker for *trans*-Golgi vesicles.

The processing of some secreted proteins by the kex2 pathway is highly conserved in eukaryotes. A similar pathway has been found in plants and animals and is utilized for the processing of specific proteins. It is generally not utilized for the secretion of most proteins. The specific role of this processing pathway in secretion appears to vary

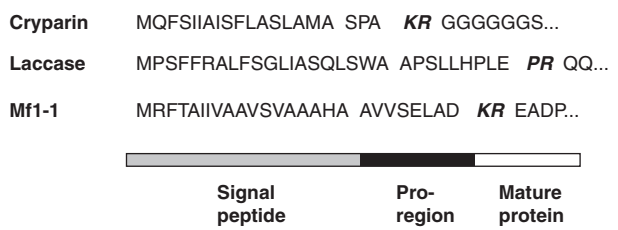


Figure 5 Amino acid N-terminal sequences derived from cloned mRNAs of three developmentally regulated host genes downregulated by CHV1. The signal peptide directs the protein to the ER for secretion and into the Golgi network for processing of the proprotein by the kex2 endoprotease at the residues lysine/arginine or proline/arginine which results in the mature form of the secreted protein.

between organisms. It was first discovered in yeast because it is involved in the processing and secretion of the viral encoded killer protein. This pathway was later found to be necessary for the secretion of the yeast alpha sex pheromone. The fact that three randomly isolated developmentally regulated host proteins, which are downregulated by the virus, are all processed by the same enzyme during secretion is an important clue in understanding how the virus perturbs development. Research has shown that not all secreted proteins of *C. parasitica* are affected by the virus; the secreted rennin-like endoprotease, endothiapepsin, for instance does not have a kex2 processing signal sequence nor it is downregulated by the virus.

The possibility that the virus directly affects secretion of some proteins, and thereby causes at least some of the symptoms associated with infection, is supported by evidence that the virus replicates using *trans*-Golgi vesicles. The vesicle fraction that contains the dsRNA genome of the virus and the viral encoded RNA polymerase and helicase co-purifies with kex2 enzymatic activity. This vesicle fraction has also been shown to contain cryparin, one of the proteins downregulated by the virus. The vesicle fraction in CHV1-infected cells that contains the virus dsRNA, viral encoded proteins, and kex2 activity are present in much greater amounts in virus-containing cells than in uninfected cells. This proliferation of vesicles in *C. parasitica* is typical of the effect of RNA viruses on their hosts, that is, the use of host membrane systems for replication by many RNA viruses cause a proliferation of these membranes within the host cells. We have estimated that in CHV1-infected cells there is at least a fivefold increase in this specific vesicle fraction when compared with noninfected cells. As previously mentioned, evidence from microscopic and cell fractionation observations suggest that CHV1 effects on membranes is specific to these *trans*-Golgi vesicles.

Recent research from our laboratory has shown that the CHV1 encoded protease p29 specifically integrates into the membranes of this *trans*-Golgi vesicle fraction. This protein is a close relative of the plant potyviral protease HC-Pro. HC-Pro is a papain-like protease that is autoprotelytic and which facilitates aphid transmission and promotes potyviral genome amplification. P29 is encoded on ORF A of CHV1 and is translated as part of the polyprotein p69 from which it is autocatalytically cleaved. Transformation of p29 into *C. parasitica* causes a loss of pigmentation, reduction in asexual sporulation, and suppression of host laccase expression, and there is evidence that it may be involved in suppression of RNA silencing by the host. Enhancement of both dsRNA accumulation and transmission of the virus through asexual spores are also functions attributed to CHV1 p29.

Recent studies show that p29 integrates into the *trans*-Golgi vesicle membranes of the host and that no other viral elements are required for this integration. While within the membrane p29 is fully susceptible to proteolytic digestion suggesting that it is primarily on the cytoplasmic side of the membrane. Deletion analysis of p29 showed that the C-terminal sequences of p29 mediate the membrane association. These transformation studies also confirmed the previous studies that showed p29 to be responsible for causing the reduced asexual sporulation and loss of pigment symptoms of virus infection. Further studies of the effect of p29 membrane integration on virus-caused host membrane proliferation and symptom production are in progress.

Based on the evidence available to date, CHV1 utilizes *trans*-Golgi vesicles of its host for replication and perhaps movement. *trans*-Golgi vesicles are critical for the final processing and secretion of proteins through the cytoplasmic membrane of the fungus, and for transport of proteins to certain cellular compartments. Studies of the viral protein p29 clearly link this protein to these vesicles and to causing some of the virus infection symptoms. The simplest hypothesis for how the virus is able to cause these and perhaps additional symptoms is that use of *trans*-Golgi vesicles for virus replication disrupts normal function of the vesicles and interferes with protein secretion or compartmentalization. The proprotein signal sequences directing kex2 endoprotease processing in a number of viral downregulated host proteins, such as the sex pheromone, cryparin, and laccase, also point to the *trans*-Golgi network as being a key to understanding the cause of virus symptom induction. The role of kex2 processing, a *trans*-Golgi vesicle function, in fungal development and its perturbation by the virus are currently under investigation.

There are precedents for RNA virus disruption of vesicle trafficking. Protein 3A of poliovirus specifically inhibits endoplasmic reticulum (ER)-to-Golgi traffic in mammalian cells, causing proteins otherwise destined for export to accumulate in ER-derived membranes. Many other viruses have been shown to have dramatic effects on the host membrane systems and a number of nonstructural virus proteins have been shown to interact with cellular factors responsible for the targeting and docking

of membranes within the various organelles and membrane systems of virus hosts. Although no vesicle membrane proteins have been isolated yet from *C. parasitica* that are involved in vesicle function, the viral protein p29 which has an extensive cytoplasmic domain upon integration into vesicle membranes is a likely candidate for interaction with any host factors that direct cellular membrane traffic. An understanding of how the virus specifically targets these *trans*-Golgi membrane vesicles, and how this targeting affects normal function of these vesicles, will likely lead to a better understanding of virus symptom production, and provide new insights into critical host developmental controls.

See also: Fungal Viruses; Hypoviruses.

Further Reading

Boland GJ (2004) Fungal viruses, hypovirulence, and biological control of *Sclerotinia* species. *Canadian Journal of Plant Pathology* 26: 6–18.

Choe SS, Dodd DA, and Kirkegaard K (2005) Inhibition of cellular protein secretion by picornaviral 3A proteins. *Virology* 337: 18–29.

Ghabrial SA (1994) New developments in fungal virology. *Advances in Virus Research* 43: 303–388.

Ghabrial SA (1998) Origin, adaptation and evolutionary pathways of fungal viruses. *Virus Genes* 16: 119–131.

Hillman BI and Suzuki N (2004) Viruses of the chestnut blight fungus, *Cryphonectria parasitica*. In: Maramorosch K and Shatkin AJ (eds.) *Advances in Virus Research, vol.* 63, pp. 423–472. San Diego, CA: Academic Press.

Jacob-Wilk D, Turina M, and Van Alfen NK (2006) Mycovirus cryphonectria hypovirus 1 elements cofractionate with *trans*-Golgi network membranes of the fungal host *Cryphonectria parasitica*. *Journal of Virology* 80: 6588–6596.

Kazmierczak P, Kim DH, Turina M, and Van Alfen NK (2005) A hydrophobin of the chestnut blight fungus, *Cryphonectria parasitica*, is required for stromal pustule eruption. *Eukaryotic Cell* 4: 931–936.

Marsh M (2005) *Current Topics in Microbiology and Immunology*, *Vol. 285: Membrane Trafficking in Viral Replication*. Heidelberg: Springer.

McCabe PM, Pfeiffer PL, and Van Alfen NK (1999) The influence of dsRNA viruses on the biology of plant pathogenic fungi. *Trends in Microbiology* 7: 377–381.

McCabe PM and Van Alfen NK (2002) Molecular basis of symptom expression by the *Cryphonectria* hypovirus. In: Tavantzis SM (ed.) *dsRNA Genetic Elements*, pp. 125–144. Washington, DC: CRC Press.

Milgroom MG and Cortesi P (2004) Biological control of chestnut blight with hypovirulence: A critical analysis. *Annual Review of Phytopathology* 42: 311–338.

Nuss DL (2005) Hypovirulence: Mycoviruses at the fungal–plant interface. *Nature Reviews Microbiology* 3: 632–642.

Turina M, Zhang L, and Van Alfen NK (2006) Effect of *Cryphonectria hypovirus 1* (CHV1) infection on Cpkk1, a mitogen-activated protein kinase kinase of the filamentous *Cryphonectria parasitica*. *Fungal Genetics and Biology* 43: 764–774.

Villanueva RA, Rouille Y, and Dubuisson J (2005) Interactions between virus proteins and host cell membranes during the viral life cycle. *International Review of Cytology – A Survey of Cell Biology* 245: 171–244.

Hypoviruses

D L Nuss, University of Maryland Biotechnology Institute, Rockville, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Anastomosis The fusion of fungal hyphae resulting in exchange of cytoplasmic material and hypovirus transmission.

Hypovirulence Virus-mediated attenuation of fungal virulence.

Vegetative incompatibility A system controlled by at lease six genetic loci that determines the ability of two fungal strains to undergo anastomosis.

Introduction

The discovery of hypoviruses, a group of RNA viruses that reduce the virulence (hypovirulence) of the chestnut blight fungus *Cryphonecria parasitica*, has stimulated intensive research into the potential of using fungal viruses for biological control of fungal diseases. Documented examples of virus-mediated hypovirulence have been reported for fungal diseases of plants that range from trees to turfgrass and involve mycoviruses, which include representatives from the *Totiviridae*, *Chrysoviridae*, *Reoviridae*, *Narnaviridae,* and *Hypoviridae* (the hypoviruses). However, the hypoviruses remain the most thoroughly studied of the hypovirulence-associated viruses, primarily due to several significant advances in hypovirus molecular biology.

A classic example of the havoc that can result from the introduction of an exotic organism, the North American chestnut blight epidemic, first reported in 1905, caused the destruction of millions of mature chestnut trees by 1950. *Cryphonecria parasitica* was subsequently introduced into Italy during the 1930s, threatening European chestnut forests and orchards. However, for reasons still to be completely understood, the European chestnut blight

epidemic was much less severe than that witnessed in North America. One clear contribution to this reduced severity was the prevalence of *C. parasitica* strains exhibiting a reduced virulence phenotype (hypovirulence), first described by an Italian forest pathologist in the 1950s. French investigators subsequently showed that the hypovirulence phenotype was transmissible following anastomosis (fusion of hyphae) between vegetatively compatible *C. parasitica* strains, implicating a cytoplasmic genetic element as the causative agent of the phenotype. The observation that a hypovirulent strain could produce a curative effect when inoculated onto existing cankers on diseased trees stimulated a successful government-sponsored biological control program using hypovirulent *C. parasitica* strains for management of chestnut blight in French chestnut plantations. Recent studies in Switzerland also support the view that natural hypovirulent strains retard disease progression in European forest ecosystems.

In 1977, Peter Day and co-workers at the Connecticut Agricultural Experiment Station reported that hypovirulent *C. parasitica* strains harbor double-stranded (ds) RNAs, providing the first indication of the nature of cytoplasmic elements responsible for the phenotype. Subsequent surveys of dsRNAs associated with different North American and European hypovirulent strains revealed considerable variations in concentration, number, and size of dsRNA components. By the late 1980s, it was clear that a detailed molecular analysis of the dsRNAs associated with a single hypovirulent strain was required to bring some measure of order to the mounting confusion generated by such surveys. This resulted in the cloning and complete sequence determination of the prototypic hypovirus, now designated CHV1-EP713, in 1991. This milestone was followed in 1992 by the construction of an infectious full-length (12 712 bp) cDNA clone of CHV1-EP713 RNA by Choi and Nuss. This development furnished direct evidence that hypoviruses are indeed the causative agents responsible for transmissible hypovirulence and provided the means for facile manipulation of the hypovirus genome. Current interest in hypoviruses extends past biological control potential to their utility as unique experimental tools for probing fundamental processes underlying fungal pathogenesis and mycovirus–-fungal host interactions.

Taxonomy and Genetic Organization

Hypoviruses are classified within the family *Hypoviridae*, consisting of the genus *Hypovirus* and four species designated *C. parasitica* hypovirus 1–4 (CHV1–CHV4). Hypovirus taxonomy is not based on virus structure, since this group of viruses does not encode a coat protein, but on genome organization, sequence similarity and symptom expression. Hypovirus genetic information is found predominantly as dsRNA associated with membrane vesicles ranging in diameter from 50 to 80 nm. As observed for fungal viruses generally, hypoviruses exhibit no extracellular phase in their life cycle. Infections cannot be initiated by inoculation with an infected cell extract or enriched fractions. Instead, these viruses are transmitted by cytoplasmic mixing as a result of fusion (anastomosis) between vegetatively compatible strains or to a variable degree in asexual spores (conidia).

The hypovirus species designations CHV1–CHV4 were assigned in the order in which their genome sequence was completed. The primary nucleotide sequence for the coding strand of the prototypic member of the genus *Hypovirus*, CHV1-EP713, specifies two large open reading frames (ORFs) designated ORF A and ORF B (**Figure 1**). A second closely related member, CHV1-Euro7, has the same organization and shares approximately 90% identity at the nucleotide level with CHV1-EP713. The type member of the CHV2 species, CHV2-NB58, shares only about 60% nucleotide sequence identity with CHV1-EP713 and lacks a portion of ORF A that, for the CHV1 species, encodes a functional *cis*-acting cysteine protease. Sequenced members of the CHV3 and CHV4 species, CHV4-GH2 and CHV4-SR2, respectively, are several kbp shorter than the CHV1 and CHV2 species, ~9.2–9.8 kbp versus 12.5–12.7 kbp, contain a single large ORF rather than two ORFs and are both more distantly related phylogenically to species CHV1 than CHV1 is to CHV2.

Hypovirus Gene Expression Strategy

Although hypovirus genetic information is readily recovered from infected cultures as linear dsRNA, the absence of a discrete virus particle and an extracellular infection phase presents some difficulties in precisely defining the hypovirus genome. Synthetic copies of the coding strand are infectious by electroporation into fungal spheroplasts and phylogenetic analyses suggest a common ancestry with the positive strand RNA plant potyviruses. Thus, one could consider hypoviruses as having a positive strand RNA genome and the dsRNA as representing accumulated replicative form RNA. Irrespective of this complication, direct analysis of hypovirus dsRNAs, cDNA cloning studies, and *in vitro* translational analyses has provided the following view of genetic organization and expression strategies for the prototypic hypovirus CHV1-EP713 shown in **Figure 2**. One strand contains a 3′-poly A tail while the complementary strand contains a 5′-poly U tract. All of the CHV1-EP713 coding information appears to reside within two contiguous ORFs on the 12 712 nt long polyadenylated strand. ORF A encodes two polypeptides, p29 and p40, that are released from a polyprotein precursor, p69, by an autoproteolytic event between Gly-248 and Gly-249, mediated by a

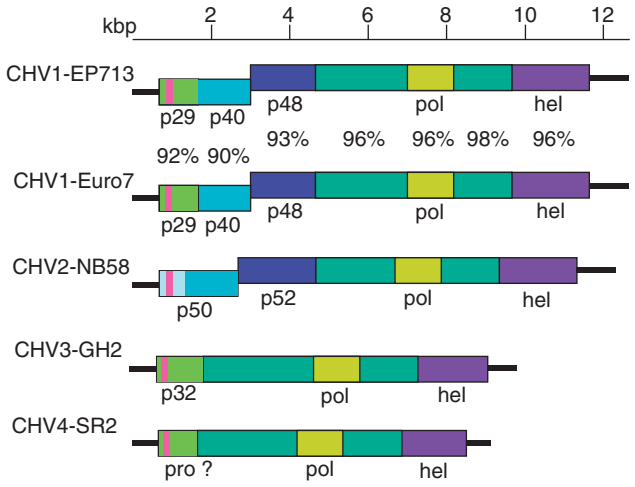


Figure 1 Genetic organization of sequenced hypovirus genomes representing the four species that comprise the genus *Hypovirus*, family *Hypoviridae*. Amino acid identity levels for coding regions of the two sequenced members of the CHV1 species, CHV1-EP713 and CHV1-Euro7, are indicated between representations of the two viral genomes. Protein coding regions homologous to CHV1-EP713 encoded p29, p40, p48, polymerase and helicase are color coded. The magenta regions represent a short conserved cysteine rich domain. Note that the genomes of CHV3-GH2 and CHV4-SR2 contain a single ORF. Modified from Dawe AL and Nuss DL (2001) Hypoviruses and chestnut blight: Exploiting viruses to understand and modulate fungal pathogenesis. *Annual Review of Genetics* 35: 1–29, with permission from Annual Reviews.

cysteine-like protease catalytic domain located within p29. ORF B has the capacity to encode a polyprotein of 3165 amino acids and contains unmistakable RNA-dependent RNA polymerase and helicase motifs. Proteolytic processing of only a portion of the ORF B polyprotein has been elucidated in the form of the autoproteolytic release of a 48 kDa protein, p48, from the N-terminus. This cleavage event occurs between Gly-418 and Ala-419 and is catalyzed by essential residues Cys-341 and His-388 within p48. The junction between ORF A and ORF B is defined by the sequence 5′-UAAUG-3′, in which the UAA portion clearly serves as the termination codon for ORF A and the AUG portion is thought to serve as the initiation codon for ORF B. While the mechanism involved in ribosome transition through the junction is not known, this unusual pentanucleotide sequence is found at the ORF A/ORF B junction for all confirmed CHV1 species. There is clearly a need for additional fine detailed mapping of the processing cascades for hypovirus-encoded polyproteins.

The genome of CHV2-NB58, type strain of species CHV2, also consists of a two ORF configuration with a UAAUG junction (**Figure 2**). However, ORF A lacks the p29 papain-like catalytic or cleavage sites and directs the translation of a 50 kDa protein product. ORF B of CHV2-NB58 does contain a p48 homolog, p52. The N-terminal portion of the single ORF of the CHV3-type strain CHV3-GH2 contains a protease, p32, with similarity to p29. A putative protease domain has been identified at the N-terminal portion of the CHV4-type strain CHV4-SR2, but protease cleavage has not been demonstrated.

In most hypovirus-infected *C. parasitica* isolates, the full-length viral dsRNA is accompanied by a constellation of shorter dsRNA species. These ancillary dsRNAs appear to be generated by internal deletion events, are replicated only in the presence of the full-length viral RNA, and are not associated with any function or phenotypic effect.

Hypovirus–Host Interactions

The phenotypic changes that are associated with hypovirus infection are not limited to hypoviurlence. Additional hypovirus-mediated phenotypic changes can include altered colony morphology, female infertility, reduced asexual sproulation (conidiation), and reduced pigmentation. The pleiotropic nature of these changes suggests that hypoviruses might perturb one or several cellular signaling pathways. Consistent with this view, alterations in G-protein, cyclic AMP-mediated-, mitogen-activated protein kinase-, and calcium/calmodulin/inositol trisphosphate-dependent-signaling pathways have been reported for hypovirus-infected fungal strains.

The construction of an ordered expressed sequence tag (EST) library representing approximately 2200 *C. parasitica* genes and development of a corresponding *C. parasitica* cDNA microarray platform has provided a view of host transcriptional responses to hypovirus infection. The modulation of transcript accumulation for approximately 13.4% of the 2200 cDNAs was shown following CHV1-EP713 infection. These transcriptional profiling studies also resulted in the identification of a

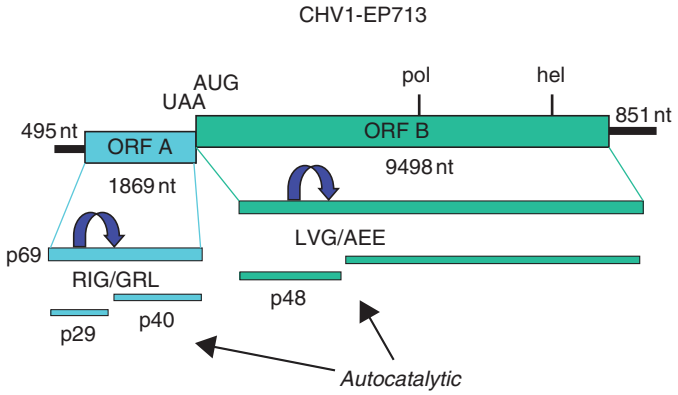


Figure 2 Expression strategy for prototypic hypovirus CHV1-EP713. The CHV1-EP713 coding strand consists of 12 712 nucleotides excluding a poly(A) tail, and contains two major coding domains designated ORF A and ORF B. Details are discussed in the text. Modified from Dawe AL and Nuss DL (2001) Hypoviruses and chestnut blight: Exploiting viruses to understand and modulate fungal pathogenesis. *Annual Review of Genetics* 35: 1–29 with permission from Annual Reviews.

subset of hypovirus responsive genes regulated through the G-protein signaling pathway and revealed a linkage between viral and mitochondrial hypovirulence.

Hypovirus-encoded symptom determinants and important replication elements have been mapped (**Figure 3**) by a combination of approaches that include (1) the construction of recombinant chimeras from hypoviruses that differ in their influence on host phenotype, (2) mutagenesis of a hypovirus infectious cDNA clone, and (3) cellular expression of viral coding domains independent of virus infection.

In analogy with plant viruses, CHV1-EP713 and CHV1-Euro7 can be viewed as severe and mild hypovirus isolates, respectively. Although these two CHV1 isolates cause quite different phenotypic changes in their fungal host, they share a high level of sequence similarity that has allowed the construction of viable chimeric viruses to begin mapping the determinants responsible for the differences in phenotypic changes.

Differences in colony morphologies were found to map to a region extending from a position just downstream of the p48 coding domain (map position 3575) to map position 9879, with clear indications of multiple discrete determinants. More specifically, the region extending from position 3575 to 5310 was able to confer a CHV1-EP713-like colony morphology when inserted into a CHV1-Euro7 genetic background. The CHV1-EP713 p48 coding region was found to be a dominant determinant contributing to suppression of asexual spore formation on the canker face.

The chimeric hypoviruses also proved to be very useful reagents when coupled with a pathway specific promoter/reporter system to map viral determinants responsible for altering G-protein/cAMP-mediated signaling. A common undesired side effect of hypovirus-mediated virulence attenuation is a significantly reduced ability of the fungal host to colonize and produce spores on the corresponding plant host. This reduces the ability of hypovirulent fungal strains to persist and spread through the ecosystem. Thus, from a practical perspective, a better understanding of the nature of viral symptom determinants and their relative effects on specific regulatory pathways and expression of gene clusters provides the means for a more rational approach for engineering hypoviruses that exhibit a desired balance between virulence attenuation and ecological fitness.

Additional insights into the functional role of viral coding regions were indirectly provided during efforts to develop hypoviruses as gene expression vectors. The nucleotide sequence corresponding to the first 24 codons of p29 was found to be required for viral replication, while the remaining 598 codons of ORF A, including all of the p40 coding region, was found to be dispensable. Substantial alterations were also tolerated in the pentanucleotide UAAUG that contains the ORF A termination codon and the overlapping putative ORF B initiation codon. For example, replication competence was maintained following either a frameshift mutation that caused a two-codon extension of ORF A or a modification that produced a single-ORF genomic organization. Further charactereization of p40 revealed a role as an accessory function in viral RNA amplication with a functional domain extending from Thr(288) to Asn(313).

Expression of the CHV1-EP713 encoded papain-like protease, p29, in the absence of virus infection was shown to cause a subset of phenotypic changes exhibited by CHV1-EP713-infected strains, for example, a white phenotype (reduction in orange pigmentation), reduced asexual sporulation and a slight reduction in the production of fungal laccase activity. By deleting all but the first 24 N-terminal codons of p29 in the context of the CHV1-EP713 infectious cDNA clone (mutant virus Δp29), it was also possible to show that the p29 protein is dispensable

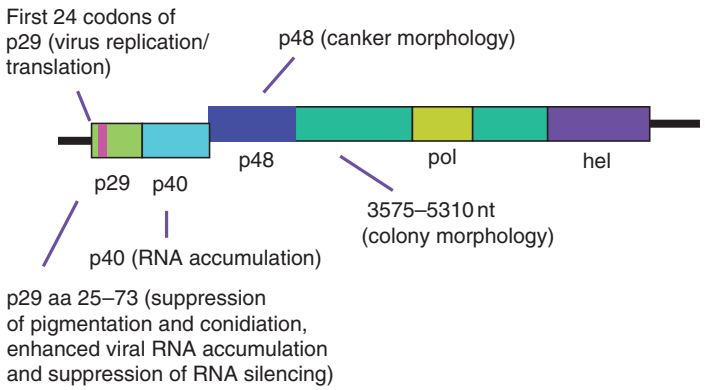


Figure 3 Emerging map of CHV1-EP713 symptom determinants and essential/dispensable replication elements as described in the text. Adapted from Dawe AL and Nuss DL (2001) Hypoviruses and chestnut blight: Exploiting viruses to understand and modulate fungal pathogenesis. *Annual Review of Genetics* 35: 1–29, with permission from Annual Reviews.

for viral replication and to demonstrate a near restoration of orange pigment production and a moderate increase in conidation levels relative to wild-type CHV1-EP713-infected fungal colonies. Deletion of p29 had no effect on virus-mediated virulence attenuation. A gain-of-function analysis involving progressive repair of the Δp29 mutant was also devised to map the p29 symptom determinant domain to a region extending from Phe-25 to Gln-73. When expressed from a chromosomally integrated cDNA copy, p29 elevated RNA accumulation and vertical transmission (through conidia) of the Δp29 mutant virus to levels observed for wild-type CHV1-EP713. Additional mutational studies indicated a linkage between p29-mediated changes in host phenotype in the absence of virus infection and p29-mediated in *trans*-enhancement of viral RNA accumulation and transmission.

The multifunctional nature of p29 was recently extended to include suppressor of RNA silencing both in the natural fungal host and in a heterologous plant system. This activity was predicted based on similarities between p29 and the well-characterized potyvirus-encoded suppressor of RNA silencing HC-Pro and has provided the first circumstantial evidence that RNA silencing in fungi may serve as an antiviral defense mechanism, a well-established function in plants.

Prospects for Biological Control

Chestnut blight cankers on American chestnut trees can be controlled by the direct application of hypovirus-infected hypovirulent *C. parasitica* strains to the canker margin, provided that the hypovirulent and resident virulent strains are vegetatively compatible. Anastomosis between the two strains results in hypovirus transmission and conversion of the canker to the hypovirulent phenotype, thereby preventing additional expansion of the canker and promoting formation of new callus tissue. However, since treated trees do not become resistant to new infections and North American *C. parasitica* populations generally exhibit a very diverse vegetative compatibility structure, this form of treatment is labor intensive and has not proved to be practical for effective control of chestnut blight in a North American forest ecosystem.

High levels of VC diversity and the severe phenotypic characteristics of the hypoviruses that have been introduced for biocontrol are thought to be two of the main contributing factors to the inability of introduced hypoviruses to spread through North America *C. parasitica* populations. In this regard, transgenic hypovirulent strains that contain a nuclear copy of the hypovirus cDNA exhibit novel hypovirus transmission properties that have been predicted to reduce these limitations. Hypovirus RNA is not transmitted to ascospores during mating. However, the hypovirus cDNA present in transgenic hypovirulent strains is inherited by a portion of the ascospore progeny, followed by the production of cDNA-derived cytoplasmically replicating viral RNA. Additionally, since the progeny represent a spectrum of vegetative compatibility groups, launching of the cytoplasmic viral RNA into these new vegetative compatibility groups is predicted to expand vegetative dissemination of hypovirus genetic information.

Three field trials with CHV1-EP713 transgenic strains have confirmed hypovirus transmission to ascospore progeny in a forest setting and also exposed the requirement for significant improvements in formulation and delivery methods. These trials also confirmed predictions that the CHV1-EP713 hypovirus performs poorly as a biological control agent because it severely reduces the ability of the fungal host to colonize, expand, and produce asexual spores on chestnut tissue. In this regard, the availability of an infectious cDNA clone for the mild hypovirus CHV1-Euro7 has provided the means to construct a transgenic hypovirulent strain that combines the properties of enhanced colonization and spore production with a novel mode of hypovirus transmission to ascospore progeny.

The infectious hypovirus cDNA clones have also been used to expand hypovirus host range to include several other pathogenic fungi that include the *Eucalyptus* canker pathogen *Cryphonectria cubensis* and the fruit tree pathogens *Phomopsis G-type* and *Valsa ceratosperma*. Hypovirus-based management of these economically important fungal diseases in controlled agricultural settings may not face the same problems of formulation and application that has been encountered for control of chestnut blight in a complex forest ecosystem.

See also: Fungal Viruses; Hypovirulence.

Further Reading

Dawe AL and Nuss DL (2001) Hypoviruses and chestnut blight: Exploiting viruses to understand and modulate fungal pathogenesis. *Annual Review of Genetics* 35: 1–29.

Hillman BI and Suzuki N (2004) Viruses of the chestnut blight fungus, *Cryphonectria parasitica*. *Advances in Virus Research* 63: 423.

Nuss DL (2005) Hypovirulence: Mycoviruses at the fungal–plant interface. *Nature Reviews Microbiology* 3: 632.

Segers GC, van Wezel R, Zhang X, Hong Y, and Nuss DL (2006) Hypovirus papain-like protease p29 suppresses RNA silencing in the natural fungal host and in a heterologous plant system. *Eukaryotic Cell* 5: 896.

Icosahedral dsDNA Bacterial Viruses with an Internal Membrane

J K H Bamford, University of Jyväskylä, Jyväskylä, Finland
S J Butcher, University of Helsinki, Helsinki, Finland

© 2008 Elsevier Ltd. All rights reserved.

Glossary

DNA packaging Energy-requiring process where the empty virus capsid is filled by the virus genome.
Protein-primed DNA replication Duplication of a linear DNA genome utilizing a protein covalently linked to the DNA terminus to initiate the reaction.
Triangulation number T number (T), a parameter for icosahedral capsids that describes the geometrical arrangement of the protein subunits.

Introduction

Bacterial viruses with an internal membrane all have double-stranded DNA (dsDNA) genomes. They are classified into two families, the *Tectiviridae* and the *Corticoviridae*. The former family consists of several viruses, whereas the latter has only one representative. The type species of the *Tectiviridae* is PRD1, which infects a wide variety of Gram-negative bacteria. Its host range is limited to bacteria that contain a conjugative antibiotic resistance plasmid, since it utilizes the plasmid-encoded cell surface DNA-transfer complex as a receptor. The type species of the family *Corticoviridae* is PM2.

The family *Tectiviridae* can be divided into two groups: viruses infecting Gram-negative bacteria and viruses infecting Gram-positive bacteria. The group infecting Gram-negative bacteria contains six extremely similar phages (PRD1, PR3, PR4, PR5, PR772, and L17) with a linear dsDNA genome. Their sequence similarity is between 91.9% and 99.8%, which is surprising since they have been isolated from different parts of the world. The other group infects Gram-positive bacteria (different *Bacillus* species). The length of the genomes (about 15 kbp) and the order of the genes is conserved in all the tectiviruses, but there is no sequence similarity between the two groups. The genomes of the tectiviruses encode about 35 proteins (**Table 1**).

The corticovirus PM2 was isolated in 1968 from seawater off the coast of Chile. The host is a marine bacterium, *Pseudoalteromonas espejiana*. PM2 has a negatively supercoiled circular dsDNA of about 10 kbp. It encodes about 17 proteins (**Table 2**). The combination of a membrane and a supercoiled DNA genome makes PM2 a unique virus. The entry and the DNA-packaging mechanisms are likely to differ from those of other viruses.

Virion Structure and Properties

Overall Structure

The virion of PRD1 is an icosahedrally symmetric particle approximately 65 nm in diameter (**Figure 1**). It is composed of about 70% protein, 15% lipid, and 15% DNA and has a mass of about 66 MDa. The structure of the virion has been studied extensively using electron microscopy and X-ray crystallography (**Figure 2**). X-ray crystallography results have indicated the roles of four proteins in controlling virus assembly. There are 240 hexagonally shaped trimers of the major capsid protein, P3 (**Figure 2(b)**), occupying the surface of the capsid on a pseudo $T = 25$ lattice, an arrangement that is also found in the human adenovirus capsid. In PRD1, a dimer of the linear glue protein P30 extends from one vertex to the next, cementing the P3 facets together (**Figure 2(c)**). At the vertex, pentamers of P31 interlock with P3 and the transmembrane protein P16.

The majority of the PRD1 vertices have two proteins attached to them: one is a trimer of P5 (**Figure 3(a)**) attached by the N-terminus, the other is a monomer of P2 (**Figure 3(b)**), the receptor-binding protein. Both P5 and P2 are elongated molecules (P5 is 17 nm long, P2 is 15.5 nm long). P2 is a club-shaped molecule with a pseudo β-propeller head and a long tail formed from extended β-sheet. The head is proposed to be the site of

Table 1 PRD1 genes, corresponding proteins, and protein functions

Gene	*Protein*	*Mass (kDa)*	*Description*[a]
I	P1	63.3	DNA polymerase (N)
II	P2	63.7	Receptor binding (S)
III	P3	43.1	Major capsid protein (C)
V	P5	34.2	Trimeric spike protein (S)
VI	P6	17.6	Minor capsid protein, DNA packaging (C, P)
VII	P7	27.1	DNA delivery, transglycosylase (L, M)
VIII	P8	29.5	Genome terminal protein (N)
IX	P9	25.8	Minor capsid protein, DNA packaging ATPase (C, P)
X	P10	20.6	Assembly (A, N)
XI	P11	22.2	DNA delivery (M)
XII	P12	16.6	ssDNA binding protein (N)
XIV	P14	15.0	DNA delivery (M)
XV	P15	17.3	Muramidase (L)
XVI	P16	12.6	Infectivity (M)
XVII	P17	9.5	Assembly (A, N)
XVIII	P18	9.8	DNA delivery(M)
XIX	P19	10.5	ssDNA binding protein (N)
XX	P20	4.7	DNA packaging (M, P)
XXII	P22	5.5	DNA packaging (M,P)
XXX	P30	9.0	Minor capsid protein (C)
XXXI	P31	13.7	Pentameric base of spike (S)
XXXII	P32	5.4	DNA delivery (M)
XXXIII	P33	7.5	Assembly (A, N)
XXXIV	P34	6.7	(M)
XXXV	P35	12.8	Holin (L)

[a](N) nonstructural early protein; (M) integral membrane protein; (S) spike complex protein; (A) assembly protein; (P) packaging protein; (C) capsid protein; (L) lysis protein.

Table 2 PM2 genes, corresponding proteins, and protein functions

Gene	*Protein*	*Mass (kDa)*	*Description*
I	P1	37.5	Spike protein
II	P2	30.2	Major capsid protein
III	P3	10.8	Membrane protein
IV	P4	4.4	Membrane protein
V	P5	17.9	Membrane protein
VI	P6	14.3	Membrane protein
VII	P7	3.6	Membrane protein
VIII	P8	7.3	Membrane protein
IX	P9	24.7	Potential ATPase
X	P10	29.0	Membrane protein
XII	P12	73.4	Replication initiation protein
XIII	P13	7.2	Transcription factor
XIV	P14	11.0	Transcription factor
XV	P15	18.1	Transcription factor
XVI	P16	10.3	Transcription factor
XVII	P17	6.0	Lysis
XVIII	P18	5.7	Lysis

receptor binding, lying distal to the virus. A specific vertex is used for packaging the phage DNA.

The overall size and structure of the phage Bam35 is very similar to that of PRD1. However, the exact counterparts of many of the PRD1 structural proteins have not yet been clearly identified. One of the major differences between PRD1 and Bam35 is the presence of a large transmembrane protein complex in Bam35 that modulates the curvature of the membrane under the capsid facets. Also, the host cell recognizing spike complex is likely to be different.

The corticovirus PM2 particle is icosahedral and measures 77 nm in diameter from spike to spike. The capsid is approximately 60 nm in diameter. Like the tectiviruses, it does not have a tail. The mass of the virion is $\sim 4.5 \times 10^7$ Da and it is composed of protein (72%), lipid (14%), and DNA (14%). The capsid is composed of 200 trimers of the major capsid protein arranged on a pseudo $T = 21$ lattice. Pentameric receptor-binding spikes protrude from the vertices.

Membrane and DNA

In PRD1, about half of the virion proteins are associated with the membrane (**Table 1**). The lipid headgroups are predominantly phosphatidylethanolamine (53%) and phosphatidylglycerol (43%) with 4% of cardiolipin. The membrane is well ordered, following the icosahedral outline of the capsid. Many interactions occur between the membrane and both the capsid and the underlying DNA. The average separation of the concentric layers of DNA is approximately 2.5 nm, similar to that found in other bacteriophages and animal viruses. Removal of the capsid and

spike proteins by heat or guanidinium hydrochloride treatment results in aggregation of the membrane vesicle.

In PM2, the membrane, lying underneath the capsid, follows the shape of the capsid as in PRD1, and there are many interactions mediated by additional minor proteins. The lipid composition is approximately 64% phosphatidylglycerol, 27% phosphatidylethanolamine, and 8% neutral lipids and a small amount of acyl phosphatidylglycerol. Release of the capsid and spike proteins from the virion by freeze–thawing or by chelation of calcium ions with ethylene glycol tetraacetic acid (EGTA) results in a soluble vesicle called the lipid core.

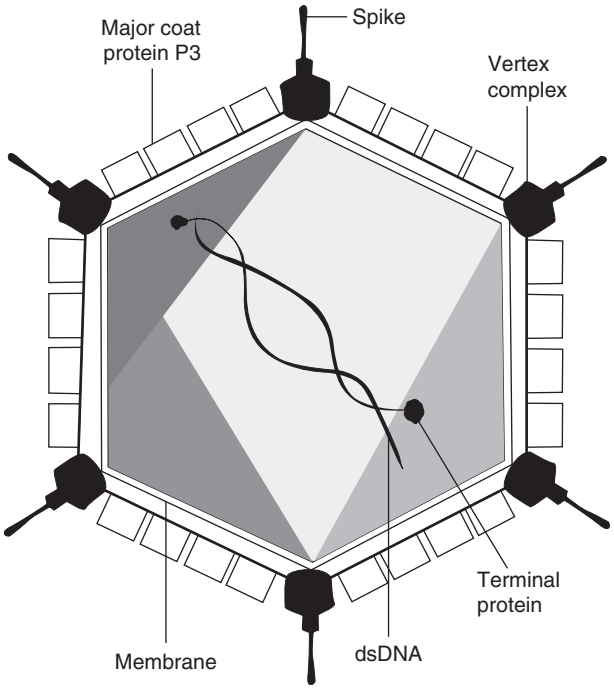


Figure 1 Schematic presentation of PRD1 virion.

Life Cycle

PRD1 is a lytic phage that exploits the transcription functions of its host. The host cell is selected by specific recognition of a plasmid-encoded receptor on the cell surface. PRD1 belongs to the class of broad-host-range, donor-specific phages, which infect cells only when an IncP-, IncN-, or IncW-type multiple-drug resistance conjugative plasmid is present. The primary function of the receptor is in bacterial conjugation. Among the hosts are several opportunistic human pathogens such as *Escherichia coli*, *Salmonella enterica*, and *Pseudomonas aeruginosa*. After adsorption, the phage genome is injected into the cell cytosol, leaving the capsid outside. After the production of the phage components, both virus- and host-encoded factors assist in particle assembly. Host cell lysis releases some 500 progeny viruses. Bam35 is a temperate phage, either growing lytically like PRD1, or existing in a dormant state within the host. In contrast to PRD1, the host range of Bam35, and its relatives GIL01 and GIL16, is limited to one species, *Bacillus thuringiensis*. AP50 infects *Bacillus anthracis*, and a defect phage replicating as a linear plasmid (pBClin15) has been described for *Bacillus cereus*. The corticovirus PM2 is lytic. Of all the dsDNA phages with an internal membrane, the life cycle of PRD1 is best understood and is described below.

Receptor Recognition

A single phage structural protein, P2, is responsible for PRD1 attachment to its host. Each of the PRD1 receptor recognition vertices is a metastable structure and possibly capable of DNA release. The injection vertex is likely to be determined by P2 binding to the receptor. The association of P2 with the receptor activates, possibly by P2 removal, the injection process. This leads to irreversible binding. Both empty and DNA-containing particles are

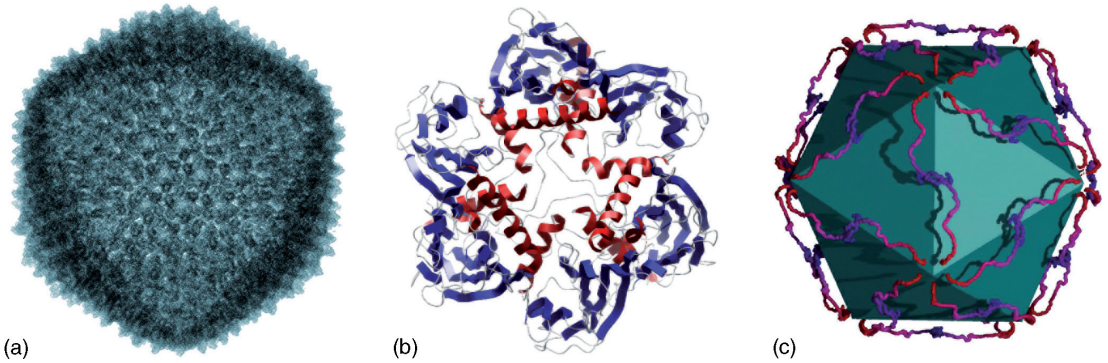


Figure 2 Structure of the icosahedral PRD1 capsid based on X-ray crystallography. (a) Electron density of the PRD1 virion at 4 Å resolution. (b) The major coat protein P3, top view. P3 has a double β-barrel fold, resulting in a hexameric shape of the trimer. (c) The structure of the virion revealed the location of the cementing protein P30 at the twofold positions. The icosahedron is representing the viral membrane. Dimers of P30 are lying underneath the capsid stabilizing the virion structure.

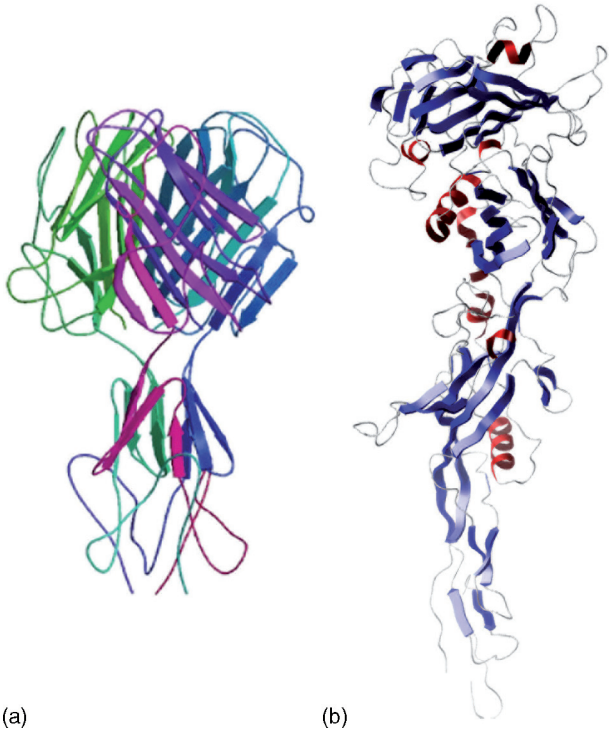


Figure 3 Structure of the vertex proteins of PRD1 based on X-ray crystallography. (a) The trimeric spike protein P5. (b) The monomeric receptor-binding protein P2. (a) Reproduced from Merckel MC, Huiskonen JT, Bamford DH, Goldman A, and Tuma R (2005) The structure of the bacteriophage PRD1 spike sheds light on the evolution of viral capsid architecture. *Molecular Cell* 18: 161–170, with permission from Elsevier.

bound equally tightly to cells, indicating that DNA injection is not a prerequisite for this tight interaction.

DNA Entry

Isolation and analysis of PRD1 mutants have resulted in the identification of eight, phage-specific, structural proteins essential for infectivity. In addition to the spike-complex proteins (P2, P5, and P31) needed for adsorption, protein P11 starts the DNA delivery process and membrane proteins P14, P18, and P32 are involved in later stages of the DNA delivery. Mutant particles missing protein P7 (a lytic transglycosylase) are infectious but the DNA entry process is delayed.

The PRD1 membrane can undergo a structural transformation from a spherical vesicle to a tubular form. A similar process has been described for Bam35 and AP50, thus occurring in all members of the *Tectiviridae*. This tube formation might be required for DNA translocation.

Genome Replication

The genome of PRD1 is a linear double-stranded DNA molecule with proteins covalently attached to both 5′ termini and having 110 bp terminal repeats. PRD1 replicates its DNA by a protein-primed replication mechanism similarly to other viruses with linear dsDNA genomes, including adenovirus and the ϕ29-type phages. PRD1 DNA replication starts with the formation of a covalent bond between the genome terminal protein, P8, and the 5′ terminal nucleotide, dGMP, in a reaction catalyzed by the phage DNA polymerase, P1. The minimal origin of replication resides in the 20 first-terminal base pairs of both genome ends, and the fourth base from the 3′ end of the template directs, by base complementation, the linking of deoxyribonucleoside monophosphate (dNMP) to the terminal protein. The 3′ end DNA sequence is maintained by sliding back of the polymerase complex.

After initiation, elongation of the initiation complex by the same DNA polymerase takes place resulting in the formation of full-length daughter DNA molecules. Two phage-encoded single-stranded DNA binding proteins, P12 and P19, are involved in replication *in vivo*.

Particle Assembly

Approximately 15 min post infection, the major capsid protein P3 and the spike-complex proteins P2, P5, and P31 are found soluble in the host cell cytosol, whereas the phage-encoded membrane proteins (e.g., P7, P11, P14, and P18) are addressed to the host cell cytoplasmic membrane (CM). Correct folding of the soluble proteins and assembly of a number of viral membrane proteins are dependent on the host GroEL/ES chaperonins. Upon assembly, a virus-specific patch from the host CM is translocated into the forming procapsid using the membrane-bound scaffolding protein P10. In addition, two small phage-encoded proteins are implicated in the assembly process: P17 and P33.

Correct assembly results in an empty capsid enclosing a membrane rich in phage-specific proteins. The linear double-stranded DNA genome is packaged into the prohead by the packaging ATPase P9. Unlike packaging ATPases of most other icosahedral dsDNA bacteriophages, P9 is part of the mature virus structure. It resides at a single vertex that also contains proteins P6, P20, and P22. P6 is a soluble protein needed for efficient DNA packaging and the latter two are integral membrane proteins connecting the portal structure to the viral membrane.

Cell Lysis

At the end of the infection cycle, the newly synthesized progeny virions are released via host cell lysis. Two genes, *XV* and *XXXV*, are involved in this step, which means that a two-component, holin–endolysin system operates in phage PRD1. The product of gene *XV*, protein P15, is a soluble β-1,4-*N*-acetylmuramidase that degrades the

peptidoglycan of the Gram-negative cell causing host cell lysis. The PRD1 particle carries another muramidase, protein P7, which has a lytic transglycosylase activity assisting in genome entry. The presence of two lytic activities probably reflects the broad host range of PRD1.

In addition to lytic enzymes, bacteriophages quite often encode helper protein factors (holins) that facilitate the access of lytic enzymes to the susceptible bond in the cell wall and control the timing of lysis. The PRD1 holin is protein P35.

Genomes and Genomics

The length of the PRD1 genome is 14 927 bp and the guanine–cytosine (GC) content is 48.1%. It has 110 bp long inverted repeat sequences at the ends, which are 100% identical. The genomes of the other Gram-negative bacteria-infecting tectiviruses are very close to that of PRD1, varying between 14 935 and 14 954 bp. They are remarkably similar in nucleotide sequence, the overall identity being 91.9–99.8%. The Bam35 genome is 14 935 bp long with a GC content of 39.7%. The inverted repeat for Bam35 is 74 bp long. These Bam35 inverted terminal repeats (ITRs) have 81% identity. PRD1 and Bam35 do not share much sequence similarity. The discovery of the almost invariant genomes for the two *Tectiviridae* groups contrasts sharply with the situation in the tailed bacteriophages. The nucleotide identity is 100% between Bam35 and GIL01 and 83% between GIL01 and GIL16.

PRD1 genome is organized into two early and three late operons (**Figure 4**). Bam35 operons have not been mapped, but the gene order and the length of the genes are similar to those of PRD1 (**Figure 4**). Although there is no overall sequence similarity between PRD1 and Bam35 genomes, Bam35 genes for DNA polymerase, packaging ATPase, and lytic enzyme can be recognized by corresponding conserved amino acid sequence motifs in the databases. The coat protein gene can be identified by comparing the N-terminal amino acid sequence of the major virion protein to the DNA sequence. The only genes of Bam35, which seem to be in different positions in the genome compared to PRD1, are those responsible for the host cell recognition (the spike protein).

The circular PM2 genome is 10 079 bp long with a GC content of 42.2%. It contains 21 putative genes, of which 17 have so far been shown to be functional. Promoter mapping by primer extension has revealed three operons, which are expressed in a timely fashion during infection. The first early operon is highly similar to the maintenance region of the *Pseudoalteromonas* plasmid pAS28. The second early operon contains genes for DNA replication and regulation of late phage functions. The PM2 genome replicates via a rolling-circle mechanism. Protein P12 has conserved sequence motifs common to superfamily I replication initiation proteins. This superfamily consists of the A proteins of certain bacteriophages, such as ϕX174 and G4, and the initiation proteins of cyanobacterial and archaeal plasmids. The function of the late operon is activated by two phage-encoded transcription factors, P13 and P14. P14 has sequence similarity to the TFIIS-type general eukaryotic transcription factors most closely resembling those of the archaeal organisms *Thermococcus celer* and *Sulfolobus acidocaldaricus*.

The structural proteins encoded by the late genes are, similar to the corresponding proteins of tectiviruses, either membrane associated or soluble. Based on the conserved amino acid sequence motifs deduced from the nucleotide sequence, one of the structural virion proteins

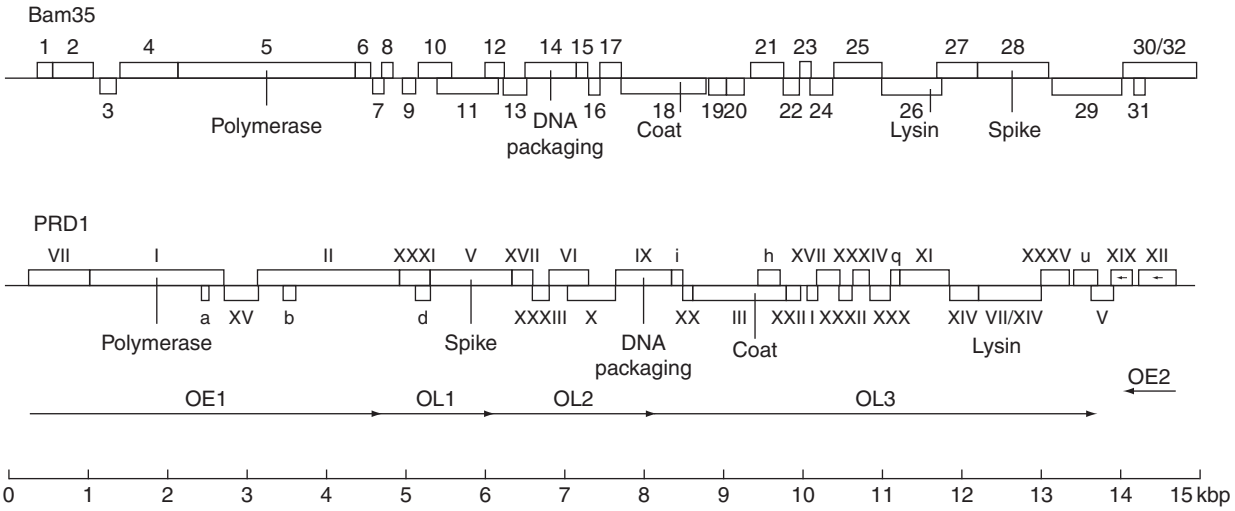


Figure 4 Alignment of the genomes of Bam35 (top) and PRD1 (middle). The two early (OE) and three late (OL) operons of PRD1 are shown at the bottom. In Bam35, the open reading frames (ORFs) are marked by Arabic numerals from left to right. PRD1 ORFs have a Roman numeral if they have been shown to encode protein, otherwise they are marked with a lowercase letter.

is a putative packaging ATPase. The packaging process of the circular supercoiled PM2 DNA is not understood at the moment.

See also: Capsid Assembly: Bacterial Virus Structure and Assembly; Genome Packaging in Bacterial Viruses; Lysis of the Host by Bacteriophage; Replication of Bacterial Viruses; Transcriptional Regulation in Bacteriophage; Virus Evolution: Bacterial Viruses.

Further Reading

Abrescia NGA, Cockburn JJB, Grimes JM, *et al.* (2004) Insights into assembly from structural analysis of bacteriophage PRD1. *Nature* 432: 68–74.

Bamford DH (2005) Family *Tectiviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 81–85. San Diego, CA: Elsevier Academic Press.

Bamford DH and Bamford JKH (2006) Lipid-containing bacteriophage PM2, the type-organism of *Corticoviridae*. In: Calendar R (ed.) *The Bacteriophages*, pp. 171–174. New York: Oxford University Press.

Bamford JKH (2005) Family *Corticoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 87–90. San Diego, CA: Elsevier Academic Press.

Benson SD, Bamford JKH, Bamford DH, and Burnett RM (1999) Viral evolution revealed by bacteriophage PRD1 and human adenovirus coat protein structures. *Cell* 98: 825–833.

Benson SD, Bamford JKH, Bamford DH, and Burnett RM (2004) Does common architecture reveal a viral lineage spanning all three domains of life? *Molecular Cell* 16: 673–685.

Cockburn JJB, Abrescia NGA, Grimes JM, *et al.* (2004) Membrane structure and interactions with protein and DNA in bacteriophage PRD1. *Nature* 432: 122–125.

Grahn AM, Butcher SJ, Bamford JKH, and Bamford DH (2006) PRD1-dissecting the genome, structure and entry. In: Calendar R (ed.) *The Bacteriophages*, pp. 161–170. New York: Oxford University Press.

Grahn AM, Daugelavicius R, and Bamford DH (2002) Sequential model of phage PRD1 DNA delivery: Active involvement of the viral membrane. *Molecular Microbiology* 46: 1199–1209.

Merckel MC, Huiskonen JT, Bamford DH, Goldman A, and Tuma R (2005) The structure of the bacteriophage PRD1 spike sheds light on the evolution of viral capsid architecture. *Molecular Cell* 18: 161–170.

Ravantti JJ, Gaidelyte A, Bamford DH, and Bamford JKH (2003) Comparative analysis of bacterial viruses Bam35, infecting a Gram-positive host, and PRD1, infecting Gram-negative hosts, demonstrates a viral lineage. *Virology* 313: 401–414.

Salas M (1991) Protein-priming of DNA replication. *Annual Review of Biochemistry* 60: 39–71.

Icosahedral Enveloped dsRNA Bacterial Viruses

R Tuma, University of Helsinki, Helsinki, Finland

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Carrier state A partial or complete viral genome is maintained inside the host cell as a stable episome without lysis or infection.
Genomic precursor ssRNA destined for packaging.
Genomic segment A piece of genomic dsRNA.
Packaging A process of genomic precursor acquisition.
Pac site A specific signal at 5' end of a genomic precursor which targets it for selective packaging.
Polymerase complex Viral assembly which contains the polymerase and is able to replicate and transcribe the viral genome. Usually it also contains the viral genomic dsRNA.
Procapsid Empty capsid assembly which is capable of acquiring the genomic precursors.
Reverse genetics RNA virus genome manipulation and rescue from cDNA clones.
Viral core dsRNA containing inner part of the virus which is capable of transcription.

Introduction – The *Cystoviridae* Family

Members of the family *Cystoviridae* are lipid-containing bacteriophages with segmented dsRNA genomes. The first recognized member, the bacteriophage φ6, was isolated in 1973. Several other members were discovered in the late 1990s. All known cystoviruses were isolated from the leaves of various plants and consequently infect primarily plant-pathogenic bacteria (pseudomonads). All share a similar architecture with a proteinaceous viral core hosting the three genomic segments (L, M, and S, approx. 6.5, 4, and 3 kbp in size, respectively). The core is enclosed in a lipid envelope which contains the host cell attachment proteins. The viral core contains an RNA-dependent RNA polymerase (RdRP) which plays a central role in RNA metabolism. Despite infecting prokaryotic hosts these phages exhibit structural and functional features that parallel those of the family *Reoviridae*. Because reverse genetics had been developed early on for φ6, the system has been a model for studying the assembly and replication of other dsRNA viruses.

An *in vitro* assembly of infectious nucleocapsids from purified constituents has been achieved for bacteriophages φ6 and φ8. Similarly, RNA packaging and replication have been extensively studied *in vitro*. Together with the reverse genetics system the *in vitro* methods have been instrumental in delineating the virus replication mechanisms. The host entry mechanism mimics that of viruses infecting eukaryotic cells and involves both membrane fusion and an endocytotic-like event. Structural and extensive biochemical characterization of individual proteins and subviral assemblies made the cystoviral system a paradigm for studying mechanisms of molecular machines in atomic details. The self-assembly and replication system shows promise in biotechnology and nanotechnology applications.

Classification and Host Range

Based on their sequences and host range five out of the recently discovered cystoviruses (φ7, φ9, φ10, φ11, φ14) are close relatives of φ6 while the remaining three (φ8, φ12, and φ13) are only distantly related to the φ6 group and among themselves. φ6 and the related phages attach to the host cell via a specific type IV pilus while the members of the second group utilize rough lipopolysaccharides (LPSs). This limits the host range to *Pseudomonas syringae* (*Pseudomonas pseudoalcaligenis* for certain mutants) for the former group while phages belonging to the latter can also infect rough LPS of other Gram-negative bacteria without forming plaques. Only φ8 form plaques on a heptose-less strain of *Salmonella typhimurium*. A common laboratory host of φ6 is *P. syringae* strain HB10Y. The φ8 phage is usually propagated on *P. syringae* strain LM2509.

Virion Structure and Properties

Physico-Chemical Properties

Virion molecular weight (MW, in dalton units, Da) $\sim 9.9 \times 10^7$ Da, sedimentation coefficient S_{20w} ~405S, density 1.24 g cm^{-3} (sucrose), nucleocapsid MW $\sim 4.0 \times 10^7$ Da. Composition: 70% (w/w) protein, 14% dsRNA, 16% phospholipids. RNA packaging density 350 g cm^{-3}.

Architecture of the Polymerase Complex and the Nucleocapsid

Figure 1 depicts schematically the architecture and localization of the proteins for the φ6 virion. The innermost structure is the polymerase complex (PC) which is composed of 120 copies of major structural protein P1 (MW 85 kDa), 12 copies of RdRP P2 (75 kDa), 12 hexamers of packaging motor P4 (subunit MW 35 kD), and 60 copies of assembly factor P7 (17 kDa). Structural details of the dodecahedral P1 skeleton are depicted in **Figure 2**. The $T = 1$ lattice is composed of 60 asymmetric P1 dimers as seen for other dsRNA viruses. However, the arrangement of dimers within the asymmetric unit is different from that seen in the reovirus or bluetongue virus cores. P2 monomers are attached to the inner surface of the PC

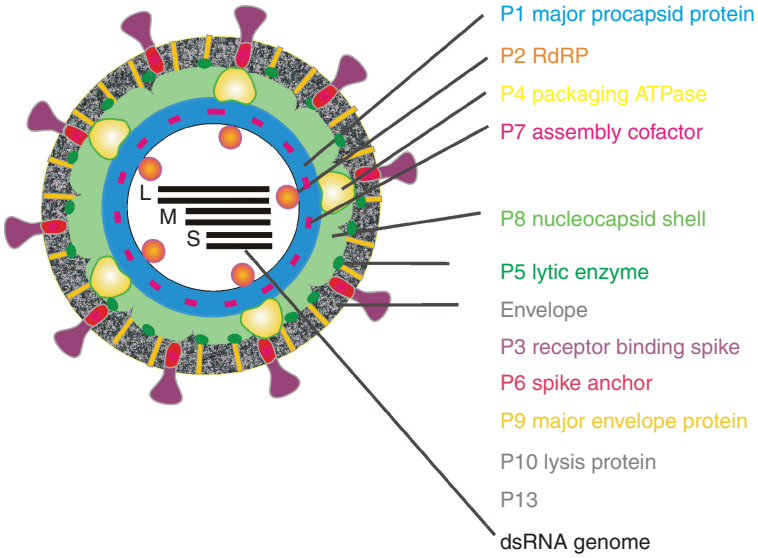


Figure 1 Schematics of φ6 virion architecture. Function of individual proteins: P1-major structural protein of PC, forms the dodecahedral skeleton of the procapsid; P2-RdRP; P3-spike protein, host cell attachment; P4-packaging ATPase, ssRNA translocation; P5-lytic enzyme (lysozyme); P6-integral membrane protein, P3 anchoring and membrane fusion; P7-assembly, packaging and replication cofactor, PC stabilization; P8-nucleocapsid coat protein; P9-membrane protein; P10-host cell lysis, perhaps holin; P11-membrane protein (most likely nonessential).

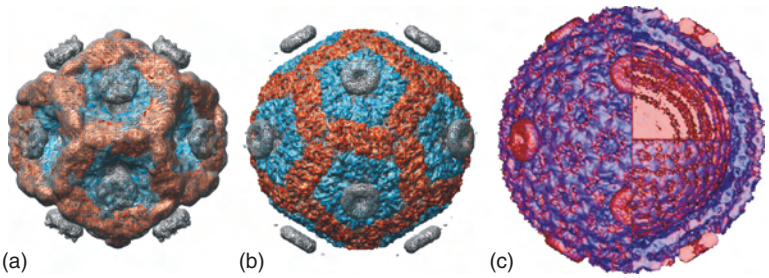


Figure 2 Structure of the polymerase complex and the nucleocapsid of φ6 as revealed by electron cryo-microscopy. (a) Modeled empty PC (void of RNA, precursor for packaging) which is also called the procapsid. (b) The $T = 1$ (triangulation number) shell of the expanded, mature, PC which contains dsRNA genome and is transcription competent. (c) The $T = 13$ P8 shell which makes the outer surface of NC together with the protruding P4 hexamers. P4 is gray, the two P1 monomers in the asymmetric dimer are colored red and blue, respectively. P8 shell is rendered purple. Reproduced from Huiskonen JT, de Haas F, Bubeck D, Bamford DH, Fuller SD, and Butcher SJ (2006) Structure of the bacteriophage φ6 nucleocapsid suggests a mechanism for sequential RNA packaging. *Structure* 14: 1039–1048, with permission from Elsevier.

shell, possibly at the fivefold vertices. P4 hexamers form turret-like protrusions on the outer surface of the fivefold vertices. Location of the P7 within the PC is not known.

PC undergoes a series of large conformational changes (expansions) during RNA packaging and genome replication. The empty dodecahedral PC (procapsid) expands into a more round, icosahedral dsRNA-filled PC, which is sometimes called the viral core. The expansion consists of motions of whole P1 subunits around hinges that run roughly parallel with the twofold edges of the dodecahedral skeleton (**Figure 2**). The expansion increases the inner volume by a factor of 2.4 and makes room for the dsRNA genome. The mature PC is subsequently enclosed in an icosahedral shell ($T = 13$) made of 200 P8 (subunit MW 16 kDa) trimers (**Figure 2**). The resulting structure is called the nucleocapsid (NC). The packaged dsRNA genome is arranged in concentric shells (layers) with average spacing 31 Å. This is larger than the 24–26 Å spacing which has been observed for other dsDNA and dsRNA viruses and reflects the lower packaging density.

The structures of P1 (PC) and P8 (NC) shells are related to the structure of dsRNA containing cores isolated from viruses belonging to the family *Reoviridae*. However, the empty packaging competent precursors have not been detected nor has a conformational change akin to the PC expansion been observed for the latter viruses.

Structure of Individual Proteins

P1

Current structural model of φ6 P1 monomer is based on high-resolution (7.5 Å) electron cryo-microscopy (**Figure 3**). The structure is composed of α-helices that are packed together to form a flat sheet. The few remaining densities have been tentatively assigned to β-sheets. The exact tracing of the polypeptide chain was not possible at this resolution; however, the two conformers in the asymmetric unit were clearly resolved.

P2

Several structures of φ6 P2 alone and in complex with various substrates were solved by X-ray diffraction. P2 is a compact, globular, monomer with α/β fold (**Figure 3**) with a domain arrangement resembling a hand (i.e., palm, fingers, and thumb domains). The fold is similar to those of other viral polymerases, for example, hepatitis C virus and HIV reverse transcriptase. RNA substrate binds in a template channel and the putative dsRNA exit site is partially occluded by the C-terminal domain in the crystal structure (**Figure 3**).

P4

The structures of φ12 P4 hexamer in apo and nucleotide-bound forms have been solved by X-ray diffraction (**Figure 3**). The hexamer has a dome-like structure with a central channel through which RNA passes during packaging. Six equivalent ATP binding sites are located between the subunits at the hexamer periphery. The subunit structure encompasses a catalytic core which is conserved among many ATPases, including Rec-A and hexameric helicases. P4 hexamer interacts with the procapsid dodecahedral framework predominantly via its C-terminal facet.

P8

The structure of the φ6 P8 trimer was derived by fragmentation of the cryo-EM density map at 7.5 Å resolution. The P8 subunit is highly α-helical. The trimer is flat (only 25 Å thick, 75 Å effective diameter) and each subunit is composed of the central, four-helix bundle core and a peripheral four helix bundle (**Figure 3**).

Membrane Envelope

Lipids are derived from the host cytoplasmic membrane but the viral envelope is enriched in phosphatidylglycerol and contains less phosphatidylethanolamine presumably due to high viral membrane curvature and charge repulsion.

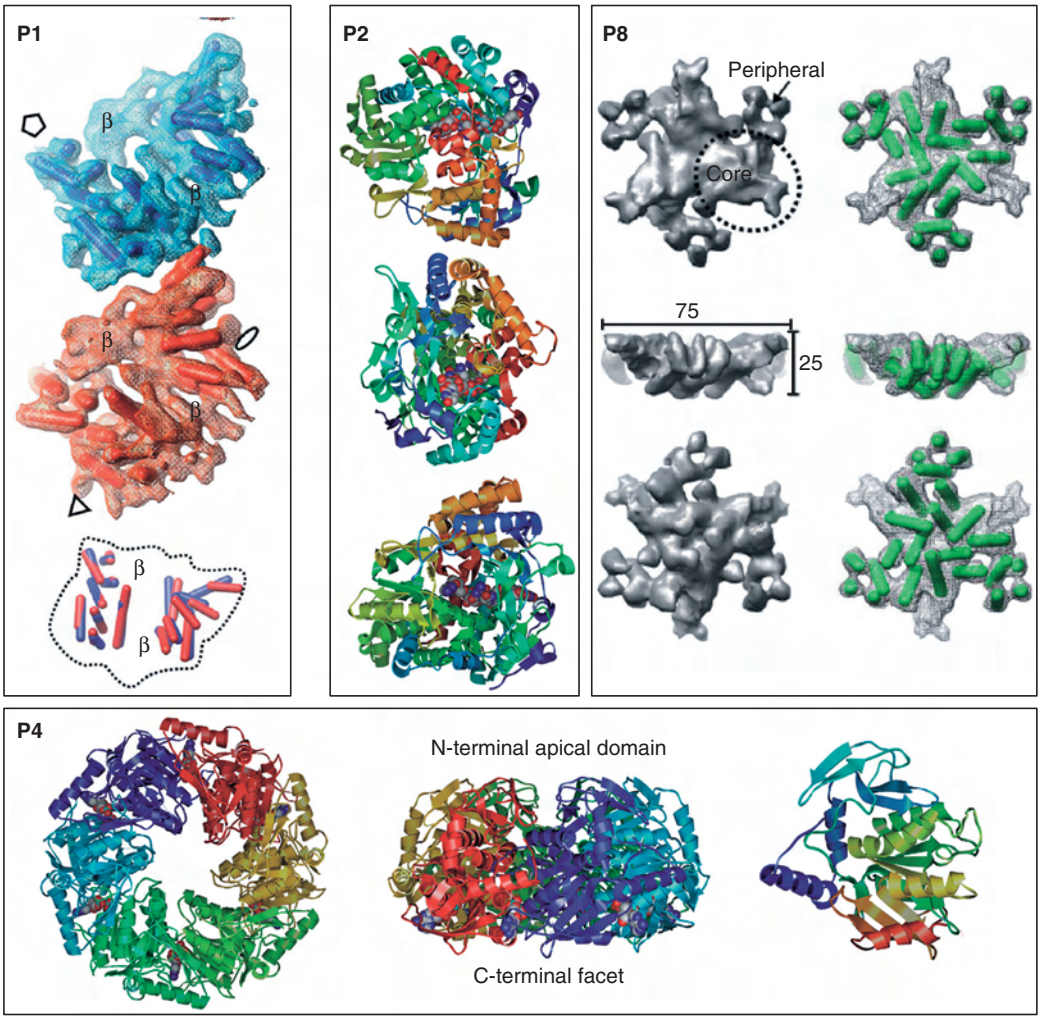


Figure 3 Structure of individual cystoviral proteins. (P1) φ6 P1 structure was derived from cryo-EM. The electron densities of the two conformers found in the asymmetric unit (see **Figure 2**) are shown in blue and red, respectively. Bottom panel shows the tentative secondary structure assignment to the electron density. Reproduced (P2) Ribbon diagram of P2 polymerase structure from phage φ6 in complex with RNA (spacefill representation). Based on PDB id 1UVI, Salgado *et al*. (2004) *Structure* 12: 307–316. Top view shows the C-terminal domain (red) blocking the RNA exit site. Middle panel displays view down the RNA template channel. Bottom panel exhibits view of the template RNA through the substrate channel. Ribbon coloring from N-terminus (blue) to C-terminus (red). (P4) P4 hexamer, the packaging motor (ATPase) structure solved by X-ray diffraction. Based on PDB id 1W44, Mancini EJ, Kainov DE, Grimes JM, Tuma R, Bamford DH, and Stuart DI (2004) Atomic snapshots on an RNA packaging motor reveal conformational changes linking ATP hydrolysis to RNA translocation. *Cell* 118: 743–755. Left panel: top view down the central channel, subunits in different colors. Middle: Side view. Right: One subunit colored from N-terminus (blue) to C-terminus (red). (P8) P8 structure derived from cryo-EM. Reproduced from Huiskonen JT, de Haas F, Bubeck D, Bamford DH, Fuller SD, and Butcher SJ (2006) Structure of the bacteriophage φ6 nucleocapsid suggests a mechanism for sequential RNA packaging. *Structure* 14: 1039–1048, with permission from Elsevier.

Lipids constitute about 40% of membrane mass while the remaining 60% are membrane proteins. The membrane is loosely associated with the nucleocapsid and the membrane-associated proteins lack icosahedral symmetry.

Genome Organization and Sequence Similarity

The φ6 genome (13385 bp) is organized into three segments (**Figure 4**): L (6374 bp in φ6) codes for the procapsid structural proteins, M (4063 bp in φ6) codes for the spike and membrane proteins, and S (2948 bp in φ6) codes for nucleocapsid and membrane proteins. The (coding) plus strand of each segment contains a short conserved sequence at the 5′-end. This sequence is followed by a packaging signal that is unique to each segment (*pac* site, about 200 nt). The dsRNA genome remains sequestered inside the PC throughout the viral life cycle and only the plus strands are extruded into the cytoplasm where they serve as messenger RNAs or packaging precursors.

Two genes, 12 and 14, code for the two nonstructural proteins identified thus far. While the former is essential for membrane morphogenesis, the latter is dispensable under laboratory conditions.

Genomic maps of the other cystoviruses are similar to φ6 with several exceptions. The viruses which attach directly to rough LPS (φ8, 12, 13) possess several separate genes for the receptor binding spike structure (3a, 3b, and 3c in φ12 and φ13). In addition, gene 7 is positioned after gene 1 in the φ8 L-segment and its expected place is occupied by ORF H. The functions of the nonessential ORFs F, G, and H are not known.

Identity at the amino acid sequence level is high among φ6, 7, 9, and 10, while φ8, 12, and 13 exhibit only limited sequence similarity to φ6 and among themselves. The limited similarity is restricted to the conserved motifs of the polymerase and the packaging ATPase. Sequence similarities for the structural proteins P1 and P7 are generally statistically insignificant for the latter group of phages despite conservation of the procapsid structure. However, high sequence identity detected in restricted regions (e.g., lysis proteins of φ12 and φ6, identical 3′-end of M segments in φ6 and φ13) indicate significant genetic interaction (e.g., recombination) among cystoviruses.

Replication Cycle

The virus life cycle is schematically illustrated in **Figure 5**. The steps were elucidated for φ6 but many features are likely to be similar for the other cystoviruses.

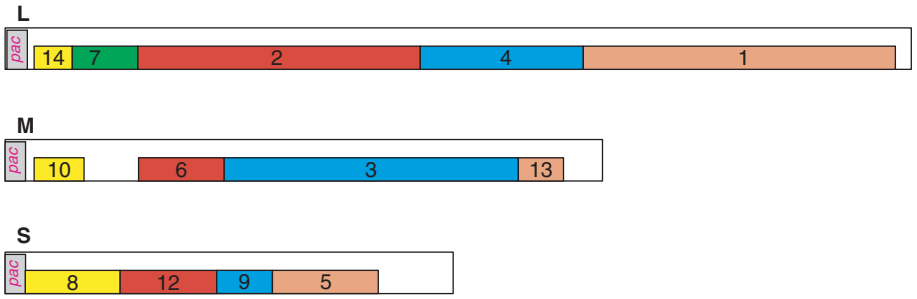


Figure 4 The phi6 genome map showing the locations of genes (gene numbers) and the packaging signal sequences (*pac*).

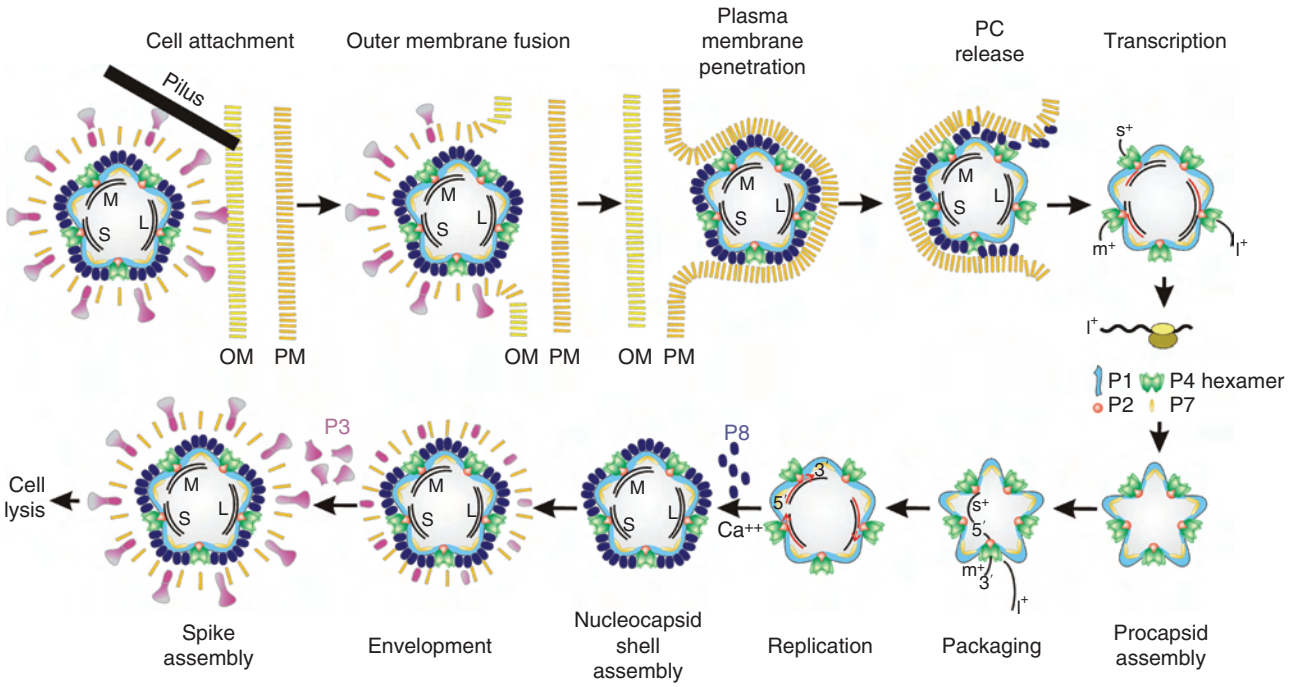


Figure 5 Life cycle diagram of φ6. See the text for details.

Host Cell Attachment and Outer Membrane Fusion

The φ6 and related phages attach to the pilus using the P3 spike protein. The pilus contracts and brings the virion into contact with the outer cell membrane (OM). The φ8, 12, 13 phages utilize a spike composed of proteins P3a, P3b, and P3c (missing in φ8) to attach directly to the rough LPS. The viral envelope then fuses with the OM using the virion fusogenic protein P6. Then the virion-associated lysozyme (P5) degrades the peptidoglycan layer to allow direct contact of the nucleocapsid with the plasma membrane (PM).

Plasma Membrane Penetration

The φ6 nucleocapsid is first enclosed in an endocytic-like vesicle which is brought into the cytoplasm. The NC coat protein P8 disassembles and disrupts the membrane vesicle in order to release the PC into the cytoplasm. The mechanism of the P8-assisted release is still elusive.

Transcription

The depolymerization of the P8 layer activates the PC to perform transcription of the dsRNA genome which produces the plus-strand copies of the three segments (s^+, m^+, l^+). These copies are made inside the PC by the P2 polymerase using a semiconservative mechanism. Several polymerase molecules may engage each segment simultaneously. The resulting RNAs are extruded via P4 hexamers into the cytoplasm where they serve as mRNA for the synthesis of phage proteins and as ssRNA precursors for packaging. Transcription of the three segments is regulated, s^+ and m^+ being produced in a several-fold excess over l^+. The regulation is due to specific interactions between the 3′-end of the minus strand and the polymerase.

PC assembly

The major structural protein P1 co-assembles with P2, P4, and P7 into empty PC (procapsid). The assembly is nucleated by a P1–P4 (P1–P2 in φ8 phage) interaction and intermediates are further stabilized by protein P7 (a 'glue' protein).

Packaging

The unique *pac* sites at the 5′-end of RNA plus strands are specifically recognized in a sequential fashion by the assembled P1 framework. This initial and selective binding brings the 5′-end to the vicinity of the packaging ATPase (P4) hexamer. The P4 ring opens and topologically encloses the RNA inside the central channel. The presence of RNA stimulates the ATPase activity. P4 translocates the ssRNA in the 5′–3′ direction into the procapsid at the expense of ATP hydrolysis. The sequential packaging order is accomplished by increasing the PC affinity for m^+ (l^+) *pac* sequence after packaging of s^+ (m^+). This switching is mediated by the expansion which is in turn driven by the increased amount of the packaged RNA (cf. the headfull packaging of dsDNA bacteriophages). The *in vivo* packaging fidelity is high. However, specific *in vitro* packaging conditions may result in acquisition of multiple s^+ segments or of m^+ segment in the absence of prior packaging of s^+. A heterologous RNA can be packaged by φ8 PCs and the sequential dependence of *in vitro* packaging is weak for this phage.

Replication and NC Maturation

During φ8 or after φ6 packaging, the polymerase performs replication (minus-strand synthesis) of ssRNA into the genomic dsRNA. P2 polymerase interacts with a specific sequence at the 3′-end of each plus strand to initiate the self-primed reaction and makes a full copy of each packaged template. This reaction doubles the amount of RNA inside the PC and yields the mature icosahedral PC. The mature PC can be coated with a layer of P8 trimers in a calcium-dependent reaction. This yields the nucleocapsid. Alternatively, PC may remain in the transcription mode and produce more plus-strand RNA. In φ8 virus the P8 protein is directly associated with the membrane and consequently the PC becomes directly enveloped. The mature φ8 PC is also infectious to host cell spheroplasts.

Membrane Acquisition and Host Cell Lysis

NC is enveloped with a lipid bilayer, which is derived from the host cell plasma membrane. It contains only the viral membrane proteins. The virion-associated lytic enzyme P5 is also incorporated at this stage. Structural protein P9 and the nonstructural protein P12 are essential and sufficient for membrane envelopment in φ6. These two proteins alone can produce lipid vesicles inside the host cell suggesting that envelopment takes place within the cytoplasm. The envelope is subsequently decorated with P3 receptor binding spike which is anchored by the integral membrane protein P6. Virions are released by host cell lysis which is assisted by phage-encoded lytic proteins P5 and P10.

Recombination

Recombination in the *Cystoviridae* is mostly heterologous requiring only about three identical nucleotides. The mechanism of recombination is template switching. The frequency of recombination is increased by truncating

the 3′-end of one of the packaged plus strands. Such truncation may be a result of cellular nuclease cleavage and yields a poor substrate for replication. Hence, recombination may serve as an effective way of correcting the nuclease damage. In addition, homologous recombination, requiring an identical sequence of about 600 nucleotides, has been demonstrated for the φ8 phage. Another mode of recombination is the exchange of whole genomic segments as also seen for other segmented genome viruses.

Genetic Tools and Applications

Reverse Genetics

A reverse genetics system allows systematic testing of the effects of engineered mutations on specific steps in the phage life cycle. Thus, it greatly facilitates delineation of the viral packaging and replication mechanisms. There are two ways to introduce a foreign gene into the dsRNA genome. (1) By constructing a recombinant ssRNA transcript containing the foreign sequences and a 5′-end *pac* site. This substrate is then packaged into procapsids, replicated and matured into NC *in vitro*. The NCs infect host cell spheroplasts to produce the engineered virus progeny. (2) A more efficient method for reverse genetics is based on electroporation of cDNA plasmids, containing the three genomic segments, into cells expressing either SP6 or T7 RNA polymerase. The foreign sequence may be introduced between the 5′-end *pac* sites and the conserved 3′-end replication sequences of each segment.

In Vitro Assembly, Packaging, and Replication System

φ6 procapsid can be efficiently produced in *Escherichia coli* by simultaneously expressing proteins P1, P2, P4, and P7 from a plasmid containing a cDNA copy of the L segment. These procapsids are capable of packaging and replicating the three genomic precursors *in vitro*. Even under optimal conditions only about 5–10% of these procapsids are successfully packaged *in vitro*. Despite the low efficiency the *in vitro* packaging system has been instrumental in delineating the sequential packaging rules and the replication specificity. Incomplete procapsids (containing proteins P1P4, P1P2P4, P1P4P7, P1P2P7) can be produced in *E. coli* and tested for packaging and polymerase activity or used for assembly of specifically labeled procapsids.

Procapsids can be assembled *in vitro* from purified proteins. The *in vitro* assembly system allowed the identification of the minimum assembly requirements and the nucleation mechanism. The *in vitro* system also allows incorporation of chemically labeled or modified subunits to facilitate biophysical and structural studies.

Carrier State

Bacteriophages φ6 and φ8 can establish a carrier state during which the viral genome (or portion of it) replicates as a stable episome inside the host cell cytoplasm. The stable episome constitutes the phage dsRNA inside polymerase complexes that assemble in the cytoplasm. Consequently, the L segment cDNA coding for the PC protein is required to establish the state. The carrier state is maintained by a selective resistance marker engineered into one of the phage genomic segments (usually M or S).

The carrier state is established by electroporating ColE1 suicide plasmids containing the cDNAs of the phage segments into a pseudomonas host that expresses T7 RNA polymerase. These plasmids cannot replicate in pseudomonads and are used to introduce the phage ssRNA into the cytoplasm.

A carrier state may be used to probe RNA packaging or to select new phage mutants. Given the high number of mutations introduced during the phage RNA replication cycle, the carrier state constitutes a good vehicle for targeted evolution of proteins or RNA with novel functions or a way to produce large quantities of siRNA.

Polymerase Applications

φ6 RdRP has the unique capacity to perform self-primed, primer-independent, replication and transcription of RNA substrates. This feature can be used, for example, to obtain complete copies of viral RNA genomes (amplification), or for primer-independent RNA sequencing. An engineered version of RdRP is commercially available.

See also: Replication of Bacterial Viruses; Virus Entry to Bacterial Cells.

Further Reading

Bamford DH, Ojala PM, Frilander M, Walin L, and Bamford JKH (1995) Isolation, purification, and function of assembly intermediates and subviral particles of bacteriophages PRD1 and φ6. In: Adolph KW (ed.) *Methods in Molecular Genetics, Vol. 6: Microbial Gene Techniques*, pp. 455–474. San Diego: Academic Press.

Butcher SJ, Grimes JM, Makeyev EV, Bamford DH, and Stuart DI (2001) A mechanism for initiating RNA-dependent RNA polymerization. *Nature* 410: 235–240.

Huiskonen JT, de Haas F, Bubeck D, Bamford DH, Fuller SD, and Butcher SJ (2006) Structure of the bacteriophage φ6 nucleocapsid suggests a mechanism for sequential RNA packaging. *Structure* 14: 1039–1048.

Kainov DE, Tuma R, and Mancini EJ (2006) Hexameric molecular motors: P4 packaging ATPase unravels the mechanism. *Cellular and Molecular Life Sciences* 63: 1095–1105.

Makeyev EV and Bamford DH (2000) The polymerase subunit of a dsRNA virus plays a central role in the regulation of viral RNA metabolism. *EMBO Journal* 19: 6275–6284.

Makeyev EV and Bamford DH (2004) Evolutionary potential of an RNA virus. *Journal of Virology* 78: 2114–2120.

Mancini EJ, Kainov DE, Grimes JM, Tuma R, Bamford DH, and Stuart DI (2004) Atomic snapshots on an RNA packaging motor reveal conformational changes linking ATP hydrolysis to RNA translocation. *Cell* 118: 743–755.
Mindich L (1999) Precise packaging of the three genomic segments of the double-stranded-RNA bacteriophage φ6. *Microbiology and Molecular Biology Reviews* 63: 149–160.
Mindich L (1999) Reverse genetics of the dsRNA bacteriophage φ6. *Advances in Virus Research* 53: 341–353.
Mindich L (2005) Phages with segmented double-stranded RNA genomes. In: Calendar R (ed.) *The Bacteriophages,* 2nd edn., pp. 197–207. New York: Oxford University Press.
Poranen MM, Paatero AO, Tuma R, and Bamford DH (2001) Self-assembly of a viral molecular machine from purified protein and RNA constituents. *Moleculer Cell* 7: 845–854.
Poranen MM, Tuma R, and Bamford DH (2005) Assembly of double-stranded RNA bacteriophages. *Advances in Virus Research* 64: 15–43.
Sun Y, Qiao X, and Mindich L (2004) Construction of carrier state viruses with partial genomes of the segmented dsRNA bacteriophages. *Virology* 319: 274–279.

Icosahedral ssDNA Bacterial Viruses

B A Fane, M Chen, and J E Cherwa, University of Arizona, Tucson, AZ, USA
A Uchiyama, Cornell University, Ithaca, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Procapsid A viral assembly intermediate containing a full complement of scaffolding proteins but devoid of genome.
Scaffolding protein A protein that directs the assembly of the virus, found in the procapsid assembly intermediate but not in the mature virion.
S Sedimentation constant in Svedberg units, measured by analytical ultracentrifugation.
T = 1 icosahedron A geometric shape consisting of 20 faces and 12 vertices. A T = 1 virion is composed of 60 viral coat proteins.

History

Bacteriophage øX174, the most well known virus of the family *Microviridae* (micro: Greek for small) was isolated in the 1920s by Sertic and Bulgakov. From the onset, this phage appeared very different from the other bacteriophages isolated during this extensive period of discovery. It was unusually tiny, readily passing through the smallest of ultrafilters. This biophysical characteristic defined its 'race': race X (Roman numeral ten) and the isolate was placed in a vial labeled #174, from which the phage derived its name, race X phage in test tube #174. And there it sat, relatively unperturbed, for decades. The first electron micrographs revealed small, vague isometric particles, vastly different from the tailed morphologies, which came to represent bacteriophages in general. Robert Sinsheimer unraveled the odd nature of the genome in 1959 and øX174 became the first recognized single-stranded (ss) DNA phage. While genetic maps of other phages consisted of orderly linear gene progressions, the øX174 map was most peculiar with genes located within genes. When Sanger and colleagues sequenced the genome, the first one ever sequenced, the complex arrangement of overlapping reading frames was confirmed, so beguiling that many suspected an extraterrestrial origin. As the *New York Times* reported the theory, an advanced race engineered øX174 and disseminated it into the cosmos where it would "persist until the evolution of intelligent life and finally of investigators interested in the genetics of phage." Although attempts were actually made to decipher the hypothesized hidden message, they all failed. However, rumors persist that the code has been broken, it reads, "Behold this marvelous little thing."

This small virus would continue to have a large impact on molecular biology. Arthur Kornberg and colleagues used it to elucidate the molecular mechanism of prokaryotic DNA replication. Masaki Hayashi and colleagues defined the øX174 assembly pathway and were the first to demonstrate that viral DNA packaging could be achieved *in vitro*. As the genome sequence initially defied imagination in 1978, the atomic structure of the viral procapsid, the first such structure solved, beguiled structural virologists, with its unusual external scaffolding protein lattice. And when events were at their most bizarre, the skeletons in the family *Microviridae* closet emerged, the gokushoviruses (Japanese for very small). These viruses escaped detection for decades, hiding out in their obligate intracellular parasitic bacterial hosts.

Virion Morphology and Genome Content

All members of the family *Microviridae* have ssDNA circular genomes of positive polarity and a T = 1 icosahedral capsid. The bulk of the capsid is composed of 60 copies of a major capsid protein (**Figure 1** and **Table 1**), which exhibits the common β-barrel motif found in most icosahedral virion capsid proteins. In addition to the major capsid protein, microviruses also contain 60 copies each of the major spike protein G and DNA binding protein J. The fourth structural protein, the DNA pilot protein, is buried within vertex channels running through the

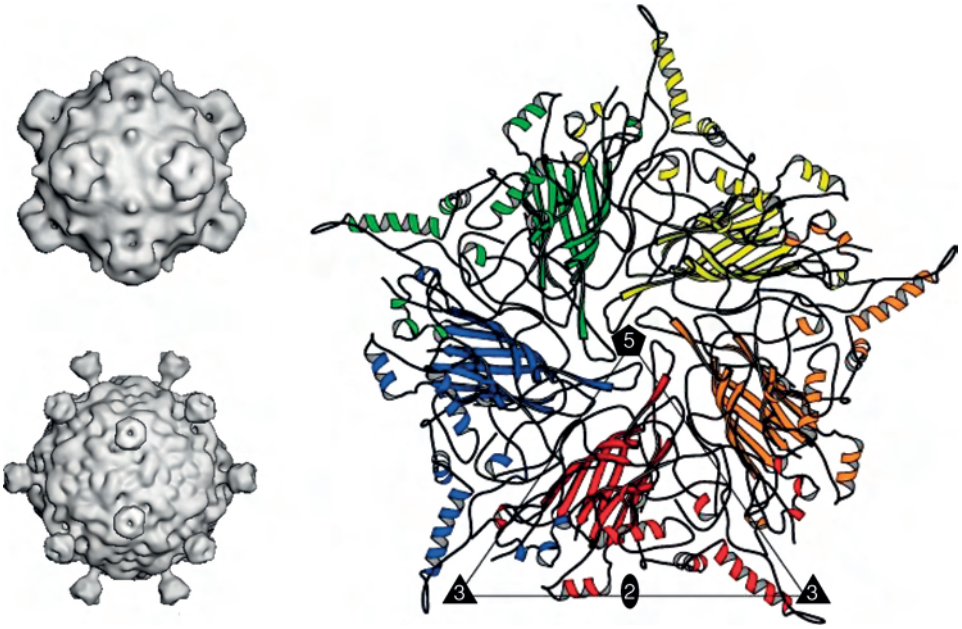


Figure 1 Virion structures. Top left, cryoelectron microscopy (cryoEM) image reconstruction of microvirus ϕX174. Bottom left, cryoEM of gokushovirus SpV4. Right, the arrangement of five ϕX174 coat proteins around a fivefold axis of symmetry. Numbers indicate axis of symmetry and the number of coat proteins that would be interacting at that axis.

Table 1 Microviridae gene products

Microvirus protein	*Gokushovirus protein*	*Function*
A	VP4	Stage II and stage III DNA replication.
A*	UD[a]	An unessential protein for viral propagation. It may play a role in the inhibition of host cell DNA replication and superinfection exclusion.
B	VP3	Internal scaffolding protein, required for procapsid morphogenesis and the assembly of early morphogenetic intermediates. Sixty copies present in the procapsid.
C	VP5?[b]	Facilitates the switch from stage II to stage III DNA replication. Required for stage III DNA synthesis.
D	NP[c]	External scaffolding protein, required for procapsid morphogenesis. 240 copies present in the procapsid.
E	UD	Host cell lysis.
F	VP1	Major coat protein. Sixty copies present in the virion and procapsid.
G	NP	Major spike protein. Sixty copies present in the virion and procapsid.
H	VP2?	DNA pilot protein needed for DNA injection, also called the minor spike protein. Twelve copies present in the procapsid and virion.
J	VP8?	DNA binding protein, needed for DNA packaging, 60 copies present in the virion.
K	UD	An unessential protein for viral propagation. It may play a role optimizing burst sizes in various hosts.

[a]UD, undetermined.
[b]indicates a hypothesized function based on bioinformatic data.
[c]NP, not present in gokushoviruses.

fivefold axes of symmetry. Particles lacking this protein extrude DNA from these locations. Protein H stoichiometry varies among the microviruses, in which there are three major clades represented by bacteriophages G4, α3, and øX174. In øX174, there are 10–12 H proteins per virion, while in bacteriophage α3 only 4–6 copies are required for viability.

Bacteriophage øX174 typifies the microvirus morphology (**Figure 1**), in which 70 Å-diameter G protein pentamers adorn the fivefold axes of symmetry, rising 30 Å from the surface of the 250 Å-diameter capsid. The gokushoviruses lack major spike proteins: hence, the fivefold axes of symmetry are not decorated. The cryoelectron microscopy image reconstruction of gokushovirus SpV4 reveals mushroom-shaped protrusions at the threefold axes of symmetry, which rise 54 Å above the surface of the 270 Å-diameter capsids (**Figure 1**). These threefold related structures appear to be composed of amino acid sequences of three interacting coat proteins. The gokushovirus VP2 is also a structural protein. Although its function has not been experimentally defined, bioinformatic approaches suggest it is analogous to the microvirus DNA pilot protein H. These proteins share a predicted N-terminal transmembrane and a central coiled-coil domain. VP8 may be analogous to the DNA binding protein J, both highly basic short peptides. However, it has not yet been detected in a gokushovirus virion. This may be due to the small size and the difficulties associated with propagating large quantities of gokushoviruses for biochemical characterization.

The genetic maps of microvirus øX174 and gokushovirus Chp2 are depicted in **Figure 2**. Gokushoviral genomes encode neither external scaffolding D nor major spike G proteins. The absence of these genes accounts for the smaller genomes. The external scaffolding protein has at least three known functions in øX174 morphogenesis. It mediates procapsid assembly, stabilizes the assembled procapsid at the two- and threefold axes of symmetry, and directs the placement of the major spike protein G pentamers into the fivefold related capsid craters. These functions are either not required or performed by different proteins in the gokushoviruses. In the gokushoviruses, procapsid assembly is most likely mediated by the internal scaffolding protein VP3, as is seen with the øX174 internal scaffolding protein B. Despite the small compact nature of microvirus genomes, two proteins A* and K with obscure or unknown functions are very strongly conserved. Three additional small ORFs are found in the H-A intercistronic region of the bacteriophage α3-like proteins. Other proteins are involved in DNA replication and lysis. These will be discussed in the context of their functions during viral replication.

Host Cell Recognition, Attachment, and Penetration

Bacteriophage øX174 attachment requires lipopolysaccharides (LPSs) containing specific terminal glucose and galactose moieties. Most microvirus research has been conducted with øX174. Six coat protein residues constitute this Ca^{2+}-dependent glucose binding site. Due to the icosahedral symmetry of the capsids, each virion has 60 glucose binding sites. Attachment is a reversible reaction. Although the molecular basis of the following irreversible penetration step remains obscure, it most likely involves the spike proteins G and H. Host range mutations map to these two proteins and to coat protein residues directly surrounding

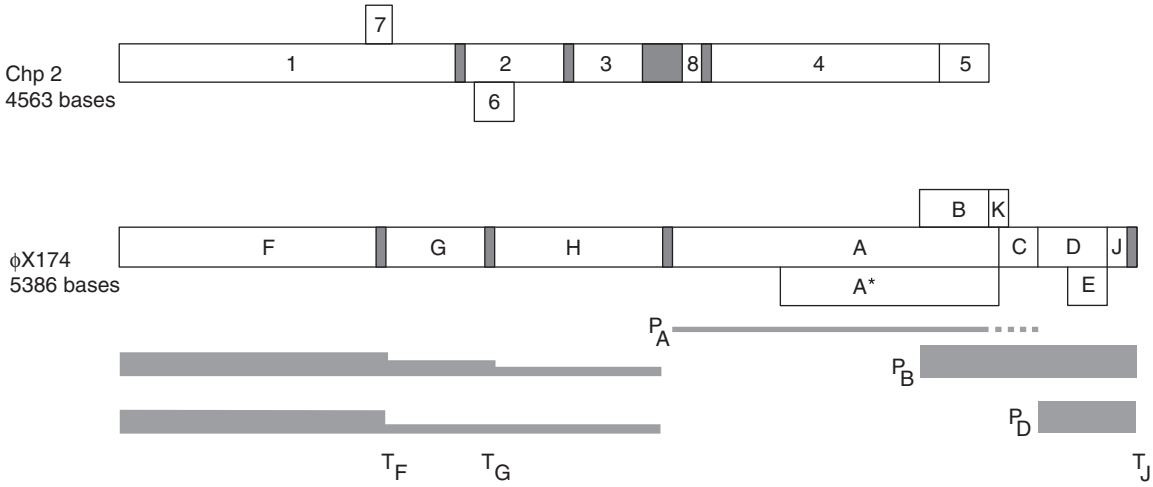


Figure 2 Genetic maps. Top, gokushovirus Chp2. Middle, microvirus øX174. Bottom, transcripts found in øX174 infected cells. The promoters and transcription terminators are indicated on the linear map of øX174. Line thickness indicates the relative abundance of the transcripts. The gene A transcript is very unstable; the terminator for this transcript is unknown. For protein functions, see **Table 1**.

the fivefold related spike complexes. Both spike proteins bind LPS of øX174 sensitive cells, but the specific details of these interactions are unknown. The location of the host range mutations argues for the existence of a second host cell penetration receptor. An analogy can be made to the large tailed bacteriophages that 'walk' along the surface of the cell, via tailspike interactions, until they find a receptor for penetration. But instead of walking, øX174 would merrily roll along.

In electron micrographs, the majority of eclipsed øX174 particles are imbedded at points of adhesion between the cell wall and inner membrane, suggesting the location of the hypothesized second receptor and indicating that the genome may be ejected directly into the cytoplasm. Penetration requires the DNA pilot protein H, which enters the cell along with the genome. Both genetic and preliminary structural data indicate that conformational changes in the capsid accompany or facilitate DNA ejection. Second-site suppressors of cold-sensitive DNA pilot proteins map to two places in the major spike protein. One set of suppressing residues lines the channel that passes through each fivefold vertex. The second set alters the interface between β-strands B and I of the G protein β-barrel, suggesting that conformational switches within this interface may mediate the channel opening.

Due to their recent discovery, the details of gokushovirus attachment and penetration have yet to be defined. The results of bioinformatic approaches combined with host range studies with the chlamydiaphages suggest that the threefold related protrusion may govern host cell specific attachment. However, there is no direct experimental evidence to support this hypothesis. Unlike microviruses, gokushoviruses appear to utilize a host cell protein, as opposed to LPS, as a receptor molecule. The exact location of the hypothesized DNA pilot protein, VP2, at the three- or fivefold axes of symmetry, is unknown as is the conduit through with the genome is ejected.

DNA Replication

The studies of øX174 DNA replication are of historical significance. Positive polarity ssDNA replication strategies are complex, occurring in three separate stages, which are described below (**Figure 3**). Kornberg and colleagues reconstituted the first two stages *in vitro* while Hayashi and colleagues reconstituted the third stage of DNA replication, which includes concurrent packaging of the single-stranded genome. Collectively, these studies established the first defined viral genome replication process on the biochemical level.

Single-stranded viral DNA is delivered into the infected cell. Stage I DNA replication involves the conversion of the ss genome into a covalently closed, double-stranded (ds), circular molecule, called replicative form one (RFI) DNA. Since purified single-stranded microvirus DNA produces progeny when transfected, host cell proteins are both necessary and sufficient for stage I replication *in vivo*. A stem–loop structure in the FG intercistronic region serves as the host cell and protein recognition site, which initiates the assembly of the primosome. After primosome assembly, the complex migrates along the ssDNA in a $5' \rightarrow 3'$ direction synthesizing the requisite RNA primers for DNA replication. Addition of the holoenzyme leads to chain elongation. The 13 host cell proteins required for this stage of synthesis are the same proteins involved in replicating the host cell chromosome.

During stage II DNA synthesis, RF1 DNA is amplified. In addition to the host cell proteins required for stage I replication, stage II replication is dependent on the viral A protein and the host cell *rep* protein, which functions as a helicase. The viral A protein binds to the origin of replication and nicks it to initiate (+) strand synthesis, which will occur via a rolling circle mechanism. After nicking, protein A forms a covalent ester bond with the DNA, forming a relaxed circular molecule called RFII. The host cell *rep* protein unwinds the helix and the host ssDNA binding protein (ssb) stabilizes the separated strands. After one round of rolling circle synthesis, protein A cuts the newly generated origin and acts as a ligase, generating a covalently closed circular molecule. Minus strand synthesis is mechanistically similar to stage I DNA synthesis.

Stage III DNA synthesis involves the concurrent synthesis and packaging of the ssDNA genome. Procapsids and viral protein C are required for this reaction along with all the proteins involved in the previous stages of replication with the exception of the ssb. Thus, a single-stranded genome is not synthesized unless there is a procapsid in which to package it. The competition between ssb and protein C for binding ssDNA most likely signals the commencement of stage III synthesis. Proteins A, C, and *rep* form a complex on the dsDNA that docks to procapsids, presumably in a groove that spans one of the twofold axes of symmetry. Procapsid binding does not occur in the absence of protein C. Mechanistically, stage III DNA synthesis is similar to the stage II (+) rolling circle synthesis. As the new (+) strand is synthesized, it is translocated into the procapsid. After one round of synthesis, protein A, which is covalently attached to the origin of replication, cuts the newly synthesized origin and acts as a ligase to generate a covalently closed circular molecule.

Gene Expression

Since microviruses contain single-stranded genomes of positive polarity, stage I DNA synthesis, which generates the negative strand, must occur before transcription.

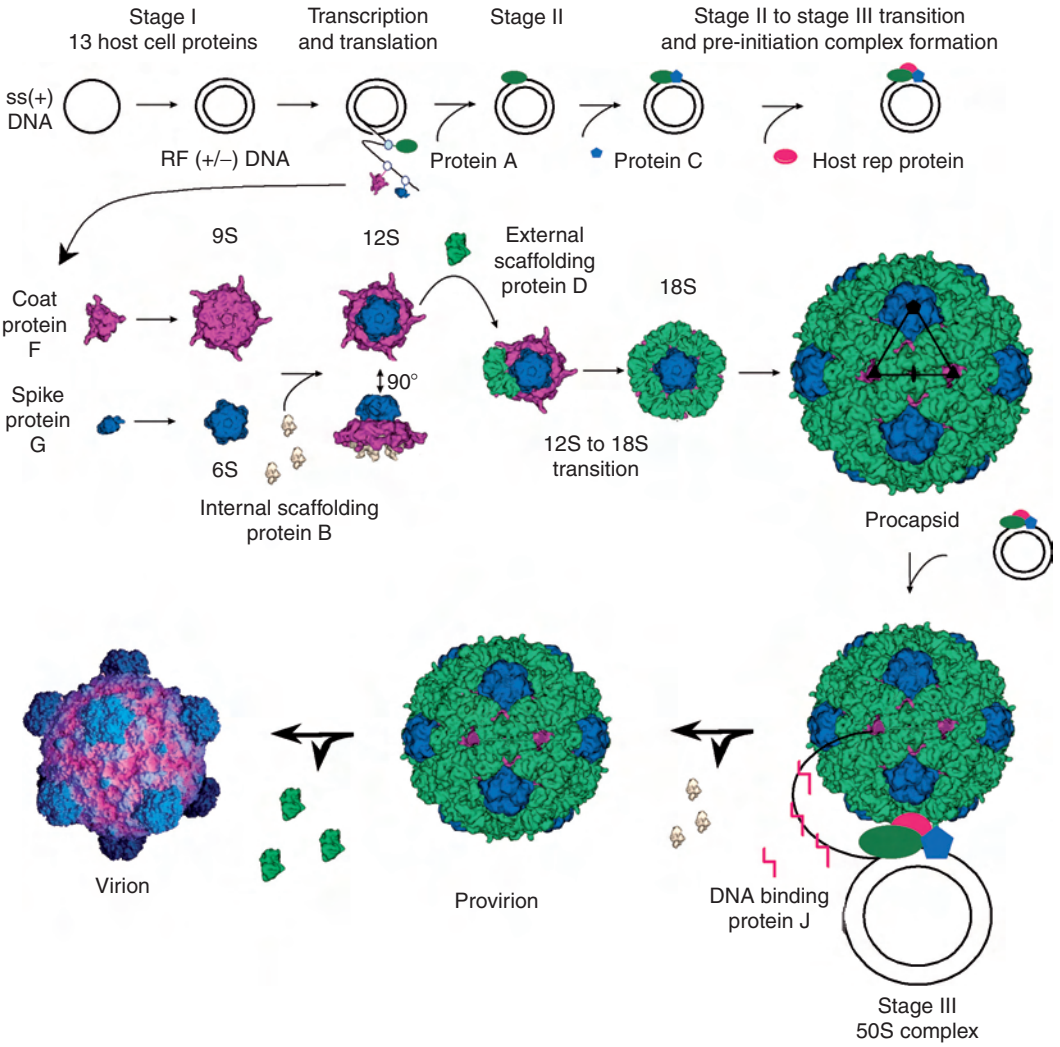


Figure 3 Morphogenesis and DNA replication of ϕX174.

Unlike large bacteriophages, gene expression in the members of the family *Microviridae* is neither temporal nor mediated by *trans*-acting mechanisms. Thus, the timing and relative production of viral proteins is entirely dependent on *cis*-acting regulation signals: promoters, transcription terminators, mRNA stability sequences, and ribosome binding sites. Promoters are found upstream of genes A, B, and D and terminators are found after genes J, F, G, and H (**Figure 2**). Since terminators are not 100% efficient, a wide variety of transcripts are produced. Yet there is a rough correlation between the abundance of a gene transcript and the amount of the encoded protein required for the viral life cycle. For example, gene D transcripts, which encode the external scaffolding protein, are the most abundant in the cell and the requirement of protein D is the greatest for progeny production. Similarly, there are more gene F, J, and G transcripts than transcripts of gene H. The relative stoichiometry of these structural proteins are 5:5:5:1, respectively. Protein expression is also affected by mRNA stability. Each mRNA species decays with a characteristic rate. Transcripts of gene A decay very rapidly, ensuring that this nonstructural protein is not overexpressed. Finally, regulation can also be achieved on the translational level. Despite gene E's location within gene D, the most abundant transcript, few E proteins, which mediate cell lysis, are translated due to an extremely ineffective ribosome binding site.

Morphogenesis

Despite extensive searches, a dependence on host molecular chaperones, such as groEL, and groES, has never been documented. Thus, chaperone independence is another factor that distinguishes the microviruses from dsDNA

bacteriophages. The first virally encoded microvirus assembly intermediates are the 9S and 6S particles, respective pentamers of the major coat F and spike G proteins (**Figure 3**). After 9S pentamer formation, five internal scaffolding proteins bind to the 9S underside, which will become the capsid's internal surface, forming the 9S* intermediate. 9S* particles then associate with spike protein pentamers to produce 12S assembly intermediates. The internal scaffolding protein also facilitates the incorporation of the DNA pilot protein H. However, the exact time protein H enters the assembly pathways remains somewhat obscure. The addition of 20 external scaffolding proteins results in the construction of the 18S particle.

The external scaffolding protein has been the focus of many research endeavors due to its inherent ability to achieve different structures. The four D subunits (D1, D2, D3, and D4) per coat protein (**Figure 4**), are arranged as two similar, but not identical, asymmetric dimers (D1D2 and D3D4). Each subunit makes a unique set of contacts with the underlying coat protein, the spike protein, and neighboring D protein subunits. Accordingly, the structure of each subunit is unique. The atomic structure of the assembly naive D protein dimer has also been determined. The subunits within that dimer, DA and DB, appear poised to achieve the four structures found in the procapsid. DA has a structure somewhat between D1 and D3, while DB has a structure midway between D2 and D4.

External scaffolding proteins are rare, only observed in parasitic satellite virus systems such as bacteriophage P4, in which the satellite virus encodes an external scaffolding protein that forces the helper's virus capsid to form a smaller capsid. Thus, microviruses are the only nonsatellite viruses known to encode an external scaffolding protein. This protein performs many of the functions typically associated with internal scaffolding proteins in one-scaffolding-protein systems, the organization of assembly precursors into a procapsid, and the stabilization of that structure. However, its function is physically and temporally dependent on the internal scaffolding protein, which can be eliminated from the pathway

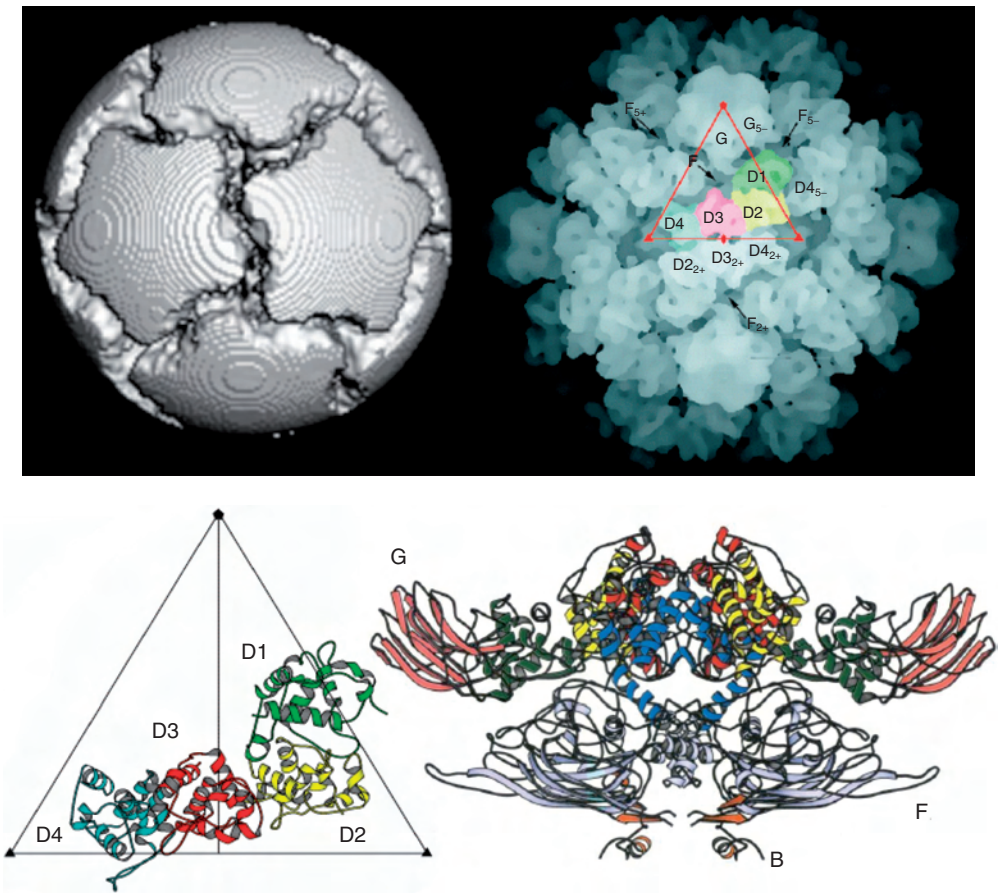


Figure 4 CryoEM and atomic renderings of the øX174 procapsid. Top left, rendering of the procapsid with the removal of the external scaffolding protein. Note that the coat protein pentamers do not contact each other. Top right, isosurface tracing of the øX174 procapsid, the four structurally unique external scaffolding proteins are depicted as D1–D4. Bottom left, the Cα backbones of the four external scaffolding protein subunits. Bottom right, cross-section of the procapsid along a twofold axis of symmetry, showing the positions of the internal B and external D scaffolding proteins relative to the coat F and spike G proteins.

(see the section titled 'Evolution and evolutionary studies' below). Procapsid morphogenesis is completed with the association of the 12 18S particles. The structure is almost exclusively held together via external scaffolding protein contacts across twofold axes of symmetry. Remarkably, there appear to be no associations between coat protein pentamers (**Figure 4**).

Gokushovirus genomes do not encode an external scaffolding protein. The details of the early assembly pathway remain to be elucidated. A gokushovirus particle containing VP3 in addition to the structural coat and DNA pilot proteins has been isolated. Unlike virions, these particles are devoid of DNA. This particle most likely represents a procapsid and indicates that VP3 functions as an internal scaffolding protein.

DNA Packaging and the DNA Binding Protein

Genome biosynthesis and packaging are concurrent processes in øX174. The pre-initiation complex, consisting of the host cell *rep*, viral A and C proteins, associates with the procapsid forming the 50S complex. As described above, the viral A protein binds the origin of replication in replicative form DNA. The results of genetic studies indicate that the pre-initiation docking site resides along a twofold axis of symmetry. The DNA binding protein J enters the procapsid during packaging and is absolutely required for genome encapsidation. Once in the procapsid, the C-terminus of the protein, which is very hydrophobic and aromatic, competes with the internal scaffolding protein for binding to a cleft in the viral coat protein. This competition results in the extrusion of the internal scaffolding protein during the packaging reaction.

Microvirus J proteins are extremely basic proteins that bind the genome via electrostatic interactions. The genome also interacts with a small cluster of basic amino acid residues in the capsid. Unlike large dsDNA bacteriophages, the øX174 genome does not exist as a dense core in the capsid. Instead, the DNA is tethered to the capsid's inner surface. In the atomic model, the protein forms an S-shaped polypeptide chain devoid of secondary structure. The C-terminus is tightly associated with the cleft located near the center of the coat protein. Each of the 60 proteins traces a path toward the fivefold axis of symmetry, crosses over to the adjacent capsid protein, and veers toward the C-terminus of the adjacent J protein. Thus, the DNA binding protein guides the incoming genome into a somewhat ordered conformation and a portion of the genome is ordered in the X-ray structure. The biophysical characterization of fully packaged particles with foreign genome-length DNA or øX174 genomes with mutant DNA binding proteins suggest that protein–DNA interactions influence the final stages of morphogenesis. Morphogenesis terminates with the provirion to virion transition: the dissociation of the external scaffolding protein and an 8.5 Å radial collapse of capsid pentamers around the genome. The tethered genome constrains the spatial orientation and secondary structure of the remaining nucleotides. Therefore, altering the tether or the base composition of the packaged nucleic acid may affect the magnitude or the integrity of the collapse. However, the role of DNA–capsid interactions in øX174 is not as dramatic as those seen in other viral systems in which abrogating genome–capsid interactions lead to severely aberrant particles.

Lysis

Unlike large double-stranded bacteriophages, microviruses do not have the genetic capacity to encode two-component endolysin and holin lysis systems. Instead, they have evolved a small protein, lysis protein E that inhibits peptidoglycan biosynthesis. Thus, lysis is dependent on host cell division, during which the cell becomes sensitive to osmotic pressure. The results of several elegant genetic studies elucidated protein E function. By selecting for lysis resistant cells, Ryland Young and colleagues first uncovered the *slyD* (*s*ensitivity to *ly*sis) gene, which encodes a peptidly-prolyl cis-transferase-isomerase, or PPIase. However, it is unlikely that the *slyD* gene product is the E protein target. Considering the function of PPIase's in protein folding and the E protein's five prolyl bonds, it seemed more likely that the E protein was a substrate for the host cell enzyme. In fact, gene E mutants, *Epos*-plates on *slyD*, can be readily isolated. The *Epos* proteins were used in a second round of selection with *slyD* cells, surviving colonies containing mutations in the *mraY* gene, which encodes tanslocase I. This enzyme catalyzes the formation of the first lipid-linked intermediate in cell wall biosynthesis and most likely the target of protein E.

Evolution and Evolutionary Studies

Due to the small genomes and the ability to interpret amino acid substitutions within the context of the virion and procapsid atomic structures, the microviruses have become one of the leading systems for molecular evolution analyses. In studies pioneered by Jim Bull, Holly Wichman, and colleagues, viruses are placed under selective conditions, and grown for numerous generations in a chemostat. At various time intervals, individual genomes are sequenced, allowing the appearance and disappearance of beneficial mutations to be monitored. Of course, the mutations obtained differ according to the selection conditions. While these studies identify beneficial changes in both structural and nonstructural proteins, many mutations are in genetic regulatory sequences, which most likely

optimize the relative level of viral proteins synthesized under the experimental conditions.

The results of an exhaustive search for new *Escherichia coli* microviruses reveal that the 47 known species can be divided into three separate clades, typified by bacteriophages øX174, G4, and α3. Although there is evidence for some horizontal gene transfer between the species, the extent is considerably lower than that observed for dsDNA viruses. The one gene that seems to be the most recent acquisition, at least in its present form, is the external scaffolding protein gene, which appears to have originated in the øX174 clade and spread to the other two.

The evolutionary relationship between the gokusho and microviruses remains somewhat obscure. There is a deep evolutionary rift between the two groups, with no known intermediate species. The rift appears to be a function of the biology of their hosts, not the hosts' evolutionary relationship. The gokushoviruses have been isolated from obligated intracellular parasitic bacteria or mollicutes. For example, the *Bdellovibrio* host for the gokushovirus øMH2K is a proteobacterium, like other microvirus hosts, but øMH2K is closely related to the phages of chlamidia. One of the primary differences between the two groups is the external scaffolding protein.

While the existence of two scaffolding proteins is unique in any system, it is particularly peculiar for the T = 1 microviruses. No other T = 1 virus requires a scaffolding protein, let alone two. An accumulation of both genetic and structural data suggests that the external scaffolding protein may be more essential for morphogenesis. The internal scaffolding protein, on the other hand, may be better viewed as en efficiency protein, facilitating several morphogenetic reactions, but not absolutely essential for any one in particular. This hypothesis has recently been tested by the isolation of a sextuple mutant strain of øX174 that no longer requires the internal scaffolding protein. Although mutations in structural and external scaffolding proteins did arise, two mutations reside in the external scaffolding gene promoters, leading to the overexpression of the mutant external scaffolding protein. These three mutations appear to have a kinetic effect on virion assembly, indicating that one function of the internal scaffolding is to lower the critical concentration of the external scaffolding protein needed to nucleate the assembly. The morphogenesis of wild-type øX174 is extremely rapid, progeny virions can be detected as quickly as 5 min postinfection, which may be critical for a small phage without the genome content to encode superinfection exclusion functions. At 5 min postinfection, most other phages are just concluding early gene expression. Rapid morphogenesis may be a consequence of having two scaffolding proteins, allowing microviruses to compete with the larger and vastly more prevalent dsDNA phages. In this evolutionary model, the external scaffolding protein is a recent acquisition. Those phages that did not acquire the gene, the gokushoviruses, persisted by finding a niche free of competition, obligate intracellular parasitic hosts like chlamidia.

Acknowledgments

The authors thank Drs. M. G. Rossmann and Timothy Baker and colleagues for assistance with figures and the support of the National Science Foundation.

See also: Plant Virus Vectors (Gene Expression Systems); Transcriptional Regulation in Bacteriophage.

Further Reading

Bernhardt TG, Struck DK, and Young R (2001) The lysis protein E of phi X174 is a specific inhibitor of the MraY-catalyzed step in peptidoglycan synthesis. *Journal of Biological Chemistry* 276(9): 6093–6097.

Brentlinger KL, Hafenstein S, Novak CR, *et al.* (2002) *Microviridae*, a family divided: Isolation, characterization, and genome sequence of phiMH2K, a bacteriophage of the obligate intracellular parasitic bacterium Bdellovibrio bacteriovorus. *Journal of Bacteriology* 184(4): 1089–1094.

Bull JJ, Badgett MR, Wichman HA, *et al.* (1997) Exceptional convergent evolution in a virus. *Genetics* 147(4): 1497–507.

Chipman PR, Agbandje-McKenna M, Renaudin J, Baker TS, and McKenna R (1998) Structural analysis of the Spiroplasma virus, SpV4: Implications for evolutionary variation to obtain host diversity among the Microviridae. *Structure* 6(2): 135–145.

Dokland T, McKenna R, Ilag LL, *et al.* (1997) Structure of a viral procapsid with molecular scaffolding. *Nature* 389(6648): 308–313.

Fane BA, Brentlinger KL, Burch AD, *et al.* (2006) øX174 *et al*. In: Calendar (ed.) *The Bacteriophages*, pp. 129–146. London: Oxford University Press.

Fane BA and Prevelige PE, Jr. (2003) Mechanism of scaffolding-assisted viral assembly. *Advances in Protein Chemistry* 64: 259–299.

Hafenstein S and Fane BA (2002) phi X174 genome-capsid interactions influence the biophysical properties of the virion: Evidence for a scaffolding-like function for the genome during the final stages of morphogenesis. *Journal of Virology* 76(11): 5350–5356.

Hayashi M, Aoyama A, Richardson DL, and Hayashi NM (1988) Biology of the bacteriophage øX174. In: Calendar R (ed.) *The Bacteriophages,* vol. 2, pp. 1–71. New York: Plenum.

Liu BL, Everson JS, Fane B, *et al.* (2000) Molecular characterization of a bacteriophage (Chp2) from Chlamydia psittaci. *Journal of Virology* 74(8): 3464–3469.

McKenna R, Xia D, Willingmann P, *et al.* (1992) Atomic structure of single-stranded DNA bacteriophage phi X174 and its functional implications. *Nature* 355(6356): 137–143.

Morais MC, Fisher M, Kanamaru S, *et al.* (2004) Conformational switching by the scaffolding protein D directs the assembly of bacteriophage phiX174. *Molecular Cell* 15(6): 991–997.

Novak CR and Fane BA (2004) The functions of the N terminus of the phiX174 internal scaffolding protein, a protein encoded in an overlapping reading frame in a two scaffolding protein system. *Journal of Molecular Biology* 335(1): 383–390.

Rokyta DR, Burch CL, Caudle SB, and Wichman HA (2006) Horizontal gene transfer and the evolution of microvirid coliphage genomes. *Journal of Bacteriology* 188(3): 1134–1142.

Uchiyama A and Fane BA (2005) Identification of an interacting coat-external scaffolding protein domain required for both the initiation of phiX174 procapsid morphogenesis and the completion of DNA packaging. *Journal of Virology* 79(11): 6751–6756.

Icosahedral ssRNA Bacterial Viruses

P G Stockley, University of Leeds, Leeds, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Pfu Plaque-forming unit; that is, the number of phage particles per host cell required to generate productive infections. Theoretically, this value could be 1, but in reality only very few of the phages that attach to the edge of a pilus will get access to the inside of a cell, so values in the 100s are not uncommon.
Polycistronic RNA The term cistron was coined a long time ago to mean any section of an mRNA that gets translated. The RNA phage genomes are 'polycistronic', because they encode multiple protein products.
Quasi-equivalent conformers These are different conformations of the identical polypeptide sequences that form at the differing symmetry-related positions of viral capsids.
Translational repression Refers to the process whereby a protein product interacts with an mRNA molecule, thus preventing its translation.
Triangulation number, *T* Defined mathematically as $T = (h^2 + hk + k^2)$, where h and k are any pair of positive integers, including zero. For instance, giving rise to triangulation numbers of 1 for $h,k = 1,0$, and 3 for $h,k = 1,1$. These h and k values can be determined by analysis of real capsids viruses by analyzing them in terms of networks of equilateral triangles that cover the surface of a sphere.

Background

Not all *Escherichia coli* cells are the same. Some of them produce a protein tube at the surface known as a pilus. It turns out that the information to build this structure is encoded by a separate genetic element, the F factor not carried by all cells. The RNA phages were initially identified because of their ability to infect *E. coli* carrying this factor. The pili encoded by F are used by the cells to swap DNA with other bacteria that lack the factor, resulting in a primitive form of sexual genetic transfer known as conjugation and the use of the term 'male' for bacteria that carry the F factor. Thus bacteria are able to swap useful genes, for example, for antibiotic resistance, rapidly within their populations. All processes in biology can be seen as opportunities ripe for exploitation, and the F pilus system, so useful to the bacteria in sharing useful genetic characteristics, presents an opening for a group of bacterial viruses to gain entry into F^+ cells. Tim Loeb working in Norton Zinder's laboratory at the Rockefeller University in New York was the first to realize that there might be male-specific bacterial viruses, bacteriophages. Together they isolated and began to characterize such phages from an initial sample of raw sewage. One intensively studied version known as MS2 is believed to have been named because it was male-specific factor 2, although there are unconfirmed stories that the MS also stands for metropolitan sewer.

Large numbers of similar phages were rapidly isolated worldwide after the initial discovery, and we now know that such phages are extremely common in the environment with up to 10^7 Pfu ml^{-1} in sewage. Both human and animal hosts appear to harbor *E. coli* populations able to support such phages, which are hence termed coliphage. In addition, virtually identical phages have been found that are specific to *Acinetobacter* (AP205) and other Gram-negative bacteria. In such phages, the infection process still occurs via pili but these are not the same as the sex pili of *E. coli*. For instance, phage PRR1 infects hosts via pili encoded by incompatibility type P plasmids which are widely dispersed, allowing the phage to infect a range of different bacterial cells.

All these bacteriophages turn out to have very closely related structural and genetic features. They form spherical capsids of ~250 Å diameter that enclose a single-stranded RNA (ssRNA) genome of ~3500–4300 nt in length. The ssRNA is the positive-sense version of the genome and acts as a messenger RNA (mRNA) while in infected cells. On the basis of immunological cross-reactivity and genome organization, the phages have been classified into two genera and these have been subdivided in turn into two groups. All these phages are members of the *Leviviridae* or the *Allelolevirida*e, the latter being distinguished by having slightly longer genomes and because of the presence of read-through products from the coat protein gene. Subgroups I and II belong to the former genus and typical members are MS2 and GA, respectively, whereas group III and IV phages belong to the latter genus with typical members being Qβ and SP, respectively.

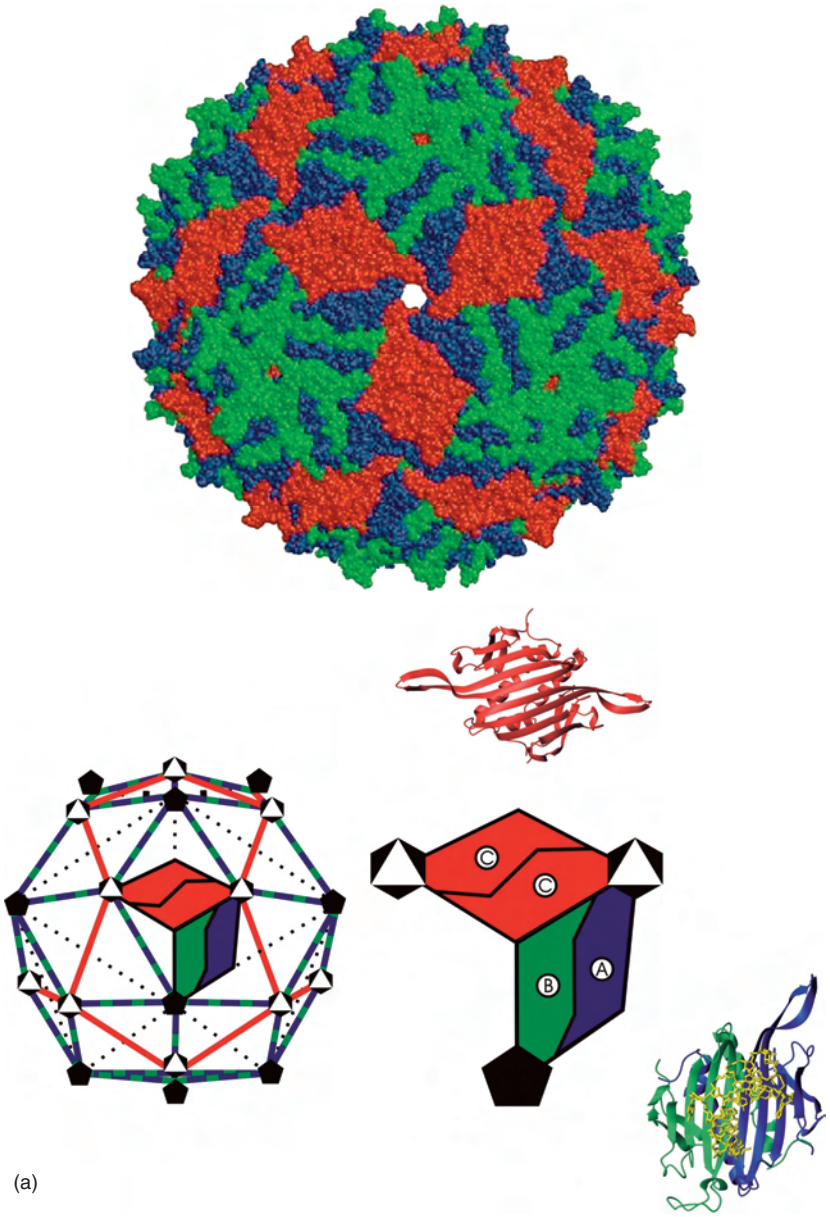
These very simple pathogens have been used since their discovery to understand many basic processes of molecular biology. They have been used to probe the structure of the bacterial pilus, to dissect the function of the protein synthesis pathway, to understand RNA replication, and to identify the initiation and termination signals on prokaryotic mRNAs. These studies led to the discovery of novel forms of genetic regulation along a polycistronic RNA, including translational repression.

Phage RNA was the first nucleic acid to be replicated in a test tube and was used as a model for the development of nucleic acid sequencing technology. Detailed insights into viral assembly processes and the molecular basis of sequence-specific RNA–protein recognition have also emerged. Research applications of these phages and their biology are still very active (see below).

Capsid Structure and Life Cycle

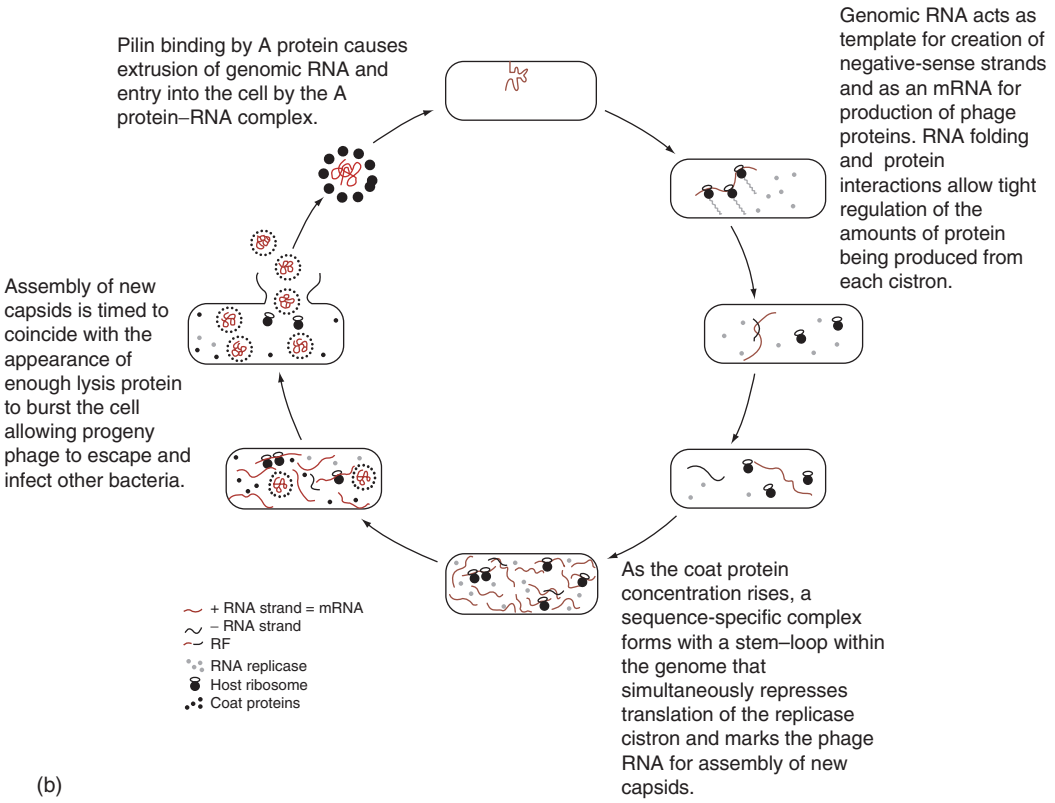
At the time of their discovery, the three-dimensional structures of viruses were not known but it was realized that the limited coding capacity of viral nucleic acids was likely to place a constraint on the number and size of gene products that could be dedicated to building a protective container, or capsid. This in turn implied that capsids are likely to be composed of multiple copies of one or very few 'coat' protein subunits necessarily resulting in highly symmetric structures. The two most 'efficient' designs for such structures, in terms of allowing multiple copies of the smallest protein units to enclose the maximum volume, and hence encompass the largest genome, are the helix and the icosahedron. Simple spherical virus capsids are therefore based on an icosahedral surface lattice (**Figure 1**).

A mathematically perfect icosahedron would have 60 facets in it so the stoichiometry for such viruses would be



(a)

Figure 1 Continued



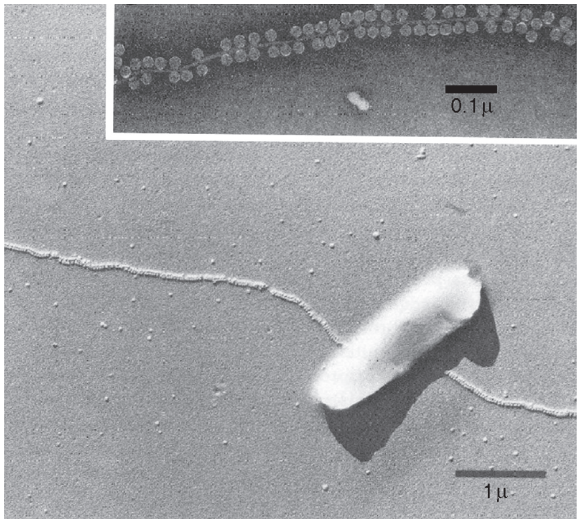


Figure 1 Capsid structure and life cycle. (a) Shows a space-filling representation of one face of the MS2 bacteriophage particle as determined using X-ray crystallography viewed along the icosahedral threefold axis. The differing quasi-equivalent protein conformers are colored red, green, and blue. There are 60 of each type accounting for the 180 coat protein copies per $T = 3$ shell. Note, the position of the A protein in the shell is not observed from the X-ray structure for technical reasons. Below is a cartoon of an icosahedron with the threefold (open triangles) and fivefold (solid pentagons) symmetry axes highlighted. The MS2 coat protein subunits form noncovalent dimers in solution and in the final capsid, and the way in which they pack around the axes is illustrated on the right both as a cartoon and as detailed three-dimensional representations of the differing quasi-equivalent conformers. Proteins are shown as ribbon diagrams representing their polypeptide backbones. The A/B dimer is shown bound to the translational repression RNA stem–loop (yellow); see also **Figure 3**. (b) Shows a schematic of the phage life cycle, including the attachment process, entry of the genomic RNA, replication, self-assembly of progeny phage particles, and subsequent lysis of the host. (c) Shows the process of infection with phage particles adhered along the length of a bacterial pilus. The magnification is ~16000× and reveals the relative sizes of the host and its virus particles. (b) Reproduced from Watson JD, Hapkins NH, Roberts JW, Steltz JA, and Winer AM (1993) *Molecular Biology of the Gene*, *Vol. I*: *General Principle*, 5th edn. New Delhi: Pearson Education, Inc., with permission. (c) Reproduced from Paranchych W (1975) Attachment, ejection and penetration stages of the RNA phage infectious process. In: Zinder ND (ed.) *RNA Phages*, p. 89. New York: Cold Spring Harbor Laboratory Press, with permission from Cold Spring Harbor Laboratory Press (NY).

60 coat proteins per shell. However, from many early studies, it was clear that viruses often had many more than 60 subunits in their capsids, although in cases where detailed biochemistry was possible it appeared that such viruses contained simple multiples of the expected value, for example, 180, 240, etc. Don Caspar and Aaron Klug offered an explanation for such protein stoichiometries via a theory they called quasi-equivalence. They argued that viral coat proteins are three-dimensional objects that are partially flexible due to the chemical properties of their constituent polypeptide backbones and amino acid side chains. It would therefore be possible to conserve inter-subunit bonding if the precise molecular interactions at any position in the capsid were flexible enough to accommodate small changes from ideality. This theory leads immediately to predictions of 'allowed' stoichiometries for capsids with subunit numbers of 180, 240, etc., derived from what is called a subtriangulation of the sphere and defined by the triangulation number, *T*. This value is allowed to vary from 1, 3, 4, etc., yielding the observed stoichiometries of 60, 180, 240, etc. subunits. The implication of this idea is that the coat proteins within the capsids with more than 60 subunits must be able to adopt slightly different conformations depending on their relative locations in the shell. These are known as quasi-equivalent conformers and this idea has subsequently been confirmed by X-ray structure determination of many viruses. The RNA phages have 180 coat protein subunits per capsid and are therefore examples of $T = 3$ shells.

As well as the major coat protein, it is known that the capsids of RNA phages contain a minor protein that is essential for infectivity known as the maturation (or A) protein. In some of the RNA phages, another minor protein is associated with the capsids due to the production of a small percentage of read-through versions of the coat protein. The function of these minor components is largely unknown. Mature infectious capsids must contain at least one copy of the maturation protein as well as the icosahedral shell composed of the major coat protein. Infection is initiated by the interaction of the A protein with the edge of a bacterial pilus, confirming that part of this protein must project on the outer surface of the virion. This interaction triggers a release of the phage RNA from inside the phage shell and proteolytic cleavage of the A protein into two major fragments. The RNA and the A protein domains then enter the cell leaving the remainder of the capsid, that is, the major coat protein, on the outside. This implies that at least one fragment of the A protein must form a tight interaction with the phage RNA. Indeed, careful measurement of the kinetics of uptake of both phage RNA and the two fragments of the A protein suggest that they enter the cell as a single complex. Two RNA-binding sites for the A protein along the genome, one toward the 5′ end and the other close to the 3′ end, have been identified. It is tempting to speculate that pilin attachment leads to conformational change in the A protein, that is made irreversible by the proteolytic cleavage, and this in turn results in expulsion of the phage genomic RNA from its capsid. The precise mechanism of how the A protein–RNA complex is taken up into the cell has not been worked out in any detail. It is assumed that these complexes can transit into the cell like DNA but this does not seem to have been firmly established.

Whatever the mechanism of cell entry, once inside the cell the phage RNA then serves as an mRNA (see below). Expression of phage proteins results in replication of the phage RNA via the formation of an RNA-dependent RNA polymerase consisting of one phage-encoded replicase subunit, the host proteins S1, and elongation factors Tu and Ts, and an additional host factor. This polymerase creates negative strand versions of the genome that in turn serve as templates for the production of progeny positive-sense genomes. In an exquisite example of timing controlled by macromolecular interactions, this replication phase of infection is reined in by a translational repression event. Translation of the replicase subunit becomes blocked by interaction of a coat protein dimer with a stem–loop operator site of just 19 nt in length that includes the start codon for that cistron.

The resultant coat protein–genomic RNA complexes then serve as the assembly initiation complexes for formation of progeny phage particles. At some point, the A protein must become a part of this complex. The final act of infection is lysis of the host cell by the action of a lysis protein leading to release of up to 10 000 progeny particles. During this process, the phage has dramatically differing needs for each of its gene products: replicase, coat protein, maturation protein, and lysis protein. Each protein also needs to act at different times. Remarkably, both the amounts and timing of the appearance of each of these products is very tightly regulated, even though each cistron is present once on every copy of the genomic RNA. The details of that regulation are described in the following sections.

Control of Phage Gene Expression

When the phage-encoded protein expression is analyzed (**Figure 2**), it can be seen that the first protein to be produced after the RNA gains entry to the cell is the coat protein, and its expression remains high throughout an infection cycle. There seems little regulation of this expression other than the sequence and stability of the initiator hairpin structure that binds the ribosome. There are also some data that suggest that upstream flanking sequences can act positively to promote translation, perhaps by forming structures that favor binding of the ribosome to this site.

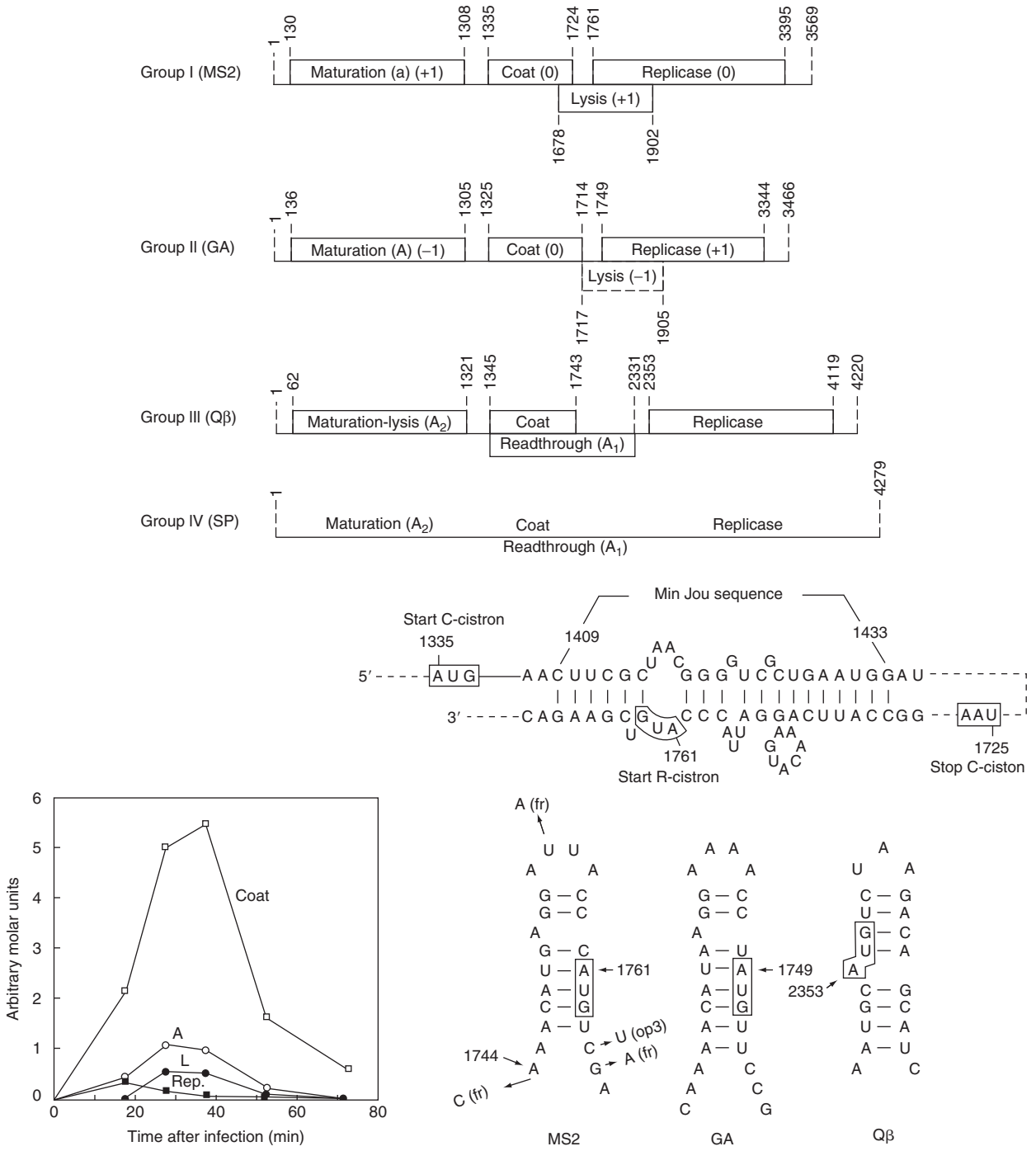


Figure 2 Genome organization and expression. The top panel shows cartoon representations of the genomes of representative members of each of the four subgroups of the RNA bacteriophages. The locations of the encoded proteins are also shown. The middle panel left shows the amounts of each of the encoded proteins produced during an infection cycle. Although each gene is present in equal amounts along each copy of every genome, there is a dramatic difference in the amounts of each protein in the cell, reflecting the requirements for different numbers of these protein products for replication, self-assembly of progeny phage, and lysis of the host. The implication is that the expression of these protein products is tightly controlled. The middle panel right shows one type of regulation that allows control of the amount of each protein product produced. At various times in the phage life cycle, the stem–loop structures shown at the bottom of the figure are sequestered by long range Watson–Crick base-pairing interactions to a segment of the coat protein gene (illustrated here for MS2). This section of the genome contains the start codon for the phage-encoded replicase subunit, so that the tertiary interaction prevents expression of replicase until the coat protein gene begins to be translated. As a ribosome transits this base-paired region, it frees up the downstream section allowing the ribosome-binding site and replicase translation to occur. At a later stage, the re-folded stem–loop is recognized by the coat protein – a coat protein dimer binds to the site and prevents further translational initiation, an example of translational repression (**Figure 3**). (a–c) Reproduced from Van Duin J (1988) Single-stranded RNA bacteriophages. In: Calendar R (ed.) *The Bacteriophages*, vol. 1, pp. 117–167. New York: Plenum, with permission.

The maturation protein is obviously needed in much smaller amounts and its appearance is slower and it is produced in much lower amounts than coat protein. It is believed that a number of regulatory features lead to this poor expression level. The start codon for the maturation gene is GUG instead of the more usual and more efficient AUG. In addition, it has been proposed that the ribosome-binding site in this cistron can be sequestered in internal base pairing with another section of the maturation protein message and, as a result, maturation protein is only expressed on nascent replicating genomic RNAs, that is, at a point when the downstream base pairing partners are yet to be replicated.

The replicase subunit is initially produced with similar kinetics and in similar amounts to the maturation protein but by 20 min after infection its levels are reduced. In fact, the phage makes more maturation protein than is strictly necessary, but the replicase is made at a level of ~5 copies/genome and its levels are always tightly controlled. This regulation is achieved in two distinct ways. Initially, the ribosome start site on the replicase subunit is sequestered in another long-range base-pairing interaction, this time with a section of the coat protein gene. Translation of the coat protein gene sequesters this complementary sequence, known as the Min Jou sequence after its discoverer, and thus frees the region around the start of the replicase gene so that it can fold to form a ribosome-binding site.

However, replication is further controlled via a translational repression complex that forms with the phage coat protein. A stem–loop operator hairpin (**Figure 3**) can form that is bound sequence-specifically by a dimer of the coat protein in a concentration-dependent fashion. This operator encompasses the start codon for replicase and formation of this repression complex effectively switches off replicase translation. The complex also acts to trigger self-assembly of the phage coat protein shell around the phage RNA. Regulated expression is obviously tightly controlled because of the importance of timing the switch in the viral life cycle from replication to assembly of progeny phage. There may also be an additional reason to ensure that large quantities of replicase are not produced because of its role(s) in creating the active replication complex.

The phage-encoded replicase subunit is the RNA-dependent RNA polymerase responsible for copying the phage genome. However, it does not function as a separate subunit. Instead, it forms complexes with at least three cellular proteins normally involved in other functions; these are the ribosomal protein S1, which functions during the loading and initiation of mRNAs on the ribosome, and translational elongation factors EF-Tu and EF-Ts. These species form a heterotypic tetramer that appears to be the active replication enzyme for positive-sense strands of the genome, that is, in the production of the negative-sense strand. The opposite reaction, that is, the generation of the positive-sense strand from the negative strand appears not to need the S1 subunit. An explanation for this altered stoichiometry in the replication machinery has been proposed based on the fact that the positive-strand must also serve as a template for peptide synthesis. This would lead to situations in which ribosomes and replication complexes, which would be moving in opposite directions on the RNA, would collide. It is believed that S1 functions in these cases by competing with the ribosome for loading onto the RNA, thus preventing replication on actively translating RNAs. The replication complexes also appear to interact with another host protein, which appears to be hexameric, but its role(s) is unclear.

A final reason for tight regulation of the expression of replicase is that the subunit or its complexes with cellular proteins are toxic to the cell. This would interrupt the phage life cycle before progeny phages are assembled. Cell death is however the ultimate goal of the infection, thus releasing progeny phage particles, but this is actively controlled by a phage-encoded lysis protein. This cistron is read from the +1 reading frame initiating within the coat protein gene. As can be seen from **Figure 2**, the lysis protein is the last to be produced, consistent with its final function in the phage life cycle. The reason for the delayed production of this gene product appears again to be a consequence of translational control. In this case, the ribosome binding site allowing initiation of the lysis protein translation appears inaccessible until the ribosome translating the coat protein completes the translation in its defined termination site. Experiments where the coat protein gene stop codons are moved further 3′ prevent lysis expression, implying that correctly regulated expression must be due to a defined RNA folding event similar to those controlling replicase translational repression. In principle, variations of the details of this mechanism may partially account for the frequency of read-through products in some of the coat proteins in some phages.

The Translational Repression/Assembly Initiation Complex

As discussed above, the expression of the phage-encoded replicase subunit is controlled by a translational repression event that occurs when a coat protein dimer binds to a 19 nt stem–loop structure (**Figure 3**), thus sequestering the AUG start codon from replicase. In a set of seminal experiments, Janette Carey and Olke Ulhenbeck were able to show that all the sequence information for the specific RNA–coat protein interaction was contained within this short fragment. Furthermore, they established rapid assays for the interaction *in vitro*, allowing extensive investigation of the effects of changes to nucleotide base sequence and the chemical functional groups on the RNA that contribute to this interaction.

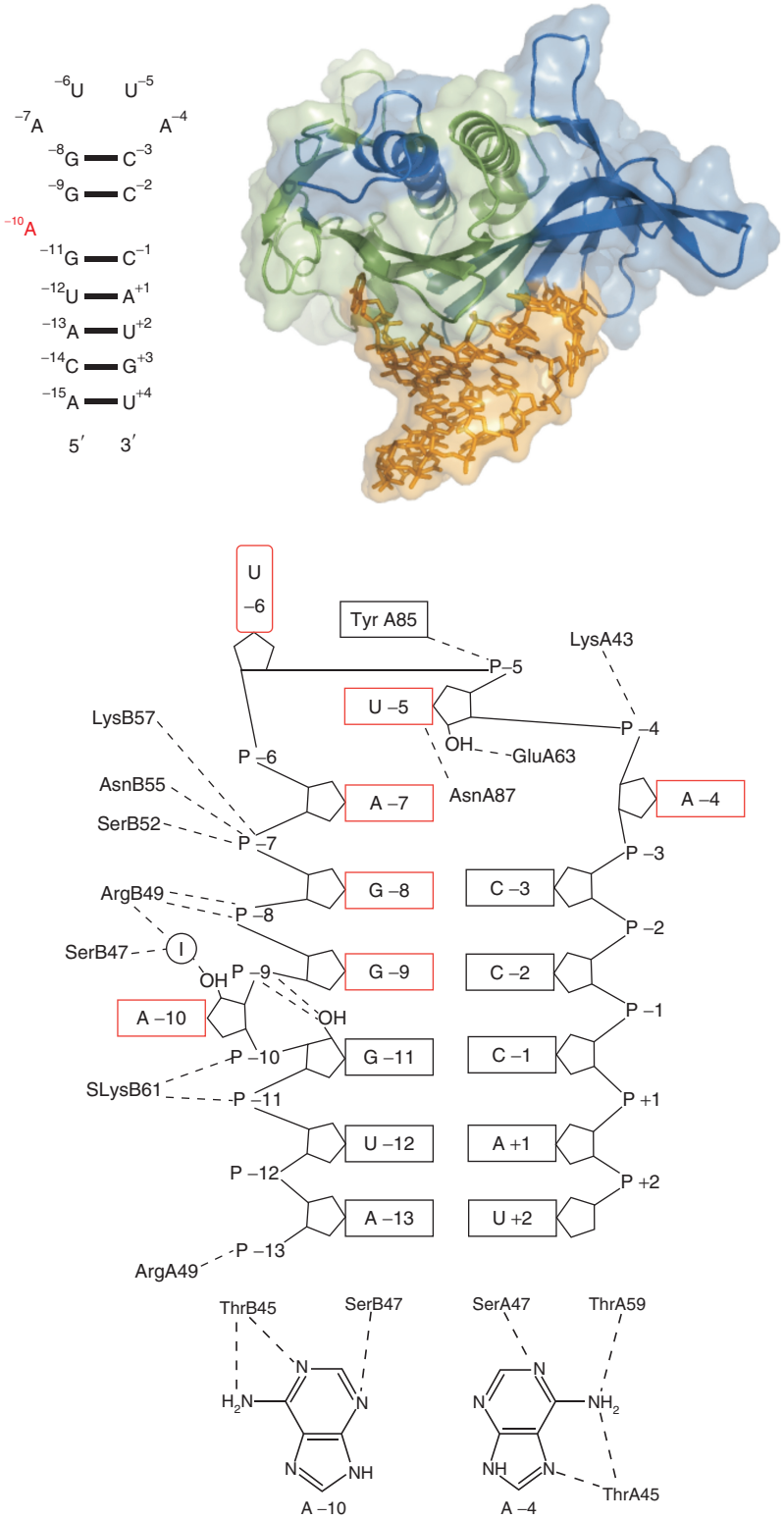


Figure 3 The MS2 translational repression complex. The top panel left shows the secondary structure of the translational operator stem–loop and the number of the bases relative to the replicase start codon. To the right is a detailed view of the X-ray structure of the interaction of the operator stem–loop with a coat protein dimer (shown as the A/B quasi-equivalent conformers). The polypeptide (blue and green) and RNA (gold) are shown as ribbon and stick models, respectively, within a transparent representation of the molecular surfaces. The bottom panel shows the intermolecular contacts that occur within the complex. The detailed contacts at A-4 and A-10 are shown below for clearity. The dotted lines indicate hydrogen bond interactions.

In addition, it has proved possible to determine X-ray crystal structures for the RNA stem–loop and over 30 of its variants in complex with the coat protein shell (**Figures 3** and **1**).

The detailed intermolecular contacts revealed and confirmed by these techniques have made this complex one of the most intensively studied examples of an RNA–protein complex. It has also been possible to determine structures for variants of the coat protein mutant at the RNA-binding site. The results of these studies suggest that the interaction is remarkably robust to simple changes outside a few key residues, principally the loop adenine at position –4 (the bases are numbered with respect to the first nucleotide of the replicase start codon). The half-life of the RNA–coat protein complex *in vitro* and presumably *in vivo* is very short (<1 min), implying that translational repression is short-lived. However, this is a simplification because the RNA–protein complex created then serves as a site of assembly initiation for formation of the intact capsid (see below). The repressive function of the coat protein reconfirms the mechanism of infection described above. If significant numbers of coat protein subunits were to enter the cell with the genomic RNA, the replicase gene would never be expressed. RNA variants that have higher affinity for coat protein can be identified *in vitro* but these would be lethal *in vivo*.

When cells are co-infected with MS2 and Qβ phages, the progeny phages that result consist of MS2 and Qβ RNAs inside their respective coat protein shells, that is, there is no evidence that the proteins can mispackage the competing genome. In part, this is because of the significant structural differences between the translational operator sites (**Figure 2**), but it also implies that there is direct coupling in the RNA–protein interaction leading to translational repression and assembly of progeny phage particles. It is believed that the RNA–coat protein complex that forms initially with a single coat protein dimer marks the phage RNA for assembly by addition of further coat protein subunits. All that is required to avoid misincorporation is that the coat proteins from different phages are unable to co-assemble and that seems to be the case even though there is a great deal of structural similarity between them.

The precise mechanism by which the phage capsid is generated is still unclear. When and how is the maturation protein incorporated into the structure, for instance? What we do know is that the stem–loop interaction appears to cause a conformational change in the coat protein subunits that bind, possibly between differing quasi-equivalent conformers, and provides a binding site for the stepwise addition of other coat protein dimers. These must somehow adjust their precise conformations in a structural context in order to produce a capsid of the correct size and symmetry. Recent evidence suggests that the folded genomic RNA may play a role in this process.

The major structural difference between quasi-equivalent conformers in a capsid occurs in a short loop of polypeptide connecting the F and G β-strands in each subunit. These FG loops have identical amino acid sequences in all subunits, but around the icosahedral fivefold symmetry axes they fold back toward the body of the subunit helping to create space to pack the subunits at this position. In contrast, at the icosahedral threefold axes, FG loops from two distinct quasi-equivalent subunits alternate in extended conformations, allowing six such loops to be packaged at these points. We now know that binding of the RNA translational operator causes a conformation change to occur at the FG loop, and it is tempting to assume that this interaction sets up the initial asymmetry to define a capsid structure via self-assembly. Precisely how the RNA is folded to fit into this structure and whether it folds during or before the assembly reaction is still a matter of debate.

Applications of RNA Phages – Three-Hybrid Assay

RNA phages have become model viruses for the development of a large number of novel scientific techniques. These are remarkably varied and include the controlled release of these phages into the wider environment as markers for the passage of viruses and of the ability of water treatment systems to remove viral particulates from drinking water. One such test was even performed on the International Space Station.

A more medically orientated set of applications has also been developed. For instance, it is known that viruses present defined epitopes to the immune system of their hosts. If those epitopes are common and unchanging, the immune response that occurs after an initial infection will render the host immune to further infection by that particular virus. Many viruses however constantly alter their outer protein surfaces presenting differing epitopes to the immune system, thus escaping immune neutralization. However, modern structural immunology techniques can identify conserved functional domains/sequences within these viral proteins that cannot be altered without also causing the virus to lose the ability to replicate or assemble. Raising immune responses against such refined constant epitopes leads to potent vaccines against a range of human and animal pathogens. In fact, work in this area has shown that the immune system is particularly well evolved to recognize repeated copies of particular epitopes, such as those that appear on the surfaces of pathogenic viruses. Therefore, researchers are busy trying to turn the tables on viruses by presenting conserved epitopes from pathogenic viruses as repeated arrays on the surfaces of harmless virus particles, such as the RNA phages. Not all such applications are directed at preventing

viral infections. One artificial construct based on the phage Qβ presents nicotine as an artificially linked epitope and it is hoped that this virus-like particle will allow 'vaccination' of smokers who otherwise have failed to break their addictions to tobacco.

Our detailed knowledge about the coat protein–RNA operator interaction that regulates replicase translation and triggers self-assembly of progeny phage particles has also been adapted to allow encapsidation of non-phage molecules ranging from large protein toxins, to peptides and DNA oligonucleotides. These potentially therapeutic species can then be directed to specific cells by decoration of the external phage capsid surface with ligands for cell-surface receptor molecules. Once complexed at the cell surface, receptor-mediated endocytosis results in internalization of the encapsidated drug into endosomes where the pH is slightly acidic, conditions that favor disassembly of phage shells, and hence release of the 'drug' entities into the cell. Encapsidation of the plant toxin ricin A chain, that normally needs an additional B chain to cross into cells, allows this highly toxic protein to be delivered into the interior of cells carrying defined external signals resulting in reagents with effective lethal doses in the picomolar range. Controls lacking the targeting ligand are essentially nontoxic, suggesting that encapsidation reduces the nonspecific uptake of ricin A chain known from other studies to create side effects. Such artificially created virus systems have additional advantages, since they can be produced carrying a range of different therapeutic cargoes that can all be targeted to the same cell via attachment of the identical targeting signals. The result is the possibility of refined multidrug therapy without the problems associated with each component having differing pharmacokinetic properties.

An important and very intensively used additional application of the translational repression complex is in the three-hybrid assay system. Macromolecular interactions *in vivo* are central to understanding the integrated functions of all gene products. However, there are relatively few techniques that can easily be used to detect such interactions. One screen, known as the two-hybrid assay, was developed to detect protein–protein interactions using a simple genetic phenotypic screen. It works by creating two fusion proteins. The first carries the information to encode a DNA-binding protein domain fused to a 'bait' protein whose cellular partners are sought. The second fusion protein carries a series of potential partners for the bait domain translationally fused to a transcriptional activation domain. Both fusion constructs are expressed in yeast that carries a binding site for the DNA-binding domain in close proximity to a reporter gene inferring resistance to some chemical challenge, for example, an antibiotic or essential metabolite. Only in those yeast cells where bait and target interact will the transcriptional activation domain drive production of the essential reporter gene. All other cells will not grow. Recovery of the live cells and sequencing of the constructs they carry thus reveals potential partners of the protein–protein interaction *in vivo*, although there is known to be a rate of false positives.

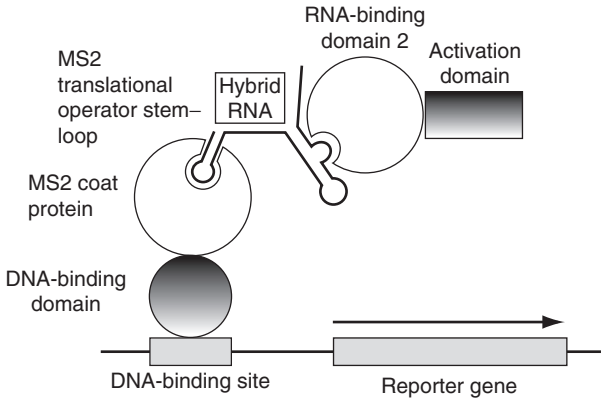


Figure 4 Three-hybrid assay. A schematic view of the components of the three-hybrid assay for detecting RNA–protein interaction *in vivo*. A DNA-binding domain is fused to the MS2 coat protein (RNA-binding domain 1). A second potential RNA-binding domain (#2) is fused to a transcriptional activation domain. Hybrid RNAs carrying the MS2 translational operator stem–loop are also expressed in yeast cells carrying the DNA-binding target adjacent to a reporter gene. Reproduced from SenGupta DJ, Zhang B, Kraemer B, Pochart P, Fields S, and Wickens M (1996) A three-hybrid system to detect RNA-protein interactions *in vivo. Proceedings of the National Academy of Sciences, USA* 93(16): 8496–8501, Copyright (1996) National Academy of Sciences, USA.

In the three-hybrid variant of this approach, the protein–protein interaction is replaced by a protein–RNA interaction. This is achieved by fusing the DNA-binding domain to the MS2 coat protein, thus producing a tethered protein that will bind to RNAs carrying the translational operator site. The translational operator sequence is then embedded in transcripts that include other potential RNA-binding domains. The transcriptional activation domain is then expressed as fusions with potential binding partners for specific RNA sites (**Figure 4**). The end result is the same as for a two-hybrid assay. Only those yeast cells in which the hybrid RNA is bound by a fusion partner will survive and the RNA sequences and or binding partners can then be identified by sequencing.

See also: Replication of Bacterial Viruses; Virus Particle Structure: Nonenveloped Viruses; Virus Particle Structure: Principles.

Further Reading

Caspar DLD and Klug A (1962) Physical principles in the construction of regular viruses. *Cold Spring Harbor Symposia on Quantitative Biology* 24: 1–24.

Paranchych W (1975) Attachment, ejection and penetration stages of the RNA phage infectious process. In: Zinder ND (ed.) *RNA Phages*, pp. 85–112. New York: Cold Spring Harbor Laboratory Press.
SenGupta DJ, Zhang B, Kraemer B, Pochart P, Fields S, and Wickens M (1996) A three-hybrid system to detect RNA–protein interactions *in vivo*. *Proceedings of the National Academy of Sciences, USA* 93: 8496–8501.
Stockley PG and Stonehouse NJ (1999) Virus assembly and morphogenesis. In: Russo VEA, Cove DJ, Edgar LG, Jaenisch R, and Salamini F (eds.) *Development: Genetics, Epigenetics, and Environmental Regulation*, pp. 3–20. Berlin: Springer.
Stockley PG, Stonehouse NJ, and Valegård K (1994) Molecular mechanism of RNA phage morphogenesis. *International Journal of Biochemistry* 26: 1249–1260.
Valegård K, Murray JB, Stockley PG, Stonehouse NJ, and Liljas L (1994) Crystal structure of a bacteriophage–RNA coat protein-operator complex. *Nature* 371: 623–626.
Valegård K, Murray JB, Stonehouse NJ, van den Worm S, Stockley PG, and Liljas L (1997) The three dimensional structures of two complexes between recombinant MS2 capsids and RNA operator fragments reveal sequence specific protein–RNA interactions. *Journal of Molecular Biology* 270: 724–738.
Van Duin J (1988) Single-stranded RNA bacteriophages. In: Calendar R (ed.) *The Bacteriophages*, pp. 117–167. New York: Plenum.
Watson JD, Hapkins NH, Roberts JW, Steltz JA, and Winer AM (1993) *Molecular Biology of the Gene*, *Vol. I*: *General Principle*, 5th edn. New Delhi: Pearson Education, Inc.
Witherell GW, Gott JM, and Uhlenbeck OC (1991) Specific interaction between RNA phage coat proteins and RNA. *Progress in Nucleic Acid Research and Molecular Biology* 40: 185–220.
Wu M, Sherwin T, Brown WL, and Stockley PG (2005) Delivery of antisense oligonucleotides to leukaemia cells by RNA bacteriophage capsids. Nanomedicine: Nanotechnology. *Biology and Medicine* 1: 67–76.

Icosahedral Tailed dsDNA Bacterial Viruses

R L Duda, University of Pittsburgh, Pittsburgh, PA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Contractile tail The tail type that defines the *Myoviridae*, composed of a baseplate that contacts the host surface, an outer protein cylinder or sheath, and an inner tail tube or core. When a host is bound the sheath contracts, driving the tube into the cell to deliver the genome to the host.
DNA packaging The energy-driven process by which a procapsid is filled by a phage chromosome to become a mature capsid.
Head or capsid The protein container for the *Caudovirales* phage genome, usually icosahedral in shape, that protects it from the environment until it is delivered to a new host.
Horizontal gene exchange Transfer of genetic material between different species.
Portal A grommet-like ring, composed of 12 copies of the portal protein, through which DNA is packaged and to which the tail is attached.
Prohead or procapsid An immature capsid that has a spherical shape and contains no DNA.
***T*-number** The triangulation number (*T*) is a formal descriptor for complex icosahedral structures which describes the geometric arrangement of subunits for a viral capsid. The number of capsid protein subunits is $\sim 60 \times T$.
Tail The part of the virion responsible for attachment to the host and genome injection. This is attached to one vertex of the capsid, at the portal.
Tail fiber An elongated protein fiber attached to a phage tail that helps recognize a host by binding to the host by the tip of the fiber.
Virion The entire viral particle composed of a capsid containing the viral genome, tail, tail fibers, and other appendages.

Introduction

The icosahedral, tailed double-stranded DNA (dsDNA) bacterial viruses or bacteriophages of the order *Caudovirales* are among the most widely recognized icons of modern molecular biology. Anyone who has participated in an advanced biology, genetics, or molecular biology class is likely to have been exposed to images of the familiar lunar-lander-shaped bacteriophage T4 virion (family *Myoviridae*) with its elongated icosahedral capsid and machine-like contractile tail (**Figure 1**(**a**)) or the plain icosahedral capsid and elegant, gently curving tail of bacteriophage λ (family *Siphoviridae*; **Figure 1**(**b**)). Both T4 and λ are bacteriophages that infect the enteric bacterial host *Escherichia coli* and have been studied extensively. Bacteriophages were originally discovered in the early twentieth century (1915–17) by English microbiologist Frederick W. Twort and French-Canadian microbiologist Felix d'Herelle. Although there was much early interest in bacteriophages because of their potential for use in treating bacterial diseases (phage therapy), research on

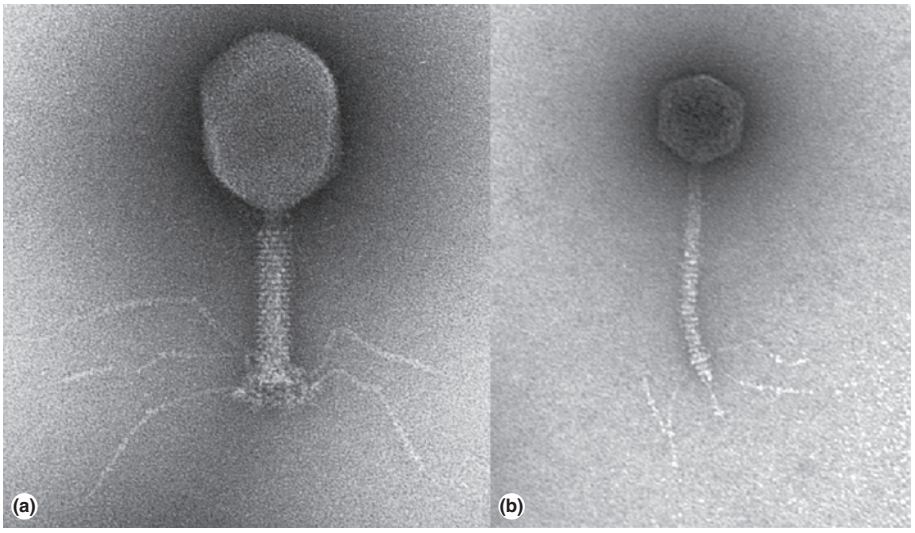


Figure 1 Electron micrographs of bacteriophage T4 and bacteriophage λ (variant Ur-λ). (a) Negatively stained electron micrograph of bacteriophage T4, the prototypical member of the *Myoviridae*. The T4 tail is 100 nm long and the T4 head has a width of 85 nm. Many of the features labeled in **Figure 2** are visible in the micrograph, including the baseplate, tail sheath, whiskers, collar, and long tail fibers. (b) Negatively stained electron micrograph of bacteriophage λ. The micrograph is of an original isolate of λ, called Ur-λ, that has long side tail fibers. Most common laboratory strains of λ carry a mutation in the side tail fiber gene that eliminates the fibers from the particle. The length of the lambda tail is ~150 nm (not including the protruding central fiber) and the lambda head is ~63 nm in diameter.

bacteriophages for disease therapy was largely abandoned after the discovery of antibiotics. Bacteriophage research was revived in the 1940s by Max Delbrück and colleagues (the well-known Phage Group that often met at the Cold Spring Harbor Laboratories on Long Island, NY), and the focused phage research started by this group of scientists led to many fundamental biological discoveries during the birth of molecular biology. The modern-day emergence of multiply antibiotic-resistant strains of human pathogens has rekindled an interest in developing phage therapy as a means of treating human diseases.

The Structure of Tailed dsDNA Bacteriophages

The invention and commercialization of the electron microscope led to the first images of phages as sperm-like particles with a head and tail using a technique called metal shadowing, in which images were formed by heavy metal atoms such as uranium that were evaporated in a vacuum and allowed to strike a dried specimen at an angle. The later introduction of negative stains (salts of heavy metals that dry as a thin layer without forming crystals and in which small particles such as phages could be embedded) for electron microscopy resulted in images that were far richer in detail than earlier methods and revealed the complexity and variety of phage morphology. **Figure 1** shows electron micrographs of phages T4 and λ made using the negative stain technique. Electron microscopy has remained a major tool in the study of bacteriophages and, in fact, the current taxonomic system for phages relies heavily on phage morphology as determined by electron microscopy as a major discriminating factor. **Table 1** shows a taxonomic table for the order *Caudovirales* and some of their characteristics. Members of the *Myoviridae* have contractile tails and include bacteriophages T4 (**Figures 1**(**a**) and **2**) and P1. Members of the *Siphoviridae* have long noncontractile tails and include phages λ (**Figure 1**(**b**)) and T5. Members of the *Podoviridae* have shorter stubby or stumpy tails and include phages P22 and T7. The utility of morphological classification as a basis for phage taxonomy has more recently come into question as new insights into phage evolution have revealed that horizontal exchange of genes is widespread among phages, and further that some structural genes of bacteriophages and viruses that infect organisms from other domains of life appear to share common ancestors.

Tailed Bacteriophage Structure and Function

Capsids

In the tailed dsDNA bacteriophages, the capsid is the container for the phage genome that protects it from the environment until it is delivered to a new host by the phage tail, the organelle of attachment and genome

Table 1 Tailed dsDNA bacteriophages: Order *Caudovirales*

Family	*Genus*	*Defining example*	*Capsid T*-number	*Genome size (bp)*
Myoviridae (phages with contractile tails)	T4-like viruses	Enterobacteria phage T4	$T=13$; $Q=20$	168 903
	P1-like viruses	Enterobacteria phage P1	$T=13$?	93 601
	P2-like viruses	Enterobacteria phage P2	$T=7$	33 593
	Mu-like viruses	Enterobacteria phage Mu	$T=7$	36 717
	SP01-like viruses	Bacillus phage SP01	$T=16$	~132 500
	ϕ-H-like viruses	Halobacterium virus ϕH	$T=7$?	~57 000
Siphoviridae (phages with thin noncontractile tails)	λ-like viruses	Enterobacteria phage λ	$T=7$	48 502
	T1-like viruses	Enterobacteria phage T1	$T=7$?	48 836
	T5-like viruses	Enterobacteria phage T5	$T=13$	121 750
	c2-like viruses	Lactococcus phage c2	$T=4$?; $Q=7$?	22 172
	L5-like viruses	Mycobacterium phage L5	$T=7$	52 297
	ψM1-like viruses	Methanobacterium ψM1	Unknown	~23 246
Podoviridae (phages with short stubby tails)	T7-like viruses	Enterobacteria phage T7	$T=7$	39 937
	ϕ-29-like viruses	Bacillus phage ϕ29	$T=3$; $Q=5$	19 366
	P22-like viruses	Enterobacteria phage P22	$T=7$	41 724

A ? denotes unknown or unconfirmed values.

injection. In many other types of viruses, the capsid is taken up by cells with the genome still inside and the virus particle uncoats or disassembles within the new host to initiate viral replication. The dsDNA bacteriophages' capsids do not have to disassemble in this manner to initiate infection, so they can be constructed to be highly stable – resistant to a wide variety of chemical and physical assaults from the environment – as they travel from one host to another. The dsDNA genome in bacteriophage capsids is packed to a very high density ($\sim 0.5\ \mathrm{g\ ml^{-1}}$) which results in high pressure, so the capsid shell has to be strong enough to withstand the high internal pressure from DNA. Most members of the *Caudovirales* have symmetric icosahedral capsids, but some have capsids with an elongated icosahedral shape, like T4 (**Figures 1(a)** and **2**) that have icosahedral ends and an elongated tubular middle section.

Tails and Tail Fibers

Phage tails function to identify and bind to the correct host and to deliver the phage genome into the new host to initiate replication. Phage tails have a large variety of morphological forms with many parts and appendages, such as those labeled on the diagram of T4 in **Figure 2** and visible in the electron micrograph in **Figure 1**. We know little about the functions of some tail parts, but others are understood in considerable detail. For example, in bacteriophage T4, both the long tail fibers and the short tail fibers (which are folded under the baseplate until after the long tail fibers are bound to the host; see **Figure 2**) recognize specific host receptors. The long tail fibers of phage T4 are the primary determinants of host range, and mutations that change the T4 host range cause alterations in the fibers near their distal tips. The whiskers that are attached to the top of the T4 tail have multiple functions. The whiskers act as assembly jigs for adding the long tail fibers to the virion, and they also act as environmental sensors that sequester the long tail fibers under unfavorable conditions and thus prevent attachment to a host.

The long tail fibers of T4 have an analog in bacteriophage λ, but in the ordinary laboratory strains of λ these fibers are not made because of a mutation in the side tail fiber gene. The electron micrograph in **Figure 1(b)** shows the long side tail fibers present on Ur-λ, a primordial λ that lacks this mutation. These long side tail fibers of Ur-λ speed up the adsorption of this phage to its host, but are not required for infection, because the primary interaction of λ with its host is mediated by the central tail fiber (a trimer of the tail protein gpJ) protruding from the end of the lambda tail. The central tail fiber of λ binds to a host maltose transport protein (called *lam*B or *mal*B) in the outer membrane of the host. Binding of phage λ to its receptor under suitable conditions acts as a signal that triggers the injection of the phage DNA genome into the host to initiate replication.

The structure and functions of many parts of the contractile tails of *Myoviridae* have been revealed in depth, especially for the phage T4 tail. After the T4 long tail fibers attach to the host, the short tail fibers unfold from the baseplate and bind to a second receptor on the host surface. The baseplate remains attached to the cell and undergoes a dramatic reorganization in which it changes from a hexagon to a star shape, causing the sheath to contract and drive the internal tail tube into the cell. As the nail-shaped tip of the inner tail tube passes through the outer membrane, tail lysozyme molecules are released to create a small hole in the cell wall peptidoglycan layer. These combined actions allow the tip of the tail tube to reach the inner membrane, where a channel is created that allows the dsDNA genome to enter the cell and initiate replication.

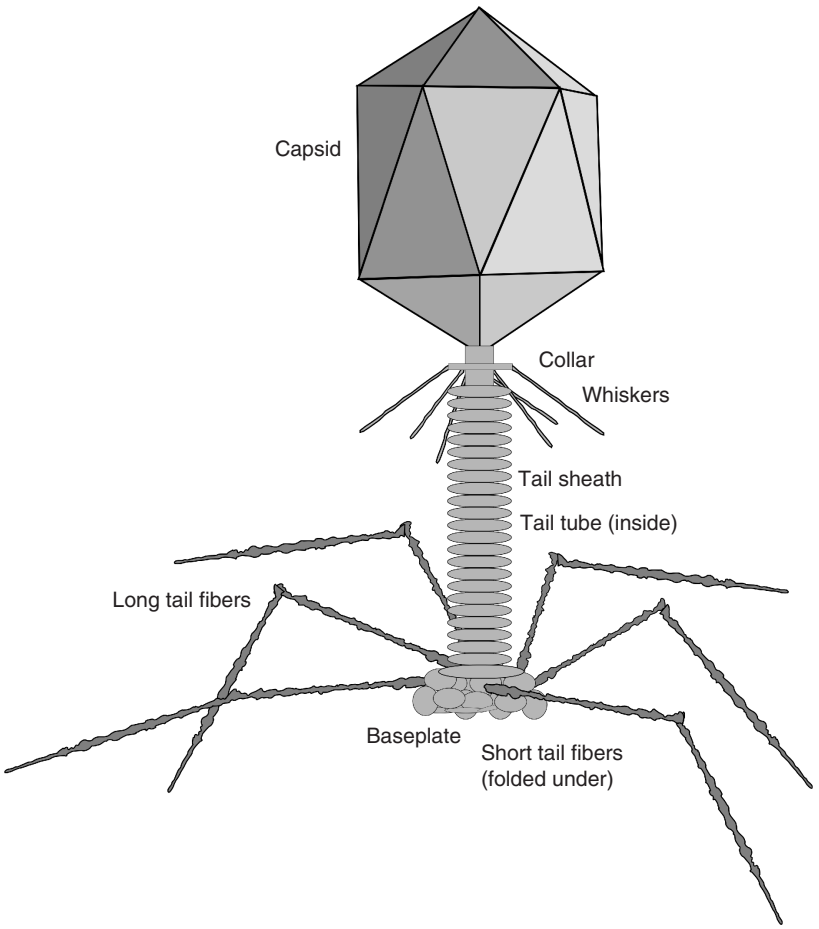


Figure 2 Schematic drawing of bacteriophage T4, with some of the structural components identified.

Temperate versus Virulent Bacteriophages

Virulent bacteriophages, once they have infected a host, are committed to go through their entire growth cycle including replication of the bacteriophage chromosome, production of progeny viral particles, and the lysis of the host cell. Temperate phages, on the other hand, are able to grow lytically like their virulent counterparts, but they are also capable of going into a dormant state within the host. This dormant state is called lysogeny, and often involves integration of the phage chromosome into the host chromosome, as occurs with phage λ, or conversion of the phage chromosome into an autonomously replicating plasmid. The seemingly normal host containing a dormant phage chromosome is called a lysogen. The dormant phage chromosome is maintained in a mostly silent state, until some change in the condition of the host (often related to stress, such as damage to the host DNA) acts as a signal to induce the phage to begin a lytic growth cycle, all the way through progeny release. The temperate phage in the lysogenic state makes a protein factor or repressor protein that can repress (nearly) all phage functions, except for the synthesis of the repressor. If the repressor is subsequently destroyed, as it may be after DNA damage occurs within the cell, the phage initiates a normal growth cycle, usually excising the phage chromosome from the host chromosome as an early step. How a temperate phage decides between a virulent and lysogenic mode of infection is a complex process that appears to depend on the conditions within the host cell at the time of infection. In a simplified version of the process, it is a balance between the production of sufficient repressor protein to shut down phage development and the antagonistic action of other factors that prevents repressor synthesis and function.

Injection

DNA Injection

As described above, all *Caudovirales* bacteriophage particles have tails consisting of highly specialized parts with specific roles to mediate attachment and infection. The

fundamental goal of attachment is the introduction of the viral DNA into the host to begin production of progeny virions. The detailed mechanism of DNA entry varies widely from phage to phage. Although this process is called DNA injection, the phage particles do not act like syringes, as the name implies. In some cases it appears that chromosome entry is largely passive, a result of the DNA being very densely packed into the virion under very high DNA pressure. The energy stored by packaging the chromosome under pressure is used to drive the DNA out of the capsid into the host; this appears to be true for many phages, including phage T4 and phage λ. Other viruses such as phage T7 (family *Podoviridae*) and phage T5 (family *Siphoviridae*), have more elaborate mechanisms of DNA injection with an initial stage that appears to be largely passive, followed by a pause after only a fraction of the chromosome has been injected. Such paused DNA injection resumes only after proteins (encoded by the genes injected in the initial stage) act to restart chromosome entry or to pull the rest of the chromosome into the host in a controlled manner.

Protein Injection

Many of these viruses inject both DNA and proteins into their hosts. The function of a few of these injected proteins is known, but in many cases remains mysterious. For example, phage T4 injects multiple copies of several proteins into the host. These include a protein that modifies the host RNA polymerase and other proteins of unknown function. Surprisingly, most of phage T4's injected proteins are not absolutely required for infection, and mutants that lack them are able to grow on many ordinary laboratory hosts. Phage T7 injects several large proteins that possibly play important roles in the injection process, for example, by forming channels for the passage of DNA and other proteins into the host. The injected proteins of phage T7 are essential for phage growth.

Host Interactions and Regulation of Gene Expression

Classes of Genes

A regulated program of gene expression begins soon after the virus has injected its DNA. The expression of phage genes can be divided by function, such as host takeover, replication, virion assembly, or lysis, but more often they are sorted by their temporal order of expression. In most cases gene expression is divided into early genes and late genes, although for some phages there may be a cascade of gene expression modes that occur sequentially. In a cascading series, early phage gene products control the expression of a set of middle gene products, which in turn control the expression of late genes.

Early Genes

Phage early genes are usually transcribed by the normal host RNA polymerase, while the expression of late genes is dependent on the expression of the early genes. The earliest genes expressed might be ones that produce proteins to counteract the host's antibacteriophage defensive systems, as occurs in phage T7. Early genes of temperate phages are those required to make the decision to become dormant or to grow normally and carry out the first steps of these processes, including, for example, the repressor gene and integration genes, or DNA replication and recombination genes. Early genes of virulent bacteriophages, such as phage T4, include genes for genome replication and genes that shut down unneeded host functions, such as host transcription and host protein synthesis, and may include other genes required to recycle host resources, for example, by specifically degrading the host chromosome to intermediates that can be reused during the replication of the phage genome.

Late Genes

The proteins encoded by the late genes include the structural proteins required for the assembly of the bacteriophage virion, as well as those needed for packaging the genome into the virion and for cell lysis. Late gene expression is often dependent on early (or middle) gene expression because the late genes have transcriptional promoters that are not recognized by the normal host polymerase. The early genes may direct the synthesis of transcription factors that either (1) change the specificity of the host RNA polymerase or (2) modify the polymerase in other ways, in order to express the whole set of late genes at the appropriate time with high efficiency. Alternatively, one of the early genes may encode for an entirely new RNA polymerase with a new specificity that recognizes late gene promoters. Expression of the structural proteins to high levels allows the production of a large number of progeny phages.

Virion Assembly

Assembly Pathways

The assembly of *Caudovirales* virions is a highly ordered process in which separate subassemblies of the virion are built and then joined to form the mature virion. The assembly of capsids, tails, and tail fibers each follows an independent assembly pathway in which individual protein components are added, usually sequentially, to a growing structure until it is complete.

Tail Assembly

The major components of a *Caudovirales* tail are a baseplate or tail tip, major tail proteins (one for a noncontractile tail,

and two for contractile tails (an inner tube protein and an outer sheath protein)), and termination or capping proteins to stabilize the completed tail and connect it to the head. Tail assembly begins with the formation of an initiator complex, which may be either a complete tail tip or a complete baseplate that is assembled via a separate pathway. The initiator complex includes a template that specifies the length of the tail, the tape measure protein complex in a compact form. The major tail proteins bind to the initiator complex and assemble into a tube around the tape measure protein until the full length of the tape measure protein is enclosed and the termination proteins can bind and stabilize the tail.

Capsid Assembly

The capsids of these viruses are built as precursor procapsids, into which the phage genome is later packed. Procapsids are initially assembled from several types of components: a portal complex (which will connect the capsid to the tail), a major capsid protein, a scaffolding protein (which may be a separate protein or may be a disposable part of the major capsid protein), and decoration or stabilization proteins (which add to and stabilize the capsid only after the genome is packed inside). The number of capsid protein subunits needed for assembly is equal to $\sim 60 \times T$ subunits, where T is the triangulation number listed in **Table 1** (or, for phages with elongated capsids, $\sim 30 \times (T + Q)$ subunits, where T and Q are specified in **Table 1**). So for a $T = 7$ virus, such as λ, ~420 copies are needed and more copies are needed for larger phages. Capsid assembly begins with the completion of the assembly initiator complex, usually the portal. The major capsid protein together with hundreds of copies of the scaffolding protein co-assembles onto the initiator to produce a complete shell with the scaffolding protein on the inside. After assembly is complete, the scaffolding protein is expelled intact or digested by a special protease that is also incorporated into the procapsid. Procapsids appear spherical and usually have a smaller diameter than mature capsids. When DNA is packaged into the procapsid, the capsid usually expands and changes shape, transforming into the typical angular, icosahedral shape of mature capsids. The decoration proteins add to the outside of the capsid after it expands and help to stabilize the structure. Once DNA is packaged, the capsid is made ready to join to a tail by the addition of proteins that bind to the portal.

Lysis

Lysis of an infected host requires two phage-encoded protein factors, an endolysin or lysozyme, and a second protein called a holin. The endolysin is a soluble enzyme with the capacity to break the bonds holding the host cell wall together. The endolysin molecules accumulate within the host cytoplasm during the late stages of infection, but are unable to attack the cell wall because they are sequestered within the cytoplasmic membrane. Holin proteins allow the endolysin to get across the cytoplasmic membrane by forming holes in the inner membrane. Holins are synthesized and inserted into the membrane in a form that does not form holes at first. As the bacteriophage infection proceeds, the holins accumulate in the membrane until a predetermined time at which they suddenly and catastrophically induce membrane breakdown. The membrane holes produced by holins allow the endolysin molecules to attack the bacterial cell wall, causing rapid cell lysis and releasing the progeny virus from the cell.

Genomes and Genomics

Chromosome Diversity and Replication

The chromosomes that are packaged into the capsids of the *Caudovirales* are linear dsDNA. *Caudovirales* virions always contain an entire genome or slightly more than an entire genome to ensure that a complete genome is packaged into each and that every particle can initiate an infection. To achieve this, a site-specific mechanism may be used, in which DNA packaging begins and ends at defined sites that are recognized by the packaging machinery to create exactly unit-genome-length DNA chromosomes. Alternatively, the amount of virion DNA may be regulated by a head-full packaging mechanism which fills a capsid that has the capacity to hold slightly more than one genome's worth of DNA. The head-full-packaged chromosomes have the same sequence at each end and are said to be terminally redundant.

After injection, the phage genomes often rearrange to form a circular chromosome that is the primary replicative form of the genome of many dsDNA phages; however, many other phages replicate without forming such DNA circles. Chromosome replication often takes place bidirectionally from a single origin, but more complex replication schemes and multiple replication origins have also been observed. Late in infection, most phages switch to a mode of replication that produces DNA concatamers, or long strings of genome copies joined end to end, either by a rolling circle mode of replication from circular chromosomes, or by recombination between multiple overlapping genome copies. Such DNA concatamers, whether linear or branched, are the forms of the genome that are the usual substrate for DNA packaging for both head-full and site-specific mechanisms. When it does occur, circularization of the phage chromosome takes place via one of the two mechanisms. The first is by the annealing of complementary single-stranded DNA sticky ends left by the packaging enzymes (in the cases where the packaged chromosomes have defined endpoints). The second is

by a recombination-like mechanism that joins the complementary regions of the overlapping, terminally redundant chromosome ends to form circular DNA molecules. Circular chromosomes are the most common form for bacterial chromosomes, but some bacteria also have linear chromosomes, and a small subset of members of this order also forms linear chromosomes that replicate as linear plasmids when in their lysogenic form.

Diversity in Genome Size and Organization

The genomes of these viruses range in size from ~20 000 bp in phage ϕ29, to ~170 000 bp in phage T4, and up to ~500 000 bp in other known examples. The smaller phages have proportionally fewer genes than the large phages, but are nonetheless functional and successful phages. Given this wide range of sizes and diversity of these viruses, it is not surprising that there is not a common conserved genome structure in the members of the order *Caudovirales*. Within a genus of phages there is often a common genetic structure, but within and across families there is often little resemblance in genome organization. There are notable exceptions to these generalizations; for example, the genus 'P22-like viruses' and the genus 'λ-like viruses' have rather closely related genomes.

Common Themes in Genome Structure

Despite the differences mentioned, there are many common general features in *Caudovirales* genomes. The genes for the structural proteins, such as those for capsids, tails, or tail fibers, tend to be found clustered together, and within these clusters, the genes that encode proteins that physically interact with each other also tend to be grouped together. For example, the capsid protein genes for the portal, the scaffolding protein, and the major capsid protein usually occur together and in the order listed. Sets of late genes are often grouped together in clusters and, in some cases, the entire set of structural genes are grouped together in clusters and transcribed together from a single promoter, as is the case for phage λ.

Horizontal Exchange of Genes Is Widespread

A large number of complete bacteriophage genomes have been fully sequenced, and hundreds of these have been deposited in GenBank and other databases. In addition, there are also many temperate phages residing in the genomes of bacterial chromosomes – some defective (or cryptic), and some complete and able to form a viable phage. Analyses of the sequences of these genomes have shown that many of the genes have highly similar counterparts in other genomes and in many cases the similarity can be inferred to be truly homologous. An important conclusion of these analyses is that there is extensive horizontal exchange of genes between phages that are not within the same species or genus or order. It appears that genetic exchange between phages by both homologous and nonhomologous recombination mechanisms is widespread and that many phages are mosaic combinations of genes found elsewhere. Many of the homologous arise genes within the *Caudovirales*, but some are from outside this order. In some cases, large modules of genes in a pair of phages are closely related (e.g., the head genes of phage HK97 (*Siphoviridae*) and phage SfV (*Myoviridae*)) despite belonging to different taxonomic groups. In other cases, a single phage tail fiber encoding gene in one phage may contain several distinct regions of homology with several other different phages.

Common Ancestry

The exact nature of the evolutionary relationships among phages and how it relates to phage taxonomy is a controversial subject, but at the level of individual protein-coding genes, it is fairly certain that proteins with a high degree of sequence similarity in analogous proteins almost certainly share a common evolutionary ancestor. The power and utility of bioinformatics to tease out weak (sequence) similarities between distantly related proteins provides the evolutionary scientist powerful tools to detect relationships that escape casual examination. However, the reliance on sequence similarity matches to detect homologous relationships breaks down when homologous proteins have diverged so far that sequence similarity is undetectable. A notable case is that of the major capsid proteins of bacteriophages and other viruses. The three-dimensional structure and protein fold of the major capsid protein of phage HK97 was determined to high resolution by X-ray crystallography. Sensitive bioinformatic techniques were able to detect weak sequence similarities between the HK97 capsid protein sequence and the capsid proteins of a large number of other members of the *Caudovirales*, suggesting that the HK97 capsid protein fold is quite common. Subsequently, the determination of the protein folds of phages T4 (*Myoviridae*), P22, ϕ29, and ε15 (*Podoviridae*) by structural methods has shown that all of these phages also have the same protein fold as HK97, despite the lack of any detectable sequence similarity.

See also: Capsid Assembly: Bacterial Virus Structure and Assembly; Genome Packaging in Bacterial Viruses; History of Virology: Bacteriophages; Replication of Bacterial Viruses; Virus Evolution: Bacterial Viruses.

Further Reading

Cairns J, Stent GS, and Watson JD (eds.) (1992) *Phage and the Origins of Molecular Biology*, expanded edition. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Calendar R (ed.) (2006) *The Bacteriophages*, 2nd edn. Oxford: Oxford University Press.
Caspar DL and Klug A (1962) Physical principles in the construction of regular viruses. *Cold Spring Harbor Symposium on Quantitative Biology* 27: 1–24.
Hendrix RW (2003) Bacteriophage genomics. *Current Opinion in Microbiology* 6: 506–511.
Hendrix RW and Casjens S (1988) Control mechanisms in dsDNA bacteriophage assembly. In: Calendar R (ed.) *The Bacteriophages*. New York: Plenum.
Hendrix RW and Duda RL (1998) Bacteriophage HK97 head assembly: A protein ballet. *Advances in Virus Research* 50: 235–288.
Hendrix RW, Roberts JW, Stahl FW, and Weisberg RA (1983) *Lambda II*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.
Karam JD, Drake JW, Kreuzer KN, *et al.* (eds.) (1994) *Molecular Biology of Bacteriophage T4*. Washington, DC: ASM Press.
Katsura I (1990) Mechanism of length determination in bacteriophage lambda tails. *Advances in Biophysics* 26: 1–18.
Leiman PG, Kanamaru S, Mesyanzhinov VV, Arisaka F, and Rossmann MG (2003) Structure and morphogenesis of bacteriophage T4. *Cellular and Molecular Life Sciences* 60: 2356–2370.

Idaeovirus

A T Jones and H Barker, Scottish Crop Research Institute, Dundee, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The genus *Idaeovirus* contains a single virus species, *Raspberry bushy dwarf virus*, named after the disease (characterized by stunting and proliferation (bushiness) of canes) with which it was first associated in red raspberry (*Rubus idaeus*). This mechanically transmissible virus was detected consistently in such diseased plants. Later, however, it was shown that raspberry bushy dwarf virus (RBDV) on its own in young raspberry plants did not induce obvious disease but, in mixed infection with black raspberry necrosis virus (BRNV), an aphid-borne virus, it intensified the degeneration in vigor caused by infection with BRNV alone. RBDV therefore does play a causal part in the total 'bushy dwarf' syndrome.

RBDV is serologically indistinguishable from loganberry degeneration virus and synonymous with raspberry yellows virus and is probably the same as raspberry line pattern virus reported from Poland.

Studies on the molecular biology of RBDV have shown it to possess a novel combination of properties and it has been assigned, as the sole known member, to the genus *Idaeovirus.* Though RBDV has a bipartite genome, its closest affinities are with viruses that have tripartite genomes in the family *Bromoviridae.*

Biological Properties

Geographical Distribution

RBDV probably occurs wherever red raspberry (*R. idaeus, R. strigosus*), black raspberry (*R. occidentalis*), or blackberry (*R. fruticosus*), is grown. The virus has been reported from Australasia, China, Eastern and Western Europe, North and South America, South Africa, and the former Soviet Union. However, at least three variants of RBDV have been characterized and some are more restricted in their geographical distribution.

Common or Scottish (S) isolates

The Scottish type isolate (S) is representative of isolates that have been identified in almost every country where raspberry is grown and are therefore sometimes referred to as isolates of the 'common strain'. They produce characteristic symptoms in herbaceous test plants and, under suitable environmental conditions, in sensitive raspberry cultivars. They are distinguished by a restricted *Rubus* host range, and many red raspberry cultivars are highly resistant, probably immune to infection. S isolates also fail to infect some hybrid berry cultivars.

Resistance-breaking (RB) isolates

These isolates are largely indistinguishable biologically from S isolates except that they are able to infect red raspberry cultivars and hybrid berry cultivars immune to S isolates. Indeed, they infect by grafting nearly all *Rubus* species and cultivars tested. RB isolates seem restricted in their distribution to Central and Eastern Europe, Russia and Siberia, and, probably through importation of infected material, to isolated areas in England and some parts of Western Europe.

Black raspberry (B) isolates

Two isolates of RBDV from the American black raspberry (*R. occidentalis*), which were serologically closely related to S isolates, were distinguishable from them by spur formation in double-diffusion serological tests in agarose gel. This is the only evidence of serological variability in field isolates of RBDV. B isolates are more difficult to maintain in culture in herbaceous plants and their particles are more difficult to purify in quantity than those of S isolates. They are also difficult to detect in *R. occidentalis*

extracts by inoculation to *Chenopodium quinoa* test plants but are readily detected by enzyme-linked immunosorbent assay (ELISA), suggesting that they may have a low specific infectivity. Only B isolates were found in naturally infected *R. occidentalis*. However, more data are needed to determine whether B isolates are restricted to *R. occidentalis* and whether S isolates can occur in *R. occidentalis* in nature. B isolates have been found in nature only in North America, the sole region where *R. occidentalis* is grown extensively.

Natural and Experimental Transmission

In red raspberry and black raspberry, RBDV is readily transmitted to progeny seedlings via either the pollen or the ovule of infected plants and transmission is greatest when both parents are infected. Transmission in association with pollen is confirmed in field and glasshouse studies. Healthy plants can be infected after the first flowering season when planted close to virus sources.

Experimentally, RBDV is readily transmitted from *Rubus* by grafting to infectible *Rubus* species and cultivars. However, graft-inoculated plants usually need to go through a dormant season before the virus becomes fully systemic. RBDV is also transmissible from infected *Rubus* to herbaceous test plants by mechanical inoculation of sap extracts in aqueous solutions of either 2% nicotine or 1% polyethylene glycol (PEG) (mol. wt. 6000 Da). Mechanical transmission from RBDV-infected herbaceous plants to some infectible red raspberry cultivars is also possible by the same procedure, but with great difficulty.

Disease Symptoms and Effects

Naturally infected plants

In nature, RBDV occurs in wild and cultivated *Rubus* species and cultivars. When infecting alone, RBDV is often symptomless in *Rubus* plants, especially in Britain. However, in sensitive raspberry cultivars and under ill-defined environmental conditions, it induces symptoms of yellows disease. This disease is characterized by an initial yellowing of the main or minor veins of the lower leaves or leaflets that progressively extends to other veins and ultimately to the interveinal lamina, sometimes affecting the whole leaf (**Figure 1**). Some raspberry cultivars may also develop chlorotic/yellow rings or line patterns in leaves.

RBDV infection also causes 'crumbly fruit' in some raspberry cultivars. This condition, arising from the abortion of some drupelets and the uneven development of others, frequently results in an abnormally shaped fruit, which, on picking, may disintegrate into individual drupelets or drupelet clusters (**Figure 2**). The same disease syndrome can be induced by other unrelated causes, such as poor pollination conditions, infection with other viruses, or genetic aberrations.

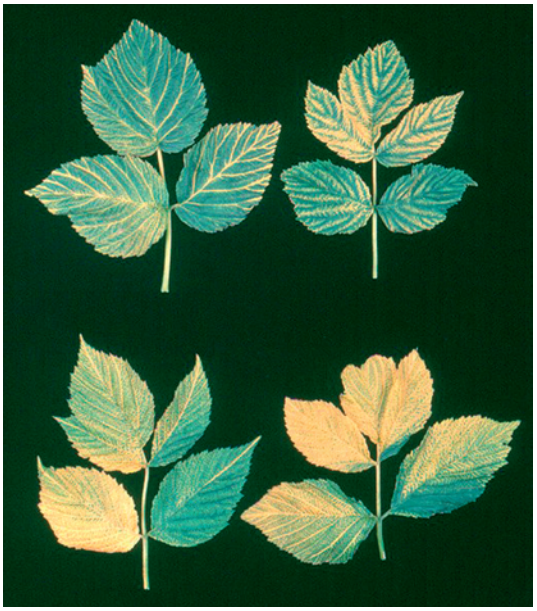


Figure 1 Leaves of RBDV-infected red raspberry showing, from upper left through to lower right, the progressive development of yellows disease symptoms. Reproduced with the permission of the Scottish Crop Research Institute (SCRI).



Figure 2 Symptoms of crumbly fruit of red raspberry caused by RBDV infection. Reproduced with the permission of the Scottish Crop Research Institute (SCRI).

Additional symptoms have been attributed to RB isolates. In field studies in England, an association was reported between infection with RBDV-RB and premature defoliation of fruiting canes, decreased vigor, leaf curling, necrosis and death of laterals, and increased winter kill in some red raspberry cultivars that are immune to S isolates of this virus. Although this evidence is only

circumstantial, it suggests that these severe symptoms are caused by infection with RBDV-RB.

Experimentally infected plants

RBDV has been transmitted by graft inoculation from *Rubus* to perennial plants in other genera. It symptomlessly infected *Fragaria vesca* and *Prunus mahaleb* seedlings and induced pronounced chlorotic veinbanding and line patterns in *Cydonia oblonga* cv. C7-1. RBDV has a moderately wide host range in herbaceous test plants, but infects most hosts symptomlessly. By mechanical inoculation of RBDV-infective sap, over 50 plant species from 12 dicotyledonous families were infected.

Particle Properties

Properties of Virus Particles

RBDV particles are best purified from leaf tissue of *C. quinoa*. Purified virus particles are about 33 nm in diameter, and appear quasi-isometric (**Figure 3**) because they tend to collapse and deform on the electron microscope grid. They appear more spherical in shape when aldehyde-fixed. Particle preparations have A260/A280 of 1.62, suggesting that RBDV particles contain about 24% RNA. The particles are unstable and readily disrupt in the presence of 0.01% sodium dodecyl sulfate, indicating that they are stabilized by protein–RNA linkages.

The particles sediment as a rather broad band in sucrose density gradients, with a sedimentation coefficient of 115 S in 0.05 M citrate buffer, pH 6. Formaldehyde-fixed virus particles, centrifuged to equilibrium at 20 °C in solutions of CsCl or RbBr, form a band at a density of 1.37 g ml^{-1}. Very rarely, preparations also contain particles *c.* 15 nm in diameter that sediment at *c.* 34 S and are serologically identical to the 33-nm-diameter particles and may be re-aggregates of viral coat protein.

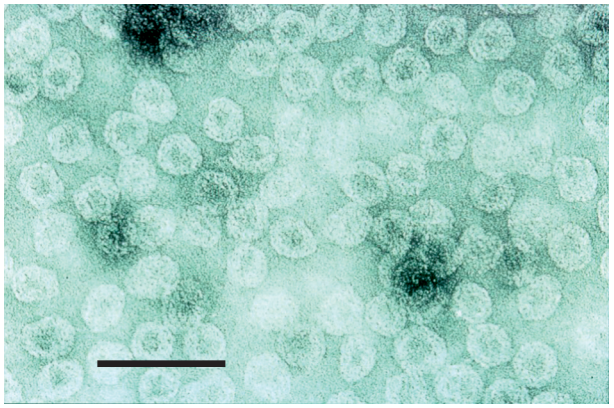


Figure 3 Electron micrograph of a purified preparation of RBDV particles stained in uranyl formate. Scale = 100 nm. Reproduced with the permission of the Scottish Crop Research Institute (SCRI).

Composition of Virus Particles

Purified preparations of RBDV particles typically yield a single major protein estimated by polyacrylamide gel electrophoresis to have an M_r of *c.* 30 kDa. However, some preparations, even highly purified ones, contain minor amounts of smaller proteins, presumably of plant origin.

The particles contain three species of single-stranded RNA (ssRNA) with M_r about 2.0×10^6 (RNA-1), 0.8×10^6 (RNA-2), and 0.3×10^6 (RNA-3). The three RNA species are sometimes present in approximately equimolar amounts but in some preparations and with some isolates, RNA-3 (or RNA material of about the same size) is often present in much greater molar concentration than RNA-1 and RNA-2. The way in which the RNA molecules are packaged in the particles is unknown, but it is unlikely that all three RNA species are contained within the same particle.

Molecular Biology

Nucleotide Sequences

The complete nucleotide sequences have been determined for the three RNA species present in particles of isolates S and RB. **Figure 4** is a diagram of the genome structure of RBDV deduced from these sequences. RNA-3 is 946 nt long and contains a single ORF, which encodes the 30 kDa coat protein. This predicted size corresponds to that of protein obtained from purified virus particles and that of the main polypeptide made by *in vitro* translation of RNA-3.

RNA-2 is 2231 nt long and its 3′ half contains the entire sequence of RNA-3; RNA-3 is thus a subgenomic mRNA. In addition, RNA-2 contains, in its 5′-half, a second ORF which encodes a 39 kDa protein. Polypeptides obtained by

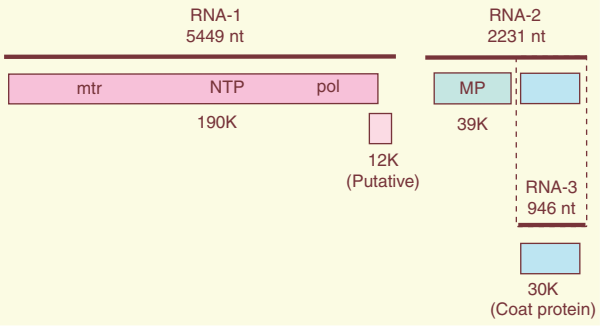


Figure 4 Diagram showing the genome structure of RBDV based on the RNA sequences. RNA molecules are represented by solid lines and the putative positions of the products of the ORFs are indicated as boxes. The positions of domains in the polyproteins are shown as: MP, residues shared among putative transport proteins; mtr, methyltransferase; NTP, NTP-binding; pol, RNA-dependent RNA polymerase. The dotted lines indicate that RNA-3 is derived from the 3′ terminal portion of RNA-2. Reproduced with the permission of the Scottish Crop Research Institute (SCRI).

in vitro translation of RNA-2 appeared to be larger than this: translation in wheat germ extracts produced a 46 kDa protein and translation in reticulocyte lysate produced a 42 kDa protein.

RNA-1 is 5449 nt long and contains one large ORF which encodes a 190 kDa protein. This protein corresponds in size to the largest polypeptide made by *in vitro* translation. A small ORF is also present in a different translation frame and overlapping the ORF for the 190 kDa protein (**Figure 4**). This small ORF encodes a 12 kDa polypeptide. All RBDV isolates sequenced to date contain this ORF in this 3′ region. No corresponding subgenomic RNA was detected in RNA extracted from RBDV-infected plants, and infectious RNA transcripts of a 12 kDa deletion mutant of RBDV were still able to infect herbaceous host plants indicating that the 12 kDa protein is not essential for infectivity. Intriguingly, the position of this gene corresponds to that of the 2b gene in the tripartite genome of cucumber mosaic virus (CMV) that encodes a 10 kDa polypeptide and overlaps the C-terminal 69 codons of ORF 2a that encodes the RNA polymerase protein. In their respective RNA molecules both the CMV 2b and putative RBDV 12 kDa proteins are encoded in the +1 reading frame of the major ORF. The CMV 2b protein is important in both long-distance systemic movement and the suppression of post-transcriptional gene silencing but it is also not necessary for infectivity. However, experiments failed to demonstrate the activity of the 12 kDa RBDV protein in suppressing post-translational gene silencing.

There are similarities among the 5′ or 3′ terminal noncoding sequences of each of the three RBDV RNA species. At the 5′ end of RBDV RNA, the first six to eight nucleotides are similar in all three RNA species, the consensus being UAUUU. At the 3′ end, RNA-3 is identical to RNA-2 (being derived from it), and RNA-1 and RNA-2 share sequence and structural similarities. The 3′-terminal 18 nucleotides are identical in RNA-1 and RNA-2, and the terminal 71 nucleotides of each RNA can be folded into similar stem–loop structures.

The noncoding region between the two ORFs in RNA-2 contains the start of RNA-3 and presumably also contains sequences responsible for the initiation and promotion of its transcription. Like the comparable region of bicistronic RNAs of other viruses, this part of RBDV RNA-2 is rich in A and U residues. No double-stranded RNA (dsRNA) was found in infected tissue which could correspond to a double-stranded form of RNA-3, and RNA-3 is therefore probably transcribed from a minus-sense copy of RNA-2.

Virus Protein Sequences

The 30 kDa protein (coat protein)

The coat protein of RBDV shows no striking similarities with the coat proteins of other viruses. In comparisons made by using CLUSTALV, RBDV coat protein had a sequence identity of between 10% (alfalfa mosaic virus, AMV) and 19% (tobacco streak virus, TSV) to coat proteins of viruses in the family *Bromoviridae*. An alternative to this sequence-based alignment program is to use secondary structure predictions to produce an alignment. The RBDV coat protein structure predicted by such an alignment was of a long N-terminal sequence containing two α-helices and followed by a region containing eight β-sheets. If correct, this arrangement would resemble that of the coat proteins of many viruses that have isometric particles. The N-terminal parts of the coat proteins of RBDV and of viruses in the family *Bromoviridae* are relatively rich in basic amino acids which are thought to be internal and to interact with the encapsidated RNA.

The 39 kDa protein

RNA-2 of RBDV corresponds in size and gene content to RNA-3 of viruses in the family *Bromoviridae*. The ORF for the 39 kDa protein therefore corresponds in position to the ORF for the putative movement protein of, for example, AMV. A small sequence identity was detected in multiple alignment tests between the 39 kDa protein and the putative or actual movement proteins of several viruses. In further alignment tests of this sort, Mushegian and Koonin have detected a match between part of the 39 kDa protein sequence and a motif conserved in the movement proteins of some other viruses, such as red clover necrotic mosaic virus and soil-borne wheat mosaic virus. These proteins were proposed to belong to a '30 kDa superfamily' of movement proteins.

The 190 kDa protein

The sequence of the 190 kDa protein has similarities with replicase proteins of viruses in the Sindbis-like supergroup. There was good correspondence between parts of the 190 kDa protein and parts of the 183 kDa protein of tobacco mosaic virus but the greatest similarity was with a hypothetical composite protein consisting of the 90 kDa protein of AMV (encoded by RNA-2) attached to the C-terminus of the AMV 125 kDa protein (which is encoded by RNA-1). The three regions of greatest similarity correspond to the locations in the AMV proteins of the methyltransferase, NTP-binding, and RNA-dependent RNA polymerase domains. The first two domains occur in the 125 kDa protein of AMV and the polymerase domain occurs in the 90 kDa protein of AMV. RBDV RNA-1 therefore corresponds in size and gene functions to RNA-1 plus RNA-2 of AMV and related viruses. Comparisons of the three domains of several viruses by using CLUSTALV suggested that RBDV is marginally more similar to viruses in the family *Bromoviridae* than to other viruses. The maximum similarity was to AMV.

Detection and Control

Detection in Plants

In *Rubus*, RBDV may induce yellowing, line patterns, or crumbly fruit, but such symptoms are unreliable for diagnosis because they may be due to infection with other disease agents or to other causes. Furthermore, RBDV often infects plants symptomlessly. Detection and diagnosis therefore depend on bioassays and/or serological tests.

RBDV can be reliably detected by one or other of the various forms of ELISA and this is the preferred detection method for B isolates, which seem to have a low specific infectivity. Other studied isolates are readily detected by mechanical transmission to *C. quinoa* test plants. However, identification of RBDV in symptom-bearing herbaceous test plants is dependent on serological tests. As the distribution of RBDV in individual *Rubus* plants can be erratic, leaves/leaflets for mechanical transmission or ELISA should be taken and pooled from at least three different nodes.

Polyclonal antisera raised to S isolates have a moderate titre of *c.* 1/512 in gel double diffusion tests. RBDV antigen in purified virus preparations or in infective sap of *C. quinoa* reacts with such antisera in microprecipitin tests and in agarose gel double-diffusion serological tests. As already mentioned, spur formation in this latter test can be used to distinguish S isolates from B isolates. In Canada, monoclonal antibodies to RBDV were able to distinguish three different epitopes of a Canadian isolate of RBDV. However, these monoclonal antibodies reacted in double antibody sandwich ELISA (DAS-ELISA) with all tested isolates from red raspberry, including B, S, and RB isolates.

Therapy

Unlike many other viruses infecting *Rubus*, RBDV is not readily eradicated from *Rubus* plants by heat treatment alone. However, heat treatment for several weeks at 36 °C followed by propagation from shoot tips or apical meristems eliminated the virus from some plants. Using only tissue culture of meristem tips, it was necessary to subculture up to three times to eliminate the virus.

Control in Crops

Because RBDV is transmitted in association with infected pollen, the only methods of controlling it in crops are to grow resistant cultivars, or to plant healthy stock of infectible cultivars and grow them in isolation from possible sources of infection. Many red raspberry cultivars are resistant, possibly immune, to S isolates (the most widespread isolates worldwide). This resistance is conferred by the presence of a single dominant gene, *Bu.* However, almost all *Bu*-containing cultivars are infectible by grafting with RB isolates. Resistance to graft inoculation with RB isolates was detected in only very few red raspberry cultivars. Studies on this form of resistance indicated that its mode of inheritance was complex but seemed to depend on the presence of gene *Bu*, together with a second resistance component whose inheritance was probably multigenic. In field studies, some raspberry cultivars, including some that do not contain gene *Bu*, are more resistant than others to natural infection with RB isolates. This might suggest that inherent resistance to field infection with RB isolates may exist in some cultivars that are infectible by grafting.

Relationships with Other Viruses

RBDV resembles viruses in the genus *Ilarvirus,* family *Bromoviridae* in having easily deformable particles that are transmitted in association with pollen. RNA-2 resembles RNA-3 of ilarviruses in the arrangement and sizes of its encoded gene products, the generation of a 3′ terminal subgenomic RNA (sgRNA) and in the structured nature of the 3′ ends of the molecules. Nevertheless, RBDV is serologically unrelated to all recognized ilarviruses tested. Also, RBDV differs from ilarviruses in the sedimentation behavior of its particles, in the number and sizes of its RNA molecules, and in having a bipartite genome. Taken together, these properties distinguish RBDV from all other well-characterized viruses and justify its classification as the sole member of the genus *Idaeovirus.* The genus has not been assigned to a family, but, because of its molecular properties, the genus *Idaeovirus* may be tentatively classified in the family *Bromoviridae*, albeit as an atypical member of a family whose four recognized genera contain viruses with tripartite genomes. The sequence of the translation product of RBDV RNA-1 resembles in different parts, sequences in the translation products of viruses in the family *Bromoviridae* and to a lesser extent the sequences of the helicase + polymerase protein ($M_r \sim 183 \times 10^3$) of tobamoviruses. Idaeoviruses, therefore, belong to the 'Sindbis-like' supergroup.

See also: Alfalfa Mosaic Virus; Brome Mosaic Virus; Bromoviruses; Cucumber Mosaic Virus; Ourmiavirus; Phycodnaviruses.

Further Reading

Jones AT (2005) Genus *Idaeovirus*. In: In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1063–1065. New York: Academic Press.

Jones AT and Mayo MA (1998) Raspberry bushy dwarf virus. In: Description of Plant Viruses No. 360, 6pp. Association of Applied Biologists. http://www.dpvweb.net/dpv/showdpv.php?dpvno=360 (accessed January 2008).

Jones AT, Mayo MA, and Murant AF (1996) Raspberry bushy dwarf idaeovirus. In: In: Harrison BD and Murant AF (eds.) *The Plant Viruses, Vol. 5: Polyhedral Virions and Bipartite Genomes*, pp. 283–301. New York: Plenum.

Iflavirus

M M van Oers, Wageningen University, Wageningen, The Netherlands

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Internal ribosome entry site (IRES) Complex secondary RNA structure that allows translation initiation independent of a 5′ cap (m^7GpppN) structure.
UTR Untranslated region in an mRNA molecule on either the 5′ or 3′ side of an open reading frame.

Introduction

Iflaviruses have been described so far in lepidopteran and hymenopteran insects as well as in bee parasitic mites. In insects these viruses can cause mortality or developmental malformations. Sacbrood virus (SBV), for instance, lethally affects honeybee larvae (*Apis mellifera*) and the first descriptions of sacbrood disease date back as early as 1913. SBV was initially characterized in 1964. Other examples of iflaviruses causing disease in honeybees are deformed wing virus (DWV), which causes morphological abnormalities, and the related Kakugo virus (KV), the causative agent of aggressiveness in infected bees. Bee parasitic Varroa mites (*Varroa destructor*) function as vectors for these (and other) bee viruses, but DWV and the related Varroa destructor virus 1 (VDV-1) can also replicate in these mites. Infectious flacherie virus (IFV) infects the silkworm *Bombyx mori* and is a major cause of cocoon loss in silk production in Japan. In 1998 IFV was the first iflavirus for which the complete genome sequence was determined. Iflaviruses were previously referred to as insect picorna-like viruses together with viruses currently classified as *Dicistroviridae*.

Characteristics and Classification

Iflaviruses are characterized by icosahedral particles, which are nonenveloped and have a size of approximately 30 nm (**Figure 1**). The virions contain a single copy of a single-stranded, linear RNA molecule of positive polarity. The RNA genome is nonsegmented and encodes one large polyprotein, which is cleaved post-translationally into structural (capsid) and nonstructural proteins.

Infections flacherie virus is the type species of the genus *Iflavirus* which is not yet assigned to a virus family. Other species with an official status in this genus are *Sacbrood virus* and *Perina nuda virus*. Tentative species in this genus according to the Universal Virus Database (ICTVdBAse, version 4) are Deformed wing virus, Kakugo virus, Varroa destructor virus 1, and Ectropis obliqua picorna-like virus. The recently discovered Venturia canescens small RNA-containing virus (VcSRV) also has typical iflavirus features.

In the past, the name insect picorna-like viruses has been used to indicate insect viruses that share major features with vertebrate picornaviruses but are now classified either in the genus *Iflavirus* or in the family *Dicistroviridae*. Since the vernacular name 'picorna-like viruses' does not refer to a definite taxonomic unit, it is better avoided. In the light of this, the species *Ectropis obliqua picorna-like virus* will probably change in the future to *Ectropis obliqua virus*, similar to the classified *Perina nuda virus* which was known as *Perina nuda picorna-like virus* in older literature. However, to prevent confusion with other viruses isolated from these insect species, such as *Perina nuda nuclear polyhedrovirus*, the addition of iflavirus in the species name needs to be considered (i.e., *Perina nuda iflavirus*).

Viral Capsid Proteins

Three major capsid proteins have been detected for iflaviruses (VP1–VP3), ranging in size between 28 and 35 kDa. Although structural analyses have not been performed for iflavirus capsids, it is anticipated that heterotrimers of these three capsid proteins serve as the building blocks of the viral capsids in a similar fashion as for mammalian picornaviruses and cricket paralysis virus (CrPV; *Dicistroviridae*).

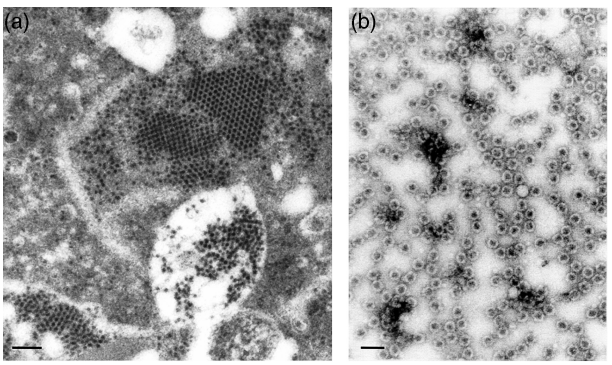


Figure 1 Virus particles of VOV-1. (a) Electron microscope photograph of a thin section of mite tissue with VDV-1 particles. (b) Purified VDV-1 particles. Scale: 150 nm (a), 50 nm (b). Photographs courtesy of Dick Peters.

A smaller less prominent structural protein is described as VP4 (4–12 kDa). The position of VP4 in the iflavirus capsids is unresolved and cannot be extrapolated from related viruses due to the variation in VP4 conformations observed in the virion structures. The various names used in the literature to refer to the structural proteins of iflaviruses are confusing. Here the nomenclature used in the *Eighth Report of the International Committee on Taxonomy of Viruses* will be followed where the capsid proteins appear in the order VP2, VP4, VP3, and VP1 from N to C in the polyprotein (**Figure 2**). The final maturation step for the iflavirus virion is proposed to be the cleavage between VP3 and VP4, thereby making the virus infectious. In picornaviruses, which have a different order of VP proteins in the polyprotein (VP4, VP2, VP3, and VP1 from N to C), this final cleavage occurs between VP4 and VP2.

The viral entry mechanism into cells is unknown, but most likely involves the release of the VP4 capsid protein as the first step after host cell recognition, similar to the situation in dicistroviruses and mammalian picornaviruses.

Genome Organization

As of February 2007, a total of seven complete iflavirus genome sequences are available (IFV, SBV, Perina nuda virus (PnV), Ectropis obliqua picorna-like virus (EoPV), DWV, KV, and VDV-1). The main characteristics of these genomes are summarized in **Table 1**. The genome size of these viruses varies between 8832 and 10 152 nt, excluding a 3′ polyA tail.

The RNA genome is nonsegmented and is translated into one large polyprotein, as shown by *in vitro* translation experiments. The polyprotein is cleaved into functional proteins by a virus-encoded protease. The information for the capsid proteins is located in the 5′ part of the genome and is followed downstream by a cluster encoding the nonstructural proteins (**Figure 2**).

For PnV and VDV-1 nucleotide sequence information was combined with N-terminal protein sequencing data for capsid proteins, allowing the demarcation of the protease cleavage sites and therefore the borders of the capsid proteins. In the iflavirus polyprotein, the structural proteins are preceded by a leader protein (L) of unknown function. This iflavirus L protein is not homologous to the leader protein of mammalian picornaviruses, which has protease activity.

The nonstructural proteins appear in the polyprotein in the order helicase (NTP-binding protein), protease, and RNA-dependent RNA polymerase (RdRp), as is the case in all members of a proposed picornavirus-like superfamily (see below). The RdRp harbors a canonical RGD motif typical for RNA polymerases of positive-sense single-stranded RNA (ssRNA) viruses.

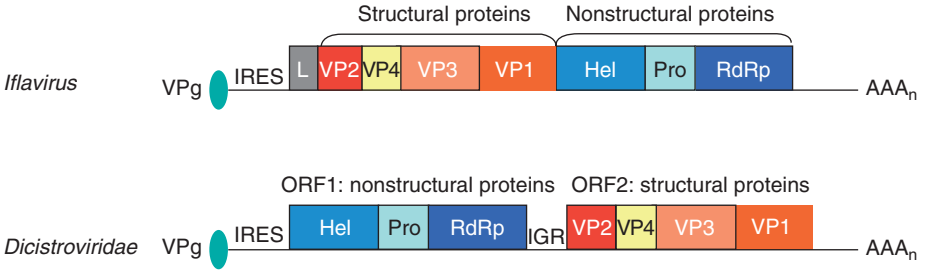


Figure 2 Genome organization of viruses in the genus *Iflavirus* compared to *Dicistroviridae*. Iflavirus genomes encode a single polyprotein with the structural proteins VP1–VP4 located in the N-terminal part and the nonstructural proteins (helicase, protease, and RNA-dependent RNA polymerase (RdRp)) in the C-terminal part. A function for the N-terminal L polypeptide has not been assigned yet. The presence of an internal ribosomal entry site (IRES) element in the 5′ untranslated region (UTR) has been confirmed for several iflaviruses. Dicistroviruses have a bicistronic genome, encoding two polyproteins separated by an intergenic region (IGR) that serves as IRES for the second open reading frame (ORF). ORF1 encodes the nonstructural proteins, ORF2 the capsid proteins.

Table 1 Iflavirus genome data[a]

Virus name	*Genome size*	*ORF size*	*5′ UTR length*	*3′ UTR length*	*Accession number*
IFV	9 650	9255	156	239	AB000906
SBV	8 832	8574	178	80	AF092924
PnV	9 476	8958	473	45	AF323747
EoPV	9 394	8961	390	43	AY365064
DWV	10 166	8682	1139	345	AY292384
KV	10 152	8682	1156	313	AB070959
VDV-1	10 112	8682	1117	313	AY251269

[a]All sizes in nt.

Comparative surveys of variation in iflavirus genomes have been performed for both DWV and VDV-1 in mites collected from beehives in distinct European locations. This study showed similarities of 96–99% between the various isolates, indicating that iflavirus genomes are not a fixed entity but subject to genetic variation in the field.

Relation to Other Taxa

Iflavirus genomes share several features with other viruses with positive-sense RNA genomes, for which a picornavirus-like superfamily has been proposed containing the families *Comoviridae*, *Picornaviridae*, *Potyviridae*, and *Sequiviridae*. The overall genome organization of iflaviruses strongly resembles that of mammalian picornaviruses, and iflaviruses have therefore been described as entomogenous picornaviruses. In these two groups of viruses the RNA genome encodes one large open reading frame (ORF) with the structural proteins followed by the nonstructural ones, although the order of the capsid proteins in the polyprotein and the number of proteases differ. The position of VP4 in iflaviruses is between VP2 and VP3, whereas in the *Picornaviridae* VP4 is the N-terminal capsid protein. The order of the capsid proteins in the polyprotein is similar in iflaviruses and dicistroviruses (**Figure 2**). Picornaviruses encode a 2C protease that is not found in iflavirus and dicistrovirus genomes.

In phylogenetic trees iflaviruses form a clade separated from dicistroviruses: their distinct genome structures justify their classification in different taxa (**Figure 3**). The major genomic difference between iflaviruses and dicistroviruses is that the latter encode separate polyproteins for nonstructural and structural proteins (hence have bicistronic genomes), while iflavirus genomes are monocistronic and encode a single polyprotein. In order to translate these ORFs into polyproteins dicistroviruses have both a 5′ and an intergenic internal ribosomal entry site (IRES) element (**Figure 2**). These two IRES elements vary from each other in terms of sequence, structure, and mechanism of action.

Another insect virus related to iflaviruses is kelp fly virus (KFV), which encodes one large polyprotein with an inhibitor of apoptosis (IAP) preceding the capsid and nonstructural protein segments. The encoded IAP protein has homology with baculovirus IAP proteins. Whether this *iap* gene is functional and plays a crucial role in the infection process is not known. Phylogenetic analysis using the RdRp sequence placed KFV in a clade separated from iflaviruses and dicistroviruses (**Figure 3**).

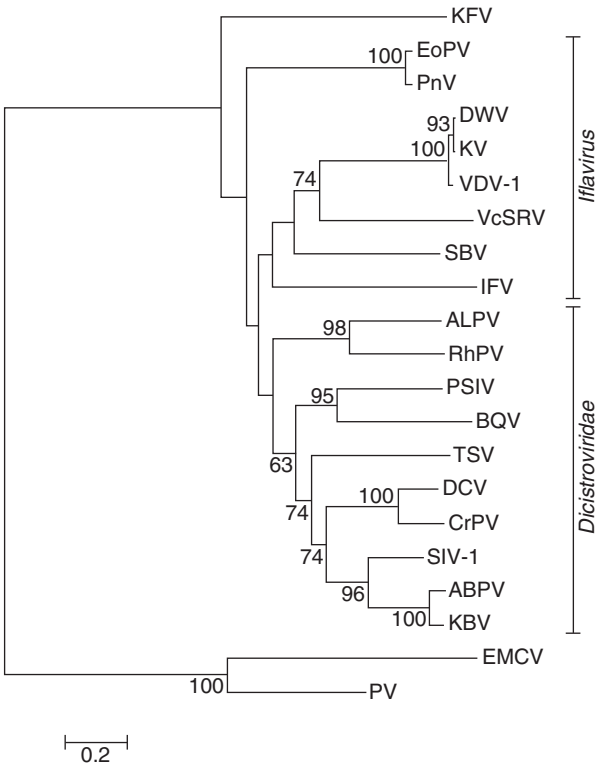


Figure 3 Phylogenetic relationship between viruses in the genus *Iflavirus* and the family *Dicistroviridae* based on RdRp sequences. Encephalomyocarditis virus (EMCV [ABC25550]) and poliovirus (PV [CAA24465]) were used as outgroups. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 3.1. Amino acid sequences were aligned using the Clustal W module and phylogenetic relationships and evolutionary distances were calculated using the neighbor-joining method. Bootstrap analysis was performed with 100 replicates. The distance marker indicates 0.2 substitutions per amino acid. The RdRp sequences were extracted form GenBank: acute bee paralysis virus (ABPV) [AAN63803], aphid lethal paralysis virus (ALPV) [NP_733845], black queen cell virus (BQV) [NP_620564], CrPV [NP_647481], DWV [AAP49283], drosophila C virus (DCV) [NP_044945], EoPV [NP_919029], IFV [NP_620559], KV [YP_015696], Kashmir bee virus (KBV) [NP_851403], KFV [YP_415507], PnV [NP_277061], plautia stali intestine virus (PSIV) NP_620555], rhipalopsiphum padi virus (RhPV) [NP_046155], SBV [AAL79021], solenopsis invicta virus 1 (SIV-1) [YP_164440], tauro syndrome virus (TSV) [ABB17263], VDV-1 [AY251269], and VcSRV [AY534885].

Tissue Tropism, Pathology, and Transmission

The primary infection sites of iflaviruses may vary. For IFV, the goblet cells of the midgut epithelium are the primary targets, suggesting virus uptake via food contamination. IFV infection finally leads to a flacherie phenotype, where excessive amounts of body fluids accumulate under the skin. This flacherie is also observed in other lepidopterans infected by iflaviruses.

Bee iflaviruses may spread by food contamination, although they also exploit other infection routes. An important site of infection for DWV and VDV-1 (and probably also KV) in honeybees are the feeding sites of *Varroa* mite nymphs and female adults on bee larvae. These mites can serve as vectors for the transmission of bee viruses. In addition, their constant feeding makes bees more susceptible to pathogens in general, due to exhaustion and immunomodulation. Direct transmission from bee to bee without the intervention of *Varroa* mites cannot be ruled out, but the incidence of infection is much lower in colonies which are not infested by these mites.

DWV and VDV-1 have been detected in head, thorax, and abdomen of bees. DWV infection may result in the development of bees with underdeveloped, malformed wings. KV, which varies only slightly from DWV in sequence, exhibits a strong neurotropism and is correlated with aggressive behavior of infected bees. Iflaviruses may also persist in insect populations in a latent symptomless state.

Replication

Replication of iflaviruses occurs in the cytoplasm where crystalline aggregates of progeny virus can be detected (**Figure 1**(**a**)). Iodination of purified IFV RNA showed that it was covalently linked to a protein of 11.5 kDa. This so-called VPg protein was removed upon translation of IFV RNA in a rabbit reticulocyte lysate, as is also the case for VPg protein of mammalian picornaviruses. It is anticipated that the 5′ ends of both the genomic RNA and the complementary strand RNA are covalently linked to a terminal VPg, implying that the iflavirus VPg protein also serves as a primer for RNA synthesis in iflaviruses, similar to the situation in the *Picornaviridae*. The location of VPg in the polyprotein is unresolved.

The presence of negative-strand iflavirus RNA is a hallmark to demonstrate replication of these positive-strand RNA viruses in insects and/or mites. For DWV and VDV-1, for example, negative-strand RNA and hence replication has been demonstrated in honeybees, as well as in bee-parasitic *Varroa* mites.

Translation

The sequenced iflaviruses can be grouped based on the length of their 5′ untranslated region (UTR) (**Table 1**). SBV and IFV have very short 5′ UTRs (156–178 nt) with hardly any predicted secondary structure. PnV and EoPV have longer 5′ UTRs (390–473 nt), which are homologous to each other and for which a mostly nonbranched secondary structure has been predicted. DWV, KV, and VDV-1 have much longer 5′ UTRs, which also have mutual homology and for which complex-branched secondary structures have been predicted. The existence of covariation in these homologous sequences, where a mutation at one side is accompanied by a second mutation to preserve secondary structures, is a measure for the likelihood of these predicted secondary structures. A three-branched or clover-leaf structure is shared between the DWV/VDV-1/KV group of iflaviruses and the entero/rhino IRES type of mammalian picornaviruses.

The presence of an IRES in the 5′ UTR, which would allow translation of the polyprotein in a cap-independent manner, has been shown for both VDV-1 and EoPV, using bicistronic plasmid constructs. For EoPV this was combined with mutational analysis to determine the functional domains in the IRES element. The EoPV core IRES was located in the third and fourth stem loop (out of six). Due to the high degree of similarity in sequence and predicted secondary structure between EoPV and PnV on the one hand and between VDV-1 and DWV/KV on the other, IRES elements are also predicted for these viruses. The presence of a functional IRES element in the very short unstructured IFV and SBV 5′ UTR sequences seems unlikely and is probably unnecessary but this awaits further analysis.

The interaction of host translation initiation factors with these IRES elements has not been studied yet. Nor is it known how iflavirus infection affects the translational machinery of the host.

Detection Methods

Iflaviruses can be detected and identified by serological methods such as enzyme-linked immunosorbent assay (ELISA) and Western blot analysis. However, this does not always result in easily interpretable data as virus preparations may contain mixtures of iflaviruses, leading to the production of nonspecific antisera. For instance, DWV and VDV-1 have been shown to coexist in a variety of European beehives. A more specific and therefore reliable method is reverse transcriptase-polymerase chain reaction (RT-PCR) with specific primers based on unique genome sequences. However, relying on single PCR primer pairs for detection is not a reliable method since iflavirus genomes are prone to variation.

RT-PCR also allows the discrimination between positive- and negative-strand RNA molecules by choosing a plus- or minus-strand-specific primer for the reverse transcription step. This makes it possible to establish if a virus is replicating in a certain insect species. Virus titers can be determined by quantitative PCR (QT-PCR), for instance to compare susceptibility of various developmental stages or to determine transmission rates.

See also: Dicistroviruses; Picornaviruses: Molecular Biology; Potyviruses; Sequiviruses.

Further Reading

Bowen-Walker PL, Martin SJ, and Gunn A (1999) The transmission of deformed wing virus between honeybees (*Apis mellifera* L.) by the ectoparasitic mite *Varroa jacobsoni* Oud. *Journal of Invertebrate Pathology* 73: 101–106.

Ghosh RC, Ball BV, Willcocks MM, and Carter MJ (1999) The nucleotide sequence of sacbrood virus of the honey bee: An insect picorna-like virus. *Journal of General Virology* 80: 1541–1549.

Hartley CJ, Greenwood DR, Gilbert RJC, *et al.* (2005) Kelp fly virus: A novel group of insect picorna-like viruses as defined by genome sequence analysis and a distinctive virion structure. *Journal of Virology* 79: 13385–13398.

Isawa H, Asano S, Sahara K, Iizuka T, and Bando H (1998) Analysis of genetic information of an insect picorna-like virus, infectious flacherie virus of silkworm: Evidence for evolutionary relationships among insect, mammalian, and plant picorna(-like) viruses. *Archives of Virology* 143: 127–143.

Lu J, Zhang J, Wang X, Jiang H, Liu C, and Hu Y (2006) *In vitro* and *in vivo* identification of structural and sequence elements in the 5′ untranslated region of Ectropis obliqua picorna-like virus required for internal initiation. *Journal of General Virology* 87: 3667–3677.

Ongus JR, Peters D, Bonmatin JM, Bengsch E, Vlak JM, and van Oers MM (2004) Complete sequence of a picorna-like virus of the genus *Iflavirus* replicating in the mite *Varroa destructor*. *Journal of General Virology* 85: 3747–3755.

Ongus JR, Roode EC, Pleij CW, Vlak JM, and van Oers MM (2006) The 5′ non-translated region of Varroa destructor virus 1 (genus *Iflavirus*): Structure prediction and IRES activity in *Lymantria dispar* cells. *Journal of General Virology* 87: 3397–3407.

Reineke A and Asgari S (2005) Presence of a novel small RNA-containing virus in a laboratory culture of the endoparasitic wasp *Venturia canescens* (Hymenoptera: Ichneumonidae). *Journal of Insect Physiology* 51: 127–135.

Rossmann MG and Tao Y (1999) Structural insight into insect viruses. *Nature Structural Biology* 6: 717–719.

Tate J, Liljas L, Scotti P, Christian P, Lin T, and Johnson JE (1999) The crystal structure of cricket paralysis virus: The first view of a new virus family. *Nature Structural Biology* 6: 765–774.

Wang X, Zhang J, Lu J, Yi F, Liu C, and Hu Y (2004) Sequence analysis and genomic organization of a new insect picorna-like virus Ectropis obliqua picorna-like virus, isolated from *Ectropis obliqua*. *Journal of General Virology* 85: 1145–1151.

Wu CY, Lo CF, Huang CJ, Yu HT, and Wang CH (2002) The complete genome sequence of Perina nuda picorna-like virus, an insect-infecting RNA virus with a genome organization similar to that of the mammalian picornaviruses. *Virology* 294: 312–323.

Yang X and Cox-Foster DL (2005) Impact of an ectoparasite on the immunity and pathology of an invertebrate: Evidence for host immunosuppression and viral amplification. *Proceedings of the National Academy of Sciences, USA* 102: 7470–7475.

Yue C and Genersch E (2005) RT-PCR analysis of deformed wing virus in honeybees (*Apis mellifera*) and mites (*Varroa destructor*). *Journal of General Virology* 86: 3419–3424.

Ilarvirus

K C Eastwell, Washington State University – IAREC, Prosser, WA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cross-protection A virus-infected plant often exhibits resistance to infection by strains of the same or closely related viruses. The degree of cross-protection has been used as a measure of the relatedness of viruses.

Dicistronic An RNA that encodes two gene products, often with a noncoding region separating them.

Recovery A phenomenon in which a virus-infected plant displays severe symptoms after initial infection, but then symptoms decline; this is often associated with a decline in virus concentration.

Subgenomic RNA A segment of RNA generated from a genomic RNA molecule through an internal promoter sequence. Since eukaryotic systems only efficiently translate the open reading frame located at the 5′ terminus of mRNA, this permits efficient translation of downstream open reading frames.

Suppressor of virus-induced gene silencing A molecule produced through virus infection that mitigates the natural plant defense system.

Virus-induced gene silencing A natural plant defense system that uses small fragments of double-stranded RNA to direct the RNA-degrading or silencing enzymes to a target RNA molecule. Double-stranded RNA results from the synthesis of complementary minus- and plus-sense RNA sequences during virus replication.

Taxonomy

The genus *Ilarvirus* was officially recognized in 1975. It is currently placed within the family *Bromoviridae* that also includes the genera *Alfamovirus*, *Bromovirus*, *Cucumovirus*, and *Oleavirus*. The quasi-isometric nature of ilarvirus particles and their relative instability in the absence of antioxidants are reflected in the sigla which originates from the descriptive phrase: isometric labile ringspot viruses. Other distinguishing features of the genus are the distribution of the genome over three plus-sense RNA segments and the absolute requirement for the coat protein to activate the RNA genome to initiate infection. These traits are shared with the genus *Alfamovirus*.

Experimental host ranges of ilarviruses are quite broad, although many have a relatively narrow range of natural hosts consisting of woody plants. Woody hosts may require several years after initial infection for the virus to uniformly infect the plant. The ringspot symptom for which the genus is named is a common symptom of many virus–host combinations. Infections by ilarviruses frequently result in a shock phase during which severe chlorotic or necrotic rings form, followed by a period of recovery with greatly reduced symptoms. This recovery phenomenon has now been described for many virus groups. No inclusion bodies have been described in tissue infected with members of the genus *Ilarvirus*.

Members of the genus *Ilarvirus* are currently divided into six subgroups (**Table 1**). Historically, this separation was based solely on serological reactivity, but it is generally supported by comparison of the amino acid sequences of structural proteins (**Figure 1**). The exception is the coat protein sequence of *Humulus japonicus* latent virus (HJLV) of subgroup 3 which is most similar to that of prune dwarf virus (PDV) of subgroup 4 rather than other members of subgroup 3. *Alfalfa mosaic virus* (AMV) is the monotypic member of the *Alfamovirus* genus; the sequence of the coat protein encoded by AMV also segregates with HJLV and PDV in this analysis. Analyses of amino acid sequences of the nonstructural proteins encoded by all three RNA segments are consistent with current subgroup designations.

In addition to the currently recognized members of the genus *Ilarvirus*, many synonyms have appeared in the literature, most of which refer to specific strains of virus species. These are listed in **Table 1**. The taxonomy of ilarvirus isolates that infect hop plants (*Humulus lupulus* L.) is still evolving. Viruses designated as hop virus C, NRSV-intermediate, and NRSV-HP-2 are related serologically to prunus necrotic ringspot virus (PNRSV) and to apple mosaic virus (ApMV) while hop virus A, apple mosaic virus-hop, and prunus necrotic ringspot virus-HP-1 are serologically related to ApMV only. Analyses of coat protein sequences of viruses obtained from hop plants of diverse geographic origins indicate that both serotypes are strains of ApMV.

Particle Structure and Genome Organization

The virions of ilarviruses are generally quasi-isometric (**Figure 2**), and the size of particle can vary within a species in relation to RNA content; particles range in diameter from 20 to 35 nm. Some ilarviruses also produce a small proportion of bacilliform particles. Virus particles contain subunits of a single structural protein (coat protein). The molecular mass of the coat protein subunits in the range of 19–30 kDa, with the majority of members having coat protein subunits within the rather narrow range of 24–26 kDa. The entire genome of ilarviruses is distributed over three RNA molecules; each is encapsidated separately. However, preparations of virions contain a fourth RNA species that is 3′-co-terminal with RNA3; this subgenomic RNA encodes the coat protein. Recently, a fifth RNA species corresponding to the 3′-untranslated region (UTR) of RNAs 1–3 was found to be encapsidated in virions of PNRSV. This is believed to represent the chance encapsidation of partially degraded RNA that is frequently found within purified virus particles. All of the major RNA species bear a 5′-7-methyl-G cap structure.

The relationship between the genera *Alfamovirus* and *Ilarvirus* is particularly close. The unique dependence on coat protein for infectivity of viral RNA is shared by members of both genera. Moreover, the coat proteins of AMV and some ilarviruses can reciprocally activate infectivity of RNA of the other. This phenomenon has led some investigators to suggest that the genera *Ilarvirus* and *Alfamovirus* should be combined. In as much as these viruses also share several features in common with other genera of the family *Bromoviridae*, the two genera remain separate. Studies of AMV have contributed much of the basic information from which the structure and replication of ilarviruses were determined and will be included here in subsequent comparisons of ilarviruses.

The type member of the genus *Ilarvirus* is *Tobacco streak virus* (TSV). The total genome of the WC isolate of TSV consists of 8622 nucleotides distributed over three RNA segments of 3491, 2926, and 2205 nucleotides (**Figure 3**). The largest segment of RNA, RNA1, contains a single open reading frame (ORF1) encoding a protein of 123.4 kDa. This protein is the replicase and contains the methyltranferase and helicase motifs. RNA2 contains ORF2a encoding a protein of 91.5 kDa. This protein is the RNA-dependent RNA-polymerase based on the presence of conserved RNA-binding motifs. RNA2 of TSV and other members of subgroups 1 and 2 contain a second potential overlapping ORF, ORF2b; AMV and ilarviruses of the remaining subgroups apparently lack this second ORF on RNA2. The function of the ORF2b product with a molecular mass of 22.4 kDa has not been determined. The dicistronic organization of RNA2 detected in ilarvirus subgroups 1 and 2 is similar to that of members of the genus *Cucumovirus*; the putative product of ORF2b of cucumoviruses has been proposed to be a key element in suppressing virus-induced gene silencing. The sequence of *Fragaria chiloensis* latent virus (FCiLV) of ilarvirus subgroup 6 is unique in that its RNA2 has the potential to encode a second small protein located toward the 5′ end of RNA2 (nucleotide positions 350–856 of RNA2). However, the putative product of this ORF bears no significant similarity to protein 2b of the cucumoviruses, nor other proteins thought to be encoded by ilarviruses.

Table 1 Virus members of the genus *Ilarvirus*

Virus (abbreviation) *Synonyms*	*GenBank accession numbers used for comparison*		
	RNA1	*RNA2*	*RNA3*
Subgroup 1			
Tobacco streak virus (TSV)	NC_003844	NC_003842	NC_003845
Annulus orae virus			
Asparagus stunt virus			
Bean red node virus			
Black raspberry latent virus			
Datura quercina virus			
Nicotiana virus 8			
Nicotiana virus vulnerans			
Sunflower necrosis virus			
Tractus orae virus			
Parietaria mottle virus (PMoV)	NC_005848	NC_005849	NC_005854
Tentative members			
Blackberry chlorotic ringspot virus (BCRSV)	DQ091193	DQ091194	DQ091195
Grapevine angular mosaic virus	—	—	—
Strawberry necrotic shock virus (SNSV)	—	AY743591	AY363228
Subgroup 2			
Asparagus virus 2 (AV-2)	—	—	X86352
Asparagus latent virus			
Asparagus virus C			
Citrus leaf rugose virus (CiLRV)	NC_003548	NC_003547	NC_003546
Citrus crinkly leaf virus			
Citrus variegation virus (CVV)	—	—	U17389
Citrus psorosis virus complex (infectious variegation component)			
Elm mottle virus (EMoV)	NC_003569	NP_619575	NC_003570
Hydrangea mosaic virus (HdMV)			AF172965
Lilac streak mosaic virus			
Lilac white mosaic virus			
Spinach latent virus (SpLV)	NC_003808	NC_003809	NC_003810
GE 36 virus			
Tulare apple mosaic virus (TAMV)	NC_003833	NC_003834	NC_003835
Subgroup 3			
Apple mosaic virus (ApMV)	NC_003464	NC_003465	NC_003480
Birch line pattern virus			S78319[a]
Birch ringspot virus			AAL84586[b]
Dutch plum line pattern virus			AAN01243[c]
Hop virus A			
Horse chestnut yellow mosaic virus			
Mild apple mosaic virus			
Mountain ash variegation virus			
Plum (Dutch) line pattern virus			
Severe apple mosaic virus			
Blueberry shock virus (BlShV)	—	—	—
Humulus japonicus latent virus (HJLV)	NC_006064	NC_006065	NC_006066
Humulus japonicus virus			
Prunus necrotic ringspot virus (PNRSV)	NC_004362	NC_004363	NC_004364
Cherry rugose mosaic virus			CAB37309[d]
Currant (red) necrotic ringspot virus			
Danish plum line pattern virus			
European plum line pattern virus			
Hop virus B			
Hop virus C			
North American plum line pattern virus			
Peach ringspot virus			
Plum (Danish) line pattern virus			
Plum (European) line pattern virus			
Plum (North American) line pattern virus			
Plum line pattern virus			
Prunus ringspot virus			

Continued

Table 1 Continued

Virus (abbreviation) *Synonyms*	*GenBank accession numbers used for comparison*		
	RNA1	*RNA2*	*RNA3*
Red currant necrotic ringspot virus			
Rose chlorotic mottle virus			
Rose line pattern virus			
Rose mosaic virus			
Rose vein banding virus			
Rose yellow mosaic virus			
Rose yellow vein mosaic virus			
Sour cherry necrotic ringspot virus			
Subgroup 4			
Prune dwarf virus (PDV)	PDU57648	AF277662	L28145
Cherry chlorotic ringspot virus			
Chlorogenus cerasae virus			
Peach stunt virus			
Prunus chlorotic necrotic ringspot virus			
Sour cherry yellows virus			
Subgroup 5			
American plum line pattern virus (APLPV)	NC_003451	NC_003452	NC_003453
Peach line pattern virosis virus			
Plum (American) line pattern virus			
Plum line pattern virus			
Prunus virus 10			
Subgroup 6			
Fragaria chiloensis latent virus (FCiLV)	NC_006566	NC_006567	NC_006568
Lilac ring mottle virus (LiRMoV)	—	—	U17391

[a]ApMV-G strain, CP sequence only.
[b]ApMV-hop strain, CP sequence only.
[c]ApMV-Fuji strain, CP sequence only.
[d]PNRSV-MRY1 isolate, CP sequence only.

RNA3 of ilarviruses is dicistronic with two potential ORFs. ORF3a encodes a protein of 31.7 kDa with cell-to-cell movement function (MP) and ORF3b encodes the structural coat protein (CP) with a molecular mass of 26.2 kDa. RNA3 of FCiLV contains a third potentially functional ORF, whose putative product does not have any sequence similarity to proteins expressed by other ilarviruses. It is unknown if this ORF is expressed *in vivo*.

Virus Replication

Members of this genus require an 'activated' RNA genome to initiate infection, that is, viral RNA must form a complex with the coat protein to be infectious. This is a distinguishing feature of ilarvirus and alfamovirus replication that differentiates them from other genera in the family *Bromoviridae*. Activation of the genome can be accomplished either through the co-inoculation of viral RNA with coat protein, or by the inclusion of subgenomic RNA4 that is then translated to produce the activating coat protein *in vivo*. This activation can be accomplished through heterologous complex formation, that is, the coat protein from one ilarvirus can bind to and potentiate the replication of RNA from several other but not all members of the genus *Ilarvirus*. Furthermore, the coat protein of AMV can activate the genome of several ilarviruses and vice versa. More recently, it has been demonstrated that RNA3 is able to fulfil the role of either coat protein or RNA4, but with much reduced efficiency. The presence of RNA4 or coat protein increases the efficiency of infection up to 1000-fold relative to the RNA activation achieved by RNA3. This is the result of very inefficient expression of the coat protein gene directly from the dicistronic RNA3.

The mechanism by which genome activation occurs is the result of the specific interaction of the coat protein with sequences located at the 3′ terminus of viral RNA. All ilarviruses for which sequences are known possess sequences at the 3′ terminus of genomic RNAs that are predicted to assume secondary structures that play a role in regulating the relative efficiency of the RNA species for transcription versus translation. Indeed, in the presence of coat protein, transcription of viral RNA is inhibited and translation is favored. The 3′-UTR of each RNA segment contains a series of conserved (A/U) (U/A/G)GC motifs that occur as single-stranded regions at the base of stem-loop structures that are recognized by and bound to coat protein molecules (**Figures 4** and **5**). The sequence and

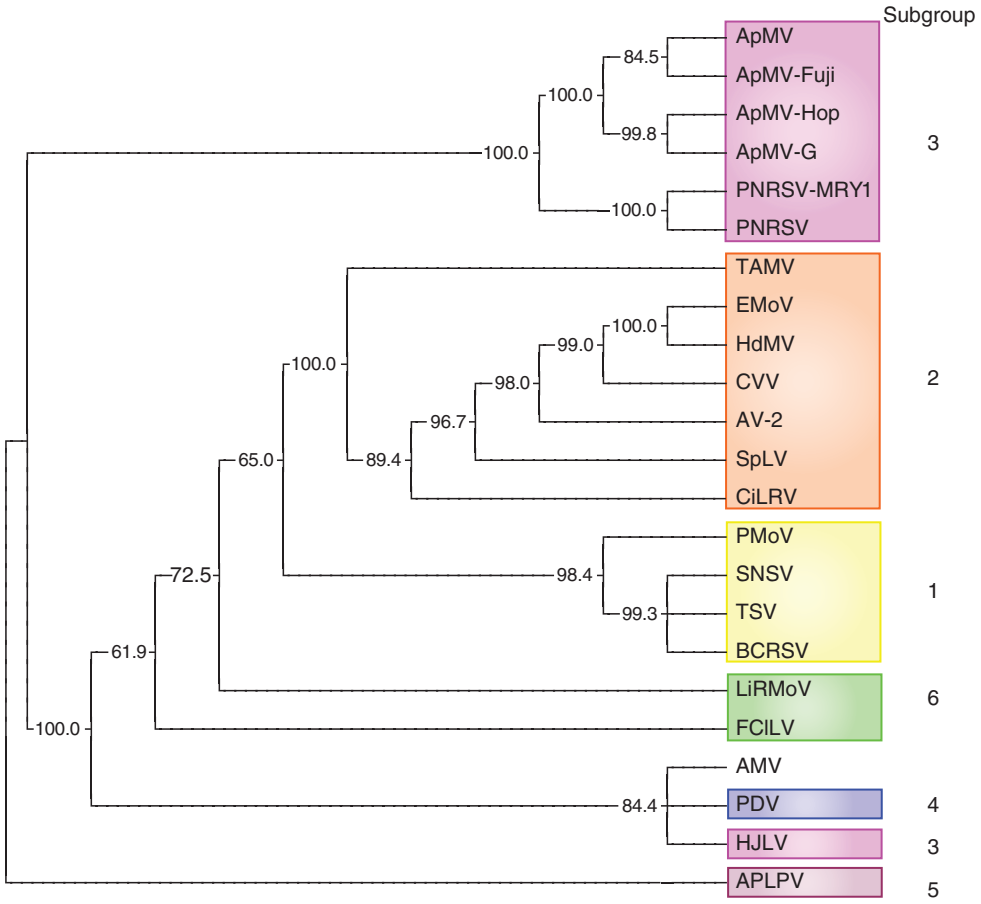


Figure 1 Cladogram illustrating the sequence relationships of ilarviruses based on the deduced amino acid sequence of the coat protein encoded by ORF3b of RNA3. Numbers at branch points indicate the percentage of bootstrap replicates in which that branch occurs. Subgroup designations are derived primarily from serological as well as molecular data and are indicated to the right of the shaded boxes. The sequence of the alfamovirus alfalfa mosaic virus is included in the analysis. See **Table 1** for abbreviations.

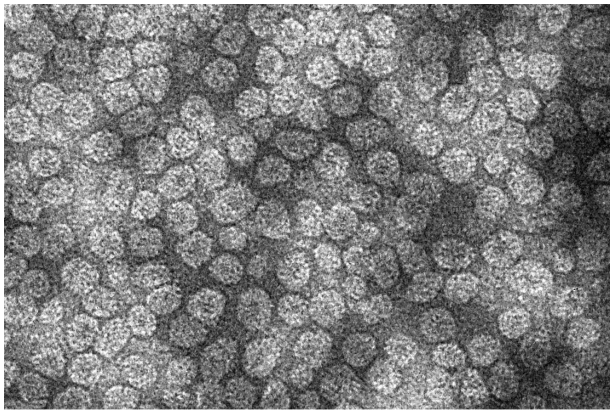


Figure 2 Electron micrograph of negative-stained particles of prune dwarf virus. In addition to quasi-isometric particles 20 and 23 nm in diameter, preparations from some ilarviruses including prune dwarf virus also contain bacilliform particles 19 × 33 nm and 19 × 38 nm.

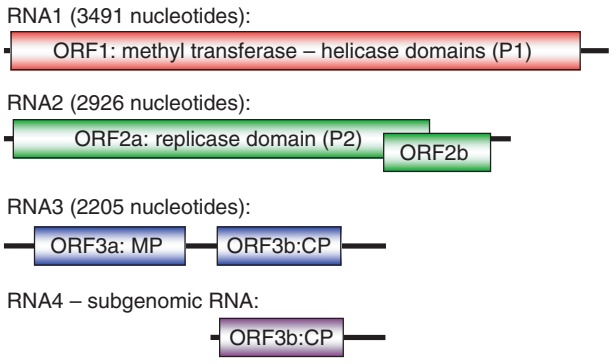


Figure 3 Genome organization of tobacco streak virus (TSV). The positive-sense RNA genome of the WC isolate of TSV is distributed between three segments. ORF2b is present only in genus *Ilarvirus* subgroups 1 and 2. ORF3a encodes a protein (MP) associated with virus movement and ORF3b encodes that coat protein (CP).The subgenomic RNA4 is transcribed from an internal promoter in the intercistronic region of minus-sense RNA3; RNA4 is 3′ co-terminal with RNA3. ORF2b is present only in genus *Ilarvirus* subgroups 1 and 2.

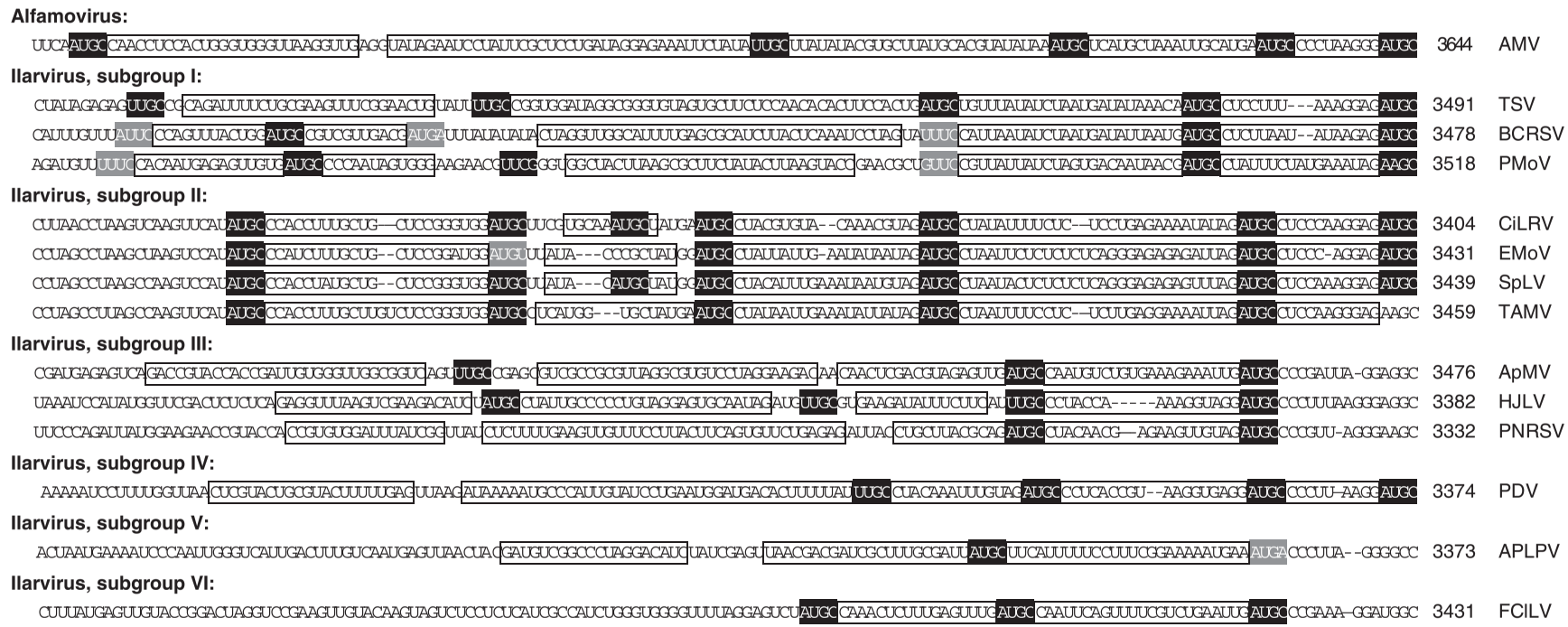


Figure 4 The 3′-untranslated region of RNA1 of ilarviruses contains a repeated (A/U) (U/A/G)GC sequence. The repeated single-stranded regions are indicated by black highlighting. Corrupted copies of (A/U) (U/A/G)GC motifs are highlighted in gray. Potential stem–loop structures are indicated by boxes.

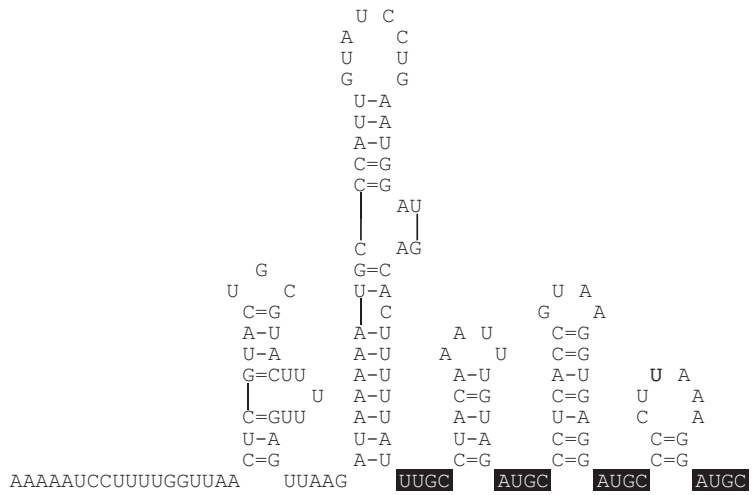


Figure 5 The 3′-untranslated region of RNA1 of ilarviruses contains a repeated (A/U) (U/A/G)GC sequence. These conserved regions are single-stranded motifs (black highlighting) that appear between base-paired stem-loops and are critical for coat protein binding and potentially for replicase binding. The secondary structure of the 3′ terminus of prune dwarf virus RNA1 illustrates the potential stem-loops configuration with intervening conserved single-stranded motifs.

the size of the stem–loop structures vary considerably when all of the ilarviruses are considered. However, within each subgroup of ilarvirus, the overall length and secondary structures of the binding region(s) are well conserved. In the case of TSV, the (A/U) (U/A/G)GC motif is repeated five times flanking four potential stem–loop structures within the 260 nucleotides located at the 3′ terminus of RNA1. This region may represent two coat protein binding sites as has been demonstrated for AMV. Ilarviruses belonging to the subgroups 4, 5, and 6 possess shorter discernable recognition sequences and may contain single coat protein binding sites. Deletion analyses of AMV RNA4 have shown that the first and third 3′-most stem loops are critical for coat protein binding and hence translation, whereas the other stem loops play a role in enhancing efficient transcription but are not strictly required for that function. There is an absolute requirement for the single-stranded (A/U) (U/A/G)GC motifs in virus function.

The role of the RNA–coat protein complex was elucidated through studies with recombinant viruses. The 3′-UTR and its ability to form a complex with coat protein subunits is functionally analogous to a poly-A tail; a poly-A tail of 40–80 residues can experimentally compensate for the absence of activating coat protein molecules. The formation of a RNA–coat protein complex or the addition of a poly-A tail both act to stimulate the translation of viral RNA resulting in the synthesis of proteins needed for virus replication. In eukaryotes, translation efficiency of mRNA is enhanced through the formation of a loop formed by the interaction of a poly-A binding protein (PABP) with poly-A, and then subsequent binding of this complex to the 5′-cap structure of RNA in combination with other cellular initiation factors. It has been suggested that the complex formed between viral RNA and coat protein mimics the complex formed by the PABP and the poly-A tail. This proposal is further strengthened by the observation that the RNA–coat protein complex binds elongation factors associated with cellular mRNA translation. Based on data from AMV, it appears that the binding of coat protein to 3′-terminal sequences of viral RNA not only facilitates translation, but also effectively blocks transcription of the RNA.

The essential complex formation between the coat protein and RNA has been examined in detail to determine critical features of the coat protein. Limited digests indicate that the N-terminus of the coat protein is primarily responsible for RNA binding. Inspection of the amino acid sequences at the N-terminus of the coat protein reveals a high percentage of basic amino acid residues that are consistent with a nucleic acid binding function. More detailed RNA protection assays and competitive binding studies suggest that a consensus sequence represented by (Q/K/R) (P/N)TXRS(R/Q) (Q/N/S) (W/F/Y)A is involved in coat protein binding to the 3′-UTR of AMV and ilarvirus genomic RNA (**Figure 6**). Although this functional domain is not strictly conserved in all ilarviruses, a core motif centered on the critical arginine residue is generally well preserved in all ilarviruses and AMV, suggesting a key role in virus function. Despite significant variation in primary sequence, the striking similarity in protein characteristics across all ilarviruses and AMV may account for the reciprocity in the ability of coat protein from several ilarviruses and AMV to activate the virus genome in heterologous combinations.

In addition to the functional domain of the coat protein described above, additional features of the coat protein may contribute to RNA binding. The critical basic arginine residue and its flanking residues are

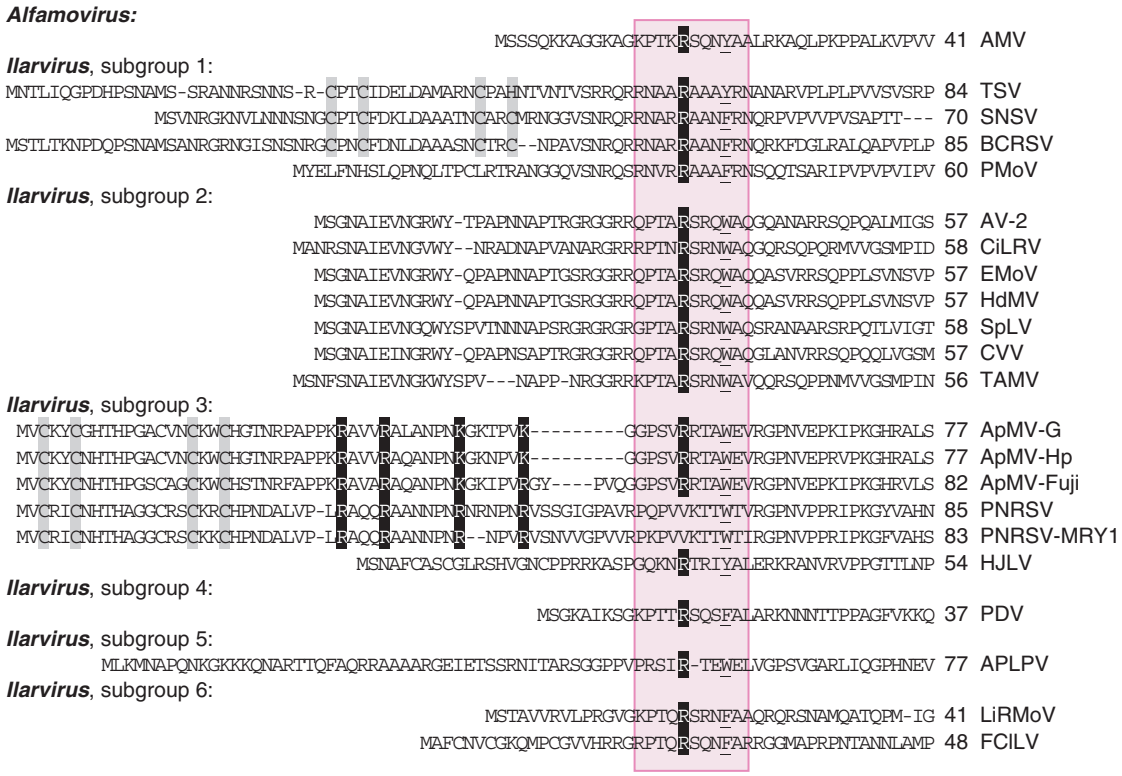


Figure 6 Alignment of the N-terminus of the coat proteins of ilarviruses. The box shaded in red indicates the putative RNA binding region with the consensus sequence (Q/K/R) (P/N)TXRS(R/Q) (Q/N/S) (W/F/Y)A and the central arginine residue highlighted in black. It has been demonstrated that in prunus necrotic ringspot virus, four arginine residues upstream of the conserved binding site are essential for RNA binding; these are highlighted with black, as are comparable residues in the apple mosaic virus coat protein sequence. Members of subgroups 1 and 3 also contain a putative zinc finger that may be involved in RNA binding; the cysteine/histidine residues potentially involved in chelating zinc cations are highlighted in gray. See **Table 1** for abbreviations.

preceded by a putative zinc finger in the coat proteins of members of subgroups 1 and 3, but not in other subgroups of ilarviruses, nor in AMV (**Figure 6**). Zinc fingers are typically involved in nucleic acid binding. Cysteine and/or hisitidine residues are critical elements of the motif represented by $CN_2CN_{10}(C/H)N_2(C/H)$; the cysteine and histidine residues are the functional amino acids capable of chelating zinc cations. The participation or requirement of this putative zinc finger in virus replication is uncertain, particularly since it is not present in all ilarviruses.

The RNA-binding region of the coat protein that is conserved in other ilarviruses appears to be absent or at least highly diverged in PNRSV. In particular, an arginine expected to be critical for RNA binding is absent in the coat protein of PNRSV in this central conserved position, although a basic lysine residue is located nearby. Moreover, electrophoretic shift assay suggests that other basic residues are involved in RNA binding. Four arginine residues of PNRSV coat protein have been experimentally determined to be involved in RNA binding and are located towards the N-terminus of the site of the conserved motif present in other ilarviruses (**Figure 6**). A similar distribution of four basic residues is observed in ApMV in addition to the conserved (Q/K/R) (P/N) TXRS(R/Q) (Q/N/S) (W/F/Y)A motif.

RNA folding predictions suggest that the 3′-UTR of some ilarviruses can fold into complex tRNA-like structures or pseudoknots that do not accommodate coat protein binding. By analogy to information obtained from AMV, the pseudoknot conformation favors transcription and hence synthesis of viral RNAs. Mutational analysis indicates that stem–loop structures located just upstream of the coat protein binding site as well as the ability to form a tRNA-like pseudoknot are required for transcription and synthesis of minus-sense RNA. Thus, the relative abundance of RNA in the pseudoknot conformation relative to that bound to coat protein provides a feedback mechanism to coordinate phases of virus replication. Translation to form nonstructural proteins is favored in the presence of coat protein, while transcription as part of the replication process is favored in the absence of the coat protein.

In addition to a requirement for regulating the use of positive-sense RNA for translation versus transcription, is the requirement for coordinating the rates of synthesis of positive-sense versus minus-sense RNA molecules. The virus replication cycle requires the switch from

minus-sense RNA synthesis to the repetitive transcription into positive-sense progeny viral RNA. The genomic features responsible for this transition are not well understood. However, recent experiments with transformed plants suggest that coat protein is not involved in this process. Secondary structural features of the minus-sense RNAs are believed to regulate the synthesis of plus-sense RNA.

The subgenomic RNA encoding the coat protein, RNA4, is transcribed from a minus-sense copy of RNA3. In brome mosaic virus, the intercistronic region between the ORFs of RNA3 contains a subgenomic promoter region consisting of sequences enriched for A/U flanking the core promoter and a poly-U tract that enhances transcription. However, the intercistronic regions of AMV and the ilarviruses are considerably shorter than that of brome mosaic virus. Moreover, although still slightly enriched in U residues, the poly-U tract and strong bias for U are absent. The transcriptional start site and a strong promoter for the synthesis of positive-sense subgenomic RNA4 occurs immediately 3′ of the position corresponding to the termination codon of ORF3a. The subgenomic promoter of AMV has been characterized; a critical component of the promoter region is a three-nucleotide loop on a base-paired stem that bears a 'bulge' at the fifth nucleotide from the loop along the base-paired stem. The stem sequence below the bulge is less critical for promoter activity. Potential stem–loop structures are evident in the intercistronic regions between ORF3a and ORF3b of ilarviruses and these may fulfill the same critical role as those in AMV and brome mosaic virus.

Based on studies of transgenic plants conducted with AMV, replication of RNA1 and RNA2 are coordinated in that a mutation leading to changes in the encoded protein of either RNA product prevents the replication of both. Moreover, this requirement is for a *cis*-encoded function since transgenic expression of the wild-type protein does not rescue the mutated RNA. In contrast, the replication of RNA3 can be supported by RNA1 and RNA2 gene products expressed in *trans*. This is a critical distinction highlighting the independence of RNA3 replication from that of RNA1 and RNA2. During the natural infection cycle, binding of the coat protein in the inoculum to the 3′ terminus of RNA1 and RNA2 is sufficient to facilitate their translation into a replication complex in the cytoplasm of the infected cell. This is accomplished by the interaction of the coat protein–RNA complex with host initiation factors. However, replication of viral RNA can only be initiated once the coat protein is dissociated from the 3′ of the RNA molecules, allowing the 3′ terminus to assume the tRNA-like pseudoknot conformation. Regulation of this dissociation process is unknown. However, it has been speculated that the association of the replication complex with membrane structures could displace the coat protein, or proteolysis of the coat protein could result in its removal from RNA. Alternatively, the coat protein could be displaced by binding of the replicase to stem–loop structures upstream of the coat protein binding sites. Replication then proceeds through synthesis of minus-sense RNA and the replication of progeny plus-sense RNA segments, including the synthesis of the plus-sense subgenomic RNA encoding the coat protein. It has been demonstrated that both the coat protein and the movement protein are required for systemic infection. It appears that the infective agent moves through modified plasmodesmata as a protein–RNA complex rather than as intact virions.

Epidemiology and Control

One or more modes of transmission have been demonstrated for each member of the genus *Ilarvirus*. All ilarviruses can be transmitted by mechanical inoculation to experimental hosts, although some with difficulty. Ilarviruses tend to have a wide experimental host range, but many have woody plants as their primary natural hosts. The role of mechanical transmission between woody plants is likely small. However, succulent hosts such as hop and asparagus plants may be more prone to field spread through mechanical transmission associated with horticultural practices.

The dissemination of viruses through vegetative propagation is one of the most critical factors affecting the distribution of ilarviruses that infect perennial crops. Budding and grafting are important means of transmission of ilarviruses that infect woody plants such as fruit trees and roses. These plants are routinely budded onto rootstock to allow rapid expansion of a particular clone into hundreds or thousands of plants. Other hosts such as hop or asparagus plants can be vegetatively propagated by stem cuttings and/or crown division. In both cases, there is high likelihood that virus will be present in the progeny plants. Chance grafting of root systems is also an important means of transmission of some viruses of perennial crops.

Many ilarviruses are transmitted through seed when the seed-bearing plant is infected. Transmission of pollen-borne virus occurs to seedlings with variable frequency, and transmission of virus from pollen to the pollinated plant has also been demonstrated for PNRSV, PDV, and TSV. Seed and pollen transmission poses a particularly high risk in fruit trees since seedling rootstocks are frequently used in production and virus infection can originate from the rootstock, the scion, or both. The most effective means of control is through the use of virus-tested material during the establishment of plantings. This approach is implemented through programs where plants are propagated from virus-tested plants and grown under conditions that minimize re-infection by viruses, including the pollen-borne ilarviruses. Many industries rely on

informal virus control programs whereas others have developed official certification programs. The use of spatial separation of the propagation source material from potentially infected host plants is a basic strategy to minimize the introduction of virus through pollen. The risk associated with seed transmission is further reduced by screening samples of seed lots for the presence of virus. Where seed is produced from perennial plants such as *Prunus* species and asparagus, routine virus testing and rouging of mother plantings is implemented to limit the distribution of virus through seed.

The association of virus transmission with pollen raises concern about the role of bees in virus epidemiology. In the case of PNRSV and PDV, it has been shown that the virus is detectable by serological assays on honeybees emerging from hives weeks after exposure to flowers with virus-laden pollen. Pollen from hives is no longer viable at this stage, but the virus is infectious. The ability of this virus to infect trees then depends on whether the virus is transmitted to the tree by fertilization or by some other physical process. This issue remains largely unresolved. Holding bees in hives for several days before allowing them to enter a new orchard has been suggested as one means of minimizing this risk.

It has been demonstrated that TSV is transmitted to natural hosts in a process known to be facilitated by thrips. The thrips species demonstrated to transmit TSV include *Frankliniella occidentalis* (Pergande), *Frankliniella schultzei* (Trybom), *Microcephalothrips abdominalis* (Crawford), *Thrips tabaci* (Lindeman), and *Thrips parvispinus* (Karny). The association of thrips with pollen transmission of TSV is one of the major factors leading to the increased importance of this virus in vegetable production in many areas of the world. The thrips facilitate transmission by mechanical abrasion of the leaf cells, allowing pollen-associated virus particles to enter cells and initiate infection. Pollen-to-leaf transmission has been demonstrated, but leaf-to-leaf transmission has not. There have been many attempts to confirm the role of thrips in the transmission of ilarviruses other than TSV. Other ilarviruses have been transmitted from pollen to the leaves of experimental hosts in a thrips-mediated manner, but transmission to natural hosts by thrips has not been convincingly demonstrated. Unlike AMV, there is no evidence of aphid transmission of any members of the genus *Ilarvirus*.

Most ilarviruses have a limited natural host range. This feature in combination with a narrow temporal window for transmission imposed by the short flowering period of many hosts means that plant-to-plant spread of ilarviruses is relatively slow. TSV is an exception where a wide natural host range sustains sources of infection throughout the year. This may explain the growing impact of TSV on production of vegetables and legumes such as soybean. Agronomic strategies such as removing perennial weeds that would otherwise provide a green bridge for the thrips vectors and the virus has helped reduce the carryover of virus inoculum in a limited number of situations. Since rainfall abates thrips populations, in some areas, sowing seed so that germination will occur during periods of increased rain also helps reduce crop losses caused by TSV. As with the ilarviruses that infect fruit trees and asparagus, the use of certified, virus-tested seed is critical for effective virus control.

Rugose mosaic is a serious debilitating disease of sweet cherry (*Prunus avium* L.) that is caused by a severe strain of PNRSV. The long latent period between infection and visible expression of disease symptoms complicates efficient control strategies. In this case, trees can be inoculated prophylactically with less severe strains of PNRSV. The inoculated plant is then resistant to subsequent infection by the severe virus strain. This phenomenon is termed cross-protection. In combination with accurate diagnosis and prompt inoculum removal, cross-protection is an effective tool in controlling rugose mosaic disease.

See also: Alfalfa Mosaic Virus; Bromoviruses; Plant Antiviral Defense: Gene Silencing Pathway; Plant Virus Diseases: Fruit Trees and Grapevine; Replication of Viruses; Vector Transmission of Plant Viruses; Vegetable Viruses; Viral Suppressors of Gene Silencing.

Further Reading

Ansel-McKinney P and Gehrke L (1998) RNA determinants of a specific RNA-coat protein peptide interaction in alfalfa mosaic virus: Conservation of homologous features in ilarvirus RNAs. *Journal of Molecular Biology* 278: 767–785.

Ansel-McKinney P, Scott SW, Swanson M, Ge X, and Gehrke L (1996) A plant viral coat protein RNA binding consensus sequence contains a crucial arginine. *EMBO Journal* 15: 5077–5084.

Aparicio F, Vilar M, Perez-Payá E, and Pallás V (2003) The coat protein of *Prunus* necrotic ringspot virus specifically binds to and regulates the conformation of its genomic RNA. *Virology* 313: 213–223.

Bol JF (2005) Replication of Alfamo- and Ilarviruses: Role of the coat protein. *Annual Review of Phytopathology* 43: 39–42.

Garrett RG, Cooper JA, and Smith PR (1985) Virus epidemiology and control. In: Francki RIB (ed.) *The Plant Viruses, Vol 1:Polyhedral Virions with Tripartite Genomes,* ch. 9, pp. 269–297. New York: Plenum.

Greber RS, Teakle DS, and Mink GI (1992) Thrips-facilitated transmission of prune dwarf and *Prunus* necrotic ringspot viruses from cherry pollen to cucumber. *Plant Disease* 76: 1039–1041.

Haasnoot PCJ, Brederode FTh, Olsthoorn RCL, and Bol JF (2000) A conserved hairpin structure in *Alfamovirus* and *Bromovirus* subgenomic promoters is required for efficient RNA synthesis *in vitro*. *RNA* 6: 708–716.

Li WX and Ding SW (2001) Viral suppressors of RNA silencing. *Current Opinion in Biotechnology* 12: 150–154.

Mink GI (1983) The possible role of honeybees in long-distance spread of *Prunus* necrotic ringspot virus from California into Washington sweet cherry orchards. In: Plumb RT and Thresh JM (eds.) *Plant Virus Epidemiology: The Spread and Control of Insect-Borne Viruses*, pp. 85–91. Oxford: Blackwell Scientific Publications.

Roossinck MJ, Bujarski MJ, Ding SW, *et al.* (2005) *Bromoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1049–1058. San Diego, CA: Elsevier Academic Press.

Scott SW, Zimmerman MT, and Ge X (2003) Viruses in subgroup 2 of the genus *Ilarvirus* share both serological relationships and characteristics at the molecular level. *Archives of Virology* 148: 2063–2075.

Swanson MM, Ansel-McKinney P, Houser-Scott F, Yusibov V, Loesch-Fries LS, and Gehrke L (1998) Viral coat protein peptides with limited sequence homology bind similar domains of alfalfa mosaic virus and tobacco streak virus RNAs. *Journal of Virology* 72: 3227–3234.

Xin H-W, Ji L-H, Scott SW, Symons RH, and Ding S-W (1998) Ilarviruses encode a cucumovirus-like 2b gene that is absent in other genera within the *Bromoviridae*. *Journal of Virology* 72: 6956–6959.

Immune Response to Viruses: Antibody-Mediated Immunity

A R Neurath, Virotech, New York, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Milestones in History

The primeval notion about immunity to disease arose in Greece about 25 centuries ago from the observation that those who recovered from an apparently contagious disease became resistant to a subsequent similar sickness. The earliest known attempts to intentionally transfer immunity to an infectious disease occurred in China in the tenth century. It involved exposing uninfected people to material from lesions caused by smallpox. This not always successful practice was introduced in the seventeenth century to the Ottoman Empire and subsequently to England and its colonies in North America.

This approach was revolutionized by replacing material from human lesions by that derived from cowpox lesions first in 1774 in England by a farmer, Benjamin Jesty, who used it on his family, and 22 years later by Edward Jenner. Thereafter, the widely used vaccination has been considered as the first example of immunization with a life-attenuated virus. Nevertheless, both the cause of the disease and the success of vaccination remained unexplained.

An understanding started to emerge about a century later when (1) Koch and Pasteur set forth the germ theory of infectious diseases, and Pasteur developed attenuated vaccines against anthrax and rabies; and (2) Von Behring and Kitasato demonstrated that immunity could be transferred by a soluble serum component(s). Several decades later, these components were shown to be antibody immunoglobulins (Heidelberger and Kabat) whose fundamental structure was elucidated by Porter and Edelman in the late 1950s, and further characterized by numerous X-ray crystallography and sequencing studies.

However, several findings indicated that antibodies are not the only mediators of specific immunity. Immune responses corresponding to delayed hypersensitivity and allograft rejections appeared unrelated to the presence of serum antibodies. Immunity could also be transferred by cells from immunized to naive animals (Landsteiner and Chase). It was shown by Gowans and colleagues in 1962 that lymphocytes are essential for immune responses.

Antibodies to very many distinct antigens can be produced upon immunization. Therefore, it was presumed that the diverse antibodies were not preformed but would be generated on demand following antigenic stimuli. In theory, there were two possibilities: that an antigen either directs or selects for the formation of a specific antibody. Further studies fully supported the clonal selection theory (Burnet, Jerne, and Talmage). The repertoire of diverse antibodies undergoing a process of maturation leading to high-affinity antibodies is generated by somatic rearrangements and hypermutation of immunoglobulin genes (Tonegawa).

Although it became evident that both immunoglobulins and cells are essential for immunity, the details of the process whereby antigens and viruses elicit immune responses were contributed to by results of studies in transplantation biology and notably the discovery of the major histocompatibility complex (MHC; HLA molecules on human cells) (Benaceraff, Dausset, and Snell). Their role in presentation of antigen fragments and interactions of specific antibody producing B-cells with T-cells, as well as the function of cytokines, chemokines, and adhesion molecules will be discussed in later sections of this article.

Immunoglobulins, lymphocytes, MHC molecules, and antigen receptors are all components of adaptative (acquired) immunity. Components of innate immunity, responding rapidly to an invading pathogen, play a key role in initiating and orchestrating the adaptive response (Janeway).

The major milestones in the history of immunology and their time lines are summarized in **Table 1**.

Table 1 History of immunology timeline

1798	Smallpox vaccination	Edward Jenner
1876	Validation of germ theory of disease by discovering bacterial basis of anthrax	Robert Koch
1879	Chicken cholera vaccine development	Louis Pasteur
1890	Discovery of diphtheria 'antitoxin' in blood	Emil Von Behring and Shibasaburo Kitasato
1882	Isolation of the tubercle bacillus	Robert Koch
1883	Delayed type hypersensitivity	Robert Koch
1884	Phagocytosis; cellular theory of immunity	Elie Metchnikoff
1891	Proposal that antibodies are responsible for immunity	Paul Ehrlich
1891	Passive immunity	Emil Roux
1894	Complement and antibody activity in bacteriolysis	Jules Bordet
1900	A, B, and O blood groups	Karl Landsteiner
1901	Cutaneous allergic reaction	Maurice Arthus
1903	Opsonization by antibody	Almroth Wright and Stewart Douglas
1907	Discipline of immunochemistry founded	Svante Arrhenius
1910	Viral immunology theory	Peyton Rous
1917	Haptens discovered	Karl Landsteiner
1921	Cutaneous reactions	Carl Prausnitz and Heinz Kustner
1924	Reticuloendothelial system	Ludwig Aschoff
1939	Discovery that antibodies are gamma globulins	Elvin Kabat
1942	Adjuvants	Jules Freund and Katherine McDermott
1942	Cellular transfer of sensitivity in guinea pigs (anaphylaxis)	Karl Landsteiner and Merill Chase
1944	Immunological hypothesis of allograft rejection	Peter Medawar
1948	Demonstration of antibody production in plasma B-cells	Astrid Fagraeus
1949	Distinguishing self vs. nonself and its role in maintaining immunological unresponsiveness (tolerance) to self	Macfarlane Burnet and Frank Fenner
1952	Discovery of agammaglobulinemia (antibody immunodeficiency)	Ogden Bruton
1953	Immunological tolerance hypothesis	Rupert Billingham, Leslie Brent, Peter Medawar, and Milan Hasek
1955–59	Clonal selection theory	Niels Jerne, David Talmage, and Macfarlane Burnet
1957	Discovery of interferon	Alick Isaacs and Jean Lindenmann
1958	Identification of first autoantibody and first recognition of autoimmune disease	Henry Kunkel
1959–62	Elucidation of antibody structure	Rodney Porter and Gerald Edelman
1959	Lymphocytes as the cellular units of clonal selection; discovery of lymphoid circulation	James Gowans
1961	Discovery of thymus involvement in cellular immunity	Jacques Miller
1968	Recognition of B- and T-cells in immunodeficiencies	Robert Good
1968	Distinction of bone marrow- and thymus-derived lymphocyte populations; discovery or T- and B-cell collaboration	Jacques Miller and Graham Mitchell
1965	Demonstration of allelic exclusion in B-cells	Benvenuto Pernis
1968–70	Elaboration of two-signal model of lymphocyte activation	Peter Bretscher and Melvin Cohn
1970	Discovery of membrane immunoglobulins	Benvenuto Pernis and Martin Raff
1971	Recognition of hypervariable regions in Ig chains	Elvin Kabat
1972	Elucidation of the major histocompatibility complex	Baruj Benacerraf, Jean Dausset, and George Snell
1974	Discovery of MHC restriction	Rolf Zinkernagel and Peter Doherty
1974	HLA-B27 predisposes to an autoimmune disease	Derek Brewerton
1975	Monoclonal antibodies used in genetic analysis	Georges Kohler and Cesar Milstein
1976	First demonstration of cross-priming	Michael Bevan
1978	Direct evidence for somatic rearrangement in immunoglobulin genes	Susumu Tonegawa
1978	Recognition that dendritic cells are distinctive and highly potent antigen-presenting cells	Ralph Steinman
1979	Discovery of leukocyte adhesion molecules and their role in lymphocyte trafficking	Eugene Butcher
1984–87	Identification of genes for the T-cell antigen receptor	Mark Davis, Leroy Hood, Stephen Hedrick, and Gerry Siu
1987	Crystal structure of MHC peptide solved	Pam Bjorkman, Jack Strominger, and Don Wiley
1989	Emerging field of innate immunity, infectious nonself model of immune recognition ('stranger hypothesis')	Charlie Janeway

Continued

Table 1 Continued

1994	Danger hypothesis of immune responsiveness	Polly Matzinger
1986	Discovery of T-helper subsets	Tim Mossmann and Bob Coffman
1989	Discovery of first chemokines	Edward Leonard, Teizo Yoshimura, and Marco Baggiolini
1991	Discovery of the first costimulatory pathway (CD28/B7) for T-cell activation	Kevin Urdahl and Mark Jenkins
1992	Cloning of CD40 ligand and recognition of its role in T-cell-dependent B-cell activation	Armitage, Spriggs, Lederman, Chess, Noelle, Aruffo, *et al*.
1996–7	Discovery of the role of Toll and Toll-like receptors in immunity	R. Medzhitov, CA. Janeway, Jr., and J. Hoffmann

Reprinted with permission from Steven Greenberg, MD, from http://www.columbia.edu/itc/hs/medical/pathophys/immunology/readings/ConciseHistoryImmunology.pdf.

Immunoglobulin Phylogeny

The adaptive immune system, based on clonally diverse repertoires of antigen recognition molecules, arose in jawed vertebrates (gnasthostomes) about 500 million years ago. Homologs of human immunoglobulins, T-cell antigen receptors (TCRs), MHC I and MHC II molecules, and recombination activating genes (RAGs) have been identified in all classes of gnasthostomes. This has provided evolutionary advantages allowing the recognition of potentially lethal pathogens, including viruses, and the initiation of protective responses against them (**Figure 1**), and represented an add-on to a preexisting innate immune system. For immunoglobulins, somatic variation was further expanded through class switching, gene conversion, and somatic hypermutation. Jawless vertebrates (agnathans) were shown to also have an adaptive immune system based on recombinatorial assembly of genetic units different from those of gnasthostomes, to generate a diverse repertoire of lymphocytes, each with distinct receptors (**Figure 2**). The assembly relies on highly variable leucine-rich repeat (LRR) protein modular units.

Most gnasthotomes generate a large part of their immunoglobulin diversity in ways similar to those in humans. However, exceptions have occurred. Two of these, relevant to passive immunotherapy and development of diagnostics using the corresponding immunoglobulins, are mentioned here. (1) Only three classes of H-chain genes, corresponding to IgM, IgY, and IgA, exist in chicken. Class switching occurs from IgM to either IgY or IgA. IgY is considered to be the ancestor of 'present-day' IgG and IgE. Unlike human IgG, IgY does not bind to cellular Fc receptors and to proteins A and G, respectively, and does not activate the complement system. (2) H-chain antibodies devoid of light chains occur in the Camelidae species (camels, dromedaries, and llamas). Single-domain fragments from such antibodies react with specific antigens with high affinity constants (nanomolar range). The binding involves longer than average third variable loops of the antibody molecules. These unique antibodies and their antigen-specific fragments are expected to become important for biotechnological and medical applications, including intrabodies (intracellular antibodies).

Innate Immunity

The innate immune system provides an early defense against pathogens and alerts the adaptive immune system when initial invasion by a pathogen has occurred. The innate system predates evolutionarily the adaptive system and relies on antimicrobial peptides and on germline-encoded pattern-recognition receptors (PRRs). Antimicrobial peptides can damage enveloped viruses and interfere with processes involved in fusion of viruses with target cell membranes. PRRs recognize microbial (viral) components defined as pathogen-associated molecular patterns (PAMPs). Different PRRs are specific for distinct PAMPs, have distinct expression patterns, activate different signaling pathways, thereby eliciting distinct responses against invading pathogens. PRRs can be subdivided into two classes: Toll-like receptors (TLRs) and non-Toll-like innate immune proteins.

Toll was originally identified as a differentiation protein in *Drosophila*, and later (1996) shown to play a role in defense against fungal infection. Searching the entire DNA sequence database for similarities with the coding sequence for *Toll* resulted in identification of Toll-like sequences, and ultimately in discovery of at least 10 functional TLRs in humans. TLRs are type 1 integral membrane glycoproteins the extracellular domain of which contains several LRR motifs and a cytoplasmic signaling domain homologous to that of the interleukin-1 receptor (IL-1R). TLRs 2, 3, 4, 7, 8, and 9 recognize PAMPs characteristic for viruses. Viral DNAs rich in CpG motifs are recognized by TLR9, leading to activation of pro-inflammatory cytokines and type 1 interferon (IFN) secretion. TLR7 and TLR8, expressed within the endosomal membrane, are specific for viral single-stranded RNA (ssRNA). Double-stranded RNA (dsRNA), generated

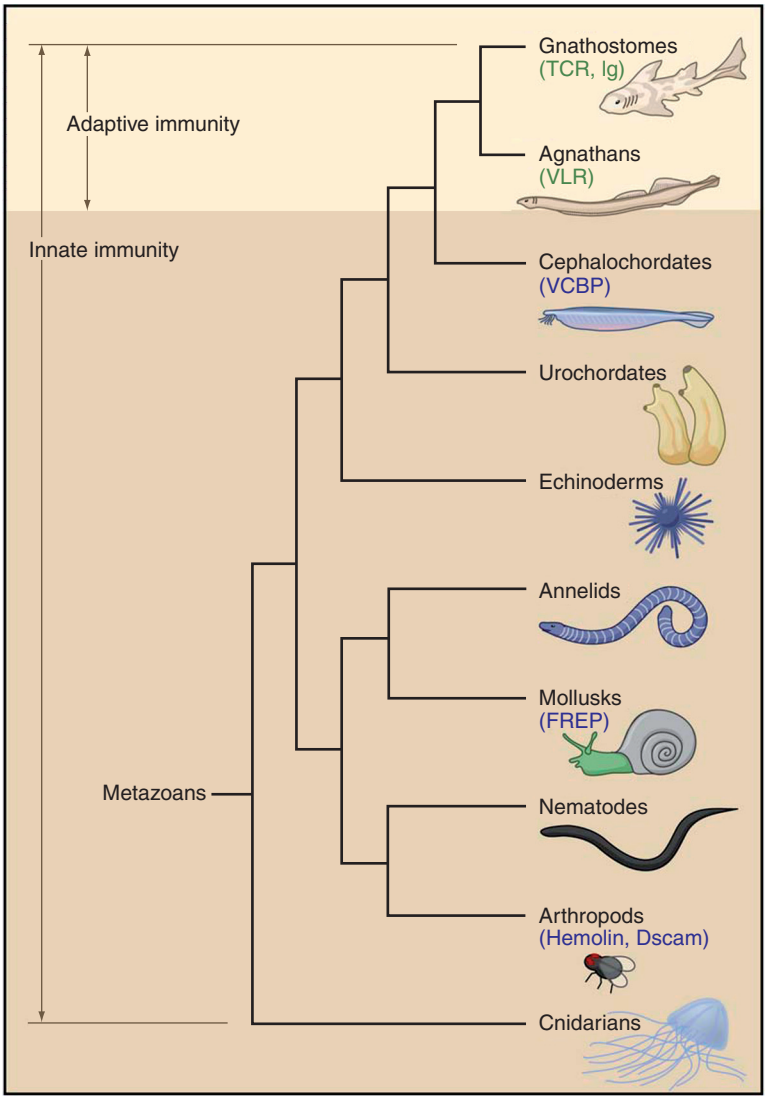


Figure 1 Phylogenetic tree indicating theoretical evolutionary relationships of metazoans and the emergence of adaptive immunity in conjunction with innate immunity. Families of immune molecules, other than Toll-like receptors (TLRs), are indicated in blue: chitin-binding domain-containing proteins (VCBPs), fibrinogen-related proteins (FREPs), hemolin and Down's syndrome cell adhesion molecule (Dscam). The recombinatorial based immune receptors are indicated in green: T-cell receptors (TCRs), immunoglobulins (Ig's), and variable lymphocyte receptors (VLRs). Reprinted from Cooper MD and Alder N (2006) The evolution of adaptive immune systems. *Cell* 124: 815–822, with permission from Elsevier.

during viral infection as an intermediate for ssRNA viruses or during transcription of viral DNA, is recognized by TLR3. TLR3 is expressed in dendritic cells (DCs) and in a variety of epithelial cells, including airway, uterine, corneal, vaginal, cervical, biliary, and intestinal cells. Cervical mucosal epithelial cells also express functional TLR9, suggesting that TLR3 and TLR9 provide an antiviral environment for the lower female reproductive tract. Some viral envelope glycoproteins are recognized by TLR2 and TLR4, each expressed at the cell surface, leading to production of pro-inflammatory cytokines. In general, the engagement of TLRs by microbial PAMPs triggers signaling cascades leading to the induction of genes involved in antimicrobial host defenses. This includes the maturation and migration of DCs from sites where infection occurs to lymphoid organs where DCs can initiate antigen-specific immune responses. Triggering distinct TLRs elicits different cytokine profiles and different immune responses. Engagement of TL3 and TRL 4, respectively, upregulates polymeric immunoglobulin receptor expression on cells. Thus, bridges between innate and adaptive immune responses are established.

TLRs are expressed either at the cell surface or in lysosomal/endosomal membranes. Therefore, they would

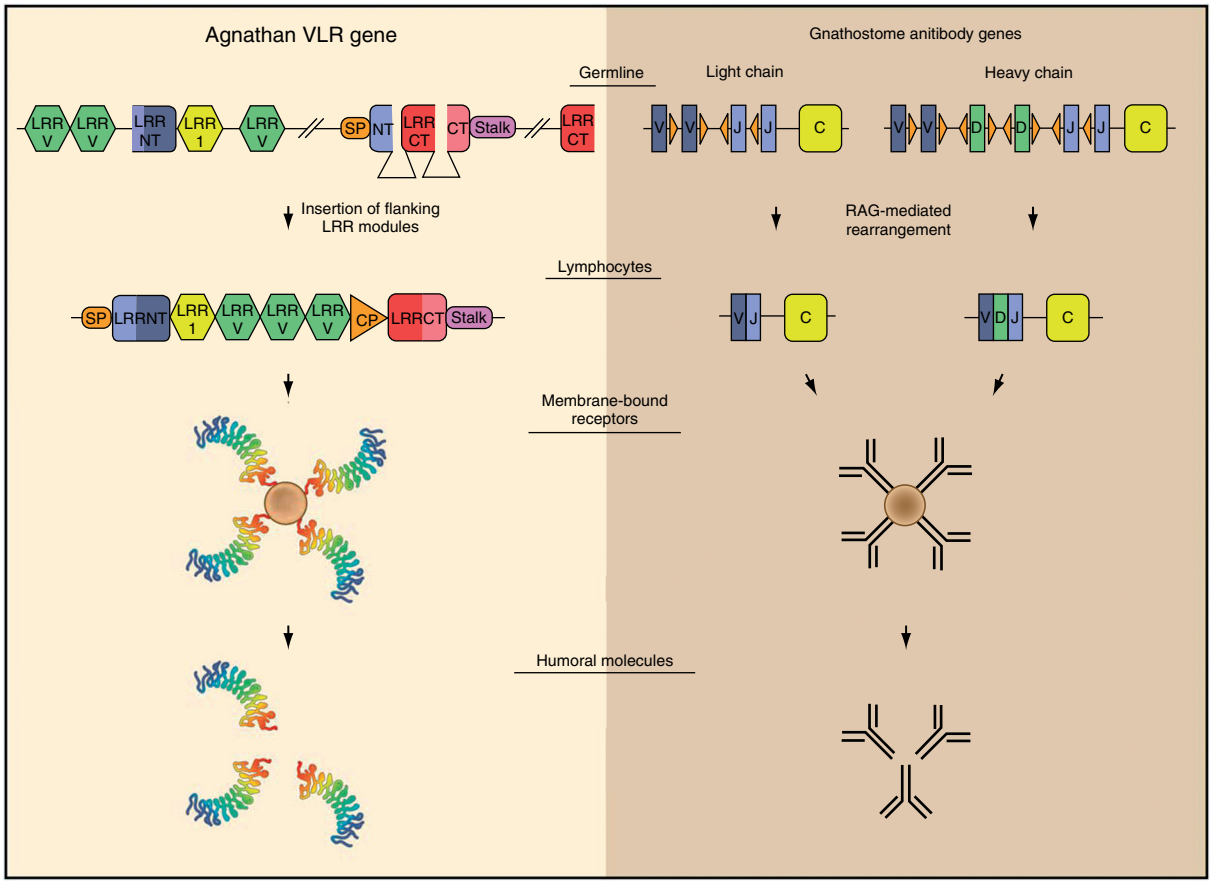


Figure 2 Two recombinatorial systems used for generating diverse antigen receptors in vertebrates. The figure compares the assembly of leucine-rich repeat (LRR) modular genetic units in agnathan lymphocytes to generate variable lymphocyte receptor (VLR) genes vs. the rearrangement of Ig gene segments in gnathostome B-lymphocytes to generate diverse antibody genes. Variable 24-amino-acid LRR (LRRV, green); N-terminal capping LRR (LRRNT, blue); variable 18-amino-acid LRR (LRR1, yellow); signal peptide (SP, orange); first six amino acids of LRRNT (NT, light blue); C-terminal capping LRR (LRRCT, red); last nine amino acids of LRRCT (CT, pink); variable 13 amino acid connecting peptide (CP, orange); and invariant VLR stalk (Stalk, purple). The small orange triangles adjacent to the representative V (blue), D (green), and J (light blue) gene segments represent recombination signal sequences (RSSs). The C (yellow) indicates the immunoglobulin constant region. Reproduced from Cooper MD and Alder N (2006) The evolution of adaptive immune systems. *Cell* 124: 815–822, with permission from Elsevier.

not recognize pathogens that succeeded in invading the cytosolic compartment. These pathogens are detected by a variety of cytoplasmic PRRs. They include retinoic acid inducible protein (RIG-1) with a helicase domain recognizing viral dsRNA, and a related protein, MDA5. Other proteins which may be involved in innate antiviral immunity include a triggering receptor on myeloid cells (TREM-1), myeloid C-type lectins and siglecs, recognizing sialic acid. One of the elements of both innate and adaptative immunity is the complement system. Some viruses or virus-infected cells can directly activate the complement cascade in the absence of antiviral antibodies.

Many viruses are endowed with properties subverting innate immune responses. Vaccinia virus produces proteins suppressing TLR- and IL-1R-induced signaling cascades. Paramyxoviruses produce proteins which associate with MDA5, and thus inhibit dsRNA-induced activation processes. Adenoviruses avoid immune surveillance by TRL9. A nonstructural protein of hepatitis C virus blocks signaling by RIG-1 and MDA5. Marburg and Ebola viruses, members of the family *Filoviridae*, elicit direct activation of TREM-1 on neutrophils. This can lead to vigorous inflammatory responses contributing to fatal hemorrhagic fevers in infected humans. Thus, some viruses have developed strategies to overcome either innate or adaptive (see next section) immune responses.

Adaptive Immunity

Adaptive immunity is a complex anticipatory system triggered by exposure to antigens, including viruses. Its hallmarks are selectivity, diversification, specificity, and memory. The principal effector molecules of the system

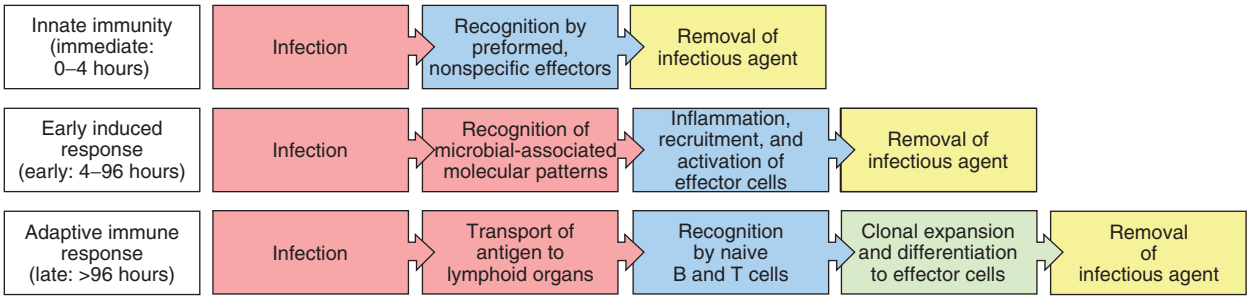


Figure 3 Three phases of a response to an initial infection. Copyright 2005 from Janeway CA, Jr., Travers P, Walport M, and Shlomchik MJ (2005) *Immunobiology*. *The Immune System in Health and Disease*, 6th edn, figure 2.1, p. 37. New York: Garland Science. Reproduced by permission of Garland Science/Taylor & Francis LLC.

are antigen-binding receptors (immunoglobulin (Ig) and T-cell receptors (TCR)). The following simplified overview (see **Figure 3**) will be limited to Ig's and humoral immune responses.

Differentiation of B-cells into antibody-generating plasma cells occurs through distinct pathways. Two pathways lead to rapid IgM and IgA antibody production by B-1 cells against T-cell-independent antigens. These antibodies have low antigen-binding constants, and immunological memory does not evolve. The third pathway involves clonal selection. The B-cells synthesize IgM and IgD low affinity antibodies which are expressed as antigen receptors on the surface of cells. Each B-cell produces a different receptor recognizing a distinct epitope. Encounter with the appropriate epitope on an antigen elicits cell division, and generation of selected clones with identical receptor specificities. The clones further differentiate into specific antibody-secreting cells. The remarkable diversity of antibodies is attributable to the fact that genes coding for Ig variable regions are inherited as sets of gene fragments, each encoding a portion of the variable region of a particular Ig polypeptide chain. The fragments are joined together to form a complete gene in individual lymphocytes. The joining process involves addition of DNA sequences to the ends of fragments to be joined, thus increasing diversity. Further diversity arises from the assembly of each Ig protein from pairs of H- and L-chains, each B-cell producing only one kind of either chain (**Figure 4**). In addition, the assembled genes for Ig's mutate rapidly when B-cells are activated by binding an antigen. These hypermutations lead to new receptor variants representing the process of affinity maturation of an immune response. Moreover, B-cells and their progeny can produce an additional variation by altering the constant part of the H chain due to gene rearrangements (=Ig isotype and subclass switching) (**Figures 5–7**). These antibodies have identical paratopes but distinct effector functions (complement activation, phagocytosis, transcytosis, etc.). As a consequence of the aforementioned processes, there are B-lymphocytes of at least 100 million distinct specificities in every human individual at any given time.

The majority of Ig is produced in mucosa-associated tissues, predominantly in the intestine, rather than in the bone marrow, spleen, and lymph nodes, in the form of IgA. This harmonizes with the fact that mucosal surfaces represent the predominant sites for entry of pathogens, including viruses. Coincidentally, it has been demonstrated that the gut is the major site for HIV-1 replication and depletion of CD4+ cells. There are two subclasses of IgA, IgA1 and IgA2, which occur in monomeric, dimeric, tetrameric, and polymeric (pIgA) forms. A distinguishing feature of secretory IgA is its association with another glycoprotein, the secretory component (SC) (**Figure 8**). SC is also the extracellular portion (which can be generated by proteolytic cleavage) of an integral epithelial cell membrane protein, the polymeric Ig receptor (pIgR) mediating transcytosis of pIgA and IgM. The latter process allows neutralization of pathogens within intracellular vesicular compartments.

An essential aspect of adaptive immunity is its ability to recall past encounters with a pathogen for decades or even an entire lifetime. This fundamental feature is the foundation of successful vaccination. Germinal center-derived memory B-cells have the following attributes: antigen specificity; hypermutated Ig variable gene segments; and ability to bestow immunological memory following their adoptive transfer to immunologically naïve recipients. The CD27 surface antigen is a marker for memory B-cells.

The multifaceted performance of B-cells is the result of a thoroughly orchestrated ensemble involving CD4+ helper T-cells, DCs, MHC class II antigens, cell-differentiation antigens (CDs), cytokines, etc. In summary, in addition to occupancy of the Ig receptor, B-cells must interact with antigen-specific T-cells. The T-cells, through specific TCRs, recognize peptide fragments generated from the antigen internalized by the B-cell, and displayed on the surface of the B-cell as a peptide–MHC class II complex.

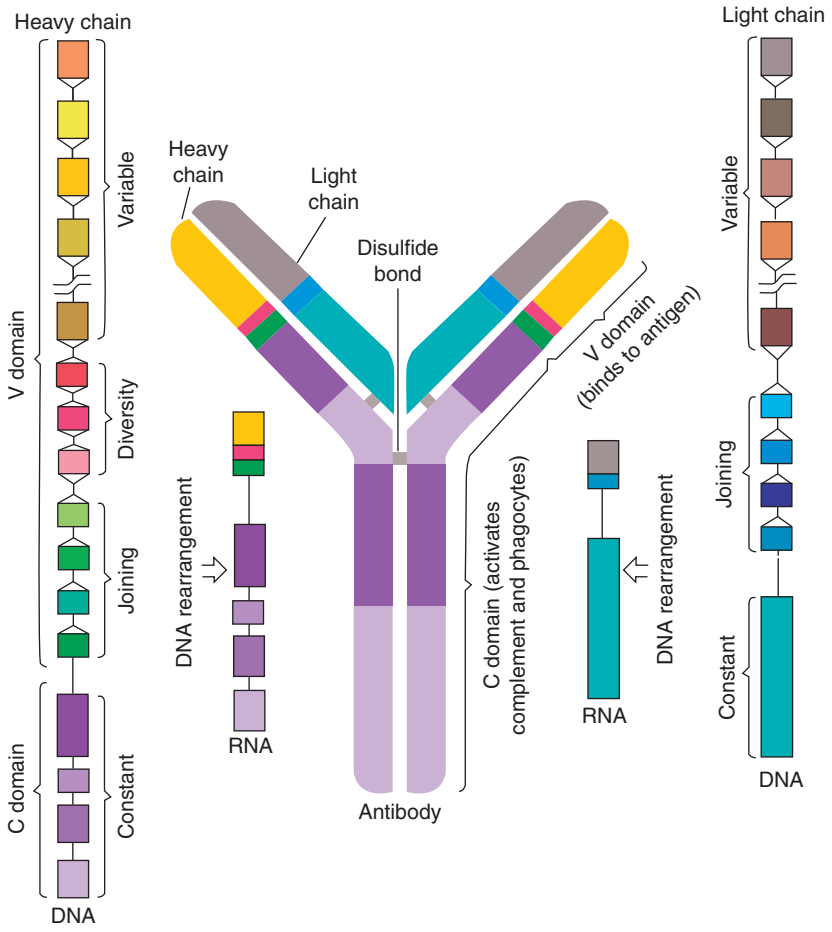


Figure 4 Antibody molecule consisting of pairs of H- and L-chains each encoded by genes assembled from different DNA segments. The segments rearrange to generate genes for chains that are distinct in each B-cell. The joining is variable so that the gene segments can encode the estimated 100 million specific antibodies each human is capable of producing. Reprinted from Janeway CA, Jr. (1993) How the immune system recognizes invaders. *Scientific American* (Sep.): 73–79, with permission of Scientific American and Ian Worpole (artist).

Helper T-cells stimulate the B-cell following binding of the CD40 ligand on the T-cell to CD40 on the B-cell; the interaction of tumor necrosis factor (TNF)–TNF receptor family ligand pairs; and the release of specific cytokines. Further details of these interactions are shown in **Figures 9–11**.

The intricacies of all these tightly coordinated events seem to minimize the possibility that 'rationally designed' synthetic vaccines will be able to successfully recreate the specificity of virus neutralization B-cell epitopes or neotopes.

Biological Functions of Antiviral Antibodies

The surface of viruses is represented by a mosaic cluster of protein or glycoprotein subunits. The subunits correspond to a single or two or more species. The pattern of repetitiveness is usually a key factor responsible for the efficiency of early and rapid B-cell responses, potent IgM antibody production, and efficient downstream antibody class switching. However, the immune response is not restricted to antigenic sites (epitopes and neotopes) on the surface of viruses. Virus particles, following initial infection or provided as a vaccine, also separate into constituent parts. Consequently, unassembled surface subunits (their epitopes and cryptotopes) and internal virus components become exposed to the immune system, ultimately resulting in the production of antibodies having multiple specificities. Only some of these are directed against intact viruses, and may have virus-neutralizing properties. The formation of antibodies with distinct specificities may not be simultaneous but rather sequential. Especially in case of some not directly cytopathic viruses (hepatitis B and C, lymphocytic choriomeningitis,

	Immunoglobulin								
	IgG1	IgG2	IgG3	IgG4	IgM	IgA1	IgA2	IgD	IgE
Heavy chain	γ_1	γ_2	γ_3	γ_4	μ	α_1	α_2	δ	ε
Molecular weight (kDa)	146	146	165	146	970	160	160	184	188
Serum level (mean adult mg ml^{-1})	9	3	1	0.5	1.5	3.0	0.5	0.03	5×10^{-5}
Half-life in serum (days)	21	20	7	21	10	6	6	3	2
Classical pathway of complement activation	++	+	+++	–	+++	–	–	–	–
Alternative pathway of complement activation	–	–	–	–	–	+	–	–	–
Placental transfer	+++	+	++	–/+	–	–	–	–	–
Binding to macrophage and phagocyte Fc receptors	+	–	+	–/+	–	+	+	–	+
High-affinity binding to mast cells and baseophils	–	–	–	–	–	–	–	–	+++
Reactivity with staphylococcal protein A	+	+	–/+	+	–	–	–	–	–

Figure 5 Properties of human immunoglobulin isotypes. The molecular mass of IgM corresponds to that of a pentamer. IgE is associated with immediate hypersensitivity. When attached to mast cells, it has a much higher half-life than in plasma. Copyright 2005 and permission as shown in legend for **Figure 3** (source = figure 4.17).

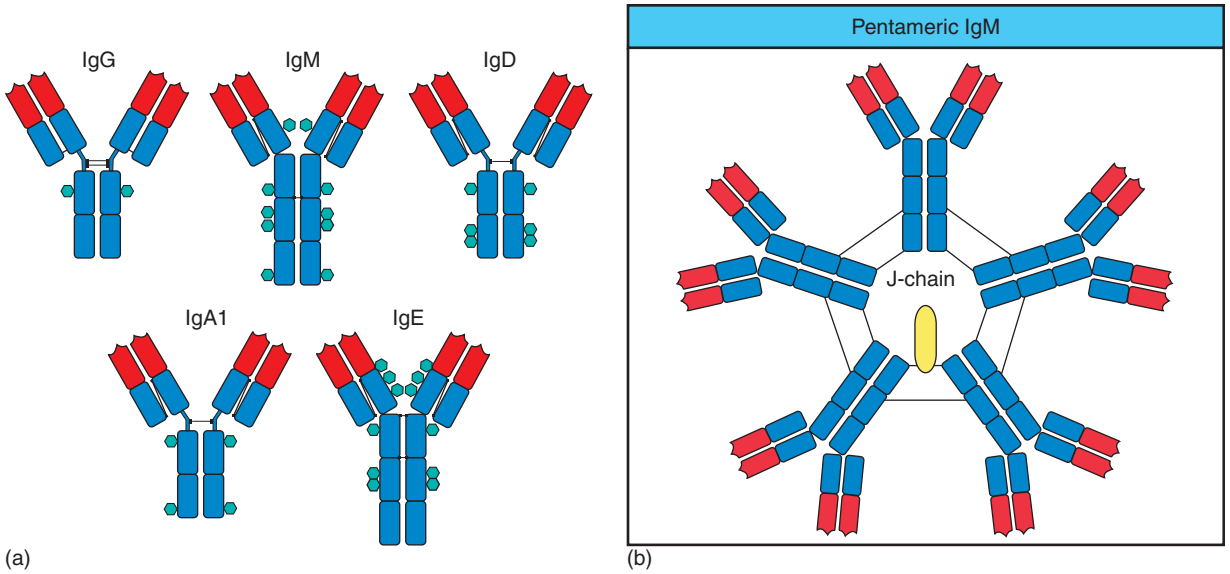


Figure 6 (a) Structural organization of human immunoglobulin isotype monomers. Both IgM and IgE lack a hinge region and each contains an additional heavy chain domain. Disulfide bonds linking the chains are indicated by black lines. N-linked glycans are shown as turquoise hexagons. (b) Pentameric IgM is associated with an additional polypeptide, the J-chain. The monomers are cross-linked by disulfide bonds to each other and to the J-chain. Copyright 2005 and permission as shown in legend for **Figure 3** (source figures 4.18 and 4.23).

Functional activity	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Neutralization	+	–	++	++	++	++	++	–
Opsonization	+	–	+++	*	++	+	+	–
Sensitization for killing by NK cells	–	–	++	–	++	–	–	–
Sensitization of mast cells	–	–	+	–	+	–	–	+++
Activates complement system	+++	–	++	+	+++	–	+	–

Distribution	IgM	IgD	IgG1	IgG2	IgG3	IgG4	IgA	IgE
Transport across epithelium	+	–	–	–	–	–	+++ (dimer)	–
Transport across placenta	–	–	+++	+	++	+/–	–	–
Diffusion into extravascular sites	+/–	–	+++	+++	+++	+++	++ (monomer)	+
Mean serum level (mg ml^{-1})	1.5	0.04	9	3	1	0.5	2.1	3×10^{-5}

Figure 7 Functions and distribution of human immunoglobulin isotypes. Copyright 2005 and permission as shown in legend for **Figure 3** (source figure 9.19).

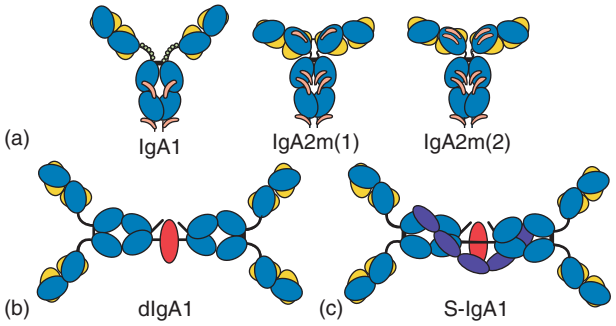


Figure 8 Structural organization of different molecular forms of human IgA. Heavy chains are shown in mid-blue, light chains in yellow, J-chain in red, and the secretory component (SC) in navy blue. (a) Monomeric mIgA. N-linked glycans are shown in orange, and O-linked glycans by small green circles. (b) Dimeric IgA1. (c) Dimeric secretory IgA1 (S-IgA1). For clarity, glycans are not shown for (b) and (c). Reprinted from Woof JM and Mestecky J (2005) Mucosal immunoglobulins. *Immunological Reviews* 206: 64–82, figure 1, with permission from Blackwell Publishing.

and HIV-1, prone to elicit a chronic carrier state), virus-neutralizing antibodies (VNAbs) appear with a delay after non-neutralizing antibodies. The latter may function as a 'decoy' if they are targeted like VNAb to virus surface epitopes.

Virus-Neutralizing and Protective Antibodies

VNAbs are crucial for protection against reinfection by a virus the VNAbs are specific for. Protection by efficacious vaccines correlates closely with *in vitro* determined VNAb titers of sera from immunized individuals. Protection by passive immunization relies on VNAb recognizing neutralization epitopes (or neotopes) on the virus surface. Coating of virus particles by antibodies is necessary but not always sufficient for virus neutralization. The effectiveness of virus neutralization correlates with the rate of antibody binding to critical epitopes and is augmented by slow dissociation of the formed antigen–antibody complexes. These kinetic parameters can be determined experimentally.

Spatial adaptive complementarity, electrostatic interactions, hydrogen bonds, and van der Waals forces contribute to the binding. Experimentally determined virus neutralization can depend on the target cells used. Virus neutralization is a multihit process and is successful when the number of unencumbered viral molecules, essential for initiation of the virus replicative cycle, is brought below a minimum threshold level. The mechanism of neutralization depends on processes obligatory for reproduction of a particular virus, and may involve the following steps: attachment to cell receptors; post-attachment events, internalization (endocytosis); fusion with cell

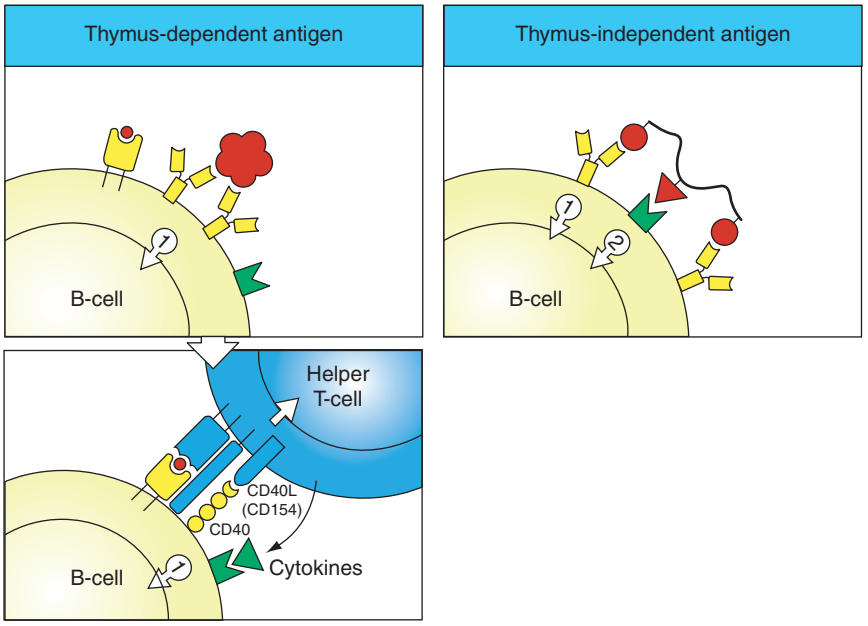


Figure 9 T-helper-cell-dependent initiation of the humoral immune response (two left panels). The first signal required for B-cell activation is delivered by binding of antigen (virus) (large red particle) to Ig cell receptors corresponding to monomeric IgM. Internalization and degradation of the antigen, and complex formation of the resulting peptide(s) (small red circle) with MHC class II molecules on the B-cell, allow the second signal to be delivered, that is, by the interaction between CD40 on the B-cell and the CD40 ligand (=CD154) on the CD4+ helper T-cell, and the engagement of the T-cell receptor (TCR) with the peptide-MHC class II complex on the B-cell. The activation is promoted by binding of cytokines to their specific receptors (see **Figure 10**). For comparison (right panel), in case of T-helper-cell-independent antigens, the second signal can be delivered by the antigen itself, either through binding of a part of the antigen to a receptor of the innate immune system (e.g., TLRs; green), or by extensive cross-linking of the membrane IgM by a polymeric antigen. Copyright 2005 and permission as shown in legend for **Figure 3** (source figure 9.2).

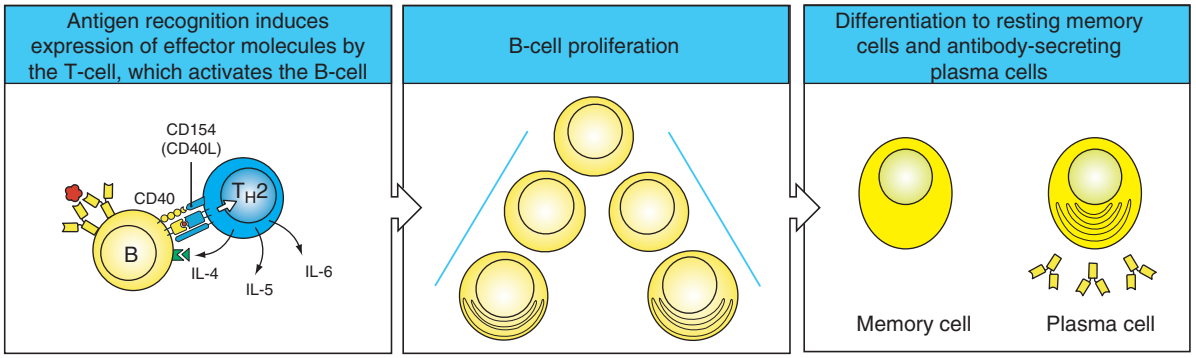


Figure 10 Antigen recognition induces the expression of B-cell stimulatory interleukins IL-4, IL-5, and IL-6 (and/or others) by the T-cell, driving the proliferation and differentiation of B-cells into antibody-secreting plasma cells. Activated B-cells can alternatively become memory B-cells. Copyright 2005 and permission as shown in legend for **Figure 3** (source figure 9.3).

membranes or endosomal vesicles; uncoating and/or intracellular localization; and enzymatic activities (e.g., transcription). Direct occupancy by VNAb of cell receptor binding sites on the virus surface might not be obligatory for neutralization. Steric hindrance or induction of deleterious conformational changes may be sufficiently effective. A unique feature of several anti-HIV-1 human monoclonal VNAb, having distinct specificities, is a very long finger-like third complementarity determining region of the immunoglobulin heavy chain allowing access to a recessed critical site on HIV-1 gp120. Such feature is rare in the human immunoglobulin repertoire but is common in the Ig's of Camelidae.

The mechanism and magnitude of VNAb neutralizing effects are influenced by their immunoglobulin isotype and subtype which affect interactions with complement,

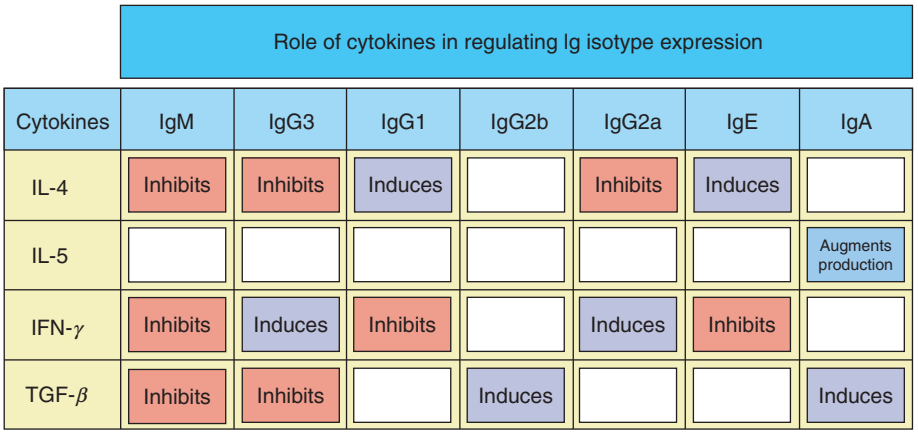


Figure 11 Role of cytokines in regulating Ig isotype switching. The individual cytokines either induce (violet) or inhibit (red) the production of particular Ig isotypes. IFN, interferon; TGF, transforming growth factor. Copyright 2005 and permission as shown in legend for **Figure 3** (source figure 9.7).

Fc receptors, and transcytosis through mucosal epithelia. Non-neutralizing virus-surface-binding antibodies sometimes enhance the effectiveness of VNAb, limit viral spread in the early phases of infection, and contribute to its suppression through antibody-mediated cellular cytotoxicity (ADCC). Antibodies directed to epitopes (or neotopes) on distinct surface proteins may act synergistically in virus neutralization.

The principle that VNAbs specific for virus surface components provide protection against disease is not absolute. The flavivirus nonstructural protein NS1 elicits a protective immune response against yellow fever, dengue, and tick-borne encephalitis viruses. The paratope binding site containing F(ab′)2 fragments are ineffective. Thus the immunoglobulin Fc portion is obligatory for the protective effect.

Antibody-Dependent Enhancement of Viral Diseases

Some viruses make use of antiviral antibodies to gain entry into target cells thus widening cell receptor usage to initiate infection. The infectious virus–antibody complexes rely upon the Fc portion of IgG antibodies to gain entry into monocytes/macrophages and granulocyte through Fc (FcR) or complement receptors (CR) expressed on these cells.The characteristic feature of the viruses is their propensity to establish persistent infections and their antigenic diversity. Antibody-dependent enhancement (ADE) has been demonstrated to occur *in vitro* with members of the families *Bunyaviridae*, *Coronaviridae*, *Flaviviridae*, *Orthomyxoviridae*, *Paramyxoviridae*, *Retroviridae*, *Rhabdoviridae*, and *Togaviridae*. A link between *in vitro* ADE and clinical manifestations cannot be always established. A relationship between ADE and disease exacerbation has been observed for dengue, measles, yellow fever, and respiratory syncytial viruses (RSVs). ADE may occur in children infected at a time when the level of transferred maternal antiviral antibodies declines to insufficient levels. ADE could represent an obstacle for development of vaccines, as has been the case for anti-RSV vaccines. A vaccine consisting of formaldehyde-treated measles virus hemagglutinin (ineffective to elicit antibodies to the virus fusion protein) induced antibodies causing ADE and led to aggravated atypical disease following infection with measles virus.

FcR- and CR-independent ADE was shown to occur following binding to HIV-1 of antibodies eliciting conformational changes in the gp120 envelope glycoprotein, allowing direct virus binding to cellular co-receptors while bypassing the primary binding to the primary CD4 cell receptor.

Immune System Evasion by Viruses

Persistence in an infected host and repeated reactivation of many viruses rely on several specific evasion strategies of adaptive immunity. Thus common protective responses are redirected or altered to the advantage of the infectious agent. This includes antiviral antibody responses and involves (1) specific paratope–epitope interactions (Fab fragments) and (2) effector mechanisms mediated by the Fc portion of antibodies.

The first mechanism is provided by genetically determined amino acid replacements leading to changes of virus epitopes (or neotopes) involved in virus neutralization. The sites of these escape mutations are usually on the viral surface, result in structural changes in antibody/virus contact sites, and lead to much less favorable kinetic parameters for antibody binding or completely abrogate binding. Presentation of new glycan chains on enveloped viruses or elimination of these chains due to mutations of N-glycosylation sites may cause substantial epitope alterations. The rate of escape mutation appearance is promoted

by error-prone replication of the viral genome. Antibodies with new specificities must be produced to bring the mutant viruses under control. The process is repeated, and if not successful, persistent infection is established. A similar process leads to evasion from T-cell-mediated protective responses.

Secondly, several viruses bypass clearance processes facilitated by the Fc portion of bound antibodies by encoding and expressing Fc receptor analogs. Subversion of the complement cascade provides another way how to block clearance of cell-free virus and infected cells.

Additional scenarios for escaping immune surveillance include: interference with MHC class I restricted antigen presentation involving also inhibition of MHC class I cell surface expression or synthesis of viral MHC class I homologs; blocking MHC class II restricted antigen presentation; downregulation of cellular CD4 or its degradation; interference with cytokine effector functions; and other strategies.

Immunoglobulins for Passive Immunization against Human Viruses

Transfer of immunity from an immune donor to an unprotected recipient by serum is one of the early landmarks in the history of immunology (Von Behring). The active serum components have later been identified as immunoglobulins. The half-life of immunoglobulin isotypes in serum is 6–21 days (**Figure 5**). Consequently, administered antivirus immunoglobulins can provide only short term prophylactic and therapeutic benefits, respectively, unless they are administered repeatedly or incorporated into a slow-release medical device. The most common applications are pre-exposure (travel, protection against community-wide infection(s), medical professionals, immunosuppressed individuals, combination with live vaccines to minimize their potential side effects) and post-exposure prophylaxis (passive immunization may provide immediate protection while the benefits from vaccination are delayed). Local mucosal applications of immunoglobulins 'as needed' appear promising against perinatal and sexual transmission, respectively, of several viruses (e.g., herpesviruses and HIV-1).

The immunoglobulins are isolated from serum of individuals pre-screened for high levels of antibodies against a particular virus or from vaccinated individuals. The immunoglobulins are purified, treated to remove or inactivate infectious agents which might be present in the pooled serum source, notwithstanding rigorous screening of the individual sera entering the pool. The products are further processed depending on their intended intramuscular or intravenous applications. All these immunoglobulins are polyclonal with respect to the 'indicated' virus and contain other antibodies originally present in the pooled sera. Oral administration of antibodies produced in bovine colostrums or chicken yolk has been recently suggested.

Alternatively, monoclonal antibodies (mAbs) specific for epitopes, known to elicit virus-neutralizing and protective immune responses, are used. They are prepared using hybridoma technologies, immortalized human peripheral B-cells, transgenic mice and bacteriophage expression libraries. If derived from animal species, the mAbs are 'humanized' using recombinant DNA techniques by replacing amino acid sequences outside the antigen-binding sites with sequences corresponding to human immunoglobulins. By an *in vitro* directed evolution process allowing manipulation of antigen-binding kinetics, mAb variants having much faster antigen association rates and much slower dissociation rates can be produced. Such antibodies have a much improved capacity to neutralize the target virus and may have a great clinical potential. Their production in high yield in plants offers an economically advantageous approach applicable to both IgG and secretory IgA antibodies.

While polyclonal antibodies from human sera provide immunological diversity, mAbs are highly specific for a single virus epitope. Potential alterations of such epitopes may generate virus neutralization escape mutants and decrease or eliminate the effectiveness of mAb prophylactics/therapeutics. Polyclonal antibody preparations prepared from pooled sera are not uniform and vary with the source of serum pools.

This problem can be overcome by the development of human recombinant antigen-specific polyclonal antibodies by a novel Sympress technology (Symphogen, Lyngby, Denmark).

Immunoglobulins, either already in clinical use or in development, are directed against one of the following viruses: hepatitis A and B; cytomegalovirus; rabies; respiratory syncytial virus; smallpox; vaccinia; varicella zoster; measles; mumps; rubella; parvovirus B19; Epstein–Barr virus; herpes simplex; tick-borne encephalitis; poliovirus; Hantavirus; West Nile virus; rotavirus; poliovirus; HIV-1; Ebola virus; and severe acute respiratory syndrome-associated coronavirus.

Unlike vaccines, passive immunization can rapidly deliver protective levels of antibodies directly to susceptible mucosal sites where many virus infections are initiated. Secretory IgA because of its polyvalency and relative stability may have advantages over IgG for passive immunization at these sites.

Antibodies function also as immunomodulators which can bridge innate and acquired, and cellular and humoral immune responses, respectively. Infected host cells can be targeted by linking anticellular toxins to antiviral antibodies or by bispecific antibodies in which one Fab fragment of the antibody is virus specific and the other one recognizes a host cell component or receptor.

Research on antibody-mediated immunity and understanding of antibody-based prophylaxis and therapies for virus diseases have provided a foundation for research on and development of antivirus vaccines.

Vaccines against Human Viral Diseases

Vaccination is the most successful medical intervention against viral diseases. Vaccines prevent or moderate illnesses caused by virus infection in an individual and prevent or diminish virus transmission to other susceptible persons, thus contributing to herd immunity. This effect is expected to be long term, depends on establishment of immunological memory at both the B- and T-cell levels, and may require consecutive or repeated vaccinations. The effectiveness of vaccination might be diminished or compromised for viruses occurring in the form of simultaneous quasi-species or undergoing time-dependent changes of antigenic properties (antigenic drift and antigenic shift). However, in some cases, vaccination predisposes to aggravated disease elicited by infection with a virus identical or related to that used for vaccination, that is, antibody enhancement of virus infection occurs (dengue and respiratory syncytial viruses and HIV-1).

The following categories of vaccines can be distinguished (**Table 2**): (1) live attenuated; (2) whole virus (inactivated); (3) glycoprotein subunit vaccines; and (4) protein vaccines based on recombinant DNA technologies. One vaccine in category (3), hepatitis B surface antigen (HBsAg), is derived from plasma of hepatitis B virus carriers. It is remarkable that vaccines in categories (3) and (4) correspond to multi-subunit self-assembled particles having antigenic specificities closely similar or identical to those expressed on the surface of virus particles. On the other hand, individual virus protein or glycoprotein subunit molecules or their peptide fragment have been less suitable candidates for vaccine development because of insufficient similarities with intact viruses.

Table 2 Past and present vaccines against human viral diseases

Live attenuated	*Killed whole virus*	*Glycoprotein subunit*	*Genetically engineered*
Influenza (nasal)	Influenza	Influenza	Papillomavirus
Rabies	Rabies	Hepatitis B	Hepatitis B
Poliovirus	Poliovirus		
Yellow fever	Japanese encephalitis		
Measles	Tick-borne encephalitis		
Mumps	Hepatitis A		
Rubella			
Adenovirus			
Varicella zoster			
Rotavirus			
Smallpox (vaccinia)			

Vaccine formulations require the incorporation of an immunological adjuvant to enhance their immunogenicity. Adjuvants are designed to optimize antigen delivery and presentation, enhance the maturation of antigen-presenting dendritic cells, and induce immunomodulatory cytokines.

Currently, most vaccines are administered parenterally using syringes with needles.The procedure is disliked by many, and is questionable for mass vaccination programs in developing countries. Therefore, efforts are being made to produce vaccines which can be delivered onto mucosal surfaces, that is, mostly orally or nasally. In addition, high-workload needle-free injection devices are being developed. At this juncture, the bifurcated needle, developed by Benjamin Rubin over 40 years ago, must be mentioned. It proved to be essential for the successful campaign to eradicate smallpox worldwide.

Veterinary Vaccines

The development and use of veterinary vaccines has the following aims: cost-effective prevention and control of virus diseases in animals; induce herd immunity; improve animal welfare and food production for human consumption; decrease the usage of veterinary drugs, thereby minimizing their environmental impact and food contamination; and decrease the incidence of zoonoses (e.g., infections by avian influenza, rabies, West Nile, Rift Valley fever viruses, respectively).

Research, development, and production of some veterinary vaccines have been on the forefront of the general field of vaccinology. The foot-and-mouth disease virus vaccine was the first one produced at an industrial scale (Frenkel method). A vaccinia-rabies virus G protein recombinant vaccine was among the first biotechnology-based vaccines licensed. The world's first DNA vaccine (against West Nile virus in horses) was approved by the US Department of Agriculture in July 2005. DIVA (Differentiating Infected from Vaccinated Animals; also termed marker) veterinary recombinant vaccines and companion diagnostic tests have been developed. They can be applied to programs to control and eradicate virus infections. These are examples to be considered for the development of human vaccines. The latter will require rigorous evaluations for safety and efficacy which are more difficult to obtain than in veterinary settings. A list of licensed veterinary vaccines is shown in **Table 3**.

Table 3 Current veterinary vaccines

Species	*Live*	*Killed*	*Recombinant*	*DNA*
Avian	Encephalomyelitis		Encephalomyelitis (fowl pox vector)	
	Influenza		Influenza (fowl pox vector)	
	Pneumovirus	Paramyxovirus Polyomavirus Reovirus		
	Bursal disease	Bursal disease	Bursal disease (Marek's disease vector)	
	Marek's disease		Marek's disease (Marek's disease vector)	
	Fowl pox		Fowl pox	
	Newcastle disease	Newcastle disease Bronchitis	Newcastle disease (fowl pox vector)	
	Anemia			
	Laryngotracheitis		Laryngotracheitis (fowl pox vector)	
	Duck enteritis			
	Duck hepatitis			
	Canary pox			
Feline	Calicivirus	Calicivirus immunodeficiency virus		
		Leukemia virus	Leukemia virus (canarypox vector)	
	Infectious peritonitis			
	Rhinotracheitis	Rhinotracheitis		
	Panleukopenia	Panleukopenia		
		Rabies	Rabies (canary pox vector)	
Canine	Adenovirus 2			
	Parvovirus	Parvovirus		
	Coronavirus	Coronavirus		
	Parainfluenza		Parainfluena (canary pox vector)	
	Canine distemper		Canine distemper (canary pox vector)	
	Measles			
	Hepatitis			
	Rabies	Rabies		
Sheep and goat	Bluetongue			
	Ovine ecthyma			
	Poxviruses			
		Louping ill		
Equine	Influenza	Influenza	Influenza (canary pox vector)	
	Rhinopneumonitis	Rhinopneumonitis Rotavirus		
	Arteritis			
	African horse sickness	African horse sickness Encephalomyelitis		
	West Nile virus Flavivirus chimera	West Nile virus	West Nile virus (canary pox vector)	West Nile virus
Bovine	Respiratory syncytial virus	Respiratory syncytial virus		
	Rhinotracheitis	Rhinotracheitis		
	Diarrhea	Diarrhea		
	Bronchitis	Bronchitis		
	Parainfluenza 3			
	Rotavirus	Rotavirus		
	Coronavirus	Coronavirus		
	Herpes 1	Herpes 1		
	Foot-and-mouth disease	Foot-and-mouth disease		
	Rinderpest			

Continued

Table 3 Continued

Species	*Live*	*Killed*	*Recombinant*	*DNA*
Porcine	Pseudorabies	Pseudorabies		
	Enterovirus	Influenza		
	Parvovirus	Circovirus		
	Rotavirus	Rotavirus		
	Transmissible gastroenteritis	Transmissible gastroenteritis		
	Reproductive and respiratory syndrome	Reproductive and respiratory Syndrome		
	Hog cholera	Hog cholera		

Several of the described vaccines are being administered as combination vaccines.

See also: AIDS: Vaccine Development; Antigen Presentation; Antigenic Variation; Antigenicity and Immunogenicity of Viral Proteins; Cytokines and Chemokines; Diagnostic Techniques: Serological and Molecular approaches; DNA Vaccines; Immune Response to viruses: Cell-Mediated Immunity; Neutralization of Infectivity; Vaccine Production in Plants; Vaccine Strategies.

Further Reading

Ahmed R (ed.) (2006) Immunological memory. *Immunological Reviews* 211, 5–337.

Burton DR (ed.) (2001) Antibodies in viral infection. *Current Topics in Microbiology and Immunology* 260, 1–300.

Casadevall A, Dadachova E, and Pirofsky LA (2004) Passive antibody therapy for infectious diseases. *Nature Reviews Microbiology* 2: 695–703.

Cooper MD and Alder N (2006) The evolution of adaptive immune systems. *Cell* 124: 815–822.

Frank SA (2002) *Immunology and Evolution of Infectious Disease.* Princeton: Princeton University Press.

Hangartner L, Zinkernagel RM, and Hengartner H (2006) Antiviral antibody responses: The two extremes of a wide spectrum. *Nature Reviews Immunology* 6: 231–243.

Janeway CA, Jr. (1993) How the immune system recognizes invaders. *Scientific American* (Sep.): 73–79.

Janeway CA, Jr., Travers P, Walport M, and Shlomchik MJ (2005) *Immunobiology*. *The Immune System in Health and Disease,* 6th edn. New York: Garland Science.

Levine MP, Kaper JB, Rappuoli R, Liu M, and Good MF (eds.) (2004) *New Generation Vaccines* 3rd edn. New York: Dekker.

O'Neill LA (2005) Immunity's early-warning system. *Scientific American* (Jan.): 38–45.

Paul WE (ed.) (2003) *Fundamental Immunology* 5th edn. Philadelphia: Lippincott Williams and Wilkins.

Plotkin SA and Orenstein WA (2004) *Vaccines,* 4th edn. Philadelphia: Saunders–Elsevier.

Pulendran B and Ahmed R (2006) Translating innate immunity into immunological memory: Implications for vaccine development. *Cell* 124: 849–863.

Van Regenmortel MHV (2002) Reductionism and the search for structure–function relationships in antibody molecules. *Journal of Molecular Recognition* 15: 240–247.

Weissman IL and Cooper MD (1993) How the immune system develops. *Scientific American* (Sep.): 65–71.

Woof JM and Mestecky J (2005) Mucosal immunoglobulins. *Immunological Reviews* 206: 64–82.

Immune Response to Viruses: Cell-Mediated Immunity

A J Zajac and L E Harrington, University of Alabama at Birmingham, Birmingham, AL, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Antigen presentation The process by which proteins are degraded into peptides that are loaded onto MHC molecules and these complexes are targeted to the cell surface.

Central-memory T cell A population of memory T cells which primarily reside in secondary lymphoid organs; characterized by the expression of CD62L and CCR7.

Chemokines Chemotactic cytokines which stimulate the migration of cells.

Cytokines Secreted proteins that regulate cellular actions by signaling via specific receptors.

Cytotoxic T lymphocyte (CTL) T cells which can kill virus-infected cells upon activation.

Effector T cell Cells capable of immediate functional activity resulting in pathogen removal.

Effector-memory T cell A population of memory T cells poised for immediate effector

function that primarily resides outside the lymphoid organs.
Immune homeostasis Maintenance of lymphocyte populations at steady-state levels.
Immunodominance The hierarchy of T-cell responses to the array of individual epitopes which are presented during any given viral infection.
Immunological memory The ability of the host to mount rapid recall responses upon re-exposure to the inducing antigen.
Immunopathology Tissue damage that results from the actions of the host's immune response.
Major histocompatibility complex (MHC) A cluster of genes involved in immune recognition and regulation; MHC class I molecules couple with β2 microglobulin to present peptides to CD8 T cells; MHC class II molecules present peptides to CD4 T cells.
T-cell exhaustion The progressive loss of antiviral T-cell functions which can culminate in the complete deletion of specific T-cell populations during chronic viral infections.
T-cell receptor (TCR) Heterodimeric receptor expressed by T cells that binds specific peptide–MHC complexes.
T helper 1 (Th1) cell Effector CD4 T-cell subset characterized by the production of IFN-γ; associated with immune responses to intracellular bacteria and viruses.
T helper 2 (Th2) cell Effector CD4 T-cell subset characterized by the secretion of IL-4, IL-5, and IL-13; important for helminth infections; linked to allergies and asthma.

General Overview

Cell-mediated immune responses play a critical role in combating viral infections. They are comprised of T-cell responses, which fundamentally differ from antibody (humoral) responses in the way they bring about infection control. The cardinal trait of cell-mediated responses is that the physical presence of reactive T cells is required for immunity, whereas humoral responses are conferred by the presence of soluble antibodies. T cells, together with B cells, form the adaptive immune response to viral infections. The hallmarks of adaptive immunity include antigen specificity and memory. These features allow T cells to elaborate responses which specifically target the numerous viruses which may infect the host. The ability to establish long-lived immunological memory provides a unique mechanism to better protect the host during subsequent viral exposures.

Due to their importance in controlling pathogens, cell-mediated immune responses are widely studied. Significantly, much of our understanding of cell-mediated immunity, including the fundamental concepts of major histocompatibility complex (MHC) restriction, tolerance, T-cell diversity, and immunological memory, has been determined by analyzing immune responses to viruses. Cell-mediated immune responses are dynamic, diverse, and display a broad range of phenotypic and functional properties.

T-Cell Recognition

T cells differ from antibodies (humoral responses) in the way they recognize viral antigens. Antibodies are capable of binding to intact viral proteins, including structural components of viral particles and also viral proteins present at the surface of infected cells. By being able to bind to conformationally complex structures, antiviral antibodies have the unique ability to neutralize the infectivity of viral particles present in the circulation or at mucosal surfaces, a function that cannot be performed by T cells. T cells cannot recognize intact viral proteins and therefore play no role in directly neutralizing whole viral particles. Instead, T cells recognize short peptide fragments presented at the cell surface in a noncovalent association with MHC molecules (see **Figure 1**). Thus, T-cell recognition is referred to as being MHC-restricted, since an individual T cell will only bind strongly to one particular MHC molecule, and is also peptide specific, as a T cell will predominately only recognize one specific short antigenic peptide.

T-Cell Receptors

CD4 and CD8 T cells express a unique surface receptor, the T-cell receptor (TCR) that determines the MHC restriction and peptide specificity of an individual T cell. In the vast majority of T cells, this is a heterodimeric receptor comprised of the TCR α- and β-chains. A smaller population of T cells (~5% of circulating T cells in humans) express an alternative form of the TCR, formed by the noncovalent association of TCR γ- and δ-chains; however, the roles of γδ T cells in controlling viral infections are not well defined. Each T cell expresses only one unique version of the TCR whose precise sequence and structure represents the end result of a series of gene rearrangements. This recombinatorial process, which occurs during T-cell ontogeny in the thymus, generates a massive repertoire of T cells with tremendous diversity between their individual TCR sequences. Estimates of the size of the T-cell repertoire suggest that 2.5×10^7 different TCRs are detectable in human blood. This large ensemble of

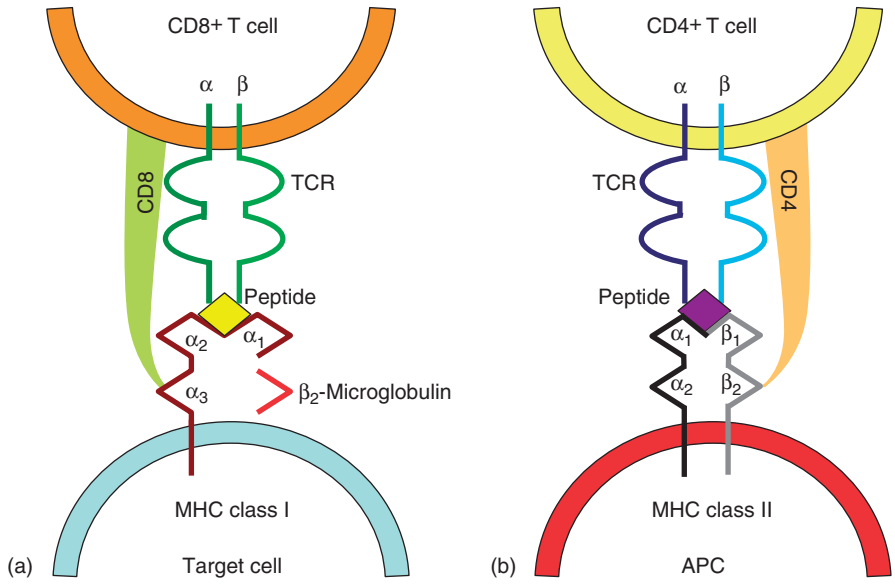


Figure 1 Similarities and differences between CD4 and CD8 T-cell recognition. Both CD4 and CD8 T cells express unique T-cell receptors at the cell surface which determine their antigen specificity and MHC restriction. (a) CD8 T cells recognize MHC class I molecules together with a non-covalently associated antigenic peptide, typically of 8–10 amino acids in length. MHC class I complexes are widely expressed and usually present endogenously synthesized antigens, including peptides derived from the degradation of viral proteins. (b) CD4 T cells recognize peptide antigens presented by MHC class II molecules. These antigenic peptides are typically 13–17 amino acids in length. These peptides are usually derived from extracellular antigens which have been endocytosed into professional antigen-presenting cells where they are proteolytically processed and re-presented at the cell surface bound to MHC class II molecules. MHC class II complexes have a much more limited tissue distribution than MHC class I molecules and are primarily expressed by macrophages, dendritic cells, and B cells.

individual T-cell clones is collectively capable of recognizing and responding to the vast array of antigens, including virally encoded antigens, which may be encountered during the lifespan of the host.

Antigen Processing and Presentation

CD8 T cells recognize MHC class I complexes (**Figure 1**). MHC class I molecules are expressed on virtually all cell types and usually present peptides derived from endogenously synthesized proteins. Antigen processing occurs continuously as newly synthesized proteins become degraded into peptide fragments by proteasomes. These fragments, typically of 8–10 amino acids in length, enter the endoplasmic reticulum and, if they have sufficient binding affinity, associate with MHC class I heavy chains together with the nonpolymorphic protein β2-microglobulin. These assembled MHC peptide complexes are then transported to the cell surface. This process allows MHC class I molecules to sample peptide fragments derived from proteins which are produced within the cell, including normal cellular proteins as well as virally encoded proteins, and present them for inspection by CD8 T cells. This ongoing immunological surveillance allows CD8 T cells to detect, respond to, and remove host cells which express 'non-self' viral proteins.

Although the endogenous pathway of antigen presentation provides a valuable mechanism for revealing the presence of virally infected cells to CD8 T cells, an alternative 'cross-presentation' pathway also operates. During cross-presentation, viral particles (or other antigens) are endocytosed by professional antigen-presenting cells and then undergo proteolytic degradation. The resulting peptide fragments can then bind to MHC class I molecules, and traffic to the cell surface. This enables antigen-presenting cells, such as dendritic cells, to display virally derived peptides to CD8 T cells even if the antigen-presenting cell itself is not capable of supporting virus replication.

CD4 T cells differ from CD8 T cells as they recognize peptides presented by MHC class II rather than MHC class I complexes (**Figure 1**). Unlike MHC class I complexes, which are ubiquitously expressed, MHC class II molecules are only presented by certain cell types such as dendritic cells, macrophages, and B cells. This limits CD4 T-cell recognition to professional antigen-presenting cells, since it is these specialized cells that have the capacity to display peptide–MHC class II complexes to CD4 T cells. Due to structural differences in the peptide-binding groove of MHC class I and MHC class II complexes, the viral peptides that are presented to CD4 T cells are typically longer (~13–17 amino acids in length) compared to those displayed by MHC class I molecules. In addition, whereas MHC class I complexes primarily present endogenously synthesized antigens, MHC class II molecules usually present antigens derived from

extracellular sources. These exogenous antigens can include viral particles and also remnants of virally infected cells. Following uptake by professional antigen-presenting cells, these antigens are degraded into peptide fragments within acidified endosomes. Alternatively, if the antigen-presenting cell is actively infected with the virus, then intracellular vesicles containing viral proteins can serve as a source of peptides for associating with MHC class II complexes. Once at the cell surface these presented antigens can be detected by, and activate, CD4 T cells which express TCRs that are capable of specifically recognizing the peptide–MHC class II combination.

Immunodominance

Individual viruses encode multiple potential T-cell epitopes; therefore T-cell responses elicited during viral infections are not monoclonal or monospecific. Instead, oligoclonal subsets of cells are induced and, although each individual T cell is responsive to only one particular peptide–MHC combination, the overall pool of cells is sufficiently diverse to ensure that numerous epitopes can be detected. The kinetics, magnitudes, phenotypic and functional traits, as well as the stability of T responses to each individual virally encoded epitope can differ. Consequently, an ordered hierarchy can emerge as certain epitopes elicit more abundant, or immunodominant responses, whereas others are less prevalent and give rise to subdominant responses (**Figure 2**).

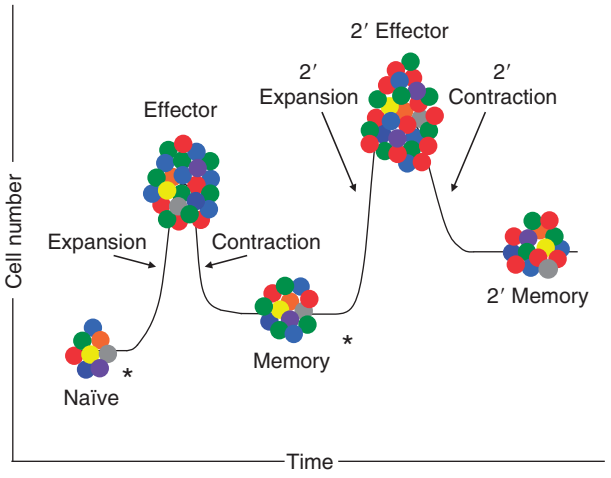


Figure 2 Changes in T-cell immunodominance can occur during primary and secondary immune responses following acute viral infections. Viruses elicit T-cell responses to a range of individual viral epitopes. These responses are not necessarily equal and in the example depicted the T cells specific for the 'green' epitope are immunodominant following the primary infection. This pattern of immunodominance is maintained during the memory phase but secondary exposure to the virus results in an anamnestic recall response during which the 'red' epitope-specific T cells predominate. Asterisk indicates when exposure to the virus occurred.

Our understanding of the precise determinants of immunodominance is incomplete; however, the hierarchy of T-cell responses is likely to be shaped by many factors. The magnitude of responses to individual viral epitopes is influenced by properties of the host's T cells including precursor frequencies and the avidity of the T cells for the presented viral antigen, as well as viral related factors including the ability of viral peptides to bind MHC complexes, the abundance of presented antigen, the kinetics of viral protein synthesis, and the types of cells which present the viral antigens. Changes in the patterns of immunodominance have been reported most notably during the course of persistent viral infections, as well as following secondary exposures to viruses which have been previously controlled (**Figure 2**). In the case of lymphocytic choriomeningitis virus (LCMV) infection of C57BL/6 mice, the NP396 epitope is co-dominant following well-controlled acute infections, and CD8 T cells specific for this epitope respond most vigorously during secondary exposures to LCMV. By contrast, during persistent LCMV infections, responses to this usually dominant epitope can become completely undetectable. During Epstein–Barr virus (EBV) infections shifting patterns of immunodominance are observed as responses to immediate early and early viral proteins are initially detected, but as viral latency becomes established responses to lytic cycle proteins decline and responses to latent viral proteins predominate. These observations demonstrate that not all antiviral T cells respond equally and suggest that certain specificities of T cells may be more effective at combating particular viral infections.

Induction of Cell-Mediated Immunity during Viral Infections

As a virus infection becomes established in the host, a series of molecular and cellular signals are initiated which activate cell-mediated immune responses. These signals include the production of interferons, other cytokines, and inflammatory mediators, in addition to the mobilization of local dendritic cells. Dendritic cells are thought to provide a critical cellular link for priming naive CD4 and CD8 T cells (**Figure 3**). It has been proposed that these cells are especially prone to infection by viruses which facilitate their role as cellular sensors for signaling the occurrence of an infection. Even if dendritic cells are not permissive to active infection with particular viruses, they can also present antigens through cross-priming to CD8 T cells, as well as to CD4 T cells via the classical exogenous antigen processing pathway.

The primary activation events which induce cell-mediated immunity predominately occur in secondary lymphoid organs including regional lymph nodes and the

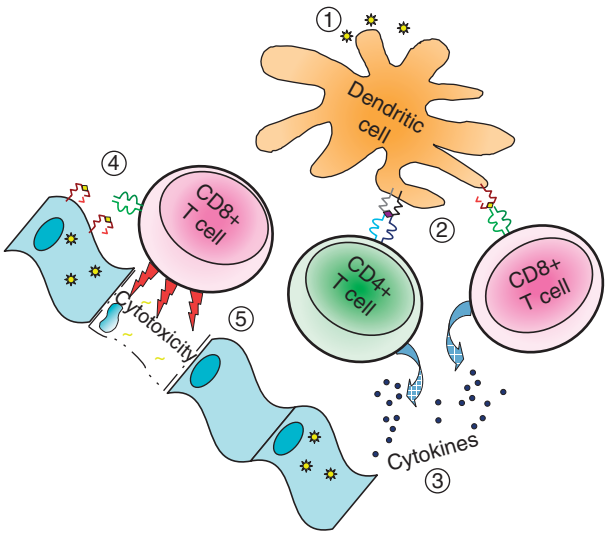


Figure 3 The induction and function of cell-mediated immune responses. (1) The presentation of viral antigens by professional antigen-presenting cells is a critical step in initiating cell-mediated immune responses. (2) Recognition of cognate peptide–MHC complexes by CD4 and CD8 T cells stimulates their proliferation and differentiation into effector cells. (3) Antigen-activated effector CD4 and CD8 T cells can express cytokines such as IL-2, TNF-α, and IFN-γ. These cytokines have important roles in coordinating the antiviral immune response and can also have direct antiviral effects. (4) Once activated antiviral T cells disperse into tissues where can they respond locally, at the sites of viral infection. (5) If viral peptide–MHC complexes are recognized, then effector CD8 T cells can elaborate cytotoxic effector functions which kill the infected cells. CD4 T cells can also become cytotoxic during certain infections; however, their impact is less promiscuous as they recognize the MHC class II complexes which are only expressed by professional antigen-presenting cells.

spleen. During the early stages of viral infections dendritic cells residing at the initial sites of infection take up viral antigens, become activated, and migrate to regional lymph nodes. Within the lymph nodes these dendritic cells encounter naïve T cells which are circulating through these organs as part of their normal immunosurveillance protocol. Engagement of TCRs on the naive T cells with viral–peptide MHC complexes presented by the dendritic cells results in sequestration of the T cells and launches the antiviral T-cell response (**Figure 2**). The ensuing proliferation and differentiation of virus-specific T cells also occur in conjunction with inflammatory mediators such as interferons and other danger signals. Many parameters, including the duration and strength of antigenic stimulation, co-stimulatory interactions, the presence of cytokines, and the provision of CD4 T cells help guide the developing response. These early events play a critical role in driving the generation of both the effector T cells as well as the subsequent establishment of the memory T cell pool.

CD8 T Cells

One of the most impressive aspects of CD8 T-cell responses is the massive proliferation of these cells which occurs during the initial phase of many viral infections (**Figures 2** and **4**). Experimental studies of acute LCMV infection of mice have demonstrated that antiviral CD8 T cells can increase over 10 000-fold during the first week of infection; over 50%, and perhaps even more, of the host's CD8 T cells are LCMV-specific at the peak of the response! Marked expansions of virus-specific CD8 T cells are a common feature of many virus infections including influenza, vaccinia virus, EBV, yellow fever virus, and early following human immunodeficiency virus (HIV) infection. During this expansion phase the patterns of gene expression change promoting the synthesis of cytokines and cytotoxic effector molecules as well as alterations in surface molecules including cytokine receptors and adhesion molecules. This results in an expanded pool of virus-specific effector cells with functional attributes necessary to control the infection. The ensemble of virus-specific effector cells which emerge during the acute phase of the infection is remarkably heterogeneous and comprises of subsets which differ in their epitope specificity, clonal abundance, effector potential, expression of adhesion molecules and cytokine receptors, and ultimate fate. Although the initial activation of T-cell responses occurs in secondary lymphoid organs, the effector cells become dispersed throughout the host. In this way the T cells are available locally, at the sites of infection, where they operate to eliminate the host of virally infected cells.

CD8 T cells are potent antiviral effector cells due to their ability to produce both inflammatory mediators as well as cytotoxic effector molecules (**Figure 3**). CD8 T cells are commonly referred to as cytotoxic T lymphocytes (CTLs), which emphasizes their ability to kill virally infected target cells. These killing functions are triggered as the effector T cell become activated following engagement with a virally infected target cell displaying an appropriate peptide–MHC complex. The subsequent release of perforin and granzyme molecules by the T cells ensures the swift destruction of the infected cell. Ideally, this targeted removal of the infected cell occurs before progeny virus is released. Alternative Fas and TNF-dependent cytotoxic mechanisms have been reported but their *in vivo* significance in killing virus infected cells is not well defined. In addition to their direct killing functions, CD8 T cells also produce a range of cytokines and chemokines. In the laboratory the production of these effector molecules is often used to detect the presence of antiviral T cells. Most importantly, within the infected host the production of these soluble mediators, such as IFN-γ and TNF-α, can also help clear viral infections without causing death

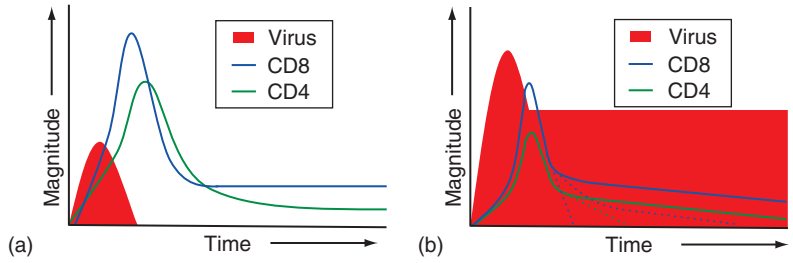


Figure 4 Successful and unsuccessful T-cell responses during acute and chronic viral infections. (a) During acute viral infections massive T-cell responses can be induced which play a principal role in clearing the infection. Following the resolution of the infection, the responding T-cell pool is downregulated but a long-lived pool of memory T cells becomes established which helps protect against subsequent viral exposures. (b) During chronic viral infections T-cell responses are elicited but a variety of phenotypic and functional defects manifest as these responses succumb to exhaustion. A gradation of exhausted phenotypes is often observed, ranging from an inability to produce effector cytokines to the complete deletion of virus-specific T cells.

of infected cells. This cytokine-mediated purging of infected cells has been most convincingly shown during viral hepatitis.

CD4 T Cells

CD4 T cells are traditionally known as helper T cells because of their ability to provide help to B cells and CD8 T cells, resulting in antibody production, class switching, cytotoxic T cell activity, and memory development. In addition to assisting cells of the adaptive immune system, CD4 T cells produce an array of cytokines and chemokines that stimulate cells of the innate immune system, such as macrophages and neutrophils, to traffic to the sites of infection and elaborate their effector activities. It has also been demonstrated that CD4 T cells are directly capable of antiviral functions, through the production of IFN-γ and, in some circumstances, by inducing lysis of virally infected cells (**Figure 3**). Thus, CD4 T cells are critical constituents of the cell-mediated immune response to viral infections; however, it should be emphasized that innate immunity as well as humoral immune responses, which are helped by CD4 T cells, are key components of the host overall antiviral response.

Like CD8 T cells, naive CD4 T cells circulate through secondary lymphoid organs in a relatively quiescent state. Following recognition of antigen in the context of MHC class II, a cascade of signaling events is initiated within the CD4 T cell which results in activation, proliferation, and differentiation into an effector CD4 T cell (**Figure 3**). Classically effector CD4 T cells have been divided into two polarized subsets based on their cytokine production profile. T helper 1 (Th1) cells primarily produce IFN-γ and are critical for the immune responses to various viral infections, as well as infections with intracellular bacteria. This subclass of effector cells is typically associated with antiviral cell-mediated immunity. Conversely, T helper 2 (Th2) cells predominantly secrete the cytokines IL-4, IL-5, and IL-13, assist with the eradication of helminth infections, and have historically been linked with the production of antibodies and humoral immune responses.

In recent years, the definition of CD4 T-cell subsets has expanded beyond Th1 and Th2 cells, with the importance of unique populations of regulatory CD4 T cells and also IL-17 producing 'Th17' cells becoming evident. Regulatory cells are pivotal for preventing autoimmunity by suppressing the activation of autoreactive T cells. Regulatory T cells are typically characterized by the expression of the transcription factor Foxp3 and by the production of the suppressive cytokines IL-10 and TGF-β. Relatively little is known regarding the significance of these CD4 T-cell populations during viral infections; however it has been proposed that regulatory T cells are both beneficial to the host, by limiting immunopathology, and detrimental, by dampening effector functions.

T-Cell Memory

Ideally, the primary immune response overwhelms the infection and results in the complete eradication of the virus from the host. If the infection is successfully resolved then the expanded pool of effector T cells does not remain constitutively activated. Instead, a downregulation phase ensues during which typically the majority (<90%) of the virus-specific T cells present at the peak of the immune response die by apoptosis. The remaining 5–10% of T cells survive the contraction phase and constitute a long-lived pool of memory T cells (**Figures 2** and **4**). In this way, a beneficial memory of past infections is established as, by comparison with naïve hosts, an increased number of virus-specific T cells are maintained which are tuned to rapidly respond if they re-encounter infected cells. The population of memory T cells which emerges following the resolution of the infection and restoration of homeostasis is not uniform, as phenotypic and functional

diversity is apparent even within subsets of memory T cells which recognize the same viral epitope. This is well illustrated by the classification of memory T cells into broad categories termed effector- and central-memory T cells. These subsets have been defined based upon their anatomical location, functional quality, proliferative potential, and expression of surface molecules.

Since T cells neither recognize nor neutralize the infectivity of viral particles they do not confer sterilizing immunity and cannot prevent secondary infections. Nevertheless, as the host cells become infected, preexisting memory T cells which developed following prior viral exposures can mount robust recall responses. These anamnestic responses are characteristically more rapidly induced, greater in magnitude, and possibly more functionally competent than primary T-cell responses. Such pronounced secondary responses help protect the host by contributing to the swift control of the infection thereby reducing the morbidity and mortality. Memory T-cell responses are not the only components of secondary immune responses as these cells act in conjunction with antiviral antibodies to protect the host during viral reexposures.

Analysis of both clinical specimens and experimental animal models has demonstrated that acute viral infections can induce very long-lived memory T-cell responses (**Figure 4**). Studies using experimental mice have demonstrated that memory CD8 T cells reactive against various infections such as LCMV, vaccinia virus, and influenza are maintained at remarkably stable levels for over 2 years following infection. By contrast, CD4 T-cell responses are not as consistent and have been reported to gradually decay over time. Although natural exposures to viruses lead to the formation of immunological memory, these advantageous responses are also induced following vaccinations. Vaccines are successful in protecting the host against subsequent infections because of their ability to promote long-lived memory responses. In humans the longevity of T-cell responses has been investigated in detail following smallpox vaccination. Smallpox-specific T-cell responses are detectable in individuals who received a single dose of the vaccine 75 years previously! Notably, the findings suggested that the responses do decline slowly with predicted half-lives of 8–15 years.

Persistent Viral Infections

Although T-cell responses can be highly effective at controlling acute infections and contribute to protective secondary responses, persistent viral infections do arise and are often associated with the development of phenotypically and functionally inferior responses (**Figure 4**). These types of infections include many viral pathogens which are of significant public health importance such as HIV and hepatitis C virus (HCV). A common feature of these infections is that T-cell responses are initially induced but qualitative and quantitative defects become apparent as the generation of robust sets of effector cells, as well as the progression of memory T-cell development are subverted. By comparision with successful T-cell responses elaborated during acute viral infections, a spectrum of phenotypic and functional defects have been detected during persistent infections. The production of cytokines including IL-2, TNF-α, and IFN-γ, as well as cytotoxic effector molecules such as perforin may be diminished or abolished, and decreased proliferative potential has also been observed. The severe loss of effector activity as well as the physical deletion of antiviral T cells which can occur during persistent infections has been termed exhaustion (**Figure 4**).

The parameters which contribute to T-cell exhaustion are not fully understood. Comparative analysis of T-cell responses to viral infections which result in different levels of antigenic exposure, such as influenza, cytomegalovirus, EBV, HCV, and HIV, indicate that antiviral T cells may adopt different preferred phenotypic and functional set points. Experimental studies suggest that many factors including, but not limited to, viral targeting and destruction of dendritic cells, the production of immunosuppressive cytokines such as IL-10, the depletion of CD4 T cell subsets, and the induction of weak neutralizing antibody responses can all contribute inferior cell-mediated immune responses. Changing viral loads may also impact the functional quality of the T-cell response. During acute HCV infection antiviral CD8 T cells transiently lose the ability to produce IFN-γ, but recover from this 'stunned' state as the viral loads are brought under control. Importantly, this suggests that under certain conditions the exhaustion of virus-specific T cells may be prevented or even reversed. Several reports have now demonstrated that during persistent LCMV, HIV, and HCV infections, antiviral T cells can express the inhibitory receptor PD-1. Antibody-based therapeutic treatments to block this receptor have been shown to promote proliferation of previously exhausted T cells and restore their functional activities. This is a promising experimental observation; however, the jury is still out on whether this approach will be a beneficial treatment for persistent infections of humans.

Immunopathology

Since viruses are obligate intracellular pathogens they must infect permissive host cells in order to replicate. Infected cells die as a direct result of the virus' lytic replication cycle or are killed as a consequence of the

actions of the antiviral immune response. Although immune-mediated destruction of virally infected cells is necessary to contain the infection, it can also result in immunopathology, which represents collateral damage to the host caused by the actions of the immune response. A classical example of immunopathology occurs following intracranial infection of adult mice with LCMV. Mice infected by this route succumb to a characteristic lethal disease and expire approximately 1 week following infection. Death can be prevented by immunosuppression of the mice, which has shown that the disease is a consequence of a vigorous CD8 T-cell response rather than due to the infection *per se*. HBV-associated viral hepatitis is another instance where anti-viral CD8 T-cell responses, which are attempting to clear the infection, cause liver damage in the infected individual.

Most viral infections are associated with the development of an IFN-γ-producing Th1-CD4 T cell response. In various animal models, the absence of CD4 T cell help during viral infection results in impaired clearance of the infectious agent. However, not all CD4 T-cell responses are beneficial as the induction of inappropriate types of CD4 T-cell responses can be deleterious to the host, due to immunopathology. In the 1960s, a group of young children were administered a formalin-inactivated vaccine for respiratory syncytial (RS) virus and following exposure to live RS virus, these children exhibited enhanced infection rates and immunopathology linked to increased frequencies of eosinophils and neutrophils within the airways. Animal studies have indicated that the vaccine was associated with a Th2-biased virus-specific immune response (increased levels of IL-4 and IL-13, as well as eosinophil recruitment to the lungs) that upon live infection displayed many of the signatures of immunopathology which manifested in these vaccinated children. Supporting experiments suggest that immunization to promote the Th1 responses or ablation of Th2 responses prevents the development of these pathological effects following live viral infection.

Immune Evasion

Arguably, one of best indications of the importance of cell-mediated immunity in controlling viral infections is the observation that many viruses have evolved strategies to evade the actions of the host immune response. There is, however, no one universal evasion mechanism; instead, viruses have adopted various preferred approaches to escape antiviral T-cell responses. A common strategy is to change the amino acid sequence of T-cell epitopes or nearby flanking residues that impede the recognition or processing of the antigenic peptide. Many viruses rely on error-prone polymerases in order to replicate, which favor the incorporation of mutations in progeny viral genomes. The resulting variant viruses will have a selective advantage if the amino acid substitution abolishes the ability of the epitope to associate with MHC molecules, negatively impacts recognition of the epitope by T cells, or prevents antigen processing.

In addition to mutating epitope sequences, many viruses encode specific molecules which function to interfere with the antiviral immune response. Both MHC class I and class II antigen-presenting pathways are targeted by several viral proteins. These immune evasion molecules block antigen presentation in various ways, ranging from preventing the transport of antigenic peptides into the endoplasmic reticulum to inhibiting the egress of peptide-loaded MHC complexes. The end result of these inhibitory strategies is to impair immunological surveillance. Although there is much anecdotal evidence that interference with antigen processing diminishes cell-mediated immune responses, experimental studies using murine cytomegalovirus (MCMV) question this notion. Infection of mice with mutants which lack several viral genes known to block antigen processing did not effect the ability of the host to elaborate an anti-MCMV CD8 T-cell response.

See also: Antigen Presentation; Antigenic Variation; Antigenicity and Immunogenicity of Viral Proteins; Cytokines and Chemokines; Immune Response to viruses: Antibody-Mediated Immunity; Immunopathology; Innate Immunity: Defeating; Innate Immunity: Introduction; Persistent and Latent Viral Infection; Vaccine Strategies; Viral Pathogenesis.

Further Reading

Alcami A and Koszinowski UH (2000) Viral mechanisms of immune evasion. *Immunology Today* 9: 447–445.

Doherty PC and Christensen JP (2000) Accessing complexity: The dynamics of virus-specific T cell responses. *Annual Review of Immunology* 18: 561–592.

Ertl HC (2003) Viral immunology. In: Paul WE (ed.) *Fundamental Immunology,* 5th edn., pp. 1021–1227. Philadelphia, PA: Lippincott Williams and Wilkins.

Finlay BB and McFadden G (2006) Anti-immunology: Evasion of the host immune system by bacterial and viral pathogens. *Cell* 124: 767–782.

Frelinger JA (2006) *Immunodominance – The Choice of the Immune System.* Weinheim, Germany: Wiley-VCH.

Klenerman P and Hill A (2005) T cell and viral persistence: Lessons from diverse infections. *Nature Immunology* 6: 873–879.

Oldstone MBA (2006) Viral persistence: Parameters, mechanisms and future predictions. *Virology* 344: 111–118.

Seder RA and Ahmed R (2003) Similarities and differences in CD4+ and CD8+ effector and memory T cell generation. *Nature Immunology* 4: 835–842.

Yewdell JW and Bennink JR (1999) Immunodominance in major histocompatibilty complex class I-restricted T lymphocyte responses. *Annual Review of Immunology* 17: 51–88.

Zinkernagel RM and Doherty PC (1997) The discovery of MHC restriction. *Immunology Today* 18: 14–17.

Immunopathology

M B A Oldstone, The Scripps Research Institute, La Jolla, CA, USA
R S Fujinami, University of Utah School of Medicine, Salt Lake City, UT, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Adaptive immune system Comprises T cells, B cells, and antibodies. Elements of the innate immune system are required for initiation of the adaptive immune response. The adaptive immune system recognizes specific antigenic epitopes. Immunopathology is mainly mediated by the adaptive immune response to viral infection.
Antibody Antibodies are secreted by plasma cells and can neutralize viruses, bind to the surface of infected cells, activate the complement system, and form immune complexes. Antibodies are an important part of the adaptive immune response.
B cell Each B cell has a specific type of antibody molecule on its surface (B cell receptor). Viral proteins are recognized by B cell receptors. B cells mature into plasma cells that secrete large amounts of antibodies.
Complement A protein cascade system mainly found in serum. The cascade can be activated by immune complexes of viral proteins and antibodies. The protein cascade generates chemotactic peptides for phagocytic cells, proteins that can coat viral particles, and lyse viral membranes.
Cytokines Proteins that can be produced by various cells within the body. These proteins are produced in response to infection. Particularly, immune cells but other cell types have cytokine receptors. Cytokines mediate inflammation and can modulate the immune response through cytokine receptors.
Immune complex A complex between antibody molecules and antigens. These complexes can activate the complement system through the Fc receptor on the antibody molecules and can lodge in glomeruli and small vessels causing immunopathology.
Immunopathology Disease resulting from the immune response to infection causing tissue injury and damage.
Innate immune system Comprising phagocytic cells (macrophages and neutrophils), complement system, natural killer (NK) cells, and various cytokines that can act within minutes to hours after infection. Recognition is to patterns of microbial structures, not specific epitopes.
Major histocompatibility complex (MHC) A region encoding highly polymorphic proteins that are involved in immune recognition of T cells, some cytokines and proteins of the complement cascade. This region is coded for by genes on human chromosome 6 and on mouse chromosome 17.
Natural killer (NK) cells A population of lymphocytes that can kill virus-infected cells. NK cells can secrete large amounts of the antiviral cytokine IFN-γ. IFN-γ can also activate macrophages. NK cells are part of the innate immune system.
T cell T cells are mature in the thymus. These lymphocytes recognize viral peptides in the context of MHC molecules through the T-cell receptor on the surface of T cells. T cells can kill virus-infected cells and release potent antiviral cytokines such as interferons.

Introduction

When a virus infects a vertebrate host, an immune response is rapidly generated against the virus. This immune response will often determine the survival or death of the host in acute infections or the establishment of a persistent infection. Tissue damage or pathology can result due to the antiviral immune response. This is different from direct viral effects where virus infection of cells can lead to tissue damage and apoptosis. A vigorous antiviral immune response is responsible for the immunopathology associated with acute and persistent/chronic virus infections. There is a necessary balance between viral clearance by the immune system and pathology induced by the immune system in attempting to clear the virus (**Figure 1**).

One important component of the adaptive immune system is the lymphocyte. Due to specialized receptors on the surface of lymphocytes (T cell receptor (TCR), B cell receptor (BCR)/antibody molecule), these cells are able to recognize or discriminate between different antigenic peptides derived from microbes or self-proteins. Lymphocytes can be divided into several broad populations. These cells can act directly to lyse or kill virus-infected cells (discussed below) or produce antiviral substances such as cytokines (interferons (IFNs), tumor necrosis factor (TNF)) that have antiviral activity but also can disturb the host's cell function or by themselves cause cellular and tissue injury. Lymphocytes in collaboration with cells of the innate

immune system can also kill virus-infected cells or secrete antibodies with antiviral neutralizing capacity (discussed below).

One population of lymphocytes are the B cells which are bursa or bone marrow-derived lymphocytes. These lymphocytes express BCRs on their surfaces called immunoglobulins or antibodies (**Figure 2**). The antibodies are secreted from differentiated B cells called plasma cells. Different antibodies can bind to specific viral proteins or antigens; but in general, one specific antibody is originally derived from one B cell and can recognize a unique epitope. Antibodies can neutralize viruses by attaching to the surface of the virion, thus inhibiting the attachment, penetration, or uncoating phases of viral replication (**Figure 2**, arrow 1). Antibodies can also bind to virions in the blood (viremia) or to viral proteins (such as hepatitis B virus surface antigen), and form antigen–antibody complexes (immune complexes) (**Figure 2**, arrow 2). If the complexes are of the appropriate size, glomerulonephritis can occur due to immune complex deposition (discussed below). Immune complexes are also engulfed by Fc receptor bearing cells of the innate immune system found in the liver and spleen. However, since immune complexes are often infectious, by this means, Fc or complement expressing cells can also be infected. However, this process aids in clearance of virus from the blood, thereby limiting the viremia. Infected cells can be killed by antibodies specific for viral proteins expressed on the surface of virus-infected cells. This requires the presence of complement proteins. Complement proteins binding to the Fc portion of the immunoglobulin (**Figure 2**, arrow 3) activate the complement cascade leading to the formation of the complement membrane attack complex on the cell membrane and eventually cell lysis. Antibodies via the Fc portion of the immunoglobulin molecule can bind to Fc receptor positive cells (macrophages and NK cells – two cell types of the innate immune system) (**Figure 2**, arrows 4 and 5). The Fab portion of the immunoglobulin (antigen recognition site) gives specificity for the killing of the virus-infected cells. Therefore, antibodies can participate in clearing/neutralizing circulating free virus, as well as

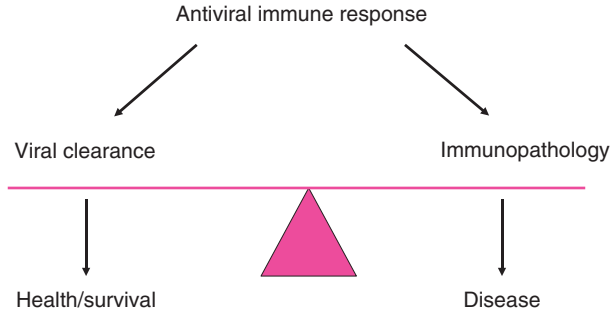


Figure 1 The antiviral immune response is a balance between viral clearance and immunopathology. One direction leads to survival of the host, whereas the other can lead to disease.

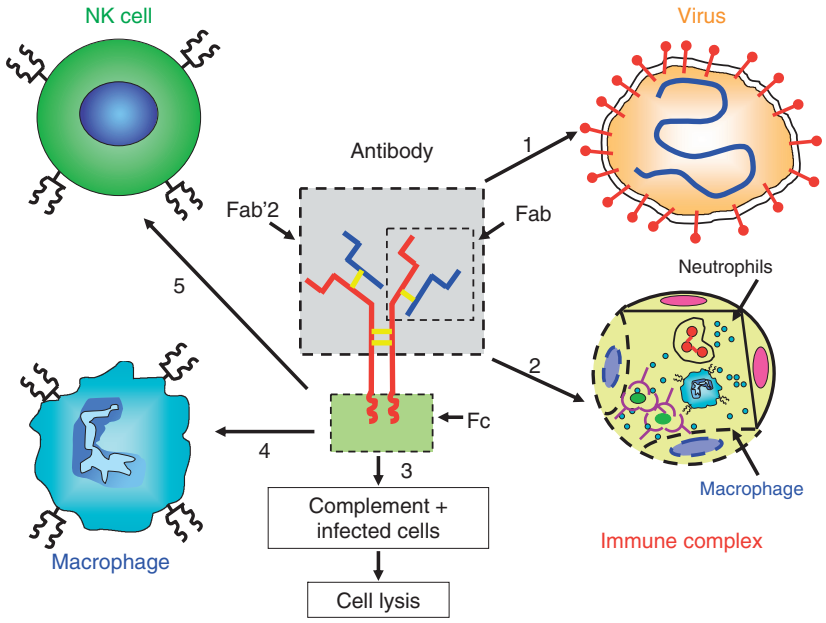


Figure 2 Antiviral antibodies can play an important role in viral clearance and/or disease. The Fab portion of the antibody molecule can bind and neutralize the virus (arrow 1). Antibodies binding to viral proteins or virions can form immune complexes that can lead to immune complex disease (arrow 2). Antibody binding to virus-infected cells through the Fc portion of the antibody molecule can activate the complement system leading to lysis of the infected cell (arrow 3). The Fc portion of the antibody molecule can also bind to Fc receptors in macrophages and NK cells. The antibody-coated macrophages and NK cells can kill infected cells via antibody-dependent cell-mediated cytotoxicity (arrows 4 and 5).

killing virus-infected cells with the help of complement and cells of the innate immune system. However, the number of antibody molecules required to kill a cell is usually in excess of 1×10^6, which makes this an inefficient process.

T cells represent another population of lymphocytes that participates in viral clearance and immunopathology. T cells can be further divided into two subpopulations, $CD4^+$ and $CD8^+$ T cells (**Figure 3**). T cells with the surface molecule CD4 are called T-helper cells (Th) due to their ability to secrete certain cytokines that 'help' B cells to differentiate into antibody producing plasma cells and 'help' the subpopulation of T cells expressing CD8 molecules to acquire cytotoxic or killer cell activity. $CD4^+$ T cells are 'restricted' by major histocompatibility complex (MHC) class II molecules. The MHC class II molecule presents viral peptides as a complex that is recognized by TCRs on $CD4^+$ T cells. The $CD4^+$ T cells can be further subdivided, depending on the types of cytokines they secrete, into $CD4^+$ Th1 T cells that are involved in delayed-type hypersensitivity immune responses and $CD4^+$ Th2 T cells that help B cells. When activated $CD4^+$ Th1 T cells secrete IFN-γ that has antiviral activity and can also in turn activate macrophages. These activated macrophages, cells of the innate immune system, can kill virus-infected cells indirectly by releasing reactive oxygen species and toxic cytokines, such as TNF or lymphotoxin (LT) that lyse cells by what is known as bystander killing. The $CD4^+$ T-cell population is also involved in the maintenance of antiviral T-cell memory. T-cell memory is key for immune individuals to respond to subsequent infections with the same or similar viruses. The $CD4^+$ T-cell population also contains a subset of T cells that also express a surface protein, CD25, and an internal protein called FoxP3. These T cells have the ability to modulate the adaptive immune response, and therefore are known as T-regulatory cells.

The $CD8^+$ T-cell population is intimately involved in viral clearance and immunopathology. $CD8^+$ T cells kill virus-infected cells by two mechanisms and are very efficient usually requiring recognition of ten or fewer viral peptide molecules in association with the MHC class I complex (see below). The first mechanism is by release of perforin molecules that form pores in the target cell's membrane similar to the complement membrane attack complex. The second mechanism involves the interaction of FasL-Fas on the T cell and the infected cell, respectively. Fas is a member of the TNF receptor family. Fas binding to its ligand leads to activation of caspase 8, a death signal in the infected cells that results in apoptosis. The cytotoxic T-lymphocyte (CTL) killing of virus infected cells occurs when infected cells are killed in a MHC class I restricted manner (TCR on $CD8^+$ T cells recognizing viral peptide complexed to class I molecules) prior to the maturation of infectious virus thus reducing the virus' ability to replicate and disseminate within the body. This supplements the action of antiviral antibodies that can neutralize infectious virus. Memory $CD8^+$ T cells form during the antiviral immune response and are long-lived T cells that can protect animals in the event of subsequent infections with the same virus.

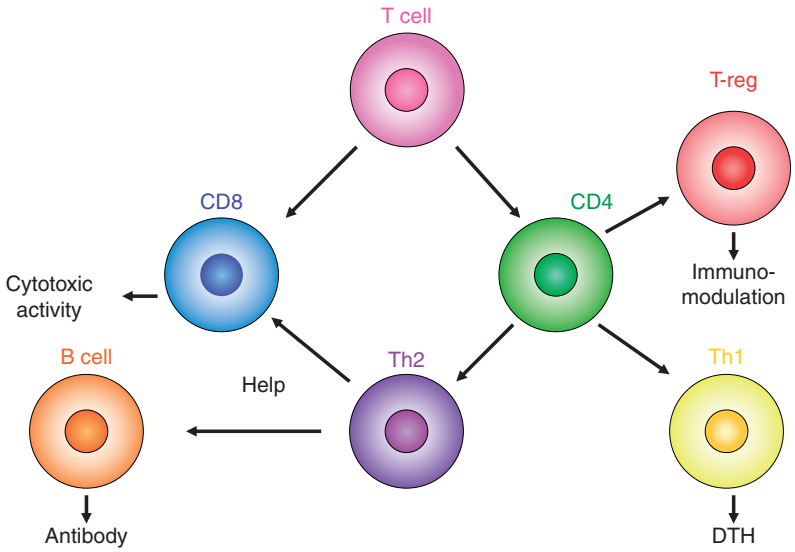


Figure 3 T cells can be divided into different subpopulations depending on their function. $CD8^+$ T cells have the ability to kill virus-infected cells and are restricted by MHC class I molecules. $CD4^+$ T cells can be further subdivided. Some $CD4^+$ T cells have the ability to provide 'help' to other T cells and B cells. Others can act as regulatory cells modulating the function of other T cells. $CD4^+$ T cells can also be directly involved in inflammatory responses through the production of various pro-inflammatory cytokines.

Thus B cells, $CD4^+$ and $CD8^+$ T cells, in conjunction with complement and cells (NK, macrophage, neutrophil) of the innate immune system, are responsible for viral clearance. These are also the cellular participants that are involved in immunopathology associated with virus infections.

Immunopathology

Antiviral immune responses are responsible for the immunopathology associated with acute and persistent/chronic virus infection. Immunopathology is determined by several factors. These involve contributions from both the host and the virus. Host factors include the age of the individual, genetic makeup of the host (MHC and non-MHC genes), route and/or site of infection, and whether the immune system is intact or immunocompromised. Characteristics of the virus consist of the strain or type and the size of the inoculum/dose.

Age of the host can play an important role in determining whether an individual survives, becomes immune, or acquires a persistent infection. For example, infection with lymphocytic choriomeningitis virus (LCMV), an arenavirus, has different outcomes depending on the age of the host. The natural host of the virus is the mouse. Its genome is comprised of two segments of RNA and is ambisense. The two RNA segments are encased by a nucleocapsid protein forming the nucleocapsid that is surrounded by a lipid envelope embedded with two viral glycoproteins. For the most part LCMV is noncytolytic and does not induce cell death after infection. Neonatal infection, infection of newborn mice with LCMV, or of adult mice with immunosuppressive variant(s) results in a persistent infection where many tissues are infected and mice survive. At this early age, the mouse's immune system is not fully developed and the mouse is not able to mount a functional CTL response. It was first proposed by Burnet that mice persistently infected with LCMV were immunologically tolerant to the virus (the host's immune system is not able to recognize the virus and mount an immune response). Accordingly, this purported immune tolerance to LCMV was why the virus was able to persist. However, seminal studies by Oldstone and colleagues demonstrated that mice persistently infected with LCMV were not tolerant. These mice produced antivirus antibodies and routinely developed glomerulonephritis. The inflammatory lesions in the kidneys were due to LCMV–anti-LCMV immune complex deposition in the glomeruli activating the complement cascade and recruiting of cells of the innate immune system leading to disease (to be discussed below) (**Figure 2**, arrow 2). Similar findings followed for congenital murine retrovirus infections.

In contrast, if mice are infected as adults with a moderate dose of LCMV (nonimmunosuppressive variant) by an extraneural route of infection (outside the central nervous system (CNS)) little or no immunopathologic disease is observed. Anti-LCMV CTLs are induced that are able to clear virus-infected cells. A portion of these LCMV-specific T cells go on to become memory T cells. Subsequent intracerebral (ic) infection of an adult immunocompetent immune mouse results in far less inflammation and immunopathology in the CNS and the animal survives. Interestingly, after ic LCMV infection of adult nonimmune mice, virus is able to replicate in certain epithelial cells. After about 5–7 days post infection the infected mouse mounts a robust anti-LCMV CTL immune response which is responsible for inflammation in the choroid plexus, ependyma, and leptomeninges causing choriomeningitis. Death is due to the action of the anti-LCMV CTL immune response altering the blood–brain barrier and initiating brain edema. This infection illustrates several of the factors involved in immunopathology. The age of the host is critical, since in very young animals the immune systems are not fully developed. These animals are immunocompromised, that is, unable to mount an effective CTL response allowing a noncytolytic virus-like LCMV to persist. Other studies have shown the importance of CTL in initiating immunopathology and death. Adult mice whose T cells are ablated or inhibited and then infected with LCMV via the ic route of infection develop a persistent infection and do not die of the choriomeningitis. Adoptive transfer of immune spleen cells, immune T cells, or LCMV-specific $CD8^+$ T cells into LCMV carrier mice leads to clearance of viral infection. Thus, this experiment complements studies of acute infection and substantiates the role of the antiviral T cells in clearance of virus and termination of infection.

Mice that are infected as newborns or earlier with LCMV develop a persistent infection, but are not without immunopathology. As mice infected at birth or earlier develop into adults, they produce anti-LCMV antibodies that can bind to viral antigens (present due to the persistent infection) forming immune complexes. Depending on the size of the immune complexes and numbers formed, the complexes deposit in the glomeruli of the kidneys due to the filtering action within the glomeruli. As a consequence of immune complex deposition in the glomeruli, the complement cascade is initiated releasing chemotactic peptides that recruit and activate polymorphonuclear leukocytes (PMNs) and macrophages into the glomeruli. These activated cells release inflammatory cytokines, such as TNF and interleukin (IL)-1, and reactive oxygen intermediates, as well as lysosomal enzymes that induce inflammation and immunopathology resulting in glomerulonephritis. The extent of glomerulonephritis induced by persistent LCMV infection varies and depends

both on the strain of mouse (genetic influences discussed below) and strain of virus.

The genetic composition of the host can play an important role in whether immunopathology occurs. For example, infection with Theiler's murine encephalomyelitis virus (TMEV), a picornavirus related to the cardioviruses, has different outcomes depending on the strain of the host. As with LCMV the mouse is the natural host. Because of the availability of inbred mice and mapping of their molecules, particularly MHC and background genes involved in innate immunity, the mouse has been an exquisite model to measure not only host genetics but influence of viral genetics as well. TMEV is a positive stranded RNA virus. The genome is approximately 8000 bases in length. The icosohedral capsid is comprised of four viral proteins. The virion is not encapsidated by a membrane. TMEV can be divided into two groups depending on neurovirulence. The GDVII subgroup is comprised of viruses that are highly neurovirulent. Members of the TO subgroup are less neurovirulent. Ic infection with the DA strain of TMEV (TO subgroup) into adult C57BL/6 mice leads to an antiviral $CD8^+$ T-cell response that clears the virus from the CNS and other tissues of the body. There is limited inflammation in the CNS but mice survive and are immune to subsequent infections with TMEV.

In contrast, ic infection of SJL/J mice with the DA strain of TMEV induces an antiviral $CD8^+$ T-cell response which is not able to clear the virus. Therefore, DA virus is able to establish a persistent infection in the CNS. Immunopathology results as a consequence of a chronic anti-TMEV immune response attempting to clear virus-infected cells in the CNS. Infected SJL/J mice eventually develop large areas of inflammation and demyelination in the spinal cord with a spastic paralysis. Genetic differences in the MHC complex between the two strains of mice are implicated in whether one mouse strain is able to clear the virus and the other mouse strain is not able to clear the virus resulting in disease. In addition, innate immune responses are also genetically determined. Therefore, the early inflammation in response to infection is encoded within the genome and varies from individual to individual.

The initial site of infection is important. A peripheral infection with LCMV in an adult mouse generates a robust anti-LCMV CTL response and immunity. Since the virus is not present in the CNS or only a few cells are infected, the CTL response clears the virus and little pathology ensues. Infection by the ic route also induces a vigorous CTL response. However, a significant number of meningial cells are infected with the virus. CTLs recognize virus-infected cells in the meninges lysing the cells via a perforin-mediated mechanism. This contributes to the death of the mouse. Therefore, the site or route of infection and, which cells are infected by the virus, are important factors.

The strain of the virus can play a major role in immunopathology, as shown by the following two examples. As mentioned previously TMEV can be divided into two groups depending on neurovirulence. The GDVII subgroup comprises viruses that are highly neurovirulent. An intravenous (IV) or ic infection of mice with the GDVII virus will result in the virus killing the mouse in 7–10 days. The death is due to the virus' ability to infect large numbers of neurons and directly kill these cells. In contrast, infection of mice (SJL/J) with viruses from the TO subgroup with 100 000–1 000 000 times more virus leads to mild acute disease with TMEV establishing a persistent infection in the CNS. The chronic anti-TMEV immune response results in extensive demyelination and inflammation. The pathology is caused by the antiviral immune response comprising antibodies capable of neutralizing the virus, $CD4^+$ and $CD8^+$ T cells recognizing virus-infected cells and either directly killing the infected cells, as in the case of antiviral $CD8^+$ T cells or TMEV antibody and complement, or killing the infected cells via bystander mechanisms which are mediated by $CD4^+$ T cells and the effector/killer cells are macrophages (as in delayed-type hypersensitivity responses).

CNS infection of adult mice with LCMV leads to the generation of antiviral $CD8^+$ T cells and infected mice die about 7–10 days later as the $CD8^+$ T-cell response develops. As mentioned above there is extensive infiltration of T cells and mononuclear cells in the meninges, alterations in the blood–brain barrier, and edema. In contrast, infection of adult mice with a variant of LCMV, clone 13, that differs in two amino acids from wild-type Armstrong LCMV, induces an immunosuppression due to infection of dendritic cells which are then not able to arm and expand T- and B-viral-specific cells. The variant LCMV is able to persist in adult mice. These persistently infected mice also develop immune complexes in their kidneys. Different strains of virus, such as clone 13, can initiate different types of disease leading to immunopathology.

The amount of virus the host first encounters can set the stage for later disease. For example, high doses of certain strains of LCMV when given by the IV route results in dissemination to the various lymphoid tissues. Virus can replicate to high titers at these sites constantly stimulating the antiviral T cells leading to 'CTL exhaustion'. This is likely due to both the continual stimulation of the TCR by antigen presenting cells leading to the apoptosis of LCMV-specific CTLs and to infection of dendritic cells. With the depletion or failure to generate virus-specific CTLs, LCMV is able to establish a persistent infection. This is in contrast when low or moderate amounts of LCMV are used to infect mice. The infected mice are able to mount an effective CTL response, virus is cleared, and animals are immune.

In summary, immunopathology results from an imbalance between the immune system's ability to clear the

virus and resulting tissue damage due to the antiviral immune response. These can take the form of antibody and complement, $CD4^+$ T cells and $CD8^+$ CTLs causing tissue damage in the process of eliminating virus and virus-infected cells.

See also: Antigen Presentation; Immune Response to viruses: Antibody-Mediated Immunity; Immune Response to viruses: Cell-Mediated Immunity.

Further Reading

Borrow P and Oldstone MBA (1997) Lymphocytic choriomeningitis virus. In: Nathanson N, Ahmed R, Gonzalez-Scarano F, *et al.* (eds.) *Viral Pathogenesis*, pp. 593–627. Philadelphia: Lippincott-Raven Publishers.

Buchmeier MJ and Oldstone MBA (1978) Virus-induced immune complex disease: Identification of specific viral antigens and antibodies deposited in complexes during chronic lymphocytic choriomeningitis virus infection. *Journal of Immunology* 120: 1297–1304.

Henke A, Huber S, Stelzner A, and Whitton JL (1995) The role of $CD8^+$ T lymphocytes in coxsackievirus B3-induced myocarditis. *Journal of Virology* 69: 6720–6728.

Oldstone MBA (1982) Immunopathology of persistent viral infections. *Hospital Practice (Office Education)* 17: 61–72.

Oldstone MBA and Dixon FJ (1969) Pathogenesis of chronic disease associated with persistent lymphocytic choriomeningitis viral infection. I. Relationship of antibody production to disease in neonatally infected mice. *Journal of Experimental Medicine* 129: 483–505.

Selin LK, Cornberg M, Brehm MA, *et al.* (2004) CD8 memory T cells: Cross-reactivity and heterologous immunity. *Seminars in Immunology* 16: 335–347.

Tsunoda I and Fujinami RS (1996) Two models for multiple sclerosis: Experimental allergic encephalomyelitis and Theiler's murine encephalomyelitis virus. *Journal of Neuropathology and Experimental Neurology* 55: 673–686.

Infectious Pancreatic Necrosis Virus

Ø Evensen and N Santi, Norwegian School of Veterinary Science, Oslo, Norway

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Fingerlings A life-cycle stage when young salmonids are one finger in length.
Fry Newly spawned fish that have fully absorbed their yolk sac.
Milt The sperm from the male fish.
Parr A young salmonid with parr marks (dark blotches) on the sides, before migration to the sea.
Smolts Salmonid fish going through smoltification, physiological changes that will allow fish to change from life in freshwater to life in the sea. The smolt state follows the parr state.

Introduction

Infectious pancreatic necrosis (IPN) was originally recognized as an acute and highly contagious disease of juvenile salmonids which typically occurs at the time of commencement of feeding of fry. The clinical manifestation of the disease has, however, changed over the last two decades. IPN outbreaks are still observed during the first few weeks after commencement of feeding of fry and also later in the freshwater phase, typically in 10–20 g fish. The most remarkable change in the clinical manifestation of the disease, observed since the mid-1980s, is the number of outbreaks in post-smolts after seawater transfer, and this has emerged as a significant problem, particularly in salmon farming. The disease pattern is seen in many of the major Atlantic salmon-producing countries such as Chile, UK, and Norway. The mortality rates in the freshwater stage vary considerably from negligible to almost 100%, while disease outbreaks in seawater typically result in 10–20% cumulative mortality, and can reach 70% in individual sea cages. This variation in mortality has been ascribed to factors related to the host species (e.g., the age and/or genetic resistance of fish) and environmental stressors. Virus characteristics such as variations between virus serotypes and strains and viral infection loads can also play an important role in determining mortality rates.

Virus Classification

Infectious pancreatic necrosis virus is the type species of the genus *Aquabirnavirus* within the family *Birnaviridae*. The disease caused by infectious pancreatic necrosis virus (IPNV) in salmonid fish is characterized by necrosis of the exocrine pancreas, which is the origin of the name of both the disease and the type species. However, aquabirnaviruses have been isolated from a wide variety of freshwater and marine fish species, as well as from marine invertebrates worldwide, and are associated with a wide range of host pathologies. Aquatic birnaviruses are

an antigenically diverse group of virus, and somewhat difficult to classify.

Serological classification based on neutralizing antibodies against surface epitopes separates isolates into serogroups A and B. Serogroup A contains nine serotypes (A1–A9) comprising most of the isolates, whereas serogroup B consists of only one serotype. Due to a lack of standardization of the methods, serotyping has been supplemented with, and partly replaced by, genetic classification. Phylogenetic analysis based on the VP2 coding region of genomic segment A suggests that aquabirnaviruses may be clustered into six genogroups that correspond to the previously established serotypes. Genogrouping based on 310 basepairs (bp) at the VP2/NS junction region has identified seven genogroups, the additional genogroup comprising Japanese aquabirnavirus isolates (**Figure 1**). More distantly related is the blotched snakehead virus which is currently an unassigned member of the family *Birnaviridae* and it has been proposed to be classified in a new genus.

There are no agreed species demarcation criteria for the genus *Aquabirnavirus*, and this has led to difficulty in nomenclature. Distantly related strains may cause similar pathologies, whereas closely related strains may range from avirulent to very virulent. Closely related strains are found in both farmed fish and marine species in the same geographical region. Therefore, the species concept does not apply very well to members of the genus *Aquabirnavirus*. Another aspect contributing to this is the intrinsic variability of RNA viruses such as the aquabirnaviruses, is the error-prone process of nucleic acid replication.

Genomic Organization and Virus Structure

The IPNV virion is 60 nm in diameter and a single-shelled, icosahedral structure with no envelope (**Figure 2**). The virus genome consists of two segments of double-stranded (ds) RNA. For serotype Jasper, segment A comprises 3097 bp and the smaller segment B comprises 2784 bp.

Genome segment A (**Figure 3**) contains a large open reading frame (ORF) encoding a 106 kDa polyprotein (NH_2-VP2-protease (VP4)-VP3-COOH), which is cotranslationally cleaved to generate pVP2 (the precursor of VP2), VP4, and VP3. The processing of the large polyprotein is controlled by VP4, which is a viral protease with a serine/lysine catalytic dyad. The cleavage sites have been identified at the pVP2–VP4 and VP4–VP3 junctions, and the structure of VP4 has recently been elucidated by X-ray crystallography. The pVP2 precursor is processed further into the mature VP2 which is the major capsid protein and, in addition, three small peptides are generated that can be detected in virus particles. VP2 spontaneously self-assembles into particles with a diameter of about 25 nm, suggesting that late maturation is to avoid premature assembly during particle morphogenesis. Although studies have indicated that some elements of VP3 may be exposed on the surface of the virion, serotype-specific and neutralizing antibodies are almost exclusively directed against continuous and discontinuous epitopes of VP2. VP3 is an internal capsid protein associated with the viral genome. By applying the yeast two-hybrid system in combination with co-immunoprecipitation, VP3 has recently been shown to bind to VP1 and to self-associate strongly. In addition, VP3 has been shown to bind specifically to dsRNA in a sequence-independent manner. The binding of VP3 and VP1 is not dependent on the presence of dsRNA.

Genome segment A also contains a small ORF that proceeds and partly overlaps the large ORF encoding the 106 kDa polyprotein. The smaller ORF encodes a highly basic, arginine-rich 17 kDa polypeptide that has been detected in infected cells but has not, to date, been conclusively demonstrated to be present in purified virus particles. This apparently nonstructural protein, designated VP5, is encoded by most but not all IPNV strains that have been examined. In an Asian IPNV strain, an anti-apoptotic effect has been demonstrated for VP5 and this may aid viral replication in the initial phase of infection. However, the amino acid sequence of VP5 is not well conserved and the anti-apoptotic effect does not occur in all IPNV strains encoding the polypeptide.

Segment B encodes a 90 kDa protein (VP1) which functions as an RNA-dependent RNA polymerase (RdRp). This enzyme directs the transcription of nonpolyadenylated mRNA from genome segments A and B. Within the virus particle, VP1 occurs as both a free polypeptide and covalently linked to the 5′ terminus of each genome segment.

Antigenic Structure and Neutralization Sites of the Virus

The VP2 is the type-specific antigen in which neutralizing sites reside within the hypervariable region. Several attempts have been made to identify the neutralization domains of VP2 and the findings reported are summarized in **Table 1**. The region comprising amino acid residues 183–335 has been found by several authors to contain neutralizing epitopes.

Virus Propagation and Cytopathic Effects

IPNV replicates in the cytoplasm and a single cycle of replication takes 16–20 h. IPNV infects a wide range of cultured fish cells, including CHSE-214, RTG-2, and BF-2 cells, all of which are routinely used for laboratory propagation of the virus. IPNV replicates well at temperatures ranging from 15 to 22 °C, resulting in a characteristic cytopathic effect. Available information on attachment, penetration, and uncoating is limited. Purified virus

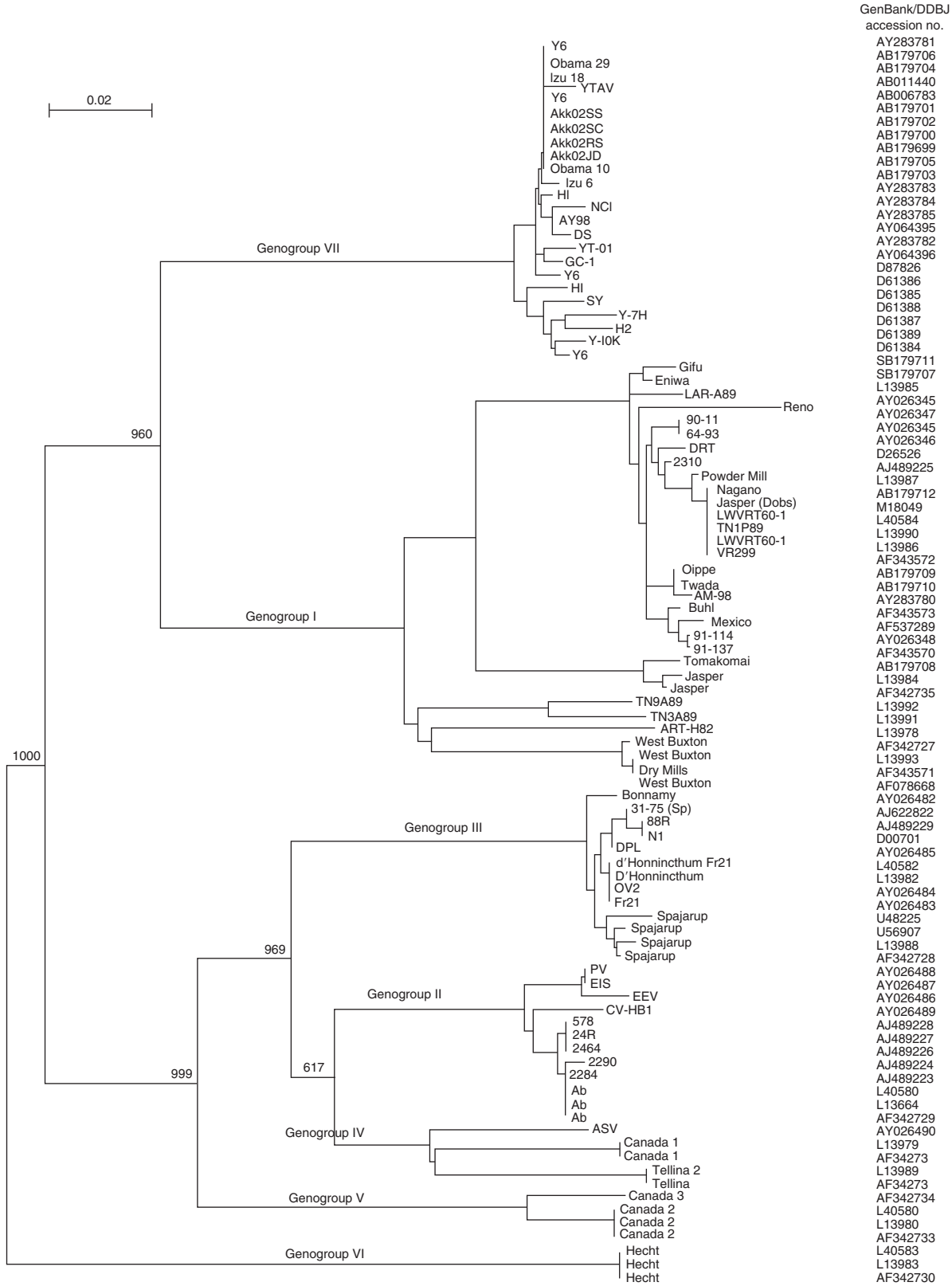


Figure 1 Molecular phylogenetic tree based on nucleotide sequences of the VP2/NS junction among 93 worldwide isolates of IPNV and other aquabirnaviruses, and corresponding GenBank/DDBJ accession numbers. Bootstrap values form 100 replicates are shown at major nodes. Scale: 0.02 replacement nucleotides per site. Reproduced from Nishizawa T, Kinoshita S, and Yoshimizu M (2005) An approach for genogrouping of Japanese isolates of aquabirnaviruses in a new genogroup, VII, based on the VP2/NS junction region. *Journal of General Virology* 86: 1973–1978, with permission from Society for General Microbiology.

attaches to CHSE-214 cells by specific and nonspecific binding to cell membrane components. After binding, IPNV can be observed in endosomes after 20–30 min, suggesting that viral entry occurs by endocytosis. Entry does not appear to be dependent on the acidic pH of the endosomes, and the virion RdRp (VP1) is active without proteolytic treatment of the virion, indicating that uncoating may not be a precondition for virus replication. VP1 can be guanylylated *in vitro* whereupon it becomes a primer for *in vitro* RNA synthesis. Unlike other viral VPg polypeptides, VP1 self-guanylylates *in vitro* in a template-independent manner, and the guanylylation site has been mapped to serine 163. Segment A mRNA is synthesized in larger amounts (2–3 times) than segment B, reflecting the relative abundance of the viral proteins. Assembly of virions takes place in the cytoplasm of infected cells, and virus release occurs via cell lysis.

Apoptosis is induced in CHSE-214 cells following IPNV infection and the so-called 'McKnight' cells associated with sloughing of mucosal cells in the intestines of juvenile salmonids suffering from IPN resemble apoptotic cells morpologically. Similar apoptotic bodies are also found in the liver of Atlantic salmon post-smolts suffering from IPN (**Figure 4**).

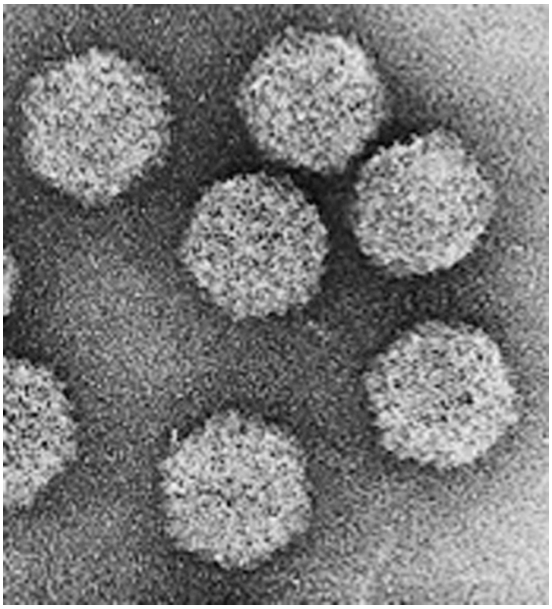


Figure 2 Negative staining of IPNV serotype Sp grown on Chinook salmon embryonic (CHSE-214) cells and purified by density gradient centrifugation.

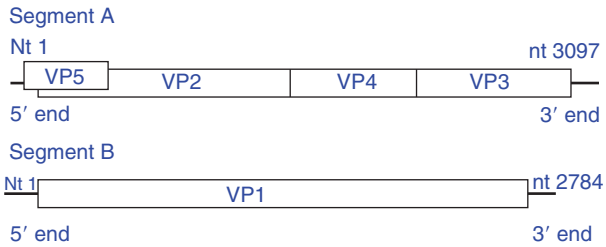


Figure 3 Schematic presentation of the genomic organization of IPNV depicting the proteins encoded by genome segments A and B.

Acute Infections

IPNV causes acute disease in juvenile salmonids and is the major pathogen of concern in brook trout (*Salvelinus fontinalis*), rainbow trout (*Oncorhyncus mykiss*), and Atlantic salmon (*Salmo salar* L.) hatcheries. Outbreaks also occur in fingerlings and parr later in the freshwater phase, as well as in post-smolts after transfer to seawater.

Salmonid fish suffering acute IPNV infection display darkened skin, abnormal behavior such as whirling or swimming on the side, and a visibly distended abdomen. Whitish threads of debris are often seen hanging from the anal orifice. At necropsy, common findings are ascites and hemorrhages in perivisceral adipose tissue, and the liver is often pale. Histopathologically, IPN is characterized by acute multifocal necrosis of the exocrine pancreas. In cases of more protracted disease, evidence of a cellular immune response can be observed. In addition to pancreatic lesions, multifocal hepatic necrosis and acute catarrhal enteritis with necrosis and sloughing of the intestinal epithelium have been reported. Viral antigen can be detected within lesions by immunohistochemistry or indirect fluorescent antibody tests.

Persistent Infections

A high proportion of fish surviving an IPN outbreak become persistently infected without apparent signs of disease. These subclinically infected individuals are of

Table 1 Summary of studies addressing the neutralizing part of IPNV VP2 protein

Author(s)	*Year*	*Reference*	*VP2 region*
Mason C	1992	PhD Thesis, Oregon State University	207–314
Liao and Dobos	1995	*Virology* 209: 684–687	200–350 (243–357) 83
Heppell *et al.*	1995	*Virology* 214: 40–49	183–335
Tarrab *et al.*	1995	*Journal of General Virology* 76: 551–558	183–337
Frost *et al.*	1995	*Journal of General Virology* 76: 1165–1172	204–330
Blake *et al.*	2001	*Diseases of the Aquatic Organisms* 45: 89–102	243–335

major importance as sources of horizontal transmission of the virus. In the carrier state, there is no direct negative impact on infected individuals, although there are some indications of suppressed immune response. However, recent studies have shown that there are differences in the ability of different strains of the Sp serotype of IPNV to establish persistent infections in Atlantic salmon fry. Carrier fish shed virus in the feces but titers fluctuate over time and increase during periods of stress. In addition, persistently infected brood fish can transfer IPNV to their progeny.

Virus can be isolated from peripheral leukocytes a short time after experimental infection but not during chronic stages. The virus is located in head kidney leukocytes in fish that recover from an acute infection and most likely in macrophages or macrophage-like cells of the head kidney in persistently infected fish. It has also been shown that IPNV multiplies in adherent leucocytes isolated from carriers, although a lytic infection is not seen and, concordantly, virus is not found in the supernatant medium from cultures of isolated head kidney macrophages. Similarly, IPNV is able to induce persistent, non-lytic infections in cell cultures in which a high proportion of the cells are infected but produce relatively low quantities of virus. Different strains of IPNV appear to vary in their ability to induce persistence in cell culture. Infected cells are resistant to superinfection with either homologous or heterologous strains or serotypes, and cultures can be cleared of IPNV infection by several passages in the presence of virus neutralizing antiserum. The underlying mechanisms of the establishment and maintenance of IPNV persistence *in vivo* and *in vitro* are largely unknown.

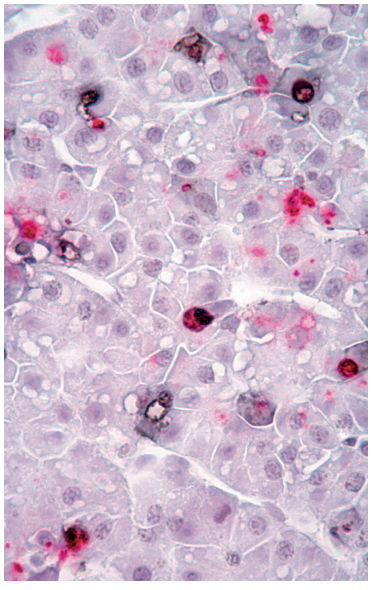


Figure 4 Liver tissue from IPNV-infected Atlantic salmon, fixed in 10% buffered formalin and embedded in paraffin wax. The tissue section was double-stained for IPNV antigen using a rabbit immune serum to IPNV Sp (red color) and for apoptotic cells based on the TUNEL method (black coloration). Note double-staining in some of the hepatocytes.

Molecular Determinants of Virulence

The mortality rate during an IPN outbreak can vary and this has been ascribed to factors related both to the host and to the virus. Viral factors influencing mortality rates are the dose of infection as well as virulence variations between serotypes and strains. IPNV strains of different serotypes are highly heterogenous and the Sp serotype is considered to be more virulent than the Ab serotype. Within the Sp serotype, different genotypes exhibit different virulence characteristics which have been studied by the use of reverse genetics.

In vitro transcribed positive-sense cRNAs of IPNV are infectious when transfected together into permissive cell cultures, allowing the recovery of genetically modified isolates. The reverse genetics technique is a valuable tool for studying the IPNV life cycle and the role of individual viral proteins in the pathogenesis of infection. Experimental studies using such recombinant IPNV strains have demonstrated that VP5 expression is not required for efficient replication *in vitro* or *in vivo*, or for virulence, or persistence of the virus. Reassortant viruses containing segment A from a high-virulence strain and segment B from a low-virulence strain, prepared by either reverse genetics or co-infection techniques, have also been used to demonstrate that segment B has no direct influence on IPNV virulence. For IPNV serotype Sp, it has been shown that certain amino acid sequence motifs in VP2 strongly correlate with virulence. Residues 217 and 221 appear to be critical determinants of virulence, and genetic signatures involving these amino acids are strongly associated with avirulent, moderately and highly virulent strains, as shown in **Table 2**. Furthermore, serial passage of the Sp serotype in cell culture leads to the attenuation of virulence as a result of an A→ T mutation at amino acid residue 221.

Table 2 Point mutations in VP2 result in a marked reduction to almost complete loss of virulence whereby 217 and 221 are key determinants

Amino acid residues of VP2			*In vivo virulence characteristics*
217	221	247	
T	A	T	Highly virulent
T	T	T	Avirulent
P	A	A	Moderately virulent
P	T	A	Avirulent

Substitution of threonine for proline at residue 217 (T217P) reduces the virulence of the strain, producing a mortality rate approx. 50 % lower than the high-virulent variant (TAT). Similarly, single amino acid substitutions of alanine for threonine at residue 221 render highly virulent strains (TAT) almost avirulent. Passage in culture of high virulent strains is associated with motif changes in position 221 (A to T) for TAT ant PAA variants, each of which results in avirulence.

Table 3 Summary of neutralizing domain, virulence determinants and the cell culture adaptation characteristics of IPN virus

Characteristics	*VP2 amino acid residues*
Immunogenic domain	183–337
Virulence determinants	217, 221, 247 (Sp serotype)
Cell culture adaptation	221 (Sp serotype)
Antibody neutralization	Not known

Serotype-specific findings have been indicated.

In summary, the neutralizing domain, the virulence determinants, and the cell culture adaptation with subsequent loss of *in vivo* virulence seem to be located in the VP2 hypervariable region for IPNV, although there is a limited number of serotypes and strains of the IPN virus that have been characterized for their virulence signatures and cell culture adaptation traits (**Table 3**).

Epidemiology

It has long been known that survivors of an IPN outbreak become carriers and surviving fish continue to shed virus without any apparent clinical symptoms, and subclinical infection is important for horizontal transmission of virus. The efficiency by which the virus is spread in a population of susceptible individuals varies between strains and there are indications that this might correlate with virulence characteristics whereby highly virulent variants are more efficient at spreading. Transmission of the virus to progeny has been shown to occur vertically via eggs in brook trout and rainbow trout. The virus is possibly present inside the eggs since there are indications that surface disinfection of the eggs is not fully effective in preventing the introduction of IPNV to hatcheries. One possible route of intra-ovum introduction is via the milt, by adsorption of virus to the sperm head.

Surveys for the prevalence of IPNV have concluded that most North Sea salmon farms harbor IPNV carrier fish, making selection for IPNV-free brood fish difficult (prevalence in Scotland is 80%). Once introduced to a hatchery, the virus is difficult to eliminate, as it is stable in water and relatively resistant to many disinfectants. It is also still not clear whether seawater outbreaks of IPN are the result of persistent infection obtained during the freshwater period or exposure from a marine source.

Control of IPN through Management

Good management includes the environment of fish to minimize stress and to reduce the likelihood of transmission of the pathogen. This is particularly important at the time of first feeding of fry, when smolt are transferred to sea and during the first few months after sea water transfer.

Transfers of fish between farms are considered as one of the highest risks in the transmission of IPNV. Another factor is the movement of eyed eggs and fry between hatcheries, common in Norway and other salmon-producing countries.

Vertical transmission has not been conclusively demonstrated in Atlantic salmon but there is evidence from other salmonid species to indicate that vertical transmission is a strategy utilized by IPNV.

Disease Control through Vaccination

There are few scientific publications addressing or documenting the effect of vaccination against IPN and most of what has been published has focused on the immune responses elicited by vaccination. Vaccination with an *Escherichia coli*-expressed VP2 recombinant protein or insect baculovirus-expressed subunit vaccine based on all structural proteins of IPNV induces specific antibody responses in Atlantic salmon. For *E. coli*-expressed proteins, it has also been shown that vaccination will confer higher secondary antibody titers in immunized fish compared to nonvaccinated controls. Similar observations have been made for inactivated vaccines based on whole virus. Reliable challenge models for IPN in Atlantic salmon post-smolts have only recently been established and this partly accounts for the lack of scientific data on protective immunity. It has been shown that partial protection can be induced in Atlantic salmon immunized with a baculovirus/insect larvae-expressed subunit vaccine. However, the level of protection was below that required under field conditions in a commercial setting.

DNA vaccination against IPNV has also been attempted in Atlantic salmon. Fish were immunized with plasmids encoding the entire polyprotein of segment A and shorter variants of the protein, and subsequently challenged with virulent IPNV strains. A reasonable protection was attained with constructs expressing the entire segment A but it is difficult to draw a firm conclusion as to the potential that lies in the use of DNA vaccines for protection against IPNV infection since the number of published studies is few.

Despite the sparse knowledge of the immunity to IPNV, the first commercial, recombinant subunit (VP2) vaccine was introduced in Norway in 1995 and has come into use throughout the industry. By 2 years after introduction of the vaccine, 68% of the fish transferred to sea were vaccinated and the national coverage had increased to 85% in 2002. Vaccines based on killed, whole-virus are also currently available in Norway, the UK, and Chile. The extent to which the use of the vaccine has influenced the prevalence of IPNV has not been ascertained.

Disease Control through Breeding

There are few published papers related to the genetic resistance to IPN in Atlantic salmon or other fish species. In Norway, IPNV challenge experiments in Atlantic salmon fry (around 0.20 g size) have shown 15–30 % of the variation in mortality between family groups can be explained by genetic factors (heritability). Therefore, there is a potential for improving resistance to IPNV through breeding programs where brood fish are selected on the basis of off-spring performance.

Another approach is to select brood fish based on survival following disease outbreaks, but the latter strategy presents a risk of bringing the infection over to offspring. One option is to expose pedigreed populations of fish to the field challenge, with some of the unchallenged full-siblings remaining in biosecure conditions for breeding of unchallenged brood-stock.

See also: Necrovirus.

Further Reading

Blake S, Ma J-Y, Caporale DA, Jairath S, and Nicholson BL (2001) Phylogenetic relationships of aquatic birnaviruses based on deduced amino acid sequences of genome segment A cDNA. *Diseases of Aquatic Organisms* 45: 89–102.

Coulibaly F, Chevalier C, Gutsche I, *et al.* (2005) The birnavirus crystal structure reveals structural relationships among icosahedral viruses. *Cell* 120: 761–772.

Dobos P (1995) The molecular biology of infectious pancreatic necrosis virus (IPNV). *Annual Review of Fish Diseases* 5: 25–54.

Duncan R, Nagy E, Krell PJ, and Dobos P (1987) Synthesis of the infectious pancreatic necrosis virus polyprotein, detection of a virus-encoded protease, and fine structure mapping of genome segment A coding regions. *Journal of Virology* 61: 3655–3664.

Feldman AR, Lee J, Delmas B, and Paetzel M (2006) Crystal structure of a novel viral protease with a serine/lysine catalytic dyad mechanism. *Journal of Molecular Biology* 358: 1378–1389.

Frost P, Håvarstein LS, Lygren B, Ståhl S, Endresen C, and Christie KE (1995) Mapping of neutralization epitopes on infectious pancreatic necrosis viruses. *Journal of General Virology* 76: 1165–1172.

Heppell J, Tarrab E, Lecomte J, Berthianme L, and Arella M (1995) Strain variability and localization of important epitopes on the major structural protein (VP2) of infectious pancreatic necrosis virus. *Virology* 214: 40–49.

Hill BJ and Way K (1995) Serological classification of infectious pancreatic necrosis (IPN) virus and other aquatic birnaviruses. *Annual Review of Fish Diseases* 5: 55–77.

Hong JR and Wu JL (2002) Induction of apoptotic death in cells via bad gene expression by infectious pancreatic necrosis virus infection. *Cell Death and Differentiation* 9: 113–124.

Liao L and Dobos P (1995) Mapping of a serotype specific epitope of the major capsid protein VP2 of infectious pancreatic necrosis virus. *Virology* 209: 684–687.

Nishizawa T, Kinoshita S, and Yoshimizu M (2005) An approach for genogrouping of Japanese isolates of aquabirnaviruses in a new genogroup, VII, based on the VP2/NS junction region. *Journal of General Virology* 86: 1973–1978.

Pedersen T, Skjesol A, and Jorgensen JB (2007) VP3, a structural protein of the infectious pancreas necrosis virus, interacts with the RNA-dependent RNA polymerase VP1 and with double-stranded RNA. *Journal of Virology* 81: 6652–6663.

Rodriguez Saint-Jean S, Borrego JJ, and Perez-Prieto SI (2003) Infectious pancreatic necrosis virus: Biology, pathogenesis, and diagnostic methods. *Advances in Virus Research* 62: 113–165.

Santi N, Sandtro A, Sindre H, *et al.* (2005) Infectious pancreatic necrosis virus induces apoptosis *in vitro* and *in vivo* independent of VP5. *Virology* 342: 13–25.

Santi N, Song H, Vakharia VN, and Evensen O (2005) Infectious pancreatic necrosis virus VP5 is dispensable for virulence and persistence. *Journal of Virology* 79: 9206–9216.

Shivappa RB, McAllister PE, Edwards GH, Santi N, Evensen O, and Vakharia VN (2005) Development of a subunit vaccine for infectious pancreatic necrosis virus using a baculovirus insect/larvae system. *Developments in Biological Standardization* 121: 165–174.

Song H, Santi N, Evensen O, and Vakharia VN (2005) Molecular determinants of infectious pancreatic necrosis virus virulence and cell culture adaptation. *Journal of Virology* 79: 10289–10299.

Tarrab E, Berthianme L, Grothé S, O'Connor-McCourt M, Heppell J, and Lecomte J (1995) Evidence of a major neutralizable conformational epitope region on VP2 of infectious pancreatic necrosis virus. *Journal of General Virology* 76: 551–558.

Xu HT, Si WD, and Dobos P (2004) Mapping the site of guanylylation on VP1, the protein primer for infectious pancreatic necrosis virus RNA synthesis. *Virology* 322: 199–210.

Infectious Salmon Anemia Virus

B H Dannevig, National Veterinary Institute, Oslo, Norway
S Mjaaland and E Rimstad, Norwegian School of Veterinary Science, Oslo, Norway

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ascites Accumulation of serous fluid in the peritoneal cavity.
Coprophagy Eating of feces.
Exophthalmia Protrusion of the eyeballs.
Fry Young offsprings, presmolt stage.
Hemagglutinin-esterase A surface glycoprotein responsible for both receptor-binding (hemagglutinin) and receptor-destroying (esterase) activities.
Pathognomonic Pathological changes typical for a specific disease.

Smolt The stage in the life cycle of young anadromous salmonids when the fish is physiologically adapted to seawater, that is, farmed salmonids can be moved from freshwater to seawater.
Well boats Boats that are used for transportation of live fish.

Introduction

Infectious salmon anemia virus (ISAV) is the causative agent of infectious salmon anemia (ISA), a disease of farmed Atlantic salmon (*Salmo salar*). ISA primarily affects fish held in or exposed to seawater. The disease appears as a systemic condition characterized by severe anemia and hemorrhages in several organs. Mortality during an outbreak of ISA varies significantly. Daily mortality in affected net pens ranges from 0.2% to 1%, but may increase during an outbreak and the cumulative mortality may exceed 90% in severe cases.

ISA was recognized in 1984 in Norway, and was soon identified as a contagious viral disease. The disease increased in prevalence and showed a peak in 1990. In the following years, the incidence of ISA was greatly reduced by the implementation of legislatory measures or husbandry practices based on general hygiene. These included mandatory health control in hatcheries and health certification for fish, restrictions on transportation of live fish, regulations of disinfection of wastewater from fish slaughterhouses and of water supplies to hatcheries. Approximately 10 years after the first recognition of ISA, the causative virus, ISAV, was isolated in cell culture using the SHK-1 cell line established from Atlantic salmon head kidney. The subsequent investigations showed that the morphological, physiochemical, and genetic properties of ISAV are consistent with classification in the *Orthomyxoviridae*.

Virus Properties and Classification

ISAV is a pleiomorphic, enveloped virus, 100–130 nm in diameter, with 10–12 nm surface projections (**Figure 1**). The virus hemagglutinates erythrocytes of several fish species and it has receptor-destroying and membrane fusion activities. Endothelial and leukocytic cells are the main target cells and the virus replicates by budding from the cell membrane. ISAV has two main surface glycoproteins: the hemagglutinin-esterase (HE) responsible for the receptor-binding and receptor-destroying activities, and a fusion protein (F).

The buoyant density of virus particles in sucrose or cesium chloride is 1.18 g ml^{-1}. The virus is stable at pH 5.7–9.0. The virus replicates in the salmon cell lines SHK-1, TO, and ASK, with a replication optimum of 10–15 °C. Some strains also replicate in the CHSE-214 cell line. There is no replication in SHK-1 cells at 25 °C or higher and, even at 20 °C, the yield of virus in SHK-1 cells is only 1% of the yield at 15 °C.

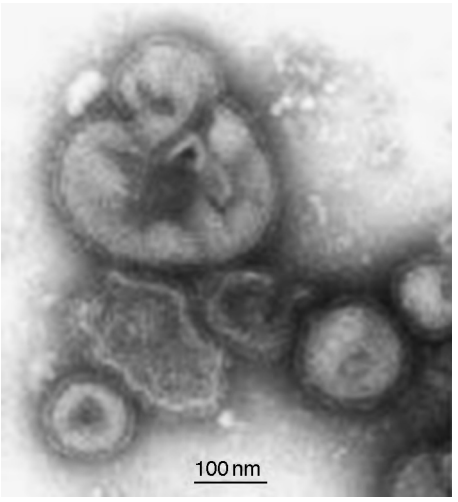


Figure 1 Electron micrograph of negative-stained ISAV particles purified from infected cell culture medium. Photo by Ellen Namork, The Norwegian Institute of Public Health, Oslo, Norway.

The 14.3 kb genome consists of eight single-stranded RNA segments of negative polarity in the size range of 1.0–2.4 kb. The amino acid identity between the ISAV proteins and those of other orthomyxoviruses is low (13–25%), and the organization of ISAV genes is unique. ISAV is therefore classified as the type species of the genus *Isavirus* within the family *Orthomyxoviridae*.

Geographic Distribution and Host Range

After the first recorded outbreak in 1984, ISA was considered to be a uniquely Norwegian disease. In 1996, a disease in Canada (New Brunswick) designated as hemorrhagic kidney syndrome was verified as ISA. The disease has thereafter been reported in Scotland (1998), the Faroe Islands (2000), and in the USA (Maine, 2001).

While natural outbreaks of ISA have only been described in Atlantic salmon, the virus may survive and replicate in other salmonid fish under experimental conditions. ISAV has been detected in wild Atlantic salmon and brown trout (*Salmo trutta*). Outbreaks of ISA in farmed Atlantic salmon have occurred mainly during the seawater stage, but indications of disease outbreaks in the freshwater stage have been reported. Disease and transmission are readily induced experimentally in Atlantic salmon kept in either freshwater or seawater. Wild Atlantic salmon are susceptible to ISA and show the same clinical signs as

farmed fish when experimentally infected. The virus has been isolated from apparently healthy rainbow trout (*Oncorhynchus mykiss*) in Ireland (2002). ISAV has also been reported to have been isolated from Coho salmon (*Oncorhynchus kitsutch*) in Chile, but this observation needs to be confirmed. Subclinically infected feral salmonids (Atlantic salmon, brown trout, and sea brown trout) have been identified in Scotland and Norway by reverse transcriptase-polymerase chain reaction (RT-PCR). However, reports of RT-PCR-positive samples from marine, nonsalmonid fish (Atlantic cod (*Gadus morhua*) and pollock (*Pollachius virens*)) need to be corroborated as samples were collected from the vicinity of cages holding ISA-diseased Atlantic salmon.

ISAV replication has been demonstrated in experimentally infected brown trout and in rainbow trout, but disease and mortality have only been produced in rainbow trout by experimental infection. ISAV has also been detected by RT-PCR in experimentally infected Arctic charr (*Salvelinus alpinus*) but neither virus replication nor clinical signs of disease have been demonstrated. ISAV could be reisolated from Pacific salmon species (*O. mykiss*, *Oncorhynchus keta*, *O. kitsutch*, *Oncorhynchus tshawytscha*) injected intraperitoneally with various Canadian and Norwegian virus isolates but no mortality was observed. Pacific salmon are therefore considered more resistant to ISA than Atlantic salmon but should not be ignored as potential virus carriers. Among marine fish, ISAV is able to propagate in herring (*Clupea harengus*) after bath challenge, but attempts to induce infection in pollock have failed. There are no indications that ISAV can infect mussel (*Mytilus edulis*) or scallops (*Pecten maximus*).

Transmission, Vectors and Reservoir Hosts

Epidemiological and experimental studies have shown that ISA may spread by water-borne transmission. The risk of ISA is closely linked to geographical proximity to farms with ISA outbreaks or to slaughterhouses and processing plants. The spread of the disease over long distances may be caused by transportation of infected fish by well boats.

The virus may be shed into the water by various routes such as skin, mucus, feces, and urine. The most likely route of virus entry is through the gills and skin lesions but transmission by coprophagy has also been proposed. ISAV may retain infectivity for long periods outside the host. No significant loss in virus titer was observed after incubation of virus supernatants for 14 days at 4 °C and 10 days at 15 °C, and infectivity of tissue preparations is retained for at least 48 h at 0 °C, 24 h at 10 °C, and 12 h at 15 °C. A 3-$\log_{10}$ reduction in virus titer after 4 months storage in sterile seawater at 4 °C has been observed, but ISAV survival time in natural seawater may be shorter.

There are indications that ISAV may be transmitted vertically. ISAV has been detected by real-time RT-PCR in fertilized eggs from ISA-diseased brood fish. However, it is not known if this represents infective virus as recovery of virus by isolation in cell culture was not performed. Eggs, fry, and juveniles from ISAV-infected parents have been demonstrated ISAV-positive by real-time RT-PCR. On the other hand, ISAV could not be detected in progeny from healthy but virus-positive brood fish. Furthermore, ISAV has been detected by real-time RT-PCR in Atlantic salmon parr and smolt sampled from hatcheries, that is, from the freshwater stage. However, there are no verified field observations that can confirm vertical transmission of ISA disease. Nevertheless, the detection of virus in progeny from infected brood fish and the few reported outbreaks in the freshwater stage indicate that this route of transmission cannot be excluded.

The sea louse (*Lepeophtheirus salmonis*) has been suggested as a possible vector for ISAV, but it is not clear if this is by passive transfer or active virus replication. Reservoir hosts of ISAV have not been identified, but the virus replicates in sea trout, which are abundant in Norway in fiords and coastal areas in the vicinity of the fish farms. However, the possible role of sea trout as a reservoir host for ISAV can only be speculated at this time.

Genetics

Nucleotide sequences of all eight ISAV genome segments have been described. The genome encodes at least 10 proteins (**Table 1**). Segments 1, 2, and 4 encode the viral polymerase subunits PB1, PB2, and PA, respectively. Segment 3 encodes the 68 kDa nucleoprotein (NP). Segments 5 and 6 encode the two major surface glycoproteins: the 50 kDa fusion (F) protein, and the 42 kDa HE responsible for receptor-binding and receptor-destroying activities. The two smallest ISAV genomic segments each contain two overlapping reading frames (ORFs). Two mRNAs are transcribed from genome segment 7 – one is collinear with the viral RNA (vRNA) and the other is spliced in an arrangement similar to the two smallest gene segments of influenza A virus. The unspliced mRNA of segment 7 encodes a nonstructural protein that has been suggested to interfere with the interferon type 1 response of the cell. The protein encoded by the spliced mRNA has not yet been characterized. No splicing of transcripts from segment 8 ORFs has been detected, indicating that ISAV uses a bicistronic coding strategy for this genomic segment. The smaller ORF1 encodes a 22 kDa matrix protein, while the collinear mRNA transcript from the larger ORF2 encodes an RNA binding structural protein of about 26 kDa with putative interferon antagonistic properties. ISAV gene segments and the respective encoded proteins are summarized in **Figure 2**.

Table 1 Genome segments and encoded proteins of ISA virus

Segment (kb)	*Encoded protein*	*ORF (bp)*	*Protein (kDa)*
1 (2.3)	Polymerase, PB2	2127	80.5[a]
2 (2.3)	Polymerase, PB1	2169	79.5[a]
3 (2.2)	Nucleoprotein, NP	1851	66–74[b]
4 (2.0)	Polymerase, PA	1840	65.3
5 (1.7)	Fusion, F	1332	53
6 (1.5)	Hemagglutinin-esterase, HE	1176[c]	38–46[b]
7 (1.3)	Two open reading frames		
	• ORF1 = nonstructural (NS)	903	34
	• ORF2 = spliced protein	369	17.5
8 (1.0)	Two open reading frames		
	• Matrix, M	588	22–24
	• as RNA-binding protein	726	26

[a]Estimations based on amino acid sequence.
[b]The estimated molecular masses of some of the proteins differ slightly in the literature, probably due to differences in experimental conditions. For HE, this could also be due to differences in glycosylation as well as in the highly polymorphic region (HPR).
[c]Length of ORF for the reference ISAV isolate Glesvaer/2/90. The length varies between isolates due to the HPR characterized by deletions/gaps. The proposed ancestral ORF sequence is estimated to 1236 bp.

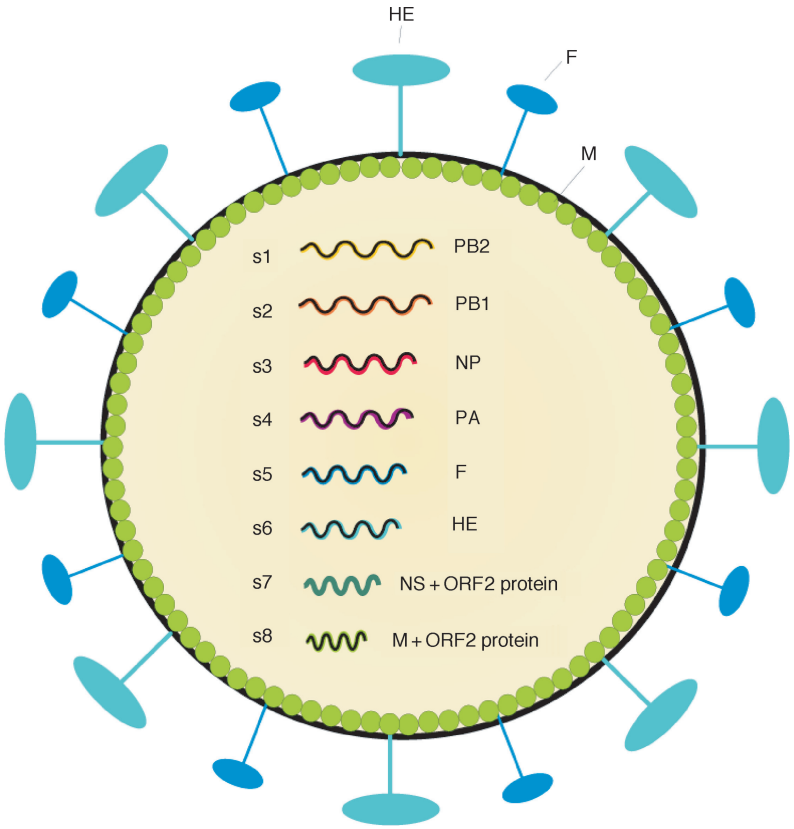


Figure 2 Schematic diagram of the ISAV virion. The two smallest genomic segments (segments 7 and 8) each contain two open reading frames (ORFs). The proteins encoded by each ORF is yet to be characterized. s, Genomic segment; PB2/PB1/PA, polymerase subunits; NP, nucleoprotein; F, fusion protein; HE, hemagglutinin-esterase; NS, nonstructural protein; M, matrix protein. Adapted from Ida Skaar.

The 3′- and 5′-terminal structure of vRNAs, and the ISAV transcription strategy, resemble those of influenza viruses. As for all members of the *Orthomyxoviridae*, each ISAV genome segment contains partially self-complementary termini that are essential for the replication process. However, compared to the 12–13 nucleotides conserved in the influenza A virus, only 8–9 nucleotides are conserved in ISAV, most likely reflecting the lower replication temperature optimum for this virus. As for the influenza viruses, the terminal 21–24 nucleotides are predicted to form self-complementary panhandle structures important for transcriptional regulation of viral RNA. ISAV also exerts cap-stealing in the host cell's nucleus, transferring 8–18 nucleotide 5′-cap structures from host mRNAs to the viral mRNAs and affecting the normal transcription and protein synthesis in the cell. ISAV mRNAs are polyadenylated, a process that is regulated from a signal 13–14 nucleotides downstream the 5′-terminus of the vRNAs. Altogether, the ISAV replication strategy is similar to those of the other orthomyxoviruses.

ISAV isolates vary in virulence, as observed by differences in disease development and clinical signs in field outbreaks and in experimental trials using genetically standardized fish. However, analysis of important virulence factors is still in its infancy. The ISAV genome is highly conserved, both over time and geographical distance, with 98–99% overall nucleotide similarity between Norwegian isolates. However, small, but relevant, differences between isolates both within and between defined geographical areas do exist, and analysis of the 5′-terminal end of the HE gene has demonstrated the presence of two major groups of isolates, one European and one North American. The European group may be further divided into three major subgroups.

The surface glycoprotein HE gene displays the highest sequence variation (94–97%) which is mainly concentrated in a small highly polymorphic region (HPR) of the HE protein that is predicted to lie immediately outside the viral envelope. Variation in this region is characterized by the presence of gaps rather than single-nucleotide substitutions. The polymorphism has been suggested to arise from differential deletions of a full-length precursor gene (HPR0) as a consequence of strong functional selection, possibly related to a recent or ongoing crossing of a species barrier. All HPRs described to date can be derived from such a full-length precursor sequence. Furthermore, the presence of a long HPR0 gene has been detected by RT-PCR in wild Atlantic salmon. The variation in HPR is most likely an important virulence factor as virus isolated from diseased fish always contains a deletion in this region.

Other genes are also most certainly important for virulence as isolates with identical HPR induce large differences in mortality in genetically standardized (major histocompatibility complex (MHC)-compatible) fish. Candidate genes include genome segment 5 encoding the surface glycoprotein with fusion activity and genome segment 7 and 8 encoding the putative IFN I antagonists in alternative reading frames. The synthetic IFN I inducer poly I:C induces no or only minor protection against ISAV infection in Atlantic salmon or cell culture, demonstrating the efficiency of the ISAV IFN I antagonistic properties. In orthomyxoviruses, reassortment of gene segments occurs frequently and is a major contributor to the evolution of these viruses and the emergence of new virulent strains. Alignments and phylogenetic studies of full-length sequenced ISAV isolates provide evidence for both genome segment reassortment and recombination. A 30 bp insert found in close proximity to the putative cleavage site of the fusion protein in several unrelated isolates combined with extensive sequence internal homology in this region suggests the presence of a recombinational hot-spot in this gene.

Pathogenesis

The mortality rate during an ISA outbreak may vary significantly. Variation in the seriousness in disease outbreaks may be influenced by the environmental and management parameters, genetics of the fish, and the virus strain.

In affected populations of farmed fish, individuals may harbor the virus for weeks or months before the development of disease. Prior to an outbreak, a slightly increased mortality over a period of 1–3 weeks is often seen. Outbreaks are often restricted initially to one or two net pens and up to 12 months can pass before clinical ISA spreads to neighboring pens in a farm. The signs exhibited by infected fish range from none to severe. The disease may appear throughout the year. The course of the disease may vary from an acute form with rapid development and high mortality, to a chronic form in which a slow increase in mortality is observed over several months, but several immediate forms may exist.

Experimentally, ISA can be induced in Atlantic salmon following intraperitoneal injection with tissue preparations or purified virus, or through infected cohabitants. The incubation period is usually 10–20 days. Studies indicate that the major portal of ISAV entry is the gills but oral entry cannot be excluded. Viral spread within the body is detectable from 5 days post-infection and cumulates at approximately 15 days. This is followed by a temporary decrease in viral load, reaching a minimum at around 25 days post-infection, followed by a second rise that continues to the terminal stage of ISA.

ISAV is found budding from endothelial cells that seem to be the main target cells. Their ubiquitous presence enables a systemic infection. By *in situ* hybridization, the most prominent ISAV-specific signals are detected in the endothelial cells of the heart. The presence of infectious virus has also been reported in leukocytes. ISAV specifically binds to glycoproteins containing 4-*O*-acetylated sialic acids. The viral esterase is specific for this sialic acid, indicating that it may be a receptor determinant for ISAV. ISAV infects cells via the endocytic pathway and fusion between virus and cell membrane takes place in the acidic environment of endosomes.

Clinical Features and Pathology

Fish infected with ISAV show a range of pathological changes, from none to severe. None of the described lesions is considered pathognomonic. Diseased fish appear lethargic and, in terminal stages, often sink to the bottom of the cage. The disease appears as a systemic condition characterized by severe anemia (hematocrit values below 10) and hemorrhages in several organs. The most prominent external signs are pale gills, exophthalmia, distended abdomen, and petechia in the eye chamber; skin hemorrhages in the abdomen, and scale edema. The major gross postmortem findings are circulatory disturbances in several organs caused by endothelial injury in peripheral blood vessels. These pathological manifestations of ISA are mainly recognized in the liver, kidney, gut, and gills, but not all of these organs are affected to the same extent during a disease outbreak. In some cases, the pathological manifestations are more clearly seen in one single organ than in others.

Abundant ascitic fluid is often present. A fibrinous layer may cover the liver capsule and the liver may be partly or diffusely dark red. The spleen and kidney may appear dark and swollen and the intestinal wall congested and dark. Petechial hemorrhages are seen on the surface of several organs as well as the adipose tissue and skeletal muscle. The major histopathological findings have been observed in the liver, kidneys, and intestine. The lesions result in extensive congestion of the liver with dilated sinusoids and, in later stages, the appearance of blood-filled spaces. Multifocal to confluent hemorrhages and/or necrosis of hepatocytes at some distance from large vessels in the liver are often seen. In the kidney, interstitial hemorrhage with tubular necrosis in the hemorrhagic areas, and accumulation of erythrocytes in the glomeruli may occur. Accumulation of erythrocytes in blood vessels of the intestinal lamina propria and hemorrhage into the lamina propria may be observed. In the spleen, accumulation of erythrocytes and distention of stroma may be found, and numerous erythrocytes may be present in central venous sinus and lamellar capillaries of the gills.

In ISA outbreaks on the American East Coast in the 1990s, histopathological changes were prominent in the kidneys. The major pathological findings included renal interstitial hemorrhage with tubular necrosis and casts, branchial lamellar and filamental congestion, and congestion of the intestine and pyloric cecae. The disease therefore initially became known as hemorrhagic kidney syndrome (HKS). The gross appearance of the fish was somewhat similar to that reported for ISA in Norway. However, liver congestion was a rare finding in fish from outbreaks in New Brunswick. HKS was later confirmed to be ISA by RT-PCR and cell culture isolation of the virus. Similar kidney and intestinal manifestations of the disease has now also been reported in Norway.

Diagnosis

Diagnosis of ISA was initially based on macroscopic, histological, and hematological findings. A dark liver was considered as a typical finding and the presence of multifocal, hemorrhagic liver necrosis with a 'zonal' appearance and hematocrit values below 10 confirmed the diagnosis. However, following the isolation of ISAV in SHK-1 cells, a number of methods for detection of virus in tissue samples were established.

Detection of ISAV antigens in kidney imprints from Atlantic salmon exhibiting clinical signs with the use of an indirect fluorescent antibody technique (IFAT) has been an important method for verification of ISA. The production of an anti-ISAV HE monoclonal antibody to be used as the primary antibody in the IFAT was an important step in the development of specific diagnostic tools. Polyclonal antibodies suitable for immunohistochemistry have also been developed, and this method has recently been implemented in the routine diagnostics of ISA.

ISAV was first isolated in the salmonid cell line SHK-1, but other salmonid cell lines such as CHSE-214, ASK, and TO also support viral propagation. Development of cytopathic effect (CPE) may be observed in infected cell cultures, but the extent of CPE may vary dependent on cell type and virus strain. The isolated virus may be identified using IFAT on infected cell cultures. Strain variations with respect to cell susceptibility have been observed. The Atlantic salmon cell lines SHK-1, TO, and ASK do not support growth of all ISAV variants as virus cannot be recovered from some RT-PCR-positive samples. The CHSE-214 cell line supports growth of some ISAV isolates, but others, such as the European variants of ISAV, do not replicate in this cell line at all. This limits the utility of the CHSE-14 cell line for virus isolation. Currently available fish cell lines appear to be either not sensitive enough or not permissive for all ISAV strains.

RT-PCR techniques have been widely in use since the RT-PCR for ISAV was described in 1997, and several

different primer sets have been found to be suitable for detection of ISAV. Real-time RT-PCR has also been established for ISAV, allowing a further increase in specificity and sensitivity.

Immune Response, Prevention and Control

Due to lack of standardized molecular and experimental tools in fish, detailed knowledge of immune responses to viral agents is limited. This represents a major problem for the development of effective vaccines against fish viral diseases. For ISA, the ability to induce a strong lymphocyte proliferative response correlates with survival and virus clearance, while induction of a humoral response is less protective, as shown in experimental trials using MHC-compatible fish.

Currently available vaccines against ISA contain inactivated whole virus grown in cell culture added to mineral oil adjuvants. Vaccines are approved by the United States Department of Agriculture (USDA) and the Canadian Food Inspection Agency (CFIA) and are commercially available in Canada and the USA. Vaccination has also been used in the Faroe Islands. The use of vaccines against ISA in the rest of Europe is subject to EU/European Fair Trade Association approval. The present policy is that control of ISA should normally be based on a nonvaccination strategy. However, due to the severe disease problems in the Faroe Islands, exceptions to these rules have been made. The efficacy of ISAV vaccines has been evaluated using a vaccination-challenge model in the laboratory. Data on the evaluation of vaccines in field situations have so far not been presented. Currently available vaccines do not result in total clearance of virus in immunized fish.

The incidence of ISA may be greatly reduced by geneal husbandry practices and control of the movement of fish, mandatory health controls, and transport and slaughterhouse regulations. Specific measures including health restrictions on affected, suspected, and neighboring farms, enforced sanitary slaughtering, segregation of different generations of fish, as well as disinfection of offal and wastewater from fish slaughterhouses and fish-processing plants, also contribute to reduction in the incidence of the disease.

See also: Orthomyxoviruses: Molecular Biology; Orthomyxoviruses: Structure of antigens.

Further Reading

Falk K, Aspehaug V, Vlasak R, and Endresen C (2004) Identification and characterization of viral structural proteins of infectious salmon anemia virus. *Journal of Virology* 78: 3063–3071.

Falk K, Namork E, Rimstad E, Mjaaland S, and Dannevig BH (1997) Characterization of infectious salmon anaemia virus, an orthomyxo-like virus isolated from Atlantic salmon (*Salmo salar* L.). *Journal of Virology* 71: 9016–9023.

Koren CWR and Nylund A (1997) Morphology and morphogenesis of infectious salmon anaemia virus replicating in the endothelium of Atlantic salmon *Salmo salar*. *Diseases of Aquatic Organisms* 29: 99–109.

Krossoy B, Devold M, Sanders L, *et al.* (2001) Cloning and identification of the infectious salmon anaemia virus haemagglutinin. *Journal of General Virology* 82: 1757–1765.

Mjaaland S, Hungnes O, Teig A, *et al.* (2002) Polymorphism in the infectious salmon anemia virus hemagglutinin gene: Importance and possible implications for evolution and ecology of infectious salmon anemia disease. *Virology* 302: 379–391.

Mjaaland S, Rimstad E, and Cunningham C (2002) Molecular diagnosis of infectious salmon anaemia. In: Cunningham CO (ed.) *Molecular Diagnosis of Salmonid Diseases*, pp. 1–22. Boston: Kluwer Academic.

Mjaaland S, Rimstad E, Falk K, and Dannevig BH (1997) Genomic characterization of the virus causing infectious salmon anaemia in Atlantic salmon (*Salmo salar* L.): An orthomyxo-like virus in a teleost. *Journal of Virology* 71: 7681–7686.

Rimstad E and Mjaaland S (2002) Infectious salmon anaemia virus. An orthomyxovirus causing an emerging infection in Atlantic salmon. *Acta Pathologica Microbiologica et Immunologica Scandinavica* 110: 273–282.

Influenza

R A Lamb, Howard Hughes Medical Institute at Northwestern University, Evanston, IL, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Antigenic drift The gradual accumulation of amino acid changes in the surface glycoproteins of influenza viruses.

Antigenic shift The sudden appearance of antigenically novel surface glycoproteins in influenza A viruses.

Pandemic The rapid spread of an infection involving many countries.

Reassortment The exchange of genome RNA segments among influenza A viruses.

Recombinant virus A virus generated through reverse genetics techniques.

Reverse genetics Techniques which allow the introduction of specific mutations into the genome of an RNA virus.
Subtype Classification of influenza A virus strains according to the antigenicity of their HA and NA proteins.

Introduction: The Disease

The disease influenza is caused by influenza virus (family *Orthomyxoviridae*). Symptoms include fever, headache, cough, nasal congestion, sneezing, and whole-body aches. Influenza virus remains an important viral pathogen of significant medical importance causing mortality statistics comparable to human immunodeficiency virus (HIV) and producing considerable morbidity in the population. There were about 22 000 deaths from acquired immune deficiency syndrome (AIDS) in the US in 1993 compared with an estimated 20 000 influenza-associated deaths in the US in each of the epidemic years between 1972–73 and 2005–06. Influenza epidemics continue to infect large numbers of people worldwide, despite the availability of inactivated vaccines derived from the current circulating strains, because of frequent natural variation of the hemagglutinin (HA) and neuraminidase (NA) envelope proteins of the virus. This variation allows the virus to escape neutralization by preexisting circulating antibody in the blood stream, present as a result of either previous natural infection or immunization.

Taxonomy and Nomenclature

The *Orthomyxoviridae* comprises five genera: *influenzavirus A*; *influenzavirus B*; *influenzavirus C*; *thogotovirus* which are tick-borne viruses; and *isavirus* which includes infectious salmon anemia virus. Influenza A viruses are further classified into subtypes based on the antigenic properties of their surface spike glycoproteins, the HA, and NA. There are 16 known HA subtypes (H1–H16) and nine NA subtypes (N1–N9). Both influenza A and B virus strains cause disease, but influenza A virus strains, in most epidemic years, are usually more widespread. All human pandemics have been caused by influenza A virus strains. Retrospective seroepidemiology indicates that the 1889–91 pandemic was caused by an H3-like virus. The 1918–19 pandemic was caused by an H1N1 virus, the 1957 pandemic by an H2N2 virus, and the 1967–68 pandemic by an H3N2 virus. In 1977, there was a reintroduction in the human population of a co-circulating H1N1 virus, and since 2001 the reassortant virus H1N2 has been isolated. Since 1997 a highly pathogenic avian virus (H5N1) has infected millions of domestic fowl, and as of 12 March 2007 is known to have infected 278 humans resulting in 168 deaths.

Transmission and Tissue Tropism

Influenza viruses of humans and other mammals are spread by aerosols, including sneezing. The virus replicates in the cells of the upper and lower respiratory tract, reaching a peak at 2–3 days after infection, and the virus is usually cleared in 7 days. Children experiencing their first infection can shed virus up to 13 days.

Influenza viruses of birds are usually spread by fecal contamination of water but can also be spread by aerosols (e.g., high pathogenicity H5N1). Avian influenza viruses replicate in both the respiratory tract and the lower intestinal tract, hence the shedding of high concentrations of virus into feces.

Virus Isolation and Propagation

The first influenza virus was isolated from pigs in 1930 and the first human virus in 1933 (influenza A virus). Influenza B virus was isolated in 1940 and influenza C virus in 1946. Influenza viruses can either be propagated in tissue culture cells (particularly Madin–Darby canine kidney cells) or in the allantoic cavity of embryonated chicken eggs.

The Virus – Structure, Genome, and Proteins

The *Orthomyxoviridae* are enveloped viruses of 150–200 nm diameter. In the electron microscope, the shape of the viruses ranges from roughly spherical to pleomorphic, and filamentous viruses over a micron in length are observed from some hosts (**Figure 1**). The lipid envelope of influenza viruses is derived from the plasma membrane of the host cell in which the virus is grown. Influenza A and B virions are morphologically indistinguishable, whereas influenza C virions can be distinguished from the other genus as the glycoprotein spike is organized into orderly hexagonal arrays.

The influenza A, B, and C viruses can be distinguished on the basis of antigenic differences between their nucleocapsid (NP) and matrix (M) proteins. Influenza A viruses are further divided into subtypes based on the antigenic nature of their HA and NA glycoproteins. Other important characteristics that distinguish influenza A, B, and C viruses are given as follows.

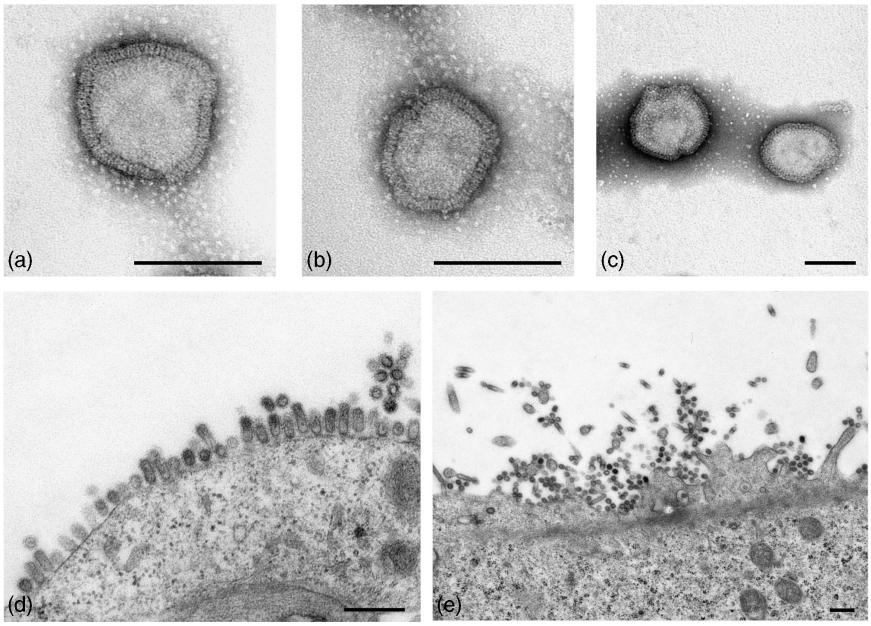


Figure 1 Electron micrographs of purified influenza virus virions (A/Udorn/72) (a–c) and A/WSN/33 virions budding from the surface of infected MDCK cells (d, e). Scale = 100 nm (a–c), 500 nm (d, e). Data provided by Dr. George Leser, Northwestern University, Evanston, IL, USA.

1. Influenza A viruses infect naturally a wide variety of avian species, humans, and several other mammalian species including swine and horses. Influenza B virus appears to naturally infect only humans, but influenza C virus has been isolated mainly from humans and also from swine in China.
2. The HA and NA of influenza A viruses exhibit much greater amino-acid-sequence variability than their counterparts in the influenza B viruses. Influenza C virus has only a single multifunctional glycoprotein, HA-esterase-fusion protein (HEF).
3. Although influenza A, B, and C viruses possess similar proteins, each virus type has distinct mechanisms for encoding proteins.
4. Influenza A and B viruses each contain eight distinct RNA segments whereas influenza C viruses contain seven RNA segments.

The most striking feature of the influenza A virion is a layer of about 500 spikes radiating outward (10–14 nm) from the lipid envelope. These spikes are of two types: rod-shaped spikes of HA and mushroom-shaped spikes of NA. The ratio of HA to NA varies but is usually 4–5 to 1. Influenza A, B, and C viruses also encode other integral membrane proteins, the M2 (influenza A virus), NB and BM2 (influenza B virus), and CM2 (influenza C virus) proteins, respectively. Biochemical evidence indicates that these proteins are only present in a few copies in virions. The viral matrix protein (M1) is thought to underlie the lipid bilayer and to associate with the cytoplasmic tails of the glycoproteins and the ribonucleoprotein (RNP) core of the virus.

Inside the virus, observable by thin sectioning of virus or by disrupting particles, are the RNP structures which can be separated into different size classes and contain eight different segments of single-stranded RNA. The RNPs have the appearance of flexible rods. The RNP strands often exhibit loops on one end and a periodicity of alternating major and minor grooves, suggesting that the structure is formed by a strand that is folded back on itself and then coiled on itself to form a type of twin-stranded helix. The RNPs consist of four protein species and RNA. NP is the predominant protein subunit of the nucleocapsid and coats the RNA, approximately 20 nucleotides per NP subunit. Associated with the RNPs is the RNA-dependent RNA polymerase complex consisting of the three P (polymerase) proteins – PB1, PB2, and PA – which are only present at 30–60 copies per virion. The NS2/NEP protein is present at 130–200 molecules per virion (**Figure 2**).

Influenza A and B viruses each contain eight segments of single-stranded RNA (for influenza A virus 2341 nucleotides to 890 nucleotides chain length), and influenza C viruses contain seven segments of single-stranded RNA (influenza C viruses lack an NA gene). The gene assignment for influenza A virus is as follows: RNA segment 1 codes for PB2, 2 for PB1 and in some strains also PB1-F2, 3 for PA, 4 for HA, 5 for NP, 6 for NA, 7 for M1 and M2, and 8 for NS1 and NS2/NEP. PB1, PB2, and PA form the RNA-dependent RNA polymerase and together with the NP protein form the RNPs. HA has receptor (cell surface expressed sialic acid (SA)) binding activity and HA through multivalent interactions attaches the virus to the surface of

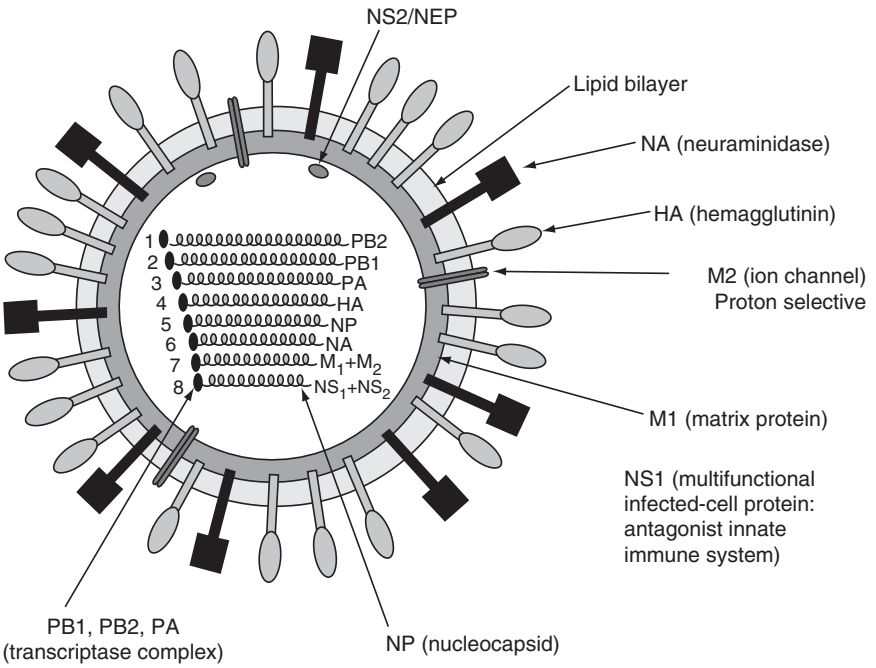


Figure 2 A schematic diagram of the structure of the influenza A virus particle. Three types of integral membrane protein – hemagglutinin (HA), neuraminidase (NA), and small amounts of the M2 ion channel protein – are inserted through the lipid bilayer of the viral membrane. The virion matrix protein M1 is thought to underlie the lipid bilayer and also to interact with the helical RNPs. The NS2/NEP protein associates with the M1 protein and is required for export of the RNPs out of the nucleus. Within the envelope are eight segments of single-stranded genome RNA (ranging from 2341 to 890 nucleotides) contained in the RNP. Associated with the RNPs are small amounts of the transcriptase complex, consisting of the proteins PB1, PB2, and PA. The coding assignment of the eight RNA segments are also illustrated. RNA segments 7 and 8 each code for more than one protein (M1 and M2, and NS1 and NS2/NEP, respectively). NS1 is found only in infected cells and is not thought to be a structural component of the virus. NS1 is a multifunctional protein and its functions include being an antagonist of the innate immune system. Adapted from Lamb RA and Krug RM (2001) *Orthomyxoviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology*, 4th edn., pp. 1487–1531. Philadelphia, PA: Lippincott Williams and Wilkins.

a cell. HA also mediates the entry of the virus into cells by causing virus–cell membrane fusion. NA has neuraminidase activity that cleaves SA from complex carbohydrate molecules. Although seemingly an opposing activity to HA-binding SA, HA binding and NA activity work at different pHs and at different times in the virus life cycle. Thus, NA activity is the receptor-destroying activity necessary for virus release from cells. The viral matrix protein (M1) is thought to underlie the lipid bilayer and to associate with the cytoplasmic tails of the glycoprotein and the RNP core of the virus. The NS2 protein forms an association with the M1 protein, which is thought to be an essential interaction in the virus life cycle for export of the RNP complex from the nucleus. The NS1 protein is a multifunctional protein. It mediates the block of transport of host cell mRNAs from the nucleus, it interacts with phosphatidylinositol-3-kinase blocking phosphorylation of the downstream effector molecule Akt, and NS1 has major roles in defeating the innate immune system and limiting the production of interferons. PB1-F2 is thought to be involved in promoting apoptosis of host cells. The influenza A virus M2 protein and the influenza B virus BM2 protein are proton-selective ion channels that cause acidification of the interior of the virus particle during uncoating of the virion in endosomes. M2 protein is the target of the antiviral drug amantadine. The function of the influenza B virus NB protein is not known. NB is not essential for replication of the virus in tissue culture cells but it appears to confer a growth advantage in infections of mice. The influenza C virus CM2 protein has been suggested to be the counterpart of M2 and BM2 and to have ion channel activity, but this has not been shown rigorously (**Figure 3**).

Evolution of Influenza Virus

The finding that all known HA and NA subtypes are maintained in avian species together with phylogenic analysis of genome nucleotide sequences led to the hypothesis that all mammalian influenza A viruses are derived from the avian influenza virus pool. The evolutionary rates for avian virus both at the nucleotide and amino acid level are significantly lower than for human viruses, a finding which suggests that the influenza viruses

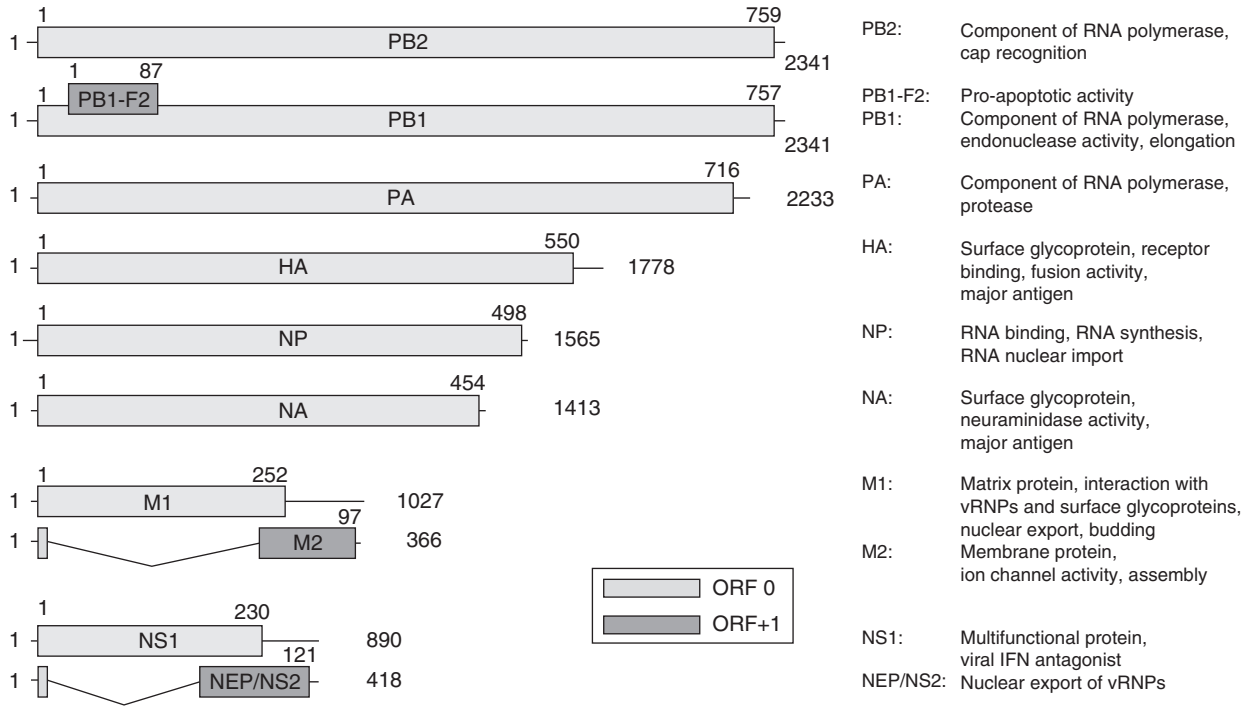


Figure 3 Genome structure of influenza A/Puerto Rico/8/34 virus. RNA segments (in nucleotides) shown in positive sense and their encoded proteins (in amino acids). The lines at the 5′ and 3′ termini represent the noncoding regions. The PB1 segment contains a second ORF in the +1 frame resulting in the PB1-F2 protein. The M2 and NEP/NS2 proteins are encoded by spliced mRNAs (the introns are indicated by the V-shaped lines). Adapted from Palese P and Shaw ML (2006) *Orthomyxoviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology*, 5th edn., pp. 1647–1689. Philadelphia, PA: Lippincott Williams and Wilkins.

of aquatic birds are in evolutionary stasis and thus are optimally adapted to their hosts. Thus, although mutations occur, they do not lead to amino acid changes. In contrast, mammalian and domestic poultry viruses continue to accumulate amino acid changes. Among human influenza viruses, the genes accumulate mutations at different rates despite the RNA polymerase having a constant mutation rate, for example, HA is evolving faster than the genes for the internal proteins, probably because of selection pressure. The H3 HA gene has evolved in a single lineage since 1968 when it was first introduced into humans with a mutation rte of 4×10^{-3} substitutions per nucleotide per year and 5×10^{-3} substitutions per residues per year in HA1. Most of these amino acid changes occur in the antigenic epitopes (sites) and it is for this reason that the influenza virus vaccine has to be reformulated each year. In contrast to influenza A virus, type B and C viruses appear to be at or near an evolutionary equilibrium in humans.

Influenza Virus Genetics

Reassortment

Reassortment is the switching of viral RNA (gene) segments in cells infected with two different influenza viruses. The term recombination is often used incorrectly for the process of reassortment. Although reassortment occurs for influenza A, B, and C viruses, it does not occur among the different types. The 1957 and 1968 pandemic viruses have been determined to be reassortments between HA and NA (1957) and HA and PB1 (1967) of avian virus origin into a human virus genetic background, and the H5N1 viruses circulating between 1997 and 2007 arose from multiple reassortment events among avian influenza viruses.

Recombination

Recombination is a rare event caused by the virion-associated RNA polymerase switching templates. Recombination has led to the insertion of nonviral nucleotide sequences into the HA gene and to the generation of defective-interfering (DI) RNAs.

Reverse Genetics

Several systems are available for the generation of influenza viruses from cloned DNA. The process is highly efficient and novel viruses, including reconstruction of 1918 influenza virus, have been generated. The reverse genetic systems for influenza virus have permitted a detailed structure–function analysis of the viral proteins and their role in the virus life cycle, pathogenesis, and host range.

Influenza Pandemics

Pandemics are outbreaks that occur in very large geographic areas, usually involving more than one continent, and affecting large percentages of the population in a short period of time.

The Pandemic of 1918–19 – Spanish Influenza

The 1918–19 pandemic killed more than 25 million people worldwide and reduced life expectancy in the US by 10 years. Approximately one-third of the US population became sick and the mortality rate is estimated to be greater than 2.5%. The mortality pattern was different from that observed for other influenza virus pandemics. Usually the highest death rates are found in very young children and in the elderly. However, for 1918, a large number of deaths occurred in young adults (15–35 years old). Analysis of the reconstructed 1918 virus indicates that this virus is highly pathogenic for mice and nonhuman primates and the available data suggest that the 1918 virus causes a 'cytokine storm' leading to inflammatory cell infiltration and hemorrhage.

The Pandemics of 1957 (Asian Influenza) and 1968 (Hong Kong Influenza)

In 1957, a newly identified H2N2 influenza virus that is a reassortment between avian and human genes (see the section titled 'Reassortment') was identified as the causative agent of influenza. Although the ensuing pandemic was not extraordinarily pathogenic, the increased mortality (70 000 deaths in the US and over 1 million worldwide) is attributed to the lack of preexisting immunity for HA and NA among humans. In 1968 the H2N2 Asian influenza virus was completely replaced by an H3N2 virus and this virus was also a reassortment between avian and human viruses. This virus was moderate in its pathogenicity (in the US, 33 800 excess mortality) but the attack rate (40%) was highest in 10–14 year olds. Probably, preexisting antibodies to the N2NA moderated the disease in older humans.

The Re-Emergence of H1N1 Viruses in 1977 – Russian Influenza

In May 1977, an outbreak of influenza occurred on the Russian–Chinese border that spread rapidly in the former Soviet Union and China, and by 1978 reached the US. The virus was identified as an H1N1 virus that was very closely related to H1N1 viruses that circulated in the 1950s. It was suggested at the time, and it is still the prevailing view, that an accidental release of virus from a laboratory started the pandemic. In 2007, this H1N1 virus is still co-circulating with the H3N2 virus.

The H5N1 'Bird Flu' Outbreak

In Hong Kong, in May 1997 a 3-year-old boy died of influenza that was identified as being caused by an H5N1 virus that was entirely of avian origin. This was the first known occurrence of transmission of an avian virus to humans with a fatal outcome. In the fall of 1997, 17 additional cases were reported with five fatal outcomes. However, importantly, there was no evidence of human-to-human transmission. Culling of all poultry in Hong Kong's live bird markets is attributed to preventing further human infections. Nonetheless, the potential for avian influenza viruses transmitting to humans was not fully realized until these cases occurred. The observed mortality rate of 33% is atypical for influenza virus infections and this formed the basis for the worldwide concern that there may be a looming pandemic of grave consequences.

In July 2003 a new outbreak of H5N1 virus started in Thailand, Vietnam, and Indonesia. Since then the virus has spread over much of Asia, reached southeast Europe, and Africa, particularly Nigeria. The virus had led to the depopulation or death through disease of over 100 million poultry. These more recent H5N1 viruses have caused severe disease in limited numbers of humans. As of 12 March 2007, there have been 278 known human infections resulting in 168 deaths. These more recent H5N1 viruses cause systemic infections in humans, with virus being recovered from the stool and cerebrospinal fluid in addition to respiratory organs. Again, to date, there is no conclusive evidence for extensive human-to-human spread of disease.

Influenza Virus in Humans

Antigenic Drift

Antigenic drift is caused by point mutations and is defined as the minor gradual antigenic changes in the HA or NA protein. Influenza A virus drift variants result from the positive selection of spontaneously arising mutants by neutralizing antibodies, that is, antibody escape mutants. Mutations on the human virus HA or NA amino acid sequence occur at a frequency of less than 1% per year. Nonetheless, antigenic drift variants can cause epidemics and often prevail for 2–5 years before being replaced by a different variant.

HA is the major antigenic spike glycoprotein and from the atomic structure of prefusion HA and mapping of both natural virus HA variants and laboratory-derived monoclonal antibody escape mutants, it was possible to determine that HA possessed five antigenic sites (epitopes). These antigenic sites are all located in HA1 at or near the top of the molecule and mostly are found in protein loops. Similarly, antigenic drift has been found for NA and the sites of antigenic drift mapped to specifc regions of the NA atomic structure.

Antigenic Shift

Antigenic shift involves major antigenic changes and it occurs through the introduction, in a new human virus, of immunologically distinct HA and/or NA molecules. Antigenic shift leads to high infection rates in an immunologically naïve population and is the cause of influenza pandemics. As discussed above (reassortment) antigenic shift occurs through mixing of human and avian genes.

Influenza Virus in Animals

The natural host (reservoir) for influenza A viruses are aquatic birds, but influenza A viruses infect a wide variety of animals in addition to humans. These include land birds and poultry, swine, horses, dogs, cats, whales, and seals. In the hosts, apart from humans and poultry, influenza viruses usually cause asymptomatic infections and the viruses are in evolutionary stasis. Even H5N1 viruses are in evolutionary stasis in aquatic birds (with notable exceptions). However, on introduction into land-based poultry or mammalian species, they evolve rapidly.

In aquatic birds, influenza viruses replicate in the epithelial cells of the intestinal tract and these birds shed influenza viruses in high concentration in feces. Influenza viruses have been isolated routinely from lakes or ponds where migratory birds have congregated. In contrast, human influenza viruses replicate in the upper respiratory tract of ducks, but not in their intestinal tract. In part, this is thought to be due to the difference in linkage of receptor molecules used by avian and human viruses (SA linked to galactose via α2,3 vs. α2,6 linkages) and corresponding amino acids located in the receptor-binding sites on the tip of the HA molecule.

Avian influenza viruses are classified as highly pathogenic (HPAI) or low pathogenicity (LPAI) viruses. LPAI viruses cause mild respiratory disease, whereas HPAI viruses cause extensive mortality. Although multiple genes are involved in virulence, high pathogenicity is always associated with the HA molecule containing a series of basic residues at the cleavage site such that the HA molecules are cleaved to the two chains HA1 and HA2 intracellularly in the *trans*-Golgi network by the endogenous enzyme furin. In contrast, low pathogenicity viruses contain an HA molecule that contains a single basic residue in the HA cleavage site and HA is cleaved/activated by extracellular trypsin-like enzymes.

There have been many outbreaks of HPAI since 1955 and these have always involved the H5 or H7 subtypes. Although LPAI viruses had been thought to be innocuous, it is now clear that HPAI arises from LPAI of the same H5 and H7 subtypes.

Several studies have indicated a role for pigs in the emergence of pandemic influenza. This includes the finding that pigs can be naturally or experimentally infected with avian influenza viruses and that the pig trachea contains both avian (α2,3-linked SA) and human-type receptors (α2,6-linked SA). Replication of avian viruses in pigs leads to variants that prefer human-type receptors. Taken together, these finding suggest that pigs can host genetically diverse viruses and lead to the notion that pigs may be the 'mixing vessel', that is, pigs can be infected simultaneously with avian and human viruses which permits the generation of reassortant viruses that could cause pandemic influenza.

H7N7 and H3N8 viruses have historically been associated with equine influenza. The horse-racing industry has promoted the use of both killed and live-attenuated vaccines for these equine-tropic viruses.

In the laboratory, although not a natural host, mice are used extensively as an animal model. Except for mouse adapted strains (e.g., A/WSN/33 and A/PR/8/34) that have been passaged multiple times through mouse brains, most human viruses do not cause mouse lethality, but they do replicate in mice and cause transient weight loss. Interestingly, recently isolated H5N1 viruses are lethal for mice without prior adaptation. Most inbred strains of mice lack the interferon-induced Mx gene and thus although mice may be a convenient small animal model it is not a perfect model.

Ferrets are readily infected by influenza A or B viruses and they develop a febrile rhinitis. The long trachae of the ferret is thought to resemble the human trachea and pathological changes of bronchitis and pneumonia resemble those seen in humans. The drawback of ferrets is that they are much more difficult to handle than mice.

Both Old and New World primates can be infected with influenza viruses. Recently, cynomolgus macaques have been used as a model system for H5N1 viruses and found to develop acute respiratory distress syndrome with fever, analogous to the disease observed in H5N1-infected humans.

Molecular Determinants of Host Range Restriction and Pathogenesis

Three proteins, HA, PB2, and NS1, have been identified as major determinants in host range restriction and pathogenicity of influenza viruses.

The HA Protein

Cleavage of HA

HA is synthesized as a precursor polypeptide chain that for HA to be active in mediating virus-cell fusion, the precursor HA0 has to be cleaved at a specific site – the cleavage site – in two chains HA1 and HA2. The atomic structure of HA shows that HA1 contains the receptor-binding site and the sites of the antigenic epitopes and

HA2 is the domain of the protein-mediating membrane fusion. The cleavage site of HPAI H5 and H7 viruses contains mutiple basic residues and are recognized by proteases resident in the *trans*-Golgi network such as furin or PC6 and thus can be cleaved in multiple different organs. In contrast, HA of LPAI and human viruses contain a single arginine residue in the cleavage site that has to be cleaved by extracellullar trypsin-like proteases and is cleaved in only a few organs.

Receptor specificity

The HA of human viruses bind preferentially to SA that is linked to the penultimate galactose (Gal) residue by an α2,6-linkage, whereas most avian and equine viruses have a higher binding affinity for SA linked to galactose via an α2,3-linkage. Nonciliated epithelial cells express SAα2,6 Gal oligosaccharides and are predominantly infected by human influenza virus. It has also been found that whereas SAα2,6Gal oligosaccharides are dominant on epithelial cells in nasal mucosa, trachea, and bronchi, SAα2,3Gal oligosaccharides are found on nonciliated bronchiolar cells at the junction between the respiratory bronchiole and alveolus and also on type cells lining the alveolar wall.

Receptor specificity is determined by the nature of the amino acids that form the receptor-binding pocket. The residues at positions 226 and 228 play an important role in receptor specificity for the H2 and H3 HAs and receptor specificity can be switched by mutations of these amino acid residues. For H1 HAs receptor specificity is determined in large part by the nature of the residues found at position 190 (H3 residue numbering).

The NS1 Protein

In addition to affecting the transport of host mRNAs out of the nucleus, NS1 functions as an interferon (INF) antagonist. Probably, all viruses need a system to defeat INF action in INF-competent hosts. The NS1 protein targets both IFN β production and the activation of INF-induced genes that are required for establishing the antiviral state. Although there are 200–300 IFN-stimulated genes, the biological properties of the encoded proteins are known only for a few, for example, dsRNA-activated protein kinase (PKR), Mx proteins, 2′,5′ oligoadenylate synthetase and RNAase L. NS1 interferes with PKR and RNase L.

NS1 protein from different influenza viruses appears to have different abilities to counteract the IFN system and thus this affects pathogenicity of the virus. Viruses that defeat the IFN system well are more pathogenic than those that only cause partial inactivation of the IFN system. Several findings suggest that the NS1 protein of highly pathogenic viruses may cause the cytokine imbalance and that has been observed in patients infected with H5N1 virus or macaques infected with 1918 H1N1 virus.

The PB2 Protein

The PB2 protein is part of the RNA-dependent RNA polymerase complex. PB2 binds to ^{7}MeGpppG cap structures on host mRNAs; the cap structure and 10–13 nucleotides of the host mRNA are cannibalized by the influenza virus RNA polymerase to act as primers for mRNA transcription. The PB2 protein and the residue found at position 627 (glutamic acid in LPAI and lysine in HPAI) have emerged as important determinants of virulence. How the mechanism by which these residue changes affect the polymerase activity is not fully elucidated. However, it appears that viruses with lysine at position 627 grow faster than those with glutamic acid at this position.

The NA Protein

HA of the A/WSN/33 human influenza virus contains a single arginine residue at the HA cleavage site; yet, for plaque formation in tissue culture unlike other human influenza virus strains, WSN does not require trypsin for multiple rounds of virus replication and plaque formation. However, the ability of A/WSN/33 to form plaques without trypsin requires the A/WSN/NA gene. It was determined that the WSN NA protein has lost a carbohydrate chain as compared to other N1NA proteins and that the lack of the carbohydrate chain permits the WSN NA to bind plasminogen, the precursor to the protease plasmin. It is envisaged that the tethered plasmin cleaves HA.

Antivirals

Amantadine and Rimantadine: M2 Ion Channel Blockers

Amantadine, and its methyl derivative rimantadine, were discovered as anti-influenza viral drugs in the 1960s by workers at DuPont. Rimantadine is licensed in the US for both the treatment and prophylaxis of influenza and at one time was being used extensively for prophylaxis in nursing homes. The drugs target the pore (transmembrane domain) of the M2 proton-selective ion channel. A drawback to the use of rimantadine is that drug-resistant variant viruses arise in humans within 4 days of drug treatment. Quite remarkably in 2005–06, the circulating H3N2 viruses were almost all rimantadine resistant despite little use of rimantadine. It has been postulated that the rimantadine-resistance mutation in M2 piggy-backed with another change in HA which conferred viral fitness.

NA Inhibitors

Two neuraminidase inhibitors are licensed in the US for the treatment and prophylaxis of influenza. Zanamivir is a derivative of the transition state intermediate 2-deoxy-2, 3-dihydro-*N*-acetylneuraminic acid and the drug binds

in the NA active site and has a K_i of 2×10^{-10}. The drug has to be administered intranasally or inhaled. Oseltamivir is another derivative of the transition state intermediate 2-deoxy-2,3-dihydro-*N*-acetylneuraminic acid and it contains a lipophilic group, which improves its bioavailability, and the drug can be taken orally. Although when used for treatment of influenza these drugs only reduce the duration of illness by a day or two, this may be highly significant (beneficial) in infections of high pathogenicity viruses.

Resistant variants to the NA inhibitors have been selected both *in vitro* and *in vivo* but at a much lower frequency than for amantadine-resistant variants. In part, this is because the drugs interact with several residues in the active site and each of these residues is important for enzyme activity. Thus, mutation of a residue in the active site leads to a lowered K_i and a lowered enzyme activity. Interestingly, emergence of resistance is first detected by finding mutations in the SA-binding site of HA, which compensates for reduced NA activity; the affinity of HA for its receptor is lowered. Later mutations occur in NA in the framework residues that surround the active site and stabilize its structure.

Vaccines

Inactivated Vaccines

Inactivated influenza A and B virus vaccines are licensed for administration in humans. The vaccine is reformulated each year to include the strains thought most likely to be prevailing. The choice of seed virus is made by the World Health Organization (WHO) and the US Centers for Disease Control (CDC). The vaccine virus is currently grown in embryonated eggs but because many human influenza virus isolates do not grow to high yield in eggs, a reassortment virus is made using the high egg-yielding PR/8/34 genetic backbone and the HA and NA genes of the candidate virus. Reverse genetics procedures speed up the time needed to produce a high-yielding reassortment over the former method of mixed infection and selecting the virus from random plaques. Vaccine is manufactured by harvesting allantoic fluid and concentrating the virus by zonal centrifugation and inactivation of infectivity with formalin or beta-propiolactone. One egg yields one to three doses of vaccine. The vaccine is administered intramuscularly. Vaccination with inactivated virus has been shown to consistently confer resistance to illness (reduced frequency and severity of disease), and to a somewhat lesser extent infection with influenza A and B viruses.

Live Virus Vaccines

A live attenuated vaccine (Flumist) has been licensed. The vaccine virus is based on the genetic backbone of a cold-adapted virus (A/Ann Arbor/6/60) and a reassortment made to incorporate the current HA and NA genes. The cold-adapted virus replicates efficiently in the nasopharynx to induce protective immunity. However, replication is restricted at higher temperatures, including those present in the lower airways and lungs. In clinical studies with matched strains, the live virus vaccine demonstrated 87% efficacy in children and 85% in adults. There is continued interest in the use of a live influenza virus vaccine because infection of the respiratory tract stimulates both systemic and local immunity, and in principle should stimulate cell-mediated immunity. Thus, all components of the human immune response are brought into action. Furthermore, there is the added advantage of acceptance of a nasal spray rather than a needle injection in young children.

See also: Antigenic Variation; Antiviral Agents; Assembly of Viruses: Enveloped Particles; Defective-Interfering Viruses; Diagnostic Techniques: Microarrays; Electron Microscopy of Viruses; Emerging and Reemerging Virus Diseases of Vertebrates; History of Virology: Vertebrate Viruses; Immune Response to viruses: Antibody-Mediated Immunity; Innate Immunity: Defeating; Innate Immunity: Introduction; Membrane Fusion; Orthomyxoviruses: Molecular Biology; Orthomyxoviruses: Structure of antigens; Replication of Viruses; Vaccine Strategies; Viral Membranes; Viral Pathogenesis; Viral Receptors; Viruses and Bioterrorism; Zoonoses.

Further Reading

Fouchier RA, Schneeberger PM, Rozendaal FM, *et al.* (2004) Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proceedings of the National Academy of Sciences, USA* 101: 1356–1361.

Gamblin SJ, Haire LF, Russell RJ, *et al.* (2004) The structure and receptor binding properties of the 1918 influenza hemagglutinin. *Science* 303: 1838–1842.

Garcia-Sastre A (2004) Indentification and characterization of viral antagonists of type 1 interferon in negative-strand RNA viruses. *Current Topics in Microbiology and Immunology* 283: 249–280.

Goto H and Kawaoka Y (1998) A novel mechanism for the acquisition of virulence by a human influenza A virus. *Proceedings of the National Academy of Sciences, USA* 95: 10224–10228.

Guan Y, Poon LL, Cheung CY, *et al.* (2004) H5N1 influenza: A protean pandemic threat. *Proceedings of the National Academy of Sciences, USA* 101: 8156–8161.

Gubareva LV, Kaisr L, and Hayden FG (2000) Influenza virus neuraminidase inhibitors. *Lancet* 355: 827–836.

Kawaoka Y and Webster RG (1988) Sequence requirements for cleavage activation of influenza virus hemagglutinin expressed in mammalian cells. *Proceedings of the National Academy of Sciences, USA* 85: 324–328.

Lamb RA, Holsinger LJ, and Pinto LH (1994) The influenza A virus M_2 ion channel protein and its role in the influenza virus life cycle. In: Wimmer E (ed.) *Receptor-Mediated Virus Entry into Cells*, pp. 303–321. Cold Spring Harbor, NY: Cold Spring Harbor Press.

Lamb RA and Krug RM (2001) Orthomyxoviridae: The viruses and their replication. In: Knipe DM and Howley PM (eds) *Fields Virology*,

4th edn., pp. 1487–1531. Philadelphia, PA: Lippincott Williams and Wilkins.
Mastrosovich MN, Mastrosovich TY, Gray T, *et al.* (2004) Human and avian influenza viruses target different cell types in cultures of human airway epithelium. *Proceedings of the National Academy of Sciences, USA* 101: 4620–4624.
Neumann G, Watanabe T, Ito H, *et al.* (1999) Generation of influenza A viruses entirely from cloned cDNAs. *Proceedings of the National Academy of Sciences, USA* 96: 9345–9350.
Palese P and Shaw ML (2006) *Orthomyxoviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 5th edn, pp. 1647–1689. Philadelphia, PA: Lippincott Williams and Wilkins.
Skehel JJ and Wiley DC (2000) Receptor binding and membrane fusion in virus entry: The influenza hemagglutinin. *Annual Review of Biochemistry* 69: 531–569.
Tumpey TM, Basler CF, Aguilar PV, *et al.* (2005) Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 310: 77–80.
Varghese JN, Laver WG, and Colman PM (1983) Structure of the influenza virus glycoprotein antigen neuramindase at 2.9 A resolution. *Nature* 303: 35–40.
Wright PF, Neumann G, and Kawaoka Y (2006) Orthomyxoviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 5th edn, pp. 1691–1740. Philadelphia, PA: Lippincott Williams and Wilkins.

Innate Immunity: Defeating

C F Basler, Mount Sinai School of Medicine, New York, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Complement A system of serum proteins that function to promote the phagocytosis or lysis of pathogens or pathogen-infected cells.
Conventional dendritic cell (cDC) A phagocytic cell type that serves as a sentinel for the immune system and that links innate and adaptive immune responses. Upon encountering an antigen, cDCs can be induced to undergo maturation, such that they produce cytokines, upregulate to their surface co-stimulatory molecules, and promote T-cell activation.
Cytokine A secreted protein that promotes or modulates immune responses.
Interferon α/β (IFN-α/β) A family of structurally related cytokines the expression of which can be induced by virus infection and other stimuli. IFN-α/β proteins bind to the IFN-α/β receptor activating a signaling pathway that modulates the expression of hundreds of genes and induces in cells an antiviral state.
Natural killer (NK) cell A lymphocyte that can kill target cells in a non-antigen-specific manner. Killing by NK cells is tightly regulated by positive and negative signals.
Opsonization The coating of a particle so as to promote its phagocytosis.
Pathogen-associated molecular patterns (PAMPs) Molecular patterns found on pathogens that are recognized as 'foreign' and that trigger innate immune responses.
Pattern recognition receptor (PRR) A host molecule that recognizes PAMPs and signals to stimulate innate immune responses.
Toll-like receptor (TLR) A member of a family of type I transmembrane proteins that serve as pattern-recognition receptors. TLRs signal through association with cytoplasmic adaptor proteins.

Innate Immunity

Immune responses have evolved, in part, to eliminate or to contain infectious agents such as viruses. Innate immunity consists of a variety of relatively nonspecific and short-lived responses typically triggered soon after the appearance of an antigen (such as a virus or other pathogen). Innate immune mechanisms can be contrasted with adaptive immune responses in which responses are antigen specific and are characterized by immunological memory.

Innate immunity can be mediated by proteins, for example, interferons (IFNs), other cytokines, or complement; and by specific cell types such as macrophages, dendritic cells (DCs), and natural killer (NK) cells. These innate responses act rapidly to suppress infection. Given that viruses have co-evolved with their hosts, it is perhaps not surprising that viruses have evolved ways to defeat innate immune responses. For virtually any effector of the innate immune response, an example can be found whereby a virus overcomes or counteracts this host response. The strategies employed by viruses to evade these responses will vary. In general, however, viruses with large genomes, such as are found in many DNA viruses, may produce 'accessory proteins' that specifically carry out immune-evasion functions. In contrast, viruses with small genomes, such as are common among many RNA viruses,

often encode multifunctional proteins. These may carry out both immune-evasion functions and functions essential for virus replication. However, regardless of the general strategy employed, suppression of innate immunity is generally critical for viral pathogenesis.

The IFN-α/β System

The IFN-α/β response serves as a major component of the innate immune response to virus infection, can be activated in most cell types, and triggers in cells an 'antiviral state'. It should be noted that there is also an IFN-γ which is structurally distinct from IFN-α/β and which is produced mainly by immune cells. Despite these differences, IFN-γ can activate signaling pathways similar to those activated by IFN-α/β and can also induce in cells an antiviral state. The IFN-α/β system can be activated in most cell types and can be viewed, in simplified terms, in two phases (**Figure 1**). The first is an induction phase in which virus infection or another stimulus activates latent transcription factors. These induce expression of the IFN-α/β genes which encode multiple IFN-α proteins and, in humans, a single IFN-β protein. In the second phase, the secreted, structurally related IFN-α/β proteins bind to the IFN-α/β receptor. This activates a Jak-STAT signaling pathway that induces expression of numerous genes, some of which have antiviral properties. There is only a single IFN-β gene but multiple IFN-α genes, and, in most cell types, it is primarily IFN-β that is produced after initial virus infection. However, in a positive-feedback loop, IFN-α/β

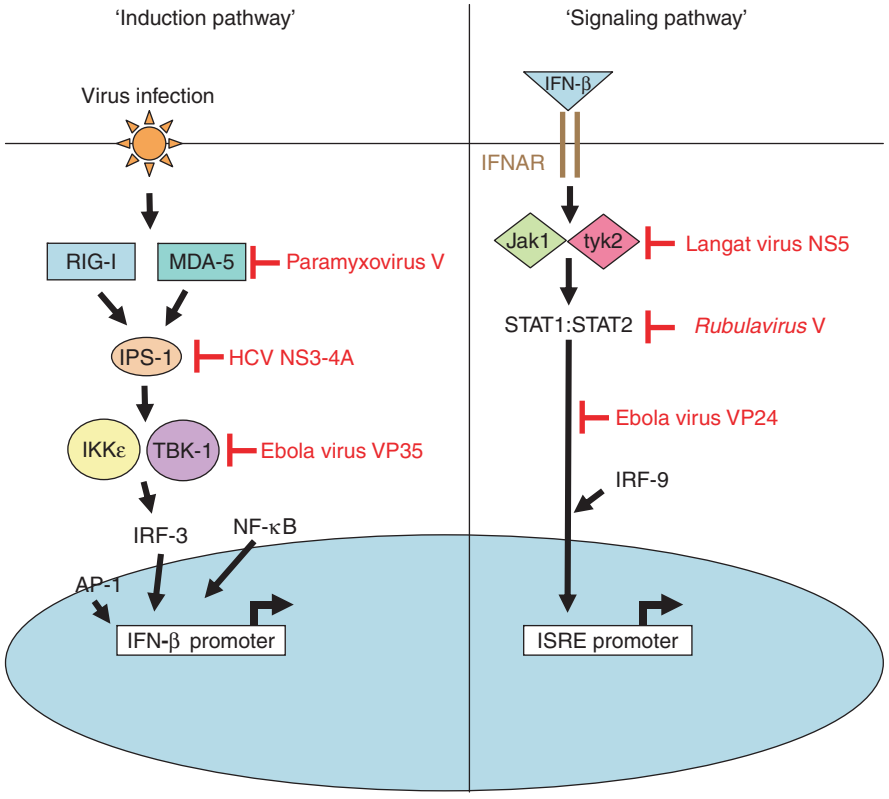


Figure 1 A simplified, schematic diagram of the IFN-α/β system and examples of viral proteins that disrupt the IFN-α/β response. Here the IFN-α/β system is depicted in two phases, each subject to virus intervention. First, the 'induction pathway' is activated by virus infection. Products of the infection activate signaling pathways, either through cytoplasmic sensors of virus infection such as retinoic acid inducible gene I protein (RIG-I) or melanoma differentiation antigen 5 protein (MDA-5) or through select Toll-like receptor (not shown). Signaling through RIG-I or MDA-5 requires IPS-1 and results in activation of the interferon regulatory factor 3 (IRF-3) kinases IKKε and TBK-1. These signaling pathways trigger activation of the transcription factors IRF-3, nuclear factor-kappa B (NF-κB), and AP-1. These cooperate to induce IFN-β gene expression. Examples of viral proteins that target the induction phase include paramyxovirus V proteins that bind and inhibit MDA-5, the hepatitis C virus (HCV) NS3-4A protease that cleaves IPS-1, and the Ebola virus VP35 protein that may act at the level of the IRF-3 kinases. Second, in the 'signaling pathway', secreted IFN-α/β binds to the IFN-α/β receptor, activating a Jak-STAT signaling cascade, resulting in the expression of numerous genes. Examples of viral proteins that target the IFN signaling pathways are the NS5 protein of Langat virus that prevents Jak1 and tyk2 kinase activation, the V proteins of the paramyxovirus genus *Rubulavirus*, which target STAT proteins for proteasome-dependent degradation, and the Ebola virus VP24 protein that prevents nuclear accumulation of activated STAT1. A third aspect of the response is the production of IFN-induced genes that encode antiviral gene products (not shown). Viral inhibitors of specific IFN-induced antiviral effector proteins also exist. Examples of inhibitors described in the text are depicted in red font in the figure.

can prime cells to produce numerous IFN-α types and larger overall amounts of IFN-α/β. The consequence is an amplification of the antiviral response.

Pathogen-Associated Molecular Patterns and Pattern-Recognition Receptors

Innate immune mechanisms, including IFN-α/β responses, may be triggered by diverse stimuli. Therefore, mechanisms have evolved to recognize as foreign structures and molecules that are conserved among groups of pathogens but distinct from host molecules. The pathogen structures and molecules recognized by the host are generically referred to as pathogen-associated molecular patterns (PAMPs), while the host molecules that recognize such structures are referred to as pattern-recognition receptors (PRRs). PRRs important for detection of virus infection include select members of the Toll-like receptor (TLR) family of transmembrane PRRs, including TLR3, TLR4, TLR7, TLR8, and TLR9, and particular cellular RNA helicases including the retinoic acid inducible gene I protein (RIG-I) and the melanoma differentiation antigen 5 protein (MDA-5).

TLRs are expressed primarily on cells, such as DCs and macrophages, which function to trigger innate immune responses and to act as antigen-presenting cells, initiating adaptive immune responses. Several TLRs have the capacity to recognize viral nucleic acids. TLR3 recognizes double-stranded RNA (dsRNA), such as polyI:polyC experimentally added to cells or dsRNA produced during the course of virus infection. TLR7 and TLR8 can be activated by virus-derived single-stranded RNAs (ssRNAs) rich in guanosine or uridine, such as those derived from influenza virus or human immunodeficiency virus (HIV). TLR9, in contrast recognizes dsDNA; and TLR9 signaling can be stimulated by CpG DNA motifs or by virus-derived dsDNA such as that from herpes simplex virus. There are also examples of other, non-nucleic acid, viral products signaling through TLRs, although such observations are often viewed as controversial because it is difficult to completely exclude the possibility that the virus preparations used contain some low level contamination with a bacterial TLR ligand. Examples of viral protein recognition by TLRs include the recognition of respiratory syncytial virus (RSV) fusion (F) protein by TLR4 and measles virus hemagglutinin protein activation of TLR2 signaling.

In general, activation of TLR signaling would seem to be a disadvantage for a virus, as it will promote innate antiviral responses. However, in some cases, viruses may take advantage of the innate response. For example, mouse mammary tumor virus (MMTV) infects B cells early during the course of infection *in vivo*. This virus can also activate TLR4 signaling. Because replication of this retrovirus requires that its host cell be dividing, the activation of B cells via TLR4 may facilitate establishment of virus infection. Similarly, activation of DCs, another early target of MMTV infection, via TLR4 signaling increases DC numbers and increases expression of CD71 the viral receptor on these cells, again promoting the infection process.

Different TLRs have different distributions among cell types, and the consequences of TLR activation will vary, depending both upon the specific TLR in question as well as the cell type on which it is activated. For example, conventional dendritic cells (cDCs) function as sentinels which, in an immature state, constitutively sample their environment for foreign antigen. Upon encountering antigen and activation of PRRs, the cDCs mature and become potent activators of T-cell responses. Of the IFN-α/β-inducing TLRs, human cDCs express TLRs 3 and 4. Their activation results in IFN-β production and the maturation of the cDC, thereby providing a link between the relatively nonspecific innate response to infection and the antigen-specific adaptive immunity of T-cell responses. In contrast, human plasmacytoid DCs (pDCs) express TLRs 7, 8, and 9, and activation of pDCs results in the production of copious amounts of IFN-α.

Many cell types do not express TLRs. However, such cells typically have the capacity to mount an IFN-α/β response to virus infection. In such cells, intracellular PRRs detect and signal in response to virus infection. Two such intracellular PRRs are RIG-I and MDA-5. RIG-I binds to and is activated by RNA molecules possessing 5′ triphosphate groups, although activation of signaling may be influence by other properties of the RNAs such as secondary structure or modifications to the RNAs. MDA-5 can be activated by intracellular dsRNA, such as transfected polyI:polyC. These properties appear to fit with data obtained from mice in which either RIG-I or MDA-5 were knocked out. Thus, viruses that produce RNAs with 5′ triphosphates during the course of their replication, including influenza viruses, paramyxoviruses, and flaviviruses, trigger IFN-β production through RIG-I. In contrast, RIG-I was not essential for detection of the picornavirus encephalomyocarditis virus in MDA-5 knockout cells, presumably because picornaviruses produce RNAs where the 5′ end is occupied by the covalently linked viral protein, VPg. MDA-5, in this case, probably activates IFN-α/β production due to recognition of viral dsRNA. It is notable however that expression from viruses of inhibitors of RIG-I or MDA-5 might influence the outcome of such analyses. For example, multiple paramyxoviruses have been found to encode 'V' proteins (discussed further below) that inhibit MDA-5 function. Thus, it is possible that paramyxovirus infection yields products that activate not only RIG-I but also MDA-5. However, because the presence of the V proteins blocks MDA-5 function, it may appear that paramyxoviruses can only activate RIG-I. The evolutionary pressure to retain an inhibitor of MDA-5 may in fact suggest a role for detection of negative-strand RNA viruses by MDA-5.

Signaling Leading to IFN-α/β Production

The cellular molecules immediately downstream of TLRs differ, depending upon which TLR is engaged. Similarly, RIG-I and MDA-5 share common downstream signaling molecules but these differ to some extent from the TLR-activated pathways.

TLR signaling is mediated by specific downstream 'TIR-domain'-containing adaptor proteins. TLR3 signaling to induce IFN-α/β expression appears to require the adapter TRIF, while TLR4 signaling appears to require TRIF and a second adaptor, TRAM. For the RIG-I and MDA-5 helicases, recognition of the appropriate nucleic acid molecule is thought to induce structural rearrangements in the helicase, exposing an otherwise repressed caspase recruitment domain (CARD, a protein:protein interaction domain), allowing the helicase to interact with downstream signaling molecules. A mitochondria-localized protein IPS-1 (also called MAVS, Cardif, or VISA) lies downstream of RIG-I and MDA-5; it is essential for the signaling from RIG-I and MDA-5. Each of these pathways leads to the activation of the cellular kinases IKKε and TBK-1 which phosphorylate interferon regulatory factor 3 (IRF-3) and also activate nuclear factor-kappa B (NF-κB), transcription factors critical for IFN-β gene expression. In contrast, TLRs 7, 8, and 9 utilize signaling pathways that are dependent upon the adapter MyD88, which involve activation of IRF-7 and which leads to the production of IFN-α.

IRF-3 and IRF-7

In most cell types, IFN-β is the primary form of IFN that is first produced. The IFN-β promoter transcription occurs following the coordinated activation of IRF-3, NF-κB, and the ATF-2/c-Jun form of AP1. The initial production of IFN-β, as well as select IFN-α genes, induces expression of a similar IRF family member, IRF-7. IRF-7, like IRF-3, is activated by phosphorylation in response to virus infection. However, unlike IRF-3, IRF-7 is able to participate in the activation of numerous IFN-α genes, thus providing a mechanism by which IFN-α/β production can be greatly amplified in response to infection. In contrast to this model, in pDCs, IRF-7 appears to be constitutively expressed and this contributes to the copious production of IFN-α by these cells in response to TLR 7, 8, or 9 agonists.

IFN Signaling

IFN-α/β and IFN-γ bind to two distinct receptors but activate similar signaling pathways. For both pathways, ligand binding activates receptor-associated Jak family tyrosine kinases. These undergo auto- and transphosphorylation and phosphorylate the cytoplasmic domains of the receptor subunits. The receptor-associated phosphotyrosine residues then serve as docking sites for the SH2 domains of STAT proteins. The receptor-associated STATs then undergo tyrosine phosphorylation and form homo- or heterodimers via reciprocal SH2 domain–phosphotyrosine interactions. Signaling from the IFN-α/β receptor results predominately in the formation of STAT1:STAT2 heterodimers which additionally interact with IRF-9. IFN-γ signaling results predominately in the formation of STAT1:STAT1 homodimers. Upon dimerization, the STAT1:STAT2 heterodimer or the STAT1:STAT1 homodimer interacts with a specific member of the karyopherin α (also known as importin α) family of nuclear localization signal receptors, karyopherin α1 (importin α5). This interaction with karyopherin α1 mediates the nuclear accumulation of these STAT1-containing complexes. The consequence of the activation and nuclear accumulation of these complexes is the specific transcriptional regulation of numerous genes, some of which have antiviral properties.

IFN-Induced Genes Encoding Antiviral Proteins

Treatment of cells with interferon induces expression of more than 100 genes and induces in cells an 'antiviral state', meaning that the cells become resistant to virus infection and/or replication. Our understanding of the IFN-induced antiviral state is very incomplete. However, the products of several IFN-induced genes have been demonstrated to exert antiviral properties. Examples include the dsRNA-activated protein kinase, PKR, which upon activation, phosphorylates the translation initiation factor eukaryotic initiation factor 2α (eIF2α). This leads to a general inhibition of translation which can suppress virus replication. Similarly, 2′-5′ oligoadenylate synthetases (OASs) are a family of IFN-induced, dsRNA-activated enzymes that catalyze formation of 2′-5′ oligoadenylates. These activate RNase L, an enzyme that destroys mRNAs and cellular RNAs and suppresses translation of proteins. Mx proteins, including Mx1 in mice or MxA in humans, belong to the dynamin family of GTPases. Their expression is IFN-inducible, and members of this family have been shown to inhibit replication of specific virus families. For example, human MxA can inhibit replication of bunyaviruses, orthomyxoviruses, paramyxoviruses, rhabdoviruses, togaviruses, picornaviruses, and hepatitis B virus. At least part of the antiviral effect of these proteins is exerted by their ability to alter the intracellular trafficking of viral proteins; for example, human MxA can sequester orthomyxovirus nucleocapsids in the cytoplasm, preventing entry into the nucleus where virus RNA synthesis takes place.

Viral Evasion of the Host IFN Response

It is likely that most, if not all, viruses successfully maintained in nature have evolved mechanisms to counteract IFN responses.

Examples of the mechanisms by which viruses defeat the IFN system include inactivation of the PRR signaling pathways, inactivation of the IFN-induced signaling pathways, and the targeting of IFN-induced antiviral 'effectors'.

Targeting Components of the PRR Signaling Pathways

Numerous examples now exist of viruses targeting the signaling pathways activated by TLRs, RIG-I, or MDA-5. Mechanisms range from targeting of the PRRs themselves, to the targeting of the transcription factors that these pathways activate, to the targeting of kinases that activate these transcription factors. The RIG-I pathway is targeted by several viruses, including Ebola virus and hepatitis A virus. In poliovirus-infected cells, the cytoplasmic PRR MDA-5 is cleaved in a caspase- and proteosome-dependent manner, presumably due to poliovirus activation of apoptosis. This observation is consistent with the idea that MDA-5 is particularly relevant to picornavirus infection. Hepatitis C virus serves as an example of a virus that can target both a TLR (TLR3) and intracellular PRR (RIG-I) pathways. HCV is an enveloped, positive-strand RNA virus of the flavivirus family associated with persistent liver infections, cirrhosis, and liver cancer. The HCV genome encodes NS3-4A, a noncovalent complex with protease and RNA helicase activity. NS3-4A has been found to inhibit, in a protease-dependent manner, virus-induced activation of IRF-3, which, as noted above, plays a major role in activating IFN-α/β responses. TLR3 signaling requires the function of the adaptor TRIF, and the protease activity of NS3-4A cleaves and inactivates TRIF, to prevent TLR3-induced IFN-α/β expression. NS3-4A was also found to cleave IPS-1, the mitochondria-localized adaptor downstream of both RIG-I and MDA-5, similarly preventing RIG-I-induced IFN-α/β expression.

Targeting Components of the IFN Signaling Pathways

Numerous examples also exist of virus-mediated inhibition of the IFN-activated Jak-STAT signaling cascades. There are many examples among the members of the paramyxovirus family itself. It should be noted that several of these also inhibit IFN-α/β production and might therefore also be classified as proteins that target multiple aspects of the IFN system (see the section titled 'Viruses targeting multiple aspects of the IFN system'). Viruses of the genus *Rubulavirus* of the paramyxovirus family encode V proteins which target STAT proteins for ubiquitin-dependent degradation. However, the specific STAT targeted by a particular V protein will vary. For example, the simian virus 5 (SV5) V protein targets STAT1 while human parainfluenza virus type 2 (HPIV2) encodes a V protein that promotes STAT2 degradation. In these cases, the V proteins appear to direct the STAT protein to a ubiquitin ligase complex that includes the proteins DDB1 and cullin 4A. In contrast, Nipah virus, an emerging zoonotic paramyxovirus that causes highly lethal encephalitis in humans, encodes proteins (P, V, and W) that share a common N-terminal domain. This domain contains sequences that mediate an interaction with STAT1, and for the V protein, also STAT2. These Nipah virus proteins inhibit STAT1 not by targeting it for degradation; rather, the P, V, and W appear to sequester STAT1 preventing the tyrosine phosphorylation that would otherwise activate it.

Upstream and downstream steps in the IFN signaling pathways are also subject to interruption by virus infection. The tyrosine kinases that activate STAT1 and STAT2 typically undergo tyrosine phosphorylation following exposure of cells to IFN. However, in cells infected with Langat virus, a flavivirus of the tick-borne encephalitis virus complex, neither STAT1/2 nor tyk2/Jak1 phosphorylation is seen. This inhibition is mediated by as association of the viral polymerase protein NS5 with both the IFN-α/β receptor and the IFN-γ receptor. In contrast, Ebola virus infection does not prevent STAT1 tyrosine phosphorylation. However, in Ebola virus-infected cells, tyrosine-phosphorylated STAT1 fails to enter the nucleus. The failure of otherwise activated STAT1 to traffic to the nucleus appears to be due to the action of the Ebola virus VP24 protein. Tyrsoine-phosphorylated STAT1 has been reported to enter the nucleus through an interaction with the nuclear localization signal receptor karyopherin alpha 1 (importin α5). VP24 interacts with this specific karyopherin alpha, and in doing so, inhibits STAT1–karyopherin alpha 1 interaction, thus explaining the defect in STAT1 nuclear import.

Targeting of Antiviral Effector Proteins

Among the IFN-induced genes with demonstrated antiviral activity, PKR has been the most heavily studied, and antagonists of PKR function were among the earliest described viral inhibitors of the IFN response. Upon activation by dsRNA, PKR phosphorylates the translation factor eIF2α, a subunit of the translation initiation factor eIF2 that brings methionyl (Met) initiator tRNA to the ribosome. Phosphorylation of eIF2α on serine 51 inhibits a GDP-to-GTP exchange reaction required for the continuing function of eIF2, and therefore this phosphorylation arrests translation at the initiation phase. As a consequence, numerous viruses have devised diverse ways to preserve the translation of their mRNAs in the face of PKR activation. Examples include the adenovirus VA_I RNA and the vaccinia virus E3L protein, which impede PKR activation. By inhibiting PKR function, these molecules not only

help preserve translation of viral mRNAs but also facilitate virus replication in the presence of an IFN response. Another notable counterbalance to PKR function is the herpes simplex virus $\gamma_1$34.5 protein. In this case, the viral protein recruits cellular phosphatase 1 alpha (PP1α) to dephosphorylate eIF-2α. A functional $\gamma_1$34.5 has been found to be required for HSV-1 neurovirulence, demonstrating its importance *in vivo*, although subsequent studies have identified additional herpes simplex virus proteins, including US11, that also facilitate translation of viral mRNAs.

Viruses Targeting Multiple Aspects of the IFN System

As was noted above, it has become apparent that viruses will often target multiple components of the IFN response. Influenza viruses serve as such an example. The influenza A virus NS1 protein is a multifunctional inhibitor of innate immune responses. Evidence suggests that NS1 directly targets RIG-I, the PRR that detects influenza viruses in most cell types. This has the consequence of preventing signaling that would lead to the activation of IRF-3 and NF-κB and to IFN-α/β production. However, NS1 proteins also can affect, in a more global way, the expression of cellular gene expression. This occurs because NS1 can interfere with host cell pre-mRNA processing and can inhibit the nuclear export of cellular mRNAs. The ability of NS1 to suppress gene expression in this way has the potential to inhibit expression of IFN-α/β genes. In addition, this function may also suppress expression of genes induced by IFN thus preventing induction of an antiviral state. Finally, NS1 is able to inhibit the activation of PKR and therefore can directly target the function of at least one IFN-induced, dsRNA-activated antiviral protein. That the NS1 protein is critical for viral evasion of IFN responses is highlighted by *in vivo* experiments. Mutant influenza viruses either lacking or encoding altered NS1s display attenuated phenotypes in mice able to mount an IFN response. However, this attenuation can be largely reversed in animals lacking a fully competent IFN system, demonstrating that the attenuation in the wild-type mice is directly related to the absence of an IFN-antagonist protein.

Defeating IFN Responses – Consequences for Adaptive Immune Responses

It should be recognized that innate cytokine responses, including IFN-α/β responses, not only serve to suppress virus replication, but also act to promote adaptive immune responses. IFN-α/β can, for example, affect T-cell responses, influencing the ability of CD8+ T-cell populations to expand and generate memory cells, although the magnitude of the IFN-effect depends upon the pathogen administered. Similarly, pathogen-specific effects of IFN-α/β upon clonal expansion of CD4+ T cells have also been described. Connections between the IFN-α/β response and the function of antigen-presenting cells have also been identified. For example, the same stimuli that activate IFN-α/β production can also activate DC maturation, promoting the upregulation of major histocompatibility complex molecules and co-stimulatory molecules, such as CD80 and CD86, and the production of cytokines. DC maturation then promotes activation of T-cell responses. Therefore, the virus-encoded mechanisms that tend to suppress IFN-α/β responses, particularly those that inhibit IFN-α/β production, may serve to suppress adaptive immune responses as well. This hypothesis is supported in part by the observation that expression of the influenza virus NS1 protein, a suppressor of IFN-α/β production, rendered viruses less able to promote human DC maturation and function compared with viruses that lacked the IFN-antagonist NS1 protein.

Other Cellular Antiviral Proteins

Other cellular proteins may also serve as innate defense against virus infection. Prime examples are members of the APOBEC3 family of cytidine deaminases which function as a defense against retrovirus infection. APOBEC3 proteins deaminate cytidines to uridines in single-stranded DNA. Family member APOBEC3G can incorporate into budding HIV-1 particles and, during reverse transcription in the subsequently infected cell, deaminate cytidines. Because this results in introduction of dC to dU changes on minus-strand DNA, the result is the dG to dA hypermutation of the positive, coding-strand of the virus genome. This can result in the introduction of lethal (to the virus) mutations as well as susceptibility to cleavage by uracil DNA glycosylase and apurinic-apyrimidinic (AP) endonuclease enzymes. However, HIV-1 can defeat the antiviral effect of APOBEC3G through the function of its protein, viral infectivity factor (Vif). Vif is required for HIV-1 replication in many cell types. This is due, in part, to the ability of Vif to bind APOBEC3G and target it for ubiquitin-dependent degradation via a ubiquitin ligase complex. The ability of Vif to eliminate APOBEC3G from cells of a particular species can determine its ability to replicate in that species. Thus, HIV-1 Vif can target APOBEC3G in humans and chimpanzees. However, the inability of HIV-1 to target APOBEC3G in African green monkeys or rhesus macaques restricts the ability of HIV-1 to infect these species, thus providing a demonstration of the critical role of Vif in overcoming the innate antiviral effect of APOBEC3G.

Cellular Components of Innate Immunity to Virus Infection

As noted above, cellular components of the innate immune response also exist. Included among such cell types are DCs (discussed above), macrophages, and NK cells. NK cells function to produce cytokines or to lyse cells in a non-antigen-specific manner. Cytokines released by NK cells may exert antiviral effects or regulate other aspects of innate or adaptive immune responses. NK killing of target cells can occur through exocytosis of cytotoxic granules or through engagement of death receptors. A role for NK function in controlling virus replication has been described for several virus types including arenaviruses, paramyxoviruses, HIV, and, most notably, herpes viruses. NK activity is regulated via a series of positive and negative regulatory signals, and it is the balance of these regulatory signals that appears to determine NK cell activation. The best-studied examples of virus evasion of NK responses are those used by cytomegalovirus (CMV). One classic mechanism of NK cell activation is recognition of a cell with low levels of class I major histocompatibility complex (MHC-I) on its surface. Because NK cells express upon their surface receptors that recognize MHC-I and negatively regulate their function, targets lacking MHC-I drive this balance toward NK cell activation. CMV-infected cells typically express low levels of MHC-I upon their surface. This is due, at least in part, to virus-encoded proteins designed to downregulate MHC-I expression, so as to permit evasion of antigen-specific T-cell responses. CMV must therefore also evade NK responses that would otherwise be activated by low MHC-I. One such mechanism is the production by CMV of MHC-I-like proteins. In addition, CMV infection downregulates ligands on the infected cell that would trigger activating signals in NK cells.

Complement

Complement is a group of proteins, both soluble and cell associated, that serve multiple innate immune functions. Antiviral properties of the complement system include its ability to enhance activation (priming) of adaptive immune responses, to lyse membrane-bound viruses and infected cells, and to promote phagocytosis of viruses and infected cells. Three major complement pathways exist and are referred to as the classical, the alternate, and the lectin-binding pathways. Although activated by different means, each pathway can, through a cascade of events, lead to the deposition of the complement protein C3b on a target. This process, termed opsonization, promotes phagocytosis. Deposition of C3b can also lead to the formation of a 'membrane attack complex' which can lyse the coated particle or cell. The classical pathway is activated by binding of a complement protein, C1q, which attaches to the Fc portion of an antibody. Thus, virus or virus-infected cells can be targeted by complement and subject to C3b deposition and subsequent lysis or phagocytosis when they become bound by antibody. Activation of the alternative pathway, in contrast, occurs in the absence of antibody and occurs when an activated form of the complement protein C3 recognizes a (usually) foreign surface. In the lectin pathway, lectins may bind to carbohydrates on antigens such as viruses, triggering a pathway similar to the classical pathway.

Because complement is strongly pro-inflammatory and cytolytic, it must be tightly regulated to protect the host, and some of these host-encoded protections can either be mimicked or co-opted by viruses to evade the antiviral effects of complement. For example, several either encode 'regulators of complement activation (RCA)' proteins or package host-encoded RCA proteins. For example, gamma herpes viruses (γHVs), large DNA viruses that establish lifelong infection, encode RCA homologs. One well-studied example is the murine γHV68 RCA which can inhibit C3 and C4 deposition *in vitro*. Deletion of this γHV68-encoded RCA decreased virulence during acute infection, but virulence was restored by deletion of host C3, demonstrating the *in vivo* importance of the viral RCA as an antagonist of the complement system. An alternate strategy can be employed by HIV-1 which is able to incorporate into virus particles two glycosylphosphatidylinositol (GPI)-linked complement control proteins, CD55 and CD59. These make virus resistant to complement-mediated lysis. Finally, viruses may also produce complement inhibitors with no obvious homology to the host complement system. West Nile virus, for example, produces a secreted glycoprotein NS1. NS1 can bind to cell surfaces but it also accumulates in serum. NS1 binds to and recruits a complement regulatory protein, factor H, and the ability of NS1 to interact with factor H correlates with its ability to impair complement activation and to suppress deposition of C3 and formation of membrane attack complexes on infected cells.

Conclusion

As is illustrated by the selected examples provided above, innate mechanisms targeting virus infection are numerous, and the manner in which viruses overcome such responses are equally diverse. Defeating such mechanisms is also critical for maintenance of viruses in nature and for viral pathogenesis. It should be recognized, however, that it may not be evolutionarily advantageous for a virus to absolutely defeat host immune responses, as this might result in the rapid demise of the host with reduced opportunity for virus amplification and transmission. Therefore, viruses do not necessarily fully block innate

immune responses. Rather many viruses will have evolved mechanisms to attenuate innate immunity only to such a degree that their spread to new hosts is facilitated.

See also: Immune Response to viruses: Antibody-Mediated Immunity; Immune Response to viruses: Cell-Mediated Immunity; Innate Immunity: Introduction.

Further Reading

Andoniou CE, Andrews DM, and Degli-Esposti MA (2006) Natural killer cells in viral infection: More than just killers. *Immunological Reviews* 214: 239–250.

Basler CF and Garcia-Sastre A (2002) Viruses and the type I interferon antiviral system: Induction and evasion. *International Reviews of Immunology* 21: 305–337.

Chiu YL and Greene WC (2006) Multifaceted antiviral actions of APOBEC3 cytidine deaminases. *Trends in Immunology* 27: 291–297.

Finlay BB and McFadden G (2006) Anti-immunology: Evasion of the host immune system by bacterial and viral pathogens. *Cell* 124: 767–782.

Gale M, Jr., and Foy EM (2005) Evasion of intracellular host defence by hepatitis C virus. *Nature* 436: 939–945.

Hilleman MR (2004) Strategies and mechanisms for host and pathogen survival in acute and persistent viral infections. *Proceedings of the National Academy of Sciences, USA* 101(supplement 2): 14560–14566.

Horvath CM (2004) Weapons of STAT destruction. Interferon evasion by paramyxovirus V protein. *European Journal of Biochemistry* 271: 4621–4628.

Kawai T and Akira S (2007) Antiviral signaling through pattern recognition receptors. *Journal of Biochemistry (Tokyo)* 141(2): 137–145.

Levy DE and Garcia-Sastre A (2001) The virus battles: IFN induction of the antiviral state and mechanisms of viral evasion. *Cytokine and Growth Factor Reviews* 12: 143–156.

Lopez CB, Moran TM, Schulman JL, and Fernandez-Sesma A (2002) Antiviral immunity and the role of dendritic cells. *International Reviews of Immunology* 21: 339–353.

Mastellos D, Morikis D, Isaacs SN, Holland MC, Strey CW, and Lambris JD (2003) Complement: Structure, functions, evolution, and viral molecular mimicry. *Immunologic Research* 27: 367–386.

Innate Immunity: Introduction

F Weber, University of Freiburg, Freiburg, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Apoptosis A form of programmed cell death.
Apoptotic bodies Remnants of cells which underwent apoptosis.
Complement system A pathogen-triggered cascade of biochemical reactions involving more than 20 soluble and cell-bound proteins. Complement activation results in opsonization, priming of humoral immune responses, and perforation of membranes.
Cytokines Proteins which mediate cell–cell communication related to pathogen defense. Secreted by immune cells or tissue cells.
Innate immunity Physical and chemical barriers, cells, cytokines, and antiviral proteins which exclude, inhibit, or slow down infection with little specificity and without adaptation or generation of a protective memory.
Interferons (**IFNs**) Cytokines mediating antiviral activity. Distinguished into type I (IFN-α/β), type II (IFN-γ), and type III (IFN-λ). Type I and type III IFNs directly mediate antiviral activity in responding cells, whereas type II IFN is more immunomodulatory.
Interferon-stimulated response element (**ISRE**) A promoter element common to all type I IFN-stimulated genes.
Opsonization Tagging of infected cells or pathogens for destruction by phagocytic cells.
Pathogen-associated molecular patterns (**PAMPs**) Molecular signatures of pathogens used by the innate immune system to distinguish self from non-self. Often highly repetitive patterns.
Pattern recognition receptors (**PRRs**) Intracellular and extracellular receptors recognizing specific PAMPs.
Phagocytosis Uptake of particles by cells.

Introduction

Viruses attempting to conquer a mammalian body are faced with an array of problems. 'Innate immunity' in a wider sense means all sorts of factors which exclude, inhibit, or slow down infections in a rapid manner but with little specificity and without adaptation or generation of a protective memory. Many of these efficient and not at all primitive defenses are evolutionarily old and can be found in all metazoans. For the sake of brevity, however, the discussion in this article is restricted to mammals as these are the best investigated organisms in that respect. RNA interference, the innate immune system of plants and nonvertebrates, is not covered here.

Mammalian innate immune defenses against virus infections can be divided into several distinct parts such as mechanical and chemical barriers (not further mentioned here), complement system, phagocytic/cytolytic cells of the immune system which act in a nonspecific manner, and cytokines (most prominently the type I interferons).

The Complement System

The complement system (which 'complements' the adaptive immune system in the defense against pathogens) primes the adaptive immune response and is also directly effective against pathogens. Complement activation is achieved by specific receptors recognizing pathogens or immunocomplexes. Three different pathways are being distinguished which are termed the classical pathway (triggered by antigen–antibody complexes), the mannan-binding lectin pathway (triggered by lectin binding of pathogen surfaces), and the alternative pathway (triggered by complement factor C3b-coated pathogen surfaces). They all activate a cascade of reactions involving more than 20 soluble and cell-bound proteins, thus resulting in a rapid and massive response. The complement system is able to (1) tag infected cells and pathogens for destruction by phagocytic cells (opsonization), (2) prime humoral immune responses, and (3) perforate membranes of infected cells by the membrane-attack complex. In response, viruses have evolved effective countermeasures such as incorporation of cellular complement-regulatory proteins into particles or expressing specific inhibitors in infected cells.

Cellular Innate Immunity

Macrophages/monocytes, granulocytes, natural killer cells, and dendritic cells belong to the cellular branch of the innate immune system. Monocytes circulate in the bloodstream for several hours before they differentiate into macrophages. These potent phagocytic cells either continue patrolling or they permanently settle in particular tissues (i.e., the Kupffer cells of the liver), being able to rapidly remove viral particles and apoptotic bodies. Activated macrophages also synthesize inflammatory cytokines such as interferon (IFN)-γ and tumor necrosis factor (TNF)-α, thus triggering an adaptive immune response. Granulocytes are also able to remove viral particles and apoptotic bodies by phagocytosis. They are rapidly attracted to inflammatory sites and enter the tissue by transendothelial migration. Both macrophages and granulocytes cleave the ingested viral proteins into fragments and present them to T lymphocytes.

Natural killer (NK) cells are able to recognize infected cells in an antigen-independent manner and destroy them by their cytotoxic activity. Also, they rapidly produce large amounts of IFN-γ to activate the adaptive immune system. NK cells are regulated by a fine balance between stimulatory and inhibitory receptors. One of their prominent features is their ability to destroy cells which lack MHC I molecules on their surface. As many viruses downregulate MHC expression in order to avoid an adaptive immune response, NK surveillance represents an important early warning and attack system against virus infections.

A key connection between the innate and the adaptive immune system is provided by dendritic cells (DCs). These specialized immune cells sample antigen at the site of infection, activate themselves and the surrounding tissue cell by cytokine synthesis, and then migrate to secondary lymphatic organs in order to mobilize T cells against the presented antigen. The differentiation into efficient antigen-presenting cells (APCs) is achieved by cytokine production which, in turn, is triggered by stimulation of receptors recognizing pathogen-specific molecular patterns (PAMPs). Two main types of DCs are being distinguished: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). mDCs are an early split-off of the myeloid bone marrow precursors, that is, the stem cells which are also giving birth to macrophages/monocytes and granulocytes, among others. Depending on the location, several subsets of mDCs such as Langerhans cells or interstitial cells are being distinguished. pDCs, which are not segregated into subpopulations, are thought to be derived from lymphatic precursor cells. Both mDCs and pDCs can sense viral infection by several intra- and extracellular PAMP receptors (see below). Depending on the DC type, high levels of interleukins or interferons are being produced which coin the subsequent immune reaction. pDCs are potent producers of the main antiviral cytokines, the type I interferons.

Antiviral Cytokines: The Type I Interferons

Isaacs and Lindenmann discovered in 1957 that cells which had been in contact with virus particles secrete a soluble factor which confers resistance to influenza viruses, a phenomenon called 'interference'. In the subsequent years, it became more and more clear that the so-called type I interferon (IFN-α/β) system is our primary defense mechanism against viral infections. In fact, humans with genetic defects in the IFN signaling pathway have a bad prognosis as they die at an early age of viral diseases which would otherwise pose little problems. Similarly, knockout mice with a defective IFN system quickly succumb to viral pathogens of all sorts although they have an intact adaptive immune system.

In response to virus infection, pDCs are particularly well equipped to synthesize and secrete IFN-α/β, but in principle all nucleated cells are able to do so. In an autocrine and paracrine manner, IFNs trigger a signaling

chain leading to the expression of potent antiviral proteins which limit further viral spread. In addition, IFNs initiate, modulate, and enhance the adaptive immune response. The signaling events which culminate in the direct IFN-dependent restriction of virus growth can be divided into three steps, namely (1) transcriptional induction of IFN synthesis, (2) IFN signaling, and (3) antiviral mechanisms.

Interferon Induction

A number of pattern recognition receptors (PRRs) recognize conserved PAMPs of viruses and initiate induction of IFN genes (see **Figure 1**). PRRs can be divided into the extracellular/endosomal toll-like receptors (TLRs) and the intracellular receptors RIG-I, MDA-5, and PKR. The main PAMPs of viruses appear to be nucleic acids, namely double-stranded RNA (dsRNA), single-stranded RNA (ssRNA), and double-stranded DNA (dsDNA).

dsRNA is an almost ubiquitous transcriptional by-product of RNA and DNA viruses. It is recognized by TLR3, the related RNA helicases RIG-I and MDA-5, and the protein kinase PKR. A third dsRNA-binding member of the RIG-I helicase family, LGP2, acts as a negative-feedback inhibitor.

Viruses with a negative-strand ssRNA genome (e.g., influenza virus) are unique in that they do not produce substantial amounts of intracellular dsRNA. Their genomic ssRNA is recognized in the endosome by TLR7 and -8. Interestingly, in the cytoplasm, RIG-I recognizes

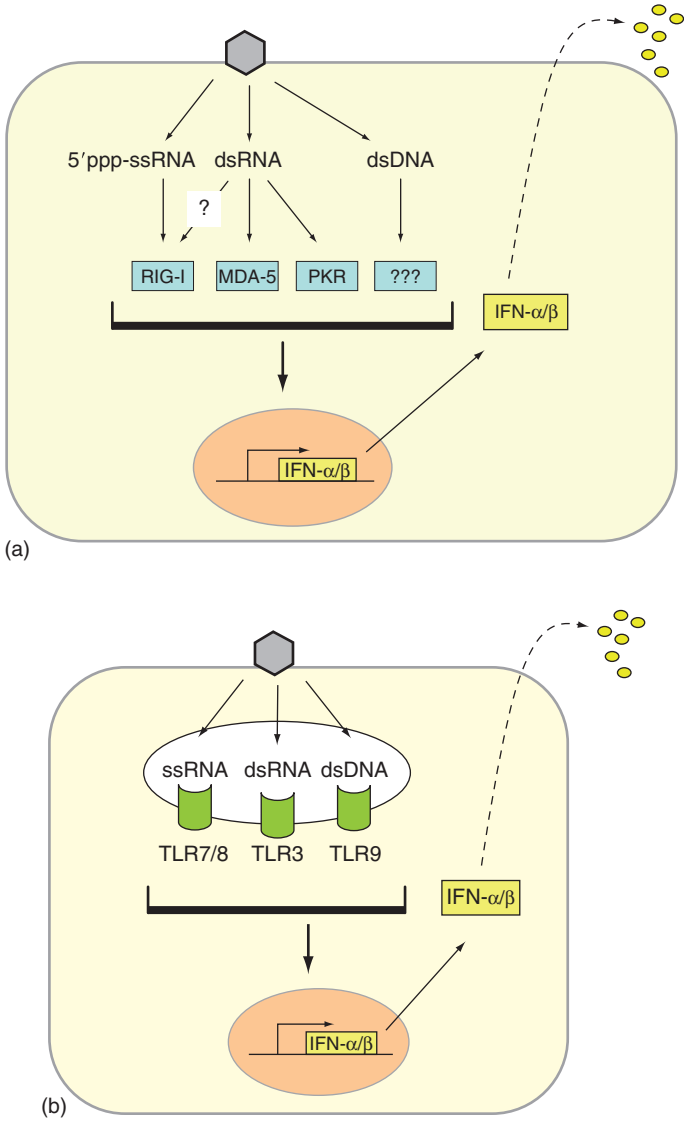


Figure 1 Depending on the virus, ssRNA, dsRNA, dsDNA, or combinations thereof represent characteristic by-products of infection which lead to induction of IFN-α/β genes. (a) These signature molecules are recognized by the intracellular PRRs RIG-I, MDA-5, PKR, and an unknown receptor for viral dsDNA. RIG-I recognizes dsRNA, but was shown to be important for recognition of 5′-triphosphate-containing ssRNA *in vivo* (see text). (b) Intracellular PAMP recognition is mirrored by the endosomal TLR pathways recognizing the same characteristics, except that ssRNAs do not need to be 5′-triphosphorylated.

the influenza virus genome in a 5′-triphosphate-dependent manner. The question how much the well-documented dsRNA-binding and unwinding activity of RIG-I contributes to its 5′-triphosphate-dependent recognition of viral genomes remains to be solved.

The third important PAMP, viral dsDNA, is again recognized both by an endosomal receptor, TLR9, and an unknown intracellular receptor. Thus, for all three nucleic acid-based PAMPs of viruses, there are specific PRRs present both in the endosomal and the intracellular compartment.

Besides nucleic acids, some viral proteins can provoke a TLR response such as the envelope proteins of respiratory syncytial virus and measles virus by activating TLR4 and TLR2, respectively.

All PRR-triggered signaling pathways eventually culminate in a strong activation of type I IFN transcription. The 'classic' intracellular pathway of IFN-β gene expression involves RIG-I and MDA-5 which both contain two N-terminal caspase-recruiting domain (CARD)-like regions and a C-terminal DExD/H box RNA helicase domain (**Figure 2(a)**). RNA binding to the helicase domain

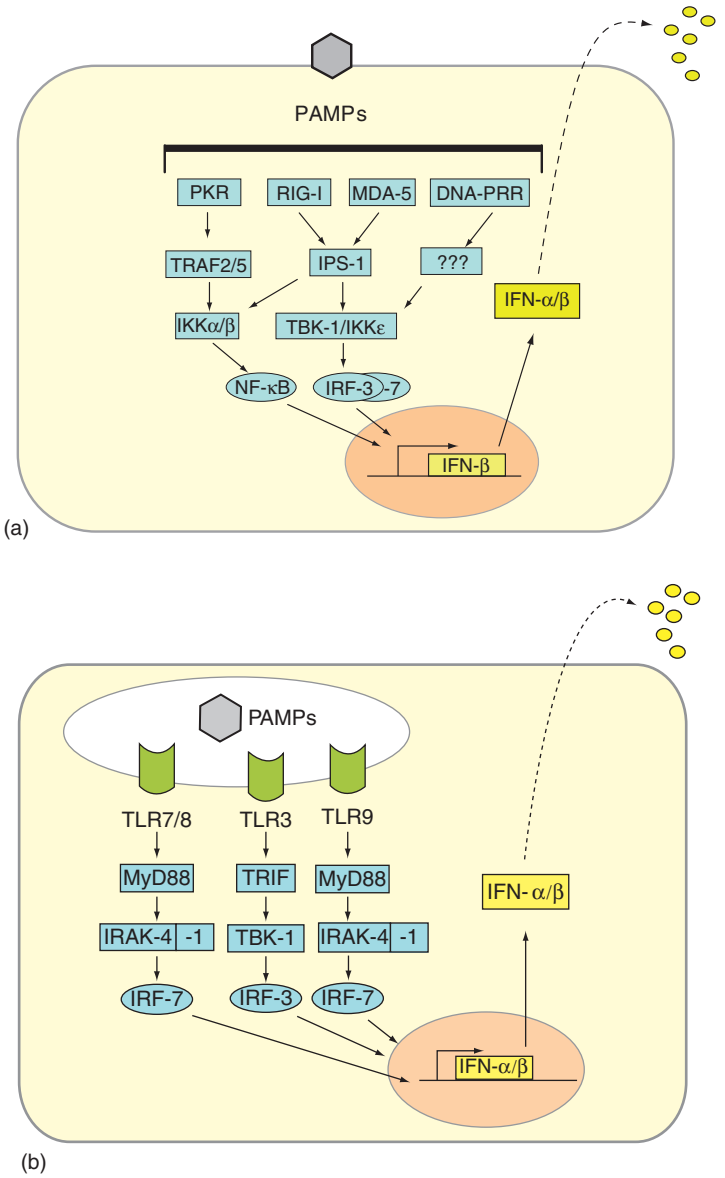


Figure 2 (a) PAMP recognition by intracellular PRRs leads to activation of the transcription factors NF-κB, IRF-3, and AP-1 (not shown). The cooperative action of these factors is required for full activation of the IFN-β promoter. IRF-3 is phosphorylated by the kinases TBK-1 and IKKε which in turn are activated by RIG-I and MDA5 via IPS-1. NF-κB is activated by the PKR pathway as well as by IPS-1. The IFN-induced IRF-7 later enhances IFN gene transcription, but is also essential for immediate early IFN-β transcription. (b) PAMP recognition by endosomal PRRs. IRF-7 is activated by IRAK-1, which in turn is phosphorylated by IRAK-4 in an MyD88-dependent manner. Both TLR7/8 and TLR9 use the MyD88 adaptor, whereas TLR3 activates IRF-3 via TRIF and TBK1.

induces a conformational change which liberates the CARD domain to interact with the signaling partner IPS-1 (also called Cardif, MAVS, or VISA). This adaptor mediates RIG-I and MDA-5 signaling and needs to be located at the mitochondrial membrane. IPS-1 has a CARD-like domain which binds to RIG-I and MDA5 and a C-terminal region which activates the kinases IKKε and TBK-1. These kinases are known to phosphorylate the transcription factor IFN regulatory factor (IRF)-3, a member of the IRF family. Phosphorylated IRF-3 homodimerizes and is transported into the nucleus. In addition, the transcription factor nuclear factor-kappa B (NF-κB) is recruited in a PKR/TRAF- and IPS-1-dependent way. Together, IRF-3 and NF-κB strongly upregulate IFN gene expression. This leads to a 'first wave' of IFN production (IFN-β and IFN-α4 in mice) which triggers the expression of the transcription factor IRF-7. Recent evidence has shown that IRF-7 is a master regulator of IFN gene expression and that IRF-3 seems to cooperate with IRF-7 for full activity. IRF-7 can be activated in the same way as IRF-3 and is responsible for a positive-feedback loop that initiates the synthesis of several IFN-α subtypes as the 'second-wave' IFNs.

mDCs can sense dsRNA by the classic intracellular pathway and, in addition, by TLR3 (**Figure 2(b)**). dsRNA-induced triggering of endosomal TLR3 proceeds via TRIF and TRAF3 which activate the kinase TBK-1, leading to phosphorylation of IRF-3 and, subsequently, to the activation of IFN-β gene expression.

pDCs sense the presence of viral ssRNA or dsDNA by TLR7, TLR8, and TLR9 (**Figure 2(b)**). Upon activation, TLR7, -8, and -9 signal through their adaptor molecule MyD88, the IRAK kinases, and IRF-7 to transcriptionally activate multiple IFN-α genes. In contrast to other cell types, pDCs contain considerable amounts of constitutively expressed IRF-7. IRF-7 is further upregulated in response to IFN and generates a positive-feedback loop for high IFN-α and IFN-β production. Furthermore, TLR7 and TLR9 are retained in the endosomes of pDCs to allow prolonged IFN induction signaling.

Type I IFN Signaling

IFN-β and the multiple IFN-α subspecies activate a common type I IFN receptor (IFNAR) which signals to the nucleus through the so-called JAK–STAT pathway (**Figure 3**). The STAT proteins are latent cytoplasmic transcription factors which become phosphorylated by the Janus kinases JAK-1 and TYK-2. Phosphorylated STAT-1 and STAT-2 recruit a third factor, IRF-9, to form a complex known as IFN-stimulated gene factor 3 (ISGF-3) which translocates to the nucleus and binds to the IFN-stimulated response element (ISRE) in the promoter region of interferon-stimulated genes (ISGs). Specialized proteins serve as negative regulators of the JAK–STAT pathway. The suppressor of cytokine signaling

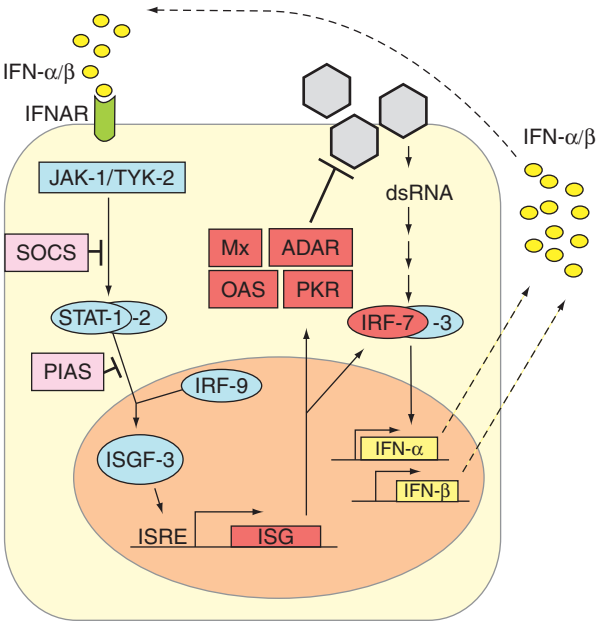


Figure 3 IFN-α and IFN-β bind to the type I IFN receptor (IFNAR) and activate the expression of numerous ISGs via the JAK/STAT pathway. IRF-7 amplifies the IFN response by inducing the expression of several IFN-α subtypes. Mx, ADAR, OAS, and PKR are examples of proteins with antiviral activity. Modified from Haller O, Kochs G, and Weber F (2006) The interferon response circuit: Introduction and suppression by pathogenic viruses. *Virology* 344: 119–130, with permission from Elsevier.

(SOCS) proteins prevent STAT activation whereas protein inhibitor of activated STAT (PIAS) family members function as small ubiquitin-like modifier (SUMO) E3 ligases and inhibit the transcriptional activity of STATs.

Direct Antiviral Effects of Type I IFNs

Type I IFNs activate the expression of several hundred IFN-stimulated genes (ISGs) with multiple functions. To date, five antiviral pathways have been studied in great detail, namely the protein kinase R (PKR), the RNA-specific adenosine deaminase 1 (ADAR 1), the 2–5 OAS/RNaseL system, the product of the ISG56 gene (p56), and the Mx proteins. PKR, ADAR1, and 2–5 OAS are constitutively expressed in normal cells in a latent, inactive form. Basal mRNA levels are upregulated by IFN-α/β and these enzymes need to be activated by viral dsRNA. PKR is a serine-threonine kinase that phosphorylates – among other substrates – the α-subunit of the eukaryotic translation initiation factor eIF2. As a consequence, translation of cellular and viral mRNAs is blocked. PKR also plays a role in virus-induced NF-κB activation, as described above. ADAR 1 catalyzes the deamination of adenosine on target dsRNAs to yield inosine. As a result the secondary structure is destabilized due to a change from an AU base pair to the less stable IU base pair and mutations accumulate within the viral

genome. The 2–5 OAS catalyzes the synthesis of short 2′–5′ oligoadenylates that activate the latent endoribonuclease RNaseL. RNaseL degrades both viral and cellular RNAs, leading to viral inhibition. P56 binds the eukaryotic initiation factor 3e (eIF3e) subunit of the eukaryotic translation initiation factor eIF3. It functions as an inhibitor of translation initiation at the level of eIF3 ternary complex formation and is likely to suppress viral RNA translation. Mx proteins belong to the superfamily of dynamin-like large GTPases and have been discovered as mediators of genetic resistance against orthomyxoviruses in mice. They most probably act by enwrapping viral nucleocapsids, thus preventing the viral polymerase from elongation of transcription.

The antiviral profiles of the IFN effectors listed above are distinct but often overlapping. Mx proteins, for example, mainly inhibit segmented negative-strand RNA viruses and also Semliki Forest virus, whereas the 2–5 OAS/RNaseL system appears more important against positive-strand RNA viruses. Moreover, only rarely the presence of one particular IFN effector determines host resistance. Rather, it is the sum of antiviral factors affecting, for example, genome stability, genetic integrity, transcription, and translation that confers the full antiviral power of IFN.

Indirect Antiviral Effects of Type I IFNs

Besides the effector proteins listed above, several ISGs contribute in a more indirect manner to the enhancement of both innate and adaptive immune responses. Virus-sensing (and in part antiviral) PRRs such as TLR3, PKR, RIG-I, and MDA5 are by themselves upregulated in a type-I-IFN-dependent manner. Similarly, IRF-7 and and STAT1, the key factors of type I IFN and ISG transcription, respectively, are ISGs. The strong positive-feedback loop mediated by the upregulation of these PRRs and transcription factors is counterbalanced by several negative regulators such as LGP2, SOCS, and PIAS, which are either ISGs or depend on IFN signaling for their suppressive action.

Type I IFNs can directly enhance clonal expansion and memory formation of $CD8^+$ T cells. Also, IFNs promote NK cell-mediated cytotoxicity and trigger the synthesis of cytokines such as IFN-γ or IL-15 which modulate the adaptive immune response, enhance NK cell proliferation, and support $CD8^+$ T-cell memory. Moreover, by upregulating TLRs, MHCs, and costimulatory molecules, IFNs enable APCs (most prominently DCs) to become competent in presenting viral antigens and stimulating the adaptive immune response.

Good Cop–Bad Cop

Given their massive impact on the cellular gene expression profile, it is quite expected that type I IFNs have not only antiviral, but also antiproliferative and immunomodulatory effects. Treatment with IFNs is an established therapy against several viral and malignant diseases such as hepatitis B, hepatitis C, Kaposi's sarcoma, papillomas, multiple sclerosis, and several leukemias and myelomas. However, the strong and systemic effects of IFNs do not come without a price. Administration of IFN can locally produce inflammation, and systemically cause fever, fatigue, malaise, myalgia, and anemia. It is no coincidence that these latter are 'flu-like' symptoms, since in many acute infections IFNs play a dominant role. The effects of IFN which are desired and beneficial if restricted to the site of first infection can turn into a life-threatening 'cytokine storm' if it becomes systemic. Severe acute respiratory syndrome (SARS) and human infections with H5N1 influenza viruses are examples of such out-of-control innate immune responses. Another 'dark side' aspect is that patients with autoimmune diseases have chronically elevated levels of IFNs, and that IFN therapy can aggravate autoimmune disorders. It is thought that pDCs (and in part B cells) are autostimulated by self-DNA via TLR9 and by small nuclear RNA complexes (snRNPs) via TLR7. Chronic production of IFNs causes maturation of mDCs, which in turn activate autoreactive T and B cells.

Concluding Remarks

The concept of innate immunity certainly comprises more than the IFN system (see above), but type I IFNs represent a central part. These cytokines not only have direct antiviral effects but also orchestrate the first defense reactions and the subsequent adaptive immune response, thus determining the course of infection. The recent findings that basically every virus appears to have evolved one or several countermeasures for controlling the IFN response is testament to its importance. In addition, IFNs are not only antiviral, but also effective tumor suppressors. Tumor cells often eliminate the IFN system during the transformation process. The payoff is an increased susceptibility to infection, an Achilles heel which is exploited by the therapeutic concept of oncolytic viruses. Tumor selectivity of such viruses can be even more increased by using IFN-sensitive mutants. The inability of those mutants to fight the IFN response is complemented by the mutations of the tumor cells, thus allowing virus growth. At the same time, these viruses are unable to infect the IFN-competent body cells.

Recently, it became apparent that there exists a hitherto unnoticed parallel world called the type III IFN system. The cytokines IFN-λ1, -λ2, and -λ3 are induced by virus infection or dsRNA and signal through the JAK/STAT cascade, but use a separate common receptor. They are able to activate antiviral gene expression and have been shown to inhibit replication of several viruses.

Thus, the IFN response has a backup system to enforce the first line of defense against virus infections.

Future studies will have to address the relative contribution of type I and type III IFNs to antiviral protection and the coming years may have even more surprises in stock. The innate immune system may be old, but as long as there are viruses and tumors, it will never come out of fashion.

See also: Immune Response to viruses: Antibody-Mediated Immunity; Innate Immunity: Defeating; Interfering RNAs; Polydnaviruses: Abrogation of Invertebrate Immune Systems.

Further Reading

Akira S and Takeda K (2004) Toll-like receptor signalling. *Nature Reviews Immunology* 4: 499–511.

Ank N, West H, and Paludan SR (2006) IFN-λ: Novel antiviral cytokines. *Journal of Interferon and Cytokine Research* 26: 373–379.

Colonna M, Trinchieri G, and Liu YJ (2004) Plasmacytoid dendritic cells in immunity. *Nature Immunology* 5: 1219–1226.

Diefenbach A and Raulet DH (2003) Innate immune recognition by stimulatory immunoreceptors. *Current Opinion in Immunology* 15: 37–44.

Garcia MA, Gil J, Ventoso I, *et al.* (2006) Impact of protein kinase PKR in cell biology: From antiviral to antiproliferative action. *Microbiology and Molecular Biology Reviews* 70: 1032–1060.

Garcia-Sastre A and Biron CA (2006) Type 1 interferons and the virus–host relationship: A lesson in detente. *Science* 312: 879–882.

Haller O and Kochs G (2002) Interferon-induced mx proteins: Dynamin-like GTPases with antiviral activity. *Traffic* 3: 710–717.

Haller O, Kochs G, and Weber F (2006) The interferon response circuit: Induction and suppression by pathogenic viruses. *Virology* 344: 119–130.

Hoebe K, Janssen E, and Beutler B (2004) The interface between innate and adaptive immunity. *Nature Immunology* 5: 971–974.

Honda K, Takaoka A, and Taniguchi T (2006) Type I interferon gene induction by the interferon regulatory factor family of transcription factors. *Immunity* 25: 349–360.

Reis e Sousa C (2006) Dendritic cells in a mature age. *Nature Reviews Immunology* 6: 476–483.

van Boxel-Dezaire AH, Rani MR, and Stark GR (2006) Complex modulation of cell type-specific signaling in response to type I interferons. *Immunity* 25: 361–372.

Vilcek J (2006) Fifty years of interferon research: Aiming at a moving target. *Immunity* 25: 343–348.

Volanakis JE (2002) The role of complement in innate and adaptive immunity. *Current Topics in Microbiology and Immunology* 266: 41–56.

Inoviruses

L A Day, The Public Health Research Institute, Newark, NJ, USA

© 2008 Published by Elsevier Ltd.

Glossary

Everted DNA Inside-out DNA helix with phosphates at the center.
Integrative phage A phage capable of having its genome integrated into that of its host.
Productive state Of a bacterium actively exporting progeny filamentous phage without lysis.
Prophage state Of a bacterium with a repressed phage genome inserted in its genome or in one of its plasmids.

Discovery, Ecology, and Host Range

In 1960, T. Loeb reported finding phages that formed plaques on *Escherichia coli* carrying the F transfer factor but not on those without it. His finding was confirmed by workers around the world, and publications in 1963 by H. Hoffmann-Berling and D. A. Marvin, by Loeb and N. Zinder and co-workers, and by H. P. Hofschneider established that such plaque formers, independently isolated, were small spherical RNA phages (fr, f2, and M12), and small filamentous DNA phages (fd, f1, and M13), the latter now collectively referred to as Ff phage (**Figure 1**). The capital F is for the F factor specifying the host, and the small f stands for filamentous. These new DNA viruses had intriguing characteristics. Surprisingly, they did not lyse their hosts in culture yet formed the plaques on bacterial lawns, their filamentous virions contained DNA genomes that were both single stranded and circular, and it was observed, by E. Trenkner, that capsid protein of the infecting phage is incorporated into progeny virions. The fd, f1, and M13 genomes were among the first viral genomes sequenced. They each have either 6407 or 6408 nucleotides encoding the same 11 genes but fewer than 100 nucleotide differences even though the isolates were from different places in the world. This near constancy seemed remarkable, and has become more so in view of the plasticity of the Ff genome made evident in a plethora of subsequent mutagenesis studies.

Now over 50 different species of filamentous DNA phages are known, and their bacterial hosts occupy many habitats. Taxonomically the phages comprise the genus *Inovirus* in the family *Inoviridae,* the terms coming from the Greek word for filament. Infections by many



Figure 1 A uranyl acetate negative stain electron micrograph of fd showing one complete virion and the ends of others. One end is more rounded or blunt, and the other is tapered with additional tassel-like mass sometimes perceptible extending from the tip. Ff (fd) particles are 890 nm long under these dry conditions, but atomic force micrographs and cryoelectron micrographs indicate that lengths of fully hydrated phage might be slightly longer.

individual species lead to productive carrier states that extrude virus, sometimes copiously, while host cells continue to divide, yet infections by many other species lead to prophage carrier states with repressed genomes integrated into the bacterial genomes. **Tables 1** and **2** list the amino acid sequences of the major coat proteins for several selected species, as well as the names of known phages and those of some of the bacterial hosts. The protein sequences show, on the one hand, enormous diversity, yet, on the other, extensive homology as, for example, the common sequences for Pf1 and Pf4, and for CUS-1 and CUS-2, as well closely related sequences for CUS-2 (YP01Φ), X, and C2. The homologies reflect extensive horizontal genetic transfer, as well as the effects of evolution on sequences perfoming the same functions. Most of the phage/host systems are in Gram-negative bacteria, such as coliforms, pseudomonads, xanthomonads, vibrios, and many others, but two of the systems are Gram positive. Filamentous phages have been discovered through (1) characterizations of plaque formers on specific bacterial strains, (2) applications of phage concentration procedures to culture supernatants, (3) sequence studies of plasmid populations, (4) screens for virulence factors in pathogenic bacteria, and (5) *in silico* analyses of bacterial genomic sequences. Like other phages, inoviruses are ubiquitous, and their number on Earth must be astronomical.

Host ranges of the Gram-negative systems are determined usually by the multidomain gene 3 protein located at one end of the virion interacting with a specific, type IV pilus and the Tol QRA protein complex. The combined receptor systems are highly efficient, approaching one infection per physical particle in some cases. The pili receptors are encoded chromosomally or on plasmids of different incompatibility groups, that is, phage Ff adsorbs to IncF pili, IKe to IncN pili, Pf3 to IncP pili, etc., and transmission of the plasmids to new bacterial species can transfer phage sensitivity. Some phages can infect, at low efficiency, cells without pili by reaching the tolQRA co-receptors some other way. Some host ranges are quite broad such as that of phage X. Transfections of non-natural hosts with naked phage DNA are sometimes possible, as shown by the transfection of female strains of *E. coli* with DNA from Pf1, a phage of *Ps. aeruginosa.*

Many, but not all, inoviruses form plaques on bacterial lawns even though they do not lyse their hosts in culture. Plaque formation and the size and appearance of plaques depend on relative rates of bacterial growth and phage production. These can be affected by phage mutations and/or the presence of prophages in the host, as well as details of plate conditions.

Morphology

Except for differences in length, filamentous phage virions are morphologically similar to the fd virions shown in **Figure 1**. Most appear to have a tapered end, with additional structures extending from it, and a rounded or blunt end. Diameters of the Gram-negatives are invariably about 7 nm, but a value of 12 nm was reported for the Gram-positive phage B5. The length range for Gram-negative wild types is 700 nm (Pf3) to 3500 nm (Pf4). The length depends upon both the size and the conformation of the packaged genome, and this conformation depends on the chemical nature of the DNA interaction domain (**Tables 1** and **2**). For example, in Ff, the DNA–protein interface is largely electrostatic, and reduction of the number of lysine residues in DNA interaction domain from 4 to 3 leads to stable virions approximately 4/3 longer than wild type, hence a more extended conformation for a DNA of unaltered size. On the other hand, DNA insertions or deletions, *in vitro* or *in vivo*, can lead to virions having unaltered DNA conformations but lengths that are longer or shorter than wild-type. Measurements of lengths have been by conventional electron microscopy (EM), scanning transmission electron microscopy (STEM), cryo-EM, atomic force microscopy, and hydrodynamic methods. In electron micrographs of static particles on grids, some phages appear more flexible than others, and

Table 1 Amino acid sequences of major capsid proteins for several strains

Phage/prophage	*Solvent interaction*	*DNA interaction*
KSF-1Φ	AIALGLGWYPWYLEVIVMVAFVSDALTVVVAVAYFMAFAYGFYTGVNAS	
Pf3	MQSVITDVTGQLTAVQADITTIGGAIIVLAAVVLGIRWIKAQFF	
Pf1, (**Pf4**)	GVIDTSAVESAITDGQGDMKAIGGYIVGALVILAVAGLIYSMLRKA	
f237, Vf04K68	EVDITGAINSAVSGGQANVSLVVAGLIGMAALGFGVTMVVGFLRR	
ΦSMA9	MVTVIADAFQPPNKEQLAVWAGGPFGLLLFLFVAGRIAGSVATFFDKNR	
CTXΦ	ADAGLVTEVTKTLGTSKDTVIALGPLIMGVVGAIVLIVTVIGLIRKAK	
VSK	MPLCKKFSLQSTLLVSLLSLLLLVLPSLASTWLSKASLSANALSTKPNR	
CUS-1, CUS-2	AEGAASSGVDLSPLTNSIDFSTVLVAIMAVAASLVTLYAGVAGVRWVLRTVKSA	
X	AEGDVVGGKGIDLTPLTNSVNFGSVLTGIMAVAGSLIVLYAGSAGVRWILRMVRGA	
C2	MGPTAPTDIASLASSVDFSSVGLGILAIAGTVITLYVTWKGAQFVIRQVRGA	
Ff (f1,fd)	AEGDDPAKAAFDSLQASATEYIGYAWAMVVVIVGATIGIKLFKKFTSKAS	
I2-2	ADDGTSTATSYATEAMNSLKTQATDLIDQTWPVVTSVAVAGLAIRLFKKFSSKAV	
B5	MVILEAPSDVGGTVSAAITALGPQITPIIGVAIGVSLIPFAAKWIFRKAKSLVS	
Xf	SGGGGVDVGDVVSAIQGAAGPIAAIGGAVLTVMVGIKVYKWVRRAM	
Cf1	SGGGGDFDGTAIIGKVTTYTAIGVTILAPSRSVVGRFATRSDRRQVSQSAA	

The names of integrative strains are in bold typeface, and potential charges are indicated for acidic (D, E) and basic residues (K, R). The sequences are right justified to compare DNA interaction domains, and are ordered on the basis of the number of basic residues in these domains. KSF-1Φ protein has no basic residues at all, but there are six aromatic residues (F, Y) in the DNA domain. Pf3 protein has only two basic residues, but there are 2.5 phosphate charges per subunit in the virion. Pf1 protein has two basic residues, yet one phosphate per subunit in the virion. VSK has only basic residues, even in the solvent interaction domain. Many sequences have proline (P) residues, often near the middle. Xf is much less α-helical than the others, having a proline in the middle as well as ten glycines (G). The basic residues in Cf1c are farther from its C terminus than is the case for the others. Over 50 inoviruses are known, and genomic sequences are available for most. Listed alphabetically, with integrative strains in bold and Ff phages in italics, they include: **493**, *AE2,* B5(Gram +), CAK-1(Gram +), **Cf1c, Cf16**, **Cf1tv**, **CTXΦ, CUS-1, CUS-2**, *δA*, *Ec9, F12*, **f237,** fs-1, fs-2, FXP, *HR*, I2–2, **If1**, IKe, Ivpf5, **KSF-1Φ, Lf,** *M13*, **MDA, Nf1-A, Nf4-G3,** Pf1, Pf2, Pf3, Pf4, Pf5, PH75, **ΦRSS1, ΦRSM1**, ΦSMA9, Pr64FS, SF, SW1, tf-1, V6, **Vf04K68, VGJΦ,** VSK, X, X2, Xf, Xo, **Vf12, Vf33**, Xv, **Xf1fΦ**, **Yp01Φ** & **Ypf1** (alternate names for CUS-2), and *ZJ/2*.

C2 and X show distinct waviness. On the other hand, phages fd, Xf, and Pf3 appear to flex only gently by EM and their lengths deduced from rotational and translational diffusion rates indicate rodlike behavior. It is worth noting that filament morphology is dynamic. The lateral and torsional flexing modes can be quite fast so it is best to consider these structures as dynamic entities exhibiting motions on many timescales. A new aspect of inovirus morphology has come from recent liquid crystal studies that indicate overall large length-scale chiralities superimposed on filament flexing for several species. This type of chirality, called coiling, has been shown to reflect diversity in the structural details of the DNA packaging. So far only one virus, Pf1, out of eight studied shows no tendency to coil.

Virion Structure

There is as yet no consensus detailed structure model for any of the virions. Nevertheless, there are many constraints defining the structures of the DNA and capsids of several of them, especially Ff, Pf1, and Pf3. First, none contains lipids or carbohydrates, only DNA and protein. Second, the structures of the packaged genomes and the protein capsids are mutually interdependent. Empty capsids do not exist, and conformations DNA and protein have in the intact virions have not been observed under other conditions. The DNA contents range from 6% to ∼15% of the total virion mass. The major coat proteins that form the shell constitute 97–99% of the protein mass, and the small remainder is in proteins at the ends critical for assembly and infection. The virions are put together in complex, multicomponent reactions at the membranes, so they are not self-assembly systems like tobacco mosaic virus (TMV). Also, they are taken apart by multicomponent reactions of a different sort at the membrane of the cells being infected.

The most extensive information is for the Ff group, and the ideas from Ff structure apply to the others as well, although there are remarkable differences in the details. Some of these ideas are presented here in conjunction with the schematic diagram in **Figure 2**. The gene names

Table 2 Bacterial hosts for phages that are in **Table 1** and/or are relevant to the text

B5	*Proprionibacterium freudenreichii (Gm +)*
C2	*Escherichia coli, IncC*
CAK-1	***Clostridium acetobutylicum (Gm +)***
Cf1	***Xanthamonas campestris pv citri***
CTXΦ	***Vibrio cholera***
CUS-1	***Escherichia coli 018:K1:H7***
CUS-2 (Yp01Φ)	***Yersinia pestis***
f237	***Vibrio parahaemolyticus***
Ff (f1, fd, M13)	*Escherichia coli, IncF*
I2–2	*Escherichia coli, $IncI_2$*
KSF-1Φ	***Vibrio cholera***
MDA, Nf1-A	***Neisseria meningitidis***
Nf4-G3	***Neisseria gonorrhea***
Pf1	*Pseudomonas aeruginosa K (PAK)*
Pf3	*Pseudomonas aeruginosa 01 (PA01)*
Pf4	***Pseudomonas aeruginosa 01 (PA01)***
PH75	*Thermus thermophilus*
ΦRSS1, ΦRSM1	***Ralstonia solanacearum***
SW1	***Shewanella piezotolerans* WP3**
tf-1	*Escherichia coli, IncT*
Vf04K68	***Vibrio parahaemolyticus***
Vf12, Vf33	***Vibrio parahaemolyticus***
VGJΦ, VSK	***Vibrio cholera***
X	*Escherichia coli, IncX*
Xf	*Xanthamonas oryzea pv oryzea*
ΦSMA9	*Stenotrophomaltophilia*

Integrative phages and their hosts are in bold. Only two systems, B5 and CAK-1, are Gram positive. Genomic sequences of bacteria in many taxonomic families contain filamentous prophage genomes.

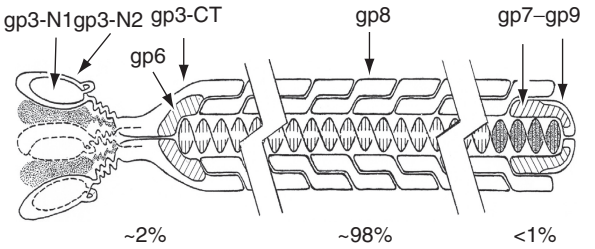


Figure 2 A scale diagram of an end-to-end section through a conjectural model of Ff. The phage diameter is 6 nm, the DNA diameter is 2 nm, and the shell thickness is also 2 nm. The structure has fivefold rotational symmetry. The diagram was drawn as if a blade angled at 144° were passed from end to end. Axial displacements of 3.2 nm align gp8 subunits next to each other, and the azimuthal angle of 144° puts two subunits opposite to each other in this particular diagram. The gp8 subunits are largely α-helical with diameters of 1 nm in the inner and outer portions. The overlaps, which are complex, are merely adumbrated by the gp8 shape, and the bend in the middle is exaggerated. The gp8 C-terminal regions are near the DNA and are the positively charged. The DNA pitch is 2.8 nm and the nucleotide rise 0.28 nm. The DNA helix has stacked bases with H-bonding between bases on opposite strands, but only about 25% can be of the Watson–Crick (W–C) type. The shaded part of the DNA represents a W–C base-paired packaging signal (PS). All five proteins are accessible to the outside as required by results from phage display studies. The gp3 part of the diagram is adapted from Lubkowski J, Hennecke F, Pluckthun A, and Wlodawer A (1998) The structural basis of phage display elucidated by the crystal structure of the N-terminal domains of g3p. *Nature Structural Biology* 5: 140–147. Overall diagram modified from Day LA and Maniloff J (2000) *Inoviridae*. In: van Regenmortel MHV, Fauquet CM, Bishop DHL, *et al*. (eds.) *Virus Taxonomy*: *Seventh Report of the International Committee on Taxonomy of Viruses*, pp. 267–275. San Diego, CA: Academic Press, with permission from Elsevier.

for Ff are based on numbered complementation groups assigned in early f1 genetic studies. Here Arabic numbers are used and gene products are labeled gp3, gp6, etc. X-ray crystallographic studies of gp3 have revealed that both its N2 and its N1 domains are accessible for interactions with the pilus and the Tol QRA receptor proteins, respectively. The gp7/9 end has the DNA packaging signal (PS), which has a DNA hairpin with a stem of two helical turns of almost perfect base pairing. Compensating mutations in the proteins and the DNA provided genetic evidence for direct interaction between the DNA of PS and the gp7/9 complex.

In Ff phages the genomes (6407 or 6408 nt) are packaged by approximately 2700 subunits of gp8, corresponding to about 2.4 nt per subunit. This noninteger ratio is the consequence of a meshing of nonequivalent helical symmetries of the DNA and of the capsid to form the structure. As the DNA helix winds within the core of the capsid, it contacts different points on the successive subunits, as indicated in **Figure 2**. Note that while the two DNA strands point in opposite directions the subunits point in the same direction. For these reasons, subunit structures are inherently nonequivalent (polymorphic) at the DNA–protein interface. The nonequivalence, or polymorphism, was proposed to be the source of large length-scale coiling effects on overall morphology deduced from the liquid crystal behaviors and micrographs of several phages. Subunit polymorphism is present in filamentous phages in which the DNA and capsid helical symmetries are not the same, and can be subtle or pronounced depending on the details of the chemical nature of the protein–DNA interface, whether more electrostatic or more hydrophobic.

Protein Subunit Conformation

Electronic spectroscopy (ultraviolet absorbance, linear and circular dichroism, and fluorescence), vibrational spectroscopy (infrared, Raman, resonance Raman, and Raman optical activity), and solid-state nuclear magnetic resonance (NMR) have been used to determine the protein conformations of many strains, especially Ff, Pf1, and Pf3. These three are 85% or more α-helical, and the α-helices are tilted about 20° on average with respect to the virion axes. However, there are several exceptions to the α-helix rule for inovirus capsids. **Tables 1** and **2** shows many prolines, either alone or in combination with small

glycine residues and sometimes near the middle of the sequence, allowing bends or kinks. Solid-state NMR provides information about such kinks as well as atomic level information on backbone and side chain structure and dynamics, and hence provides essential constraints for full structure determinations.

Capsid Helical Symmetry

X-ray fiber diffraction patterns have established two basic types of capsid helical symmetry. The patterns for Ff, If1, and IKe, all show layer spacings of 32 Å (3.2 nm) and sharp diffraction on the meridian at 16 Å. It was eventually realized that these patterns indicated two pentamers of subunits every 32 Å. This symmetry characterizes class I capsid structures. Each pentamer is interdigitated with the pentamers before it and after it in such a way that DNA interaction domains of ten subunits contact the DNA at any point along the filament. Early radiochemical results indicated five copies of gp3 in fd, and one expects the fivefold rotational symmetry to extend into the two ends of the capsid.

Class II capsid symmetry is characteristic of Pf1, Xf, Pf3, and PH75. These viruses all have X-ray patterns with layer spacings of 75–80 Å and intensity close to the meridian on every fifth layer and certain other layers. These patterns indicate a helix of 5.4 subunits per turn repeating every five turns, but the patterns change with hydration and are difficult to interpret in detail. However, patterns for fibers of Pf1 prepared at temperatures below about 10 °C reliably define a capsid of 5.46 subunits per turn of a one-start helix. Subunits in successive turns interdigitate with those in the preceding and following turns so that the DNA interaction domains of 11 subunits contact the DNA at any point along the filament.

Theoretical studies of the packing of α-helical subunits around DNA have helped clarify why these two packing symmetries are preferred over a few other possible symmetries. However, the molecular mechanisms determining choice symmetry types are not understood.

Virion Stoichiometry and DNA Conformations

The DNA structures in filamentous phages differ dramatically, and three types have been proposed for the DNA helices in Ff, Pf1, and Pf3. The first type is much like classical double-stranded DNA (dsDNA) with H-bonded and stacked bases, although only about 25% of the H-bonding can be of the Waston–Crike type. This type is in Ff, in which the axial distance between nucleotides is 2.8 Å and off-layer X-ray diffraction for a sample held at low pH indicated a pitch of 28 Å. At face value, these data indicate a tenfold DNA helix, in accord with the fivefold rotational aspect of the capsid symmetry. Many spectroscopic measurements, including, a chiral method, established the type of right-handed DNA structure in **Figure 2**. Several other phages in both capsid symmetry classes I and II have been shown to have this type of DNA helix, with pitches near 30 Å and stacked bases facing in with the possibility of ~25% Watson–Crick (W–C) hydrogen bonding. Stoichiometric ratios lie between 2.0 and 2.5 nt per subunit.

The second type is that proposed for the DNA in the Pf1 virion. The Pf1 virion has 1.0 nt per subunit and the axial distance between bases 6.1 Å. Although some debate remains as to the exact structure, these two constraints and several others from various spectroscopies are satisfied by an everted DNA helix model having paraxial phosphates only 2 Å from the center. The bases are unstacked, strongly tilted, and extended outward into the capsid. The DNA has the 16 Å pitch of the capsid and 2.73 nucleotides per turn in each DNA strand to match exactly the 5.46 subunits per helical turn of the capsid helix. The helical symmetry of the DNA was established by the positions of phosphorus and two nondiester oxygens of the nucleotides as deduced from the Pf1 electron density map, and from theoretical analysis of the positioning forces exerted by electrostatic interactions between the phosphates. Further support is from single-molecule studies of DNA twist, and from Raman and infrared spectra of oriented Pf1. Pf1-like DNA structures are expected in f237 and Vf04K68, as well as in FXP, and certainly in Pf4, based on sequence (**Tables 1** and **2**).

The third type of DNA helix is that in the Pf3 virion. It is proposed to have 16 Å pitch but the axial base separation is only 2.4 Å yet the bases are not stacked. The Pf3 stoichiometry is 2.5 nt per subunit but the Pf3 subunit can provide only two positive charges. The constraints have produced an everted DNA model with phosphates at about 5 Å radius, leaving a hole in the center for metal ions to neutralize the charge. These three types of DNA packing are thus very different. The differences are reflected in their central mass densities which are high for fd, very high for Pf1, but low for Pf3, as shown by STEM analysis. Other types of DNA packing are anticipated. It seems certain that there are critical factors in addition to the sequences in **Tables 1** and **2** that determine DNA structure type but none are yet known.

Genome Organization

Inovirus genomes range from just below 6 kbp to over 12 kbp, with 6–8 kbp required for productive infections. With few exceptions, the genes for productive infections are ordered according to their functions in the life cycle. Two or three DNA replication genes are followed in

order by five virion structure genes, then by two virion morphogenesis genes, and finally by DNA sequences for controlling DNA packaging and DNA replication. All five structure proteins are membrane proteins prior to their incorporation into virions, while other gene products function enzymatically as inner membrane proteins and another forms a pore in the outermembrane through which virions are released. On other genomes, the patterns of virion structural genes are invariant, but the relative positions of morphogenesis genes can switch or be absent. For CTXF, the outer membrane pore function is provided by a host secretin and its position on the genome is taken by cholera toxin genes *ctxA* and *ctxB.* In the Gram-positive B5 system, there is no outer membrane, and hence a gene for a pore forming protein is missing completely from the phage genome.

Integrative strains encode integrases and repressors together with operator sequences, and the genes for them are located upstream and downstream of the core genes just described. Their sizes and detailed arrangements vary. Prophage sequences, partial or complete, are found integrated at one or many chromosomal sites. In several systems, the principal site is the bacterial *dif* site and the enzymes involved are the host XerC/D recombinases. In the CTXΦ system, integration at *dif* places one or more phage genomes juxtaposed with a smaller helper phage element called RS1. This arrangement allows complete phage genomes of circular topology to be generated without excision of the integrated prophage.

Life Cycles

Carrier States That Extrude Virions

Inoviruses infect their natural hosts without lysing or otherwise killing them, and the infected cells divide and produce virus indefinitely. Three groups of complex processes occur to produce virions: attachment of the phage and import of its ssDNA, conversion of it to a circular dsDNA for initial viral gene expression followed by viral dsDNA replication leading to progeny ssDNA sequestered in cytoplasm and structure proteins ready in the membrane, and finally virion assembly and export. These processes are delicately regulated to produce the appropriate numbers of the various proteins for continual cell division and copious phage production. The most extensive information is from the Ff/*E. coli* systems.

Phage attachment and DNA penetration

Phage attaches initially to the end of the F pilus through domain N2 of gp3 (**Figure 2**) which facilitates contact of domain N1 of gp3 with Tol A, a protein anchored in the inner membrane that extends into the periplasm. This interaction in turn facilitates interactions between gp3 and membrane proteins Tol Q and Tol R that translocate the ssDNA into the cytoplasm and insert the protein subunits of the incoming phage into the membrane, and these subunits are later found in progeny virions. The Tol QRA proteins are absolutely required for DNA entry. The pili receptors, whether nonconjugative, like a toxin co-regulated pilus receptor for CTXΦ and a mannose-sensitive hemagglutinin pilus receptor for KSF-1 and other vibriophages, or conjugative like the F-pilus, enhance the specificity and efficiency of infection but they are not always essential. The receptors in Gram-positive systems have not been established.

DNA replication and gene expression

The ssDNA is converted to a parental supercoiled dsDNA by cellular enzymes starting with RNA polymerase which creates an RNA primer for the initial complementary strand synthesis. The DNA site for the priming has an unusual hairpin structure with a high affinity for the RNA pol holoenzyme. Priming is followed by complementary strand synthesis by a DNA polymerase, then displacement of the RNA primer and ligation, and finally gyrase action, to produce the parental supercoiled dsDNA. Transcripts are generated from promoters of various strengths and various rho-dependent and -independent terminators. Translation is controlled by overlaps of genes in different frames or alternate starts in the same frame as well as by codon usage. Replication of this dsDNA begins with a nick at a specific, high-symmetry site once a viral endonuclease is expressed from it. Progeny dsDNA circles are produced via ssDNA intermediates in rolling circle replication. The dsDNA circles lead to further mRNA and protein synthesis. Increasing amounts of another replication protein and a ssDNA-binding protein eventually downregulate the nicking activity of the nuclease. Large amounts of the DNA binding protein (gene 5 protein) accumulate to form complexes with nascent plus strands being displaced in the rolling circle replication. About 100 progeny ssDNA plus strands become sequestered within flexible unbranched filamentous structures having stoichiometric ratios of 9 or 10 nt per gp5 dimer. The crystal structure of gp5 has been determined and the structures and properties of gp5/nucleic acid complexes have been extensively studied. Nevertheless, the complex mechanism of gp5 slippage on the lengthening DNA to form such structures, which involves the gp5 folding the nascent single strand back on itself and continually adjusting the fold point, is not established. The mechanism leaves the tight hairpin of the packaging site hairpin exposed at one end and the rest of the loop of ssDNA covered with about 700 gp5 dimers. While this is happening, thousands of copies of the major coat protein and smaller numbers of the four other capsid proteins are accumulating in the inner membrane. The morphogenesis proteins are also inserted in the inner membrane, and about 14 molecules of another morphogenetic protein forms a closed pore in the outer membrane.

Assembly and export

Assembly nucleates with the formation of a complex between gp7 and gp9 (**Figure 2**) and the exposed packaging signal at one end of the gp5/ssDNA complex, anchoring it to the membrane. Concerted nucleoside triphosphate (NTP)-driven interactions move the DNA from the complexes into the membranes where gp8 coat protein displaces gp5. The gp8 are present as specific dimers and higher multimers in the membrane and they might remain with the same neighbors but with altered contacts during assembly and in the virion. Virions are completed by additions of gp6 and gp3 which recognize the reverse turn of the DNA when the whole genome is packaged. Mutants of these two latter proteins lead to long virions containing multiple genomes. Adhesions of the inner-membrane assembly sites and the outer-membrane pores create the complete assembly/extrusion sites.

Virus is produced at about 100 sites per cell, without lysis and without preventing the cell from dividing.

Prophage Carrier States

Integrative filamentous phages which enter prophage states infect the cell as described above by means of attachment to specific type IV pili and DNA penetration via Tol QRA proteins. The circular ssDNA is then converted to circular dsDNA which can replicate autonomously or become integrated. It is conceivable that both processes take place in the same cell, or even that integration is initiated on the ssDNA. Whether integrated or not, the phage genomes are subject to repression by their own repressors, like the master regulator Rst R of CTXΦ, or by cellular repressors like Lex A, and the degree of suppression depends on several factors. Total suppression of the integrated prophage blocks all phage replication and export as well as the production of co-regulated or independently regulated toxins carried on the phage genomes. The regulatory state is shifted when the bacterium infects its plant or animal target, or when it is exposed to UV radiation or to mitomycin C, all of which can induce virion production at low or high levels. The filamentous prophage states differ from classic lysogeny of the lambdoid phages in that lysis does not occur, and the cells are less immune to superinfection by more of the same phage. Although widely used, the classic terms lytic and lysogenic are not exactly correct for these systems.

Filamentous Phages in Bacterial Pathogens

Innumerable bacteria inhibit Earth and most cause no harm to plants or animals. Those that do cause harm are variants carrying virulence factors of various types, principally toxins, and these are present in genetic elements or islands in the chromosomes or plasmids in the otherwise harmless strains. They can be transmitted vertically to progeny or transmitted horizontally to nonpathogenic or other pathogenic strains by way of transposons, plasmids, or prophages or infectious phage particles. Detailed characterizations of virulence factors in *Vibrio cholera* strains, coupled with the prior observation that filamentous phage Cf1 can enter prophage states, set the stage for the discovery that cholera toxin genes are encoded on the genome of an integrative filamentous phage/prophage they named CTXΦ. Transmission of the virulence genes via filamentous phage particles converted nonpathogenic vibrio strains to pathogenic ones. Detailed studies of CTXΦ, have made it the paradigm for filamentous phage-borne virulence factors.

A comprehensive search for principal virulence factors of *E. coli* 018:K1:H7, which is a K1 pathogenic strain responsible for human neonatal meningitis and cystitis in women, led to the discovery of CUS-1, an inducible prophage integrated at the *E. coli dif* site. The 9.5 kbp genome of CUS-1 has five or so open reading frames (ORFs) not in the 6.4 kbp genome of Ff and their functions probably include an integrase and a repressor. Searches by polymerase chain reaction (PCR) of pathogenic and nonpathogenic strains established that prophage CUS-1 is tightly correlated with virulence. A prophage of virtually identical sequence integrates into the *dif* site of *Yersinia pestis* (biovar orientalis), and it was given the name CUS-2. An ORF present in CUS-1 is absent in CUS-2. The presence of CUS-2Φ filamentous particles in supernatants was observed, and PCR analyses of over 50 different *Y. pestis* strains established the wide distribution of CUS-2 in modern pathogenic strains. This same *Yersinia* prophage has also acquired the names Yp01Φ and Ypf1 in separate studies.

A meningococcal disease-associated prophage (MDA phage) of 8 kbp was recently identified through DNA microarray comparisons of the whole genomes of 29 highly invasive *Neisseria meningitis* strains with 20 noninvasive strains. The bacterial isolates were from different worldwide occurrences of disease, and the comparisons showed a single 8 kbp cluster of genes that were in 29/29 of the invasive strains but only in 2/29 of the noninvasive strains. The genes were clearly those of a filamentous phage, and filamentous phage virions were found in the culture supernatants. Statistical analyses pointed unequivocally to the phage as a principal virulence factor. Computer-based genomics of the *Neisseria* strains identified this same gene cluster in *N. memingitidis*, as well as many clusters of filamentous prophage-like genes in the *N. gonorrhea* genome. These were Nf2 through Nf4.

Pf4 is a prophage in the genome of *Pseudomonas aeruginosa*, strain 01 (PA01), that is induced and exported when this bacterium forms biofilms on different substrates. PA01 is a treacherous pathogen that causes infections in burns and nosarcomial infections in hospitals, as well as in the clogged lungs of cystic fibrosis patients. The Pf4

genome of 12.5 kbp contains a 7.5 kbp portion virtually identical to the Pf1 genome so one can project a 3500 nm long virion on the basis of a Pf1-like DNA conformation and a Pf1 length of 2100 nm. Another phage likely to have Pf1-like DNA structure and that is tightly associated with pathogenicity is f237. This virus has been found with high frequency as a prophage in *V. parahaemolyticus* pandemic strains of servar 03:K6 obtained from patients suffering from acute gastroenteritis and septicema caused by shellfish in Japan and North America. Its genome is similar to the CTXΦ genome with a potential virulence factor located in the position of the cholera toxin genes.

Filamentous Phage Display

The now highly developed and expanding technology of displaying libraries of protein structures from which selections are made for desired properties began over 20 years ago when G. P. Smith published his paper on displaying libraries of antigens fused to the gp3 of filamentous phage Ff(f1). The fundamental idea is that the selected property is directly coupled to the gene determining the sequence of the protein with that property. Through thoughtful library design of and efficient selection, it has become routine to obtain peptides or proteins of high specificity and affinity. Thousands of applications of display in many areas of research and biotechnology as well as nanotechnology have brought with them many ingenious solutions to technical problems such as bias in the supposedly random sequences, limitations on the sizes and orientations of the inserts, and their functionalities that can be presented. The work has led to better understanding of filamentous phage structure which in turn has led to new display applications. In addition to Smith's initial unexpected result, it was fully unexpected that all five structural proteins of Ff can be used to display libraries, and that enormous proteins could be fused to properly modified gp8 subunits, and also that even heterodimeric proteins, such as Fab light and heavy chains, can be presented on the gp7/9 end of the virions. These results and many others are due to the great plasticity of the filamentous phage structure. This plasticity itself was unexpected based on the constancy in sequence among the three Ff's mentioned at the beginning of this article and the complexity of the assembly and extrusion processes. The detailed mutagenesis studies of S. S. Sidhu have shown just how plastic the gp8 protein structures can be. Among the countless applications, the display of antibody libraries following the contributions of G. Winter and co-workers and the development of homing peptides following the work of E. Rouslahti seem particularly noteworthy. These types of applications are the subjects of many general and specialized reviews. Display technology using filamentous phage has become routine and powerful, and it has spawned other display technologies based on other phages and on cell surface display in yeast and *E. coli* that extend the possibilities for highly specific diagnostic methods, tissue and tumor specific drugs, therapeutic antibodies, and vaccines. Much of these developments will be based on continued refinements of filamentous phage display systems.

See also: Filamentous ssDNA Bacterial Viruses; History of Virology: Bacteriophages; Icosahedral ssDNA Bacterial Viruses.

Further Reading

Bille E, Zahar JR, Perrin A, *et al.* (2005) A chromosomally integrated bacteriophage in invasive meningococci. *Journal of Experimental Medicine* 201: 1905–1913.

Bradley DE, Coetzee JN, Bothma T, and Hedges RW (1981) Phage X: A plasmid-dependent, broad host range, filamentous bacterial virus. *Journal of General Microbiology* 126: 389–396.

Campos J, Martinez E, Marrero K, *et al.* (2003) Novel type of specialized transduction for CTX phi or its satellite phage RS1 mediated by filamentous phage VGJΦ in *Vibrio cholerae*. *Journal of Bacteriology* 185: 7231–7240.

Davis BM and Waldor MK (2005) Virulence-linked bacteriophages of pathogenic vibrios. In: Waldor MK, Freidman DI, and Adhya SL (eds.) *Phages: Their Role in Bacterial Pathogenesis and Biotechnology*, pp. 187–205. Washington, DC: ASM Press.

Day LA and Maniloff J (2000) *Inoviridae*. In: van Regenmortel MHV, Fauquet CM, Bishop DHL, *et al*. (eds.) *Virus Taxonomy*: *Seventh Report of the International Committee on Taxonomy of Viruses*, pp. 267–275. San Diego, CA: Academic Press.

Day LA, Marzec CJ, Reisberg SA, and Casadevall A (1988) DNA packing in filamentous bacteriophages. *Annual Review of Biophysics and Biophysical Chemistry* 17: 509–539.

Goldbourt A, Gross BJ, Day LA, and McDermott AE (2007) Filamentous phage studied by magic-angle spinning NMR: Resonance assignment and secondary structure of the coat protein in Pf1. *Journal of the American Chemical Society* 129: 2338–2344.

Gonzalez MD, Lichtensteiger CA, Caughlan R, and Vimr ER (2002) Conserved filamentous prophage in *Escherichia coli* O18:K1:H7 and *Yersinia pestis* biovar orientalis. *Journal of Bacteriology* 184: 6050–6055.

Liu DJ and Day LA (1994) Pf1 virus structure: Helical coat protein and DNA with paraxial phosphates. *Science* 265: 671–674.

Lubkowski J, Hennecke F, Pluckthun A, and Wlodawer A (1998) The structural basis of phage display elucidated by the crystal structure of the N-terminal domains of g3p. *Nature Structural Biology* 5: 140–147.

Model P and Russel M (2006) Filamentous bacteriophage. In: Calendar R (ed.) *The Bacteriophages,* 2nd edn., pp. 375–456. New York: Oxford.

Sidhu SS (2000) Phage display in pharmaceutical biotechnology. *Current Opinion in Biotechnology* 11: 610–616.

Tomar S, Green MM, and Day LA (2007) DNA–protein interactions as the source of large-length-scale chirality evident in the liquid crystal behavior of filamentous bacteriophages. *Journal of the American Chemical Society* 129: 3367–3375.

Tsuboi M, Kubo Y, Ikeda T, Overman SA, Osman O, and Thomas GJ, Jr. (2003) Protein and DNA residue orientations in the filamentous virus Pf1 determined by polarized Raman and polarized FTIR spectroscopy. *Biochemistry* 42: 940–950.

Wang YA, Yu X, Overman S, Tsuboi M, Thomas GJ, Jr., and Egelman EH (2006) The structure of a filamentous bacteriophage. *Journal of Molecular Biology* 361: 209–215.

Welsh LC, Symmons MF, Sturtevant JM, Marvin DA, and Perham RN (1998) Structure of the capsid of Pf3 filamentous phage determined from X-ray fibre diffraction data at 3.1 Å resolution. *Journal of Molecular Biology* 283: 155–177.

Insect Pest Control by Viruses

M Erlandson, Agriculture & Agri-Food Canada, Saskatoon, SK, Canada

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Biological control The use of microbial pathogens to control pest organisms.
Integrated pest management Multifaceted approach in designing insect control strategies.
Optical brighteners Stilbene compounds used to enhance infectivity of biological control agents.
Melanoplus sanguinipes Species of migratory grasshopper.

Introduction

Insects take a tremendous toll on food and fiber production in the agricultural and forestry sectors, with estimates as high as 30% of crop production lost to insect damage in the field and in storage. Insects also act as vectors for many disease organisms infecting plants of agricultural importance and animals and humans. For some 60 years, insect control has relied primarily on the use of broad-spectrum chemical pesticides. This heavy usage has led to the emergence of secondary pest problems due to the loss of arthropod natural enemies and the development of resistance in insect pest populations to chemical insecticides. In addition, negative human health and environmental effects have been noted due to the broad target spectrum of these chemicals and their persistence in the environment. As a result, more environmentally sustainable pest control strategies have been proposed, notably integrated pest management (IPM). IPM incorporates a range of control tactics including cultural methods, judicious use of chemical insecticides, and biological control agents to suppress insect pest populations below levels that cause economic damage.

Biological control agents include microbial pathogens of insects and these are attractive for IPM systems due to their narrow host range. Currently microbial pest control products constitute only about 1.5% of the worldwide pesticide market, and microbial insecticide products are dominated by *Bacillus thuringiensis* (Bt)-based formulations. Much of the developmental research on microbial pathogens of insects has focused on their use in the same fashion as chemical insecticides. However, the potential for microbial pathogens to amplify in hosts and persist in pest populations following application allows the implementation of alternate use strategies. Four potential strategies for the use of microbial pathogens as insect control agents have been suggested: classical biological control, conservation, inoculation, and inundation. Although variable terminology has been used by researchers working on different groups of biological control agents to describe the four strategies, virus examples can be cited for each approach. The suitability of a virus for any of these strategies depends on the basic biology of the virus–host interaction and the specific attributes of the agricultural or forest production system.

Classical biological control involves the introduction of a microbial pathogen from a foreign source into a new ecosystem with an aim to permanent establishment and long-term suppression of an insect pest, most commonly a non-native species. A good example is the European spruce sawfly, *Gilpinia hercyniae*, a major forest pest of European origin, which became established in eastern North America in the 1930s through early 1940s. Following the introduction of a highly specific and efficacious baculovirus originating from northern Europe, the pest population was reduced to below an economically significant level and remains so to the present time.

Conservation aims to conserve or enhance naturally occurring populations of microbial pathogens of insects either by environmental manipulation or changes to existing insect control strategies, including use of chemical insecticides. There are few examples of the implementation of this strategy with microbial pathogens of insects in general and no good virus examples can be cited.

The inoculative release strategy involves the application of a microbial pathogen to an insect pest population at the early stage of an outbreak, based on the expectation of season-long control with the potential for carry-over to multiyear control. This approach has been referred to augmentation when indigenous microbial pathogens are used and seasonal colonization when non-native species are introduced. There are a number of examples of this approach in forestry, including the baculovirus product TM Biocontrol-1 (Orgyia pseudotsugata multiple nucleopolyhedrovirus (MNPV)) for control of Douglas fir tussock moth. For this and the classical approach to work effectively the virus needs a mechanism for efficient spread through the host population and is often most successful for pest species that exhibit a colonial or gregarious behavior that increases the interaction between individuals.

The strategy of inundation is similar to the use of conventional chemical insecticides in that the microbial insecticide is applied to a pest insect population that has exceeded its economic threshold with the expectation

of rapid control. This approach has been used mostly against pests of greenhouse and horticultural crops as, for example, the use of Spodoptera exigua nucleopolyhedrovirus for control of beet armyworm in greenhouse crops in Europe or the codling moth granulovirus (GV) in orchards. To succeed as a microbial insecticide, a virus must be sufficiently infectious and virulent to produce high rates of infection when applied in the field and kill insects in a timely fashion so as to limit economic plant damage or the vectoring potential of the insect pest. In addition, the virus must be relatively cheap to produce, in quantities required for the market, and be in a form that is readily stored and applied. The host specificity must be sufficiently broad to target pests that constitute an economically viable market and yet have enough target specificity to be advantageous in an IPM system. Finally, a virus's target specificity, biology, and ecology need to be well understood in order for government regulatory agencies to be convinced of its environmental and human health safety as a prerequisite for registration as a microbial insecticide.

Viruses are associated with insects in a wide range of ecological relationships: as pathogens, as plant and animal viruses relying on insect vectors for transmission, and as specialized symbionts that allow hymenopteran parasitoids to fully develop in their hosts (e.g., polydnaviruses). The current taxonomy of viruses includes 22 families whose hosts include an insect or other arthropod for at least some members of the group. At least 14 virus families have representatives pathogenic in invertebrates (**Table 1**) and at least some of these families have representative viruses that have been investigated for potential as biological control agents of insects.

In the following sections, those virus families whose members have been investigated most extensively for potential as insect control agents will be described. Research has focused on virus groups with readily observable symptomatology (baculoviruses and iridescent viruses), those producing impressive epizootics in insect populations (baculoviruses), and those easily detected by light microscopy as a consequence of being occluded in relatively large protein crystals (baculoviruses, cypoviruses (CPVs), and entomopoxviruses) within infected host cells. As well, economically important insect orders, notably Lepidoptera, Diptera, Coleoptera, and Orthoptera, have been more thoroughly screened for viruses than other arthropod and insect groups. Although a number of virus groups have been investigated, those in the family *Baculoviridae* show the most promise for development as insect biological control agents.

Table 1 Virus families associated with insects

				Related viruses in	
Family/genus	*Nucleic acid*	*Virion shape*	*Occlusion body*	*Vertebrates*	*Plants*
Reoviridae					
Cypovirus	dsRNA, linear 10 segments	Isometric	+	Reovirus (e.g., blue tongue)	Rice dwarf virus Wound tumor virus
Tetraviridae				None	None
Betatetravirus	ssRNA+, 1-linear	Isometric	–		
Omegatetravirus	ssRNA+, 2-linear	Isometric	–		
Dicistroviridae				None	None
Cripavirus	ssRNA+, linear	Isometric	–		
Nodaviridae				Stripe jack virus	None
Alphanodavirus	ssRNA+, 2-linear	Isometric	–		
Picornaviridae	ssRNA+	Isometric	–	Polio, cold virus	Many
Rhabdoviridae	ssRNA–	Bullet-shaped	–	Rabies	
Baculoviridae				None	None
Nucleopolyhedrovirus	dsDNA, circular	Rod	+		
Granulovirus	dsDNA, circular	Rod	+		
Poxviridae				Smallpox	None
Entomopoxvirus	dsDNA, linear	Ovoid	+		
Iridoviridae				African swine fever virus	None
Iridovirus and *Chloridovirus*	dsDNA	Isometric	–		
Ascoviridae				None	None
Ascovirus	dsDNA, circular	Rod–ovoid	–		
Polydnaviridae				None	None
Bracovirus	dsDNA, m-circular	Rod	–		
Ichnovirus	dsDNA, m-circular	Fusiform	–		
Parvoviridae				Canine distemper virus	None
Densovirus	ssDNA	Isometric	–		

RNA Viruses

Tetraviridae

Tetraviruses are small icosahedral, nonenveloped viruses with linear single-stranded RNA (ssRNA) genomes. Tetraviruses have been isolated only from Lepidoptera. Infection appears to be restricted to the midgut and horizontal transmission by ingestion of infectious virus is likely the major route of infection; however, there is some evidence for vertical transmission. There is much variation in pathogenicity among tetravirus isolates, with symptoms ranging from inapparent to acutely lethal infections. Helicoverpa armigera stunt virus (HaSV) is the most extensively studied virus in this group and may have some potential as a biocontrol agent for pests in the *Helicoverpa* group. The *Helicoverpa* group contains some of the most damaging pests of agricultural crops, including the cotton bollworm, tobacco budworm, and corn earworm. HaSV is highly pathogenic for early instar larvae but has almost no impact on the last two larval stages. HaSV produces rapid cessation of feeding, significant delays in larval growth, and very characteristic stunting or shrinkage of the larval body. With one exception, no tetravirus replicates in cell culture and these viruses are difficult to produce in quantities required for insect control. Recently, HaSV virion assembly was demonstrated in plant protoplasts cotransformed with a plasmid expressing the HaSV capsid gene and plasmids carrying the complete cDNAs of the two HaSV genomic RNAs, suggesting novel methods of tetravirus production are possible.

In a recent field test, HaSV applied against *H. armigera* in sorghum achieved control comparable to that of commercial preparations of Gemstar (a registered strain of the baculovirus Heliothis zea single nucleopolyhedrovirus and standard chemical insecticides. These data along with the possibility of producing infectious HaSV virus particles in transgenic plants suggest that tetraviruses may have potential as viral insecticides or in more novel approaches comparable to transgenic crops expressing *B. thurinigensis* σ-endotoxins.

Reoviridae

Reoviruses are characterized by icosahedral virions with genomes consisting of 10–12 linear dsRNA molecules. The family *Reoviridae* contains a number of genera that replicate in both vertebrate hosts and arthropod vectors such as ticks and mosquitoes, as well as plant viruses that replicate both in plant hosts and arthropod vectors such as leafhoppers. The family also contains a single genus *Cypovirus* whose member viruses exclusively infect insects.

CPVs, like baculoviruses and entomopoxviruses (see below), are occluded within crystalline proteinaceous structures referred to as occlusion bodies (OBs) or polyhedra. The OB provides some stability for the virus particles in the environment. CPVs are transmitted orally and upon ingestion the OB dissolves in the alkaline conditions of the host insect gut, releasing virions to infect gut cells. Typical CPV infections are limited to midgut epithelial cells and disease symptoms are chronic in nature, mimicking those of starvation. These include reduced feeding and larval growth, increased development times, and often malformed adults that have reduced longevity and fecundity.

CPVs have been isolated most frequently from Lepidoptera but a few isolates from Diptera and Hymenoptera have been described. Although CPVs are highly infectious and persist in insect populations, they have received limited attention for development as viral insecticides due to their generally chronic rather than acute symptomology. To date, a single CPV product, Matsukemin, has been registered for use against *Dendrolimus spectabilis*, a pest of pine trees in Japan and China. It is significant that this virus is targeted against a pest of forests where substantial defoliation can be tolerated without economic damage. The other proposed use for CPV is in combination with chemical insecticides for control of insect populations resistant to these specific chemicals. There is evidence that CPV infection of the insect gut is able to suppress chemical insecticide detoxification based on cytochrome P450 pathways.

DNA Viruses

As previously mentioned, some of the most studied insect-specific DNA viruses are those readily detected due to the presence of OB and those producing observable epizootics in insect pests and thus are considered to have good potential as biological control agents of insects. In the following sections those virus families with species showing the best potential will be discussed, including *Parvoviridae*, *Poxviridae*, and *Baculoviridae*. The unassigned species *Oryctes rhinocerus virus* will also be discussed as it is an important example of a virus used successfully in an inoculative strategy.

Parvoviridae: Subfamily *Densovirinae*

Members of the subfamily *Densovirinae* exclusively infect arthropods. These viruses are small, nonenveloped, icosahedral virions with a single linear 4–6 kbp single-stranded DNA (ssDNA) genome. Densoviruses (DNVs) have been isolated from Lepidoptera, Diptera, Homoptera, Hemiptera, and Orthoptera. DNVs were discovered in laboratory colonies of the wax moth, *Galleria mellonella*, and in insects reared commercially such as wax moth and silkworm. DNVs infect larvae and produce a range of symptoms that include alterations in cuticular pigmentation, progressive paralysis, and death. Some DNVs appear to infect a single host while others have broad

experimental host ranges. The virulence of DNVs varies considerably depending on the virus species.

Some of the DNVs infecting economically important pests are quite virulent and host specific, and have been investigated as biocontrol agents. Early studies showed that introductions of *Galleria mellonella* DNV into beehives controlled heavy infestations of wax moth. The potential of several DNVs for the control of the oil palm insect pests *Sibine fusca* and *Casphalia extranea* was tested in spray trials in South America, Africa, and Egypt using homogenates of infected insects. The results were promising but as yet no information is available on the development of commercial products based on these DNVs. The Aedes aegypti densovirus was investigated for mosquito control in the Soviet Union, and a commercial product called Viroden was developed.

Although there is considerable genome sequence homology between DNVs and some of the vertebrate parvoviruses, there is no evidence for DNV-related pathology in mammalian injection trials. However, more extensive safety tests would be required before any DNV could be registered as a viral insecticide under the current regulations in most of the developed nations.

Poxviridae: Subfamily *Entomopoxvirinae*

Entomopoxviruses, subfamily *Entomopoxvirinae,* are typical poxviruses with large brick-shaped enveloped virions and a linear double-stranded DNA (dsDNA) 130–375 kbp genome. These large complex viruses replicate in the cytoplasm of infected cells and mature virions are typically occluded within proteinaceous OBs called spheroids. There are three recognized genera in the *Entomopoxvirinae*, distinguished by virion morphology, genome size, and host range: *Alphaentomopoxvirus* has been isolated exclusively from Coleoptera, *Betaentomopoxvirus* from Lepidoptera and Orthoptera, and *Gammaentomopoxvirus* from Diptera.

Entomopoxviruses have been investigated for their potential use as biological control agents against orthopteran insects from which other insect virus groups, including baculoviruses, have not been described. Melanoplus sanguinipes entomopoxvirus (MSEV) and Oedaleus senegalensis entomopoxvirus are of interest because they infect many of the major grasshopper and locust pest species. MSEV, in particular, has a broad host range among grasshoppers and locusts. This virus is infectious upon ingestion. It initially infects midgut epithelium cells and eventually produces a systemic infection in the host. MSEV infections develop slowly in grasshoppers with mortality occurring only after 3 or more weeks, though other symptoms such as inhibition of pigment production (**Figure 1**), developmental delay, and reduced food consumption occur more quickly. Preliminary field trials in which 1.0×10^{10} MSEV spheroids per hectare, formulated in starch granules or on wheat bran, were applied to rangeland plots produced 25–30% infection levels among grasshoppers within 2 weeks. However, for grasshopper and locust pests, entomopoxviruses are not likely virulent enough to be considered for use as microbial insecticides for crop protection but may have the potential in an inoculative strategy to suppress grasshopper populations over a number of seasons in rangeland habitats where plant damage is not as critical as it is for cereal crops.

The morphological and biochemical similarities of entomopoxviruses to poxviruses infecting mammals have raised potential safety concerns. However, as more genome sequence data have become available, it is clear that entomopoxviruses have a set of collinear core genes

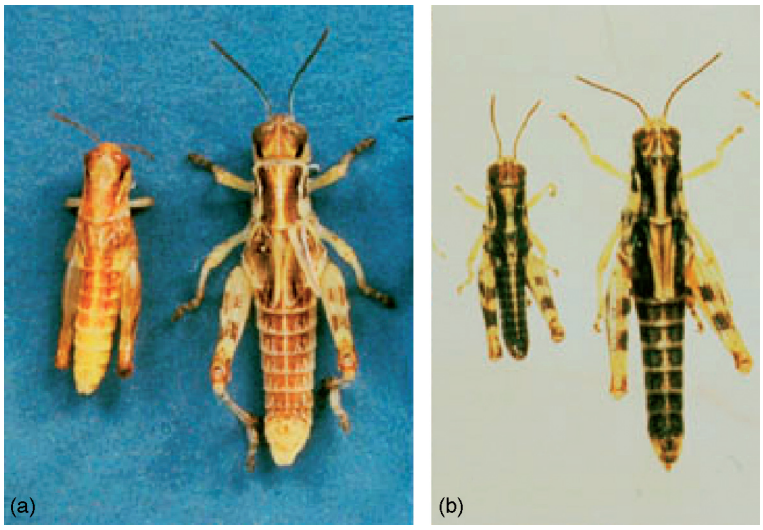


Figure 1 Nymphs of the migratory grasshopper, *Melanoplus sanguinipes*, infected with Melanoplus sanguinipes entomopoxvirus (a) compared to uninfected nymphs (b).

that distinguish them from vertebrate poxviruses. In addition, no serological relationship exists between insect and vertebrate poxviruses, and the evidence for host restriction of entomopoxviruses to insects is strong.

Baculoviridae

The *Baculoviridae* is the most studied group of insect-specific viruses due to their long-recognized potential as biological control agents and their use as eukaryotic expression vector systems. Baculoviruses are characterized by rod-shaped enveloped virions and a circular, dsDNA genome of 80–180 kbp. As with CPVs and entomopoxviruses, virions are contained within OB (0.5–5 μm diameter). The virus is infectious orally and occlusion-derived virions (ODVs) infect midgut epithelial cells after ingestion and dissolution of the OB in the alkaline condition of the midgut. Typically, two virion phenotypes occur in baculovirus infections: ODVs and budded virions (BVs), which spread the infection to tissues throughout the host. Thus the ODVs are responsible for horizontal transmission between insect hosts and BVs for systemic spread of infection within a host.

Baculoviridae contains two genera, *Nucleopolyhedrovirus* and *Granulovirus*, which are distinguished on the basis of OB morphology. GVs have been described only from Lepidoptera whereas NPVs have been isolated from Lepidoptera, Hymenoptera, Diptera, and possibly crustaceans. To date, baculoviruses have been isolated from over 700 species of arthropods.

Baculoviruses are often associated with spectacular epizootics, particularly among lepidopteran and sawfly forest pests. At late stages of baculovirus infection, insects may climb to the top of host plants where they die and are thus quite obvious (**Figure 2**). These infected cadavers serve as a source of inoculum as they degrade and release OB onto the plant canopy or into the soil. Although the virions within OB are quite susceptible to ultraviolet (UV) inactivation, when OBs are protected within intact cadavers or in upper layers of soil, the virus can remain viable for extended periods. In this way, the OBs can serve as a reservoir for infection of subsequent host generations, potentially for several years as the virus is returned to the plant canopy. This pattern of virus cycling is particularly evident for insect populations in more stable ecosystems such as forests.

The obvious nature of baculovirus epizootics led to an early interest in their potential as biological control agents. Baculoviruses have a number of attributes that are potential advantages for a microbial insecticide, particularly in the current context of increased interest in more environmentally sustainable pest control in an IPM system. In general, baculoviruses are quite virulent compared to other insect viruses and kill their hosts in 5–14 days depending on the specific virus–host interaction, virus dose, stage of insect development, food plant, and abiotic factors (notably temperature). Baculoviruses often replicate to high levels, and it is not uncommon for 1.0×10^{8}–1.0×10^{9} OBs to be produced in a single host, resulting in large quantities of inoculum for secondary infection beyond the initial virus application. Baculoviruses are typically quite host-specific, infecting a few closely related species. There are, however, exceptions. For example, Autographa californica multiple nucleopolyhedrovirus and Mamestra brassicae multiple nucleopolyhedrovirus infect upward of 100 species in several lepidopteran families. Narrow host specificity limits the risk of negative nontarget effects and makes baculoviruses attractive for use in IPM systems. The fact that baculoviruses are occluded in OB leads to increased environmental stability and enables baculovirus-based insecticides to be applied using existing spray application technology. Most current strategies for production of baculovirus-based insecticides rely on *in vivo* production in insects, allowing for relatively low technology production systems, which can be advantageous in the developing world. It is also generally considered that the cost of registration of baculoviruses, as low-risk pest control alternatives, may be less than that for registration of chemical insecticides.



Figure 2 Bertha armyworm, *Mamestra configurata*, larva infected with Mamestra configurata nucleopolyhedrovirus.

However, some of the perceived advantages can also be a liability. For example, the host specificity of a baculovirus may limit its market size to one or a few pest

species, and yet significant development and production costs are incurred. In contrast, costs are spread over a broader spectrum of target species in the case of a chemical insecticide. Although relatively virulent, baculoviruses need to be ingested by and replicate in the host before feeding stops and mortality occurs. Thus, the lag time between application and control is significantly longer than for contact chemical insecticides whose knockdown effect occurs very rapidly. Education of agricultural producers and/or pest control companies on strategies for the use of baculovirus insecticides will be essential for successful adoption of this technology. There is also a perceived high cost of production for baculoviruses whether done *in vivo* in cultured insects, largely due to labor costs, or in insect cell culture systems which can be capital intensive and operationally expensive due to media components required for their maintenance. However, one of the major limitations of baculoviruses is their limited field stability in aqueous spray suspensions due to UV inactivation. The half-life of a spray application of unformulated baculovirus may be as little as half a day under certain field conditions, and novel approaches to virus formulation are required to overcome this drawback.

Currently, a number of baculoviruses are registered as insect control products worldwide (**Table 2**). Most of the baculoviruses registered for control of lepidopteran and sawfly forest pests were developed and registered by governmental agencies; for example, Gypcheck and Neocheck-S were produced by the US Forest Services. These viruses have typically been used to treat pest populations before they reach their maximum outbreak phase, in essence creating an epizootic before it would naturally occur in a delayed density-dependent manner. This use pattern is an example of an inoculative release strategy. As many of these forest pest populations are cyclical in nature, the production and stockpiling of baculovirus-based insecticides has not been taken up by commercial ventures but rather by government agencies to be used when required.

A limited number of baculoviruses are used as microbial insecticides or inundative control agents in relatively large scale applications. The Cydia pomonella granulovirus (CpGV) for control the codling moth in orchard

Table 2 Registered and experimental virus-based bioinsecticides

Virus	*Product names*	*Target pest*	*Production/crop system*
Baculoviruses			
Lymantria dispar multiple nucleopolyhedrovirus	Gypcheck	Gypsy moth	Forestry
Orgyia pseudotsugata multiple nucleopolyhedrovirus	TM Biocontrol	Douglas fir tussock moth	Forestry
Neodiprion sertifer nucleopolyhedrovirus	Neocheck-S, Virox	European spruce sawfly	Forestry
Neodiprion lecontei nucleopolyhedrovirus	Lecontivirus	Redheaded pine sawfly	Forestry
Adoxphyes orana granulovirus	Capex 2	Summer fruit tortrix moth	Orchard
Cydia pomonella granulovirus	Madex 3, CYD-X, Granupom, Carposin Virosoft CP-4, Virin-Gyap	Codling moth	Orchard
Autographa californica multiple nucleopolyhedrovirus	VPN 80	Multiple pest targets	Horticulture, Glasshouse and field crops
Anagrapha falcifera multiple nucleopolyhedrovirus		Multiple pest targets	Horticulture, Glasshouse and field Crops
Anticarsia gemmatalis multiple nucleopolyhedrovirus	Polygen, multigen	Velvet bean caterpillar	Soybean
Heliocoverpa armigera single nucleopolyhedrovirus		*Heliothis*/*Helicoverpa* complex	Cotton
Helicoverpa zea multiple nucleopolyhedrovirus	Gemstar	*Heliothis*/*Helicoverpa* complex	Cotton, Horticulture
Mamestra brassicae multiple nucleopolyhedrovirus	Mamestrin	Multiple pest target	
Mamestra configurata multiple nucleopolyhedrovirus		Bertha Armyworm (*M. configurata*)	Canola
Spodoptera exigua multiple nucleopolyhedrovirus	Spodex	Beet Armyworm (*S. exigua*)	Horticulture, glasshouse, and field crops
Cypoviruses			
Dendrolimus spectabilis cypovirus 1	Matsukemin	Pine caterpillar	Forestry

crops has been successfully marketed under a number of product names. CpGV is an attractive control alternative for producers both to combat insecticide-resistant populations and where there is pressure to reduce chemical pesticide applications in the orchard industry. There are also a few examples of baculovirus products being used to control lepidopteran pests of field crops. These include: Helicoverpa zea nucleopolyhedrovirus and Helicoverpa armigera single nucleopolyhedrovirus (HaSNPV) for control of members of the *Helicoverpa/Heliothis* pest complex on cotton; Spodoptera exigua multiple nucleopolyhedrovirus for control of beet armyworm, *S. exigua,* in greenhouse ornamental and vegetable crops, as well as field crops; and Anticarsia gemmatalis multiple nucleopolyhedrovirus (AgMNPV) for control of velvetbean caterpillar, *Anticarsia gemmatalis*, in soybean. Although not used extensively, AcMNPV and Anagrapha falcifera multiple nucleopolyhedrovirus (a strain of AcMNPV) have great potential due to their much wider host range. Both HaSNPV and AgMNPV are used extensively. Each year, HaSNPV is applied to more than 100 000 ha of cotton in China. AgMNPV is the most successful example of a baculovirus-based insecticide, being applied annually to more than 2 million ha of soybean in Brazil. This virus is produced from infected field-collected and/or laboratory-reared caterpillars. A number of factors have been cited for the success of AgMNPV as a viral insecticide: (1) it is relatively virulent and gives good control when applied at 1.5×10^{11} OB/ha, a rate 5–10 times lower than that for some other baculovirus products; (2) it is efficiently transmitted within the pest population following application (thus, one AgMNPV application gives control equivalent to that from 1.8 applications of conventional chemical insecticides); (3) soybean can withstand substantial defoliation without yield reductions; (4) formulated AgMNPV costs producers less than chemical insecticides; and (5) an IPM program and education strategy for producers was developed during its introduction in Brazilian agriculture.

Despite the success of AgMNPV as a viral insecticide, the delay between virus application and pest mortality and the rapid inactivation of virus by UV radiation are still considered major drawbacks. The latter problem is being addressed for baculoviruses in general by formulation research. Previous formulation efforts were based on approaches typically used with chemical pesticides in terms of wetting, sticker and emulsifying agents, as well as UV-blocking and absorption agents. Recently, more novel UV protection agents have been investigated, including stilbene-derived optical brighteners. One unexpected outcome of this research was the demonstration of increased infectivity of baculoviruses in the presence of optical brighteners, possibly due to their interaction with the peritrophic matrix, a protective barrier that lines the insect midgut. This has led to increased research efforts to identify other synergistic compounds that increase efficacy when included in a formulation cocktail for baculoviral insecticides.

Significant research effort, based on baculovirus molecular biology and from the development of baculovirus expression vectors, has gone into the genetic manipulation to enhance speed of kill. Two lines of investigation have been pursued: (1) insertion and expression of exogenous genes that impact negatively on normal insect function; and (2) manipulation or deletion of baculovirus genes that affect infectivity or virulence. Exogenous genes that have been expressed in baculoviruses include insect-specific neurotoxin genes from invertebrate sources, and insect genes that encode peptide hormones, enzymes that affect hormones or hormonally regulated processes, and proteases (**Table 3**). Recombinant baculoviruses expressing insect-specific neurotoxins from scorpion and mite sources using baculovirus late and very late gene promoters (*p6.9*, *p10*, and *polyhedrin*) have produced the best results, decreasing the time to kill host insects by 30–50%, thus killing hosts in 48–72 h post-infection. The expression of peptide hormones or enzymes regulating hormone levels, for example juvenile hormone esterase, has met with less success, possibly because of the complex nature of insect endocrine regulation. Significant increases in the speed of kill, up to 50% faster, have been observed for a recombinant AcMNPV virus expressing the flesh fly protease, cathepsin L, an enzyme known to digest proteins associated with the basal lamina of the insect midgut. It is postulated that protease degradation of the midgut basal lamina allows for more rapid spread of virus infection to the rest of the insect.

Table 3 Genetic manipulation of baculoviruses for enhanced efficacy

Invertebrate toxins (insect selective neurotoxins)
Androctonus australis (North African scorpion) insect toxin 1 (AaIT)
Leiurus quinquestriatus hebraeus (Israeli scorpion) insect toxin 2 (LqhIT2)
Bethus eupeus (Central Asian scorpion) insect toxin 1 (BeIT)
Pyemotes tritici, straw-itch mite insect-selective neurotoxin (tox34)
Agelenopsis aperta (American funnel web spider) insect – selective neurotoxin (μ-*Aga-IV*)
Anemonia sulcata (sea anemones) insect-selective neurotoxin (AsII)
Other toxins
Bacillus thuringiensis σ-endotoxin
Insect hormones
Diuretic hormone – *Manduca sexta* DH
Eclosion hormone – *M. sexta* EH
Prothoracicotropic hormone
Pheromone biosynthesis activating neuropeptide – *Helicoverpa zea* PBAN
Enzymes
Juvenile hormone esterase – JHE – from multiple species
Baculovirus enhancin (metalloprotease) – TnGV and MacoNPV
Basal lamina degrading enzymes – human gelatinase A, rat stromelysin-1, and flesh fly cathepsin L

Manipulation of baculovirus genes for increased efficacy has for the most part focused on deletion strategies, particularly deletion of the gene for *ecdysteroid UDP-glucosyl transferase* (*egt*). *egt* encodes an enzyme that inactivates the insect hormone ecdysone which controls the timing of the molt. This gene is thought to confer an advantage on baculoviruses because delaying the molt, an energy- and resource-intensive process, leads to prolonged larval development and higher yields of virus. *egt*-deletion viruses kill host insects up to 30% more quickly than the wild-type virus.

Among other baculovirus genes that increase efficacy is *enhancin*, a gene found only in GVs and a few NPVs. *enhancin* encodes a metalloprotease that degrades the peritrophic matrix, and its expression in baculoviruses that do not naturally contain the gene has been demonstrated to lower the dose required for infection of host larvae.

A number of recombinant baculoviruses have been tested in small-scale field trials to evaluate efficacy, persistence, nontarget insect impacts, and safety. The most commonly used approach is a combination of the *egt*-deletion strategy and scorpion toxin gene expression. AcMNPV and HaSNPV recombinants of this type have been field-tested in different crop systems and have demonstrated significant increases in efficacy over wild-type viruses, in some cases giving equivalent crop protection as standard chemical insecticides.

To date, none of the recombinant baculoviruses has been registered or commercialized, but they appear to hold good promise as effective insect control agents. There is some question as to whether wild-type or recombinant baculoviruses will be successfully commercialized using a similar model to that for chemical. Perhaps more innovative strategies for commercially producing and marketing viral insect control agents will need to be devised.

Oryctes Rhinoceros Virus

Oryctes rhinoceros virus (OrV) was previously classified as a nonoccluded baculovirus based on structural similarities including an enveloped, rod-shaped virion and a circular dsDNA genome of approximately 130 kbp. However, there are morphological differences, notably nucleocapsid structure and the lack of an OB. In addition, genomic sequence data indicate that OrV is not related to baculoviruses. OrV was first discovered in the 1960s during a search for biological control agents for adults of the rhinoceros beetle, *Oryctes rhinoceros*, which caused severe damage to coconut palm plantations on South Pacific islands where the insect had been accidentally introduced. Originally discovered in rhinoceros beetle larvae in Malaysia, the subsequent utilization of this virus in an IPM system is credited with saving the palm industry in the South Pacific.

Larvae become infected with OrV by ingestion and produce a systemic infection with significant mortality occurring within 1–4 weeks. Larvae do not cause significant economic damage to palms as they typically feed on decaying vegetation in palm plantations. Thus initial control attempts with OrV targeted the larval stage by applying homogenized infected larvae mixed with sawdust to leaf litter in palm plantations. This strategy significantly reduced the buildup of adult beetle populations and feeding damage on palm shoots. In addition, infected adult beetles disseminated OrV to neighboring plantations. The early stage of infection in adult beetles is restricted to the midgut epithelium; thus, large quantities of OrV are voided in the fecal material, resulting in very efficient horizontal transmission to other adult beetles as well as to larvae. Virus infection of adults also leads to significantly reduced fecundity. Thus, it became common practice to release infected beetles into infested palm plantations as means of introducing the virus into the system. Initial trials conducted in Samoa showed reductions in palm damage of up to 95% in treatment versus control plots. Subsequently, introductions of virus-infected adults were made on other South Pacific islands leading to significantly lower economic damage to palms. This utilization strategy for OrV has led to the long-term control of this important pest with only occasional re-introductions of infected adults being required. This is an example of a very successful inoculative release strategy. The success is largely due to specific knowledge of the virus–pest interaction; the adult beetle produces large quantities of OrV inoculum in fecal material leading to infection of other beetles and the gregarious nature of the pest also contributes to the efficacy by which the virus spreads naturally.

Recently, changes in agriculture practices have led to resurgence in the rhinoceros beetle as an economic pest in Malaysia. This has spurred renewed interest in OrV as a biological control agent and led to additional studies on the ecology and genetics of the virus. OrV strains of differing virulence have been identified in natural populations; thus there are opportunities for selecting the most efficacious strains for particular beetle populations. The ongoing successful application of this virus for beetle control will require more basic knowledge of OrV genetics and full understanding of the environmental factors that determine the virus prevalence and dispersal in the pest population. Finally, it is noteworthy that in the 40 years since the discovery of OrV no similar viruses have been found in any arthropod other than beetles in the family Scarabaeidae.

Several other families of insect-specific DNA viruses have been investigated for their potential as biological control agents but due to their lack of virulence and poorly understood modes of transmission they are not discussed here. These include *Ascoviridae* and *Iridoviridae (Iridovirus* and *Chloriridovirus).*

Conclusions

Insect-specific viruses have proven potential for development as biological control agents of insect pests in the context of sustainable IPM systems. Their utilization faces several hurdles that include the lag time between virus application and insect control, virus production technology, and limited field stability, but these issues are being addressed by further research and development. To date, baculoviruses have been the most successfully exploited insect viruses in terms of the number of species registered and commercially developed for use in specific markets. There is potential for other virus groups including CPVs, tetraviruses, and entomopoxviruses to be developed as more detailed information on their genetics and biology is forthcoming. For all these virus groups the possibility of genetic engineering holds promise for increased efficacy. However, it is quite likely that innovative approaches that diverge from the chemical insecticide model may be needed to fully exploit the potential of viruses as insect biological control agents.

See also: Ascoviruses; Baculoviruses: Molecular Biology of Granuloviruses; Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Baculoviruses: Molecular Biology of Sawfly Baculoviruses; Entomopoxviruses; Oryctes Rhinocerous Virus; Parvoviruses of Arthropods; Tetraviruses.

Further Reading

Bonning BC (2005) Baculoviruses: Biology, biochemistry, and molecular biology. In: Gilbert L, Iatrou K, and Gill S (eds.) *Comprehensive Molecular Insect Science,* vol. 6, pp. 233–270. Amsterdam: Elsevier.

Christian PD, Murray D, Powell R, Hopkinson J, Gibb NN, and Hanzlik TN (2005) Effective control of a field population of *Helicoverpa armigera* by using the small RNA virus Helicoverpa armigera stunt virus (*Tetraviridae*: *Omegatetravirus*). *Journal of Economic Entomology* 98: 1839–1847.

Copping LG and Menn JJ (2000) Biopesticides: A review of their action, applications and efficacy. *Pest Management Science* 56: 651–676.

Huger AM (2005) The *Oryctes* virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). *Journal of Invertebrate Pathology* 89: 78–84.

Kamita SG, Kang K-D, Hammock BD, and Inceoglu AB (2005) Genetically modified baculoviruses for pest insect control. In: Gilbert L, Iatrou K, and Gill S (eds.) *Comprehensive Molecular Insect Science,* vol. 6, pp. 271–322. Amsterdam: Elsevier.

Lacey LA, Frutos R, Kaya HK, and Vail P (2001) Insect pathogens as biological control agents: Do they have a future? *Biological Control* 21: 230–248.

Moscardi F (1999) Assessment of the application of baculoviruses for control of Lepidoptera. *Annual Review of Entomology* 44: 257–289.

Vail PV, Hostetter DL, and Hoffmann DF (1999) Development of multi-nucleocapsid nucleopolyhedroviruses (MNPVs) infectious to loopers (Lepidoptera: Noctuidae: Plusiinae) as microbial control agents. *Integrated Pest Management Reviews* 4: 231–257.

Insect Reoviruses

P P C Mertens and H Attoui, Institute for Animal Health, Pirbright, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Diptera (also known as true flies) An order of insects that comprises mosquitoes, gnats, midges, sand flies, and other flies. They possess a single pair of wings on the mesothorax (the middle of the three segments of the thorax of an insect) and a pair of halters which are derived from the hind wings present on the metathorax (the posterior of the three segments of the thorax of an insect). The name is derived from the number of wings (*di* means two in Greek and *pteron* refers to the wings).

Hymenoptera This order of insects comprises the sawflies, wasps, bees, and ants. The name refers to the membranous wings of the insects, and is derived from ancient Greek (*humen*: membrane and *pteron*: wings). The hind wings of hymenopterans are connected to their forewings by hooks.

Lepidoptera This order of insects comprises butterflies, skippers, and moths. The name is derived from ancient Greek and refers to the minute scales (*lepidon*) that cover the membranous lanceolate wings (*pteron*) of adults.

Introduction

Insects can become infected by many diverse DNA or RNA viruses, including members of the families *Baculoviridae*, *Poxviridae* (genus *Entomopoxvirus*), *Iridoviridae*, *Polydnaviridae*, *Ascoviridae*, *Birnaviridae*, and *Reoviridae*. The family *Reoviridae* (the reoviruses) includes 15 recognized genera of icosahedral, nonmembranous viruses, with genomes composed of 9–12 segments of linear double-stranded RNA (dsRNA). Many animal and plant

reoviruses are transmitted by insect or arthropod vectors that they also infect (genera: *Orbivirus*, *Fiivirus*, *Phytoreovirus*, *Oryzavirus*, *Seadornavirus*, and *Coltivirus*). However, three established genera of reoviruses (*Cypovirus*, *Idnoreovirus*, and *Dinovernavirus*) contain viruses that only infect insects.

The genus *Cypovirus* (sigla: *cy*toplamic *po*lyhedrosis *virus*) was recognized by the International Committee for the Taxonomy of Viruses (ICTV) in 1978. Cypovirus (CPV) particles are ~70 nm in diameter, contain a ten segmented dsRNA genome, and belong to the 'turreted' reoviruses, with surface projections (turrets) situated at each of the 12 'fivefold' vertices. Unlike most other reoviruses, CPVs are single-shelled, with no outer capsid layer. However, they characteristically produce crystalline protein inclusion bodies (polyhedra, usually within the cytoplasm of infected cells), in which virus particles become singly or multiply embedded. More than 230 CPV isolates have been described, with evidence for at least 20 distinct species (based on differences in the migration pattern of their genome segments during electrophoresis–electropherotype and nucleotide sequence divergence). However, very few CPVs have so far been isolated from Africa, and it appears likely that many more species will be recognized in the future.

The genus *Idnoreovirus* (sigla: *i*nsect-*d*erived *n*on*o*cluded *reovirus*) was formally recognized by ICTV in 2005. Idnoreoviruses have ten segmented dsRNA genomes, are icosahedral, ~70 nm in diameter with turreted 'core' particles. However, unlike the CPVs, they also have an outer capsid protein layer and do not produce polyhedra. Idnoreoviruses have been isolated from various flies including the fruit fly, the small fruit fly (drosophila), the olive fly, and housefly. They have also been isolated from wasps.

The recently created genus *Dinovernavirus* (sigla: *d*ouble-stranded *i*nsect, *nove*m (nine in Latin, indicating the number of genome segments) *RNA virus*) has initial support from ICTV and includes the first nine-segmented dsRNA virus. Like the CPVs, dinovernaviruses are single-shelled, turreted, and lack an outer capsid layer, but do not produce polyhedra or a 'polyhedrin' protein.

Table 1 summarizes the recognized species within the three genera described above.

Table 1 List of species (and tentative species) within genera *Cypovirus*, *Idnoreovirus*, and *Dinovernavirus*

Species	*Representative isolate*	*Abbreviation*
Genus *Cypovirus*		
Cypovirus 1	Bombyx mori cypovirus 1	BmCPV-1
Cypovirus 2	Aglais urticae cypovirus 2	AuCPV-2
Cypovirus 3	Anaitis plagiata cypovirus 3	ApCPV-3
Cypovirus 4	Actias selene cypovirus 4	AsCPV-4
Cypovirus 5	Euxoa scandens cypovirus 5	EsCPV-5
Cypovirus 6	Aglais urticae cypovirus 6	AuCPV-6
Cypovirus 7	Mamestra brassicae cypovirus 7	MbCPV-7
Cypovirus 8	Abraxas grossulariata cypovirus 8	AgCPV-8
Cypovirus 9	Agrotis segetum cypovirus 9	AsCPV-9
Cypovirus 10	Aporophyla lutulenta cypovirus 10	AlCPV-10
Cypovirus 11	Heliothis armigera cypovirus 11	HaCPV-11
Cypovirus 12	Autographa gamma cypovirus 12	AgCPV-12
Cypovirus 13	Polistes hebraeus cypovirus 13	PhCPV-13
Cypovirus 14	Lymantria dispar cypovirus 14	LdCPV-14
Cypovirus 15	Trichoplusia ni cypovirus 15	TnCPV-15
Cypovirus 16	Choristoneura fumiferana cypovirus 16	CfCPV-16
Tentative cypovirus species	Heliothis armigera cypovirus (B strain)	HaCPV-B
	Plutella xylostella cypovirus	PxCPV
	Maruca vitrata cypovirus (A strain)	MvCPV-A
	Maruca vitrata cypovirus (B strain)	MvCPV-B
Genus *Idnoreovirus*		
Idnoreovirus 1	Diadromus pulchellus idnoreovirus 1	DpIRV-1
Idnoreovirus 2	Hyposoter exiguae idnoreovirus 2	HeIRV-2
Idnoreovirus 3	Musca domestica idnoreovirus 3	MdIRV-3
Idnoreovirus 4	Dacus oleae idnoreovirus 4	DoIRV-4
Idnoreovirus 5	Ceratitis capitata idnoreovirus 5	CcIRV-5
	Drosophila melanogaster idnoreovirus 5	DmIRV-5
Tentative idnoreovirus species	Drosophila S virus	
Genus *Dinovernavirus*		
Dinovernavirus 1	Aedes pseudoscutellaris dinovernavirus 1	ApDNV-1

Historical Overview

CPVs were first recognized by Ishimori in 1934, who observed polyhedra in the midgut cells of diseased silkworm larvae. Cytoplasmic polyhedrosis has subsequently been recognized as an important cause of economic losses in the Japanese sericulture industry. CPVs have a wide host range, infecting many lepidopterous insect species (221 host species have been identified). Recent reports of CPV infections in mosquitoes confirm that they can also infect Diptera. The high levels of sequence divergence between equivalent genome segments of different CPV species, and the large numbers of distinct species, suggest that this is an ancient group. Indeed, CPVs have been detected in biting midges trapped in amber, indicating that they existed almost 100 million years ago.

The first idnoreovirus was isolated from the housefly *Musca domestica* in 1978. Several other idnoreoviruses have been isolated; the most recent (Diadromus pulchellus idnoreovirus 1 (DpIRV-1) has a genome composed of ten dsRNA segments in functional male insects, with an additional segment (derived from genome segment 3) in particles isolated from females or sterile males.

The genus *Dinovernavirus* currently contains a single species (*Aedes pseudoscutellaris dinovernavirus*; the virus Aedes pseudoscutellaris dinovernavirus 1 (ApDNV-1) was isolated from the AP61 cell line established by Varma *et al.* from the mosquito *Aedes pseudoscutellaris*.

Host Range, Diseases, Transmission, and Distribution

Cypoviruses

CPVs have only been isolated from arthropods, including members of over 45 genera of Lepidoptera. However, they have also been isolated from Hymenoptera, including the wasps *Polistes hebraeus* and *Diadromus pulchellus*, and from Diptera, including the mosquitoes *Culex restuans* and *Uranotaenia sapphirina*. One isolate was reported from the freshwater daphind *Simocephalus expinosus*. Experimental studies indicate that CPVs cannot infect mammals or mammalian cell cultures.

The majority of CPV infections produce chronic disease, often without extensive larval mortality. Consequently, many individuals become adults even though they are heavily diseased. Infected larvae can stop feeding as early as 2 days post infection, producing symptoms of starvation. Their body size and weight are often reduced and diarrhea is common. The duration of the larval stage can also increase by ~1.5 times. Infected pupae are frequently smaller and many diseased adults do not emerge correctly, can be malformed, and flightless. Infected females may exhibit a reduced egg-laying capacity.

CPVs can be transmitted on the surface of eggs, producing high levels of infection in the subsequent generation. However, disinfection of the egg surface destroys the virus, indicating that CPVs are not transovarially transmitted. The minimum infectious dose increases dramatically in later larval instars, although virulence varies significantly with virus strain and host species. Larvae can sometimes recover from CPV infections, possibly because the gut epithelium has considerable regenerative capacity and infected cells are shed at each larval moult.

CPVs are normally transmitted by an oral–fecal route. Ingested polyhedra on contaminated food materials dissolve in the high pH environment of the insect gut, releasing occluded virus particles, which can then infect the cells lining the gut wall. Infection is generally restricted to the columnar epithelial cells of the larval midgut, although goblet cells may also become infected. CPV infection spreads throughout the midgut region (and sometimes infect the fat body), although infection of the entire gut has occasionally been observed in some species. The production of very large numbers of polyhedra gives the gut a characteristically creamy-white appearance. The endoplasmic reticulum of infected cells is progressively degraded, mitochondria enlarged, and the cytoplasm becomes highly vacuolated. In most cases, the nucleus shows few pathological changes. However a CPV strain has been detected that produces polyhedra within the cell nucleus. In the later stages of infection, cellular hypertrophy is common and microvillae are reduced or absent. Polyhedra are released into the gut lumen by cell lysis and are excreted. The gut pH is lowered during infection preventing dissolution of progeny polyhedra in the gut fluid.

Idnoreoviruses

All known members of the genus *Idnoreovirus* infect insects, with host species that include *Diadromus pulchellus* and *Hyposoter exiguae* (wasps: Hymenoptera); *Musca domestica* (housefly); *Dacus oleae* (olive fly); *Ceratitis capitata* (Mediterranean fruit fly); and *Drosophila melanogaster* (small fruit fly) (flies: Diptera).

Unlike the CPVs, idnoreoviruses appear to cause few pathological effects in their hosts, although they may significantly influence the biological properties of individual insects. Drosophila S virus appears to be associated with the 'S' phenotype in *D. simulans* (a maternally inherited morphological trait associated with abnormal bristles). The presence of an additional 3.33 kbp dsRNA segment in DpIRV-1 is related to the sex and ploidy of the host, and may play a role in the biology of the host wasp species, possibly by providing information necessary for larval development.

Dinovernavirus

ApDNV-1, the only representative of this genus, was isolated from persistently infected *A. pseudscutellaris* cells.

Virion Properties, Genome, and Replication

Cypoviruses

Large proteinaceous inclusion bodies are characteristically produced during CPV infections, usually within the cytoplasm of infected insect cells. The crystalline matrix of these 'polyhedra' is primarily composed of a single viral 'polyhedrin' protein that is often encoded by the smallest genome segment (Seg-10). The polyhedrin structure appears to protect the occluded virus particles, possibly filling the role of outer capsid proteins found in other reoviruses. Polyhedra are frequently symmetrical (cubic, icosahedral, or irregular) depending on both the virus strain (polyhedrin sequence) and the host species. The polyhedrin molecules appear to be arranged as a face-centered cubic lattice with center-to-center spacing varying between 4.1 and 7.4 nm. In some virus species, the virus particles are occluded singly within small occlusion bodies, which can also aggregate. However, some CPVs produce large polyhedra containing large numbers of particles, often regularly spaced within the polyhedrin matrix. 'Empty' polyhedra containing no virus particles have also been observed.

CPV particles have a single-layered capsid, composed of a central shell, ~57 nm in diameter (by cryoelectron microscopy), which extends to 71.5 nm when the 12 'turrets' or 'spikes' on the icosahedral fivefold vertices are included. These turrets are hollow, up to 20 nm in length and 15–23 nm wide (conventional electron microscopy and negative staining). The CPV capsid has a central compartment, ~35 nm in diameter, which normally contains the genomic dsRNA.

CPV virions are structurally comparable to the cores of other reoviruses, particularly those from the genera with 'turreted' cores (e.g., *Orthoreovirus*, *Aquareovirus*, and *Oryzavirus*) (**Figures 1(a)** and **2**). CPV particles generally contain five to six distinct proteins, three of which have been identified as the T2 protein (120 copies, equivalent to the VP3(T2) subcore shell protein of bluetongue virus, or orthoreovirus lambda1); 'large protrusion protein' (LPP, 120 copies, comparable to orthoreovirus sigma2); and 'turret protein' (TP, 60 copies, comparable to orthoreovirus lambda2). CPVs also contain transcriptase enzyme complexes attached to the inner surface of the capsid shell at the icosahedral fivefold vertices. Two or three of the CPV structural proteins are usually >100 kDa.

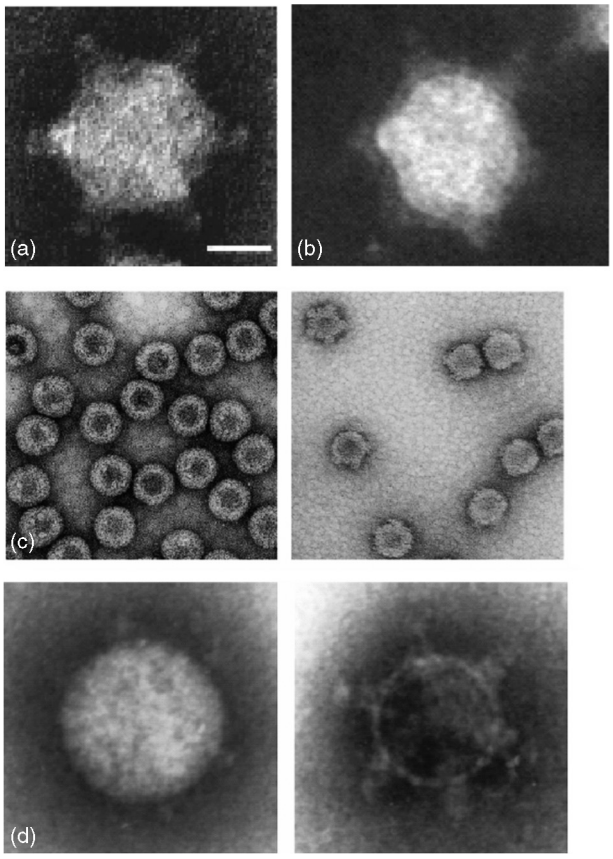


Figure 1 Electron micrographs of CPV, dinovernavirus, and idnoreovirus particles. (a) Contrast electron micrograph of nonoccluded Orgyia pseudosugata cypovirus 5 (OpCPV-5) virion. (b) Contrast electron micrograph of APDNV particles. The bars represent 20 nm. (c) Electron micrographs of purified virus particles (left) and core particles (right) of Hyposoter exiguae idnoreovirus-2 (HeIRV-1) stained with uranyl acetate. (d) Electron micrographs of a virus particle (left) and core particle (right) (stained with sodium phosphotungstate) of purified Dacus oleae idnoreovirus-4 (DoIRV-4). DoIRV-4 virions have small icosahedrally arranged surface projections (probably up to 12 in number), while the cores show much larger 'spikes' or 'turrets', which may lose a portion near to the tip (like those of the CPVs). (a) Courtesy of C. L. Hill. (c) Courtesy of Andrea Makkay and Don Stoltz. (d) Courtesy of Max Bergoin.

The structural proteins of Bombyx mori cypovirus 1 (BmCPV-1) are 148 (VP1), 136 (VP2), 140 (VP3), 120 (VP4), 64 (VP6), and 31 kDa (VP7) (see **Table 2**). Polyhedra also contain the 25–37 kDa 'polyhedrin protein' (28.5 kDa for BmCPV-1) which represents approximately 95% of the polyhedra protein (dry weight). The buoyant density of virions in CsCl is 1.44 g cm^{-3}, *c.* 1.30 g cm^{-3} for empty particles, and 1.28 g cm^{-3} for polyhedra. Due to the high level of variation between different CPVs, it is unlikely that their homologous proteins can be identified simply by their migration order during polyacrylamide gel electrophoresis (PAGE).

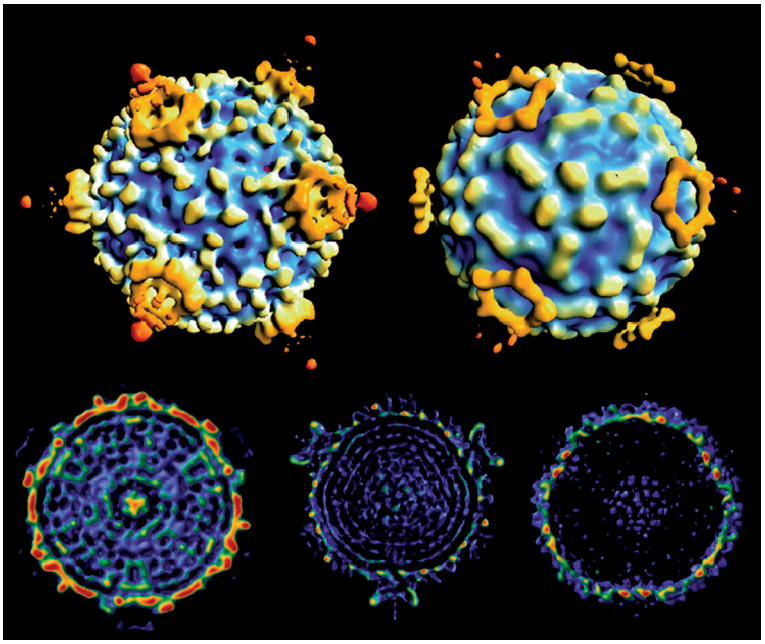


Figure 2 Cryoelectron microscopy reconstruction of a virion of Orgyia pseudosugata cypovirus-5 (OpCPV-5) to 26 Å resolution. Top left: Reconstruction of a nonoccluded virion; top right: reconstruction of an occluded virion. Bottom left: Cross section of a cryoelectron microscopy reconstruction of a fully occluded virion; bottom center: cross section of a full nonoccluded virion; and bottom right: cross section of an empty virion.The cross sections show evidence of dsRNA packaged as distinct layers and suggest localization of the transcriptase complexes at the fivefold axes of symmetry. Courtesy of C. L. Hill.

Table 2 Coding assignments of the CPV type 1 genome segments

Segment	*Size (bp)*	*Protein*	*Function/location*
Seg-1	4190	VP1 (VP1)	Major core (virion)
Seg-2	3854	VP2 (VP2)	RdRp (virion)
Seg-3	3846	(VP3)	(Virion)
Seg-4	3262	VP3 (VP4)	Possible methyltransferase (virion)
Seg-5	2852	NS1* (NS5)	Nonstructural, similar to FMDV 2Apro
Seg-6	1796	VP4 (VP6)	Leucine zipper ATP/GTP binding protein (virion)
Seg-7	1501	NS3, NS4 (VP7)	Nonstructural, with 'structural' cleavage products
Seg-8	1328	VP5 (NSP8)	Unknown (anomalous PAGE migration at 55 kDa)
Seg-9	1186	NS5 (NSP9)	Nonstructural, dsRNA binding
Seg-10	994	Polyhedrin	Polyhedrin protein (Pod)

NS1* (NS5): cleaved into NS2 (NS5a) and NS6 (NS5b).
RdRp, RNA-dependent RNA polymerase.
From information available at: http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/BmCPV-1.htm.
Under the 'Protein' heading, an alternative nomenclature for the viral proteins is given in brackets. The nomenclature that is used in the Eighth Report of the ICTV and presented on the website: http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/protein-comparison.htm.

CPVs retain infectivity for several weeks at −15, 5, or 25 °C. The virus retains full enzymatic activity (dsRNA-dependent single-stranded RNA (ssRNA) polymerase and capping activity) even after repeated freeze–thawing (up to 60 cycles), even though this disrupts the virus particle into ten active but distinct enzyme/template complexes. Each complex containing one genome segment and a complete transcriptase complex that includes one of the 'spike' structures derived from the fivefold axes of the virion capsid.

Cations have relatively little effect on CPV structure. Heat treatment of virions at 60 °C for 1 h leads to degradation and release of genomic RNA. Virus particles are relatively resistant to treatment with trypsin, chymotrypsin, ribonuclease A, deoxyribonuclease, or phospholipase. Virion enzyme functions also show some resistance

to treatment with proteinase K. However, this may reflect retention of enzyme activities despite particle disruption, particularly during the early stages of digestion. CPV particles are resistant to detergents including sodium deoxycholate (0.5–1%), but are disrupted by 0.5–1% sodium dodecyl sulfate (SDS), which releases the genomic dsRNA. Treatment with triton X-100, NP40, or urea also causes disruption of the virus particle structure. One or two fluorocarbon treatments have little effect on virus infectivity, though treatment with ethanol leads to release of RNA from virions. Viruses and polyhedra are readily inactivated by ultraviolet (UV) irradiation which dissociates the dsRNA template from the transcriptase complexes. Polyhedra remain infectious for years at temperatures below 20 °C. Virions can be released from polyhedra by treatment with carbonate buffer at pH greater than 10.5 but are disrupted below pH 5. As in permissive insects' midguts, high pH treatment completely dissolves the polyhedral protein matrix. This process is partly due to increased solubility of polyhedrin at high pH but is also aided by alkaline-activated proteases associated within the polyhedra.

Polyhedra (but not virions) contain significant amounts of adenylate-rich oligonucleotides. In the majority of cases, CPV particles contain ten dsRNA genome segments. However, virus particles containing an 11th small segment have been detected (e.g., Trichoplusia ni cypovirus 15, TnCPV-15). The genome segments of BmCPV-1 vary from 4190 to 994 bp with a total genome size of 24 809 bp. The genome segments of other CPVs have been estimated by electrophoretic comparisons, and have calculated sizes between 5.6 and 0.6 kbp, with a total genome length of 29.2–33.3 kbp.

The size distribution of the genome segments varies widely between different CPVs (e.g., the smallest dsRNA has an estimated size that varies between 0.53 and 1.44 kbp). These size differences formed the initial basis for recognition and classification of distinct species (electropherotypes) of CPVs (which differ significantly in the migration of at least three genome segments during electrophoresis using 1% agarose or 3% SDS-PAGE). The genome segment migration patterns of types 1, 12, and 14 have some overall similarity, suggesting that they are more closely related than some other CPVs (**Figure 3**).

The termini of different genome segments within a single CPV species are often highly conserved but differ from those reported for other species (**Table 3**). Choristoneura fumiferana cypovirus 16 (CfCPV-16) shows high levels of sequence variation when compared to CPV-1, -2, -5, -14, or -15 (**Table 3**) and is therefore considered to be a distinct species, even though it has a similar 5′ end but different 3′ ends to representatives of CPV-5. These data demonstrate that different CPV electropherotypes are likely to have different conserved RNA terminal sequences.

Large size variations in the genome segments of most CPV species (apart from CPV-1, -12, and -14) indicate that the gene assignments for one 'type' or species cannot simply be applied to the other CPVs. Genome segment coding assignment by *in vitro* translation of individual genome segments have been published for isolates of CPV-1 and -2. These data and subsequent sequencing studies indicate that the polyhedrin protein is often encoded by the smallest segment.

CPV uptake appears to be a relatively inefficient process in insect cell cultures, but can be significantly improved by lipofection. Liposomes deliver CPV particles into the cytoplasm where replication occurs. CPVs do not require particle modification to activate transcriptase and capping enzymes. The outer coat proteins

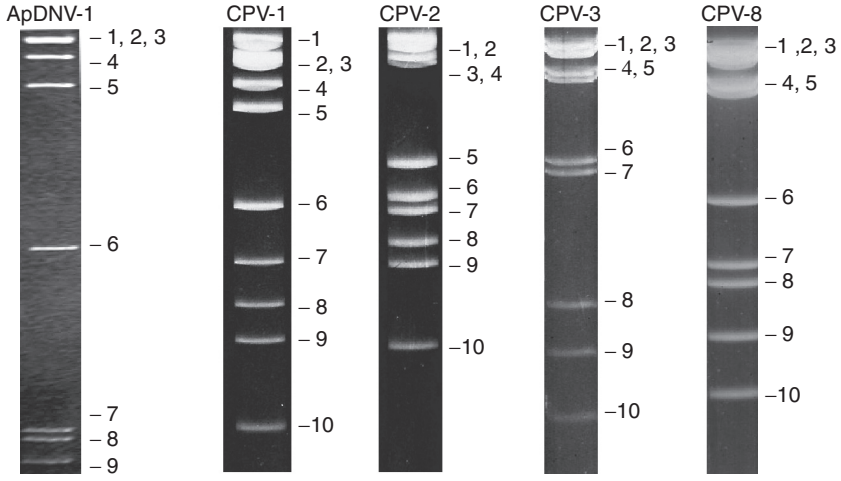


Figure 3 The genomes of dinovernaviruses and CPVs. Genome electropherotypes of members of genera *Dinovernavirus* (ApDNV-1) and *Cypovirus* (CPV-1, -2, -3, and -8). The segment position is indicated to the right of each profile.

Table 3 The conserved terminal sequences of member viruses of genera *Cypovirus*, *Idnoreovirus*, *Dinovernavirus*, and their comparison to those of genera *Fijivirus* and *Oryzavirus* (both are plant viruses)

Virus	*(Isolate)*	*Conserved RNA terminal sequences*
Cypovirus 1	(BmCPV-1)	5′-AGUAA.....GUUAGCC-3′
	(DpCPV-1)	5′-AGTAA.....GUUAGCC-3′
	(LdCPV-1)	5′-AGU$^{A}/_{G}^{A}/_{G}$.....G$^{u}/_{c}$UAGCC-3′
Cypovirus 2	(LiCPV-2)	5′-AGUUUUA.....UAGGUC-3′
Cypovirus 4	(ApCPV-4)	5′-AAUCGACG.....GUCGUAUG-3′
	(AaCPV-4)	5′-AAUCGACG.....GUCGUAUG-3′
	(AmCPV-4)	5′-AAUCGACG.....GUCGUAUG-3′
Cypovirus 5	(OpCPV-5)	5′-AGUU.....UUGC-3′
Cypovirus 14	(LdCPV-14)	5′-AGAA.....CAGCU-3′
Cypovirus 15	(TnCPV-15)	5′-AUUAAAAA.....GC-3′
Cypovirus 16	(CfCPV-16)	5′-AGUUUUU.....UUUGUGC-3′
Idnoreovirus 1	DpIRV-1	5′-$^{A}/_{G}$U/CAAUUU.....AGUAAAAAAAUn$^{A}/_{G}$G-3′
Dinovernavirus	APDNV	5′-AGUU$^{A}/_{U}$.....$^{A}/_{U}$AGU-3′
Fijivirus	NLRV	5′-AGU.....GUUGUC-3′
	MRCV	5′-AAGUUUUUU.....GUC-3′
	FDV	5′-AAGUUUUUU.....GUC-3′
	RBSDV	5′-AAGUUUUUU.....GUC-3′
Oryzavirus	RRSV	5′-GAUAAA.....GUGC-3′

Data concerning the terminal regions of members of the *Reoviridae* are listed at: http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/CPV-RNA-Termin.htm.

of other reoviruses need to be removed to activate transcription. CPV polymerase activity can show very pronounced dependence on the presence of *S*-adenosyl-L-methionine or related compounds, although this varies between different CPV species.

Virus replication and assembly occur within the host cell cytoplasm, although viral RNA synthesis can sometimes occur within the nucleus. Replication is accompanied by the formation of viroplasm (virogenic stroma or 'VIB') within the cytoplasm, containing large amounts of viral proteins and virus particles. The mechanism used to select the individual genome segments for packaging and assembly into progeny particles (exactly one copy of each segment) is unknown. The importance of the conserved terminal regions in this process is indicated by packaging and transcription of a mutant Seg10 of CPV-1 that contained only 121 bp from the 5′ end and 200 bp from the 3′ end. Particles are occluded within polyhedra apparently at the periphery of the VIB, from about 15 h post-infection onward. The polyhedrin protein is produced late in infection and in large excess compared to the other viral proteins. It is unknown how polyhedrin synthesis is regulated.

Idnoreoviruses

Electron microscopy and negative staining of the idnoreovirus particles show that they have no prominent features, are spherical in appearance (icosahedral symmetry), and ~70 nm in diameter. However, they do have a clearly defined outer capsid layer that can be removed to reveal the virus core. Unlike the CPVs, idnoreoviruses do not encode a polyhedrin protein, and the virus particles are 'nonoccluded'. Idnoreovirus core particles have an estimated diameter of ~60 nm, with 12 icoahedrally arranged surface 'turrets' or 'spikes'.

Limited studies of some viruses within the genus indicate that they are resistant to freon (trichlorotrifluoroethane) and CsCl. They may also be resistant to chymotrypsin. Intact virus particles and cores of the prototype isolate Diadromus pulchellus idnoreovirus-1 (DpIRV-1) have densities of 1.370 and 1.385 g ml^{-1}, respectively, while intact virions and empty particles of Dacus oleae idnoreovirus-4 (DoIRV-4) have a density of ~1.38 and ~1.28 g ml^{-1}, respectively (determined by CsCl gradient centrifugation). The outer capsid layer of Hyposoter exiguae idnoreovirus-2 (HeIRV-2) can be disrupted by brief exposure to 0.4% sodium sarcosinate, releasing the virus core.

The genome of most idnoreoviruses consists of ten linear segments of dsRNA that are conventionally identified as 'genome segment 1' to 'genome segment 10' (Seg-1 to Seg-10) in order of reducing molecular weight (and increasing electrophoretic mobility during agarose gel electrophoresis (AGE)). The total genome of DpIRV-1 contains an estimated 25.15 kbp of dsRNA, with individual segments ranging between ~4.8 and ~0.98 kbp, and an electrophoretic migration pattern (by 1% AGE) showing five larger and five smaller segments (a '5/5' pattern). However, the virions of DpIRV-1 may be atypical since they can sometimes also contain an 11th, 3.33-kbp genome segment, the presence of which is related to the sex and ploidy of the individual wasp host. This additional dsRNA (migrating between genome segments 3 and 4) contains sequences similar to and therefore possibly derived from Seg-3 (3.8 kbp).

The genome segments of HeIRV-2 range in size from ~3.9 to ~1.35 kbp, with a '4/6' electrophoretic migration pattern (by PAGE). DoIRV-4 contains an estimated 23.4 kbp, with segments estimated from ~3.8 to ~0.7 kbp and a '5/3/2' electrophoretic migration pattern (by PAGE). Ceratitis capitata idnoreovirus-5 (CcIRV-5) has a '3/3/4' genome segment migration pattern when analyzed by PAGE and has clear similarities to Drosophila melanogaster idnoreovirus-5 (DmIRV-5), suggesting that despite some serological differences, they belong to the same virus species. It is unclear how closely drosophila S virus is related to the other drosophila viruses. It is therefore, currently, classified as a 'tentative species' within the genus.

By analogy with other reoviruses (e.g., the orbiviruses), genome segment migration patterns (by AGE) are likely to be characteristic of each *Idnoreovirus* species. Initial sequencing studies suggest that the 3′ termini of DpIRV-1 genome segments are more variable than most other reoviruses with little sign of conservation. However, conserved sequences were detected at the 5′ termini (**Table 3**), which are different from those of other reovirus species. No sequence data are currently available for other members of this genus.

Although the proteins of DpIRV-1 have not been extensively characterized, purified virions contain 11 structural proteins with M_r 21–140 kDa (as analyzed by SDS-PAGE). Three of these appeared to be glycosylated (M_r approximately of 21, 15, and 35 kDa). Some of the viral genome segments have been sequenced (as indicated in **Table 4**). The viral proteins are named as VP1–VP10 based on the molecular weight of the genome segment (segment number) from which they are translated.

Table 4 Coding assignments for the DpIRV-1 genome segments

Segment	*Size (bp)*	*Protein*	*Function/location*
Seg-1	4800	VP1	
Seg-2	4230	VP2	
Seg-3	3812	VP3	Contains RdRp motifs
Seg-x	3333		Sequence closely related to Seg-3. Presence related to sex and ploidy of the host
Seg-4	3000	VP4	
Seg-5	2700	VP5	
Seg-6	1750	VP6	
Seg-7	1652	VP7	
Seg-8	1318	VP8	
Seg-9	1240	VP9	
Seg-10	985	VP10	

Seg-x: the supernumerary segment that is linked to the sex and ploidy of the insect host.
RdRp, RNA-dependent RNA polymerase.
More information is available at: http://www.iah.bbsrc.ac.uk/dsRNA_ virus_proteins/Idnoreovirus.htm.

On the basis of their structural and biochemical similarity, it seems likely that many aspects of the genome organization and replication of the idnoreoviruses will show similarities to those of other reoviruses (particularly the other turreted viruses). On this basis it is likely that the virus core will contain transcriptase complexes that synthesize mRNA copies of the individual genome segments. These will be exported and translated to produce the viral proteins within the host cytoplasm. These positive-sense RNAs are also likely to form templates for negative-strand synthesis during progeny virus assembly and maturation. The genome segments that have been characterized represent single genes, with a large ORF, and relatively short noncoding terminal regions.

Dinovernavirus

Transmission electron microscopy of ApDNV (the prototype dinovernavirus) showed that purified virions have a structure similar to core particles of turreted reoviruses (**Figure 1(b)**), with particular similarities to the single-shelled CPVs, with no outer capsid layer. The mean diameter of the virus particle is ~49–50 nm, with a central space (for the viral genome) that is 36–37 nm in diameter. Turrets were clearly visible projecting from the particle surface.

The infectivity of ApDNV-1 particles is destroyed by treatment with 0.1% SDS but unaffected by 1% deoxycholate or repeated treatments with Freon 113 or Vertrel-XF. Freezing at −20 or −80 °C, or heating to 50–60 °C for 30 min abolished ApDNV infectivity, even in presence of 50% fetal bovine serum (FBS). However, infectivity was stable for up to 3 weeks at room temperature and for at least 5 months at 4 °C. ApDNV is stable at pH 6–8, but infectivity was reduced tenfold at pH 4–5 or pH 9–10. The virion morphology (observed by electron microscopy) becomes distorted below pH 5 and is completely disrupted below pH 3.5.

ApDNV persistently infects AP61 cells without any visible cytopathic effects and has been detected in these cells from several different sources. The number of copies of the ApDNV genome in each persistently infected AP61 cell was estimated by quantitative polymerase chain reaction (PCR) as between 1 and 5, although treatment with 2-aminopurine (2-AM) can increase the number to 60–80 genome copies per cell. The virus can also infect and replicate in mosquito cells, including AA23 and C6/36 (from *Aedes albopictus*) cells and A20 cells (from *Aedes aegypti*), but not in AE cells (*A. aegypti*) or Aw-albus (*Aedes w-albus*). ApDNV failed to replicate in mammalian cells or in mice.

Unlike other reoviruses, the genome of ApDNV only contains nine segments of dsRNA, with a 4/1/3 AGE migration profile (**Figure 3**). The termini of ApDNV contain conserved terminal sequences (**Table 3**). The first three nucleotides 'AGU' are conserved between ApDNV,

some fijiviruses, and some CPVs; though, unlike the CPVs and fijiviruses, the first and last nucleotides of the ApDNV genome segments are complementary (A and U). The mean G+C content of the ApDNV genome is 34.4%, as compared to 34.8% for fijiviruses, 44.7% for oryzaviruses, 43% for the CPVs, and 63% for idnoreoviruses.

The putative functions of ApDNV proteins VP1–VP9 (based on sequence similarities to other reovirus proteins) are shown in **Table 5**. These comparisons suggest that there is no equivalent to the CPV polyhedrin gene and appear to confirm the status of ApDNV as a distinct and authentic nine-segmented virus.

Antigenic and Genetic Relationships

Cypoviruses

CPVs are currently classified within 16 species that were initially identified by their distinctive dsRNA migration patterns. Cross-hybridization analyses of the dsRNA, serological comparisons of the viral proteins, and comparison of RNA sequences confirmed the validity of this classification and have identified five further tentative species within the genus. However, only relatively few CPV isolates have been characterized, suggesting that there may be many more species that are as yet unidentified.

Virus isolates within a single CPV electropherotype exhibit high levels of antigenic cross-reaction, as well as efficient cross-hybridization of their denatured genomic dsRNA, even under high-stringency conditions. In contrast, there is little or no evidence of serological cross-reaction between viruses from different electropherotypes (representing different CPV species). However, CPV-1 and CPV-12 are exceptions, showing a low but significant level of serological cross-reaction. These viruses also show some overall similarity in their electropherotype pattern and detectable levels of cross-hybridization of their genomic RNA. CPV-14 also shows some similarity in its RNA electropherotype pattern to both CPV-1 and CPV-12, suggesting that it may also show some antigenic relationship and RNA sequence homology with these viruses.

The nomenclature currently used to identify different CPV isolates takes account of both the virus species and the host species from which the virus was originally isolated (e.g., an isolate of CPV-1 from *Bombyx mori* would be identified as BmCPV-1). The sequence data that are available for isolates of CPV-1, -2, -5, -14, -15, and -16 allow a comparison of some genes from these viruses. There is significantly higher conservation in the largest genome segments (possibly as a result of functional constraints) although the level of variation is relatively uniform across the whole genome. Earlier cross-hybridization studies suggested that the level of nucleotide sequence variation is also relatively uniform across the whole CPV genome, possibly reflecting the absence of neutralizing antibodies (and therefore antibody selective pressure) in their insect hosts. Different isolates within a single CPV species usually show very high levels of nucleotide sequence identity. For example, different isolates of CPV-5 show >98% identity in genome Seg 10 (the polyhedrin gene), while different isolates of CPV-1 show 89–98% nucleotide sequence identity in this gene. In contrast, comparisons of unrelated species showed only relatively low levels (20–23%) of sequence identity (**Figure 4**).

The level of amino acid (aa) identity in the putative viral polymerase varied from 92.9% to 99.5% within a single CPV type, and 42.3–43.3% between different types (**Figure 5**); see **Table 6** for abbreviations and accession numbers).

Table 5 Coding assignments for the ApDNV-1 genome segments

Segment	*Size (bp)*	*Protein*	*Function by similarity*
Seg-1	3817	VP1	Possible cell attachment
Seg-2	3752	VP2	RdRp
Seg-3	3732	VP3	T2
Seg-4	3375	VP4	Nonstructural
Seg-5	3227	VP5	Possible capping enzyme
Seg-6	1775	VP6	Possible NTPase
Seg-7	1171	VP7	Nonstructural
Seg-8	1151	VP8	Possible translational regulation
Seg-9	1147	VP9	Viral inclusion bodies

More information is available at: http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/Dinovernavirus.htm.

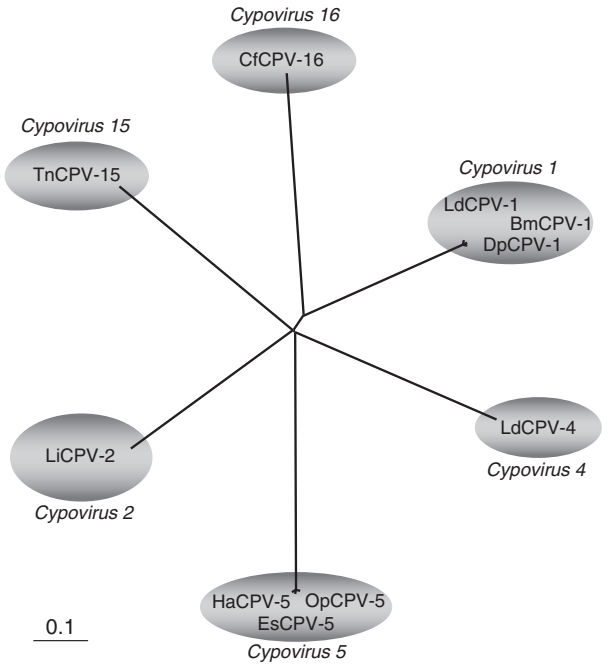


Figure 4 Phylogenetic tree for polyhedrin proteins from isolates belonging to six *Cypovirus* species. The scale bar indicates the number of substitutions per site.

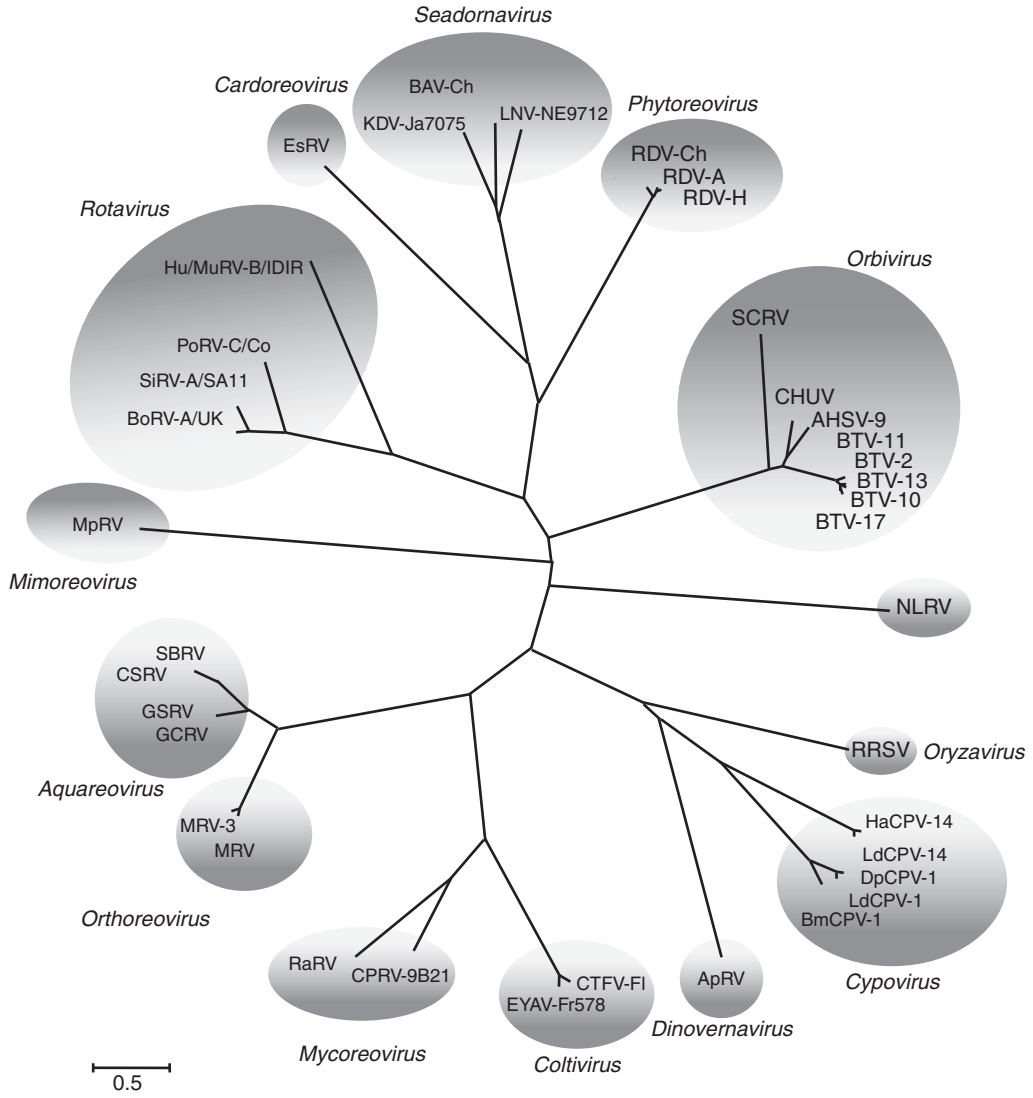


Figure 5 Neighbor-joining phylogenetic tree, built with available polymerase sequences for representative members of 15 recognized genera of family *Reoviridae*. The abbreviations and accession numbers are those provided in **Table 6**. The scale bar indicates the number of substitutions per site.

Idnoreoviruses

Antigenic relations between different idnoreoviruses have not been analyzed. The phylogenetic relationship between different idnoreoviruses is also unknown (only DpRV has been sequenced). However, the sequence of DpRV shows homologies to other reoviruses (aa identities reaching up to 20% with fijiviruses, oryzaviruses, rotaviruses, seadornaviruses, and orbiviruses).

Dinovernaviruses

Sequence comparison of the Aedes pseudoscutellaris sdinovernavirus genome to other reoviruses has shown significant aa identities in each of the viral proteins. Members of the genera *Cypovirus*, *Oryzavirus*, and *Fijivirus*, which are also turreted, showed the highest aa identities (19–31%). Amino acid identity values <30% between RdRps of the various reoviruses can be used to distinguish the members of different genera. The relationship of ApDNV to other reoviruses is illustrated by a phylogenetic tree for the RdRp gene (**Figure 5**). Although these sequence comparisons confirm the validity of ApDNV classification within a separate genus, they also indicate significant relationships with the turreted CPVs, fijiviruses, and oryzaviruses (aa identities of 26%, 23%, and 22%, respectively). Lower levels of aa identity (20%) were also detected with the mycoreoviruses (which are also turreted).

Table 6 Sequences of the RNA-dependent RNA polymerases (RdRps) used in phylogenetic analysis of different reoviruses (**Figure 4**)

Species	*Isolate*	*Abbreviation*	*Accession number*
Genus *Seadornavirus* (12 segments)			
Banna virus	Ch	BAV-Ch	AF168005
Kadipiro virus	Java-7075	KDV-Ja7075	AF133429
Liao ning virus	LNV-NE9712	LNV-NE9712	AY701339
Genus *Coltivirus* (12 segments)			
Colorado tick fever virus	Florio	CTFV-Fl	AF134529
Eyach virus	Fr578	EYAV-Fr578	AF282467
Genus *Orthoreovirus* (10 segments)			
Mammalian orthoreovirus	Lang strain	MRV-1	M24734
	Jones strain	MRV-2	M31057
	Dearing strain	MRV-3	M31058
Genus *Orbivirus* (10 segments)			
African horse sickness virus	Serotype 9	AHSV-9	U94887
Bluetongue virus	Serotype 2	BTV-2	L20508
	Serotype 10	BTV-10	X12819
	Serotype 11	BTV-11	L20445
	Serotype 13	BTV-13	L20446
	Serotype 17	BTV-17	L20447
Palyam virus	Chuzan	CHUV	Baa76549
St. Croix river virus	SCRV	SCRV	AF133431
Genus *Rotavirus* (11 segments)			
Rotavirus A	Bovine strain UK	BoRV-A/UK	X55444
	Simian strain SA11	SiRV-A/SA11	AF015955
Rotavirus B	Human/murine strain IDIR	Hu/MuRV-B/IDIR	M97203
Rotavirus C	Porcine Cowden strain	PoRV-C/Co	M74216
Genus *Aquareovirus* (11 segments)			
Golden shiner reovirus	GSRV	GSRV	AF403399
Grass carp reovirus	GCRV-873	GCRV	AF260511
Chum salmon reovirus	CSRV	CSRV	AF418295
Striped bass reovirus	SBRV	SBRV	AF450318
Genus *Fijivirus* (10 segments)			
Nilaparvata lugens reovirus	Izumo strain	NLRV-Iz	D49693
Genus *Phytoreovirus* (10 segments)			
Rice dwarf virus	Isolate China	RDV-Ch	U73201
	Isolate H	RDV-H	D10222
	Isolate A	RDV-A	D90198
Genus *Oryzavirus* (10 segments)			
Rice ragged stunt virus	Thai strain	RRSV-Th	U66714
Genus *Cypovirus* (10 segments)			
Bombyx mori cytoplasmic polyhedrosis virus 1	BmCPV-1	BmCPV-1	AF323782
Dendrolimus punctatus cytoplasmic polyhedrosis 1	DpCPV-1	DpCPV-1	AAN46860
Lymantria dispar cytoplasmic polyhedrosis 1	LdCPV-1	LdCPV-1	NC_003017
Lymantria dispar cytoplasmic polyhedrosis 14	LdCPV-14	LdCPV-14	AAK73087
Heliothis armigera cypovirus 14	HaCPV-14	HaCPV-14	DQ242048
Genus *Mycoreovirus* (11 or 12 segments)			
Rosellinia anti-rot virus	W370	RaRV	AB102674
Cryphonectria parasitica reovirus	9B21	CPRV	AY277888
Genus *Mimoreovirus* (11 segments)			
Micromonas pusilla reovirus	MpRV	MPRV	DQ126102
Genus *Dinovernavirus* (9 segments)			
Aedes pseudoscutellaris dinovernavirus	ApDNV	ApDNV	DQ087277
Genus *Cardoreovirus* (12 segments)			
Eriocheir sinensis reovirus	Isolate 905	EsRV	AY542965

See also: African Horse Sickness Viruses; Aquareoviruses; Bluetongue Viruses; Coltiviruses; Insect Pest Control by Viruses; Mycoreoviruses; Orbiviruses; Plant Reoviruses; Reoviruses: General Features; Reoviruses: Molecular Biology; Rotaviruses; Seadornaviruses.

Further Reading

Attoui H, Jaafar MF, Belhouchet M, *et al.* (2005) Expansion of family *Reoviridae* to include nine-segmented dsRNA viruses: Isolation and characterization of a new virus designated Aedes pseudoscutellaris reovirus assigned to a proposed genus (Dinovernavirus). *Virology* 343: 212–223.

Mertens PPC, Attoui H, Duncan R, and Dermody TS (2005) *Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 447–454. San Diego, CA: Elsevier Academic Press.

Mertens PPC, Makkay A, Stoltz D, Duncan R, Bergoin M, and Dermody TS (2005) Idnoreovirus, *Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 517–521. San Diego, CA: Elsevier Academic Press.

Mertens PPC, Rao S, and Zhou ZH (2005) Cypovirus, *Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 522–533. San Diego, CA: Elsevier Academic Press.

Payne CC and Mertens PPC (1983) Cytoplasmic polyhedrosis viruses. In: Joklik WK (ed.) *The Reoviridae*, pp. 425–504. New York: Plenum.

Plus N, Gissman L, Veyrunes JC, Pfister H, and Gateff E (1981) Reoviruses of drosophila and ceratitis populations and of drosophila cell lines: A new genus of the *Reoviridae* family. *Annales de Virologie (Institut Pasteur)* 132E: 261–270.

Plus N, Veyrunes JC, and Cavalloro R (1981) Endogenous viruses of *Ceratitis capitata* Wied. 'J.R.C. Ispra strain' and *C. capitata* permanent cell lines. *Annales de Virologie (Institut Pasteur)* 132E: 91–100.

Varma MG, Pudney M, and Leake CJ (1974) Cell lines from larvae of *Aedes* (*Stegomyia*) *malayensis* Colless and *Aedes* (S) *pseudoscutellearis* (Theobald) and their infection with some arboviruses. *Transactions of the Royal Society for Tropical Medicine and Hygiene* 68: 374–382.

Insect Viruses: Nonoccluded

J P Burand, University of Massachusetts at Amherst, Amherst, MA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Agonadal A condition describing insects that have malformed reproductive tissues.
Oryctes rhinoceros The rhinoceros beetle that is an economic insect pest of coconut and palm plantations.

History

The unassigned nonoccluded, insect viruses (NOIVs) are a loosely associated group of rod-shaped, enveloped, DNA-containing, insect viruses that have structural and biological similarities but with possible genetic relatedness. It has been proposed that this group of viruses be classified as a new virus family, the *Nudiviridae*. These viruses were often referred to as nonoccluded baculoviruses since they resemble baculoviruses in their size and shape as well as due to the fact that they contain a double-stranded circular DNA genome. Unlike members of the family *Baculoviridae*, these viruses are not found associated with a protein crystal occlusion body associated with baculoviruses and as such have evolved modes of transmission involving a close association between the virus and the host which often involves the virus becoming latent or persistent.

Only three NOIVs have been studied to a significant extent. The first of these to be identified was the oryctes virus (Or-1V), which was initially isolated from larvae of the rhinoceros beetle, *Oryctes rhinoceros*, in Southeast Asia in 1963 and is currently used throughout the palm-growing tropics of the Asia-Pacific region for the control of this beetle, an economically important pest of oil palm. The successful use of Or-1V for the control of this insect pest is a landmark in classical biological control.

The best-characterized NOIV is Hz-1 virus (Hz-1V), which was first identified in 1978 as a persistent agent in the insect cell line IMC-Hz-1. The IMC-Hz-1 cell line was established from adult ovarian tissues of the corn earworm *Helicoverpa zea*. The first indications that this cell line was persistently infected with a virus came from electron micrographs of these cells in which long, virus-like particles were visible. The virus harbored in these cells was eventually recovered and propagated in a number of lepidopteran cell lines of ovarian origin. Interestingly, not only could the virus undergo productive replication in these cell lines but it could also establish persistent infections in these lines.

The virus Hz-2V, formerly known as gonad-specific virus (GSV), is presently the only known sexually transmitted insect virus (**Figure 1**). This NOIV was first observed in electron micrographs of ovarian tissues of insects from a colony of *H. zea* established at the

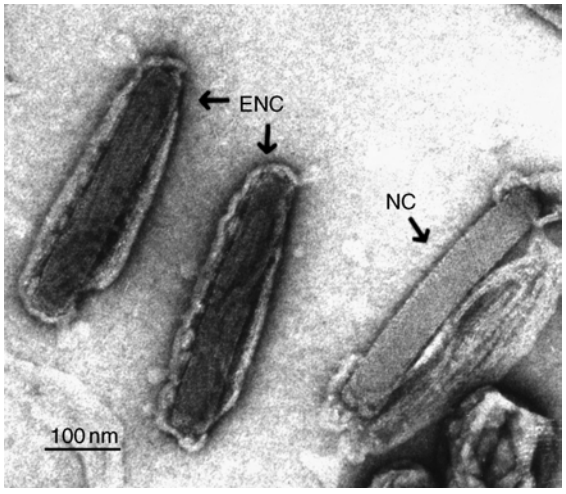


Figure 1 Electron micrograph of Hz-2V virus particle showing enveloped nucleocapsids (ENC) and a nucleocapsid from which the envelope has been partially removed (NC). Reproduced from Burand JP and Lu H (1997) Replication of a gonad-specific insect virus in TN-368 cells in culture. *Journal of Invertebrate Pathology* 70: 88–95, with permission from Elsevier.



Figure 2 A micrograph showing Or-1V nucleocapsids to permit readers to compare and contrast structure. Reproduced from Huger AM (2005) The *Oryctes* virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). *Journal of Invertebrate Pathology* 89(1): 78–84, with permission from Elsevier.

USDA-ARS Southern Insect Management Laboratory in Stoneville, MS. A portion of the insects from this colony were sterile and had malformed reproductive tissues, a condition referred to as being agonadal (AG). Some individual females from the Stoneville colony were fertile asymptomatic carriers of the virus and when mated with healthy males gave rise to virus-infected, sterile, AG progeny. AG males from the Stoneville colony were also sterile and could transmit virus to healthy females which also gave rise to AG progeny.

Physical Properties

All three viruses in this group have supercoiled, double-stranded DNA genomes ranging in size from 130 kbp for Or-V1 to approximately 230 kbp for Hz-1V and Hz-2V. The genomes of both Hz-1V and Hz-2V have been sequenced and are 99% identical, indicating that these two viruses are very closely related. Both of these viruses code for over 100 open reading frames (ORFs), several of which share homology with baculovirus genes. This homology with baculovirus genes, including the *per os* infectivity or *pif* genes and inhibitor of apopotosis or *IAP* genes, probably represents domains in these proteins that perform functions which are common in the infection pathway of these viruses in their insect hosts. The absence of the four linked, baculovirus 'central core' genes (*helicase*, *lef-5*, *ac96*, and *38K*) in the Hz-1V and Hz-2V genomes suggests that these two viruses are not members of the family *Baculoviridae*.

The enveloped Or-1V virus particle is 220 nm × 120 nm and is composed of 27 structural proteins ranging in size from 9.5 to 215 kDa with 14 of these proteins residing in the viral envelope (**Figure 2**). Hz-1V and Hz-2V are longer and narrower particles with enveloped nucleocapsids measuring 414 nm × 80 nm. These virus particles are also complex being comprised of 14–16 proteins of between 190 and 14 kDa, five of which are thought to be components of the viral envelope. The viral envelope of Hz-2V itself is distinctive, consisting of a large number of folds or ridges, each of which is associated with an individual filament located between the envelope and nucleocapsid giving the envelope a 'rope-like' appearance (**Figure 1**).

Replication

The replication of Or-1V has been studied *in vitro* using the cell line DSIR-HA-1179 which was derived from the black beetle, *Heteronychus arator*. The virus apparently enters these cells by pinocytosis with the first signs of viral cytopathic effect (CPE) are visible by 18–24 h post infection (pi), with cell lysis and viral release occurring by 3–4 days pi. Nuclear hypertrophy and migration of nuclear chromatin occur in infected cells by about 7 h pi followed by an accumulation of envelope material in chromatin-free areas of the nucleus. The assembly of the virus begins with the formation of envelopes and nucleocapsid shells in these chromatin-free areas; then the shells are filled with viral DNA which comprises the electron-dense core of the virus. Virus particles enter

the cytoplasm between 12 and 36 h pi at which time virus replication peaks followed by the release of virus particles that bud from the cell membrane.

Only eight of the 27 Or-1V structural proteins have been detected in *in vitro* replication studies in which infected, DSIR-HA-1179 cells were pulse-labeled with [^{35}S]-methionine. Two of the structural proteins, p4.6 and p10, were first detected at 4 h pi while the p11.5 protein was first detected at 10 h pi, and six viral proteins, p46.5, p40, p27, p25, p22, and p13, all were first detected at 6 h pi. The inability to detect the synthesis of the remaining virus structural proteins or any additional virus-induced intracellular proteins may be a consequence of Or-1V not actively shutting off cell protein synthesis during replication and/or the method of labeling used in these experiments.

Hz-1V and Hz-2V replication has been examined in the ovarian cell line (TN-368) derived from the cabbage looper, *Trichoplusia ni*. Replication of these two viruses proceeds very rapidly in this cell line culminating in cell lysis by approximately 24 h pi. The first signs of viral replication are rounding of the cells followed by nuclear hypertrophy. As replication proceeds in these cells, membrane vesicles appear in the nucleus, which become filled with electron-dense capsids containing viral DNA and develop into single, mature enveloped nucleocapsids. The replication of Hz-2V in the gypsy moth ovarian cell line Ld652Y proceeds more slowly and more closely resembles *in vivo* replication culminating in the formation of bundles of enveloped virus particles in the cell nucleus.

The Hz-1V replication cycle consists of the sequential expression of virus-specific intracellular proteins and it has been divided into three stages: an early stage, from 0 to 4 h pi, which is prior to the onset of viral DNA replication; an intermediate phase, from 4 to 8 h pi; and a late phase, beyond 8 h pi. The pattern of expression of viral-specific intracellular proteins in cells productively infected with standard virus particles differs from the pattern of protein synthesis in cells infected with defective interfering particles, which has been shown to lead to the establishment of cell lines persistently infected with Hz-1V.

A total of 101 viral-specific transcripts expressed from dispersed regions along the viral genome have been detected during productive Hz-1V replication. These transcripts range in size from 0.8 to over 9.5 kbp in length and follow a cascade of temporal expression similar to that found for virus-specific intracellular protein synthesis. Only three of the 24 viral transcripts detected at 2 h pi are highly expressed and only a single 2.9 kbp transcript is constitutively expressed (CE 2.90) throughout virus replication. At 4 h pi 30 new transcripts are detected with an additional 21 detected at 6 h pi and 16 more transcripts detected at 8 h pi. No new viral transcripts were detected later than 8 h pi. The expression of a single 2.9 kbp viral transcript known as the persistently associated transcript (PAT1) has been found in cells persistently infected with Hz-1V. PAT1 is constitutively expressed in cells persistently infected with the virus and its expression appears to be required for the establishment and maintenance of Hz-1V persistent infections.

Pathology and Transmission

Or-1V infection of rhinoceros beetles occurs *per os* and although the virus was initially discovered in beetle larvae, insects in breeding sites in this stage of development are generally virus free. Transmission of the virus is thought to occur primarily between adults through cooperative nest building and other social activities including mating that are part of the adult behavior of this insect. In both larvae and adults, midgut epithelial cells are the primary site of virus replication from which the infection spreads to other tissues including the fat body. Infected larvae appear translucent during the initial phases of infection as the infected fat body disintegrates. With the progression of viral infection, chalky-white bodies become visible in the midgut epithelium under the insects' integument and the diseased larvae appear shiny, beige, and waxen, dying within 1–4 weeks after infection.

Virus replication in adult beetles results in the proliferation of infected midgut cells to the point where within 1–2 weeks after the onset of infection they fill the midgut lumen, with virus being released from infected cells into the gut and eventually excreted in the feces of infected beetles. This proliferation of cells and the swelling of the midgut is a diagnostic symptom of viral infection in adults. Virus infection in adults is often chronic, lasting several weeks before the insects dies. During this time infected individuals continue to shed virus in their feces as they visit breeding sites, contaminating these sites while they attempt to mate and in this way ultimately contributing to the spread of Or-1V within the beetle population.

Once it was established that infected adults could act as effective vectors and spread the virus to other beetles through the contamination of breeding sites, Or-1V was introduced as a control agent by simply releasing virus-infected beetles into pest populations. Successful releases of infected beetles and the self-spreading of the viral disease resulting in the long-term suppression of the rhinoceros beetle have been demonstrated in areas throughout the South Pacific including Fiji, Tonga, Wallis Island, the Tokelau and Palau Islands, as well as Papua New Guinea and American Samoa.

Hz-1V is not known to cause any pathology *in vivo*; Hz-2V on the other hand shows a very interesting and unique pathology in insects. Hz-2V replication in insects does not result in mortality but rather occurs only in adult reproductive tissues resulting in the malformation of these tissues and the sterility of infected moths. Infected

insects appear normal as larvae but emerge as AG adults with abnormal reproductive systems.

The reproductive tissues of Hz-2V-infected male moths are grossly malformed into a 'Y-shaped' structure. In infected males many of the reproductive tissues normally responsible for producing sperm, pheromonostatic peptide (PSP), and the spermatophore are absent or grossly malformed. Interestingly, reproductive tissues essential for the initiation of copulation and transfer of reproductive fluids to female moths during mating appear to be intact, suggesting that AG males may be able to mate with healthy female moths and transfer Hz-2V particles, without fertilizing these individuals or altering their sexual receptivity to further mating with other male moths. Virus-infected female moths lack ovaries as well as other reproductive tissues and have grossly deformed and enlarged common and lateral oviducts. EM observations of these reproductive structures shows a significant amount of virus replication in cells which have proliferated in these tissues. Upon emergence, many Hz-2V-infected females have a clearly visible, virus-filled 'plug' covering the reproductive opening at the end tip of the abdomen (**Figure 3**).

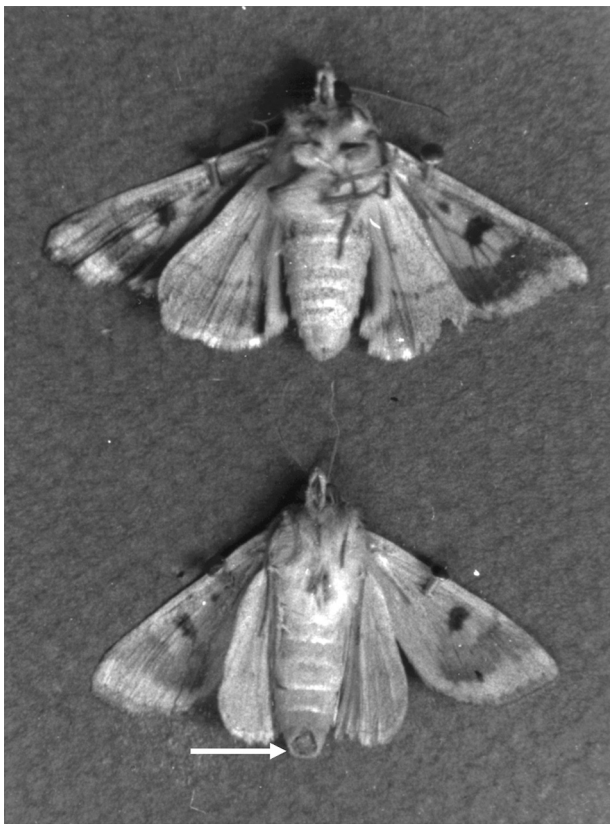


Figure 3 Hz-2V-infected *H. zea* female moth. Arrow indicates presence of virus plug. Reproduced from Burand JP and Lu H (1997) Replication of a gonad-specific insect virus in TN-368 cells in culture. *Journal of Invertebrate Pathology* 70: 88–95, with permission from Elsevier.

Hz-2V-infected female moths produce 5–7 times more of the male-attracting pheromone and as a result attract more males than their healthy counterparts. Healthy male moths attracted to infected females can become contaminated with virus upon coming into contact with the 'virus' plug at the tip of the female's abdomen while attempting to mate. Unlike healthy females that stop calling for mates and are nonreceptive to males after mating, Hz-2V-infected females continue to call and attempt to mate with males, serving as very efficient vectors in facilitating the spread of the virus within the insect population.

Persistence

With Or-1V transmission occurring primarily through fecal contamination of nesting sites and with Hz-2V being sexually transmitted to healthy individuals primarily during matings with infected moths, it is clear that the inability of NOIVs to survive outside their insect host for any extended period of time has led to these viruses evolving modes of transmission which involve a close association between them and their insect host. In the case of Hz-2V the existence of the virus in both a persistent and latent state in the insect host appears to be a part of the normal replication cycle of the virus. In infected insects Hz-2V can be persistent in the early larval stage with virus particles being virtually undetectable. Although viral DNA and some viral gene transcripts can be detected in these individuals, they appear normal and are physically indistinguishable from uninfected insects. For some of infected insects, particularly females, the virus remains persistent and these individuals are fertile and able to pass the virus on transovarially to their progeny, some of which become sterile, AG adults. In other infected insects Hz-2V is latent, becoming activated into productive replication with the onset of the development of adult reproductive tissues and causing the malformation of these tissues. The fate of Hz-2V-infected insects becoming fertile asymptomatic carriers of the virus or sterile AG adults appears to be determined by the amount of virus each individual egg receives from the female parent.

The close association which has evolved between Or-1V and the rhinoceros beetle has proven to be the key to the successful use of this virus as a biological control agent and has resulted in the persistence of this virus in beetle population throughout the Asia-Pacific region. The successful use of this virus in controlling this beetle pest suggests that other NOIVs may also have potential for use in insect pest control programs (**Figure 4**). The ability of Hz-2V to establish persistent infections in *H. zea* insects and be transmitted to the progeny of these insects suggests that this NOIV may

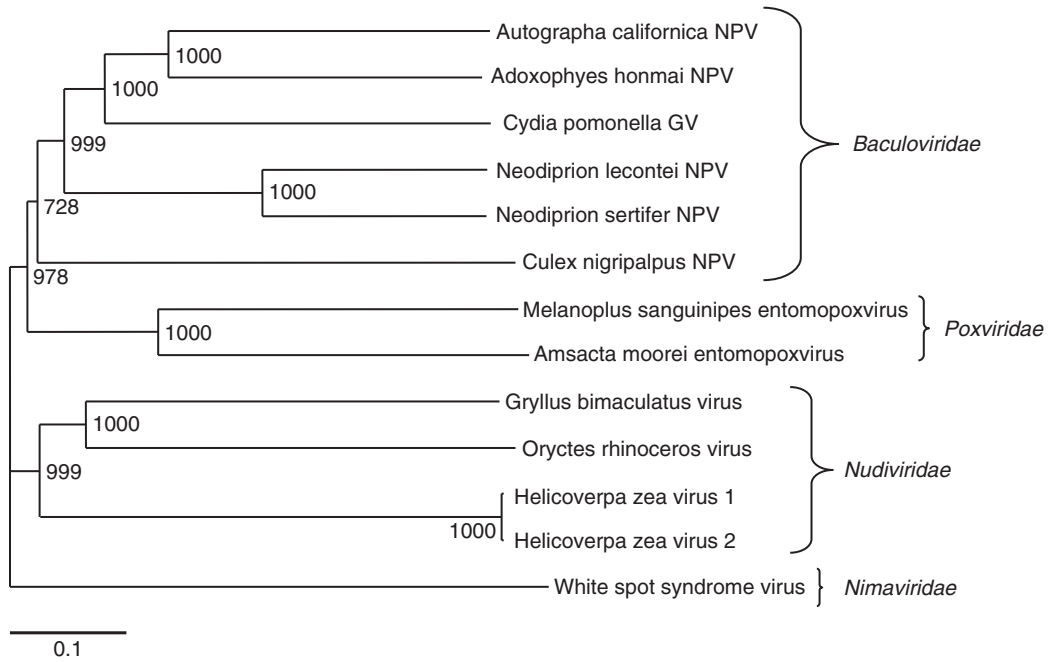


Figure 4 A dendogram showing phylogenetic relationships.

have the potential to serve both as model to study the nature of viral persistence and as a tool for altering the genetic make-up of insects.

See also: Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Herpesviruses: Latency; Insect Pest Control by Viruses; Oryctes Rhinocerous Virus; Persistent and Latent Viral Infection.

Further Reading

Burand JP (1998) *Nudiviruses*. In: Miller LK and Ball A (eds.) *The Insect Viruses*, 69pp. New York: Plenum.

Burand JP, Kawanishi CY, and Huang Y-S (1986) Persistent baculovirus infections. In: Granados RR and Federici BA (eds.) *The Biology of Baculoviruses,* vol. 1, pp. 159–177. Boca Raton, FL: CRC Press.

Burand JP and LU H (1997) Replication of a gonad-specific insect virus in TN-368 cells in culture. *Journal of Invertebrate Pathology* 70: 88–95.

Burand JP, Rallis CP, and Tan W (2004) Horizontal transmission of Hz-2V by virus infected *Helicoverpa zea* moths. *Journal of Invertebrate Pathology* 85: 128–131.

Hamm JJ, Carpenter JE, and Styer EL (1996) Oviposition day effect on incidence of agonadal progeny of *Helicoverpa zea* (Lepidoptera: Noctuidae) infected with a virus. *Annals of the Entomological Society of America* 89: 266–275.

Huger AM (2005) The *Oryctes* virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle, *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). *Journal of Invertebrate Pathology* 89: 78–84.

Huger AM and Kreig A (1991) *Baculoviridae*: Nonoccluded baculoviruses. In: Adams JR and Bonami JR (eds.) *Atlas of Invertebrate Viruses*, pp. 287–319. Boca Raton, FL: CRC Press.

Wang Y, van Oers MM, Crawford AM, Vlak JM, and Jehle JA (2007) Genomic analysis of Oryctes rhinoceros virus reveals genetic relatedness to Heliothis zea virus 1. *Archives of Virology* 152(3): 519–531.

Interfering RNAs

K E Olson, K M Keene, and C D Blair, Colorado State University, Fort Collins, CO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

dsRNA Double-stranded RNA, the trigger for RNAi and intermediate in RNA virus replication.

miRNA MicroRNA; small RNAs, about 21–23 bases in length, that post-transcriptionally regulate the expression of genes by binding to the 3′ untranslated regions (3′ UTR) of specific mRNAs.

RISC RNA-induced silencing complex; a multiprotein complex that brings the guide strand of the siRNA duplex and the cellular mRNA together and cleaves

the mRNA with associated endonuclease activity. mRNA is then degraded.

RNAi An evolutionarily conserved, sequence-specific antiviral pathway triggered by double-stranded RNA (dsRNA) that leads to degradation of both the dsRNA and mRNA with homologous sequence.

siRNA Small interfering RNAs; 21–25 bp duplexes that provide guides for sequence-specific cleavage of mRNA. siRNAs are considered the hallmark of RNAi.

Introduction

Antiviral innate immune responses in vertebrate hosts restrict viral invasion until humoral and cell-mediated acquired immune responses specifically clear virus infection. In mammals, innate immune responses are induced when infected cells recognize viral components such as nucleic acids through host pattern-recognition receptors. Toll-like receptors are important pattern-recognition sensors that recognize viral components (e.g., double-stranded RNA (dsRNA)) and signal induction of type I interferons and inflammatory cytokines that lead to an antiviral state. Organisms such as insects probably depend entirely on antiviral innate immune responses to overcome viral infection, since acquired immune responses have not been identified in invertebrates. Interferon-like molecules also have not been found in insects, making it unclear how insects cope with viral infections. In 1998, researchers first described an intracellular response in the worm *Caenorhabditis elegans* that could be triggered by dsRNA and efficiently silenced the expression of genes having sequence identity with the dsRNA trigger. This response, termed RNA interference or RNAi, is now known to be an ancient antiviral innate immune response in eukaryotic organisms including worms, plants, insects, and mammals and is a common evolutionary link among these organisms in their fight against virus invasion.

RNA Interference

RNAi, post-transcriptional gene silencing (PTGS), quelling, and sense suppression are terms for related pathways that have been described in different organisms. All are RNAi responses triggered by dsRNA that result in degradation of both dsRNA and mRNA with cognate sequence. These pathways are highly conserved evolutionarily and exist in many organisms including plants, fungi, and animals. RNAi and related pathways have several functions, including regulation of development, silencing and regulation of gene expression, and defense against viruses and transposable elements. In this article, we focus on RNAi as an antiviral, innate immune pathway.

RNAi Mechanism of Action

The RNAi pathway is divided into an initiator phase and an effector phase. The initiator phase consists of the recognition and processing of long dsRNA molecules by the RNaseIII enzyme Dicer into small interfering RNAs (siRNAs) of 21–25 bp. siRNAs, considered the hallmark of the RNAi response, are duplexes with 3′ overhangs of 2 nt. Each strand has a 5′-PO_4 and 3′-OH. In the fruitfly *Drosophila melanogaster* (drosophila), the siRNAs are unwound and incorporated into the RNA-induced silencing complex, or RISC, with the assistance of Dicer-2 and R2D2, to start the effector phase of the pathway. In the effector phase, one strand of the siRNA duplex acts a as guide sequence to target the RISC to complementary mRNAs and determine the cleavage site on the mRNA. The RISC is known to include products of the following genes: *Argonaute2* (*Ago2*), *Vasa intronic gene* (*VIG*), *fragile X mental retardation* (*FXR*), and *Tudor Staphylococcal nuclease* (*Tudor-SN*). Other genes in drosophila encode proteins having RNA helicase activity associated with RNAi and include *spn-E*, *Rm62*, and *armi*. The latter gene products have been implicated with heterochromatin and transposon silencing.

RNAi Components and Function: Dicers, the Sensors of dsRNA and Initiators of siRNA Production

The majority of biochemical information related to Dicer proteins has been elucidated from studies in drosophila, showing that Dicer enzymes are intracellular sensors of dsRNA that initiate RNAi in drosophila cultured cell and embryo lysates. Candidate genes from three families encoding RNase III motifs were expressed in drosophila S2 cells, immunoprecipitated, and tested *in vitro* for their ability to transform long dsRNA molecules (>30 bp) into small RNAs of ~21 nt (**Figure 1**). The enzyme capable of producing siRNAs was termed Dicer and contained a helicase domain as well as two RNase III domains. The production of siRNAs by Dicer-2 in drosophila was ATP-dependent and the enzyme was inactive in degrading single stranded RNAs. Dicer depletion by immunoprecipitation from drosophila cell lysates resulted in decreased siRNA production. Dicer is a ~200 kDa protein with an N-terminal RNA helicase domain, a PAZ (PIWI/Argonaute/Zwille) domain, a conserved domain of unknown function (DUF283), two C-terminal RNase III domains, and an RNA binding motif. In its active form, Dicer is a homodimer. Dicer is evolutionarily conserved, and is found in drosophila, *C. elegans*, humans, mice, trypanosomes, zebrafish, the fungi *Magnaporthe oryzae* and *Neurospora crassa*, budding yeast (*Schizosaccharomyces pombe*), plants, and many other organisms. The number

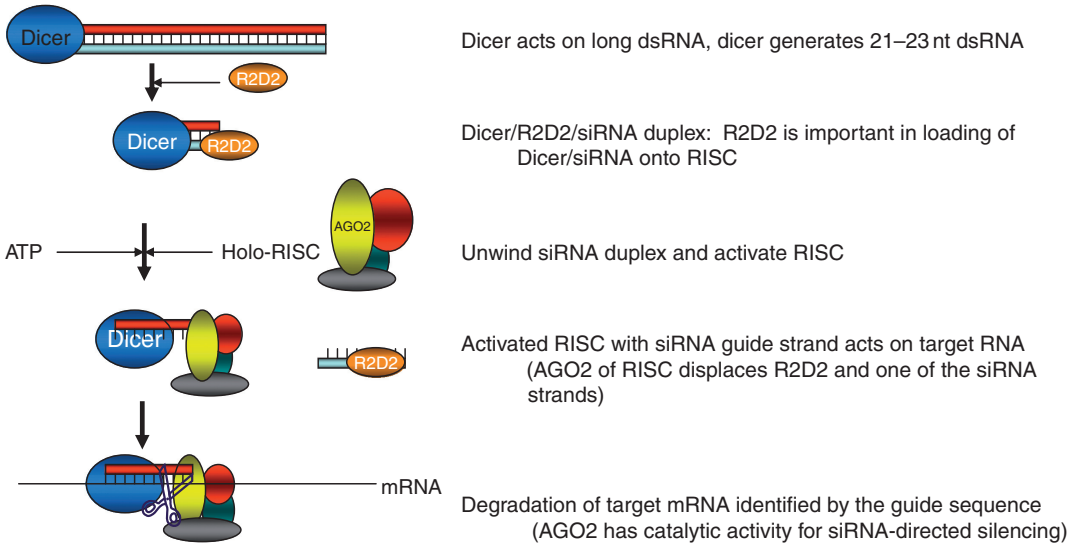


Figure 1 General scheme for RNAi leading to degradation of a specific mRNA.

of *dicer* (*dcr*) genes varies in different organisms. In drosophila there are two *dcr* genes (*dcr1* and *dcr2*); in the plant *Arabidopsis thaliana* there are four, each of which processes siRNAs from different dsRNA sources; however, in the genomes of humans and *C. elegans*, there is only one *dcr* gene.

Dicers and miRNA Biogenesis

The RNAi pathway has two branches leading to production of either siRNA or microRNA (miRNA). The miRNAs *lin-4* and *let-7* were first discovered in *C. elegans* and play a crucial role in development. Subsequently, hundreds of different miRNAs have been found in most eukaryotic organisms and exploring their expression and function is now an important topic of research in a wide range of invertebrates and vertebrates. miRNAs are produced from endogenous pre-miRNA transcripts that form stem–loop precursors. Silencing of genes by the miRNA pathway occurs not by degradation of the mRNA, but rather by translational arrest during protein synthesis. Also, unlike the siRNA pathway, miRNA silencing does not require complete base pairing between the miRNA and the target sequence to be silenced. Distinct RISCs process small RNAs for the effector phase of the two gene-silencing mechanisms. It is not known how the distinction is made between siRNA production and miRNA production with a single Dicer enzyme in *C. elegans*. In humans, interferon and other innate immune responses are induced by long dsRNA, so Dicer-like activity generating siRNAs may not be as crucial. In organisms such as drosophila that encode two Dicer proteins, Dicer-1 is the enzyme that produces miRNAs from endogenous transcripts.

R2D2 Protein and RNAi

R2D2 is a 36 kDa protein with two dsRNA binding domains that bridges the initiator and effector stages of the RNAi pathway. R2D2 co-purifies with Dicer-2 from an siRNA-generating extract of drosophila S2 cells. R2D2 association does not affect the enzymatic activity of Dicer-2, but is required to load the newly formed siRNAs into RISC. Dicer-2 forms a heterodimer complex with R2D2 and the siRNA duplex that appears to detect the thermodynamic asymmetry of the siRNA duplex. The passenger strand of the siRNA duplex is separated from the guide strand, which interacts at its 5′ and 3′ ends with the PIWI (possessing RNase H-like activity) and PAZ domains of Argonaute-2 (AGO2), respectively. Strand selection (passenger strand vs. guide strand) depends on the thermodynamic stability of the first four nucleotides of the 5′ terminus of an siRNA duplex. The siRNA strand whose 5′ end has lower base-pairing stability becomes the guide strand, leaving the more stable strand as the passenger strand. R2D2 binds to the thermodynamically more stable end of an siRNA whereas Dicer-2 binds on the opposite end. Release of the degraded passenger strand is believed to be an ATP-dependent reaction. The guide siRNA strand remains associated with RISC and guides AGO2 to the target mRNA containing the complementary sequence. After hybridization, AGO2 cleaves the phosphodiester backbone of the target mRNA. Target RNA cleavage occurs between the 10th and 11th nucleotides of the guide siRNA measured from its 5′ phosphate group. The phosphate group of the 5′ end of the guide siRNA influences the fidelity of the cleavage position as well as the stability of RISC. Recently it was confirmed that the RNAi pathway of drosophila functions as an antiviral

immunity mechanism. Drosophila with null mutations for genes encoding Dicer-2, Argonaute-2, or R2D2 were highly susceptible to RNA viruses such as Flock house virus (FHV) (*Nodaviridae*), Drosophila C virus (*Dicistroviridae*), or Sindbis virus (*Togaviridae*) which in some cases were lethal for the flies.

Argonaute Proteins and RNAi

Another family of genes that has already been discussed in this review as important to RNAi is the *argonaute* gene family. In drosophila, there are five *argonaute* genes. Two of the genes, *argonaute1* (*ago1*) and *argonaute2* (*ago2*), have been implicated in RNAi. Argonaute-2 is an important component of the RISC complex. AGO2 has been termed 'Slicer' and is the only component of human RISC that is required for the degradation of mRNA molecules, and its PIWI domain may contain the endonuclease activity. Drosophila embryo mutants lacking *ago2* are unable to perform siRNA-directed mRNA cleavage, although miRNA-directed cleavage is still possible. The embryos also lack the capacity to load siRNAs into RISC.

Drosophila Argonaute-1 (AGO1) is not involved in siRNA-directed cleavage as is AGO2. AGO1 is believed to function downstream of the production of the siRNAs and not as a component of RISC. These studies also demonstrated that *Drosophila* mutants lacking AGO1 are embryonic lethals, implicating AGO1 in *Drosophila* development. AGO1 is required for miRNA-directed cleavage and dispensable for siRNA-directed cleavage, showing divergent roles for different Argonaute proteins. This functional differentiation of the Argonaute proteins has also been noted in plants.

Argonaute proteins are characterized by the PAZ domain and the PIWI domain. The crystal structures of the PAZ domain of AGO2 from *Drosophila* and the thermophile *Pyrococcus furiosus* were determined and shown to have structural properties similar to proteins that bind single-stranded nucleic acid. The PAZ domain recognizes the 3′ overhangs of the siRNA duplexes. Interestingly, this is the same region that is recognized by certain viral suppressors of RNAi. In addition to its endonuclease motif, the PIWI domain is involved with protein–protein interactions between Argonaute and Dicer and may play a role in siRNA loading onto RISC. Recently, researchers have shown that both human AGO1 and AGO2 proteins reside in intracellular structures known as 'cytoplasmic bodies'. These areas of the cell are believed to be sites of regulation of cellular mRNA turnover. Several other AGO proteins from drosophila are associated with the RNAi pathway. These proteins include Piwi, implicated in transposon silencing, Aubergine, associated with germline gene repression, and AGO3, an argonaute-like protein of unknown function. As already mentioned, a number of other proteins have now been associated with the RNAi pathway, including Vig, Tudor-SN, and Fmr-1, all associated with RISC.

Unique Properties of RNAi in Plants and Animals

In *C. elegans* and plants, an amplification of the RNAi response occurs. In these systems, the original siRNAs act as primers for synthesis of new intracellular dsRNA by the endogenous RNA-dependent RNA polymerase (RdRP). These newly produced dsRNAs are processed by Dicer and generate an additional and potentially more diverse pool of siRNAs. This phenomenon is termed 'transitive RNAi' and allows for the degradation of a full-length mRNA even when the initial trigger sequence represents only a portion of the gene or genome. Amplification of the siRNA signal is bidirectional along the transcript in plants; however, in *C. elegans* the signal can only spread 3′–5′ along the transcript. Transitive RNAi does not occur in species such as *D. melanogaster*, where RdRP genes are absent from the genome. Gene knockdown experiments in organisms lacking transitive RNAi require design of dsRNA for specific disruption of gene expression.

One unique aspect of PTGS in plants is the ability of the silencing signal to spread throughout the organism. The siRNAs, complexed with host proteins, are part of a silencing complex that can move to other tissues in the plant through phloem tissues. The distance that the siRNAs travel is dependent on their exact length and the Dicer enzyme by which they were generated. The exact mechanism for long-distance movement has yet to be elucidated. RNAi in *C. elegans* can spread from the point of induction, especially if the dsRNA is introduced into the intestine either by injection or by feeding.

RNAi has become an important reverse genetics tool for studying gene function. Systematic knockdown of all genes has been accomplished in both drosophila and *C. elegans* with great success, identifying previously unknown functions of genes in various biological pathways and revealing differences in the RNAi pathways of each organism. In drosophila, the siRNA signal appears not to be amplified (transitive RNAi) and does not spread as seen in *C. elegans* and plants. This means that RNAi activity is most likely confined to those cells in which the dsRNA trigger is detected by Dicer. RNA silencing has been used in many other invertebrates, including mosquito disease vectors. Several studies have shown that RNAi can be used to knock down endogenous and exogenous gene expression in mosquitoes that transmit medically important pathogens to animals and humans. Studies of RNAi in mosquitoes have also shown that it is possible to silence RNAi complex genes using RNAi. As an example, the ability to silence the RNAi pathway in a hemocyte

cell line of *Anopheles gambiae* was tested by transfecting dsRNA derived from exon sequences of the *A. gambiae dcr1* and *dcr2* and *argonaute 1–5* (*ago1–5*) genes. RNAi in *A. gambiae* cells required expression of Dicer-2, AGO2, and AGO3 proteins. This study also demonstrated that RNAi in the mosquito, as in drosophila, does not spread from the target cells, suggesting that RdRP-mediated transitive amplification is absent in the mosquito.

Viruses and RNAi

Since the discovery of RNA silencing, a number of researchers have hypothesized that RNAi plays an important role in antiviral defense. RNA viruses with positive-sense genomes, in particular, form dsRNA intermediates as they replicate in host cells. Whether this is the general viral trigger of RNAi in infected cells is still controversial, since viruses with negative-strand genomes generate little detectable dsRNA during their replication. Studies to elucidate a mechanism termed pathogen-derived resistance (PDR) in plants were among the first to show that viruses could be targeted by RNA silencing. Transgenic plants were engineered to express a portion of the tobacco etch virus (TEV) coat protein. Upon challenge with TEV, the transgenic plants were found to be resistant to the virus. No overt symptoms were seen, and no virus could be recovered from the leaf tissue. PDR was shown to be virus specific, as the transgenic plants could be infected with a genetically unrelated virus; however, any RNA that was introduced into plant cells that shared homology with the RNA in the transgene was degraded. Plant virologists also observed that the delivery of a fragment of viral RNA to plant cells by a heterologous virus expression vector made the cells resistant to challenge by the first virus, and termed this related phenomenon virus-induced gene silencing. Soon after the discovery that plants used an RNA-mediated defense mechanism against viruses, it was shown that many plant viruses encoded protein suppressors of RNA silencing. This would be expected as the virus and host develop a biological arms race to express countermeasures that limit the ability of one to overcome the other.

Viral Encoded Suppressors of RNAi

Several suppressors have been found that interrupt the RNA-silencing pathway at different steps, indicating that evasion of RNA silencing has evolved more than once. One protein, helper component-proteinase, or HC-Pro, from potyviruses of plants is thought to be the most potent suppressor of RNA silencing found to date. HC-Pro functions at a step that prevents the accumulation of siRNAs by interacting with the RNase III enzyme Dicer. HC-Pro also has the capability to reverse established silencing of a transgene, suggesting that the protein inhibits a mechanism required for the maintenance of silencing. The 19 kDa protein (p19) from tombusviruses acts as a suppressor of PTGS in plants in a different manner from HC-Pro. p19 does not block production of the 21–25 nt siRNAs; rather it binds them via the 2-nt 3′ overhangs. Notably, the protein will bind only double-stranded 21 nt sequences; single-stranded RNAs of the same length are not recognized by p19. In binding the siRNAs, p19 forms a homodimer and sequesters the guide sequences that are required for RISC incorporation and targeting mRNA degradation. Another virus, cucumber mosaic virus, encodes a protein 2b that interferes with the spread of siRNA signal in the plant host, allowing systemic spread of the virus.

In addition to the PTGS suppressors encoded by plant viruses, the insect virus Flock house virus encodes a protein, B2, which can suppress RNAi activity in both drosophila S2 and plant cells. In transgenic plants containing a green fluorescent protein (GFP) gene, in which transient expression of siRNAs targeting GFP mRNA had silenced its expression, the presence of B2 protein reversed silencing of GFP. siRNAs were still detected in the tissues, indicating that the suppression of gene silencing did not occur before the production of siRNAs. It is likely that this protein sequesters the siRNAs from the RNAi machinery, possibly in a manner similar to p19. Finally, two other animal viruses encode suppressors of interferon induction that also have apparent RNAi suppressor activity. These are the influenza virus NS1 protein and the vaccinia virus E3L protein.

Viruses may employ other strategies to evade the RNAi pathway. These strategies include (1) sequestration of the viral dsRNA replicative intermediates in viral cores or in double-membrane structures in the host cell formed during viral replication; (2) viral replication and spread outpacing the RNAi pathway; and (3) replication in tissues that are resistant to RNAi.

miRNAs and Viruses

In the last couple of years, a number of viral-encoded miRNAs have been discovered. The functions of most viral-derived miRNAs are unknown; however, DNA viruses such as polyomaviruses and herpesviruses transcribe miRNAs in infected vertebrate cells that appear to regulate expression of critical viral and host genes during infection. The location of miRNAs within different virus genomes are not conserved, suggesting that miRNAs are likely to be recent acquisitions in viral genomes that help adapt the virus to the host during the virus lifecycle. For instance, a viral miRNA in SV40 virus was recently discovered that regulates the viral T antigen. The SV40

miRNA accumulates in late stages of infection and targets the early T antigen mRNA for degradation, thus reducing its expression. Studies of many families of RNA viruses have failed to identify miRNAs in RNA genomes and this finding is consistent with the prominent role of the cellular DNA-dependent RNA polymerase II in the biogenesis of miRNA precursors.

Use of RNAi for Disease Control

Virus diseases of plants cause extensive economic losses. Control of plant virus diseases has usually been associated with control of insects or nematodes that transmit viruses, or through sanitation and quarantine of infected plants. The transformation of plants with effector genes designed to transcribe inverted-repeat RNA (dsRNAs) that target plant viruses can provide novel virus-resistant varieties. Transgenic plants expressing inverted repeats of viral sequences exhibit varying degrees of resistance to the virus or viruses with genome sequences closely related to the source of the transgene. This resistance is due to PTGS wherein viral mRNA is degraded in the cytoplasm soon after synthesis.

In a similar genetic approach to that described previously in plants, we have genetically modified *Aedes aegypti* mosquitoes to exhibit impaired vector competence for dengue type 2 virus (DENV-2) transmission. DENVs are normally transmitted by *A. aegypti* mosquitoes to humans during epidemic outbreaks of dengue diseases. If a DENV-derived dsRNA trigger is expressed in the cell prior to viral translation and replication, an antiviral state can be induced in the mosquito that blocks virus infection. To do this, mosquitoes were genetically modified to express an inverted-repeat (IR) RNA derived from the premembrane protein coding region of the DENV-2 RNA. The IR RNA formed a 560 bp dsRNA in infected midgut epithelial cells of the mosquitoes to induce the RNAi pathway. A transgenic family, Carb77, was selected that expressed IR RNA in the midgut after a blood meal. Carb77 mosquitoes ingesting an artificial blood meal with 10^7 pfu ml^{-1} of DENV-2 exhibited marked reduction of viral envelope antigen in midguts and salivary glands after infection. Transmission of virus by the Carb77 line was significantly diminished when compared to control mosquitoes. As evidence that the resistance was RNAi mediated, DENV-2-derived siRNAs were readily detected in RNA extracts from midguts following ingestion of a blood meal with no virus. In addition, loss of the resistance phenotype was observed when the RNAi pathway was interrupted by injecting *ago2* dsRNA 2 days prior to induction of IR-RNA transgene, confirming that DENV-2 resistance was caused by an RNAi response.

Targeting of replicating animal viruses using RNAi has prompted discussion about whether RNAi can be used as an antiviral therapy. In mammalian cells, siRNAs, rather than long dsRNAs, are required to induce RNAi as an antiviral therapy because these cells possess interferon and other antiviral innate immune pathways that are triggered by dsRNA >30 bp in length. Numerous studies in cell culture have shown that HIV replication can be halted when cells are treated with siRNAs that target the viral genome. West Nile virus (WNV) replication also can be reduced in cultured cells by treatment with siRNAs targeting the virus RNA. These studies showed a significant reduction in levels of WNV RNA if cells were pretreated with siRNAs; however, the cells that were treated subsequent to the establishment of viral replication did not show the same reduction in viral RNA, suggesting that the RNA may be sequestered from the RNAi machinery after replication is established in the cell. Studies investigating RNAi as a therapy for hepatitis C virus (HCV) infection have used siRNAs to effectively target HCV replicon RNAs in cultured human cells as well as in a mouse model.

Model Systems for Studying Role of RNA in Virus Infections

While the genetics and biochemistry of RNAi in *C. elegans* and *D. melanogaster* have been investigated in detail, there have been no virus infection models of these animals until very recently. RNAi-based innate immunity has been detected in *C. elegans*-derived cultured cells infected with vesicular stomatitis virus (VSV; *Rhaboviridae*). FHV replication in *C. elegans* triggered potent antiviral silencing that required RDE-1, an argonaute protein essential for RNAi mediated by siRNAs. This antiviral innate immunity was capable of rapid virus clearance in *C. elegans* in the absence of FHV RNAi suppressor protein B2. Two recent papers have shown that successful infection and killing of *Drosophila* by FHV was strictly dependent on expression of the viral suppressor protein B2. *Drosophila* with a knockout mutation in the gene encoding Dicer-2 showed enhanced susceptibility to infection by FHV, cricket paralysis, and *Drosophila* C viruses (*Dicistroviridae*) and Sindbis virus (*Alphavirus*; *Togaviridae*). These data demonstrate the importance of RNAi for controlling virus replication *in vivo* and establish *dcr2* as a drosophila susceptibility locus for virus infections. *C. elegans* and drosophila are important models for studying virus–RNAi interactions. The drosophila model system allows advanced genetic approaches such as generation of null mutants of RNAi components in a system that has few other dsRNA-triggered defensive responses to complicate mechanistic studies. The availability of the annotated drosophila genome sequence and established genetic approaches is critical for understanding RNAi mechanisms and continues to make the drosophila model important to our understanding of innate immune responses to viruses.

Mosquitoes, RNAi, and Arboviruses

Mosquitoes and arboviruses provide an important naturally occurring insect–virus system to study the potential role of RNAi in host defense. Arboviruses are RNA viruses that must replicate in their arthropod vector for amplification before they can be transmitted to a vertebrate host, such as humans. Obviously, arboviruses must somehow evade the RNAi pathway to successfully replicate in the mosquito prior to transmission. There are several advantages to studying RNAi in mosquitoes. First, the complete genome sequence is now available for at least two medically important vectors, *A. gambiae* and *A. aegypti*. Second, the RNAi pathway of mosquitoes is similar in structure and function to the pathway in drosophila and many of the component genes of RNAi have been identified. Third, new genetic approaches are allowing researchers to manipulate genes that affect innate immune responses in the mosquito. Fourth, infectious cDNA clones of arbovirus genomes from at least three virus families are available to allow manipulation of the viral genes and identify determinants of RNAi modulation. Fifth, RNAi–virus interactions studies can occur in systems directly relating to medically important pathogens.

Mosquitoes, like drosophila, do not have responses comparable to interferon induction, so they can be injected with long dsRNAs (300–500 bp) to trigger the RNAi pathway and efficiently silence specific vector genes that may participate in innate immune pathways, including RNAi. As an example, to determine whether RNAi conditions the vector competence of *A. gambiae* for O'nyong-nyong virus (ONNV; *Alphavirus*), a genetically modified ONNV expressing GFP (eGFP) was developed to readily track virus infection. After intrathoracic injection, ONNV-eGFP slowly spread to other *A. gambiae* tissues over a 9 day period. Mosquitoes were co-injected with virus and dsRNA derived from the ONNV nsP3 gene. Treatment with nsP3 dsRNA inhibited virus spread significantly, as determined by GFP expression patterns. ONNV-GFP titers from mosquitoes co-injected with nsP3 dsRNA also were significantly lower at 3 and 6 days after injection than in mosquitoes co-injected with non-virus-related β-galactosidase (β-gal) dsRNA. However, mosquitoes co-injected with ONNV-GFP and dsRNA derived from the *A. gambiae ago2* gene displayed widespread GFP expression and virus titers 16-fold higher than β-gal dsRNA controls at 3 or 6 days after injection. These observations provided direct evidence that RNAi is an antagonist of ONNV replication in *A. gambiae* and suggest that this innate immune response plays a role in conditioning vector competence. These types of experiments could be vital to understanding why some mosquito species are excellent vectors of disease and others are not.

The Biological Arms Race between Viruses and Hosts

RNA virus–host interactions are often characterized as an escalating arms race between two mortal enemies. The host evolves innate and acquired immune pathways to counter the destructive effects of virus invasion and the virus adapts to these defense measures by rapidly evolving new ways of evading the host's attempts at pathogen control. RNA–virus interactions with the host's RNAi pathway exemplify this struggle for dominance. Many RNA viruses evolve rapidly because their RNA-directed RNA polymerases are error prone, providing significant variation in virus genome populations needed to probe weaknesses in the host's defense and allow selection of new virus variants that have an advantage in their replication. As we have described earlier, a number of families of plant RNA viruses have evolved RNAi suppressors. Each family has developed a different strategy to down-regulate RNAi, as shown by the fact that their suppressors attack different steps in the pathway. Still other viruses may have adapted to host RNAi by sequestering their replicative intermediate dsRNA triggers in double-membrane structures derived from host endoplasmic reticulum. Rapid evolution of RNA viruses also produces significant challenges to developing therapeutic strategies for humans using siRNAs to target and destroy viruses. Strategies that use siRNAs to trigger RNAi can be thwarted by point mutations in the target sequence. This has prompted development of siRNAs that target multiple regions of the viral RNA, highly conserved regions of the viral genome, or host genes essential for virus infection. However, RNA viruses are constrained in the amount of genetic variation they can tolerate and remain genetically fit for cell entry, replication, packaging, and egress.

Viruses that have complex life cycles, such as arthropod-borne viruses that must replicate in both vertebrate and invertebrate cells, are further constrained in their evolutionary potential. Finally, there is evidence that the host can evolve to counter the threat posed by RNA viruses. A recent finding emphasized the critical role of RNAi as an innate immune mechanism in *Drosophila* when researchers showed that RNAi pathway genes (*dcr2, ago2, r2d2*) are among the 3% fastest evolving genes among drosophila species, confirming the biological arms race between viruses and insects. The antiviral role of RNAi has been studied for less than a decade and is only now being exploited as a mechanism to fight viral diseases. Knowledge gained since the discovery of RNAi in 1998 should allow researchers to fully exploit RNAi as a means of controlling a number of infectious agents that cause disease in plants, animals, and humans.

Acknowledgments

Our research is funded by NIH grants AI34014 and AI48740 and the Grand Challenges in Global Health through the foundation for NIH.

See also: Satellite Nucleic Acids and Viruses.

Further Reading

Akira S, Uematsu S, and Takeuchi O (2006) Pathogen recognition and innate immunity. *Cell* 124: 783–801.
Baulcombe D (2004) RNA silencing in plants. *Nature* 431: 356–363.
Franz AWE, Sanchez-Vargas I, Adelman ZN, *et al.* (2006) Engineering RNA interference-based resistance to dengue virus type 2 in genetically modified *Aedes aegypti*. *Proceedings of the National Academy of Sciences, USA* 103: 4198–4203.
Galiana-Arnoux D, Dostert C, Schneemann A, Hoffmann JA, and Imler JL (2006) Essential function *in vivo* for dicer-2 in host defense against RNA viruses in *Drosophila*. *Nature Immunology* 7: 590–597.
Hammond SM, Boettcher S, Caudy AA, Kobayashi R, and Hannon GJ (2001) Argonaute2, a link between genetic and biochemical analyses of RNAi. *Science* 293: 1146–1150.
Kavi HH, Fernandez HR, Xie W, and Birchler JA (2005) RNA silencing in *Drosophila*. *FEBS Letters* 579: 5940–5949.
Keene KM, Foy BD, Sanchez-Vargas I, Beaty BJ, Blair CD, and Olson KE (2004) RNA interference as a natural antiviral response to O'nyong-nyong virus (*Alphavirus; Togaviridae*) infection of *Anopheles gambiae*. *Proceedings of the National Academy of Sciences, USA* 101: 17240–17245.
Leonard JN and Schaffer DV (2006) Antiviral RNAi therapy: Emerging approaches for hitting a moving target. *Gene Therapy* 13: 532–540.
Li WX, Li H, Lu R, *et al.* (2004) Interferon antagonist proteins of influenza and vaccinia viruses are suppressors of RNA silencing. *Proceedings of the National Academy of Sciences, USA* 101(5): 1350–1355.
Voinnet O (2005) Induction and suppression of RNA silencing: Insights from viral infections. *Nature Reviews Genetics* 6: 206–220.
Zamore PD (2002) Ancient pathways programmed by small RNAs. *Science* 296: 1265–1269.

Iridoviruses of Vertebrates

A D Hyatt, Australian Animal Health Laboratory, Geelong, VIC, Australia
V G Chinchar, University of Mississippi Medical Center, Jackson, MS, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Anemia Reduced number (below normal) of erythrocytes (red blood cells).
Ectothemic Animals whose temperature varies with the surrounding environment. Also known as poikliotherms.
Epitheliotropic Having a special affinity for epithelial cells.
Hyperplasia Abnormal increase in the volume of a tissue or organ caused by an increase in the number of normal cells.
Karyolysis Dissolution of a cell nucleus.
Karyorrhexis Rupture of the cell nucleus in which the chromatin disintegrates and is extruded from the cell.
Pyknosis Degeneration of a cell in which the nucleus shrinks in size and the chromatin appears as a solid, structureless feature.
Urodeles Amphibians belonging to the order Caudata, including the salamanders and newts, in which the larval tail persists in adult life.

Introduction

The family *Iridoviridae* encompasses five recognized genera, two of which infect invertebrates (*Iridovirus*, *Chloriridovirus*), and three of which infect ectothermic vertebrates (*Ranavirus*, *Lymphocystivirus*, and *Megalocytivirus*). In addition, two other viruses that infect cold-blooded vertebrates (*Erthrocytic necrosis virus* and *White sturgeon iridovirus* (WSIV) remain unassigned members of the family. Iridoviruses that infect 'cold-blooded' vertebrates (fish, amphibians, and reptiles) have become the focus of recent interest. These viruses are being identified and isolated with increasing frequency and their importance is being measured in terms of their impact on farmed production and trade in fish and amphibians. There are also significant impacts of iridoviruses on biodiversity, most notably the decline of local amphibian populations.

The history of research into these viruses extends back to the nineteenth century for lymphocystis virus, the 1940s for *Frog virus 3* (FV3, genus *Ranavirus*) and 1980s for the first description of a highly infectious fresh water piscine ranavirus, *Epizootic haematopoietic necrosis virus* (EHNV, genus *Ranavirus*) and saltwater megalocytiviruses

such as red sea bream iridovirus (RSIV, species *Infectious spleen and kidney necrosis virus*, genus *Megalocystivirus*). Recent interest in the ranaviruses and megalocytiviruses is associated with disease epizootics of freshwater and salt water finfish, die-offs of frogs and salamanders, and illegal trade in wildlife. Fish, amphibians, and reptiles are being bought and sold illegally and transported across national and/or international borders without appropriate certification and quarantine. Increasing reports of disease involving vertebrate iridoviruses suggests expansion of the geographic distribution and host range, and, as such, they are considered to represent a significant group of emerging viruses.

Structure

Each of the three recognized genera of chordate iridoviruses shares common structural, replicative, genomic, and protein characteristics, and each genus contains several distinct species and isolates/strains. The virions of vertebrate iridoviruses range in size from 100–300 nm. They are comprised of four concentric layers: an outer envelope composed of a lipid bilayer and virus-encoded transmembrane proteins; an icosahedral protein shell comprised of the major capsid protein (MCP), an inner lipid membrane; and a central dsDNA core (approximately 170 kbp) and associated proteins. The outer membrane is acquired as the virus buds through the plasma membrane (**Figure 1(a)**). Lymphocystiviruses and megalocytiviruses differ from the above in that they are seldom observed budding from the host cell plasma membranes. Lymphocystiviruses differ from ranaviruses, megalocytiviruses, and erythrocytic necrosis viruses in that the capsids contain an outer fringe of external, fibril-like protusions (**Figure 1(g)**). Megalocytiviruses also differ from ranaviruses in that assembly sites within cells infected *in vivo* are membrane bound (**Figure 1(e)**).

Geographical Distribution and Host Range

Table 1 illustrates the diversity of hosts and geographic locations from which vertebrate iridoviruses have been identified and/or isolated. It is important to note that while many iridoviruses have been observed and isolated, they cannot be assigned to a specific taxonomic group until specific demarcation criteria relating to their molecular, structural, and host-disease characteristics are determined and interpreted as prescribed by the International Committee on Taxonomy of Viruses (ICTV).

Differences in virion size and topography, host range, and geographic locations distribution are generally restricted to specific isolates. Lymphocystis viruses have been reported worldwide and infect both freshwater and marine finfish. Similarly, ranaviruses have been isolated from most continents and have been reported to infect freshwater finfish, anurans (frogs and toads), urodeles (salamanders), and reptiles (turtles and snakes). Megalocytiviruses infect more than 30 species of cultured marine and freshwater finfish belonging mainly to the orders Perciformes and Pleuronectiformes (**Table 1**).

Hosts for vertebrate iridoviruses include all classes of ectothermic vertebrates. However, to date, only one vertebrate iridovirus, namely Bohle iridovirus (BIV), has been shown, under experimental conditions, to infect multiple vertebrate species, genera, and classes. While cross-species/class transmission has been demonstrated following experimental exposure, BIV is yet to be isolated from any epizootic. Moreover, the maximum permissive temperature for vertebrate iridovirus replication (approximately 15–25 °C) precludes replication in mammals or in mammalian cells incubated at 37 °C.

Phylogeny

At the genomic level, megalocytiviruses, ranaviruses, and lymphocystiviruses differ markedly in genomic organization, GC content, and sequence identity. The GC content of lymphocystiviruses (27–29%) is markedly lower than that of ranaviruses or megalocytiviruses (49–55%). Construction of a phylogenetic tree based on the inferred amino acid sequence of the major capsid protein indicates that three genera of vertebrate iridoviruses form separate clusters that are distinct from each other and from the invertebrate viruses (**Figure 2**). Sequence similarity/identity between different vertebrate iridovirus genera is typically <50%, whereas it is greater than 70% within a genus. Moreover, there is more sequence diversity among ranaviruses, which infect all classes of ectotherms, than among megalocytiviruses, which infect only teleost fish.

Clinical Features and Pathology

Iridoviruses of fish, amphibians, and reptiles are often highly virulent and can cause fatal infections in their hosts. Epizootics leading to mass mortalities with death rates approaching 100% have been reported in fish and amphibians.

Lymphocystosis in fish is, in the main, chronic and benign and not life-threatening; its impact is mainly

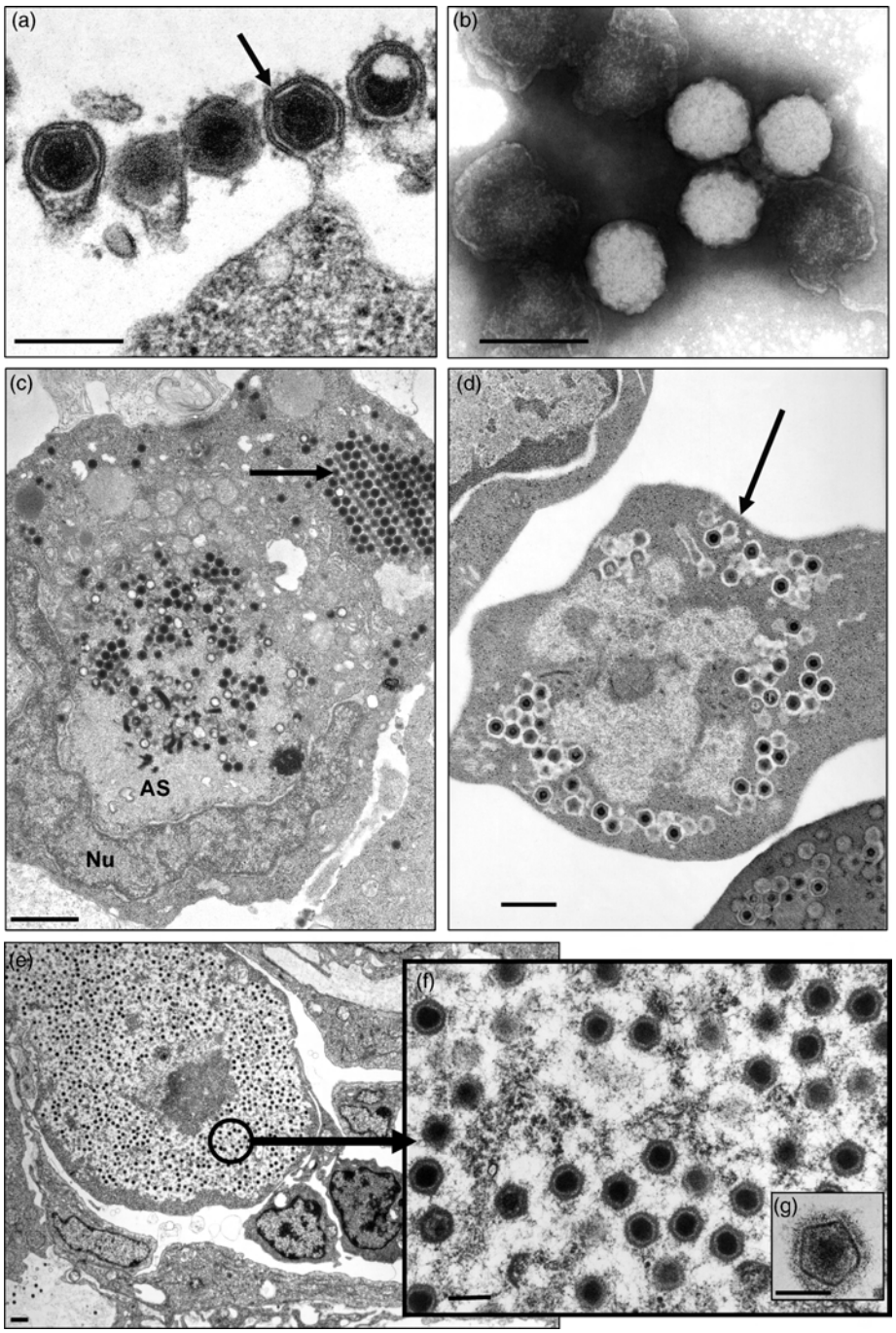


Figure 1 Electron micrographs of iridoviruses identified within or isolated from fish, amphibians, and reptiles. (a) Transmission electron micrograph of an ultrathin section from an EHNV-infected cell. The host-derived membrane is indicated (arrow). (b) Micrograph of a negatively stained EHNV illustrating the difference in appearance when the whole virus is imaged. (c) Low magnification image of a single cultured cell (Chinook salmon embryo cell) infected with EHNV. The image is from an ultrathin section and illustrates the presence of paracrystalline arrays (arrow), virus assembly sites (AS), and a distorted nucleus (Nu). (d) Image of an ultrathin section of a reptilian erythrocyte (arrow) infected with erythrocytic necrosis iridovirus. Bar represents 1 μm. (e) An ultrathin section of an unknown hypertrophied cell from a dwarf gourami infected with an unknown megalocytivrus. The enlarged cell is apparent. (f) Enlargement of the indicated region from panel (e). Large icosahedral viruses are apparent. (g) Image of a single lymphocystivirus from an ultrathin section of an infected cell. The virus differs from the others due to the presence of surface-associated fibrils (arrow). Scale = 200 nm (a, b, g); 1 μm (c–e); 300 μm (f).

Table 1 Vertebrate iridoviruses[a]

Host	*Virus*	*Country or region where isolated*
Fish		
Examples of ranaviruses		
Red-fin perch (*Perca fluviatilis*) and rainbow trout (*Onchorhynchus mykiss*)	Epizootic hemotopoietic necrosis virus (EHNV)	Australia
Catfish (*Ictalurus melas*)	European catfish virus (ECV)	Europe (France)
Largemouth bass (*Micropterus salmonides*)	Largemouth bass virus (LMBV)	North America (USA)
Guppy fish (*Poecilia reticlata*)	Guppy fish iridovirus (GV6)	Southeast Asia
Examples of meglacytiviruses		
Red sea bream (*Pagrus major*)	Red seabream iridovirus (RSIV)	Japan
Sea bass (*Lateolabrax sp.*)		Japan
Brown spotted grouper (*Epinephelus tauvina*)	Grouper sleepy disease virus (GSIV)	Southeast Asia
Cultured mandarin fish (*Siniperca chuatsi*)	Infectious spleen and kidney necrosis virus (ISKNV)	Southeast Asia
Examples of lymphocystiviruses		
Infect a large range of fish including flounder (*Platichthys flesus*), plaice (*Pleuronectes platessa*), and dab (*Limanda limanda*)	Lymphocystis disease virus 1 (LCDV-1) (also referred to as Flounder lymphocystis disease virus, FLDV), Dab lymphocystis disease virus (LCDV-2, tentative species of genus *Lymphocystivius*)	Ubiquitous
Amphibians		
Examples of ranaviruses		
Leopard frog (*Rana pipiens*)	Frog virus 3	North America (USA)
Leopard frog (*Rana pipiens*)	Leopard frog iridoviruses (LT1-LT4)	North America (USA)
Red eft (*Diemictylus viridescens*)	T6–20	North America (USA)
North American bullfrog (*Rana catesbeiana*)	Tadpole edema virus (TEV)	North America (USA)
Edible frog (*Rana esculenta*)	*Rana esculanta* iridovirus (REIR)	Europe (Croatia)
Ornate burrowing frog (*Limnodynastis ornatus*)	Bohle iridovirus (BIV)	Australia
Cane toad (*Bufo marinus)*	*Bufo marinus* Venezuelan iridovirus 1 (GV)	South America (Venezuela)
Common frog (*Rana temporaria)*	*Rana temporaria* United Kingdom iridovirus 1 (RUK 11)*	Europe (UK)
Tiger salamander (*Ambystoma tigrinum stebbensi*)	*Ambystoma tigrinum* iridovirus (ATV)	North America (USA)
Reptiles		
Examples of ranaviruses		
Box turtle (*Terrapene c. Carolina*)	Tortoise virus 3 (TV3)	North America (USA)
Central Asian tortoise (*Testudo horsefieldi*)	Tortoise virus 5 (TV5)	North America (USA)
Gopher tortoise *(Gopherus polyphemus*)		North America (USA)
Green tree python (*Morelia viridis*)	Wamena virus (WV)*	Australia (origin Irian Jaya)

[a]Listed viruses are examples of vertebrate iridoviruses; the list is not exhaustive. Not all listed viruses are included within the *Eighth Report of the International Committee on Taxonomy of Viruses* (Fauquet *et al*.); those which are not assigned to genera are indicated (*).

cosmetic. Infection causes cellular hypertrophy with individual cells reaching 100 μm to 1 mm in diameter. Infected cells develop a thick hyline capsule, a central, enlarged nucleus and basophilic inclusions which correspond to the comparatively large size (up to 300 nm) of the viruses. Megalocytiviruses, on the other hand, cause a darkening of body color and lethargy which has led to the disease being referred to as 'sleepy disease'. Infected animals also exhibit severe anemia, petechia of the gill, and enlargement of the spleen. Enlarged, inclusion body-bearing cells which function as viral assembly sites are characteristic structures of the spleen, kidney, liver, and other internal organs. WSIV, which is currently an unassigned member of the family, causes significant mortalities among farm-raised juvenile white sturgeon (*Acipenser transmontanus*) in North America. The virus is epitheliotropic, infecting the skin, gills, and upper alimentary tract. Affected tissues display hyperplasia with characteristic amphophilic to basophilic enlarged Malpighian cells filled with virus particles. Erthrocytic necrosis iridoviruses of fish, amphibians, and reptiles are associated with intracytoplasmic inclusion bodies within erythrocytes. Infection is characterized by nuclear degeneration, margination of chromatin, pyknosis, karyorrhexis, and karyolysis. A major clinical feature of such animals is anemia.

The clinical outcome of ranavirus infections varies from benign to fatal. Infections can lead to ulceration and/or systemic hematopoietic necrosis in amphibians and fish, and skin polyps, skin sloughing, and systemic hematopoietic necrosis in urodeles. The pathology of

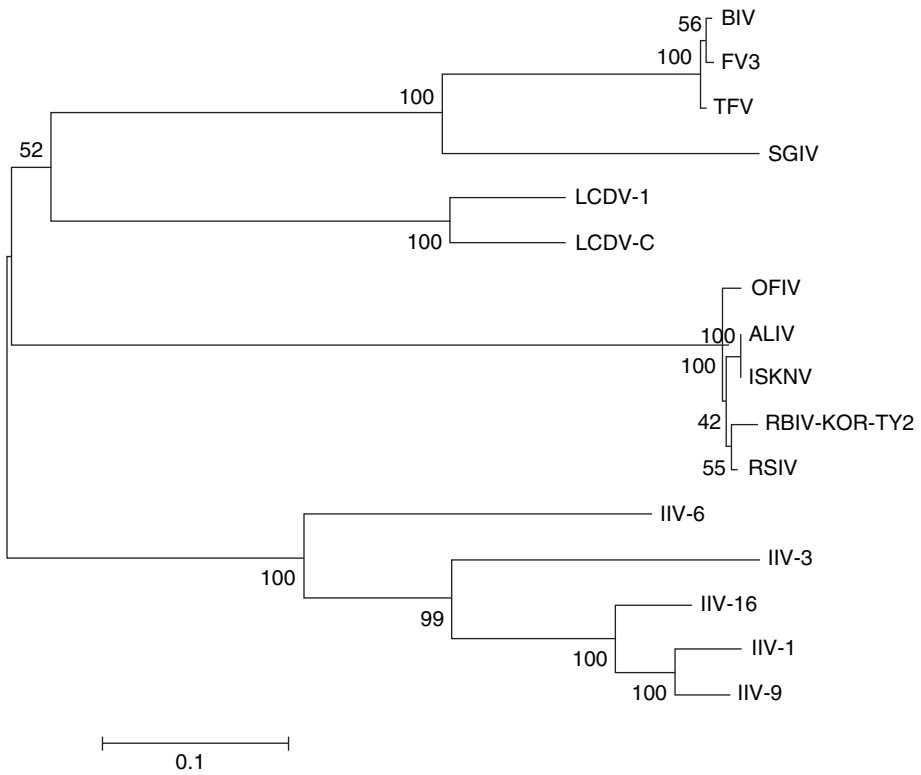


Figure 2 Phylogenetic relationships among iridoviruses. The inferred amino acid sequences of the MCP of 16 iridoviruses, representing all five currently recognized genera, were aligned using the CLUSTAL W program and used to construct a phylogenetic tree using the Neighbor-Joining algorithm and Poisson correction within MEGA version 3.1. The tree was validated by 1000 bootstrap repetitions. Branch lengths are drawn to scale and a scale bar is shown. The number at each node indicates bootstrapped percentage values. The sequences used to construct the tree were obtained from the following viruses: genus *Megalocytivirus* – ISKNV, infectious skin and kidney necrosis virus (AF370008); ALIV, African lampeye iridovirus (AB109368); OFIV, olive flounder iridovirus (AY661546); RSIV, red sea bream iridovirus (AY310918); RBIV, rock bream iridovirus (AY533035); genus *Ranavirus* – SGIV, Singapore grouper iridovirus (AF364593); TFV, tiger frog virus (AY033630); BIV, Bohle iridovirus (AY187046); FV3, frog virus 3 (U36913); genus *Lymphocystivirus* – LCDV-1, lymphocystis disease virus (L63545); LCDV-C, lymphocystis disease virus – China (AAS47819.1); genus *Iridovirus* – IIV-6, invertebrate iridescent virus 6 (AAK82135.1); IIV-16 (AF025775), IIV-1 (M33542), and IIV-9 (AF025774); genus *Chloriridovirus* – IIV-3 (DQ643392).

vertebrate iridovirus infections is best described for the ranavirus EHNV. The associated disease is referred to as epizootic hemaopoietic necrosis and this designation is applicable to most ranavirus infections. Infection results in the degeneration of hematopoietic cells and damage to the vascular endothelium within most organs. For example, in infected kidneys (**Figure 3**) destruction of the blood-forming cells, termed acute hematopoietic necrosis, occurs. Within the liver and spleen, multifocal necrosis is common. Other organs are also affected, including the pancreas and the vascular endothelium within the liver, spleen, kidney, gill, and heart.

Emerging Infectious Pathogens

Although lymphocystis disease has been known since the nineteenth century, other vertebrate iridoviruses are more recently recognized and the incidence of reports of ranavirus and meglocytivirus infections has increased in recent years with respect to the number of new hosts and new geographic locations. Ranavirus and megalocytivirus epizootics have been reported in finfish in Asia, North America, South America, the United Kingdom, and Australia. However, long-term declines in finfish populations have been attributed to over-fishing rather than infectious disease. Dramatic fluctuations of amphibian populations due to ranavirus infections have been reported but these have not been recognized as causative agents for reported global amphibian population declines; these are due, in the main, to the fungus Batrachochytrium dendrobatidis.

Transmission and Control

Lymphocystiviruses are transmitted horizontally via abraded lesions, *Aedes aegypti*, *Aedes albopictus* (C6/36),

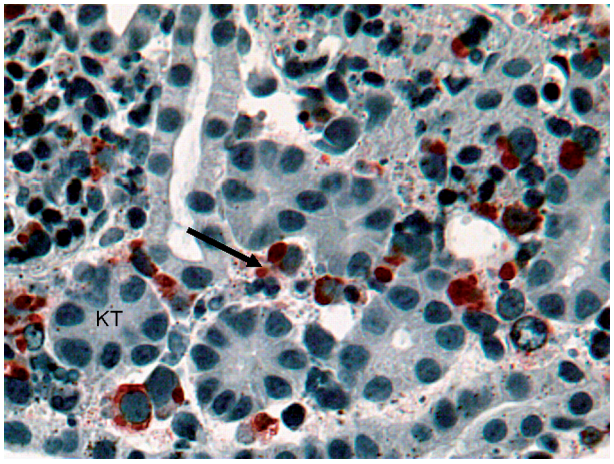


Figure 3 Photomicrograph (light microscopy) of the kidney of an infected *Bufo marinus* tadpole infected with a South American ranavirus. Extensive necrosis of the hematopoietic cells is indicated by the presence of chromogen (brick-red color) following an immunoperoxidase procedure using a primary antibody against EHNV. KT, kidney tubules. The image was viewed at ×100 magnification.

and *Drosophila melanogaster*. The principal mode of transmission of megalocytiviruses (e.g., RSIV) and ranaviruses is horizontally via virus-containing water. Ranaviruses remain viable in water, as dried culture medium, in frozen carcasses, and at various temperatures (4 °C, −20 °C, and −70 °C) for prolonged periods, indicating that they can exist between epizootics both within and outside their biological host(s). The resistant nature of these viruses also indicates their potential for translocation via fomites such as boots, boat hulls, and fishing tackle, in live fish used for stocking aquaculture ponds, via bait fish, and on the skin surfaces of predatory animals such as birds. The international trade in wildlife, a considerable portion of which is illegally performed, is also a recognized mechanism for the effective global transport of vertebrate iridoviruses.

To date, vaccines have only been developed against the megalocytiviruses of Asia. For RSIV, a formalin-inactivated vaccine and a DNA vaccine have been used for the protection of finfish including red sea bream, yellowtail, and amberjack. Control of other diseases such as epizootic hematopoietic necrosis is by containment using diagnosis, surveillance, and management strategies documented by the Office Internationale des Epizooties (OIE). No control measures exist for other ranaviruses or erythrocytic necrosis viruses.

Concluding Remarks

Iridoviruses of fish, amphibians, and reptiles represent a potential health risk to both free-ranging and captured ectothermic vertebrates and are recognized as agents of economical importance by commercial fishing and aquaculture industries; they are not a direct health risk to humans. Over the past two decades, there has been an increase in the number of reported disease incidents involving these large dsDNA viruses. In some reports, surveillance has indicated that these viruses have increased their geographical range and may, therefore, be regarded as emerging viruses. While iridovirus-associated diseases can cause mass mortalities of some species, they are not recognized as a primary cause of the reported global population declines of amphibians or finfish.

The patterns of disease and details on viral structure for the recognized genera are known. However, detailed knowledge of the cellular pathways associated with the pathogenesis of the various diseases is limited.

See also: Iridoviruses of Invertebrates.

Further Reading

Chinchar VG (2002) Ranaviruses (family *Iridoviridae*): Cold-blooded killers. *Archives of Virology* 147: 447–470.

Chinchar VG, Essbauer S, He JG, *et al.* (2005) Family *Iridoviridae*. In: Fauquat C, Mayo MA, Maniloff M, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 145–162. San Diego, CA: Elsevier Academic Press.

Chinchar VG, Hyatt AD, Miyazaki T, and Williams T (in press) Family *Iridoviridae*: Poor viral relations no longer. *Current Topics Microbiology and Immunology* .

Daszak P, Berger L, Cunningham AA, Hyatt AD, Green DE, and Speare R (1999) Emerging infectious diseases and amphibian population declines. *Emerging Infectious Diseases* 5: 735–748.

Daszak P, Cunningham AA, and Hyatt AD (2000) Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science* 287: 443–449.

Eaton HE, Metcalf J, Penny E, Tcherepanov V, Upton C, and Brunetti CR (2007) Comparative genomic analysis of the family *Iridoviridae*: Re-annotating and defining the core set of iridovirus genes. *Virology Journal* 4: 11.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy. Classification and Nomenclature of Viruses. Eighth Report of the International Committee on the Taxonomy of Viruses*, San Diego, CA: Elsevier Academic Press.

Hyatt AD, Gould AR, Zupanovic Z, *et al.* (2000) Comparative studies of piscine and amphibian iridovirues. *Archives of Virology* 145: 301–331.

Office Internationale des Epizooties (2006) Epizootic haematopoietic necrosis. In: *Aquatic Animal Health Code*, p. 2.1.1. Paris: OIE.

Robert J, Morales H, Buck W, Cohen N, Marr S, and Gantress J (2005) Adaptive immunity and histopathology in frog virus 3-infected Xenopus. *Virology* 332: 667–675.

Schock DM, Bollinger TK, Chinchar VG, Jancovich JK, and Collins JP (2008) Experimental evidence that amphibian ranaviruses are multi-host pathogens. *Copeia* 2008: 133–143.

Tan WGH, Barkman TJ, Chinchar VG, and Essani K (2004) Comparative genomic analyses of frog virus 3, type species of the genus *Ranavirus* (family *Iridoviridae*). *Virology* 323: 70–84.

Williams T, Barbosa-Solomieu V, and Chinchar VG (2005) A decade of advances in iridovirus research. *Advances in Virus Research* 65: 173–248.

Iridoviruses of Invertebrates

T Williams, Instituto de Ecología A.C., Xalapa, Mexico

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Circular permution Genome is a linear molecule of DNA with different terminal sequences at the population level. Physical maps of such genomes are circular.
Covert infection A sublethal infection that causes no obvious changes in the appearance or behavior of the host.
Cytopathic effect Alteration in the microscopic appearance of cultured cells following virus infection.
MCP Major capsid protein. A highly conserved structural polypeptide of *c.* 50 kDa that represents about 40% of the total protein content of the virion.
Patent infection Invertebrates that display iridescent hues due to an abundance of virus particles in crystalline arrays in infected cells.

Introduction

Invertebrate iridescent viruses (IIVs) (aka invertebrate iridoviruses) are icosahedral particles of approximately 120–200 nm in diameter that infect invertebrates, mostly insects, in damp or aquatic habitats. These viruses cause two types of disease: one patent and the other covert (inapparent). An abundance of virus particles in the cells of patently infected insects causes them to develop an obvious iridescent color that typically ranges from violet, blue, green, or orange. Patent disease is usually fatal in the larval or pupal stages. In contrast, covert infections are not lethal; covertly infected insects appear healthy and may develop to the adult stage and reproduce. Interest in these viruses has been limited by the perception that they have little potential as biological control agents against insect pests. However, there is now growing awareness of the potential impact of sublethal IIV disease on the dynamics of insect populations, including insect vectors of medical importance worldwide.

History

Originally discovered in 1954 infecting soil-dwelling populations of cranefly larvae (*Tipula paludosa*) in England, IIVs were subsequently reported in insects and other invertebrates worldwide. Detailed electron microscope observations in the late 1960s and early 1970s confirmed the icosahedral nature of the particle and revealed a complex ultrastructure including an internal lipid membrane and an external fringe of fibrils extending from the capsid of certain isolates. Serological relationships among IIVs and with iridoviruses from vertebrates were revealed during the 1970s and the genome of IIV-6 was shown to be circularly permuted and terminally redundant in 1984. Abundant covert IIV infections in insect populations were detected using molecular techniques and comparative genetic studies broadly supported previous serological findings on the relationships among these viruses in the 1990s. High-resolution ultrastructural studies in 2000 built on the previous model and the first complete genome of an IIV was sequenced in 2001.

Classification

IIVs are currently assigned to one of two genera in the family based on particle size and genetic characteristics. Small IIVs with dehydrated particle sizes in ultrathin section typically around 120 nm diameter have been isolated from several different orders of insects and terrestrial isopods and are assigned to the genus *Iridovirus* (**Table 1**). Due to the limited genome sequence data available, only two IIVs have been assigned species status, *Invertebrate iridescent virus 1* (IIV-1) and *Invertebrate iridescent virus 6* (IIV-6), that is the type species of the genus. Tipula iridescent virus and chilo iridescent virus are recognized synonyms of each of these names, respectively. An additional 11 viruses are presently considered as tentative species in the genus. The viruses of this genus can be further divided into three distinct complexes based on genetic and serological characteristics: one large complex containing IIV-1 and at least nine other IIVs, and two smaller complexes, one containing IIV-6, and the other containing IIV-31 and an IIV from a beetle (*Popillia japonica*).

The genus *Chloriridovirus* is comprised of a single species, *Invertebrate iridescent virus 3* (IIV-3), with a particle size of ~180 nm diameter in ultrathin section, isolated from a mosquito. There are a great many additional records of iridoviruses from invertebrate hosts but these have not been characterized.

Geographical Distribution

IIVs have been observed infecting invertebrates in tropical and temperate regions on every continent except Antarctica. A number of marine invertebrates have also been reported as

Table 1 Classification of iridoviruses isolated from invertebrates

Genus, virus (alternative name)	*Abbreviation*	*Host species (order)*[a]	*Location*	*Accession numbers*
Iridovirus				
Invertebrate iridescent virus 1 (Tipula iridescent virus)	IIV-1 (TIV)	*Tipula paludosa* (D)	UK	M33542, M62953
Invertebrate iridescent virus 6 (Chilo iridescent virus)	IIV-6 (CIV)	*Chilo suppressalis* (L)	Japan	AF303741, NC_003038
Anticarsia gemmatalis iridescent virus[b]	AGIV	*Anticarsia gemmatalis* (L)	USA	AF042343
Invertebrate iridescent virus 2[b]	IIV-2	*Sericesthis pruinosa* (C)	Australia	AF042335
Invertebrate iridescent virus 9[b]	IIV-9	*Wiseana cervinata* (L)[c]	New Zealand	AF025774, AY873793
Invertebrate iridescent virus 16[b]	IIV-16	*Costelytra zealandica* (C)	New Zealand	AF025775, AY873794
Invertebrate iridescent virus 21[b]	IIV-21	*Helicoverpa armigera* (L)[d]	Malawi	
Invertebrate iridescent virus 22[b]	IIV-22	*Simulium variegatum* (D)	UK	AF042341, M32799
Invertebrate iridescent virus 23[b]	IIV-23	*Heteronychus arator* (C)	South Africa	AF042342
Invertebrate iridescent virus 24[b]	IIV-24	*Apis cerana* (Hy)	Kashmir	AF042340
Invertebrate iridescent virus 29[b]	IIV-29	*Tenebrio molitor* (C)	USA	AF042339
Invertebrate iridescent virus 30[b]	IIV-30	*Helicoverpa zea* (L)	USA	AF042336
Invertebrate iridescent virus 31[b]	IIV-31	*Armadillidium vulgare* (Is)[e]	USA	AF042337, AJ279821, AF297060
Chloriridovirus				
Invertebrate iridescent virus 3	IIV-3	*Ochlerotatus (Aedes) taeniorhynchus* (D)	USA	AJ312708

[a]Insect orders Coleoptera (C), Diptera (D), Homoptera (H), Hymenoptera (Hy), Lepidoptera (L), and terrestrial isopods (Is) (Crustacea).
[b]Tentative member.
[c]Also isolated from sympatric insect species *Witlesia sabulosella* (L) and *Opogonia* sp. (C).
[d]Also isolated from *Lethocerus colombiae* (H) in Lake Victoria, Uganda, but may have been contaminated.
[e]Also isolated from *Porcellio dilatatus* and probably several other species of terrestrial isopods.

hosts to iridoviruses. Humidity appears to be the principal factor limiting the distribution of these viruses. Records of IIV infections are most common in aquatic or soil-dwelling arthropods during periods of rainfall and absent in species that inhabit arid or desiccated habitats.

Host Range and Virus Propagation

IIVs replicate in many types of insect cells and may even replicate in reptilian cells *in vitro*. Host range *in vivo* depends very much on the route of infection. Most IIVs show a remarkably broad host range when the inoculum is injected compared to a reduced host range following oral administration of inoculum. For example, injection of particles of IIV-6 results in patent infections in species from all major insect orders and a number of other arthropods isopods and a centipede. Other IIVs, such as IIV-3, IIV-16, or IIV-24, appear restricted to one or two closely related host species. Certain IIVs are capable of infecting multiple host species in their natural habitat, including IIV-9 that infects soil-dwelling species of Lepidoptera and Coleoptera in New Zealand, and IIV-31 that infects several species of terrestrial isopods in the USA. IIVs are also capable of replication in host species that do not develop signs of disease, but the range of species susceptible to such asymptomatic infections is largely unknown.

Virus propagation *in vitro* is most readily achieved in cell lines from dipteran (*Aedes aegypti*, *Ae. albopictus*, *Drosophila* DR1, DL2, etc.) and lepidopteran species (Sf-9, Sf-21, Cf-124, etc.), although recently, cell lines from species of Homoptera and Coleoptera have also been successfully used. Most IIVs can be grown in massive quantities in the standard laboratory host, *Galleria mellonella* (Lepidoptera: Pyralidae). The mosquito virus IIV-3 can only be produced *in vivo* in larvae of *Ochlerotatus taeniorhynchus*.

Properties of the Virion

IIV particles comprise an electron dense core of DNA and associated proteins, surrounded by a lipid membrane encased by an exterior protein capsid (**Figure 1**). Virions released by budding may have an additional outer envelope but this is not essential for infectivity. Detailed studies using cryo-electron microscopy and three-dimensional image reconstruction have examined particles of IIV-6 in closely packed quasi-crystalline hexagonal arrays with an interparticle distance of 40–60 nm. Particle diameter was calculated to be 162 nm along the two- and threefold axes of symmetry and 185 nm along the fivefold axis, considerably larger than the diameter in ultrathin section. The outer capsid is composed of trimeric capsomers, each approximately 8 nm diameter and 7.5 nm high, arranged in a pseudo-hexagonal array. A thin fiber projects radially from the surface of each capsomer that probably regulates interparticle distance, a key characteristic for the iridescence of infected hosts. At the base, the

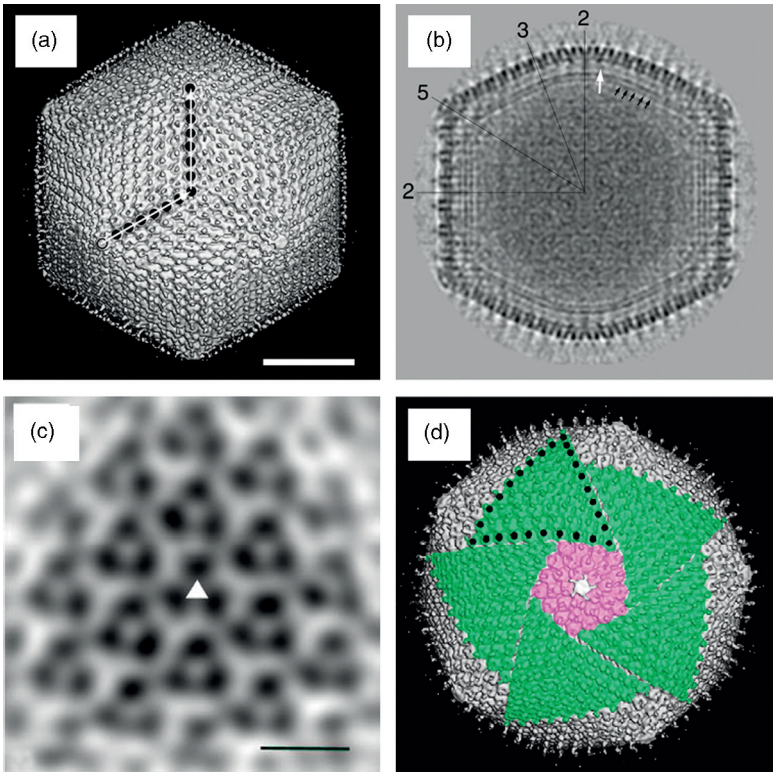


Figure 1 (a) 3-D reconstruction of an IIV-6 particle viewed down threefold axes of symmetry by cryoelectron microscopy. Circles indicate the position of capsomers along the h and k lattice used to calculate the triangulation number (*T*). The proximal end of surface fibers are visible, whereas the flexible distal ends have become lost during the reconstruction process (white bar = 50 nm applies to images (a)–(c)). (b) Central section of the reconstruction density map viewed along twofold axes indicating two-, three-, and fivefold axes of symmetry. A lipid bilayer is observed beneath the capsid shell (black arrows) with numerous connections to an additional shell (white arrow) beneath the outer shell. (c) Close-up view of the trimers comprising each capsomer shown as a planar section through a reconstruction density map viewed along threefold axes (triangle) (black bar = 10 nm). (d) Facets of capsid with five trisymmetrons (highlighted in green) arranged around one pentasymmetron (in pink). The central capsomer of the pentasymmetron (uncolored) is pentavalent. The edge of each trisymmetron comprises 10 capsomers (black dots). Reprinted by permission from Macmillan Publishers Ltd: Nature Structural Biology, Yan X, Olson NH, Van Etten JL, Bergoin M, Rossmann MG, Baker TS (2000) Structure and assembly of large lipid-containing dsDNA viruses. *Nature Structural Biology* 7: 101–103, copyright (2000).

interconnected capsomers form a contiguous icosahedral shell ~2.5 nm thick. The major capsid protein (MCP) exists externally as a noncovalent trimer and internally as a trimer linked by disulfide bonds. The capsomers are arranged into trisymmetron and pentasymmetron facets. Each particle consists of 20 trisymmetrons, composed of 55 capsomers, and 12 pentasymmetrons composed of 30 capsomers and one hexavalent capsomer of uncertain composition. Pentavalent capsomers are located at the vertices of the particle in the center of each pentasymmetron. This gives a total of 1460 capsomers + 12 pentavalent capsomers per particle. The triangulation number (T) is 147. Larger IIVs that infect mosquitoes and midges have larger trisymetrons, probably comprising 78 subunits giving a likely 1560 subunits per particle. A lipid bilayer, 4 nm thick, surrounds the DNA core, and is intimately associated with an additional inner shell beneath the fused layer of the capsid. Core and capsid polypeptides are likely interconnected by intermembrane proteins passing through the lipid layer. The core is a highly hydrated entity in which the DNA–protein complex appears to be arranged in a long coiled filament of some 10 nm diameter.

IIVs are structurally complex: one-dimensional PAGE resolves 20–32 polypeptides with weights typically from 11 to 200 kDa. Much of the polypeptide diversity of IVs appears to be associated with the core and lipid membrane. At least six polypeptide species are associated with the DNA within the core, the major component being a 12.5 kDa species in IV-6. The MCP comprises about 470 amino acids (~50 kDa) and represents 40–45% of the total particle polypeptide.

Properties of the Genome

Each IIV genome is comprised of a linear molecule of DNA that ranges in size from 140 to 210 kbp, and is circularly permuted and terminally redundant. Circular permution means that the terminal sequences differ for each genome in

a population, whereas terminal redundancy means that part of the sequence at one end of the DNA molecule is repeated at the other end. For example, if a complete genome is represented by the numbers 0–9, the DNA molecules from individual virus particles may be represented by the following combinations: 012345678901, 234567890123, 4567890123456, etc., where terminal redundancy is indicated by the underlined numbers. In IIV-6, the degree of terminal redundancy has been estimated as 12% and the genome contains six origins of replication.

The IIV genome is either not methylated, or methylated at very low or undetectable levels. In contrast, high levels of methylation of cytosine residues are seen in virtually all vertebrate iridoviruses. Currently, only two IIV genomes have been sequenced in their entirety: IIV-3 and IIV-6. The genome of IIV-6 is 212 kbp (unique portion) with 28.6% G+C content and comprises 468 open reading frames (ORFs), of which 234 are nonoverlapping. The genome of IIV-3 is 191 kbp (unique portion) with a 48% G+C content and comprises 453 ORFs, of which 126 are nonoverlapping. No collinearity is observed between the genomes of IIV-3 and IIV-6.

Core IIV genes include those involved in (1) replication, including DNA polymerase (037L), RNA polymerase II (α-subunit 176R, β-subunit 428L), Rnase III (142R), a helicase (161L), and a DNA topoisomerase II (045L); (2) nucleotide metabolism, such as ribonucleotide reductase (α-subunit 085L, β-subunit 376L), dUTPase (438L), thymidylate synthase (225R), thymidylate kinase (251L), and thymidine kinase (143R); and (3) other proteins of known function including IAP inhibitor of apoptosis (157L, 193R), PCNA (436R), and the MCP (274L).

Other notable putative genes identified in IIV-6 include an NAD+-dependent DNA ligase (205R) and a putative homolog of sillucin (160L), a cysteine-rich peptide antibiotic. The promoter regions of the MCP and DNA polymerase genes have been located to essential sequences at 29–53 and 6–27 positions upstream of the transcriptional start site, respectively (**Figure 2**). An ORF (100L) has been detected in IIV-6 with truncated homology to the nuclear polymerizing (ADP-ribosyl) transferase from eukaryotic organisms. Interestingly, the large subunit of the IIV-6 ribonucleotide reductase appears to contain an intein, a form of selfish genetic element that removes itself from the protein by post-translational autocatalytic splicing. Genes unique to IIV-3 include two putative transmembrane proteins, a protein similar to fungal DNA polymerase, and a protein similar to a fungi RNA Pol II subunit.

IIVs have been shown to have extensive regions of repetitive DNA that account for 20% (IIV-3) to over 25% (IIV-9) of the genome. The coding function of these regions is unknown although transcription of these regions has been detected late in the infection cycle. The pattern of repetitive DNA in the genome of IIV-6 is complex and involves boxes of tandem repeat sequences and others with a number of different interdigitated repeat sequences of variable size and homology.

Replication

The model for iridovirus replication is that of frog virus 3 (FV-3). Virions display cytotoxic properties and are capable of nongenetic reactivation. Like all other members of the family, IIVs do not replicate at temperatures above 30 °C.

Evolution

Iridoviruses are members of a monophyletic clade of large, nucleocytoplasmic DNA viruses that includes the families *Poxviridae, Phycodnaviridae*, *Asfarviridae* and the recently discovered giant icosahedral mimivirus from an ameba. The clade shares a total of 41 ancestral genes including structural components, and those involved in DNA packaging, replication, transcription and RNA modification including subunits of RNA polymerase and transcription factors, many of which appear to have been acquired from eukaryotic host cells.

Sequence comparisons of the virus-encoded δ DNA polymerase and MCP indicated putative evolutionary relationships between IIVs and ascoviruses of lepidopteran insects. Homologs to about 40% of the proteins encoded by *Spodoptera frugiperda ascovirus 1a* are found in IIV-6 with lower percentages seen among vertebrate iridoviruses, phycodnaviruses, and African swine fever virus (ASFV). Additional analyses based on *bro* genes (Baculovirus repeated ORFs), a multigene family of unknown function, support the conclusion that ascoviruses are more closely related to invertebrate iridoviruses than to vertebrate iridoviruses. Like IIVs, lepidopteran ascoviruses have very low oral infectivity but are highly infectious by injection and depend on parasitoid wasps for transmission. Structural similarities between iridoviruses and the allantoid particles of ascoviruses are not immediately apparent although molecular evidence indicates clear relationships between these viruses.

Signs and Characteristics of Disease

The principal sign of patent IV infection is the iridescent hues which arise from the paracrystalline arrangement of virus particles in host cells. Light is reflected from the surface of close packed particles and causes interference with incident light known as 'Bragg reflections'.

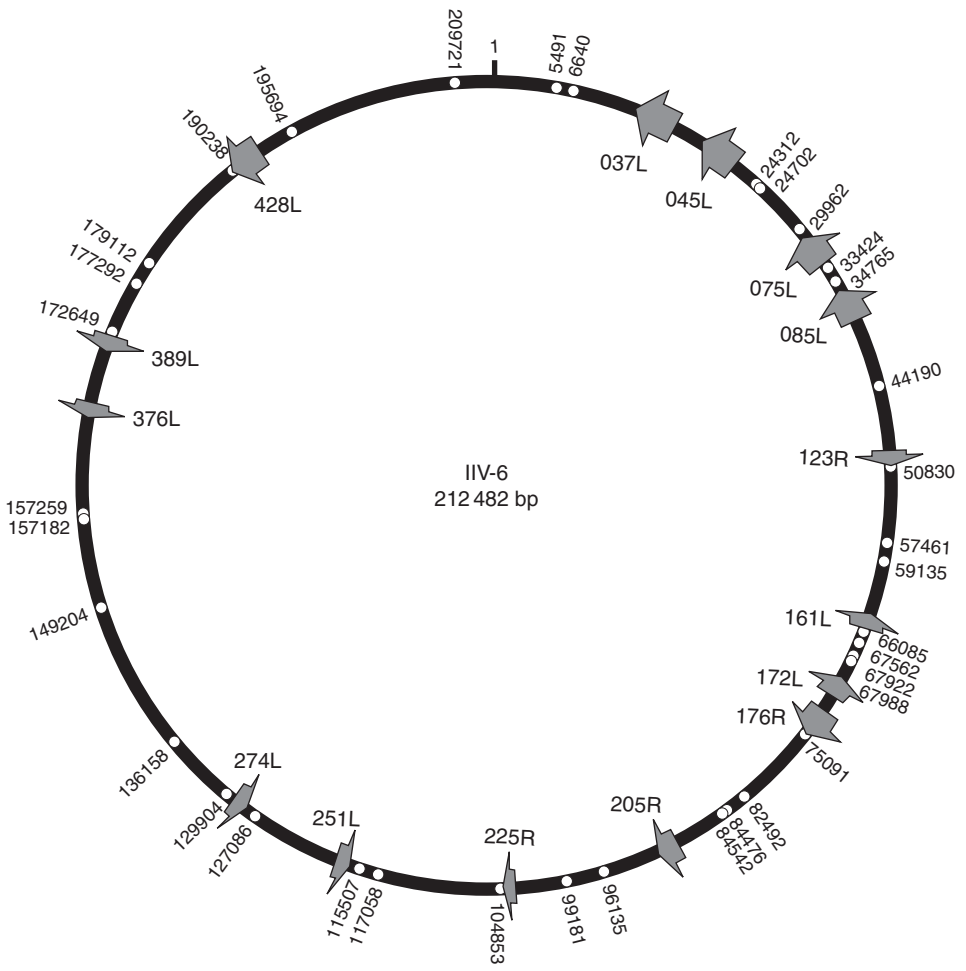


Figure 2 Genetic map of IIV-6. Position of selected ORFs (arrows) identified in the complete genome sequence of IIV-6 that encode the following putative proteins: DNA polymerase (037L), topoisomerase II (045L), ATPase (075L), ribonucleoside diphosphate reductase large subunit (085L), protein-tyrosine phosphatase (123R), helicase (161L), global transactivator homolog (172L), DNA-dependent RNA polymerase 1 (176R), DNA ligase (205R), thymidylate synthase (225R), thymidylate kinase (251L), major capsid protein (274L), ribonucleoside diphosphate reductase small subunit (376L), serine-threonine protein kinase (389L), DNA-dependent RNA polymerase 2 (428L). White dots accompanied by figures indicate the nucleotide positions of *Eco*RI cleavage sites. Reproduced from Jakob N, Darai G, and Williams T (2002) Genus *Iridovirus*. In: Christian T and Darai G (eds.) *Springer Index of Viruses*, Berlin: Springer, with kind permission of Springer Science and Business Media.

The small IIVs of the genus *Iridovirus* usually display violet, blue, or turquoise colors, whereas large IIVs from mosquitoes (genus *Chloriridovirus*) commonly display colors such as green, yellow, or orange. Purified pellets of IIVs also iridesce. Some isolates have unusually long external fibrils attached to the capsid and these isolates do not iridesce.

Covert infections have been detected in natural populations of blackflies (*Simulium variegatum*) and a mayfly (*Ecdyonurus torrentis*), and in laboratory populations of a mosquito (*Ae. aegypti*) and a lepidopteran (*G. mellonella*). Covert infections have been detected by polymerase chain reaction (PCR) amplification of the MCP gene, electron microscope observations, and insect bioassay techniques (**Figure 3**). Studies on IIV-6 in *Ae. aegypti* have revealed clear costs of covert infection including an increase in larval development time and reductions in adult body size, longevity, and fecundity. Overall the reproductive capacity of covertly infected mosquitoes is reduced by 22–50% compared to healthy mosquitoes, depending on the number of cycles of blood meals followed by oviposition.

Pathology

IIVs replicate extensively in most host tissues, especially the epidermis, muscles, fat body, nerves, hemocytes, and areas of the gut. IIV-1 caused the formation of epidermal

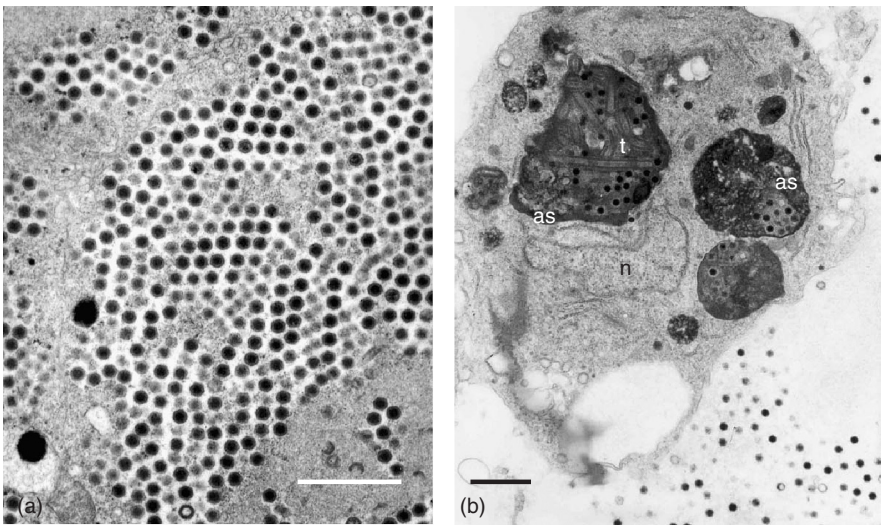


Figure 3 IIV particles in cytoplasm of cells from insects with patent or covert infection. (a) Arrays of closely packed particles of IIV-3 in an epidermal cell from a patently infected mosquito larva. Scale = 1 μm. (b) Low density of particles in a hemocyte from a covertly infected mayfly larva. Cells from covertly infected insects show the characteristic cytoplasmic virus assembly sites (as) close to the nucleus (n), and also the presence of tubular structures (t) likely to be aberrant forms of virus capsids. Scale = 1 μm. (a) Photo courtesy of J. J. Becnel. (b) Reproduced from Tonka T and Weiser J (2000) Iridovirus infection in mayfly larvae. *Journal of Invertebrate Pathology* 76: 229–231, with permission from Elsevier.

tumors in silkworm larvae but such pathology is not observed in other hosts. In mosquito larvae infected by IIV-3, the fat body, epidermis, imaginal disks, hemocytes, trachea, muscle, visceral nerves, gonads, and esophagus were infected but not the remaining gut or malpighian tubules. Individuals with patent infections that survive to pupate frequently show marked deformations of the pupa, particularly of the wing buds.

Pathological changes at the cellular level include cell rounding and the appearance of extensive areas of finely granulated material devoid of cell organelles, the cytoplasmic virus assembly sites. Marked contraction of the cells followed by cell detachment are also common cytopathic effects. Rapid cell–cell fusion is influenced by the multiplicity of infection in cells infected by IIV-6. A virion-associated protein appears to be responsible. The formation of numerous vesicles arising from blebbing of cell membranes followed by loss of cell adhesion and cell–cell fusion has also been observed in lepidopteran cells infected by IIV-1. Changes in the position and morphology of mitochondria have been reported.

Ecology

Ecological studies of IIVs are sparse, probably because the incidence of patent disease is typically very low. The majority of studies have used iridescence as the sole criterion for diagnosing infection, although PCR and insect bioassay have also been employed successfully to detect and quantify covert infections. Studies on backflies and Lepidoptera have indicated that there exists considerable genotypic variation in IIV populations such that individual insects collected at the same place and time may harbor genetically distinct variants.

Studies on transmission have been hindered because the route of infection is unknown or uncertain for most IIV–host systems. Cannibalism or predation of infected individuals involves the consumption of massive doses of particles and appears to be the principal mechanism of transmission in populations of mosquitoes, isopods, tipulids, mole crickets, and cannibalistic species of Lepidoptera. Hymenopteran parasitoids and entomopathogenic nematodes have been shown capable of transmitting IIV infections during the act of oviposition or host penetration, respectively. Survival of IIV-3 in mosquito populations appears to depend on alternating cycles of horizontal transmission between cannibalistic larvae and vertical transmission from adult female mosquitoes that acquire infection shortly before pupating. Horizontal transmission is also favored in high-density populations of some hosts wherein the frequency of aggressive encounters between conspecifics and the probability of wounding is greater than at low densities.

There is clear evidence of seasonality in many IIV–host associations due to seasonal fluctuations in precipitation and/or host densities. The persistence of IIV-6 in soil depends on moisture, whereas persistence in water is markedly affected by solar ultraviolet radiation.

Occasional epizootics of patent disease have been reported in lepidopteran species, *Helicoverpa zea* and *Anticarsia gemmatalis*, the cricket *Scapteriscus borellii*, as well as tipulid, mosquito, and blackfly larvae.

Economic Importance

IIVs have been observed to infect natural populations of major insect pests and numerous species of insect vectors of medical or veterinary importance (mosquitoes, midges, and blackflies). However, the low prevalence of patent infections and relatively broad host range of most IIVs means that these viruses are not considered as likely agents for programs of biological control. An IIV is believed to be responsible for periodically devastating populations of mopane worms (*Gonimbrasia belina*, Lepidoptera) that represent a multimillion dollar food industry in several southern African countries. Iridovirus infections are also associated with severe diseases and mass mortalities in oyster populations, but the relationship between these marine iridovirus and those infecting terrestrial and freshwater arthropods is unknown.

See also: Ascoviruses; Baculoviruses: General Features; Iridoviruses: General Features; Iridoviruses of Vertebrates.

Further Reading

Darai G (ed.) (1990) *Molecular Biology of Iridoviruses.* Dordrecht, The Netherlands: Kluwer.

Delhon G, Tulman ER, Afonso CL, *et al.* (2006) Genome of Invertebrate iridescent virus type 3 (mosquito iridescent virus). *Journal of Virology* 80: 8439–8449.

Jakob N, Darai G, and Williams T (2002) Genus *Iridovirus*. In: Christian T and Darai G (eds.) *Springer Index of Viruses*, Berlin: Springer.

Jakob NJ, Muller K, Bahr U, and Darai G (2001) Analysis of the first complete DNA sequence of an invertebrate iridovirus: Coding strategy of the genome of Chilo iridescent virus. *Virology* 286: 182–196.

Tonka T and Weiser J (2000) Iridovirus infection in mayfly larvae. *Journal of Invertebrate Pathology* 76: 229–231.

Webby R and Kalmakoff J (1998) Sequence comparison of the major capsid protein gene from 18 diverse iridoviruses. *Archives of Virology* 143: 1949–1966.

Williams T (1998) Invertebrate iridescent viruses. In: Miller LK and Ball LA (eds.) *The Insect Viruses*, pp. 31–68. pp. 31–68. New York: Plenum.

Williams T, Barbosa-Solomieu V, and Chinchar VG (2005) A decade of advances in iridovirus research. *Advances in Virus Research* 65: 173–248.

Yan X, Olson NH, Van Etten JL, Bergoin M, Rossmann MG, and Baker TS (2000) Structure and assembly of large lipid-containing dsDNA viruses. *Nature Structural Biology* 7: 101–103.

Iridoviruses: General Features

V G Chinchar, University of Mississippi Medical Center, Jackson, MS, USA
A D Hyatt, Australian Animal Health Laboratory, Geelong, VIC, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Anuran An amphibian of the order Salientia (formerly Anura or Batrachia) which includes frogs and toads. Also called, salientians.

Introduction

Members of the family *Iridoviridae*, hereafter referred to as iridovirids to distinguish them from members of the genus *Iridovirus*, are large (120–200 nm), double-stranded DNA viruses that utilize both the nucleus and the cytoplasm in the synthesis of viral macromolecules, but confine virion formation to morphologically distinct, cytoplasmic assembly sites. Virus particles display icosahedral symmetry but, unlike other virus families, infectious virions can be either nonenveloped (i.e., naked) or enveloped, although the latter show a higher specific infectivity. The viral capsid is composed primarily of the major capsid protein (MCP), a ~50 kDa protein that is highly conserved among all members of the family. An internal lipid membrane, that is essential for infectivity, underlies the capsid and encloses the viral DNA core. Approximately 30 virion-associated proteins have been identified by gel electrophoresis, but the functions of most of these proteins are unknown. The viral genome is linear, double-stranded DNA and ranges in size from 103 to 212 kbp, depending upon the viral species. As a likely consequence of its mode of packaging, viral DNA is terminally redundant and circularly permuted. The size of the repeat regions range from 5% to 50% of the genome and, like the genome size, appears to vary with the specific viral species.

Iridovirus Taxonomy

Members of the family *Iridoviridae* are classified into five genera, two of which infect invertebrates (*Iridovirus*, *Chloriridovirus*), and three that infect ectothermic vertebrates (*Ranavirus*, *Lymphocystivirus*, and *Megalocytivirus*). In addition to differences in host range, viruses within the three vertebrate iridovirus genera, with one known exception, contain highly methylated genomes in which every cytosine present within a CpG motif is methylated.

Viral isolates within the genus *Megalocytivirus* show high levels of sequence identity and it is not clear if they represent strains of the same viral species, or a small number of closely related species. To a lesser extent, the same is true within the genus *Ranavirus*, although here differences in host range and clinical presentation, along with a higher level of sequence variation, allows identification of individual viral species (**Table 1**).

Recently, genomic analysis validated taxonomic divisions made earlier on the basis of physical characteristics, clinical presentation, and host range. A phylogenetic tree constructed using the inferred amino acid sequences of the major capsid proteins (MCPs) of 16 iridovirids, representing all five genera, supports the division of the family into three genera of vertebrate iridoviruses (*Megalocytivirus*, *Ranavirus*, and *Lymphocystivirus*), and a phylogenetically diverse cluster of invertebrate iridescent viruses (IIVs) composed of the existing *Chloriridovirus* and *Iridovirus* genera (**Figure 1**). Recently, a maximum likelihood (M-L) tree, constructed using a concatenated set of 11 protein sequences conserved among all iridoviruses, confirmed this finding. Moreover, a M-L tree constructed using MCP sequences from 14 invertebrate iridescent viruses (many of which had been tentatively classified as members of the genus *Iridovirus*) has shown that IIV-6 clusters with two tentative members of the genus *Iridovirus* on one branch, whereas IIV-3 is linked with the remaining 12 isolates on a separate branch. Given this admixture of putative chloriridovirus and iridovirus species, it has been suggested that the status of the existing invertebrate genera may require revision. However, the lack of collinearity between IIV-3 and IIV-6, the low levels of amino acid identity between IIV-3 and IIV-6, along with differences in host range, virion size, and GC content, indicate that IIV-3 and IIV-6 are likely members of different viral genera. On a larger taxonomic scale, recent work has linked the family *Iridoviridae* with other large DNA virus families such as the *Poxviridae*, *Asfarviridae*, *Phycodnaviridae*, the newly discovered mimiviruses, and the *Ascoviridae*. However, although it is clear that these large DNA viruses share a set of common genes, it is not known if iridoviruses are more closely related to ascoviruses, the recently discovered mimivirus, or another virus family.

Viral Genome

The 12 completely sequenced iridovirid genomes range in size from 103 to 212 kbp (**Table 2**). The genomes of vertebrate iridovirids are found at the lower end of this size range, whereas the genomes of IIV-3 and IIV-6 (invertebrate iridovirids) occupy the high end. Consistent with the differences in size, vertebrate iridovirids encode ~100 putative ORFs, whereas invertebrate iridovirids code for

Table 1 Taxonomy of the family *Iridoviridae*

Genus	*Viral species [strains]*[a]	*Tentative species*
Iridovirus	*Invertebrate iridescent virus 6* (IIV-6) [Gryllus iridovirus, Chilo iridescent virus]	Anticarsia gemmatalis iridescent virus (AGIV), IIV-2, -9, -16, -21, -22, -23, -24, 29, -30, -31
	Invertebrate iridescent virus 1 (IIV-1) [Tipula iridescent virus]	
Chloriridovirus	*Invertebrate iridescent virus-3* (IIV-3) [Aedes taeniorhynchus iridescent virus, mosquito iridescent virus]	
Ranavirus	*Frog virus 3* (FV3), [tadpole edema virus, TEV; tiger frog virus, TFV]	Singapore grouper iridovirus (SGIV), grouper iridovirus (GIV), Rana esculenta iridovirus, Testudo iridovirus, Rana catesbeiana virus Z (RCV-Z)
	Ambystoma tigrinum virus (ATV), [Regina ranavirus, RRV]	
	Bohle iridovirus (BIV)	
	Epizootic haematopoietic necrosis virus (EHNV)	
	European catfish virus (ECV), [European sheatfish virus, ESV]	
	Santee-Cooper ranavirus, [largemouth bass virus, LMBV; doctor fish virus, DFV; guppy virus 6, GV-6]	
Megalocytivirus	*Infectious spleen and kidney necrosis virus* (ISKNV) [Red Sea bream iridovirus, RSIV; African lampeye iridovirus, ALIV; orange spotted grouper iridovirus, OSGIV; rock bream iridovirus, RBIV]	
Lymphocystivirus	*Lymphocystis disease virus 1* (LCDV-1) [flounder lymphocystis disease virus, flounder virus]	LCDV-2, LCDV-C, LCDV-RF; [Dab lymphocystis disease virus]
Unclassified		White sturgeon iridovirus (WSIV)
		Erythrocytic necrosis virus (ENV)

[a]Recognized viral species are italicized and likely virus strains or isolates are enclosed within brackets. Tentative species are listed, as are commonly used abbreviations for species, strains, isolates, and tentative species.

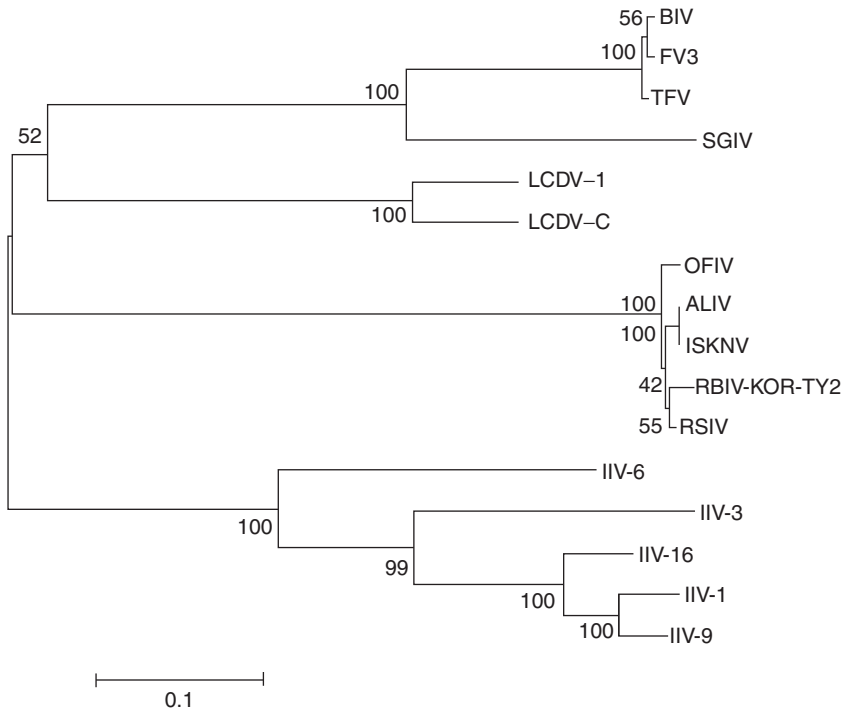


Figure 1 Phylogenetic relationships among iridovirids. The inferred amino acid sequences of the MCP of 16 iridovirids, representing all five currently recognized genera, were aligned using the CLUSTAL W program and used to construct a phylogenetic tree using the neighbor-joining algorithm and Poisson correction within MEGA version 3.1. The tree was validated by 1000 bootstrap repetitions. Branch lengths are drawn to scale. The number at each node indicates bootstrapped percentage values. The sequences used to construct the tree were obtained from the following viruses: genus *Megalocytivirus* – ISKNV, infectious skin and kidney necrosis virus (AF370008); ALIV, African lampeye iridovirus (AB109368); OFIV, olive flounder iridovirus (AY661546); RSIV, red sea bream iridovirus (AY310918); RBIV, rock bream iridovirus (AY533035); genus *Ranavirus* – SGIV, Singapore grouper iridovirus (AF364593); TFV, tiger frog virus (AY033630); BIV, Bohle iridovirus (AY187046); FV3, frog virus 3 (U36913); genus *Lymphocystivirus* – LCDV-1, lymphocystis disease virus (L63545); LCDV-C, lymphocystis disease virus (China) (AAS47819.1); genus *Iridovirus* – IIV-6, invertebrate iridescent virus 6 (AAK82135.1); IIV-16 (AF025775), IIV-1 (M33542), and IIV-9 (AF025774); genus *Chloriridovirus* – IIV-3 (DQ643392).

Table 2 Coding potential of iridovirid genomes

Genus	*Virus*	*bp*[a]	*No. of genes*[b]	*%GC*	*GenBank Acc. No.*
Iridovirus	IIV-6	212 482	234	29	AF303741
Chloriridovirus	IIV-3	191 132	126	48	DQ643392
Lymphocystivirus	LCDV-1	102 653	110	29	L63545
	LCDV-C	186 250	176	27	AY380826
Megalocytivirus	ISKNV	111 362	105	55	AF371960
	OSGIV	112 636	121	54	AY894343
	RBIV	112 080	118	53	AY532606
Ranavirus	FV3	105 903	98	55	AY548484
	TFV	105 057	105	55	AF389451
	ATV	106 332	96	54	AY150217
	SGIV	140 131	162	49	AY521625
	GIV	139 793	120	49	AY666015

[a]The value shown represents the unique genome size in base pairs (bp) minus the length of the terminal repeats.
[b]The value shown is an estimate of the total number of nonoverlapping genes encoded by a given virus. It is generally lower than the total number of putative ORFs which includes putative genes encoded on opposing DNA strands and overlapping genes.

126–234 putative proteins. BLAST analysis of the various viral genomes indicates that about a quarter to a third of the viral ORFs share sequence identity/similarity to eukaryotic and viral proteins of known function including the two largest subunits of RNA polymerase II, a viral DNA polymerase, the two subunits of ribonucleotide reductase, dUTPase, thymidylate synthase, thymidylate kinase, a major capsid protein, and other proteins likely to

be directly involved in viral replication. In addition to these replicative proteins, iridovirids also encode one or more putative 'immune evasion' proteins such as a viral homolog of eukaryotic translational initiation factor 2α (vIF-2α), a steroid oxidoreductase, a caspase activation and recruitment domain (CARD)-containing protein, and a homolog of the TNF receptor. Dot plot analyses have shown that virus species within the genus *Ranavirus* display some degree of collinearity in their gene order, but that inversions occur even between closely related viruses such as Ambystoma tigrinum virus (ATV) and tiger frog virus. Moreover, between more distantly related ranaviruses (e.g., Singapore grouper iridovirus and FV3) and between viruses from different genera, collinearity appears to break down completely, indicating that, although iridovirids share many genes in common, their precise arrangement within the viral genome does not appear to be critical for successful virus replication. Furthermore, although viral genes are temporally expressed, there is no clustering of immediate early (IE), delayed early (DE), or late (L) viral genes. These observations suggest that the marked differences in gene order between different viral species may be due to the propensity of iridovirids to undergo high levels of recombination.

DNA repeats are present within the intergenic regions of all iridovirids, but differ between genera in their extent, arrangement, and sequence motifs. For example, 15 distinct repeats, comprising two distinct groups, 0.8–4.6 kbp in length, are found within IIV-3. The repeats are composed of a ~100 bp sequence that is present in 2–10 copies/genome. They show 80–100% identity within a group and ~60% identity between groups. The function(s) of the repeat regions is unknown but, by analogy to similar regions in other DNA viruses, they may play roles in genome replication and gene expression. Among members of individual vertebrate iridovirid genera, sequence identity within particular coding regions, for example, the MCP, is relatively high (>70%). However, there is considerable diversity among the MCPs of invertebrate viruses, with some members of the genus *Iridovirus* showing only 50% identity to others. As expected, between genera amino acid identity/similarity is only ~50%.

Virus Replication Cycle

Most of what is known about the replication of iridovirids is based on studies conducted with *Frog virus 3* (FV3), the type species of the genus *Ranavirus*. Thus, any discussion of iridovirid life cycles will have a strongly 'ranacentric' orientation. By necessity, that bias will continue here, but with the caveat that other members of the family, especially viruses from different genera, may not follow the FV3 pathway. **Figure 2** summarizes the salient features of FV3 replication.

Virus Entry

The cellular receptor for FV3 is apparently a quite common and highly conserved molecule as a range of mammalian, avian, piscine, and amphibian cell lines can be infected *in vitro*. However, since the host range *in vivo* is restricted mainly to anurans, the receptor molecule may not be expressed as widely in whole animals, at least in cells in which infection may be initiated. In addition, since FV3 does not replicate at temperatures above 32 °C, mammals and birds are nonpermissive hosts. The host range varies with the particular virus. Some iridovirids, such as RSIV and LCDV, possess a broad host range and infect a wide variety of different fish species, whereas other members of the family, such as ATV, infect a limited number of host species. Although both naked and enveloped virions are infectious, early work suggests that these two forms of the virus enter target cells via different mechanisms. Non-enveloped virions are thought to bind to the plasma membrane and inject their viral cores into the cytoplasm whence it is transported to the nucleus. In contrast, enveloped virions enter the cell by receptor-mediated endocytosis.

Early Viral RNA Synthesis

Following entry of the viral DNA into the nucleus, IE viral RNA synthesis begins in a process catalyzed by host cell RNA polymerase II (Pol II). Naked viral DNA is not infectious and IE transcription is dependent upon one or more virion-associated proteins, referred to as virion-associated transcriptional transactivators (VATTs). However, it is not known if VATTs modify the viral DNA template or interact with cellular RNA polymerase II (Pol II) itself. Subsequently, one or more newly synthesized IE proteins, designated virus-induced transcriptional transactivators (VITTs), are required for the transcription of delayed early (DE) viral genes. Although its mechanism of action is unknown, VITTs are likely required to overcome the inhibitory effect of high levels of DNA methylation on Pol II-catalyzed transcription. Like other DNA viruses, transcription of both invertebrate and vertebrate iridovirid genes is a highly regulated process in which the three classes of viral genes (IE, DE, and late) are synthesized in a coordinated manner. In general, IE transcripts likely encode regulatory and catalytic proteins required for the synthesis of DE and late viral gene products, whereas late mRNAs are translated to yield viral structural proteins.

Viral DNA Synthesis and Methylation

Following its synthesis in the cytoplasm and translocation to the nucleus, viral DNA polymerase synthesizes genome-sized DNA molecules. Subsequently, viral DNA is transported to the cytoplasm where the second stage of viral DNA replication takes place. In this stage, large,

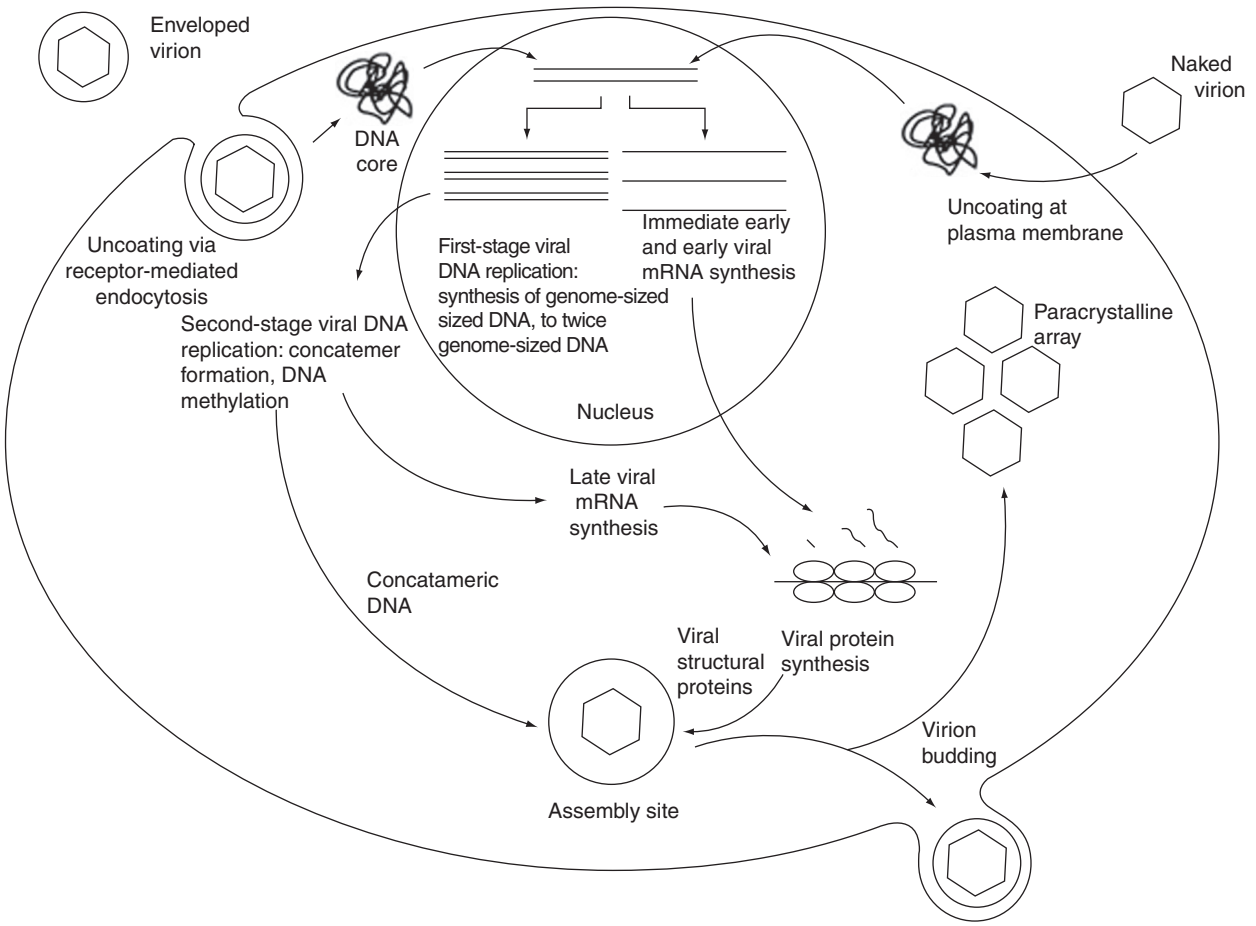


Figure 2 FV3 replication cycle. See text for details. Reproduced from Chinchar VG (2002) Ranaviruses (family *Iridoviridae*): Cold-blooded killers. *Archives of Virology* 147: 447–470, with permission.

concatameric structures are generated that contain more than 10 times the unit length of viral DNA. The mechanics of concatamer formation are not known, but likely involve multiple recombination events and may occur within viral assembly sites.

Following transport to the cytoplasm, viral DNA is methylated by a virus-encoded cytosine DNA methyltransferase. It is not known whether unit length or concatameric DNA is the template for methylation, or whether both serve equally well. The precise role of DNA methylation in the virus life cycle has not yet been elucidated. It has been postulated that methylation protects viral DNA from degradation mediated by a virus-encoded endonuclease similar to those found in bacterial restriction-modification systems. While this scenario is plausible, the rationale for encoding the endonuclease is not clear, unless one postulates that breakdown products of host cell DNA are used to synthesize viral DNA. Regardless of the reason, methylation is important for successful virus replication since treatment of cells with 5-azacytidine, an inhibitor of DNA methylation, reduces virus yields 100-fold. Analysis of viral DNA following sucrose gradient centrifugation suggests that the reduction in viral yields is likely due to the increased sensitivity of unmethylated DNA to nucleolytic attack and the inability of nicked DNA to be properly packaged into virions. An alternative hypothesis that is currently being tested suggests that viral DNA is methylated to block activation of a Toll-like receptor 9 (TLR-9) response. According to this hypothesis, unmethylated FV3 DNA, like that of herpes simplex virus DNA, binds TLR9 and triggers a pro-inflammatory response. In contrast, methylated DNA may not trigger as rapid an immune response and thus permit higher levels of virus replication *in vivo*.

Late Viral RNA Synthesis

Although early viral transcription is catalyzed by host Pol II, late viral transcription is likely catalyzed by a virus-encoded or virus-modified enzyme, designated vPol II. Supporting this view is the observation that all iridovirids sequenced to date encode homologs of the two largest subunits of Pol II which either form a unique, wholly virus-encoded polymerase, or a chimeric polymerase composed of host and viral components. It is likely that vPol II catalyzes the synthesis of late viral messages within viral assembly sites. However, it is not known if

some vPol II returns to the nucleus where it contributes to the synthesis of early transcripts, or if viral transcription remains spatially and temporally separated into nuclear (i.e., early transcription catalyzed by host Pol II) and cytoplasmic (i.e., late transcripts synthesized by vPol II) compartments. Recent studies indicate that cells treated with an antisense morpholino oligonucleotide (asMO) targeted to the largest subunit of vPol II show ~80% reduction in late, but not early, viral message expression, and >95% drop in virus yields. These results support the hypothesis that late viral gene transcription is likely catalyzed by a virus-encoded or virus-modified Pol II-like molecule.

Virion Assembly

Following synthesis, late viral proteins, which are likely structural components of the virion, are transported into assembly sites where they participate in virion formation. Unfortunately, the mechanics of virion assembly are poorly understood. Packaging of viral DNA appears to occur via a headful mechanism in which a unit length of viral DNA, plus an additional variable amount (depending upon the virus species), is inserted into the developing virion. As a consequence of this mechanism, viral DNA is terminally redundant and circularly permuted. Completed (but not yet enveloped) virions are often seen scattered within assembly sites or present within paracrystalline arrays (**Figure 3**). It is unclear whether virions are transported from assembly sites to the arrays, or whether, as virion morphogenesis continues, the original assembly site is transformed into a paracrystalline array. In contrast to the accumulation of naked virions within the cytoplasm, enveloped virions form by budding at the plasma membrane. However, most virions remain cell associated and are only released from the cell upon lysis.

Virus assembly sites are one of the most striking features seen in virus-infected cells following visualization by either fluorescent antibody staining or transmission electron microscopy (**Figure 3**). In ranavirus-infected cells, assembly sites are electrolucent areas within the

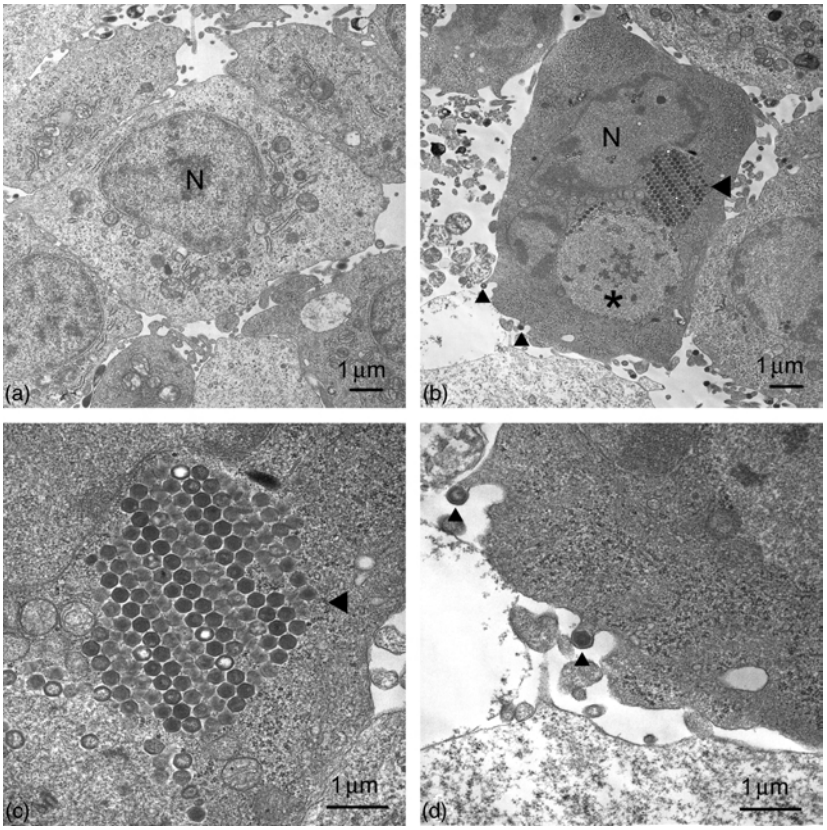


Figure 3 Electronmicrographic analysis of RCV-Z-infected FHM cells. FHM cells were infected with RCV-Z, a tentative member of the genus *Ranavirus*, at an MOI ~20 PFU/cell and fixed for electron microscopy at 9 h post infection. (a) Mock-infected FHM cells; (b) RCV-Z-infected cells showing chromatin condensation within the nucleus (N), a single large paracrystalline array within the cytoplasm (arrowhead), and a viral assembly site (asterisk); (c) enlargement of the paracrystalline array seen within (b); (d) enlargement of a virion in the process of budding from the plasma membrane. Arrowheads in (c) and (d) indicate virions within a paracrystalline array (c) or in the process of budding (d). Reproduced from Majji S, LaPatra S, Long SM, *et al*. (2006) Rana catesbeiana virus Z (RCV-Z): A novel pathogenic ranavirus. *Diseases of Aquatic Organisms* 73: 1–11, with permission from Inter-Research Science Center.

cytoplasm that are devoid of cellular organelles such as mitochondria, endoplasmic reticulum, ribosomes, or cytoskeletal elements. Assembly sites contain viruses in various stages of formation ranging from partially formed capsids to complete, nonenveloped capsids with electron-dense cores. The matrix is granular and frequently contains smooth-surfaced vesicles and diffuse electron-dense material which may be nucleic acid. FV3 assembly sites are surrounded by intermediate filaments. It has been suggested that the filaments may play a direct role in virus replication, perhaps by anchoring assembly sites and facilitating entry of viral components, or by excluding cellular organelles that might interfere with virion morphogenesis. Furthermore, the association of ranaviruses with the cytoskeleton suggests that intermediate filaments may be involved in virion envelopment and release. Disruption of intermediate filaments by treatment of FV3-infected cells with taxol or microinjection of anti-vimentin antibodies has been shown to interfere with the ability of intermediate filaments to encompass assembly sites. It has also been shown to lead to intrusion of cellular components into the assembly site, reduced accumulation of viral proteins, and 70–80% reduction in virus yields. Moreover, assembly sites are observed in the absence of late gene expression, suggesting that one or more early viral gene products and the viral DNA are sufficient for their formation.

The morphological appearance of cells from fish infected *in vivo* with megalocytiviruses such as RSIV is markedly different from the assembly site formation observed in ranavirus-infected cells. RSIV-infected splenocytes contain large inclusion bodies that are surrounded by a membrane and enclose not only the viral assembly site, but also mitochondria, rough and smooth endoplasmic reticulum, and electron-dense amorphous structures that likely contain viral DNA. Inclusion body-bearing cells are also found within the kidney, liver, heart, and gills, and are a hallmark of megalocytivirus infection. Interestingly, *in vitro* infection of cultured cells with RSIV does not result in the formation of membrane-bound inclusions, and the resulting histology is similar to that seen following ranavirus infection.

Effects of Virus Infection on Host Cell Function and Viability

FV3-infection results in a marked inhibition of cellular DNA, RNA, and protein synthesis and culminates in apoptotic cell death. The mechanisms responsible for these outcomes are slowly being resolved. Whereas the inhibition of cellular DNA synthesis appears to be a secondary consequence of the prior inhibition of host RNA and protein synthesis, the latter are likely to be the direct effect of virus infection. Inhibition of translation appears to be due to a series of events that include the synthesis of abundant amounts of highly efficient viral mRNAs, degradation of preexisting host transcripts, inhibition of host transcription, and the induction of a dsRNA-activated protein kinase (PKR) that phosphorylates and thereby inactivates eIF-2α. However, as viral translation persists in the face of host translational shutdown, FV3, like many other viruses, may encode factors that antagonize PKR-mediated effects. Most ranaviruses encode an eIF-2α homolog (vIF-2α) that, like its vaccinia virus counterpart (K3L), is thought to act as a pseudosubstrate that binds PKR, preventing phosphorylation of eIF-2α. Surprisingly, in at least one strain of FV3, vIF-2α is truncated by a deletion that removes the region homologous to eIF-2α and K3L, rendering it unable to bind to PKR. However, these strains maintain high levels of viral protein synthesis, suggesting that, as in the case of vaccinia virus, other viral proteins also play key roles in maintaining viral translation.

Apoptosis, as evidenced by DNA fragmentation, chromatin condensation, and membrane reversal, takes place in FV3-infected cells from ~6 to 9 h post infection. The immediate trigger for apoptosis is not known, but may involve PKR activation, translational shutdown, or another viral insult. Interestingly, both host cell shutdown and apoptosis can be triggered by productive infections as well as nonproductive infections with heat- or ultraviolet (UV)-inactivated virus, suggesting that a virion-associated protein is responsible. Receptor binding by virions may be sufficient to trigger these effects.

Concluding Remarks

For most of the 40 years since its discovery, FV3 has been studied, not for its impact on the infected host, but because it is the type species of a novel virus family that possesses certain features that can best (or only) be studied in this system. However, as other iridovirids are now emerging as important agents of disease in commercially and ecologically important animal species, it is likely that we will see concerted efforts to study their life cycles and, in the process, uncover novel aspects of iridovirus biogenesis. It is anticipated that the pioneering studies of the 1970s and 1980s will be extended using contemporary techniques such as asMOs and small interfering RNAs to elucidate the function of key viral proteins. In addition, study of the role of the host immune system in combating iridovirus infections in fish and amphibians may provide a way, not only to uncover potential virus-encoded immune evasion proteins, but also to elucidate key elements in antiviral immunity in lower vertebrates.

See also: Iridoviruses of Invertebrates; Iridoviruses of Vertebrates.

Further Reading

Allen MJ, Schroeder DC, Holden MTG, and Wilson WH (2005) Evolutionary history of the *Coccolithoviridae*. *Molecular Biology and Evolution* 23: 86–92.

Chinchar VG (2002) Ranaviruses (family *Iridoviridae*): Cold-blooded killers. *Archives of Virology* 147: 447–470.

Chinchar VG, Hyatt AD, Miyazaki T, and Williams T (in press) Family *Iridoviridae*: Poor viral relations no longer. *Current Topics in Microbiology and Immunology*.

Delhon G, Tulman ER, Afonso CL, *et al.* (2006) Genome of invertebrate iridescent virus type 3 (mosquito iridescent virus). *Journal of Virology* 80: 8439–8449.

Hyatt AD, Gould AR, Zupanovic Z, *et al.* (2000) Comparative studies of piscine and amphibian iridovirues. *Archives of Virology* 145: 301–331.

Iyer LM, Aravind L, and Koonin EV (2001) Common origin of four diverse families of large eukaryotic DNA viruses. *Journal of Virology* 75: 11720–11734.

Jancovich JK, Mao J, Chinchar VG, *et al.* (2003) Genomic sequence of a ranavirus (family *Iridoviridae*) associated with salamander mortalities in North America. *Virology* 316: 90–113.

Lua DT, Yasuike M, Hirono I, and Aoki T (2005) Transcription program of Red Sea bream iridovirus as revealed by DNA microarrays. *Journal of Virology* 79: 15151–15164.

Majji S, LaPatra S, Long SM, *et al.* (2006) Rana catesbeiana virus Z (RCV-Z): A novel pathogenic ranavirus. *Diseases of Aquatic Organisms* 73: 1–11.

Murti KG, Goorha R, and Chen M (1985) Interaction of frog virus 3 with the cytoskeleton. *Current Topics in Microbiology and Immunology* 116: 107–131.

Sample RC, Bryan L, Long S, *et al.* (2007) Inhibition of iridovirus protein synthesis and virus replication by antisense morpholino oligonucleotides targeted to the major capsid protein, the 18 kDa immediate early protein, and a viral homologue of RNA polymerase II. *Virology* 358: 311–320.

Stasiak K, Renault S, Demattei M-V, Bigot Y, and Federici BA (2003) Evidence for the evolution of ascoviruses from iridoviruses. *Journal of General Virology* 84: 2999–3009.

Tan WGH, Barkman TJ, Chinchar VG, and Essani K (2004) Comparative genomic analyses of frog virus 3, type species of the genus *Ranavirus* (family *Iridoviridae*). *Virology* 323: 70–84.

Williams T, Barbosa-Solomieu V, and Chinchar VG (2005) A decade of advances in iridovirus research. *Advances in Virus Research* 65: 173–248.

J

Jaagsiekte Sheep Retrovirus

J M Sharp, Veterinary Laboratories Agency, Penicuik, UK
M de las Heras, University of Glasgow Veterinary School, Glasgow, UK
T E Spencer, Texas A&M University, College Station, TX, USA
M Palmarini, University of Glasgow Veterinary School, Glasgow, UK

© 2008 Elsevier Ltd. All rights reserved.

History

Jaagsiekte sheep retrovirus (JSRV) is the causative agent of a naturally occurring lung adenocarcinoma of sheep known as ovine pulmonary adenocarcinoma (OPA, also known as jaagsiekte or sheep pulmonary adenomatosis). OPA was recognized for the first time in South Africa in the nineteenth century as a cause of dyspnea in herded sheep, hence the origin of the Afrikaans name 'jaagsiekte', meaning driving (=jaagt) sickness (=ziekte). OPA is one of the original 'slow diseases' of sheep (along with scrapie, maedi-visna and paratuberculosis) originally described in the 1930s by the Icelandic physician Björn Sigurdsson. The slow diseases of sheep were of great biological importance as they allowed, for the first time, the recognition that an infectious agent could cause clinical disease many months or years after the initial infection of the host.

Studies on JSRV were hampered for years by the lack of a suitable tissue culture system for the cultivation of JSRV. The isolation of full-length JSRV molecular clones ($JSRV_{21}$ and JS_7) allowed the *in vitro* generation of infectious viral particles by a transient transfection system, which has sparked a variety of studies that have elucidated many aspects of the molecular biology of JSRV. JSRV is a remarkable virus in many respects. It is the only known virus that induces a naturally occurring lung adenocarcinoma. In addition, the JSRV envelope glycoprotein is an oncoprotein. This is a unique example among retroviruses and oncogenic viruses in general in which a structural protein functions also as a dominant oncoprotein.

Classification

JSRV belongs to the genus *Betaretrovirus* within the family of the *Retroviridae*. Retroviruses are divided on the basis of their modality of transmission as 'exogenous' and 'endogenous' viruses. Exogenous retroviruses are horizontally transmitted between infected and uninfected hosts. Endogenous retroviruses are stably integrated in the genome of the host species from which they derive, are usually defective, and are transmitted vertically like any other Mendelian gene. JSRV is an exogenous retrovirus and it is highly related to enzootic nasal tumor virus (ENTV) of sheep (ENTV-1) and goats (ENTV-2). JSRV and ENTV have common pathogenic characteristics, and both cause low-grade adenocarcinomas of secretory cells in different portions of the respiratory tract of small ruminants. Interestingly, sheep, goats, and other members of the *Caprinae* have several copies of nonpathogenic JSRV-related 'endogenous' retroviruses (commonly referred as enJSRVs) stably integrated in their genome. The phylogenetic relationship between JSRV, ENTV, and enJSRVs is indicated in **Figure 1**.

Genetic Organization and Virion Proteins

The JSRV virions are enveloped particles of approximately 100 nm in diameter and a density by isopycnic centrifugation in sucrose gradients of 1.15 g ml^{-1} for particles obtained from cell cultures. Virions purified from lung secretions of OPA-affected sheep have a slightly higher density (1.16–1.18 g ml^{-1}).

JSRV has the typical genomic organization of a simple retrovirus; the genomic RNA of 7455 nt (in the $JSRV_{21}$ infectious molecular clone) contains the canonical retroviral genes *gag*, *pro*, *pol*, and *env* (**Figure 2**). Apart from Env, few studies have been undertaken to assign functions to the JSRV proteins and these are assumed to be the same as other retroviruses. The *gag* gene encodes the structural proteins of the viral core. Gag is expressed as an immature polyprotein that is cleaved upon exit by the viral protease. The JSRV Gag is cleaved into at least five proteins: MA

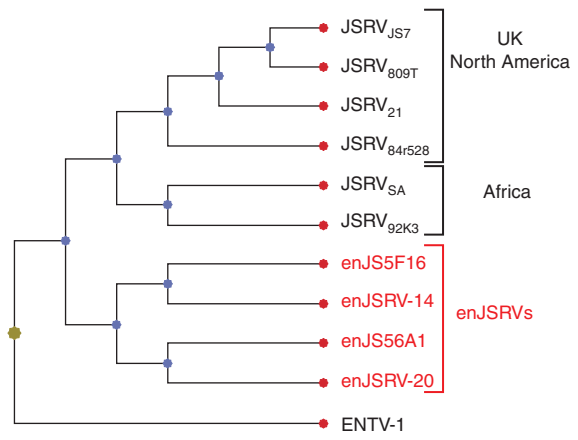


Figure 1 Phylogenetic analysis of sheep betaretroviruses. A phylogenetic tree of representative small ruminant betaretroviruses was derived by the neighbor-joining method using JSRV, ENTV-1, and enJSRVs *env* aligned using Clustal W. JSRV, enJSRVs, and ENTV-1 cluster in three distinct phylogenetic groups. Note that JSRV isolates from Africa cluster in separate branches from European and North American isolates.

(p23), p15, CA (p26), NC, and p4. MA is myristoylated and presumably interacts with the cell membrane during viral egress. CA is the major capsid protein, while NC interacts tightly with the genomic RNA although no specific studies have been conducted on the JSRV NC. The *pro* gene overlaps *gag* and encodes most probably a dUTPase (DU, deoxyuridine tryphosphatase) and the viral protease (PR). The main function of DU is to avoid misincorporation of uracil into DNA during reverse transcription (see below). As mentioned above, PR cleaves the Gag polyprotein upon exit and it is absolutely required in order to obtain mature infectious viral particles. The *pol* gene overlaps *pro* and is predicted to encode the viral reverse transcriptase (RT) and integrase (IN). Both RT and IN are virion-associated enzymes; RT copies the viral single-stranded RNA genome into double-stranded DNA, while IN serves to join the proviral DNA into the host genome. RNaseH is a subdomain of RT and serves to degrade the viral genomic RNA once has been copied into DNA.

An additional open reading frame (*orf-x*) overlaps *pol* and has some unusual features, including a codon usage different from other genes within JSRV, and a very hydrophobic predicted amino-acid sequence that shows no strong similarities to any known protein and only weak homology to a member of the G protein coupled receptor family. The role of this open reading frame is unknown. Orf-x is conserved among all of the exogenous JSRVs examined to date, but it does not seem to be required for JSRV replication *in vitro* nor for cell transformation either *in vitro* or *in vivo*.

The *env* gene encodes the glycoproteins of the viral envelope (Env). The JSRV Env is formed by two subunits, the surface domain (SU) which interacts with the cellular receptor and mediates viral entry, and the transmembrane domain (TM), which fixes SU to the lipid bilayer. A unique feature of the JSRV *env* is that it functions essentially as a dominant oncogene, and its sole expression is sufficient to induce cell transformation *in vitro* and *in vivo* (see below).

Noncoding regions are present at the 5′ and 3′ end of the genome. The R region is repeated at the 5′ and 3′ end of the genome. U5 is present uniquely at the 5′ end, and the U3 is present at the 3′ end of the genome. The viral promoter and enhancer regions are present in the U3 (see below).

Genomic sequence variability among JSRV strains is very low. For instance, the infectious molecular clones JSRV$_{21}$ and JSRV$_{JS7}$ are 99.3% identical along the entire genomic sequence, and they were derived from naturally occurring OPA cases from Scotland collected many years apart. JSRV$_{21}$ and JSRV$_{JS7}$ Env proteins are 100% identical. JSRV isolates from Africa can be distinguished phylogenetically from the UK and North American isolates, although there is still a high degree of homology (~93% along the entire nucleotide genomic sequence) among the two groups.

Replication Cycle

In general, the replication cycle of JSRV is not thought to be markedly different from other betaretroviruses. The lack of a suitable tissue culture system for the propagation of JSRV has not allowed detailed studies on the replication of this virus.

JSRV interacts with a specific cellular receptor to enter the cell. The use of retroviral pseudotypes identified hyaluronidase 2 (HYAL2) as the cellular receptor for JSRV entry. HYAL2 is a glycosylphosphatidylinositol (GPI) linked membrane protein with a low hyaluronidase activity and is widely expressed on many different cell types. Besides ovine HYAL2, the human ortholog also allows JSRV entry, while mouse and rat Hyal2 do not. Detailed steps of JSRV entry and post-entry, such as membrane fusion and the dependence or independence from acidic pH have not been investigated, but are likely to be similar to those of other retroviruses including uncoating, reverse transcription of the viral genome into double-stranded DNA, entry into the nucleus of the preintegration complex, and stable integration of the proviral DNA into the host DNA. During the process of reverse transcription, the noncoding regions at the 5′ and 3′ ends of the genome (R, U5, and U3) are duplicated and give origin to the viral long terminal repeats (LTRs).

The retroviral LTRs are major determinants of retrovirus tropism, as the 5′ LTR of the provirus initiates transcription and the U3 region contains the majority of *cis*-acting sequences interacting with the cellular RNA polymerase II and with cellular transcription factors. The exogenous JSRV LTRs are particularly active in reporter

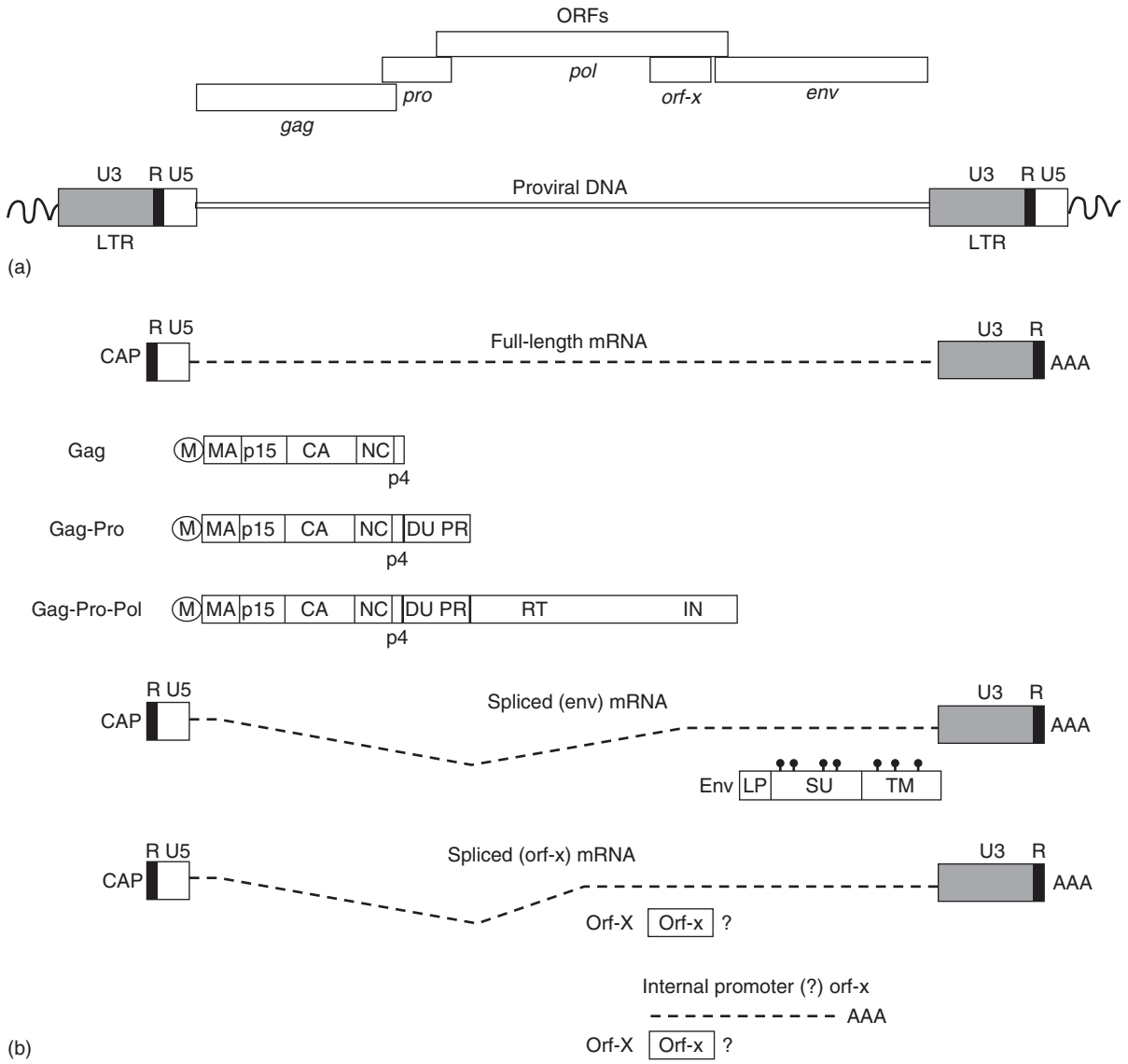


Figure 2 Genomic organization, mRNAs and viral proteins of JSRV. (a) Schematic organization of the JSRV open reading frames (ORFs) and their relative position in the JSRV provirus. The JSRV LTR, repeated at both ends of the provirus genome, is divided into U3, R and U5. (b) Major JSRV RNAs and proteins. The JSRV provirus transcribes various mRNAs. A full-length mRNA encodes for the Gag, Pro and Pol proteins and serves as genome for the newly synthesized viral particles. A spliced mRNA encodes the viral Env. The Env glycoprotein is glycosylated , and putative glycosylated sites are indicated by full circles. Two additional mRNAs have been detected and encompass the *orf-x* reading frame, but their biological significance is unknown at present. MA, matrix; CA, capsid; NC, nucleocapsid; DU, dUTPase; PR, protease; RT, reverse transcriptase; IN, integrase; LP, leader peptide; SU, surface; TM, transmembrane.

assays using type II pneumocytes/Clara cell lines and interact with lung-specific transcription factors such as forkhead box A2 (FOXA2; alias HNF-3β). These features may explain the preferential expression of exogenous JSRV in the transformed cells of the lungs, which have phenotypic characteristics of type II pneumocytes and Clara cells.

The expression of JSRV, as in all retroviruses, is believed to follow the basic transcriptional events of cellular mRNAs including capping of the 5′ end and polyadenylation at the 3′ end. JSRV encodes a full-length mRNA that serves as genome of the viral progeny and is also translated into the Gag polyprotein and the proteins encoded by *pro* and *pol*. The latter are expressed as fusion proteins with Gag. As in all betaretroviruses, *pro* and *pol* are in different open reading frames to *gag* and are expressed by ribosomal frameshifting. The viral Env is produced from a mRNA which is spliced using the splice donor in the untranslated *gag* region and a splice acceptor immediately before *env*.

Another spliced mRNA has been found to use the same splice donor of the *env* mRNA and a splice acceptor before the *orf-x* reading frame. This mRNA presumably expresses a protein encoded by *orf-x*, but there are no published data supporting this assumption. A possible mRNA expressing *orf-x* has been found to lack the R-U5 regions (typical of all other mRNAs starting from the U3) and is thought to derive from the expression of an internal promoter

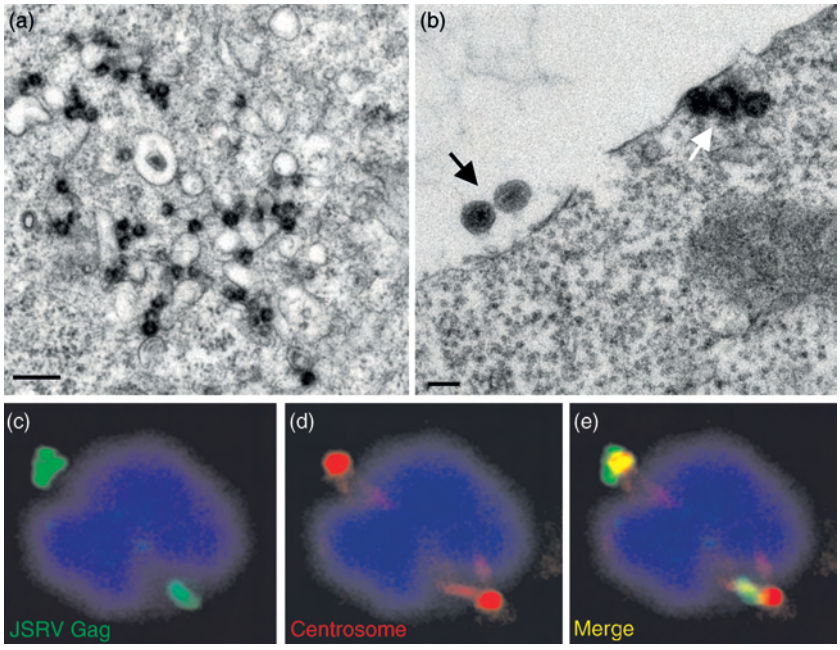


Figure 3 Electron and confocal microscopy of cells expressing JSRV. Electron microscopy in 293T cells transiently transfected with the $JSRV_{21}$ infectious molecular clone showing intracytoplasmic particles (a; scale = 200 μm). In panel b, intracellular particles in the vicinity of the cell membrane (white arrow) and extracellular particles complete with viral envelope (black arrow) are also visible (scale = 100 μm). (c–e) Confocal microscopy in HeLa cells transiently transfected with JSRV and probed with an antiserum towards the JSRV matrix (c) and γ-tubulin (d). The JSRV Gag concentrates in the pericentrosomal area (e).

that has not been characterized. Other mRNAs deriving from the use of secondary splice acceptors and non-conventional polyadenylated sites have been detected but their biological significance, if any, is unknown.

JSRV assembles in the cytoplasm (**Figures 3(a)** and **3(b)**) like all betaretroviruses, most likely in the pericentriolar region (**Figures 3(c)–3(e)**). Other retroviruses such as lentiviruses and gammaretroviruses assemble instead mostly at the cell membrane. The JSRV intracellular particles interact with Env at the cell membrane or in a cellular compartment not yet fully identified and egress from the cell. Upon exit, the Gag polyprotein is cleaved by the viral protease into the mature proteins described above. A more detailed description of the replication cycle of retroviruses is discussed elsewhere in this encyclopedia.

enJSRVs

The sheep genome contains at least 27 copies of endogenous retroviruses highly related to JSRV (hence the name enJSRVs). Endogenous retroviruses are believed to derive from integration events of ancestral exogenous retroviruses into the germline of the host. enJSRVs have a high degree of homology with JSRV. For example, $JSRV_{21}$ and enJS56A1 are 92% identical at the nucleotide level along the entire genome. Major differences are located in the U3 region, in two regions in Gag (termed variable regions 1 and 2), and in the cytoplasmic tail of the transmembrane domain of the Env (termed variable region 3). At least some of these highly divergent regions are the basis of important biological differences between JSRV and enJSRVs.

enJSRV transcripts have been detected in most tissues by sensitive reverse transcriptase polymerase chain reaction (RT-PCR) assays. However, high levels of enJSRVs (both mRNA and proteins) are found specifically in the epithelia of the genital tract of the ewe and, particularly, in the epithelia of the uterus (**Figure 4**). In the placenta, enJSRVs are expressed in the mononuclear trophectoderm cells of the conceptus (embryo/fetus and associated extra-embryonic membranes), but are most abundant in the trophoblast giant binucleate cells (BNCs) and multinucleated syncytial plaques of the placentomes. The temporal expression of enJSRVs envelope (*env*) gene in the trophectoderm is coincident with key events in the development of the sheep conceptus. Indeed, using a morpholino antisense oligonucleotide to induce loss-of-function *in utero*, enJSRVs Env knockdown caused a reduction in trophoblast outgrowth and inhibited/prevented trophoblast giant binucleate cell differentiation during blastocyst elongation and formation of the conceptus. Thus, enJSRVs have been proposed to be essential in sheep for peri-implantion growth and differentiation.

enJSRVs expression appears to be regulated by progesterone and expression of the progesterone receptor. However, the specific enJSRV loci that are transcriptionally active are not known at present. enJSRVs are also expressed in the sheep fetus, supporting the idea that sheep are tolerized toward JSRV. Indeed, JSRV-infected naïve sheep (with or without lung adenocarcinoma) have

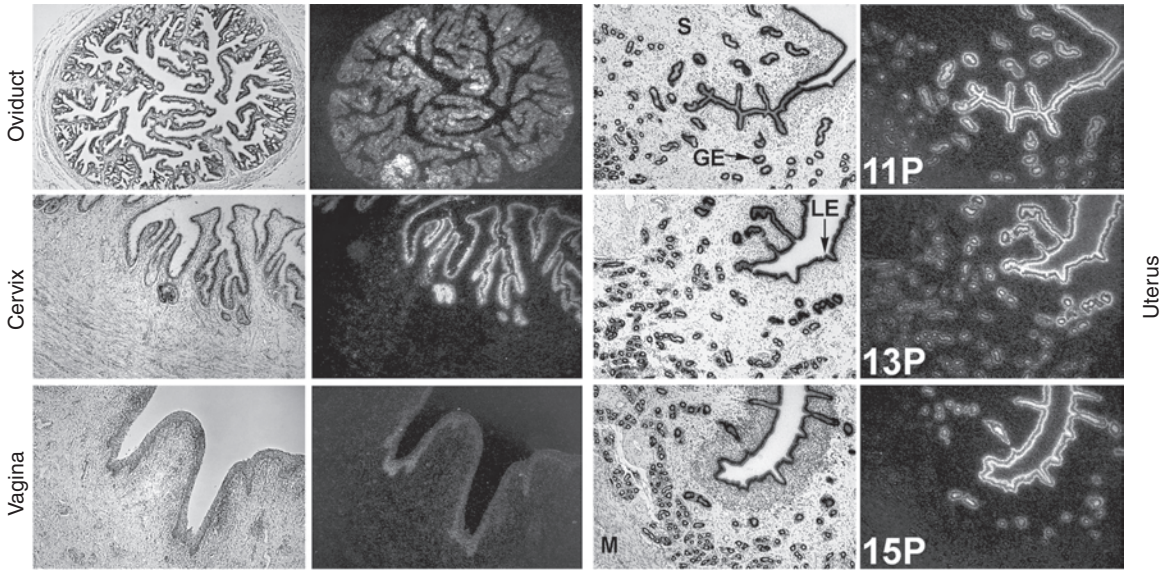


Figure 4 enJSRVs are highly expressed in epithelia of the genital tract of the ewe. *In situ* hybridization analysis of enJSRVs *env* mRNA in the oviduct, uterus, cervix, and vagina. Note the specific expression of enJSRVs *env* mRNA in the epithelia lining the oviduct, cervix, and vagina of the cycling ewe. During the estrous cycle and pregnancy (P), enJSRVs mRNA is particularly abundant in the luminal and glandular epithelia of the uterus. LE, luminal epithelium; GE, glandular epithelium; M, myometrium; S, stroma. Modified from Palmarini M, Gray CA, Carpenter K, Fan H, Bazer FW, and Spencer TE (2001) Expression of endogenous betaretroviruses in the ovine uterus: Effects of neonatal age, estrous cycle, pregnancy, and progesterone. *Journal of Virology* 75(23): 11319–11327.

no detectable specific humoral or cellular immune responses, although recombinant JSRV CA, in the presence of adjuvants, can induce antibody production and specific T-cell responses in vaccinated sheep.

Three full-length enJSRV loci have been cloned and characterized (enJS56A1, enJS5F16, and enJS59A1). All three loci have deletions or stop codons that make them replication incompetent. However, enJS56A1 and enJS5F16 maintain intact ORFs for *gag* and *env*. By using retroviral vectors pseudotyped by the enJSRVs Env, it was found that they too use HYAL2 as a cellular receptor and interfere by receptor competition with JSRV entry.

One of the enJSRVs loci, enJS56A1, is defective for viral exit when overexpressed in transfected cells, although abundant intracytoplasmic Gag is detected and intracytoplasmic viral particles are visible by electron microscopy. The replication defect of enJS56A1 is determined by its Gag protein, which is *trans*-dominant over the exogenous JSRV Gag if co-expressed in the same cell. A tryptophan residue in position 21 of the enJS56A1 Gag (replacing an arginine in JSRV) is the main determinant for the block induced by enJS56A1. Thus, enJS56A1 exerts a unique mechanism of retroviral interference, which occurs at a late step of the replication cycle. The mechanism and timing of the block induced by enJS56A1 are not yet understood. However, the observation of viral particles by electron microscopy in cells expressing enJS56A1 (or co-expressing enJS56A1 and JSRV) suggests that enJS56A1-induced interference depends on a defect in Gag trafficking. Recent studies suggest that enJS56A1 appears to block JSRV, most likely in *trans*, by hampering the ability of the latter to reach the centrosome, the proposed site of assembly for betaretroviruses.

enJSRVs are not expressed in the differentiated epithelial cells of the lungs but are expressed in the epithelium of the genital tract. As mentioned above, enhancer regions are located in the U3 and indeed enJSRVs transcription is regulated by progesterone via the progesterone receptor. Interestingly, the enJSRVs LTRs do not respond to lung transcription factors (such as FOXA2) unlike the exogenous JSRV LTRs. Thus, the LTR appears to be a major contributor to the different tropisms (genital tract vs. respiratory tract) shown by enJSRVs and JSRV.

The enJSRVs Env (or at least the Env of those enJSRVs loci cloned so far) is not able to transform cells *in vitro*, unlike the highly related JSRV Env. Indeed, the cytoplasmic tail of the JSRV Env is where major determinants of transformation are located. In particular, an SH2 binding domain is present in the exogenous JSRV Env, but is absent in the enJSRVs Env sequenced to date. It is hypothesized that enJSRVs have been selected in the sheep for their ability to confer resistance to infection of the host by the related exogenous betaretroviruses. This innate resistance could have provided a selection pressure for betaretroviruses with tropism towards the respiratory tract (i.e., the current JSRV) rather than the genital tract.

Ovine Pulmonary Adenocarcinoma

OPA is a naturally occurring lung cancer of sheep that has been reported in most sheep-rearing countries. It is absent

from Australia and New Zealand and has been eradicated from Iceland. The disease is characterized by a progressive respiratory condition caused by the growth of the lung adenocarcinoma. The tumor appears to originate from two types of differentiated epithelial secretory cells of the distal respiratory tract, the type II pneumocyte and the Clara cell, which retain their phenotype (**Figure 5**). OPA is invariably fatal once the disease is diagnosed, and affected sheep die as a result of compromised respiratory function caused by tumor enlargement or from secondary bacterial infections. The incubation period of the naturally occurring disease can be very long lasting several months to years.

Susceptibility/resistance to JSRV of some breeds has been suggested but not proved. OPA can be transmitted experimentally only with material that contains JSRV, such as lung secretions collected from affected animals or virions obtained by transiently transfecting cells with JSRV infectious molecular clones. The experimental OPA model is highly reproducible. JSRV infection can be induced in most, if not all, inoculated lambs aged 1–6 months at the time of inoculation and a high proportion of them develop clinical signs and OPA lesions. Neonatal lambs are most susceptible and, in contrast to naturally occurring OPA, the incubation period for experimentally induced OPA can be as short as a few weeks.

Although most experimentally inoculated lambs develop clinical signs and OPA lesions, under natural conditions the majority of JSRV-infected sheep do not develop OPA during their commercial lifespan. Within an endemically infected flock, lambs appear to become infected at a very early age although the routes of transmission have not been determined yet. Interestingly, most infected animals harbor the virus as a disseminated infection of their lymphoid tissues and do not show detectable pulmonary lesions. Although lymphoreticular cells appear to serve as the principal reservoir of virus infection, viral antigens are detected only rarely in this compartment where sensitive PCR assays are necessary to detect viral RNA or proviral DNA.

The short incubation period in young lambs experimentally infected with JSRV may be explained by the combination of high virus infectious doses present in the inoculum and the higher abundance of the permissive target cells (type 2 pneumocytes and Clara cells) in lambs compared to adult animals. In natural infections, the mechanisms involved in converting the stable persistent lymphoid infection in peripheral tissues to a progressive pulmonary epithelial tumor are not clear. In addition, it is not known whether and how efficiently sheep infected by JSRV, but with no neoplastic lesions, are able to transmit the virus to uninfected sheep. In an affected flock, control of OPA is very difficult as no vaccines or effective diagnostic tests are available.

Mechanisms of Virus-Induced Cell Transformation

The mechanisms used by JSRV to induce cell transformation are different from those followed by the majority of oncogenic retroviruses. Most of the tumors caused by

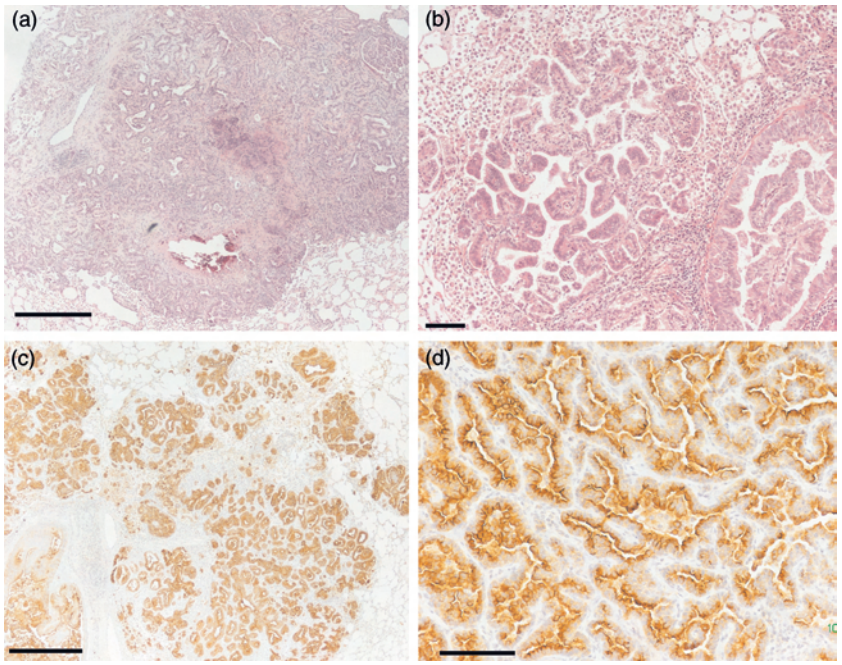


Figure 5 JSRV-induced tumours *in vivo*. (a, b) Histology from a lung tumor section of a naturally occurring case of OPA. Sections were stained with hematoxylin and eosin. Note the presence of papillary to acinar neoplastic lesions that replace the normal alveolar structure of the lungs. (c, d) Immunohistochemistry in lung sections from a JSRV experimentally inoculated lamb show expression of the viral Env in tumor cells (characterized by the intracytoplasmic brown color). Scale = 500 μm (a, c); 100 μm (b, d).

retroviruses are due to insertional activation of cellular oncogenes nearby the integrated proviruses. As a result, tumors originated by retroviral insertional activation are monoclonal or oligoclonal and common proviral integration sites are detected in tumors from different animals. Another mechanism of retrovirus transformation is transduction of cellular proto-oncogenes by the retroviral genome that are transmitted as a mixture of replication competent 'helper' viruses and replication defective viruses that have captured the oncogene. Tumors caused by retroviral transduction are in general multiclonal.

In contrast, JSRV follows neither of the mechanisms mentioned above. OPA tumors are multiclonal, and common proviral integration sites have been rarely observed. However, no cellular oncogenes have been found to be transduced by JSRV. The oncogenic properties of JSRV are due directly to one of its structural proteins.

Transfection of a variety of fibroblast and epithelial cell lines with expression plasmids for the JSRV Env leads to efficient cell transformation (**Figure 6**). Moreover, experimental infection of mice and lambs, with replication incompetent vectors expressing JSRV Env, induced tumors in the inoculated animals. Thus, the JSRV Env is a dominant oncoprotein both *in vitro* and *in vivo* and viral spread is not necessary for tumorigenesis.

The mechanisms involved in JSRV Env-induced transformation have not been fully elucidated; however, signal transduction involving the PI3K-Akt and H/N Ras-MEK-MAPK pathways are important. Conflicting reports are available on the involvement of other cellular oncogenes such as the Stk/Ron tyrosine kinase in the onset of JSRV Env-induced cell transformation.

One of the major determinants of JSRV Env transformation is the transmembrane domain (TM), although other regions may be important. In particular, a putative docking site (Y-X-X-M) for phosphatidylinositol 3-kinase (PI-3K) is critical for JSRV-Env induced cell transformation. Within this motif, Y590 is crucial for JSRV Env-induced cell transformation, although Y590 mutants maintain a reduced ability to induce cell transformation in some cell lines. In summary, the JSRV Env acts as a dominant oncogene *in vitro* and *in vivo* and its expression is sufficient to induce lung adenocarcinoma in the target species.

Enzootic Nasal Tumor Virus

ENTVs of sheep and goats are distinct betaretroviruses highly related to each other and JSRV. ENTV causes a contagious tumor of the mucosal nasal glands, as well as respiratory and olfactory mucosa, in their respective target species known as enzootic nasal adenocarcinoma (ENT). Clinically, ENT is characterized by respiratory distress caused by the enlargement of the tumor, nasal discharge, and skull deformation. Like OPA, ENT has a long incubation period.

The molecular biology of ENTV has not been studied in great detail, but the virus displays many features in common

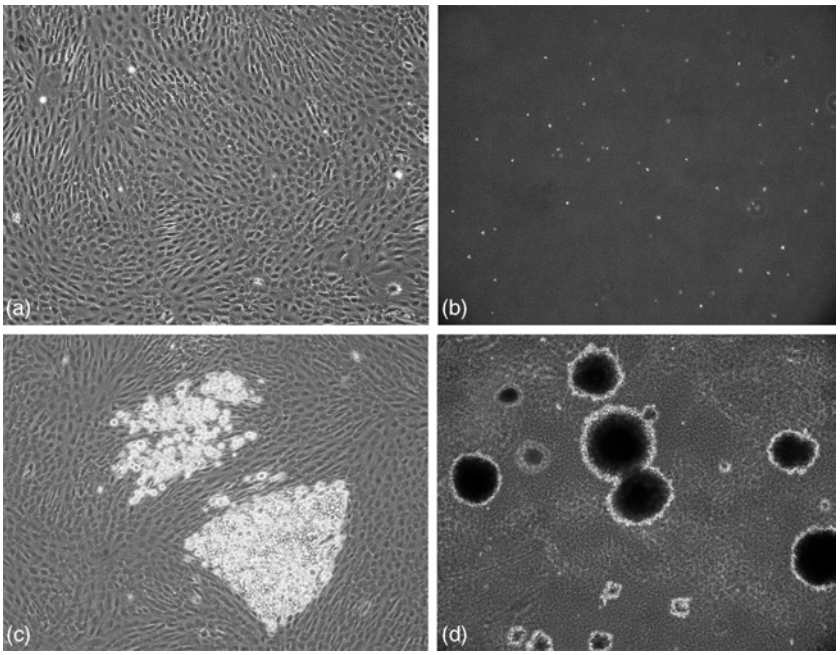


Figure 6 Expression of the JSRV Env transforms cells *in vitro*. Transformation of the rat fibroblast cell line 208F by the JSRV envelope glycoprotein. 208F cells were mock-transfected (a) or transfected with an expression plasmid for the JSRV Env (c). Mock-transfected 208F cells are morphologically flat, possess a strong contact-inhibition and do not grow in soft agar (b). On the other hand, 208F cells transfected with an expression plasmid for the JSRV Env show the onset of foci of transformed cells (c), which are able to form colonies in soft agar (d).

with JSRV. The cellular receptor for ENTV is also HYAL2 and the ENTV Env, like its JSRV counterpart, is a dominant oncogene that appears to follow the same mechanisms of cell transformation used by JSRV. The main differences between the ENTVs and JSRV appear to be concentrated in the U3 region of the viral LTRs. The ENTV LTR, unlike the JSRV LTR, does not bind lung-specific transcription factors such as FOXA2, which is likely the basis of tropism differences between the two viruses.

Future Perspectives

Small ruminant betaretroviruses are a fascinating group of viruses with unique characteristics that are of broad interest through their veterinary, comparative medical and biological importance. The veterinary importance arises from the economic impact in many sheep rearing countries of the diseases induced by JSRV and ENTV combined with the absence of any effective control tools or mechanisms. Their comparative medical interest stems from the striking similarity of some forms of human lung adenocarcinoma to OPA, which is considered an excellent outbred large animal model for these tumors with opportunities to investigate issues that are not available from other systems. Lung cancer is the main cause of death among cancer patients and effective therapeutic strategies are greatly needed to improve patient survival and well-being. OPA is a large animal model that can identify and test the efficacy of new therapeutic interventions in a highly reproducible system.

enJSRVs are an especially active group of endogenous retroviruses and offer insights into several areas of general biological interest, such as viral replication, interference, and reproductive biology. Understanding the mechanisms of the enJS56A1-induced block could inspire the design of novel anti-retroviral strategies and shed light on early events in retroviral assembly and/or trafficking. In particular, this unique viral block provides additional clues on the variety of mechanisms shaping co-evolution of endogenous/exogenous retroviruses and their hosts.

enJSRVs are highly expressed in the genital tract of the ewe and are intimately involved in early placental development in this animal species. The sheep/enJSRVs model can be useful to experimentally address the hypothesis that endogenous retroviruses have shaped and are essential for mammalian biology.

See also: Retroviruses: General Features; Simian Retrovirus D.

Further Reading

Fan H (ed.) (2003) *Jaagsiekte Sheep Retrovirus and Lung Cancer.* Berlin: Springer.

Maeda N, Palmarini M, Murgia C, and Fan H (2001) Direct transformation of rodent fibroblasts by jaagsiekte sheep retrovirus DNA. *Proceedings of the National Academy of Sciences, USA* 98: 4449–4454.

Palmarini M, Gray CA, Carpenter K, Fan H, Bazer FW, and Spencer TE (2001) Expression of endogenous betaretroviruses in the ovine uterus: Effects of neonatal age, estrous cycle, pregnancy, and progesterone. *Journal of Virology* 75(23): 11319–11327.

Palmarini M, Sharp JM, De las Heras M, and Fan H (1999) Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. *Journal of Virology* 73: 6964–6972.

Wootton SK, Halbert CL, and Miller AD (2005) Sheep retrovirus structural protein induces lung tumours. *Nature* 434: 904–907.

Japanese Encephalitis Virus

A D T Barrett, University of Texas Medical Branch, Galveston, TX, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arbovirus Virus which replicates in hematophagous insects and which then may be transmitted to vertebrates.
Flavivirus Any virus in the genus *Flavivirus*, family *Flaviviridae*.
Japanese encephalitis Disease caused by Japanese encephalitis virus.
Viremia Multiplication of virus in the blood of animals.

Introduction

Japanese encephalitis (JE) is a rural, zoonotic viral disease and is a major public health problem in many Asian countries. It is the most important of the arthropod-borne virus encephalitides and has replaced polioviruses as the major cause of human epidemic encephalitis in some parts of the world. Although a disease resembling JE was described in the late nineteenth century, the virus causing it, Japanese encephalitis virus (JEV), was first isolated in Japan in 1935. This prototype strain is known

as Nakayama. Originally, JEV was termed 'Japanese B encephalitis virus' and was classified on the basis of antigenic characteristics as a member of the group B arboviruses. Subsequently it was reclassified as JEV in the family *Togaviridae*, before being reclassified as a member of the family *Flaviviridae* (species *Japanese encephalitis virus*). The virus is transmitted between vertebrate hosts by mosquitoes. Humans, as are most vertebrates, are 'dead-end' hosts, due to production of a low viremia, such that mosquitoes cannot be infected while feeding. JE is characterized by infection of the central nervous system. There are at least 50 000 clinical cases of JE reported each year but it is thought that the actual number is much higher. The majority of cases occur in children below the age of 10 years. The case–fatality rate is 15–25% and up to 70% of those who survive infection develop neurological sequelae. In addition, JE is the most important flavivirus disease of livestock. Horses and pigs are considered to be of veterinary importance. Horses become encephalitic and are dead-end hosts, whereas JEV can induce abortion in pigs, which are considered a major amplifying host.

Virus

Japanese encephalitis virus is a species in the genus *Flavivirus*, family *Flaviviridae*. The genus contains approximately 70 viruses. JEV is a member of the JEV complex, viruses that are closely related on the basis of cross-reactivity in neutralization tests and at the nucleotide level. The JEV complex includes Cacipacore, JEV, Koutango, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, Usutu, West Nile, and Yaounde viruses. JEV replicates in a wide range of cell cultures derived from various animals and mosquitoes. Monkey kidney-derived Vero and LLC-MK2 cells usually are used for infectivity titrations, and these cell lines as well as C6-36 cells from *Aedes albopictus* mosquitoes usually are used to grow the virus.

Physical Properties

JEV virions are approximately 50 nm in diameter, icosahedral in shape, and have a lipid envelope. The envelope is derived from the host cell. Infectivity is lost after treatment with heat, detergents, lipid solvents, or acidic pH. Lipid solvents (e.g., ether and chloroform) and ionic detergents (e.g., sodium dodecyl sulfate) inactivate both infectivity and hemagglutination activity, while the milder nonionic detergents (e.g., nonidet P40, triton X-100, or X-113) only destroy infectivity. The sedimentation coefficient of the virion is approximately 200S and it sediments at a density of 1.20–1.23 g cm^{-3} in potassium tartrate-glycerol or sucrose gradients.

Virions contain three structural proteins. The small capsid (C) protein surrounds the genome of the virus and the envelope contains two proteins known as envelope (E) and membrane (M). The E protein is the viral hemagglutinin (i.e., the protein that binds to red blood cells) and contains most of the epitopes recognized by neutralizing antibodies. The E protein is the major virion protein and has one glycosylation site, at residue 154. Two types of virions are recognized; mature extracellular virions containing M protein, and immature intracellular virions containing precursor M (prM) protein, which is proteolytically cleaved during maturation to yield M protein. The genome comprises one positive-sense, single-stranded RNA molecule of *c.* 11 000 nt and is infectious. The 5′ terminus of the genome possesses a type I cap ($m\text{-}^{7}GpppAmp$) followed by the conserved dinucleotide AG. There is no terminal poly(A) tract at the 3′ terminus. The gene order is C-prM-E-NS1-NS2A-NS2B-NS3-NS4A-NS4B-NS5. There are 95 nucleotides in the 5′ noncoding region, and the single open reading frame has 10 296 nucleotides. The 3′ noncoding region is variable in length, depending on the strain.

In addition to the mature virion, two additional physical entities have been described, namely the slowly sedimenting hemagglutinin and soluble complement-fixing antigen. The former is associated with immature particles and the latter with secreted NS1 protein.

Replication Cycle

The replication cycle involves virion binding to cell receptor(s), mediated by the viral E protein. Uptake of the virion into cells is via receptor-mediated endocytosis followed by pH-dependent membrane fusion to release the virus nucleocapsid into the cytoplasm. The input virus does not contain viral RNA-dependent RNA polymerase. Thus, the positive-sense genomic RNA is translated to generate the nonstructural proteins required for replication of the virus, including the RNA-dependent RNA polymerase. RNA replication is associated with membranes and begins with transcription of the input genomic RNA to synthesize complementary negative-sense strands, which are then used as templates to transcribe positive-sense genomic RNA. The genomic RNA is synthesized by a semiconservative mechanism involving replicative intermediates (i.e., containing double-stranded regions as well as nascent single-stranded molecules) and replicative forms (i.e., duplex RNA molecules). Synthesis of negative-sense RNA continues throughout the replication cycle. All viral proteins are produced as a single polyprotein that is co- and post-translationally processed by cellular proteases and by the viral NS2B-NS3 serine protease to generate individual structural and nonstructural proteins. In addition to the three structural proteins C, prM, and E, seven nonstructural (NS) proteins

are found in virus-infected cells: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5. Few of the nonstructural proteins have been studied in detail. NS3 is a multifunctional protein whose N-terminal one-third forms the viral serine proteinase complex together with NS2B. The C-terminal portion of NS3 contains an RNA helicase domain involved in RNA replication, as well as an RNA triphosphatase activity involved in the formation of the 5′ terminal cap structure of the viral RNA. Two enzymatic activities have been assigned to NS5: the RNA-dependent RNA polymerase and the methyltransferase activity necessary for methylation of the 5′ cap structure. NS1 is an unusual nonstructural protein as it is glycosylated at two sites: residues 130 and 207. The functions of NS2A, NS4A, and NS4B are poorly understood, but current evidence suggests that NS2A, NS2B, NS4A, and NS5 are all part of the replication complex, and that NS1 is involved in RNA synthesis and virus assembly. The function in NS4B is not clear but may be an interferon antagonist. Other studies suggest that NS5 is involved in this function.

Virions are first observed in the rough endoplasmic reticulum, which is believed to be the site of virus assembly (i.e., interaction of genomic positive-sense RNA molecules with structural proteins C, prM, and E). Progeny virions assemble by budding through intracellular membranes into cytoplasmic vesicles. These immature virions (i.e., containing prM (which is thought to act as a chaperone) rather than M protein) are then transported through the membrane systems of the host secretory pathway to the cell surface where exocytosis occurs. Shortly before virion release, the prM protein is cleaved by furin or a furin-like cellular protease to generate mature virions that contain M protein. Immature virions have low infectivity compared to mature virions. Host-cell macromolecular synthesis is not shut-off during virus replication and is not decreased until cytopathic effect is evident late in the infection process.

Geographic Distribution

JEV is found throughout much of Asia. It is epidemic in temperate regions of Asia (e.g., Japan, Taiwan, People's Republic of China, Korea, eastern Russia, northern Vietnam, northern Thailand, Burma, Nepal, Sri Lanka, and India) and endemic in tropical regions (e.g., Malaysia, Indonesia, southern Vietnam, southern Thailand, and the Philippines). JE was not described in Australia until 1995, when cases of JE were reported on the island of Badu in the Torres Strait. The first human case of JE was reported on mainland Australia in 1998. This recent introduction into the Torres Straits and northern Australia is thought to be due to wind-blown mosquitoes.

Epidemiology

JE is reported in nearly all Asian countries. It is endemic in tropical areas of Asia and epidemic in temperate regions of Asia. Endemicity in tropical areas is thought to be due to the availability of mosquito vectors throughout the year, while there is seasonal incidence in temperate climates. Specifically, the disease is reported annually with seasonal (peaking in June and July during the rainy season) and age (mainly children between the ages of 1 and 15 but peaking in those 3–5 years of age) distributions. Some endemic countries that utilize childhood immunization have seen the age distribution of cases move toward older children and adults. Human infections are related to increased vector densities associated with rainfall or with irrigation practices. It is estimated that 3 billion people live in JE endemic areas. Of this population, approximately 700 million are children under the age of 15 years with an annual birth cohort of 70 million per year. The disease incidence varies greatly between countries, ranging from <10 to >100 per 100 000 population. On the basis of these figures, it is estimated that there are approximately 175 000 cases of JE per year, of which approximately 50 000 have clinical encephalitis. Only 1 in 1000 JEV infections are symptomatic. The estimated case–fatality rate is 25% (5–40% have been reported), and approximately 45% (plus reports of up to 70%) of surviving patients have neurological and/or psychiatric sequelae. These include neurological and psychomotor retardation, motor deficits, convulsions, memory impairment, optic nerve atrophy, limb paralysis, parkinsonism, and also psychological and behavioral disorders. Much of the information on neurological sequelae comes from case reports rather than detailed studies incorporating controls. Nonetheless, it appears that a large proportion of patients with neurological sequelae do recover completely over time.

The effect of concurrent infection with human immunodeficiency virus on JEV infection is unknown and there are few reports of JEV infection of pregnant women, given that the virus predominantly causes disease in children. However, there are reports from India that JEV infection can cause abortion during the first two trimesters of pregnancy.

JE appears to be age related, with the majority of patients being children and the elderly tending to have encephalitis. It is thought that this is due to the high prevalence of antibody in adults. All evidence indicates that there is one serotype of JEV and that infection provides lifelong protection from reinfection against all known antigenic and genetic variants of the virus.

Swine are important amplifying hosts of JEV. Infected pigs develop viremias sufficient to infect mosquitoes that subsequently feed on them. Importantly, adult pigs do not show clinical signs of JE. In terms of veterinary disease, infection of pregnant sows results in abortion and stillbirth

due to transplacental infection and causes aspermia in boars. Infected equids can succumb to JE and cattle seroconvert; however, both are considered dead-end hosts.

Molecular Epidemiology

Strain variation has been recognized for many years. Initially antigenic variation was detected with polyclonal antisera in neutralization, complement fixation, agar gel diffusion, and hemagglutination inhibition tests and subsequently with monoclonal antibodies. Overall, two major immunotypes of JEV have been differentiated: Nakayama and Beijing-1/JaGAr-01. Other antigenically distinct groups of strains have been identified, including those from northern Thailand and Malaysia, while studies with monoclonal antibodies suggested that at least five antigenic groups could be identified. Oligonucleotide mapping provided the first evidence for genetic variation among strains of JEV. Subsequent studies used direct nucleotide sequencing of portions of the genome to identify four genotypes (genetic clusters) of JEV. Genotype I includes isolates from Cambodia and northern Thailand; genotype II includes isolates from Indonesia, southern Thailand, and Malaysia; and genotype III includes isolates from temperate regions of Asia (Japan, China, Taiwan, India, Nepal, Sri Lanka, and the Philippines). Subsequent studies showed that isolates from Japan, Korea, and India clustered within genotype III. Genotype IV includes certain isolates from Indonesia. Interestingly, recent studies have shown that during the past 25 years, genotype I is replacing genotype III in China, Japan, Korea, and Vietnam.

Overall, molecular epidemiologic studies of JEV have identified at least five antigenic groups and four genotypes. The relationship of the antigenic groups to the genotypes is not clear at present. The practical significance of these differences has been the subject of much debate. In particular, should strains from one or more antigenic groups/genotypes be included in vaccines to give effective protection against all JEV strains found in nature (see below)? Also, there is very little information on the molecular basis of neurovirulence of JEV or on biological differences between genotypes of JEV (see below). However, it is noteworthy that JEV isolates from Thailand can be distinguished genetically: isolates from northern Thailand are of genotype I while isolates from southern Thailand are of genotype II. The incidence of encephalitis in humans in northern Thailand is 12.2 per 100 000 while it is only 0.3 per 100 000 in southern Thailand. There are many potential explanations for this situation, but genetic differences between the viruses may contribute to these epidemiologic differences. Finally, the entire genome of a number of strains of JEV has been sequenced, including representatives of all four genotypes.

Pathogenicity and Virulence

Vertebrates of many species are susceptible to JEV. The virus is lethal for newborn mice and rats by all routes of inoculation. As the animals get older, age-related resistance to disease is observed following inoculation by peripheral routes, while adult mice are still susceptible following direct inoculation of virus into the brain, that is, neuroinvasiveness decreases with age while the virus is still neurovirulent for all ages of mice. The virus causes a lethal disease in primates inoculated intracerebrally, while some strains are lethal also by intranasal inoculation; peripheral inoculation of JEV causes only asymptomatic infection.

In nature, the virus readily infects individuals of a large number of vertebrate and invertebrate species. Many vertebrates are considered 'dead-end' hosts. Horses and humans develop encephalitis, and swine have inapparent infections, while the virus causes aspermia in boars and stillbirth or abortion in pregnant swine. Persistent infections have been reported in experimental infection of pregnant mice and virus has been passed vertically to offspring.

Although it is known that the virus appears in the blood before invading the central nervous system, the host and viral determinants of disease are poorly understood. It is not known whether the virus directly crosses the blood–brain barrier or uses the olfactory nerve route to invade the brain, nor is it known why the virus targets particular regions of the brain. The cell receptor for the virus has not been identified.

Strains of the virus differ in neuroinvasiveness and this appears to be related to the level of viremia, but the exact molecular determinants of JEV that control neuroinvasion have not been identified. However, studies on the attenuated vaccine strain SA14-14-2 have shown that the E protein is a major determinant of neurovirulence in the mouse model, with residue 138 dominant, and residues 107, 176, and 279 also contributing to neurovirulence.

Clinical Symptoms

Following the bite of a JEV-infected mosquito, there is an incubation period of 4–16 days before clinical symptoms are observed. These take the form of a febrile illness with headache, aseptic meningitis, or encephalitis. The most important form of the disease is acute meningomyeloencephalitis. Onset is rapid, with 1–4 days of fever, headache, chills, drowsiness, mental confusion, stupor, anorexia, nausea and vomiting. Subsequently, patients show symptoms of nuchal rigidity, photophobia, tremors, involuntary movements, focal motor nerve impairment involving the central and peripheral nervous systems, or coma. Generalized motor seizures are seen in children.

Examination of the cerebrospinal fluids of patients may provide indicators of infection, including one or more of increased pressure, increased concentration of protein, an increased number of lymphocytes, anti-JEV IgM antibody, and virus. Fatal cases usually have respiratory complications, seizures, virus in cerebrospinal fluid, and low levels of anti-JEV IgM antibody. Pathological examination of brain tissue from fatal cases and imaging studies of patients show that infection of neurons is widespread in the central nervous system, although the thalamus, basal ganglia, and anterior horns of the spinal cord appear to be particularly involved. Microscopic lesions include perivascular inflammation, neuronal degeneration, and necrosis, rather than apoptosis. Nonfatal cases recover in 1 to 2 weeks. However, neurological and/or psychiatric sequelae are seen in a large proportion of patients who survive the acute disease (see the section titled 'Epidemiology').

Diagnosis

The virus is rarely isolated from peripheral blood during the acute stage of the disease in humans. This is thought to be due to a combination of a low viremia and clinical symptoms of the disease, which are not normally seen until after the virus has invaded the central nervous system by which time the viremia has finished. Virus can be isolated from cerebrospinal fluid early in the course of acute encephalitis, but this is consistent with a poor prognosis. Most virus isolates have been obtained from brains of patients at autopsy, or from mosquito pools. Viral antigen can also be detected by immunohistochemical techniques applied to neurons of patients at autopsy.

Many procedures have been used to detect serum antibodies against JEV: hemagglutination-inhibition, complement fixation, immunofluoresence, and enzyme-linked immunosorbent assay (ELISA). It is necessary to show at least a fourfold increase or decrease in titers of antibody to JEV between paired serum samples for any of these tests to be used to make a presumptive diagnosis of JE. Such a 'presumptive' diagnosis is required because of the extensive serological cross-reactions with antibodies against other flaviviruses, and all such serodiagnoses must be confirmed by neutralization tests. Many other flaviviruses overlap geographically with JEV, including the dengue and West Nile viruses, and can result in misinterpretation of test results. Detection of IgM antibodies is considered a relatively (JE complex-specific) specific test for JEV infection and is the serologic method of choice. An IgM-capture ELISA is usually used to detect IgM antibody to JEV in blood or cerebrospinal fluid within 7 days of onset of clinical symptoms. Recently, a dot-blot IgM assay has become available. Detection of IgM antibody in cerebrospinal fluid is associated with clinical JE and is predictive of a poor outcome.

Transmission

The virus is transmitted between vertebrate hosts by mosquitoes. The natural cycle involves rice-field-breeding mosquitoes and domestic pigs or wading ardeids (e.g., egrets and herons). The most important vector is *Culex tritaeniorhynchus*, found in most parts of Asia and which breeds in water pools and flooded rice fields. The virus also has been demonstrated to infect mosquitoes of other species, including *Cx. fuscocephala*, *Cx. gelidus*, *Cx. pipiens*, *Cx. bitaeniorhynchus*, *Cx. epidesmus*, *Cx. vishnui*, *Mansonia uniformis*, *M. bonneae/dives*, *Aedes curtipes*, *Ae. albopictus*, *Armigeres obturans*, *Anopheles hyrcanus*, and *An. barbirostris*. In temperate regions, the 'JE season' is considered to start in April–June with detection of virus in mosquitoes; this peaks in July. During July and August, virus is detected with increasing frequency in pig and bird amplifying hosts. The 'season' usually ends in September–October. Human infections are concurrent with the increased frequency in amplifying hosts. The exact timing of the 'JE season' will vary depending on geographic location in Asia, rainfall, and migration of birds. It is not clear how JEV survives between 'JE seasons' in temperate areas. There is no evidence that JE epidemics follow heavy rains, major floods, etc.; intervals between outbreaks vary from 2 to 15 years. There is evidence to support vertical transmission by *Culex* and *Aedes* spp. mosquitoes, sexual transmission between male and female mosquitoes, and a potential role for migratory birds in long-distance movement of JEV. However, none of these possibilities has been conclusively demonstrated.

Culex tritaeniorhynchus preferentially feeds on animals other than humans. Consequently, high seroprevalence rates are seen in a wide range of animals including dogs, ducks, chickens, cattle, bats, water buffaloes, donkeys, monkeys, snakes, and frogs. The role, if any, that these animals play in the ecology of JEV is not known. However, birds and pigs are considered to be the major viremic amplifying hosts of the virus. Among birds, ducks, chickens, water hens, egrets, and herons seroconvert.

Swine are important amplifying hosts in the epidemiology of the virus. Infected pigs develop sufficiently high viremias to infect mosquitoes. Importantly, adult pigs do not show clinical signs of JEV infection.

Infected equids can succumb to JE, while cattle seroconvert; however, both are considered dead-end hosts as viremias are too low to infect mosquitoes.

Treatment

There are no antiviral treatments to control flavivirus infections; rather, supportive therapy is the norm.

Control

As with most mosquito-borne diseases, transmission of JEV can be blocked by mosquito control measures. In addition, immunization of pigs, a major vertebrate amplifying host, blocks the transmission cycle. However, given that JE is usually found in rural areas, human immunization is the method of choice. Although vaccination is used to control JE, socioeconomic development of Asian countries is also contributing to control. Smaller areas of rice fields, centralized pig production, use of agricultural pesticides, and reduction of the rural population at the expense of increased urbanization have all contributed to a decrease in cases of JE.

There are licensed vaccines available to control JE. Inactivated vaccines are based on strains Nakayama, Beijing-1, or P3. The former two are based on formalin-inactivated mouse brain preparations that are semipurified to remove brain materials while strain P3 is also formalin-inactivated, but grown in primary hamster kidney cell cultures in the People's Republic of China. Inactivated mouse brain-derived vaccines are approved by the World Health Organization for international use. The above vaccines require two doses given on days 0 and 7–28 to induce protective immunity with a booster dose at 1 year, and subsequently every 3–4 years to maintain immunity. Initial formalin-inactivated mouse brain vaccines were based on strain Nakayama; however, in 1989, this strain was replaced with strain Beijing-1 in most markets because the latter is antigenically closer to recent Japanese isolates of JEV, a high potency being retained following purification, and immunogenicity is superior to that of strain Nakayama. This resulted in a vaccine that required a dose equivalent to half that needed for strain Nakayama vaccines. Inactivated vaccines are manufactured in India, Japan, Republic of Korea, People's Republic of China, Thailand, Taiwan, and Vietnam. The mouse-brain inactivated vaccine has been reported to cause occasional adverse events (estimated to be one in a million doses), including allergic mucocutaneous reactions. The majority of the reactions take place after the second or subsequent dose of vaccine. There were a number of adverse events reported during the period 1989–92 of which 15% were hospitalized and two-thirds required medical treatment. In addition, there have been occasional reported cases of acute disseminated encephalomyelitis since 1983. Studies to date suggest there is no reduced seroconversion rate or an increase in adverse events when mouse brain-derived JE vaccine is given simultaneously with vaccines against measles, DPT, and polio. Due to the adverse events, consideration is being given in Japan to employ an inactivated vaccine produced in Vero cells, and this second-generation vaccine is undergoing clinical trials to demonstrate noninferiority of immunogenicity compared to the mouse brain product.

There are no contraindications to the use of this vaccine, other than a history of hypersensitivity reactions to previous doses. However, vaccination is not recommended during pregnancy and pregnant women are only vaccinated when at high risk of exposure to the infection. Mouse brain-derived vaccine has been given safely in various states of immunodeficiency, including HIV infection.

There is also a live Japanese encephalitis vaccine based on strain SA14-14-2 grown in primary hamster kidney cell culture. Until recently, this vaccine was only licensed for use in the People's Republic of China. However, the World Health Organization has now developed criteria for the production of the vaccine, and it is now licensed in Nepal, India, and Republic of Korea, and clinical trials are being undertaken in Sri Lanka, the Philippines, Thailand, and Indonesia. This vaccine appears to be very efficacious and over 300 million doses of this vaccine have been administered in the People's Republic of China since 1988 with no known reports of serious adverse events.

The success of human vaccination has been demonstrated by the decrease in the number of cases in Japan. Prior to 1966, there were 1000–5000 cases per year with mortality up to 50%. Following the introduction of vaccination, the number of cases has steadily decreased to the point where fewer than 10 cases have been reported each year during the 1990s. A similar situation has taken place in the Republic of Korea and Taiwan, and all three countries have reported a shift from cases in children to cases in adults, particularly in the elderly. Since the vast majority of JE cases are children, vaccination has focused on children.

Vaccination is used to reduce the incidence of abortions in JEV-infected pigs. A live vaccine has been used in the People's Republic of China to protect horses.

The mechanism of protective immunity is poorly understood for most flaviviruses, although production of neutralizing antibodies ($\geq$1:10) appears to correlate with immunity. Most neutralizing antibodies recognize epitopes of the E protein. Significantly, in passively immunized mice, detectable neutralizing antibodies will protect against a wild-type challenge administered by the intracerebral route. Cell-mediated immunity, in particular T-cell epitopes, have been mapped to nonstructural proteins, in particular NS3.

See also: Flaviviruses: General Features; Flaviviruses of Veterinary Importance; Vaccine Safety; Vaccine Strategies.

Further Reading

Burke DS and Leake CJ (1988) Japanese encephalitis. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. 3, pp. 63–92. Boca Raton, FL: CRC Press.

Burke DS, Tingpalapong M, Ward GS, Andre R, and Leake CJ (1986) Intense transmission of Japanese encephalitis to pigs in a region free

of epidemic encephalitis. *Japanese Encephalitis and Haemorrhagic Renal Syndrome Bulletin* 1: 17–26.

Chen W-R, Tesh RB, and Rico-Hesse R (1990) Genetic variation of Japanese encephalitis virus in nature. *Journal of General Virology* 71: 2915–2922.

Halstead HB and Jacobson J (2003) Japanese encephalitis. *Advances in Virus Research* 61: 103–138.

Halstead SB and Tsai TF (2004) Japanese encephalitis vaccines. In: Plotkin SA and Orenstein WA (eds.) *Vaccines,* 4th edn., pp. 919–958. Philadelphia: W B Saunders.

Huang C (1982) Studies of Japanese encephalitis in China. *Advances in Virus Research* 27: 72–100.

Mackenzie JS, Deubel V and Barrett ADT (eds.) (2002) *Japanese Encephalitis and West Nile Viruses, Vol. 267*: *Current Topics in Microbiology and Immunology*, pp. 1–416. Vienna: Springer.

Mackenzie JS, Gubler DJ, and Petersen LR (2004) Emerging flaviviruses: The spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nature Medicine* 10(supplement): S98–S109.

Solomon T (2003) Recent advances in Japanese encephalitis. *Journal of Neurovirology* 9: 274–283.

Solomon T and Winter PM (2004) Neurovirulence and host factors in flavivirus encephalitis – Evidence from clinical epidemiology. *Archives of Virology Supplement* 18: 161–170.

K

Kaposi's Sarcoma-Associated Herpesvirus: General Features

Y Chang and P S Moore, University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Kaposi's sarcoma (KS) was first described by Moritz Kaposi in 1872 as a rapidly fatal, 'idiopathic, pigmented sarcoma' of the skin. Although initially associated with men of Eastern European, Ashkenazi Jewish and Mediterranean ancestry, by the 1950s KS was recognized to be the third most common malignancy in sub-Saharan Africa. The unusual geographic distribution of this cancer led to speculation about a viral cause, speculation that intensified with the onset of the acquired immune deficiency syndrome (AIDS) epidemic in the early 1980s. AIDS patients were 100 000-fold more likely to develop KS than the general population and the tumor took on a more aggressive and frequently lethal form in AIDS patients.

Two epidemiologists, Harold Jaffe and Valerie Beral, examined patterns among AIDS surveillance data and concluded that KS is caused by a viral agent other than human immunodeficiency virus (HIV) and that the agent is uncommon in the general population but sexually transmitted efficiently among gay men. These predictions were later found to be strikingly accurate. Over 20 possible culprits were described as 'the KS agent'. As early as 1972, Giraldo and colleagues suggested that human cytomegalovirus might cause KS. These findings were discounted after further study, but in 1984 Walter and colleagues succeeded in directly observing herpesvirus particles in KS tumor specimens, thus reviving the possibility that KS is a herpesvirus disease.

In 1993, Chang and Moore used representational difference analysis to isolate two small DNA fragments from a KS lesion similar to, but distinct from, known herpesvirus sequences. Collaborating with Ethel Cesarman, they were then able to show that this putative new virus was present in virtually all KS tumors, was more likely to be found at the tumor site than distal tissues, and was not present in control tissues from patients without AIDS. The virus was given the common name Kaposi's sarcoma-associated herpesvirus (KSHV) and recognized to be the eighth human herpesvirus (HHV-8).

A B-cell lymphoma, called primary effusion lymphoma (PEL), was quickly identified as harboring KSHV. These lymphomas were known to be common secondary malignancies among KS patients, and isolation of PEL cells allowed the first *in vitro* culture of KSHV. These early cell lines were coinfected with both Epstein–Barr virus (EBV) and KSHV, but nonetheless accurate first generation serologic assays were developed using them. A second neoplastic disorder called multicentric Castleman's disease (MCD) was also recognized to be frequently infected with KSHV by Soulier and colleagues.

Once the virus was identified, its characterization proceeded rapidly. Studies demonstrated that all forms of KS, both HIV-positive and HIV-negative, are uniformly infected with KSHV. Extensive HIV/AIDS cohort databanks were used for serologic test development and showed that KSHV infection preceded onset of disease. These early studies also revealed that KSHV infection is uncommon in the general population of the USA and Europe – despite technical confusion resulting from highly cross-reactive assays – and patterns of infection mirror KS tumor rates among high risk populations, following closely the Beral-Jaffe predictions for the KS agent. Parravincini and colleagues showed that KS arising among transplant patients could either be due to reactivation of preexisting infection or to *de novo* infection from the organ allograft, helping to explain the association of this tumor with transplantation.

Virologic characterization was equally rapid. In 1996, Ganem's group isolated a KSHV-infected PEL cell line free of EBV infection, and performed the first studies on viral replication. While KSHV had been previously cultured *in vitro* in EBV-coinfected cell lines, this was a major breakthrough allowing unambiguous characterization of the virus. That same year, Chang and Moore finished sequencing the 165 kbp viral genome, almost exactly 2 years after reports of its initial discovery. These studies revealed that the virus makes extensive use of molecular piracy, encoding homologs to multiple known cellular oncogenes. Shortly thereafter, research groups

identified KSHV inhibitory proteins targeting p53 and retinoblastoma protein, interferon regulatory proteins, and KSHV paracrine-signaling molecules regulating cell growth control, providing key molecular clues on how this virus might initiate tumorigenesis.

Shortly after its initial description, KSHV was widely recognized to be the infectious cofactor causing KS and related diseases. With the onset of the AIDS epidemic, KS is now the most common tumor reported in sub-Saharan Africa, resulting in an enormous, unappreciated morbidity and mortality. In contrast, the introduction of highly effective retroviral therapy into developed countries in the mid-1990s has reduced KS incidence among AIDS patients by nearly 90% – a remarkable public health success story.

Taxonomy, Classification and Evolution

KSHV is a gammaherpesvirus (subfamily *Gammaherpesvirinae*) belonging to the genus *Rhadinovirus*, which is distinct from the genus *Lymphocryptovirus* containing EBV (**Figure 1**). While rhadinoviruses are distributed among both primates and nonprimates (e.g., mice, cattle, and horses), EBV-like viruses are found exclusively among primates.

The genus *Rhadinovirus* contains three evolutionarily distinct lineages of primate viruses. One lineage is found among New World monkeys and two parallel but distinct lineages (including KSHV) infect Old World primates including humans. KSHV was first recognized to be closely related to the New World squirrel monkey virus, herpesvirus saimiri. In the search for KSHV-like viruses among nonhuman primates, similar viruses were found among rhesus macaques as well as baboons, gorillas, and chimps, suggesting an evolutionary codivergence for these viruses with their hosts.

A second rhadinovirus lineage found in rhesus macaques (and hence commonly called rhesus rhadinovirus, RRV) was quickly extended to other Old World primate hosts. The importance of this finding is that no human RRV-like virus has been described. If it exists, and evolutionary evidence strongly suggests that it might, then it would become HHV-9.

Molecular epidemiology studies based on variation of the K1 gene and the right-hand end of the genome reveal at least four unique viral clades called A–D that largely match patterns of human migration from Africa. There are several conserved alleles of the right-hand end of the genome (called M, N, O, and P) found across clades suggesting that these viruses are chimeric and have arisen by recombination.

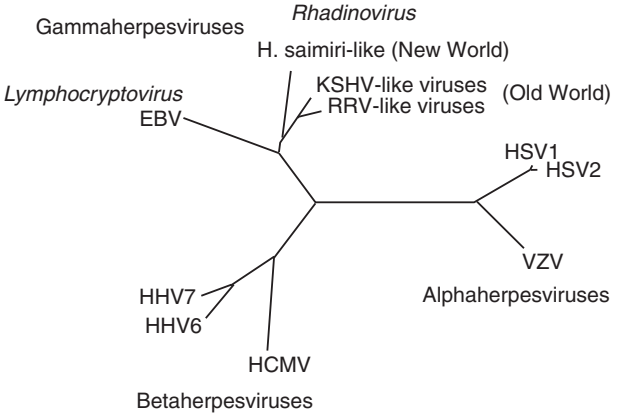


Figure 1 Relationship of KSHV to other herpesviruses. KSHV belongs to a lineage of rhadinoviruses infecting Old World primates. A second rhadinovirus lineage, exemplified by rhesus rhadinovirus (RRV), has been discovered infecting Old World primates. No human infections with an RRV-like virus have been described.

Geographic Distribution

Unlike most human herpesviruses, KSHV is not a ubiquitous human infection. KSHV infection is hyperendemic throughout sub-Saharan Africa with estimated adult infection rates of 30–60%. Other isolated populations, including indigenous Amazonian tribes, may also have infection rates at this level or higher. The prevalence of infection declines among Mediterranean basin countries such that general adult infection rates in Sicily average about 10% and continue to decline at more northern latitudes in Italy. In the USA and Northern Europe, approximately 2% of blood donors are KSHV infected. While general infection rates are low in the USA and Europe, gay men are recognized to have a rate of infection 10–20 times higher than heterosexuals.

Transmission

In the absence of underlying immunosuppression, KSHV infection appears to be asymptomatic, or perhaps associated with transient fever and rash. KSHV is recognized to have several patterns of transmission, and modes of transmission differ dramatically between developed and developing countries. It is secreted from the oral mucosa through salivary secretions. In developed countries, there is a strong sexual component to transmission among gay and bisexual men but the precise sexual behavior responsible for transmission is unknown. Two possibilities include deep kissing and use of saliva as a sexual lubricant. In contrast, heterosexual transmission is apparently rare.

In developing countries, and possibly endemic regions of Europe and the Middle East, casual transmission is common. Again precise mechanisms of transmission are

unknown, but KSHV transmission is much more efficient in sub-Saharan Africa where, unlike Northern Europe and the USA, significant rates of infection occur among children and adolescents. While intrauterine transmission and transmission through breast milk is uncommon, maternal-child transmission does occur during infancy and early childhood.

Of increasing concern, KSHV is also transmitted both through organ allografts and through blood transfusion. KS arising during transplantation is an important clinical problem with rates among all transplant patients between 0.2% and 6% depending on the geographic locale and background endemicity for viral infection. KS in this setting has a high mortality and morbidity since primary treatment involves removing immunosuppression. The bulk of KS among transplant recipients derives from reactivation of preexisting, quiescent infection which subsequently flares during immunosuppression, but transmission from donor allografts has been documented. Interestingly, one study has demonstrated that KS tumor cells rather than virus itself can be transmitted during transplantation.

Blood transfusion has in the past been considered a minor component of KSHV transmission. Several studies have shown a relationship between non-AIDS KS and past transfusion history, but this relationship has been obscured by the overwhelming risk of sexual transmission among HIV/AIDS patients. Transfusion transmission has recently been documented to occur among a fraction of infected blood samples. Whether a public health response to this is needed remains unknown.

Host Range and Virus Propagation

KSHV is grown in the laboratory using PEL-derived cell lines and can be activated into lytic virus production by treatment with chemicals such as protein kinase C activators or histone deacetylase inhibitors. The virus cannot be maintained in cultured cells from KS tumor explants and is rapidly lost within a few passages.

KSHV can, however, be readily transmitted to a range of human and nonhuman cell lines in culture, although it is lost unless it is kept under some form of selection. The virus is commonly transmitted in culture to endothelial cell lines, which undergo transformation into a spindle growth pattern resembling KS. Receptors for virus binding and entry include extracellular heparan, integrin $\alpha 3\beta 1$, the cystine transporter xCT, and DC-SIGN. It is not yet clear which of these receptors act in concert or independently of each other to allow different steps of binding, internalization, and entry. Transmission to nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice has been documented, although the consequences of infection have not been fully examined.

Although whole virus transmission to animal models is limited, single gene expression studies have proved valuable. The K1 protein, for example, has been placed into a herpesvirus saimiri backbone and caused lymphatic tumors in rhesus macaques. Many KSHV genes, including those encoding viral interleukin 6 (vIL6), viral interferon regulatory factor 1 (vIRF1), and kaposin, transform rodent cell lines and cause tumors in mice. In addition, expression of the viral G protein-coupled receptor (vGPCR) in transgenic mice results in paracrine development of endothelial cell tumors that closely resemble human KS tumors.

Virus Genetics and Molecular Biology

KSHV is a ~165 kbp double-stranded DNA virus with a long unique region (LUR) containing all protein-coding sequences that is flanked by high G + C content direct terminal repeats (TRs) on both ends. The TRs are 801 bp in length each, with 20 or more units in a typical virus, and are the site for circularization (latency) or linearization (lytic replication). One KSHV protein, latency-associated nuclear antigen 1 (LANA1), acts similarly to a corresponding EBV protein, EBNA1, to bridge between the viral episome and host cell chromosomes during latency, allowing equal segregation of virus to daughter cells. During latent replication, in which the virus is in a circular state, the origin of DNA replication is located within the TR region.

The LUR encodes over 90 independent protein-coding open reading frames (ORFs), and alternative splicing may markedly increase the expressed number of viral proteins. During latency, the KSHV genome is largely quiescent with only a few genes constitutively expressed, primarily from the right-hand latency locus and including LANA1, viral cyclin (vCYC), viral FLICE-inhibitory protein (vFLIP), and approximately 12 micro-RNAs.

KSHV, like other herpesviruses, has a highly choreographed cascade of gene expression during lytic replication. Lytic replication is initiated by a single transactivator protein, RTA, expressed from ORF50. This leads to sequential early, delayed-early, and late gene expression. Ultimately, the viral polymerase is expressed and linear KSHV chromosomes are replicated through a rolling circle mechanism.

KSHV genes are commonly divided into 'latent' and 'lytic' classes based on whether or not they are activated during the lytic replication cycle. This is clearly too simplistic a categorization to be meaningful. A large group of nonstructural protein genes have long been known to be expressed at low levels during viral latency but are upregulated during lytic replication (so called type II genes). Notch signaling, which activates the transcription factor RBP-Jk that acts in concert with RTA to initiate lytic

replication, has recently been found to activate expression of these genes without late gene expression or virion production. Increased notch signaling is present in PEL cells and appears to be responsible for low level expression of type II genes during viral latency.

Molecular Piracy by KSHV

Rhadinoviruses show the most extensive piracy of host genes among the herpesviruses. KSHV genes that have been directly acquired from the host genome include those encoding proteins involved in DNA synthesis (thymidine kinase, dihydrofolate reductase, thymidylate synthase, ribonucleotide reductase subunits, and formyglycinamide ribotide amidotransferase (FGARAT)), deoxyuridine metabolism (dUTPase), paracrine signaling (viral complement control protein, vIL6, viral chemokine ligands 1, 2 and 3 (vCCL1, vCCL2, and vCCL3)) and vGPCR, interferon signaling (vIRF1, vIRF2, and LANA2), major histocompatibility class (MHC) regulation (modulator of immune recognition 1 and 2 (MIR1 and MIR2)), and cell cycle (vCYC) and apoptosis control (vBCL-2, vFLIP, and viral inhibitor of apoptosis (vIAP)). These proteins frequently fall into the type II expression pattern described above.

All of these proteins have recognizable sequence similarity to their human counterparts and appear to act in the same regulatory pathways. The primary difference between the viral and human homologs is at the level of protein regulation, in which the viral protein generally escapes from normal regulatory controls imposed on the human protein. vCYC, for example, targets the retinoblastoma protein for phosphorylation by recruiting cellular cyclin-dependent kinases. This allows the viral protein to initiate unscheduled S-phase entry of the cell cycle. vCYC resembles cellular D-type cyclins but differs in a key characteristic in that it is resistant to cyclin-dependent kinase inhibitors that control cellular cyclin function. Expression of these proteins, together with viral proteins that have no cellular counterparts, such as LANA1, is thought to be responsible for the tumorigenic capacity of this virus.

While the range of these homologous nonstructural proteins is unique to KSHV among the human herpesviruses, careful inspection reveals that the functions of these proteins are similar to those found in other viruses. There is a close functional correspondence between latent EBV proteins and latent KSHV proteins despite the fact that there is little or no sequence similarity. In general, EBV nonstructural proteins act as signaling molecules to activate specific cellular pathways, whereas KSHV has pirated members of these pathways to act in a similar fashion.

Immune Response

Infection with KSHV generates a strong antibody response that can be measured by indirect immunofluorescence assays (IFA), ELISA, and western blotting. In the absence of lytic stimulation, the nuclear antigen LANA1 commonly elicits antibody titers exceeding 1:50 000. This protein migrates aberrantly as a 223–234 kDa doublet band on western blotting. While LANA1 immunoreactivity is highly specific for KSHV infection, approximately 20% of AIDS-KS patients do not have immunoreactivity. For this reason, attempts to increase test sensitivity using lytic genes have been made. Whole cell lytic immunoassays can be highly sensitive but have demonstrated cross-reactivity that can be measured using adsorption with formalin-fixed KSHV-negative cell line antigen. Use of lytic cell assays led to considerable confusion in early studies about the prevalence of KSHV infection in various low-risk populations.

Two recombinant structural antigens, the ORF65 and K8.1 proteins, have been valuable in maintaining high sensitivity with low levels of cross-reactivity. Assays based on these antigens have been further refined using peptide epitopes in ELISA assays. Together with a recombinant LANA1, ELISA sensitivity and specificity of greater than 90% for KSHV detection can be routinely achieved. Complicating the serologic detection of KSHV is the fact that much of this work is based on patients with end-stage AIDS-KS. These patients routinely lose antibody reactivity due to loss of CD4+ helper activity.

The surge in KS among AIDS patients revealed the importance of cellular immunity, particularly CD4+ based immunity, to KS tumor control. Cell-mediated immunity assays have measured cellular immune responses against a number of KSHV protein epitopes, including those found on both structural and latent antigens. Among AIDS patients, these responses closely follow regression of KS tumors during highly active antiretroviral therapy (HAART).

Surprisingly, cellular immunity to the constitutively expressed LANA1 protein is generally low. The corresponding EBV protein, EBNA1, escapes immune surveillance by inhibiting its own proteosomal degradation and retarding its own translational synthesis. The latter effect appears to inhibit the formation of misfolded EBNA1 defective ribosomal products that are rapidly processed into MHC class I (MHC I) presented peptides. A similar mechanism seems to be at work for KSHV LANA1. Although the two proteins are not homologous, the nucleotide sequence of a central repeat region of both viral proteins is similar but is frameshifted between the two viruses. It remains to be seen whether the nucleotide sequence or the individual protein sequences are responsible for this immunoevasion effect.

KSHV also evades MHC I immune processing by expressing two proteins, MIR1 and MIR2, that ubiquitinate MHC I and other immune accessory proteins, causing them to be internalized and degraded. Although MIR1 and MIR2 are generally expressed during lytic replication, MIR1 is expressed at low levels during latency and can be upregulated by notch signaling.

In addition to cell-mediated immune response evasion, KSHV encodes a number of proteins that act to overcome innate immune signaling pathways. vIRF1 and the ORF45 protein act to sequester interferon-regulated transcription factors, effectively shutting off interferon signaling during infection. vIL6 signals in a similar manner to human IL-6 but it can bind directly to the signal transducer molecule gp130 to initiate signaling. One consequence of this effect is to abrogate signaling from interferon receptors to downstream signaling machinery.

KSHV-Associated Diseases

Kaposi's Sarcoma

KS is an endothelial tumor having molecular markers suggesting that it originates from lymphatic endothelium (**Figure 2**). Microscopically, the tumor is characterized by tumor spindle cells forming disorganized vascular clefts that fill with blood cells, giving the tumor a bruised like appearance. KSHV is detected in virtually all spindle cells, but tumors also show active neoangiogenesis in which more organized vessels lace the tumor mass. KSHV is generally absent from these cells and the intrusion of neovascular and immune cell components give the tumors a mixed cellular origin. Thus, the pathology of KS strongly suggests that the tumor has both neoplastic and hyperplastic components. Conflicting data exist as to whether or not KS tumors are cellularly monoclonal, and studies of viral TRs suggest that KS tumors may originate from both polyclonal and monoclonal infection. It is likely that tumor cells evolve from polyclonal populations and gradually evolve into monoclonal or oligoclonal entities over time, helping to explain conflicting studies on the origin of the tumor.

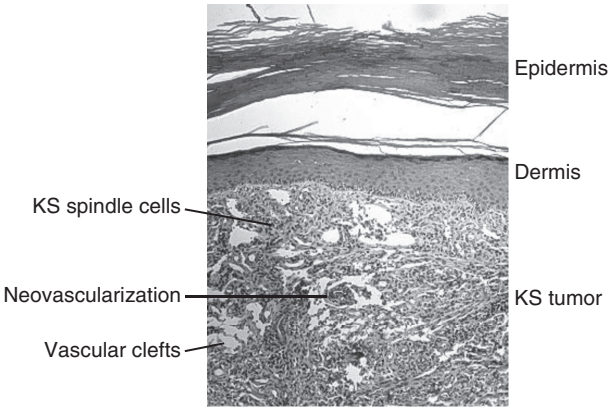


Figure 2 Photomicrograph of a KS tumor showing spindle tumor cells forming irregular vascular clefts that fill with blood. While near-universal KSHV infection of spindle cells is generally seen, the virus is generally absent from the neovascularization that can also be present in tumors. Frequently an inflammatory infiltrate is also seen in KS tumors.

Similarly, there is mixed evidence for the virus contributing to KS tumor growth through virus-transforming and paracrine activities. In tumors, virtually all tumor spindle cells are infected with KSHV, suggesting that the virus drives cell multiplication through intracellular expression of specific oncoproteins while the overwhelming majority of tumor cells are in a tight latency expression pattern. In line with this, antiherpesvirus drugs, such as ganciclovir, are effective in preventing KS but, once tumors are established, have little impact on disease. In contrast, KS tumors show marked hyperplasia and cellular cytokine overexpression. Since the virus is rapidly lost from tumor cells once the tumor is disaggregated and put into culture, there is strong evidence that the tumor environment maintains KSHV infection through paracrine signaling mechanisms. The best animal model for KS involves transgenic expression of vGPCR, which is known to induce factors such as VEGF in T cells, resulting in disorganized proliferation of endothelial cells. Taken together, these data suggest that the virus contributes to tumorigenesis through both neoplastic and hyperplastic mechanisms.

KS tumors from all sites and geographic settings are nearly universally infected with KSHV. While KS in the past has been split into categories depending on the epidemiologic setting, such as endemic KS, classical KS, iatrogenic KS, and epidemic KS, all forms of the tumor are pathologically indistinguishable. Cellular immune status appears to be key to the clinical expression of the tumor among infected persons. In the absence of HIV, KS primarily occurs in those with immune compromise such as the extreme elderly and patients on immunosuppressive therapy. The incubation period from infection to KS has been reported to average 3 years among HIV/AIDS patients, but this appears to be determined by degree of underlying immunosuppression at time of infection. In one case, transient KS was detected in an AIDS patient weeks after initial infection. While KSHV is largely asymptomatic in immunologically intact individuals, it has a very high degree of tumor expression among those who are immunosuppressed. Up to half of early AIDS patients develop KS and among transplant patients infected with KSHV, and 20–60% have been reported to develop clinical disease.

Primary Effusion Lymphoma

PEL is a rare, postgerminal center B-cell tumor accounting for less than 3% of B-cell lymphomas from various clinical series in developed countries. The tumor can

form malignant effusions in body cavities, and among AIDS patients it generally has an aggressive course. Unlike KS, PELs are monoclonal. Tumor cells lack most B-cell markers, although V(D)J rearrangement studies confirm their B-cell origin and they express syndecan-1. PEL tumor cells have been reported to be autocrine-dependent on human IL10 and viral IL6. The latter cytokine is peculiar since it appears to form a 'xenocrine' loop in which vIL6 signaling abrogates cellular interferon responses resulting from virus infection that would otherwise stop growth of the tumor cell. PEL cells are uniformly infected with KSHV and can be grown stably in culture with each cell maintaining 40–150 copies of the viral episome. Cell lines derived from PEL tumors, particularly from AIDS patients, frequently have EBV coinfection, but EBV-negative, KSHV-positive tumors are also found.

Multicentric Castleman's Disease

Unlike PEL, MCD is considered a hyperproliferative lymphoid disorder, with affected individuals displaying a high rate for subsequent development of other lymphoid malignancies. Among HIV-negative persons, only about 50% of MCD tumors show evidence of KSHV infection whereas >90% of HIV-positive MCD patients have KSHV present.

The primary characteristic of MCD is the formation of adventitious germinal centers with marked hyperplasia in lymphoid organs. Examination of these KSHV-positive tumors reveals scattered KSHV infected cells in the marginal zone of the germinal centers, with the bulk of the cells being uninfected. Aberrant IL6 signaling has been demonstrated to be central to the pathogenesis of MCD, and KSHV-positive tumors have marked expression of vIL6, with little human IL6 expression, while KSHV-negative tumors show extensive overexpression of the human cytokine.

Unlike KS and PEL, KSHV-infected tumor cells in MCD show a broad range of viral gene expression and perhaps full lytic viral replication. While standard treatment of KSHV-positive tumors has a high mortality, recent studies have reported marked success in treatment using anti-CD20 antibodies together with ganciclovir.

Other KSHV-Related Disorders

Transplant patients have been reported to develop bone marrow failure after graft infection with KSHV. The extent and consequences of this nonmalignant complication are unknown. In addition, a plasmacytic post-transplant lymphoproliferative disorder from KSHV has been reported to occur among transplant patients.

KSHV has been reported to be associated with a number of other malignant and autoimmune diseases, including multiple myeloma, sarcoidosis, post-transplant skin tumors, and idiopathic pulmonary hypertension. Despite initial enthusiasm, subsequent studies have largely failed to demonstrate any association between the virus and these diseases. It remains formally possible that KSHV causes a subset of one or more of these conditions with the majority of disease cases caused by other agents or conditions.

Diagnosis, Treatment, and Prevention

At present, there are no clinically certified tests to detect KSHV infection, although individual research laboratories have the capacity to test samples under special conditions. This presents a major public health problem, particularly in the setting of transplantation and in blood transfusions in highly endemic areas of the world. Treatment of KSHV-related diseases also generally does not directly target underlying viral infection, instead relying on traditional surgical and chemotherapy. An exception to this are ongoing clinical studies of MCD in which the lytic form of the virus is controlled with ganciclovir. Similarly, blinded clinical trials among high-risk AIDS patients show that antiherpesviral treatment can be highly effective in preventing KS. The introduction of HAART therapy has dramatically reduced the risk of KS among these patients, and thus attempts to extend these results have been limited. Since HAART does not target KSHV, it is expected that a resurgence of KS may occur with aging of the AIDS population.

There is currently no development of vaccines that might be useful to developing country populations. The exquisite sensitivity of KSHV-associated tumors to immune reconstitution and the loss of KSHV from developed populations throughout the world provides evidence that an appropriate vaccine might be extremely effective in controlling this virus infection.

In summary, the public health importance of this virus is clear: KS is the most common malignancy in sub-Saharan Africa and remains an important cause of cancer death among transplant and immunocompromised patients. It remains distressing that the wealth of basic science data available for this virus has yet to be used in a significant fashion to prevent or treat diseases caused by KSHV.

See also: Epstein–Barr Virus: General Features; Herpesviruses: Discovery; Herpesviruses: General Features; Kaposi's Sarcoma-Associated Herpesvirus: Molecular Biology; Simian Gammaherpesviruses.

Further Reading

Beral V, Peterman TA, Berkelman RL, and Jaffe HW (1990) Kaposi's sarcoma among persons with AIDS: A sexually transmitted infection? *Lancet* 335: 123–128.

Chang Y, Cesarman E, Pessin MS, *et al.* (1994) Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 265: 1865–1869.

Ganem D (2006) KSHV-induced oncogenesis. In: Arvin A, Campadelli-Fiume G, Moore PS, Mocarski E, Roizman B, Whitley R, and Yamanishi K (eds.) *The Human Herpesviruses: Biology, Therapy and Immunoprophylaxis*, ch. 56, pp. 1007–1030. Cambridge, UK: Cambridge University Press.
Jarviluoma A and Ojala PM (2006) Cell signaling pathways engaged by KSHV. *Biochimica Biophysica Acta* 1766: 140–158.
Kaposi M (1872) Idiopathic multiple pigmented sarcoma of the skin. *Archives of Dermatology and Syphilology* 4: 265–273.
Martin J (2006) Epidemiology of KSHV. In: Arvin A, Campadelli-Fiume G, Moore PS, Mocarski E, Roizman B, Whitley R, and Yamanishi K (eds.) *The Human Herpesviruses: Biology, Therapy and Immunoprophylaxis*, ch. 5, pp. 960–985. Cambridge, UK: Cambridge University Press.
Mendez JC and Paya CV (2000) Kaposi's sarcoma and transplantation. *Herpes* 7: 18–23.
Russo JJ, Bohenzky RA, Chien MC, *et al.* (1996) Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8). *Proceedings of the National Academy of Sciences, USA* 93: 14862–14867.

Kaposi's Sarcoma-Associated Herpesvirus: Molecular Biology

E Gellermann and T F Schulz, Hannover Medical School, Hannover, Germany

© 2008 Elsevier Ltd. All rights reserved.

History

Kaposi's sarcoma (KS) is an endothelial cell-derived tumor. It is common in acquired immunodeficiency syndrome (AIDS) patients, and is known to have occurred before the arrival of human immunodeficiency virus (HIV). Today, it is one of the most frequently occurring tumors in Africa.

In 1994, following epidemiological studies that suggested the involvement of a sexually transmissible agent other than HIV in the pathogenesis of epidemic (AIDS-associated) KS, Y. Chang, and P. S. Moore used representational difference analysis to identify two sequence fragments of a then unknown rhadinovirus (or γ_2-herpesvirus). Epidemiological studies have now established KS-associated herpesvirus (KSHV, also known as human herpesvirus 8, HHV-8) as a necessary and indispensable causative factor in the pathogenesis of all forms of KS. In addition to 'epidemic' KS, these include the 'classic' form that is more common mainly in HIV-negative elderly men in some Mediterranean countries, the 'endemic' form known to have existed in East and Central Africa long before the spread of HIV, and 'iatrogenic' KS as seen, for example, in transplant recipients. KSHV is also essential in the pathogenesis of primary effusion lymphoma (PEL) and frequently found in the plasma cell variant of multicentric Castleman's disease (MCD). PEL is a rare lymphoma characterized by effusions in the pleural or abdominal cavity. MCD is a lymphoproliferative disorder characterized by multiple lesions that involve lymph nodes and spleen and featuring expanded germinal centers with B-cell proliferation and vascular proliferation.

KSHV (species *Human herpesvirus 8*) is a member of the genus *Rhadinovirus* in subfamily *Gammaherpesvirinae* of the family *Herpesviridae*. It was the first rhadinovirus discovered in humans and thereby also the first in an Old World primate. In the 12 years since this discovery, it has become apparent that most, if not all, Old World primate species, including the great apes (chimpanzee, gorilla and orangutan) harbor related rhadinoviruses. Of these, the genomes of two distinct rhesus macaque viruses have been sequenced completely (or nearly completely), and found to represent two major rhadinovirus lineages. Short-sequence fragments of rhadinovirus genomes representing these lineages have been found in most Old World primates, with the exception of humans, who appear to harbor only KSHV.

Virion Structure

KSHV has the characteristic morphological appearance of a herpesvirus, with a core containing the double-stranded, linear viral DNA, an icosahedral capsid (100–110 nm in diameter) containing 162 capsomers, the tegument that surrounds the capsid, and the envelope, in which glycoproteins are embedded. The virion contains at least 24 proteins, among which are five capsid proteins, eight envelope proteins, six tegument proteins, and five other proteins such as the regulator of transcriptional activation (RTA) and replication-associated protein (RAP or K-bZIP) that are not usually considered as structural virion proteins. The presence of RTA may account for the transient lytic gene expression program that occurs soon after virus entry into the cell. Among the envelope glycoproteins are gB and gpK8.1, which interact with cellular receptors like heparan sulphate or integrin molecules, thereby mediating entry of the virus into the host cell.

Genome Organization

The KSHV genome consists of a long unique region of approximately 140 kbp flanked by multiple, variable

numbers of a tandemly repeated 801 bp terminal repeat (TR) (**Figure 1**). All the protein-coding open reading frames (ORFs) are located in the long unique region, and around 90 have been described so far. These are named ORF4 through ORF75 and K1 through K15. In addition, a noncoding RNA and an alternatively spliced RNA that is processed into 12 microRNAs (miRNAs) has been reported. miRNAs are a class of evolutionarily conserved, noncoding RNAs of about 22 nucleotides that are presumed to have regulatory functions.

The majority of KSHV genes are homologous to genes in the alpha- and betaherpesviruses. Their functions as structural virion proteins or nonstructural players during the lytic (productive) replication cycle are therefore assumed to be similar to those of their counterparts. These genes are not discussed in further detail in this article.

In addition, several genes are only found in KSHV or closely related rhadinoviruses of Old World primates. These are shaded gray in **Figure 1** and include genes encoding latency-associated nuclear antigen 1 (LANA-1; encoded by ORF73) and several homologs of cellular proteins, including D-type cyclin (vcyc; encoded by ORF72), a homolog of a cellular FLICE-inhibitory protein (vFLIP; encoded by ORF71), interleukin 6 (vIL6; encoded by K2), macrophage inflammatory proteins (vMIP1, vMIP2, and vMIP3; encoded by K6, K4.1, and K4.2), interferon regulatory factors (vIRF1, vIRF2, vIRF3, and vIRF4; encoded by K9, K11, K10.5, and K10), complement regulatory protein (KCP; encoded by ORF4), and two members of a class of membrane-associated ubiquitin E3 ligases involved in the downregulation of major histocompatability class I (MHC I) and adhesion molecules from the cell surface (MIR1 and MIR2; encoded by K3 and K5). Ten of the miRNAs are located in the intergenic region between K12 and ORF71 and two are within K12.

The KSHV genome contains two lytic origins of DNA replication (Ori-Lyt), which show extensive sequence similarity to each other but are oriented in opposite directions. Each copy of TR contains a latent origin of DNA replication (Ori-Lat) that consists of two binding sites for LANA-1 and an adjacent region that is essential for replication.

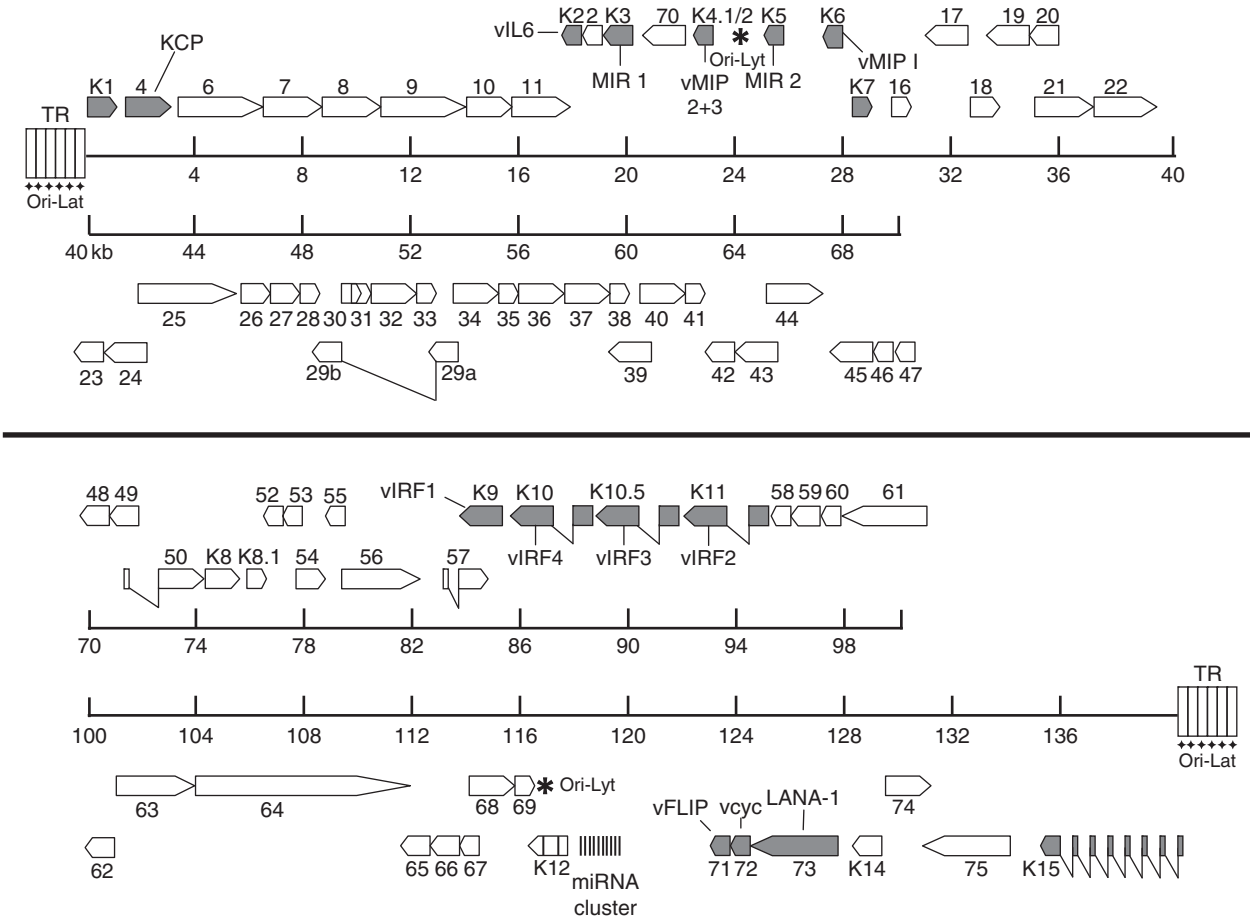


Figure 1 Schematic map of the KSHV genome. Protein-coding ORFs are denoted ORF4–ORF75 and K1–K15, and the names of encoded proteins are given for some (see RefSeq NC_003409 and NC_009333). ORFs typical of Old World or New World Primate rhadinoviruses are shaded gray and other marked features are described in the text. The scale is in kbp.

Evolution

Rhadinoviruses closely related to KSHV appear to exist in many, if not all, Old World primate species. The phylogenetic relationships between DNA fragments from these viruses suggest the co-evolution of primate rhadinoviruses with their host species. Modern KSHV isolates can be grouped into five subtypes (A–E), three of which are still associated with particular human populations (B, Africa; D, Australasia; E, Native Americans), while for two (A and C) such an association is no longer easily discernable, presumably as a result of extensive population mixing in Europe and in Near and Middle Eastern countries. However, one variant of subtype A (A5) is often seen in populations with links to Africa. With the exception of the K1 gene and the right end of the viral genome, variability between subtypes does not exceed a few percent.

Extensive recombination has evidently occurred between KSHV subtypes, and many modern isolates have chimeric genomes. At the right end of the genome, which includes the K15 gene, two types of very divergent and slowly evolving sequences have been found, suggesting that recombination events with rhadinoviruses other than KSHV could have taken place. By contrast, at the left end of the genome, the K1 gene shows evidence of extensive variation and evolution under selective pressure. It is possible that selective pressure exerted by T cells recognizing epitopes may have shaped the marked variability of the encoded membrane glycoprotein, but other explanations are also conceivable.

Tropism and Cell Entry

In vivo, KSHV has been found in human peripheral blood B and T cells, monocytes/macrophages, and endothelial cells. *In vitro*, a wide range of cell lines and primary cell cultures have been infected with KSHV, including human primary and immortalized endothelial cell cultures, epithelial cell lines and primary epithelial cell cultures, fibroblast cultures, monocytes/macrophages, and dendritic cells. Cell cultures from some other host species can also be infected *in vitro*, as can a strain of non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mouse. These observations indicate that the restriction of KSHV for humans in the context of 'natural' infections is not determined at the level of cell entry.

The viral membrane glycoproteins gB (encoded by ORF8) and gpK8.1 (K8.1) are involved in entry of KSHV into target cells. Both interact with heparan sulfate, and gB also binds to $\alpha_3\beta_1$ integrin via an arginine-glycine-aspartate (RGD) motif. In addition, DC-SIGN (CD209) serves as a receptor for KSHV on macrophages and dendritic cells. xCT, the 12-transmembrane light chain of the human cystine/glutamate exchange transporter system x-c, serves as a receptor for KSHV fusion and entry.

The interaction of KSHV with heparan sulfate is thought to mediate the first, 'low-affinity' contact with the target cell, and subsequent binding of individual viral proteins to particular receptors then initiates the next stages of fusion and endocytosis of viral particles. Different entry routes may predominate, depending on the lineage of the infected cell. The importance of the interaction of gB with $\alpha_3\beta_1$ integrin may lie in the integrin-mediated activation of focal adhesion kinase (FAK), whose phosphorylation has been shown to play an important role during the early stages of KSHV entry, such as cellular uptake of viral DNA. The related kinase Pyk2 may also contribute to this process. The subsequent activation of Src, phosphatidylinositol 3-kinase (PI-3K), protein kinase C (PKC), RhoGTPase, mitogen-activated protein kinase (MEK), and extracellular signal-regulated kinase 1/2 (ERK1/2) facilitates virus uptake and immediate-early gene expression. KSHV-activated RhoGTPase in turn contributes to microtubular acetylation and leads to the modulation of microtubule dynamics required for movement of KSHV in the cytoplasm, and for delivery of viral DNA into the cell nucleus.

Latent Persistence and Replication

One of the characteristic features of KSHV is its propensity of quickly establishing a nonproductive, persistent state of infection following cell entry. Akin to its gammaherpesvirus (lymphocryptovirus) cousin, Epstein–Barr virus (EBV), but in contrast to alpha- and betaherpesviruses, this latent state of infection appears to be the default option for KSHV in most cell types, and presumably reflects its mode of long-term persistence *in vivo*. Latency is characterized by a restricted pattern of viral gene expression. Latent viral genes are depicted in **Figure 2** and include those for LANA-1, vcyc, vFLIP, and kaposin A and B, as well as the miRNAs. Several promoters, indicated in **Figure 2**, direct expression of cognate transcripts, of which two (P1 and P3) are strictly latent whereas two others (P2 and P4) become active during the lytic replication cycle. In addition, vIRF3 (also known as LANA-2) is part of the latent gene expression pattern in latently infected B cells. *In vivo*, expression of many of these latent genes has been demonstrated in the majority of KSHV-infected endothelial and spindle cells of KS, and the neoplastic B cells of PEL and MCD, using immunohistochemical or *in situ* hybridization techniques.

During latency, the KSHV genome persists as a circular episome, of which multiple copies (up to 100 or more) have been detected in latently infected PEL cell lines, although it is likely that the copy number varies widely

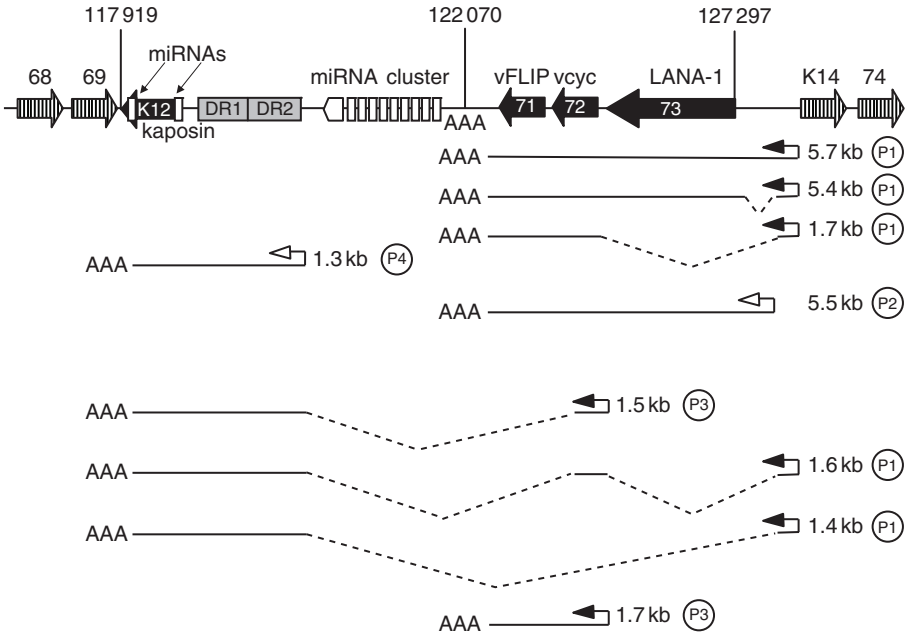


Figure 2 The latency gene cluster in the KSHV genome. The KSHV latency locus encodes four proteins indicated by black arrows (K12/kaposin, ORF71/vFLIP, ORF72/vcyc, and ORF73/LANA-1), as well as 12 miRNAs (white boxes), and is flanked by lytic genes (striped arrows). Lytic promoters are indicated by white arrows and latent promoters by black arrows. Readthrough of a viral polyadenylation signal located at nucleotide 122 070 leads to miRNA expression. P1 (127 880/86) gives rise to a precursor RNA that is spliced to produce 5.7 and 5.4 kb tricistronic (ORF71, ORF72, and ORF73) and 1.7 kb bicistronic (ORF71 and ORF72) mRNAs. Additionally, P1 drives two transcripts that could function as precursors for the miRNAs. As soon as RTA is expressed, P2 (127 610) is induced and gives rise to a 5.5 kb mRNA encompassing all three ORFs. Lytic transcription from P4 (118 758) leads to a 1.3 kb transcript coding for K12/kaposin. P3 (123 751/60) controls a 1.7 kb transcript encoding vFLIP and vcyc and a 1.5 kb transcript that has the potential to encode the miRNAs. Sequence coordinates are derived from GenBank NC_003409.

in other cell types. Replication of the genome during S-phase is ensured by LANA-1, which binds to viral episomes via its C-terminal region and recruits a range of cellular factors, including the 'origin-recognition complex', which is involved in replication of cellular DNA. Two binding sites for LANA-1, spaced 39 bp apart, are located in each of the multiple 801 bp TRs that flank the viral genome. In addition, LANA-1 attaches viral episomes to mitotic chromosomes via its N-terminal domain, thus ensuring their segregation to daughter cells during mitosis as well as their replication during S-phase.

Possibly linked to its role in the replication of viral episomes is the ability of LANA-1 to promote the transition of the G1/S cell cycle checkpoint, thereby enabling viral DNA synthesis. The physical interaction of LANA-1 with the retinoblastoma protein pRB and with GSK-3β is thought to contribute to this property. In addition, LANA-1 binds to p53 and may thereby inactivate cell cycle checkpoint functions that could interfere with successful viral DNA replication, although this can also lead to genomic instability in the infected cell.

A further facet of the role of LANA-1 during latent persistence is its inhibition of the central activator of the lytic replication cycle, RTA, thereby contributing to maintenance of the latent infection. The ability of LANA-1 to interact with components of silenced and transcriptionally active chromatin, such as MeCP2 (which binds to methylated CpG motifs and thereby promotes chromatin silencing) or brd2/RING3, brd4/MCAP, brd4/HUNK, and brd3/orfX (which bind to acetylated histones in transcriptionally active chromatin regions), may be related to its role as a transcriptional repressor and activator, but such a link is currently still tenuous.

Among the other latent viral proteins is vcyc, which, although a homolog of a D-type cyclin, has a more pleiotropic range of functions. The ability of vcyc to promote the transition of the G1/S cell cycle checkpoint is linked to its phosphorylation of pRB (in concert with cdk4/6) and perhaps its inactivation of p27. This property may be important in the context of establishing S-phase conditions required for viral episome replication. However, recent findings clearly indicate that vcyc also contributes to the enhanced proliferation of virus-infected cells.

Translated from the same bicistronic mRNA as vcyc, vFLIP has anti-apoptotic functions and is a potent activator of the NFκB pathway. It is currently thought that the contribution of this protein to maintaining latency may involve protection against apoptosis, induced either by intracellular surveillance mechanisms detecting the

replication of an extraneous agent or by cytotoxic T cells directed against latent viral proteins. Another role could be the repression of the lytic cycle via inhibition of the RTA promoter, which has been shown in several rhadinoviruses to be downregulated by the NFκB pathway. In PEL-derived cell lines, continuous activation of the NFκB pathway is required for the survival of these latently infected cells, and in endothelial cells NFκB activation may contribute to the formation of spindle cells, one of the histological hallmarks of KS lesions.

The fourth strictly latent protein is vIRF-3 (or LANA-2), which is expressed in a strictly B-cell-specific manner by K10.5 (**Figure 1**). Its contribution to latency is incompletely understood, but may involve its ability to interact with p53.

Unlike the latency proteins described above, expression of the transcript encoding kaposin A and B increases after initiation of the lytic replication cycle. It is currently not clear whether these two proteins play a role during latent persistence or whether they contribute to early stages of the lytic cycle. Both proteins influence intracellular signaling pathways, kaposin A by associating with cytohesin-1, a guanine nucleotide exchange factor for ARF GTPases, and regulator of integrin signaling that triggers the Erk/MAPK signaling cascade, and kaposin B by binding to MK2 and increasing the stability of cellular cytokine mRNAs that are regulated by the p38 MAPK pathway. Likewise, the roles of the KSHV miRNAs during latency, if any, are not known precisely, but may involve regulation of cellular and viral mRNAs.

Lytic Replication

Lytic replication results in the production of new KSHV virions. The central regulator of the lytic replication cycle is RTA, which is encoded by ORF50. Overexpression of RTA in latently infected cells is sufficient to trigger reactivation from latency. However, in many experimental systems additional inhibition of histone deacetylases will augment this process. As in other herpesviruses, lytic replication involves a 'rolling circle' mechanism to synthesize new viral DNA concatamers, from which individual linear viral genomes are cleaved during packaging into newly assembled capsids. This cleavage occurs in the TR region, thus ensuring the packaging of the complete long unique region of the genome flanked by a variable number (within limits) of copies of TR. Synthesis of new viral DNA occurs in nuclear replication compartments in the vicinity of ND10/POD domains, which undergo morphological changes during this process. As in other herpesviruses, the exact role of ND10/POD domains in productive herpesviral replication is not fully understood. KSHV proteins recruited to lytic replication compartments include the single-stranded DNA-binding protein (encoded by ORF6), the polymerase processivity factor (ORF59), the DNA polymerase (ORF9), the primase-associated factor (ORF40/41), the primase (ORF56), and the DNA helicase (ORF44), which together mediate viral DNA replication. Other viral proteins are involved in aspects of this process. Thus RAP (K-bZIP; encoded by K8), a structural but not functional homolog of EBV ZTA, is also associated with viral replication compartments and blocks the cell cycle at the G1/S transition by increasing expression levels of p21 and C/EBPα as well as by associating with and downmodulating the activity of cyclin-dependent kinase 2 (cdk-2). The ability to block the cell cycle at G1/S appears to be an important early step in the lytic cycle for most herpesviruses, presumably because it facilitates diversion of the nucleotide pools from cellular to viral DNA replication.

Immune Evasion

To establish an infection in higher vertebrates, viruses must interfere with the host cell immune system. Mechanisms of immune evasion are especially important for viruses that establish long-term infections (e.g., herpesviruses). Like other herpesviruses, KSHV employs several mechanisms to protect infected cells from attacks by the immune system.

Latency represents one strategy to escape from the immune system. During latency only a minimal number of viral proteins are expressed, thus reducing the number of antigens that are presented to the immune system. Another strategy favored by herpesviruses is to downregulate MHC I proteins on the surface of infected cells, thereby decreasing antigen presentation and the recognition of infected cells by cytotoxic T lymphocytes.

KSHV encodes two proteins, modulator of immune recognition 1 and 2 (MIR1 and MIR2; encoded by K3 and K5), which are involved in protecting virus-infected cells against natural killer (NK) cells or cytotoxic T lymphocytes. They are both expressed immediately after viral reactivation. While K3 efficiently modulates multiple MHC alleles, K5 affects the expression not only of HLA-A, but also of ICAM-1 and B7.2. These membrane proteins downregulate MHC I molecules by increasing their endocytosis and degradation rate. This mechanism differs from that of other known viral inhibitors of MHC I expression, which either interfere with the synthesis of MHC I chains or retain them in the endoplasmic reticulum. MIR1 and MIR2 are members of the family of enzyme type 3 (E3) ubiquitin ligases, which regulate the last step of ubiquitination, and thereby increase the degradation rate of MHC I proteins.

The complement system represents a potentially important antiviral mechanism. KSHV encodes a lytic

protein called KSHV complement-control protein (KCP; encoded by ORF4), which is incorporated into virions and inhibits activation of the complement cascade, thereby protecting virions and virus-infected cells from complement-mediated opsonisation or lysis.

Kaposin B, noted above for its ability to modulate the p38 MAPK pathway through its interaction with MK2, has an immunomodulatory function. It is able to increase the expression of certain cytokines by stabilizing their mRNAs. The turnover of some cytokine transcripts is regulated via AU-rich elements (AREs) present in the 3′ nontranslated region, which target the RNAs for degradation. The p38 pathway, via MK2, regulates this turnover. By binding to MK2, kaposin B inhibits the decay of cytokine mRNAs containing AREs. This leads to the enhanced production of certain cytokines, such as IL6 and granulocyte macrophage colony-stimulating factor (GM-CSF).

The viral homolog of IL6, vIL6, binds directly to gp130, the signal-transducing chain of the cellular IL6 receptor and a variety of other cellular cytokine receptors. Unlike cellular IL6, which requires binding to the IL6 receptor α-chain specifically to trigger only the α-chain/gp130 receptor complex, vIL6 has a much broader range of cellular targets and more pleiotropic effects. It is a potent stimulant for B-cell proliferation and also acts on other bone-marrow-derived cells.

Finally, three other KSHV genes show significant homologies to cellular chemokines. Their encoded proteins (vMIP1, vMIP2, and vMIP3) modulate the immune system. They are able to bind to chemokine receptors, act as chemo-attractants, and presumably affect the composition of leukocyte infiltrates around KSHV-infected cells. vMIP1 and vMIP2 have also been shown to have angiogenic properties and may therefore contribute to angiogenesis in KS lesions.

Inhibition of Apoptosis

Apoptosis is necessary for the elimination of cells that are no longer required or have become damaged. Many viruses modulate apoptotic pathways to prevent the premature death of an infected cell, which must be avoided in order to complete the replication cycle.

In PEL cells, pharmacological inhibition of the NFκB pathway leads to apoptosis, suggesting that NFκB, which is activated by several viral proteins, plays an important role in preventing apoptosis in KSHV-infected cells. Several viral proteins have been shown to have an anti-apoptotic function, including vFLIP, which blocks Fas- and Fas-ligand-induced apoptosis at the level of procaspase 8 activation and which is also a potent inducer of NFκB (see above).

Among the KSHV vIRFs, vIRF-1 and vIRF-2 both inhibit interferon signaling and subsequently prevent induction of apoptosis. Both vIRF-1 and vIRF-3 inhibit the activation of p53-dependent promoters.

The product of K7, which is the viral inhibitor of apoptosis (vIAP), is thought to operate as a molecular adaptor, bringing together Bcl-2 and effector caspases. The viral homolog of human Bcl-2, vBcl-2 (encoded by ORF16) is expressed only in late-stage KS lesions. It inhibits virus-induced apoptosis and the pro-death protein BAX.

KSHV-Associated Diseases

KSHV is responsible for KS, a tumor of endothelial origin, as well as for PEL, a rare B-cell lymphoma. It is also found in the plasma cell variant of MCD and thought to play an essential role in the pathogenesis of this B-cell-derived lymphoproliferative disorder. Additional sightings of KSHV in a variety of other proliferative or neoplastic disorders have not been confirmed. However, KSHV may play a role in the occasional case of hemophagocytic syndrome and bone marrow failure in immunosuppressed individuals.

Kaposi's Sarcoma

With very few exceptions, KSHV sequences have been detected by PCR in all KS cases. The few exceptions are held to reflect technical problems or the variable load of KSHV DNA in this pleomorphic tumor, which, in addition to KSHV-infected spindle and endothelial cells, contains many other (uninfected) cellular lineages. KSHV adopts a latent gene-expression pattern in the majority of infected endothelial or spindle cells. As shown by immunohistochemistry and *in situ* hybridization studies, the latent genes discussed above (i.e., LANA-1, vcyc, vFLIP, and kaposin) are expressed in the majority of cells, whereas immediate-early, early, or late genes or proteins are expressed only in a small percentage. Most KS tumors also show a uniform pattern of TR lengths in the circular viral episome, consistent with viral latency. This could suggest that viral latent proteins play a key role in the atypical differentiation of endothelial cells into spindle cells, shown by gene expression array studies to represent an intermediate endothelial cell differentiation stage between vascular and lymphatic endothelial cells. However, it is conceivable that certain viral proteins of the lytic cycle, although apparently only expressed in a few cells, could also contribute to pathogenesis by either exerting a paracrine effect (if secreted, as in the case of vIL6 or vMIPs) or inducing the expression of cellular growth factors that could then act on neighboring latently infected cells (e.g., the viral chemokine receptor homolog encoded by ORF74 induces the secretion of VEGF). Given the inefficient persistence of KSHV in latently infected endothelial cell cultures *in vitro*, it is also

conceivable that periodic lytic reactivation of KSHV is required to infect new endothelial cells and thereby maintain KSHV in this population. On the other hand, *in vitro* experiments suggest that, in spite of being lost rapidly from most infected dividing cells in culture, a small percentage of such cells can remain stably infected, possibly as a result of epigenetic modifications of the viral genome. It is therefore equally conceivable that the latently infected spindle cells in KS tumors are derived from cells that harbor such epigenetically modified latent viral genomes.

Primary Effusion Lymphoma

The presence of KSHV DNA is a defining feature of PEL, a rare lymphoma entity characterized by a lymphomatous effusion in the pleural or abdominal cavity containing malignant B cells that have a rearranged immunoglobulin gene and often express syndecan-1/CD138 and MUM1/IRF4, two markers characteristic for postgerminal center B cells. The majority of PEL samples are dually infected with KSHV and EBV, but 'KSHV-only' cases also occur. KSHV adopts a latent gene-expression pattern in the majority of PEL cells and in permanent cell lines derived from PEL samples. In addition to the latent proteins found in KS (LANA-1, vcyc, vFLIP; see above and **Figure 2**), vIRF3 is expressed in latently infected PEL cells, indicating a B-cell-specific pattern of latent gene expression. A minority of PEL cells, or PEL-derived cell lines, can spontaneously undergo productive viral replication, or be induced to do so by treatment with phorbol esters, histone deacetylase inhibitors such as sodium butyrate, or overexpression of RTA, the central regulator of the productive viral replication cycle. Such PEL-derived cell lines have for a long time been a source of virus for *in vitro* experiments.

Multicentric Castleman's Disease

KSHV is frequently found in the B-cell population of the plasma cell variant of MCD. As in KS and PEL, the majority of virus-infected cells are in a latent stage of infection, expressing characteristic latency proteins such as LANA-1, but also the B-cell-specific latent protein vIRF3. However, unlike KS and PEL, in which only a very small proportion of virus-infected cells appear to undergo productive viral replication at any point in time (see above), a sizable number of KSHV-infected B cells in MCD spontaneously express viral proteins that are thought to reflect productive viral replication. In addition, case reports suggest that MCD activity is reflected by the level of peripheral blood viral load, indicating that MCD may be the manifestation of, or be accompanied by, productive viral reactivation in B cells. Among the 'lytic' viral proteins expressed in MCD B cells is vIL6, a potent stimulator of B-cell growth (see above).

See also: Herpesviruses: General Features; Kaposi's Sarcoma-Associated Herpesvirus: General Features.

Further Reading

Ablashi DV, Chatlynne LG, Whitman JE, Jr., and Cesarman E (2002) Spectrum of Kaposi's sarcoma-associated herpesvirus, or human herpesvirus 8, diseases. *Clinical Microbiology Reviews* 15: 439–464.

Brinkmann MM and Schulz TF (2006) Regulation of intracellular signalling by the terminal membrane proteins of members of the *Gammaherpesvirinae. Journal of General Virology* 87: 1047–1074.

Damania B (2004) Oncogenic gamma-herpesviruses: Comparison of viral proteins involved in tumorigenesis. *Nature Reviews Microbiology* 2: 656–668.

Dourmishev LA, Dourmishev AL, Palmeri D, Schwartz RA, and Lukac DM (2003) Molecular genetics of Kaposi's sarcoma-associated herpesvirus (human herpesvirus-8) epidemiology and pathogenesis. *Microbiology and Molecular Biology Reviews* 67: 175–212.

Edelman DC (2005) Human herpesvirus 8 – A novel human pathogen. *Virology Journal* 2: 78.

Kempf W and Adams V (1996) Viruses in the pathogenesis of Kaposi's sarcoma – A review. *Biochemical and Molecular Medicine* 58: 1–12.

Moore PS and Chang Y (2001) Kaposi's sarcoma-associated herpesvirus. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 2803–2833. Philadelphia, PA: Lippincott Williams and Wilkins.

Rezaee SA, Cunningham C, Davison AJ, and Blackbourn DJ (2006) Kaposi's sarcoma-associated herpesvirus immune modulation: An overview. *Journal of General Virology* 87: 1781–1804.

Schulz TF (2006) The pleiotropic effects of Kaposi's sarcoma herpesvirus. *Journal of Pathology* 208: 187–198.

L

Lassa, Junin, Machupo and Guanarito Viruses

J B McCormick, University of Texas, School of Public Health, Brownsville, TX, USA

© 2008 Elsevier Ltd. All rights reserved.

This article is reproduced from the previous edition, volume 2, pp 887–897, © 1999, Elsevier Ltd., with an update by the Editor.

History

Lymphocytic choriomeningitis (LCM) virus, the first identified arenavirus, was isolated by Lillie and Armstrong in 1933 from the cerebrospinal fluid of a patient suspected of having St Louis encephalitis. The virus was again isolated in 1935 from patients with aseptic meningitis, and finally by Traub in 1935 from laboratory mice. More than 20 years passed before Junin virus, the next member of this taxon to be identified, was isolated from patients with Argentine hemorrhagic fever in 1957. Machupo virus from patients with Bolivian hemorrhagic fever was similarly identified in 1964. A third arenavirus from South America which is pathogenic for humans is Guanarito virus, isolated from patients in Venezuela in 1991. Yet another pathogenic arenavirus, Sabia virus, this time from Brazil, was isolated from a fatally ill individual. Several other arenaviruses have also recently been isolated from rodent species in South America, but none of these have yet been associated with human illness. Lassa virus was the first arenavirus isolated from Africa, in 1969, and remains the only arenavirus pathogenic for humans from Africa, although several other arenaviruses have also been identified from Africa. In total, 23 arenaviruses have been identified worldwide, but only six are known to be pathogenic for humans.

Classification

The 23 members in the *Arenavirus* genus of the *Arenaviridae* have traditionally been placed in categories of Old and New World arenaviruses (**Table 1**), based on geographic locations. More recently, Tacaribe complex viruses (in the Western hemisphere) have been more completely placed in phylogenetic relationship with each other based on a sequence of about 600+nucleotides in the nucleoprotein. The present information suggests three related groups: lineage A contains Pichinde, Parana, Flexal and Tamiami, Allpahuayo, Pirital, Whitewater Arroyo, and Bear Canyou viruses; lineage B contains Junin, Machupo, Amapari, Guanarito, Sabia, Cupixi, Chapare, and Tacaribe viruses; lineage C contains Latino and Oliveros viruses. More recently still, further genetic analysis has led to the suggestion that Pichinde and Oliveros viruses are most closely associated with Old World arenaviruses. The likelihood that arenaviruses and their rodent hosts have coevolved was suggested sometime ago, and now seems a possible hypothesis to begin to test through the parallel use of rodent genetics, alongside the understanding of the genetic relationships between arenaviruses.

The six arenaviruses presently known to be pathogenic for humans (LCM, Lassa, Junin, Machupo, Guanarito, and Sabia viruses) will be the primary subjects of this article.

Properties of the Virion

The arenaviruses are enveloped, pleomorphic, membrane viruses ranging in diameter from 50 to 300 nm, with a mean diameter of 110–130 nm. The virion density in sucrose is 1.17 g ml^{-1}. They contain two segments of single-stranded RNA tightly associated with a nucleocapsid protein. This is enclosed in a membrane consisting of two glycosylated proteins (or in some cases a single glycosylated protein). The genome consists of two segments of single-stranded RNA both containing two genes encoded in an ambisense structure. The small RNA segments encode for the glycoprotein precursor (GPC) and for the nucleoprotein (NP). The NP and GPC genes are encoded in nonoverlapping reading frames with origins at the 3′ and 5′ ends of the molecule, respectively. The N gene is encoded by the 5′ half of the viral complementary RNA sequence corresponding to the 3′ half of the viral RNA molecule. The GPC gene is encoded by the 5′ half of the viral RNA

Table 1 Arenaviruses: basic biological information

Virus	*Human disease*	*Geographic distribution*
LCM	Choriomeningitis	Europe, Asia, Western Hemisphere
Lassa	Lassa fever	West Africa
Mopeia	Human infection, no disease known	Southem Africa/Mozambique, Zimbabwe, Rep. of South Africa
Mobala	Human infection, no known disease	Central African Republic
Ippy	Unknown	Central African Republic
Lineage B[a]		
Cupixi	None	Brazil
Chapare	One human hemorrhagic fever	Bolivia
Junin	Argentine hemorrhagic fever	Argentina
Machupo	Bolivian hemorrhagic fever	Bolivia
Guanarito	Venezuelan hemorrhagic fever	Venezuela
Sabia	Hemorrhagic fever	Brazil
Amapari	None	Brazil
Tacaribe	None	Trinidad
Lineage A[a]		
Parana	None	Paraguay
Tamiami	None	Florida
Pichinde	None	Colombia
Flexal	None	Brazil
Pirital	None known	Venezuela
Whitewater Arroyo	None known	North America
Allpahuayo	None	Peru
Bear Canyou	None	California
Lineage C[a]		
Latino	None	Bolivia
Oliveros	None known	Argentina

[a]In current phylogenetic scheme suggested for Tacaribe complex viruses.

molecule. Similarly, the large strand of RNA codes for the RNA-dependent viral RNA polymerase and a smaller ring-finger protein involved in replication. The arenaviruses are virtually indistinguishable from each other morphologically, and all share the characteristic granules noted in electron micrographs. These granules appear to be the results of the binding of the zinc-binding ring-finger protein of arenaviruses with the nuclear fraction of ribosomal proportion. The *nuclear ribosomal* protein (PO) appears in the virion, while other ribosomal proteins do not. This suggests that the granules, believed to be nonspecific inclusion of ribosomes into the virion, may rather be a specific process related to virion replication and assembly. These granules give rise to the family name *Arenaviridae,* derived from *arenos,* the Latin word for sand, based on their electron micrographic appearance as grains of sand.

Geographic and Seasonal Distribution

Junin, Machupo, and Guanarito viruses occur in Argentina, Bolivia, and Venezuela, respectively. Junin and Guanarito are endemic in their respective areas. Junin virus primarily infects workers during the corn harvesting season, by disturbance of the rodent host, *Calomys callosus,* which lives in the corn fields. Guanarito virus infection is endemic, and its epidemiology may resemble that of Lassa virus in Africa, which occurs throughout the year; however, insufficient data presently exist to confirm this impression. Machupo virus occurred in epidemic fashion in the 1960s in a circumscribed area of Bolivia. It was associated with the transient marked increase in the population of *Calomys* rodents, which are normally field rodents but because of overpopulation moved into human dwellings in search of food. Elimination of the rodents in the towns stopped the epidemic, and few further cases have since been reported (though a few cases were reported in 1996). Chapare virus has been isolated from a human fatal hemorrhagic fever patient in Bolivia in 2004. No reservoir has been identified and nothing is known about its epidemiology or geographic distribution. Lassa virus infection is endemic in West Africa from Senegal to Cameroon and perhaps other areas not yet explored. There are increases in Lassa infection during the dry seasons, perhaps because of increased virus stability in lower humidity, but other, as yet unknown, factors may also be involved.

Host Range and Virus Propagation

All of the New World arenaviruses have rodent reservoir hosts with the exception of Tacaribe virus, which was isolated from bats in Trinidad (**Table 1**). A hallmark of the arenaviruses is their intimate biological relationship with rodents, resulting in lifetime infection and chronic virus excretion. Many arenaviruses have more than one rodent host, although usually a single species will predominate as the reservoir in nature.

The hosts of LCM virus (LCMV) have included *Mus* species and hamsters. Guinea pigs are also capable of transmitting the virus in laboratory settings. Machupo virus often renders its major natural host, *Calomys callosus,* essentially sterile by causing the young to die *in utero.* Machupo virus also induces a hemolytic anemia in its rodent host, with significant splenomegaly, often an important identifier of infected rodents in the field. The

major rodent hosts for Junin virus are *Calomys* species. Transmission of Junin virus from rodent to rodent is generally horizontal, and not vertical, and is believed to occur through contaminated saliva and urine. The *Calomys* rodents are affected by the virus, with up to 50% fatality among infected suckling animals, and stunted growth in many others. Both Junin and Machupo viruses induce a humoral immune response when transmitted to their suckling natural rodent hosts, which may have neutralizing antibody in the face of persistent infection. Guanarito virus has been isolated from *Zygodontomys brevicauda,* though its detailed biology remains to be learned.

The only known reservoir of Lassa virus in West Africa is *Mastomys natalensis,* one of the most commonly occurring rodents in Africa. At least two species of *Mastomys* (diploid types with 32 and 38 chromosomes) inhabit West Africa, and both have been found to harbor the virus. All species are equally susceptible to silent persistent infection, as seen when LCMV infects mice. This presumably occurs as in LCM infection, from a selective deletion of the thymic T cell response to the virus. All of the arenaviruses pathogenic for humans will also infect and produce illness in a wide range of primates. However, it is not known whether such infections occur in nature, as is known for Ebola virus for example. In addition, human infection plays no biological role in the life cycle and ecology of the arenaviruses.

Virus Propagation

The original isolation of LCMV was made in suckling mice, which have been important in isolation and characterization of several of the arenaviruses. The arenaviruses are, however, easily cultivated in a wide variety of mammalian cell monolayers. The Vero E6 remains the cell of choice for primary isolation and cultivation, but arenaviruses also replicate in baby hamster kidney cells, as well as in a number of specialized cells such as continuous macrophage lines, endothelial cells, fibroblasts and a variety of mouse cell lines, with specific MHC markers used as targets for immunological studies. The infected cells may produce a cytopathic effect (CPE) beginning on days 4–7 of incubation. However, not all arenaviruses produce CPE, especially on primary isolation. For diagnosis, cells may be harvested after 48–72 h and assayed for antigen by immunofluorescent antibody (IFA) or ELISA. Virus plaquing techniques may also be used for the arenaviruses.

Genetics

While advances have been made in determining the genetic relationship between different arenaviruses (see Classification), the level of genetic variability within species of arenaviruses is not well characterized, though it must be added that little genetic data on field isolates exist on which to make this judgment. It would appear that the frequency of variability at the amino acid level, as judged by B cell epitope variability, is not high. Thus the variability in B cell epitopes among Lassa viruses isolated from humans or rodents over a 10-year period in a circumscribed area was not substantial, suggesting that B cell epitopes are under limited immune pressure in their rodent hosts. Knowledge of the epitopes recognized by T cells may be crucial for the development of recombinant Lassa virus vaccines. A human T-helper cell epitope, highly conserved between Old and New World arenaviruses, as well as HLA-A2-restricted CD8 T cell protective Lassa virus epitope have been described. The South American arenaviruses may be under both B and T cell immune pressure in their rodent hosts, though no data are available on this issue. Reassortment, demonstrated only in the laboratory, may also be a means of genetic variability, but its occurrence in nature and therefore its importance is unknown.

Evolution

The *Arenaviridae* are distributed over five continents and can be divided into three 'coevolutionary' groups: Lassa complex in Africa, LCM in North America, Europe, and South America, and the Tacaribe complex in South America. Today's arenaviruses probably descended from an ancestral virus which subsequently differentiated in parallel with the evolution of the Cricetid rodents persistently infected by arena viruses. It seems likely, therefore, that the present distribution and evolution of these viruses are directly related to the distribution and evolution of the earliest Cricetid rodents and their descendants, which now make up the natural hosts of most of this family of viruses (Tacaribe virus has a bat host). The coevolution of these viruses will undoubtedly continue and depend primarily on mutations, selected by the persistently infected host's immune pressure, and perhaps on reassortment in the rodent host.

Epidemiology

The fundamental determinant of the ecology of hemorrhagic fevers is the occurrence of persistent virus infection in rodents. Who becomes infected and what are the functions of the behavior of the persistently infected rodent, and the cultural and occupational patterns of human populations. The arenavirus hemorrhagic fevers are primarily rural and semirural diseases; however, some evidence exists that under certain conditions (poverty, overcrowding), their rodent reservoirs may also establish urban habits. The rodents infect the environment via

urine, feces and saliva. Interactions between rodents and humans are peridomestic or in agricultural areas. However, details of the rodent population dynamics, behavior, and the natural history of the persistent virus infection in the feral rodent hosts are only poorly understood. The Old World arenaviruses, LCM and Lassa, produce persistent infection in their rodent hosts without significant detrimental effects. The South American viruses, in contrast, may cause illness and death in newborn rodents, or may induce persistence. The modes of transmission from rodents to humans are not precisely known. Direct contact by humans, with cuts and scratches on hands and feet, with articles and surfaces contaminated by virus may be a more important and consistent mode of transmission. Transmission through mucosal surfaces may also occur under some circumstances.

Lassa Fever

Lassa fever occurs in West Africa, but with a wide geographic area from Northern Nigeria to Guinea, encompassing perhaps 100 million population. At least two species of *Mastomys* occupy West Africa, and both have been found to harbor the virus. These rodents, especially the species with 32 chromosomes, are highly commensal with humans. The movement of *Mastomys* within a village is very limited, and their average lifespan is about 6 months, with little seasonal fluctuation in their breeding pattern. From 5% to as many as 70% have been found to be infected with virus in some village houses. Therefore, most virus transmission takes place in and around the homes. All age groups and both sexes are affected and antibody prevalence increases with age. Risk factors for human infection include contact with rodents, direct contact with ill persons infected with Lassa virus, presence of a large household rodent population, and human practices such as catching, cooking and eating rodents, and indiscriminant storage of food. In many endemic areas, Lassa fever is a common cause of hospitalization. The death rate in systematically studied, untreated hospitalized patients with Lassa fever is 16%, very similar to that described for Junin and Machupo infections. Nosocomial transmission occurs in Africa from contact with infected patients, or through improper use or sterilization of needles, sometimes leading to an outbreak. Person-to-person spread of Lassa virus in households is common, a unique characteristic of Lassa virus in relation to other arenaviruses (no data are yet available on Guanarito virus from Venezuela, or Sabia virus from Brazil), and is usually associated with direct contact or care of someone with a febrile illness, or possibly with sexual contact with the spouse during the incubation or convalescent phases of illness. Illness to infection ratios are 10–25% in some endemic areas of West Africa. Antibody prevalence ranges from less than 1% to over 40% in some villages.

Argentine Hemorrhagic Fever (AHF)

AHF, caused by Junin virus, was recognized in the 1950s in the northwestern part of the Buenos Aires Province in Argentina, an area of very fertile farmland and therefore of great economic importance. The total number of cases reported over a 30 year period is about 21 000. AHF is a seasonal disease with peak yearly incidence in May. The average number of cases from 1981 to 1986 was 360 per year. Although all ages and sexes are susceptible, nevertheless the major group affected is the male working population, explained by the habits of the rodent hosts for Junin virus, *Calomys musculinus* and *Calomys laucha*. These animals are not peridomestic, but rather occupy grain fields, and this is the major reason for the affected population of field workers. Infection also occurs infrequently in other rodents: *Mus musculus, Akodon azarae,* and *Oryzomys flavecens*. Transmission from rodent to rodent is horizontal, not vertical, and is thought to occur via contaminated saliva and urine. It is believed that the major routes of virus transmission from humans is through contact with virus-infected dust and grain products and subsequent infection through cuts and abrasions on the skin, or through airborne dust generated primarily by killing and scattering of rodents during mechanized farming. The disease has spread over the 30 years or so since its recognition from an area of 16 000 km^2 and a quarter of a million persons to an area greater than 120 000 km^2 containing a population of over 1 million persons. Furthermore, the incidence in the older affected areas appears to wane after 5–10 years. Overall antibody prevalence is 12%, with a typical predominance in agricultural workers. One-third of the seropositive individuals have no history of typical illness, suggesting that the case: infection ratio is about 2:3. The incidence of AHF is now very low due to the extensive vaccination campaign in the endemic area.

Bolivian Hemorrhagic Fever (BHF)

The only known reservoir for the virus is *Callomys callosus,* a Cricetid rodent that is found in the highest density at the borders of tropical grassland and forest. The distribution of this rodent appears to include the eastern Bolivian plains, as well as northern Paraguay and adjacent areas of western Brazil. The disease was recognized in 1959, and by 1962 more than 1000 cases had been identified in a confined area of two provinces, with a 22% case fatality ratio. The largest known epidemic of BHF, involving several hundred cases, occurred in the town of San Joaquin in 1963 and 1964. This outbreak occurred because of a marked increase in the *Callomys* population, and the subsequent invasion of homes in the town by these rodents. Although the *Callomys* appears to be capable of living both in the areas surrounding the towns and in the

towns themselves (where the most efficient transmission of virus would appear to occur), they favor a nonperidomestic habitat where contact with humans is much reduced. It appears that the situation in San Joaquin was unusual, and such an event has not been observed again after nearly 25 years. There has not appeared to be any increase in the geographic areas affected by BHF recently, and few cases have been reported.

Venezuelan Hemorrhagic Fever

The epidemiologic pattern of this disease has not yet been well characterized. In one survey the antibody prevalence in the population in the endemic area in central Venezuela was 2.6%. Guanarito virus was isolated from the cane rat *Zygodontomys brevicauda*. The occurrence of person-to-person transmission has not yet been demonstrated. The low frequency of infection in family contacts and lack of disease in hospital workers caring for patients suggest that person-to-person spread is uncommon. The pattern of infection includes all ages and sexes, suggesting that transmission occurs in and around houses, similar to Lassa fever and BHF and unlike AHF.

Transmission and Tissue Tropism

The primary mode of arenavirus transmission is from human contact with rodent urine or blood. This probably occurs primarily when individuals come into contact with surfaces or materials recently contaminated by rodent urine, or when they trap a rodent and handle the carcass. Some evidence suggests that rodent blood or urine might be aerosolized by machinery during mechanized harvesting of corn in Argentina, with consequent transmission of Junin virus to people working near the machinery; however, no detailed studies of specific risk factors exist. In Africa, the rodents are highly commensal and probably deposit virus-laden urine in many areas of the houses they inhabit. The people tend to walk barefooted, and, based on the epidemiologic pattern of somewhat sporadic infection in households, it seems likely that contact with infectious urine is the primary source of contamination. In addition, in parts of Africa people catch and eat rodents, putting them in direct contact with rodent tissue, blood and secretions during the preparation. Finally, for some of the viruses, particularly Lassa virus, there is person-to-person transmission primarily through contact with the blood or secretions of an infected, ill person. Whatever the mode of transmission, it would seem that the reticuloendothelial system is probably a primary target of replication of the arenaviruses, though they replicate well in many organs, including liver, adrenal gland, placenta, lung and many other organs.

Pathogenesis

The most common sites of initiation of human infection by the arenaviruses are not yet known, although they seem likely to be cuts and abrasions in the skin. Following the initiation of infection, all arenaviruses progress to generalized multiorgan infections, especially of the reticuloendothelial system. Thereafter, however, the pathogenesis of the different infections is variable. Cellular receptors for both New and Old world arenaviruses have recently been described.

Lassa fever

The 1–3 week incubation that follows infection suggests an unknown primary replication site, probably within the reticuloendothelial system. Route and titer of infecting dose may be important determinants of outcome, as may the virus strain. For example, death rates in nosocomial outbreaks where parenteral exposure is substantial are usually higher than community-acquired infections.

The degree of organ damage in fatal human infections is mild, which is sharply at variance with the clinical course and collapse of the patient. Indeed, there are few clues to the pathogenesis of Lassa fever in standard pathological studies. Liver damage is variable, with concomitant cellular injury, necrosis and regeneration. Nevertheless, serum aspartate aminotransferase (AST) levels over 150 iu ml $^{-1}$ are correlated with poor outcome, and an ever-increasing level is also associated with increased risk of death. Alanine aminotransferase (ALT) is only marginally raised, and the ratio of AST:ALT in natural infections and in experimentally infected primates is as high as 11:1. Furthermore, prothrombin times, glucose and bilirubin levels are near normal, excluding biochemical hepatic failure. An increasing Lassa viremia is also associated with increasing case fatality. In addition to the liver, high virus titers occur in many other organs without significant pathologic or functional lesions, perhaps reflecting blood rather than parenchymal levels of virus.

Some patients develop severe pulmonary edema and adult respiratory distress syndrome, gross head and neck edema, pharyngeal stridor and hypovolemic shock. This pattern is consistent with edema due to capillary leakage, rather than cardiac failure and impaired venous return. Endothelial cell dysfunction has been demonstrated in primates experimentally infected by Lassa fever, in that there is apparently a marked decrease in prostacyclin production by endothelial cells. Loss of integrity of the capillary bed presumably causes the leakage of fluids and macromolecules into the extravascular spaces and the subsequent hemoconcentration, hypoalbuminemia, and hypovolemic shock. Proteinuria is common, occurring in two-thirds of patients.

Edema and bleeding may occur together or independently. Since there is a minimal disturbance of the intrinsic, and almost none of the extrinsic, coagulation system, and there is no increase in fibrinogen breakdown products, disseminated intravascular coagulation (DIC) is excluded. Furthermore, platelet and fibrinogen turnover in experimental primate infections are normal. Though platelet numbers are only moderately depressed, in severe disease platelet aggregation is almost completely abolished by a circulating inhibitor. The origin of this inhibitor is not known; however, it cannot be reproduced with viral material nor can it be blocked by antibodies to Lassa virus. In the platelet, it blocks dense granule and ATP release and thus abolishes the secondary wave of *in vitro* aggregation, while sparing the arachidonic acid metabolite-dependent primary wave. The inhibitor of platelet function also interferes with the generation of the FMLP-induced superoxide generation in polymorphonuclear leukocytes probably through a similar mechanism.

AHF and BHF

Despite the different degrees of bleeding, there are sufficient similarities between the course of disease in AHF, BHF, and Lassa fever to speculate that there exists a similar pathophysiologic pathway underlying all of the diseases. Organ function, other than the endothelial system, appears to remain intact, and the critical period of shock is brief, lasting only 24–48 h. Hepatitis is mild, and renal function is also well maintained. Bleeding is more pronounced with AHF and BHF than Lassa fever, but it is not the cause of shock and death. Capillary leakage is significant, with loss of protein and intravascular volume being much more pronounced than loss of red cells. Proteinuria is significant, and dehydration with hemoconcentration appears to be an important process. The shock is not associated with evidence of disseminated intravascular coagulation, and even though there are petechiae suggesting some direct endothelial damage, no clear evidence of virus replication in, and damage of, endothelium has been demonstrated. Thus, clinical observations suggest that vascular endothelial dysfunction may be the basis of subsequent circulatory failure in AHF and BHF. Persistent hypovolemic shock in the face of intravascular volume expanders suggests that it is due to the loss of endothelial function and leakage of fluid into extravascular spaces. This is supported by the tissue edema frequently observed, and more directly by the pulmonary edema which may result from vigorous fluid therapy of the shock. These events lead to irreversible shock and death in the most severely ill patients. Two other observations have been made in AHF: high levels of interferon in severely/fatally ill patients, and a decrease in complement. These are general phenomena observed in other severe infectious processes and are consistent with the events described above. Neither would dictate a substantially different pathophysiologic explanation of these diseases.

Clinical Features

Lassa Fever

Lassa fever begins after 7–18 days of incubation, with fever, headache, and malaise. Aching in the large joints, pain in the lower back, a nonproductive cough, severe headache, and sore throat are common. Many patients also develop severe retrosternal or epigastric pain. Vomiting and diarrhea occurs in 50–70% of patients. In more severely ill patients, complete prostration may occur by the 6th to 8th day of illness. Patients with Lassa fever appear toxic and anxious, and in the absence of shock, the skin is usually moist from diapheresis. There is an elevated respiratory rate and pulse. The systolic blood pressure may be low. There is no characteristic skin rash; petechiae and ecchymoses are rare, nor is jaundice a feature of Lassa fever. Conjunctivitis is common, but rare conjunctival hemorrhages portend a poor prognosis. Seventy percent of patients have pharyngitis, often exudative, but few if any petechiae, and ulcers are rare. Mucosal bleeding occurs in 15–20% of all patients, and although associated with severe disease it is almost never of a magnitude to produce shock by itself. Edema of the face and neck are commonly seen in severe disease. About 20% of patients have pleural or pericardial rubs heard late in disease at the beginning of convalescence. The abdomen may be diffusely tender. The ECG may be abnormal, primarily with elevated T waves, but without a characteristic pattern. Moderate or severe diffuse encephalopathy with or without general seizures is characteristic in severe disease. Severe Lassa fever can progress rapidly between the 6th and 10th day to *respiratory distress* with stridor due to laryngeal edema, central cyanosis, hypovolemic shock and clinical signs of encephalopathy, sometimes with coma and seizures. Tremors are often seen in the hours just before death. Acute, focal neurological signs rarely occur, with the exception of VIII nerve deafness seen in convalescence. Residual ataxia is common. Lassa fever is also a pediatric disease affecting all ages of children. The disease appears to be even more difficult to diagnose by clinical criteria in children than in adults because its manifestations are so general. In very young babies marked edema may be seen, associated with very severe disease. In older children the disease may manifest as a primary diarrheal disease or as pneumonia or simply as an unexplained prolonged fever. The case fatality in children is 12–14%.

The mean white count in early Lassa fever may be low or normal, with a relative lymphopenia, but late neutrophilia may supervene in severe disease. Proteinuria is very

characteristic. A serum AST (SGOT) level of >150 iu ml^{-1} is associated with a case fatality of 50%, and there is a correlation between an ever-increasing level and an increased risk of death. A viremia of $>1 \times 10^3$ $TCID_{50}$ ml^{-1} is associated with an increasing case fatality. Both factors together carry a risk of death of 80%.

Pharyngitis, proteinuria, and retrosternal chest pain have a predictive value for Lassa fever in febrile hospitalized patients of 81% and a specificity of 89%. Likewise, a triad of pharyngitis, retrosternal chest pain, and proteinuria (in a febrile patient) correctly predicted Lassa fever 80% of the time (in an endemic area). Both triads have sensitivities of 50% for detecting cases of Lassa fever. Significant complications of Lassa fever include a two- to threefold increased risk of maternal death from infection in the third trimester, and a fetal/perinatal loss of 84% that does not seem to vary by trimester. Another significant complication is that of acute VIII nerve deafness, with nearly 30% of patients suffering an acute loss of hearing in one or both ears. Other complications which appear to occur much less frequently are uveitis, pericarditis, orchitis, pleural effusion and ascites.

AHF and BHF

These diseases have an insidious onset of malaise, fever, general myalgia, and anorexia. Lumbar pain, epigastric pain, retro-orbital pain, often with photophobia, and constipation occur commonly. Nausea and vomiting frequently occur. Temperature is high, reaching 40 °C or above. Unlike LCM and Lassa fever, AHF and BHF do not usually lead to respiratory symptoms and sore throat. On physical examination, patients appear toxic. Conjunctivitis, erythema of the face, neck, and thorax are prominent. Petechiae may be observed in the axillae by the 4th or 5th day of the illness. There may be a pharyngeal enanthem, but pharyngitis is uncommon. Relative bradycardia is often observed. The disease may begin to subside after 6 days of illness; if not, the second stage of illness supervenes, and most commonly manifests as epistaxis and/or hematemesis or acute neurological disease. The bleeding may be from mucosal surfaces or into the skin, with petechiae and hemorrhagic rash, with preceding increase in packed red cell volume. Pulmonary edema is common in severely ill patients. The appearance of intractable shock is a serious sign which becomes irreversible in some patients, and accounts for the majority of deaths from AHF and BHF. Fifty percent of AHF and BHF patients also have neurologic symptoms during the second stage of illness, such as tremors of the hands and tongue, progressing in some patients to delirium, oculogyrus, and strabismus.

A low white blood cell count (under 1000 mm^{-1}) and a platelet count under 100 000 are invariable. Proteinuria is common and microscopic hematuria also occurs. Alterations in clotting functions are minor, and DIC is not a significant part of the diseases. Liver and renal function tests are only mildly abnormal.

Venezuelan Hemorrhagic Fever

Little information is available on the spectrum of disease caused by Guanarito virus infection. Hospitalized patients with severe diseases are febrile on admission, with prostration, headache, arthralgia, cough, sore throat, nausea/vomiting, diarrhea, and hemorrhage. The bleeding includes epistaxis, bleeding gums, menorrhagia, and melena. On physical examination, patients with severe disease appear toxic, and usually dehydrated. They are described as having one or more of a series of signs, including pharyngitis, conjunctivitis, cervical lymphadenopathy, facial edema, and petechiae. Thrombocytopenia and neutropenia are common at admission. Case fatality of a single group of 15 hospitalized patients was over 60%; however, the single serum survey available suggests that overall mortality:infection ratio is much lower.

Pathology and Histopathology

Lassa Fever

The most frequently and consistently observed microscopic lesions in fatal human Lassa fever are focal necrosis of the liver, adrenal glands, and spleen. The liver damage is variable in the degree of hepatocytic necrosis. The liver demonstrates cellular injury, necrosis, and regeneration, with any or all present at death. A substantial macrophage response occurs, with little if any lymphocytic inflammatory response. Nevertheless, fatal cases do not exhibit sufficient hepatic damage to implicate hepatic failure as a cause of death. Similarly, moderate splenic necrosis is a consistent finding, primarily involving the marginal zone of the periarteriolar lymphocytic sheath. Diffuse focal adrenocortical cellular necrosis has been less frequently observed. Although high virus titers occur in other organs, such as brain, ovary, pancreas, uterus, and placenta, no significant lesions have been found. Thus, few clues to the pathogenesis of Lassa fever are found in standard pathological studies. It is clear that the outcome in Lassa fever is associated with the degree of virus replication; nevertheless the effect of replication is not major tissue destruction, but a more subtle biological effect on vascular endothelium, and perhaps other key cells or organs.

AHF and BHF

AHF and BHF are typical hemorrhagic diseases and have very similar pathologic features, some of which differ from Lassa fever. Patients with AHF manifest a skin rash and petechiae, and gross examination of organs at necropsy

also show petechiae on the organ surfaces. Ulcerations of the digestive tract have been described, but the bleeding is not massive. Microscopic examination shows a general alteration in endothelial cells, mild edema of the vascular walls, with capillary swelling, and perivascular hemorrhage. Large areas of intra-alveolar or bronchial hemorrhage are often seen with no evidence of inflammatory process. Pneumonia with necrotizing bronchitis or pulmonary emboli is observed in half of the fatal necropsied cases. Hemorrhage and a lymphocytic infiltrate have been observed in the pericardium, occasionally with interstitial myocarditis. The lymphnodes are enlarged and congested with reticular cell hyperplasia. Splenic hemorrhage is common. Medullary congestion with pericapsular and pelvic hemorrhages are frequently seen. Acute tubular necrosis occurs in about half of the fatal cases, but adrenal necrosis has not been reported.

Venezuelan Hemorrhagic Fever

Only a limited number of post-mortem dissections have been performed. Observations included pulmonary edema with diffuse hemorrhages in the parenchyma, and subpleural, focal hepatic hemorrhages with congestion and yellow discoloration, cardiomegally with epicardial hemorrhages, splenic and renal swelling, and bleeding into numerous cavities, including stomach, intestines, bladder, and uterus.

Immune Response

There is a brisk B cell response to Lassa virus, with a classic primary IgG and IgM antibody response to virus early in the illness. This event does not, however, coincide with virus clearance, and high viremia and high IgG and IgM titers often coexist in both humans and primates. Indeed, virus may persist in the serum and urine of humans for several months after infection, and possibly longer in occult sites, such as renal tissue. Neutralizing antibodies to Lassa virus are absent in the serum of patients at the beginning of convalescence, and in most people they are never detectable. In a minority of patients some low titer serum neutralizing activity may be observed several months after resolution of the disease. Passive protection with antibody to Lassa virus has been demonstrated in animals given selected antiserum at the time or soon after inoculation with virus, but clinical trials of human plasma given after onset of illness have shown little or no protective effect. Thus, the clearance of Lassa virus in acute infection appears to be less dependent of antibody formation, and presumably depends more on the cell immune response. This is supported by recent experience with experimental Lassa vaccines in primates. In recent studies of LCM infection there is clearly apoptosis of T cells and probably B cells during acute infection in the mouse model. Such a scenario must be entertained for acute Lassa infection, and could help explain the fulminant course in some patients. Such work will need to be performed in primate models or during studies of human disease in endemic areas. Neutralizing and complement fixing antibody to Junin and Machupo are usually detectable 3–4 weeks after the onset of illness. Indirect fluorescent antibodies may be detected at the end of the second week of illness. The efficacy of convalescent plasma has been demonstrated in the therapy of Junin infection (see Therapy). The effectiveness of the plasma has been demonstrated to be associated with the level of Junin virus-neutralizing antibodies. The IFA test is the most commonly performed test for diagnosis by antibody detection for AHF and BHF. Little data are available on Guanarito virus, which appear to induce an antibody response. However, more like AHF and BHF, antibodies to Guanarito virus seem to appear later in illness.

Prevention and Control

Rodent Control

The ideal method of prevention for these rodent-borne diseases is to prevent contact between rodents and humans. The effectiveness of this was shown in the outbreaks of BHF in the 1960s. However, the prospects of rodent control in preventing AHF are not so bright. The human–rodent encounter resulting in AHF occurs during the crop harvests, and with the present technology it is difficult to imagine how control of noncommensal feral rodents could be accomplished. The best choice may be better protection of the agricultural worker from contact with rodent secretions and blood. Similarly, the control of rodents as a broad approach to preventing Lassa fever is not realistic. The improvement of housing and food storage could reduce the domestic rodent population, but such changes are not easily made. Rodent trapping in an individual village where transmission is high has demonstrated as much as a fivefold reduction in the rate of virus transmission. However, such a program is only applicable in villages with exceptional transmission rates, and would certainly not be applicable to large areas.

Vaccines

The live attenuated Junin vaccine has now been shown to be not only effective but also has had a dramatic effect in reducing the number of cases of AHF seen each year in the endemic area. A vaccine against Lassa fever has been made by cloning and expressing the Lassa virus glycoprotein gene into vaccinia virus. This vaccine has proved highly successful in preventing severe disease and

death in challenged monkeys. Only the glycoprotein gene is protective in the two species of monkeys thus far tested, and the basis of protection is not neutralizing antibody, which does not develop, but more likely the cell-mediated immune response. Vaccines using the glycoprotein vectored by VSV prevent disease and death in the nonhuman primates model.

Drug Prophylaxis

In the event of identifiable exposure to Lassa virus (and possibly other pathogenic arenaviruses) in a hospital or laboratory setting, the prophylactic use of orally administered ribavirin is recommended, although efficacy data are missing.

Therapy

Significant advances have been made in the therapy of Junin and Lassa virus infections since the early 1990s. Convalescent plasma is effective in the treatment of Junin virus during the first 8 days of illness, but not Lassa virus. Ribavirin is effective in reducing the viremia and mortality of Lassa fever particularly when given in the first week of illness.

Lassa Fever

Ribavirin can prevent death in Lassa fever when given at any point in the illness, but it is more effective when given early and intravenously. Thus, patients with risk factors for severe disease who were treated within the first 6 days of illness experienced a 5–9% case fatality. Those with the same risk factors, in whom treatment was initiated more than 6 days after the onset of illness, had a case fatality of 20–47%. Regardless, the case fatality was significantly less for ribavirin-treated patients in all categories than for either nontreated patients or for those treated with plasma alone. As such it seems reasonable to assume that it would also be an effective measure in the event of laboratory or hospital exposure to the disease. The pathogenesis of the infection is less reversible later in illness. Furthermore, patients treated with ribavirin had significant declines in viremia regardless of outcome, whereas patients who were untreated or treated with plasma and who died showed no decrease in viremia, consistent with the observation that outcome, and presumably the result of therapy, is closely related to the inhibition of virus replication. Therefore, patients coming late in disease will require more effective clinical management of physiologic dysfunction, and perhaps other drugs which may be used to stabilize the shock state sufficiently long to allow recovery and improve survival (see Pathogenesis).

AHF

A randomized trial of patients with AHF demonstrated that convalescent-phase plasma reduced the mortality from 16% to 1% in the patients who were treated in the first 8 days of illness. The efficacy of the plasma was directly related to the concentration of neutralizing antibodies of the plasma.

The success of this therapy was not without a price, however, which was the development of a late neurological syndrome in about 10% of cases. Thus far, there is no correlation between either the day of therapy or the dose of neutralizing antibodies given and the occurrence of late neurological syndrome. Passive antibody therapy depends on collection of plasma from persons known to have had the disease, testing the plasma (or screening the donor) for antibodies to blood-borne agents such as hepatitis, and proper storage until its use. In addition, the advent of the acquired immunodeficiency syndrome (AIDS) and the other diseases transmissible by blood products means that further screening is required before use.

BHF

Recent experience of successful treatment of two patients with intravenous ribavirin suggests that further efforts to evaluate the effectiveness of ribavirin in this disease will be worthwhile.

Future Perspectives

An important area of future research includes a more through understanding of the viral and host components of the virus-clearing and protective immune response in humans. A second area of substantial interest is the nature of the persistent infection in the rodent host. A more complete understanding of the pathogenesis of Lassa fever may provide insight not only to that disease, but to basic elements of how the host response may be detrimental as well as beneficial to the host. Finally, a more through comprehension of how to control the more widespread arenavirus diseases, either through vaccination, or preferably rodent control, is essential.

See also: Lymphocytic Choriomeningitis Virus: General Features.

Further Reading

Buchmeier MJ, Bowen MD, and Peters CJ (2001) Arenaviridae: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 1635–1668. Philadelphia: Lippincott Williams and Wilkins.

Cajimat MNB and Fulhorst CF (2004) Phylogeny of the Venezuelan arenaviruses. *Virus Research* 102: 199–206.
Clegg JCS (2002) Molecular Phylogeny of the Arenaviruses. *Current Topics in Microbiology and Immunology* 262: 1–24.
Damonte EB and Coto CE (2002) Treatment of arenavirus infections: from basic studies to the challenge of antiviral therapy. *Advances in Virus Research* 58: 125–155.
Enria D, Mills JA, Flick R, *et al.* (2006) Arenavirus infections. In: Guerrant RL, Walker DH, and Weller PF (eds.) *Tropical Infectious Diseases. Principles, Pathogens and Practice,* 2nd edn., pp. 734–755. Philadelphia, PA: Elsevier Churchill Livingstone.
Fisher-Hoch SP and McCormick JB (2004) Lassa fever vaccine. *Expert Review of Vaccines* 3: 189–197.
Gunther S and Lenz O (2004) Lassa virus. *Critical Reviews in Clinical Laboratory Sciences* 41: 339–390.
Kunz S, Borrow P, and Oldstone MBA (2002) Receptor structure, binding, and cell entry of arenaviruses. *Current Topics in Microbiology and Immunology* 262: 111–137.
McCormick JB, Webb PA, Scribner CL, *et al.* (1986) Lassa fever: Effective therapy with ribavirin. *New England Journal of Medicine* 314: 20.
McCormick JB, Webb PA, Krebs JW, Johnson KM, and Smith K (1986) A prospective study of the epidemiology and ecology of Lassa fever. *Journal of Infectious Diseases* 155: 437.
Rojek JM, Spiropoulou CF, and Kunz S (2006) Characterization of the cellular receptors for the South American hemorrhagic fever viruses Junin, Guanarito, and Machupo. *Virology* 349: 476–491.
Salazar-Bravo J, Ruedas LA, and Yates TL (2002) Mammalian Reservoirs of Arenaviruses. *Current Topics in Microbiology and Immunology* 262: 25–63.

Legume Viruses

L Bos, Wageningen University and Research Centre (WUR), Wageningen, The Netherlands

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ecology Highly complex interaction of organisms with each other and with their physical environment and its study.
Epidemiology (**quantitative ecology**) Rapid quantitative spread of parasites and pathogens in populations of their host(s) and its study.
Vector Organism or other agent that can spread a virus or any parasite and introduce it to or into other organisms including plants so that attack or infection results.
Virus identification The acts of (1) describing the particular identity or independence of a new virus (i.e., virus characterization) and (2) recognizing a virus as an entity earlier described, classified, and named, with the aim of etiological disease diagnosis.

Introduction

Legume viruses and those of any other type of crop must be dealt with in relation to the crop concerned. Initially, plant viruses drew attention in crops because they were injurious to plants and reduced crop productivity. Legumes were among the first crops studied for virus infections. Also, plants were for long the sole medium to study the viruses and distinguish between them from the view point of the species they infected the symptoms they produce. Most plant viruses still derive their names from crops on which they were first detected and from the symptoms they caused, for example, bean yellow mosaic virus (BYMV). Plants were also used as differential hosts for separating viruses from mixtures by selective passage. Natural hosts affect the evolution of the viruses that infect them. Selected test or indicator plants soon helped for detecting viruses in plants and in vector organisms, and later in fractions during purification. Laboratory studies of viruses for their intrinsic properties made virological interest gradually shift from their effects on plants to molecular biology. But plants remain indispensable for propagating most plant viruses, and basic information on plant viruses must merge in relation to crops such as legumes for elucidating the biological and societal impact of viruses, and showing how to combat them in agricultural practice.

Legumes

Legumes are botanical species in the pod-bearing family Leguminosae or Fabaceae (order Fabales) of which the subfamily Papilionoideae is the largest and economically most important. Root-nodule bacteria (*Rhizobium* spp.) that bind nitrogen from the air make legumes do well under poor growing conditions and improve soil fertility. Legumes are protein-rich sources of food and fodder of high nutritive value to man and animals. The seeds of grain legumes serve as a meat substitute in developing countries. Those of soybean and groundnut contain valuable oil for industrial processing, and they are increasingly produced on large holdings as cash crops for export, as of soybean in Brazil. Green seeds or whole pods and sometimes green leaves or sprouted seeds are also eaten as a vegetable. Some tropical legume species are grown for

their edible swollen roots. Clovers are forage or fodder crops, or serve as green manure and cover crops used in crop rotation for maintaining soil fertility. Leguminous tree species are often grown in the tropics for shading and sometimes for timber.

Plants, including leguminous species, keep playing important roles in studying plant viruses for their effects on plants and crops, and also for their separation from natural mixtures, their propagation, and even their detection. Common bean (French bean or bush bean; *Phaseolus vulgaris*) and cowpea (*Vigna unguiculata*) are still used as test plants for detecting several viruses or measuring their infectivity by the local lesions they produce on uniform opposite primary leaves either on plants or detached.

History of Legume Virology

The history of research on legume viruses illustrates how, through trial and error, plant virology has evolved from its middle ages concentrating on their effects on plants and crops into the present high-technology era dominated by molecular biology of the viruses themselves. Legumes were among the first crops studied for virus diseases. Some early examples were bean common mosaic (1917, 1921) (**Figure 1**) and soybean mosaic (1921). Viruses were originally distinguished by their hosts or host ranges and by symptoms, and later also by their ways of spread, as in seed (bean common mosaic potyvirus, BCMV, 1919; **Figure 2**) and by specific insects such as aphids or beetles. Later, further distinction was by the so-called 'physicochemical properties' studied in expressed sap, especially since 1930 when sap transmission was facilitated with abrasives. Viruses of annual legumes, such as peas and beans, were also found in perennial legumes such as clovers, and viruses described from legumes and those from nonlegumes increasingly appeared to cross-infect their respective hosts. For example, beet curly-top curtovirus (BCTV) was found to cause severe disease in beans, and BYMV often infected gladiolus. With information accruing in the 1930s, efforts to devise keys for the recognition of viruses of legumes were first made in 1939 and 1945, and order slowly began to emerge.

After Wold War II, viruses were increasingly recognized as worldwide production constraints, and legume virus workers were among the first to standardize the techniques for identifying the viruses dealt with (1960). An International Working Group on Legume Viruses (IWGLV) was established in 1961 for the exchange of seeds of test plants and antisera and of information. New techniques such as serology and electron microscopy were advocated for further laboratory characterization of the viruses themselves, and collaboration between researchers across national barriers was stimulated. A resulting tentative list of viruses reported from naturally infected leguminous plants (1964) animated further worldwide assemblage of information in computerized form as by the Australian Virus Identification Data Exchange project (VIDE). Its microfiche publication on *VIDE Viruses of Legumes* was soon followed by a printed version (1983). Similar books on viruses of plants in Australia (1988) and of tropical plants (1990) were succeeded by *Viruses of Plants* (1996), which was also distributed on the Internet. It later contributed to the database of the International Committee on Taxonomy of Viruses (ICTV), but it all began with IWGLV.

The IWGLV has in 2001 merged with the International Working Group on Vegetable Viruses, set up in 1970 with similar means and objectives and partially dealing with the same crops. The major aim remains surveying of the crops for viruses and their economic importance, understanding their ecology, and learning



Figure 1 Dramatic effect of common mosaic, caused by bean common mosaic potyvirus (BCMV) in some cultivars of common bean (*Phaseolus vulgaris*). The disease was described during the decade starting 1910 as one of the first recognized, now globally distributed legume virus diseases. (Left, resistant cultivar.)



Figure 2 BCMV in common bean with primary infection from the seed in plant at left and secondary infection by aphids in adjacent plant at right.

how they can be controlled for safeguarding crop productivity. But first of all comes virus identification.

Legume Viruses

In early plant virology viruses were thought to be rather specific in host range, for example, limited to Leguminosae or even to single species. Examples of narrow host specialization were BCMV and soybean mosaic virus (SMV) on beans (**Figure 1**) and soybean, respectively. They are highly seed transmitted and are introduced into crops every year by the seed that is sown, whereupon they are further spread from infected seedlings by aphids (**Figure 2**). Alternative hosts then are not needed for survival from one growing season to another. The aphid-transmitted wisteria vein mosaic potyvirus (WVMV) has so far been found worldwide in a few ornamental *Wistera* spp. only, possibly from a single vegetative origin.

Several viruses, of legumes, soon appeared to be much more polyphagous than supposed before. BYMV, for example, infects several annual legumes, but is prevalent in ornamental gladiolus as well, and occurs in other Iridaceae and some 14 other plant families. The related cowpea aphid-borne potyvirus (CABMV) also causes woodiness disease in passionfruit, and BCMV has meanwhile been found naturally in at least 26 leguminous species.

Some 'nonlegume viruses', other than the beet virus BCTV, also infecting legumes are the potyviruses beet mosaic virus (BtMV), lettuce mosaic virus (LMV), and turnip mosaic virus (TuMV). Extremely polyphagous viruses like the aphid-borne cucumber mosaic cucumovirus (CMV) and the soil-borne and fungus-transmitted tobacco necrosis necrovirus (TNV) include legumes among their many natural hosts (**Figure 3**). Presumed host barriers are increasingly transgressed, and novel types of disease emerge in cases of often haphazard new encounters between plants and viruses – as through the global movement of plant species and cultivars and of viruses and their strains distributed worldwide in plant propagation materials. Legume viruses are therefore increasingly difficult to define as a special category, but over 14% of the plant viruses still derive their name from legumes.

Most taxonomic groups of plant viruses are represented in legumes, and a 1991 monograph by Edwardson and Christie lists 279 different legume species reported naturally infected by 171 viruses then thought to be distinct. Perennial legumes, such as clovers, host many viruses, often in mixtures, and in plants the viruses may interact, either directly or via their host. BYMV, for example, helps multiply CMV and increase disease severity as in cowpea stunt. Groundnut rosette in Africa is caused by a complex of groundnut rosette umbravirus (GRV) together with an associated satellite RNA and groundnut rosette assistor luteovirus (GRAV), that helps aphid transmission. In nature, leguminous hosts and the viruses infecting them vary greatly and the result of infection may range from symptomless infection to severe disease up to premature plant death. Symptoms may often be mistaken for mere physiological disturbances as by mineral deficiency (**Figure 4**). Concise literature on virus diseases of specific legume crops is given by the compendia



Figure 3 Severe pod necrosis in common bean caused by the soil-borne and sap- and fungus-transmitted tobacco necrosis necrovirus (TNV).



Figure 4 Beginning chlorosis in top of faba-bean plants with bean leafroll luteovirus (BLRV), often mistaken for a mere effect of mineral deficiency.

of crop diseases published by the American Phytopathological Society, St. Paul, MN, USA. These and other reviews up till 1996 have been listed by Bos in 1996.

Variation of Legume Viruses

BCMV was one of the first viruses of which 'strains' were detected on new cultivars of common bean bred for resistance. The same was later found for SMV on soybean where resistance breeding was also prominent. Genetic change of crops is known to exert selection pressure on the viruses allowing these to co-evolve. Of BCMV, at least seven pathogenicity genes have been postulated and four of them interact with the bean's susceptibility or resistance genes in a gene-for-gene relationship.

Genetic adaptation to plant species and host cultivars where the viruses happen to land may also account for the wide variation that exists within the virus genera, explaining the evolution of virus species. Legume potyviruses (genus *Potyvirus*, now part of the family *Potyviridae*) have many variants and intermediates as between and around BCMV, SMV, and BYMV. Their expanding number made biological properties, such as host ranges, symptoms, cross-protection, and serology, increasingly inadequate for distinction and specific detection, and a semblance of continuity started to blur the originally postulated boundaries. It suggested progressive evolution through mutation, recombination, and selective adaptation in niches often created by man. Molecular genetics, recording percentages of nucleotide and amino acid sequence identities, has helped artificially to draw boundaries between different 'viruses' and devise phylogenetic dendrograms (**Figure 5**). This allows more final identification but entails increasing reliance on molecular techniques. Similar evolutionary developments have been observed among, for example, legume luteoviruses and begomoviruses.

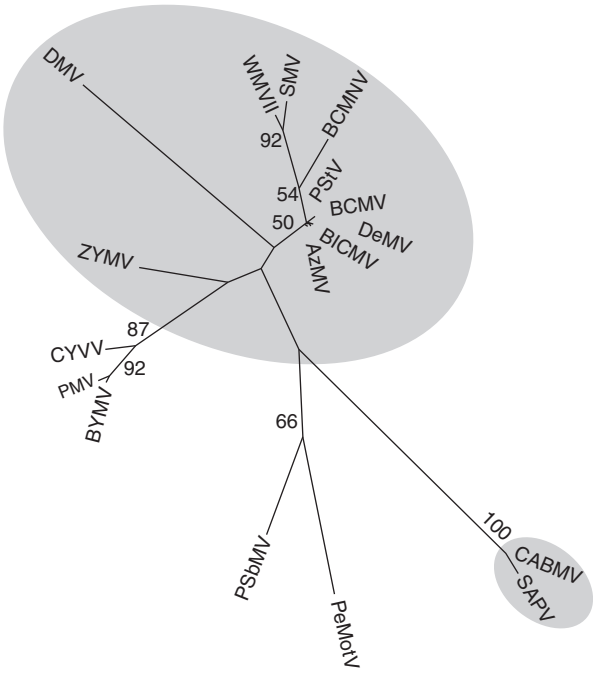


Figure 5 Phylogenetic tree of the legume-infecting viruses in the family *Potyviridae* based on analysis of the nucleotide sequences of the 3′-end noncoding region (NCR) of their ss (+) RNA genome. Branch lengths are proportional to genetic distance. Reproduced from Berger PH, Wyatt SD, Shiel PJ, Silbernagel MJ, Druffel K, and Mink GI (1997) Phylogenetic analysis of the *Potyviridae* with emphasis on legume-infecting potyviruses. *Archives of Virology* 142: 1979–1999, with permission from Springer-Verlag.

Ecology

What happens to the legume viruses in legume crops is a matter of an intriguingly complex ecology. Factors to consider are: (1) the viruses already mentioned, (2) the sources of infection, (3) the vectors that spread the viruses, (4) the crops that are subject to infection and differ in genetic vulnerability, and finally (5) the growing conditions, including soil, climate, and the cropping system that influence host plants and vectors. These factors may capriciously interact, and the outcome is often hard to predict. Several of the factors listed largely depend on the grower's decisions, as for his/her choice of place and time of cultivation and the choice of crop and cultivar and of cultural practices. Legume viruses illustrate the widest known array of ecological relationships between viruses and plants. The quantitative involvement of the factors mentioned is the subject of epidemiology. Comprehension of the ecology of the viruses and of their epidemiology explaining to what extent and with what speed the viruses spread is essential for devising ways and means to control the viruses or limit their effects.

Sources of Infection

Many legume viruses are seed-borne, and commercial seed lots, even when usually only partially infested, provide infected seedlings throughout a newly sown crop. For BCMV (**Figure 2**) and SMV in common bean and soybean, respectively, these are the almost only, but efficient and early, within-crop sources of infection. BYMV that is seed-transmitted in yellow lupin and at low rates in pea and faba bean, has other sources of infection as well. Pea seed-borne mosaic potyvirus (PSbMV), which caused considerable concern in the pea-growing and -processing industry in the USA during the late 1960s and 1970s, is now known to often occur in seed of several other legumes worldwide. Notorious and vast other sources of infection for annual legumes are nearby perennial clovers and medics in pastures or grown as pure crops. BYMV readily moves from clovers (**Figure 6**) and also from nonleguminous ornamental gladiolus to bean when grown nearby.

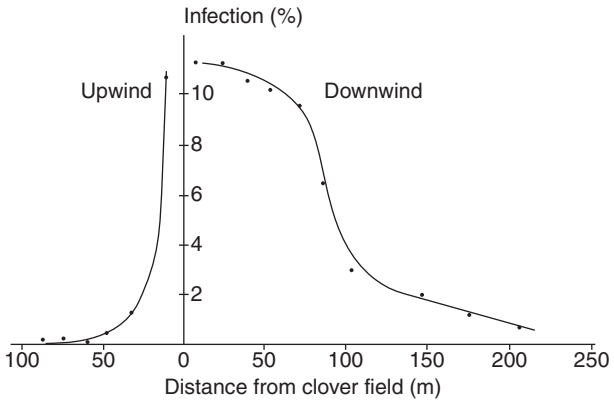


Figure 6 Spread of bean yellow mosaic potyvirus (BYMV) that is nonpersistently transmitted by aphids from red clover into field of common bean, influenced by prevailing wind. Reproduced from Hampton RO (1967) Natural spread of viruses infectious to beans. *Phytopathology* 57: 476–481, with permission from American Physopathological Society.

Infection of wild plants, as by CMV, pea early-browning tobravirus (PEBV) and TNV, is often symptomless and the wild sources of infection may then be hard to find.

Means and Ways of Spread

By contact

For legumes, spread from one plant to another when plants touch or are touched, seems to be limited to white clover mosaic potexvirus (WClMV), TNV, and the beetle-transmitted red clover mottle comovirus (RCMV). They occur in plants in high concentration and have very stable virions. RCMV may even be spread with a lawn mower. Some stable plant viruses, such as the fungus-transmitted red clover necrotic mosaic dianthovirus (RCNMV) may also be released into soil and water from decaying roots and may then get mechanically into plants when these are wounded. Tomato bushy stunt tombusvirus (TBSV), reported from *Robinia pseudoacacia* and *Wisteria floribunda*, may be transmitted directly from living roots.

Via seed

Many legume viruses (69 of 171 listed) have been reported to be distributed via seed, although often in such low percentages (below 1% or less) that infected seedlings easily escape notice. Such seed may still spread viruses over long distances to places where they did not occur before. Many legume viruses have already been spread worldwide in seed, and they continue to threaten legume crop improvement in developing countries. However, rates of seed-lot infestation with infected seeds may be high as of PSbMV where up to 90% of the seeds of infected mother plants may contain the virus, so that crops grown from such seed will directly suffer economically. Most seed-transmitted viruses of legumes are carried in the embryo, which remains infective for the seed's life. The legume viruses that in their plant hosts are limited to the phloem, as a rule, do not pass via the seed. Most mechanically transmissible viruses are detectable for a while in the seed coats from where they usually cannot reach the seedling.

By living organisms

Most spread of legume viruses is by living organisms, called vectors. It usually is rather, if not highly, specific, and most viruses have complicated relationships with their vectors so that viruses transmitted by one type of vector usually are not transmitted by any other type, and the vectors have complex ecologies of their own as well. Seed-borne viruses, with the exception of cryptoviruses which have no known vector, have either an aerial or subsoil vector for further dissemination.

Aboveground, most legume viruses are readily insect transmitted.

1. 'Nonpersistent and noncirculative transmission', as of many carla-, caulimo-, cucumo-, and fabaviruses, and the large group of potyviruses that are all readily transmissible in expressed sap, is by mere mouthpart contamination and usually is not specific. It is rapid but over short distances only (**Figure 6**). These viruses usually move in zones from a nearby other crop or do so spotwise from infected seedlings or weeds.

2. 'Persistent transmission' particularly holds for phloem-limited viruses that usually are not sap-transmissible. Examples are the luteoviruses of bean leafroll (BLRV) (**Figure 4**) and soybean dwarf (SbDV) by aphids, the mastrevirus of chickpea chlorotic dwarf (CpCDV) and the curtovirus BCTV in common beans by leafhoppers, the bean golden mosaic begomovirus (BGMV) by whiteflies, and the tospoviruses of tomato spotted wilt (TSWV) and groundnut bud necrosis (PBNV) in groundnut by thrips. Their relationship with the vectors is highly specific, that is, circulative. Some viruses even multiply in their vector (propagative transmission) and transovarially pass to offspring. The vectors then remain viruliferous for extended periods or for life. Propagative viruses are viruses of animals (insects) as well as of plants. Persistently transmitted plant viruses may be transferred over very long distances. Leafhoppers are strong flyers and they may cover hundreds of kilometres in high-level wind currents.

3. 'Semipersistent transmission' as of clover yellows closterovirus (CYV) and related criniviruses often is by whiteflies. Virus uptake is from the sieve tubes, and the virus is only adsorbed in the insect's foregut.

4. A variety of viruses that are artificially transmissible in plant sap is naturally transmitted by beetles (*Coleoptera*) including blister beetles (bean pod mottle comovirus; BPMV), lady beetles (southern bean mosaic sobemovirus; SBMV), leaf beetles (cowpea mosaic comovirus; CPMV; and BPMV) and some weevils (broad bean mottle

bromovirus; BBMV). These have biting mouthparts, and virus acquisition and introduction by these vectors is immediate and after a single bite. Virus retention in the beetles is in the hemolymph for days or weeks, and specificity of transmission is due to specific inactivation of most of the viruses in the regurgitant fluid that is produced by the beetles during feeding.

5. Exceptional among legume viruses is the possibly persistent transmission of pigeon pea sterility mosaic virus (PPSMV) in India by the eryophyid mite *Aceria cajani* that is invisible to the naked eye and mostly moves passively as by wind.

Within the soil, a number of legume viruses are transmitted by either nematodes or fungi.

1. The trichodorid nematodes that spread PEBV are of low mobility. Patches of infected plants in fields of pea and common bean hardly enlarge. The transmission resembles semipersistence, and retention in the vector may be for months especially at low temperatures. Disease recurrence the next season, as well as long-distance dissemination of the viruses, is especially explained by high rates of seed transmission and by occurrence in weeds. Trichodorid nematodes and the viruses they transmit do best on sandy soils or in sandy patches.

2. (a) Chytrid fungi (*Olpidium brassicae* and *O. bornovanus*) transmit the necrovirus TNV (**Figure 3**) and the dianthovirus RCNMV, respectively. These viruses are carried externally on the fungal zoospores, and transmission is nonpersistent. The virus-carrying spores move for short distances in soil water or over longer distances in irrigation water. (b) The plasmodiophorid fungus *Polymyxa graminis* spreads peanut clump pecluvirus (PCV) and broad bean necrosis pomovirus (BBNV). The viruses are carried within the hard-walled resting spores of the fungus and may persist there for many years. These spores may be transferred over long distances in drainage water and much farther in dry soil on seeds, vegetative propagation material, tools and transport vehicles, and in wind-blown dry soil and seeds. Fungus-transmitted viruses are favored by rainfall and irrigation.

The Role of Man

Viruses are nearly always and often invisibly present in or around crops or in wild species. Whether disease results greatly depends on susceptibility and sensitivity (vulnerability) of the crop and thus on the crop species or cultivar that the growers choose. A change of farming system may also create new niches for viruses and their vectors. Large-scale commercial cropping of groundnut in Northern Nigeria, for example, led to a dramatic epidemic of groundnut rosette in 1975. Dense populations of the vectoring aphids resulted from their survival and multiplication on volunteer groundnut plants and on numerous other plant species available throughout the year due to increased irrigation. In Brazil, the acreage of soybean for the world market tremendously increased and its growing period extended, creating vast areas of an excellent food and breeding plant for the whitefly *Bemisia tabaci*. This led to the enormous upsurge of bean golden mosaic virus (BGMV) in common bean. Where soybean cultivation had also expanded in traditional bean-producing areas, the seed-borne SMV, not usually infecting bean, also showed up in that crop because of high infection pressure from soybean and the cultivation of susceptible bean varieties. In the Latin Americas and other parts of the world, whitefly-transmitted viruses have also increased enormously in soybean and a range of crops including many nonlegumes such as cotton, tobacco and tomato. New niches may also result from the introduction of alien crops or new cultivars, and alien viruses inadvertently introduced in germplasm for breeding programs and in commercial seed may well be pathogenic to local crops or crop cultivars.

Most of the many seed-borne viruses of legumes have already shown up everywhere. Legume improvement programs in developing countries often entailed new diseases by viruses introduced in germplasm. In developed countries the introduction of virus-resistant cultivars, as of bean and soybean, has evoked the emergence of resistance-breaking virus strains. Continuing agricultural modernization and further internationalization of trade and traffic are therefore bound to lead to newly emerging diseases. In fact, most if not all virus diseases of crops are 'man made'.

Epidemiology

Accurate quantitative information about epidemic development of virus infection in crops usually is scarce. Epidemic disease development in crops when beginning with a limited number of infected plants is known to be a polycyclic process and to proceed according to an S-curve. This also holds for legume viruses (**Figure 7**; curve *V*). The speed of development greatly depends on distance between source and subject of infection, on the number of infection sources, on vector population density (**Figure 7**; curve *A*) and efficiency, and on crop and cultivar susceptibility (or resistance). When seeds are major if not sole primary sources of infection as of BCMV and SMV in bean and soybean, respectively, initial disease incidence determined by seed infestation below 0.1% mostly does not lead to incidences at the time of harvest that cause appreciable loss. In resistant cultivars, virus multiplication in individual plants usually is lower than in susceptible ones so that it takes longer for plants to become efficient secondary sources of infection. Epidemic development in crops of such cultivars is then delayed and crops are resistant as a whole.

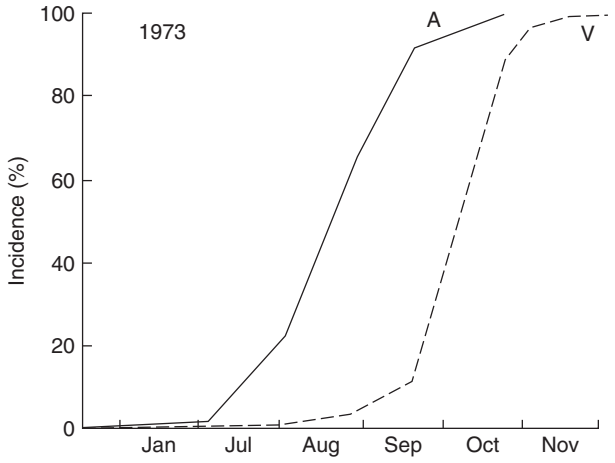


Figure 7 The increase in incidence with time of subterranean clover red leaf virus (a strain of soybean dwarf virus, SbDV, an unassigned virus of the family *Luteoviridae*) (**V**), and of its aphid vector *Aulacorthum solani* (**A**) in crop of faba bean in Tasmania. Reproduced from Thresh JM (1983) Progress curves of plant virus disease. *Advances in Applied Biology* 8: 1–85, with permission from Elsevier.

Persistently transmitted viruses such as the luteoviruses usually move in from far away and many plants may become infected around the site where the viruliferous aphids land and move about. Then, infection may soon evenly invade an entire crop. Nematode-transmitted viruses like PEBV in pea and bean occur spotwise depending on irregularity of soil type and nematode distribution. Plants usually are invaded with such viruses early when the nematodes become active, and the spots may slowly enlarge over the years if the soil permits the nematodes to extend their territory.

Economic Importance

Crop loss depends on incidence of diseased plants, on time of infection during crop development, and on symptom severity. Many viruses of legumes are highly contagious and often rapidly reach all plants of a crop. Losses then are high if the symptoms are severe as of faba bean with faba bean necrotic yellows nanovirus (FBNYV) where plant death often leads to total crop failure (e.g., **Figure 8**). Early infections usually are most severe, particularly when symptoms accumulate as of the phloem-limited luteoviruses viruses in chickpea, faba bean (**Figure 4**), pea, and several other legumes. These viruses primarily cause degeneration of the sieve tubes and impede transport in vascular bundles. This then is followed by a cascade of secondary and tertiary symptoms such as poor root growth and leaf yellowing, plant stunting, and even premature death. Late infections usually are less harmful than early ones because plant susceptibility and sensitivity often decrease toward plant maturity. Necrosis directly affects yield and quality particularly when the pods are



Figure 8 Total crop loss in faba bean (*Vicia faba*) over vast area in Egypt in 1992 caused by faba bean necrotic yellows nanovirus (FBNYV).

involved. Examples are certain genetic host/virus combinations of soybean with SMV and of bean with BCMV and BCMNV, of bean with TNV (**Figure 3**), and of the same and pea with PEBV.

The effects of viruses on yield are often overlooked or neglected. Plant yellowing (**Figure 4**) may be mistaken for noninfectious mineral deficiency, but final losses may still be dramatic. Chlorophyl defects, as in various mosaics and yellows diseases (**Figures 1** and **4**), always reduce assimilation capacity and adversely affect yield. Even when visible symptoms are absent, root nodulation, plant vitality and productivity may considerably be diminished, and plant susceptibility and sensitivity to other pathogens increased. In perennial clovers, longevity of stands may be reduced by increased infection by root rot fungi and by raised sensitivity to drought and winter injury. One virus may also support another virus. In cowpea stunt, caused by a mixture of CMV and blackeye cowpea mosaic virus (BCMV or a variant), experiments have shown that together they caused a reduction in seed yield of 86%, whereas single infections only led to seed losses of 14 and 2.5%, respectively.

In practice, severe crop losses have been reported in legumes. The dramatic 1975 epidemic of groundnut rosette in Northern Nigeria occurred on over 1 million ha of groundnut and destroyed an estimated 0.7 million ha worth over $250 million. The same year, the whitefly-transmitted BGMV had become the main production constraint in the traditional cultivation of bean in Brazil. In Latin America, where beans is one of the main staple foods, more than 2.5 million ha was under attack by the virus during the 1990s. The related bean dwarf mosaic begomovirus (BDMV) showed up in Argentina in 1981 and at least one million ha previously planted with bean in South America was abandoned because of the risk of total yield loss. In 1992 in the Nile Valley, Egypt, the then newly recognized FBNYV wiped out entire crops of faba bean on *c.* 16 000 ha in the Beni Suef Governorate (**Figure 8**).

Financial losses that mostly are not taken into account but may be substantial are the costs of hygienic measures to be taken by growers, and of the more expensive price of seed of resistant cultivars and of seed certified for low rates of infection. Nationwide, costs of research, education, extension, and quarantine must also be considered.

Control

Virus-diseased plants cannot be cured once they are infected, and control nearly always is preventive. Either one or, mostly, a combination of mainly hygienic measures are to be taken based on profound knowledge of the ecology and epidemiology of the viruses in their relation to crop ecology. But this, first of all, requires proper disease diagnosis, that is, reliable identification of the causal virus for allowing a goal-directed strategy of control.

Often highly effective, even when applied singly, is the avoidance or removal of the sources of infection. For example, soil infested with soil-borne and nematode- or fungus-transmitted viruses, such as PEBV, TNV, or PCV, should not be used for growing peas, bean, and groundnut. Commercial seed used for growing crops must be relatively free of seed infected with seed-borne viruses, and possibly be certified for low rates of infection. Certification schemes have been developed for the production of commercial seed of legume crops that often harbor aphid-transmitted seed-borne viruses such as BCMV and SMV. The commercial seed must be produced under relatively vector-free conditions away from sources of infection, while infected plants must be regularly removed. The harvested product must be tested and certified for low rates of virus infection so that infection of crops to be grown from the seed is likely to remain below damage thresholds. For most legumes, commercial seed should not contain more than 0.1–0.5% of infected seeds. Germplasm used for breeding purposes and as basic material for the production of commercial seed, must be multiplied under vector-proof conditions from seed of plants that have been tested individually for virus freedom, and suspected plants must be removed. Annual legume crops must not overlap with nearby older crops or with other perennial crops such as clovers that may harbor several legume viruses (**Figure 6**).

Epidemic buildup must be prevented by avoiding high densities of vector insects by choosing growing conditions or a season with less vector insects. Systemic insecticides are helpful to reduce the spread of persistently insect-transmitted viruses such as the legume luteoviruses. Virus dissemination over long distances must be avoided or prevented also by the use of clean seed, which is especially important for legumes because of the many viruses that are seed-borne in them.

The use of resistant cultivars, and breeding for resistance, has been the most widely used means of managing virus disease incidence and severity, and has a long tradition for legumes. It should be an ongoing effort because of the selection pressure that new cultivars exert on viruses for developing new resistance-breaking strains. Breeding for resistance to seed transmission is also possible as found in beans and lupin for BCMV and CMV, respectively. For developing countries, International Agricultural Research Institutes (IARCs) as CIAT in Cali, Colombia, ICARDA in Aleppo, Syria, ICRISAT in Hyderabad, India, and IITA in Ibadan, Nigeria, that are mandated for legume crops, are instrumental in improving resistance of important leguminous food and fodder crops by breeding, often in concert with national institutes in their respective regions. They also provide information and survey the respective crops in their regions of outreach, study the identity and ecology of the viruses that are important there, and develop techniques and means for their proper identification and for testing of plant genetic materials for resistances to the viruses concerned. The institutes have immense collections of germplasm in their gene banks that are also sources of genes for resistance to viruses. Genetic engineering is increasingly replacing original methods of gene transfer by pollination.

The ever-modernizing agriculture, including the continuing change of crop genetic makeup as well as of farming systems, however, keeps entailing new risks. Even resistance of cultivars practically never means immunity, and resistance hardly ever is durable. For the many seed-borne viruses of legumes, in particular, the continuing globalization of trade and traffic create a special hazard. Certification of commercial seed, which must be grown in the open with a view to the immense quantities needed, and quarantine of germplasm, which cannot handle individual seeds of the large quantities that are distributed worldwide, is unlikely to ever guarantee absolute freedom from the many known and still unknown and often latent seed-borne viruses.

Most virus control in crops, therefore, involves interference through crop management with the ecology of the viruses. Most measures are imperfect and are likely to entail new problems. Hence, man will never be ready to combat viruses in legumes and whatever other crops. Since the mostly preventive measures of control must be taken outside the field-grown crops, for example, at the source of infection, and what individual growers are doing or neglecting has a bearing on the health of crops of other growers, public interests are at stake. That is why public institutions, governments, and international organizations must remain involved as for regulation, quarantine, certification, advice, teaching, and continuing research. Such research, as on legume viruses, is of great relevance to human society. The study of legume viruses provides an outstanding example of the dynamics and complexity of viruses in their relation to crops.

See also: Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus; Luteoviruses; Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Geminiviruses; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes; Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Potyviruses; Virus Species.

Further Reading

Berger PH, Wyatt SD, Shiel PJ, *et al.* (1999) Phylogenetic analysis of the *Potyviridae* with emphasis on legume-infecting potyviruses. *Archives of Virology* 142: 1979.

Berger PH, Wyatt SD, Shiel PJ, Silbernagel MJ, Druffel K, and Mink GI (1997) Phylogenetic analysis of the *Potyviridae* with emphasis on legume-infecting potyviruses. *Archives of Virology* 142: 1979–1999.

Bos L (1970) The identification of three new viruses isolated from *Wisteria* and *Pisum* in the Netherlands, and the problem of variation within the potato virus Y group. *Netherlands Journal of Plant Pathology* 76: 8.

Bos L (1983) Plant virus ecology: The role of man, and the involvement of governments and international organizations. In: Plumb RT and Thresh JM (eds.) *Plant Virus Epidemiology; the Spread and Control of Aphid-Borne Viruses*, 7pp. Oxford: Blackwell.

Bos L (1992) New plant virus problems in developing countries: A corollary of agricultural modernization. *Advances in Virus Research* 41: 349.

Bos L (1996) *Research on Viruses of Legume Crops and the International Working Group on Legume Viruses; Historical Facts and Personal Reminiscences*, 151pp. Aleppo, Syria: International Working Group Legume Viruses.

Bos L, Hagedorn DJ, and Quantz L (1960) Suggested procedures for international identification of legume viruses. *Tijdschr. Plantenziekten* 66: 328.

Boswell KF and Gibbs AJ (1983) *Viruses of Legumes 1983. Descriptions and Keys from VIDE*, 139pp. Canberra: Research School of Biological Sciences, The Australian National University.

Edwardson RE and Christie RG (1986) *Viruses Infecting Forage Legumes,* monograph No.14, 742pp + appendices. Gainesville: University of Florida Agricultural Experiment Station.

Edwardson RE and Christie RG (1991) *CRC Handbook of Viruses Infecting Legumes*, 504pp. Boca Raton, FL: CRC Press.

Hampton RO (1967) Natural spread of viruses infectious to beans. *Phytopathology* 57: 476.

Jones RAC (2000) Determining 'threshold'levels for seed-borne virus infection in seed stocks. *Virus Research* 71: 171.

Summerfield RJ and Roberts EH (eds.) (1985) *Grain Legume Crops.* 859pp. London: Collins.

Thresh JM (1983) Progress curves of plant virus disease. *Advances in Applied Biology* 8: 1–85.

Van der Maesen LJG and Sadikin S (eds.) (1989) *Pulses. Plant Resources of South-East Asia No. 1.* 105pp. Wageningen: Pudoc.

Weiss F (1945) Viruses described primarily on leguminous vegetable and forage crops. *Plant Disease Reporter, Supplement* 155: 32.

Leishmaniaviruses

R Carrion Jr., Southwest Foundation for Biomedical Research, San Antonio, TX, USA
Y-T Ro, Konkuk University, Seoul, South Korea
J L Patterson, Southwest Foundation for Biomedical Research, San Antonio, TX, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Polyprotein An expressed protein comprising two functionally distinct domains.
Ribosomal frameshift Translation of a protein from an alternate reading frame.
Shine–Dalgarno sequence A consensus sequence (AGGAGG) that helps recruit the ribosome to the mRNA to initiate protein synthesis by aligning it with the start codon.

length and encodes two major proteins – the capsid protein and the RNA-dependent RNA polymerase (RdRp). Its capsid protein has a unique endoribonuclease activity that targets the viral genome in a site-specific manner. In addition to primary structure, a hairpin loop upstream of the cleavage site is essential for processing of the viral RNA. The polymerase is expressed as a polyprotein that is processed by a host-encoded cysteine protease. Over time, this ancient virus has elaborated a variety of mechanisms to control copy number that has allowed it to co-evolve with its protozoan host.

Introduction

Leishmania RNA virus (LRV) is the type species of the genus *Leishmaniavirus* in the family *Totiviridae*. The virus persistently infects the protozoan *Leishmania*. It possesses an icosahedral capsid of approximately 32–33 nm in diameter. The double-stranded (ds) RNA genome of leishmaniaviruses ranges in size from 5.2 to 5.3 kbp in

History

The first documentation that some strains of *Leishmania* were infected with a virus came in 1974 when virus-like particles (VLPs) were observed in the cytoplasm of *Leishmania hertigi*. However, over a decade would pass before molecular data were reported. Initially, a 6.0 kbp band was visualized on an ethidium bromide-stained gel of

parasite extracts from *Leishmania braziliensis guyanensis*. A probe generated from cDNA of nucleic acid moiety, termed LR1, failed to hybridize to the parasites genomic DNA confirming its existence as an extrachromosomal entity. Electron microscope examination of sucrose gradient fractions of parasite lysates confirmed the existence of 32 nm VLPs in fractions that contained LR1 RNA (**Figure 1**).

Additional virus-infected isolates were identified on the basis of RdRp activity. Those isolates exhibiting RdRp activity also harbored a 6 kbp nucleic acid that was determined to be dsRNA. The products generated from polymerase assays showed no hybridization to *Leishmania* genomic DNA, but did hybridize to the 6 kbp dsRNA, thus providing conclusive evidence that the dsRNA served as template. Soon after, electron microscopy correlated the polymerase activity with $CsCl_2$ purified virus particles. To date, 14 leishmaniavirus species comprising both New World and Old World viruses have been identified (**Table 1**).

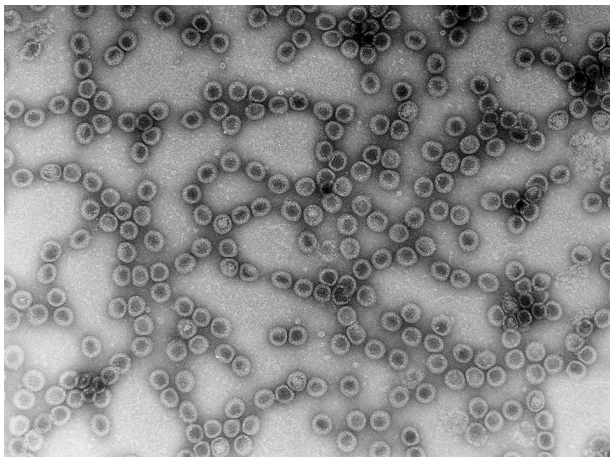


Figure 1 An electron micrograph of LRV1-4 particles isolated from a sucrose gradient of *Leishmania* lysate. Reproduced from Cadd TL and Patterson JL (1994) Synthesis of viruslike particles by expression of the putative capsid protein of Leishmania RNA virus in a recombinant baculovirus expression system. *Journal of Virology* 68: 358–365, with permission from American Society for Microbiology.

Initial studies to characterize the LRV polymerase revealed that the RdRp was associated with viral particles. *In vitro* polymerase assays resulted in the production of genome length RNA as well as a faster migrating RNA species. RNase protection assays confirmed that the genome length was dsRNA and the faster migrating species ssRNA. This evidence supports a dual function for the LRV polymerase as both replicase and transcriptase. The LRV replication cycle appears to undergo a strategy similar to the dsRNA totivirus that infects yeast. LRV replication involves generation of a full-length positive-sense RNA species that is encapsidated and serves as a template for negative-sense RNA synthesis. Positive-sense RNA transcripts are then produced from the parental dsRNA in a conservative manner and extruded into the cytoplasm where they are translated into viral proteins, or encapsidated to serve as template for viral replication.

Genome Organization

The genome of leishmaniaviruses ranges from 5.2 kbp (**Figure 2**) for the Old World viruses to 5.3 kbp for New World viruses. Open reading frames (ORFs) are encoded on the positive-sense strand of New World viruses (**Figure 3**). ORF1 and ORF X encode small ORFs that share no homology to any known protein sequence. These small ORFs correspond to the 5′-untranslated region (UTR), which is 449 nt in length in LRV1-4 showing greater than 90% identity to all known leishmaniaviruses at the nucleotide level. ORF2 encodes the 82 kDa viral capsid protein that has been shown to spontaneously assemble into VLPs when expressed

Table 1 Known leishmaniaviruses and associated sequences

Viruses	*Host range*	*Sequences available*	*Accession numbers*
LRV1-1	*Leishmania* CUMC1	Complete genome (1–5284)	M92355
LRV1-2	*Leishmania* CUMC3	Partial 5′-UTR (1–253)	AF230881
LRV1-3	*Leishmania* M2904		
LRV1-4	*Leishmania* M4147	Complete genome (1–5283)	U01899
LRV1-5	*Leishmania* M1142		
LRV1-6	*Leishmania* M1176		
LRV1-7	*Leishmania* BOS12	Partial 5′-UTR (1–251)	AF230882
LRV1-8	*Leishmania* BOS16	Partial 5′-UTR (1–253)	AF230883
LRV1-9	*Leishmania* M6200	Partial 5′-UTR (1–253)	AF230884
LRV1-10	*Leishmania* LC76	Partial 5′-UTR (1–251)	AF230885
LRV1-11	*Leishmania* LH77	Partial 5′-UTR (1–251)	AF230886
LRV1-12	*Leishmania* LC56		
LRV1-13	*Leishmania* NC	Partial 5′-UTR/RdRp	U23810/U39069
LRV2-1	*Leishmania* 5ASKH	Complete genome (1–5241)	U32108

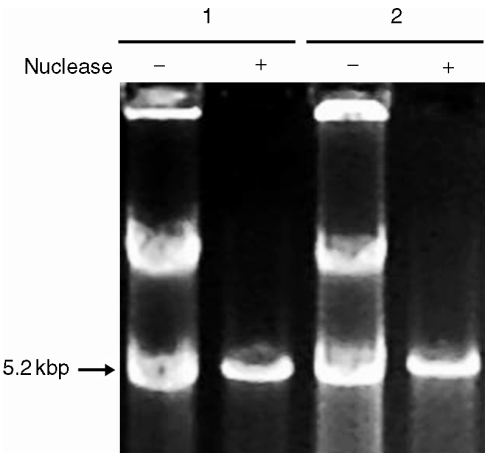


Figure 2 New World parasite strains containing a dsRNA moeity. Equal concentrations of total RNA from late-log phase parasites were incubated in the presence (+) or absence (–) of mung bean nuclease/DNase then resolved on a 1% agarose gel. The gel was stained with ethidium bromide then visualized on a UV transilluminator. Note the presence of dsRNA at approximately 5.2 kbp.



Figure 3 Schematic representation of genome organization of New World and Old World leishmaniaviruses. Reproduced from Scheffter S, Widmer G, and Patterson JL (1994) Complete sequence of Leishmania RNA virus 1-4 and identification of conserved sequences. 199: 479–483, with permission from Elsevier.

in vitro. The viral polymerase is encoded by ORF3, which contains the conserved six consensus RdRp motifs and overlaps the capsid domain by 71 nt. *In vitro* data and sequence analysis support the notion that the viral polymerase is expressed by a ribosomal frameshift to yield a capsid–polymerase polyprotein in a manner similar to several other members of the family *Totiviridae*.

Endoribonuclease Activity

A unique feature of LRV1-4 is that it encodes endoribonuclease activity within its assembled capsid particle. The cleavage event is site specific requiring a minimal essential sequence, and is mediated by both purified wild-type virus particles, as well as *in vitro* expressed VLPs. Later it was shown that the Old World LRV2-1 virus particles also possessed site-specific endoribonuclease activity.

Mutational analysis of the viral genome suggests that the minimal essential sequence required for site-specific targeting of the capsid endoribonuclease resides within viral nucleotides 249–342 in the 5′-UTR. This region contains conserved secondary structure, which was hypothesized to contain determinants for capsid-mediated cleavage. Nuclease mapping and site-specific mutagenesis identified a hairpin structure 40 nt upstream of the cleavage site that, when eliminated, abrogated cleavage. Reconstituting the hairpin through compensatory mutations allowed for precise cleavage of the substrate. Characterization of endoribonuclease activity associated with baculovirus expressed LRV1-4 VLPs reveals an absolute requirement for divalent cations such as Mg^{2+} and Ca^{2+}. This requirement is probably for stabilizing substrate secondary structure since electrophoretic mobility shift assay (EMSA) showed that the divalent cation itself changed the substrate RNA mobility. Furthermore, endoribonuclease activity was greatly inhibited by salt concentrations greater than 25 mM, indicating that high concentration of salts may interfere with enzyme-substrate interactions.

Processing of a Viral Polyprotein by a Cellular Protease

The inability to observe a capsid–polymerase fusion protein *in vivo* is explained by a proteoytic cleavage event that separates the two domains. Host cell extract from either virus infected or uninfected *Leishmania* strains produced a cleavage event in an *in vitro* expressed capsid–polymerase polyprotein. In both cases, a specific cleavage event was observed to yield both 95 and 82 kDa fragments, which matched well the predicted sizes of the individual polymerase and capsid products, respectively. The presence of specific proteolytic cleavage activities in both virus-infected and -uninfected parasite extracts indicates that the protease is host-encoded. It has been recently demonstrated that the protease responsible for cleavage is a unique trypsin-like cysteine protease. The purified protease is related to the cysteine proteases of *Leishamnia mexicana* found to be important for survival within macrophages. They are part of a large array of cathepsin L-like proteases that are differentially expressed. That processing of the Gag-Pol polyprotein is linked to an essential gene for parasite survival suggests that it plays a role in ensuring maintenance the LRV infection. The role of the proteolytic processing in generating functional viral proteins and/or its role in the virus replication has yet to be deciphered.

It is hypothesized that excessive production of viral proteins may adversely affect host macromolecule synthesis potentially leading to cell death, and ultimately a selective pressure to eliminate the virus. Targeting a fusion protein is an effective approach to maintain a low copy number. It is believed that the LRV replication cycle

is similar to the yeast LA virus. From *gag-pol* overlapping reading frames, capsid protein is produced primarily until a ribosomal frameshifting event occurs. The Gag-Pol fusion proteins dimerize, enabling the binding of positive-sense viral RNA, which primes Gag polymerization and genome packaging. It can be envisioned that by cleaving the fusion protein at the junction of the Gag-Pol domains, packaging would ultimately be aborted. This scenario would require tight regulation of protease activity, since indiscriminate proteolysis would lead to loss of virus.

Mechanisms of Viral Persistence

Expression of proteins in *Leishmania*, as well as other trypanosomes, requires a highly conserved 39 nt splice leader. Early studies have shown that LRV1-4 transcripts do not possess the leader sequence and also lack a cap structure. Therefore, LRV1-4 must translate its proteins by a cap-independent mechanism. Computer sequence analysis of LRV1-4 clones has revealed that the 5′-UTR contains extensive secondary structure similar to those that promote internal initiation in poliovirus. In fact, it has been demonstrated that the 5′-UTR can function as a *cis*-element in promoting ribosomal entry. These observations, in addition to its similarity to the internal initiation sites of picornaviruses, suggest that the 5′-UTR functions as an internal ribosome entry site (IRES) in LRV1-4.

If the 5′-UTR of LRV1-4 functions as an IRES, how does one reconcile the observed removal of the UTR with its presumed role as a *cis*-acting internal initiation element? The conundrum may be explained by the hypothesis that the endoribonuclease activity plays a regulatory role in maintaining a persistent infection. Phylogenetic evidence has confirmed that LRV1-4 is an ancient virus that co-evolved with its protozoan host. Since the parasite's reproductive cycle is predominately asexual, and there is no evidence horizontal transfer between parasites, viral persistence is dependent on maintaining a copy number compatible with host survival. Excessive production of viral proteins may adversely affect host macromolecule synthesis potentially leading to cell death, and ultimately, a selective pressure to eliminate the virus. Cleavage of the LRV1-4 transcripts could alter the functionality of the message by affecting translation, RNA stability, RNA packaging, and/or viral replication. These events are not exclusive as LRV1-4 is likely to employ a variety of regulatory mechanisms to maintain a persistent infection. It has been demonstrated that cleavage of LRV1-4 RNA correlates with the growth of the parasite. In addition, it has been shown that overexpression of capsid protein results in a suppression of LRV replication. These observations support a tightly regulated model of viral translation in which viral transcripts are being translated efficiently by cap-independent mechanisms involving the 5′-UTR. As the molar concentration of VLPs increases, endoribonuclease cleavage increases, thus maintaining a low-level viral persistence by decreasing efficiency of viral translation.

Alternatively, cleavage of the 5′-UTR may represent removal of translation-attenuating *cis*-elements. Other viral systems, such as coronaviruses, have translation-attenuating elements which are selected for during persistent infection. The extensive secondary structure present in the LRV1-4 UTR may function in the same respect. Cleavage of the LRV1-4 5′-UTR exposes a cryptic protein-binding site on transcripts that permits complexing with *Leishmania* cytoplasmic proteins. Although the translation machinery of *Leishmania* has not been well characterized, the speculation is that these factors are accessory proteins which may facilitate translation. Analysis of LRV1-4 UTR sequences has identified a pyrimidine-rich region complementary to a pyrimidine-rich sequence of *Leishmania* 18S rRNA. It is postulated that binding of ribosomal RNA would facilitate translation analogous to the Shine–Dalgarno sequence in prokaryotes. In this case, the cleavage of the 5′-UTR would be removing translation attenuation factors and noninitiating AUGs to allow for efficient viral gene expression through the binding of translation accessory proteins as well as rRNA to cleaved transcripts.

Another manner in which the virus may interact with its host is through the cleavage of host RNA. Sequence analysis of *Leishmania* rRNA and the endoribonuclease cleavage site of both New World and Old World viruses have identified several regions of the rRNA sequence that possesses similarity to the cleavage site. If the hypothesis were validated, cleavage of rRNA would likely have an effect on host translation machinery. Since the translation of host genes is predicated upon the presence of a splice-leader sequence, host genes may have an advantage over viral transcripts in recruiting translation machinery. LRV has adapted by allowing for efficient internal initiation via an IRES; the endoribonuclease may have evolved to provide an additional role in promoting viral gene expression by decreasing translation of cellular mRNAs.

It is entirely possible that the cleavage event benefits the virus in multiple ways. When virus copy number is low, translation is allowed to proceed via a cap-independent mechanism using the LRV IRES. As copy number increases, transcripts are cleaved in addition to *Leishmania* rRNA and a slower more regulated form of translation occurs. Cryptic sites are exposed on the cleaved transcript and bind host translation accessory proteins. The short 5′-cleaved fragment also binds the cleaved transcript blocking assembly of translation accessory proteins, thus decreasing the

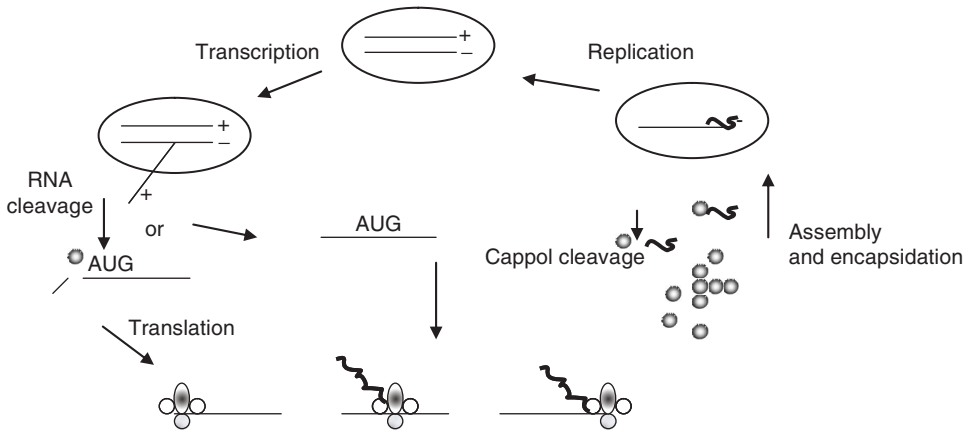


Figure 4 A model of LRV replication. Reproduced from MacBeth KJ and Patterson JL (1998) Overview of the leishmaniavirus endoribonuclease and functions of other endoribonucleases affecting viral gene expression. *Journal of Experimental Zoology* 282(1–2): 254–260 (review), with permission from Research Trends.

rate of viral protein synthesis. The result is a tightly regulated persistent infection compatible with parasite survival.

Leishmaniavirus: A Model

Recent advances in studies on leishmaniavirus have permitted the creation of a model for the LRV-replication cycle (**Figure 4**). As described above, there are many opportunities for regulation that have potentially aided in maintaining a persistent infection as the virus coevolved with its host. Beginning with a mature particle containing dsRNA and viral transcriptase, dsRNA is transcribed in a conservative fashion and the resultant transcript is extruded from capsid. At a low molar abundance of assembled particles, viral proteins are translated by a cap-independent mechanism utilizing the LRV IRES located within the 5′-UTR. As the number of viral particles increases, the endoribonuclease activity of the LRV capsid cleaves the viral transcripts slowing the rate of translation. The cleaved 5′-UTR and the 3′-cleaved product compete for host proteins, which may be essential for translation of viral transcripts lacking a cap structure and now an IRES. Translation results in the production of a large number of capsid proteins, then occasionally through a ribosomal frameshift, a less abundant capsid–polymerase polyprotein is expressed. A host encoded-cysteine protease quickly cleaves the polyprotein in a process that is probably required for regulating viral copy number or generating functional viral proteins. Full-length viral RNA then presumably binds to a yet-to-be-determined binding site on the RdRp ensuring encapsidation of the genome. Replication follows through an unknown mechanism, thus reconstituting the dsRNA genome.

See also: Giardiaviruses; Totiviruses; Yeast L-A Virus.

Further Reading

Cadd TL and Patterson JL (1994) Synthesis of virus like particles by expression of the putative capsid protein of Leishmania RNA virus in a recombinant baculovirus expression system. *Journal of Virology* 68: 358–365.

Carrion R, Jr., Ro YT, and Patterson JL (2003) Purification, identification, and biochemical characterization of a host-encoded cysteine protease that cleaves a leishmaniavirus gag-pol polyprotein. *Journal of Virology* 77(19): 10448–10455.

Lee SE, Suh JM, Scheffter S, Patterson JL, and Chung IK (1996) Identification of a ribosomal frameshift in Leishmania RNA virus 1-4. *Journal of Biochemistry* (*Tokyo*) 120(1): 22–25.

MacBeth KJ and Patterson JL (1995) Single-site cleavage in the 5′-untranslated region of Leishmaniavirus RNA is mediated by the viral capsid protein. *Proceedings of the National Academy of Sciences, USA* 92(19): 8994–8998.

MacBeth KJ and Patterson JL (1995) The short transcript of Leishmania RNA virus is generated by RNA cleavage. *Journal of Virology* 69(6): 3458–3464.

MacBeth KJ and Patterson JL (1998) Overview of the leishmaniavirus endoribonuclease and functions of other endoribonucleases affecting viral gene expression. *Journal of Experimental Zoology* 282(1–2): 254–260 (review).

Ro YT and Patterson JL (2000) Identification of the minimal essential RNA sequences responsible for site-specific targeting of the Leishmania RNA virus 1-4 capsid endoribonuclease. *Journal of Virology* 74(1): 130–138.

Ro YT, Scheffter SM, and Patterson JL (1997) Specific *in vitro* cleavage of a Leishmania virus capsid-RNA-dependent RNA polymerase polyprotein by a host cysteine-like protease. *Journal of Virology* 71(12): 8983–8990.

Scheffter S, Widmer G, and Patterson JL (1994) Complete sequence of Leishmania RNA virus 1-4 and identification of conserved sequences. 199: 479–483.

Weeks RS, Patterson JL, Stuart K, and Widmer G (1992) Transcribing and replicating particles in a double-stranded RNA virus from Leishmania. *Molecular and Biochemical Parasitology* 52(2): 207–213.

Widmer G, Comeau AM, Furlong DB, Wirth DF, and Patterson JL (1989) Characterization of a RNA virus from the parasite Leishmania. *Proceedings of the National Academy of Sciences, USA* 86(15): 5979–5982.

Widmer G and Dooley S (1995) Phylogenetic analysis of Leishmania RNA virus and Leishmania suggests ancient virus–parasite association. *Nucleic Acids Research* 23(12): 2300–2304.

Leporipoviruses and Suipoxviruses

G McFadden, University of Florida, Gainesville, FL, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Poxviruses of leporids and swine cause a broad range of symptoms varying from mild lesions of the skin right up to the lethal systemic diseases (**Table 1**). The agent of myxomatosis, a virulent disease of domestic rabbits described originally by G. Sanarelli in 1896, was in fact the first viral pathogen discovered for a laboratory animal. The close similarity of myxoma virus (MYX) with other members of the poxvirus family, such as variola and fowlpox viruses, was first recognized by Aragão in 1927. MYX is notable because, although it causes rather benign lesions in the native *Sylvilagus* rabbit (the brush rabbit in North America and the tropical forest rabbit in South America), when introduced to the European (*Oryctolagus*) rabbit it causes an invasive disease syndrome called myxomatosis with up to 100% mortality. MYX was the first viral agent ever introduced into the wild for the purpose of eradicating a vertebrate pest, namely the feral European rabbit population in Australia in 1950 and, 2 years later, in Europe. The resulting genetic selection of virus isolates with lesser pathogenicity and upsurgence of rabbits with greater resistance to the viral disease was studied intensively by Frank Fenner and his colleagues as a model system to investigate the ecological consequences of virus/host evolution in an outbred population.

Also, of interest to the history of animal virology is the fact that the first DNA virus associated with transmissible tumors was Shope fibroma virus (SFV), described in 1932 by Richard Shope as an infectious agent of fibroma-like hyperplasia in cottontail rabbits (*Sylvilagus floridanus*) in the eastern USA. It is likely that the agent of 'hare sarcoma', described first in Germany in 1909, was also a poxvirus, now called hare fibroma virus (HFV). HFV remains the only leporipoxvirus to have arisen outside the Americas but its biology closely resembles that of SFV.

Very little is known about the remaining leporipoxviruses. Subcutaneous fibromatosis in gray squirrels of the eastern USA and western gray squirrels in California, caused by poxviruses now collectively called squirrel fibroma virus (SqFV), has been observed since 1936, but their rigorous classification with the MYX–SFV group was not made until 1951 by L. Kilham. Similarly, HFV, described first in 1959 in the European hare (*Lepus europaeus*), was also shown to be a closely related poxvirus in 1961. In 1983, an outbreak of a disease resembling

Table 1 Members of the genera *Leporipoxvirus* and *Suipoxvirus*

Member	*Abbreviation*	*Natural host*	*Major arthropod vector*	*Natural host disease*	*Disease in domesticated European rabbit (Oryctolagus cuniculus)*
Leporipoxvirus					
Myxoma	MYX	California brush rabbit[a], S. American tapeti[b] (*Sylvilagus* sp.)	Mosquito, flea	Localized benign fibroma	Systemic lethal myxomatosis
Rabbit fibroma (Shope fibroma)	SFV	N. American cottontail rabbit (*Sylvilagus floridans*)	Mosquito, flea	Localized benign fibroma	Localized benign fibroma
Malignant rabbit fibroma[c]	MRV	Lab. rabbit[d] (*Oryctolagus cuniculus*)		Not observed in wild	Systemic lethal syndrome similar to myxomatosis
Squirrel fibroma	SqFV	Gray squirrel (*Sciurus* sp.)	Probably mosquito	Localized or multiple fibromas	Occasional nodular dermal lesions
Hare fibroma	HFV	Wild hares (*Lepus* sp.)	Probably mosquito	Localized benign fibroma	Localized benign fibroma
Suipoxvirus					
Swinepox	SPV	Domestic pigs (Suidae sp.)	Hog lice	Localized cutaneous lesions	Intradermal lesions but no serial propagation

[a]Also called Marshall–Regnery myxoma.
[b]Also called Aragão's (or Brazilian) myxoma.
[c]Laboratory recombinant between MYX and SFV.
[d]MRV has been propagated only by serial inoculation of laboratory rabbits and in cultured cells.

myxomatosis in laboratory rabbits in San Diego was caused by a novel leporipoxvirus later shown to be a genetic recombinant between SFV and a still-undefined strain of MYX. This virus, called malignant rabbit fibroma virus (MRV), has never been observed in wild rabbit populations but is of interest as an experimental model for poxvirus-induced immunosuppression and tumorigenesis. In most respects, MRV can be considered to be a substrain of MYX.

Based on landmark experiments with pneumococcus in the 1920s, the very first example of what was believed to be genetic interaction between viruses was reported in 1936 with the discovery that heat-inactivated myxoma could be reactivated with live SFV (Berry–Dedrick transformation), but later work showed this to be a genome rescue phenomenon rather than true recombination.

The only known member of the *Suipoxvirus* genus, swinepox virus (SPV), has been observed sporadically in pig populations throughout the world, but is not considered a serious pathogen because infected animals usually have only moderate symptoms and recover completely.

Taxonomy and Classification

The genera *Leporipoxvirus* and *Suipoxvirus* are in the subfamily *Chordopoxvirinae* of the family *Poxviridae*. The prefix 'lepori' comes from Latin *lepus* or *leporis* ('hare') and 'sui' from Latin *sus* ('swine'), to denote the relatively restricted host range of these viruses. All the viruses in the genus *Leporipoxvirus* can be shown to be closely related to each other by serology, immunodiffusion, and fluorescent antibody tests, although antigenic differences can be detected in strains of MYX. SPV (genus *Suipoxvirus*) is antigenically unique and is not known to have any closely related members. In terms of broad features, all are typical poxviruses, with characteristic brick-shaped virions containing a double-stranded DNA (dsDNA) genome with covalently closed hairpin termini and terminal inverted repeat (TIR) sequences. Like other poxviruses, viral macromolecular synthesis takes place exclusively in the cytoplasm of infected cells.

Properties of the Virion

As for all other members of the poxvirus family, the virions have a characteristic brick-shaped morphology with dimensions of approximately 250–300 nm × 250 nm × 200 nm. The leporipoxviruses are uniquely sensitive to ether and chloroform but otherwise the virions are very stable at ambient temperatures and in skin lesions. In all other respects, such as chemical composition and physical properties, the virus particles are very similar to those of vaccinia virus (VACV).

Properties of the Viral DNA and Protein

Complete genomic sequences are available for SFV, MYX, and SPV. The leporipoxviruses have dsDNA genomes of 160–163 kbp, with hairpin termini and TIR sequences of 10–13 kbp. SPV DNA is somewhat smaller (146.5 kbp) but otherwise the genome has similar characteristics. Each virus encodes from 150 to 160 proteins. A web-based resource is available that records the predicted proteins expressed by these three poxviruses (see 'Relevant website' section). Viral DNA of leporipoxviruses cross-hybridize at moderate stringencies only with other members of the genus and SPV DNA is unique and is not known to cross-hybridize with any other poxvirus DNA. The MRV DNA genome is 95% identical to that of MYX, except that it possesses five genes derived from SFV plus three SFV/MYX fusion genes.

The nucleotide composition of the leporipoxviruses (44% G + C for MYX) is higher than that of the orthopoxviruses (35% for VACV) but there is evidence that many of the viral genes important for virus replication, gene expression, and viral assembly are conserved between the genera. These conserved genes are clustered near the central regions of the viral genome. In contrast, viral genes mapping near the genomic termini show considerable variability, and are believed to encode many of the specific determinants of pathogenesis, host range, and disease characteristics.

The protein complexities of these viruses are comparable to those of most poxviruses, although the profiles are unique for each member. In general, about 80–90 poxvirus genes are relatively well conserved among the various member poxviruses, whereas the remainder (usually in excess of 50–60 genes) are more diverged and specify the unique features of virus–host interactions, such as host tropism, immunomodulation, and virulence.

DNA Replication, Transcription, and Translation

All of the major features of macromolecular synthesis by these viruses are very similar to those deduced for the prototype poxvirus, VACV. Viral DNA synthesis is restricted to cytoplasmic sites, although replication for leporipoxviruses tends to be initiated somewhat more slowly than for VACV. The virus-encoded transcriptional apparatus is well conserved between the poxvirus genera, and many of the important regulatory signals that are

utilized by VACV, such as promoters and transcription termination sequences, are also utilized with comparable efficiency in the leporipoxviruses. Thus, viral genes from one genus can be introduced to another by recombination or by DNA transfection technologies to generate chimeric virus constructs that maintain the correct regulation of the new genetic information. As in the case of VACV, transcriptional units can be of different kinetic classes (early/intermediate/late) and there is no splicing of viral mRNA.

The leporipoxviruses replicate in cytoplasmic factories that appear by microscopic analysis as eosinophilic B-type inclusion bodies. These factories, also called virosomes, can be visualized by Feulgen, Giemsa, or fluorescent antibody staining. SPV produces nuclear inclusions and vacuolations in addition to cytoplasmic bodies but these nuclear alterations are not believed to be sites of viral replication.

Molecular Mechanisms of Pathogenesis

Since these viruses are of only minor veterinary importance, recent research has focused on the elucidation of the determinants for viral virulence, particularly with respect to the strategies that these viruses employ to subvert the immune system of the infected host. Particular attention has been paid to the mechanism(s) underlying the immune dysfunction caused by MYX infection in *Oryctolagus* rabbits. To date, at least two classes of viral gene products have been directly implicated in the immunomodulation induced by these viruses:

1. 'Virokines' are secreted virus-encoded proteins that are targeted to host-specific pathways outside the infected cell. For example, SFV and MYX encode growth factors related to epidermal growth factor and transforming growth factor α that participate in stimulating fibroblastic proliferation at primary and secondary tumors.
2. 'Viroceptors' are viral proteins that mimic cellular receptors and function by sequestering important host cytokines that normally participate in the antiviral immune response. Leporipoxviral-encoded receptor-like molecules have been discovered for tumor necrosis factor (TNF) and interferon γ (IFN-γ), and may exist for other antiviral lymphokines as well. SPV encodes a novel homolog of cellular chemokine receptors, and the leporipoxviruses express secreted chemokine-binding proteins that are important for virus pathogenesis.

Interference with antigen presentation by MYX is also believed to play a role in circumventing T-cell recognition during early stages of virus infection. One MYX gene product responsible for evading immune clearance, designated Serp1, is an extracellular inhibitor of cellular serine proteinases, and the purified protein exhibits potent anti-inflammatory properties in a variety of animal model systems of inflammatory disease.

Geographic and Seasonal Distribution

All three major species of *Sylvilagus* rabbits in the Americas have endemic fibroma-like poxviruses, and myxomatosis is now established in wild *Oryctolagus* rabbit populations of South America, Europe, and Australia. SqFV and HFV have been reported to date only in North America and Europe, respectively. The leporipoxviruses in the wild undergo seasonal fluctuations that correlate well with increased populations of arthropod vectors in summer and autumn, most prominently mosquitoes. An exception to this is found in Britain, where the major vector of MYX is the flea, which is not as seasonally variable.

In the case of SPV, outbreaks are not tied to seasonal cycles but are generally associated with the degree of hog lice infestation.

Host Range and Virus Propagation

These viruses demonstrate a very restricted host range in terms of ability to cause disease, although viral replication can also occur in cultured cells from some nonsusceptible hosts as well. In some cases, viral replication in tissue culture monolayers or chicken chorioallantoic membranes produces 'foci' in which infected cells manifest minimal cytopathic effects, thus permitting macroscopic cell aggregations to develop. The extent of cytopathology is markedly influenced by both the cell type and the virus strain, and in some instances the infected cells may detach from the monolayer to produce visible plaques. When viral replication is relatively slow and the toxicity to the target cell sufficiently moderate, a chronically infected carrier culture can be established in which progeny virus production persists for extended passages. Although poxviruses cannot permanently transform primary cells into an immortalized state, cells persistently infected with the fibroma-inducing leporipoxviruses can assume many of the phenotypic characteristics associated with the transformed phenotype, such as novel morphology, growth in reduced serum, and ability to form colonies in soft agar. It is likely that some of these phenotypic characteristics are facilitated by secreted poxviral proteins (virokines) that mimic cellular mitogens, such as epidermal growth factor, and trigger neighboring cells into excessive proliferation.

In the cases of the benign leporipoxviruses and SPV, replication is restricted to dermal and subcutaneous sites, with occasional involvement of draining lymph nodes. However, MYX and MRV are unique in

that they also replicate efficiently in lymphoid cells, such as macrophages, B cells, and T cells. MYX, like human immunodeficiency virus type 1, replicates in either resting or stimulated T cells, and can be readily isolated from splenocyte cultures. The molecular basis for the uniquely permissive nature of MYX replication in lymphocytes is unknown, but is unquestionably an important factor in the extreme virulence of myxomatosis. Several MYX genes have been identified (e.g., M-T2, M-T4, M-T5, and M11L) that express host range determinants that block the cellular apoptosis response to infection of lymphocytes.

Evolution and Genetic Variability

The deliberate release of MYX into rabbit populations of Australia, France, and Britain in the early 1950s provided a unique opportunity to study the natural selection pressures exerted on a particularly virulent virus/host interaction. There is an extensive literature on the ecological consequences of the feral rabbit eradication program, and the rapid evolution of myxomatosis in the wild is well documented. Although the original South American MYX virus strain that was introduced left very few survivors in selected populations, within a few years attenuated viral strains with reduced virulence took over and more resistant rabbits became predominant.

In terms of the categories of viral virulence, some strains of MYX are classified as highly virulent (e.g., Moses and Lausanne), and attenuated variants exist down to relatively nonpathogenic (e.g., neuromyxoma and the Nottingham strains). Little is known about the extent of genetic variation in other leporipoxviruses, although different isolates of SFV show marked variations in tumorigenicity. Generally, leporids that recover from infection with one member either become resistant or undergo partial protection from infection by another member.

SPV shares some antigenic crossreactivity with VACV, but neutralizing antibody does not confer cross-protection for secondary infection by members of different genera.

Transmission and Tissue Tropism

The principal mode of transmission is by biting arthropod vectors, and the major inoculation route is dermal. Since these viruses do not replicate in the vector, the transmission is purely mechanical and hence virus spread can be readily accomplished by alternative routes. Thus, mosquitoes, fleas, blackflies, ticks, lice, mites, and even thistles and the claws of predatory birds have all been implicated in leporipoxvirus transmission. The efficiency of transmission by arthropods is quite variable, and is related to viral titers in skin lesions as well as the size of the vector populations. There are no known respiratory or oral routes of infection with members of either genus, but in some infections, such as MYX in domestic rabbits, the disease can be transmitted by direct contact with ocular discharges or open cutaneous lesions.

The sui- and leporipoxviruses in their native hosts are specific for the epidermis or subdermis and usually do not progress to secondary sites, although draining lymph nodes can be affected. However, in the specific case of MYX infection of the domestic rabbit, the virus can propagate efficiently in lymphocytes and migrate via infected leukocytes through lymphatic channels to establish secondary sites of infection. Recently, it was shown that MYX can productively infect and kill a variety of human cancer cells, likely due to cell signaling changes associated with cellular transformation in the tumor cells.

Pathogenicity

The leporipoxviruses are restricted to rabbits, squirrels, and hares, and swinepox is found only in domestic pigs. For SFV infection of *Sylvilagus* rabbits, tumors can last for many months before regressing, whereas in *Oryctolagus* rabbits recovery is usually complete within a few weeks. Only MYX manifests dramatic alterations in pathogenicity when the European rabbit is infected. For all of these viruses, the immune status of the host rabbit plays a critical role; for example, in adult rabbits, SFV rarely causes disease symptoms except for the primary fibroblastic lesion, but in newborn or immunocompromised animals the infection can lead to invasive tumors and much higher titers of infectious virus in infected tissues. Agents such as cortisone, X-rays, or immunosuppressants can dramatically increase SFV tumor development, and chemical promoters like 3,4-benzopyrene or methylcholanthrene can predispose progression to invasive fibromatosis or even metastatic fibrosarcoma.

The ability to cause collapse of the host immune response, replicate in lymphocytes, and spread efficiently to secondary sites is a unique property of MYX in *Oryctolagus* rabbits. The myxomatosis syndrome can be associated with multiple external signs (e.g., South American MYX) or may have relatively fewer gross symptoms (e.g., California MYX) and mortalities can range up to 100%. Supervening Gram-negative bacterial infections in the respiratory tract and conjuctiva are often observed concomitantly during myxomatosis, particularly by the adventitious pathogens *Pasteurella multocida* or *Bordetella bronchoseptica*, and contribute to the lethality of the disease.

SPV is only mildly pathogenic in pigs although it can cause a minor level of mortality, usually associated with milk-feeding reduction in younger animals.

Clinical Features of Infection

The cutaneous tumors induced by the different leporipoxviruses in their natural hosts are clinically very similar

to each other. The fibromas are rarely associated with any other symptoms, such as fever or appetite loss, and invariably regress as long as the animal is not otherwise immunocompromised. In the case of MYX in *Oryctolagus* rabbits, however, the symptoms rapidly become severe as the tumors fail to regress and the concomitant immunosuppression contributes to the lethal myxomatosis syndrome. The clinical features of myxomatosis are influenced by the genetic background of both the virus strain and the rabbit host. In the preacute form of the disease caused by California MYX, the rabbits succumb in less than 1 week, and often have only minor external symptoms, such as inflammation and edema of the eyelids. Skin hemorrhages can be observed in some cases and convulsions often precede death. In the acute form caused by South American strains of MYX, the rabbits survive 1–2 weeks and develop more distinctive symptoms. The primary tumor can be either flat and diffuse or protuberant, and secondary site tumors around the nose, eyes, and ears become prominent by 6–7 days, at which time purulent exudates from the nose and eyes frequently develop. The cutaneous tumors often become necrotic and a generalized immune dysfunction exacerbates the progressive secondary bacterial infestation of the respiratory tract. In the case of the more attenuated MYX isolates, such as neuromyxoma, the disease course is less severe and may be associated with little or no mortality.

The disease course of SPV in pigs is rather different, and resembles that of VACV in humans. Inoculation results in localized dermal papules, which progress to vesicles and pustules, after which the lesions crust and scab over. The only clinical symptom is occasional minor fever and the animals recover within 3 weeks.

Pathology and Histopathology

The primary tumors caused by leporipoxviruses in *Sylvilagus* rabbits, squirrels, and hares all closely resemble proliferant fibromas. Following inoculation, an acute inflammatory reaction occurs with infiltration of polymorphonuclear and mononuclear cells and proliferation of fibroblast-like cells of uncertain origin. The 'tumor' consists of pleomorphic cells imbedded in a matrix of intercellular fibrils of collagen. Unlike the transformed cells induced by other DNA tumor viruses, cells from poxviral tumors are not immortalized and cannot be propagated independently. Instead they appear to require secreted virus-encoded proteins in order to sustain the hyperproliferative state. Inclusion bodies characteristic of poxviral replication can be observed in the cytoplasms of epithelial and some fibroma cells. As the tumor develops, mononuclear leukocyte cuffing of adjacent vessels is observed and at the base of the tumor there is accumulation of lymphocytes, plasma cells, macrophages, and neutrophils. The ratio between influx of inflammatory cells and fibroblast proliferation is variable but generally there is little or no necrosis. The speed with which immune cells clear the viral infection and reverse the hyperproliferation can range from 1–2 weeks to 6 months, depending on both the virus and the host.

The principal difference between the benign fibroma syndrome described above and the devastating disease caused by MYX in *Oryctolagus* rabbits is that the latter virus efficiently propagates in host lymphocytes and is able to circumvent the cell-mediated immune response to the viral infection. The subcutaneous tumors consist of proliferating undifferentiated mesenchymal cells, which become large and stellate with prominent nuclei ('myxoma' cells). In surrounding tissue there can be extensive proliferation of endothelial cells of the local capillaries and venules, often to the point where complete occusion leads to extensive necrosis of the infected site. The overlying epithelial cells can show hyperplasia or degeneration, depending on the virus strain, and poxviral inclusion bodies are frequently observed in the prickle-cell layer. In some MYX strains, primary and secondary skin tumors can undergo extensive hemorrhage and internal lesions may be found in the stomach, intestines, and heart. The virus readily migrates to secondary sites within infected immune cells and concomitant cellular proliferation can be detected in the reticulum cells of lymph nodes and spleen, as well as the conjunctival and pulmonary alveolar epithelium. The nasal mucosa and conjunctiva overlying secondary tumors undergo squamous metaplasia such that the epithelia become nonciliated and nonkeratizing. Disruption of the ciliary architecture may be one of the factors that facilitate the extensive Gram-negative bacterial infections of the eyes, nose, and respiratory tract. Varying degrees of inflammatory cell infiltration by polymorphonuclear heterophils occur soon after infection but there is only a limited and ineffective cellular immune response. The lymph nodes and spleen show evidence of aberrant T-cell activation and hyperplasia, and infectious virus can be isolated from all lymphoid organs except the thymus. Death is believed to be caused by a combination of tissue damage from the increasing tumor burden, generalized immunosuppression, and debilitating bacterial colonization of the respiratory tract.

Little is known about SPV pathogenesis but gross features closely resemble those of the noninvasive orthopoxviruses in their native hosts.

Immune Response

The benign fibromas caused by SFV/SqFV/HFV regress, albeit slowly, due to a combined cellular and humoral immune response. These viruses are excellent antigens, and neutralizing antibody produced during recovery will also cross-react with other members of the genus. All of the leporipoxviruses are strongly cell associated, and

cell-mediated immunity is probably the single most important mechanism of viral clearance. Other immune mechanisms are also activated, including interferon production, antibody-mediated cell lysis, sensitized macrophages, and natural killer cells. Neutralizing antibody can last for many months after viral clearance and immunity is usually cross-protective to the other leporipoxviruses.

In the unique case of MYX in *Oryctolagus* rabbits the picture is very different. Although circulating antibody can be detected against virions, as determined by neutralization or agglutination, and against soluble antigens, as determined by complement fixation and precipitin tests, the antibody provides little protection against the disease progression. Instead, cellular immunity is severely compromised, and by day 6–7 lymphocytes (especially splenocytes) are demonstrably dysfunctional in their response to mitogens and lose the ability to secrete critical cytokines such as interleukin-2. Unlike the case of SFV, there is a notable absence of virus-specific T cells in either the spleen or draining lymph nodes. Immune dysfunction is common for viruses that replicate in lymphocytes, but the precise levels at which MYX intervenes in cellular immunity remain to be clarified. There is some evidence that these viruses interfere with the function of cell surface major histocompatibility complex (MHC) class I molecules, which could prevent proper viral antigen presentation and hence interfere with immune recognition of infected cells. Also, several virus-specific gene products have been shown to be secreted homologs of the cellular receptors for TNF and IFN-γ that are believed to bind and sequester these extracellular ligands in the vicinity of virus-infected cells and thus short-circuit immune pathways dependent on TNF and IFN-γ.

SPV-infected pigs generally recover from the infection and become immune to secondary challenge. There are few data on the nature of this immunity, but it bears close resemblance to that of VACV immunization in humans.

Prevention and Control

Since these viruses are spread principally by biting arthropods, vector control is the single most effective method of disease prevention. The viruses are susceptible to standard anti-poxvirus chemical agents, such as phosphonoacetic acid, arabinosyl cytosine, and rifampicin, but these are of limited utility in infected animals. Immunization against myxomatosis can be accomplished with live SFV or attenuated strains of MYX.

Future Perspectives

Now that DNA sequencing studies have revealed the genomic repertoire of so many poxviruses, it is likely that more viral proteins which determine the clinical characteristics of their diseases will be characterized. Studies on viral gene products that stimulate fibroblastic and endothelial cells to proliferate will likely provide information on how mitogenesis is regulated by surface receptors on these target cells. Some of the secreted virokines and viroceptors have the potential to be used as drugs to treat inflammatory diseases. The ability of MYX to replicate in lymphocytes offers an important system in which to elucidate the mechanisms of cellular tropism by which these viruses suppress the innate apoptosis response to virus infection. Furthermore, the analysis of virus-induced immunosuppression should shed light on the various immune strategies used by the host to combat viral infections in general. The restricted host ranges of the lepori- and suipoxviruses suggest the potential for the genetic manipulation of these viruses such that heterologous foreign antigen genes can be expressed for the purpose of developing novel vaccines against important pathogens of domestic leporids and swine. Finally, the ability of MYX to infect and kill many human tumor cells offers the potential as a therapy against human cancer.

See also: History of Virology: Vertebrate Viruses; Poxviruses; Vaccinia Virus; Viral Pathogenesis.

Further Reading

Barrett JW, Cao J-X, Hota-Mitchell S, and McFadden G (2001) Immunomodulatory proteins of myxoma virus. *Seminars in Immunology* 13: 73–84.

DiGiacomo RF and Maré CJ (1994) Viral diseases. In: Manning P, Ringler DH, and Newcomer CE (eds.) *The Biology of the Laboratory Rabbit,* 2nd edn., p. 171. San Diego, CA: Academic Press.

Fenner F and Radcliffe FN (1965) *Myxomatosis.* Cambridge: Cambridge University Press.

Johnston JB and McFadden G (2004) Technical knockout: Understanding poxvirus pathogenesis by selectively deleting viral immunomodulatory genes. *Cellular Microbiology* 6: 695–705.

Kerr PJ and Best SM (1998) Myxoma virus in rabbits. *Revue Scientifique et Technique de l'office International des Épizooties* 17: 256–268.

Kerr P and McFadden G (2002) Immune responses to myxoma virus. *Viral Immunology* 15: 229–246.

Lucas A and McFadden G (2004) Secreted immunomodulatory proteins as novel biotherapeutics. *Journal of Immunology* 173: 4765–4774.

Nazarian SH and McFadden G (2006) Immune evasion by poxviruses. *Future Medicine* 1: 129–132.

Seet BT, Johnston JB, Brunetti CR, *et al.* (2003) Poxviruses and immune evasion. *Annual Review of Immunology* 21: 377–423.

Sypula J, Wang F, Ma Y, Bell J, and McFadden G (2004) Myxoma virus tropism in human tumor cells. *Gene Therapy and Molecular Biology* 8: 103–114.

Relevant Website

http://www.poxvirus.org – Poxvirus Bioinformatics Resource Center.

Luteoviruses

L L Domier, USDA–ARS, Urbana, IL, USA
C J D'Arcy, University of Illinois at Urbana-Champaign, Urbana, IL, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Hemocoel The primary body cavity of most arthropods that contains most of the major organs and through which the hemolymph circulates.
Hemolymph A circulatory fluid in the body cavities (hemocoels) and tissues of arthropods that is analogous to blood and/or lymph of vertebrates.

Introduction

Viruses of the family *Luteoviridae* (luteovirids) cause economically important diseases in many monocotyledonous and dicotyledonous crop plants, including barley, wheat, potatoes, lettuce, legumes, and sugar beets. Yield reductions as high as 30% have been reported in epidemic years, although in some cases crops can be totally destroyed. Diseases caused by the viruses were recorded decades and even centuries before they were associated with the causal viruses. In many cases, the stunted, deformed, and discolored plants that result from luteovirid infection were thought to be the result of abiotic factors, such as mineral imbalances or stressful environmental conditions, or of other biotic agents. This, along with their inabilities to be transmitted mechanically, delayed the initial association of the symptoms with plant viruses. For example, curling of potato leaves was first described in Lancashire, UK, in the 1760s, but was not recognized as a specific disease of potato until 1905 and to be caused by an aphid-transmitted virus until the 1920s. The causal agent, potato leaf roll virus (PLRV), was not purified until the 1960s. Similarly, widespread disease outbreaks in cereals, probably caused by barley yellow dwarf virus (BYDV), were noted in the United States in 1907 and 1949. In 1951, a virus was proposed as the cause. Other diseases caused by luteovirids, like sugarcane yellow leaf, which is caused by sugarcane yellow leaf virus (ScYLV), were not described until the 1990s.

Taxonomy and Classification

Members of the family *Luteoviridae* were first grouped because of their common biological properties. These properties included persistent transmission by aphid vectors and the induction of yellowing symptoms in many infected host plants. 'Luteo' comes from the Latin *luteus*, which translates as yellowish. All luteovirids have small (*c.* 25 nm diameter) icosahedral particles, composed of one major and one minor protein component and a single molecule of positive-sense single-stranded RNA of approximately 5600 nt in length.

The family *Luteoviridae* is divided into three genera – *Luteovirus*, *Polerovirus* (derived from potato leaf roll), and *Enamovirus* (derived from pea enation mosaic) – based on the arrangements, sizes, and phylogenetic relationships of the predicted amino acid sequences of the open reading frames (ORFs). In some plant virus families, a single gene can be used to infer taxonomic and phylogenetic relationships. Within the family *Luteoviridae,* however, different taxonomic relationships can be predicted depending on whether sequences of the replicase (ORF2) or coat protein (CP; ORF3) genes are analyzed (**Figure 1**). ORFs 1 and 2 of the luteoviruses are most closely related to the polymerase genes of viruses of the family *Tombusviridae*, while ORFs 1 and 2 of the poleroviruses and enamoviruses are related to those of the genus *Sobemovirus.* These polymerase types are distantly related in evolutionary terms. Consequently, it has been suggested that luteovirid genomic RNAs arose by recombination between ancestral genomes containing the CP genes characteristic of the family *Luteoviridae* and genomes containing either of the two polymerase types. For taxonomic purposes, the polymerase type has been the primary determinant in assigning a virus to a genus. For this reason, viruses for which only CP sequences have been determined have not been assigned to a genus. The current members of the family are listed in **Table 1**. The genus *Luteovirus* contains five species, and the *Polerovirus* genus has nine species. The genus *Enamovirus* contains a single virus, pea enation mosaic virus 1 (PEMV-1). The family also contains 11 virus species that have not been assigned to a genus. Of these, recently determined sequences of genomic RNAs of BYDV-GAV and carrot red leaf virus (CtRLV) suggest that BYDV-GAV is a strain of BYDV-PAV and that CtRLV is a unique species in the genus *Polerovirus.*

Virion Properties

The sedimentation coefficients $S_{20,w}$ (in Svedberg units) for luteoviruses and poleroviruses range from 106S to 127S. Buoyant densities in CsCl are approximately 1.40 g cm^{-3}. The particles formed as result of the mixed

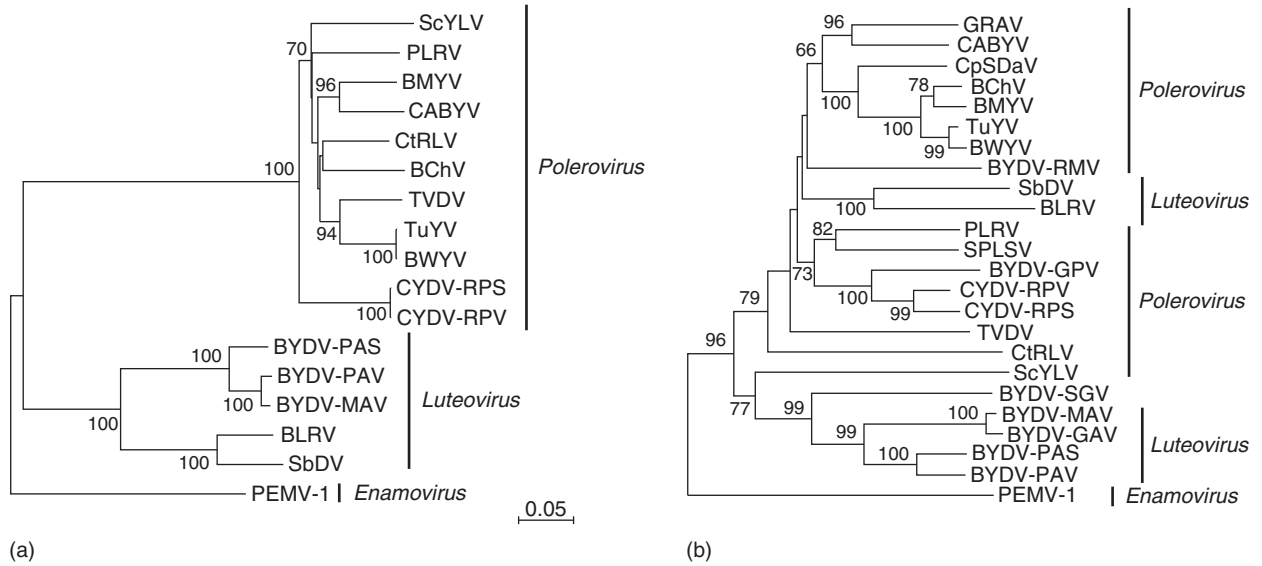


Figure 1 Phylogenetic relationships of the predicted amino acid sequences of the (a) RNA-dependent RNA polymerase (ORF2) and (b) major capsid protein (ORF3). When predicted amino acid sequences from ORF2 are used to group virus species the genera form three distinct groups. Using predicted amino acid sequences from ORF3, species of the genera *Luteovirus* and *Polerovirus* are intermingled in the tree. The resulting consensus trees from 1000 bootstrap replications are shown. The numbers above each node indicate the percentage of bootstrap replicates in which that node was recovered. For virus abbreviations, see **Table 1**.

Table 1 Virus members in the family *Luteoviridae*

Genus	*Virus*	*Abbreviation*	*Accession number*
Luteovirus	Barley yellow dwarf virus – MAV	BYDV-MAV	NC_003680[a]
	Barley yellow dwarf virus – PAS	BYDV-PAS	NC_002160
	Barley yellow dwarf virus – PAV	BYDV-PAV	NC_004750
	Bean leafroll virus	BLRV	NC_003369
	Soybean dwarf virus	SbDV	NC_003056
Polerovirus	Beet chlorosis virus	BChV	NC_002766
	Beet mild yellowing virus	BMYV	NC_003491
	Beet western yellows virus	BWYV	NC_004756
	Cereal yellow dwarf virus – RPS	CYDV-RPS	NC_002198
	Cereal yellow dwarf virus – RPV	CYDV-RPV	NC_004751
	Cucurbit aphid-borne yellows virus	CABYV	NC_003688
	Potato leafroll virus	PLRV	NC_001747
	Turnip yellows virus	TuYV	NC_003743
	Sugarcane yellow leaf virus	ScYLV	NC_000874
Enamovirus	Pea enation mosaic virus 1	PEMV-1	NC_003629
Unassigned	Barley yellow dwarf virus – GAV	BYDV-GAV	NC_004666
	Barley yellow dwarf virus – GPV	BYDV-GPV	L10356
	Barley yellow dwarf virus – RMV	BYDV-RMV	Z14123
	Barley yellow dwarf virus – SGV	BYDV-SGV	U06865
	Carrot red leaf virus	CtRLV	NC_006265
	Chickpea stunt disease associated virus	CpSDaV	Y11530
	Groundnut rosette assistor virus	GRAV	Z68894
	Indonesian soybean dwarf virus	ISDV	
	Sweet potato leaf speckling virus	SPLSV	
	Tobacco necrotic dwarf virus	TNDV	
	Tobacco vein distorting virus	TVDV	AJ575129

[a]Accession numbers beginning with NC_ represent complete genomic sequences.

infections by PEMV-1 and PEMV-2 sediment as two components. The $S_{20,w}$ are 107–122S for B components (PEMV-1) and 91–106S for T components (PEMV-2, an umbravirus). Virions are moderately stable and are insensitive to treatment with chloroform or nonionic detergents, but are disrupted by prolonged treatment with high concentrations of salts. Luteovirus and polerovirus particles are insensitive to freezing.

Virion Structure and Composition

All members of the *Luteoviridae* have nonenveloped icosahedral particles with diameters of 25–28 nm (**Figure 2**). Capsids are composed of major (21–23 kDa) and minor (54–76 kDa) CPs, which contain a C-terminal extension to the major CP called the readthrough domain (RTD). According to X-ray diffraction and molecular mass analysis, virions consist of 180 protein subunits, arranged in $T = 3$ icosahedra. Virus particles do not contain lipids or carbohydrates.

Virions contain a single molecule of single-stranded positive-sense RNA of 5300–5900 nt. The RNAs do not have a 3′ terminal poly(A) tract. A small protein (VPg) is covalently linked to the 5′ end of polerovirus and enamovirus genomic RNAs. Cereal yellow dwarf virus RPV (CYDV-RPV) also encapsidates a 322 nt satellite RNA that accumulates to high levels in the presence of the helper virus. Complete genome sequences have been determined for 17 members of the *Luteoviridae* (**Table 1**). For several viruses, genome sequences have been determined from multiple isolates.

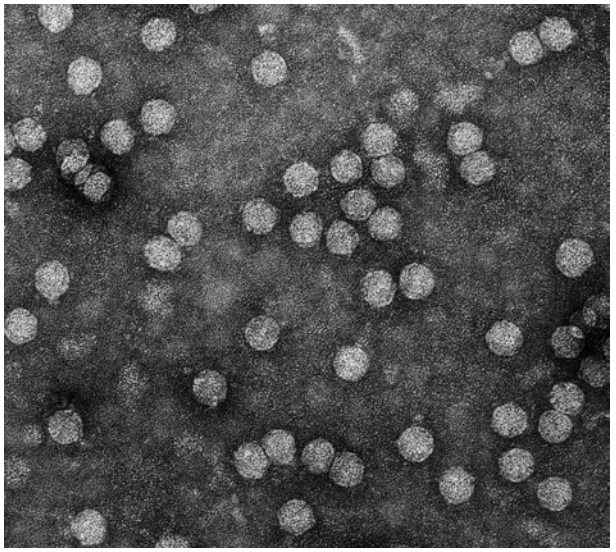


Figure 2 Transmission electron micrograph of soybean dwarf virus particles magnified 240 000×. Virions (stained with uranyl acetate) are *c*. 25 nm in diameter, hexagonal in appearance, and have no envelope.

Genome Organization and Expression

Genomic RNAs of luteovirids contain five to eight ORFs (**Figure 3**). ORFs 1, 2, 3, and 5 are shared among all members of the *Luteoviridae*. Luteoviruses lack ORF0. Enamoviruses lack ORF4. The luteo- and polerovirus genomes contain one or two small ORFs, ORFs 6 and 7, within or downstream of ORF5. An additional ORF, ORF8, has been discovered in ORF1 of PLRV. In the enamo- and poleroviruses ORF0 overlaps ORF1 by more than 600 nt, which also overlaps ORF2 by more than 600 nt. In the luteoviruses, ORF1 overlaps ORF2 by less than 50 nt. In all luteo- and polerovirus genome sequences (except for cucurbit aphid-borne yellows virus (CABYV) and GRAV), ORF4 is contained within ORF3. A single, in-frame amber (UAG) termination codon separates ORF5 from ORF3.

Luteovirids have relatively short 5′ and intergenic noncoding sequences. The first ORF is preceded by 21 nt in CABYV RNA and 142 nt in soybean dwarf virus (SbDV) RNA. ORFs 2 and 3 are separated by 112–200 nt of noncoding RNA. There is considerable variation in the length of sequence downstream of ORF5, which ranges from 125 nt for CYDV-RPV to 650 nt for SbDV.

Luteovirids employ an almost bewildering array of strategies to express their compact genomes. ORFs 0, 1, 2, and 8 are expressed directly from genomic RNA. Downstream ORFs are expressed from subgenomic RNAs (sgRNAs) that are transcribed from internal initiation sites by virus-encoded RNA-dependent RNA polymerases (RdRps) from negative-strand RNAs and are 3′-co-terminal with the genomic RNA. Since the initiation codon for ORF0 of polero- and enamoviruses is upstream of that of ORF1, translation of ORF1 is initiated by 'leaky scanning' in which ribosomes bypass the AUG of ORF0 and continue to scan the genomic RNA until they reach the ORF1 AUG. The protein products of ORF2 are expressed as a translational fusion with the product of ORF1. At a low but significant frequency during the expression of ORF1, translation continues into ORF2 through a –1 frameshift that produces a large protein containing sequences encoded by both ORFs 1 and 2 in a single polypeptide. ORF8, which has only been identified in PLRV, resides entirely within ORF1 in a different reading frame and encodes a 5 kDa replication-associated protein. To express ORF8, sequences within the ORF fold into a structure called an internal ribosome entry site (IRES), which recruits ribosomes to initiate translation about 1600 nt downstream of the 5′ terminus of PLRV RNA.

ORFs 3, 4, and 5 are expressed from sgRNA1, the 5′ terminus of which is located about 200 nt upstream of ORF3 at the end of ORF2, and extends to the 3′ terminus of the genome. Luteo- and poleroviruses produce a second sgRNA that expresses ORFs 6 and 7. Luteoviruses produce a third sgRNA, which does not appear to encode

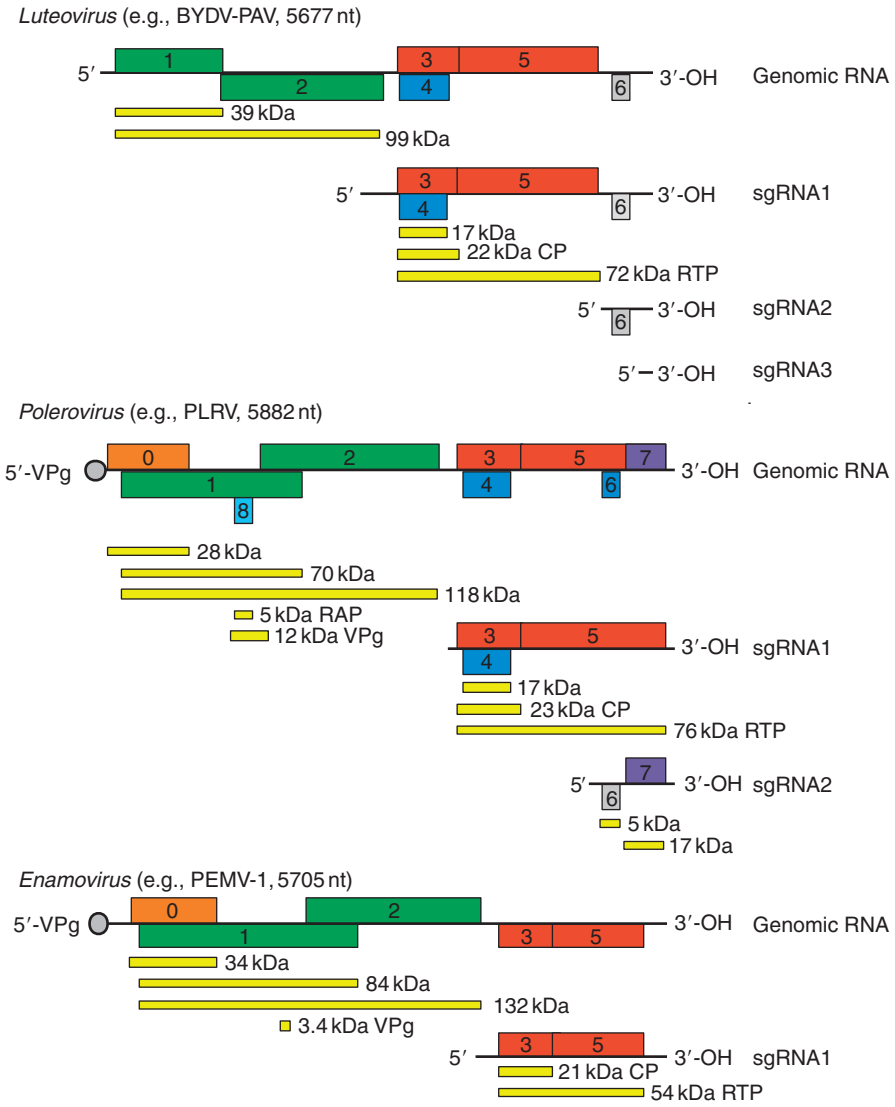


Figure 3 Maps of the virus genomes of genera in the family *Luteoviridae*. Individual ORFs are shown with open boxes. The ORFs are staggered vertically to show the different reading frames occupied by each ORF. The yellow boxes indicate protein products with the predicted sizes listed to the right of each. The polyproteins encoded by ORF1 of enamo- and poleroviruses contain the protease and the genome-linked protein (VPg). The predicted amino acid sequences of proteins encoded by ORF2 are similar to RNA-dependent RNA polymerases. ORF3, which encodes the major coat protein, is separated from ORF5 by an amber termination codon. ORF4, when present, is contained within ORF3 and encodes a protein required for virus cell-to-cell movement.

a protein. ORF3 is translated from the 5′ terminus of sgRNA1. ORF4 of luteo- and poleroviruses, which encodes a 17 kDa protein, is contained within ORF3, and is expressed from the same sgRNA as ORF3 through a leaky scanning mechanism much like that used to express ORF1 in polero- and enamoviruses. In all luteovirids, ORF5 is expressed only as a translational fusion with the products of ORF3 by readthrough of the UAG stop codon at the end of ORF3. This produces a protein with the product of ORF3 at its N-terminus and the product of ORF5 at its C-terminus.

While enamo- and polerovirus RNAs contain 5′ VPgs that interact with translation initiation factors, luteovirus RNAs contain only a 5′ phosphate. Unmodified 5′ termini are recognized poorly for translation initiation. To circumvent this problem, a short sequence located in the noncoding region just downstream of ORF5 in the BYDV-PAV genome acts as a potent enhancer of cap-independent translation by interacting with sequences near the 5′ termini of the genomic and sgRNAs to promote efficient translation initiation.

Research into the functions of the proteins encoded by luteovirids has shown that the 28–34 kDa proteins encoded by ORF0 are effective inhibitors of post-transcriptional gene silencing (PTGS). PTGS is an innate and highly adaptive antiviral defense found in all eukaryotes that is

activated by double-stranded RNAs (dsRNAs), which are produced during virus replication. Consequently, viruses that contain mutations in ORF0 show greatly reduced accumulations in infected plants.

The ORF1-encoded proteins of enamo- and poleroviruses contain the VPg and a chymotrypsin-like serine protease that is responsible for the proteolytic processing of ORF1-encoded polyproteins. The protease cleaves the ORF1 proteins in *trans* to liberate the VPg, which is covalently attached to genomic RNA. The protein expressed by ORF8 of PLRV is required for virus replication. Luteovirid ORF2s have a coding capacity of 59–67 kDa for proteins that are very similar to known RdRps and hence likely represent the catalytic portion of the viral replicase.

ORF3 encodes the major CP of the luteovirids, which ranges in size from 21 to 23 kDa. ORF5 has a coding capacity of 29–56 kDa. However, ORF5 is expressed only as a translational fusion with the product of ORF3 when, about 10% of the time, translation does not stop at the end of ORF3 and continues through to the end of ORF5. The ORF5 portion of this readthrough protein has been implicated in aphid transmission and virus stability. Experiments with PLRV and BYDV-PAV have shown that the N-terminal region of the ORF5 readthrough protein determines the ability of virus particles to bind to proteins produced by endosymbiotic bacteria of aphid vectors. Interactions of virus particles with these proteins seem to be essential for persistence of the viruses in aphids. Nucleotide sequence changes within ORF5 of PEMV-1 abolish aphid transmissibility. The N-terminal portions of ORF5 proteins are highly conserved among luteovirids while the C-termini are much more variable.

The luteo- and polerovirus genomes possess an ORF4 that is contained within ORF3 and encodes proteins of 17–21 kDa. Viruses that contain mutations in ORF4 are able to replicate in isolated plant protoplasts, but are deficient or delayed in systemic movement in whole plants. Hence, the product of ORF4 seems to be required for movement of the virus within infected plants. This hypothesis is supported by the observation that enamoviruses lack ORF4. While luteo- and poleroviruses are limited to phloem and associated tissues, the enamovirus PEMV-1 is able to move systemically through other plant tissues in the presence of PEMV-2, which under natural conditions invariably coexists with PEMV-1.

Some luteo- and polerovirus genomes contain small ORFs within and/or downstream of ORF5. In luteoviruses, no protein products have been detected from these ORFs in infected cells. BYDV-PAV genomes that do not express ORF6 are still able to replicate in protoplasts. The predicted sizes of the proteins expressed by ORFs 6 and 7 of PLRV are 4 and 14 kDa, respectively. Based on mutational studies, it has been proposed that these genome regions may regulate transcription late in infection.

Evolutionary Relationships among Members of the *Luteoviridae*

Viruses in the family *Luteoviridae* have replication-related proteins that are similar to those in other plant virus families and genera. The luteovirus replication proteins encoded by ORFs 1 and 2 resemble those of members of the family *Tombusviridae*. In contrast, polymerases of poleroviruses and enamoviruses resemble those of viruses in the genus *Sobemovirus*. The structural proteins of some sobemoviruses also are similar to the major CP of luteovirids. Using an X-ray crystallography-derived structure of virions of the sobemovirus rice yellow mottle virus, which shares a CP amino acid sequence similarity of 33% with PLRV, it was possible to predict the virion structure of PLRV and other luteovirids.

Host Range and Transmission

Several luteovirids have natural host ranges largely restricted to one plant family. For example, BYDV and CYDV infect many grasses, BLRV infects mainly legumes, and CtRLV infects mainly plants in the family Apiaceae. Other luteovirids infect plants in several or many different families. For example, beet western yellows virus (BWYV) infects more than 150 species of plants in more than 20 families. As techniques for infecting plants with recombinant viruses have improved, the experimental host ranges of viruses have been expanded to include plants on which aphid vectors would not normally feed. For example, BYDV, CYDV, PLRV, and SbDV have been shown to infect *Nicotiana* species that had not been described previously as experimental hosts for the viruses when inoculated biolistically with viral RNA or using *Agrobacterium tumefaciens* harboring binary plasmids containing infectious copies of the viruses. These results suggest that feeding preferences of vector aphids play important roles in defining luteovirid host ranges.

Luteovirids are transmitted in a circulative manner with varying efficiencies by at least 25 aphid species. With the exception of the enamovirus PEMV-1, members of the family *Luteoviridae* are transmitted from infected plants to healthy plants in nature only by the feeding activities of specific species of aphids. There is no evidence for replication of the viruses within aphid vectors. *Myzus persicae* is the most common aphid vector of luteovirids that infect dicots. Several different species of aphids transmit luteovirids that infect monocotyledenous plants (BYDV and CYDV) in a species-specific manner.

Circulative transmission of the viruses is initiated when aphids acquire viruses from sieve tubes of infected plants during feeding. The viruses travel up the stylet, through the food canal, and into the foregut (**Figure 4**). The viruses then are actively transported across the cells

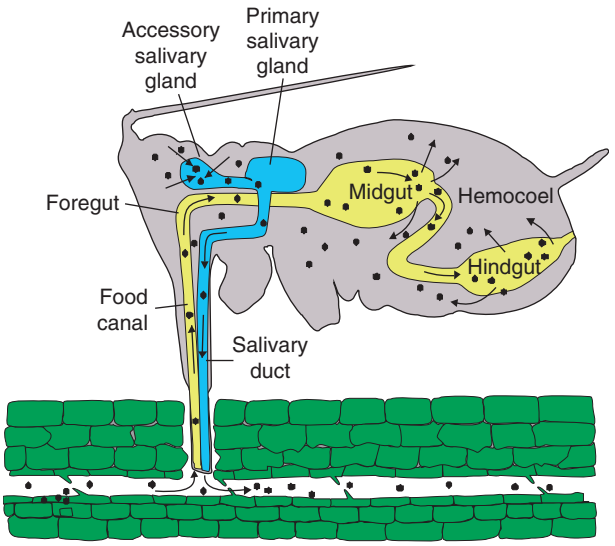


Figure 4 Circulative transmission of viruses of the family *Luteoviridae* by vector aphids. While feeding from sieve tubes of an infected plant, an aphid (shown in cross section) acquires virus particles, which travel up the stylet, through the food canal, and into the foregut. The virions are actively transported across cells of the posterior midgut and/or hindgut into the hemocoel in a process that involves receptor-mediated endocytosis. Virions then passively migrate through the hemolymph to the accessory salivary gland where they are again transported by a receptor-mediated process to reach the lumen of the gland. Once in the salivary gland lumen, the virions are expelled with the saliva into the vascular tissue of host plants. Aphids can retain the ability to transmit virus for several weeks. Hindgut membranes usually are much less selective than those of the accessory salivary glands, which is why viruses that are not transmitted by a particular species of aphid often accumulate in the hemocoel, but do not traverse the membranes of the accessory salivary gland.

of the alimentary tract into the hemocoel in a process that involves receptor-mediated endocytosis of the viruses and the formation of tubular vesicles that transport the viruses through the epithelial cells and into the hemocoel. Luteovirids are acquired at different sites within the gut of vector aphids. PLRV and BWYV are acquired in the posterior midgut. BYDV, CYDV, and SbDV are acquired in the hindgut. CABYV is taken up at both sites. Viruses then passively migrate through the hemolymph to the accessory salivary gland where the viruses must pass through the membranes of the accessory salivary gland cells in a similar type of receptor-mediated transport process to reach the lumen of the gland. Once in the salivary gland lumen, viruses are expelled with saliva into vascular tissues of host plants. Since large amounts of virus can accumulate in the hemocoel of aphids, they may retain the ability to transmit virus for several weeks. Typically hindgut membranes are much less selective than those of the accessory salivary glands. Consequently, viruses that are not transmitted by a particular species of aphid often are transported across gut membranes and accumulate in the hemocoel, but do not traverse the membranes of the accessory salivary gland.

The RTD of the minor capsid protein plays a major role in aphid transmission of luteovirids. The RTD interacts with symbionin produced by endosymbiotic aphid-borne bacteria, which may protect virions from degradation by the aphid immune system. The specificity of aphid transmission and gut tropism has been linked to the RTD in multiple luteovirids.

Unlike other luteovirids, PEMV-1 can be transmitted by rubbing sap taken from an infected plant on a healthy plant, in addition to being transmitted by aphids. This difference in transmissibility is dependent on its multiplication in cells co-infected with PEMV-2, but aphid transmissibility can be lost after several mechanical passages.

Replication

Luteovirids infect and replicate in sieve elements and companion cells of the phloem and occasionally are found in phloem parenchyma cells. PEMV-1 is able to move systemically into other tissues in the presence of PEMV-2. Virus infections commonly result in cytopathological changes in cells that include formation of vesicles containing filaments and inclusions that contain viral RNA and virions. The subcellular location of viral RNA replication has not been determined unequivocally. However, early in infection, negative-strand RNAs of BYDV-PAV are first detected in the nucleus and later in the cytoplasm, which suggests that at least a portion of luteovirus replication occurs in the nucleus. Synthesis of negative-strand RNA, which requires tetraloop structures at the 3′ end of BYDV-PAV genomic RNAs, is detected in infected cells before the formation of virus particles. Late in infection, BYDV-PAV sgRNA2 inhibits translation from genomic RNA, which may promote a switch from translation to replication and packaging of genomic RNAs.

Virus–Host Relationships

While some infected plants display no obvious symptoms, most luteovirids induce characteristic symptoms that include stunting, leaves that become thickened, curled or brittle, and yellow, orange, or red leaf discoloration, particularly of older leaves of infected plants. These symptoms result from phloem necrosis that spreads from inoculated sieve elements and causes symptoms by inhibiting translocation, slowing plant growth, and inducing the loss of chlorophyll. Symptoms may persist, may vary seasonally, or may disappear soon after infection. Temperature and light intensity often affect symptom severity and development. In addition, symptoms can

vary greatly with different virus isolates or strains and with different host cultivars.

Yield losses caused by luteovirids are difficult to estimate because the symptoms often are overlooked or attributed to other agents. US Department of Agriculture specialists estimated that yield losses from BWYV, BYDV, and PLRV infections were over $65 million during the period 1951–60. Plants infected at early stages of development by luteovirids suffer the most significant yield losses, which often are linearly correlated with the incidence of virus infection.

Epidemiology

Luteovirid infections have been reported from temperate, subtropical, and tropical regions of the world. Some of the viruses are found worldwide, such as BWYV, BYDV, and PLRV. Others have more restricted distribution, such as tobacco necrotic dwarf virus, which has been reported only from Japan, and groundnut rosette assistor virus, which has been reported only in African countries south of the Sahara.

Most luteovirids infect annual crops and must be reintroduced each year by their aphid vectors. Some viruses are disseminated in infected planting material, like PLRV where infected potato tubers are the principal source of inoculum for new epidemics. Consequently, programs to produce clean stock are operated around the world to control these viruses. Alate, that is, winged, aphid vectors may transmit viruses from local cultivated, volunteer, or weed hosts. Alternatively, alate aphids may be transported into crops from distant locations by wind currents. These vectors may bring the virus with them, or they may first have to acquire virus from locally infected hosts. The agronomic impact of luteovirid diseases depends both on meteorological events that favor movement and reproduction of vector aphids and susceptibility of the crop at the time of aphid arrival. Only aphid species that feed on a particular crop plant can transmit virus. Aphids that merely probe briefly to determine a plant's suitability will not transmit the viruses. Secondary spread of the viruses is often primarily by apterous, that is, wingless, aphids. The relative importance of primary introduction of virus by alate aphids and of secondary spread of virus by apterous aphids in disease severity varies with the virus, aphid species, crop, and environmental conditions.

Some members of the family *Luteoviridae* occur in complexes with other members of the family or with other plant viruses. For example, BYDV and CYDV often are found co-infecting cereals; BWYV and SbDV are often found together in legumes; and PLRV is often found co-infecting potatoes with potato virus Y and/or potato virus X. Some other plant viruses depend on luteovirids for their aphid transmission, such as the groundnut rosette virus, carrot mottle virus, and bean yellow vein banding virus (all umbraviruses), which depend on groundnut rosette assistor virus, carrot red leaf virus, and PEMV-1, respectively.

Diagnosis

An integral part of controlling luteovirid diseases is accurate diagnosis of infection. Because symptoms caused by luteovirids often resemble those caused by other biotic and abiotic factors, visual diagnosis is unreliable and other methods have been developed. Initially, infectivity, or biological, assays were used to diagnose infections. These techniques also have been used to identify species of vector aphids and vector preferences. In bioassays, aphids are allowed to feed on infected plants and then are transferred to indicator plants. These techniques are very sensitive, but can require several weeks for symptoms to develop on indicator plants. The strong immunogenicity of luteovirids has facilitated development of very specific and highly sensitive serological tests that can discriminate different luteovirids and sometimes even strains of a single virus species. Poly- and monoclonal antibodies for virus detection are produced by immunizing rabbits and/or mice with virus particles purified from infected plants. Techniques also have been developed to detect viral RNAs from infected plant tissues by reverse transcription-polymerase chain reaction (RT-PCR), which can be more sensitive and discriminatory than serological diagnostic techniques. Even so, serological tests are the most commonly used techniques for the detection of infections because of their simplicity and speed.

Control

Because methods are not available to cure luteovirid infections after diagnosis, emphasis has been placed on reducing losses through the use of tolerant or resistant plant cultivars and/or on reducing the spread of viruses by controlling aphid populations. Many luteovirids are transmitted by migrating populations of aphids that occur at similar times each year. For those virus–aphid combinations, it is sometimes possible to plant crops so that young, highly susceptible plants are not in the field when the seasonal aphid migrations occur. Insecticides have been used in a prophylactic manner to reduce crop losses. While insecticide treatments do not prevent initial infections, they can greatly limit secondary spread of aphids and therefore of viruses. In some instances biological control agents such as predatory insects and parasites have reduced aphid populations significantly. Genes for resistance or tolerance to infection by luteovirids have been identified in most agronomically important plant

species infected by the viruses. For BYDV, PLRV, and SbDV, transgenic plants that express portions of the virus genomes have been produced through DNA-mediated transformation. In some cases, the expression of these virus genes in transgenic plants confers higher levels of virus resistance than resistance genes from plants.

See also: Barley Yellow Dwarf Viruses; Cereal Viruses: Wheat and Barley; *Sobemovirus*; Tombusviruses.

Further Reading

Brault V, Perigon S, Reinbold C, *et al.* (2005) The polerovirus minor capsid protein determines vector specificity and intestinal tropism in the aphid. *Journal of Virology* 79: 9685–9693.

Falk BW, Tian T, and Yeh HH (1999) Luteovirus-associated viruses and subviral RNAs. *Current Topics in Microbiology and Immunology* 239: 159–175.

Gray S and Gildow FE (2003) Luteovirus–aphid interactions. *Annual Review of Phytopathology* 41: 539–566.

Hogenhout SA, van der Wilk F, Verbeek M, Goldbach RW, and van den Heuvel JF (2000) Identifying the determinants in the equatorial domain of Buchnera GroEL implicated in binding potato leafroll virus. *Journal of Virology* 74: 4541–4548.

Lee L, Palukaitis P, and Gray SM (2002) Host-dependent requirement for the potato leafroll virus 17-kDa protein in virus movement. *Molecular Plant–Microbe Interactions* 10: 1086–1094.

Mayo M, Ryabov E, Fraser G, and Taliansky M (2000) Mechanical transmission of potato leafroll virus. *Journal of General Virology* 81: 2791–2795.

Miller WA and White KA (2006) Long-distance RNA–RNA interactions in plant virus gene expression and replication. *Annual Review of Phytopathology* 44: 447–467.

Moonan F, Molina J, and Mirkov TE (2000) Sugarcane yellow leaf virus: An emerging virus that has evolved by recombination between luteoviral and poleroviral ancestors. *Virology* 269: 156–171.

Nass PH, Domier LL, Jakstys BP, and D'Arcy CJ (1998) *In situ* localization of barley yellow dwarf virus PAV 17-kDa protein and nucleic acids in oats. *Phytopathology* 88: 1031–1039.

Nixon PL, Cornish PV, Suram SV, and Giedroc DP (2002) Thermodynamic analysis of conserved loopstem interactions in P1–P2 frameshifting RNA pseudoknots from plant *Luteoviridae*. *Biochemistry* 41: 10665–10674.

Pfeffer S, Dunoyer P, Heim F, *et al.* (2002) P0 of beet western yellows virus is a suppressor of posttranscriptional gene silencing. *Journal of Virology* 76: 6815–6824.

Robert Y, Woodford JA, and Ducray-Bourdin DG (2000) Some epidemiological approaches to the control of aphid-borne virus diseases in seed potato crops in Northern Europe. *Virus Research* 71: 33–47.

Taliansky M, Mayo MA, and Barker H (2003) *Potato leafroll virus*: A classic pathogen shows some new tricks. *Molecular Plant Pathology* 4: 81–89.

Terradot L, Souchet M, Tran V, and Giblot Ducray-Bourdin D (2001) Analysis of a three-dimensional structure of potato leafroll virus coat protein obtained by homology modeling. *Virology* 286: 72–82.

Thomas PE, Lawson EC, Zalewski JC, Reed GL, and Kaniewski WK (2002) Extreme resistance to potato leafroll virus in potato cv. Russet Burbank mediated by the viral replicase gene. *Virus Research* 71: 49–62.

Lymphocytic Choriomeningitis Virus: General Features

R M Welsh, University of Massachusetts Medical School, Worcester, MA, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Lymphocytic choriomeningitis virus (LCMV), of the family *Arenaviridae*, is an etiological agent for human acute aseptic meningitis and grippe-like infections and is maintained in nature by lifelong persistent infections of mice (*Mus musculus*). Three strains still being studied today were initially isolated in the USA in the 1930s. During an attempt to recover and passage virus from a suspected human case of St. Louis encephalitis, Armstrong and Lillie isolated LCMV (Armstrong strain) from a monkey that developed a lymphocytic choriomeningitis – hence the name. Traub then reported the isolation of a serologically indistinguishable virus contaminating his mouse colony (Traub strain), and Rivers and Scott isolated similar viruses from human meningitis patients (one being the WE strain). Subsequently, many other isolations from man and animals were made, and a clear cause–effect relationship between LCMV and about 8% of the US cases of human nonbacterial meningitis was established.

The LCMV infection of the mouse soon became an important model system for studying viral immunology. A distinguishing feature of the LCMV infection was that it established a long-term persistent infection in mice infected *in utero* or shortly after birth, whereas adult mice inoculated with LCMV either cleared the virus with lasting immunity or, in the case of an intracranial infection, died of a lethal meningoencephalitis (**Figure 1**). Antiviral antibody was difficult to detect in the persistently infected mice, and this led Burnet and Fenner to postulate that exposure to viral antigens before the maturation of the immune system resulted in mice becoming immunologically tolerant to LCMV and thus unable to clear the infection. This was one of the systems that provided the basis for Burnet's Nobel Prize-winning theories of immunological tolerance. Subsequent work by Oldstone and Dixon, however, demonstrated that persistently infected mice did make antiviral antibody, but it was difficult to detect because of excess viral antigen. The antiviral antibody traveled in the circulation complexed

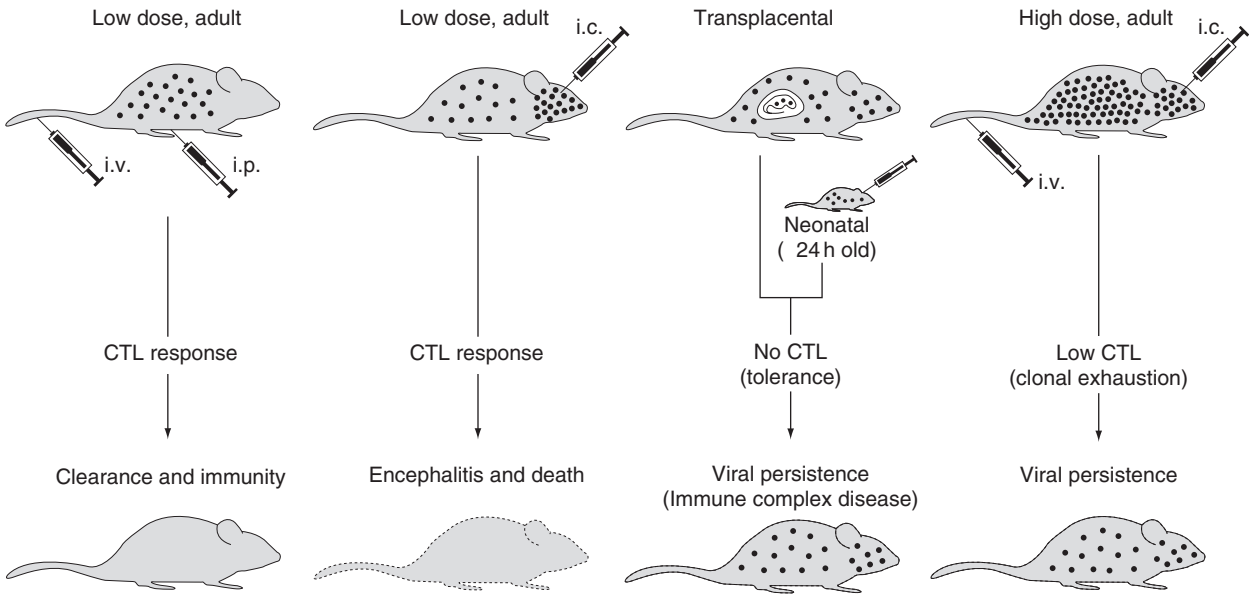


Figure 1 Pathogenesis of the LCMV infection is dependent on the age of the host, the route of infection, and the dose of the virus.

to virus and complement, and these circulating as well as tissue-bound immune complexes contributed to a progressive degenerative disease involving glomerulonephritis, arteritis, and chronic inflammatory lesions. In the acute infection both the clearance of the virus and the lethal meningoencephalitis were shown to be due mostly to the cytotoxic T lymphocyte (CTL) response. Antiviral CTLs were first demonstrated in the LCMV model, and Zinkernagel and Doherty used this model to demonstrate the Nobel Prize-winning concept of major histocompatibility complex (MHC) restriction in CTL recognition. Persistently infected mice were eventually shown to have split-tolerance to LCMV, in that, although they could mount an antibody response to LCMV, they could not generate the LCMV-specific CTLs which were needed to clear the infection.

Taxonomy and Classification

LCMV is the prototype virus of the *Arenaviridae* family of RNA viruses, and, although it has some homology with all arenaviruses, it is most closely related to the African virus *Lassa* and is classified as an Old World arenavirus. Its ambisense genome consists of two single-stranded RNAs, each encoding two genes of opposite polarity and separated by intergenic regions with strong RNA secondary structure. The small S RNA (1.1×10^3 kDa) in the virion encodes in the negative-sense a 63 kDa nucleoprotein (NP) and in the positive- or message-sense a 75 kDa cell-associated glycoprotein (GPC), which is cleaved into two virion glycoproteins, GP1 (44 kDa) and GP2 (35 kDa). The large L RNA (2.3×10^3 kDa) in the virion encodes in the negative-sense a 200 kDa RNA-dependent RNA polymerase (L) and in the positive-sense a smaller 11 kDa zinc-binding protein (Z) which binds to the ribonucleoprotein complex and is involved in transcriptional regulation. LCMV replicates in the cytoplasm and buds from the plasma membrane, incorporating host lipids into the viral membrane. It is pleomorphic, with sizes reported from 50 to 300 nm. Some of the virions, which may or may not be infectious, contain ribosome-like structures, giving the virion the characteristic arenavirus appearance.

Geographic and Seasonal Distribution

LCMV infections of mice and man have been well established in Europe and in North and South America, but they are not well documented elsewhere. LCMV is considered an Old World arenavirus brought to the Americas by its host, *Mus musculus*. There is some evidence that human infections occur more commonly in the winter and spring.

Host Range and Viral Propagation

The natural host for LCMV is the mouse, but it can be transmitted to man, hamsters, guinea pigs, rats, dogs, swine, monkeys, chimpanzees, and chick embryos. It commonly causes long-term persistent infections in mice and hamsters, and this has provided a source for human infections. The receptor for LCMV is alpha-dystroglycan, and LCMV strains binding to this receptor at high affinity tend to disseminate *in vivo* more than those that bind at lower affinity. LCMV grows in a wide variety of tissues *in vivo* and in most cells tested in culture. For instance, LCMV has been propagated and plaque assayed in 3T3, baby hamster kidney

(BHK), Detroit-98, HeLa, JLSV-9, L-929, MDBK, MDCK, Vero, and vole cell lines. Vero, L-929, and BHK cells are most commonly used for plaque assays, and BHK cells have been the choice for most biochemical analyses because of the relatively high yields of virus (*c.* 10^8 plaque-forming units (PFU) ml^{-1}) and the lack of secreted endogenous retrovirus contaminants. LCMV also grows in lymphocytes and macrophages, and the latter can be used as stimulator or target cells in T-cell proliferation or cytotoxicity assays, respectively.

Genetics

Like other single-stranded RNA viruses, LCMV mutates frequently, and these mutants vary in their tropism and disease-producing potential. A single passage of a cloned LCMV variant into mice will soon segregate into clear neurotropic and turbid viscerotropic plaque variants, which can be recovered from the brain and spleen, respectively. A single amino acid change in the LCMV glycoprotein (residue 260) can convert the immunostimulatory Armstrong strain of LCMV into an immunosuppressive (clone 13) variant, and these genotypes rapidly intraconvert during *in vivo* passage. Several strains of LCMV have been sequenced, and the highest level of sequence homology is at the 5′ and 3′ termini of the S and L virion RNA. These are presumed polymerase-binding sites well conserved throughout the arenavirus family. Different arenaviruses cross-interfere via a defective-interfering virus mechanism. The preservation of these polymerase-binding sites may allow for this heterotypic interference. The NP and GP of the Armstrong and WE strains share 90% amino acid homology.

The presence of two virion RNAs allows for high-frequency recombination due to reassortment of viral genomes. The technique of generating reassortants has led to the assignment of viral-encoded proteins to the appropriate RNA and has facilitated the mapping of genes required for disease-producing potential. The ease of producing reassortants in the laboratory suggests that they also occur in nature and probably play roles in enhancing the genetic diversity of arenaviruses.

Evolution

Arenaviruses do not show substantial sequence homology with any other virus group, but the homology within members of this family suggests a common origin for all arenaviruses. LCMV is most closely related to Lassa virus, which, like LCMV, has its origins in the Eastern Hemisphere. Each arenavirus favors a specific rodent host, and selective evolutional pressure on these viruses must have been conferred by their adaptation to their respective hosts in forms that established persistent infections. Of interest is that LCMV and other arenaviruses undergo rapid evolution as they form persistent infections. These persistent infections *in vitro* and *in vivo* result in the extensive production of defective-interfering virus and of attenuated relatively noncytopathic plaque variants which may help in the maintenance of persistent infections by preventing virus-induced cell death.

Serological Relationships and Variability

Antisera to Lassa virus and to all members of the Tacaribe complex cross-react to some extent with LCMV by complement fixation and immunofluorescence assays but not at the level of viral neutralization. Few monoclonal antibodies show cross-reactivity between LCMV and other Tacaribe complex viruses, but several cross-react with the more closely related Lassa virus. Infection of guinea pigs with LCMV immunizes them against a lethal dose of Lassa virus. Different strains of LCMV are not easily distinguishable by antisera but can be distinguished by some monoclonal antibodies and molecular methods.

Epidemiology

Human LCMV infections occur without sexual bias and at all ages, but most frequently in the 20–30-year age group. A longitudinal study in the USA from 1941 to 1958 implicated LCMV infections in about 8% of patients diagnosed with suspected viral meningitis, and serological studies have suggested up to a 10–15% incidence of LCMV infection in the general population. Most of these infections are probably mild or subclinical. Laboratory infections with LCMV are relatively common, and several cases have occurred in laboratories working with the WE strain, which was re-isolated from one laboratory worker and identified serologically with monoclonal antibodies.

Transmission and Tissue Tropism

LCMV has been experimentally transferred to man by intramuscular injection, but the normal route of infection is probably via the respiratory tract after exposure to mouse secretions. LCMV is shed at high titer in mouse feces and urine and is probably not transmitted by arthropod vectors. Another source of infection is the Syrian hamster (*Mesocricetus auratus*), which, like the mouse, can harbor a long-term persistent infection. Several cases of LCMV in different geographic areas have been linked to a colony of persistently infected hamsters distributed throughout the

USA. Recently, several patients developed severe LCMV infections after receiving transplanted organs from a deceased individual who had contracted LCMV from a pet hamster. Horizontal man-to-man transmission is rare, but LCMV can cross the placenta and infect the fetus.

Pathogenicity

LCMV strains appear to differ in their pathogenicity in man, but conclusive analyses of strain virulence differences in humans have not been carried out. Several human infections have occurred in laboratories working with the WE strain, and the WE strain has been reisolated from a laboratory worker with meningitis and clearly identified. There appear to be fewer (if any) anecdotal reports of human infection with the parent Armstrong strain. However, the more rapidly disseminating clone 13 variant of the Armstrong strain of LCMV has also been linked to human infection. The WE strain is much more virulent than the parent Armstrong strain in hamsters and guinea pigs, and reassortant analyses have mapped the ability to cause lethal infections in guinea pigs to the L RNA of the WE strain. The United States Centers for Disease Control recommends Biosafety Level 2 practices for most studies with LCMV in mice but Biosafety Level 3 practices for work with hamsters. This is based on the presumption that LCMV becomes more virulent for humans as it passes through hamsters. Although this has not been formally proven, this precaution would appear necessary due to the high number of clinical infections in individuals exposed to persistently infected hamsters.

LCMV has been used in a number of pathogenesis studies in mice. Viral strain variant differences exist in the encephalitis model, in which 'docile' or viscerotropic variants fail to kill mice, whereas 'aggressive' or more exclusively neurotropic variants do. However, a docile variant for one strain of mouse may be aggressive to another strain of mouse, and the susceptibility of mice to these variants seems to be linked to both MHC- and non-MHC genes. One reason for 'docility' is the immunosuppressive nature of some LCMV strains, particularly when inoculated into mice at high dose. Lethal encephalitis does not occur when the T-cell response is severely compromised. Immunosuppressive variants of LCMV tend to replicate to very high levels in the visceral organs. This high-dose antigen load can clonally exhaust the T cells by either driving them into apoptosis or into a functional anergy. This clonal exhaustion may limit the encephalitis but can result in a long-term persistent infection (**Figure 1**).

One interesting pathogenic feature of LCMV is its ability to cause a loss in cellular specialized or 'luxury' functions required not for cell survival but for homeostasis of the whole organism. Persistent LCMV infection results in reduced neurotransmitter enzyme activity in cultured neuroblastoma cells *in vitro* and reduced levels of growth and thyroid hormones in mice. Reduced growth hormone synthesis is associated with a runting syndrome in young infected mice. LCMV infects *in vivo* the cells that produce growth hormone and thyroid hormone and causes significant reductions in levels of mRNA encoding these hormones but not other hormones and proteins such as thyroid-stimulating hormone, actin, and collagen.

Clinical Features of Infection

The LCMV infection of man can be an inapparent or subclinical infection, or it can present as a nonmeningeal grippe-like ailment, an aseptic meningitis, or a more severe meningoencephalitis. The incubation period is 1–2 weeks or longer, and the disease may come in two or three waves. The grippal type is characterized by fever, malaise, lethargy, weakness, myalgia, arthralgia, fever, headache, photophobia, anorexia, and nausea. Some patients develop a rash, arthritis, parotitis, or orchitis. The grippal type is likely the most common form of the disease. The meningeal type is often preceded by the grippal type and presents with stiff neck, vomiting, irritability, and Brudzinski's and Kernig's signs. The meningoencephalomyelitic form, which is relatively rare, is associated with confusion, hallucinations, papilledema, and weakness progressing to paralysis. Patients usually recover without lasting sequelae. Death is very rare. There have been only nine fatal cases documented between 1942 and 1992, but two deaths were more recently seen in infected transplant recipients. There are also some reports of human transplacental infection, resulting in fetal abortion or malformation.

Pathology and Histopathology

LCMV can be recovered from the blood, cerebrospinal fluid (CSF), urine, and nasopharyngeal secretions during the human LCMV disease. Leukopenia is a common feature of the infection, but there is a pleocytosis in the CSF in the meningeal stages, and histological analyses of diseased brain tissue in lethal cases of LCMV have revealed meningeal perivascular inflammation and many lymphocytes and monocytes in the arachnoid. This is consistent with studies in the mouse which have demonstrated a lymphocytic infiltration of the meninges. Extensive studies in the mouse model of LCMV-induced acute meningoencephalitis have indicated that virus-specific MHC class I-restricted CD8+ CTLs are the major mediators of the lethal disease, and this may also be the mechanism for the human disease. The mouse model has also shown that antibody–virus–complement immune complexes

can be pathological entities, and these could be involved in some of the human arthritic symptoms and the rash, when present.

Immune Response

Human cases of LCMV are characterized by a rise in antibody titer after infection, followed by lasting immunity. Little work has been done on human cellular immunity to infection. However, the LCMV infection of the mouse has provided the most extensive analyses of the cellular immune response to any virus disease. The infection is characterized by an early stage (1–5 days post infection) and later stage (6–10 days post infection). The early stage involves the log-phase replication of the virus and a variety of antigen-nonspecific responses including the liberation of type 1 interferon (IFN)-α and -β and tumor necrosis factor (TNF)-α, the activation and proliferation of natural killer (NK) cells, and a depression in bone marrow hematopoiesis. The type 1 IFN and probably some of the TNFα is directly and rapidly induced in cells by the LCMV infection. Type 1 IFN and IL-15 stimulate the activation and proliferation of NK cells, which substantially increase in number and accumulate in virus-infected tissue. There is initially a type 1 IFN-induced attrition of memory T cells and other cell types, resulting in a leukopenia. Depressed bone marrow function, which is likely due to the effects of inhibitory cytokines like IFN and TNFα and to the ability of activated NK cells to lyse or suppress hematopoietic precursor cells, may contribute to the leukopenia. Antibodies to IFN-α and -β (as well as γ) enhance the synthesis of LCMV, but antibodies to NK cells do not alter the course of the infection. During this early stage of infection there is a pronounced type 1 IFN-induced increase in class I MHC antigen expression throughout the body, an increase in the susceptibility of the cells to CTLs, and a decrease in their susceptibility to NK cells. IFN-mediated protection of uninfected and LCMV-infected target cells may make them resistant to surveillance by NK cells.

The second phase of the response is associated with the expansion of clones of virus-specific T and B cells, a decrease in the production of virus, an increase in the production of IFN-γ and interleukin (IL)-2, and an increase in the activation of macrophages. The major factor in the control of the LCMV infection and in the development of encephalitis and immunopathological lesions throughout the body is the generation of CD8+ CTLs, which can develop in CD4+ T-cell-depleted mice. CD4 T cells and CD40/CD40L interactions, however, seem to be needed for the maintenance of long-term memory CD8 T-cell responses. The IFN-induced upregulation of class I MHC antigens conditions cells in the host to be good targets for CD8+ T cells, which recognize viral peptides in the context of class I antigens. Many LCMV-encoded immunodominant and subdominant T-cell epitopes have been identified, and, in the context of synthetic peptides or vaccinia virus recombinants, they can immunize mice against LCMV.

LCMV infection is so profound at inducing CD8 T-cell responses in the mouse that over 20% of the CD8 T cells can remain LCMV-specific in the memory pool. This has enabled the LCMV infection to be very useful in the discovery and analysis of T-cell-dependent heterologous immunity, where T cells specific for one pathogen are recruited into the response to an unrelated pathogen and alter protective immunity and immunopathology. For example, a complex network of T-cell cross-reactivities between LCMV and vaccinia virus causes LCMV-immune mice to partially resist vaccinia virus infection, while at the same time developing severe T-cell-associated immunopathological lesions.

Antiviral antibody plays only a minor role in the acute LCMV infection and is not needed to clear the infection. However, in the absence of antibody, virus may eventually recrudesce in immune mice. In the absence of a CTL response, such as in congenitally infected mice, antibody–virus–complement immune complexes mediate the development of glomerulonephritis, arteritis, and other inflammatory lesions. Usually the LCMV infection *per se* is relatively mild, and it is the immune response to the virus that causes the damage in the infected mouse.

Prevention and Control of LCMV

LCMV has not been a sufficiently significant human pathogen to warrant special public health measures for its control. However, the disease can be reduced in frequency by ridding houses of mice, by ensuring that pet hamsters are not infected, and by adhering at least to Biosafety Level 2 procedures in the laboratory. Supportive therapy without antiviral chemotherapy is recommended for patients experiencing LCMV infection.

Future Perspectives

Studies with LCMV have in the past provided much of the conceptual basis for viral immunology and pathogenesis, including (1) the theory of immunological tolerance, (2) the concept of virus-induced immunopathology, (3) the analysis of immune complex disease, (4) the discovery of virus-specific CTLs and demonstration of their roles in viral clearance and immunopathology, (5) the discovery of MHC restriction in T-cell recognition, (6) the discovery and analysis of NK cell activation and proliferation, (7) the concept that sublethal viral infections can abrogate specialized 'luxury' functions of cells and lead to metabolic disturbances, (8) the observation that high antigen loads can cause T-cell clonal exhaustion

and lead to persistent infection, and (9) the phenomenon of T-cell-dependent heterologous immunity. In the future, the LCMV infection will likely continue to be an important model used for the development of vaccines directed against T-cell epitopes, for examining the clearance of virus by T-cell immunotherapy, for elucidating mechanisms of virus-induced immunosuppression and T-cell tolerance that contribute to persistent infections, for analyzing the regulatory roles of cytokines in the development of the NK-cell and T-cell responses, and for clarifying regulatory mechanisms of heterologous immunity. LCMV variants can produce in mice syndromes that very closely resemble acquired immunodeficiency syndrome (AIDS), and it is possible to manipulate this system to get a persistent infection without a CTL response at all, a persistent infection associated with immunosuppression, and a low-level CTL response, or an acute infection with a strong CTL response which clears the infection. Exploiting the biology of these systems should continue to provide basic concepts fundamental to viral immunology and pathogenesis.

See also: Defective-Interfering Viruses; History of Virology: Vertebrate Viruses; Immune Response to viruses: Antibody-Mediated Immunity; Lassa, Junin, Machupo and Guanarito Viruses; Persistent and Latent Viral Infection.

Further Reading

Buchmeier MJ, Welsh RM, Dutko FJ, and Oldstone MB (1980) The virology and immunobiology of lymphocytic choriomeningitis virus infection. *Advances in Immunology* 30: 275–331.

Lehmann-Grube F (1986) Lymphocytic choriomeningitis virus. In: Braude AL, Davis CE, and Fierer J (eds.) *Infectious Diseases and Medical Microbiology*, 1076pp. Philadelphia, PA: W.B. Saunders.

Oldstone MBA (2002) Biology and pathogenesis of lymphocytic choriomeningitis virus infection. *Current Topics in Microbiology and Immunology* 263: 83–117.

Sevilla N and de la Torre JC (2006) Arenavirus diversity and evolution: Quasispecies *in vivo*. *Current Topics in Microbiology and Immunology* 299: 315–335.

Welsh RM (1999) Lymphocytic choriomeningitis virus as a model for the study of cellular immunology. In: Cunningham MW and Fujinami RS (eds.) *Effects of Microbes on the Immune System*, pp. 280–312. Philadelphia, PA: Lippincott Williams and Wilkins.

Lymphocytic Choriomeningitis Virus: Molecular Biology

J C de la Torre, The Scripps Research Institute, La Jolla, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Genome Organization

Arenaviruses have a bi-segmented negative single-stranded RNA genome and a life cycle restricted to the cell cytoplasm. Individual arenaviruses exhibit some variability in the lengths of the two genomic RNA segments, L (*c.* 7.2 kbp) and S (*c.* 3.5 kbp), but their overall organization is well conserved across the virus family. As with other negative-strand (NS) RNA viruses, arenaviruses are characterized by a lack of infectivity of their purified genome RNA species and the presence of a virion-associated RNA-dependent-RNA polymerase (RdRp). However, the arenavirus coding strategy has unique features compared to prototypical NS RNA viruses. Each arenavirus genome segment uses an ambisense coding strategy to direct the synthesis of two polypeptides in opposite orientation, separated by a non-coding intergenic region (IGR) (**Figure 1**). The S RNA encodes the viral glycoprotein precursor, GPC (*c.* 75 kDa), and the nucleoprotein, NP (*c.* 63 kDa), whereas the L RNA encodes the viral RdRp, or L polymerase (*c.* 200 kDa), and a small RING finger protein Z (*c.* 11 kDa). The NP and L coding regions are transcribed into a genomic complementary mRNA, whereas the GPC and Z coding regions are not translated directly from genomic RNA, but rather from genomic sense mRNAs that are transcribed using as templates the corresponding antigenome RNA species, which also function as replicative intermediates.

Terminal Nucleotide Sequences

Arenaviruses exhibit high degree of sequence conservation at the 3′-end of the L and S RNA segments (17 out of 19 nucleotides (nt) are identical). Likewise, similar to other NS RNA viruses, arenaviruses also exhibit complementarity between the 5′- and 3′-ends of their genomes and antigenomes. The almost exact inverted complement of the 3′-end 19 nt is found at the 5′-termini of genomes and antigenomes of arenaviruses. Thus, the 5′- and 3′-ends of both L and S genome segments are predicted to form panhandle structures. This prediction is supported by electron microscopy data showing the existence of circular ribonucleoprotein (RNP) complexes within arenavirus virion particles. This terminal complementarity may reflect the presence at the 5′-ends of *cis*-acting signal sequences that provide a nucleation site for RNA encapsidation, required to generate the nucleocapsid (NC) templates recognized by the virus polymerase. Terminal complementarity may also be a consequence of strong similarities between the genome

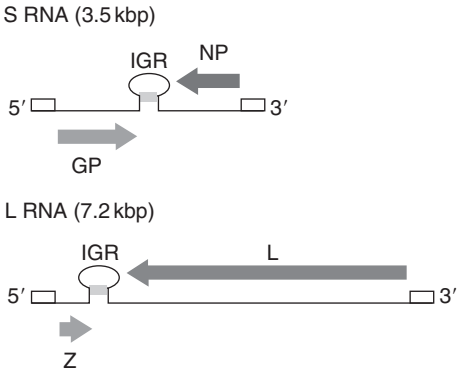


Figure 1 Scheme of the LCMV genome organization.

and antigenome promoters used by the virus polymerases. Studies using reverse genetics approaches have shown that both conserved terminal sequence elements and the panhandle structure are essential for the activity of the virus genome. This terminal complementarity has been proposed to favor the formation of both intra- and intermolecular L and S duplexes that might be part of the replication initiation complex, but its mechanistic aspects remain undefined. For several arenaviruses, an additional nontemplated G residue has been detected on the 5′-end of their genome and antigenome RNAs.

Intergenic Regions

Arenavirus IGRs are predicted to fold into a stable hairpin structure. Transcription termination of the S-derived NP and GP occurs at multiple sites within the predicted stem of the IGR suggesting that a structural motif rather than a sequence-specific signal promotes the release of the arenavirus polymerase from the template RNA. Some arenaviruses, including lymphocytic choriomeningitis virus (LCMV), Lassa fever virus (LFV), and Pichinde virus (PCV), contain one single predicted stem loop within the S IGR, whereas the S IGR of others like Tacaribe virus (TACV) and Mopeia are predicted to contain two distinct stem loops located downstream to the translation termination codons from NP and GPC.

Virion Structure

Virions are pleomorphic but typically spherical with an average diameter of 90–110 nm, and surrounded by a lipid envelope covered with surface glycoprotein spikes. Virions contain the L and S genomic RNAs as helical NC structures that are organized into circular configurations, with lengths ranging from 400 to 1300 nm. The L and S genomic RNA species are not present in equimolar amounts within virions (L:S ratios $\sim$ 1:2), and low levels of both L and S antigenomic RNA species are also present within virions. Host ribosomes can be incorporated into virions, but the biological implications of this remain to be determined. Virus particles have a 'sandy' appearance from which the family name is derived.

Arenavirus Gene Products

Viral Nucleoprotein

The NP (M_r 60–68 kDa) is the most abundant viral polypeptide both in infected cells and virions (about 1530 NP molecules/virion particle). The NP is the main structural element of the viral NC and associates with the genome RNA to form beadlike structures. Phosphorylated forms of the NP are usually detected at late stages of acute infection and their abundance increases in persistently infected cells, but the functional implications of these changes in the stage of NP phosphorylation have not been established.

Viral Glycoproteins

The viral GPC (70–80 kDa) is post-translationally proteolytically processed to generate the two mature virion glycoproteins GP-1 (40–46 kDa) and GP-2 (35 kDa), and a stable 58-amino-acid signal peptide (SP). Evidence indicates that in addition to its predicted role in targeting the nascent polypetide to the endoplasmic reticulum, the SP of the arenavirus GPC likely serves additional roles in the biosynthesis, trafficking, and function of the viral envelope glycoproteins. Cleavage of GPC into GP-1 and GP-2 is mediated by the SKI-1/S1P cellular protease. GP-1 is located at the top of the spike, away from the membrane, and is held in place by ionic interactions with the N-terminus of the transmembrane GP-2, that forms the stalk of the spike. GP-1 is the virion attachment protein that mediates virus interaction with host cell-surface receptors and subsequent virus cell entry via receptor mediated endocytosis. Alpha-dystroglycan (α-DG) has been identified as the receptor for LCMV, LFV, and several other arenaviruses. Evidence indicates that unprocessed arenavirus GPC can traffic to the cell surface, but the correct processing of the virus GPC into GP1 and GP2 is strictly required for the production of infectious particles that bud from the plasma membrane. Accordingly, growth of LCMV was severely impaired in cells deficient in SK1–1/S1P. Notably, proteolytic processing of GP requires the structural integrity of its GP-2 cytoplasmic tail.

L Polymerase

The arenavirus L protein has the characteristic sequence motifs conserved among the RdRp (L proteins), of NS RNA viruses. The proposed polymerase module of L is located within domain III, which contains highly

conserved amino acids within motifs designated A and C. Mutation-function analysis showed that sequence serine-aspartic acid-aspartic acid (SDD) characteristic of motif C of segmented NS RNA viruses, as well as the presence of the highly conserved D residue within motif A of L proteins, are strictly required for the function of the LCMV L polymerase. Several mutant L proteins exhibit a strong dominant negative (DN) under assay conditions where wt and mutant L proteins do not compete for template RNA or other *trans*-acting proteins, viral or cellular, required for polymerase activity. These results suggested that L–L interaction is required for LCMV polymerase activity. Intragenic complementation has been documented for the L genes of several NS RNA viruses. Consistent with this, direct L–L physical interaction has been demonstrated for the paramyxoviruses Sendai and parainfluneza virus 3 (PIV3), and this L oligomerization was required for polymerase activity. Likewise, biochemical evidence supports that the LCMV L protein has also the property of forming oligomeric structures.

Detailed sequence analysis and secondary structure predictions have been documented for the LFV L polymerase. These studies unequivocally identified only one functional domain corresponding to the putative RNA polymerase domain that exhibited a similar folding as those found in the corresponding domains of determined crystal structures of viral RdRp. In addition, several regions, outside the polymerase region, of strong alpha-helical content were identified, as well as a putative coiled-coil domain at the N-terminus. Secondary structure-assisted alignment of the RNA polymerase region indicated that arenaviruses are most closely related to nairoviruses.

Z Protein

The arenavirus small RING finger protein Z (*c.* 11 kDa) has no homolog among other known NS RNA viruses. Z is a structural component of the virion, and in LCMV-infected cells Z has been shown to interact with several cellular proteins including the promyelocytic leukemia (PML) protein, and the eukaryote translation initiation factor 4E (eIF4E). The former has been proposed to contribute to the noncytolytic nature of LCMV infection, whereas the latter has been proposed to repress cap-dependent translation. Early studies suggested a role of Z in arenavirus transcriptional regulation. However, more recent studies using reverse genetic approaches have shown that Z is not required for virus RNA replication and transcription, but rather it exhibits a dose-dependent inhibitory effect on RNA synthesis mediated by the arenavirus polymerase. The linear amino acid structure of Z could be seen as composed of three modules: the N-terminal part, the RING finger domain RD, and the C-terminal part including conserved proline motifs that constitute canonical late (L) domains. Despite some minor differences on protein size, the same overall structure is also seen among different arenavirus Z proteins. The Z RD domain exhibits the highest levels of conservation. The N-terminus contains a conserved canonical mirystoylation motif, whereas the C-terminus of Z contains conserved proline motifs that are similar to those present within the L domains of several Gag and matrix viral proteins and that have been identified as key functional elements during budding of enveloped viruses from the plasma membrane.

Arenavirus Life Cycle

Cell Attachment and Entry

Consistent with a broad host range and cell type tropism, a highly conserved and widely expressed cell-surface protein, alpha-dystroglycan (aDG) has been identified as a main receptor for LCMV, Lassa fever, and several other arenaviruses. Upon receptor binding, arenavirus virions are internalized by uncoated vesicles and released into the cytoplasm by a pH-dependent membrane fusion step. The pH-dependent fusion between viral and cell membranes in the acidic environment of the endosome is mediated by the GP2 portion of arenavirus GP, which is structurally similar to the fusion active membrane proximal portions of the GP of other enveloped viruses. Recent molecular modeling studies revealed the presence of two antiparallel alpha-helices separated by a glycosylated peptide loop within the ectodomain of arenavirus GP2, which is a characteristic feature of the proposed viral transmembrane protein superfamily.

RNA Replication and Transcription

The fusion between viral and cellular membranes results in the release of the viral RNP into the cytoplasm, which is followed by viral RNA synthesis. The encapsidated vRNA present in the vRNP does not serve as a direct template for protein synthesis, but rather as a template for both transcription and replication. The viral NP and L polypeptides were identified as the minimal viral *trans*-acting factors required for efficient RNA synthesis, both transcription and replication, mediated by the polymerase of LCMV, as well as LFV and the New World arenavirus TACV.

Z is not required for intracellular transcription and replication of an LCMV MG, but rather Z exhibits a dose-dependent inhibitory effect on both transcription and replication of LCMV MG. This inhibitory effect of Z has also been reported for TAV. Moreover, cells transduced with a recombinant, replication-deficient, adenovirus expressing Z (rAd-Z) become resistant to LCMV and

LFV. Z-mediated resistance to virus infection was not due to a blockade of virus entry, but rather to a strong inhibitory effect of Z on both viral transcription and RNA replication that in the case of TAV appears to involve Z–L interaction. Mutation-function studies showed that the N- and C-terminal residues of Z were not required for its inhibitory activity on RNA synthesis. In contrast, the structural integrity of the Z RING domain (RD) was strictly required, but not sufficient for the Z inhibitory activity. Many RING finger proteins play a key role in the control of intracellular protein degradation by acting as ubiquitin (Ub)–protein ligases (or E3s). A subset of RING proteins implicated in protein degradation has been shown to have a conserved tryptophan (W) where other hydrophobic amino acids are otherwise found. Notably, arenavirus Z proteins contain a highly conserved W residue located next to the second conserved cystein (C) residue of its RD. Substitution of this W residue by A resulted in a Z protein with reduced inhibitory activity, an effect that might be related to its potential E3 activity. Nevertheless, experimental evidence has failed to support, yet, an E3 activity associated with Z.

It is plausible that increased expression of Z protein during the virus life cycle might contribute to block replication of an additional infection by a genetically closely related arenavirus, which is known as homotypic viral interference. Superinfection exclusion could also influence arenavirus evolution and contribute to explain the observed population partitioning in the field, resulting in the maintenance of independent evolutionary lineages of the same strain within a small geographic range.

A widely accepted model for the control of arenavirus RNA replication and gene transcription (**Figure 2**) proposes that at early times of infection, low levels of NP prevent the virus polymerase to go across the IGR, and hence transcription is favored over replication. Viral mRNAs have extra nontemplated nucleotides (nt) and a cap structure at their 5′-ends, but the origin of both the cap and 5′-non-templated nt extensions remain to be determined. The 3′-termini of the subgenomic nonpolyadenylated viral mRNAs has been mapped to multiple sites within the distal side of the IGR, which is reminiscent of 'intrinsic' or 'rho-independent' termination in prokaryotes and represents strong support for the IGR as termination signal for transcription complexes. Direct experimental data has confirmed that the IGR is a *bona fide* transcription termination signal. Intriguingly, the IGR appears to also play a key role in packaging or cell egress, or both, of infectious viral particles. Increasing intracellular NP levels during the course of the infection would unfold secondary RNA structures within the IGR. This would attenuate structure-dependent transcription termination at the IGR, thus promoting replication of genome and antigenome RNA species. However, studies using a helper virus-free minigenome (MG) rescue

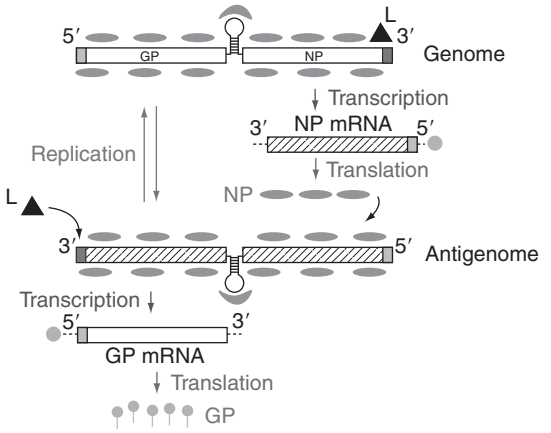


Figure 2 Basic aspects of arenavirus RNA replication and gene transcription illustrated for the S segment. Once the virus RNP is delivered in to the cytoplasm of the infected cell, the polymerase associated with the virus RNP initiates transcription from the genome promoter located at the genome 3′-end. Primary transcription results in synthesis of NP and L mRNA from the S and L, respectively, segments. Subsequently, the virus polymerase can adopt a replicase mode and moves across of the IGR to generate a copy of the full length antigenome RNA (agRNA). This agRNA will serve as template for the synthesis of the GP (agS) and Z (agL) mRNAs. The agRNA species serve also as templates for the amplification of the corresponding genome RNA species.

system showed that although transcription and replication were strictly dependent on NP, both were however equally enhanced by incrementally increasing amounts of NP up to levels in the range of LCMV-infected cells. These findings, similar to those described for the paramyxovirus respiratory syncytial virus, are consistent with a central role for NP in transcription and replication of the LCMV genome, but they do not support a participation of NP levels in balancing the two processes. These observations would be consistent with a model where two distinct polymerase complexes may exist, which are committed to either transcription or replication. This view is supported by the finding that LCMV mRNAs are capped while genomes and replicative intermediates are not, suggesting that the replicase is committed to either process already when initiating at the 3′-end of the template using either a cap-oligonucleotide primer or a prime and realign mechanism.

The activity of the genomic promoter recognized by virus polymerase requires both sequence specificity within the highly conserved 3′-terminal 19 nt of arenavirus genomes, and the integrity of the predicted panhandle structure formed via sequence complementarity between the 5′- and 3′-termini of viral genome RNAs. A prime and realign mechanism has been proposed for the initiation of arenavirus RNA replication. This model accounts for the presence of a nontemplated G at the 5′-ends of the arenavirus genomic and antigenomic RNAs. This model assumes that arenavirus polymerases, like many other

viral RdRps, can initiate RNA synthesis *de novo* only with ATP or GTP. Therefore, arenavirus RNA initiation is thought to take place from an internal (+2) templated cytidilate. Once the first phosophodiester bond has been formed, the pppGpC will slip backwards on the template and realign, creating a nascent chain whose 5′-end is at position −1 with respect to the template, before the polymerase can continue the downstream synthesis. The length of the RNA is kept constant despite a nontemplated nucleotide being added at the 5′-end, because the polymerase is supposed to terminate RNA synthesis by removing the last base. A similar mechanism has been proposed for RNA initiation of Hantaviruses (*Bunyaviridae*).

Genomic and antigenomic viral RNA species with terminal deletions, or nontemplated nucleotide extensions, have been documented during persistent LCMV infections. These truncated RNAs have been proposed to be a new type of defective interfering (DI) genome that contributes to the establishment and maintenance of LCMV persistence. It has been hypothesized that truncated RNAs are replication competent but are not used as templates for transcription. Increased ratio of truncated to full-length RNAs would be expected to cause a decrease in viral gene expression. On the other hand, addition of nontemplated nucleotides could repair some truncated RNAs to full-length sequence, thus leading to a transient increase in virus gene expression. This hypothesis is compatible with the observations that LCMV persistence is characterized by cyclical changes in numbers of antigen-positive cells and production of infectious virus. A corollary of this hypothesis would be that the arenavirus' highly conserved 3′-terminal 19 nt sequence is not strictly required for initiation of replication, which appears to be in conflict with mutation-function analysis of the arenavirus genomic promoter using reverse genetic approaches. One possible explanation for this apparent discrepancy would be that truncated RNAs detected in LCMV persistence are continuously generated *de novo* from full-length templates by an unknown mechanism. On the other hand, cellular factors induced or modified by LCMV persistence might influence the activity of the virus polymerase.

Assembly and Budding

Z has been postulated to participate in virion morphogenesis. For most enveloped viruses, a matrix (M) protein is involved in organizing the virion components prior to assembly. Interestingly, arenaviruses do not have an obvious counterpart of M. However, cross-linking studies showed complex formation between NP and Z, suggesting a possible role of Z in virion morphogenesis. Recent data have shown that Z is the main driving force of arenavirus budding and that this process is mediated by the Z proline-rich late (L) domain motifs PT/SAP and PpxY, similar to those known to control budding of several other viruses including HIV and Ebola, via interaction with specific host cell proteins. Consistent with this observation Z exhibits features characteristic of bona fide budding proteins: (1) Z has ability to bud from cells by itself, and (2) Z can efficiently substitute for the RSV L domain. Targeting of Z to the plasma membrane, the location of arenavirus budding, strictly required its myristoylation. Accordingly, the myristoylation inhibitor 2-hydroxymyristic acid (2-OHM) inhibited Z-mediated arenavirus budding without affecting RNA synthesis mediated by the LCMV polymerase. Intriguingly, the IGR appears to also play a key role in packaging or cell egress, or both, of infectious viral particles.

Arenavirus Reverse Genetics

The development of reverse genetic systems for several arenaviruses including the prototypic LCMV has provided investigators with a novel and powerful approach for the investigation of the *trans*-acting factors and *cis*-acting sequences that control arenavirus replication and gene expression, as well as assembly, and budding. Likewise, the implementation of reverse genetic approaches for arenaviruses has opened new avenues for the development of novel vaccine and antiviral strategies to combat arenavirus infections. Moreover, the ability to generate recombinant LCM viruses with predetermined specific mutations and analyze their phenotypic expression in its natural host, the mouse, would significantly contribute to the elucidation of the molecular mechanisms underlying LCMV–host interactions including virus persistence and associated disease.

Molecular Phylogeny

The family *Arenaviridae* comprises two distinct complexes: the LCMV–Lassa complex, which includes the Old World arenaviruses; and the TACV complex, which includes all known New World arenaviruses. Early sequence analysis of laboratory adapted arenaviruses revealed a significant degree of genetic stability with amino acid sequence homologies of 90–95% among different strains of the prototypic arenavirus LCMV, whereas significant higher levels of genetic diversity (37–56%) were observed for homologous proteins of different arenaviruses species. More recent, genetic studies on arenavirus field isolates (including Lassa, Junin, Guanarito (GTO), Pirital (PIR), and Whitewater Arroyo) have revealed a high degree of genetic variation among geographical and temporal isolates of the same virus species. Notably, a remarkably high level of genetic divergence (26% and 16% at the nt and amino acid level, respectively) has been documented among PIR isolates

within very small geographic regions. The substantial degree of inter- and intraspecies genetic variation among arenaviruses appear to have important biological correlates, as suggested by the significant variation in biological properties observed among LCMV strains. Dramatic phenotypic differences among genetically closely related LCMV isolates indicate that a few amino acid replacements in LCMV proteins could suffice to produce important alterations in the virus biological properties.

See also: Lymphocytic Choriomeningitis Virus: General Features.

Further Reading

Bowen MD, Rollin PE, Ksiazek TG, *et al.* (2000) Genetic diversity among Lassa virus strains. *Journal of Virology* 74: 6992–7004.

Buchmeier MJ, de la Torre JC, and Peters CJ (2006) *Arenaviridae*: The virus and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1791–1827. Philadelphia, PA: Lippincott Williams and Wilkins.

Cornu TI, Feldmann H, and de la Torre JC (2004) Cells expressing the RING finger Z protein are resistant to arenavirus infection. *Journal of Virology* 78: 2979–2983.

Eichler R, Lenz O, Strecker T, Eickmann M, Klenk HD, and Garten W (2003) Identification of Lassa virus glycoprotein signal peptide as a *trans*-acting maturation factor. *EMBO Reports* 4: 1084–1088.

Hass M, Golnitz U, Muller S, Becker-Ziaja B, and Gunther S (2004) Replicon system for Lassa virus. *Journal of Virology* 78: 13793–13803.

Kunz S, Borrow P, and Oldstone MB (2002) Receptor structure, binding, and cell entry of arenaviruses. *Current Topics in Microbiology and Immunology* 262: 111–137.

Lee KJ, Novella IS, Teng MN, Oldstone MB, and de la Torre JC (2000) NP and L proteins of lymphocytic choriomeningitis virus (LCMV) are sufficient for efficient transcription and replication of LCMV genomic RNA analogs. *Journal of Virology* 74: 3470–3477.

Meyer BJ, de la Torre JC, and Southern PJ (2002) Arenaviruses: Genomic RNAs, transcription, and replication. In: Oldstone MB (ed.) *Arenaviruses I,* vol. 262, pp. 139–149. Berlin: Springer.

Perez M, Craven RC, and de la Torre JC (2003) The small RING finger protein Z drives arenavirus budding: Implications for antiviral strategies. *Proceedings of the National Academy of Sciences, USA* 100: 12978–12983.

Perez M and de la Torre JC (2003) Characterization of the genomic promoter of the prototypic arenavirus lymphocytic choriomeningitis virus (LCMV). *Journal of Virology* 77: 1184–1194.

Pinschewer DD, Perez M, Sanchez AB, and de la Torre JC (2003) Recombinant lymphocytic choriomeningitis virus expressing vesicular stomatitis virus glycoprotein. *Proceedings of the National Academy of Sciences, USA* 100: 7895–7900.

Sanchez AB and de la Torre JC (2005) Genetic and biochemical evidence for an oligomeric structure of the functional L polymerase of the prototypic arenavirus lymphocytic choriomeningitis virus. *Journal of Virology* 79: 7262–7268.

Sanchez AB and de la Torre JC (2006) Rescue of the prototypic arenavirus LCMV entirely from plasmid. *Virology* 350: 370–380.

Strecker T, Eichler R, Meulen J, *et al.* (2003) Lassa virus Z protein is a matrix protein and sufficient for the release of virus-like particles [corrected]. *Journal of Virology* 77: 10700–10705.

York J, Romanowski V, Lu M, and Nunberg JH (2004) The signal peptide of the Junin arenavirus envelope glycoprotein is myristoylated and forms an essential subunit of the mature G1–G2 complex. *Journal of Virology* 78: 10783–10792.

Lysis of the Host by Bacteriophage

R F YoungIII and R L White, Texas A&M University, College Station, TX, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Antiholin Phage-encoded protein that inhibits the holin by directly interacting with it; may be produced from the same coding sequence as the holin, as in the case of the lambda dual-start motif, or may be encoded by a completely separate gene, as in the case of the T4 holin *t* and antiholin *rI* genes.

Dual-start motif Motif at the beginning of a holin gene consisting of two start codons separated by one or two codons, at least one of which specifies a positively charged residue; from each of these two start codons, a separate protein product is translated, and the products differ only by two or three N-terminal residues but possess opposing functions (the shorter polypeptide is the active holin and the longer polypeptide is the antiholin).

Endolysin Enzyme with muralytic activity; that is, it degrades the host cell wall by cleaving one or more of three chemical bonds in the peptidoglycan.

Holin Small phage-encoded membrane protein responsible for the release or activation of the endolysin to accomplish lysis and for the timing of the lysis event.

Holin triggering The onset of lysis as caused by lesion formation by the holin.

Holin–endolysin lysis This two-component lysis strategy relies on a small, phage-encoded membrane protein (the holin) to time the lytic event and activate or release second component, the endolysin, a phage-encoded muralytic enzyme that destroys the host cell wall. It is used by essentially all phages with dsNA genomes.

Lysis Destruction of host cells to release progeny virions at the end of the phage vegetative cycle; two

fundamental lysis strategies are the 'holin-endolysin' and the 'single-gene' lysis systems.

Pinholin Holin, such as S^{21}, that makes small holes that depolarize the membrane but are too small to allow a fully folded, soluble endolysin to escape into the periplasm.

Protein antibiotic Single-gene lysis system, such as the ϕX174 E protein or the Qβ A_2 protein, which causes lysis in a manner that is indistinguishable from lysis induced by antibiotics inhibiting any of the steps in the pathway of cell wall biogenesis.

Rz–Rz1 Type II cytoplasmic membrane protein and outer-membrane lipoprotein, respectively, encoded by most phages with Gram-negative hosts. Through specific C-terminal interactions, Rz and Rz1 form a complex that spans the periplasm. In some cases, the Rz–Rz1 complex is required for lysis under conditions that stabilize the outer membrane.

SAR endolysin Signal anchor release endolysin; the endolysin is initially secreted as a type II integral membrane protein (single transmembrane domain or TMD; N-in, C-out topology), with the TMD acting as a signal anchor domain. The SAR domain is not cleaved like a normal signal sequence but is able to escape from the membrane. The exit of the SAR domain from the bilayer is accelerated when the membrane is depolarized. SAR domains are rich in nonhydrophobic residues, especially glycine and alanine. These endolysins are inactive in the membrane tethered form and become active upon release from the membrane, which occurs slowly and spontaneously in the absence of holin function or rapidly upon holin-mediated membrane depolarization.

Single-gene lysis This strategy effects lysis with a single protein by inhibiting a step in cell wall synthesis and without inducing any detectable muralytic enzyme activity; it is used by non-filamentous lytic ssDNA and ssRNA phages.

Spanin A phage-encoded outer membrane lipoprotein that contains a C-terminal TMD and thus spans the entire periplasm. It replaces the Rz–Rz1 proteins in some phages and can complement an *Rz–Rz1* defect.

Preface: Host Lysis and Release of Progeny Virions

In eubacterial systems, there are two modes by which bacteriophages effect release of the progeny from the infected host: 'extrusion' and 'lysis'. Extrusion is limited to the male-specific filamentous phages and can be viewed as a chronic process that is not fatal to the host cell. Essentially, each filamentous phage particle is extruded through the envelope as its DNA is coated with coat proteins that have accumulated in the cytoplasmic membrane. There is no concomitant disruption of the membrane and thus the process is compatible with continued cell growth. This process is intimately related to the morphogenesis of the virion and is thus treated in detail elsewhere in this encyclopedia. All nonfilamentous phages cause complete 'lysis' of the host, requiring that the murein component of the cell envelope be at least partially destroyed. Thus, this article is devoted to the molecular basis of host lysis.

Lysis versus 'Rotting'

It is important at the outset to make a distinction between phage lysis and the cell lysis that can occur as a result of antibiotic intoxication, developmental processes, or fatal cellular dysfunction. Phage lysis is generally a much more rapid and saltatory event. Under ideal physiological conditions in the laboratory, where a culture is uniformly and synchronously infected, lysis of the entire culture can be observed within a time interval that is usually small compared to the length of the total infection cycle. In most cases, cell lysis not deriving from phage infection is essentially a passive event; the cell wall deteriorates as a result of a failure or lack of coordination of murein synthesis and turnover processes. An exception to this may be in the autolysis of bacteria that occurs as a result of a developmental pathway, such as lysis of the mother cell during sporulation, where murein degradation is actively promoted by expression of muralytic enzymes. There is some evidence that lysis systems were evolutionarily late additions to the genetic architecture of phages (see below). It seems likely that primitive phages did not directly promote lysis of the host; instead, liberation of the progeny virions was eventually effected as a result of the general derangement of cellular growth and maintenance systems caused by massive viral-specific reprogramming of gene expression. However, the rapid, actively programmed lysis now universally characteristic of all nonfilamentous phages presumably conferred a great competitive advantage, allowing the progeny virions an early opportunity to diffuse into the environment and initiate new infection cycles, instead of being trapped in a dead host and relying on 'rotting' of the corpse for eventual release.

Implicit in this argument, however, is an intriguing question; if lysis is an active programmed process, what determines when it will be initiated? The simplest expectation might be that lysis would be triggered when the cell was exhausted in terms of its macromolecular synthesis capacity, so that the assembly of virions was significantly diminished or halted. This turns out not to be the case.

Instead, at least for almost all dsDNA and, perhaps, dsRNA phages (hereafter: dsNA) the timing of lysis is determined by a small phage-encoded membrane protein called a 'holin' (see below). The holin triggers lysis at a time built into its primary structure, irrespective of the ability of the host cell to maintain or even increase the rate of intracellular assembly of virions. Holin-mediated lysis can be changed by mutations in the holin sequence or expression signals or by the direct inhibitory effects of another phage-encoded protein called the 'antiholin'. Irrespective of the molecular details, it must be kept in mind that there is no direct link between the progress of the morphogenetic program of the infecting phage and lysis timing. Lysis timing is an independent fitness parameter. In fact, the only decision made during the vegetative cycle of any phage is when to terminate the infection and effect lytic release of the progeny virions. It should be noted that this is not a purely basic science matter; phage-mediated lysis has a direct role in many disease processes. Some bacterial toxins are actually proteins or polypeptides encoded by prophages in the pathogenic bacterium. The expression of genes encoding products like the Shiga-like toxins of hemorraghic *Escherichia coli* and release of the toxin itself occurs as a result of the lysogenic induction of a fraction of the bacterial population and the subsequent phage-mediated lysis of these host cells. The study of phage lysis may thus reveal opportunities for therapeutic intervention in these diseases.

Modes of Phage Lysis

There are at least two fundamental strategies by which host lysis is accomplished by bacteriophages: 'holin–endolysin' lysis and 'single gene' lysis. The former strategy relies on a viral-encoded muralytic enzyme to destroy the host cell wall and is used by phages with genome sizes above about ~10 kb; that is, essentially all phages with dsNA genomes. The latter mode causes lysis without inducing any detectable muralytic enzyme activity and is used by nonfilamentous phages with genomes under this limit, including the lytic ssDNA and ssRNA phages. The molecular bases of these two fundamental strategies are the substance of the remainder of this article.

Holin–Endolysin Lysis

The lytic strategy adapted by most phages other than the smallest ones involves, at a minimum, two proteins: a membrane protein called a 'holin', and a muralytic enzyme called an 'endolysin'. The endolysin is an enzyme that degrades the cell wall (also known as murein or peptidoglycan). The 'holin', mentioned above, is a small membrane protein that controls the enzymatic activity of the endolysin or its access to its murein substrate; the timing of the onset, or 'triggering', of lysis is entirely determined by the holin's primary structure. In addition, most dsDNA phages of Gram-negative bacteria also either a pair of interacting secreted proteins called Rz and Rz1, or a 'spanin' protein, which has both cytoplasmic membrane and OM localization signals. The Rz–Rz1 complex and the spanin protein both span the entire periplasm. Finally, in many phages, a regulatory protein called an 'antiholin' is also produced. As its name implies, the antiholin binds to and inhibits the function of the holin and can delay, or, in some circumstances, completely block lysis.

The holin–endolysin systems thus involve a minimum of two genes, a holin and an endolysin. However, some phage lysis systems use as many as five proteins; one example is the well-studied coliphage P2, which encodes a holin, endolysin, antiholin, and Rz–Rz1 proteins. It must be noted here, however, that there has been little study of the lysis systems of phages with Gram-positive hosts, although the existence of the simplest two-gene holin–endolysin systems has been demonstrated, as in the case of phage ϕ29, which is the prototype of a very diverse and widespread family of phages of Gram-positive bacteria. In addition, the emerging field of phage genomics suggests that some phages of Gram-positive bacteria may have lysis components that have no counterpart in the better-studied Gram-negative systems. This is especially likely in phages of mycobacteria and similar microbes, where there is a unique waxy outer surface that in some ways serves the purpose of an outer membrane for these important Gram-positive organisms.

Holins

Holins may be the most diverse functional family in biology. The last comprehensive survey, more than 5 years ago, found more than 250 holin or putative holin genes among the sequenced phages and in the prophages found in bacterial genomes. Homology analysis found that these sequences could be grouped into 50 unrelated families. Primary structure analysis by membrane topology algorithms indicates that most holins can be grouped into three topology classes, based on the number of transmembrane helical domains (TMDs): class I: 3 TMDs, with the N-terminus out and C-terminus in (the cytoplasm); class II: 2 TMDs, N-in, C-in; and class III, 1 TMD, with N-in and C-out. In many cases, the membrane topology is not obvious from the primary structure, and there is no reason to doubt that there may be more topological classes in this large and diverse functional group. The prototype class I, II, and III members are the holins from phages lambda, phage 21, and phage T4, respectively (**Figure 1**); all have been subjected to genetic analysis, and the holin from lambda has also been studied by biochemical means. Only one other holin has had extensive genetic study, the class I holin of the tectivirus PRD1.

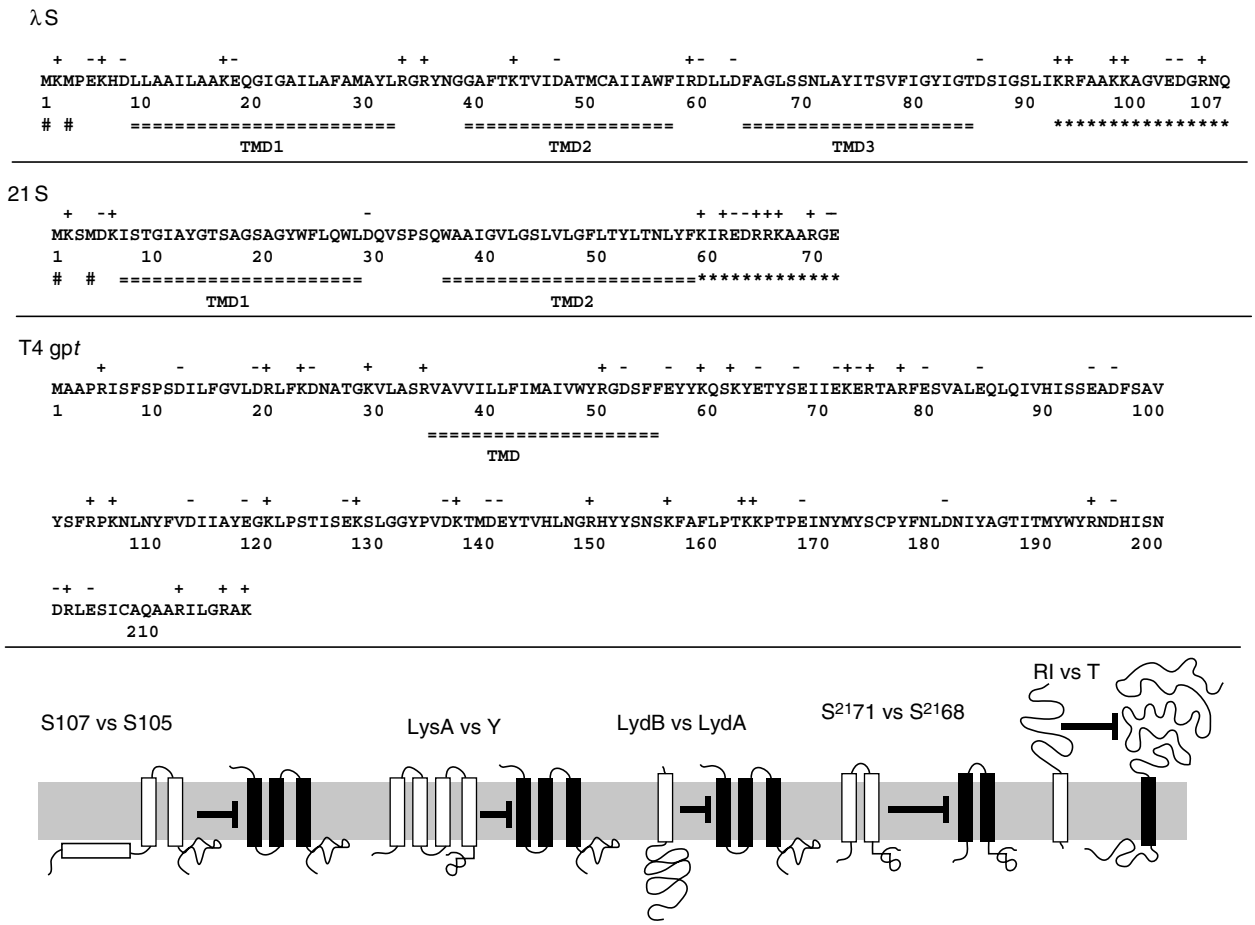


Figure 1 Holins and antiholins: sequence and topology. Upper 3 panels: Primary structure and membrane topology of the products of lambda *S*, phage 21 S^{21}, and T4 *t*; ===, transmembrane domains (numbered for each protein) as predicted by TMHMM program (http://www.cbs.dtu.dk/services/TMHMM-2.0/); ***, the highly charged, dispensable C-terminal region of lambda S and S^{21} gene products; #, the two start codons of lambda *S* and S^{21}. Lower panel: Membrane topologies of coliphage holins and their cognate antiholins; interaction between holin/antiholin pair is indicated. From left to right: antiholin S107 and holin S105 of lambda; antiholin LysA and holin Y of P2; antiholin LydB and holin LydA of P1; antiholin S^{21}71 and holin S^{21}68 of lambdoid phage 21; antiholin RI and holin gp*t* of T4.

Endolysins

There are three chemical bonds that constitute the covalent polymer of the peptidoglycan: glycosidic bonds between the amino-sugars, amide bonds between between the linking oligopeptide and the lactyl group of MurNAc, and peptide bonds within the linking oligopeptide. Lambda R is a 17 kDa muralytic enzyme that cleaves the MurNac–GlcNac glycosidic linkage of the peptidoglycan, releasing a disaccharide digestion product lacking a reducing end. Three other different enzyme activities are associated with other endolysins, each associated with the breakage of one of the three different types of covalent bonds that link the subunits of the murein polymer. (Note the historical term lysozyme is also associated with three different enzymatic entities.)

Glycosidases. Also known as true lysozymes or, less precisely, as muramidases, these enzymes cleave the same peptidoglycan bonds as the transglycosylases but do so by hydrolysis, freeing disaccharide products with reducing ends. Strictly, glycosidases can also be called either muraminidases or glucosaminidases, depending on whether they cleave after MurNac or GlcNac residues. The phage T4 lysozyme, E (product of the T4 *e* gene), is perhaps the most intensively studied protein structure, in terms of the energetics and mechanics of its tertiary structure, and is a 164 residue monomeric, soluble globular enzyme.

Amidases. These enzymes hydrolyze the amide bond. The best-characterized phage amidase is the T7 lysozyme, or gp*3.5*. Gene *3.5* is expressed as an early gene because it has a second important function beyond the endolysin role: it complexes with and inhibits T7 RNA polymerase.

Endopeptidases. These enzymes hydrolyze a peptide bond or isopeptide bond in the cross-linking polypeptide moieties. For example, the *S. aureus* phage A500 has an L-alanyl-D-glutamate endopeptidase as its endolysin. Currently, no endolysin with endopeptidase activity has been characterized structurally.

There does not appear to be any linkage between the enzymatic type of endolysin and the topology class of the

holin. For example, the holins of lambda and the *Salmonella* phage P22 are nearly identical class I holins, but the P22 endolysin is a T4 E glycosidase homolog, whereas lambda R is a transglycosylase. Moreover, the lambda holin has been shown to function with the T4 glycosidase E and the T7 amidase gp*3.5*, at least to the extent that co-expression of the holin and either endolysin results in host lysis.

Holin–Endolysin Lysis: The Lambda Paradigm

The paradigm system for studying lysis has been the classic phage lambda, and the functions of the holin, antiholin, endolysin, and Rz–Rz1/spanin are best described with reference to this system. For studying lysis, the main advantage of lambda, besides its unparalleled genetics, is that one can use thermo-inducible lysogens of *E. coli* carrying a single lambda prophage to obtain the synchronous initiation of the vegetative cycle in all the cells of a culture in balanced growth. The extent and timing of lysis can then easily be monitored by following the turbidity of the culture. Under carefully controlled physiological conditions, such lysogenic inductions result in a synchronous and saltatory lysis of all the cells in the culture at about 50 min after induction, releasing ~100 progeny virions per induced cell. Noninvasive methods to assess the energy state of the cell have shown that, up to the instant of lysis triggering, the induced cells have normal energy metabolism and an intact, energized membrane. This rigidly scheduled lysis program of lambda can be subverted by any disturbance of the energy metabolism of the cells; for example, addition of an energy poison like cyanide or the uncoupler dinitrophenol (DNP) triggers 'premature' lysis, indicating that holin–endolysin systems rely on the energized state of the membrane to achieve temporal scheduling.

The lambda lysis genes, *SRRzRz1,* are clustered in the 'lysis cassette' immediately downstream of the lambda late gene promoter (**Figure 2**). This promoter is activated about 8 min after induction and serves not only the lysis

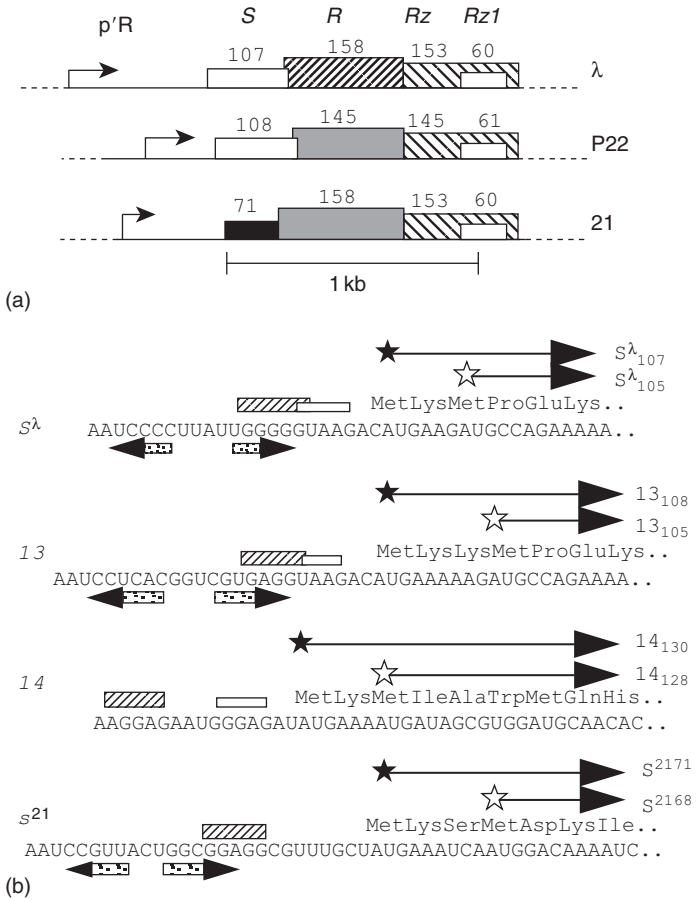


Figure 2 The lambda lysis cassette and the dual start motif. (a) The late promoter and *S*, *R*, *Rz* and *Rz1* genes of lambda, P22 and phage 21; amino acid lengths of each gene product are indicated above each gene. (b) The dual start motifs of the *S* gene of coliphage lambda, gene *13* of *Salmonella* phage P22, gene *14* of *B. subtilis* phage ϕ29, and the *S* gene of lambdoid coliphage 21; the boxed sequences (diagonal striped box for the antiholin, white box for the holin) indicate the Shine–Dalgarno sequences for the dual translational starts; the start codon for each protein is marked with a star and arrow (filled stars indicate antiholin start codons, white stars indicate holin start codons) in the direction of translation above the sequence and the length of both protein products is given in amino acid (aa) residues. Stem–loop structures (if present) are marked with arrows below the sequence in the direction of base of the stem–loop.

genes but also the approximately 25 essential genes downstream of the lysis cassette, which are required for DNA packaging and the synthesis and assembly of heads, tails, and tail fibers. Thus, the lysis genes are expressed simultaneously with all the other genes needed for viral morphogenesis. Genes *S* and *R* are required for lysis under all conditions; *R* encodes the endolysin, a transglycosylase, which accumulates fully folded and active in the cytoplasm. *S* is a 107 codon reading frame which encodes two products, named S105 and S107 for their length in amino acid residues, as a consequence of dual translational starts at Met codons 1 and 3 (**Figures 1** and **2**). S105 is the essential holin; the properties of S107, which is nonessential and functions as an antiholin, will be described below. In the absence of functional S105 protein, the lambda vegetative cycle, begun either from an induction of a lysogen or from infection, continues unabated for several hours, far beyond the 50 min lysis time observed for phages carrying the parental *S* allele. During this extended latent period, progeny virions and lambda endolysin accumulate in the cytoplasm. Lysis can be elicited almost instantaneously if the host membranes are disrupted by chloroform or a detergent, reflecting the endolysin's requirement for the *S* holin to achieve access to the cell wall. In contrast, null mutations in *R* do not result in an extended latent period, if the *S* gene is functional; instead, cellular respiration, ATP synthesis, all macromolecular events, and phage assembly stop at the normal time of lysis, but the progeny virions are left within the dead cell.

The lambda holin S105

These physiological effects and other observations indicate that the *S* holin functions by forming a hole in the membrane. This conclusion is reinforced by the ability of purified S105 protein to form holes in artificial membranes; moreover, missense mutations that block hole formation *in vivo* also block *in vitro* hole formation. The holes that are formed appear to be nonspecific, since null alleles in the lambda *S* gene can be complemented by completely unrelated cloned holin genes from other phages of Gram-negative hosts, such as T4, P2, and PRD1 and also from phages of Gram-positive hosts. Nothing has been published about the structure of the S holes except that they must be very large; fusions of *R* to β-galactosidase (resulting in a tetrameric *R*-βgal chimera with a molecular mass exceeding 500 kDa) are fully functional as endolysins and support plaque formation.

Genetic and biochemical studies focused on the unique ability of S105, an integral membrane protein, to form large nonspecific holes in the membrane, and on the equally unique capacity to accomplish this at a programmed time. The S105 protein has three transmembrane domains, with an N-out, C-in topology (**Figure 1**). The C-terminal cytoplasmic domain is nonessential, although the number of basic residues in this sequence is important for lysis timing. *S* represents an extreme case of genetic malleability. Missense mutations in all three TMDs, including in series of positions all the way around each helical domain, affect lysis timing unpredictably and dramatically. For example, substitutions of Gly or Leu at Ala52 result in catastrophically early lysis at about 20 min, before the first progeny virion is assembled, whereas Ala52 to His or Trp result in delayed lysis (and increased burst size) and Ala52 to Val, Ile, or Thr are functionally nonlytic, null alleles. The fact that all surfaces of all three TMDs are sensitive to substitution suggests that helical packing, both intramolecular and intermolecular, is involved in the *S* holin function and timing. This notion is reinforced by cross-linking studies that show S oligomerizes in the membrane. Some of the mutants that block lysis also block oligomerization, such as A52V, which yields only dimers, but others do not.

Based on these properties, a model for S105 function has been proposed in which it assembles into large multimeric 'death rafts' within which lipid is completely or largely excluded. At some point dependent on the overall helical packing properties of the particular S105 allele, the raft develops a lesion, leading to a local collapse of the membrane potential. In this normal triggering event, and also in the case of premature lysis triggering with an energy poison, this local collaspse of membrane potential triggers a conformational shift at the tertiary or quaternary level, leading to immediate hole formation. It is thought that the remarkable malleability of S105 for its lysis timing phenotype reflects the effects of changing side chain mass and shape on the helical packing involved in formation and stability of the rafts. In support of this notion is the interesting observation that some defective *S* alleles have an unusual form of partial dominant character. In lysogens carrying two prophages, one wild type for *S* and the other an absolute-defective missense *S* allele, lysis timing is actually earlier than with a lysogen carrying two S^+ prophages.

The antiholin S107

Compared to S105, without which lysis does not occur, S107 normally has a much more subtle null phenotype for lysis. Elimination of the start codon for S107 advances the timing of lysis by 5–7 min. By altering RNA secondary structures flanking the translation initiation region, alleles with different S105:S107 ratios can be constructed. Strikingly, any allele generating a S105:S107 ratio <1 does not spontaneously trigger until very late after induction and has dominant-negative character.

The only distinction between S105 and S107 is the latter's N-terminal additional dipeptide Met_1–Lys_2, and the critical feature is the Lys residue. It has been shown that the N-terminal TMD of S107 is prevented from integrating into the membrane as long as the bilayer is energized; thus, S107 and S105 have different topologies. Depolarization by an energy poison, or by holin-dependent hole formation, relaxes the barrier, allows the

N-terminal TMD of the S107 molecule to integrate into the bilayer, and converts the antiholin into a functional holin. S107 binds S105, and it is thought that S107:S105 complexes are inactive for hole formation; the net effect is that, when triggering does occur, thereby collapsing the membrane potential, all the S105:S107 complexes are converted into active holin complexes, which may increase the size or number of the holes.

It is important to note that the dependence of the inhibitory activity of S107 on the energized state of the membrane is distinct from the susceptibility of S105 to being triggered by the collapse of the membrane potential. In the former case, a topological change is caused by the de-energization of the bilayer, whereas S105 has all three TMDs integrated into the membrane from the outset.

Rz–Rz1 encodes the spanin complex

The most distal genes of the lambda lysis cassette, *Rz* and *Rz1*, have an unusual relationship in that the smaller gene, *Rz1*, is entirely embedded within *Rz* in the +1 reading frame. Although there are a few other examples of such out-of-frame nested genes (see below for two examples in other phage lysis systems), *Rz* and *Rz1* are unique in biology because the two genes are required for the same biological function. The products of the two reading frames are components of a complex that spans the entire periplasm, from the cytoplasmic membrane to the outer membrane (OM). This function of this complex is unknown, but it appears to be important for efficient disruption of the envelope. The evidence for this is that, in the absence of either *Rz* or *Rz1*, lysis is blocked if the OM is stabilized by ~10 mM divalent cations in the medium. The terminal phenotype is a mechanically fragile spherical cell, presumably bounded by the stabilized OM.

Rz–Rz1 equivalents are found in almost all phages of Gram-negative bacteria. The genes are highly diverse, both in terms of sequence and gene arrangement. In many cases, the *Rz1* gene begins within the *Rz* gene but extends beyond its end. In other cases, the *Rz* and *Rz1* genes are completely separated, although always in the order *Rz–Rz1*. Rz proteins are type II 'signal anchor' proteins, with an N-terminal TMD tethering a periplasmic domain to the bilayer. Rz1 proteins are lipoproteins that are attached to the inner surface of the OM by the lipid and fatty acid groups of the modified N-terminal Cys residue. Rz and Rz1 form complexes by a specific C-terminal interaction. In the classic coliphage T1, the *Rz–Rz1* genes are replaced by a single gene encoding a larger OM lipoprotein with the unique feature of a C-terminal TMD. Because this lipoprotein spans the entire envelope, it has been designated as a 'spanin'. The more common Rz–Rz1 pairs comprise a functional equivalent to the spanin, albeit with different topology.

A New Paradigm: Signal Sequences, Pinholins, and SAR Endolysins

Until recently, the lambda lysis paradigm, where the holin controls lysis by gating the escape of the muralytic endolysin from the cytoplasm, was thought to apply to all dsNA phages. However, recent findings have established that there are completely different modes of holin–endolysin lysis. The first hint of this came from experiments with the lysis system of a phage, fOg44, which grows on the Gram-positive host *Oenococcus oeni*. The lysozyme of this phage was shown to have a bona fide signal sequence, such that it is exported by the *sec* translocon of the host, concomitant with proteolytic removal of the signal. Nevertheless, fOg44 also encodes what appears to be a canonical holin, suggesting that the holin plays a different role in the control of lysis in this phage system. Studies from the more genetically accessible phages of *E. coli* have provided unequivocal evidence of a new paradigm. Genetic and molecular analysis revealed that the holin of lambdoid phage 21 does not form holes large enough to allow exit of a soluble endolysin. Instead, the class II holin, called S^{21} because it is the product of the *S* gene of phage 21, forms small holes that cause depolarization of the membrane but are not large enough to allow an endolysin to cross the membrane. For lambda, this result would be the worst possible outcome of a lysis program; the host cell would be dead, stopping the further accumulation of progeny virions and leaving the existing progeny, along with the cytoplasmic endolysin, trapped in a corpse. Phage 21, and many other phages, however, encode an endolysin with a *sec*-specific secretory signal in the form of an N-terminal TMD appended to a glycosidase catalytic domain. The TMD functions as 'signal anchor' domain, so that the endolysin is initially secreted as type II integral membrane proteins (single TMD, N-in, C-out). This would seem to be inconsistent with the notion of specifically timed lysis, since the exported endolysin activity would begin appearing in the periplasm immediately after the onset of late protein synthesis. Remarkably, however, these enzymes are inactive in the membrane-tethered form, so there is no catastrophically early lysis. In addition, the signal anchor TMDs have a special character, in that they can completely escape from the membrane, although they are not cleaved off like normal signal sequences. When this happens, the endolysins become active and murein degradation ensues. Endolysins with this feature are called SAR (signal anchor release) endolysins. It is not known yet what allows these membrane-embedded helical domains to have the unprecedented ability to be extracted efficiently from the bilayer; at least from primary structure analysis, it is clear that most SAR domains are rich in Ala, Gly, Ser, and other minimally hydrophobic neutral residues. This feature suggests that the SAR domains are localized to the

membrane by 'tricking' the signal recognition machinery, but are metastable in the environment of the bilayer, requiring the polarization of the membrane to remain integrated.

In support of this notion, SAR endolysins are found to spontaneously release from the membrane at a low rate, even when the membrane remains energized. Thus, the induction of a cloned SAR endolysin gene can eventually cause lysis as a result of spontaneous release and activation of the induced protein. Moreover, in the unrelated phages P1 and 21, both with SAR endolysins, neither holin gene is required for plaque formation, since, in the absence of holin triggering, the infected cell will eventually lyse and release progeny virions. Even so, the release of the SAR endolysin from the membrane is quantitative and rapid if the membrane is de-energized, as happens when the S^{21} holin triggers to form its small holes. This elegant variation on the classic lambda paradigm serves to emphasize the real role of the holin in lysis – to provide timing. In the lambda/T4 system, the holin effects timing by controlling the escape of the already active phage endolysin from the cytoplasm. In contrast, in the phage 21 system and many others, the holin determines timing by depolarizing the membrane, thus causing the concerted activation of all the membrane-tethered, inactive endolysin molecules.

To distinguish holins like lambda S105 and T4 T, which form holes sufficiently large to let fully folded enzymes transit the membrane nonspecifically, from holins like S^{21}, which make small holes that serve to depolarize the bilayer, the latter type of holin has been termed a 'pinholin'. Phage genomes are highly mosaic, and the lysis cassettes are no exception; in general, then, there is no significant barrier to recombination events that pair different holin and endolysin genes. However, pinholin genes are obviously more limited in this respect, compared to the canonical holins, in that they can only function with SAR endolysins, which do not require large holes to allow escape from the cytoplasm.

Diversity in Antiholin Strategies

The dual start motif which allows the *S* gene to produce both the S105 holin and S107 antiholin has been found in a number of holin genes, including the unrelated class I holin gene from the *B. subtilis* phage ϕ29 and in the class II holin genes of the S^{21} family (**Figures 1** and **2**). In the latter, the extra positive charge in the longer antiholin form of the protein still influences the membrane topology of the first TMD, as in lambda S107, but in this case, it inhibits TMD1 from exiting the membrane, rather than entering it. Remarkably, S^{21} TMD1 has the properties of an SAR domain, spontaneously leaving the bilayer at a certain rate, which is accelerated if the membrane is depolarized. As long as it stays within the bilayer, TMD1 binds TMD2 and prevents it from oligomerizing to form the pinhole. In effect, TMD1 is an internal antiholin of the S^{21} class II holin, and in the longer form produced from the dual start, the internal antiholin sequence is retarded from escaping the membrane.

In other lysis systems, the holin and antiholin are encoded by separate genes, with a striking number of different topologies (**Figure 1**). The well-studied coliphage P2 has a putative antiholin gene, *lysA*, sandwiched in its lysis gene cluster between the holin and endolysin genes, *Y* and *K*, and the *Rz–Rz1* equivalents, *lysB–lysC*. LysA is predicted to be an integral membrane protein with four predicted TMDs. In the classic phage T4, the antiholin gene is *rI*, one of the first genetic markers identified in storied history of phage biology; the RI protein is a type II signal anchor protein with a 72 residue periplasmic domain. The T4 holin, encoded by gene *t*, is the prototype class III holin, with a single TMD and a large periplasmic domain. RI inhibits gp*t* through interactions of the periplasmic domains. In infected cells, the RI protein is activated to be an inhibitor of gp*t* by secondary infections that occur about 2 min after the primary infection. T4 gene product, gp*imm*, blocks passage of the secondary phage's DNA into the cytoplasm, resulting in ectopic localization of the capsid contents in the periplasm. It is not known whether it is the mislocalized DNA or internal capsid proteins that activate RI, or what the activating event is. Interestingly, RI is also an SAR protein, releasing from the membrane at a certain rate, and its N-terminal SAR TMD confers instability on the protein. This instability ensures that RI inhibition of the holin is not permanent. The concept is that holin triggering should be postponed as long as the infected cell continues to be superinfected with more T4 phage particles, which is an indication that the current environment is host poor. When superinfection ceases, the instability of RI ensures that the gp*t* holin will be allowed to trigger and cause the release of the progeny virions. Overexpression of *rI* bypasses the need for activation, just as altering the ratio of S105 to S107 in favor of S107 blocks lysis in the lambda system.

Antiholins have also been identified in P1, where the *lydB* gene encodes a small type I integral membrane protein, that inhibits the LydA holin, a class I holin unrelated to lambda S105. Interestingly, in P1, the holin is not required for lysis or plaque-formation, since the P1 Lyz is a SAR endolysin. Instead, the antiholin LydB is essential, because in its absence, the LydA holin triggers lysis too early, before the first virion has been completed (on average). LydB binds to LydA, but other details of its inhibitory function have not been elucidated. In general, except for the T4 system, nothing is known about how antiholin function is regulated during infection. Indeed, it is not clear why antiholins exist at all in cases like the lambda and phage 21 systems. The loss of the antiholin product in these cases has only a slight effect – the

acceleration of lysis by a few minutes. Considering the genetic malleability of the lambda *S* gene, it is easy to isolate mutations which would cause S105 to trigger at almost any time, so the presence of the elaborate dual start system to generate a slight delay in lysis would seem to be unnecessary. As noted above, altering the 5′-end of the *S*-gene to cause synthesis of S107 in excess of S105 results in a block in lysis; it is possible that some physiological conditions may exist which cause the S107:S105 ratio to change, which would thus achieve real-time control of lysis timing.

'Single-Gene' Lysis

Although the holin–endolysin system is nearly universal among dsNA phages, it is not found in all lytic phages. A second well-studied mode for host lysis is found in two other major classes of icosahedral phages in Gram-negative bacteria, the ssDNA *Microviridae* and the male-specific ssRNA *Allolevivirida*e. In both of these cases, a single gene is necessary and sufficient for lysis – gene *E* in the prototype *Microvirus* ϕX174 and gene A_2 in the prototype *Allolevivirus*, Qβ. Moreover, both lytic proteins cause lysis in the same way, by inhibiting an enzyme in the synthesis pathway of peptidoglycan precursors. In eubacteria, the committed step for synthesis of murein is the reaction catalyzed by MurA, which adds the 3C backbone of PEP to the amino-sugar ring of the sugar nucleotide UDP-GlcNac (**Figure 3**). A series of further modifications to this sugar nucleotide yields UDP-MurNac-pentapeptide. The aminosugar-pentapeptide is transferred to a C55 lipid carrier by a membrane-embedded enzyme, MraY, converted to a disaccharide-pentapeptide by the addition of a GlcNac moiety, and then flipped to the outside surface of the cytoplasmic membrane by a yet unidentified 'flippase' component. Linkage of the disaccharide-pentapeptide to the murein is catalyzed by the transglycosylase activity of a multicomponent machinery involving the penicillin-binding proteins (PBPs). The formation of the new glycosidic bond results in the liberation of the undecaprenol carrier and is followed, in Gram-negative bacteria, by the formation of isopeptide cross-links between the peptidoglycan chains, a process mediated by transpeptidase activities of the PBP complex. The two lysis proteins E and A_2 were found to be specific inhibitors of MraY and MurA, respectively. Thus, infection with ϕX174 or Qβ results in the cessation of the flow of precursors to the periplasmic murein synthesis complex. It is not completely clear why this results in lysis. Most cells are seen to undergo lysis during septation, when new cell wall material is required to separate the daughter cells. Apparently, bacterial cells are unable to stop the septation process if the flow of murein precursors is interrupted. In any case, E- and A_2-mediated lysis is indistinguishable from lysis induced by antibiotics that inhibit any of the steps in the pathway

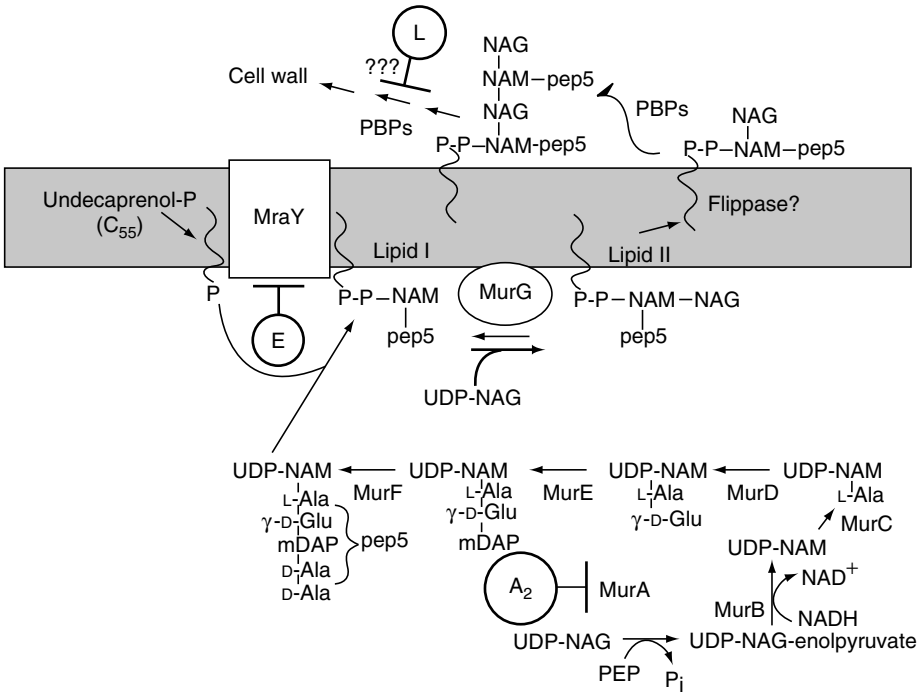


Figure 3 Phage-encoded 'protein antibiotics' inhibit the synthesis of cell wall precursors. The murein synthesis pathway beginning with the first committed step to peptidoglycan synthesis, which is catalyzed by MurA. Inhibition by E and A_2 is shown at the step that they inhibit, and inhibition by the L protein is indicated after the incorporation of the disaccharide oligopeptide subunit into the peptidoglycan (the specific target of L-mediated lysis is not currently known).

for precursor synthesis or by β-lactams, which inhibit the PBPs. E and A_2 have been called 'protein antibiotics' because of this similarity of action.

E: The First Embedded Gene

The molecular mechanisms of E and A_2 function are of interest at least in part because both MraY and MurA are universally conserved in bacteria and are thus attractive antibiotic targets. The *E* gene of ϕX174 was originally defined as an essential cistron in ϕX174 that gave rise to nonsense mutants with defects in lysis but not in virion production. Strangely, mutations in an essential morphogenesis gene, *D*, mapped to both sides of the *E* mutations. ϕX174 was the first DNA genome to be sequenced, and part of the interest in this ground-breaking work was the revelation that *E* was entirely embedded within *D*, in the +1 reading frame. (Note, however, that the *D-E* embedded relationship is very different from that of *Rz–Rz1*, because in the latter case, the two protein products are required for the same biological function.) *E* encodes a 91 residue type I integral membrane protein. The C-terminal cytoplasmic domain is highly basic (predicted +11 net charge) and completely dispensable. Fusions of the N-terminal domain of *E* to heterologous protein domains such as β-galactosidase and GFP result in chimeras with unimpaired lytic function. Mutations conferring resistance to *E* have been mapped to two of the ten TMDs of MraY. In *in vitro* assays, MraY activity has been shown to be inhibited in membranes containing E. However, to date, the inhibition has not been demonstrated in a purified system. A host gene, *slyD*, encoding a cytoplasmic *cis–trans* peptidylprolyl *cis–trans* isomerase (PPIase), is required for E-mediated lysis. In the absence of SlyD, the E protein is extremely unstable, suggesting that the PPIase is needed for proper folding of E. Mutations that restore the ability of the phage to plate on *slyD* hosts have been mapped to N-terminal residues of E. These turn out to be translation-up mutations that bypass the *slyD* block by increasing the rate of synthesis without affecting the proteolytic instability. While it is unclear if there is a regulatory relationship between E-mediated lysis and the host *slyD* gene, the strict dependence of ϕX174 plating on the *slyD* function remains the strongest ligand-independent phenotype ever found for PPIases, which, despite their ubiquity in most compartments of both eukaryotic and prokaryotic cells, have not been associated with essential functions.

A_2: A Virion-Borne Lysis Protein

Unlike *E*, the *A_2* gene occupies a unique reading frame, in this case the first cistron of the Qβ genome (**Figure 4**), encoding a 420 residue polypeptide. However, the lysis function is just one of several essential functions of A_2, which is present in one copy in the Qβ virion. In addition to lysis, A_2 is required for adsorption to the male sex pilus and protection of the 5′-end of the ssRNA genome from external RNase attack. Unlike the membrane-embedded MraY, MurA, the target of A_2, is a soluble enzyme that has been extensively studied at both the structural and mechanistic levels. Thus far, however, the only soluble form of A_2 that has been obtained has been in the form of purified Qβ virions, and these have been shown to inhibit MurA *in vitro*. A misssense mutation in MurA, *rat1* (resistance to A-two), that blocks the inhibitory action of A_2 and abolishes Qβ plaque formation, has been found to be a Leu to Gln change in residue 138; this residue is on the surface of MurA near the active site cleft. Qβ *por* (plates on rat) mutations that overcome the *rat1* block and restore

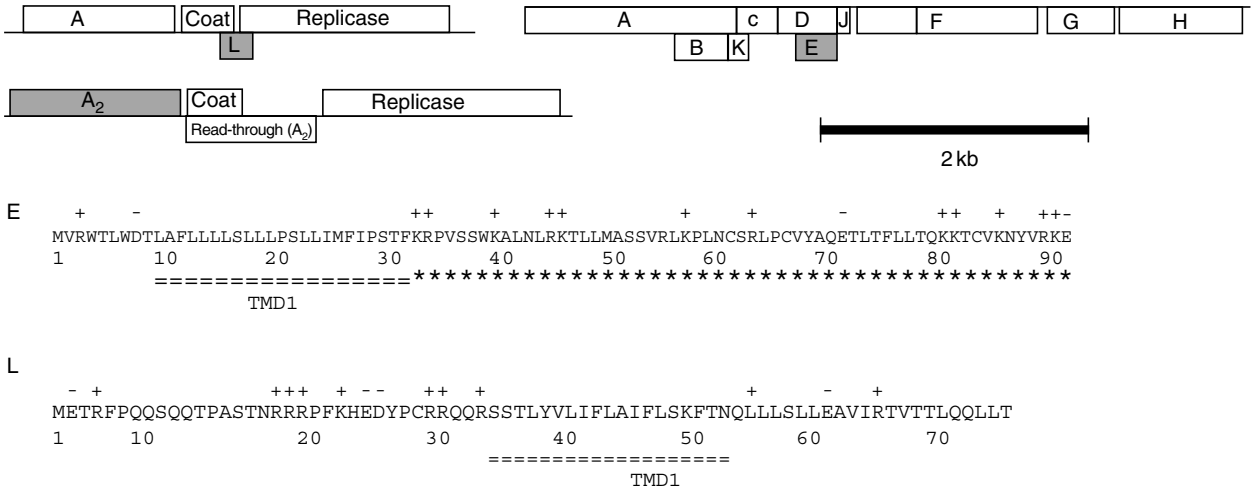


Figure 4 Single gene lysis systems. Upper panel: Genome maps of phages MS2, ϕX174, and Qβ; the lysis gene of each phage is colored gray. Lower panel: Primary sequence and membrane topology of the lysis proteins L, E; ===, transmembrane domains as predicted by TMHMM (see above.); ***, the highly charged, dispensable C-terminal region of E.

plaque-forming ability map to several positions in the N-terminal domain of A_2. Among the issues not yet resolved, besides the molecular details of the MurA-A_2 interaction, is whether or not the actual *in vivo* inhibitor of MurA is A_2 on the Qβ virion or free A_2 protein.

L: Another Embedded Gene and an Enduring Mystery

Operationally, the fundamentals of how the holin-endolysin and the two 'protein antibiotic' systems described above effect lysis are now established, even though the molecular mechanisms are still a matter of investigation. The remaining mystery in phage lysis, in terms of the general mode of action, is that of the male-specific ssRNA *Leviviridae*. These viruses have nearly the same genome organization as the *Alloleviviridae*, except that the lysis gene is a separate, small (75 codon) reading frame designated as *L* (**Figure 4**). Expression of *L* is necessary and sufficient for lysis, but, unlike the case with E and A_2, synthesis of murein is undisturbed, at least up to the initial incorporation of the disaccharide oligopeptide subunit into the peptidoglycan. The simplest notion would be that L interferes with cell wall synthesis or maturation in some step downstream of the initial glycosidic bond formation. Alternatively, L may be triggering an autolytic response analogous to those triggered in many Gram-positive systems as a result of starvation or developmental processes.

The *L* gene is yet another example of an embedded gene; in this case, the *L* cistron overlaps the end of the *coat* gene and the beginning of the *rep* gene encoding the RNA-dependent RNA polymerase. The predicted L protein spans only 75 residues, with a highly charged basic N-terminal domain and a hydrophobic C-terminal domain. Although no topological studies have been done, it has been reported that the L protein accumulates in zones of adhesion between the inner and outer membranes. The expression of *L* is regulated by RNA structures that control access to its translation initiation site and are influenced by ribosomes transiting the end of the *coat* gene.

Perspective

Lysis was once considered a rather trivial and inevitable consequence of bacteriophage infection, an indirect consequence of the extensive re-direction of the host biosynthetic and maintenance functions to the purposes of the virus. However, it is now clear that lysis is a carefully orchestrated and regulated process programmed by the phage. Moreover, the molecular basis of the lytic event and its regulation are compellingly interesting, involving fundamental issues of the behavior of membrane proteins and also of the function of the bacterial cell wall biosynthesis pathway. The availability of the traditionally powerful genetic facility of phages, coupled with modern genomic, proteomic, and structural approaches, gives promise of rapid progress in our understanding of this fundamental biological process.

See also: Ecology of Viruses Infecting bacteria; Virus Entry to Bacterial Cells.

Further Reading

Bernhardt TG, Wang IN, Struck DK, and Young R (2002) Breaking free: 'Protein antibiotics' and phage lysis. *Research in Micriobiology* 153: 493–501.

Bläsi U and Young R (1996) Two beginnings for a single purpose: The dual-start holins in the regulation of phage lysis. *Molecular Microbiology* 21: 675–682.

Rydman PS and Bamford DH (2003) Identifcation and mutational analysis of bacteriophage PRD1 holin protein P35. *Journal of Bacteriology* 185: 3795–3803.

Sao-Jose C, Parreira R, and Santos MA (2003) Triggering of host-cell lysis by double-stranded DNA bacteriophages: Fundamental concepts, recent developments and emerging applications. In: Pandalais G (ed.) *Recent Research Developments in Bacteriology,* vol. 1, pp. 103–130. Kerala, India: Transworld Research Network.

Sao-Jose C, Parreira R, Vieira G, and Santos MA (2000) The N-terminal region of the *Oenococcus oeni* bacteriophage fOg44 lysin behaves as a bona fide signal peptide in *Escherichia coli* and as a cis-inhibitory element, preventing lytic activity on oenococcal cells. *Journal of Bacteriology* 182: 5823–5831.

Steiner M, Lubitz W, and Bläsi U (1993) The missing link in phage lysis of Gram-positive bacteria: Gene 14 of *Bacillus subtilis* phage ϕ29 encodes the functional homolog of lambda S protein. *Journal of Bacteriology* 175: 1038–1042.

Tran TAT, Struck DK, and Young R (2005) Periplasmic comains define holin–antiholin interactions in T4 lysis inhibition. *Journal of Bacteriology* 187: 6631–6640.

Wang IN, Deaton J, and Young R (2003) Sizing the holin lesion with endolysin-beta-galactosidase fusion. *Journal of Bacteriology* 185: 779–787.

Wang IN, Smith DL, and Young R (2000) Holins: The protein clocks of bacteriophage infections. *Annual Reviews of Microbiology* 54: 799–825.

Xu M, Struck DK, Deaton J, Wang IN, and Young R (2004) A signal-arrest-release sequence mediates export and control of the phage P1 endolysin. *Proceedings of the National Academy of Sciences* 101: 6415–6420.

Young R (2005) Phage lysis. In: Waldor MK, Friedman DI, and Adhya SA (eds.) *Phages: Their Role in Pathogenesis and Biotechnology*, pp. 92–127. Washington, DC: ASM Press.

Young R and Wang IN (2006) Phage lysis. In: Calendar R (ed.) *The Bacteriophages*, pp. 104–126. Oxford: Oxford University Press.

Young R, Wang IN, and Roof WD (2000) Phages will out: Strategies in host cell lysis. *Trends in Microbiology* 8: 120–128.

M

Machlomovirus

K Scheets, Oklahoma State University, Stillwater, OK, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Maize chlorotic mottle virus (MCMV) was initially found in maize (*Zea mays*) and sorghum (*Sorghum bicolor*) fields in Peru in 1973. In 1976, the virus appeared in Kansas (USA) maize fields, alone and as part of a synergistic disease. In 1978, the second component of the synergistic disease, corn lethal necrosis (CLN), was identified as any maize-infecting virus of the family *Potyviridae*. MCMV and CLN spread to Nebraska, and both MCMV and CLN appeared in Mexico in 1982. On the island of Kauai, Hawaii (USA), a severe outbreak of MCMV appeared in the winter seed nurseries in 1989–90. MCMV is endemic in Peru and along the Kansas–Nebraska border. In 2004, MCMV and CLN were first detected in Thailand where MCMV continues to spread.

Taxonomy and Classification

The only known member of the genus *Machlomovirus* is the species *Maize chlorotic mottle virus*. Its inclusion in the family *Tombusviridae* is based on the high degree of homology of the encoded viral replicase. The genome organization of the single viral RNA of MCMV is most similar to panicum mosaic virus (PMV), species *Panicum mosaic virus,* genus *Panicovirus,* family *Tombusviridae*. Key taxonomic features of the genus *Machlomovirus* are a unique open reading frame (ORF) at the 5′ end of the genome that largely overlaps the pre-readthrough portion of the viral replicase gene, and a readthrough ORF preceding and overlapping the capsid protein (CP) gene near the 3′ end of the genome.

Virion Structure and Properties

Transmission electron microscopy of negatively stained MCMV virions shows a smooth sphere or hexagonal structure approximately 28 nm in diameter (**Figure 1**). The virion consists of a 4437 nt single-stranded RNA surrounded by 25.1 kDa CP subunits that lack the protruding domain found on CPs of viruses from many genera in the family *Tombusviridae*. Sequence similarity to the CPs of PMV, tobacco necrosis virus genus *Necrovirus*, family *Tombusviridae*, and southern bean mosaic virus genus *Sobemovirus* suggest that MCMV is a $T = 3$ icosahedral virion with 180 copies of its CP in the viral shell. The estimated weight of the virion is 5.95 mDa. Purified MCMV has a sedimentation coefficient ($s_{20,w}$) of 109S and a buoyant density in CsCl of 1.365 g ml^{-1}. The thermal inactivation point in maize sap is 85–90 °C. The virion is stable at pH 5.

Genome Structure and Organization

The genome organization of the genus *Machlomovirus* is based on the complete sequence of MCMV. The plus-sense RNA is 4437 nt long and contains seven overlapping ORFs that encode proteins of 7 kDa or larger (**Figure 2**). The RNA was reported to have no poly(A) tail and an ^{m7}G cap at the 5′ end. However, in common with other viruses in the family, the encoded MCMV replicase does not have any motifs characteristic of the methyltransferase domain found in viral replicases of capped RNA viruses. None of the other MCMV-encoded proteins contain a methyltransferase domain, so it is likely that the RNA in MCMV, like in other members of the family, is in fact uncapped. The 5′ untranslated region (UTR) is 117 nt long, and ORF1 encodes a 32 kDa highly acidic protein (estimated pI = 3.83). ORF2 begins 19 nt downstream and encodes a 50 kDa highly basic protein (estimated pI = 10.59) so the migration of these two proteins in sodium dodecyl sulfate (SDS) polyacrylamide gels is likely to be anomalous. Suppression of the UAG stop codon of ORF2 would produce a 111 kDa protein. A cluster of four ORFs is encoded in the 3′ third of the viral RNA downstream of the transcription start site for the 1467-nt-long subgenomic RNA1 (sgRNA1). ORF4 encodes a 7.5 kDa protein (p7a), and suppression of its UGA stop codon would produce a 31 kDa protein. The second AUG of sgRNA1 begins ORF7 which encodes the

viral CP. ORF6 was identified by similarity of its gene product p7b to small peptides encoded in similar locations on carmoviruses, necroviruses, and PMV, and it begins with a noncanonical start codon. *In vitro* translation of MCMV virion RNA in rabbit reticulocyte lysate produces p32, p50, p111, and p25. The two 7 kDa peptides and p31 were not detected in rabbit reticulocyte lysate translations. The 3′ UTR is 343 nt long and encodes a 337-nt-long sgRNA (sgRNA2).

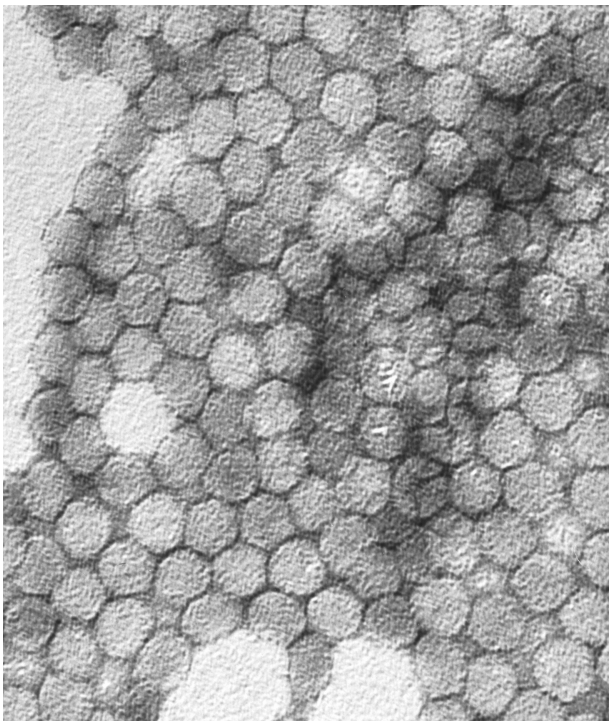


Figure 1 Purified maize chlorotic mottle virus negatively stained with uranyl acetate.

Replication

The replication strategy of MCMV has not been completely determined, but inoculation of maize protoplasts with transcripts from wild type and mutant versions of an infectious cDNA has provided some information. Transcripts with mutations in the 3′ third of the genome that stop expression of one or more of the proteins encoded on sgRNA1 are capable of replication. Additionally, mutations just upstream of the sgRNA1 transcription start site that stop expression of sgRNA1 but do not alter the sequence of p111 are capable of replication, indicating that none of the proteins encoded on sgRNA1 are necessary for replication. Based on the replication mechanisms of other tombusvirus family members it is likely that after virion disassembly, MCMV viral RNA is translated to produce the viral replicase which then synthesizes the negative strand of genomic RNA after recognizing sequences and structures located at the viral 3′ terminus that have sequence and structural similarities to the promoters of carmoviruses. The complementary strand is then used as template for synthesis of progeny viral RNA strands. sgRNA synthesis mechanisms differ between genera in the family *Tombusviridae*, and it is not known which mechanism is used by MCMV. sgRNA1 synthesis may initiate by replicase binding internally to the sgRNA promoter on the genomic complementary strand. Alternatively, occasional premature termination

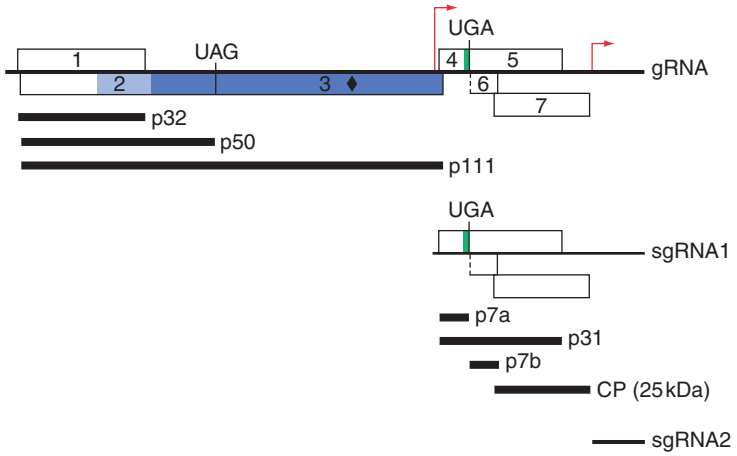


Figure 2 Genome organization and protein products of maize chlorotic mottle virus. The seven ORFs are marked as boxes on the genomic RNA and subgenomic RNA1 (sgRNA1) in each reading frame. The two suppressible stop codons are indicated (UAG and UGA), and the noncanonical start codon for ORF6 is marked with a dashed line. The proteins are indicated as heavy black bars beneath their mRNAs. The dark blue box marks the region encoding high protein sequence similarity to replicases of other members of the family *Tombusviridae* with a monopartite genome, and the light blue box indicates the area encoding protein similarity only to PMV replicase. The diamond marks the location encoding the 'GDD' motif. The green box in ORF4 marks the coding region for a conserved peptide sequence also found in PMV, carmoviruses, and necroviruses. Bent red arrows mark the sgRNA transcription start sites. sgRNA2 does not contain any significant ORFs.

of viral complementary strand synthesis at a specific location may produce separate complementary strand copies of sgRNA1 that are used as templates to synthesize many copies of sgRNA1. Although sgRNA2 accumulates in infected maize plants and inoculated protoplasts, its function and method of transcription are not known.

Geographic and Seasonal Distribution

Originally MCMV was found only in the Western Hemisphere. Peru, Argentina, Mexico, and the USA have reported infections, and it is possible that MCMV is present in other countries in the Western Hemisphere anywhere the beetle vectors are found in maize-growing areas. In Peru, MCMV is found in northern coastal areas where maize is grown as a seasonal crop as well as in the southern coastal areas where it is grown year-round. MCMV is endemic in several valleys in the Department of Lima. In the continental USA, where maize is a seasonal crop, MCMV was initially found in Kansas near the Nebraska border, then spread along the Republican and Big Blue River Valleys. The virus continued spreading along river valleys and is endemic in Nebraska and Kansas. MCMV initially appeared in the state of Guanajuato, Mexico, and has spread from there to additional nearby states. After its appearance in Kauai, Hawaii, MCMV has not remained as a problem. In Thailand, maize production is year-round, and MCMV was first detected in the province of Saraburi. It is now found in Saraburi, Nakohn Ratchasrima, Tak, and Petchaboon provinces.

Host Range and Virus Propagation

Susceptibility has been tested for 73 plant species (19 dicots) representing 35 genera, and only members of the family Poaceae are hosts. Although some cultivars of hexaploid wheat (*Triticum aestivum*), durum wheat (*Triticum durum*), barley (*Hordeum vulgare*), and sorghum (*S. bicolor*) are susceptible to infection, maize is the only crop in which MCMV infection is of economical importance, with many susceptible cultivars in all maize types (sweet, floury, field, and popcorn). MCMV can be readily propagated by mechanical inoculation in the inbred lines N28Ht, N28, and Oh28, but no reliable local lesion host has been identified.

Genetics

Viral RNA or uncapped transcripts of a full-length cDNA are sufficient for replication in protoplasts and infection of plants. The first ORF encodes a highly acidic 32 kDa protein with no similarity to any other protein in databases. The location of ORF1 suggests that it is a protein needed early in the viral life cycle, but its function is not known. ORF2 and ORF3 comprise a 50 kDa protein and a readthrough protein of 111 kDa containing the 'GDD' motif found in almost all RNA-dependent RNA polymerases (**Figure 2**). Sequence similarities of the carboxyl end of p50 and the p111 readthrough region to viral replicase proteins of other members of the family *Tombusviridae* suggest that these are similarly involved in replication of MCMV. p50 is much larger than the pre-readthrough proteins encoded by most of the monopartite viruses in the same family (27–33 kDa) and the sequence similarity only extends to the carboxyl third of p50. The similarity between p50 and the 48 kDa protein from PMV encompasses about 60% of the carboxyl end. Along with other members of the family, no regions identifiable as a helicase domain or a methyltransferase domain are found in p111. Like PMV and oat chlorotic stunt virus (genus *Avenavirus*), MCMV produces a single sgRNA to express a cluster of ORFs in the 3′ third of the genome, and none of the proteins encoded in this region are required for replication. The genes for p7a and p7b are upstream of the CP ORF, similar to the location of small ORFs in PMV, necroviruses, and carmoviruses. p7a and p7b have similar hydrophobic/hydrophilic characteristics and some sequence similarity to the peptides encoded in the corresponding locations in PMV, necroviruses, and carmoviruses, with the greatest sequence similarity occurring in the C-terminal region of p7a. These small peptides are required for cell-to-cell movement for PMV, necroviruses, and carmoviruses, so it is likely that p7a and p7b have similar functions. Database searches with the protein encoded in ORF5 do not identify any proteins with related sequence. The most 3′ ORF encodes the viral CP.

Serologic Relationships and Variability

The complete relationship of MCMV isolates from countries where MCMV has been found on a noticeable scale has not been determined. Two serotypes from Kansas (K1 and K2) and one from Peru have been compared, and they can be differentiated by agar double-diffusion assays. Most isolates from Nebraska and Kansas are similar to MCMV-K1, which is the source used for the MCMV sequence in GenBank (X14736) and the infectious transcript cDNA. Sequence comparisons have been done using reverse transcription-polymerase chain reaction (RT-PCR) amplification of the CP shell domain ORF from 47 isolates collected from Nebraska/Kansas, Hawaii, and Peru. Alignment of the 200 bp fragments indicates that each geographic region can be identified by a predominant unique genotype. The Hawaiian isolates show the least intrapopulational divergence, consistent with the recent appearance of the virus in Hawaii before the sample collection period. The Hawaiian isolates are more closely related to Peru isolates than to those from Nebraska/Kansas. The complete sequence of the CP gene of MCMV isolated in Thailand (AY587605) shows 96%

identity to MCMV-K1 at the nucleotide level and 97% identity at the protein level. When the corresponding 200 nt region is compared to the data in the larger study, the Thai sequence groups with Nebraska/Kansas sequences. These data suggest that Peru was the source for MCMV in Hawaii while the source of virus in Thailand was Nebraska/Kansas.

Transmission

MCMV is readily transmitted by mechanical inoculation of leaves or roots and by vascular puncture inoculation of seeds. Six species of beetles of the family *Chrysomelidae* found in the continental USA can transmit MCMV: the southern corn rootworm beetle (*Diabrotica undecimpunctata howardi*), the northern corn rootworm beetle (*Diabrotica barberi*), the western corn rootworm beetle (*Diabrotica virgifera virgifera*), the cereal leaf beetle and larvae (*Oulema melanopus*), the corn flea beetle (*Chaetocnema pulicaria*), and the flea beetle (*Systena frontalis*). The southern corn rootworm beetle is the vector in Mexico. In Peru, *Diabrotica viridula* (adult and larvae) and *Diabrotica decempunctata sparsella* transmitted MCMV-P. MCMV was spread by a large infestation of thrips (*Frankliniella williamsi*) in Hawaii. MCMV-K1 epidemiology indicates a soil and water connection, so it is possible that MCMV is soil borne, water borne, or transmitted by a fungus as is seen with various other viruses in the tombusvirus family. It is possible that the different major isolates of MCMV are predominantly transmitted by either a soil route or insects. Seed transmission occurs at a very low rate (0.008–0.04%).

Epidemiology

MCMV reappears in previously infected fields in Nebraska and Kansas, and there is little indication that the virus spreads very rapidly during the maize-growing season, suggesting that beetles are not the major source of viral spread there. MCMV does not overwinter in grasses surrounding the fields, suggesting that soil is the reservoir for future infections. Although it was hypothesized that infected crop residue might be a food source for beetle larvae which would infect new seedlings, it was later shown that MCMV loses its infectivity over the winter, and its loss in infectivity correlates with drying. Additionally, larvae do not feed on dead infected plant material. Infected fields are in river valleys and likely to be irrigated suggesting a water-borne mechanism. In the regions of Peru where maize is planted year-round, infection patterns correlate with emergence and spread of the beetle vectors. The mechanism of spread in Thailand has not yet been determined. The large geographic jumps in MCMV outbreaks suggest that initial infections occur via seed transmission. Although all the known beetle vectors are native to the New World, the western corn rootworm beetle was introduced to Serbia in 1992 and has since spread to additional European countries. This increases the potential for an outbreak of MCMV in Europe if it is introduced there via seeds.

Pathogenicity

MCMV infections can reduce yields of maize in experimental plots by up to 59%, but natural infections are seldom as severe. Average losses of 10–15% of sweet and floury maize crops occur in Peru. The more devastating disease CLN is caused by a synergistic interaction with potyviruses. CLN has been reported in Peru, Kansas, Nebraska, Mexico, and Thailand. In Kansas and Nebraska, maize dwarf mosaic virus-A (MDMV-A; genus *Potyvirus*), sugarcane mosaic virus MD-B (SCMV MD-B) (genus *Potyvirus*), and wheat streak mosaic virus (WSMV) (genus *Tritimovirus*) are found in natural CLN infections. In Mexico and Thailand, the potyvirus is usually SCMV MD-B. CLN has caused crop losses as high as 91%. If both viruses are present when plants are young, they initially develop a chlorotic mottle, then become extremely chlorotic and stunted. Leaves become necrotic starting at the leaf margins, then necrosis spreads inward causing rapid plant death. If the viral combination infects the plants at later stages, plants may appear green and healthy except for early drying of husks, producing ears that are fully developed except for wrinkled and shriveled seeds. As well as the synergistic increase in symptoms in CLN, the concentration of MCMV increases dramatically similarly to most synergisms involving potyviruses. With SCMV MD-B present, MCMV concentrations averaged more than fivefold higher than in singly infected plants, and in WSMV/MCMV synergisms the MCMV levels increased up to 11-fold higher than in singly infected plants. Although most potyviral synergisms do not cause an increase in potyvirus concentrations, the WSMV/MCMV synergism increased the WSMV infection rate and caused a two- to threefold increase in WSMV concentrations.

Pathology and Histopathology

Disease symptoms vary depending on maize genotype and age at infection. The mildest symptoms are a light chlorotic mottle on leaves with little effect on corn yield. In more susceptible varieties, these symptoms progress to chlorotic stripes parallel to the midvein that may coalesce forming elongated chlorotic blotches that may further turn necrotic. Severe symptoms include necrosis, stunting, decreased male inflorescence with few spikes, decreased number of ears, and malformed and partially filled ears. Plants infected at earlier stages usually have stronger symptoms and lower yields than later infections. MCMV has been found in all parts of the plant including leaves, stem, roots, cob, silk, pollen, ear sheaves, bracts,

and in all seed parts (embryo, cotyledon, endosperm, and pericarp). Electron microscopy of cross sections of MCMV-infected maize showed virus-like particles in xylem vessels, often filling the entire vessel lumen. Some xylem tubes were filled with an electron dense matrix that appeared to be virus-like particles embedded in viroplasm. Some parenchyma cells contained vacuolated viroplasms. Chloroplasts were highly disorganized.

Prevention and Control

MCMV-resistant maize lines are being developed, and perennial diploid teosinte (*Zea diploperennis*) is a source for additional resistance genes. If maize lines resistant to either MCMV or the potyviral components of CLN are used, the potential for CLN outbreaks is diminished. Interestingly, a transgenic maize line expressing the CP gene from SCMV MD-B showed fewer disease symptoms when inoculated singly with SCMV MD-B, MDMV-A, and MCMV, as well as showing fewer symptoms when inoculated with MCMV + SCMV-MD-B or MCMV + MDMV-A. In Kansas and Nebraska, crop rotation with soybeans or MCMV-resistant sorghum in fields previously infected with MCMV decreases the incidence of MCMV in the following year. In Kauai, Hawaii, MCMV was essentially eliminated as a detectable disease by completely destroying all maize on the island and not replanting for 6 months. This was followed by including a 90 day fallow period each year for 2 years, and has continued with a 60 day fallow period every year.

Evolution

Based on genome organization and protein sequence similarities, MCMV is most closely related to PMV and vice versa. MCMV CP and the putative viral replicase proteins are markedly most similar to the PMV equivalents, and p7b is most similar to the 6.6 kDa peptide encoded by PMV. Both MCMV and PMV encode four overlapping ORFs on large sgRNAs that have similar start codon characteristics; the second ORFs begin with noncanonical start codons, the CP ORFs begin with the second AUGs, and a fourth ORF overlaps the CP ORFs. Additionally, the host range for both viruses is restricted to monocots. Since there is no similarity in the proteins encoded by the ORFs overlapping the CP ORFs, it suggests that MCMV and PMV evolved from a virus lacking those ORFs and the 5′ ORF encoding p32. The origins of the p32 ORF and p31 readthrough region are unknown. MCMV and the other members of the family *Tombusviridae* belong to virus supergroup II based on their viral replicases along with umbraviruses, a subgroup of luteoviruses, hepatitis C virus, pestiviruses, flaviviruses, and the positive-strand RNA coliphages.

See also: Carmovirus; Cereal Viruses: Maize/Corn; *Necrovirus*; Tombusviruses.

Further Reading

Batten JS and Scholthof K-BG (2004) Genus *Panicovirus*. In: Lapierre H and Signoret PA (eds.) *Viruses and Virus Diseases of Poaceae (Gramineae)* pp. 411–412. Paris: INRA.

Castillo J and Hebert TT (1974) A new virus disease of maize in Peru. *Fitopatologia* 9: 79–84.

Chiemsombat P, Larprom A, See-Tou W, and Patarapuwadol S (2006) Mixed infection of maize chlorotic mottle virus and sugarcane mosaic virus in sweet corn. In: Pohsoong T (ed.) *Proceedings of the Second Kasetsart University Corn and Sorghum Research Program Workshop*, pp. 214–219. Nakhon Nayok: Thailand Kasetsart University.

Gordon DT, Bradfute OE, Gingery RE, Nault LR, and Uyemoto JK (1984) Maize chlorotic mottle virus. In: *CMI/AAB Description of Plant Viruses*, No. 284. Kew, UK: Commonwealth Mycological Institute.

Lommel SA, Martelli GP, Rubino L, and Russo M (2005) Genus *Machlomovirus*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 932–934. San Diego, CA: Academic Press.

Scheets K (2000) Maize chlorotic mottle machlomovirus expresses its coat protein from a 1.47-kb subgenomic RNA and makes a 0.34-kb subgenomic RNA. *Virology* 267: 90–101.

Scheets K (2004) Maize chlorotic mottle. In: Lapierre H and Signoret PA (eds.) *Viruses & Virus Diseases of Poaceae (Gramineae)*, pp. 642–644. Paris: INRA.

Shafer KS (1992) *Molecular Evolution of Maize Chlorotic Mottle Virus: Isolates from Nebraska, Hawaii and Peru*. MD Thesis, University of Nebraska, Lincoln.

Maize Streak Virus

D P Martin, D N Shepherd, and E P Rybicki, University of Cape Town, Cape Town, South Africa

© 2008 Published by Elsevier Ltd.

Glossary

Agroinfection Technique used for the infection of host plants with cloned virus genomes involving transfer of virus DNA into the nuclei of host cells by the bacterium *Agrobacterium tumefaciens*.

Agroinoculation See agroinfection.

Bicistronic Contains two protein-coding regions within a single mRNA transcript.

Capsomer A subunit of the mature virus particle containing an ordered series of polymerized coat protein molecules.
***Cicadulina* spp.** A group of leafhopper species involved in transmission of MSV.
C-*ori* Complementary strand (i.e., the half of the DNA duplex that is not packaged into virus particles) origin of replication.
CP Coat protein. The only protein component of the virus particle also believed to be involved in nuclear trafficking and cell-to-cell movement of viral DNA.
LIR Long or large intergenic region containing the origin of virion strand replication and gene promoters.
MP Movement protein. A small (*c.* 10 kDa) protein believed to be involved in intercellular virus movement via plasmodesmata.
Oviposition Egg-laying. To oviposit means to lay eggs.
Plastochron The time interval between successive leaf primordia, or the attainment of a certain stage of leaf development.
Rep Replication-associated protein involved in the initiation of virion strand replication.
RepA A truncated version of Rep with a unique C-terminal domain believed to be involved in regulation of host and/or virus gene expression.
SIR Short or small intergenic region containing gene polyadenylation signals and the origin of complementary strand replication.
Viruliferous A state in which an MSV vector species is carrying and is capable of transmitting the virus.
V-*ori* Virion strand (i.e., the half of the DNA duplex that is packaged into virus particles) origin of replication.

Introduction

Maize streak virus (MSV) is the causal agent of maize streak disease (MSD), the most serious viral disease of maize in Africa. It is a major contributor to the continent's food security problems and is endemic throughout Africa south of the Sahara. It is also found on the Indian Ocean islands of Madagascar, Mauritius, and La Réunion. There is no obvious barrier to spread of the virus outside of this region; hence it should be considered as a serious potential problem for other as yet unaffected maize-growing areas.

History and Taxonomy

"The disorder of the mealie plant, locally described as 'Mealie Blight', 'Mealie Yellows', or 'Striped Leaf Disease', belongs to a group of plant troubles arising from obscure causes . . ." was how MSD was first described by Claude Fuller in 1901 in Natal, South Africa. Fuller mistakenly attributed the disease to a soil disorder, but in retrospect it is quite clear that the 'mealie (a local word for maize or corn) variegation' he described and drew in minute detail can be attributed to MSV.

The first milestone in MSD research was reached in 1924, when H. H. Storey determined that a virus obligately transmitted by leafhopper species of the genus *Cicadulina* (**Figure 1**) was the causal agent of MSD. Storey named the virus 'maize streak virus', and was also the first to determine both the genetic basis of MSV transmission by *Cicadulina mbila*, and that resistance to MSD in maize could be inherited.

When MSV particles were first purified in 1974 they were found to have a novel twinned quasi-icosahedral (geminate) shape (**Figure 2**), from which the name 'geminivirus' was derived. This was followed by the unexpected discovery in 1977 that geminivirus particles contain circular single-stranded DNA (ssDNA), a genome type never before observed in plant viruses. These novel characteristics



Figure 1 The leafhopper vector of MSV, *Cicadulina mbila* Naudé. Photograph courtesy of Dr. Benjamin Odhiambo, Kenyan Agricultural Research Institute (KARI).



Figure 2 MSD symptoms on a maize leaf: note characteristic veinal streaks. Photograph courtesy of Dr. Frederik Kloppers, PANNAR (Pty) Ltd., Greytown, KwaZulu-Natal, South Africa.

led to the proposal of a new virus group – the geminiviruses – consisting of MSV and other viruses with geminate particle morphology and ssDNA genomes. *Maize streak virus* is now recognized as the type species of the genus *Mastrevirus*, in the family *Geminiviridae*.

Host Range and Symptoms

While most notorious for the yield losses it causes when infecting maize, MSV also infects over 80 other grass species including the economically important crops wheat, barley, and rye. In susceptible maize and grass genotypes, the virus first causes symptoms between 3 and 7 days after inoculation. These first appear as almost circular pale spots of 0.5–2 mm diameter in the lowest exposed portions of the youngest leaves. Later, fully emerged symptomatic leaves show veinal streaks from a few millimeters long to the entire length of the leaf and between 0.5 and 3 mm wide. These streaks often fuse laterally and symptomatic leaves may become >95% chlorotic (**Figure 2**).

Plants are worst affected when infected within a few days of coleoptile emergence; symptoms only develop above the site of inoculation on newly emerging leaves. Susceptible varieties may display severe stunting as well as very severe streaking, and cob development may be abolished. Yield losses can reach 100% when susceptible varieties are infected early.

Of the nine major MSV strains so far identified (designated MSV-A through MSV-I; (**Figure 3**)), only MSV-A produces economically significant infections in maize. The 'grass-adapted' MSV strains (MSV-B, -C, -D, -E, -F, -G, -H, and -I) differ from MSV-A types by 5–25% in nucleotide sequence, and produce substantially milder symptoms in maize than do MSV-A viruses and are often incapable of producing symptomatic infections in MSV-resistant maize genotypes. They may also have distinct but overlapping host ranges.

Diversity and Evolution

MSV is closely related to the other distinct 'African streak' mastreviruses, panicum streak virus, sugarcane streak virus, sugarcane streak Egypt virus, and sugarcane streak Reunion virus, with which it shares ~65% genome sequence identity. It is, however, most similar to, although not necessarily more closely related to, an isolate of digitaria streak virus from the Pacific island of Vanuatu, with which it shares ~67% genome sequence identity (**Figure 3**).

The full genome nucleotide sequences of MSV-A isolates display relatively low degrees of diversity, with any two MSV-A isolates obtained from anywhere in Africa invariably having genome sequences that are more than 97% identical. MSV isolates from La Réunion share

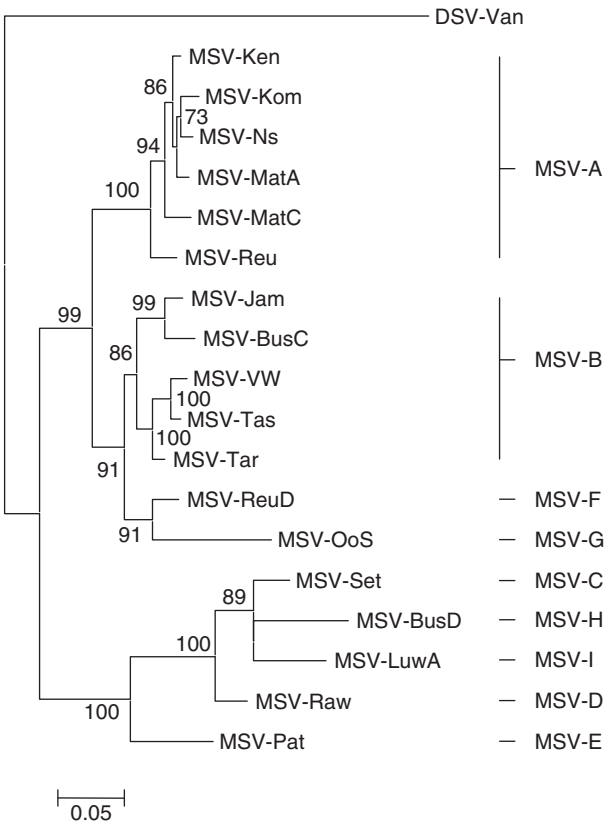


Figure 3 Phylogenetic relationships between the full genome sequences of different MSV strains. The tree is constructed using the maximum-likelihood method (HKY model transition and transversion weight determined from the data and 100 bootstrap replicates) and numbers associated with branches indicate degrees of bootstrap support for those branches. Branches with less than 70% support have been collapsed and the genome sequence of a digitaria streak virus (DSV) from Vanuatu is included as an outgroup. Only viruses in the MSV-A group have been isolated from maize. All viruses in the other groups have been isolated from wheat, barley, or wild grass species.

~95% identity with mainland isolates. Given that maize is a crop introduced at multiple points into Africa and its neighboring islands less than 400 years ago, and that the virus is not seed-borne, this may indicate either that the rate at which MSV-A is evolving is fairly slow, or that continent-wide spread and dominance of new MSV genotypes with enhanced fitness is very rapid. Experimental assessments of the rates at which the MSV-A-type isolate MSV-Reu evolves when maintained in a susceptible maize genotype, a resistant maize genotype, and a non-maize host (*Coix lacryma-jobi*) are, respectively, 9.5×10^{-5}, 17.3×10^{-5}, and 26.5×10^{-5} nucleotide substitutions per site per year. These evolutionary rates are relatively low when compared with RNA viruses and imply that genome-wide only one nucleotide becomes fixed every ~1.5–4 years, depending on the selection pressures exerted by the host species. Despite these low nucleotide fixation rates, it has been demonstrated that

certain artificially induced mutations will revert to wild-type states at an unexpectedly high rate of approximately 36 substitutions per site per year. This combination of a low evolution rate and high mutation rate implies that MSV is currently occupying a fitness peak in maize. Despite the virus' apparent evolutionary sluggishness it may therefore be able to adapt very quickly and break any inbred or transgenic MSV resistance traits it is exposed to. In any case, the fact that there is only a very narrow range of sequence variants that can cause severe disease in maize over the whole geographical range of the virus, compared to the wide range of sequence variation in viruses adapted to other grasses, indicates that there is a high degree of sequence selection by the host genotype.

Transmission

In nature, MSV and other African streak viruses are neither seed nor contact transmissible and rely instead on transmission by cicadellid leafhoppers in the genus *Cicadulina* (including among others *C. mbila, C. storeyi, C. bipunctella zeae, C. latens*, and *C. parazeae*). Of these *C. mbila* is considered the most important MSV vector as it is the most widely distributed. Also, a greater proportion of *C. mbila* individuals are capable of transmitting the virus than is found with other *Cicadulina* species. The virus may be acquired by leafhoppers at any developmental stage in less than 1 h of feeding with a minimum acquisition time of 15 s. A latent period within the vector during which the virus cannot be transmitted lasts between 12 and 30 h at 30 °C. Once this latent period is over (signaled by the appearance of virus within the leafhopper's body fluids) the virus can be transmitted within 5 min of feeding. The so-called viruliferous leafhoppers can then transmit the virus for the rest of their lives.

Particle Structure

MSV particles (**Figure 4**) consist of two incomplete icosohedra with a $T = 1$ surface lattice, comprising 22 pentameric capsomers each containing five coat protein (CP) molecules. Particle dimensions are 38 nm × 22 nm with the 110 CP molecules in each virion packaging a ~2690 nt covalently closed mostly single-stranded circular DNA genome. The packaged DNA has annealed to it a complementary ~80 nt sequence believed to act as a primer for complementary strand synthesis following infection and uncoating.

Genome Organization

As with all other mastreviruses discovered to date, the MSV genome encodes four proteins: a movement protein (MP) and a CP in the virion sense, and a replication-associated protein (Rep) and a regulatory protein (RepA) that is expressed from the same transcript as Rep in the complementary sense. MSV genomes also contain two intergenic regions: these are a short or small one (SIR), and a long or large one (LIR), which are the complementary sense strand and virion sense strand origins of replication, respectively (**Figure 5**).

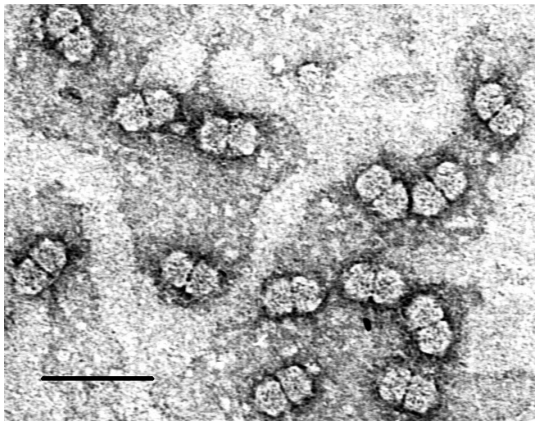


Figure 4 Electron micrograph of MSV purified from infected maize, showing particles of 18 nm × 30 nm stained with uranyl acetate. Scale = 50 nm. Photograph courtesy of Kassie Kasdorf; copyright EP Rybicki.

The Long Intergenic Region

Besides containing divergent RNA polymerase II-type promoters and other transcriptional regulatory features necessary for the expression of the complementary and virion sense genes, the LIR also contains sequence elements that are essential for replication. The most striking of these is an inverted repeat sequence that is capable of forming a stable hairpin loop structure. All geminiviruses sequenced to date have the highly conserved nonanucleotide sequence TAATATT↓AC within the loop sequence of similar hairpin structures: this sequence contains the virion sense strand origin of replication (V-*ori*;↓).

A sequence 6–12 nt long occurring in all known mastreviruses between the TATA box that directs *rep*/*repA* transcription and the *repA* initiation codon is directly repeated in the stem near the V-*ori* hairpin, and is probably involved in Rep and/or RepA binding during replication.

The hairpin and two GC boxes on the 5′ side of the stem also forms part of an upstream activator sequence (UAS) required for efficient CP expression. The GC boxes bind nuclear factors to the UAS and are known as the rightward promoter element (*rpe1*).

The Short Intergenic Region

The MSV SIR occurs between the termination codons of the *CP* and *rep* genes (**Figure 5**) and contains the

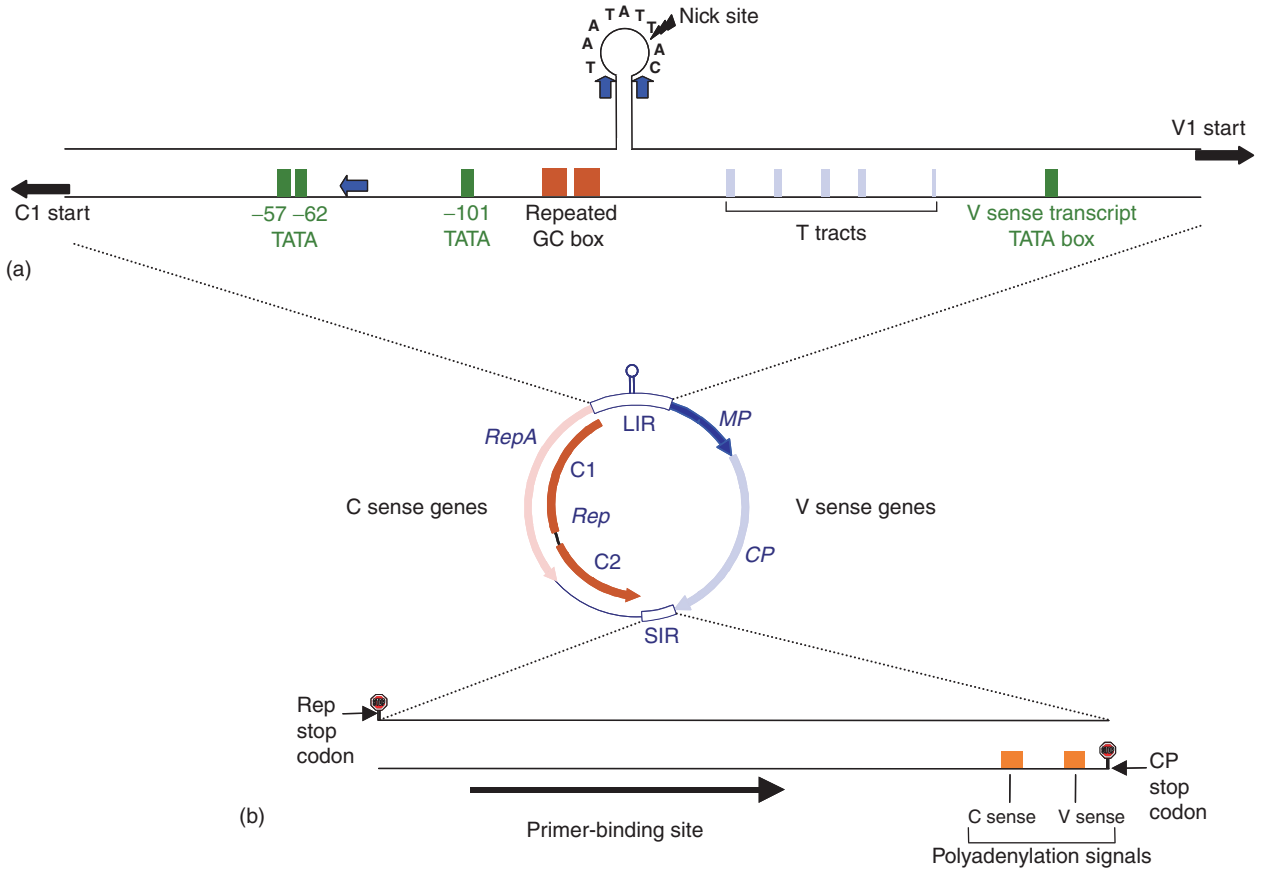


Figure 5 A schematic representation of the MSV LIR (a) and SIR (b), shown in context with the MSV genome. In (a) the main features of the MSV LIR are shown. These include a stem–loop structure with the loop's nonanucleotide sequence conserved among all geminiviruses and other rolling-circle systems. The site at which Rep introduces an endonucleolytic nick to initiate virion strand replication is shown. Iterated sequences (iterons) are shown in the V sense, with blue arrows indicating their location in the LIR. Iterons are potentially specific Rep-recognition sequences via which Rep may bind to the LIR. 5′ of the stem–loop is a repeated GC-box, which binds host transcription factors. A series of T tracts 3′ of the stem–loop may be involved in DNA bending of this region of the LIR. TATA boxes 5′ and 3′ of the stem–loop are potential C sense and V sense transcription initiation sites, respectively. In (b), the main features of the SIR include polyadenylation signals for V and C sense transcripts, and a primer-binding site on the plus strand. An ~80 bp DNA primer-like molecule, encapsidated with the viral genome and annealed to this site, is thought to be involved in initiating complementary strand replication. Both the MSV LIR and SIR are essential for viral replication.

polyadenylation and termination signals of the virion and complementary sense transcripts. The SIR also contains the origin of complementary strand synthesis (C-*ori*). A small 80-nt-long primer-like molecule is bound to the SIR of encapsidated MSV DNA and, at the onset of an infection, probably enables synthesis of double-stranded DNA (dsDNA) replicative forms (RFs) of the genome from newly uncoated virion strand DNA.

The Complementary Sense Genes (*rep* and *repA*)

The replication initiator/associated protein (Rep) is the only MSV gene product that is absolutely required for virus replication. In mastreviruses, *repA* (the *C1* open reading frame (ORF)) and the *C2* ORF (also called *repB*), respectively, encode the N- and C-terminal portions of Rep (**Figure 5**). Beginning at the same transcription initiation site, two C sense transcripts (1.5 and 1.2 kbp in size) are produced during MSV infections. Splicing of the larger transcript removes an intron, which permits expression of full-length Rep from the two ORFs.

It is very probable, although as yet unproven *in vivo*, that RepA is translated from both the unspliced 1.5 and 1.2 kbp C sense transcripts. If expressed in infected cells, MSV RepA would have the same N-terminal 214-amino-acid sequence as Rep, but would have a different C-terminus. RepA is possibly a multifunctional protein that modifies the nuclear environment to favor viral replication.

The N-terminal portions of Rep and RepA contain three conserved amino acid sequence motifs commonly found in replication-associated proteins of many extremely diverse rolling-circle replicons. Other significant landmarks include a plant retinoblastoma-related

protein (pRBR) interaction motif, via which RepA but not Rep binds to the host pRBR which is involved in host cell cycle regulation, and oligomerization domains via which Rep and RepA bind to other Rep/RepA molecules (Rep activities are influenced by the aggregation state of Rep and/or RepA homo- and heterooligomers). It is also likely that the approximately 100 N-terminal amino acids of both Rep and RepA are involved in binding of the proteins to the viral LIR.

The C-terminal portion of Rep contains a dNTP-binding domain with motifs similar to those found in proteins with kinase and helicase activities. The dNTP-binding domain also sits within a region with similarity to the DNA-binding domains of the *myb*-related class of plant transcription factors: this domain may be functional in the induction of virus and/or host gene transcription.

The C-terminal portion of RepA, which is different from that of Rep, contains another potential transactivation domain also possibly involved in regulation of virus and/or host gene expression. A second domain within the C-terminal portion of RepA possibly interacts with host proteins involved in developmental regulation.

The Virion Sense Genes (MP and CP)

Transcription of the MSV virion (V) sense genes is directed by two TATA boxes within the LIR 26 and 214 nucleotides 5′ of the *MP* start codon. Each TATA box directs the production of different-sized transcripts, both of which terminate at the same place. Splicing of an intron within the *MP* portion of V sense transcripts appears to be an important determinant of relative MP and CP expression levels. Whereas CP is expressed from both long and short, spliced and unspliced V sense transcripts, MP is most likely only expressed from unspliced long transcripts.

The MP is post-translationally modified and contains a hydrophobic domain that may either facilitate its interaction with host cell membranes or be involved in homo- or heterooligomerization with the CP.

The N-terminal ~100 amino acids of the CP contain both nuclear localization signals and a sequence-nonspecific dsDNA- and ssDNA-binding domain. The CP and MP interact with one another and it is possible that this interaction is involved in trafficking of naked and/or packaged virus DNA from nuclei through nuclear pores, to the cell periphery, through plasmodesmata, and into the nuclei of neighboring cells.

Molecular Biology of MSV

Replication

As with other geminiviruses, MSV replicates by a rolling-circle mechanism (rolling-circle replication, or RCR; (**Figure 6**)) within the nuclei of infected cells. Replication may also occur by recombination-dependent mechanisms but this has not been conclusively demonstrated for MSV. As with other rolling-circle replicons, MSV replication is discontinuous with virion strand replication being initiated from the hairpin structure in the LIR and complementary strand synthesis being initiated from a short 80 nt primer-like molecule synthesized on the SIR of newly replicated virion strands.

Particle Assembly and Movement

Besides being the primary location of replication, the nucleus is also the site of virus particle assembly. CP molecules in the nucleus nonspecifically bind virion strands released during RCR (there is no known encapsidation signal in mastrevirus genomes), arresting the synthesis of new RF DNAs. Viral ssDNA molecules are packaged into particles that aggregate to form large paracrystalline nuclear inclusions. Crystalline arrays of MSV particles have also been detected outside nuclei within physiologically active phloem companion cells, and inside the vacuoles of dead and dying cells within chlorotic lesions. These lesions are caused by an as yet unexplained degeneration of chloroplasts in infected cells.

The mechanistic details of MSV cell-to-cell movement are still obscure, but it seems to involve an interaction between the CP, MP, and viral DNA. Besides requiring the coordinated interactions of viral gene products and DNA, the successful movement of MSV genomes from infected to uninfected cells is strongly dependent on the extent of plasmodesmatal connections between neighboring cells. Also, in maize it appears as though certain cell types are more sensitive to MSV infection than others. For example, in maize leaves the virus infects all photosynthetic cell types (e.g., mesophyll and bundle sheath cells) but despite abundant plasmodesmatal connections between photosynthetic, epidermal, and parenchyma cells, MSV is only rarely detectable in the latter two cell types.

It is unknown whether systemic movement of geminiviruses within plants simply relies on normal cell-to-cell movement to deliver genomic DNA into the phloem, or whether viral DNA is specifically packaged for long-distance transport. It is possible that cell-to-cell movement might involve unencapsidated ss- or dsDNA but that long-distance movement in the phloem might require encapsidation.

Long-distance movement of MSV within infected plants occurs via phloem elements and it is believed that MSV is incapable of invading the root apical, shoot apical, and reproductive meristems due to the absence of developed vasculature in these tissues. Thus, the virus is not found in tissue which develops into gametes and is not seed-borne. It also does not appear to travel down plants from the site of inoculation to older tissue.

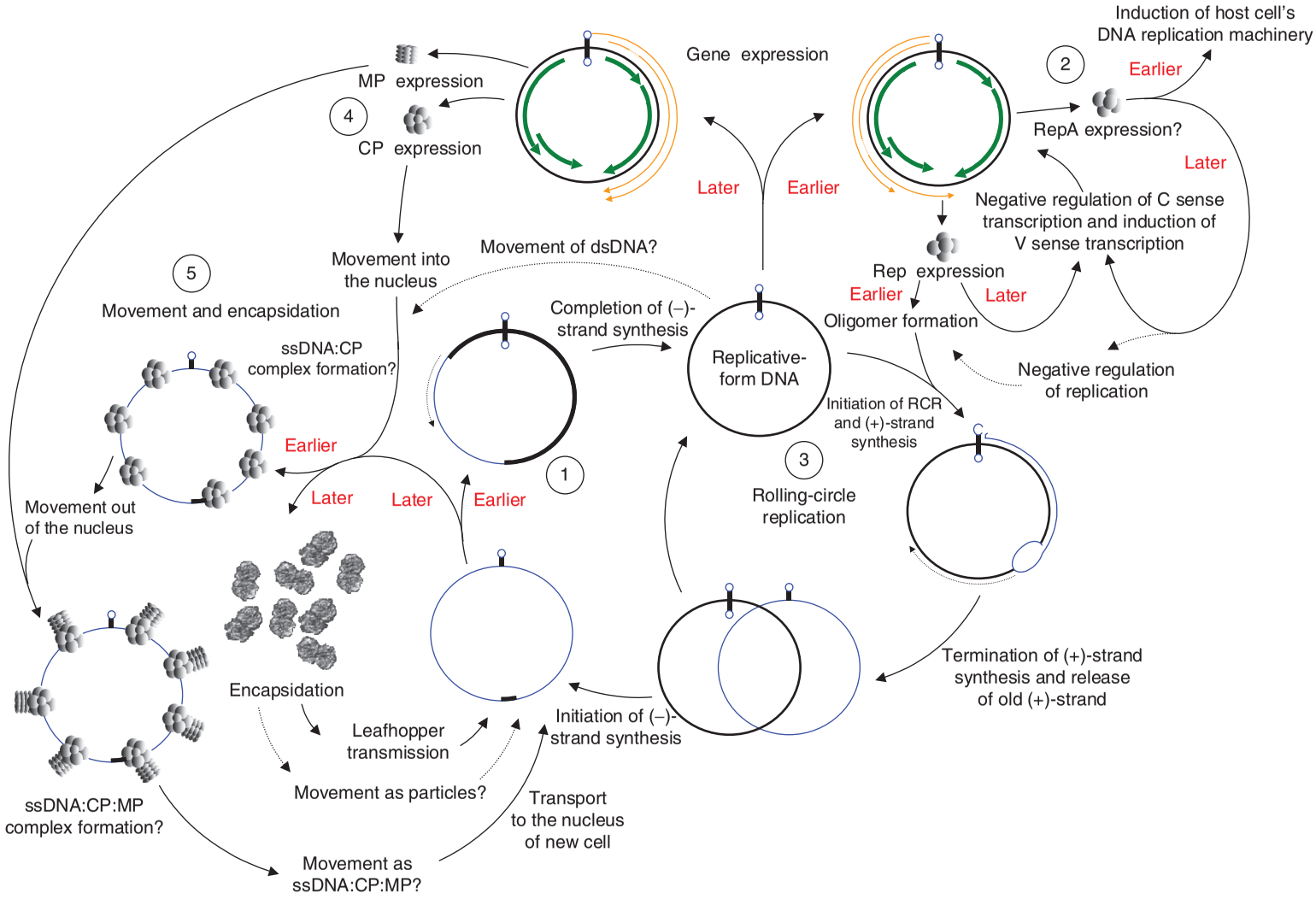


Figure 6 Summary of the MSV infection process. Early during an infection following the synthesis of a dsDNA replicative form (RF; 1) RepA is most likely expressed and induces a cellular state in which viral DNA replication can occur (2). Rep is also expressed early and RCR begins (3). At a later point in the infection process, following genome amplification and possibly Rep and/or RepA induction of the V sense promoter, MP and CP are expressed (4), and movement and encapsidation occur (5). Represented here is movement of unencapsidated ssDNA, but it should be noted that it is possible that dsDNA and/or encapsidated ssDNA may also be moved either cell to cell or systemically within the phloem of plants. Whereas the involvement of MSV CP and MP in movement has been demonstrated, the mechanics of the process are obscure. While the probable timing of events is indicated, it is unlikely, for example, that absolutely no MP and CP expression occurs during the earlier stages of the infection process. ssDNA is represented by blue lines, dsDNA by bold black lines, and RNA by orange lines.

Within the shoot apex where most productive MSV replication occurs, MSV first enters developing leaves at approximately plastochron five. While the virus is restricted to the developing leaf vasculature before plastochron 12, it is likely that the development of metaphloem elements at approximately plastochron 12 provides an opportunity for the virus to escape the vasculature into the photosynthetic cells of the leaf. Metaphloem develops with the abundant plasmodesmatal connections required for efficient loading of photoassimilates once the leaf emerges from the whorl. Before emergence, however, the developing photosynthetic tissues are still net importers of photoassimilates and the virus most likely moves into these cells through their plasmodesmatal connections with the metaphloem.

On the leaves, the pattern of chlorotic streak-like lesions that characterizes MSV infections is directly correlated with the pattern of virus accumulation within the leaves and the virus can only be acquired by leafhoppers from these lesions. The degree of chlorosis that occurs within lesions can differ between MSV isolates and is related to the severity of chloroplast malformation that occurs in infected photosynthetic cells.

Control of MSD

Although effective control of MSD in cultivated crops is possible with the use of carbamate insecticides, and it is possible to avoid the worst of leafhopper infestations by varying planting dates, the fact that small farmers cannot generally use these options means that the development and use of MSV-resistant crop genotypes is probably the best way to minimize the impact of MSD on African agriculture. MSV resistance is associated with up to five separate alleles conferring a mixture of both dominant and recessive traits, none of which is sufficient by itself. Despite great successes achieved in the development of MSV-resistant maize genotypes that tolerate infection without significant yield loss, there has been only limited success in the field. For example, severe infections of the so-called MSV-tolerant genotypes can occur when they are grown under environmental conditions different from those in which the plants were selected, meaning each distinct geographical growing area requires specific varieties to be bred for maximum resistance. Another problem facing breeders is that natural genetic resistance is not usually associated with desirable agronomic traits such as good yield and it can therefore be difficult to transfer resistance traits without also transferring undesirable characteristics. Moreover, the number of alleles involved means that successful breeding takes years for each release. Even in the absence of any predictive modeling of sporadic MSD outbreaks, most farmers would still prefer to gamble on the use of higher-yielding MSV-sensitive genotypes. Efforts are currently underway to introduce MSV resistance traits into commercial maize genotypes by genetic engineering. This technique has the advantage of enabling the direct transfer of single-gene resistance, without linkage to undesirable characteristics, to many different breeding lines suited to different environmental conditions. However, up till now this strategy has been limited by negative public perception of genetically modified organisms, and the expensive and time-consuming risk assessment necessary to ensure a safe feed and food product.

MSD Epidemiology

There are loose correlations between MSD incidence and both environmental conditions and agricultural practices. Environmental influences on MSD epidemiology are mostly driven by a strong correlation between rainfall and leafhopper population densities. For example, drought conditions followed by irregular rains at the beginning of growing seasons tend to be associated with severe MSD outbreaks. Also, maize planted later in the growing season tends to get more severely infected than that planted at the beginning of the season, probably due to steady increases in leafhopper numbers and inoculum sources over the course of the season. As is the case with most insect-borne virus diseases, however, the incidence of MSD is erratic. Whereas MSD can devastate maize production in some years, in others it has only a negligible effect. The reason for this is that apart from MSD epidemiology being strongly dependent on environmental variables it is also the product of extremely complex interactions between the various MSV leafhopper vector and host species, and an as yet unknown number of virus strains.

Leafhopper Vectors

Serious MSD outbreaks are absolutely governed by leafhopper acquisition and movement of severe MSV isolates from infected plants (wild grasses or crop plants) to sensitive, uninfected crop plants. The distance that MSV spreads from a source of inoculum is determined by the movement behavior of leafhoppers. Distinct long- and short-distance flight morphs have been detected among certain *Cicadulina* populations. It is believed that the long-flight morphs are a migratory form and as such they may play an important part in the rapid long-distance spread of virulent MSV variants. Migratory movement is more common in certain *Cicadulina* species than in others and it is probably influenced by environmental conditions.

The dynamics of primary infection following leafhopper invasion of a susceptible maize crop are influenced by leafhopper population densities, the proportions of viruliferous individuals in populations, and the virus titer

within these individuals. Disease spread within individual maize fields is apparently linear when only a few viruliferous leafhoppers are involved in transmission, but becomes exponential once the number of insects exceeds one individual per three plants.

Plant Hosts

Although attempts to understand the dynamics of MSD epidemics have focused primarily on vector population dynamics and behavior, an important component of MSD epidemiology is the population density, turnover, and demographics of the over 80 grass species that are both MSV and vector hosts. Because *Cicadulina* species favor certain annual grass hosts for mating and oviposition, the species composition of grass populations that vary seasonally in any particular area will directly influence leafhopper population densities and feeding behaviors in that area.

The species composition and age distribution of grasses (including cultivated crops) in an area may also affect the amount of MSV inoculum available for transmission in that area. While MSV infects at least 80 of the 138 grass species that leafhoppers feed on, both the susceptibility of these grasses to MSV infection and the severity of symptoms that occur following their infection may be strongly influenced by a number of factors. While sensitivity to infection can vary substantially from species to species, it can also vary within a species with genotype and plant age at the time of inoculation: for example, plants from many species, including maize, generally become more resistant to MSV infection with age, thereby reducing the inoculum available for transmission to other plants.

The Virus

While efforts are underway to promote the widespread cultivation of MSV-resistant maize in Africa, surprisingly little is known about the MSV populations that will confront these new genotypes. Although to date nine major MSV strain groupings have been discovered, it is unknown whether any other than the maize-adapted MSV-A strain play an important role in the epidemiology of MSD. MSV-B, -C, -D, and -E isolates only produce very mild symptoms in MSV-sensitive maize genotypes and are therefore unlikely to pose any significant direct threat to maize production. Mixed MSV-A and -B infections have, however, been detected in nature and there is also strong evidence of recombination occurring between these strains. It is therefore possible that MSV-B, and possibly other MSV strains, may indirectly influence MSD epidemiology through recombination with MSV-A-type viruses. Recombination has been linked with the emergence of a number of geminivirus diseases and it is quite conceivable that it may have already contributed to the emergence of MSV and may also eventually contribute to the evolution of MSV genotypes with elevated virulence in resistant maize varieties.

It seems highly probable that MSV isolates, strains, and even distinct MSV-related mastreviruses travel with the leafhoppers as a 'swarm' of virus types through a variety of grasses, both perennial and annual, each of which has a virus genotype or group of genotypes most suited to replication in it. Thus, the dominant virus in any particular host at any one time will probably be different, but the swarm diversity is preserved – in part because the dominant type in any case may facilitate the replication of other, less fit virus types in any one host. Consequently, it is possible to see very different dominant viruses in maize and wheat grown in consecutive summer and winter growing seasons in the same field, as a result of independent selection by the host genotype of the most fit virus genotype.

Future Threat

MSV is rightly regarded as a significant potential threat to maize production outside of Africa: while the vectors do not occur outside of the current range, there is no obvious reason that they would not survive in other, climatically similar areas such as the southern USA and South America and Eurasia, and it is a distinct possibility that they could inadvertently spread or be deliberately taken there. If one or more vector species did become established, and were viruliferous, spread of MSV and its relatives into native grasses and cultivated maize and other cereals would be inevitable. It is worth noting here that as none of the maize varieties grown outside of Africa has any but the weakest resistance to MSV, the probability of severe economic consequences would be very high.

See also: Plant Resistance to Viruses: Geminiviruses.

Further Reading

Bosque-Pérez NA (2000) Eight decades of maize streak virus research. *Virus Research* 71: 107–121.

Boulton MI (2002) Functions and interactions of mastrevirus gene products. *Physiological and Molecular Plant Pathology* 60: 243–255.

Damsteegt V (1983) Maize streak virus. Part I: Host range and vulnerability of maize germ plasm. *Plant Disease* 67: 734–737.

Efron Y, Kim SK, Fajemisin JM, *et al.* (1989) Breeding for resistance to maize streak virus – A multidisciplinary team-approach. *Plant Breeding* 103: 1–36.

Fuller C (1901) *First Report of the Government Entomologist, Natal, 1899–1900.* http://www.mcb.uct.ac.za//msv/fuller.htm (accessed February 2008).

Harrison BD, Barker I, Bock K, *et al.* (1977) Plant viruses with circular single-stranded DNA. *Nature* 270: 760.

Martin DP, Willment J, Billharz R, *et al.* (2001) Sequence diversity and virulence in *Zea mays* of maize streak virus isolates. *Virology* 288: 247.
McLean AP (1947) Some forms of streak virus occurring in maize, sugarcane and wild grasses. *Science Bulletin of Department of Agriculture for Union of South Africa* 265: 1–39.
Palmer KE and Rybicki EP (1998) The molecular biology of mastreviruses. *Advances in Virus Research* 50: 183–234.
Storey HH (1925) The transmission of streak disease of maize by the leafhopper. *Balclutha mbila naudé. Annals of Applied Biology* 12: 422–443.
Zhang W, Olson NH, Baker TS, *et al.* (2001) Structure of the maize streak virus germinate particle. *Virology* 279: 471–477.

Relevant Website

http://www.mcb.uct.ac.za – The Maize Streak Virus Home Page at Online Resources of the University of Cape Town Department of Molecular and Cell Biology.

Marburg Virus

D Falzarano, University of Manitoba, Winnipeg, MB, Canada
H Feldmann, Public Health Agency of Canada, Winnipeg, MB, Canada

© 2008 Elsevier Ltd. All rights reserved.

History

In 1967 the first noted cases of a viral hemorrhagic fever (VHF) caused by a new family of viruses, the *Filoviridae*, occurred when laboratory workers in Germany contracted severe VHF after handling tissues from African green monkeys (*Cercopithecus aethiops*) imported from Uganda. Shortly after, two cases were identified in the former Yugoslavia, where a veterinarian was infected during the necropsy of a dead monkey. In total there were 32 cases, including six secondary infections and a single retrospective case. Overall, there were seven fatalities in the primary infections resulting in a case–fatality rate of 23%. The new virus was named Marburg virus (MARV) after the German city which had reported the first cases (see **Figure 1** for a map of all known occurrences of MARV).

Following these initial cases of Marburg hemorrhagic fever (MHF) there were only a small number of isolated cases noted until 1998. In 1975, three cases of MHF were reported in Johannesburg, South Africa. The index case, who did not survive, had traveled to Zimbabwe immediately before becoming ill. Shortly afterward, his travel companion and a nurse who cared for them also became ill but both later recovered. Cases of MHF also occurred in Kenya in 1980 and 1987. In 1980 the index case became ill in western Kenya and died in Nairobi where a physician also got infected but survived. In 1987, a fatal case occurred in the same region of western Kenya. The index cases in both 1980 and 1987 had traveled to the Mt. Elgon region, which is located close to Lake Victoria and was the source of the monkeys that initiated the original 1967 outbreak (trapped near Lake Kyogo, Uganda).

The first large community outbreak of MHF occurred in 1998 in central Africa. The community of Durba/Watsa, located in the northeastern region of the Democratic Republic of the Congo (DRC), had 149 cases with an 83% fatality rate. The response to this outbreak was limited due in part to the remote location and an ongoing conflict in the region. Sporadic cases continued after the end of the outbreak with most cases being linked either directly or indirectly to illegal mining in an underground gold mine. Typically, the index cases were gold miners who initiated multiple, short chains of human-to-human transmission within their families. The Durba outbreak was somewhat unusual compared to previous MARV and Ebola virus (EBOV) outbreaks in that it continued for almost 2 years, during which multiple distinct genetic lineages were found to be circulating, indicating several independent introductions of virus into the human population from the unknown natural reservoir.

To date, the largest outbreak of MHF occurred in the Uíge Province of Angola in 2004–05. At the conclusion of the outbreak, the Ministry of Health in Angola reported a total of 252 cases, including 227 deaths. This case–fatality rate of 90% is the highest observed during an MHF outbreak thus far and is more typical of past severe Ebola hemorrhagic fever (EHF) outbreaks. A large proportion of the cases were found among young children, which is unusual for filovirus outbreaks. The high case–fatality rate and fast progression of the disease were suggestive of a more virulent strain. In contrast to the Durba/Watsa outbreak, the viruses isolated from patients were highly conserved, indicating a single introduction with little evolution of the virus during the outbreak similar to what has been reported for previous EHF outbreaks. The most recent occurrence of MHF was in 2007 in Uganda, where there were three confirmed cases.

In addition to the initial outbreak and those that have occurred in the natural setting there have also been at least three laboratory-acquired infections of MARV since the mid-1980s, with one fatality occurring in Russia.

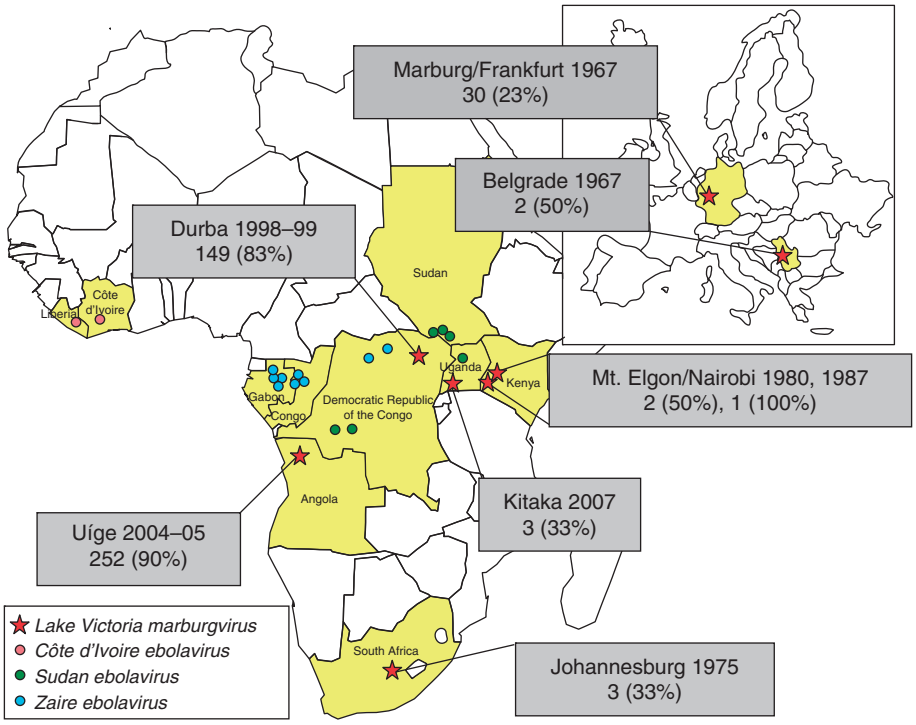


Figure 1 Location of known filovirus outbreaks. Countries that have experienced occurrences of filoviruses are indicated in yellow. For MARVs, the location of the outbreak is indicated by a red star. The main center (city/village) of the outbreak is indicated, along with the year of the outbreak, the number of cases, and the case–fatality rate (indicated in brackets). Incidents of the various EBOV species are also indicated.

Taxonomy and Classification

All MARVs are classified as a single species, *Lake Victoria marburgvirus*, which comprise the genus *Marburgvirus*, family *Filoviridae*, within the order *Mononegavirales*. They share unique morphologic, physicochemical, genetic, and biological features with members of the genus *Ebolavirus*. Due to their high fatality rates, frequency of person-to-person transmission, the potential for aerosol infectivity, and the absence of vaccines and treatments, these viruses are considered to be 'biosafety level 4' (BCL-4) agents and have been placed on the 'category A' and 'select agent' list.

Virus Structure and Composition

Electron microscopy of MARV reveals distinctive pleomorphic filamentous particles (**Figure 2(a)**) that can appear in U-shaped, 6-shaped, or circular (torus) configurations, or as elongated filamentous forms of varying length (up to 14 000 nm). Filamentous particles may also form branched structures. The length of peak infectivity for MARV particles is between 790 and 860 nm with a uniform diameter of 80 nm for all particles. Virus particles contain a helical ribonucleoprotein (RNP) complex, which contains the negative-sense viral RNA genome, the polymerase (L) protein, the nucleoprotein (NP), and viron protein (VP) 35 and 30. The RNP is surrounded by the matrix protein (VP40) and a closely apposed outer envelope derived from the host cell plasma membrane (**Figure 2(b)**). The surface of the particle has membrane-anchored protein spikes, made up by the virus glycoprotein (GP), which gives the virus particle a rough appearance. These spikes are approximately 7 nm in length and are spaced at approximately 10 nm intervals. Virus particles have a molecular weight of approximately $3–6 \times 10^8$ Da and a density of $1.14\,g\,ml^{-1}$ as determined by centrifugation in a potassium tartrate gradient; uniform, bacilliform particles have a sedimentation coefficient of 1300–1400 S. Virus infectivity is rather stable at room temperature. Inactivation can be performed by ultraviolet (UV) light or gamma irradiation, 1% formalin, β-propiolactone or brief exposure to phenolic disinfectants and lipid solvents, like deoxycholate and ether, as well as ionic detergents such as sodium dodecyl sulfate.

Genome Organization and Expression

The MARV genome consists of a single, linear molecule of negative-stranded RNA that is just over 19 000 bp in length. The genome is slightly larger than that of EBOV but is organized in a similar manner (**Figure 2(b)**). The

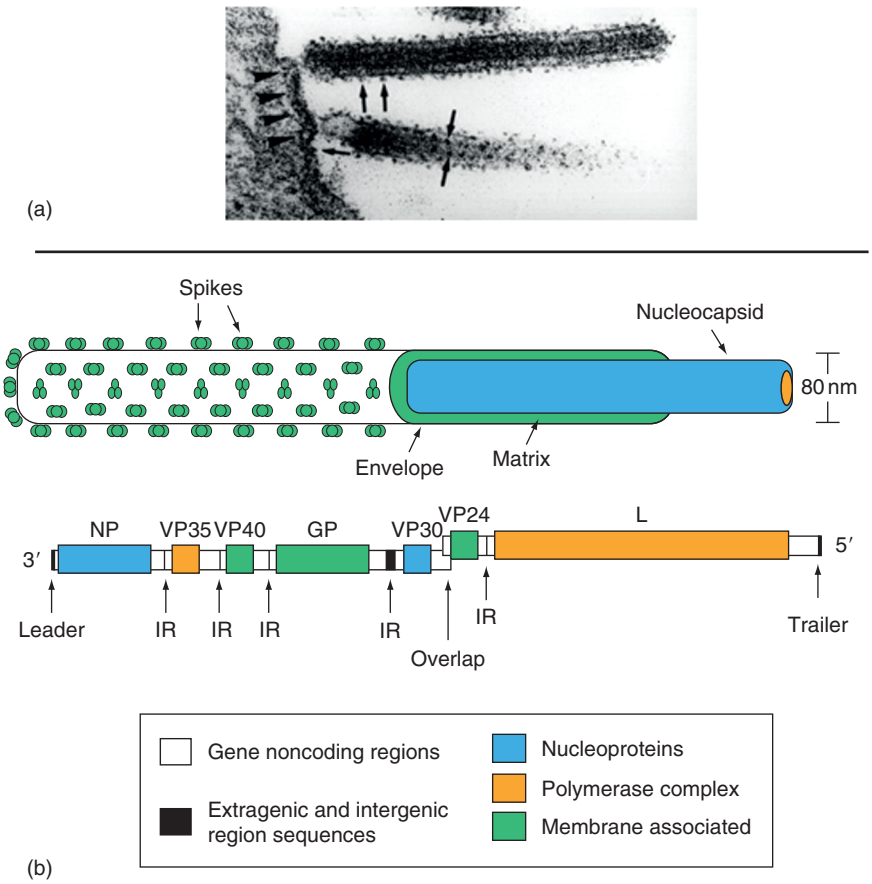


Figure 2 Virus particle morphology. (a) Transmission electron micrograph (negative stain) of MARV particles budding from an infected cell. The arrows indicate the glycoprotein spikes. (b) Schematic representation of the MARV particle structure (upper portion) and genome (lower portion) indicating open reading frames, noncoding regions, a single gene overlap (VP30/VP24), and intergenic regions (IRs).

MARV genome is noninfectious, rich in adenosine and uridine residues, not polyadenylated and complementary to viral-specific messenger RNA. Complete genome sequences are available from isolates covering six episodes of MHF (1967, 1975, 1980, 1987, 1999, and 2005). The genome amounts to 1.1% of the total virion weight with a sedimentation coefficient of 46 S (0.15 M NaCl, pH 7.4).

The genes are arranged in a linear fashion in the following order: 3′ leader; NP; viral structural protein (VP)-35; VP40; GP; VP30; VP24; polymerase (L); 5′ trailer (**Figure 2(b)**). All genes are flanked at their 3′ and 5′ ends by highly conserved transcriptional start and stop signal sequences that almost always include the pentamer 3′-UAAUU-5′. Most genes are separated by intergenic sequences that are variable in length and nucleotide composition. An unusual feature of the MARV genome is the presence of a gene overlap, between VP30 and VP24, a feature that is shared with EBOV, which have two or three gene overlaps (VP35–VP40, GP–VP30, and or VP24–L). Extragenic sequences that are complementary at their very extremities are present at the 3′ and 5′ end of the genome. This complementarity favors formation of a panhandle structure between the genomic termini, but it is unclear if such a structure can actually form due to co-transcriptional encapsidation. Both genomic ends are self-complementary and modeling suggests that almost identical hairpin-like structures are present at the 3′ ends of the genome and antigenome.

All of the MARV proteins are incorporated into the virus and are either part of the RNP complex or associated with the envelope. For a summary of protein functions, see **Table 1**. RNP-associated proteins are involved in transcription and replication while envelope-associated proteins are primarily involved in assembly/budding or entry. NP and VP30 are phosphoproteins and are considered the major and minor nucleoproteins, respectively. They interact strongly with the genomic RNA molecule, forming the viral RNP complex, in combination with VP35 and L which form the polymerase complex. EBOV VP30 is thought to be a transcriptional activator that is regulated by phosphorylation but MARV VP30 does not appear to function in this manner.

The polymerase complex transcribes and replicates the MARV genome. L is the RNA-dependent RNA

Table 1 MARV proteins: Function(s) and localization

Gene order [a]	*Marburg virus proteins*	*Protein function (localization)*
1	Nucleoprotein (NP)	Major nucleoprotein, RNA genome encapsidation (component of RNP[b])
2	Virion protein 35 (VP35)	Polymerase complex cofactor, type I interferon antagonist (component of RNP)
3	Virion protein 40 (VP40)	Matrix protein, virion assembly and budding (membrane associated)
4	Glycoprotein (GP)	Receptor binding and membrane fusion (membrane associated)
5	Virion protein 30 (VP30)	Minor nucleoprotein (component of RNP)
6	Virion protein 24 (VP24)	Minor matrix protein, virion assembly (membrane associated)
7	Polymerase (L)	RNA-dependent RNA polymerase, enzymatic portion of polymerase complex (component of RNP)

[a]Gene order refers to 3′–5′ gene arrangement as shown in **Figure 2(b)**.
[b]Ribonucleoprotein complex.

polymerase and contains motifs that are linked to RNA (template), phosphodiester (catalytic site), and ribonucleotide triphosphate binding. MARV L is larger than EBOV L and while there are areas of conservation between the two, nearly 25% of the C-terminal quarter is not conserved, in addition to stretches of sequences that are totally unique to MARV L. VP35 is thought to act as an essential cofactor of L that affects the mode of RNA synthesis (transcription or replication) similar to that of the P proteins of other negative-stranded viruses. In addition, VP35 also acts as a linker between L and NP as well as having an antagonistic effect on the type I interferon pathway.

The surface of MARV particles is covered with spike structures (**Figures 2(a)** and **2(b)**) that are composed of the structural glycoprotein, GP, which is anchored in the membrane as a timer in a type I orientation. These spike proteins play a role in entry and are thought to influence pathogenesis. MARV GP is encoded in a single open reading frame in contrast to EBOV GP, which is encoded in two open reading frames and is only expressed after RNA editing. Sequence analysis of MARV GP genes indicates that a nucleotide sequence that corresponds to the editing region of EBOV GP genes is absent. MARV GP is processed similar to EBOV GP but in contrast to EBOV GP the glycans on MARV GP lack terminal sialic acids if propagated in specific cell lines. This may be caused by differences in processing as the protein is directed through the *trans*-Golgi apparatus.

VP40 functions as the matrix protein in combination with VP24, which acts as a minor matrix protein. VP40 is the most abundant protein in the virion; however, only small amounts of VP24 are incorporated into virus particles. Both VP40 and VP24 are hydrophobic, have an affinity for membranes, and are associated with the virion envelope. VP40 is essential for virus budding as it initiates and drives the envelopment of the RNP by the plasma membrane. The role of VP24 in the replication of MARV is still unclear and direct interactions with other virus proteins have not been described.

Replication

MARVs are thought to replicate in a similar manner to EBOV; however, most of the research on replication has been performed on EBOV. Only the NP, VP35, and L proteins are required for transcription and replication of MARV, in contrast to EBOV, which also requires VP30. This indicates that there may be differences in the factors required for transcription/replication. It also appears that there might be differences in receptor binding for MARV, which appear to have a higher affinity for asialoglycoprotein, whereas this interaction does not seem to be important for EBOV infection. For a detailed description of MARV replication, the reader is referred to the replication section of Zaire ebolovirus (*Filoviridae*) which is covered elsewhere in this encyclopedia.

Evolutionary Relationship between Viruses

MARV and EBOV are clearly related in sequence, morphology, and the general disease that they cause; however, it is also clear that they represent different genera of filoviruses. The nucleotide and amino acid differences between MARV and EBOV are both *c.* 55%. Phylogenetic analysis indicates that within MARV there are two distinct lineages of viruses (**Figure 3**) that show a diversity of up to 21%, with the 1987 isolate from Kenya (Ravn) being the most divergent from the rest. This distinct lineage was also seen again in the Durba/Watsa outbreak, along with other isolates that cover most, if not all, of the MARV repertoire.

The outbreak in 2005 in the Uíge Province of northern Angola was a surprise as the origins of all earlier outbreaks were linked directly to East Africa. What is somewhat surprising is that the Angola strain of MARV was less different (~7%) from the main group of East African MARV than one would expect given the large geographic separation (**Figure 3**). This suggests that the

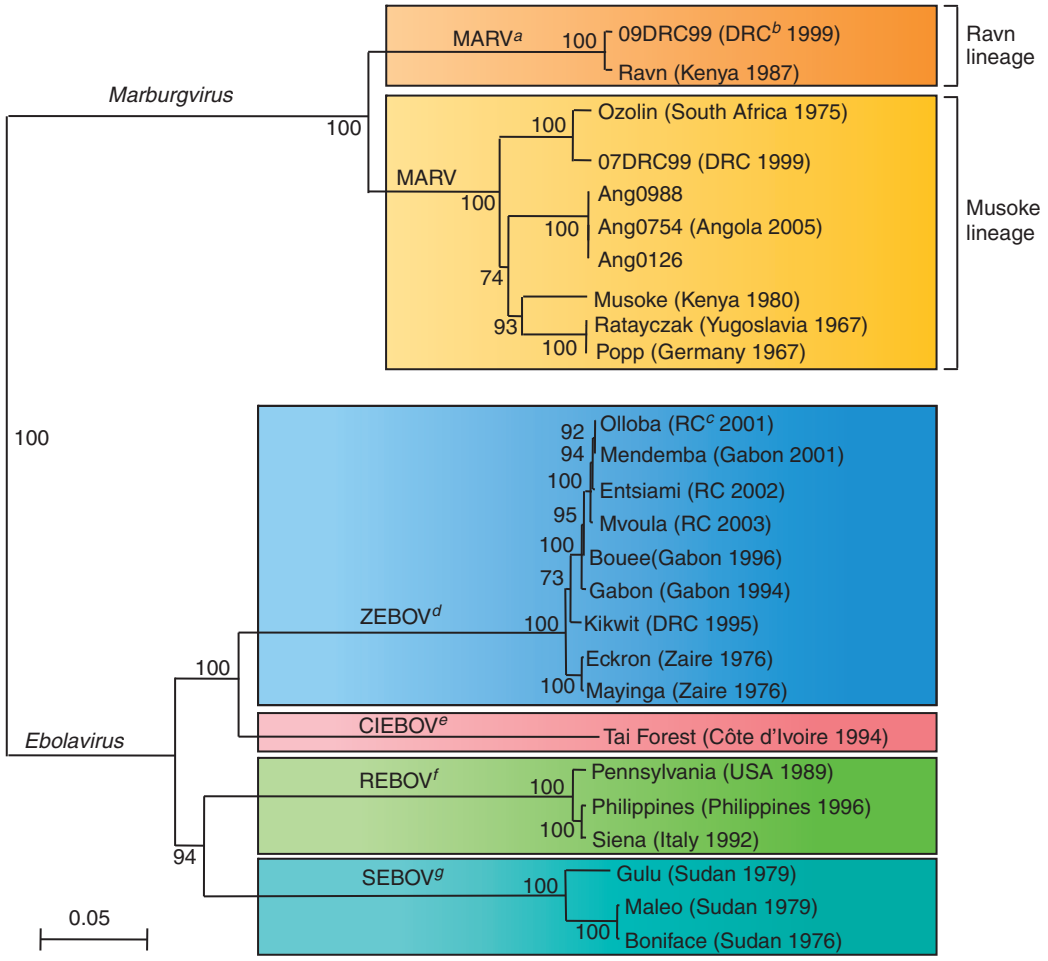


Figure 3 Phylogeny of the family *Filoviridae*. Neighbor-joining analysis of the nucleotide sequence of the GP gene of Marburg and Ebola virus isolates, indicating the two lineages of MARV and the different species of EBOV. Sequences were obtained from GenBank. Confidence values at branch point were obtained from 1000 bootstraps. The bar length equals 5% nucleotide difference. [a]MARV – *Lake Victoria marburgvirus*, [b]Democratic Republic of the Congo, [c]Republic of the Cango, [d]ZEBOV – *Zaire ebolavirus*, [e]CIEBOV – *Côte d'Ivore ebolavirus*, [f]REBOV – *Reston ebolavirus*, [g]SEBOV – *Sudan ebolavirus*.

virus reservoir in these regions may not be substantially different. However, an index case was never identified, and thus the importation of the virus from East Africa cannot be ruled out. Given the length of the outbreak (from fall of 2004 to July 2005) it seemed possible that multiple introductions of MARV could be possible. However, remarkably few nucleotide differences were found among the Angolan clinical specimens (0–0.07%), which is consistent with a single introduction of virus into the human population, followed by person-to-person transmission with little accumulation of mutations. Typically, RNA viruses evolve rapidly due to positive selection in combination with the large number of errors that are made and cannot be corrected during replication (missing proof-reading activity of the polymerase). However, disease progression is so rapid that most individuals seem to die before an effective immune response can be mounted. Therefore, the positive selection of viruses may not occur.

Transmission and Host Range

The reservoir for MARV remains unknown, but its emergence in Angola extends the scope of the reservoir search beyond East Africa. Humans and nonhuman primates serve as natural hosts and it is unclear if other animals are infected. In Durba/Watsa, epidemiological data linked over 70% of the cases with mines or caves, suggesting that the natural reservoir could well be associated with such environments and that bats have been a favorable species for a reservoir. With the Angola outbreak, difficulties in surveillance and contact tracing, combined with the delay in the identification of the outbreak, led to poor epidemiological linkage of MARV cases and ultimately to a lack of success in identifying a point source or mounting any ecological study. Filovirus outbreaks in general are relatively rare events. If the natural reservoir of MARV is similar to that of EBOV, the emergence of MARV in

western Africa should not be surprising, as the sites of multiple large EHF outbreaks are less than 500 miles away (**Figure 1**), including areas which have experienced almost yearly activity over the last decade. EBOV has recently been linked to fruit bats in Gabon and the Republic of the Congo. It is thought that MHF outbreaks start with the rare introduction of the virus into the human population followed by waves of human-to-human transmission (usually through close contact with infected individuals or their body fluids), with little if any evolution of the virus during the course of the outbreak.

Clinical Features

MARVs cause severe hemorrhagic fever in both humans and nonhuman primates. The incubation period lasts from 2 to 21 days (average 4–10), after which there is a sudden onset of nonspecific flu-like symptoms that can include fever, chills, malaise, headache, and myalgia (**Figure 4**). This is followed 2–10 days later by the development of more severe symptoms that include systemic (i.e., prostration, anorexia), gastrointestinal (nausea, vomiting, abdominal pain, diarrhea), respiratory (chest pain, shortness of breath, cough), vascular (conjunctival injection, postural hypotension, edema), and neurologic (headache, confusion, coma) manifestations. The presence of a macropapular rash associated with varying degrees of erythema is also frequently observed and is a useful differential diagnostic.

In cases where coagulation abnormalities develop, hemorrhagic manifestations can include petechiae, ecchymoses, bleeding from venipuncture sites, mucosal bleeding (typically involving the gastrointestinal tract), and visceral hemorrhagic effusions. Patients that develop coagulation abnormalities usually have a bad prognosis. The late stages of the disease are characterized by the development of shock with convulsions, severe metabolic disturbances, and coagulopathy. The onset of shock, with or without obvious bleeding, leads to multiple organ failure with death typically occurring between days 7 and 16. Nonfatal cases have fever for approximately 5–9 days with improvement typically occurring around days 7–11 – the time that the humoral antibody response is noted (**Figure 4**). In patients who survive, convalescence is prolonged and is sometimes associated with myelitis, recurrent hepatitis, psychosis, or uveitis in addition to psycho-social difficulties integrating back into their community.

The mortality for MARV seems to average around 70–85% with the exception of the outbreak in Europe (only 23%). The reason for the large difference in mortality in the European versus the African cases is unknown and may be the result of important host-genetic differences, genetic difference between virus strains, or the standard of care available to infected individuals.

Pathogenesis and Pathology

Clinical investigations of outbreaks of human MARV infections have provided most of the descriptive information on the pathology and pathogenesis of these viruses. However, studies in laboratory animals are much more

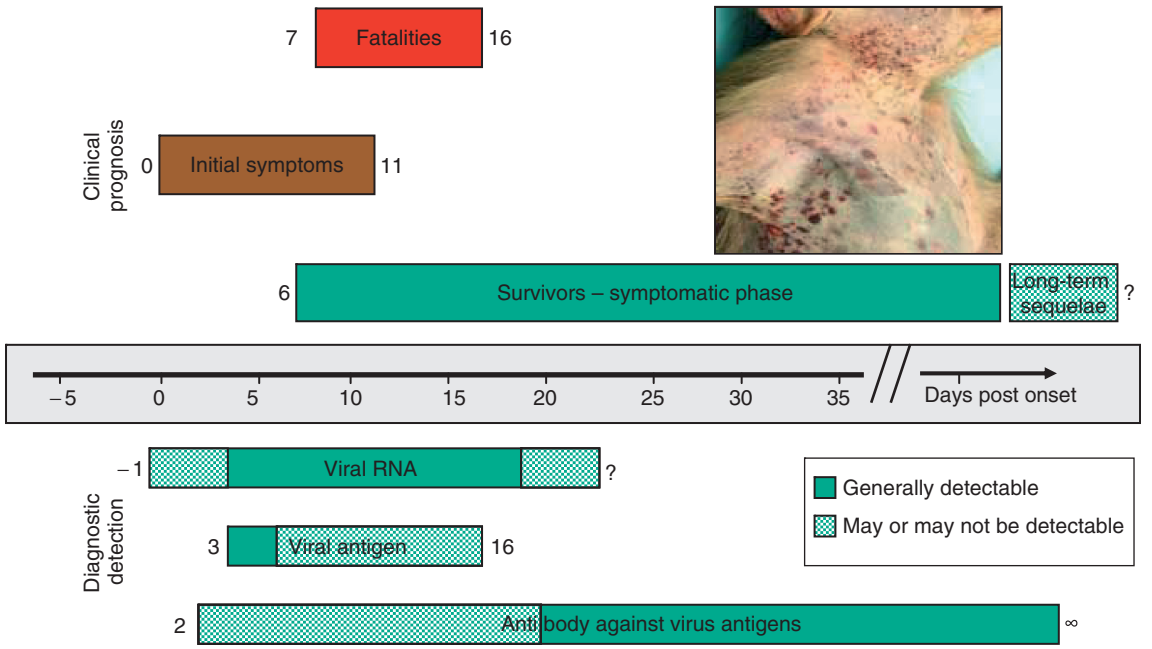


Figure 4 Clinical presentation and diagnostic window. The upper portion describes the appearance of clinical symptoms in humans. Also shown are the characteristic petechiae, seen here on the neck and upper body, of a nonhuman primate with MHF (inset). The lower portion illustrates the timeframe for the appearance of diagnostic targets (viral RNA, viral antigen, and host antibody response).

comprehensive and consistent. Guinea pigs have been employed to study MHF. While guinea pigs have served as effective early screens for evaluating antiviral drugs and candidate vaccines, the disease pathogenesis seen in these animals is not nearly as representative of the human clinical picture as nonhuman primates are.

The different strains of MARV appear to have different levels of virulence. Initially, it appeared that MARV strains were more comparable to *Sudan ebolavirus*; however, virulence of the recently isolated Angola strain appears to be similar to *Zaire ebolavirus*. Most strains of MARV produce uniformly lethal infections in cynomolgus and rhesus macaques. Infections of macaques with the Angola strain appear to progress more rapidly than infections with other strains. For example, challenge of rhesus macaques by intramuscular injection with 1000 pfu of the Musoke or Angola strains produces a uniformly lethal infection, but death occurs within 10–12 days versus 6–8 days, respectively.

While the pathology of MARV is less extensively studied than that of EBOV, it appears to be similar. The pathological changes seen in patients fatally infected with any of the filoviruses include extensive necrosis of parenchymal cells of many organs, including the liver, spleen, kidney, and gonads with little infiltration in infected tissues. However, no single organ is sufficiently damaged to explain the fatal outcome. Infection of cells leads to intracytoplasmic vesiculation and mitochondrial swelling which is followed by a breakdown of organelles and terminal cytoplasmic rarification or condensation. MARV appears to cause more severe liver damage than EBOV. Hepatocellular necrosis is widespread with extremely high infectivity titers present in infected liver samples. Elevations in liver enzymes are prominent findings in most filovirus infections and the hepatocellular degeneration and necrosis observed during MARV infections is extensive. Liver function impairment could contribute to the overall pathogenesis because hemorrhagic tendencies in some cases may be related to decreased synthesis of coagulation factors and other plasma proteins as a result of hepatocellular necrosis. In the late stages of the disease, hemorrhage occurs in the gastrointestinal tract, pleural, pericardial, and peritoneal spaces, as well as the renal tubules with deposition of fibrin. Abnormalities in coagulation parameters include fibrin split products and prolonged prothrombin and partial thromboplastin times. Clinical and biochemical findings support anatomical observations of extensive liver involvement, renal damage, changes in vascular permeability, and activation of the clotting cascade.

Fluid distribution and platelet abnormalities indicate dysfunction of endothelial cells and platelets. In addition to direct vascular involvement in infected hosts, active host mediator molecules probably play a significant role in these disorders. Infected monocytes/macrophages are probably responsible for producing different proteases, peroxide, and other mediators, such as tumor necrosis factor alpha (TNF-α) that may have a negative effect on the infected host. The increased production of TNF-α can result in secondary activation of mediators with important protective as well as deleterious effects. For example, supernatants of MARV-infected monocytes/macrophages cultures are capable of increasing paraendothelial permeability, thus exacerbating the development of the shock syndrome seen in severe and lethal cases. The endothelium is also directly targeted by the virus and endothelial cells support cytolytic MARV replication in culture. The bleeding disorders observed during infection could be due to direct endothelial damage caused directly by virus replication or indirectly by cytokine-mediated processes. The combination of viral replication in endothelial cells and virus-induced mediator release from infected leukocytes may also promote a distinct pro-inflammatory phenotype of the endothelium that triggers, most likely via tissue factor, the coagulation cascade.

Diagnosis

Diagnostics for MARV and EBOV infections use the same principles. As these infections typically occur in isolated regions of Africa that do not have the diagnostic capabilities to identify filovirus infections, the initial diagnosis of MHF and EHF will most likely be based on clinical symptoms. Diagnosis of single cases is very difficult due to similarity of symptoms to other diseases also present in the endemic areas. Due to the rarity of filovirus infections, diagnostic testing is usually performed at national and/or international reference laboratories that are capable of performing the required tests under suitable containment conditions. During outbreaks, healthcare workers who have direct contact with patients are at high risk for infection and adequate barrier nursing precautions need to be implemented during the collection of samples and treatment of patients. Laboratory diagnosis is based on either detection of virus-specific antibodies, virus particles, or particle components. Inactivation of samples is necessary when testing is not done under BSL-4 conditions.

Currently, reverse transcriptase-polymerase chain reaction (RT-PCR) and antigen detection enzyme-linked immunosorbent assay (ELISA) are the primary test systems to diagnose acute infections; RT-PCR, however, is more sensitive than antigen detection ELISA. Viral antigen and/or nucleic acid can be detected in blood from 3 to 18 days post onset of symptoms (**Figure 4**). Most laboratories currently favor RT-PCR because of its sensitivity, specificity, and rapidity. RT-PCR is also readily used in a mobile lab setting and has proved to be accurate

and effective in both MHF and EHF outbreaks. Due to the seriousness of a positive test for filoviruses, the diagnosis of index cases or of single imported cases should not be solely based on a single technology. Confirmation by an independent assay and/or laboratory should always be attempted.

Serological assays are second choice for acute diagnosis as patients often succumb to the disease before an antibody response is generated. Alternatively, immunohistochemistry, direct immunofluorescence on tissues, or electron microscopy can be used for diagnostics, but these methods lack sensitivity, are time consuming, or require expensive equipment. Virus isolation from clinical specimens in tissue culture and/or animals is easily achieved but takes time and requires BSL-4. Filoviruses grow well in a number of cell lines, including Vero and Vero E6, which are the most frequently used. The most commonly used laboratory animals for virus isolation are inbred guinea pig strains; however, it should be kept in mind that often several passages are required to obtain a lethal infection.

Treatment

The current treatment of MARV infections mainly involves supportive therapy, which is directed toward the maintenance of effective blood volume and electrolyte balance. Shock, cerebral edema, renal failure, coagulation disorders, and secondary bacterial infections may be life threatening and have to be managed. Most experimental treatment strategies have been studied for EBOV, but it is expected that several of these approaches would have similar effects on MARV.

Therapeutic antibodies are still considered a valuable short-term solution. This strategy might perhaps be more realistic for the treatment of MHF than EHF based on the observation that humoral responses seemed to be more effective against MARV. The use of recombinant nematode anticoagulant protein c2 (rNAPc2), which seems to be a promising approach for EBOV, did not result in a similar positive effect for MARV infections (Angola strain). Studies on novel antiviral strategies such as viral gene silencing through specific siRNA or functional domain interference with small peptides have been very limited for MARV. In contrast, more focus has been given to strategies targeting host responses. Treatment with TNF-α neutralizing antibodies has been partially successful in guinea pig models of MHF, but has yet to be evaluated in nonhuman primates. MARV-infected guinea pigs are partially protected by Desferal, an interleukin 1 (IL-1) and TNF-α antagonist. In a separate study, treatment of animals with IL-1 receptor antagonist (IL-1RA) or anti-TNF-α decreased the concentration of circulating TNF-α and allowed 50% survival.

As with EBOV, ribavirin is not indicated for MHF treatment. In general, it seems plausible that combination therapy for MHF and EHF will be superior over any single treatment form.

Prevention

Protective MARV vaccines would be extremely valuable for at-risk medical personnel, first responders, military personnel, researchers, and high-risk contact groups during outbreaks (such as family members). Past vaccine approaches were based on either inactivated virus preparations, which were of limited protective efficacy and considered unsafe, or subunit vaccines, which showed efficacy in the rodent but not nonhuman primate model. Protective efficacy in nonhuman primates could first be demonstrated with a system based on Venezuelan equine encephalitis virus replicons expressing the MARV GP and/or NP. Currently, the most promising vaccine approach seems to be a live-attenuated vector based on vesicular stomatits virus expressing the MARV GP (strain Musoke). The protective efficacy and safety of this vaccine vector has been demonstrated in two animal models, the guinea pig and nonhuman primate. Protective efficacy could also be achieved against challenge with heterologous MARV strains as well as homologous aerosol challenge. In addition, the vector showed astonishing efficacy in postexposure treatment of rhesus macaques; single, high-dose treatment 30 min after high-dose challenge protected all animals from lethal disease. Despite the success it remains questionable if a replication-competent vector will be granted approval for human use.

Acknowledgments

The Public Health Agency of Canada (PHAC), Canadian Institutes of Health Research (CIHR), and CBRNE (chemical, biological, radiological, and nuclear) Research and Technology Initiative (CRTI), Canada, supported work on filoviruses at the National Microbiology Laboratory of the Public Health Agency of Canada.

See also: Ebolavirus.

Further Reading

Bausch DG and Geisbert TW (2007) Development of vaccines for Marburg hemorrhagic fever. *Expert Reviews of Vaccines* 6: 57–74.

Bausch DG, Nichol ST, Muyembe-Tamfum JJ, *et al.* (2006) Marburg hemorrhagic fever associated with multiple genetic lineages of virus. *New England Journal of Medicine* 355: 909–919.

Becker S and Muhlberger E (1999) Co- and posttranslational modifications and functions of Marburg virus proteins. *Current Topics in Microbiology and Immunology* 235: 23–34.

Bray M and Paragas J (2002) Experimental therapy of filovirus infections. *Antiviral Research* 54: 1–17.
Feldmann H, Geisbert TW, Jahrling PB, *et al.* (2005) *Filoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 645–653. San Diego, CA: Elsevier Academic Press.
Hensley LE, Jones SM, Feldmann H, Jahrling PB, and Geisbert TW (2005) Ebola and Marburg viruses: Pathogenesis and development of countermeasures. *Current Molecular Medicine* 5: 761–772.
Martini GA and Siegert R (eds.) (1971) *Marburg Virus Disease.* New York: Springer.
Mohamadzadeh M, Chen L, Olinger GG, Pratt WD, and Schmaljohn AL (2006) Filoviruses and the balance of innate, adaptive, and inflammatory responses. *Viral Immunology* 19: 602–612.
Paragas J and Geisbert TW (2006) Development of treatment strategies to combat Ebola and Marburg viruses. *Expert Reviews of Anti-Infective Therapy* 4: 67–76.
Sanchez A, Geisbert TW, and Feldmann H (2007) Marburg and Ebola viruses. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1409–1448. Philadelphia, PA: Lippincott Williams and Wilkins.
Siegert R, Shu HL, Slenczka W, Peters D, and Muller G (1967) On the etiology of an unknown human infection originating from monkeys. *Deutsche Medizinische Wochenschrift* 92: 2341–2343.
Slenczka WG (1999) The Marburg virus outbreak of 1967 and subsequent episodes. *Current Topics in Microbiology and Immunology* 235: 49–75.
Zaki SR and Goldsmith CS (1999) Pathologic features of filovirus infections in humans. *Current Topics in Microbiology and Immunology* 235: 97–116.

Marnaviruses

A S Lang, Memorial University of Newfoundland, St. John's, NL, Canada
C A Suttle, University of British Columbia, Vancouver, BC, Canada

© 2008 Elsevier Ltd. All rights reserved.

The first, and currently the only, virus species classified within the family *Marnaviridae* is *Heterosigma akashiwo RNA virus* (HaRNAV). This virus was isolated from the Strait of Georgia in coastal British Columbia and infects and causes the lysis of the marine, toxic bloom-forming, unicellular, photosynthetic alga *Heterosigma akashiwo*. The host organism, *H. akashiwo*, forms blooms in the Strait of Georgia waters every year, and the blooms of this organism are toxic to fish.

When the virus is added to a growing culture of susceptible *H. akashiwo*, complete lysis of the culture generally occurs within 1 week or less. Only some strains of *H. akashiwo* are susceptible to infection by HaRNAV, and the pattern of susceptibility does not appear to relate to geographic origin of the strain in question. Therefore, HaRNAV causes lysis of some, but not all, *H. akashiwo* strains isolated from coastal British Columbia waters as well as some, but not all, strains from other locations in the Pacific such as coastal Japan.

HaRNAV particles are small (approximately 25 nm in diameter) and spherical, and appear to have an icosahedral symmetry (**Figure 1**). Signs of viral-induced intracellular cytopathology are visible within 48 h of infection of *H. akashiwo* by HaRNAV. One notable effect is the appearance of membranous vesicles within the cytoplasm; such structures are the site of RNA replication for other positive-stranded ssRNA viruses and are well characterized for picornaviruses such as poliovirus. This is followed by the appearance of assembled viral particles throughout the cytoplasm, and these can be at sufficient density for the particles to form crystalline arrays.

The genome of HaRNAV (strain SOG263) has been completely sequenced. The single-stranded RNA (ssRNA) genome is 8587 nucleotides long with positive polarity and a poly (A) tail. The 5′ and 3′ untranslated regions (UTRs) total 9.8% of the genome sequence and the 5′ UTR contains sequence features believed to be important for protein translation; for example, there is a

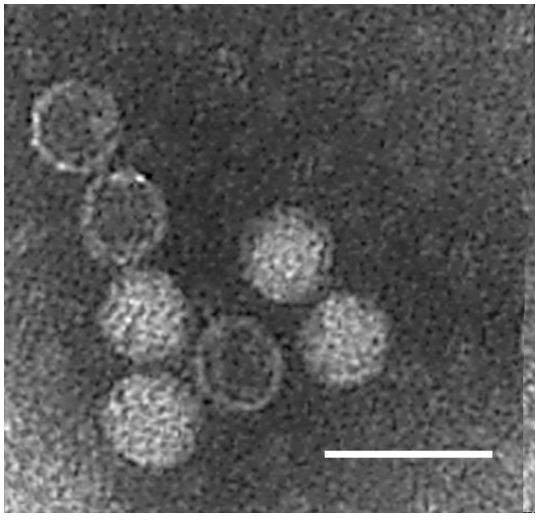


Figure 1 Transmission electron micrograph of HaRNAV particles negatively stained with phosphotungstic acid. Scale = 50 nm. Reprinted from Tai V, Lawrence JE, Lang AS, *et al*. (2003) Characterization of HaRNAV, a single-stranded RNA virus causing lysis of *Heterosigma akashiwo* (Raphidophyceae). *Journal of Phycology* 39: 343–352, with permission from Blackwell Publishing.



Figure 2 Map of the HaRNAV genome. The approximate predicted location of the single open reading frame is shown as a box on top of the positive-polarity ssRNA genome. Regions within the polyprotein sequence that have homology to known ssRNA viral protein domains are colored and annotated: Hel, 'RNA helicase'; RdRp, RNA-dependent RNA polymerase; VP1, VP2, and VP3, capsid protein subunits. For the structural proteins, the locations of N-termini found by sequencing are shown as vertical lines. The N-terminus marked with an asterisk (*) was only found in immature virus particles. Modified from Lang AS, Culley AI, and Suttle CA (2004) Genome sequence and characterization of a virus (HaRNAV) related to picorna-like viruses that infects the marine toxic bloom-forming alga *Heterosigma akashiwo*. *Virology* 320: 206–217, with permission from Elsevier.

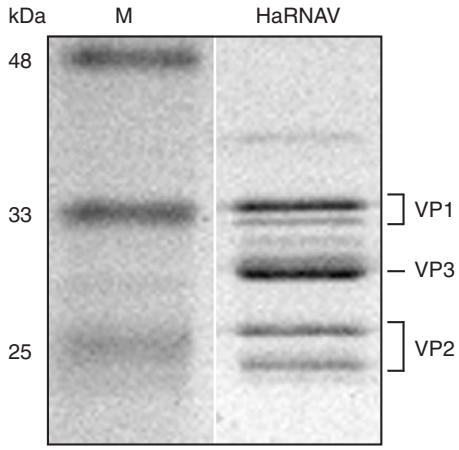


Figure 3 SDS-PAGE analysis of HaRNAV particles. The identities of the major bands are labeled, where known, corresponding to the designations in **Figure 2**. The lane marked M contains molecular weight markers with sizes indicated in kilodaltons on the left. Modified from Lang AS, Culley AI, and Suttle CA (2004) Genome sequence and characterization of a virus (HaRNAV) related to picorna-like viruses that infects the marine toxic bloom-forming alga *Heterosigma akashiwo*. *Virology* 320: 206–217, with permission from Elsevier.

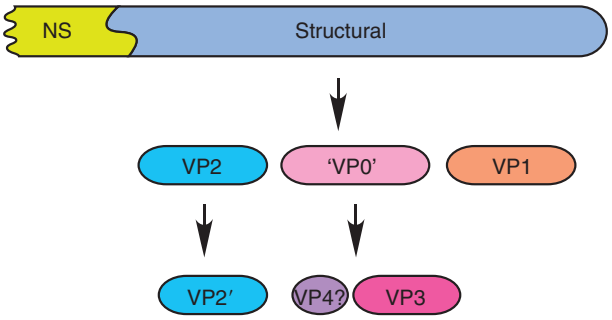


Figure 4 Organization of the structural protein subunits within the polyprotein. NS, nonstructural protein domains.

pyrimidine-rich tract of sequence immediately preceding the predicted start codon and it is also predicted to contain extensive secondary structure. The genome sequence predicts a single open reading frame (ORF) encoding a protein that contains conserved +ssRNA virus protein domains, as indicated by comparisons with sequence databases. Conserved 'RNA helicase', RNA-dependent RNA polymerase (RdRp), and structural protein domains are recognizable by these comparisons (**Figure 2**).

When subjected to SDS-PAGE, HaRNAV particles appear to contain at least six major protein bands varying in size between 24 and 39 kDa (**Figure 3**). Sequence analyses of these protein bands indicate that they represent multiple 'versions' of only three different polypeptides. Other than the two bands corresponding to the VP2 protein which have different N-termini (**Figures 2–4**), the differences between protein bands that migrate to different positions in the gel but that appear to contain the same polypeptide sequences are not known. All three of the known structural protein sequences (VP1, 2, and 3) have significant similarity with the structural proteins of viruses classified in the family *Dicistroviridae*, including cricket paralysis virus (CrPV) for which a crystal structure has been determined. Also, the HaRNAV organization of the structural protein subunits within the larger (structural portion of the) polyprotein is the same as found in the family *Dicistroviridae* (**Figure 4**). Note however that a VP4-like protein has not been identified associated with HaRNAV particles.

The N-terminal sequences of several structural proteins were determined and this showed that two of the proteins have a conserved sequence at the cleavage site (ST–SEI). Beyond this, there is surprisingly little recognizable conservation at the protein processing sites. Based on computer searches of the sequence databases, there is no conserved putative protease domain recognizable in the HaRNAV polyprotein sequence, but it is reasonable to expect that there is one, as found in well-studied members of the proposed viral order *Picornavirales*.

Phylogenetic analyses that have been performed with the recognizable conserved domains from the HaRNAV polyprotein sequence and the corresponding regions of other related virus sequences clearly indicate that HaRNAV does not fall within the previously characterized groups within the proposed order *Picornavirales*. A phylogenetic analysis of viral (putative) RdRp domains is shown in **Figure 4**. All defined groups are found to cluster in these analyses.

In the time since the characterization of the HaRNAV genome, other positive ssRNA viruses that infect marine

protists have been isolated and these viruses (HcRNAV, RsRNAV, and SssRNAV) all differ from HaRNAV in that their genomes are dicistronic. Phylogenetic analyses indicate that RsRNAV and SssRNAV likely belong in the proposed order *Picornavirales* while HcRNAV does not belong in this group. Our analyses with the RdRp domains (the complete sequence spanning regions I–VIII) do not suggest that these viruses are closely related to the family *Marnaviridae* (**Figure 5**, **Table 1**), although HaRNAV does give the highest scoring match of any cultured virus when the putative RdRp sequences from RsRNAV were used in a BLAST search against the GenBank database. (The overall top-scoring matches to the RsRNAV sequence were from unidentified picorna-like RdRp sequences amplified from marine environments by degenerate RT-PCR.) The same search with the putative RdRp sequence from SssRNAV gave viruses in the family *Dicistroviridae* as the top-scoring matches.

Analyses performed with the VP3-like capsid protein sequences from these viruses tell a different story. BLAST searches with the VP3-like sequence from any of the three cultured marine viruses (HaRNAV, RsRNAV, and SssRNAV) give the other two viruses as the top-scoring matches. Pairwise alignments show that they are all >30% identical: 34% for HaRNAV versus RsRNAV; 33% for HaRNAV versus SssRNAV; and 32% for RsRNAV versus SssRNAV. Phylogenetic analyses performed with the capsid protein sequences (**Figure 6**, **Table 1**) clearly indicate a closer relationship between these marine RNA viruses than the analyses with the RdRp domain. If we extend the amount of sequence used for the phylogenetic analyses and use the (putative) RNA helicase, RdRp, and VP3-like sequences concatenated into one sequence, the same groupings are strongly supported (**Figure 6**). However, there are other significant differences between HaRNAV and these viruses, such as monocistronic (HaRNAV) versus dicistronic (RsRNAV and SssRNAV) genome organization. Furthermore, although all analyses we have conducted support the idea that RdRp phylogenies are robust at discriminating established genus and family-level relationships between picorna-like viruses, a relationship between these marine viruses is not supported (**Figure 5**). Clearly more work is required and more related viruses need to be cultured and characterized to allow

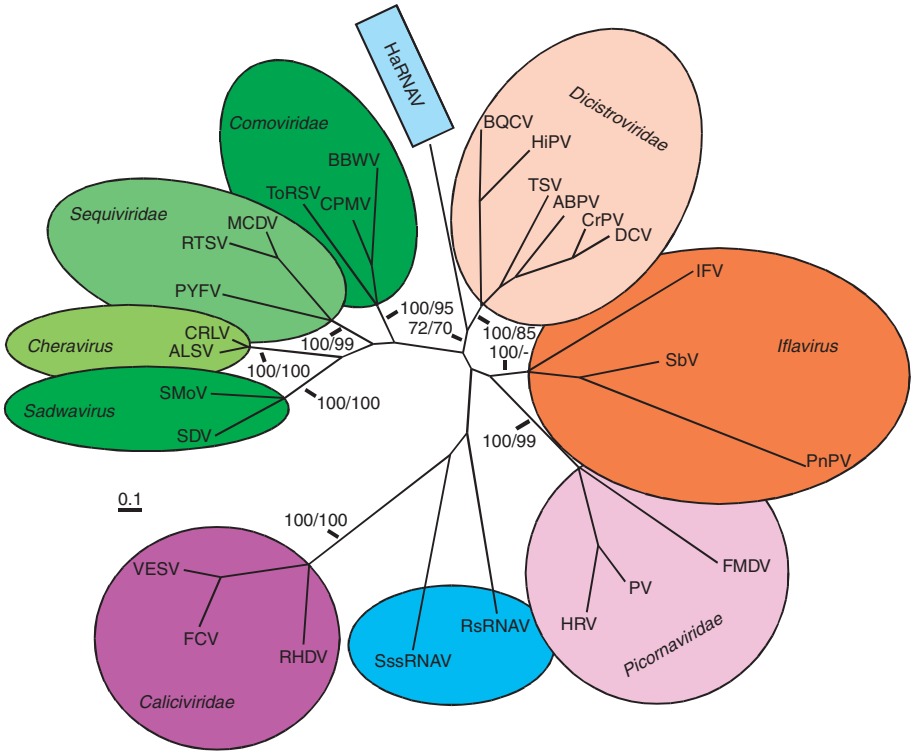


Figure 5 Phylogenetic analyses of RdRp sequences. Bayesian and neighbor-joining analyses were performed with aligned complete (putative) RdRp domain regions I through VIII. The Bayesian maximum likelihood tree is shown with the Bayesian scale bar. Important branches are labeled with the Bayesian clade credibility values (percentages based on 250 000 generations), followed by the neighbor-joining bootstrap values (percentages based on 10 000 replicates). A dash indicates that the branch was not supported by the neighbor-joining analysis. Defined viral groups are labeled by their family or genus designation where appropriate and highlighted together by color. The colors reflect the host organism species as follows: orange, insects/arthropods; pink, mammals; green, plants; blue, marine protists. HaRNAV represents the family *Marnaviridae* and the other two marine viruses (RsRNAV and SssRNAV) are not yet classified. Full names of the viruses and sequence database accession numbers are in **Table 1**.

Table 1 Viruses used in genome comparisons with HaRNAV

Virus	*Abbreviation*	*Accession number*
Acute bee paralysis virus	ABPV	NC_002548
Apple latent spherical virus	ALSV	NC_003787
Black queen cell virus	BQCV	NC_003784
Broad bean wilt virus 2	BBWV	AF225953, AF225954
Cherry rasp leaf virus	CRLV	NC_006271
Cowpea mosaic virus	CPMV	NC_003549
Cricket paralysis virus	CrPV	NC_003924
Drosophila C virus	DCV	NC_001834
Feline calicivirus	FCV	NC_001481
Foot-and-mouth disease virus	FMDV	NC_004004
Heterosigma akashiwo RNA virus	HaRNAV	AY337486
Himetobi P virus	HiPV	NC_003782
Human rhinovirus 14	HRV	NC_001490
Human poliovirus	PV	NC_002058
Infectious flacherie virus	IFV	NC_003781
Maize chlorotic dwarf virus	MCDV	NC_003626
Parsnip yellow fleck virus	PYFV	NC_003628
Perina nuda picorna-like virus	PnPV	NC_003113
Rabbit hemorrhagic disease virus	RHDV	NC_001543
Rhizosolenia setigera RNA virus	RsRNAV	AB243297
Rice tungro spherical virus	RTSV	NC_001632
Sacbrood virus	SbV	NC_002066
Satsuma dwarf virus	SDV	NC_003785
Schizochytrium single-stranded RNA virus	SssRNAV	AB193726
Strawberry mottle virus	SMoV	NP_733954
Taura syndrome virus	TSV	NC_003005
Tomato ringspot virus	ToRSV	NC_003840
Vesicular exanthema of swine virus	VESV	NC_002551

the phylogenetic the relationships among these viruses to be understood better. Given the extreme evolutionary distance between the hosts of these viruses (and the viruses themselves based on RdRp analyses), it seems unlikely that they would fall within the same family.

Although HaRNAV (strain SOG263) is the only marnavirus to be cultured and fully described so far, there is clear evidence that closely related viruses occur in marine systems. A degenerate RT-PCR approach that targeted picorna-like virus RdRp genes in marine virus communities produced 11 distinct RdRp sequences that grouped very closely with HaRNAV in phylogenetic analyses. These 11 sequences show variation at a total of 17 sites in the amplified 450-nucleotide region and each different sequence has between 1 and 5 nucleotide polymorphisms relative to the HaRNAV sequence (**Table 2**). The differences across all of these sequences (relative to HaRNAV) at the nucleotide level result in four polymorphic sites in the amino acid sequences, and two of these sites have two different polymorphisms at the same site in different clones (**Table 2**). Although these variations are in one of the most conserved regions of all +ssRNA viruses, the sequences have a very high level of identity with HaRNAV suggesting that these different sequences represent different strains of HaRNAV.

The RT-PCR probing of natural marine virus community RdRp genes further showed that there are many more unknown picorna-like viruses present in these communities. These virus sequences are more closely related to RsRNAV than to any of the other currently known and cultured marine viruses. We speculate that these sequences represent viruses from completely distinct genera, if not families, relative to marnaviruses, and so are beyond the scope of this article. One notable point about the sequences recovered with this approach is the vast diversity of these types of viruses that are likely to be present in the marine environment, even within a single water sample.

Work in the ocean shows that marine systems harbor a whole new world of ssRNA viruses. Culturing of some of these marine ssRNA viruses has shown that several have features that recognizably link them with known groups, such as picornaviruses. HaRNAV, representing the family *Marnaviridae*, shares many features with known groups of ssRNA viruses, particularly with viruses in the proposed order *Picornavirales*. Despite these shared characteristics, however, a large part of the HaRNAV genome has no recognizable similarity to any other known sequences, viral or otherwise. More work is clearly needed with the individual marine RNA viruses, and even more viruses need to be brought into culture, sequenced, and characterized.

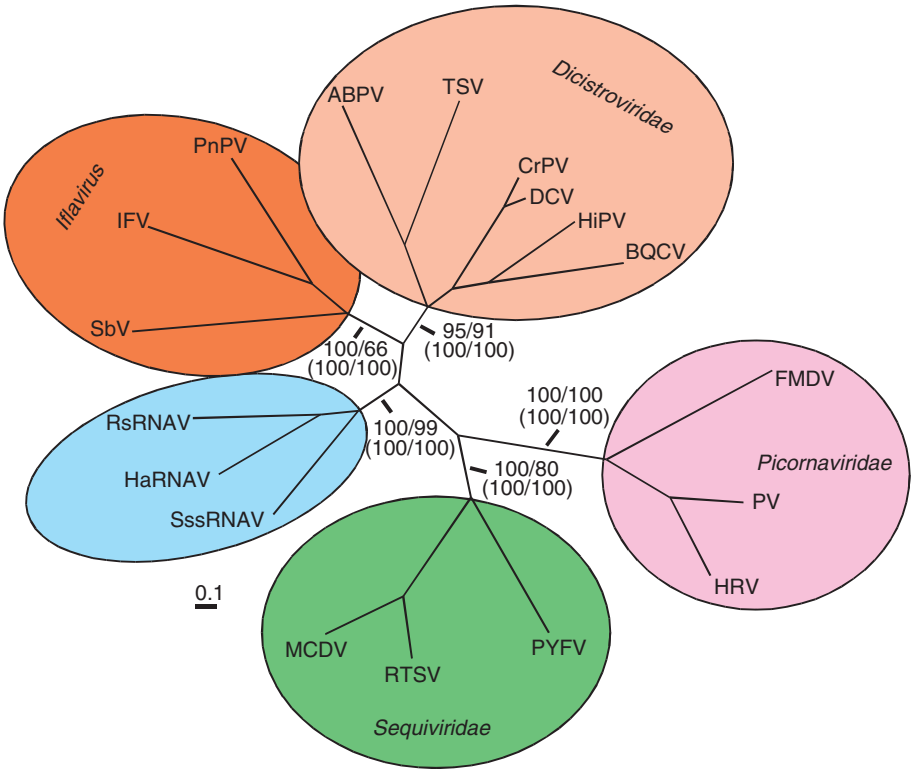


Figure 6 Phylogenetic analyses of VP3-like capsid sequences. Bayesian and neighbor-joining analyses were performed with aligned complete (putative) VP3-like sequences. The Bayesian maximum likelihood tree is shown with the Bayesian scale bar. Important branches are labeled with the Bayesian clade credibility values (percentages based on 175 000 generations), followed by the neighbor-joining bootstrap values (percentages based on 10 000 replicates). The corresponding support values for the branches from analyses of the (putative) 'RNA Helicase', RdRp, and VP3-like sequences concatenated into one long sequence are shown in brackets with Bayesian clade credibility values (percentages based on 175 000 generations). Defined viral groups are labeled by their family or genus designation where appropriate and highlighted together by color. The colors reflect the host organism species as follows: orange, insects/arthropods; pink, mammals; green, plants; blue, marine protists. HaRNAV represents the family *Marnaviridae* and the other two marine viruses (RsRNAV and SssRNAV) are not yet classified. Full names of the viruses and sequence database accession numbers are in **Table 1**.

Table 2 Polymorphisms, relative to HaRNAV, in RdRp sequence fragments amplified from natural marine virus communities

	Position[a]																
Sequence	*63*	*72*	*78*	*134*	*212*	*223*	*258*	*267*	*285*	*316*	*317*	*363*	*379*	*380*	*396*	*432*	*438*
HaRNAV[b]	A	C	G	T	C	A	A	C	T	A	A	C	T	T	A	G	A
JP500-1	.[c]	.	.	.	**T**[d]	T	.	.	.	.	.	.	.	.	.	.	.
JP800-1	.	.	.	**C**	**T**	.	.	.	.	.	.	.	.	**C**	.	A	.
JP800-4	.	.	.	.	**T**	.	.	.	.	.	.	.	.	.	.	.	.
JP800-8	.	.	.	.	**T**	.	.	.	.	.	.	T	.	.	G	.	.
JP800-11	.	.	.	.	**T**	.	G	.	.	C	.	.	**C**	.	.	.	.
FRP896-2	.	.	.	.	**T**	.	.	.	.	.	**C**	.	.	.	G	.	T
FRP896-3	G	.	.	.	**T**	.	.	.	.	.	.	.	.	.	G	.	T
FRP896-4	.	.	A	.	**T**	.	.	.	.	.	.	.	.	.	G	.	T
FRP896-5	.	.	.	.	**T**	.	.	.	C	.	.	.	.	.	G	.	T
FRP896-6	.	T	.	.	**T**	.	.	T	.	.	.	.	.	.	G	.	T
FRP896-7	.	.	.	.	**T**	.	.	.	.	.	**G**	.	.	.	G	.	T

[a]Sequences are 450 bases long.
[b]The cultured and sequenced strain HaRNAV SOG263.
[c]'.' indicates the sequence is the same as the sequenced strain HaRNAV SOG263 at this position.
[d]Changes that result in different amino acid sequences are in bold.

The ssRNA viruses isolated from marine systems infect basal eukaryotes, protists, and so these viruses may be ancestral to the related viruses infecting higher organisms such as mammals. Study of these viruses will further our understanding of RNA virus evolution.

See also: Algal Viruses.

Further Reading

Christian P, Fauquet CM, Gorbalenya AE, *et al.* (2005) A proposed *Picornavirales* order. In: Fauquet CM (ed.) *Microbes in a Changing World*. San Francisco: International Unions of Microbiological Societies.

Culley AI, Lang AS, and Suttle CA (2003) High diversity of unknown picorna-like viruses in the sea. *Nature* 424: 1054–1057.

Culley AI, Lang AS, and Suttle CA (2005) Taxonomic structure of the family *Marnaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 789–792. San Diego, CA: Elsevier Academic Press.

Culley AI, Lang AS, and Suttle CA (2006) Metagenomic analysis of coastal RNA virus communities. *Science* 312: 1795–1798.

Hellen CUT and Sarnow P (2001) Internal ribosome entry sites in eukaryotic mRNA molecules. *Genes and Development* 15(13): 1593–1612.

Jackson WT, Giddings TH, Taylor MP, *et al.* (2005) Subversion of cellular autophagosomal machinery by RNA viruses. *PLoS Biology* 3(5): e156.

Koonin EV and Dolja VV (1993) Evolution and taxonomy of positive-strand RNA viruses: Implication of comparative analysis of amino acid sequences. *Critical Reviews in Biochemistry and Molecular Biology* 28: 375–430.

Lang AS, Culley AI, and Suttle CA (2004) Genome sequence and characterization of a virus (HaRNAV) related to picorna-like viruses that infects the marine toxic bloom-forming alga *Heterosigma akashiwo*. *Virology* 320: 206–217.

Nagasaki K, Shirai Y, Takao Y, *et al.* (2005) Comparison of genome sequences of single-stranded RNA viruses infecting the bivalve-killing Dinoflagellate *Heterocapsa circularisquama*. *Applied and Environmental Microbiology* 71(12): 8888–8894.

Nagasaki K, Tomaru Y, Katanozaka N, *et al.* (2004) Isolation and characterization of a novel single-stranded RNA virus infecting the bloom-forming diatom *Rhizosolenia setigera*. *Applied and Environmental Microbiology* 70(2): 704–711.

Shirai Y, Takao Y, Mizumoto H, Tomaru Y, Honda D, and Nagasaki K (2006) Genomic and phylogenetic analysis of a single-stranded RNA virus infecting *Rhizosolenia setigera* (Stramenopiles: Bacillariophyceae). *Journal of the Marine Biological Association of the UK* 86: 475–483.

Tai V, Lawrence JE, Lang AS, *et al.* (2003) Characterization of HaRNAV, a single-stranded RNA virus causing lysis of *Heterosigma akashiwo* (*Raphidophyceae*). *Journal of Phycology* 39: 343–352.

Takao Y, Mise K, Nagasaki K, Okuno T, and Honda D (2006) Complete nucleotide sequence and genome organization of a single-stranded RNA virus infecting the marine fungoid protist *Schizochytrium* sp. *Journal of General Viology* 87: 723–733.

Takao Y, Nagasaki K, Mise K, Okuno T, and Honda D (2005) Isolation and Characterization of a novel single-stranded RNA virus infectious to a marine fungoid protist *Schizochytrium* sp. (*Thraustochytriaceae, Labyrinthulea*). *Applied and Environmental Microbiology* 71(8): 4516–4522.

Tate J, Liljas L, Scotti P, *et al.* (1999) The crystal structure of cricket paralysis virus: The first view of a new virus family. *Nature Structural Biology* 6: 765–774.

Measles Virus

R Cattaneo, Mayo Clinic College of Medicine, Rochester, MN, USA
M McChesney, University of California, Davis, Davis, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Oncolytic virotherapy The experimental treatment of cancer patients based on the administration of replication-competent viruses that selectively destroy tumor cells but leave healthy tissue unaffected.

RNA editing The introduction into a RNA molecule of nucleotides that are not specified by the gene; the measles virus polymerase introduces a single G nucleotide in the middle of the phosphoprotein messenger RNA by reading twice over a C template (polymerase stuttering).

Subacute sclerosing panencephalitis (SSPE) A rare but always lethal brain disease caused by measles virus.

Syncytia Fused cells with multiple nuclei characteristic of measles virus infection.

Introduction and Classification

Measles virus (MV) is an enveloped nonsegmented negative-strand RNA virus of the family *Paramyxoviridae*, genus *Morbillivirus*. The *Paramyxoviridae* are important agents of disease, causing age-old diseases of human and animals (measles, mumps, respiratory syncytial virus (RSV), the parainfluenza viruses), and newly recognized emerging diseases (Nipah, Hendra, morbilliviruses of aquatic mammals).

Among negative-strand RNA viruses, the *Paramyxoviridae* are defined by having a protein (F) that causes fusion of viral and cell membranes at neutral pH. The organization and expression strategy of the nonsegmented genome of *Paramyxoviridae* including MV is similar to that of the *Rhabdoviridae*.

The defining characteristics of the genus *Morbilliviruses* are the lack of neuraminidase activity, and cell entry

through the primary receptor signaling lymphocyte activation molecule (SLAM, CD150): MV, canine distemper virus, and rinderpest virus all enter cells through SLAM (human, canine, or bovine, respectively). The cellular distribution of SLAM overlaps with the sensitivity of different cell types to wild type MV infection, and explains immunosuppression.

Viral Particle Structure and Components

MV particles are enveloped by a lipid bilayer derived from the plasma membrane of the cell in which the virus was grown. They have been visualized as pleomorphic or spherical, depending on the methods used for their purification. Their diameter ranges from 120 to 300–1000 nm, implying that their cargo volume may differ by a factor 30 and that the large particles are polyploid. Inserted into the envelope are glycoprotein spikes that extend about 10 nm from the surface of the membrane and can be visualized by electron microscopy (**Figure 1**). A schematic diagram of an MV particle is shown in **Figure 2**(**a**).

Inside the viral membrane is the nucleocapsid core, typically including several genomes. Each genome has 15 894 nucleotides tightly encapsidated by a helically arranged nucleocapsid (N) protein (**Figure 2**(**a**)). Two other proteins, a polymerase (L for large) and a polymerase cofactor (P for phosphoprotein), are associated with the RNA and the N protein to form a replicationally active ribonucleoprotein (RNP) complex. The MV RNP is condensed by the matrix (M) protein and then selectively covered by the two envelope spikes, consisting of oligomers of the F and H proteins.

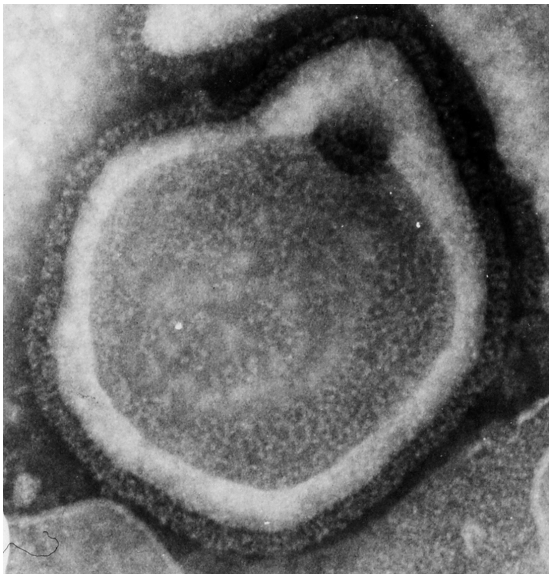


Figure 1 MV particle. Electron micrograph from Claire Moore and Shmuel Rozenblatt, Tel-Aviv University, Israel.

The F protein spike is trimeric, whereas the H protein forms covalently linked dimers that may form noncovalently linked tetramers. The H protein contacts the cellular receptors, whereas F executes fusion. This protein is cleaved and activated by the ubiquitous intracellular protease furin. In the assembly process viral proteins are preferentially incorporated into nascent viral particles, whereas the majority of host proteins are excluded.

Genome, Replication Complex, and Replication Strategy

The MV negative-strand genome begins with a 56 nt 3′ extracistronic region known as leader, and ends with a 40 nt extracistronic region known as trailer. These control regions are essential for transcription and replication and flank the six genes. The term gene refers here to contiguous, nonoverlapping transcription units separated by three untranscribed nucleotides. There are six genes coding for eight viral proteins, in the order (positive strand): 5′-N-P/V/C-M-F-H-L-3′ (**Figure 2**(**b**)). The P gene uses overlapping reading frames to code for three proteins, P, V, and C. Complete sequences of several MV wild type and vaccine strains have been obtained.

The first gene codes for the N protein. Each N protein covers 6 nt, and about 13 N proteins may constitute a turn in the RNP helix. RNPs are formed when N is expressed in the absence of other viral components, suggesting that N–N interactions drive RNP assembly. Two domains have been identified in the N protein: a conserved N-terminal N core (about 400 amino acids) and a variable C-terminal N tail (about 100 amino acids). N core is essential for self-assembly, RNA binding, and replication activity, whereas N tail interacts with a C-terminal domain of the P protein. N protein exists in at least two forms in infected cells: one associated with RNA in a RNP structure and a second unassembled soluble form named N^0 that may encapsidate the nascent RNA strand during genome and antigenome replication.

The second gene codes for three proteins implicated in transcription or innate immunity control: P, V, and C. Two of these proteins have a modular structure: the 231 N-terminal residues of V are identical with those of P, but its 68 C-terminal residues are translated from a reading frame accessed by insertion of a nontemplated G residue through co-transcriptional editing (polymerase stuttering). This V domain is highly conserved in paramyxoviruses, with cysteine and histidine residues binding two zinc molecules per protein. The main function of the V protein is to counteract the innate immune response; V interferes with intracellular signaling pathways supporting the interferon response and sustains virus spread in the host immune system.

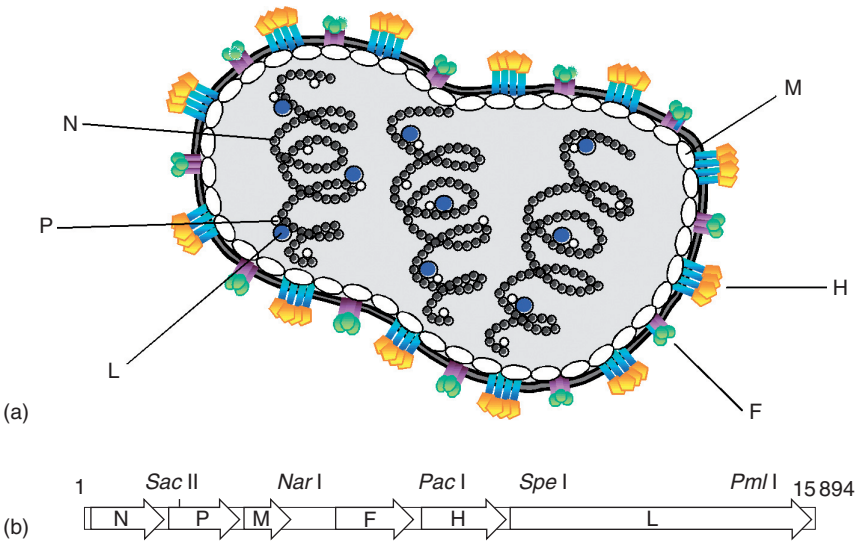


Figure 2 (a) Diagram of a polyploid MV particle containing three genomes. The viral nucleocapsid (N), phosphoprotein (P), polymerase (large, L), matrix (M), fusion (F), and hemagglutinin (H) proteins are indicated with different symbols. (b) Schematic representation of the MV antigenome (plus strand). The open reading frames of the six largest proteins are indicated with open arrows. The P gene codes for three proteins, P, V, and C. Unique restriction enzyme sites used for reverse genetics are indicated above the genome.

The main function of the P protein is to support viral replication and transcription; it is an essential component of the polymerase, and of the protein complex mediating RNA encapsidation by N^0 protein. The P protein N-terminal segment, identical with the V protein N-terminus, is phosphorylated on serines and threonines and contains regions of high intrinsic disorder, possibly facilitating interactions with multiple viral and cellular proteins. P self-assembles as a tetramer through a central region in its unique domain, and interacts with N tail through its C-terminus.

The third protein expressed from the P gene is named C. Its reading frame is accessed by ribosomes initiating translation 22 bases downstream of the P AUG codon. The MV C protein not only inhibits the interferon response but also has infectivity factor function, and a role in virus particle release has been demonstrated for the C protein of the related Sendai virus.

The third, fourth, and fifth genes code for envelope-associated proteins that are discussed below in the context of receptor recognition and membrane fusion. The large (L) sixth and last gene codes for the RNA-dependent RNA polymerase, believed to possess all enzymatic activities necessary to synthesize mRNA: nucleotide polymerization, capping and methylation, and polyadenylation. L adds poly-A tails to nascent viral mRNAs cotranscriptionally, by stuttering on a stretch of U residues occurring at the end of each viral gene. Sequence comparison identified six highly conserved domains in the L protein that were tentatively assigned different catalytic functions, whereas interaction of this 200 kDa protein with the P and other viral proteins was mapped to nonconserved regions.

The N, P, and L proteins are associated with the RNA to form the replicationally active RNP complex that starts primary transcription after cell entry. The negative-strand genome is then used to synthesize positive-strand 'anti-genomes' that produce more genomes, completing one amplification cycle. Amplification produces the genomic templates for secondary transcription.

The polymerase transcribes the viral genome with a sequential 'stop–start' mechanism. It accesses it through an entry site located near its 3′ end, transcribes the first gene (N) with high processivity, polyadenylates the N mRNA, and reinitiates P mRNA synthesis. The frequency of reinitiation is less than 100%, resulting in a gradient of transcript levels; N is transcribed at the highest levels, the most promoter-distal L gene (coding for a catalytic enzyme) at the lowest. The gene order and transcription strategy are fundamental characteristics that MV shares with all other paramyxoviruses.

Envelope Proteins and Cellular Receptors

The M protein, coded by the third gene, is a somewhat hydrophobic protein visualized as an electron-dense layer underlying the lipid bilayer. M is not an intrinsic membrane protein but can associate with membranes. It also binds RNPs, associates with the F and H protein cytoplasmic tails and modulates cell fusion. Thus, it is considered the assembly organizer and may also drive virus release/budding.

The two spike glycoproteins F and H are primarily responsible for membrane fusion and receptor attachment,

respectively. An interesting peculiarity of the F gene is the long (almost 500 nt) 5′ untranslated region, whose function has not yet been characterized. The trimeric F protein, which is cleaved and activated by the ubiquitous intracellular protease furin, executes membrane fusion. This process is necessary for MV not only to enter cells, but also to spread through cell–cell fusion forming giant cells (syncytia). Membrane fusion activity depends not only on F protein proteolytic activation, but also on receptor recognition by the cognate H protein, which transmits a signal to F eliciting a conformational change and membrane fusion.

Since the MV H protein can hemagglutinate certain nonhuman primate red cells but lacks neuraminidase activity, it is named H and not HN. Other *Paramyxoviridae* use sialic acid as receptor and need neuraminidase to destroy receptor activity while budding from a host cell. Morbilliviruses do not use sialic acid as a receptor and do not need neuraminidase.

Two MV receptors have been characterized: Wild-type and vaccine MV target SLAM, whose expression is limited to immune cells. This protein was originally identified on activated B and T lymphocytes, but it is also expressed constitutively on immature thymocytes, memory T cells, and certain B cells. Other cell types including monocytes and dendritic cells (DCs) express SLAM only following activation. This cellular distribution overlaps with the sensitivity of different cell types to wild-type MV infection. Another strong argument for the central role of SLAM in MV tropism is the fact that three morbilliviruses (MV, canine distemper virus, and rinderpest virus) enter cells through SLAM (human, canine, or bovine, respectively).

The live attenuated MV vaccine strain Edmonston can also use the regulator of complement activation membrane cofactor protein (MCP; CD46) as a receptor. CD46's primary function is to bind and promote inactivation of the C3b and C4b complement products, a process protecting human cells from lysis by autologous complement, a function that requires ubiquitous expression. The question of the relevance of an ubiquitous receptor for the pathogenesis of a lymphotropic virus was raised when it was shown that the H protein of the attenuated Edmonston strain interacts much more efficiently with CD46 than the proteins of certain wild-type MV strains, and then again when the MV receptor function of human SLAM was discovered. The relevance of CD46 has been discussed in the context of both MV virulence and attenuation.

Clinical Features

Measles, one of the most contagious infectious diseases of man, was recognized clinically by the rash and other signs from early historical times and it was differentiated from small pox by Rhazes in the tenth century, who noted that it could be more fatal than small pox. Transmitted by aerosol droplets, the infection has an incubation period of 7–10 days, with onset of fever, cough, and coryza, followed in about 4 days by the skin rash which begins on the face and spreads to the whole body. The skin rash fades after about 5 days and clinical resolution is usually uneventful. Measles is typically an infection of childhood and protective immunity is lifelong, such that a second case of measles in a child or adult would be highly unusual. Prior to widespread vaccination against measles in the 1960s, the infection had a case–fatality rate of less than 5% in children, higher in infants and in children in developing countries, where fatality rates of up to 20% can still occur. Complications include pneumonia, encephalitis, otitis media, blindness, and secondary infections by common bacteria and viruses. In developing countries, common complications are diarrheal illness and wasting. 'Atypical measles', a severe MV infection that occurred following the use of whole, inactivated virus vaccine from 1963 to 1967, is characterized by high and persistent fever, a different body rash that resembles Rocky Mountain spotted fever, and lobar pneumonia with effusions. This unusual clinical manifestation is discussed in the section titled 'Pathology and histopathology'.

Compared to infections by other viruses of the *Paramyxoviridae*, measles has more serious clinical potential than infection by RSV or the parainfluenza viruses, possibly because the latter infections are usually confined to the respiratory tract and not systemic; but measles in man causes less disease and death than infections due to the most closely related morbilliviruses, rinderpest in cattle, or canine distemper in dogs.

Latent tuberculosis may be reactivated following a case of measles. A regular complication of measles virus-induced immunosuppression was initially studied by von Pirquet, who observed, in 1908, that the tuberculin skin test response is suppressed during measles. MV-induced immunosuppression has an onset near to the peak of viremia, such that the primary immune responses to MV are not impaired, but the immunosuppression is global and lasts for several months. Several distinct mechanisms of virus–cell interaction have been elucidated to account for impaired functions of lymphocytes and antigen presenting cells by MV *in vitro*, but the global and sustained nature of immunosuppression *in vivo* is not understood. Infections by other morbilliviruses are also associated with immunosuppression and this complication was a hallmark of epidemic disease in harbor seals that resulted in the discovery of a new morbillivirus, the phocine distemper virus. In the extreme case, immunosuppression with massive lymphocyte depletion is fatal in canine distemper virus-infected ferrets.

Immune Response and Complications

The primary immune responses to MV, initially IgM antibody, type 1 CD4 and CD8+ T-cell responses, followed by neutralizing IgG antibody, are completely effective in controlling viral replication and resolution of the infectious process. Both primary and secondary immunodeficiencies that impair T-cell responses, for example, the DiGeorge syndrome or advanced HIV infection, are a significant risk for failure of the host to control MV infection, resulting in persistent infection and death or serious disease in the lower respiratory tract and central nervous system. In contrast, deficiency of the antibody response does not impair the immune control of MV replication.

These fundamental insights about antiviral immunity, based upon the tragic consequences of immunodeficiency diseases or childhood leukemia, were confirmed by the experimental infection of rhesus monkeys with MV. CD8+ T-cell depletion of monkeys at the time of viral inoculation resulted in prolonged viremia until the T cells repopulated, but depletion of B cells had no significant effect on MV infection. Persistent and/or fatal MV infection has occurred in HIV-infected children who contracted measles and this happened in a young, HIV-infected man who was vaccinated with live, attenuated MV. However, the risk of serious disease with measles in the immunocompromised host (50% or greater case fatality) outweighs the risk of vaccination, and measles vaccination is recommended for HIV-infected infants and children unless they have severe immunodeficiency with low, age-adjusted CD4+ T-cell counts in blood.

Persistent MV infection can result in giant cell pneumonia or two neurological diseases: measles inclusion body encephalitis and subacute sclerosing panencephalitis (SSPE). Another rare neurologic complication, acute demyelinating encephalomyelitis, not due to continuing viral replication in the brain, is associated with autoimmunity to myelin. Several other diseases, including multiple sclerosis, inflammatory bowel disease, and autism, have been linked to MV infection, either anecdotally or by inference or deduction, but causal connections to MV infection or vaccination have never been established.

Pathology and Histopathology

The histopathological hallmark of MV infection is the formation of syncytia, or multinucleated giant cells. Mediated by the viral fusion protein, syncytial cells are not unique to MV infection but they are characteristic. MV infects cells of ectodermal, endodermal, and mesenchymal origin and synctial cells have been observed in all of these cell types. Multinucleated giant cells are readily observed in the organized lymphoid organs and in mononuclear cell aggregates in many tissues, including the inflamed lung, where they are referred to as Warthin–Finkeldy giant cells. These syncytia are of lymphocyte, macrophage, dendritic, or reticular cell origin. Syncytial cells are also readily observed in many epithelia, including the columnar epithelieum of the trachea and bronchi, the stratified squamous epithelium of the skin and buccal mucosa, and the transitional epithelium of the urinary bladder and urethra. Endothelial syncytial cells were observed in small pulmonary arteries of monkeys infected with MV. Eosinophilic cytoplasmic and nuclear inclusion bodies can be seen in measles giant cells. The syncytial cells of measles are not long lived and they disappear with resolution of the infection.

The major pathologic changes of measles are due to inflammation and necrosis followed by tissue repair without fibrosis. Secondary infections by bacterial or other viral agents are common and they alter the pathologic process accordingly, especially in the respiratory and gastrointestinal tracts. Pathology in the lower respiratory tract is mainly peribronchiolar inflammation and necrosis with a mild exudate, but interstitial pneumonitis with mononuclear cell infiltrates may occur. In the brain and central nervous system, perivascular mononuclear infiltrates can occur with a few necrotic endothelial cells, microglia, and neurons. In lymph nodes, spleen, and thymus, the major changes are mild to moderate lymphocyte depletion and multinucleated giant cells. Lymph node and splenic follicular hyperplasia are not seen in primary measles but are present if the host was previously infected or vaccinated against measles.

The unexpected occurrence of atypical measles following vaccination with whole-inactivated virus and subsequent infection with live, attenuated vaccine virus or wild-type virus was thought to be due to an aberrant, anamnestic host immune response resulting in an Arthus reaction or delayed hypersensitivity. Similar immunopathology was seen in children exposed to RSV following vaccination with whole-inactivated RSV. Atypical measles was experimentally induced in monkeys vaccinated with whole-inactivated MV and then challenged with wild type virus. Compared to monkeys vaccinated with live, attenuated MV, the aberrant immune responses associated with atypical measles resulted in immune complex deposition and eosinophilia. A marked skewing of T-cell cytokine responses toward type 2, with abnormal interleukin 4 production was also observed.

Diagnosis, Prevention and Control

As measles becomes a rare illness in regions with high vaccine coverage where the virus has been eliminated or controlled, the diagnosis of MV infection will be a challenge. The typical punctate, maculopapular skin rash, and the prodromal signs and symptoms are not specific for this viral infection and measles diagnosis requires differentia-

tion from other pathogens causing exanthems of children, including infectious mononucleosis (Epstein–Barr virus) and cytomegalovirus infections, rubella, scarlet fever, typhus, toxoplasmosis, meningococcus, and staphylococcus. An enanthem with small white lesions on the buccal, labial, or gingival mucosal (Koplik's spots) is considered specific for MV infection and it typically occurs a few days before the skin rash. A buccal swab smeared on a glass slide, fixed and stained (e.g., Wright–Giemsa), may show multinucleated syncytia of epithelial cells. Secondary infections due to MV-induced immunosuppression are caused by the common pathogens in a geographic region. Detection of the early IgM response in blood by antibody-capture ELISA is diagnostic. MV can be cultured from peripheral blood mononuclear cells and from oral or nasal aspirates. Rescue of wild-type MV is readily done using B95a cells or Vero cells transfected with human SLAM. The virus can be detected by reverse transcription polymerase chain reaction (RT-PCR) from these samples and from urine. Filter paper spot assays of blood, buccal, or nasopharyngeal swabs have recently been developed for the detection of viral antibodies or viral RNA. The World Health Organization (WHO) hosts a website with descriptions of measles diagnostic samples and assays.

Measles remains the leading cause of vaccine-preventable illness and death in the world. The WHO and other international bodies have proposed the global eradication of MV in the twenty-first century, following the current campaign to eradicate poliovirus. This is an achievable goal as humans are the only known host species for this virus, although nonhuman primates are susceptible hosts. Regional elimination of MV has been achieved. Endogenous, circulating MV has been eliminated from the Americas in the last 20 years by the vaccination of children with the live, attenuated vaccines in current usage.

Many countries are planning to increase vaccination coverage as they progress toward targeted reductions in measles mortality or elimination of transmission. Recent experience in both developed and developing countries has shown that the maintenance of effective herd immunity against MV requires a two-dose vaccination strategy. Because of the highly contagious nature of this infection, about 95% of susceptibles will require vaccination to prevent the circulation of MV in a population of more than several hundred thousand. Greater than 90% measles vaccine coverage has been achieved in most countries but more than 25% of countries have not achieved this level of vaccination (**Figure 3**). The HIV pandemic

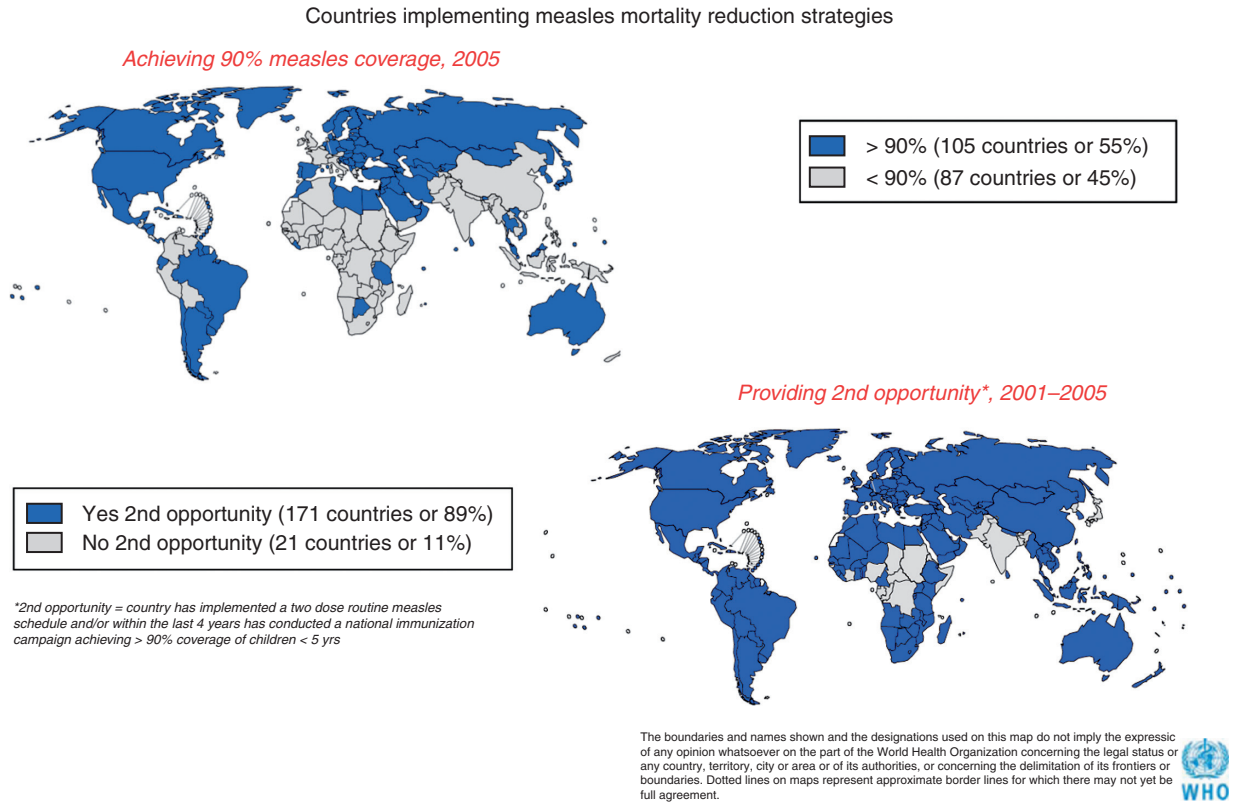


Figure 3 Global MV vaccine coverage, 2005. Reproduced from World Health Organization (2005) *Progress Towards Global Immunization Goals – 2005: Summary presentation of key indicators*, with permission.

presents a potential obstacle to MV eradication globally, especially in Africa and Asia, but HIV-infected children that are not immunocompromised can be safely vaccinated with live, attenuated MV (see above). The importation of MV into countries where elimination has been achieved is a very real challenge for diagnosis and control which has been successfully met by molecular genetic methods of rapid viral genomic sequencing and taxonomy.

The New Frontiers: MV-Based Multivalent Vaccines and Oncolysis

The live attenuated measles vaccine, which has an outstanding efficacy and safety record, is being developed as pediatric vaccine eliciting immunity against additional pathogens. An infectious cDNA with the identical coding capacity of a vaccine strain but with regularly spaced restriction sites has been engineered, and vectored MV expressing the hepatitis B surface antigen (HBsAg) generated by reverse genetics. One of these vectored MV vaccines induced protective levels of HBsAg antibodies while protecting Rhesus macaques against measles challenge. Another vectored MV expressing the West Nile virus envelope protein protected mice from West Nile virus encephalitis. A distinctive advantage of immunization with a di- or multivalent MV-based vaccine is delivery of an additional immunization safely without additional cost.

The knowledge gained from basic research has also been applied to the development of vectors for targeting and eliminating cancer cells. Oncolytic virotherapy is the experimental treatment of cancer patients based on the administration of replication-competent viruses that selectively destroy tumor cells but leave healthy tissue unaffected. MV is one of several human vaccine strains, or apathogenic animal viruses, currently being genetically modified to improve oncolytic specificity and efficacy. These modifications include targeting cell entry through designated receptors expressed on cancer cells like CD20 (lymphoma), CD38 (myeloma), or carcinoembryonic antigen (colon cancer).

Moreover, cancer cell specificity can be enhanced by silencing the expression of proteins that counteract the interferon system, often inactivated in cancer cells. Finally, proteolytic activation of the F protein has been engineered for exclusive cleavage through matrix metalloproteinases, enzymes degrading the extracellular matrix and promoting cancer invasiveness. Clinical trials of MV-based oncolysis for ovarian cancer, myeloma, and glioma are ongoing. These trials will soon profit from second generation targeted oncolytic MV with enhanced cancer specificity.

See also: Paramyxoviruses of Animals; Parainfluenza Viruses of Humans; Mumps Virus; Paramyxoviruses.

Further Reading

Cathomen T, Mrkic B, Spehner D, *et al.* (1998) A matrix-less measles virus is infectious and elicits extensive cell fusion: consequences for propagation in the brain. *EMBO Journal* 17: 3899–3908.

Cattaneo R (2004) Four viruses, two bacteria, and one receptor: Membrane cofactor protein (CD46) as pathogens' magnet. *Journal of Virology* 78: 4385–4388.

Griffin DE (2007) Measles virus. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., vol. 2, pp. 1551–1586. Philadelphia, PA: Lippincott Williams and Wilkins.

Katz SL (2004) Measles (rubeola). In: Gershon AA, Hotez PJ, and Katz SL (eds.) *Krugman's Infectious Diseases of Children*, 11th edn., pp. 353–372. Philadelphia: Mosby.

Lamb RA and Parks GD (2007) *Paramyxoviridae*: The viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., vol. 1, pp. 1449–1496. Philadelphia, PA: Lippincott Williams and Wilkins.

McChesney MB, Miller CJ, Rota PA, *et al.* (1997) Experimental measles. Part I. Pathogenesis in the normal and the immunized host. *Virology* 233: 74–84.

Moss WJ and Griffin DE (2006) Global measles elimination. *Nature Reviews Microbiology* 4: 900–908.

Panum P (1939) Observations made during the epidemic of measles on the Faroe Islands in the year 1846. *Medical Classics* 3: 829–886.

Rager M, Vongpunsawad S, Duprex WP, and Cattaneo R (2002) Polyploid measles virus with hexameric genome length. *EMBO Journal* 21: 2364–2372.

Rima BK and Duprex WP (2006) Morbilliviruses and human disease. *Journal of Pathology* 208: 199–214.

Springfeld C and Cattaneo R (in press) Oncolytic virotherapy. In: Schwab M (ed.) *Encyclopedia of Cancer*, 2nd edn. Heidelberg: Springer.

von Messling V and Cattaneo R (2004) Toward novel vaccines and therapies based on negative-strand RNA viruses. *Current Topics in Microbiology and Immunology* 283: 281–312.

von Messling V, Svitek N, and Cattaneo R (2006) Receptor (SLAM [CD150]) recognition and the V protein sustain swift lymphocyte-based invasion of mucosal tissue and lymphatic organs by a morbillivirus. *Journal of Virology* 80: 6084–6092.

von Pirquet C (1908) Das Verhalten der kutanen Tuberkulinreaktion während der Masern. *Deutsche medizinische Wochenschrift* 34: 1297–1300.

World Health Organization (2005) *Progress Towards Global Immunization Goals – 2005: Summary Presentation of Key Indicators.* http://www.who.int/immunization_monitoring/data/SlidesGlobalImmunization.pdf.

World Health Organization (2007) *Manual for the Laboratory diagnosis of Measles and Rubella virus Infections*, 2nd edn., http://www.who.int/immunization_monitoring/LabManualFinal.pdf (accessed December 2007).

Yanagi Y, Takeda M, and Ohno S (2006) Measles virus: Cellular receptors, tropism and pathogenesis. *Journal of General Virology* 87: 2767–2779.

Relevant Website

http://www.ncbi.nlm.nih.gov – National Center for Biotechnological Information.

Membrane Fusion

A Hinz and W Weissenhorn, UMR 5233 UJF-EMBL-CNRS, Grenoble, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Fusion pore Small opening at the site of two merged lipid bilayers, which allows the exchange of fluids. Fusion pores expand gradually to complete membrane fusion.
Hemifusion Membrane fusion intermediate state with the two proximal leaflets of two opposed bilayers merged to one.
Lipid raft Small membrane microdomain enriched in cholesterol and glycosphingolipids. These domains are resistant to solubilization by Triton X-100.
Type 1 TM protein A glycoprotein composed of an N-terminal external domain and a single transmembrane region followed by a cytoplasmic domain.

Introduction

Enveloped viruses contain a lipid bilayer that serves as an anchor for viral glycoproteins and protects the nucleocapsid containing the genetic information from the environment. The lipid bilayer is derived from host cell membranes during the process of virus assembly and budding. Consequently, infection of host cells requires that enveloped viruses fuse their membrane with cellular membranes to release the nucleocapsid and accessory proteins into the host cell in order to establish a new infectious cycle. Glycoproteins from enveloped viruses evolved to combine two main features. Firstly, they contain a receptor-binding function, which attaches the virus to the host cell. Secondly, they include a fusion protein function that can be activated to mediate fusion of viral and cellular membranes. Both tasks can be encoded by a single glycoprotein or by separate glycoproteins, which act in concert.

Three different classes of viral fusion proteins have been identified to date based on common structural motifs. These include class I fusion proteins, characterized by trimers of hairpins containing a central alpha-helical coiled-coil structure, class II fusion proteins, characterized by trimers of hairpins composed of beta structures, and class III proteins, forming trimers of hairpins by combining structural elements of both class I and class II fusion proteins (**Table 1**).

Viral glycoproteins interact with distinct cellular receptors by initiating conformational changes in the fusion protein leading to membrane fusion. Fusion occurs either at the plasma membrane, where receptor binding triggers conformational changes in the glycoprotein, or in endosomes upon virus uptake by endocytosis. In the latter case the low pH environment of the endosome leads to protonation (key histidine residues have been specifically implicated in the process), which induces conformational changes that lead to fusion of viral and cellular membranes.

The biophysics of membrane fusion is dominated by the stalk hypothesis. According to this view, fusion of two lipid bilayers in an aqueous environment requires that they come into close contact associated with a significant energy barrier. This process involves local membrane bending creating a first site of contact. Complete dehydration of the initial contact site induces monolayer rupture that allows mixing of lipids from the two outer leaflets, resulting in a hemifusion stalk. In a next step, the model predicts that radial expansion of the stalk leads to either direct fusion pore opening or to the formation of another intermediate, the hemifusion diaphragm, an extended bilayer connecting both membranes. The hemifusion diaphragm may also expand into a fusion pore. Fusion pore formation, which is characterized by an initial opening and closing ('flickering') of the pore may be mediated by several factors such as lateral tension in the hemifusion stalk or bilayer and the curvature at the edges of the hemifusion state. Finally the fusion pore extends laterally until both membranes form a new extended lipid bilayer (**Figure 1**).

The applicability of the stalk model to viral membrane fusion processes is supported by a number of observations. Labeling techniques allow to distinguish between merging of lipid bilayers and content mixing thus visualizing intermediate steps in membrane fusion. This has been applied to several liposome fusion systems demonstrating that membrane fusion steps can be arrested at different stages. Furthermore, certain lipids such as inverted cone-shaped lysophospholipids induce spontaneous positive bilayer curvature and inhibit hemifusion, while cone-shaped phosphatidylethanolamines induce negative curvature and promote hemifusion. In contrast, the lipid effect on the opening of the fusion pore is the opposite. Finally, electron microscopy images of influenza virus particles fused with liposomes reveal structures resembling stalk intermediates.

These observations are consistent with the hypothesis that viral fusion proteins generate initial contacts between two opposing membranes and their extensive refolding

Table 1 Crystal structures of viral fusion proteins

Virus family	*Virus species*	*PDB code*
Class I		
Orthomyxoviridae	*Influenza A virus HA*	1HA0, 3HMG, *1HTM, 1QU1*
	Influenza C virus HEF	1FLC
Paramyxoviridae	*Simian parainfluenza virus 5* F	2B9B,*1SVF*
	Human Parainfluenza virus F	1ZTM
	Newcastle disease virus F	*1G5G*
	Respiratory syncytial F	*1G2C*
Filoviridae	*Ebola virus* gp2	*1EBO, 2EBO*
Retroviridae	*Moloney Murine leukemia virus* TM	*1AOL*
	Human immunodeficiency virus 1 gp41	*1ENV, 1AIK*
	Simian immunodeficiency virus gp41	*2SIV, 2EZO*
	Human T cell leukemia virus 1 gp21	*1MG1*
	Human syncytin-2 TM	*1Y4M*
	Visna virus TM	1JEK
Coronaviridae	*Mouse hepatitis virus* S2	*1WDG*
	Sars corona virus E2	*2BEQ, 1WYY*
Class II		
Flaviviridae	*Tick-borne encephalitis virus* E	*1URZ,* 1SVB
	Dengue 2, and 3 virus E	*1OK8 IUZG, 10AN, 1TG8*
Togaviridae	*Semliki forest virus* E1	1E9W *1RER*
Class III		
Rhabdoviridae	*Vesicular stomatitis virus G*	2GUM
Herpesviridae	*Herpes simplex virus* gB	*2CMZ*

regulates and facilitates fusion via lipidic intermediate states by lowering the energy to form stalk-like intermediate structures.

Class I Fusion Glycoproteins

Biosynthesis of Fusion Proteins

Class I fusion proteins are expressed as trimeric precursor glycoproteins that are activated by proteolytic cleavage with subtilisin-like enzymes such as furin. This produces a receptor-binding subunit that is either covalently or noncovalently attached to the membrane fusion protein subunit, which anchors the heterotrimer to the viral membrane. The endoproteolytic cleavage positions a hydrophobic fusion peptide at or close to the N-terminus of the fusion domain. Subtilisin-like proteases recognize a conserved multibasic recognition sequence R-X-K/R-R or a monobasic cleavage site present in various glycoproteins. The nature of the cleavage site and its efficient cleavage (e.g., influenza virus hemagglutinin) has been associated with pathogenicity. The multibasic recognition sequences present in influenza virus HA, *SV5* F protein, *HIV*-1 gp160, and Ebola virus GP lead to mostly intracellular processing, whereas monobasic cleavage sites in Sendai virus F protein or influenza virus HA are efficiently cleaved extracellularly, resulting in a more tissue-restricted distribution of these viruses.

Cleavage activates the fusion potential of the viral -glycoproteins and is required for most class I glycoprotein-mediated fusion events. Although some evidence suggests Ebola virus processing by furin is not required for entry, it still requires the activity of endosomal cysteine proteases for efficient entry. Proteolytic cleavage thus generates in most cases a metastable glycoprotein structure that can switch into a more stable structure upon cellular receptor interaction including proton binding in the acidic environment of endosomes. This metastability was first recognized to play an important role in influenza virus hemagglutinin-mediated entry and has since been associated with all class I glycoproteins.

Structure of Native Influenza Virus Hemagglutinin

Since the structure solution of influenza virus hemagglutinin (HA), HA has served as the prototype of a class I fusion protein. The HA_1 domain, which contains the receptor-binding domain, folds into a beta structure that binds sialic acid-containing cellular receptors at the top of the molecule. In addition, both N- and C-termini of HA_1 interact with the stem of fusion domain HA_2 in an extended conformation. HA_2 anchors hemagglutinin to the viral membrane and folds into a central triple-stranded coiled-coil structure that is followed by a loop region and an antiparallel helix, which extends towards the N-terminal fusion peptide that is buried within the trimer interface (**Figure 2**).

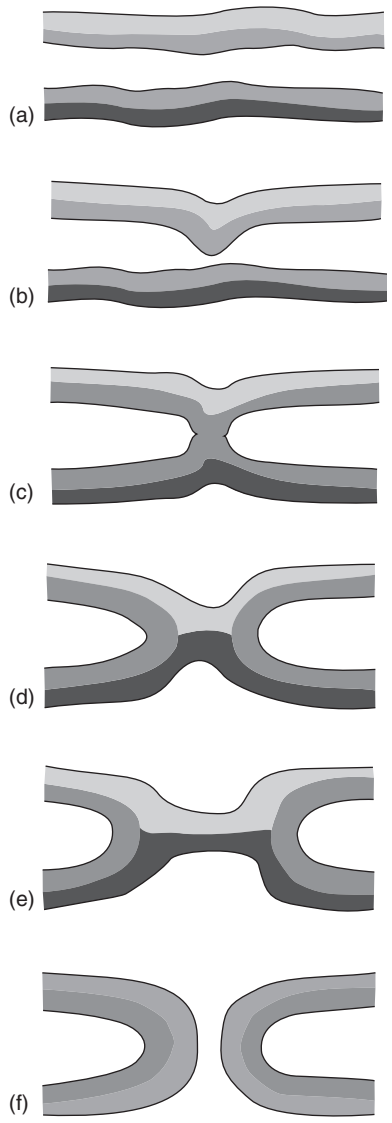


Figure 1 Fusion of two lipid bilayers. (a) Two parallel lipid bilayers do not approach closely. (b) Close contact mediated by local membrane bending. Hemifusion stalks with contact of outer leaflets (c) and inner leaflets (d). Fusion pore opening (f) may proceed directly from the stalk structure (d) or via a hemifusion diaphragm (e).

Structure of the Precursor Influenza Virus Hemagglutinin HA_0

The structure of uncleaved influenza virus HA_0 shows that only 19 residues around the cleavage site are in a conformation, which is different from the one seen in the native cleaved HA structure. This difference entails an outward projection of the last residues of HA_1 (323–328) and the N-terminal residues of HA_2 (1-12), resulting in the exposure of the proteolytic cleavage site (**Figure 2**). Upon cleavage, HA_2 residues 1–10 fill a mostly negatively charged cavity adjacent to the cleavage site, which leads to the sequestering of the fusion peptide within the trimeric structure (**Figure 2**).

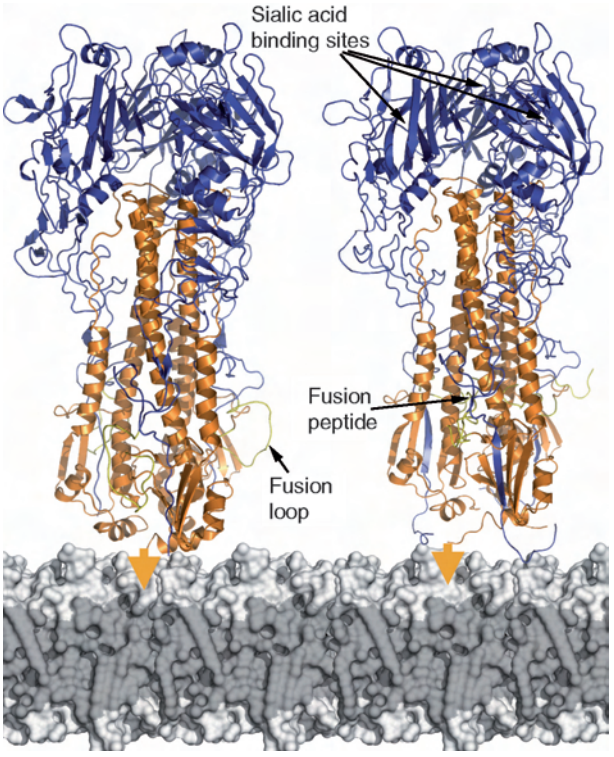


Figure 2 Ribbon diagram of trimeric conformations of influenza A virus hemagglutinin before (left, pdb code 1HA0) and after (right, pdb code 3HMG) proteolytic processing. The HA_1 receptor-binding domain is shown in blue and the positions of the sialic acid-binding sites are indicated. The fusion protein subunit HA_2 is shown in orange. The orientation towards the lipid bilayer is indicated by the orange triangle.

Structure of the Low pH-Activated Conformation of Hemagglutinin HA_2

Low pH destabilizes the HA_1 trimer contacts, which causes the globular head domains to dissociate. This movement facilitates two major conformational changes. (1) A loop region (residues 55–76) refolds into a helix (segment B in HA) and extends the central triple-stranded coiled-coil in a process that projects the fusion peptide approximately 100 Å away from its buried position in native HA. (2) Another dramatic change occurs toward the end of the central triple-stranded coiled-coil, where a short fragment unfolds to form a reverse turn which positions a short helix antiparallel against the central core. This chain reversal also repositions a β-hairpin and the extended conformation that leads to the transmembrane region (**Figure 3**). Although its orientation changes, the core structure of the receptor-binding domain HA_1 does not change upon acidification.

Since both the neutral pH structure and the core of the low pH structure from hemagglutinin have been solved, a number of class I fusion protein structures have been determined and a common picture has emerged for their mode of action (**Table 1**). The characteristic of all class I

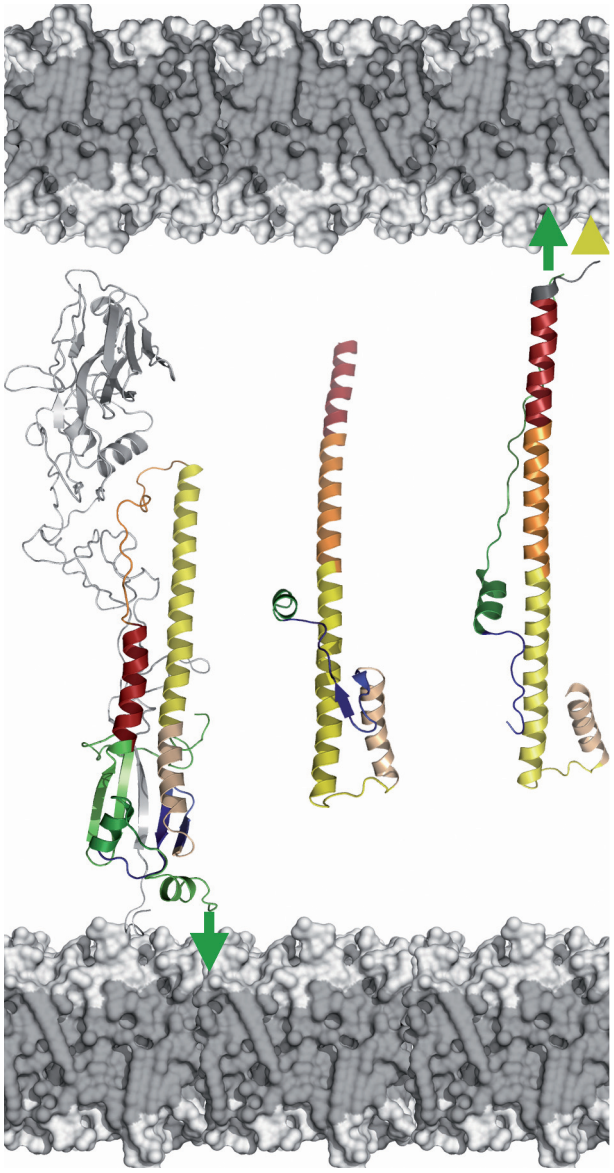


Figure 3 Ribbon representations of the conformational changes in HA_2 upon low pH exposure. Only one monomer is shown for clarity. Left panel: Native cleaved HA (pdb code 3HMG), HA_1 in gray, the secondary structure elements for HA_2 that change are shown in different colors. Middle panel: Low pH HA_2 (pdb code 1HTM) projecting the N-terminus leading to the fusion peptide towards the target cell membrane. Right panel: The C-terminal region has completely zipped up against the N-terminal coiled-coil domain (pdb code 1QU1). The membrane orientations of the TM region and the fusion peptide are indicated by green arrows and yellow triangles, respectively.

fusion protein cores is their high thermostability suggesting that they represent the lowest energy state of the fusion protein. Secondly, they all contain a central triple-stranded coiled-coil region with outer C-terminal antiparallel layers that are either mostly helical or adopt extended conformations, thus forming trimers of helical hairpins. Since they resemble the low pH form of HA, it is assumed that they all represent the postfusion conformation. Although there is only structural evidence for extensive conformational rearrangement of the fusion protein subunit in case of hemagglutinin HA and the paramyxovirus F protein (**Figure 4**), it is assumed that all known class I fusion protein core structures are the product of conformational rearrangements induced by receptor binding.

Class I-Mediated Membrane Fusion

The positioning of the N- and C-terminal ends containing the fusion peptide and the transmembrane region at the same end of a core structure, which was first established for the *HIV*-1 gp41 core structure, led to the proposal of the following general fusion model. (1) Proteolytic cleavage activation transforms the glycoprotein into a metastable conformation. (2) Receptor binding induces conformational changes in the fusion protein that exposes the fusion peptide and allows fusion peptide interactions with the target membrane (**Figures 5**(**a**) and **5**(**b**)). This generates a prehairpin intermediate structure that can be targeted by fusion inhibitors such as the HIV-1-specific T-20 peptide (**Figure 5**(**b**)). (3) Extensive refolding of the fusion domain most likely requires the dissociation of the C-terminal regions (**Figures 5**(**b**) and **5**(**c**), indicated by blue lines) and leads to the apposition of the two membranes, concomitantly with the zipping up of the C-terminal region against the N-terminal coiled-coil domain ultimately forming the hairpin structure (**Figures 5**(**c**) and **5**(**d**)). The complete refolding process is thought to pull the two membranes into close-enough proximity to concomitantly allow membrane fusion. The extensive rearrangement of the fusion protein is thought to control the formation of different intermediate bilayer structures such as the hemifusion stalk (**Figure 5**(**d**)), and or the hemifusion diaphragm, followed by fusion pore opening and expansion (**Figure 5**(**e**)). It is generally assumed that membrane fusion occurs while the helical hairpin structure is formed and the core fusion protein structures represent postfusion conformations. Refolding of the fusion protein might produce defined stable intermediate structures, as suggested by the two low pH structures of influenza virus HA_2. One indicates that most of the outer layer has not yet zipped up to form the hairpin structure (**Figure 3**, middle panel: the C-terminal ends could extend back to the transmembrane region), while the other one reveals the extended conformation of the outer layer which forms together with the N-terminal coiled-coil a stable N-capped structure (**Figure 3**, right panel). Stepwise refolding may thus lock the fusion process irreversibly at distinct steps in agreement with a general irreversibility of class I-mediated fusion processes. The two membrane anchors, which are not present in the fusion conformation structures, also play an active

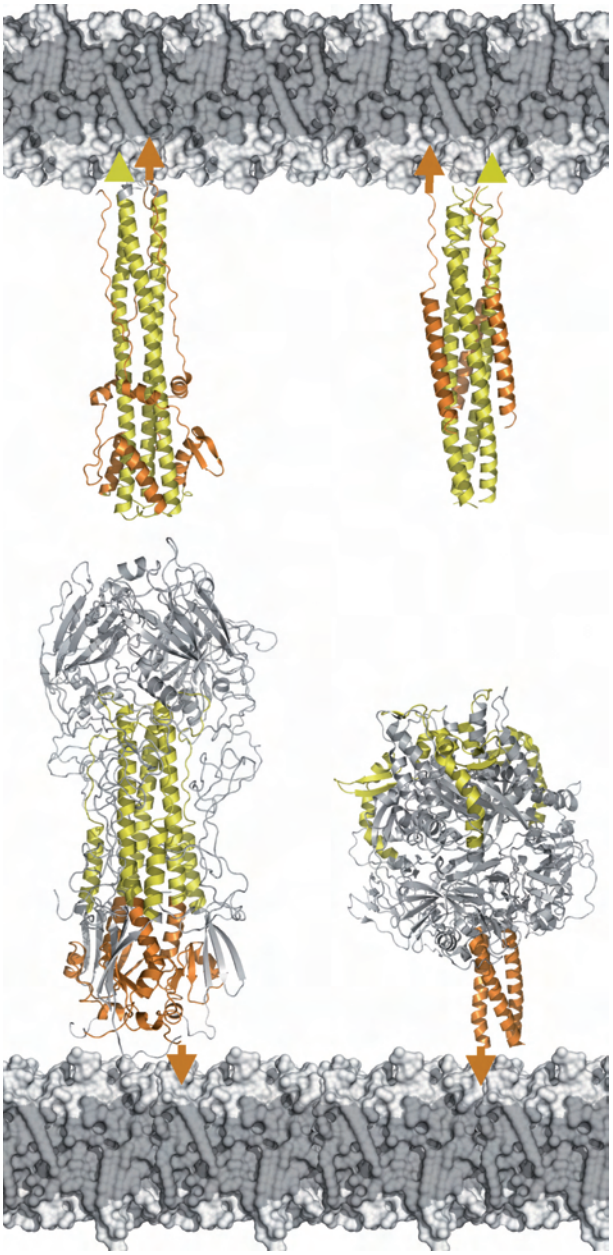


Figure 4 Comparison of the conformational changes induced upon receptor binding of two class I fusion proteins, influenza A virus hemagglutinin (left panel, pdb codes 3HMG and 1QU1) and paramyxovirus F protein (right panel, Simian parainfluenza virus 5 F, pdb codes 2B9B and 1SVF). The lower panel shows ribbon diagrams of native HA and F with secondary structure elements that change conformation upon activation highlighted in two colors (the inner triple-stranded coiled-coil region of the postfusion conformation in yellow and the C-terminal layers in orange). Although both native structures differ quite substantially, the conformational changes result in similar hairpin structures (upper panel) orienting both membrane anchors toward the target cell membrane. The membrane orientations of the TM region and the fusion peptide are indicated by orange arrows and yellow triangles, respectively.

role in the fusion reaction. Replacement of the transmembrane region by a glycophosphatidylinositol anchor leads to a hemifusion phenotype in case of hemagglutinin-driven membrane fusion, highlighting the role of the transmembrane region. Furthermore the C-terminal membrane proximal region plays an important role in fusion as shown in the case of *HIV*-1 gp41-mediated fusion.

Fusion Peptide

Class I fusion peptide sequences vary between virus families and are usually characterized by their hydrophobicity and a general preference for the presence of glycine residues. Although most fusion peptides of class I fusion proteins locate to the very N-terminus of the fusion protein, a few are found within internal disulfide-linked loops (e.g., filovirus Gp2 and the Avian sarcoma virus fusion proteins). NMR studies on the isolated influenza virus hemagglutinin fusion peptide revealed a kinked helical arrangement, which was suggested to insert into one lipid bilayer leaflet. This mode of bilayer interaction was proposed not only to mediate membrane attachment but also to destabilize the lipid bilayer. A further important function of fusion peptide sequences might be their specific oligomerization at the membrane contact site, which might constitute sites of initial membrane curvature.

Cooperativity of Fusion Proteins

A number of studies suggest that more than one class I fusion protein trimer is required to promote class I-driven membrane fusion. It has been suggested that activated hemagglutinin glycoproteins interact with each other in a synchronized manner and cooperativity of refolding allows the synchronized release of free energy required for the fusion process. This implies that activated fusion proteins assemble into a protein coat-like structure that helps to induce membrane curvature, possibly also by inserting the fusion peptides into the viral membrane outside of the direct virus–cell contact site. However, it should be noted that no clearly ordered arrays of activated class I glycoproteins have yet been observed experimentally.

Role of Lipids in Fusion

The lipid content of a viral envelope such as that of *HIV*-1 was shown to contain mostly lipids normally present in lipid raft microdomains at the plasma membrane. Lipid rafts are small ordered lipid domains that are enriched in cholesterol, sphingomyelin, and glycosphingolipids. A number of enveloped viruses (e.g., influenza virus, HIV-1, Ebola virus, measles virus) use these platforms for assembly and budding, and some evidence suggest

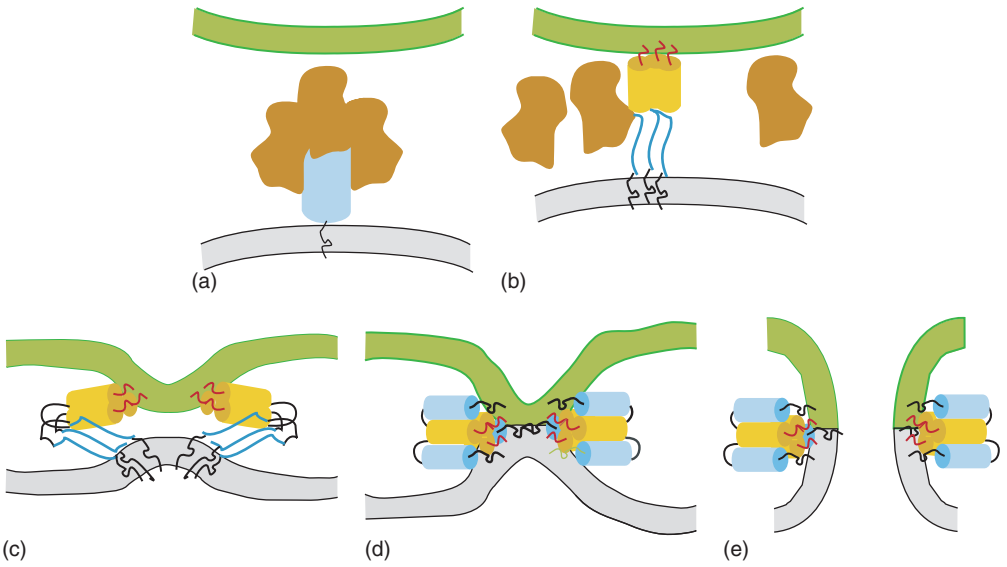


Figure 5 Model for class I glycoprotein-mediated membrane fusion. See text for explanation. The receptor-binding domains are indicated in brown and the fusion protein as cylinders. Note that some fusion proteins such as F from paramyxoviruses associate with an attachment protein (HN, H, or G). The latter interacts with F and cellular receptors triggering F-mediated fusion at the plasma membrane.

that raft platforms are also required for virus entry. Since the fusion activity of viral glycoproteins such as *HIV*-1 Env is affected by cellular receptor density as well as Env glycoprotein density, it has been suggested that both ligands have to be clustered efficiently to cooperatively trigger productive Env-mediated membrane fusion. This observation is consistent with the sensitivity of *HIV*-1 entry to cholesterol depletion.

Class II Fusion Proteins

Biosynthesis of Fusion Proteins

Class II fusion proteins comprise the fusion proteins from positive-strand RNA viruses such as the *Togaviridae* family, genus *Alphaviruses* (e.g., *Semliki Forest virus* (SFV)), and the *Flaviviridae* (e.g., *Dengue*, *Yellow fever*, *and Tick-borne encephalitis* virus (TBE)) (**Table 1**). Flaviviruses express the glycoprotein E that associates with a second precursor glycoprotein prM, while alphaviruses express two glycoproteins, the fusion protein El and the receptor-binding protein E2. E1 associates with the regulatory precursor protein p62. Both E-prM and E1-p62 heterodimerization are important for folding and transport of the fusion proteins. Cleavage of the fusion protein chaperones p62 and prM by the cellular protease furin in the secretory pathway is a crucial step in the activation of E and E1 fusion proteins.

Structure of the Native Fusion Protein

The native conformations of the flavivirus E glycoproteins and that of the alphavirus E1 glycoproteins are similar and fold into three domains primarily composed of β-sheets, with a central domain I, flanked by domain III connecting to the transmembrane region on one side and domain II on the other side (**Figure 6**, lower panel). Domain II harbors the fusion loop that is stabilized by a disulfide bridge and mostly sequestered within the antiparallel flavivirus E glycoprotein homodimer. In analogy, the fusion loop might be sequestered within the *SFV* E1–E2 heterodimer. Dimeric E–E and E1–E2 interactions keep the glycoproteins in an inactive, membrane-parallel conformation that covers the viral membrane. *SFV* E1–E2 heterodimers form an icosahedral scaffold with $T=4$ symmetry. Similarly, flavivirus E homodimers completely cover the viral membrane surface. The arrangement of the class II glycoproteins is thus completely different from the appearance of class I glycoprotein spikes, which do not form a specific symmetrical protein coat. In addition to forming the outer protein shell, flavivirus E and alphavirus E2 interact with cellular receptors, which direct the virion to the endocytotic pathway.

Structure of the Activated Fusion Protein

There are only minimal changes in secondary structure during the low pH-induced rearrangement of *TBE* E and *SFV* E1. However, the conformational changes result in an approximate 35–40 Å movement of domain III and a rotation of domain II around the hinge axis connecting domains I and II. This rearrangement produces a hairpin-like structure with a similar functional architecture as class I fusion proteins (**Figure 6**, upper panel). The outside of the trimer reveals a groove that was suggested to

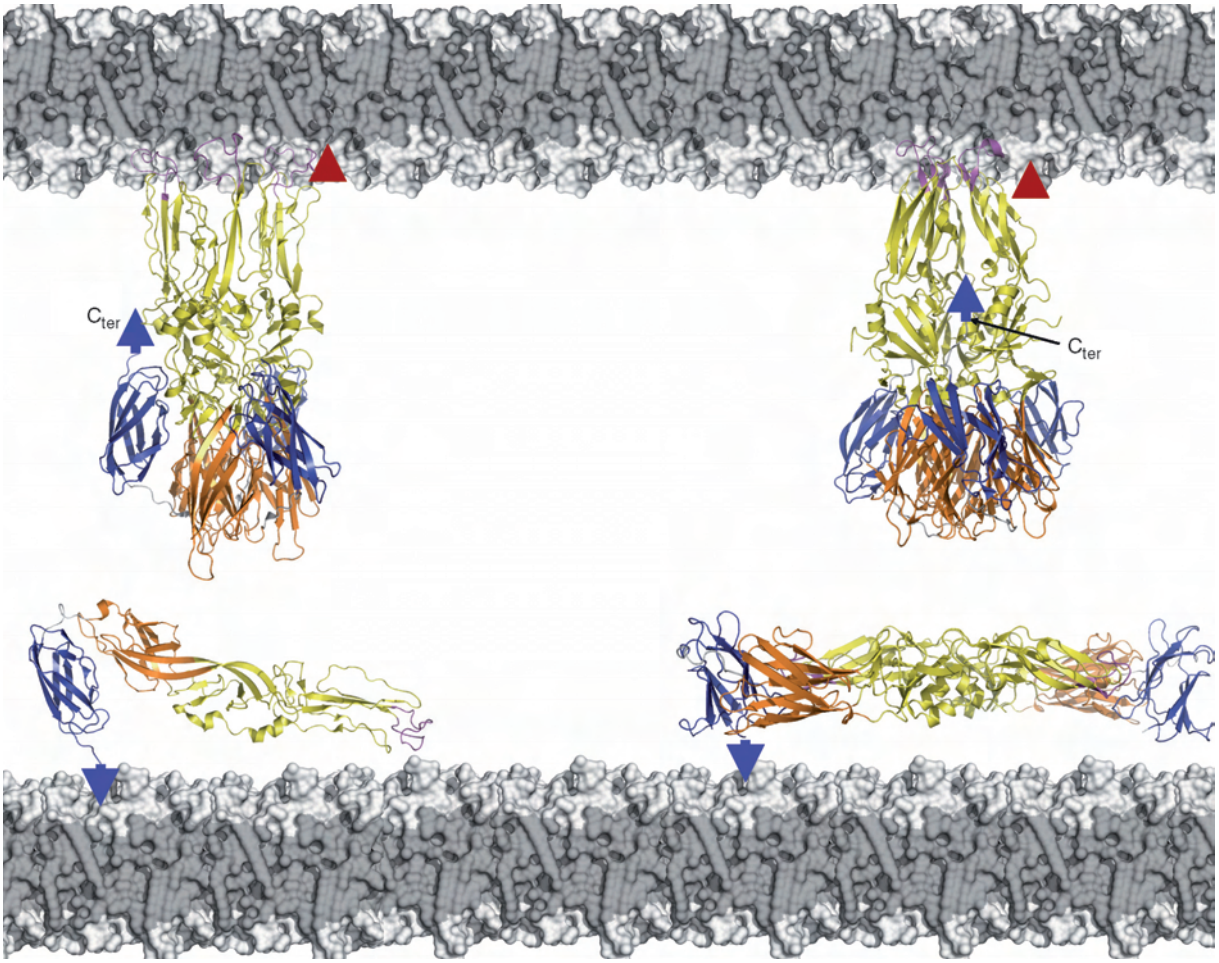


Figure 6 Ribbon diagram of the structures of *SFV* E1 (left panel, pdb codes 2ALA and 1RER) and of the *TBE* E (right panel, pdb codes 1URZ and 1SVB) in their native dimeric state (lower panel) and low-pH-activated trimeric conformation (upper panel). The three main domains of E1 and E are colored differently: domain I in blue, domain II in orange, and domain III in yellow. In both cases, activation of the conformational changes leads to trimeric hairpin structures. The membrane orientations of the TM region and the fusion peptide are indicated by blue arrows and red triangles, respectively.

accommodate the segment, which connects to the transmembrane anchor and thus positions the fusion loops next to the membrane anchors. One significant difference between the *TBE* E and *SFV* E1 low pH conformations are the orientations of the fusion loops. *TBE* E fusion loops undergo homotrimer interactions, while *SFV* E1 fusion loops do not interact within trimers (**Figure 6**).

Class II-Mediated Membrane Fusion

At the low pH of endosomes E and E1 undergo conformational rearrangements that involve three major steps. Firstly, the homo- or heterodimers dissociate from the membrane-parallel conformation in a reversible manner assuming monomeric fusion proteins that expose their fusion loop to the target membrane (**Figures 7(a)** and **7(a′)**). This seems to be a main difference between class I and class II fusion, since trimer dissociation into monomers has not been implicated in any class I fusion pathway. Secondly, fusion loop membrane interaction leads to the formation of homotrimers with an extended conformation. Trimerization is irreversible and tethers the fusion protein to the target membrane (**Figure 7(b)**). It is comparable to the postulated prehairpin structure of class I fusion proteins such as *HIV*-1 gp41 (**Figure 7(b)**). Notably, both fusion intermediates can be targeted by either fusion protein peptides (T-20 in case of *HIV*-1 gp41) or recombinant fusion protein domains (such as the E3 domain in case of *TBE*), to block membrane fusion. Further refolding, namely the reorientation of domain I, then pulls the two membranes into closer apposition that ultimately leads to the formation of a hemifusion stalk-like structure (**Figures 7(c)** and **7(d)**). Finally complete zipping up of the C-terminal ends against the N-terminal core domains allows fusion pore opening and its expansion (**Figure 7(e)**). Similar to the case of class I fusion protein-driven fusion, refolding is thought to provide the energy for fusion (**Figure 7**).

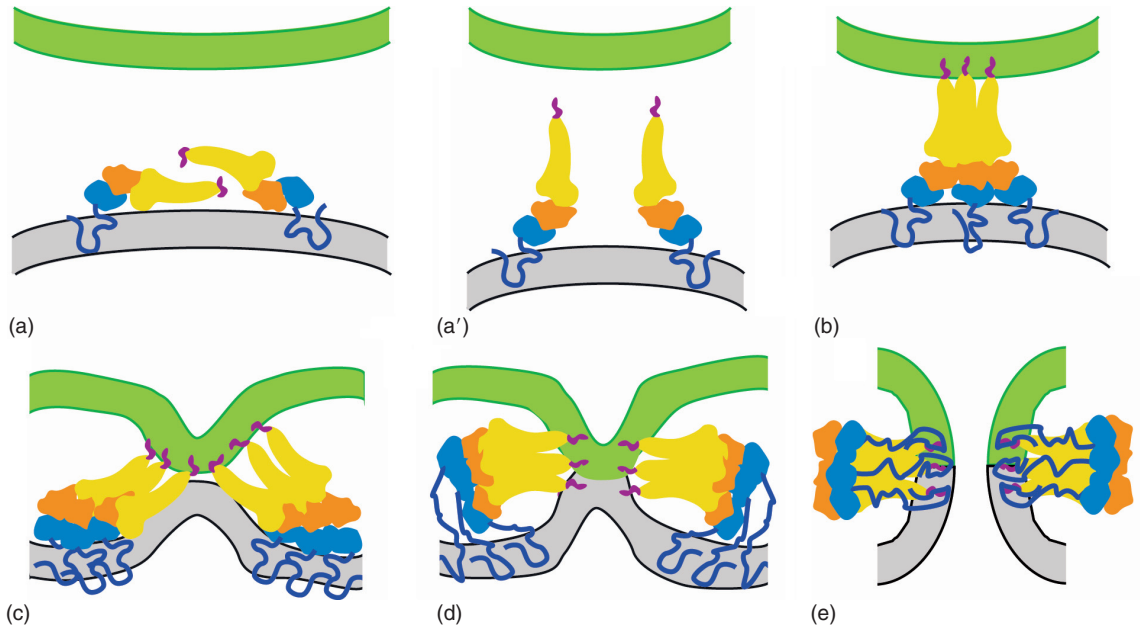


Figure 7 Model for membrane fusion of class II fusion proteins. See text for explanation. The three domains are colored as in **Figure 6**.

Fusion Peptide

The native and low-pH-induced crystal structures of the *TBE* virus E, dengue fever virus E, and *SFV* E1 proteins reveal that the conformation of the fusion loop changes upon acidification. The low-pH structures indicate that only hydrophobic side chains of the loop insert into the hydrocarbon chains of the outer leaflet of a target membrane. This is sufficient to anchor the fusion protein to the host cell membrane. Further oligomerization of fusion loops, as shown in the case of the low pH form of *SFV* E1, where crystal packing analysis revealed fusion loop interactions between trimers, was suggested to induce local membrane deformation, such as induction of a nipple-like membrane deformation (**Figure 7(c)**). This has been predicted in the stalk model to play an important role in the generation of lipidic intermediates during membrane fusion. Therefore the *SFV* E1 conformation might reflect an intermediate fusion state preceding the suggested post-fusion conformation of flavivirus E trimers with homotrimeric fusion loop interactions. *In vivo*, the latter conformation might be induced by the final refolding of the C-terminal membrane proximal region and thus determining 'open' and 'closed' conformations of *SFV* E1 trimers and *TBE virus* E trimers, respectively (**Figure 6**).

Fusion Protein Cooperativity in Membrane Fusion

Homo- or heterodimeric class II fusion proteins already form a protein shell covering the complete viral membrane in the native state. Upon activation *in vitro*, both, soluble *SFV* E1 protein and flavivirus E protein insert their fusion loops into liposomes and form arrays of trimers organized in a lattice composed of rings of five or six. The E protein lattice on liposomes contains preferentially rings of five, which seems to affect the curvature of coated liposomes. In contrast rings of six form mostly flat hexagonal arrays *in vitro*. E1 pentameric rings can also be reconstructed from the crystal packing of E1 trimers. This strongly suggests that formation of a distinct fusion protein lattice might exert a cooperative effect on the fusion process.

Role of Lipids in Fusion

Although heterodimer dissociation exposes the *SFV* E1 fusion loop, its insertion into a lipid bilayer requires low pH triggering and cholesterol, which is consistent with the observation that *SFV* fusion depends on cholesterol and sphingolipids. The lipids required for E1 activation and fusion imply indirectly that lipid raft microdomains might be targeted for fusion. Flavivirus fusion, however, seems to be less dependent on cholesterol than alphavirus fusion.

Class III Fusion Proteins

Biosynthesis of Fusion Proteins

The glycoprotein G from vesicular stomatitis virus (VSV), a member of the *Rhabdoviridae* (e.g., *VSV* and *Rabies virus*), negative-strand RNA viruses, and gB from Herpesvirus,

a member of the *Herpesviridae*, double-stranded DNA viruses, constitute a third class of viral fusion proteins based on the structural similarity of the postfusion conformation of their respective glycoproteins. Unlike class I and II envelope proteins both, *VSV* G and herpesvirus gB, are neither expressed as precursor proteins nor are they proteolytically activated.

Rhabdoviruses express a single trimeric glycoprotein G, which acts as receptor-binding domain to induce endocytosis and as the fusion protein that controls fusion with endosomal membranes upon acidification. However, different from class I and class II fusion machines the conformational changes induced by low pH are reversible. Changes in pH can easily revert the three proposed conformations of G, the native conformation as detected on virions, an activated state that is required for membrane interaction, and an inactive postfusion conformation.

Herpesvirus entry and fusion is more complex since it requires four glycoproteins, namely gD, gH, gL, and gB. Glycoprotein gD contains the receptor-binding activity and associates with gB as well as gH and gL. While gB seems to constitute the main fusion protein, the others are thought to be required for activation of the fusion potential of gB, which is pH independent.

Structure of the Low-pH-Activated VSV Glycoprotein and Herpesvirus gB

VSV G is composed of four domains that, interestingly, show similarities to both class I and class II fusion protein structures. It contains a β-sheet-rich lateral domain at the top, a central α-helical domain that mediates trimerization, and resembles the α-helical hairpin structure of class I fusion molecules, a neck domain containing a pleckstrin homologous (PH) domain, and the fusion loop domain that builds the trimeric stem of G. This stem-like domain exposes two loops at its very tip containing aromatic residues constituting the membrane-interacting motif of G. The stem domain resembles that of class II fusion proteins, albeit its different strand topology, which could be the result of convergent evolution. Although the complete C-terminus is not present in the structure, it points towards the tip of the fusion domain, indicating that both membrane anchors, the fusion loops and the transmembrane region, could be positioned at the same end of an elongated hairpin structure (**Figure 8**). The overall similarity of the structural organization of *VSV* G with that of herpesvirus gB indicates a strong evolutionary relationship between the rhabdovirus G and herpesvirus gB fusion proteins (**Figure 8**).

The Fusion Loops

The fusion loops extending from the stem-like domain of *VSV* G is similar to those observed in class II fusion proteins. The architecture is such that only few hydrophobic side chains can intercalate into one lipid bilayer leaflet, potentially up to 8.5 Å. Intercalation of side chains into one leaflet may induce curvature of the outer leaflet with respect to the inner leaflet, which would satisfy the stalk model. The role of lipidic intermediate states, including the hemifusion state, has been confirmed experimentally in case of rhabdovirus G-mediated fusion.

Since both *VSV* G and herpesvirus gB resemble class I and class II fusion proteins which adopt a hairpin conformation with both membrane anchors at the same end of the molecule, it is most likely that they follow very similar paths in membrane fusion as suggested for class I and class II fusion proteins.

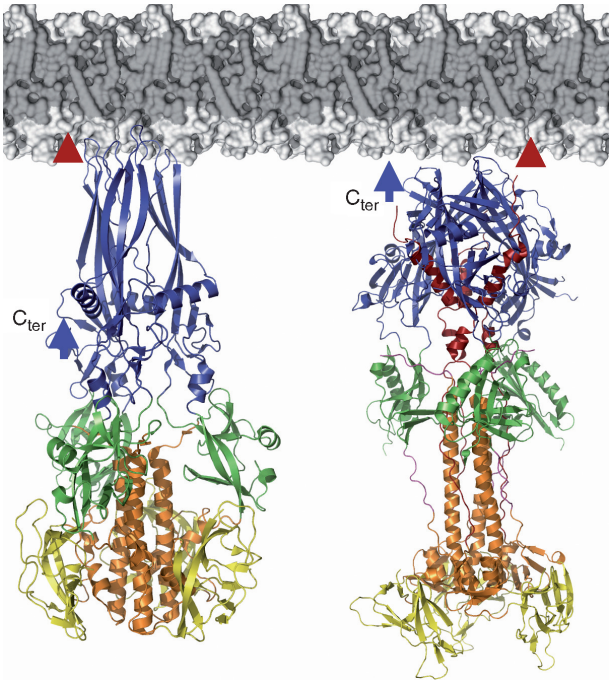


Figure 8 Ribbon diagram of class III fusion glycoproteins from *VSV* G (left panel, pdb code 2CMZ) and Herpesvirus gB (right panel, pdb code 2GUM). The individual domains are colored as follows: domain I in yellow, domain II in orange, domain III in green, and domain IV in blue. Their orientation toward the target membrane is indicated and shows the attachment of the putative fusion loops to one leaflet of the bilayer (red triangles) and the putative orientation of the TM region toward the same end of the fusion loop region (blue arrow).

Summary

Although accumulating structural evidence suggests that the structural motifs used by viral fusion proteins and the mode of their extensive refolding varies substantially, the final product, namely the generation of a hairpin-like structure with two membrane anchors at the same end

of an elongated structure, is maintained in all known postfusion conformations of viral glycoproteins. Thus the overall membrane fusion process is predicted to be the same for class I, II, and III fusion proteins, although the kinetics of refolding and fusion might vary to a large extent due to the involvement of different structural motifs to solve the problem of close apposition of two membranes.

See also: Metaviruses.

Further Reading

Chernomordik LV and Kozlov MM (2005) Membrane hemifusion: Crossing a chasm in two leaps. *Cell* 123(3): 375–382.

Earp LJ, Delos SE, Park HE, and White JM (2005) The many mechanisms of viral membrane fusion. *Current Topics in Microbiology and Immunology* 285: 25–66.

Gallo SA, Finnegan CM, Viard M, *et al.* (2003) The HIV Env-mediated fusion reaction. *Biochimica Biophysica Acta* 1614: 36–50.

Harrison SC (2005) Mechanism of membrane fusion by viral envelope proteins. *Advances in Virus Research* 64: 231–261.

Kelian M and Rey FA (2006) Virus membrane-fusion proteins: More than one way to make a hairpin. *Nature Reviews Microbiology* 4(1): 67–76.

Lamb RA, Paterson RG, and Jardetzky TS (2006) Paramyxovirus membrane fusion: Lessons from the F and HN atomic structures. *Virology* 344(1): 30–37.

Roche S and Gaudin Y (2003) Pathway of virus-induced membrane fusion studied with liposomes. *Methods in Enzymology* 372: 392–407.

Skehel JJ and Wiley DC (2000) Receptor binding and membrane fusion in virus entry: The influenza hemagglutinin. *Annual Review of Biochemistry* 69: 531–569.

Metaviruses

H L Levin, National Institutes of Health, Bethesda, MD, USA

Published by Elsevier Ltd.

Glossary

Erranti Comes from the Latin *errans*, meaning to wander.
Integration The insertion of cDNA into the genome of a host cell.
Long terminal repeats (LTRs) Sequence repeats on both ends of retroviruses and many retrotransposons that play a critical role in reverse transcription.
LTR-retrotransposon A form of transposable element that propagates by the reverse transcription of an RNA intermediate and the subsequent integration of the cDNA.
LTR-retroelements Include any genetic element with LTRs. They are retroviruses and LTR-retrotransposons regardless of whether they possess env or env-like proteins.
Meta Comes from the Greek metathesis for 'transposition'. Also to indicate uncertainty regarding whether these are true viruses.
Semoti Comes from the Latin *semotus*, meaning 'distant' or 'removed'. This refers to the large evolutionary distance between semotiviruses and members of the other two genera of the family *Metaviridae*.

Introduction

The family *Metaviridae* includes a vast number of genetic elements that populate the genomes of eukaryotes. They possess two long terminal repeats (LTRs) that flank coding sequences for the capsid protein Gag, and the enzymes protease (PR), reverse transcriptase (RT), and integrase (IN) (**Figure 1**). The assignment of elements to the *Metaviridae* versus other families with the same structure is based on phylogenetic relationships. Seven regions of RT sequence with strong homology are aligned and elements with similar sequence patterns are grouped into families such as the *Metaviridae*. In addition, elements belonging to the *Metaviridae* are distinguished from those in the family *Pseudoviridae* by the order of the coding sequences for RT and IN. While any element belonging to the *Metaviridae* contains RT sequence upstream of IN (**Figure 1**), members of the family *Pseudoviridae* encode IN before RT. Before the nomenclature for viruses and retrotransposons was standardized by the ICTV, the *Metaviridae* was named after two of its founding elements, gypsy/Ty3.

It is of great interest that many members of the *Metaviridae* possess envelope (env) proteins with similar structure to retrovirus env's, known for their role in particle release and infection (**Figure 1**). This presents the possibility that many elements first thought to be retrotransposons may actually be infectious viruses. These potential

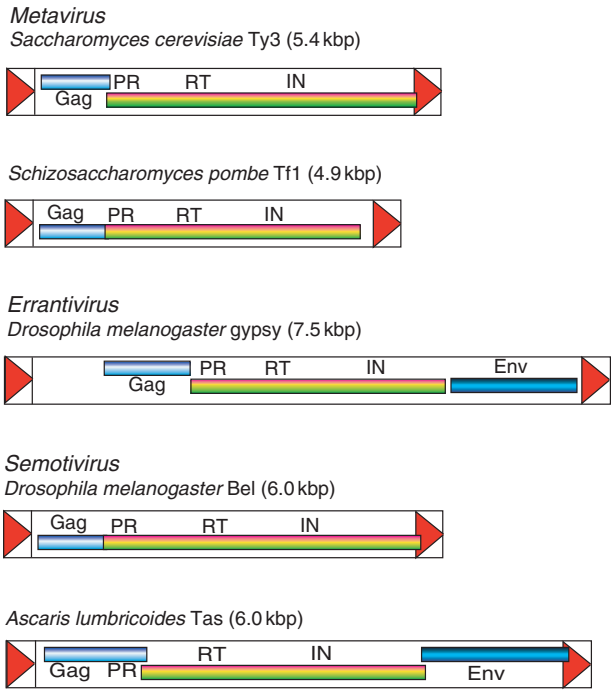


Figure 1 Genome structure of representative members of the family *Metaviridae*. The genomes in their integrated forms are shown with LTRs (red triangles) and the coding sequences for their proteins (colored rectangles). Overlap between two coding sequences indicates the position of a ribosomal frameshift.

viruses might have been classified as members of the family *Retroviridae* but their env proteins are unrelated to those of *Retroviridae* and their RT sequences firmly place them within the *Metaviridae*. Many of the env-containing elements belong to a single clade that constitutes the genus *Errantivirus*, within the family *Metaviridae*. The two other genera in the *Metaviridae*, the genera *Metavirus* and *Semotivirus* (**Figure 2**), were defined based on the separate phylogenies of their RT sequences. The semotiviruses are notable for their extensive divergence from the other genera of *Metaviridae*. LTR-retrotransposons and their env-containing relatives are collectively referred to as LTR-retroelements.

The life cycle of members of the family *Metaviridae* relies on the reverse transcription of their mRNA followed by the integration of this cDNA into the genome of the host cell (**Figure 3**). Integrated copies of the transposons are transcribed by RNA pol II and the mRNA is translated into the Gag, PR, RT, and IN proteins. Gag assembles into particles that encapsidate the RT, IN, and the transposon mRNA. The particles produced by errantiviruses, and some of the metaviruses and semotiviruses, are unique in their ability to interact with an env-like protein and bud from the plasma membrane. Once these particles have escaped they are similar to retroviruses in their ability to infect other cells. All the members of *Metaviridae* form a complex between RT and the mRNA that results in the synthesis of a full-length cDNA that is then inserted by IN into the host genome. This life cycle as described for members of the metavirus family is general and applies to the propagation of all LTR-containing transposons and viruses.

The early discovery of LTR-retrotransposons and their env-containing derivatives resulted from genetic studies of model organisms such as *Saccharomyces cerevisiae* and *Drosophila melanogaster*. *Gypsy*, Tom, 17.6, 297, Idefix, Tirant, and Zam were discovered in *D. melanogaster* and form the basis of the errantivirus lineage (**Table 1**). The retrotransposons 412, Blastopia, Mdg1, Mdg3, Micropia, and 412 were also identified in *D. melanogaster*, and these, based on the phylogeny of their RT sequences, are classified as metaviruses. The study of yeast sequences led to the discovery of other metaviruses such as Ty3 of *Sa. cerevisiae* and Tf1 of *Schizosaccharomyces pombe*. The original members of the genus *Semotivirus* were Pao of *Bombyx mori* and TAS of *Ascaris lumbricoides*. Although these contain mutations and are poorly described, other semotiviruses have been identified including BEL, an abundant and active element of *D. melanogaster*.

The completion of genome sequence projects has contributed considerably to the discovery of transposons that belong to the family *Metaviridae*. For example, the completed sequence of the *D. melanogaster* genome revealed the presence of at least 20 clades of retroelements within the family *Metaviridae*; 15 are clearly errantiviruses. Similarly, analyses of the genome sequences of *Arabidopsis thaliana* and *Caenorhabditis elegans* revealed nine and six metavirus lineages, respectively. For the most part, the increased sequence data show that the members of any given clade propagate in a limited range of host species. For example, the nine clades of metaviruses in *A. thaliana* are only observed in other plant species. However, exceptions to this pattern have recently been observed. Although the clade of metaviruses that contains Tf1 was only found in fungi, Sushi and Sushi II were discovered in the puffer fish *Fugu rubripes*. Another example of a metavirus clade that exists in distant host species is that of Osvaldo, an element of *Drosophila buzzatii*. The genome of the Atlantic cod *Gadus morhua* contains a metavirus that clearly falls in the same lineage as Osvaldo. These examples of clades that bridge distantly related host species indicate that our current collection of sequenced genomes is still insufficient to document the breadth of hosts that possess transposon lineages.

Expression

The survival and propagation of retrotransposons depends on a carefully balanced relationship with their host. High levels of transposition would damage the host genome and jeopardize the existence of the element. In general, the

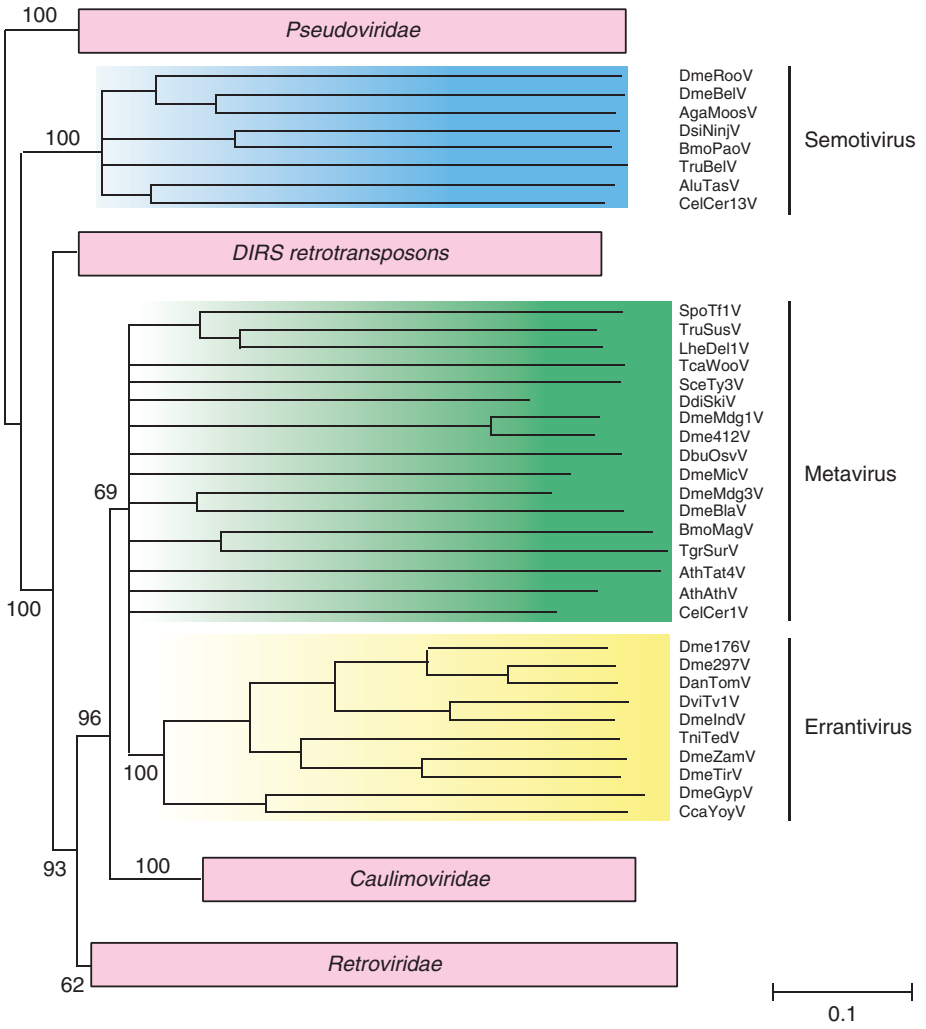


Figure 2 Phylogeny of the family *Metaviridae* and related groups based on their reverse transcriptase domain. The portion of the reverse transcriptase domain used in this analysis spans approximately 250 aa and includes the most conserved residues found in all retroelements. The phylogram is a 50% consensus tree of the transposons based on neighbor-joining distance algorithms and was rooted using sequences of members of the family *Pseudoviridae*. Bootstrap values (percentage of the time all elements are located on that branch) are shown for the major branches only. Transposons that are included in the various divisions of *Metaviridae* are indicated by the vertical lines to the right of each systematic name. Each group of elements that are not part of the family *Metaviridae* is represented by a box with the length of the box related to the sequence diversity within that group. DIRS retrotransposons are mobile elements that utilize a reverse transcriptase closely related to that of the family *Metaviridae* but lack many structural features of this group and integrate by a different mechanism. Scale bar at bottom represents divergence per site. Reproduced from Eickbush T, Boeke JD, Sandmeyer SB, and Voytas DF (2005) *Metaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 409–420. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

LTR-retrotransposons of yeast and *D. melanogaster* are dormant and exhibit very low levels of transposition. These low frequencies of spontaneous insertions suggest that the transcription of LTR-retrotransposons is heavily controlled. For example, the expression of most LTR-retrotransposons is induced by stress or specific stages of development. The gypsy element in wild-type stocks has very low transposition activity. However, stocks with mutant versions of the transcription factor *flamenco* possess substantially higher levels of transposition. The high rates of integration are only observed in female progeny of homozygous *flamenco* females. The mutations in *flamenco* cause 10- to 20-fold increases in full-length gypsy transcripts and this RNA occurs only in the follicle cells surrounding oocytes at stages 8–10 of oogenesis. A hypothesis that accounts for how increased transcription in follicle cells results in germline transposition in the progeny is that the gypsy env allows particles assembled in the follicle cells to infect the oocyte. Thus, transposition rates of gypsy in the progeny appear to be regulated at the level of transcription in the follicle cells of the mother. This program of regulated expression benefits gypsy by promoting transmission into the germ line

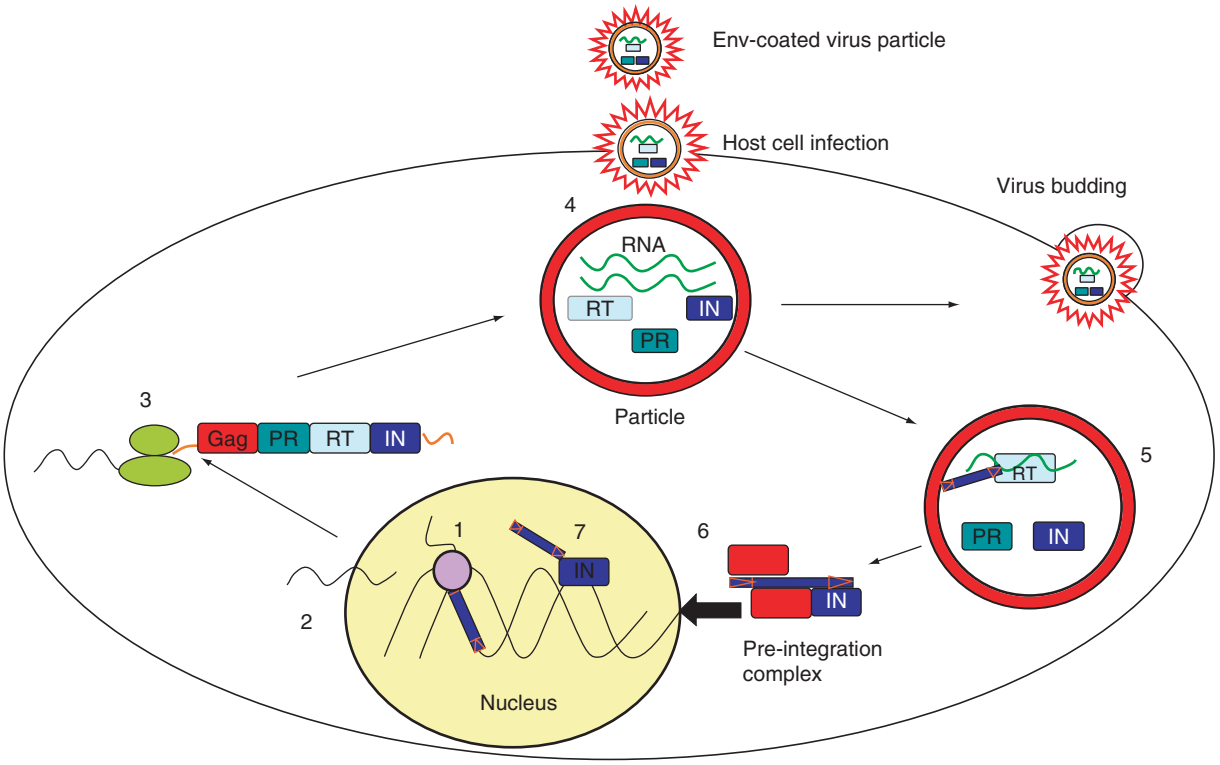


Figure 3 Life cycle of retrotransposons. The retrotransposon life cycle is contained entirely within the host cell. A seven-step life cycle is shown. First, cellular RNA polymerase transcribes an endogenous DNA copy of the transposon (blue) (1), producing an RNA copy (green). Next, that RNA transcript is exported from the nucleus (2) and then translated by host-cell ribosomes (green ovals) (3). The proteins of the retrotransposon then assemble into a virus-like particle (4). Reverse transcription takes place within this particle (5), producing a new DNA copy of the transposon. The pre-integration complex then enters the nucleus (6). Finally, the integrase protein mediates the integration of the transposon into a new location in the genome (7). The members of the family *Metaviridae* that encode an env-like protein have a life cycle that is quite similar to that of retroviruses. They have the additional ability to enter and leave the host cell. The ruffled circles indicate the particles with env-like proteins. Reproduced from **figure 1** of Kelly FD and Levin HL (2005) The evolution of transposons in *Schizosaccharomyces pombe*. *Cytogenetic and Genome Research*. 110: 566–574, with permission from S. Karger AG. Basel.

while minimizing the damage to the fly that would result from somatic events.

Perhaps the most extensively studied regulation of expression is that of Ty3. Its transcription is low in diploid cells but is dramatically induced in haploids exposed to mating factor. In cells with the mating type *a*, Ty3 is induced by α-factor treatment while cells with the mating type α are induced by a-factor. This developmental control is mediated by regulatory sequences within the LTR. Deletion analyses of the Ty3 LTR in a plasmid system identified a 101 nt segment that restricts transcription in diploids and is required for the mating-type regulation. Additional studies with a *lacZ* reporter fused to the *CYC1* promoter found that nucleotides 56–97 of the Ty3 LTR conferred a tenfold increase in expression in *a* cells treated with α-factor and a 100-fold decrease in expression in diploid cells. Inspection of the sequence between nucleotides 56 and 97 identified two matches to the binding site for Ste12p, a transcription factor that binds to pheromone response elements. This highly regulated expression of Ty3 is likely a mechanism that restricts transposition activity to cells in the process of mating. The benefit of this strategy for the transposon is not immediately clear.

Microarray studies of *Sc. pombe* revealed that Tf2, a species of the genus *Metavirus* closely related to Tf1, is a typical example of a transposon that is induced by conditions of stress. Cells challenged with hydrogen peroxide to induce oxidative stress produced as much as 20-fold more Tf2 RNA. This increase requires Atf1p, the transcription factor responsible for inducing a large number of environmental stress response genes. Other experiments showed that cells exposed to elevated temperatures (39 °C) induced Tf2 RNA by tenfold. The increase in Tf2 RNA that results from environmental stress is typical of many transposons. Although the function of this response to stress is not clear, an increase in transposition may help the elements to survive conditions that damage DNA.

Once the RNA of LTR-containing elements is produced, its translation presents particular challenges that must be overcome to allow the assembly of virus-like particles (VLPs). For all classes of LTR-containing elements, the coding sequences for Gag, PR, RT, and IN are

Table 1 Virus names, their abbreviations, the accession numbers (in square brackets), and discerning features (sor some cases)

Metaviruses			
Arabidopsis thaliana Athila virus	AthAthV	[AC007209]	Has env-like protein
Arabidopsis thaliana Tat4	AthTat4V	[AB005247]	Has env-like protein
Bombyx mori Mag virus	BmoMagV	[X17219]	
Caenorhabditis elegans Cer1 virus	CelCer1V	[U15406]	Gag, PR, RT, and IN are in single ORF
Cladosporium fulvum T-1 virus	CfuT1V	[Z11866]	Has self-priming structure
Dictyostelium discoideum Skipper virus	DdiSkiV	[AF049230]	PR is encoded in a separate ORF
Drosophila buzzatii Osvaldo virus	DbuOsvV	[AJ133521]	Has env-like protein
Drosophila melanogaster Blastopia virus	DmeBlaV	[Z27119]	
Drosophila melanogaster Mdg1 virus	DmeMdg1V	[X59545]	
Drosophila melanogaster Mdg3 virus	DmeMdg3V	[X95908]	
Drosophila melanogaster Micropia virus	DmeMicV	[X14037]	
Drosophila melanogaster412 virus	Dme412V	[X04132]	
Drosophila virilis Ulysses virus	DviUlyV	[X56645]	
Fusarium oxysporum Skippy virus	FoxSkiV	[L34658]	Has self-priming structure
Lilium henryi Del1 virus	LheDel1V	[X13886]	
Saccharomyces cerevisiae Ty3 virus	SceTy3V	[M34549]	Incerts 1 to 4 nt upstream of pol III transcribed genes
Schizosaccharomyces pombe Tf1 virus	SpoTf1V	[M38526]	Has self-primed reverse transcription
Schizosaccharomyces pombe Tf2 virus	SpoTf2V	[L10324]	
Takifugu rubripes Sushi virus	TruSusV	[AF030881]	Has self-priming structure
Tribolium castaneum Woot virus	TcaWooV	[U09586]	
Tripneustis gratilla SURL virus	TgrSurV	[M75723]	
Errantiviruses			
Ceratitis capitata Yoyo virus	CcaYoyV	[U60529]	Has env-like protein
Drosophila ananassae Tom virus	DanTomV	[Z24451]	Has env-like protein
Drosophila melanogaster virus	DmeZamV	[AJ000387]	Has env-like protein
Drosophila melanogaster Gypsy virus	DmeGypV	[M12927]	Is infectious and has env-like protein
Drosophila melanogaster Idefix virus	DmeIdeV	[AJ009736]	Has env-like protein
Drosophila melanogaster Tirant virus	DmeTirV	[X93507]	Has env-like protein
Drosophila melanogaster 17.6 virus	Dme176V	[X01472]	Has env-like protein
Drosophila melanogaster 297 virus	Dme297V	[X03431]	Has env-like protein
Drosophila virilis Tv1 virus	DviTv1V	[AF056940]	Has env-like protein
Trichoplusia ni TED virus	TniTedV	[M32662]	Has env-like protein
Semotiviruses			
Anopheles gambiae Moose virus	AgaMooV	[AF060859]	
Ascaris lumbricoides Tas virus	AluTasV	[Z29712]	Env-like protein
Bombyx mori Pao virus	BmoPaoV	[Z79443]	
Caenorhabditis elegans Cer13 virus	CelCer13V	[Z81510]	Has env-like protein
Drosophila melanogaster Bel virus	DmeBelV	[U23420]	
Drosophila melanogaster Roo virus	DmeRooV	[AY180917]	
Drosophila simulans Ninja virus	DsiNinV	[D83207]	
Fugu rubripes Suzu virus	FruSuzV	[AF537216]	

contained within a single transcript. The proteins are first expressed as polyproteins that are then cleaved by PR to produce the mature polypeptides. However, the Gag protein functions as the particle capsid and must be present in substantial excess to allow functional particles to assemble. Typically, the high levels of Gag relative to RT and IN result from programmed frameshifts positioned after Gag but before the coding sequences for RT and IN. The frameshifts are relatively inefficient and allow translation of RT and IN at levels 10- to 20-fold lower than Gag. The expression of the Ty3 proteins is a good example of this regulation. The coding sequence for Gag is separated from the ORF for PR, RT, and IN by a +1 frameshift. Fusions of these coding sequences to *lacZ* show that 20 times more Gag is translated than RT and IN. The +1 frameshift is also observed in the translation of retroviruses such as human immunodeficiency virus 1 (HIV-1) and Rous sarcoma virus (RSV). However, detailed studies of the translation mechanisms reveal fundamental differences in the frameshift of Ty3. The programmed frameshift of HIV-1 and RSV depends on a so-called slippery site where the ribonucleotides in a codon are repeated allowing the charged tRNA to rebind in the +1 frame. The sequence that causes frameshifting in Ty3 is GCG AGU U. The first codon is GCG (Ala), a sequence that is not repeated so the tRNA is not predicted to rebind in the +1 frame. Instead evidence indicates that the AGU is decoded by a low-abundance tRNA and this allows a near-cognate $tRNA^{Ala}_{IGC}$ to decode the GCG. The resulting I:G pairing in the wobble position of the

peptidyl-tRNA coupled with the ribosomal pausing caused by low availability of the tRNA decoding AGU, mispositions the incoming $tRNA^{Val}$ in the +1 frame.

Although most elements in the family *Metaviridae* have +1 or −1 frameshifts that regulate the ratios of Gag versus RT and IN, the semotiviruses BEL1 and Cer13 and the metavirus Tf1 are examples that encode Gag, PR, RT, and IN all within a single ORF. Extensive studies of the Tf1 proteins in *Sc. pombe* found that they are all expressed within a single primary translation product. Cells in log phase cultures contain equal amounts of Gag and IN. But when cells become stationary in saturated cultures, most of the IN is degraded. This regulated degradation of IN provides the excess levels of Gag that are typically required for assembly of particles. Once the excess IN is degraded, VLPs form that contain 25 times more Gag than IN.

All three genera of the family *Metaviridiae* contain elements that have the additional challenge of expressing an env protein. The source of the env proteins appears to be spliced copies of the retroelement RNA that lack the sequences for Gag, PR, RT, and IN. In the case of gypsy, Zam, and Tom, experimental data show correlations between the expression of spliced RNAs and transposition.

Reverse Transcription

After VLPs mature, the encapsidated RNA is reverse transcribed into double-stranded DNA copies of the retroelement. This process of reverse transcription is a complex series of reactions that rely on two catalytic activities of RT, the synthesis of DNA and the degradation of RNA annealed to DNA known as RNase H activity. Despite the great differences between LTR-retroelements of the *Retroviridae*, *Pseudoviridae*, and *Metaviridae*, their mechanisms of reverse transcription are extremely similar (**Figure 4**). A full-length RNA begins and ends with sequence from the R portion of the LTRs. It is called R because this sequence is repeated on each end of the transcript. Upstream of R is U3 (unique on the 3′ end), a segment of the LTR that is only found on the 3′ end of the transcript. U5 (unique on the 5′ end) is the segment of the LTR present only on the 5′ end of the transcript. Reverse transcription is primed by specific tRNA species that are also packaged in the particles. The tRNA anneals to the retroelement RNA just after the upstream LTR at a position called the primer-binding site (PBS). The tRNA primes the reverse transcription of a short minus-strand species of DNA referred to as the minus-strand-strong-stop (−SSS). As this cDNA is being synthesized, the RNase H activity of RT degrades the RNA after it is reverse transcribed. Degradation of the 5′ end of the RNA releases the −SSS from the 5′ end of the RNA and allows it to anneal to the R sequence at the 3′ end of the RNA. This positions the −SSS so it can be extended by RT to generate the minus-strand of the LTR-retroelement. During the synthesis of the minus-strand cDNA, the remainder of the RNA is degraded by RNase H. The exception to this is RT does not degrade a polypurine tract (PPT) that functions as the plus-strand primer for reverse transcription. A short segment of plus-strand cDNA is produced and RNase H removes the RNA sequence from the 5′ end of the minus-strand DNA. Circularization is then mediated by the annealing of PBS sequences. The formation of a circle allows RT to extend the plus-strand intermediate into a full-length DNA. The final section of the minus strand is also generated.

Priming of Reverse Transcription

The features of reverse transcription as described above have been observed experimentally during the replication of elements in the *Metaviridae*. Based on sequence complementarity, the primer for gypsy is thought to be $tRNA^{Lys}$ while the majority of the other errantiviruses are predicted to use $tRNA^{Ser}$. The metavirus Ty3 is thought to be primed by the initiator $tRNA^{Met}$ because there are 8 nt after the upstream LTR which are complementary to 8 nt at the 3′ end of the tRNA. This prediction was demonstrated experimentally in strains of *Sa. cerevisiae* lacking the four genomic genes for the initiator $tRNA^{Met}$. The strain contains a plasmid copy of the initiator $tRNA^{Met}$ that supports the initiation of translation. Mutations in the plasmid-encoded $tRNA^{Met}$ that specifically alter the complementarity with Ty3 restricted reverse transcription but not protein translation.

Another mechanism of priming that has been studied extensively is that of the metavirus Tf1. In this case, there is a surprising difference in the nature of the primer. No tRNA candidate with complementarity to the Tf1 mRNA is known. Instead, the first 11 nt of the Tf1 mRNA itself has perfect complementarity to its PBS. This observation led to the discovery of an unusual self-priming mechanism in which the first 11 nt of the Tf1 mRNA function as the primer of the minus-strand strong stop cDNA (**Figure 5(a)**). Evidence for this mechanism includes the result that single nucleotide mutations in the PBS and in the 11 nt at the 5′ end of the mRNA cause substantial reductions in Tf1 transposition. Importantly, the combination of mutations in the PBS and the self-primer that reestablish complementarity returns transposition frequencies to near wild-type levels. Further evidence that the first 11 nt prime reverse transcription is the Tf1 mRNA in VLPs is cleaved between nucleotides 11 and 12. The cleavage depends on the complementarity with the PBS and the cleavage is required for production of −SSS. Another surprising feature of the self-priming mechanism is that the cleavage requires the RNase H activity of RT. One initial question that the self-priming mechanism poses is how can the 11 nt sequence removed from the Tf1 mRNA be restored during reverse transcription. The answer is that the sequence of

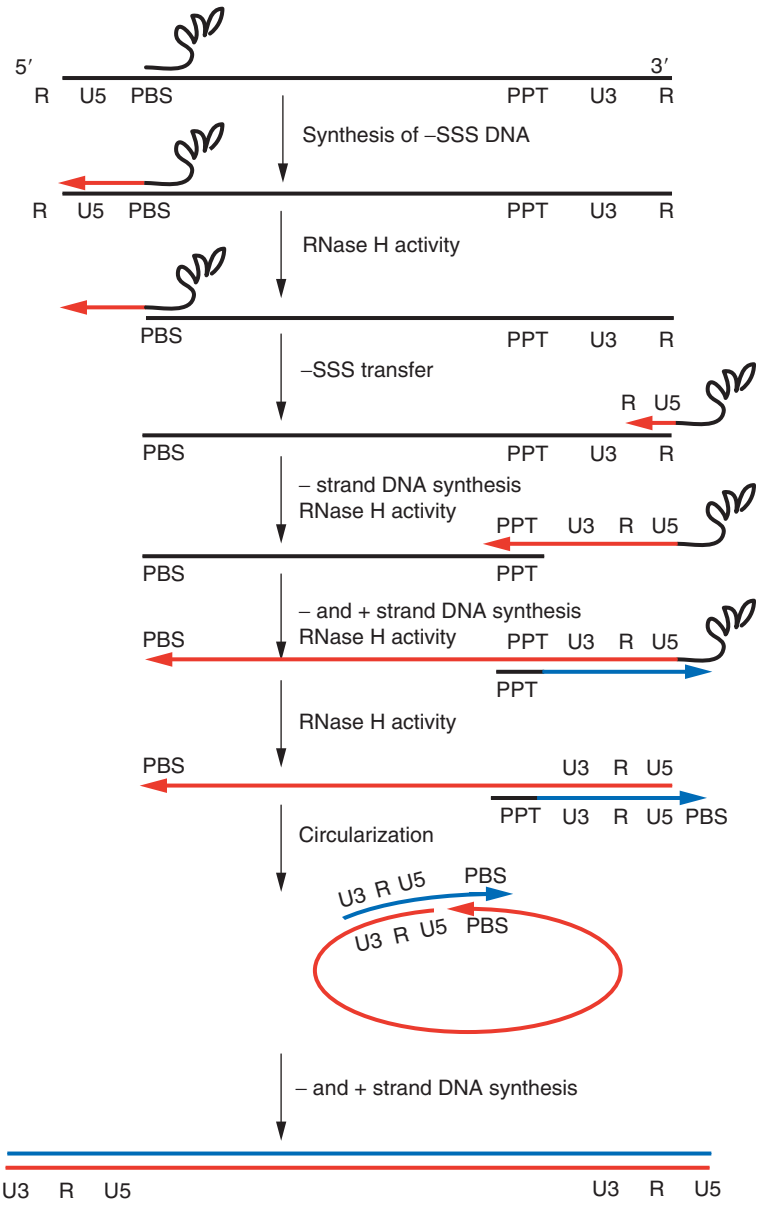


Figure 4 The reverse transcription of LTR-retrotransposons. The process of reverse transcription of LTR-retrotransposons is the same as for retroviruses. RNA is shown in black. The plus-strand and minus-strand cDNAs are shown in blue and red, respectively. The first intermediate of cDNA produced is the minus-strand strong-stop (–SSS). RNase H degrades the R and U5 regions from the 5′ end of the transcript and the –SSS transfers to the 3′ end of the RNA. The bulk of the minus-strand cDNA is then produced while RNase H degrades the rest of the transcript except for the plus-strand primer (PPT). The plus-strand strong-stop is produced and RNase H removes the tRNA primer from the 5′ end of the minus-strand cDNA. Once circularized, the plus-strand and minus-strand cDNAs are extended to generate their full-length forms.

the first 11 nt is present on both ends of the Tf1 mRNA and during reverse transcription of the downstream LTR, the 11 nt are restored (**Figure 4**). Although the self-priming mechanism appears quite different from the priming of other LTR-retroelements, experiments showed the cleavage of the Tf1 mRNA depends on its formation of a complex structure surrounding the PBS that is quite similar to the priming structure found in the RNA of RSV (**Figures 5(b)** and **5(c)**). The characterization of the complex structure in the Tf1 mRNA made it possible to identify many other LTR-retrotransposons that likely use self-priming to initiate reverse transcription.

Integration

The INs of retroviruses catalyze two reactions critical for integration, 3′ processing and strand transfer. The processing step removes two or three nucleotides from the

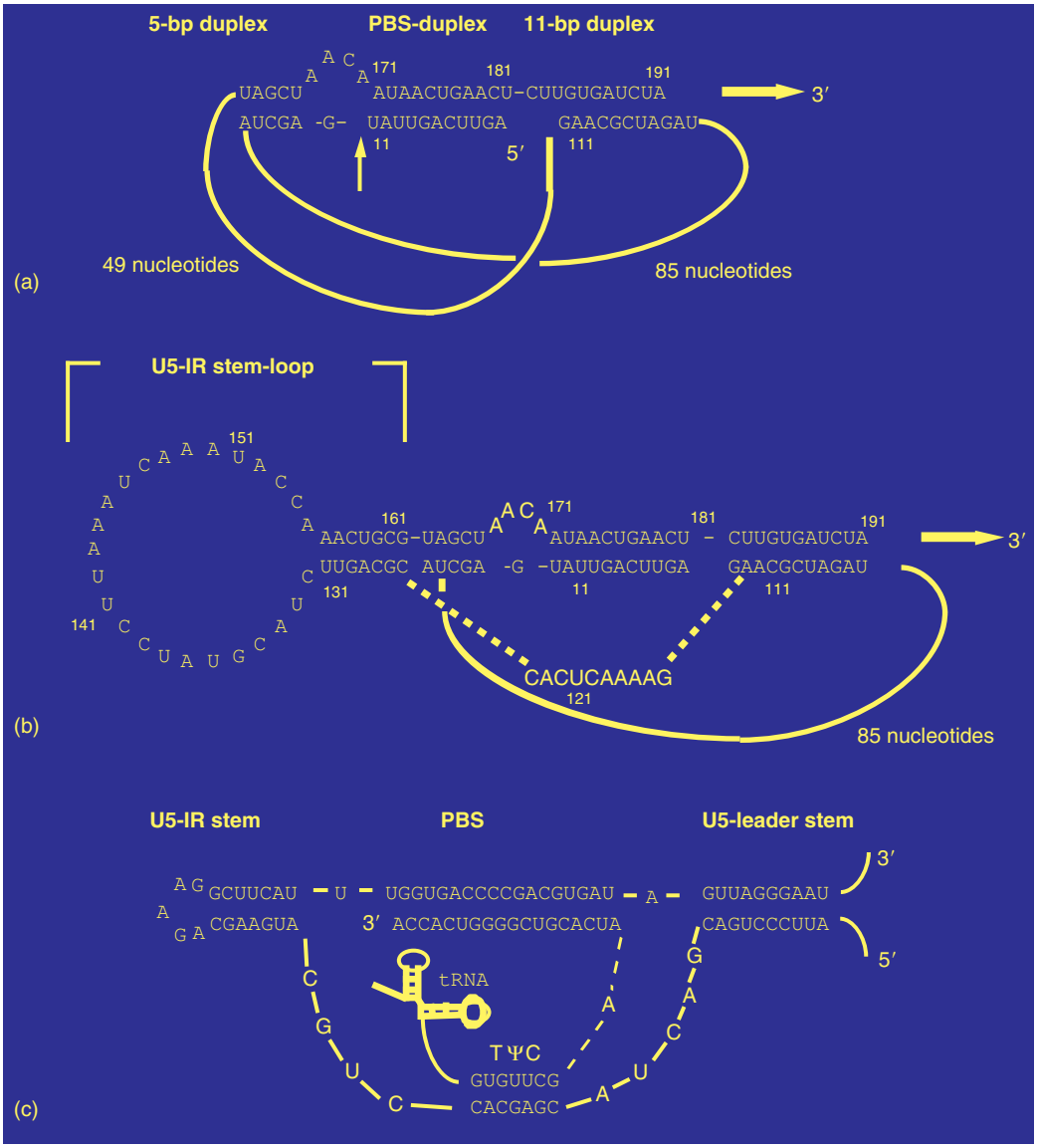


Figure 5 The self-priming structure of the Tf1 mRNA. (a) The self-priming mechanism of Tf1 requires an RNA structure consisting of three duplexes. The regions of duplex structure are labeled 5-bp duplex, PBS-duplex, and 11-bp duplex. The 5′ end of the RNA is labeled and the yellow lines represent extended stretches of sequence. The arrow labeled 3′ indicates that the majority of the transcript is 3′ of the sequence shown. The numbers shown in small type indicate the nucleotide position relative to the first base of the transcript. (b) Additional base pairing exists between nucleotides (nt 124–130 and nt 162–156) within a 49 nt loop. The resulting structure is a U5-IR stem–loop with a 7 bp stem and a 25 nt loop. The dashed line indicates that the nucleotides shown connect directly to the indicated regions of the diagram. (c) The U5-IR stem–loop of RSV. The three regions of duplex structure in the transcript of RSV are U5-IR, PBS, and U5-leader stem. These structures show considerable similarity to the self-priming RNA of Tf1. The tRNA primer is shown in diagrammatic form and all the lines between nucleotides indicate a direct connection to adjacent nucleotides. The 3′ and 5′ labels indicate the polarity of the RNA and the position of the transcript sequences that are not shown. Reproduced from Lin JH and Levin HL (1998) Reverse transcription of a self-primed retrotransposon requires an RNA structure similar to the U5-IR stem-loop of retroviruses. *Molecular and Cell Biology* 18: 6859-6869, with permission from American Society for Microbiology.

3′ ends of the cDNA in order to position the highly conserved CA dinucleotide at the 3′ end of the cDNA. The processing step allows the 3′ hydroxyls of the terminal 'A' to act as nucleophiles in a pair of strand-transfer reactions that cleave the phosphodiester bonds in the target DNA and make a covalent bond between the 3′ ends of the viral DNA and the 5′ ends of the target DNA. In retroviruses, and some LTR-retrotransposons, the tRNA primers initiate reverse transcription two or three nucleotides from the LTR and this results in the additional deoxynucleotides at the cDNA termini that IN removes during the processing reaction. This is the case for some metaviruses such as Ty3. Direct support for 3′ processing is that cDNA isolated from Ty3 particles terminates with the conserved

CA dinucleotide. However, mutations that inactivate IN cause cDNA to accumulate that retains two additional nucleotides 3′ of the CA.

In the case of other LTR-retrotransposons including Tf1, the minus-strand primers initiate reverse transcription with no extra nucleotides added before the LTR. These LTR-retrotransposons were thought to proceed to the strand-transfer reaction without a processing step. This assumption was tested for Tf1 and was found to be incorrect. Despite the position of the self-primer adjacent to the upstream LTR, cDNA in Tf1 particles has nucleotides 3′ of the CA dinucleotide. The sequences of these 1–4 nt is untemplated and thought to be added by the terminal transferase activity of RT. Because these extra nucleotides must be removed before strand transfer, Tf1 IN was tested for 3′ processing activity. Experiments with oligonucleotide substrates show that Tf1 IN as a recombinant protein does possess 3′ processing activity that specifically removes the nucleotides 3′ of the CA. The finding that Tf1 IN has processing activity brings into question the predicted lack of processing activity in the INs of the other LTR-retrotransposons.

Target Site Specificity

The integration of cDNA can disrupt gene function and has the potential to reduce the viability of host cells. Since the survival of transposons depends entirely on the well being of the host, mechanisms have evolved that specifically target integration to noncoding portions of the host genome. The integration of Ty3 is one of the best-studied examples of this. Ty3 expressed from the Gal promoter generates insertions 1–4 nt upstream of tRNA, 5S, and U6 genes, all of which are expressed by pol III RNA polymerase. The promoters of tRNA, U6, and 5S genes bind transcription factors TFIIIC downstream and TFIIIB upstream of the position that transcription initiates. Both of these factors do play a role in targeting Ty3 integration. In *in vitro* assays that measure integration of Ty3 into a plasmid copy of a tRNA gene, fractions containing TFIIIB and TFIIIC are required. However, the role of TFIIIC in transcription is to load TFIIIB onto the DNA of the tRNA gene. This suggests the function of TFIIIC in integration could be indirect. In *in vitro* reactions with purified factors, the U6 gene binds TFIIIB without the requirement of TFIIIC. Thus, the role of TFIIIC in Ty3 integration can be tested independently from its function in loading TFIIIB. The results of integration assays using a plasmid copy of the U6 gene as a target show that TFIIIB, and not TFIIIC, is necessary for insertion. TFIIIB is composed of three subunits, TBP, Brf, and B″. Further analysis reveals that TBP and Brf are the minimum number of subunits that support Ty3 integration. When the *in vitro* integration experiments are performed using truncated segments of Brf, just the N-terminal 282 amino acids are necessary. Taken together, these results implicate Brf as a key transcription factor responsible for positioning Ty3 insertions.

Tf1 is another metavirus that has a strong preference for a specific type of target site. The genome of *Sc. pombe* contains 186 inserts of Tf1 or transposons closely related to Tf1. Ninety-six percent of the inserts occur in intergenic sequences that contain a pol II promoter. It is significant that the inserts cluster within 400 nt of the 5′ end of ORFs. This accumulation of insertions near the 5′ end of genes could be the result of a mechanism that targets integration to these locations or, conversely, they could represent an evolutionary equilibrium where insertions into the ORFs or near the 3′ end occur but are lost due to selective pressure. However, when a *neo* marked version of Tf1 is expressed from a plasmid, the insertions also cluster within 400 nt of the start of ORFs. Insertions do not occur within ORFs. These data confirm that the positioning of Tf1 near the 5′ end of ORFs is due to an insertion mechanism. The preference for the 5′ end of genes suggests that the integration mechanism may recognize components of the pol II transcription machinery.

The location of Ty3 and Tf1 in chromatin that is actively expressed is representative of transposons in compact genomes such as yeast. The transposons of complex eukaryotes commonly cluster in heterochromatin where the bulk of transcription is off. For example, the great majority of gypsy elements are located in the pericentromeric regions of the *D. melanogaster* genome. The location of gypsy elements is conserved in strains from different geographic origins. This suggests that a high number of gypsy elements are nonmobile and were already present in the pericentromeric sequences of the common ancestor of the *D. melanogaster* strains.

The accumulation of metaviruses in centromeric sequences is also observed in plants. Transposons belonging to the *Metaviridae* in *A. thaliana* form a diverse set of retroelements that fall into three distinct lineages, *Athila*, *Tat,* and *Metavirus*. Together the *Metaviridae* of *A. thaliana* constitute as much as 2.34% of the genome and the retroelements of each of the three lineages cluster in the pericentromeric heterochromatin. Interestingly, *Tat* and *Athila* exhibited significantly stronger associations with heterochromatin than metaviruses. In addition, members of the family *Pseudoviridae* make up 1.25% of the *A. thaliana* genome and these elements are only loosely associated with pericentromeric regions. The pronounced differences in distribution patterns of the retroelements in *A. thaliana* indicate that targeted integration plays an important role in positioning the elements.

Env-Mediated Infection

The vertebrate retroviruses make up a relatively young clade that has a close relationship to members of the family

Metaviridae. They are thought to have originated from a member of the *Metaviridae* that acquired a third ORF, the *env* gene. Env is a transmembrane protein that confers infectivity by mediating receptor recognition and membrane fusion. It is glycosylated and has an N-terminal signal peptidase cleavage site. It is surprising that among the members of the families *Pseudoviridae* and *Metaviridae,* six lineages have independently acquired *env*-like genes. In each of these cases the third ORFs have structural features typical of the vertebrate retroviruses. The *env*-like proteins are predicted to possess N-terminal leader peptides, N-glycosylation sites, and transmembrane domains. Each of the three genera of the *Metaviridae* contains *env*-like lineages. Two lineages in the metavirus family with *env*-like genes are the clades represented by Athila and Osvaldo. One lineage with *env*-like genes in the errantiviruses is represented by gypsy and two lineages in the semotiviruses are represented by Cer13 and TAS.

Efforts to identify the source of the retroviral *env* genes have been difficult because their sequences have no similarity to the *env*-like genes of other retroelements or to any other genes known. Fortunately, sequences similar to the *env*-like proteins of members of the family *Metaviridae* have been identified. More importantly, the sequence similarities suggest that the env-like proteins were acquired from unrelated viruses and these proteins in their original viruses already had env activities. The *env*-like genes of the errantiviruses have highly significant homology to genes from three insect baculoviruses, indicating that the *env* genes from errantiviruses and the baculoviral ORFs share common ancestry. Although the function of the baculoviral proteins is not known, their structural features indicate they are analogous to the env proteins of vertebrate retroviruses.

The env-like protein of the semotiviruses also has homology to proteins of unrelated viruses. The third ORF of Cer13 has significant homology to a group of proteins (G2) from phleboviruses, a class of single-stranded RNA viruses. The G2 proteins are thought to have env-like function because they are glycoproteins with a predicted transmembrane domain. It is interesting that despite the similarity between the semotivirus Cer13 and Tas, their env-like proteins are unrelated. It appears that the env-like protein of TAS resulted from a different acquisition. The Tas protein has a modest similarity to gB glycoproteins from viruses in the family *Herpesviridae*, a class of double-stranded DNA viruses. The glycoprotein gB has been implicated in the viral attachment and fusion of herpesviruses. It is significant that the segment of gB glycoproteins thought to be responsible for viral attachment to the cell surface is exactly the segment that has similarity to Tas.

Despite the many examples of env-like proteins encoded by LTR-retroelements, only the VLPs of gypsy are known to be infectious. Stocks of *D. melanogaster* that produce high levels of gypsy transposition can be used to make preparations of VLPs. Extracts of pupae from these stocks can be fed to strains that lack active gypsy elements. The result is the horizontal transmission of active gypsy. In addition, extracts from the stocks with active gypsy can be used to fractionate VLPs on sucrose gradients. The fractions contain a clear peak with high levels of RT activity and immunoelectron micrographs show these fractions contain gypsy VLPs. When these fractions are fed to larvae that lacked active gypsy, 1.5% of the flies that emerged contained new insertions of gypsy. These results show that gypsy has the properties expected for an infectious virus. One surprising result is that mutations that inactivate env in an expressed copy of gypsy do not reduce transposition activity. One explanation is that the infectious behavior of the particles is due to env-like protein expressed from a related transposon in the genome of *D. melanogaster*.

Conclusions

LTR-retroelements have been highly successful throughout the evolution of eukaryotes. Analyses of the available genome sequences indicate that members of the family *Metaviridae* are the most abundant class of LTR-retroelements. One significant distinction of the family *Metaviridae* is its close relationship to the disease-causing retroviruses such as HIV-1. As a result, various members of the *Metaviridae* are actively being studied as models for the vertebrate retroviruses. Perhaps the most significant result from the study of elements belonging to the *Metaviridae* is the discovery that structures responsible for specific mechanisms are modular and can be readily substituted within relatively short periods of evolution. For example, LTR-retroelements that use the self-priming mechanism exist in the same genus as elements that use tRNA priming. Just a limited modification of transposon sequence rendered them able to self-prime. The *env*-like genes are an example of how modules that promote infection can be acquired multiple times during independent events. Whether self-priming PBSs and e*nv*-like genes are the only forms of modular evolution for LTR-retroelements is unlikely. As increasing numbers of genomes are sequenced from a broad swath of organisms, we are likely to learn much more about modular evolution and the diversity of LTR-retroelements.

See also: Pseudoviruses; Retroviruses: General Features.

Further Reading

Bowen NJ, Jordan I, Epstein J, Wood V, and Levin HL (2003) Retrotransposons and their recognition of pol II promoters: A comprehensive survey of the transposable elements derived from the complete genome sequence of *Schizosaccharomyces pombe*. *Genome Research* 13: 1984–1997.

Eickbush T, Boeke JD, Sandmeyer SB, and Voytas DF (2005) Metaviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of International Committee on Taxonomy of Viruses*, pp. 409–420. San Diego, CA: Elsevier Academic Press.

Eickbush TH and Malik HS (2002) Newly identified retrotransposons of the gypsy/Ty3 class in fungi, plants, and vertebrates. In: Craig NL, Craigie R, Gellert M, and Lambowitz AL (eds.) *Mobile DNA II*, pp. 1111–1144. Washington, DC: American Society of Microbiology.

Kelly FD and Levin HL (2005) The evolution of transporons in *Schizosaccharomyces pombe*. *Cytogenetic and Genome Research*. 110: 566–574.

Kirchner J, Connolly CM, and Sandmeyer SB (1995) Requirement of RNA polymerase III transcription factors for *in vitro* position-specific integration of a retroviruslike element. *Science* 267: 1488–1491.

Le Grice SFJ (2003) 'In the beginning': Initiation of minus strand DNA synthesis in retroviruses and LTR-containing retrotransposons. *Biochemistry* 42: 14349–14355.

Levin H (2002) Newly identified retrotransposons of the gypsy/Ty3 class in fungi, plants, and vertebrates. In: Craig NL, Craigie R, Gellert M, and Lambowitz AL (eds.) *Mobile DNA II*, pp. 684–704. Washington, DC: American Society of Microbiology.

Levin HL (1995) A novel mechanism of self-primed reverse transcription defines a new family of retroelements. *Molecular and Cell Biology* 15: 3310–3317.

Lin JH and Levin HL (1998) Reverse transcription of a self-primed retrotransposon requires an RNA structure similar to the U5-IR stem-loop of retroviruses. *Molecular and Cell Biology* 18: 6859–6869.

Malik HS, Henikoff S, and Eickbush TH (2000) Poised for contagion: Evolutionary origins of the infectious abilities of invertebrate retroviruses. *Genome Research* 10: 1307–1318.

Pelisson A, Mejlumian L, Robert V, Terzian C, and Bucheton A (2002) Drosophila germline invasion by the endogenous retrovirus gypsy: Involvement of the viral *env* gene. *Insect Biochemistry and Molecular Biology* 32: 1249–1256.

Sandmeyer S (2003) Integration by design. *Proceedings of the National Academy of Sciences, USA* 100: 5586–5588.

Sandmeyer SB, Aye M, and Menees T (2002) Newly identified retrotransposons of the gypsy/Ty3 class in fungi, plants, and vertebrates. In: Craig NL, Craigie R, Gellert M, and Lambowitz AL (eds.) *Mobile DNA II*, pp. 663–683. Washington, DC: American Society of Microbiology.

Song SU, Gerasimova T, Kurkulos M, Boeke JD, and Corces VG (1994) An env-like protein encoded by a *Drosophila* retroelement – Evidence that gypsy is an infectious retrovirus. *Genes and Development* 8: 2046–2057.

Mimivirus

J-M Claverie, Université de la Méditerranée, Marseille, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

16S rDNA The gene encoding the small ribosomal RNA molecule, a universal component of all cellular prokaryotic organisms, the sequence of which is used for identification and classification purposes.

Aminoacyl-tRNA synthetases The highly specific enzymes responsible for the loading of a given amino acid onto its cognate tRNA(s). These enzymes are at the center of the use of the genetic code, together with the specific tRNA/mRNA recognition mediated by the anticodon/codon pairing on the ribosome.

COG Cluster of orthologous groups. Families of evolutionarily conserved genes, usually associated with well-defined protein functions and 3-D structures.

NCLDV Nucleocytoplasmic large DNA viruses, a group of large dsDNA viruses, the replication of which involves a stage the host cell cytoplasm and the host cell nucleus. Although presently very diverse in size, host range, and morphology, these are suspected to have originated from a common ancestor, contemporary to the emergence of the eukaryote lineage.

Paralogs Copies of similar genes (originated by duplication) found in the same genome.

Proteomics New methodological approaches allowing the whole protein complement of an organism (or a virus) to be identified.

Introduction

The recent discovery in 2003 of acanthamoeba polyphaga mimivirus and the analysis of its complete genome sequence sent a shock wave through the community of virologists and evolutionists. Its particle size (750 nm), genome length (1.2 million bp), and large gene repertoire (911 protein coding genes) blur the established boundaries between viruses and parasitic cellular organisms. In addition, the analysis of its genome sequence identified many types of genes never before encountered in a virus, including aminoacyl-tRNA synthetases and other central

components of the translation machinery previously thought to be the signature of cellular organisms. The information available on this giant double-stranded DNA (dsDNA) virus mostly consists of electron microscopy (EM) images, its genome sequence, and proteomic data. Very little is yet known about its pathogenicity.

History and Classification

In 1992, following a pneumonia outbreak in the West Yorkshire mill town of Bradford (England), a routine investigation for legionella (a pneumonia causing intracellular parasitic bacteria) within the amoeba colonizing the water of a cooling tower led Timothy Rowbotham from the Britain's Public Health Laboratory Service to discover a microorganism resembling a small Gram-positive coccus (initially called Bradford coccus). Cultivation attempts failed, and no amplification product was obtained with universal 16S rDNA bacterial primers at that time (**Figure 1**). The mysterious sample was stored in a freezer for about 10 years, until it reached the laboratory of Prof. Didier Raoult, at the school of medicine in Marseilles, France. There, EM of infected *Acanthamoeba polyphaga* cells provided the first hints that Bradford coccus was in fact a giant virus, with mature icosahedral particules *c.* 0.7 µm in diameter, a size comparable to that of mycoplasma cells (**Figure 2**). The viral nature of the agent was definitively established by the demonstration of an eclipse phase during its replication, and the analysis of several gene sequences exhibiting a clear phylogenetic affinity with nucleocytoplasmic large DNA viruses (NCLDVs), a group of viruses including members of the *Poxviridae*, the *Iridoviridae*, the *Phycodnaviridae*, and the *Asfarviridae*. This new virus was named mimivirus (for mimicking microbe) and is now classified by ICTV as the first member of the *Mimiviridae*, a new family within the NCLDV. The size of its particles makes mimivirus the largest virus ever described.

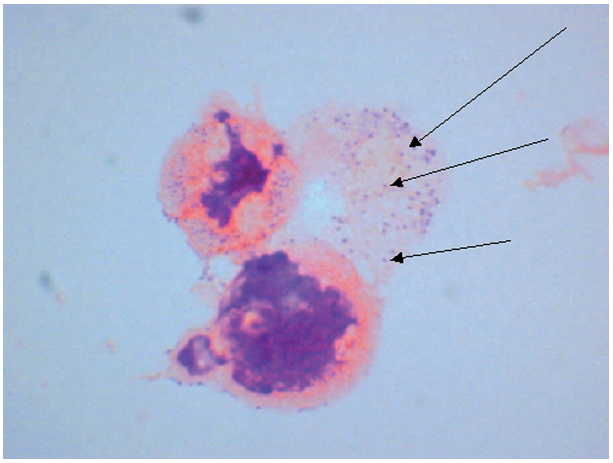


Figure 1 Coccus-like appearance of mimivirus particles (indicated by arrows) under the light microscope after Gram coloration in infected amoeba. Reproduced from La Scola B, Audic S, Robert C, *et al*. (2003) A giant virus in amoebae. *Science* 299: 2033, with permission from American Association for the Advancement of Science.

Host Range and Pathogenicity

Among a large number of primary or established cell lines from vertebrates or invertebrates that were tested for their ability to support mimivirus infection and replication, only cells from the species *Acanthamoeba polyphaga, A. castellanii*, and *A. mauritaniensis* could be productively infected by a cell-free viral suspension. This narrow range of target cell specificity, restricted to protozoans, apparently conflicts with other reports suggesting that mimivirus might be an amoebal pathogen-like legionella, causing pneumonia in human. Numerous seroconversions to mimivirus have been documented in patients with both community- and hospital-acquired pneumonia. In addition, mimivirus DNA was found in respiratory samples of a patient with hospital-acquired pneumonia and a laboratory infection of a technician by mimivirus has also been reported. The patient's serum sample was found to strongly react with many mimivirus proteins, most of them unique to the virus, making cross-reactions with other pathogens unlikely. Isolation of the mimivirus from an infected patient is yet to be accomplished in order to formally establish that it is indeed pathogenic to human. In the meantime, as a precautionary measure, mimivirus should be considered a potential pneumonia agent and manipulated as a class 2 pathogen. In this context, it is worth noticing that mimivirus particles have been shown to remain infectious for at least 1 year at temperature of 4–32 °C in a neutral buffer.

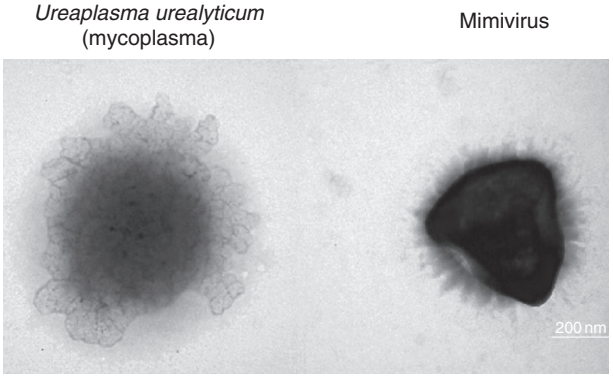


Figure 2 Comparable size of a mimivirus particle and a mycoplasma cell. EM picture of a co-culture. Reproduced from La Scola B, Audic S, Robert C, *et al*. (2003) A giant virus in amoebae. *Science* 299: 2033, with permission from American Association for the Advancement of Science.

Replication Cycle

Upon infection of *A. polyphaga* cells, mimivirus exhibits a typical viral replication cycle with an eclipse phase of 4 h post infection (p.i.), followed by the appearance of newly synthesized virions at 8 h p.i. in the cytoplasm, leading to the clustered accumulation of viral particles filling up most of the intracellular space, until infected amoebae are lysed at 24 h p.i. Little more is known about the details of the various stages of the replication cycle. Combining various hints from genomic and proteomic analysis as well as from EM suggests the following infection scenario (**Figure 3**):

1. free virus particles mimicking bacteria (by their micron size and perhaps the lipopolysaccharide (LPS)-like layer surrounding the capsid) are taken up as putative food by the amoeba;
2. the LPS-like layer is digested within the amoeba endocytic vacuole, making protein at the surface of the capsid accessible for a specific interaction at the vacuole membrane;
3. the content of the capsid is then injected into the amoeba cytoplasm, eventually using a specialized apparatus visible as a 600 Å 'vertex' (see below) leaving the empty capsid in the endocytic vacuole (documented by EM); and
4. early transcription events occur in the cytoplasm, as suggested by the proteomic content of the capsid.

It is not clear if, how, and when the virus genome gets into the amoeba nucleus (for its replication) or if the synthesis of particle components and capsid assembly only proceeds from 'virus factories' located in the cytoplasm.

Genome Organization

The mimivirus genome (**Figure 4**) consists of a single ds-DNA, 1 181 404 bp long, within which 911 protein-coding genes are predicted. Two inverted repeats of about 900 nt are found near both extremities of the sequence, suggesting that the genome might adopt a circular topology as result of their annealing. The genome nucleotide composition is 72% A + T and exhibits some level of local strand asymmetry revealed by plotting the A + C strand excess as well as the gene excess (number of genes expressed from one strand minus the number of genes expressed from the

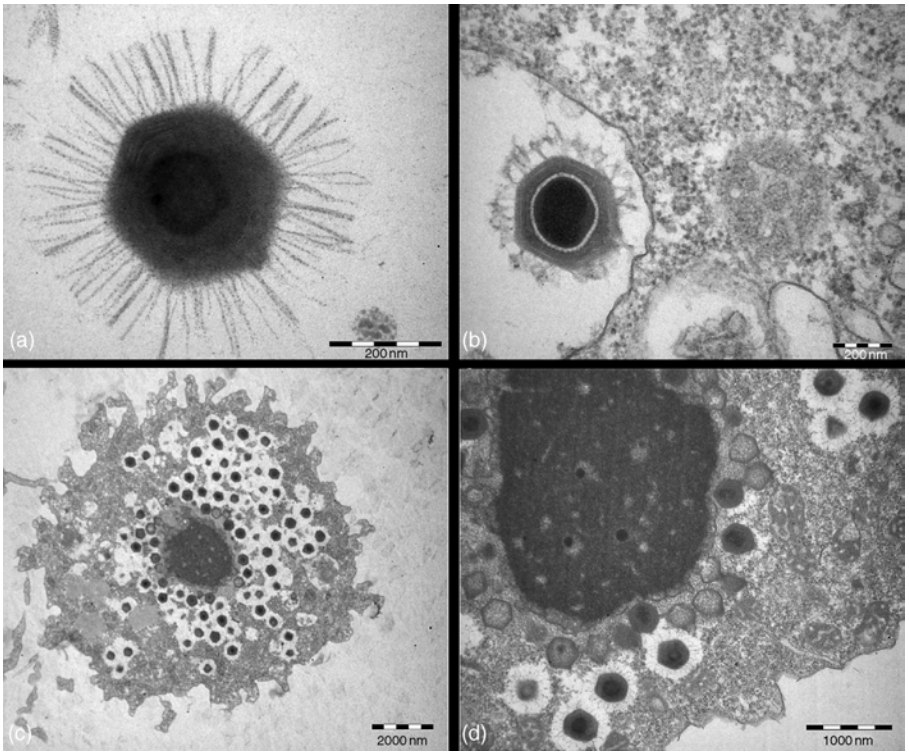


Figure 3 EM pictures of mimivirus particles at various maturation stages. (a) Free particle (see **Figure 6** for a different aspect of the fibers recovering the capsid). (b) Mimivirus particle in an amoeba vacuole, exhibiting a damaged (digested) fiber layer. (c) Clustered particles in the cytoplasm of an amoeba, 8 h p.i. (d) Close-up of a virus factory surrounded by particles at various stages of maturation: empty particles, filled particle (with a dark central core) without their translucent capsular material, mature particles (central dark core, white halo of cross-linked fibers). Reproduced from Suzan-Monti M, La Scola B, and Raoult D (2006) Genomic and evolutionary aspects of mimivirus. *Virus Research* 117: 145–155, with permission from Elsevier.

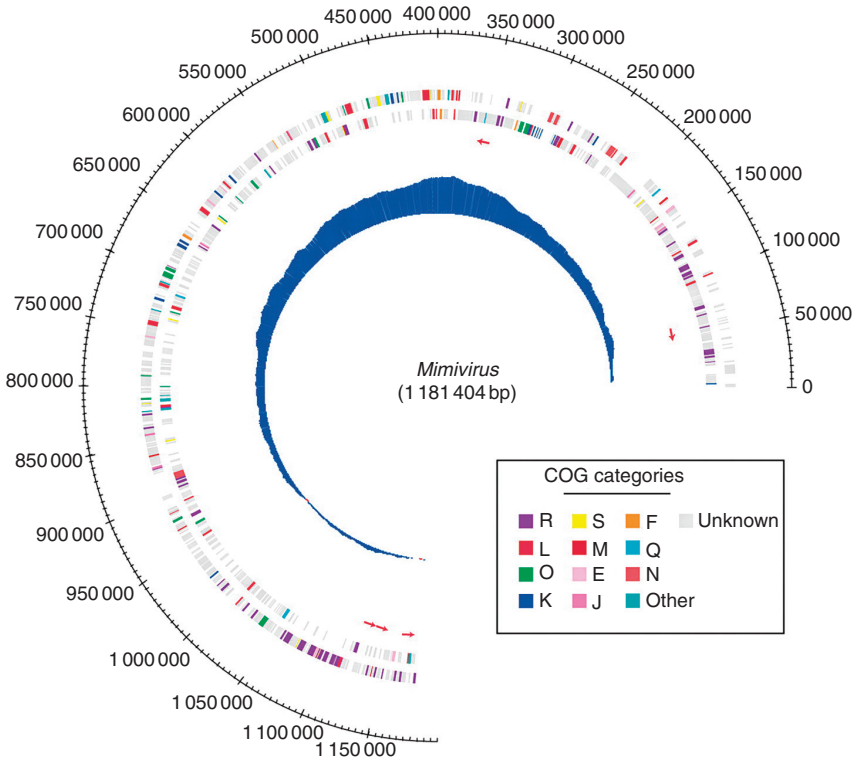


Figure 4 Map of the mimivirus chromosome. The predicted protein-coding sequences are shown on both strands and colored according to the function category of their matching COG. Genes with no COG match are shown in gray. Abbreviations for the COG functional categories are as follows: E, amino acid transport and metabolism; F, nucleotide transport and metabolism; J, translation; K, transcription; L, replication, recombination, and repair; M, cell wall/membrane biogenesis; N, cell motility; O, post-translational modification, protein turnover, and chaperones; Q, secondary metabolites biosynthesis, transport, and catabolism; R, general function prediction only; S, function unknown. Small red arrows indicate the location and orientation of tRNAs. The A + C excess profile is shown on the innermost circle, exhibiting a peak around position 380 000. The leading strand is defined as the one directed outward, from this point. Reproduced from Raoult D, Audic S, Robert C, *et al.* (2004) The 1.2-megabase genome sequence of mimivirus, *Science* 306: 1344–1350, with permission from American Association for the Advancement of Science.

other strand). Both graphs exhibit a slope reversal near position 380 000 of the genome, as found in bacterial genomes, and associated with the location of the origin of replication. Mimivirus genes are preferentially transcribed in the orientation going away from this putative origin of replication, thus defining a 'leading strand' (with 578 genes) and a 'lagging strand' (with 333 genes). Such a strong asymmetrical distribution of genes is unique among known nucleocytoplasmic large DNA viruses. Despite this local asymmetry, the total number of genes on both strands are very similar (450 'R' vs. 461 'L' genes). The overall amino acid composition of the predicted proteome is strongly biased in favor of residues encoded by codons rich in A + T such as isoleucine, asparagine, and tyrosine. The relative usage of synonymous codons for any given amino acid is also biased by the high A + T percentage. This has made the production of recombinant mimivirus proteins difficult in traditional *Escherichia coli* expression systems. Paradoxically, the preferred codon usage in mimivirus genes is almost the opposite to the one exhibited by its host *Acanthamoeba castellanii* or *A. polyphaga* (the most frequently used codons being the rarest in the amoebal genes). It is also quite different from the more even distribution observed in human and vertebrate genes.

Mimivirus genome contains multiple traces of gene duplications, including a few ancient en bloc duplication events, many dispersed individual gene duplications, and recurrent tandem duplication events leading to large families of co-localized paralogs. Overall, one-third of mimivirus genes have at least one paralog. The analysis of gene collinearity indicates that genome segments [1–110 000] and [120 000–200 000] have been duplicated at positions approximately symmetrical with respect to the chromosome center. The largest paralogous families include an ankyrin-repeat-containing protein (with 66 members), BTB/POZ-domain-containing proteins (26 members), and a 14-paralog family of proteins of unknown function, of which 12 are arranged in a perfect tandem repeat. In contrast to parasitic/endosymbiotic bacteria with genome of comparable size (such as *Rickettsia*), the mimivirus genome exhibits no pseudogene, and thus no sign of an ongoing genome reduction/degradation

process. Mimivirus does not appear to be under any evolutionary pressure to decrease the size of its genome. The segmental duplication and massive individual gene duplication explain the origin of a large part of the mimivirus genome, without the need to invoke an exceptional propensity for horizontal gene transfers.

Promoter Structure

A unique feature of the mimivirus genome is the presence of the motif AAAATTGA in the 150 nt upstream region of nearly 50% of the protein-coding genes. From its strong preferential occurrence in the 5′ upstream region (mostly at positions ranging from −50 to −75 from the initiator ATG), it is assumed that this motif corresponds to a specific promoter signal. The presence of the AAAATTGA motif significantly correlates with genes transcribed from the leading strand (313/578 = 54% vs. 133/313 = 40%). It also correlates with predicted gene functions normally associated with the early and late/early phase of the virus replication (nucleotide metabolism, transcription, translation). As the AAAATTGA motif is not prevalent in the genome sequence of amoebal organisms, it is hypothesized that this motif is a core promoter element specifically recognized by the transcription preinitiation complex encoded by the mimivirus genome (mainly the large and small RNA polymerase subunits and a remote homolog of the TFIID 'TATA box-binding' initiation factor). The reasons behind the lack of variability of this mimivirus promoter element, usually quite degenerate among eukaryotes and eukaryotic viruses, are unknown.

Gene Content

As is usual for viruses, only a third of the 911 protein-coding genes of mimivirus have been associated with functional attributes. About 200 of these genes exhibit significant matches to 108 distinct COG families. The genome also encodes six tRNAs (three $tRNA_{leu}$, one $tRNA_{trp}$, one $tRNA_{cys}$, and one $tRNA_{his}$).

Typical NCLDV Core Genes in Mimivirus

The comparative genomic study of NCLDV suggested the monophyletic origin of four viral families: the *Asfarviridae*, the *Iridoviridae*, the *Phycodnaviridae*, and the *Poxviridae*. These studies identified sets of core genes (shared across these viral families) with various levels of conservation: class I core genes are found in all species from all families, class II core genes are absent in some species from a given family, while class III ones are shared by three families.

A strong argument for classifying mimivirus as a *bona fide* member of the NCLDV is the presence of 9/9 class I core gene homologs in its genome, 6/8 of the class II core genes, and 11/14 of the class III. Remarkably, the two class II core genes missing from mimivirus genomes encode two enzymes central to DNA synthesis: thymidylate kinase (catalyzing the formation of dTDP from dTMP) and the quasi-universal detoxifying enzyme dUTPase (dUTP pyrophosphatase, transforming dUTP into dUMP, to avoid the misincorporation of uracyl in DNA).

Phylogenetic analyses using class I core gene sequences let to mimivirus being assigned to a new family, the *Mimiviridae*, distinct from the four previously defined NCLDV families (**Figure 5**).

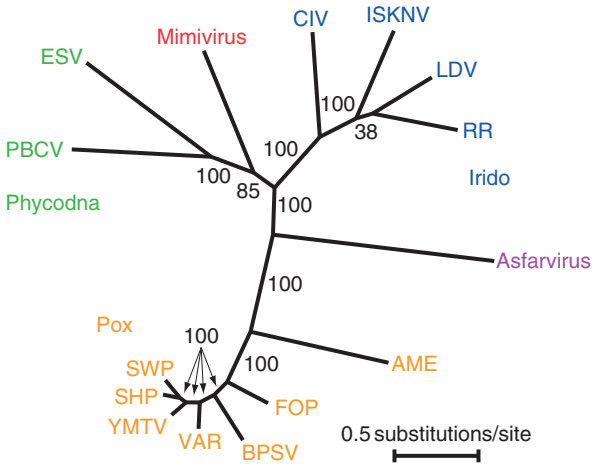


Figure 5 Phylogenetic position of mimivirus among established NCLDV families. Viral species representing the diverse families of NCLDV are included as follows: *Acanthamoeba polyphaga mimivirus, Phycodnaviridae* (*Paramecium bursaria chlorella virus* (PBCV) and *Ectocarpus siliculosus virus* (ESV)), *Iridoviridae* (*Chilo iridescent virus* (CIV), *Regina ranavirus* (RR), *Lymphocystis disease virus type 1* (LDV), and *Infectious spleen and kidney necrosis virus* (ISKNV)), *Asfarviridae* (*African swine fever virus*), and *Poxviridae* (*Amsacta moorel entomopoxvirus* (AME), *Variola virus* (VAR), *Fowlpox virus* (FOP), *Bovine papular stomatitis virus* (BPSV), *Yaba monkey tumor virus* (YMTV), *Sheeppox virus* (SHP), and *Swinepox virus* (SWP)). This tree was built with the use of maximum likelihood and based on the concatenated sequences of eight conserved proteins (NCLDV class I genes): vaccina virus (VV) D5-type ATPase, DNA polymerase family B, VV A32 virion packaging ATPase, capsid protein, thiol oxidoreductase, VV D6R helicase, serine/threonine protein kinase, and A1L transcription factor. One of the class I genes (VV A18 helicase) is absent in LDV and was not included. The alignment contains 1660 sites without insertions and deletions. A neighbor-joining tree and a maximum-parsimony tree exhibited similar topologies. Bootstrap percentages are shown along the branches. Reproduced from Raoult D, Audic S, Robert C, *et al.* (2004) The 1.2-megabase genome sequence of mimivirus, *Science* 306: 1344–1350, with permission from American Association for the Advancement of Science.

Remarkable Genes Found in Mimivirus

Translation-related genes

The mimivirus genome exhibits numerous genes encoding central components of the protein translation system. This was most unexpected, as viruses are traditionally distinguished from cellular organisms by their inability to perform protein synthesis independently from their host. This incomplete virally encoded translation subsystem includes four amynoacyl-tRNA synthetases (ArgRS, TyrRS, CysRS, and MetRS). These enzymes, responsible for the accurate loading of a given amino acid on its cognate tRNAs, are the true enforcers of the genetic code. The enzymatic activity of the TyrRS has been experimentally validated, as well as its strict specificity for tyrosine and its eukaryotic $tRNA_{Tyr}$.

In addition, mimivirus genome encodes two translation initiation factors: a GTP-binding elongation factor (EF-Tu) and a peptide chain release factor. Finally, mimivirus exhibits the first virus-encoded tRNA (Uracil-5)-methyltransferase. This enzyme is responsible for the U to T modification characterizing the T-loop in all tRNAs, a region involved in ribosome recognition, as well as aminoacylation and the binding of EF-Tu. Besides these unique translation-related enzymes, mimivirus encodes six tRNAs, a common feature in some NCLDV, such as phycodnaviruses.

DNA repair-related genes

The mimivirus genome encodes a comprehensive set of DNA repair enzymes, covering all types of DNA damage: alkylating agents, ultraviolet (UV) light, or ionizing radiations. In particular, mimivirus is the first virus to exhibit a formamidopyrimidine-DNA glycosylase (used to excise oxidized purines), a UV-damage endonuclease, a 6-*O*-methylguanine-DNA methyltransferase (used to get rid of O^6-alkylguanine), and a homolog to the DNA mismatch repair enzyme MutS. Mimivirus also possesses DNA topoisomerases, the enzymes required for solving entanglement problems associated with DNA replication, transcription, recombination, and chromatin remodeling. There are three mimivirus-encoded topoisomerases: first the type IA topoisomerase identified in a virus, the usual type IIA topoisomerase (such as found in most NCLDVs), and a type IB topoisomerase as found in the family *Poxviridae*.

DNA synthesis

The mimivirus genome exhibits the only known virally encoded nucleoside diphosphate kinase (NDK) protein. This enzyme catalyzes the synthesis of nucleoside triphosphate (other than ATP) from ATP. Its activity has been demonstrated experimentally, and exhibits a specific affinity for deoxypyrimidine nucleotides. This enzyme may help alleviate limited supplies in dTTP and dCTP for DNA synthesis. The genome also encodes a deoxynucleoside kinase (DNK), a thymidylate synthase (dUMP to dTMP), as well as the salvage enzyme thymidine kinase (TMP synthesis from thymidine).

Host signaling interfering pathways

The mimivirus genome encodes a large number of putative proteins exhibiting ankyrin repeats (66), a BTB/POZ domain (26), an F-box domain (10), and a kinase domain (9). Genes harboring these features are frequently involved in protein–protein interactions (ankyrin repeat), ubiquitin-mediated interactions (BTB/POZ, F-Box), and intracellular signaling (kinases). Remarkably, mimivirus exhibits three proteins exhibiting both a cyclin and a cyclin-dependent kinase (CDK) domain. It is likely that these proteins, by interfering with the cell signaling pathways, play a central role in turning the amoebal host into an efficient virus factory. On the other hand, identifying the cellular targets of these viral proteins, and their precise mode of action, might reveal original and valuable new directions for the therapeutic manipulation of eukaryotic cell metabolism.

Miscellaneous metabolic pathways

Previous analyses of NCLDV genomes, in particular from phycodnaviruses, revealed that these viruses possess biosynthetic abilities going well beyond the minimal requirements of viral DNA replication, transcription, and virion-packaging systems.

Mimivirus builds on this established trend by exhibiting a wealth of amino acid-, lipid-, and sugar-modifying enzymes, albeit all of them in the form of apparently incomplete (virus-encoded) pathways. For instance, mimivirus encodes three enzymes related to the metabolism of glutamine: an asparagine synthase (glutamine hydrolyzing), a glutamine synthase, and a glutamine-hydrolyzing guanosine 5′-monophosphate synthase. The reasons behind the special affinity of the virus for glutamine are unknown. Mimivirus also encodes three lipid-modifying enzymes: a cholinesterase, a lanosterol 14α-demethylase, and a 7-dehydrocholesterol reductase. Although these enzymes might be involved in the disruption of the host cell membrane, none of them have been described as participating in the infection process in other viruses. Finally, mimivirus exhibits an impressive array of enzymes normally involved in the synthesis of complex polysaccharides, such as perosamine, a high molecular weight capsular material found in bacteria. Mimivirus particles (see below) retain the Gram stain, a unique phenomenon that might be related to the presence of a densely reticulated (lipo)polysaccharide layer at their surface. In this context, the mimivirus-encoded procollagen-lysine, 2-oxoglutarate 5-dioxygenase, an enzyme that hydroxylates lysine residues in collagen-like peptides, might be central both to the attachment of carbohydrate moieties and to the

formation of intermolecular cross-links. It is tempting to speculate that the unique hairy-like appearance of the virion, together with its resistance to chemical and mechanical disruption, is directly linked to the presence of a layer of heavily cross-linked lipo-proteo-polysaccharide material covering the 'regular' capsid.

Intein and introns

Inteins are protein-splicing domains encoded by mobile intervening sequences. They catalyze their own excision from the host protein. Although found in all domains of life (Eukarya, Archaea, and Eubacteria), their distribution is highly sporadic. Mimivirus is one of the few dsDNA viruses exhibiting an intein, inserted within its DNA polymerase B gene. Mimivirus intein is closely related to the one found in the DNA polymerase of heterosigma akashiwo virus (HaV), a large dsDNA virus infecting the single-cell bloom-forming raphidophyte (golden brown alga) *H. akashiwo*. Both appear monophyletic to the archaeal inteins.

Type I introns are self-splicing intervening sequences that are excised at the mRNA level. One type IB intron has been identified in several chlorella viruses species, but they remain rare in eukaryotic viruses. Mimivirus exhibits four self-excising (predicted) introns: one in the largest RNA polymerase subunit gene, the other three in the second-largest subunit. Given that introns are mostly detected when they interrupt the coding sequence of known proteins, some additional instances located within anonymous open reading frames (ORFs) might have escaped detection.

Particle Structure

Morphology

The discovery of the characteristic (i.e., icosahedral) viral morphology exhibited by mimivirus particles (initially mistaken as small Gram-positive intracellular bacteria) under the electron microscope was the turning point in its correct identification as a *bona fide* virus. Despite its unprecedented size, the icosahedral symmetry of the particle is good enough to allow a computer-generated three-dimensional (3-D) reconstruction at a resolution of about 75 Å, from series of cryoelectron microscopy (cryo-EM) images. Mimivirus has a capsid with a diameter of *c.* 0.5 μm, covered by 0.125-μm-long, closely packed fibers (**Figure 6**). The total diameter of a free particle is thus about 0.75 μm, consistent with its visibility in the light microscope following Gram staining (**Figure 1**). The pseudo-triangulation for mimivirus particles is estimated at $T \approx 1180$, predicting that the whole capsid is constituted of *c.* 70 000 individual molecules of the L425 ORF-encoded major capsid protein. Inside a 70-Å-thick protein shell, cryo-EM images suggest the presence of two 40-Å-thick lipid membranes, a structure also found in other NCLDVs such as the African swine fever virus and some poxviruses.

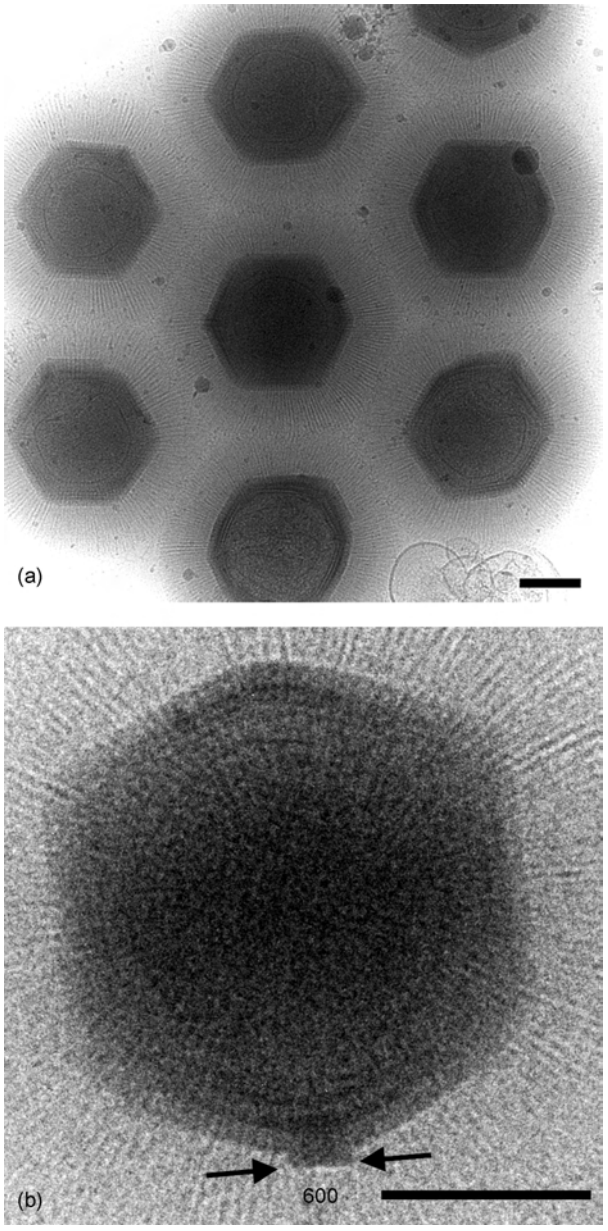


Figure 6 Cryo-EM high-quality images of mimivirus particles. (a) Cluster of mature particles, exhibiting a solid and compact fiber layer. (b) Close-up of one particle (0.75 μm across) exhibiting a densely packed layer of cross-linked fibers, and a unique vertex. Reproduced from Xiao C, Chipman PR, Battisti AJ, *et al*. (2005) Cryo-electron microscopy of the giant mimivirus. *Journal of Molecular Biology* 353: 493–496, with permission from Elsevier.

Virion Proteomics

A detailed study of purified isolated virions using various electrophoresis separation methods followed by mass spectrometry analysis indicated that 114 different mimivirus

genes are present in the viral particle. Many of these genes correspond to multiple products, exhibiting a variety of post-translational modifications including glycosylation (such as observed for the major capsid protein L425), proteolysis, and, most likely, phosphorylation.

Besides the expected *bona fide* 'structural' proteins (i.e., capsid proteins, major core protein, A16L-like virion associated protein, lipocalin-like lipoprotein, and a 'spore coat' assembly factor), a large number of proteins with enzymatic functions have been identified. For instance, mimivirus particles appear to possess a complete transcriptional apparatus including all virus-encoded DNA-directed RNA polymerase subunits (five), as well as four transcription factors, its mRNA capping enzyme, and two helicases. Nine gene products all related to oxidative pathways constitute the next largest functional group of particle-associated enzymes. These enzymes are probably important to cope with the oxidative stress generated by the host defense. Five proteins associated to DNA topology and damage repair are also present in the particle. Finally, seven enzymes associated to lipid (lipase and phosphoesterase) or protein modification (kinases and phosphatase) complete the pool of particle enzymes, together with the two tRNA methyltransferases. Yet, the majority of particle-associated proteins are the products of genes (65) of unknown function, of which 45 exhibit no convincing similarity in databases. It is worth noticing that only a small fraction (13/114) of the products associated with the particle are encoded by genes harboring the AAAATTGA promoter signal, confirming its correlation with early and late/early viral functions.

The functions predicted for the particle-associated gene products suggest a possible scenario for the early stage of the virus replicative cycle. First, it is likely that the lipolytic and proteolytic enzymes are part of the process by which the virus penetrates the amoeba cytoplasm from its vacuolar location. The two topoisomerases (IA and IB) might then be involved in the unpacking of the DNA from the particle, and its injection into the cytoplasm. The DNA repair enzymes might then get into action prior to starting transcription. The complete transcription machinery found in the viral particle is very similar to that in poxviruses. This suggests that some transcription could take place in the host cytoplasm immediately upon mimivirus infection.

Particle-Associated mRNAs

In a way reminiscent of members of the family *Herpesviridae*, a number of virus-encoded mRNAs are found associated with the virus particle. They include the messengers for DNA polymerase (R322), the major capsid protein (L425), the TFII-like transcription factor (R339), the tyrosyl-, cysteinyl-, and arginyl-tRNA synthetase (L124, L164, and R663), and four proteins of unknown function. These immediate early gene transcripts may be needed to initiate the first step of the replicative cycle, prior to any viral gene expression.

Evolution: The Position of Mimivirus in the Tree of Life

Of the 63 homologous genes common to all known unicellular organisms from the three domains of life (Eukarya, Eubacteria, and Archaea), seven have been identified in the mimivirus genome: three aminoacyl-tRNA synthetases, the two largest RNA polymerase subunits, the sliding clamp subunit of the DNA polymerase (PCNA), and a 5′-3′ exonuclease. The unrooted phylogenetic tree built from the concatenated sequences of the corresponding proteins indicates that mimivirus branches out near the origin of the Eukaryota domain (**Figure 7**). This position is consistent with several competing hypotheses proposing that large dsDNA viruses either predated, participated in, or closely followed, the emergence of the eukaryotic cell.

Other Possible Members of the Family *Mimiviridae*

Mimivirus was serendipitously discovered within *A. polyphaga*, a free-living ubiquitous amoeba, prevalent in aquatic environments. Many of the mimivirus core genes exhibit a

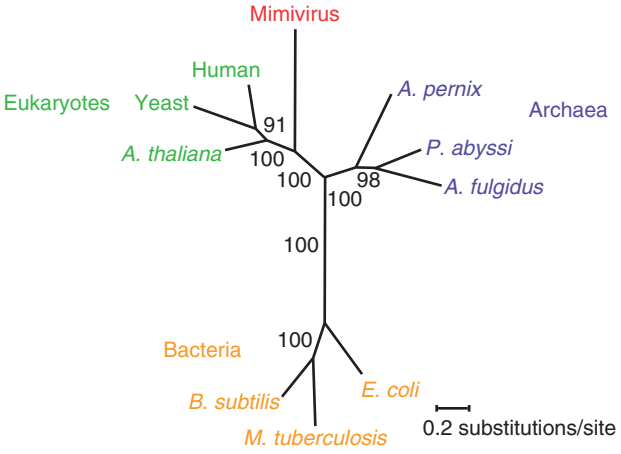


Figure 7 A phylogenetic tree of species from the three domains of life (Eukaryota, Eubacteria, and Archaea) and mimivirus. The tree was inferred with the use of a maximum-likelihood method based on the concatenated sequences of seven universally conserved protein sequences: arginyl-tRNA synthetase, methionylt-RNA synthetase, tyrosyl-tRNA synthetase, RNA polymerase II largest subunit, RNA polymerase II second largest subunit, PCNA, and 5′-3′ exonuclease. Bootstrap percentages are shown along the branches. Reproduced from Raoult D, Audic S, Robert C, *et al.* (2004) The 1.2-megabase genome sequence of mimivirus. *Science* 306: 1344–1350, with permission from American Association for the Advancement of Science.

phylogenetic affinity with members of the family *Phycodnaviridae* (algal and phytoplankton viruses). It can be expected therefore that additional viruses belonging to the family *Mimiviridae* will be found in aquatic/marine environments, for instance in marine protists. The bioinformatic analysis of environmental (metagenomics) DNA sequences strongly suggests that large viruses evolutionarily closer to mimivirus than any known virus species may be found among randomly collected bacteria-sized (passing through 3 μm but retained on 0.3 μm filters) marine microorganisms. Close ecological encounters of mimivirus ancestors with gorgonian octocorals (genus *Leptogorgia*) are also suggested by clear evidence of a lateral transfer of a mismatch repair (MutS) gene. Since very little is known about viruses infecting coral-associated microbial populations, it seems likely that new species of *Mimiviridae* might be hiding within this complex ecological niche.

See also: Origin of Viruses; Nature of Viruses; Virus Evolution: Bacterial Viruses.

Further Reading

Abergel C, Chenivesse S, Byrne D, Suhre K, Arondel V, and Claverie JM (2005) Mimivirus TyrRS: Preliminary structural and functional characterization of the first amino-acyl tRNA synthetase found in a virus. *Acta Crystallographica Section F* 61: 212–215.

Benarroch D, Claverie J-M, Raoult D, and Shuman S (2006) Characterization of mimivirus DNA topoisomerase IB suggests horizontal gene transfer between eukaryal viruses and bacteria. *Journal of Virology* 80: 314–321.

Berger P, Papazian L, Drancourt M, La Scola B, Auffray JP, and Raoult D (2006) Amoeba-associated microorganisms and diagnosis of nosocomial pneumonia. *Emerging Infectious Diseases* 12: 248–255.

Claverie JM, Ogata H, Audic S, Abergel C, Suhre K, and Fournier P-E (2006) Mimivirus and the emerging concept of 'giant' virus. *Virus Research* 117: 133–144.

Ghedin E and Claverié JM (2005) Mimivirus relatives in the Sargasso Sea. *Virology Journal* 2: 62.

La Scola B, Audic S, Robert C, *et al.* (2003) A giant virus in amoebae. *Science* 299: 2033.

La Scola B, Marrie TJ, Auffray JP, and Raoult D (2005) Mimivirus in pneumonia patients. *Emerging Infectious Diseases* 11: 449–452.

Ogata H, Raoult D, and Claverie JM (2005) A new example of viral intein in mimivirus. *Virology Journal* 2: 8.

Raoult D, Audic S, Robert C, *et al.* (2004) The 1.2-megabase genome sequence of mimivirus. *Science* 306: 1344–1350.

Raoult D, Renesto P, and Brouqui P (2006) Laboratory infection of a technician by mimivirus. *Annals of Internal Medicine* 144: 702–703.

Renesto P, Abergel C, Decloquement P, *et al.* (2006) Mimivirus giant particles incorporate a large fraction of anonymous and unique gene products. *Journal of Virology* 80: 11678–11685.

Suhre K (2005) Gene and genome duplication in Acanthamoeba polyphaga mimivirus. *Journal of Virology* 79: 14095–14101.

Suhre K, Audic S, and Claverie JM (2005) Mimivirus gene promoters exhibit an unprecedented conservation among all eukaryotes. *Proceedings of the National Academy of Sciences, USA* 102: 14689–14693.

Suzan-Monti M, La Scola B, and Raoult D (2006) Genomic and evolutionary aspects of mimivirus. *Virus Research* 117: 145–155.

Xiao C, Chipman PR, Battisti AJ, *et al.* (2005) Cryo-electron microscopy of the giant mimivirus. *Journal of Molecular Biology* 353: 493–496.

Molluscum Contagiosum Virus

J J Bugert, Wales College of Medicine, Heath Park, Cardiff, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Acanthoma A tumor composed of epidermal or squamous cells.
Anamnesis The complete history recalled and recounted by a patient.
Epicrisis A series of events described in discriminating detail and interpreted in retrospect.

History and Classification

The typical molluscum lesion, a smooth, dome-shaped, flesh-colored protrusion of the skin with a typical central indentation, was first described by Edward Jenner (1749–1823) as a 'tubercle of the skin' common in children. Thomas Bateman (1778–1821) first used the term 'molluscum contagiosum' (MC). In 1841, 'molluscum bodies', intracytoplasmic inclusions in the epidermal tissues of MC lesions, were independently observed by Henderson and Paterson. Similarities of molluscum bodies to the 'Borrel' bodies in fowlpox-infected tissues were noted later by Goodpasture, King, and Woodruff. At the beginning of the last century, Juliusberg demonstrated that the etiological agent of MC cannot be removed by filtration through Chamberland filters. Filtrates were used to infect human volunteers, who developed MC between 25 and 50 days after inoculation. Short of fulfilling all the Koch postulates, an animal model for MC has not been established to this day. Smaller elementary bodies inside the molluscum bodies were observed by Lipschütz in 1911. Electron microscopy revealed these elementary bodies to be poxvirus particles with dimensions of 360 nm × 210 nm.

Virion, Genome, and Evolution

Molluscum contagiosum virus (MCV) particles have a typical poxviral morphology (**Figure 1**). The virions are enveloped, pleomorphic, but generally ovoid to brick-shaped, with a dumbbell-shaped central core and lateral bodies similar to those in orthopoxvirus virions. MCV cores show complex structural patterns. Virions are often found to have membrane fragments loosely attached to them, indicating a noncontinuous lipid envelope wrapping the core.

The genome of MCV is a double-stranded DNA molecule of 190 289 bp (GenBank accession U60315: MCV type 1/80) with covalently closed termini (hairpins) and about 4.2 kbp of terminally inverted repeats (**Figure 2**). This excludes 50–100 bp of terminal hairpin sequences that could not be cloned or sequenced because replicative intermediates are not apparent in DNA from MCV biopsy specimens. The genomes of MCV (genus *Molluscipoxvirus*, subfamily *Chordopoxvirinae*), crocodilepox virus, and parapoxviruses stand out in the family *Poxviridae* because they have G+C contents of over 60%.

The MCV genome encodes 182 nonoverlapping open reading frames of more than 45 codons (**Figure 2**), almost half of which have no similarities to known proteins. Hypothetical MCV structural proteins and proteins encoding enzymes of the replication and transcription apparatus share obvious homologies to other poxvirus proteins. Less-obvious homologies exist between MCV and avipoxviruses (MC130, MC133, and MC131 A-type inclusion body-like proteins) and notably between MCV, parapoxviruses, and crocodilepox virus (MC026 modified RING protein and a number of proteins shared between only two of the above poxviruses). Unique MCV nonstructural proteins that are not involved in replication or transcription can be divided into two functional classes: (1) proteins dealing with the host immune system (host-response-evasion factors), such as the MCV chemokine antagonist (MC148) and the interleukin-18 (IL18)-binding protein (MC054), and (2) proteins supporting MCV replication in the host cell or the host tissue (host cell/tissue-modulating factors), such as the antiapoptotic selenoprotein MC066 and the Hrs-binding protein MC162.

An epidermal growth factor (EGF) homolog similar to the ones expressed by other poxviruses was not found in the genome of MCV. The only other poxvirus that does not encode this factor is swinepox virus. However, MCV-infected basal keratinocytes seem to increase EGF receptor and transferrin receptor expression, in comparison to uninfected epidermis. Inducing EGF receptor expression may be an indirect mechanism causing epidermal hyperproliferation. MCV is the only chordopoxvirus that does not encode a J2R-like thymidine kinase.

In a phylogenetic analysis of 26 poxvirus genomes, MCV (representing the molluscipoxviruses) formed a group by itself among the subfamily of chordopoxviruses, separate from avipoxviruses (fowlpox virus), orthopoxviruses (vaccinia and variola viruses), and all other genera.

Four main genetic subtypes of MCV have been identified by DNA fingerprinting. MCV type 1 prototype (p) is the most common genetic type (98%) in immune-competent hosts in Western Europe. MCV type 1 (including variants) is the most common genetic type worldwide. MCV types 2–4 are relatively more commonly seen in immunocompromised individuals. One type has only been described in Japan. MCV genotypes discernible by DNA fingerprinting do not change when the viruses are transmitted between family members or in larger contact groups, indicating a low overall mutation rate.

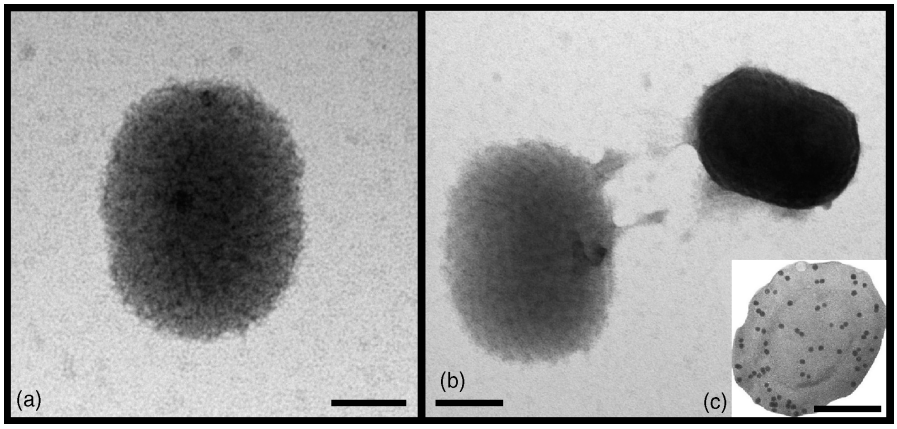


Figure 1 Electron microscope images of MCV particles negatively stained using the ammonium molybdate technique. (a) MCV particle showing the typical core protein pattern. (b) MCV particles: one that has lost the envelope appears larger and shows the typical core protein pattern, while the other is still wrapped in membrane and appears smaller. (c) Five nanometer gold particles bound to mouse antihuman antibodies, detecting human polyclonal patient antibodies binding to the surface of an MCV particle. Scale = 100 nm (a–c). Electron microscopy: Bugert and Hobot, Cardiff University School of Medicine, 2005.

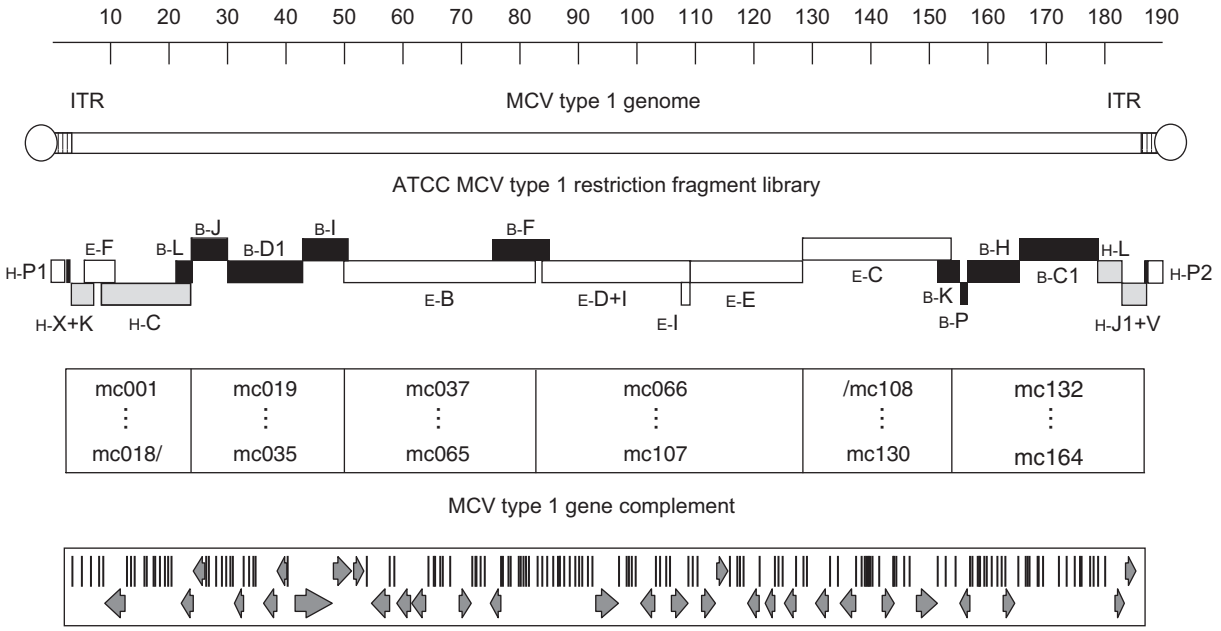


Figure 2 The MCV genome. The scale at the top is in kbp. The inverted terminal repeats (ITRs) are shown as vertical stripes flanking the genome, pointing inward from both covalently closed ends. The ITRs do not contain any complete coding sequences. The area covered by the ATCC MCV genome fragment library is shown as white (EcoRI restriction fragments), gray (HindIII restriction fragments), and black (BamHI restriction fragments) boxes representing the respective size of the viral sequence insert of each clone. The terminal HindIII restriction fragments P1 and P2 do not contain protein-coding regions and were not cloned. MCV genes are given the lowercase prefix 'mc', whereas (in the text) MCV proteins are given the uppercase prefix 'MC'. The MCV genes encoded in different parts of the genome, as defined by the boundaries of defined restriction fragments, are indicated in white boxes. The slashes signify ORFs that straddle a restriction site used for cloning and that are therefore not completely contained in any one plasmid on either side of the restriction site. Two MCV genes (mc036 and mc131) are split between two cloned restriction fragments, and therefore not listed. The numbering of MCV genes ends at mc164, the original number of genes larger than 90 codons. An additional 18 genes have been identified as being larger than 45 codons and are numbered as appendices of preceding genes (e.g., mc004.1). Protein-coding regions of the MCV type 1 gene complement are shown at the bottom as arrows (larger than 1.5 kbp) or small rectangles.

Host Range and Virus Propagation

MCV, like smallpox virus, is considered to be an exclusive pathogen of man. Reports of MCV in a number of animals, including horses, chimpanzees, and kangaroos, have not been supported by DNA sequence confirmation. Many pox-like infections of vertebrates, most of them caused by orthopoxviruses, can be confused with MCV by their clinical appearance. Conventional immune-competent laboratory animals, including mice, rats, guinea pigs, and tree shrews, do not support MCV replication in their skin. MCV-infected human keratinocytes have been transplanted into mice with severe combined immune deficiency (SCID) and typical MCV lesions have subsequently developed in these non-natural hosts. Attempts to passage the virus in SCID mice were unsuccessful. However, despite the absence of molecular evidence, an animal reservoir of MCV cannot be excluded.

MCV has so far not been grown in conventional human cell lines, including immortalized tumorigenic/virus-transformed and nontumorigenic skin keratinocytes (HaCaT, NIKS). Experiments with *ex vivo* cultures of human skin cells (raft cultures) are ongoing. MCV may use a vegetative mechanism for replicating in differentiating keratinocytes.

In the absence of culturable virus, classical virological research on MCV is severely restricted. All progress so far has been made by studying MCV genes in isolation, based on the complete MCV genome sequence gained from an overlapping redundant MCV genome fragment library. This reagent has been made available to the ATCC. The entire MCV gene complement is covered by 18 recombinant bacterial plasmid clones harboring viral sequences from the EcoRI, BamHI, and HindIII restriction fragment libraries of MCV type 1/80 (**Figure 2**).

Further research is being carried out using abortive cell culture systems. MCV induces a remarkable cytopathogenic effect (CPE) in human fibroblasts, both in primary cells (MRC5) and in telomerase-transduced immortal cell lines (hTERT-BJ-1). The CPE starts 4 h post infection (p.i.) and reaches a maximum at 24 h p.i., with the cells looking as if they have been trypsinized, partially detaching from the monolayer, rounding, and clumping. Cells settle down at 48–72 h p.i., but show a morphological transformation from an oblong fibroblast to a more square epithelial-looking cell type. MCV transcribes early mRNA in these cells. The

CPE is not induced by UV-inactivated virions or in the presence of cycloheximide, indicating that expression of viral proteins is required. mRNA transcription can be detected by reverse transcriptase-polymerase chain reaction (RT-PCR) for months in serially passaged infected cells. A productive MCV infection cannot be rescued nongenetically by co-infection with other chordopoxviruses in these cells. MCV can infect human HaCaT keratinocytes and transcribes mRNA in these cells, but does not induce a CPE. MCV cannot infect nonhuman cells. It induces type 1 interferons in mouse and human embryo fibroblasts and IL8 in human lung epithelial cells, which it cannot infect, suggesting involvement of surface pathogen-associated molecular pattern (PAMP) receptors like TLR2 or TLR4. Removal of interferon pathways from cell lines susceptible for MCV infection would be worth investigating, in order to exclude interference causing abortive MCV infections. MCV is currently isolated from human-infected skin biopsies. MCV purified from biopsy material can be used for infection studies, electron microscopy, viral DNA extraction, and analyses of early mRNA synthesized by *in vitro* transcription of permeabilized virions.

Clinical Features and Pathology

The mean incidence of MC in the general population is 0.1–5%. Seroepidemiological studies have shown that antibody prevalence in persons over 50 years of age is 39%. This indicates that MC is a very common viral skin infection, which is supported by its common occurrence in dermatological practice. MCV outbreaks occur in crowded populations with reduced hygienic standards. Outbreaks with more than 100 cases have been described in kindergartens, military barracks, and public swimming pools. MC was found to be very common in the Fiji Islands.

MC is most often seen as a benign wart-like condition (German: *dellwarze*) with light pruritus in preadolescent children, but can occur in immuncompetent adults. MC is more severe in immunocompromised people or individuals with atopic dermatitis, where it can lead to giant molluscum and eczema molluscum. The lesions generally occur on all body surfaces, but not on the palm of the hand or on mucous membranes. They may be associated with hair follicles. MC lesions can grow close to mucous membranes on the lips and eyelids. When situated near the eye, they can lead to conjunctivitis. MC is transmitted by smear-infection with the infectious fluids discharged from lesions and by direct contact with contaminated objects. MC is not very contagious and therefore infection depends on a high inoculating dose. It is a sexually transmitted disease when lesions are located on or in the vicinity of sexual organs.

MC lesions are generally globular, sometimes ovate, 2–5 mm in diameter and sit on a contracted base. Cellular semiliquid debris can be expressed from the central indentation at the top of the lesion. The infection spreads via contact with this fluid. Histologically, the tumors are strictly limited to the epidermal layer of the skin and have a resemblance to hair follicles. They are therefore classified as acanthomas.

If not mechanically disturbed, MC lesions will persist for months and even years in immune-competent hosts, but can disappear spontaneously, probably when virus-infected tissue is exposed to the immune system. To expose the infection by limited (sterile) trauma is a way of treatment.

Scratching or disturbing the lesions leads to a quicker resolution but can complicate the condition through bacterial superinfection. This must be avoided in severely immunocompromised hosts, who develop widespread MC with hundreds of lesions of larger size and can succumb to sepsis following bacterial superinfection. MCV is a marker of late-stage disease in human immunodeficiency virus (HIV)-infected individuals, and in HIV-infected populations the incidence of MC was 30% before the onset of human cytomegalovirus (HCMV) prophylaxis with cidofovir.

MCV probably enters the epidermis through microlesions. The typical MCV lesion contains conglomerates of hyperplastic epithelial cells organized in follicles and lobes, which all develop into a central indentation toward the surface of the skin in a process similar to holocrine secretion. The whole lesion has the appearance of a hair follicle where the hair is replaced by the virus-containing plug. The central indentation is filled with cellular debris and is rich in elementary viral particles in a waxy plug-like structure. This plug becomes mobilized and spreads the infection to other areas of surrounding skin or contaminates objects. The periphery of the MCV lesion is characterized by basaloid epithelial cells with prominent nuclei, large amounts of heterochromatin, slightly basophilic cytoplasm, and increased visibility of membranous structures. These cells are larger than normal basal keratinocytes, they divide faster than normal basal cells, their cytoplasm contains a large number of vacuoles, and they are sitting on top of an intact basal membrane. The lesion is a strictly intraepidermal hyperplastic process (acanthoma). Distinct poxviral factories (molluscum bodies or Henderson–Patterson bodies) appear about four cell layers away from the basal membrane in the stratum spinosum. The inclusion bodies grow and obliterate cellular organelles. Cells with inclusion bodies do not divide further. The cytoplasm of MCV-producing cells shows keratinization, which is not expected at that stage of keratinocyte differentiation and indicates dyskeratinization in the sense of abnormal differentiation.

Immune Response

In undisturbed MC lesions, histological studies have shown a conspicuous absence of effectors of the cellular immune system, in particular skin-specific tissue macrophages (Langerhans cells). This is in contrast to papillomata, where a vigorous cellular immune response, including cytotoxic T lymphocytes (CTLs), is mounted immediately. The absence of macrophages has been attributed to the activity of various MCV genes that are suspected to make the MC lesion immunologically 'invisible'. This includes a biologically inactive IL8 receptor-binding beta-chemokine homolog (MC148), which may suppress the immigration of neutrophils; a major histocompatibility complex (MHC) class I homolog that may upset MHC class I antigen presentation on the surface of infected cells, or natural killer cell recognition; and an IL18-binding protein, which underlines the importance of this cytokine for the local immune response in human skin. As for the humoral response, MCV-specific antibodies have been detected in several studies, showing a seroprevalence of MCV of up to 40% in the general population, much higher than previously expected. However, these antibodies do not seem to confer a neutralizing immunity. MCV genes were expressed in a cowpox virus expression system and two antigenetically prominent MCV proteins identified: mc133L (70 kDa protein: MC133) and mc084L (34 kDa protein: MC084). These proteins are presumably glycosylated and present on the surface of MCV virions, where they allow binding of antibodies and detection by immune electron microscopy (**Figure 1(c)**).

Diagnosis, Treatment, and Prevention

MCV is readily diagnosed by its clinical appearance and by the typical histopathology found in sections of lesion biopsies. After the eradication of smallpox, MCV is the most commonly diagnosed poxviral infection. Ortho- and parapoxviral zoonoses are rare. In the differential diagnosis of MC, smallpox must be considered along with other ortho- and parapoxviruses. The anamnesis must cover contacts with pets, especially gerbils and chipmonks of African origin (monkeypox) as well as local rodents and cats (cowpox). Always looming in the background is the possibility of a smallpox bioterrorist attack. An actual example for the management of such a contingency is the epicrisis of a monkeypox outbreak in Wisconsin, USA, in 2003 published by the Centers for Disease Control and Prevention (CDC). The most likely nonpoxviral differential diagnosis is varicella-zoster virus. Other viral agents can be excluded by - electron microscopy and PCR. Further differential diagnoses include syphilis, papilloma, and skin malignancies such as melanoma.

MC lesions are generally self-limiting, with an average of 6 months to 5 years for lesions to disappear. Patients with immune dysfunction or atopic skin conditions have difficulty clearing lesions. Therapy is recommended for genital MC to avoid sexual transmission, and should include antipruritics to prevent scratching and bacterial superinfection.

Treatment options cover a wide range of invasive and topical treatment strategies (**Table 1**). Topical application of salicylic acid or removal by curettage emerge as the two most successful strategies for large and small numbers

Table 1 Suggested MC treatments, side effects, and success rates

Symptom	*Therapy*	*Side effects*	*Success rates*
Pruritus	Antihistamine ointments	Tiredness	High
Acanthoma	Curettage (surgical) with sharp spoon. Local anesthetics required for children	Scar, pain impractical for large lesion numbers	High
	Lancing of lesion with needle	Pain, infection	High
	Cryosurgery with topical anesthesia	Pain, no scars	Medium
	Topical salicylic acid colloid, for example, occlusal (26% salicylic acid in polyacrylic vehicle)		High
	Immune modulators Tacrolimus 0.1% ointment Pimecrolimus 0.5% ointment Imiquimod 0.5% ointment Podophyllotoxin 0.5% ointment	Predisposition for bacterial and fungal skin infections	Low
	Antivirals (high cost) DNA polymerase inhibitors Acyclic nucleoside phosphonates (e.g., topical cidofovir) Topoisomerase inhibitors Lamellarin Coumermycin Cyclic depsipeptide sansalvamide A	Allergy	High

of lesions, respectively. Needling of lesions has been reported to work and may expose MCV to immune effectors. In immune-suppressed individuals with widespread MC, topical immune modulators like imiquimod have been tried with limited success. Topical antivirals work but are not cost-effective.

MC is best prevented by exposure prophylaxis. The vaccine against smallpox (vaccinia virus) does not protect against MCV, underlining the fundamental antigenic differences between ortho- and molluscipoxviruses.

See also: Capripoxviruses; Cowpox Virus; Electron Microscopy of Viruses; Entomopoxviruses; Fowlpox virus and other avipoxviruses; Leporipoviruses and Suipoxviruses; Mousepox and Rabbitpox Viruses; Parapoxviruses; Poxviruses; Smallpox and Monkeypox Viruses; Vaccinia Virus; Virus Databases; Yatapoxviruses.

Further Reading

Brown T, Butler P, and Postlethwaite R (1973) Non-genetic reactivation studies with the virus of molluscum contagiosum. *Journal of General Virology* 19: 417–421.

Bugert JJ (2006) Molluscipoxviruses. In: Mercer A, Schmidt A, and Webber O (eds.) *Advances in Infectious Diseases*, pp. 89–112. Basel: Birkhauser.

Bugert JJ and Darai G (1991) Stability of molluscum contagiosum virus DNA among 184 patient isolates: Evidence for variability of sequences in the terminal inverted repeats. *Journal of Medical Virology* 33: 211–217.

Bugert JJ and Darai G (2000) Poxvirus homologues of cellular genes. *Virus Genes* 21: 111–133.

Buller RM, Burnett J, Chen W, and Kreider J (1995) Replication of molluscum contagiosum virus. *Virology* 213: 655–659.

Konya J and Thompson CH (1999) Molluscum contagiosum virus: Antibody responses in persons with clinical lesions and seroepidemiology in a representative Australian population. *Journal of Infectious Diseases* 179: 701–704.

McFadden G, Pace WE, Purres J, and Dales S (1979) Biogenesis of poxviruses: Transitory expression of molluscum contagiosum early functions. *Virology* 94: 297–313.

Melquiot NV and Bugert JJ (2004) Preparation and use of molluscum contagiosum virus from human tissue biopsy specimens. *Methods in Molecular Biology* 269: 371–384.

Porter CD and Archard LC (1992) Characterisation by restriction mapping of three subtypes of molluscum contagiosum virus. *Journal of Medical Virology* 38: 1–6.

Postlethwaite R and Lee YS (1970) Sedimentable and non-sedimentable interfering components in mouse embryo cultures treated with molluscum contagiosum virus. *Journal of General Virology* 6: 117–125.

Reed RJ and Parkinson RP (1977) The histogenesis of molluscum contagiosum. *American Journal of Surgical Pathology* 1: 161–166.

Scholz J, Rosen-Wolff A, Bugert J, *et al.* (1998) Molecular epidemiology of molluscum contagiosum. *Journal of Infectious Diseases* 158: 898–900.

Senkevich TG, Bugert JJ, Sisler JR, Koonin EV, Darai G, and Moss B (1996) Genome sequence of a human tumorigenic poxvirus: Prediction of specific host response-evasion genes. *Science* 273: 813–816.

Thompson CH, Yager JA, and Van Rensburg IB (1998) Close relationship between equine and human molluscum contagiosum virus demonstrated by *in situ* hybridisation. *Research in Veterinary Science* 64: 157–161.

Viac J and Chardonnet Y (1990) Immunocompetent cells and epithelial cell modifications in molluscum contagiosum. *Journal of Cutaneous Pathology* 17: 202–205.

Vreeswijk J, Leene W, and Kalsbeek GL (1976) Early interactions of the virus molluscum contagiosum with its host cell. Virus-induced alterations in the basal and suprabasal layers of the epidermis. *Journal of Ultrastructural Research* 54: 37–52.

Mononegavirales

A J Easton and R Ling, University of Warwick, Coventry, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Antigenome An RNA molecule complementary in sequence to the single-stranded genomic nucleic acid of a virus.

Nucleocapsid Internal structure of a virus containing its genetic material and one or more proteins. It may exist in infected cells independently of virus particles.

Introduction

The order *Mononegavirales* comprises four families of viruses, the *Bornaviridae*, *Rhabdoviridae*, *Filoviridae*, and *Paramyxoviridae*. This order of viruses includes the agents of a wide range of diseases affecting human, animals, and plants. The disease may be highly characteristic of the virus (e.g., measles, mumps) or of a more general nature (e.g., respiratory disease caused by various paramyxoviruses). Disease and host range vary to some extent between the viral families; these and other differences between the families are summarized in **Table 1**. The feature which unites these viruses is the presence of a genome consisting of a single RNA molecule which is of opposite polarity to the mRNAs that they encode. The genomic RNA, which has inverted terminal complementary repeats, ranges in size from about 9 to 19 kbp and is usually present in a helical nucleocapsid. Typically, 93–99% of the genomic sequence encodes proteins with the genes encoding the structural proteins common to all members of the order arranged in the same relative position in the genome with respect to each other.

Table 1 Distinguishing features of viruses belonging to the indicated families

	Bornaviridae	*Filoviridae*	*Paramyxoviridae*	*Rhabdoviridae*
Genome size (approximate, nt)	8900	19 000	13 000–18 000	11 000–15 000
Virion morphology	90 nm, spherical	Filamentous, circular, 6-shaped	Pleomorphic, spherical, filamentous	Bullet-shaped or bacilliform
Replication site	Nucleus	Cytoplasm	Cytoplasm	Cytoplasm except nucleorhabdoviruses
Host range	Horses, sheep, cats, ostriches	Primates, (bats?)	Vertebrates	Vertebrates, vertebrates + invertebrates, plants + invertebrates
Growth in cell culture	Noncytopathic	Noncytopathic	Lytic, often with syncytium formation	Lytic for animal viruses, rapid for vesiculoviruses
Pathogenic potential	Behavioral disturbances to severe encephalomyelitis	Hemorrhagic fever	Respiratory or neurological illness	Mild febrile illness to fatal neurological disease

The genome RNA is present in the form of a nucleocapsid complex with at least one structural protein and this complex in turn is enclosed in a lipid membrane which in all cases except the bornaviruses has spikes of about 5–10 nm comprised of the viral glycoproteins. The nucleocapsids are incorporated into virions via interactions mediated by a viral matrix protein which acts as a bridge between the complex and the virus glycoprotein(s). The lipid membrane of the virus is derived from the host cell plasma membrane or internal membranes during a budding process. The shape of the viral particles varies although certain forms predominate in different families of viruses.

Taxonomy

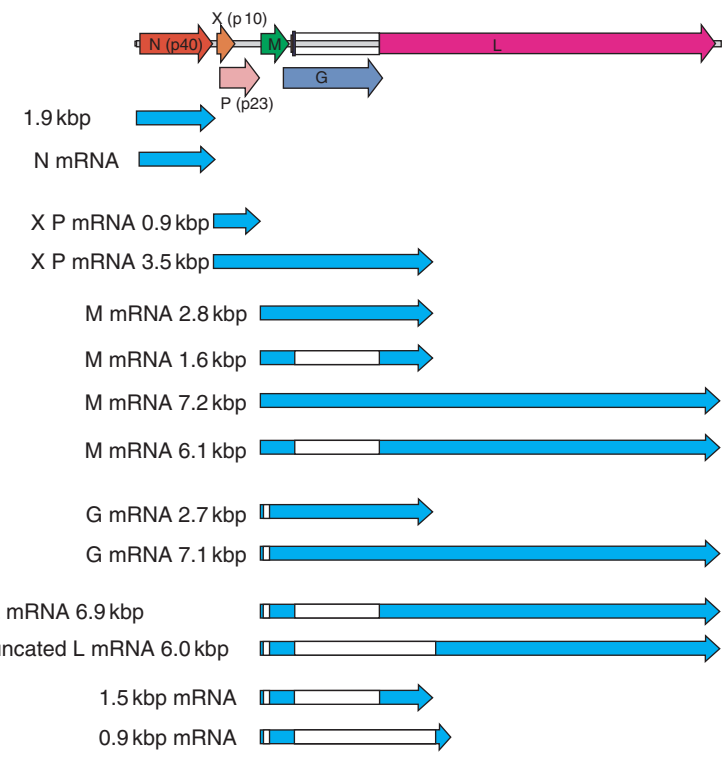
The order *Mononegavirales* is divided into four families on the basis of features such as those listed in **Table 1** and common aspects of the genetic organization, shown in **Figures 1** and **2**. There is little conserved sequence between viruses in different families except for regions of the L (polymerase) protein. A phylogenetic tree based on part of the L protein sequence is shown in **Figure 3** in which the different families can be separated. The taxonomic structure of the order is based on a range of attributes including morphological features of the virion and shape of nucleocapsid rather than the sequence similarities. Consequently, the pneumoviruses are classified as a subfamily of the *Paramyxoviridae* despite the L protein sequence apparently being more similar to that of the filoviruses. A large number of viruses that belong to the order have not been classified as yet in a particular genus. Examples of these are tupaia rhabdovirus and Flanders virus in the *Rhabdoviridae*, various paramyxoviruses isolated from rodents such as tupaia paramyxovirus, Mossman virus, Beilong virus, and J virus, whose L gene sequences cluster near the henipaviruses and morbilliviruses, and fer-de-lance virus with an L gene sequence most similar to that of the respiroviruses.

Virus Structure

All mononegaviruses have an enveloped virus particle which contains glycoprotein spikes projecting from the surface, though Borna disease virus has a less well defined structure with no spikes and an electron-dense center. The shapes of the viruses vary considerably. Viruses in the family *Paramyxoviridae* are highly pleomorphic with the major form usually being roughly spherical, with filamentous and irregular shaped forms also being present. Rhabdoviruses have a more defined shape than the viruses in the other families, being bullet shaped or bacilliform and with a uniform width and length determined by the genome length. Filoviruses such as Ebola virus have a basic bacilliform shape of relatively uniform width (~80 nm) but are often folded into branched, circular, and 6-shaped forms and may form long filaments. Internally, several layers are apparent, the helical outer layer surrounding an electron-dense layer and an axial channel (**Figure 4**).

The nucleocapsid complex structures of the mononegaviruses are all helical which in viruses in the subfamily *Paramyxovirinae* give rise to a herringbone pattern. In contrast, rhabdoviruses and pneumoviruses have a more flexible and less regular structure. Even the more regular structures are nevertheless believed to exist in dynamic states allowing structural changes during RNA synthesis. In the pneumoviruses the width of the nucleocapsid is generally narrower and the helical pitch greater than in other paramyxoviruses, enabling these viruses to be distinguished prior to their molecular characterization.

Bornaviridae
Borna disease virus
AJ311524 8908 nt



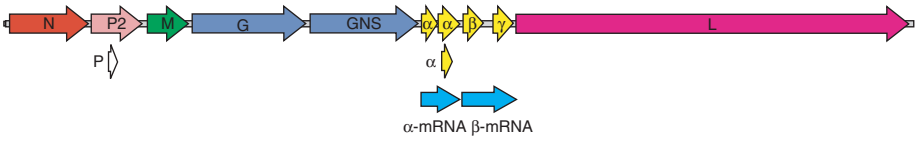
Rhabdoviridae
Vesiculovirus
Vesicular stomatitis virus
NC_001560 11 161 nt



Lyssavirus
Rabies virus
DQ875051 11 863 nt



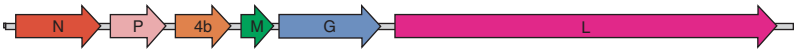
Ephemerovirus
Bovine ephemerovirus
AF234533 14 900 nt



Novirhabdovirus
Infectious hematopoietic
necrosis virus
NC_001652 11 131 nt



Cytorhabdovirus
Lettuce necrotic yellows virus
NC_007642 12 807 nt



Nucleorhabdovirus
Sonchus yellow net virus
NC_0016513 13 720 nt

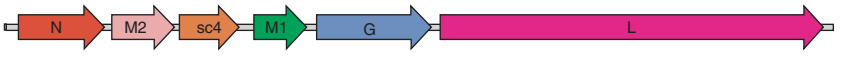


Figure 1 Organization of the genomes of viruses in the families *Bornaviridae* and *Rhadbdoviridae*. Open reading frames are colored to indicate proteins that are believed to have similar functions in the different viruses. Messenger RNAs are only shown where they differ from the monocistronic transcripts normally observed for viruses in the order *Mononegavirales*. Introns in Borna disease virus are indicated by unshaded regions. All the rhabdoviruses have potential second open reading frames in the second (phosphoprotein) gene although this is only shown for bovine ephemerovirus. The P gene was formely known as NS in vesiculoviruses and is sometimes known as M1 in lyssaviruses and novirhabdoviruses and M2 in nucleorhabdoviruses.

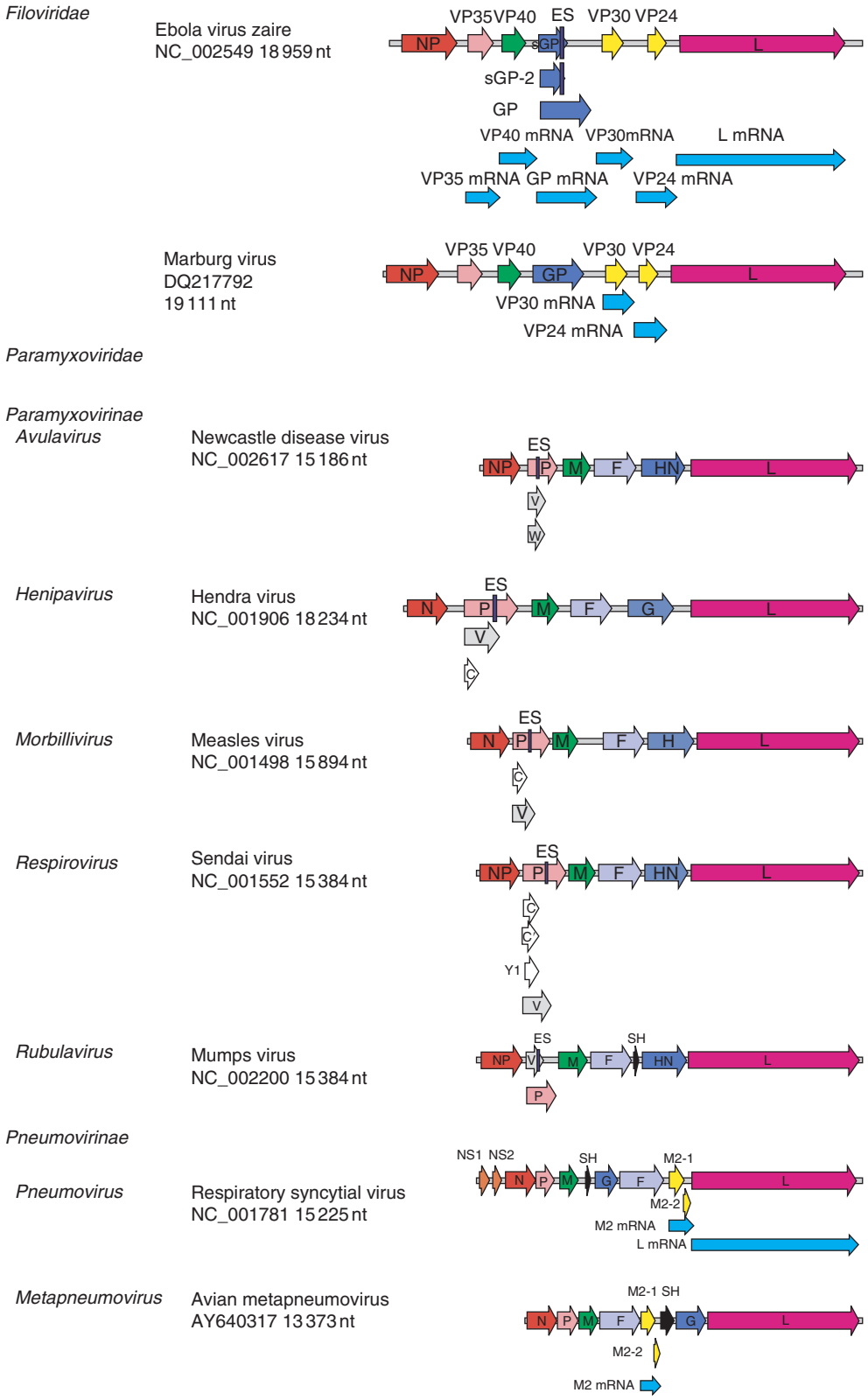


Figure 2 Genomic organization of viruses in the families *Filoviridae* and *Paramyxoviridae*. Open reading frames (ORFs) are colored as in **Figure 1**. Messenger RNAs are again indicated only where they have unusual features such as overlaps between adjacent genes or encoding two ORFs. However, all P genes of viruses in the *Paramyxovirinae* subfamily encode more than one protein, so these mRNAs are not shown. ES indicates an editing site where nontemplated residues are added to generate additional proteins. The ORF in the same line as the ORFs from other genes is the one encoded by the unedited RNA.

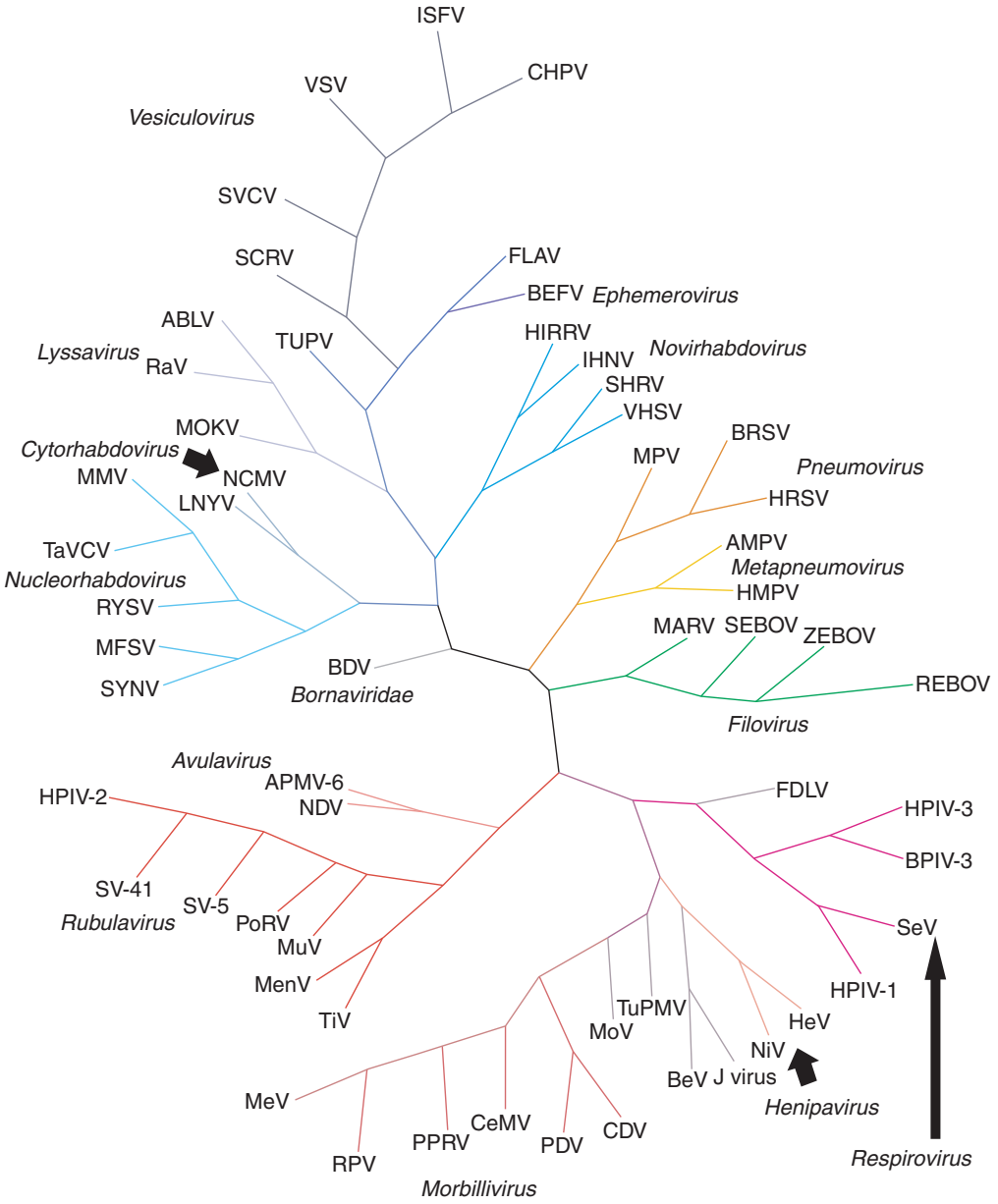


Figure 3 Phylogenetic tree of the largest region of the L protein shared by viruses in each of the mononegavirus genera. Tree branches of members in the same subfamily or family are shaded in similar colors. Virus abbreviations are those in the *Eighth Report of the International Committee on the Taxonomy of Viruses*. Borna viruses: BDV, Borna disease virus. Filoviruses: MARV, Lake Victoria marburgvirus; REBOV, Reston ebolavirus; SEBOV, Sudan ebolavirus; ZEBOV, Zaire ebolavirus Metapneumoviruses: AMPV, avian metapneumovirus; HMPV, human metapneumovirus. Pneumoviruses: BRSV, bovine respiratory syncytial virus; HRSV, human respiratory syncytial virus; MPV, murine pneumonia virus. Vesiculoviruses: CHPV, Chandipura virus; ISFV, Isfahan virus; SVCV, spring viremia of carp virus; SCRV, Siniperca chuatsi virus; VSV, vesicular stomatitis virus (San Juan). Lyssaviruses: ABLV, Australian bat lyssavirus; MOKV, Mokola virus; RaV, rabies virus. Novirhabdoviruses: HIRRV, hirame rhabdovirus; IHNV, infectious hematopoietic necrosis virus; SHRV, snakehead rhabdovirus; VHSV, viral hemorrhagic septicaemia virus. Ephemerovirus: BEFV, bovine ephemeral fever virus. Unassigned animal rhabdoviruses: FLAV, Flanders virus; TUPV, Tupaia rhabdovirus. Cytorhabdoviruses: LNYV, lettuce necrotic yellows virus; NCMV, northern cereal mosaic virus. Nucleorhabdoviruses: MFSV, maize fine streak virus; MMV, maize mosaic virus; RYSV, rice yellow stunt virus; SYNV, Sonchus yellow net virus; TaVCV, taro vein chlorosis virus. Henipaviruses: HeV, Hendra virus; NiV, Nipah virus. Avulaviruses: NDV, Newcastle disease virus; APMV-6, avian paramyxovirus 6. Rubulaviruses: HPIV-2, human parainfluenza virus 2; MenV, menangle virus; MuV, mumps virus; PoRV, porcine rubulavirus; SV-5, simian virus 5; SV-41, simian virus 41; TiV, Tioman virus. Respiroviruses: BPIV-3, bovine parainfluenza virus 3; HPIV-1(3), human parainfluenza virus 1(3); SeV, Sendai virus. Morbilliviruses: CDV, canine distemper virus; CeMV, cetacean morbillivrus; MeV, measles virus; PPRV, peste-des-petit-ruminants virus; PDV, phocine distemper virus; RPV, rinderpest virus. Unclassified paramyxoviruses: BeV, Beilong virus; FDLV, fer-de-lance virus; MoV, Mossman virus; TuPMV, Tupaia paramyxovirus.

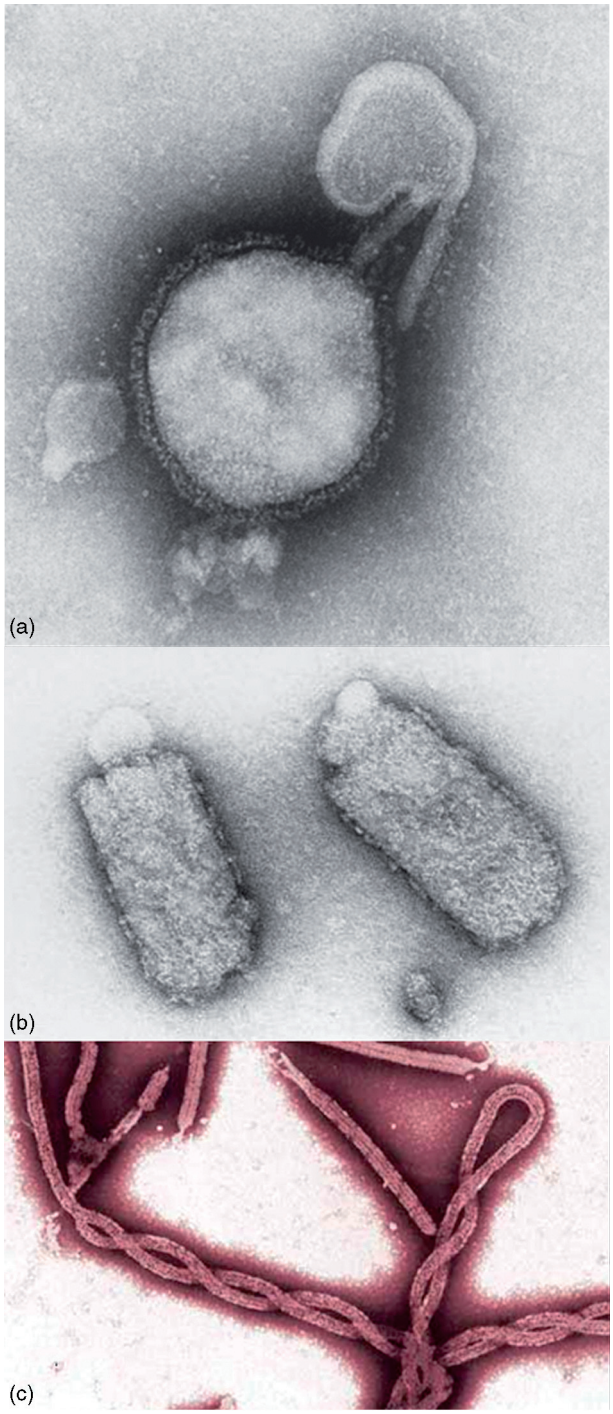


Figure 4 Electron micrographs showing the differing virus particle morphologies seen within the order *Mononegavirales*. (a) Electron micrograph of human parainfluenza virus type 3, a member of the family *Paramyxoviridae*. (b) Electron micrograph of rabies virus, a member of the family *Rhabdoviridae*. (c) Electron micrograph of Ebola virus, a member of the family *Filoviridae*. (a) Reproduced from Henrickson KJ (2003) Parainfluenza viruses. *Clinical Microbiology Reviews* 16: 242–264, with permission from American Society for Microbiology. (b) Reproduced from Mebatsion T, Weiland F, and Conzelmann K-K (1999) Matrix protein of rabies virus is responsible for the assembly and budding of bullet-shaped particles and interacts with the transmembrane spike glycoprotein G. *Journal of Virology* 73: 242–250, with permission from American Society for Microbiology. (c) Reprinted by permission from Macmillan Publishers Ltd: Nature Medicine, vol. 10, pp. S110–S121, Geisbert TW and Jahrling PB, Exotic emerging viral diseases: Progress and challenges, copyright (2004).

Rhabdovirus nucleocapsids are assembled into a more structured skeleton form in virus particles due to association with a matrix protein.

Features of the Viral Genome RNA

The precise details of the genome RNA vary between viruses with a wide range of lengths possible. An unusual feature of some paramyxoviruses is that the genome length is always a multiple of six nucleotides, referred to as the 'rule of six'. This is due to the nucleoprotein which binds the RNA associating with units of six nucleotides. However, most viruses in the order do not conform to this rule.

Promoter Sequences

The virus RNA contains three functional promoter sequences, the genomic promoters at the 3′ ends of the genome and the antigenome which directs replication of the virion RNA, and the transcriptional promoter which directs transcription from the genome. The genomic and antigenomic promoters share at least a partial sequence similarity due to the inverse complementarity of the virion RNA ends. To date the precise sequence requirements for the genomic and transcriptional promoters have not been defined precisely for most viruses and it is likely that they overlap. In some viruses, a short leader RNA transcript is produced from the genomic promoter. These transcripts, like the full-length antigenome and genome, are neither capped nor, other than for the nucleorhabdoviruses, polyadenylated. Originally it was believed that the transcriptional promoter initiated transcription from the genomic 3′ terminus producing the short leader RNA and then reinitiated transcription from the first gene to produce the first capped mRNA with a termination and reinitiation cycle at each gene junction to produce the remaining mRNAs as described below. More recently this has become less certain and the transcriptional promoter may cause initiation of transcription to occur directly from the first mRNA start signal.

Gene Start and Gene End Signals

Each gene is flanked by a transcription start and termination sequence. The transcription start and transcription termination/polyadenylation signals, though differing

between viruses, are conserved between different genes of the same virus, each being *c.* 10 nt in length. The gene end/polyadenylation signals have a series of uridine residues at the end of the gene (the 5′ end in the genomic sense) that are copied by a 'stuttering' mechanism to give a poly(A) tail.

Intergenic Regions

Most viruses of the order *Mononegavirales* have untranscribed regions between the gene end and gene start signals. These occur between all genes in most of the viruses. However, in the *Bornaviridae*, most of the transcription start sites are located upstream of the transcription end/polyadenylation site of the upstream gene so the only intergenic region is between the N and P genes. In the filoviruses and respiratory syncytial viruses, some transcripts also overlap. In the latter case, the overlap is confined to the L gene transcript starting upstream of the M2 gene, in Marburg virus the mRNAs for the VP30 and Vp24 proteins overlap, and in Ebola virus the VP35/VP40, GP/VP30, and VP24/L transcripts overlap. In some viruses, the size of the intergenic regions is conserved between all the genes, for example, two nucleotides in vesicular stomatitis virus (VSV) and three nucleotides in respiroviruses and henipaviruses, whereas in most viruses the length of the intergenic regions is variable.

Transcription

Transcription is initiated from the single promoter near the 3′ end of the genome and requires the N, P, and L proteins in addition to the genome as a minimal requirement. In pneumoviruses, transcription of longer mRNAs is enhanced by the M2-1 protein and in nucleorhabdoviruses, nucleocapsid complexes isolated from nuclei, but not virus particles, are transcriptionally active, thus suggesting a requirement for cellular proteins. The involvement of cellular proteins in virus transcription has been suggested for some other viruses whereas transcription can be achieved *in vitro* with nucleocapsids isolated from VSV and cytorhabdoviruses.

The precise start point of transcription is not known. It is now considered possible that transcription is initiated directly from the start of the first gene, though it is possible for some viruses that transcription begins at the 3′ terminus of the genome with production of a short nonpolyadenylated leader RNA. The polymerase initiates mRNA synthesis at the first transcription initiation signal sequence. Having transcribed the gene the polymerase encounters the conserved transcription termination sequence at which point it iteratively adds a polyadenylate tail at the sequence of U residues and ceases transcription. The polymerase then either detaches from the template and has to reinitiate at the 3′ transcriptional promoter or reinitiates transcription from the next gene start signal. In the case of the L gene of respiratory syncytial virus and most genes in Borna disease virus this gene start signal may be upstream of the gene end signal. This overlapping organization may reduce the level of expression of the downstream gene. More typically an intergenic sequence separates the two genes and the polymerase reinitiates transcription from the downstream gene. In a small proportion of cases the polymerase fails to terminate transcription as normal and continues to the end of the next gene generating a polycistronic transcript. The overall effect of this transcription process is to produce a gradient of transcription with those genes closer to the promoter being expressed at higher levels than those located further away. The steepness of this gradient varies considerably between viruses and between the cell infected (e.g., in measles vs. subacute sclerosing panencephalitis (SSPE)). The attenuation may vary between different genes, so the decline in transcript levels may be in a series of steps rather than a smooth gradient.

Splicing of mRNA

Most viruses in this order replicate in the cytoplasm and produce only unspliced RNA molecules. Nucleorhabdoviruses replicate in the nucleus but splicing has not been observed in these viruses. However, Borna disease virus replicates in the nucleus and three introns have been identified (**Figure 1**). The smallest and most 5′ terminal (mRNA sense) is spliced out to remove the part of the M open reading frame (ORF) and allow translation of the RNA to give the G glycoprotein. A doubly spliced RNA with both the first intron and a second one removing most of the G ORF produces the L mRNA. A third longer intron shares the splice donor site with the second one but has a different splice acceptor site resulting in larger intron that could result in a truncated L protein or proteins in either of the other two ORFs but these have not been observed. The use of spliced RNAs results in a larger number of transcripts beyond that normally observed for a virus in the order *Mononegavirales* with five gene start sites.

Replication of the Viral RNA

Replication of the viral RNA occurs via a positive-sense antigenome intermediate. This RNA, like the genome, is encapsidated as it is synthesized. The factors determining whether a genomic template is used for transcription or replication are not well understood and may vary between viruses. In some cases, the stoichiometry of the N, P, and L proteins constituting the polymerase are known to

differ between the two processes. The antigenome RNA is subsequently used as the template for the synthesis of multiple genomic RNA molecules. The inverse complementarity of the terminal sequences is presumably related to their functions in synthesis of full-length antigenomic and genomic RNAs and their encapsidation.

Viral Proteins

A set of five proteins have counterparts in all the viruses of the order whereas others are unique to viruses in specific families or genera.

Nucleocapsid (N) Proteins

All members of the order possess a protein that coats the genomic and antigenomic RNAs to form a nucleocapsid complex along with the phosphoprotein and polymerase protein. In paramyxoviruses conforming to the 'rule of six', the N protein binds six nucleotides of RNA. The N protein is essential for transcription and replication and is usually encoded by the promoter proximal gene transcribed at the highest level (the exception is in the pneumoviruses where there are two short genes encoding nonstructural proteins). In the nucleorhabdoviruses there is a nuclear localization signal near the C-terminus and the N protein is directed to subnuclear structures by association with the P protein. In Borna disease virus, two forms of N protein, p40 and p38, are translated from a single mRNA using an internal translation initiation event. Both are localized in the nucleus, the p38 protein lacking a nuclear localization signal and being translocated in association with other virus proteins.

Phosphoprotein (P)

This protein is encoded by the gene following that encoding the nucleocapsid protein in all the viruses. It is phosphorylated and required for transcription and replication. In some viruses, the ratio of the N and P proteins is believed to influence whether the viral polymerase carries out transcription or replication. The nucleorhabdovirus P protein has a nuclear export signal and is expressed throughout the cell when expressed alone but localized to the nucleus in the presence of N protein. In Borna disease virus, the P protein has a nuclear localization signal and probably has a role in retaining the N and X proteins in the nucleus. The rabies virus P protein inhibits the production of β-interferon by interfering with the function of the TBK kinase that activates IRF3 that in turn activates transcription from the β-interferon promoter. The P protein of Ebola virus (VP35) also inhibits IRF3-mediated activation of the β-interferon promoter but acts upstream of kinases such as TBK.

Other Proteins Encoded by the P mRNA

The P mRNA encodes additional ORFs in the *Bornaviridae* and members of the subfamily *Paramyxovirinae* by use of alternative initiation codons, which are not always the standard AUG. Rhabdoviruses and filoviruses also have additional ORFs that, in the case of some VSV strains, appear to be used to produce C and C′ proteins which may enhance transcription. In Borna disease virus, leaky scanning results in the P protein being produced from an ORF different from that of the first which encodes the X or p10 protein. The X protein is localized to the nucleus with other viral proteins and has a nuclear export signal that is blocked by association with P protein. A second AUG codon in the same frame as the P protein results in an N-terminally truncated form of the P protein P′ or p16 that associates with the other viral proteins.

In the viruses belonging to the subfamily *Paramyxovirinae* there are two mechanisms for expressing of additional genes from the P mRNA. The C proteins (C, C′, Y1, and Y2) are translated in a different ORF from alternative initiation codons that are sometimes not the standard AUG codon. The V proteins (V and W) share a common N-terminus with the P protein but have an editing site where additional G residues are sometimes inserted by the transcriptase (one for V proteins and two for W) into the mRNA giving the protein an alternative C-terminus. In most cases the unedited mRNA transcript encodes the P protein and the edited ones the V or W proteins but in the rubulaviruses the V protein is encoded by the unedited transcript and P by the edited one. These proteins play a role in the inactivation of interferon induction and signaling, although the details of how this occurs vary between viruses.

Matrix Proteins

The matrix protein is typically encoded by the third transcript (except for the pneumoviruses, nucleorhabdoviruses, and cytorhabdoviruses that have additional genes, as described below). It is a major structural component of the viruses and is important for particle morphogenesis. In some cases, virus-like particles can be obtained by expression of M protein alone and the morphology of rabies virus lacking an M gene loses the characteristic bullet shape in addition to there being a greatly reduced yield of virus particles. Different subpopulations of the M protein are believed to be involved in different aspects of morphogenesis (see below). In the case of vesiculoviruses, the M protein inhibits cellular transcription and mRNA export from the nucleus, thus preventing the interferon response. In addition, the M protein is largely responsible for the cytopathic effect seen in cells infected with VSV. In the case of Borna disease virus, there are conflicting reports as to the glycosylation status and subcellular localization of M protein.

Viral Glycoprotein(s)

All the viruses encode between one and three surface glycoproteins that are responsible for attachment of the virus to infected cell membranes and subsequent fusion either with the plasma membrane or in the case of rhabdoviruses with internal membranes. In addition, some rubulaviruses and all pneumoviruses have a less well characterized small hydrophobic protein that is also membrane associated and sometimes glycosylated.

The G proteins of rhabdoviruses and filoviruses and the fusion proteins of the viruses in the family *Paramyxoviridae* form trimers at the surface of infected cells and on virions. These proteins are type I membrane proteins with an N-terminal signal peptide that is cleaved off in the endoplasmic reticulum, a C-terminal membrane anchor, and a number of glycosylation sites on the ectodomain. The Ebola virus G protein is encoded by an edited mRNA with the unedited RNA and a second edited RNA encoding shorter soluble glycoproteins lacking a transmembrane anchor. The fusion proteins of viruses in the families *Paramyxoviridae* and the filovirus G proteins have a protease cleavage site that results in the mature form of each monomer having two disulfide-linked chains comprising a smaller N-terminal chain, F2, and a larger F1 chain. The N-terminus of the F1 chain has the fusion peptide which is located at the end of an extended α-helix following activation by binding to a receptor or exposure to low pH. This peptide inserts into the target membrane and a hairpin-like structure is formed that results in the C-terminal viral membrane anchor being located at the same end of the molecule as the fusion peptide along with their associated heptad repeats.

Viruses in the family *Paramyxoviridae* have a second membrane glycoprotein variously known as H if it has hemagglutination activity, HN if it has hemagglutination and neuraminidase activity, and G if it has neither (although the murine pneumonia virus G protein was named by analogy with that of the other pneumoviruses despite having hemagglutination activity). This protein is a type II membrane protein with an N-terminal, uncleaved signal-anchor and a C-terminal ectodomain that has N-linked glycosylation sites and in pneumoviruses extensive O-linked glycosylation. The proteins form a tetramer in the cases where the structure has been studied and is conventionally regarded as the attachment protein, although in some cases virus lacking this protein can still infect cells. In some viruses (e.g., Newcastle disease virus, human parainfluenza virus type 3, mumps virus, and canine distemper virus), it is also required to obtain full fusion activity, although in others (e.g., simian virus 5, measles virus, and avian metapneumovirus) F protein alone is sufficient.

The rubulaviruses SV5 and mumps along with all the pneumoviruses have a third small type II membrane protein. This is glycosylated in the case of the pneumovirus proteins and in the metapneumoviruses has an additional C-terminal region with conserved cysteine residues. In the pneumoviruses several forms exist, viz. unglycosylated, glycosylated, and a polylactosamine-modified glycosylated form. Little is known about its function although the avian metapneumovirus SH protein suppresses F-protein-mediated cell–cell fusion.

The ephemeroviruses have an additional nonstructural, highly glycosylated protein of unknown function.

The L protein

The L protein is the largest viral protein and is encoded by all viruses in the order *Mononegavirales* at the 5′ end of the genome and is therefore produced from the least abundant mRNA transcript. It contains conserved motifs and is presumed to contain the major enzymatic activities of the RNA polymerase in the nucleocapsid complex in which, together with the N and P proteins (and in pneumoviruses M2-1 protein), it carries out transcription (including capping and polyadenylation) and replication of the viral RNA.

Additional Virus-Encoded Proteins

Several members of the order encode proteins in addition to those described above. Two nonstructural proteins, NS1 and NS2, are encoded by the pneumoviruses and are not found in the metapneumoviruses or any of the other viruses in the order *Mononegavirales*. They are encoded by the most abundant transcripts and appear to have a role in counteracting the host interferon response. Both proteins are required to block the effects of interferon presumably by acting on downstream effectors because they do not interfere with JAK/STAT signaling. They also interfere with IRF3 activation which is required for interferon β-induction.

The pneumoviruses also contain an M2 gene containing two ORFs located immediately upstream of that encoding the L protein. The first ORF encodes the M2-1 protein which shows marked similarity between all these viruses and has been shown to enhance transcription, particularly of longer genes. The second ORF of the M2 gene shows no similarity to other proteins or conservation between viruses. The M2-2 protein is thought to inhibit virus RNA synthesis.

Three plant viruses (the nucleorhabdovirus, rice yellow stunt virus, and the cytorhabdoviruses, northern cereal mosaic virus and strawberry crinkle virus) have poorly characterized ORFs prior to the polymerase gene which may encode additional proteins. There is tentative evidence that these may be related to sequences likely to be involved in RNA replication/transcription. Novirhabdoviruses have an ORF encoding the nonvirion (NV) protein that appears to enhance virus growth.

Cytorhabdoviruses and nucleorhabdoviruses encode between one and four additional proteins at a location

between the phosphoprotein and matrix protein genes. These are not well characterized but appear to have similarities to proteins in plants involved in transport between cells via plasmodesmata and are involved in the spread of nucleocapsids between plant cells via the plasmodesmata.

Viral Entry

Mononegaviruses infecting mammalian cells typically attach to receptors on the plasma membrane of cells and fuse either directly with the plasma membrane or, after uptake into endosomal vesicles and exposure to a more acidic environment, with endosomal membranes. These processes are achieved using one or more virus glycoproteins. In viruses of the family *Paramyxoviridae*, these two activities appear to reside on different proteins with the G, H, or HN protein mediating attachment and the F protein mediating fusion. However, in cell culture, viruses lacking the attachment protein gene are often able to grow, suggesting that viral attachment is possible in its absence. The paramyxoviruses generally fuse with the plasma membrane at neutral pH whereas animal rhabdoviruses fuse with endosomal membranes following activation by low pH. Primary infection of plants by the cytorhabdoviruses and nucleorhabdoviruses involves physical injection into plant tissue by invertebrate vectors such as planthoppers, leafhoppers, and aphids, with the latter responsible for viral entry of all but two of these viruses that infect dicotylydenous plants. Fusion of the viral membrane with cellular membranes results in the release of viral nucleocapsids into either the cytoplasm or, in the case of the nucleorhabdoviruses, the nucleus. The animal viruses, with the exception of the bornaviruses, replicate in the cytoplasm where the viral nucleocapsids are released following fusion of the viral membrane with the plasma membrane or endosomal membrane.

Viral Assembly and Budding

The process of viral assembly has been most thoroughly studied in VSV and the general features are thought to be common to all members of the order though the details will differ. The overall process of VSV assembly involves early stages where nucleocapsids are assembled by N protein being released from N–P dimers to bind the genomic RNA as it is being synthesized. At the same time, the G glycoprotein forms microdomains at the plasma membrane. Different populations of M protein are believed to be involved in different steps of assembly. These are, first, binding to regions of plasma membrane enriched in G protein, second, recruitment of the nucleocapsids to these regions, and third, condensation of the nucleocapsids into helical structures which may have M protein at the center as well as between the membrane and the nucleocapsid. While G protein facilitates viral assembly it is not essential, and the M protein is believed to be the key component in assembly with virus-like particles being produced by expression of M protein alone. As the M protein associated with the membrane binds to the condensing nucleocapsid structures, bullet-shaped protrusions occur at the membrane surface. Cellular proteins are believed to associate with the M protein via a so-called late domain (PPPY in the case of VSV) to directly or indirectly cause fusion of the membrane at the base of the protrusions to release virus particles.

The matrix protein is also the main determinant of virus assembly and budding in viruses of the family *Paramyxoviridae* although the precise mechanisms probably vary between viruses. For example, Sendai virus M protein alone can form virus-like particles whereas SV5 M protein cannot. Interaction of viral and cellular proteins probably varies as well since measles and respiratory syncytial virus production are dependent on the presence of cellular actin whereas human parainfluenza virus 3 release is dependent upon the presence of microtubules. These cellular components are required for efficient RNA synthesis as well as virus production. In measles virus, interaction of the M protein and the glycoproteins may occur in lipid rafts to which M protein is recruited independently of the glycoproteins. However recruitment of the hemagglutinin to the rafts may be dependent upon its interaction with the fusion protein. In contrast, the M proteins of Sendai virus and respiratory syncytial virus may require interaction with viral glycoproteins, in particular the fusion protein, for recruitment into lipid rafts. This association is believed to occur during transport to the cell surface.

Budding of most of the animal viruses in the order occurs at the plasma membrane. However, rabies virus buds into internal membrane compartments as do the plant rhabdoviruses. In the case of the nucleorhabdoviruses, there appears to be extensive rearrangement of the intracellular membranes with enlarging of the nuclei due to the insertion of extra membranes through which the virus buds. These membranes are contiguous with the nuclear membrane resulting in the virus ending up in the perinuclear space. Virus is transmitted from here via insect vectors. The matrix protein has an additional role in the polarity of virus release from cells in measles virus where, in contrast to most viruses in the family *Paramyxoviridae*, the HN protein is not transported in a polarized manner and the F protein is directed to the basolateral surface. In the presence of M protein, complexes of M and the glycoproteins are sorted to the apical surface. In other members of this virus family, the glycoproteins are targeted to the apical surface from which the viruses bud, whereas in VSV sorting of G protein and virus budding occur at the basolateral surfaces.

The cytoplasmic tails of the glycoproteins of the viruses in the family *Paramyxoviridae* have roles in enhancing the efficiency and specificity of virus production, recombinant viruses with truncated cytoplasmic tails showing a lack of

localization at the membrane surface, lower virus yields, and virus preparations with reduced amounts of viral glycoproteins and increased levels of cellular proteins. VSV G protein also requires a minimal length of cytoplasmic tail to enhance budding efficiency but there does not appear to be any requirement for a specific sequence. Some paramyxovirus fusion proteins can produce virus-like particles.

All the viruses in the order *Mononegavirales* with the possible exception of Borna disease virus therefore seem to have the matrix protein and at least one glycoprotein as major determinants of virus release but the exact details may vary even within members of the same family or subfamily. Budding to release progeny virions of most of the animal viruses occurs at the plasma membrane. However, rabies virus buds into internal membrane compartments as do the plant rhabdoviruses. In the case of the nucleorhabdoviruses, there appears to be extensive rearrangement of the intracellular membranes with enlarging of the nuclei due to the insertion of extra membranes through which the virus buds. These membranes are contiguous with the nuclear membrane resulting in the virus ending up in the perinuclear space, from where they are transmitted via insect vectors.

See also: Animal Rhabdoviruses; Bornaviruses; Bovine Ephemeral Fever Virus; Chandipura Virus; *Ebolavirus*; Filoviruses; Fish Rhabdoviruses; Human Respiratory Syncytial Virus; Marburg Virus; Measles Virus; Mumps Virus; Paramyxoviruses of Animals; Parainfluenza Viruses of Humans; Paramyxoviruses; Plant Rhabdoviruses; Rabies Virus; Rinderpest and Distemper Viruses; Sigma Rhabdoviruses; Vesicular Stomatitis Virus.

Further Reading

Feldmann H, Geisbert TW, Jahrling PB, *et al.* (2005) Family *Filoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 645–653. San Diego, CA: Elsevier Academic Press.

Geisbert TW and Jahrling PB (2004) Exotic emerging viral diseases: Progress and challenges. *Nature Medicine* 10: S110–S121.

Henrickson KJ (2003) Parainfluenza viruses. *Clinical Microbiology Reviews* 16: 242–264.

Jackson AO, Dietzgen RG, Goodin MM, Bragg JN, and Deng M (2005) Biology of plant rhabdoviruses. *Annual Reviews of Phytopathology* 43: 623–660.

Jayakar HR, Jeetendra E, and Whitt MA (2004) Rhabdovirus assembly and budding. *Virus Research* 106: 117–132.

Lamb RA, Collins PL, Kolakofsky D, *et al.* (2005) Family *Paramyxoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 655–668. San Diego, CA: Elsevier Academic Press.

Lamb RA and Parks GD (2006) *Paramyxoviridae*: The viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1449–1496. Philadelphia: Lippincott Williams and Wilkins.

Lipkin WI and Briese T (2006) *Bornaviridae*. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1829–1851. Philadelphia: Lippincott Williams and Wilkins.

Lyles DS and Rupprecht CE (2006) *Rhabdoviridae*. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1363–1408. Philadelphia: Lippincott Williams and Wilkins.

Mebatsion T, Weiland F, and Conzelmann K-K (1999) Matrix protein of rabies virus is responsible for the assembly and budding of bullet-shaped particles and interacts with the transmembrane spike glycoprotein G. *Journal of Virology* 73: 242–250.

Pringle CR (2005) Order *Mononegavirales*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 609–614. San Diego, CA: Elsevier Academic Press.

Sanchez A, Geisbert TW, and Feldmann H (2006) *Filoviridae*: Marburg and Ebola viruses. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1409–1448. Philadelphia: Lippincott Williams and Wilkins.

Schwemmle M, Carbone KM, Tomongo K, Nowotny N, and Garten W (2005) Family *Bornaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 615–622. San Diego, CA: Elsevier Academic Press.

Tomonga K, Kobayashi T, and Ikuta K (2002) Molecular and cellular biology of Borna disease virus infection. *Microbes and Infection* 4: 491–500.

Tordo N, Benmandour A, Calisher C, *et al.* (2005) Family *Rhabdoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 623–644. San Diego, CA: Elsevier Academic Press.

Mouse Mammary Tumor Virus

J P Dudley, The University of Texas at Austin, Austin, TX, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

HRE Hormone response elements found in several viral genomes that bind the activated forms of steroid hormone receptors to increase transcription in the presence of a steroid ligand.

Rem A Rev-like RNA-binding protein that is responsible for efficient nuclear export of unspliced mouse mammary tumor virus RNA.

Superantigen A protein that interacts with entire classes of T cells primarily through the variable region of the beta-chain of the T-cell receptor, leading to signal transduction and the release of cytokines.

History

Mouse mammary tumor virus (MMTV) was first reported in 1933 by the Jackson Memorial Laboratory and by Korteweg in 1934 as an extrachromosomal influence on the incidence of breast cancer in inbred mouse strains. Initial crosses between strains with a high mammary cancer incidence and strains with a low mammary cancer incidence revealed that female progeny invariably had the mammary cancer incidence of the female parent. Subsequently, Bittner showed that an extrachromosomal factor was transmitted through maternal milk, and this factor was later associated with viral particles, called 'B-particles', which caused breast cancer in susceptible mice.

Taxonomy and Classification

MMTV is a prototype species of the genus *Betaretrovirus* in the family *Retroviridae*. These viruses previously were referred to as type B retroviruses based on their appearance by electron microscopy (a characteristic acentric core within particles of *c.* 100 nm). Milk-borne MMTVs are often named for the inbred mouse strain from which they are derived, for example, C3H MMTV or MMTV (C3H). Multiple double-stranded DNA copies are found in the chromosomal DNA of most commonly used laboratory strains of mice (called integrated or endogenous proviruses). These endogenous proviruses presumably represent viral insertions into chromosomal DNA of germline cells and are referred to as *Mtv* followed by an Arabic number, for example, *Mtv8*. Most endogenous *Mtv*s have defects in one or more genes and, therefore, these proviruses often fail to produce infectious virus. Currently, MMTV is classified with other betaretroviruses including Mason–Pfizer monkey virus (MPMV), Jaagsiekte sheep retrovirus (JSRV), and human endogenous retrovirus type-K (HERV-K).

Properties of the Virion

MMTV particles contain a single-stranded positive-sense RNA, which exists as a dimer, and is encapsidated as a helical ribonucleoprotein (RNP) by the nucleocapsid (NC) protein; reverse transcriptase (RT) and integrase (IN) are closely associated with the RNP. The RNP is surrounded by an icosahedral shell (although the exact structure has not been carefully studied) composed of capsid (CA) protein. There is considerable variation in the size of cores, similar to that observed with human immunodeficiency virus 1 (HIV-1). MMTV capsids are bound via the matrix (MA) protein to the viral envelope, a portion of the cellular plasma membrane that has been modified by the insertion of the surface (SU) and transmembrane (TM) proteins.

Properties of the Genome

The viral RNA is bound at either end by a short direct repeat (R) of 15 bp (**Figure 1**). The R regions are adjacent to unique regions of approximately 120 and 1200 bp, respectively, present at the 5′ (U5) or 3′ (U3) ends of the RNA. A cellular tRNA (tRNA3Lys) is bound through 18 bp of complementarity to each copy of the viral RNA

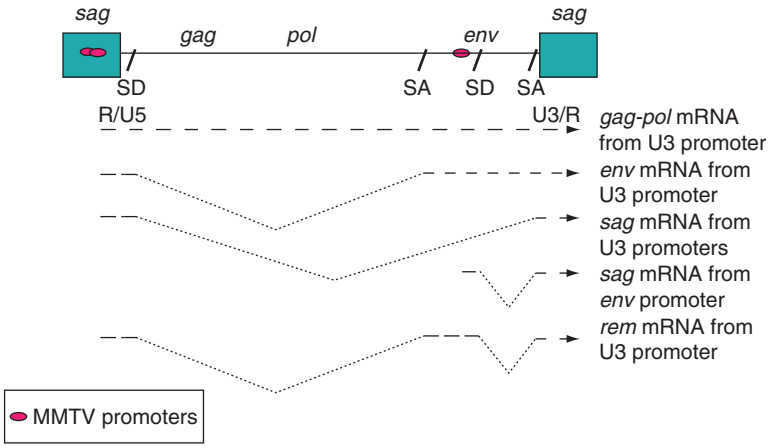


Figure 1 Diagram of the MMTV proviral genome and structure of viral mRNAs. The boxed regions represent the LTRs with two described promoters. The standard promoter gives unspliced transcripts that start with the first base of the R region in the 5′ LTR and end at the last base of the R region of the 3′ LTR. Unspliced transcripts may be directly exported from the nucleus for Gag, Gag-Pro, and Gag-Pro-Pol translation. Alternatively, such transcripts may be spliced to give two singly spliced mRNAs encoding either envelope or superantigen. A fourth transcript from the LTR promoter gives a doubly spliced mRNA that encodes the RNA export protein, Rem. An internal promoter in the envelope gene also allows production of a singly spliced RNA encoding the superantigen. The positions of known splice donors (SD) and acceptors (SA) are also shown.

at the primer-binding site (PBS) located just downstream of U5. Although the RNA packaging site (often referred to as ψ) has not been defined, this site is likely to include the region between the splice donor (SD) and splice acceptor (SA) sites for the envelope (*env*) mRNA. Packaging of viral mRNAs, other than genomic RNA, is prevented by exclusion of the ψ region. The first SD site precedes the group-specific antigen (*gag*) region that encodes a Gag precursor with the nonglycosylated proteins of the virion in the order NH2-MA-p21-CA-NC-COOH. Interestingly, the virus also encodes two other precursor polypeptides, Gag-Pro and Gag-Pro-Pol, from the genomic RNA by ribosomal frameshifting. Gag-Pro encodes the Gag proteins, a dUTPase (DU), and the viral protease (PR), whereas the Gag-Pro-Pol protein also encodes RT, including a ribonuclease H (RNase H) activity, and IN. Both the DU and RT are trans-frame proteins that contain sequences from the preceding protein, NC and PR, respectively. At the 3′ end of the genome, the envelope (Env) proteins are specified in the order NH2-SU-TM-COOH from a singly spliced mRNA. Recent findings indicate that MMTV encodes another protein called regulator of export of MMTV mRNA or Rem from a doubly spliced mRNA. The *rem* mRNA is translated from the open reading frame that also specifies SU and TM. Such data emphasize the efficiency with which MMTV and other viruses use their genetic information. Furthermore, unlike most other retroviruses, which have multiple stop codons within the long terminal repeat (LTR), the MMTV U3 region has yet another gene that encodes a superantigen (Sag) from one or more singly spliced mRNAs.

Virus Replication

Recent evidence indicates that MMTV uses the transferrin receptor 1 (Tfr1) to mediate infection. Tfr1 is ubiquitously expressed in many rodent cells, and this observation may explain infection of numerous rat and mouse cell lines in culture. Differences in mouse Tfr1 and primate Tfr1 appear to be sufficient to prevent MMTV infection of monkey and human cells. Despite numerous reports of MMTV sequences in human tumors, failure of human Tfr1 to allow viral entry is one argument against the validity of these data. However, use of other receptors for MMTV entry, particularly in specific cell types, is possible. Entry through Tfr1 appears to occur through adsorptive endocytosis, and receptors are recycled to the cell surface where they allow entry of additional MMTV particles into previously infected cells. Thus, unlike many retroviruses, MMTV is not susceptible to superinfection resistance, and many chronically infected mouse lines have large numbers of integrated proviruses.

Following entry and partial uncoating in the cytoplasm, the virally encoded RT is activated. Using the cellular lysyl-tRNA primer bound to genomic RNA, RT synthesizes a partial minus-strand DNA bound to plus-stranded RNA. Using the RT-associated RNase H activity and several strand transfers, the RNA template is degraded and a double-stranded provirus is synthesized. However, the product of reverse transcription is different from the starting template so that the U5 and U3 sequences present uniquely in viral RNA are duplicated to give longer repeats at each end of the provirus (LTRs). The LTRs have the structure U3-R-U5 (**Figure 1**). Because nuclear entry of the preintegration complex (PIC) containing the provirus is thought to require nuclear envelope breakdown during mitosis, it is generally believed that MMTV must infect dividing cells. However, MMTV encodes DU, a protein found in many nonprimate lentiviruses that infect nondividing cells. DU prevents misincorporation of uracil and mutation of newly synthesized proviruses in nondividing cells where the ratio of dUTP to TTP is high.

After nuclear entry of the PIC, the provirus integrates using the MMTV-encoded IN protein cleaved from the Gag-Pro-Pol precursor. IN protein introduces an asymmetric cut 2 bp from the linear ends of the provirus as well as an asymmetric break exactly 6 bp apart on opposite DNA strands of host DNA. Proviral integrations are not site specific and may occur at transcriptionally active sites, although this idea has not been tested directly. Following the joining reaction, the repair of virus–cell junctions by cellular enzymes generates a 6 bp direct repeat of cell DNA that flanks the viral LTRs. Such a structure resembles those formed by the transposable elements of bacteria, yeast, and *Drosophila*.

The integrated provirus contains all the signals necessary for recognition by RNA polymerase II, and many of these signals are present in the U3 region of the LTR. Transcription from the standard promoter is initiated in the 5′ LTR starting at the U3/R junction and terminating at the R/U5 junction (**Figure 1**). However, several other promoters have been described, including one approximately 500 bp upstream of the U3/R junction and another within the envelope gene. Termination appears to be reasonably inefficient, and some MMTV transcripts probably terminate in the adjacent cellular DNA. A portion of genome-length MMTV RNA is processed into singly spliced *env* and *sag* mRNAs.

Surprisingly, the *sag* gene appears to be expressed by at least two singly spliced mRNAs from independent promoters (**Figure 1**), possibly allowing different levels of Sag in various cell types. MMTV also produces a doubly spliced mRNA that encodes the RNA export protein, Rem. In most cell types, the levels of *gag-pol* and *env* mRNAs greatly exceed *sag* and *rem* mRNAs.

The unspliced RNA (8.7 kb) is translated into Gag, Gag-Pro, and Gag-Pro-Pol precursor proteins. Since *pro* (the viral protease gene) and *pol* (the polymerase/integrase

gene) are out-of-frame with respect to *gag* and each other, one ribosomal frameshift is required for Gag-Pro synthesis, and a second frameshift is necessary to produce Gag-Pro-Pol (**Figure 2**). Because the first frameshift is quite efficient, MMTV RT levels in infected cells are equivalent to those produced by other retroviruses. The *env* mRNA is translated into a precursor protein on membrane-bound polyribosomes. This precursor is modified by glycosylation in both the endoplasmic reticulum and the Golgi, and protein cleavage to SU and TM also occurs in the latter compartment. The *sag* mRNA appears to be translated into a type II transmembrane protein of 36 kDa; this protein is glycosylated and reportedly cleaved to generate a C-terminal fragment of 18 kDa. Sag is associated with major histocompatibility complex (MHC) class II protein at the surface of antigen-presenting cells. Unlike the previously described proteins, Sag is not a known structural component of virions. The Rem protein is translated in the same frame as the envelope protein, including the Env signal peptide (**Figure 2**). The nascent protein escapes signal recognition particle, and the full-length Rem protein (33 kDa) localizes to the nucleolus using motifs found in the signal peptide. Rem synthesis is required for efficient export of unspliced genomic RNA and must precede production of infectious viral particles. The N-terminal one-third of Rem appears to encode all functions necessary for RNA export, and deletion of the C-terminus increases the ability of Rem to function in export assays. These data indicate that unspliced MMTV RNA export and particle production are negatively regulated, at least in some cell types.

The precursors for Gag, Gag-Pro, and Gag-Pro-Pol aggregate within the cell cytoplasm into procapsids called intracytoplasmic A particles. This process is distinct from the maturation of C-type particles that assemble Gag precursors at the cell surface concomitant with the budding process. Presumably, the precursor proteins are folded so that NC and RT proteins are sequestered inside the particle to interact with a dimer of viral RNA and the lysyl tRNA. The viral PR, which is present in a fraction of the Gag precursors, is apparently responsible

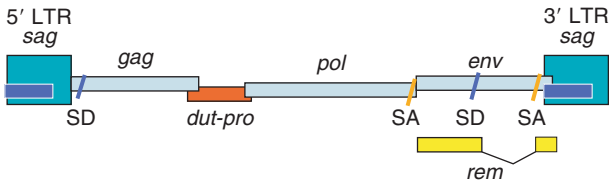


Figure 2 Positions of the open reading frames within MMTV proviral DNA. The large boxes represent the 5′ and 3′ LTRs, whereas the smaller elongated boxes represent the reading frames for the genes shown in italics. The *rem* gene is in the same reading frame as the envelope gene, which is altered by splicing (V shape). The *sag* coding sequence at the 5′ end of the genome is not expressed because it is located upstream of the viral promoters.

for the cleavage events that produce the mature virion proteins, MA, p21, p3, p8, CA, and NC. The functions of p21, p3, and p8 are currently unknown. Like HIV, the MMTV Gag precursor contains a P(S/T)AP sequence that likely functions as a late (L) domain; such domains interact with cellular protein complexes (known as ESCRTs) to promote budding. Further cleavages by PR to give functional RT and IN, and mature cores apparently occur after budding.

Transmission and Tissue Tropism

MMTV is transmitted horizontally through maternal milk (called exogenous or milk-borne virus) or vertically through the germline (endogenous viruses). The exogenous viruses are responsible primarily for the high mammary cancer incidence of mouse strains such as RIII and C3H. However, some strains, such as GR, carry an endogenous virus (in this case, *Mtv2*) that is also transmitted through milk. Although most common inbred mouse strains carry endogenous *Mtv*s, some recently inbred strains lack such proviruses. Interestingly, strains that lack *Mtv*s often show resistance to disease induced by exogenous MMTV regardless of the route of transmission.

MMTV particles ingested by newborn mice in maternal milk survive passage through the stomach during the first weeks of life prior to maturation of the intestinal tract (**Figure 3**). These particles enter the small intestine where they cross the epithelium through M cells. Subsequently, MMTV virions encounter and infect dendritic cells and B cells. Following reverse transcription, integration, and transcription, Sag protein is produced and presented at the surface of such cells in association with MHC class II protein. Sag signaling through the T-cell receptor (TCR) leads to the release of cytokines, leading to a pool of dividing lymphoid cells for MMTV infection as well as division of previously infected cells. Both B and T cells are required for MMTV transmission since knockout or transgenic animals lacking either lymphoid subset cannot be infected efficiently by the milk-borne route. This lymphoid cell reservoir is necessary to preserve MMTV infectivity prior to the onset of puberty in mice when a source of susceptible and dividing mammary cells becomes available. Sag-mediated stimulation of lymphoid cells also improves the efficiency of viral transfer within the mammary gland. MMTV production increases during lactation when hormone levels elevate transcription from the hormone-responsive element (HRE) in the LTR U3 region (**Figure 4**). High virion production during lactation ensures that large amounts of particles will be produced at a time when newborn offspring can be infected.

Horizontal transmission of MMTV by seminal or salivary fluid has been reported. The low infectivity of

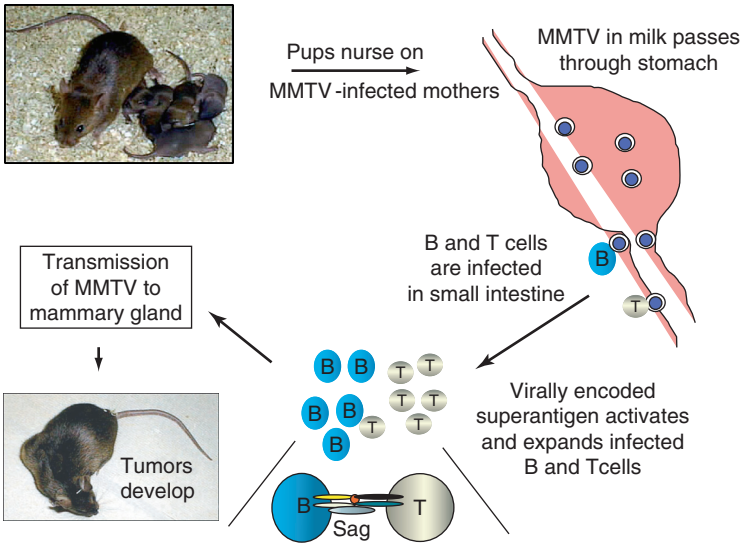


Figure 3 The MMTV life cycle. Infected mothers transmit virus to their pups through milk. In the first few weeks of life, the pups ingest the virus, which passes through the stomach into the small intestine. MMTV then transverses the M cells to deliver virus to gut-associated lymphoid cells. B cells in the gut are infected and express Sag protein at the cell surface in conjunction with MHC class II protein. Recognition of the Sag C-terminal amino acids by the variable portion of the β-chain of the T-cell receptor results in signal transduction and the release of cytokines. Cytokines then stimulate the proliferation of adjacent B and T cells, which act as a reservoir for the virus until transmission to mammary epithelial cells occurs during puberty. Virus expression and release is highest during lactation to ensure milk-borne transmission. Mammary tumors arise after multiple rounds of pregnancy and lactation due to insertional mutagenesis.

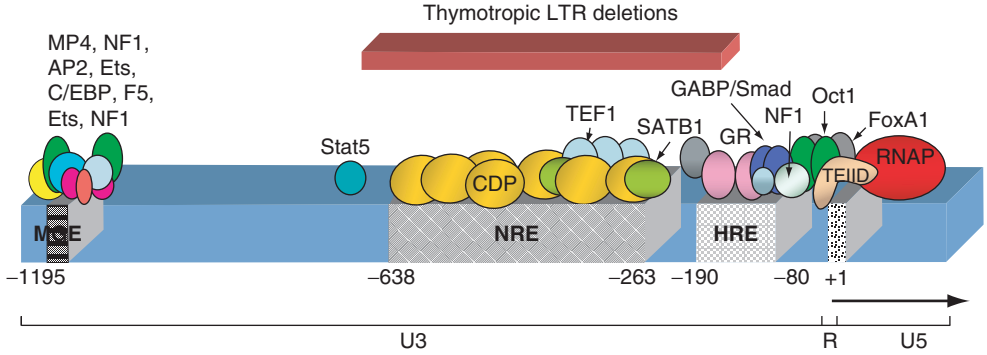


Figure 4 Transcriptional control elements in the MMTV LTR. The major transcriptional start site is located at the U3/R border in the 5′ LTR of integrated proviral DNA. The mammary gland enhancer (MGE) is located near the 5′ end of the LTR. The negative regulatory element (NRE) contains binding sites for Cutl1/CDP, SATB1, and TEF1, but different cell types vary in the level of these factors. The hormone response element (HRE) contains multiple binding sites for the activated forms of several steroid receptors, including glucocorticoid receptor (GR). The GR sites appear to be flanked by binding sites for FoxA1. The region spanning deletions found in thymotropic strains of MMTV is shown above the LTR. TFIID, transcription factor IID; RNAP, RNA polymerase II.

most body fluids (other than milk) may be attributable to virally infected lymphoid cells and the absence of large amounts of infectious MMTV particles.

Genetics and Disease Susceptibility

MMTV causes mammary adenocarcinomas by infection of the mammary epithelium. The genetic factors that influence mammary tumor incidence in mice include (1) the presence or absence of milk-borne virus, (2) the presence of an infectious endogenous MMTV, (3) cellular factors that determine virus entry and replication, and (4) host factors that may influence the immune response (including the Sag response) or hormonal levels in the animals. The presence of milk-borne virus has been demonstrated in mouse strains with a high mammary cancer incidence by foster nursing experiments as originally described by Bittner. Shortly thereafter, it was shown that such high-cancer-incidence strains (e.g., C3H) permanently

lost this trait if nursed on mothers with a low mammary cancer incidence (e.g., BALB/c). Such strains, known as C3Hf, have a mammary tumor incidence between 38% and 47% (average latency of 600 days) in multiparous animals, whereas C3H strains expressing milk-borne MMTV have a tumor incidence of 88–95% (average latency of 300 days) in breeding females. Mammary tumors appearing in C3Hf mice are the result of expression of a replication-competent endogenous provirus known as *Mtv1*. Strains, such as BALB/c and C57BL, which have a mammary tumor incidence of 1% or less with long latencies, appear to lack a replication-competent MMTV.

A number of cellular and host factors also influence the appearance of mammary tumors in different mouse strains. Most of these factors have not been defined, but many of them appear to directly or indirectly affect the ability of MMTV to replicate. For example, the resistance of C57BL to MMTV infection appears to be the result of failure to express MHC class II I–E molecules, one of two types of class II antigens expressed in mice. Although many MMTV Sag proteins apparently interact with class II I–A proteins, this interaction is less efficient than the Sag/MHC class II I–E complexes in the stimulation of lymphoid cells. Loss of specific class II molecules is not the only defense against MMTV infection. The presence of endogenous MMTV proviruses that express Sag proteins results in the deletion of specific subsets of T cells. If these endogenous viruses encode Sags with the same TCR reactivity as exogenous MMTVs, milk-borne infection is blocked by preventing viral amplification in lymphoid cells (**Figure 3**). For example, endogenous *Mtv7* expresses a Sag protein that reacts with and causes deletion of Vβ6+ T cells; therefore, the milk-borne MMTV (SW) strain that encodes TCR Vβ6-reactive Sag cannot infect mouse strains that carry *Mtv7* because these mice lack the T-cell subset required for MMTV transmission.

Resistance to exogenous MMTV infection is usually a recessive characteristic, which has been used to determine if various mouse strains have the same or different resistance genes. For example, C57BL and I strain mice are both resistant to C3H MMTV infection, yet F1 hybrids of these strains have a high incidence of mammary tumors when infected by C3H virus. This result suggests that I strain mice ($H\text{-}2^{i}$) encode a functional MHC class II I–E molecule that complements the defect in C57BL mice and overcomes resistance to MMTV infection. More recently, disease resistance in I strain mice has been attributed to the development of strong neutralizing antibodies to MMTV. Further, development of neutralizing antibodies appears to be dependent on the MMTV strain. MMTVs with weak Sag activities (e.g., C3H MMTV) appear to elicit few neutralizing antibodies, whereas the opposite applies to MMTVs with strong Sag function (e.g., SW MMTV). Another host resistance mechanism is mediated by Toll-like receptor 4 (TLR4) signaling, which normally triggers an innate immune response. Wild-type C3H MMTV is lost during passage in C3H/HeJ animals, which lack functional TLR4, but not in related TLR4+ mice. C3H MMTV interactions with TLR4 trigger the immunosuppressive cytokine IL-10, allowing the virus to subvert the innate immunity by elimination of cytotoxic T cells. Further, YBR/Ei mice exhibit a dominant resistance to MMTV that depends on an adaptive T-cell response to the infection. However, not all MMTV resistance mechanisms may depend on the immune response; NH mice are resistant to the virus, perhaps related to hormonal changes that lead to early reproductive difficulties in this strain.

Traditional types of genetic experiments with MMTV have been difficult for two reasons. First, since MMTV does not form plaques or foci in cultured cells, cloning of viral stocks has not been possible. Second, molecular cloning of an intact MMTV provirus has been difficult for some MMTV strains because of selection against a specific part of the *gag* region during growth of proviral clones in *Escherichia coli*. This difficulty has been overcome by combining the 5′ end of an endogenous provirus (e.g., *Mtv1*) with the 3′ end of an exogenous provirus (e.g., C3H MMTV). Such hybrid proviruses have been used to produce infectious virions that retain oncogenicity for the mammary gland and to show that the MMTV *gag* region contributes to development of mammary tumors.

Pathogenicity

MMTV induces primarily type A and B mammary adenocarcinomas. Insertional mutagenesis is presumed to be the mechanism of MMTV-induced mammary tumors and is consistent with the relatively long latent period for tumor development (6–9 months). However, the MMTV envelope protein may act as a tumor initiator for mammary cells.

Common integration sites can be identified from MMTV-induced tumors. Current data have implicated more than 10 different loci (designated *int* or integration site genes) in MMTV-induced mammary tumors (**Table 1**). Most of these integration sites share the following characteristics:

1. The majority of the MMTV integrations are outside of the gene-coding regions, and the MMTV promoter is rarely used to initiate *int* gene transcription. Thus, an unmodified protein product is produced.
2. The MMTV provirus can activate target gene transcription over considerable distance (in excess of 15 kb). There may be activation of multiple genes in a cluster.
3. Proviruses often are integrated upstream in the opposite transcriptional orientation or downstream in the same orientation as the target gene.

Table 1 Common integration sites found in MMTV-induced mammary tumors

Locus	*Gene family/function*	*Mouse chromosome*	*Integration frequency*
Wnt1 (*Int-1*)	Wingless/growth factor	15	80% in C3H; 70% in BR6; 30% in GR
Wnt3 (*Int-4*)	Wingless/growth factor	11	10% in GR
Wnt10b	Wingless/growth factor	15	23% of *Fgf3* transgenic
eIF3-p48 (*Int-6*)	Eukaryotic translation initiation factor	15	6% in *Fgf3* transgenic
Rspo2 (*Int-7*)	R-spondin homolog/ growth factor receptor	15	13% in Czech II
Fgf3 (*Int-2*)	Fibroblast growth factor	7	65% in BR6; 5% in C3H; 20% in GR
Fgf4 (*Fgfk/hst/hst-1/ Hstf-1*)	Fibroblast growth factor	7	10% in BR6
Fgf8 (*Aigf*)	Fibroblast growth factor	19	80% in *Wnt1* transgenic
Notch1 (*Mis6/Tan1*)	Notch	2	8% of *Erbb2* transgenic
Notch4 (*Int-3*)	Notch	17	20% in Czech II; 8% in BR6
Cyp19a1 (*Cyp19/Int-5/ Int-H*)	Cytochrome P450/aromatase	9	3 chemically induced BALB/c hyperplasias

4. Target gene transcription is low or undetectable in normal adult mammary glands.
5. Transcription of the *int* genes is regulated developmentally.
6. The *int* genes appear to be conserved evolutionarily, and a number of these genes encode growth factors or truncated growth factor receptors.

Such observations are consistent with the transcriptional activation of conserved genes in the mammary gland by their proximity to MMTV LTR enhancers. Transgenic animals overexpressing the *int* genes often show mammary hyperplasias and sporadic tumors; this observation confirms the involvement of multiple genes during oncogenesis. Mating of animals with different transgenes accelerates this process, indicating cooperation between *int* genes.

MMTV variants that are expressed preferentially in T cells induce T-cell lymphomas. Interestingly, these thymotropic MMTV appear to activate a different set of oncogenes, for example, c-*myc* and *Rorc*, relative to milk-borne MMTVs as a result of LTR alterations.

Transcriptional Regulation

Studies of the MMTV HRE have been a paradigm for hormone-regulated gene expression. MMTV RNA levels increase *c.* 10–50-fold in the presence of glucocorticoids, progesterone, or androgens at the level of transcriptional initiation. Hormone inducibility is conferred by multiple binding sites to allow cooperative binding with the consensus TGTTCT in the region from –80 to –190 (**Figure 4**). Glucocorticoids are believed to bind to their receptors (GRs) in the cytoplasm and to subsequently translocate to the nucleus where they exchange rapidly with their binding sites. The integrated MMTV LTR is occupied by six nucleosomes (A–F) (not shown) with the A nucleosome located at the transcription initiation site (+1). The standard MMTV promoter contains a TATA element (−30 bp), which binds transcription factor IID (TFIID). Hormone-bound GR is believed to recruit remodeling factors to increase accessibility at nucleosome B and to allow binding of transcription factors Oct1 and NF1 between the TATA box and the HRE.

The tissue distribution of MMTV expression is tightly linked to viral transcriptional control, yet steroid receptors are present in many tissues in which MMTV transcription is repressed. The importance of negative regulation in MMTV transcription and disease specificity is exemplified by the isolation of MMTV variants, such as type B leukemogenic virus (TBLV), which induce T-cell lymphomas rather than mammary tumors. Such variants lack all or part of the negative regulatory element (NRE) present upstream of the HRE and acquire a T-cell specific enhancer. The NRE binds at least two related homeodomain-containing proteins called special AT-rich binding protein 1 (SATB1) and Cut-like protein1/CCAAT displacement protein (Cutl1/CDP), which have different tissue distributions and act as repressors of MMTV transcription. The highest levels of SATB1 are expressed not only in T cells but also in B cells explaining transcriptional repression in most lymphoid tissues. Mutations of the promoter-proximal SATB1-binding site elevate MMTV expression of LTR-reporter constructs in cultured cells and in lymphoid tissues of transgenic mice. Interestingly, CDP expression is high in undifferentiated B cells and mammary cells and decreases during differentiation. In mammary epithelial cells, where SATB1 is also absent, CDP is cleaved during differentiation to yield a dominant-negative protein that interferes with full-length CDP binding to the MMTV LTR. Therefore, the levels of functional CDP repressor are lowest when MMTV particle production is highest at lactation, the period when virus transmission occurs in milk.

MMTV specifies several enhancer elements, including the HRE and a mammary gland enhancer (MGE) near the 5′ end of the LTR. The MGE contains binding sites for multiple nuclear factors, including MP4, NF1, AP2, Ets, and C/EBP. Synthesis of some factors is inducible by prolactin, epidermal growth factor, or tumor necrosis factor α. The absence of NRE-binding repressors and ligand-bound steroid receptors presumably cooperate with the HRE to maximize virus production in milk.

Immune Response

Endogenous MMTVs, like exogenous viruses, express Sag proteins, initially known as minor lymphocyte stimulating (Mls) antigens. Unlike conventional antigens where a peptide associates with the groove formed by the MHC class II α- and β-chains, the central portion of Sag binds to class II protein (**Figure 5**). Since Sag is a type II transmembrane protein, the C-terminal portion is available to interact with the variable region of the β-chain (Vβ) of the TCR on a subset of CD4+ or CD4+CD8+ cells. Therefore, Sag recognizes entire classes of T cells (up to 30% of the entire T-cell repertoire), in contrast to conventional antigens that recognize TCR on less than 1 in 10^4 T cells. Sag stimulation leads to the rapid deletion of reactive cells if Sag is expressed in the thymus, whereas extrathymic deletion appears to occur more slowly.

Sag is relatively conserved among different MMTV strains, except at the C-terminus, which mediates TCR interactions. C-terminal polymorphisms correlate with reactivity with specific TCR β-chains; for example, C3H MMTV reacts with Vβ14 and 15 chains. Molecular switching experiments have shown that the C-terminal half is sufficient to specify TCR reactivity, and virtually any mutation within this region is sufficient to abolish function. Deletion of specific T-cell subsets in various feral and laboratory mouse strains is believed to provide immunity to specific milk-borne MMTVs, and there is evidence that endogenous *Mtv*s shape the immune response against other pathogens.

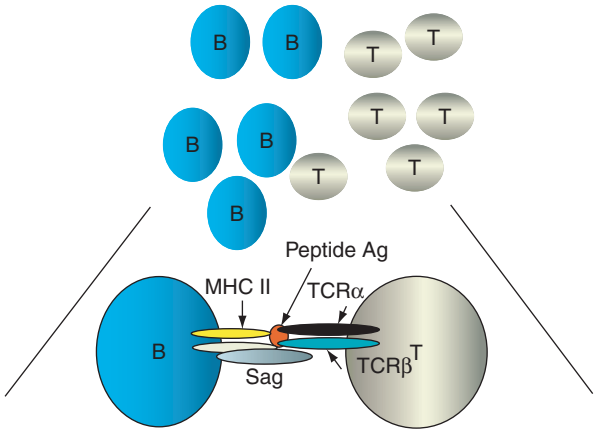


Figure 5 Interaction of MMTV Sag with the TCR. The α and β chains of the TCR on CD4+ cells combine to recognize a peptide (foreign or self) (small circle) residing in the binding pocket of the MHC class II molecule on MMTV-infected antigen-presenting cells. The C-terminal end of Sag interacts with the variable region of the TCR β-chain, although the TCR α-chain has been reported to affect Sag binding. Accessory molecules such as CD4 probably affect the stability of the Sag–TCR interaction.

Future Perspectives

MMTV has been valuable for studies of hormone-regulated gene expression, tissue-specific transcription, immune response, and mechanisms of oncogenesis. The recent discovery that MMTV is a complex retrovirus suggests that this virus will provide a useful model for understanding pathogenesis by human retroviruses, such as HTLV and HIV.

See also: Immune Response to viruses: Antibody-Mediated Immunity; Retroviruses: General Features; Viral Pathogenesis.

Further Reading

Bhadra S, Lozano MM, and Dudley JP (2005) Conversion of mouse mammary tumor virus to a lymphomagenic virus. *Journal of Virology* 79: 12592–12596.

Callahan R and Smith GH (2000) MMTV-induced mammary tumorigenesis: Gene discovery, progression to malignancy and cellular pathways. *Oncogene* 19: 992–1001.

Czarneski J, Rassa JC, and Ross SR (2003) Mouse mammary tumor virus and the immune system. *Immunologic Research* 27: 469–480.

Golovkina TV, Chervonsky A, Dudley JP, and Ross SR (1992) Transgenic mouse mammary tumor virus superantigen expression prevents viral infection. *Cell* 69: 637–645.

Held W, Waanders GA, Shakhov AN, Scarpellino L, Acha-Orbea H, and MacDonald HR (1993) Superantigen-induced immune stimulation amplifies mouse mammary tumor virus infection and allows virus transmission. *Cell* 74: 529–540.

Jude BA, Pobezinskaya Y, Bishop J, *et al.* (2003) Subversion of the innate immune system by a retrovirus. *Nature Immunology* 4: 573–578.

Kinyamu HK, Chen J, and Archer TK (2005) Linking the ubiquitin-proteasome pathway to chromatin remodeling/modification by nuclear receptors. *Journal of Molecular Endocrinology* 34: 281–297.

Liu J, Bramblett D, Zhu Q, *et al.* (1997) The matrix attachment region-binding protein SATB1 participates in negative regulation of tissue-specific gene expression. *Molecular and Cellular Biology* 17: 5275–5287.

Mertz JA, Simper MS, Lozano MM, Payne SM, and Dudley JP (2005) Mouse mammary tumor virus encodes a self-regulatory RNA export protein and is a complex retrovirus. *Journal of Virology* 79: 14737–14747.

Ross SR, Schofield JJ, Farr CJ, and Bucan M (2002) Mouse transferrin receptor 1 is the cell entry receptor for mouse mammary tumor virus. *Proceedings of the National Academy of Sciences, USA* 99: 12386–12390.

Zhu Q, Maitra U, Johnston D, Lozano M, and Dudley JP (2004) The homeodomain protein CDP regulates mammary-specific gene transcription and tumorigenesis. *Molecular and Cellular Biology* 24: 4810–4823.

Mousepox and Rabbitpox Viruses

M Regner, F Fenner, and A Müllbacher, Australian National University, Canberra, ACT, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Pyknotic Exhibiting a degenerate nucleus by contraction of nuclear contents; a sign of cell death.

Introduction

Ectomelia virus (ECTV), which is the agent of mousepox, and rabbitpox virus (RPXV) share two features; they are both orthopoxviruses and both are known only as infections of laboratory animals, the mouse and rabbit, respectively.

Ectromelia Virus

History

ECTV was discovered in 1930 by J. Marchal, as a spontaneous infection of laboratory mice at the National Institute of Medical Research in London. It was called infectious ectromelia because of the frequent occurrence of amputation of a foot in animals that had recovered from infection. Soon after, J. E. Barnard showed, by ultraviolet (UV) microscopy, that it had oval virions about the same size as those of vaccinia virus (VACV). The only other experiments done with the virus at that time involved studies of experimental epidemics by W. W. C. Topley and his colleagues.

In 1946, F. M. Burnet, in Melbourne, showed that ECTV was serologically related to VACV. During experimental epidemics carried out in Burnet's laboratory, F. Fenner found that in animals that did not die of acute hepatitis there was a rash, and he named the disease mousepox. Subsequent studies led to the development of a classical model explaining the spread of virus around the body in generalized viral infections with rash.

In laboratories in Europe and the USA, the virus was regarded as a major menace to colonies of laboratory mice, and stringent steps were taken to prevent its entry to the USA. Only after extensive outbreaks in several cities of that country in 1979 were studies of the virus undertaken in the USA, in high-security laboratories.

Classification

Ectromelia virus is a species within the genus *Orthopoxvirus* of the family *Poxviridae*, as evidenced by the morphology of the virion, cross-protection tests, and restriction endonuclease mapping. Most strains of *Ectromelia virus* (e.g., Moscow, Hampstead) recovered from naturally infected mouse colonies are highly virulent. However, a substrain of Marchal's original strain was attenuated by serial passage on the chorioallantoic membrane of chicken eggs (Hampstead egg).

Genetics

The ECTV genome is typical for an orthopoxvirus in size and composition. It is A + T-rich and comprises 210 kbp. Almost half of the genome consists of a central region of high homology to other orthopoxviruses and this is flanked by significantly more variable terminal regions. The genome comprises 175 potential genes encoding proteins, including numerous predicated host range proteins and host response modifiers. ECTV does not cluster phylogenetically with any other member of the virus family.

Host Range and Virus Propagation

ECTV produces disease in *Mus musculus* and several other species of mice, and is considered a natural mouse pathogen. The rabbit, guinea pig, and rat can be infected by intradermal or intranasal inoculation, with the production of small skin lesions or an inapparent infection. ECTV will grow on the chorioallantoic membrane of the developing chick embryo, and also in cell cultures derived from a variety of species. Quantitation of virus stocks is determined by growth on cell culture monolayers (plaque assay).

Geographic Range and Seasonal Distribution

ECTV has been spread around the world inadvertently by scientists working with laboratory mice, and has been repeatedly reported from laboratories in several countries of Europe and from Japan and China. Mousepox has never been enzootic for prolonged periods in mouse colonies in the USA, but accidental importations sometimes occurred with mice or mouse tissues from European laboratories, with devastating consequences. In contrast, there are extremely limited data regarding the occurrence of ECTV in wild animals.

Epidemiology

In laboratory mice, ECTV is infectious by all routes of inoculation. It always produces a generalized infection but there are local lesions in the lungs after intranasal

inoculation and in the peritoneal cavity after intraperitoneal injection. The response of mice is strongly conditioned by mouse genotype (see below).

The usual source of natural infection is via minor abrasions of the skin, which may occur from contaminated bedding or during manipulations by animal handlers. Infection may also occur by the respiratory route, but probably only between mice in close proximity to each other. A primary lesion usually develops at the site of infection. Since mice are readily infected by inoculation, virus-contaminated mouse serum, ascites fluid or mouse cells, tumors or tissues constitute a risk to laboratory colonies previously free of infection.

Enzootic Mousepox

Until the introduction of rigorous screening techniques in the 1960s, mousepox was enzootic in many mouse-breeding establishments in Europe and Japan. A variety of mechanisms probably operated to maintain the virus, without disrupting the mouse-breeding program as to make control mandatory. One important factor was probably the high level of genetic resistance and trivial symptomatology exhibited by many mouse genotypes. Another may have been maternal antibody. Another possible mechanism for maintaining enzootic infection is chronic, clinically inapparent infection, which sometimes occurs after oral administration, when some mice show infection of Peyer's patches, excretion of virus in the feces, and lesions in tail skin.

Susceptibility of Different Strains of Mice

Analysis of spontaneous epizootics and deliberate experiments in the 1980s showed that C57BL/6, 129J, and AKR mice were highly resistant to mousepox, CBA/J and SJL/J intermediate, and BALB/c and A/J mice were highly susceptible. Strain differences are best demonstrated after footpad inoculation or in natural epizootics, since C57BL/6 mice are relatively susceptible by intranasal, intracerebral, or intraperitoneal infection.

Pathogenesis

Fenner's work in the 1930s to 1940s established mousepox as a model system for the study of generalized viral infections. Müllbacher has more recently used the mousepox model for the analysis of immune effector molecules required for resistance to ECTV infections, using specific gene knockout mutant mouse strains. Mousepox is generally considered an excellent mouse model of human smallpox and is now used increasingly by the bioterrorism defense research community.

Mice are usually infected in the footpad and, after an incubation period of *c.* 7 days (as found in natural infections), a local ('primary') lesion develops at the inoculation site. A few days later some mice die, with no other visible skin lesions but with acute necrosis of the liver and spleen, and in those that survive a rash develops which goes through macular and pustular stages before it scabs.

During the incubation period the virus passes through the mouse body in a stepwise fashion: infection, multiplication and liberation, usually accompanied by cell necrosis, first in the skin and then the regional and possibly the deeper lymph nodes, until it reaches the bloodstream (primary viremia).

C. A. Mims showed in the 1960s that, during the primary viremia, virus is ingested by the phagocytic littoral cells of the liver and spleen. After a day or so, much larger amounts of virus are liberated into the circulation (secondary viremia). Next follows an interval during which the virus multiplies to high titer before visible changes are produced, so that 2 or 3 days usually elapse between the appearance of the primary lesion and the secondary rash. Some animals die before skin lesions appear, but titration experiments and histological examination showed that early skin lesions are present.

Clinical Features of Infection

Early workers described two forms of the disease, a rapidly fatal form in which apparently healthy mice die within a few hours of the first signs of illness and show extensive necrosis of the liver and spleen at autopsy, and a chronic form characterized by ulcerating lesions of the feet, tail, and snout. Fenner found that in natural infections most mice develop a primary lesion, usually on the snout, feet, or belly. Subsequently, virus multiplies to high titer in the liver and spleen. Some mice die at this stage, but if they survive they almost invariably develop a generalized rash (**Figure 1**).

Age affects the response of genetically susceptible mice. Both virulent and attenuated strains produce higher mortalities in suckling mice and in mice about a year old than in 8-week-old mice.

Pathology and histopathology

The pathological changes in naturally occurring mousepox in susceptible mice are quite characteristic. Additional lesions in the peritoneal cavity or lung occur after intraperitoneal or intranasal inoculation, respectively; these are important because mousepox sometimes occurs after unwitting passage of ECTV by these routes.

Intracytoplasmic inclusion bodies

ECTV produces two types of intracytoplasmic inclusion body in infected cells, A-type and B-type. The latter occur in all poxvirus infections and are the sites of viral multiplication; more characteristic of mousepox are the

prominent acidophilic inclusion bodies (A-type), which are always found in infected epithelial cells but rarely in liver cells (**Figure 2**).

Skin lesions

The earliest primary lesions that can be recognized macroscopically are the seat of advanced histological changes, for viral multiplication has then been in progress for several days. There is no macroscopic breach of the skin surface, but the dermis and subcutaneous tissue are edematous and there is widespread lymphocytic infiltration of the dermis. Inclusion bodies can be seen in the epidermal cells at the summit of the lesion. Necrosis of these epidermal cells is followed by ulceration of the surface. The exudate forms a scab beneath which healing occurs. Histologically, the changes of the rash are similar to those in the primary lesion.

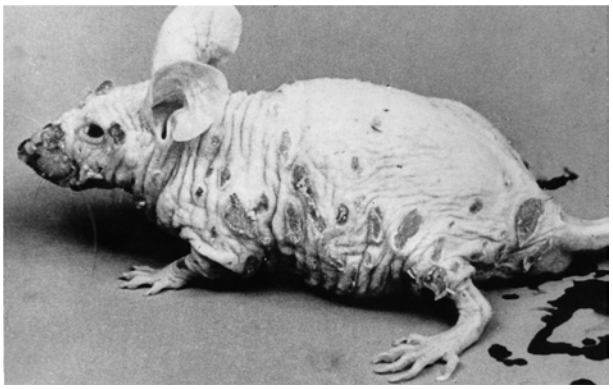


Figure 1 The rash of mousepox as it appears 14 days after infection, in a naturally infected genetically hairless mouse (not athymic). Similar lesions occur beneath the hair of other strains of susceptible mice and can be clearly demonstrated by epilation. Reproduced from Fenner F (1982) Mousepox. In: Foster HL, Small JD, and Fox JG (eds.) *The Mouse in Biomedical Research*, vol II, pp. 209–330. New York: Academic Press, with permission from Elsevier.

Lesions of the liver

The liver and spleen are invariably invaded during the incubation period and virus multiplies to high titer here. The liver remains macroscopically normal until within 24 h of death, when it appears enlarged and studded with minute white foci. The necrotic process extends rapidly and at the time of death the liver is enlarged with many large semiconfluent necrotic foci. In animals that survive, the liver usually returns to its normal macroscopic appearance, but occasionally numerous white foci occur.

Histologically, little change is apparent until macroscopic changes have appeared, although immunofluorescence techniques have shown that infection always occurs first in the littoral cells of the hepatic ducts, from which the virus spreads to contiguous parenchymal cells. Numerous scattered foci of necrosis then appear throughout the liver parenchyma and in fatal cases these rapidly extend until they became semiconfluent. The portal tracts show slight infiltration with lymphoid cells. Liver regeneration commences early and is active, especially in nonfatal cases, and fibrosis does not occur.

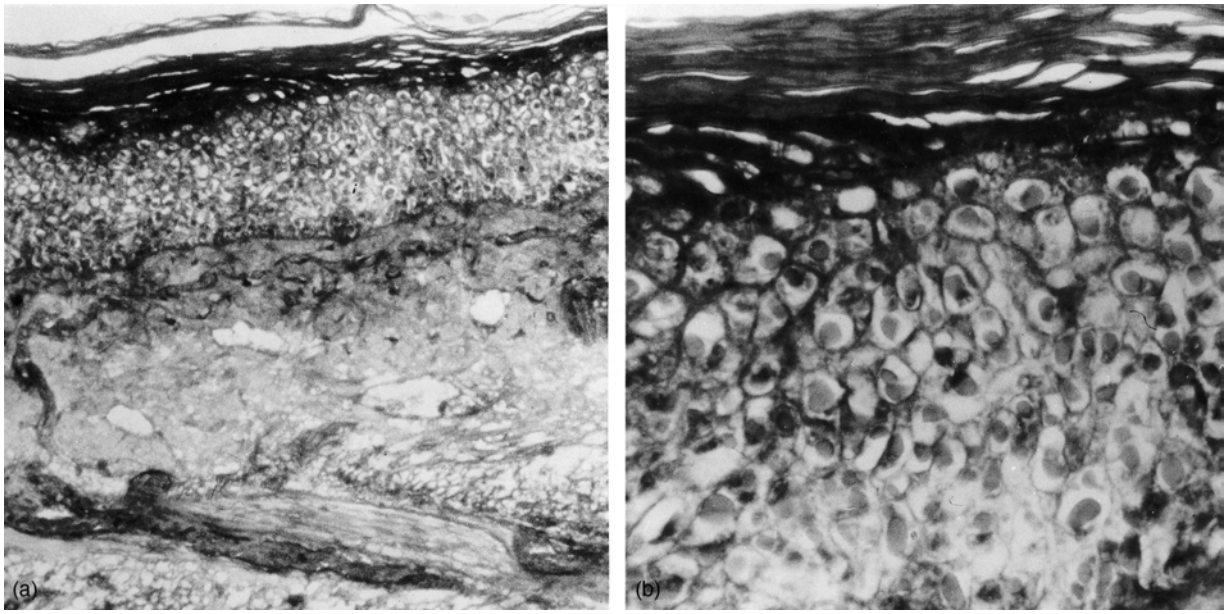


Figure 2 Section of the skin of the foot of a mouse injected with ECTV in the footpad six days earlier. (a) Low power. (b) High power, Mann's stain. Almost every epithelial cell contains an eosinophilic A-type inclusion body. With most strains of ECTV, these A-type inclusion bodies contain large numbers of mature virions. Reproduced from Fenner F (1982) Mousepox. In: Foster HL, Small JD, and Fox JG (eds.) *The Mouse in Biomedical Research*, vol II, pp. 209–330. New York: Academic Press, with permission from Elsevier.

Lesions of the spleen

The spleen shows macroscopic changes at least a day earlier than the liver and higher titers of virus are found in the spleen. Virus reaches the spleen in infected lymphocytes, which initiate infection in the substance of the follicles. While infected follicles are destroyed by the spreading infection, neighboring follicles show the proliferative response characteristic of antibody production.

In surviving mice, lesions of the spleen vary from small raised plaques about a millimeter in diameter to areas of fibrous tissue that, after severe attacks, almost completely replace the normal splenic tissue. These changes constitute reliable autopsy evidence that a mouse has recovered from an attack of mousepox.

Lesions of other organs

The regional lymph nodes draining the site of the primary lesion are enlarged from the time the primary lesions can be detected, and they usually show localized areas of necrosis, with pyknotic nuclear debris in a featureless background. In fatal cases, the gut is often engorged and the lymphoid follicles enlarged. Small necrotic foci with typical inclusion bodies occur in the intestines in most acutely fatal cases of mousepox. Occasionally, especially in very young mice, there are hemorrhagic foci in the kidneys.

Lesions after intraperitoneal inoculation

There is no primary skin lesion, but in acutely fatal cases the necrosis of the liver and spleen resembles that found after natural infection. In addition, there is usually some increase in intraperitoneal fluid and a considerable amount of pleural fluid, and the pancreas is often grossly edematous. In animals that survive the acute infection there is a great excess of peritoneal and pleural fluid, the peritoneal surfaces of the liver and spleen are covered with a white exudate, the walls of the gut are thickened and rigid, and there is often fat necrosis in the intraperitoneal fat. Extensive adhesions between the abdominal viscera develop later.

Lesions after intranasal inoculation

When small doses of virus are inoculated intranasally, there is usually little change in the lungs except patchy congestion; the changes in the liver and spleen are those characteristic of naturally acquired mousepox. With larger doses of virus, congestion of the lungs is more pronounced and consolidation may occur, and when very large doses are given death occurs with patchy or complete consolidation of the lungs and little change in the liver and spleen. The apparent pneumotropism is due to the fact that the local reaction, which occurs after the intranasal inoculation of very large doses of virus, kills the animal before there is time for the characteristic changes in the liver and spleen to occur.

Immune Response

Two weeks after infection mice are solidly immune to reinfection by footpad inoculation of the virus. This immunity declines slowly but even a year after recovery multiplication of the virus after footpad challenge is confined to the local skin lesion.

Humoral immunity

Antibodies generated by a primary infection protect from subsequent challenge. Newborn mice receive maternal antibody via the placenta and in the milk during the first 7 days after birth. Until titers decline to undetectable levels by the seventh week after birth, this maternal antibody confers protection against death, but not against infection, with moderate doses of ECTV. Furthermore, in the absence of functional B cells, clearance of primary infection with ECTV may be deficient, leading to viral persistence and eventual death.

Cell-mediated immunity

Work by Blanden in the early 1970s showed that T cells are critical in recovery from primary infection with ECTV, and mice pre-treated with anti-thymocyte serum die from otherwise sublethal doses of virus due to uncontrolled viral growth in target organs. These mice have impaired cell-mediated responses but normal neutralizing antibody responses, elevated interferon levels in the spleen, and unchanged innate resistance in target organs. The active cells in the immune population are cytotoxic T cells, although natural killer cells probably also play an important role early in the infection.

Virus-specific cytotoxic T cells are detectable 4 days after infection and reach peak levels in the spleen 1–2 days later, while delayed hypersensitivity is detectable by the footpad test 5–6 days after inoculation. In contrast, significant neutralizing antibody is not detectable in the circulation until the eighth day.

Cytotoxic T cells employ two different mechanisms to destroy virus-infected cells before the release of viral progeny: one, the granule exocytosis pathway, is mediated by perforin and granzymes that are stored in cytolytic granules and secreted toward, and enter, the infected cell, inducing apoptosis; the other one via triggering of death receptors (e.g., Fas) on the surface of infected cells, inducing a cascade of caspase activation that also leads to apoptosis. However, poxviruses encode inhibitors of the death receptor pathway of killing (e.g., SPI-2, see below), rendering the granule pathway indispensable for recovery. Consequently, mice genetically deficient in perforin or both principal granzymes (A and B) are highly susceptible to ECTV infection.

T-cell-secreted cytokines are also important factors determining the outcome of infection. Whereas interferon-gamma (IFN-γ) critically contributes to viral clearance,

an imbalanced cytokine response of the so-called Th2 type, characterized by lack of IFN-γ and excess of interleukin-4 (IL-4), predisposes to greater susceptibility. A recombinant ECTV expressing IL-4 was found to be highly virulent even in resistant strains of mice and those vaccinated with attenuated virus, prompting concerns about the possible creation of recombinant poxviruses with increased virulence in humans for the purpose of bioterrorism.

Host response modulation

As do other poxviruses, ECTV encodes numerous host-response modifiers in order to evade or suppress the immune response to allow for maximal viral replication. Broadly, these can be divided into inhibitors of the inflammatory response, and anti-apoptotic proteins. Several ECTV-encoded proteins have been shown to neutralize or inhibit key inflammatory cytokine pathways, for example, an IL-18-binding protein and homologs of the IL-1β, tumor necrosis factor (TNF) and IFN receptors. On the other hand, virally encoded inhibitors of components of the caspase cascade inhibit apoptotic pathways. Examples are the ECTV protein p28, a RING finger-domain protein, which is a potent ECTV virulence factor that inhibits UV-induced, but not Fas- or TNF-induced apoptosis; and SPI-2, a serine proteinase inhibitor, which blocks TNF-α-mediated apoptosis via caspase 1/8 inhibition.

Future

Mousepox is now very rare as a natural infection in laboratory colonies of mice but it is likely that, despite the strict controls necessary to protect mouse colonies, mousepox will be investigated more extensively in the future. Workers in Australia, the USA, and the UK are now using it as a model for studies of problems such as immunocontraception and the role of the many homologs of mammalian host response modifier genes that are found in all poxviruses. In addition, the recognition of mousepox as a good mouse model for human smallpox has, in the light of increased interest in poxvirus immunobiology, seen a resurgence of research into its pathologies, immune evasion strategies, and the mechanisms of recovery from this disease.

Rabbitpox Virus

History and Classification

Rabbitpox is a laboratory artifact, due to the infection of laboratory rabbits with VACV, usually with neuro-VACV variants; hence this account of RPXV will omit reference to those aspects that are covered in the article on VACV.

The name rabbitpox was originally given to devastating outbreaks of a generalized disease, likened to smallpox in man, in a colony of laboratory rabbits at the Rockefeller Institute of Medical Research in New York in 1932–34, when other scientists had been working with neuro-VACV in rabbits in an adjacent room prior to the outbreak. The virus recovered from the outbreak was called RPXV and was shown to be very similar to neuro-VACV in its biological properties. Subsequently, the restriction map of the Utrecht strain (see below) was found to be almost identical with that of VACV.

Another outbreak occurred in the Netherlands in 1941. It began among rabbits bought from a dealer a few days after they were introduced into the laboratory colony, and spread among the stock rabbits. The disease was usually lethal, death occurring before there was time for the development of a rash. The virus that caused this outbreak, designated RPXV-Utrecht, caused similar highly lethal epizootics when it 'escaped' in the Institut Pasteur in Paris in 1947; other outbreaks have been described in laboratory rabbits in the USA in the 1960s.

Epidemiology

In all outbreaks, spread appeared to occur by the respiratory route, and experiments confirmed that infection occurs readily by this route. Rabbits infected by contact are not infectious for other rabbits until the second day of illness, which is usually 5 days after infection. Actual contact is not necessary; transmission can occur across the width of a room, and air sampling revealed the presence of RPXV in the air of rooms housing infected rabbits.

Genetics

Since 1960, the Utrecht strain of RPXV virus has been used for genetic studies on poxviruses, since it was found to give rise to white pock mutants on the chorioallantoic membrane of chicken eggs and host range mutants in a pig kidney cell line, both of which entail deletions and transpositions of DNA. Recombination experiments with the white pock mutants were used to construct the first crude 'genetic map' produced for an animal virus.

When the complete coding sequence of the Utrecht strain was reported, it was confirmed that it is most closely related to VACV, with more than 95% sequence similarity. It was also established that RPXV is not a direct evolutionary descendant from VACV, as it contains several genes present in smallpox virus but not VACV.

Pathogenesis

A good deal of experimental work has been carried out on the pathogenesis of rabbitpox as an animal model of smallpox, with results that were largely confirmatory of those obtained with mousepox. In rabbits infected by the intranasal instillation of a small dose of virus, by aerosol, or after intradermal infection or contact infection, there is a stepwise spread of virus through the organs,

although the incubation period is shorter than in mousepox and there seems to be little delay at the regional lymph nodes. Viremia is leukocyte associated.

Clinical Features of Infection

RPXV causes an acute generalized disease in which a rash appears in animals that survive long enough, presenting as pocks on the skin and mucous membranes (**Figure 3**). Rabbits dying of hyperacute infection show no obvious skin lesions, the so-called 'pockless' rabbitpox. Such infections are analogous to acutely lethal cases of mousepox and perhaps to early hemorrhagic-type smallpox, in which death occurs before there is time for pustular skin lesions to develop.

Pathology and Histopathology

The most distinctive lesions are the pocks on the skin and mucous membranes and occasionally small areas of focal necrosis are found in the internal organs (liver, spleen, lung, testes, ovaries, uterus, adrenals, and lymph nodes). In the so-called 'pockless' form, a few pocks may occur around the mouth and they may be visible on the shaved skin. The most prominent gross lesions are pleuritis, focal necrosis of the liver, enlarged spleen, and edema and hemorrhage of the testes.

RPXV, being a strain of VACV, produces B-type inclusions (Guarnieri bodies) in infected cells, but not the prominent A-type inclusions found in cells infected with ECTV.

Immune Response

Rabbits that have recovered from rabbitpox are immune to infection with VACV, but in very severe infections rabbits die before there is time for an effective immune response. The importance of enveloped virions in the pathogenesis and immunology of orthopoxvirus infections was fist demonstrated in experiments with RPXV. Passive immunization with sera that did not contain antibody to the viral envelope failed to protect rabbits against challenge infection, even though the neutralization titer of the ineffective antiserum (produced by immunization with inactivated VACV) was much higher, as judged by conventional neutralization tests. This work helped explain the failure of inactivated VACV to provide protection against infection with orthopoxviruses.

Future

Since rabbitpox appears to be a laboratory artifact, due to the introduction of strains of VACV that can spread from one rabbit to another in rabbit colonies, prophylaxis appears to be a matter of preventing such events. These events were rare even when VACV, and especially neuro-VACV, were extensively used in animal experiments several decades ago, and it is unlikely that further episodes will occur now that most research with VACV utilizes cultured cells rather than intact animals. Nevertheless, laboratory managers who use rabbits should be aware of the possibility that some strains of VACV can spread naturally from one rabbit to another.

Because of the higher sensitivity of rabbits to low doses with RPXV compared to that of mice to ECTV, and efficient spread via the aerosol and respiratory routes, RPXV has also been recently revisited as a model for human smallpox.

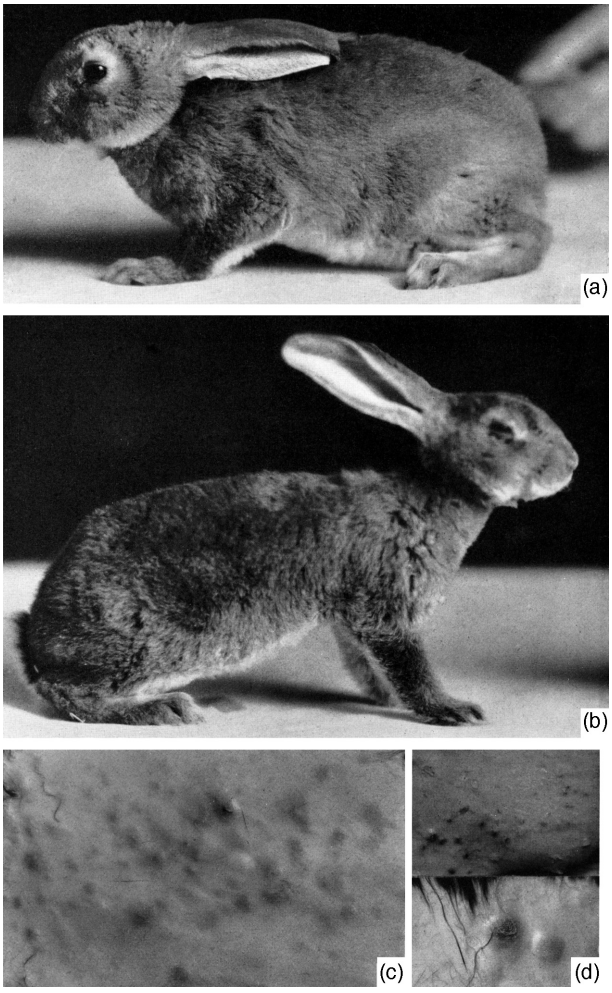


Figure 3 Rabbitpox. (a) and (b) Littermates with different types of disease. The course of infection was mild in the rabbit shown in (a) and external lesions were limited in the skin; the animal in (b) was seriously ill and its posture is a manifestation of acute respiratory distress resulting from extensive mouth lesions. (c) Cutaneous lesions on the trunk. The coat in this area was loose and easily plucked by hand. (d) Skin of a pregnant doe self-plucked for nest fur, showing both dry crusted pustules and others in earlier stages of development. Reproduced from Greene HSN (1934) Rabbit pox: I. Clinical manifestations and course of disease *Journal of Experimental Medicine* 60: 427–440. Copyright 1934 The Rockefeller University Press.

See also: Poxviruses; Smallpox and Monkeypox Viruses; Vaccinia Virus.

Further Reading

Adams MM (2007) Rabbitpox and vaccinia virus infections of rabbits as a model for human smallpox. *Journal of Virology* 81: 11084–11095.
Esteban DJ and Buller RM (2005) Ectromelia virus: The causative agent of mousepox. *Journal of General Virology* 86: 2645–2659.
Fenner F (1982) Mousepox. In: Foster HL, Small JD, and Fox JG (eds.) *The Mouse in Biomedical Research,* vol. II, pp. 209–230. New York: Academic Press.
Fenner F and Buller RML (1996) *Mousepox*. In: Nathanson N (ed.) *Viral Pathogenesis*, 535pp. Philadelphia: Lippincot-Raven.
Fenner F, Wittek R, and Dumbell KR (1989) *The Orthopoxviruses.* San Diego: Academic Press.
Greene HSN (1934) Rabbit pox. I: Clinical manifestations and course of disease. *Journal of Experimental Medicine* 60: 427–440.
Müllbacher A (2003) Cell-mediated cytotoxicity in recovery from poxvirus infections. *Reviews in Medical Virology* 13: 223–232.
Müllbacher A and Lobigs M (2001) Creation of killer poxvirus could have been predicted. *Journal of Virology* 75: 8353–8355.

Movement of Viruses in Plants

P A Harries and R S Nelson, Samuel Roberts Noble Foundation, Inc., Ardmore, OK, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ancillary viral proteins Virus-encoded proteins that do not meet the definition of a movement protein, but are required for virus movement.
Intercellular movement Movement between two cells.
Intracellular movement Movement within a single cell.
Microfilaments A component of the cytoskeleton formed from polymerized actin monomers.
Microtubules A component of the cytoskeleton composed of hollow tubes formed from α–β tubulin dimers.
Molecular chaperones A family of cellular proteins that mediate the correct assembly or disassembly of other polypeptides.
Movement protein Virus-encoded proteins that can transport themselves cell to cell, bind RNA, and increase the size exclusion limits of plasmodesmata.
Phloem Vascular tissue that transports dissolved nutrients (e.g., sugars) from the photosynthetically active leaves to the other parts of the plant. In most plants there is only one phloem class, but for some plant families this tissue is divided into two classes: (1) internal phloem (internal or adaxial to xylem) and (2) external phloem (external or abaxial to xylem).
Systemic movement Movement through vascular tissue to all parts of the plant.
Viroid A plant pathogen containing nucleic acid that encodes no proteins.
Xylem Vascular tissue that transports water and minerals through the plant.

Introduction

In order for a plant virus to infect its host systemically, it must be capable of hijacking the host's cellular machinery to replicate and move from the initially infected cell. Plant viruses require wounding, usually by insect or fungal vectors or mechanical abrasion, for an infection to begin. Once inside a cell, the virus initiates transcription (DNA viruses) and translation and replication (DNA and RNA viruses) activities. Some of these viral products are required for virus movement and often interact with host factors (proteins or membranes) to carry out this function. Virus movement in plants can be broken down into three distinct steps: (1) intracellular movement, (2) intercellular movement, and (3) systemic movement. Intracellular movement refers to virus movement to the periphery of a cell and includes all metabolic activities necessary to recycle the host and viral constituents required for the continued transport of the intracellular complex. Intercellular movement refers to virus movement between cells. In order for a plant virus infection to spread between cells, viruses must move through specific channels in the cell wall, called plasmodesmata (PD), that connect neighboring cells. Once intracellular and intercellular movement is established, the virus can invade the vascular cells of the plant and then spread systemically through the open pores of modified PD within the sugar-transporting phloem sieve elements. Upon delivery by the phloem to a tissue distant from the original infection site, virus exits the vasculature and resumes cell-to-cell movement via PD in the new tissue. Although it will not be discussed further in this article, it is important to know that a few viruses utilize the water-transporting xylem vessels for systemic transport.

When contemplating plant virus movement it is critical to understand that each virus movement complex varies in viral and host factor composition over time as it travels within and between cell types. In addition, individual viruses often utilize unique host factors to support their movement. The diverse and dynamic nature of virus movement complexes makes it difficult to summarize plant virus movement in a simple unified model. However, there is evidence that some stages of virus movement, although carried out by apparently unrelated host or virus proteins, do have functional convergence.

Virus movement in plants has been studied with a wide range of virus genera, including, but not limited to, tobamoviruses, potexviruses, hordeiviruses, comoviruses, nepoviruses, potyviruses, tombusviruses, tospoviruses, and geminiviruses. In this article we do not review virus movement by all plant viruses, but rather focus on model viruses within genera that provide the most information on the subject. We review what is currently known about the three steps of virus movement in plants and attempt to convey the complexity of movement mechanisms utilized by members of different virus genera. However, we also highlight recent findings indicating that irrespective of the presence of seemingly unrelated host or viral factors, functional similarities exist for some aspects of movement displayed by viruses from different genera.

Intracellular Movement

Intracellular movement is necessary to deliver the virus genome to PD for cell-to-cell transmission. This has been an understudied area, as researchers have only recently had the ability to label and observe the movement of viral proteins and RNA in near-real-time conditions. Early studies relied on static images of immunolabeled viral proteins from light and transmission electron microscopes to determine their intracellular location. While a few of these studies related the intracellular location of the viral protein to the stage of infection, most did not and thus the importance of the intracellular location for virus movement was not understood. Other early studies of virus movement relied on the mutation of specific viral genes in virus genomic clones and the assessment of the intercellular movement of the resulting mutant virus, through the presence of local (representing intracellular and intercellular movement) or systemic (representing intracellular, intercellular and vascular movement) disease. Although these genetic experiments often determined which viral proteins were important for virus intercellular or systemic movement, they could not determine whether the mutation prevented intracellular or intercellular movement, both outcomes being visually identical. In more recent studies, fusion of viral proteins with fluorescent reporter genes such as the green fluorescent protein (GFP) have given researchers a powerful method to observe both the intracellular movement and final subcellular destination of many viral proteins in near-real-time conditions. However, it is important not to over-interpret movement studies using GFP since GFP maturation for fluorescence emission takes hours and thus the visible movement and position of the GFP or GFP:viral protein fusion may not reflect early movement activity. Additionally, the level of GFP within the movement form of the virus may be too low to detect during critical phases of movement.

Although intracellular virus movement in plants is just beginning to be elucidated, it is clear that specific viral proteins regulate this activity. Chief among these are the virally encoded movement proteins (MPs), named to indicate their genetically determined requirement for intercellular virus movement. MPs are defined based upon three functional characteristics: their (1) association with, or ability to increase, the size exclusion limit (SEL) of PD; (2) ability to bind to single-stranded RNA (ssRNA); and (3) ability to transport themselves or viral RNA cell to cell. Based upon these defining characteristics, a number of proteins have been classified as MPs (**Table 1**). Many viral MPs have similar sequences indicating a shared evolutionary history. However, a considerable number have no obvious sequence similarity between them. The absence of a shared sequence for these MPs suggests convergent evolution for movement function by unrelated predecessor proteins. MPs often interact with host proteins that modify their amino acid backbone (e.g., through phosphorylation) or host proteins associated with intracellular trafficking (e.g., cytoskeletal or vesicle-associated proteins) (**Table 1**). However, the role of MPs in intracellular movement remains largely unknown because technical limitations have prevented visualizing movement of individual viral RNA or DNA associated with MPs in real time. In addition, it is becoming clear that ancillary viral proteins (**Table 1**), which do not fulfill the classical definition of an MP, are essential for virus movement. These proteins are often associated with membranes or cytoskeletal elements and thus likely function primarily for intracellular virus movement. The interaction of MPs with host factors and the impact of the ancillary viral proteins on intracellular virus movement are discussed in detail in the following section. Models for intracellular virus movement of particular genera of viruses are presented based on some of this information (**Figure 1**).

Host factors and intracellular virus movement

Host proteins shown to interact with viral MPs include kinases, chaperones, nuclear-localized proteins (often transcription co-activators), and proteins that are associated with or are core components of the cytoskeletal or vesicle trafficking systems (**Table 1**). In addition, some MPs have been shown to associate with host membranes.

Table 1 Proteins necessary for the cell-to-cell movement of plant viruses

Virus	*MP*[a]	*Ancillary viral proteins*[b]	*Host protein interactors with MP*
Tobacco mosaic virus, Tomato mosaic virus	**30 kDa**	126 kDa	Actin, tubulin, MPB2C, PME, KELP, MBF1, calreticulin
Red clover necrotic mosaic virus	**35 kDa**		
Groundnut rosette virus	ORF4		
Cowpea chlorotic mottle virus	3a		
Brome mosaic virus	**3a**	CP	
Cucumber mosaic virus	**3a**	CP	NtTLP1
Bean dwarf mosaic virus	BC1	BV1	
Tobacco etch virus	CP	CI, HC-Pro, VPg	
Barley stripe mosaic virus	TGBp1	TGBp2, TGBp3	
Potato virus X	**TGBp1**	TGBp2, TGBp3 + CP	TIPs
Cowpea mosaic virus	**48 kDa**	CP	
Cauliflower mosaic virus	**38 kDa**	CP	MPI7, PME
Turnip crinkle virus	**p8** + p9		Atp8
Tomato bushy stunt virus	**P22**		HFi22, REF
Potato leaf roll virus	17 kDa		
Tomato spotted wilt virus	**NS_m**		DnaJ-like, At4/1
Beet necrotic yellow vein virus	TGBp1	TGBp2, TGBp3, p14	
Grapevine fanleaf virus	2B	CP	Knolle, actin, tubulin
Rice yellow stunt virus	P3		
Rice dwarf virus	Pns6		
Southern bean mosaic virus	ORF1		
Turnip yellow mosaic virus	69 kDa		
Alfalfa mosaic virus	**P3**	CP	
Prunus necrotic ringspot virus	3a		
Tobacco rattle virus	**29 kDa**		
Soil-borne wheat mosaic virus	37 kDa		
Peanut clump virus	P51	P14, P17	
Potato mop top virus	**TGBp1**	TGBp2, TGBp3	
Commelina yellow mottle virus	N-term 216 kDa		
Beet yellows virus	p6, Hsp70h, p64	CPm, CP	
Rice stripe virus	Pc4		
Apple stem grooving virus	36 kDa		
Raspberry bushy dwarf virus	39 kDa		

[a]Regular (i.e., no bold) font indicates marginal classification as MP because the protein either has not been fully tested or has some but not all of the functions classically associated with MP (see text for definition).
[b]Necessary for viral cell-to-cell movement.

For geminiviruses, whose DNA genomes replicate in the nucleus, it is not surprising that nuclear factors may be necessary to transport viral genetic components required for virus replication into or out of the nucleus. For RNA viruses, however, there must be other reasons for an interaction between a nuclear protein and viral MP since these viruses are replicated in the cytoplasm. Some of the nuclear host proteins are non-cell-autonomous factors (e.g., HiF22) and thus it has been suggested that their interaction with MPs may inadvertently aid in virus intracellular and intercellular movement. It is also possible that MP and nuclear protein interactions occur to prevent transcription of host defense proteins or enhance transcription of host proteins necessary for virus movement, either within the infected cell or after transport to uninfected cells at the infection front.

The discovery over 10 years ago that tobacco mosaic virus (TMV) MP associates with microtubules (MTs) and microfilaments (MFs) was the first evidence that the host cytoskeleton might be involved in virus movement in plants. Although results from early studies indicated that disruption of MT arrays or their association with TMV MP could inhibit TMV movement, later studies suggested this was not so. Disruption of MT arrays with pharmacological agents or by tubulin transcript knockdown using virus-induced gene silencing had no effect on TMV movement or MP localization. Other work showed that the association of the MP with MTs happened late in infection, probably after virus movement had occurred. Also, during time-course studies it was determined that the MP disappeared during late stages of infection. This finding, in combination with the discovery that a mutant virus expressing a functionally enhanced MP with limited affinity for MTs moved cell to cell better than the parental virus, led to the idea that the association of MP with MTs is critical for MP degradation rather than to aid virus

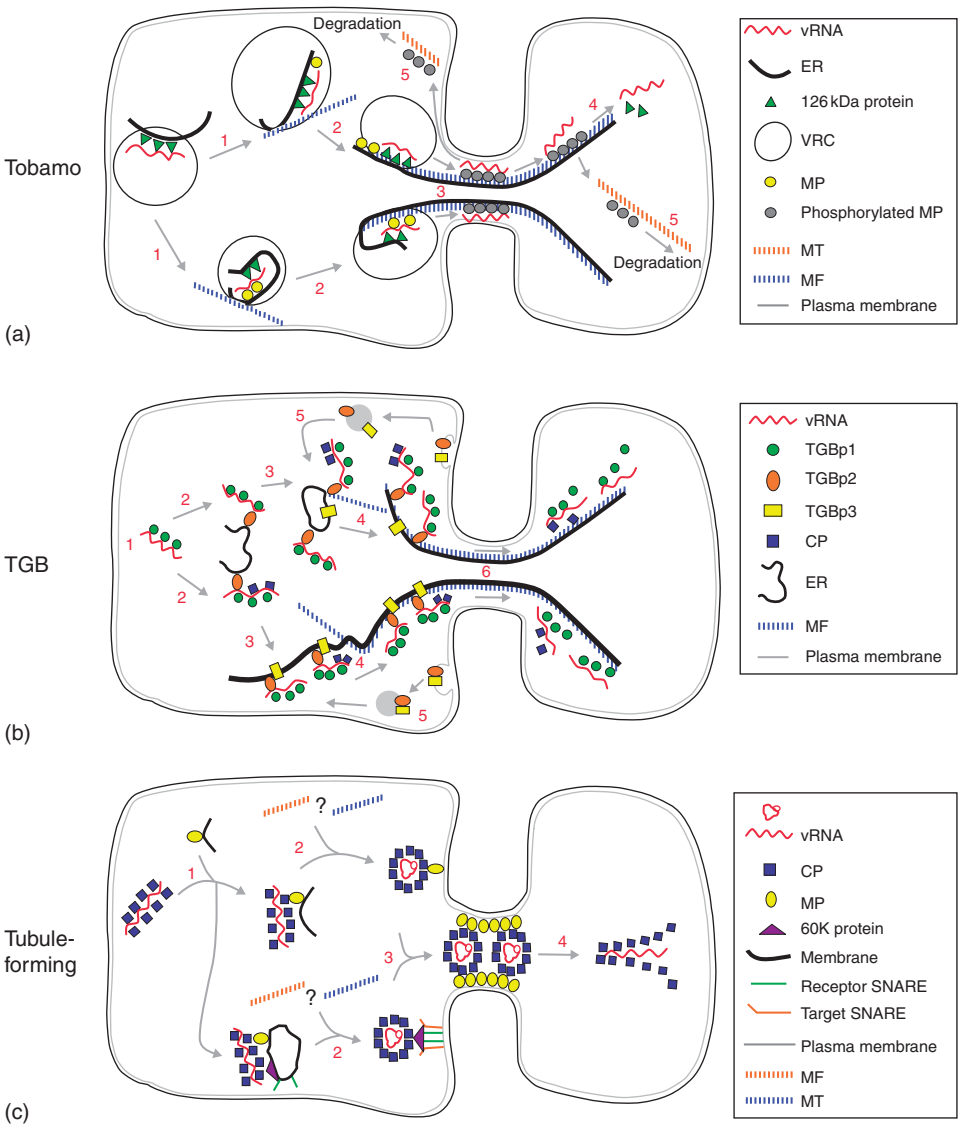


Figure 1 Models for cell-to-cell movement of plant viruses using tobamovirus triple gene blocks (TGBs), or tubule-forming strategies. (a) Viral 126 kDa protein binds both viral RNA (vRNA) and endoplasmic reticulum (ER) forming a cytoplasmic body in the cell termed a VRC. MP associates with the ER and possibly the vRNA within the VRC (step 1). VRCs associated with microfilaments (MFs) traffic toward plasmodesmata (PD; step 2). Here we show an indirect association of the VRC with actin mediated by the ER, but it is also possible that this interaction is mediated directly by the viral 126 kDa protein or MP. At the PD, vRNA is released from its association with the 126 kDa protein and is transported through the PD in association with MP (step 3). Phosphorylation of the MP occurs either within the cytoplasm, the cell wall, or both, and likely regulates transport to and through PD and subsequent translation of the vRNA in the new cell (steps 3 and 4). MP is degraded in the later stages of infection, likely via association with MTs and delivery to specific cellular sites of degradation (step 5). (b) Progeny vRNA binds to TGB protein 1 (TGBp1; step 1). The TGBp1/vRNA complex, either in the presence or absence of coat protein (CP, depending on virus genus), then binds TGBp2 attached to the ER to form a movement-competent ribonucleoprotein complex (RNP, step 2). The RNP then interacts with TGBp3, either directly or indirectly, to be positioned near the PD (steps 3 and 4). RNPs associate with actin MFs likely through an interaction with TGBp2, which may be responsible for transport to the PD. Following delivery of vRNA to the PD, TGBp2 and TGBp3 are likely recycled via an endocytic pathway (step 5). vRNA is actively transported through the PD via an unknown mechanism (step 6), although TGBp1 or CP may be involved, and is released from associated proteins in the next cell to allow replication to initiate. (c) CP-bound vRNA associates with the MP (itself associated with a membrane of unknown origin, step 1). The complex then moves, either as a vesicle directed to the PD through targeting proteins such as those from the SNARE family, or through other unknown targeting signals to the cell periphery (step 2). Interaction between SNAREs and virus may be mediated by a viral 60K protein for cowpea mosaic virus. The requirement for the cytoskeleton in transport of MP–vRNA complex is unclear since the nepovirus, grapevine fanleaf virus, requires cytoskeletal elements for proper delivery of its MP to the cell wall while cowpea mosaic virus does not. At or near the PD, the vesicular or nonvesicular membranes fuse with the plasma membrane and the attached MP directs the CP-associated vRNA through the PD (step 3). The vRNA is then released into the next cell to initiate virus replication and movement (step 4). Reproduced from Nelson RS (2005) Movement of viruses to and through plasmodesmata. In: Oparka KJ (eds.) *Plasmodesmata*, 1st edn., pp. 188–211. Oxford: Blackwell, with permission from Blackwell.

cell-to-cell movement. Further support for this idea came from the finding that the *Nicotiana tabacum* host protein, MPB2C, binds to MP and promotes its accumulation at MTs, yet acts as a negative effector of MP cell-to-cell transport. The role of MTs during TMV movement remains to be fully understood, but at this time it appears that they are more involved with MP degradation or compartmentalization than with virus movement (**Figure 1(a)**).

In contrast to the large body of work focusing on the role of the MT–MP interaction in TMV movement, studies on the role of the MFs in the movement of TMV and other viruses have only recently been published. It was demonstrated that intracellular movement of TMV viral replication complexes (VRCs; large multiprotein complexes comprised of host and viral factors) and cell-to-cell spread of the virus were blocked by MF inhibitors (pharmacological and transcript silencing agents). VRCs were later determined to physically traffic along MFs (**Figure 1(a)**). The interaction of TMV VRCs with MFs may be mediated by the TMV 126 kDa protein (a protein containing helicase, methyltransferase, and RNA silencing suppressor domains), since expression of a 126 kDa protein:GFP fusion in the absence of the virus results in fluorescent protein bodies that, like VRCs, traffic along MFs. VRC association with MFs may be mediated through a direct interaction of the 126 kDa protein with MFs or through an intermediary cell membrane. MFs are known to associate with membranes in plant cells and the 126 kDa protein binds to an integral membrane host protein, TOM1. However, the MP of TMV is long known to bind actin and associate with membranes, so the relative importance of the TMV 126 kDa protein or MP for directing intracellular VRC movement is unclear (**Figure 1(a)**).

Recently, MFs were demonstrated to co-localize with ancillary proteins required for movement of the hordeivirus, potato mop-top virus (PMTV). PMTV encodes a conserved group of proteins termed the triple gene block (TGB) that are required for cell-to-cell virus movement (see **Table 1**). Two of these TGB proteins (TGBp2 and TGBp3) co-localize with motile granules that are dependent upon the endoplasmic reticulum (ER)–actin network for intracellular movement. In addition, the TGBp2 protein from the potexvirus, potato virus X (PVX), localizes to MFs in what are likely ER-derived vesicles (**Figure 1(b)**). The association of TGBp2 from potexviruses and the 126 kDa protein from TMV with MFs and their requirement for successful virus movement provide an elegant example of convergent evolution since TGBp2 and 126 kDa protein have no sequence identity.

The role of the cytoskeleton in the intracellular transport of some plant viruses is unclear. Cowpea mosaic virus (CPMV), for example, does not require the host cytoskeleton for the formation of tubular structures containing MP on the surface of protoplasts. These tubular structures are similar to the tubules formed in modified PD (that likely do not contain cytoskeleton) which are necessary for intercellular movement of this virus (**Figure 1(c)**). The role of tubules in intercellular transport of CPMV is discussed in next section. Work with grapevine fanleaf virus (GFLV), another tubule-forming virus, has revealed the possibility that this virus may be targeted to the PD by membrane vesicle SNARE (v-SNARE)-mediated trafficking. The MP of GFLV co-immunoprecipitates with KNOLLE, a target SNARE (t-SNARE). The 60 kDa protein of CPMV has been shown to bind a SNARE-like protein. t-SNAREs such as KNOLLE act as specific receptors for targeted delivery of Golgi-derived vesicles to sites where fusion with the plasma membrane will occur. Thus, it is possible that the SNARE trafficking machinery delivers viral proteins (and possibly associated viral RNA) to the plasma membrane near PD (**Figure 1(c)**).

There is evidence that, following movement of the viral RNA to the PD, some viral factors involved in this movement may be recycled for further use. The TGBp2 and TGBp3 proteins from PMTV localize to endocytic vesicles as evidenced by labeling with FM4-64 dye, a marker for internalized plasma membrane (**Figure 1(b)**). Additionally, TGBp2 co-localizes with Ara7, a marker for early endosomes. The functional significance of this endocytic association of viral proteins remains to be determined and it is not known whether proteins from other viruses may also traffic in the host cell's endocytic pathway.

Intercellular Movement

Following intracellular movement to the cell periphery, the virus must then move through PD in order to spread into neighboring cells. PDs are plasma membrane-lined aqueous tunnels connecting the cytoplasm of adjacent cells. An inner membrane, termed the desmotubule, is a tubular form of the ER and is an extension of the cortical ER. PDs can be subdivided structurally into simple (containing a single channel) or branched (containing multiple channels) forms. The SELs of PD are increased by the disruption of MFs indicating a role for actin in PD gating and indeed both actin and myosin have been observed in PD. Thus, it is possible that cytoskeletal-mediated transport of viral components results in direct delivery of virus to and passage through PD.

Protein movement through PD is dependent on the developmental stage of the PD. For example, free GFP (27 kDa) moves through simple but not branched PDs. Branched PDs generally have smaller SELs than simple PDs and the presence of more branched PDs in mature photosynthate-exporting (source) versus immature

photosynthate-importing (sink) leaves represents a developmental change that limits transport of macromolecules through PD. This developmental change also affects the localization pattern for some viral MPs. For example, both cucumber mosaic virus and TMV MPs are observed predominantly or solely within branched PD in source leaves and not simple PD in sink leaves. The TMV MP expressed in transgenic plants, however, is sufficient to complement the movement of an MP-deficient TMV mutant in both source and sink leaves. Thus, the presence of MP in the central cavity of branched PD in source leaves may not represent a site of function for the MP, but rather the final deposition of inactive MP. Although it is possible that the level of MP binding in simple PD is below the detection limits of the current technology, questions remain about where and how the MP functions in virus movement.

A clue to TMV MP function during virus movement comes from findings showing that a TMV MP–viral RNA complex could not establish an infection in protoplasts, but could do so when introduced into plants. It was suggested that a change in the phosphorylation state of the MP at the cell wall was necessary to weaken the binding between the MP and viral RNA, thereby allowing translation of the viral genome and initiation of infection in the next cell. Indeed, a PD-associated protein kinase has been identified that phosphorylates TMV MP. Thus, the protein kinase in the cell wall may be necessary to end the involvement of MPs in virus movement and release the viral RNA for translation in the new cell (**Figure 1**(**a**)). Also, considering that there are additional phosphorylation sites on the TMV MP besides those targeted by the PD-associated protein kinase, it is likely that proper sequential phosphorylation of this protein is necessary to allow it to function in both intracellular and intercellular virus movement. For potyviruses, the eukaryotic elongation factor, eIF4E, appears to modulate both virus accumulation, likely by affecting translation, and cell-to-cell movement. Thus, as for TMV, virus accumulation and movement may be linked activities.

Chaperones of host or viral origin may be required for PD translocation of some MPs. Host-encoded calreticulin modulates TMV intercellular movement and co-localizes with TMV MP in PD. The virus-encoded virion-associated protein (VAP) of cauliflower mosaic virus (CaMV) binds MP through coiled-coil domains and co-localizes with MP on CaMV particles within PD. The mechanism by which a molecular chaperone can support intercellular virus movement is illustrated by the virally encoded Hsp70 chaperone homolog (Hsp70h) of beet yellows virus. Hsp70h requires MFs to target it to the PD. The Hsp70h is a component of the filamentous capsid and its ATPase activity is required for virus cell-to-cell movement. These findings led to a model where Hsp70h mediates virion assembly and, once localized to the PD, actively translocates the virion from cell to cell via an ATP-dependent process. The idea that viral proteins may actively participate in plasmodesmal translocation of virus is further supported by the finding that the NTPase activity of the TGBp1 helicase from PMTV is necessary for its translocation to neighboring cells and that the coat protein (CP) of PVX, necessary for virus cell-to-cell movement, has ATPase activity (**Figure 1**(**b**)). It has also been found that the helicase domain of the TMV 126 kDa protein is required for cell-to-cell movement. In these cases it seems likely that the helicase activity is necessary to remodel viral RNA, thereby easing passage of the virus through PD.

Tubule-forming viruses have adopted another strategy for intercellular movement whereby virus-induced tubules span modified PD that lack a desmotubule in order to transmit capsids from cell to cell (**Figure 1**(**c**)). Such capsid-containing tubules are known to be composed, at least in part, of MP and have been identified for a number of viruses, including commelina yellow mottle virus, CaMV, CPMV, and tomato ringspot virus.

Although the tubule-forming viruses modify PD differently than those utilizing classical MPs, it was recently determined that the tubule-forming MP from tomato spotted wilt virus can functionally substitute for the non-tubule-forming TMV MP to support TMV movement. This is likely another example where two proteins with no sequence identity and therefore no apparent evolutionary relationship have independently evolved to functionally support movement of viruses.

Systemic Movement

Some viruses are limited to the phloem of plants (i.e., phloem-limited viruses) and require inoculation, often by aphids, directly to vascular cells for infection. Systemic movement of a non-phloem-limited virus through vascular tissue, however, requires that the virus moves from nonvascular cells into veins. Veins are defined as major or minor based on their structure, location, branching pattern, and function (**Figure 2**). Whether major or minor, each vein contains many different cell types with greatly differing structures. Within *N. tabacum*, minor veins include phloem parenchyma, xylem parenchyma, and companion cells, along with sieve elements and xylem vessels (**Figure 2**). All of these cells have distinct structures and locations within the vein which present unique regulatory sites for virus entry. Between plant species, companion cell morphologies vary greatly with an obvious difference being the number of PDs between these cells and other vascular cells. This difference is functionally related to the type of photosynthate transport system exhibited by

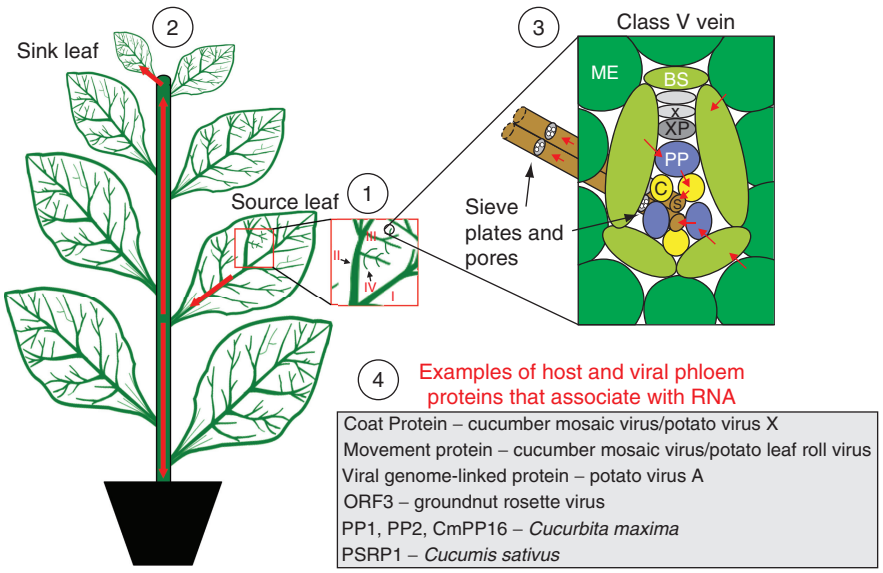


Figure 2 (1) Viral infection of a source leaf occurs by intercellular movement of the virus into the vasculature (class I–V veins indicated). (2) Virus travels through the phloem from the source leaf throughout the plant (red arrows) and exits vascular tissue to resume cell-to-cell movement in sink tissue. (3) In order to enter the phloem of a class V vein, a non-phloem-limited virus must travel through mesophyll cells (ME), bundle sheath cells (BS), and possibly phloem parenchyma cells (PP) before entering the companion cells (C) and finally the sieve elements (S). Movement through SEs requires passage of virus through pores within the sieve plates. A minority of viruses move through the xylem (X). (4) Examples of host and viral proteins that have been identified in phloem and that associate with RNA are indicated.

the plant (i.e., apoplastic versus symplastic). In addition, bundle sheath cells, which have their own unique position and structure surrounding the minor veins, must be considered as potential regulators of virus movement. These complex cell types are difficult to study because it is problematic to directly access or isolate them.

Recently, studies have been conducted that conclusively indicate which veins, minor or major, can serve as entry sites for rapid systemic infection. Using surgical procedures to isolate specific veins and TMV or CPMV modified to express GFP as a reporter, it was determined that either major or minor veins in leaves of *Nicotiana benthamiana* and *Vigna unguiculata* can be invaded directly and serve as inoculum sources for systemic infection. In addition, for TMV, direct infection of cells in transport veins in stems yielded a systemic infection. Considering that major and transport veins do not have terminal endings bounded by nonvascular tissue, it is likely that virus entered these veins by passing through bundle sheath cells and interior vein cells.

Virus transport and accumulation are regulated within vascular tissue. In plants that have internal and external phloem, potyviruses and tobamoviruses accumulate in specific tissue depending on the tissue's position relative to the inoculated leaf. In the inoculated leaf and the stem below, virus accumulates in the external phloem, whereas in the stem and leaf veins above the inoculated leaves, virus accumulates in the internal phloem.

Exit of PVX, TMV, and CPMV from vascular cells in sink tissues only occurs from major veins. For a growing number of viruses, however, exit occurs from both major and minor veins indicating that there is not a uniform exit strategy for all viruses.

The virus and host factors that control systemic virus accumulation are becoming better understood, mostly through genetic studies. Virus factors include CP, some MPs, and some nonstructural proteins such as the 126 kDa protein of TMV. Although CPs are often necessary for systemic infection, it is clear that for some viruses, such as groundnut rosette virus, a CP is not present and the virus still produces a systemic infection in the host. Also, for viruses that normally require the CP for systemic infection, the loss of the CP through mutation or deletion may still allow systemic movement of the virus in specific hosts. Lastly, viroids, which do not encode any proteins, can systemically infect plants. These results indicate that although a capsid may be required to protect viral RNA for systemic transport in some hosts, other viral or host proteins can functionally mimic the CP and allow systemic infection.

MP function during systemic infection has, in one case, been uncoupled from its role during intra- and intercellular transport. Some point mutations in the red clover necrotic mosaic virus MP still allow intercellular movement, but prevent systemic movement. Additional support that MPs function to allow systemic movement

comes from studies with the 17 kDa MP of potato leafroll virus, a phloem-limited virus. This MP, when expressed from within an infectious virus sequence in transgenic plants, is uniquely localized to PD connecting the companion cells with sieve elements, even though virus accumulated in both vascular and nonvascular cells. Thus, the PD between companion cells and sieve elements may be uniquely recognized by this MP to allow the virus to only invade vascular tissue. More recently, it has been shown that a host factor, CmPP16, that is thought to function by forming ribonucleoprotein complexes with phloem transcripts has sequence similarity with viral MPs. Thus, some MPs may function to protect RNA while in transit through the phloem.

Other viral proteins such as the 2b protein of CMV, p19 of TBSV, and the 126 kDa protein of TMV have been linked to supporting systemic movement of their respective viruses. Considering that all of these proteins are suppressors of gene silencing, it is possible that this activity is related to their function in supporting systemic movement. It is known that a member of the plant silencing pathway, specifically, the RNA-dependent RNA polymerase, RDR6, functions in sink tissue (e.g., the shoot apex) by responding to incoming signals for RNA silencing. RDR6 has also been shown to control virus accumulation in systemic, but not inoculated, leaves. Thus, it is possible that viral suppressor activity could function to specifically allow systemic accumulation of viruses.

Host factors that modulate virus systemic spread either support or restrict this activity. A protein methylesterase (PME) is involved in both intercellular and systemic movement of TMV. For systemic movement, PME is essential for virus to exit into nonvascular tissue of the uninoculated leaves. A phloem protein from cucumber, p48, was found to interact with CMV capsids and may function to protect the capsid during transport. Host factors that restrict virus systemic movement include the restricted TEV movement (RTM) proteins, which are expressed only in phloem-associated cells and accumulate in sieve elements. RTM1 is related to the lectin, jacalin, while RTM2 has a heat shock protein motif. RTM1 may function in a plant defense pathway within the veins, although the jacalin-like proteins have not been previously linked to virus defense. RTM2 may function as a chaperone to prevent unfolding of a transport form of the virus within the sieve elements. A third protein that serves as a negative regulator is a cadmium-induced glycine-rich protein, cdiGRP. This protein does not act directly to restrict systemic movement. Instead, it induces callose deposits which are thought to restrict intercellular transport of the virus. This could prevent exit of virus from the vascular tissue. Interestingly, cadmium treatment inhibits the systemic spread of RNA silencing, lending support to the idea that spread of specific viruses affected by cadmium treatment (i.e., TMV and turnip vein clearing virus) is functionally similar to that of a host silencing signal.

See also: Brome Mosaic Virus; Bromoviruses; *Carmovirus*; Citrus Tristeza Virus; Cucumber Mosaic Virus; *Furovirus*; *Hordeivirus*; Luteoviruses; *Nepovirus*; Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Geminiviruses; *Potexvirus*; Potyviruses; Tobacco Mosaic Virus; *Tobamovirus*; *Tobravirus*; Tombusviruses; *Tospovirus*; *Umbravirus*; Viral Suppressors of Gene Silencing; Viroids; Virus Induced Gene Silencing (VIGS).

Further Reading

Boevink P and Oparka KJ (2005) Virus–host interactions during movement processes. *Plant Physiology* 138: 1815–1821.

Derrick PM and Nelson RS (1999) Plasmodesmata and long-distance virus movement. In: van Bel AJE and van Kesteren WJP (eds.) *Plasmodesmata: Structure, Function, Role in Cell Communication,* 1st edn., pp. 315–339. Berlin: Springer.

Gilbertson RL, Rojas MR, and Lucas WJ (2005) Plasmodesmata and the phloem: Conduits for local and long-distance signaling. In: Oparka KJ (ed.) *Plasmodesmata,* 1st edn., pp. 162–187. Oxford: Blackwell.

Heinlein M and Epel BL (2004) Macromolecular transport and signaling through plasmodesmata. *International Review of Cytology* 235: 93–164.

Lewandowski DJ and Adkins S (2005) The tubule-forming NSm protein from *Tomato spotted wilt virus* complements cell-to-cell and long-distance movement of *Tobacco mosaic virus* hybrids. *Virology* 342: 26–37.

Lucas WJ (2006) Plant viral movement proteins: Agents for cell-to-cell trafficking of viral genomes. *Virology* 344: 169–184.

Morozov SY and Solovyev AG (2003) Triple gene block: Modular design of a multifunctional machine for plant virus movement. *Journal of General Virology* 84: 1351–1366.

Nelson RS (2005) Movement of viruses to and through plasmodesmata. In: Oparka KJ (ed.) *Plasmodesmata,* 1st edn., pp. 188–211. Oxford: Blackwell.

Nelson RS and Citovsky V (2005) Plant viruses: Invaders of cells and pirates of cellular pathways. *Plant Physiology* 138: 1809–1814.

Oparka KJ (2004) Getting the message across: How do plant cells exchange macromolecular complexes? *Trends in Plant Science* 9: 33–41.

Rakitina DV, Kantidze OL, Leshchiner AD, *et al.* (2005) Coat proteins of two filamentous plant viruses display NTPase activity *in vitro*. *FEBS Letters* 579: 4955–4960.

Requena A, Simón-Buela L, Salcedo G, and García-Arenal F (2006) Potential involvement of a cucumber homolog of phloem protein 1 in the long-distance movement of cucumber mosaic virus particles. *Molecular Plant Microbe Interactions* 19: 734–746.

Roberts AG (2005) Plasmodesmal structure and development. In: Oparka KJ (ed.) *Plasmodesmata,* 1st edn., pp. 1–32. Oxford: Blackwell.

Scholthof HB (2005) Plant virus transport: Motions of functional equivalence. *Trends in Plant Science* 10: 376–382.

Silva MS, Wellink J, Goldbach RW, and van Lent JWM (2002) Phloem loading and unloading of cowpea mosaic virus in *Vigna unguiculata*. *Journal of General Virology* 83: 1493–1504.

Verchot-Lubicz J (2005) A new cell-to-cell transport model for potexviruses. *Molecular Plant Microbe Interactions* 18: 283–290.

Waigman E, Ueki S, Trutnyeva K, and Citovsky V (2004) The ins and outs of nondestructive cell-to-cell and systemic movement of plant viruses. *Critical Reviews in Plant Sciences* 23: 195–250.

Mumps Virus

B K Rima and W P Duprex, The Queen's University of Belfast, Belfast, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Editing Process by which mRNAs are altered during or after transcription of the gene.
Mononegavirales Order of viruses consisting of four families with nonsegmented negative-stranded RNA genomes.
Nucleocapsid The ribonucleoprotein of mumps virus consisting of the RNA genome, the nucleocapsid protein, and associated proteins.
Pleomorphic From the Greek 'having many forms'.
Polyploid Having more than one genome copy per virion.
R_0 Index used in epidemiology, indicating the number of secondary cases that could originate from a single index case of viral infection.
Viremia Presence of virus in blood.
Virion The virus particle.

History

The primary clinical manifestation in mumps is swelling of the salivary glands because of parotitis. This symptom is so characteristic that the disease was recognized very early as different from other childhood illnesses which give rise to skin rashes. Hippocrates described mumps as a separate entity in the fifth century BC. He also noted swelling of the testes (orchitis) as a common complication of mumps. Infection in the central nervous system (CNS) and meninges in some cases of mumps was first noted by Hamilton in 1790. In 1934, Johnson and Goodpasture demonstrated the filterable nature of the causative agent and Koch's postulates were fulfilled by infection of human volunteers with virus propagated in the salivary glands of monkeys.

Taxonomy and Classification

Mumps virus (MuV) is sensitive to ether and other membrane-destroying reagents and has hemagglutinating and neuraminidase (HN) activity. It contains a nonsegmented negative-stranded RNA genome. MuV is thus classified in the family *Paramyxoviridae* and placed in the genus *Rubulavirus*. MuV is the prototype species, and two other human rubulaviruses, human (h) parainfluenza virus 2 (hPIV2) and hPIV4, have been identified. Other rubulaviruses infect a range of vertebrates – for example, Mapuera virus infects bats. Simian virus 5 (SV5) was originally isolated from rhesus and cynomolgus monkey kidney-cell cultures and thus designated as a primate virus. Sometimes SV5 is referred to as canine parainfluenza virus although it has a more extended host range and it has been suggested the virus be renamed PIV5. Porcine rubulavirus (PoRv) caused a fatal encephalomyelitis in neonatal pigs. Newcastle disease virus and a number of avian paramyxoviruses were included in the genus although these have recently been assigned to the genus *Avulavirus*.

Properties of the Virion

Paramyxoviruses consist of an inner ribonucleoprotein (RNP) core surrounded by a lipid bilayer membrane from which spikes protrude (**Figure 1**). The MuV virion appears to be roughly spherical and normally diameters range from 100 to 300 nm when the virus is grown in cell culture or embryonated eggs. Sometimes bizarre rod-shaped and other pleomorphic particles have been observed. Electron microscopy (**Figure 1(a)**) shows that MuV has the typical paramyxovirus structure with a lipid bilayer membrane surrounding an internal RNP complex, the nucleocapsid. The nucleocapsid displays the herring-bone structure characteristic of *Paramyxoviridae* (arrow, **Figure 1(a)**) and is approximately 1 μm in length with a diameter of 17 nm and an internal central core of 5 nm. Some of the pleomorphic particles have been reported to contain more than one RNP structure. The biological significance of such polyploid particles has not been investigated.

The RNP contains the RNA genome covered with nucleocapsid (N) protein as well as a phosphoprotein (P) and the large protein (L). The RNP is surrounded by a lipid bilayer membrane derived from the host cell in which the matrix protein (M) of the virus is embedded (**Figure 1(b)**). This protein interacts with the internal core N protein and the viral glycoproteins. Spikes (10–15 nm in length) protrude from the membrane and these contain the viral glycoproteins, the hemagglutinin–neuraminidase protein (HN) and the fusion (F) protein. The HN protein is probably a homo-tetramer, the fusion protein a homo-trimer. The ratio of HN and F protein molecules in the spike complex has not been elucidated yet. The spikes are also involved in the hemolysis of erythrocytes of different origins. The hemolysis reflects the ability of the virus to fuse with infected cells. Fusion is required for the entry of the RNP cores into cells.

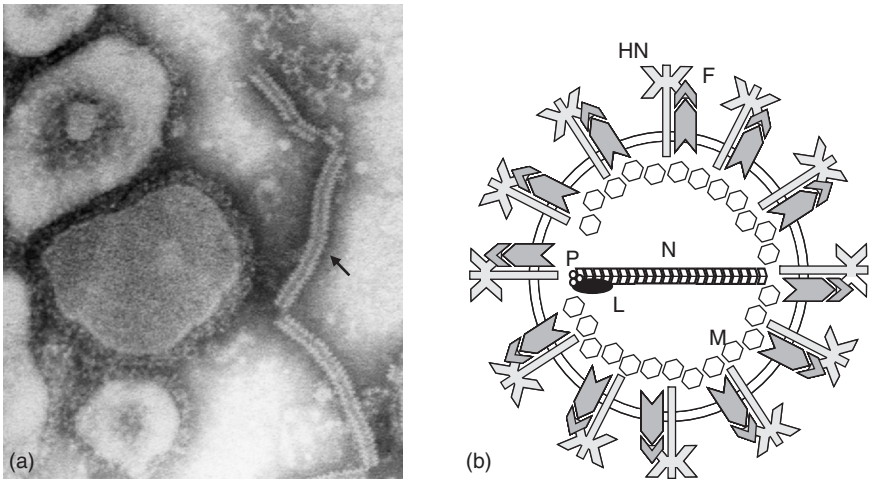


Figure 1 The structure of the mumps virion. In electron micrograph (a) the arrow points to a nucleocapsid released from a virus particle showing the characteristic herringbone structure. Schematic representation (b) indicates the location of the major viral structural proteins (see **Figure 2** for an explanation of the shapes and abbreviations).

Properties of the Genome

MuV has a single nonsegmented negative-stranded RNA genome (**Figure 2**(**a**)). The nucleotide sequence of the entire genome of several strains is known and all of these are 15 384 nt in length. The order of the genes of MuV and transcription of the genome is similar to that of other paramyxoviruses, especially to that of PIV5. There are seven transcription units which are separated by intergenic (Ig) sequences. The basic unit of infectivity is the negative-stranded, encapsidated RNP (**Figure 2**(**d**)).

Properties of the Proteins

The properties of the MuV proteins are summarized in **Table 1** and the number of amino acid residues in each viral protein is given in **Figure 2**(**c**). It should be emphasized that this assignment is largely based on analogy of the MuV proteins with those of other paramyxoviruses, as gene identifications have not been carried out directly. However, the similarities to other paramyxoviruses are so striking that this assignment is beyond dispute. The presence of six structural proteins, namely, the N, P, M, and L proteins, as well as two glycosylated membrane-spanning proteins, the HN and F proteins, has been demonstrated in MuV virions. Furthermore, the virus induces the synthesis of at least two nonstructural proteins (V and W) from transcripts of the V/W/P gene. The presence of a small hydrophobic (SH) protein has been demonstrated in MuV-infected cells using antisera to peptides derived from the deduced amino acid sequence. It is not clear whether the protein is incorporated into virions. At least one strain (Enders) expresses the SH gene as a tandem readthrough transcript with the F gene in tissue culture. It is unlikely that the SH protein is translated from such an F–SH bicistronic mRNA and growth of this strain in tissue culture may not require the expression of this protein. A recombinant virus lacking the SH protein has been shown to be unaffected in growth in several cell types, formally proving the nonessential nature of the protein.

Physical Properties

The MuV virion is very susceptible to heat and treatment with ultraviolet (UV) light. The UV target size is one genome equivalent of RNA. The virus is inactivated by 0.2% formalin and the presence of the lipid bilayer confers sensitivity to both ether and chloroform. Treatment with 1.5 M guanidine hydrochloride leads to selective inactivation of neuraminidase and not hemagglutination activity of the virus. This indicates that separate domains of the HN molecule are responsible for these two functions.

Replication

MuV is capable of infecting a variety of cells in culture although whether this is the case *in vivo* remains to be determined. The attachment of MuV is mediated primarily through the interaction of the HN protein with a sialic acid, but the exact nature of the molecule(s) to which this moiety is linked remains unknown. Hence, MuV sialoglycoconjugate receptor(s) have not been precisely defined. The cooperative binding of a number of HN

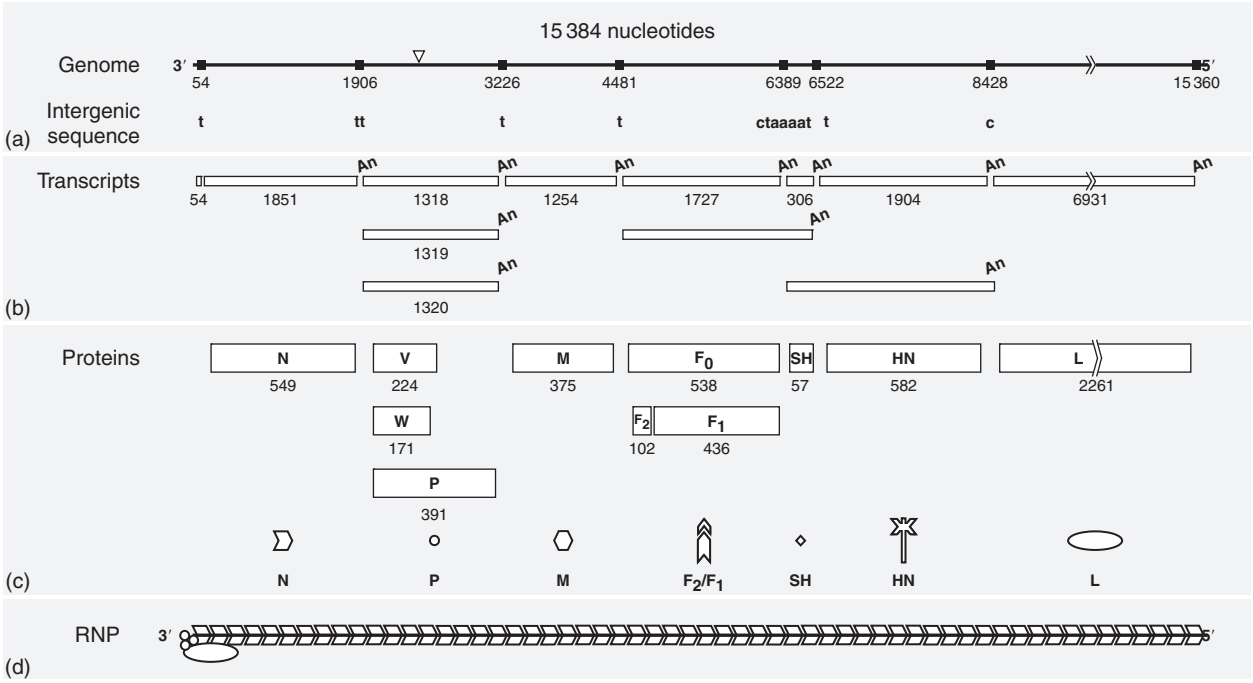


Figure 2 Transcription, replication, and translation of the mumps virus genome. In this figure are indicated: the mumps gene order and size and the position, sequence (positive strand) of the intergenic sequences (a); the major transcripts derived from the MuV genome and their sizes (b); the proteins and their sizes in numbers of amino acid residues and their schematic representations (c), and the replicative intermediates (d).

Table 1 Properties of the proteins of MuV

Protein	*Size in SDS-PAGE (kDa)*	*Function*
Nucleocapsid (N)	68–73	Phosphorylated structural protein of RNP; protects genome from RNases, possible role in regulation of transcription and replication (S antigen)
Phosphoprotein (P)	45–47	Phosphorylated protein associated with the RNP; possible role in solubilization of the N protein, role in RNA synthesis
Large (L)	>200	Protein with RdRp polymerase activity associated with the RNP; role in capping, methylation, and polyadenylation
Matrix (M)	39–42	Hydrophobic protein associated with inner side of membrane; role in budding by interactions with the N, HN, and F proteins
Fusion (F)	65–74	Acylated, glycosylated protein F_2–F_1 heterodimer activated by proteolytic cleavage; fusion of virion membrane with the plasma membrane which also involves HN (hemolysis antigen)
Hemagglutinin–neuraminidase (HN)	74–80	Acylated and glycosylated protein with hemagglutinin and neuraminidase activity; accessory role in fusion of virion membrane with the plasma membrane (major V antigen)
Small hydrophobic (SH)	6	Membrane protein with unknown function; present in infected cells but not detected in virions to date
Nonstructural V (earlier NS1)	23–28	Phosphorylated protein with a cysteine-rich domain which may be involved in metal binding; leads to the proteasomal degradation of STAT1 and targets STAT3 for ubiquitination
Nonstructural W (earlier NS2)	17–19	Phosphorylated protein with unknown role, possibly an artifact of misediting

SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; kDa, mass of the protein in kilodaltons.

molecules to cell surface molecules probably leads to invagination of the host cell membrane and this may allow the fusion protein in its proteolytically cleaved, activated state to fuse the membrane of the virus and the host cell. Whether viropexis is another mechanism of entry of MuV is not known.

After introduction of the RNP into the cell, primary transcription of the negative-stranded genome occurs.

This is probably mediated by the viral L and P proteins of the virion and leads primarily to the synthesis of positive-strand, monocistronic mRNAs (**Figure 2**(**b**)). In addition, a number of polycistronic mRNAs have been identified. RNA-dependent RNA polymerase (RdRp) activity has been demonstrated in MuV virions but the role of the various viral proteins in this has not been assessed directly. Therefore, most of what follows is analogous to other paramyxoviruses. The first gene encoding the N protein is preceded by a leader region. No reports have appeared on the presence or absence of encapsidated or unencapsidated leader transcripts in infected cells or on the question of whether the leader region is transcribed by itself, in tandem with the first gene(s), or not at all. The transcription complex recognizes the 3′ end of the genome and transcribes the genes sequentially (**Figure 2**(**c**)), stopping at each Ig sequence to synthesize the polyadenylate (An) tails of the various mRNAs by repeated transcription on a poly(U) stretch in the genome. This reiterative transcription is sometime referred to as 'polymerase stuttering'. It has not been determined if the mRNAs are capped and methylated at the 5′ end.

The Ig sequences are 1–7-nt-long sequences (**Figure 2** (**a**)) and they are flanked by highly conserved gene end (GE) and gene start (GS) signal sequences (**Figure 3**). At any intergenic sequence there is a finite chance that the RdRp complex will leave the template. This gives rise to a transcription gradient so that the 3′ proximal N gene is most frequently transcribed and the 5′ proximal L gene is transcribed very infrequently. The gradient has not been quantified in MuV-infected cells. Occasionally, the GE and GS sequences are ignored and tandem readthrough transcripts of two or more genes are generated. Recognition of transcription signals appears to be dependent on host as well as viral factors. Thus, strains which have been adapted to growth in eggs only give rise to monocistronic mRNAs in chicken embryo fibroblasts, whereas only large tandem readthrough transcripts are generated if mammalian cells in culture are infected with these strains.

Co-transcriptional editing is responsible for the generation of mRNAs encoding the P and W proteins of MuV. Insertion of one or up to five extra G residues at a site in the V/W/P gene with a sequence similar to the polyadenylation signal leads to the formation of mRNAs that encode the W and P protein or V, W, or P proteins with an extra glycine residue (**Figures 2**(**b**) and **2**(**c**)).

The mRNAs are translated by cellular ribosomes and there is no indication for shutoff of host mRNA translation or preferential translation of viral mRNAs in MuV-infected cells. Early reports of temporal translational control and control of transcription and translation of host mRNAs by the presence of viral glycoproteins in the cell membrane have not been confirmed. Some of the viral proteins are modified post-translationally (**Table 1**). The processing pathway for the HN protein differs from that for the F protein. The HN protein forms oligomers during transport through the Golgi apparatus, and carbohydrate side chains are modified slowly in the *trans*-Golgi cisternae, whereas the F protein rapidly matures with respect to its glycosylation. The proteolytic cleavage and consequent activation of the F_0 precursor into the F_1 and F_2 complex occurs in the *trans*-Golgi cisternae. Both the F and the HN proteins appear to play a role in fusion of virus-infected cells and syncytium formation. This is indicated by the observations that first, fusing as well as nonfusing strains contain cleaved activated F protein (i.e., the F_1 and F_2 complex); second, levels of neuraminidase are higher in nonfusing strains; and third, proteolytic cleavage of the HN protein can activate fusion. Co-expression of F and HN from eukaryotic expression vectors leads to the formation of multinucleated syncytia in transiently transfected cells which are indistinguishable from those generated in an MuV infection. These fusion assays have been useful in assessing the effect of site-directed mutagenesis on the biological function of the proteins and in determining where oligomerization takes place in the cell. Co-transfection of an equivalent construct containing the M gene has shown that this does not alter syncytium size.

Replication of the genome starts at the 3′ end of the (–) RNP. In replication mode the RdRp ignores the GE, Ig, and GS signals to generate one single positive-stranded RNA molecule. This RNA is concomitantly encapsidated

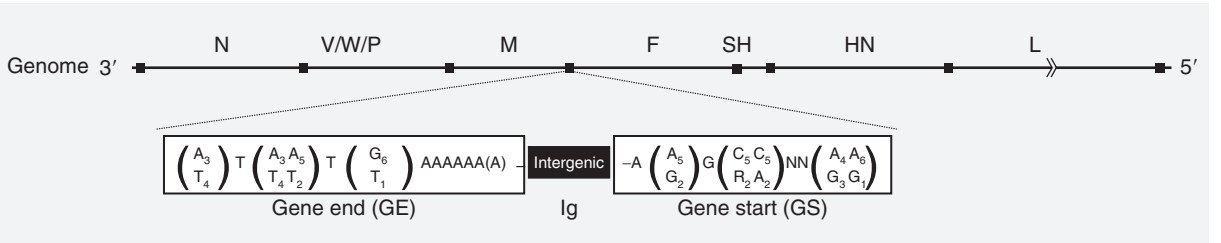


Figure 3 Consensus sequences of the seven gene start (GS) and gene end (GE) sequences. The various consensus sequences are shown as positive strand sequences. The specific intergenic sequences and their positions in the genome are shown in **Figure 2(a)**.

by N protein immediately after the start of its synthesis leading to the formation of a (+)RNP. It is not known for MuV if the transcriptase complex and the replicase complex are identical or whether they involve different host factors, as is the case for vesicular stomatitis virus, another virus in the order *Mononegavirales*. The intracellular concentration of N protein may regulate the balance between transcription and replication. The positive-stranded RNAs in the newly formed RNPs then form the template for further negative-strand synthesis, again with concomitant encapsidation by N protein. The resulting (–)RNPs reach the cell surface by an unknown mechanism where they are incorporated into progeny virions. The budding process itself and its regulation have not been studied in great detail for MuV. Virion budding probably takes place as a consequence of the interaction of the M protein with the RNP (most probably the C-terminal part of the N protein) and the cytoplasmic tails of the viral glycoproteins which accumulate in patches on the plasma membrane. Both (+)RNPs, containing the genome, and (–)RNPs, containing the antigenome, can be incorporated into MuV virions. However, whether the budding process is selective in favoring inclusion of (–)RNPs into virions is not yet clear.

There are a number of outstanding issues with respect to the life cycle of MuV which are not yet clear, some of which are specific to the virus. The role(s) of the V and W (earlier called NS1 and NS2, respectively) nonstructural proteins in the processes of replication, transcription, translation, and assembly has yet to be elucidated. Neither is it clear what role the SH protein plays. A functional role, preventing apoptosis in infected *in vitro* cells, has been suggested (see above). However, whether this is the case *in vivo* remains to be determined. Studies on the assembly of the RNP and the localization of the P and L proteins as well as the transport of the complex to the plasma membrane also remain to be carried out for MuV. Particularly, since the cell cytoskeleton appears to play a role in the replication of other paramyxoviruses, it is important to investigate this aspect of MuV replication.

Geographic and Seasonal Distribution

The virus has a worldwide distribution and requires a minimum population for it to continue to be able to circulate by continuous infection. The minimum population size is assumed to be of a similar order of magnitude to that for measles virus (*c.* 250 000) although no systematic study has been undertaken to ascertain this for MuV. Before successful control of mumps by vaccination had been achieved, outbreaks of mumps were more often observed in the winter and spring than in the summer, at least in the temperate Northern Hemisphere. Such a seasonal pattern was not observed in the tropics.

Host Range and Viral Propagation

Man is the only known host for the virus. Dogs can be naturally infected and show parotid swelling, although they do not pass on the virus. MuV can be used to infect a variety of animals experimentally, including monkeys, cats, dogs, ferrets, and a number of rodent species such as rabbits, suckling rats and mice, hamsters, and guinea pigs. Its adaptation to growth in 8-day-old embryonated eggs allowed the biological activities of the virus to be studied before the advent of tissue culture. The virus infects a wide range of cells in culture and causes a distinct cytopathic effect in most cell cultures with either rounding off or detachment of the cells from the substratum or widespread syncytium formation. Cytopathic effect varies from strain to strain and fusing and nonfusing variants have been described. The virus also readily establishes persistent infection in tissue culture systems, a property shared with many of the other viruses in the family *Paramyxoviridae*. Only low titers of virus are produced in such infections. However, the clinical significance of these observations is questionable as they do not reflect any known pathological outcome of the acute infection.

Serologic Relationships and Variability

MuV is a monotypic virus, and tests with human sera indicate the existence of only a single serotype. Polyclonal sera from infected individuals show a low level of cross-reactivity with hPIV2 and PIV5. Variability between strains has been demonstrated using monoclonal antibodies with the HN and N proteins showing the greatest diversity when single epitopes are examined. Sequence comparisons of the highly variable SH gene of MuV has demonstrated the existence of 12 genotypes. Sequence analysis of the SH gene is routinely used in molecular epidemiological studies (see below).

Evolution and Genetics

Human populations only became dense enough to sustain MuV from about 4000 years ago and, therefore, it has been suggested that the virus must have evolved from an animal pathogen. However, no closely related primate or other animal pathogen has yet been identified. Neither temperature-sensitive nor any other conditional lethal mutants of MuV have been reported nor has recombination been described in any nonsegmented negative-strand RNA virus. Although host range mutants have not been isolated, adaptation of the virus to growth in eggs or in chicken embryo fibroblasts requires a number of blind passages. Strains adapted in this way do not readily grow and fail to

generate syncytia when they are used to infect mammalian cells in culture. As MuV is a neuropathogenic virus some clinical isolates have been adapted to grow in the CNS of experimental animals to study this biological property. These viruses are currently being used in reverse genetics approaches to attempt to identify the molecular determinant of neuropathogenesis. At present neutralizing monoclonal antibody escape mutants of the HN protein are the only type of MuV mutants which have been described.

Epidemiology

Outbreaks of mumps show annual periodicities although the length of the cycle may vary from 2 to 7 years as a result of factors that are poorly understood. Historically, the virus caused severe problems when troops were assembled for war and it was observed that male recruits from rural populations were affected in greater numbers. It is, therefore, assumed that children in isolated rural or island populations are exposed to the virus later in life than those in densely populated conurbations. The infection gives rise to lifelong immunity from disease, but it is not clear whether a single dose of live-attenuated vaccine achieves the same. Based on an ongoing outbreak involving a number of vaccinated individuals some have suggested that vaccinees may indeed be susceptible. However, at present this idea should be treated with caution until a systematic retrospective analysis has been performed and the data are considered alongside the known rates of seroconversion after vaccination. The existence of a number of different genotypes has allowed the development of molecular epidemiology for mumps. Specific genotypes appear to dominate in Japan and China, respectively. In some European outbreaks, co-circulation of two genotypes has been described. Changes in the nucleotide sequences of viruses isolated during an epidemic have not been studied although differences between isolates obtained over several years from a given geographical area indicate that the virus gradually accumulates nonexpressed and, to a lesser extent, expressed mutations. However, strains isolated more than 40 years apart can still easily be recognized as belonging to a specific genotype.

Transmission and Tissue Tropism

The virus replicates in the upper respiratory tract and the salivary glands and is transmitted in salivary droplets. Patients are infectious from 3 days before until approximately 4 days after the onset of clinical symptoms. Mumps can also cause viruria but this is not considered important in transmission. Transmission occurs only in the acute phase and from the level of infection observed in naive populations, it can be concluded that the virus is highly contagious ($R_0 = 5–12$) but not as contagious as, for example, measles virus ($R_0 = 16–450$) or chickenpox viruses. The infection is systemic and the virus multiplies in a wide variety of tissues in the human host. The tropism for the pancreas, particularly the β-cells, has been suggested as an explanation for the temporal link between MuV infection and juvenile onset diabetes mellitus. However, direct evidence for such a link is missing (**Table 2**).

Clinical Features of the Infection

In humans the normal MuV incubation period ranges from 14 to 21 days although occasionally this period has been estimated to extend for over 50 days. In infected individuals as many as one-third of MuV infections are subclinical. When symptoms ensue the most common clinical manifestation is parotitis. Other complications, such as orchitis, are not infrequent (**Table 2**). Contrary to popular belief, orchitis has not been linked to an increase in male sterility. Mastitis occurs in females with the same frequency and the incidence of both these clinical features increases with the age at which MuV infection is contracted. Before the development of the currently used live-attenuated vaccine, MuV was the most common cause of viral meningitis and encephalitis in the USA. The encephalitis is usually benign although minor neurological changes, learning, and concentration impairments and sudden deafness are well-documented sequelae in a number of patients. More rare complications are oophoritis, thyroiditis, pancreatitis, otitis, retinitis, conjunctivitis, and keratitis (**Table 2**).

Pathology and Histopathology

Upon infection, the virus replicates primarily in the nasal mucosa and the epithelial layer of the upper respiratory tract. After penetrating the draining lymph nodes, a transient viremia occurs and thereafter various target organs such as the salivary glands, the kidney, pancreas, and the CNS are infected. Infection in the salivary glands produces parotitis which is the most predominant clinical feature of the virus. Viral replication leads to tissue damage and the subsequent immune response leads to inflammation and swelling of the gland. Dissemination into the kidneys can lead to prolonged infection of this organ and viruria. Virus can be isolated from throat swabs, blood, saliva, and urine. Involvement of the CNS may be as high as 50% of cases and parotitis is not required for this to take place. MuV can be readily isolated from the cerebrospinal fluid (CSF) in cases of meningoencephalitis.

Table 2 Clinical features, complications and prognosis of mumps virus infection in various organs

Organ	*Clinical features*	*Frequency*	*Pathology*	*Prognosis*
Salivary gland	Parotitis (bilateral or unilateral)	95% of symptomatic cases	Blockage of duct of Stensen	Swelling disappears usually in 3–4 days
Submaxillary and sublingual salivary glands	Swelling	Rare		Swelling subsides in 3–4 days
Testes	Orchitis (mostly unilateral; some bilateral)	25% of males (especially adult males)		Good; mostly only transient depression of sperm production
Breast	Mastitis	15% of females		Good; cause of virus in breast milk
Ovaries	Oophoritis	Rare		
Meninges	Meningitis fever/ vomiting	Mononuclear pleocytosis in CSF in 40–60% of cases	Ependymal epithelium destroyed by virus	Benign and self-limiting: ataxia (some permanent damage possible)
Brain	Encephalitis	2% of cases (<1% = fatal)	Virus spreads by neuronal pathways	Poor
Kidney				Good/cause of viruria
Pancreas	Pancreatis with nausea and vomiting	50% of cases	Could be due to interferon responses rather than specific tissue infection	Good/no established link to IDDM
Middle ear	Deafness	Very rare (<3%)	Cochlear infection	Permanent deafness is very rare
Heart	Myocarditis	Very rare	Fibroelastosis	Altered ECG; can cause myocardial infection
Blood	Immunosuppression	All cases	Infection in macrophages and lymphocytes	Viremia resolves
Fetus	Abortion in first trimester	Frequent	Virus is widespread in tissues of aborted fetus	In live births CMI response in the absence of humoral response

Pathogenicity

A number of MuV strains give rise to varying degrees of pathogenicity in experimental animal infections, for example, mice and hamsters. However, this does not seem to extend to natural human infections above and beyond some variation in the level of meningitis which tends to be associated with particular MuV strains (see below). The neurovirulence of MuV strains and vaccine batches is assessed using a monkey neurovirulence test (MNVT), but recently an alternative approach was devised based on the level of viral replication and hydrocephalus in neonatal rats. This rat neurovirulence test (RNVT) has apparently better predictive power than the MNVT. Some neutralizing monoclonal antibody escape mutants show alterations in neuropathogenicity in a hamster model.

Immune Response

It is not known whether the humoral or cell-mediated immune (CMI) response is the most important in clearing MuV from an infected host. Both play a role although neither seems to be required exclusively for successful control of the infection. Eleven days after infection, the humoral immune response is well established and the presence of neutralizing viral antibodies probably terminates viremia. Similarly, the appearance of IgA in the salivary fluid stops excretion of infectious MuV in the saliva. The precise time at which MuV reactive cytotoxic T lymphocytes first appear is unclear and these have been demonstrated in both the blood from patients with the natural disease and in vaccinees. The magnitude and effectiveness of this response may be related to the genetic human leukocyte antigen (HLA) background of the host. A complication in the development of the CMI response is the tropism of the virus for T and B cells. Reduction in CMI responses to antigens previously recognized has been observed, although the mechanism is unclear. It is less severe and of shorter duration than the immunosuppression associated with measles virus infection. The virus grows well in activated but not in resting T lymphocytes and infection of the CNS during MuV infection is thought to occur via transfer of infected monocytes into the choroid plexus. Perinatal exposure of the fetus to MuV via the placenta does not appear to lead to infection of fetal tissues. However, this can give rise to anomalous immune responses

to MuV in the newborn child. In these children, CMI but no humoral responses are detectable. Neutralizing B-cell epitopes have been defined in the HN gene of MuV but no other B- or T-cell epitopes have been delineated, as yet.

Prevention and Control

Adaptation of MuV to growth in embryonated eggs and chicken embryo fibroblasts allowed the early development of live-attenuated vaccines for mumps. In 1946, Enders observed that adaptation of MuV to chicken cells was associated with the loss of virulence for monkeys. In the past, killed virus preparations were used for human vaccination although these did not lead to lifelong protective immunity and their use has been discontinued. In general, the appearance of atypical cases of measles and respiratory syncytial virus infection after vaccination with killed virus has led to the exclusive use of live-attenuated vaccines for the control of paramyxoviruses. Mumps vaccination has substantially reduced the incidence of the disease worldwide. After successful licensing of the vaccine in 1967 in the USA, the incidence of mumps dropped from 76 to 2 cases per 100 000 population. There are now a number of live-attenuated vaccine strains, for example, the Jeryl Lynn (JL) strain, which are usually administered in a trivalent vaccine containing live-attenuated strains of measles and rubella viruses as well as MuV (MMR). At present most developed countries have chosen to adopt a two-dose schedule for MMR vaccination and the vaccine is administered at 15 months and *c.* 4 years of age. The JL strain of MuV is most frequently used in the MMR vaccine and interestingly it has been shown to be comprised of two strains, the major (JL5) and the minor (JL2) components. The Urabe strain has been removed from MMR vaccines since reports in several countries indicated a higher incidence of meningitis associated with the use of this strain of MuV.

MuV and Inclusion Body Myositis

In the past MuV has been implicated in inclusion body myositis. However, neither *in situ* hybridization nor the polymerase chain reaction or immunocytochemistry has been able to link MuV to the paramyxovirus-nucleocapsid-like structures observed in muscle cells of patients with this disease. In contrast, myocarditis in patients with fibroelastosis has been associated with the presence of mumps viral RNA with reverse transcription-polymerase chain reaction (RT-PCR). The link to arthritis is also unclear (**Table 2**).

Future Perspectives

Over the last ten years, the development of reverse genetics systems for all members of the *Mononegavirales* has had a significant impact on our understanding of these viruses. Such systems have been generated for PIV5, hPIV2, and MuV, and to date they have been used to begin to define the structural and functional relationships and the roles that various proteins play in attenuating these viruses. The opportunity to examine the contribution of individual proteins from neurovirulent strains, such as Urabe, to neuropathogenesis in animal models should help to identify virulence determinants, something which is important if it proves necessary to develop new MuV vaccines. Expression of individual proteins may also allow a dissection of the humoral and cellular immune response and this should help improve our understanding of the role that these have in virus clearance.

One of the key challenges for the next number of years is to systematically monitor the immune status of vaccines and assess their susceptibility to reinfection by currently circulating MuV genotypes. Furthermore, documenting the occurrence of sequelae after the introduction of large-scale vaccination is vital. This should be underpinned by comprehensive molecular epidemiological approaches to ensure that the currently circulating genotypes are rapidly detected. Such approaches are particularly important given the recent large number of vaccinated individuals who have been reportedly infected in recent and ongoing outbreaks in both the UK and the USA. Linking MuV genotypes to particular phenotypes, such as neurovirulence, is crucial for our understanding of this ubiquitous human pathogen and the key goal in the medium term is to move from unproven association to formal proof. It is in this arena that reverse genetics approaches for MuV should prove most useful.

See also: Central Nervous System Viral Diseases; Epidemiology of Human and Animal Viral Diseases; Measles Virus; Paramyxoviruses of Animals; Parainfluenza Viruses of Humans; Persistent and Latent Viral Infection.

Further Reading

Carbone KM and Rubin S (2006) Mumps virus. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,*, 5th edn, pp. 1527–1550. Philadelphia: Lippincott Williams and Wilkins.

Feldmann HA (1989) Mumps. In: Evans AS (ed.) *Viral Infections of Humans,* ch. 17, p471. New York: Plenum.

Gupta RK, Best J, and MacMahon E (2005) Mumps and the UK epidemic 2005. *British Medical Journal* 330: 1132–1135.

Mungbean Yellow Mosaic Viruses

V G Malathi and P John, Indian Agricultural Research Institute, New Delhi, India

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Agroinoculation Delivery of the viral genome through *Agrobacterium* inoculation.
Binary vector In these systems, the T-DNA region containing a gene of interest is contained in one vector and the *vir* region is located in a separate disarmed Ti plasmid. The plasmids co-reside in *Agrobacterium* and remain independent.
Nuclear localization signal Arrangement of basic residues like arginine or lysine in a protein that facilitates entry through nuclear pores.
Replicon Autonomously replicating DNA segments having an independent replication origin.
Rolling circle replication A mechanism for copying single-stranded circular genome by means of double-stranded intermediates.

History

The mungbean yellow mosaic virus (MYMV) and mungbean yellow mosaic India virus (MYMIV) infect a variety of leguminous crop plants and cause devastating yellow mosaic and golden mosaic diseases. A virus disease of mungbean (*Vigna radiata* (L.) Wilczek) exhibiting bright yellow mosaic symptoms was first observed in 1950s in Delhi, India by Nariani. The causal virus was easily transmissible to selected legume hosts through the whitefly vector. Nariani recorded the virus as mungbean yellow mosaic virus in 1960. Yellow mosaic viruses were reported subsequently from several hosts either as strains of MYMV or as separate entities. The annual loss due to yellow mosaic disease caused by these two viruses in blackgram, mungbean, and soybean together is estimated to be $300 million.

Taxonomy and Classification

The two viruses belong to the species *Mungbean yellow mosaic virus* and *Mungbean yellow mosaic India virus* (genus *Begomovirus*, family *Geminiviridae*). The type species *Mungbean yellow mosaic virus* is represented by a mungbean isolate from Thailand. The type species *Mungbean yellow mosaic India virus* is represented by a blackgram isolate from Delhi, India. The members of the family *Geminiviridae* have circular, single-stranded DNA genome of 2.5–2.7 kbp and are distinguished from other viruses by their characteristic geminate particle (about 30×20 nm in size) morphology. In the family *Geminiviridae,* the genus *Begomovirus* is differentiated, among other criteria, from the genera *Mastrevirus*, *Curtovirus,* and *Topocuvirus* by the fact that its members are transmitted by whiteflies (*B. tabaci* Genn) in a persistent manner.

Geographic Distribution

MYMV occurs in Thailand, Pakistan, and in western and southern states of India. MYMIV occurs in northern, eastern, and central states of India, Pakistan, Nepal, and Bangladesh. Identity of yellow mosaic viruses infecting grain legumes in Bhutan and Sri Lanka is not yet elucidated.

Symptoms in Plants

As the name implies, the characteristic symptoms caused by the infection are yellow and golden mosaic in the leaves (**Figure 1**). Symptoms start as scattered small specks or yellow spots in the leaf lamina which enlarge to irregular yellow and green patches alternating with each other on matured leaves. The yellow areas increase, coalesce resulting in complete yellowing of leaves. Plants produce fewer flowers and pods. Pod size and seed size are reduced. In some of the varieties of blackgram, necrotic mottling is seen. Sometimes the green areas are raised and the leaves show puckering and reduction in size. In French bean, affected plants show downward leaf curling and corrugated leaf lamina instead of yellow mosaic.

Virion or Particle Structure

Typical geminate particles measuring $(15–18) \times 30$ nm have been observed in leaf dip preparation of plant extracts of naturally infected plants.

In double antibody sandwich enzyme-linked immunosorbent assay (ELISA) the protein preparations from MYMV- and MYMIV-infected plants reacted to polyclonal and monoclonal antibodies to Indian and African cassava mosaic viruses, although A_{405} values obtained for field-infected plants were very low. The molecular weight of the coat protein (CP) of MYMV and MYMIV is ~28 kDa and consists of ~230 amino acids. There is one α-helix (WRKPRFY) at the N-terminus and the rest of

the protein contains potential β-sheets. The recombinant MYMIV CP protein lacking the first N-terminus 22 amino acids was expressed as maltose-binding and glutathione *S*-transferase (GST) fusion protein. The expressed CP bound preferentially to single-stranded (ss) DNA rather than double-stranded (ds) DNA. The blackgram isolate of MYMV-Vig, CP was shown to interact with nuclear import factor, α importin of plants, suggesting nuclear import of CP via α-importin-dependent pathway.

Genome Organization

The MYMV and MYMIV have a genome organization (**Figure 2**) similar to Old World bipartite begomoviruses. The genome consists of two circular ssDNA components. They are designated as DNA-A and DNA-B components which are encapsidated separately. The ~2745 bp DNA-A component codes for the CP and for viral DNA replication and transcription proteins. The ~2616 bp DNA-B encodes for proteins for movement and nuclear localization. DNA-A replicates autonomously and is dependent on DNA-B for movement functions. Replication of DNA-B component is dependent on DNA-A-encoded proteins and both components are essential for viral pathogenicity. DNA-A has two open reading frames (ORFs) in the viral sense, (ORF AV1-CP; AV2-pre-CP) and five in the complementary sense (ORF AC1-replication initiation protein, Rep; ORF AC2-transcription activator protein, TrAP; ORF AC3-replication enhancer protein, REn; ORFs AC4 and AC5). The role of ORFs AV2, AC4, and AC5 is yet to be deciphered. The ORF AV2 is present only in Old World begomoviruses. In DNA-B, there is one ORF in viral sense strand, ORF BV1-nuclear shuttle protein (NSP), and one in complementary sense, ORF BC1-movement protein (MP). The nucleotide sequence identity



Figure 1 Yellow and golden mosaic symptoms in different legumes in the field: (a) mungbean, (b) blackgram, (c) soybean, and (d) cowpea.

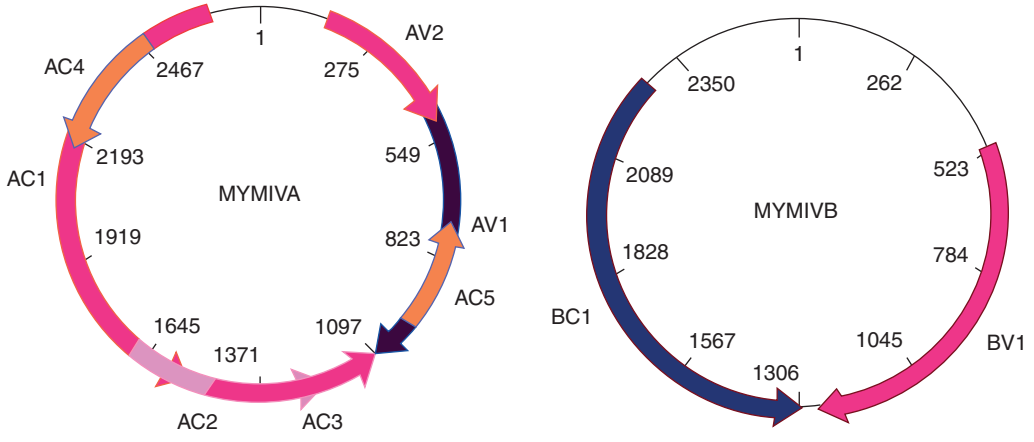


Figure 2 Genome organization of mungbean yellow mosaic India virus (MYMIV). Arrows represent open reading frames of virion and complementry sense. AV1, coat protein; AV2, pre-coat protein; AC1, replication intimation protein; AC2, transcription activator protein; AC3, replication enhancer protein; BC1, movement protein; BV1, nuclear shuttle protein.

between the DNA-A component of the two viruses is only 80%, justifying their recognition as separate species.

One characteristic feature of MYMV occurring in a southern state (Tamil Nadu) of India is the occurrence of multiple DNA-B components along with only one DNA-A component. Between rightward and leftward ORFs, in both the DNA components, there is a noncoding intergenic region. Intergenic region contains a characteristic stem–loop structure which is conserved in all geminiviruses. It lies 29 nt downstream of TATA box. The structure consists of GC-rich stem and an invariant nonanucleotide sequence, TAATATTAC. The Rep protein cleaves this nonanucleotide sequence to initiate replication. In the intergenic region there are 5–8 nt sequences that occur, as repeats and are called as iterons. Iterons represent Rep protein-binding sites, which cleaves the plus DNA strand to initiate replication. The Rep-binding repeat sequence was identified to be ATCGGTGT which occurs as invert and tandem repeat before the TATA box and one copy is also present after the nonanucleotide sequence in MYMIV. In MYMV the iterons are identified as GGTGTAxxGGTGT (x any nucleotide). The arrangement of iterons in MYMV and MYMIV is unique and does not show lineage to other begomoviruses. The term origin of replication (*ori*) refers to sequences from the tandem repeat of iteron, upstream of TATA box to the invariant nonanucleotide sequence. Intergenic region also contains promoters for rightward and leftward ORFs.

Within the intergenic region, there is a stretch of 180–210 nt sequence that is near identical between DNA-A and DNA-B components of bipartite begomoviruses; and this region called as common region (CR) is specific for a virus. In the case of MYMV and MYMIV, analysis of the CR of DNA-A and DNA-B components of more than 17 isolates revealed that there is considerable divergence in the whole CR in DNA-B compared to DNA-A component. The divergence in the CR is essentially in the origin of replication and ranges from 14% to 23% in the whole CR, 22% to 29% in the origin of replication among different isolates of MYMV and MYMIV.

Replication

The delicate mouth parts of the whitefly vector inject the virion particles in sieve tube cells while sucking the plant juice. From the geminate particles, ssDNA genome is released, whether the intact geminate particle or the genomic DNA enters the nucleus through the nuclear pore is debated. Once the ssDNA enters the nucleus, it is dependent on host DNA polymerase to synthesize a replicative dsDNA. It is the dsDNA which is the template both for transcription of various genes and for replication.

Investigations on geminiviruses have suggested that the viral DNA replicates in a rolling-circle mode (RCR). During RCR, Rep binds to specific iterons present in the CR and hydrolyzes the phosphodiester bond between the seventh and eighth residues of the invariant nonamer sequence 5′TAATATT$^{\downarrow}$ AC 3′ (arrow indicates site of cleavage). Rep remains bound covalently to the 5′ phosphate end and 3′ hydroxyl end thus generated becomes available for rolling-circle replication. After a full cycle of replication, the new origin is generated which is again hydrolyzed by Rep. Subsequently, Rep closes the nascent 3′ end of the DNA with the previously generated 5′ end. In this way one unit genome-length circular, ssDNA molecule,that is, the mature viral genome, is processed.

The Rep protein of MYMIV was overexpressed in *Escherichia coli*. The recombinant and refolded protein bound to CR, in a sequence-specific manner; binding of DNA-A was more efficient than DNA-B. The recombinant protein showed site-specific nicking/closing and type-1 topoisomerase activities. The cleavage function was especially upregulated by ATP, suggestive of ATP-mediated conformational changes required to cleave the nonanucleotides. A large oligomeric complex (approximately 24 mer) of Rep protein was shown to function as helicase.The recombinant Rep protein of MYMIV showed binding with recombinant pea, proliferating cell nuclear antigen (PCNA) protein. The site-specific cleaving and closing activity and ATPase function of Rep were also impaired when bound with PCNA. There was a strong interaction between Rep and CP of MYMIV, the domain of interaction with the CP has been mapped to the central region of Rep. The activities of Rep were downregulated by the CP indicating how geminiviral DNA replication could be regulated by the CP.

Gene Expression and Transcription Regulation

The circular ssDNA genome of geminiviruses replicate through a double-stranded intermediate which is also the template for transcription for polymerase II. Transcription in these viruses is bidirectional and both viral and complementary strand encode different proteins.

Total RNA isolated from young leaves of blackgram seedlings agroinoculated with MYMV-Vig were subjected to transcript analysis by circularized reverse transcriptase-polymerase chain reaction (RT-PCR) method. The study showed that in DNA-A there are two major transcription units, one on viral sense and another one in complementary sense. Both are dicistronic, where rightward transcription unit is used for translation of ORF AV2/AV1 and leftward transcription unit for AC1/AC4. Likewise, there are two major transcripts in DNA-B, one rightward and one in leftward side, ORF BV1 and ORF BC1, are translated from these two transcripts respectively. The two major transcripts in both the DNA are driven by the promoter in

the intergenic region and so the promoter is considered to be bidirectional. The promoter present between ORF AC1/AV2 is contained within 252 bp intergenic region in DNA-A. The promoter in DNA-B resides in the larger fragment of the intergenic region (957 bp) between ORFs BC1/BV1. Both the bidirectional promoters have direction-specific core elements located at an optimal distance from the transcription start site. Both rightward and leftward transcription are activated by the transcription factor AC2 encoded by the virus.

A new feature that was seen in MYMV-Vig for the first time is that the Rep protein showed synergistic activity with the AC2 protein and contributed to the activation of rightward promoters.

Besides these four transcripts, a fifth transcript of 1.4 kbp that hybridizes with ORF AC2 probe is predicted to be the transcript from which ORF AC2/AC3 will be translated. This transcript was driven by a strong monodirectional promoter upstream of ORF AC2.

Another unique feature was the transcription unit of the BC1 protein. There is a conserved leader-based intron which is not seen in any other begomoviruses. The 123 nt intron within BC1 transcript appears to have all the features of plant introns. Such an intron with consensus 5′ and 3′ splice sites (AG/GU,CAG/G) and one or more short ORFs (sORFs) appear at the same or nearby location in all the isolates of MYMV and MYMIV.

One characteristic feature observed in all transcripts except that of AC1 and BC1 is multiple transcription initiation sites, closely spaced (3–4 nt) from each other and multiple polyadenylation sites. Translation of ORFs AV1, AC4, and AC3 is predicted to be of leaky scanning type wherein the proximal 5′ AUG is in suboptimal context and is bypassed allowing the second product (AV1, AC4, and AC3) to be translated. Short ORFs present between AV2 and AV1 may also help in reinitiation of transcription of second product.

Infectivity of Cloned Components

Infectivity of blackgram isolate of MYMV, MYMV-[Vig] and blackgram, cowpea, mungbean and soybean isolates of MYMIV, MYMIV-[Bg], MYMIV-[Cp], MYMIV-[Mg], and MYMIV-[Sb] on leguminous hosts have been established by 'agroinoculation'. In this strategy dimers of DNA-A and DNA-B components are cloned in a binary vector, which is mobilized in *Agrobacterium tumefaciens*. The partial or complete tandem repeat constructs are amplified in *A. tumefaciens* in nutrient broth; the bacterial culture is used to deliver the viral inoculum. In all these cases, considerable divergence was seen in the origin of replication in DNA-B component, which did not impair their infectivity. An interesting feature was seen with the cowpea isolate; though it is not whitefly transmissible to hosts other than cowpea and French bean, through agro-inoculation the cowpea isolate was systemically infected and produced yellow mosaic symptoms in blackgram and mungbean. This adaptation to new hosts was maintained by the viral progeny isolated from agroinoculated plants, which could then be easily transmitted by whitefly to other hosts. However, the blackgram isolate of MYMIV did not produce yellow mosaic symptoms in cowpea even by agroinoculation. An improved agroinfection using one strain of *A. tumefaciens* was shown for MYMV-Vig. Partial tandem repeat construct of DNA-A and DNA-B was made in vectors having compatible replicons and introduced into the same *Agrobacterium* cells. When *Agrobacterium* cells having both constructs were inoculated, infectivity rate was 100% in blackgram.

Inoculation with different DNA-B components, KA22 and KA27 with DNA-A of MYMV-Vig, showed differences in symptoms severity. KA22 DNA-B (which is more closely related to MYMIV DNA-B) caused more intense mosaic symptoms with high viral DNA titer in blackgram. In contrast, KA27 DNA-B (closely related to DNA-B of Thailand isolate of MYMV) caused more intense symptoms and high viral DNA titer in mungbean. DNA-B is therefore considered as an important pathogenicity determinant of host range between blackgram and mungbean.

Host Range

The MYMV and MYMIV isolates have very narrow host range infecting only leguminous hosts. Whatever information is available on host range, they have been generated before the demarcation of isolates into two viruses. Whether differentiation into two species based on DNA-A nucleotide sequence will reflect on the biological properties need to be investigated freshly. The isolates from blackgram, mungbean, horsegram, and mothbean are easily transmissible to following leguminous species: mungbean (*V. radiata*), blackgram (*V. mungo*), mothbean (*V. aconitifolia* (Jacq) Marechal), soybean (*Glycine max*), phaemey bean (*Phaseolus lathyroides*), horsegram (*Macrotyloma uniflorum*) and black tapery bean (*P. acutifolius*), pigeonpea (*Cajanus cajan*), and French bean (*P. vulgaris*). These isolates are not transmissible to *Dolichos*. The transmission of these isolates to cowpea is inconsistent and contradictory results are reported. The cowpea isolate of MYMIV is transmissible only to cowpea (*V. unguiculata*), yard long bean (*V. unguiculata* (L.) Walp. f.sp. *sesquipedalis*), and French bean and not to any other host. The pigeonpea and soybean isolates could be transmitted to cowpea, though back transmission from cowpea to soybean or pigeonpea is inconsistent. Thus, it has been difficult to exactly assess the host range of the viruses as they are not sap transmissible and transmission by whitefly is dependent on the biotype of the vector used, its feeding preferences, and susceptibility of the genotypes tested. Weeds like *Brachiaria ramosa*, *Eclipta alba*, and *Xanthium strumarium*, and

garden plant *Cosmos bipinnatus* were reported as hosts for MYMIV. Total nucleic acid extracted from above hosts showing symptoms of begomovirus infection did not hybridize with radiolabeled MYMIV DNA-B probe, indicating that the begomoviruses infecting these hosts are different. At present, MYMIV isolates are not transmissible to any other nonleguminous host.

Transmission and Virus–Vector Relationship

All isolates of MYMV and MYMIV are neither sap transmissible (except the Thailand isolate of MYMV) nor are they transmitted through seeds. MYMV and MYMIV are transmitted by whitefly, *B. tabaci* Genn, in a persistent circulatory manner. Young expanding leaves are better sources of inoculum than old leaves. A single whitefly can transmit the virus given an acquisition (AAP) and inoculation access period (IAP) of 24 h. Transmission percentage definitely increases with increased number of whiteflies. Female whiteflies are found to transmit more effectively and retain the virus for a longer period than male whiteflies. Starvation for a duration of 15 min, before acquisition and inoculation access period increases the transmission rate to 50%. Though the vector can acquire and transmit the virus immediately after an AAP and IAP of 10–15 min, the minimum period of 4–6 h is required. There is a latent period of more than 3 h inside the vector for transmission to occur. The whiteflies can retain the virus for 10 days and transmission rate decreases gradually. The period of retention may differ from one batch of insects to another batch. There is no transovarial transmission. No evidence exists till date for association of any specific biotype of the vector with the epidemic outbreak of the disease.

Ecology and Epidemiology

Bemisia tabaci is a polyphagous vector with wide host range; however, it shows strong host preferences and its feeding behavior is a major factor in deciding the active spread of the virus from one crop species to another. For example, the cowpea isolate of MYMIV does not spread from golden mosaic disease-affected cowpea plants to adjacent blackgram or mungbean fields in northern India.

Bemisia tabaci thrives best under hot and humid conditions that prevail in the tropical and subtropical area in the Indian subcontinent. The population of the vector is influenced by temperature, relative humidity, and rainfall.

Depending on vector population build-up, spread of the virus is gradual, cumulative, and is in the direction of prevalent wind. In northern India with the onset of monsoon rain (June–July), as the population of vector increases, rate of spread of virus increases. Whereas before monsoon

Table 1 Matrix of pairwise identity percentages of complete nucleotide sequences of DNA-A of selected begomoviruses

YMV isolates	*MYMIV*	*MYMIV-Mg[Isl]*	*MYMV-Vig*	*MYMV*	*DoYMV*	*HgYMV*
MYMIV	*	95.8	80.3	80.0	60.5	80.9
MYMIV-Mg[Isl]		*	81.3	81.0	61.7	82.3
MYMV-Vig			*	96.7	62.4	84.1
MYMV				*	62.4	84.0
DoYMV					*	62.0
HgYMV						*

Acronyms are as given in text; '*' indicates homologous comparisons. Matrix was generated using the CLUSTAL algorithms.

Table 2 Matrix of pairwise identity percentages of complete nucleotide sequences of DNA-B of selected begomoviruses

YMV isolates	*MYMIV*	*MYMV-Vig*	*MYMV-Vig [Mad KA 21]*	*MYMV-Vig [Mad KA 27]*	*MYMV-Vig [Mad KA 28]*	*MYMV-Vig [Mad KA 34]*	*MYMV*	*HgYMV*
MYMIV	*	91.8	92.3	69.6	92.4	92.0	69.5	66.9
MYMV-Vig		*	96.8	69.7	96.6	98.9	86.3	66.6
MYMV			69.5	95.0	69.8	69.4	*	65.9
MYMV-Vig [Mad KA 21			*	69.7	98.2	96.9		67.1
MYMV-Vig [Mad KA 27]				*	69.7	69.7		66.6
MYMV-Vig [Mad KA 28]					*	96.7		67.5
MYMV-Vig [Mad KA 34]						*		66.7
HgYMV								*

Acronyms are as given in text; '*' indicates homologous comparisons. Matrix was generated using the CLUSTAL algorithms.

Table 3 List of viruses with their accession numbers from GenBank database used for sequence analysis and phylogenetic comparison

Virus	*Acronym*	*Accession Number*
Bean calico mosaic virus	BCaMV	AF110189
Bean dwarf mosaic virus	BDMV	M88179
Bean golden yellow mosaic virus-[Mexico]	BGYMV-[MX]	AF173555
Bhendi yellow vein mosaic virus-[301]	BYVMV-[301]	AJ002453
Bhendi yellow vein mosaic virus-[Madurai]	BYVMV-[Mad]	AF241479
Cabbage leaf curl virus	CaLCuV	U65529
Cotton leaf curl Rajasthan virus	CLCuRV	AF363011
Cowpea golden mosaic virus-[Nigeria]	CPGMV-[NG]	AF029217
Indian cassava mosaic virus-[Maharashtra]	ICMV-[Mah]	AJ314739
Maize streak virus-A[Kom] (RANDOM)	MSV-A[Kom]	AF003952
Mungbean yellow mosaic India virus	MYMIV	AF126406, AF142440
Mungbean yellow mosaic India virus-[Bangladesh]	MYMIV-[BG]	AF314145
Mungbean yellow mosaic India virus-Cowpea	MYMIV-[Cp]	AF481865, AF503580
Mungbean yellow mosaic India virus-Mungbean	MYMIV-[Mg]	AF416742, AF416741
Mungbean yellow mosaic India virus-Mungbean [Akola]	MYMIV-Mg[Akol]	AY271893, AY271894
Mungbean yellow mosaic India virus-[Soybean]	MYMIV-[Sb]	AY049772, AY049771
Mungbean yellow mosaic India virus-Mungbean [Islamabad]	MYMIV-Mg [Isl]	AY269992
Mungbean yellow mosaic India virus-Mungbean [Nepal]	MYMIV-Mg [Nep]	AY271895
Mungbean yellow mosaic India virus-[Soybean TN]	MYMIV-[SbTN]	AJ416349, AJ420331
Mungbean yellow mosaic India virus-Mungbean [Islamabad]	MYMIV-[PK;Cp]	AJ512496
Mungbean yellow mosaic India virus-[MBK-A25]	MYMIV-[MBK-A25]	AY937175, AY937196
Mungbean yellow mosaic virus-Mungbean[Haryana]	MYMV-Mg [Har]	AY271896
Mungbean yellow mosaic virus-Soybean [Islamabad]	MYMV-Sb [Isl]	AY269991
Mungbean yellow mosaic virus-Soybean [Madurai]	MYMV-Sb [Mad] MYMV-[Sb]	AJ421642, AJ867554, AJ582267
Mungbean yellow mosaic virus–Vigna	MYMV-Vig[KA22]	AJ132575, AJ132574
Mungbean yellow mosaic virus-Vigna [KA27]	MYMV-Vig [KA:27]	AF262064
Mungbean yellow mosaic virus-Vigna [Maharashtra]	MYMV-Vig [Mah]	AF314530
Mungbean yellow mosaic virus-Thailand	MYMV-Thailand	D14703, D14704
Hosegram yellow mosaic virus	HgMV-[IN:Coi]	AJ627904, AJ627905
Dolichos yellow mosaic virus	DoYMV-[IN]	AY306241
Mungbean yellow mosaic virus–Vigna [Madurai]	MYMV–[KA:34]	AJ439057
Mungbean yellow mosaic virus–Vigna[Madurai]	MYMV–[KA:28]	AJ439058
Mungbean yellow mosaic virus–Vigna [Madurai]	MYMV–[KA:21]	AJ439059
Potato yellow mosaic virus-[Venezuela]	PYMV-[VE]	D00940
Soybean crinkle leaf virus-[Japan]	SbCLV-[JR]	AB050781
Tomato golden mosaic virus-Yellow Vein	TGMV-YV	K02029
Tomato leaf curl Bangalore virus-[Ban5]	ToLCBV-[Ban5]	AF295401
Tomato leaf curl New Delhi virus-Severe	ToLCNDV-Svr	U15015
Tomato yellow leaf curl Thailand virus-[2]	TYLCTHV-[2]	AF141922

rain *B. tabaci* populations are nonviruliferous, whiteflies may pick up the inoculum from self-perpetuating weeds harboring viruses. However, the role of any weed or crop plant specifically serving as reservoir for MYMV and MYMIV has not been ascertained. A serious outbreak of yellow mosaic disease caused by MYMV in mungbean occurred in northern Thailand in 1977, resulting in reduction in mungbean cultivation and shift in cropping pattern.

Management

The main components of management of the disease are cultivation of resistant genotypes, cultural practices to ensure weed-free or inoculum-free field, and judicious use of chemicals to reduce the vector population.

The genetics of resistance to MYMIV is understood and it appears to be governed by a single recessive gene or two complementary recessive genes in mungbean. Sources of resistance to both the viruses have been identified by rigorous screening in the field; some of the resistance sources identified are being used for breeding purposes.

Cultural practices like adjustment of sowing dates to avoid the disease, rouging the weeds and plants that get infected early in the season, inter or mixed cropping of mungbean or blackgram with nonhost plants like sorghum, pearl millet, and maize have been adopted and shown effective in bringing down the disease incidence. The vector whitefly is controlled by applying phorate, disulfoton granules @ $2\,\mathrm{kg\,ha^{-1}}$ during sowing. Spray of

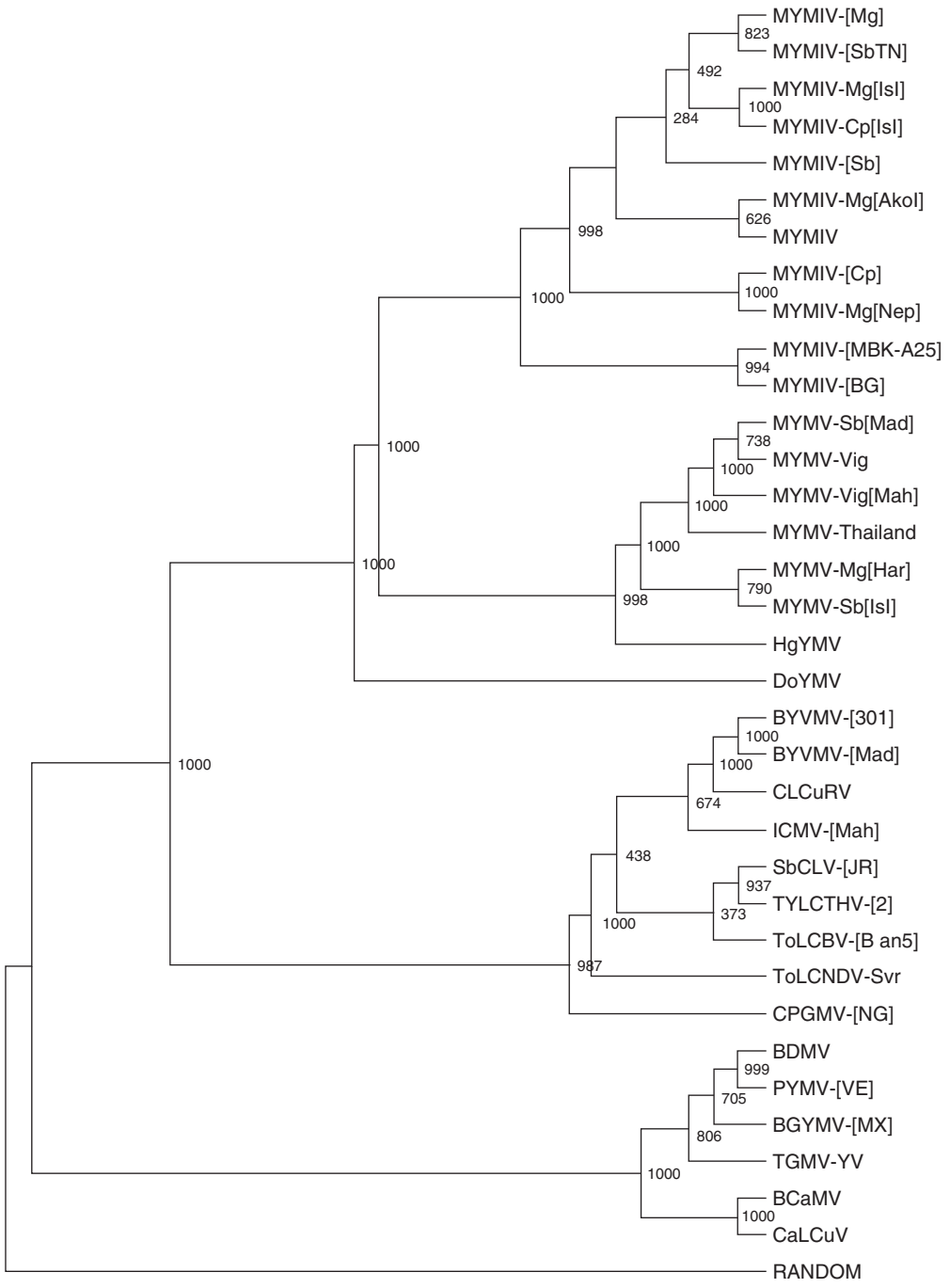


Figure 3 Phylogenetic relationship between selected begomovirus isolates based on complete nucleotide sequence of DNA-A. Dendogram was constructed using the neighbour-joining method with bootstraping (1000 replicates) in CLUSTAL X. Vertical distances are arbitary, horizontal distances are propotional to genetic distances.

metasystox malathion @ 0.1% is known to effectively reduce the virus spread and disease incidence.

Biolistic delivery of RNAi constructs targeting CR sequences of MYMV-Vig isolate resulted in complete recovery from infection (68–77%) in blackgram seedlings, offering an alternative approach for the management of the disease. Possibility of using *Paecilomyces farinosus*, an endophyte of whitefly, as biocontrol agent is being explored.

Phylogenetic Relationship

Complete nucleotide sequence of DNA-A component of 28 MYMV and MYMIV isolates from Indian subcontinent and Thailand is available in the GenBank database. Nucleotide sequence of a limited number of yellow mosaic virus isolates infecting *Dolichos* and horsegram has also been completed. There are currently four different

species comprising 'yellow mosaic legume viruses'. They are *Mungbean yellow mosaic virus*, *Mungbean yellow mosaic India virus*, *Horsegram yellow mosaic virus,* and *Dolichos yellow mosaic virus*. Percentage nucleotide identity for DNA-A and DNA-B component between the viruses of the different species is given in **Tables 1** and **2**. Comparison of nucleitide sequence of DNA-A component revealed 81% identity between MYMIV and MYMV isolates; both the viruses share only 60% identity with cowpea golden mosaic virus from Nigeria.

A phylogenetic tree based on complete nucleotide sequence (**Table 3**) of DNA-A component of selected begomoviruses revealed their distinct lineages. MYMV and MYMIV showed a clear dichotomy. Three viruses, MYMV, MYMIV, and DoYMV, occupy three branches separately (**Figure 3**).

The topology of the phylogenetic tree based on nucleotide sequence of DNA-B (**Figure 4**) component is different from DNA-A. There are three major branches: one major branch comprising DNA-B of MYMIV isolates and (four) DNA-B found associated with MYMV-Vig DNA-A. The second branch consisting of one isolate each of soybean (MYMV-Sb [Mad]B1) and blackgram (MYMV-Vig[Mad]KA27) from southern India and one mungbean isolate from Thailand (MYMV); the third branch is highly divergent from others consisting of one soybean isolate of MYMV-Sb[Mad]B2 and HgYMV. Recombination events have been identified in a mungbean isolate of MYMIV. Occurrence of multiple DNA-B components with MYMV DNA-A indicates how MYMIV DNA-B could have been captured by MYMV DNA-A during mixed infection of both MYMV and MYMIV.

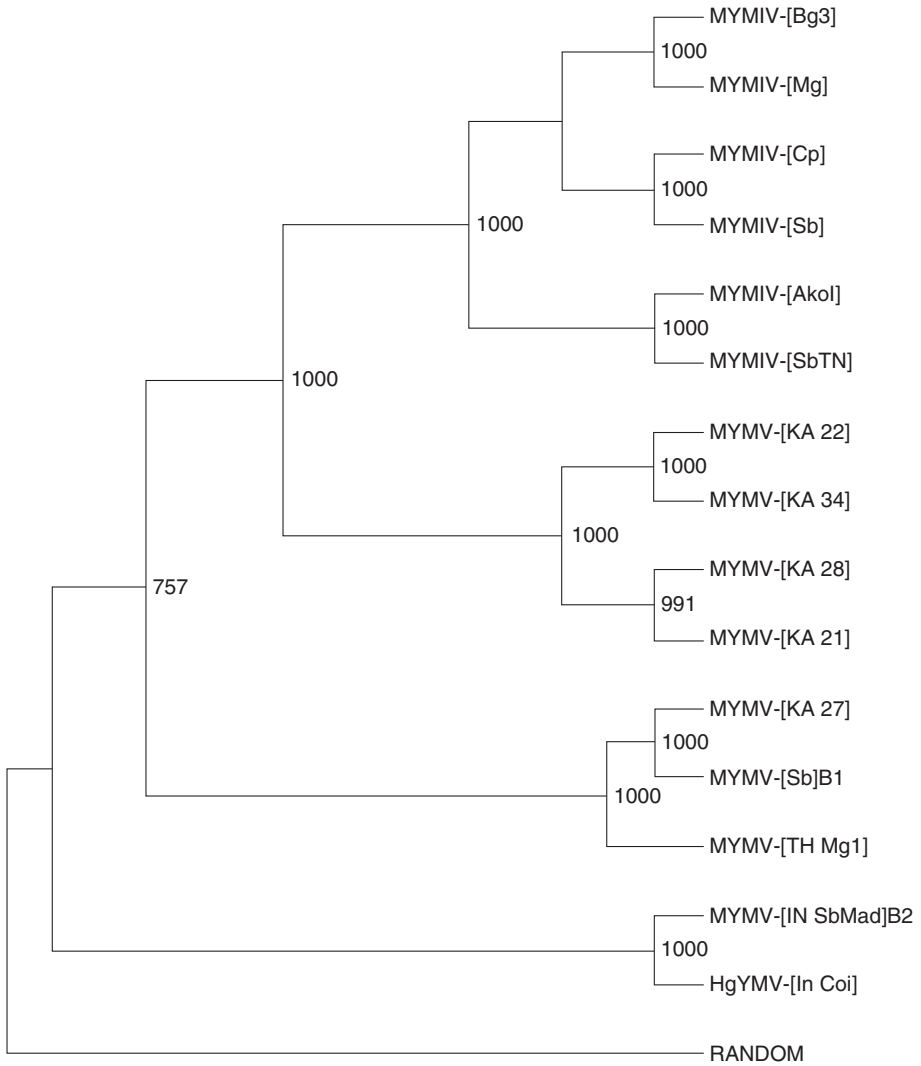


Figure 4 Phylogenetic relationship between yellow mosaic virus isolates based on complete nucleotide sequence of DNA-A dendogram was constructed using the neighbor-joining method with bootstraping (1000 replicates) in CLUSTAL X. Vertical distances are arbitary, horizontal distances are propotional to genetic distances.

A striking feature of DNA-A tree is the unique position occupied by MYMV and MYMIV. They do not cluster with typical begomoviruses of the Indian subcontinent origin, which is unusual. Begomoviruses of a specific geographical origin show high degree of conservation in CP gene, due to selection pressure exerted by the whitefly biotype. On the contrary, MYMV and MYMIV which are transmitted effectively by the same whitefly biotypes in Indian subcontinent like other begomoviruses, yet do not cluster with them. Due to their unique identity, MYMV and MYMIV are separately classified as 'legumoviruses'. They neither show host-related affinity as they are divergent from legume begomoviruses of both Old World (the virus species *Cowpea golden mosaic virus* from Nigeria, *Soybean crinkle leaf virus* from Thailand) and New World (the virus species *Bean golden yellow mosaic virus*, *Bean dwarf mosaic virus,* and *Bean calico mosaic virus*). Probably yellow mosaic begomoviruses of legumes diverged from a common ancestor virus much earlier in evolution and might have gained their unique features while adapting to legume hosts during evolution in the Indian subcontinent.

See also: Bean Golden Mosaic Virus; Tomato Leaf Curl Viruses from India.

Further Reading

Chaudhary NR, Malik PS, Singh DK, Islam MN, Kaliappan K, and Mukherjee SK (2006) The oligomeric Rep protein of Mungbean yellow mosaic India virus (MYMIV) is a likely replicative helicase. *Nucleic Acid Research* 34: 6362–6377.

Girish KG and Usha R (2005) Molecular characterization of two soybean infecting begomoviruses from India and evidence for recombination among legume infecting begomoviruses from Southeast Asia. *Virus Research* 108: 167–176.

Green SK and Kim D (eds.) (1992) *Mungbean Yellow Mosaic Disease. Proceedings of an International Workshop*, Bangkok, Thailand, 79pp. Taipei: AVRDC.

Malathi VG, Surendernath B, Naghma A, and Roy A (2005) Adaptation to new host shown by the cloned components of Mungbean yellow mosaic India virus causing golden mosaic in northern India. *Canadian Journal of Plant Pathology* 27: 439–447.

Malik PS, Kumar V, Bagewadi B, and Mukherjee SK (2005) Interaction between coat protein and replication initiation protein of Mungbean yellow mosaic India virus might lead to control of viral DNA replication. *Virology* 337: 273–281.

Mandal B, Varma A, and Malathi VG (1997) Systemic infection of *Vigna mungo* using the cloned DNAs of the blackgram isolate mungbean yellow mosaic geminivirus through agroinoculation and transmission of the progeny virus by whiteflies. *Journal of Phytopathology* 145: 503–510.

Nariani TK (1960) Yellow mosaic of mung (*Phaseolus aureus* L.). *Indian Phytopathology* 13: 24–29.

Nene YL (1968) A survey of the viral diseases of pulse crops in Uttar Pradesh. In: *First Annual Report, FG-In-358*, Pantnagar, India: Uttar Pradesh Agricultural University,

Shivaprasad PV, Akbergenov R, Trinks D, *et al.* (2005) Promoters, transcripts and regulatory proteins of Mungbean yellow mosaic geminivirus. *Journal of Virology* 79: 8149–8163.

Stanley J, Bisaro DM, Briddon RW, *et al.* (2005) Geminiviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, San Diego, CA: Elsevier Academic Press.

Trinks D, Rajeswaran R, Shivaprasad PV, *et al.* (2005) Suppression of RNA silencing by a geminivirus nuclear protein AC2 correlates with transactivation of host gene. *Journal of Virology* 79: 2517–2527.

Usharani KS, Surendranath B, Haq QMR, and Malathi VG (2004) Yellow mosaic virus infecting soybean in northern India is distinct from the species infecting soybean in southern and western India. *Current Science* 86: 845–850.

Varma A, Dhar AK, and Mandal B (1992) MYMV-transmission and its control in India. In: Green SK and Kim D (eds.) *Mungbean Yellow* Mosaic *Disease. Proceedings of an International Workshop*, pp. 1–25. Bangkok, Thailand, Taipei: AVRDC.

Murine Gammaherpesvirus 68

A A Nash and B M Dutia, University of Edinburgh, Edinburgh, UK

© 2008 Elsevier Ltd. All rights reserved.

Origins and Ecology

Murine gammaherpesvirus 68 (MHV-68) belongs to species *Murid herpesvirus 4*, genus *Rhadinovirus*, subfamily *Gammaherpesvirinae*, family *Herpesviridae*. It was originally isolated from a bank vole (*Clethrionomys glareolus*) in Slovakia. Two other related herpesviruses (MHV-60 and MHV-72) also came from bank voles and two more (MHV-76 and MHV-78) from wood mice (*Apodemus flavicollis*). The five viruses were originally isolated following the inoculation of diluted suspensions of various tissues (lung, spleen, liver, kidney, and heart) into the brains of newborn mice. Different virus isolates were obtained from the brains of mice following either the first, second, or third intracranial passage from mouse to mouse. These Slovakian viruses developed cytopathic effect in epithelial and fibroblast cell lines from a variety of species ranging from chickens to primates. One other isolate (MHV-Brest) was reported in a shrew (*Crocidura russula*). Subsequently, a number of new isolates have arisen primarily from wood mice following a survey of wild rodents in the Wirrell, Liverpool.

Virus Structure and Genetic Content

MHV-68 virion structure is similar to that of other herpesviruses. The capsid, tegument, and glycoprotein genes are homologous to those of other herpesviruses and can be predicted to fulfill the same roles in the MHV-68 virion. The composition of the MHV-68 capsid, tegument, and envelope has been investigated, revealing five capsid proteins (encoded by ORF25, ORF62, ORF26, ORF65, and ORF29), three tegument proteins (ORF75c, ORF45, and ORF11), five envelope proteins (ORF8, ORF51, ORF27, ORF28, and ORF22), and four proteins of undetermined locations (ORF20, ORF24, ORF48, and ORF52). The viral tRNA-like RNAs (vtRNAs), which are unique to MHV-68, are present in the tegument. Other structural proteins are predicted by analogy with other herpesviruses but their presence in the virion is yet to be demonstrated.

The genetic content of MHV-68 is based on the complete DNA sequences of MHV-68 strains g2.4 and WUMS. These are not independent strains, since the latter was derived by limited passage and plaque purification of the former. As a consequence, the two sequences are very similar (g2.4, GenBank accession number AF105037; WUMS, U97553). The proposed gene layout is shown in **Figure 1**.

The linear, double-stranded DNA genome of MHV-68 consists of a single unique region of 118 237 bp flanked by a variable number of 1213 bp terminal repeats. The unique region and terminal repeats have nucleotide compositions of 46 and 77.6% G+C, respectively. Two internal tandem repeats are located within the unique region: a 40 bp repeat located at genome coordinates 26 778–28 191 and a 100 bp repeat located at coordinates 98 981–10 1170.

The unique region of the MHV-68 genome is largely collinear with the genomes of other rhadinoviruses, including Kaposi's sarcoma-associated herpesvirus (KSHV) and herpesvirus saimiri. It contains 73 protein-coding open reading frames (ORFs) and also encodes eight vtRNAs and nine predicted microRNAs (miRNAs). As with other rhadinoviruses, MHV-68 possesses a number of cellular homologs, including a complement regulatory protein (CRP, encoded by ORF4), a Bcl-2 homolog (vBcl-2, M11), a cyclin D homolog (vcyclin, ORF72), and a G protein-coupled receptor (vGPCR, ORF74).

MHV-68 also contains a number of unique genes clustered at the left end of the genome. These include four genes encoding proteins (M1, M2, M3, and M4) and eight genes encoding the vtRNAs and miRNAs. In MHV-76, the first 9.5 kbp of the genome, containing M1–M4 and the vtRNAs and miRNAs, is absent;

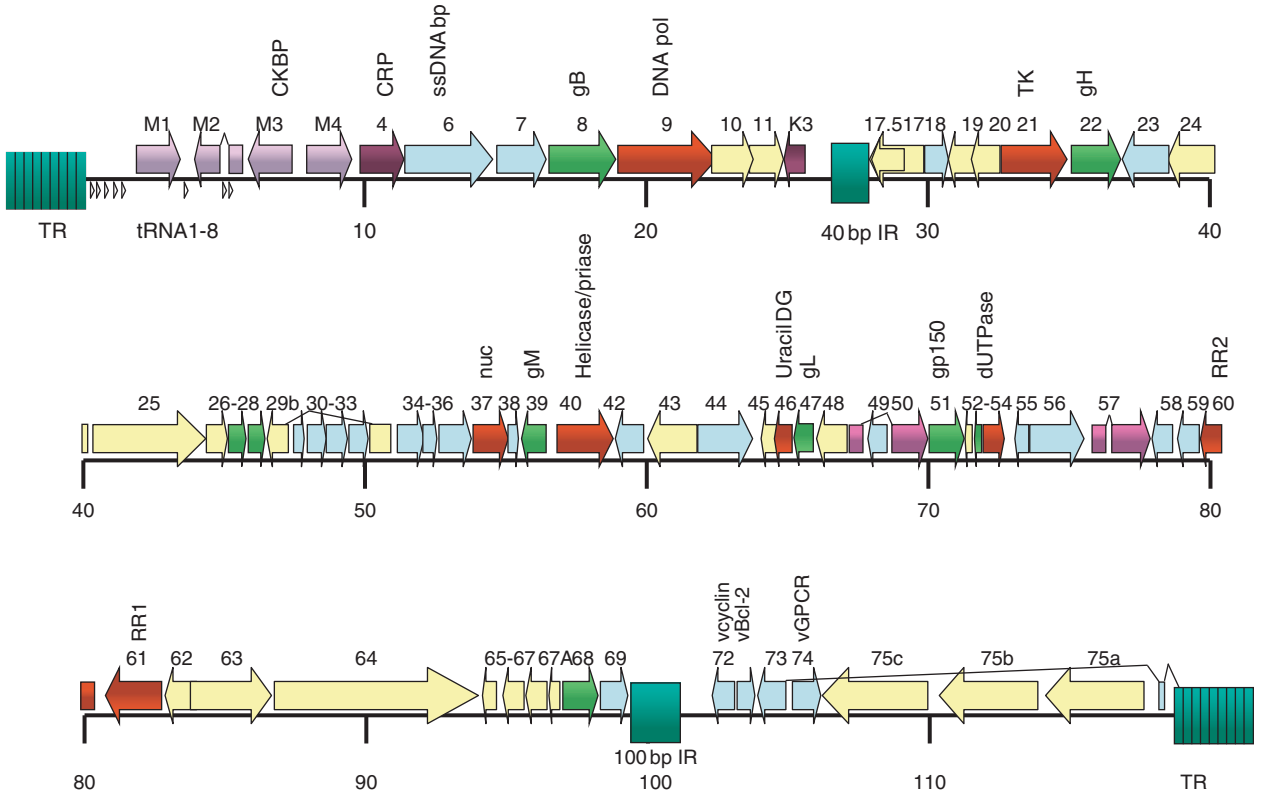


Figure 1 Organization of the MHV-68 genome. Yellow color indicates structural proteins; light green, glycoproteins; red, enzymes; violet, transcriptional transactivators; lavender, unique MHV-68 genes ; plum, immunomodulatory proteins; and dark green, repeats. TR, terminal repeat; IR, internal repeat; CKBP, chemokine-binding protein; CRP, complement regulatory protein; TK, thymidine kinase; nuc; alkaline exonuclease; uracil DG, uracil deglycosylase; RR, ribonucleotide reductase; vGPCR, viral G protein-coupled receptor.

otherwise the genome is identical to that of MHV-68. MHV-72 is deficient in the first 7 kbp of the genome and lacks M1–M3 and the vtRNAs and miRNAs; otherwise the genome is identical to that of MHV-68. It is not known whether MHV-76 and MHV-72 are deletion mutants of MHV-68 that arose during isolation or whether they exist naturally. MHV-Brest is closely related to MHV-68 but appears to represent a different virus species. The sequence of the left end region is particularly highly conserved between MHV-Brest and MHV-68, with greatest divergence in M2.

Infection and Replication

MHV-68 is able to infect and replicate in a range of mammalian epithelial and fibroblast cell lines. A productive infection occurs in the majority of cell lines, but a persistent infection is established in some lymphoid cells. MHV-68 establishes a latent infection in NS0, a myeloma cell line, but not in the thymoma cell line BW5147. The virus is maintained indefinitely in NS0 cells as a latent infection, with approximately 5% of the cells undergoing reactivation and expressing lytic cycle proteins. This scenario is similar to that seen for lymphoblastoid cell lines infected with Epstein–Barr virus (EBV). Other B cell lines can also be infected, including B cell hybridomas and the commonly used A20 cell line. One latently infected B cell line, S11, has been derived from a lymphoma obtained from a MHV-68-infected mouse.

Many MHV-68 genes have been assigned functions based on their orthology to genes of other herpesviruses whose roles are known. The use of signature-tagged transposon mutagenesis has resulted in the identification of a number of MHV-68 genes that are essential for the replication process, as well as several genes that are not essential but significantly enhance viral replication. So far, 41 genes have been shown to be essential for viral replication, of which 17 are essential for replication in all herpesviruses, including MHV-68 ORF6 (encoding the single-stranded DNA-binding protein, ssDNA bp), ORF8 (glycoprotein B, gB), ORF9 (DNA polymerase, DNA pol), ORF22 (glycoprotein H, gH), and ORF64 (large tegument protein). Some essential genes have homologs only within the gammaherpesviruses, including ORF45, which corresponds to the KSHV IRF7-binding protein.

MHV-68 transcription occurs in a temporal fashion and is detected from 3 h post infection (p.i.) *in vitro*. The replication genes ORF6 (ssDNA bp), ORF9 (DNA pol), ORF60 (ribonucleotide reductase subunit 2, RR2), and ORF61 (RR1) are transcribed with similar kinetics, with a defined early peak of transcription. ORF57, a conserved herpesvirus protein involved in RNA transport and stability that can also act as a transcriptional transactivator, shows similar kinetics. Other genes can be clustered into three general expression patterns. One exhibits a peak in transcript levels at 5 h p.i., followed by a gradual decrease thereafter, and includes ORF50 (Rta), ORF37 (alkaline exonuclease, nuc), and glycoprotein L (gL). The second group has a peak at approximately 8 h p.i., and includes the structural genes ORF25 (major capsid protein), ORF33 (a tegument protein), ORF38 (a tegument-associated membrane protein), and ORF51 (envelope glycoprotein gp150), as well as several genes of unknown function (ORF20 and ORF52). The final group shows a peak of transcription at 12 h p.i., and includes a second group of structural proteins such as ORF19 (a tegument protein), ORF66 (a capsid protein), ORF68 (a glycoprotein), and ORF65 (a capsid protein). From this temporal analysis of viral gene expression, it is possible to build up a profile of the events that take place during MHV-68 replication.

Along with the essential replication proteins, a number of nonessential genes are also expressed during infection *in vitro*. The M4, K3, and ORF73 genes are expressed with immediate early kinetics, while genes with cellular homologs (encoding vcyclin, vBcl-2, and vGPCR) are expressed with early–late kinetics. The early–late group also includes the abundantly expressed M3 gene, which encodes a chemokine-binding protein.

New virions are assembled in a manner similar to that observed for herpes simplex virus type 1. MHV-68 can exit the cell into the extracellular space or can directly infect adjacent cells. ORF51 (encoding gp150) is necessary for viral egress into the extracellular space, whereas ORF27 (gp48) has been identified in the process of egress directly into adjacent cells. gp48 must be localized to the plasma membrane for efficient movement of virions into new cells, a process that requires the ORF58 protein.

Expression of Unique Viral Genes

The unique MHV-68 genes (M1–M4 and the vtRNA genes) are important determinants of pathogenicity. They are absent from the deletion mutant MHV-76, which replicates with the same efficiency as MHV-68 *in vitro* but is attenuated *in vivo*. The functions of these genes have been the subject of intense study but currently they are not fully understood. The M1, M3, and M4 protein sequences are related to each other, but similar proteins have not been detected in sequence databases. Similarly, the M2 protein is not related to known proteins. The M1, M3, and M4 proteins are secreted from infected cells. Studies with mutant viruses have shown that M1 is involved in reactivation from latent infection and in pathogenesis in the lung. M4 is an immediate early gene involved in the establishment of latent infection. M3 encodes an abundant protein that is expressed early in the lytic cycle and found during productive infection and establishment of a latent infection. It is an important

determinant of pathogenesis during the acute stages of infection but does not appear to be transcribed during long-term latent infection. It is secreted from cells in large quantities and has a high affinity for specific members of the chemokine family, binding to all classes of chemokines (CC, CXC, C, and CX3X) and functionally inhibiting the ability of chemokines to signal through host G protein-coupled receptors.

M2 is a B-cell-specific gene that is involved in acute latent infection. It encodes a membrane-associated protein required for efficient establishment of latency and control of latent infection in B cells. In its absence, there is a defect in memory B-cell latency and increased long-term latency in germinal center B cells. M2 is likely to play a multifunctional role in MHV-68 infection and potentially interacts with a number of cellular proteins. It binds the guanine nucleotide exchange factor vav, altering the normal lymphocyte signaling process and inhibiting B cell receptor-induced cell-cycle arrest and apoptosis. M2 interacts with the DDB1/COP9/cullin repair complex and the ATM DNA damage transducer, blocking DNA damage-induced apoptosis. There is also evidence that M2 inhibits the cellular response to interferon (IFN) by binding to Stat1/Stat2 proteins.

The vtRNAs are predicted to fold into typical cloverleaf structures and, like cellular tRNAs, are thought to be transcribed by RNA polymerase III since the appropriate promoter elements are present within the genes. Following cleavage of excess nucleotides at both the 5′ and 3′ termini, the transcripts are post-transcriptionally modified by the addition of a 3′ CCA sequence, but are not aminoacylated. Only one vtRNA, vtRNA5, contains all the variant or semivariant bases present in mammalian tRNA sequences. The vtRNAs are expressed during lytic and latent infection and have been used widely as a marker for latent infection *in vivo* as their high level of expression means they are readily detectable by *in situ* hybridization. Their function is unknown. The primary vtRNA transcripts also encode miRNAs. The miRNAs are processed and expressed in infected cells, but no information is available on their specificity and function or on their expression *in vivo*. They are currently the only miRNAs known to be transcribed by polymerase III, and their association with the vtRNAs is intriguing.

Entry and Spread within the Host

The natural route of infection is uncertain, but by analogy with other animal gammaherpesviruses the respiratory tract is likely to be a primary route. Introduction of virus intranasally into inbred mice leads to a productive infection of alveolar epithelial cells, resulting in bronchiolitis. During MHV-68 infection, inflammatory responses in the lung evolve slowly compared to the response seen in MHV-76 infection, where a rapid inflammatory cell infiltrate occurs. This difference could be attributed to the chemokine-binding activity of the M3 protein in MHV-68 delaying the onset of inflammation. During MHV-68 infection, the evolution of the inflammatory response includes an initial wave of macrophages, peaking at day 3, followed by a wave of CD8 T cells peaking at day 7. Inflammation resolves by the second week, although in some mice focal accumulations of mononuclear cells are seen in the lung as late as day 30. In fact, the lung continues to be a site of virus persistence for the life of the infected animal. This indicates a potential role for the lung as a major site for virus transmission.

From the lung, the virus enters the local lymph node (mediastinal lymph node, MLN). Here, dendritic cells and macrophages are initially infected, followed by infection of B cells. Evidence supporting a role for dendritic cells in this process comes from studies using an MHV-68 recombinant expressing green fluorescent protein, in which infected cells are tracked in normal and in B cell-deficient (μMT) mice. In the presence and absence of B cells, virus is detected in CD11c-positive dendritic cells and F4/80-positive macrophages. Infection appears to be transient in the absence of B cells and there is little or no viremic phase. The MLN is considered to be the primary site for B-cell infection and B cells the principal cell population responsible for disseminating virus within the host. Tropism of MHV-68 for B cells may be related to the presence of gp150 on the virion envelope. Experiments using tagged gp150 have demonstrated binding to $CD19^+$ (B cells) and to some $CD19^-$ spleen cells. However, there was no interaction between gp150 and murine epithelial cells. During the first week of infection in the MLN, B cells undergo a rapid expansion accompanied by an increase in the number of latently infected B cells against a background of lymphadenopathy. From the MLN, infected B cells traffic to the spleen and other lymphoid tissues. By the second week of infection, a similar rapid expansion of latently infected B cells is observed in the spleen, concomitant with appearance of splenomegaly. The number of latently infected cells increases from $1/10^7$ to $1/10^4$ spleen cells in the space of a few days, and then the numbers decline to between $1/5x10^5$ and $1/10^6$ by the third or fourth week of infection.

B-cell proliferation, and hence the number of latently infected cells observed during lymphadenopathy and splenomegaly, is controlled by CD4 T cells. This indicates that the virus exploits T–B cell collaboration to its advantage, for example, by utilizing B-cell proliferation as a means to maximize the number of latently infected B cells. However, the virus does not depend completely on this strategy, since in the absence of CD4 T cells a long-term latent infection is still established, suggesting that the virus has the capability directly to

manipulate B-cell growth and differentiation in order to establish a latent infection (a possible role for the M2 protein). A remarkable feature of MHV-68 latency is the constant number ($1–2/10^6$ latent cells) found in the spleen for the life of the animal. This number is established whether or not CD4 T cells are present in the host.

The presence of B cells and CD4 T cells is absolutely required for the evolution of splenomegaly, which suggests that cognate interactions occur between these cells similar to those for any other antigenic response. During splenomegaly, there are large increases in both B- and T-cell populations. Germinal centers increase in number and size and act as the principal location for latently infected cells. By the third week of infection there is an increase in the number of circulating lymphocytes, dominated by $V\beta4^+$ CD8 T cells. This phase of infection is similar to the infectious mononucleosis caused by EBV. The mechanism for this selective increase in Vβ4 usage is not known, but indicates a form of super-antigen-driven proliferation. A summary of the processes involved in entry and spread is shown in **Figure 2**.

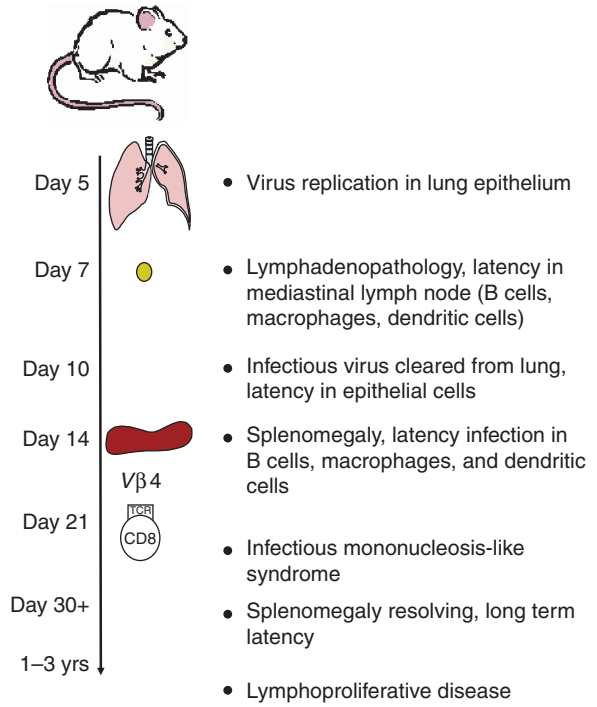


Figure 2 Pathogenesis of MHV-68 infection following intranasal infection. Virus replicates in lung epithelium before spreading to the mediastinal lymph nodes (MLN) which drain the lung. Latent infection is established in the MLN and the virus trafficks throughout the lymphoid system causing transient splenomegaly and expansion of Vβ4+ CD8 T cells. After about 30 days, asymptomatic long-term latency is established. At later times, infection may lead to lymphoproliferative disease.

Host Factors Influencing Infection

The age and immunological status of animals influence the spread of infection and tissue tropism. Infection of young (2–3-week old) or immunocompromised mice results in spread of virus via the bloodstream to a number of tissues, resulting in a productive infection. These include the heart, kidney, liver, adrenal gland, and peripheral nervous system (e.g., trigeminal ganglion). The central nervous system can also become infected when virus is introduced intracranially. Both glial and neuronal cells are observed to undergo a productive infection. These data argue in favor of a promiscuous virus infection, which is supported by observations of the infection of peritoneal exudate cells and epithelial cells in the gut.

Molecular and Cellular Basis of Latency

Viral gene expression changes dramatically from infection in the lung to infection in the spleen. As the virus enters into the latent state, there is a progressive shutdown of gene expression. In MHV-68 infection, this depends to a large extent on the cell type infected. Four cell types have been implicated in maintaining the latent state: B cells, macrophages, dendritic cells, and epithelial cells in the lung. Different populations of B cells are involved in the latent infection. Naive B cells are the initial source of latent virus and, as these cells differentiate following signals from CD4 T cells, they form Germinal Centre B cells (a major site of latency), which may undergo isotype switching and develop into plasma cells and memory B cells, another source of latent infection. Epithelial cells probably represent the main site of persistence, based on the evidence from wood mice where virus is readily isolated from lung tissue, indicating that the respiratory tract may be the principal site of virus shedding.

A number of genes have been linked with MHV-68 latency. These include the vtRNA genes, M2, M3, M4, K3, ORF72 (vcyclin), ORF73, ORF74 (vGPCR), M11 (vBcl-2), and ORF65.

As discussed above, M2 is important for the establishment and maintenance of the initial phases of B cell latency in splenic follicles. However, long-term latency is maintained in the absence of M2 expression. Many of the other genes associated with latency do so by improving the efficiency of the latent infection and/or the reactivation of virus from latency. An exception is ORF73, which is essential for the establishment of MHV-68 latency *in vivo*. The encoded protein is conserved among the gammaherpesviruses and shares with KSHV LANA-1 structural and sequence homology and similar functions such as the ability to tether the viral episome to chromatin

to enable the viral genome to be carried to daughter cells during mitosis. Another key function of the ORF73 protein is in inhibiting the activity of Rta, a replication and transcription activator encoded by ORF50, which is a key protein in the reactivation of virus from latency. The balance between the ORF73 protein and Rta is pivotal in defining whether latency or reactivation to a productive infection prevails.

Pathogenesis

Lymphoproliferative disorders are a feature of gammaherpesvirus infection. In MHV-68 infection, a spectrum of disorders of increasing severity occurs, with splenomegaly at one end and lymphomas at the other. The development of lymphoproliferative disease is dependent on mouse strain and host immune status. Lymphomas have been reported in BALB/c mice infected for periods of 9 months or longer (median 14 months). In one study, approximately 10% of infected mice developed tumors in both lymphoid and nonlymphoid tissue (lung, liver, kidney, and heart), of which 50% were classified as high-grade lymphomas. In a separate investigation, the frequency of mice with tumors increased to over 50% following treatment with the immunosuppressive drug, cyclosporin A. The tumors in both experiments were of mixed cell phenotype with CD3+ T cells interspersed among B220+ B cells. The B cells were either kappa or lambda light chain restricted, suggesting a clonal origin of the B-cell population. MHV-68 DNA-positive lymphocytes were found interspersed in the tumor cell mass or on the fringes of lymphomas. In some animals, the number of virus-positive cells was low, whereas in others there were huge numbers of genome-positive cells. The infected cells were not positive for lytic cycle proteins, suggesting that virus reactivation was not occurring in these mice.

BALB/c mice infected with MHV-72 also develop tumors with a frequency similar to MHV-68. The number of tumor-bearing mice increased following immunosuppression with the antifungal agent, FK-506. In 5 of 13 neoplasia-positive mice, virus was isolated directly from the tumors. Lymphoproliferative disease in BALB/c mice has also been reported for MHV-60 and another Slovakian isolate, MHV-Sumava.

BALB/c mice lacking β2-microglobulin (β2m) develop lymphomas and an atypical lymphoid hyperplasia (ALH), with lymphocytosis resembling EBV-associated post-transplant lymphoproliferative disease at a higher rate than wild type BALB/c mice. A total of 67% of the BALB/c β2m mice developed lymphoproliferative disease compared to 22% of mock-infected controls. MHV-68 infected cells were common in the ALH cells but, again, scarce in the lymphomas, suggesting that, while MHV-68 may instigate lymphomagenesis, continued presence of the virus is not necessary in transformed cells.

A number of B cell lines have been established from MHV-68-infected tumor-positive mice, of which S11 is the best characterized. This immunoglobulin M-positive, major histocompatibility complex class II-positive B cell line harbors the virus in a latent form as demonstrated by the presence of a circular genome, a marker of latent infection. As with lymphoblastoid cell lines derived from EBV infection, S11 has around 2–5% of cells expressing lytic antigens. A transcript analysis of S11 revealed that vtRNA and M2, but not M3, were expressed in virtually all latently infected cells. S11 establishes tumors when transferred to nude mice, and has been used to dissect the immunological mechanisms involved in targeting tumor cell growth. In a series of adoptive transfer experiments of MHV-68-specific CD8 and CD4 T cells into S11 tumor-bearing nude mice, regression of tumor cell growth was effectively achieved by CD4 T cells but surprisingly not with CD8 T cells.

The molecular basis for tumor cell induction is not known. Cell lines adapted from lymphomas in mice have multiple chromosome rearrangements, and in the situation where viral DNA was detected in such cells, it is tempting to speculate that virus could initiate tumorigenesis by a hit-and-run mechanism. A number of candidate viral genes could initiate cell transformation, including ORF72 (encoding vcyclin), ORF74 (vGPCR), and M11 (vBcl-2). Transgenic mice expressing vcyclin under the control of the lck promoter, which is active early in thymocyte development, showed increased numbers of immature thymocytes. High-grade lymphoblastic lymphomas developed in the thymuses of 45% of these mice. These mice also show a decrease in the number of mature T cells and an increase in thymic apoptosis, supporting the notion that vcyclin may require the involvement of other factors to promote cell survival and tumor formation. Transfection of MHV-68 ORF74 into 3T3 cell lines leads to the establishment of stable transformed cells. These cells do not, however, develop into tumors in nude mice. vBcl-2 is expressed during the latent phase and is highly efficient at preventing cell death via such immunological mechanisms as tumor necrosis factor α and Fas–Fas ligand interaction. It therefore seems likely that *in vivo* a number of genes act in concert to promote cellular proliferation, survival, and tumor formation.

MHV-68 is also involved with the genesis of other pathologies. In young or immunologically compromised mice (e.g., deficient in the IFN-γ receptor), the virus is associated with arteritis, where persistently infected macrophages colonize the media of the major blood vessels, leading eventually to rupture of the arterial wall and cardiac arrest. Infection of mice deficient in an IFN-γ response results in fibrosis of lymphoid tissue, liver, and lung, from which the animals recover.

See also: Epstein–Barr Virus: General Features; Epstein–Barr Virus: Molecular Biology; Herpesviruses: Discovery; Herpesviruses: General Features; Herpesviruses: Latency; Kaposi's Sarcoma-Associated Herpesvirus: General Features; Kaposi's Sarcoma-Associated Herpesvirus: Molecular Biology; Simian Gammaherpesviruses.

Further Reading

Blaskovic D, Stancekova M, Svobodova J, and Mistrikova J (1980) Isolation of five strains of herpesviruses from two species of free living small rodents [letter]. *Acta Virologica* 24: 468.

Nash AA, Dutia BM, Stewart JP, and Davison A (2001) Natural history of murine gammaherpesvirus infection. *Philosophical Transactions of the Royal Society of London, Series B* 356: 569–579.

Speck SH and Virgin HW (1999) Host and viral genetics of chronic infection: A mouse model of gammaherpesvirus pathogenesis. *Current Opinion in Immunology* 16: 456–462.

Stevenson PG and Efstathiou S (2005) Immune mechanisms in murine gammaherpesvirus-68 infection. *Viral Immunology* 18: 445–456.

Sunil-Chandra NP, Efstathiou S, Arno J, and Nash AA (1992) Virological and pathological features of mice infected with murine gammaherpesvirus 68. *Journal of General Virology* 73: 2347–2356.

Virgin HW, Latreille P, Wamsley P, *et al.* (1997) Complete sequence and genomic analysis of murine gammaherpesvirus 68. *Journal of Virology* 71: 5894–5904.

Mycoreoviruses

B I Hillman, Rutgers University, New Brunswick, NJ, USA

© 2008 Elsevier Ltd. All rights reserved.

The 8th Report of the International Committee for the Taxonomy of Viruses (ICTV) which lists 12 genera of reoviruses that infect mammals, invertebrates, and plants, was the first to also include the fungus-infecting genus *Mycoreovirus*. Members of many of the reovirus genera in the 8th Report replicate in organisms representing more than one kingdom or phylum: for example, all plant reoviruses replicate in and persistently infect their insect vectors, and similarly many of the mammalian reoviruses replicate in their invertebrate vectors. Three fungal virus species have been confirmed to be members of the genus *Mycoreovirus* of the family *Reoviridae*. Two of these viruses were isolated from the filamentous ascomycete fungus *Cryphonectria parasitica,* and the other is from the soil-borne fungus *Rosellinia necatrix*, also an ascomycete but representing a different fungal family.

All of the mycoreoviruses cause disease in their infected hosts, resulting in greatly reduced virulence, reduced growth in culture, reduced laccase accumulation, and reduced sporulation relative to wild-type, virus-free cultures of the same genetic background (**Figure 1**). The mycoreoviruses are most closely related to the genus *Coltivirus* of tick-borne reoviruses (**Figure 2**). Both Colorado tick fever virus (CTFV) and the closely related Eyach virus (EyaV) cause mammalian diseases, and both viruses replicate in their arthropod vectors as well as in their mammalian hosts. The coltiviruses are not well understood at the molecular level and so have shed only a little light on the study of mycoreoviruses. The genomic sequences of both CTFV and EyaV have been published, and they have been found to be more closely related to the genus *Orthoreovirus*, which includes the common human pathogen mammalian reovirus (MRV), than to members of the other two genera of the family *Reoviridae* (*Orbivirus* and *Rotavirus*) that have been well studied at the structural and molecular levels.

Filamentous fungi are valuable subjects for investigation of eukaryotic viruses. This has been especially true for ascomycetes, which usually are haploid throughout their vegetative phases and thus readily amenable to tools of classical genetics and to relatively simple gene knockout and knockdown experiments. *Cryphonectria parasitica* has been exceptional as a host for examination of fungal viruses. The fungus caused the greatest pandemic of a tree species in recorded history, killing 3–4 billion American chestnut trees. The fungus is very stable in culture, showing a consistent morphology upon continued maintenance and subculture, is easily transformed and transfected, and can be examined through classical genetics. Because of its historical importance as a pathogen and the potential for its control using natural or genetically engineered viruses, more viruses that result in stable morphological changes and/or changes in virulence have been identified and characterized in this fungus than in any other.

Structure–Function Relationships

Relatively little is known of the details of mycoreovirus structure, but all indications from electron micrographs of

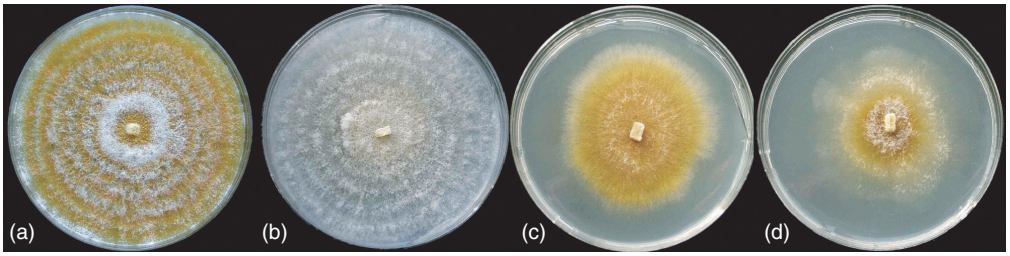


Figure 1 Morphologies of isogenic *Cryphonectria parasitica* colonies infected with three mycoviruses. (a) Uninfected colony, strain EP155; (b) strain EP155 infected with hypovirus CHV-1/EP713; (c) strain EP155 infected with mycoreovirus MyRV-1/Cp9B21; (d) Strain EP155 infected with mycoreovirus MyRV-2/CpC18.

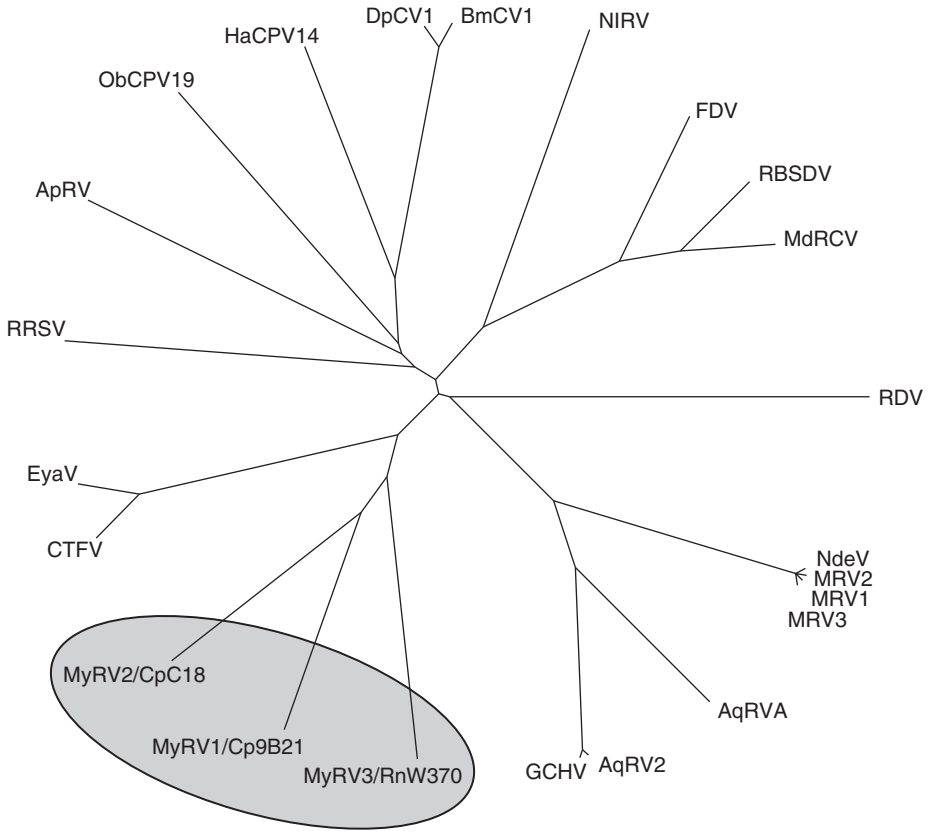


Figure 2 Unrooted neighbor-joining tree based on ClustalW alignments of complete deduced amino acid sequences of RNA-dependent RNA polymerase genes of the reoviruses most closely related to the mycoreoviruses (shaded).

negatively stained virus particles and genome sequence analysis are that it belongs to the orthoreovirus subgroup. The orthoreoviruses are distinct from the orbiviruses and rotaviruses in that they have identifiable pentameric turrets on top of each fivefold axis of the core particle, and these have been demonstrated to be involved in capping of nascent mRNA. The protein that was experimentally demonstrated to be the mycoreovirus guanylyltransferase is homologous to turret proteins of orthoreoviruses that have the same function, supporting the grouping of mycoreoviruses within the orthoreovirus subgroup of the *Reoviridae*.

Relatively little is known about the movement of fungal viruses in general within the mycelium and during horizontal or vertical virus transmission. Viruses that have been investigated are generally found in hyphal tip cells and move with the growing mycelium; however, location of mycoreoviruses within the mycelium has not been investigated. The steps in the infection process of the mammalian orthoreovirus have been investigated in considerable detail. Virus entry into cells via vesicles occurs by receptor-mediated endocytosis, whereupon the μ1 protein, which is myristoylated at its N-terminus and inserted into the inner membrane of the vesicle cleaves

autocatalytically, resulting in pore formation in the vesicle membrane and subsequent virus release into the cytoplasm. Both of the *C. parasitica* mycoreoviruses have homologs of the orthoreovirus μ1 protein, with strong myristoylation signals at the amino termini of the corresponding proteins and putative sites for autocatalytic cleavage. This suggests that a similar mechanism of egress from vesicles is a component of the infection cycle of these reoviruses. Interestingly, although the Rosellinia virus, MyRV-3/RnW370, contains a homolog of the μ1 protein, it does not contain a glycine residue at the penultimate position of the N-terminus of this deduced protein, and based on its sequence it has a very low predicted probability of myristoylation. It does, however, contain the N/P putative cleavage motif at a similar position and in a similar environment as those in the other viruses.

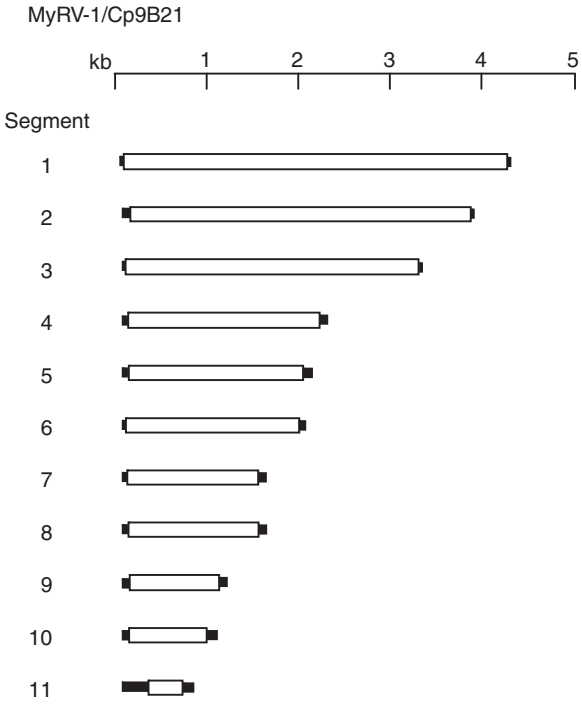
It is relatively easy to cure *C. parasitica* and *R. necatrix* from their associated mycoreoviruses. In *C. parasitica*, single asexual spores are usually virus free, and in *R. necatrix*, cultures initiated from excised hyphal tips (the terminal 2–8 cells) are also virus free. These are in contrast to cultures infected with the well-studied hypovirus of *C. parasitica,* cryphonectria hypovirus-1 (CHV-1), in which most or sometimes all conidia contain virus and the virus-infected cultures cannot be cured by hyphal tip isolation. It is likely that details of mycoreovirus movement within the mycelium as well as horizontal and vertical transmission are quite different from those properties of hypoviruses, which have no capsid protein, are more closely related to positive-sense single-stranded RNA viruses such as plant potyviruses and animal picornaviruses, and whose replication is associated with the fungal *trans*-Golgi network.

Taxonomy and Nomenclature

Naming of fungal viruses is often problematic because infectivity studies are very difficult with fungal viruses and have not been done with most. In nature, fungal viruses do not exit completely from an infected isolate and enter an uninfected one exogenously. Instead, they move from one isolate to another only after the hyphae of the two isolates have fused (anastomosed), in which case the contents of one or usually more cells from the infected and uninfected isolate are mixed. Mixed infections are common and symptomless infections are the norm for fungal viruses. For these reasons, fungal virus names usually contain reference not only to the host genus and species, but to the host strain of origin as well. The genus name *Mycoreovirus* was a natural one to use because it is descriptive . For the sake of simplicity and consistency with other viral nomenclature, species are numbered progressively as they are described. The fungal species and isolate in which a particular virus was identified is provided after the species number. In this system, the first mycoreovirus species described was designated *Mycoreovirus-1.* The only isolate of this virus species identified to date was from *C. p*arasitica strain 9B21, so the virus is designated mycoreovirus-1/Cp9B21 or MyRV-1/Cp9B21. The reason that MyRV-2 represents a separate virus species even though the two were isolated from the same fungal species and only a few miles away is because it shares much less sequence similarity at both the nucleotide and amino acid levels (<50%) than expected for two viruses in a single species. Surprisingly, both of these species are monotypic: these two virus isolates, each representing a different species, are the only two mycoreoviruses isolated from *C. parasitica*, even though thousands of isolates infected with members of the family *Hypoviridae* have been identified worldwide. In contrast, dsRNA or virus from dozens of isolates of *Rosellinia necatrix* from different parts of Japan have been isolated, but all are closely related to each other, indicating that they represent strains of a single virus species, MyRV-3.

Genome Structures, Organizations, and Relationships

The three fungal reoviruses examined to date appear to have 11 segments of dsRNA that are required for infection. Isolates of one of the viruses, MyRV-3, have been found to have either 12 or 11 segments (see below). There is significant sequence similarity among the larger segments of the three mycoreovirus species and between homologous segments of the mycoreoviruses and their closest relatives, the coltiviruses, but this similarity becomes less apparent in the middle segments and is not apparent at all in the small segments. This feature is common with other members of the family *Reoviridae*, in which the more distant relationships are generally revealed in only the large segments. Each of the 11 required segments of mycoreoviruses appears to contain a single open reading frame. **Figure 3** Segments 1–5 of the mycoreoviruses are homologous to segments 1–5 of the coltiviruses (segments 4 and 5 of MyRV2-CpC18 are transposed relative to the others). Based largely on similarity with other reoviruses, their predicted functions are: S1: RNA-dependent RNA polymerase (core protein); S2: dsRNA-binding (core protein); S3: guanylyltransferase (turret protein); S4: Myristoylated membrane penetration protein (outer capsid); S5: cytoskeleton-interacting (core). Segment 6 of the mycoreoviruses is predicted to encode a nucleic acid binding core protein and is homologous to segment 10 of the two coltiviruses. Segment 7 of MyRV-1/Cp9B21 encodes a proline-rich protein with similarity to several viral and nonviral proline-rich domains. Homologies among the smaller mycoreovirus proteins (from S8–11 or 12) and other reovirus deduced proteins, including those of coltiviruses, are unknown.



Segment	Size, nt	ORF aa	ORF kDa	Putative function
1	4127	1354	151.8	RdRp
2	3826	1238	138.5	Rep. assoc.
3	3258	1065	120.8	Guanylyltransferase; methyltransferase?
4	2269	720	79.8	Major capsid? Myristoylated
5	2023	647	72.8	Similar to coltivirus VP5
6	2056	649	73.4	NTP-binding core protein
7	1536	481	54.1	Proline rich, leucine zipper
8	1539	469	51.2	?
9	1072	297	32.9	Similar to MyRV-3/RnW370 VP11
10	975	247	27.8	?
11	732	101	11.5	?

Figure 3 Diagram of the genome segments of mycoreovirus-1/Cp9B21 shown with segment size, deduced open reading frame (ORF) in number of amino acid residues and predicted protein product size, and putative protein function. Only the guanylyltransferase function of segment 3 has been demonstrated experimentally.

One of the indications of close relationship among reoviruses is conserved terminal sequences. This also is a good indication of whether or not pseudorecombinants can be generated by co-infection with two related viruses. In the case of the mycoreoviruses, the three species have different conserved terminal sequences, consistent with their taxonomic separation. Furthermore, although co-infection of a single colony with the two reoviruses has been achieved, pseudorecombinants have not yet been recovered from these doubly infected isolates.

Mycoreovirus-1/Cp9B21

Although MyRV-1/Cp9B21 was not the first of the two *Cryphonectria* reoviruses identified, fungal cultures infected with this virus proved to be more stable and easily studied than those infected with MyRV-2/CpC18 (see below), so early molecular investigations have focused on this virus. When grown on solid media in petri dishes (e.g., a defined complete medium or potato dextrose agar (PDA)), MyRV-1/Cp9B21-infected colonies are deep orange in color and have little aerial hyphae compared to their uninfected counterparts. As with other *C. parasitica* viruses, the phenotype of the infected culture has very little to do with the host isolate but is determined almost entirely by the virus.

Fungal isolates infected with MyRV-1/Cp9B21 are much less virulent than uninfected isolates, and are among the most debilitated of any virus-infected *C. parasitica* cultures studied to date. Although the virus accumulates to reasonably high concentrations in infected colonies, it is transmitted very poorly through conidia, at rates of only 2–5%. This may account in part for the rarity of the virus in nature.

Recently, double infections of the well-characterized hypovirus CHV-1/EP713 and MyRV-1/Cp9B21 were examined. In these analyses, it was found that presence of the hypovirus increased both the concentration and vertical transmission rate through conidia of the reovirus, but that the reovirus did not affect the concentration or transmission rate of the hypovirus. Furthermore, transgenic expression of only the hypovirus protein p29 also resulted in increased accumulation and transmission of MyRV-1/Cp9B21. This is consistent with the prediction that p29 serves as a suppressor of RNA silencing (interference) during hypovirus infection, and that this effect acts *in trans* to support enhanced mycoreovirus replication.

Expression of MyRV-1/Cp9B21 Gene Products

Functional analysis of the MyRV-1/Cp9B21 genome was initiated by cloning the 11 individual segments into a baculovirus expression vector and expressing them in

insect cells. All 11 segments were expressed, resulting in 11 identifiable protein products on polyacrylamide gels. Only one of the proteins, the segment 3 product, has been studied functionally. This protein was found to be active in autoguanylylation assays, confirming it as the viral guanylyltransferase. Deletion and site-directed mutational analysis determined that the amino acid sequence EPAGY**H**PRPSIVVP**H**YFVFR constituted the catalytically active site of the MyRV-1/Cp9B21 guanylyltransferase. The Hx_8H motif was identified as absolutely conserved in all three members of the genus *Mycoreovirus*, as well as in the structurally related genera *Coltivirus*, *Orthoreovirus*, *Aquareovirus*, *Cypovirus*, *Dinovernavirus*, *Oryzavirus*, and *Fijivirus*. In all of the above genera in which the guanylyltransferase has been identified functionally, the Hx_8H motif has been found within the sequence. The core consensus sequence for the guanylyltransferase within this group of genera was a/vxxHxxxxxxxHhyf/lvf, with only the H residues being absolutely conserved.

Mycoreovirus-2/CpC18

The first virus that was tentatively identified as a mycoreovirus was MyRV-2/CpC18. Like many of the virus-infected *C. parasitica* cultures, the one that was found to contain this virus was isolated from a canker on an American chestnut tree. The circumstances of the discovery were somewhat unusual in that only one of 36 fungal isolates from that particular canker was virus infected; the rest were virus free. Although it is not unusual to find mixed fungal infections within a canker or to isolate virus-containing and virus-free cultures from one canker, the ratio of 1/36 is extraordinary. The phenotype of the infected culture, designated C-18 (the 18th isolate from canker C on that particular tree) was distinct from the phenotype of MyRV-1/Cp9B21-infected cultures described above in that it was light brown in color and had more aerial mycelium (**Figure 1**). The two viruses have very similar, dramatic negative effects on fungal virulence.

A major difference between the two *C. parasitica* mycoreoviruses is their stability and transmissibility. MyRV-2/CpC18 is easily lost upon subculture of the fungus, while this has never been observed with MyRV-1/Cp9B21. Furthermore, MyRV-2/CpC18 is extremely difficult to transmit from an infected isolate to an isogenic uninfected isolate by hyphal anastomosis, a property that is not seen with other *C. parasitica* viruses. It is presumed that these properties are related, but their reasons have not been elucidated. Both MyRV-1/Cp9B21 and MyRV-2/CpC18 have been transmitted to uninfected fungal isolates by inoculating *C. parasitica* protoplasts with purified virus particle preparations and allowing the protoplast to regenerate cell walls, form a hyphal network, and grow into a single colony. The ability to infect protoplasts efficiently using purified virus particle preparations is an interesting and useful feature of *C. parasitica* reoviruses. This has allowed for infection of different genotypes of *C. parasitica* regardless of vegetative incompatibility group and potential for transmission by hyphal anastomosis.

Mycoreovirus-3/RnW370

White rot is a root disease of fruit trees that can be limiting to production. Control of the fungus that causes the disease, *Rosellinia necatrix*, by chemical means is difficult and not economically feasible. Pathologists in Japan, where the disease is particularly severe, have sought to use virus-infected strains to control the disease, leading to the identification of several viruses. This plant/fungus interaction represents an interesting contrast to the chestnut/*C. parasitica* interaction. As a root disease, there are challenges and opportunities for biological control of a fungal pathogen with viruses that do not apply to aerial diseases. One of the viruses under investigation for biocontrol of *R. necatrix* is the mycoreovirus MyRV-3, which causes reduced virulence of the fungus. Unlike the monophyletic *C. parasitica* reoviruses, different strains of MyRV-3 have been isolated from a variety of *R. necatrix* strains from around Japan.

Of the *R. necatrix* viruses, the virus isolate that has been most thoroughly characterized is MyRV-3/RnW370. In surprising contrast to the *C. parasitica* viruses, MyRV-3/RnW370 was found to contain 12 rather than 11 segments. However, the presence of 12 segments is not a consistent feature of all MyRV-3 isolates. Examination of different virus-infected isolates of *R. necatrix* shows that they may have either 12 or 11 segments. Experiments to investigate virus composition and transmission have been performed on hyphal tip cultures from infected *R. necatrix* isolates, resulting in demonstration that these viruses behave like the *Cryphonectria* mycoreoviruses.

When only 11 segments are present in MyRV-3 isolates, segment 8 is the one that is absent from the full complement. With most reoviruses, sequence conservation among species and genera is evident in the larger segments, but much less so in the smaller segments, and this is true in the mycoreoviruses and related genera. Consistent with this general trend, sequence comparison between the 12 segments of MyRV-3 and the 12 segments of the two coltiviruses has suggested nothing about possible function of the apparently dispensable segment 8.

It is intriguing to think that perhaps segment 8 is vestigial for reoviruses in fungi and is required only in another host, past or present. This would be similar to the leafhopper-transmitted phytoreovirus, wound tumor virus (WTV), in which deletion mutations in any of three segments may be found upon successive serial, insect-free virus passage or long-term virus maintenance in plants, whereupon resulting mutant viruses become defective in their transmission

properties and incapable of replicating in their leafhopper vectors. An apparent major difference between MyRV-3 and WTV is that in MyRV-3 there is no evidence for remnants of segment 8; it appears to be either present or entirely absent. In the well-characterized mutants of WTV, the deleted segments are not completely gone, but shorter segments containing the two termini and varying amounts of adjacent sequence remain, ensuring that a total of 12 segments remain. Whether this represents a difference between the dsRNA segment sorting and packaging mechanisms of phytoreoviruses and mycoreoviruses is not known. In this line of inquiry, the close association of fungi with mites in natural settings may be significant to the evolutionary biology of mycoreoviruses: it may be no coincidence that their closest relatives are the tick-borne coltiviruses, with ticks and mites both in the arachnid subclass Acari. Unfortunately, mites are very difficult experimental subjects and cell cultures are not currently available. Furthermore, much less is known about coltivirus gene function than is known about many of the other members of the family *Reoviridae* that are pathogenic to humans, making it more difficult to pursue this line of research from a strictly bioinformatic standpoint.

Effects of Mycoreoviruses on Fungal Gene Expression

Considerable information has been amassed on the impact of CHV-1, a positive-sense RNA virus, on its fungal host. In contrast, studying the mechanisms of mycoreovirus infection of fungi is in its infancy. The first study addressing these questions was done by microarray analysis using mRNA isolated from isogenic fungal isolates of *C. parasitica* infected with either MyRV-1 or MyRV-2, and comparing results to the same strain that was uninfected or infected with either of two different CHV-1 strains, and with fungal mutants defective in virulence characteristics. To date, these experiments have been performed only on EST-based arrays representing ~20% of the total *C. parasitica* gene complement. Overall, there was consistency in the effects of the two *C. parasitica* mycoreoviruses on host gene expression: MyRV-1 infection resulted in differential expression of 6.5% of the genes on the array, whereas MyRV-2 infection affected expression of 5.8% of those genes. As might be expected based on their phenotypes, similar but distinct suites of genes were up- or downregulated in isogenic fungal isolates infected with the two reoviruses. Approximately 60% of the genes whose expression was affected were the same whether infection was by MyRV-1 or MyRV-2, and all but one of those genes were altered in the same direction. Some of these groups of genes are in common with those that are differentially regulated in cultures infected with the unrelated hypoviruses, but there are predictable differences. For example, hypovirus infection of *C. parasitica* results in female infertility, whereas mycoreovirus infection does not, and this is reflected in the expression of two genes predicted to be involved in the *C. parasitica* mating response. Both *mf2-1*, which encodes the fungal pheromone precursor, and *Csp12*, which encodes a homolog of the yeast *Ste12*-like transcription factor, were substantially downregulated in hypovirus-infected fungal isolates, which are female sterile, whereas there was much less effect on expression of these genes in either of the mycoreovirus-infected *C. parasitica* isolates. Virus is transmitted to ascospores at a rate of ~50% or less when the female parent is infected, but there is no virus transmission to ascospore progeny if the male parent in a mating is infected. Sequencing the complete genome of *Cryphonectria parasitica* is now underway. This will allow for the complete set of genes to be represented in an oligo array for more thorough investigation of differential gene expression.

See also: Coltiviruses; Fungal Viruses; Hypoviruses.

Further Reading

Enebak SA, Hillman BI, and MacDonald WL (1994) A hypovirulent *Cryphonectria parasitica* isolate with multiple, genetically unique dsRNA segments. *Molecular Plant-Microbe Interactions* 7: 590–595.

Hillman BI, Supyani S, Kondo H, and Suzuki N (2004) A reovirus of the fungus *Cryphonectria parasitica* that is infectious as particles and related to the *Coltivirus* genus of animal pathogen. *Journal of Virology* 78: 892–898.

Hillman BI and Suzuki N (2004) Viruses of *Cryphonectria parasitica*. *Advances in Virus Research* 63: 423–472.

Kanematsu S, Arakawa M, Oikawa Y, *et al.* (2004) A reovirus causes hypovirulence of *Rosellinia necatrix*. *Phytopathology* 94: 561–568.

Mertens P and Hillman BI (2005) Genus mycoreovirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 556–560. San Diego, CA: Elsevier Academic Press.

Nibert ML and Schiff LA (2001) Reoviruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 2nd ed., vol. 2, pp. 1679–1729. Philadelphia, PA: Lippincott Williams and Wilkins.

Nuss DL (2005) Hypovirulence: Mycoviruses at the fungal-plant interface. *Nature Reviews Microbiology* 3: 632–642.

Osaki H, Wei CZ, Arakawa M, *et al.* (2002) Nucleotide sequences of double-stranded segments from hypovirulent strain of the white root rot fungus *Rosellinia necatrix*: Possibility of the first member of the *Reoviridae* from fungus. *Virus Genes* 25: 101–107.

Supyani S, Hillman BI, and Suzuki N (2006) Baculovirus expression of the 11 *Mycoreovirus*-1 genome segments and identification of the guanylyltransferase-encoding segment. *Journal of General Virology* 88: 342–350.

Suzuki N, Supyani S, Maruyama K, and Hillman BI (2004) Complete genome sequence of *Mycoreovirus 1*/Cp9B21, a member of a new genus in the family *Reoviridae* isolated from the chestnut blight fungus, *Cryphonectria parasitica*. *Journal of General Virology* 85: 3437–3448.

Wei CZ, Osaki H, Iwanami T, Matsumoto N, and Ohtsu Y (2003) Molecular characterization of dsRNA segments 2 and 5 and electron microscopy of a novel reovirus from hypovirulent isolate, W370, of the plant pathogen *Rosellinia necatrix*. *Journal of General Virology* 84: 2431–2437.

Wei CZ, Osaki H, Iwanami T, Matsumoto N, and Ohtsu Y (2004) Complete nucleotide sequences of genome segments 1 and 3 of Rosellinia anti-rot virus in the family *Reoviridae*. *Archives of Virology* 149: 773–777.

N

Nanoviruses

H J Vetten, Federal Research Centre for Agriculture and Forestry (BBA), Brunswick, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Rep (**replication initiator protein**) It initiates replication only of its own DNA molecule and possesses origin-specific DNA cleavage, nucleotidyl transferase activity, and ATPase activity.
Satellite Satellites are subviral agents composed of nucleic acids; they depend for their multiplication on co-infection of a host cell with a helper virus. When a satellite encodes the coat protein in which its nucleic acid is encapsidated it is referred to as a satellite virus.

Introduction

Until the late 1980s, yellowing and dwarfing diseases of legumes and banana whose causal agents were persistently transmitted by aphids, not transmitted by sap and difficult to isolate, were generally thought to be caused by single-stranded RNA (ssRNA) viruses of the family *Luteoviridae*. In attempts to elucidate the etiology of these diseases, unusually small, icosahedral particles measuring only 18–20 nm in diameter were consistently isolated from infected plants. These particles did not contain one type of linear ssRNA but several circular ssDNA molecules, all of which were about 1 kb in size. Because of the disease symptoms (dwarfing) and the small size of the virions and genome components these viruses were referred to as nanoviruses. They differ from geminiviruses, the only other known group of ssDNA viruses of plants, in particle morphology, genome size, number and size of DNA components, genomic organization, mode of transcription, and vector species.

Meanwhile, four of these viruses, namely banana bunchy top virus (BBTV), faba bean necrotic yellows virus (FBNYV), milk vetch dwarf virus (MDV) and subterranean clover stunt virus (SCSV), have been formally described. Because of striking differences to other ssDNA viruses of plants, bacteria, and vertebrates, these viruses have recently been assigned to the family *Nanoviridae*. On the basis of differences in biology (host range, aphid vectors) and both genome size and organization, members of this family ('nanovirids') are subdivided into the genera *Nanovirus* and *Babuvirus*. Moreover, increasing evidence suggests that these viruses are not only quite variable in their biological and molecular properties but also that crops in tropical and subtropical countries of the Old World harbor further nano- and babuviruses, such as abaca bunchy top virus (ABTV) in the Philippines, cardamom bushy dwarf virus (CBDV) in India, and faba bean necrotic stunt virus (FBNSV) in Ethiopia and Morocco. BBTV occurs widely on Pacific Islands (including Hawaii), in Australia and Indochina but has an erratic geographic distribution in South Asia and Africa. SCSV and MDV have been reported only from Australia and Japan, respectively, whereas FBNYV appears to have a much wider geographic distribution (West Asia, Middle East, North and East Africa, and Spain). Nanovirids are not known to occur in the New World.

Particle Properties

Virions of the nanovirids are not enveloped, 17–20 nm in diameter, and presumably of an icosahedral T = 1 symmetry structure containing 60 subunits. Capsomeres may be evident, producing an angular or hexagonal outline (**Figure 1**). Virions are stable in Cs_2SO_4 but may not be stable in CsCl. The buoyant density of virions is about 1.24–1.30 g cm^{-3} in Cs_2SO_4, and 1.34 g cm^{-3} in CsCl. They sediment as a single component in sucrose rate-zonal and Cs_2SO_4 isopycnic density gradients.

Virions have a single capsid protein (CP) of about 19 kDa. No other proteins have been found associated with virions. Up to 12 distinct DNA components each of about 1 kb have been isolated from virion preparations of different species and their isolates. Each ssDNA component appears to be encapsidated in a separate particle.

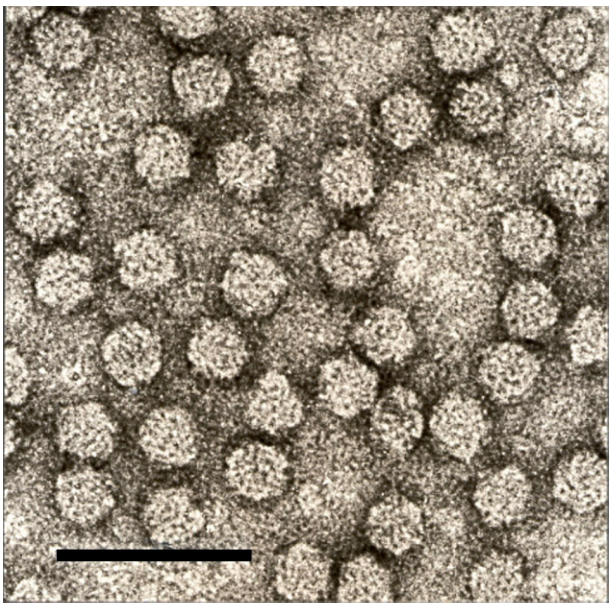


Figure 1 Negative constrast electron micrograph of particles of an isolate of *Faba bean necrotic yellows virus.* Scale = 50 nm. Courtesy of D. E. Lesemann and L. Katul.

Virions are strong immunogens. Most nanovirid species are serologically distinct from one another. However, antisera and some monoclonal antibodies (MAbs) to BBTV cross-react fairly strongly with ABTV and CBDV. On the contrary, antisera to FBNYV and SCSV cross-react weakly with SCSV and FBNYV, respectively, in Western blots and immunoelectron microscopy but not at all in double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). However, MDV antigen reacts strongly not only with an antiserum to FBNYV but also with the majority of MAbs to FBNYV. Therefore, species-specific MAbs are required for the differentiation and specific detection of not only FBNYV and MDV (CP amino acid sequence identity of about 83%), but presumably also BBTV, ABTV, and CBDV.

Biological Properties

Economic Importance

Based on symptomatology and transmission characteristics, all diseases now known to have a nanovirid etiology were earlier suspected to be caused by luteoviruses. Unlike luteoviruses, however, nanovirids generally cause more severe symptoms. In many virus–host combinations, early infections lead to very severe effects and even premature plant death. The disease caused by BBTV is considered the most serious viral disease of banana worldwide. SCSV and FBNYV are also thought to be of great economic importance as they have caused serious diseases of subterranean clover in Australia and of faba bean in Egypt, respectively, leading to repeated crop failures.

Host Range

Individual species have narrow host ranges. FBNYV, MDV, and SCSV naturally infect a range of leguminous species, whereas BBTV has been reported only from *Musa* species and closely related species within the Musaceae, such as abaca (*M. textilis* Née) and *Ensete ventricosum* Cheesem. There are no confirmed non-*Musa* hosts of BBTV. Symptoms of BBTV include plant stunting, foliar yellowing, and most characteristic dark green streaks on the pseudostem, petioles, and leaves. All economically important natural hosts of FBNYV, MDV, and SCSV are legumes, in which these viruses generally cause plant stunting and a range of foliar symptoms, such as leaf deformations and chlorosis or reddening. Although FBNYV infects >50 legume species and only few nonlegume species (*Stellaria media, Amaranthus*, and *Malva* spp.) under experimental and natural conditions, major legume crops naturally infected by FBNYV are faba bean, lentil, chickpea, pea, French bean, and cowpea. Likewise, SCSV experimentally infects numerous legume species, but its economically important natural hosts include only subterranean clover, phaseolus bean, faba bean, pea, and medics. MDV is known to cause yellowing and dwarfing in Chinese milk vetch (*Astragalus sinicus* L.), a common green manure crop in Japan, as well as in faba bean, pea, and soybean.

Tissue Tropism and Means of Transmission

SCSV and FBNYV have been shown to replicate in inoculated protoplasts. All members of assigned species are restricted to the phloem tissue of their host plants and are not transmitted mechanically and through seeds. Apart from graft transmission, vector transmission had been the only means of experimentally infecting plants with nanovirids, until infectivity of purified FBNYV virions by biolistic bombardment was demonstrated.

Transmission by Aphids

Under natural conditions, all viruses are transmitted by certain aphid species, in which they can persist for many days or weeks without replicating in their vectors. Whereas only one aphid species (*Pentalonia nigronervosa*) has been reported as vector of BBTV, several aphid species transmit FBNYV, MDV, and SCSV. *Aphis craccivora* appears to be the major natural vector of these viruses as it is the most abundant aphid species on legume crops in the afflicted areas and was among the most efficient vectors under experimental conditions. Other aphid vectors of FBNYV are *Aphis fabae* and *Acyrthosiphon pisum* but in no case were *Myzus persicae* and *Aphis gossypii* able to transmit this virus. SCSV has been reported to be vectored also by *Ap. gossypii, M. persicae*, and *Macrosiphum euphorbiae*, but some of these accounts now appear questionable.

Transmission studies showed that aphids are able to transmit FBNYV and SCSV following short acquisition and inoculation access feeding periods of about 30 min each. Although viruliferous aphids often retain transmission ability for life, nanovirids do not multiply in their insect vectors. Together with the observation that longer acquisition and inoculation access feeding periods resulted in higher transmission rates, the strikingly long persistence of nanovirids in their insect vectors indicates that they are transmitted in a circulative persistent manner similar to that of luteoviruses.

For FBNYV it has been demonstrated that purified virions alone are not transmissible by its aphid vector, regardless of whether they are acquired from artificial diets or directly microinjected into the aphid's hemocoel. However, faba bean seedlings biolistically inoculated with intact virions or viral DNA developed symptoms typical of FBNYV infections and were efficient sources for FBNYV transmission by aphids. These observations together with results from complementation experiments suggest that FBNYV (and other nanovirids) require a virus-encoded helper factor for its vector transmission that is either dysfunctional or absent in purified virion preparations.

Genome Organization and Protein Functions

DNA Structure

Up to 12 distinct DNA components each of about 1 kb in size have been isolated from virion preparations of different nanovirid species and their isolates. The majority of them seem to be structurally similar in being positive sense, transcribed in one direction, and containing a conserved stem–loop structure (and other conserved domains) in the noncoding region. Each coding region is preceded by a promoter sequence with a TATA box and followed by a polyadenylation signal (**Figure 2**). By analogy to the BBTV

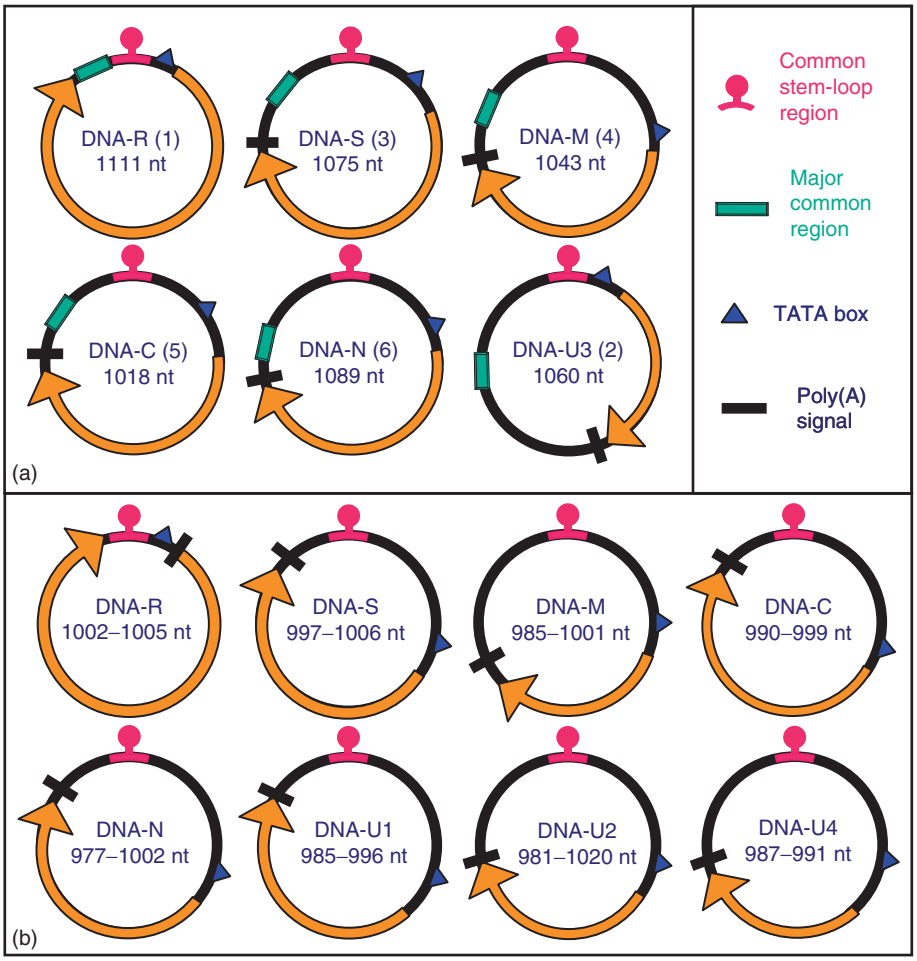


Figure 2 Diagram illustrating the putative genomic organization of the assigned species of the genera *Babuvirus* (a) and *Nanovirus* (b) and depicting the structure of the six and eight identified DNA components identified from the genomes of BBTV (a) and the three nanoviruses (b) FBNYV, MDV, and SCSV (see also **Table 1**). Each DNA circle contains its designated name and its size (range). Arrows refer to the location and approximate size of the ORFs and the direction of transcription. Note that DNA-U2 and -U4 have not been identified for SCSV.

DNA-U3, -S, -C, -M, and -N, each of which has been shown to yield only one mRNA transcript, the majority of the nanovirid DNAs contain only one major gene. Only for BBTV DNA-R two mRNA transcripts were detected, a large one mapping to the major replication initiator protein (Rep)-encoding open reading frame (ORF) and a small one completely internal to the major *rep* ORF.

Viral DNA Types

The understanding of the genomic organization of nanovirids was initially complicated by the fact that several DNAs encoding different replication initiator (Rep) proteins had been found associated with BBTV, FBNYV, MDV, and SCSV isolates. However, it was soon demonstrated that only one of the Rep-encoding DNAs of each nanovirid is an integral part of its genome and required for the replication of the other DNAs that encode other types of viral proteins. In addition to being consistently associated with a nanovirid infection and capable of initiating replication of the other viral DNAs, master Rep-encoding DNAs share with the other DNAs of a nanovirid species, a highly conserved sequence encompassing the stem loop.

The genomic information of each nanovirid is distributed over at least six or eight molecules of circular ssDNA (**Table 1**). The fact that a typical set of six and eight distinct DNAs has been consistently identified from a range of geographical isolates of BBTV and FBNYV (and MDV), respectively, suggests that the babuvirus genome consists of six DNAs and the nanovirus genome of eight DNA components. DNA-R, -S, -C, -M, and -N have been identified from all four assigned species of the family *Nanoviridae* (**Table 1**). DNA-U1 is shared by all three nanovirus species but is absent from the BBTV genome. The apparent absence of DNA-U2 and -U4 in the SCSV genome appears to be due to the fact that these genome components have not been identified yet from this nanovirus. DNA-U1, -U2, and -U4 seem to be absent from the BBTV genome and specific components of the nanovirus genome. In contrast, DNA-U3 appears to be specific of the babuvirus genome and absent from the nanovirus genome. However, the question as to whether DNA-U3 and -U4, which potentially encode similar-sized proteins (~10 kDa), are functionally distinct, remains to be determined (**Table 1**).

Integral Genome Segments

Despite the aforementioned circumstantial evidence, the number and types of ssDNA components constituting the integral parts of the nanovirid genome are still enigmatic. There has been only one (reported) attempt to use cloned DNAs to reproduce a nanovirid infection. Characteristic symptoms of FBNYV infection were obtained in faba bean, the principal natural host of FBNYV, following biolistic DNA delivery or agroinoculation with full-length clones of all eight DNAs that have been consistently detected in field samples infected with various geographical isolates of FBNYV. However, experimental infection with different combinations of fewer than these eight DNAs also led to typical FBNYV symptoms. Only five genome components, DNA-R, -S, -M, -U1, and -U2, were sufficient for inducing disease symptoms in faba bean upon agroinoculation. Symptomatic plants agroinoculated or bombarded with eight DNAs contained typical FBNYV virions; however, the virus produced after agroinoculation of cloned viral DNAs has not yet been transmitted by *Ap. craccivora* or *Ac. pisum*, two efficient aphid vectors of FBNYV.

Satellite-Like Rep DNAs

In addition to the putative genomic DNAs, a large number of additional DNAs encoding Rep proteins have been described from nanovirid infections. These DNAs are very diverse and phylogenetically distinct from the DNA-R of the nanovirids (**Figure 3**). They are structurally similar and phylogenetically closely related to nanovirid-like *rep* DNAs that have recently been found associated with some begomoviruses (e.g., ageratum yellow vein virus DNA 1 (AJ238493) and DNA 2 (AJ416153); cotton leaf curl Multan virus DNA 1 (AJ132344-5). However, due to the inclusion of an A-rich sequence within the intergenic region, the begomovirus-associated DNAs are larger (~1300 nt) than the nanovirid-associated DNAs (~930 to ~1100 nt). In contrast to the genomic DNA-R which encodes the only known Rep protein essential for the replication of the multipartite genome of the nanovirids, these additional *rep* DNAs are only capable of initiating replication of their cognate DNA but not of any heterologous genomic DNA. Since they are, moreover, only erratically associated with nanovirid infections, they are regarded as satellite-like DNAs that depend on their helper viruses for various functions, such as encapsidation, transmission, and movement.

There are no data as to whether these additional *rep* DNAs are of any biological significance to the helper virus. However, recent agroinoculation experiments with eight FBNYV DNAs or with the same eight FBNYV DNAs in combination with a satellite-like *rep* DNA (FBNYV-C11) suggest that FBNYV-C11 can reduce the number of symptomatic (42/77 vs. 21/74) and severely infected faba bean plants (16/42 vs. 2/21) and, thus, interfere with establishment of disease. This may be due to competition between the additional *rep* DNAs and the genomic nanovirid DNAs for factors required for replication, systemic movement, or encapsidation. It is also noteworthy that the protein encoded by another additional *rep* DNA (C1) of FBNYV was about ten times more active in

Table 1 Key properties of assigned and tentative members of the genera *Babuvirus* and *Nanovirus* of the family *Nanoviridae*

	Genus *Babuvirus*			Genus *Nanovirus*			
	Assigned species	Tentative species		Assigned species			Tentative species
	BBTV	ABTV	CBDV	FBNYV	MDV	SCSV	FBNSV
Geographic distribution	Pacific Islands, Australia, southern Asia, Africa	Borneo, Philippines	India	Near East, North Africa, Ethiopia	Japan	Australia, Tasmania	Ethiopia, Morocco
Biological properties							
Major host plants	*Musa* spp.	*Musa* spp. (abaca, banana)	Large cardamom (*Amomum subulatum*)	Legumes	Legumes	Legumes	Legumes
Aphid vectors	*Pentalonia nigronervosa*	*Pentalonia nigronervosa*	*Micromyzus kalimpongensis*	*Aphis craccivora, A. fabae, Acyrthosiphon pisum*	*Aphis craccivora, Acyrthosiphon pisum*	*Aphis craccivora, A. gossypii* and other aphid spp.	*Aphis craccivora*
Virion properties							
Morphology	Icosahedral	No data	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Icosahedral
Particle diameter (nm)	18–20	No data	17–20	18	18	17–19	18
Sedimentation coefficient	46 S	No data	No data	No data	No data	No data	No data
Density (g cm^{-3})	1.28	No data	No data	1.245	No data	1.24	No data
Capsid protein (kDa)	20	No data	No data	20	No data	19	20
Genome properties							
Number of components[a]	6 (9)	6	1	8 (12)	8 (12)	6 (8)	8
Component sizes (nts)	1018–1111	1013–1099	No data	985–1014	977–1022	988–1022	923–1003
Integral genome components[b]							
DNA-R (M-Rep, 33.1–33.6)[c]	+ (1)[d]	+ (1)	+ (1)	+ (2)	+ (11)	+ (8)	+ (2)
(U5, 5.0)	+ (1)	− (1)	− (1)	− (2)	− (11)	− (8)	− (2)
DNA-S (CP, 18.7–19.3)	+ (3)	+ (3)	No data	+ (5)	+ (9)	+ (5)	+ (5)
DNA-C (Clink, 19.0–19.8)	+ (5)	+ (5)	No data	+ (10)	+ (4)	+ (3)	+ (10)
DNA-M (MP, 12.7–13.7)	+ (4)	+ (4)	No data	+ (4)	+ (8)	+ (1)	+ (4)
DNA-N (NSP, 17.3–17.7)	+ (6)	+ (6)	No data	+ (8)	+ (6)	+ (4)	+ (8)
DNA-U1 (U1, 16.9–18.0)	—	—	No data	+ (3)	+ (5)	+ (7)	+ (3)
DNA-U2 (U2, 14.2–15.4)	—	—	No data	+ (6)	+ (7)	—	+ (6)
DNA-U3 (U3, 10.3)	+ (2)[e]	+ (2)[e]	No data	—	—	—	—
DNA-U4 (U4, 10 or 12.5)	—	—	No data	+ (12)	+ (12)	—	+ (12)
Additional (satellite-like) Rep-encoding DNAs	DNA-S1, -S2, -S3, -Y1, -W2	No data	No data	DNA-C1, -C7, -C9, and -C11	DNA-C1, -C2, -C3, and -C10	DNA-C2 and -C6	No data

[a]Numbers of identified genome components possibly forming the viral genome. Numbers in parentheses give the total number of distinct ssDNA components described from one or various isolates of each virus. For details see text.

[b]DNA(s) encoding proteins that are either functionally equivalent and/or share significant levels of sequence similarities were placed on the same line.

[c]Assigned and tentative functions of the protein encoded by the genome components is given in parentheses: master replication initiator protein (M-Rep), capsid protein (CP), cell-cycle link protein (Clink), movement protein (MP), putative nuclear shuttle protein (NSP), and proteins of unknown functions (U1–U4). The deduced molecular mass (in kDa) of the protein(s) encoded by each genome component is given in parenthesis (behind the protein designation).

[d]A plus (+) and dash (–) indicates as to whether a component (or ORF) encoding a similar protein has been identified from a virus species or not, respectively. Numbers in parentheses give the DNA-component numbering used originally by the research group studying this virus.

[e]A U3-encoding ORF has been identified only from several South Pacific isolates of BBTV, but not from South Asian isolates of BBTV and the two known ABTV strains.

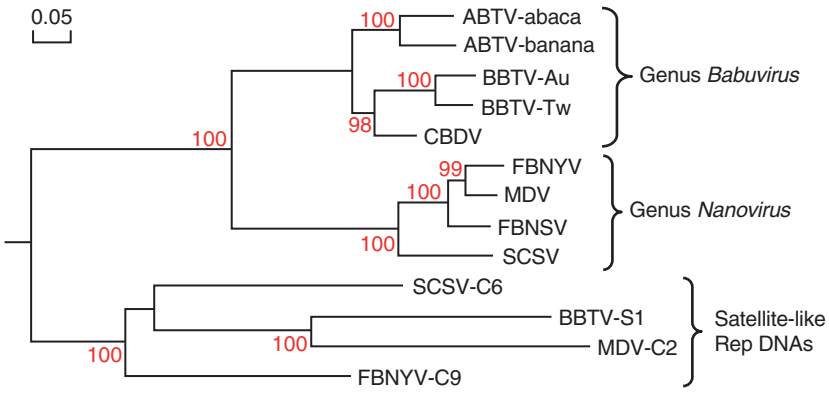


Figure 3 Neighbor-joining dendrogram illustrating the nucleotide sequence relationships in the DNA-R within and between the genera *Babuvirus* and *Nanovirus* of the family *Nanoviridae*. For comparison, four diverse representatives of the numerous satellite-like Rep-encoding DNAs frequently found associated with nanovirid infections were included to demonstrate their phylogenetic distinctness from the DNA-R of the nanovirids. DNA-R sequences used are those of members of the four assigned nanovirid species, *Banana bunchy top virus* (BBTV), *Faba bean necrotic yellows virus* (FBNYV), *Milk vetch dwarf virus* (MDV), and *Subterranean clover stunt virus* (SCSV), and the tentative species, abaca bunchy top virus (ABTV), cardamom bushy dwarf virus (CBDV), and faba bean necrotic stunt virus (FBNSV). Since some species appear to be particularly diverse, the DNA-R sequences of ABTV isolates from abaca and banana as well as BBTV isolates from Australia (Au) and Taiwan (Tw) representing the South Pacific and Asian groups, respectively, were also included in the comparison. Vertical branch lengths are arbitrary and horizontal distances are proportional to percent sequence differences. Sequence alignments and dendrograms were produced using DNAMAN (version 6, Lynnon Corporation, Quebec, Canada) which uses a CLUSTAL-type algorithm. The dendrograms were bootstrapped 1000 times (scores are shown at nodes).

an *in vitro* origin cleavage and nucleotidyl-transfer reaction than the master Rep protein of FBNYV. Moreover, from many nanovirid infections the additional *rep* DNAs were identified often prior to the master Rep-encoding DNA-R, suggesting that they attain higher concentrations than DNA-R in nanovirid-infected plants.

Proteins

In addition to the CP (about 19 kDa) coded for by the DNA-S transcript, at least 5–7 nonstructural proteins are encoded by the mRNA(s) transcribed from the genomic ssDNAs (**Table 1**, **Figure 2**). The large transcript from DNA-R encodes the master Rep protein (33.1–33.6 kDa). Although a second smaller transcript from the BBTV DNA-R contains a virion-sense ORF completely nested within the master Rep-encoding ORF, there is no experimental evidence that this small ORF potentially encoding a 5 kDa protein (U5) of unknown function is expressed. In addition, a small ORF similar in size and location has not been identified from the DNA-R of both nanoviruses and the tentative babuviruses ABTV and CBDV. DNA-C encodes a 19.0–19.7 kDa protein ('Clink') which contains a conserved LxCxE motif and has been shown to interact with plant proteins involved in cell-cycle regulation. A 12.7–13.7 kDa protein described from all four nanovirid species contains a stretch of 25–30 hydrophobic residues at its N terminus. This together with other experimental evidence obtained for the BBTV DNA-M-encoded protein (MP) indicates that it is involved in cell-to-cell movement of nanovirids. DNA-N encodes a 17.3–17.7 kDa protein which has been identified from all four nanovirids and proposed to act as a nuclear shuttle protein (NSP). Based on significant levels of amino acid sequence identities among some of the other nanovirid proteins, they appear to have similar but unknown functions and are provisionally referred to as U1–U4 proteins (**Table 1**).

The most conserved nanovirid proteins are the master Rep protein (54–97% identity) and NSP (41–91%), followed by the CP (20–84%), Clink (18–72%), and MP (14–76%). Consequently, the babuvirus BBTV shares significant levels of amino acid sequence identity with the nanoviruses only in the M-Rep (54–56%) and NSP (41–45%), whereas the amino acid sequence similarities between the two genera are negligible in the CP (20–27%), the MP (20–23%), and the Clink protein (18–23%). One of the least conserved proteins of the babuviruses and nanoviruses appears to be the protein U3 and U4, respectively.

Nanovirid Replication

Since the nanovirids DNAs and some of the biochemical events determined for nanovirid replication resemble those of the geminiviruses, their replication is also thought to be completely dependent on the host cell's DNA replication enzymes and to occur in the nucleus through transcriptionally and replicationally active double-stranded DNA (dsDNA) intermediates by a rolling-circle type of replication mechanism. Upon decapsidation of viral ssDNA,

one of the first events is the synthesis of viral dsDNA with the aid of host DNA polymerase. As the virus DNAs have the ability to self-prime during dsDNA synthesis, it is likely that preexisting primers are used for dsDNA replicative form (RF) synthesis, as has been shown for BBTV. From these dsDNA forms, host RNA polymerase then transcribes mRNAs encoding the M-Rep and other viral proteins required for virus replication. Viral DNA replication is initiated by the M-Rep protein that interacts with common sequence signals on all the genomic DNAs. Nicking and joining within the conserved nonanucleotide sequence TAT/GTATT-AC by the Rep proteins of BBTV and FBNYV has been demonstrated *in vitro*. The nonanucleotide sequence is flanked by inverted repeat sequences with the potential to form a stem–loop structure, a common feature of every nanovirid DNA. Replication of the viral DNAs by the cellular replication machinery is enhanced by the action of Clink, a nanovirid-encoded cell-cycle modulator protein.

Relationships to Other Families of ssDNA Viruses

All Rep proteins of the nanovirid species have most of the amino acid sequence domains characteristic of Rep proteins of ssDNA viruses of other taxa, such as the families *Geminiviridae* and *Circoviridae*. However, the nanovirid Rep proteins differ from those of members of the family *Geminiviridae* in being smaller (about 33 kDa), having a slightly distinct dNTP-binding motif (GPQ/NGGEGKT), and in sharing amino acid sequence identities of only 17–22% with them. A particularly noteworthy feature of the nanovirid M-Rep protein is the lack of the Rb-binding (LxCxE) motif and, thus, the apparent absence of cell-cycle modulation functions from this protein. In contrast to some geminiviruses, whose monopartite genome encodes a Rep protein with a conserved Rb-binding motif, the nanovirids have a separate DNA segment encoding an LxCxE-containing protein involved in cell-cycle regulation. Moreover, nanovirids are clearly distinct from geminiviruses in particle morphology (isometric vs. geminate) and dimensions (18–20 nm vs. 18 × 30 nm), genome size (6.5 or 8.0 vs. 2.6–3.0 or 5.0–5.6) and segments (6 or 8 vs. 1 or 2), mode of transcription (uni- vs. bidirectional) as well as in vector species (aphids vs. whiteflies, leafhoppers, or a treehopper). Although nanovirids and circoviruses share similar particle morphologies, the most notable differences between these two virus taxa are that the circoviruses infect vertebrates (pigs and birds) and have a monopartite genome which is only 1.8–2.0 kb in size and from which the *rep* and *cap* genes are bidirectionally transcribed. All of these viruses have a conserved nonanucleotide motif at the apex of the stem–loop sequence which is consistent with the operation of a rolling-circle model for DNA replication.

See also: Banana Bunchy Top Virus; Circoviruses; Luteoviruses; Maize Streak Virus; Plant Resistance to Viruses: Geminiviruses.

Further Reading

Aronson MN, Meyer AD, Györgyey J, *et al.* (2000) Clink, a nanovirus encoded protein binds both pRB and SKP1. *Journal of Virology* 74: 2967–2972.

Boevink P, Chu PWG, and Keese P (1995) Sequence of subterranean clover stunt virus DNA: Affinities with the geminiviruses. *Virology* 207: 354–361.

Burns TM, Harding RM, and Dale JL (1995) The genome organization of banana bunchy top virus: Analysis of six ssDNA components. *Journal of General Virology* 76: 1471–1482.

Chu PWG and Helms K (1988) Novel virus-like particles containing circular single-stranded DNA associated with subterranean clover stunt disease. *Virology* 167: 38–49.

Chu PWG and Vetten HJ (2003) *Subterranean clover stunt virus*. AAB Descriptions of Plant Viruses, No. 396. http://www.dpvweb.net/dpv/showadpv.php?dpvno=369 (accessed June 2007).

Franz AW, van der Wilk F, Verbeek M, Dullemans AM, and van den Heuvel JF (1999) Faba bean necrotic yellows virus (genus *Nanovirus*) requires a helper factor for its aphid transmission. *Virology* 262: 210–219.

Karan M, Harding RM, and Dale JL (1994) Evidence for two groups of banana bunchy top virus isolates. *Journal of General Virology* 75: 3541–3546.

Katul L, Timchenko T, Gronenborn B, and Vetten HJ (1998) Ten distinct circular ssDNA components, four of which encode putative replication-associated proteins, are associated with the faba bean necrotic yellows virus genome. *Journal of General Virology* 79: 3101–3109.

Mandal B, Mandal S, Pun KB, and Varma A (2004) First report of the association of a nanovirus with foorkey disease of large cardamom in India. *Plant Disease* 88: 428.

Sano Y, Wada M, Hashimoto T, and Kojima M (1998) Sequences of ten circular ssDNA components associated with the milk vetch dwarf virus genome. *Journal of General Virology* 79: 3111–3118.

Timchenko T, de Kouchkovsky F, Katul L, David C, Vetten HJ, and Gronenborn B (1999) A single rep protein initiates replication of multiple genome components of faba bean necrotic yellows virus, a single-stranded DNA virus of plants. *Journal of Virology* 73: 10173–10182.

Timchenko T, Katul L, Aronson M, *et al.* (2006) Infectivity of nanovirus DNAs: Induction of disease by cloned genome components of *Faba bean necrotic yellows virus*. *Journal of General Virology* 87: 1735–1743.

Timchenko T, Katul L, Sano Y, de Kouchkovsky F, Vetten HJ, and Gronenborn B (2000) The master rep concept in nanovirus replication: Identification of missing genome components and potential for natural genetic reassortment. *Virology* 274: 189–195.

Vetten HJ, Chu PWG, Dale JL, *et al.* (2005) Nanoviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 343–352. San Diego, CA: Elsevier Academic Press.

Wanitchakorn R, Hafner GJ, Harding RM, and Dale JL (2000) Functional analysis of proteins encoded by banana bunchy top virus DNA-4 to -6. *Journal of General Virology* 81: 299–306.

Narnaviruses

R Esteban and T Fujimura, Instituto de Microbiología Bioquímica CSIC/University de Salamanca, Salamanca, Spain

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ribozyme RNA with a catalytic activity.

Introduction

The narnaviruses 20S RNA and 23S RNA (ScV20S and ScV23S, respectively) are positive-strand RNA viruses found in the yeast *Saccharomyces cerevisiae*. Currently only these two viruses are ascribed to the genus *Narnavirus* of the family *Narnaviridae*. Like most fungal viruses, they have no extracellular transmission pathway. They are transmitted horizontally by mating, or vertically from mother to daughter cells. It is believed that the high frequency of mating or hyphal fusion that occurs in the host life cycle makes an extracellular route of transmission dispensable for the viruses. The thick cell wall of fungi may also form a formidable barrier. The lack of extracellular transmission may, in turn, explain two prominent features found in narnaviruses. First, they are persistent viruses and do not kill the host cells. If their infection caused damages or disadvantages to the host, then the viruses might have perished during the course of evolution because of the lack of an escape route. Second, because there is no extracellular phase, the viruses do not need to form virions to protect their RNA genomes in the extracellular environment. In addition, they do not need machinery to ensure exit or reentry to a new host. The lack of a virion structure may sound peculiar to those who are familiar with infectious viruses. Considering that viruses are selfish parasites, however, it will be natural for them to shed genes or functions unnecessary for their existence. Consequently, the genomes of narnaviruses are simple and small: they only encode a single protein, the RNA-dependent RNA polymerase (RdRp). This may contribute to their persistence by reducing a number of viral proteins that might interfere with metabolism vital for the host. The simplicity of their RNA genomes encoding a single protein, together with the recent development of 20S and 23S RNA virus launching systems from yeast expression vectors, makes narnaviruses a good model system to investigate replication and the molecular basis for intracellular persistence of RNA viruses.

Historical Background

20S RNA was first described in 1971 as a single-stranded RNA (ssRNA) species accumulated in yeast cells transferred to 1% potassium acetate, a standard procedure to induce sporulation in yeast under nitrogen-starvation conditions. Because of its mobility relative to 25S and 18S rRNAs, the species was named 20S RNA. Later it was found, however, that the accumulation of 20S RNA was not related with the sporulation process because haploid cells that do not sporulate also accumulate 20S RNA under nitrogen-starvation conditions. It was also found that 20S RNA is a cytoplasmic genetic element. The realization of 20S RNA as a viral entity, however, had to wait several years, until the characterization of 20S RNA by cloning and sequencing in 1991. 23S RNA was reported first time in 1992. Both viruses were placed in the genus *Narnavirus* of the new family, *Narnaviridae* (naked RNA virus), a taxonomic group that appeared for the first time in the seventh edition of the International Committee on Taxonomy of Viruses (ICTV). The other genus in the family is *Mitovirus*, whose members are found in mitochondria of fungi, many of them pathogenic to plants. The members of the family have small RNA genomes (2–3 kb) that encode single proteins, their RdRps, and reside either in the cytoplasm (members of the genus *Narnavirus*; narnaviruses) or in the mitochondria (mitoviruses) of the host.

Viral Genomes

Many laboratory strains of *S. cerevisiae* harbor 20S RNA virus and fewer strains contain 23S RNA virus. Both viruses are compatible in the same host. The presence of 20S and 23S RNA viruses does not render phenotypic changes to the host. Under nitrogen-starvation conditions, the amounts of the viral genomes become almost equivalent to those of rRNAs (>100 000 copies/cell; **Figure 1**). In contrast, vegetative growing cells contain much lower amounts of the viral RNAs (5–20 copies/cell). **Figure 2(a)** shows the genome organization of narnaviruses. Both 20S and 23S RNAs are small (2514 and 2891 nt, respectively) and each genome encodes a single protein: a 91 kDa protein (p91) by 20S RNA and a 104 kDa protein (p104) by 23S RNA. The 5′ untranslated regions in both RNAs are extremely short: 12 nt in the case of 20S RNA and

only 6 nt in 23S RNA. These RNAs lack poly(A) tails at the 3′ ends and have perhaps no 5′ cap structures. The same RNA can serve as template for translation and also for negative-strand synthesis. The antigenomic (or negative-strand) RNAs have no coding capacity for protein and are present at much lower copy numbers compared to the genomic (or positive-strand) RNAs under the induction conditions. The double-stranded forms of 20S and 23S RNAs are known and called W and T, respectively. These double-stranded RNAs (dsRNAs) accumulate when the cells are grown at 37 °C, a rather high temperature for yeast (the optimal temperature for growth is about 28 °C). These dsRNAs are not intermediates of replication but by-products. Replication proceeds from a positive strand to a negative strand and then to a positive strand.

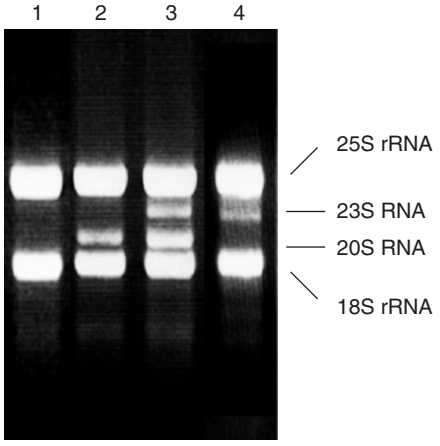


Figure 1 Agarose gel electrophoresis of RNA extracted from virus-free and virus-infected nitrogen-starved yeast cells. RNA from virus-free yeast cells (lane 1) or cells infected with 20S RNA virus alone (lane 2), 23S RNA virus alone (lane 4), or both viruses together (lane 3), was separated in an agarose gel and visualized by ethidium bromide staining. The positions of the 20S and 23S RNAs together with the rRNAs are indicated to the right.

The proteins encoded in the viral genomes are not processed to produce smaller fragments with distinct functional domains. Both proteins contain amino acid motifs well conserved among RdRps from positive-strand and dsRNA viruses (**Figure 2**(**a**)) In addition, p91 and p104 share stretches of amino acid sequences (denoted by 1–3 in **Figure 2**(**a**)) in the same order throughout the molecules, indicating a close evolutionary relationship between these two viruses. Remarkably, their RdRp consensus motifs are most closely related to those of RNA bacteriophages such as Qβ.

Ribonucleoprotein Complexes as Viral Entities

Yeast is also a natural host for dsRNA totiviruses, called L-A and L-BC. Like narnaviruses, totiviruses have no extracellular transmission pathway. However, these viruses have *gag* and *pol* genes, and their dsRNA genomes are encapsidated into intracellular viral particles. In contrast, 20S and 23S RNA viruses have no capsid genes to form virion structures. Then, how do these viruses exist inside

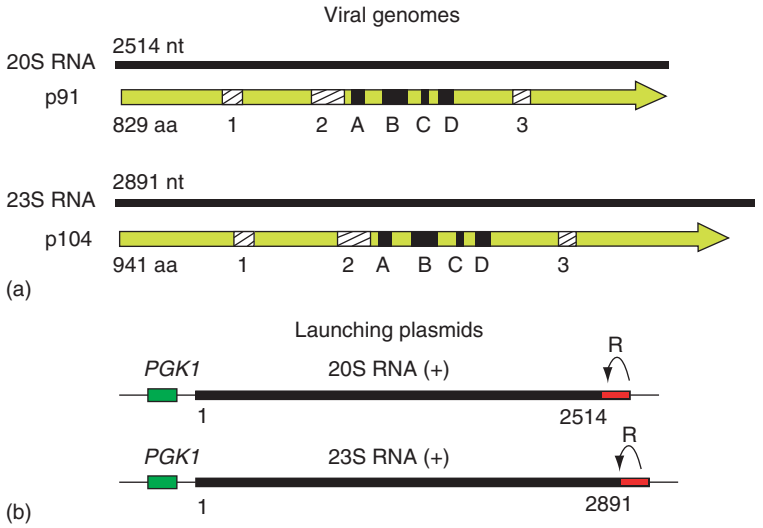


Figure 2 Genomic organization of 20S and 23S RNA viruses (a) and their launching plasmids (b). (a) Diagrams of 20S and 23S RNAs and the proteins encoded by them, p91 and p104, respectively. A–D represents motifs conserved among RdRps from positive strand and dsRNA viruses and 1–3 indicates amino acid stretches conserved between p91 and p104. (b) The complete cDNA of 20S or 23S RNA genome is inserted downstream of the constitutive *PGK1* promoter in a yeast expression vector in such a way that positive strands are transcribed from the promoter. The HDV ribozyme (R) is fused directly to the 3′ end of the viral genome.

the cell and establish a persistent infection without a protective coat? Earlier studies demonstrated that 20S RNA migrated as 'naked RNA' in sucrose gradients. Furthermore, deproteination with phenol had no apparent effect on its mobility. Because protein provides a large part of the molecular mass in virions, these data clearly indicate that narnaviruses lack a virion structure. When specific antibodies against their RdRps became available, however, it was realized that each RdRp is associated with its RNA genome and this interaction is specific; that is, p91 is associated only with 20S RNA and p104 only with 23S RNA. These ribonucleoprotein complexes reside in the cytoplasm and are not associated with the nucleus, mitochondria, or intracellular membranous structures. Further studies indicated that most of the positive strands of 20S and 23S RNA viruses under induction conditions are associated with their own RdRps in a 1:1 stoichiometry. It is not known whether host proteins are present in the complexes. These complexes are called 'resting complexes' to distinguish them from the 'replication complexes' described in the following section. Negative strands are present at much lower amounts compared to positive strands, and available data indicate that they also form complexes with their own RdRps. These findings suggest that the formation of ribonucleoprotein complexes between the viral RNA and its RdRp is important for the life cycles of 20S and 23S RNA viruses.

Replication Intermediates

Lysates prepared from virus-induced cells have an RdRp activity. The activity is insensitive to actinomycin D or α-amanitin, thus independent of a DNA template. The majority of *in vitro* products are positive strands of 20S RNA. Synthesis of negative strands accounts for a small fraction of the RNA products compared to that of positive strands, thus reflecting the high positive/negative-strand ratio in the lysates. There is no, or very little, *de novo* synthesis *in vitro*. Therefore, radioactive nucleotides are unevenly distributed into 20S RNA positive-strand products with more incorporation into the 3′ end region. Replication complexes that synthesize 20S RNA positive strands have a ssRNA backbone and migrate in native agarose gels as a broad band corresponding to an ssRNA in the size ranging from 2.5 to 5 kbp long. These complexes consist of a full-length negative-strand template (2.5 kbp) and a nascent positive strand of less than unit-length, probably held together by the polymerase machinery. Deproteination with phenol converts them to dsRNA. Therefore, W dsRNA is not a replication intermediate but a byproduct. It is likely that the high temperature (37 °C) for growth may destabilize replication complexes, thus resulting in the accumulation of dsRNA. Upon completion of RNA synthesis *in vitro*, the positive-strand products as well as the negative-strand templates are released from replication complexes. It is likely that, in the cell, the released negative strands are immediately recruited to another round of positive-strand synthesis, because the majority of negative strands in lysates are present in replication complexes engaging in the synthesis of positive strands. Interestingly, both positive and negative strands released from replication complexes are associated with protein. Because replication complexes contain at least one p91 molecule per complex, p91 is a good candidate for the protein.

Generation of Narnaviruses *In Vivo*

As mentioned earlier, the presence of narnaviruses does not render phenotypic changes to the host. This has hindered studies on replication or virus/host interactions using yeast genetics. This obstacle has been overcome by recent developments in generating 20S and 23S RNA viruses *in vivo* from a yeast expression vector (**Figure 2(b)**). In either case, the complete viral cDNA was inserted in the vector downstream of a constitutive promoter in such a way that positive strands can be transcribed from the promoter. The 3′ end of the viral sequence was directly fused to the hepatitis delta virus (HDV) antigenomic ribozyme. Therefore, intramolecular cleavage by the ribozyme will create transcripts *in vivo* having the 3′ termini identical to the viral 3′ end. The efficiency of virus launching is high. The 20–70% of the cells transformed with the vector generated the virus. The primary transcripts expressed from the vectors have nonviral sequences (about 40 nt) at the 5′ ends. The generated viruses, however, possessed the authentic viral 5′ ends without the extra sequences. It is likely that the 5′ nonviral extension was eliminated by a 5′ exonuclease. Using these launching systems, it has been demonstrated that each RdRp is essential and specific for replication of its own viral RNA. p91 is essential for 20S RNA replication and does not substitute p104 for replication of 23S RNA virus. Similarly, p104 is essential for 23S RNA replication and does not support 20S RNA replication. Because negative strands cannot be decoded to the RdRps, vectors in which the viral cDNAs were reversed failed to generate the virus. These negative-strand-expressing vectors, however, successfully generated narnaviruses, if active polymerases were provided in *trans* from a second vector. Therefore, both 20S and 23S RNA viruses can be generated from either positive or negative strands expressed from a vector.

cis-Acting Signals for Replication

20S and 23S RNA genomes share the same 5 nt inverted repeats at the 5′ and 3′ termini (5′-GGGGC. . .GCCCC-OH). Extensive analysis was done modifying each nucleotide at the 3′ ends. It was found that the third and fourth

C's from the 3′ termini are essential for replication in both viruses. While the 3′ terminal and penultimate C's can be eliminated or changed to other nucleotides without affecting virus generation, the generated viruses recovered the wild-type C's at the termini. Therefore, the consecutive four C's at the 3′ terminus are essential for these viruses (**Figure 3**). In contrast, the G at position 5 from the 3′ end is dispensable for replication in both viruses. 23S RNA virus requires an additional 3′ *cis*-signal for replication. The stem–loop structure proximal to the 3′ end contains a mismatched pair of purines in the stem (**Figure 3**). This mismatched pair is essential for replication but the virus tolerates any combination of purines at this position. On the other hand, changing the purines to pyrimidines or eliminating one of the purines at the mismatched pair blocked virus generation. The distance between the mismatched pair and the 3′ terminal four C's and/or their spatial configuration appears to be critical, because shortening or increasing the length of the stem between the two sites by more than 1 bp abolished virus launching. It is not known whether 20S RNA virus has a similar *cis*-signal in the stem–loop structure proximal to the 3′ end. The G at position 5 from the 3′ end is located at the bottom of the stem structure. This G, as mentioned earlier, can be changed to another nucleotide without impairing replication, as long as the modified nucleotide is hydrogen-bonded at the bottom of the stem.

The negative strands of 20S and 23S RNA viruses also possess four consecutive C's at the 3′ ends. Using the two-vector system mentioned above, it has been found that the third and fourth C's from the 3′ end are essential for replication. Similar to the positive strands, the 3′ terminal

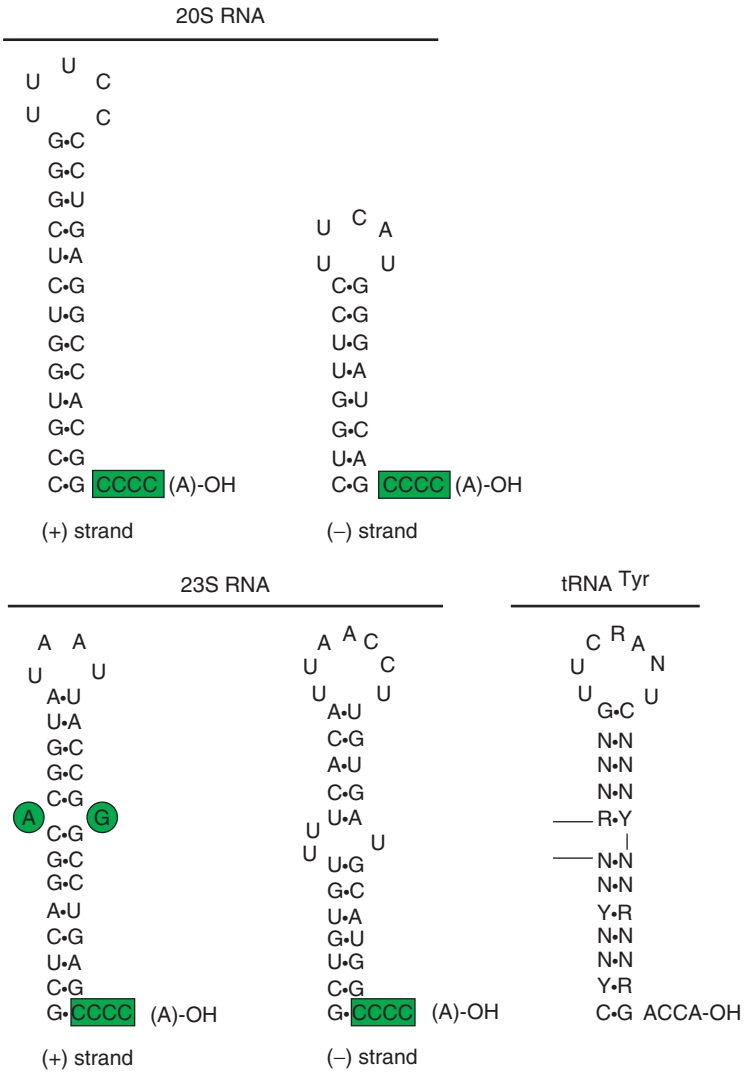


Figure 3 Comparison of the 3′ terminal secondary structures in the positive (+) and negative (–) strands of 20S and 23S RNA viruses, with the top half domain of $tRNA^{Tyr}$. The nontemplated A residues at the viral 3′ termini are indicated by parenthesis. The consecutive four C's essential for replication are boxed (green). A second *cis*-signal (the mismatched pair of purines) present in the positive strand of 23S RNA virus is circled (green). Y, R, and N stand for pyrimidine, purine, and any base, respectively.

and penultimate C's can be eliminated or changed to other nucleotides without affecting virus generation and the generated viruses recovered the wild-type four C's at the 3′ ends. Therefore, the consecutive four C's at the 3′ end of the negative strand are again a *cis*-signal for replication. The 5′ ends of viral positive strands have not been analyzed extensively. Elimination of the 5′ terminal G or changing it to other nucleotide had no effect on virus generation and the generated viruses recovered this G at the 5′ ends.

cis-Signals for Formation of Ribonucleoprotein Complexes

Narnaviruses, as mentioned earlier, exist as ribonucleoprotein complexes in the host cytoplasm. In the absence of the HDV ribozyme, RNA transcribed from the launching vectors failed to generate viruses because of the presence of nonviral extensions at the 3′ end. The transcripts, however, can be decoded to viral polymerases and the polymerases can form complexes *in vivo* with the transcripts, thus providing an assay system to analyze *cis*-signals for formation of ribonucleoprotein complexes. By immunoprecipitation with antiserum specific to p104, it has been found that the bipartite 3′ *cis*-signal for replication (more specifically, the mismatched pair of purines and the third and fourth C's from the 3′ end) is essential for 23S RNA positive strand to form complexes with the polymerase. This highlights the importance of formation of ribonucleoprotein complexes for the life of narnaviruses. The cytoplasm is filled with host RNAs, including a great variety of mRNAs. Formation of complexes between the viral polymerase and its template RNA will therefore facilitate replication and increase its fidelity by discriminating against nonviral RNAs as templates. The importance of the third and fourth C's from the 3′ end for formation of complexes with p104 suggests that these nucleotides are in close contact with p104. Because the 23S RNA virus has no coat protein to protect the viral RNA, it is likely that such interaction protects the 3′ ends of 23S RNA from exonuclease cleavage. In the case of 20S RNA virus, a similar *in vivo* assay indicates that the 3′ *cis*-signal for replication (in particular the third and fourth C's from the 3′ end) is also important for formation of ribonucleoprotein complexes with p91. When isolated resting complexes of 20S RNA virus were analyzed *in vitro*, however, it was found that p91 interacts with 20S RNA not only at the 3′ end but also at the 5′ terminal region of the molecule. The 5′ binding site is located at the second stem–loop structure from the 5′ end. Computer-predicted analysis indicates that the 5′ and 3′ termini of 20S RNA (and 23S RNA) are brought together into close proximity by a long-distance RNA/RNA interaction (**Figure 4**). This may allow a single molecule of p91 to interact simultaneously with both ends of 20S RNA genome in a resting complex. This protein/RNA interaction may provide a clue to understand the molecular basis of narnaviruses persistence (see below).

Narnavirus Persistence in the Host

mRNA degradation in yeast, like in other eukaryotes, is initiated by shortening the 3′ poly(A) tail followed by decapping at the 5′ end. Then the decapped mRNA is degraded by the potent Xrn1p/Ski1p 5′ exonuclease as well as by a 3′ exonuclease complex called exosome. The RNA genomes of narnaviruses, as mentioned earlier, have no 3′ poly(A) tails and perhaps no cap structures at the 5′ ends, thus resembling intermediates of mRNA degradation. This suggests that these RNA genomes are vulnerable to the exonucleases involved in mRNA degradation. In fact, the copy numbers of 20S and 23S RNAs increase several-fold in strains having mutations in *SKI* genes such as *SKI2*, *SKI6*, and *SKI8*. These mutations were originally identified by their failure in lowering the copy numbers of L-A dsRNA totivirus and its satellite RNA M. It is known that the *SKI2*, *SKI6*, and *SKI8* gene products are components or modulators of the exosome. These observations suggest that the 3′ end of the viral genome is constantly nibbled by 3′ exonucleases. Therefore, one of the reasons for narnaviruses to form ribonucleoprotein complexes may be to protect their 3′ ends from exonuclease cleavage. The fact that the third and fourth C's from the 3′ end are important to form complexes in both viruses fits this hypothesis because binding of the RdRp to these nucleotides would block progression of the exonuclease and protect the internal region. As described earlier, mutations introduced at the terminal and penultimate positions at the 3′ end had no deleterious effects on virus launching and the generated viruses recovered the wild-type sequences. This suggests that the terminal and penultimate positions at the 3′ ends are not only vulnerable to cleavages but also accessible to the repair machinery. The 3′ ends of these viruses may undergo constant turnover at these positions.

As regards the 5′ end, the first four nucleotides in both 20S and 23S RNAs are consecutive G's (**Figure 4**). It is known that oligo G tracts inhibit progression of the Xrn1/Ski1 5′ exonuclease. Furthermore, these consecutive G's are buried at the bottom of a long stem structure in both viruses. These features thus suggest that 20S and 23S RNAs by themselves are quite resistant to the 5′ exonuclease. The initiation codon of p91 is located in the middle of the long stem structure proximal to the 5′ end. If p91 binds to this stem in the ribonucleoprotein complex, then such a stable binding may interfere with translation of new p91 molecules from the RNA. In this context, it may make sense that the 5′ binding site of p91 in the complexes is located at the second stem–loop structure from the 5′ end. By binding simultaneously to the 3′ end and also

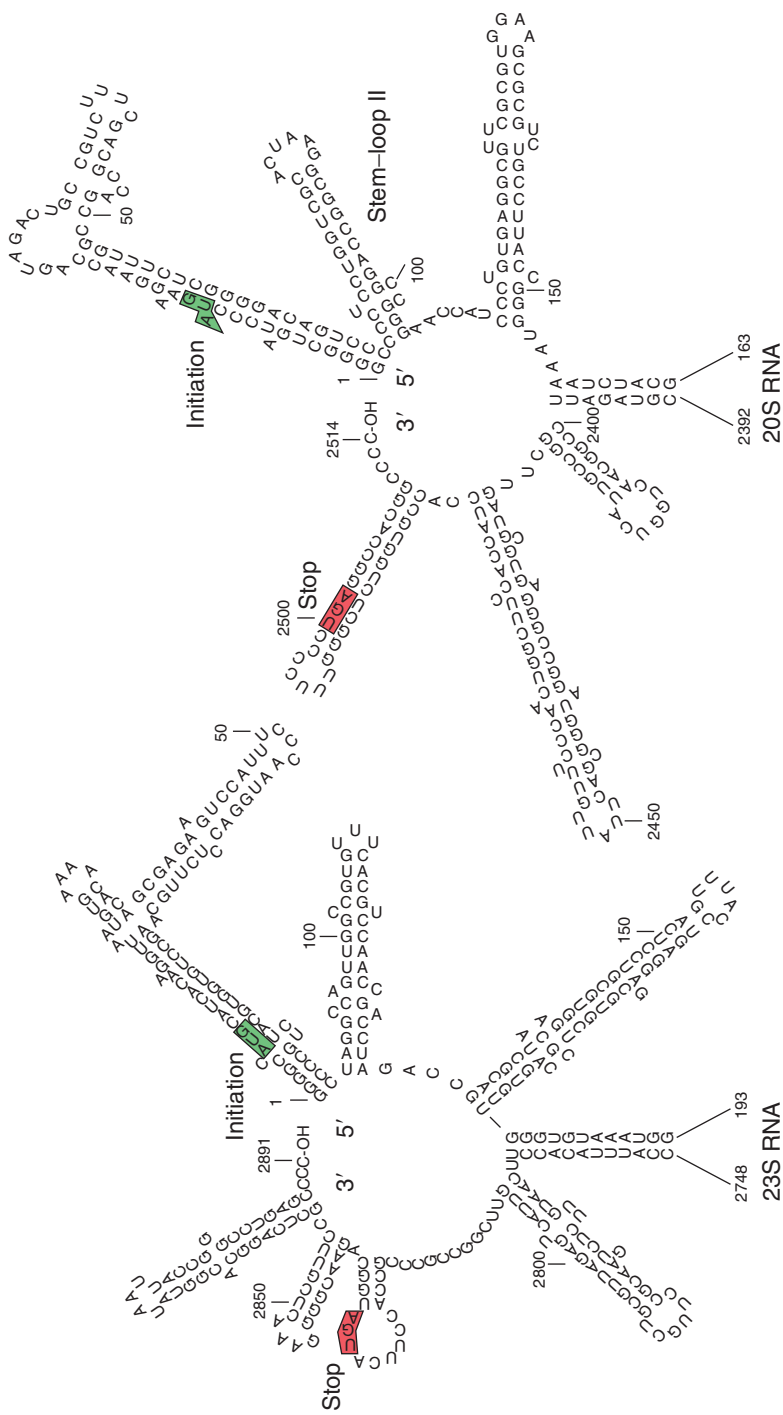


Figure 4 Secondary structures at the 5′ and 3′ end regions of 23S and 20S RNA positive strands, as predicted by the MFOLD program. The AUG initiation codons (green) and the stop codons (red) for p104 and p91 are boxed. Stem–loop II where the 5′ binding site for p91 is located is shown. Note that about 150 nt from the ends in each viral genome there are inverted repeats of 8–12 nt long that bring both 5′ and 3′ ends to a close proximity.

to the region close to the 5′ end of the same RNA molecule, p91 may stabilize the long-distance RNA–RNA interactions that bring the 5′ and 3′ ends of the RNA into proximity, and thus helps the RNA to form an organized structure in resting complexes.

It is not known whether the 3′ end repair is carried out by the replicase machinery during the replication process, or by host enzymes. However, the following evidence favors the latter case. The 3′ terminal structures of 20S and 23S RNAs resemble a half of tRNA, the so-called 'top-half' domain, consisting of the acceptor stem and T stem (**Figure 3**). The domain provides the determinants necessary for specific interactions with tRNA-related enzymes such as the tRNA nucleotidyltransferase (CCA-adding enzyme). This raises the possibility that 20S and 23S RNAs nibbled at the 3′ ends by 3′ exonucleases are repaired to the wild-type sequences by the CCA-adding enzyme. The fact that 15–30% of both positive and negative strands of 20S and 23S RNAs possess an unpaired A at the 3′ ends supports this possibility. Furthermore, that the 3′ repair is confined to the terminal and penultimate positions is consistent with the catalytic activity expected for the CCA-adding enzyme. Given that narnaviruses are persistent viruses, the underlying mechanism(s) to maintain the integrity of the viral 3′ ends will, therefore, have considerable significance for a long-term infection.

See also: Fungal Viruses; Yeast L-A Virus.

Further Reading

Buck KW, Esteban R, and Hillman BI (2005) *Narnaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 751–756. San Diego, CA: Elsevier Academic Press.

Esteban LM, Rodríguez-Cousiño N, and Esteban R (1992) T double-stranded (dsRNA) sequence reveals that T and W dsRNAs form a new RNA family in *Saccharomyces cerevisiae*: Identification of 23S RNA as the single-stranded form of T dsRNA. *Journal of Biological Chemistry* 267: 10874–10881.

Esteban LM, Fujimura T, García-Cuéllar MP, and Esteban R (1994) Association of yeast viral 23 S RNA with its putative RNA-dependent, RNA polymerase. *Journal of Biological Chemistry* 269: 29771–29777.

Esteban R and Fujimura T (2003) Launching the yeast 23S RNA narnavirus shows 5′ and 3′ *cis*-acting signals for replication. *Proceedings of the National Academy of Sciences, USA* 100: 2568–2573.

Esteban R and Fujimura T (2006) Yeast narnavirus replication. In: Hefferon KL (ed.) *Recent Advances on RNA Virus Replication*, pp. 171–194. Trivandrum, India: Research Ringspot.

Esteban R, Vega L, and Fujimura T (2005) Launching of the yeast 20S RNA narnavirus by expressing the genomic or anti-genomic viral RNA *in vivo*. *Journal of Biological Chemistry* 280: 33725–33734.

Fujimura T and Esteban R (2004) Bipartite 3′ *cis*-acting signal for replication in yeast 23 S RNA virus and its repair. *Journal of Biological Chemistry* 279: 13215–13223.

Fujimura T, Solórzano A, and Esteban R (2005) Native replication intermediates of the yeast 20S RNA virus have a single-stranded RNA backbone. *Journal of Biological Chemistry* 280: 7398–7406.

Kadowaki K and Halvorson HO (1971) Appearance of a new species of ribonucleic acid synthesized in sporulation cells of *Saccharomyces cerevisiae*. *Journal of Bacteriology* 105: 826–830.

Matsumoto Y and Wickner RB (1991) Yeast circular RNA replicon: Replication intermediates and encoded putative RNA polymerase. *Journal of Biological Chemistry* 266: 12779–12783.

Rodríguez-Cousiño N, Esteban LM, and Esteban R (1991) Molecular cloning and characterization of W double-stranded RNA, a linear molecule present in *Saccharomyces cerevisiae*: Identification of its single-stranded RNA form as 20S RNA. *Journal of Biological Chemistry* 266: 12772–12778.

Solórzano A, Rodríguez-Cousiño N, Esteban R, and Fujimura T (2000) Persistent yeast single-stranded RNA viruses exist *in vivo* as genomic RNA polymerase complexes in 1:1 stoichiometry. *Journal of Biological Chemistry* 275: 26428–26435.

Wejksnora PJ and Haber JE (1978) Ribonucleoprotein particle appearing during sporulation in yeast. *Journal of Bacteriology* 134: 246–260.

Wesolowski M and Wickner RB (1984) Two new double-stranded RNA molecules showing non-Mendelian inheritance and heat inducibility in *Saccharomyces cerevisiae*. *Molecular and Cellular Biology* 4: 181–187.

Widner WR, Matsumoto Y, and Wickner RB (1991) Is 20S RNA naked? *Molecular and Cellular Biology* 11: 2905–2908.

Nature of Viruses

M H V Van Regenmortel, CNRS, Illkirch, France

© 2008 Elsevier Ltd. All rights reserved.

The Discovery of Viruses

By the end of the nineteenth century, it had been established that many infectious diseases of animals and plants were caused by small microorganisms which could be visualized in the light microscope and could be cultivated as pure cultures on synthetic nutritive media. However, in the case of several infectious diseases, it had not been possible to identify the causative agent in spite of many carefully executed experiments. These results suggested that some other type of infectious agent existed which was not identifiable by light microscopy and could not be cultured on conventional bacteriological media.

The first evidence that such infectious agents, later called viruses, were different from pathogenic bacteria was obtained by filtration experiments with porcelain Chamberland filters used for sterilization. These bacteria-retaining filters, which had been used by Louis Pasteur to

remove pathogenic microorganisms from water, were used by Dmitri Ivanovsky in St. Petersburg in 1892 in his study of the tobacco mosaic disease. Ivanovky showed that when sap from a diseased tobacco plant was passed through the filter, the filtrate remained infectious and could be used to infect other tobacco plants. Although Ivanovsky was the first person to show that the agent causing the tobacco mosaic disease passed through a sterilizing filter, all his publications show that he did not grasp the significance of his observation and that he remained convinced that he was dealing with a small bacterium rather than with a new type of infectious agent. More than 10 years after his initial experiment he still believed that the filter he used might have had fine cracks which allowed small spores of a microorganism to pass through it.

The same filtration experiment was repeated on 1898 by Martinus Beijerinck in Delft, Holland, who, unaware of Ivanovsky's papers, again showed that the filtered tobacco sap from diseased plants was infectious. However, Beijerinck went further and demonstrated that the infectious agent was able to diffuse through several millimeters of an agar gel. From this, he concluded that the infection was not caused by a microbe but by what he called a *contagium vivum fluidum* or contagious living liquid. He established that the agent could reproduce itself in a tobacco plant and called it a virus. In the same year, a similar type of filtration experiment was done by Friedrich Loeffler and Paul Frosch who were investigating the important foot-and-mouth disease of cattle. These German investigators reported that although the causative agent of the disease passed through a Chamberland-type filter, it did not go through a Kitasato filter which had a finer grain than the Chamberland filter. From this result, they concluded that the causative virus, which was multiplying within the host, was a corpuscular particle and not a soluble agent as claimed by Beijerinck. Within a few years, it became generally accepted that filterable viruses that remained invisible in the light microscope represented a new class of pathogenic agent different from bacteria.

Although all historical accounts of the beginnings of virology mention the work of Ivanovsky, Beijerinck, and Loeffler, there is disagreement among various authors about who should be credited with the discovery that viruses were a new type of infectious agent. This is an interesting debate since it concerns the nature of what is a scientific discovery. It is indeed not sufficient to make a novel observation such as the filterability of an infectious agent but it is also necessary to interpret the observation correctly and to grasp the significance of an unexpected experimental finding. Ivanovsky was the first to observe the filterability of a virus but he did not recognize that he was dealing with a new type of infectious agent. Beijerinck realized that he was dealing with something different from a microbe but he thought that the virus was an infectious liquid rather than a small corpuscular particle. Only Loeffler correctly concluded that the virus causing foot-and-mouth disease was a small particle stopped by a fine-grain Kitasato filter and he therefore came closest to the modern concept of a virus. It was only the work of William Elford with graded collodion membranes, done more than 30 years later, which established that different viruses had particle diameters in the range of 20–200 nm. Only with the advent of the electron microscope was it finally possible to determine the actual morphology of virus particles.

Viruses as Chemical Objects

The perception of what viruses are changed dramatically in 1935 when Wendel Stanley, working at the Rockefeller Institute in Princeton, showed that tobacco mosaic virus (TMV), the agent studied by Ivanovsky and Beijerinck, could be crystallized in the form of two-dimensional paracrystals. This led to the view that viruses were actually chemical objects rather than organisms and it stimulated an intense interest in viruses which now seemed to be entities at the borderline between chemistry and biology. Many scientists became fascinated with viruses and viewed them as 'living molecules' since they seemed to be able to reproduce themselves. The needle-shaped crystals of TMV, visualized by Stanley, suggested that a dead protein could be a living infectious agent and it was believed by some that viruses might hold the key to the origin of life. Stanley had initially reported that TMV was a pure protein, but in 1936 Frederick Bawden and Norman Pirie in Britain showed that TMV also contained phosphorus and carbohydrate and was in fact an RNA-containing nucleoprotein. However, it took nearly another 20 years before it was established that it was the RNA in the virus that was the infectious entity.

Whether or not viruses should be regarded as living organisms has been regarded by some to be only a matter of taste. A definite answer to this question requires that one has a clear understanding of what is meant by 'life'.

What Is Life?

Life is not a material entity, nor a force, nor a property, but a conceptual object made up of the collection of all living systems, past, present, and future. All living systems possess the property of 'being alive' and the concept 'life' corresponds to the abstract, mental representation of this property. Philosophers say that 'life' is the extension of the predicate 'is alive', the extension of a concept being the objects that the concept refers to. Instead of analyzing the concept 'life', it is thus more relevant to ask which characteristics of biological organisms give them the property of being alive. One needs to ascertain to what objects the concept of 'life' refers to and thus to provide an answer to the question: what is a living organism?

What Is an Organism?

A simple answer to this question would be to say that organisms are living agents but this is unsatisfactory for several reasons, one of them being that it would rule out dead organisms. A better answer would be to say that organisms are living agents at some point of their existence. The related claim that all living agents are organisms is equally problematic since organs like hearts or kidneys are clearly not organisms although they are considered to be alive. Equating organisms with living agents is thus not satisfactory and these two concepts need to be differentiated.

A useful approach is to consider the class of living agents as a cluster concept. Such a concept is defined by a cluster of properties, the majority of which have to be present in all members of the class although some properties can be absent in individual members. Many of the properties tend to be present simultaneously because of the existence of underlying relationships between them: this means that if an individual member possesses any one of these properties, it increases the probability that it also possesses some of the others. Such a cluster concept, known as a polythetic class, is useful to group biological entities that show inherent variation and it has also been used to define virus species.

Living agents can be defined by a cluster of properties that include:

1. compositional and structural properties such as the presence of nucleic acids and proteins, and of heterogenous and specialized parts;
2. functional properties such as the capacity to grow and develop, to reproduce, and to repair themselves; and
3. properties such as metabolism and environmental adaptation that arise from the interaction of a living agent with its environment.

Biosystems interact selectively with the environment through a membrane boundary which restricts the types of exchanges that can occur between components of the system and items in its environment. The environment of a system consists of those things that can be influenced by the system or that may act upon the system. The notion of environment is thus limited to the immediate environment of the system and it exists only relative to a given system.

Any agent in order to be living must necessarily possess a sufficient subset of this cluster of properties, although it need not possess them all. For instance, sterile organisms that do not reproduce or plant seeds with a completely dormant metabolism can be included in the class of living agents.

In addition to having many of the properties listed above, living agents in order to qualify as organisms must also belong to a reproductive lineage characterized by a life cycle. Organs, therefore, are not organisms since they do not reproduce themselves as members of a lineage. Organs are replicated when the entire organism reproduces but they lack a life cycle. The various entities that comprise the life cycle of an organism correspond to developmental stages in the life of that organism.

Finally, in order to qualify as organisms, living agents must also possess a functional autonomy that allows them to exercise control over themselves and to be at least partly independent from other organisms and environmental influences. Organs, tissues, or the leaves of a plant, for instance, are living things but they are not organisms since they are not functionally autonomous, their life being dependent on that of the organisms they belong to.

It should be clear from the preceding discussion that things such as DNA molecules and other biochemical constituents of cells, as well as organelles such as ribosomes are neither living agents nor organisms. For the same reasons, viruses, although they are biological systems, are neither living agents nor *a fortiori* microorganisms.

Viruses Should Not Be Confused with Virus Particles or Virions

A virus has both intrinsic properties such as the size of the virus particle and relational properties such as having a host or vector, the second type of property existing only by virtue of a relation with other objects. Relational properties are also called emergent properties because they are possessed only by the viral system as a whole and are not present in its constituent parts. During its multiplication cycle, a virus takes on various forms, for instance, as a replicating nucleic acid in the host cell. One stage of what is metaphorically called the 'life cycle' of a virus corresponds to the virus particle or virion which can be characterized by intrinsic, structural, and chemical properties such as size, mass, chemical composition, and sequence of both coat proteins and nucleic acids.

Compared to a virion, a virus also possesses a number of relational properties that become actualized during transmission and infection, for instance, when the virus becomes integrated into the host cell during the viral replication cycle. A virus cannot be thus reduced to the physical constituents and chemical composition of a virion and it is necessary to include in its description the various biotic interactions and functional activities that make the virus a biological system. Confusing 'virus' with 'virion' is somewhat similar to confusing the entity 'insect', which includes several different life stages, with a single one of these stages such as a pupa, a caterpillar, or a butterfly.

When Eckhard Wimmer of Stony Brook University in New York gives the chemical formula of poliovirus as $C_{332,652}\ H_{492,288}\ N_{98,245}\ O_{131,196}\ P_{7,501}\ S_{2,340}$, he provides only the chemical composition of one poliovirus particle. However, this reductionist chemical description of a

virion does not amount to a characterization of the entity poliovirus.

The chemical composition or even the sequence of the coat protein of the virion does not give information on the tertiary and quaternary structural elements in the outer capsid that form the three-dimensional assembly of atoms that will be recognized by host cell receptors, allowing the virus to infect its host. The receptor-binding site of the virion is actually a relational, emergent biological entity defined by its ability to be recognized by the complementary receptor molecules present in certain host cells. The viral receptor-binding site is thus not an intrinsic feature of the virion that could be defined independently of an external cellular receptor since its identity as a site depends on the existence of a specific relationship with a host cell. This relationship arose during the process of biological evolution that culminated in the capacity of the virus to infect certain host cells. Such a specific functional relationship with a host is the essential feature that gives viruses their unique status as molecular genetic parasites capable of using cellular systems for their own replication.

The Nature of Viruses

Although viruses are not living organisms, they are considered to be biological entities because they possess some of the properties of living systems such as having a genome and being able to adapt to particular hosts and biotic habitats. However, viruses do not possess many of the essential attributes of living organisms such as the ability to capture and store free energy and they lack the characteristic autonomy that arises from the presence of integrated, metabolic activities. Viruses do not replicate or self-replicate themselves, but are 'being replicated' (i.e., passively rather than actively) through the metabolic activities of the cells they have infected. The replication of viruses involves a process of copying done by certain constituents of host cells. In contrast, cells do not replicate but reproduce themselves by a process of fission.

A virus becomes part of a living system only after its genome has been integrated in an infected host cell and it is not more alive than other cellular constituents such as genes, macromolecules, and organelles. The consensus among biologists is that the simplest system that can be said to be alive is a cell. Viruses are thus nonliving, infectious agents which can be said, at best, to lead a kind of borrowed life. Duncan McGeoch has described viruses metaphorically as "mistletoes on the Tree of Life."

The difference between viruses and various types of organisms becomes obvious when the functional roles of the proteins found in viruses and in organisms are compared. When proteins are divided into three broad functional categories corresponding to energy utilization, information carriers, and communication mediators, the proportion of each protein class found in viruses is markedly different from that found in living organisms.

Viruses have the highest proportion of proteins involved in information processes related to the control and expression of genetic information but have very few proteins of the energy and communication classes (**Figure 1**). This distribution is due to the fact that viruses utilize the metabolic machinery of the host cell and rely entirely on the energy supply systems of the host they infect. In contrast, bacteria have the highest proportion of proteins of the energy class involved in small-molecule transformation, whereas animals have a high proportion of proteins involved in intra- and intercellular communication.

Some authors have argued that viruses are living microorganisms because they share with certain parasitic organisms the property of being obligate parasites. However, the way viruses depend on their cellular hosts for replication is a type of molecular parasitism that is totally different from the metabolic dependency shown, for instance, by rickettsia or by bacteria that colonize the gut of certain animals. Obligate parasitism on its own is clearly not a sufficient criterion for establishing that an entity is a living organism.

Viruses are subcellular infectious agents which at one stage of their replication cycle in the infected cell are reduced to their nucleic acid component. The role of the virion is to allow transmission to new hosts while it also protects the viral genome from degradation by nucleases and from other environmental attack.

It is customary to distinguish acute viral infection which is associated with active replication and production of virions from the asymptomatic type of specific

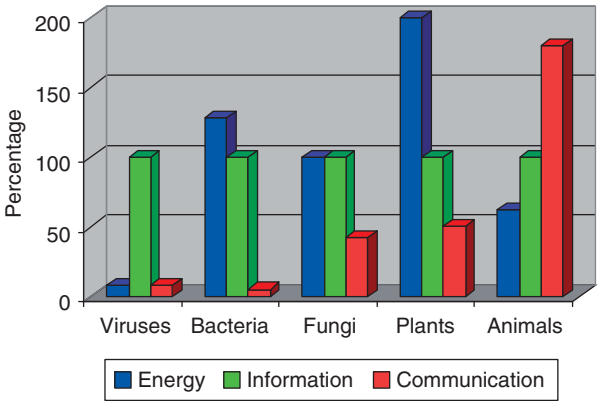


Figure 1 The proteins of viruses, microorganisms, plants, and animals have different functional roles. The vertical bars represent the proportion of proteins in the categories of energy utilization and carrier of information, relative to those in the category of mediator of information. Viruses have the highest proportion of proteins involved in information processes related to the control and expression of genetic information. Reproduced from Patthy L (1999) *Protein Evolution*. Oxford: Blackwell Science, with permission from Blackwell Publishing.

virus–host relationship characterized by latency, persistence, and absence of disease. In the latter case the viral genome is maintained in the host but no virions are produced for long periods of time and no antiviral host immune response is elicited. Small amounts of virions are produced episodically which is sufficient for transmission of the virus to new hosts. If the virus happens to switch to a new type of host, this may involve a changeover from no disease in the latent host to the appearance of disease following reactivation in the new host. This host-switching phenomenon is sometimes responsible for the appearance of new emerging viral diseases.

Fitness and Viral Evolution

Fitness is usually taken to be the property of an organism that ensures its survival and reproductive success in a variable and unpredictable environment. Since viruses are not living organisms, the fitness of a virus is usually measured in terms of virus progeny, that is, the number of virions produced during an acute virus infection. In the case of a latent, persistent viral infection, viral fitness has no equivalent quantitative definition. Whereas for acute virus infections, fitness is measured at the expense of the host (since infected cells die), in the case of latent infections, fitness of the virus merges with fitness of the host and the concept loses its usefulness for describing a differential capacity of the virus.

Even in the case of living organisms, the concept of fitness is of limited value for explaining Darwinian evolution. Natural selection has been defined as the process whereby organisms of a given variety outnumber those of other varieties and prevail in the long run, mainly due to their greater fertility and adaptness to changing environments. In fact, selection amounts only to differential survival and since fitness is defined as anything that promotes the chances of survival, survival of the fittest and natural selection amounts to no more than survival of the survivors.

Because of the small number of phenotypic characters that can be studied with viruses, virus evolution has always been difficult to study. Most studies have concentrated on phylogenetic analyses based on the comparison of genome sequences, with the expectation that genes will diverge in sequence as they evolve from a common ancestor. Like other genomes, viral genomes evolve through such mechanisms as mutation, recombination, and gene reassortment. Molecular analysis of viral evolution has concentrated on measurements of variation in the genotype, without much clarity about what this variation means in terms of survival value for the virus. In many cases, it seems that viruses may in fact have co-evolved with their hosts or vectors, due to the maintenance of host- or vector-restricted molecular constraints on otherwise much higher rates of evolution of the virus itself.

Viral Isolates, Strains, and Serotypes

The term virus isolate refers to any particular virus culture that is being studied and it is thus simply an instance of a given virus.

A viral strain is a biological variant of a virus that is recognizable because it possesses some unique phenotypic properties that remain stable under natural conditions. Characteristics that allow strains to be recognized include (1) biological properties such as a particular disease symptom or a particular host, (2) chemical or antigenic properties, and (3) the genome sequence when it is known to be correlated with a unique phenotypic character. If the only difference between a 'wild type' virus taken as reference and a particular variant is a small difference in genome sequence, such a variant or mutant is not given the status of a separate strain in the absence of a distinct phenotypic characteristic.

Strains that possess unique, stable antigenic properties are called serotypes. Serotypes necessarily also possess unique structural, chemical, and genome sequence properties that are related to the differences in antigenicity. Serotypes constitute stable replicating lineages which allow them to remain distinct over time. The infectivity of individual serotypes of animal viruses can be neutralized only by their own specific antibodies and not by antibodies directed to other serotypes. This inablility of serotype-specific antibodies to cross-neutralize other serotypes is important in the case of animal viruses that are submitted to the immunological pressure of their hosts.

See also: Origin of Viruses; Tobacco Mosaic Virus; Virus Particle Structure: Principles; Virus Species.

Further Reading

Grafe A (1991) *A History of Experimental Virolgy.* Heidelberg: Springer.
Mahner M and Bunge M (1997) *Foundations of Biophilosophy*. Berlin, Springer.
Patthy L (1999) *Protein Evolution*. Oxford: Blackwell Science.
Van Regenmortel MHV (2003) Viruses are real, virus species are man-made, taxonomic constructions. *Archives of Virology* 148: 2481–2488.
Van Regenmortel MHV (2007) The rational design of biological complexity. A deceptive metaphor. *Proteomics* 7: 965–975.
Villarreal LP (2005) *Viruses and the Evolution of Life.* Washington, DC: ASM Press.
Waterson AP and Wilkinson L (1978) *An Introduction to the History of Virology.* Cambridge: Cambridge University Press.
Wilson RA (2005) *Genes and the Agents of Life.* Cambridge: Cambridge University Press.
Witz J (1998) A reappraisal of the contribution of Friedrich Loeffler to the development of the modern concept of virus. *Archives of Virology* 143: 2261–2263.

Necrovirus

L Rubino, Istituto di Virologia Vegetale del CNR, Bari, Italy
G P Martelli, Università degli Studi, Bari, Italy

© 2008 Elsevier Ltd. All rights reserved.

Taxonomy and Classification

As the representative of a monotypic group, *Tobacco necrosis virus A* (TNV-A) was among the 16 groups of plant viruses described in 1971, and became the type species of the genus *Necrovirus* when it was established in 1995. Currently, *Necrovirus* is a genus in the family *Tombusviridae* and comprises seven definitive member species (i.e., *Beet black scorch virus* (BBSV), *Chenopodium necrosis virus* (ChNV), *Leek white stripe virus* (LWSV), *Olive latent virus 1* (OLV-1), *Olive mild mosaic virus* (OMMV), *Tobacco necrosis virus A* (TNV-A), and *Tobacco necrosis virus D* (TNV-D)) and two tentative species (i.e., Carnation yellow stripe virus (CYSN) and Lisianthus necrosis virus (LNV)).

Virion Properties

Necroviruses have very stable particles which resist temperatures in excess of 90 °C. TNV-A virions sediment a single component with a coefficient ($S_{20,w}$) of 118S and have buoyant density of 1.399 g ml^{-1} at equilibrium in CsCl.

Virion Structure and Composition

Virions are approximately 28 nm in diameter, have angular profile and a capsid made up of 60 copies of a trimer consisting of three chemically identical but independent protein subunits (A, B, and C) stabilized by Ca^{2+} ions, arranged in a $T = 3$ lattice. Subunit size ranges from 24–27 kDa (BBSV and LWSV, respectively) to 29–30 kDa (other viral species). The capsid has a smooth appearance as protein subunits lack the protruding domain proper of members of the majority of the other genera in the family *Tombusviridae*.

Virions encapsidate a molecule of single-stranded, positive-sense RNA, approximately 3.7 kb in size, constituting *c.* 19% of the particle weight. The genome of five definitive viral species has been fully sequenced: TNV-A, 3684 nt (accession NC001777); TNV-D, 3762 nt (NC003487, U62546); LWSV, 3662 nt (X94660), OLV-1, 3699 nt (X85989); and OMMV, 3683 nt (NC006939).

Genome Organization and Expression

The monopartite genome contains four open reading frames (ORFs) which, in the order from the 5′ to the 3′ terminus, code for replication-associated proteins (ORF1 and ORF1-RT), movement proteins (ORF2 and ORF3), and the coat protein (CP) (ORF4). Some species possess a fifth ORF either located in the 3′ terminal region (TNV-A) or in the middle of the genome (TNV-D^{H} and BBSV), partially overlapping the C-terminus of ORF1-RT and the N-terminal region of ORF2. The genome is very compact, having noncoding regions of limited size (**Figure 1**).

Genomic RNA acts as messenger for the translation of a protein of 22–24 kDa from ORF1. By translational readthrough of the UAG termination codon of ORF1, a protein of 82–83 kDa is synthesized (**Figure 1**), which contains, in the readthrough portion, the GDD motif of RNA-dependent RNA polymerases (RdRp). This

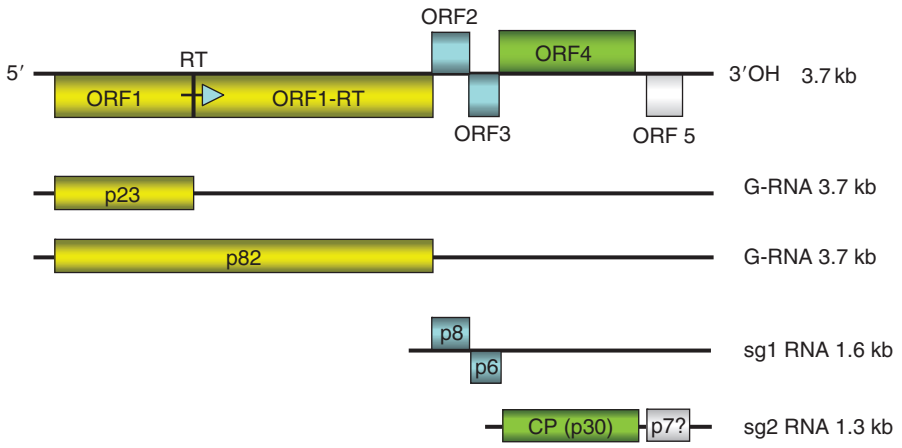


Figure 1 Genome organization and replication strategy of tobacco necrosis virus A.

protein, together with the expression product of ORF1, is indispensable for virus replication.

Genes downstream ORF1-RT are expressed via the synthesis of two subgenomic RNAs, 1.4–1.6 nt and 1.1–1.3 nt in size (**Figure 1**).

ORFs 2 and 3 are two small centrally located ORFs which, depending on the viral species, encode proteins of 7–8 and 6–7 kDa, respectively. Both of these proteins are involved in cell-to-cell transport but are dispensable for viral replication. LWSV ORF2 differs in size from that of other sequenced necroviruses, for it codes for a protein of 11 kDa. The additional central ORFs of TNV-D^H and BBSV code for a 7 kDa and a 5 kDa protein, respectively which, like the products of ORFs 2 and 3, are necessary for cell-to-cell movement.

ORF4 is the CP gene encoding a 24–30 kDa protein, the building block of the capsid, which is required also for efficient systemic virus spreading in the host. No experimental evidence is available of the expression of TNV-A ORF5.

Interspecific Relationships

Necroviruses are serologically distinguishable from one another. Some are unrelated (e.g., LWSV with TNV-A and TNV-D), whereas others share antigenic determinants that result in weak relationships. This is the case of TNV-A and TNV-D that are distantly related with OLV-1 (serological differentiation index from 6 to 9), and of OMMV or BBSV with certain TNV isolates.

The level of molecular similarity varies very much with the viral species. Thus, the RdRp of OMMV, a putative recombinant between TNV-D and OLV-1, shows 91.2% identity at the amino acid level with the comparable protein of OLV-1, whereas the identity level of CP is highest (86.2%) with that of TNV-D. Among the other sequenced species in the genus, the amino acid identity of RdRps and CPs is much lower, ranging from 32% to 34% and 35% to 50%, respectively.

Satellites

TNV-A and TNV-D activate the replication of a satellite virus (TNSV) present in nature as four biologically and serologically distinct strains, supported by different viral isolates. TNSV has isometric particles *c.* 17 nm in diameter, sedimenting as a single component with a coefficient of 50S. Its capsid is constructed with 60 identical subunits *c.* 22 kDa in size, arranged in a $T = 1$ lattice, and contains a single-stranded, positive-sense RNA molecule 1239 nt in size. TNSV RNA accounts for *c.* 20% of the particle weight, and comprises a single ORF encoding the CP.

Whereas satellite viruses are not associated with any other member of the genus, BBSV supports the replication of a small, linear, single-stranded, noncoding satellite RNA 615 nt in size, which is encapsidated in the virions in monomeric or, more rarely, dimeric form. Monomers are thought to be produced from multimeric templates.

TNSV interferes to some extent with helper virus infections for its presence in TNV inocula reduces slightly the virus concentration and the size but not the number of local lesions. By contrast, BBSV satRNA enhances the aggressiveness of the helper virus for more lesions are produced on infected hosts when mixed inocula are used.

Transmission and Host Range

All necroviruses are readily transmitted by mechanical inoculation to experimental herbaceous hosts, which usually react with necrotic local lesions, not followed by systemic infection. Under natural conditions, infection is often restricted to the roots. Some members are transmitted through the soil either by the chitrid fungus *Olpidium brassicae* (TNV-A, TNV-D, BBSV), or without the apparent intervention of a vector (OLV-1). Particles of species transmitted by *O. brassicae* are acquired by the vector from the soil where they are released from roots of infected plants through sloughing off epidermal cell layers and/or following decay of plant debris. Virions are bound tightly to the plasmalemma and the axoneme (flagellum) of the fungal zoospores and are transported inside the zoospore cytoplasm when the flagellum is retracted prior to encystment that precedes penetration into the host. The same mechanism is exploited by TNSV to gain entrance into both vector and host. Since necroviruses and their satellites (TNSV and BBSV satRNA) multiply in the plant cells but not in the fungal plasmodium, zoospores released from infected roots are virus free. Transmission can therefore occur only if they come again in contact with and adsorb virus particles.

Seed transmission of necroviruses has not been reported, except for OLV-1, which was detected in the integuments and internal tissues of 82% olive seeds, and transmitted to 35% of the seedlings.

The natural host range and geographical distribution of necroviruses varies with the species. Thus, TNV-A and TNV-D are ubiquitous and infect a wide range of cultivated and wild plants. OLV-1 was recorded from olive in several Mediterranean countries, citrus in Turkey, and tulip in Japan, OMMV from olive in Portugal, BBSV from beet in China, and LWSV from leek in France.

Virus–Host Relationships

Necroviruses replicate very actively in their hosts, which translates into the production of a large number of virus particles in infected cells of all tissue types, including vessels. Virions are either scattered in the cytoplasm,

gathered in bleb-like evaginations of the tonoplast into the vacuole, or arranged in crystalline arrays of various sizes. TNSV particles can also give rise to crystals that can be found in the same cells along with those of the helper virus. Cells infected by TNV and OLV-1 also contain two types of inclusions, that is, clumps of electron-dense amorphous material resembling accumulations of excess CP and fibrous bundles made up of thin filaments with a helical structure. In OLV-1 infected cells, these bundles were identified as accumulations of the 8 kDa movement protein expressed by ORF2. The same protein and the 6 kDa movement protein coded for by ORF3 were detected by immunogold labeling near plasmodesmata. Cytoplasmic clusters of membranous vesicles with fibrillar material, derived from the endoplasmic reticulum or lining the tonoplast, were observed in cells infected by OLV-1 and LWSV.

See also: Carmovirus; Plant Virus Diseases: Ornamental Plants; Tombusviruses.

Further Reading

Castellano MA, Loconsole G, Grieco F, *et al.* (2005) Subcelluar localization and immunodetection of movement proteins of olive latent virus 1. *Archives of Virology* 150: 1369.

Gua L-H, Cao H-E, Li D-W, *et al.* (2005) Analysis of nucleotide sequences and multimeric forms of a novel satellite RNA associated with beet black scorch virus. *Journal of Virology* 79: 3664.

Lommel SA, Martelli GP, Rubino L, *et al.* (2005) Necrovirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, 926pp. San Diego, CA: Elsevier Academic Press.

Molnar H, Havelda Z, Dalmay T, *et al.* (1997) Complete nucleotide sequence of tobacco necrosis virus D^{H} and genes required for RNA replication and virus movement. *Journal of General Virology* 78: 1235.

Oda Y, Saeki K, Takahashu Y, *et al.* (2000) Crystal structure of tobacco necrosis virus at 2.25 Å resolution. *Journal of Molecular Biology* 300: 153.

Russo M, Burgyan J, and Martelli GP (1994) Molecular biology of Tombusviridae. *Advances in Virus Research* 44: 381.

Uyemoto JK (1981) Tobacco necrosis and satellite viruses. In: Kurstak E (ed.) *Handbook of Plant Virus Infections and Comparative Diagnosis*, 123pp. Amsterdam, The Netherlands: Elsevier/North Holland Biochemical Press.

Nepovirus

H Sanfaçon, Pacific Agri-Food Research Centre, Summerland, BC, Canada

Crown Copyright © 2008 Published by Elsevier Ltd. All rights reserved.

Glossary

Odonstyle Anterior section of the stylet of a longidorid nematode. The odonstyle consists of a needle-like mouth spear used to penetrate plant root cells.

Odontophore Posterior section of the stylet of a longidorid nematode. The odontophore is located at the base of the nematode mouth. Stylet protactor muscles are attached to the ondotophore.

Triradiate lumen Posterior region of the lumen (or food channel) of the esophagus of a longidorid nematode. The triradiate lumen is located in a muscular bulb at the base of the esophagus. The radial muscles attached to the triradiate lumen are used for food ingestion.

Introduction and Historical Perspective

The genus *Nepovirus* was among the first 16 groups of viruses recognized by the International Committee on Taxonomy of Viruses (ICTV). The name stands for nematode-transmitted viruses with polyhedral particles. Although nematode transmission was one of the original defining characteristics of the genus, the primary criteria for inclusion of viruses in the genus are now the structure of the RNA genome and of the particles. As a result, not all viruses belonging to the genus *Nepovirus* are nematode transmitted (**Table 1**), and some nematode-transmitted polyhedral viruses have been reclassified in distinct genera (e.g., strawberry latent ringspot virus is now considered a tentative member of genus *Sadwavirus*).

Taxonomy and Relation to Other Viruses

The genus *Nepovirus*, along with the genera *Comovirus* and *Fabavirus*, belongs to the family *Comoviridae*. Nepoviruses are also related to the unassigned genera *Sadwavirus* and *Cheravirus* which include viruses previously considered as tentative nepoviruses. Common characteristics among members of these five genera include: small polyhedral particles, a bipartite positive-strand RNA genome, and a conserved arrangement of protein domains within the polyproteins encoded by RNA1 and -2. Nepoviruses are distinguished from viruses of the other four genera by their single large coat protein (CP). This classification is supported by phylogenetic comparisons of the RNA

Table 1 Some properties of nepoviruses

			Database accession numbers			
			RNA1	*RNA2*	*Satellite RNAs*	
Virus name	*Abbreviation*	*Vector*[a]			*Type B*[b]	*Type D*
Subgroup A (*RNA2 M_r 1.3–1.5 × 10^6*)						
Arabis mosaic virus	ArMV	*Xiphinema diversicaudatum*	NC006057	NC006056	NC003523	NC001546
Arracacha virus A	AVA					
Artichoke Aegean ringspot virus	AARSV					
Cassava American latent virus	CsALV					
Grapevine fanleaf virus	GFLV	*X. index*; *X. italiae*[c]	NC003615	NC003623	NC003203	
Potato black ringspot virus	PBRSV		AJ616715			
Raspberry ringspot virus	RpRSV	*Longidorus elongatus*; *L. macrosoma; Paralongidorus maximus*	NC005266	NC005267		
Tobacco ringspot virus	TRSV	*X. americanum*	NC005097	NC005096		NC003889
Subgroup B (*RNA2 M_r 1.4–1.6 × 10^6*)						
Artichoke Italian latent virus	AILV	*L. apulus*; *L. fasciatus*		X87254		
Beet ringspot virus[d]	BRSV	*L. elongatus*	NC003693	NC003694		
Cocoa necrosis virus	CNV					
Crimson clover latent virus	CCLV					
Cycas necrotic stunt virus	CNSV		NC003791	NC003792		
Grapevine chrome mosaic virus	GCMV		NC003622	NC003621		
Mulberry ringspot virus	MRSV	*L. martini*			+	
Myrobalan latent ringspot virus	MLRSV					
Olive latent ringspot virus	OLRSV			AJ277435		
Tomato black ring virus	TBRV	*L. attenuatus*	NC004439	NC004440	NC003890	
Subgroup C (*RNA2 M_r 1.9–2.2 × 10^6*)						
Apricot latent ringspot virus	ALRSV			AJ278875		
Artichoke yellow ringspot virus	AYRSV					
Blackcurrant reversion virus	BRV	*Cecidophyopsis ribis* (mite)	NC003509	NC003502	NC003872	
Blueberry leaf mottle virus	BLMoV		U20622	U20621		
Cassava green mottle virus	CsGMV					
Cherry leaf roll virus	CLRV		Z34265	U24694		
Chicory yellow mottle virus	ChYMV				NC006452	NC006453
Grapevine Bulgarian latent virus	GBLV				+	
Grapevine Tunisian ringspot virus	GTRSV					
Hibiscus latent ringspot virus	HLRSV					
Lucerne Australian latent virus	LALV					
Peach rosette mosaic virus	PRMV	*X. americanum; L. diadecturus*[e]	AF016626			
Potato virus U	PVU					
Tomato ringspot virus	ToRSV	*X. americanum*; *X. bricolensis* *X. californicum*; *X. rivesi*	NC003840	NC003839		

[a]Only cases for which a specific association with a nematode vector has been verified experimentally are reported here. Nonvalidated associations with nematode vectors have been reported for the following viruses: CLRV – *Xiphinema* sp., GCMV – *X. index*, PBRSV – *X. americanum*, PVU – *Longidorus* sp.

[b]The sequence accession number of satellite RNAs is given when known. In the case of MLRSV and GBLV, the + symbol indicates that a type B satellite is known to be associated with the virus but has not been sequenced.

[c]Transmission of GFLV by *X. italiae* has been reported in only one instance. Many other populations of *X. italiae* did not transmit the virus. Thus, *X. index* is considered the main vector of GFLV.

[d]BRSV was previously known as TBRV-S or TBRV-Scottish.

[e]Transmission of PRMV by *L. diadecturus* was reported for only one location in spite of the widespread distribution of the vector in North America. Thus, *X. americanum* is considered the main vector of PRMV.

genomes. Nepoviruses are similar to members of the family *Picornaviridae* in that they share the same modular arrangement of replication proteins on the polyproteins, conserved motifs in these replication proteins, and a similar capsid structure. Nepoviruses have been divided into three subgroups based on the length and packaging of RNA2, sequence similarities, and serological properties (subgroups A, B, and C; see **Table 1**).

Virus Particle Structure

Nepoviruses have isometric particles of 26–30 nm in diameter with sharp hexagonal outlines (**Figure 1(a)**). Equilibrium centrifugation in CsCl of purified virus particles typically reveals the presence of three types of particles. T-particles (top component) sediment at 50S and do not contain an RNA molecule. In electron microscopy, these empty particles are penetrated by a negative stain. B-particles (bottom component) sediment at 115–134S and contain a single molecule of RNA1. In the case of subgroup A nepoviruses, B-particles can also contain two molecules of RNA2. M-particles (middle component) sediment at 86–128S and contain a single molecule of RNA2. M- and B-particles of subgroup C nepoviruses are often difficult to separate due to the larger size of RNA2.

Nepovirus particles contain 60 molecules of a single CP with an M_r of $(53–60) \times 10^3$. Tobacco ringspot virus (TRSV) (see **Table 1** for abbreviations) is the only nepovirus for which the atomic structure of the virus particle has been solved. The pseudo $T = 3$ structure was found to be very similar to that of comoviruses and picornaviruses. The single CP contains three functional domains with one β-barrel each. Each functional domain corresponds to one of the three smaller CPs found in picornaviruses. Comoviruses also share a similar structure with one large CP containing two β-barrels and one small CP with a single β-barrel. It was suggested that the CP(s) of nepoviruses, comoviruses, and picornaviruses have evolved from a common ancestor.

Genome Structure

The two RNA molecules of nepoviruses are polyadenylated at the 3′ end and are covalently linked to a small viral protein (VPg) at the 5′ end. Each RNA codes for one large polyprotein which is cleaved by a viral

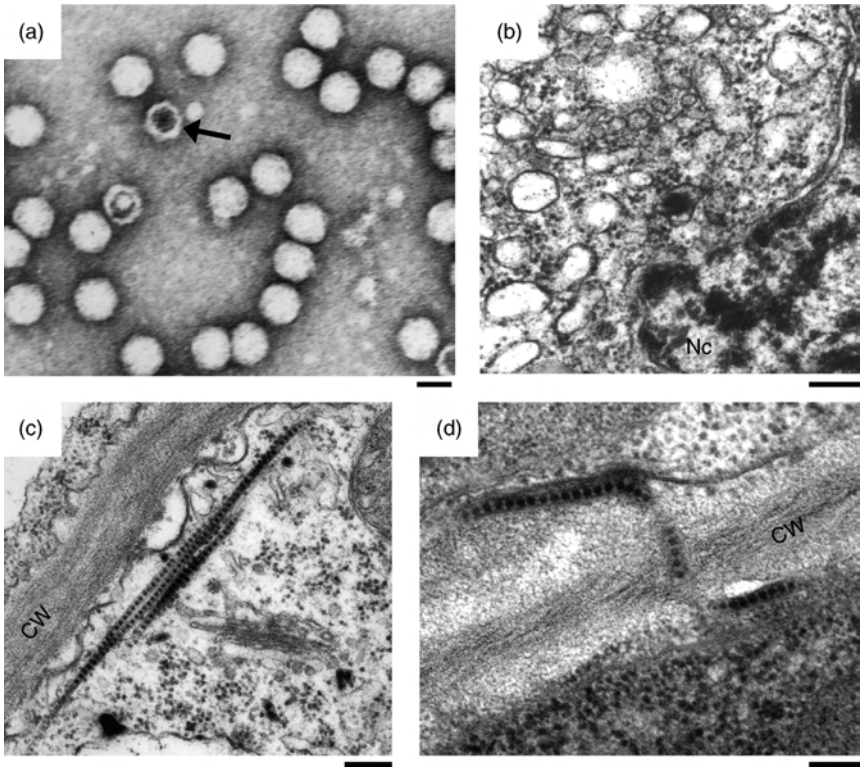


Figure 1 Electron micrograph depicting purified nepovirus particles and cytopathological structures typical of nepovirus-infected cells. (a) Purified ToRSV particles in negative staining. Note the empty particle (T-particle) which is penetrated by the negative stain (arrow). (b) Proliferation of membrane vesicles observed in the vicinity of the nucleus (Nc) in ToRSV-infected cells. (c) Tubular structures containing virus-like particles accumulating near the cell wall (CW) in PRMV-infected cells. (d) Tubular structure traversing the cell wall in ArMV-infected cells. Scale = 25 nm (a), 200 nm (b–d).

proteinase (Pro). RNA1 encodes replication proteins and can replicate independently of RNA2. RNA1 and -2 are both required for cell-to-cell movement of the virus. The identification of conserved sequence motifs and the characterization of cleavage sites recognized by the viral Pro have led to the definition of protein domains within the polyproteins. The genomic organization of a representative virus from each subgroup is shown in **Figure 2**. The RNA2-encoded polyprotein includes the domains for the CP and movement protein (MP) at its C-terminus as well as one (for subgroup A and B nepoviruses) or two (for ToRSV, a subgroup C nepovirus) additional protein domains at its N-terminus. The C-terminal portion of the RNA1-encoded polyprotein contains the domains for the putative helicase (also termed NTB protein because it contains a conserved nucleoside triphosphate-binding sequence motif), VPg, Pro, and RNA-dependent RNA polymerase (Pol). In the case of ToRSV, characterization of cleavage sites recognized *in vitro* by the viral Pro has resulted in the identification of two protein domains upstream of the NTB domain: X1 of unknown function and X2. X2 is a highly hydrophobic protein that shares conserved sequence motifs with the RNA1-encoded 32 kDa protein of comoviruses. An equivalent domain is present in the N-terminal region of the RNA1-encoded polyprotein of subgroup A and B nepoviruses (shown in orange in **Figure 2**). Further characterization of cleavage sites will be necessary to determine whether this region constitutes an independent protein domain in these viruses or whether it is included as part of a larger NTB protein (in the case of GFLV) or 1a protein (in the case of BRSV).

In addition to the coding region, each RNA includes untranslated regions (UTRs) at its 5′ and 3′ ends. The 5′ UTR is 70–300 nt long while the 3′ UTR varies in length from 200–400 nt for subgroup A and B nepoviruses to 1300–1600 nt for subgroup C nepoviruses. A short conserved sequence is present at the immediate 5′ end of the RNAs of many but not all nepoviruses. In addition, conserved structural features have been identified in the 5′ UTRs of subgroup A and B nepovirus RNAs (i.e., a series of stem and loop structures). The presence of conserved sequences and/or structural motifs within the UTRs points to a possible role for these elements in the viral replication cycle. However, this needs to be confirmed experimentally. In addition to these short conserved motifs, RNA1 and -2 often share regions of complete or partial sequence identity in the UTRs (**Figure 2**). In subgroup B and C nepoviruses, the 3′ UTRs are identical between RNA1 and -2 while the 5′ UTRs share 68–100% sequence identity. For example, the 5′ UTRs of ToRSV RNA1 and -2 share a region of 100% sequence identity which extends into the coding region. On the other hand, the 5′ UTRs of the BRV RNAs share only 78% sequence identity and do not extend beyond the UTR. In subgroup A nepoviruses, the 5′ and 3′ UTRs of RNA1 and -2 share homology but are not identical (70–97% sequence identity). It was suggested that the extensive regions of sequence identity detected in the 5′ and 3′ ends of ToRSV RNAs are the result of recombination events occurring during replication of the viral RNAs. Experimental support for this suggestion was provided using pseudorecombinants consisting of GCMV RNA1 and BRSV RNA2. Sequencing of the viral progeny revealed that the 3′ UTR of GCMV RNA1 was transferred to BRSV RNA2 after three passages. In contrast, recombination was not readily observed between the 3′ UTRs of BLMV RNA1 and -2. It was suggested that selection rather than recombination played a role in the conservation of sequence identity in BLMV RNAs.

So far, the production of infectious cDNA clones has only been reported for subgroup A nepoviruses (GFLV, ArMV, RpRSV). Thus, reverse genetics is only possible

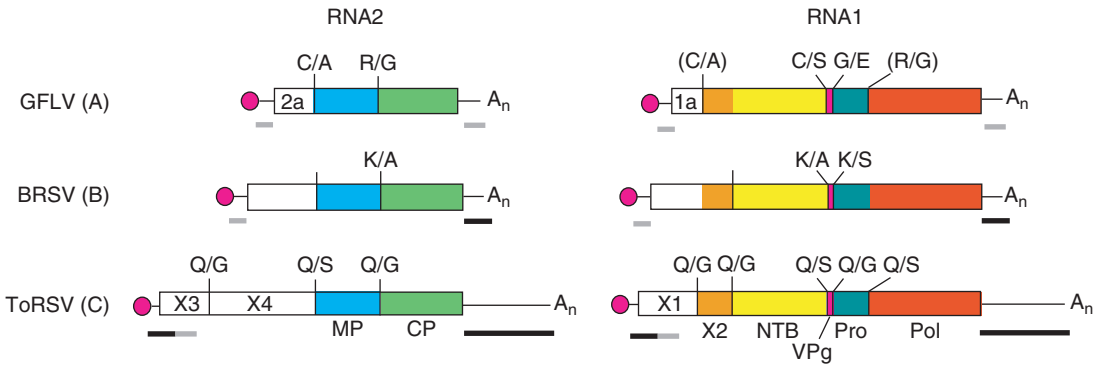


Figure 2 Genomic organization of representative nepoviruses of subgroup A (GFLV), B (BRSV), and C (ToRSV). Each RNA is represented with the covalently attached VPg (pink circle) and the polyA tail (A_n). The coding regions are represented by the boxes. Cleavage sites confirmed by *in vitro* processing experiments or by the detection of viral proteins in infected plants are indicated by the continuous vertical lines. Identified or putative (in parentheses) cleavage sites are indicated above each line when known. The function of each protein domain is indicated at the bottom of the figure. Thick bars below each RNA indicate regions with high degree of sequence identity between RNA1 and -2. The black portions indicate regions with 100% sequence identity and the gray portions represent regions with 75–83% sequence identity.

for these viruses. Removal of the covalently linked VPg from purified viral RNAs either decreased (ArMV, RpRSV) or abolished (TRSV, ToRSV, BRSV) their infectivity, although it did not affect the ability of these RNAs to be translated *in vitro*. Possibly, the requirement for an intact VPg is an obstacle for the production of infectious clones for some nepoviruses.

Regulated Polyprotein Processing

Nepoviruses encode a single proteinase which is responsible for processing the two polyproteins. Nepovirus proteinases are related to the 3C-Pro of picornaviruses. The catalytic triad consists of a histidine, aspartic acid, and cysteine. The proteinase also contains a substrate-binding pocket which determines its cleavage site specificity. A conserved histidine is found in the substrate-binding pocket of subgroup C nepovirus, comovirus, and picornavirus proteinases. The cleavage sites recognized by subgroup C nepovirus proteinases contain a glutamine, asparagine, or aspartate at the −1 position (**Table 2**). This is similar to the conserved glutamine or glutamate found at the −1 position of picornavirus cleavage sites. In contrast, the proteinases of subgroup A and B nepoviruses contain a leucine instead of a histidine in their substrate-binding pocket and recognize very different cleavage sites that have a lysine, cysteine, arginine, or glycine at the −1 position.

The RNA1-encoded polyprotein is cleaved predominantly intramolecularly (*cis*-cleavage) although intermolecular processing (*trans*-cleavage) of the N-terminal cleavage site has been reported for GFLV. The RNA2-encoded polyprotein is cleaved *in trans* by the proteinase. The processing cascade results in the release of mature proteins as well as stable processing intermediates containing two or more protein domains. These intermediate polyproteins accumulate in infected plant cells and may have different activities from the mature proteins. In ToRSV-infected cells, several polyprotein intermediates containing the NTB domain are detected in addition to the mature NTB protein. In BRSV-infected cells, an intermediate containing the Pro and Pol domains accumulates rather than the mature Pro and Pol proteins. This suggests preferential recognition of some cleavage sites by the viral Pro. Slow release of mature proteins by processing of stable intermediates at suboptimal cleavage sites may provide a regulatory mechanism to control the accumulation of specific protein species during the replication cycle. In fact, the activity of the proteinase itself is regulated by its release from larger polyprotein precursors. Indeed, the mature proteinase of GFLV and ToRSV cleaves cleavage sites on the RNA2-encoded polyprotein more efficiently than the VPg-Pro precursor.

Table 2 Identified cleavage sites in the polyprotein of nepoviruses

Virus	*Cleavage site*
Subgroup A	
ArMV	R/G
GFLV	R/G, C/A, C/S, G/E
RpRSV	C/A
TRSV	C/A
Subgroup B	
BRSV	K/A, K/S
GCMV	R/A
OLRSV	K/A
CNSV	K/S
Subgroup C	
BRV	D/S
BLMoV	N/S
CLRV	Q/S
ToRSV	Q/G, Q/S

Viral RNA Replication

Infection of plant cells by nepoviruses results in membrane proliferation and the formation of cytoplasmic inclusion bodies which contain membrane vesicles (**Figure 1(b)**). The use of cerulenin, an inhibitor of *de novo* phospholipid synthesis, has confirmed that membrane proliferation is required for the replication of GFLV RNAs. Brefeldin A also inhibits GFLV replication suggesting a requirement for intact vesicle trafficking between the endoplasmic reticulum (ER) and the Golgi apparatus. The replication complex of two nepoviruses (GFLV and ToRSV) has been shown to co-localize with ER-derived membranes in infected cells. Double-stranded RNA replication intermediates, viral replication proteins, and replication activity are associated with the membrane-bound complexes. GFLV VPg antibodies were used to isolate membrane vesicles that had a rosette-like structure similar to that associated with picornavirus replication complexes.

In the case of ToRSV, the mature NTB protein or a larger intermediate polyprotein containing the NTB domain has been proposed to play a role in anchoring the replication complex to the membranes. The mature NTB and the intermediate NTB-VPg polyprotein are integral membrane proteins that co-fractionate with the replication complex. The NTB protein is targeted to ER membranes when expressed individually and contain two membrane-binding domains: a C-terminal transmembrane domain and a putative N-terminal amphipathic helix. The ToRSV X2 protein is also an ER-targeted integral membrane protein and may play a role in viral replication although its association with the replication complex in infected cells remains to be confirmed. Other replication proteins (Pro and Pol) are soluble when expressed individually but are found in association with

the membrane-bound replication complex in infected cells. Thus, they are probably brought to the replication complex either as part of a larger polyprotein that includes the NTB domain or through protein–protein interaction with the viral membrane anchors. By analogy with picornaviruses, the VPg protein may act as a primer for viral replication, although this has not been demonstrated experimentally for nepoviruses. In the context of the NTB-VPg polyprotein, the VPg domain is translocated in the lumen of the membranes. Because replication presumably takes place on the cytoplasmic side of the membranes, it is unlikely that the luminally oriented VPg present in the NTB-VPg protein plays an active role in replication. Therefore, other intermediate precursors containing the VPg domain (e.g., VPg-Pro or VPg-Pro-Pol) probably act as donors for a replication-active VPg protein. In addition to RNA1-encoded replication proteins, the GFLV RNA2-encoded 2a protein is also associated with the replication complex. The 2a protein is required for the replication of RNA2 but not RNA1 and probably interacts with RNA1-encoded replication proteins.

Cell-to-Cell and Long-Distance Movement in the Plant

Nepovirus-infected cells are characterized by the presence of tubular structures containing virus-like particles in or near the cell wall (**Figures 1(c)** and **1(d)**). These tubules are similar to the ones found in comovirus-infected cells and have been suggested to direct the cell-to-cell movement of intact virus particles. The viral MP is a structural component of the tubular structure. Expression of the GFLV MP alone is sufficient to induce the formation of empty tubular structures in intact plant cells or protoplasts. In the latter case, tubular extensions are found projecting from the surface of the protoplasts, a phenomenon also induced by comovirus MP. The GFLV MP is an integral membrane protein. The secretory pathway and the cytoskeleton are involved in the intracellular targeting of the GFLV MP from its site of synthesis (probably in association with the ER-bound replication complex) to specific foci in the cell wall where it assembles into tubules. The presence of specific sites of tubule formation within the cell wall suggests an interaction between MP and a cellular receptor but this has not been confirmed experimentally. By analogy with comoviruses, it is likely that an interaction between MP and CP is necessary to enable nepovirus cell-to-cell movement. Long-distance movement of TRSV occurs through the phloem resulting in the invasion of most tissues of the plant including meristematic tissues. The virus probably reaches the phloem through cell-to-cell movement from inoculated cells to phloem sieve tubes.

Host Range, Symptomatology, and Interaction of Nepoviruses with the Plant Post-Transcriptional Gene Silencing Pathway

Most nepoviruses have a wide host range that includes woody and herbaceous hosts. In nature, many hosts remain symptomless while others display symptoms such as necrotic or chlorotic rings which can appear as concentric rings (ringspots) or lines. Other symptoms can include leaf flecking and mottling, vein necrosis, plant stunting, and in some cases death. Common experimental hosts used for virus propagation are *Chenopodium quinoa* (in which most nepoviruses induce obvious symptoms), *C. amaranticolor*, *C. murale*, *Cucumis sativus*, *Nicotiana clevelandii*, *N. benthamiana*, *Petunia hybrida,* and *Phaseolus vulgaris.* The intensity of symptoms produced by nepovirus infection depends on the specific virus–host combination and to a large extent on environmental conditions. In many herbaceous hosts and in particular in *Nicotiana* species, symptoms develop on the inoculated leaves and on the first upper systemic leaves. Later in infection, new leaves remain free of symptoms although the virus is present. This phenomenon is termed recovery. Recovered leaves often contain somewhat reduced titer of the virus compared to symptomatic leaves and are resistant to secondary viral infection in a sequence-specific manner. This suggests that induction of the plant RNA silencing machinery plays a role in virus clearance and symptom recovery. It was recently shown that although recovery of *N. benthamiana* from necrotic symptoms induced by ToRSV is accompanied with induction of RNA silencing, the virus titer is not significantly reduced in recovered leaves. Thus, the relationship between symptom recovery and RNA silencing may be more complex than first envisaged. While many plant viruses encode potent suppressors of RNA silencing, analysis of two nepoviruses (TBRV and ToRSV) did not reveal significant silencing suppression activity.

Satellites

Two classes of satellite RNAs (satRNAs) have been found in association with some but not all nepoviruses (**Table 1**). SatRNAs depend on the helper virus for their replication and are encapsidated in virus particles. Type B satRNAs are 1100–1500 nt in length. They are linked to a VPg molecule at their 5′ end, polyadenylated at their 3′ end, and encode a nonstructural protein which is essential for their replication. The exact function of the encoded protein is not known but it has been suggested that it interacts with the viral replication complex. Sequences at the 5′ and 3′ ends of type B satRNAs are also important for their replication. The 5′ ends of type B satRNAs often have short sequence motifs that are

identical or nearly identical to the 5′ ends of the viral RNAs and are likely recognized by the viral replication complex. One or several copies of type B satRNAs are packaged in the viral particles, either alone or together with one molecule of RNA2. As a general rule, type B satRNAs are replicated specifically by the virus with which they are associated although there are exceptions (e.g., the replication of GFLV satRNA is supported by ArMV). Type B satRNAs are usually found in low concentration in the field or in experimental systems and do not seem to affect significantly the replication of the helper virus or the symptomatology of the disease. For example, type B satRNAs have been found to be associated with only 15–17% of ArMV or GFLV isolates.

Type D satRNAs are less than 500 nt long. They are not linked to a VPg molecule or polyadenylated and do not encode a protein. Type D satRNAs are encapsidated as monomeric or multimeric linear molecules. A circular form of the molecule is present in infected cells and serves as the polymerase template for replication through a rolling-circle mechanism. The multimeric linear forms produced during replication are cleaved in an autocatalytic reaction which allows the release of linear monomers. These monomers are circularized to form new templates of positive or negative polarity. Type D satRNAs have been shown to either attenuate (TRSV) or intensify (ArMV) symptoms associated with the helper virus.

Diseases and Economic Considerations

Nepoviruses cause a wide range of diseases on a variety of crops including: grapevine (ArMV, GBLV, GCMV, GFLV, GTRSV, RpRSV, TBRV, ToRSV, TRSV), soft fruits such as strawberry, raspberry, blueberry, black currant, and red currant (ArMV, BLMV, BRV, CLRV, RpRSV, TBRV, ToRSV, TRSV), fruit trees such as peach, apricot, almond, cherry, plum, walnut, and apple (CLRV, MLRSV, PRMV, RpRSV, ToRSV), and horticultural crops including but not limited to hop, soybean, potato, beet, and tobacco (AILV, ArMV, AVA, BRSV, CGMV, PBRSV, and TRSV). Many nepoviruses also infect and induce diseases in ornamental species. Most nepoviruses are restricted geographically by the natural distribution of their nematode vector. An exception to this is GFLV which has been disseminated worldwide along with its vector. GFLV is the most significant nepovirus at the economic level and can reduce yield in grapevine by as much as 80%. Other nepoviruses can cause significant diseases where they occur.

Transmission

Many nepoviruses are transmitted by soil-inhabiting nematodes belonging to three closely related genera *Xiphinema*, *Longidorus,* or *Paralongidorus* in the order Dorylaimida, family Londigoridae (**Table 1**). The nematodes feed ectoparasitically on the roots using long mouth stylets. There is no evidence that nepoviruses replicate in the nematodes. The acquired viruses remain transmissible for varying periods of time (9 weeks for species *Longidorus* and up to 4 years for species *Xiphinema*), suggesting different modes of retention and release of the virus. Because of the restricted movement of the nematodes through the soil, the spread of nematode-transmitted nepoviruses through an infected field is slow and often occurs in patches. The interaction between nepoviruses and nematodes is usually specific with only one or two species of nematodes transmitting a given nepovirus. A notable exception is RpRSV which is transmitted by nematodes from the genera *Longidorus* and *Paralongidorus*. The viral determinant for the specificity of nematode transmission has been mapped to the CP in the case of GFLV. It is not known whether specific nematode receptors recognize the viral CP. Earlier studies suggested that carbohydrates may be involved in the retention of ArMV particles in its vector. However, further experiments will be required to determine if the viral CP has lectin properties. It has been suggested that pH changes may be involved in the release of viral particles from their site of retention within the nematode. Nepoviruses transmitted by *Longidorus* sp. are usually associated with the odontostyle, while viruses transmitted by *Xiphinema* sp. are found associated with the cuticle lining the lumen of the odontophore and the esophagus. Interestingly, while TRSV and ToRSV are both transmitted by *X. americanum*, immunofluorescence labeling of these viruses in the nematode vector revealed different sites of retention. TRSV is retained predominantly in the lining of the lumen of the stylet extension and the anterior esophagus, while ToRSV is localized only in the triradiate lumen.

Although the predominant vector for transmission of TRSV is a nematode, possible aerial vectors have been suggested including *Thrips tabaci* and *Epitrix hirtipennis* (flea beetle). The importance of these vectors in natural epidemics of TRSV-induced diseases needs to be confirmed. Other nematode-transmitted nepoviruses do not have known aerial vectors. BRV is transmitted by the eriophyid gall mite of black currant (*Cecidophyopsis ribis*) and possibly other *Cecidophyopsis* species but not by nematodes. Plant-to-plant transmission can occur rapidly (in only 4 h). Virus particles have not been found inside mites, suggesting that the transmission may be nonpersistent or semipersistent. Two surface-exposed amino acid triplets are conserved between the CP of BRV and other mite-transmitted viruses from unrelated genera, suggesting that they may play a role in the interaction between the virus and its vector. However, this remains to be determined experimentally.

Seed transmission has been reported for most but not all nepoviruses. For example, BRV is apparently not seed

transmitted. Infection can occur through the ovule or the pollen, although nepovirus-infected pollen may not compete effectively with healthy pollen. An exception to this is BLMoV and CLRV which are efficiently transmitted by pollen, even to the mother plant. Many nepoviruses are readily transmitted through grafting and mechanical inoculation. The propagation of infected seed and plant stocks plays an important role in the long-distance movement of nepoviruses. Because many nepoviruses have a wide host range including many common weeds, dormant weed seeds may constitute an important reservoir for the virus in the field.

Population Structures

Examination of nepovirus isolates recovered from different hosts or geographic locations has revealed a degree of sequence diversity of 2–20% at the nucleotide level. Isolates may also differ in their serological properties. Several nepovirus isolates have arisen through recombination implying that mixed infections occur in nature. A detailed analysis of GFLV population structure in an infected vineyard demonstrated the presence of mixed infections and a high degree of recombination between the various isolates. Evidence for mixed infections was also provided from the analysis of an ArMV isolate which revealed the presence of two species of RNA2, each encoding a distinct polyprotein. Analysis of a number of CLRV isolates revealed that the degree of diversity was defined primarily by the host rather than the geographic location. This is an unusual situation among plant viruses and may reflect the fact that this virus is pollen transmitted.

Control

Nepoviruses are mainly controlled through the removal of infected plants and replanting with resistant cultivars (when available) or with virus-free, certified plant material. In the case of nematode-transmitted nepoviruses, soil can be fumigated with broad-range nematicides. However, this method is not always effective because nematode populations can occur at considerable depths in the soil (1 m or more). Further, nematicides are costly and toxic to the environment. Bait plants can be used to test for the presence of viruliferous nematodes in the soil. Careful weed control is recommended to eliminate potential reservoirs for further nepovirus infection. Since few resistant cultivars have been reported for nepoviruses, the usefulness of transgenic approaches has been investigated. Resistance to various nepoviruses has been engineered in herbaceous hosts using the coding region for the viral CP in the sense or anti-sense orientation. Other regions of the viral genome have also been used to engineer resistance to nepoviruses through the induction of RNA silencing. Resistance to GFLV has been reported in transgenic grapevines transformed with the CP coding region. The resistance was effective in a field situation heavily infected with viruliferous nematodes. It is of interest to note that the presence of susceptible and resistant GFLV-CP transgenic grapevine in the field did not increase the occurrence of recombination events in the virus population. It is likely that the next generation of transgenic lines will be aimed at increasing the efficiency of induction of sequence-specific RNA silencing using transgenes that contain only very small portions of the viral genome.

Nepovirus Research in the Future

The next phase of nepovirus research will undoubtedly address several fundamental questions. First, the function and mode of action of nepovirus proteins, in particular protein domains in the N-terminal region of the two polyproteins, require further investigation. Although a putative function has been assigned in some cases (e.g., the GFLV 2a protein was shown to play a role in RNA2 replication), in other cases the role of the protein in the virus replication cycle is unknown (e.g., ToRSV X1, X3, and X4). Second, the role played by host factors in the translation, replication, and cell-to-cell (or systemic) movement of nepoviruses needs to be characterized. Large-scale studies of the interaction of the plant and virus proteomes will be necessary to address this question. In addition, it will be useful to analyze the ability of nepoviruses to infect collections of plants mutated or silenced for the expression of specific genes. Third, further work is necessary to understand the specificity of nepovirus–vector interactions. These questions are not only important to satisfy scientific curiosity, but also for the rational design of alternative approaches for the control of nepoviruses.

See also: Picornaviruses: Molecular Biology; Plant Virus Diseases: Fruit Trees and Grapevine; Plant Virus Diseases: Ornamental Plants; Sadwavirus; Satellite Nucleic Acids and Viruses.

Further Reading

Andret-Link P, Schmitt-Keichinger C, Demangeat G, Komar V, and Fuchs M (2004) The specific transmission of grapevine fanleaf virus by its nematode vector *Xiphinema index* is solely determined by the viral coat protein. *Virology* 320: 12–22.

Brown JF, Trudgill DL, and Robertson WM (1996) Nepoviruses: Transmission by nematodes. In: Harrison BD and Murant AF (eds.) *The Plant Viruses, Vol. 5: Polyhedral Virions and Bipartite RNA Genomes*, pp. 187–209. New York: Plenum.

Chandrasekar V and Johnson JE (1998) The structure of tobacco ringspot virus: A link in the evolution of icosahedral capsids in the picornavirus superfamily. *Current Biology* 6: 157–171.
Gaire F, Schmitt C, Stussi-Garaud C, Pinck L, and Ritzenthaler C (1999) Protein 2 A of grapevine fanleaf nepovirus is implicated in RNA2 replication and colocalizes to the replication site. *Virology* 264: 25–36.
Harrison BD and Murant AF (1996) Nepoviruses: Ecology and control. In: Harrison BD and Murant AF (eds.) *The Plant Viruses, Vol. 5: Polyhedral Virions and Bipartite RNA Genomes*, pp. 211–228. New York: Plenum.
Jovel J, Walker M, and Sanfacon H (2007) Recovery of *Nicotiana benthamiana* plants from a necrotic response induced by a nepovirus is associated with RNA silencing but not with reduced virus titer. *Journal of Virology* 81: 12285–12297.
Laporte C, Bettler G, Loudes AM, *et al.* (2003) Involvement of the secretory pathway and the cytoskeleton in intracellular targeting and tubule assembly of grapevine fanleaf virus movement protein in tobacco BY-2 cells. *Plant Cell* 15: 2058–2075.
Le Gall O, Iwanami T, Karasev AV, *et al.* (2005) Family *Comoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 807–818. San Diego, CA: Elsevier Academic Press.
Mayo MA and Robinson DJ (1996) Nepoviruses: Molecular biology and replication. In: Harrison BD and Murant AF (eds.) *The Plant Viruses Vol. 5: Polyhedral Virions and Bipartite RNA Genomes*, pp. 139–185. New York: Plenum.
Murant AF, Jones AT, Martelli GP, and Stace-Smith R (1996) Nepoviruses: General properties, diseases and virus identification. In: Harrison BD and Murant AF (eds.) *The Plant Viruses, Vol. 5: Polyhedral Virions and Bipartite RNA Genomes*, pp. 99–137. New York: Plenum.
Rebenstorf K, Candresse T, Dulucq MJ, Buttner C, and Obermeier C (2006) Host species-dependent population structure of a pollen-borne plant virus, cherry leaf roll virus. *Journal of Virology* 80: 2453–2463.
Ritzenthaler C, Laporte C, Gaire G, *et al.* (2002) Grapevine fanleaf virus replication occurs on endoplasmic reticulum-derived membranes. *Journal of Virology* 76: 8808–8819.
Susi P (2004) Black currant reversion virus, a mite-transmitted nepovirus. *Molecular Plant Pathology* 5: 167–173.
Vigne E, Bergdoll M, Guyader S, and Fuchs M (2004) Population structure and genetic variability within isolates of grapevine fanleaf virus from a naturally infected vineyard in France: Evidence for mixed infection and recombination. *Journal of General Virology* 85: 2435–2445.
Zhang SC, Zhang G, Yang L, Chisholm J, and Sanfacon H (2005) Evidence that insertion of tomato ringspot nepovirus NTB-VPg protein in endoplasmic reticulum membranes is directed by two domains: A C-terminal transmembrane helix and an N-terminal amphipathic helix. *Journal of Virology* 79: 11752–11765.

Neutralization of Infectivity

P J Klasse, Cornell University, New York, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Affinity The strength of an intermolecular interaction, for example, of antibody (Ab) binding to antigen (Ag).
Dissociation constant K_d [M], in the law of mass action for a bimolecular association, it corresponds to the concentration of an unbound molecule that yields half-maximal binding to the other molecule. It quantifies affinity: the lower the K_d, the higher the affinity.
Epitope The site on an antigen that makes contact with the paratope of an Ab.
Molecularity The number of molecules involved in the rate-limiting step of a chemical reaction; here it refers to the number of Abs that must bind to a virus particle in order to neutralize it.
Occupancy θ, the degree to which one molecule is ligated by another, for example, the percentage of Ag sites bound by Ab.
Paratope The part of an Ab molecule that makes contact with the epitope of an Ag.
Stoichiometry In the context of neutralization, it is the study of integer ratios of molecules in Ab–virion complexes.
Valency The number of separate, usually identical, areas of contact in bimolecular association.

Introduction

Miscellaneous agents inhibit viral infection. Among them are antibodies (Abs), which have disparate effects on viral infection both *in vivo* and *in vitro*. One such effect is virus neutralization, which can be stringently defined as the reduction in infectivity by interference before the first biosynthetic step in the viral replicative cycle, through the binding of antibodies to epitopes on the surface of the virion. The definition delimits neutralization from other effects of Abs on viral replication and on infected cells. For example, Abs to neuraminidase of influenza virus inhibit viral release from the cell surface, a late step in the replicative cycle. That does not qualify as neutralization. Another example is Abs to viral receptors. Such Abs

can block infection, but they do not fulfill the criterion of binding to the virion.

Maybe the definition too restrictively specifies Abs as the neutralizing agents. Obviously, some loosening of this criterion will allow a comparison with the neutralizing effects of fragments of Abs, peptide mimics of paratopes, fragments of receptors and their mimics, or even other ligands, as long as they bind to the viral surface.

What the definition implicitly does include is also noteworthy. Enveloped viruses carry cellular passenger antigens (Ags). If Abs to these inhibit infection, that counts as neutralization. Under certain circumstances, Abs aggregate virions. If that diminishes infectivity, the definition includes it as neutralization – although some researchers have chosen not to. Similarly, the definition can accommodate for effects of complement, in addition to that of the Abs, as enhancement of neutralization. Whether or how such enhancement occurs is a matter for investigation. As a case in point, a report found neutralization through virolysis to be more complement-enhanced when mediated by Abs to a retroviral transmembrane protein than by Abs to the surface unit of the same envelope glycoprotein complex. Notably, the definition stipulates little about mechanism. That is left to hypotheses and their testing by experiment.

A closer look at the defining terms 'reduction', 'first biosynthetic step', and 'binding' raises some questions. First, does any degree of 'reduction' count as neutralization? Does neutralization entail the complete abrogation of the infectivity of at least some virions? Or can it be partial, a mere reduction in the propensity to infect? Second, the 'first biosynthetic step' occurs after viral entry. Why does the definition put the limit at so late a step? How could Abs affect events occurring after the viral genome or core has entered the cytoplasm from the cell surface or from an endosomal vesicle? Controversial cases of postentry neutralization have been promulgated. Therefore, the definition should not exclude them; they must be refuted or corroborated by experiment. Empirically then, which replicative steps does neutralization block? Third, the definition makes Ab 'binding' a necessary condition for neutralization. But is it also sufficient? Or are there Abs that can bind to surface epitopes on virions but still not neutralize? And if so, why?

Experimental Measurement

Passive immunization, that is, transfer of neutralizing antibodies (NAbs), can protect an organism against some viral infections. Many successful vaccines induce high titers of NAbs and neutralization is, quite plausibly, often crucial to immune defenses *in vivo*. But it is measured *in vitro*. Classically, a neutralization assay consists of four steps: First, virus is incubated with test and control Abs. Then these reaction mixes are added to target cells, and the virus is left to adsorb. After free virus is washed off, virus that has entered the cells is allowed to replicate. Finally, the degree of replication is measured.

This basic scheme comes in different forms. A key distinction is between single- and multicycle assays. Early studies measured inhibition of replication in plaque- and focus-forming assays. These quantal assays were adapted for both bacteriophage and animal viruses. Such tools have a theoretical advantage: if the inoculum is dilute, a primary plaque or focus most likely will represent the local spread from a single infectious event. Furthermore, if progeny virus is prevented from forming secondary plaques and foci, the number of infectious units measured will correspond to the first cycle of replication. Then the degree of neutralization can be expressed as the proportion of initial infectious units prevented from infecting.

Today neutralization studies often employ defective viruses: a viral gene has been deleted and replaced with a reporter gene. Envelope glycoprotein genes can be supplied in *trans*. Hence, one can make pseudotype particles with neutralization targets of choice. Like quantal assays, this method limits the infectivity to the first replicative cycle.

Other neutralization assays measure the reduction in viral Ag production during the culture. If multiple replicative cycles are allowed, though, the reduction in Ag may not be proportional to the fraction of virus that was originally inactivated by the binding of Abs.

Antibody–Antigen Binding and Epitope Occupancy on Virions

The binding of Abs to epitopes on virions is the *sine qua non* of neutralization. The concentration of the antibody, together with its affinity for the epitope, determines the occupancy, θ, of Ab on the virion, that is, the proportion of specific epitopes that the Ab ligates. If the occupancy is too low, the virion will not be neutralized. Instead, the infectivity may even be enhanced by subneutralizing occupancies.

The occupancy can be estimated from the law of mass action, provided the molar excess of Ab over Ag is vast. Usually, this is the case since NAbs tend to neutralize in the nM, or higher, concentration range, which viral Ags in inocula rarely reach. This is reflected in the percentage law: the proportion neutralized by an Ab at a certain concentration is approximately constant when the amount of virus is varied.

Suppose we know the number of epitopes on the virion, and the K_d of the Ab for binding to those epitopes. In theory, we can then estimate how many Abs per virion are bound at any given Ab concentration. In practice, however, this is often impossible. Notably, virions do not always have a constant number of epitopes. The virions of some viruses vary in size. Also, enveloped viruses can incorporate varying numbers of envelope glycoprotein molecules; subunits of these can be lost from the virions after budding. Furthermore, the relevant K_d is seldom known. Often the K_d is measured for Ab binding to a form

of the neutralization Ag that differs from the functional one on the infectious virion: the binding studies may have used an unprocessed precursor or a truncated, immobilized monomeric subunit of an oligomer. In addition, the oligomeric context of the epitope strongly affects its antigenicity. The spacing of Ag sites on virions, as well as in binding assays, can also affect the avidity, and therefore the functional affinity, of Ab binding. Clearly, the relevant affinity of NAbs is central to an understanding of neutralization.

The IC_{50} of a NAb, that is, the concentration at which it neutralizes half of the inoculum, is also a notable variable. According to the reasoning above, it should stand in a definite relation to the K_d for Ab binding to virions. If the minimum neutralizing occupancy happens to be 50%, the IC_{50} and the K_d will be similar. With higher occupancies required, we get $IC_{50} > K_d$; with lower, $IC_{50} < K_d$ – all according to the formula $\theta = ([Ab]/K_d)/(([Ab]/K_d) + 1)$.

The maximal extent of neutralization is intriguing. As mentioned, some Abs fail entirely to neutralize. Others have only marginally inhibitory effects. Yet others may give a plateau of neutralization at around, say 75% or 90%. We can imagine a whole spectrum. At one extreme end of the spectrum, where we find the most effective NAbs, no residual infectivity is detectable with low-infectivity inocula. But with higher viral doses, even a strong neutralization leaves a detectable residual infectivity. Furthermore, when neutralization is studied over time, it may reach a plateau. Such residual infectivity, called the 'persistent fraction', has been studied extensively. Usually, the persistent and the neutralized fractions do not differ genetically. Nor does spontaneous aggregation of virus explain all persistence. No comprehensive explanation has been found, but some contributing factors deserve mention. Because of both steric hindrance and the stochastic nature of binding, the Ab may not yield a sufficient occupancy for a complete abrogation of the infectivity of all virions. Conformational, and therefore antigenic, heterogeneity among epitopes may leave some unoccupied. Moreover, the Ab binding is in principle reversible, and at the cell surface Abs and receptors may compete for binding to the virion. Finally, NAbs act in parallel with, and may differentially affect, spontaneous decay of the virus. A better understanding of these dynamics may suggest which properties of NAbs render them most effective. Suffice it to say that the maximal extent of neutralization is not predictable from the IC_{50} value of an Ab. And *in vivo,* that maximum may be more decisive than a titration midpoint.

Mechanism

The first replicative step prevented by the Ab is one aspect of the neutralization mechanism. Another is how, at the intermolecular level, the Ab effects this block.

The initial step in the replicative cycle is attachment to a susceptible cell. NAbs to widely different viruses block this step. For example, Abs to distinct epitopes of influenza virus hemagglutinin (HA), when occupying one in four HA trimers, neutralize by blocking viral attachment. Some viruses, however, interact with ancillary attachment molecules on the surface of cells before docking onto their major receptors. In such cases, the NAb may not interfere with the initial attachment but with the subsequent receptor interactions. Moreover, assortments of NAbs to HIV-1, rotavirus, and papilloma virus show widely different capacities to block the attachment of each virus. These differences may stem from how distally on a viral spike or other protrusion the epitope is located, and at what angle to the virus particle the Ab binds; other contributing factors may be the maximum number of epitopes that can simultaneously be occupied, the size of the Ab, and the valency of its binding.

After attachment to the cell, the next obligatory step for many viruses is internalization by endocytosis. Hence, if endocytic uptake is blocked by the Ab, the virus is neutralized. Failing that, neutralization can still occur inside the endosome. By delaying fusion or penetration, the Ab will promote the eventual degradation of the virus in lysosomes.

We can also hypothesize the converse. Some viruses cannot use the endosomal route for productive entry. Therefore shunting the virion–Ab complex onto that route, for example, through Fc–receptor interactions, would instead lead to neutralization.

After docking onto a major receptor, many viruses interact with co-receptors as a necessary entry step. If that interaction is blocked by an Ab, the virus is neutralized. At this stage, however, steric constraints may prevent access to the relevant epitopes, as multimolecular complexes form. Indeed, a Fab binding adjacently to the co-receptor binding site of HIV-1 neutralizes more efficiently than the corresponding immunoglobulin G (IgG).

We can study post-attachment neutralization (PAN) by letting the virus adsorb to cells at a temperature too low to allow entry. After that, the Ab is allowed to bind to the adsorbed virus. As the cells are warmed up, the non-neutralized virus will infect. At which steps, then, can PAN intervene? In spite of the name and experimental setup, PAN does not necessarily affect only pure post-attachment events. In fact, Abs that interfere directly with virus–receptor interactions often mediate PAN. That may be explained by the necessity of recruiting multiple receptors into a fusion or entry complex. The lower temperature prevents collateral diffusion of receptors; the Ab covers remaining sites not engaged by the receptors; then, at the warm-up, the Ab precludes the requisite additional receptor interactions. Or else PAN occurs because the Ab induces the dissociation of the virus particle. Most importantly, we cannot declare that PAN involves major neutralization mechanisms just because we can make it happen. For when Abs are instead first allowed to bind to virions, attachment may be blocked as the complexes later encounter the cells, which pre-empts any further replicative steps. It also pre-empts PAN.

Particularly the neutralization of picornaviruses, such as poliovirus and rhinovirus, was long thought to interfere with a late penetration step and to depend on the induction of conformational changes in their antigens. Many NAbs can change the isoelectric point, pI, of the picornavirus particle. But their capacity to do so correlates poorly with their neutralizing efficacy. Besides, many of these Abs effectively block virus attachment.

Structural studies have shown that sometimes the paratope of a NAb changes more than the epitope upon binding. An extreme example is one of the most potent NAbs against rhinovirus, which remolds its paratope but leaves the Ag conformationally intact. Furthermore, if neutralization depended on conformational changes in the Ag, there ought to be Abs that block neutralization by competing with NAbs, thereby preventing conformational changes. Such antibodies, though, are not readily found.

Of course, viral proteins, while mediating entry, change conformation or even refold. Therefore, hampering such changes may promote neutralization at the postattachment stage. Or blocking interaction with a receptor, the role of which is to trigger conformational changes, will prevent them by default.

The block of receptor interactions is not necessarily direct. On the contrary, because of the relative sizes of surface viral Ags and Abs, indirect steric interference is potentially strong. Furthermore, when an irrelevant epitope is tagged onto an attachment- and entry-mediating viral protein, at least in some cases, Abs to that epitope can neutralize the virus. What seems most crucial, therefore, is simply the Ab occupancy on functional viral proteins.

Kinetics

The dissociation constant, K_d, is equal to the ratio of the two rate constants for the binding reaction, k_{off}/k_{on}. Yet how the kinetics of NAb binding influences the potency and extent of neutralization is largely unexplored. It is known, though, that the higher the density of target cells and receptors, the greater the demands on neutralization. Thus, the kinetic constants for the Ab, as well as for the receptor binding may influence neutralization in the competitive situation at the cell surface.

One kind of kinetic study takes advantage of PAN. When cells with attached virus are warmed up, NAbs differ in the length of time during which they can still neutralize. Several factors may be responsible for such differences: how fast the on-rate is, whether the epitopes are only transiently exposed, and precisely when the Ab interferes in the chronology of molecular entry events.

Kinetic studies of a different sort have influenced the theory of neutralization profoundly. If the reaction between Ab and virus can be stopped, and the mixture rapidly transferred to target cells, the decline in infectivity can be monitored as a function of reaction time. Much debate has focused on the first part of such kinetic plots of declining relative infectivity. The absence of a shoulder on the curve was interpreted as evidence of neutralization through the very first Ab binding events. It has been questioned, though, whether Ab binding is sufficiently slow to allow the recording of the infectivity of virions with single Abs bound. In any case, at low temperatures, with low concentrations of low-affinity Abs, such kinetic neutralization curves do evince shoulders. Even if the first few Abs reduce the infectivity, they may not abrogate the entire infectious potential of a virion. Perhaps they only dent it. Such interpretations depend on whether neutralization and infectivity are all-or-nothing phenomena or more gradual and incremental. Another complication is the need to estimate stochastic, rare multihits when on average only few Abs have bound.

In a seminal study, Dulbecco *et al.* demonstrated that the rate of neutralization of poliovirus and Western equine encephalitis virus, expressed as a function of Ab concentration, follows approximate first-order kinetics. They took this as evidence that each virion is neutralized by a single Ab, so-called 'single-hit neutralization'. The order of a reaction, however, does not have to be an integer; it is not equivalent to the number of Abs required for neutralization of a virion (see section 'Molecularity'). In addition, the discussion above of the molar ratio of Ab to Ag in an ordinary neutralization reaction makes it clear that what Dulbecco and followers have observed are reactions of 'pseudo'-first order. This is still of practical value. For it allows a calculation of the 'neutralization rate constant'. But the kinetic study of reaction order cannot determine whether neutralization requires single or multiple hits.

Molecularity

Molecularity is the minimum number of molecules involved in the rate-determining step of a reaction. In the case of neutralization, the two reactants are Ab and virus. The molecularity is then the minimum number of Abs a virion must bind in order to be neutralized. Stoichiometry describes only relative amounts, but since the goal is to find the number of Abs on each virion, that term is also often used in this context.

The average number of Abs bound to virus particles, at different recorded degrees of neutralization, has been ascertained through radio labeling and electron microscopy. Such data allow us to calculate the minimal number of Abs required for neutralization, if we assume that Ab binding follows a Poisson distribution (**Figure 1**). For example, if a virus were neutralized by the single hit of an Ab, then with an average of 1 Ab per virion, 37% of the initial viral infectivity would remain. That is merely a special case. It therefore makes no sense to identify the number of Ab molecules on the x-axis at 37% residual infectivity as the number of hits required for neutralization, except on the single-hit curve. Instead, a proper Poisson analysis involves

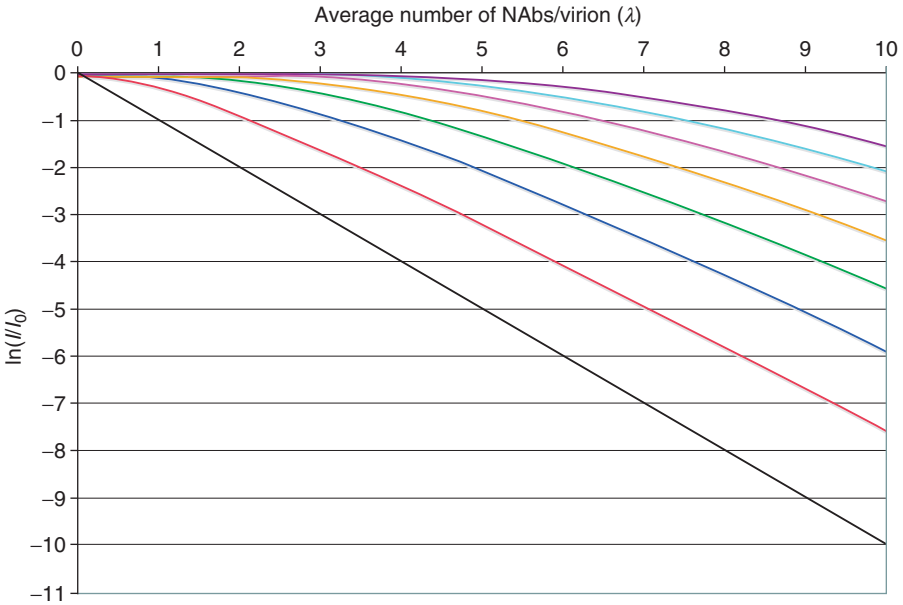


Figure 1 Molecularity of neutralization. The diagram shows the virus infectivities that can be predicted from a Poisson distribution of low numbers of Abs bound to virions. Different absolute thresholds for neutralization are assumed. The natural logarithm of the still infectious fraction of virus, $\ln(I/I_0)$ is plotted on the *y*-axis as a function of the average number of NAbs per virion, λ, on the *x*-axis. The latter can be determined, for example, by radio-labeling techniques or electron microscopy. If the minimum number of NAbs per virion required for neutralization is *L*, then the infectious fraction of virions will be equal to the cumulated fractions with fewer than *L* NAbs bound to them: $I/I_0 = \Sigma_{r=0}^{L-1}(\lambda^r e^{-\lambda})/r!$ Thus with $L = 1$, the contested single-hit molecularity, and at an average of 1 NAb per virion, $\lambda = 1$, $I/I_0 = e^{-1}$ so that $\ln(I/I_0) = -1$: if the single-hit hypothesis were true, approximately 37% of the infectivity would remain when the virions have on average one NAb bound to them. As can be seen in the diagram only the curve for $L = 1$ (black) goes through the point with the coordinates [1,−1]. The other curves represent other molecularities: $L = 2$ (red), $L = 3$ (blue), $L = 4$ (green), $L = 5$ (orange), $L = 6$ (magenta), $L = 7$ (cyan), and $L = 8$ (purple). These curves do not go through [*L*,−1]. It has been a common error in the literature to read the x value at $y = -1$, or 37% infectivity on a nonlogarithmic scale, and assume that to be the value of *L*, the number of hits required for the neutralization. Instead, the diagram shows that the best fitting molecularity can be obtained by comparing the empirical with the theoretical curves.

the plotting of many relative infectivities as a function of the number of bound Abs per virion. Then these empirical data should be compared with the Poisson-derived molecularity curves. The closest one best represents the molecularity.

An additional premise of this Poisson analysis is that a virion is either infectious or not. In other words, there is an absolute threshold of neutralization: with a minimum number of Abs bound, the virion loses all its infectivity at one fell swoop. A radical alternative to this view is that every Ab bound diminishes the infectious propensity of the virion by an equal amount. In fact, there is much evidence of partial reductions in infectivity by NAbs. But a plausible model of neutralization might locate the effects of Abs somewhere in between the two models: there may be a zone of occupancies over which the virion suffers drastic but not total losses in infectivity.

Much direct evidence supports multihit molecularities of neutralization. Four to five Abs are required to give significant neutralization of poliovirus, 36–38 for papillomavirus, 70 for influenza virus, and 225 for rabies virus. Accordingly, Burton and co-workers found an approximately linear relationship between surface area of the virion and the number of Abs required for neutralization. Neutralization could then be seen as the coating of virus particles by Abs, which could interfere with attachment or entry directly or indirectly. The theory can be refined. For example, two enveloped viruses with the same surface area but different numbers of envelope glycoprotein spikes may not require the same number of Abs for neutralization. There is much evidence to support this theory and little to refute it.

Escape and Resistance

Viral genes vary in sequence to widely different extents. The most variable ones encode neutralization antigens; within such antigens surface-exposed elements, often loops containing some of the neutralization epitopes, vary most (**Figure 2**). If a mutation in such a region reduces the affinity of a NAb, then the virus gains a replicative advantage in the presence of the Ab, *in vivo* or *in vitro*. The selection of such mutations constitutes viral escape from neutralization.

Suppose neutralization did not result directly from Ab binding, but indirectly from the induction of conformational changes. Then viral escape from neutralization might sometimes be mediated by mutations blocking those secondary effects of the Abs rather than diminishing the Ab

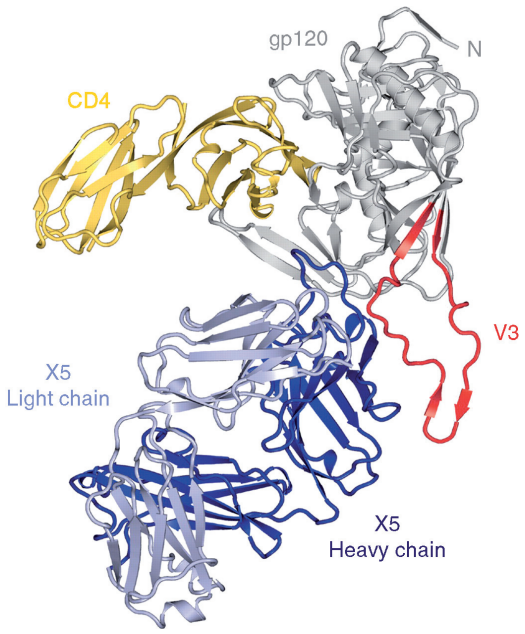


Figure 2 NAbs to HIV-1 are directed to its trimeric envelope glycoprotein, both to the surface unit, gp120, and to the transmembrane protein, gp41. Here, a three-dimensional model of the core of gp120 (gray) is shown. The structure was obtained by high-resolution crystallography of a trimolecular complex. The second molecule in the complex is a two-domain fragment of CD4 (yellow), the major receptor for HIV. Conserved neutralization epitopes overlap the site where CD4 binds. Abs to such epitopes compete with CD4 for binding to gp120 and can thereby directly block a necessary docking event in the viral attachment and entry process. The third molecule in the complex is a neutralizing Fab of the Ab X5. This binds to an epitope generated when CD4 interacts with the full-length gp120; it is located adjacent to the co-receptor binding site. Interestingly, the Fab of X5 neutralizes more efficiently than the whole Ab because its epitope is not induced until the envelope glycoprotein complex docks onto CD4, at which stage access to the epitope is scarce for the larger whole Ab but not for the Fab. Lastly, a variable region, V3, of the gp120 molecule is shown jutting out like a hook (red). This region also contains important neutralization epitopes, but because of the high sequence variability of V3, Abs to these epitopes generally do not cross-neutralize strains. V3 and more conserved adjacent surfaces make contacts with the co-receptor. Reproduced from Huang CC, Tang M, Zhang MY, *et al.* (2005) Structure of a V3-containing HIV-1 gp120 core. *Science* 310 (5750): 1025–1028. Reprinted with permission from AAAS.

binding itself. Yet, a study of rhinovirus found that all mutations conferring neutralization escape also reduced Ab binding. The routes of escape taken by viruses thus shed light on the mechanisms of neutralization.

Some NAbs bind to more conserved epitopes, which are so intricately involved in attachment or entry functions that the virus can accommodate no great changes there. To produce such Abs has a fitness value for the host; to minimize their elicitation has a fitness value for the virus. A result of these evolutionary forces pitted against each other can be seen in influenza virus. As influenza epidemics rage, the viral HA protein keeps varying residues located on a rim surrounding a receptor-binding pocket. The mutations abrogate the binding of, and hence the neutralization by, Abs that the previous serotype of the virus elicited. But the receptor-binding capacity of the virus, guarded from Ab access in the depth of a pocket, remains intact.

The abrogation of NAb binding does not have to be so direct; mutations outside the epitope can confer resistance; they do not even have to be close in space to the epitope surface. When a neutralization-escape mutant of foot-and-mouth-disease virus was compared with the wild type structurally and serologically, the resistant phenotype was attributed to residues well outside the epitope in the VP1 protein. The mutated residues seem to affect the crucial orientation of the epitope loop from a distance in space. An escape mutant of HIV-1 provides another intriguing example. The subtle substitution of a Thr for an Ala in a highly conserved region of the transmembrane protein confers resistance to NAbs directed to the CD4-binding site on the other, noncovalently linked, moiety of the envelope glycoprotein. Perhaps most intriguing of all, substitutions in the cytoplasmic tail of the HIV-1 transmembrane protein can reduce the sensitivity to NAbs that bind on the other side of the viral membrane, to epitopes on the outer subunit of the envelope glycoprotein complex. This antigenic change must involve intricate conformational effects across the membrane and from subunit to subunit. Even so, the end result points to the most straightforward mechanism of resistance: the affinities of the NAbs are reduced.

Although the molecular basis thus varies for how NAb binding is reduced, such reduction is the route of escape generally taken. One provocative exception has been found. An escape mutant of rabies virus can still infect when coated by NAb; hypothetically, the virus has switched to a new entry mechanism, independent of its highly NAb-occupied envelope glycoprotein.

How does tissue culture affect sensitivity to neutralization? The isolation of a virus removes it from the immune system of the infected host; it entails the lifting of any selective neutralization pressure exerted *in vivo*. Since resistance *in vivo* may have come at a price in basic fitness, when there is no longer any gain from such sacrifices, the replicative functions will evolve anew toward optimum. When propagated *in vitro* the virus may therefore become artificially sensitive to neutralization. That seems to have happened to some strains of HIV.

HIV and many other viruses that survive the onslaught of the host immune system have developed a panoply of phenotypic traits protecting them specifically against neutralization: much of the Ab response is misdirected to epitopes that are exposed only on disassembled oligomers and denatured or degraded protein. Neutralization may require a high occupancy. Glycan shields can render surfaces on envelope glycoproteins nonimmunogenic; the glycan moietites may shift from site to site as a result of escape mutations, providing a malleable shield.

Essential interactions with receptors may be limited to a subset of pocket-lining residues, so that the virus can tolerate variation not just around the receptor-binding site but also within it.

As mentioned, some potent NAbs induce barely any changes in the epitopes. Indeed, less potent Abs to overlapping sites sometimes generate greater changes. When such changes are entropically unfavorable, the adverse effect on affinity and occupancy favor the virus. Thus, entropic masking constitutes yet another viral defense.

Few viruses may exploit the entire repertoire of potential defenses. But these traits attest to the importance of neutralization in natural infection. When combined and richly developed, they also posit a formidable challenge to the induction of NAbs through vaccination.

See also: Antigenic Variation; Immune Response to viruses: Antibody-Mediated Immunity; Persistent and Latent Viral Infection; Vaccine Strategies; Viral Receptors.

Further Reading

Andrewes CH and Elford WJ (1933) Observations on anti-phage sera. I: The percentage law. *British Journal of Experimental Pathology* 14(6): 367–376.

Burnet FM, Keogh EV, and Lush D (1937) The immunological reactions of the filterable viruses. *Australian Journal of Experimental Biology and Medical Science* 15: 227–368.

Burton DR (ed.) (2001) *Antibodies in Viral Infection. Vol. 260. Current Topics in Microbiology and Immunology.* Berlin: Springer.

Burton DR, Desrosiers RC, Doms RW, *et al.* (2004) HIV vaccine design and the neutralizing antibody problem. *Nature Immunology* 5(3): 233–236.

Della-Porta AJ and Westaway EG (1978) A multi-hit model for the neutralization of animal viruses. *Journal of General Virology* 38(1): 1–19.

Dulbecco R, Vogt M, and Strickland AGR (1956) A study of the basic aspects of neutralization. Two animal viruses. Western equine encephalitis virus and poliomyelitis virus. *Virology* 2: 162–205.

Fazekas de St. GS (1961) Evaluation of quantal neutralization tests. *Nature* 191: 891–893.

Gollins SW and Porterfield JS (1986) A new mechanism for the neutralization of enveloped viruses by antiviral antibody. *Nature* 321 (6067): 244–246.

Huang CC, Tang M, Zhang MY, *et al.* (2005) Structure of a V3-containing HIV-1 gp120 core. *Science* 310(5750): 1025–1028.

Jerne NK and Avegno P (1956) The development of the phage-inactivating properties of serum during the course of specific immunization of an animal: Reversible and irreversible inactivation. *Journal of Immunology* 76: 200–208.

Klasse PJ and Moore JP (1996) Quantitative model of antibody- and soluble CD4-mediated neutralization of primary isolates and T-cell line-adapted strains of human immunodeficiency virus type 1. *Journal of Virology* 70(6): 3668–3677.

Klasse PJ and Sattentau QJ (2002) Occupancy and mechanism in antibody-mediated neutralization of animal viruses. *Journal of General Virology* 83(Pt 9): 2091–2108.

Mandel B (1978) Neutralization of animal viruses. *Advances in Virus Research* 23: 205–268.

Nybakken GE, Oliphant T, Johnson S, Burke S, Diamond MS, and Fremont DH (2005) Structural basis of West Nile virus neutralization by a therapeutic antibody. *Nature* 437(7059): 764–769.

Skehel JJ and Wiley DC (2000) Receptor binding and membrane fusion in virus entry: The influenza hemagglutinin. *Annual Review of Biochemistry* 69: 531–569.

Nidovirales

L Enjuanes, CNB, CSIC, Madrid, Spain
A E Gorbalenya, Leiden University Medical Center, Leiden, The Netherlands
R J de Groot, Utrecht University, Utrecht, The Netherlands
J A Cowley, CSIRO Livestock Industries, Brisbane, QLD, Australia
J Ziebuhr, The Queen's University of Belfast, Belfast, UK
E J Snijder, Leiden University Medical Center, Leiden, The Netherlands

© 2008 Elsevier Ltd. All rights reserved.

Glossary

3CL^{pro} or M^{pro} 3C-like proteinase, or main proteinase.
ADRP ADP-ribose-1″-phosphatase.
CS TRS Core sequence.
ExoN 3′ to 5′ exoribonuclease.
NendoU Nidovirus endoribonuclease.
O-MT Ribose-2′-O-methyltransferase.
PL^{pro} Papain-like cysteine proteinase.
TRS Transcription-regulating sequence.

Taxonomy and Phylogeny

The order *Nidovirales* includes the families *Coronaviridae*, *Roniviridae*, and *Arteriviridae* (**Figure 1**). The *Coronaviridae* comprises two well-established genera, *Coronavirus* and *Torovirus*, and a tentative new genus, *Bafinivirus*. The *Arteriviridae* and *Roniviridae* include only one genus each, *Arterivirus* and *Okavirus*, respectively. All nidoviruses have single-stranded RNA genomes of positive polarity that, in the case of the *Corona-* and *Roniviridae* (26–32 kbp), are the largest presently known RNA virus genomes. In contrast, members of the *Arteriviridae* have a smaller genome ranging

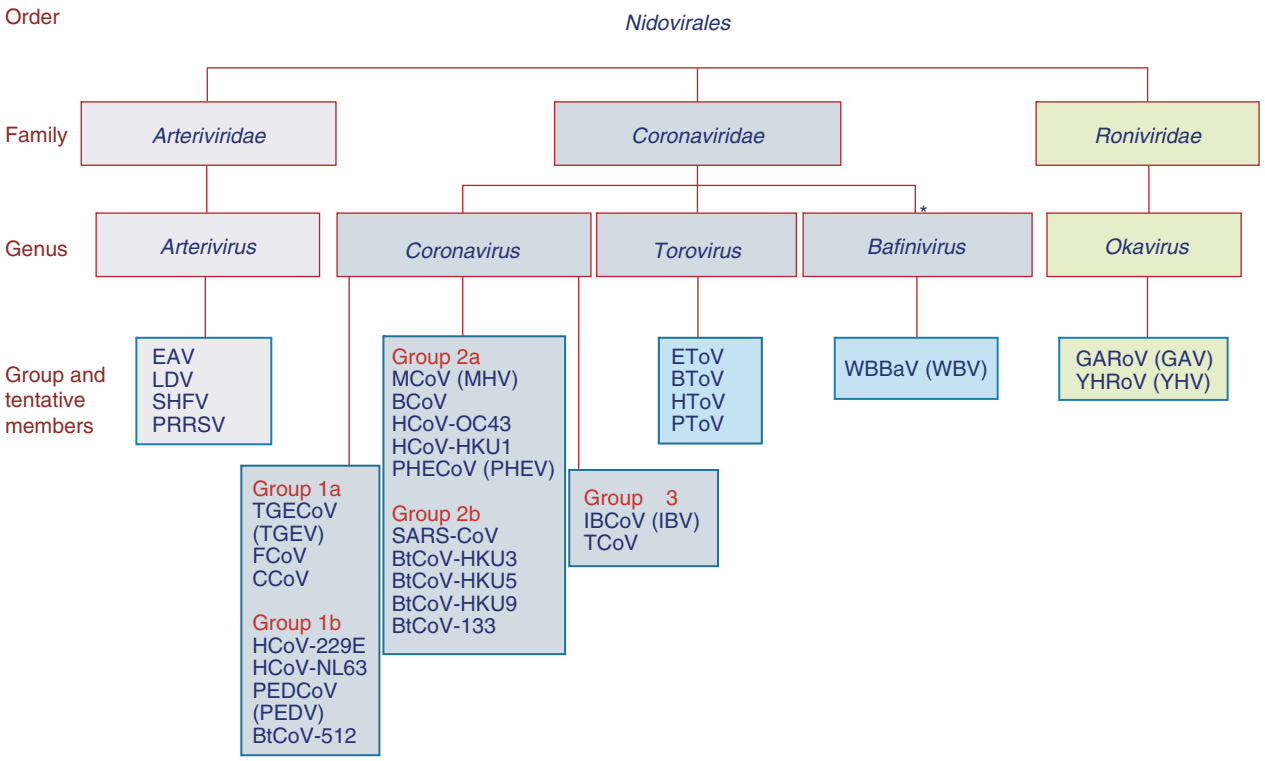


Figure 1 Nidovirus classification and prototype members. The order *Nidovirales* containing the families *Coronaviridae* (including the established genera *Coronavirus* and *Torovirus*, and a new tentative genus *Bafinivirus*), *Arteriviridae*, and *Roniviridae*. Phylogenetic analysis (see **Figure 2**) has confirmed the division of coronaviruses into three groups. In arteriviruses, four comparably distant genetic clusters have been differentiated. To facilitate the taxonomy of the different virus isolates, the types Co, To, Ba, Ro, standing for coronavirus, torovirus, bafinivirus, or ronivirus, respectively, have been included. The following CoVs are shown: human coronaviruses (HCoV) 229E, HKU1, OC43 and NL63, transmissible gastroenteritis virus (TGEV), feline coronavirus (FCoV), porcine epidemic diarrhoea virus (PEDV), mouse hepatitis virus (MHV), bovine coronavirus (BCoV), bat coronaviruses (BtCoV) HKU3, HKU5, HKU9, 133 and 512 (the last two isolated in 2005), porcine hemagglutinating encephalomyelitis virus (PHEV), avian infectious bronchitis virus (IBV), and severe acute respiratory syndrome coronavirus (SARS-CoV); ToV: equine torovirus (EToV), bovine torovirus (BToV), human torovirus (HToV), and porcine torovirus (PToV); BaV: white bream virus (WBV); Arterivirus: equine arteritis virus (EAV), simian haemorrhagic fever virus (SHFV), lactate dehydrogenase-elevating virus (LDV), and three (Euro, HB1, and MLV) porcine reproductive and respiratory syndrome viruses (PRRSV); RoV: gill-associated virus (GAV) and yellow head virus (YHV). Human viruses are highlighted in red. Some nodes are formed by a pair of very closely related viruses (e.g., SARS-CoV and BtCoV-HKU3). Asterisk indicates tentative genus.

from about 13 to 16 kbp. The data available from phylogenetic analysis of the highly conserved RNA-dependent RNA polymerase (RdRp) domain of these viruses, and the collinearity of the array of functional domains in nidovirus replicase polyproteins, were the basis for clustering coronaviruses and toroviruses (**Figure 2**). The more distantly related roniviruses also group with corona- and toroviruses, thus forming a kind of supercluster of nidoviruses with large genomes. By contrast, arteriviruses must have diverged earlier during nidovirus evolution. The current taxonomic position of coronaviruses and toroviruses as two genera of the family *Coronaviridae* is currently being revised by elevating these virus groups to the taxonomic rank of either subfamily or family.

A comparative sequence analysis of coronaviruses reveals three phylogenetically compact clusters: groups 1, 2, and 3. Within group 1, two subsets can be distinguished: subgroup 1a that includes transmissible gastroenteritis virus (TGEV), canine coronavirus (CCoV), and feline coronavirus (FCoV), and subgroup 1b that includes the human coronaviruses (HCoV) 229E and NL63, porcine epidemic diarrhoea virus (PEDV), and bat coronavirus (BtCoV) 512 which was isolated in 2005. Within group 2 coronaviruses, two subsets have been recognized: subgroup 2a, including mouse hepatitis virus (MHV), bovine coronavirus (BCoV), HCoV-OC43, and HCoV-HKU1; and subgroup 2b, including severe acute respiratory syndrome coronavirus (SARS-CoV) and its closest circulating bat coronavirus relative, BtCoV-HKU3. A growing number of other bat viruses has been recently identified in groups 1 and 2. It is currently being debated whether some of these viruses (e.g., BtCoV-HKU5, BtCoV-133 (isolated in 2005), and BtCoV-HKU9) may in fact represent novel subgroups or groups. Avian infectious bronchitis virus (IBV) is the prototype of coronavirus group 3, which also includes several other bird coronaviruses. In arteriviruses, there are four comparably distant genetic clusters, the prototypes of which are equine arteritis

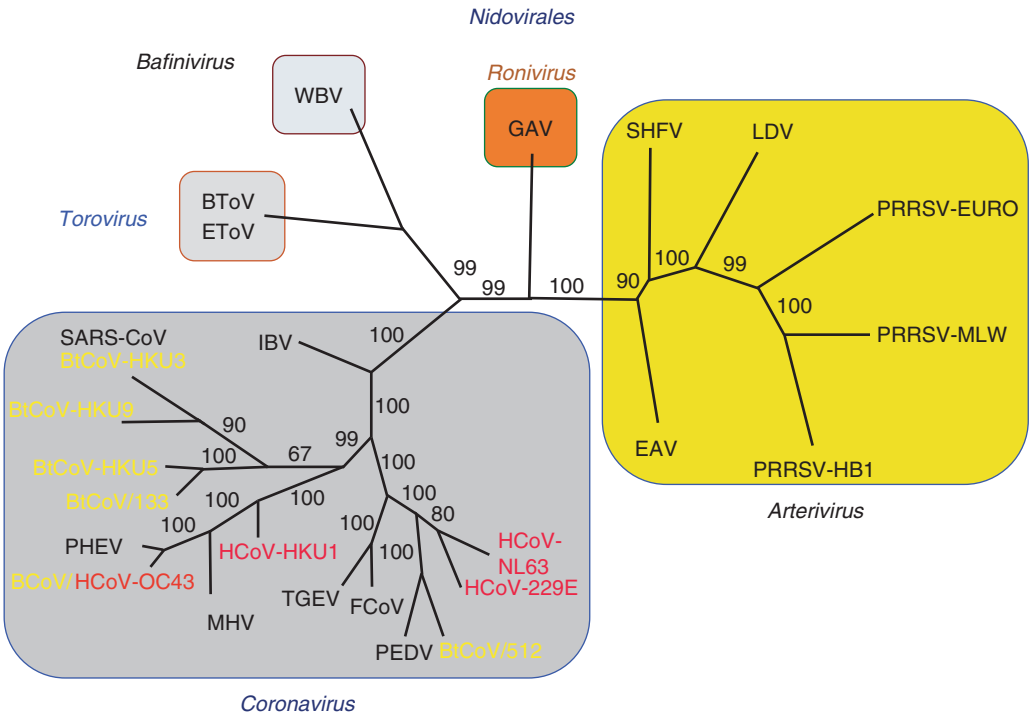


Figure 2 Nidovirus phylogeny. Tree depicting the evolutionary relationships between the five major groups of nidoviruses as shown in **Figure 1** (*Coronavirus, Torovirus, Bafinivirus, Ronivirus*, and *Arterivirus*). This unrooted maximum parsimonious tree was inferred using multiple nucleotide alignments of the RdRp-HEL region of a representative set of nidoviruses with the help of the PAUP*v.4.0b10 software (AEG, unpublished). Support for all bifurcations from 100 bootstraps performed is indicated. The phylogenetic distances shown are approximate. For acronyms, see **Figure 1**.

virus (EAV), lactate dehydrogenase-elevating virus (LDV) of mice, simian hemorrhagic fever virus (SHFV) infecting monkeys, and porcine reproductive and respiratory syndrome virus (PRRSV) which infects pigs and includes European and North American genotypes.

Roniviruses are the only members of the order *Nidovirales* that are known to infect invertebrates. The family *Roniviridae* includes the penaeid shrimp virus, gill-associated virus (GAV), and the closely related yellow head virus (YHV).

More than 100 full-length coronavirus genome sequences and around 30 arterivirus genome sequences have been documented so far, whereas only very few sequences have been reported for toroviruses, bafiniviruses, and roniviruses. Therefore, information on the genetic variability of these nidovirus *taxa* is limited.

Diseases Associated with Nidoviruses

Coronavirus infections are mainly associated with respiratory, enteric, hepatic, and central nervous system diseases. In humans and fowl, coronaviruses primarily cause upper respiratory tract infections, while porcine and bovine coronaviruses establish enteric infections, often resulting in severe economic losses. In 2002, a previously unknown coronavirus that probably has its natural reservoir in bats crossed the species barrier and caused a major outbreak of SARS, which led to more than 800 deaths worldwide.

Toroviruses cause gastroenteritis in mammals, including humans, and possibly also respiratory infections in older cattle. Bafiniviruses have been isolated from white bream fish but there is currently no information on the pathogenesis associated with this virus infection. Roniviruses usually exist as asymptomatic infections but can cause severe disease outbreaks in farmed black tiger shrimp (*Penaeus monodon*) and white pacific shrimp (*Penaeus vannamei*), which in the case of YHV can result in complete crop losses within a few days after the first signs of disease in a pond. Infections by arteriviruses can cause acute or persistent asymptomatic infections, or respiratory disease and abortion (EAV and PRRSV), fatal age-dependent poliomyelitis (LDV), or fatal hemorrhagic fever (SHFV). Arteriviruses, particularly PRRSV in swine populations, cause important economic losses.

Virus Structure

In addition to the significant variations in genome size among the three nidovirus families mentioned above, there are also major differences in virion morphology (**Figure 3**) and host range. Nidoviruses have a lipid envelope which protects the internal nucleocapsid structure and contains

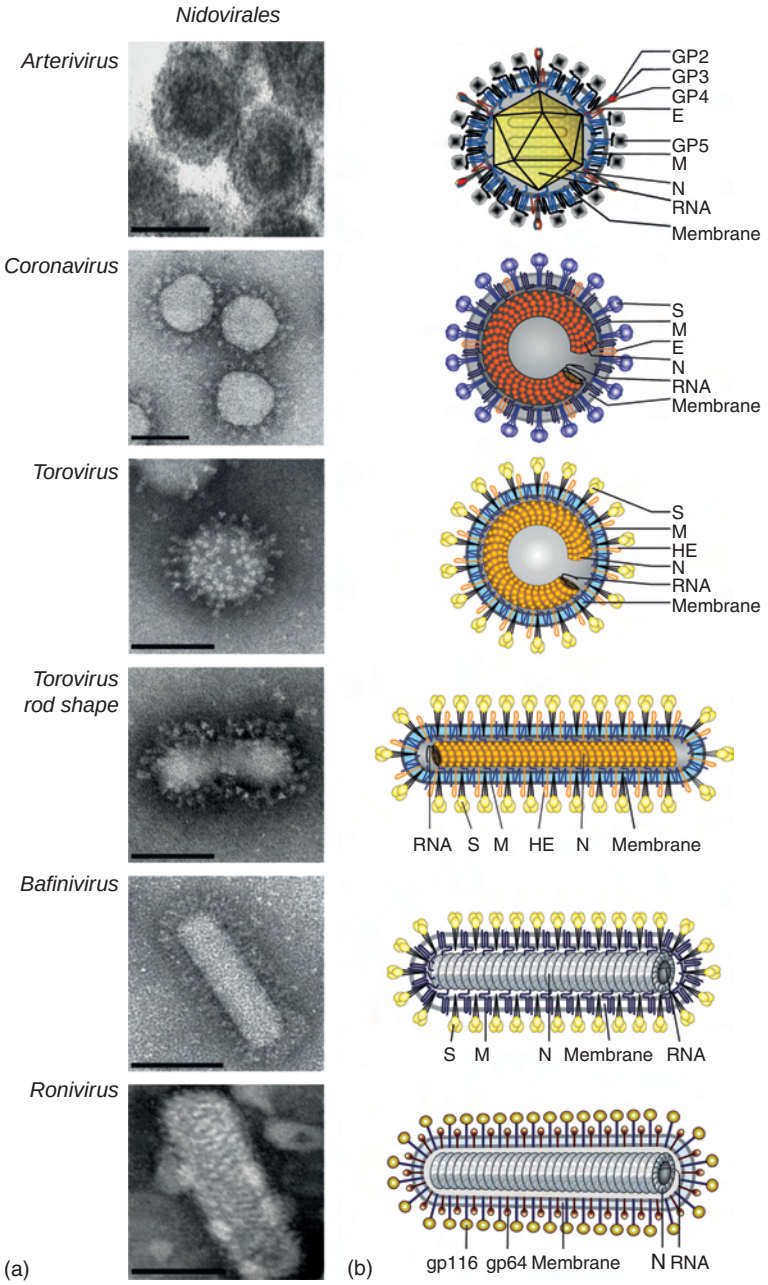


Figure 3 Nidovirus structure. Architecture of particles of members of the order *Nidovirales*: electron micrographs (a) and schematic representations (b) are shown. N, nucleocapsid protein; S, spike protein; M, membrane protein; E, envelope protein; HE, hemagglutinin-esterase. Coronavirus M protein interacts with the N protein. In arterivirus, GP_5 and M are major envelope proteins, while GP_2, GP_3, GP_4, and E are minor envelope proteins. Toro-, bafini-, and roniviruses lack the E protein present in corona- and arteriviruses. Proteins gp116 and gp64, ronivirus envelope proteins. Different images were reproduced with permission from different authors: arterivirus, E. Snijder (Leiden, The Netherlands); ronivirus, P. J. Walker (CSIRO, Australia); bafinivirus, J. Ziebuhr (Queen's University, Belfast) torovirus, D. Rodriguez (CNB, Spain); coronavirus, L. Enjuanes (CNB, Spain).

a number of viral surface proteins (**Figure 3**). Whereas coronaviruses and the significantly smaller arteriviruses have spherical particle structures, elongated rod-shaped structures are observed in toro-, bafini-, and ronivirus-infected cells. The virus particles of the *Corona-* and *Roniviridae* family members carry large surface projections that protrude from the viral envelope (peplomers), whereas arterivirus particles possess only relatively small projections on their surface. Coronaviruses have an internal core shell that is formed by a nucleocapsid featuring a helical symmetry. The nucleocapsid (N) protein interacts with the carboxy-terminus of the envelope membrane (M) protein. The intracellular forms of torovirus, bafinivirus, and ronivirus nucleocapsids have extended rod-shaped (helical)

morphology. By contrast, mature (extracellular) toroviruses (but not bafini- and roniviruses) feature a remarkable structural flexibility, which allows them to adopt crescent- and toroid-shaped structures also. Unlike other nidoviruses, arteriviruses have an isometric core shell. In all nidoviruses, the nucleocapsid is formed by only a single N protein that interacts with the genomic RNA.

Both the number and properties of structural proteins vary between viruses of the three families of the *Nidovirales* and may even vary among viruses of the same family. Nidoviruses usually encode at least three structural proteins: a spike (S) or major surface glycoprotein, a trans-membrane (M) or matrix protein, and the N protein (**Figure 3**). Ronivirus particles are unique in that they possess two envelope glycoproteins, gp116 (S1) and gp64 (S2), but no M protein. Coronavirus and arterivirus particles possess another envelope protein called E that is not conserved in toroviruses, bafini-, and roniviruses. Toroviruses and subgroup 2a coronaviruses, such as MHV, have a hemagglutinin esterase (HE) as an additional structural protein, whereas the SARS-CoV has at least four additional proteins that are present in the viral envelope (encoded by ORFs 3a, 6, 7a, and 7b). The proteins may promote virus growth in cell culture or *in vivo*, but they are dispensable for virus replication.

The major envelope proteins are the S and M proteins in coronaviruses and toroviruses, the GP_5 and M proteins in arteriviruses, and the S1 and S2 proteins in roniviruses. Among these, only the corona-, toro-, and bafinivirus S and M proteins share limited sequence similarities, possibly indicating a common origin. Whereas S proteins can differ in size, they share an exposed globular head domain and, with the exception of roniviruses, a stem portion containing heptad repeats organized in a coiled-coil structure. The S proteins of corona- and toroviruses (and most likely those of bafiniviruses) form trimers that bind the cell surface receptor whereas receptor binding in roniviruses is probably mediated by gp116 (S1). The arterivirus envelope proteins form two higher-order complexes: one is a disulfide-linked heterodimer of GP_5 and the M protein; and the other is a heterotrimer of the minor structural glycoproteins GP_2, GP_3, and GP_4. Except for the E and M proteins, all arterivirus structural proteins are glycosylated. By contrast, the M proteins of corona- and toroviruses (and, most likely, bafiniviruses) are glycosylated, and they share a triple-spanning membrane topology with the amino-terminus exposed on the outside of the virions and the carboxy-terminus facing the nucleocapsid. In TGEV, a proportion of the M proteins has a tetra-spanning membrane topology leading to the exposure of both termini on the virion surface.

In the virion, the coronavirus E protein has a low copy number (around 20) and deletion of the E protein gene from the genome of the group 1 coronavirus TGEV blocks virus maturation, preventing virus release and spread. In the group 2 coronaviruses MHV and SARS-CoV, deletion of the E protein results in a dramatic reduction, of up to 100 000-fold, of virus infectivity. The coronavirus E and SARS-CoV 3a proteins are viroporins, that is, they belong to a group of proteins that modify membrane permeability by forming ion channels in the virion envelope.

Genome Organization

Nidovirus genomes contain variable numbers of genes, but in all cases the 5′ terminal two-thirds to three-quarters of the genome is dedicated to encoding the key replicative proteins, whereas the 3′ proximal genome regions generally encode the structural and, in some cases, accessory (group- and virus-specific) proteins (**Figure 4**). Nidovirus genome expression is controlled at the translational and post-translational levels. Thus, for example, ribosomal frameshifting is required for the expression of ORF1b, and the two replicase polyproteins (pp1a and pp1ab) are proteolytically processed by viral proteases. The proteolytic processing occurs in a coordinated manner and gives rise to the functional subunits of the viral replication–transcription complex. By contrast, the expression of the structural and several accessory proteins is controlled at the level of transcription. It involves the synthesis of a nested set of 3′ co-terminal sg mRNAs that are produced in nonequimolar amounts. As in cellular eukaryotic mRNAs, in general only the ORF positioned most closely to the 5′ end of the sg mRNA is translated.

The Replicase

The nidovirus replicase gene is comprised of two slightly overlapping ORFs, 1a and 1b. In corona-, toro-, bafini-, and roniviruses, ORF1a encodes a polyprotein (pp1a) of 450–520 kDa, whereas a polyprotein of 760–800 kDa (pp1ab) is synthesized from ORF1ab. Expression of the ORF1b-encoded part of pp1ab involves a ribosomal frameshift mechanism that, in a defined proportion of translation events, directs a controlled shift into the −1 reading frame just upstream of the ORF1a stop codon (**Figure 4**). In arteriviruses, pp1a (190–260 kDa) and pp1ab (345–420 kDa) are considerably smaller in size. Proteolytic processing of coronavirus pp1a and pp1ab generates up to 16 nonstructural proteins (nsps 1–16), while processing of the arterivirus replicase polyproteins generates up to 14 nsps. It is generally accepted that most of the replicase nsps assemble into a large protein complex, called the replication–transcription complex. The complex is anchored to intracellular membranes and likely also includes a number of cellular proteins. Nidoviruses replicase genes include a conserved array of protease, RNA-dependent RNA polymerase (RdRp), helicase (HEL), and endoribonuclease (NendoU) activities. In contrast to other positive-strand RNA viruses,

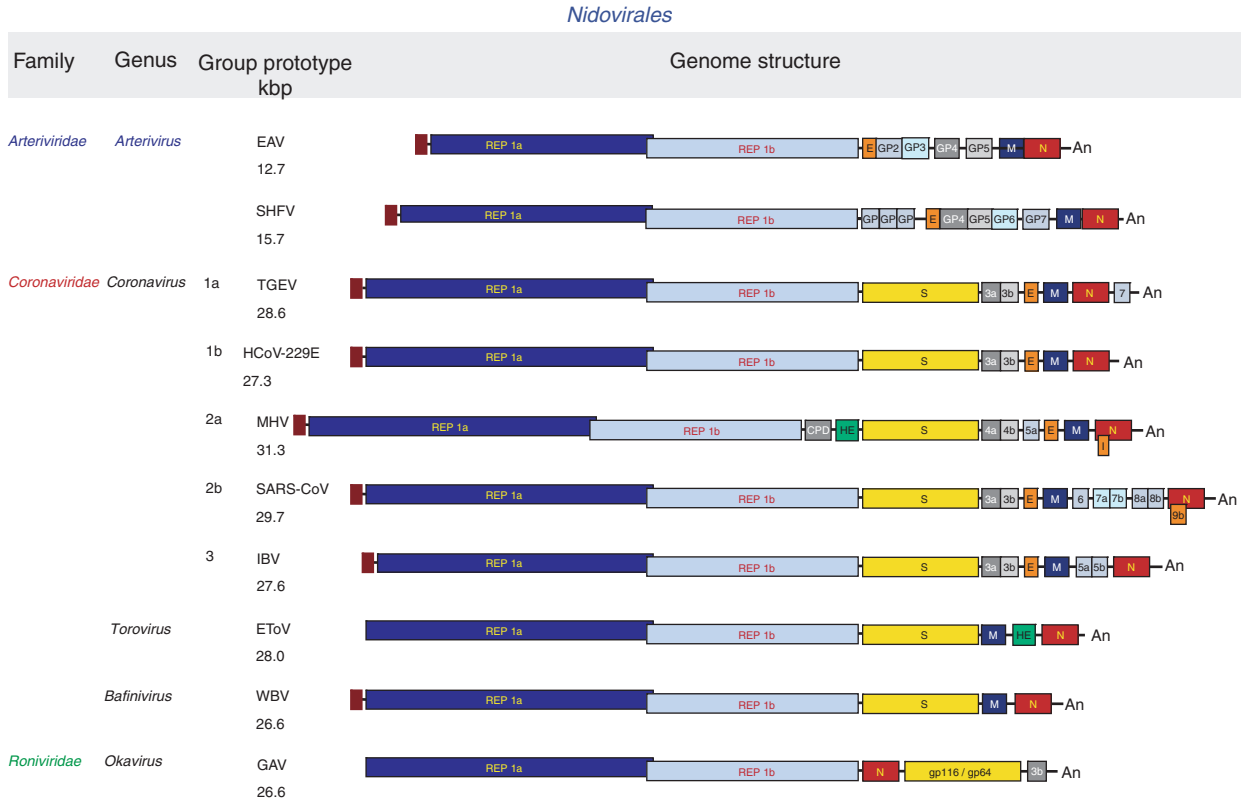


Figure 4 Nidovirus genome structure. Genome organization of selected nidoviruses. The genomic ORFs of viruses representing the major nidovirus lineages are indicated and the names of the replicase and main virion genes are given. References to the nomenclature of accessory genes can be found in the text. Genomes of large and small nidoviruses are drawn to different scales. Red box at the 5′ end refers to the leader sequence. Partially overlapping ORFs have been drawn as united boxes. Spaces between boxes representing different ORFs do not mean noncoding sequences.

they employ an RdRp with a characteristic SDD rather than the usual GDD active site.

The vast majority of proteolytic cleavages in pp1a/pp1ab are mediated by an ORF1a-encoded chymotrypsin-like protease that, because of its similarities to picornavirus 3C proteases, is called the 3C-like protease (3CL^{pro}). Also the term 'main protease' (M^{pro}) is increasingly used for this enzyme, mainly to refer to its key role in nidovirus replicase polyprotein processing (**Figure 5**). Nidovirus–M^{pro}share a three-domain structure. The two N-terminal domains adopt a two-β-barrel fold reminiscent of the structure of chymotrypsin. With respect to the principal catalytic residues, there are major differences between the main proteases from different nidovirus genera. The presence of a third, C-terminal domain is a conserved feature of nidovirus main proteases, even though these domains vary significantly in both size and structure. The C-terminal domain of the coronavirus M^{pro} is involved in protein dimerization that is required for proteolytic activity *in trans*. Over the past years, a large body of structural and functional information has been obtained for corona- and arterivirus main proteases which, in the case of coronaviruses, has also been used to develop selective protease inhibitors that block viral replication, suggesting that nidovirus main proteases may be attractive targets for antiviral drug design.

In arteri-, corona-, and toroviruses, the M^{pro} is assisted by 1–4 papain-like ('accessory') proteases (PL^{pro}) that process the less well-conserved N-proximal region of the replicase polyproteins (**Figure 5**). Nidovirus PL^{pro} domains may include zinc ribbon structures and some of them have deubiquitinating activities, suggesting that these proteases might also have functions other than polyprotein processing. Bafini- and roniviruses have not been studied in great detail and it is not yet clear if these viruses employ papain-like proteases to process their N-terminal pp1a/pp1ab regions (**Figure 5**).

The replicase polyproteins of 'large' nidoviruses with genome sizes of more than 26 kb (i.e., corona-, toro-, bafini-, and roniviruses) include 3′–5′ exoribonuclease (ExoN) and ribose-2′-*O*-methyltransferase (MT) activities that are essential for coronavirus RNA synthesis but are not conserved in the much smaller arteriviruses (**Figure 5**). The precise biological function of ExoN has not been established for any nidovirus but the relationship with cellular DEDD superfamily exonucleases

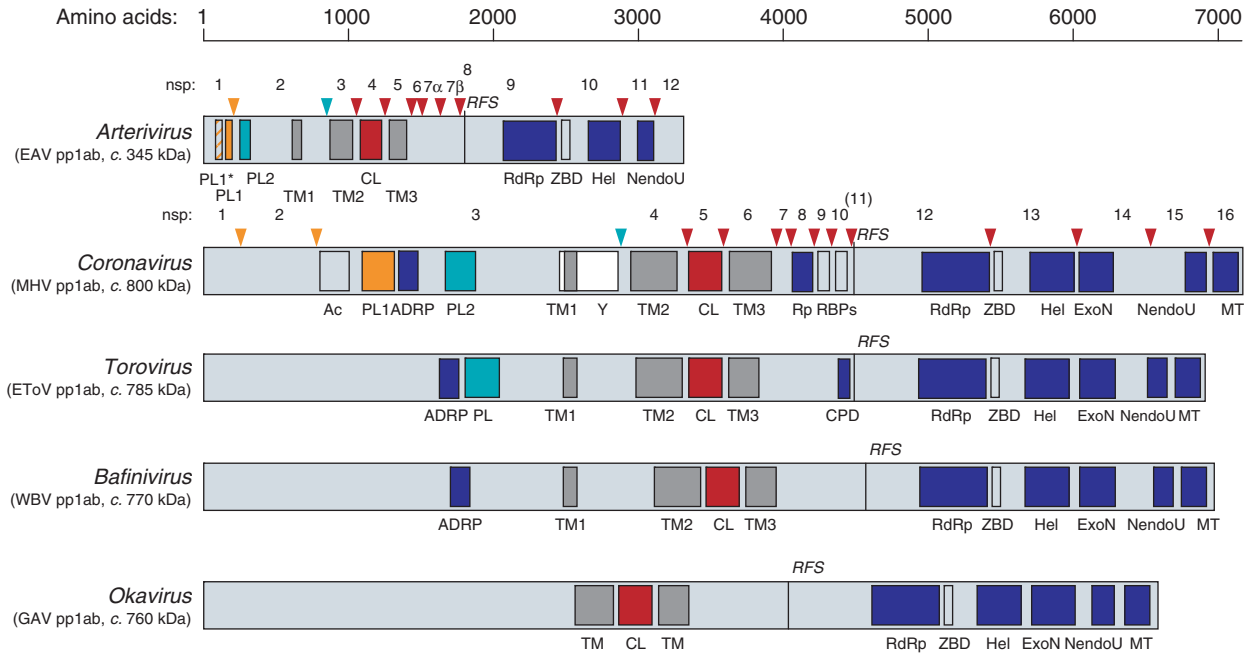


Figure 5 Nidovirus replicase genes. Polyprotein (pp) 1ab domain organizations are shown for representative viruses from the five nidovirus genera. Acronyms as in **Figure 1**. Arterivirus and coronavirus pp1ab processing pathways have been characterized in considerable detail and are illustrated here for EAV and MHV. N-proximal polyprotein regions are cleaved at two or three sites by viral papain-like proteases 1 (PL1) and 2 (PL2), whereas the central and C-terminal polyprotein regions are processed by the main protease, M^{pro}. PL1 domains are indicated by orange boxes and cognate cleavage sites are indicated by orange arrowheads. PL2 domains and PL2-mediated cleavages are shown in green and CL domains and CL-mediated cleavages are shown in red. Note that EAV encodes a second, but proteolytically inactive PL1 domain (PL1*; orange-striped box). For the genera *Torovirus*, *Bafinivirus*, and *Okavirus*, the available information on pp1ab proteolytic processing is limited and not shown here. Other predicted or proven enzymatic activities are shown in blue: ADRP, ADP-ribose 1′-phosphatase; Rp, noncanonical RNA polymerase ('primase') activity; RdRp, RNA-dependent RNA polymerase; HEL, NTPase/RNA helicase and RNA 5′-triphosphatase; ExoN, 3′-to-5′ exoribonuclease; NendoU, nidoviral uridylate-specific endoribonuclease; MT, ribose-2′-O methyltransferase; CPD, cyclic nucleotide phosphodiesterase. Regions with predicted transmembrane (TM) domains are indicated by gray boxes. Other functional domains are shown as white boxes: Ac, acidic domain; Y, Y domain containing putative transmembrane and zinc-binding regions; ZBD, helicase-associated zinc-binding domain; RBPs, RNA-binding proteins. Expression of the C-terminal part of pp1ab requires a ribosomal frameshift, which occurs just upstream of the ORF1a translation stop codon. The ribosomal frameshift site (RFS) is indicated.

and recently published data suggest that ExoN may have functions in the replication cycle of large nidoviruses that, like in the DEDD homologs, are related to proofreading, repair, and recombination mechanisms.

NendoU is a nidovirus-wide conserved domain that has no counterparts in other RNA viruses. It is therefore considered a genetic marker of the *Nidovirales*. The endonuclease has uridylate specificity and forms hexameric structures with six independent catalytic sites. Cellular homologs of NendoU have been implicated in small nucleolar RNA processing whereas the role of NendoU in viral replication is less clear. Reverse genetics data indicate that NendoU has a critical role in the viral replication cycle.

Two other RNA-processing domains, ADP-ribose-1″-phosphatase (ADRP) and nucleotide cyclic phosphodiesterase (CPD), are conserved in overlapping subsets of nidoviruses (**Figure 5**). Except for arteri- and roniviruses, all nidoviruses encode an ADRP domain that is part of a large replicase subunit (nsp3 in the case of coronaviruses). The coronavirus ADRP homolog has been shown to have ADP-ribose-1′-phosphatase and poly(ADP-ribose)-binding activities. Although the highly specific phosphatase activity is not essential for viral replication *in vitro*, the strict conservation in all genera of the *Coronaviridae* suggests an important (though currently unclear) function of this protein in the viral replication cycle. This may be linked to host cell functions and, particularly, to the activities of cellular homologs called 'macro' domains which are thought to be involved in the metabolism of ADP-ribose and its derivatives.

The CPD domain is only encoded by toroviruses and group 2a coronaviruses. In toroviruses, the CPD domain is encoded by the 3′ end of replicase ORF1a (**Figure 5**), whereas in group 2a coronaviruses, the enzyme is expressed from a separate subgenomic RNA. The enzyme's biological function is not clear. Coronavirus CPD mutants are attenuated in the natural host whereas replication in cell culture is normal, suggesting some function *in vivo*. The available information suggests that nidovirus replicase

polyproteins (particularly, those of large nidoviruses) have evolved to include a number of nonessential functions that may provide a selective advantage in the host.

ORF1a of all nidoviruses encodes a number of (putative) transmembrane proteins, like the coronavirus nsps 3, 4, and 6 and the arterivirus nsps 2, 3, and 5. These have been shown or postulated to trigger the modification of cytoplasmic membranes, including the formation of unusual double-membrane vesicles (DMVs). Tethering of the replication–transcription complex to these virus-induced membrane structures might provide a scaffold or subcellular compartment for viral RNA synthesis, possibly allowing it to proceed under conditions that prevent or impair detection by cellular defense mechanisms, which are usually induced by the double-stranded RNA intermediates of viral replication.

Finally, recent structural and biochemical studies have yielded novel insights into the function of a set of small nsps encoded in the 3′-terminal part of the coronavirus ORF1a. For example, nsp7 and nsp8 were shown to form a hexadecameric supercomplex that is capable of encircling dsRNA. The coronavirus nsp8 was also shown to have RNA polymerase (primase) activity that may produce the primers required by the primer-dependent RdRp residing in nsp12. For nsp9 and nsp10, RNA-binding activities have been demonstrated and crystal structures have been reported for both proteins. Nsp10 is a zinc-binding protein that contains two zinc-finger-binding domains and has been implicated in negative-strand RNA synthesis.

Structural and Accessory Protein Genes

In contrast to the large genome of *Coronaviridae*, which can accommodate genes encoding accessory proteins (i.e., proteins called 'nonessential' for being dispensable for replication in cell culture (**Figure 6**)), the smaller genomes of arteriviruses only encode essential proteins (**Figure 4**). Coronaviruses encode a variable number of accessory proteins (2–8), while the torovirus genome contains a single accessory gene encoding a hemagglutinin-esterase (HE). Coronavirus accessory genes may occupy any intergenic position in the conserved array of the four genes encoding the major structural proteins (5′-S-E-M-N-3′), or they may reside upstream or downstream of this gene array. Roniviruses are unique among the presently known nidoviruses in that the gene encoding the N protein is located upstream rather than downstream of the gene encoding the glycoproteins. Several members of the coronavirus group 1a are exceptional in that they contain genes downstream of the N protein gene, which has not been reported for other coronaviruses. The ronivirus glycoprotein gene is also unique in that it encodes a precursor polyprotein with two internal signal peptidase cleavage sites used to generate the envelope glycoproteins S1 and S2 as well as an amino-terminal protein with an unknown function.

The accessory genes are specific for either a single virus species or a few viruses that form a compact phylogenetic cluster. Many proteins encoded by accessory

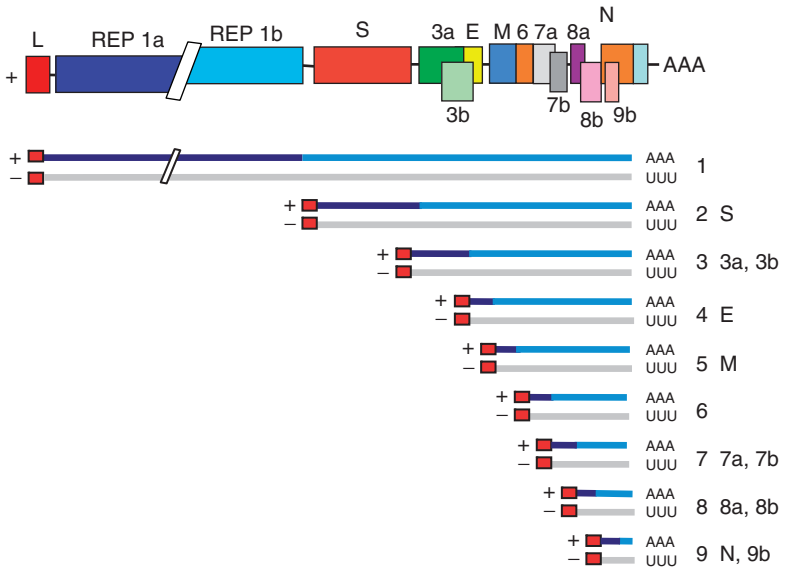


Figure 6 SARS-CoV genome organization and sg mRNA expression. A diagram of CoV structure using SARS-CoV as a prototype. Below the top bar a set of positive- and negative-sense mRNA species synthesized in infected cells is shown. Dark and light blue lines (+), mRNA sequences translated and nontranslated into viral proteins, respectively. Light gray lines (–), RNAs complementary to the different mRNAs. L, leader sequence. Poly(A) and Poly(U) tails are indicated by AAA or UUU, respectively. Rep 1a and Rep 1b, replicase genes. Other acronyms above and below the top bar indicate structural and nonstructural proteins. Numbers and letters to the right of the thin bars indicate the sg mRNAs.

genes may function in infected cells or *in vivo* to counteract host defenses and, when removed, may lead to attenuated virus phenotypes. Group 1 coronaviruses may have 2–3 accessory genes located between the S and E genes and up to two other genes downstream of N gene. Viruses of group 2 form the most diverse coronavirus cluster, and they may have between three and eight accessory genes. In this cluster, MHV, HCoV-OC43, and BCoV form the phylogenetically compact subgroup, 2a, that is characterized by the presence of (1) two accessory genes located between ORF1b and the S gene encoding proteins with CPD and HE functions, (2) two accessory genes located between the S and E protein genes, and (3) an accessory gene, I, that is located within the N protein gene. Of this set of five accessory proteins, only three homologs are encoded by the recently identified HCoV-HKU1, which is the closest known relative of the cluster formed by MHV, HCoV-OC43, and BCoV. In contrast, the most distant group 2 member, SARS-CoV, has seven or eight unique accessory genes, two between the S and E protein genes, four to five between the M and N protein genes, and ORF9b which entirely overlaps with the N protein gene in an alternative reading frame. In group 3 avian coronaviruses, of which IBV is the prototype, several accessory genes, which are expressed from functionally tri- or bicistronic mRNAs, have been identified in the region between the S and E protein genes (gene 3) and between the M and N protein genes (gene 5).

Some functionally dispensable ORF1a-encoded replicase domains may also be considered as accessory protein functions. For instance, MHV and SARS-CoV nsp2 turned out to be nonessential for replication in cell culture.

Replication

Like in all other positive-stranded RNA viruses, nidovirus genome replication is mediated through the synthesis of a full-length, negative-strand RNA which, in turn, is the template for the synthesis of progeny virus genomes. This process is mediated by the viral replication complex that includes all or most of the 14–16 nsps derived from the proteolytic processing of the pp1a and pp1ab replicase polyproteins of arteriviruses and coronaviruses. The replication complex, which is likely to include also cellular proteins, is associated with modified intracellular membranes, which may be important to create a microenvironment suitable for viral RNA synthesis as well as for recruitment of host factors. Electron microscopy studies of cells infected with arteriviruses (EAV) and coronaviruses (MHV and SARS-CoV) have shown that RNA synthesis is associated with virus-induced, DMVs. The origin of DMVs is under debate and different intracellular compartments including the Golgi, late endosomal membranes, autophagosomes, and the endoplasmic reticulum have been implicated in their formation.

Studies of *cis*-acting sequences required for nidovirus replication have mainly relied on coronavirus defective-interfering (DI) RNAs replicated by helper virus. Genome regions harboring minimal *cis*-acting sequences have been mapped to around 1 kb domains of the genomic 5′ and 3′ ends. Studies with MHV DI RNAs have indicated that both genome ends are necessary for positive-strand synthesis, whereas only the last 55 nt and the poly (A) tail at the genomic 3′ end are required for negative-strand synthesis. It has been postulated that the 5′ and 3′ ends of the genome may interact directly during RNA replication, as predicted by computer-aided simulations of MHV and TGEV genomic RNA interactions in protein-free media. There is, however, some experimental evidence supporting protein-mediated cross-talk between both genome ends in the form of RNA–protein and protein–protein interactions.

Several experimental approaches have implicated, in addition to the nsps encoded by the replicase gene, the N protein in coronavirus RNA synthesis. Early in infection, the coronavirus N protein colocalizes with the site of viral RNA synthesis. In addition, the N protein can enhance the rescue of various coronaviruses from synthetic full-length RNA, transcribed *in vitro* or from cDNA clones. In contrast, arterivirus RNA synthesis does not require the N protein.

Host factors that may participate in nidovirus RNA synthesis have been identified mainly from studies of coronaviruses and arteriviruses. In coronaviruses (MHV and TGEV), heterogeneous nuclear ribonucleoprotein (hnRNP) A1 has been identified as a major protein binding to genomic RNA sequences complementary to those in the negative-strand RNA that bind another cellular protein, polypyrimidine tract-binding protein (PTB). hnRNP A1 and PTB bind to the complementary strands at the 5′ end of coronavirus RNA and could mediate the formation of an RNP replication complex involving the 5′ and 3′ ends of coronavirus genomic RNA. The functional relevance of hnRNP A1 in coronavirus replication was supported by experiments showing that its overexpression promotes MHV replication, whereas replication was reduced in cells expressing a dominant-negative mutant of hnRNP A1. There is also experimental evidence to suggest that the poly(A)-binding protein (PABP) specifically interacts with the 3′ poly(A) tail of coronavirus genomes, and that this interaction may affect their replication. Other cellular proteins found to bind to coronavirus genomic RNA, such as aconitase and the heat shock proteins HS40 and HS70, might be involved in modulating coronavirus replication. Similarly, interactions of cellular proteins such as transcription cofactor p100 with the EAV nsp1, or of PTB or fructose bisphosphate aldolase A with SHFV genomic RNA, suggest that, in arterivirus replication also, a number of cellular proteins may be involved.

Transcription

RNA-dependent RNA transcription in some members of the *Nidovirales* (coronaviruses, bafiniviruses, and arteriviruses), but not in others (roniviruses), includes a discontinuous RNA-synthesis step. This process occurs during the production of subgenome-length negative-strand RNAs that serve as templates for transcription and involves the fusion of a copy of the genomic 5′-terminal leader sequence to the 3′ end of each of the nascent RNAs complementary to the coding (body) sequences (**Figure 6**). The resulting chimeric sg RNAs of negative polarity are transcribed to yield sg mRNAs that share both 5′- and 3′- terminal sequences with the genome RNA. Genes expressed through sg mRNAs are preceded by conserved 'transcription-regulatory sequences' (TRSs) that presumably act as attenuation or termination signals during the production of the subgenome-length negative-strand RNAs. In arteriviruses and coronaviruses, the TRSs preceding each ORF are presumed to direct attenuation of negative-strand RNA synthesis, leading to the 'jumping' of the nascent negative-strand RNA to the leader TRS (TRS-L). This process is guided by a base-pairing interaction between complementary sequences (leader TRS and body TRS complement) and it has been proposed that template switching only occurs if the free energy (ΔG) for the formation of this duplex reaches a minimum threshold. This process is named 'discontinuous extension of minus strands' and can be considered a variant of similarity-assisted template-switching that operates during viral RNA recombination. The genome and sg mRNAs share a 5′-leader sequence of 55–92 nt in coronaviruses and 170–210 nt in arteriviruses.

Toroviruses are remarkable in that they employ a mixed transcription strategy to produce their mRNAs. Of their four sg mRNA species, the smaller three (mRNAs 3 through 5) lack a 5′ common leader and are produced via nondiscontinuous RNA synthesis. In contrast, sg mRNA2 has a leader sequence that matches the 5′-terminal 18 nt of the genomic RNA and its production requires a discontinuous RNA-synthesis step reminiscent of, but not identical, to that seen in arteri- and coronaviruses.

Synthesis of torovirus mRNAs 3 through 5, and possibly of the two mRNAs in roniviruses, is thought to require the premature termination of negative-strand RNA synthesis at conserved, intergenic, TRS-like sequences to generate subgenome-length negative-strand RNAs that can be used directly as templates for sg mRNA synthesis. In the case of torovirus mRNA2, a TRS is lacking. Fusion of noncontiguous sequences seems to be controlled by a sequence element consisting of a hairpin structure and 3′ flanking stretch of 23 residues with sequence identity to a region at the 5′ end of the genome. It is thought that during negative-strand synthesis, the hairpin structure may cause the transcriptase complex to detach, prompting a template switch similar to that seen in arteri- and coronaviruses.

In addition to regulatory RNA sequences, viral and host components involved in protein–RNA and protein–protein recognition are likely to be important in transcription. For example, the arterivirus nsp1 protein has been identified as a factor that is dispensable for genome replication but absolutely required for sg RNA synthesis. The identification of host factors participating in nidovirus transcription is a field under development and specific binding assays have recently identified a limited number of cellular proteins that associate with *cis*-acting RNA regulatory sequences. For example, differences in affinity of such factors for body TRSs might regulate transcription in nidoviruses by a mechanism similar to that of the DNA-dependent RNA-polymerase I termination system, in which specific proteins bind to termination sequences.

Origin of Nidoviruses

The complex genetic plan and the replicase gene of nidoviruses must have evolved from simpler ones. Using this natural assumption, a speculative scenario of major events in nidovirus evolution has been proposed. It has been speculated that the most recent common ancestor of the *Nidovirales* had a genome size close to that of the current arteriviruses. This ancestor may have evolved from a smaller RNA virus by acquiring the two nidovirus genetic marker domains represented by the helicase-associated zinc-binding domain (ZBD) and the NendoU function. These two domains may have been used to improve the low fidelity of RdRp-mediated RNA replication, thus generating viruses capable of efficiently replicating genomes of about 14 kbp. The subsequent evolution of much larger nidovirus genomes may have been accompanied by the acquisition of the ExoN domain. This domain may have further improved the fidelity of RNA replication through its 3′–5′ exonuclease activity, which might operate in proofreading mechanisms similar to those employed by DNA-based life forms. It has been suggested that the ORF1b-encoded HEL, ExoN, NendoU, and *O*-MT domains may provide RNA specificity, whereas the relatively abundantly expressed CPD and ADRP might control the pace of a common pathway that could be part of a hypothetical oligonucleotide-directed repair mechanism used in the present coronaviruses and roniviruses. The expansion of the replicase gene may have been associated with an increase in replicase fidelity, thus also supporting the further expansion of the 3′-proximal genome region to encode the structural proteins required to form complex enveloped virions.

Effect of Nidovirus Infection on the Host Cell

Compared to other viruses, the interactions of nidoviruses with their hosts have not been studied in great detail. In many cases, information is based on relatively few studies performed on a limited number of viruses from the families *Coronaviridae* and *Arteriviridae*. Also, most studies have been performed with viruses that have been adapted to cell culture and therefore may have properties that differ from those of field strains. Coronaviruses and arteriviruses are clearly the best-studied members of the *Nidovirales* in terms of their interactions with the host.

Coronavirus infection affects cellular gene expression at the level of both transcription and translation. Upon infection, host cell translation is significantly suppressed but not shut off, as is the case in several other positive-RNA viruses. The underlying mechanisms have not been characterized in detail, but data obtained for MHV and BCoV suggest that they may involve the 5′-leader sequences present on coronavirus mRNAs. The viral N protein was reported to bind to the 5′-common leader sequence and it has been speculated that this might promote translation initiation, leading to a preferential translation of viral mRNAs. Furthermore, host mRNAs were reported to be specifically degraded in MHV- and SARS-CoV-infected cells, further reducing the synthesis of cellular proteins. Another mechanism affecting host cell protein synthesis may be based on specific cleavage of the 28S rRNA subunit, which was observed in MHV-infected cells.

Studies on cellular gene expression following nidovirus infections have mainly focused on the coronaviruses MHV and SARS-CoV. For example, SARS-CoV infection was reported to disrupt cellular transcription to a larger extent than does HCoV-229E. Differences in cellular gene expression have been proposed to be linked to differences in the pathogenesis caused by these two human coronaviruses. Apart from the downregulation of genes involved in translation and cytoskeleton maintenance, genes involved in stress response, proapoptotic, pro-inflammatory, and procoagulating pathways were significantly upregulated. Both MHV and SARS-CoV induce mitogen-activated phosphate kinases (MAPKs), especially p38 MAPK. In addition, activation of AP-1, nuclear factor kappa B (NF-κB), and a weak induction of Akt signaling pathways occur after SARS-CoV infection and the N and nsp1 proteins were suggested to be directly involved in inducing these signaling pathways.

Nidoviruses have also been reported to interfere with cell cycle control. Infection by the coronaviruses TGEV, MHV, SARS-CoV, and IBV was reported to cause a cell cycle arrest in the G0/G1 phase and a number of cellular proteins (e.g., cyclin D3 and hypophosphorylated restinoblastoma protein) and viral proteins (MHV nsp1, SARS-CoV 3b 7a, and N proteins) have been proposed to be involved in the cell cycle arrest in G0/G1.

Many viruses encode proteins that modulate apoptosis and, more generally, cell death, which allows for highly efficient viral replication or the establishment of persistent infections. Infection by coronaviruses (e.g., TGEV, MHV, and SARS-CoV) and arteriviruses (e.g., PRRSV and EAV) have been reported to induce apoptosis in certain cell types. Apoptosis has also been reported in shrimp infected with the ronivirus YHV and is thought to be involved in pathogenesis. Both apoptotic and antiapoptotic molecules have been found to be upregulated, suggesting that a delicate counterbalance of pro- and antiapoptotic molecules is required to ensure cell survival during the early phase of infection, and rapid virus multiplication before cell lysis occurs. Coronavirus-induced apoptosis appears to occur in a tissue-specific manner, which obviously has important implications for viral pathogenesis. For instance, SARS-CoV was shown to infect epithelial cells of the intestinal tract and induce an antiapoptotic response that may counteract a rapid destruction of infected enterocytes. These findings are consistent with clinical observations of a relatively normal endoscopic and microscopic appearance of the intestine in SARS patients. Furthermore, SARS-CoV causes lymphopenia which involves the depletion of T cells, probably by apoptotic mechanisms that are triggered by direct interactions of the SARS-CoV E protein with the antiapoptotic factor Bcl-xL. Also the MHV E protein has been reported to induce apoptosis. The SARS-CoV 7a protein was found to induce apoptosis in cell lines derived from lung, kidney, and liver, by a caspase-dependent pathway. Apoptosis has also been associated with arterivirus infection but information on underlying mechanisms and functional implications is limited.

Coronavirus and arterivirus infections trigger proinflammatory responses that often are associated with the clinical outcome of the infection. Thus, for example, there seems to be a direct link between the IL-8 plasma levels of SARS patients and disease severity, similar to what has been described for pulmonary infections caused by respiratory syncytial virus. In contrast, despite the upregulation of IL-8 in intestinal epithelial cells, biopsy specimens taken from the colon and terminal ileum of SARS patients failed to demonstrate any inflammatory infiltrates, which may be the consequence of a virus-induced suppression of specific cytokines and chemokines, including IL-18, in the intestinal environment.

Innate immunity is essential to control vertebrate nidovirus infection *in vivo*. The induction of type I IFN (IFN-α/β) varies among different coronaviruses and arteriviruses. Whereas some coronaviruses such as TGEV are potent inducers of type I IFN, other coronaviruses (MHV and SARS-CoV) or arteriviruses (PRRSV)

do not stimulate its production, thus facilitating virus escape from innate immune defenses. Type I interferon is a key player in innate immunity and in the activation of effective adaptive immune responses. Upon viral invasion, IFN-α/β is synthesized and secreted. IFN-α/β molecules signal through the type I interferon receptor (IFNR), inducing the transcription of several antiviral mediators, including IFN-γ, PKR, and Mx. IFN-γ is critical in resolving coronavirus (MHV and SARS-CoV), and also arterivirus (EAV, LDV, and PRRSV) infections. Like many other viruses, coronaviruses have developed strategies to escape IFN responses. For example, it has been shown that the SARS-CoV 3b, 6, and N proteins antagonize interferon by different mechanisms, even though all these proteins inhibit the expression of IFN by interfering with the function of IRF-3.

In arteriviruses such as PRRSV, IFN-γ is produced soon after infection to promote Th1 responses. However, PRRSV infections or vaccination with attenuated-live PRRSV vaccines cause only limited IL-1, TNF-α, and IFN-α/β responses. This then leads to IFN-γ and Th1 levels that fail to elicit strong cellular immune responses.

See also: Arteriviruses; Coronaviruses: General Features; Coronaviruses: Molecular Biology; Severe Acute Respiratory Syndrome (SARS); Torovirus; Yellow Head Virus.

Further Reading

de Groot RJ (2007) Molecular biology and evolution of toroviruses. In: Snijder EJ, Gallagher T, and Perlman S (eds.) *The Nidoviruses*, pp. 133–146. Washington, DC: ASM Press.

Enjuanes L (ed.) (2005) *Current Topics in Microbiology and Immunology, Vol. 287: Coronavirus Replication and Reverse Genetics.* Berlin: Springer.

Enjuanes L, Almazan F, Sola I, and Zuniga S (2006) Biochemical aspects of coronavirus replication and virus–host interaction. *Annual Review of Microbiology* 60: 211–230.

Gorbalenya AE, Enjuanes L, Ziebuhr J, and Snijder EJ (2006) *Nidovirales*: Evolving the largest RNA virus genome. *Virus Research* 117: 17–37.

Masters PS (2006) The molecular biology of coronaviruses. *Advances in Virus Research* 66: 193–292.

Sawicki SG, Sawicki DL, and Siddell SG (2007) A contemporary view of coronavirus transcription. *Journal of Virology* 81: 20–29.

Siddell SG, Ziebuhr J, and Snijder EJ (2005) Coronaviruses, toroviruses, and arteriviruses. In: Mahy BWJ and ter-Meulen V (eds.) *Virology,* 10th edn. vol.1, pp. 823–856. London: Hoddeer-Arnold.

Snijder EJ, Siddell SG, and Gorbalenya AE (2005) The order *Nidovirales*. In: Mahy BWJ and ter-Meulen V (eds.) *Virology,* 10th edn., vol. 1, pp. 390–404. London: Hodder-Arnold.

Snijder EJ and Spaan WJM (2007) *Arteriviruses*. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* vol. 1, pp. 1205–1220. Philadelphia: Lippincott Williams and Wilkins.

Spaan WJM, Cavanagh D, de Groot RJ, *et al.* (2005) Nidovirales. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 937–945. San Diego, CA: Elsevier Academic Press.

van Vliet ALW, Smits SL, Rottier PJM, and de Groot RJ (2002) Discontinuous and non-discontinuous subgenomic RNA transcription in a nidovirus. *EMBO Journal* 21(23): 6571–6580.

Walker PJ, Bonami JR, Boonsaeng V, *et al.* (2005) *Roniviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 975–979. San Diego, CA: Elsevier Academic Press.

Ziebuhr J and Snijder EJ (2007) The coronavirus replicase: Special enzymes for special viruses. In: Thiel V (ed.) *Molecular and Cellular Biology: Coronaviruses*, pp. 31–61. Norfolk, UK: Caister Academic Press.

Zuñiga S, Sola I, Alonso S, and Enjuanes L (2004) Sequence motifs involved in the regulation of discontinuous coronavirus subgenomic RNA synthesis. *Journal of Virology* 78: 980–994.

Nodaviruses

P A Venter and A Schneemann, The Scripps Research Institute, La Jolla, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Viruses belonging to the family *Nodaviridae* are small (28–37 nm), nonenveloped, and isometric. These viruses characteristically package bipartite positive-sense RNA genomes that are made up of RNA1 (3.0–3.2 kb) and RNA2 (1.3–1.4 kb). RNA1 encodes protein A, the RNA-dependent RNA polymerase (RdRp), and RNA2 encodes the capsid protein which is required for formation of progeny virions. The family is subdivided into two genera: *Alphanodavirus*, whose members infect insects, and *Betanodavirus*, whose members infect fish. Alphanodaviruses have become model systems for studies on RNA replication, specific genome packaging, virus structure, and assembly, and for studies on virus–host interactions that are required to suppress RNA silencing in animal cells. Betanodaviruses, on the other hand, cause high mortalities in hatchery-reared fish larvae and juveniles, and are therefore economically important pathogens to the marine aquaculture industry.

Taxonomy

Table 1 lists the definitive and tentative nodavirus species, their natural hosts, and geographic origin. A comparison between the capsid protein sequences of alpha- and betanodaviruses shows that these genera are distantly related with an approximate similarity of only 10%. Viruses within each genus share antigenic determinants but also show distinct immunological reactivities. Within the genus *Alphanodavirus*, the species *Flock house virus*, *Black beetle virus*, and *Boolarra virus* are relatively closely related to each other while *Nodamura virus* (the type species of this genus) and *Pariacoto virus* are evolutionarily the most distant alphanodaviruses. Conversely, available sequences for the RdRp and capsid protein of three betanodaviruses show high levels of identity (*c.* 80% and 90%, respectively). Phylogenetic analysis based on alignments of a variable region within the betanodavirus capsid protein sequence grouped the virus species into four genotypes: *Tiger puffer nervous necrosis virus*, *Striped jack nervous necrosis virus*, the type species of the genus *Betanodavirus*; *Barfin flounder nervous necrosis virus*; and *Redspotted grouper nervous necrosis virus*. Viruses belonging to the *Striped jack nervous necrosis virus* genotype exhibit subtle but distinct serological differences to viruses belonging to the other genotypes.

Host Range and Geographic Distribution

All known alphanodaviruses were originally isolated in Australasia, except for Pariacoto virus (PaV), which was isolated in Peru. Several alphanodaviruses can multiply in a wide range of insect species in addition to their natural insect hosts (**Table 1**) including bees, beetles, mosquitoes, moths, tsetse flies, and ticks. In the laboratory, larvae of the common wax moth (*Galleria mellonella*) are convenient hosts for most of these viruses. Nodamura virus (NoV) is not only infectious to insects, but also has the unique ability to cause hind-limb paralysis and 100% mortality in suckling mice. Flock house virus (FHV), black beetle virus (BBV), and Boalarra virus (BoV) can readily be propagated to very high yields in cultured *Drosophila melanogaster* cells. PaV is not infectious to *D. melanogaster*

Table 1 Natural hosts and geographic origin of viruses belonging to the family *Nodaviridae*

Virus species	*Virus abbreviation*	*Host*	*Geographic origin*
Genus *Alphanodavirus*			
Black beetle virus	BBV	Scarab beetle (*Heteronychus arator*)	New Zealand
Boolarra virus	BoV	Underground grass grub (*Oncopera intricoides*)	Australia
Flock house virus	FHV	Grass grub (*Costelytra zealandica*)	New Zealand
Gypsy moth virus	GMV	Gypsy moth (*Lymantria ninayi*)	Papua New Guinea
Manawatu virus	MwV	Grass grub (*Costelytra zealandica*)	New Zealand
New Zealand virus	NZV	Unknown	Unknown
Nodamura virus	NoV	Mosquitoes (*Culex tritaeniorhynchus*)	Japan
Pariacoto virus	PaV	Southern armyworm (*Spodoptera eridania*)	Peru
Genus *Betanodavirus*			
Atlantic cod nervous necrosis virus	ACNNV	Atlantic cod (*Gadus morhua*)	Canada
Atlantic halibut nodavirus	AHNV	Atlantic halibut (*Hippoglossus hippoglossus*)	Norway
Barfin flounder nervous necrosis virus	BFNNV	Barfin flounder (*Verasper moseri*)	Japan
Dicentrarchus labrax encephalitis virus	DIEV	Sea bass (*Dicentrarchus labrax*)	France
Dragon grouper nervous necrosis virus	DGNNV	Dragon grouper (*Epinephelus lanceolatus*)	Taiwan
Greasy grouper nervous necrosis virus	GGNNV	Greasy grouper (*Epinephelus tauvina*)	Singapore
Grouper nervous necrosis virus	GNNV	Grouper (*Epinephelus coioides*)	Taiwan
Japanese flounder nervous necrosis virus	JFNNV	Japanese flounder (*Paralichthys olivaceus*)	Japan
Lates calcarifer encephalitis virus	LcEV	Barramundi (*Lates calcarifer*)	Israel
Malabaricus grouper nervous necrosis virus	MGNNV	Grouper (*Epinephelus malabaricus*)	Taiwan
Redspotted grouper nervous necrosis virus	RGNNV	Redspotted grouper (*Epinephelus akaara*)	Japan
Seabass nervous necrosis virus	SBNNV	Sea bass (*Dicentrarchus labrax*)	France
Striped jack nervous necrosis virus	SJNNV	Striped jack (*Pseudocaranx dentex*)	Japan
Tiger puffer nervous necrosis virus	TPNNV	Tiger puffer (*Takifugu rubrides*)	Japan
Umbrina cirrosa nodavirus	UCNNV	Shi drum (*Umbrina cirrosa*)	Italy

cells, but a number of insect cell lines that are susceptible to infection by this virus have been identified, including *Helicoverpa zea* FB33 cells. FHV, NoV, and PaV can also be propagated in baby hamster kidney cells when the genomic RNAs or cDNA clones of these viruses are transfected into these cells. Other heterologous expression systems that have been shown to support FHV replication include plant cells, the yeast *Saccharomyces cerevisiae,* and the worm *Caenorhabditis elegans*.

The incidence of virus nervous necrosis, the disease caused by betanodaviruses, has been reported in Asia, Australia, Europe, Japan, and North America. The natural hosts are predominantly hatchery-reared larvae and juveniles of fish species, but mortalities in adult fish have also been reported. In the laboratory, some betanodaviruses are infectious to a number of fish cell lines, including cultured cells from striped snakehead fish (SNN-1), sea bass larvae (SBL), and orange spotted grouper (GS). Striped jack nervous necrosis virus (SJNNV) also replicates when *in vitro* synthesized transcripts corresponding to its genomic RNAs are introduced into SSN-1 cells.

Virion Properties

The capsids of nodaviruses consist of 180 copies of a single gene product (or protomer) arranged with $T = 3$ icosahedral symmetry. The protomers adopt three slightly different conformations based on the three quasi-equivalent positions within the capsid shell. Sixty of these protomers are arranged into 12 pentamers at the icosahedral fivefold axes, while 120 are arranged into 20 hexamers at the threefold axes. In alphanodaviruses, a significant percentage of the packaged genomic RNA is organized as duplex RNA. Specifically, in the case of PaV, X-ray crystallography visualized 25 bp of double-stranded RNA (dsRNA) at each of the 60 icosahedral twofold contacts of the virion (**Figure 1**). Together, these regions of duplex RNA represent 35% of the genome and they give the impression of a dodecahedral cage in the interior of the particle. RNA cage structures are not observed for betanodaviruses. High-resolution X-ray crystal structures and cryoelectron microscopy image reconstructions of several alphanodaviruses as well as a cryoelectron microscopy image reconstruction of the betanodavirus malabaricus grouper nervous necrosis virus (MGNNV) show that capsid structures are not conserved between the two genera (**Figure 2**).

Alphanodavirus Capsids

Alphanodavirus capsids are approximately 32–33 nm in diameter (**Figure 1(a)**). Each protomer in the viral capsid is initially composed of protein alpha (~44 kDa), which spontaneously cleaves into mature capsid proteins beta (~39 kDa) and gamma (~4 kDa) following assembly of virus particles. Beta represents the N-terminal portion of alpha protein, whereas gamma represents a short C-terminal peptide that remains associated with mature virions. Alpha protein (as well as beta protein) contains a central β-barrel motif that forms the spherically closed shell of the capsid. Loops between the β-strands form the exterior surface of the capsid. The N- and C-termini of alpha protein form the interior surface, which harbors the beta–gamma cleavage site and has predominate α-helical secondary structure. Biophysical studies on the dynamic behavior of FHV particles in solution have shown that the termini are transiently exposed at the exterior surface of the capsid.

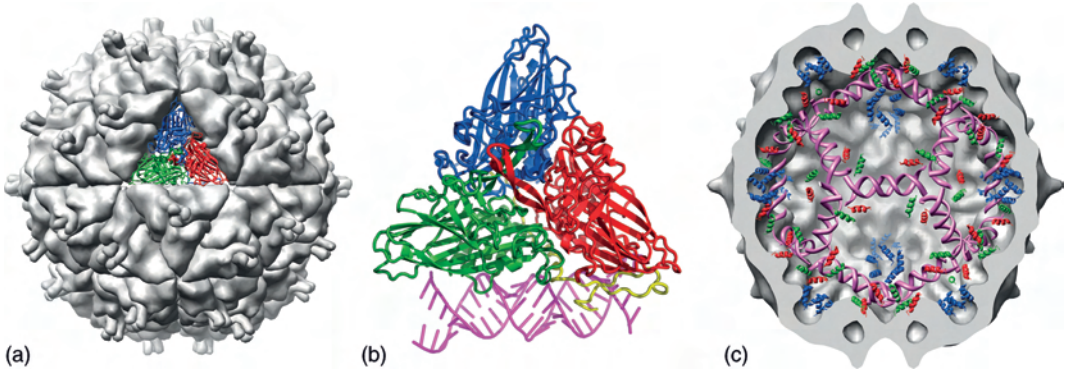


Figure 1 Structure of the alphanodavirus PaV. (a) Molecular surface rendering of PaV based on atomic coordinates. The coat protein subunits or protomers (shown in gray) adopt three different quasi-equivalent positions within the viral capsid. A-subunits are related by fivefold symmetry, while the B- and C-subunits are related by threefold symmetry. The high-resolution structure of one A-, B-, and C-protomer, which together represent one of the 60 icosahedral asymmetric units of the particle, is shown in blue, red, and green, respectively. (b) Top-down view of one icosahedral asymmetric unit showing the interaction between the extended N-terminus (yellow) of the A-subunit (blue) and the duplex RNA (magenta) located at the twofold contacts of the virion. (c) Cut-away view of the PaV virion showing the organization of the internal icosahedrally ordered duplex RNA and the gamma-peptides associated with the A-, B-, and C-subunits. Color coding is as described in panels (a) and (b). The bulk of the coat protein shell (gray) is shown at 22 Å resolution. Panels (a) and (c) were generated with the program Chimera. Courtesy of Dr. P. Natarajan.

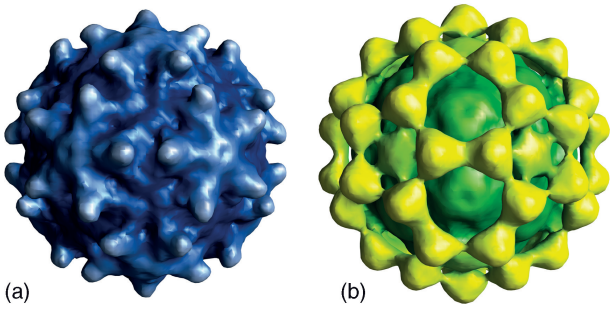


Figure 2 Three-dimensional cryoelectron microscopy reconstruction of (a) native PaV at 22 Å resolution and (b) MGNNV virus-like particle at 23 Å resolution. The inner capsid shell of MGNNV is shown in green, whereas outer protrusions are shown in yellow. Courtesy of Dr. P. Natarajan

The quaternary structure of the termini associated with protomers at the fivefold axes is markedly different from those at the threefold axes:

- Specifically, in PaV, the N-termini of threefold associated protomers are structurally disordered, while the N-termini of fivefold associated protomers are well ordered. Positively charged residues within these termini make extensive neutralizing contacts with the packaged RNA (**Figure 1(b)**). The resultant RNA–protein complexes play an important role in controlling the $T = 3$ symmetry of the capsid. This is illustrated by aberrant assembly of FHV capsid proteins with deleted N-termini. These mutants assemble into multiple types of particles, including smaller 'egg'-shaped particles with regions of symmetry that are similar to those of $T = 1$ particles.
- The C-terminal α-helices on the gamma-cleavage products of threefold-associated protomers interact with the duplex RNA, while their fivefold-associated counterparts do not contact RNA, but are grouped together into helical bundles along the fivefold axes of the capsid (**Figure 1(c)**). The positioning of these pentameric helical bundles within the capsid and the amphipathic character of each helix makes them ideal candidates for membrane-disruptive agents that are released from the virion during cell entry to facilitate the translocation of genomic RNA into host cells. These helices have in fact been shown to be highly disruptive to artificial membranes *in vitro* as judged by their ability to permeabilize liposomes to hydrophilic solutes.

Betanodavirus Capsids

MGNNV capsids are approximately 37 nm in diameter and thus slightly larger than alphanodavirus capsids. In addition, each of the protomers of the capsid consists of two structural domains as compared to the single domain structures of alphanodavirus protomers. The domains that are more internally located in the capsid form a contiguous shell around the packaged RNA while the outer domains form distinct surface protrusions on MGNNV capsids (**Figure 2**). Each betanodavirus protomer is composed of a single capsid protein (42 kDa) that does not undergo autocatalytic cleavage.

Virion Assembly and Specific Genome Packaging

The assembly pathway of alphanodaviruses is much better characterized than that of betanodaviruses. Alphanodavirus assembly proceeds through a precursor particle, the provirion, while an equivalent assembly intermediate is not evident for betanodaviruses. For alphanodaviruses, 180 copies of newly synthesized capsid precursor protein alpha assemble rapidly in the presence of excess viral RNA into the provirion intermediate, which has identical morphology to that of a mature virion. The assembly of provirions serves as a trigger for a maturation event in which alpha is autocatalytically cleaved into beta and gamma with a halftime of about 4 h. Maturation is required for acquisition of particle infectivity. A dependence on cleavage for infectivity is in agreement with the role of gamma as a membrane disruption agent for RNA translocation during cell entry.

Although mature virions display increased chemical stability compared to provirions, it has been shown that the capsid proteins of mature virions more readily unfold under high pressure. This characteristic combined with the dynamic properties of mature virions could also be critical for their infectivity, because it could accelerate virus uncoating during cell entry.

The two genomic strands of alphanodaviruses are co-packaged into a single virion during assembly. Compelling evidence for co-packaging comes from the discovery that the entire complement of packaged RNA1 and RNA2 forms a stable hetero-complex within FHV virions when these particles are exposed to heat. The mechanism by which FHV co-packages its genome is still unknown, but a number of molecular determinants for specific genome packaging have been elucidated:

- Mutants of capsid protein alpha lacking N-terminal residues 2–31 are unable to package RNA2. The N-terminal residues of this protein are therefore bifunctional for virion assembly, because they control both the specific packaging of RNA2 and the $T = 3$ symmetry of capsids.
- The C-terminal residues carried on the gamma-region of alpha protein are not only critical for virus assembly and infectivity, but also for the specific packaging of both viral RNAs.
- A predicted stem–loop structure proximal to the 5′ end of RNA2 (nt 186–217) has been proposed to represent a packaging signal for this genomic segment.

Despite the specificity for genomic RNA under native conditions, random cellular RNAs are readily packaged into virus-like particles (VLPs) when alpha is synthesized in a heterologous expression system. This is also true for the betanodaviruses MGNNV and dragon grouper nervous necrosis virus (DGNNV), whose capsid proteins have been synthesized in insect cells and *Escherichia coli*, respectively. The random RNAs packaged within alphanodavirus VLPs are organized into dodecahedral cages that are very similar to those of wild-type virions. Alpha protein is therefore the sole determinant for RNA cage formation, because specific RNA sequences and lengths are not important for this organization of the packaged nucleic acid.

Genome packaging is not only controlled by specific interactions between alpha protein and the viral RNAs, but also by a coupling between RNA replication and virion assembly. Only alpha proteins synthesized from replicating RNAs, as opposed to nonreplicating RNA, can package viral genomic RNA and partake in the assembly of infectious virions. This is exemplified by an ability to synthesize two distinct populations of FHV particles when alpha is co-synthesized from both replicating and nonreplicating RNAs within the same cell: (1) a population of infectious virions derived from the synthesis of alpha from replicating viral RNA, and (2) a population of VLPs containing random cellular RNA derived from the synthesis of alpha from nonreplicating mRNA.

Genome Structure and Coding Potential

The nodaviral genome consists of RNA1 and RNA2, and both RNAs are co-packaged into single virions. The 5′ ends of these RNAs are capped but their 3′ ends are not polyadenylated. Interestingly, the 3′ ends of alphanodavirus RNAs are unreactive with modifying enzymes to the 3′-hydroxyl groups of RNA molecules, while those of betanodavirus RNAs are marginally more reactive. It therefore appears as if these 3′ ends are blocked by an as-yet unidentified moiety or secondary structure.

A schematic representation of the genomic organization and replication strategy of FHV is shown in **Figure 3**. An open reading frame for the 112 kDa RdRp (protein A) nearly spans the entire length of FHV RNA1. RNA3, a 387 nt subgenomic RNA that is not packaged into virions, is synthesized from RNA1. RNA3 corresponds to the 3′ end of RNA1 and carries two open reading frames. One of these is in the +1 reading frame relative to protein A and

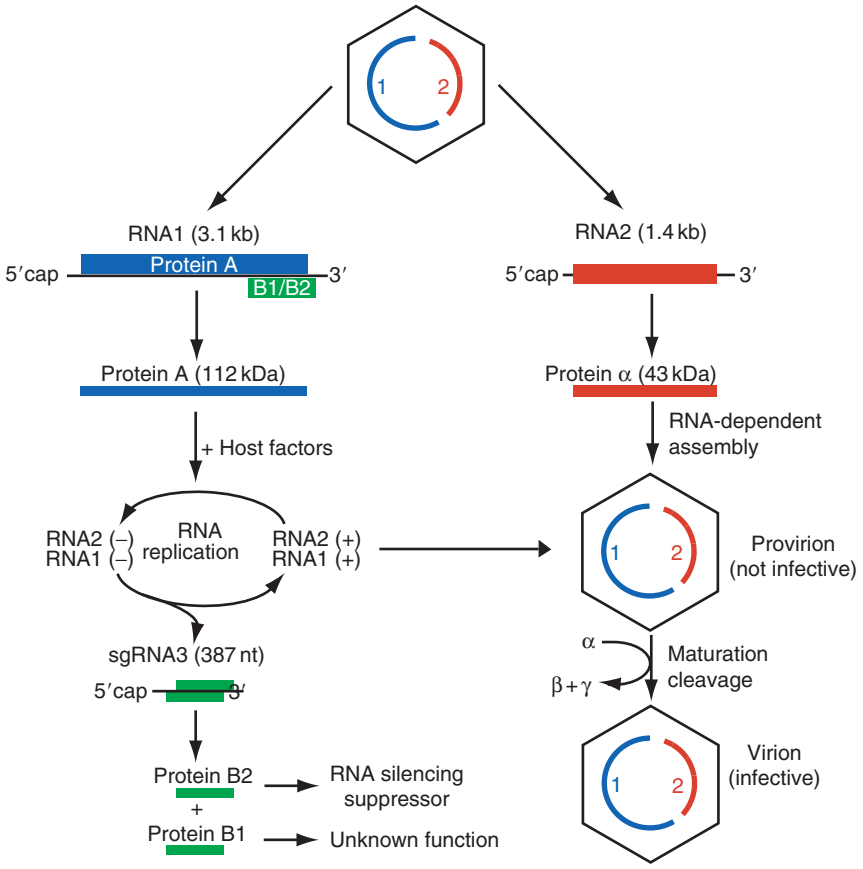


Figure 3 Genomic organization and replication strategy of the alphanodavirus FHV. Adapted from Ball LA and Johnson KL (1998) Nodaviruses of insects. In: Miller LK and Ball LA (eds.) *The Insect Viruses*, pp. 225–267. New York: Plenum.

encodes B2 (106 residues), which is required for the suppression of RNA silencing in infected host cells. The other matches the protein A reading frame and encodes B1 (102 residues) whose function is unknown. The coding potential of RNA1 and RNA3 is conserved within the family *Nodaviridae* with the exception that BoV RNA3 does not encode B1. RNA2 of all the species within this family has a single open reading frame for the viral capsid protein. In the genus *Alphanodavirus*, this protein is in the form of capsid precursor protein alpha, which undergoes cleavage to produce beta and gamma, while in the genus *Betanodavirus* it is in the form of a capsid protein that is not cleaved.

Regulation of Viral Gene Expression

The information on the replication cycle of nodaviruses in this and the next section pertains primarily to alphanodaviruses, which have been studied in much greater detail than betanodaviruses.

Following virion entry, RNA and protein synthesis are temporally regulated with the net effect of dividing the cellular infection cycle of nodaviruses into two phases. In the early phase, replication complexes are established to enable the amplification of viral RNAs, and in the following phase, the synthesis of capsid protein alpha is boosted to high levels to promote virion assembly. As described in the following section, RNA3 fulfills an important role in regulating this process, which is best characterized for FHV among all the nodaviruses.

RNA Synthesis

RNA1 and RNA2 are detectable at 5 h post infection. Rates of synthesis for these RNAs peak at about 16 h, but the RNAs continue to accumulate to very high levels within the cell throughout the remainder of the infection cycle which lasts 24–36 h. RNA3, on the other hand, is co-synthesized with RNA1 in the early hours of infection, but levels of this RNA decrease dramatically after 10 h. Interactions between RNA3 and RNA2 coordinate the synthesis of RNA1 and RNA2 in two ways. First, RNA3 is required as a transactivator for the replication of RNA2, which is a unique function for a subgenomic RNA. Second, RNA2 suppresses RNA3 synthesis, which accounts for the diminished levels of RNA3 at the later stages of the replication cycle.

Protein Synthesis

During the early stages of virus infection, proteins A and B2 accumulate within the cells to establish replication complexes and to suppress the RNA-silencing defenses within the cell, respectively. The rate of protein A synthesis peaks at 5 h post infection and B2 is optimally synthesized between 6 and 10 h. Subsequent to this, the synthesis of these proteins is suppressed, but both proteins are stable and remain detectable at the very late stages of infection. The shutdown of protein A synthesis is partly due to the increased translation efficiency for RNA2 as compared with RNA1. Conversely, the decreased rate of B2 synthesis is due to the RNA2-mediated suppression of RNA3 synthesis. The synthesis of capsid protein alpha lags 2–3 h behind the synthesis of proteins A and B2. Hereafter, alpha is synthesized at a remarkable rate, which peaks at 16 h, and it accumulates to cellular levels that are significantly greater than those of proteins A and B2.

RNA Replication

The replication cycle of nodaviruses occurs exclusively within the cytoplasm of infected cells and leads to the accumulation of RNA1 and RNA2 to levels that approximate those of ribosomal RNAs. The majority of these RNAs are positive stranded, but *c.* 1% are in the form of negative strands. Protein A is the only virus-encoded protein that is required for RNA replication, because it directs the autonomous replication of RNA1 in the absence of RNA2. In addition, the other proteins encoded on FHV RNA1 (B1 and B2) are redundant for replication in cells that do not inactivate FHV via the RNA-silencing pathway. Consistent with its role in RNA replication, protein A of both alpha- and betanodaviruses contains a GlyAsp Asp motif, as well as a number of other conserved motifs for RdRps.

Replication Complexes

The replication of nodaviral RNA occurs within spherules that have a diameter of 40–60 nm and are formed by the outer membranes of mitochondria in infected cells (**Figure 4**). Within each spherule, protein A functions as an integral membrane protein that is exposed on the cytoplasmic face of the outer membrane. The N-terminus of protein A acts both as a transmembrane domain that anchors it into the membrane and as a signal peptide for the targeting of this protein to mitochondria. It is presumed that multiple copies of protein A are associated with each spherule, because several copies thereof are visualized in spherules by immunoelectron microscopy, and this protein was shown to self-interact *in vivo* by co-immunoprecipitation and fluorescence resonance energy transfer.

Several alphanodaviruses are able to replicate their RNAs in diverse cellular environments, including those of insects, yeast, plant, worm, and mammalian cells. This shows that the formation of active replication complexes does not rely on factors that are specific to a particular host. The type of intracellular membrane is also not critical, because protein A mutants with N-terminal targeting sequences to endoplasmic reticulum membranes

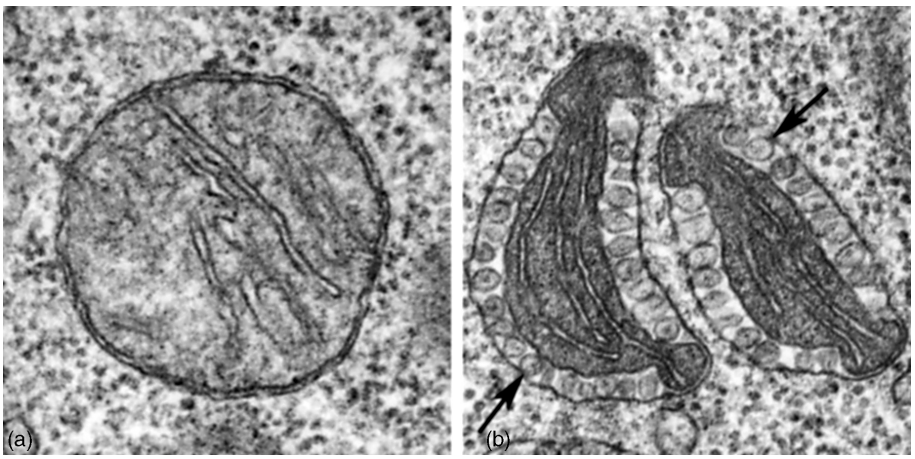


Figure 4 Electron micrographs of mitochondria in (a) uninfected and (b) FHV-infected *D. melanogaster* cells. Arrows point to spherules in which RNA replication occurs. Reproduced from Miller DJ, Schwartz MD, and Ahlquist P (2001) Flock house virus RNA replicates on outer mitochondrial membranes in drosophila cells. *Journal of Virology* 75: 11664–11676.

relocate to these membranes and support robust RNA replication. However, membrane association of protein A is important for complete replication of RNA1, RNA2, and RNA3 as gauged by the *in vitro* activities of partially purified preparations of this protein. Apart from its dependence on intracellular membranes, only one cellular host factor that supports RNA replication has been identified. This factor, the cellular chaperone heat shock protein 90, does not facilitate RNA synthesis *per se*, but plays an important role in the assembly of FHV RNA replication complexes.

cis-Acting Sequences Controlling Replication

During the replication of RNA1 and RNA2, the synthesis of negative strands is governed by approximately 50–58 nt at the 3′ end of positive-strand templates. Conversely, synthesis of positive strands requires fewer than 14 nt at the 3′ ends of negative-strand templates. Internally located *cis*-acting elements on the positive strands have been identified on both FHV RNA1 (nucleotides 2322–2501) and RNA2 (nucleotides 520–720). Furthermore, it is likely that a predicted secondary structure at the 3′ end of positive-strand RNA2 from FHV, BBV, BoV, and NoV acts as a *cis*-acting signal for the synthesis of RNA2 negative strands. This structure consists of two stem loops and is preceded by a conserved C-rich motif. No conserved sequences or secondary structures are discernible at the 3′ termini of RNA1.

A long-distance base-pairing interaction between two *cis*-acting elements on positive strands of RNA1 controls the transcription of the subgenomic RNA3. The base pairing occurs between a short element (approximately 10 nt) located 1.5 kbp upstream of the RNA3 start site and a longer element (approximately 500 nt) that is located proximal to the start site. It is highly likely that this interaction promotes the premature termination of negative-strand RNA1 synthesis, which would result in the synthesis of negative-strand RNA3. Evidence that the synthesis of negative-strand RNA3 precedes the synthesis of positive-strand RNA3 supports this hypothesis. Following its transcription from RNA1, RNA3 is able to replicate in an RNA1-independent manner.

Genetics

The nodavirus RdRp switches templates to generate two types of minor RNA species by RNA recombination: (1) defective interfering RNAs (DI-RNAs) and (2) RNA dimers. DI-RNAs are internally deleted products of the genomic RNAs and often contain sequence rearrangements. RNA dimers, on the other hand, contain head-to-tail junctions between two identical copies of RNAs 1, 2, or 3, or between a copy each of RNA2 and RNA3. Both positive- and negative-strand versions of these RNAs are generated in infected cells and it is unknown whether dimers play a role in RNA replication.

Reassortment between RNA1 and RNA2 could possibly play a role in the evolution of nodaviruses. However, evidence for this is only experimental in nature, as it was shown that the RdRps from FHV, BBV, and BoV can replicate each other's RNAs and that all six of the possible reassortants are infectious.

Suppression of RNA Silencing by Protein B2

B2 plays a critical role in the life cycle of alphanodaviruses as a suppressor of RNA silencing, which acts as an antiviral defense mechanism in the insect hosts of these

viruses. RNA silencing is triggered by dsRNA, which may be present at the onset of an alphanodavirus infection as RNA replication intermediates or as structured regions within single-stranded viral RNAs. During the RNA-silencing pathway, this dsRNA is processed into small (21–25 bp) interfering RNAs (siRNAs) by the endoribonuclease Dicer, and the siRNAs, in turn, guide the RNA-induced silencing complex (RISC) to specifically degrade the viral genome.

B2 has been proposed to suppress RNA silencing at two stages of this pathway. First, it binds to the siRNA cleavage products of Dicer and can therefore prevent their incorporation into RISC. Second, it is able to bind to long dsRNAs and may therefore inhibit the formation of siRNAs from full-length viral RNAs. B2 is a homodimer that forms a four-helix bundle (**Figure 5**). The dimer interacts with one face of the RNA duplex and makes several contacts with two minor grooves and the intervening major groove. These contacts are exclusively with the ribose phosphate backbone of the RNA helix, which explains the sequence-independent binding of B2 to dsRNA.

The role of alphanodaviral B2 as a suppressor of RNA silencing is not limited to insects, but extends to the RNA silencing pathways of plants, worms (*C. elegans*), and mammalian cells. Preliminary studies on betanodaviral B2 suggest that this protein functions as an inhibitor of RNA silencing in fish.

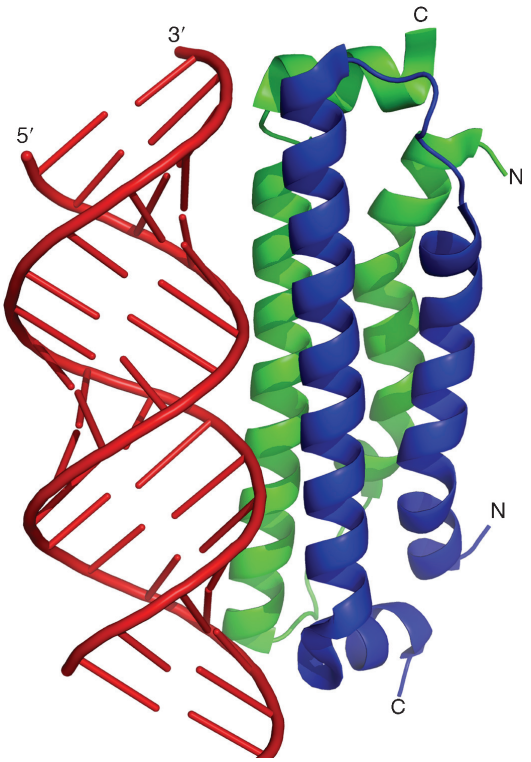


Figure 5 Structure of FHV B2 bound to dsRNA. Blue and green: B2 dimer; red: RNA. B2 forms a homodimer that binds dsRNA in a sequence-independent manner. The interactions occur between the flat face of the B2 molecule and the phosphodiester backbone of the RNA. Courtesy of Dr. P. Natarajan.

Virus–Host Cell Interactions

A general phenomenon of nodavirus infection is the presence of large cytoplasmic paracrystalline arrays of virus particles. In addition, betanodavirus-infected cells exhibit extensive vacuolation, while alphanodavirus-infected cells characteristically show a clustered distribution of mitochondria. The mitochondria are markedly elongated and deformed, and show the presence of numerous spherules on their surface (see **Figure 4**). In cell culture, alphanodavirus infections are accompanied by a progressive shutdown of host protein synthesis and the accumulation of virus particles to very high yields. Despite the high virus yields, most cell types permissive to infection by these viruses remain intact. The exceptions are *D. melanogaster* line 1 cells infected with either FHV or BBV, because these cells undergo cytolysis at 3 days post infection.

Both FHV and BBV are also able to establish persistent infections in *D. melanogaster* cells, rendering these cells immune to superinfection by FHV and BBV. Persistence is not established by mutations within the viral genes and could therefore only be attributed to cellular changes that affect interactions between the virus and its host.

Epidemiology and Pathogenesis

Disease symptoms for the natural insect hosts of alphanodaviruses have not been characterized, but are presumed to be similar to those of experimentally infected *G. mellonella* larvae, which suffer stunted growth, severe paralysis, and 100% mortality. In general, these viruses show a wide histopathological distribution within insects, and are readily detectable in neural and adipose tissues, midgut cells, salivary glands, muscle, trachea, and fat body. NoV causes severe hind limb paralysis in suckling mice, making it the only alphanodavirus pathogenic to a mammalian host. This disease symptom is caused by the replication of this virus in muscle cells and by the degeneration of spinal cord neurons.

Betanodaviruses are the causative agents of virus nervous necrosis in larvae and juveniles of both marine and freshwater fish, and cause significant problems to the marine aquaculture industry. The ability of these viruses to be transmitted both horizontally and vertically is most probably a contributing factor to the epizootic infections that have been caused by these viruses among hatchery-reared fish. Infected fish larvae show abnormal swimming behavior, which is caused by the development of vacuolating encephalopathy and retinopathy. Like alphanodaviruses, betanodaviruses are also able to establish

persistent infections in their fish hosts. This is evident in the detection of these viruses in large populations of marine fish that show no clinical signs of viral nervous necrosis. The development and instatement of screening methods for the detection of these subclinical infections are therefore of great importance to the marine aquaculture industry.

See also: Fish Viruses; Viral Suppressors of Gene Silencing.

Further Reading

Ball LA and Johnson KL (1998) Nodaviruses of insects. In: Miller LK and Ball LA (eds.) *The Insect Viruses*, pp, 225–267. New York: Plenum.

Chao JA, Lee JH, Chapados BR, *et al.* (2005) Dual modes of RNA-silencing suppression by flock house virus protein B2. *Nature Structural and Molecular Biology* 12: 952–957.

Delsert C, Morin N, and Comps M (1997) Fish nodavirus lytic cycle and semipermissive expression in mammalian and fish cell cultures. *Journal of Virology* 71: 5673–5677.

Li H, Li WX, and Ding SW (2002) Induction and suppression of RNA silencing by an animal virus. *Science* 296: 1319–1321.

Lindenbach BD, Sgro JY, and Ahlquist P (2002) Long-distance base pairing in flock house virus RNA1 regulates subgenomic RNA3 synthesis and RNA2 replication. *Journal of Virology* 76: 3905–3919.

Miller DJ, Schwartz MD, and Ahlquist P (2001) Flock house virus RNA replicates on outer mitochondrial membranes in Drosophila cells. *Journal of Virology* 75: 11664–11676.

Nishizawa T, Furuhashi M, Nagai T, Nakai T, and Muroga K (1997) Genomic classification of fish nodaviruses by molecular phylogenetic analysis of the coat protein gene. *Applied and Environmental Microbiology* 63: 1633–1636.

Schneemann A, Ball LA, Delsert C, Johnson JE, and Nishizawa T (2005) Family *Nodaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 865–872. San Diego, CA: Elsevier Academic Press.

Schneemann A, Reddy V, and Johnson JE (1998) The structure and function of nodavirus particles: A paradigm for understanding chemical biology. *Advances in Virus Research* 50: 381–446.

Tang L, Johnson KN, Ball LA, *et al.* (2001) The structure of pariacoto virus reveals a dodecahedral cage of duplex RNA. *Nature Structural Biology* 8: 77–83.

Tang L, Lin CS, Krishna NK, *et al.* (2002) Virus-like particles of a fish nodavirus display a capsid subunit domain organization different from that of insect nodaviruses. *Journal of Virology* 76: 6370–6375.

Venter PA, Krishna NK, and Schneemann A (2005) Capsid protein synthesis from replicating RNA directs specific packaging of the genome of a multipartite, positive-strand RNA virus. *Journal of Virology* 79: 6239–6248.

Noroviruses and Sapoviruses

K Y Green, National Institutes of Health, Bethesda, MD, USA

© 2008 Published by Elsevier Ltd.

Glossary

Fomites An object that in itself is not harmful, but that can harbor a pathogenic organism and therefore serve as an agent of its transmission.

Gastroenteritis An inflammation of the stomach and intestines, often characterized by symptoms of vomiting and diarrhea.

Introduction

Diarrheal illnesses have a major impact on public health. The association of bacteria with such illness was recognized over a century ago with the discovery of bacterial pathogens such as *Vibrio cholerae* and *Shigella dysenteriae*. The role of viruses in diarrheal illness was established much later. Volunteer studies in the 1940s showed that bacteria-free filtrates of feces obtained from individuals with diarrheal disease could induce a similar illness in volunteers who were challenged orally with the inoculum. In 1972, the Norwalk virus was identified in human feces and shown to be the etiologic agent of an outbreak of gastroenteritis that occurred in an elementary school in Norwalk, Ohio in 1968. The Norwalk virus became the prototype strain for a large group of related caliciviruses known now as the noroviruses. A second group of distantly related caliciviruses, the sapoviruses, was discovered soon afterwards.

The family *Caliciviridae* includes several important human and animal pathogens, including the noroviruses and sapoviruses associated with acute gastroenteritis (**Table 1**). The human noroviruses, named for the prototype strain *Norwalk virus*, are now recognized as a major cause of gastroenteritis in all age groups. Norovirus outbreaks often occur in settings such as communities, nursing homes, schools, hospitals, cruise ships, camps, social gatherings, families, and military personnel. Norovirus illnesses can also occur sporadically in the community. A common name for norovirus gastroenteritis is 'stomach flu', but this is a misnomer because the noroviruses are not related to influenza virus. The acute, symptomatic phase of the illness, which often includes either vomiting or diarrhea, or both, generally lasts from 24 to 48 h. In most cases, norovirus gastroenteritis is mild and self-limiting, but it can be

Table 1 Taxonomy of the *Caliciviridae*

Genus	*Species*	*Type strain*
Norovirus (NoV)	*Norwalk virus* (NV)	Hu/NoV/GI.1/Norwalk/1968/US
Sapovirus (SaV)	*Sapporo virus* (SV)	Hu/SaV/GI.1/Sapporo/1982/JP
Lagovirus (LaV)	*Rabbit hemorrhagic disease virus* (RHDV)	Ra/LaV/RHDV/GH/1988/DE
	European brown hare syndrome virus (EBHSV)	Ha/LaV/EBHSV/GD/1989/FR
Vesivirus (VeV)	*Vesicular exanthema of swine virus* (VESV)	Sw/VeV/VESV/VESV-A48/1948/US
	Feline calicivirus (FCV)	Fe/VeV/FCV/F9/1958/US

Calicivirus strains are written in a cryptogram format that is organized as follows: Host species from which the virus was obtained/genus/species (or genogroup)/strain name/year of occurrence/country of origin. Abbreviations for the host species are: Fe, feline; Ha, Hare; Hu, Human; Sw, Swine; Ra, Rabbit. Country abbreviations are: DE, Germany; FR, France; JP, Japan; US, United States. GenBank Accession numbers of representative viruses: Norwalk virus, M87661; Sapporo virus, U65427; RHDV, M67473; VESV, AF181082; FCV, M86379

incapacitating during the symptomatic phase. The illness can sometimes be severe and prolonged in certain individuals such as either the very old or young, or those compromised by pre-existing illness or immunosuppressive therapy.

The sapoviruses, named for the prototype strain, *Sapporo virus*, are characteristically associated with pediatric gastroenteritis, but outbreaks and illness in older individuals can occur. Although illness is characteristically mild, severe disease has been reported. The sapoviruses do not appear to be a major cause of epidemic gastroenteritis, in contrast to the noroviruses. It should also be noted that although sapoviruses and noroviruses have both been associated with pediatric gastroenteritis, and the noroviruses are considered to be the second most important cause of such illnesses, the major cause by far of severe nonbacterial diarrhea in infants and young children is the rotavirus, which belongs to a different virus family, the *Reoviridae*.

Caliciviruses have been detected in a large number of animal species and are associated with a diverse range of clinical syndromes (**Table 2**). Veterinary pathogens of note in the *Caliciviridae* include feline calicivirus (FCV), an important cause of respiratory illness in cats, and rabbit hemorrhagic disease virus (RHDV), an often-fatal acute infection in rabbits.

Table 2 Natural host range and disease syndromes associated with caliciviruses

Calicivirus Genus	*Host*	*Disease syndrome*
Norovirus	Human	Gastroenteritis
	Porcine	Gastroenteritis
	Bovine	Gastroenteritis
	Murine	Asymptomatic enteric infection in wild type mice severe systemic disease in mice lacking STAT1
Sapovirus	Human	Gastroenteritis
	Porcine	Gastroenteritis
	Mink	Gastroenteritis
Vesivirus[a]	Porcine	Vesicular exanthema, abortion
	Sea lion	Vesicular lesions
	Chimpanzee	Vesicular lesions
	Canine	Gastroenteritis
	Walrus	None described
	Mink	None described
	Bovine	None described
	Reptilian	None described
	Skunk	None described
	Amphibian	None described
	Cetacean	None described
	Feline	Upper respiratory, pneumonia, oral ulceration
Lagovirus	Rabbit	Hepatitis, disseminated intravascular coagulation
	Hare	Hepatitis, disseminated intravascular coagulation

[a]There is a report documenting human infection with a vesivirus isolated from a sea lion.

Taxonomy and Classification

The taxonomy of the noroviruses and sapoviruses is based primarily on the phylogenetic relationships among strains (**Figure 1**). Members of the family *Caliciviridae* apparently share a common ancestor, and the four major phylogenetic groups define the four genera: *Norovirus*, *Sapovirus*, *Lagovirus*, and *Vesivirus* (**Table 1**). Members within each genus share common features in their genomic organization, but host range and disease manifestations may vary among members of each genus. Each genus is further divided into one or more species, with each species represented by a 'type' strain (**Table 1**). The genus *Lagovirus* is comprised of two species, *Rabbit hemorrhagic disease virus* and *European brown hare syndrome virus*, and the genus *Vesivirus* is subdivided into two species, *Vesicular exanthema of swine virus* and *Feline calicivirus*. Presently, the genera *Norovirus* and *Sapovirus* have only one species each, designated as *Norwalk virus* and *Sapporo virus*, respectively.

The official classification system of viruses by the International Committee on Taxonomy of Viruses (ICTV) does not address classification below the species level. However, several genetic typing systems have been developed to facilitate communication among researchers in tracking the spread of epidemic strains. These genetic typing systems are based on sequence relatedness in a selected

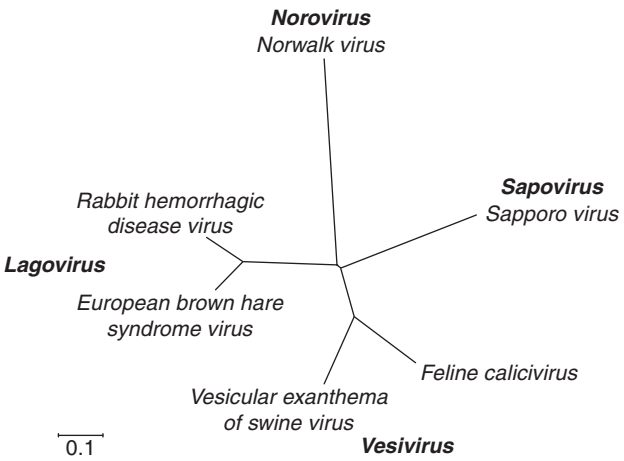


Figure 1 Phylogenetic relationships of the *Caliciviridae*. Caliciviruses in the four genera (*Norovirus*, *Sapovirus*, *Lagovirus*, and *Vesivirus*) cluster into four major groups when the nucleotide sequences of full-length genomes are compared in a phylogenetic analysis (Neighbor-joining, Jukes-Cantor distance parameter). Each genus can be further divided into groups that represent distinct species.

region of the viral genome. One such approach is based on comparisons of the major capsid protein-encoding gene as illustrated for the noroviruses and sapoviruses in **Table 3**. In these genetic typing systems, the noroviruses and sapoviruses are first divided by sequence relatedness into major branches within the genus termed 'genogroups'. For example, noroviruses are presently divided into five distinct genogroups designated I, II, III, IV, and V. Each genogroup is further divided into genetic clusters that are designated as 'genotypes'. For example, genogroup I of the noroviruses is divided into eight genotypes, and genogroup II is divided into 19 genotypes (**Table 3**). The role of genetic diversity in the natural history of the noroviruses and sapoviruses, and the correlation of genotypes with antigenicity is not yet known. However, the classification and genetic typing systems allow the utilization of a cryptogram to describe strains. The cryptogram is organized as follows: Host species from which the virus was obtained/genus/species or genetic type/strain name/year of occurrence/country of origin. For example, Norwalk virus would be designated as: Hu/NoV/GI.1/Norwalk/1968/US.

Virion Structure and Composition

Virus particles are isometric, ranging from 27 to 35 nm in diameter, with icosahedral symmetry. Human caliciviruses such as Norwalk virus (representing the noroviruses) and Parkville virus (representing the sapoviruses) can be observed in stool specimens by negative-stain-electron microscopy (EM) as shown in **Figure 2**. Cryo-EM and computer-generated reconstructions of recombinant virus-like particles produced by expression of the major capsid protein (VP1) of the Norwalk or Parkville virus illustrates the presence of the cup-shaped depressions on the surface, a common feature of caliciviruses that inspired the family *Caliciviridae* (*calix* means 'cup' in Latin) (**Figure 2**). The mature virion contains two structural proteins, VP1 and VP2. VP1 (approximately 60 kDa in most strains) is the predominant capsid protein and is present in 180 copies per virion. The VP1 contains the major antigenic and receptor binding sites of the virus. The VP2 (ranging in size from approximately 8 to 29 kDa) is present in an estimated one or two copies per virion, and may play a role in particle assembly and stability. A third protein found in virions is the VPg, an approximately 15 kDa protein that is covalently linked to the viral RNA genome. The presence of the VPg protein on the viral RNA is required for the efficient initiation of an infection following entry of the viral RNA into cells, most likely because of the proposed role of VPg in mediating interactions between the viral RNA and the host cell translation machinery.

Genome Organization and Expression

The genome of the caliciviruses is a positive-sense RNA molecule ranging in length from approximately 7.4 to 8.3 kbp. The 5′-end of the genome is covalently linked to a protein, VPg, and the 3′-end of the genome contains a poly (A) tract. There is a relatively short nontranslated region (NTR) at both the 5′- and 3′-ends of the genome that flank the open reading frames (ORFs). Genomes of the caliciviruses are organized into either two or three major ORFs, depending on the genus (**Figure 3**). The first ORF (beginning near the 5′-end) of all caliciviruses encodes the nonstructural (NS) proteins of the virus, whereas the terminal ORF encodes the minor structural protein, VP2. The major difference in reading frame usage among the caliciviruses relates to the coding sequence of the major capsid protein, VP1. In the noroviruses and vesiviruses, the VP1 is encoded in a separate reading frame (ORF2), whereas in the sapoviruses and lagoviruses, the VP1 is encoded in the same ORF (ORF1) as the NS proteins. Thus, the norovirus genome is organized into three major ORFs, and the sapovirus genome is organized into two major ORFs.

Caliciviruses encode six to seven mature NS proteins, depending again on the genus. This variation is due to differences among the proteolytic processing strategies used by viruses to cleave the ORF1 polyprotein. In the lagoviruses, the virus-encoded cysteine proteinase cleaves the ORF1 polyprotein at six cleavage sites to release seven mature NS proteins, designated as NS1 through NS7 (**Table 4**). In some genera, cleavage between certain NS proteins has not been detected during virus replication. This includes the NS1 and NS2 precursor of the noroviruses (designated as NS1–2 in this nomenclature system, and known also as the N-terminal protein or p48) and the

Table 3 Norovirus and sapovirus genetic typing systems

Reference virus	*Genogroup*	*Genotype*	*GenBank accession number*
Noroviruses			
Hu/NoV/GI.1/Norwalk/1968/US	I	1	M87661
Hu/NoV/GI.2/Southampton/1991/UK	I	2	L07418
Hu/NoV/GI.3/Desert Shield 395/1990/SA	I	3	U04469
Hu/NoV/GI.4/Chiba 407/1987/JP	I	4	AB042808
Hu/NoV/GI.5/Musgrove/1989/UK	I	5	AJ277614
Hu/NoV/GI.6Hesse 3/1997/DE	I	6	AF093797
Hu/NoV/GI.7/Winchester/1994/UK	I	7	AJ277609
Hu/NoV/GI.8/Boxer/2001/US	I	8	AF538679
Hu/NoV/GII.1/Hawaii/1971/US	II	1	U07611
Hu/NoV/GII.2/Melksham/1994/UK	II	2	X81879
Hu/NoV/GII.3/Toronto 24/1991/CA	II	3	U02030
Hu/NoV/GII.4/Bristol/1993/UK	II	4	X76716
Hu/NoV/GII.5/Hillingdon/1990/UK	II	5	AJ277607
Hu/NoV/GII.6/Seacroft/1990/UK	II	6	AJ277620
Hu/NoV/GII.7/Leeds/1990/UK	II	7	AJ277608
Hu/NoV/GII.8/Amsterdam/1998/NL	II	8	AF195848
Hu/NoV/GII.9/VA97207/1997	II	9	AY038599
Hu/NoV/GII.10/Erfurt546/2000/DE	II	10	AF427118
Sw/NoV/GII.11/Sw918/1997/JP	II	11	AB074893
Hu/NoV/GII.12/Wortley/1990/UK	II	12	AJ277618
Hu/NoV/GII.13/Fayetteville/1998/US	II	13	AY113106
Hu/NoV/GII.14/M7/1999/US	II	14	AY130761
Hu/NoV/GII.15/J23/1999/US	II	15	AY130762
Hu/NoV/GII.16/Tiffin/1999/US	II	16	AY502010
Hu/NoV/GII.17/CS-E1/2002/US	II	17	AY502009
Sw/NoV/GII.18/OH-QW101/2003/US	II	18	AY823304
Sw/NoV/GII.19/OH-QW170/2003/US	II	19	AY823306
Bo/NoV/GIII.1/Jena/1980/DE	III	1	AJ011099
Bo/NoV/CH126/1998/NL	III	2	AF320625
Hu/NoV/GIV.1/Alphatron 98–2/1998/NL	IV	1	AF195847
Mu/NoV/GV.1/MNV-1/2003	V	1	AY228235
Sapoviruses			
Hu/SaV/GI.1/Sapporo/1982/JP	I	1	U65427
Hu/SaV/GI.2/Parkville/1994/US	I	2	U73124
Hu/SaV/GI.3/Stockholm/1997/SE	I	3	AF194782
Hu/SaV/GII.1/London/1992/UK	II	1	U95645
Hu/SaV/GII.2/Mex340/1990/MX	II	2	AF435812
Hu/SaV/GII.3/Cruise ship/2000/US	II	3	AY289804
Sw/SaV/GIII/PEC-Cowden/1980/US	III	1	AF182760
Hu/SaV/GIV/Hou7–1181/1990/US	IV	1	AF435814
Hu/SaV/GV/Argentina39/AR	V	1	AY289803

Note: Norovirus and sapovirus typing systems from Zheng *et al.*, and Farkas *et a*l., respectively.

NS6 and NS7 precursor of the vesiviruses (NS6–7, also known as ProPol). The calicivirus NS proteins and their functions, if known, are summarized in **Table 4**. Key enzymes include an NTPase (NS3), a cysteine proteinase (NS6), and an RNA-dependent RNA polymerase (NS7).

Replication

Cell culture systems are not available for all caliciviruses, but those that grow in cell culture, such as FCV and murine norovirus characteristically grow efficiently and form plaques (**Figure 4**). Like other positive-strand RNA viruses, caliciviruses replicate in the cytoplasm of infected cells. The positive-strand RNA genome functions as a messenger RNA that is translated following entry of the virus particle into the cell. This initial translation event produces NS proteins that are used in the replication of the viral RNA genome and the production of new progeny. Replication of the RNA occurs on intracellular membranes and extensive membrane rearrangements are observed in infected cells (**Figure 4**). The viral capsids can sometimes be seen in infected cells as paracrystalline arrays (**Figure 4**). There are two major positive-strand

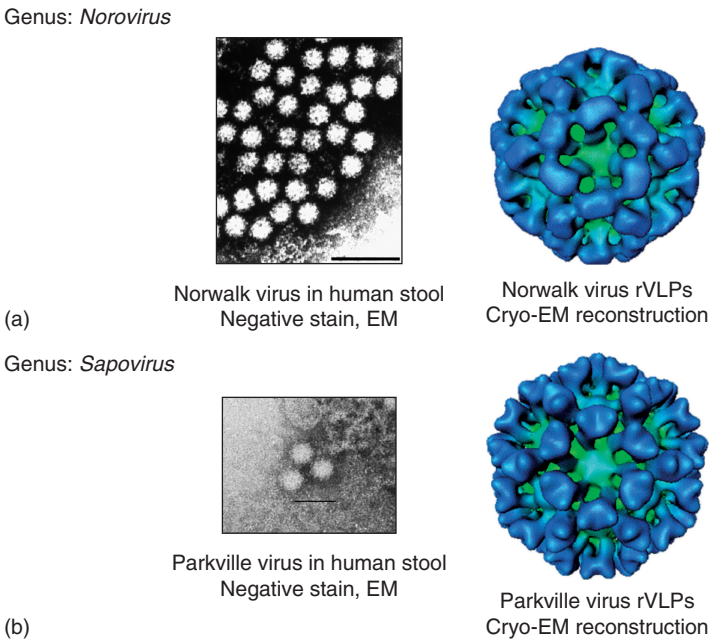


Figure 2 Structural features of noroviruses and sapoviruses. (a) Genus: *Norovirus*. (Left panel) Norwalk virus, a norovirus, as seen in human stool material by negative-staining and immune EM (IEM). The size of Norwalk virus in stool material has been described as 27–32 nm in diameter. Scale = 100 nm. (Right panel) Norwalk virus recombinant virus-like particles (rVLPs) were expressed in the baculovirus system, purified, and analyzed with the technique of cryo-EM. (b) Genus: *Sapovirus*. (Left panel) Parkville virus, a sapovirus, as seen in human stool material by negative staining and EM. Scale = 50 nm. (Right panel) Parkville virus rVLPs were analyzed by cryo-EM. The cryo-EM computer-generated images of both Norwalk virus and Parkville virus rVLPs show that the surfaces of the particles have cup-shaped depressions, a structural hallmark of the caliciviruses. (a, left panel) Image provided by A. Z. Kapikian, National Institutes of Health. (a, b, right panel) Image provided by B. V. Prasad, Baylor College of Medicine. (b, left panel) Image provided by C. D. Humphrey, Centers for Disease Control and Prevention.

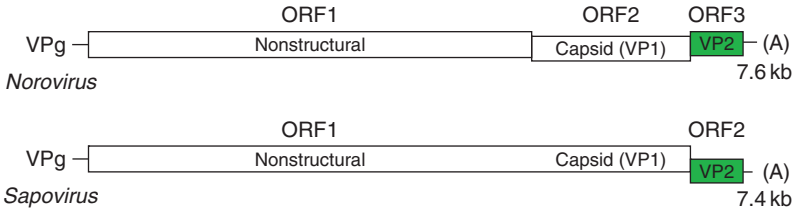


Figure 3 Genome organization of noroviruses and sapoviruses. The positive sense RNA genome of Norwalk virus (representing the noroviruses) is organized into three major ORFs. The genome of Manchester virus (representing the sapoviruses) is organized into two major ORFs, with the VP1capsid coding sequence in frame with that of the NS proteins. (The Norwalk virus and Manchester virus genome sequences correspond to GenBank accession numbers M87661 and X86560, respectively.)

RNA species detected in infected cells – one corresponds to the full-length genome, and the other corresponds to an abundant subgenomic RNA co-terminal with the 3′-end region of the genome. The NS proteins are translated from the full-length RNA. The subgenomic RNA serves as a messenger RNA for translation of the VP1 and VP2, the two structural proteins.

Evolution

Comparative sequence analysis suggests that members of the *Caliciviridae* have a common ancestor. A distant

Table 4 Calicivirus proteins

Virion proteins (VP)	
VP1	Major structural protein of virion (~60 kDa)
VP2	Minor structural protein, function unknown
VPg	Covalently linked to genomic RNA
Nonstructural proteins (NS)	
NS1	N-terminal protein of ORF1, function unknown
NS2	Function unknown
NS3	NTPase
NS4	Function unknown
NS5	Becomes linked to RNA as VPg, role in translation and replication
NS6	Proteinase (Pro)
NS7	RNA-dependent RNA Polymerase (Pol)

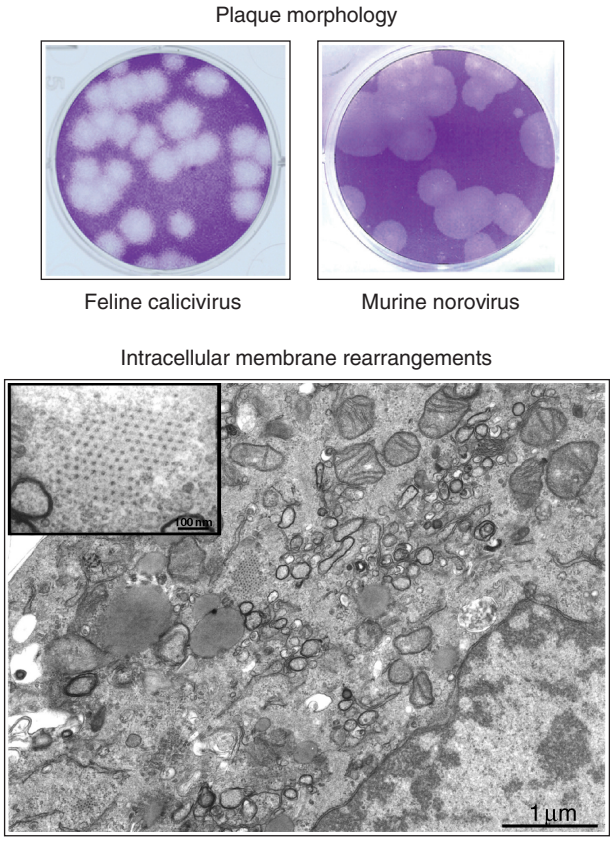


Figure 4 (Upper panel) Plaque morphology and membrane rearrangements in an infected cell. Feline calicivirus and murine norovirus induce a rapid cytopathic effect in permissive cell culture. Plaques can be detected in a cell monolayer by 24 h post infection. (Lower panel) Caliciviruses, like other positive strand RNA viruses, induce membrane rearrangements and vesicles in infected cells. The inset shows a paracrystalline array of capsid proteins produced in an FCV-infected cell. Images provided by A. Weisberg.

evolutionary relationship has been proposed between the *Caliciviridae* and members of the family *Picornaviridae*. A striking feature among the caliciviruses is their extensive genetic variation. This variation is due, in part, to the high error rate inherent in positive-strand RNA virus replication, which employs an RNA-dependent RNA polymerase without a known editing function for correcting mistakes. There is compelling evidence also that recombination occurs between related strains, which would then allow the emergence of new viruses. Potential recombination sites have been identified in norovirus genomes at several positions, including the junction between the ORF1 and ORF2, which would allow a virus to emerge with a new subgenomic region that would encode a different VP1 capsid protein.

Transmission and Host Range

Transmission of the noroviruses and sapoviruses occurs by several modes, including direct person-to-person contact, ingestion of contaminated food or water, or exposure to contaminated fomites. Noroviruses are a major cause of foodborne gastroenteritis. The route of transmission is predominantly fecal–oral, and viruses can be shed in feces for days to weeks following infection. The virus has been detected in vomitus, and transmission may occur also via exposure to aerosolized droplets of vomitus. Good personal hygiene and frequent hand washing is important in controlling the spread of these highly infectious viruses.

Noroviruses have been found in several mammalian species, including humans, pigs, cattle, and mice, and it is likely that additional hosts will be identified. Sapoviruses have also been found in several hosts thus far, including humans, pigs, and mink. There is little evidence that zoonotic transmission of animal caliciviruses to humans (or humans to animals) plays a role in the natural history of these viruses, but such transmission might be possible considering that certain human norovirus strains can infect chimpanzees and pigs in experimental challenge studies.

Pathogenicity

Noroviruses and sapoviruses replicate in the enteric tract, and viral antigen-expressing cells (for both groups of viruses) have been identified in the small intestinal epithelial cells of swine. The cell types supporting the replication of the human noroviruses and sapoviruses have not been verified *in vivo*, but intestinal biopsies have shown blunting of the intestinal villi following infection of adult volunteers with Norwalk virus. The mechanisms responsible for diarrhea are not known, but the lesion caused by blunting of the intestinal villi may affect absorption and the osmotic balance. Murine norovirus is shed in feces and can be found in multiple organs of asymptomatic normal mice, including the intestine, liver, spleen, and mesenteric lymph nodes. However, certain strains of murine norovirus can cause a highly lethal infection in immunodeficient mice lacking key components of the innate immune system. In these mice, a lethal, disseminated infection occurs leading to encephalitis, meningitis, vasculitis, hepatitis, and pneumonia.

Clinical Features of Infection

Norovirus gastroenteritis is characterized by a short incubation period and an acute onset of illness. Symptoms include one or more of the following: diarrhea, vomiting, fever, malaise, and abdominal cramps. The illness is generally considered mild and self-limiting, but severe illness can occur. Treatment is aimed toward the prevention of dehydration, and includes either oral rehydration or intravenous administration of fluids. Noroviruses have been identified in several studies as the second

most important viral agent (but considerably less than rotaviruses) associated with severe gastroenteritis in infants and young children.

The sapoviruses have been associated predominantly with diarrhea that can range from mild to severe. The illness is usually self-limiting, but the prevention of dehydration is important.

Noroviruses, sapoviruses, and other caliciviruses have been associated with asymptomatic infection, and persistent infection has been documented. Shedding of the virus can occur for prolonged periods (weeks to months) following resolution of symptoms in some patients.

Immune Response

Immunity to the noroviruses is poorly understood, because it has been difficult to study the role of neutralizing antibodies in the absence of a cell culture system. Adult volunteer challenge studies have been the only available approach for the study of resistance to illness in humans. It was noted early in these volunteer studies that the presence of pre-existing serum or local intestinal antibodies to the virus did not correlate with resistance to illness, but rather was associated with susceptibility to infection and illness. In contrast, later studies suggested that local immunity in the intestine played an important role in mediating protection, but immunity to Norwalk virus was short term. Susceptibility to infection may also be associated with host genetic factors relating to the presence or absence of receptors for the virus on intestinal epithelial cells. There is evidence that noroviruses bind to histo-blood group antigens on intestinal epithelial cells, and these antigens might serve as receptors or binding ligands for the virus. Because histo-blood group antigens vary among individuals, and variation among norovirus strains has been detected in the recognition of these antigens, it has been postulated that this might, in part, provide an explanation for varying host susceptibility. The understanding of immunity to the noroviruses is complicated by the marked antigenic diversity among circulating strains. Limited cross-challenge studies have found evidence for at least two distinct serotypes, represented by the Norwalk and Hawaii norovirus strains, but the number of serotypes is presently unknown.

The predominant association of sapovirus gastroenteritis with individuals in younger age groups suggests that immunity to sapoviruses may be acquired early in life.

Prevention and Control

There are presently no vaccines or antiviral therapies for the control of norovirus and sapovirus gastroenteritis. Prevention and control strategies currently consist of standard infection control procedures, such as frequent hand washing, avoidance of exposure, and disinfection of contaminated areas.

Diagnosis

Norovirus outbreaks characteristically have the following epidemiological features, known as the 'Kaplan criteria': (1) a mean (or median) illness duration of 12–60 h, (2) a mean (or median) incubation period of 24–48 h, (3) more than 50% of people with vomiting, and (4) no bacterial agent previously found. Although these features are generally highly specific for the provisional diagnosis of a norovirus outbreak, laboratory tests are recommended for ruling out bacteria and confirming a viral etiology. The most widely used method for detection of noroviruses and sapoviruses is reverse transcriptase-polymerase chain reaction (RT-PCR) analysis of viral RNA in stool specimens. The extensive genetic variation among the noroviruses has been problematic in the design of broadly reactive primer pairs, and many laboratories use more than one primer pair for detection.

Noroviruses and sapoviruses can be observed in stool specimens by EM, but this technique often requires the use of antibodies to facilitate detection (immune EM). Commercial immunoassays are becoming available for clinical use in which broadly reactive antibodies are used to detect norovirus antigen in stool specimens.

Vaccination

Vaccines are not available for the noroviruses, but studies are in progress to identify and evaluate potential vaccine candidates. Most vaccine candidates are based on the production of recombinant norovirus VP1, the major capsid protein. The VP1 will spontaneously self-assemble into virus-like particles (VLPs) that are antigenically similar to native virions and can be produced in high yields in expression systems such as baculovirus. The VLPs are immunogenic when administered orally to humans.

Vaccines have been developed and are in use for caliciviruses such as FCV (for use in cats) and RHDV (for use in rabbits). Because FCV grows efficiently in cell culture, a live attenuated vaccine has been developed, as well as an inactivated vaccine.

Future Perspectives

Noroviruses are the major cause of nonbacterial gastroenteritis outbreaks, and large numbers of illnesses occur each year. The sapoviruses play a lesser role in epidemic gastroenteritis, but further studies are needed. Efforts will continue to focus on the development of improved

diagnostic tests and in the establishment of a cell culture system for the human noroviruses and sapoviruses. It will also be important to determine whether vaccines or antiviral drugs will be effective in controlling norovirus disease. A norovirus vaccine candidate based on the expression of recombinant virus-like particles is under investigation in early clinical trials. Promising new tools for study of the noroviruses include the first efficient cell culture system (murine norovirus), a human norovirus infectivity assay using a three-dimensional cell culture system, a cell-based human norovirus replicon system (Norwalk virus), a reverse genetics system for the murine noroviruses, and an animal disease model (pigs) for the human noroviruses. In addition, advances in the elucidation of the structure and function of individual viral proteins may lead to a better understanding of replication in this important group of viruses.

See also: Caliciviruses; Enteric Viruses.

Further Reading

Asanaka M, Atmar RL, Ruvolo V, Crawford SE, Neill FH, and Estes MK (2005) Replication and packaging of Norwalk virus RNA in cultured mammalian cells. *Proceedings of the National Academy of Sciences, USA* 102: 10327–10332.

Atmar RL and Estes MK (2006) The epidemiologic and clinical importance of norovirus infection. *Gastroenterology Clinics of North America* 35: 275–290.

Chang KO, Sosnovtsev SV, Belliot G, King AD, and Green KY (2006) Stable expression of a Norwalk virus RNA replicon in a human hepatoma cell line. *Virology* 353: 463–473.

Cheetham S, Souza M, Meulia T, Grimes S, Han MG, and Saif LJ (2006) Pathogenesis of a genogroup II human norovirus in gnotobiotic pigs. *Journal of Virology* 80: 10372–10381.

Chen R, Neill JD, Noel JS, *et al.* (2004) Inter- and intragenus structural variations in caliciviruses and their functional implications. *Journal of Virology* 78: 6469–6479.

Estes MK, Prasad BV, and Atmar RL (2006) Noroviruses everywhere: Has something changed? *Current Opinion in Infectious Diseases* 19: 467–474.

Farkas T, Zhong WM, Jing Y, *et al.* (2004) Genetic diversity among sapoviruses. *Archives of Virology* 149: 1309–1323.

Green KY, Ando T, Balayan MS, *et al.* (2000) Taxonomy of the caliciviruses. *Journal of Infectious Diseases* 181: S322–S330.

Hansman GS, Natori K, Shirato-Horikoshi H, *et al.* (2006) Genetic and antigenic diversity among noroviruses. *Journal of General Virology* 87: 909–919.

Hardy ME (2005) Norovirus protein structure and function. *FEMS Microbiology Letters* 253: 1–8.

Jiang X, Graham DY, Wang K, and Estes MK (1990) Norwalk virus genome cloning and characterization. *Science* 250: 1580–1583.

Kapikian AZ (2000) The discovery of the 27-nm Norwalk virus: An historic perspective. *Journal of Infectious Diseases* 181: S295–S302.

Kapikian AZ, Wyatt RG, Dolin R, Thornhill TS, Kalica AR, and Chanock RM (1972) Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious nonbacterial gastroenteritis. *Journal of Virology* 10: 1075–1081.

Le Pendu J, Ruvoen-Clouet N, Kindberg E, and Svensson L (2006) Mendelian resistance to human norovirus infections. *Semin Immunology* 18: 375–386.

Noel JS, Liu BL, Humphrey CD, *et al.* (1997) Parkville virus: A novel genetic variant of human calicivirus in the Sapporo virus clade, associated with an outbreak of gastroenteritis in adults. *Journal of Medical Virology* 52: 173–178.

Sosnovtsev SV, Belliot G, Chang KO, *et al.* (2006) Cleavage map and proteolytic processing of the murine norovirus nonstructural polyprotein in infected cells. *Journal of Virology* 80: 7816–7831.

Straub TM, Höner zu Bentrup K, Orosz-Coghlan P, *et al.* (2007) *In vitro* cell culture infectivity assay for human noroviruses. *Emerging Infectious Diseases* 13(3): 396–403.

Tacket CO, Sztein MB, Losonsky GA, Wasserman SS, and Estes MK (2003) Humoral, mucosal, and cellular immune responses to oral Norwalk virus-like particles in volunteers. *Clinical Immunology* 108: 241–247.

Ward VK, McCormick CJ, Clarke IN, *et al.* (2007) Recovery of infectious murine norovirus using pol II-driven expression of full-length cDNA. *Proceedings of the National Academy of Sciences, USA* 104: 11050–11055.

Wobus CE, Thackray LB, and Virgin HW (2006) Murine norovirus: A model system to study norovirus biology and pathogenesis. *Journal of Virology* 80: 5104–5112.

Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, and Monroe SS (2006) Norovirus classification and proposed strain nomenclature. *Virology* 346: 312–323.

O

Ophiovirus

A M Vaira and R G Milne, Istituto di Virologia Vegetale, CNR, Turin, Italy

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Bipartite nuclear targeting sequence Signal involved in nuclear translocation of proteins characterized by (1) two adjacent basic amino acids (Arg or Lys), (2) a spacer region of ten residues, (3) at least three basic residues (Arg or Lys) in the five positions after the spacer region.

Mononegavirales Order of viruses comprising species that have a non-segmented, negative-sense RNA genome. The order includes four families: *Bornaviridae*, *Rhabdoviridae*, *Filoviridae*, and *Paramyxoviridae*.

Introduction

The genus *Ophiovirus* does not fit into any existing virus family, and a new family (*Ophioviridae*) has been proposed but is not yet official. The genus comprises a number of viral species, all plant-infecting viruses, relatively recently discovered. In some cases ophioviruses are now known to be the cause of well-known and 'classical' major plant diseases, but in other cases the presence of the virus has not been linked to any specific symptom, because of invariably mixed infections with other viruses. Ophioviruses occur in monocots and dicots, in vegetables, ornamentals, and trees, in the New and Old World, suggesting a well-adapted group of viruses containing more species than the ones already discovered. Where identified, the vectors of ophioviruses have proved to be *Olpidium brassicae*, a soil-inhabiting fungus. Ophioviruses have been slow to emerge mainly because the virions are not easy to see in the electron microscope; indeed, the first ophiovirus particle was observed only in 1988 and its morphology understood in 1994. The generic name derives from the Greek word *ophis*, meaning snake, in reference to the serpentine appearance of the virus particles.

Taxonomy and Classification

There are currently five species in the genus *Ophiovirus*, and at least one tentative species (**Table 1**). The accepted species in the genus are: *Citrus psorosis virus* (CPsV), the type species, *Lettuce ring necrosis virus* (LRNV), *Mirafiori lettuce virus* (MiLV), *Ranunculus white mottle virus* (RWMV), and *Tulip mild mottle mosaic virus* (TMMMV). The family *Ophioviridae*, containing a single genus listing all the species has recently been proposed but is not official. To date, the genomes of three species, CPsV, LRNV, and MiLV, are fully sequenced.

Phylogenetic analysis of the complete RNA-dependent RNA polymerase (RdRp) domain of RWMV and of the RdRp core modules of CPsV, MiLV, and RWMV, provide evidence of a relationship with *Rhabdoviridae* and *Bornaviridae* species, both belonging to the *Mononegavirales*. However, ophioviruses appear to form a monophyletic group, separate from the other negative-stranded RNA viruses; and of course the *Ophiovirus* genome is multipartite, excluding membership in the *Mononegavirales*.

For species demarcation within the genus, different criteria have been considered: the different coat protein (CP) sizes, absence of, or distant serological relationship between the CPs, differences in natural host range and different number, organization, and/or size of genome segments. From available data, there is 94–100% identity among complete CP amino acid sequences of isolates belonging to the same species, in particular for MiLV and CPsV, and alignments between incomplete but considerable parts of CP sequences of two RWMV isolates also show almost 100% identity. The percentage of identity falls to 30–52% for interspecies alignments, with the exception of the identity between MiLV and TMMMV CP sequences, which is about 80%, confirming the closeness of these two viruses. They are currently considered as two different species at least because of different host ranges; more information on TMMMV sequences is needed prior to any revision of their taxonomic position.

The percentage of identity/similarity between CP amino acid sequences may also be proposed as a further

Table 1 Virus members in the genus *Ophiovirus*

Virus (alternative name)	*Abbreviations*	*Accession numbers*[a]
Citrus psorosis virus (Citrus ringspot virus)	CPsV (CRsV)	NC006314, NC006315, NC006316
Lettuce ring necrosis virus	LRNV	NC006051, NC006052, NC006053, NC006054
Mirafiori lettuce virus (Mirafiori lettuce big-vein virus)	MiLV (MLBVV)	NC004779, NC004781, NC004782, NC004780
Ranunculus white mottle virus	(RWMV)	AF335429, AF335430, AY542957
Tulip mild mottle mosaic virus	(TMMMV)	AY204673, AY542958
Freesia ophiovirus (freesia sneak virus)	(FOV-FreSV)	AY204676, DQ885455

[a]Complete genome sequences are reported when available.

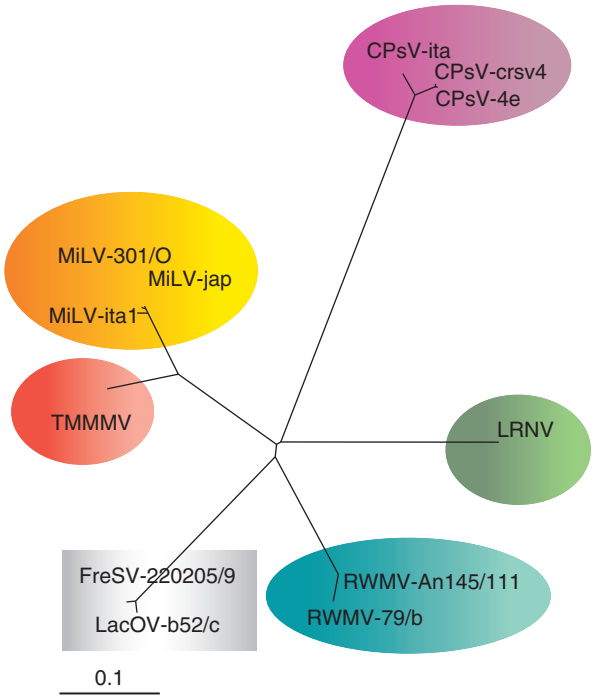


Figure 1 Unrooted phylogenetic tree of established and putative members of genus *Ophiovirus* based on their coat protein amino-acid sequences. The tree was generated by the neighbor-joining method and bootstrap values were estimated using 1000 replicates. All bootstrapping values were above 500. Branch lengths are related to the evolutionary distances. Viral isolates included in the analysis (accession numbers are reported when available): CPsV-4e (AAC41022), CPsV-CRSV4 (AAF00018), CPsV-ita (CAJ43825), MiLV-301/O (AAN60449), MiLV-ita1 (AAU12873), MiLV-jap (AAO49152), TMMMV (AAT08133), LRNV (AAT09112), RWMV-79/b (AAT08132) partial sequence, RWMV-an145/111 partial sequence, FreSV-220205/9 (DQ885455), LacOV-b52/c from *Lachenalia*, partial sequence. Ovals grouping isolates represent established viral species, the rectangle the proposed new species.

tool for species demarcation when complete sequences are available. A phylogenetic tree based on alignment of representative CP amino acid sequences is shown in **Figure 1**.

There has been some further discussion on the position of CPsV, as it is presently the only ophiovirus with a woody natural host, and there is no evidence of soil transmission, contrary to the apparent rule for ophioviruses; Western blotting has shown no serological cross-reaction between CPsV coat protein and other ophiovirus CPs and there are several molecular differences, the main one being the presence of three, not four genome segments. These differences, if confirmed and reinforced, could lead to considering placement of CPsV in a different genus within the proposed family *Ophioviridae*.

Reverse transcriptase-polymerase chain reaction (RT-PCR) amplification, using degenerate primers, of a 136 bp fragment from the RdRp gene, located outside the sequence coding the polymerase domain, is currently the best tool for detecting and identifying species within the genus. Sequencing and phylogenetic analysis of the 45 amino acid string deduced from the amplified fragment of several isolates belonging to different species, fully supports the present species classification and gives an indication of the taxonomic positioning of newly diagnosed isolates. An ophiovirus in freesia provisionally named freesia sneak virus (FreSV), considered a tentative species, was diagnosed and proposed as a new ophiovirus species owing to this procedure, when no other viral genome sequences were known; the complete CP sequence later obtained confirmed the hypothesis. A positive reaction with the genus-specific RT-PCR has recently been obtained for several samples from *Lachenalia*, a monocot in the family *Hyacinthaceae*; in this case the RT-PCR and subsequent analysis of the 45-amino-acid string suggest placing the isolate within the freesia sneak virus tentative species. Also in this case, subsequent CP gene sequencing showed nearly 100% identity with the homologous portion of FreSV at amino acid level, confirming this position.

Properties of Particles

The virions are naked filamentous nucleocapsids about 3 nm in diameter forming circularized structures of different lengths. The virions appear to form internally coiled circles that in some cases can collapse into pseudolinear duplex structures (**Figure 2**). There is no evidence of an

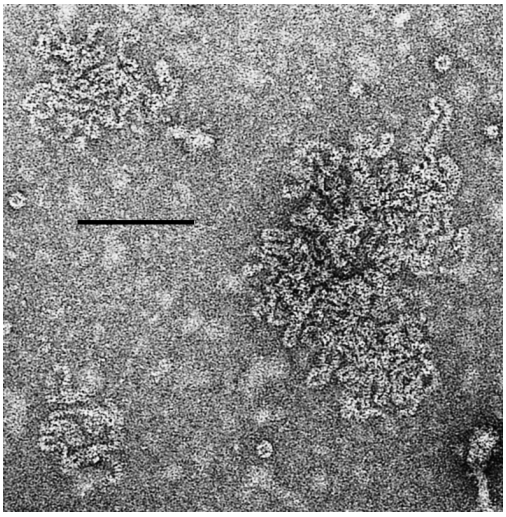


Figure 2 Negative contrast electron micrograph (uranyl acetate) of partially purified virion preparation from field lettuce showing big-vein symptoms. Note large and small ophiovirus particles. The bar represents 100 nm.

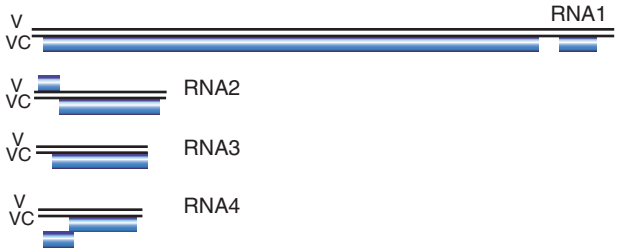


Figure 3 Genomic organization of ophioviruses. The four genomic RNAs are represented. The RNA4 is not described in all species, see text and **Table 2** for details; v-RNA and vc-RNA indicate the viral and the viral complementary RNAs, respectively. Boxes indicate ORFs.

envelope. The 5′ and the 3′ ends of the RNAs have been structurally analyzed in the complete genome sequences available for CPsV and MiLV, to assess the probability of panhandle-like or similar structures. In CPsV, secondary structure predictions do not support significant complementarity between the termini of the viral RNAs to afford panhandle formation. On the contrary, in MiLV, the terminal sequences do carry partial inverted repeats, potentially allowing panhandles to form; the terminal sequences also contain palindromes potentially able to fold into stem-loops, so far not found for CPsV sequences. Thus, the apparent circularization of the particles of all ophioviruses remains incompletely explained.

Genome Organization and Replication

The ophiovirus genome is ssRNA, 11.3–12.5 kbp in size, divided into three or four segments (RNAs 1–4) and mainly negative sense. Positive-sense RNA is also encapsidated, to some extent with CPsV or in nearly equimolar amounts, with MiLV. The 3′ termini of the viral RNAs show sequences of 9–12 nt conserved within the RNAs of each species: A_7GUAUC for CPsV, A_{4-6}UAAUC for MiLV, and A_7GUAUCA and A_3UA_3GUAUCA for LRNV; these may be involved in the recognition of the RNAs by the RdRp.

The genome organization of ophioviruses is as follows (**Figure 3** and **Table 2**). RNA1 is negative sense, shows short 5′ and 3′ untranslated regions, and contains two open reading frames (ORFs) separated by an AU-rich intergenic region. The putative product of ORF1 did not show any significant similarity with others available in databases and its function remains unknown. The product of the large ORF2 contains the core polymerase module with the five conserved motifs proposed to be part of the RdRp active site and shows low but significant amino acid sequence similarities with the L protein of several rhabdoviruses.

RNA2 contains one ORF in the negative strand in all three sequenced viruses, encoding a putative nonstructural protein with no significant sequence similarity to other known proteins and therefore of unknown function. In the amino acid sequence of the CPsV protein, two motifs bear similarity to a putative 'bipartite nuclear targeting sequence' considered to be a nuclear localization signal. An additional minor ORF, in the virus-sense strand, is present in MiLV RNA2, but to date there is no evidence for expression of the predicted 10 kDa protein.

RNA 3 contains one ORF in the vc RNA in all three viruses which has been identified as coding for the nucleocapsid protein.

An RNA4 has been found only in MiLV and LRNV. MiLV RNA4 contains two overlapping ORFs in the negative strand in different reading frames: the first putatively encodes a 37 kDa protein, but the second, overlapping the first by 38 nt, appears to lack an initiation codon. This second ORF has a theoretical coding capacity of 10.6 kDa and is suggested to be expressed by a +1 frameshift. A slippery sequence, GGGAAAU, can be recognized immediately in front of the UGA stop codon of the 37 kDa ORF. This unique feature of ORF positioning has been shown for two different isolates of MiLV, and therefore seems not to be caused by cloning artifacts.

RNAs 1, 2, and 3 of the other partially sequenced species are similar in size to those described. No RNA4 has been found in CPsV, RWMV, and TMMMV, but the possible existence of an RNA4, perhaps in very low concentration or very similar in size to RNA3, cannot be ruled out.

Analysis for the presence of subgenomic RNAs has not extensively been pursued for all species, even though several studies have used minus-strand and plus-strand probes in Northern blot hybridization using RNA extracted from infected tissues as well as virus particles, to obtain information regarding the polarity of encapsidated viral

Table 2 Details of genomic organization of fully sequenced viruses inside the genus *Ophiovirus*

Virus	*RNA1*	*RNA2*	*RNA3*	*RNA4*
CPsV	8186 nt 2 ORFs in vc RNA ORF1: 24 kDa ORF2: 280 kDa proteins	1645 nt 1 ORF in vc RNA 54 kDa protein	1447 nt 1 ORF in vc RNA 49 kDa	
MiLV	7794 nt 2 ORFs in vc RNA ORF1: 25 kDa ORF2: 263 kDa proteins	1788 nt 1 ORF in vRNA: 10 kDa protein 1 ORF in vc RNA: 55 kDa protein	1515 nt 1 ORF in vc RNA 48.5 kDa	1402 nt 2 ORFs in vc RNA: ORF1: 37 kDa ORF2: 10.6 kDa proteins
LRNV	7651 nt 2 ORFs in vc RNA ORF1: 22 kDa ORF2: 261 kDa proteins	1830 nt 1 ORF in vc RNA 50 kDa protein	1527 nt 1 ORF in vc RNA 48 kDa protein	1417 nt 1 ORF in vc RNA 37 kDa protein

RNA. With CPsV in such studies no subgenomic RNAs were ever found for any of RNA1, 2, or 3.

A putative nuclear localization sequence present in the 280 kDa protein coded by RNA1 and in the 54 kDa protein coded by RNA2 in CPsV, and also observed in available analogous sequences of MiLV and RWMV, together with the polymerase similarities found with isolates from the genera *Nucleorhabdovirus* and *Bornavirus*, may suggest nuclear replication also for ophioviruses; in any case, gold immunolabeling and electron microscopy of RWMV-infected tissues showed accumulation of CP in the cytoplasm.

Viral Proteins

The most studied ophiovirus protein is the CP. There is only one CP, varying somewhat in size according to species. For CPsV, MiLV, and LRNV, this is 48.6, 48.5, and 48 kDa, respectively; Western blotting has given apparent sizes of 43 and 47 kDa for RWMV and TMMMV CPs, respectively. FreSV CP is 48.4 kDa. Ophiovirus CPs are relatively poor antigens. The antisera or, in one case, monoclonal antibodies, have been produced using purified virus preparations or recombinant protein. Western blots show that the CPs of RWMV, TMMMV, MiLV, and LRNV are slightly to moderately related; CPsV CP appears to be unrelated to the others. No clear information is available for FreSV. Genetic variability in the CP genes has been extensively studied in CPsV and MiLV.

Variability of the CP gene of CPsV was assessed serologically and by sequence analysis of two regions located in the 3′ and 5′ halves of the gene. Variability assessed by a panel of monoclonal antibodies to the protein resulted in 14 reaction patterns but no correlation was found between serogroups and specific amino acid sequences, field location, or citrus cultivar. Results from sequence analysis showed limited nucleotide diversity in the CP gene within the population. Diversity was slightly higher in the 5′ region. The ratio between nonsynonymous and synonymous substitutions (d_N/d_S) for the two regions indicated a negative selective pressure for amino acid changes, more intense at the 3′ end. When the entire CP sequences were considered, two clusters were identified, one comprising 19 isolates from Italy and an isolate from Spain and a second one containing only the Florida CPsV isolate.

Phylogenetic analysis of MiLV CP genes has revealed two distinct subgroups; however, this grouping was not correlated with symptom severity on lettuce or the geographic origin of the isolates; whether these two subgroups show different characteristics with regard to virulence in indicator plants or serological relationships remains to be determined; furthermore also in this case, a low value of d_N/d_S ratio was estimated for all MiLV isolates and also between the two subgroups, supporting a negative selection pressure for amino acid changes. In general, under natural conditions, genetic stability seems to be the rule rather than the exception.

The other viral protein, considered mainly for taxonomic purposes, is the putative RdRp, encoded by RNA1 in all species, described earlier.

No information is available regarding the synthesis or the function of other putative ORF products.

Pathogenicity and Geographic Distribution

Formerly a so-called citrus psorosis group of diseases included some of the most widespread graft-transmissible disorders of citrus, in some cases of undemonstrated etiology. Characterization of CPsV has shown that it is the cause of the great majority of psorosis symptoms but that a small but still undefined proportion of trees with 'psorosis-like' bark-scaling shows no evidence of carrying

the virus, and the idea of a non-CPsV psorosis-like disease of unknown etiology is emerging. In the past, different kinds of symptoms were described: 'psorosis A', characterized by causing bark-scaling in trunk and limbs of infected field trees; 'psorosis B', causing rampant scaling of thin branches in field trees and chlorotic blotches in old leaves with gummy pustules in the underside; 'ringspot', characterized by presence of chlorotic blotches and rings in the old leaves of inoculated seedlings but apparently no specific symptoms on infected field trees or in other cases, chlorotic flecks and ringspots on leaves, and trunk and fruit symptoms (**Figure 4**). All these appear to be caused by CPsV but in the former 'psorosis group' there are other non-CPsV diseases with symptoms such as chlorotic leaf-flecking and oak-leaf patterns; while graft-transmissible, these diseases have not yet yielded to further analysis. Psorosis is an ancient disease; bark-scaling of citrus was first observed in Florida and California in the 1890s. The disease has been brought under control in most advanced citrus-growing countries due to rigorous indexing and quarantine. In Argentina it remains a severe problem, in Mediterranean areas it is also reported, and in citrus-growing parts of Asia the disease may well be widespread, although rigorous testing for the presence or absence of CPsV has generally been lacking.



Figure 4 Bark scaling in citrus, typical of severe psorosis.

RWMV has been reported in two species, ranunculus (*Ranunculus asiaticus* hyb.) and anemone (*Anemone coronaria*) in northwest Italy since the 1990s. The pathogenic impact is uncertain as it was almost always found in mixed infection with other viruses commonly infecting the two species. The symptom description 'white mottle' present in its name derives from the bright white mottling symptoms consistently observed on *Nicotiana benthamiana* leaves mechanically infected by RWMV. In some cases the bright mottle has also been observed in naturally infected ranunculus, always in mixed infection. Indexing for the presence of RWMV in ranunculus crops done in 1996 showed an incidence of 2.5% among symptomatic plants; furthermore, the very few plants apparently infected only with RWMV did not show any distinctive symptoms. Infection of ranunculus seedlings by mechanical inoculation results in limited necrosis and deformation of stems and leaves. The pathogenic potential of RWMV is thus still unclear.

Tulip mild mottle mosaic disease, caused by TMMMV, is one of the most serious diseases in some bulb-producing areas of Japan (which produces bulbs on a large scale for the Southeast Asian market) and has been reported since 1979. Symptoms on tulips include color-attenuating mottle on flower buds and color-increasing streak on petals (**Figure 5**); mild chlorotic mottle and mosaic slightly appear along the leaf veins. TMMMV infection is up to now restricted to *Tulipa* species and cultivars, and has not been reported in other geographical areas.

Lettuce big-vein disease was first reported in the United States in 1934 and occurs in all major lettuce-producing areas in the word. The disease becomes serious during cooler periods of the year. The main symptoms are vein-banding in the leaves, due to zones parallel to the vein cleared of chlorophyll (**Figure 6**); there is associated leaf distortion, delayed head formation, and decreased head size.

The causal agent of the disease, long known to be soil-transmitted, was first identified as 'lettuce big-vein virus' (LBVV) (genus *Varicosavirus*); recently, re-evaluation of the etiology has been necessary as a second less easily detected virus, MiLV, was found in lettuces with big-vein symptoms. Following experimental inoculation of the two viruses together and separately, MiLV was shown to be the etiological agent of big-vein, while LBVV, now renamed as lettuce big-vein associated virus (LBVaV),



Figure 5 TMMMV symptoms on tulip (healthy tulip on the left). Courtesy of T. Morikawa, Toyama Agric. Res. Center, Tonami, Toyama, Japan.



Figure 6 Leaf of butterhead lettuce showing big-vein symptoms. Close-up showing vein-banding.

apparently plays no part in the disease, although it is almost always present, and is, like MiLV, transmitted by *Olpidium*. Studies on symptom development in the field have confirmed that both viruses very commonly occur together in lettuce crops. MiLV likely occurs worldwide; it is reported in California (USA), Chile, France, Germany, Italy, Spain, the Netherlands, England, Denmark, Japan, Australia, and New Zealand. To date, natural infection has been reported only in cultivars of *Lactuca sativa*.

LRNV is closely associated with lettuce ring necrosis disease, first described in the Netherlands and in Belgium as 'kring necrosis' and also in France as *maladie des taches orangées*, in the 1980s. It is an increasingly important disease of butterhead lettuce crops in Europe. Definitive proof that LRNV is the cause of ring necrosis is still awaited. In southern France the disease is observed primarily in winter lettuces (September–January) under plastic or glass, when the crop is maturing, the day length is short, and both light intensity and temperature are low. Symptoms depend on the lettuce type and environmental conditions, and mainly consist of necrotic rings and ring-like patterns on leaves, which may render the product unmarketable. LRNV is often found together with MiLV (and, of course, LBVaV) with which it shares host and vector. The virus has also been reported in California.

Although the ophiovirus isolated from freesia has not yet been recognized as an official new ophiovirus species, we would like to say a few words about the freesia disease. Necrotic disorders of freesia (*Freesia refracta* hyb., family Iridaceae), known as 'leaf necrosis' and 'severe leaf necrosis', were first described in freesia crops in the Netherlands before 1970, and a similar disease, named 'freesia streak', was reported in England and Germany in the same years. The 'severe leaf necrosis' appeared to be caused by mixed infection with the potyvirus freesia mosaic virus and a virus with varicosavirus morphology and mode of transmission, that has been tentatively named Freesia leaf necrosis virus. In recent years, in northwest Italy, a necrotic disease of freesia has spread and caused considerable economic losses, even though it was present since 1989. Both in Italy and in the

Netherlands, the disease seems to be linked to the presence of an ophiovirus. Typical symptoms are chlorotic spots and streaks at the leaf tips that expand downwards and turn necrotic, and may vary according to cultivar and climate. Freesia sneak virus has been proposed as the name of this new ophiovirus species.

Experimental Symptoms and Host Ranges

All ophiovirus are, though sometimes not easily, mechanically transmissible to a limited range of test plants, including Chenopodiaceae and Solanaceae, inducing local lesions and in some cases systemic mottle.

Cytopathology

Thin sections of *Nicotiana clevelandii* leaves mechanically infected by RWMV have been examined by electron microscopy but no distinctive inclusions were observed and no virus particles were seen. In classical thin sections, viral nucleic acids stain up well but protein coats are faintly contrasted, so it is not surprising that very thin nucleoprotein threads in random orientations should escape detection. After gold immunolabeling with RWMV-specific polyclonal antibodies, sections of the cytoplasm of parenchyma cells were seen in the EM to be clearly labeled, but nuclei, chloroplasts, mitochondria, and microbodies were unlabeled. No other studies on ophiovirus cytopathology are till now available.

Transmission, Prevention, and Control

Most ophiovirus species are soil-transmitted through the obligately parasitic soil-inhabiting fungus *Olpidium brassicae*; this has been proved for TMMMV, MiLV, and LRNV; FreSV is known to be soil-transmitted, and the freesia leaf necrosis complex (presumably containing FreSV and the varicosavirus freesia leaf necrosis virus) has been transmitted by *O. brassicae*. The virus–vector relationship was recognized as the *in vivo* type, at least for MiLV-*O. brassicae*; virus-free zoospores acquire the virus during the vegetative part of their life cycle in the roots of virus-infected host plants. Zoospores released from infected sporangia apparently carry the virus internally in their protoplasts and transmit it to healthy roots. The virus enters vector resting spores, and can survive for years in the soil in the absence of a growing host plant. Furthermore, disease control is difficult because of the lack of safe and effective treatments against the fungal vector. As ophioviruses occur in low concentration and are physically labile *in vitro*, it would be interesting to establish whether they multiply in the vector.

No information on natural vectors is available for RWMV. CPsV is commonly transmitted by vegetative propagation and no natural vectors have been identified; in some cases natural spread of psorosis in limited citrus areas has been reported, but the spatial patterns would suggest a hypothetic aerial vector instead of a soil-borne one. The data are however based on symptom observation, not on analysis for the spread of CPsV.

For disease control the use of resistant or tolerant crops may be the best choice. In Japan, the use of resistant tulip cultivars is the most important component of managing TMMMV disease, as it can be highly effective and has no deleterious effect on the environment; resistance assays have allowed researchers to identify highly resistant tulip lines and use them for breeding new resistant cultivars. For lettuce in soil-less cultivation, using ultraviolet (UV) sterilization of nutrients has shown good results in prevention of MiLV and LRNV infection, although for field lettuces the prospect is less good as no classical sources of resistance or tolerance have yet been identified. In the case of CPsV, control of the sanitary status of mother plants for producing propagating material is essential. Shoot-tip grafting *in vitro* associated with thermotherapy or somatic embryogenesis from stigma and style cultures have been successfully used to eliminate CPsV from plant propagating material. Several transgenic citrus lines exist carrying parts of the CPsV genome, and promising resistance may emerge from these.

See also: Plant Rhabdoviruses; Varicosavirus.

Further Reading

Derrick KS, Brlansky RH, da Graça JV, Lee RF, Timmer LW, and Nguyen TK (1988) Partial characterization of a virus associated with citrus ringspot. *Phytopathology* 78: 1298–1301.

García ML, Dal Bo E, Grau O, and Milne RG (1994) The closely related citrus ringspot and citrus psorosis viruses have particles of novel filamentous morphology. *Journal of General Virology* 75: 3585–3590.

Martín S, López C, García ML, *et al.* (2005) The complete nucleotide sequence of a Spanish isolate of citrus psorosis virus: Comparative analysis with other ophioviruses. *Archives of Virology* 150: 167–176.

Morikawa T, Nomura Y, Yamamoto T, and Natsuaki T (1995) Partial characterization of virus-like particles associated with tulip mild mottle mosaic. *Annals of the Phytopathological Society of Japan* 61: 578–581.

Roggero P, Ciuffo M, Vaira AM, Accotto GP, Masenga V, and Milne RG (2000) An ophiovirus isolated from lettuce with big-vein symptoms. *Archives of Virology* 145: 2629–2642.

Roggero P, Lot H, Souche S, Lenzi R, and Milne RG (2003) Occurrence of mirafiori lettuce virus and lettuce big-vein virus in relation to development of big-vein symptoms in lettuce crops. *European Journal of Plant Pathology* 109: 261–267.

Vaira AM, Accotto GP, Costantini A, and Milne RG (2003) The partial sequence of RNA 1 of the ophiovirus ranunculus white mottle virus

indicates its relationship to rhabdoviruses and provides candidate primers for an ophio-specific RT-PCR test. *Archives of Virology* 148: 1037–1050.
Vaira AM, Accotto GP, Gago-Zachert S, *et al.* (2005) Ophiovirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 673–679. San Diego, CA: Elsevier Academic Press.
Van der Wilk F, Dullemans AM, Verbeek M, and Van den Heuvel JFJM (2002) Nucleotide sequence and genomic organization of an ophiovirus associated with lettuce big-vein disease. *Journal of General Virology* 83: 2869–2877.

Orbiviruses

P P C Mertens, H Attoui, and P S Mellor, Institute for Animal Health, Pirbright, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arbovirus Viruses that are transmitted between their vertebrate host species, by insects or other arthropod vectors, replicating in both host and vector species.
Culicoides Blood-feeding dipterous insects also known as biting midges.
Ecchymosis Area of haemorrhage larger than petechiae.
Petechiae Pinpoint- to pinhead-sized red spots under the skin that are the result of small hemorrhages.

Introduction

The reoviruses (a term used here to indicate any member of the family *Reoviridae*) have genomes composed of 9–12 separate segments of linear double-stranded RNA (dsRNA), packaged as exactly one copy of each segment per icosahedral virion. The family *Reoviridae* contains a total of 12 established genera (*Orthoreovirus*, *Orbivirus*, *Cypovirus*, *Aquareovirus*, *Rotavirus*, *Coltivirus*, *Seadornavirus*, *Fijivirus*, *Phytoreovirus*, *Oryzavirus*, *Idnoreovirus*, and *Mycoreovirus*) as well as three proposed 'new' genera of viruses (*Mimoreovirus*, *Cardoreovirus,* and *Dinovernavirus*). Closely related reoviruses that infect the same cell can exchange genome segments by a process known as reassortment, generating new progeny virus strains. This ability to 'reassort' is regarded as a primary indication that different virus strains belong to the same virus species, within each of the genera of the *Reoviridae*. The largest genus *Orbivirus* contains 21 distinct virus species and 12 further unassigned viruses (**Table 1**), each of which has a ten-segmented dsRNA genome. The orbiviruses are transmitted between their vertebrate hosts by ticks or hematophagous insects (e.g., mosquitoes or biting midges (*Culicoides* spp.)) in which they also replicate, and they are therefore regarded as 'arthropod-borne viruses' or 'arboviruses'. Some orbiviruses cause severe diseases of domesticated and wild animals, including members of the species: *African horse sickness virus* (AHSV), *Bluetongue virus* (BTV), *Epizootic hemorrhagic disease virus* (EHDV), and *Equine encephalosis virus* (EEV). The prototype *Orbivirus* species, *Bluetongue virus,* has been extensively studied and provides a useful paradigm for other members of the genus.

Historical Overview

Bluetongue (BT) was originally recognized as a disease of sheep and cattle in South Africa in the late eighteenth century and was initially reported in the scientific literature, as 'malarial catarrhal fever'. In 1905, Spreull suggested the name 'bluetongue' to reflect a significant, although infrequent, clinical sign of the disease. He also showed that the agent was filterable and caused an inapparent infection in goats and cattle.

BT was initially regarded as a disease of ruminants that was exclusive to Africa. However, in 1943 an outbreak occurred in Cyprus, killing approximately 2500 sheep (70% mortality in infected animals), and it was suggested that less virulent strains had caused earlier but unrecognized outbreaks on the island. Subsequently outbreaks occurred in 1946, 1951, 1965, and 1977, and have continued sporadically to the present day. BT was recorded in the 1940s in Palestine and Turkey, and by 1950 was present in Israel. In 1948, an apparently new disease known as 'sore muzzle' was recognized in Texas and, in 1952, BTV serotype 10 was isolated from infected sheep in California. BTV serotypes 11, 17, 13, and 2 were subsequently isolated in New Mexico (1955), Wyoming (1962), Idaho/Florida (1967), and Florida (1983), respectively, and these types are now regarded as endemic in North America. More recently, BTV-1 was identified in Louisiana (2004), BTV-3 in Florida and Mississippi (1999–2006), and BTV types 5, 6, 14, 19, and 22 were isolated in Florida (2002–05).

Table 1 Species in the genus *Orbivirus*

Virus species (virus abbreviation)	*Number of serotypes/strains*	*Vector species*	*Host species*
African horse sickness virus (AHSV)	9 numbered serotypes (AHSV-1 to AHSV-9)	*Culicoides* spp. (biting midges)	Equids, dogs, elephants, camels, cattle, sheep, goats, humans (in special circumstances) predatory carnivores (by eating infected meat)
Bluetongue virus (BTV, *Orbivirus* type species)	24 numbered serotypes (BTV-1 to BTV-24)	*Culicoides* spp. (biting midges)	All ruminants, camelids and predatory carnivores (by eating infected meat)
Changuinola virus (CGLV)	12 named serotypes	Phlebotomines, culicine mosquitoes	Humans, rodents, sloths
Chenuda virus (CNUV)	7 named serotypes	Ticks	Seabirds
Chobar Gorge virus (CGV)	2 named serotypes	Ticks	Bats
Corriparta virus (CORV)	6 named serotypes/strains[a]	Culicine mosquitoes	Humans, rodents
Epizootic hemorrhagic disease virus (EHDV)	10 numbered or named serotypes/strains[a] (EHDV-1 to EHDV-8, EHDV 318, Ibaraki virus (atypical EHDV-2))	*Culicoides* spp. (biting midges)	Cattle, sheep, deer, camels, llamas, wild ruminants, marsupials
Equine encephalosis virus (EEV)	7 numbered serotypes (EEV-1 to EEV-7)	*Culicoides* spp. (biting midges)	Equids
Eubenangee virus (EUBV)	4 named serotypes	*Culicoides* spp., anopheline and culicine mosquitoes	Unknown hosts
Ieri virus (IERIV)	3 named serotypes	Mosquitoes	Birds
Great Island virus (GIV)	36 named serotypes/strains[a]	Argas, Ornithodoros, Ixodes ticks	Seabirds, rodents, humans
Lebombo virus (LEBV)	1 numbered serotype (LEBV-1)	Culicine mosquitoes	Humans, rodents
Orungo virus (ORUV)	4 numbered serotypes (ORUV-1 to ORUV-4)	Culicine mosquitoes	Humans, camels, cattle, goats, sheep, monkeys
Palyam virus (PALV)	13 named serotypes/strains[a]	*Culicoides* spp., culicine mosquitoes	Cattle, sheep
Peruvian horse sickness virus (PHSV)	1 numbered serotype (PHSV-1)	Mosquitoes	Horses
St. Croix River virus (SCRV)	1 numbered serotype (SCRV-1)	Ticks	Hosts unknown
Umatilla virus (UMAV)	4 named serotypes	Culicine mosquitoes	Birds
Wad Medani virus (WMV)	2 named serotypes	Boophilus, Rhipicephalus, Hyalomma, Argas ticks	Domesticated animals
Wallal virus (WALV)	3 serotypes/strains	*Culicoides* spp.	Marsupials
Warrego virus (WARV)	3 serotypes/strains	*Culicoides* spp., anopheline and culicine mosquitoes	Marsupials
Wongorr virus (WGRV)	8 serotypes/strains	*Culicoides* spp., mosquitoes	(Cattle, macropods)
Tentative species			
Andasibe virus (ANDV)		Mosquitoes	Unknown hosts
Codajas virus (COV)		Mosquitoes	Rodents
Ife virus (IFEV)		Mosquitoes	Rodents, birds, ruminants

Continued

Table 1 Continued

Virus species (virus abbreviation)	*Number of serotypes/strains*	*Vector species*	*Host species*
Itupiranga virus (ITUV)		Mosquitoes	Unknown hosts
Japanaut virus (JAPV)		Mosquitoes	Unknown hosts
Kammavanpettai virus (KMPV)		Unknown vectors	Birds
Lake Clarendon virus (LCV)		Ticks	Birds
Matucare virus (MATV)		Ticks	Unknown hosts
Tembe virus (TMEV)		Mosquitoes	Unknown hosts
Tracambe virus (TRV)		Mosquitoes	Unknown hosts
Yunnan orbivirus (YUOV)		*Culex tritaeniorhyncus*	Unknown hosts

[a]In some species the serological relationships between strains has not been fully determined. For more information concerning individual named types, see Mertens PPC, Duncan R, Attoui H, and Dermody TS (2005) *Reoviridae.* In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses,* pp. 466–483. San Diego, CA: Elsevier Academic Press.

Several BTV serotypes are also present in Central and South America and, although less well characterized, these include BTV-1, 3, 4, 6, 8, 11, 12, 13, 14, and 17.

BT virus serotype 10 caused a single epizootic in Spain and Portugal in 1956, and was reported in West Pakistan in 1958 and 1960, and in India in 1961. The disease now occurs regularly and is regarded as endemic on the Indian subcontinent, involving many different serotypes. Although Australia was initially considered to be free of BT, in 1978 a virus that was collected in the Northern Territory (during 1976), was identified as BTV. Eight serotypes have subsequently been isolated in Australia (BTV-1, 3, 9, 15, 16, 20, 21, and 23). Initially outbreaks of BT in Europe were infrequent, involving a single virus strain on each occasion, and were generally short lived (4–5 years). However, since 1998, eight distinct BTV strains from six different serotypes (BTV-1, 2, 4, 8, 9, and 16) have invaded Europe, with new introductions almost every year , resulting in the deaths of >1.8 million animals.

African horse sickness (AHS) was recognized as early as 1780, during the early days of the European colonization and importation of horses into southern Africa, resulting in epizootics with high rates of mortality in infected animals. AHSV is only considered to be enzootic in sub-Saharan Africa but has caused occasional major epizootics, with very high levels of associated mortality in infected horses, in the Middle East, the Indian subcontinent, North Africa, and the Iberian Peninsula.

Epizootic hemorrhagic disease (EHD) has occurred as periodic outbreaks in the south-eastern United States since 1890, where it causes a fatal disease of deer known by 'back-woods men' as 'black tongue'. The New Jersey and South Dakota strains were isolated in 1955 and 1956, respectively, and a second serotype was isolated in the Canadian province of Alberta in 1962. EHDV has been isolated from a range of animal species including cattle (a possible 'reservoir' host). Ibaraki virus, which causes an acute febrile disease of cattle, was first recorded in Japan in 1959 and, although it is classified as EHDV serotype 2, it is regarded as atypical. There are also at least 5 serotypes of EHDV in Australia, which are known to infect cattle, buffalo, and deer without causing clinical disease. In 2006, outbreaks of the disease were recorded in cattle in Israel and Morocco.

Members of other orbivirus species are widely distributed around the world and have been isolated in Australia, North and South America, Africa, Asia, and Europe.

Host Range and Transmission

Orbiviruses are transmitted between their vertebrate hosts by a variety of hematophagous arthropods. BTV, AHSV, EHDV, and EEV are all transmitted by adult females of certain *Culicoides* species which bite the mammalian host in order to obtain proteins, prior to laying eggs. BTV is only enzootic in areas where these vectors are present and active for the majority of the year, thus maintaining a continuous infection cycle in the vector and vertebrate host species. Although there are in excess of 1000 species of *Culicoides* worldwide, only 17 have been connected with BTV and 11 are known to be capable of transmitting the virus. These are *C. sonorensis*, *C. imicola*, *C. fulvus*, *C. actoni*, *C. wadai*, *C. nubeculosus*, *C. dewulfi, C. brevitarsis, C. obsoletus, C. pulicaris,* and *C. insignis*. Many of the remaining *Culicoides* species may be refractory to infection, although environmental factors (particularly higher temperatures) can increase vector competence, even in species that are usually refractory. BTV vectors are most active between 18 and 29 °C, and are almost inactive below 10 °C or above 30 °C. The viral polymerase, which is responsible for all viral RNA synthesis, has a temperature optimum between 27 and 35 °C but is almost inactive below 15 °C. Relatively small rises in temperature within the range 15–30 °C can significantly increase both the activity of insect vectors, and the rate of virogenesis within infected individuals, significantly pincreasing their efficiency as vectors.

Infections caused by BTV and EHDV are normally restricted to domesticated and wild ruminants, although there is also evidence for BTV infection of shrews and some rodents. AHS is considered to be a disease primarily of horses and other equids. However, dogs infected with BTV (caused by a contaminated vaccine) developed a fatal viral pneumonia. AHSV infection of dogs (caused by ingestion of infected meat) can be fatal. BTV and AHSV infections have also been reported in large carnivores in Africa, and may significantly affect their numbers. The epidemiological significance of BTV or AHSV infection in dogs and other carnivores, caused by eating infected meat or by other routes, has not been fully assessed. It is unclear if they can develop a high level of viraemia, or can act as a source of infection to adult *Culicoides*. There is serological evidence that AHSV can infect elephants and isolates have occasionally been obtained from camels, cattle, sheep, and goats. Under unusual circumstances (involving inhalation of a freeze dried neurotropic vaccine strain), AHSV has also infected humans, causing encephalitis and retinitis. The potential risk to human health posed by consumption of AHSV infected meat has not yet been fully evaluated.

Orbiviruses can infect marsupials, humans, rodents, bats, monkeys, sloths, and particularly birds (*Great Island virus*, *Ieri virus,* and *Umatilla virus* species). Under experimental conditions, many orbiviruses can infect mice or embryonated chicken eggs, and these hosts are routinely used for virus isolation from infected tissue samples (e.g., blood) or from insect vectors. Adult mice can show high mortality levels when infected via a nasal route with some

strains of AHSV. This may have relevance to the reported cases of AHSV in humans infected via the same route by neurotropic vaccine strains. Experimental animal systems can provide useful models for studies of the immune response to these viruses and for identification of virulence factors, as shown for members of the *Great Island virus* (GIV) and AHSV species.

Epidemiology and Disease

Infection with BTV, EHDV, or AHSV is accompanied by pyrexia and edema/inflammation, particularly around the face, mouth, and nose. BTV replicates in hematopoietic and lymphoid tissues, including the spleen, bone marrow, monocytes, macrophages, neutrophils, and draining lymphoid tissues. Infection of vascular endothelial cells causes vascular thrombosis and hemorrhages that vary from petechiae to ecchymosis and is accompanied by leakage of fluids, into surrounding tissues or the lungs (causing frothing). Clinical signs of BT may be severe, particularly in sheep and can include epithelial lesions or ulceration of the mouth, coronitis, degeneration of skeletal muscle, lameness, and vomiting, leading to pneumonia (which is frequently fatal), hemorrhages of the skin horn junction, torticollis (usually fatal), and occasionally to a cyanotic appearance of the tongue.

BT is effectively restricted to a band around the world between 53° N and 30° S, determined largely by the distribution of vector insects. Temperatures below 0 °C, when maintained for approximately 2 h or more, will kill adult *Culicoides*. Therefore, in those areas at relatively greater latitudes (which experience frosts), adult vectors may not be present or active in significant numbers throughout the entire year. Consequently, the virus may be absent, or maintained at only low levels in line with vector populations, for part of the year (usually in winter). However, in some areas there is evidence for a mechanism that allows the virus to persist through the winter (over-wintering). The exact nature of this mechanism is unknown; it has been suggested that it could involve persistent infection of either the mammalian host or larvae of vector insects, although over-wintering by these mechanisms has not been demonstrated. Seasonal variation in the incidence of disease is illustrated by the outbreaks of AHS in Spain, Portugal, and Morocco (1987–91), where cases were detected only in late summer and autumn, and by the outbreak of BT in northern Europe during the mid to late summer of 2006. The effects of high temperatures, together with relatively low humidity, may also be particularly significant in Africa, where it is evident that the Sahara represents at least a partial barrier to the spread of BT and other orbiviruses to the Mediterranean region.

The outbreaks of BTV in Europe (since 1998) collectively represent one of the clearest examples of disease distribution being affected by climate change. Increases in average temperatures in the region have been reflected by changes in the distribution of *C. imicola* (a major vector species) in southern Europe. This has not only allowed *C. imicola* to transmit BTV in new areas, it also generated a partial overlap with European populations of *C. obsoletus*, *C. pulicaris*, and *C. dewulfi* that are widespread across most of central and northern Europe, allowing the virus to be passed to at least one of these species as a new vector. The more northerly *Culicoides* species can clearly transmit the virus, particularly during the very warm summers that are now affecting much of Europe, as demonstrated by the outbreak of BTV-8 in the Netherlands, Belgium, Germany, France, and Luxemburg during the mid-to late summer of 2006, when record temperatures were experienced in the region. The whole of Europe must now be considered at risk to outbreaks of BT and some other orbivirus diseases that are transmitted by the same vectors (e.g., AHS or EHDV). This conclusion was confirmed by the further spread of BTV-8 and to the UK, Denmark, and Switzerland during 2007.

Although BTV and some of the other orbiviruses are widely distributed, not all serotypes of each virus species are present at each location. Introduction of orbiviruses (e.g., BTV or AHSV) into areas that are usually free of the disease, and which therefore contain immunologically naive populations of susceptible host species, can result in high mortality rates in infected animals. As an example, during the disease outbreak caused by BTV-8 in northern Europe during 2006, it was estimated that up to 50% of those sheep showing clinical signs of infection, eventually died from the disease. Although much smaller numbers of cattle showed clinical signs of infection, approximately 10–15% of these affected animals also died. Even the introduction of a new serotype of BTV into enzootic areas can result in disease in host animals that have neutralizing antibodies against the types already present.

The mortality rate caused by African horsesickness virus (AHSV) in naive horses is frequently cited as above 90%, making it the most lethal and most dangerous of the equine pathogens. The epidemiology of AHSV is discussed elsewhere in this encyclopedia.

BTV can cause a significant reduction in the productivity of domesticated animals in endemic areas. It has been estimated that annual losses in the USA are of the order of $120 million. Infectious BTV has been detected in bull semen and some early experiments (although not reproducible and therefore of uncertain significance) indicated the possibility of long-term persistence of virus and immunotolerance in cattle that were naturally infected *in utero*. Restrictions are imposed on the movement or importation of live animals or germ line materials (semen and ova) from infected areas, although the exact regulations, involving testing, quarantine periods, or even complete import/export bans differ from country to country. These barriers

to trade, along with the associated surveillance and testing programs, also represent important causes of financial loss associated with outbreaks of orbivirus diseases.

Viral RNAS, Proteins, Virion Structure, and Properties

The structure, components, properties, and assembly of the BTV particle have been studied extensively by many techniques, including electron microscopy, cryoelectron microcopy, and X-ray crystallography.. The properties of the orbivirus particles and individual viral proteins described here are derived primarily from studies of BTV and AHSV.

Mature orbivirus particles are relatively featureless when viewed by negative-contrast electron microscopy (**Figure 1**). They are icosahedral, nonenveloped, approximately 90 nm in diameter and are composed of three layers of proteins, the subcore (VP3), the core-surface layer (VP7), and the outer capsid (VP2 and VP5) (**Figure 2**). Progeny orbivirus particles can leave infected cells by budding through the outer cell membrane, acquiring an envelope in the process, that can be lost soon after release. This may explain why unpurified virus is usually associated with cellular membranes and cell debris.

The buoyant densities of purified orbivirus particles in CsCl are $1.36\,g\,ml^{-1}$ (intact virions) and $1.40\,g\,ml^{-1}$ (cores). Virus infectivity is stable at pH 8–9 but virions exhibit a marked decrease in infectivity outside the pH range 6.5–10.2. This reflects the loss of outer capsid proteins at ~pH 6.5. Virus infectivity is abolished at pH 3.0, reflecting further disruption of the virus core. Viruses held *in vitro* at less than 15 °C in blood samples, serum, or albumin can remain infectious for decades, while virus infectivity is rapidly inactivated at 60 °C. Orbiviruses are relatively resistant to treatment with solvents, and nonionic detergents, or weak anionic detergents (such as sodium *N*-lauroyl sarcosine), although sensitivity varies with virus species. However, strong anionic detergents such as sodium dodecyl sulfate (SDS) will disrupt the particle and destroy infectivity. Freezing reduces virus infectivity by ~90%, possibly due to particle disruption. However, once frozen, virus infectivity remains stable at −70 °C.

The orbivirus capsid contains seven structural proteins, arranged as three concentric capsid layers. The outer capsid layer of BTV and the related orbiviruses is composed of proteins VP2 and VP5 (encoded by genome segment 2 and segment 6, respectively). VP5 is involved in membrane penetration and can cause cell–cell fusion, while VP2 is primarily involved in cell attachment and is the major target for neutralizing antibodies generated by the infected host. VP2 is also the most variable of the virus proteins and the specificity of its interactions with these neutralizing antibodies (as determined by serum neutralization assays) can be used to identify 24 distinct BTV serotypes. The other orbivirus species also contain a variable number of distinct virus 'serotypes' (**Table 1**). The identification of the BTV, AHSV, or EHDV serotype involved in an outbreak of disease is an important aspect of virus diagnosis and would have direct relevance to the selection of an appropriate vaccine 'type'.

The orbivirus outer capsid can be modified by proteolytic enzymes (e.g., cleavage of BTV-VP2 by trypsin or chymotrypsin) and can be completely removed by treatment with divalent metal ions, or reduced pH, to release the virus core. The surface of the orbivirus core has ring-shaped capsomers, composed of the VP7 protein that are readily observed by electron microscopy (**Figure 1**) (hence orbivirus from Latin: *orbis*, meaning 'ring' or 'circle'). The BTV core is also infectious in its own right, for some mammalian cells, and particularly for adult *Culicoides* or *Culicoides* cell cultures, indicating that outer core protein VP7 can also mediate cell attachment and penetration in the absence of either VP2 or VP5. The core structural proteins are more conserved than those of the outer capsid and are serologically cross-reactive (e.g., by enzyme-linked immunosorbent assay (ELISA)) between different serotypes belonging to the same orbivirus species, but not between isolates from different species, providing

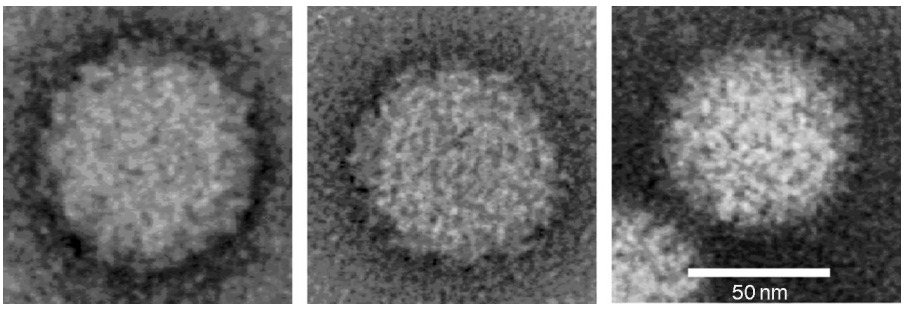


Figure 1 Electron micrographs of bluetongue virus serotype 1. Preparations of purified BTV-1 particles (strain – RSArrrr/01) were stained with 2% uranyl acetate: (left) virus particle, showing the relatively featureless surface structure; (center) infectious subviral particle (ISVP), in which outer capsid protein VP2 has been cleaved by treatment with chymotrypsin, showing some discontinuities in the outer capsid layer; (right) core particle, from which the entire outer capsid has been removed to reveal the structure of the VP7 (T13) core-surface layer and showing characteristic ring-shaped capsomeres.

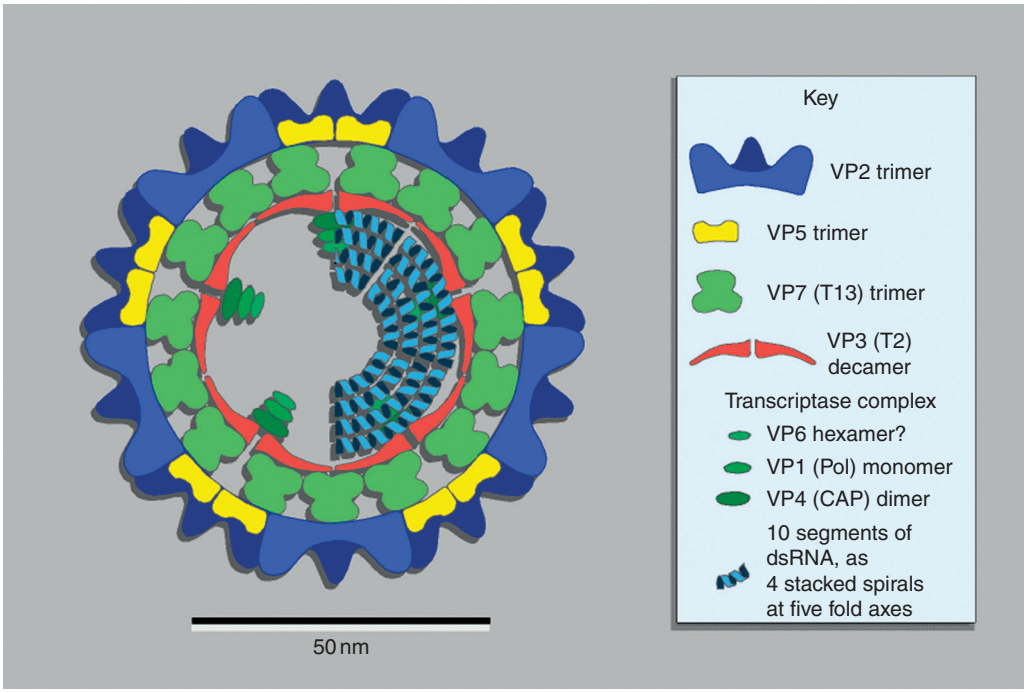


Figure 2 Diagram of the bluetongue virus (BTV) particle structure. A cross-section diagram of the orbivirus particle structure (viewed down a fivefold axis) was constructed using data from biochemical analyses, electron microscopy, cryoelectron microscopy and X-ray crystallography generated for bluetongue virus. Courtesy of P.P.C. Mertens and S. Archibald. Reproduced from Mertens PPC, Maan S, Samuel A, and Attoui H (2005) *Orbivirus, Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy*: *Eighth Report of the International Committe on Taxonomy of Viruses*, pp. 466–483. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

a basis for serogroup-(virus-species-) specific assays. VP7 of BTV is particularly immunodominant and represents the major 'serogroup-specific' antigen detected in BTV-specific diagnostic assays, although the other core and nonstructural proteins also show virus-species/serotype-specific cross-reactions.

Orbivirus-infected cells also synthesize three nonstructural proteins (NS1, NS2, and NS3, encoded by BTV genome segments 5, 8, and 10, respectively).

Genome Organization and Replication

The BTV genome represents 12% and 19.5% of the total mass of intact orbivirus particles and cores, respectively. The genome segments range in size from 3954 to 822 bp (total of 19.2 kbp – **Figure 3**) and are identified as 'segments 1 to 10' (Seg-1 to Seg-10) in order of decreasing molecular weight and, hence, increasing electrophoretic mobility in 1% agarose gels. Different isolates belonging to the same orbivirus species usually have genome segments with a uniform size distribution, generating a uniform migration pattern (electropherotype) by agarose gel electrophoresis (AGE) (**Figure 4**), and this can be used to help identify individual virus species. However, variations in primary sequence often cause significant variations in migration patterns during polyacrylamide gel electrophoresis (PAGE) so that in most cases Seg-5 and Seg-6 of BTV migrate in the reverse order. This method can frequently be used to distinguish different virus strains or reassortants within the same virus species.

Orbivirus genome segments usually have a single major open reading frame (ORF) which is always on the same strand of the RNA (see **Table 2**). However, some ORFs can have more than one functional initiation site near to the 5′-end of the RNA, resulting in production of two related proteins (e.g., segment 10 of BTV encoding NS3 and NS3a).

The 5′-untranslated regions (UTRs) of BTV type 10 genome segments range from 8 to 34 bp, while the 3′-UTR are 31–116 bp in length. For other BTV serotypes and other virus species, these lengths can vary. However, in general, the 5′-UTRs are shorter than the 3′-UTRs. The UTRs of almost all of the orbivirus genome segments that have been sequenced (**Table 2**) contain two conserved base pairs at each terminus (5′-GU. . . AC-3′, in the positive sense). The six terminal base pair sequences at both the 3′- and 5′-UTRs of the ten BTV genome segments are almost invariably conserved in different BTV isolates (**Table 2**). Other orbiviruses have terminal sequences which are comparable to, but not always the same as, those of BTV, although they may not be conserved in all ten genome segments.

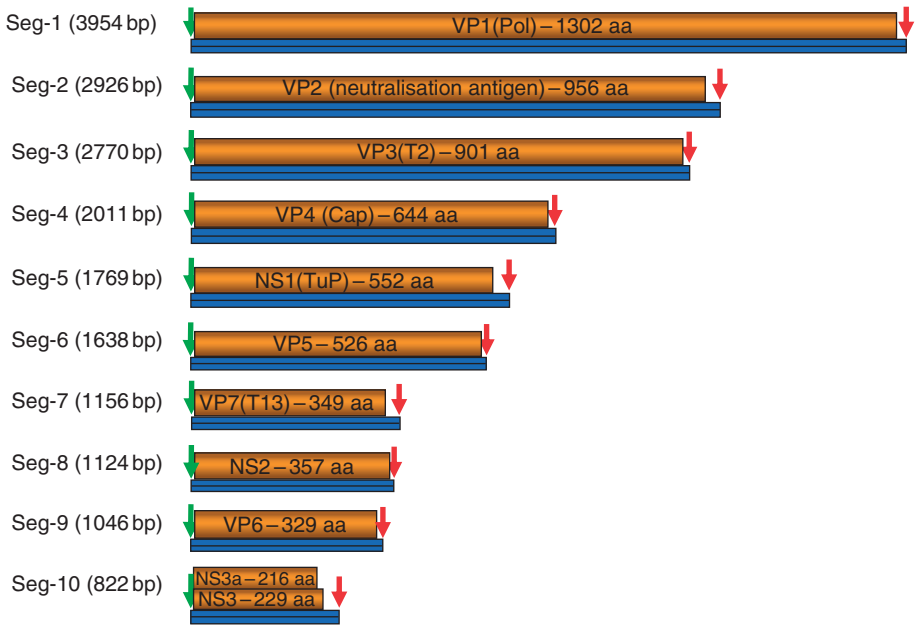


Figure 3 Organization of the BTV genome. The organization of the ten linear, dsRNA, genome segments of BTV-10. With the exception of Seg-10, each genome segment encodes a single viral protein. Seg-10 has two in-frame and functional initiation codons near to the upstream end of the segment (see http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/BTV.htm). Like other members of the family, each AHSV genome segment contains conserved terminal sequences immediately adjacent to the upstream and downstream termini ((green arrow), 5′-GUUAAA.....ACUUAC-3′ (red arrow) in the positive-sense strand) (see **Table 2**).

Orbiviruses replicate in a variety of mammalian and insect cell lines, including BHK21 (baby hamster kidney), Vero (African green monkey kidney), KC (*Culicoides sonorensis*), and C6/36 (*Aedes albopictus*) cells. Intact orbivirus particles bind to the host cell surface via their outer capsid proteins, leading to endocytosis and cell penetration. However, BTV core particles are also infectious, indicating that the core surface protein (VP7 of BTV) can also mediate cell attachment and entry. Cell-surface receptors for BTV have not yet been identified, although core particles can bind to glycosaminoglycans. VP2 is the BTV hemagglutinin (binds sialic acid residues) and (along with VP5, NS1, and NS3) appears to determine virulence of AHSV, which may reflect its role in cell entry and initiation of infection. VP5 can induce cell fusion, suggesting an involvement in cell membrane penetration (i.e., release from endosomes).

The details of cell infection and the intracellular replication cycle of BTV represent a paradigm for other orbiviruses and are discussed elsewhere in this encyclopedia.

Evolutionary Relationships among the Orbiviruses

Structural similarities, serological cross-reactions, and significant levels of nucleotide or amino acid sequence identities in specific genes or proteins clearly demonstrate that the different orbiviruses have a common ancestry. However, the members of distinct orbivirus species can be distinguished by a failure to cross-react in 'serogroup specific' serological assays, which target the more conserved core or nonstructural proteins (e.g., VP7). Different orbivirus species also show relatively large sequence differences even in their most conserved RNAs/proteins. These can be used to distinguish them and have been used to design species-specific primers for diagnostic reverse transcriptase-polymerase chain reaction (RT-PCR) assays. The nonstructural proteins and structural proteins of the orbivirus core are usually the most conserved components of each virus species (serogroup), while showing significantly higher levels of variation between different species. For example, the subcore shell protein (VP3 of BTV) is very highly conserved between members of the same orbivirus species (showing >73% amino acid identity between BTV strains) but shows significantly lower levels of conservation between virus species (serogroups 21.4% to 72%), reflecting more distant evolutionary relationships (**Figure 5**).

The protein components of the outermost capsid layer of the virus interact with the host defenses (including antibodies and cellular components of the mammalian immune system) and are therefore subjected to selective pressure, to change and avoid recognition. The outer surface components of different orbivirus species have also evolved to mediate transmission, cell attachment, and penetration in different host and vector species. Consequently, these outer capsid proteins (and the genome segments from which they are translated) usually show a greater degree of diversity

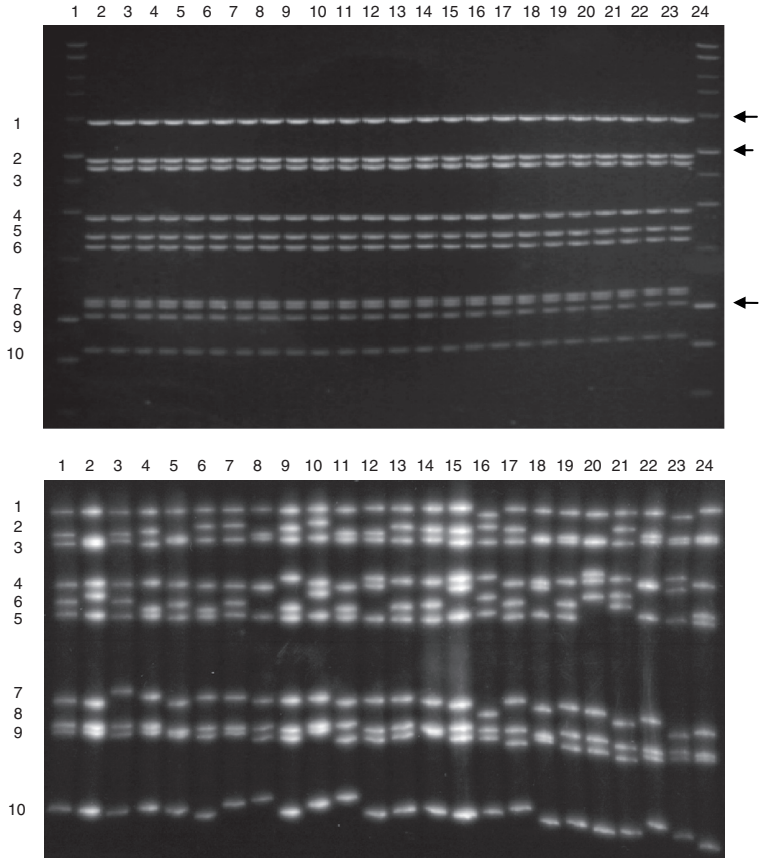


Figure 4 Electrophoretic analysis of BTV genomic dsRNAs. The genomic dsRNAs from the 24 reference strains of bluetongue viruses (BTV-1 to BTV-24: lanes 1–24) were analyzed by electrophoresis in a 1% agarose gel (top panel). Genome segments are referred to by numbers, in order of increasing electrophoretic mobility as indicated at the sides of gel. A consistent electropherotype was observed, which (despite similarities to that of some EHDV isolates) is different from the majority of other orbiviruses. The same set of dsRNAs were also analyzed by 11% polyacrylamide gel electrophoresis (PAGE) (bottom panel). Genome segments 5 and 6 migrate in a reverse order during PAGE, for most BTV isolates. Some of the more intense bands contain two genome segments which co-migrate. Differences were detected in the RNA migration pattern (PAGE-electropherotype) of each of the BTV isolates, which reflect variations in their primary nucleotide sequences.

Table 2 Conserved terminal sequences of orbivirus genome segments

Virus isolate	*Conserved RNA termini (positive strand)*
Bluetongue virus (BTV)	5′-GUUAAA.....UUAC-3′
African horse sickness virus (AHSV)	5′-GUU $^{A}/_{U}$ A$^{A}/_{U}$.....AC$^{A}/_{U}$UAC-3′
Epizootic hemorrhagic disease virus (EHDV)	5′-GUUAAA.....$^{A}/_{G}$CUUAC-3′
Great Island virus (BRDV)	5′-GUAAAA.....A$^{A}/_{G}$GAUAC-3′
Palyam virus (CHUV)	5′-GU $^{A}/_{U}$ AAA.....$^{A}/_{G}$CUUAC-3′
Equine encephalosis virus (EEV)[a]	5′-GUUAAG.....UGUUAC-3′
St. Croix River virus (SCRV)	5′-$^{A}/_{G}$UAAU$^{G}/_{A/U}$.....$^{G}/_{A/U}$$^{C}/_{U}$$^{C}/_{A}$UAC-3′
Peruvian horse sickness virus (PHRV)	5′-GUUAAAA.....$^{A}/_{G}$$^{C}/_{G}$$^{A}/_{G}$UAC-3′
Yunan orbivirus (YUOV)	5′-GUUAAAA.....$^{A}/_{G}$UAC-3′

[a]Based on genome segment 10 (only) of the seven different serotypes.

within each species, than the protein components of the core or the nonstructural proteins.

In BTV, AHSV, or EHDV, the larger of the two outer capsid proteins (VP2 – encoded by genome segment 2) mediates cell attachment and is the major target for neutralizing antibodies. VP2 shows up to 27% amino acid sequence variation within a single serotype, while different BTV types can show as much as 73% variation in the sequence of this protein. Variations in the amino acid sequence of VP2 also reflect the serological relationships (cross-reactions) between different serotypes (**Figure 6**). The other orbivirus outer coat protein (VP5) can also

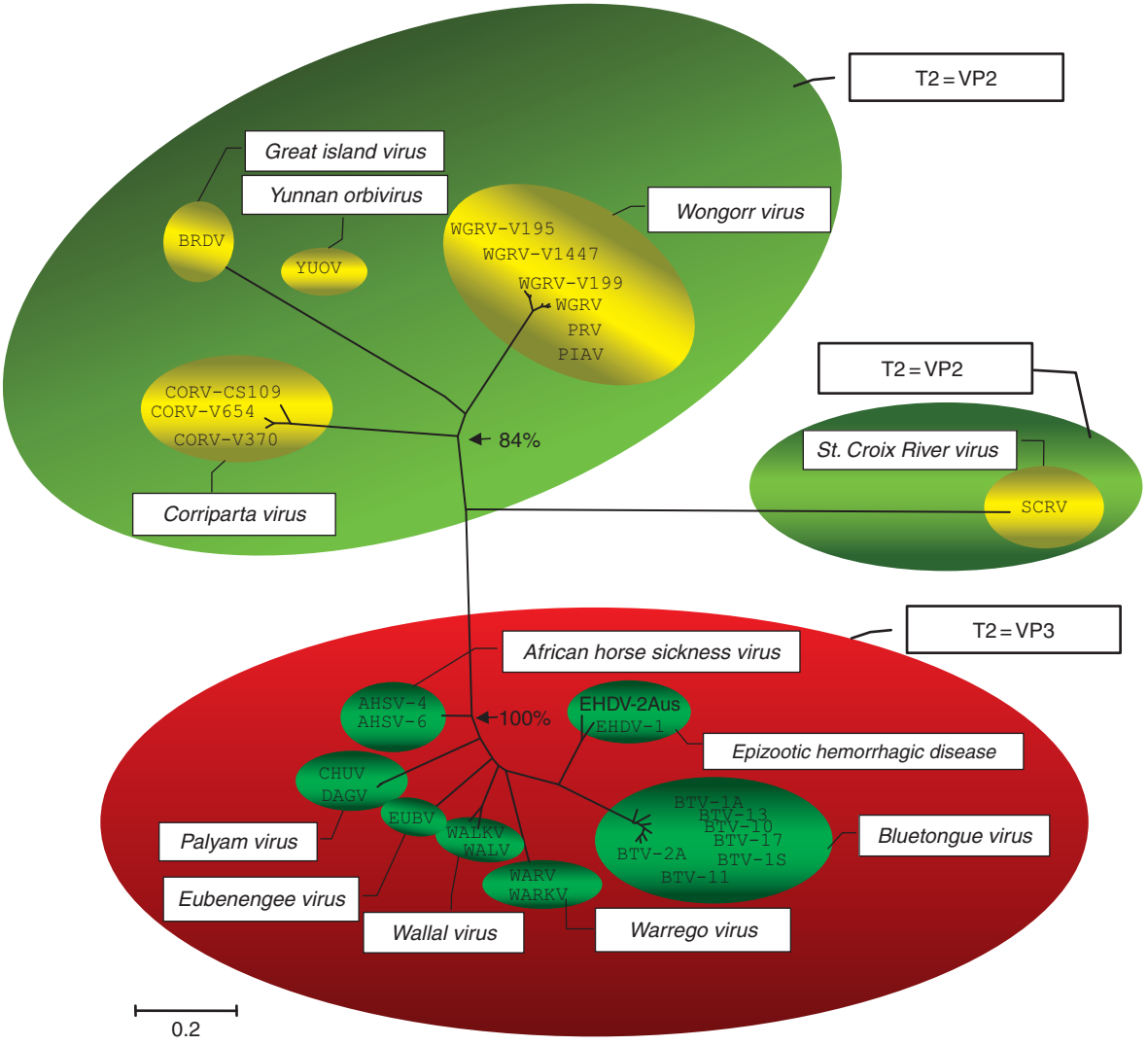


Figure 5 Phylogenetic comparison of sub-core shell (T2) proteins of different orbiviruses. Unrooted neighbor-joining tree showing relationships between the deduced amino acid sequences of the sub-core shell protein (T2) of different orbivirus species. This NJ tree was constructed using MEGA program version 3.1 and the p-distance algorithm based on partial sequences for VP3/VP2, as indicated (amino acids 393–548 relative to BTV-10 sequence). Similar trees were obtained with the Poisson correction and the gamma distance. Two major clusters of virus species were detected, one group with T2 proteins (VP2) encoded by genome segment 2 (supported by bootstrap values of >84%), and a second group with T2 protein (VP3) encoded by genome segment 3 (e.g., BTV and AHSV). These two groups appear to represent distinct evolutionary lineages. SCRV was the most divergent virus from either insect-borne or the other tick-borne orbiviruses and forms a separate small cluster.

influence serotype, although variations in the amino acid sequence of BTV VP5 show only a partial correlation with virus serotype. In some other orbiviruses (e.g., the Great Island viruses), it is the smaller outer capsid protein that exerts a greater influence over serotype. Despite its biological significance and at least partial control of cross-protection between different virus strains, the serotype of the virus currently has no formal taxonomic significance.

Within a single virus species, many of the genome segments also display sequence variations that reflect the geographic origin of the virus isolate (topotypes). For example, BTV and EHDV isolates can be divided into eastern and western groups, based on sequence comparisons of several genome segments/proteins (i.e., 'eastern' viruses from Australia, India, and Asia, and 'western' viruses from Africa and America). Since 1998, both eastern and western strains of BTV have invaded Europe, providing a potentially unique opportunity for these viruses to co-infect the same host and exchange (reassort) genome segments. Regional variations in VP2/Seg-2 and VP5/Seg-6 indicate that different BTV serotypes evolved before they became geographically dispersed, and then acquired further point mutations that distinguish these different topotypes.

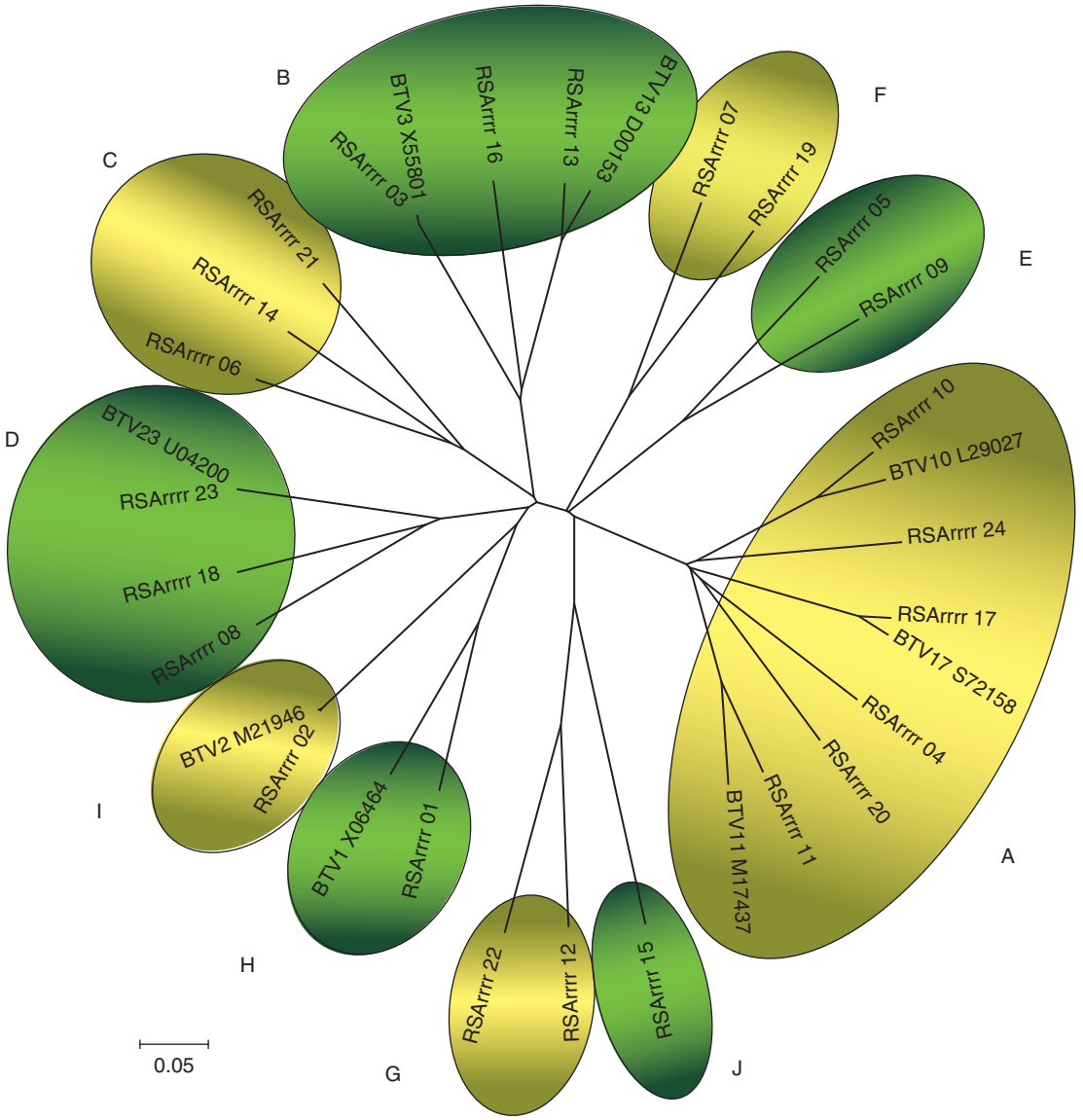


Figure 6 Phylogenetic relationships of bluetongue virus outer capsid protein VP2. Unrooted neighbor-joining tree showing the relationships between deduced amino acid sequences of VP2 (encoded by genome segment 2) from the 24 BTV serotypes. This NJ tree was constructed using MEGA program version 3.1 using the p-distance algorithm and the full-length VP2 sequences of the 24 BTV types. The different serotypes are distinct but show some relationships (green bubbles) that mirror the serological relatedness (cross-reactions) that are known to exist between different serotypes. These groupings are reflected in the nucleotide sequences of genome segment 2 and the ten distinct groups have previously been identified as nucleotypes A–J.

Sequence analyses of BTV Seg-2/VP2 provide rapid and reliable diagnostic methods to determine both the serotype and origin of individual isolates (molecular epidemiology).

However, VP7/genome segment 7 and NS3/genome segment 10 of BTV, EHDV, and AHSV show significant variations that do not appear to reflect virus serotype, or the geographic origins of the isolate. Indeed NS3 of AHSV is the second most variable of the viral proteins (after VP2), showing greater diversity than the smaller outer capsid protein VP5. As VP7 and NS3 can play roles in infection and virus release/dissemination that may be essential within the insect vector, it has been suggested that these proteins and genome segments might show variations that relate to the species or population of vector insects by which they are transmitted.

Orbiviruses of Humans

Orbiviruses and antibodies indicative of orbivirus infection have been isolated from and/or detected in humans. Changuinola virus (one of twelve 'named' serotypes within

the *Changinola virus* species) was isolated in Panama from a human with a brief febrile illness. The virus has also been isolated from phlebotomine flies, and antibodies have been detected in rodents. Changuinola virus replicates in mosquito cells (C6/36) without producing CPE and is pathogenic for newborn mice or hamsters following intracerebral inoculation.

Kemerovo, Lipovnik, and Tribec viruses are among 36 tick-borne virus serotypes from the *Great island virus* species. These viruses were implicated as causes of a nonspecific fever, or neurological infection in the former USSR (Kemerovo) and Central Europe (Lipovnik and Tribec). More than 20 strains of Kemerovo virus were isolated in 1962 from patients with meningoencephalitis, and from *Ixodes persulcatus* ticks in the Kemerovo region of Russia. The virus was also isolated from birds and infects Vero or BHK-21 cells. Lipovnik and Tribec viruses may be involved in Central European encephalitis (CEE) and >50% of CEE patients had antibodies to Lipovnik virus. The virus is also suspected in the etiology of some chronic neurological diseases including polyradiculoneuritis and multiple sclerosis. Antibodies against a Kemerovo-related virus have been detected in Oklahoma and Texas, in patients with Oklahoma tick fever. Sixgun City virus is one of seven tick-borne serotypes of the *Chenuda virus* species isolated from birds. Several Oklahoma tick fever patients also had antibodies to Sixgun City virus, although no virus was isolated.

Lebombo virus type 1 (the only serotype of the *Lebombo virus* species) was isolated in Ibadan, Nigeria, in 1968, from a child with fever. The virus replicates in C6/36 cells without CPE and lyses Vero and LLC-MK2 (Rhesus monkey kidney) cells. It is pathogenic for suckling mice and has also been isolated from rodents and mosquitoes (*Mansonia* and *Aedes* species) in Africa.

The *Orungo virus* species (ORV) contains four distinct serotypes (ORV-1 to ORV-4), transmitted by *Anopheles*, *Aedes*, and *Culex* mosquitoes. ORV is widely distributed in tropical Africa where it has been isolated from humans, camels, cattle, goats, sheep, monkeys, and mosquitoes. ORV was first isolated in Uganda during 1959 from the blood of a human patient with fever and diarrhoea, who developed weakness of the legs and generalized convulsion. The weakness progressed to flaccid paralysis. Only a few clinical cases were reported (involving fever, headache, myalgia, nausea, and vomiting), despite a high prevalence of virus infection and three deaths. ORV causes lethal encephalitis in suckling mice and hamsters. It also causes CPE and plaques in Vero and BHK-21 cells, and it replicates when inoculated into the thorax of adult *Aedes aegypti* mosquitoes. High rates of co-infection with yellow fever and Orungo viruses have been reported, reflecting their similar geographic distribution and transmission by Aedes mosquitoes, as the principal vectors.

See also: African Horse Sickness Viruses; Bluetongue Viruses; Reoviruses: General Features; Reoviruses: Molecular Biology.

Further Reading

Anthony S, Jones H, Darpel KE, *et al.* (2007) A duplex RT-PCR assay for detection of genome segment 7 (VP7 gene) from 24 BTV serotypes. *Journal of Virological Methods* 141: 188–197.

Grimes JM, Burroughs JN, Gouet P, *et al.* (1998) The atomic structure of the bluetongue virus core. *Nature* 395: 470–478.

Maan S, Maan NS, Samuel AR, Rao S, Attoui H, and Mertens PPC (2006) Analysis and phylogenetic comparisons of full-length vp2 genes of the twenty-four bluetongue virus serotypes. *Journal of General Virology* 88: 621–630.

Mertens PPC and Attoui H (eds.) ReoID: Orbivirus reference collection. http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/ReoID/viruses-at-iah.htm (accessed July 2007).

Mertens PPC, Attoui H, and Bamford DH (eds.) The RNAs and proteins of dsRNA viruses. http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/ (accessed July 2007).

Mertens PPC and Diprose J (2004) The Bluetongue virus core: A nano-scale transcription machine. *Virus Research* 101: 29–43.

Mertens PPC, Duncan R, Attoui H, and Dermody TS (2005) *Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 447–454. San Diego, CA: Elsevier Academic Press.

Mertens PPC, Maan NS, Prasad G, *et al.* (2007) The design of primers and use of RT-PCR assays for typing European BTV isolates: Differentiation of field and vaccine strains. *Journal of General Virology* 88: 621–630.

Mertens PPC, Maan S, Samuel A, and Attoui H (2005) *Orbivirus, Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 466–483. San Diego, CA: Elsevier Academic Press.

Mertens PPC and Mellor PS (2003) Bluetongue. *State Veterinary Journal* 13: 18–25.

Mo CL, Thompson LH, Homan EJ, *et al.* (1994) Bluetongue virus isolations from vectors and ruminants in Central America and the Caribbean. Interamerican Bluetongue Team. *American Journal of Veterinary Research* 55: 211–215.

Owens RJ, Limn C, and Roy P (2004) Role of an arbovirus nonstructural protein in cellular pathogenesis and virus release. *Journal of Virology* 78: 6649–6656.

Purse BV, Mellor PS, Rogers DJ, Samuel AR, Mertens PPC, and Baylis M (2005) Climate change and the recent emergence of bluetongue in Europe. *Nature Reviews Microbiology* 3: 171–181.

Sellers RF (1980) Weather, host and vectors: Their interplay in the spread of insect-borne animal virus diseases. *Journal of Hygiene (Cambridge)* 85: 65–102.

Shaw AE, Monaghan P, Alpar HO, *et al.* (2007) Development and validation of a real-time RT-PCR assay to detect genome bluetongue virus segment 1. *Journal of Virological Methods*. 145: 115–126.

Relevant Websites

http://www.rcsb.org/pdb – Information concerning protein structures, RCSB Protein Data Bank.

http://viperdb.scripps.edu – Information concerning virus structure, virus particle explorer database(viperdb).

http://www.oie.int – OIE data on AHSV outbreaks, OIE homepage.

Organ Transplantation, Risks

C N Kotton, Massachusetts General Hospital, Boston, MA, USA
M J Kuehnert, Centers for Disease Control and Prevention, Atlanta, GA, USA
J A Fishman, Massachusetts General Hospital, Boston, MA, USA

© 2008 Elsevier Ltd. All rights reserved.

Donor-Derived Infections

Donor-derived viral infections represent a significant risk to recipients in the solid organ and hematopoietic stem cell transplantation (SOT and HSCT) setting. Viruses can be transmitted due to latent or active infection in the donor at the time of donation; rarely, donor infection is very acute and transmission occurs during the incubation period of infection, before symptoms or other evidence of infection can arise, for example, when the donor is transfused with infected blood near the time of death. Clinical consequences of infection in the transplant recipient depend on the virulence of the infecting pathogen, immune status of the recipient to the pathogen in question, and level of host immunosuppression, among other factors. Immunocompromised patients may have unusual presentations of infection, and even organisms of low virulence may cause high morbidity and mortality in these recipients.

Routine donor screening is in place for cytomegalovirus (CMV), Epstein–Barr virus (EBV), herpes simplex virus (HSV), varicella zoster virus (VZV), hepatitis B (HBV), hepatitis C (HCV), and human immunodeficiency virus (HIV), although positive donor testing does not preclude organ transplantation, with the exception of HIV. Transplantation of an organ from an infected donor is at the discretion of the transplant center and transplanting surgeon. The most commonly recognized transplant-transmitted pathogens are the herpesviruses (e.g., HSV, VZV, CMV, EBV, human herpes virus 8 (HHV-8)), hepatitis viruses (e.g., HBV, HCV), and retroviruses (e.g., human T-cell leukemia virus (HTLV)), although transmission of HIV is now unusual given current screening using nucleic acid testing (NAT). Most notable are infections that are either emerging in donor populations, or are otherwise unusual in causing illness in transplant patients. Many of these pathogens are zoonotic in nature. Some examples include West Nile virus (WNV), rabies, and lymphocytic choriomeningitis virus (LCMV) (**Table 1**).

WNV transplant-associated transmission was recognized during the first large epidemic seasonal activity in the US in 2002, when four organ recipients developed infection from a common donor (three complicated by neuroinvasive disease, the other by febrile illness), and was associated with a donor who contracted the infection from blood transfusion. The risk of infection through transfusion has been greatly reduced by blood donor screening initiated by NAT in 2003, although rarely, breakthrough infections can still occur. Subsequently, there has been an additional report of transplant transmission from a donor who likely acquired infection by mosquito in 2005; in that investigation, the donor was found to be IgM positive, indicating evidence of recent acquisition of infection, but viremia could not be detected by NAT. WNV infection has also been suspected to be transmitted through HSCT. Meningitis and encephalitis of unknown etiology in a patient recently post-transplant should cause clinicians to consider transplant transmission of WNV. Although WNV neuroinvasive disease occurs in less than 1% of WNV infections overall, transplant patients who acquire infections have an estimated 40-fold risk for developing neuroinvasive disease compared with the general population.

In 2004, four recipients of kidneys, a liver, and an arterial segment from a common organ donor died of encephalitis later diagnosed as rabies. Encephalitis developed in all four recipients within 30 days of transplantation and was associated with delirium, seizures, respiratory failure, and coma. Antibodies against rabies virus were present in three of the four recipients along with the donor, who had reportedly been bitten by a bat. This investigation also outlines the importance of careful accounting and management of tissue and organ-associated conduits in hospitals; during the investigation, the source of rabies infection in the arterial segment recipient was not clear.

Two separate occurrences of transplant transmission of LCMV have also been reported in 2003 and 2005. The transplant recipients had abdominal pain, altered mental

Table 1 Viral pathogens in transplantation recipients

Herpes simplex virus
Varicella zoster virus
Epstein–Barr virus
Cytomegalovirus
HHV-6
HHV-7
HHV-8/KSHV
Parvovirus B19
West Nile virus
Rabies
Hepatitis B and C virus
Papillomavirus
Polyomavirus BK/JC
Adenovirus, RSV, influenza, parainfluenza viruses
Lymphocytic choriomeningitis virus (LCMV)
Metapneumovirus
HIV
SARS-associated coronavirus

status, thrombocytopenia, elevated transaminases, coagulopathy, graft dysfunction, and either fever or leukocytosis within 3 weeks after transplantation. Seven of the eight recipients died. In both investigations, LCMV could not be detected in the organ donor, requiring further epidemiologic investigation to try to confirm the source. No source of LCMV infection was found in the 2003 cluster; in the 2005 cluster, the donor had had contact in her home with a pet hamster infected with a viral strain identical to the LCMV detected in the transplant recipients.

Transplant-transmitted infection is rare and might be difficult to recognize, but physicians should consider the possibility, particularly when unexplained neurologic complications occur. These investigations underscore the challenge in detecting and diagnosing infections that occur in recipients of organs or tissues from a common donor. The potential for disease transmission from donor source may not be considered in recipient evaluation. In these investigations, the ability to connect illnesses to a common organ donor was facilitated by the fact that multiple recipients were hospitalized at the same facility. As organ and tissue transplantation becomes more common, the potential risks of disease transmission may also increase.

Because of improved diagnostic assays, donor-transmitted infections are increasingly recognized, although often times, the impact of infection is not well understood. The advent of polymerase chain reaction (PCR) and other genetic material-based tests have allowed for detection of active viremia, in contrast to serologic tests, which reflect past acquisition of infection. This is important not only for recipient diagnosis, but also for recognition of donor infection retrospectively; donor diagnosis is only possible if appropriate specimens are stored postmortem.

Recognition of viral infection transmitted through transplantation is increasing, likely both due to increased recognition of unexpected symptoms consistent with transmission, and due to increasingly sophisticated diagnostic testing in both the recipient and the donor. Investigation of potential donor-transmitted infection requires rapid communication among physicians in transplant centers, organ procurement organizations, and public health authorities. An immediate system for tracking and disseminating pertinent patient data to evaluate donor-derived infection and associated adverse event outcomes is needed. Until such a system can be established, clinicians should report unexpected outcomes or unexplained illness in transplant recipients to their local organ and tissue procurement organizations.

Reactivation of Latent Infections

Given the frequency of latent viral infection, notably among herpesviruses, reactivation of latent infection provides a major source of infection after transplantation. The specific virus, the tissue infected, stimuli for activation, and the nature of the host immune response impact the nature of viral latency. Some viruses are metabolically inactive when latent, while others continue to replicate at low levels that may be determined by the effectiveness of the host's immune response. Multiple factors contribute to viral reactivation after transplantation, including graft rejection and therapy, immune suppression (especially reduction of T-cell mediated, cytotoxic immunity), inflammation, and tissue injury. Numerous cellular pathways are involved in the control of viral replication and are activated after transplantation, such as nuclear factor κB, IκB, and JAK-STAT (the Janus family of protein tyrosine kinases (JAKS) and signal transducers and activators of transcription (STAT) proteins). Antirejection therapy can also result in a significant release of pro-inflammatory cytokines which may increase viral replication. In general, reactivation of viruses, especially late after transplantation, should suggest new immune defects (e.g., cancer) or relative over-immunosuppression.

Latency and reactivation has been best studied in the herpesviruses, which establish lifelong, latent infection after primary infection. In general, latency is considered to be the absence of viral replication, with viral genomes present in the cell without replication or spread. Studies of other viruses, such as Friend virus in mice, suggest that protective antiviral immunity is an active process mediated by 'leaky' (low-level) viral replication. The existence of true latency, as opposed to low-level replication, remains controversial. Herpesviruses make 'latency' proteins that both control viral persistence within the target cell and influence other cellular processes. The latent state is characterized by low levels or the absence of detectable viral antigens, minimal transcription of productive or lytic cycle genes, and expression of the latency-associated viral transcripts. Viral latency may be occasionally interrupted, leading to reactivation and spread of infectious virus with or without recurrent disease. EBV establishes latency in B lymphocytes in association with expression of a limited set of viral genes. Immune control of HSV infection and replication occurs at the level of skin or mucosa during initial or recurrent infection and in the dorsal root ganglion, where latency and reactivation are controlled by immune mechanisms mediated by interferons, myeloid and plasmacytoid dendritic cells, CD4(+) and CD8(+) T cells, and other cytokines. Despite similarities, the molecular details and mechanisms of latency and reactivation vary considerably among the herpesviruses. Mechanisms responsible for maintenance of latency are unclear.

Reactivation of CMV has been extensively studied. CMV viral genomes can be found in CD14+ monocytes and CD34+ progenitor cells, although the primary reservoir for latent CMV and the mechanisms by which latency is maintained are unknown. Allogeneic immune responses

and fever (via tumor necrosis factor-α (TNF-α)) have been shown *in vitro* to increase both CMV promoter activity and viral replication. Immune suppression is not essential for the reactivation of latent CMV, but serves to perpetuate such infections once activated. Subclinical activation of CMV is common and increasing diagnosed by sensitive molecular assays.

For other viruses such as BK polyomavirus, specific types of tissue damage such as warm ischemia and reperfusion injury may precipitate viral activation; they have been linked to an inflammatory state in grafts (via activation of TNF-α, nuclear factor kappa B (NF-κB), neutrophil infiltration, and nitric oxide synthesis), tubular-cell injury, and enhanced expression of cell-surface molecules, all of which may contribute to viral activation. Thus, immune injury, inflammatory cytokines, and ischemia-reperfusion injury stimulate viral replication and change expression of virus-specific cell-surface receptors. The hosts' direct pathway antiviral cellular immune response within allografts is less effective due to mismatched major histocompatibility antigens between the organ donor and host with dependence on indirect pathways of antigen presentation. These factors may render the allograft more susceptible to viral infection.

Common reactivation infections after transplantation include CMV, HBV, HCV, HIV, HSV-1 and HSV-2, HPV, and VZV (as zoster). Other less clinically common viral infections related to reactivation include the polyoma viruses BK and JC, human herpes virus 6 (HHV-6), human herpes virus 7 (HHV-7), and HHV-8. Reactivation of one virus may lead to reactivation of others; multiple studies have shown that infection with HHV-6 and/or HHV-7 are risk factors for CMV disease and CMV infection may trigger HHV-6 and HHV-7 reactivation. While some reactivation infections routinely cause significant clinical disease, such as CMV, HSV, and VZV, others may cause more variable illness. HHV-6, for example, commonly reactivates with immunosuppression, especially after HSCT, often with clinically significant infection. By contrast, the role of both HHV-6 and HHV-7 in SOT recipients is less well defined; while reactivation is common, clinical disease is generally not evident.

New Infections

Based on epidemiologic exposures, new infections from the environment are commonly acquired after transplantation. The respiratory viruses are the most common new infections after transplantation, including RSV, influenza, parainfluenza, and adenovirus. New respiratory pathogens (metapneumovirus and SARS coronavirus) also cause major infections in immunocompromised hosts. Gastrointestinal viruses such as rotavirus or Norwalk virus are common and can cause significant diarrhea and dehydration; diarrheal syndromes may alter absorbance of calcineurin inhibitors (e.g., cyclosporine and tacrolimus), with unexpectedly elevated levels of tacrolimus. Nonimmune patients can acquire primary EBV, CMV, VZV, parvovirus B19, and other infections in the post-transplantation period. In the absence of previous immunity and with the attenuation of immunity due to the immunosuppressive regimen, new infections are often more severe and prolonged than in the general population. For example, parvovirus B19 infection is often more persistent and relapsing in transplantation patients, occasionally complicated by the unusual findings of hepatitis, myocarditis, pneumonitis, glomerulopathy, arthritis, or transplantation graft dysfunction.

'Direct Effects' and 'Indirect Effects' of Viral Infection

The effects of viral infection are conceptualized as 'direct' and 'indirect' (see **Table 2**). This classification serves to separate the tissue-invasive viral infection (cellular and tissue injury) from effects mediated by inflammatory responses (e.g., cytokines) or by alterations in host immune responses. Syndromes such as fever and neutropenia (e.g., with CMV infection) or invasive disease resulting in pneumonia, enteritis, meningitis, and encephalitis are considered direct effects. Indirect effects of viral infections are generally thought to be immunomodulatory responses to viral infections mediated by cytokines, chemokines, and/or growth factors. The impact of these

Table 2 Direct and indirect effects of CMV infection

Direct effects of CMV infection	*Indirect effects of CMV infection*
Fever and neutropenia syndrome (leukopenia, fever, myalgia, fatigue, thrombocytopenia, hepatitis, nephritis)	Increases risk of secondary infection by bacteria, fungi, and viruses
Myelosuppression	Increases risk of graft rejection
Pneumonia	Increases risk of PTLD
Gastrointestinal invasion with colitis, gastritis, ulcers, bleeding, or perforation	May increase risk of HHV-6 and HHV-7 infection
Hepatitis	
Pancreatitis	
Chorioretinitis	

effects is diverse and includes systemic immune suppression predisposing to other opportunistic infections (notably with CMV or HCV infections). In addition, viral infection may alter the expression of cell-surface antigens (e.g., major histocompatibility antigens) provoking graft rejection and/or cause disregulated cellular proliferation (contributing to atherogenesis in cardiac allografts, obliterative bronchiolitis in lung transplantation, or to oncogenesis). Increased viral replication and persistence may contribute to allograft injury (fibrosis) or chronic rejection. Infection with one virus may stimulate replication of other viruses in a form of viral 'cross talk'. As was noted above, infection with HHV-6 and/or HHV-7 serve as risk factors for CMV disease and vice versa. The direct and indirect effects of HHV-6 reactivation can be significant: HHV-6 infection is associated with high levels of IL-6 and TNF-α, and in pediatric renal or bone marrow transplantation, HHV-6 reactivation is strongly associated with acute rejection. Co-infection with HCV and CMV predicts an accelerated course for hepatitis. Co-infection with CMV and EBV increases the risk for post-transplantation lymphoproliferative disorders (PTLD) by 12–20-fold. A more theoretical concern is that T-cell responses against viral infections are thought to produce cross-reacting immune responses against graft antigens, possibly via 'alternative recognition' within the T-cell receptor. This cross-reactivity is termed 'heterologous immunity' and may provoke abrogation of graft tolerance.

Virus Specific Syndromes

The HHV family has eight human members, all of which can cause significant disease in transplantation recipients. The risk of many of these infections is reduced by the use of valganciclovir or acyclovir after transplantation. HSV-1 and -2 (HHV-1 and -2) usually cause oral and genital ulceration, although it may occur in more unexpected areas as well. Recurrent disease can be problematic for some patients and warrants consideration of secondary prophylaxis with antiviral therapy. VZV (HHV-3) is common with an incidence of herpes zoster among 869 patients after SOT of 8.6% (liver 5.7%, renal 7.4%, lung 15.1%, and heart 16.8%), with a median time of onset 9.0 months, as reported by Razonable *et al.* in 2005. After allogeneic HSCT, in one study by Koc *et al.* 41 of 100 (41%) developed VZV reactivation a median of 227 days (range 45–346 days) post-transplantation. Both primary and disseminated VZV infection can be lethal in these populations. Nonimmune transplantation patients should be monitored carefully after exposure to clinical varicella and the use of antiviral therapy or varicella immunoglobulin for prophylaxis should be considered. EBV (HHV-4) can cause febrile systemic illness, lymphocytosis, leukopenia, hepatitis, and mediates post-transplantation lymphoproliferative disease (PTLD). PTLD constitutes a spectrum of disease, which is often responsive to reduced immunosuppression in previously immune hosts. EBV-seronegative individuals with primary infection after transplantation are at increased risk for EBV-mediated PTLD. The clinical presentation of CMV (HHV-5) can range from a 'CMV syndrome' including fever, malaise, leukopenia, to a 'flu-like' illness with myalgias and fatigue, to a more significant end-organ disease with pneumonitis, colitis, encephalitis, hepatitis, or chorioretinitis. CMV is the single most important pathogen in transplantation recipients due to direct and indirect effects (see above).

HHV-6 commonly reactivates after transplantation, especially after HSCT where it is associated with hepatitis, pneumonitis, CMV reactivation, bone marrow suppression, and encephalitis. HHV6 causes less symptomatic clinical infection after SOT, although the indirect effects of reactivation have not been studied. HHV-7 commonly reactivates after transplantation. The clinical symptoms caused by HHV-7 are uncertain, although neurological symptoms seem to be significant, especially in children. HHV-8 causes Kaposi's sarcoma and is seen in SOT recipients at a rate 500–1000 higher than the general population, with a prevalence of 0.5–5% depending on the patient's (and donor's) country of origin.

Hepatitis B and C are among the most common indications for liver transplantation, and can complicate other transplantations as well. Hepatitis C is currently the most common indication for liver transplantation, accounting for 40–45% of cases in recent times. Recurrent post-transplantation hepatitis C infection poses a conundrum between treating the hepatitis C and reducing immunosuppression without precipitating rejection. Given the risk of precipitating graft dysfunction, hepatitis C treatment with interferon and ribavirin is often deferred in extrahepatic transplant recipients. For hepatitis B, the goal is complete viral suppression before and after transplantation, using hepatitis B immunoglobulin as well as antiviral agents with lower-dose immunosuppression. At this time, it is unclear which antivirals and immunosuppressive regimens are optimal for this population. Liver transplantation for HBV with combination viral prophylaxis and hepatitis B immunoglobulin results in survival rates equivalent to other indications for liver transplantation.

Respiratory viruses are the most common community-acquired infections in transplantation recipients. Given the increased rates of pneumonia and bacterial and fungal superinfection, prevention (vaccination, avoidance of sick individuals) is essential. Diagnosis of respiratory viruses within a few hours via enzyme-linked immunosorbent assay (ELISA) or immunofluorescent staining is available in most medical centers. Viral cultures are time consuming and expensive. Respiratory syncytial virus and parainfluenza are the most common community-acquired

respiratory viruses, followed by influenza and adenovirus. Antiviral medications (rimantidine, amantidine, or oseltamivir) may prevent or reduce the severity of illness. The use of ribavirin or RSV immune globulin in adults to prevent RSV infection is unproven. Ribavirin is commonly used for documented RSV infections of the lower respiratory tract.

Metapneumovirus and severe acute respiratory syndrome-associated coronavirus (SARS-CoV) are emerging pathogens in transplantation patients. The clinical spectrum of disease from metapneumovirus ranges from symptomatic (even fatal) to asymptomatic cases. Some groups have suggested a possible correlation with graft rejection in lung transplant recipients. Diagnosis is often made using molecular assays; the full impact of this infection in transplantation is yet to be realized. SARS, caused by a zoonotic coronaviruses, is a highly contagious and rapidly progressive form of viral pneumonia, which spread from Asia to many parts of the world in early 2003. A number of transplantation patients were infected, some of whom died. The impact of SARS and resulting infection control issues was significant for both active organ transplantation (i.e., concerns about transmitting donor-derived infections) as well as routine follow-up care for transplantation patients, some of who deferred healthcare visits.

Gastrointestinal viruses such as rotavirus or Norwalk virus may cause significant diarrhea and dehydration. Enteroviral infections in the summer months in the northern hemispheres are common and can have a more complicated and prolonged course in renal transplantation recipients.

BK virus is associated with a range of clinical syndromes in immunocompromised hosts: viruria and viremia, ureteral ulceration and stenosis, and hemorrhagic cystitis. The majority of patients with BK virus infections are asymptomatic. Infection by JC polyomavirus has been observed in renal allograft recipients as both nephropathy (in association with BK virus or alone) and/or progressive multifocal encephalopathy (PML). JCV establishes renal latency but receptors are present in multiple tissues including the brain. Infection of the central nervous system generally presents with focal neurologic deficits or seizures and may progress to death following extensive demyelination.

Human papilloma virus (HPV) infections can cause significant disease in renal transplantation recipients, including oral, skin, genital, and rectal lesions ranging from warts and dysplasia to malignancy (especially squamous cell carcinoma). The recent arrival of a vaccine for genital HPV infections may help reduce these infections.

In transplantation recipients, parvovirus B19 infection can cause erythropoietin-resistant anemia, pancytopenia, myocarditis, or pneumonitis. Direct renal involvement with glomerulopathy and allograft dysfunction has been reported in renal transplantation recipients. Clinical and virologic responses to treatment with intravenous immunoglobulin are usually excellent.

Several zoonotic viruses have caused major illness and death in the transplantation setting, including WNV, rabies, and lymphocytic choriomeningitis virus (LCMV). All have been recently reported as donor-derived infections related to SOT, with clinically subtle infections in the donor and often deadly infections in the recipients. WNV is more morbid after SOT; the risk of meningoencephalitis in a SOT patient infected with WNV has been estimated to be 40%, compared with <1% in normal hosts. Donors in endemic areas should be screened for WNV, as the prevalence can be high. Aside from donor-derived infections, rabies and LCMV have not been reported.

Although HIV-infected patients are living longer and dying less often from complications related to acquired immunodeficiency syndrome (AIDS), they are experiencing significant morbidity and mortality related to end-stage liver and renal disease. Preliminary studies suggest that both patient and graft survival are similar in HIV-negative and HIV-positive kidney and liver transplantation recipients. However, HCV infection appears to be accelerated even in controlled HIV infection. Ongoing multicenter trials of transplantation in HIV infections are continuing. Drug interactions between the immunosuppressive regimen and antiretroviral drugs necessitate careful monitoring. The profound and long-lasting suppression of the CD4+ T-cell count in patients who receive thymoglobulin induction therapy has been associated with an increased risk of infections requiring hospitalization.

Diagnostic Assays

Rapid and sensitive molecular biology-based assays for many of the common viruses after transplantation have replaced, for the most part, serologic testing and *in vitro* cultures for the diagnosis of infection. Serologic assays are generally less sensitive in transplant patients, as humoral immune responses may be delayed or absent. In one series, 29% of patients with parvovirus B19 infection as shown by PCR assay had a negative IgM assay. Quantitative molecular tests allow the optimization and individualization of antiviral therapies for prevention and treatment of infection. This advance is most significant in the management of CMV, EBV, hepatitis B, and hepatitis C viruses, where quantitative assays (such as viral loads or antigenemia tests) guide antiviral therapy. Nonquantitative (i.e., qualitative) assays are less useful in management as they do not assess responses to therapy and cannot differentiate primary infection, from reactivation or reinfection. For example, chromosomal integration of latent HHV-6 DNA (which happens in 1–3% of immunocompetent subjects) leads to high levels of viral DNA, whether or not the infection is active; one group concludes that any diagnosis of HHV-6 encephalitis should not be made without first excluding chromosomal HHV-6 integration by measuring

DNA load in CSF, serum and/or whole blood. Latent infections due to EBV and CMV may be qualitatively positive by PCR, confirming the need for quantitative assays.

Blood tests may not always accurately reflect the level of end-organ diseases; thus, it may be useful to test specific affected tissues as well the blood. In this regard, histologic evaluation of tissues using pathogen-specific immunohistochemistry may augment systemic assays. Patients with CMV colitis, for example, may have negative molecular or antigenemia blood assays for CMV. In addition, patients may shed CMV in secretions without true infection, limiting the diagnostic capacity of a positive culture. BK polyomavirus may be detectable in the urine (either by cytology, looking for the classic decoy cells, or by PCR) before it is detectable in the blood, providing a window of opportunity for reducing immunosuppression possibly in advance of invasive disease. Adenovirus may be detectable in local infections, such as cystitis, with negative blood assays.

Therapy

The treatment of viral infections in the renal transplantation recipient includes: the reduction of immunosuppression, antiviral therapy, diagnosis and treatment of co-infections (such as CMV, EBV, HHV-6, or -7), and use of adjunctive therapies such as immunoglobulins or colony stimulating factors. The overall level of immunosuppression has a major impact on both the risk of reactivation of latent infection and the ability to clear such an infection. Reducing the immunosuppressive regimen during active viral infection can be a major contribution toward clearing infection, although it presents a risk of graft rejection. As protective cytotoxic immunity to viruses is generally T-cell (CD8+) mediated, an initial reduction of antimetabolites (if neutropenic) and calcineurin inhibitors merits consideration. In contrast, a reduction of the steroid dose during the acute phase of a febrile illness may cause acute adrenal insufficiency.

When available, antiviral therapies (such as acyclovir, ganciclovir, ribavirin, lamivudine, and oseltamivir) are often essential. The toxicity of some agents (such as cidofovir, foscarnet, and ribavirin) may complicate management, notably in the face of reduced renal function in patients receiving calcineurin inhibitors as part of the immunosuppressive regimen. The duration of therapy is often longer in transplant patients than in normal hosts, and often reflects the ability to reduce the overall level of immunosuppression, that is. less immunosuppression may result in a shorter treatment time. The increased information provided by molecular diagnostics may allow for more directed treatment regimens.

Antiviral therapy is often used for prophylaxis in the post-transplantation period, especially for individuals at risk for primary infection. In general, acyclovir and related agents are used to prevent HSV and VZV and ganciclovir or valganciclovir to prevent CMV as well as HSV and VZV. The relative advantages of universal prophylaxis (i.e., the use of antiviral medication in all susceptible patients for a period after transplantation) or monitoring with preemptive (early) therapy remain to be established. Meta-analyses suggest that universal prophylaxis with antiviral medications in SOT recipients reduces CMV disease and CMV-associated opportunistic infections, graft rejection, and mortality; their use is recommended for high risk individuals (CMV-positive recipients and in CMV-negative recipients of CMV-positive organs). Some studies indicate that universal prophylaxis and preemptive therapy are effective in reducing the incidence of CMV disease. In the HSCT setting, where the risks of bone marrow toxicity from valganciclovir are higher, many programs choose to use preemptive monitoring and therapy for CMV.

Various adjunctive therapies have been helpful in treating and preventing viral infections. Immunoglobulins (i.e., intravenous immune globulins (IVIG), CMV, and HBV hyper-immune globulins (both prepared from plasma preselected for high titer antibodies to CMV and HBV, respectively), as well as monoclonal antibodies such as those for RSV) have been helpful in preventing and treating viral infections, likely due to both direct and indirect immunomodulatory properties. A significant percent of patients have post-transplantation hypogammaglobulinemia, which has been linked to increased mortality and may benefit from globulin repletion. This may be most apparent in the setting of active infections, as well as prophylaxis. Immunostimulatory agents, such as interferon-alpha used to treat hepatitis C, can be helpful at treating the viral infection but may perturb the relationship between the graft and the host, precipitating rejection. Reversal of neutropenia can be done using colony stimulating factors such as G-CSF, which is generally well tolerated in sold organ transplantation patients.

Vaccination

Vaccines should be given to patients as early as possible in the course of organ failure and well in advance of transplantation to optimize immune responses. In general, a response to vaccination is more likely to occur when given pre-transplantation rather than after transplantation. Nonlive viral vaccines such as hepatitis B, hepatitis A, injectable influenza, rabies, HPV, injectable polio may be given both pre- and post-transplantation (see **Table 3** for classifications). Live viral vaccines such as attenuated influenza (delivered by nasal spray), measles, mumps, rubella, varicella (both Varivax, for protection against varicella in nonimmune subjects, as well as Zostavax, for protection against zoster), yellow fever, oral polio, and

Table 3 Viral vaccines, classified by type of vaccine

Virus	*Live attenuated*[a]	*Killed*	*Subunit/protein*
Measles	x		
Mumps	x		
Rubella	x		
Varicella-zoster (both for varicella and zoster)	x		
Yellow fever	x		
Smallpox	x		
Influenza	x (intranasal)	x (injectable)	x (injectable)
Japanese encephalitis	x	x	
Poliovirus	x (oral)	x (injectable)	
Hepatitis A virus		x	
Rabies virus		x	
Hepatitis B			x
Human papilloma virus			x

[a]Live-attenuated vaccines are generally contraindicated in immunocompromised hosts.

vaccinia (smallpox) should generally be avoided after SOT and in patients who are chronically immunosuppressed (such as those with GVHD); some may be given after HSCT. Consideration of future travel or trips home should also be considered in the pre-transplantation period. Vaccines for CMV, EBV, RSV, and other viral pathogens are under further investigation.

See also: Transmissible Spongiform Encephalopathies.

Further Reading

Anonymous (2004) Guidelines for vaccination of solid organ transplant candidates and recipients. *American Journal of Transplantion* 4(supplement 10): 160–163.

Cervera C, Marcos MA, Linares L, *et al.* (2006) A prospective survey of human herpesvirus-6 primary infection in solid organ transplant recipients. *Transplantation* 82: 979–982.

Eid AJ, Brown RA, Patel R, and Razonable RR (2006) Parvovirus B19 infection after transplantation: A review of 98 cases. *Clinical Infectious Disease* 43: 40–48.

Fischer SA, Graham MB, Kuehnert MJ, *et al.* (2006) Transmission of lymphocytic choriomeningitis virus by organ transplantation. *New England Journal of Medicine* 354: 2235–2249.

Fishman JA and Rubin RH (1998) Infection in organ-transplant recipients. *New England Journal of Medicine* 338: 1741–1751.

Hirsch HH, Knowles W, Dickenmann M, *et al.* (2002) Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. *New England Journal of Medicine* 347: 488–496.

Hodson EM, Barclay PG, Craig JC, *et al.* (2005) Antiviral medications for preventing cytomegalovirus disease in solid organ transplant recipients. *Cochrane Database of Systematic Reviews* CD003774.

Kotton CN (2007) Zoonoses in solid-organ and hematopoietic stem cell transplant recipients. *Clinical Infectious Disease* 44: 857–866.

Kotton CN and Fishman JA (2005) Viral infection in the renal transplant recipient. *Journal of the American Society of Nephrology* 16: 1758–1774.

Kotton CN, Ryan ET, and Fishman JA (2005) Prevention of infection in adult travelers after solid organ transplantation. *American Journal of Transplantion* 5: 8–14.

Kumar D, Drebot MA, Wong SJ, *et al.* (2004) A seroprevalence study of West Nile virus infection in solid organ transplant recipients. *American Journal of Transplantion* 4: 1883–1888.

Ljungman P, Engelhard D, de la Camara R, *et al.* (2005) Vaccination of stem cell transplant recipients: Recommendations of the Infectious Diseases Working Party of the EBMT. *Bone Marrow Transplantion* 35: 737–746.

Mylonakis E, Goes N, Rubin RH, Cosimi AB, Colvin RB, and Fishman JA (2001) BK virus in solid organ transplant recipients: An emerging syndrome. *Transplantation* 72: 1587–1592.

Small LN, Lau J, and Snydman DR (2006) Preventing post-organ transplantation cytomegalovirus disease with ganciclovir: A meta-analysis comparing prophylactic and preemptive therapies. *Clinical Infectious Disease* 43: 869–880.

Ward KN, Leong HN, Thiruchelvam AD, Atkinson CE, and Clark DA (2007) HHV-6 DNA level in CSF due to primary infection differs from that in chromosomal viral integration and has implications for the diagnosis of encephalitis. *Journal of Clinical Microbiology* 45: 1298–1304.

Origin of Viruses

P Forterre, Institut Pasteur, Paris, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Archaea A domain of prokaryotic microorganisms whose informational mechanisms (DNA replication, transcription, and translation) are closely related to those of eukaryotes.

Homology Two biological structures are homologs if they originated from a common ancestral structure.

The homology cannot be quantified (a protein is or is not homologous to another one). Many biologists without an evolutionary background still confuse homology and similarity (only the latter can be quantified).

LUCA (Last Universal Common Ancestor) The most recent common ancestor shared by all modern cellular organisms. The modern genetic code was already established in LUCA. Other features of LUCA (cellular vs. acellular, RNA vs. DNA genome) are still highly controversial.

RNA world The period of life evolution before the appearance of DNA. Depending on the authors, the RNA world is viewed as either cellular (a world of RNA cells) or acellular (a world of free-living macromolecules).

Universal tree of life The tree based on 16S/18S rRNA comparison, in which the cellular living world is divided into three domains: Archaea, Bacteria, and Eukarya. The evolutionary relationships between these domains are controversial.

The Classical View of Virus Origin and Its Consequences

The origin of viruses and their evolutionary relationships with cellular organisms are still enigmatic, but recent advances from comparative genomics and structural biology have produced a new framework to discuss these issues on firmer grounds. Historically, three hypotheses have been proposed to explain the origin of viruses: (1) they originated in a precellular world ('the virus-first hypothesis'); (2) they originated by reductive evolution from parasitic cells ('the reduction hypothesis'); and (3) they originated from fragments of cellular genetic material that escaped from cell control ('the escape hypothesis'). All these hypotheses had specific drawbacks. The virus-first hypothesis was usually rejected firsthand, since all known viruses require a cellular host. The reduction hypothesis was difficult to reconcile with the observation that the most reduced cellular parasites in the three domains of life, such as Mycoplasma in Bacteria, Microsporidia in Eukarya, or Nanoarchaea in Archaea, do not look like intermediate forms between viruses and cells. Finally, the escape hypothesis failed to explain how such elaborate structures as complex capsids and nucleic acid injection mechanisms evolved from cellular structures, since we do not know any cellular homologs of these crucial viral components.

Because of these drawbacks, the problem of virus origin was for a long time considered untractable and not worth of serious consideration (similarly, the study of bacterial evolution was considered a hopeless and futile task prior to the pioneering work of Carl Woese). However, since the problem of the origin is so entrenched in the human mind, it was never completely ignored. Much like the concept of prokaryotes became the paradigm on how to think about bacterial evolution, the escape hypothesis became the paradigm favored by most virologists to solve the problem of virus origin. This scenario was chosen mainly because it was apparently supported by the observation that modern viruses can pick up genes from their hosts. In its classical version, the escape theory suggested that bacteriophages originated from bacterial genomes and eukaryotic viruses from eukaryotic genomes (**Figure 1(a)**). This led to a damaging division of the virologist community into those studying bacteriophages and those studying eukaryotic viruses, 'phages' and viruses being somehow considered to be completely different entities. The artificial division of the viral world between 'viruses' and bacteriophages also led to much confusion on the nature of archaeal viruses. Indeed, although most of them are completely unrelated to bacterial viruses, they are often called 'bacteriophages', since archaea (formerly archaebacteria) are still considered by some biologists as 'strange bacteria'. For instance, archaeal viruses are grouped with bacteriophages in the drawing that illustrates viral diversity in the last edition of the *Virus Taxonomy Handbook*. Hopefully, these outdated visions will finally succumb to the accumulating evidence from molecular analyses.

Viruses Are Not Derived from Modern Cells

Abundant data are now already available to discredit the escape hypothesis in its classical adaptation of the prokaryote/eukaryote paradigm. This hypothesis indeed predicts that proteins encoded by bacterial viruses (avoiding the term bacteriophage here) should be evolutionarily related to bacterial proteins, whereas proteins encoded by viruses infecting eukaryotes should be related to eukaryotic proteins. This turned out to be wrong since, with a few exceptions (that can be identified as recent transfers from their hosts), most viral encoded proteins have either no homologs in any cell or only distantly related homologs. In the latter cases, the most closely related cellular homolog is rarely from the host and can even be from cells of a domain different from the host. More and more biologists are thus now fully aware that viruses form a world of their own, and that it is futile to speculate on their origin in the framework of the old prokaryote/eukaryote dichotomy. As for all other aspects of microbiology, the problem of the nature and origin of viruses made a great leap forward when this dichotomy was successfully challenged by the trinity concept introduced

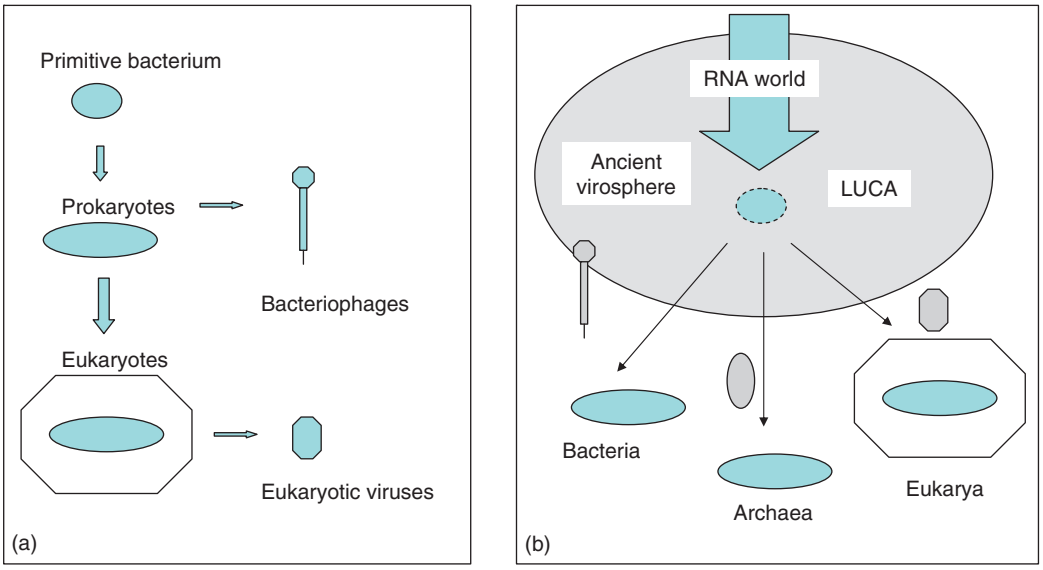


Figure 1 Two conflicting views of virus origin and evolution.

by Carl Woese, that is, the division of the living cellular world into three domains, Archaea, Bacteria, and Eukarya.

The building of a universal tree of life based on rRNA sequence comparisons, and the idea that all living organisms did not diverge from a 'primitive bacterium', as previously assumed in most texbooks, but from a less-defined last universal common ancestor (LUCA) opened the way to think about the origin and evolution of viruses in a new context (**Figure 1(b)**). Indeed, the main problem with the initial formulation of the three major hypotheses for the origin of viruses was that all of them were based on our knowledge of modern cells (themselves viewed as either prokaryotes or eukaryotes). Hence, modern viruses need modern cells to replicate, modern cells cannot regress to viral forms, and free RNA or DNA does not recruit today's proteins from modern cells to form capsids and other elaborated viral structures. The major innovation introduced by the work of Carl Woese was to open a window on the possibility of ancient worlds populated by cells most likely very different from modern ones. As early as 1980, Woese and co-workers coined the term urkaryote to name the cellular lineage that gave rise to modern eukaryotic cells (prior to the mitochondrial endosymbiosis and possibly before the origin of the nucleus itself). Later on, Woese discussed in detail the fact that the organisms that populated the basal branches of the universal tree were probably not yet modern cells, and suggested that some proteins with puzzling phylogenetic patterns could have originated in ancestral cell lineages that have now disappeared. These theoretical considerations laid the ground to the interpretation of observations that could not have been understood in the classical linear and dichotomic view of cellular evolution, such as the discovery that viruses infecting either prokaryotic or eukaryotic hosts could share homologous features (see below). In the new framework introduced by Woese's universal tree of life, these observations led to the idea that viruses might be relics of lost domains, or else that viruses predated LUCA. As a result,viruses now appear in the literature as additional branches in the universal tree or the universal tree itself is immersed in a 'viral ocean'.

The metaphor of the viral ocean, proposed by Dennis Bamford, illustrates both the concept of virus antiquity and their predominance in the modern biosphere. Another major breakthrough in recent viral research was indeed the realization that viruses are much more abundant than cells and much more diverse than previously suspected. It is thus currently assumed that viral genomes represent the major throve of genetic diversity on Earth. All these trends make it irrelevant to ask whether viruses are alive. As recently pointed out by Jean-Michel Claverie, the question of the nature of viruses has been for a long time obscured by the confusion between virus and virus particle, whereas the major components of the virus life cycle correspond to the intracellular viral factory. All this concurs to put again the question of virus origin on the agenda. Thus a brief summary of the main data that presently point to an ancient origin of viruses and the discussion of how the three major hypotheses explaining the origin of viruses have been rejuvenated in this new framework are presented here.

Viruses Are Ancient

For a long time, virologists thought that the various virus families were evolutionary unrelated, indicating a polyphyletic origin. In recent years, this view has

progressively changed with the identification of more and more relationships (sometimes totally unexpected) between different viral lineages, making it possible to define a limited number of large viral groups whose hosts encompass the three cellular domains. For example, it has been clearly established that some double-stranded RNA viruses infecting bacteria are homologous to those infecting eukarya, suggesting that they predated the divergence between bacteria and eukaryotes. Since the RNA replicases/transcriptases of these double-stranded RNA viruses are homologous to those of single-stranded RNA viruses, all RNA viruses presently known seem to be evolutionary related (at least in term of their replication apparatus). In the case of DNA viruses, structural analyses of their capsid proteins have revealed an unexpected close evolutionary relationship between some bacterial spherical viruses, eukaryotic viruses of the nucleocytoplasmic-large-DNA viruses (NCLDV) superfamily, and an archaeal virus isolated at Yellowstone, USA. Structural comparative analyses also indicate that head and tailed bacterial viruses (*Caudavirales*) share homologous features with herpes viruses. These data clearly indicate that some viral specific proteins originated before the divergence between the three domains (hence before LUCA). It is tempting to suggest that a similar ancient origin also explains why most viral proteins either have no cellular homologs (except for plasmid versions or viral remnants in cellular genomes) or are only very distantly related to their cellular homologs. All these considerations thus push back the origin of viruses before the emergence of modern cells. We will now discuss how the three classical hypotheses for the origin of viruses have been revisited in this new context.

The Virus-First Hypothesis

The virus-first hypothesis was for a long time politically incorrect. It clashes with the cellular theory of life and the traditional assumption that viruses are nonliving entities. This hypothesis was first revived in the 1980s by Wolfram Zillig, who suggested that viruses originated in a prebiotic word, using the 'primitive soup' as a host. Such hypothesis has gained strength in recent years, in parallel with the suggestion that cellular organisms originated only at a late stage of life evolution. The idea that 'life' first evolved in an acellular context can be traced back to the first version of the RNA world theory. More recently, it was boosted by the discovery that archaeal lipids are dramatically different from the bacterial ones (with an opposite stereochemistry of the glycerol backbone linkages and a different type of carbon chains). To explain this dichotomy, several authors have proposed that LUCA was not a cellular entity and that cellular membranes originated independently after the divergence of Archaea and Bacteria. A more elaborate version of this scenario has been proposed by William Martin and Eugene Koonin, who suggested that life originated and evolved in the cell-like mineral compartments of a warm hydrothermal chimney. In that model, viruses emerged from the assemblage of self-replicating elements using these inorganic compartments as the first hosts. The formation of true cells occurred twice independently only at the end of the process (and at the top of the chimney), producing the first archaea and bacteria. The latter escaped from the same chimney system as already fully elaborated modern cells. In the model, viruses first co-evolved with acellular machineries producing nucleotide precursors and proteins (**Figure 2(a)**). This acellular 'life' evolved by competition between different machineries and associated viruses to infect more and more compartments of the hydrothermal system.

Cellular versus Acellular Evolution of Early Life

The acellular model of early life evolution (up to LUCA) raises several problems. First, comparative genomics analyses indicate that some membrane proteins (ATP synthetases, signal recognition particle receptors) are homologous and ubiquitous in the three domains of life, hence were probably already present in LUCA. Some authors have further stressed that the emergence of the RNA world involves at least the existence of complex mechanisms to produce ATP, RNA, and proteins. This means an elaborated metabolism to produce ribonucleotide triphosphate (rNTP) and amino acids, RNA polymerases and ribosomes, as well as an ATP-generating system. If such a complex metabolism was present, it appears unlikely that it was unable to produce lipid precursors, hence membranes. If this is correct, then 'modern' viruses did not predate cells, but originated in a world populated by primitive cells. The proponents of this scenario consider that it fits better with the contention that Darwinian selection requires competition between well-defined individual entities. It has been often assumed that RNA viruses are relics of the RNA world. In that case, viruses might have originated in a world of primitive cells with RNA genomes. In that context, it is even possible that cellularization occurred before the emergence of the modern protein synthesizing machinery and that RNA cells existed that contained no proteins (at least no proteins produced by an RNA machinery related to modern ribosomes). Modern viroids may be relics of this stage, whereas true viruses might have only appeared after the establishment of the ribosome-based mechanism for protein synthesis. In such a cellular scenario, now one has to explain how RNA viruses originated from RNA cells. Interestingly, this has led to a revival of the reduction and escape hypothesis, but in a new context.

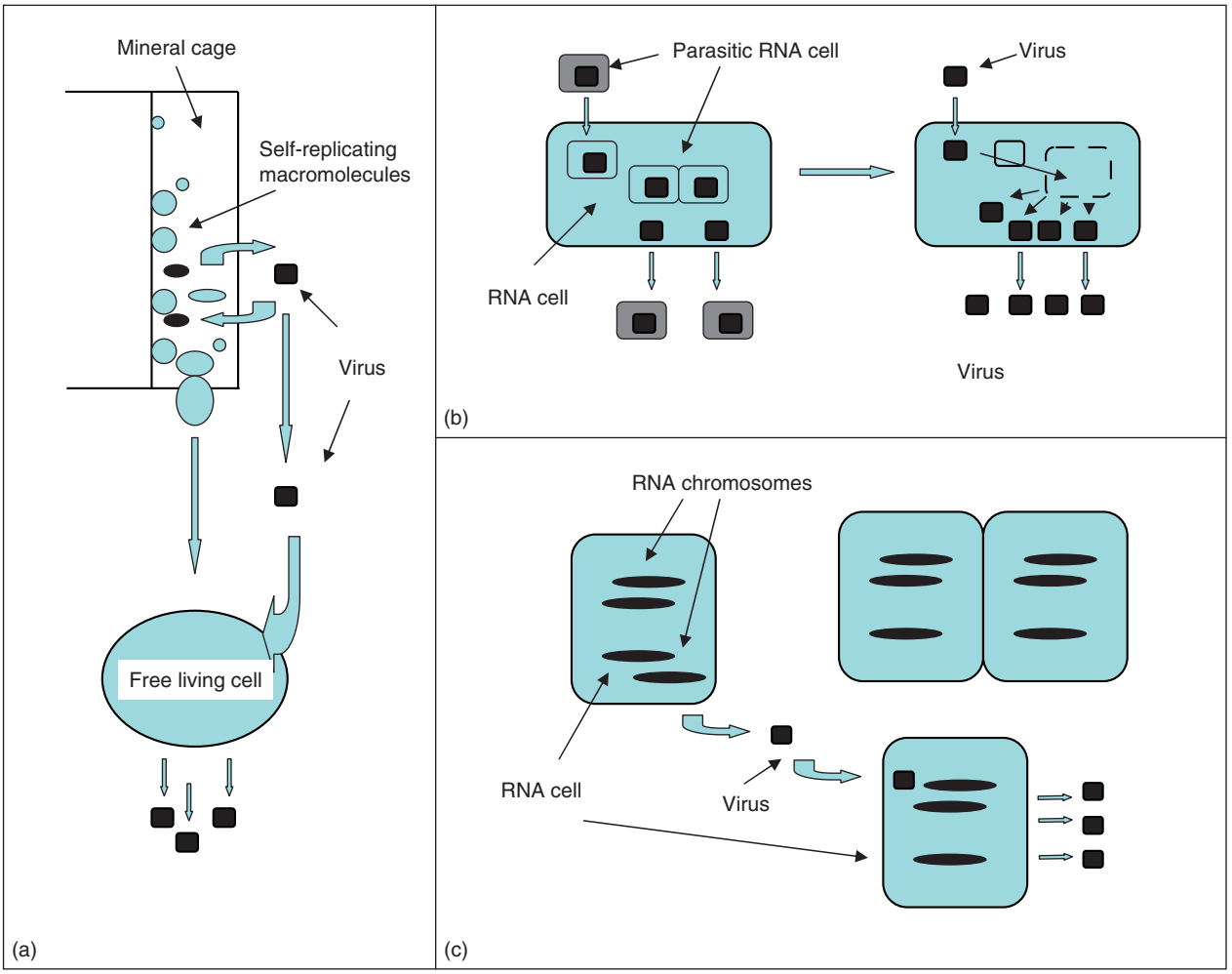


Figure 2 The three revisited hypotheses for virus evolution: (a) the virus-first hypothesis; (b) the reduction hypothesis; (c) the escape hypothesis.

The Reduction Hypothesis

The reduction hypothesis revisited in the context of pre-LUCA cells posits that RNA viruses originated by reduction from parasitic RNA cells, by losing progressively their own machinery for protein synthesis and for energy production (**Figure 2(b)**). An analogy for the possible mechanism of reduction can be seen in the reductive evolution that led to modern *Chlamydia*. Indeed, an interesting parallel can be drawn between the viral particles (the virion) and the infectious form of this bacterium (the elementary body) that is small and metabolically inactive. The main difference between viruses and *Chlamydia* is that the intracellular form of the latter, called the 'reticulate body', is a fully developed intracellular bacterium that uses its own ribosomes for protein synthesis, whereas the intracellular viral factories (although often physically separated from the host cytoplasm) have somehow a direct access to the host ribosomes. A first step in the evolution of a parasitic RNA cell toward a viral state might thus have been the division of its cell cycle between an inert extracellular stage (the protovirion) and an intracellular stage. The second step would have been the dissolution of the membrane of the intracellular parasitic cell to gain access to the protein machinery of the host. One can argue that the transformation of an intracellular parasitic cell into a viral-type factory became impossible with modern cells (such as *Chlamydia*) because, as stated by Carl Woese, the latter are too complex and too integrated to be 'deconstructed' into free-living subentities. In contrast, small parasitic RNA cells reproducing inside larger RNA cells were probably much simpler and could have been easily reduced into a viral factory by loss of their own membrane and translation machinery.

The Escape Hypothesis

The escape hypothesis is also easier to defend in the context of an ancestral world of RNA cells. It has been often argued that the genomes of ancestral RNA cells may have been fragmented and composed of semiautonomous chromosomes that were replicated independently

and transferred randomly from cell to cell. The coupling between the segregation of the cellular genome and of the cellular machinery for protein synthesis (the genotype and the phenotype) was probably not so efficient in these RNA cells than it is in modern cells. The reproduction of such primitive RNA cells could have produced a mixture of progenies, some of them containing both systems (chromosomes plus ribosomes) but others containing only either RNA chromosomes or ribosomes. The latter two types of progenies would have died, except if a cell containing only chromosomes turned out to be able to infect a complete cell (or a cell containing only ribosomes). The RNA chromosomes carrying genes facilitating specifically their infectious ability and/or protecting their integrity during their resting stages would have been selected in such a situation (**Figure 2(c)**).

Relationships between RNA and DNA Viruses

If the first viruses were RNA viruses infecting RNA cells, one is left with a major question; did DNA viruses originate independently from RNA viruses or did they evolve from RNA viruses? One possibility is that DNA viruses originated either by escape or reduction from primitive DNA cells, much like RNA viruses could have originated from RNA cells. Such hypothesis supposes the existence of primitive DNA cells (less integrated than modern ones) that lived either before LUCA (if the latter was already a cellular organism) or shortly after LUCA (corresponding to early branches of the universal tree which have disappeared without descendants). The possibility that some large DNA viruses originated by reduction from extinct DNA cells evolutionarily related to early eukaryotic cells was recently boosted by the discovery of the giant mimivirus whose genome size (1.2 Mb) is three times larger than the smallest genomes of parasitic archaea or bacteria. This virus encodes a few components of the translation system that could be relics of ancient cellular lineages now extinct. On the other hand, the huge diversity of DNA viruses suggests that different lineages of DNA viruses could have originated at different periods and by different mechanisms. Indeed, there are arguments to suggest that at least some DNA viruses originated from RNA viruses. In particular, the RNA replicases/transcriptases of RNA viruses are homologous to the reverse transcriptase of retroviruses and to DNA polymerases of the A family encoded by many DNA viruses. Similarly, RNA and DNA viruses encode homologous RNA/DNA helicases. The hypothesis of an evolutionary transition from RNA to DNA viruses could explain the existence of intermediate forms such as retroviruses (with an RNA genome and an RNA–DNA–RNA cycle) and hepadnaviruses (with a DNA genome and a DNA–RNA–DNA cycle). Interestingly, retroviruses and hepadnaviruses are evolutionarily related, suggesting that the transition from RNA to DNA occurred in the virosphere.

Viruses and the Origin of DNA

Considering the possibility that at least some DNA viruses originated from RNA viruses, it has been suggested that DNA itself could have appeared in the course of virus evolution (in the context of competition between viruses and their cellular hosts). Indeed, DNA is a modified form of RNA, and both viruses and cells often chemically modify their genomes to protect themselves from nucleases produced by their competitor. It is usually considered that DNA replaced RNA in the course of evolution simply because it is more stable (thanks to the removal of the reactive oxygen in position 2′ of the ribose) and because cytosine deamination (producing uracil) can be corrected in DNA (where uracil is recognized as an alien base) but not in RNA. The replacement of RNA by DNA as cellular genetic material would have thus allowed genome size to increase, with a concomitant increase in cellular complexity (and efficiency) leading to the complete elimination of RNA cells by the ancestors of modern DNA cells. This traditional textbook explanation has been recently criticized as incompatible with Darwinian evolution, since it does not explain what immediate selective advantage allowed the first organism with a DNA genome to predominate over former organisms with RNA genomes. Indeed, the newly emerging DNA cell could not have immediately enlarged its genome and could not have benefited straight away from a DNA repair mechanism to remove uracil from DNA. Instead, if the replacement of RNA by DNA occurred in the framework of the competition between cells and viruses, either in an RNA virus or in an RNA cell, modification of the RNA genome into a DNA genome would have immediately produced a benefit for the virus or the cell. It has been argued that the transformation of RNA genomes into DNA genomes occurred preferentially in viruses because it was simpler to change in one step the chemical composition of the viral genome than that of the cellular genomes (the latter interacting with many more proteins). Furthermore, modern viruses exhibit very different types of genomes (RNA, DNA, single-stranded, double-stranded), including highly modified DNA, whereas all modern cellular organisms have double-stranded DNA genomes. This suggests a higher degree of plasticity for viral genomes compared to cellular ones. The idea that DNA originated first in viruses could also explain why many DNA viruses encode their own enzymes for deoxynucleotide triphosphate (dNTP) production, ribonucleotide reductases (the enzymes that produce deoxyribonucleotides from ribonucleotides), and thymidylate synthases (the enzymes that produce deoxythymidine monophosphate (dTMP) from deoxyuridine monophosphate (dUMP). Because, in modern

cells, dTMP is produced from dUMP, the transition from RNA to DNA occurred likely in two steps, first with the appearance of ribonucleotide reductase and production of U-DNA (DNA containing uracil), followed by the appearance of thymidylate synthases and formation of T-DNA (DNA containing thymine). The existence of a few bacterial viruses with U-DNA genomes has ben taken as evidence that they could be relics of this period of evolution.

If DNA first appeared in the ancestral virosphere, one has also to explain how it was later on transferred to cells. One scenario posits the co-existence for some time of an RNA cellular chromosome and a DNA viral genome (episome) in the same cell, with the progressive transfer of the information originally carried by the RNA chromosome to the DNA 'plasmid' via retro-transposition.

New Hypotheses about the Role of Viruses in the Origin of Modern Cells

The idea that viruses 'invented' DNA implies that they have been major players in the origin of modern cells. Indeed, several provocative hypotheses have been proposed in recent years that put viruses as central players of various evolutionary scenarios In the context of an ancient DNA virosphere, it has been argued that different lineages of DNA viruses would have 'invented' different enzymatic activities to replicate, repair, and recombine their DNA, explaining why the proteins dealing with DNA are now so diverse, often belonging to several nonhomologous protein families. It has thus been proposed that many (possibly all) cellular enzymes involved today in cellular DNA replication, repair, and/or recombination first originated in viruses before being transferred to cells. More specifically, several authors suggested that either the bacterial DNA replication mechanism, the eukaryotic/archaeal ones, or both, are of viral origin, in order to explain why the major proteins of the DNA replication machineries in eukaryotes and archaea (DNA polymerase, helicase, and primase) are not homologous to their functional analogs in bacteria (suggesting that LUCA had still an RNA genome). In order to explain why the archaeal and eukaryotic DNA replication machineries also exhibit some crucial differences (besides a core of homologous proteins), it was even suggested that the three cellular domains (Archaea, Bacteria, and Eukarya) originated from the independent fusions of three RNA cells and three large DNA viruses. In the latter scenario, the replacement of an RNA genome by a DNA genome at the onset of domain formation would have produced a drastic reduction in the rate of protein and rRNA evolution, explaining why proteins evolved apparently much less rapidly after the formation of the three domains than during the period between LUCA and the last common ancestor of each domain. The formation of each domain from three different types of RNA cells could have also selected three groups of RNA and DNA viruses specific for each domain, those that were able to infect these three ancestral RNA cells and their immediate descendants. This could explain a paradox in the modern biosphere, that each domain is characterized by its own set of viruses (for instance, NCLDVs are specific for eukaryotes) despite the fact that these viruses probably originated from a virosphere that predated LUCA. It must be remembered that NCLDVs share homologous capsid proteins with some bacterial and archaeal viruses.

Another area in which evolutionists have now recruited viruses for help is the problem of eukaryote origin. The 'viral eukaryogenesis' hypothesis posits that the eukaryotic nucleus originated from a large DNA virus, possibly related to NCLDV. This hypothesis was inspired by the analogies between the life cycle of the nucleus and those of poxviruses. In particular, both the eukaryotic nucleus and poxviruses build their membrane from the endoplasmic reticulum. Again, the discovery of the mimivirus (a member of the NCLDV family) led credence to such hypothesis. The relationships between the eukaryotic nucleus and giant viruses might have been even more complex. Hence, it was also recently proposed that a bidirectional evolutionary pathway was operating early on, with both large DNA viruses producing nuclei by infecting ancestral proto-eukaryotic cells, and also infectious nuclei producing new large DNA viruses. Finally, it was suggested that several different viruses might have been involved in eukaryogenesis to explain the presence of multiple RNA and DNA polymerases in eukaryotic cells.

Although most hypotheses previously discussed will probably always lack definitive proof, comparative genomics analyses have recently revealed a clear case of viral intervention in the formation of modern eukaryotic cells, that is, the viral origin of the DNA transcription and replication apparatus of mitochondria. This was inferred from the discovery that the RNA polymerase, DNA polymerase, and DNA helicase operating in mitochondria are of viral origin. These enzymes probably originated from a provirus that was integrated into the genome of the α-proteobacterium at the origin of mitochondria, since proviruses encoding homologs of these enzymes have been detected in the genome of several proteobacteria.

Conclusions

The idea that modern viruses are not simple extensions of prokaryotic or eukaryotic cells but derived from an ancient virosphere whose evolution encompassed the RNA world and the period of the RNA-to-DNA transition has far-reaching consequences. One of the most important in terms of practical consequences for all biologists is that modern viruses (and plasmids, which most

likely originated from them) would have inherited from this ancient virosphere many molecular mechanisms that have disappeared from modern DNA cells. This would explain why the molecular biology of the viral world for transcription, replication repair, and recombination is more diverse than that of the cellular world (despite the fact that we have only explored a tiny fraction of the modern virosphere). If this view is correct, many still unknown molecular mechanisms (and their associated proteins) remain to be discovered in viruses. The exploration of viral diversity will be for sure one of the major challenges of biology in this new century.

See also: Evolution of Viruses; Nature of Viruses; Virus Evolution: Bacterial Viruses.

Further Reading

Bamford DH, Grimes JM, and Stuart DI (2006) What does structure tell us about virus evolution? *Current Opinion in Structural Biology* 15: 655–663.
Bell PJL (2001) Viral eukaryogenesis: Was the ancestor of the nucleus a complex DNA virus? *Journal of Molecular Evolution* 53: 251–256.
Claverie JM (2006) Virus takes center stage in cellular evolution. *Genome Biology* 7: 1–10.
Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.* San Diego, CA: Elsevier Academic Press.
Filée J and Forterre P (2005) Viral proteins functioning in organelles: A cryptic origin? *Trends in Microbiology* 13: 510–513.
Forterre P (2005) The two ages of the RNA world, and the transition to the DNA world, a story of viruses and cells. *Biochimie* 87: 793–803.
Forterre P (2006) Three RNA cells for ribosomal lineages and three DNA viruses to replicate their genomes: A hypothesis for the origin of cellular domain. *Proceedings of the National Academy of Sciences, USA* 103: 3669–3674.
Forterre P and Krish H (2003) Viruses: Origin, evolution and biodiversity. *Research in Microbiology (Special Issue)* 154: 223–311.
Hamilton G (2006) Virology: The gene weavers. *Nature* 441: 683–685.
Koonin EV and Martin W (2005) On the origin of genomes and cells within inorganic compartments. *Trends in Genetics* 21: 647–654.
Ortmann AC, Wiedenheft B, Douglas T, and Young M (2006) Hot crenarchaeal viruses reveal deep evolutionary connections. *Nature Reviews Microbiology* 4: 520–528.
Prangishvili D, Forterre P, and Garrett RA (2006) Viruses of the Archaea: A unifying view. *Nature Reviews Microbiology* 4: 837–848.
Raoult D, Audic S, Robert C, *et al.* (2004) The 1.2-megabase genome sequence of mimivirus. *Science* 306: 1344–1350.
Villarreal LP (2005) *Viruses and the Evolution of Life.* Washington: ASM Press.
Woese CR, Kandler O, and Wheelis ML (1990) Towards a natural system of organisms: Proposal for the domains Archaea, Bacteria, and Eucarya. *Proceedings of the National Academy of Sciences, USA* 12: 4576–4579.

Orthobunyaviruses

C H Calisher, Colorado State University, Fort Collins, CO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arbovirus A virus transmitted to vertebrates by hematophagous (blood-feeding) insects.
Orthobunyavirus A virus in the genus *Orthobunyavirus*.
Reassortant A virus having genomic RNAs of two different viruses.
Transovarial transmission Vertical transmission, from mother to offspring.
Sympatrically Occupying the same or overlapping geographic areas without interbreeding.
Teratogenic Causing malformations of an embryo or fetus.

Introduction

From the late 1950s and onward, Jordi Casals, Robert Shope and co-workers at the World Health Organization's Collaborating Centre for Arbovirus Reference and Research collected and studied the antigenic relationships among the many viruses that had been and were being collected by Rockefeller Institute workers investigating epidemics of yellow fever and other virus diseases. Through their meticulous studies, they were able to show that some of these viruses were related to each other serologically (antigenically) and formed 'groups' of viruses. However, at least one virus in each of certain groups reacted with antibody to at least one virus in another group or groups. This was confusing because a group logically comprises certain viruses and not others, else the 'others' would be considered in the group. Casals then proposed that these tenuously interrelated viruses formed a 'supergroup', the Bunyamwera supergroup, and suggested that there might be other supergroups to be found. Subsequently, many viruses were shown to have similarities antigenically, by size, morphogenetics, and morphology, and by molecular means, yet also could be distinguished by these methods, such that groups, supergroups, and, eventually, genera, were included in a family of viruses, the *Bunyaviridae* (named after *Bunyamwera virus*).

This family comprises five genera: O*rthobunyavirus*, *Nairovirus*, *Phlebovirus*, *Hantavirus*, and *Tospovirus*. Viruses of the first three are transmitted by hematophagous arthropods and infect vertebrates; hantaviruses are not known to be transmitted by arthropods but infect vertebrates; and tospoviruses are transmitted by plant-feeding thrips and do not infect vertebrates. Any virus in the family is 'a bunyavirus' so, to avoid confusion, the original genus name *Bunyavirus* was changed to *Orthobunyavirus*.

According to the International Committee on Taxonomy of Viruses, 48 species are recognized within the genus and these 48 species include 160 viruses, including various strains, plus three viruses that are, at this time, considered 'tentative species' (**Table 1**). As the current ICTV list is somewhat confusing (it lists strains and synonyms), **Table 1** provides a slightly modified list of species and viruses placed in genus *Orthobunyavirus*.

Many studies of the orthobunyavirus-type species (prototype), *Bunyamwera virus*, have yielded a commensurate amount of information. Much that is known about the orthobunyaviruses is known by extrapolation from studies of one or more viruses of the genus or of viruses in a particular group of viruses. However, whereas all orthobunyaviruses share some characteristics, they are distinct. The differences, which are relatively or seemingly trivial, may be biologically significant. For example, La Crosse and Snowshoe hare viruses share considerable RNA sequence similarities and are considered 'subtypes' or 'varieties' of the same virus. Their geographic ranges overlap and their natural cycles include being transmitted between small mammals by mosquitoes and they cause disease in humans, usually young humans. However, the small mammals they employ as principal vertebrate hosts for amplification differ (chipmunks [*Tamias* spp.] and squirrels [*Sciurus* spp.] for La Crosse virus, hares [*Lepus americanus*] for Snowshoe hare virus); their principal mosquito vectors differ (*Aedes triseriatus* for La Crosse virus, other *Aedes* spp. for Snowshoe hare virus); La Crosse virus is the primary cause of pediatric arboviral encephalitis in the US, Snowshoe hare virus is a rare cause of such infections; and La Crosse virus has been isolated as far south as Texas and Louisiana, Snowshoe hare virus has been found in Alaska, and in Canada in the Yukon and Northwest Territories, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario and Quebec in Canada, but only as far south as Montana, Minnesota, Wisconsin, Ohio, Pennsylvania, New York and Massachusetts in the US. Clearly, differences between 'species' (a taxonomic term) and 'virus' (a nomenclatural term) may be confusing but they are epidemiologically and diagnostically relevant.

Most orthobunyaviruses were discovered during routine or epidemic surveillance efforts. Many have been isolated only once or, at most, a few times; some have been isolated many times in a single location or a few times in many locations; some are isolated with considerable frequency; orthobunyaviruses are found worldwide. Most have not been associated with disease in humans, livestock, or wildlife but those that have been cause uncomplicated illnesses (fever, headache). However, certain orthobunyaviruses are recognized as the etiologic agents of severe disease, for example, the aforementioned La Crosse virus.

La Crosse Virus

In 1964, this virus was isolated from brain tissue of an encephalitic child from Minnesota who had died in 1960 in a hospital in nearby La Crosse, Wisconsin. Till then, the only recognized human pathogenic California serogroup virus in North America was California encephalitis virus, but La Crosse virus was soon shown to differ from that virus. Most infections are subclinical or cause mild illnesses, but the more severe infections can lead to illnesses characterized by frank encephalitis progressing to seizures with coma. The case–fatality rate is $<1\%$, with neurological sequelae often requiring several years to resolve, if they do resolve and there are individual and social costs from the adverse effects on IQ and school performance. Short-term to long-term hospitalization costs can exceed $450 000. Since its recognition, La Crosse virus has been shown to cause childhood encephalitides each year. In the period 1964–2003, 3190 (a mean of 80 per year) human California group (mostly La Crosse virus infections) have been diagnosed in the US. In comparison, 4632 infections with St. Louis encephalitis (a flavivirus), 640 infections with Western equine encephalitis, and 215 infections with Eastern equine encephalitis (both togaviruses) occurred in the same period. Inapparent:apparent infection rates as high as 26:1 have been determined. Other California group viruses occasionally cause febrile illnesses with infrequent central nervous system involvement in Europe and North America.

Akabane Virus

Epizootics of congenital defects and abortion 'storms' had been observed in cattle, sheep, and goats in Japan and Australia since the 1930s, but an etiologic agent was not identified until 1959, when the orthobunyavirus Akabane virus was isolated from *Aedes vexans nipponii* and *Culex tritaeniorhynchus* mosquitoes in Japan; in Australia, the principal arthropod vector is the biting midge *Culicoides brevitarsis*. In other places, other vectors have been identified.

This virus now is known to occur widely in Africa and the Middle East and, wherever it occurs, has been responsible for malformations of the fetus. The range and

Table 1 Species and viruses[a] placed in the genus *Orthobunyavirus*

Species	*Viruses*
Acara virus	Acara, Moriche
Akabane virus	Akabane, Sabo, Tinaroo, Yaba-7
Alajuela virus	Alajuela, San Juan
Anopheles A virus	Anopheles A, Las Maloyas, Lukuni, Trombetas
Anopheles B virus	Anopheles B, Boraceia
Bakau virus	Bakau, Ketapang, Nola, Tanjong Rabok, Telok Forest
Batama virus	Batama
Benevides virus	Benevides
Bertioga virus	Bertioga, Cananeia, Guaratuba, Itimirim, Mirim
Bimiti virus	Bimiti
Botambi virus	Botambi
Bunyamwera virus	Batai, Birao, Bozo, Bunyamwera, Cache Valley, Fort Sherman, Germiston, Iaco, Ilesha, Lokern, Maguari, Mboke, Ngari, Northway, Playas, Potosi, Santa Rosa, Shokwe, Tensaw, Tlacotalpan, Tucunduba, Xingu
Bushbush virus	Benfica, Bushbush, Juan Diaz
Bwamba virus	Bwamba, Pongola
California encephalitis virus	California encephalitis, Inkoo, Jamestown Canyon, Keystone, La Crosse, Lumbo, Melao, San Angelo, Serra do Navio, Snowshoe hare, Tahyna, Trivitattus
Capim virus	Capim
Caraparu virus	Apeu, Bruconha, Caraparu, Ossa, Vinces
Catu virus	Catu
Estero Real virus	Estero Real
Gamboa virus	Gamboa, Pueblo Viejo
Guajara virus	Guajara
Guama virus	Ananindeua, Guama, Mahogany Hammock, Moju
Guaroa virus	Guaroa
Kairi virus	Kairi
Kaeng Khoi virus	Kaeng Khoi
Koongol virus	Koongol, Wongal
Madrid virus	Madrid
Main Drain virus	Main Drain
Manzanilla virus	Buttonwillow, Ingwavuma, Inini, Manzanilla, Mermet
Marituba virus	Gumbo Limbo, Marituba, Murututu, Nepuyo, Restan
Minatitlan virus	Minatitlan, Palestina
M'Poko virus	M'Poko, Yaba-1
Nyando virus	Nyando, Eret-147
Olifantsvlei virus	Bobia, Dabakala, Olifantsvlei, Oubi
Oriboca virus	Itaqui, Oriboca
Oropouche virus	Facey's Paddock, Oropouche, Utinga, Utive
Patois virus	Abras, Babahoya, Pahayokee, Patois, Shark River
Sathuperi virus	Douglas, Sathuperi
Simbu virus	Simbu
Shamonda virus	Peaton, Sango, Shamonda
Shuni virus	Aino, Kaikalur, Shuni
Tacaiuma virus	Tacaiuma, Virgin River
Tete virus	Bahig, Matruh, Tete, Tsuruse, Weldona
Thimiri virus	Thimiri
Timboteua virus	Timboteua
Turlock virus	Lednice, Turlock, Umbre
Wyeomyia virus	Anhembi, BeAr-328208 (unnamed), Macaua, Sororoca, Taiassui, Wyeomyia
Zegla virus	Zegla
Tentative species in the genus	
Leanyer, Mojui dos Campos, Termeil	

[a] The International Committee for Taxonomy of Viruses lists additional strains of certain of these viruses. Strain designations have been omitted from this list.

severity of its effects are related to the stage of gestation at infection of the dam. In adult animals, infection is subclinical and preexisting immunity in the dam provides protection to the fetus, such that the presence of Akabane virus may go undetected in areas enzootic for the virus. When it does cause congenital malformations, they may present as increased incidence of abortions and premature births occurring during seasons when arthropods are most abundant. The principal malformations are hydranencephaly (congenital absence of the cerebral hemispheres in which the space in the cranium that they normally occupy is filled with fluid) and arthrogryposis (permanent fixation of a joint in a contracted position), but dystocia (slow or difficult labor or delivery) may occur, necessitating cesarian section to save the dam. Surviving offspring manifest various developmental deficits and do not thrive. Other orthobunyaviruses have been shown to be teratogenic and Cache Valley virus has been associated with congenital defects in ruminants and, perhaps, in humans.

Oropouche Virus

Another example of the variations among orthobunyaviruses is Oropouche virus, isolated in 1955 from a febrile forest worker in Trinidad. Since then, a great deal has been learned as a result of data accrued during numerous epidemics. Oropouche disease is not fatal, but patients suffer from a mélange of signs and symptoms, including abrupt onset, fever, headache, myalgias, arthralgias, anorexia, dizziness, chills, photophobia, and an assortment of other symptoms. The acute phase of illness lasts 2–5 days but recurrence of symptoms can occur in patients who resume strenuous activities prior to complete resolution of their illness. Repeated epidemics of

thousands of cases of Oropouche disease have occurred in urban population centers throughout the Brazilian states of Para, Amapa, Amazonas, Tocantins, Maranhao, Rondonia, and Acre. Epidemics of Oropouche fever were also reported in Panama in 1989 and in the Amazon region of Peru in 1992 and 1994.

The principal vector is thought to be the biting midge *Culicoides paraensis,* although isolations of Oropouche virus have also been made from mosquitoes. During epidemics, transmission of Oropouche virus can be maintained in a vector-human cycle. Oropouche disease transmission does not occur year-round and investigators have shown that monkeys, sloths, and mosquitoes comprise a sylvatic maintenance cycle.

Reassortment

Among the many orthobunyaviruses causing uncomplicated febrile illness, the group C viruses are notable. These viruses (Apeu, Bruconha, Caraparu, Ossa, Vinces, Madrid, Gumbo Limbo, Marituba, Murutucu, Nepuyo, Restan, Itaqui, and Oriboca) are related one to another in various ways. They have been detected only in the Americas, from Florida to South America and have been isolated from mosquitoes, bats, rodents, marsupials, and humans. When the first group C viruses were isolated in Brazil in the 1950s antigenic analyses demonstrated complex patterns of relationships. That is, whereas pairs of group C viruses might be closely related by complement-fixation (CF) tests, they were not related, much less closely related, by hemagglutination-inhibition (HI) and neutralization (N) tests. Using CF tests, Apeu and Marituba, Caraparu and Itaqui, and Oriboca and Murutucu were shown to be related but Apeu and Itaqui, Oriboca and Caraparu, and Marituba and Murutucu were shown to be related by HI and N tests. Because these viruses occur sympatrically and are transmitted between vertebrate hosts by mosquitoes of the same species, it was thought that these results were an indication of a series of natural reassortments and that these viruses might serve as a model of viral evolution.

As detailed elsewhere in this volume, viruses of the family *Bunyaviridae* have tripartite genomes consisting of three RNA segments: designated small (S), medium (M) and large (L). The S segment encodes the nucleocapsid (N) protein and a nonstructural protein (NSs); the M segment encodes a polypeptide that is post-translationally cleaved to produce surface glycoproteins Gn and Gc, as well as a nonstructural protein (NSm); the L segment encodes a large protein containing the RNA-dependent RNA polymerase for replication and transcription of the genomic RNA segments. We now know that the group C viral CF antigen is the N protein and that the group C hemagglutinin is a surface glycoprotein, critical for virus attachment and, therefore, for neutralization. Recent molecular genetic studies of these viruses have corroborated earlier antigenic, ecologic, and genetic studies, and have shown that many of these viruses are genetic reassortants. That is, that they have various combinations of S, M, and L RNAs and that the various combinations lead to the various antigenic characteristics, patterns of cross-protection, and enzootic persistence in nature.

Naturally occurring reassortants of La Crosse virus and Patois group viruses have been documented; a Simbu group virus, Jatobal virus has been shown to contain the S RNA of Oropouche virus; Tinaroo and Akabane viruses are naturally occurring reassortants; and Ngari virus, a Bunyamwera group virus, is a reassortant comprising the S and L segments of Bunyamwera virus and the M segment from Batai virus. The Ngari virus strains (named Garissa virus at the time) had been isolated from human hemorrhagic fever patients, one in Kenya and one in Somalia, during a large outbreak of Rift Valley fever (Rift Valley fever virus is a phlebovirus) in East Africa. Thus, reassortment of orthobunyaviral RNAs produced a virus with characteristics atypical of Bunyamwera virus, which has not been associated with severe disease, and which mimic the clinical characteristics of Rift Valley fever. It is likely that other orthobunyaviruses will be shown to be reassortants, that reassortment is an on-going evolutionary process, and that reassortment will provide us with emerging diseases into the future.

Indeed, laboratory studies of experimental reassortants of La Crosse, Snowshoe hare, and other California group orthobunyaviruses have been useful in providing us with insights to understand the structure–function relationships of the various RNAs (neurovirulence or neuroinvasiveness and neuroattenuation map to the L RNA (which encodes the viral polymerase)). Lifelong infection of the arthropod vector, as well as transovarial transmission and venereal transmission, provide substantial opportunity for the virus to evolve by genetic drift or, under suitable circumstances of mixed infections, by segment reassortment. Orthobunyavirus reassortment, however, is limited to closely related viruses, reducing the possibility of unrestricted orthobunyaviral evolution.

Diagnosis and Treatment

Diagnosis of orthobunyavirus infections is by detection of viral RNA by PCR, as well as serologic assays including HI, CF, N, enzyme-linked immunosorbent assays, and immunofluoresence. Identification of orthobunyaviruses is by PCR, and the serologic assays mentioned above, the most specific of which is the N test. Treatment of illnesses caused by orthobunyaviruses is not needed for the more mild illnesses but when called for is merely symptomatic.

See also: Bunyaviruses: General Features; Tomato Spotted Wilt Virus.

Further Reading

Anderson CR, Spence L, Downs WG, and Aitken THG (1961) Oropouche virus: A new human disease agent from Trinidad, West Indies. *American Journal of Tropical Medicine and Hygiene* 10: 574–578.

Casals J (1963) New developments in the classification of arthropod-borne animal viruses. *Anais de Microbiologia* 11: 13–34.

Edwards JF, Karabatsos N, Collisson EW, and de la Concha Bermejillo A (1997) Ovine fetal malformations induced by *in utero* inoculation with Main Drain, San Angelo, and La Crosse viruses. *American Journal of Tropical Medicine and Hygiene* 56: 171–176.

Endres MJ, Griot C, Gonzalez-Scarano F, and Nathanson N (1991) Neuroattenuation of an avirulent bunyavirus variant maps to the L RNA segment. *Journal of Viology* 65: 5465–5470.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 699–716. San Diego, CA: Elsevier Academic Press.

Gerrard SR, Li L, Barrett AD, and Nichol ST (2004) Ngari virus is a Bunyamwera virus reassortant that can be associated with large outbreaks of hemorrhagic fever in Africa. *Journal of Virology* 78: 8922–8926.

Hammon WMcD and Reeves WC (1952) California encephalitis virus, a newly described agent. Part I. Evidence of natural infection in man and other animals. *California Medicine* 77: 303–309.

Iroegbu CU and Pringle CR (1981) Genetic interactions among viruses of the Bunyamwera complex. *Journal of Virology* 37: 383–394.

Nunes MRT, Travassos da Rosa APA, Weaver SC, Tesh RB, and Vasconcelos PFC (2005) Molecular epidemiology of group C viruses (*Bunyaviridae*, *Orthobunyavirus*) isolated in the Americas. *Journal of Virology* 79: 10561–10570.

Saeed MF, Wang H, Suderman M, *et al.* (2001) Jatobal virus is a reassortant containing the small RNA of Oropouche virus. *Virus Research* 77: 25–30.

Shope RE and Causey OR (1962) Further studies on the serological relationships of group C arthropod-borne viruses and the association of these relationships to rapid identification of types. *American Journal of Tropical Medicine and Hygiene* 11: 283–290.

Thompson WH, Kalfayan B, and Anslow RO (1965) Isolation of California encephalitis group virus from a fatal human illness. *American Journal of Epidemiology* 81: 245–253.

Ushijima H, Clerx-van Haaster M, and Bishop DH (1981) Analyses of the Patois group bunyaviruses: Evidence for naturally occurring recombinant bunyaviruses and existence of viral coded nonstructural proteins induced in bunyavirus-infected cells. *Virology* 110: 318–332.

Watts DM, Pantuwatana S, DeFoliart GR, Yuill TM, and Thompson WH (1973) Transovarial transmission of La Crosse virus (California encephalitis group) in the mosquito *Aedes triseriatus*. *Science* 182: 1140–1141.

Relevant Website

http://www.cdc.gov – Centers for Disease Control and Prevention (accessed on 11 December 2006).

Orthomyxoviruses: Molecular Biology

M L Shaw and P Palese, Mount Sinai School of Medicine, New York, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Antigenic drift The gradual accumulation of amino acid changes in the surface glycoproteins of influenza viruses.

Antigenic shift The sudden appearance of antigenically novel surface glycoproteins in influenza A viruses.

Cap snatching The process whereby the influenza virus polymerase acquires capped primers from host mRNAs to initiate viral transcription.

Recombinant virus A virus generated through reverse genetics techniques.

Reverse genetics Techniques that allow the introduction of specific mutations into the genome of an RNA virus.

Subtype Classification of influenza A virus strains according to the antigenicity of their HA and NA genes.

Classification and Nomenclature

Influenza viruses are members of the family *Orthomyxoviridae* which is divided into five genera: *Influenzavirus A, Influenzavirus B, Influenzavirus C, Thogotovirus*, and *Isavirus*. These viruses share many common features but are defined by having a segmented, single-stranded, negative-sense RNA genome that replicates in the nucleus of infected cells. Influenza A and B viruses both have eight genome segments which can encode 11 proteins while influenza C viruses have seven genome segments that encode nine proteins. Division of influenza viruses into the *Influenzavirus A*, *B*, or *C* genera is based on cross-reactivity of sera to the internal viral antigens, whereas different strains within each genus are distinguished by the antigenic characteristics of their surface glycoproteins. On this basis, 16 different hemagglutinin (HA) subtypes (H1–H16) and nine different neuraminidase (NA) subtypes (N1–N9) have been described for influenza A viruses. In theory, any combination of H and N subtypes is possible but the only

subtypes known to have circulated in the human population are the H1N1, H2N2, and H3N2 subtypes. All remaining H and N subtypes have been identified in viruses isolated from animals, particularly avian species. Individual influenza virus strains are named according to their genus (i.e., A, B, or C), the host from which the virus was isolated (omitted if human), the location of the isolate, the isolate number, the year of isolation, and, for influenza A viruses, the H and N subtypes. As an example, the first isolate of a subtype H3N8 influenza A virus isolated from a duck in Ukraine in 1963 is named A/duck/Ukraine/1/63 (H3N8). Influenza A viruses can undergo antigenic shift through reassortment of their glycoprotein genes. In contrast, influenza B and C viruses have only one antigenic subtype and do not undergo antigenic shift; however, all influenza viruses are characterized by minor antigenic differences, known as antigenic drift.

Virion Structure and Composition

Influenza virions are pleomorphic, enveloped particles (**Figure 1**). Spherical influenza virus particles have a diameter of approximately 100 nm but long filamentous particles (from 300 nm to several micrometers in length) have also been observed, particularly in fresh clinical isolates. The viral glycoproteins are embedded in the host-derived lipid envelope and are visible as spikes that radiate from the exterior surface of the virus when particles are viewed under the electron microscope. For influenza A and B viruses, the glycoproteins consist of the HA protein, which is the major surface protein and the NA protein. Influenza C viruses have only one surface glycoprotein, the HEF (hemagglutinin/esterase/fusion) protein. Additional minor components of the viral envelope are the M2 protein (influenza A viruses), NB and BM2 proteins (influenza B viruses), and the CM2 protein (influenza C viruses). The most abundant virion protein, M1, makes up the matrix which lies beneath the lipid membrane and surrounds the ribonucleoprotein (RNP) complexes. RNPs consist of the viral RNAs which are coated with the nucleoprotein (NP) and associated with the heterotrimeric polymerase complex (PB1, PB2, and PA proteins). Finally, small amounts of the nuclear export protein (NEP/NS2) have also been found within influenza A and B virus particles.

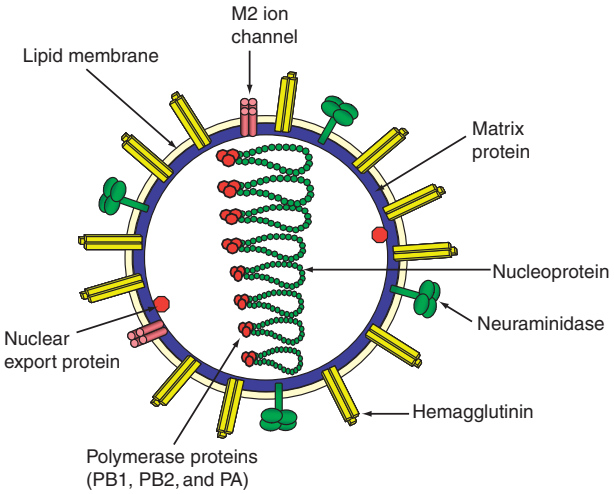


Figure 1 The viral proteins are indicated by arrows. The HA is a trimer while the NA and M2 proteins are both homotetramers. The matrix protein lies beneath the host-derived lipid envelope. Eight ribonucleoproteins form the core of the particle. These consist of the RNA genome segments coated with the nucleoprotein and bound by the polymerase complex. Small amounts of the nuclear export protein are also found within the virus particle.

Physical Properties

Because influenza viruses contain a lipid membrane, they are highly sensitive to delipidating and other denaturing agents. Differences in pH, ionic strength, and ionic composition of the surrounding medium influence the viral resistance to physical and chemical agents. The infectivity can be preserved in saline-balanced fluid of neutral pH at low temperatures. Influenza viruses are also relatively thermolabile, and are rapidly inactivated at temperatures higher than 50 °C. Agents affecting the stability of membranes, proteins, or nucleic acids, such as ionizing radiation, detergents, organic solvents, etc., reduce or completely destroy the infectivity of the virus.

Properties of the Viral Genome and Proteins

The genomes of influenza A and B viruses consist of eight separate negative-sense, single-stranded RNA segments known as vRNA. The influenza C virus genome has seven such segments. Each viral RNA segment exists as an RNP complex in which the RNA is coated with nucleocapsid protein (NP) and forms a helical hairpin that is bound on one end by the heterotrimeric polymerase complex. Noncoding sequences are present at both 5′ and 3′ ends of the viral gene segments; at the extreme termini are partially complementary sequences that are highly conserved between all segments in all influenza viruses (5′ end: AGUAGAAACAAGG and 3′ end: UCG(U/C) UUUCGUCC). When base-paired, these ends function as the viral promoter which is required for replication and transcription. The additional noncoding sequences contain the polyadenylation signal for mRNA synthesis as well as parts of the packaging signals required during virus assembly.

The eight segments of the influenza A virus genome code for the viral proteins (**Table 1**). The three largest segments each encode one of the viral polymerase subunits, PB2, PB1, and PA. The second segment also encodes an accessory protein, PB1-F2, from an alternate open reading frame within the PB1 gene. PB1-F2, which is unique to influenza A viruses, localizes to mitochondria and has pro-apoptotic activity. Segment 4 codes for the HA protein. The mature HA protein is a trimeric type I integral membrane glycoprotein which is found in the lipid envelope of virions and on the surface of infected cells. HA undergoes several post-translational modifications including glycosylation, palmitoylation, proteolytic cleavage, disulfide bond formation, and conformational changes. Cleavage of the precursor HA0 molecule into its HA1 and HA2 subunits (which are linked by a disulfide bridge) is mediated by host cell proteases and is essential for the fusion activity of HA. HA is also responsible for binding to host cell-surface receptors (sialic acid) and is the major target of neutralizing antibodies. Segment 4 of the influenza B virus genome also encodes HA but in influenza C virus, this segment codes for the HEF protein. HEF has attachment, fusion, and receptor-destroying activity and therefore incorporates the functions of HA and NA (see below) into one protein, explaining why influenza C viruses possess one less genome segment. HEF also recognizes a different cellular receptor, namely 9-*O*-acetylneuraminic acid.

The NP is encoded by segment 5. This is a highly basic protein whose main function is encapsidation of the viral RNA (an NP monomer binds approximately 24 nt of RNA) which is necessary for recognition by the polymerase. NP also plays a crucial role in transporting the viral RNPs into the nucleus which it achieves through interaction with the host nuclear import machinery. Segment 6 of influenza A and B viruses encodes the NA protein which is the second major viral glycoprotein (**Table 1**). This type II integral membrane protein is a tetramer and its sialidase (neuraminidase) activity is required for efficient release of viral particles from the infected cell. In influenza B viruses, the NA gene also encodes the NB protein from a second open reading frame. Little is known about this protein apart from the fact that it is a membrane protein and is required for efficient virus growth *in vivo*.

Segment 7 of influenza A viruses encodes two proteins, the matrix protein, M1, and the M2 protein. M1 is expressed from a collinear transcript, while M2 is derived from an alternatively spliced mRNA. M1 associates with lipid membranes and plays an essential role in viral budding. It also regulates the movement of RNPs out of the nucleus and inhibits viral RNA synthesis at late stages of viral replication. M2 is a tetrameric type III membrane protein that has ion channel activity. It functions primarily during virus entry where it is responsible for acidifying the core of the virus particle which triggers dissociation of M1 from the viral RNPs (uncoating). The BM2 protein of influenza B virus plays a similar role but it is expressed from the M1 transcript via a 'stop/start' translation mechanism. The role of CM2 in uncoating of influenza C viruses is less well established. This protein is encoded on segment 6 and is produced by signal peptide cleavage of a precursor protein (p42). The matrix protein of influenza C virus, CM1, is expressed from a spliced transcript.

The shortest RNA (segment 8 in influenza A and B viruses and segment 7 in influenza C viruses) encodes the NS1 protein from a collinear transcript and the NEP/NS2 protein from an alternatively spliced transcript. NS1 is an RNA-binding protein that is expressed

Table 1 Influenza A virus[a] genes and their encoded proteins

Genome segment	*Length in nucleotides*	*Encoded proteins*	*Protein size in amino acids*	*Function*
1	2341	PB2	759	Polymerase subunit, mRNA cap recognition
2	2341	PB1	757	Polymerase subunit, endonuclease activity, RNA elongation
		PB1-F2[b]	87	Pro-apoptotic activity
3	2233	PA	716	Polymerase subunit, protease activity
4	1778	HA	550	Surface glycoprotein, receptor binding, fusion activity, major viral antigen
5	1565	NP	498	RNA binding activity, required for replication, regulates RNA nuclear import
6	1413	NA	454	Surface glycoprotein with neuraminidase activity, virus release
7	1027	M1	252	Matrix protein, interacts with vRNPs and glycoproteins, regulates RNA nuclear export, viral budding
		M2[c]	97	Integral membrane protein, ion channel activity, virus assembly
8	890	NS1	230	Interferon antagonist activity, regulates host gene expression
		NEP/NS2[c]	121	Nuclear export of RNA

[a]Influenza A/PR/8/34 virus.
[b]Encoded by an alternate open reading frame.
[c]Translated from an alternatively spliced transcript.

at high levels in infected cells. Gene knockout studies have shown that NS1 is required for virus growth in interferon-competent hosts but not interferon-deficient hosts, indicating that NS1 acts to inhibit the host antiviral response. Influenza A virus NS1 has also been shown to interfere with host mRNA processing. Influenza B virus NS1 also has interferon antagonist activity and inhibits the conjugation of ISG15 to target proteins. The NEP/NS2 protein mediates the nuclear export of newly synthesized RNPs, corresponding with its expression at late times during viral infection.

Viral Replication Cycle

Virus Attachment and Entry

Influenza viruses bind to neuraminic acids (sialic acids) on the surface of cells via their HA proteins. The receptors are ubiquitous on cells of many species and this is one of the reasons why influenza viruses are able to infect many different animals. There appears to be some specificity associated with certain HAs, which recognize sialic acid bound to galactose in either an α-2,3- or an α-2,6-linkage. The latter linkage is more common in human cells while the α-2,3-linkage is more common in avian cells. Consequently, the HAs of human influenza viruses have a preference for the α-2,6-linkage and the HAs of avian influenza viruses are more likely to bind to α-2,3-linked sialic acids (**Figure 2**).

Entry of influenza viruses into cells involves internalization by endocytic compartments. This process is thought to occur via clathrin-coated pits, but non-clathrin-, non-caveolae-mediated internalization of influenza viruses has also been described. The actual entry process requires a low-pH-mediated fusion of the viral membrane with the endosomal membrane. The fusion activity can only occur after a pH-mediated structural change in the HA of the virus has occurred and if the HA was previously cleaved into an HA1 and an HA2 subunit. The fusion peptide at the N-terminus of the HA2 subunit interacts with the endosomal membrane, and through the acid pH-mediated conformational change the viral and endosomal membranes are brought together and fuse, opening up a pore that releases the viral RNPs into the cytoplasm. This uncoating step also depends on the presence of the viral M2 protein, an ion channel protein. The uncoating can be inhibited by amantadine (and rimantadine), which are FDA-approved anti-influenza drugs targeting the M2 protein. These compounds interfere with the influx of H^+ ions from the endosome into the virus particle, which

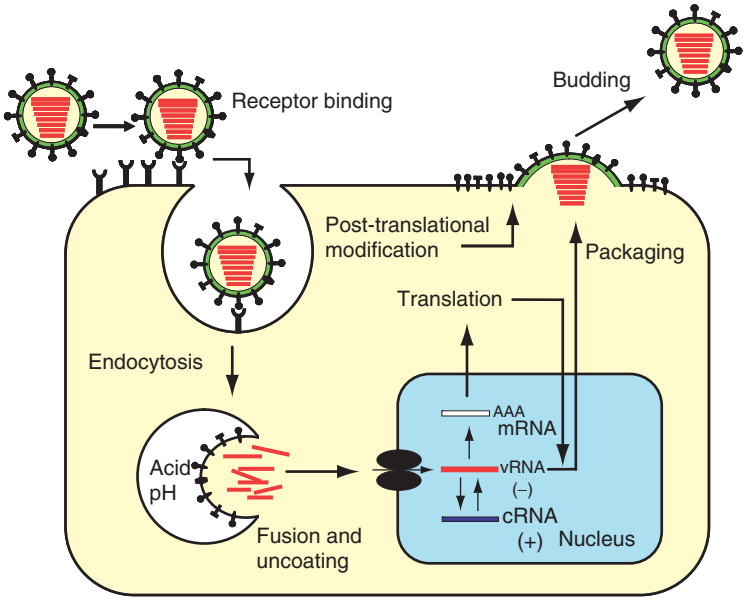


Figure 2 Virus attachment to the host cell is mediated by interaction of the HA protein with sialic acid-containing receptors. During endocytosis the interior of the endocytic vesicle becomes acidified. This induces conformational changes in HA which triggers its fusion activity and leads to fusion between the viral and endosomal membranes. The interior of the virus also becomes acidified due the ion channel activity of the M2 protein. The low pH dissociates the M1 protein from the RNP complexes, which are then transported into the nucleus. Viral RNAs are transcribed into mRNA and are replicated through a cRNA intermediate. Following export into the cytoplasm, the mRNAs are translated into viral proteins. The HA, NA, and M2 proteins are transported to the surface via the endoplasmic reticulum and Golgi (where they undergo post-translational modification). The remaining viral proteins are transported into the nucleus where they are required for either replication or nuclear export of newly synthesized vRNA. Virus assembly takes place at the apical plasma membrane which involves packaging of the eight RNPs into budding virus particles. Efficient release of budding particles requires the activity of the viral neuraminidase.

seems to be required for the disruption of protein–protein interactions, resulting in the release of RNPs into the cytoplasm, free of the other viral proteins.

An important characteristic of the influenza virus life cycle is the dependence on the nuclear functions of the cell. Once the RNP has been released from the endosome into the cytoplasm, it is then transported into the nucleus, where RNA transcription and replication take place (**Figure 2**). This cytoplasmic nuclear transport is an energy-driven process involving the presence of nuclear localization signals (NLSs) on the viral proteins and an intact cellular nuclear import machinery.

Viral RNA Synthesis

All influenza virus RNA synthesis occurs in the nucleus of the infected cell. The viral polymerase uses the incoming negative-sense vRNA as a template for direct synthesis of two positive-sense RNA species, mRNA and complementary RNA (cRNA). The viral mRNAs are incomplete copies of the vRNA and are capped and polyadenylated. cRNAs are full-length copies of the vRNA and in turn serve as a template for the generation of more negative-sense vRNA. Both vRNA and cRNA promoters are formed by a duplex of the partially complementary 5′ and 3′ ends, which increases the affinity for polymerase binding. Several studies have also indicated the importance of secondary structure in this region leading to the proposal of a corkscrew configuration. The stem–loop structures in this model have been shown to be critical for transcription.

Transcription

The viral transcription reaction is dependent on cellular RNA polymerase II activity because initiation requires a 5′ capped primer which is cleaved from host cell pre-mRNA molecules. This unique mechanism is known as cap snatching. Initiation of transcription begins with the binding of the vRNA promoter to the PB1 subunit. PB2 then recognizes and binds the cap structure on host pre-mRNAs which stimulates the endonuclease activity of PB1. The host mRNA transcripts are cleaved 10–13 nt from their 5′ caps, and the addition of a G residue which is complementary to the C residue at position 2 of the vRNA 3′ end serves to initiate transcription. Elongation is catalyzed by the PB1 protein and proceeds until the polyadenylation signal is encountered. This signal is located approximately 16 nt from the 5′ end of the vRNA and consists of five to seven uridine residues. On reaching this position the polymerase stutters, thereby adding a poly(A) tail to the viral mRNA transcripts.

Certain influenza virus proteins are expressed from alternatively spliced transcripts. The virus uses the cellular splicing machinery for this process but unlike host mRNAs which undergo complete splicing, the splicing of viral transcripts is relatively inefficient (1:10 ratio of spliced to unspliced transcripts). Of course this is necessary to ensure the production of proteins from both spliced and unspliced mRNAs and consequently several regulatory mechanisms are in place. These include the rate of nuclear export, the activity of host cell splicing factors, as well as *cis*-acting sequences within the NS1 transcript.

Replication

The replication step involves the synthesis of positive-sense cRNA from the incoming vRNA and subsequent generation of new negative-sense vRNA for packaging into progeny virions. In contrast to viral mRNAs, cRNAs are not prematurely terminated (and not polyadenylated) and do not possess a 5′ cap. Therefore the initiation and termination reactions for these two positive-sense RNAs are very different. Initiation of cRNA (and vRNA) synthesis is thought to be primer independent but it is not well understood and we also do not know how the polymerase manages to bypass the polyadenylation signal when it is in replication mode. In contrast to mRNAs, newly synthesized cRNAs and vRNAs are encapsidated and it has been proposed that the availability of soluble NP (i.e., not associated with RNPs) controls the switch between mRNA and cRNA synthesis. Other theories are that there are conformational differences between the transcription- and replication-competent polymerases and also that cellular proteins may be involved.

Packaging, Assembly, and Release of Virus

Influenza viruses assemble their components at (and in) the cytoplasmic membrane. The M1 protein has been shown to play an important role in recruiting the viral RNPs to the site of assembly at the cytoplasmic membrane, and together with the HA, NA, and M2 proteins it is the driving force in particle formation. Several influenza virus proteins, including the HA and NA, have apical sorting signals, which partially explains the observation that influenza viruses bud from the apical side of polarized cells. This asymmetric process may also provide the mechanism by which influenza viruses are largely restricted to the surface side of the respiratory tract rather than reaching the internal side, which would result in a systemic disease.

Assembly of an infectious particle requires the packaging of a full complement of the RNA genome. The precise mechanism by which this is achieved remains unclear. The first model, the random incorporation model, suggests that influenza virus RNAs, by virtue of their 5′ and 3′ termini, are recognized by viral proteins at the cytoplasmic membrane and that the correct eight RNAs are randomly packaged. If, for example, an average of 12 RNAs gets packaged, a reasonable percentage of particles would then possess a full set of the eight

influenza virus RNAs. The other model, the selective incorporation model, suggests that the newly made particle has only eight RNA segments because specific signals on each RNA result in the incorporation of one segment each of the eight different RNAs. There is increasing evidence to support the validity of the latter model: there appear to be unique packaging signals on each gene segment which include both coding and noncoding sequences near the 5′ and 3′ ends of the eight different RNAs. However, it is still not clear how (or even whether) such sequences define structural elements required for the packaging of each different RNA.

Infectious particles are only formed after budding is complete. Infectious influenza virus is not found inside of cells, as is the case for poliovirus, adenovirus, and others. As influenza virus particles bud off the cytoplasmic membrane, the viral NA is needed to remove sialic acid from the cell surface as well as from the carbohydrate-carrying viral glycoproteins themselves. In the absence of a functional NA or in the presence of NA inhibitors, virus particles are not released but instead form aggregates on the surface of infected cells. The NA is thus a releasing factor which is needed in order for the newly formed virus to break free and move on to infect neighboring cells. Oseltamivir and zanamivir are two FDA-approved neuraminidase inhibitors which have strong prophylactic and therapeutic activity against influenza A and B viruses.

Reverse Genetics of Influenza Viruses

Influenza viruses are negative-strand RNA viruses, and thus their genomic RNA, when transfected into cells, does not result in the formation of infectious virus. (In contrast, the RNA of poliovirus, a positive-strand RNA virus, after transfection into cells gives rise to infectious poliovirus particles.) Initial experiments involving the transfection of a synthetic influenza virus RNA mixed with purified viral polymerase and superinfection with helper virus resulted in the formation of infectious virus which had at least one cDNA-derived gene (into which mutations could be introduced). More recently, transfection of plasmids expressing the viral RNAs and viral proteins led to a helper-free virus rescue system (**Figure 3**). The term reverse genetics refers to the fact that viral RNA is first reverse-transcribed into DNA, which can be modified. This mutated DNA is then used in the plasmid-only transfection system to create infectious influenza virus. The advances of reverse genetics have been invaluable for the study of the structure/function relationships of the different influenza virus genes and proteins. In many cases, the definitive role of a protein (or domain of a protein) can only be explored by making the appropriate knockout mutant or by changing the genes in a virus and then studying its effect on virus replication.

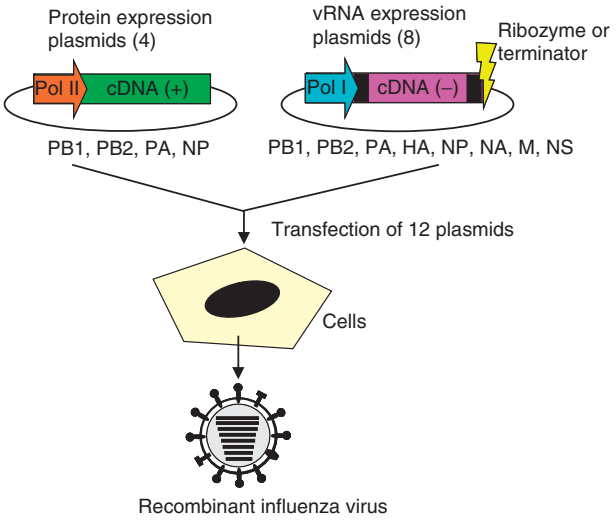


Figure 3 The negative-sense cDNA for each viral segment is cloned between a polymerase I promoter and the hepatitis delta virus ribozyme or polymerase I terminator. These plasmids will give rise to exact copies of the eight viral RNA segments. These eight plasmids are transfected into mammalian cells along with four expression plasmids for the polymerase proteins and NP, which are required for replication of the viral RNAs. The viral RNAs are replicated and transcribed by the reconstituted viral polymerase and result in the formation of infectious recombinant influenza virus.

Reverse genetics has also been used to reconstruct the extinct 1918 influenza virus using sequence data from tissue samples of victims of the 1918 pandemic. The study of this virus showed that the 1918 virus was intrinsically more virulent than other human influenza viruses and that this fact, more than any other, was responsible for the high morbidity and mortality observed in the 1918–19 pandemic.

Another application of reverse genetics techniques relates to making improved influenza virus vaccines. In the case of the avian H5N1 viruses, most strains are highly virulent by virtue of their having a basic cleavage peptide between the HA1 and HA2 subunits of the HA. The presence of this sequence of basic amino acids in the cleavage peptide allows for rapid cleavage of the HA in a variety of cells and has been found to be associated with rapid growth and high virulence. For vaccine-manufacturing purposes, it is advisable to remove this sequence signature of virulence by reverse genetics techniques. Also, the master seed strains for use in killed and live influenza virus vaccines could be constructed by reverse genetics, which would greatly accelerate the time-consuming process of selecting the right strains during interpandemic years.

Reverse genetics also allows the construction of influenza viruses which can express foreign genes. Such constructs are not only helpful in the laboratory for studies of the effects of the foreign genes in the context of an

infectious virus, but they may also be useful for the development of combination vaccines. Reverse genetically modified influenza viruses represent a potential platform to express foreign antigens which can induce protective immunity against several disease agents. Genetically modified influenza viruses might also be used in the fight against cancer, as influenza viruses have been shown to be oncolytic and to induce a vigorous T-cell response. The latter could be taken advantage of by expressing tumor antigens from an influenza virus vector, which then would result in the induction of a vigorous cytolytic T-cell response against cancer cells expressing such antigens.

See also: Antiviral Agents; Human Respiratory Viruses; Orthomyxoviruses: Structure of antigens.

Further Reading

Cros JF and Palese P (2003) Trafficking of viral genomic RNA into and out of the nucleus: Influenza, Thogoto and Borna disease viruses. *Virus Research* 95: 3–12.

Deng T, Vreede FT, and Brownlee GG (2006) Different *de novo* initiation strategies are used by influenza virus RNA polymerase on its cRNA and viral RNA promoters during viral RNA replication. *Journal of Virology* 80: 2337–2348.

Fodor E, Devenish L, Engelhardt OG, *et al.* (1999) Rescue of influenza A virus from recombinant DNA. *Journal of Virology* 73: 9679–9682.

Gamblin SJ, Haire LF, Russell RJ, *et al.* (2004) The structure and receptor binding properties of the 1918 influenza hemagglutinin. *Science* 303: 1838–1842.

Garcia-Sastre A and Biron CA (2006) Type 1 interferons and the virus–host relationship: A lesson in *détente*. *Science* 312: 879–882.

Hayden FG and Palese P (2002) Influenza virus. In: Richman DD, Whitley RJ, and Hayden FG (eds.) *Clinical Virology,* 2nd edn., pp. 891–920. Washington, DC: ASM Press.

Krug RM, Yuan W, Noah DL, and Latham AG (2003) Intracellular warfare between human influenza viruses and human cells: The roles of the viral NS1 protein. *Virology* 309: 181–189.

Neumann G, Watanabe T, Ito H, *et al.* (1999) Generation of influenza A viruses entirely from cloned cDNAs. *Proceedings of the National Academy of Sciences, USA* 96: 9345–9350.

Noda T, Sagara H, Yen A, *et al.* (2006) Architecture of ribonucleoprotein complexes in influenza A virus particles. *Nature* 439: 490–492.

Palese P (2007) Influenza and its viruses. In: Engleberg NC, DiRita V, and Dermody TS (eds.) *Schaechter's Mechanisms of Microbial Disease,* 4th edn., pp. 363–369. Philadelphia, PA: Lippincott Williams and Wilkins.

Palese P and Shaw ML (2007) *Orthomyxoviridae*: The viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, Lamb RA, and Martin MA (eds.) *Fields Virology,* 5th edn., pp. 1647–1689. Philadelphia, PA: Lippincott Williams and Wilkins.

Pinto LH and Lamb RA (2006) Influenza virus proton channels. *Photochemical and Photobiological Sciences* 5: 629–632.

Schmitt AP and Lamb RA (2005) Influenza virus assembly and budding at the viral budozone. *Advances in Virus Research* 64: 383–416.

Stevens J, Blixt O, Tumpey TM, *et al.* (2006) Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science* 312: 404–410.

Tumpey TM, Basler CF, Aguilar PV, *et al.* (2005) Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 310: 77–80.

Orthomyxoviruses: Structure of Antigens

R J Russell, University of St. Andrews, St. Andrews, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Hemagglutinin The surface glycoprotein responsible for receptor binding and cell fusion.
Influenza The prototypical member of the family *Orthomyxoviridae* of viruses.
Neuraminidase The surface glycoprotein necessary for viral release.

Introduction

The family *Orthomyxoviridae* is defined by viruses that have a negative-sense, single-stranded, and segmented RNA genome. There are five different genera in the family: *Influenzavirus A*, *B*, and *C*, *Thogotovirus*, and *Isavirus*.

Influenza viruses are classified into three types: A, B, and C. Type A influenza viruses are further classified into subtypes based on the antigenic properties of their surface antigens, hemagglutinin (HA) and neuraminidase (NA). There are 16 different HA subtypes (H1–H16) and nine different NA subtypes (N1–N9) of influenza A. Viruses containing HAs from each of the 16 HA subtypes and NAs of the nine NA subtypes, in different combinations, have been isolated from avian species. Human viruses that caused pandemics in the last century were of the combination H1N1 in 1918, H2N2 in 1957, and H3N2 in 1968. Viruses that are currently circulating are of the H1N1, H3N2 combination and B. Influenza viruses also infect a range of other animals including swine and equine, with H1N2 and H3N2 currently infecting the former and H3N8 the latter.

Different influenza virus strains are named according to their type, the species from which the virus was isolated

(omitted if human), place of virus isolation, the number of the isolate and the year of isolation, and in the case of the influenza A viruses, the HA and NA subtypes. For example, the 282nd isolate of an H5N1 subtype virus from chickens in Hong Kong in 2006 is designated: influenza A virus (A/chicken/Hong Kong/282/2006(H5N1)).

The genus *Thogotovirus* contains two different species, *Dhori virus* and *Thogoto virus*. Viruses from both these species were isolated from ticks, and therefore are different from influenza viruses with respect to their host range. The genus *Isavirus*, with the type species *Infectious salmon anemia virus*, is also distinct from influenza viruses A, B, and C, although numerous studies identify these isolates as members of the family *Orthomyxoviridae*.

The majority of the research into the family *Orthomyxoviridae* of viruses has been concerned with influenza viruses and therefore this article will focus exclusively on the structures of the influenza viral antigens.

Influenza Virus Surface Glycoproteins

The surface of type A and B influenza viruses has two major surface glycoproteins, HA and NA, whereas influenza C viruses have a single glycoprotein, hemagglutinin–esterase-fusion (HEF). Antibodies are raised to both HA and NA, although HA elicits the dominant antigenic response, and it is the natural variation in amino acid sequence (antigenic drift) and subsequent structural changes that limits the long-term effectiveness of an anti-influenza vaccine. Consequently, annual updates to the vaccine composition are required to maintain a match to the viruses that are currently circulating.

Influenza A and B Hemagglutinin

In type A and B influenza viruses, HA mediates attachment of the virus to the host cell via binding to sialic acid residues on the termini of cell surface glycoproteins. HA is also responsible for the fusion of viral and host cell membranes.

HA is synthesized as a single-chain polypeptide (HA0) of approximately 550 amino acids, which is subsequently proteolytically cleaved into two chains, HA1 and HA2. An interchain disulfide bond exists between cys-14 (HA1) and cys-137 (HA2). The monomers then associate noncovalently to form the functional trimer. The highly conserved and hydrophobic N-terminal residues of HA2, known as the fusion peptides (see below), are buried from solvent upon trimerization. It is known that these residues insert into the host membrane to facilitate virus membrane–cell membrane fusion. The C-terminal end of HA2 has a transmembrane anchor that tethers the molecule to the viral membrane.

HA is a highly N-glycosylated protein. For example, the H1 HA from the virus that caused the pandemic of 1918 has five glycosylation sites in HA1 and one in HA2. The number of glycosylation sites varies between virus species and has a marked impact on the antigenicity of the molecule (see below). H1 HA isolated from viruses circulating in 2002 had accumulated an additional six potential glycosylation sites with respect to the 1918 H1 HA. In addition to their influence on the antigenicity of HA, glycosylation sites that are conserved in all 16 HAs play a role in the co-translational folding of HA via binding to host cell chaperones.

To facilitate structural studies of HA, the protein is proteolytically cleaved from the surface of the virus, which removes the hydrophobic transmembrane anchor from the molecule. To date, no structure has been elucidated of a full-length HA.

The first crystal structure of HA to be determined was from the A/Aichi/2/68 (H3N2) virus, and showed the protein to be composed of a membrane-proximal, triple-stranded α-helical stem-like structure, that is composed of residues predominantly from HA2, that supports a membrane-distal globular multidomain structure, that is composed of residues solely from HA1 (**Figure 1(a)**). The membrane-distal part of each monomer can be subdivided into a vestigial esterase domain and a receptor-binding domain, the latter located at the very tip of each monomer. The receptor-binding domain consists of three secondary structure elements – the 190 helix (residues 190–198), the 130 loop (residues 135–138), and the 220 loop (residues 221–228) – that form the sides of each site, with the base made up of the conserved residues Tyr-98, Trp-153, His-183, and Tyr-195.

HA mediates the first stage of virus infection via binding to terminal sialic acid sugars on host glycoproteins and glycolipids. Sialic acids are usually found in either α-2,3- or α-2,6-linkages to galactose, the predominant penultimate sugar of N-linked carbohydrate side chains. The binding preference of an HA for one of these linkage types correlates with the species that the virus infects. The avian enteric tract has predominantly sialic acid in the α-2,3-linkage and all HAs found in the 16 antigenic subtypes found in avian influenza viruses bind preferentially to this linkage. In cells of the human upper respiratory tract, however, sialic acid in the α-2,6-linkage predominates. Indeed, HAs from human influenza viruses display a binding preference for sialic acid in the α-2,6-linkage. An avian origin has been proposed for human influenza viruses and therefore a change in binding specificity is required for cross-species transfer. The precise details of the amino acid changes underlying this switch in specificity seem to be HA subtype dependent but it has been shown that for both H1 and H3 HAs, only a small number of mutations are necessary. For example, a single mutation in H3 HA of Gln-226 to Leu-226 causes a switch in specificity from

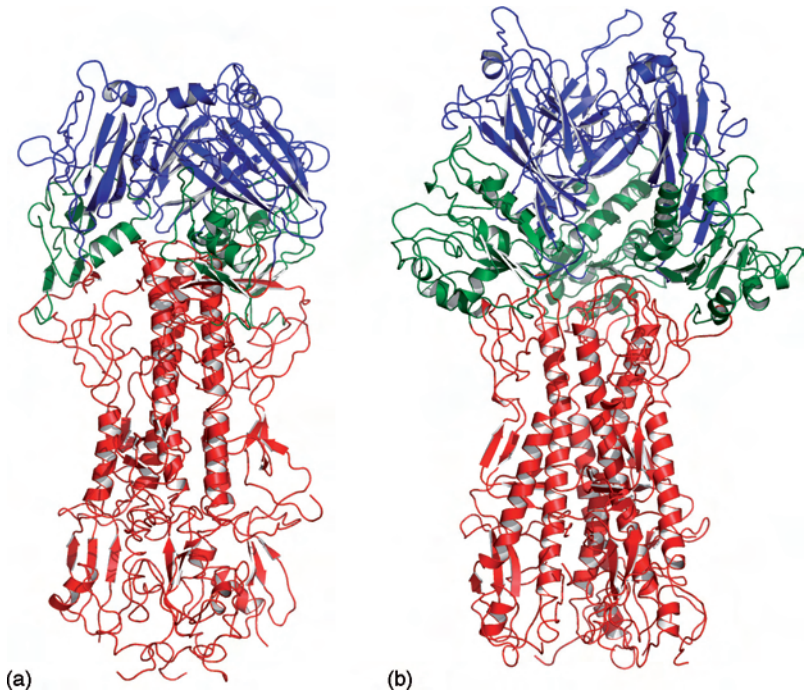


Figure 1 (a) Cartoon representation of influenza A HA. The receptor-binding domain of HA1 is colored blue and the vestigial esterase domain green. HA2, which forms the central α-helical stem, is colored red. (b) Cartoon representation of influenza C HEF. The receptor-binding domain of HEF1 is colored blue and the esterase domain green. HEF2, which forms the central α-helical stem, is colored red.

α-2,3- to α-2,6-binding preference. Insights into the amino acids involved in avian and human receptor binding have also been gained from elucidating the structure of HA in complex with receptor analogs.

The structure of HA0 has also been elucidated via crystallization of a protein in which Arg-329 had been mutated to a glutamine to prevent proteolytic cleavage of the polypeptide. The structure revealed that the cleavage site is located in a prominent surface loop which lies adjacent to a cavity which is not present in the cleaved-HA structure. There are three ionizable residues in this cavity which are buried from solvent after cleavage due to the burial of the nearly formed N-terminus of HA2 (fusion peptide). Thus it has been proposed that cleavage of HA0 results in a metastable form of the protein in which a low-pH trigger has been set due to the burial of ionizable residues. A characteristic of highly pathogenic influenza viruses such as the currently circulating H5N1 viruses is an insertion of a series of basic residues adjacent to the cleavage site. This polybasic insertion would create a much more extended surface loop which would facilitate enhanced intracellular cleavage.

In addition to sialic acid receptor binding, HA mediates virus membrane–cell membrane fusion. Subsequent to receptor binding, the influenza virus is internalized via endocytosis, exposing the virus to a low-pH environment which triggers a dramatic and irreversible conformational change in HA. Exposure of HA to low pH causes an alteration in the protonation state of residues that are buried by the fusion peptide, resulting in its removal from its buried site. Subsequently, the middle of the original long α-helix of HA2 unfolds to form a reverse turn, jack-knifing the C-terminal half backward toward the N-terminus, and results in HA2 adopting a rod-like coiled-coil structure. HA1, which was missing from the crystal structure, de-trimerizes and swings away from the HA2 but the interchain disulfide bond is maintained. As a consequence of these molecular rearrangements, the N-terminal fusion peptide, which inserts into the host membrane, and the C-terminal transmembrane anchor of HA2 are placed at the same end of the HA molecule, thereby facilitating membrane fusion (**Figure 2**).

The structure adopted by HA2 at the pH of membrane fusion shares a number of features with equivalent parts of the membrane fusion proteins of human immunodeficiency virus (HIV) and Ebola virus. In each case, the molecules contain central triple-stranded coiled coils that are surrounded by three α-helices that pack antiparallel to the core helices. As a consequence, the fusion peptides and membrane anchor regions of all of the proteins are at the same end of a rod-shaped molecule.

At present, no crystal structure of influenza B HA has been elucidated but it is expected to adopt the same conformation as influenza A HAs.

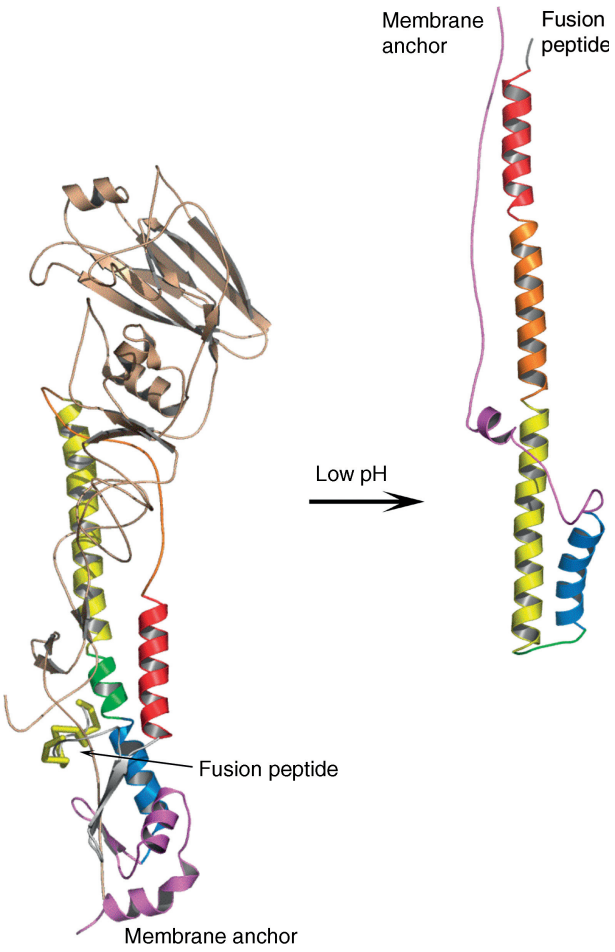


Figure 2 Schematic representation of the molecular rearrangements of HA upon exposure to low pH. The location of the fusion peptide and membrane anchor is shown in both structures. HA1 is colored in gold and HA2 is colored to highlight equivalent regions of the polypeptide. Note that only the yellow part of HA2 adopts the same position and conformation in both forms.

Influenza C HEF

In contrast to the HA of influenza A and B viruses, the major glycoprotein, HEF, of the C viruses has a receptor-destroying activity, as well as receptor-binding and fusion activities. As a consequence, influenza C viruses lack the NA glycoprotein. Despite sharing only 12% sequence identity with influenza A HAs, both HEF and HA are structurally similar (**Figure 1**). HEF has a membrane-proximal, α-helical stem-like structure and a membrane-distal globular multidomain structure. The receptor-binding domain of both HEF and HA are structurally similar despite HEF binding 9-*O*-acetyl sialic acid rather than sialic acid. The receptor-destroying domain shows structural homology to bacterial esterases, in keeping with its activity as a 9-*O*-acetylesterase. The stem region of HEF is similar to that observed in HA except that the triple-stranded, α-helical bundle diverges at both its ends and that the fusion peptide is partially exposed to solvent. The receptor-binding domain is inserted into a surface loop of the esterase domain which itself is inserted into a surface loop of the stem. Thus all three functions of the HEF are segregated into structurally distinct domains.

Influenza A and B Neuraminidase

After virus replication, NA removes sialic acid from virus and cellular glycoproteins to facilitate virus release and the spread of infection to new cells, otherwise viral aggregation would occur. Thus NA is commonly known as a receptor-destroying enzyme.

NA is synthesized as a single polypeptide chain but unlike HA no post-translational cleavage occurs. NAs have a highly conserved short cytoplasmic tail and a hydrophobic transmembrane region that provides the anchor for the stalk and the head domains. Like HA, NA is a highly N-glycosylated protein and serves to influence the antigenicity of NA.

NA suitable for structural studies is made via proteolytic cleavage from the virus surface which removes the membrane anchor and stalk domain. A deletion in the stalk domain is characteristic of highly pathogenic influenza viruses but as yet no structural information is available for this domain of NA.

The first crystal structure of the head domain of NA was of a N2 subtype, and showed that the molecule was homotetrameric with circular fourfold symmetry. Each monomer is composed of six topologically identical four-stranded antiparallel β-sheets that are themselves arranged like the blades of a propeller (**Figure 3**).

Sialic acid, the product of catalysis, binds in a deep pocket on the surface of the molecule, roughly in the middle of each monomer. No conformational changes occur upon binding of sialic acid. The amino acids in the active site that interact with sialic acid are highly conserved across all NA subtypes. The active sites of all influenza NAs contain three arginine residues, Arg 118, Arg 292, and Arg 371, that bind the carboxylate of the substrate sialic acid; Arg 152 that interacts with the acetamido substituent of the substrate; and Glu 276 that forms hydrogen bonds with the 8- and 9-hydroxyl groups of the substrate.

These properties of the active site made NA an attractive target for structure-based drug design programs, and resulted in the synthesis of oseltamivir (Tamiflu) and zanamivr (Relenza), two clinically licensed anti-influenza drugs.

The crystal structure of influenza B NA has also been elucidated and is essentially identical to influenza A NA,

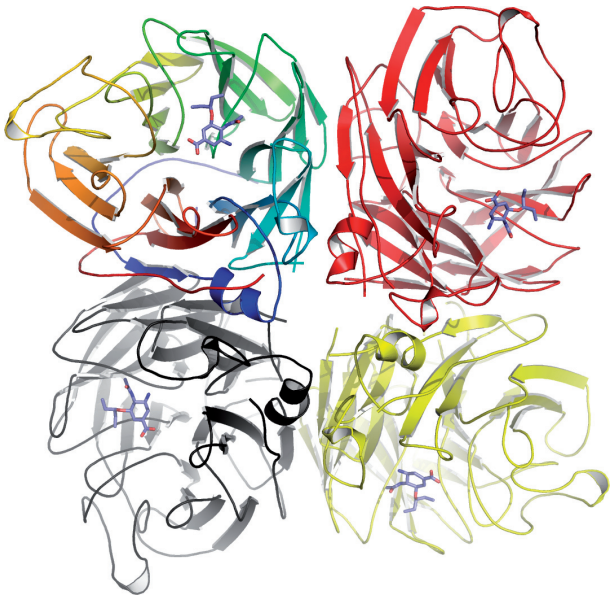


Figure 3 Cartoon representation of influenza A NA. Each monomer of the tetramer is colored differently, with the monomer at the top-left colored to emphasize the six-bladed β-propeller structure. Oseltamivir is bound to the active site and shown in stick representation and colored blue.

with the structure having the same homotetrameric arrangement and a high degree of conservation of the active site.

Influenza A Subtype Variation in Antigen Structure

To date, the crystal structures of H1, H3, H5, H7, and H9 HAs have been elucidated, which represent the four phylogenetic clades of influenza A HAs. Sequence identity between clades is between 40% and 60%. All of the HAs have the same molecular fold but HAs representative of each clade are distinguished by differences in the orientation of their membrane distal globular domains relative to the central trimeric coiled coil. For example, the receptor-binding domain of H3 HA is rotated clockwise by 24° relative to H1 HA. Also, the conformation of the interhelical loop of HA2 is different in HA from each clade. The clade-specific differences between HAs are clustered in regions that undergo conformational changes in membrane fusion, for example near to the fusion peptide, and it has been suggested that differences in the stability of HAs to pH and temperature may represent selection pressures in the evolution of influenza A HAs.

Influenza A NAs form two distinct phylogenetic groups. Crystal structures of N1, N4, and N8 have been elucidated as representatives of group 1 and N2 and N9 as representatives of group 2. All NAs have the same homotetrameric conformation but group-specific differences occur in the active site. Relative to group 2 NAs, a large cavity exists in group 1 NAs adjacent to the 4-amino group of oseltamivir. Upon binding of inhibitors to group 1 NAs, a conformational change of the 150-loop occurs which results in the active site of the two groups of NA being essentially identical. Chemical exploitation of this cavity is currently being undertaken in the development of a new generation of anti-influenza drugs to address the problem of mutations in NA that cause resistance to oseltamivir and zanamivir.

Antigenic Variation and Antibody Binding

Influenza viruses undergo a process known as 'antigenic drift' whereby natural variants of HA and NA occur, due to the error-prone nature of the viral RNA polymerase. HA is the major molecule recognized by the adaptive immune system of the host, although NA does elicit an immune response. Upon infection of a cell, the virus causes an immune response that commonly results in the production of neutralizing antibodies, in the case of HA, which acts to prevent the virus from binding to cells. Antibodies against NA, while they do not inhibit virus entry, prevent spread of the virus and afford some protection against challenge with the same or similar virus.

In the last century, three viruses were introduced into the human population from the avian reservoir of influenza viruses, and the subsequent pandemics caused millions of deaths. Following them, immune pressure causes antigenic variation that has the potential to continue until the fitness of the virus is compromised via deleterious mutations in HA which affects its receptor-binding properties.

Examination of the positions of amino acid substitutions of natural variants of HAs show that they are scattered throughout the molecule. Of the changes that are retained by circulating viruses, so-called 'fixed' substitutions, the majority are located on the surface of the molecule. Whereas, in the case of H3 HA subtype virus isolated between 1968 and 2005, two-thirds of the residues that are not retained are buried. This suggests that the 'fixed' substitutions have been selected because they alter the local structure of HA and prevent antibody binding.

This is supported by the fact that the location of amino acid substitutions in antigenic variants of HAs that have been selected by growing virus in the presence of anti-HA monoclonal antibodies (mAbs) map to the same location as the 'fixed' substitutions. Thus sites of mAb-selected mutations indicate the sites at which selecting antibodies bind. These mutants escape neutralization by the selecting antibody. The antibodies nevertheless still bind but with a much reduced affinity. There appears to be no

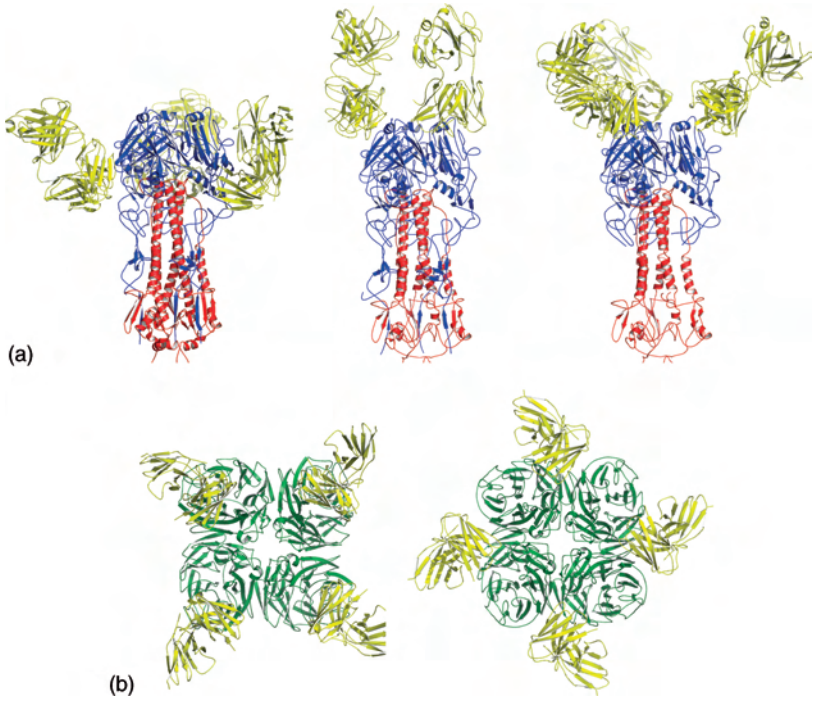


Figure 4 Antibody binding to influenza antigens. (a) Three different crystal structures of influenza HA bound to Fab fragments. (b) Two different crystal structures of influenza NA bound to Fab fragments.

preference in the type of amino acid change that occurs in selected variants.

Prevention of antibody recognition frequently occurs through the introduction of new glycosylation sites in HA. Oligosaccharide attachment at these sites prevents recognition first by covering of the protein surface and thus no antibodies being selected and second, because the sugars in the oligosaccharides are made by cellular enzymes, they are deemed by the immune system to be antigenically 'self'.

Sites of amino acid substitutions that occur in natural and antibody-selected variants are predominantly on the surface of the membrane distal part of HA, and commonly surround the receptor-binding site. This suggests that there is a link between the neutralization of viral infectivity and the prevention of virus binding to cells.

Crystal structures have been elucidated for HA and NA in complex with antibodies (**Figure 4**), and has shown that fragment antigen binding (Fab) antibody fragments do indeed bind to different parts of the molecules, supporting the observation that natural variants are scattered throughout the molecule. It is interesting to note that the stoichiometry of Fab binding is not always three molecules of Fab to one trimeric spike of HA. Of the three anti-HA antibodies that have been studied structurally, two of the binding sites overlap. All three of the antibodies have been shown to neutralize infectivity by the prevention of virus binding to cells. One of the antibodies, however, also blocks the conformational change of HA that is necessary for fusion.

Conclusion

Knowledge of the three-dimensional structures of the surface antigens of influenza viruses have had a major impact on the understanding of their role in the life cycle of influenza viruses. Insights into receptor binding, fusion mechanisms, and drug discovery have been gained and the study of these antigens from a range of influenza viruses has given insights into their structural evolution in relation to immune recognition.

See also: Orthomyxoviruses: Molecular Biology.

Further Reading

Lamb RA and Krug RM (2001) *Orthomyxoviridae*: The viruses and their replication. In: Knipe DM, Howley PM, Lamb RA, *et al.* (eds.) *Fields Virology*, 4th edn., p. 1487. Philadelphia: Lippincott Williams and Wilkins.

Palese P and Shaw ML (2006) *Orthomyxoviridae*: The viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology*, 5th edn., pp. 1647–1689. Philadelphia: Lippincott Williams and Wilkins.

Skehel JJ and Wiley DC (2000) Receptor binding and membrane fusion in virus entry: The influenza hemagglutinin. *Annual Review of Biochemistry* 69: 531.

Wright PF, Neumann G, and Kawaoka Y (2006) Orthomyxoviruses. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology*, 5th edn., pp. 1691–1740. Philadelphia: Lippincott Williams and Wilkins.

Oryctes Rhinoceros Virus

J M Vlak, Wageningen University, Wageningen, The Netherlands
A M Huger, Institute for Biological Control, Darmstadt, Germany
J A Jehle, DLR Rheinpfalz, Neustadt, Germany
R G Kleespies, Institute for Biological Control, Darmstadt, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

GC content Guanosine + cytosine content.
Gonad Male reproductive organ in insects.
Nuclear hypertrophy Enlargement and structural alterations of cell nuclei.
Oryctes rhinoceros A beetle insect that is an economic pest of coconut and palm plantations.

History

Oryctes rhinoceros virus (OrV) was first identified in 1963 in Malaysia through a field study in Southeast Asia to develop a strategy to control the rhinoceros beetle, *Oryctes rhinoceros*. This scarab beetle is native in Southeast Asia, but was accidentally introduced into some South Pacific Islands and became a major pest in coconut and palm plantations greatly affecting local economies. Since its initial discovery, OrV is found in rhinoceros and related beetles throughout Southeast Asia and used as an effective biocontrol agent of beetles in many countries, especially in Southeast Asia and the Pacific. Due to its structural similarity to baculoviruses, its replication in the nucleus, and the facultative occurrence of polyhedra-shaped occlusion bodies, OrV was originally considered a nonoccluded baculovirus. OrV is known in the literature under various names, such as rhabdionvirus, Oryctes virus (OrV), Oryctes baculovirus, Oryctes nonoccluded baculovirus, Oryctes nudibaculovirus, and abbreviations, such as OrV, Or-V1, and OrNOB.

Pathology

Larvae and adult beetles are infected by the oral route taking up OrV released from either disintegrated deceased larvae or from defecated infected midgut cells of chronically infected beetles. The first signs of OrV infection of larvae are extreme lethargy and lack of appetite. The larvae become translucent and their fat bodies in state of disintegration become visible through the integument. The midgut epithelial cells are the primary site of infection of both larvae and adult beetles. In the latter, the infected midgut is strikingly swollen and whitish in appearance. After replication of the virus in the larval midgut epithelial cells (**Figure 1**), the virions then transfer by an unknown mechanism into the interior where they infect other tissues such as fat body to establish a systemic infection. In the end, the larvae become translucent, flaccid, and finally disintegrate, often under septicemic conditions after 1–4 weeks. In beetles, the infection remains initially restricted to the midgut, where the epithelial cells are sloughed off regularly upon the infection into the gut lumen. This stimulates the crowds of regenerative crypts sitting on the midgut to start an enduring vigorous cell proliferation, by which the midgut lumen is completely filled up with myriads of cells that are packed with virus in their hypertrophied nuclei (**Figure 2**). This process can take weeks, before the infection becomes systemic and the beetles die. Strong nuclear hypertrophy of midgut and fat body cells and a ring zone in the nucleus recognize infection microscopically, which is characteristic for virus-harboring regions (**Figure 2**).

Replication and Assembly

OrV replicates in a cell line derived from the beetle *Heteronychus arator* (DSIR-HA-1179) and this allows the dissection of the replication of OrV both morphologically and biochemically. Virions derived from midgut tissue of infected beetles are used for infection. OrV enters cells most likely by pinocytosis and enters the nucleus as nucleocapsid through nuclear pores. The first signs of infection are the rounding up of the fibroblast-like *H. arator* cells followed by chromatin rearrangement and nuclear hypertrophy and finally, 3–4 days after infection, cell lysis. As evidenced by electron microscopy, virus assembly starts with the accumulation of so-called nucleocapsid shells in chromatin-free areas of the nucleus and their enwrapping by virion envelopes. Later electron-dense material, presumably viral nucleoprotein core with DNA, condenses into these preformed enveloped 'shells'. Later in infection, mature rod-shaped virions accumulate in large arrays at the nuclear periphery (**Figure 1**). The virions then enter the cytoplasm through the nuclear pores and ultimately leave the cell by budding from the cell membrane. The assembly process is a salient feature of OrV infection which also occurs during white spot syndrome virus (WSSV) morphogenesis.

Virion Properties

OrV in solution has a rod- to ovoid-shaped appearance and its nucleocapsid shows a short tail-like appendage at one end (**Figure 3**). As such, OrV looks very similar to several other helicoverpa zea viruses, HzV-1, Hz-2V, and GSV (gonad-specific virus), and WSSV of shrimp, except that the appendage is associated with the envelope rather than in the latter case the nucleocapsid as is the case with OrV. The OrV virions are about 220 nm × 120 nm in size and contain a single rod-shaped nucleocapsid, which is wrapped in an envelope consisting of two unit membranes that are sensitive to nonionic detergents (**Figure 5**). The nucleocapsid contains a circular double-stranded DNA molecule in a supercoiled form. The flexuous proteinacious tail-like appendage measures about 270 nm in length and 10 nm in width. Its function is unclear.

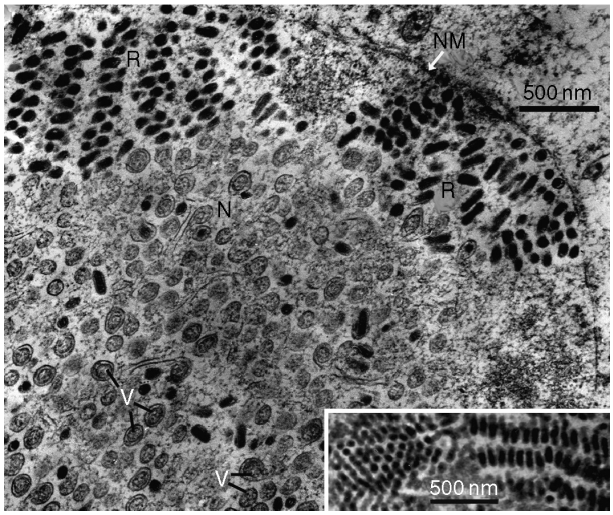


Figure 1 Electron micrograph of a thin section showing part of the nucleus (N) of a fat body cell of a third instar *Oryctes* beetle larva with heavy OrV infection. Note the accumulation of virus rods (R) at the nuclear periphery and the single- and double-membraned vesicles (V) in the nuclear centre. NM, nuclear membrane. Insert: Virus rods in cross (left) and longitudinal (right) section arranged in a pseudocrystalline pattern. Reprinted from *Journal of Invertebrate Pathology*, 89, Huger AM, The Oryctes virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), pp. 78–84, Copyright (2005), with permission from Elsevier.

Virions isolated from defecations of *Oryctes* adults showed at least 12 major structural proteins ranging in size from 10 to 76 kDa by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The smaller two, 10 and 13 kDa, are the most abundant and make up 50% of the total virion protein. At least 27 proteins have been found associated with OrV virions obtained from *H. arator* cell culture (cell line DSIR-HA-1179), ranging in size from 9.5 to 215 kDa; 14 of these are thought to be envelope proteins. The reason for this discrepancy in protein composition between OrV produced in beetles and in beetle cells is not known, but it could be the result of the production method employed. There is evidence to suggest that OrV and some baculoviruses share common antigens in immunological tests.

In OrV-infected *H. arator* cells only eight out of the 27 virion proteins were detected by pulse-labeling cells with radioactive amino acids ([^{35}S]-methionine). Two of these structural virion proteins, p4.6 and p10, were found as early as 4 h post infection (hpi), and the remaining six, p46.5, p40, p27, p25, p22, and p13, at 6 hpi. Since host protein synthesis is not shut off as a consequence of infection, the other OrV virion proteins may have been made and labeled if they contain methionine, but could then be submerged in the large amounts of labeled host-cell proteins. Immunoprecipitation of infected cell

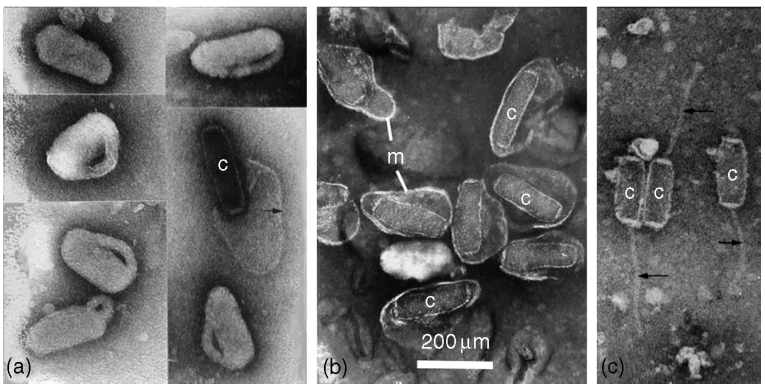


Figure 2 Electron micrographs with structural details of Oryctes rhinoceros virus rods, negatively stained with phosphotungstic acid. (A) Virions unpenetrated by stain, often being artificially mug-shaped; middle right: the virus membrane (arrow) is shedded from the nucleocapsid (c). (B) Virions with longer penetration by stain, thus displaying the nucleocapsids (c) and the surrounding viral membrane (m). (C) Three nucleocapsids (c) showing the typical thread-like appendix (arrows). Reprinted from *Journal of Invertebrate Pathology*, 89, Huger AM, The oryctes virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), pp. 78–84, Copyright (2005), with permission from Elsevier.

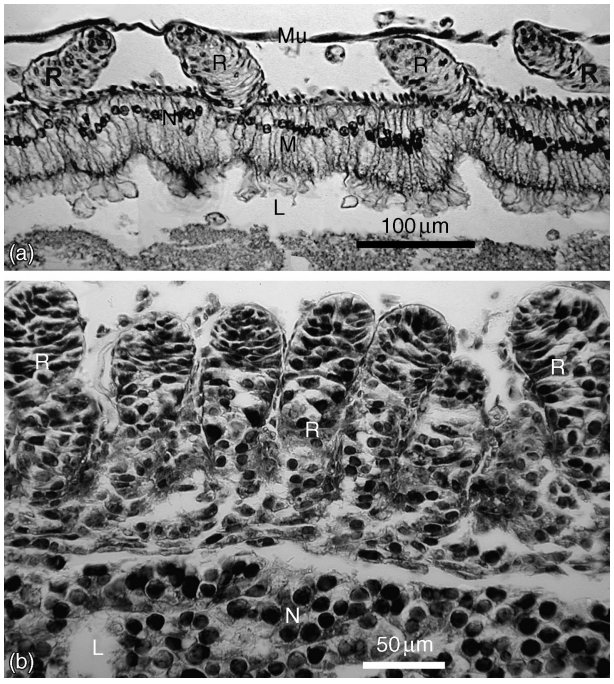


Figure 3 Light micrographs showing the midgut epithelium of rhinoceros beetle adults in longitudinal sections stained with Heidenhain's hematoxylin. (a) Midgut epithelium (M) of a healthy adult with a black-stained row of cell nuclei (N); the epithelium is outside occupied by regenerative crypts (R) and bounded by the muscularis (Mu); L, midgut lumen. (b) Midgut epithelium of an OrV-diseased adult with greatly enlarged regenerative crypts (R); the latter are massively proliferating cells from their apical region into the midgut lumen, where the hypertrophied cell nuclei (N) produce large amounts of virus. Reprinted from *Journal of Invertebrate Pathology*, 89, Huger AM, The Oryctes rhinoceros virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle *Oryctes rhinoceros* (Coleoptera: Scarabaeidae), pp. 78–84, Copyright (2005), with permission from Elsevier.

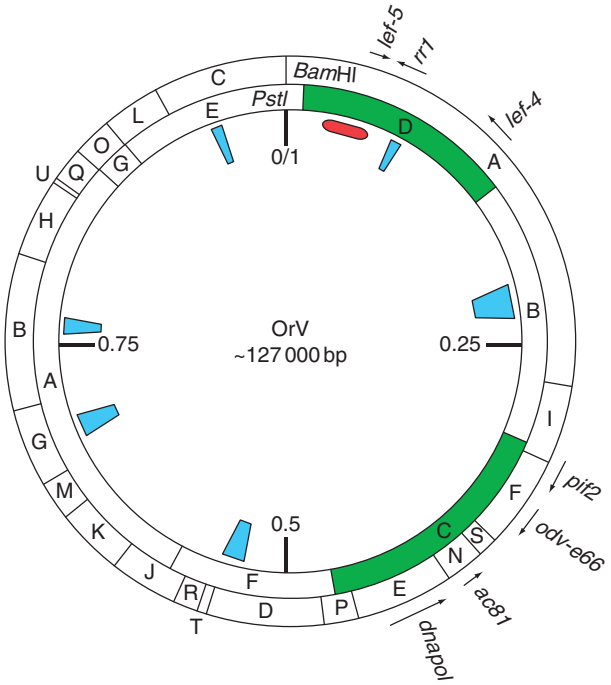


Figure 4 Genetic map of the OrV genome. The genome is drawn as a circular molecule and the *Bam*HI and *Pst*I restriction sites are indicated in the outer and inner circle, respectively. The site between *Bam*HI A and C is arbitrarily chosen as the zero point of the map. The approximate location of repeat regions is indicated (quadrangulars in blue). The genes conserved with baculoviruses and their direction of transcription are indicated (arrows) as well as the location of the primer sets for PCR identification (red bar).

proteins with OrV virion-specific antibodies is needed to reveal the remaining OrV virion proteins. OrV-specific nonstructural proteins would have remained undetected.

Genome Structure

The circular OrV genome is about 127 kbp in size with a GC content of 43–44%. Digestion of the viral DNA with restriction enzymes *Bam*HI, *Eco*RI, *Hin*dIII, and *Pst*I produced 21, 43, 23, and 7 DNA fragments, respectively, of different size. A physical map of the OrV genome has been constructed for these four restriction enzymes and six regions with reiterated sequences have been identified and mapped on the viral genome (**Figure 4**). Many of these restriction fragments have been cloned in bacterial plasmids and two of these (*Pst*I-C and *Pst*I-D) have been analyzed in detail.

Genetic variation between OrV field isolates exists as evidenced by DNA restriction fragment length polymorphism (RFLP). These variations are often small insertions and deletions in the reiterated repeat sequences. On the basis of genomic differences, at least three strains can be identified, which also differ in virulence. An OrV-specific DNA detection test based on a polymerase chain reaction (PCR) has been developed (**Figure 4**).

Portions of the OrV genome, more specifically OrV fragments *Pst*I-C (19 805 bp) and *Pst*I-D (17 146 bp), have been sequenced. Forty open reading frames of 50 or more amino acids were identified. Only 15 showed homology with other DNA viruses and these include DNA polymerase (*dnapol*), subunits of DNA-dependent RNA polymerase, ribonucleotide reductase (*rr1*), and thymidylate synthase, all enzymes involved in DNA replication and transcription. Ten open reading frames had homologs with HzV-1.

Phylogeny and Taxonomy

The OrV particle is rod-shaped and the virus replicates in the nucleus, hence it was originally considered to be a member of the family *Baculoviridae*. Since the virus

particles are mostly not occluded into occlusion bodies in infected hosts, it was assigned as a baculovirus of the nonoccluded type (subgroup C baculoviruses: nonoccluded baculoviruses). Later OrV was taken out of the family *Baculoviridae* due to the lack of genetic information and orphaned. OrV remained unassigned. Suggestions to establish a new Oryctes virus family were made, but genetic evidence was not available. Recent sequence and phylogenetic analysis provides compelling evidence that OrV deserves species status and that it can be a member of a new genus (*Nudivirus*) to be established. OrV is distantly related to baculoviruses, but not to whispoviruses despite its morphological similarities.

Phylogeny reconstructions using DNA polymerase gene (dnapol) sequences of OrV and a number of other viral DNA polymerases indicated that OrV formed a monophyletic group with HzV-1 and this might suggest that they have a common ancestor (**Figure 5**). On this basis OrV may be accommodated together with HzV-1 in a new genus, for which the name *Nudivirus* has been proposed. On the basis of the limited amount of sequence information of OrV, already ten open reading frames are closely related to HzV-1 and more are expected upon completion of the OrV genome sequence analysis. Furthermore, OrV and HzV-1 share structural and replication properties warranting inclusion in the same genus. The major difference between OrV on the one hand and the *H. zea* viruses on the other is their genome size; the HzV-1 genome is about twice the size (228 kbp) and the virus has never been isolated from insect larvae. There is a distant relationship of OrV and HzV-1 with baculoviruses at the level of the 29 conserved baculovirus genes, such as *dnapol*,

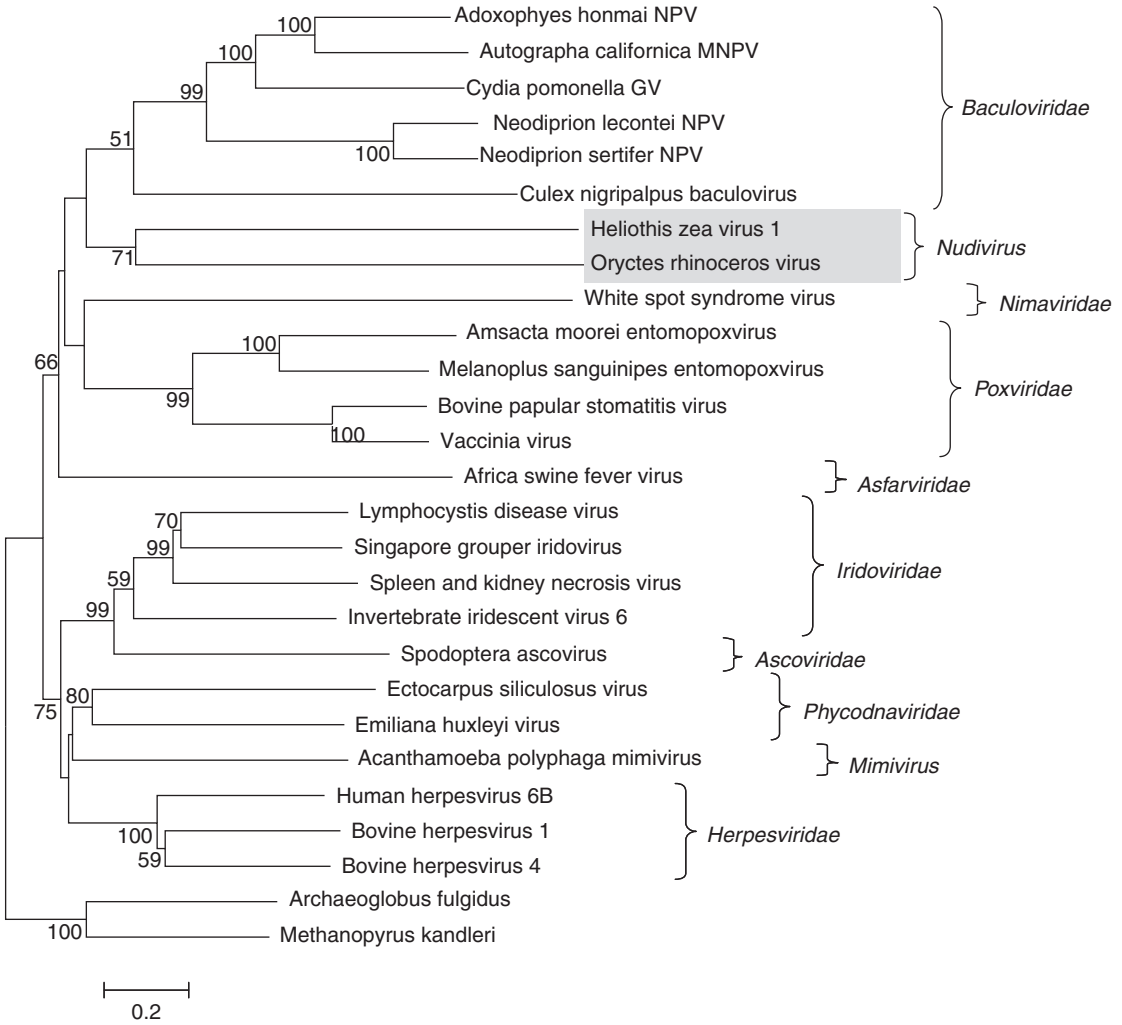


Figure 5 Phylogenetic affiliations of *Oryctes rhinoceros virus* (OrV) to other dsDNA viruses using DNA polymerase. Bootstrap values indicate the robustness of the minimal evolution tree (1000 replicates) and the values above 50% are given next to the nodes. Neighbor-joining and maximum parsimony analyses revealed a similar tree topology (not shown). Both OrV and HzV-1 are indicated on a gray background. The tree is rooted using the DNA polymerases of two archaeal species: *Archaeoglobus fulgidus* and *Methanopyrus kandleri*. The scale bar represents a distance of 20%. Adapted from Wang Y, van Oers MM, Crawford AM, Vlak JM, and Jehle JA (2006) Genomic analysis of Oryctes rhinoceros virus suggests genetic relatedness to Heliothis zea virus. *Archives of Virology* 152: 519–531.

lef4, and *lef5*. These viruses share common early (CAGT) and late (DTAAG) transcription promoter motifs. Therefore an inclusion of the proposed genus *Nudivirus* in the family *Baculoviridae* may be a future possibility. Genetically, OrV is not related to WSSV (*Nimaviridae*) despite its morphological similarities.

Transmission

OrV is taken up orally by larvae of *Oryctes* beetles at the breeding sites, which are usually located in rotting logs and stumps of palm trees and also in any decomposing organic matter. The infection in these larvae becomes systemic and at the ultimate stage they disintegrate. The virus is released at the breeding site, where it can be taken up by both new uninfected larvae or adult beetles. Horizontal disease transmission between beetles most frequently occurs by oral contact with fecal virus during mating or by co-occupation of the same habitat. In adult beetles the infection becomes chronic and develops differently; the virus initially only replicates in the midgut but not beyond. After infected midgut cells have been sloughed off into the gut lumen, large amounts of virus are produced in masses of cells proliferating from the regenerative crypts (**Figure 3**), and the virus is released through the feces. The infection of the beetles is usually without symptoms, although they stop feeding and egg laying. Such chronically infected beetles act as 'flying virus-spraying machines', flying around for weeks and defecating virus into their natural habitats and breeding sites, thus providing for an effective autodissemination and colonization of the virus disease over large areas. This horizontal transmission of OrV by autodissemination takes advantage of the ecological behavior of beetle populations and this is a key element in the success of OrV as a biocontrol agent.

Application as Biocontrol Agent

The first field trials to control the rhinoceros beetle were undertaken in 1967 in Samoa (then known as Western Samoa). Breeding sites were artificially contaminated with OrV and the disease became endemic over time. The virus spread autonomously in the beetle population and this autodissemination became the method of *Oryctes* control in coconut and oil palm until the present day. The adult beetles themselves are the natural vectors of OrV and responsible for the spread of the virus through fecal deliveries. The method of contamination of breeding sides was superseded by the release of trapped and subsequently infected beetles in affected sites. The virus became endemic and a permanent component of the *Oryctes* beetle ecology. *Oryctes* populations were effectively controlled in the Pacific Islands and other countries, and OrV provided long-term suppression. The use of OrV is a classical but single example of successful inoculative control of insects by virus. After the initial success of this strategy in the 1970s and 1980s, the interest in OrV faded away, in part due to the absence of severe beetle attacks. Recent outbreaks of the beetle in the Pacific as a consequence of careless policies for land clearance and replanting of palm trees have initiated a revival of *Oryctes* control by OrV.

See also: Baculoviruses: Molecular Biology of Granuloviruses; Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Insect Viruses: Nonoccluded; White Spot Syndrome Virus.

Further Reading

Bedford GO (1980) Biology, ecology and control of palm rhinoceros beetles. *Annual Review of Entomology* 25: 309–339.

Burand JP (1998) Nudiviruses. In: Miller LK and Ball LA (eds.) *The Insect Viruses*, pp. 69–90. New York: Plenum.

Crawford AM, Ashbridge K, Sheehan C, and Faulkner P (1985) A physical map of the Oryctes baculovirus genome. *Journal of General Virology* 66: 2649–2658.

Crawford AM and Sheehan C (1985) Replication of Oryctes baculovirus in cell culture: Viral morphogenesis, infectivity and protein synthesis. *Journal of General Virology* 66: 529–539.

Crawford AM, Zelazny B, and Alfiler AR (1986) Genotypic variation in geographical isolates of Oryctes baculovirus. *Journal of General Virology* 67: 949–952.

Hajek AE, Delalibera I, and McManus ML (2000) Introduction of exotic pathogens and documentation of their establishment and impact. In: Lacey LA and Kaya HK (eds.) *Field Manual of Techniques in Invertebrate Pathology*, pp. 339–369. Dordrecht, The Netherlands: Kluwer.

Huger AM (2005) The Oryctes virus: Its detection, identification, and implementation in biological control of the coconut palm rhinoceros beetle *Oryctes rhinoceros* (Coleoptera: Scarabaeidae). *Journal of Invertebrate Pathology* 89: 78–84.

Huger AM and Krieg A (1991) *Baculoviridae*: Nonoccluded baculoviruses. In: Adams JR and Bonami JR (eds.) *Atlas of Invertebrate Viruses*, pp. 287–319. Boca Raton, FL: CRC Press.

Jackson TA, Crawford AM, and Glare TR (2005) Oryctes virus – time for a new look at a useful biocontrol agent. *Journal of Invertebrate Pathology* 89: 91–94.

Mayo MA (1995) Unassigned viruses. In: Murphy FA, Fauquet CM, Bishop DHL, *et al.* (eds.) *Virus Taxonomy: The Sixth Report of the International Committee on Taxonomy of Viruses*, pp. 504–507. Vienna: Springer.

Mayo MA, Christian PD, Hillman BI, Brunt AA, and Desselberger U (2005) The unassigned viruses. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1129–1144. San Diego, CA: Elsevier Academic Press.

Mohan KS and Gopinathan KP (1991) Physical mapping of the genomic DNA of Oryctes rhinoceros baculovirus, KI. *Gene* 107: 343–344.

Ramle M, Wahid MB, Norman K, Glare TR, and Jackson TA (2005) The incidence and use of Oryctes virus for control of rhinoceros beetle in oil palm plantations in Malaysia. *Journal of Invertebrate Pathology* 89: 85–90.

Richards NK, Glare TR, Aloali'i I, and Jackson TA (1999) Primers for the detection of Oryctes virus from Scarabaeidae (Coleoptera). *Molecular Ecology* 8: 1552–1553.

Wang Y, van Oers MM, Crawford AM, Vlak JM, and Jehle JA (2006) Genomic analysis of Oryctes rhinoceros virus suggests genetic relatedness to Heliothis zea virus. *Archives of Virology* 152: 519–531.

Ourmiavirus

G P Accotto and R G Milne, Istituto di Virologia Vegetale CNR, Torino, Italy

© 2008 Elsevier Ltd. All rights reserved.

Introduction

This is a fascinating group of viruses with a unique virion structure but very little is known about them. Cassava virus C (CsVC) has been reported only once, in cassava from Malawi, in a mixed infection with African cassava mosaic virus. The damage it may cause is as unresearched as its molecular biology. Epirus cherry virus (EpCV) was found in a single cherry tree (since dead) showing rasp-leaf symptoms, with leaf distortion and development of enations, but this may not have been a single infection, and the virus was not isolated and returned to cherry. In a collaboration between Iranian researchers and the Turin plant virus laboratory, Ourmia melon virus (OuMV) has been detected on several occasions in melons from northwestern Iran, but generally in mixed infections with other cucurbit viruses, so the extent of economic losses ascribable to this virus is unknown.

Potentially the viruses are damaging since they have been experimentally transmitted to a rather wide range of dicot species including important crop plants, producing symptoms of mosaic, mottle, and necrosis. Data on the genome are too limited to assign the genus to any family, but if one had to guess, that family might be the *Bromoviridae*. Unless otherwise indicated, the data given below refer to OuMV.

Taxonomy

OuMV, CsVC, and EpCV have been placed within the genus *Ourmiavirus* firstly because all the virions have the same morphology, which is distinct and unique to the group. In addition, the cytopathology of cells experimentally infected with OuMV and CsVC is similar but distinct from that of other viruses (the cytopathology of EpCV has not been reported). The experimental host range of the three viruses, tested by mechanical inoculation, is similar, and the symptoms are generally systemic ringspots, mottle, mosaic, and necrosis. The coat proteins of OuMV and EpCV are of similar but not identical size and are distantly serologically related. The three positive-strand single-stranded RNAs (ssRNAs) comprising the genomes of OuMV and EpCV are of similar size (there is no information for CsVC).

The rather poor data available have suggested placement of the three viruses in separate species, for the following reasons. The viruses have nonoverlapping natural host ranges (cucurbits, cherry, cassava). OuMV and EpCV coat proteins are only distantly related serologically and are of distinct sizes. CsVC is reportedly not serologically related to OuMV. The three RNAs of EpCV are slightly but consistently smaller than those of OuMV, and the respective RNA-2's and RNA-3's show no evident sequence homologies (no data for CsVC).

Similarity with Other Taxa

The architecture of the virions differs clearly from that of other bacilliform or quasi-bacilliform particles of similar size such as alfalfa mosaic virus, olive latent virus 2, or ilarviruses, but this morphology, together with the presence of an ssRNA positive-strand genome in three segments, may indicate affinities with members of the *Bromoviridae*.

Properties of Particles

The particles (**Figure 1**) are unenveloped. They have cylindrical bodies 18 nm diameter capped by sharply conical (probably hemi-icosahedral) ends and are found in discrete lengths. The bodies of the particles are composed of a series of double disks, the commonest particles having two disks (particle length 30 nm), a second common particle having three disks (particle length 37 nm), with rarer particles having four disks (particle length 45.5 nm) and six disks (particle length 62 nm). A particle with five disks (hypothetical length 54 nm) has not been detected. The buoyant density in CsCl of particles of all sizes is 1.375 g cm^{-3}.

The particles are stable near pH 7 (infective in suspension for at least 35 days at 20–25 °C), and thermally they are relatively stable; infectivity survives in crude sap after heating for 10 min at 70 °C but not 80 °C. The particles survive treatment with chloroform but not *n*-butanol and survive treatment with 1% Triton X100 detergent.

Particle preparations contain ssRNA of three sizes, estimated as 0.9, 0.35, and 0.32×10^6 (M_r). There is one coat protein (estimated size 25.2 kDa).

Genome Organization

The ssRNA is positive stranded. RNA-1 presumably encodes the polymerase. The coat protein is encoded by either RNA-2 or RNA-3.

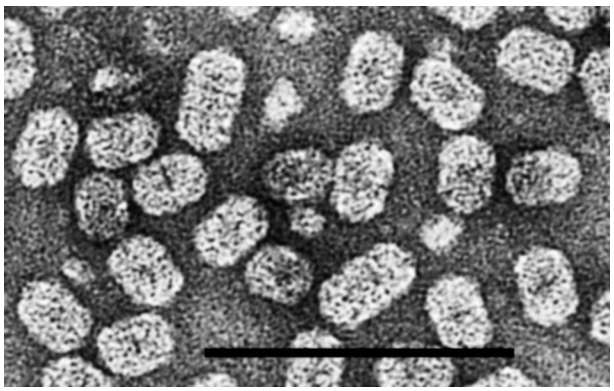


Figure 1 Purified preparation of OuMV particles negatively stained in uranyl acetate. Scale = l00 nm.

Viral Proteins

There is one coat protein. One nonstructural protein (possibly with a transport function) forms tubules in the cytoplasm of infected cells; virions are frequently found within the tubules.

Pathogenicity

The only natural host of OuMV so far detected is melon (*Cucumis melo*), which develops a mosaic-ringspot disease. The virus can easily be mechanically inoculated to many different hosts (34 species in 14 families). Local lesions appear in 3–4 days on *Chenopodium quinoa* and *Gomphrena globosa*, and these are followed, on *Nicotiana clevelandii*, *Nicotiana benthamiana*, and *Nicotiana megalosiphon*, by systemic necrotic mosaic. The virus particles accumulate to a relatively high titer. Among experimentally inoculated plants of commercial value tested, systemic disease developed in *Capsicum annuum* (two of three cultivars tested), tomato (one of two cultivars tested), *Cucumis melo* (melon; three out of three cultivars tested), *Cucumis sativus* (cucumber; four out of four cultivars tested), *Ocimum basilicum* (sweet basil), *Petunia hybrida*, *Spinacia oleracea* (spinach), *Viola tricolor* (pansy), and *Zinnia elegans*.

Geographic Distribution

OuMV have been found repeatedly in northwestern Iran; CsVC was reported from Malawi; EpCV was reported from Greece.

Cytopathology

All tissues are invaded. Membranes proliferate in the cytoplasm, with damage to chloroplasts. Many long tubules composed of protein, about 25 nm in diameter and packed with virions, develop in infected cytoplasm, giving a highly characteristic appearance to ultrathin sections viewed in the electron microscope.

Transmission, Prevention, and Control

No vector has yet been identified, and the method of field spread is unknown, although experimental seed-transmission rates of 1–2% in *N. benthamiana* and *N. megalosiphon* have been reported. Several aphid species, the whitefly *Trialeurodes vaporariorum* and the mite *Tetranychus urticae*, failed to transmit the virus experimentally.

See also: Bromoviruses.

Further Reading

Accotto GP, Riccioni L, Barba M, and Boccardo G (1997) Comparison of some molecular properties of Ourmia melon and Epirus cherry viruses, two representatives of a proposed new virus group. *Journal of Plant Pathology* 78: 87–91.

Aiton MM, Lennon AM,Roberts IM, and Harrison BD (1988) Two new cassava viruses from Africa. Abstracts, 5th International Congress of Plant Pathology, Kyoto, Japan, 20–27 August 1988, p. 43.

Avgelis A, Barba M, and Rumbos I (1989) Epirus cherry virus, an unusual virus isolated from cherry with rasp-leaf symptoms in Greece. *Journal of Phytopathology* 126: 51–58.

Lisa V, Milne RG, Accotto GP, Boccardo G, Caciagli P, and Parvizy R (1988) Ourmia melon virus, a virus from Iran with novel properties. *Annals of Applied Biology* 112: 291–302.

Milne RG (2005) Genus *Ourmiavirus*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1059–1061. San Diego, CA: Elsevier Academic Press.

P

Papaya Ringspot Virus

D Gonsalves, J Y Suzuki, and S Tripathi, USDA, Pacific Basin Agricultural Research Center, Hilo, HI, USA
S A Ferreira, University of Hawaii at Manoa, Honolulu, HI, USA

Published by Elsevier Ltd.

Introduction

The term papaya ringspot virus (PRSV) was first used in the 1940s to describe a viral disease of papaya. The name was used primarily to describe the ringspots that appeared on fruits from infected plants. Early investigations showed that the virus was transmitted by several species of aphids in a nonpersistent manner. That is, the aphid vector could acquire the virus in a short period of time while feeding on infected plants and likewise transmit the virus in a span of few seconds to less than a minute during subsequent feeding. In the same decade researchers from India and other places like Puerto Rico reported the occurrence of an aphid-transmitted disease of papaya; based on the symptoms on the leaves, it was identified as papaya mosaic virus. Work in the 1980s showed that the aphid-transmitted papaya mosaic virus and PRSV were really the same, and the name of PRSV was adopted. PRSV is a member of the family *Potyviridae*, a large and arguably the most economically important group of plant viruses. Today, the term papaya mosaic virus is reserved for a virus that is not aphid transmitted, belongs to the family *Potexviridae*, and causes the papaya mosaic disease which is seldom observed and not important commercially.

The systemic host range of PRSV is confined to plants in the families Caricaceae and Cucurbitaceae, with the primary economically important host being papaya and a range of cucurbits such as squash, watermelon, and melons. It does cause local lesions on plants of the family Chenopodiaceae such as *Chenopodium quinoa* and *C. amaranticolor*. The disease on cucurbits was, early on, referred to as being caused by watermelon mosaic virus-1 (WMV-1). Later serological and molecular characterization showed that PRSV and WMV-1 are virtually identical. Based on their close relationship, a single name was adopted to unify both viruses into one group. The name PRSV was chosen due to its being named before WMV-1. To clarify host range, 'P' (PRSV-P) or 'P type' is used to designate virus infecting papaya and cucurbits, while 'W' (PRSV-W) or 'W type' refers to virus infecting cucurbits only. The virus symptoms on cucurbits are identical to those on Caricaceae. Leaves of infected plants show severe mosaic, and chlorosis, are deformed, and often exhibit shoestring-type symptoms. The fruits are also often deformed and bumpy. In papaya, PRSV infection is characterized by mosaic and chlorosis symptoms on leaves, water-soaked streaks on the petiole, and deformation of leaves that can result in shoestring-like symptoms that resemble mite damage (**Figure 1**). The virus can cause deformation and ringspot symptoms on the fruit, hence the name PRSV. Commercial PRSV-resistant transgenic papaya expressing the coat protein (*CP*) gene of the virus has been used to control PRSV P in Hawaii, as will be discussed later.

General Properties of PRSV

The virus particles are flexuous rods about 760–800 nm $\times$ 12 nm with single RNA of about 10 326 b in length. Virus particles consist of 94.5% protein and 5.5% nucleic acid by weight. It has a single coat protein (CP) of about 36 kDa. Analysis of purified virus preparations that are stored show that the CP degrades to smaller proteins of *c.* 31–34 and 26–27 kDa proteins, possibly due to proteolytic degradation. The density of the virion in purified preparations is 1.32 g cm^{-3} in CsCl.

PRSV should not be confused with another potyvirus, papaya leaf distortion mosaic virus (PLDMV), which occurs in Okinawa and other parts of Asia, such as Taiwan. This virus causes very similar symptoms as PRSV on papaya and cucurbits but is serologically unrelated and its CP shares only 55–59% similarity to that of PRSV.

PRSV Genome

A genetic map of PRSV genome with polyprotein processing sites and products is presented in **Figure 2** and their possible functions in **Table 1**. Much of the knowledge on

the genome of PRSV has been obtained from extensive work done by the laboratory of Dr. Shyi-Dong Yeh of National Chung-Hsing University in Taiwan. The genomic RNA of PRSV is 10 326 nt in length excluding the poly(A) tract and contains one large open reading frame that encodes a polyprotein of 3344 amino acids starting at nucleotide position 86 and ending at position 10 120. A VPg protein is linked to the 5′ end of the RNA while a poly(A) tract is at the 3′ end. The polyprotein is cleaved into proteins designated (name (size in M_r)): P1 (63K), helper component (HC-Pro, 52K), P3 (46K), cylindrical inclusion protein (CI, 72K), nuclear inclusion protein a (NIa, 48K), nuclear inclusion protein b (NIb, 59K), coat protein (CP, 35K), as well as two other proteins 6K1 (6K) and 6K2 (6K). The cleaved proteins are arranged on the genome starting from the 5′ in order as: P1–(HC-Pro)–P3–6K1–CI–6K2–NIa–NIb–CP (**Figure 2**).



Figure 1 Symptoms of PRSV on papaya.

The cleavage proteins mentioned above have been identified by immunoprecipitation and dynamic precursor studies with PRSV-p as well as by extensive studies on proteolytic processing of polyproteins from other potyviruses. Three virus-encoded proteinases are responsible for at least seven cleavages: the P1 protein from N-terminus of the polyprotein autocatalytically liberates its own C-terminus, the HC-Pro also cleaves its own C-terminus, and NIa is responsible for *cis*- and *trans*-proteolytic processing to generate the CI, 6K, NIa, NIb, and CP proteins. NIa has also been shown to contain an internal cleavage site for delimitation of the genome-linked protein (VPg) and the proteinase (Pro) domains. Thus, the genomic organization and processing of the polyprotein of PRSV is similar to those of other potyviruses.

A rather interesting feature of the PRSV is that sequence analysis predicts two potential cleavage sites at the N-terminus of the CP. One of the sites (VFHQ/SKNF) predicts a CP of 33K and a NIb of 537 amino acids about 20 amino acids larger than those of other potyviruses. The second predicted cleavage site (VYHE/SRGTD) generates a CP of 35K and an NIa of 517 amino acids. There is no firm evidence to suggest that only one cleavage site is used. If both sites are used in polyprotein processing, one would expect heterogeneous products. This may explain why the analysis of purified CP preparations that are stored frequently shows the major ~36K form in addition to smaller CPs that are 2–5K smaller.

Sequence Diversity and Evolution

Knowledge of the sequence diversity among isolates of a virus has great implications in developing an effective virus disease management program and in understanding the origin and biology of the virus. Recently, numerous PRSV P and W sequences from virus isolated from different parts of the world have been reported in the sequence database. Amino acid and nucleotide sequence divergence among PRSV isolates differ by as much as 14%. These differences, interestingly, are considerably less than that found among isolates of other potyviruses, such as yam mosaic virus (YMV), that differ by as much as 28%. Although initial data from the USA and Australia suggested that there was little variation among PRSV isolates within these countries, more recent data from India and Mexico have suggested that the sequence

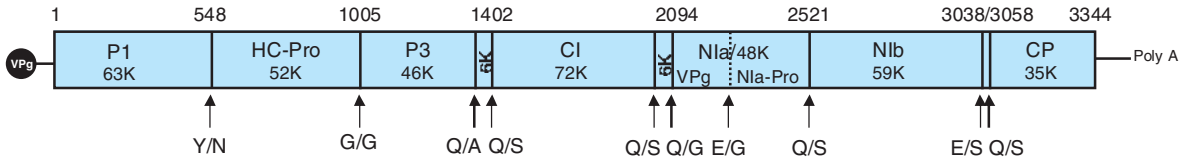


Figure 2 Genome map of PRSV. Vertical arrows indicate the proteolytic cleavage sites.

Table 1 PRSV proteins and their possible functions

Viral protein	*Size (M_r)*	*Functions*
P1	63K	Proteinase Cell-to-cell movement
HC-Pro	52K	Vector transmission Proteinase Pathogenicity Suppressor of RNA silencing Cell-to-cell movement
P3	46K	Unknown, but possible role in replication
6K1	6K	Unknown, but possible role in replication
CI	72K	Genome replication (RNA helicase) Membrane attachment Nucleic acid-stimulated ATPase activity Cell-to-cell movement
6K2	6K	Unknown, but possible roles in: • Replication • Regulation; inhibition of NIa nuclear translocation
NIaVPg	21K	Genome replication (primer for initiation of RNA synthesis)
NIaPro	27K	Major proteinase
NIb	59K	Genome replication (RNA-dependent RNA polymerase, RdRp)
CP	35K	RNA encapsidation Vector transmission Pathogenicity Cell-to-cell movement

variation between PRSV isolates in other countries may be greater than previously recognized. Heterogeneity in *CP* length ranging from 840 to 870 nt has been noted. The observed size differences in *CP* sequence occurred in multiples of three, preserving the reading frame between genes of different genomes and resulting in CPs of between 280 amino acids (Indian P isolate KA2) and 290 amino acids (VNW-38 from central Vietnam). Interestingly, the CP-coding region of all isolates from Thailand were 286 amino acids in length, while those from India and Vietnam demonstrated considerable heterogeneity in CP length, at 280–286 and 285–290 amino acids, respectively. The first 50 or so amino acids of the N-terminal region of the PRSV *CP* gene were found to be highly variable and all differences in CP length were confined to this region. The differences in this region that did exist consisted of conservative amino acid substitutions. The majority of the size differences occurred in one of two hypervariable regions and most were due to differences in the number of EK repeats.

A phylogenetic study based on *CP* sequences from 93 isolates of type P and W PRSV from different geographic locations was done by generating a phylogenetic tree using the neighbor-joining method. In the phylogenetic tree, sequences from one Sri Lankan isolate and two Indian isolates formed a sister cluster to the rest of the sequences. The other isolates formed two major lineages: I included all isolates from the Americas, Puerto Rico, Australia, and a few from South Asia; and II included isolates from Southeast Asia and the Western Pacific. Lineage I was the major of the two, containing three clusters of Brazilian isolates, two Indian isolates, and Australian, Mexican and US isolates. Within lineage I, the Brazilian and Mexican isolates were more diverse than the US and Australian isolates. Lineage II included all of the isolates from the Southeast Asia and Western Pacific, including China, Indonesia, Thailand, Vietnam, Taiwan, Japan, and the Philippines. However, the subclustering of isolates did not correlate well with their geographic origins; rather, they appeared to be a single mixed population with some well-defined subpopulations. These observations suggest that considerable movement of PRSV isolates has occurred among the Southeast Asian countries. Thai isolates of the P type diverged together, whereas PRSV W type diverged with other Southeast Asian isolates. Both P and W isolates of PRSV from Vietnam were intermingled with other Asian isolates. Sequence analysis showed that all Vietnamese isolates (except the P type from the southern part of the country) diverged from a common branch with P isolates from Japan and Taiwan while PRSV isolates from South Vietnam were diverged compared to those from the Philippines and seemed closely related to several W types from Thailand.

An interesting feature of PRSV is the origin of types P and W. As noted above, a number of viral diseases on cucurbits were historically associated with WMV-1 and not with any diseases of papaya. Did type P originate from W, or was it the reverse, or did they evolve independently? Evidences from various sources indicate that PRSV is primarily a pathogen of cucurbits, and that PRSV P originated from PRSV W. Work in Australia suggests that the recent outbreak of PRSV P came from the population of PRSV W already present in Australia. This suggestion is also supported by the diversity in cucurbit-infecting potyviruses that are phylogenetically related to PRSV.

Infectious Transcripts of Recombinant PRSV Help to Reveal Potential Determinants of Several Biological Characteristics

Determinants for Host Range Specificity

Studies utilizing the technique of producing infectious transcripts from recombinant viruses followed by bioassays of the transcripts have demonstrated that sequences of the PRSV genome responsible for determining papaya and cucurbit host specificity are not in the region of the *CP* gene. However, nucleotides 6509–7700 encoding the NIa gene and parts of the NIb gene were critical for papaya infection. Amino acids of the NIb gene of this region (nucleotides 7644–7700) between PRSV-P and PRSV-W

type are identical, whereas sequence comparison of nucleotides 6509–7643 of four type P and two type W viruses showed that two amino acids at positions 2309 (K → D) and 2487 (I → V) of PRSV are significantly different between papaya-infecting type P and non-papaya-infecting type W. Further point mutational studies in these sites indicated that these two amino acids located in the NIa proteinase are responsible for conferring the ability to infect papaya.

Determinants for Local Lesion Formation on *Chenopodium*

As noted earlier, PRSV causes local lesions on *C. amaranticolor* and *C. quinoa*. The severe strain PRSV HA strain from Hawaii causes local lesions on *C. quinoa* but a mild nitrous acid mutant of it, PRSV HA 5-1, does not. Recombinant infectious viruses were generated by exchanging genome parts between PRSV HA and PRSV HA 5-1. The study revealed that the pathogenicity-related region is present between nucleotide positions 950 and 3261 of the PRSV HA genome and mutations in the *P1* and *HC-Pro* genes resulted in the attenuation of PRSV HA symptoms and the loss of ability to produce local lesions on *C. quinoa*. The *HC-Pro* gene of PRSV is the major determinant factor for local lesion formation.

Determinants on Severity of Symptoms, Suppression of Gene Silencing, Infection of Transgenic Papaya

Virus–host interaction studies based on recombinant analyses between severe and mild strains of PRSV indicated that the *HC-Pro* gene plays an important role in viral pathogenicity and virulence and acts as a suppressor of the gene-silencing defense mechanism in the papaya host plant. In addition, the comparative reaction of recombinant PRSV with chimeric *CP* gene sequences showed that heterologous sequences and their position in the *CP* gene influences their pathogenicity on PRSV-resistant transgenic papaya.

An interesting phenomenon of strain-specific cross-protection was observed in papaya and horn melon provided by the mild strain HA 5-1 of PRSV. The PRSV mild mutant HA 5-1 provided 90–100% protection against the severe parental strain PRSV HA in greenhouse and field conditions. However, the degree of protection provided in horn melon by HA 5-1 against a PRSV type-W strain from Taiwan was only 20–30%. Studies on strain-specific cross-protection phenomenon indicated that the recombinant HA 5-1 carrying both the heterologous *CP* and the 3′ untranslated region (UTR) of the PRSV W from Taiwan significantly enhanced the protection against the Taiwan strain in cucurbits. However, chimeric HA 5-1 virus carrying either heterologous *CP* or heterologous 3′ UTR showed reduced effectiveness of protection against PRSV HA in papaya when compared to protection by the native mild HA 5-1.

Similar to other potyviruses, PRSV is also transmitted by insect vector aphids (*Myzus persicae* and *Aphis gossypii*) in a nonpersistent manner. Detailed studies with other potyviruses show that HC-Pro/virions interaction is essential for aphid transmission of potyviruses. Although not empirically tested, it would seem likely that aphid transmission of PRSV would similarly be governed by HC-Pro/virion interactions.

Pathogen-Derived Resistance for Controlling PRSV: The Hawaii Case

The control or management of PRSV has been approached through practices such as quarantine, eradication, avoidance by planting papaya in areas isolated from the virus, continual rogueing of infected plants, use of tolerant lines to reduce damage caused by PRSV, cross-protection through the use of mild virus strains, and resistance using the approach of 'pathogen-derived resistance'. The efforts to control PRSV in Hawaii are described because it involves all of the above practices. Ultimately, the most successful has been the 'pathogen-derived resistance' approach.

The state of Hawaii consists of eight main islands that are in rather close proximity to each other with the shortest and farthest distance between islands being 7 mile between Maui and Kahoolawe and 70 mile between Kauai and Oahu. Travels between the islands are prevalent with the exception of Kahoolawe, which is not inhabited, and Niihau, which is privately owned. PRSV was first detected in the 1940s on Oahu where Hawaii's papaya industry was located at that time. Efforts to control the virus on Oahu largely consisted of state officials and farmers continually monitoring for infected plants and rogueing them, especially in areas where the virus was not prevalent. However, by the late 1950s, PRSV was causing extensive damage, which caused the papaya industry to relocate to Puna on the island of Hawaii.

The relocation of the industry to Puna was timely and effective because Puna had an abundance of land that was suitable to grow Kapoho, a cultivar of excellent quality that adapted to the volcanic soil base there, allowing excellent drainage, had high rainfall and yet lots of sunshine, and the land there could be bought or leased at reasonable prices. By the 1970s, the Kapoho papaya grown in Puna accounted for 95% of the state's papaya production, making papaya the second most important fruit crop behind pineapple.

Despite strict quarantine on movement of papaya seedlings between islands, PRSV was discovered in the town of Hilo which was only about 18 miles away from the center of the papaya-growing area of Puna. However,

PRSV was indeed discovered in Puna in May 1992 (**Figure 3(a)**) and the Hawaiian papaya industry would be forever changed. By 1995, a third of the papaya growing area was completely infected and much of the rest of Puna had widespread infection (**Figure 3(b)**). By 1998, the production of papaya in Puna had dropped to 27 million pounds of papaya from 52 million pounds in 1992 when PRSV was discovered in Puna. In retrospect, the efforts of quarantine, monitoring and rogueing of infected plants in Hilo, and suppression efforts of PRSV in Puna all played key roles in helping Hawaii's papaya industry, because it gave researchers time to develop control measures for PRSV.

Research to develop tolerant varieties and cross-protection measures were started in the 1970s. Since resistance to PRSV has not been identified in *Carica papaya*, researchers have used tolerant germplasm in an attempt to develop papaya cultivars with acceptable PRSV tolerance and horticultural characteristics. However, tolerance to PRSV is apparently governed by a family of genes that is inherited quantitatively, which makes it technically difficult to develop cultivars of acceptable horticultural quality. Furthermore, the tolerant lines do become infected with PRSV, although fruit production continues still at a lower level. Indeed, in Thailand, the Philippines, and Taiwan, a number of tolerant lines have been developed and are used. However, Hawaii grows the small 'solo'-type papaya and efforts to introduce tolerance into acceptable 'solo' papaya cultivars have not been successful.

Efforts to use cross-protection were similarly started in the late 1970s to control PRSV in Hawaii. Cross-protection can be defined as the use of a mild strain of virus to infect plants that are subsequently protected against economic damage caused by a severe strain of the same virus. This practice has been used successfully for many years to minimize damage by citrus tristeza virus in Brazil, for example. In the early 1980s, a mild strain of PRSV (described above as PRSV HA 5-1) was developed through nitrous acid treatment of a severe strain, PRSV HA isolated from Oahu island. This mild strain was tested in Hawaii on Oahu island and showed good protection against damage by severe strains but produced symptoms that were very obvious on certain cultivars, such as Sunrise, especially in the winter months. This prominent symptom induction on certain cultivars and the logistics of mild strain buildup and inoculation of plants, among others, were factors that caused it not to be consistently used on the island of Oahu. There was no justification to use it on the island of Hawaii because PRSV was not yet found in Puna during the 1980s. Interestingly, the mild strain was used extensively for several years in Taiwan, but it did not afford sufficient protection against the severe strains from Taiwan and thus its use was abandoned after several years.

Transgenic Resistance

In the mid-1980s, an exciting development on tobacco mosaic virus (TMV) provided a rationale that resistance to plant viruses could be developed by expressing the viral *CP* gene in a transgenic plant. This approach was called CP-mediated protection, and, at about the same time, a report introduced the concept of 'parasite-derived resistance'. The report on transgenic resistance to TMV set off a flurry of work in many laboratories to determine if this approach could be used for developing resistance to other plant viruses. Likewise, work was initiated in 1985 to use this approach for developing PRSV-resistant transgenic papaya for Hawaii.

Key requirements for successful development and commercialization of transgenic virus-resistant plants are the isolation and engineering of the gene of interest, vectors for mobilization into and expression of the gene in the host, transformation and subsequent regeneration of the host cells into plants, effective and timely screening of transformants, testing of transformants, and the ability to deregulate and commercialize the product.

The *CP* gene of the mild strain of PRSV was chosen as the 'resistance' gene because it had been recently cloned and it was of the PRSV P type. The gene was engineered into a wide host range vector that could replicate in *Escherichia coli* as well as in *Agrobacterium tumefaciens*, the bacterium used for one of the most widely used methods of plant transformation. The commercial cultivars Kapoho, Sunrise, and Sunset were chosen for transformation. Initially, transformation of papaya was attempted using the *Agrobacterium*–leaf piece approach, where leaf pieces would be infected with *Agrobacterium* harboring the *CP* gene and transformed cells would be regenerated via organogenesis into transgenic plants. The latter is the direct regeneration of cells from an organ such as the leaf. This approach did not work due to our failure to develop plants from leaf pieces. A shift to the transformation of somatic embryos via the biolistic (often referred to as the gene gun) approach resulted in obtaining of about a dozen transgenic papaya lines, four of which expressed the *CP* gene. In 1991, tests of the R_0 lines identified a transgenic Sunset that expressed the *CP* gene of PRSV HA 5-1, and showed resistance to PRSV from Hawaii. A field trial of R_0 plants was started in April 1992 on as the island of Oahu, and a month later PRSV was discovered at Puna in May 1992, as discussed above.

The Oahu field trial showed that R_0 plants of line 55-1 were resistant, and line 55-1 was further developed to obtain the cultivar 'SunUp' which is line 55-1 that has the *CP* gene in a homozygous state, and 'Rainbow' which is an F_1 hybrid of SunUp and the nontransgenic 'Kapoho'. SunUp is red-fleshed and Rainbow is yellow-fleshed. In 1995, SunUp and Rainbow were tested in a subsequent field trial in Puna and showed excellent resistance (**Figure 3(c)**). Due to its



Figure 3 (a) Healthy Puna papaya in 1992. (b) Severely infected papaya orchards in Puna in 1994. (c) Field trial of transgenic papaya. PRSV-infected nontransgenic papaya on left and PRSV-resistant transgenic papaya on right. (d) Commercial planting of transgenic papaya one year after releasing seeds of PRSV-resistant transgenic papaya. (e) Transgenic papaya commonly sold in supermarkets. (f) Risk of growing nontransgenic papaya still exists in 2005. Foreground is PRSV-infected nontransgenic papaya that are cut, and background shows healthy PRSV-resistant transgenic papaya.

yellow flesh and good shipping qualities, Rainbow was especially preferred by the growers. Line 55-1 was deregulated by the US government and commercialized in May 1998. The deregulation also applied to plants that were derived from line 55-1. The timely commercialization of the transgenic papaya in 1998 was crucial since PRSV had decreased papaya production in Puna by 50% that year compared to 1992 production levels. The transgenic Rainbow papaya was quickly adopted by growers and recovery of papaya production in Hawaii was underway

(**Figure 3(d)**). The transgenic papaya is sold throughout Hawaii (**Figure 3(e)**) and the mainland USA, and to Canada where it was deregulated in 2003. However, several challenges remain: coexistence, exportation of nontransgenic papaya to Japan, deregulation of transgenic papaya in Japan, and the adoption of transgenic papaya in other countries that suffer from PRSV.

Hawaii still needs to grow nontransgenic papaya to satisfy the lucrative Japanese market as well as for production of organic papaya, for example. Interestingly, the islands of Kauai and Molokai do not have PRSV but grow only limited acreage of papaya. This situation illustrates the point that many factors influence decisions on the localities and crops that are grown. In Hawaii, Puna is the best place to grow papaya for the reasons mentioned above; there is a lot of land, farmers there are intuned to growing the crop, the region receives plenty of water, sunshine, and has a well-drained lava-based 'soil' structure, and there are high-quality cultivars adapted to the local growing conditions. The disadvantage is PRSV, but that disadvantage was overcome through the introduction of the PRSV-resistant Rainbow papaya that has good commercial attributes plus virus resistance. Puna accounts for 90% of Hawaii's papaya and, as of 2005, Rainbow represents 66% of the papaya grown in Puna. Growing nontransgenic papaya can be risky because PRSV is still around (**Figure 3(f)**), but judicious use of isolation from virus sources and constant rogueing can provide a means of raising nontransgenic papaya. However, a major market is Japan, which still has not deregulated the transgenic papaya. To maintain the lucrative Japanese market, Hawaii has to continue the exportation of nontransgenic papaya to Japan. The immediate solution is to concurrently grow nontransgenic and transgenic papaya, and subsequently to deregulate the transgenic papaya so it can be freely shipped into Japan. What approaches are being taken?

Currently, Japan accepts nontransgenic papaya but it needs to be free of 'contamination' by transgenic papaya. The Hawaii Department of Agriculture (HDOA) and the Japan Ministry of Agriculture, Forestry and Fisheries (MAFF) have agreed on an 'identity preservation protocol' (IPP), in which nontransgenic papaya can even be grown in close proximity (coexistence) to transgenic papaya in Puna, for example, and still be shipped to Japan. The protocol involves a series of monitoring and checkpoints in Hawaii that allow direct marketing of the papaya without delay while samples of the shipment are spot-checked by MAFF officials in Japan. The process has worked very well and allowed Hawaii to maintain its market share in Japan. This represents a practical case of 'coexistence' of transgenic and nontransgenic papaya and fruitful collaboration between governments (Hawaii and Japan) that provides mutual benefits to all parties.

The ideal situation would be, however, to freely export nontransgenic and transgenic papaya to Japan. To this end, efforts are underway to deregulate the transgenic papaya in Japan by obtaining approval from Japanese governmental agencies such as MAFF, the Ministry of Health, Labor, and Welfare (MHLW), and the Ministry of the Environment (MOE). MAFF has provisionally approved Hawaii's transgenic Rainbow and SunUp papayas, and efforts to present the final documentation to all three agencies are nearing completion. Deregulation of the transgenic papaya in Japan not only would expand the Hawaiian transgenic market but would also be a good case study for evaluating the effectiveness of commercialization and marketing of fresh transgenic products, since the transgenic papaya in Japan would be labeled, and subsequently consumers there would be given an opportunity to make a personal choice between a transgenic and nontransgenic product. The implications of this opportunity are obvious given the current 'controversial' climate over genetically modified organisms (GMOs) in the world. It is indeed rare that the previously little known PRSV could perhaps provide an example that would help us to resolve such controversies.

Summary Remarks

PRSV has been thoroughly characterized and is a typical member of the family *Potyviridae*, arguably the largest and economically most important plant virus group. The complete genome sequence has been elucidated and infectious transcripts have provided a means to determine the genetic determinants of some important biological functions such as host range and virulence. Furthermore, pathogen-derived resistance has been used to control PRSV in Hawaii through the use of virus-resistant transgenic papaya. In the US, only three virus-resistant transgenic crops have been commercialized: squash, papaya, and potato. The transgenic virus-resistant papaya provides a potential means to test the global acceptance of GMOs while presenting a plausible approach to control a disease affecting papaya worldwide.

See also: Plum Pox Virus; Potato Virus Y; Plant Resistance to Viruses: Engineered Resistance; Watermelon Mosaic Virus and Zucchini Yellow Mosaic Virus; Potyviruses.

Further Reading

Bateson M, Henderson J, Chaleeprom W, Gibbs A, and Dale J (1994) Papaya ringspot potyvirus: Isolate variability and origin of PRSV type P (Australia). *Journal of General Virology* 75: 3547–3553.

Fuchs M and Gonsalves D (2007) Safety of virus-resistant transgenic plants two decades after their introductions: Lessons from realistic field risk assessments studies. *Annual Review of Phytopathology* 45: 173–202.

Gonsalves D (1998) Control of papaya ringspot virus in papaya: A case study. *Annual Review of Phytopathology* 36: 415–437.

Gonsalves D, Gonsalves C, Ferreira S, *et al.* (2004) Transgenic virus resistant papaya: From hope to reality for controlling of papaya ringspot virus in Hawaii. *APSnet Feature, American Phytopathological Society, Aug.–Sept*. http://www.apsnet.org/online/feature/ringspot/ (accessed January 2008).

Gonsalves D, Vegas A, Prasartsee V, Drew R, Suzuki JY, and Tripathi S (2006) Developing papaya to control papaya ringspot virus by transgenic resistance, intergeneric hybridization, and tolerance breeding. *Plant Breeding Reviews* 26: 35–78.

Yeh SD and Gonsalves D (1994) Practices and perspective of control of papaya ringspot virus by cross protection. In: Harris KF (ed.) *Advances in Disease Vector Research*, pp. 237–257. New York: Springer.

Yeh SD, Jan FJ, Chiang CH, *et al.* (1992) Complete nucleotide sequence and genetic organization of papaya ringspot virus RNA. *Journal of General Virology* 73: 2531–2541.

Papillomaviruses: General Features of Human Viruses

G Orth, Institut Pasteur, Paris, France

© 2008 Elsevier Ltd. All rights reserved.

History

The recognition of different clinical types of warts and the suspicion of the venereal transmission of anogenital warts date from Greco-Roman times. Transmission of skin warts to humans by cell-free wart extracts demonstrated their viral etiology in 1907. Rabbit skin warts and derived carcinomas induced by the cottontail rabbit (Shope) papillomavirus (PV) provided the first model of viral carcinogenesis in mammals in the 1930s. The role of PVs in human cancer remained unexplored for a long time, mainly because of the lack of tissue culture systems allowing their propagation and study. It was long assumed that all types of human warts were due to a single virus, the human wart virus, until advances in DNA technology allowed the demonstration of the plurality of the human papillomavirus (HPV) in the late 1970s. It was proposed that the diversity of HPV-associated lesions resulted from distinct biological properties of the different HPV types. More than 100 HPV genotypes have been characterized so far.

The specific association of HPV type 5 (HPV-5) with the skin cancers of epidermodysplasia verruciformis (EV), a rare genetic disease, provided the first molecular evidence for the role of HPVs in human cancer. In the mid-1970s, koilocytes in cervicovaginal smears were recognized as HPV-infected cells, and their frequent association with features of cervical dysplasia suggested a role of HPV in cervical carcinogenesis. Specific HPV types (HPV-16 and HPV-18) were isolated from cervical cancers in the early 1980s. A wealth of virological, epidemiological, and molecular data now support the conclusion that specific HPV genotypes are etiologically related to over 95% of cervical carcinomas, the second most common cancer in women worldwide. For a long time, the prevention of cervical cancer relied only on the Papanicolaou (Pap) test, which involved the cytological detection of intraepithelial cancer precursors. Virological tests allowing the detection of the potentially oncogenic HPV genotypes now represent a means to improve the sensitivity of the screening of women at risk. A vaccine aimed at preventing infection with HPV-16 and HPV-18 is now available, paving the way for primary prevention of cervical cancer.

Virion Structure and Genome Organization

HPVs are characterized by a small (52–55 nm diameter), nonenveloped, icosahedral capsid composed of 72 pentameric capsomeres (**Figure 1**). Their genome is a double-stranded, covalently closed, circular DNA molecule of 7500–8000 bp. The viral DNA is associated with cellular histones to form a chromatin-like structure. The genetic information is located on a single DNA strand and consists of at least eight open reading frames (ORFs). The viral genome is divided into three regions (**Figure 2**): (1) a noncoding, long regulatory region (LRR) or upstream regulatory region (URR), which contains transcription and replication regulatory elements; (2) the early (E) region, which encodes proteins (E6 and E7) interacting with cellular proteins regulating the cell cycle, proteins (E1 and E2) involved in the replication and transcriptional regulation of the viral genome, a protein (E4) expressed late in the viral life cycle, and a small hydrophobic protein with growth-promoting properties, which is encoded by an ORF present in most HPV types either between E2 and L2 (E5) or overlapping E6 (E8); (3) the late (L) region, which encodes the capsid proteins, the major L1 protein constituting the capsomeres, and the minor L2 protein involved in viral DNA transport and encapsidation.

Taxonomy and Classification

The International Committee on the Taxonomy of Viruses recognized the family *Papillomaviridae* in 2005. More than 100 HPV types have been described so far, based on the

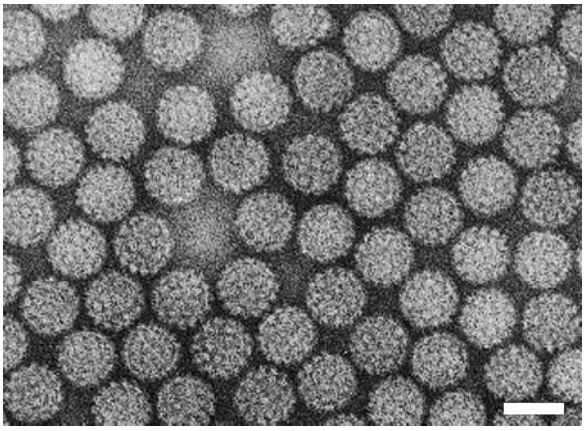


Figure 1 HPV-1 particles purified from plantar warts. Scale = 50 nm. Courtesy of Dr. O. Croissant.

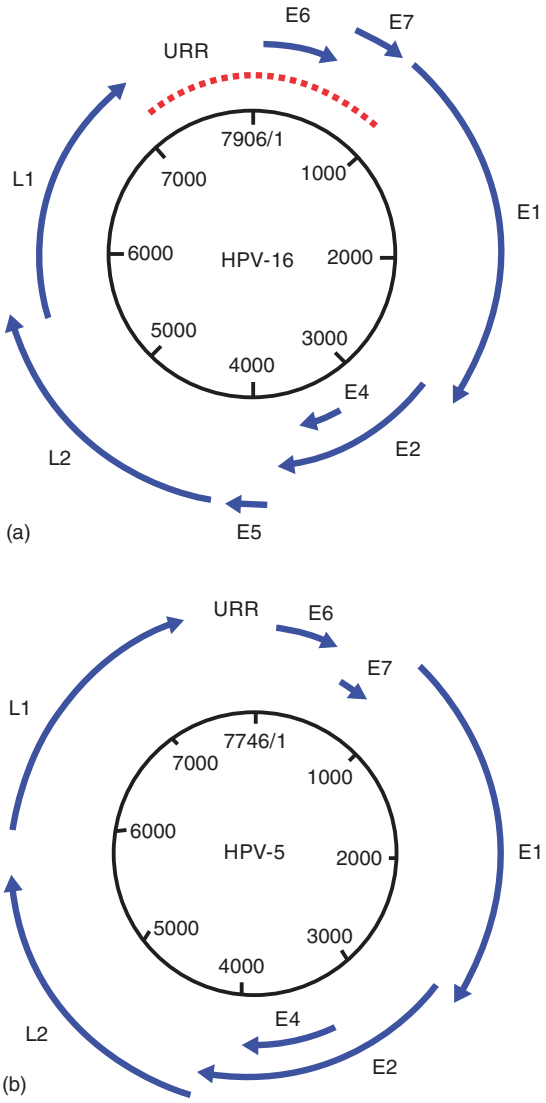


Figure 2 Genetic maps of (a) HPV-16 and (b) HPV-5. Note the lack of an E5/E8 ORF in HPV-5. The minimal HPV-16 genome fragment retained in carcinoma cells after integration into the host genome is indicated in red.

isolation of their complete genome and comparison of their nucleotide sequences. Two PV genomes are recognized as distinct types (genotypes) if they share less than 90% identical nucleotides in the L1 ORF, the most conserved genomic region. Types were designated by a number (1–106 so far), following the chronological order of their characterization. The PV taxonomy adopted recently is based on the phylogenetic relationships of the L1 ORF of animal and HPVs (**Figure 3**). Higher-order assemblages are considered as genera (identified by Greek alphabetical prefixes) and lower-order assemblages as species (each species identified by the number of the best known type). Genera share less than 60% nucleotide identity in the L1 ORF, species within a genus share 60–70%, and types within a species share 71–89%.

HPV types are distributed in five of the 16 PV genera (**Figure 3**). The genus *Alphapapillomavirus* comprises types differing by their genital, oral, or cutaneous tropism and by their pathogenicity (low-risk types associated with benign proliferations and high-risk types associated with invasive carcinomas). The genera *Gammapapillomavirus*, *Mupapillomavirus*, and *Nupapillomavirus* comprise types associated with cutaneous warts, whereas the types associated with EV belong to the genus *Betapapillomavirus* (**Figure 3** and **Table 1**). The genera differ by the presence of an E5 (*Alphapapillomavirus*) or E8 (*Gammapapillomavirus*, *Mupapillomavirus*) ORF or the absence of an E5/E8 ORF (*Betapapillomavirus*). Species comprise related PV types that often share similar biological and pathogenic properties. A large number of presumed novel HPV types, most of them belonging to the genera *Betapapillomavirus* and *Gammapapillomavirus*, have been partially characterized by sequencing short amplicons obtained with degenerate or consensus L1 primers. The number of HPV types is likely to exceed 200.

Within types, DNA sequence identities in the range of 90–98% correspond to subtypes, and isolates with more than 98% sequence identity represent variants. Whereas subtypes have only been described for a few HPV types (HPV-5, HPV-20, HPV-34, HPV-44, and HPV-68), variants have been identified for each HPV type, especially for the most clinically relevant genital types and HPV-5. Phylogenetic grouping of the HPV-16 L1 DNA variants identified in different geographic regions and ethnic groups has allowed recognition of five lineages (African-1, African-2, European, Asian, and African-American). A similar clustering of variants has also been reported for other genital types. The intratype variability does not usually exceed 2% in the coding region and 5% in the LRR. Some studies suggest that HPV-16 variants differ by their biological properties and oncogenic potential. More studies are needed to understand the impact of genetic intratypic variability on the persistence of infections with

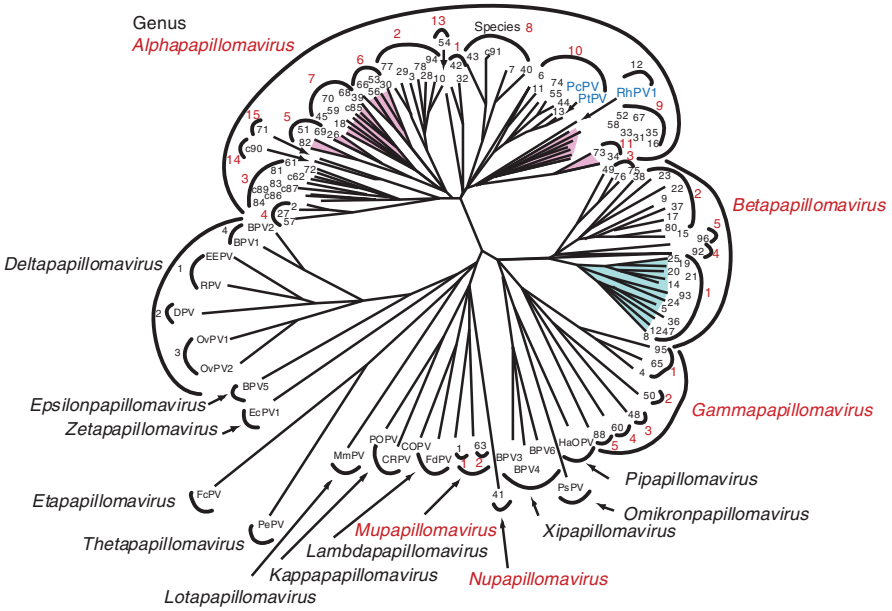


Figure 3 Phylogenetic tree of human and animal PVs based on the L1 ORF sequences of 118 PV types. Genera and species (the latter numbered according to the groups listed in **Table 1**) comprising HPV types are indicated in red. The species containing high-risk types associated with anogenital cancers are shaded in pink. The species containing HPV types associated with EV skin carcinomas is shaded in light blue. Types infecting nonhuman primates are indicated in blue. Reproduced from de Villiers EM, Fauquet C, Broker TR, Bernard HU and zur Hausen H (2004) Classification of Papillomaviruses. *Virology* 324: 20, with permission from Elsevier.

carcinogenic HPV types and the risk of malignant progression of the associated lesions. It should be stressed that HPV variants are stable entities. The observed HPV intratypic variability likely reflects the very slow co-evolution of these viruses with their host.

Host Range, Tissue Tropism, and Transmission

HPVs are highly host and tissue specific. Humans are not susceptible to infection by animal PVs, and HPVs do not infect other species. However, HPV capsids agglutinate mouse erythrocytes, and oncogenic genital HPVs are capable of transforming established rodent cell lines, which points to a ubiquitous cellular receptor. Tumors developed after grafting HPV-16-transformed cells to syngeneic mice provide models to test immunotherapeutic approaches. Transgenic mice for the HPV-16 or HPV-18 E6 and E7 genes develop benign or malignant cutaneous tumors.

HPVs infect keratinocytes of keratinizing or nonkeratinizing, pluristratified squamous epithelia. Some HPV types infect the epidermis of the skin, while others infect the epithelial component of the anogenital and oral mucous membranes. A major target for oncogenic genital HPVs is the squamous epithelium resulting from glandular metaplasia, at the squamocolumnar junction of the uterine cervix. Almost all cervical intraepithelial precancerous lesions and invasive carcinomas originate in this so-called transformation zone. The reserve cells located beneath the endocervical single-layered, columnar epithelium are likely to develop into either squamous metaplastic cells or columnar (glandular) cells, and this may account for the development of HPV-associated squamous cell carcinoma (SCC) or adenocarcinoma and their precursor lesions. Esophageal, tracheobronchial, nasal, conjunctival, and urinary epithelia are possible sites of infection with the known HPVs.

HPVs are very stable viruses, which are released from the desquamating superficial, differentiated keratinocytes of the infected epithelia. Infection of basal cells proceeds through minor abrasions of the epithelium. Transmission of cutaneous types occurs through direct contact with an infected tissue or contaminated objects and surfaces. Genital HPVs are generally transmitted by sexual contact. Transmission of HPV-6 and HPV-11 by passage through an infected birth canal is at the origin of laryngeal papillomatosis of young children.

Virus Replication

In vivo, the life cycle of low-risk and high-risk HPVs is closely linked to the biology of the keratinocyte. Keratinocytes are responsible for the renewal, cohesion, and barrier function of pluristratifying epithelia. HPVs most likely target the slow-cycling, self-renewing stem cells located in the basal layer of the epithelium and in the bulge of hair follicles, as well as their transient amplifying cell progeny.

Candidate cell-surface receptors are heparan sulfate proteoglycans and α6 integrin. Virus entry may result in a latent, asymptomatic infection, which is a still poorly understood phenomenon. The maintenance of viral DNA in stem cells, as autonomous replicating episomes, would only require the E1 and E2 proteins. Infection may also lead to the development of a productive lesion within 1–3 months. Depending on the tissue and the HPV type, these lesions are known as warts, papillomas, or low-grade anogenital intraepithelial lesions (**Table 1**). Lesions develop as a consequence of the proliferation of infected basal and parabasal cells maintaining episomal viral genomes at low copy number. This involves the expression of E1, E2, E5/E8, E6, and E7 genes. The E5/E8, E6, and E7 proteins likely release stem cells from their tight micro-environmental control and, through their interaction with host cell proteins, activate the host cellular DNA synthesis machinery and inhibit apoptosis in response to unscheduled DNA synthesis. This strategy allows the productive phase of the life cycle to proceed in the growth-arrested, terminally differentiating keratinocytes. Vegetative viral DNA replication, transcription of the E4, L1, and L2 genes from a differentiation-dependent promoter, synthesis of the late proteins, and viral particle assembly take place as the differentiating cells migrate toward the most superficial layers. Virion-containing squames are released into the environment (**Figures 4** and **5**). HPV replication provokes a cytopathic effect, such as the accumulation of cytoplasmic

Table 1 Main HPV genotypes and associated diseases

Genus and species[a]	*Group*[a]	*Types*[b]	*Tropism*	*Diseases*
Alphapapillomavirus				
Human papillomavirus 32	1	32	Oral	Focal epithelial hyperplasia
		42	Genital	Low-grade lesions
Human papillomavirus 10	2	3, 10, 28, 29, 77	Cutaneous	Flat and common warts
Human papillomavirus 61	3	61, 72, 81, 83, 84	Genital	Low-grade lesions
Human papillomavirus 2	4	2, 27, 57	Cutaneous	Common warts
Human papillomavirus 26	5	26, **51**, 69, **82**	Genital	Low- and high-grade lesions, invasive carcinomas
Human papillomavirus 53	6	30, 53, **56, 66**	Genital	Low- and high-grade lesions, invasive carcinomas
Human papillomavirus 18	7	**18, 39, 45, 59, 68**, 70	Anogenital	Low- and high-grade lesions, invasive carcinomas
Human papillomavirus 7	8	7	Cutaneous	Warts found in butchers
		40, 43	Genital	Low-grade lesions
Human papillomavirus 16	9	**16, 31, 33, 35, 52, 58**, 67	Anogenital	Low- and high-grade lesions, invasive carcinomas
Human papillomavirus 6	10	6, 11	Anogenital	Warts, cervical low-grade lesions, Buschke–Löwenstein tumors
			Laryngeal	Recurrent papillomatosis
		13	Oral	Focal epithelial hyperplasia
Human papillomavirus 34	11	34, **73**	Genital	Low- and high-grade lesions, invasive carcinomas
Human papillomavirus 54	13	54	Genital	Low-grade lesions
Human papillomavirus 71	15	71	Genital	Low-grade lesions
Betapapillomavirus				
Human papillomavirus 5	1	**5, 8**, 12, **14**, 19, **20**, 21, 24, 25, 36, **47,** 93	Cutaneous	EV
Human papillomavirus 9	2	9, 15, **17**, 22, 23, 37, 38, 80	Cutaneous	EV
Human papillomavirus 49	3	49, 75, 76	Cutaneous	EV
Human papillomavirus c and 92	4	92	Cutaneous	Premalignant/malignant lesions?
Gammapapillomavirus				
Human papillomavirus 4	1	4, 65	Cutaneous	Warts
Human papillomavirus 50	2	50	Cutaneous	Warts
Human papillomavirus 48	3	48	Cutaneous	Warts
Human papillomavirus 60	4	60	Cutaneous	Warts
Mupapillomavirus				
Human papillomavirus 1	1	1	Cutaneous	Plantar warts
Human papillomavirus 63	2	63	Cutaneous	Warts
Nupapillomavirus				
Human papillomavirus 41	1	41	Cutaneous	Warts

[a]Genera and species are defined in the *Eighth Report of the International Committee on Taxonomy of Viruses*. Groups of phylogenetically related types corresponding to species were numbered earlier according to a different scheme, which is utilized in **Figures 3** and **6**.
[b]Genotypes found in cancers are indicated in bold type.

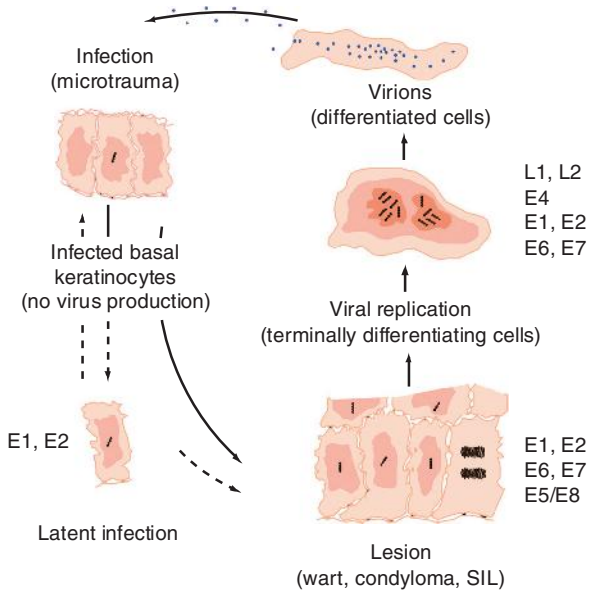


Figure 4 Life cycle of HPVs in keratinocytes. Viral genomes are retained as low-copy number episomes in the nuclei of latently infected cells and in basal and parabasal cells of benign lesions. Vegetative viral DNA replication occurs in more superficial, terminally differentiating cells. ORFs expressed are indicated.

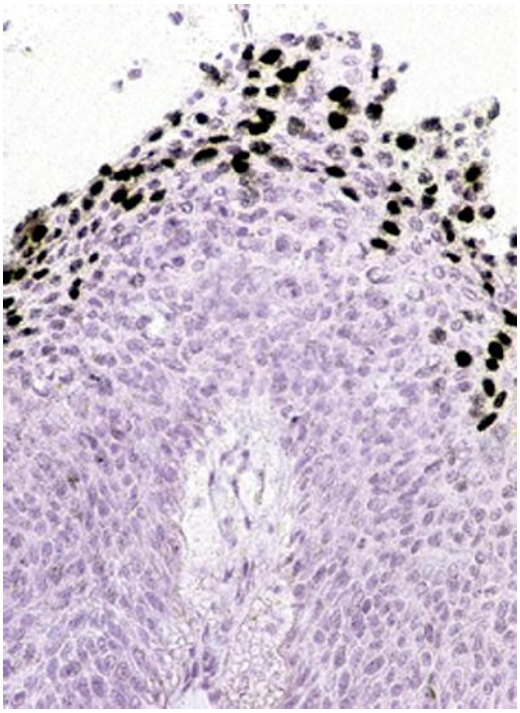


Figure 5 Vegetative replication of HPV-16 DNA in the nuclei of superficial cells of a high-grade cervical intraepithelial neoplasia, as detected by *in situ* hybridization. Courtesy of Dr. O. Croissant.

inclusions (containing the viral E4 protein) pathognomonic of HPV-1-induced skin warts, or the perinuclear cavity, binucleation, and heavily stained nuclei that characterize the koilocytes observed in anogenital lesions. In low-grade anogenital lesions, such as cervical intraepithelial neoplasia grade 1 (CIN1), basaloid cells and mitotic figures occupy the lower third of the epithelium, and koilocytosis is frequently marked. In high-grade lesions, neoplastic basaloid cells and mitotic figures occupy the lower two-thirds of the epithelium in CIN grade 2 (CIN2) or the full thickness of the epithelium in CIN grade 3 (CIN3). Koilocytes are often still identified in CIN2 but little, if any, virus production occurs in CIN3. Infection of endocervical glandular cells does not lead to the production of virions.

Routine propagation of HPVs *in vitro* has not yet been achieved. Until recently, the production of infectious viral particles has relied on cumbersome methods involving xenografts or organotypic raft cultures. Oncogenic genital HPV types are capable of immortalizing human keratinocytes in culture, and cell lines (e.g., HeLa or Caski cells) have been derived from cervical cancers. The viral genome is integrated into the cellular genome, which precludes virus multiplication in such cell lines. Cell lines harboring viral episomes have been isolated from cervical intraepithelial lesions or obtained by transfecting cloned genomes of genital HPVs into primary keratinocyte cultures. The late viral functions are expressed and viral particles are produced when cells are grown on collagen rafts at an air-liquid interface, in conditions allowing terminal differentiation of keratinocytes (organotypic cultures).

High-yield methods have been developed recently for producing HPV pseudoviruses carrying a reporter gene or for obtaining infectious HPV particles without any requirement for epithelial cell differentiation (co-transfection of codon-optimized L1 and L2 expression plasmids and full-length HPV genomic DNA). Pseudoviruses and infectious virions represent useful tools for studying the first steps of infection and performing neutralization assays.

Evolution

It is acknowledged that PVs have co-evolved with their hosts, with estimated nucleotide change rates of 1% per 100 000 to 1 000 000 years. All known HPV types are assumed to have already infected the anatomically modern humans who emerged in East Africa about 200 000 years ago and spread out of Africa some 60 000–50 000 years ago. The time required for the emergence of species, types, and subtypes can be inferred from a co-evolutionary model on the basis of the phylogenetic relationships between alphapapillomaviruses of monkeys (rhesus macaque (RhPV)), apes (common chimpanzee (PtPV) and pigmy chimpanzee (PcPV)), and humans (HPVs) (**Figure 3**). Specifically, the divergence between

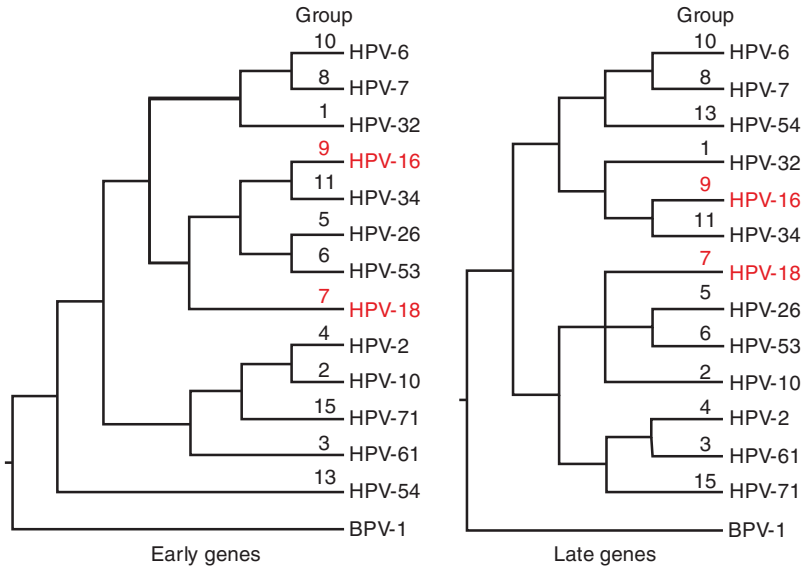


Figure 6 Phylogenetic trees of human alphapapillomaviruses based on the nucleotide sequences of early (E6, E7, E1, E2) and late (L2, L1 genes). The species corresponding to the groups shown are listed in **Table 1**. HPV-16 (group 9) and HPV-18 (group 7) show different groupings in the early and late phylogenies. From Narechania A, Chen Z, DeSalle R, and Burk RD (2005) Phylogenetic incongruence among oncogenic genital alpha human papillomaviruses. *Journal of Virology* 79: 15503–15510, with permission from the American Society for Microbiology.

Old World monkeys and the common ancestor of apes and humans, between the chimpanzee and human lineages, and between common and pigmy chimpanzees occurred approximately 25, 6, and 0.9 million years ago, respectively. Thus, emergence of a PV species would take 10–25 million years, with types diverging within a few million years and subtypes within less than 1 million years.

The mechanisms that have led to the extreme diversity among HPVs and their distinct tissue tropisms are little understood. It is likely that both selection and random genetic drift as well as founder effects were involved, as in human evolutionary history. The extent to which interspecies transmission and recombination events contributed to diversification remains an open question. Comparison of phylogenies of early and late genes of oncogenic genital HPV types disclosed a phylogenetic incongruence, which suggests that their evolution may not have been monophyletic across all genes (**Figure 6**). This may have involved an ancient recombination event in the lineage derived from a common ancestral oncogenic PV or an asymmetric genome convergence driven by selective constraints on particular genes.

Serological Relationships and Variability

Virions can be purified from lesions for some skin HPV types only, and early studies led to the identification of type-specific antigens and group-specific (type-common) antigens, using antibodies against intact or disrupted viral particles. HPV capsids are composed of 360 L1 molecules (72 capsomeres) and 12–30 L2 molecules displaying their N-terminal region on the capsid surface. When produced in various eukaryotic expression systems, L1 proteins self-assemble into virus-like particles (VLPs), which are morphologically similar to virions. When co-expressed with L1, L2 is incorporated stably into the capsids. Monoclonal antibodies raised against L1 VLPs have allowed the identification of both conformational, usually type-specific epitopes and surface or cryptic linear epitopes that show various levels of cross-reactivity when analyzed by an ELISA test using intact or denatured VLPs. Distinct serotypes have been identified only for HPV-5. Only the closely related types HPV-6 and HPV-11, or HPV-18 and HPV-45, exhibited detectable cross-reactions when the serological relatedness of genital HPV types was assessed using a hemagglutination inhibition test.

Neutralizing antibodies can be demonstrated by a xenograft infectivity assay, a cell culture focus assay (HPV/bovine PV-1 pseudovirions), assays based on HPV pseudoviruses carrying a reporter gene, or antibody displacement tests. Type-specific neutralizing antibodies develop in response to natural HPV infections and VLP immunization. They recognize conformational epitopes mapped to surface-exposed, hypervariable loops of the L1 protein. HPV-16 variants belong to a single serotype. VLPs of closely related types (HPV-6/HPV-11, HPV-16/HPV-31, HPV-31/HPV-33, and HPV-18/HPV-45) share one or more cross-neutralization epitopes that are less immunogenic than type-specific epitopes. Broadly

cross-neutralizing epitopes shared by cutaneous and mucosal human and animal PVs have been found at the N-terminus of the L2 protein.

Epidemiology

Epidemiologic studies on HPV infections have been hindered by the great multiplicity and phylogenetic diversity of HPV types and by methodological problems. The Southern blot hybridization technique (without prior amplification of viral sequences), the first gold standard for detecting and identifying HPVs, has a low sensitivity and is labor intensive. The US FDA-approved Hybrid Capture 2 test, based on liquid hybridization of HPV DNA sequences with a mixture of 13 high-risk HPV RNA probes and the chemiluminescent detection of hybrids, does not distinguish individual types. This test is useful as an adjunct to Pap smear for the management of equivocal cervical cytology and screening for cervical cancer. HPV detection and genotyping methods currently used for research purposes rely on amplification of viral DNA sequences (polymerase chain reaction (PCR)-based tests) in fresh specimens (cervical scrapes, skin swabs, plucked hairs, or biopsy specimens) or archival smears, and biopsies. These sensitive tests allow screening for the whole spectrum of HPV infections, from asymptomatic to overt infections. A broad spectrum of genital or cutaneous HPV types can be detected with degenerate or consensus primers targeting a conserved region (usually the L1 ORF). HPV amplicons are detected by hybridization with generic probes or cocktails of probes and identified by dot blot hybridization or reverse blot assays, using type-specific oligonucleotide probes. Sequencing PCR products allows identification of types and variants or putative novel HPV types. Viral loads can be determined by type-specific, real-time PCR. Seroepidemiological studies are based on ELISA using L1 VLPs and detect past or current infections.

Genital and cutaneous HPV infections are widespread and have a worldwide distribution. An impressive diversity of known human beta- and gammapapillomaviruses or related putative novel types (over 100) was identified in the normal skin of healthy adults. Skin HPVs cause highly prevalent, asymptomatic infections likely to be acquired very early in infancy, which may suggest a commensal nature. High detection rates of DNA sequences of HPV-5 (associated with EV carcinomas) and of anti-HPV-5 antibodies have been reported in patients with psoriasis and other conditions involving extensive keratinocyte proliferation. Over 40 HPV types of the genus *Alphapapillomavirus* infect the anogenital tract. Genital HPV infections are very common in sexually active young women (about one-third of normal women in their teens and early twenties) and men. The 3-year cumulative incidence of HPV infection among young women has been found to exceed 40% in several studies. The concurrent or sequential detection of more than one type is a common finding. Prevalence of cervical infections decreases with increasing age, with, sometimes, a second peak after age 55. HPV-16 is one of the more widespread types. Most infections are asymptomatic and transient. Most women appear to have cleared their infection after 1 or 2 years, with a median duration of HPV DNA detectability of approximately 1 year. Cytological signs of infection (koilocytes) are observed at three- to tenfold lower rates than HPV DNA detection. Most cytological abnormalities correspond to low-grade lesions and most regress spontaneously within a year and represent transient productive infections. Only a minority of women exposed to genital HPVs will show persistent infection (approximately 10% being still HPV DNA positive after 5 years) and have a substantial risk to develop a high-grade cervical lesion and, possibly, an invasive carcinoma when infected with high-risk types. High-grade CIN may develop from persistent low-grade lesions or arise without any history of mild cytological atypia. CIN2 lesions are virologically more heterogeneous than CIN3, and have a greater chance to regress and a lower risk to progress to invasive cancer. As a safety measure, all lesions diagnosed as CIN2 or worse have to be treated. Type-specific differences are observed in the natural history of HPV infections in terms of prevalence, regional distribution, association with cytological abnormalities, duration, and risk for the development of a high-grade lesion. HPV-16 persists longer than most other types and has a higher prevalence, and persistent HPV-16 infections are more likely to cause cervical precancerous and malignant disease.

Sexual behavior (multiple lifetime or recent sex partners) is the major risk factor for genital HPV infection. Immune factors play an important role in the natural history of HPV infections. For instance, human immunodificiency virus (HIV)-infected women display two- to fourfold higher rates of cervical HPV infection and homosexual male AIDS patients have a high prevalence (about 50%) of anal HPV infection. Genetic factors are predominantly involved in two HPV-associated diseases, oral focal epithelial hyperplasia (Heck's disease) and EV.

Pathogenicity

Each HPV type has a specific pathogenicity. A number of HPV types induce benign, self-limiting proliferations of the skin or the mucous membranes (**Table 1**), which usually regress spontaneously or after treatment. Each clinical type of skin wart described is preferentially associated with specific HPV types. HPV-6 and HPV-11 cause anogenital warts. Both types are associated with the rare

anogenital Buschke-Löwenstein tumors (verrucous carcinomas) and single cases of carcinomas of the respiratory tract, but are almost never detected in cervical carcinomas. Other HPV types are etiologically associated with the development of invasive carcinomas (**Table 1**). In 2005, the International Agency for Research on Cancer recognized HPV-16, HPV-18, HPV-31, HPV-33, HPV-35, HPV-39, HPV-45, HPV-51, HPV-52, HPV-56, HPV-58, HPV-59, and HPV-66 as carcinogenic to humans and HPV-5 and HPV-8 as carcinogenic to patients with EV.

Contrasting with the many betapapillomaviruses associated with EV benign lesions (EV-specific HPVs), only a few (HPV-5 and, occasionally, HPV-8, HPV-14, HPV-17, HPV-20, and HPV-47) are found in EV premalignant lesions and SCCs of the skin. Viral genomes are maintained as high copy number episomes and E6 and E7 transcripts are easily detected. Invalidating mutations in both alleles of either the *EVER1/TMC6* or the *EVER2/TMC8* gene, encoding transmembrane proteins of unknown function, usually cause the abnormal susceptibility of EV patients to infection by betapapillomaviruses. DNA sequences of a large variety of cutaneous HPV types are detected in low amounts (usually much less than one copy of viral genome per cell) in 25–55% of cutaneous basal cell carcinomas and SCCs in the general population, and in an even higher proportion of SCC (up to 90%) in immunosuppressed patients. Similar detection rates are found for premalignant skin lesions (actinic keratoses and Bowen's disease), and also for healthy skin. The role of HPVs in skin carcinogenesis in non-EV patients remains a matter of debate.

It is now firmly established that persistent infection by specific, sexually transmitted high-risk alphapapillomaviruses is causally associated with the development of over 95% of invasive carcinomas of the uterine cervix and their immediate intraepithelial precursors. About 85% of cervical cancers are SCCs and most of the other cases are adenocarcinomas. This casual relationship is based on virological, epidemiological, and molecular evidence. It is first supported by the consistency of HPV DNA detection in invasive cancers and the specificity of the types detected. Of the 40 HPV types associated with low-grade CIN, 16 are found in cancers. The seven most frequently detected types are, by decreasing order of frequency, HPV-16 (about 55%), HPV-18 (about 15%), HPV-45, HPV-31, HPV-33, HPV-52, and HPV-58, and account for about 87.5% of invasive cancers, worldwide. Other types are found at frequencies lower than 2% (HPV-35, HPV-39, HPV-51, HPV-56, HPV-59, HPV-66, HPV-68, HPV-73, and HPV-82). Some geographical variations have been observed, with a higher prevalence of cervical cancer associated with HPV-45 in Africa or HPV-52 and HPV-58 in China and Japan. The same HPV types are found in CIN3, the immediate precursor of SCC. While HPV-16 predominates in SCCs, HPV-16 and HPV-18 are almost as frequent in adenocarcinomas.

A strong association between the detection of high-risk HPV DNA and high-grade CIN or invasive cancer was demonstrated in case-control studies. The odds ratio values, that is, the ratio of the odds of being infected among cases and controls, ranged from 45 to 430 for 13 HPV types. Cohort studies showed that women positive at baseline for high-risk types (especially HPV-16) have a higher risk of developing CIN3 and invasive cancer and that this risk is closely linked to persistent infections. Infection thus precedes the development of precancer and cancer, a prerequisite for establishing causality.

Experimental studies support the role of high-risk HPV types in the whole process of cervical carcinogenesis. Integration of the viral genome into the cellular genome is observed in the majority of cancers (**Figure 7**), and in high-grade lesions. Disruption of the E1/E2 region inactivates the negative regulation exerted by the E2 protein on transcription of the E6/E7 region, which results in constitutive expression of E6 and E7. These multifunctional oncoproteins complex and inactivate cellular proteins, such as p53 protein (E6) and pRB protein (E7), involved in negative regulation of the cell cycle and the response to DNA-damaging agents. This leads to cell proliferation and genetic and chromosomal instability. CIN3 may evolve into invasive SCC after years or decades. This involves the accumulation of genetic and epigenetic events targeting cellular oncogenes or tumor-suppressor genes.

HPV infection is a necessary, but not sufficient, cause of cervical cancer. Long-term use of hormonal contraception, high parity, tobacco smoking, and coinfection with HIV increase the risk of cancer in HPV-infected women. Other probable cofactors are coinfection with herpes simplex virus type 2 or *Chlamydia trachomatis* and dietary factors. Viral factors, such as HPV variants, viral load, and viral integration, are likely to play a part. Host genetic factors remain little understood. The positive or negative

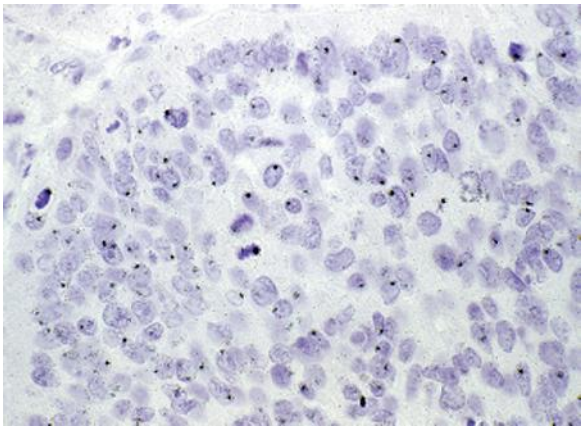


Figure 7 Integrated HPV-16 DNA sequences in an invasive cervical squamous cell carcinoma, as detected in nuclei (dots) by *in situ* hybridization. Courtesy of Dr. O. Croissant.

associations of CIN and/or invasive carcinoma with certain human leukocyte antigen (HLA) class II alleles or haplotypes suggest an immunogenetic control.

High-risk genital HPV types, mostly HPV-16, are also associated with other anogenital invasive cancers and their precursors: the great majority of vaginal and anal carcinomas and about 50% of vulvar and penile carcinomas (basaloid and warty tumors). HPV-16 DNA has been detected in approximately 20% of oropharyngeal carcinomas and in a limited number of carcinomas of the oral cavity.

Clinical Features of Infection

Skin warts (papillomas) occur predominantly in children over 5 years and in young adults. At least two-thirds of cases regress spontaneously within 2 years. Palmo-plantar myrmecia warts are deep and painful. Common warts are elevated, hyperkeratotic and located mainly on the hands, fingers, and knees. Flat warts are usually multiple, slightly raised, and are localized mainly on the hands and face. EV is a lifelong genodermatosis characterized by disseminated flat, wart-like lesions and macules. In about half of the patients, some lesions start converting to premalignant lesions and invasive SCCs, usually on light-exposed areas, about 20 years after the onset of the disease.

Genital HPV infections represent the most commonly diagnosed viral sexually transmitted diseases. Genital warts and Bowenoid papules are found mostly in young adults, on the external genitalia and in the anal region. Anogenital warts are soft, exophytic proliferations. Bowenoid papules, also recognized as vulvar, penile, and anal intraepithelial neoplasia (VIN, PIN, and AIN), are flat or somewhat elevated, often pigmented, usually multiple, and display a rather benign clinical course in spite of their usual association with HPV-16 and histological features of intraepithelial neoplasia. This is in contrast to solitary lesions of genital Bowen's disease (high-grade intraepithelial neoplasia) affecting individuals over 50 years. Anal lesions with a variety of morphological appearance and features of intraepithelial neoplasia are found at the anorectal junction in HIV-infected males. Infection of the cervix results mostly in flat, acetowhitening areas corresponding to low-grade CIN. Cytological evidence of HPV infection is found in cervicovaginal smears of 2–3% of women in mass-screening programs. Infection with genital HPVs are often multiple, and associated diseases can affect the entire anogenital region.

Oral focal epithelial hyperplasia is observed in children and adults. The disease is highly prevalent in American Indians and Eskimos but rarely observed in Caucasians. It is characterized by multiple papules, which never undergo malignant transformation. Laryngeal papillomatosis is a rare recurrent disease that can occur at any age. The most frequent site affected is the vocal cords. Trachea, lung, oral, and nasal cavities can also be involved. Respiratory papillomatosis may be life-threatening by obstructing airways.

Pathology and Histopathology

Benign lesions are characterized by variable degrees of hyperplasia of the various epithelial layers and hypertrophy of dermal papillae (papillomatosis). Some features may reflect distinct biological properties of the associated HPV types. The extent of papillomatosis is prominent for HPV-1, HPV-2, HPV-4, HPV-6, HPV-7, or HPV-11, much less pronounced for HPV-3 or HPV-10, and often absent for EV-HPVs. The cytopathic effect associated with viral replication in differentiating cells is recognized as koilocytosis for anogenital alphapapillomaviruses, and presents specific features (number, size, shape, and structure of cytoplasmic inclusions or extent of perinuclear vacuolization) for cutaneous lesions induced by alpha-, beta-, gamma-, mu-, and nupapillomaviruses.

Anogenital lesions with features of squamous intraepithelial neoplasia (CIN, VIN, PIN, AIN) may show different degrees of severity. The grading of the lesions depends on the proportion of epithelium occupied by basal-type cells. Basaloid cells restricted to the lower third of the epithelium, usually showing mild atypia, and a well-preserved maturation define low-grade (grade 1) intraepithelial neoplasia. In high-grade lesions, neoplastic basaloid cells occupy up to two-thirds of the epithelium (grade 2) or the whole epithelium (grade 3). The disorganized architecture of the epithelium includes abnormal mitotic figures and nuclear atypia. For cervical lesions, CIN1 corresponds to mild dysplasia, CIN2 to moderate dysplasia, and CIN3 to severe dysplasia and carcinoma *in situ*, according to the dysplasia-carcinoma *in situ* classification. The Bethesda System of nomenclature for the evaluation of cervicovaginal Pap smears has introduced low-grade squamous intraepithelial lesions (SIL), encompassing HPV infection (koilocytes) and CIN1, and high-grade SIL, encompassing CIN2 and CIN3. Equivocal atypical squamous cells (ASC) are labeled ASC-US (undetermined significance) or ASC-H (cannot exclude a high-grade lesion). Features specific to Bowen's disease, especially individual cell keratinization, are observed in Bowenoid papules and EV lesions undergoing malignant conversion.

Immune Response

Strong immune responses are not usually generated because HPVs most often induce asymptomatic infections and because productive infection does not lead to host-cell lysis. A major problem remains the availability of well-standardized, sensitive, and specific assays.

Humoral immune responses upon natural infections or immunization are evaluated using VLP-based ELISA tests or pseudovirus-based neutralization assays. IgG and IgA antibody responses to HPV infections are weak and not always detected (50–75% of women with HPV-6/HPV-11- or HPV-16-associated disease). Seroconversion is a slow process. IgG antibody levels are generally stable during several years. Men show lower seropositivity rates when compared to women at similar risk. Although predominantly type-specific, the VLP-based ELISA test may also recognize non-neutralizing, cross-reactive, linear epitopes disclosed by ill-assembled VLPs. The neutralization assay may be substantially more type-specific. Neutralizing antibodies are found in about 75% of women with prevalent HPV-16 or HPV-18 infections. Such antibodies are likely to prevent repeat infections. Antibodies to native E6 or E7 oncoproteins are rarely detected in patients with precancerous cervical lesions but are found in about 50% of patients with late-stage invasive cervical carcinomas. They constitute virus type- and cancer-specific markers, with no prognostic value.

HPV infections and HPV-associated lesions usually clear spontaneously. Cell-mediated immunity (CMI) is thought to play a major role in this outcome. A high incidence of cutaneous and anogenital HPV-associated diseases is observed among patients with acquired or iatrogenic CMI deficiencies. Infiltrates of CD4+ and CD8+ T-lymphocytes and macrophages are observed in regressing flat warts, genital warts, and CIN. This suggests that cytotoxic T-lymphocyte (CTL) responses and delayed-type hypersensitivity reactions are important in the regression of HPV-induced lesions. The E1 and E2 proteins (required for viral genome episomal maintenance and replication) and E6 and E7 proteins (upregulated during tumor progression) are likely targets of the adaptive CMI responses. Several HLA-restricted CTL and T-helper (Th) cell epitopes have been identified in HPV-16 early proteins. Memory CD4+ Th cell responses to HPV-16 E2 and E6 have been detected in approximately half of healthy women but found to be absent or impaired in patients with high-grade CIN or invasive cancers. CTL responses against E6 and E7 have been found occasionally in patients with cervical HPV-16 associated infection or disease but no obvious relationship has been found so far with regression or progression of the disease. There is some evidence for defective natural killer cell function in patients with HPV-16 associated disease.

Genital high-risk HPV types, especially HPV-16, have evolved mechanisms to evade innate and adaptive immunity. E6 and E7 oncoproteins interact with cellular proteins involved in the synthesis of, or response to, type 1-interferons. Unlike dermal dendritic cells, Langerhans cells, the resident epithelial antigen-presenting cells, are not activated by uptake of HPV VLPs. An altered expression of HLA class I antigens (downregulation) and class II antigens (upregulation) is observed in a substantial proportion of CIN and cervical carcinomas. Tumor cells may thus evade immune surveillance by altering their interaction with CD8+ CTLs or turn into nonprofessional antigen-presenting cells, which may result in T-cell anergy.

Patients suffering from certain primary immunodeficiency diseases are prone to chronic cutaneous and/or genital HPV disease: (1) patients with the WHIM syndrome (mutations in the gene encoding the CXCR4 receptor) and (2) late after hemopoietic stem-cell transplantation, patients with severe combined immune deficiency caused by common γc cytokine receptor subunit or Janus kinase-3 deficiency. Inactivation of *EVER* genes may result in the development of EV lesions by complementing for the lack of an E5/E8 ORF in EV-specific HPVs. EV could thus represent a primary deficiency of intrinsic immunity against certain HPV types.

Prevention and Control

Education on prevention of sexually transmitted diseases should include appropriate information on HPV infections, their role in cervical cancer, and the available prevention strategies, which makes invasive cervical cancer an almost entirely preventable disease.

There are no specific anti-HPV therapeutic agents (targeting E6, E7, E1, or E2). Treatment modalities are mostly aimed at destroying or removing lesions. Conventional treatments involve topical applications of caustic agents, physical destruction, surgical removal, or chemotherapeutic agents. Immune response modifiers could be of value (interferon or imiquimod, an agonist of Toll-like receptor 7). Therapeutic vaccines aimed at generating specific CTL responses to eradicate premalignant lesions, such as CIN2/3, have targeted the HPV E6 and E7 oncoproteins. Phase I/II trials have been performed or are in progress to test vaccines based on recombinant viruses, naked DNA, fusion proteins, peptides, or pulsed dendritic cells. Contrasting with preclinical studies in murine models, most published trials showed little efficacy in patients, but some promising approaches are currently being tested.

The implementation of population-based cervical cancer screening programs, based on the Pap test, proved to be a most successful strategy for dramatically decreasing cervical cancer incidence and mortality. To increase the sensitivity of the detection of pre-invasive lesions and cervical cancer, several US organizations recommend the use of an FDA-approved HPV DNA test for high-risk HPV types in conjunction with cytology (conventional Pap test or liquid-based cytology), as a screening option for women aged 30 years and older. Future screening strategies may combine HPV testing and a cytological triage of women found HPV positive.

Two prophylactic subunit vaccines have recently been developed, the tetravalent (Merck) and bivalent (Glaxo-SmithKline, GSK) vaccines, which contain HPV-16, HPV-18, HPV-6 and HPV-11 L1 VLPs and HPV-16 and HPV-18 L1 VLPs, respectively. Clinical trials have established their safety and consistent immunogenicity in young adult women and their high efficacy in preventing persistent infections, premalignant lesions, and genital warts caused by the vaccine HPV types. The tetravalent vaccine was licensed by the US FDA in 2006, as a vaccine for girls and women 9–26 years of age (prior to exposure to the risk of HPV infection) to prevent cervical cancer, genital warts, high-grade cervical, vulvar and vaginal lesions, and CIN1 caused by HPV-6, HPV-11, HPV-16, and HPV-18. Approval of the bivalent vaccine is expected in 2007. Both vaccines should theoretically protect against ~70% of cervical cancers, 60–70% of CIN2/3 and 25–30% of CIN1, and the tetravalent vaccine also against over 90% of genital warts. A general agreement is the need for regular screening of vaccinated women according to current guidelines, to check for the long-term efficacy of the vaccine and detect lesions associated with non-vaccine HPV types. Several issues remain to be addressed: duration of protection (need for boosters), long-term safety, degree of cross-protection against non-vaccine HPV types, and efficacy in men. A major concern is the availability of the vaccine in developing countries, where over 80% of worldwide cervical cancers occur and screening programs are not available. Incorporating additional high-risk HPV types or, for instance, taking advantage of the broadly cross-neutralizing epitopes borne by the L2 protein, should provide strategies for second generation vaccines.

See also: Papillomaviruses: Molecular Biology of Human Viruses; Papillomaviruses of Animals; Tumor Viruses: Human; Viral Pathogenesis.

Further Reading

Bosch FX, Lorincz A, Munoz N, Meijer CJ, and Shah KV (2002) The causal relation between human papillomavirus and cervical cancer. *Journal of Clinical Pathology* 55: 244–265.

Bosch FX, Schiffman M, and Solomon D (eds.) (2003) Future directions in epidemiologic and preventive research on human papillomaviruses and cancer. *Journal of the National Cancer Institute Monographs* 31: 1–130.

Campo MS (ed.) (2006) *Papillomavirus Research: From Natural History to Vaccines and Beyond*. Wymondham, UK: Caister Academic Press.

de Villiers EM, Fauquet C, Broker TR, Bernard HU, and zur Hausen H (2004) Classification of papillomaviruses. *Virology* 324: 17–27.

Frazer IH, Thomas R, Zhou J, *et al.* (1999) Potential strategies utilised by papillomaviruses to evade host immunity. *Immunological Reviews* 168: 131–141.

Harper DM, Franco EL, Wheeler CM, *et al.* (2006) Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: Follow-up from a randomised control trial. *Lancet* 367: 1247–1255.

Howley PM and Lowy DR (2007) Papillomaviruses. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 2229–2354. Philadelphia, PA: Wolters Kluwer/Lippincott Williams and Wilkins.

IARC (2006) *IARC Monographs on the evaluation of carcinogenic risks to humans, Vol. 90: Human Papillomaviruses.* Lyon: International Agency for Research on Cancer Press.

Münger K, Baldwin A, Edwards KM, *et al.* (2004) Mechanisms of human papillomavirus-induced oncogenesis. *Journal of Virology* 78: 11451–11460.

Munoz N, Bosch FX, de Sanjosé S, *et al.* (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. *New England Journal of Medicine* 348: 518–527.

Narechania A, Chen Z, DeSalle R, and Burk RD (2005) Phylogenetic incongruence among oncogenic genital alpha human papillomaviruses. *Journal of Virology* 79: 15503–15510.

Orth G (2006) Genetics of epidermodysplasia verruciformis: Insights into host defense against papillomaviruses. *Seminars in Immunology* 18: 362–374.

Orth G and Jablonska S (eds.) (1997) Papillomaviruses: Part two. *Clinics in Dermatology* 15: 303–470.

Villa LL, Costa RLR, Andrade RP, *et al.* (2006) High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 virus-like particle vaccine through 5 years of follow-up. *British Journal of Cancer* 95: 1459–1466.

zur Hausen H (2002) Papillomaviruses and cancer: From basic studies to clinical application. *Nature Reviews Cancer* 2: 342–350.

Papillomaviruses: Molecular Biology of Human Viruses

P F Lambert and A Collins, University of Wisconsin School of Medicine and Public Health, Madison, WI, USA

© 2008 Published by Elsevier Ltd.

Introduction

Papillomaviruses (PVs) are nonenveloped, double-stranded DNA tumor viruses that infect mammalian and avian hosts. There are more than 100 genotypes of human papillomaviruses (HPVs) that can either infect mucosal or cutaneous epithelia. PVs all share the common property of inducing benign proliferative lesions within the epithelial tissues they infect. In these lesions, the virus is replicated in a manner that is exquisitely tied to the differentiation program of the epithelial host cell. Within the mucosal genotypes, HPVs are further subcategorized as low- or high-risk genotypes, reflective of the latter's additional association with frank cancer. High-risk HPVs

are best known as the etiologic agent of virtually all cervical cancer, other anogenital cancers, as well as a subset of head and neck cancers. The high-risk HPV most commonly found in these cancers is HPV-16. In this article, the nature of the papillomaviral life cycle as discovered through the study of this high-risk HPV is described. We also compare and contrast its life cycle to that of other PVs, where possible.

Genome

PVs contain an ~8000 bp, circular, double-stranded DNA genome. The encapsidated genome is thought to exist in a chromatinized state within the virus particle; however, there is one report claiming an absence of cellular histones in virus particles. In the infected host cell, the viral genome is delivered to the nucleus where it remains in an extrachromosomal state throughout the viral life cycle. Integration of the viral DNA into the host chromosome is not a normal part of the viral life cycle but can occur in some PV-associated cancers.

The viral genome is organized into three regions: the long control region (LCR), which contains many *cis*-elements involved in the regulation of both DNA replication and transcription; as well as the early (E) and late (L) regions that encode subsets of genes so denoted by their onset of expression in the context of the viral life cycle (**Figure 1**). Transcription of the viral genome occurs unidirectionally and is directed from multiple promoters that are located within the LCR and the 5′ end of the E region. Multiple splicing and polyadenylation signals are used depending on the stage of the viral life cycle, leading to a variety of transcripts with differential coding capacity. The viral genome codes for eight viral genes. The early gene products, which are coded from the E region, contribute to the transcription, replication, and maintenance of the genome as a stable, nuclear plasmid within the poorly differentiated compartment of the infected tissue, as well as its amplification and encapsidation within the more terminally differentiated cellular compartment to make progeny virus. A subset of the early genes (E5, E6, and E7) possesses transforming properties in tissue culture, and tumorigenic properties in experimental animal models. The late structural genes encode for the major capsid protein, L1, and the minor capsid protein, L2. The individual role of each viral gene in the life cycle is described in this article.

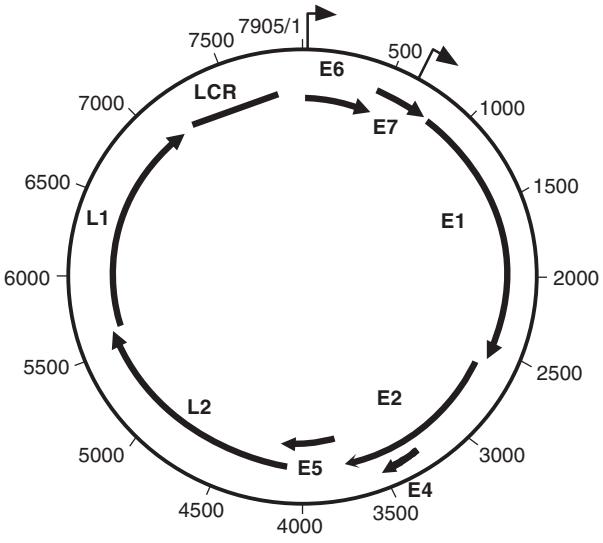


Figure 1 The HPV genome. The locations of the protein-coding sequences of early (E) and late (L) genes, and those of the long control region (LCR) and transcriptional promoters (arrows), are shown.

Pathogenesis

PVs cause warts: benign, self-regressing lesions of the skin, oral cavity, and anogenital tract. The mucosotropic HPVs are the leading viral cause of sexually transmitted disease in women, with over 50% of sexually active women having been infected in their lifetime. A subset of these mucosotropic HPVs, the so-called 'high-risk' genotypes, are the cause of virtually all cervical cancer, with approximately 500 000 new cases diagnosed yearly, and approximately half as many deaths due to this cancer, making it the second leading cause of death by cancer among women worldwide. Precursor lesions to cervical cancer, called cervical intraepithelial neoplasia (CIN) grades 1–3 (with grade 3 having the most dysplastic characteristics), can be detected by the Pap smear, a cytological screening of cervical scrapes. Pap smears have led to a threefold reduction in the incidence of cervical cancer in those countries employing it as a routine preventative screening technique.

Recently approved prophylactic vaccines that prevent infection by two of the most common high-risk HPVs, HPV-16 and HPV-18, are predicted to lead to an 80% reduction of cervical cancer worldwide beginning in 2040 if the vaccine is universally administered to the world population of women. Socioeconomic conditions may limit the utility of these vaccines.

Other less frequently observed anogenital cancers, of the anus, penis, vulva, and vagina, are also caused by these high-risk HPVs. On average, there is a latency of over a decade between infection and onset of these anogenital cancers. In these cancers, one can find retention of the HPV genome, often in the integrated state, with a subset of viral oncogenes, specifically E6 and E7, upregulated in their expression. In mouse models, the high-risk HPV E6 and E7 oncogenes have been demonstrated to predispose mice to cervical cancer.

The same high-risk HPVs, particularly HPV-16, are etiologically associated with approximately 25% of head and neck squamous cell carcinoma (HNSCC), particularly of the oropharynx where the etiological correlation is 50% or greater. Again, the continued expression of E6 and E7 is found in HPV-positive HNSCC, and, in a mouse model, the HPV-16 E6 and E7 oncogenes have been demonstrated to predispose mice to HNSCC.

Cutaneous HPVs are also associated with a subset of skin cancers. This has been demonstrated most clearly for a subset of cutaneous HPVs that cause epidermodysplasia verruciformis (EV), a skin lesion that appears to arise preferentially in certain families, arguing a genetic predisposition. Patients with EV are highly prone to develop skin cancers. In mouse models, EV-associated HPVs have been experimentally demonstrated to predispose animals to skin cancer.

Life Cycle

With the exception of the so-called fibropapillomaviruses (e.g., bovine papillomaviruses (BPVs) and other PVs that infect ungulate hosts), most PVs are specifically epitheliotropic. Their life cycle occurs within stratified squamous epithelia, which are composed of basal and suprabasal compartments (**Figure 2**). The basal cells within the three-dimensional architecture of such epithelial tissues comprise a single layer sheet of poorly differentiated, proliferative cells that are physically attached to the underlying basement membrane that separates the epithelium from the underlying stroma. The suprabasal compartment comprises the multiple layers of cells positioned above the basal compartment that are quiescent and, as they become more superficial in their position, progressively more differentiated. When basal cells undergo cell division, one daughter cell loses contact with the basement membrane, elevating it to the suprabasal compartment where it exits the cell cycle and begins a program of terminal differentiation. As these cells move upward, they become progressively more terminally differentiated and lose nuclear membrane integrity, leading to the production of terminally differentiated squames, which eventually slough off into the environment. PVs simultaneously exploit and disrupt these normal cellular programs of differentiation and quiescence for their life cycle and the production of progeny virions.

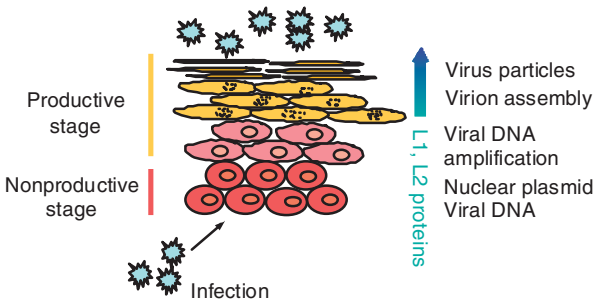


Figure 2 The HPV life cycle. A schematic cross section of stratified squamous epithelium is shown with basal cells at the bottom and terminally differentiated squames at the top. The various processes that arise within the nonproductive and productive stages of the viral life cycle are indicated at relevant positions on the periphery.

Fibropapillomaviruses differ from other PVs in that they can also infect the underlying dermal fibroblasts, leading to a hyperplasia of the cells. Lesions induced by fibropapillomaviruses can be quite large due to this underlying fibroplasia. The life cycle of these fibropapillomaviruses, however, still relies directly upon the infection of the epithelial cell, where the production of progeny virus specifically occurs.

Entry

It is presumed, though it has never been directly demonstrated, that PVs initiate infection of the host epithelia via entry into cells within the basal compartment. The specific mechanism(s) PVs use to gain access to, bind, and enter basal cells is not clear. The integrin α6:β4 heterodimer has been implicated as a cellular receptor for the virus, allowing for entry into cells upon its expression. However, in the absence of this integrin, some PVs retain the ability to infect cells. Other factors such as heparin sulfate proteoglycans are thought to play a role in cellular binding/entry by certain HPV genotypes at least in some cell types. Both the major (L1) and minor (L2) capsid proteins are required for viral particle infectivity, and both have been postulated to bind to cellular receptors. PVs have been argued to enter cells via clathrin-dependent or caveolin-dependent pathways and to require endosomal acidification. Once inside, L2 is thought to bind to β-actin and this is thought to facilitate movement of the virus particle through the cytoplasm to the nucleus. Alternatively, it has been argued that L2 binds to the microtubule motor dynein, facilitating intracytoplasmic transport. How the encapsidated DNA actually enters the nucleus is currently unknown; however, once there it appears to associate with subnuclear domains called promyelocytic leukemia protein oncogenic domains (PODs).

Establishment of the Nonproductive Infectious State

Once an HPV particle enters the host cell and translocates its DNA to the nucleus, it must rely primarily on cellular machinery to replicate its genome since HPVs only encode one component of the machinery required for initiating DNA replication, E1, a DNA helicase. It is partially for this reason that it is presumed that HPVs initiate their viral life cycle by infecting cells within the basal compartment, as these are the only cells within

the epithelia that are normally proliferating and have active cellular replication machinery. In these infected basal cells, the HPV genome becomes established as a low-copy-number nuclear plasmid. The 'early' promoter (designated P_{97} in the case of HPV-16) directs expression of the early genes, some of which are required for the replication of the viral genome, with transcripts being observed as early as 4 h post infection. This early promoter appears to stay active throughout the viral life cycle, whereas the 'late' promoter (designated P_{742} in HPV-16) is only active in cells upon their differentiation. Thus within the basal compartment, early but not late viral genes that encode the structural proteins are expressed, and therefore no progeny virus is produced. Consequently, the infectious state found in the basal cells is referred to as the 'nonproductive' stage of the infectious life cycle.

Maintenance of the Nonproductive Infectious State

A hallmark of HPV infections is their long-term persistence over many years. In the case of high-risk HPVs, this persistence is a prerequisite for the development of cancers associated with these viruses. For the virus to persist long term, it must maintain its genome within the basal compartment over multiple cell divisions, allowing for the continual regeneration of daughter cells that can provide a reservoir of proliferation-competent cells harboring the viral genome. Several early genes have been implicated in the establishment, as well as maintenance of viral genomes (see below). The virus must also affect an amplification of cells within the basal compartment that harbor the viral genome, as seen in warts where one finds a localized expansion of cells within the infected epithelia.

One possible explanation for both persistence and localized expansion of infected cells is that the virus infects epithelial stem cells. From these stem cells are derived the transiently amplifying cell population that populates the local area with HPV-positive cells. This is an attractive hypothesis; however, stem cells are infrequent. Therefore, the necessity to infect stem cells would indicate that productive infection is an inefficient process. An alternative explanation is that HPVs infect the transiently amplifying nonstem cells and extend their lifespan, thereby leading both to viral persistence and an expansion of infected cells at the original site of infection. Perhaps reflective of this alternative explanation is the observation that high-risk viruses have the unique capability among PVs to immortalize epithelial cells. This immortalization potential of high-risk HPVs, in turn, reflects the unique properties of their oncogenes E6 and E7. Whether the high-risk HPVs are unique in their capacity to establish persistent infections without the need to infect stem cells, as might be predicted by their immortalization potential, remains unclear.

Productive Stage

Normally when a basal cell divides, it produces two daughter cells, one of which separates physically from the basement membrane, transits to the suprabasal compartment, and begins a program for terminal differentiation that includes exiting the cell cycle and halting new rounds of DNA synthesis. When a daughter cell that harbors HPV genomes transits to the suprabasal compartment, it begins the so-called 'productive' stage of the HPV life cycle in which progeny viruses are made. In this stage of the viral life cycle, the HPV genome induces a delay and perturbation of the terminal differentiation program, which, importantly, includes the retention of an ability of these suprabasal cells to support DNA synthesis. This DNA synthesis-competent state is critical to allow for the amplification of the viral genome that is necessary for the production of progeny virus. Production of progeny virus within the suprabasal compartment also relies upon the selective expression of the late viral genes that encode the viral capsid proteins. These steps are spatially and temporally regulated. Specifically, induction of suprabasal DNA synthesis and a delay in early steps of epithelial cell differentiation both occur early in the productive stage and within the lower strata. Late gene expression, viral DNA amplification, and progeny virus production arise in the upper strata and are associated with further perturbations in terminal differentiation. Thus, specific life cycle events correspond intimately with different alterations in the terminal differentiation process within certain regions of the infected epithelium.

The multistage mode of replication of viral DNA genomes exemplifies the role that differentiation plays in the viral life cycle. HPV genomes harbored extrachromosomally within the basal compartment are replicated on average once per cell cycle and are stably maintained within this proliferating cell compartment at a low copy number. In contrast, the viral DNA is amplified to a much higher copy number in the upper layers of the epithelia strata. This difference in replication state in basal versus suprabasal cells correlates with a switch in the mode of replication from theta to, seemingly, rolling-circle DNA replication, and is induced by cell differentiation.

Functions of Individual Viral Genes

The Role of E1

PVs rely heavily on cellular machinery to replicate their DNA. However, PVs do encode one gene that is enzymatically involved in viral DNA replication and is required for plasmid replication, E1. E1 binds the viral origin of replication (ori) and possesses DNA helicase activity that permits the unwinding of the viral genomic template for replication. *In vivo* viral replication is mediated by E1, as well as the viral transactivator, E2.

E2 is able to bind specific DNA sequences, E2 binding sites (E2BSs), as well as the E1 protein; both of these E2 properties help drive E1-mediated plasmid replication. The current model proposes a multistep, interdependent process for viral replication in which E1 homodimers and E2 homodimers cooperatively bind to ori, then undergo conformational changes that allow E1 to assemble into a helicase-competent hexamer. Many cellular factors contribute to E1-dependent viral replication, including DNA polymerase α, chaperone proteins, histone H1, and replication factor A. In some cases, E1 has been shown to recruit these factors directly.

While E1 is required for the establishment of papillomaviral DNA replication, E1 is dispensable for plasmid maintenance of the BPV genome. In bovine papillomavirus type 1 (BPV-1)-transformed mouse C127 cells, in which that genome was already established as a nuclear plasmid, E1 was not required for stable maintenance of the viral plasmid. An E1-independent form of replication could also be detected in *Saccharomyces cerevisiae* and the *cis*-requirements for this replication were mapped. The ability of PVs to employ an E1-independent replication pathway may be a property of or reflect various modes of replication that the virus utilizes during different stages of its life cycle. In contrast, or in addition, the replication mechanism may reflect the ability of the virus to take advantage of a different host cell environment.

The Role of E2

In addition to a role in viral DNA replication, E2 also regulates viral transcription and the maintenance of the extrachromosomal viral genome. The ability of E2 to regulate transcription is mediated by its binding to E2BSs within the papillomaviral genome. In the PV genome there are multiple E2BSs that have different affinities for E2. Their relative locations are thought to determine the effects of E2 on transcription. If E2 binds an E2BS that is near to, but not overlapping, promoter elements, E2 activates transcription from that promoter. However, if the E2BS overlaps promoter elements, then it can repress transcription by sterically hindering binding of cellular factors necessary for transcription from that promoter.

In the context of the high-risk HPV genomes, E2's ability to suppress transcription from the viral promoter that drives expression of the viral early genes is thought to reflect one reason why integration of the viral genome into the host chromosome, and the consequent disruption of E2, contributes to increased expression of the viral oncogenes E6 and E7. However, these integration events are likely to have pleiotropic effects, as it has been shown that integration results in increased stability of the E6/E7 mRNAs due to the loss of an mRNA instability element located in the 3′ end of the early region. The ability of E2 to repress transcription of the viral oncogenes has been exploited to turn off expression of E6 and E7 in cervical cancer cells lines and thereby demonstrate the requirements for constitutive expression of E6 and E7 in these cancer-derived cell lines. Interestingly, E2 only appears to repress transcription of viral genomes when they are integrated into the host genome. This may reflect the fact that E2BSs in extrachromosomal HPV genomes can be methylated, and this methylation is known to prevent E2 binding. It is likely that epigenetic events also modulate the expression of integrated copies of the viral genome in cervical cancers by altering the chromatin state of the viral genome. In addition to the full-length E2 gene product, E2-mediated transcriptional regulation can also be mediated by N-terminally truncated forms of E2, E8^E2, and E2TR, which are synthesized from alternative transcripts. These E2 proteins can act as dominant negative factors to suppress E2-mediated transcriptional activation either by competing themselves for binding to the E2BSs or through heterodimerization with the full-length E2 protein.

PV genomes are maintained as nuclear plasmids throughout the viral life cycle. Therefore, there is a need for viral plasmids to be segregated efficiently to daughter cells during cell division. For BPV-1, the full-length E2 protein has been shown to mediate this process and is thought to reflect E2's ability to associate with host chromosomes during mitosis. E2 can bind to the chromosomal attachment protein, bromodomain 4 (Brd4), and, in yeast, the ability of BPV-1 E2 to mediate plasmid maintenance relies upon the co-expression of mammalian Brd4. These data suggest that Brd4 contributes to BPV-1 E2's plasmid maintenance function. Further study is required to ascertain whether this proposed mechanism is relevant to HPVs. Of concern is the observation that mutant HPV-31 genomes that contain amino acid substitutions in the E2 protein predicted to disrupt Brd4 binding, based upon prior studies in BPV-1 E2, retain an ability to replicate stably as a nuclear plasmid, though less efficiently than wild-type HPV-31 genomes.

The Role of E1^E4

As with the other PV proteins, numerous functions are ascribed to the E1^E4 gene product. The small (10–20 kDa) regulatory viral protein is generated from a spliced mRNA that fuses the E1 and E4 translational open reading frames (see **Figure 1**). E1^E4 is poorly expressed in undifferentiated monolayer cultures containing HPV, as well as within basal cells of raft cultures. However, E1^E4 expression is increased substantially in the upper strata of papillomas. Within raft cultures the greatest E1^E4 expression is seen in the more superficial layers of epithelium that also contain amplified viral genomes. Owing to colocalization of E1^E4 and amplified viral genomes, it was proposed that E1^E4 functions in the productive stage of the viral life cycle, specifically viral DNA amplification. Indeed, when severe truncation

mutations of E1^E4 were made in the context of the entire cottontail rabbit papillomavirus (CRPV), HPV-16, or HPV-31 genomes, defects in viral DNA amplification were observed, indicating that E1^E4 contributes to the amplification of viral genomes. However, effects in viral genome establishment were also seen with some HPV-16 E1^E4 mutants. How E1^E4 mediates viral genome establishment is not clear.

E1^E4 contains three major motifs postulated to contribute to the role of E1^E4 in the viral life cycle. A leucine-rich motif in the N-terminus is required for association with keratins and perhaps to contribute to viral DNA amplification. A proline-rich region in the central section, containing threonine residues, is required for induction of cell cycle arrest at the G2/M phase and sequestering of cyclinB/cdk1 complexes in the cytoplasm. The C-terminus contains a mucosal homology domain, and regulates the ability of E1^E4 to oligomerize, bind the DEAD-box RNA helicases, and induce collapse of keratin filamentous networks. In addition, the association of E1^E4 with cornified cell envelopes (CCEs) and PODs has not been mapped, but both interactions are postulated to contribute to the role of E1^E4 in the PV life cycle.

The association of E1^E4 with both CCEs and keratin filaments is thought to aid egress of the virus. PVs are not lytic; rather, they assemble their progeny virions within the granular and cornified layers of the epithelia. The progeny virions are thought to be released in the context of the natural process of epithelial sloughing. These E1^E4 interactions are proposed to aid the disruption of cellular structure and thus the ability of progeny virions to be released into the environment.

The associations of E1^E4 with cyclinB/cdk1, PODs, and the E1^E4-associated DEAD-box RNA helicase are each postulated to mediate a role for E1^E4 in viral replication or amplification. Overexpression of E1^E4 leads to a cell cycle profile consistent with cells that are arrested at G2/M. It is thought that E1^E4 mediates G2 arrest via decreasing the soluble nature of the cyclin complex and preventing it from translocating to the nucleus. It is hypothesized that these cells retain an ability to support viral DNA amplification. In HPV-1-induced warts, E1^E4 associates with promyelocytic leukemia protein (PML) and the *in vitro* overexpression of E1^E4 induces relocalization of PML to E1^E4 nuclear inclusion bodies. These data suggest that PODs, with E1^E4, may play a role in the life cycle of HPVs.

The Role of E5

E5 is a small, dimeric, hydrophobic membrane protein that localizes to the Golgi membrane and endoplasmic reticulum. Initially, most studies to understand the function and contribution of E5 to transformation, replication, or oncogenesis by PVs were performed within the context of BPV. In the case of BPV-1, E5 is the major transforming protein and can induce immortalization and transformation of rodent fibroblasts and keratinocytes. In contrast, E6 and E7 are the major transforming proteins of HPV. Though, in the context of the HPV genome, E5 is not absolutely required for the induction of DNA synthesis or immortalization, it contributes quantitatively to both of these processes. Furthermore, E5 can independently transform rodent keratinocytes, induce anchorage-independent growth, and override cellular growth arrest signals to promote proliferation. Therefore, E5 appears to play an important yet secondary role in transformation and oncogenesis induced by HPVs, whereas it plays a primary role in BPV-induced transformation.

The ability of E5 to transform cells is attributed to its ability to activate growth factor receptors and to inhibit the 16 kDa vacuolar ATPase (vATPase). BPV-1 E5 can induce the activation of platelet-derived growth factor receptor β (PDGFRβ) in a ligand-independent fashion. This activation occurs through direct binding of BPV-1 E5 to PDGFRβ, which induces receptor oligomerization and activation. In contrast, HPV E5 increases the signaling capacity of the epidermal growth factor receptor (EGFR). This difference in target growth receptors is presumably mediated by tissue tropism specificity. Fibroblasts, which BPVs infect along with keratinocytes, contain abundant amounts of PDGFR. In keratinocytes, which are the normal host cells of HPVs, EGFR is a key growth factor receptor. EGFR phosphorylation, a marker of its activation, is increased in the presence of E5, at least when it is overexpressed. Expression of EGF in concert with E5 leads to greater proliferation of keratinocytes. However, when HPV E5 was studied in the context of the complete viral genome, HPV E5 did not induce a detectable increase in either levels or phosphorylation of EGFR. E5 contributes with E6 and E7 to immortalize keratinocytes and contributes quantitatively to the proliferative capacity of HPV and the ability of HPV to override growth arrest signals. Therefore, while *in vitro* and heterologous system studies predicted that HPV E5 contributes to the viral life cycle through upregulation of EGFR signaling, EGFR-independent pathways may actually be responsible for the proliferative contribution of E5 to the HPV life cycle. Alternatively, E5 may mediate its effects via upregulation of EGFR but the threshold level modified by E5 may not be distinguishable with current technical methodologies.

While E5 does not contribute to the nonproductive stage of the HPV-16 or HPV-31 life cycle, it does contribute quantitatively to the productive stage of both HPV-16 and HPV-31. In the absence of a full-length E5 gene, HPV genomes are defective in their ability to override the normal differentiation program. Normally, HPV-16 and HPV-31 can induce DNA synthesis and proliferation markers in cells induced to undergo differentiation, which are normally quiescent. However, in the absence of E5, DNA synthesis and proliferation marker expression was

quantitatively less than the induction of these markers by wild-type HPV genomes. E5 was also shown to be required for efficient viral DNA amplification (HPV-16 and HPV-31), late viral gene expression, and re-entry into the cell cycle (HPV-31). These data illustrate that E5 contributes quantitatively to many aspects of the productive stage of the HPV life cycle. Further support for a role of E5 in productive infection lies in *in vivo* studies that examine papilloma formation by E5 alone or E5 mutant genomes. In the context of CRPV, E5 mutant genomes are less effective at inducing papillomas than the wild-type CRPV genome. In addition, HPV-16 E5 transgenic mice develop spontaneous papillomas and hyperplasia. Thus, E5 contributes to proliferation both in the context of animal models and organotypic raft culture viral life cycle studies.

The Role of E6

E6, an approximately 150 amino acid residue protein, is encoded by the first translational open reading frame (ORF) within the HPV genome and is one of the major HPV oncoproteins. High-risk HPV E6 is able to transform rodent fibroblasts and immortalize keratinocytes, as well as other epithelial cells, in conjunction with E7, the other major HPV oncogene. In addition, *in vivo* E6 can inhibit epithelial differentiation, induce hyperplasia, suppress DNA damage-induced cell cycle arrest, and contribute to carcinogenic promotion and progression. All of these activities of E6 are thought to contribute to its oncogenicity. E6 does not possess any intrinsic enzymatic activity; rather, its oncogenic capabilities are mediated through its ability to act as a scaffold and regulate protein–protein interactions. Within E6 there are two motifs that are generally thought to mediate such interactions: two zinc finger domains and an α-helix-binding domain. High-risk E6 also contains a C-terminal PSD-95/Dlg/ZO-1 (PDZ) domain as well. Some of the protein interactions that have been mapped to these E6 domains include: p53, E6-associated protein (E6AP), E6-binding protein (E6BP), c-myc, p300/CBP, paxillin, PDZ proteins, interferon regulatory factor 3, and Bcl-2 homologous antagonist/killer (Bak).

The most studied of these interactions is the E6–p53 interaction. p53 is a transcription factor and tumor suppressor that is activated upon aberrant DNA replication, cellular stress, or cellular damage signals. As a response to cellular damage signals, the tumor suppressor p53 is upregulated and can induce cell cycle arrest or apoptosis through its transcriptional activity. p53 is a major regulator of DNA synthesis inhibition through cell cycle arrest and induction of cell death through apoptosis, which is presumably the reason that p53 is one of the most commonly mutated proteins in human cancer. Through the association of E6 with E6AP, a ubiquitin ligase, E6 is able to bind and induce the degradation of p53, though recent studies argue that other factors might also mediate E6's ability to induce p53 degradation. The ability of E6 to deregulate p53 correlates, at least in part, with its ability to transform cells in tissue culture and induce tumors in mice.

Clearly, the E6–p53 interaction is important for the oncogenic capabilities of E6. However, *in vivo* studies of the high-risk HPV-16 E6 using HPV transgenic mouse models indicate that an E6 mutant that retains the ability to inactivate p53 is partially defective for oncogenic phenotypes observed in mice transgenic for wild-type HPV-16 E6, including its abilities to induce skin tumors and cervical cancer. Thus, p53 inactivation is not sufficient to account for E6's oncogenic properties. The E6 mutant studied, competent to inactivate p53, is defective in its interaction with PDZ proteins. The ability of E6 to bind PDZ proteins also correlates with its ability to transform baby rat kidney cells. Only E6 proteins encoded by high-risk HPVs can bind PDZ proteins; E6 proteins encoded by low-risk HPVs do not contain the four-residue C-terminal, PDZ interaction domain. PDZ proteins are named for the protein family members that were initially identified to possess copies of a motif of approximately 80–100 residues, termed PDZ domains. These proteins are scaffolding proteins that generally interact with the C-terminus of their protein partners, which possess a charged four-residue motif, the PDZ interaction domain. The evolutionarily conserved PDZ proteins are required for development, cell adhesion, cellular growth and differentiation, and cell cycle processes. The E6 interaction with PDZ proteins is reported to lead to degradation of the PDZ proteins. Data indicate that the E6–PDZ interaction allows for the dysregulation of normal cellular growth controls and E6-induced hyperproliferation, transformation, and carcinogenesis. Therefore, while the E6–p53 interaction is required for E6-induced transformation and proliferation, the E6–PDZ interaction also contributes to E6 oncogenicity in a fashion that is distinct from p53.

E6 contributes to the viral life cycle. In HPV-31, as well as HPV-16, E6 is required for viral episome maintenance. In transient replication assays, HPV-31 E6-null genomes are able to replicate, indicating that E6 is not required for the initial establishment of HPV genomes. However, these E6-null genomes are not able to maintain the viral episome in long-term assays. HPV-31 genomes that are specifically defective in their ability to interact with PDZ proteins are still able to exhibit properties of intact nonproductive and productive stages, yet are slightly defective at each step of the life cycle. In contrast, there are clear defects in the viral life cycle when E6–p53 interactions are disrupted. Although the ability to bind p53 is not required for the establishment of viral episomes, the ability of viral episomes to be maintained correlates with the ability of E6 to induce degradation of p53. How the degradation of p53 contributes to maintenance of the viral episome is not clear. E6-mediated p53

degradation may be needed for viral maintenance to protect against induction of cellular damage signals induced during viral replication, especially as a consequence of the dysregulation of the tumor-suppressor protein, retinoblastoma (pRb), by E7.

The Role of E7

E7 is a 98-residue protein composed of two N-terminal conserved regions and a zinc finger domain in its C-terminus. The C-terminus regulates E7 multimerization. The N-terminus is partially homologous to two other virally coded oncoproteins, adenovirus (Ad) E1A and simian virus 40 T antigen (SV40TAg). E7 contains a pocket protein-binding motif, LXCXE, that confers an ability for E7 to bind pRb, as well as the two other 'pocket protein' family members, p107 and p130. The LXCXE motif is required for the ability of E7 to transform rodent fibroblasts and, in conjunction with E6, to immortalize human fibroblasts and keratinocytes efficiently. In addition, the LXCXE motif is required for E7 repression of pRb-induced senescence of cells harboring HPV genomes; and, in mice, the ability of E7 to bind pRb is essential for E7's induction of epithelial hyperplasia. This conserved region within high-risk E7s, and their interaction with the pocket proteins, correlate with their oncogenic properties. However, E7 is a multifunctional protein that is reported to bind not only the pocket proteins but also as many as 100 other cellular factors. Other potentially relevant partners include: the pocket proteins (pRb, p107, and p130), the cyclin-dependent kinase inhibitors p21 and p27, CK2 (formerly casein kinase II), and histone deacetylase (HDAC).

The pocket proteins are cell cycle regulators that modulate cell cycle progression and DNA synthesis. pRb is a tumor suppressor and is part of one of the most frequently mutated regulatory pathways in human cancers. p107 and p130 are closely related to pRb and possess similar biochemical activities. Together the pocket proteins regulate overlapping and distinct parts of the cell cycle. Both low- and high-risk E7 are able to bind the pocket proteins, although the relative binding preference for the pocket proteins appears to differ between low- and high-risk E7 proteins. In addition, high-risk HPV E7s can induce degradation of the pocket proteins. Two other DNA tumor viruses, Ad and SV40, also bind and inactivate the pocket proteins and affect the cell cycle.

The most studied E7–pocket protein interaction is the E7–pRb interaction. Normally, pRb is hypophosphorylated early in the cell cycle. In its hypophosphorylated state, pRb binds the transcription factor E2F/DP complex. The E2F/DP complex is a transcriptional activation complex that drives the expression of S phase genes. In early G1 phase of the cell cycle, hypophosphorylated pRb binds to E2F/DP and consequently inactivates the transcriptional complex. In late G1, pRb is phosphorylated by G1-specific cyclin/cdk complexes composed of cyclin D and cyclin E, and this leads to release of the E2F/DP complex from pRb. The free E2F/DP complex then becomes transcriptionally active and S-phase promoting genes are transcribed. In the context of HPV infection, E7 expression negates the requirement for pRb phosphorylation to activate the E2F/DP transcriptional complex. Specifically, E7 binding preferentially to hypophosphorylated pRb leads to the release of E2F/DP complexes from pRb and the consequent activation of the E2F complex. Therefore, E7 inactivation of pRb bestows on HPV the ability to override the pRb inhibition of the cell cycle.

In the context of the entire genome, the overall mechanistic requirements for E7 differ between genotypes. In HPV-16, E7 is required for the productive stage of the viral life cycle. Specifically, an HPV-16 genome carrying a null mutation of E7, while able to become established stably as a nuclear plasmid in human keratinocytes, is unable to support many aspects of the life cycle that arise in the differentiating compartment of stratified epithelium, including the inhibition of differentiation, reprogramming of suprabasal cells to support DNA synthesis, and viral DNA amplification. A similar requirement for E7 specifically in the productive stage of the life cycle was reported for HPV-18, and subsequent studies with HPV-16 demonstrated the importance of the E7–pocket protein interactions in mediating its role in the life cycle. An HPV-16 mutant genome carrying a mutation within the E7 ORF that disrupts the ability of E7 to bind pRb and the other pocket proteins displayed all of the defects in the productive life cycle that were observed with the E7-null HPV-16 genome. In contrast, a mutant genome in which E7 is disrupted in its ability to induce the degradation of the pocket proteins retained a partial ability to induce suprabasal DNA synthesis and viral DNA amplification but was completely defective in inhibiting cellular differentiation. Thus, there are differential requirements for E7's binding versus degradation of pRb and the other pocket proteins in E7's contribution to the various stages of the viral life cycle.

In contrast to what was observed with HPV-16 and HPV-18, the intermediate-risk HPV-31 genome appears to require E7 during the nonproductive stage of the viral life cycle as well as during the productive stage. E7-null HPV-31 genomes are unable to be maintained stably as nuclear plasmids in cultures of poorly differentiated human keratinocytes, whereas E7-null HPV-16 and HPV-18 genomes do stably replicate as nuclear plasmids. This difference apparently reflects genotype-specific differences.

The Role of L1 and L2

L1 and L2 are late viral genes encoding the major and minor capsid proteins, respectively. The PV capsid

contains 72 pentameric capsomeres arranged on a $T=7$ icosahedral lattice and is approximately 55 nm in diameter, as determined by cryoelectron microscopy. The majority of the viral capsid is composed of the major capsid protein, L1, with L2 composing a minor fraction. Expression of L1 alone allows for the formation of pseudovirions or virus-like particles, VLPs, which are visually indistinguishable from native virions. If L2 is expressed in conjunction with L1, it is also incorporated into VLPs, but L2 is not required for capsid formation. L1 and L2 are specifically expressed in the most superficial layers of stratified keratinocytes, where progeny virus production arises.

Although L2 is not required for capsid formation, it does possess activities that likely contribute to a role in the viral life cycle. Some of these have been described above, under the section on virus entry, and include binding to cell surface receptors, actin, and PML, all of which have been implicated in early steps of virus infection. In addition, L2 is thought to be required for viral DNA encapsidation, although this requirement is obviated in some pseudovirion assembly systems. In life cycle studies carried out with HPV-31, L2-null genomes were able to support the nonproductive stage and most aspects of the productive viral life cycle in transfected human keratinocytes, with the exception that the level of encapsidated DNA in the progeny virions was reduced significantly and these progeny viruses were further reduced in their infectivity. Therefore, L2 seems to have a role in the viral life cycle in at least two distinct steps, encapsidation and infectivity. Whether the role of L2 in infectivity is at the level of receptor recognition or viral trafficking or both is something that should be determined in the future.

See also: Papillomaviruses: General Features; Papillomaviruses: General Features of Human Viruses; Papillomaviruses of Animals.

Further Reading

Bernard HU (2002) Gene expression of genital human papillomaviruses and potential antiviral approaches. *Antiviral Therapy* 7: 219–237.

Collins A, Nakahara T, and Lambert PF (2005) Contribution of pocket protein interactions in mediating HPV16 E7's role in the papillomavirus life cycle. *Journal of Virology* 79: 14769–14780.

Demeret C, Desaintes C, Yaniv M, and Thierry F (1997) Different mechanisms contribute to the E2 mediated transcriptional repression of human papillomavirus type 18 viral oncogenes. *Journal of Virology* 71: 9343–9349.

Evander M, Frazer IH, Payne E, Qi YM, Hengst K, and McMillan NA (1997) Identification of the α6 integrin as a candidate receptor for papillomaviruses. *Journal of Virology* 71: 2449–2456.

Lambert PF, Ozbun MA, Collins A, Holmgren S, Lee D, and Nakahara T (2005) Using an immortalized cell line to study the HPV life cycle in organotypic ''raft'' cultures. *Methods in Molecular Medicine* 119: 141–155.

Longworth MS and Laimins LA (2004) Pathogenesis of human papillomaviruses in differentiating epithelia. *Microbiology and Molecular Biology Reviews* 68: 362–372.

Munger K, Baldwin A, Edwards KM, *et al.* (2004) Mechanisms of human papillomavirus-induced oncogenesis. *Journal of Virology* 78: 11451–11460.

Nakahara T, Doorbar J, and Lambert PF (2005) HPV16 E1^E4 contributes to multiple facets of the papillomavirus life cycle. *Journal of Virology* 79: 13150–13165.

Ozbun MA (2002) Human papillomavirus type 31b infection of human keratinocytes and the onset of early transcription. *Journal of Virology* 76: 11291–11300.

Pyeon D, Lambert PF, and Ahlquist P (2005) Production of infectious human papillomavirus independently of viral replication and epithelial cell differentiation. *Proceedings of the National Academy of Sciences, USA* 102: 9311–9316.

Sanders CM and Stenlund A (2001) Mechanism and requirements for bovine papillomavirus, type 1, E1 initiator complex assembly promoted by the E2 transcription factor bound to distal sites. *Journal of Biological Chemistry* 276: 23689–23699.

Schiller JT and Lowy DR (2006) Prospects for cervical cancer prevention by human papillomavirus vaccination. *Cancer Research* 66: 10229–10232.

Skiadopoulos MH and McBride AA (1998) Bovine papillomavirus type 1 genomes and the E2 transactivator protein are closely associated with mitotic chromatin. *Journal of Virology* 72: 2079–2088.

zur Hausen H (2002) Papillomaviruses and cancer: From basic studies to clinical application. *Nature Reviews. Cancer* 2: 342–350.

Papillomaviruses of Animals

A A McBride, National Institutes of Health, Bethesda, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Warts or papillomas have been recognized in animals for centuries. The first description of transmissible animal papillomas was in the ninth century in *Al-Kheyl wal Beytareh*, a book of horse medicine by Ibn Akhi Hazam, the stablemaster for the Caliph of Baghdad. By the late 1800s, there were several examples of experimental transmission of warts in animals such as dogs and horses and in 1907 Ciuffo demonstrated that human warts could be transmitted by sterile filtrates, indicating a viral etiology. Cottontail rabbit or Shope papillomavirus (CRPV) was isolated in 1933 by Richard Shope and this was the first papillomavirus (PV) to be studied in detail.

Papillomas are normally benign, but there are several examples of animal warts undergoing malignant

transformation to carcinomas. This was one of the earliest indications that viruses could be involved in the development of cancer and it helped initiate the field of viral oncology. This potential for malignant progression was later important in the recognition that human papillomaviruses (HPVs) were associated with cervical cancer. Relative to other viruses, PV research was hampered because of difficulties in propagating the virus; papillomaviruses (PVs) are species specific and epitheliotrophic and require a stratified and differentiated epithelium for productive infection. In the 1980s, research progressed more rapidly with the advent of molecular cloning because the small viral genomes could be readily propagated, and genetically modified, in a bacterial plasmid. Bovine papillomavirus type 1 (BPV-1) became a very popular prototype because of its unusual ability to transform and maintain its genome in rodent cells in culture. This allowed extensive analysis of the transformation, replication, and transcriptional properties of BPV-1. By the 1990s, several systems had been developed that allowed limited propagation of PVs in culture in artificial skin equivalents or epithelial xenografts in laboratory animals.

Much PV research has centered on the functions of the viral proteins. These small viruses encode only a handful of proteins and most function by interacting with a plethora of cellular proteins. Because viruses target pivotal regulatory functions in the cell, this research has led not only to a detailed understanding of the PV life cycle but has provided great insight into the function of key cellular proteins.

Taxonomy, Classification, and Evolutionary Relationships

Initially, PVs were grouped with polyomaviruses in the family *Papovaviridae*. However, it was later recognized that these viruses are not closely related and the International Committee on the Taxonomy of Viruses (ICTV) now recognizes the PVs as a new family, the *Papillomaviridae*. The *Papillomaviridae* have been further classified into genera and species based upon phylogenetic clustering of viral capsid protein L1 gene sequences (see **Figure 1**). Different genera have less than 60% and species have 60–70% nucleotide sequence identity. Individual PV types have 71–89% nucleotide identity. Differences of 2–10% identity define a subtype and less than 2%, a variant. Each genus is identified by a Greek letter, for example, *Alphapapillomavirus*. Each PV type is designated according to the host species and sometimes the site of infection, PV for papillomavirus, and finally, if several PVs have been isolated from the same species, a number. Examples are ROPV (rabbit oral PV) and BPV-1.

PVs have co-evolved with their hosts over millions of years and all types have most likely existed since the speciation of their host. The slow evolution of PVs is probably due to their use of the high-fidelity host DNA polymerase to replicate their genomes. It has been estimated that the most variable parts of PV genomes change at a rate of 0.25% per 10 000–20 000 years and there is no evidence for recombination between different viral types.

Host Range and Tissue Tropism

PVs are widespread. There are hundreds of different human types and PVs have been isolated from a diverse range of animals. To date, most animal PVs have been isolated from mammals, but viruses have also been identified in birds (see **Table 1**). PVs have been isolated from rodents, such as Syrian hamsters, the European harvest mouse, and the African multimammate rat. Unfortunately, no virus has yet been found that can infect laboratory mice. Multiple viral types have been found in those species that have been examined carefully (e.g., primate, cow, and rabbit) and many subclinical infections have been identified in primates (human and nonhuman). Presumably, more extensive investigation of each host would reveal a number of PV types that might rival the hundreds of PVs found in humans. Therefore, it would not be unreasonable to predict that there might exist over 100 000 different PV types.

All PVs are epitheliotrophic; each virus is species specific and infects and replicates in either cutaneous or mucosal epithelium, often at particular sites in the host. The reason for this tropism is not well understood, but it does not seem to depend on cell surface receptors for the virus and is thought to be due to very precise interactions between viral and host proteins that are required for a successful, productive infection. An exception to this is the fibropapillomaviruses. In addition to the productive infection of the epithelium, these viruses also nonproductively infect the underlying dermal fibroblasts, resulting in a fibroma. Probably because of this, these viruses have a less restricted host range and can nonproductively infect related host species and can transform and nonvegetatively replicate in cells from other hosts. For example, BPV-1 can cause equine sarcoids and can transform rodent cells.

Transmission, Clinical Features, Pathology, and Pathogenicity

PV-associated disease ranges from clinically inapparent infections, through a variety of benign warts, to malignant carcinoma. These differences in pathology are due to different viral types, different epithelial host cells, and the immune response of the host. The complete viral life cycle requires a stratified, differentiating epithelium

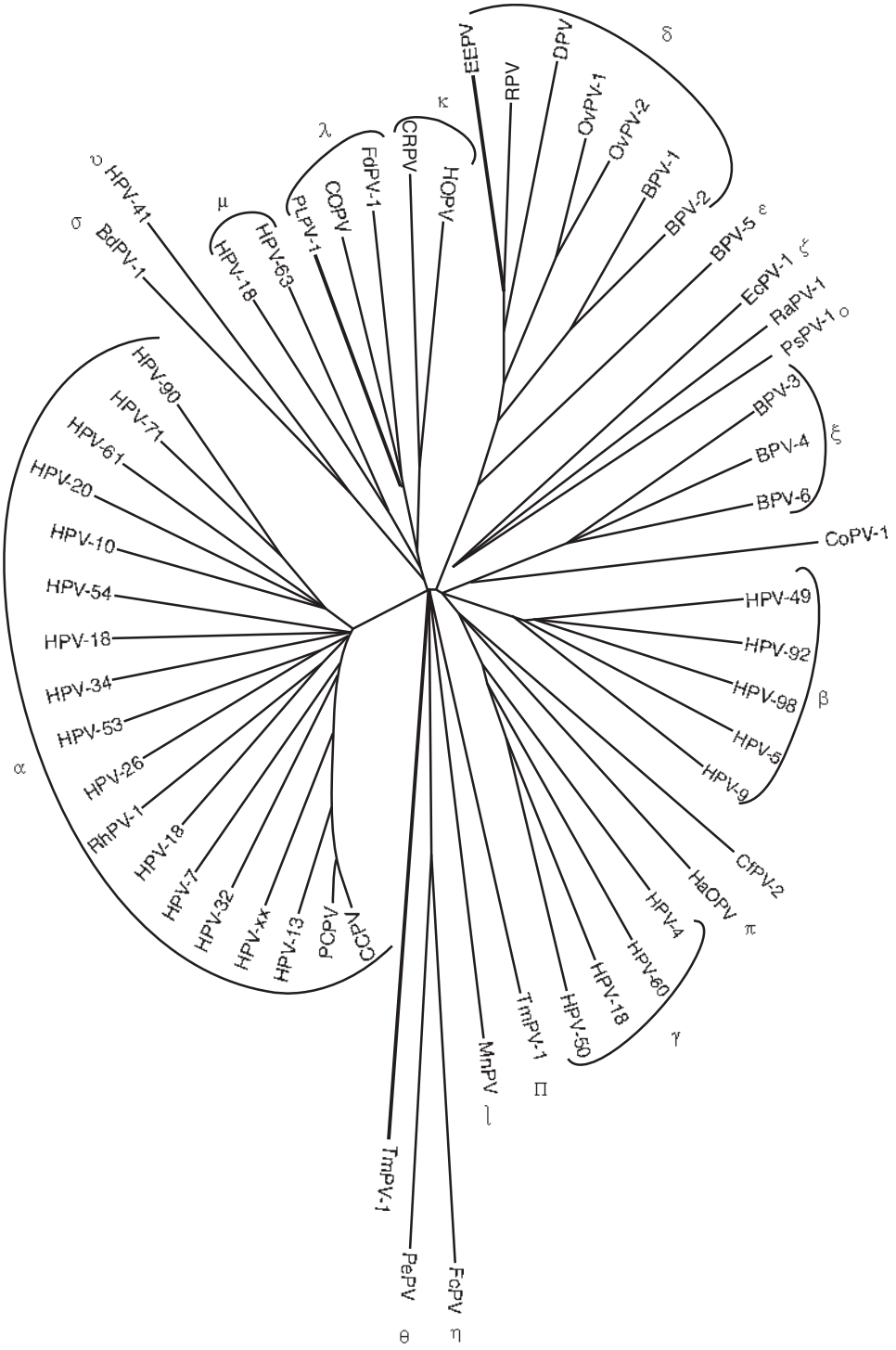


Figure 1 The evolutionary relationships between animal and a subset of human papillomaviruses as represented by a phylogenetic tree. Adapted from Van Doorslaer K, Rector A, Vos P, and Van Ranst M (2006) Genetic characterization of the *Capra hircus* papillomavirus: A novel close-to-root artiodactyl papillomavirus. *Virus Research* 118: 164–169, with permission from Elsevier.

(see **Figure 2**). In normal skin, only the basal cells can divide; after each division, one daughter cell remains in the basal layer and the other is pushed upward to begin the differentiation process. The latter cells withdraw from the cell cycle and begin to synthesize proteins that provide strength and barrier function to the epithelium.

To initiate infection, PVs usually require direct contact with the appropriate epithelia and must access the

Table 1 Notable animal papillomaviruses (by genus)

Alphapapillomavirus: mucosal and cutaneous lesions in order Primates
PCPV-1: Pygmy chimpanzee (*Pan paniscus*) papillomavirus
RhPV-1: Rhesus monkey (*Macaca mulatta*) papillomavirus type 1
Deltapapillomavirus: fibropapillomas in superorder Ungulata
BPV-1, BPV-2: Bovine (*Bos taurus*) papillomavirus types 1 and 2
DPV: Deer (*Cervus*) papillomavirus
EEPV: European elk (*Alces alces*) papillomavirus
OvPV-1, OvPV-2: Ovine (*Ovis aries*) papillomavirus types 1 and 2
RPV: Reindeer (*Rangifer tarandus*) papillomavirus
Epsilonpapillomavirus: cutaneous lesions in family *Bovidae*
BPV-5: Bovine (*Bos taurus*) papillomavirus type 5
Zetapapillomavirus: cutaneous lesions in order Perissodactyla
EcPV-1: Equus caballus (horse) papillomavirus type 1
Etapapillomavirus: cutaneous lesions in class Aves (Passeriformes)
FcPV: Fringilla coelebs (chaffinch) papillomavirus
Thetapapillomavirus: cutaneous lesions in class Aves (Psittaciformes)
PePV: Psittacus erithacus timneh (African grey parrot) papillomavirus
Iotapapillomavirus: cutaneous lesions in order Rodentia
MnPV: Mastomys natalensis (multimammate rat) papillomavirus
Kappapapillomavirus: cutaneous and mucosal lesions in order Lagomorpha
CRPV: Cottontail rabbit (*Sylvilagus*) papillomavirus
ROPV: Rabbit (*Oryctolagus cuniculus*) oral papillomavirus
Lambdapapillomavirus: cutaneous and mucosal lesions in order Carnivora
COPV: Canine (*Canis familiaris*) oral papillomavirus
FdPV: Felis domesticus (cat) papillomavirus
Xipapillomavirus: cutaneous and mucosal lesions in family *Bovidae*
BPV-3, BPV-4, BPV-6: Bovine (*Bos taurus*) papillomavirus types 3, 4, and 6
Omikronpapillomavirus
PsPV: Phocoena spinipinnis (porpoise) papillomavirus
Pipapillomavirus
HaOPV: Hamster (*Mesocricetus auratus*) oral papillomavirus
Unassigned
BPV-7: Bovine (*Bos taurus*) papillomavirus type 7
CCPV-1: Common chimpanzee (*Pan troglodytes*) papillomavirus type 1
CfPV-2, CfPV-3: Canine (*Canis familiaris*) papillomavirus types 2 and 3
CgPV-1, CgPV-2: Colobus monkey (*Colobus guereza*) papillomavirus types 1 and 2
ChPV-1: Capra hircus (goat) papillomavirus type 1
EdPV: Erethizon dorsatum (porcupine) papillomavirus
MfPV: Macaca fasicularis (long-tailed macaque) papillomavirus
MmPV: Micromys minutus (European harvest mouse) papillomavirus
PlPV-1: Procyon lotor (raccoon) papillomavirus type 1
RaPV-1: Rousettus aegyptiacus (Egyptian fruit bat) papillomavirus type 1
TmPV-1: Trichechus manatus latirostris (manatee) papillomavirus type 1
TtPV-1, TtPV-2: Tursiops truncates (bottlenose dolphin) papillomavirus types 1 and 2
TvPV: Trichosurus vulpecula (brushtall possum) papillomavirus

dividing cells in the basal layer of the epithelium through micro-abrasions or wounds. Viral DNA is established and maintained in the nuclei of dividing basal cells as an extrachromosomal replicating element. The infected basal cells serve as a reservoir of infected cells in the continual, progressive vertical differentiation that occurs in the maturation of the epidermis. Viral genome amplification, capsid protein synthesis, and particle production are restricted to the overlying, terminally differentiated cells. PVs disrupt the normal differentiation process, primarily because they must maintain cells in an S-phase-like state so that DNA replication enzymes are available to amplify the viral genome in differentiated cells. The infectious process is associated with a proliferation of the epidermal layers and in acanthosis (hyperplasia of the spinous layer), parakeratosis (persistence of the nuclei into the stratum corneum), hyperkeratosis (thickened cornified layer), and papillomatosis (undulating epithelium). Koilocytes (large, round, vacuolated cells with pyknotic nuclei) appear in the stratum spinosum and granulosum and abnormal keratohyalin granules are produced.

This process can result in a broad spectrum of papilloma-induced morphologies. For example, BPV-1-induced fibropapillomas in cattle can be sessile or pedunculate and lobate, fungiform, or verrucate. Other BPV types cause flat or filiform teat papillomas or alimentary papillomas. CRPV-induced papillomas occur as dark, highly keratinized masses. They range in size from 0.5 to 1 cm in

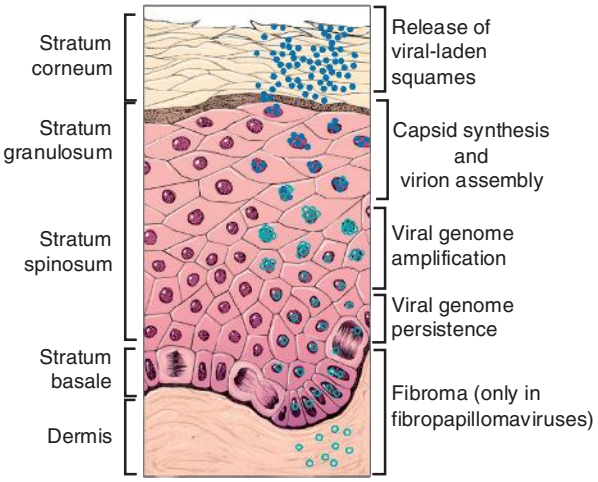


Figure 2 Life cycle of papillomaviruses. The diagram shows a model of stratified epithelium. The lowermost basal layer provides the germinal cells necessary for regeneration of the epidermis. In the stratum spinosum, desmosomes can be observed between cells giving them a characteristic spiny appearance. The cells of the stratum granulosum accumulate dense basophilic keratohyalin granules that contain lipids, which help form a waterproof barrier function. The outermost layer consists of dead cells filled with mature keratin. The stages of the viral replicative life cycle that occur in each cell layer are indicated. Fibropapillomaviruses also infect fibroblasts in the dermis, resulting in a fibroma.

diameter and can reach several centimeters in height, resulting in cutaneous 'horns'. In fact, it is thought that these 'horns' inspired the legend of the jackalope, a mythical creature that is a cross between a jackrabbit and an antelope. At the other end of the spectrum, healthy appearing skin from many animals has been found to harbor PVs.

Replication Cycle

All PVs have similar life cycles, closely linked to keratinocyte differentiation, although there are variations in the timing of each stage of infection with respect to differentiation that are probably linked to the host cell, the viral type, and the immune response. PVs infect the basal cells and probably use a common cell surface receptor. $\alpha 6\beta 4$ integrin and heparan sulfate proteoglycans are candidate receptors, but there is evidence to suggest that other molecules may be important. In the basal cells, the viral genome is maintained in a low-copy, extrachromosomal state and is replicated along with cellular DNA in a cell cycle-dependent manner. PV infections are usually long-lived and persistent and these cells must provide a reservoir of infected cells for the overlying virus producing tissue. As the infected cells differentiate and migrate upward to the stratum spinosum, there are changes in viral gene expression and vegetative viral DNA replication begins. Expression of the viral capsid proteins, L1 and L2, is first detected in cells of the stratum spinosum and virus-specific cytopathic effects are most pronounced in the stratum granulosum. Virions are assembled in the upper differentiated layers of the papilloma and are found throughout the nuclei, frequently organized into paracrystalline arrays, in cells which are destined to be sloughed from the epidermis. Viral transcription, translation, and replication are regulated through both positive and negative cellular processes that change during terminal differentiation. In fibropapillomas, there is also a proliferation of infected fibroblasts in the underlying dermis. Although the viral genome is maintained in the infected fibroblasts, there is no late gene expression or virion production.

Genome Organization and Expression

All PVs have a double-stranded, circular DNA genome of 7–8 kbp. There is a region of approximately 1 kbp that is called the long control region (LCR), upstream regulatory region (URR), or noncoding region (NCR). This region contains transcriptional enhancers and promoters, the DNA replication origin, and sequences required for genome maintenance (MME; minimal maintenance element). The viral promoters are regulated by cellular factors and the viral E2 proteins. In most viruses, the genes are organized in the order LCR–E6–E7–E2/E4–(E5)–SIR–L2–L1, where SIR represents the short intergenic region (see **Figure 3**). The coding region is divided into the early and late regions. The early region is expressed in the lower, more undifferentiated layers of a papilloma and proteins expressed from this region are designated E1 through E8 (see below). The capsid antigens, L1 and L2, are encoded by the late region and are expressed in the more superficial, differentiated cells of a papilloma. One exception is the E4 protein, which is encoded by the early region but is expressed abundantly in the upper layers of a wart. All viral RNA species are transcribed from one strand and are extensively processed to give rise to alternatively spliced mRNA species. The early polyadenylation site is located between the early and late regions while the late transcripts use a second site at the end of the late region.

Each viral protein is relatively well conserved from one virus to another. However, proteins from different viral types interact with distinct cellular proteins as well as those common to many viral types, and this likely explains their highly selective species and tissue tropism. One exception to the common genomic organization is the E5 gene, which is not present in all viruses. Another is genus *Xipapillomavirus*, which contains certain bovine papillomaviruses (BPV-3, BPV-4, BPV-6), that do not have an E6 gene and instead have a different gene in this position that has been designated E8. E8 is a membrane

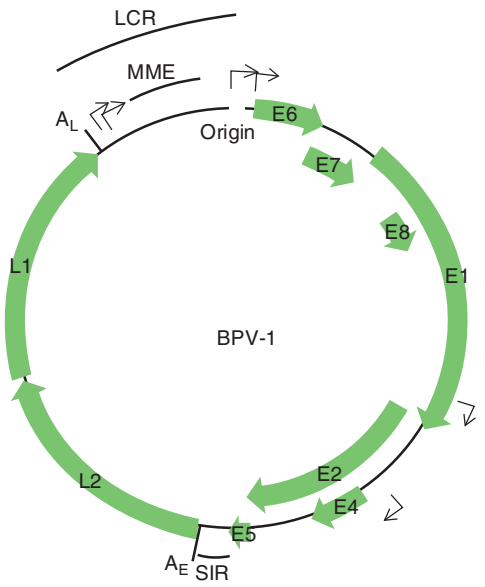


Figure 3 The BPV-1 genome. E and L designate the early and late open reading frames, respectively; promoters are indicated by arrows. LCR, long control region; SIR, short intragenic region; A_E and A_L, early and late polyadenylation sites, respectively; MME, minichromosome maintenance element.

protein with properties analogous to the E5 proteins of other PVs but there is no evidence that they are evolutionarily related. PePV, the African grey parrot PV, also has two genes at the beginning of the early region that have little similarity with the classical E6 and E7 genes and have instead been designated E8 and E9.

Functions of Viral Proteins

The functions of the individual viral proteins encoded by animal viruses are somewhat analogous to their HPV counterparts. The E5 protein of BPV-1 is the primary transforming protein and promotes proliferation of infected cells. It is a membrane protein that can transform fibroblasts by inducing constitutive, ligand-independent activation of the β-type platelet-derived growth factor receptor (PDGF-Rβ), and by interfering with Golgi acidification. BPV-4 E5 also transforms fibroblasts and disrupts gap junction-mediated intercellular communication. Many PV E5 proteins downregulate surface expression of major histocompatibility complex (MHC) class I molecules, which helps the virus to evade the host immune system.

The E1 protein is the primary replication protein. It is an ATP-dependent helicase that specifically binds and unwinds the viral DNA replication origin to allow access of the cellular replication proteins. It binds cooperatively to the origin in concert with the E2 protein. The E1 protein then converts to a double hexamer that encircles the DNA and unwinds the origin. The structure and function of the E1 protein is analogous to that of the polyomavirus SV40 T antigen. Much of our understanding about PV replication originates from key studies in BPV-1.

The E2 gene encodes multiple proteins that are the result of expression from multiple promoters and alternative RNA splicing. In BPV-1, there are three E2 proteins (E2TA, E2TR, and E8/E2). They share sequence-specific DNA binding and dimerization activities and the longest protein, E2TA, also contains a transcriptional activating domain. E2TA stimulates viral promoters by binding to multiple 12 bp palindromic sequences in E2-specific enhancers within the LCR. This activity is modulated by the E8/E2 and E2TR proteins which antagonize the functions of E2TA. The E2TA protein also represses transcription from viral promoters when the E2 binding sites overlap essential promoter elements. The E2 protein has an additional role in maintaining and partitioning the genome in the dividing basal cells. The E2 protein is a sequence-specific binding protein that binds to multiple E2 binding sites in the viral genome (in the MME) and tethers them to the cellular mitotic chromosomes through the transactivation activation domain. This has been best characterized for BPV-1, and the cellular bromodomain protein, Brd4, which binds to acetylated histones on chromatin, is an important cellular protein in this tethering complex. E2 also has a role at late stages of infection because expression of BPV-1 E2 proteins is greatly increased in cells that are vegetatively amplifying viral DNA.

Restricting high levels of viral DNA replication and antigens to the more superficial layers of an epithelium and releasing virus by the natural process of desquamation might be important for immune evasion by the virus. However, this means that the virus needs to replicate its DNA in cells that have normally withdrawn from the cell cycle and are undergoing differentiation. One of the main functions of the E6 and E7 proteins seems to be to sustain cells in an S-phase-like state so that the virus has available the enzymes to replicate its own DNA. This aberrant state induces cell cycle sensors that would normally arrest cells and perhaps cause them to undergo apoptosis. The molecular mechanism by which the E6 and E7 proteins of the 'high-risk' HPVs avert this process, by inactivating the retinoblastoma (pRb) and p53 proteins, is well understood. However, it is not yet clear how many of the animal viruses and 'low-risk' HPVs fulfill this function. The BPV-1 E6 protein disrupts the actin cytoskeleton and binds a number of cellular proteins, including ERC-55, the focal adhesion protein paxillin, the E3 ubiquitin ligase E6AP, and the clathrin adaptor complex AP-1. BPV-1 E7 lacks the binding motif that mediates direct binding of the HPV E7 proteins to pRb. However, both BPV and HPV E7 proteins bind a cellular protein, p600, a unique pRb- and calmodulin-binding protein. In the nucleus, p600 and pRB, seem to act as a chromatin scaffold and, in the cytoplasm, p600 forms a meshwork structure with clathrin. Viral-induced

hyperplasia is believed to be induced by the viral early gene products, and results from both increased division of the basal cells and delayed maturation of the committed keratinocytes of the spinous layer (acanthosis). An unintended result of this cell cycle deregulation in some viruses is the immortalization of the infected cells and the continual division of cells that have sustained DNA damage, which can lead to a malignant phenotype.

The E4 protein, usually expressed from a spliced mRNA as E1^E4, is expressed at very high levels in the productively infected cells of a wart. It can interfere with the cell cycle, which may be important for deregulation of cell division and differentiation. E4 can also disrupt keratin filaments and induce abnormalities in the cornified envelope. These functions may be important for egress of the virus from the outer layers of a wart.

The L1 protein is the major capsid protein. The minor capsid protein, L2, is important late in infection for packaging the viral genome into the capsid. L2 is also important early in infection to transport the viral DNA to the nucleus and to establish a permissive site within the nucleus to initiate viral transcription and replication.

Virion Structure and Properties

PV virions form a nonenveloped, icosahedral structure of 55–60 nm diameter (see **Figure 4**). The capsid is composed of the major capsid protein, L1, and the minor capsid protein, L2, which form 72 pentameric capsomeres with an icosahedral symmetry of $T = 7$. The viral genome is packed in a nucleohistone complex. The L1 protein can self-assemble into virus-like particles (VLPs); VLPs present the conformational epitopes required for generating high-titer neutralizing antibodies and are the basis of very successful vaccines in humans and animals.

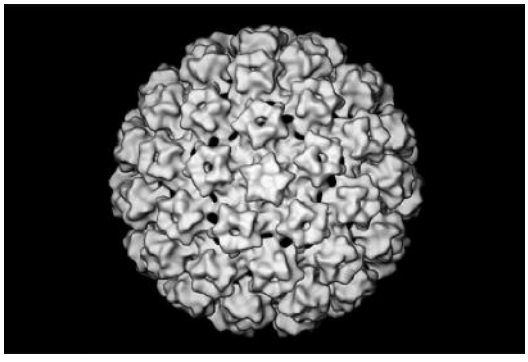


Figure 4 BPV-1 capsid. Reproduced from Trus BL, Roden RB, Greenstone HL, Vrhel M, Schiller JT, and Booy FP (1997) Novel structural features of bovine papillomavirus capsid revealed by a three-dimensional reconstruction to 9 Å resolution. *Nature Structural Biology* 4: 413–420.

Virus Propagation

The complete life cycle of PVs requires a stratified, differentiated epithelium because vegetative viral DNA replication and late gene expression can only take place in differentiated keratinocytes. To reproduce this in the laboratory, several xenograft techniques have been developed. Small pieces of epithelial tissue infected with virus and implanted in the renal capsule of an immunocompromised mouse will produce viral particles. Skin from various species can also be grafted onto immunocompromised mice and either infected with virus or transfected with viral DNA. The infected xenograft will form a papilloma-like lesion that will produce virion particles. Artificial skin equivalents (organotypic rafts) can also be established in tissue culture from keratinocytes and fibroblasts of various species. These rafts support the viral life cycle and produce viral particles. Relatively large quantities of infectious virions can also be purified from cells that have been co-transfected with the viral genome along with expression vectors for the L1 and L2 capsid proteins.

Notable Animal Papillomaviruses

PVs have been isolated and characterized from a multitude of animals including cattle, sheep, deer, horses, rabbits, dogs, mice, birds, and nonhuman primates. Many are listed in **Table 1** and a few of the better-studied viruses are described in detail below.

BPV-1. BPV-1 causes fibropapillomas on the cutaneous epithelium of cattle. It is readily transmitted among herd animals through direct contact of abraded skin. BPV-1 belongs to an unusual class of PVs that cause fibropapillomas in ungulates. These viruses have a broader host range than most PVs; BPV-1 can naturally infect horses, giving rise to sarcoids, can cause tumors in hamsters, and can morphologically transform mouse fibroblasts in culture. The infection is nonproductive in each of these cases.

In 1980, Lowy and co-workers demonstrated that cloned BPV-1 DNA could morphologically transform mouse cells in culture. The viral DNA replicated as an extrachromosomal element within these cells. Because of this, BPV-1 became the molecular prototype of the PVs and viral functions responsible for transformation, DNA replication, and transcriptional regulation were first characterized for BPV-1.

BPV-4. BPV-4 causes benign papillomas of the alimentary tract in cattle. In certain regions of Scotland, when cattle graze on bracken, these papillomas progress at a high rate to malignant carcinoma. Bracken grown in these regions contains at least one identified co-carcinogen,

a flavenoid called quercetin, which promotes malignant progression of the papillomas.

CRPV. Cottontail rabbit papillomavirus (CRPV) naturally infects the cutaneous epithelium of wild cottontail rabbits and is also able to infect jackrabbits and snowshoe rabbits. In contrast, experimental infection of domestic rabbits results in nonproductive papillomas that support normal early viral gene expression and genome replication, but are unable to support late gene expression and virus particle production. CRPV-induced papillomas can either persist or regress, depending on host genetic factors, and persistent papillomas can progress to carcinomas in both wild and domestic rabbits. CRPV DNA can induce papillomas on scarified rabbit skin and this has allowed a genetic assessment of which viral functions are required to induce papillomas. The CRPV model has also been used for the development of preventive and therapeutic PV vaccines.

COPV. Canine oral PV (COPV) induces warts on the oral mucosa of dogs. Infection is normally followed by spontaneous immune-mediated regression. COPV is a good model for mucosal PV infection and has been very useful in studying the immune response and the development of PV vaccines.

RhPV-1. Rhesus papillomavirus type 1 (RhPV-1) is a sexually transmitted PV associated with genital disease that was first isolated from a penile squamous cell carcinoma. RhPV-associated disease progression closely resembles that seen in human genital HPV infections and RhPV-1 is phylogenetically closely related to the 'high-risk' HPVs that are associated with cervical cancer. RhPV-1 will likely become an important model to study human genital PV infections.

Detection and Diagnosis

Serological tests for PV infection have been unreliable and detection usually relies on testing for viral DNA. Specific viral types can be detected using either hybrid capture or highly sensitive molecular techniques such as nested polymerase chain reaction (PCR) with the use of degenerate primers.

Immune Response, Prevention, and Control

PV infections can be prolonged and persistent, but usually regress spontaneously. This immune-mediated regression is effected by T-cells while reinfection is prevented by humoral immunity. This phenomenon was noted in 1898 by M'Fadyean and Hobday who concluded after experiments with canine oral papillomas that, "the animal is left in a measure protected against an infection of the same kind." This spontaneous and simultaneous regression of papillomas by systemic immunity has been noted in many animals.

Much of our understanding of the immunology of PV infection comes from studies of animal PVs. Humoral immunity to subsequent infections is due to neutralizing antibodies directed against the capsid antigens. This immunity is type specific and can be bypassed by using viral DNA to induce papillomas in rabbit skin. Effective animal vaccines were produced that consist of crude wart extract, and these have been quite effective in cattle and dogs. The use of highly purified VLPs of COPV, CRPV, BPV, and Equus caballus papillomavirus (EcPV) as effective prophylactic vaccines laid the groundwork for the recently licensed HPV vaccines.

Cellular immunity is crucial for regression of papillomas. Dense infiltrates of T-lymphocytes can be observed in regressing warts in many animal species and immunosuppression can result in severe papillomatosis. The early, noncapsid proteins are important antigens for cell-mediated immunity and these might prove to be effective therapeutic vaccines for existing infections. Vaccination with CRPV early viral gene products has been shown to clear papillomas in rabbits. However, one complication is that PVs encode several functions that enable them to evade the immune system; they are able to inhibit interferon-dependent innate immunity and disrupt viral antigen presentation, which might also interfere with therapeutic vaccination.

See also: Papillomaviruses: General Features; Papillomaviruses: General Features of Human Viruses; Papillomaviruses: Molecular Biology of Human Viruses; Simian Virus 40.

Further Reading

Campo MS (2002) Animal models of papillomavirus pathogenesis. *Virus Research* 89: 249–261.

de Villiers EM, Fauquet C, Broker TR, Bernard HU, and zur Hausen H (2004) Classification of papillomaviruses. *Virology* 324: 17–27.

Nicholls PK and Stanley MA (2000) The immunology of animal papillomaviruses. *Veterinary Immunology and Immunopathology* 73: 101–127.

Peh WL, Middleton K, Christensen N, *et al.* (2002) Life cycle heterogeneity in animal models of human papillomavirus-associated disease. *Journal of Virology* 76: 10401–10416.

Trus BL, Roden RB, Greenstone HL, Vrhel M, Schiller JT, and Booy FP (1997) Novel structural features of bovine papillomavirus capsid revealed by a three-dimensional reconstruction to 9 Å resolution. *Nature Structural Biology* 4: 413–420.

Van Doorslaer K, Rector A, Vos P, and Van Ranst M (2006) Genetic characterization of the *Capra hircus* papillomavirus: A novel close-to-root artiodactyl papillomavirus. *Virus Research* 118: 164–169.

Papillomaviruses: General Features

H U Bernard, University of California, Irvine, Irvine, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Papillomaviruses (PVs) are small DNA viruses that infect mucosal and cutaneous epithelia (skin). More than 100 PV types have been isolated from humans, and one or some few PV types have been found in virtually every carefully studied mammal and bird. PVs are strictly host species specific – human papillomaviruses (HPVs) cannot infect any other mammals, and no animal PV infects humans. Historically, PV research began with certain mammalian PV types (cottontail rabbit papillomavirus and bovine papillomavirus), as the large lesions caused by these viruses were sources for substantial virus preparations. Today's refined techniques led to the isolation and analysis of numerous PV types from a variety of human lesions, notably carcinomas of the cervix uteri (HPV-16), genital (HPV-6), and common warts (HPV-2). PVs cause benign and malignant neoplasia as they express oncoproteins with pleiotropic functions resulting from interactions with numerous cellular proteins. These oncoproteins induce continuing cell divisions in peripheral epithelial layers, while in the absence of PV oncoproteins, cell divisions are restricted to cells of the basal layer of epithelia. The consequence of this deregulation is a localized growth of the epithelium, leading to a papilloma (or wart), which gave this virus family its name. Within the broad spectrum of PV research, most efforts were directed to understand HPV-mediated carcinogenesis. These molecular studies have an increasing impact on medical practice in the form of DNA diagnosis and prophylactic vaccination.

Papillomavirus Particles and Genomes

PVs have icosahedral, nonenveloped particles (capsids or virions) with a diameter of approximately 55 nm. The particle is composed of 72 capsomers, and each of these capsomers consists of five identical L1 proteins, which are encoded by the PV genome. The virions also contain the minor capsid protein L2, which, however, is not part of the capsomers and is not required to generate a complete virion.

The PV particle contains the circular, double-stranded DNA genome in the form of chromatin. Many important PV types have genome sizes very close to 7900 bp; other viral genomes have sizes of a few hundred basepairs below or above this number. Most PV types encode eight open reading frames (ORFs): E1, E2, E4, E5, E6, E7, L1, and L2 (**Figure 1**). These ORFs are considered genes, as each of them is sufficient to encode a protein, although differential splicing also leads to alternative uses of some ORFs. The exact role and regulation of some alternative splices are still insufficiently understood. PV mRNAs are normally polycistronic (i.e., contain several ORFs), a dramatic deviation from the monocistronic mRNAs typical for eukaryotes and their viruses. The efficient translation of ORFs that are located 3′ of other ORFs is still little understood. Some few PVs lack the E5 or E6 genes. The L1 and E6 genes are separated by a genomic segment constituting about 10% of the PV genome, which contains many *cis*-responsive elements required for viral transcription and replication. This segment is called the long control region (LCR).

The Most Commonly Studied Papillomaviruses

More than 200 PV types exist in humans, and half of these have been isolated and formally described. The number of additional PV types in mammals is probably unlimited, although so far only a few dozen have been described. The tremendous diversity of PVs is not such a formidable barrier to understand PV biology as one might fear, as most research was based on only nine HPVs and three PVs from other mammals. Human papillomavirus 1 (HPV-1) induces plantar (foot-sole) warts and HPV-2 common (hand or face) warts. HPV-5 and HPV-8 are associated with epidermodysplasia verruciformis, a skin neoplasia linked to a genetic risk factor. HPV-6 and HPV-11 cause genital and laryngeal warts. HPV-16, HPV-18, and HPV-31 are the most prominent types causally linked to anogenital and some head and neck carcinomas. Bovine papillomavirus 1 (BPV-1) causes fibropapillomas and BPV-4 mucosal lesions in cattle, and the cottontail rabbit papillomavirus (CRPV) cutaneous lesions in rabbits. Owing to its medical importance and high prevalence, HPV-16 has probably been the object of more research than all remaining types together. The information in this article dealing with PV proteins and gene regulation is therefore biased in favor of HPV-16. Many – but clearly not all – functions of this virus are shared with other PVs.

Taxonomy and Evolution

PVs and polyomaviruses share two properties, namely small circular double-stranded DNA genomes and nonenveloped isosahedral capsids. As a consequence, they had once been

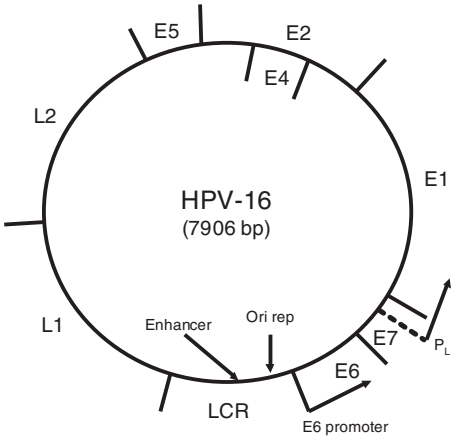


Figure 1 Genome organization of human papillomavirus type 16 (HPV-16). The genome consists of double-stranded DNA with a size of 7906 bp. LCR: Long control region, containing numerous *cis*-responsive elements for the viral transcription and replication, including the epithelial specific enhancer and the replication origin (ori rep). P_L: late promoter; E6 and E7: the two principal oncogenes; E1: replication initiation protein; E2: transcription factor, support of replication, segregation of viral DNA; E4: virion assembly and release; E5: minor oncogene; L2: minor capsid protein, intracellular transport of viral DNA after infection; L1: major capsid protein.

lumped into one virus family, *Papovaviridae*. When it became clear that they have different genome organizations and no genomic similarity, they were split into the two families *Papillomaviridae* and *Polyomaviridae*.

The PV literature uses the term 'type' to identify unrelated PV isolates, and the terms 'subtype' and 'serotype' are inappropriate. There are presently more than 100 formally described HPV types. New PV types have been found in all carefully examined mammal species, but so far in only two birds, and none in other animal taxa. Only one or some very few PV types have been detected in each animal host, and it is unclear whether the high number of HPV types has resulted from the more intense study of humans, or whether humans had been a more 'fertile' environment for PV evolution than other mammals.

The taxonomy of PVs was built on the comparison of genomes rather than on amplifiable viruses or on serology, since PVs do not multiply efficiently in cell culture, and since no consistent serology could be established based on natural infections. The genome-based taxonomy of PVs was founded in the 1980s with DNA hybridization data, and was later shifted to nucleotide sequence comparisons. By definition, a PV type is unique when the nucleotide sequence of its L1 gene differs by at least 10% from the L1 sequence of any other PV type. As a consequence, PV types are genotypes and not 'serotypes', although a retrospective study would likely reveal that most PV types are serologically distinct. PV types are designated by one or two capital letters identifying their host, and numbered according to the sequence of isolation, for example, HPV-16 for human papillomavirus type 16.

In order to classify PV types following traditional taxonomic terminology, the term 'type' has been placed in the taxonomic hierarchy below the term 'species'. In other words, phylogenetically closely related PV types with similar or identical biological and pathogenic properties have become lumped into PV species. While this procedure was justified to arrange PVs taxonomically in a manner similar to other virus families, it has created species with potentially confusing numeric designations that may not become generally used in the literature. In order to give an example, HPV types 16, 31, 33, 35, 52, 58, and 67 form the species *Human papillomavirus 16*. At the taxonomic level between species and the family (*Papillomaviridae*), PV species form genera that are designated by Greek prefixes (*Alpha-* through *Pi-papillomavirus*). Most medically important HPV types belong to genus *Alphapapillomavirus*.

Repeated isolates of the same PV type differ by up to 2% of their genomic sequence, and are termed variants of the original prototype isolate. Evidence suggests that this diversity evolved over a period of several hundred thousand years in linkage to the evolution of *Homo sapiens*. The term 'subtype' is used for rare PV isolates that show diversity in between the type and the variant level. PVs do not rapidly diversify like many RNA viruses, and do not form quasi-species.

Host Range

PVs are host species-specific, with the exception of a few PVs that were found in several different domestic hoofed animals, possibly transmitted by close contact between these animals. It has not been carefully studied whether host specificity is based on the lack of efficient contact or, more likely, on molecular incompatibility between PVs and heterologous hosts.

Within a particular host, notably humans, PVs often have a preferential target tissue. For example, HPV-1 is typically found in flat warts of the foot sole, HPV-2 in common warts of the skin elsewhere, HPV-6 in mucosal but also cutaneous lesions of the genitals, and HPV-16 mostly in anogenital and head and neck mucosal epithelia. This may be based either on molecular restrictions to replication in other types of epithelia, or on the lack of symptoms of established viral infections in inappropriate target epithelia. An example for the latter scenario is the fact that HPV-16 and some related types can cause cancer in women, but infect men normally asymptomatically. This possibility is also supported by the observation of HPV types that cause 'epidermodysplasia verruciformis' in patients with a genetic risk factor, while these same types can be detected in the skin of many individuals of

the general human population in the absence of any symptoms.

While nearly all PV types are only found in epithelia, certain PV types replicate in cattle (like BPV-1) and other hoofed animals; also in mesenchymal tissue (fibropapillomaviruses).

Functions of Papillomavirus Proteins

The PV proteins that participate in viral replication, transcription, and transformation are identified by the letter E (for early), and those found in the capsid by the letter L (for late), each followed by a number according to its size in the first genome characterized (E1 and L1 being the two largest proteins). In the following paragraphs, these proteins will be discussed in the order of their numbers.

The 649-amino-acid residue E1 protein controls the duplication of the PV genome. It initiates DNA replication at the single viral replication origin about 100 bp 5′ of the E6 promoter. By convention, PV genomes are numbered in such a way that the E1 binding site spans the genome position 1. The E1 binding site is only loosely conserved as a 20 bp A+T-rich segment, and is therefore also only poorly recognized by the E1 protein. This failure is compensated by the E2 protein, which has binding sites close to the E1 target. E2 binding sites are much more sequence-specific than those for E1, and by forming a complex with E1, E2 is crucial for binding the replication origin.

E1 binds initially as a dimer and then undergoes polymerizations during the subsequent steps of replication initiation. Central to E1 function is an ATP-dependent helicase, and E1 hexamers melt and unwind the double-stranded DNA of the replication origin and translocate DNA single strands. Beyond these basic E1 functions, PV DNA duplication depends on the host replication machinery. E1 recruits cellular replication proteins to the PV origin of replication, including DNA polymerase α, replication protein A, and topoisomerase I. There is also evidence that E1 is a target for cellular signals that couple PV replication to the cell cycle.

The E2 protein has a size of 365-amino-acid residues and can be divided into three domains, an N-terminal transcription activation domain, a central hinge region, and a C-terminal DNA-binding domain. E2 proteins have at least three functions: stimulation of replication, modulation of transcription, and attachment of PV genomes to cellular chromosomes during mitosis.

All three functions require the DNA-binding domain. E2 proteins form dimers, which bind the palindromic sequence ACCGNNNNCGGT or slightly degenerated targets. The genomes of HPV-16 (and those of many related medically important HPV types) have four E2 binding sites, two of them positioned close to the E6 promoter, one 5′ of the E1 binding site, and one in the center of the LCR. The two E2 binding sites at the E6 promoter overlap with binding sites for TFIID and the Sp1 factor, and E2 represses the promoter by displacing these activators. The E2 binding site 5′ to the E1 binding sites, and one of the two E2 sites at the E6 promoter, lead to cooperation between E1 and E2 in origin function, as described above. The function of the E2 binding site in the center of the LCR is not well understood.

The E2 protein was for the first time studied in BPV-1 and described as an activator of transcription. Occupation of E2 binding sites in the LCR of BPV-1 strongly stimulates the E6 promoter, as these sites form an E2-dependent enhancer. This E2 function depends on the N-terminal transcription activation domain. The N-terminal domain of the HPV-16 E2 protein is homologous to the BPV-1 E2 domain, and the HPV-16 protein functions as a transcriptional activator in the context of BPV-1 genomes. Strangely, however, no enhancer activation function could ever be detected in HPV-16 genomes, and, consequentially, E2 is known only as a repressor of this virus. Many PVs also express shortened transcripts of E2 that solely encode the C-terminal DNA-binding domain and function as repressors by competing with full-size E2 proteins for binding to E2 *cis*-responsive elements.

The E4 ORF is positioned within the E2 gene, overlapping with the E2 hinge. Its 95-amino-acid residues are translated in a different reading frame from E2. A splice (E1^E4) fuses some N-terminal amino acids of the E1 ORF to E4. Some E4 transcripts terminate downstream of the early genes, while others extend into the late genes and are spliced to L2 or L1. E4 is sometimes considered a late protein due to the resulting co-expression with L2 and L1 and the fact that E4 is highly expressed in differentiating cells. E4 is known to support viral genome amplification in the productive part of the PV life cycle, capsid protein expression, virion assembly, and virion release, although the detailed mechanisms behind these E4 functions are still poorly understood. They include interactions with and reorganization of the cytokeratin network, reorganization of nuclear ND10 domains, and effects on cell cycle regulators.

E5 proteins are small (83-amino-acid residues in HPV-16 and only 44 residues in BPV-1) and do not show inter-type sequence similarities. They have functional homologies, however, as they are highly hydrophobic and thereby localized to the cell membrane and the endoplasmatic reticulum. Cell culture experiments defined E5 proteins as transforming proteins, although they are not expressed in many anogenital carcinomas due to interruption of the viral genome. They are pleiotropic and share at least three functions: (1) association with the 16 kDa subunit of the vacuolar H^+-ATPase, influencing the half-life of tyrosine kinase receptors; (2) direct interactions with tyrosine kinase receptors including the platelet-derived

growth factor and epidermal growth factor receptors, and (3) downreguation of major histocompatibility complex class I molecules.

E6 and E7 are the two principal transforming proteins of the oncogenic HPV types as judged by cell culture transformation, continued expression in carcinomas, reversion of the transformed phenotype of cancer-derived cell lines after annihilation of E6/E7 expression, and complex pleiotropic molecular functions. They are encoded in the 5′ segment of polycistronic mRNAs transcribed from the E6 promoter, although many transcripts lack the ability to translate E6 due to an internal (E6*) splice.

The E6 protein of HPV-16 has a size of 149-amino-acid residues. The position of eight cysteine residues suggests the formation of two zinc fingers, which are larger than zinc fingers reported from cellular proteins. Although one would expect that these would help to induce a strong three-dimensional conformation, there is no information yet about the structure of E6. E6 shares this problem with the E7 protein, which has one single zinc finger with similar sequence properties. The lack of information about the structure of E6 and E7 proteins has remained a major obstacle in efforts to design drugs that would specifically interfere with these important oncoproteins.

More than 20 different cellular targets of E6 proteins have been described, and the contributions of these diverse interactions to the viral life cycle and carcinogenic processes are still much debated. It should be noted that PV oncoprotein functions evolved to support the latent and productive HPV life cycle, that is, the creation of a molecular environment favorable to virus replication. In spite of the medical importance of HPV-induced carcinogenesis, this pathological outcome should be considered an aberration and a fortuitous byproduct of the viral biology.

The most prominent target of E6 is the cell-cycle regulator and tumor suppressor p53. E6 and p53 form a trimeric complex including a protein called E6AP. E6AP is a ubiquitin ligase and the trimeric association results in p53 degradation. As a consequence, p53 is lost as inducer of the cdk inhibitor p21CIP, and the cell cycle of the infected cell is set free to undergo G1/S transition, creating an environment favorable to viral DNA replication, and establishing a prerequisite for oncogenic transformation. p53 is also known to be an inducer of apoptosis, and p53 elimination by E6 protects against this mechanism, which would otherwise eliminate infected cells and terminate PV replication. Yet other functions of E6 include the modulation of transcription by affecting the cofactor CBP/p300, effects on the immune response by interactions with the interferon regulatory factor-3, and alterations of cell shape and signaling by reaction with hDlg in a manner similar to a pathway that is induced by mutations during carcinogenesis of the colon.

The E7 oncoprotein of HPV-16 has a size of only 99 amino acid residues. E7 binds the retinoblastoma (RB) cell cycle regulator and tumor suppressor. In its normal function, RB represses the transcription factor E2F, which, in the absence of RB, induces genes required for G1/S transition of the cell cycle. The normal RB and E2F interactions are controlled by cyclin-dependent kinases. When E7 binds RB, E2F is released from the RB-E2F complex in an uncontrolled manner. Elimination of p53 and RB cell cycle control is a fundamental event in carcinogenesis, as many cancers whose etiology does not depend on viruses carry p53 and RB mutations. E7 is pleiotropic, and its functions include an affinity with the centromere. This leads to chromosomal abnormalities and genomic instability, resulting in a 'mutator phenotype' and promoting establishment of the malignant phenotype.

The 531-amino-acid residue L1 protein is the principal building block of the virion by forming 72 pentamers, which arrange into structurally complete icosahedral capsids in the absence of viral DNA or any other viral protein. This property has led to the production of HPV-6, HPV-11, HPV-16, and HPV-18 particles in heterologous expression systems as prophylactic anti-HPV vaccines. The L1 protein is central to the first step of PV infections by binding to proteoglycans and integrins at the surface of epithelial target cells. The infection does not select specific target cells, and the epithelial specificity of PV infections is established by the transcriptional environment in epithelial cells.

The L2 protein is part of the viral particle, where it forms a complex with the PV DNA. After uptake into endosomes, L2 accompanies the viral DNA by contact to the microtubule network via the motor protein dynein to the nucleus and subsequently to the subnuclear promyelocytic leukemia protein bodies, suggesting that it may be involved in the intracytoplasmic and nuclear transport of the PV DNA.

Gene Expression

The expression of PV genes, that is, ultimately the availability of each viral protein in the infected cell, is influenced by numerous mechanisms, such as frequency of transcription, recognition of transcription termination sites, differential splicing, mRNA stability, translation efficiency, and protein stability. There are many similarities of these mechanisms among different PVs, notably among the medically important HPV types, but strict generalizations are not possible. An example of exceptions is the transcription factor E2, which is in BPV-1 an enhancer-activating factor, but a negative regulator in HPV-16.

Transcription of all eight PV genes starts a few nucleotides 5′ of the ATG of E6 (E6 promoter), and continues unidirectionally around the whole viral genome. The E6 promoter has a TATA box that binds the general

transcription factors including TFIID, and a promoter element, bound by Sp1. Among the numerous mRNAs generated from the E6 promoter are those that encode E2. E2 has two binding sites at the E6 promoter, and E2 proteins bound to these sites displace Sp1 and TFIID. Increasing mRNA levels derived from the E6 promoter lead to increased E2 expression and decreased use of this promoter, a perfect negative feedback loop, one of several adaptions of PV genomes to an inefficient (latent) rather than fulminant infection. HPV-16 genomes often recombine with chromosomal DNA in carcinomas such that the E2 gene is disconnected from the E6 promoter, releasing this repression mechanism.

The E6 promoter of HPVs has a low activity and requires activation by an enhancer centered 300 bp upstream of the promoter. This enhancer is only active in epithelial cells and determines the epithelial specificity of PVs. It does not bind E2, but depends on a variety of transcription factors including AP1, NFI, and glucocorticoid and progesterone receptors. Interestingly, the stimulation of PV oncogene expression by progesterone correlates with epidemiological data pointing to a high risk of cancer progression in women with multiple pregnancies and long-term anti-ovulant usage. A second promoter (p_L), relevant for the expression of L1 and L2, is positioned within the E7 gene. Activation of the TATA-less p_L is reminiscent of an early-late switch and still little understood.

PV transcription is strongly regulated by epigenetic mechanisms, that is, conformational changes of the PV chromatin, specifically of two nucleosomes that bind the HPV-16 enhancer and promoter. Histone acetylation and deacetylation can permit or restrict transcription. A variety of mechanisms can influence these parameters, notably the antagonistic factors CDP and AP1, which couple PV transcription to epithelial differentiation, and DNA methylation, which may determine whether an infection takes a latent or productive course.

Transmission and Epidemiology

PV infections are only stable when the pathogen reaches basal layers of an epithelium, for example, in wounds. PV infections result from physical contact between healthy and infected epithelia that peripherally release PV particles (e.g., during desquamation of the skin). As sexual intercourse leads to physical contact between genital epithelia, infections by many HPV types are considered sexually transmitted diseases. Epidemiological data support this mechanistic concept and document a rapid increase of genital PV infections in male and female individuals after commencement of sexual activity. Epidemiological evidence also supports the view that the risk to develop cervical carcinomas increases with young age at the start of sexual activity and with the number of sexual partners per lifetime. Additional risk factors are long-term use of anti-ovulants, multiparity, and tobacco smoking. Epidemiology provided a foundation for the concept of 'high-risk' and 'low-risk' HPV types. Both groups of HPVs are regularly found in exfoliated cells from anogenital sites, but only high-risk HPVs are frequent in cancer. 18 of the roughly 40 HPV types found in anogenital mucosas are considered high-risk types (e.g., HPV-16, HPV-18, and HPV-31), while HPV-6 and HPV-11, the cause of genital warts, are low-risk types, as they are rare in malignancies.

PVs are frequently transferred from mother to child during birth. So far, however, there is no evidence for efficient establishment of infections with high-risk HPV types toward anogenital carcinogenesis by this infection route. It is very possible, however, that the relatively high fraction of juvenile patients affected by laryngeal papillomatosis have acquired their HPV-6 and HPV-11 infections from their mothers.

Pathogenicity

PVs are well established as the cause of a variety of cutaneous and mucosal neoplastic lesions. As discussed above, the molecular pathology of such lesions is based on changes of epithelial homeostasis affected by the viral oncoproteins. In spite of the detailed knowledge of many molecular mechanisms, many aspects of PV pathogenicity are not as obvious as it is often assumed.

An example is the fact that many PV types have only been found in latent infections, and even those viruses typically associated with benign and malignant neoplasia can regularly be detected in healthy tissue. It is not well understood whether every PV infection leads to neoplasia, unless suppressed by an immune response, or whether the viral biology includes latent stages, maintenance of PV DNA without changes of the infected cell. This is exemplified by the fact that the same HPV types that can induce carcinogenesis of the cervix infect the penis without easily detectable symptoms. While male individuals function efficiently as transient hosts, they are rarely affected by disease. Yet another example is the fact that HPV-associated genital carcinogenesis in women is rare in the vagina, but typically arises from the transformation zone of the cervix, a tissue where adjacent endocervical columnar epithelia and ectocervical squamous epithelia change in a process called squamous metaplasia. As HPV tumors at yet other sites (anal carcinomas and laryngeal papillomas) are also associated with boundaries between different epithelia, one may speculate that yet poorly understood differentiation processes may synergize with PV molecular biology in order to induce neoplastic changes. Lastly, it is well confirmed that progression of precancerous lesions to malignant carcinomas is not just the result of PV-encoded mechanisms, but requires the accumulation of additional

genomic changes, which are documented in extensive databases of chromosomal aberrations detected in cervical carcinomas. The molecular identity of these mutations is still a matter of research.

Diagnosis

HPV infections are diagnosed by the detection of a cutaneous or mucosal neoplasia, that is, a wart. Wart-like lesions, condylomata acuminata, can also occur at the cervix under the influence of the low-risk HPV-6 and HPV-11. High-risk HPVs induce flat condylomas which can be diagnosed by visual inspection through a colposcope. The traditional diagnosis of cervical PV infections is the Papanicolaou test ('pap test'), a technique developed in the 1950s and predating all knowledge about PVs. The Pap test aims to detect and classify dysplasia by microscopic observation of stained cervical exfoliated cells. Diagnostic criteria include a perinuclear halo (believed to result from accumulation of E4 protein) and nuclear enlargement (a consequence of polyploidies). In recent years, Pap tests have become complemented by DNA diagnostic detection of PVs.

Treatment

The treatment of many benign cutaneous or mucosal PV lesions will typically be a 'wait and see' approach. For those cases where treatment is necessary, surgical procedures can be based on excision by knife, laser, cryotherapy, or caustic substances. The application of concentrated solutions of salicylate is a traditional treatment of common warts, and podophyllin has been used in the treatment of genital warts. Antiviral drugs targeting PVs include interferons and imiquimode. Cervical precancerous lesions are surgically removed by excision of a cone-shaped wedge from the cervix or loop excision of the transformation zone. For information about treatment of malignancies, appropriate handbooks about gynecological oncology, etc., should be consulted.

Immunology and Vaccination

Anti-PV immune responses can be directed against the viral capsid, that is, targeting the L1 protein, or against virally infected cells, targeting any of the six early proteins.

Particles of HPV-16 and HPV-18 consisting only of L1 protein have been developed as vaccines by Merck and GlaxoSmithKline, the product of the former company also including HPV-6 and HPV-11 capsids. The vaccines became available in 2006/07, and have been cleared by the Food and Drug Administration of the United States for prophylactic vaccination of women aged 9–26. Vaccination during extensive clinical studies efficiently protected PV-uninfected women against *de novo* PV infections over periods exceeding 5 years. The vaccinations led to the stimulation of humoral immune responses by more than an order of magnitude beyond levels found in natural infections. The success of this approach is apparently based on anti-PV immune globulin concentrations in cervical mucus that suffice to neutralize PV particles before they inject the viral DNA into target cells.

Induction of immunity against the early PV proteins could form the basis of therapeutic vaccination, a potentially splendid strategy for anticancer immune therapy. Unfortunately, while scientists are well aware of this possibility, no major success has yet been achieved, and evidence points to numerous major obstacles. Investigations of naturally infected individuals generally showed weak immune responses against early PV proteins, and those humoral or cellular responses that were detectable correlated poorly with pathology or detection of PV DNA. A reason for this fact may be that PV early proteins are poor antigens, as suggested by comparison in animal systems with various other antigens. In addition, many PV proteins are only expressed at low concentrations, notably the oncoprotein E6 present in all cervical carcinomas, and rarely enter the circulation, as they are shed from the epithelium with the infected cell population.

See also: Papillomaviruses: General Features of Human Viruses; Papillomaviruses: Molecular Biology of Human Viruses; Papillomaviruses of Animals; Polyomaviruses.

Further Reading

Bernard HU (2002) Gene expression of genital human papillomaviruses and potential antiviral approaches. *Antiviral Therapy* 7: 219–237.

Campo MS (ed.) (2006) *Papillomavirus Research: From Natural History to Vaccines and Beyond*. Wymondham, UK: Caister Academic Press.

Davy C and Doorbar J (eds.) (2005) *Human Papillomaviruses, Methods and Protocols*. Totowa, NJ: Humana Press.

de Villiers EM, Fauquet C, Broker TR, Bernard HU, and zur Hausen H (2004) Classification of papillomaviruses. *Virology* 324: 17–27.

Lowy DR and Schiller JT (2006) Prophylactic human papillomavirus vaccines. *Journal of Clinical Investigation* 116: 1167–1173.

Mantovani F and Banks L (2001) The human papillomavirus E6 protein and its contribution to malignant progression. *Oncogene* 20: 7874–7887.

Munger K, Basile JR, Duensing S, *et al.* (2001) Biological activities and molecular targets of the human papillomavirus E7 oncoprotein. *Oncogene* 20: 7888–7898.

Munoz N, Bosch FX, de Sanjosé S, *et al.* (2003) Epidemiological classification of human papillomavirus types associated with cervical cancer. *New England Journal of Medicine* 348: 518–527.

zur Hausen H and de Villiers EM (1994) Human papillomaviruses. *Annual Review of Microbiology* 48: 427–447.

Paramyxoviruses of Animals

S K Samal, University of Maryland, College Park, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Emerging virus A virus that has never before been recognized.
Phenotype The collective structural and biological properties of a cell or an organism.
Reverse genetics A technique whereby infectious virus is produced entirely from complementary DNA.
Syncytia Formation of fused or multinucleated cells.
Viremia The presence of a virus in the blood.
Zoonotic diseases Diseases that can be transmitted from animals to humans.

Introduction

The family *Paramyxoviridae* contains a large number of viruses of animals (**Table 1**), including a number of major animal pathogens (such as Newcastle disease virus (NDV), canine distemper virus, and rinderpest virus), zoonotic pathogens (such as Hendra and Nipah viruses), and a number of somewhat obscure viruses whose natural histories are poorly understood. New paramyxoviruses are being isolated on an ongoing basis from a wide variety of animals. For example, new paramyxoviruses have emerged that are pathogenic for marine mammals such as seals, dolphins, and porpoises (e.g., cetacean morbillivirus). Other paramyxoviruses that have been identified from various sources during the last few decades, such as Salem virus, Mossman virus, J-virus, and Beilong virus, are not associated with known diseases and are poorly understood. The recently identified Hendra and Nipah viruses came to light when they crossed species barriers and infected humans, causing severe, often fatal, zoonotic diseases. There are many animal paramyxoviruses, but only a few effective vaccines are currently available. Previously, genetic manipulation of paramyxoviruses was not possible because the genome is not infectious alone and RNA recombination is essentially nonexistent. This posed an impediment to the molecular and biological characterization of these viruses. However, in the last decade, methods of producing virus entirely from cDNA clones (reverse genetics) have been developed and have allowed manipulation of the genome of paramyxoviruses. This has greatly improved our understanding of the functions of each gene in replication and pathogenesis of these viruses. Another important aspect of this new technology is that vaccines can now be designed for some of the animal paramyxoviruses for which either vaccines are not currently available or the available vaccines are not satisfactory.

Taxonomy and Classification

Paramyxoviruses (some of which are sometimes also called parainfluenza viruses) belong to the family *Paramyxoviridae* of the order *Mononegavirales*. The order contains four families of enveloped viruses possessing linear, nonsegmented, negative-sense, single-stranded RNA genomes. The family *Paramyxoviridae* is further divided into two subfamilies: *Paramyxovirinae* and *Pneumovirinae* (**Table 1**). The two subfamilies differ in several features, most notably: (1) differences in nucleocapsid diameter (18 nm in *Paramyxovirinae* and 13–14 nm in *Pneumovirinae*); (2) possession of six to seven transcriptional units in *Paramyxovirinae*, and eight to ten transcriptional units in *Pneumovirinae*; (3) presence of an additional nucleocapsid-associated protein (M2-1) and an RNA regulatory protein (M2-2) in *Pneumovirinae*; (4) structural differences in the attachment protein; and (5) lack of RNA editing of the P mRNA in *Pneumovirinae*. The subfamily *Paramyxovirinae* comprises five genera, *Rubulavirus*, *Avulavirus*, *Respirovirus*, *Henipavirus,* and *Morbillivirus*, as well as a number of unclassified viruses that might become the basis of one or more additional future genera, in *Paramyxovirinae* (**Table 1**). The division of this subfamily into five genera and the unclassified group is based on: (1) amino acid sequence relationship between the corresponding proteins; (2) the number of transcriptional units; (3) RNA editing products of the P gene; and (4) the presence of neuraminidase and hemagglutinin activities in the attachment protein. The subfamily *Pneumovirinae* contains two genera: *Pneumovirus* and *Metapneumovirus*. These two genera differ by (1) presence of two additional genes, NS1 and NS2, in pneumovirus; (2) the pneumovirus gene order SH–G–F–M2, as opposed to metapneumovirus gene order F–M2–SH–G; and (3) amino acid sequence relationship between the corresponding proteins.

Host Range and Virus Propagation

Animal paramyxoviruses have been isolated from many different vertebrate animal hosts including mice, rats, bats, dogs, dolphins, seals, birds, cattle, pigs, horses, reptiles, tree shrews, and monkeys. In general, paramyxoviruses

Table 1 The genera and species of animal paramyxoviruses

Subfamily	*Genus*	*Animal virus*	*Animal host*	*Disease*
Paramyxovirinae	*Rubulavirus*	Parainfluenza virus 5 (formerly simian virus 5)	Dogs, pigs, monkeys	Respiratory disease
		Simian virus 41	Monkeys	Respiratory disease
		Porcine rubulavirus (La-Piedad-Michoacan-Mexico virus)	Pigs	Encephalitis, reproductive failure, corneal opacity
		Mapuera virus	Bats	Unknown
		Menangle virus (tentative species in the genus)	Pigs, bats	Reproductive failure
		Tioman virus (tentative species in the genus)	Bats	Unknown
	Avulavirus	Newcastle disease virus (avian paramyxovirus 1)	Domestic and wild fowl	Respiratory and neurological disease
		Avian paramyxoviruses 2–9	Domestic and wild fowl	Respiratory disease
	Respirovirus	Bovine parainfluenza virus 3	Cattle, sheep, and other mammals	Respiratory disease
		Sendai virus (murine para influenza virus 1)	Mice, rats, and rabbits	Respiratory disease
		Simian virus 10	Monkeys	Respiratory disease
	Henipavirus	Hendra virus	Bats, horses, humans	Severe respiratory disease
		Nipah virus	Bats, pigs, humans	Encephalitis
	Morbillivirus	Canine distemper virus	Carnivora species	Severe generalized and central nervous system disease
		Cetacean morbillivirus	Dolphins and porpoises	Severe respiratory and generalized disease
		Peste des petits ruminants virus	Sheep and goats	Severe generalized disease
		Phocine distemper virus	Seal	Severe generalized and central nervous system disease
		Rinderpest virus	Cattle, wild ruminants	Severe generalized disease
	Unclassified	Nariva virus		Unknown
		J-virus		Unknown
		Mossman virus		Unknown
		Tupia paramyxovirus	Tree shrews	Unknown
		Salem virus	Horses	Unknown
		Fer de lance virus	Snakes	Fatal disease
		Beilong virus	Rodents (?)	Unknown
Pneumovirinae	*Pneumovirus*	Bovine respiratory syncytial virus	Cattle	Respiratory disease
		Pneumonia virus of mice	Mice	Respiratory disease
	Metapneumovirus	Avian metapneumovirus	Turkeys, chickens	Severe respiratory disease in turkeys Swollen head syndrome in chickens

are restricted in host range. However, in recent years, some animal paramyxoviruses have been found to cross species barriers and infect other animal species and humans. In some cases, the animal viruses are highly virulent in the new host, as exemplified by Nipah and Hendra viruses, and pose a major public health concern. Interestingly, fruit bats in the genus *Pteropus* have been implicated as a reservoir of a number of new and emerging zoonotic animal paramyxoviruses. Other paramyxoviruses, such as NDV and bovine parainfluenza virus 3, can experimentally infect a variety of non-natural hosts, including rodents and monkeys, but typically are highly attenuated in these hosts. Many different primary and established cell cultures are used to grow animal paramyxoviruses. Some viruses do not readily grow in cell culture (e.g., avian metapneumovirus) and require adaptation by several passages in the cell cultures. Cell cultures derived from homologous species are generally used for cultivation of morbilliviruses and pneumoviruses. However, a number of paramyxoviruses grow well in cells of different host origin. For example, avian metapneumoviruses grow well in monkey kidney (Vero) cells, and bovine parainfluenza virus-3 grows well in monkey kidney (LLC-MK2 and Vero) cells and in baby hamster kidney (BHK_{21}) cells. Avian paramyxoviruses grow well in embryonated chicken eggs or cells derived from avian species. Some paramyxoviruses require

the addition of protease, such as trypsin, α-chymotrypsin, or allantoic fluid (as a source of secreted protease), to the medium for growth in cell culture. This is necessary for cleavage activation of the viral fusion F protein (see below). Characteristic cytopathic effects of paramyxoviruses include the formation of syncytia (multinucleated giant cells) and eosinophilic cytoplasmic inclusion bodies.

Properties of Virion

The virions are 150–350 nm in diameter, pleomorphic, but usually spherical in shape. They consist of a nucleocapsid surrounded by a lipid envelope. Virion M_r is around 500×10^6. Virion buoyant density in sucrose is 1.18–1.20 g cm^{-3}. Some viruses (particularly of *Pneumovirinae*) are also produced in long filamentous form. Virions are highly sensitive to dehydration, heat, detergents, lipid solvents, formaldehyde, and oxidizing agents. Virus stability varies from stable (NDV) to very labile (rinderpest, canine distemper, bovine respiratory syncytial virus, and avian metapneumovirus). The schematic of a typical paramyxovirus is shown in **Figure 1**.

Genome

The genome consists of a single segment of negative-sense RNA (i.e., complementary to mRNA) that is 13–19 kbp in length and contains six to ten genes encoding up to 12 different proteins. The genome contains neither a 5′ cap nor a 3′ end poly(A) tail. At the 3′ and 5′ ends of the genome are short extragenic (noncoding) regions known as the 'leader' and 'trailer' region, respectively. The length of the leader is approximately 50 nt, whereas the length of the trailer is 23–161 nt. The leader region (*Pneumovirinae*) or the leader region and adjacent upstream end of the adjacent N gene (*Paramyxovirinae*) contains a single genomic promoter that is involved in the synthesis of the mRNAs as well as a complete positive-sense replicative intermediate called the antigenome. Generally, the first 10–12 nt of the leader and trailer are complementary, reflecting a conservation of promoter sequences present at the end of the genome and antigenome. At the beginning and end of each gene are conserved transcriptional control signals involved in initiation and termination/polyadenylation of the mRNAs. These conserved sequences are known as 'gene-start' and 'gene-end' sequences. The genes are separated by short intergenic regions that are not copied into mRNA. There is one exception in bovine respiratory syncytial virus where the L gene-start sequence is located upstream of the gene-end sequence of the upstream M2 gene, resulting in overlapping genes. The intergenic region is a conserved trinucleotide for respiroviruses, morbilliviruses, and henipaviruses, but is variable in length for all other paramyxoviruses. Thus, this might be a potential signal in some viruses, but not in others. The gene map of a representative member of each genus is shown in **Figure 2**. The nucleotide lengths of the genomes of members of subfamily *Paramyxovirinae* are even multiples of six, which is required for efficient RNA replication and is known as the 'rule of six'. However, the rule of six does not apply to the members of the subfamily *Pneumovirinae*. The genome size of a number of animal paramyxoviruses has been determined: 15 384 nt for Sendai virus; 15 456 nt for bovine parainfluenza virus 3; 15 246 nt for simian virus 5; 15 450 nt for simian virus 41; 15 186 nt for NDV; 15 882 nt for rinderpest virus; 15 948 nt for peste des petits ruminants virus; 15 690 nt for canine distemper virus; 15 702 nt for cetacean morbillivirus; 18 234 nt for Hendra virus; 18 246 nt for Nipah virus; 15 140 nt for bovine respiratory syncytial virus; 14 886 nt for pneumonia virus of mice; 15 522 nt for Tioman virus; 16 236 nt for avian paramyxovirus type 6; 13 373 nt for avian metapneumovirus type A; and 14 150 nt for avian metapneumovirus type C.

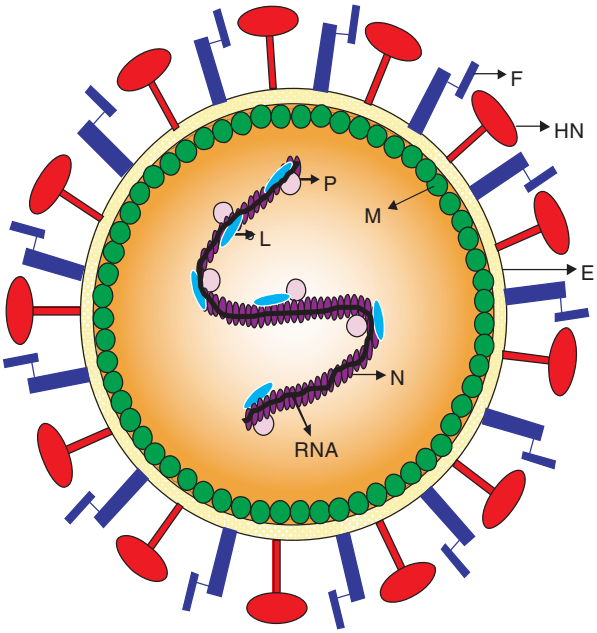


Figure 1 Schematic diagram of a paramyxovirus. N, nucleocapsid protein; P, phosphoprotein; L, large polymerase protein; M, matrix protein; F, fusion protein; HN, hemagglutinin-neuraminidase protein; E, envelope.

Proteins

All paramyxoviruses contain two glycosylated surface envelope proteins, a fusion protein (F) and an attachment protein (G or H or HN). The F protein mediates viral penetration by inducing fusion between the viral envelope and the host cell plasma membrane. In paramyxoviruses,

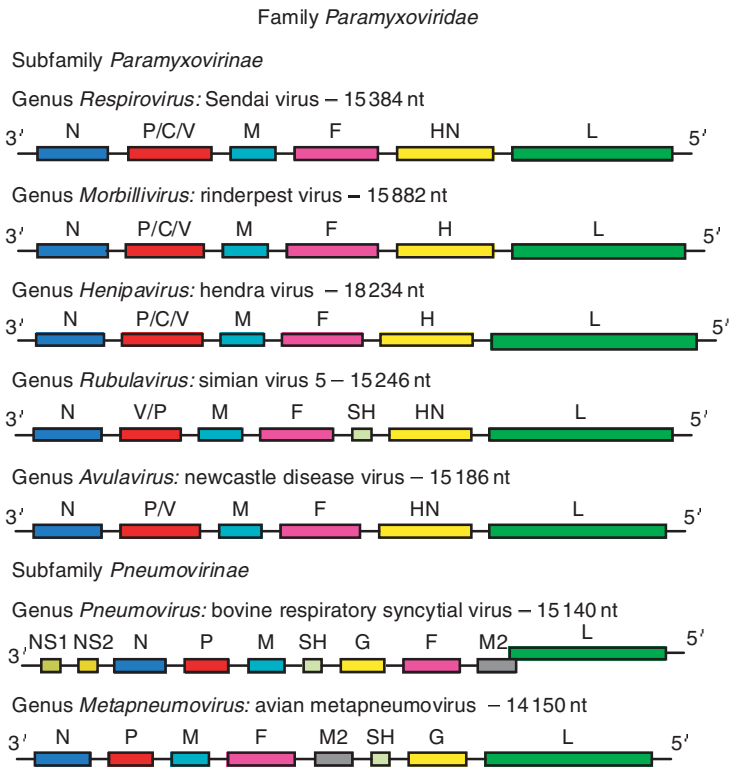


Figure 2 Map of genomic RNA (3′ to 5′) of animal paramyxoviruses representing the seven genera of the family *Paramyxoviridae*. Each box represents a separate gene; multiple distinct open reading frames (ORFs) within a single gene are indicated by slashes. For the P gene, the product encoded by the unedited mRNA is given first. In bovine respiratory syncytial virus of genus *Pneumovirus*, there is a transcriptional overlap at M2 and L genes.

the fusion event occurs at neutral pH. The F protein is synthesized as an inactive precursor (F_0), which is activated following cleavage by cellular protease(s) to generate two disulfide-linked F_1 and F_2 subunits. Some paramyxoviruses have multiple basic residues (arg and lys) at the cleavage site and thus are readily cleaved by furin-like proteases found intracellularly in most tissue types. Other paramyxoviruses have few, or only one, basic residues at the cleavage site and thus are cleaved extracellularly by a trypsin-like protease secreted in the respiratory and intestinal tracts, which limits virus replication. Hence, the number of arg and lys residues at the F protein cleavage site is a major determinant of paramyxovirus virulence. However, other viral proteins also contribute to the virulence of paramyxoviruses. The attachment protein binds to cell surface receptor and facilitates viral penetration. The attachment proteins of rubulaviruses, avulaviruses, and respiroviruses are designated HN since they possesses both hemagglutination activity, which is due to binding to sialic acid, and neuraminidase activity, which cleaves sialic acid on the cell surface and facilitates release. The attachment protein of morbilliviruses is designated H because it possesses hemagglutination activity, which is due to binding to the signaling lymphocyte activation molecule (SLAM) receptor, but not neuraminidase activity. Attachment proteins that generally lack hemagglutination and neuraminidase activities are designated G (glycoprotein). These occur in members of genera *Henipavirus*, *Pneumovirus*, and *Metapneumovirus*. The envelopes of the genera *Rubulavirus*, *Pneumovirus*, and *Metapneumovirus* contain a third integral membrane protein called small hydrophobic (SH) protein. The SH protein might play a role in cell fusion or in morphogenesis and also has been reported to interfere with cytokine-mediated intracellular signaling. The viral matrix protein (M) forms the inner layer of the virus envelope and plays an important role in virus assembly.

Inside the virion envelope lies the helical nucleocapsid. The genome and antigenome of paramyxoviruses are never found as free RNA either intracellularly or in the virion, but rather are tightly associated with the viral nucleocapsid protein (N) in the form of a ribonucleoprotein core. Virion nucleocapsids contain two other proteins, the phosphoprotein (P) and the large protein (L) that together constitute the viral RNA-dependent RNA polymerase complex. Virion nucleocapsids of the subfamily *Pneumovirinae* contain an additional protein (M2-1), which is a transcription elongation factor. A nonabundant protein (M2-2) is also produced from the second open reading frame (ORF) of the M2 gene and is involved in the balance between genome replication and transcription. The RNA within the nucleocapsid is resistant to nucleases.

Members of subfamily *Paramyxovirinae* encode multiple proteins from the P gene, due in part to a mechanism called 'RNA editing'. The P gene contains an editing site at which nontemplated G residues are added into the P mRNA by stuttering during transcription. The inclusion of additional G residues has the potential to shift the reading frame to access alternate frames, thus creating one or more chimeric proteins in which N-terminal domain is encoded by the P ORF upstream of the editing site and the C-terminal domain is encoded by the alternative ORF downstream of the editing site. In almost all members of the subfamily *Paramyxovirinae*, two of the major products of the P gene are the P and V proteins. For the respiroviruses, morbilliviruses, avulaviruses, and henipaviruses, the unedited mRNA of the P gene produces the P protein. Addition of one G nucleotide at the editing site produces an mRNA that encodes the V protein. In rubulaviruses, the unedited P mRNA encodes the V protein and addition of two G nucleotides produces the P mRNA. The V proteins of respiroviruses and morbilliviruses are nonstructural; whereas, the V proteins of rubulaviruses and avulaviruses are structural components of the virions. The respiroviruses, henipaviruses, and morbilliviruses also encode a third major protein from the P gene, namely the C protein. The C protein is synthesized from a +1 reading frame that overlaps the P and V reading frames. The V and C accessory proteins play important roles counteracting host cell antiviral defense mechanisms, especially the interferon system, and have been reported to be involved in other activities such as RNA synthesis and virion morphogenesis. *Pneumovirinae* lacks RNA editing. The members of genus *Pneumovirus* produce two additional nonstructural proteins (NS1 and NS2) from separate, promoter-proximal genes, which play a role in counteracting host cell antiviral defense mechanisms.

Replication and Virus Assembly

Paramyxovirus gene expression and RNA replication occur in the cytoplasm of infected cells, and progeny virions bud from the plasma membrane. Various cell surface molecules serve as receptors. Respiroviruses, rubulaviruses, and avulaviruses utilize sialic acid residues on various cellular glycoproteins (e.g., glycophorin) and gangliosides as receptors. Morbilliviruses utilize SLAM (also known as CD150) as a receptor. Infection by respiratory syncytial virus *in vitro* involves glycosaminoglycans, and Hendra and Nipah viruses use ephrin-B2 for infection of human cells. The F protein mediates fusion of the viral envelope and the plasma membrane of the host cell. As a result of the fusion, the viral nucleocapsid is released into the cytoplasm. Once in the cytoplasm, the nucleocapsid initiates transcription. The viral polymerase enters at the promoter located at the 3′-end of the genome. This promoter serves the dual function of mRNA and antigenome synthesis. Transcription is linear, sequential, and involves a stop-start mechanism guided by the gene-start and gene-end signals. As polymerase molecules progress along the genome, there is some dissociation at each gene junction, leading to a gradient of mRNA abundance that decreases according to distance from the 3′ end of the genome. The viral mRNAs are 5′-capped by the viral polymerase and contain a 3′ poly(A) tail that is produced by stuttering on the gene-end sequence. The intracellular accumulation of viral nucleocapsid-associated proteins results in the initiation of RNA replication. During RNA replication, the gene-start and gene-end signals are ignored and an exact complementary copy of the genome (antigenome) is synthesized. RNA synthesis is tightly linked to encapsidation of the progeny molecule. A promoter located at the 3′ end of the antigenome is used to synthesize genome.

The viral M protein plays a major role in mediating association of the nucleocapsids with patches in the plasma membrane where the viral envelope proteins have accumulated. It is thought that the M protein assembles the virion by forming a bridge between the cytoplasmic tails of envelope proteins and the nucleocapsids. Both the final assembly and budding of the virus occur at the plasma membrane of infected cells.

Reverse Genetics

Reverse genetics refers to the generation of subviral particles or complete infectious virus entirely by expression of cloned cDNAs. This provides a method for introducing desired changes into the viral genome. A number of animal paramyxoviruses have been recovered from cDNAs using reverse genetics, including simian virus 5, NDV, bovine parainfluenza virus 3, Sendai virus, canine distemper virus, rinderpest virus, bovine respiratory syncytial virus, and avian metapneumovirus. The basic method involves transfecting cultured cells with plasmids encoding the viral N, P, and L proteins, as well as the viral antigenome, all under the control of the T7 promoter. The positive-sense antigenome typically is expressed rather than the negative-sense genome to avoid hybridization with the positive-sense mRNAs, but virus has also been recovered (less efficiently) by expressing the genome. The bacteriophage T7 RNA polymerase is provided either by infection with a recombinant vaccinia virus expressing T7 RNA polymerase or by transfecting into cell lines that constitutively express T7 RNA polymerase. The recovery of bovine respiratory syncytial virus requires expression of an additional plasmid encoding the transcription elongation factor M2-1. Intracellular synthesis of the viral N, P, and L proteins and antigenome RNA results in the assembly of a biologically viral nucleocapsid that launches an

infection leading to production of infectious virus. It is now feasible to genetically engineer attenuated viruses for use as live virus vaccines for several animal paramyxoviruses for which effective vaccines are not currently available. Perhaps even more exciting is the potential to use animal paramyxoviruses as vaccine vectors to design multivalent vaccines or to use more stable vectors to express antigens from less stable pathogens. At present, several animal paramyxoviruses, such as Sendai virus, NDV, and bovine parainfluenza virus 3, are being evaluated as vaccine vectors for other animal pathogens and also for use in humans as host-range-restricted vectors expressing antigens of human pathogens.

Genetic and Serologic Relationships

The relationships among paramyxoviruses can be deduced from nucleotide and amino acid sequence relatedness and serological analysis. Paramyxoviruses show very little amino acid sequence conservation among members of different genera. The sequence relatedness varies greatly within a genus, some members showing higher levels of sequence relatedness than others. The overall sequence conservation of paramyxovirus structural proteins in descending order seems to be L>M>F>N>H/HN/G>P. The L protein has five short regions of high homology near the center of the protein, which are not only conserved among paramyxoviruses, but are also conserved among all nonsegmented negative-strand RNA viruses. The C-terminal, domain of the V protein, is also conserved among all paramyxoviruses and contains seven invariant cysteine residues. Some animal paramyxoviruses show high levels of relatedness by sequence and serology with human paramyxoviruses. This implies that they have close evolutionary relationships and may have arisen by crossing species boundaries. Examples of pairs of related animal and human viruses include bovine and human parainfluenza virus 3, bovine and human respiratory syncytial virus, Sendai virus and human parainfluenza virus 1, simian virus 5 and human parainfluenza virus 2, and avian and human metapneumoviruses. In addition, all viruses within the genus *Morbillivirus* are related by sequence and serology. Rinderpest virus is more closely related to measles virus than to peste des petits ruminants virus and canine distemper virus. It is thought that the rinderpest virus is the archetype from which the other members of the genus morbillivirus have probably evolved, a process that involved crossing species boundaries.

Epidemiology

Some paramyxoviruses, such as NDV, canine distemper virus, bovine parainfluenza virus 3, and Sendai virus, have a worldwide distribution. Peste des petits ruminants virus is widespread in all countries lying between the Sahara and the Equator, in the Middle East, and in Southeast Asia. Avian metapneumovirus subtypes A, B, and D are present in Europe, but only subtype C is prevalent in the US. Nipah and Hendra viruses have emerged as new pathogens in Malaysia and Australia, respectively. Outbreaks of Menangle virus infection have been reported only in Australia.

The diseases caused by animal paramyxoviruses depend in part on their tissue tropism: as described below, some remain restricted to the respiratory tract and cause disease at that site, whereas others can disseminate by viremia to other tissues and cause disease that depends on the site of viral replication and pathogenesis. Immunity against viruses whose pathogenesis involves viremia tends to be relatively strong and long-lived, likely reflecting the long life of the serum antibody response. For example, rinderpest virus, which was once present on most continents, has been eradicated from Europe, America, and most of Asia. It remains enzootic only in parts of Asia and Africa. In contrast, immunity against viruses that remain localized in the superficial epithelium of the respiratory tract, such as bovine parainfluenza virus 3 and respiratory syncytial virus, is less effective and long-lived, and reinfection is common.

Transmission and Pathogenesis

Paramyxoviruses such as NDV and the morbillliviruses are highly infectious. The respiratory tract is the primary portal of entry for most paramyxoviruses and, for many, is the major site of viral replication; a few paramyxoviruses also infect via the enteric tract. Infection occurs by several different routes, including aerosols (NDV, bovine respiratory syncytial virus, avian metapneumovirus) and contaminated feed and water (Newcastle disease, canine distemper, and rinderpest viruses). Transmission of paramyxoviruses from fruit bats to animals is thought to occur by the fecal–oral route. In some viruses, the replication is confined to the respiratory mucosal surface (bovine parainfluenza virus 3, bovine respiratory syncytial virus, avian metapneumovirus), while in others, the initial replication on the respiratory tract is followed by systemic spread. Virulent strains of NDV initially infect the upper respiratory tract and then spread via the blood in the spleen and kidney, producing a secondary viremia. This leads to infection of other target organs, such as lung, intestine, and central nervous systems. In morbilliviruses, after initial replication in the respiratory tract, the virus multiplies further in regional lymph nodes, then enters the bloodstream, carried within lymphocytes, to produce primary viremia that spreads the virus to reticuloendothelial systems. Viruses produced from these sites are carried by

lymphocytes to produce secondary viremia, which leads to infection of target tissues, such as lung, intestine, and central nervous systems.

Diseases

Paramyxoviruses are responsible for a wide variety of diseases in animals. Many paramyxoviruses primarily cause respiratory disease (bovine parainfluenza virus 3, bovine respiratory syncytial virus, avian metapneumovirus), while others cause serious systemic disease (rinderpest, virulent strains of Newcastle disease, canine distemper). Many diseases caused by animal paramyxoviruses also have a neurological component (canine distemper, Newcastle disease, Nipah virus) or a reproductive disease component (parainfluenza virus 5 in pigs and Menangle virus). Interestingly, the type of disease caused by Newcastle disease virus can vary, depending on the strain of the virus. Some strains cause only respiratory tract disease, some cause generalized hemorrhagic lesion, while others cause neurological disease. Most Newcastle disease virus strains replicate in the respiratory tract, while some predominantly replicate in the intestinal tract. Certain members of the genus *Morbillivirus*, canine distemper virus, phocine distemper virus, and cetacean viruses, cause high levels of central nervous system (CNS) diseases in their natural hosts, but CNS diseases are not associated with other members of genus *Morbillivirus*, such as rinderpest and peste des petits ruminant viruses. The severity of clinical disease also varies among animal paramyxoviruses. Some viruses cause asymptomatic or mild respiratory disease (bovine parainfluenza virus 3, simian virus 5, avian paramyxovirus types 2–9), while other viruses can cause severe disease leading to 90–100% mortality in susceptible hosts (rinderpest, Newcastle disease virus, canine distemper virus). There is also extreme variation in the pathogenicity of strains of some paramyxoviruses. For example, Newcastle disease virus strains range from avirulent to highly virulent (causing 100% mortality in chickens).

Immune Response

Paramyxoviruses induce both local and systemic antibody-mediated and cell-mediated immunity. Secretory IgA and cytotoxic T-lymphocytes play major roles in resolving infection and protecting against reinfection, but are somewhat short-lived, especially following a primary infection. Serum antibodies can also contribute to resolving infection and usually provide durable protection against reinfection. As already noted, serum antibodies are particularly effective against viruses whose pathogenesis involves viremia. Local immune factors play a greater role against viruses that remain localized in the respiratory tract. The envelope glycoproteins, H/HN/G and F, are the major neutralization and protective antigens of paramyxoviruses, although all of the viral proteins have the potential to contain epitopes for cellular immune responses. In some viruses (e.g., bovine parainfluenza virus 3), HN protein is the major protective antigen, while in other viruses (e.g., Newcastle disease virus), F protein is the major protective antigen. Most or all paramyxoviruses have evolved mechanisms that suppress the synthesis of interferon and the establishment of an interferon-mediated antiviral state.

Prevention and Control

Vaccination is a very effective means of controlling paramyxovirus infections. Both live-attenuated and inactivated vaccines have been developed for major animal paramyxovirus pathogens. Live-attenuated vaccines typically are more effective than the inactivated vaccines. Currently, effective live-attenuated vaccines are available for rinderpest, canine distemper, and Newcastle disease. However, satisfactory live-attenuated or inactivated vaccines are not available for diseases caused by bovine respiratory syncytial virus, Nipah virus, Hendra virus, and avian metapneumoviruses. Although the live-attenuated vaccines for rinderpest, canine distemper, and Newcastle diseases are generally very effective, there have been concerns about their potential safety and reversion to virulence. Furthermore, these vaccines cannot serologically distinguish vaccinated animals from naturally infected animals. Therefore, new and highly effective animal paramyxovirus vaccines are being engineered using reverse genetics techniques.

Future Perspectives

Some of the animal paramyxoviruses cause devastating diseases of animals, while others appear to be nonpathogenic. Many of the animal paramyxoviruses lack an effective vaccine. Development of reverse genetics systems has not only improved our understanding of the biology of these viruses, but has also provided methods for engineering effective vaccines. It is now possible to adjust the attenuation phenotype of a vaccine, introduce genetic markers into the vaccine viruses for differentiation between vaccine and wild-type strains, and to engineer thermostable vaccines for use in developing countries. The next steps will be to test these vaccines using a large number of animals, and to have them commercially available for vaccination purposes. Another advantage of reverse genetics is the use of animal paramyxoviruses as vectors to express foreign genes. This makes possible the use of one animal paramyxovirus vaccine to protect from multiple animal diseases. Since recombination involving members of *Paramyoxoviridae* is essentially nonexistent, they will be particularly valuable as vectors to express the antigens of recombination-prone viruses such as

coronaviruses. Some animal paramyxovirus-based vectors can be useful for the development of vaccines against emerging human infections such as H5N1 avian influenza, severe acute respiratory syndrome (SARS), and those caused by Ebola, Marburg, Nipah, and Hendra viruses. Since animal paramyxoviruses can be chosen that are serologically unrelated to common human pathogens, the general human population is susceptible to immunization with animal paramyxovirus-vectored vaccines. Reverse genetics systems are currently available for many but not all animal paramyxoviruses. Therefore, there is a great need to develop reverse genetics systems for the remaining animal paramyxoviruses. Furthermore, it is necessary to develop reverse genetics systems of local paramyxovirus strains for development of effective vaccines against the prevailing virus strains. In addition to vaccine development, it is also important to understand the pathogenesis and determinants of virus virulence and the mechanisms of interspecies transmission of the viruses. Due to the availability of reverse genetics systems for these viruses, we are confident that the next decade will bring a significant improvement in our understanding of their biology and we will witness development of better and safer vaccines against animal diseases.

See also: Measles Virus; Mumps Virus; Parainfluenza Viruses of Humans; Viral Pathogenesis; Human Respiratory Syncytial Virus; Rinderpest and Distemper Viruses.

Further Reading

Conzelmann KK (2004) Reverse genetics of mononegavirales. *Current Topics in Microbiology and Immunology* 283: 1–41.

Easton AJ, Domachowske JB, and Rosenberg HF (2004) Animal pneumoviruses: Molecular genetics and pathogenesis. *Clinical Microbiology Reviews* 17: 390–412.

Kurath G, Batts WN, Ahme W, and Winton JR (2004) Complete genome sequence of fer-de-lance virus reveals a novel gene in reptilian paramyxoviruses. *Journal of Virology* 78: 2045–2056.

Lamb RA, Collins PL, Kolakofsky D, *et al.* (2005) *Paramyxoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 655–668. San Diego, CA: Elsevier Academic Press.

Lamb RA and Parks GD (2006) *Paramyxoviridae*: The viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology*, 5th edn., pp. 1449–1496. Philadelphia: Lippincott Williams and Wilkins.

Li Z, Yu M, Zhang H, *et al.* (2006) Beilong virus, a novel paramyxovirus with the largest genome of non-segmented negative-stranded RNA viruses. *Virology* 346: 219–228.

Wang LF, Harcourt BH, Yu M, *et al.* (2001) Molecular biology of Hendra and Nipah viruses. *Microbes and Infection* 3: 279–287.

Parainfluenza Viruses of Humans

E Adderson and A Portner, St. Jude Children's Research Hospital, Memphis, TN, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The human parainfluenza viruses (hPIVs) are an important cause of respiratory disease in infants and children. Four types were discovered between 1956 and 1960. hPIV-1, hPIV-2, and hPIV-3 were first isolated from infants and children with lower respiratory tract (LRT) disease and subsequently shown to be a major cause of croup (type 1) and pneumonia and bronchiolitis (type 3). hPIV-4 was initially isolated from young adults and has been associated with mild upper respiratory tract disease of children and adults. Other viruses antigenically and structurally related to the human paramyxoviruses have been isolated from animals. Sendai virus, a natural pathogen of mice and not of humans, was the first PIV isolated and is antigenically related to human PIV-1. Simian virus (SV5) now PIV-5, recovered from primary monkey kidney cells, causes croup in dogs and is related to human type 2, and bovine shipping fever virus is a subtype of type 3.

Taxonomy and Classification

The PIVs belong to two genera, human parainfluenza virus types 1 and 3 to *Respirovirus* and human parainfluenza virus types 2, 4a, and 4b to *Rubulavirus*, of the subfamily *Paramyxovirinae* in the family *Paramyxoviridae*. Some other species found in the *Rubulavirus* are mumps virus, which causes disease in humans and in the genus *Respirovirus* Sendai virus in mice and Bovine parainfluenza type 3. The family *Paramyxoviridae* belongs to the order *Mononegavirales*, the distinctive feature of which is a negative-stranded RNA genome and a similar strategy of replication, suggesting that all negative-stranded viruses may have evolved from an archetypal virus.

Virion Structure, Genome Organization, and Protein Composition

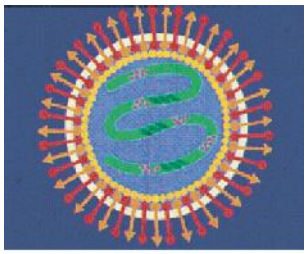
The hPIVs are roughly spherical, lipoprotein enveloped particles 150–250 nm in diameter with an internal helical

nucleocapsid containing the negative-sense single-stranded RNA genome. Projecting from the surface of the virion are the hemagglutinin–neuraminidase (HN) and fusion (F) glycoproteins. These glycoproteins are anchored to the plasma membrane of the infected cell or the virion envelope by a hydrophobic transmembrane region. In paramyxoviruses the transmembrane domain of HN is located near the N-terminus of the molecule and F near the C-terminus. Extending into the cytoplasm of the infected cell or inside the virion membrane is a short hydrophilic tail region, which plays a role in viral assembly through its interaction with the matrix (M) protein which lines the inner surface of the plasma membrane and itself interacts with the nucleocapsid. Inside the lipid bilayer of the viral particle is the RNA nucleocapsid which houses the nonsegmented negative-stranded RNA genome. The genomes of hPIV-1, hPIV-2, and hPIV-3 are approximately 15 000 nt and all genes except L have been sequenced for hPIV-4a and -4b and are certain to fall in this range once the sequencing is complete. The RNA genome serves as a template for transcription of mRNAs specifying six virion structural proteins linked in tandem in the order of 3′-nucleoprotein (NP)–polymerase-associated protein (P/V)–matrix protein (M)–fusion protein (F)–hemagglutinin-neuraminidase (HN)–large protein (L)-5′. The P gene alone is unique in its capacity to express, in addition to the P protein, various other proteins by utilizing internal initiation codons in the same or different reading frames or by RNA editing of the P gene mRNA through insertion of nontemplated G residues. Besides P, these other proteins have been designated V, C, and D, depending on the PIV. The helical nucleocapsid is 18 nm in diameter, contains approximately 2000 molecules of NP bound to the RNA genome, about 200 P and 20 L molecules. The P protein, which forms a polymerase complex with L, is essential for the enzymatic processes of viral RNA transcription and replication including the 3′ addition of poly(A), modification of the 5′ end, and nucleotide polymerization of viral transcripts. The approximate molecular weights in daltons of the PIV proteins as exemplified by PIV-3 are: NP, 58 000; P, 68 000; M, 40 000; F, 63 000; HN, 72 000; and L, 256 000. A cartoon and an electron micrograph of a naturally occurring PIV are shown in **Figure 1**.

Attachment Protein

The process of infection is initiated by the action of the HN glycoprotein, which binds the virion to sialic acid-containing glycoprotein or glycolipid receptors on the host cell surface. The same process is responsible for the hemagglutination of avian and mammalian erythrocytes. HN also causes the enzymatic (neuraminidase) cleavage of sialic acid residues from the carbohydrate moiety of glycoproteins and glycolipids, which functionally serves to prevent the self-aggregation of virus during release and likely aids in the spread of virus from infected cells. Besides attachment, HN provides an unknown function that is either essential or enhances the fusion activity of the F protein. It is proposed that HN directly interacts with F, possibly altering its conformation, thereby stimulating the fusion activity of F.

The morphology of HN based on studies of PIVs such as Sendai virus is envisioned as an N-terminal stalk region



Membrane proteins
- HN: receptor-binding, neuraminidase, fusion promotion
- F: membrane fusion
- M: assembly



Nucleocapsid
- NP: encapsidate viral RNA
- P: forms polymerase complex with L
- L: RNA synthesis, capping, polyadenylation

Figure 1 Parainfluenza virus structure.

of approximately 130 amino acids anchoring a large glycosylated hydrophilic globular head region to the viral envelope. A small uncharged hydrophobic peptide located near the N-terminus spans the viral envelope, and a small hydrophilic domain is internal to the membrane. The globular head contains the active site for virus attachment, neuraminidase activity, and antigenic determinants that induce neutralizing antibodies for hPIVs, as well as the other members of the *Respirovirus* and *Rubulavirus* such as Sendai virus. HN exits on the surface of the virion as disulfide-linked homodimers or tetramers.

Solution of the three-dimensional structure of the HN protein of NDV and more recently of hPIV-3 and SV5 has resolved many of the previous issues concerning the structure and functions of HN. A significant advance was the determination that the HN sialic acid-binding site and the neuraminidase active site were the same and for NDV that conformational change of the site switches HN activity from sialic acid binding (attachment) to sialic acid hydrolysis (neuraminidase activity). However, similar conformational changes may not occur for hPIV-3 and SV5 HN.

Fusion Protein

Following virus attachment, F, the other surface glycoprotein, mediates the fusion of the virion and host cell-surface membranes, which allows the nucleocapsid to be deposited in the cell cytoplasm where gene expression begins. F expressed on the surface of infected cells also mediates fusion, allowing the extension of infection to uninfected cells.

All F proteins are synthesized as inactive precursors (FO) which are post-translationally cleaved by a host cell trypsin-like protease to form the biologically active molecule. For the paramyxoviruses in general, the cleavage site is located about 100 amino acids from the N-terminus of FO. The cleavage site is characterized by a short span of basic amino acids on the amino side of the site and a longer stretch of about 30 hydrophobic residues on the carboxyl side. The number and location of basic amino acids on the amino side of the cleavage site varies with individual PIVs. hPIV-1 and Sendai virus have one basic amino acid immediately adjacent to the cleavage site (Arg), whereas PIV-2 and PIV-3 have two (Arg–Lys) and PIV-5 has five (all Arg). The motif of paired basic amino acids at the cleavage site increases the efficiency of cleavage, host and tissue tropism, and pathogenicity, as the dibasic motif is recognized by a ubiquitous protease, whereas the enzymes that cleave at a monobasic site are found in a limited number of tissues. Cleavage of FO results in two disulfide-linked fragments; the larger one, F1, forms the new hydrophobic N-terminus, which causes membrane fusion. The smaller product, F2, is the original approximately 100 N-terminal residues of FO.

Recent solutions of the crystal structure of the F in its prefusion and postfusion conformations reveals dramatic alterations in the F protein structure during membrane fusion and offers insight into the mechanism of F activation.

M Protein

The M protein lines the inner surface of the viral envelope and is thought to play a role in virus maturation by interacting with the envelope glycoproteins and the nucleocapsid. During infection M associates with the inner leaflet of the plasma membrane where it orchestrates the release (budding) of progeny virus by interacting with specific sites on the cytoplasmic tail of the viral glycoproteins and the nucleocapsid and in transport of viral components to the budding site.

Transcription, Translation, and Replication

The negative-strand strategy of viral replication involves the synthesis of unique mRNA species of each paramyxovirus gene during infection. After introduction of the infecting nucleocapsid into the host cell, transcription is the first step in gene expression. Transcriptional regulation of mRNA abundance is determined by the gene order; the closer the gene is to the 3′ end of the genome, the more efficient is the transcription. Thus, the abundance of paramyxovirus proteins is determined mainly by the polarity of the genome. Once protein synthesis is underway, replication of the genome begins, which provides additional templates for transcription and replication.

Development of 'reverse genetics' systems for SV and hPIV-3 as well as other members of the paramyxovirus family, in which the RNA genomes of these viruses are expressed from a DNA template, has provided tools and facilitated our understanding of PIV protein structures and function, virus assembly, regulation of RNA transcription and replication, and viral pathogenesis.

Geographic and Seasonal Distribution

All of the hPIV types 1–4 have a wide geographic distribution. PIV-1–PIV-3 have been identified in most areas where facilities are available for the study of childhood

respiratory tract diseases. PIV-4 has been isolated in fewer areas but this is likely to be due to the difficulty of isolation and it is probably widely distributed.

Host Range and Viral Propagation

The four hPIV types were originally isolated from humans: they cause disease in humans and humans are the primary host. Other laboratory animals can serve as experimental hosts; hamsters, guinea pigs, and ferrets can be infected with hPIV-1, hPIV-2, and hPIV-3 but these infections are usually asymptomatic. hPIV-3 can also infect cotton rats, rhesus, and patas monkeys and chimpanzees, but these animals are not good model systems for studying disease caused by PIVs.

Embryonated hen's eggs can support the growth of some strains of hPIV-1, hPIV-2, and hPIV-3, but are much less sensitive than monkey kidney cells for primary isolation. Sendai virus, a murine subtype of PIV-1, is an exception in that it grows exceedingly well in eggs.

All four hPIV types grow well in primary monkey or human kidney cells, which are also used in the isolation of virus from clinical samples. LLC-MK2, a rhesus monkey kidney cell line, offers an efficient system for the isolation of PIVs and an experimental tissue culture system. PIV-2 and PIV-1 require trypsin in the medium to cleave the F glycoprotein for cell growth, but not PIV-3 strains. Viral infection of tissue culture can be detected by hemadsorption with chicken and guinea pig erythrocytes and the cytopathic effects produced by the viruses.

Genetics and Evolution

The entire genomic sequences of hPIV-1, hPIV-2, and hPIV-3 have been completed. The genomes of other members of the genera *Respirovirus* and *Rubulavirus* (Sendai virus, bPIV-3, and PIV-5) have been sequenced as well. In general, the genome organization is remarkably similar, but differences exist in sequence, intergenic regions, and nonstructural proteins expressed from the P gene. Sequence and immunological analyses suggest that human hPIV-1 and Sendai virus are closely related type 1 PIVs, as is PIV-3 of human and bovine origin. hPIV-1 and -3 are more closely related to each other than to hPIV-2, PIV-5, mumps virus and NDV, which in turn show a closer evolutionary relationship to each other. Evolutionary divergence of hPIV-3 and hPIV-1 is greatest for the P protein (a phenomenon of paramyxovirus P proteins in general), less for the HN and F glycoproteins, and least for M, NP, and L. Similarly, hPIV-1 and Sendai virus, and human and bovine PIV-3, show the greatest divergence in the P protein and least for NP.

Serologic Relationships and Variability

The human paramyxoviruses can be divided into two antigenic groups, comprised of PIV-1/PIV-3 and PIV-2/PIV-4/mumps virus. Human PIVs share certain antigenic determinants, but structural and antigenic variation occurs between serotypes and, to a lesser degree, within strains belonging to the same type. Antigenic diversity is not generally progressive, but may contribute to PIVs' propensity to cause recurrent infection. Antibody to F and HN correlates with neutralizing antibody and protective immunity.

Epidemiology and Transmission

The hPIVs are an important cause of respiratory tract infections (**Table 1**). hPIV-3 infections are endemic, whereas infections caused by other PIVs occur in outbreaks. Infections are common before 2 years, especially with hPIV-3, and almost universal by 5 years. hPIVs are highly communicable by respiratory droplets and by contact with surfaces contaminated by respiratory secretions. The incubation period is 2–6 days. Virus is typically shed for 4–21 days.

Pathogenicity

hPIVs bind to specific receptors on upper respiratory tract epithelial cells and may subsequently spread to the LRT. Optimal fusion efficiency requires the coordinated action of both F and HN. The virus and host factors contributing to disease pathogenesis are not completely understood. Both viral (F and HN structure) and host features (genetic susceptibility, host cell proteases, immunocompetence) are likely to influence the severity of infection.

Table 1 Proportion of viral respiratory tract infections caused by PIV

	Proportion (%)	*Most common type*
Rhinitis (common cold)	20	
Pharyngitis	20	
Laryngotracheitis (croup)	50–70	PIV-1
Bronchiolitis	20	PIV-3
Pneumonia	10	PIV-1, PIV-3
Otitis media (middle ear)	10	

Clinical Features

hPIVs cause between 15% and 65% of viral respiratory infections, most notably laryngotracheitis (croup) and bronchiolitis (**Table 1**). Although infections are generally mild, PIVs are responsible for over 10% of pediatric hospitalizations for respiratory infection. Life-threatening LRT infection occurs in patients with cellular immunodeficiencies, particularly those with severe combined immunodeficiency and hematopoietic stem cell and solid organ transplants. Rare cases of parotiditis, myocarditis, aseptic meningitis, and encephalitis have been reported.

The gold standard for diagnosis of hPIV infections is culture on primary monkey kidney, human embryonic kidney, or human lung carcinoma cells, which typically requires incubation for 4–7 days. Detection of epithelial cell-associated viral antigens in nasopharyngeal or bronchoalveolar washings is rapid and has a sensitivity and specificity of >80% compared to culture. Polymerase chain reaction (PCR) is rapid and more sensitive, but is not widely available. Detection of specific IgM and IgG in paired acute and convalescent sera has limited utility in the diagnosis of acute infections.

Pathology

hPIV has direct cytopathic effects, causing ciliary damage, epithelial necrosis, and recruitment of a mononuclear inflammatory response. Recent studies suggest that host inflammatory responses, rather than the direct effects of viral replication, are most accountable for the signs and symptoms of PIV infection. Alterations in HN receptor-binding or -activity influence host inflammatory responses independent of viral replication or the ability to infect epithelial cells. PIV infection may trigger long-term bronchial hyperreactivity in genetically susceptible persons.

Immune Response

Viral shedding is more prolonged and disease severity increased in persons with T-cell immunodeficiencies, implying cellular immunity is important in the control of acute hPIV infection. Protection from reinfection is associated with the development of serum and secretory antibody against F and HN. The duration of protection after acute illness is relatively short however, especially in infants, and protection from infection by heterotypic stains is incomplete.

Prevention and Control

Most hPIV infections require supportive care only. Children with moderate-to-severe croup benefit from inhaled vasoconstrictors or inhaled or systemic corticosteroids, which reduce airway edema. Anecdotal reports describe the successful use of ribavirin in immunocompromised patients. There are currently no licensed vaccines to prevent hPIV. Early formalin-inactivated vaccines were poorly immunogenic and had the potential to enhance pulmonary inflammatory responses to infection with wild-type virus.

Future Perspectives

Novel antiviral therapies being developed for PIV infections include selective inhibitors of HN, fusion, and IMP dehydrogenase, and siRNA against phosphoprotein mRNA. Vaccines currently in early clinical trials include live attenuated human PIV-3, bovine PIV-3, human-bovine chimeras, and bovine PIV or Sendai virus expressing heterologous human PIV F and HN proteins. Besides vaccines, antiviral drugs may be designed that curtail viral replication or prevent viral spread. Knowledge of the three-dimensional structure of HN and F, especially those regions involved in viral attachment, penetration, and release, will be important in drug design.

See also: Paramyxoviruses of Animals; Human Respiratory Viruses; Viral Membranes.

Further Reading

Karron RA and Collins PL (2006) Parainfluenza viruses. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1497–1526. Philadelphia, PA: Lippincott Williams and Wilkins.

Lamb RA and Parks GD (2006) *Paramyxoviridae*: The viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1449–1496. Philadelphia, PA: Lippincott Williams and Wilkins.

Lamb RA, Paterson RG, and Jardetzky TS (2006) Paramyxovirus membrane fusion: Lessons from the F and HN atomic structures. *Virology* 344(1): 30–37.

Morrison T and Portner A (1991) Structure, function and intracellular processing of the glycoproteins of the *Paramyxoviridae*. In: Kingsbury DW (ed.) *The Paramyxoviruses*, pp. 347–382. New York: Plenum.

Subbarao K (2003) Parainfluenza viruses. In: Long SS, Pickering LK, and Prober CG (eds.) *Principles and Practice of Pediatric Infectious Diseases,* 2nd edn., p. 1131. Philadelphia, PA: Churchill Livingstone.

Takimoto T and Portner A (2004) Molecular mechanism of paramyxovirus budding. *Virus Research* 106: 133–145.

Paramyxoviruses

R E Dutch, University of Kentucky, Lexington, KY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Antigenome A complementary copy of the entire viral genome.
RNA-dependent RNA polymerase An enzyme that synthesizes new strands of RNA from an RNA template.
Zoonotic virus Virus that can be transmitted between animals and humans.

Introduction

The family *Paramyxoviridae* includes numerous important viral pathogens for both animals and humans. For humans, measles virus remains a significant cause of mortality, particularly in children, causing approximately 500 000 deaths annually. Human respiratory syncytial virus (HRSV), human metapneumovirus (HMPV), and the human parainfluenza viruses (HPIVs) are important causative agents of respiratory disease, especially in the very young. Paramyxoviruses such as Newcastle disease virus and rinderpest virus are potent animal pathogens with significant economic consequences. Finally, two recently identified paramyxoviruses, Hendra virus and Nipah virus, infect multiple species, and are thus the first zoonotic members of the family. The lethality of Hendra and Nipah viruses in humans and the lack of effective antiviral treatment led to their classification as biosafety level 4 (BSL-4) level pathogens. Thus, members of this important viral family infect a wide variety of species, and many of the members cause potentially lethal disease.

Taxonomy and Classification

The family *Paramyxoviridae* contains two subfamilies: the *Paramyxovirinae* and the *Pneumovirinae*. Differentiation between subfamilies is based on morphological characterization, genomic differences, and variability in function of viral proteins. *Paramyxovirinae* genomes typically encode either six or seven genes, with RNA editing of the P gene leading to additional proteins. Five genera are present in the *Paramyxovirinae*: *Rubulavirus*, which includes mumps virus; *Avulavirus*, which contains the avian Newcastle disease virus; *Respirovirus*, whose members include Sendai virus and hPIV 1 and 3; *Morbillivirus*, including measles virus; and the recently created *Henipavirus* genus, which is comprised of the Hendra and Nipah viruses. Viruses in the subfamily *Pneumovirinae* encode eight to ten genes and are morphologically distinct from those in the *Paramyxovirinae*. Two genera are present in the *Pneumovirinae*: *Pneumovirus*, which includes HRSV; and *Metapneumovirus*, which contains the recently discovered human respiratory pathogen, HMPV. In addition, a number of unclassified paramyxoviruses exist, including J-virus, Fer de lance virus, and Beilong virus.

Virion Structure and Genome Organization

Paramyxoviruses are enveloped particles with the lipid envelope derived from the plasma membrane of the infected cells which produced the progeny virus (**Figure 1**). Viral particles range in size from 150 to 350 nm in diameter. While the majority of viruses are spherical, varying shapes including filamentous forms have been observed. The major viral glycoproteins, the fusion (F) protein, and the attachment (HN, H, or G) protein, extend as spike-like projections from the virion envelope, and thus are positioned to mediate attachment and entry. Contained within the envelope is the viral nucleocapsid core, 15 000–19 000 nt of negative-sense RNA wrapped with the viral nucleocapsid (N) protein. Attached to this are the L (large-polymerase) and P (phosphoprotein) proteins. The viral RNA, N, L, and P proteins are referred to as the nucleocapsid core. The nucleocapsid is helical, with the nucleocapsids of the subfamily *Paramyxovirinae* having an average diameter of 18 nm, and those of the subfamily *Pneumovirinae* having a 13–14 nm diameter.

The *Paramyxoviridae* genome is a single segment of negative-sense RNA. This genome contains between six and ten genes, depending on the virus, but the presence of overlapping reading frames in the P gene results in the production of up to 12 proteins. Noncoding regions are present at the 3′ end (the approximately 50 nt 'leader') and the 5′ end (the 23–161 nt 'trailer'); they are essential for the transcription and replication of the genome. The gene order for the subfamily *Paramyxovirinae* is N–P/C/V–M–F–HN(H)–L, with the exception of the genus *Rubulavirus*, which contains an additional gene encoding the SH protein between the F and HN genes. The subfamily *Pneumovirinae* contains additional genes, with the gene order NS1–NS2–N–P–M–SH–G–F–M2–L for the genus *Pneumovirus* and N–P–M–F–M2–SH–G–L for the genus *Metapneumovirus*. Intergenic regions not present in the

transcribed mRNA are present between each set of genes. The one exception is the overlapping M2 and L genes of the genus *Pneumovirus*, as the gene start for L is present within the M2 gene. Sequences at the beginning ('gene start') and end ('gene end') are critical for the initiation and termination/polyadenylation of each gene (**Figure 2**).

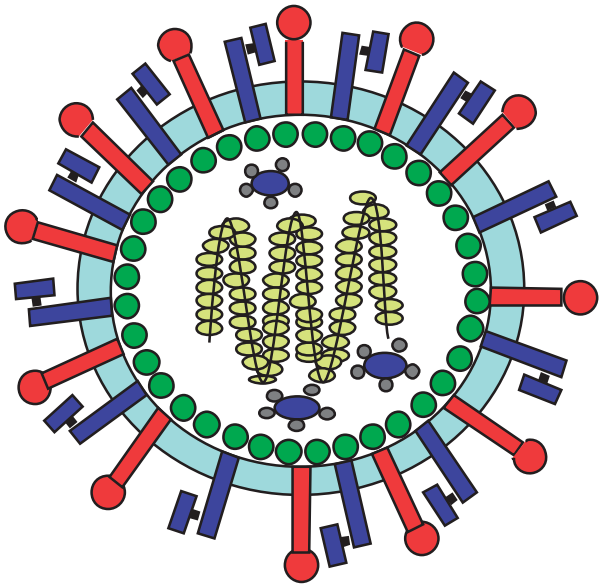


Figure 1 Schematic diagram of a paramyxovirus. The lipid bilayer (envelope) is shown in light blue. The fusion (blue) and attachment (red) glycoproteins extend outward from the bilayer. Lining the interior of the envelope is the matrix protein (green). The RNA genome is coated with nucleocapsid protein (yellow). The RNA-dependent RNA polymerase composed of the large polymerase protein (blue) and phosphoprotein (gray) is also packed in the interior, and interacts with the ribonuclecapsid particle.

Viral Proteins

Attachment (HN, H, or G) Protein

The paramyxovirus attachment proteins perform the critical function of primary attachment of the virus to the target cell via interactions with cell surface receptors. Paramyxovirus attachment proteins are termed HN, H, or G depending on their ability to bind and cleave sialic acid. Paramyxovirus HN proteins, such as those from Newcastle disease virus and mumps virus, both bind cell surface sialic acid moieties during attachment and cleave sialic acid molecules from the carbohydrates of other viral glycoproteins and from the surface of the infected cell during assembly and budding. Viruses with HN attachment proteins utilize sialic acid on cell surface molecules as the receptor for virus binding. Paramyxovirus H proteins, such as those from the *Morbillivirus* measles, can promote sialic acid binding, but lack sialic-acid cleaving neuraminidase activity. The primary receptor for the measles attachment protein is not sialic acid moities, however. Instead, measles H protein binds the cell surface proteins CD46 and SLAM, providing an explanation for the restriction of measles infection to higher primates, which contain these molecules. Finally, paramyxovirus attachment proteins which neither bind nor cleave sialic acid are termed G, for glycoprotein. Viruses with G attachment proteins include the Hendra and Nipah viruses in the *Paramyxovirinae* and all members of the *Pneumovirinae*. The Hendra and Nipah G proteins utilize Ephrin B2 (and Ephrin B3) as the viral receptor. *Paramyxovirinae* attachment proteins also play a second important role in entry, as the triggering of the membrane fusion activity of the fusion protein generally requires the presence of the homotypic attachment protein for members of this subfamily. *Pneumovirinae* G proteins vary greatly from

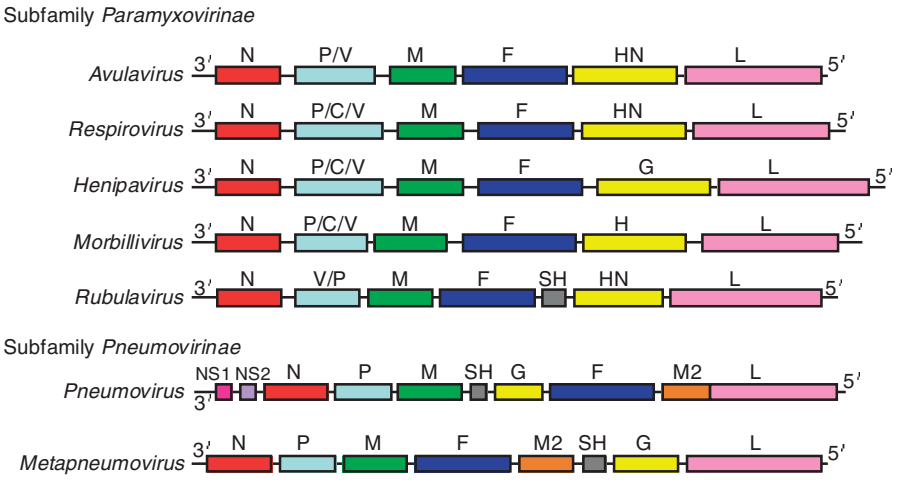


Figure 2 Genetic maps showing gene position for each genus of the *Paramyxoviridae*. Genes are indicated by colored boxes, with gene name designated above the box. For genes encoding more than one open reading frame, the name of each is shown above, separated by a '/'. For the *Pneumovirus* genus, the M2 and L genes overlap.

those of the *Paramyxovirinae*, as they are considerably smaller in size and are not essential for fusion protein function. In fact, recombinant HRSV and HMPV lacking their respective G proteins are both competent for replication, suggesting that the F protein can facilitate attachment to the target cell.

Fusion (F) Protein

Paramyxovirus F proteins are critical for viral entry, as they promote fusion of the viral membrane with the target cell membrane, enabling release of the nucleocapsid core into the cytoplasm of the target cell. F proteins can also promote fusion of infected cells with neighboring cells, leading to the formation of multinucleated giant cells, termed syncytia. Paramyxovirus F proteins are functional trimers which undergo both N-linked glycosylation and post-translational proteolytic processing. Proteolytic processing of the precursor form F_0 to the disulfide-linked heterodimer F_1+F_2 is necessary for the fusogenic activity of all F proteins, but the mechanisms of cleavage differ. F proteins from the majority of paramyxoviruses are cleaved by the host enzyme furin at a position following a series of multibasic amino acids. Furin cleavage occurs in the *trans*-Golgi network, resulting in cleaved, activated F protein on the plasma membrane and on the surface of budded virions. A small number of F proteins, including those from Sendai virus, hPIV1, and HMPV, have a single basic residue at the proteolytic cleavage site and are processed by extracellular proteases following viral release. This mechanism of proteolytic processing restricts viral propagation to tissue containing the required protease. Finally, Hendra and Nipah virus F proteins are proteolytically processed after a single basic residue by the cellular protease cathepsin L, in a mechanism that requires endocytosis from and subsequent recycling to the plasma membrane. The majority of F proteins require their homotypic attachment protein for fusion activity, and it is hypothesized that receptor binding by the attachment protein triggers the F protein to undergo a series of conformational changes which lead to fusion. Some members of the subfamily *Paramyxovirinae* (SV5 F and measles F) and of the subfamily *Pneumovirinae* (HRSV F and HMPV F) can promote fusion in the absence of the attachment protein. The mechanism for triggering of fusion for these F proteins is less clear, though HMPV F protein-promoted fusion can be triggered by low pH. Following the triggering event, a series of conformational changes leads to formation of a six-helix bundle between conserved heptad repeat regions, an event that is intimately tied to the membrane fusion process. Recent structural analysis of the prefusion and postfusion conformations of paramyxovirus F proteins has shed considerable light on this process, but many details of the mechanism remain unclear. Interestingly, paramyxovirus F proteins contain many conserved elements seen in fusion proteins from other viruses, including the HIV env protein, the Ebola virus glycoprotein, and the influenza virus hemagglutinin, suggesting a common ancestry for these important viral proteins.

Matrix (M) Protein

The M protein of *Paramyxoviridae* is the most abundant protein in the virion, and plays a central role in virus assembly. M protein interacts with multiple components of the budding virus, and these interactions are likely critical for M protein function. M is a peripheral membrane protein, and evidence suggests that it underlies the viral membrane. M also interacts with nucleocapsids and with the cytoplasmic tails of the attachment and fusion proteins. In addition, the M protein self-associates. These key interactions make the M protein the central player in orchestrating viral budding. Interestingly, the inactivation of M protein may contribute to persistent paramyxovirus infections, such as subacute sclerosing panencephalitis, a persistent, fatal measles virus infection of the brain.

Nucleocapsid (N) Protein

The paramyxovirus N protein plays a number of critical roles in the viral life cycle. The viral RNA genome is encapsidated by N proteins, protecting the genome from degradation. This tight N protein–RNA association is maintained during transcription and replication. Paramyxovirus N proteins do not contain previously identified RNA-binding motifs, but N protein RNA-binding activity has been localized to the conserved N-terminus of the protein. The N protein also facilitates interactions with the P and L proteins that are critical for transcription and replication. It is hypothesized that the concentration of free N protein in the cell is important for relative rates of transcription and replication. Finally, N protein interactions with the viral M protein likely assist in proper viral assembly.

Polymerase (L) Protein and Phosphoprotein (P)

Paramyxovirus RNA-dependent RNA polymerase activity, needed for transcription and replication, is promoted by a complex between the viral L and P proteins. The L protein, the largest of the paramyxovirus proteins at approximately 2200 amino acids, contains a number of small domains homologous to those found in other viral RNA-dependent RNA polymerases, indicating that L likely contains the catalytic activity for RNA polymerization. Experimental evidence suggests that the L protein also promotes polyadenylation of viral mRNA, and L has been implicated in phosphorylation of the viral N and P proteins. The L protein is present in only approximately

50 copies per virion, making it the least abundant of the paramyxovirus structural proteins. This low abundance is at least partly due to the position of the L gene in the genome, as it is the last to be transcribed, and thus has the lowest amount of mRNA produced. The active P–L complex contains five to ten P proteins per L protein. While the L protein likely contains the catalytic domains required for polymerization and polyadenylation, the P protein plays the critical role of tethering L to the N protein – RNA template.

For members of the subfamily *Paramyxovirinae*, multiple protein products are produced from the P gene through a fascinating mechanism known as RNA editing. In this process, the L–P RNA-dependent RNA polymerase adds, at a specific location known as the editing site, one, two, or four G nucleotides not encoded in the P gene. This addition of nontemplated nucleotides results in production of additional mRNAs and protein products, including the V, W, D, and I proteins. While many of the functions of these proteins remain unclear, V has been shown to interact with a cellular protein DDB1 that in turn forms a protein complex, a viral degradation complex (VDC) that mediates proteasomal destruction of the transcription factor, STAT 1. STAT 1 is a critical factor in establishing the interferon-induced antiviral state. Viruses from the genera *Morbillivirus*, *Respirovirus*, and *Henipavirus* also contain two overlapping reading frames in the P gene region. The viral C protein, also shown to play roles in countering the antiviral response, is produced from this alternative reading frame.

Additional Proteins

Several proteins are produced only by a subset of the *Paramyxoviridae*. SH (small hydrophobic) is a small transmembrane domain containing protein produced by members of the genera *Rubulavirus*, *Pneumovirus*, and *Metapneumovirus*. The SV5 and mumps SH proteins have been demonstrated to block apoptosis (programmed cell death) during infection, and are suggested to interfere with tumor necrosis factor alpha (TNFα) signaling. The SH proteins of the pneumoviruses and metapneumoviruses differ considerably from each other and from those of the rubulaviruses, and the role of these proteins is currently unclear.

Members of the subfamily *Pneumovirinae* also contain an M2 gene, which encodes two proteins, M2-1 and M2-2. M2-1 is a transcription elongation factor which, recent evidence suggests, interacts with the viral P protein. The M2-2 protein has been implicated in controlling the balance between transcription and replication. Finally, members of the genus *Pneumovirus* contain two additional genes at the 3′ end of the genome which encode the NS1 and NS2 proteins. Both NS1 and NS2 have also been implicated in counteracting antiviral defense within infected cells.

Replication

Paramyxovirus replication (**Figure 3**) begins with viral attachment to the plasma membrane of the target cell, a process mediated by the attachment protein through interactions with sialic acid moieties or other specific receptors. Subsequent fusion of the viral envelope with the target cell plasma membrane is promoted by the viral F protein, and results in delivery of the viral ribonucleocapsid core into the cytoplasm. Both transcription and replication occur exclusively in the cellular cytoplasm. As the RNA genome is negative sense (anti-coding sense), transcription of the genome to generate mRNA is then performed. The RNA-dependent RNA polymerase, composed of the L and P proteins, binds to a promoter present at the 3′ end of

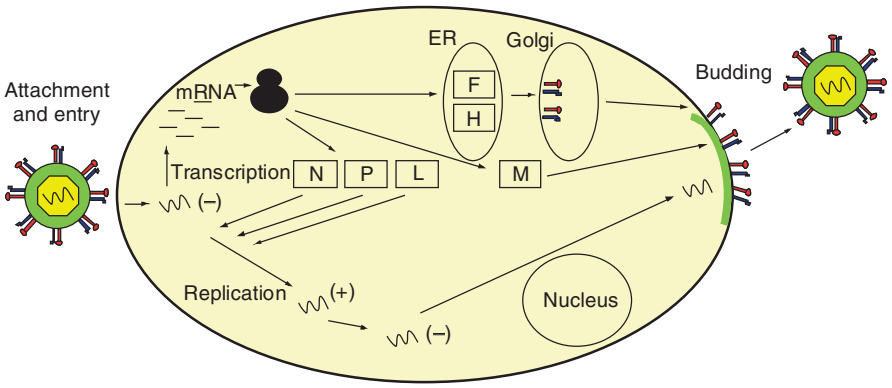


Figure 3 Life cycle of a paramxyovirus infection. Attachment and entry leads to release of the nucleocapsid (wavy line) into the cytoplasm. Transcription then occurs, generating mRNA which is translated to viral proteins by the host translation machinery. The F (fusion) and H (attachment) proteins are co-translationally transported into the endoplasmic reticulum (ER), followed by transport through the Golgi to the plasma membrane. Newly synthesized N (nucleocapsid), P (phosphoprotein), and L (large polymerase) proteins allow for replication of the genome, first to antigenome (+), then to genome ( ) forms. M (matrix) protein assists in viral assembly, when the newly synthesized ribonucleoprotein core is incorporated into an enveloped particle containing the viral glycoproteins.

the genome, with a second internal promoter needed for efficient initiation of transcription for members of the subfamily *Paramyxovirinae*. The viral RNA polymerase begins transcription at this 3′ end, synthesizing a leader RNA and then sequentially transcribing the genes in the 3′-to-5′ direction. After termination of transcription of each gene, the polymerase moves on to reinitiation of transcription at the next gene, always moving in a 3′-to-5′ direction. However, reinitiation of transcription is not completely efficient, and this process therefore results in a sequential decrease in the mRNA levels from genes at the 3′ end to those at the 5′ end, with the final L gene having the lowest level of mRNA synthesized. Newly synthesized viral mRNAs are then translated to viral protein using the host translation machinery. The viral glycoproteins are co-translationally transported in the endoplasmic reticulum, where they are folded, modified by addition of N-linked carbohydrates, and transported through the secretory pathway to the plasma membrane. The same viral RNA polymerase which carries out transcription of mRNA is also responsible for viral replication, and the 3′ end promoter is also critical for this process. Many important questions remain concerning the switch from transcription (generating separate mRNAs) to replication (which ignores gene start and end regions, thus generating a complete copy of the genome). However, evidence does suggest that the accumulation of viral proteins, in particular N and M2-2, is critical for the switch to replication. Replication results first in an antigenome, a complementary copy of the entire viral genome. The newly synthesized antigenome can then be used as a template for synthesis of new negative-sense genomes. Replication of the viral genome is closely linked to encapsidation, where the newly created viral ribonucleocapsid cores are packaged into new virion particles. This assembly process is thought to occur at the plasma membrane for all members of the *Paramyxoviridae*. The M protein is a key player in the proper assembly of new progeny virus, as it interacts with the cytoplasmic tail of the glycoproteins, with the lipid bilayer of the plasma membrane, and with the ribonucleocapsid cores. The budding virus takes its envelope from the plasma membrane of the cell, which now contains viral glycoproteins.

Reverse Genetics

Analysis of specific mutations within the context of viral infection allows for a detailed molecular dissection of events in the viral life cycle. An important breakthrough which facilitated this type of detailed molecular analysis for the study of paramxyoviruses was the development of reverse genetics systems which allow synthesis of new viral progeny with specifically introduced mutations. This process was technically challenging as the paramyxovirus genome is a negative-sense RNA, and thus cannot be utilized by cellular machinery for direct translation of proteins. Reverse genetics methods allow for synthesis of viral mRNA, proteins, and eventual viral progeny by introduction of a plasmid containing the viral genome along with plasmids expressing the viral N, P, and L proteins, with a T-7 promoter present on all four plasmids. Expression of the T-7 polymerase leads to synthesis of the viral N, P, and L proteins along with synthesis of antigenome RNA. These elements then drive viral mRNA and protein synthesis, and eventual assembly of new viral particles containing the desired mutations. Experiments utilizing recombinant viruses generated using these techniques have led to significant new understanding of this important viral family. In addition, reverse genetics techniques are now being utilized for creation of new potential vaccines.

Host Range and Transmission

Paramyxoviruses infect a wide range of vertebrates, but to date no infection of invertebrates by members of this family has been described. A large number of paramyxoviruses are human pathogens, including measles virus, mumps virus, HRSV, and HMPV. The family also includes viruses which infect a variety of animal species. Newcastle disease virus and a series of avian paramyxoviruses infect both domestic and wild fowl, while avian metapneumovirus targets both chickens and turkeys. A wide range of animals, including dogs, raccoons, and skunks, are infected by canine distemper virus. Bats are infected by a number of paramyxoviruses, including Menangle virus and Tioman virus, and are likely the major animal reservoir for both Hendra and Nipah virus. Rinderpest virus infects cattle, as does bovine respiratory syncytial virus. Even sea creatures and reptiles are not immune from paramyxovirus infection. Phocine distemper virus causes severe disease in seals, while Cetacean morbillivirus infects both dolphins and porpoises. Fatal disease in snakes is associated with Fer de lance virus.

The majority of paramyxoviruses are respiratory pathogens, and transmission of virus generally occurs via virus-containing aerosols or contact with surfaces contaminated with virus-containing secretions. A small number of paramyxoviruses, including Newcastle disease virus, can infect through the gastrointestinal tract. Transmission of Hendra and Nipah virus from bats appears to involve contact with contaminated feces.

Disease, Prevention, and Treatment

Paramyxoviruses most commonly cause respiratory disease, but a number of members of the family also cause systemic infections, and some have been shown to cause encephalitis. Measles virus, which is still responsible

for approximately 500 000 deaths per year, causes a systemic infection with fever, rash, and respiratory symptoms, and measles-related complications include pneumonia and encephalitis. HRSV is the leading cause of bronchiolitis and pneumonia in infants. HMPV and the HPIVs are also major pathogens causing severe respiratory disease in infants and young children. Nipah virus was first identified in 1999, and has already been the cause of a number of outbreaks in Southeast Asia, with mortality rates as high as 75% and evidence of human-to-human transmission. Nipah is associated with encephalitis resulting in fever, brain inflammation, and seizures, but respiratory symptoms have also been observed. Animal paramyxoviruses can also cause localized respiratory infection or systemic infections, depending on the animal and virus.

Vaccines have been effective at preventing diseases caused by a number of paramyxoviruses. The first vaccine for measles became available in 1963, and routine vaccination with MMR vaccine (measles-mumps-rubella) has decreased incidence of these infections in the developed world to extremely low levels. A number of vaccines for animal paramyxoviruses are routinely utilized, including vaccines for canine distemper, rinderpest, and Newcastle disease virus. However, development of successful vaccines for a number of important paramyxoviruses, including human and bovine respiratory syncytial viruses, has not been successful to date, and this remains a significant challenge for scientists in the field. In addition, few antiviral agents are approved for treatment of infection by these viruses. Development of effective antiviral therapies will likely be aided by our growing understanding of the molecular details of paramyxovirus infection.

See also: Henipaviruses; Human Respiratory Syncytial Virus; Measles Virus; Mumps Virus; Paramyxoviruses of Animals; Parainfluenza Viruses of Humans; Rinderpest and Distemper Viruses.

Further Reading

Eaton BT, Broder CC, Middleton D, and Wang L-F (2006) Hendra and Nipah viruses: Different and dangerous. *Nature Reviews Microbiology* 4(1): 23–35.

Lamb RA and Parks GD (2006) *Paramyxoviridae:* The viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1449–1496. Philadelphia, PA: Lippincott Williams and Wilkins.

Loughlin GM and Moscona A (2006) The cell biology of acute childhood respiratory disease: Therapeutic implications. *Pediatric Clinics of North America* 53(5): 929–959.

Pekosz A, He B, and Lamb RA (1999) Reverse genetics of negative-strand RNA viruses: Closing the circle. *Proceedings of the National Academy of Sciences, USA* 96: 8804–8806.

van den Hoogen BG, de Jong JC, Groen J, *et al.* (2001) A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nature Medicine* 7(6): 719–724.

Parapoxviruses

D Haig, Nottingham University, Nottingham, UK
A A Mercer, University of Otago, Dunedin, New Zealand

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Parapoxviruses (PPVs) are epitheliotropic viruses found worldwide. The individual viruses generally exhibit a narrow host range and infect via scarified or damaged skin and give rise to pustular lesions of the skin and occasionally the buccal mucosa. These lesions are associated with low mortality and high morbidity. In addition to a narrow host range, most of the PPVs can also infect humans.

There are numerous historical references to diseases of domesticated animals such as sheep and cattle that we would now suspect to be the result of infection by PPVs. These references include Jenner's 'spurious' cowpox which is likely to have been caused by the PPV, pseudocowpox virus (PCPV). In the latter part of last century, reports appeared in the scientific literature which recognized the distinct identities of the diseases caused by members of this genus. Following an extensive study of contagious pustular dermatitis of sheep, Aynaud produced a report in 1923 which included the observation that the disease could be transmitted by a 'filterable' agent. The isolation of each of the viruses in cell culture was reported in the period from 1957 to 1963. Detailed reports of the transmission of each disease to humans appeared in 1933 (orf virus (ORFV)), 1963 (PCPV), and 1967 (bovine papular stomatitis virus (BPSV)). The first molecular analyses of PPV genomes appeared in 1979 with publication of restriction endonuclease cleavage site maps and reports of G + C contents. These were followed, in 1989, by the

first description of the DNA sequence of a region of a PPV genome and in 2004 with full genome sequence for two strains of ORFV and one of BPSV.

Taxonomy and Classification

The genus *Parapoxvirus* belongs to the subfamily *Chordopoxvirinae* of the family *Poxviridae*. The type species of the genus is *Orf virus*, and the other species recognized as members are *Bovine papular stomatitis virus*, *Pseudocowpox virus*, *Squirrel parapoxvirus*, and a recently identified member, *Parapoxvirus of red deer in New Zealand*. Synonyms for the viruses include contagious pustular dermatitis virus and contagious ecthyma virus for ORFV and milker's nodule virus and paravaccinia virus for PCPV. Tentative species of this genus are Auzduk disease virus (camel contagious ecthyma virus), chamois contagious ecthyma virus, and sealpox virus. A virus that infects red squirrels and induces a pustular skin disease was thought to be a PPV, but recent DNA sequence data suggest that this is not the case.

The three original members of the genus were classified as separate species on the basis of the host animal and/or the pathology of the disease. Likewise, the observation of a parapox-like virus in red deer first suggested that this might represent another species. These separations have been supported by later studies which employed DNA/DNA hybridization, restriction endonuclease profiling, sequence data, or serology.

Host Range, Epidemiology, and Virus Propagation

Natural infection by ORFV has been reported in domestic, bighorn, and thinhorn sheep, domestic and Rocky Mountain goat, chamois, Himalayan thar, musk-ox, reindeer, steenbook, and humans. Experimental inoculations have shown that monkeys are susceptible to ORFV but a wide range of other animals including mouse, rabbit, dog, cat, and domesticated chicken are resistant. BPSV and PCPV both establish infection in cattle and humans but all other species tested, including sheep, are resistant. The PPV of red deer induces only very mild lesions on sheep and has not been tested in other species. The PPV of seals has been reported in a range of seals and sea lions. PPVs do not produce lesions on the chorioallantoic membrane of the developing chick embryo.

The PPVs of cattle and sheep are found throughout the world, essentially wherever their host animal occurs. The viruses are maintained in populations by a combination of chronic infection, frequent reinfection, and the environmentally resistant nature of the viruses.

ORFV shed in scab material can remain infective under dry conditions for lengthy periods (at least 4 months and possibly years) and infection of naive animals by virus persisting in heavily contaminated areas such as barns, yards, and sheep camps is likely to play a major role in maintaining the disease. One study has shown that if the scab material is ground up so as to release the virus, then exposure to field conditions quickly results in inactivation of ORFV. Several studies have shown that a productive infection can be established in animals which have recovered from a previous infection. Such reinfections result in lesions that are smaller and resolve more quickly than primary infections. This short-lived immunity is likely to contribute to the persistence of the disease.

In the case of PCPV, it is apparent that infection can be spread within dairy herds by contamination of milking machinery and milkers' hands. The introduction of procedures which reduce damage to teats and improve general hygiene at milking time can control the spread of the disease.

The most widely used cell culture systems have been primary ovine or bovine cells derived from sources such as testis, skin biopsy and embryonic kidney, lung, and muscle. There have also been reports of ORFV isolates adapted to growth in established cell lines. Yields of infectious ORFV from cell culture tend to be 10- to 100-fold lower than those achieved with vaccinia virus.

Serologic Relationships

PPVs show extensive antigenic cross-reactivity, although monoclonal antibodies have been able to distinguish each of the species. There are also antigens shared with other poxvirus genera but there is no cross-protection between PPVs and either orthopoxviruses or capripoxviruses.

Clinical Features

PPVs cause proliferative lesions that are confined to the skin and oral mucosa with no evidence of systemic spread (**Figure 1**). Infection is initiated in abrasions and generally proceeds through an afebrile, self-limiting lesion that resolves within 3–9 weeks without leaving a scar. ORFV lesions are most generally seen around the mouth and nares; hence, the infection is commonly referred to as scabby mouth or sore mouth. Lesions are also observed on other parts of the body, for example, the coronet, udder, or vulva. Following experimental inoculation of scarified skin, lesions progress through erythema, papule, vesicle, pustule, and scab before resolving. Large, proliferative, tumor-like lesions have been observed. It is likely that these are a result of an immune impairment of the host animal.



Figure 1 Orf in *Ovis aries*. Note the pustular lesions around the mouth and nares.

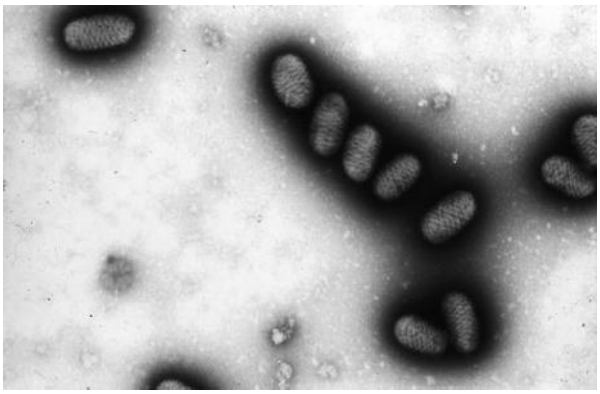


Figure 2 Electron micrograph of a cluster of negatively stained orf virus (ORFV) particles.

Lesions around the mouth can interfere with feeding or suckling and especially in young animals result in failure to thrive. Teat lesions can have similar effects through the inhibition of suckling. Lesions on growing deer antler can affect antler growth and severely affect marketability of the product.

It is probable that all PPVs are able to infect humans, although a human case of the PPV of red deer has not been reported. Transmission to humans occurs readily although there is little evidence of human to human transmission. Progression of the lesions is essentially as seen in sheep and cattle such that the infection is benign and confined to pustular lesions on the skin at the points of infection. More severe progressive disease can occur in immune-compromised individuals. Severe reactions have also been recorded in otherwise normal individuals in cases of burns and in cases of atopic dermatitis. Erythema multiformae reactions in the form of rashes on the backs of the hands and on the legs and ankles are common.

Properties of the Virion

PPV particles are ovoid in shape and measure 220–300 nm × 147–170 nm (**Figure 2**). In these characteristics they resemble other poxviruses except that PPVs are a little smaller than most other *Chordopoxvirinae*, which are also more commonly described as brick-shaped rather than ovoid. A distinctive feature of PPV virions is their 'ball-of-yarn' appearance when negatively stained specimens are viewed by electron microscopy. This results from a single 10–20 nm wide thread arranged as spiral coil around the particle. This unique morphology has been the basis for confirming a suspected PPV infection. However, this feature has recently been observed in poxviruses that are not members of the genus *Parapoxvirus* and DNA analysis is required to provide definitive identification.

Analysis of thin sections of virions has revealed a lipoprotein bilayer surrounding a biconcave core and two associated lateral bodies. Some particles have an external membranous structure. It seems likely that this is equivalent to the Golgi-derived membrane which forms the outer layer of the extracellular enveloped form of vaccinia virus.

Physical Properties

PPVs are resistant to desiccation and, in a dried state within scab material, the viruses retain infectivity for at least 4 months and possibly years. Under laboratory conditions, infectivity may be maintained over many years. UV light, γ-irradiation, or heating at 56°C for 1 h will inactivate PPVs.

PPV Genomes

The PPV genome is a single, linear, double-stranded (ds) DNA molecule of 130–140 kbp with inverted terminal repeats of about 3.5 kbp in the case of ORFV (**Figure 3**). The ends of the genome are cross-linked. The G+C content of the genome is high (average of 64%). Restriction endonuclease cleavage site maps have been produced for each of the PPVs except that infecting red deer. These revealed some variability between isolates of the same species but conserved patterns, consistent with the classification of the genus, were apparent.

Full genome sequences have been obtained for three strains of tissue-culture adapted ORFV (OV-IA82 and NZ2 isolated from sheep and OV-SA00 isolated from a goat) and a tissue-culture-adapted strain of BPSV (BV-AR02). These encode an estimated 133 BPSV or 132 ORFV genes, of which 129 are collinear in the viral genomes and 88 conserved in all *Chordopoxvirinae* studied to date. The majority of these are essential genes involved in virus replication, packaging, and export. There are

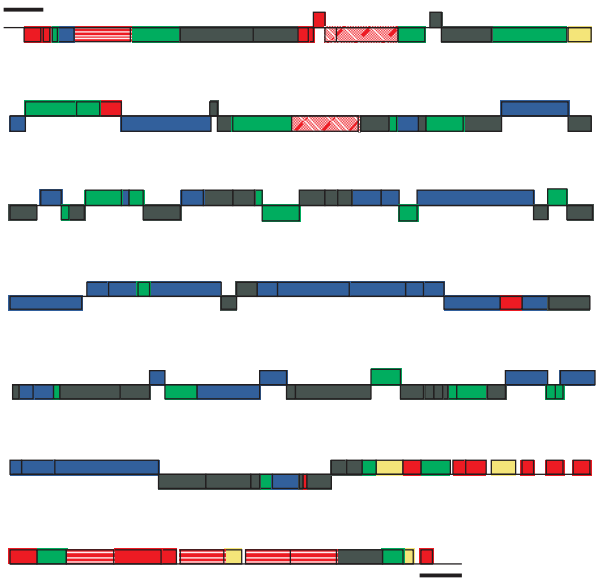


Figure 3 Orf virus (ORFV) genetic map. Boxes above the line represent genes transcribed rightward and those below the line genes transcribed leftward. Each line except the last corresponds to 20 kbp. The boxes are colored to indicate predicted functions. Those involved in virion structure or assembly are black, DNA replication or RNA transcription blue, host response modulation yellow, and ankyrin-repeat proteins are indicated with horizontal red lines. Genes of unknown function are further subdivided by their presence in most poxviruses (green), in parapoxviruses only (red), or in parapoxviruses and molluscipoxviruses only (red diagonal stripes).

fewer nucleotide metabolism genes compared with other poxviruses. Seventeen BPSV and eighteen ORFV open reading frames (ORFs) lack amino acid similarity to other poxvirus or cellular proteins. The PPV genomes have some similar features (including three orthologous genes) to that of molluscum contagiosum virus – a skin-tropic poxvirus pathogen of humans. Putative and known pathogenesis-related genes are concentrated in the terminal regions of the genome. In ORFV there are ~25 such genes with unknown structure or function. In one study, the early transcribed genes of ORFV were mapped to identify candidate pathogenesis genes. cDNAs for 38 genes were identified, seven of these unrelated to any poxvirus or cellular gene in public databases.

The identification of transcriptional start points of PPV early genes highlighted adjacent sequences which are very similar to sequences shown to act as transcriptional promoters in vaccinia virus. These observations have been supported by data indicating that A+T-rich transcriptional control sequences characterized in vaccinia virus function in very similar ways in PPVs. Analysis of expression of ORFV antigens expressed from vaccinia virus recombinants carrying large multigene fragments of ORFV DNA suggests that ORFV late genes are also faithfully transcribed from their own promoters by vaccinia virus.

Passage of ORFV in cell culture results in genomic rearrangements. A detailed study of one such rearranged isolate showed that 19.3 kbp from the right end of the genome had been duplicated and replaced 6.6 kbp at the left end, resulting in a net deletion of 3.3 kbp at the left end. This recombination occurred between nonhomologous sequences and caused the deletion of three genes. These alterations to the genome attenuated the growth of the virus in sheep skin. Similar rearrangements have been observed in other isolates.

Properties of Viral Proteins

SDS-polyacrylamide gel electrophoresis analyses of ORFV and PCPV virions have detected 30–40 polypeptides ranging in size from 10 to 220 kDa. Controlled degradation of the virions into core and surface fractions indicated the presence of 10–13 surface polypeptides. Prominent among these were a polypeptide of 39 kDa (ORFV) or 42–45 kDa (PCPV), which was suggested to be the subunit of the virion surface tubule protein, and a 15 kDa polypeptide. The 39 kDa ORFV polypeptide appears to be a dominant antigen and several independently derived monoclonal antibodies are directed against it.

The genes encoding two major structural proteins of ORFV have been identified. One of these is a 42 kDa protein with strong amino acid sequence similarity to the vaccinia virus protein encoded by gene F13L. This vaccinia virus polypeptide is the major, nonglycosylated, 37 kDa protein specific for the extracellular enveloped form of the virus. Cloning and expression of the ORFV gene has shown that both antibody and T-cell responses are directed against this protein during infection by ORFV. A second ORFV protein, p10k, is a homolog of the vaccinia virus 14 kDa fusion protein encoded by gene A27L. This vaccinia virus protein has a surface location and is associated with intracellular mature virus but is required for the formation of extracellular enveloped virus. The ORFV protein may be involved in the expression of the characteristic basket-weave pattern on the virions. The F1L product of ORFV is a major heparin-binding protein and may be involved in virus binding to cells to initiate the infection cycle.

Pathogenesis-related proteins are discussed in a later section.

Replication

As with all poxviruses, the replication of PPVs occurs in the cytoplasm of infected cells. Studies in cell culture have shown very similar patterns in the replication of ORFV and PCPV. DNA replication begins 4–8 h post infection (h.p.i.) and reaches a plateau between 25 and

30 h.p.i. The first viral or viral-induced polypeptides appear from 10 h.p.i. Viral particles appear 16–24 h.p.i. and continue to be produced until at least 40 h.p.i. Viral replication is accompanied by inhibition of host DNA and protein synthesis. Some ORFV genes have been shown to be transcribed when infection occurs in the presence of an inhibitor of DNA synthesis and therefore fit the definition of early poxvirus genes. The transition between early and late replication events occurs about 8–10 h.p.i. The presence in ORFV of genes with homology to vaccinia virus intermediate genes encoding late gene transactivators suggests that expression of ORFV genes follows a regulated cascade (early–intermediate–late) similar to that reported for vaccinia virus.

Pathogenesis and the Host Immune Response to Infection

The histological sequence of events in the skin of sheep after ORFV infection is similar in primary and reinfection lesions, in spite of differences in the magnitude of the lesions and the time taken to resolve. Antibodies to ORFV envelope proteins have been used to detect ORFV antigen in epidermal keratinocytes, particularly those regenerating the damaged skin. Basal keratinocytes at the root of hair follicles can also contain virus. Some infected cells show evidence of a ballooning-like degeneration. There is no evidence that ORFV infects other, nonepithelial cell-types *in vivo*. ORFV lesions often exhibit epidermal downgrowths (rete formation) into the dermis. This is particularly marked in primary lesions. Another characteristic feature is extensive capillary dilation and proliferation.

ORFV lesions contain a dense accumulation of immune and inflammatory cells underneath and adjacent to virus-infected cells. These include neutrophils, lymphocytes (T and B cells), and dendritic cells that stain intensely with major histocompatibility complex class II antigens. This dense network of dendritic cells is characteristic of orf lesions in sheep. The function of these cells is not known. The accumulating cells increase and decrease in number in parallel with the presence of virus in epidermal cells. The histology of human ORFV lesions is generally similar to that described in sheep. A comparison of ORFV and PCPV lesions in humans has not revealed any histopathological differences.

Immune Response to Infection

PPVs, in common with other poxviruses, stimulate a vigorous immune and inflammatory response in their hosts, and have evolved to replicate in the presence of this response. In sheep experimentally infected with ORFV, studies in the skin and lymph draining into (afferent lymph) and out of (efferent lymph) local lymph nodes have demonstrated that activated CD4+ (helper) and CD8+ (cytotoxic) T cells, B cells, and antibodies are generated as part of the sheep-acquired immune response to infection. The cytokines generated in lymph in response to virus reinfection are typical of type 1 antiviral cell-mediated immune responses and include interleukin (IL)-1β, IL-2, tumor necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-α, and IFN-γ. Studies of ORFV reinfection in sheep depleted of specific lymphocyte subsets or treated with the immunosuppressant drug cyclosporin-A indicated that at least CD4+ T cells and interferons are important components of the host-protective-response against infection. These studies also indicated that the cutaneous damage sustained during ORFV infection is due in large part to the virus rather than host immune-mediated.

Sheep infected with ORFV mount detectable antibody responses to a small number of viral antigens but there is considerable individual qualitative and quantitative variation in the response. There is a lack of neutralizing antibody. There is no apparent correlation between antibody titers and severity of viral lesions and passive transfer of antibody does not confer protection against virus challenge.

Immunomodulation by ORFV

In general, PPV infection is mild and localized with more severe disease only occurring in stressed or otherwise immune-impaired individuals. An intriguing feature of PPVs is the ability to repeatedly infect animals despite an apparently typical antiviral immune response to infection. This may in part be a result of the action of viral-encoded immune modulators that are a feature of large DNA viruses. There are now several examples of these that have been identified within the terminal regions of the genome of ORFV.

Viral Vascular Endothelial Growth Factor

The first of these to be reported was a homolog of mammalian vascular endothelial growth factor (VEGF). The viral VEGF is expressed early, shows 16–27% amino acid sequence identity to mammalian VEGFs (VEGF-A,-B, -C,-D) and has been classified as VEGF-E. It is unique among the VEGF family in that it interacts with VEGF receptor 2 (VEGFR-2) but not with either VEGFR-1 or VEGFR-3. VEGFs are specific mitogens for endothelial cells and regulate normal and pathological angiogenesis, including the vascularization of solid tumors. Extensive capillary proliferation and dilation is a feature of ORFV lesions and the viral VEGF plays a major role in generating these features. It may also indirectly enhance

proliferation of the epithelium, the target tissue for virus replication, as epidermal proliferation was reduced in lesions with a VEGF gene knock-out virus compared to wild-type virus lesions. A report that human VEGF can inhibit the functional maturation of dendritic cells hints at a possible immune modulating role for the viral VEGF. A VEGF-like gene has not been reported in any other poxvirus.

Viral Interferon Resistance Protein

ORFV encodes an interferon resistance protein (OVIFNR) produced by the 020 gene early in infection, which inhibits the interferon-induced shutdown of cellular protein translation (via PKR) by binding to viral dsRNA, thus preventing activation of the protein kinase PKR. 020 is a homolog of the vaccinia virus E3L gene (31% predicted amino acid sequence identity) that has the same function.

Viral IL-10

ORFV IL-10 (vIL-10) is the product of an early viral gene and the predicted protein is very similar to ovine IL-10 over the C-terminal two-thirds of the molecule while differing substantially at the N-terminus (80% amino acid sequence identity overall). The vIL-10 exhibited similar anti-inflammatory and immunostimulatory activities to ovine IL-10. Both cytokines suppressed TNF-α production from macrophages and suppressed IFN-γ production from peripheral blood cells. Both vIL-10 and ovine IL-10 stimulated mast cell proliferation provided co-stimulatory cytokines were present (IL-3 or IL-4). vIL-10 is a virulence protein as recombinant virus lacking the vIL-10 gene is attenuated compared to wild-type or vIL-10 gene-reconstituted virus in sheep infection experiments. The ORFV IL-10 may act to suppress elements of the antiviral immune response, thereby delaying virus clearance. IL-10-like genes have been reported in two other poxvirus genera (*Capripoxvirus* and *Yatapoxvirus*) although these proteins show markedly less sequence similarity to mammalian IL-10 than does ORFV IL-10.

Viral GM-CSF Inhibitory Factor

The protein product of ORFV ORF 117 encodes an early virus protein with novel function. Viral GM-CSF inhibitory Factor (GIF) binds to and inhibits the biological activity of the cytokines GM-CSF and IL-2. Among other properties, GM-CSF regulates the recruitment, differentiation, and activation of macrophages, neutrophils, and dendritic cells. Activated T cells are an important source of GM-CSF during immune responses to pathogens. IL-2 is produced predominantly by T cells and stimulates the expansion and activation of T cells and natural killer cells among other cell types. GIF has probably evolved to perform its dual function from an ancestral type-1 cytokine receptor, as it shares important structure–function features of this receptor family while exhibiting an otherwise divergent primary amino acid sequence. A GIF protein is also expressed in other PPVs, and is conserved in ORFV strains though there is only ~40% amino acid identity between these and BPSV GIF. The role of GIF in ORFV pathogenesis is currently not known.

Viral Chemokine-Binding Protein

ORFV encodes a chemokine-binding protein, the product of ORF 112, which is expressed early in the virus life cycle. It is structurally and functionally related to the type II CC chemokine-binding proteins of the *Orthopoxvirus* and *Leporipoxvirus* genera. Viral chemokine-binding protein (vCBP) binds CC chemokines, including monocyte-chemotactic protein-1, macrophage inflammatory protein-1α and RANTES (regulated upon activation, normal T-cell-expressed and secreted). These regulate T cell and monocyte/macrophage recruitment to sites of infection. In addition, and uniquely among poxvirus CBPs, vCBP binds to lymphotactin, a C-chemokine that recruits T and B cells, and neutrophils. vCBP may well function to inhibit key aspects of an antiviral immune response.

PPVs as Vectors and Immunomodulators

ORFV could prove useful as a vector for delivering microbial antigens to the immune system. Proof of concept for this has been obtained with protective immunity generated to pseudorabies virus and Borna disease virus infection of rodents. The restricted host ranges of the PPVs and lack of systemic spread even in immuno-compromised animals make them good viral vector candidates. Furthermore, inactivated ORFV particles have been shown to exhibit nonspecific immuno-modulatory effects that enhance immunity to a variety of pathogens in several species. This is thought to be mediated by IFN and a type 1 immune response that is downregulated at later stages by IL-10, among other cytokines. This feature of ORFV continues to be exploited commercially.

Prevention and Control

ORFV vaccines have been available for many years and are widely used to protect lambs against the debilitating effects of natural infection. These vaccines consist of live and essentially nonattenuated virus that is applied to a scratch on the skin of a leg. The ensuing infection does not interfere with feeding and provides significant

protection against infection for some months. However, the scab derived from vaccination lesions is likely to contaminate the environment and contribute to the perpetuation of the disease. New vaccines that induce protection but do not shed infectious virus are highly desirable. This might be achieved by deleting genes encoding viral virulence determinants or by delivering the protective antigens of the virus in an appropriate way.

Humans may be infected by PPVs following contact with animal lesions or scab. The viruses in scab associated with animal products such as wool or farm equipment can remain infectious for lengthy periods. Care should be taken to avoid contact between any skin wound and potentially contaminated material.

Future Perspectives

Recent studies with ORFV have raised the possibility of developing a vaccine able to protect animals against infection by this virus without generating significant amounts of infectious virus. Such a vaccine would also be likely to reduce the frequency of human infections.

The use of ORFV and perhaps other PPVs as vaccine vectors is proving a useful and robust strategy. The generation of ORFV recombinants and other PPVs lacking pathogenesis-related genes will continue to advance our understanding of the pathogenesis of PPV diseases. An understanding of PPV pathogenesis and the viral proteins involved, coupled to recombinant virus generation, should lead to improved control strategies for this important group of viruses.

See also: Capripoxviruses; Poxviruses; Vaccinia Virus.

Further Reading

Delhon G, Tulman ER, Afonso CL, *et al.* (2004) Genomes of the parapoxviruses orf virus and bovine papular stomatitis virus. *Journal of Virology* 78: 168–177.

Haig DM (2006) Orf virus infection and host immunity. *Current Opinion in Infectious Diseases* 19: 127–132.

Haig DM and McInnes CJ (2002) Immunity and counter-immunity during infection with the parapoxvirus orf virus. *Virus Research* 88: 3–16.

Mercer AA, Ueda N, Friederichs SM, *et al.* (2006) Comparative analysis of genome sequences of three isolates of orf virus reveals unexpected sequence variation. *Virus Research* 116: 146–158.

Partitiviruses of Fungi

S Tavantzis, University of Maine, Orono, ME, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The vast majority of mycoviruses (viruses infecting fungi) are 'cryptoviruses', that is, they are not usually associated with an overt pathology of their fungal hosts. So, it is not surprising that the first mycovirus paper, by M. Hollings published in *Nature* (London) in 1962, was about virus-like particles (VLPs) thought to be the cause of a serious dieback disease of the cultivated mushroom *Agaricus bisporus*. Following Hollings' groundbreaking paper, progress in mycovirology was slow, as in addition to their cryptic nature, fungal viruses can be transmitted only through intracellular routes. Most of the characterized mycoviruses possess double-stranded RNA (dsRNA) genomes but this high proportion might be biased since most of the published data refer to fungal screenings for the presence of dsRNA followed by transmission electron microscopy or virus characterization studies.

Mycoviruses are classified into nine families (*Barnaviridae, Chrysoviridae, Hypoviridae, Metaviridae, Narnaviridae, Partitiviridae, Pseudoviridae, Reoviridae, Totiviridae*), and one genus (*Rhizidiovirus*) not classified into a specific family. Viruses belonging to the family *Partitiviridae* usually cause cryptic infections in fungi and plants, and are classified into three genera, *Partitivirus*, *Alphacryptovirus*, and *Betacryptovirus*. The name *Partitiviridae* originates from the Latin *partitus*, which means 'divided' and refers to the fact that the genome of these viruses is bipartite (separately encapsidated) and consists of two monocistronic dsRNAs that are similar in size.

Taxonomy and Classification

This section focuses on the genus *Partitivirus*, which consists of viruses that infect filamentous fungi. The genera *Alphacryptovirus* and *Betacryptovirus* include viruses that are found in plants. The recognized species of the genus *Partitivirus* are listed in **Table 1** whereas tentative members are shown in **Table 2.** The sequence accession numbers refer to the RNA-dependent RNA polymerase (RdRp) genes of the respective partitiviruses.

Table 1 Virus members in the genus *Partitivirus*

Virus	*Abbreviation*	*Accession number*
Agaricus bisporus virus 4	AbV-4	
Aspergillus ochraceous virus	AoV	
Atkinsonella hypoxylon virus	AhV	L39125
Discula destructiva virus 1	DdV-1	AF316992
Discula destructiva virus 2	DdV-2	AY033436
Fusarium poae virus	FpV-1	AF047013
Fusarium solani virus 1	FsV-1	
Gaeumannomyces graminis virus 019/6-A	GgV-019/6-A	
Gaeumannomyces graminis virus T1-A	GgV-T1-A	
Gremmeniella abietina RNA virus MS1	GarV-MS1	AY08993
Helicobasidium mompa virus	HmV	
Heterobasidion annosum virus	HaV	
Penicillium stoloniferum virus S	PsVS	AY156521
Rhizoctonia solani virus 717	RhsV-717	AF133250

Table 2 Tentative members of the genus *Partitivirus*

Virus	*Abbreviation*	*Accession number*
Ceratocystis polonica partitivirus	CpPV	
Ceratocystis resinifera partitivirus	CrPV	
Diplocarpon rosae virus	DrV	
Gremmeniella abietina RNA virus MS2	GaRV-MS2	AY615211
Helicobasidium mompa partitivirus	HmPV	
Ophiostoma partitivirus 1	OPV-1	AM087202
Penicillium stoloniferum virus F	PsV-F	AY758336
Phialophora radicicola virus 2-2-A	PrV-2-2-A	
Pleurotus ostreatus virus	PoV	AY533038
Rosellinia necatrix virus 1-W8	RnV-1-W8	AB113347

Virion Properties

The virion M_r estimates range from 6×10^6 to 9×10^6 and the $S_{20,w}$ values (in Svedberg units) range from 101 to 145, whereas the $S_{20,w}$ values of particles lacking nucleic acid range from 66 to 100S. Virion buoyant density in CsCl is 1.34–1.37 g cm^{-3} for particles with nucleic acid and 1.29–1.30 g cm^{-3} for particles devoid of nucleic acid. Purified virion preparations contain, in addition to mature virions and empty virions, sedimenting and density components that are thought to be replicative intermediates containing single-stranded RNA (ssRNA) and particles with both ssRNA and dsRNA.

Virion Structure and Composition

Viruses of the genus *Partitivirus* have isometric particles with icosahedral symmetry ranging from 30 to 35 nm in diameter (**Figure 1**). Negatively stained virions that are devoid of nucleic acid have capsids with dark centers as they are penetrated by stain (**Figure 1**). The virion capsid is not enveloped and consists of 12 capsomers and 120 capsid protein (CP) subunits with a molecular mass ranging from 57 to 76 kDa. Virion-associated RNA polymerase

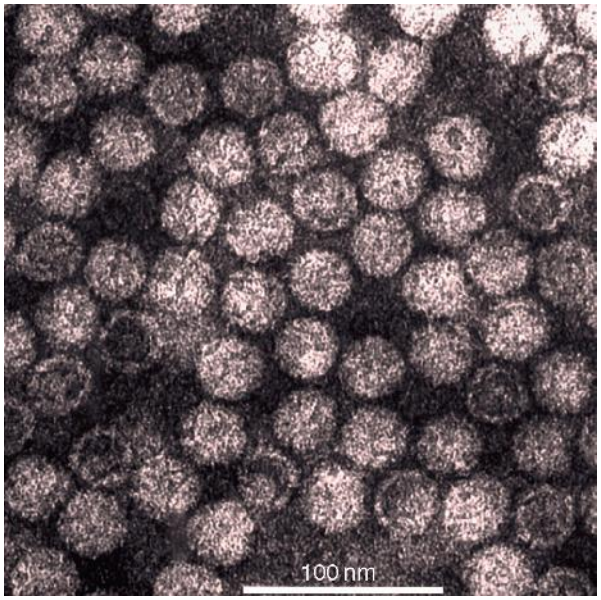


Figure 1 Electron micrograph of negatively stained virions of Rhizoctonia solani virus 717 (RhsV 717), a representative species of the genus *Partitivirus*. Adapted from Tavantzis SM and Bandy BP (1988) Properties of a mycovirus from *Rhizoctonia solani* and its virion-associated RNA polymerase. *Journal of General Virology* 69: 1465–1477, with permission from Society for General Microbiology.

activity is attributed to RdRp with sizes ranging from 77 to 86 kDa in fungal partitiviruses sequenced to date.

Antigenic Properties

Antisera to purified virus preparations appear to contain antibodies to the virion RdRp polypeptide. This argument is supported by two lines of evidence: (1) antiserum to the rhizoctonia solani virus 717 (RhsV 717) reacted to both virion proteins CP and RdRp and (2) antiserum to the aspergillus ochraceous virus (AoV) reacted to the AoV CP and RdRp as well as the RdRp of the 'slow' component (PsV-S) of penicillium stoloniferum virus complex. Unlike the respective CPs, the RdRp polypeptides of AoV and PsV-S share a high degree of similarity, and this is congruent with the phylogenetic analysis of CPs and RdRps of members of the genus *Partitivirus* which shows a higher rate of evolutionary changes occurring in the *CP* genes (**Figure 4** (**b**)) as compared to the rate in the *RdRp* genes (**Figure 4**(**a**)).

Genome Organization

Virions contain two separately encapsidated linear dsRNAs, dsRNA1 and dsRNA2, that are 1.5–2.4 kbp in size. The dsRNA components of a particular virus are of similar size (differing by 20–200 bp). Additional dsRNAs (satellite or defective) may be present in purified fungal partitivirus particles. The larger segment, dsRNA1, codes for an RdRp, whereas the smaller segment, dsRNA2, codes for a CP (**Table 3**). The 5′ ends of the coding strands of dsRNA1 and dsRNA2 are often highly conserved, and usually contain inverted repeats that may form stable stem–loop structures (**Figure 2**).

It has been hypothesized that partitiviruses may have evolved from totiviruses by dividing their genomes between two dsRNA segments. Like the totiviruses, the partitivirus genome consists of two genes (CP and RdRp) but, unlike the totiviruses, these genes are located on separate dsRNAs (bipartite genomes). In contrast to partitiviruses, many totiviruses express their RdRp as a CP–RdRp fusion protein. Totiviruses, such as the helminthosporium victoriae 190S virus (Hv190SV), that express RdRp as a separate nonfused protein might be evolutionary intermediates between ancestral totiviruses and partitiviruses.

Genome Expression and Virus Multiplication

As described above, the bisegmented genome of fungal partitiviruses consists of two dsRNA segments of similar

Table 3 Genome organization of the RhsV 717 partitivirus

Genome	*ORF*	*Frame*	*Nucleotide coordinates*	*Amino acids*	*Mol. mass (kDa)*	*Putative function*	*Kozak sequence*	*Inverted repeats*[a]
dsRNA1 (2363 bases)	1	2	86–2275	730	85.8	RdRp[b]	502–510	736–747
dsRNA2 (2206 bases)	1	1	79–2130	683	76.4	CP	None	None

[a]Inverted repeats longer than 12 bases.

[b]The Rhs 717 partitivirus RdRp (dsRNA 1,ORF 1) contains the dsRNA RdRp motifs according to Bruenn (1993). The residue coordinates of these motifs are as follows: Motif I, 242-LVTGT-246; Motif II, 315-FLKSFPTMM-323; Motif III, 363-ARKPECCIMYG-373; Motif IV, 397-IDWSGYDQRL-406; Motif V, 479-GVPSGMLLTQFLDSFGNLY-LII-500; Motif VI, 519-FIMGDDNSIF-528; Motif VII, 569-IETLSYRC-576; Motif VIII, 584-DVEK-587.

Reproduced from Strauss EE, Lakshman DK, and Tavantzis SM (2000) Molecular characterization of the genome of a partitivirus from the basidiomycete *Rhizoctonia solani*. *Journal of General Virology* 81: 549–555, with permission from Society for General Microbiology.

```
dsRNA1   – GUAGUCUUUUAGUAUCGAUCCCUCGACUCUCGACCGCACUAAAUCUCAUC   – 50
           : ::::::::::::::::::::::::::       ::::::::::::::
dsRNA2   – G-AGUCUUUUAGUAUCGAUCCCUCGACU- - - - -CGCACUAAAUCUCAUU   – 43

dsRNA1   – GUUAUUACGAACGAACUCUCUUCAAUCAACACACAAUGCUCUACAACUUC   – 100
           ::: :::::::: :::::: : :: :: :::: :
dsRNA2   – GUUUUUAAGAACAAACUCUCAACUCUCGCAACCUGAUGCCUUCGCCAAAG   – 93
```

Figure 2 Homology at the 5′ ends of the coding strands of the two genomic segments of the RhsV 717. If a 6-base gap is allowed in dsRNA2, the two dsRNAs are highly conserved. The identity drops to 65% around the region that includes the translation initiation sites. AUG codons are shown in bold letters and the CAA motifs (possibly involved in translational enhancement) are double-underlined. Identical positions are shown with colons beneath the sequence. The underlined bases are complementary and are capable of forming stable stem–loop structures. Adapted from Strauss EE, Lakshman DK, and Tavantzis SM (2000) Molecular characterization of the genome of a partitivirus from the basidiomycete *Rhizoctonia solani*. *Journal of General Virology* 81: 549–555, with permission from Society for General Microbiology.

size. The larger segment, dsRNA1, encodes an RdRp, whereas the smaller component, dsRNA2, encodes a CP. Northern blot analysis of purified virions as well as total RNA from partitivirus-infected mycelial tissue revealed no subgenomic RNA species in the form of discrete RNA bands with sizes smaller than that of full-length dsRNA1 or dsRNA2. This is consistent with analysis of the coding potential of these genomic RNAs, showing that the long open reading frames encoding the RdRp and CP polypeptides cover the entire length of dsRNA1 and dsRNA2, respectively.

Mycoviruses, including members of the genus *Partitivirus*, have been difficult to transfect using an inoculum consisting of purified virions. Thus, methods of synchronous viral infection and multiplication have not been available for studying partitivirus gene expression or virion replication *in vivo*. The recent transfection of the rosellinia necatrix virus W8 (RnV-W8) into protoplasts of its fungal host is a positive development with regard to unveiling the life cycle of fungal partitiviruses. Current knowledge of partitivirus gene expression, dsRNA replication, and virus multiplication is based on studies of RdRp activity in purified virus fractions, virus-related RNA content in infected mycelia, and *in vitro* translation studies of denatured dsRNA1 or dsRNA2 or full-length cDNA clones representing dsRNA1 or dsRNA2.

Populations of virions include particles of different $S_{20,w}$ values depending on their RNA content. Thus, purified virus preparations consist of mature virions (L1 or L2) containing a single molecule of dsRNA, virus particles with a single molecule of ssRNA corresponding to the plus-strand of the respective dsRNAs (M1, M2), a heterogeneous subpopulation of heavy (H) particles representing various replication stages and containing particles with a dsRNA genomic component and ssRNA tails or one molecule each of dsRNA and ssRNA; particles with two molecules of dsRNA (P) one of which is a product ('P') of replication (**Figure 3**). Data from *in vitro* RdRp activity studies in purified preparations of PsV-S and RhsV 717 are congruent with the replication scheme presented in **Figure 3**. However, experimental evidence for a number of the steps depicted in this replication scheme is currently lacking, and its verification awaits *in vivo* studies involving transfection of host mycelia with purified virions (or cDNAs representing dsRNAs 1 and 2) resulting in synchronous virus replication.

Phylogenetic Relationships among Fungal Partitiviruses

Phylogenetic analyses of the predicted amino acid sequences of fungal partitivirus RdRps and CPs (**Figures 4(a)** and **4(b)**, respectively) show that these viruses are closely related. Both analyses suggest that fungal partitiviruses form two clusters. The RdRp clusters have 100% bootstrap support (**Figure 4(a)**), whereas the CP clusters are also well supported (**Figure 4(b)**). These results are in agreement with

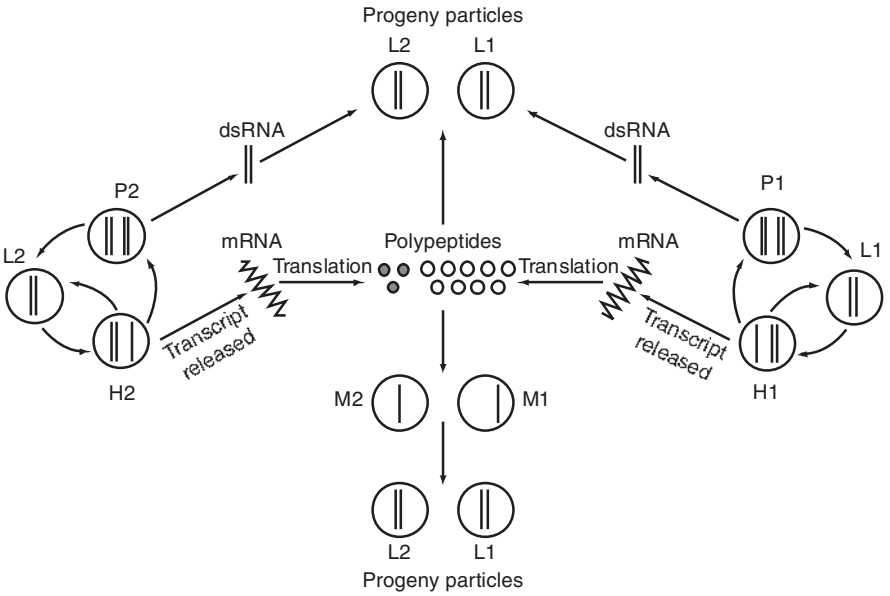


Figure 3 Model for replication of penicillium stoloniferum S virus (PsV-S). The open circles represent capsid protein subunits and the closed circles represent RNA polymerase subunits. Solid lines represent RNA strands whereas wavy lines represent mRNA. Adapted from Seventh Report of the International Committee on Taxonomy of Viruses, http://www.virustaxonomyonline.com/virtax/lpext.dll/vtax/agp-0013/dr06/dr06-fg/dr06-fig-0002, with permission from Elsevier.

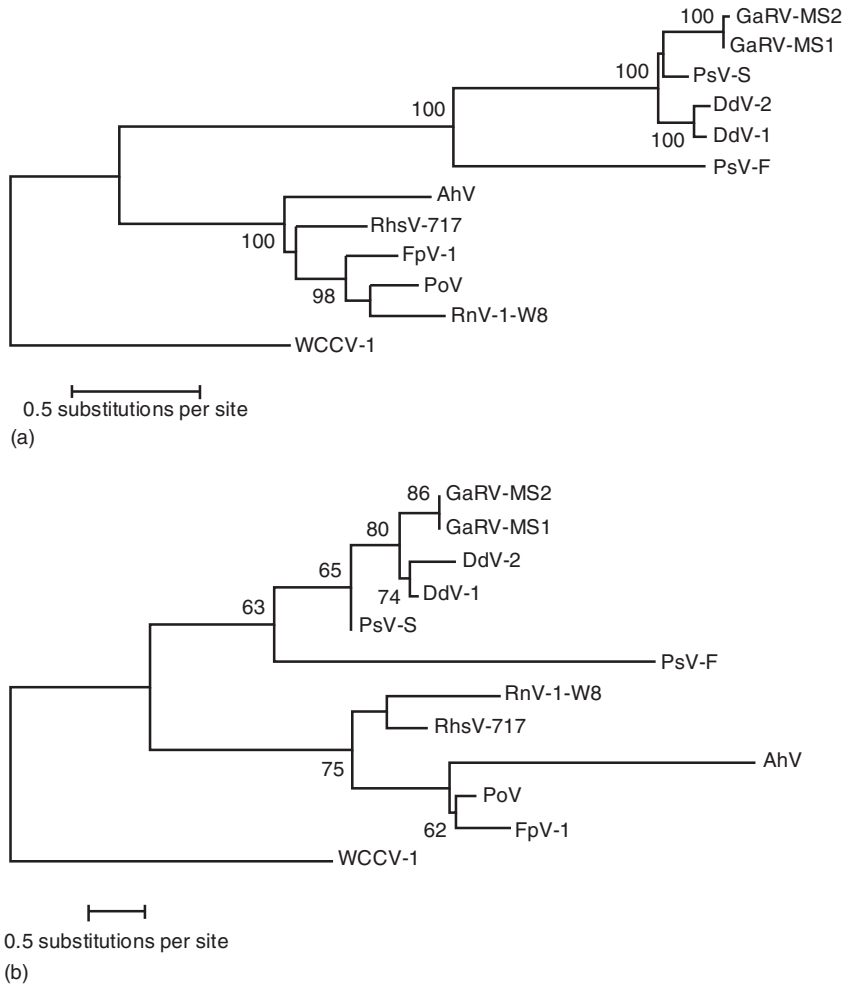


Figure 4 A neighbor-joining phylogram showing phylogenetic relationships among fungal partitivirus (a) RdRp and (b) CP amino acid sequences. The JTT model of amino acid evolution was applied in phylogenetic reconstruction using the software MEGA3.1. The tree was outgroup-rooted with the respective proteins of white clover cryptic virus 1 (WCCV-1), a plant partitivirus. Numbers above branches represent bootstrap support values and bar represents amino acid substitutions per site. See **Tables 1** and **2** for abbreviations of fungal partitivirus names.

recently reported phylogenetic analyses showing that the evolutionary rate of CPs is higher than that of the RdRps, and are in congruence with data suggesting a cross-serological relationship between the RdRps of AoV and PsV-S.

Transmission of Partitiviruses

Like most mycoviruses, partitiviruses may be transmitted by anastomosis between hyphae of genetically related fungal genotypes. Moreover, the ineffectiveness of hyphal tip isolation to eliminate partitivirus particles or dsRNA in conjunction with the detection of partitiviruses in apical hyphal sections by electron microscopy suggest that these viruses are transmitted vertically by sexual spores (basidiospores or rarely ascospores) as well as asexual spores (conidiospores) derived from virus-infected fruit bodies. The proportion of spores containing partitivirus dsRNAs varies widely even within the same species and apparently depends on the combination of fungal host and virus genotypes. For example, in different isolates of the basidiomycete *Heterobasidion annosum*, 3–55% of germinated conidia, and 10–84% of germinated basidiospores, contained partitivirus dsRNAs.

Partitivirus Pathogenesis

Partitiviruses occur in all parts of an infected mycelial mat, but virion concentrations are higher in the older hyphal sectors than in hyphal tips. This adaptation to

multiply mainly in areas of the fungal mycelium that are less involved in fungal growth might be one of the reasons that partitiviruses, although they replicate to relatively high levels, are usually associated with a symptomless infection of their respective fungal hosts. Recently, the latency of a partitivirus infection was demonstrated by direct experimental evidence in the case of ascomycete *Rosellinia necatrix* and the RnV-1-W8 partitivirus. Protoplasts of *R. necatrix* were transfected with purified particles of RnV-1-W8. The resulting mycelium contained transmissible RnV-1-V8 virions but showed no observable symptoms associated with the presence and replication of this virus. In contrast, in the *H. annosum* study (see previous section), germination frequency of basidiospores was significantly reduced ($P < 0.05$) by the presence of partitivirus dsRNA in the parental fruit bodies. Use of isogenic fungal lines may be needed to verify these results.

See also: Fungal Viruses; Chrysoviruses; Partitiviruses: General Features; Totiviruses.

Further Reading

Bruenn JA (1993) A closely related group of RNA-dependent RNA polymerases from double-stranded RNA viruses. *Nucleic Acids Research* 21: 5667–5669.

Ghabrial SA (1998) Origin, adaptation and evolutionary pathways of fungal viruses. *Virus Genes* 16: 119–131.

Ghabrial SA, Buck KW, Hillman BI, and Milne RG (2005) *Partitiviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 581–590. San Diego, CA: Elsevier Academic Press.

ICTVdB Management (2006) 00.049.0.01. Partitivirus. In: Büchen-Osmond C (ed.) *ICTVdB – The Universal Virus Database, version 4*, New York: Columbia University.

Kim JW, Choi EY, and Kim YT (2006) Intergeneric relationship between the aspergillus ochraceous virus F and the penicillium stoloniferum virus S. *Virus Research* 120: 212–215.

Strauss EE, Lakshman DK, and Tavantzis SM (2000) Molecular characterization of the genome of a partitivirus from the basidiomycete *Rhizoctonia solani*. *Journal of General Virology* 81: 549–555.

Tavantzis SM and Bandy BP (1988) Properties of a mycovirus from *Rhizoctonia solani* and its virion-associated RNA polymerase. *Journal of General Virology* 69: 1465–1477.

Partitiviruses: General Features

S A Ghabrial, University of Kentucky, Lexington, KY, USA
W F Ochoa and T S Baker, University of California, San Diego, La Jolla, CA, USA
M L Nibert, Harvard Medical School, Boston, MA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cryo-transmission electron microscopy Transmission electron microscopy of unstained, unfixed, frozen-hydrated (vitrified) specimens, preserved as close as possible to their native state.
Hyphal anastomosis The union of a hypha with another resulting in cytoplasmic exchange.
Icosahedral symmetry Arrangement of 60 identical objects or asymmetric units adopted by many isometric (spherical) viruses, with a combination of two-, three-, and fivefold rotations equivalently relating the units about a point in space.
Mycoviruses Viruses that infect and multiply in fungi.
'$T = 2$' symmetry The so-called 'forbidden' triangulation symmetry, in which 120 chemically identical protein monomers, form 60 identical, asymmetric dimers arranged with icosahedral symmetry. Monomers in a '$T = 2$' structure occupy two distinct, nonequivalent positions and therefore differ from the 'allowed' symmetries where monomers are either equivalently or quasi-equivalently related.
Virus capsid Generally a protective shell, composed of multiple copies each of one or more distinct protein subunits, that encapsidate the viral genome.

Introduction

In the early 1960s, interest in the antiviral activities associated with cultural filtrates of *Penicillium* spp. led to the discovery of double-stranded RNA (dsRNA) isometric viruses in these and other filamentous fungi. Fungal viruses, or mycoviruses, are now known to be of common occurrence. The isometric dsRNA viruses isolated from *Penicillium* spp. were among the first to be molecularly characterized and were shown to have segmented genomes. Those with bipartite genomes are currently classified in the genus *Partitivirus* in the family *Partitiviridae*. Interestingly, plant viruses (also called cryptoviruses) with

bipartite dsRNA genomes and very similar properties to the fungal partitiviruses were discovered in the late 1970s and are presently classified in the genera *Alphacryptovirus* and *Betacryptovirus* in the family *Partitiviridae*. The fungal partitiviruses and plant partitiviruses are discussed elsewhere in this encylopedia. The goals of this article are to examine the similarities and differences between these two groups of viruses and to discuss future perspectives of partitivirus research.

Biological Properties

Fungal and plant partitiviruses are both associated with latent infections of their respective hosts. There are no known natural vectors for any partitivirus. Fungal partitiviruses are transmitted intracellularly during cell division and sporogenesis (vertical transmission) as well as following hyphal anastomosis, that is, cell fusion, between compatible fungal strains (horizontal transmission). In some ascomycetes (e.g., *Gaeumannomyces graminis*), virus is usually eliminated during ascospore formation. Experimental transmission of fungal partitiviruses has been reported by transfecting fungal protoplasts with purified virions. The plant cryptoviruses, on the other hand, are not horizontally transmitted by grafting or other mechanical means, but are vertically transmitted by ovule and/or pollen to the seed embryo. Thus, whereas sexual reproduction of the host is required for the survival of plant partitiviruses, it is detrimental to the continued existence of the fungal partitiviruses that infect some ascomycetes. Transmission of fungal partitiviruses through asexual spores, however, can be highly efficient, with 90–100% of single conidial isolates having received the virus. In summary, transmission of fungal partitiviruses by asexual spores and plant partitiviruses by seed provide the primary or only means for disseminating these viruses.

Both fungal and plant partitiviruses are generally associated with symptomless infections of their hosts. While cryptoviruses are present in very low concentrations in plants (e.g., 200 μg of virions per kg of tissue for white clover cryptic virus), fungal partitiviruses can accumulate to very high concentrations (at least 1 mg of virions per g of mycelial tissue for penicillium stoloniferum virus F (PsV-F)). Mixed infections of fungal or plants hosts with two distinct partitiviruses are not rare. PsV-S and PsV-F represent one example in which two partitiviruses infect the same fungus, *Penicillium stoloniferum*. Interestingly, a significant increase in cryptovirus concentration has been observed in mixed infections with unrelated viruses belonging to other plant virus families.

Virion Properties

The buoyant densities of virions of members of the partitivirus family range from 1.34 to 1.39 gm cm ^{3}, and the sedimentation coefficients of these virions range from 101S to 145S (S_{20w} in Svedberg units). Generally, each virion contains only one of the two genomic dsRNA segments. However, with some viruses like the fungal partitivirus PsV-S, purified preparations can contain other distinctly sedimenting forms that include empty particles and replication intermediates (see the next section).

All fungal and plant partitiviruses examined to date have been shown to possess virion-associated RNA-dependent RNA polymerase (RdRp) activity, which catalyzes the synthesis of single-stranded RNA (ssRNA) copies of the positive strand of each of the genomic dsRNA molecules. The *in vitro* transcription reaction occurs by a semi-conservative mechanism, whereby the released ssRNA represents the displaced positive strand of the parental dsRNA molecule and the newly synthesized positive strand is retained as part of the duplex.

Genome Organization and Replication

Virions of members of the partitivirus family contain two unrelated segments of dsRNA, in the size range of 1.4–2.3 kbp, one encoding the capsid protein (CP) and the other encoding the RdRp. The two segments are usually of similar size and are encapsidated separately, that is, each particle generally contains only one dsRNA segment. The genomes of at least 16 members of the genus *Partitivirus* have recently been completely sequenced (**Table 1**). In contrast, the complete genome sequences of only three alphacryptoviruses and no betacryptoviruses have yet been determined (**Table 1**). The genomic structure of Atkinsonella hypoxylon virus (AhV-1), the type species of the genus *Partitivirus*, comprising segment 1 (2180 bp, encoding the RdRp) and segment 2 (2135 bp, encoding the CP), is schematically represented in **Figure 1**.

The presence of one or more additional dsRNA segments is common among members of the family *Partitiviridae*. For example, in addition to the two genomic segments, preparations of AhV-1 contain a third dsRNA segment of 1790 bp. With the exception of the termini, this third segment is also unrelated to the other two. The absence of any long open reading frame (ORF) on either strand of segment 3 of AhV-1 suggests that it is a satellite segment, not required for replication. Satellite dsRNAs are often associated with infections by members of the family *Partitviridae* (**Table 1**).

Limited information on how fungal viruses in the genus *Partitivirus* replicate their dsRNAs is derived from *in vitro* studies of virion-associated RdRp and the isolation from naturally infected mycelium of particles that represent various stages in the replication cycle. The RdRp is believed to function as both a transcriptase and a replicase. The transcriptase activity within an assembled virion catalyzes the synthesis of progeny positive-strand

Table 1 List of viruses in the family *Partitiviridae* with sequenced genomic dsRNAs

Virus[a]	*Abbreviation*	*dsRNA segment no. (size in bp; encoded protein, size in kDa)*	*GenBank accession no.*
Genus: *Partitivirus*			
*Atkinsonella hypoxylon virus**	AhV	1 (2180; RdRp, 78)	L39125
		2 (2135; CP, 74)	L39126
		3 (1790; satellite)	L39127
Ceratocystis resinifera virus	CrV	1 (2207; RdRp, 77)	AY603052
		2 (2305; CP, 73)	AY603051
*Discula destructiva virus 1**	DdV1	1 (1787; RdRp, 62)	NC_002797
		2 (1585; CP, 48)	NC_002800
		3 (1181; satellite)	NC_002801
		4 (308; satellite)	NC_002802
*Discula destructiva virus 2**	DdV2	1 (1781; RdRp, 62)	NC_003710
		2 (1611; CP, 50)	NC_003711
*Fusarium poae virus 1**	FpV-1	1 (2203; RdRp, 78)	NC_003884
		2 (2185; CP, 70)	NC_003883
*Fusarium solani virus 1**	FsV-1	1 (1645; RdRp, 60)	D55668
		2 (1445; CP, 44)	D55669
*Gremmeniella abietina virus MS1**	GaV-MS1	1 (1782; RdRp, 61)	NC_004018
		2 (1586; CP, 47)	NC_004019
		3 (1186; satellite)	NC_004020
*Helicobasidium mompa virus**	HmV	V1–1 (2247; RdRp, 83)	AB110979
		V1–2 (1776; RdRp, 63)	AB110980
		V-70 (1928; RdRp, 70)	AB025903
*Heterobasidion annosum virus**	HaV	1 (2325; RdRp, 87)	AF473549
Ophiostoma partitivirus 1	OPV-1	1 (1744; RdRp, 63)	AM087202
		2 (1567; CP, 46)	AM087203
Oyster mushroom virus	OMV	1 (2038; RdRp, 70)	AY308801
Penicillium stoloniferum virus F*	PsV-F	1 (1677; RdRp, 62)	NC_007221
		2 (1500; CP, 47)	NC_007222
		3 (677; satellite)	NC_007223
*Penicillium stoloniferum virus S**	PsV-S	1 (1754; RdRp, 62)	NC_005976
		2 (1582; CP, 47)	NC_005977
Pleurotus ostreatus virus	PoV	1 (2296; RdRp, 82)	NC_006961
		2 (2223; CP, 71)	NC_006960
*Rhizoctonia solani virus 717**	RhsV-717	1 (2363; RdRp, 86)	NC_003801
		2 (2206; CP, 76)	NC_003802
Rosellinia necatrix virus 1	RnV-1	1 (2299; RdRp, 84)	NC_007537
		2 (2279; CP, 77)	NC_007538
Genus: *Alphacryptovirus*			
*Beet cryptic virus 3**	BCV-3	2 (1607; RdRp, 55)	S63913
*Vicia cryptic virus**	VCV	1 (2012; RdRp, 73)	NC_007241
		2 (1779; CP, 54)	NC_007242
*White clover cryptic virus 1**	WCCV-1	1 (1955; RdRp, 73)	NC_006275
		2 (1708; CP, 54)	NC_006276
Unclassified viruses in the family *Partitiviridae*			
Cherry chlorotic rusty spot associated partitivirus	CCRSAPV	1 (2021; RdRp, 73)	NC_006442
		2 (1841; CP, 55)	NC_006443
Fragaria chiloensis cryptic virus	FCCV	1 (1743; RdRp, 56)	DQ093961
Pyrus pyrifolia cryptic virus	PpV	1 (1592; RdRp, 55)	AB012616
Raphanus sativus cryptic virus 1 (or Radish yellow edge virus*)	RasV-1 (RYEV)	1 (1866; RdRp, 67)	NC_008191
		2 (1791; CP, 56)	NC_008190
Raphanus sativus cryptic virus 2	RasV-2	1 (1717; RdRp, 55)	DQ218036
		2 (1521; unknown)	DQ218037
		3 (1485; unknown)	DQ218038

[a]An asterisk next to the virus name indicates it is presently recognized by ICTV as a member or a tentative member in the family *Partitiviridae*. Family members or tentative members that have not been sequenced to date are not included.

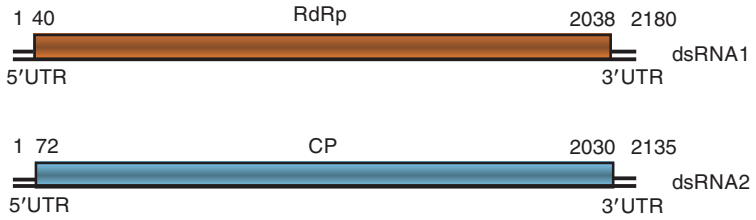


Figure 1 Genome organization of atkinsonella hypxylon virus (AhV), the type species of the genus *Partitivirus*. dsRNA1 contains the RdRp ORF (nt positions 40–2038) and dsRNA2 codes for the CP ORF (nt positions of 72–2030). The RdRp and CP ORFs are represented by rectangular boxes. Reproduced from Ghabrial SA, Buck KW, Hillman BI, and Milne RG (2005) *Partitiviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 581–590. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

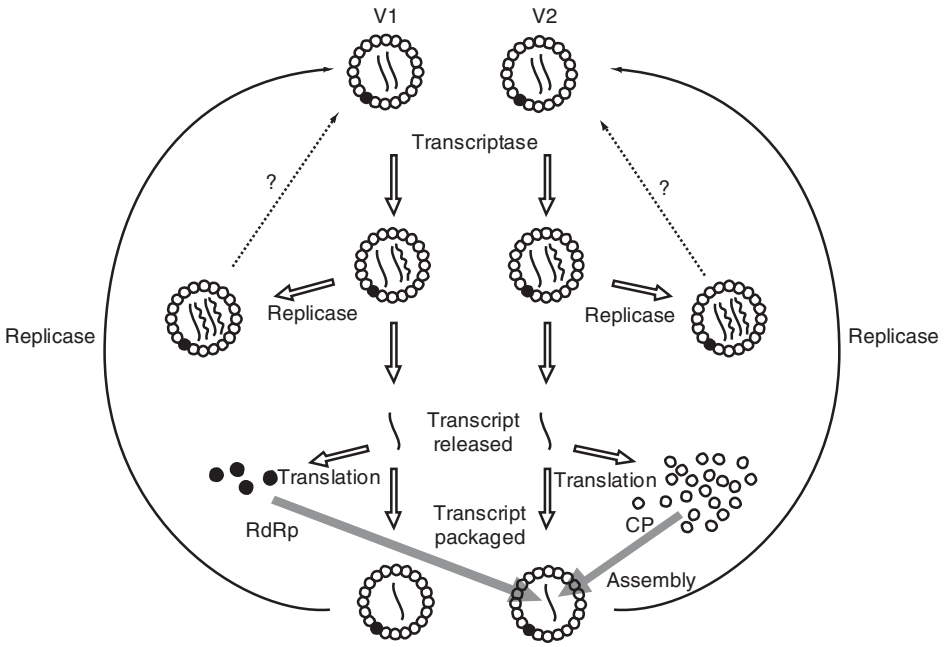


Figure 2 Model for replication of penicillium stoloniferum virus S (PsV-S). The open circles represent capsid protein (CP) subunits and the closed circles represent RNA-dependent RNA polymerase (RdRp) subunits. Solid lines represent parental RNA strands whereas wavy lines represent newly synthesized progeny RNA strands. Reproduced from Ghabrial SA and Hillman BI (1999) Partitiviruses-fungal (*Partitiviridae*). In: Granoff A and Webster RG (eds.) *Encyclopedia of Virology*, 2nd edn., pp. 1477–1151. San Diego: Academic Press, with permission from Elsevier.

RNA from the parental dsRNA template, accompanied by the displacement of the parental positive strand and its release from the virion. This can presumably occur through repeated rounds, giving rise to multiple positive-strand copies. Each released positive strand can then be consecutively or alternatively (1) used as a template for protein (CP or RdRp) translation by the host machinery or (2) packaged by CP and RdRp into an assembling progeny virion. The replicase activity within this assembling virion then catalyzes the synthesis of negative-strand RNA on the positive-strand template, reconstituting a genomic dsRNA segment. The replication of plant partitiviruses is presumed to mimic that described for the fungal viruses.

Partially purified virion preparations are known to include a small proportion of particles that contain only one ssRNA molecule corresponding to the genomic positive strand. These may be particles in which the replicase reaction is defective or has not yet occurred. In addition, partially purified virion preparations are known to contain a relatively large proportion of a heterogeneous population of particles more dense than the mature virions. These dense particles may represent various stages in the replication cycle including particles containing

the individual genomic dsRNAs with ssRNA tails of varying lengths, particles with one molecule of dsRNA and one molecule of its ssRNA transcript and particles with two molecules of dsRNA (**Figure 2**).

Structures of Partitivirus Virions

The isometric dsRNA mycoviruses are classified within the families *Totiviridae*, *Chrysoviridae*, and *Partitiviridae*. The capsid structures of representative members of each of the first two families have been determined, at least one at near atomic resolution using X-ray crystallography, and the others at low to moderate resolutions (~1.5–2.5 nm) using cryo-transmission electron microscopy (cryo-TEM) combined with three-dimensional (3D) image reconstruction. We have recently initiated systematic cryo-TEM and image reconstruction studies of three viruses, PsV-S, PsV-F, and FpV-1, within the genus *Partitivirus*. Based on phylogenetic analysis of partitivirus CPs or RdRps, PsV-S, and PsV-F form sister clades in the same cluster, whereas FpV-1 is placed in a separate and distinct cluster within this genus (see section on 'Taxonomic and phylogenetic considerations'). These viruses are structurally distinguished primarily on the basis of significant differences in the sizes of the respective CPs and percent nucleotide and amino acid sequence identity of their RdRps and CPs. Structures of partitviruses from the genera *Alphacrypto-* and *Betacryptovirus*, all of which are plant viruses, have yet to be reported.

Preliminary studies of PsV-S, PsV-F, and FpV-1 strongly suggest that all members of the genus *Partitivirus* share a number of common features. The structure of PsV-S is used to highlight these features, and significant differences are identified below.

PsV-S represents one of the simplest of the dsRNA viruses. Each virion comprises of one of the two genome segments (S1, 1754 bp and S2, 1582 bp), one or two copies of the 62 kDa RdRp, and 120 copies of the 47 kDa CP. Virions exhibit an overall spherical morphology in negative stain or in vitrified solution (**Figure 3**). Reports of virion diameters based on measurements from stained specimens likely underestimate the true virion size, owing to distortions and other potential artifacts associated with the imaging of negatively stained specimens. Based on cryo-TEM images, the maximum diameter of fully hydrated PsV-S virions is 35 nm. 3D reconstruction of the PsV-S structure at ~21 Å resolution demonstrates that the capsid is a contiguous shell (average thickness ~2 nm) of subunits arranged with icosahedral symmetry (**Figure 4**(**a**)). The external surface of PsV-S displays 60 prominent protrusions that rise approximately 3 nm above an otherwise relatively featureless, spherical shell. Each protrusion and a portion of the underlying shell, constitutes one asymmetric unit (1/60th) of the capsid. The dimeric morphology of each protrusion is

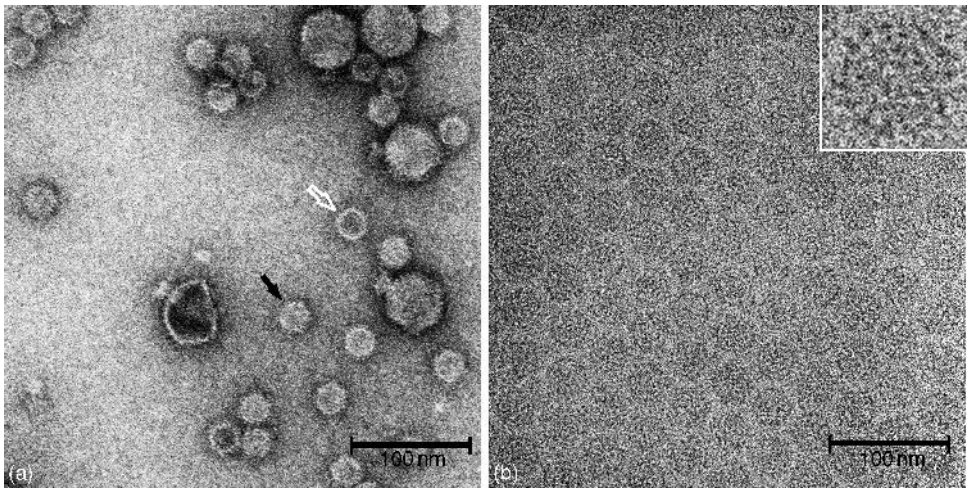


Figure 3 Electron micrographs of penicillium stoloniferum virus S (PsV-S). Samples were negatively stained in 2% uranyl acetate (a) or prepared unstained and vitrified (b). Micrographs were recorded on a CCD detector in an FEI Polara transmission electron microscope operated at 200 keV with samples at (a) room or (b) liquid nitrogen temperatures. In (a) bacteriophage P22 (five largest particles) was mixed with PsV-S to serve as a calibration reference. Heavy metal stain surrounds virions (e.g., black arrow) and contrasts their surfaces against the background carbon support film. Stain penetrates into the interior of 'empty' capsids (e.g., white arrow), resulting in particle images in which only a thin, annular shell of stain-excluding material (capsid) is seen. In (b) the unstained PsV-S sample was vitrified in liquid ethane. Here particles appear dark (higher density) against a lighter background of surrounding water (lower density). The inset shows a three-times enlarged view of an individual particle, in which several knobby surface features are clearly visible.

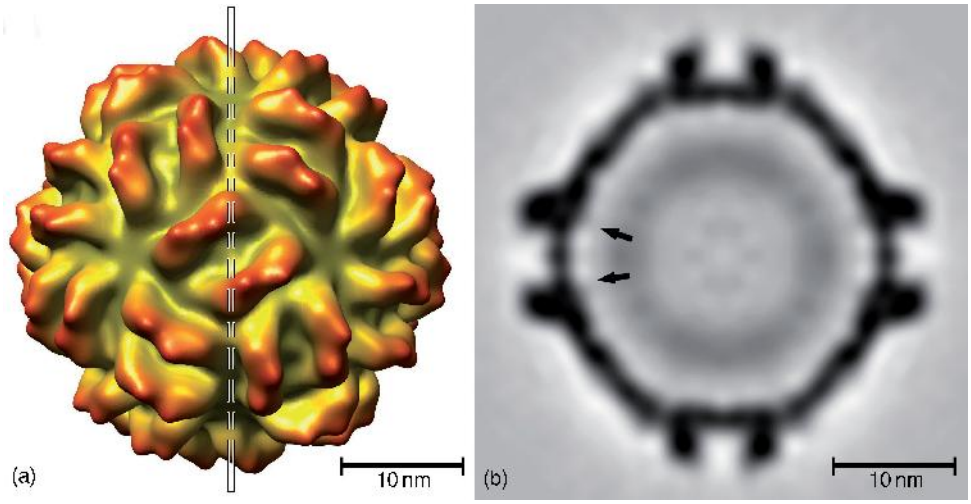


Figure 4 Three-dimensional (3D) structure of PsV-S. (a) Shaded, surface representation of PsV-S 3D reconstruction viewed along an icosahedral twofold axes. The 3D map is color-coded to emphasize the radial extent of different features (yellows and greens highlight features closest to the particle center, and oranges and reds those farthest from the center). A total of 60 prominent protrusions extend radially outward from the capsid surface. Each protrusion exhibits an approximate dyad symmetry, which is consistent with the expectation that the partitivirus capsid consists of 120 capsid protein monomers, organized as 60 asymmetric dimers in a so-called 'T=2' lattice. (b) Density projection image of a central, planar section through the PsV-S 3D reconstruction (from region marked by dashed box in (a)). Darker shades of gray correspond to higher electron densities in the map section and lighter shades represent low-density features such as water outside as well as inside the particles. The capsid shell appears darkest because it contains a closely packed, highly ordered (icosahedral) arrangement of capsid subunits. The genomic dsRNA on the inside appears at lower density, in part because the RNA is not as densely packed and in part because the RNA adopts a less ordered arrangement. The protrusions seen in (a) appear as large 'bumps' in the central section view that decorate the outside of a contiguous, ~2 nm thick, shell. Arrows point to faint density features that appear to form contacts between the inner surface of the protein capsid and the underlying RNA. These contacts occur close to the fivefold axes of the icosahedral shell.

consistent with an asymmetric unit being composed of two CP monomers. Hence, there are 120 protein subunits arranged as 60 asymmetric dimers packed in a so-called '$T = 2$' ('forbidden') lattice, which requires that the monomers do not interact equivalently or quasiequivalently as often occurs, especially in smaller, simpler virus capsids (e.g., with triangulation symmetries such as $T = 1, 3, 4$).

An additional, symmetric ball of weak density within the capsid is attributed to the genomic dsRNA, and does not appear to adopt any regular structure at the limited resolution of this initial 3D reconstruction (**Figure 4**(**b**)). Lack of detectable genome organization may, in part, be attributed to averaging effects that occur when images of both PsV-S particle types are combined to produce the reconstructed 3D density map. Potential interactions between the genome and the inner wall of the capsid are suggested by faint lines of density observed in thin, planar sections through the density map (arrows; **Figure 4**(**b**)).

PsV-F and PsV-S co-infect and are co-purified from the fungal host, *Penicillium stoloniferum*. The coat protein of PsV-F (420 aa; 47 kDa) is very similar in size to that of PsV-S, but they only share 17% sequence identity. PsV-F virions are ~37 nm in diameter and the gross morphology and $T = 2$ organization of the capsid is remarkably similar to that of PsV-S (not shown). However, the 60 protrusions in PsV-F are narrower, they extend further above the shell (~6.5 nm), and their long axes are rotationally aligned ~18° in a more anti-clockwise orientation compared to those in PsV-S.

Fusarium poae virus (FpV-1) also exhibits a '$T = 2$' arrangement of its more massive coat protein subunits (637 aa; 70 kDa) (not shown). These subunits assemble to form ~42 nm diameter virions, significantly larger than both PsV-S and PsV-F. The average thickness of the FpV-1 shell varies considerably and is much less uniform than the shells of the smaller partitiviruses. The dimeric protrusions in FpV-1 extend ~3 nm above the capsid shell and have a wide base that appears to form more extensive interactions or connections to the underlying shell compared with the other two partitviruses. Central sections through a low-resolution density map of FpV-1 exhibit three concentric layers of weak density, with a 3 nm spacing consistent with a relative close packing of nucleic acid as found in other dsRNA virus capsids.

Current studies are aimed at obtaining higher-resolution 3D reconstructions for each of the fungal partitiviruses mentioned above. In the absence of any crystallographic structure determinations, higher-resolution cryo-TEM reconstructions should enable more detailed comparisons to be made, and potentially provide a means to locate the

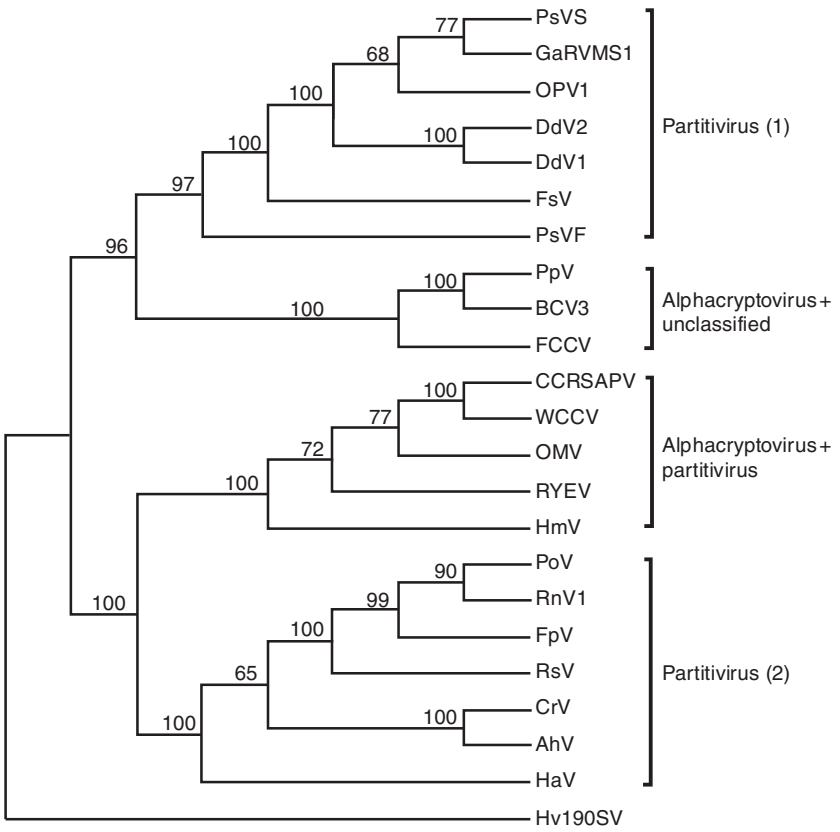


Figure 5 Phylogenetic analysis of the RdRp conserved motifs and flanking sequences derived from aligned, deduced amino acid sequences of members of the family *Partitiviridae* using the program CLUSTAL X. Motifs 3 through 8 and the sequences between the motifs, as previously designated by Jiang and Ghabrial (*Journal of General Virology* 85, 2111–2121, 2004) were used. See **Table 1** for virus name abbreviations. Bootstrap numbers out of 1000 replicates are indicated at the nodes. The tree was rooted with the RdRp of Helminthosporium victoriae 190S virus (Hv190SV), a member of the genus *Totivirus* in the family *Totiviridae* (GenBank accession no. NC_003607), which was included as an outgroup.

RdRps that are presumed to be fixed inside the capsid shell and possibly associated with a specific recognition site on each of the separately encapsidated genomic dsRNA segments. Such studies will also allow evolutionary links to the '$T=2$' cores of other well-studied dsRNA viruses that infect both fungal and nonfungal hosts to be explored. Structural studies expanded to include members of the genera *Alphacrypto-* and *Betacryptovirus* also have the potential to systematically characterize the similarities as well as differences in the life cycles of fungal and plant partitiviruses.

Taxonomic and Phylogenetic Considerations

Recent phylogenetic analyses based on amino acid sequences of RdRps (conserved motifs) of members of the family *Partitiviridae* led to the identification of four clusters, two of which are large and comprise only members of the genus *Partitivirus* (**Figure 5**). One large cluster with strong bootstrap support includes the partitiviruses DdV1, DdV2, FsV, GaV-MS1, OPV, and PsV-S (see **Table 1** for virus abbreviations). The CPs of these viruses share significant amino acid sequence identities (34–62%), but less than the RdRps (55–70% identity), suggesting that the CPs have evolved at a faster rate. The second partitivirus RdRp cluster consists of AhV, CrV, FpV, PoV, RhsV, and RnV1. These two large clusters were proposed to comprise two subgroups (subgroups 1 and 2) of the genus *Partitivirus* (**Figure 5**). Interestingly, the CPs of these two subgroups differ significantly in size with average sizes of 47 and 74 kDa for subgroups 1 and 2, respectively. Of the remaining two RdRp clusters (**Figure 5**), one consists of BCV (genus *Alphacryptovirus*) and two other, unclassified plant cryptoviruses (FCCV and PpV). The fourth cluster consists of a mixture of plant viruses (WCCV and RYEV, genus *Alphacryptovirus*) and fungal viruses (OMV and HmV, genus *Partitivirus*), together with CCRSAV, which may be either a fungal or a plant virus, as yet to be determined. This raises the interesting possibility of horizontal transfer of members of the

family *Partitiviridae* between fungi and plants. This is a reasonable possibility because some of the viruses in these clusters have fungal hosts that are pathogenic to plants. Based on our current knowledge of partitviruses, the taxonomy of the family *Partitiviridae* will probably need to be reconsidered. As additional molecular and structural information from a wider range of plant and fungal partitiviruses is gathered, the need for new or revised taxonomic classifications may become even more apparent.

Future Perspectives

Development of Infectivity Assays for Fungal Partitiviruses

The recent success of using purified RnV-1 virions to infect virus-free isolates of the fungal host *Rosellinia necatrix* is very promising since there is considerable need to advance our knowledge of the biology of fungal partitiviruses (e.g., host-range, virus–host interaction, and molecular basis of latent infection). The natural host range of partitiviruses is restricted to the same or closely related vegetative compatibility groups that allow lateral transmission. At present, there are no known experimental host ranges for fungal partitiviruses because suitable infectivity assays have yet to be developed. With the success of the *Rosellinia necatrix*-RnV-1 infectivity assays, it is expected that future research would explore the potential experimental host range of RnV-1 including other fungal species in the genus *Rosellinia* and related genera. It is also anticipated that comparable infectivity assays using purified virions would be developed for other fungal partitiviruses. Because of the possibility alluded to earlier, of horizontal transfer of partitiviruses between fungi and plants, it is interesting to test the infectivity, using fungal protoplasts, of plant partitiviruses such as the alphacryptoviruses WCCV-1 and RYEV, which are known to be more closely related to certain fungal partitiviruses (e.g., OMV, HaV, and HmV) than to other plant partitiviruses. Alternatively, it would likewise be interesting to explore the infectivity of fungal partitiviruses such as OMV, HaV, and HmV to appropriate plant protoplasts.

Fungal partitiviruses are known to be associated with latent infections of their natural hosts. But, conceivably, some partitiviruses may induce phenotypic changes and/or virulence attenuation in one or more of their experimental host fungi. If true, this would provide excellent opportunities for exploiting fungal partitiviruses for biological control and for basic studies on host–pathogen interactions. Furthermore, transfection assays using fungal protoplasts electroporated with full-length, *in vitro* transcripts of cloned cDNA to viral dsRNAs should also be applicable to partitiviruses. This would allow for the development of partitivirus-based vectors for expressing heterologous proteins in fungi. Because many partitiviruses are known to support the replication and encapsidation of satellite dsRNA, this property would facilitate the construction of recombinant vectors. Genes of interest could be inserted into the satellite molecule between the conserved termini that are presumed to be required for replication and encapsidation. Considering the high level of partitivirus accumulation in their fungal hosts, the use of partitiviral vectors provides an attractive and cost-effective means for the overproduction of valuable proteins in filamentous fungi.

Reconsideration of the Taxonomy of the Family *Partitiviridae*

There is an urgent need to characterize at the molecular level, a broad range of plant partitiviruses that should include representatives of the genus *Betacryptovirus*. Taxonomic considerations of partitiviruses may benefit greatly from elucidating the capsid structure of representative members of each of the four clusters delineated by phylogenetic analysis. It may, however, require concerted efforts to generate purified virions of alphacryptoviruses and betacryptoviruses in quantities needed for structural studies since they generally occur at very low concentrations in their hosts.

See also: Alphacryptovirus and *Betacryptovirus*; Fungal Viruses; Partitiviruses of Fungi.

Further Reading

Antoniw JF (2002) *Alphacryptovirus* (*Partitiviridae*). In: Tidona CA and Darai G (eds.) *The Springer Index of Viruses*, pp. 676–679. New York: Springer.

Antoniw JF (2002) *Betacryptovirus* (*Partitiviridae*). In: Tidona CA and Darai G (eds.) *The Springer Index of Viruses*, pp. 680–681. New York: Springer.

Crawford LJ, Osman TAM, Booy FP, *et al.* (2006) Molecular characterization of a partitivirus from ophiostoma himal-ulmi. *Virus Genes* 33: 33–39.

Ghabrial SA (2001) Fungal viruses. In: Maloy O and Murray T (eds.) *Encyclopedia of Plant Pathology,* pp. 478–483. New York: Wiley.

Ghabrial SA (2002) *Partitivirus* (*Partitiviridae*). In: Tidona CA and Darai G (eds.) *The Springer Index of Viruses*, pp. 685–688. New York: Springer.

Ghabrial SA and Hillman BI (1999) Partitiviruses-fungal (*Partitiviridae*). In: Granoff A and Webster RG (eds.) *Encyclopedia of Virology*, 2nd edn., pp. 1477–1151. San Diego: Academic Press.

Ghabrial SA, Buck KW, Hillman BI, and Milne RG (2005) *Partitiviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 581–590. San Diego, CA: Elsevier Academic Press.

Sasaki A, Kanematsu S, Onoue M, Oyama Y, and Yoshida K (2006) Infection of *Rosellinia necatrix* with purified viral particles of a member of *Partitiviridae* (RnPV1-W8). *Archives of Virology* 151: 697–707.

Parvoviruses of Arthropods

M Bergoin, Université Montpellier II, Montpellier, France
P Tijssen, Université du Québec, Laval, QC, Canada

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Densonucleosis virus Original name of densovirus.
Densovirus Insect parvovirus.

History

The discovery of invertebrate parvoviruses goes back to the early 1960s when an epizootic decimated a mass rearing of the greater wax moth, *Galleria mellonella*, larvae reared as fishing bait on the French side of Lake of Geneva. Electron microscopic examination of thin sections from diseased larvae revealed hypertrophied nuclei containing an electron-dense viroplasm with thousands of tiny viral particles. After purification, these particles were observed as small isometric, nonenveloped virions, 22–25 nm in diameter, and their biochemical analysis showed that they contained single-stranded DNA. The virus causing the disease was designated as densonucleosis virus, which later was simplified to the name densovirus (DNV). Diseases presenting the same type of histopathological features, and caused by the same type of viruses, were later described not only in Lepidoptera but also in other insect orders and in the Decapoda phylum of Arthropoda with the discovery of DNV infections in crabs and shrimps. The cloning and sequencing of several DNV genomes during the last two decades, while confirming their relatedness to the vertebrate parvoviruses, revealed an extraordinary diversity, reflecting very likely the evolutionary diversity of their hosts. Presently, parvoviruses of arthopods are recognized as *bona fide* members of the family *Parvoviridae* in which they form the subfamily *Densovirinae*.

Classification of DNVs

The family *Parvoviridae* musters all viruses that contain a linear, single-stranded DNA genome and has been subdivided into two subfamilies. Vertebrate parvoviruses are classified into the subfamily *Parvovirinae*, whereas the *Densovirinae* groups all parvoviruses from arthropods. Consequently, arthropod parvoviruses are usually named DNVs. Vertebrate and arthropod parvoviruses share hallmark features such as a $T=1$ capsid structure, usually possess a structural protein with a phospholipase A2 (PLA2) motif, and contain similar-sized genomes of 4–6 kb that replicate by self-priming and hairpin transfer. However, there is little sequence identity, except for a few enzyme motifs, between the viruses of these subfamilies.

Another isometric, single-stranded DNA virus, bombyx mori densovirus type 2 (BmDNV-2), has been isolated from the silkworm. Despite its morphological similarity with DNVs, the genome of BmDNV-2 is much larger (about 12.5 kb), segmented, and contains, in contrast to parvoviruses, its own DNA polymerase motif. Because of these differences, the ICTV has rejected its inclusion in the *Densovirinae* and the naming of this virus as a DNV is unfortunate.

The grouping of the DNVs into genera is based on a limited number of viruses and becomes strained with an expanding number of characterized viruses and will probably undergo a continuous revision. So far, molecular characterization of DNVs allowed their classification into at least four genera: *Densovirus*, *Iteravirus*, *Pefudensovirus*, and *Brevidensovirus*. Many DNVs remain unclassified since only roughly half of the *c.* 30 reported DNV isolates are sufficiently characterized with respect to their molecular biology. On the other hand, many more DNVs remain to be isolated from the million-odd insect species.

DNV genera are currently distinguished in the ICTV classification according to the genome organization by the following criteria: (1) monosense versus ambisense; (2) presence or absence of inverted terminal repeats (ITRs); (3) split or single open reading frame (ORF) coding for the capsid proteins. Sequence identities may be very low despite overall similarity in genome organization and expression strategies. The members of the genus *Densovirus* have an ambisense genome, organized with cassettes for VP (structural proteins) and NS (nonstructural or Rep proteins) on the 5′ halves of the complementary strands and with long ITRs (*c.* 550 nt). Examples are the junonia coenia densovirus (JcDNV) and galleria mellonella densovirus (GmDNV). The viruses in the genus *Iteravirus* have a monosense 5 kb genome with *c.* 230 nt ITRs (hence the name of the genus) and are so far only found in the order Lepidoptera. Examples are the casphalia extranea densovirus (CeDNV) and bombyx mori densovirus type 1 (BmDNV-1). The members of the genus *Brevidensovirus* have a 4 kb genome with a monosense organization and terminal hairpins but lacking ITRs.

Dedicated to the memory of Alan Simpson, who, under the supervision of Prof. Michael Rossmann at Purdue University, solved the first densovirus structure by X-ray crystallography. Alan, 38, sadly drowned after getting into difficulties while on a solo kayaking trip in Scotland on March 2007.

The members of this genus are found in Diptera, such as *Aedes aegypti* and *Aedes albopictus*, but at least some DNVs from shrimps, such as infectious hypodermal and hematopoietic necrosis virus (IHHNV), are closely related. The VPs of the members of both genera *Iteravirus* and *Brevidensovirus* are coded from a single ORF.

Previously, all ambisense DNVs isolated from different insect orders were classified into the single genus *Densovirus* making this genus quite heterogeneous. Presently, we distinguish the DNV members into three subgroups (A, B, and C; see **Table 1**) with increased uniformity. Subgroup A contains the prototypical DNV genus members such as GmDNV and JcDNV. Although the viruses in this genus have high sequence identities, they have different ecological niches. The ORF for VP of subgroup B members is split and requires splicing of the ORFs for expression of the largest VP. Moreover, their genomes are shorter (5.5 kb), and their ITRs have an I- instead of a Y-shape. Although subgroup B members share a common genome organization, that is distinct from subgroup A members, they have very low sequence identities (10–30%) among each other and with the subgroup A members. One of them, periplaneta fuliginosa densovirus (PfDNV), was reclassified in the genus *Pefudensovirus* in the latest ICTV report, but not enough is known about the expression strategies at the molecular level of other members of this subgroup for co- or separate classification. The ITRs of PfDNV, acheta domesticus densovirus (AdDNV), and blatella germanica densovirus (BgDNV) of this subgroup vary greatly in size (125–215 nt) and these viruses have been isolated from Orthoptera, Hemiptera, and Dictyoptera but not from Lepidoptera as the prototypical *Densovirus* members. So far, there is a single member in subgroup C, culex pipiens densovirus (CpDNV), from the mosquito *Culex pipiens*, that uses, in addition to two promoters on the strand encoding NS polypeptides, a complex splicing in the NS genes.

Table 1 Names of viruses, their abbreviations, hosts and accession numbers

Ambisense densoviruses			
Subgroup A densoviruses (genus *Densovirus*, 6 kb genome with 0.55 kb ITRs, single ORF for VP)			
Junonia coenia densovirus (JcDNV)	*Junonia coenia*	Lepidoptera	NC_004284
Galleria mellonella densovirus (GmDNV)	*Galleria mellonella*	Lepidoptera	NC_004286
Diatraea saccharalis densovirus (DsDNV)	*Diatraea saccharalis*	Lepidoptera	NC_001899
Helicoverpa armigera densovirus (HaDNV)	*Helicoverpa armigera*	Lepidoptera	Sequenced, not submitted
Mythimna loreyi densovirus (MlDNV)	*Mythimna loreyi*	Lepidoptera	NC_005341
Pseudoplusia includens densovirus (PiDNV)	*Pseudoplusia includens*	Lepidoptera	Sequenced, not submitted
Subgroup B densoviruses (5.5 kb genome with 0.2 kb ITRs, split ORFs for VP; PfDNV is classified in a new genus, *Pefudensovirus*, while others remain to be classified)			
Acheta domesticus densovirus (AdDNV)	*Acheta domesticus*	Orthoptera	Dictyoptera NC_005041
Blattella germanica densovirus (BgDNV)	*Blattella germanica*	Dictyoptera	
Myzus persicae densovirus (MpDNV)	*Myzus persicae*	Hemiptera	NC_005040
Planococcus citri densovirus (PcDNV)	*Planococcus citri*	Hemiptera	NC_004289
Periplaneta fuliginosa densovirus (PfDNV)	*Periplaneta fuliginosa*	Dictyoptera	AB028936
Subgroup C densoviruses (6 kb genome with 0.3 kb ITRs, split ORFs for VP and NS)			
Culex pipiens densovirus (CpDNV)	*Culex pipiens*	Diptera	Sequenced, not submitted
Monosense densoviruses			
Genus *Iteravirus* (5 kb genome, 0.25 kb ITRs)			
Casphalia extranea densovirus (CeDNV)	*Casphalia extranea*	Lepidoptera	AF375296
Bombyx mori densovirus (BmDNV-1)	*Bombyx mori*	Lepidoptera	AY033435
Dendrolimus punctatus (DpDNV-1)	*Dendrolimus punctatus*	Lepidoptera	NC_006555
Genus *Brevidensovirus* (4 kb genome, no ITRs but terminal hairpins, no phospholipase A2 motif in VP)			
Aedes aegypti densovirus (AaeDNV)	*Aedes aegypti*	Diptera	
Aedes albopictus densovirus (AalDNV-1)	C6/36 cell line	Diptera	NC_004285
Aedes albopictus densovirus (AalDNV-2)	C6/36 cell line	Diptera	AY095351
Penaeus stylirostris densovirus (PstDNV)	*Penaeus stylirostris*	Decapoda	AF273215
Unassigned (6.3 kb, no ITRs but hairpins, no phospholipase A2 motif in VP)			
Penaeus monodon densovirus (PmoDNV)	*Penaeus monodon*	Decapoda	DQ002873
Noncharacterized densoviruses			
Agraulis vanillae densovirus (AvDNV)	*Agraulis vanillae*	Lepidoptera	
Euxoa auxilliaris densovirus (EaDNV)	*Euxoa auxilliaris*	Lepidoptera	
Lymantria dispar densovirus (LdiDNV)	LPC Ld-D52 cell line	Lepidoptera	
Pieris rapae densovirus (PrDNV)	*Pieris rapae*	Lepidoptera	
Sibine fusca densovirus (SfDNV)	*Sibine fusca*	Lepidoptera	
Haemagogus equinus densovirus (HeDNV)	GML-HE-12 cell lines	Diptera	
Toxorhynchites amboinensis densovirus (TaDNV)	TRA-171 cell lines	Diptera	
Simulium vittatum densovirus (SvDNV)	*Simulium vittatum*	Diptera	
Leucorrhinia dubia densovirus (LduDNV)	*Leucorrhinia dubia*	Odonata	

Access number for the most recently complete DNA sequences are indicated.

Biophysical and Structural Features of Virions

All the capsids of DNVs are built on the same $T=1$ model with 60 structural proteins but the relative number of constituent polypeptides involved in virus assembly varies from one genus to another. The virions of the members of the genus *Densovirus* contain four clearly distinct polypeptide bands upon sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), ranging in size between 45 and 110 kDa and with varying relative amounts. These structural proteins were shown to be N-extended isoforms. As exemplified by BmDNV-1 and CeDNV, iteravirus particles contain five structural proteins, which are also N-extended isoforms, with molecular masses between 48 and 76 kDa. Both the capsids of the members of the genera *Iteravirus* and *Densovirus* possess, like virtually all vertebrate parvoviruses, phospholipase A2 activity. In contrast, the viruses of the third genus, *Brevidensovirus*, do not display phospholipase A2 activity and have only two structural proteins (N-extended isoforms) of about 40 kDa. The brevidensoviruses have all been isolated from mosquitoes and shrimps.

The virions of the various DNVs share with those of the vertebrate parvoviruses a high buoyant density in CsCl of *c.* 1.40–1.44 g cm^{-3}, linked to a high nucleic acid content (about 20–30%), and sedimentation coefficients of about 110S. GmDNV, JcDNV, and BmDNV-1 particles were shown to contain polyamines (spermine, spermidine, and putrescine) that could partially neutralize the viral DNA, whereas so far no reports of polyamines in vertebrate parvoviruses have been published.

The near-atomic structure of the particle of GmDNV was solved by X-ray crystallography and revealed some striking differences with the structure of vertebrate parvoviruses (**Figures 1(a)** and **1(b)**). The β-barrel of the GmDNV VP4 capsid protein must be rotated and radially translated to superimpose it on the β-barrel of the capsid protein of canine parvovirus (CPV). Another difference with the vertebrate parvoviruses was the absence of loop 4 in GmDNV capsid protein, resulting in a β-annulus-type structure instead of a spike around the threefold axes. The βA strand folds back to its own subunit in vertebrate parvovirus structures thus far solved. In contrast, in GmDNV, the βA strand is linearly extended across the twofold axes, allowing it to hydrogen bond with the βB strand of the neighboring subunit ('swapping domain'). Overall, the outside of the GmDNV capsid is much smoother than that of vertebrate parvoviruses, perhaps as a result of a different evolutionary pressure (**Figure 1(a)**).

The three-dimensional (3-D) structure of aedes albopictus densovirus type 2 (AalDNV-2), a member of the genus *Brevidensovirus*, has been solved to a resolution of about 16 Å by cryoelectron microscopy. The sequence identity of the structural proteins of this virus with those of GmDNV is less than 20% which is translated into some distinctive structural features. For example, AalDNV-2 particles display density ridges around the threefold axes and prominent protusions at the fivefold axes that are both absent in the GmDNV particle. Nevertheless, the twofold proximal depression as well as the inner surfaces and large cavities under the fivefold vertices are conserved. Similarly, inner surfaces were most conserved among vertebrate parvoviruses.

Finally, X-ray diffraction data recently collected will allow solving of both the IHHNV shrimp parvovirus and the BmDNV-1 atomic structure to about 3 Å in the near

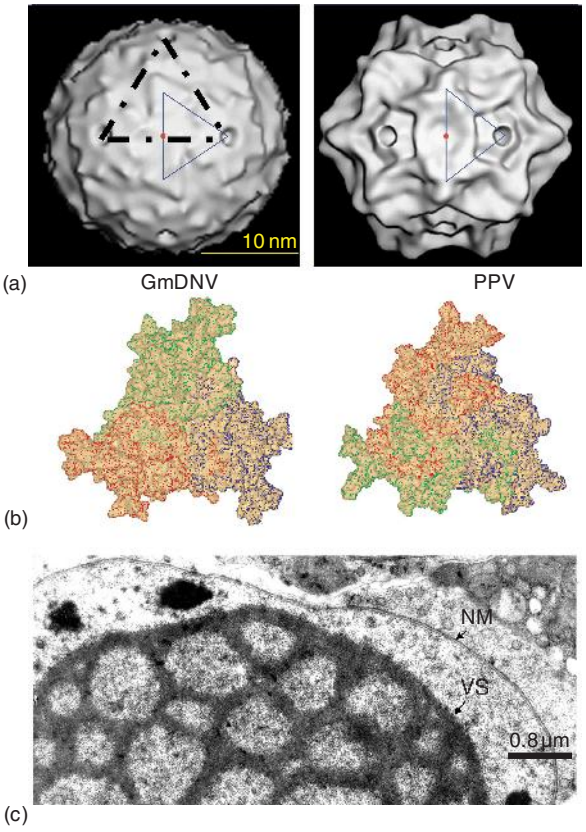


Figure 1 Comparative surface topology (a) and capsid protein interactions (b) between GmDNV and PPV (Porcine parvovirus) as representatives of subfamilies *Densovirinae* and *Parvovirinae*. The large triangle (broken line) connecting three fivefold axes represents the size of a trimer (surface representation in (b)) and the small triangle the approximate size occupied by a single structural protein. The most conserved sequences/structures are on the inside face of the trimers, whereas the outside faces, as shown here by the GmDNV and PPV trimers, differ considerably in structure sequence and protein interactions. (c) Thin section through the nucleus (NM, nuclear membrane) of a heavily infected *Sibine fusca* midgut cell. The viral particles assemble from a reticulated electron-dense virogenic stroma (VS). Photo J. L. Duthoit.

future. Interestingly, the IHHNV capsid seems to lack both the A and G β-strands that are involved, in the case of GmDNV, in inter-subunit interactions.

Host Range and Pathology

As illustrated in **Figure 1(c)**, the hypertrophy of infected nuclei where thousands of viral particles are produced from electron-dense virogenic stroma is the striking histopathological feature in all DNV infections.

Most of the DNVs isolated so far are known to be highly pathogenic and fatal for their natural host and to be responsible for epizootics in natural populations of noxious or commercially reared insects. Examples of hosts are phytophagous members of the Lepidoptera or the Orthoptera, scavengers belonging to the Dictyoptera, and vectors like aphids and mosquitoes. Unfortunately, they also appeared to be highly pathogenic in mass-reared arthropods, such as the silkworm *Bombyx mori* or shrimps, causing serious economic losses.

The host range of the different DNVs varies considerably. Some, like GmDNV, are monospecific, whereas others, such as the closely related mythimna loreyi densovirus (MlDNV) or JcDNV, infect a large range of different hosts. Similarly, PfDNV infects many different members of the genus *Periplaneta*, whereas AdDNV infects readily the European cricket but not the American cricket. BmDNV-1 infects certain strains of *B. mori* whereas other strains are resistant while BmDNV-2 shows the opposite tropism. The mosquito DNVs of the genus *Brevidensovirus* seem all to have a wide host range.

The symptoms of viral infection vary from host to host. Some DNVs, such as GmDNV and JcDNV, infect all tissues except the midgut. Others, such as BmDNV-1, SfDNV, and CeDNV, infect only the midgut, whereas PfDNV infects the hindgut and shows a hypertrophied fat body. Not surprisingly, external signs of infection vary greatly. Larvae infected with a member of the DNV genus become lethargic and anorexic and become progressively whitish and paralyzed, followed by a slow melanization. Moulting and metamorphosis is also inhibited. Infection of the susceptible silkworms with BmDNV-1 also leads to flaccidity and is usually fatal within a week. Infection of slug caterpillar pests of the oil palm, *Casphalia extranea* and *Sibine fusca* (Lepidoptera *Limacodidae*), with their respective DNVs induces intensive proliferation and progressive thickening and opacity of the midgut wall, leading to tumor-like lesions. The *Casphalia* larvae become anorexic and their color changes from green to yellowish brown after infection. Infection of the cockroach, *Periplaneta fuliginosa*, with PfDNV leads to paralysis of the hind legs and their movements become uncoordinated. Females are particularly affected by PfDNV; the abdomen is swollen due to the hypertrophied fat body, which is milky white in contrast to the brownish tissues in uninfected specimens. The most striking feature of the infection of European crickets with AdDNV is their decreasing ability to jump and they end up lying motionless on their back. The most obvious pathological change was seen in the gut. Infected females always had less food in the gut, and especially striking was the completely empty digestive ceca. They usually die within 10 days. Infection of *Aedes aegypti* larvae with DNV led to paralysis and their bodies were distorted and curved. They usually hung near the water surface.

Potential Use of DNVs for Biological Control of Insect Pests

Despite their high virulence for their natural hosts, their usually limited host range, and their lack of pathogenicity for vertebrates, DNVs have so far not received the attention they deserve for their potential in controlling economically and medically important insects. Indeed, attempts to use SfDNV and CeDNV to control outbreaks of their host, *C. extranea* and *S. fusca*, two major pests of oil palm industrial plantations in Côte d'Ivoire and Columbia, respectively, were very successful. Mortalities above 90% were recorded 2 weeks after a single airplane spraying over hundreds of hectares with a suspension containing 5–100 heavily infected larval homogenate per hectare. Similarly, a commercial formulation (Viroden) of AeDNV was used for the control of *A. aegypti* larvae in different areas of the former Soviet Union and a PfDNV formulation is presently produced in China for the control of cockroaches. The homologies found in some specific regions of the NS and VP genes between DNV and vertebrate parvovirus genomes raised a concern about safety for the use of DNVs as biopesticides. However, a number of data indicate that DNVs can be safely used. These include (1) the complete lack of pathological symptoms in mice and rabbits inoculated with milligrams of different types of infectious DNVs used to raise antisera; (2) the lack of pathogenicity and normal development of suckling mice following intracranian inoculation of GmDNV, AaeDNV, and AalDNV, and (3) the inability of GmDNV and MlDNV genomes to replicate in vertebrate cells. These data are certainly good arguments for the safety toward animals and humans of DNVs, but their use as pesticides will require more studies before approval by safety agencies.

Genome Organization and Expression Strategies

The overall structure and organization of coding sequences of members in the different genera of DNVs is depicted in **Figure 2**. DNVs have a small, compact

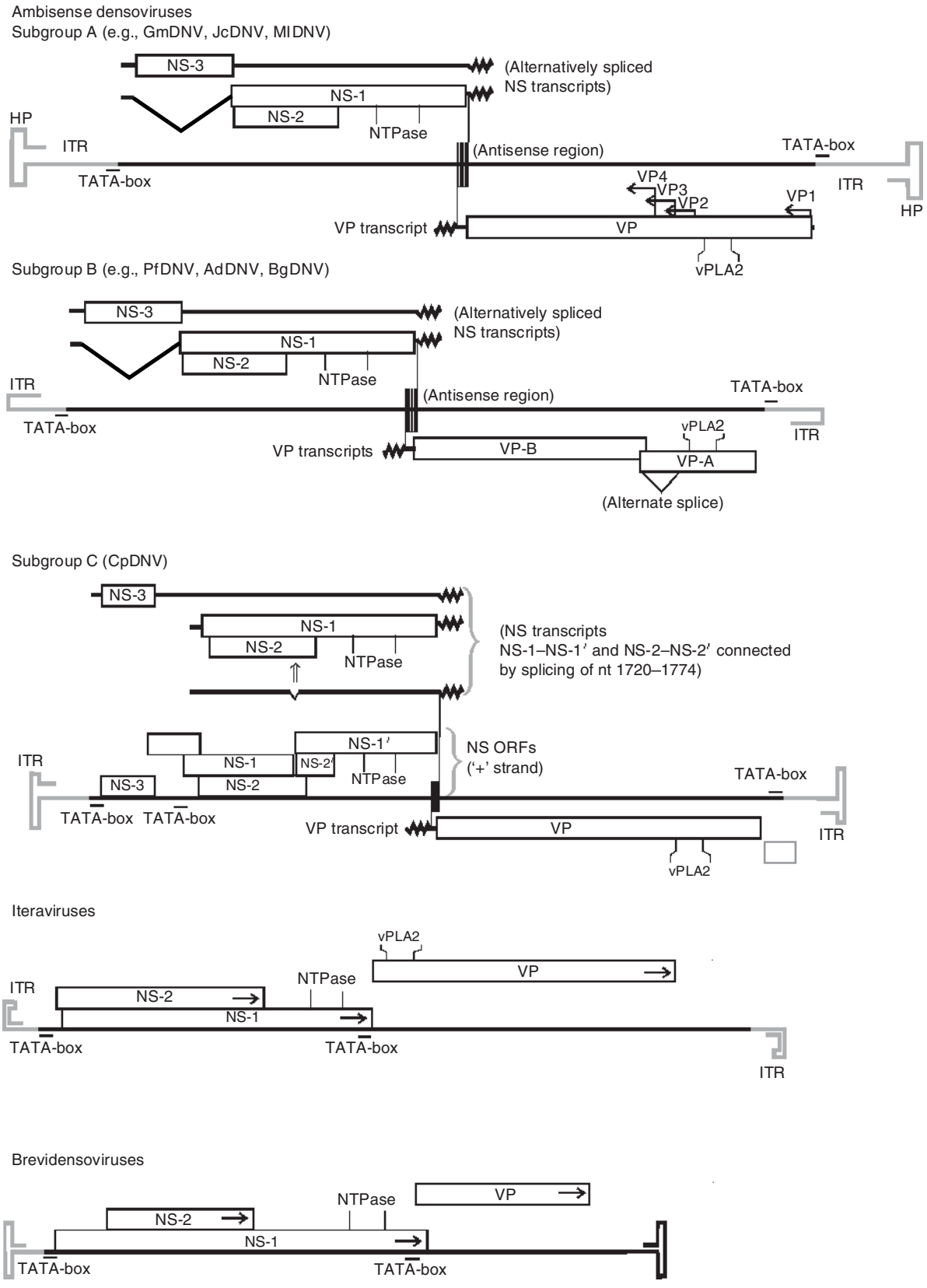


Figure 2 Continued

genome with limited coding capacities but well-developed complex strategies of alternative splicing and leaky scanning mechanisms for regulation of the expression of sets of overlapping genes in a timely and coordinated quantitative fashion. Leaky scanning expression strategy is widely used in invertebrate parvoviruses; it dictates the generation of N-terminal extended isoforms of the structural proteins from the same reading frame or of alternative nonstructural proteins from different reading frames. Leaky scanning of DNV mRNAs has not yet been studied in detail but is remarkable in that it often results in the coding of four to five isoforms of their structural proteins with scanning often exceeding 1000 nt for the most downstream initiation AUG. It is not clear whether this process includes ribosome shunting (discontinuous ribosomal scanning).

Another widely used expression strategy of DNVs to express their NS proteins NS-1 and NS-2 is the presence of two overlapping ORFs which have their initiation codons separated by a few nucleotides (usually four). These initiation codons, of which the 5′-proximal AUG is usually in a less favorable context, are thus completely covered by the footprint of about 13–15 nt of the small ribosomal subunit.

The VP cassette can be recognized by PLA2 motif for all parvoviruses except for those belonging to the genera *Amdovirus* and *Brevidensovirus*. This motif consists of a GPG calcium-binding site followed after about 18 amino acids by the HDxxY catalytic site motif (**Figure 3**).

Although DNVs from other genera have two NS proteins, NS-1 and NS-2, most ambisense DNVs have a third NS protein (NS-3). Sequence identity of NS-3 with proteins from non-parvoviruses (# AY293731 from ClGV granulosis virus and S78547 from BmDNV-2) suggests a horizontal transmission of this gene early in evolution of the ambisense DNVs. So far, the knowledge of expression strategies of DNVs is limited to those with an ambisense genome.

DNVs with an Ambisense Genome

Subgroup A

The subgroup A DNV members have 6 kb genomes with long ITRs of *c.* 550 nt representing about 20% of the viral genome (**Figure 2**). The terminal ~130 nt of the ITRs can be folded into typical Y-shaped hairpins occurring in two orientations 'flip' and its reverse complement 'flop', as described for vertebrate parvoviruses of the genus *Dependovirus*. Transcript mapping revealed that the rest of the ITRs contain the TATA-boxes at the border of the unique sequence and upstream promoter sequences for the NS and VP gene cassettes, respectively. These genomes have about 80–90% sequence identity and their proteins show strong serological cross-reactivities. Their structural proteins are N-terminally extended isoforms of 45, 53, 58, and 89 kDa synthesized by a leaky scanning mechanism from an unspliced 2.6 kb transcript. Their nonstructural proteins are synthesized from two species of mRNAs. An unspliced 2.5 kb transcript is translated from its 5′-most proximal initiation codon into NS-3, a 20 kDa protein, whereas the precise excision of NS-3 coding sequence from the 2.5 kb transcript gives rise to a spliced 1.8 kb mRNA which is translated into NS-1 (60 kDa) and NS-2 (30 kDa) by alternative initiation at the tandem initiation codons of NS-1/-2 cassette. In this subgroup, ORFs of the NS and VP genes are separated by *c.* 30 nt only. As a consequence, the NS and VP transcripts of GmDNV, JcDNV, and MlDNV have a terminal antisense sequence of about 60 nt.

Subgroup B

So far all ambisense DNVs of subgroup B have shorter genomes than those of subgroup A, about 5.5 kb, and use splicing and leaky scanning to generate the structural proteins. The ITRs are about 125–215 nt long, and occupy only about 7% of the total genome length. Their folding by self-complementarity generates I-shaped hairpin structures. As for subgroup A, the 3′ extremities of ITRs of subgroup B DNVs contain the TATA-boxes and their upstream promoter elements for both the NS and VP genes. The precise mapping of AdDNV NS transcripts revealed a great similarity with the transcription pattern of subgroup A genomes with an unspliced 2.5 kb and a spliced 1.8 kb transcript for NS-3 and NS-1–NS-2 translation, respectively. In contrast, expression of VP gene combines a complex splicing/frameshift strategy with leaky scanning

Figure 2 Schematic representation of the structure and expression strategies of DNVs. Two main groups can be distinguished, the ambisense DNVs with thus far three subgroups, and the monosense DNVs with thus far two genera, the iteraviruses and the brevidensoviruses. The genomes are represented with their 5′ and 3′ hairpin termini, whereas transcripts from left to right (NS coding strand) are depicted above the genome and transcripts from right to left (VP coding strand) below the genome. The positions of splicing, as so far known, is shown by a V (thin line) in or below the transcripts. Members of subgroups A and B produce two NS transcripts, one unspliced that yields NS-3 and one that undergoes splicing to remove NS-3 and allows translation of both NS-1 and NS-2 by leaky scanning. Whereas VPs of subgroup A viruses are produced by leaky scanning from an unspliced mRNA, the VPs of subgroup B viruses are coded by two ORFs that need to be connected in frame. For subgroup C, the position of NS ORFs is shown for both the primary transcripts as well as the mature spliced mRNAs. The two NS transcripts are not generated by splicing, as for subgroups A and B, but by using two distinct promoters. Although the large VP ORF for this virus is preceded by a small ORF, VPs are only generated from the large ORF by leaky scanning. Transcript mapping is still lacking for the iteraviruses but genome analysis indicates that NS and VP use different promoters and that no splicing is used. Brevidensoviruses use essentially the same strategy.

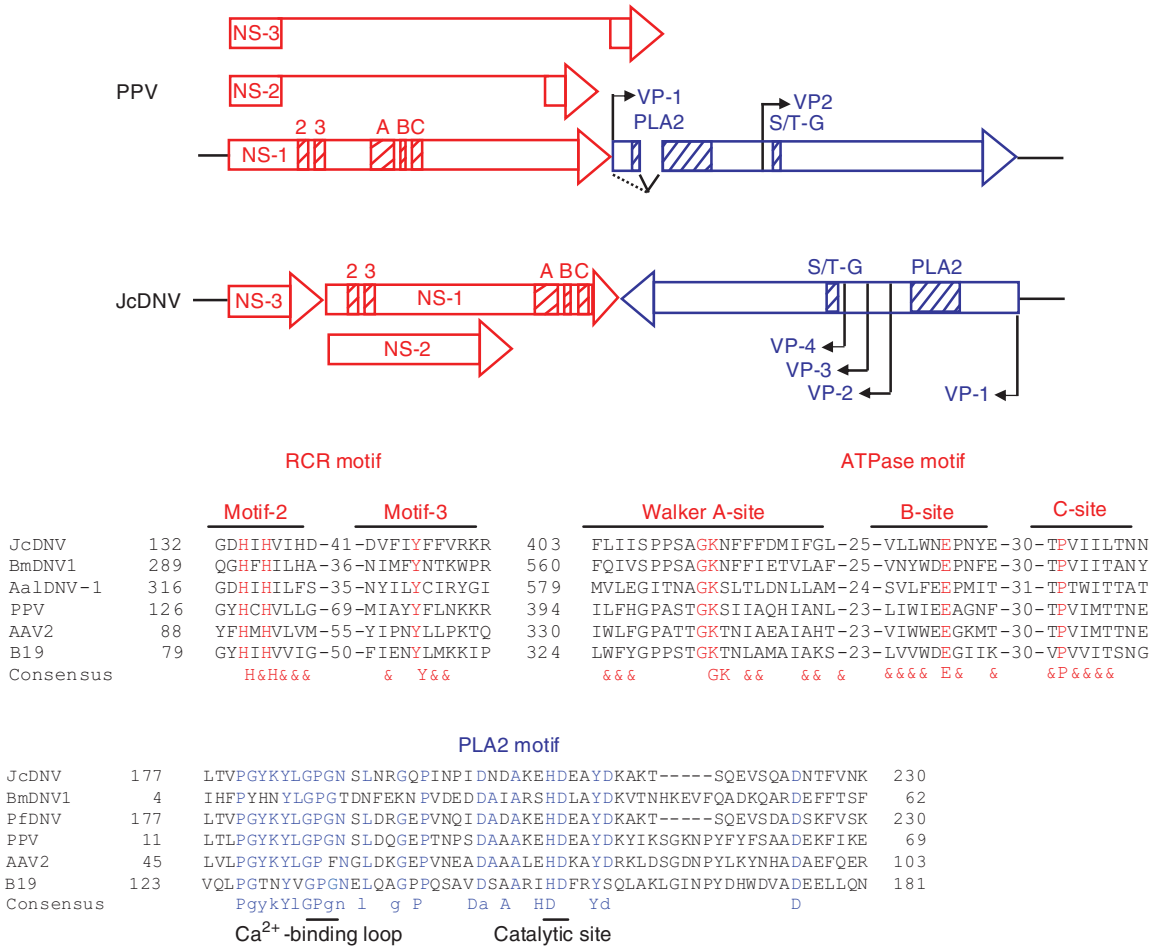


Figure 3 Conserved sequence motifs in viral proteins of vertebrate and invertebrate parvoviruses. The most-conserved motifs between members of the subfamilies *Parvovirinae* and *Densovirinae* are the rolling-circle replication (RCR) and ATPase (helicase) motifs of NS-1 and phospholipase A2 (PLA2) motifs of VP1. Consensus sequences are indicated below the motifs and represent bulky, hydrophobic amino acids (M, V, L, F, Y, V, W).

to generate four isoforms of the structural proteins. Surprisingly, splicing does not remove an initiation codon or put the two ORFs in frame, its most common objective in parvoviruses, but removes the sequences required for frameshifting. The VP and NS ORFs are separated by 3 nt for BgDNV, 15 nt for panonychus citri densovirus (PcDNV), 24 nt for AdDNV, and 30 nt for PfDNV. The overlap of NS and VP AdDNV transcripts generates a 32 antisense sequence.

Subgroup C

With a 6 kbp genome and *c.* 400 nt ITRs, able by folding to form an asymmetric T-shaped structure, the CpDNV is the only representative of this subgroup. The analysis of the unique sequence of CpDNV genome (unpublished) revealed several striking features in the strand encoding NS proteins. These features include both the organization of coding sequences and transcription modalities of the genome, and clearly differentiate CpDNV genome from those of subgroup A and B members. Indeed, instead of being in the same frame and separated from NS-1 coding sequence by a single stop codon, as in the other subgroups, NS-3 is in another frame and separated from NS-1–NS-2 tandem sequences by a 256 nt gap. Furthermore, the NS-1–NS-2 coding sequences are not continuous but split into four ORFs: two upstream encompassing the N-terminal and two downstream encompassing the C-terminal moieties, respectively (see **Figure 2**). Finally, expression of NS genes is not regulated by a single promoter as in subgroups A and B but by two. One promoter with a TATA-box located at the limit of the left ITR generates a 2.3 kb transcript that is used for NS-3 translation, whereas a second promoter located in the gap between NS-3 and NS-1 5′-most ORF drives expression of NS-1 and NS-2 proteins through a 1.7 kb transcript. A consequence of this organization is that by removing a

short intron (53 nt) the N- and C-terminal sequences of both NS-1 and NS-2 are spliced in frame. It is not yet clear whether this small intron serves a particular role or that removal of corresponding intron sequence in the virus genome would be without consequence. The three VP proteins of CpDNV capsid (85, 64, and 57 kDa) are generated by leaky scanning from an unspliced 2.2 kb transcript. As for members of subgroups A and B, the overlap of NS and VP transcripts generates a 14 nt antisense sequence. Taken together, the properties of CpDNV genome justify, in our opinion, the creation of an additional genus for DNVs with an ambisense genome.

DNVs with a Monosense Genome

Members of two genera of DNVs, *Iteravirus* and *Brevidensovirus*, share a genomic organization similar to that of vertebrate parvoviruses by having their sequences coding for NS and VP proteins located on the same strand. They possess only two overlapping NS ORFs encoding NS-1 and NS-2 (NS-3 ORF is lacking) and a single VP ORF located in the 5′ half and 3′ half of the genome, respectively (**Figure 2**). In addition to their host range, iteraviruses differ from brevidensoviruses by their genome size and structure of the genome termini.

Genus Iteravirus

This genus is presently represented by only three viruses – BmDNV-1, CeDNV, and dendrolimus punctatus densovirus (DpDNV) – all isolated from Lepidoptera. Owing to its deleterious impact on silkworm production, BmDNV-1 was discovered early and has been studied in detail, particularly in Japan. Successful genetic selection of silkworm strains resistant to this virus has been achieved. The structure of the 5 kb genome is highly conserved among the three iteravirus representatives. They possess ITRs *c.* 230 nt in length, the first 159 of which are able to fold into a typical J-shaped terminal hairpin occurring in two flip/flop orientations.

The expression of iteraviruses has not yet been studied by transcript mapping but two putative TATA-boxes, one upstream from NS-1 ORF the other overlapping its 3′ extremity, and polyadenylation signals have been identified. Furthermore, the ORFs in BmDNV, CeDNV, and DpDNV are in identical positions and share 80–90% homologies, suggesting that they represent actual genes. The putative initiation codons for both CeDNV, DpDNV, and BmDNV NS-1 and NS-2 are all in a favorable context. As previously shown for BmDNV-1 by peptide mapping, and more recently for CeDNV by expression in a baculovirus system, the five structural proteins of iteravirus capsid are five N-terminal extended isoforms of VP generated by leaky scanning.

Genus Brevidensovirus

With the shortest (4 kb) genomes among parvoviruses, the lack of ITRS, and a natural host range encompassing several species of medically important mosquitoes (*Aedes* and *Anopheles* species) and of economically important shrimp species (*Paeneus stylirostris*), brevidensoviruses constitute an original group of invertebrate parvoviruses. Recently, noninfectious IHHNV-related sequences were reported in the genome of *Penaeus monodon*. The fact that larval stages of most mosquitoes are aquatic and that many species can afford to develop in salty waters support the hypothesis of a common ancestor for mosquito and shrimp DNVs. The aedes aegypti densovirus (AaeDNV) and aedes albopictus densovirus type 1 (AalDNV-1) from cell line C6/36 are the prototypes. AalDNV strains have been isolated from several laboratories around the world handling C6/36 or other mosquito cell lines.

All the genomes of brevidensoviruses so far sequenced have terminal hairpins able to fold into perfect T-shaped structures of *c.* 60 nt, but with different sequences at 5′ and 3′ extremities. Their unique sequences are very divergent from the other DNVs, and like members of the genus *Amdovirus* (Aleutian disease virus) of vertebrate parvoviruses their VP ORF does not contain the PLA2 motif. This may be one of the reasons why the small (*c.* 1.2 kb) VP ORF codes for only two structural proteins, VP-1 and VP-2, of *c.* 40 kDa each, that is, a size much smaller than those of other parvoviruses.

Two putative promoter TATA-boxes at map units 7 and 61 are conserved among these viruses. Northern blot analysis of AeDNV transcripts revealed two species of apparently unspliced mRNAs of 3.5 and 1.2 kb, encoding NS and VP proteins, respectively. These sizes indicate that the VP and NS transcripts co-terminate in the vicinity of the polyadenylation signal near the 3′ end of the genome, a property shared by most vertebrate parvovirus transcripts, except those of erythroviruses. Furthermore, primer extension and mutational analyses of the structural gene promoter of AaeDNV revealed that the TATA-sequence at map unit 61 is apparently not involved in gene expression but a CAGT initiator (Inr) sequence 60 nt upstream of this sequence and an upstream TATA-box at map unit 60 were found to be critical for efficient gene expression. The same CAGT box is also present upstream of P61 TATA box of AalDNV-1 genome and has been shown to be important for many arthropod and mammalian promoters.

Unclassified DNVs

In addition to a number of DNV isolates that have not been further characterized (**Table 1**), the genome of the hepatopancreatic parvovirus (renamed penaeus monodon densovirus, PmDNV) of the black tiger prawn *P. monodon*

was recently cloned and sequenced. The virus packages (–)-polarity single-stranded DNA with a length of 6321 nt and terminal hairpins of 222 (5′) and 215 nt (3′), respectively. The genome has a monosense organization with three major predicted coding regions (left, mid-, and right ORF). Sequence identities indicated that these ORFs code for NS-1 (mid-ORF), NS-2 (left ORF), and VP (right ORF). Surprisingly, the coding capacity of the right ORF is approximately 92 kDa, whereas the two capsid proteins are only about 57 and 54 kDa. N-terminal protein sequencing suggested a proteolytic cleavage but it is not clear whether this cleavage is maturation related or that the N-terminal 35 kDa protein serves a nonstructural function.

Phylogenetic Comparison of DNVs

Several motifs in the proteins of DNVs having essential enzymatic functions can serve to establish phenetic relationships among these viruses and their vertebrate counterparts. In particular, the rolling-circle replication (RCR) and ATPase (Walker) motifs in NS-1 present through the whole family *Parvoviridae* and the PLA2 motif found in the N-terminal sequence of most of the VP1 structural polypeptide (**Figure 3**) are useful for these comparisons. On the other hand, only some members of subgroup A of ambisense DNVs contain an NS-3 gene. This gene is, however, found in other viruses such as the granuloviruses. Obviously, the choice of NS-3 for phylogenetic purposes may show a closer relationship of such viruses to granulosis viruses than to other NS-3-less DNVs. Analysis of relationships using the NS-1 motifs revealed three distinct virus subgroups (**Figure 4**) corresponding to ambisense DNVs, brevidensoviruses, and iteraviruses. Differences can still be substantial within each group, for example, sequence identities among the ambisense DNVs often are less than 20%.

Conclusions

The genomes of DNVs, while fitting the main criteria of those of vertebrate parvoviruses, have revealed a remarkable diversity in both their noncoding terminal palindromic sequences, gene organization, and transcription modalities. It is particularly noteworthy that viruses with a similar genome organization that have evolved in different insect orders have strikingly different genome sequences. It is far from clear whether the lack of sequence identities led to essential different expression strategies and classification according to overall genome organization.

The forthcoming data on genome sequence and organization of several insects from different orders could provide keys for a better understanding of the evolution and adaptation of DNVs to such a variety of hosts.

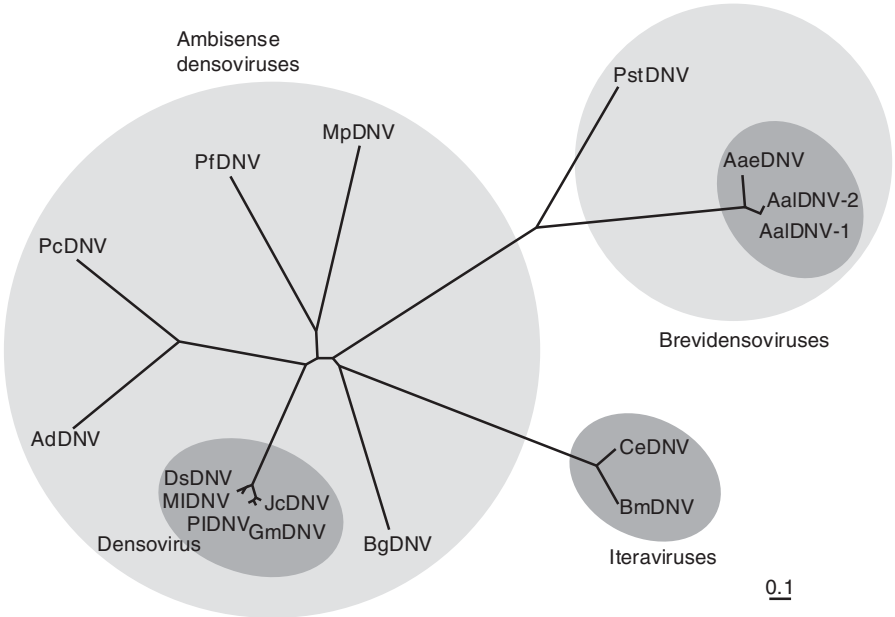


Figure 4 The RCR and ATPase motifs of the NS-1 (**Figure 3**) were used to construct an unrooted tree. The scale bar represents 10 mutations per 100 sequence positions. The length of the branch thus represents the phylogenetic distance between the various DNVs. Three distinct DNV groups are recognized, one ambisense virus group and two monosense virus groups. The tree was generated by distance matrix analysis (Phylip program package PROTDIST), using Dayhoff PAM 001 scoring matrix followed by FITC, applying the global search option.

See also: Insect Pest Control by Viruses; Parvoviruses: General Features.

Further Reading

Bergoin M and Tijssen P (2000) Molecular biology of *Densovirinae*. In: Faisst S and Rommelaere J (eds.) *Parvoviruses: From Molecular Biology to Pathology and Therapeutic Uses*, pp. 12–32. Basel: Karger.

Simpson AA, Chipman PR, Baker TS, *et al.* (1998) The structure of an insect parvovirus (galleria mellonella densovirus) at 3.7 Å resolution. *Structure* 15: 1355–1367.

Tattersal P, Bergoin M, Bloom E, *et al.* (2005) Family *Parvoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 353–369. San Diego, CA: Elsevier Academic Press.

Tijssen P, Bando H, Li Y, *et al.* (2006) Evolution of densoviruses. In: Kerr JR, Cotmore SF, Bloom ME, Linden RM, and Parrish CR (eds.) *Parvoviruses*, pp. 55–68. London: Hodder Arnold.

Tijssen P and Bergoin M (1995) Densonucleosis viruses constitute an increasingly diversified subfamily among the parvoviruses. *Seminars in Virology* 6: 347–355.

Tijssen P, Szelei J, and Zadori Z (2006) Phospholipase A2 domains in structural proteins of parvoviruses. In: Kerr JR, Cotmore SF, Bloom ME, Linden RM, and Parrish CR (eds.) *Parvoviruses*, pp. 96–105. London: Hodder Arnold.

Parvoviruses of Vertebrates

C R Parrish, Cornell University, Ithaca, NY, USA

© 2008 Published by Elsevier Ltd.

Glossary

Inverted terminal repeats Sequences at the termini of the viral genomes that form imperfect palindromes, which are required for DNA replication.

Panleukopenia Reduction in the numbers of white blood cells of all classes.

History

Parvoviruses are the cause of a variety of diseases that range from severe and even lethal to subclinical and in some cases cryptic diseases, and so their discovery was delayed by the relative lack of specific clinical signs and in some cases by the difficulties of culturing the agents. Diseases of cats caused by the feline panleukopenia virus (FPV) were first recognized in the early part of the twentieth century, and a similar virus was recognized as the cause of enteritis in mink in the late 1940s. Other diseases caused by parvoviruses were recognized in the 1950s, and those included the Kilham rat virus isolated from rats, and the H1 virus isolated from tissues grown in rats. The adeno-associated viruses (AAVs) were identified in the early 1960s as contaminants of adenovirus isolates, and those were soon shown to be dependent on the adenovirus as a helper for their replication. FPV was isolated in the early 1960s in tissue culture once it was recognized that its replication depended on dividing cells and that the virus itself was only poorly cytopathic, both common properties of parvoviruses. Many different viruses were identified over the next decades, including the minute virus of mice (MVM) that was isolated in the 1960s originally as a contaminant of cultured mouse cells, and which was subsequently found to be related to a number of different parvoviruses that are widespread in rodents. The porcine parvovirus was identified in the 1970s as the cause of porcine fetal infections, and the human B19 parvovirus was also identified in the 1970s as the cause of the erythema contagiosum (fifth disease) in children and anemia in people with underlying problems with erythropoiesis. The canine parvovirus (CPV) emerged in the late 1970s as the cause of new diseases of dogs; it was closely related to the FPV, and it seemed likely that CPV was a mutant of the feline virus with mutations that gave it an expanded host range. The list of parvoviruses and their diseases has continued to grow over the years due to the discovery of viruses in vertebrates as well as invertebrates and crustacea, and those have included viruses of rabbits, horses, cows, and other species which are not clearly the cause of specific diseases. DNA analysis seeking cryptic virus sequences has also identified a number of new parvoviruses in humans and other animals. Those human viruses are mostly not directly associated with any disease, and the recently described human bocavirus appears to be associated with respiratory disease in neonates who are infected with other agents. It appears likely that there are parvoviruses of most animals, but that most are the cause of only very mild disease or subclinical infections.

The biology and distribution of the viruses vary widely, and each virus has its own specific properties and natural history. Among the human parvoviruses related to B19 in the genus *Erythrovirus*, three genetically distinct lineages have been identified which have different temporal or

geographic distributions. The B19 viruses have a global distribution and are found to infect people of all ages. The type 2 viral sequences are primarily found in the tissues of people born before *c.* 1973, suggesting that they were infecting people prior to that year, but that they are not currently circulating. The type 3 viruses have been mostly found in people in Africa or people who migrated to other countries from Africa.

Taxonomy and Classification

All of the viruses fall into the family *Parvoviridae*, which includes viruses with linear single-stranded DNA (ssDNA) genomes, and small nonenveloped capsids around 25 nm in diameter. The family is divided into two subfamilies, *Parvovirinae* and *Densovirinae*, to host respectively vertebrate and invertebrate viruses. The invertebrate viruses are not considered in this article. The subfamily *Parvovirinae* includes the genera *Parvovirus*, *Erythrovirus*, *Dependovirus*, *Amdovirus*, and *Bocavirus*. The viruses are classified based on their structure, serological relationships, and their genome organization and sequence relationships.

Geographical and Seasonal Distribution

The viruses are relatively resistant in the environment and are shed in various secretions depending on their specific pathogenesis and tissue tropism. Some infect the intestinal epithelial cells and are shed in the feces (CPV and FPV), while others infect cells in the kidneys and are shed in the urine (many rodent parvoviruses), or replicate in a limited number of tissues in the body and are likely spread by respiratory routes (human B19 parvovirus). This results in a variety of different means of spread and the epidemiology of the viruses can differ significantly. Some viruses require close contact between animals to get efficient transmission, as appears to be the case with the Aleutian mink disease virus (AMDV) which has been controlled in many cases by serological testing and culling infected animals. Other viruses can spread widely and rapidly with no direct contact between hosts. While the tracking of viruses and their spread can be difficult, there is clear evidence for the rapid and extensive spread of some parvoviruses. For example, the CPV spread around the world during 1978 after it first emerged, and variants of the original CPV infected animal strain also spread globally within a year of their first emerging. Global distribution of viral genotypes of B19 has also been observed. Although there is no strict seasonality to the infections by most parvoviruses, the fact that many of the viruses infect fetal or neonatal animals means that the distribution of the infection and disease may be seasonal for animals that breed at particular times of the year.

Host Ranges and Virus Propagation

Most of the parvoviruses appear to have relatively narrow natural host ranges, although they can sometimes be shown to infect cells from a broader range of hosts when those are inoculated in tissue culture. DNA replication of the autonomous parvoviruses occurs during S phase in mitotically active cells, and hence the tissue tropism of the infection depends on the age of the animal, which will affect the populations of dividing cells that are available. This also leads to age-related diseases, with many viruses showing a severe infection of fetuses or neonates, but only a mild or subclinical infection of older animals. Within the host some of the viruses may have a narrow tissue tropism, as is seen in the case of the human B19 virus which is largely restricted to the erythroid precursor cells in the bone marrow in humans after birth. The mechanisms that control host range or tissue tropisms of the viruses can vary depending on the virus, but have been associated with receptor binding by the capsids, with intracellular blocks to replication that are dependent on the function of the viral NS2 protein, or to the regulation of specific gene expression or splicing in different differentiated host cells.

Viral Capsids, Their Activities, and Cell Infection

The viral capsid is nonenveloped and around 25 nm in diameter (**Figure 1**). The capsid is assembled from two or three versions of a single protein. The main capsid protein is designated VP2 or VP3 (depending on the virus), and it contains the major structural motif that allows assembly to occur and makes up ~90% of the protein in the capsid. Longer versions of the capsid protein are also included and required for infection, most likely because they contain a phospholipase A2 enzyme activity, as well as basic sequences that control the nuclear transport of the capsids and capsid proteins during infection and replication. The phospholipase A2 enzymatic activity can vary significantly depending on the virus being examined, but that appears to be required for successful cell infection, most likely by modifying the endosomal membrane to allow viral release into the cytoplasm. The capsid is assembled from a total of 60 copies of the combined VP1 and -2 (and -3 where that is present) proteins. The N-terminus of the largest (VP1) protein is sequestered or buried within the capsid when they are produced within the cell, and this can become exposed to the outside during the process of infection by an unknown mechanism. The capsid packages the viral ssDNA genome, and in some viruses capsids package mainly the minus strand, while others can package either the plus or minus DNA strands with similar efficiency.

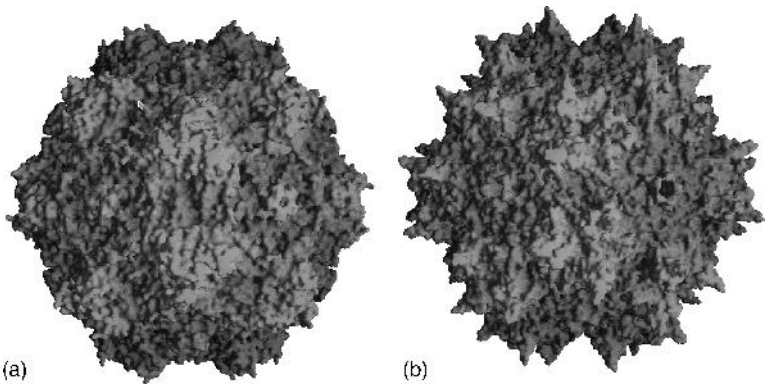


Figure 1 A surface view of (a) the CPV and (b) the AAV-2 virus capsid as derived from the atomic structure of the viruses. The diameter of the particles is ~25 nm, and they are assembled from 60 copies of a combination of the VP2 protein (CPV) or VP3 protein (AAV) (about 55 copies) and the slightly longer VP1 protein (about five copies). The binding sites of the cell receptor and many neutralizing antibodies are located on the raised regions found around the threefold axes of icosahedral symmetry. A pore that penetrates through the capsid is seen at the fivefold axes of symmetry.

The capsids can bind a variety of different receptors on cells leading to infection. Receptors range from sugars such as specific sialic acids (MVM) and heparan sulfate proteoglycan (many serotypes of AAV), to cell surface glycoprotein receptors of a variety of different types. Receptor or co-receptor proteins identified so far include the basic fibroblast growth factor receptor (AAV type 2), αV β2 integrins (AAV type 2), and transferrin receptor (canine and feline parvoviruses).

Cell Infection and Viral Replication

Although all of the parvoviruses appear to enter and infect cells through receptor-mediated endocytic processes, the specific pathways and mechanisms involved are still being defined, and it appears that many viruses are able to use multiple endosomal routes of infection. Uptake often involves clathrin-mediated pathways, but for many viruses other pathways may also be used. While signaling by the receptor or receptor-associated molecules may occur and in some cases enhances the infection, it is not clear that it is required for infection by all viruses. The viral particles traffic within the endosomal system of the cell, and in many cases remain in endosomes for long periods. The particle in an intact or mostly intact form enters the cytoplasm in a process that is controlled or assisted by the PLA2 activity of the VP1 unique region. Within the cytoplasm, the capsid may be able to associate with microtubular-based molecular motors such as dynein, and become localized in the pericentreolar region of the cell near the microtubular organizing center. Exactly how the virus or the viral DNA enters the cell nucleus is not clear. The small parvovirus particle could likely pass through the nuclear pore intact, and some evidence suggests that basic sequences in the N-terminal region of the VP1 protein are functional nuclear localization motifs. However, other studies suggest that the capsid may also be able to directly affect the nuclear envelope, perhaps allowing the virus to enter the nucleus for replication. It is also possible that both mechanisms can apply under different circumstances. In either case, the capsid and the associated ssDNA enters the nucleus. How the DNA is removed from the capsid is not known, but possibilities are that the capsid comes apart in the nucleus, or that the DNA is extracted from the capsid, possibly by the activity of the host cell DNA polymerase initiating replication on the 3′ end of the DNA.

The Viral Genome

The viruses of vertebrates have many of their genomic features in common, and although there is wide divergence between the sequences of many of the viruses, shared sequences in a conserved region of the nonstructural protein suggest that all the viruses share a single common ancestor and have many properties in common. The genomes are all between ~4500 and ~5200 bases in length, and each contains two large open reading frames, as well as short sequences near the 3′ and 5′ end of the genome, and either inverted terminal repeats or terminal palindromes (**Figure 2**). The terminal sequences consist of imperfect palindromes, in some cases with specific mismatched sequences that are involved in the control of DNA replication. In the standard genome orientation, the left-hand open reading frame encodes the nonstructural proteins (called NS1 (and -2) or Rep) and the right-hand open reading frame encodes the structural protein genes (in AAVs termed the Cap genes) (VP1 and -2, or VP1–3, depending on the virus). Genomes may contain

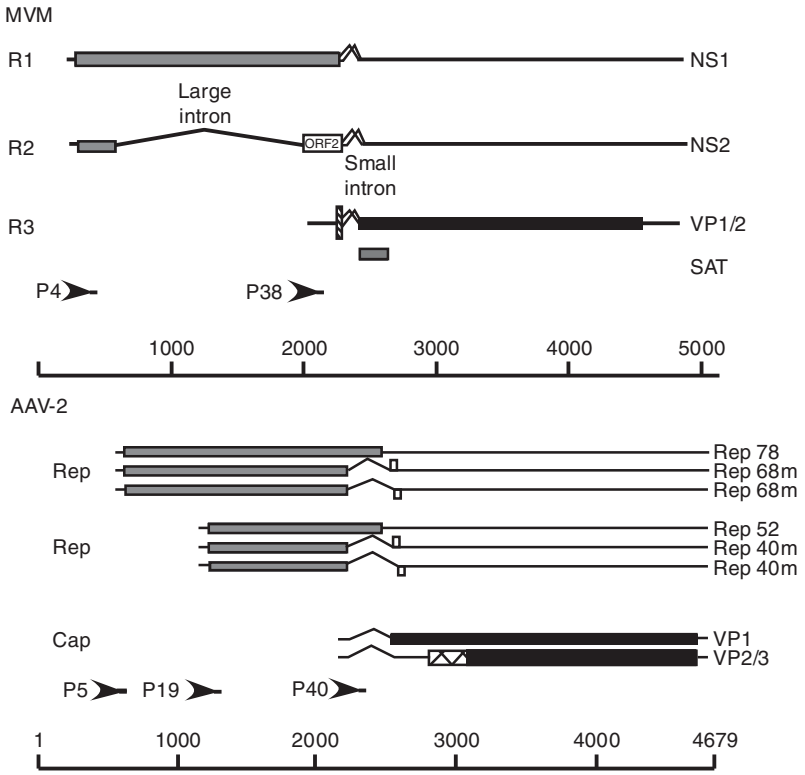


Figure 2 Diagrammatic representations of the genome structures and gene organization of representatives of the autonomous parvoviruses (MVM) and the AAVs (AAV type 2). The nonstructural 1 and 2 (NS1 and -2) and replication (Rep) proteins of the viruses are encoded by the left half of the genome, while the viral proteins 1 and 2 (VP1 and VP2), and, in the case of the AAV, the capsid (Cap) genes, are encoded in the right half of the genome.

between one and three functional promoters for RNA transcription. Whereas often there is only a single promoter that is found near the left of the genome, additional promoters may be used to express the capsid proteins (in many viruses, including the viruses related to MVM) or smaller versions of the nonstructural proteins (as seen in AAV). Each gene comes in multiple versions, through the use of alternative transcription start sites, splicing to give different transcripts, and by internal initiation of translation. In some viruses, there are short additional open reading frames besides the NS and capsid proteins, and in many viruses a short open reading frame (called SAT) that overlaps the beginning of the major capsid protein has been identified, while an 11 kDa protein is encoded in the sequence of the B19 parvovirus after the capsid protein gene open reading frame.

The larger NS1 or Rep are multifunctional proteins that serve various functions involved in viral replication. The protein serves as a DNA helicase, site-specific nickase, and it becomes covalently attached to the 5′ end of the ss- and double-stranded (ds)-forms of the viral DNA. The activities of the proteins are regulated by site-specific phosphorylation. The functions of the NS2 protein are not completely understood, but it interacts with the Crm1 protein and appears to regulate the transport and assembly of capsids within the cells of some hosts. As mentioned above, the VP or Cap proteins spontaneously assemble into capsids when expressed in cells.

Evolution and Variation

As mentioned, the parvoviruses appear to be related through one or a few common ancestors, and phylogenetic analysis shows that the viruses can be divided into several major clades or related groups. The timeline of the evolution of the viruses is unclear as the molecular clock of sequence variation over long time periods is not known. When CPV and B19 sequences with known dates were analyzed, the rates of evolution proved to be surprisingly high, with around 10^{-4}–10^{-5} substitutions per nucleotide per year, similar to the rates observed for some RNA viruses. Intrahost variation of the viral sequences has been seen during persistent infections by MVM in immunodeficient mice, and by B19 in humans. The viral DNA is replicated by host cell DNA polymerases, and while the true fidelity of the replication is not known, it appears that it must be considerably lower than the fidelity seen for

the replication of the host genome. This probably results from the mode of DNA replication, the single-stranded nature of the viral genome, and the rapid turnover of the viruses in nature.

Pathogenesis

The parvoviruses cause a wide variety of diseases, ranging from subclinical or inapparent to severe and lethal diseases. When infected alone, the AAVs do not complete their replication or cause any disease in animals. The tissue tropisms of the autonomous parvoviruses require the presence of mitotically active cells, and so there are many more tissues available for virus replication in a fetus or neonatal animal, and fewer in older animals. For some viruses such as porcine parvovirus, the disease seen is primarily in fetuses that are infected *in utero*, and after birth infections cause only a mild disease. More severe disease associated with PPV is seen in a co-infection with the porcine circovirus type 2, where infection by the parvovirus apparently causes immune stimulation that enhances the replication and pathogenicity of the circovirus. It appears that the human bocavirus may cause only a mild infection by itself, but it is associated with more severe disease in cases of co-infection with other viruses. The CPV and FPV infect the dividing cells in various lymphoid organs which are most active in younger animals, while in the intestine they also infect the rapidly proliferating cells in the crypts of Leiberkuhn, and therefore cause both a lymphopenia and lymphoid depletion, and also diarrhea resulting from loss of epithelial cells in the gut and subsequent loss of osmoregulation. The human B19 has a tropism for the rapidly dividing erythroid precursor cells in the bone marrow, and can cause a loss of erythrocyte production. Since erythrocytes generally have a long life span, the loss of production only results in anemia where there is an underlying condition that causes loss of erythrocytes, or chronic loss of production. However, the large amounts of viral antigen produced in the circulation result in a transient antibody–antigen immune complex disease which is seen as erythema infectiosum (fifth disease) in children, or as arthritis in adults presumably due to deposition of immune complexes in the joints.

Some viruses cause a more chronic disease, the best understood being AMDV. Infection of mink generally results in a chronic persistent infection, where the virus is only partly neutralized by the antibody responses that develop, and so there is a continuing production of viral antigen–antibody complexes. The disease is more prominent in the background of the Aleutian coat color phenotype minks, which have a condition similar to human Chédiak–Higashi syndrome; they are therefore deficient in the ability of removing the immune complexes from circulation and show increased levels of circulating immune complexes and a more rapid course of disease.

Immune Responses, Diagnosis, Prevention, and Control

The immune responses to parvoviruses develop rapidly after infection, and in most cases the virus is largely or completely controlled. Where they have been tested, antibodies are quite protective and also are effective at controlling most virus infections, and those would work in conjunction with the cell-mediated responses. Maternal immunity is effective at protecting neonatal animals from infection in many cases. Most of the parvoviruses do not have obvious mechanisms for manipulating or evading the host immune responses, and how the viruses that establish persistent infections do so in face of a strong antibody response is not known. In the case of Aleutian mink disease, the virus is not effectively neutralized by the antibodies that develop and so it continues to infect and spread even in the face of strong immune responses.

Vaccines are effective against most of the parvoviruses (with the exception of AMDV where disease can be enhanced by vaccination). Both modified live and inactivated vaccines have been developed, and both can be effective at protecting against the disease as long as they can generate an effective immune response. Experimental subunit vaccines have been tested and shown to be effective at protecting animals against disease, and in one case the vaccine contained peptides derived from the sequence of the N-terminus of the VP2 protein, and the antibodies generated both neutralized the virus and protected the animals. Vaccination in most cases is interfered with by preexisting maternal immunity, and hence when vaccinating young animals it is necessary to vaccinate when the maternal immunity has waned to such a low level that the immune responses to the vaccine can be developed.

Diagnostic approaches vary depending on the purpose of the test. Antibodies are long-lived, and serological tests are generally sensitive and will show the existence of prior infections, but those may not be a reflection of the current infection status of the animal. Polymerase chain reaction (PCR) is frequently used to detect the presence of virus, but it will also detect the presence of persistent viral DNA left over from a prior viral infection which may not be actively replicating at the time of testing. Virus isolation can be used for some viruses, but others grow poorly in tissue culture (e.g., AMDV) or grow only in specific differentiated cells that are difficult to maintain in routine culture (B19). The wild-type viruses are often poorly cytopathic in culture; hence, other methods may be required to detect the presence of any viruses isolated.

Control is difficult for many parvoviruses, and in some cases the low incidence of clinical disease makes vaccination unnecessary. Some viruses such as the canine and feline parvoviruses appear to be widespread and to infect animals readily, and those are controlled only with difficulty. In those cases, quarantine may be used to prevent infection of young susceptible animals with wild-type virus before the animals are vaccinated. Vaccines to PPV are given to the sow before breeding to prevent infection of the fetuses. Parvoviruses that infect rodents in experimental colonies are difficult to control due to their being persistently shed from infected animals. In this case, control involves cesarean derivation of uninfected animals and establishment of a clean colony.

See also: Parvoviruses: General Features; Vaccine Strategies.

Further Reading

Brown KE and Young NS (1997) The simian parvoviruses. *Reviews in Medical Virology* 7: 211–218.

Farr GA, Zhang LG, and Tattersall P (2005) Parvoviral virions deploy a capsid-tethered lipolytic enzyme to breach the endosomal membrane during cell entry. *Proceedings of the National Academy of Sciences, USA* 102: 17148–17153.

Girod A, Wobus CE, Zadori Z, *et al.* (2002) The VP1 capsid protein of adeno-associated virus type 2 is carrying a phospholipase A2 domain required for virus infectivity. *Journal of General Virology* 83: 973–978.

Kaufmann B, Simpson AA, and Rossmann MG (2004) The structure of human parvovirus B19. *Proceedings of the National Academy of Sciences, USA* 101: 11628–11633.

Kerr JR, Cotmore SF, Bloom ME, Linden RM, and Parrish CR (2006) *Parvoviruses*. London: Hodder Arnold.

Young NS and Brown KE (2004) *Parvovirus B19*. *New England Journal of Medicine* 350: 586–597.

Parvoviruses: General Features

P Tattersall, Yale University Medical School, New Haven, CT, USA

© 2008 Elsevier Ltd. All rights reserved.

The Family *Parvoviridae*: An Overview

The family *Parvoviridae* comprises small, isometric, nonenveloped viruses that contain single-stranded DNA genomes that are linear, a set of properties unique in the known biosphere. The parvovirus virion contains a single genomic molecule 4–6 kbp long, which terminates in short palindromic sequences that can fold back on themselves to create duplex hairpin telomeres. These terminal hairpins are different from one another, both in sequence and predicted structure, in heterotelomeric genomes, while in homotelomeric genomes they form part of a terminal repeat (TR) that can be inverted or direct. These terminal hairpins are essential for the unique rolling-hairpin strategy of parvovirus DNA replication, and are therefore an invariant hallmark of the family.

The bulk of the parvovirus genome lies between these hairpins, and comprises two gene cassettes, with transcripts from one half of the genome, by convention the left-hand side, programming synthesis of the DNA replication initiator protein(s), while the right-hand encodes an overlapping set of capsid polypeptides. All parvovirus initiator proteins incorporate two separate enzymatic cores. The N-terminal of these is a site-specific single-strand nuclease domain, comprising active-site motifs common to all rolling-circle initiator proteins, which recognize and nick the replication origin(s). Carboxy-terminal to this nickase lies a helicase domain, which belongs to the superfamily III (SF3) group of viral 3′-to-5′ helicases. Parvovirus genomes replicated using a unidirectional, strand-displacement mechanism called rolling-hairpin replication (RHR), which appears to be an evolutionary adaptation of the ancient rolling-circle replication mechanism used by bacteriophages with circular single-stranded DNA genomes. The parvovirus genome contains two viral origins of DNA replication, one embedded in each hairpin telomere. During RHR, these hairpins can unfold to be copied, then refold to allow continuous amplification of the linear template. Viral replication exhibits two distinct phases, the first of which is an amplification phase, during which high-molecular-weight duplex replicative form (RF) DNA intermediates are generated and subsequently processed back down to monomeric duplexes by the action of the viral nickase. Later in the infectious process, a genome displacement phase predominates, in which individual progeny single strands are displaced from duplex forms and encapsidated.

To initiate the first phase of RHR, the 3′ nucleotide of incoming virion DNA pairs with an internal base to create a DNA primer, which allows a host polymerase to initiate synthesis of a complementary DNA strand. This generates a monomer-length, duplex intermediate in which the two strands, designated plus or minus with respect to transcription, are covalently linked, or hairpinned, by a single

copy of the original viral telomere. For homotelomeric parvoviruses, these hairpinned structures create replication origins that can be acted upon by NS1 or Rep proteins, in a process called terminal resolution, which converts them into duplex palindromic telomeres. For heterotelomeric viruses, however, the left-end telomere in its hairpin form can be refractory to nicking by NS1, and replication proceeds through an obligatory dimer RF. For both types of parvovirus, further DNA amplification proceeds by unidirectional strand displacement, where the initiator protein also serves as the 3′-to-5′ replicative helicase, while all other replication proteins are commandeered from the host cell. Replication continues to the end of the genome where the hairpin is displaced and copied to create a palindromic, or extended form, telomere. These terminal palindromes can be melted and rearranged in hairpins such that the 3′ end can act as a primer for further replication, using the newly synthesized product as a template. Where the template ends in a hairpinned telomere, this is copied by the replication fork, switching the direction of synthesis back toward the initiating telomere, creating a dimer. Thus the RHR process creates a series of palindromic duplex dimeric and tetrameric concatemers, in which the unit-length genomes are fused in left-end:left-end and right-end:right-end combinations.

Site-specific single-strand nicks are subsequently introduced, by the viral initiator protein, into the duplex telomeric origins embedded in these concatemers, allowing successive rounds of replication to be initiated, or progeny single strands to be excised and ultimately displaced. Nicking involves a trans-esterification reaction that generates a base-paired 3′ nucleotide and leaves the initiator nuclease covalently attached, by a phosphotyrosine bond, to the 5′ nucleotide at the nick. Displaced progeny single strands re-enter the replication pool unless sequestered by the packaging machinery and encapsidated into empty particles. Thus packaging appears to be driven by ongoing viral DNA synthesis, and is entirely dependent upon the availability of preformed capsids. The efficiency of excision, displacement, and packaging of each strand sense depends upon the efficiency of the replication origin producing it. Thus it follows that all homotelomeric parvoviruses generate equal numbers of each strand as packaging precursors, and therefore produce virion populations containing equal numbers of plus and minus strands, each packaged in a separate particle. The ratio of plus to minus strands packaged by the heterotelomeric parvoviruses depends upon the relative efficiencies of the two different DNA replication origins embedded in their disparate telomeres. In all cases where this has been determined, the origin at the 5′ end of the transcription template strand, by convention the right-hand end of the minus strand, is the predominant origin, and drives the displacement and packaging of the minus strand at ratios between 10:1 and 100:1 over that of the plus strand. Thus, all of the heterotelomeric parvoviruses examined to date are effectively negative-strand DNA viruses.

Independent of the sense of strand they contain, parvovirus particles are physically very stable and are antigenically and structurally quite simple. A combination of protein chemistry, X-ray crystallography and cryo-electron microscopy, has established that the virion is an icosahedral structure exhibiting $T = 1$ symmetry, constructed from two to four species of structural protein. These polypeptides are encoded as a nested sequence set in the right half of the genome, and the capsid shell comprises 60 copies of the common, usually C-terminal, 60–70 kDa region of the polypeptide set. Each polypeptide contains an eight-membered β-barrel, or jelly-roll, fold, found in most viral capsid proteins. The surface of the particle is formed by several of the loops between these β-strands, such that differences in length and primary amino acid sequence of these loops gives rise to the marked differences in topology observed between members of separate genera, as shown in **Figure 1**. Virions are resistant to inactivation by organic solvents, thus lack essential lipids, and there is no evidence that any of the capsid polypeptides are glycosylated, although they may be modified post-translationally by phosphorylation. The N-terminal region of the largest structural polypeptide, VP1, is an extension of the common structural region, and, in most parvoviruses, contains a functional phospholipase A_2 (PLA_2) enzymatic core whose activity is essential for escape from endosomal compartment into the cytosol, early in viral entry.

Only two antigenic sites have been identified on the virion surface, as defined by mutations that allow escape from neutralization by monoclonal antibodies. Since this relatively simple antigenic structure is very stable, serotype has been a useful adjunct to sequence-based phylogenetic analysis for taxonomic classification, particularly for the parvoviruses of vertebrates. Parvoviruses encode several well-recognized functional protein domains that might serve as linearly descended evolutionary 'tags'. Foremost among these is the relatively contiguous set of well-defined functional subdomains, called Walker boxes, within the SF3 helicase domain of the replication initiator protein of all known parvoviruses. Interestingly, SF3 helicases have also been identified within genes encoded by DNA viruses as diverse as members of the families *Poxviridae, Baculoviridae, Papillomaviridae, Polyomaviridae*, and *Circoviridae,* as well as in the genomes of small RNA viruses such as members of the families *Picornaviridae* and *Comoviridae*. However, this class of helicase has not been found encoded in cellular genomes, and their phylogenetic branch within the AAA + ATPase superfamily diverged from the rest of the tree of life before the separation of the archaea, bacteria, and eukarya. This suggests that the SF3 class of helicases might have originally evolved in primitive replicons that are only represented by viruses in the present biosphere, and that the SF3 helicase

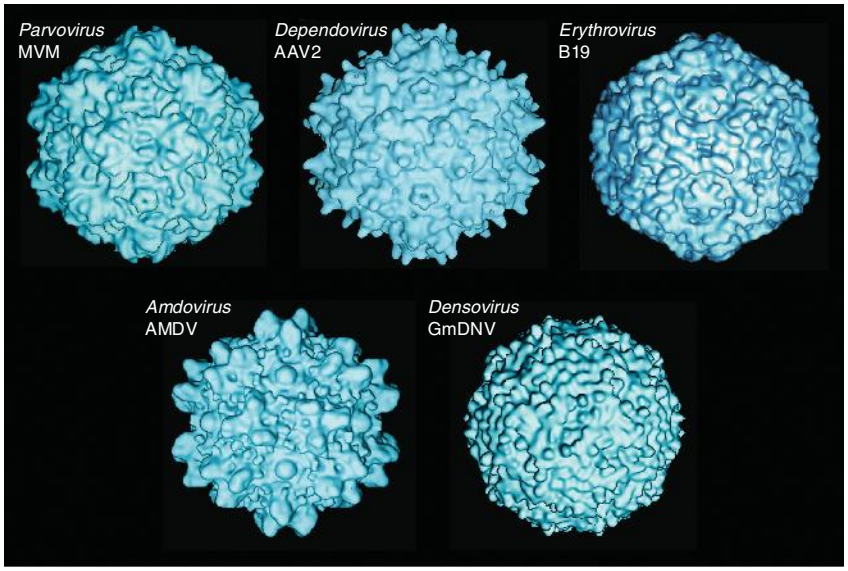


Figure 1 Molecular topography of representative virions from the family *Parvoviridae*. Low-resolution surface maps of minute virus of mice (MVM), adeno-associated virus-2 (AAV2), B19, mature aleutian mink disease virus (AMDV), and galleria mellonella densovirus (GmDNV), at 13 Å resolution. The surface map image of AMDV was generated from a pseudoatomic model built into cryo-EM density, while the others were generated directly from atomic coordinates, as described by Agbandje-McKenna and Chapman in 2006, and were graciously provided by Lakshmanan Govindasamy and Mavis Agbandje-McKenna.

domain evident in present-day parvoviruses might represent an uninterrupted vertical link to the first common ancestor of the entire family *Parvoviridae*.

The family *Parvoviridae* is divided into two subfamilies, the subfamily *Parvovirinae*, whose members infect vertebrate hosts, and the subfamily *Densovirinae*, whose members infect arthropods. Using DNA sequence-based phylogenetic analysis, members of the subfamily *Parvovirinae* have been divided into five genera, and those of the subfamily *Densovirinae* into four genera, as listed in **Table 1**. As listed in **Tables 2** and **3**, these nine genera contain 34 species accepted as such by the International Committee for the Taxonomy of Viruses, with 25 species tentatively assigned to individual genera and a further nine virus isolates currently unassigned within the subfamily *Densovirinae*.

Table 1 The taxonomic structure of the family *Parvoviridae*

Subfamily	*Genus*
Parvovirinae	*Parvovirus*
	Dependovirus
	Erythrovirus
	Amdovirus
	Bocavirus
Densovirinae	*Densovirus*
	Pefudensovirus
	Iteravirus
	Brevidensovirus

Genera in the Subfamily *Parvovirinae*

Genus *Parvovirus*

Viruses belonging to species within the genus *Parvovirus* have heterotelomeric genomes ~5 kbp in length. The packaged strands are predominantly negative-sense with respect to their monosense transcription strategy, although parvovirus LuIII virus packages both strands, in separate particles, in an approximately equimolar ratio. The terminal hairpin at the 3′-end of the negative strand, by convention the left-hand end of the genetic map, is 115–121 nucleotides (nt) long, whereas their right-hand hairpins vary between 200 and 248 nt in length. As shown in **Figure 2**, there are two transcriptional promoters, at map units ~4 and ~40, from the left-hand end, and transcripts co-terminate at a single polyadenylation site near the right-hand end of the genome. Many viruses belonging to this genus encode a second, smaller, nonstructural protein, NS2, in addition to the major DNA replication initiator protein NS1. NS2 is involved in several aspects of viral replication and capsid assembly, but is dispensable in some cell types. Infecting virions lack accessory proteins, chromatin or even a duplex transcription template, and therefore remain silent within their host cell nucleus until the cellular synthetic machinery manufactures a complementary DNA strand, creating the first transcription template. Typically, this occurs when the host cell enters S-phase, of its own volition, and it is followed rapidly by expression of viral transcripts driven from the left-hand promoter. These viruses can persist both in nondividing cells in

Table 2 Subfamily *Parvovirinae*

Genus *Parvovirus*	
Species	
Minute virus of mice – type species	(MVM)
Feline panleukopenia virus	(FPV)
H-1 parvovirus	(H-1PV)
Kilham rat virus	(KRV)
LuIII virus	(LuIIIV)
Mouse parvovirus 1	(MPV-1)
Porcine parvovirus	(PPV)
HB parvovirus[a]	(HBPV)
Lapine parvovirus[a]	(LPV)
RT parvovirus[a]	(RTPV)
Tumor X virus[a]	(TXV)
Tentative species[b]	
Hamster parvovirus	(HaPV)
Rat minute virus 1	(RMV-1)
Rat parvovirus 1	(RPV-1)
Genus *Dependovirus*	
Species	
Adeno-associated virus-1	(AAV1)
Adeno-associated virus-2 – type species	(AAV2)
Adeno-associated virus-3	(AAV3)
Adeno-associated virus-4	(AAV4)
Adeno-associated virus-5	(AAV5)
Avian adeno-associated virus	(AAAV)
Bovine adeno-associated virus	(BAAV)
Duck parvovirus	(BDPV)
Goose parvovirus	(GPV)
Canine adeno-associated virus[a]	(CAAV)
Equine adeno-associated virus[a]	(EAAV)
Ovine adeno-associated virus[a]	(OAAV)
Tentative species[b]	
Adeno-associated virus-7	(AAV7)
Adeno-associated virus-8	(AAV8)
Adeno-associated virus-9	(AAV9)
Adeno-associated virus-10	(AAV10)
Adeno-associated virus-11	(AAV11)
Adeno-associated virus-12	(AAV12)
Serpentine adeno-associated virus	(SAAV)
Caprine adeno-associated virus	(Go.1 AAV)
Bovine parvovirus type 2[c]	(BPV2)
Genus *Erythrovirus*	
Species	
Human parvovirus B19 – type species[d]	(B19V-Au)
Tentative species[b]	
Chipmunk parvovirus	(ChpPV)
Pig-tailed macaque parvovirus	(PmPV)
Rhesus macaque parvovirus	(RmPV)
Simian parvovirus	(SPV)
Bovine parvovirus type 3[c]	(BPV3)
Genus *Amdovirus*	
Species	
Aleutian mink disease virus – type species	(ADV-G)
Genus *Bocavirus*	
Species	
Bovine parvovirus 1 – type species	(BPV1)
Canine minute virus	(CnMV)
Tentative species[b]	
Human bocavirus[c]	(HBoV)

[a]Formally accepted by ICTV, but no representative genomes have been sequenced.
[b]Representative genome sequenced, but not formally accepted as a species by ICTV.

culture and within the intact host animal, and, in the latter case, frequently re-emerge following immunosuppression. Despite convincing serologic and PCR evidence for long-term persistence by many parvoviruses, at present essentially nothing is known of the mechanisms underlying this type of latency. Members of many species within this genus, particularly those whose natural host are rodents, have been found to be markedly oncolytic in transformed human cells, both in culture and in xenotransplanted tumor models.

Genus *Dependovirus*

The genus *Dependovirus*, as the name implies, originally comprised only the helper-dependent adeno-associated viruses. However, phylogenetic analysis now places the autonomously replicating viruses from the *Goose parvovirus* and *Duck parvovirus* species solidly within this genus. Except for members of these two avian parvovirus species, all other viruses belonging to this genus that have been isolated to date are dependent upon helper adenoviruses or herpes viruses for efficient replication. Most primate adeno-associated virus (AAV) serotypes isolated to date appear to be simian viruses. On the other hand, sero-epidemiologic evidence clearly indicates that AAV2 and AAV3 are human viruses, while AAV5, which is most closely related to caprine AAV, has been isolated from humans only once.

So far, all members of this genus have been found to be homotelomeric, and their virions contain equivalent numbers of positive or negative DNA strands, between 4.7 and 5.1 kbp in size. Typically, the AAV genome has TRs of ~145 nt, the first ~125 nt of which form a palindromic hairpin sequence, while members of the autonomously replicating avian parvovirus species have ITRs that are much larger, between 444 and 457 nt. As shown in **Figure 2**, three distinct transcriptional strategies have been elucidated for members of this genus. Two subtypes, represented by AAV2 and AAV5, have three transcriptional promoters (P5, P19, and P40), but differ in the positions of their functional polyadenylation sites. For both of these subtypes there is one polyadenylation site at the right-hand end of the genome, but for the AAV5-like viruses another site, located in the middle of the genome, is also used. The GPV-like viruses have a polyadenylation site arrangement like that of AAV5, but differ from the other two subtypes by not having a functional middle, P19, promoter.

[c]Genome sequenced or partially sequenced by PCR-based virus discovery techniques, but virus not yet isolated physically or biologically.
[d]Three major genotypes, represented by B19V-Au, B19-LaLi, and B19-V9. Species names are italicized, abbreviations for individual viruses are given in parentheses.

Table 3 Subfamily *Densovirinae*

Genus *Densovirus*	
Species	
Junonia coenia densovirus – type species	
Junonia coenia densovirus	(JcDNV)
Galleria mellonella densovirus	
Galleria mellonella densovirus	(GmDNV)
Tentative species[a]	
Diatraea saccharalis densovirus	(DsDNV)
Mythimna loreyi densovirus	(MlDNV)
Toxorhynchites splendens densovirus	(TsDNV)
Pseudoplusia includens densovirus	(PiDNV)
Genus *Pefudensovirus*	
Species	
Periplaneta fuliginosa densovirus – type species	
Periplaneta fuliginosa densovirus	(PfDNV)
Genus *Iteravirus*	
Species	
Bombyx mori densovirus – type species	
Bombyx mori densovirus	(BmDNV)
Tentative species[a]	
Casphalia extranea densovirus	(CeDNV)
Sibine fusca densovirus	(SfDNV)
Genus *Brevidensovirus*	
Species	
Aedes aegypti densovirus – type species	
Aedes aegypti densovirus	(AaeDNV)
Aedes albopictus densovirus	
Aedes albopictus densovirus	(AalDNV)
Tentative species[a]	
Penaeus stylirostris densovirus[b]	(PstDNV)
Aedes pseudoscutellaris densovirus	(ApDNV)
Simulium vittatum densovirus	(SvDNV)

[a]Representative genome sequenced, but not formally accepted as a species by ICTV.
[b]This is also called infectious hypodermal and hematopoietic necrosis virus (IHHNV) of penaeid shrimps.
Species names are italicized, acronyms for individual viruses are given in parentheses.

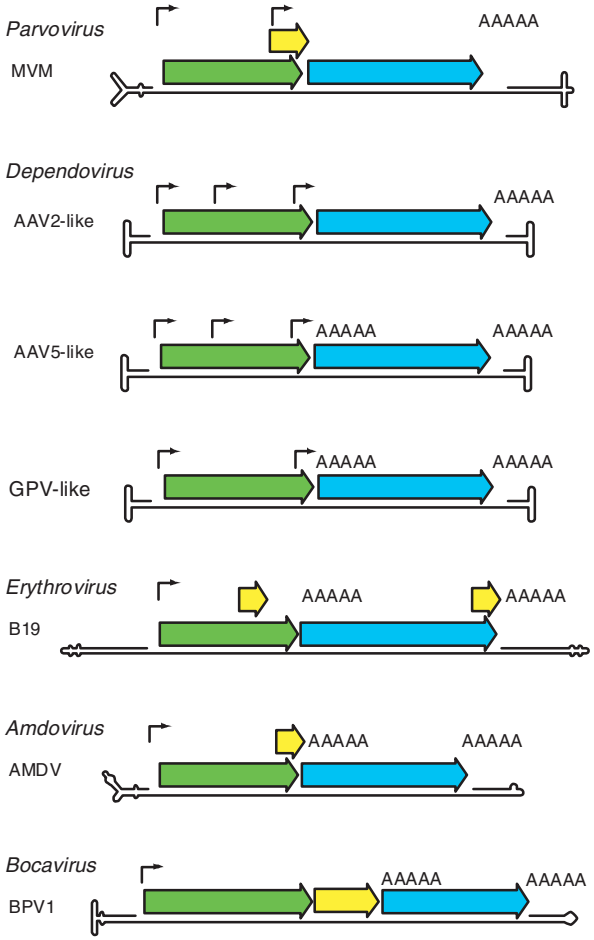


Figure 2 Genetic strategies of representative viruses from genera in the subfamily *Parvovirinae*. Genomes from viruses belonging to the type species of each genus, and an additional two subtypes from the genus *Dependovirus*, are denoted as a single line terminating in hairpin structures. The hairpins are drawn to represent their predicted structures, and are scaled about 20× with respect to the rest of the genome. Open reading frames are represented by arrowed boxes, colored green for the major SF3 domain-containing replication initiator protein, blue for the structural proteins of the capsid, and yellow for the ancillary nonstructural proteins. Transcriptional promoters are indicated by solid arrows and polyadenylation sites by the AAAAA sequence block.

Under certain conditions, such as the treatment of host cells with mutagens or hydroxyurea, AAV DNA replication can be detected in the absence of helper viruses. However, infections without helper virus generally result in a persistent latent infection. Three modes of persistence have been demonstrated for AAV. First, AAV2 and some other serotypes can integrate their genomes site-specifically into a 4 kbp locus on human chromosome 19q13-qter, designated AAVS1. This occurs by a replication-dependent mechanism, requiring low-level expression of Rep, and depends upon sequences in AAVS1 that can function as an AAV DNA replication origin. The second mode, which is independent of Rep gene expression, is primarily seen with recombinant AAV (rAAV) genomes used in gene transduction scenarios, and proceeds through nonspecific integration. Typically, a transgene, flanked by AAV ITRs and packaged in an AAV capsid, is used to infect target tissues in a host animal. Integration of rAAVs occurs at multiple positions throughout the host genome, with a bias toward actively transcribed loci, and is enhanced by the presence of double-strand breaks in host DNA. A third form of persistence, again exploited in rAAV-based gene transduction strategies, results from the establishment of monomeric, and later concatemeric, circular duplex episomes, following vector delivery at high copy number to postmitotic tissues, such as liver or skeletal muscle. Similar episomes have also been detected *in vivo* in latently infected human

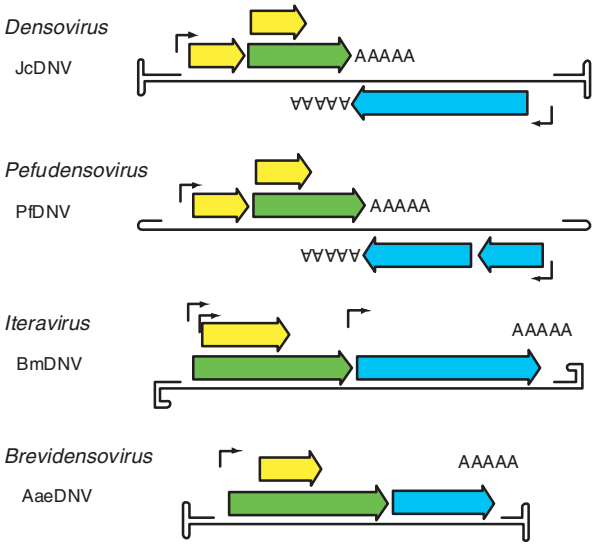


Figure 3 Genetic strategies of a representative of the type species from genera in the subfamily *Densovirinae*. Genomes from viruses belonging to the type species of each genus are diagrammed as in the legend to **Figure 2**.

tissues, although it is not known whether they are created by cellular repair mechanisms or by annealing of complementary strands. Recent studies have shown that the tissue most efficiently targeted by rAAV vectors depends upon the serotype of AAV providing the capsid gene, although a consensus hierarchy of target preference across all of the serotypes continues to evolve.

Genus *Erythrovirus*

Until recently, the only known human pathogenic member of the family *Parvoviridae* was human parvovirus B19 (B19V). This virus is responsible for transient aplastic crisis in patients with a variety of hemolytic anemias, and is the causative agent of *Erythema Infectiosum*, a widespread childhood rash-like disease also referred to as 'fifth disease'. Intrauterine transmission of B19V from an infected mother to her fetus can result in *hydrops fetalis*, particularly in the second trimester of pregnancy. Recently it has become apparent that there are at least three distinct genotypes of B19V, circulating in different human subpopulations. While these are all serotypically equivalent, and thus are considered members of the same species, retrospective PCR analysis of archival tissues has shown that they have predominated in the human population during different periods in the recent past. Additional erythroviruses have been identified in other primate species, which share B19V's specificity for erythroid progenitor cells and cause a similar disease in their hosts.

The erythrovirus genome is homotelomeric, with inverted terminal repeats (ITRs) of 383 nt, and populations of mature B19V virions contain equivalent numbers of positive- and negative-sense DNA strands, each ~5.5 kbp in size. As shown in **Figure 2**, viral transcription is driven by a single promoter at map unit 6 and terminates at alternative polyadenylation sites, one near the middle of the genome, the other at the right-hand end, which generates transcripts encoding NS1 and VP1, respectively. The B19V genomes contain two additional small open reading frames (ORFs), which are accessed by alternatively splicing and translation initiation. One of these encodes an 11 kDa protein containing three proline-rich regions containing consensus Src homology 3 domains, while the other encodes a 7.5 kDa polypeptide of unknown function.

Genus *Amdovirus*

The genus *Amdovirus* contains a single species, *Aleutian mink disease virus*. Mature Aleutian mink disease virus (AMDV) virions contain a predominantly negative-strand, heterotelomeric genome of 4748 nt whose sequence is markedly divergent from other members of the subfamily *Parvovirinae*. As shown in **Figure 2**, the ADMV transcription strategy resembles that of the erythroviruses, in having a single promoter and two alternative polyadenylation sites. The major distinguishing feature of AMDV is its VP1 N-terminus, which is quite short and completely lacks a phospholipase 2A enzymatic core. As seen in **Figure 1**, AMDV virion structure is also somewhat distinctive. Permissive replication has only been achieved in cell culture for the ADV-G isolate, which replicates in Crandell feline kidney cells and is relatively apathogenic in mink. Several highly pathogenic strains of AMDV exist and differ in their disease potential depending upon the age and genetic background of the infected host. Thus infected mink kits develop interstitial pneumonia involving direct attack on type II pneumocytes, while adult mink develop an autoimmune disease characterized by massive hypergammaglobulinemia and fatal glomerulonephritis. As the virus' name suggests, the latter condition is exacerbated in mink carrying the Aleutian coat color allele, which is tightly linked to an antigen presentation disorder in this genetic background. While mostly studied in mink, serologic evidence suggests that this virus also circulates naturally in several other species within the superfamily *Musteloidea*.

Genus *Bocavirus*

The bocaviruses package heterotelomeric genomes ~5.5 kbp in length, and the bovine virus, bovine parvovirus 1 (BPV1), has been shown to package negative-sense and positive-sense DNA strands at a 10:1 ratio. The bocavirus transcription strategy resembles that of the erythroviruses and AMDV in having a single promoter at the left-hand end, and two alternative polyadenylation sites. The bocaviruses differ, however, from all other members of the

subfamily *Parvovirinae* in encoding a 22.5 kDa nuclear phosphoprotein, NP1, that is distinct from any other parvovirus-encoded polypeptide. As shown in **Figure 2**, the ORF encoding NP1 sits immediately downstream of that encoding NS1, and immediately upstream of the middle polyadenylation site. NP1, whose function is currently unknown, is abundantly expressed from transcripts that have been spliced to remove the NS1 ORF, and can be polyadenylated at either site. Human bocavirus (HBoV) was discovered in 2005 and subsequent PCR-based surveys have indicated that it is a common human respiratory pathogen, mostly of infants and young children. Although sero-epidemiologic assays remain to be developed, studies so far indicate that HBoV is likely the cause of a substantial proportion of undiagnosed respiratory tract disease, and appears to be distributed worldwide.

Genera in the Subfamily *Densovirinae*

Genus *Densovirus*

The homotelomeric densovirus genome is typically about 6 kbp in length, with long ITRs. The genome contains a >500 nt long ITR, the first ~100 nt of which are predicted to be able to fold as a T-shaped structure. These viruses, along with the pefudensoviruses described below, represent a paradigm shift for the members of the family *Parvoviridae*, since they exhibit ambisense organization. Although they appear to maintain the division of the genome into separate nonstructural and structural gene cassettes typical of all other family members, these cassettes are inverted with respect to one another, as shown in **Figure 3**. Populations of virions encapsidate equal numbers of positive and negative strands. The positive strand, by convention the strand encoding the nonstructural proteins, contains three ORFs, which are predicted to encode three NS proteins, and which are accessed by alternative splicing of mRNAs transcribed rightward from a promoter just within the ITR at the left-hand end. The negative, or complementary, strand expresses four structural proteins by alternative translation initiation, from an mRNA transcribed leftward from the homologous promoter just inside the right-hand ITR.

Genus *Pefudensovirus*

Like the members of the genus *Densovirus*, viruses belonging to the genus *Pefudensovirus* exhibit an ambisense organization. The homotelomeric genome is ~5.5 kbp in length, with a rightward promoter at 3 map units and a leftward promoter at 97 map units, located within the opposing 201 nt ITRs. As can be seen in **Figure 3**, the VP gene is located in the 5′ half of the complementary strand, and is split into a small upstream ORF and a large downstream ORF, which appear to be spliced in order to code for the largest VP, expression of which also appears to require frameshifting after splicing. Unlike other members of the family *Parvoviridae*, in which the PLA2 domain is expressed in the N-terminus of the largest structural protein, in pefudensoviruses this motif is centered 60–70 amino acids from the C-terminus of the ORF predicted to encode the small VP protein. The ORFs predicted to encode the three pefudensovirus nonstructural proteins are organized in the same way as for those of the genus *Densovirus*, and are of similar sizes.

Genus *Iteravirus*

The homotelomeric iteravirus genome is ~5 kbp in length, and populations of virions encapsidate equal numbers of plus and minus strands, in separate particles. The monosense genome, diagrammed in **Figure 3**, has an ITR of 230 nt, the first 159 of which are predicted to fold in a 'J-shaped' hairpin structure. There are two ORFs for nonstructural proteins and one ORF encoding the structural proteins, located on the same strand. A predicted transcriptional promoter resides upstream of each nonstructural ORF, such that each is expressed from its own transcript. A further putative promoter, lying downstream of the nonstructural genes, is predicted to drive production of the structural gene transcript, from which the synthesis of four or five overlapping structural proteins is proposed to occur by a leaky scanning translational initiation process.

Genus *Brevidensovirus*

The monosense genomes of members of the genus *Brevidensovirus* are the smallest within the family, being about 4 kbp in length. This is the only genus within the subfamily *Densovirinae* that have heterotelomeric genomes, and their virions encapsidate predominantly (85%) negative-sense strands. The left-hand end of a representative of the type species, *Aedes aegypti densovirus*, comprises a palindromic sequence of 146 nt while the right-hand end is a palindromic sequence of 164 nt. While different in sequence, both terminal sequences are predicted to fold into a T-shaped structure. As shown in **Figure 3**, the genome contains two transcriptional promoters, at map units 7 and 60. Two overlapping ORFs encoding the nonstructural proteins occupy more than half of the genome, the balance being occupied by a single ORF encoding the structural polypeptides. The genome does not contain any translated sequence recognizable as a PLA2 domain. Viruses belonging to this genus are evolutionarily extremely remote from all other members of the family *Parvoviridae*, and infect arthropod species as diverse as mosquito and shrimp. The latter include viruses that cause hypodermal and hematopoietic necrosis, infecting all multiple organs of ectodermal and mesodermal origin

of the shrimp, but not the midgut. These viruses quite closely resemble the mosquito viruses in both genome size and organization. Recently, however, a number of parvoviruses have been isolated from shrimp that infect hepatopancreatic epithelial cells. While these cluster phylogenetically with the brevidensoviruses when their common sequences are compared, they are more than 50% larger, and differ from them substantially in genome organization. Namely, their two nonstructural ORFs are arranged in tandem, and their structural protein genes are also substantially longer, although they are still devoid of a recognizable PLA2 domain. Thus, in contrast to the founding members of this genus, these shrimp viruses have the largest genomes in the family *Parvoviridae*, and it is not yet clear whether they represent a new genus separate from the genus *Brevidensovirus*.

Acknowledgments

The author would like to thank Susan Cotmore, David Pintel, and Peter Tijssen for much helpful discussion, and Lakshmanan Govindasamy and Mavis Agbandje-McKenna for providing the images for **Figure 1**. The author was supported by PHS grants AI26109 and CA29303 from the National Institutes of Health.

See also: Parvoviruses of Vertebrates.

Further Reading

Agbandje-McKenna M and Chapman M (2006) Correlating structure with function in the viral capsid. In: Kerr J, Cotmore SF, Bloom ME, Linden RM, and Parrish CR (eds.) *The Parvoviruses*, ch. 10, pp. 125–139. London: Hodder Arnold.

Carter BJ, Burstein H, and Peluso RW (2004) Adeno-associated virus and AAV vectors for gene delivery. In: Templeton NS (ed.) *Gene and Cell Therapy: Therapeutic Mechanisms and Strategies*, ch. 5, pp. 71–102. New York: Dekker.

Cotmore SF and Tattersall P (2006) Genome structure and organization. In: Kerr JR, Cotmore SF, Bloom ME, Linden RM, and Parrish CR (eds.) *The Parvoviruses*, ch. 7, pp. 73–94. London: Hodder Arnold.

Cotmore SF and Tattersall P (2006) Parvoviruses. In: DePamphilis M (ed.) *DNA Replication and Human Disease*, ch. 29, pp. 593–608. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Qiu J, Yoto Y, Tullis G, and Pintel DJ (2006) Parvovirus RNA processing strategies. In: Kerr J, Cotmore SF, Bloom ME, Linden RM, and Parrish CR (eds.) *The Parvoviruses*, ch. 18, pp. 253–273. London: Hodder Arnold.

Tattersall P (2006) The evolution of parvoviral taxonomy. In: Kerr J, Cotmore SF, Bloom ME, Linden RM, and Parrish CR (eds.) *The Parvoviruses*, ch. 1, pp. 5–14. London: Hodder Arnold.

Tattersall P, Bergoin M, Bloom ME, *et al.* (2005) *Parvoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 353–369. San Diego, CA: Elsevier Academic Press.

Tijssen P, Bando H, Li Y, *et al.* (2006) Evolution of densoviruses. In: Kerr J, Cotmore SF, Bloom ME, Linden RM, and Parrish CR (eds.) *The Parvoviruses*, ch. 5, pp. 55–68. London: Hodder Arnold.

Wu Z, Asokan A, and Samulski RJ (2006) Adeno-associated virus serotypes: Vector toolkit for human gene therapy. *Molecular Therapy* 14: 316–327.

Young NS and Brown KE (2004) Parvovirus B19. *New England Journal of Medicine* 350: 586–597.

Pecluvirus

D V R Reddy, Hyderabad, India
C Bragard, Université Catholique de Louvain, Leuven, Belgium
P Sreenivasulu, Sri Venkateswara University, Tirupati, India
P Delfosse, Centre de Recherche Public-Gabriel Lippmann, Belvaux, Luxembourg

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Coiled-coil motif A protein structure in which two to six α-helices of polypeptides are coiled together like the strands of a rope.
Hetero-encapsidation Partial or full coating of the genome of one virus with the coat protein of a differing virus. Also termed transcapsidation or heterologous encapsidation.
Leaky scanning mechanism Mechanism by which the ribosomes fail to initiate translation at the first AUG start codon, and scan downstream for the next AUG codon.
Post-transcriptional gene silencing Mechanism for sequence-specific RNA degradation in plants.
t-RNA-like structure Structure mimicking a t-RNA.
Virus-like particles Consist of the structural proteins of a virus. These particles resemble virions meaning that they are not infectious.

History

Pecluviruses, responsible for the 'clump' disease in peanut (=groundnut, *Arachis hypogaea*), have been reported from West Africa and the Indian subcontinent and contribute

globally to annual losses estimated to exceed 38 million US dollars. Pecluviruses also cause economic losses also to other dicotyledonous crops such as pigeonpea, chilli, cowpea, and monocotyledonous crops such as wheat, barley, sorghum, pearl millet, foxtail millet, maize, and sugarcane.

Taxonomy and Classification

The genus *Pecluvirus* comprise two species: isolates from West Africa were grouped under the species name *Peanut clump virus* (African peanut clump virus), and those that occur in India were grouped under *Indian peanut clump virus*. Once the molecular features of the genomes of Indian peanut clump virus (IPCV) and peanut clump virus (PCV) were reported, the ICTV in 1997 assigned them to the newly established genus *Pecluvirus* (siglum from peanut clump). The two viruses differ in host range, antigenic properties, and genomic sequences (see below). Furthermore, the viruses can be distinguished from their geographical location.

Geographic and Seasonal Distribution

IPCV occurs in India in the states of Andhra Pradesh, Gujarat, Punjab, Rajasthan, and Tamil Nadu, and in Pakistan in the provinces of Sindh and Punjab. In Africa, PCV has been reported from Benin, Burkina Faso (Saria, Kamboinsé, Bobo Dioulasso, Niangoloko), Chad, Congo, Côte d'Ivoire, Niger (Sadoré, Maradi), Mali (Segou, Koutiala, Bamako), Gabon, Senegal (Bambey, Cap-Vert, Thies, Sine, Saloum, Pout, Mbour, Kirene), and Sudan. IPCV occurs mainly in the rainy season (July–November) in peanut crops. In post-rainy season (December–March), crops escape the disease. In West Africa, the majority of the peanut crops are grown during the rainy season.

Biological Properties

Host Range and Symptoms

PCV infects economically important crops such as peanut, sorghum, sugarcane, maize, pearl millet, finger millet, and cowpea, whereas IPCV infects peanut, cowpea, pigeon pea, chilli, wheat, barley, pearl millet, finger millet, sorghum, and maize. Both the viruses also infect several monocotyledonous weeds (*Cynodon dactylon, Cyperus rotundus*) that can play a vital role in the survival and dissemination.

Affected plants are conspicuous in the field as a result of their dark green appearance, stunting, and occurrence in patches. IPCV occurs at a high incidence in Rajasthan, India, where peanuts are grown on over 250 000 ha in rotation with irrigated winter wheat and barley and with rain-fed pearl millet during summer. Early-infected plants will not yield and even late-infected crops showed reduction in crop yields up to 60%.

The cereal hosts act as reservoirs of inoculum. The Hyderabad isolate of IPCV (IPCV-H) in young wheat plants up to 3 weeks old induced symptoms similar to rosette caused by soil-borne wheat mosaic virus (SBWMV). Diseased plants are stunted with poorly developed root system. Grain yield losses up to 58% were recorded. Wheat CV RR-21 infected with Durgapura isolate (IPCV-D) showed reduced growth without any overt symptoms on leaves. IPCV-infected barley plants were stunted and bushy, with chlorotic or necrotic leaves, and the majority of these plants died. Those plants that reached maturity produced poorly developed spikes. IPCV-H could also infect finger millet, foxtail, or Italian millet, pearl millet, and sorghum plants. In maize, IPCV-H is responsible for aerial biomass losses up to 33% and grain loss up to 36%.

PCV caused red mottle and chlorotic streaks or stripes on sugarcane with incidence up to 50%. The symptoms varied considerably depending on the cultivar. Yield reductions up to 6% were recorded and the sugar yield was reduced by 14%. In Niger, PCV caused yield losses to sorghum grain up to 62%, to sorghum straw up to 45%, and to pearl millet straw up to 15%, whereas no effect on millet grain yield was observed.

Various of PCV and IPCV were readily detected in the cells of roots, stems, and leaves of systemically infected hosts. PCV particles in wheat cells were found in the cytoplasm, near the nucleus or along the plasmalemma, and arranged in angled-layer aggregates. IPCV and PCV have wide experimental host ranges which include both dicotyledonous and monocotyledonous plants. *Nicotiana clevelandii* × *Nicotiana glutinosa* hybrid and *Phaseolus vulgaris* (Top Crop) are suitable for propagation and as assay/diagnostic hosts, respectively, for IPCV. The isolates of IPCV collected from clump-diseased peanut crops from different locations differed slightly in their host ranges. *Canavalia ensiformis* and *N. clevelandii* × *N. glutinosa* hybrid were found to be useful for distinguishing the IPCV isolates. *Chenopodium amaranticolor, Nicotiana benthamiana, N. glutinosa, P. vulgaris*, and *Triticum aestivum* are of diagnostic value for PCV. The symptoms induced in *C. amaranticolor* by various PCV isolates collected from Senegal, Burkina Faso, and Niger were shown to differ markedly. *Nicotiana benthamiana* and *P. vulgaris* are the propagation species of PCV.

Transmission

Pecluviruses can be transmitted by sap and through peanut seed (PCV up to 6% and IPCV up to 24%). IPCV is transmissible through the seed of pearl millet, finger millet, foxtail millet, wheat, and maize generally at rates of $<2\%$. IPCV has been shown to be transmitted by the plasmodiophorid, *Polymyxa graminis*. *Sorghum arundinaceum*

(bait plant) is a suitable host for testing vector transmission of PCV to roots. Convincing evidence for PCV transmission by *Polymyxa* is still lacking. The thick-walled resting spores of the vector probably carry the virus and contribute to its survival. *P. graminis* from tropical and subtropical regions differed in ribotype and temperature requirements. For North American and European *P. graminis* f. sp. *temperata*, optimum temperature for survival is between 15 and 20 °C as opposed to the narrow optimum temperature range (close to 30 °C) for the Indian *P. graminis* f. sp. *tropicalis*. *Polymyxa graminis* in the tropics has a wide host range, including monocotyledonous and dicotyledonous plants, as opposed to the narrow host range (largely restricted to monocotyledons) for *P. graminis* f. sp. *temperata*. The plasmodiophorid needs a cereal host for completing its life cycle.

Serological Diagnosis

PCV is highly immunogenic and its polyclonal antibodies did not react with IPCV, barley stripe mosaic (BSMV), tobacco mosaic (TMV), beet necrotic yellow vein, potato mop top (PMTV), and SBWMV viruses. Wide serological diversity exists among isolates of PCV and IPCV. Therefore serological tests have limitations to detect more than one isolate from a single antibody source. Antisera to different isolates of IPCV facilitated the grouping of isolates into three serotypes, IPCV-H, IPCV-D, and IPCV-Ludhiana (IPCV-L). All IPCV serotypes are serologically distinct from PCV isolates, and vice versa. Utilizing four different formats of enzyme-linked immunosorbent assay (ELISA) and a panel of monoclonal antibodies raised against a PCV isolate, a number of PCV isolates were grouped into five serotypes. Interestingly, one of the monoclonal antibodies reacted with IPCV-D in triple antibody sandwich ELISA.

Molecular Diagnosis

To detect pecluvirues in disease surveys, to eliminate virus-contaminated sources in quarantine, and to devise strategies for disease management, it is essential to utilize diagnostic tools that are highly sensitive and broadly specific. Nucleic acid probes (both radioactive and nonradioactive) for the conserved regions were found to be ideal for pecluvirus diagnosis. Initially probes derived from the 742 nt at the 3′ end of IPCV-RNA1 were used. Subsequently, probes targeting the P14 gene and a probe (CGAGCCATAGAGCACGGTTGTGGG) derived from the conserved 3′ terminal ends of both RNA1 and RNA2 of IPCV facilitated highly sensitive detection of a range of IPCV and PCV isolates. Of the various methods tested, reverse transcription-polymerase chain reaction (RT-PCR) was found to be the most suitable one.

Molecular Properties

Virus Particles

ICPCV virions are nonenveloped rigid rods, 24 nm in diameter, with two predominant lengths of *c.* 250 and *c.* 180 nm. PCV particles contain two predominant rigid rods measuring *c.* 245 and *c.* 190 nm and 21 nm in diameter with a clear axial canal. They contain a single coat protein (CP) of 23 kDa.

Genome

The pecluvirus genome is bipartite. PCV and IPCV virions contain positive-sense, single-stranded RNAs (4% by weight) RNA1 is *c.* 5900 nt long and RNA2 is *c.* 4500 nt long. The RNAs of PCV showed little sequence identity with those of IPCV, with the exception for the 3′ terminal 273 nt, which are conserved between the two RNAs (**Figure 1**).

Coding Sequences

RNA1 encodes two proteins involved in viral RNA replication (P131 and P191). P191 is a C-terminally extended form of P131 produced by translational readthrough of the UGA termination codon (P131) (**Table 1**). Additionally RNA1 codes for P15, a suppressor of post-transcriptional gene silencing (PTGS). The P15 open reading frame (ORF) is downstream of the P191 ORF and separated from it by a noncoding region of about 60 nt. The amino acid sequence of P191 contains methyltransferase, helicase, and RNA-dependent RNA polymerase domains. P15 on RNA1 is a cysteine-rich protein (CRP), translated from a relatively abundant sub-genomic RNA1 (**Figure 1**). P15 resembles CRPs of BSMV, poa semilatent virus, and SBWMV. These proteins have been suggested to act as regulatory factor during virus replication as well as for long-distance movement and contribute to virulence factor. RNA1 is able to replicate in the absence of RNA2 in protoplasts of tobacco BY-2 cells. However, both RNA1 and RNA2 are needed for infection. Experiments using enhanced 5′ green fluorescent protein and 5′-bromouridine 5′-triphosphate labels have suggested that PCV replication complexes co-localize with endoplasmic reticulum green fluorescent bodies accumulating around the nucleus during infection. P15 does not act directly at sites of viral replication but intervenes indirectly to control viral accumulation levels. It acts as a suppressor of PTGS, though it shares no sequence similarities with previously described anti-PTGS molecules encoded by other viruses. The P15 possesses four C-terminal proximal heptad

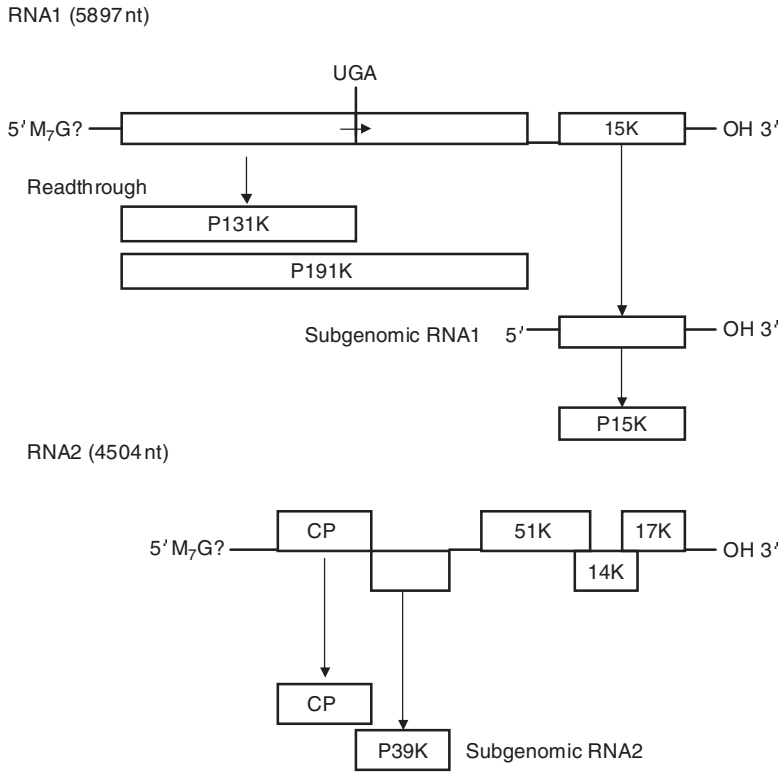


Figure 1 Illustrates the organization of the pecluvirus genome.

repeats that can generate a coiled-coil interaction and is targeted to peroxisomes via a C-terminal SKL motif. Such a motif is conserved among pecluviruses from both Africa and India. It has been demonstrated that a coiled-coil motif is necessary for the anti-PTGS activity of P15, but the peroxisomal localization signal is not, although it is required for efficient intercellular movement of the virus.

RNA2 is relatively more complex and encodes five polypeptides (**Figure 1**). The 5′-proximal ORF (23K) encodes the CP. The following ORF (ORF2) encodes P39, a putative vector transmission factor, which is expressed by a leaky scanning mechanism *in vitro*. ORF2 starts 1 nt upstream of the first residue of UGA stop codon of the CP cistron. The remaining three ORFs encode P51, P14, and P17 and form a triple gene block (TGB) (**Table 1**). TGB plays a role in virus movement. P51 is translated from a relatively abundant subgenomic RNA2, but subgenomic RNAs responsible for the synthesis of P14 and P17 have not yet been detected. However, analogy with other TGB-containing viruses suggests that both proteins are probably translated from a low-abundance subgenomic RNA with a 5′ terminus upstream of the P14 gene.

Noncoding Sequences

The 5′ and 3′ noncoding regions (NCRs) of RNA1 are about 130 and 300 nt in length, respectively (**Figure 1**). Those of the RNA2 are more diverse, between *c.* 390 and *c.* 500 nt in length. There is no distinct 5′ sequence feature common to all pecluviruses. RNA1 and RNA2 have similar 5′ NCRs except for six to seven nucleotides and these sequences are shared among pecluvirus species. The 3′ NCRs are *c.* 300 nt in length, and *c.* 100 terminal nucleotides are identical among pecluvirus RNAs sequenced so far. Such sequence similarity has enabled the development of a hybridization probe corresponding to the 3′-terminal 700 nt of IPCV-H. This probe detected all the currently known IPCV serotypes as well as an isolate of PCV.

The 3′ NCR of pecluviruses, as in the case of furoviruses, forms a t-RNA-like structure (TLS) that is capable of high-efficiency valylation and aids in the replication of both PCV RNAs. The internal NCRs in RNA1 are present between P191 and P15 (60 nt) and in RNA2 between P39 and P51 (145 nt).

Sequence Comparisons

The RNA1-encoded polypeptides of PCV and IPCV share identities ranging from 75% (P15) to 95% (readthrough part of P191) and show significant similarities with furoviruses (e.g., 56% identity with polymerase of SBWMV). The proteins encoded by RNA2 are 39% (P39) to 89% (P14) identical between species. The CPs are

Table 1 Pecluvirus open reading frames, polypeptides, and their functions

Genomic RNA	*ORF*	*M_r of polypeptide (kDa)*	*Function*
RNA1	P131	131	Methyltransferase, helicase, replicase
	P191	191	
	P15	15	Suppressor of PTGS
RNA2	P23	23	Coat protein
	P39	39	Putative vector transmission factor
	P51	51 Triple gene block	
	P14	14	Virus movement
	P17	17	

c. 60% identical and also have significant similarity (*c.* 30% identity) with the CP of BSMV (genus *Hordeivirus*). The TGB proteins resemble those of PMTV (genus *Pomovirus*).

The nucleotide sequence of IPCV-H RNA1, is similar to that of PCV and the polypeptides encoded by this are 60–95% identical. Comparison of the P15 gene shows a close relationship between IPCV isolates and a relatively high diversity among PCV isolates (**Figure 2**).

The five RNA2-encoded ORFs of IPCV-L are between 32% and 93% identical to those encoded by PCV RNA2. The partial nucleotide sequences of RNA2 of IPCV-H and -D showed that the polypeptides encoded by the two 5′-proximal ORFs (CP and P39) are similar to those of the IPCV-L serotype. A conserved motif 'F-E-X_6-W' is present near the CP C-terminus of all three IPCV serotypes and PCV, as in the CPs of other rod-shaped viruses (TMV and tobacco rattle virus).

The full-length sequence comparison between RNA2 of four isolates of PCV and two isolates of IPCV have revealed a high degree of variability in size (between 58% and 79%). Amino acid sequence alignments of each of the five ORFs of RNA2 showed that ORF4, encoding P14 of TGB, is highly conserved (90–98% identical), whereas the P39 encoded by ORF2 is less conserved (25–60% identical). The CP of eight isolates showed amino acid sequence identities between 37% and 89%. Phylogenetic comparisons, based on complete RNA2 sequences, showed that the eight isolates could be grouped into two distinct clusters with no geographical distinction between PCV and IPCV isolates. Phylogenetic tree topologies for individual ORFs revealed an overall similarity with that obtained from complete RNA2 sequences, but the relative positions of individual isolates varied within each cluster. Further, such studies indicate that there is substantial divergence among the RNA2's of pecluviruses and suggest that different polypeptides have evolved differently, possibly due to different selection pressures.

Several PCV isolates propagated in *N. benthamiana* contain an RNA2 shorter than that of the type isolate. Partial characterization of two such isolates revealed that their RNA2′s have undergone deletions in ORF2. The impact of deletions in ORF2, implicated in vector transmission, is to be established.

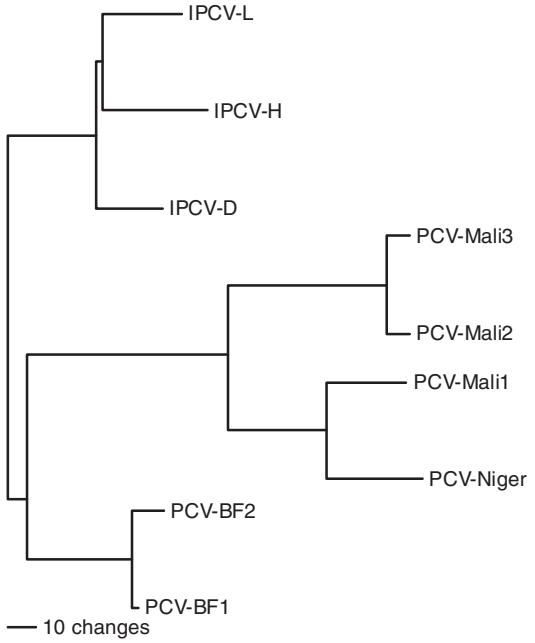


Figure 2 Maximum parsimony phylogenetic tree obtained by a heuristic search from 352 characters of the P15 ORF of IPCV and PCV. Multiple alignments of nucleotide sequences of the P15 ORF from different sources of IPCV (D, H, L serotypes) and PCV isolates from Mali, Burkina Faso (BF), and Niger were obtained using CLUSTAL W 2.08b with the suggested default settings. Phylogenetic analyses were performed using PAUP 4.0 beta 1 (Sinauer Associates, Inc., Sunderland, MA) (Dieryck and Bragard, unpublished data).

Assembly of Virus Particles

The origin of assembly sequence (OAS) positions has been identified in the ssRNA genomes of several rod-shaped viruses. By testing the ability of different RNA1 and RNA2 deletion mutants to be encapsidated *in vivo*, the RNA1 and RNA2 sequences required for assembly into PCV virions have been established. A putative OAS was mapped in the 5′-proximal part of the P15 gene of RNA2. Nevertheless, the nonencapsidation of subgenomic RNA that encodes P15 raises questions about the mechanism underlying the encapsidation process. Two sequence positions that could drive encapsidation of RNA2

have been identified. One is in the 5′-proximal CP gene and the other in the P14 gene near the RNA2 3′ terminus. No obvious sequence similarities between different assembly initiation sequences have been noted. The initiation of PCV assembly, like that of TMV, probably involves interaction of CP with a relatively short sequence which is presented by an RNA secondary structure in a special configuration.

Interestingly, the possible localization of an OAS in the CP gene of IPCV was realized as a result of formation of virus-like particles (VLPs) in *Escherichia coli* and *N. benthamiana*, confirming the results observed for PCV.

The assembly of VLPs in either bacteria or plants has been proposed as a way to protect and accumulate specific mRNA, as a means to study the molecular assembly of the capsid and for the production of oral vaccines. In genetically transformed *N. benthamiana* or *E. coli* cells, the cloned IPCV-H CP gene is expressed and assembled into VLPs. The monomer VLP size approximately corresponds to the one expected, according to the length of the encapsidated CP gene transcript RNA. Using immunocapture RT-PCR(IC-RT-PCR), such VLPs have been demonstrated to contain RNA encoding IPCV-H CP. When transgenic *N. benthamiana* expressing IPCV-H CP gene inoculated with the serologically distinct IPCV-L serotype, accumulated virus particles that contained both types of CP, the possibility of hetero-encapsidation in transgenic plants was suggested.

Epidemiology and Management

Introduction

The control of peanut clump depends upon the accurate and sensitive detection of IPCV and PCV in plants, seeds, and soil. Peanut clump disease is largely restricted to sandy soils and sandy loams. Detection is needed to identify infested fields and implement appropriate management strategies; second, to eliminate seed lots infected by the viruses and to assess the resistance of peanut, pearl millet, sorghum, and sugarcane breeding lines.

Life Cycle

Dicotyledonous hosts restrict the multiplication of the plasmodiophorid vector and hence are considered as fortuitous hosts that may not contribute to perpetuation of virus inoculum. Indeed, either virus-infected peanut roots or seed could transmit or establish the disease. Monocotyledonous plants such as maize, pearl millet, and sorghum are 'preferred' hosts of the vector and contribute to the build-up of vector inoculum potential in the soil. Seed of millets, maize, and wheat and rhizomatous grasses (e.g., *Cynodon dactylon*) are likely to contribute to the disease establishment in new areas by supporting the multiplication of both the virus and plasmodiophorid vector.

The role of rainfall and temperature in the dynamics of infection by IPCV-H and its vector were analyzed on various crops. Wheat followed by barley showed the highest virus incidence, although *P. graminis* was rarely observed in the roots of wheat and was not detected in those of barley. The roots of maize, pearl millet, and sorghum plants, colonized by *P. graminis*, showed the presence of the virus. Peanut is a systemic host for the virus but no *P. graminis* was found in its roots. High rainfall soon after summer months resulted in high incidences of the disease. Weekly rainfall of 14 mm is sufficient for the vector to initiate infection. Temperatures (27–30 °C) prevailing during the rainy season were found to be conducive to virus transmission. At temperatures below 23 °C, infection did not occur and the development of the plasmodiophorid was delayed. This appears to be the major reason for the absence of clump disease on crops raised during the post-rainy season in India.

Cultural Practices

Continuous cropping with fortuitous hosts such as peanut, cowpea, or pigeonpea is likely to reduce the plasmodiophorid population in soil. Seed-borne inoculum from dicotyledonous hosts does not aid in disease establishment. However, seed-borne inoculum from cereal hosts and rhizomatous grasses can contribute to disease establishment.

Initial experiments showed that application of soil biocides (e.g., dibromochloropropane, DD, fumigant nematicides that also have fungicidal action) and soil solarization were effective in reducing disease incidence. Nonetheless, they are not economical to adopt and additionally are hazardous to use.

The following cultural practices can reduce disease incidence of IPCV:

1. early planting of groundnut before the onset of the monsoon under judicious irrigation;
2. trap cropping with pearl millet, that is, sowing a pearl millet crop at a high density and then ploughing the entire crop, 2 weeks after germination, into the soil and then planting with peanut;
3. avoiding rotation of peanut with such highly susceptible crops as sorghum, wheat, and maize; and
4. maintain continuous cropping with dicotyledonous crops (peanut, pigeonpea, cowpea, and marigold) for at least three growing seasons.

The above-recommended measures are ecofriendly and economical and are practicable even under marginal farming conditions.

Host Plant Resistance

No resistance to IPCV was found in over 9000 cultivated *Arachis* germplasm lines. Resistance to IPCV was identified

in a wild *Arachis* sp., but it is yet to be incorporated into cultivated peanut. Transgenic peanut lines carrying virus genes (CP and replicase) are currently being evaluated. However, they are unlikely to be available in the near future for cultivation.

Future Perspectives

The suspected role of P39 in vector transmission of the virus needs to be confirmed probably by mutational analysis to exploit this ORF in transgenic research. The presence of subgenomic RNAs encoding P14 and P17 in infected tissues needs to be verified. The cultural control measures tested for IPCV are worth exploiting in West Africa to minimize the impact of PCV.

The most economical way to control pecluviruses is by developing virus-resistant cultivars. The organizations involved must seek approvals from appropriate licensing agencies so that the transgenic plants will become available for cultivation.

See also: Furovirus.

Further Reading

Bragard C, Doucet D, Dieryck B, and Delfosse P (2006) Detection of pecluviruses. In: Rao GP, Kumar PL, and Holguin-Peña RJ (eds.) *Characterization, Diagnosis & Management of Plant Viruses: Vegetable and Pulse Crops,* 1st edn., vol. 3, pp. 125–140. Houston: Studium Press.

Bragard C, Duncan GH, Wesley SV, Naidu RA, and Mayo MA (2000) Virus-like particles assemble in plants and bacteria expressing the coat protein gene of *Indian peanut clump virus*. *Journal of General Virology* 84: 267–272.

Delfosse P, Reddy AS, Thirumala Devi K, *et al.* (2002) Dynamics of *Polymyxa graminis* and *Indian peanut clump virus* (IPCV) infection on various monocotyledonous crops and groundnut during the rainy season. *Plant Pathology* 51: 546–560.

Dunoyer P, Pfeffer S, Fritsch C, Hemmer O, Voinnet O, and Richards KE (2002) Identification, subcellular localization and some properties of a cystein-rich suppressor of gene silencing encoded by peanut clump virus. *Plant Journal* 19: 555–567.

Fritsch C and Dollet M (2000) Genus *Pecluvirus*. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, *et al.* (eds.) *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, pp. 913–917. San Diego: Academic Press.

Hemmer O, Dunoyer P, Richards K, and Fritsch C (2003) Mapping of viral RNA sequences required for assembly of peanut clump virus particles. *Journal of General Virology* 84: 2585–2594.

Herzog E, Guilley H, Manohar SK, *et al.* (1994) Complete sequence of peanut clump virus RNA-1 and relationships with other fungus transmitted rod shaped viruses. *Journal of General Virology* 75: 3147–3155.

Herzog E, Hemmer O, Hauser S, Meyer G, Bouzoubaa S, and Fritsch C (1998) Identification of genes involved in replication and movement of peanut clump virus. *Virology* 248: 312–322.

Legrève A, Delfosse P, and Maraite H (2002) Phylogenetic analysis of *Polymyxa* species based on nuclear 5.8S and internal transcribed spacers ribosomal DNA sequences. *Mycological Research* 106: 138–147.

Legrève A, Delfosse P, Vanpee B, Goffin A, and Maraite H (1998) Differences in temperature requirements between *Polymyxa* sp. of Indian origin and *Polymyxa graminis* and *Polymyxa betae* from temperate areas. *European Journal of Plant Pathology* 104: 195–205.

Manohar SK, Dollet M, Dubern J, and Gargani D (1995) Studies on variability of peanut clump virus: Symptomatology and serology. *Journal of Phytopathology* 143: 233–238.

Miller JS, Wesley SV, Naidu RA, Reddy DVR, and Mayo MA (1996) The nucleotide sequence of RNA-1 of Indian peanut clump furovirus. *Archives of Virology* 141: 2301–2312.

Naidu RA, Miller JS, Mayo MS, Wesley SV, and Reddy AS (2000) The nucleotide sequence of Indian peanut clump virus RNA 2: Sequence comparisons among pecluviruses. *Archives of Virology* 145: 1857–1866.

Reddy AS, Hobbs HA, Delfosse P, Murthy AK, and Reddy DVR (1998) Seed transmission of Indian peanut clump virus (IPCV) in peanut and millets. *Plant Disease* 82: 343–346.

Reddy DVR, Mayo MA, and Delfosse P (1999) Pecluviruses. In: Granoff A and Webster RG (eds.) *Encyclopedia of Virology,* 2nd edn., vol. 2, pp. 1196–1200. New York: Academic Press.

Pepino Mosaic Virus

R A A Van der Vlugt, Wageningen University and Research Centre, Wageningen, The Netherlands
C C M M Stijger, Wageningen University and Research Centre, Naaldwijk, The Netherlands

© 2008 Elsevier Ltd. All rights reserved.

Glossary

IEM (immunosorbent electron microscopy) Identification of a virus by visualization in the electron microscope of the reaction of the virus coat with a specific antiserum.

RFLP (restriction fragment length polymorphism) A technique whereby DNA fragments of approximately the same length but with different sequences can be differentiated by the patterns of fragments that are generated by digestion with restriction enzymes.

RT-PCR (reverse transcriptase polymerase chain reaction) A PCR reaction whereby first a cDNA copy of the viral RNA has to be generated by reverse transcriptase (RT) before PCR-amplification is possible.

Virus symptomatology Typical local or systemic symptoms on natural host plants or experimental test plants that can be used to characterize and differentiate plant viruses and plant virus strains.

Introduction

Pepino mosaic virus (PepMV) was first found in 1974 in field samples of pepino plants (*Solanum muricatum*) located near some potato fields, collected in the Canete valley in coastal Peru. These plants showed some yellow mosaic in young leaves. Electron microscope (EM) investigations showed the presence of typical filamentous potexvirus particles of approximately 500 nm, which did not react with antisera against potato virus X (PVX). Serologically, it appeared most closely related to narcissus mosaic virus (NaMV) but the host ranges of both viruses differ considerably. It was concluded that pepino mosaic virus was a new and distinct potexvirus.

After its initial description in 1980 the virus was not again reported and was not considered to be of any agricultural significance until it was again found in 1999 in commercial protected tomato crops (*Lycopersicon esculentum*) in the UK and the Netherlands. Since then the virus has spread very rapidly through commercial tomato crops worldwide and was reported from Belgium, Canada, Chile, China, France, Germany, Italy, Finland, Morocco, Norway, Peru, Poland, Portugal, Spain, the Netherlands, UK, Ukraine, and the USA.

Host Range and Symptomatology

The host range of PepMV is quite narrow and seems mainly restricted to the Solanaceae of which many species are infected systemically. Its main hosts appear to be *Lycopersicon* sp. and *Solanum* sp. The originally described pepino strain was found to infect wild and commercial tuber-bearing *S. tuberosum* sp., mostly with a symptomless systemic infection or with mild mosaic symptoms, but in two local Peruvian *S. tuberosum* cultivars and *S. stoloniferum* PI 230557 it caused a severe systemic necrosis. The virus was shown to be transmitted through the tubers.

Its best-known natural hosts are pepino (*S. muricatum*) and cultivated tomato (*L. esculentum*) but surveys showed infection with PepMV of several related wild *Lycopersicon* sp. These include *L. peruvianum*, *L. parviflorum*, *L. chilense*, *L. chmielewskii*, and *L. pimpinellifolium*. Infections in these wild species were generally symptomless.

Several weed hosts were found to be hosts for PepMV. The original virus description reports weed hosts in Peru, including *Datura stramonium*, *Nicandra physaloides*, and *Physalis peruvianum*. More recent studies on material from mainland Spain and the Canary Islands showed that a large number of weeds species were latently infected (i.e., showing no symptoms).

The initially described PepMV caused a distinct yellow mosaic on young leaves of pepino and most infected plants also showed dark green enations on the lower surface of some leaves. In *Lycopersicon* sp., it caused a symptomless systemic infection as shown by back-inoculation on a sensitive indicator plants such as *Nicotiana glutinosa*. Only *Datura metel*, *D. stramonium*, and a number of *Nicotiana* sp. showed distinct symptoms upon systemic infection. The virus failed to infect 13 plant species in six other families (see **Table 1**).

In contrast to the original pepino strain, the virus found in 1999 in commercial tomato crops in the UK and the Netherlands did cause distinct symptoms in tomato. Symptoms may depend on the tomato cultivar, the age of the plant when first infected, and growing conditions, and included yellow leaf spots, yellow-green mosaic, mottle on the older leaves and/or slight curling in the top leaves (nettlehead symptom), or a grayish appearance of the top of the plant. Fruit symptoms range from alteration in color, mild sometimes concentric yellow/orange mottling, and uneven ripening to netting and cracking and shape distortion. Basically, the tomato strain causes much more pronounced plant symptoms: more severe leaf chlorosis, bright yellow mosaic, leaf distortions ('bubbling'), green striation on the stem and sepals. Some isolates are reported to cause more or less pronounced leaf and stem necrosis. Because of fruit symptoms, fruit quality can be affected which may result in economic damage. Symptom expression is reported to depend on environmental conditions with low temperatures and low light conditions favoring more pronounced symptoms. Symptoms are also reported to become less pronounced when conditions change. There are indications that the different virus strains may induce different symptoms.

Inoculation studies on sets of indicator plants clearly showed a number of differences between the original Pepino strain and tomato isolates (see **Table 1**). Especially, *D. metel, D. stramonium*, and *N. glutinosa* can be used to distinguish between strains.

Virion Properties

PepMV has filamentous particles typical of potexviruses with a normal length of 510 nm. Particles are comprised of a single capsid protein (CP) of approximately 26 kDa. Ultrathin section of infected leaf material may contain inclusions consisting of arrays of filamentous virus-like particles. The virus particles are less clearly cross-banded than those of PVX.

PepMV is fairly stable. In endpoint dilution studies, sap from infected *N. glutinosa* was always infectious at

Table 1 Host range and symptoms of different pepino mosaic virus strains and isolates

	Pepino isolate	*Dutch tomato type isolate*	*Italian tom isolate*	*Spanish tom isolate (Sp13)*
Capsicum annuum	/(vc,vn)	/	/	/
Chenopodium amaranticolor	/	/	+,cl/	/
C. quinoa	/	/	+, cl/	/
Cucumis sativus	/	/	/	/
Cucurbita pepo			/	
Datura stramonium	(nl)/nl	/c,m	+, cs/+, m	
D. metel	nl/l	/m		
Lycopersicon esculentum	/l	/(m)	+, cs/+, m	/
Nicotiana benthamiana	/cl	/c	+, cs/+, m	?/m, c,ld
N. bigelovii	nr/m	/l		
N. clevelandii			+, cl	?/m, c,ld
N. debneyi	/l	/l		
N. glutinosa	/vc,cr,m	/	/	/
N. hesperis-67A	nl,n/d,nl,n	nl,n/d,nl,n		
N. megalosiphon			+, cs, n/+, m.n	
N. occidentalis-P1	cl,nl/c,d,n,nl	cl,nl/c,d,n,nl		
N. rustica	nl/l	/m	+, cl	/
N. tabacum 'WB'	/(vc)	/	+, l/	/
Petunia hybrida	/	/	/	
Phaseolus vulgaris	/	/		/
Physalis floridana	/(cl,m,nl)	/		
Solanum melongena	/l	/m	+, cs/+, m	
S. muricatum	/(m)	/(m)	+, l/ +, l	
S. nigrum			+, cs/ +, m	
S. tuberosum	/	/	/	
Vicia faba	/	/		

The pepino isolate is the original Peruvian pepino isolate. The Dutch tomato type isolate is the isolate first described from the Netherlands. The Italian tomato isolate is the deviant 'chenopodium' isolate originally isolated from tomato plants. The Spanish tomato isolate is a typical Spanish tomato isolate.
Abbreviations: cl, chlorotic lesions; m, mosaic; n, necrosis; l, latent infection; c, chlorosis; cr, chlorotic rings; d, dwarfing; ld, leaf deformation; mo, mottle; nl, necrotic lesions; vc, veinal chlorosis; vn, veinal necrosis; ( ), symptoms observed occasionally.

dilutions of 10^{-4}, occasionally at 10^{-5} but never at 10^{-6}. Sap lost most of its infectivity after 10 min at 65 °C and was no longer infectious at 70 °C. Sap stored at 20 °C still shows some infectivity after 3 months while leaves of *N. glutinosa* desiccated over silica gel were still infectious after 6 months. Under practical conditions in greenhouses and fields, the virus may easily survive for several weeks in plant debris and on surfaces that have come in contact with virus-infected leaves or fruits.

A polyclonal antiserum raised against the original pepino isolate showed no reaction with PVX and potato aucuba mosaic virus (PAMV), the only two other potexviruses known to infect tomato. Both in immunosorbent electron microscopy (IEM) and in ELISA, it clearly reacted with different tomato isolates. Comparisons of antisera raised against the pepino and tomato strains showed differences in heterologous titers between them.

Genome Organization and Expression

The genome of PepMV resembles that of typical potexviruses. Its positive single-stranded RNA is 6410 nucleotides (nt) long, capped at the 5′ end, polyadenylated at the 3′ end and contains 5′- and 3′-nontranslated regions. It encodes five putative partly overlapping open reading frames (ORFs 1–5, see **Figure 1**).

The 5′-nontranslated region (5′-NTR) of the virus is 85 nt longs and like all other potexviruses it starts with the 5′-GAAAA pentanucleotide.

ORF1 (nt 86–4406) encodes a 164 kDa protein of 1439 amino acids (aa). It contains a putative methyltransferase domain (aa 59–224) specific for the supergroup of 'Sindbis-like' viruses, a NTPase/helicase domain (aa 708–934) with the NTP-binding motifs GCGGSGKS and VVIFDD and an RNA-dependent RNA polymerase (RdRp) domain (aa 1217–1374) characterized by the SGEGPTFDANT-X22-GDD motif.

The stop codon of the first ORF is followed by a short intergenic region (IR1) of 25 nt and a set of three partially overlapping ORFs typically known as the triple gene block (TGB).

ORF2 (nt 4432–5136) encodes the first TGB protein (TGBp1), a 234 aa protein of 26 kDa. This protein contains a typical NTPase/helicase motif (aa 26–233) characterized by seven conserved motifs, two of which may be involved in NTP binding. TGBp1 belongs to the superfamily I of RNA helicases.



Figure 1 Schematic organization of the RNA genome of pepino mosaic virus with its five open reading frames (ORFs). M7G = 5′ cap, AAAAA$_n$ = poly(A) tail.

ORF3 (nt 5117–5488) overlaps 19 nucleotides with the 3′-end of ORF2 and extends 148 nt past the start codon of ORF4. It encodes a small 14 kDa protein of TGBp2 which contains a potexvirus-specific consensus motif: PxxGDxxHxL/FPxGGxYxDGTKxxxY.

ORF4 (nt 5340–5594) encodes the third TGB protein (TGBp3), a 85 aa protein of 9 kDa. This protein is the most variable among potexviruses. It contains a CxV/IxxxG consensus motif among potexvirus TGBp3 proteins.

The second intergenic region (IR2) of 38 nt (nt 5595–5632) precedes ORF5 (nt 5633–6346) which encodes the 238 aa coat protein (CP) of 25 kDa. This CP contains the amphipathic core sequence KFAAFDFFDGVT. A similar sequence is also found in the CP of other potexviruses and might be responsible for binding of virus RNA to the CP through hydrophobic interactions.

The 64 nt long 3′-NTR (nt 6347–6410) precedes the poly(A) tail and contains the hexamer 5′-ACUUAA sequence which is also present in the 3′-NTR of all potexviruses sequenced so far. This motif is proposed to be a *cis*-acting element involved in the positive and negative viral RNA synthesis. The 5′-AAUAAA polyadenylation signal terminates the RNA genome, whereby the AAA portion forms the first A residues of the poly(A) tail.

Virus Isolates and Strains

The tomato isolate of PepMV described in 1999 clearly differed in a number of characteristics from the original pepino isolate from Peru. The differences are most pronounced in the reactions of both isolates on tomato (*L. esculentum*) in which the pepino isolate is symptomless and the tomato isolate causes distinct symptoms. Also on a number of test plants, the two isolates can be distinguished, most clearly on *N. glutinosa* and *D. stramonium* (see **Table 1**).

Since 1999 a large number of different PepMV isolates have been described. These were either isolated from commercial tomato crops in different countries or from wild *Lycopersicon* sp. Most show the typical characteristics of the tomato strain but some deviant isolates were observed. A number of isolates show more severe symptoms (including leaf and stem necrosis) in tomato or wild *Lycopersicon* sp.

Sequence data of most PepMV isolates are available from DNA fragments generated by RT-PCR reactions using degenerate primer sets based either on conserved potexvirus motifs or on PepMV specific sequences. Sequence comparisons of RT-PCR DNA fragments obtained from the RdRp region of the pepino isolate and a number of tomato isolates from different geographical origins show approximately over 99% sequence identity between the tomato isolates and only around 95% between all tomato isolates and the pepino isolate. Other studies confirmed the genetic distance between the original pepino isolate and the tomato isolates. Based on the available molecular and biological data, the tomato and pepino isolates are now considered two distinct strains of PepMV.

Comparison of full-length nucleotide sequences and deduced amino acid sequences of four tomato isolates from France and Spain confirmed the very high levels of sequence identity (over 99%) within the tomato isolates. Full-length sequence data were also obtained from an isolate collected from a wild *L. peruvianum* during a survey in Peru. This isolate induced only very mild mosaic and leaf distortions in *L. peruvianum* and was symptomless in *L. esculentum* upon mechanical inoculation. This isolate shows slightly lower levels of sequence identity with the isolates from the tomato strain (95.2–98.1%).

Interestingly, comparisons of sequence data obtained from RT-PCR fragments from many different tomato isolates identified a number of aberrant isolates. One Italian isolate obtained in 2000 from symptomatic tomato plants in Sardinia is divergent from the tomato and pepino strain in a 295 nt PCR-product derived from the methyltransferase motif in ORF1. Additionally, this isolate was described to infect *Chenopodium quinoa* and *C. amaranticolor*, both of which are not infected by either the tomato or pepino strain, and it also shows a distinctive single-stranded conformation polymorphism (SSCP) pattern. It is not clear whether this chenopodium-infecting isolate truly constitutes a new PepMV-strain.

In the USA the full sequences of two isolates collected in 2000 were obtained directly from dried symptomatic tomato leaves. The sequence of the 3′-end of a third isolate was determined directly from infected fresh tomato fruits. Direct nucleotide sequence comparisons showed only 79–82% overall identity between European tomato isolates from France and Spain and US isolates and 86% overall nucleotide identity between the RNA genomes of both US isolates. The 3′-end of the genome of the third US isolate showed between 92.9% (TGBp3) and 100% (CP) nt sequence identity to the two European tomato strain sequences.

An restriction fragment length polymorphism (RFLP) analysis of PCR products of the RdRp region obtained from 102 tomato samples collected at various locations in Spain

had already identified three distinct virus types: the typical tomato and pepino isolates and a clearly distinct third type. The cloned PCR product of isolate 3253, representative of the third type, had 80.5% identity with a tomato strain isolate and 79.3% with the previously mentioned Peruvian LP2001 isolate. Phylogenetic analysis showed it had only 60–69% identity to sequences of other potexviruses and it was most closely related but distinct from both the tomato and pepino PepMV strains. Interestingly, US isolates showed 98% identity to the sequence of Sp3253 indicating a very close relationship between these isolates.

Currently available biological data (i.e., host and indicator plant symptoms), serological relationships, and multiple nucleotide sequence alignments suggest at least three clusters of relationship between the PepMV isolates investigated so far:

1. The tomato (*L. esculentum*) type strain isolates, all highly similar.
2. The pepino (*S. muricatum*) type strain isolates, originally from pepino and wild *Lycopersicon* sp.
3. Isolates US1, US2, and Sp3253.

The status of the Italian *Chenopodium* isolate remains unclear by lack of sufficient biological and molecular data.

Virus Epidemiology

PepMV clearly originates from South America. Studies showed that the virus was widespread in *Lycopersicon* sp., in Peru, even in isolated wild populations, suggesting that Peru might be its center of origin. Interestingly, most infected *Lycopersicon* sp. showed no distinct symptoms. Several studies have confirmed that the original pepino isolate is characterized by the absence of, or only very mild symptoms on commercial tomato crops. In contrast, the strain now known as the tomato strain of PepMV causes more distinct symptoms in tomato. These symptoms were first noticed in commercial tomato crops in 1999 in the UK and the Netherlands and soon after that in many other countries worldwide.

Studies employing either strain-specific primers or comparisons of viral gene sequences revealed that the tomato strain is prevalent in all countries where the virus has been found. All tomato isolates found are highly similar and cannot be classified according to geographic origin, or symptoms on naturally or experimentally infected plants. These data indicate a fairly recent introduction and expansion of the tomato strain into commercial tomato crops, as the most likely cause for the virus epidemic.

Detailed population studies from Spain on material collected between 1998 and 2004 confirmed the prevalence and homogeneity of the tomato strain isolates as well as their presence since 1998. However, there were clear indications for several independent introductions of this virus strain, both on the Canary Islands and the Spanish mainland. Interestingly in addition to the tomato strain, the original pepino strain and the US2 strain were also found, but always in mixed infections with the tomato strain. Both strains have been shown to be present in Spain since 2000.

Although the first known occurrence of PepMV in North America can be traced back to 2000, the virus seems to differ from that in the EU. In addition to US3, an isolate highly homologous to the tomato strain, two distinct strains were found; US1 and US2. Both strains were identified from pooled symptomatic leaf material collected in 2000 in Arizona. No reports are known about the presence of the pepino strain in North America.

The data above suggest that different strains of PepMV, at different occasions, have spread from South America, most likely from different host plants, either pepino, wild *Lycopersicon* sp. or other as yet unidentified hosts, resulting in different introductions of the virus in different parts of the world. A number of virus characteristics are likely to have contributed to this:

- The original pepino strain is (nearly) symptomless in commercial tomato.
- Proper diagnostic tests for the virus only became available after the recognition of the tomato strain in commercial tomato crops.
- As a potexvirus, PepMV is easily mechanically transmissible and remains infectious for several weeks in plant debris or on contaminated surfaces.
- Tomato fruits harvested from infected plants contain high concentrations of virus.

PepMV introductions in commercial tomato crops may thus have remained largely unnoticed until the tomato strain manifested itself. At this moment no data are available on the possible occurrence of this strain in pepino or wild *Lycopersicon* sp. in South America and the origin of this strain remains unknown. It has been suggested that the tomato isolates show an increased fitness and virulence in commercial tomato. This might explain the fast worldwide spread of the virus.

Several studies report mixed infections of the tomato strains with either the pepino strain or US2 strain or of the pepino and US2 strains and in Spain recombinants between the tomato and US2 strain have been identified. It is not known to what extent these mixed infections and recombination events have contributed or will contribute to the spread of the virus.

Virus Damage and Control

Initially, PepMV infections in commercial tomato crops were reported as relatively insignificant with no apparent

plant or fruit symptoms and no or very limited yield reduction. Studies from the UK, however, reported significant effects on fruit quality. Smaller-sized fruits with different grades of uneven ripening and discoloration and occasionally mis-shaped fruits, led to production unsuited for the fresh UK market.

Studies indicate that symptoms induction by PepMV is highly dependent on environmental conditions. In Spain, PepMV-infected tomato plants showed only symptoms from autumn through spring and symptoms disappeared with higher temperatures in late spring. Similar effects of high light conditions and high temperatures have been reported from other countries. However, symptoms of PepMV-infected plants can be highly variable, ranging from very mild leaf symptoms to severe leaf and stem necrosis and fruit symptoms. Mild, chlorotic, or necrotic PepMV isolates have been reported and it seems that the capacity to induce different symptoms is a property of each isolate. Mixed infections of different strains of PepMV occur and are likely to be relatively common. The precise effects of the mixed infections on the symptomatology of the virus are unknown, as are the occurrences of these mixed infections. In addition, it should be noted that it is not always clear what the possible contribution of other plant pathogens in the expression of symptoms may be.

PepMV is a mechanically transmitted virus. The most important transmission routes are through contaminated tools, clothes, and surfaces. The virus is relatively stable at room temperature and can survive and stay infectious for several weeks in plant debris and on contaminated surfaces. Fruits from infected plants can contain high concentrations of virus and do not necessarily show symptoms. Long-distance transmission of the virus is likely to occur. Implementation of strict hygiene protocols during the growing season and thorough cleaning of greenhouses at the end of the growing season can effectively control the introduction and spread of the virus.

There are conflicting reports on the possibility of seed transmission. Sensitive techniques like real-time RT-PCR have detected the virus in seed lots harvested from infected plants, but transmission to progeny plants has not been conclusively demonstrated. In Europe, the EC directive 2004/200/EC prohibits the trade of seeds collected from PepMV-infected plants or not officially tested for the absence of PepMV. This effectively eliminates any possible chance of the transmission of the virus through seed lots.

Effective control of the virus in commercial tomato crop should come from the use of virus-free seeds and planting material, strict hygiene measures, and a constant monitoring for possible infections.

See also: Potexvirus.

Further Reading

Jones RAC, Koenig R, and Lesemann D-E (1980) Pepino mosaic virus, a new potexvirus from pepino (*Solanum muricatum*). *Annals of Applied Biology* 94: 61.

Pagán I, Córdoba-Sellés M, Martinéz-Priego L, *et al.* (2006) Genetic structure of the population of *Pepino mosaic virus* infecting tomato crops in Spain. *Phytopathology* 96: 274.

Van der Vlugt RAA, Cuperus C, Vink J, *et al.* (2002) Identification and characterization of *Pepino mosaic potexvirus* in tomato. *EPPO Bulletin* 32: 503.

Persistent and Latent Viral Infection

E S Mocarski and A Grakoui, Emory University School of Medicine, Atlanta, GA, USA

© 2008 Elsevier Ltd. All rights reserved.

Concepts

By nature of severity and ready association of symptoms with infection, acute viral diseases have played a dominant role in developing concepts of viral pathogenesis. Acute viral diseases were the first to succumb to vaccines that were based largely on the knowledge that initial exposure to a pathogen leaves the host with immune memory, reducing levels of secondary infection and disease. As a result, vaccination now controls a variety of acute viral diseases. Chronic viral infection underlies a wide variety of other medically important diseases, that either follow directly from primary infection or that require months, years, or decades to develop. Chronic viral infections are often widespread or even universal within a host species; however, disease rarely occurs in more than a small fraction of the infected population. Also, acute as well as chronic disease can follow infection with a single pathogen. For chronic disease, proof of etiology may only become evident once vaccination has successfully controlled the acute disease. Thus, measles vaccination provides protection from acute measles as well as the chronic disease, subacute sclerosing panencephalitis (SSPE). Vaccination against hepatitis B virus

(HBV) prevents acute and chronic hepatitis as well as hepatocellular carcinoma arising from chronic infection. Similarly, vaccination against varicella-zoster virus (VZV) prevents chickenpox (varicella) as well as shingles (zoster) and the neuropathic pain that often follows disease recurrence. Most recently, a human papillomavirus (HPV) vaccine specifically targeting chronic disease states, condyloma and cervical carcinoma, has been developed. Other pathogens associated with significant disease, such as human immunodeficiency virus (HIV), hepatitis C virus (HCV), and a number of herpesviruses (**Table 1**), have not yet surrendered to vaccination. Antiviral therapies have succeeded in controlling some of these; however, the chronic nature of the underlying infection complicates antiviral strategies and favors selection of drug-resistant progeny within the chronically infected host. The list of chronic diseases associated with long-term viral infection has grown, with experimental models suggesting that chronic virus infection contributes to certain cancers, as well as to chronic diseases such as diabetes and atherosclerosis. Many of these chronic diseases remain uncontrolled, and a link to viral (or microbial) pathogens might open avenues to therapy as well as vaccination.

Acute, Persistent, and Latent Infection

Chronic viral infection is distinguished from acute infection by timing and completeness of clearance. Initial

Table 1 Persistent and latent human viral infection and disease

Virus	*Genome maintenance*	*Cell and tissue tropism*	*Chronic disease syndrome*
Flavivirus			
HCV	Persistent infection	Hepatocyte	Chronic hepatitis[b] Hepatocellular carcinoma
Retrovirus			
HIV-1 and -2	Integrated provirus-persistent infection	$CD4^+$ T lymphocyte Mono/Macs/DCs	AIDS[b]
HTLV-1 and -2	Integrated provirus-persistent infection	T lymphocyte	Leukemia
Hepadnavirus			
HBV	Persistent infection	Hepatocyte	Chronic hepatitis[a,b] and hepatocellular carcinoma[a]
Herpesvirus			
HSV-1 and -2	Latent episome	Sensory neuron	Recurrent vesicular lesions[b]
VZV	Latent episome	Sensory ganglion	Zoster (shingles)[a,b]
EBV	Latent episome	B lymphocyte	Lymphoproliferative disease, nasopharyngeal carcinoma, African Burkitt's lymphoma and Hodgkin's lymphoma
CMV	Latent episome	Myeloid progenitor	Congenital disease, opportunistic disease[b]
HHV-6A,-6B, and -7	Latent episome	$CD4^+$ T Lymphocyte	Opportunistic disease
KSHV (HHV-8)	Latent episome	B lymphocyte (?)	Kaposi's sarcoma
Adenovirus			
Adenoviruses	Persistent infection	Adenoid lymphocyte and other sites	(None)
Papovavirus			
JC	Latent episome	Ductal epithelial, lymphoid, astrocyte	Progressive multifocal leukencephalopathy[b]
BK	Latent episome	Ductal epithelial	(None)
HPV	Latent episome	Basal epithelial	Warts, condyloma[a], and cervical cancer[a]
Poxvirus			
Molluscum contagiousum	Persistent infection	Basal epithelial	Skin lesion
Parvovirus			
AAV	Integrated-persistent infection	Epithelial cell and lymphocyte	(None)
B19	Persistent infection	Erythroid progenitor	Erythroblast crisis and persistent anemia
Paramyxovirus			
Measles	Persistent infection	CNS neuron	Subacute sclerosing panencephalitis and inclusion body encephalitis
Prion	Autocatalytic protein	CNS neuron	Transmissible spongiform encephalopathies

[a]Controlled by vaccination.
[b]Antiviral.

exposure to any virus results in primary infection that may be resolved in any of the three general infection patterns (**Figure 1**). One common pattern for viruses is 'acute infection', which may be accompanied by acute disease (**Figure 2**), and is typically controlled by the host adaptive immune response such that the pathogen is completely eliminated by the host response to infection. Immunological memory that initiates following primary infection provides protection from a subsequent, secondary infection. Immunological memory is the reason why vaccines substitute for primary infection and provide long-lived immunity. Exceptions where immunity is not sufficient to protect from reinfection (**Figure 2**), such as the well-known example of annual influenza virus epidemics, may be due to genetic changes in the virus (so-called antigenic 'drift' and 'shift') that allows this RNA virus to escape from existing immunity. Due to the nature of vaccines, immunity from vaccination often wanes with time, such as has been observed with measles, mumps, and, most recently, VZV. A booster vaccination can raise the levels of immunity. Continuous replication, or 'persistent infection' following primary infection (**Figure 1**), follows from incomplete immune clearance, sometimes occurring because infection occurs at an immune-privileged host site, and may continue without disease or in a chronic or progressive disease pattern (**Figure 2**). A biological reservoir of quiescent virus is called 'latent infection' (**Figure 1**). Latent infection is associated with maintenance of viral genome without active viral replication, and occurs in a cellular lineage specific for the particular virus. Latent infection is accompanied by host immune control that completely suppresses continuous replication; however, this surveillance does not prevent latent infection or the sporadic reactivation and recurrent virus replication that is a hallmark of latency (**Figure 1**). Such patterns may be associated with recurrent, chronic, or opportunistic disease patterns (**Figure 2**). Persistence and latency/recurrence patterns sustain pathogens within individuals and improve transmission within populations. The propensity of a particular virus to initiate chronic infection in either of the two general patterns (persistent or latent infection) depends on the type of virus as well as host cell tropism and immune response determinants. The replication scheme and particular cellular niche of the virus are important. A wide range of immunomodulatory viral functions have been identified in latent viruses, and these functions contribute to escape from host immunity. Immunological memory to latent viruses may be dysregulated as has been shown for persistent viruses leaving the host response ineffective in preventing recurrent infection.

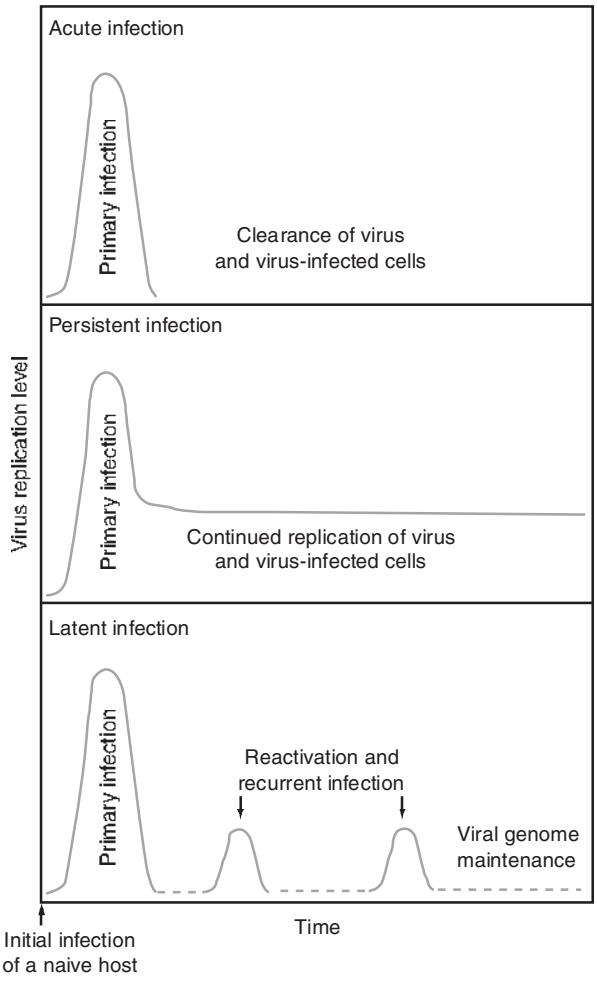


Figure 1

Lifelong latent infection depends upon the participation of host cells that are not susceptible to productive replication but that form a biologic reservoir for reactivation, and reactivation can be dictated by changes in status or differentiation state of host cells. It is easy to imagine that latent infection may be established as soon as a virus reaches the appropriate cell lineage, and that this occurs while active replication continues at other body sites. This view of latency focuses on cells rather than the intact host, and follows from the fact that host cell populations may be permissive or nonpermissive. While a reservoir of latently infected cells may certainly be established concurrent with primary infection, the events of acute infection may leave many cells that are abortively infected or that are transiently nonpermissive and do not survive to become a biological reservoir. The term 'latency' best describes the period when actively replicating virus is no longer present anywhere in the host. Latency is a property of some virus groups (**Table 1**) and may be determined through the way the viral genome is maintained in host cells. The nature of genome maintenance allows precise distinction between latent and persistent infection. Finally, it is sometimes difficult

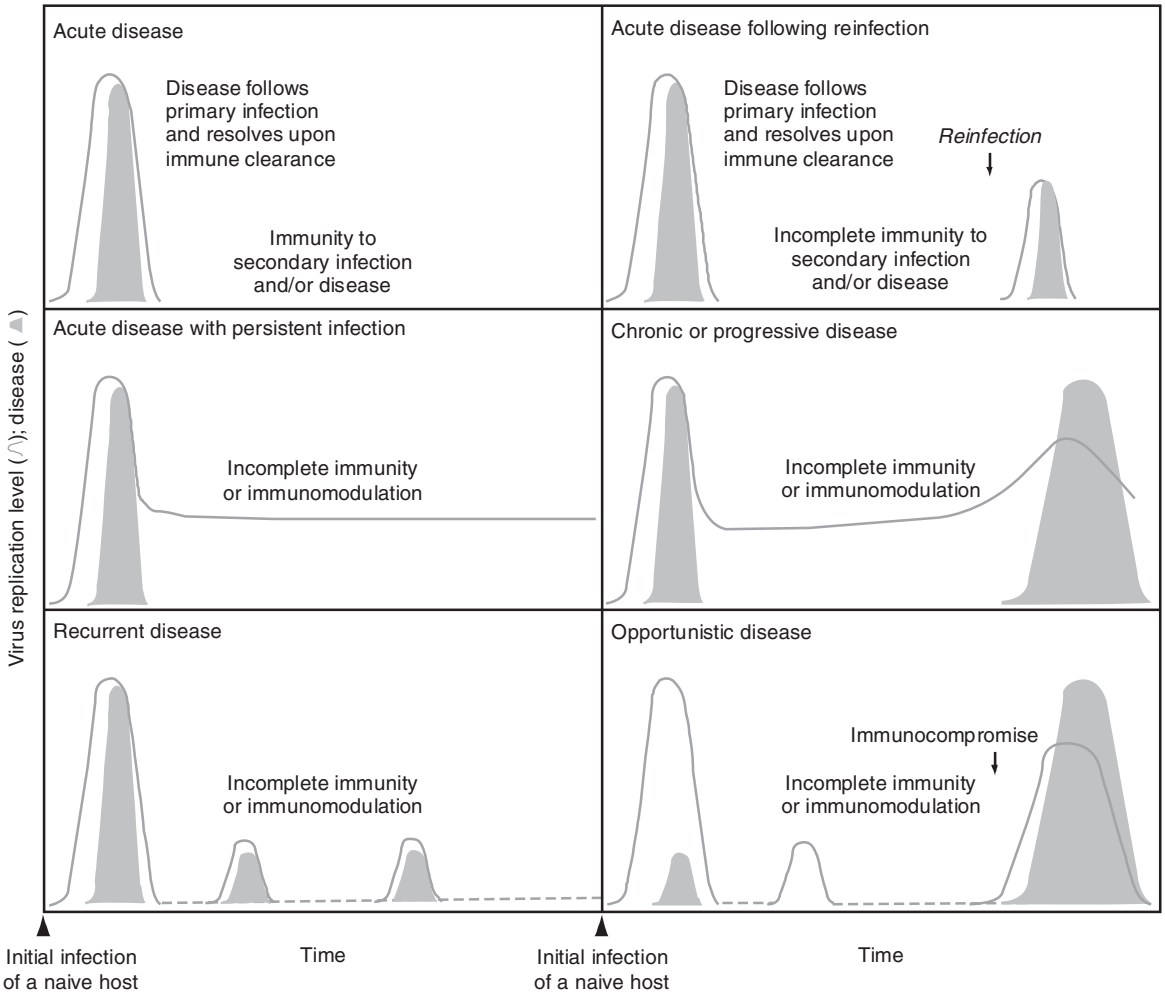


Figure 2

to distinguish abortive infection from latent infection. Abortive infection leaves either the complete viral genome or genome fragments deposited in host cells but these do not constitute a biological reservoir for reactivation, although abortive infection of nonpermissive cells may underlie pathogenesis such as the contribution of some DNA viruses to cancer.

Pathogenesis of Chronic Infection

Pathogenesis refers to the complex biological process of infection in the host animal and is influenced by the levels and distribution of virus during primary infection, just as characterized for acute viral infections. The diversity of viruses is immense; however, every virus in Nature infects only a defined set of susceptible host animals, which represents its species range. Although exceptions exist, human chronic viral pathogens tend to be species-restricted and exhibit evidence of a long co-evolution with the host species. This co-existence and co-evolution as the host species evolve enables these pathogens to reach a balance (or *détente*) with host-clearance mechanisms (cell-intrinsic, innate immune, and adaptive immune responses). The pathogenesis of chronic virus infection depends on the interplay of genetic and environmental determinants that are specific to a particular virus type as well as to the host animal species. Further, there may also be susceptibility determinants that vary between individuals within a particular species. Reactivation and recurrent viral replication are important components of pathogenesis of latent virus infections. Long-term persistent replication or latency may have a dramatic impact on the physiology of cells, altering normal cellular function. Thus, chronic viral damage underlies a variety of known conditions, including immunosuppression, organ dysfunction, and certain cancers, and has been suggested to play a role in many others based on experimental models.

Every member of some animal virus groups, including retroviruses, papovaviruses, adenoviruses, hepadnaviruses, and herpesviruses, relies on persistent and/or latent infection patterns. Some members of other virus groups rely only on persistent infection, sometimes in a host-dependent fashion, including flaviviruses, iridoviruses, and a range of others. In other cases, properties of the host dictate outcome, as with the regulatory mechanisms first characterized in lysogenic bacteriophages, or the persistent infection patterns that are intrinsic to plant and insect viruses. Disease pathogenesis (**Figure 2**) may follow various patterns. (1) Persistent infection may drive disease, such as with HIV-associated acquired immune deficiency syndrome (AIDS) or HCV-associated chronic liver damage and hepatocellular carcinoma. (2) Latent viral infection may alter host cell behavior such as with HPV-associated cervical cancer or Epstein–Barr virus (EBV) lymphoproliferative disease. (3) Reactivation of latent infection may lead to disease such as in herpes simplex virus (HSV)-recurrent cold sores or cytomegalovirus (CMV)-associated opportunistic infections in immunocompromised hosts. With various acute or chronic viral pathogens, reinfection may also contribute to disease patterns; however, existing immunity generally reduces the likelihood of significant disease. Common principles of chronic viral pathogenesis as well as in the diseases that arise from chronic infection have become more widely appreciated.

Immune Control

Immune control of chronic infections relies on cell-intrinsic, innate, and adaptive immunity in mammalian hosts, just the same as acute infections, although the biology of chronic infection relies on pathogen functions that can deflect or modulate host clearance. Cell-intrinsic and innate immunity reduce or delay acute replication levels, and are sometimes even sufficient to prevent disease through well-known mediators such as interferons and natural killer (NK) lymphocytes. The innate immune system is also responsible for recognition and processing of antigens to prime the adaptive immune response to infectious agents. Immune control of infection is influenced by the way in which a pathogen interacts with antigen-presenting cells (APCs): dendritic cells (DCs) and macrophages (Macs). This may occur through direct infection and antigen presentation, or indirectly through pathogen phagocytosis and cross-presentation to lymphocytes in secondary lymphoid tissues to dictate the ultimate breadth and effectiveness of the adaptive immune response.

All host cells sense and mount intrinsic responses to intracellular pathogens such as viruses, triggering cellular signaling cascades, programmed cell death, induction of interferon responses, and repression of viral genome transcription or replication. DCs and Macs, classically considered part of the reticuloendothelial system, produce an abundance of interferons, other cytokines, and a range of physiologically active mediators such as prostaglandins, vasoregulators, and hormones that may all be considered part of the innate response to infection. Cytokines and interferons initially drive the activation of NK lymphocytes, cytotoxic cells that recognize infected cells through reduced major histocompatibility complex (MHC) class I antigen levels, and increased MHC-like stress protein expression at the cell surface. This process is independent of the particular pathogen, and leads to the production of important cytokines that regulate the behavior of other leukocytes. DCs and Macs are considered crucial to innate responses because they acquire virus in the periphery and migrate to secondary lymphoid organs (lymph nodes, tonsils, and spleen) where an adaptive immune response unfolds. As the major producers of interferons and cytokines during viral infection, various subsets of DCs (and likely Macs) carry out the range of pattern recognition, antigen presentation, and co-stimulation activities with lymphocytes to guide both innate and adaptive immune responses. The antigen-specific adaptive immune response plays a crucial role in control of primary infection, as well as in establishing a reservoir of primed lymphocytes that constitute a memory response to secondary infection and prevents disease upon re-exposure to pathogens. Antibodies, produced by B lymphocytes following antigen-driven differentiation into plasma cells with the help of CD4+ T lymphocytes, form one critical arm of the adaptive response. Antibodies bind to and neutralize viruses, trigger complement-dependent lysis of virus-infected cells, and facilitate phagocytosis. Passive antibodies, or vaccines that only induce humoral immunity, are sufficient to control various acute viral pathogens, including influenza virus, paramyxoviruses, rubella virus, picornaviruses, rotaviruses, and vector-borne viruses. The B-lymphocyte response is central to control and clearance and occurs via neutralization of viral infectivity. Adaptive cellular immunity carried out by CD8+ T lymphocytes functioning as cytotoxic cells, often in collaboration with CD4+ cells, form the other critical arm. Control of persistent and latent viral infection, which is highly cell associated, relies on cytotoxic T-cell as well as antibody clearance mechanisms. In these viruses, cellular immunity is typically critical. CD4+ and CD8+ T lymphocytes also produce cytokines that contribute to suppressing replication and may induce immunopathology. There is sufficient evidence to say that both arms of the adaptive immune response facilitate long-lived immunity to reinfection, sometimes dependent on transmission route or virus type, so generalities may not apply to particular viral pathogens. For example, there is a strong interest in antibody-based immunity in control of HIV infection.

Immune Regulation and Immunopathology

The immune response is regulated, distinguishing self from non-self, while responding to infectious agents. Immune response processes may contribute to disease, by the direct recognition of viral antigen or by breaking the natural tolerance to self. Both antigen-specific and nonspecific damage to tissues as well as collateral damage to uninfected tissues may underlie disease. The damage to liver from chronic HBV (or chronic HCV) replication as well as the stromal keratitis due to recurrent HSV infection is triggered by viral infection but proceeds as a result of active antiviral immunity driving immunopathology. Although antibodies and lymphocytes are important effectors of immune control, dysregulation of the immune response during chronic infection may miscue processes that are normally beneficial to the host. Chronic infection can confound one important property of the adaptive immune response and cause a breakdown in the ability of the immune response to discriminate self from non-self. Thus, a balance of immunologic memory and the regulation of immunity, currently believed to be the job of regulatory CD4+ T lymphocytes as well as cell surface proteins that regulate memory T-lymphocytes, can be achieved. The capacity of persistent and latent viruses to deflect or avoid host immune clearance through immunomodulatory functions likely also contributes to infection and disease patterns. Immunologic memory associated with B or T lymphocytes contributes to secondary (anemnestic) responses upon subsequent exposures to the same or closely related viruses. Incompleteness in the immune response opens the way to reactivation as well as reinfection. Chronic viruses (papillomaviruses, retroviruses, herpesviruses, adenoviruses) rely on reactivation, but often benefit from transmission patterns that include reinfection with closely related strains. Immunopathology may be promoted by chronic infection but is by no means a requisite of chronic infection; this depends on the type of virus. Even acute infections that are typically cleared by adaptive immune effector mechanisms may trigger immunopathology upon re-exposure and this may manifest as chronic disease.

Immunomodulation

Like the immune response that the host mounts to control chronic infection, viruses carry an arsenal of defensive functions that undermine the effectiveness of immune clearance. Some of these functions go beyond defense and actively subvert or even exploit intrinsic and innate responses to infection. Although the main challenge to vaccination is often viewed as antigenic variation, such as characterizes HIV, or the number of viral strains in circulation, such as characterizes HCV, viral functions that deflect, subvert, or exploit host responses themselves may undermine vaccine strategies to control chronic infections. When viewing the large number of immunomodulatory functions encoded by large DNA viruses (poxviruses and herpesviruses), or the more restricted numbers encoded by smaller viruses (retroviruses and flaviviruses), the overwhelming impression is that viruses have found diverse ways to deal with a set of common challenges for chronic infection (**Table 2**). In many cases, the pathways that viruses target are critical to clearance, so neither the pathogen nor the host ultimately wins. The standoff that ensues is what characterizes chronic infection. The presence of immunomodulatory functions in a particular virus reveals the importance of specific immune pathways in control of virus infection. Remarkably, viruses with small genomes that encode fewer than 20 genes (retroviruses, papovaviruses, hepadnaviruses, flaviviruses, paramyxoviruses) have succeeded as well as viruses encoding greater than 20 genes (adenoviruses, poxviruses, herpesviruses), suggesting that there are many successful ways to reach this balance and achieve persistent or latent infection. Here, we will discuss a subset of these processes to provide a perspective on this growing field.

Certain cell-intrinsic and innate responses to infection are used throughout invertebrate and vertebrate hosts to detect and eliminate pathogens. Cell stress resulting from sensing an intruder and initiating pattern recognition and signaling cascades are initially important. Intrinsic stress leading to programmed cell death (apoptosis, autophagy, and other forms) is an important and ancient mechanism to get rid of a host of intracellular pathogens. Extrinsic apoptosis due to signaling via tumor necrosis factor (TNF) family ligands is a development of vertebrates to expand the ways that apoptosis can be induced. The induction of apoptosis stops viruses before they have a chance to replicate and disseminate. Virus-encoded cell death suppressors are common (**Table 2**) in persistent and latent virus infection, likely delaying cell death.

The sensing of virus infection by cellular receptors that detect pattern recognition, such as Toll-like receptors (TLRs), are important to induce interferons and subversion of TLR signaling is common in persistent and latent viruses. Various components of TLR signaling pathways, including IRF-3, IRF-7, RIG-I, and NF-κB, may be targeted.

A variety of cellular enzymes may block steps in viral replication. These need to be overcome by viral or cellular functions in order for replication to proceed, so these have been found to play roles in establishing latency. The impact of histone deactylases (HDACs) on herpesviruses is one well-established example, and these likely also play into papillomavirus and adenovirus infection. APOBEC, a cytoplasmic cytidine deaminase, is a potent inhibitor

Table 2 Immunomodulation in chronic viral infection

Virus	*Cell-intrinsic*	*Innate*	*Adaptive*
Flavivirus			
HCV	TLR, RIGI, IRF3, APOP	IFN, NK, APC	VAR, CD8,
Retrovirus			
HIV-1 and -2	TLR, APOP, AUTO, APOBEC	IFN, NK, PKR, CYTK, APC	VAR, MHC-I, CD4
Hepadnavirus			
HBV	APOP	IFN, APC	
Herpesvirus			
HSV-1 and -2	TLR, IRF3, APOP, AUTO, HDAC	IFN, NK, PKR, APC	MHC-I, MHC-II, Ab, C′, CD8, CD4
VZV	TLR, IRF3, APOP, HDAC	IFN, NK, APC	MHC-I, MHC-II, Ab, CD8, CD4
EBV	TLR, IRF3, IRF7, APOP, AUTO, HDAC	IFN, NK, CYTK, APC	MHC-I, MHC-II, Ab, CD8, CD4
CMV	TLR, IRF3, APOP, AUTO, HDAC	IFN, NK, PKR, CYTK, APC	MHC-I, MHC-II, Ab, CD8, CD4
HHV-6A, -6B, and -7	TLR, IRF3, APOP, HDAC	IFN, NK, PKR, CYTK	MHC-I, Ab, CD8
KSHV (HHV-8)	TLR, IRF3, IRF7, APOP, HDAC	IFN, NK, PKR, CYTK	MHC-I, MHC-II, Ab, C′, CD8, CD4
Adenovirus			
Adenoviruses	TLR, IRF3, APOP, AUTO, HDAC	IFN, NK, PKR, CYTK	MHC-I, CD8, CD4
Papillomavirus			
HPV	APOP, HDAC	IFN, NK, CYTK, APC	MHC-I
Poxvirus			
Molluscum contagiousum	APOP	IFN, NK, PKR, CYTK	MHC-I

Cell-intrinsic: TLR, Toll-like receptor; RIG-I, retinoic acid-inducible gene-I; IRF3/IRF7, interferon-regulated factor-3 and -7; APOP, apoptosis; AUTO, autophagy; APOBEC, family of cytosine deaminases; HDAC, histone deacetylases. Innate: IFN, interferon; PKR, protein kinase R; CYTK, cytokines; NK, natural killer lymphocytes; APC, antigen-presenting cell. Adaptive: MHC-I, major histocompatability class I; MHC-II, major histocompatability class II; Ab, antibody; C′, complement; CD8, CD8+ T lymphocytes; CD4, CD4+ T lymphocytes; VAR, variation in genome sequence.

of HIV and restricts viral replication by introducing mutations into the viral genome.

Persistent and latent infections are controlled over the course of the first few days by innate clearance, making subversion of innate clearance mechanisms an important area. Inflammatory cytokines, the most ancient of which are in the interleukin-1 (IL-1) family, but joined by a range of pro-inflammatory cytokines (IL-6, TNF family, interferons), are induced by viral infection with a range of specific as well as nonspecific inhibitors of these pathways.

In addition to the impact on cytokines, NK cells effectively reduce levels of virus-infected cells within the first few days post infection, and some viruses, particularly in the herpesvirus family, have a number of NK subversion functions.

A variety of these subversion pathways can be imagined to be active in infected APCs. For example, some proteins produced by replicating EBV directly inhibit the antigen-presentation pathway by specifically preventing their own presentation on the cell surface, thereby influencing how well the adaptive immune response to the virus is primed. However, many immune-evasion strategies that have been studied in detail have been found to be effective only in somatic cells and are more important in reducing the potency of adaptive immune clearance mechanisms that are induced.

Persistent and latent viruses have found myriad ways to subvert the effectiveness of host immune effector functions, ranging from deflecting antibody and complement to reducing MHC-I and MHC-II levels to make infected cells less recognizable by T lymphocytes. Many viruses make cytokine/cytokine receptor and chemokine/chemokine receptor mimics that likely subvert both innate and adaptive phases of the immune response by altering cell differentiation or migration.

Chronic Disease Manifestations

Latent and persistent viral infections contribute to chronic disease manifestations in a variety of ways including causing (1) congenital infection with long-term sequelae, (2) transformation of infected cells into malignant cells, (3) virus-mediated organ damage, (4) immune-mediated organ damage. Viruses that are able to infect a newborn during delivery or cross the placenta during pregnancy pose both acute and chronic disease risks. Such pathogens may initiate spontaneous abortion or cause progressive diseases in newborns. The herpesvirus, HSV, causes a rare but fatal (if untreated) systemic infection with neurological damage in newborns exposed at delivery. Cytomegalovirus (CMV) is a common congenital viral infection worldwide that may cause severe

neurological disease, but more often causes subtle, progressive hearing, eyesight, and learning disabilities. This virus causes congenital infection with chronic progressive disease consequences. Neonates born to mothers acutely infected with CMV acquire the infection through transplacental transmission. Although the mother's infection is often asymptomatic, the infant with severe congenital CMV disease (also called symptomatic infection) may have microencephaly (an abnormally small head), hepatosplenomegaly (an enlarged spleen and liver), a bruise-like rash, retinitis, and progressive central nervous system complications including deafness, blindness, and psychomotor retardation. More than half of those infants with symptomatic CMV infections at birth will have neurologic complications later in life. Immunocompromised patients are susceptible to reactivation of a wide variety of latent viruses, with CMV most frequently observed. Other herpesviruses, adenoviruses, and papovaviruses may also cause chronic disease manifestations.

In some viruses, proliferative diseases and malignancies may result directly during latent infection, without any requirement for reactivation or replication. Viruses such as EBV and HPV directly transform cells in which they reside latently. Thus, EBV specifically targets naive B lymphocytes, converts them to a memory phenotype as if they had encountered antigen, and immortalizes these cells such that they remain latent hosts for life. EBV has several distinct viral latency 'programs' characterized by differential viral protein expression and associated chronic lymphoproliferative diseases as well as malignancies including endemic Burkitt's lymphoma, some varieties of Hodgkin's lymphoma, NK/T-cell lymphomas, and nasopharyngeal lymphoma. HPV is a second example of a virus causing both transformation of infected cells into malignant cells and virus-mediated organ damage depending on the viral genotype. For example, HPVs 16 and 18 are considered high-risk genotypes with propensity to lead to malignant transformation and are implicated in the development of cervical cancer. The E6 and E7 proteins from these high-risk genotypes are oncogenic, whereas the E6 and E7 proteins from lower-risk genotypes are not. Some HPV types have virtually no oncogenic potential but cause chronic damage to the skin in the form of plantar warts (HPV 1) or common warts (HPV 2). Similarly, HSV can be a chronic problem as a cause of recurrent aphthous stomatitis or painful genital mucosal ulcerations. Although the chronic damage by low-risk HPV or mucosal ulcerations of HSV may not be life threatening, other viruses can directly and indirectly cause grave chronic illnesses. Although the mechanism is unclear, parvovirus B19 has been associated with cessation of red blood cell, white blood cell, and platelet production by the bone marrow. This aplastic anemia can be fatal. Certainly HIV, known to rapidly deplete the host of its important CD4+ T lymphocytes, progresses to life-endangering AIDS when untreated. Even when the virus itself is not implicated in causing cytopathic damage to host organ systems, the immune response to the viral infection may instead wreak havoc on critical tissue. For example, HBV and HCV, while not strictly cytopathic themselves, lead to persistent inflammation in the liver with the host immune response largely responsible for the eventual development of cirrhosis and end-stage liver disease. HCV-related end-stage liver disease is now the single leading indication for liver transplantation in the United States illustrating the impact of persistent infection on public health.

Many viruses are controlled adequately in the immunocompetent host only to cause significant disease in immunocompromised hosts, and in this instance are referred to as opportunistic infections. There are many reasons why a host may be immunocompromised including HIV disease in which there is a progressive loss of CD4+ T-cell function, iatrogenic immunosuppression to maintain organ viability following transplantation, or chemotherapy to suppress cancerous cell growth. HIV patients with very low CD4+ T-cell counts, rendering them severely immunocompromised with AIDS, often manifest with multiple opportunistic infections. For example, whereas HSV usually causes self-limited disease processes in most immunocompetent hosts, in immunosuppressed hosts, HSV can cause an AIDS-related encephalitis. Similarly, HHV 8 has been implicated in the development of Kaposi's sarcoma in AIDS patients. CMV is an example of a virus that is usually well controlled by immunocompetent hosts but presents life-threatening infection in immunosuppressed transplant patients. HPV can similarly lead to development of verruca and condyloma recalcitrant to treatment in the immunosuppressed host with more frequent development of HPV-related carcinoma; and the poxvirus responsible for molluscum contagiosum generally causes skin lesions only in children, whose immune systems are still developing, and adults who are immunocompromised. Finally, hosts co-infected with HIV and HCV demonstrate more rapid development of HCV-related liver disease than immunocompetent patients.

In summary, the interplay between viruses and the infected host determines whether a virus is acutely resolved, becomes latent or persistent, and causes minimal or significant disease manifestations. Many challenges lie ahead to determine strategies sufficient to alter viral pathogenesis, augment host immunity, and change the course of virus-induced disease manifestations. These strategies may ultimately be in the form of prophylactic or therapeutic vaccination or may capitalize on host immune response modulation.

Acknowledgments

The authors acknowledge support from the Cancer Research Institute Investigator Award (A. Grakoui), Woodruff Health Sciences Fund (A. Grakoui and E. S. Mocarski), the Georgia Cancer Coalition (E. S. Mocarski), and the USPHS (A. Grakoui and E. S. Mocarski).

See also: Apoptosis and Virus Infection.

Further Reading

Biron C and Sen G (2006) Innate immune responses to viral infection. In: Knipe DM, Howley PM Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 249–278. Philadelphia: Lippincott Williams and Wilkins.

Braciale T, Hahn YS, and Burton D (2006) Adaptive immune responses to viral infection. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., 279–325. Philadelphia: Lippincott Williams and Wilkins.

Virgin S (2006) Pathogenesis of viral infection. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 2701–2772. Philadelphia: Lippincott Williams and Wilkins.

Phycodnaviruses

J L Van Etten, University of Nebraska–Lincoln, Lincoln, NE, USA
M V Graves, University of Massachusetts–Lowell, Lowell, MA, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Since the early 1970s, viruses or virus-like particles (VLPs) have been been reported in at least 44 taxa of eukaryotic algae, which include members in 10 of the 14 classes of algae. However, most of the early reports described single accounts of microscopic observations. The VLPs were not characterized because they were difficult to obtain in reasonable quantities. Several factors contributed to the low virus concentrations: (1) often only a few algal cells contained particles; (2) usually the cells only contained particles at one stage of the algal life cycle; (3) cells containing particles tended not to lyse; (4) in most cases the particles were not infectious; and (5) some hosts could not be cultured easily.

However, this situation began to change with the discovery of a family of large double-stranded DNA (dsDNA)-containing viruses that infect and replicate in certain strains of unicellular, eukaryotic, exsymbiotic, chlorella-like green algae. The first such 'chlorella viruses' were discovered in 1978 in chlorella symbiotic with *Paramecium bursaria* and in 1981 in chlorella symbiotic with the green coelenterate *Hydra viridis*. The algae from *P. bursaria* can be grown free of the paramecium in culture, and these cultured, naturally endosymbiotic *Chlorella* strains (NC64A and Pbi or their equivalents) serve as hosts for many similar viruses. The lytic chlorella viruses can be produced in large quantities and assayed by plaque formation using standard bacteriophage techniques. Recently, a plaque-forming virus that infects chlorella symbiotic with the heliozoon *Acanthocystis turfacea* was described. This virus does not infect *Chlorella* NC64A or *Chlorella* Pbi. The prototype chlorella virus is PBCV-1, which stands for *Paramecium bursaria* chlorella virus. The genomes (313–370 kbp) of several of the chloroviruses have either been sequenced or are in the process of being sequenced.

Large polyhedral, dsDNA-containing viruses that infect certain marine algae are also under active investigation. These include viruses that infect filamentous brown algae, *Ectocarpus* sp. (EsV viruses) and *Feldmannia* sp. (FsV viruses) (**Table 1**), and viruses that infect *Emiliania huxleyi* (EhV viruses). The genomes of some of these viruses have also been sequenced recently. Although all of these algal viruses arose from a common ancestor, they can have different lifestyles. For example, EsV and FsV viruses have a lysogenic phase in their life cycle and are only expressed as virus particles in sporangial cells of their host. In contrast, the chlorella viruses and EhV viruses are lytic.

The first algal viruses to be discovered were large dsDNA viruses; consequently, it was assumed for several years that algae were only infected by large dsDNA viruses. However, this scenario is changing rapidly. A positive-sense 9.1 kbp single-stranded RNA (ssRNA) virus has been discovered that infects a toxic bloom-forming alga, *Heterosigma akashiwo* (called HaRNAV) that is related to the picorna-like virus superfamily. A dsRNA, reo-like virus that infects a microalga, *Micromonas pusilla*, has been reported and finally a virus (CsNIV) with an unusual genome structure that infects diatoms in the genus *Chaetoceros* has been described. The CsNIV genome consists of a single molecule of covalently closed circular single-stranded DNA (ssDNA) (6005 nucleotides) as well as a segment of linear ssDNA (997 nucleotides). These recently discovered algal viruses are described in other articles in this encyclopedia.

Table 1 Taxonomy and general characteristics of some phycodnaviruses

Genus[a]	*Type species*[a]	*Known host range*[a]	*Source*	*Particle diameter (nm)*	*Genome size (kbp) and conformation*	*Latent period (h)*	*Burst size*
Chlorovirus	*Paramecium bursaria* chlorella virus 1 (PBCV-1)	*Chlorella* NC64A *Chlorella* Pbi *Chlorella* SAG 3.83	FW	190	313–370 Closed linear dsDNA, hairpin termini	6–8	200–350[b]
Coccolithovirus	*Emiliania huxleyi* virus 86 (EhV-86)	*Emiliania huxleyi*	MW	160–200	407–415 Circular	4–6	400–1000
Phaeovirus	*Ectocarpus siliculousus* virus 1 (EsV-1)	*Phaeophycea* *Ectocarpus siliculousus* *Ectocarpus fasciculatus* *Feldmannia simplex* *Feldmannia irregularis* *Feldmannia species* *Hincksia hincksiae* *Myriotrichia clavaeformis* *Pilayella littoralis*	MW	150–170	170–340 Open linear, single stranded regions	ND	$>1\times10^6$
Prasinovirus	*Micromonas pusilla* virus SP1 (MpV-SP1)	*Micromonas pusilla*	MW	115	200	7–14	1800–4100
Prymnesiovirus	*Chrysochomulina brevifilum* virus PW1 (CbV-PW1)	*Haptophyceae (aka Prymnesiophyceae)* *Chrysochomulina brevifilum* *Chrysochomulina strobilus* *Chrysochomulina globosa*	MW	145–170	510	ND	800–1000
Raphidovirus	*Heterosigma akashiwo* virus 01 (HaV01)	*Heterosigma akashiwo*	MW	202	294	30–33	770

[a]Data abstracted from http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm.
[b]PFU/cell.
FW, freshwater; MW, marine/coastal water; ND, not determined.
Reproduced from Dunigan DD, Fitzgerald LA, and Van Etten JL (2006) Phycodnaviruses: A peek at genetic diversity. *Virus Research* 117: 119–132, with permission from Elsevier.

Taxonomy and Classification

Members and prospective members of the family *Phycodnaviridae* constitute a genetically diverse, but morphologically similar group of viruses with eukaryotic algal hosts from both fresh and marine waters. Accumulating genetic evidence indicates that the phycodnaviruses together with the poxviruses, iridoviruses, asfarviruses, and the 1.2 Mbp mimivirus have a common evolutionary ancestor, perhaps, arising at the point of eukaryogenesis, variously reported to be 2.0–2.7 billion years ago. All of these viruses share nine gene products and 33 more gene products are present in members of at least two of these five viral families. Collectively, these viruses are referred to as nucleocytoplasmic large DNA viruses (NCLDV).

Phycodnaviruses are large (mean diameter 160 ± 60 nm) icosahedrons, which encapsidate 160–560 kbp dsDNA genomes. Where known, the viruses have an internal-membrane that is required for infection. Phylogenetic analyses of their δ-DNA polymerases indicate that they are more closely related to each other than to other dsDNA viruses and that they form a monophyletic group, consistent with a common ancestor. However, the phycodnaviruses fall into six clades which correlate with their hosts and each has been given genus status. Often the genera can be distinguished by additional properties, for example, lytic versus lysogenic lifestyles or linear versus circular genomes. Members of the genus *Chlorovirus* infect freshwater algae, whereas, members of the other five genera (*Coccolithovirus*, *Phaeovirus*, *Prasinovirus*, *Prymnesiovirus*, and *Raphidovirus*) infect marine algae.

Structure and Composition

Chlorella virus particles are large (molecular weight $\sim 1 \times 10^9$ Da) and complex. The PBCV-1 virion contains more than 100 different virus-encoded proteins. The PBCV-1 54 kDa major capsid protein is a glycoprotein and comprises ~40% of the total protein. The major capsid protein consists of two eight-stranded, antiparallel β-barrel, jelly-roll domains related by pseudo sixfold rotation. This structure resembles the major coat proteins from some other dsDNA viruses that infect all three domains of life including bacteriophage PRD1, human adenoviruses, and a virus STIV infecting the Archaea, *Sulfolobus solfataricus*. This finding led to the suggestion that these three viruses may also have a common evolutionary ancestor with the NCLDVs, even though there is no significant amino acid sequence similarity among their major capsid proteins.

Cryoelectron microscopy and three-dimensional image reconstruction of the PBCV-1 virion (**Figure 1**) indicate that the outer capsid is icosahedral and covers a lipid bilayered membrane. The membrane is required for infection because the virus loses infectivity after exposure to organic solvents. The outer diameter of the virus capsid ranges from 1650 Å along the two- and threefold axes to 1900 Å along the fivefold axis. The capsid shell consists of 1680 doughnut-shaped trimeric capsomers plus 12 pentameric capsomers at each icosahedral vertex. The trimeric capsomers are arranged into 20 triangular facets (trisymmetrons, each containing 66 trimers) and 12 pentagonal facets (pentasymmetrons, each containing 30 trimers and one pentamer at the icosahedral vertices). Assuming all the trimeric capsomers are identical, the outer capsid of the virus contains 5040 copies of the major capsid protein. The virus has a triangulation number of 169. However, PBCV-1 is not the largest phycodnavirus; phaeocystis pouchetti virus (PpV01) has an icosahedral capsid with a triangulation number of 219.

Structural proteins of many viruses, such as herpesviruses, poxviruses, and paramyxoviruses, as well as the chlorella viruses, are glycosylated. Typically, viral proteins are glycosylated by host-encoded glycosyltransferases located in the endoplasmic reticulum (ER) and Golgi and then transported to a host membrane. Nascent viruses acquire the glycoprotein(s) and only become infectious by budding through the membrane, usually as

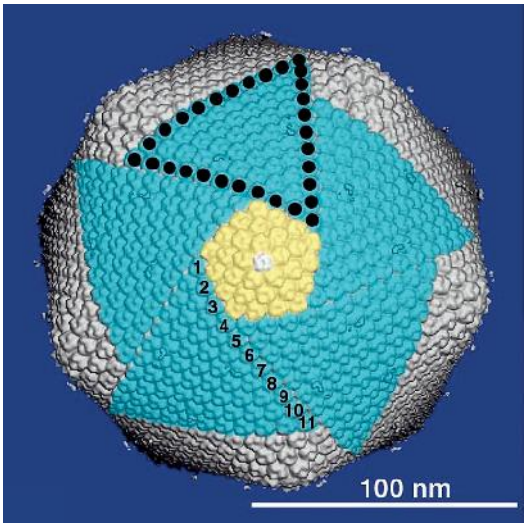


Figure 1 Three-dimensional image reconstruction of chlorella virus PBCV-1 from cryoelectron micrographs. The virion capsid consists of 12 pentasymmetrons and 20 trisymmetrons. Five trisymmetrons are highlighted in the reconstruction (blue) and a single pentasymmetron is colored yellow. A pentavalent capsomer (white) lies at the center of each pentasymmetron. Each pentasymmetron consists of one pentamer plus 30 trimers. Eleven capsomers form the edge of each trisymmetron (black dots) and therefore each trisymmetron has 66 trimers. Reprinted, with permission, from Van Etten JL (2003) Unusual life style of giant chlorella viruses. *Annual Review of Genetics* 37: 153–195, ©2003 by Annual Reviews.

they are released from the cell. Consequently, the glycan portion of virus glycoproteins is host specific.

However, glycosylation of PBCV-1 major capsid protein differs from this paradigm. Accumulating evidence indicates that PBCV-1 encodes most, if not all, of the enzymes involved in constructing the complex oligosaccharides attached to its major capsid protein and that the process occurs independently of the ER and Golgi. Furthermore, five of six putative PBCV-1-encoded glycosyltransferases are predicted to be located in the cytoplasm. PBCV-1 also encodes several additional proteins involved in post-translational modification that may alter virus structural proteins. These include a prolyl-4-hydroxylase and several protein kinases and a phosphatase.

Genomes

The 331 kbp PBCV-1 genome is linear and nonpermuted. The genome termini consist of 35 nucleotide long, incompletely base-paired, covalently closed hairpin loops that exist in one of two forms (flip and flop). Each hairpin loop is followed by an identical 2.2 kbp inverted repeat sequence; the remainder of the genome consists primarily of single-copy DNA. The PBCV-1 genome has ~695 open reading frames (ORFs) that have 65 or more codons, of which ~366 are probably protein encoding. The putative protein-encoding genes are evenly distributed on both strands and intergenic space is minimal, 275 ORFs are separated by less than 100 nucleotides (**Figure 2**). One exception is a 1788 bp sequence near the middle of the genome. This sequence has a polycistronic gene containing 11 tRNAs (**Figure 2**).

Approximately 40% of the 366 PBCV-1 gene products resemble proteins in the databases, many of which have not previously been associated with viruses (some are listed in **Figure 3**). Eighty-four ORFs have paralogs within PBCV-1, forming 26 groups. The size of these groups ranges from two to six members.

Some PBCV-1 genes are interrupted by introns; a gene encoding a transcription factor-like protein contains a self-splicing type I intron, whereas the δ-DNA polymerase gene contains a spliceosomal-processed type of intron. In addition, one of the PBCV-1 tRNA genes is predicted to have an intron.

One unusual feature of PBCV-1 DNA, as well as the other chlorella virus DNAs, is that they contain methylated bases. Chlorella virus genomes contain 5-methylcytosine in amounts varying from 0.1% to 48% of the total cytosines. Many viral DNAs also contain N^6-methyladenine with concentrations up to 37% of the total adenines. This led to the discovery that many chlorella viruses encode multiple DNA methyltransferases, as well as site-specific (restriction) endonucleases.

The 407 kbp circular dsDNA genome of one of the EhV viruses is predicted to have 472 protein-encoding genes. Only 66 (14%) of these 472 gene products match a sequence in GenBank. The EhV virus encodes several unexpected genes never found in a virus before, including four gene products involved in sphingolipid biosynthesis and two additional gene products which encode desaturases.

The structure of the 335 kbp EsV genome is unknown. Although several experiments suggest that the genome is circular, DNA sequencing indicates that it has defined ends with inverted repeats. The virus is predicted to contain 231 protein-encoding genes, 48% of which resemble various proteins in the public databases. About 12% of the EsV genome consists of tandem repeats and portions of the genome have ssDNA regions. Collectively, PBCV-1, EsV, and EhV viruses have in excess of 1000 unique ORFs. A total of 123 putative ORFs from these three viruses is organized into metabolic domains (**Figure 3**). Interestingly, these three viruses only have 14 gene products in common. Not surprisingly, several of these common ORFs are involved in DNA replication, such as the δ-DNA polymerase, large and small subunits of ribonucleotide reductase, proliferating cell nuclear antigen (PCNA), superfamily II and III helicases, and the newly recognized archaeo-eukaryotic primases. Another common ORF among the three phycodnaviruses is the major capsid protein.

Virus Replication

PBCV-1 attaches rapidly, specifically, and irreversibly to the external surface of cell walls, but not to protoplasts, of host *Chlorella* NC64A. Attachment always occurs at a virus vertex, followed by degradation of the wall at the attachment point. Following wall degradation, the internal membrane of the virus probably fuses with the host membrane resulting in entry of the virus DNA and virion-associated proteins into the cell, leaving an empty capsid on the surface. Several observations suggest that the infecting DNA, plus associated proteins, is rapidly transported to the host cell nucleus and the host transcription machinery is reprogrammed to transcribe virus RNAs. This process occurs rapidly because early PBCV-1 transcripts can be detected within 5–10 min post infection (p.i.). PBCV-1 translation occurs on cytoplasmic ribosomes and early PBCV-1-encoded proteins can be detected within 10 min p.i.

PBCV-1 DNA synthesis and late virus transcription begins 60–90 min p.i. Ultrastructural studies of PBCV-1-infected chlorella suggest that the nuclear membrane remains intact, at least during early stages of virus replication. At approximately 2–3 h p.i., assembly of virus

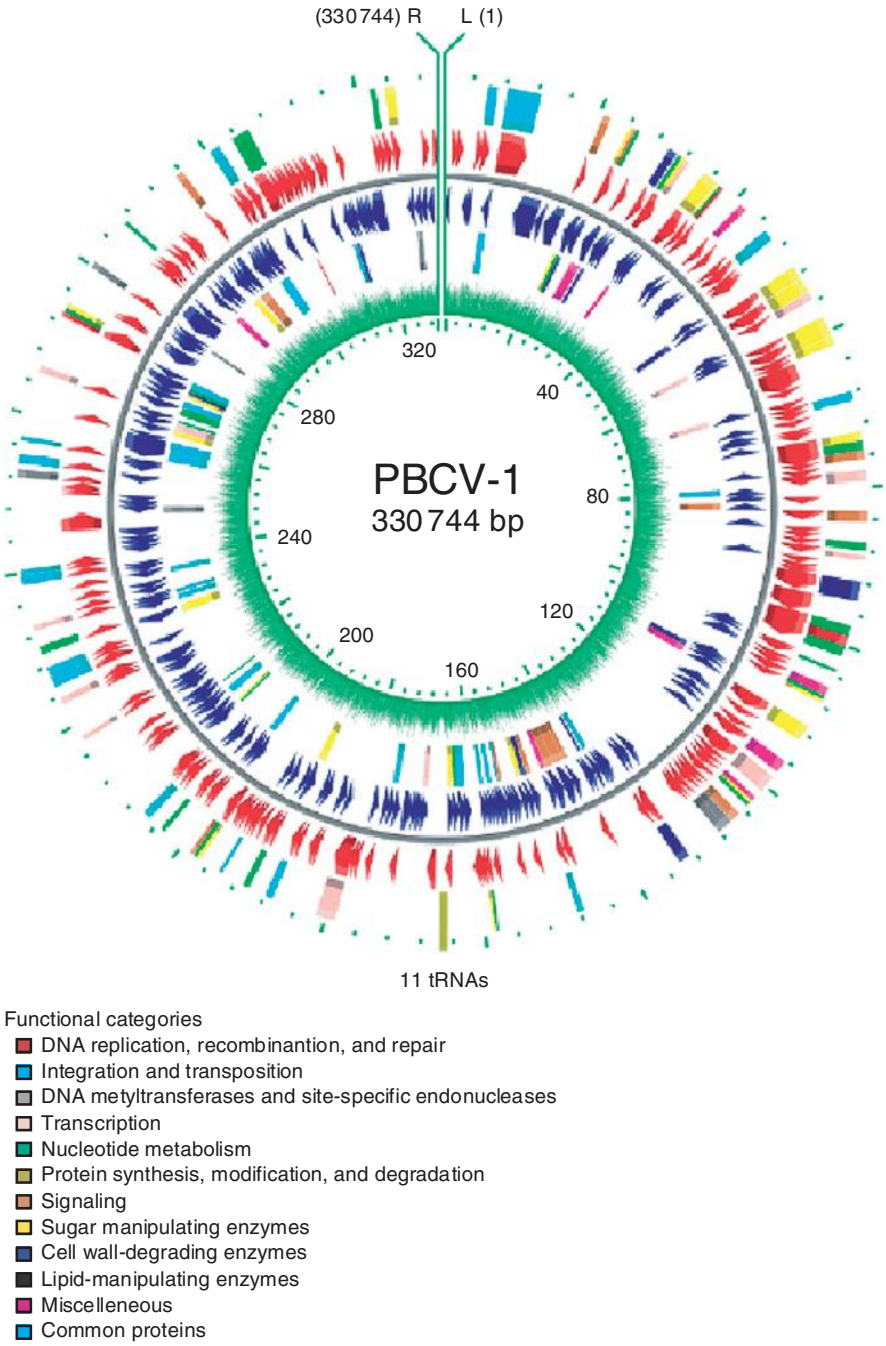
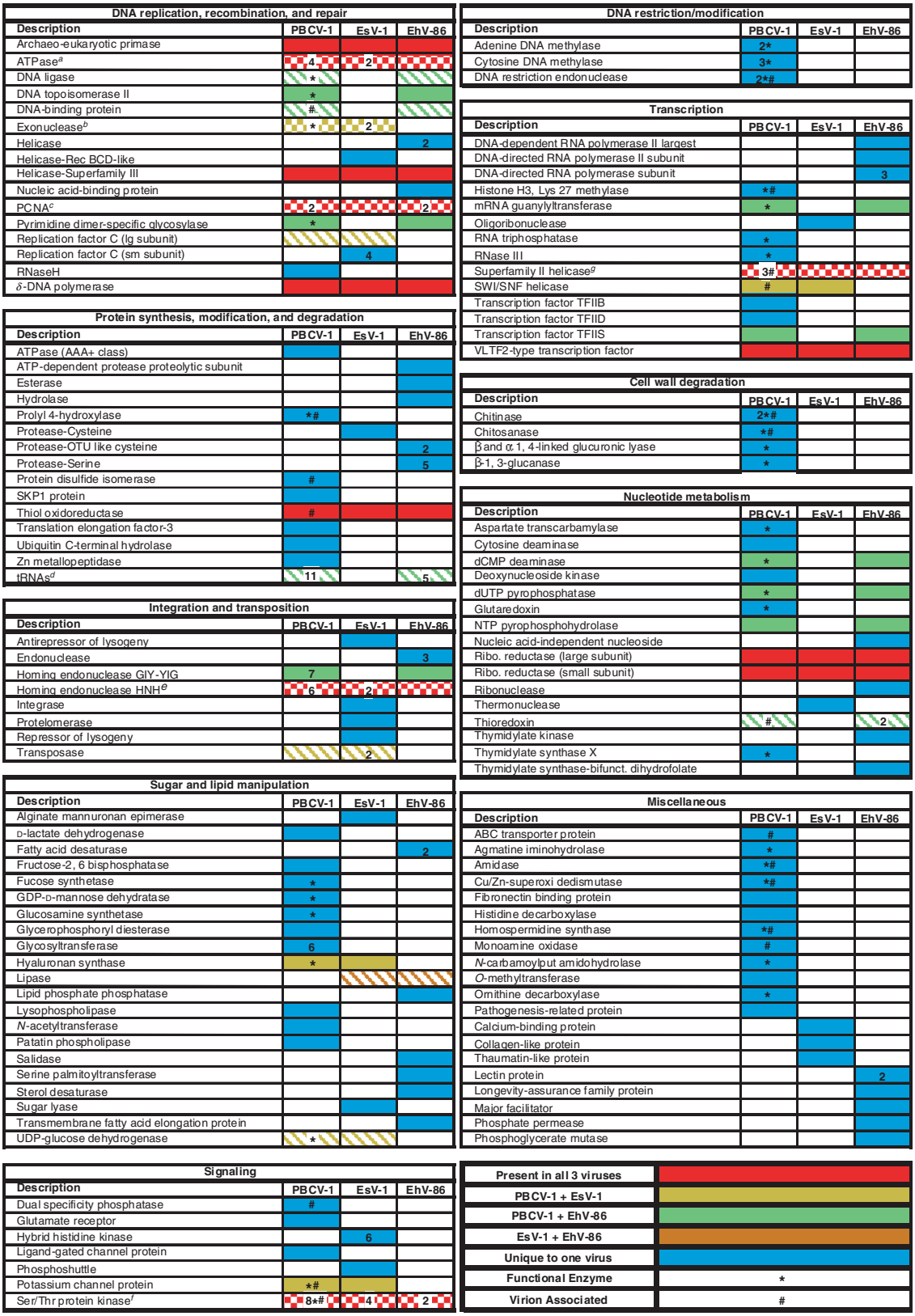


Figure 2 Map of the chlorella virus PBCV-1 genome visualized as a circle using Circular Genome Viewer (Paul Stothard, Genome Canada). However, the genome is a linear molecule and the ends are depicted at the top of the figure as green lines (L and R represent the left and right ends of the genome, respectively). The red and blue arrows represent the 366 protein-encoding genes; red arrows depict genes transcribed in the rightwards direction and blue arrows genes transcribed in the leftwards direction. The two rings that flank the protein-encoding genes show the predicted functions of the proteins, color-coded by function (see insert in the figure). The location of the polycistronic gene encoding the 11 tRNAs is indicated in the outermost ring (i.e., this gene is transcribed in the rightwards direction). The innermost ring (in green) represents the A + T content determined using a 25 bp window. Note that the A + T content is fairly constant over the genome (60% A + T).

capsids begins in localized regions in the cytoplasm, called virus assembly centers, which become prominent at 3–4 h p.i.. By 5 h p.i., the cytoplasm is filled with infectious progeny virus particles (~1000 particles/cell) and by 6–8 h p.i. localized lysis of the host cell releases progeny. Of the progeny released, 25–50% of the particles are infectious. Mechanical disruption of cells releases infectious virus 30–50 min prior to cell lysis, indicating the



DNA replication, recombination, and repair			
Description	**PBCV-1**	**EsV-1**	**EhV-86**
Archaeo-eukaryotic primase			
ATPase[a]	4	2	
DNA ligase	*		
DNA topoisomerase II	*		
DNA-binding protein	#		
Exonuclease[b]	*	2	
Helicase			2
Helicase-Rec BCD-like			
Helicase-Superfamily III			
Nucleic acid-binding protein			
PCNA[c]	2		2
Pyrimidine dimer-specific glycosylase	*		
Replication factor C (lg subunit)			
Replication factor C (sm subunit)		4	
RNaseH			
δ-DNA polymerase			

Protein synthesis, modification, and degradation			
Description	**PBCV-1**	**EsV-1**	**EhV-86**
ATPase (AAA+ class)			
ATP-dependent protease proteolytic subunit			
Esterase			
Hydrolase			
Prolyl 4-hydroxylase	*#		
Protease-Cysteine			
Protease-OTU like cysteine			2
Protease-Serine			5
Protein disulfide isomerase	#		
SKP1 protein			
Thiol oxidoreductase	#		
Translation elongation factor-3			
Ubiquitin C-terminal hydrolase			
Zn metallopeptidase			
tRNAs[d]	11		5

Integration and transposition			
Description	**PBCV-1**	**EsV-1**	**EhV-86**
Antirepressor of lysogeny			
Endonuclease			3
Homing endonuclease GIY-YIG	7		
Homing endonuclease HNH[e]	6	2	
Integrase			
Protelomerase			
Repressor of lysogeny			
Transposase		2	

Sugar and lipid manipulation			
Description	**PBCV-1**	**EsV-1**	**EhV-86**
Alginate mannuronan epimerase			
D-lactate dehydrogenase			
Fatty acid desaturase			2
Fructose-2, 6 bisphosphatase			
Fucose synthetase	*		
GDP-D-mannose dehydratase	*		
Glucosamine synthetase	*		
Glycerophosphoryl diesterase			
Glycosyltransferase	6		
Hyaluronan synthase	*		
Lipase			
Lipid phosphate phosphatase			
Lysophospholipase			
N-acetyltransferase			
Patatin phospholipase			
Salidase			
Serine palmitoyltransferase			
Sterol desaturase			
Sugar lyase			
Transmembrane fatty acid elongation protein			
UDP-glucose dehydrogenase	*		

Signaling			
Description	**PBCV-1**	**EsV-1**	**EhV-86**
Dual specificity phosphatase	#		
Glutamate receptor			
Hybrid histidine kinase		6	
Ligand-gated channel protein			
Phosphoshuttle			
Potassium channel protein	*#		
Ser/Thr protein kinase[f]	8*#	4	2

DNA restriction/modification			
Description	**PBCV-1**	**EsV-1**	**EhV-86**
Adenine DNA methylase	2*		
Cytosine DNA methylase	3*		
DNA restriction endonuclease	2*#		

Transcription			
Description	**PBCV-1**	**EsV-1**	**EhV-86**
DNA-dependent RNA polymerase II largest			
DNA-directed RNA polymerase II subunit			
DNA-directed RNA polymerase subunit			3
Histone H3, Lys 27 methylase	*#		
mRNA guanylyltransferase	*		
Oligoribonuclease			
RNA triphosphatase	*		
RNase III	*		
Superfamily II helicase[g]	3#		
SWI/SNF helicase	#		
Transcription factor TFIIB			
Transcription factor TFIID			
Transcription factor TFIIS			
VLTF2-type transcription factor			

Cell wall degradation			
Description	**PBCV-1**	**EsV-1**	**EhV-86**
Chitinase	2*#		
Chitosanase	*#		
β and α 1, 4-linked glucuronic lyase	*		
β-1, 3-glucanase	*		

Nucleotide metabolism			
Description	**PBCV-1**	**EsV-1**	**EhV-86**
Aspartate transcarbamylase	*		
Cytosine deaminase			
dCMP deaminase	*		
Deoxynucleoside kinase			
dUTP pyrophosphatase	*		
Glutaredoxin	*		
NTP pyrophosphohydrolase			
Nucleic acid-independent nucleoside			
Ribo. reductase (large subunit)			
Ribo. reductase (small subunit)			
Ribonuclease			
Thermonuclease			
Thioredoxin	#		2
Thymidylate kinase			
Thymidylate synthase X	*		
Thymidylate synthase-bifunct. dihydrofolate			

Miscellaneous			
Description	**PBCV-1**	**EsV-1**	**EhV-86**
ABC transporter protein	#		
Agmatine iminohydrolase	*		
Amidase	*#		
Cu/Zn-superoxi dedismutase	*#		
Fibronectin binding protein			
Histidine decarboxylase			
Homospermidine synthase	*#		
Monoamine oxidase	#		
N-carbamoylput amidohydrolase	*		
O-methyltransferase			
Ornithine decarboxylase	*		
Pathogenesis-related protein			
Calcium-binding protein			
Collagen-like protein			
Thaumatin-like protein			
Lectin protein			2
Longevity-assurance family protein			
Major facilitator			
Phosphate permease			
Phosphoglycerate mutase			

Present in all 3 viruses	
PBCV-1 + EsV-1	
PBCV-1 + EhV-86	
EsV-1 + EhV-86	
Unique to one virus	
Functional Enzyme	*
Virion Associated	#

Figure 3 Continued

virus does not acquire its glycoprotein capsid by budding through the host plasma membrane as it is released from the cell. Other chlorella viruses have longer replication cycles than PBCV-1. For example, virus NY-2A requires approximately 18 h for replication and consequently forms smaller plaques.

Virus EsV initiates its life cycle by infecting free-swimming, wallless gametes of its host. Virus particles enter the cell by fusion with the host plasma membrane and release a nucleoprotein core particle into the cytoplasm, leaving remnants of the capsid on the surface. The viral core moves to the nucleus within 5 min p.i. One important feature that distinguishes the EsV life cycle from the other phycodnaviruses is that the viral DNA is integrated into the host genome and is transmitted mitotically to all cells of the developing alga. The viral genome remains latent in vegetative cells until it is expressed in the algal reproductive cells, the sporangia or gametangia. Massive viral DNA replication occurs in the nuclei of these reproductive cells, followed by nuclear breakdown and viral assembly that continues until the cell becomes densely packed with virus particles. Virus release is stimulated by the same factors that induce discharge of gametes from the host, that is, changes in temperature, light, and water composition. This synchronization facilitates interaction of viruses with their susceptible host cells.

In contrast to the chlorella and EsV viruses, very little is known about the EhV life cycle, including how it infects its host. One property that clearly distinguishes EhV from the other phycodnaviruses is that it encodes six RNA polymerase subunits; in contrast, neither PBCV-1 nor EsV encodes a recognizable RNA polymerase component. Thus like the poxviruses, EhV may carry out its entire life cycle in the cytoplasm of its host. The latent period for EhV is 4–6 h with a burst size of 400–1000 particles per cell.

Virus Transcription

Detailed studies on transcription are lacking for all of the algal viruses and only a few general statements can be made about PBCV-1 transcription:

1. PBCV-1 infection rapidly inhibits host RNA synthesis.
2. Viral transcription is programmed and early transcripts appear within 5–10 min p.i. Late viral transcription first begins about 60–90 min p.i.
3. Some early viral transcripts are synthesized in the absence of *de novo* protein synthesis. As expected, the synthesis of later transcripts requires translation of early virus genes.
4. Early and late genes are dispersed in the PBCV-1 genome.
5. PBCV-1 ORFs are tightly packed on both DNA strands and the coding regions of some of the genes overlap. The largest distance between PBCV-1 ORFs is a 1788-nucleotide stretch in the middle of the genome. This sequence has a polycistronic gene containing 11 tRNAs (**Figure 2**).
6. Consensus promoter regions for early and late genes have not been identified, although the 50 nucleotides

Figure 3 Selected ORFs in the PBCV-1, EsV, and EhV genomes are arranged by their metabolic domains. If a genome encodes a putative protein more than once, a number in the box indicates the number of genes of this type per genome. Color-coding is indicated on the figure and is used to depict the relationship between viruses. Red indicates proteins that are encoded by all three viruses; yellow indicates proteins that are encoded by PBCV-1 and EsV, but not EhV; green indicates proteins that are encoded by PBCV-1 and EhV, but not EsV; orange indicates proteins that are encoded by EsV and EhV, but not PBCV-1; and blue indicates there are no shared homologs. Solid colored boxes indicate that the putative proteins are homologs. A diagonally stripped box indicates that the putative proteins are nonhomologous, and a checkered box indicates that the putative proteins are a mix of homologous, nonhomologous, or unique ORFs. In this case, a footnote has been added to clarify the specific differences; in parentheses the ORF has been defined by the gene number and any ORF beginning with an 'A' is from the PBCV-1 genome, an 'EsV' is from the EsV-1 genome, and an 'EhV' is from the EhV-86 genome. Proteins known to be functional are indicated with a star (*) and proteins known to be associated with the virion are indicated with a pound sign (#). Superscript 'a' indicates ATPase – One homolog between all three viruses (A392R, EsV-26, and EhV072), one homolog between PBCV-1 and EsV (A565R and EsV-171), and two PBCV-1 ATPases which have no homologs in EsV or EhV (A561L and A554/556/557L). Superscript 'b' indicates Exonuclease – One homolog between PBCV-1 and EsV (A166R and EsV-64) and one unique to EsV (EsV-126). Supercript 'c' indicates PCNA – One homolog between all three viruses (A193L, EsV-132, and EhV020), one homolog between PBCV-1 and EhV (A574L and EhV020). However, EhV-440, another EhV-encoded PCNA, has no homologs in PBCV-1 or EsV. Superscript 'd' indicates tRNAs – PBCV-1 has 11 tRNA genes, encoding AA Leu (2), Ile, Asn (2), Lys (3), Tyr, Arg, and Val. EhV has five tRNA genes, encoding AA Leu, Ile, Gln, Asn, and Arg. Supercript 'e' indicates Homing endonuclease HNH – One homolog between two viruses (A422R and EhV087). PBCV-1 (A87R) and EsV (EsV-119) are homologous. Four other HNH endonucleases are unique to PBCV-1 (A267L, A354R, A478R, and A490L) and one is unique to EsV (EsV-1–16). Superscript 'f' indicates Ser/Thr protein kinase – Four PBCV-1, two EsV-1, and one EhV-86 S/T kinases ORFs are homologous (A248R, A277L, A282L, A289L, EsV-82, EsV-111, and EhV451). The remaining S/T kinase ORFs from the three genomes (A34R, A278L, A614L, A617R, EsV-104, EV-156, and EhV-402) are unique. Suerscript 'g' indicates that superfamily II helicase, PBCV-1 (A153R), and EsV (EsV-66) are homologous. The two additional PBCV-1-encoded helicases (A241R and A363R) and EhV104 are unique. Reproduced from Dunigan DD, Fitzgerald LA, and Van Etten JL (2006) Phycodnaviruses: A peek at genetic diversity. *Virus Research* 117: 119–132, with permission from Elsevier.

preceding the ATG start codon of most functional PBCV-1 genes are at least 70% A+T.
7. Transcription of some PBCV-1 genes appears to be complex. For example, some gene transcripts exist as multiple bands and these patterns change between early and late times in the virus life cycle.

Additional *Chlorella* Viruses

Several hundred plaque-forming chloroviruses have been characterized to various degrees. They infect either *Chlorella* NC64A cells (NC64A viruses), an endosymbiont of *P. bursaria* isolated from North America, or *Chlorella* Pbi cells (Pbi viruses) that are endosymbiotic with a paramecium isolated in Europe. Like PBCV-1, each of these viruses contain many structural proteins, a large (>300 kbp) dsDNA genome, and they are chloroform sensitive. The DNAs of some of these viruses hybridize strongly with PBCV-l DNA, while others hybridize poorly.

Three additional chlorella virus genomes have been sequenced recently and others are nearing completion. The largest, the 370 kbp genome of virus NY-2A, contains ~400 protein-encoding genes. Most common genes are colinear in viruses PBCV-1 and NY-2A, which infect the same host chlorella. However, almost no colinearity exists between common genes in Pbi virus MT325 and those in PBCV-1, NY-2A, and AR158 suggesting plasticity in the chlorella virus genomes. Additionally, the G + C contents of the three NC64A viruses range from 40% to 41% whereas the G + C contents of the Pbi viruses are approximately 45%. These last two observations suggest that these two virus groups have been separated for considerable evolutionary time. Viruses morphologically similar to the NC64A and Pbi viruses have also been isolated from chlorella symbiotic in the coelenterate *Hydra viridis* and very recently from the heliozoon *Acanthocystis turfacea*. The symbiotic hydra chlorella have not been cultured and so very little is known about these viruses. However, the *A. turfacea* viruses can be isolated by plaque formation.

Other Algal Viruses

Field isolates representing at least six genera of filamentous brown algae contain virus particles that are morphologically similar to EsV and FsV. Virus expression is variable; virions are rarely observed in vegetative cells but often are common in unilocular sporangia (FsV) or both unilocular and plurilocular sporangia (EsV). EsV viruses only infect the free swimming, zoospore stage of *Ectacarpus* sp. All natural isolates of *Feldmania* sp. are infected with virus and so infection studies cannot be conducted.

Viruses that infect *Emiliania huxleyi*, *Micromonas pusilla*, and *Chrysochromulina brevfilum* have been isolated from many marine environments. These viruses can be distinguished by DNA restriction patterns.

Phycodnavirus Genes Encode Some Interesting and Unexpected Proteins

Many chlorella virus-encoded enzymes are either the smallest or among the smallest proteins of their class. In addition, homologous genes in the chloroviruses can differ in nucleotide sequence by as much as 50%, which translates into amino acid differences of 30–40%. Therefore, comparative protein sequence analyses can identify conserved amino acids in proteins as well as regions that tolerate amino acid changes. The small sizes and the finding that many chlorella virus-encoded proteins are 'user friendly' have resulted in the biochemical and structural characterization of several PBCV-1 enzymes. Examples include: (1) The smallest eukaryotic ATP-dependent DNA ligase, which is the subject of intensive mechanistic and structural studies. (2) The smallest type II DNA topoisomerase. The virus enzyme cleaves dsDNAs about 50 times faster than the human type II DNA topoisomerase; consequently, the virus enzyme is being used as a model enzyme to study the mechanism of topoisomerase II DNA cleavage. (3) An RNA guanylyltransferase that was the first enzyme of its type to have its crystal structure resolved. (4) A small prolyl-4-hydroxylase that converts Pro-containing peptides into hydroxyl-Pro-containing peptides in a sequence-specific fashion. (5) The smallest protein (94 amino acids) to form a functional potassium ion channel. These minimalist enzymes may represent evolutionary precursors of contemporary proteins, but it is also possible that they are products of evolutionary optimization during viral evolution.

The chloroviruses are also unusual because they encode enzymes involved in sugar metabolism. Three PBCV-1 encoded enzymes, glutamine:fructose-6-phosphate amindotransferase, UDP-glucose dehydrogenase, and hyaluronan synthase, are involved in the synthesis of hyaluronan, a linear polysaccharide composed of alternating β-1,4-glucuronic acid and β-1,3-*N*-acetylglucosamine residues. All three genes are transcribed early in PBCV-1 infection and hyaluronan accumulates on the external surface of the infected cells.

Two PBCV-1-encoded enzymes, GDP-D-mannose dehydratase and fucose synthase, comprise a three-step pathway that converts GDP-D-mannose to GDP-L-fucose. The function of this putative pathway is unknown. However, fucose, a rare sugar, is present in the glycans attached to the major capsid protein.

PBCV-1 encodes four enzymes involved in polyamine biosynthesis: ornithine decarboxylase (ODC), homospermidine synthase, agmatine iminohydrolase, and *N*-carbamoyl-putrescine amidohydrolase. ODC catalyzes the decarboxylation of ornithine to putrescine, which is the first and the rate-limiting enzymatic step in polyamine biosynthesis. Not only is the PBCV-1-encoded ODC the smallest known ODC, the PBCV-1 enzyme is also interesting because it decarboxylates arginine more efficiently than ornithine.

The genome sequences of other phycodnaviruses have revealed several interesting and unexpected gene products. However, except for an aquaglyceroporin encoded by chlorella virus MT325, none of these products have been expressed and tested for enzyme function.

Ecology

Eukaryotic algae are important components of both freshwater and marine environments; however, the significance of viruses in these systems is only beginning to be appreciated. The chlorella viruses are ubiquitous in freshwater collected throughout the world and titers as high as 100 000 infectious particles per mililiter have been reported in native waters. Typically, the titer is 1–100 infectious particles per milliliter. The titers are seasonal with the highest titers in the spring. It is not known whether chlorella viruses replicate exclusively in algae symbiotic with paramecia or if the viruses have another host(s). In fact, it is not known if paramecium chlorellae exist free of their hosts in natural environments. However, the chlorellae are protected from virus infection when they are in a symbiotic relationship with the paramecium.

The concept that viruses might have a major impact on the marine environment began about 15 years ago with the discovery that seawater contains as many as 10^7 VLPs per milliliter. This huge population consits of both bacterial and algal viruses and is important because phytoplankton, consisting of cyanobacteria and eukaryotic microalgae, fix 50–60% of the CO_2 on Earth. At any one time, 20–40% of these photosynthetic organisms are infected with a virus. Consequently, these viruses contribute to microbial composition and diversity, as well as, nutrient cycling in aqueous environments. Thus viruses, including the phycodnaviruses, have a major impact on global carbon/nitrogen cycles that is only beginning to be appreciated by scientists, including those who model such cycles.

Algal viruses are also believed to play major roles in the termination of marine algal blooms and so there are active research efforts to understand the natural history of these algal/virus systems. For example, the coccolithophorid *Emiliania huxleyi* is a unicellular alga found throughout the world; the alga can form immense coastal and mid-oceanic blooms at temperate latitudes that cover 10 000 km^2 or more. One of the primary mechanisms for terminating *E. huxleyi* blooms is lysis by the EhV viruses described above.

The filamentous brown algae, *Ectocarpus* sp. and *Feldmania* sp. isolated from around the world, are infected with lysogenic EsV and FsV viruses, respectively. Lysogeny is consistent with the observation by early investigators that VLPs appear infrequently in eukaryotic algae and only at certain stages of algal development. The apparent lack of infectivity by many of the previously observed VLPs in eukaryotic algae is also consistent with a lysogenic lifestyle. The VLPs might either infect the host and resume a lysogenic relationship or be excluded by preexisting lysogenic viruses.

Perspectives

Sequence analyses of three phycodnaviruses suggest that this family may have more sequence diversity than any other virus family. These three viruses only have 14 homologous genes, which means that there are in excess of 1000 unique ORFs in just these three viruses. Despite the large genetic diversity in these three sequenced phycodnaviruses, phylogenetic analyses of δ-DNA polymerases and DNA primases indicate that the phycodnaviruses group into a monophyletic clade within the NCLDVs. A recent analysis using eight concatenated core NCLDV genes also indicates that the phycodnaviruses cluster together and are members of the NCLDV 'superfamily'. However, it is obvious that the identification and characterization of phycodnaviruses is in its infancy. Metagenomic studies, such as DNA sequences from the Sargasso Sea samples, indicate that translation products of many unknown sequences are more similar to PBCV-1 proteins than the next known phycodnavirus.

See also: African Swine Fever Virus; Algal Viruses; Iridoviruses of Invertebrates; Marnaviruses; Poxviruses.

Further Reading

Brussaard CPD (2004) Viral control of phytoplankton populations – A review. *Journal of Eukaryotic Microbiology* 51: 125–138.

Dunigan DD, Fitzgerald LA, and Van Etten JL (2006) Phycodnaviruses: A peek at genetic diversity. *Virus Research* 117: 119–132.

Iyer ML, Balaji S, Koonin EV, and Aravind L (2006) Evolutionary genomics of nucleocytoplasmic large DNA viruses. *Virus Research* 117: 156–184.

Muller DG, Kapp M, and Knippers R (1998) Viruses in marine brown algae. *Advances in Virus Research* 50: 49–67.

Van Etten JL (2003) Unusual life style of giant chlorella viruses. *Annual Review of Genetics* 37: 153–195.

Van Etten JL, Graves MV, Muller DG, Boland W, and Delaroque N (2002) Phycodnaviridae – large DNA algal viruses. *Archives of Virology* 147: 1479–1516.

Wilson WH, Schroeder DC, Allen MJ, *et al.* (2005) Complete genome sequence and lytic phase transcription profile of a Coccolithovirus. *Science* 309: 1090–1092.
Yamada T, Onimatsu H, and Van Etten JL (2006) Chlorella viruses. *Advances in Virus Research* 66: 293–336.

Relevant Websites

http://www.giantvirus.org – Giantvirus.org.
http://greengene.uml.edu – Greengene at UML.

Phylogeny of Viruses

A E Gorbalenya, Leiden University Medical Center, Leiden, The Netherlands

© 2008 Elsevier Ltd. All rights reserved.

Introduction: Evolution, Phylogeny, and Viruses

Biological species, including viruses, change through generations and over time in the process known as evolution. These changes are first fixed in the genome of successful individuals that give rise to genetic lineages. Due to either limited fidelity of the replication apparatus copying the genome or physico-chemical activity of the environment, nucleotides may be changed, inserted, or deleted. Genomes of other origin may also be a source of innovation for a genome through the use of specially evolved mechanisms of genetic exchange (recombination). Accepted changes, known as mutations, may be neutral, advantageous, or deleterious, and depending on the population size and environment, the mutant lineage may proliferate or go extinct. Overall, advantageous mutations and large population size increase the chances for a lineage to succeed. The lineage fit is constantly reassessed in the ever-changing environment and lineages that, due to mutation, became a success in the past could be unfit in the new environment. Due to the growing number of mutations accumulating in the genomes, lineages diverge over time, although occasionally, due to stochastic reasons or under similar selection pressure, they may converge.

The relationship between biological lineages related by common descent is called phylogeny; the same term also embodies the methodology of reconstructing these relationships. Phylogeny deals with past events and, therefore, it is reconstructed by quantification of differences accumulated between lineages. Due to the lack of fossils and (relatively) high mutation rate, viruses were not considered to provide a recoverable part of phylogeny until the advent of molecular data proved otherwise. Comparison of nucleotide and amino acid sequences, and, occasionally, other quantitative characteristics such as distances between three-dimensional structures of biopolymers, have been used to reconstruct virus phylogeny. Results of phylogenetic analysis are commonly depicted in the form of a tree that may be used as a synonym for phylogeny. For instance, all-inclusive phylogeny of cellular species is depicted as the Tree of Life (ToL).

With few exceptions, virus phylogeny follows the theory and practice developed for phylogeny of cellular life forms. For inferring phylogeny, differences between the sequences of species members, assumed to be of a discernable common origin, are analyzed. If species in all lineages evolve at a uniform constant rate, like clock ticks, their evolution conforms to a molecular clock model. The utility of this model in relation to viruses may be very limited. Rather, related virus lineages may evolve at different and fluctuating rates and some sites may mutate repeatedly with each new mutation erasing a record about the prior change. As a result, the accumulation of inter-species differences may progress nonlinearly with the time elapsed. At present, our understanding of these parameters of virus evolution is poor and this limits our ability to assess the fit between a reconstructed phylogeny and the true phylogeny, with the latter practically remaining unknown for most virus isolates. This gap in our knowledge does not eliminate the conceptual strength of phylogentic analysis for reconstructing the relationships between biological species.

The ultimate goal of virus phylogeny is reconstructing the relationships between 'all' virus isolates and species. In contrast to cellular species, which form three compact domains (kingdoms) and whose origin is traced back to a common ancestor in the ToL, major virus classes may combine species that have originated from different ancestors. Thus, reconstructing the comprehensive virus phylogeny requires comparisons that involve genomes of virus and cellular origins. This formidable task remains largely 'work in progress'. In fact, most efforts in virus phylogeny are invested in reconstructing the relationships at the micro, rather than grand, scale and they focus on well-sampled lineages that have practical (e.g., medical) relevance. Phylogeny itself or in combination with other data may provide a deep insight into virus evolution and diverse aspects of virus life cycles, including virus interactions with their hosts.

Our knowledge about contemporary virus diversity has been steadily advancing with new viruses being

constantly described by systematic efforts as well as occasional discoveries. These developments indicate that only a small part of virus diversity has so far been unraveled and has become available for phylogenetic studies. It is also likely that many more lineages existed in the past; some of these lineages are likely to have ancestral relationships with contemporary lineages.

Tree Definitions

Species share similarity that varies depending on the rate of evolution and time of divergence. The entire process of generating contemporary species diversity from a common ancestor is believed to proceed through a chain of intermediate ancestors specific for different subsets of the analyzed species. The relationship between the common ancestor, intermediate ancestors, and contemporary species may be likened to the relationship between, respectively, root, internal nodes, and terminal nodes (leaves) of a tree, an abstraction that is widely used for the visualization of this relationship. Trees are also part of graph theory, a branch of mathematics, whose apparatus is used in phylogeny. Formally and due to a strong link between phylogeny and taxonomy, leaves may be called operational taxonomy units (OTUs) and internal nodes and roots, since they have not been directly observed, are known as hypothetical taxonomy units (HTUs). Nodes are connected by branches or edges.

The tree may be characterized by topology, length of branches, shape, and the position of the root. The topology is determined by relative position of internal and terminal nodes; it defines branching events leading to contemporary species diversity. If two or more trees obtained for different data sets feature a common topology, these trees are called congruent. The branch length of a tree may define either the amount of change fixed or the time passed between two nodes connected in a tree, and is known as 'additive' or 'ultrametric', respectively. The tree shape may be linked to particulars of evolutionary process and reflect changes in the population size and diversity due to genetic drift and natural selection. The position of the root at the tree defines the direction of evolution. Species that descend from an internal node in a rooted tree form a cluster and the node is called most recent common ancestor (MRCA) of the cluster that thus has a monophyletic origin. The branch lengths and the root position may be left undefined for a tree that is then called 'cladogram' and 'unrooted tree', respectively.

Phylogenetic Analysis

Multiple alignment of polynucleotide or amino acid sequences representing analyzed species and maximized for similarity is traditionally used as input for phylogenetic analysis. The quality of alignment is among the most significant factors affecting the quality of phylogenetic inference. Due to the redundancy of the genetic code, changes in polynucleotide sequences are accumulated at a higher rate than those in amino acid sequences. In viruses, including RNA viruses, this difference is not counterbalanced by other constraints linked to dinucleotide frequency and RNA secondary (tertiary) structure. Because of these differences, phylogeny of closely related species is commonly inferred using polynucleotide sequences, while protein sequences, preserving better phylogenetic signal, may be used to infer phylogeny of distantly related species.

Differences between species, as calculated from alignment, may be quantified as either pairwise distances forming a distance matrix or position-specific substitution columns (discrete characters of states of alignment), the latter preserving the knowledge about location of differences. The respective methods dealing with these quantitative characteristics are known as distance and discrete (character state). The distance methods are praised for their speed and are considered a technique of choice for analysis of large data sets. They are often designed to converge on a unique phylogeny, with none others being even considered. The unweighted pair group method with arithmetic means (UPGMA) in which a constantly recalculated distance matrix is used to define the hierarchy of similarities through systematic and stepwise merging of most similar pairs at a time was the first technique introduced for clustering. The neighbor-joining (NJ) method uses a more sophisticated algorithm of clustering that minimizes branch lengths, and is the most popular among distance methods. Although different trees may be compared in how they fit a distance matrix, it is distance character-based methods that are routinely used to assess numerous alternative phylogenies in search for the best one in a computationally very intensive process. Due to the calculation time involved, assessing all possible phylogenies is found to be impractical for data sets including more than 10 sequences; for larger data sets different heuristic approximations are used that may not guarantee a recovered phylogeny to be the best overall. There are two major criteria for selecting the best phylogeny using character-state based information through either maximum parsimony (MP) or maximum likelihood (ML). In MP analysis, a phylogeny with a minimal number of substitutions separating analyzed species is sought. The ML analysis offers a statistical framework for comparing the likelihood of fitting different trees into the data in search for one with the best fit. The latter approach is mathematically robust and its statistical power may also be used in combination with other techniques of tree generation. Most recently, a Bayesian variant of the ML

approach has gained popularity. It utilizes prior knowledge about the evolutionary process in combination with repeated sampling from subsequently derived hypotheses.

After a tree is chosen, it is common to assign support for internal nodes through assessing nodes' persistence in trees related to the chosen tree. One particular technique, called bootstrap analysis, in which trees are generated for numerous randomly modified derivatives of the original data set, is most frequently used. Each internal node in the original tree is characterized by a so-called bootstrap value that is equal to the number of nodes appearing in all tested trees. Although the relationship between bootstrap and statistical values is not linear, nodes with very high bootstrap values are considered to be reliable. If species evolve according to a molecular clock model, the root position in a tree could directly be calculated from the observed inter-species differences as a midpoint of cumulative inter-species differences. Alternatively, the root position may be assigned to a tree from knowledge about analyzed species that was gained independently from phylogenetic analysis. Commonly, this knowledge comes in the form of a single or more species which are assumed (or known) to have emerged before the 'birth' of the analyzed cluster. These early diverged species are collectively defined as 'outgroup', while the analyzed species may be called 'in-group'. Also, a tree may be generated unrooted, a common practice in phylogenetic analysis of viruses for which the applicability of the molecular clock model remains largely untested and reliable outgroups may not be routinely available. In the unrooted tree, grouping of species in separate clusters may be apparent, although these clusters may not be treated as monophyletic as long as the direction of evolution has not been defined. These challenges are addressed by the development of new approaches that infer rooted trees without artificially restricting species evolution to a constant rate (known as relaxed molecular clock models).

Virus phylogeny can be inferred using genomes or distinct genes and each of these approaches, standard in phylogenomics, may be considered as complementary. Under the first approach, genome-wide alignments are used for analysis. Due to complexities of the evolutionary process that may be region specific, reliable genome-wide alignments can routinely be built only for relatively closely related viruses whose analysis, however, may be further complicated by recombination events (see below). Using the second approach, genes with no evidence for recombination may be merged (concatenated) in a single data set that may be used to produce a superior phylogenetic signal compared to those generated for distinct genes or entire genomes. For viruses with small genomes or for a diverse set of viruses, it is common practice to use a single gene to infer virus phylogeny. Although the results produced may be the best models describing evolutionary history of a group of viruses, the validity of this gene-based approach for the genome-wide extrapolation remains a point of debate.

When the tree is reconstructed for (part of) genomes, an underlying assumption is that the analyzed data set has a uniform phylogeny. This condition may be violated due to homologous recombination between (closely) related viruses. In phylogenetic terms, recombination may be revealed through incongruency of trees built for a genome region, where recombination occurs, and other regions. Trees may also become incongruent due to various technical reasons related to the size and diversity of a virus data set and deviations of the evolutionary process among lineages. These characteristics complicate interpretation of the congruency test, which is widely used in different programs to identify recombination in viruses.

Applications of Phylogeny in Virology

Phylogenetic analysis is used in a wide range of studies to address both applied and fundamental issues of virus research, including epidemiology, diagnostics, forensic studies, phylogeography, origin, evolution, and taxonomy of viruses. First question to be answered during an outbreak of a virus epidemic concern the virus identity and origin. Answers to these questions form the basis for implementing immediate practical measures and prospective planning enabling specific and rapid virus detection and epidemic containment, which may include the use and development of antiviral drugs and vaccines. Among different analyses performed for virus identification at the early stage of a virus epidemic, the phylogenetic characterization is used for determining the relationship of a newly identified virus with all other previously characterized and sequenced viruses.

Results of this analysis may be sufficient to provide answers to the questions posed, as regularly happens with closely monitored viruses that include most human viruses of high social impact, for example, influenza, human immunodeficiency virus (HIV), hepatitis C virus (HCV), poliovirus, and others. For these viruses, there exist large databases of previously characterized isolates and strains that comprehensively cover the natural diversity. Should a newly identified virus belong to one of these species, chances are that it has evolved from a previously characterized isolate or a close variant and this immediately becomes evident in the clustering of these viruses in the phylogenetic tree. Combining the results of gene-specific and genome-wide phylogenetic analysis allows one to determine whether recombination contributed to the isolate origin. For instance, recombination was found to be extremely uncommon in the evolution

of HCV, but not for poliovirus lineages that recombine promiscuously, also with closely related human coxsackie A viruses, both of which belong to human enteroviruses.

When an emerging infection is caused by a new never-before-detected virus, the phylogenetic analysis is instrumental for classification of this virus and in the case of a zoonotic infection, for determining the dynamic of virus introduction into the (human) population and initiating the search for the natural virus reservoir. This was the case with many emerging infections including those caused by most recently introduced Nipah virus, a paramyxovirus, and SARS coronavirus (SARS-CoV). With the latter virus, poor sampling of the coronavirus diversity in the SARS-CoV lineage at the time, some uncertainty over the relationship between phylogeny and taxonomy of coronaviruses, and the complexity of phylogenetic analysis of a virus data set including isolated distant lineages led to considerable controversy over the exact evolutionary position of SARS-CoV among coronaviruses. Since then, the matter has largely been resolved but this experience illustrates some challenges in inferring virus phylogeny.

The search for a zoonotic reservoir of an emerging virus may involve a significant and time-consuming effort that requires numerous phylogenetic analyses of ever-expanding sampling of the virus diversity generated in pursuit of the goal. In this quest, phylogenetic analysis canalizes the effort and provides crucial information for reconstructing parameters of major evolutionary events that promoted the virus origin and spread. For instance, intertwining HIV and simian immunodeficiency virus (SIV) lineages in the primate lentivirus tree led to postulation that the existing diversity of HIV in the human population originated from several ancestral viruses independently introduced from primates over a number of years. Similar phylogenetic reasoning was used to trace the origin of a local HIV outbreak to a common source of HIV introduction through dental practice (known as 'HIV dentist' case). These are typical examples illustrating the utility of phylogenetic analysis for epidemiological and forensic studies.

Geographic distribution of places of virus isolation is another important characteristic relative to which virus phylogeny may be evaluated. This field of study belongs to phylogeography. The evolution of human JC polyomavirus provides an example of confinement of circulation of virus clusters to geographically isolated areas, represented by three continents. Recent identification of West Nile virus in the USA illustrates geographical expansion of an Old World virus into the New World. Analysis of phylogenies of field isolates of rabies virus of the family *Rhabdoviridae* sampled from different animals over Europe led to the recognition that interspecies virus expansion is occuring faster when compared to geographical expansion.

Phylogenies also reveal information about the relative strength of the virus–host association over time. In some virus families (e.g., the *Coronaviridae*) host-jumping events may be relatively frequent, including the emergence of at least two human viruses, dead-end SARS-CoV and successfully circulating human coronavirus OC43 (HCoV-OC43). At the other end of the spectrum one finds the family *Herpesviridae*. Extensive phylogenetic analysis of herpesviruses and their hosts showed a remarkable congruency of topologies of trees indicating that this virus family may have emerged some 400 million years ago and that herpesviruses cospeciate with their hosts.

Phylogenetic analysis becomes increasingly important in virus classification (taxonomy) and relies on complex multicharacter rules applied to separate virus families by respective 'study groups'. For viruses united in high-rank taxa above the genus level, phylogenetic clustering for most conserved replicative genes is commonly observed and used in the decision making process. For instance, human hepatitis E virus, originally classified as a calicivirus using largely virion properties, was eventually expelled from the family due to poor fit of genome characteristics, including results of phylogenetic analysis. Phylogenetic considerations also played an important role in forming recently established families, for example, the *Marnaviridae* and *Dicistroviridae*. In contrast, phylogenetic analysis has been of relatively little use in the taxonomy of large DNA phages which has been developed in such a way that existing families may unite phages with different gene layouts and phylogenies. The relationship between phylogeny and taxonomy is evolving and in future one might hope for important advancements that improve cross-family consistency in relation to phylogeny.

See also: Emerging and Reemerging Virus Diseases of Vertebrates; Evolution of Viruses; Origin of Viruses; Taxonomy, Classification and Nomenclature of Viruses; Virus Classification by Pairwise Sequence Comparison (PASC); Virus Databases; Virus Evolution: Bacterial Viruses; Virus Species.

Further Reading

Dolja VV and Koonin EV (eds.) (2006) Comparative genomics and evolution of complex viruses. *Virus Research* 117: 1–184.

Domingo E (2007) Virus evolution. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology*, 5th edn., pp. 389–421. Philadelphia, PA: Wolters Kluwer, Lippincott Williams and Wilkins.

Domingo E, Webster RG, and Holland JJ (eds.) (1999) *Origin and Evolution of Viruses.* San Diego: Academic Press.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.* San Diego, CA: Elsevier Academic Press.

Felsenstein J (2004) *Inferring Phylogenies.* Sunderland, MA: Sinauer Associates, Inc.

Gibbs AJ, Calisher CH, and Garcia-Arenal F (eds.) (1995) *Molecular Basis of Virus Evolution.* Cambridge: Cambridge University Press.

Moya A, Holmes EC, and Gonzalez-Candelas F (2004) The population genetics and evolutionary epidemiology of RNA viruses. *Nature Reviews Microbiology* 2: 279–288.
Page RD and Holmes EC (1998) *Molecular Evolution. A Phylogenetic Approach.* Boston: Blackwell Publishing.
Salemi M and Vandamme AM (eds.) (2003) *The Phylogenetic Handbook. A Practical Approach to DNA and Protein Phylogeny.* Cambridge: Cambridge University Press.
Villarreal LP (2005) *Viruses and Evolution of Life*. Washington, DC: ASM Press.

Picornaviruses: Molecular Biology

B L Semler and K J Ertel, University of California, Irvine, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

***Cre* (*cis*-acting replication element)** First found in human rhinovirus genomic RNA and subsequently identified in other picornavirus genomes, the *cre* acts as template for the viral RNA polymerase $3D^{pol}$ to uridylylate VPg to VPg-pU-pU. Evidence suggests that this *cre* can function in *trans* as well.
IRES (internal ribosome entry site) An RNA sequence typically characterized by extensive nucleic acid secondary structure. The 40S ribosomal subunit of the cellular translation machinery interacts with RNA stem loops/sequence, and subsequently allows translation of downstream RNA sequence of the IRES. Translation, therefore, proceeds without recognition of a 5′ cap. Utilized by some virus families (including *Picornaviridae*) and cellular messenger RNAs.
Polyprotein In the context of the discussion of *Picornaviridae*, this refers to the long protein resulting from translation of the single open reading frame of picornavirus RNA. The polyprotein is processed by viral proteinases to yield mature viral proteins.
Positive-strand RNA A single molecule of picornavirus RNA that encodes functional viral protein when translated in the 5′–3′ direction. This is the 'sense' orientation of picornavirus RNA as it enters the cell that is also encapsidated in progeny virions.
RNP complex Ribonucleoprotein complex. Describing a stable interaction of RNA and protein(s), either *in vivo* or *in vitro*.
Uridylylation Refers to the addition of two uridylate residues to the VPg molecule by the picornavirus RNA-dependent RNA polymerase $3D^{pol}$ (and other viral proteins) using a viral RNA template.
VPg Virus protein, genome-linked. Also known as 3B, the function of VPg is to act as a protein primer for the picornavirus RNA-dependent RNA polymerase. Following uridylylation, VPg-pU-pU is covalently attached to the 5′ end of picornavirus RNAs (positive- and negative-strands).

Overview of Virus Family *Picornaviridae*

Introduction

Viruses belonging to the family *Picornaviridae* are small (Latin *Pico*) RNA (*rna*) viruses whose host range is typically restricted to mammals. Genera associated with *Picornaviridae* include erbovirus, teschovirus, kobuvirus, aphthovirus, cardiovirus, enterovirus, hepatovirus, parechovirus, and rhinovirus. The first three of these genera are relatively recent additions to the picornavirus family, and the last four contain pathogens that are the most extensively studied picornaviruses capable of infecting humans. Particularly, poliovirus of the enterovirus family is widely considered to be the 'prototypical' picornavirus, and perhaps the most feared by humans due to the potential for poliovirus infection to result in paralytic poliomyelitis. This debilitating affliction can result in paralysis of one or more limbs in an infected individual, and in rare cases even death. Human rhinovirus infection in humans results in the common cold, and is one of the most prevalent diseases throughout the world. While in the developed world the common cold is often seen merely as an inconvenience at worst, it is the most important cause of asthma exacerbations, and there is no effective vaccine for the virus nor any effective medical treatment for infection.

Due to the intense interest in *Picornaviridae* based upon the diseases associated with picornavirus infections, the virus family has received extensive scientific attention to understand the mechanisms of gene expression and replication of its members. In turn, insights into the manner in which members of the *Picornaviridae* propagate have allowed a better understanding of how these viruses cause disease and also how other unrelated virus families

replicate within their own host cell systems. The aim of this article is to discuss the general mechanisms of gene expression and propagation of the *Picornaviridae* as well as to highlight some of the differences between the individual picornavirus family members.

Classification

From a disease perspective, individual virus species of the family *Picornaviridae* are grouped by genus based on their pathogenic properties and/or route of infection (**Table 1**). For example, the enterovirus genus includes poliovirus and coxsackievirus based upon the natural oral route of entry into the host and replication in gut tissue. The genus cardiovirus includes encephalomyocarditis virus (EMCV), which infects heart and nervous tissues in rodents. Based upon the ability of neutralizing antibody to recognize capsid antigens on the surface of the virion the species of *Picornaviridae* are further subclassified on the basis of serotype. This can range from a single serotype in the case of hepatitis A virus (HAV), or, in the case of rhinovirus, to ~100 identified serotypes.

In terms of molecular genetics, picornaviruses are broadly classified according to the internal ribosome entry site (IRES) in the 5′ noncoding region (NCR). Based upon similarity in RNA secondary structure and sequence studies, three distinct types of picornavirus IRES elements have been characterized. The genomes of enteroviruses (poliovirus and coxsackievirus) and rhinovirus contain type I IRESs, while type II IRESs are found in the genomes of cardiovirus, aphthovirus, erbovirus, and parechovirus. The HAV IRES is considered an outlier of the type II IRES and is classified as a type III IRES. The recently identified teschovirus and kobuvirus IRES elements are yet to be examined in enough detail for placement into a specific type. The function and characteristics of picornavirus IRES types are discussed in the section on 'Translation' of this article.

The other primary difference that distinguishes the picornavirus genera relates to structure and function of the proteins encoded by the polyprotein. For example, the genomes of enteroviruses, rhinoviruses, parechoviruses, and hepatoviruses do not code for a 'leader' (L) protein. Instead, many of these viruses encode a proteinase in the P2 region of the genome designated 2A. The 2A proteinase is structurally similar among the enteroviruses and rhinoviruses. Cardiovirus, aphthovirus, erbovirus, teschovirus, and kobuvirus genomes code for an L protein at the amino terminus of the uncleaved polyprotein, but only the aphthovirus L protein has been reported to be a proteolytically active proteinase. Parechovirus and HAV RNA genomes do not encode an L protein and the 2A regions of the genomes of these viruses are not proteolytically active.

Genome Structure and Features

All picornaviruses contain a single molecule of RNA of messenger RNA (mRNA) sense (hereafter referred to as positive-sense) within the capsid (**Figure 1**). The nucleotide (nt) length of the RNA genome found in virion particles is quite short compared to most other viruses, ranging from 6500 to 9500 nt in length. The 5′ end of the viral genome is covalently linked to the viral polypeptide VPg (virus protein, genome linked). The 5′ RNA terminus also contains RNA secondary structures important for viral RNA synthesis (see the section titled 'Viral RNA replication'). In comparison to cellular mRNAs, the picornavirus 5′ NCR is quite long often in excess of 700 nt and up to 1200 nt. Encompassing nearly 10% of the total RNA genome length, the 5′ NCR contains the picornavirus IRES, which allows initiation of translation of the viral genome. The 3′ NCRs of picornaviruses are, in contrast to many cellular mRNAs, quite short, ranging from 50 to 100 nt in length. Lastly, a homopolymeric poly(A) tract encoded by the viral genome follows the 3′ NCR and may participate in the formation of stable RNA secondary structure(s) with the heteropolymeric 3′ NCR. The poly(A) tract likely stabilizes and protects the viral RNA

Table 1 Members of *Picornaviridae*

Genus	*Representative species*	*Associated disease*
Aphthovirus	*Foot-and-mouth disease virus (FMDV)*	Foot-and-mouth disease (livestock)
Cardiovirus	*Encephalomyocarditis virus (EMCV), Theiler's murine encephalomyocarditis virus (TMEV)*	Encephalomyelitis, myocarditis (mice and livestock), demyelination in mice
Enterovirus	*Poliovirus, coxsackievirus*	Poliomyelitis; hand, foot, and mouth disease; myocarditis
Erbovirus	*Equine rhinitis virus*	Upper respiratory infection (horses)
Hepatovirus	*Hepatitis A virus*	Acute liver disease
Kobuvirus	*Aichi virus*	Gastroenteritis
Parechovirus	*Human parechovirus*	Gastroenteritis, paralysis, encephalitis, neonatal carditis
Rhinovirus	*Human rhinovirus*	Common cold
Teschovirus	*Porcine teschovirus*	Encephalitis, paralysis (swine)

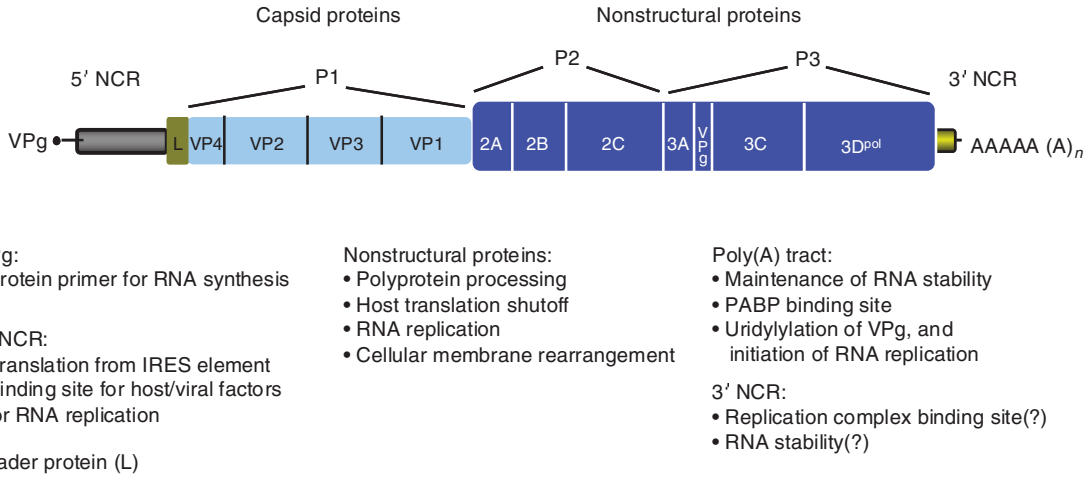


Figure 1 Organization of a typical picornavirus RNA genome. VPg is covalently attached to the 5′ terminus of genomic RNA. The 5′ NCR contains RNA sequences forming RNA structural elements used in virus RNA translation and replication. The coding region is divided into three sections consisting of the P1, P2, and P3 regions. The RNA region of P1 is translated and processed to virion capsid proteins. Some of the functions of the nonstructural proteins contained in the P2 and P3 regions are listed above. Following the P3 region, the 3′ NCR and poly(A) tract function in replication and RNA stability.

genome from intracellular degradation enzymes, much as the poly(A) tracts of cellular mRNAs protect their cognate RNAs. Despite the genetic and phenotypic differences that distinguish the members of the family *Picornaviridae*, all members utilize similar strategies of genome replication and production of infectious virus particles in their respective host-cell environments. Due to a positive-sense RNA genome, picornavirus RNA can immediately be translated into polyprotein upon entry into the cell cytoplasm, and does not require the inclusion of viral proteins such as polymerase in the capsid (as in the case of negative-strand RNA viruses). Moreover, nearly all picornaviruses inhibit cap-dependent translation of host cell mRNAs while utilizing a cap-independent mechanism to translate their own genome, reducing the competition for translation factors between the virus and host cell mRNAs in favor of the virus.

Picornavirus Entry and Gene Expression

Capsid

Picornavirus genomes are packaged into icosahedral virion structures consisting of 60 individual protomers formed by the structural proteins in the P1 coding region: VP1, VP2, VP3, and VP4. A single copy of VP1, VP2, and VP3 is organized into a triangular subunit on the surface of the capsid. Five of these subunits are organized around a fivefold symmetrical axis to form a pentameric protomer (**Figure 2**). Twelve of these pentameric subunits (a total of 60 triangular protomers) form the icosahedron, with twofold and threefold axes of symmetry located at the connection points. The small structural protein VP4 is not present on the outside of the mature virion; it is located in the interior of the capsid structure and interacts with the encapsidated RNA genome. During assembly, inclusion of the RNA genome coincides with the processing of a precursor polypeptide (VP0) into VP4 and VP2. The mechanism for the maturation of VP0 to VP4 and VP2 is currently unknown. What is known is that the viral proteinases 2A, L, and 3C/3CD do not appear to be involved, suggesting a cellular factor may be required for virion maturation or that viral RNA/capsid proteins effect this cleavage.

The virion capsid proteins VP1, VP2, and VP3 of enteroviruses and rhinoviruses protrude from the surface of the capsid structure at the axes of symmetry, forming the 'mesa' at the fivefold axes and the 'propeller' at the threefold axes (**Figure 2**). Observations of these extrusions using X-ray crystallography give the impression of clefts between the axes of symmetry, termed 'canyons'. The canyon region of *Picornaviridae* was initially proposed to be the site of virion binding to cell receptors prior to entry into the cytoplasm, based upon its predicted inaccessibility to neutralizing antibody. This hypothesis has been supported by cryoelectron micrograph studies of cell receptor bound to the canyon region of rhinovirus and coxsackievirus virion particles. While the canyon region of cardioviruses and aphthoviruses is not nearly as pronounced as in enteroviruses and rhinoviruses, neutralization studies investigating the virion capsid of foot-and-mouth disease virus (FMDV) indicate that the G–H loop of the VP1 capsid protein is important for cell receptor recognition.

A summary of some of the cellular molecules identified as cellular receptors for picornaviruses is listed in

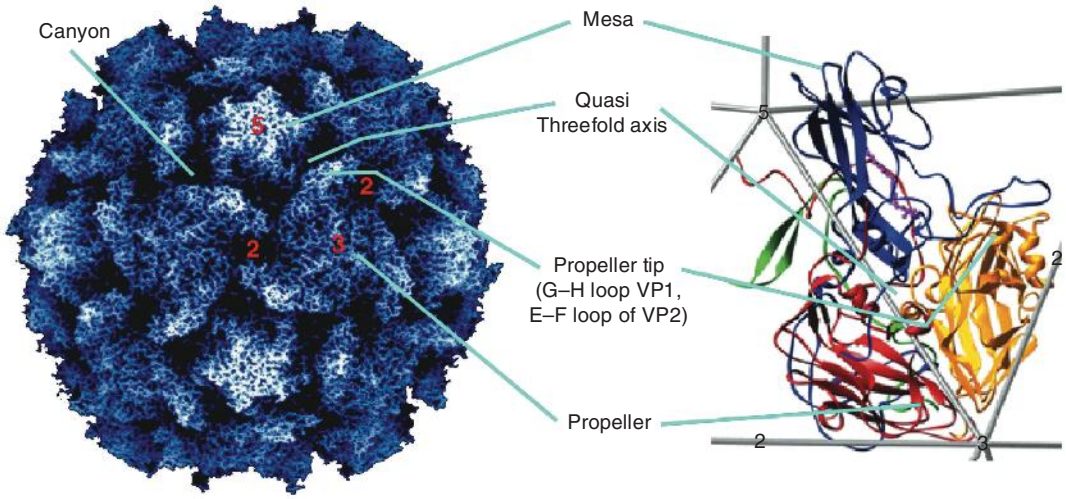


Figure 2 Atomic structure of poliovirus capsid. Left panel: A radially depth-cued rendering of the atomic model of the structure of the 160S poliovirus virion particle. Right panel: An expanded representation of a single protomer showing ribbon diagrams of VP1 (blue), VP2 (yellow), VP3 (red), and VP4 (green) overlaid on an icosahedral framework. The fivefold, threefold, and twofold axes are indicated by numbers. Cyan lines point to prominent surface features in the two panels. Reproduced from Bubeck D, Filman DJ, Cheng N, Steven AC, Hogle JM, and Belnap DM (2005) The structure of the poliovirus 135S cell entry intermediate at 10-angstrom resolution reveals the location of an externalized polypeptide that binds to membranes. *Journal of Virology* 79: 7745–7755, with permission from the American Society for Microbiology.

Table 2 Examples of cell receptors for picornavirus entry

Genus	*Species*	*Cellular receptor*
Aphthovirus	*Foot-and-mouth disease virus*	Integrin (strains A12), heparan sulfate (strain O1)
Cardiovirus	*Encephalomyocarditis virus*	VCAM-1
Enterovirus	*Coxsackievirus B1-6*	CAR, DAF (CD55)
	Poliovirus 1–3	CD155 (Pvr)
Hepatovirus	*Hepatitis A virus*	HAVcr-1
Rhinovirus	*Major rhinovirus group*	ICAM-1
	Minor rhinovirus group	LDL

VCAM, Vascular cell adhesion molecule; CAR, coxsackie-adenovirus receptor; DAF, decay-accelerating factor (CD55); Pvr, poliovirus receptor (CD155); HAVcr-1, hepatitis A virus cell receptor; ICAM-1, intercellular adhesion molecule; LDL, low-density lipoprotein.

Table 2. Some of the receptors, such as the rhinovirus major group receptor ICAM-1, have known cellular functions, while the normal cellular function of other virus receptors, such as the HAV receptor HAVcr-1, have not been determined. For poliovirus, entry of the virion RNA into the cell is not fully understood but may occur via a variation of receptor-mediated endocytosis; however, acidification of the endosome may not be necessary as release of the viral RNA is not pH dependent. Whether the mature virion is internalized wholly in a membrane-bound vesicle or whether the RNA exits at the plasma membrane surface is also unknown. Once bound to CD155, the virion capsid induces a conformational change and the internal VP4 capsid protein is extruded to the external surface to contact the plasma membrane along with the amino terminus of VP1 around the fivefold axis of symmetry, forming a channel by which the RNA has been proposed to enter the cytoplasm and begin the replicative cycle through translation of the RNA genome.

Translation of Genomic RNA

Once the virus has been uncoated and its RNA released into the host cell cytoplasm, the positive-strand RNA genome is translated. This viral mRNA contains *cis*-acting RNA sequence and structural elements distinct from those of host cell mRNAs. Eukaryotic mRNAs contain a 7-methyl guanosine cap that is attached to the nascent transcript in the nucleus during RNA processing. In addition to protecting the transcript from degradation, recognition of the cap structure by the eIF-4F complex serves as a scaffold for the assembly of other initiation factors and the 40S ribosomal subunit. Picornavirus genomes lack anything resembling a cap structure. Instead, the 5′ end of

genomic RNA (as well as negative-strand RNA intermediates) is covalently bound to the viral protein VPg. This protein does not appear to have a role in translation of the polyprotein. Studies carried out with picornavirus mRNAs associated with translating polysomes have not detected VPg bound to viral RNAs, and further evidence indicates an unknown cellular factor as capable of cleaving the RNA–protein bond.

Translation of picornavirus positive-strand RNAs does not follow the conventional scanning model of most cellular mRNAs. Analysis of the first completely sequenced picornavirus, poliovirus, indicated that the 5′ NCR contains numerous AUG start codons in contexts favorable for initiation of translation that were not utilized by the virus. Further, biochemical analysis of the 5′ NCR determined that this region of viral RNA contains several RNA secondary structures with sufficient thermodynamic stability to most likely inhibit the 40S ribosomal subunit from scanning toward a start codon. Subsequent studies demonstrated that an IRES is utilized to translate the poliovirus RNA genome. All picornaviruses contain an IRES in their 5′ NCRs. IRES elements have also been identified in the genomes of other RNA viruses as well as in cellular mRNAs. An example of the mechanism of picornavirus RNA translation is shown in **Figure 3** for the poliovirus IRES.

In contrast to host cell cap-dependent translation, cap-independent translation initiation from an IRES does not use a cap structure at the 5′ end of the RNA to interact with the eIF-4F complex. In fact, many picornaviruses will actually proteolytically cleave or otherwise sequester eukaryotic initiation factors with high efficiency. The notable exception is HAV; cleavage of the eIF-4G component of the eIF-4F complex inhibits IRES activity, suggesting that an intact eIF-4F complex may interact with a site within the IRES to initiate HAV translation. While cleaved eIF-4G is rendered nonfunctional for cellular mRNA translation, eIF-4A and eIF-4B may participate with the cleaved portion of eIF-4G to bring the eIF-1A/eIF-2-met tRNA/eIF-3/40S ribosome complex to the viral IRES for enteroviruses, rhinoviruses, and aphthoviruses.

By convention, the RNA structure elements of the picornavirus type I IRES (enteroviruses and rhinoviruses) and HAV (denoted as having a type III IRES) are described by roman numerals, while the distinct 5′ NCR secondary structure elements of the type II IRES (including those of cardioviruses and aphthoviruses) are denoted by letters.

Chemical and enzymatic RNA structure probing aided by computer modeling studies have determined that the enterovirus and rhinovirus 5′ NCRs (approximately 700–800 nt in length) contain six stem–loop structures, with the region containing the IRES encompassing stem loops II through VI (**Figure 4**). Extensive genetic analysis of type I stem–loop structures involving point mutations, substitutions, or complete deletions of specific stem–loop structures indicates that specific RNA sequence elements

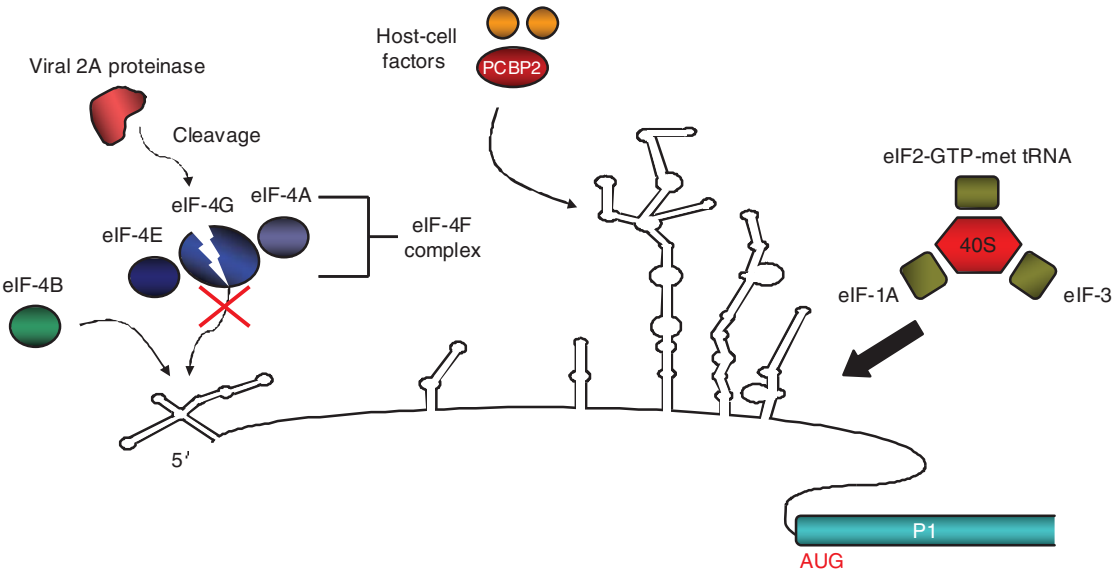


Figure 3 Cap-independent RNA translation of poliovirus RNA. Binding of cellular protein PCBP2 and other host cell factors to RNA secondary structures in the IRES element stabilizes the RNA, allowing association of the 40S ribosomal subunit with eIF-1A, eIF2-GTP-met, and eIF-3 bound. Following binding of the 60S subunit to form the complete 80S ribosome, initiation of RNA translation begins at the AUG start codon preceding the P1 coding region. Translation of the genomic RNA leads to the generation of the 2A proteinase, which will shut off host cell cap-dependent RNA translation through inactivation of the eIF-4F complex via cleavage of eIF-4G. Also note the absence of a cap structure at the 5′ end of the RNA.

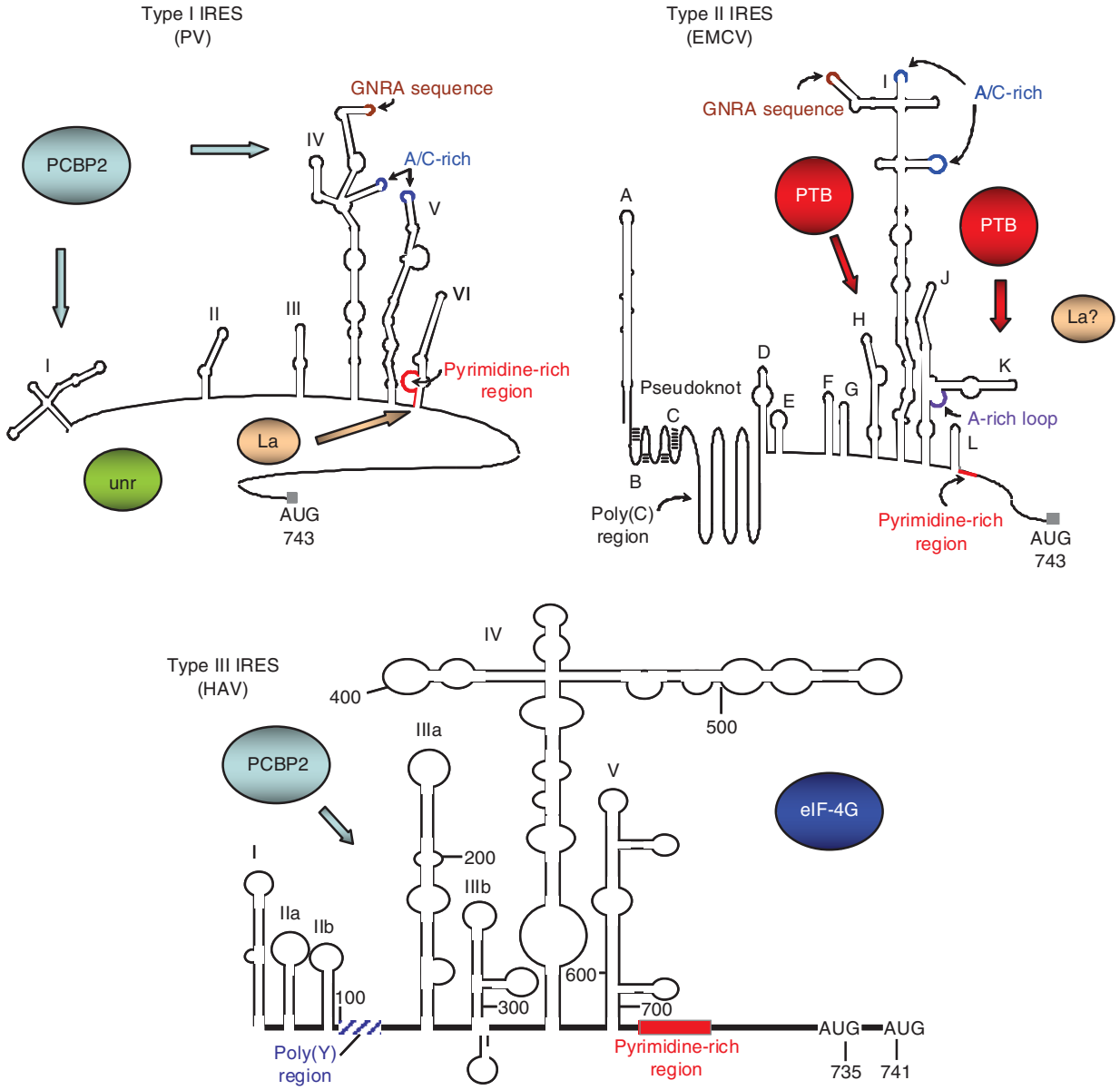


Figure 4 Comparison of IRES elements in the 5′ NCR of picornavirus RNA genomes. The predicted RNA secondary structure motifs associated with type I, II, and III IRES elements are represented by poliovirus, encephalomyocarditis virus, and hepatitis A virus, respectively. The secondary structure of the RNA sequence for each of the 5′ NCRs has been predicted by computer modeling and confirmed by RNA structure probing. Also depicted are some of the cellular factors known to bind to each particular IRES type, and their putative binding sites. Reproduced from Stewart SR and Semler BL (1997) RNA determinants of picornavirus cap-independent translation initiation. *Seminars in Virology* 8: 242–255, with permission from Elsevier.

are also important for IRES function. In particular, the poliovirus stem–loop IV contains a short region of single-stranded cytidines and a GNRA tetra-loop sequence important for cellular protein binding and efficient viral genome translation. Similar sequence and structural elements have also been predicted for the IRES of the closely related enterovirus coxsackievirus B.

The type II IRES elements of FMDV, EMCV, and the recently classified IRES of parechoviruses are bounded between stem-loops D through L (**Figure 4**) and are contained within a 5′ NCR of greater length than their enterovirus counterparts, from over 800 nt for EMCV to 1200 nt for FMDV. In addition to a greater number of RNA stem–loop structures within the 5′ NCR, some type II IRES elements also include a poly(C) region preceding stem–loop D that is missing in picornaviruses with a type I IRES (Theiler's murine encephalomyocarditis virus, a cardiovirus related to EMCV, does not contain a poly(C)

tract). The function of this homopolymeric stretch of cytosine residues (ranging from 60 to over 400 nt) is unknown. Despite the structural differences compared to type I IRES elements, the type II IRES also contains a GNRA tetra-loop sequence in the stem–loop I, as well as A/C-rich regions that are found in type I IRES elements. The IRES of HAV is contained within stem loops IIIa to V, and although its stem–loop structures more closely resemble those of the type II IRES, the requirements for efficient IRES function are different than the type I or type II class of elements.

Picornavirus IRES elements operate to direct the assembly of initiation complexes using the host cell protein synthesis machinery. This assembly is mediated by the interactions between IRES RNA elements and cellular factors, including cellular proteins that do not appear to have a role in cap-dependent cellular translation. These RNA–protein interactions between viral RNA and host cell proteins have been identified as either being crucial for optimal translation efficiency specifically for one IRES type, or important for translation of more than one IRES type, perhaps acting to stabilize the RNA secondary structure that allows internal ribosome entry. For example, rabbit reticulocyte lysate (RRL) is a commonly used and efficient *in vitro* system for the translation of mRNA. However enterovirus, rhinovirus, and HAV IRES elements initiate translation aberrantly in RRL. In particular, the efficiency and fidelity of poliovirus translation in RRL can be increased significantly by the addition of crude extracts from HeLa cells or by high concentrations of the human La protein. In contrast, the type II IRES elements of EMCV, FMDV, and Theiler's virus RNA direct translation efficiently in RRL without the addition of La or other factors. Another cellular protein, polypyrimidine tract binding protein (PTB), interacts with poliovirus, human rhinovirus, EMCV, and FMDV IRES elements (**Figure 4**). The high-affinity RNA binding of PTB to the type II IRES elements of EMCV and FMDV suggests a role for the protein in directing viral RNA translation. Mutations introduced into the EMCV IRES designed to abrogate binding of PTB to the IRES result in a loss of IRES activity. **Table 3** lists some of the known interactions of cellular proteins with picornavirus IRES elements.

The cellular protein poly(rC) binding protein (PCBP) binds to the 5′ NCR (specifically stem–loop I and IV) of enterovirus and rhinovirus genomic RNAs and is necessary for the activity of the IRES element. Depletion of the PCBP isoform PCBP2 from HeLa cytoplasmic extracts drastically reduces the translation efficiency of poliovirus RNA, which can be restored with the addition of recombinant PCBP2. Intriguingly, while FMDV and EMCV IRES elements bind PCBP2, depletion of PCBP2 does not affect the ability of these IRESs to direct translation *in vitro*. A substantial amount of research has focused on the role of PCBP in poliovirus infection, as binding of PCBP (either as PCBP1 or PCBP2) to stem-loop I of poliovirus is necessary for RNA replication (described below).

Table 3 Examples of host proteins interacting with picornavirus 5′ NCRs

Cell proteins	*Virus RNA binding*
La autoantigen	PV, HRV, CVB, EMCV, FMDV
PCBP2	PV, HRV, CVB, FMDV, HAV
unr	PV, HRV
PTB	PV, HRV, EMCV, FMDV
ITAF-45	FMDV
eIF-4A	EMCV
eIF-4B	EMCV, PV, FMDV
eIF-4E	EMCV
eIF-4G	EMCV, FMDV
eIF-2	EMCV, PV

Polyprotein Processing

Ribosome recognition and initiation within the picornavirus IRES allows the translation of the long polyprotein (approximately 250 kDa) encoded by the single open reading frame (ORF) encoded by picornavirus genomes. The viral polyprotein is proteolytically cleaved by the viral proteinases L (encoded by aphthoviruses), or 2A (encoded by enteroviruses and rhinoviruses), and 3C and 3CD (encoded by all picornaviruses) (**Figure 5**). Viral protein processing yields the precursor molecules P1, P2, and P3. The P1 region of the polyprotein is further processed to three or four distinct proteins, which are the structural components of the viral capsid, and in the case of cardioviruses, aphthoviruses, erboviruses, teschoviruses, and kobuviruses, the nonstructural protein L. The proteolytically processed P2 precursor contains proteins necessary for restructuring of cytoplasmic membranes and vesicles utilized in virus replication, for the shutoff of host cell translation, and for viral RNA replication. The P3 precursor is processed to produce major proteins necessary for RNA replication, including the RNA-dependent RNA polymerase $3D^{pol}$, and the viral proteinases 3C and 3CD, both of which are responsible for the majority of viral polyprotein cleavage events. The translation stop codon for picornavirus genomes terminates synthesis of the polyprotein immediately after synthesis of the P3 polyprotein, at the carboxy terminus of the $3D^{pol}$ sequence.

Substrates of Viral Proteinases

Picornavirus Capsid Proteins

The P1 sequence encodes the capsid proteins VP4, VP2, VP3, and VP1. Initial processing of the viral polyprotein generates VP0 (a precursor to VP4 and VP2), VP3, and VP1. Parechoviruses and kobuviruses do not process the

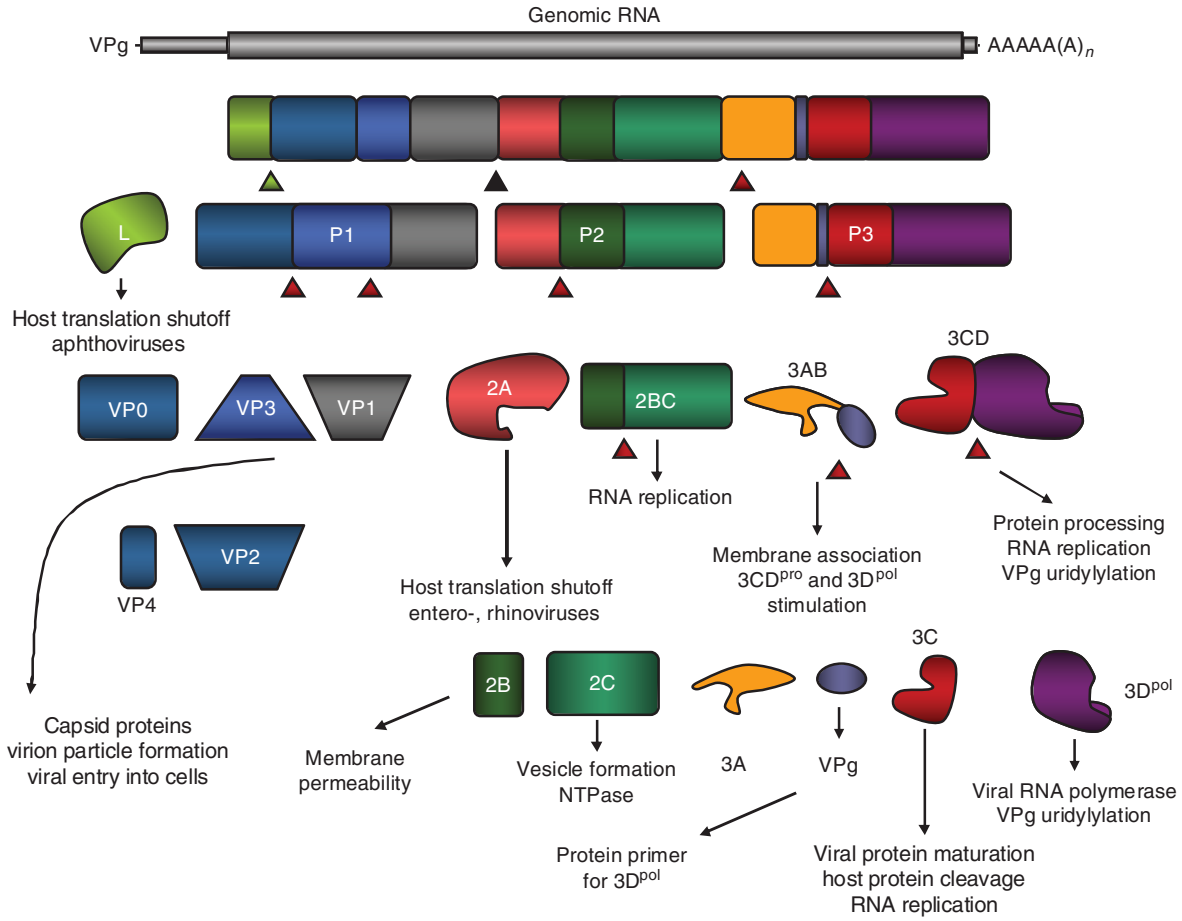


Figure 5 Picornavirus polyprotein processing. As the polyprotein is translated, autocatalysis by viral proteinases 2A/L or 3C/3CD results in the P1, P2, and P3 precursors. (The L protein of cardioviruses is not a proteinase and thus is not released by autocatalysis, and is instead released by 3C/3CD mediated cleavage from the P1 precursor.) These precursors are further processed into the virus structural proteins (P1) or nonstructural proteins (P2 and P3). The functions of the precursor and mature virus proteins are described above. The color-coded triangles correspond to specific cleavage events by the viral proteinases (green, L; black, 2A; red, 3C/3CD).

P1 capsid precursor in a manner analogous to how poliovirus processes VP0; therefore, complete processing of the capsid proteins results in only three proteins used in the formation of the virion capsid. For poliovirus, cleavage of the P1 region into the mature capsid components requires the proteolytic activity of 3CD. For all picornaviruses that process VP0 into VP4 and VP2, an unknown host or viral factor may effect this novel cleavage event.

Viral Proteins Involved in RNA Replication

Viral protein 2B is known to alter and increase the permeability of intracellular membranes including the endoplasmic reticulum. The 2B protein of coxsackievirus B3 (CVB3) contains a region of amino acids that resemble a cationic α-helix. Infection of tissue culture cells with a CVB3-containing mutations in the 2B α-helix predicted to disrupt the cationic character of the domain resulted in a decrease in virion progeny being released into the extracellular space, suggesting that 2B, possibly in conjunction with the viral proteins 2C/2BC, may facilitate virion release during later stages of picornavirus infection. While the precise function of picornavirus 2B is unknown, insertion mutations into the hydrophobic region of the poliovirus 2B produced a virus with defects in viral RNA synthesis that was incapable of rescue by complementation with wild-type virus, suggesting a *cis*-acting role for the 2B protein in picornavirus RNA replication.

During poliovirus infection, 2C and its precursor molecule 2BC are the viral factors responsible for induction of membranous vesicles that are rearranged by the 2B protein (see above) creating a vesicle scaffold for the viral replication complex to begin RNA synthesis. Both proteins are known to interact with the vesicles associated with the virus RNA replication complexes, and 2BC and 2C (but not 2B) induce the formation of these membranous vesicles when expressed alone in tissue culture cells.

The 2C coding region contains several putative structural domains that point to its role as a multifunctional

protein in the picornavirus replication pathway. Poliovirus 2C protein is predicted to contain two amphipathic α-helices at its amino- and carboxy-termini and a cysteine-rich motif resembling a DNA zinc-binding finger domain. The middle of the coding region contains a domain with sequence elements found in RNA helicases; while helicase activity is yet to be reported, the 2C protein does have NTP-binding and hydrolysis functions. Although its precise function in RNA synthesis is currently unknown, the 2C protein of picornaviruses clearly has a role in viral RNA replication based upon studies using guanidine hydrochloride (Gu–HCl). In the presence of low concentrations of Gu–HCl (2 mM), picornavirus negative-strand RNA replication is specifically inhibited. Sequence analysis of poliovirus mutants resistant to the addition of Gu–HCl revealed lesions in the 2C coding region, indicating that poliovirus sensitivity to Gu–HCl mapped to the 2C protein. Poliovirus 2C and 2BC viral proteins also bind to the RNA stem–loop structure at the 3′ end of negative-strand RNA, suggesting that these proteins may also have a role in positive-strand RNA synthesis. Recent evidence has also suggested that poliovirus 2C may regulate the catalytic activity of the 3C proteinase to process viral precursor molecules.

The carboxy-terminal hydrophobic domain of the viral 3A protein anchors this small (approximately 130 amino acids) polypeptide and its precursor 3AB into cellular membranes. Mutations within the hydrophobic region predictably disrupt the interaction of the poliovirus 3AB precursor with cellular membranes, but more importantly they also disrupt efficient cleavage of viral P2-P3 or P2-3AB protein precursor molecules by the 3C. Thus, 3AB may provide a means of sequestering viral factors required for RNA synthesis and protein processing in the same cytoplasmic location as the vesicles formed from intracellular membranes, providing at the same time the VPg protein primer for RNA replication.

The viral proteinases 3C and 3CD cleave the polyprotein encoded by the genomic RNA to produce mature viral proteins. These proteinases also cleave cellular proteins, perhaps acting to disrupt host cell translation machinery or an antiviral response mounted by the cell. In addition, 3CD also participates in processes critical to viral RNA replication. Interaction of poliovirus 3CD, the precursor of 3C and the polymerase $3D^{pol}$, is required to form an RNP complex with PCBP2 and the 5′ end of genomic RNA. This complex has been shown to be required for poliovirus RNA replication, possibly by facilitating negative-strand RNA synthesis via communication of the 5′ and 3′ termini of the genomic RNA. In addition, poliovirus 3CD enhances the rate of uridylylation of VPg from the *cre* element *in vitro*, suggesting that 3CD may assist the $3D^{pol}$ through either direct interaction or binding the RNA sequence of the *cre* to alter its conformation in a manner suitable to serve as template.

The $3D^{pol}$ of picornaviruses is the RNA-dependent RNA polymerase responsible for the synthesis of negative- and positive-strand RNAs. *In vitro* studies examining the requirements for poliovirus $3D^{pol}$ RNA synthesis determined that although the viral polymerase cannot initiate RNA synthesis without a primer sequence, it can elongate a nascent RNA strand following initiation without need of additional viral or cellular proteins. Interestingly, it has been proposed that 3AB may have a stimulatory role in the elongation step.

Host Cell Protein Cleavage and Translation Shutoff

In addition to their role in the maturation of viral proteins, the 2A (enteroviruses, rhinoviruses) and L (aphthoviruses) proteinases also have a role in influencing the translation activity of the host cell by cleaving factors involved in cap-dependent translation initiation. Both proteinases shut down host cell protein synthesis by cleaving the eIF-4G component of the eIF-4F initiation factor complex, resulting in an inability of the amino-terminal domain of eIF-4G to recognize and bind eIF-4E that is associated with the 7-methyl cap structure on the 5′ end of cellular mRNAs. Enterovirus and rhinovirus protein synthesis is upregulated as a consequence of this increase of free ribosomes that are available for internal ribosome entry. EMCV uses an alternate method to inhibit host cell translation by activating a cellular translational repressor, 4E-BP1, which binds cellular eIF-4E and therefore inhibits host cap-dependent translation.

Viral RNA Replication

Genomic RNA Elements Involved in Picornavirus RNA Replication

Like all RNA viruses, picornavirus replication uses a viral-encoded RNA-dependent RNA polymerase to specifically synthesize RNA from a viral RNA template. Picornavirus genomic RNA is released from the capsid into the cytoplasm without replication proteins (save for the viral VPg linked to the 5′ end); therefore, replication of the initial viral genome must follow translation of its coding region to generate the $3D^{pol}$ and other viral replication proteins. The polymerase uses the protein VPg as a primer to initiate RNA synthesis by catalyzing the addition of two uridylate residues to generate VPg-pU-pU. The initiation site of negative-strand RNA synthesis is at the 3′ poly(A) tract of the genomic positive-sense RNA. Viral negative-strand RNA exists in a duplex with its positive-sense RNA template. This double-stranded RNA intermediary is termed the replicative form, or RF. Negative-strand RNA in turn acts as template for the

synthesis of positive-strand RNAs. The ratio of positive- to negative-strand RNA in infected cells has been observed to be approximately 50:1, suggesting that a single negative-strand RNA intermediate acts as a template for the production of multiple positive-strand RNAs. Newly synthesized viral positive-sense RNAs will be translated to produce additional viral proteins, used as additional template RNAs for negative-strand RNA synthesis, or packaged into virions for infection of other host cells (**Figure 6**). This section discusses the RNA elements and viral proteins utilized by picornaviruses and their functions in the replicative cycle, the contributions of cellular proteins and structures, and the unanswered questions regarding picornavirus RNA replication.

In addition to the IRES, picornavirus 5′ NCRs contain additional stable RNA secondary structural elements that are critical for replication of viral positive- and negative-strand RNAs. For entero- and rhinovirus genomic RNAs, this structure is termed stem–loop I or cloverleaf, so named for the cruciform secondary structure predicted to form within the first ~100 nt of the RNA. The genomes of other picornaviruses including aphthoviruses and cardioviruses also contain an RNA structure at their 5′ termini, termed stem–loop A, which is analogous in function but not in structural conformation to the cloverleaf. For simplicity, the discussion below will primarily refer to the poliovirus stem–loop I RNA structure at the 5′ terminus. An insertion mutation in stem–loop I resulted in a deficiency in viral RNA synthesis. More importantly, a revertant virus containing a mutation in the genomic region encoding the viral proteinase 3C was isolated and found to have rescued the RNA synthesis defect. Binding of precursor 3CD to stem–loop I was confirmed genetically via mutations of stem–loop I nucleotides designed to destabilize the cruciform. Using electrophoretic mobility shift analysis, the RNA affinity of 3CD for stem–loop I was found to be increased when another protein was bound to the RNA cruciform. This protein was later identified as the cellular protein PCBP2, and it associated with stem–loop I and 3CD to produce a ribonucleoprotein

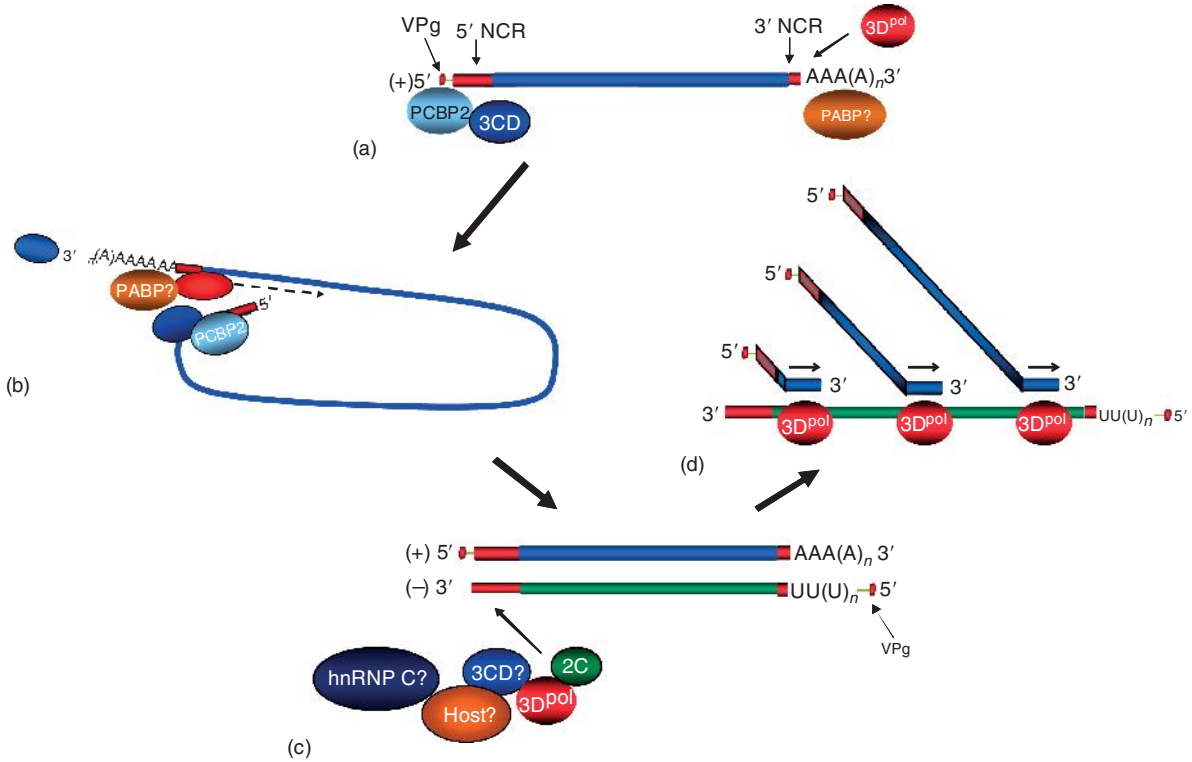


Figure 6 Picornavirus RNA replication. Shown here is a model of the general mechanism of RNA replication of picornaviruses, with the cellular and viral proteins depicted being specific for what is known for poliovirus. (a) The input genomic RNA (positive-sense) associates with viral and cellular factors (including PABP and PCBP2). Viral 3CD and cellular PCBP2 is proposed to interact with the RNA structure at the terminus of the 5′ NCR and contact PABP bound to the poliovirus poly(A) tract. The RNA-dependent RNA polymerase $3D^{pol}$ begins synthesis of a complementary negative-strand RNA. (b) The resulting double-stranded RNA intermediate (termed the replicative form or RF) forms part of the positive-strand RNA replication complex. (c) RNA replication complexes hypothesized to include host proteins (e.g., hnRNP C), viral proteins 3CD, 2C, and $3D^{pol}$. Multiple initiations of $3D^{pol}$ RNA synthesis from the negative-strand RNA template result in the formation of the partially double stranded replicative intermediate, RI. (d) Positive-strand progeny RNAs will then re-enter the replication cycle for translation of additional viral proteins, or to serve as template for the replication of negative-strand RNA, or to be packaged into progeny virion particles.

(RNP) structure called the ternary complex. Formation of this RNA–protein complex has been confirmed *in vitro* and in poliovirus-infected cells. Depletion of PCBP2 from HeLa cell S10 extracts reduces levels of poliovirus RNA synthesis, supporting the hypothesis that PCBP2 is required for RNA replication. However, the question remained as to what the 3CD/PCBP2/stem–loop I ternary complex contributed to picornavirus RNA replication.

An intriguing possible answer to the role of the 3CD/PCBP2/stem–loop I complex involves another cellular protein, poly(A) binding protein (PABP). In the cell, PABP stabilizes and protects cellular mRNA transcripts by binding the poly(A) tract at the 3′ end of the mRNA. It also participates in translation through interactions with initiation factors at the 5′ end of the mRNA. *In vitro* binding studies using purified PABP and an RNA probe corresponding to the 3′ NCR and poly(A) tract of poliovirus correlated an ability of PABP to interact with this region with a minimal poly(A) tract. Furthermore, purified PABP could interact with the 3CD/PCBP2/stem–loop I ternary complex *in vitro*. PABP and poliovirus 3CD could be co-immunoprecipitated from extracts of poliovirus-infected HeLa cells, suggesting that PABP and the viral ternary complex may also interact during a poliovirus infection. These interactions would suggest that the 5′ and 3′ ends of positive-strand RNA are in close proximity through a 'protein–protein' bridge formed by viral and cellular factors. End-to-end communication of the positive-strand RNA could facilitate initiation of negative-strand RNA synthesis.

An internal RNA sequence within the picornavirus genome is also important in the picornavirus replicative cycle. This sequence has been termed the *cis*-acting replication element, or *cre*. First discovered in human rhinovirus 14 genomic RNA and later identified for other picornaviruses, the *cre* forms a short (50–100 nt) RNA hairpin structure in the positive-strand RNA of picornaviruses, with a conserved AAACAC sequence in the loop portion of the hairpin. Nucleotide mutations that disrupt the conserved sequence inhibit viral RNA synthesis and are therefore lethal to the virus. The position of the *cre* varies widely among picornaviruses; in enteroviruses, it is located within the 2C coding region, while in cardioviruses the *cre* maps to the VP2 coding region. The *cre* of aphthoviruses is unique among picornaviruses in that it is located outside the coding region in the 5′ NCR. The position of the *cre* varies even among serotypes of the same genus. For example, the human rhinovirus 2 *cre* is found in the 2A proteinase coding region, while human rhinovirus 14 *cre* is found in the VP1 RNA sequence. Studies of lethal mutations introduced in the poliovirus *cre* demonstrated that viral RNA replication could be rescued when a second, wild-type sequence was introduced elsewhere in the genome. The *cre* is considered to be a site at which viral replication proteins bind and uridylylate the VPg protein primer. The poly(A) tract at the end of the 3′ NCR was originally thought to be the template RNA used in VPg uridylylation; however, *in vitro* studies using synthetic VPg indicated that the poliovirus *cre* hairpin as a source of RNA template was much more efficient in stimulating the uridylylation of VPg than was the viral RNA poly(A) tract. Mutational analysis of poliovirus RNA has indicated that the *cre* may only be used in positive-strand RNA synthesis, and that the poly(A) tract is the primary source of template RNA for VPg uridylylation during negative-strand RNA synthesis.

Following the $3D^{pol}$ coding region, the 3′ NCR and the 3′ poly(A) tract are thought to be involved in RNA synthesis by acting as a *cis*-acting signal for negative-strand RNA replication. The poly(A) tract encoded by the viral genome may contribute to stable RNA secondary structures consisting of one to three stem–loop structures in the heteropolymeric 3′ NCR. The poly(A) tract likely stabilizes and protects the viral RNA genome from intracellular degradation enzymes, much as the poly(A) tracts of cellular mRNAs do. The poly(A) tract, in conjunction with bound cellular PABP, $3D^{pol}$, and other viral/cellular proteins may also function in negative-strand RNA synthesis. Human rhinovirus and poliovirus RNA genomes lacking the 3′ NCR (but leaving the genome encoded poly(A) tract intact) are still infectious in tissue culture, indicating the 3′ NCR is dispensable for viral replication. Mutant poliovirus lacking a 3′ NCR displays an RNA replication defect following infection of HeLa cells. This defect was shown to occur at the level of positive-strand RNA synthesis. Interestingly, the RNA replication defect exhibited by the poliovirus 3′ NCR deletion mutant was exacerbated in neuronal cells. Such a cell-specific defect suggested that the presence (or absence) of a cellular factor was important for proper poliovirus 3′ NCR function. The cellular protein nucleolin has been reported as being capable of interaction with the poliovirus 3′ NCR; however, its function in viral RNA replication is unknown. Viral proteins including the 3AB and 3CD protein have been reported to bind to the poliovirus 3′ NCR, and the $3D^{pol}$ of EMCV has been shown to bind to 3′ NCR sequences of the EMCV genome.

Synthesis of Positive- and Negative-Strand Viral RNA

Initiation of viral RNA synthesis requires the uridylylation of VPg by the viral polymerase $3D^{pol}$ and may also involve other viral proteins, including 3CD and the VPg precursor 3AB. Utilizing the 3′ poly(A) tract as a source of template, VPg is uridylylated to form VPg-pU-pU, and the $3D^{pol}$ synthesizes negative-strand RNA complementary to the genomic positive-sense RNA. The double-stranded RNA intermediate (RF) then serves as template for multiple initiation events by the RNA polymerase to

synthesize positive-strand RNAs. In the infected cell, negative-strand RNA has only been observed either in a duplex with the positive-strand RNA or with several elongating positive-strand RNAs existing in a partially double-stranded RNA complex termed the replicative intermediate (RI). Similar to negative-strand RNA synthesis, positive-strand RNA synthesis requires the uridylylation of VPg and as noted earlier, *in vitro* studies have indicated that during synthesis of positive-strand RNAs uridylylation of VPg was more effective using a *cre* RNA hairpin as the source of template rather than the poly(A) tract. During positive-strand RNA synthesis, multiple positive-strand RNAs are initiated using a single negative-strand template; therefore, the more efficient *cre* acting as the site of uridylylation may be necessary to add VPg-pU-pU to newly synthesized positive-strand RNAs. RNA secondary structures within the 5′ and 3′ ends of positive- and negative-strand RNAs, respectively, may sufficiently destabilize base-pairing of the RNAs to allow the $3D^{pol}$ access to the negative-strand RNA template. The $3D^{pol}$ of poliovirus has also been reported to have duplex unwinding activity. Interestingly, the poliovirus 2C protein contains NTPase activity and has protein structural similarity to RNA helicases. It has also been shown to bind to the 3′ end of negative-strand RNA, suggesting a role in viral RNA replication. Perhaps $3D^{pol}$ and 2C form a complex capable of simultaneous unwinding and chain elongation of the RNA.

In addition to interacting with picornavirus genomic RNAs to promote virus translation and RNA replication, cellular proteins have also been shown to interact with negative-strand RNA intermediates. Cellular protein hnRNP C can interact with the RNA secondary structures formed by the 3′ end of poliovirus negative-strand RNA as well as with viral proteins 3CD and 2C. Complexes of hnRNP C and poliovirus replication proteins may promote initiation of positive-strand RNA synthesis via such RNA–protein complexes.

Unanswered Questions and Conclusions

Despite decades of research invested into the mechanisms by which members of the family *Picornaviridae* propagate their genome in infected cells, many questions remain unanswered. The discovery of the *cre* RNA structures helped address the mechanism of how VPg is primed for positive-strand RNA synthesis. However, how the negative strand acts as template for positive-strand RNA and what viral and/or cellular factors are necessary for RNA initiation are largely unanswered questions. The question pertaining to the role of cellular factors in this process is further complicated by the tissue tropism primarily determined by virus-specific cell receptor usage and by RNA structure/sequence differences among picornaviruses. Cap-independent RNA translation from the IRES element is known to occur for all picornaviruses, yet distinct differences exist even between IRES classes in the ability of the IRES to direct translation. For example, poliovirus and human rhinovirus utilize a type I IRES and share some similarities in the ability to bind cellular factors to promote translation; yet their tissue tropisms differ. What are the sequence and structural differences in the IRES element responsible for virus species-specific differences in translation, and what are the cellular proteins responsible for these differences?

One long-standing question for picornavirus researchers is how template utilization is controlled for translation versus viral RNA replication. Specifically, translating ribosomes on genomic RNA in the 5′ to 3′ direction would interfere with RNA polymerase synthesizing negative-strand RNA (proceeding along the genomic RNA in a 3′ to 5′ manner). Is there a molecular switch to turn off translation to allow RNA replication? Are viral and/or cellular factors involved in translation modified or relocated to permit RNA replication? Or is it an effect of increased levels of local concentrations of proteins that shifts the template RNA from translation to replication competency. Such mechanistic questions underscore the intricate molecular processes through which these pathogens have evolved to maximize the reproduction of their genomes. Study of these mechanisms of viral gene expression has provided insights into the design of vaccines and therapeutic drugs not only for picornaviruses, but also for other virus families.

See also: Rhinoviruses; Poliomyelitis.

Further Reading

Agol VI (2006) Molecular mechanisms of poliovirus variation and evolution. *Current Topics in Microbiology and Immunology* 299: 211–259.

Agol VI, Paul AV, and Wimmer E (1999) Paradoxes of the replication of picornaviral genomes. *Virus Research* 62: 129–147.

Bedard KM and Semler BL (2004) Regulation of picornavirus gene expression. *Microbes and Infection* 6: 702–713.

Belsham GJ (2005) Translation and replication of FMDV RNA. *Current Topics in Microbiology and Immunology* 288: 43–70.

Bubeck D, Filman DJ, Cheng N, Steven AC, Hogle JM, and Belnap DM (2005) The structure of the poliovirus 135S cell entry intermediate at 10-angstrom resolution reveals the location of an externalized polypeptide that binds to membranes. *Journal of Virology* 79: 7745–7755.

Hogle JM (2002) Poliovirus cell entry: Common structural themes in viral cell entry pathways. *Annual Review of Microbiology* 56: 677–702.

Jang SK (2006) Internal initiation: IRES elements of picornaviruses and hepatitis C virus. *Virus Research* 119: 2–15.

Semler BL and Wimmer E (eds.) (2002) *Molecular Biology of Picornaviruses.* Washington, DC: ASM Press.

Stewart SR and Semler BL (1997) RNA determinants of picornavirus cap-independent translation initiation. *Seminars in Virology* 8: 242–255.

Plant Antiviral Defense: Gene Silencing Pathway

G Szittya, Agricultural Biotechnology Center, Godollo, Hungary
T Dalmay, University of East Anglia, Norwich, UK
J Burgyan, Agricultural Biotechnology Center, Godollo, Hungary

© 2008 Elsevier Ltd. All rights reserved.

Introduction

One of the most striking biological discoveries of the 1990s is RNA silencing, which is an evolutionarily conserved gene-silencing mechanism among eukaryotes. Initially, it was identified in plants as a natural defense system thought to have evolved against molecular parasites such as viruses and transposons. However, it has become clear that RNA silencing also plays a very important role in plant and animal development by controlling gene expression.

RNA silencing operates through diverse pathways; however it uses a set of core reactions, which are triggered by the presence of long, perfect or imperfect double-stranded (ds) RNA molecules that are cleaved by Dicer or Dicer-like (DCL), an RNase III-type enzyme, into short 21–26 nucleotide (nt) long molecules, known as short interfering RNAs (siRNA) and microRNAs (miRNA). These small RNAs are then incorporated into a protein complex called RNA-induced-silencing complex (RISC) containing members of the Argonaute (AGO) family. RISCs are guided by the short RNAs to target RNAs containing complementary sequences to the short RNAs and this interaction results in cleavage or translational arrest of target RNAs. In fungi, nematodes, and plants, the silencing signal is thought to be amplified by RNA-dependent RNA polymerases (RDRs); however, RDRs have not been identified in other systems.

Recent discoveries have shown that the RNA-silencing machinery also affects gene function at the level of genomic DNA. The first observation of RNA-guided epigenetic modification was the RNA-directed DNA methylation (RdDM) of chromosomal DNA in plants resulting in covalent modifications of cytosins. RdDM was shown to require a dsRNA that was processed into short 21–24 nt RNAs and it also required some of the components of the silencing machinery such as RDR, DCL, and AGO proteins. RNA-silencing-mediated heterochromatin formation is also an epigenetic process and results in covalent modifications of histones (usually methylation of k9H3) and has been reported in fission yeast, animals, and plants.

It is now clear that RNA silencing is diverse and is involved in a variety of biological processes that are essential in maintaining genome stability, development, and adaptive responses to biotic and abiotic stresses.

Diverse Gene-Silencing Pathways in Plants

Most of the current knowledge about RNA silencing in plants has arisen from analysis of the model plant *Arabidopsis thaliana*. To date, there are several different, yet partially overlapping gene-silencing pathways known in plants. These pathways operate through the production of small RNAs and these small RNA molecules guide the effector complexes to homologous sequences where they exert their effect. Small RNAs are diverse and are involved in a variety of phenomena. They can be classified on the basis of their biogenesis and function as: microRNAs (miRNA), *trans*-acting siRNAs (ta-siRNA), natural *cis*-antisense transcript-derived siRNAs (nat-siRNA), heterochromatin siRNAs (hc-siRNA), and *cis*-acting siRNAs. In *Arabidopsis* the existence of multiple paralogs of RNA-silencing-associated proteins such as ten AGOs, six RDRs and four DCLs may explain the diversification of small RNAs and gene-silencing pathways.

MicroRNA Pathway

miRNAs are mainly 21 nt long and regulate endogenous gene expression during development and environmental adaptation. Important features of miRNAs are that one miRNA duplex is produced from one locus and that they are encoded by miRNA genes that are distinct from the genes that they regulate. Primary miRNAs (pri-miRNAs) are transcribed by RNA polymerase II (Pol II) and possess 5′ caps and poly(A) tails. These long single-stranded primary transcripts with an extensive fold-back structure are cleaved during a stepwise process in the cell nucleus by DCL1. DCL1 interacts with HYL1 (a double-stranded RNA binding protein) to produce the mature miRNA duplex (miRNA:miRNA*). Both strands of the miRNA duplex are methylated (specific to plants) at the 2′ hydroxyl group of the 3′ terminal ribose by HEN1 (an S-adenosyl methionoine (SAM)-binding methyltransferase). Methylation protects miRNAs from polyuridylation and probably from degradation at the 3′ end. The plant exportin-5 homolog HASTY exports the miRNA duplexes to the cytoplasm, where the miRNA strand is incorporated into the RISC complex and the miRNA* strand is degraded.

Plant miRNAs have near-perfect complementarity to their target sites and they bind to these sites to guide the cleavage of target mRNAs. AGO1 is part of the plant RISC, since plant miRNAs are associated with AGO1 and immuno-affinity-purified AGO1 cleaves miRNA targets *in vitro*.

In *Arabidopsis* miRNAs also have a role in RNA-directed DNA methylation, a mechanism generally associated with siRNA-mediated gene silencing. It was shown that PHABULOSA (PHB) and PHAVOLUTA (PHV) transcription factor coding sequences are heavily methylated several kilobases downstream of the miRNA-binding site. It was proposed that DNA methylation of PHB/PHV occurs *in cis* and depends on the ability of the miR165/166 to bind to the transcribed RNAs.

Currently, more than 100 miRNAs have been identified in *Arabidopsis*, but many more are likely to exist. The comparison of miRNAs from different plant species has shown that many of them are conserved; however, there are species-specific miRNAs. These 'young' miRNA genes were proposed to evolve recently and show a high degree of homology to the target genes even beyond the mature miRNA sequence. The list of the currently known plant miRNAs can be found in miRBase.

Trans-Acting siRNA Pathway

ta-siRNAs are 21 nt long endogenous siRNAs and they require components of both the miRNA and *cis*-acting siRNA pathways for their biogenesis. They are produced from noncoding (nc) TAS genes transcribed by Pol II. TAS ncRNAs contain an miRNA-binding site and are cleaved by an miRNA-programmed RISC. The production of cleaved TAS ncRNA therefore requires DCL1, HYL1, HEN1, and AGO1. The cleaved TAS ncRNA is converted into dsRNA by SGS3 (coiled-coil putative Zn^{2+} binding protein) and RDR6. This long dsRNA is then cleaved in a phased dicing reaction by DCL4 to generate 21 nt ta-siRNAs. The phase of the dicing reaction is determined by the initial miRNA cleavage site. Ta-siRNAs are methylated by HEN1 and guide mRNA cleavage through the action of AGO1 or AGO7 RISC. Some of the ta-siRNAs regulate juvenile-to-adult transition but other functions remain to be identified.

Natural *cis*-Antisense Transcript-Derived siRNA Pathway

Ten percent of the *Arabidopsis* genome contains overlapping gene pairs, also known as natural *cis*-antisense gene pairs. Nat-siRNA is a recently discovered class of endogenous siRNAs that are derived from natural *cis*-antisense transcripts regulating salt tolerance in *Arabidopsis*. It was reported that both 24 and 21 nt siRNAs (nat-siRNAs) were generated from a locus consisting of two overlapping antisense genes *P5CDH* and *SRO5*; *P5CDH* mRNA is expressed constitutively, whereas *SRO5* is induced by salt stress. When both transcripts are present, a dsRNA can form by the annealing of *P5CDH* and *SRO5* transcripts, which in turn initiates the biogenesis of a 24 nt nat-siRNA by the action of DCL2, RDR6, SGS3, and NRPD1A (RNA polymerase IVa subunit). This 24 nt nat-siRNA guides the cleavage of the constitutively expressed *P5CDH* transcript and sets the phase for a series of secondary, phased 21 nt nat-siRNAs produced by DCL1. The function of these secondary nat-siRNAs is unclear since the *P5CDH* transcript is also downregulated in their absence (in *dcl1* background) as well. However, in wt plants 21 nt nat-siRNAs mediate the cleavage of *P5CDH* transcript. The presence of many overlapping genes in eukaryotic genomes suggests that other nat-siRNAs may be generated from other natural *cis*-antisense gene transcripts.

Heterochromatin siRNA Pathway

The large majority of endogenous small RNAs is derived from transposons and repeated sequences. To explain their biogenesis, it is proposed that tandem repeats or multiple copies of transposable elements generate dsRNAs. Analysis of mutant plants suggests that these molecules are produced by one of the plant RNA-dependent RNA polymerases (RDR2). The long dsRNAs are cleaved by DCL3 to produce 24 nt long hc-siRNAs that are methylated by HEN1. The accumulation of hc-siRNAs also requires NRPD1a and NRPD2, putative DNA-dependent RNA polymerases collectively called PolIV. The PolIV complex is proposed to be involved in an amplification loop together with the *de novo* methyl-transferases (DRMs), the maintenance methyl-transferase (MET1), and the SWI2/SNF2 chromatin remodeling factor DDM1 that are also required for hc-siRNA production. The resulting hc-siRNAs are incorporated into an AGO4-containing complex which then guides DNA methylation and heterochromatin formation. An overview of these and other proteins with roles in *Arabidopsis* small RNA pathways is shown in **Table 1**.

cis-Acting siRNAs

Exogenous nucleic acid invaders such as viruses and transgenes trigger the production of *cis*-acting siRNAs, which are both derived from and target these molecular invaders. This pathway was the first RNA-silencing pathway identified in plants more than 15 years ago. Since this pathway is activated upon virus infection, it was proposed to be an RNA-based immune system against molecular parasites.

In plants, the *cis*-acting siRNA pathway can be used to induce sequence-specific silencing of endogenous mRNA

Table 1 Plant proteins involved in small RNA pathways

Gene	*Alternative name*	*Protein name*	*Gene code*	*Biochemical activity and function*	*Pathway*
AGO1	*DND*	ARGONAUTE 1	At1g48410	Ribonuclease activity; RNA slicer	miRNA ta-siRNA *cis*-acting siRNA
AGO4		ARGONAUTE 4	At2g27040	Unknown; RNA-directed DNA methylation	hc-siRNA
AGO7	*ZIPPY*	ARGONAUTE 7	At1g69440	Ribonuclease activity; RNA slicer	ta-siRNA
DCL1	*CAF/SIN1/ SUS1/EMB76*	DICER-LIKE 1	At1g01040	RNase III; miRNA synthesis	miRNA nat-siRNA
DCL2		DICER-LIKE 2	At3g03300	RNase III; 22 nt or 24 nt siRNA synthesis	nat-siRNA
DCL3		DICER-LIKE 3	At3g43920	RNase III; 24 nt siRNA	hc-siRNA *cis*-acting siRNA
DCL4		DICER-LIKE 4	At5g20320	RNase III; 21 nt siRNA	ta-siRNA *cis*-acting siRNA
DDM1	*SOM*	DECREASE IN DNA METHYLATION 1	At5g66750	SWI2/SNF2-like chromatin remodeling enzyme; maintenance of CG DNA and histone methylation	hc-siRNA
DRM1		DOMAINS REARRANGED METHYLASE 1	At5g15380	Cytosine DNA methyltransferase; *de novo* DNA methylation and maintenance of asymmetric methylation of DNA sequences	hc-siRNA
DRM2		DOMAINS REARRANGED METHYLASE 2	At5g14620	Cytosine DNA methyltransferase; *de novo* DNA methylation and maintenance of asymmetric methylation of DNA sequences	hc-siRNA
HEN1		HUA ENHANCER 1	At4g20910	S-adenosyl methionine(SAM)-binding methyltransferase; miRNA and siRNA methylation	miRNA ta-siRNA nat-siRNA hc-siRNA *cis*-acting siRNA
HST		HASTY	At3g05040	Exportin; miRNA transport	miRNA

Continued

Table 1 Continued

Gene	*Alternative name*	*Protein name*	*Gene code*	*Biochemical activity and function*	*Pathway*
HYL1	*DRB1*	HYPONASTIC LEAVES 1	At1g09700	dsRNA biding; assisting in efficient and precise cleavage of pri-miRNA through interaction with DCL1	miRNA
MET1	*DDM2/RTS2*	METHYLTRANSFERASE 1	At5g49160	Cytosine DNA methyltransferase; maintenance of CG DNA methylation	hc-siRNA
NRPD1a	*SDE4*	NUCLEAR DNA-DEPENDENT RNA POLYMERASE IVa	At1g63020	DNA-dependent RNA polymerase; 24 nt siRNA production and siRNA-directed DNA methylation	nat-siRNA hc-siRNA
NRPD2a	*DRD2*	NUCLEAR DNA-DEPENDENT RNA POLYMERASE IVb	At3g23780	DNA-dependent RNA polymerase; 24 nt siRNA production and siRNA-directed DNA methylation	hc-siRNA
RDR2		RNA-DEPENDENT RNA POLYMERASE 2	At4g11130	RNA-dependent RNA polymerase; endogeneous 24 nt siRNA production	hc-siRNA
RDR6	*SDE1/SGS2*	RNA-DEPENDENT RNA POLYMERASE 6	At3g49500	RNA-dependent RNA polymerase; 21 nt and 24 nt siRNA biogenesis, natural virus resistance	ta-siRNA nat-siRNA *cis*-acting siRNA
SDE3		SILENCING DEFECTIVE 3	At1g05460	RNA helicase; Unclear	nat-siRNA *cis*-acting siRNA
SGS3	*SDE2*	SUPPRESSOR OF GENE SILENCING 3	At5g23570	Unknown; RNA stabilizer	ta-siRNA nat-siRNA *cis*-acting siRNA
WEX		WERNER SYNDROME-LIKE EXONUCLEASE	At4g13870	RNaseD 3′-5′ exonuclease; Unclear	*cis*-acting siRNA

through post-transcriptional gene silencing (PTGS). For this, sequences homologous to the endogenous gene are expressed ectopically in sense, antisense, or inverted-repeat orientation causing S-PTGS, AS-PTGS, and IR-PTGS, respectively. During IR-PTGS, IR transgenes direct the production of long dsRNAs which are processed to 21 and 24 nt siRNAs and methylated by HEN1. Twenty-one nt siRNAs are produced by DCL4 and guide AGO1 RISC to cleave the homologous endogenous mRNAs. Most likely, DCL3 produces 24 nt siRNAs that mediate DNA or histone modification at homologous loci. In *Arabidopsis* forward-genetic screens have identified many components of the S-PTGS pathway. During S-PTGS single-stranded transgene RNAs can be converted into dsRNAs by the combined action of RDR6, SGS3, SDE3 (putative DEAD box RNA helicase), and possibly WEX (RNase D 3′–5′ exonuclease). The resulting dsRNA is likely to be processed by DCL4 to 21 nt siRNAs, which are then methylated by HEN1. These siRNAs are incorporated into AGO1 RISC and guide the cleavage of homologous mRNAs. It is likely that this pathway also operates as an RNA-based immune system against viruses, where the dsRNA could be a replication intermediate or an internal secondary structure feature of the viral RNA. However, it is very likely that different viruses activate different siRNA pathways.

Gene Silencing and Antiviral Defense

The phenomenon that plants are able to recover from virus infection was first described almost 80 years ago. During recovery the initially infected leaves and first systemic leaves showed severe viral symptoms. However, the upper leaves were symptom free and became immune to the virus. As a consequence the plant became resistant to secondary infection against the inducing virus or close relatives. The discovery of recovery led to the concept of cross-protection. Cross-protection is a type of induced resistance and its basis is that prior infection with one virus provides protection against closely related viruses. Although recovery and cross-protection were described a very long time ago, it was not clear until recently how they operated. However, cross-protection demonstrated that plants harbor an adaptive antiviral defense system. During the last decade it became clear that this adaptive antiviral defense system was similar to RNA silencing and suggested that RNA silencing served as a natural defense system against viruses.

Successful virus infection in plants requires replication in the infected cells, cell-to-cell movement, and long-distance spread of the virus. However, plants try to protect themselves against virus infection and RNA silencing was evolved to detect and degrade foreign RNA molecules. The vast majority of plant viruses have RNA genomes with dsRNA replication intermediates and also with short imperfect hairpins in their genome that are ideal targets of the RNA-silencing mechanism. Moreover, plant viruses with DNA genome also serve as a target for the antiviral RNA-silencing pathway. In these cases, during replication the DNA is transcribed bi-directionally and overlapping transcripts form dsRNA and induce RNA silencing. It has become evident that plant viruses are potent inducers and targets of RNA silencing. In addition, as a part of co-evolution, plant viruses have developed silencing suppressor proteins to defend themselves against RNA silencing.

Many different types of plant viruses exist. They have different nucleic-acid content, genome organization, replication strategies, and have very different host ranges. However, it seems to be a common feature that the virus infection of plants results in the accumulation of viral siRNAs, yet the size profile of viral siRNAs varies depending on the virus. It has also been demonstrated that plants, mutant in different components of the RNA-silencing pathway, showed different responses to virus infection depending on the virus suggesting that different siRNA-mediated pathways are activated in plants in response to infection by different viruses.

Dicer-Like Proteins in Antiviral Defense

Arabidopsis encodes four DCL enzymes. DCL1 produces both miRNAs and siRNAs, while the three other DCLs produce only siRNAs. During the biogenesis of endogenous small RNAs DCL1 produces predominantly 21 nt miRNAs and 21 nt nat-siRNAs. DCL2 synthesizes a 24 nt nat-siRNA, DCL3 generates 24 nt hc-siRNA, and DCL4 produces 21 nt ta-siRNA and *cis*-acting siRNA. Analysis of Arabidopsis *dcl* mutants has revealed that there are some redundancies among the four DCL enzymes.

When a virus infects a plant, the virus-derived dsRNA or, as was shown with tombusvirus infection, the partially self-complementary structures of its genomic RNA, become substrates of DCLs and viral infection results in the accumulation of viral siRNAs. However, it has been shown that the size profile of viral siRNAs varies depending on the virus. Many viruses trigger the generation of 21 nt long viral siRNAs while others induce the production of both 21 and 24 nt siRNAs, with some viruses generating mostly 24 or 22 nt long viral siRNAs.

In *Arabidopsis* cucumber mosaic virus (CMV) siRNAs accumulate as 21 nt long species, tobacco rattle virus (TRV) produces virus-specific 21 and 24 nt siRNAs and turnip crinkle virus (TCV) produces only 22 nt long viral siRNAs. However, these viral siRNA size profiles were altered in different *Arabidopsis dcl* mutants. For example, in *dcl4* the accumulation of 21 nt CMV siRNAs was undetectable, but 22–24 nt viral siRNAs were present. Further analysis of *dcl* mutants has revealed that CMV 21 nt

siRNAs are produced by DCL4, whereas the TRV 21 and 24 nt long siRNAs are generated by DCL4 and DCL3, respectively. However, the DCL3-dependent 24 nt long siRNAs are neither necessary nor sufficient to mediate defense against TRV. Thus, the DCL3-dependent RNA-silencing pathway alone cannot limit TRV infection. It was also shown that TCV 22 nt siRNAs are produced by DCL2 and they are sufficient to direct destruction of the virus. Further analysis has revealed that both DCL4 and DCL2 mediate TCV RNA silencing, with DCL2 providing redundant viral siRNA processing function, likely because TCV suppresses the activity of DCL4. It was concluded that viral siRNAs are produced by DCL4; however, DCL2 can substitute for DCL4 when the activity of the latter is reduced or inhibited by viruses. Furthermore, it was proposed that DCL4 might be the first line of antiviral defense, with DCL2-mediated activity coming to the fore when DCL4 is deactivated by a viral silencing suppressor protein. Whether all viral siRNAs are produced primarily by DCL4 from viruses with RNA genomes remains to be determined.

DNA viruses are also targets of RNA silencing, and virus-derived siRNAs have been detected in plants infected with DNA geminiviruses. *Arabidopsis* infected with Cabbage leaf curl begomovirus (CaLCuV), a geminivirus, generates 21, 22, and 24 nt viral siRNA of both polarities, with the 24 nt species being a predominant size-class. The accumulation of three different size-classes of viral siRNAs suggests that more than one DCL is involved in the biogenesis of these RNAs. Genetic evidence has shown that DCL3 is required to generate the 24 nt viral siRNAs and DCL2 is necessary for the production of a substantial fraction of the 22 nt viral siRNAs. Other yet unidentified DCLs or combination of DCLs generate the 21 nt and the remaining of 22 nt viral siRNAs. It has been shown that at least two RNA-silencing pathways are involved in DNA virus–plant interactions.

HEN1

HEN1 encodes an S-adenosyl methionine(SAM)-binding methyl-transferase that methylates the 2′-OH of the 3′-terminal nucleotide of miRNAs and siRNAs. It is suggested that all known endogenous small RNAs in *Arabidopsis* are methylated by HEN1. Infection of *Arabidopsis* with CaLCuV shows that the 3′-terminal nucleotide of viral siRNA is methylated by HEN1. However, mutations in HEN1 gene do not increase plant susceptibility to DNA viruses. Conversely, during the infection of a cytoplasmic RNA virus (oilseed rape mosaic tobamovirus), a major fraction of virus-derived siRNAs are not methylated in *Arabidopsis*. Moreover, it was also demonstrated that viral RNA-silencing suppressors interfere with miRNA methylation in *Arabidopsis*. However, the understanding of the exact biochemical process of viral siRNA methylation and its biological relevance together with the effect of viral silencing suppressors remains a challenge in plant virus interaction.

RISC

The existence of viral siRNA-programmed antiviral RISC that cleaves viral target RNA is widely accepted. However, in theory, DCL-mediated dicing of the viral genome would be sufficient to repress virus infection. Recently, it was shown that efficient accumulation of DCL-3-dependent (in *dcl2–dcl4* double mutant) TRV-derived 24 nt siRNAs was not sufficient to mediate viral defense against TRV. This may argue for an antiviral RISC to promote defense against TRV. In plants, AGO1 was shown to have RISC activity and it was physically associated with miRNAs, ta-siRNAs, and *cis*-acting siRNAs. However, the AGO1 slicer did not contain virus-derived siRNAs when *Arabidopsis* plants were infected with three different viruses (CMV, TCV, tobacco mosaic virus (TMV)). Unfortunately, the experiment did not examine more viruses and had not tested the effect of the viral suppressors on RISC assembly. Furthermore, no other viral siRNA-containing RISC complex has been identified, yet. However, the Arabidopsis genome encodes 10 AGOs and it is possible that some of them may have diversified to become the antiviral slicer of RISC.

The Role of RNA-Dependent RNA Polymerase 6 in Virus Infection

Plants contain many *RDR* genes, for example, the model plant *Arabidopsis* has six RDR paralogs. *Arabidopsis* RDR6 is necessary for ta-siRNA, nat-siRNA, and S-PTGS pathways and in some cases also in antiviral defense. A likely biochemical role of RDR6 in RNA silencing is to produce dsRNA that is cleaved by DCLs. *Arabidobsis rdr6* mutants showed hypersusceptibility to CMV, a cucumovirus, but not to TRV, a tobravirus, TMV, a tobamovirus, turnip vein clearing virus (TVCV), also a tobamovirus, TCV, a carmovirus, and turnip mosaic virus (TuMV), a potyvirus. In *Nicotiana benthamiana*, a species often used in plant virus research, RDR6 plays a role in defense against potato virus X (PVX), a potexvirus, potato virus Y (PVY), a potyvirus but is not involved in resistance against TMV, TRV, TCV, and CMV. The role of NbRDR6 in viral defense against PVX has been studied in detail. During PVX infection, NbRDR6 has no effect on replication, cell-to-cell movement, and virus-derived siRNA accumulation. However, NbRDR6 is implicated in systemic RNA silencing. In plants, there is a mobile silencing signal that spreads through the plasmodesmata and phloem. The exact nature of the silencing signal is unknown, but it is likely to be a 24 nt siRNA species. Both RDR6 and SDE3

are involved in the long-distance spread of the silencing signal generated during PVX infection. During infection NbRDR6 prevents meristem invasion by PVX and is required for the activity but not for the production of a systemic silencing signal. A model for NbRDR6 function in antiviral defense against PVX is that RDR6 uses the incoming silencing signal to produce dsRNA precursors of secondary siRNAs which mediate RNA silencing as an immediate response to slow down the systemic spread of the virus. However, it remains obscure why *rdr6* mutant plants are not hypersusceptible to all tested viruses.

SDE3

Arabidopsis SDE3 encodes a DEAD box putative RNA helicase. SDE3, in addition to RDR6, is involved in long-range but not cell-to-cell signaling of RNA silencing. *Arabidopsis* SDE3, like RDR6, is implicated in defense against CMV but has no effect on TMV, TCV, and TRV infection. It is very likely that they act together on the same RNA-silencing pathway during viral defense.

Many proteins have been identified with a role in RNA silencing. However, only a few have known function in viral defense. A simplified model summarizes the antiviral role of RNA silencing during virus infection (**Figure 1**). In the inoculated cell the entering viruses start to replicate using the virus-encoded replicase protein (Viral RdRP). During the replication cycle the virus forms dsRNA replication intermediates. The plant antiviral defense system recognizes the dsRNA and DCL4 uses it as a substrate to produce virus-derived 21nt siRNAs. The viral siRNAs may be methylated at the 3′-nucleotide by HEN1; however, the extent and general appearance of methylation on viral siRNAs remains undetermined. Virus-derived dsRNAs are diced mainly through DCL4. However, in some cases the invading virus-encoded silencing suppressor protein can repress DCL4 activity. In these cases other DCLs with redundant function become the major viral siRNA producer. Another source of virus-derived siRNAs is the partially self-complementary structures of the viral genomic RNA. These secondary structures can also be the substrate of a DCL. According

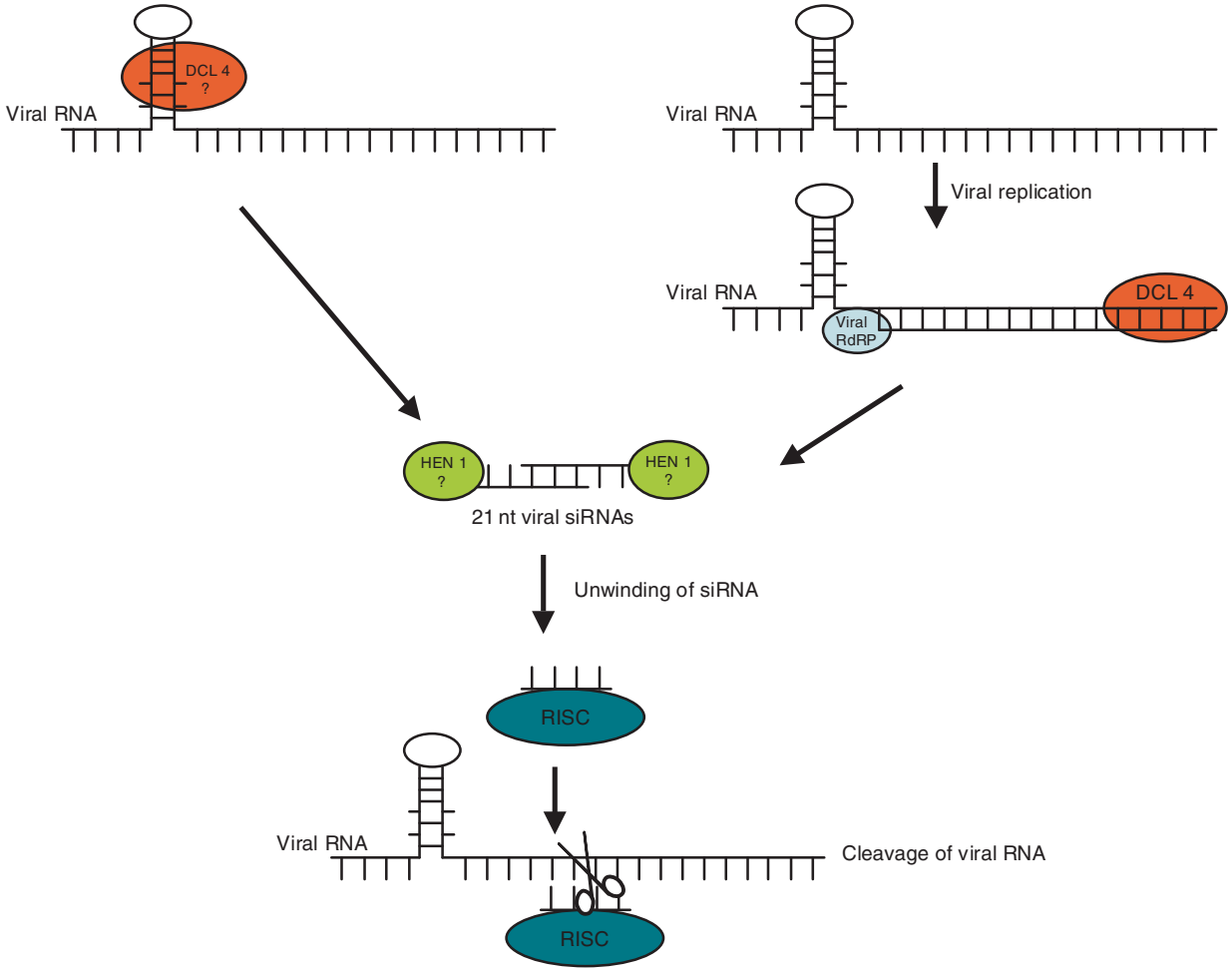


Figure 1 Simplified model of plant antiviral defence against RNA viruses in *Arabidopsis*.

to the size of these viral siRNAs, they may be DCL4 products. However, further investigation is needed to determine their exact origin. The viral siRNAs from both origins are unwound and one strand of the siRNA duplex is incorporated into RISC. The siRNA-loaded RISC recognizes and destroys complementary target viral RNAs. In some cases RDR6 and SDE3 are implicated in antiviral defense by slowing down the accumulation of certain viruses in systemically infected leaves.

Many viral genomes encode silencing suppressors that can inhibit different points of the plant defense system. They can repress HEN1 function or bind to the siRNA duplexes and sequester them from RISC assembly.

Environmental Influence on Gene-Silencing Pathways

It has long been known that plant–virus interactions are strongly modified by environmental factors, especially by temperature. Higher temperature is frequently associated with milder symptoms, low virus content of the infected plants, and rapid recovery from virus disease. In contrast, at cold air temperatures plants become more susceptible to virus infection, develop severe symptoms, and have higher virus content. The effect of temperature on virus-induced RNA silencing has been tested by infecting *N. benthamiana* plants with cymbidium ringspot virus (CymRSV) and Cym19stop virus (an RNA-silencing suppressor mutant of the same virus) and growing the infected plants at different temperatures. It has been found that CymRSV symptoms were attenuated at 27 °C and that the attenuated symptoms were associated with reduced virus level and that the amount of virus-derived siRNAs gradually increased with rising temperature. The CymRSV-encoded p19 protein acts as a viral suppressor of RNA silencing; thus, Cym19stop-infected plants recover from viral infection at a standard temperature (21 °C). However, at low temperature (15 °C) Cym19stop-infected plants display strong viral symptoms, and the level of virus-derived siRNAs is dramatically reduced. At low temperature, RNA silencing fails to protect the plants, even when the virus lacks a silencing suppressor. Therefore, RNA-silencing-mediated plant defense is temperature dependent and temperature regulates this defense pathway through the control of siRNA generation.

In *N. benthamiana*, NbRDR6 participates in the antiviral RNA silencing pathway that is stimulated by rising temperatures, suggesting that the function of NbRDR6 may be closely related to the temperature sensitivity of the RNA-silencing pathway. Importantly, it has also been demonstrated that temperature does not influence the accumulation of miRNAs, thus ensuring normal development at lower temperatures.

Viral Counterdefense: Silencing Suppressors

RNA silencing in plants prevents virus accumulation. To counteract this defense mechanism, most plant viruses express silencing suppressor proteins. More than a dozen silencing suppressors have been identified from different types of RNA and DNA viruses. These proteins probably evolved independently in different virus groups, because they are structurally diverse and no obvious sequence homology has been detected between distinct silencing suppressors. An interesting feature common to many viral silencing suppressors is that they were initially identified as pathogenicity or host range determinants. Suppressor activity has been identified in structural as well as nonstructural proteins involved in almost every viral function.

Viral silencing suppressor proteins operate through a variety of mechanisms. The molecular basis for suppressor activity has been proposed for several viruses, including p19 of tombusviruses, HC-Pro of potyviruses, p21 of closteroviruses, and B2 protein of flock house virus, a nodavirus infecting both plants and animals. Currently, the suppressor activity of p19 has been studied in most detail. The high-resolution crystal structures of two different tombusvirus p19 proteins, combined with molecular and biochemical data, have indicated precisely how silencing is blocked. It has been found that a tail-to-tail p19 homodimer specifically binds to siRNAs and recognizes their characteristic size, thus sequestering the products of Dicer and suppressing the silencing effect. Other studies have demonstrated that the molecular basis of silencing suppression of p21 and HC-Pro is also siRNA sequestration. B2 has been shown to bind to dsRNAs and inhibit siRNA formation. Analysis of many silencing suppressor proteins that bind dsRNA, either size selectively or size independently, has suggested that dsRNA binding is a general plant viral silencing suppression strategy which has evolved independently many times.

Viral infection leads to various symptoms, and many viral silencing suppressors have been previously described as pathogenicity determinants. Overexpression of viral silencing suppressors can result in developmental abnormalities in plants because they affect miRNA accumulation and function. Whether viral silencing suppressors inhibit the miRNA pathway because the miRNA and siRNA pathways share common components or because the miRNA pathway directly or indirectly influence virus infection is unknown.

Application of Virus-Induced Gene Silencing

Replicating plant viruses are both strong inducers and targets of the plant RNA-silencing mechanism.

This strong RNA-silencing-inducing ability of plant viruses is used in virus-induced gene silencing (VIGS). VIGS is an RNA-silencing-based technique used to reduce the level of expression of a gene of interest and study the function of the knocked-down gene. Full-length viral clones can be modified to carry a fragment of an endogenous gene of interest and these are known as VIGS vectors. DsRNA of the inserted fragment is generated during viral replication and mediates the silencing of the target gene. Both RNA and DNA viral genomes have been successfully developed into VIGS vectors and have been used in reverse genetics studies in many different plants. The most widely used VIGS vectors are based on TRV, TMV, and cabbage leaf curl geminivirus (CbLCV).

See also: Plant Resistance to Viruses: Engineered Resistance; Virus Induced Gene Silencing (VIGS); Viral Suppressors of Gene Silencing.

Further Reading

Akbergenov R, Si-Ammour A, Blevins T, *et al.* (2006) Molecular characterization of geminivirus-derived small RNAs in different plant species. *Nucleic Acids Research* 34: 462–471.

Baulcombe D (2004) RNA silencing in plants. *Nature* 431: 356–363.

Bouche N, Lauressergues D, Gasciolli V, and Vaucheret H (2006) An antagonistic function for Arabidopsis DCL2 in development and a new function for DCL4 in generating viral siRNAs. *EMBO Journal* 25: 3347–3356.

Brodersen P and Voinnet O (2006) The diversity of RNA silencing pathways in plants. *Trends in Genetics* 22: 268–280.

Chan SW, Henderson IR, and Jacobsen SE (2005) Gardening the genome: DNA methylation in *Arabidopsis thaliana*. *Nature Reviews Genetics* 6: 351–360.

Deleris A, Gallego-Bartolome J, Bao J, Kasschau KD, Carrington JC, and Voinnet O (2006) Hierarchical action and inhibition of plant Dicer-like proteins in antiviral defense. *Science* 313: 68–71.

Herr AJ (2005) Pathways through the small RNA world of plants. *FEBS Letters* 579: 5879–5888.

Qu F and Morris TJ (2005) Suppressors of RNA silencing encoded by plant viruses and their role in viral infections. *FEBS Letters* 579: 5958–5964.

Silhavy D and Burgyan J (2004) Effects and side-effects of viral RNA silencing suppressors on short RNAs. *Trends in Plant Science* 9: 76–83.

Szittya G, Silhavy D, Molnar A, *et al.* (2003) Low temperature inhibits RNA silencing-mediated defence by the control of siRNA generation. *EMBO Journal* 22: 633–640.

Vaucheret H (2006) Post-transcriptional small RNA pathways in plants: Mechanisms and regulations. *Genes and Development* 20: 759–771.

Vazquez F (2006) Arabidopsis endogenous small RNAs: Highways and byways. *Trends in Plant Science* 11: 460–468.

Voinnet O (2005) Induction and suppression of RNA silencing: Insights from viral infections. *Nature Reviews Genetics* 6: 206–220.

Voinnet O (2005) Non-cell autonomous RNA silencing. *FEBS Letters* 579: 5858–5871.

Wang MB and Metzlaff M (2005) RNA silencing and antiviral defense in plants. *Current Opinion in Plant Biology* 8: 216–222.

Relevant Website

http://microrna.sanger.ac.uk – miRBase::Sequences.

Plant Reoviruses

R J Geijskes and R M Harding, Queensland University of Technology, Brisbane, QLD, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Open reading frame A sequence of nucleotides in DNA that can potentially translate as a polypeptide chain.
Icosahedral Having twenty equal sides or faces.
Capsid The protein shell that surrounds a virus particle.
Monocotyledon A flowering plant that has only one cotyledon or seed leaf in the seed.
Dicotyledon A flowering plant that has two cotyledons or seed leaves in the seed.
Transovarial Transmission from one generation to another through eggs.

Introduction

The family *Reoviridae* comprises a diverse group of viruses which can infect vertebrates, invertebrates, and plants. Despite their large host range, all members of the family *Reoviridae* share common properties including an icosahedral shaped virion and segmented double-stranded RNA (dsRNA) genome. The family *Reoviridae* consists of nine genera of which three genera, *Fijivirus*, *Oryzavirus,* and *Phytoreovirus* are plant-infecting reoviruses. These reoviruses generally replicate in both plant hosts and insect vectors. Infection of the insect vector is non-cytopathic and persists often throughout the life of the insect. Infection of the host plant is tissue specific and can cause severe disease. Fiji leaf gall disease, caused by Fiji disease

virus (FDV), has caused yield losses of up to 90% in susceptible varieties of sugarcane in Australia. Rice ragged stunt virus (RRSV) is reported to reduce yield of rice by up to 100% in severe infections (generally 10–20%). Rice dwarf disease, caused by rice dwarf virus (RDV), can also cause significant losses as infected plants often fail to bear seeds. The genera of plant-infecting reoviruses are differentiated according to the number of genomic dsRNA segments and their electrophoretic profile, hosts, serological relationships, and capsid morphology (**Table 1**).

Taxonomy and Classification

Currently there are three genera of the family *Reoviridae* which are classed as plant-infecting reoviruses, *Fijivirus*, *Oryzavirus*, and *Phytoreovirus*. These reoviruses replicate both in plant hosts (except for one fijivirus: *Nilaparvata lugens* reovirus) and in their insect vectors (**Table 1**). Infection of the host plant is species specific, although the host range can often be extended under experimental conditions, and can produce various symptoms, including severe disease. The complete genome sequence has been obtained for a number of viruses and at least partial sequence information is now available for all plant reoviruses. This has allowed detailed comparisons within these genera and across all of the *Reoviridae*, thus providing a basis for the classification of these viruses into species and genera.

Within the genus *Fijivirus*, individual species have considerable similarities to the type species, Fiji disease virus. Classification into separate species is based on unique characteristics such as capacity to exchange genome segments, relatively high amino acid sequence similarity, serological cross-reaction, cross-hybridization of RNA or cDNA probes, host species, and insect vector species. In addition to these commonly used identifiers, analysis of the available genome sequences has assisted in identification of *Fijivirus* species. Gross genome characteristics for fijivirus members include a genome size of approximately 29 kbp and a characteristically low G+C content of 34–36%. Unique, and highly conserved, 5′ and 3′ terminal sequences are present in different plant reovirus species; in all RNA segments, the 3′ terminal trinucleotide is conserved across all species within the genus *Fijivirus* (**Table 2**). Inverted repeats are found adjacent to the terminal sequences and these differ from those of other plant reoviruses.

Members of the genera *Oryzavirus* and *Phytoreovirus* have significant similarity to type members rice ragged stunt virus and wound tumor virus, respectively.

Table 1 Characteristics of plant reoviruses

Genus	*Virus*	*dsRNA genome segments*	*Host*	*Vector/s*
Fijivirus	Fiji disease virus (FDV)	10	Monocot (Gramineae)	Planthoppers: *Perkinsiella saccharicida*, *P. vastatrix*, *P. vitiensis*
	Rice black-streaked dwarf virus (RBSDV)	10	Monocot (Gramineae)	Planthoppers: *Laodelphax striatellus*, *Ribautodelphax albafascia*, *Unkanodes sapporona*
	Maize rough dwarf virus (MRDV)	10	Monocot (Gramineae)	Planthopper: *Ribautodelphax notabilis*
	Pangola stunt virus (PaSV)	10	Monocot (Gramineae)	Planthoppers: *Sogatella furcifera*, *S. kolophon*
	Mal del Rio Cuarto virus (MRCV)	10	Monocot (Gramineae)	Planthopper: *Delphacodes kuscheli*
	Oat sterile dwarf virus (OSDV)	10	Monocot (Gramineae)	Planthoppers: *Javesella pellucidia*, *J. discolour*, *J.dubia*, *J.obscurella*, *Dicranotropis hamata*
	Garlic dwarf virus (GDV)	10	Monocot (Liliaceae)	Planthopper: Unknown
	Nilaparvata lugens reovirus (NLRV)	10	No plant host reported	Planthoppers: *Nilaparvata lugens*, *Laodelphax striatellus*
Oryzavirus	Echinochloa ragged stunt virus (ERSV)	10	Monocot (Gramineae)	Planthoppers: *Sogatella longifurcifera*, *S. vibix*
	Rice ragged stunt virus (RRSV)	10	Monocot (Gramineae)	Planthopper: *Nilaparvata lugens*
Phytoreovirus	Wound tumor virus (WTV)	12	Dicots	Leafhoppers: *Agallia constricta*, *A.quadripunctata*, *Agalliopsis novella*
	Rice dwarf virus (RDV)	12	Monocot (Gramineae)	Leafhoppers: *Nephotettix cincticeps*, *N. nigropictus*, *Recillia dorsalis*
	Rice gall dwarf virus (RGDV)	12	Monocot (Gramineae)	Leafhoppers: *Nephotettix cincticeps*, *N. nigropictus*, *N. virescens*, *N. malayanus*, *Recillia dorsalis*

Demarcation of species within the oryzaviruses and phytoreoviruses are primarily based on the ability to exchange genome segments although other characteristics, as mentioned for fijiviruses above, are also used. When available, genomic sequences are examined to reveal distinguishing features to support the classification. Oryzaviruses have a total genome size of approximately 26 kbp and specific 5′ and 3′ terminal sequences in all RNA segments: $^{5'}$GAUAAA—GUGC$^{3'}$. Phytoreoviruses have a total genome size of approximately 25 kbp, a G+C content between 38% and 48% and specific 5′ and 3′ terminal sequences, ($^{5'}$GG(U/C)A—(U/C)GAU$^{3'}$) in all RNA segments.

Virion Structure and Genome Organization

Fijivirus

The virions have a complex double icosahedral capsid construction and consist of a capsid, a core, and a nucleoprotein complex. Virions are fragile structures and readily break down *in vitro* to give cores. The outer capsid is 65–70 nm in diameter with 12 'A' type spikes located at the vertices of the icosahedron. The inner core is about 55 nm in diameter, with 12 'B' type spikes located at the vertices. The viral nucleic acid is located at the center of the virus particle, within the inner core capsid. Each virion contains a single full-length copy of the genome. Fijivirus genomes contain ten dsRNA genomic segments varying from approximately 1.8–4.5 kbp (**Figure 1**). The total genome is approximately 29 kbp with a low G + C content of 34–36%. Highly conserved unique 5′ and 3′ terminal sequences are found on all RNA segments (**Table 2**). Segment-specific inverted repeats are found adjacent to these terminal sequences. Segments 1–6, 8, and 10 are monocistronic, containing one open reading frame (ORF), while segments 7 and 9 each contain two ORFs. NLRV is the only fijivirus identified to date which differs from this structure, with one ORF on segment 7. The functions of proteins encoded by most ORFs are still unconfirmed; gene functions of segments 1–4 and 8–10 have been predicted based on protein expression studies or sequence similarities to related reoviruses (**Table 3**).

Oryzavirus

The virions have a double-shelled icosahedral capsid and consist of an outer capsid, an inner capsid, and a core. Virions are fragile and readily break down *in vitro* to give subviral core particles unless pre-treated. The outer capsid is 75–80 nm in diameter with 12 'A' type spikes located at the

Table 2 Conserved 5′ and 3′ sequences identified in fijiviruses

Virus	*5′ conserved sequence*	*3′ conserved sequence*[a]
Fiji disease virus	$^{5'}$AAGUUUUU—$^{3'}$	$^{5'}$—CAGCNNNN*GUC*$^{3'}$
Rice black-streaked dwarf virus	$^{5'}$AAGUUUUU—$^{3'}$	$^{5'}$—AGCUNN(C/U)*GUC*$^{3'}$
Maize rough dwarf virus	$^{5'}$AAGUUUUUU—$^{3'}$	$^{5'}$—U*GUC*$^{3'}$
Mal del Rio Cuarto virus	$^{5'}$AAGUUUUU—$^{3'}$	$^{5'}$—CAGCUNNN*GUC*$^{3'}$
Oat sterile dwarf virus	$^{5'}$AACGAAAAAAA—$^{3'}$	$^{5'}$—UUUUUUUUA*GUC*$^{3'}$
Nilaparvata lugens reovirus	$^{5'}$AGU—$^{3'}$	$^{5'}$—GUU*GUC*$^{3'}$

[a]Italicized trinucleotide is conserved in all fijivirus sequences reported to date.

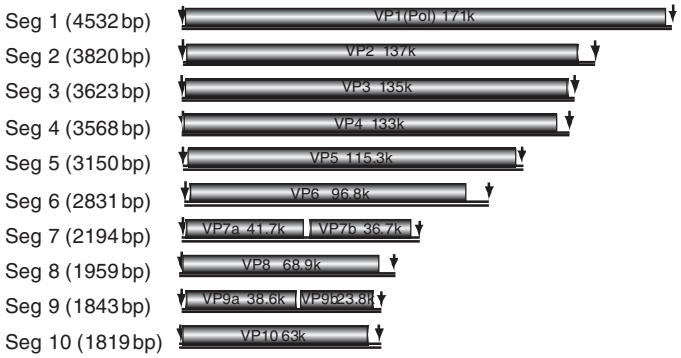


Figure 1 Genome organization of Fiji disease virus (FDV) containing 10 dsRNA segments. Each segment contains one ORF except for Seg 7 and Seg 9 which contain two ORFs. The arrows indicate the location of the 5′ and 3′ conserved sequences, respectively. Reproduced from Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (2005) *Virus Taxonomy – Classification and Nomenclature of Viruses: Eighth Report of the International Committee on the Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

Table 3 Genome organization of FDV and predicted gene function

Segment	*Size (bp)*	*Protein nomenclature*	*MW (kDa)*	*Predicted function (location)*
S1	4532	VP1	170.6	RdRp (core)
S2	3820	VP2	137.0	Possible core protein (core)
S3	3623	VP3	135.5	Possible B spike (capsid)
S4	3568	VP4	133.2	Possible core protein (core)
S5	3150	VP5	115.3	Unknown
S6	2831	VP6	96.8	Unknown
S7	2194	VP7a	41.7	Unknown
		VP7b	36.7	Unknown
S8	1959	VP8	68.9	Possible NTP-binding (core)
S9	1843	VP9a	38.6	Structural protein (unknown)
		VP9b	23.8	Nonstructural
S10	1819	VP10	63.0	Outer capsid protein (capsid)

Reproduced from Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (2005) *Virus Taxonomy – Classification and Nomenclature of Viruses*: *Eighth Report of the International Committee on the Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

five fold axis of the icosahedron. The core capsid is about 57–65 nm in diameter, with 12 'B' type spikes. The viral nucleic acid is located at the center of the viral particle, within the core capsid. The virus genome consists of ten dsRNA segments ranging in size from 1162–3849 bp (RRSV) with a total length of 26 kbp (**Figure 2**). Genome segments 1–3, 5, 7–10 of RRSV each contain a single ORF, while segment 4 contains two ORFs. Segment 8 encodes a polyprotein which is cleaved into two proteins. **Table 4** summarizes the organization of the RRSV dsRNA segments and the predicted function of the encoded proteins.

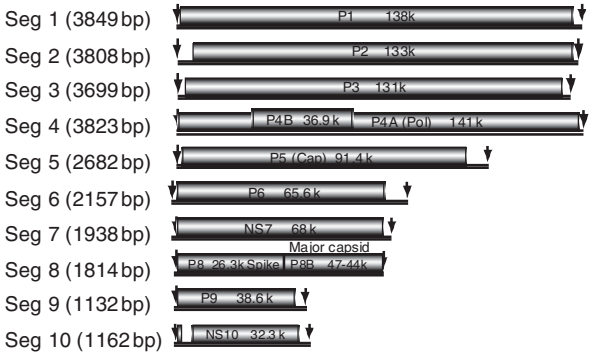


Figure 2 Genome organization of rice ragged stunt virus (RRSV) containing 10 dsRNA segments. Each segment contains one ORF except for Seg 4 which contains two ORFs. The arrows indicate the location of the 5′ and 3′ conserved sequences, respectively. Reproduced from Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (2005) *Virus Taxonomy – Classification and Nomenclature of Viruses*: *Eighth Report of the International Committee on the Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

Phytoreovirus

The virions have a double-shelled icosahedral capsid construction and consist of an outer capsid, a core capsid, and a smooth core. Virions are approximately 70 nm in diameter with 12 spikes located at the fivefold vertices of the icosahedron and generally remain intact when purified. WTV, the type member, possesses three protein shells: an outer amorphous layer made up of two proteins, an inner capsid made up of two proteins, and a smooth core made up of three proteins that is about 50 nm in diameter. Each virion contains a single full-length copy of the genome. Phytoreoviruses have 12 segments of dsRNA which range in size from approximately 1–4.5 kbp with a total genome length of approximately 25 kbp (**Figure 3**) and a G+C content of 38–44%. Each segment of RDV contains a single ORF except for segment 12, which contains two ORFs. **Table 5** summarizes the organization of the RDV dsRNA segments and the putative function of the encoded proteins.

Replication and Gene Expression

The replication and gene expression of plant-infecting reoviruses is thought to be similar to that of other reoviruses. The best described of these is bluetongue virus (BTV), type member of the genus *Orbivirus*. If the BTV model is accurate for the plant-infecting reoviruses, replication occurs after virions (or viral cores) are delivered into the host cell. Replication is initiated when the viral capsid layer is removed and the core enters the cytoplasm of the cell. The viral genome (10–12 segments) remains packaged in the central cavity of the viral core to ensure host cell defense responses to dsRNA are not activated. The core is biochemically active with RNA-dependent RNA polymerase (RdRp), capping enzyme, and helicase enzyme. The viral core contains a number of channels, the largest of which is at the fivefold axis of the icosahedral structure. Smaller channels allow the entry of nucleotides into the core which are required for transcription. The large channel is located adjacent to the

Table 4 Genome organization of RRSV and predicted gene function

Segment	*Size (bp)*	*Protein nomenclature*	*MW (kDa)*	*Predicted function (Location)*
S1	3849	P1	137.7	Virion associated (B spike)
S2	3810	P2	133.1	Inner core capsid (core capsid)
S3	3699	P3	130.8	Major core capsid (core capsid)
S4	3823	P4a	141.4	RdRp
		P4b	36.9	(unknown)
S5	2682	P5	91.4	Capping enzyme
S6	2517	P6	65.6	Unknown
S7	1938	NS7	68	Nonstructural protein (unknown)
S8	1814	P8	67.3	Precursor polyprotein/protease
		P8a	25.6	Spike
		P8b	41.7	Major capsid protein
S9	1132	P9	38.6	Vector transmission (spike)
S10	1162	NS10	32.3	Nonstructural protein

Reproduced from Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (2005) *Virus Taxonomy – Classification and Nomenclature of Viruses*: *Eighth Report of the International Committee on the Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

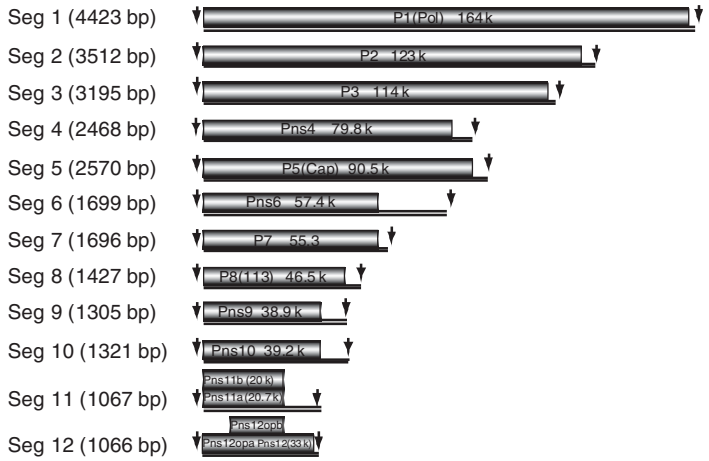


Figure 3 Genome organization of rice dwarf virus (RDV) containing 12 dsRNA segments. Each segment contains one ORF except for Seg 11 and Seg 12 which contain two ORFs. The arrows indicate the location of the 5′ and 3′ conserved sequences, respectively. Reproduced from Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (2005) *Virus Taxonomy – Classification and Nomenclature of viruses*: *Eighth Report of the International Committee on the Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

replicase/transcriptase complex which has helicase activity for the unwinding and rewinding of the dsRNA genome during transcription of negative RNA strand. The newly formed positive strand mRNA molecules are modified to form a Cap1 structure by the guanylyltransferase, nucleotide phosphohydrolase, and transmethylase activity of the capping enzyme prior to the extrusion of mRNA, from the major pore, into the cytoplasm. These mRNA molecules released into the cytoplasm can be translated to produce viral proteins. Nonstructural viral proteins aggregate to form inclusion bodies or viroplasms. The viroplasm is the site of most of the mRNA production and assembly of core proteins. The mRNA molecules, one of each segment, are assembled with these viral proteins to form new virus core particles. Once a copy of the mRNA is inside a new viral core, the negative strand is synthesized completing the replication of the dsRNA genome. The complete viral core containing dsRNA then moves to the periphery of the viroplasm where capsid proteins are assembled to form the complete new viral particle.

Control of gene expression of a multisegmented genome is complex and not fully understood. Each genome segment contained within the viral core is associated with a single replicase/transcription complex, located adjacent to the major pore in the vertices of the icosahedron, and is transcribed separately to make full-length positive sense RNA copies. The location of the replicase/transcription complex also restricts the number of genome segments to a maximum of 12. These 10–12 mRNAs are produced in different molar amounts based largely on segment size

Table 5 Genome organization of RDV and putative gene function

Segment	*Size (bp)*	*Protein nomenclature*	*MW (kDa)*	*Predicted function (location)*
S1	4423	P1	170	RdRp (core)
S2	3512	P2	130	Capsid structural protein (outer capsid)
S3	3195	P3	110	Major core protein (core capsid)
S4	2468	Pns4	83	Nonstructural protein
S5	2570	P5	89	Guanylyltransferase (core)
S6	1699	Pns6	56	Nonstructural protein
S7	1696	P7	58	Nucleic acid binding protein (core)
S8	1427	P8	43	Major outer capsid protein
S9	1305	Pns9	49	Nonstructural protein
S10	1321	Pns10	53	Nonstructural protein
S11	1067	Pns11a	23	Nonstructural protein
		Pns11b	24	
S12	1066	Pns12	34	Nonstructural protein
		Pns12OPa	8	
		Pns12OPb	7	

Reproduced from Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (2005) *Virus Taxonomy – Classification and Nomenclature of Viruses*: *Eighth Report of the International Committee on the Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

resulting in more copies of smaller mRNAs. This interaction between mRNA molecules of varying length and the replicase/transcription complex provides some control over the expression levels of individual virus genes. Translation of mRNA segments is largely independent of mRNA length although some segments are translated more efficiently resulting in a secondary method of control over expression. A third level of control results from the use of multiple or overlapping ORFs on one mRNA strand which are translated at different efficiencies. Lastly, some ORFs encode a polyprotein which must be processed to form functional proteins.

Distribution

Plant-infecting reoviruses are seasonally distributed as a result of plant host/crop cycles and presence of insect vector. Plant reoviruses have been isolated from every continent but some genera are more widespread than others. Fijiviruses are the most widely distributed, which is not surprising given that they are the most numerous. Fijiviruses occur in Africa, Europe, South America, Asia, Australia, and South Pacific Islands. Oryzaviruses have only been isolated from the Indian subcontinent and Asia while phytoreoviruses have been isolated from North America, Asia, and Africa.

Host Range and Virus Transmission

Fijiviruses

The genus *Fijivirus* contains eight species whose members infect a range of monocotyledonous plants of the families Gramineae and Liliacae. Common plant hosts include the Gramineae: *Avena sativa, Oryza sativa*, *Saccharum officinarum, Zea mays,* and the Liliacae: *Allium sativum*. However, this natural host range can be extended significantly by experimental virus infection. Virus is transmitted by delphacid planthoppers (**Table 1**). Virus can be acquired in juvenile stages, replicates in the vector, and, following a two week latent period, is transmitted to plants in a persistent manner. No transovarial transmission of virus has been reported. In addition to transmission by insect vectors, mechanical transmission of the virus to susceptible hosts has been achieved for some members with difficulty.

Oryzaviruses

The genus *Oryzavirus* contains two species whose members infect monocotyledonous plants of the family Gramineae. Common plant hosts include *Oryza sativa* and *Echinochloa crus-galli*. However, this natural host range can be extended by experimental virus infection to include other economically important species such as *Hordeum vulgare, Triticum aestivum,* and *Zea mays*. Virus is transmitted by delphacid planthoppers (**Table 1**). An acquisition period of 3 h is required followed by a 9-day latent period prior to transmission at all life stages in an intermittent manner. No transovarial transmission or mechanical transmission of virus has been reported.

Phytoreoviruses

The genus *Phytoreovirus* contains two species whose members infect monocotyledonous plants of the family Gramineae and one species which infects dicotyledonous plants. Common plant hosts include the Gramineae: *Oryza*

sativa and the dicot – *Melilotus officinalis*. However, the natural host range of the dicot-infecting WTV can be extended significantly by experimental virus infection. Virus is transmitted by cicadellid leafhoppers (**Table 1**). Virus can be acquired after a short feeding period, replicates in the vector, and, following a 10–20 day latent period, is transmitted to plants throughout the life of the vector. Transovarial transmission of virus has been reported. Attempts to transmit the virus to susceptible hosts by mechanical methods have been unsuccessful.

Pathogenicity

The pathogenicity of plant reoviruses is particularly interesting as most viruses replicate in both insects and plant hosts. Most of these viruses do not appear to cause any disease in the insect host and pathogenicity of these viruses is restricted to the plant host. The pathogenicity of fijiviruses varies considerably. Fiji leaf gall disease (caused by FDV) has been reported to cause losses of up to 90% in susceptible sugarcane varieties, while NLRV has no known plant host and, therefore, no pathogenicity. Oryzaviruses can also cause important yield losses. Rice ragged stunt disease (caused by RRSV) has been reported to cause losses of 10–20% but sometimes as high as 100% in severe infections of susceptible varieties. The pathogenicity of phytoreoviruses is much milder although rice dwarf disease (caused by RDV) can be severe as infected plants often fail to bear seeds. There is currently little information on the molecular basis for pathogenicity and it is not known if different isolates of the same virus cause diseases of varying severity.

Diagnosis and Control

Diagnosis of plant reovirus infections can be done on the basis of symptoms or by use of molecular tests. Symptoms vary in different virus/host complexes but symptoms such as plant stunting, increased numbers of side shoots, and tumors or gall formation in phloem tissue are commonly observed. Given the variability in time to symptom expression and symptom severity, alternative tests are often used. Molecular and serological tests have been developed to assist in the diagnosis of viral infection in nonsymptomatic plant material and vector insects. Serological tests are usually in enzyme-linked immunosorbent assay (ELISA) format and rely on polyclonal antisera raised against virions or expressed viral proteins. Recently, molecular tests such as reverse transcriptase-polymerase chain reaction (RT-PCR), which are faster and more specific than serology, have become the most common method of diagnosis. Species-specific primers are now commonly available as increasing numbers of plant reovirus genomes are being sequenced.

Control strategies for plant reoviruses can be focused on either the host plant or insect vector. Plant-based control through breeding to develop resistant plant species is most commonly utilized in combination with removal of susceptible varieties and infected plants which provide a source of inoculum. This approach has provided robust control of RDV in rice and FDV in sugarcane. Genetic engineering of plant hosts has also been explored as an alternative control strategy. Pathogen-derived resistance approaches using either coat protein or other viral genes to control RDV, RRSV, and FDV have not proved as successful as those used to control other RNA plant viruses. However, this may improve in the future as more information on virus infection and replication becomes available providing new resistance targets. Control of insect vector numbers with insecticide has provided some additional disease control. Unfortunately, chemical control appears to be of limited use in cases of high vector pressure. The current combination of diagnosis and control measures is already relatively effective and has resulted in reduced disease incidence and impact.

Future

Although our understanding of plant reoviruses is increasing, many of the molecular and biological properties of these viruses are still unknown. The complete sequence information is now available for a number of these viruses which will allow production of cDNA probes to further elucidate the infection and replication processes in both plant and insect cells. The potential to produce infectious clones also holds promise for detailed studies of both plant and insect host ranges and methods of resistance employed by nonhost species. This information combined with knowledge gained from comparison to animal reoviruses may assist in further development of control strategies for diseases caused by these viruses in plants.

See also: Insect Reoviruses; Reoviruses: General Features; Reoviruses: Molecular Biology.

Further Reading

Bamford DH (2000) Virus structures: Those magnificent molecular machines. *Current Biology* 10: R558–R561.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (2005) *Virus Taxonomy – Classification and Nomenclature of Viruses: Eighth Report of the International Committee on the Taxonomy of Viruses.* San Diego, CA: Elsevier Academic Press.

Hull R (2002) *Matthews Plant Virology.* London: Academic Press.

Mertens P (2004) The dsRNA viruses. *Virus Research* 101: 3–13.

Mertens PPC and Diprose J (2004) The Bluetongue virus core: A nano-scale transcription machine. *Virus Research* 101: 29–43.

Plant Resistance to Viruses: Engineered Resistance

M Fuchs, Cornell University, Geneva, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Plant viruses can cause severe damage to crops by substantially reducing vigor, yield, and product quality. They can also increase susceptibility to other pathogens and pests, and increase sensitivity to abiotic factors. Detrimental effects of viruses can be very costly to agriculture. Losses of over $1.5 billion are reported in rice in Southeast Asia, $5.5 million in potato in the United Kingdom, $63 million in apple in the USA, and $2 billion in cassava worldwide. Also, grapevine fanleaf virus (GFLV) alone is responsible for losses of over $1.0 billion in grapevines in France.

A number of strategies are implemented to mitigate the impact of plant viruses. Quarantine measures and certification of seeds and propagation stocks limit the introduction of virus diseases in virus-free fields and areas. Cultural practices, rouging, control of vectors, and cross-protection based on mild virus strains or benign satellite RNA reduce the transmission rate of viruses in areas where epidemics can be problematic. However, the ideal and most effective approach to control viruses relies on the use of resistant crop cultivars. Host resistance genes have been extensively exploited by traditional breeding techniques. In recent years, cloning, sequencing, and functional characterization of some plant resistance genes have provided new insights into their structure and effect on virus multiplication at the cell and plant level. Notwithstanding, host resistance genes have been identified for a few viruses and only a limited number of commercial elite crop cultivars exhibit host resistance to viruses.

The advent of biotechnology through plant transformation and the application of the concept of pathogen-derived resistance (PDR) has opened new avenues for the development of resistant crop cultivars, in particular when resistant material with desired horticultural characteristics has not been developed by conventional breeding or when no host resistance sources are known. The past two decades have witnessed an explosion in the development of virus-resistant transgenic plants. Some crop plants expressing viral genes or other antiviral factors have been tested successfully in the field and a few have been commercialized. Extensive field evaluation experiments and commercial releases have demonstrated the benefits offered by virus-resistant transgenic plants to agriculture. Risk assessment studies have been conducted to address environmental safety issues associated with the release of transgenic plants expressing viral genes. Field experiments have provided a level of certainty of extraordinarily limited, if any, hazard to the environment. Also, studies on RNA silencing have provided new insights into the molecular and cellular mechanisms underlying engineered resistance to viruses in plants. Overall, the safe deployment of virus-resistant transgenic plants has become an important strategy to implement effective and sustainable control measures against major virus diseases.

Development of Virus-Resistant Transgenic Plants

Historical Perspectives

Over the past two decades, biotechnology expanded the scope of innovative approaches for virus control in plants by providing new tools to engineer resistance. The first approach to confer resistance to viruses in plants resulted from the development of efficient protocols for plant transformation and the application of the concept of PDR. This concept was initially conceived to engineer resistance by transferring and expressing a dysfunctional pathogen-specific molecule into the host genome in order to inhibit the pathogen. Therefore, a segment of the virus' own genetic material was hypothesized to somehow protect a plant against virus infection by acting against the virus itself.

The first demonstration of PDR in the case of plant viruses was with tobacco mosaic virus (TMV) in 1986. Transgenic tobacco plants expressing the TMV coat protein (CP) gene either did not display symptoms or showed a substantial delay in symptom development upon inoculation with TMV particles. Early experiments suggested a direct correlation between expression level of TMV CP and the degree of resistance. This type of PDR was known as CP-mediated resistance. It was successfully applied against numerous plant viruses. In the early 1990s, transgenic expression of the viral replicase domain was also shown to induce resistance. This approach was referred to as replicase-mediated resistance. Meanwhile, other virus-derived gene constructs, including sequences encoding movement protein, protease, satellite RNA, defective interfering RNAs, or noncoding regions, were also described to confer virus resistance in plants. It soon became evident that more or less any viral sequence could provide some level of resistance to virus infection when expressed in transgenic plants. Interestingly, in many cases, resistance was shown to require transcription of the virus-derived transgene rather than expression of a protein product. Subsequently, at the turn of the twenty-first century, tremendous progress has been made at unraveling the molecular and cellular mechanisms underlying virus resistance.

The discovery of RNA silencing as a key mechanism in antiviral defense in plants and regulation of gene expression in eukaryotes has been a major scientific breakthrough.

Resistance to viruses has also been achieved by transforming susceptible plants with antiviral factors other than sequences derived from viral genomes. Production of antibodies or antibody fragments, 2′–5′ oligoadenylate synthase, ribosome inactivating proteins, double-stranded RNA (dsRNA)-specific RNases, dsRNA-dependent protein kinases, cystein protease inhibitors, nucleoprotein-binding interfering aptamer peptides, and pathogenesis-related proteins has been reported to protect plants against virus infection.

Over the past two decades, many plant species and crop plants have been successfully engineered for resistance against numerous viruses with diverse taxonomic affiliation and various mode of transmission. The majority of virus-resistant transgenic plants result from the application of PDR. Virus-derived transgene constructs include full-length, untranslated, and truncated coding and noncoding fragments, in sense or antisense orientation. The CP gene is the most commonly used viral sequence segment to engineer resistance.

Control of Virus Diseases

Virus resistance is evaluated in transgenic plants upon mechanical inoculation, agro-infiltration, grafting, or vector-mediated infection and expressed as immunity, restricted infection, delay in the onset of disease symptoms, or recovery. As an example, squash expressing the CP genes of zucchini yellow mosaic virus (ZYMV), watermelon mosaic virus (WMV), and cucumber mosaic virus (CMV) are highly resistant to mechanical inoculation with a mixture of these three viruses while control plants inoculated with only one of these three viruses are readily infected and exhibit severe symptoms (**Figure 1(a)**).

Some transgenic crop plants, such as cereal, vegetable, legume, flower, forage, and fruit crops, expressing virus-derived gene constructs or other antiviral factors have been tested under field conditions (**Table 1**). So far, a total of 58 virus species that belong to 26 distinct genera have been the target of field resistance evaluation in 37 different plant species.

In the USA, virus resistance represented 10% (1242 of 11 974) of the total applications approved for field releases between 1987 and early August 2006. Other target traits were herbicide tolerance (30%), insect resistance (26%), improved product quality (19%), agronomic properties (9%), and fungal resistance (6%). In the USA, field tests with virus-resistant transgenic crops started in 1988 and accounted for 13–37% of the approved releases from 1988 to 1999.

A high level of resistance to virus infection has been well documented for numerous transgenic crops expressing viral genes even under conditions of very stringent disease pressure and vector-mediated virus infection. For example, in the case of virus-resistant squash, transgenic cultivars expressing the CP gene of ZYMV and WMV are highly resistant to mixed infection by these two aphid-transmitted potyviruses (**Figure 1(b)**). Transgenic plants have a lush canopy and vigorous growth, whereas control plants show a chlorotic and distorted canopy, and stunted growth (**Figure 1(f)**). Transgenic plants produce fruits of marketable quality throughout the growing season (**Figure 1(h)**), whereas most fruits of control plants are malformed and discolored (**Figure 1(g)**), hence not marketable (**Figure 1(e)**). Remarkably, transgenic squash plants expressing the CP gene of ZYMV, WMV, and CMV exhibit identical levels of resistance to mechanical inoculation (**Figure 1(c)**) and aphid-mediated infection (**Figure 1(d)**) by these three viruses under field conditions in which no insecticides were sprayed to control aphid vector populations. Some of the 37 transgenic crops that have been field-tested or commercialized so far are also engineered for resistance to viruses that are not mentioned in **Table 1**. Similarly, in addition to the crops listed in **Table 1**, other virus-resistant transgenic plant species, including cabbage, cassava, grapevine, ryegrass, and petunia, among others, will likely be extensively field-tested in the future based on their promising performance under greenhouse conditions.

A few transgenic crops expressing viral CP genes have been commercially released in the USA and the People's Republic of China (**Table 1**). In the USA, virus resistance represented 10% (9 of 87) of the petitions granted for deregulation by the regulatory authorities until August 2006. Squash expressing the CP gene of ZYMV and WMV received exemption status in 1994 and was released thereafter. This was the first disease-resistant transgenic crop to be commercialized in the USA. Squash expressing the CP gene of ZYMV, WMV, and CMV has been deregulated and commercialized in 1995. Subsequently, numerous squash types and cultivars have been developed by crosses and back crosses with the two initially deregulated lines. The adoption of virus-resistant squash cultivars is steadily increasing since 1995. In 2004, the adoption rate was estimated to be 10% (2500 ha) across the country with an average rate of 20% in the states of Florida, Georgia, and New Jersey. Papaya expressing the CP gene of papaya ringspot virus (PRSV) was deregulated in 1998 and commercialized in Hawaii. PRSV-resistant papaya was the first transgenic fruit crop to be commercially released in the USA. Its adoption rate has been very high from the start of its release in 1998 because PRSV had devastated the papaya industry in Hawaii. Transgenic papaya cultivars were planted on more than half of the total acreage (480 ha) in 2004. Several potato cultivars expressing the replicase gene of potato leafroll virus (PLRV) or the CP gene of potato virus Y (PVY) were deregulated in 1998 and 2000.



Figure 1 Reaction of transgenic squash ZW-20 expressing the coat protein (CP) gene of zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV), and transgenic squash CZW-3 expressing the CP gene of ZYMV, WMV, and cucumber mosaic virus (CMV) to virus infection. (a) Resistance of transgenic CWZ-3 to mechanical inoculation with ZYMV, WMV, and CMV (upper right), and susceptibility of nontransgenic plants to single infection by mechanical inoculation of CMV (lower right), WMV (upper left), and ZYMV (lower left); (b) fields of transgenic ZW-20 (foreground) and nontransgenic plants (background) surrounded by a border row of mechanically inoculated nontransgenic plants; (c) transgenic ZW-20 (right) and nontransgenic (left) plants mechanically inoculated with ZYMV and WMV; (d) transgenic CZW-3 (right) and nontransgenic (left) plants subjected to aphid-mediated inoculation of ZYMV and WMV; (e) close-up of a fruit from transgenic ZW-20 (left) and fruits from nontransgenic plants (five fruits on the right), all subjected to aphid-mediated inoculation of ZYMV and WMV; (f) nontransgenic squash mechanically inoculated with ZYMV and WMV (left) and transgenic ZW-20 (right) exposed to aphid inoculation of ZYMV and WMV; and fruit production of (g) nontransgenic and (h) transgenic squash following aphid-mediated inoculation of ZYMV and WMV.

Table 1 Examples of virus-resistant transgenic crops that have been tested in the field or commercially released

Crop		*Resistance to*	
Category/common name	*Scientific name*	*Virus*	*Genus*
Cereals			
Barley	*Hordeum vulgare*	Barley yellow dwarf virus	*Luteovirus*
Canola	*Brassica napus*	Turnip mosaic virus	*Potyvirus*
Corn	*Zea mays*	Maize dwarf mosaic virus	*Potyvirus*
		Maize chlorotic dwarf virus	*Waikavirus*
		Maize chlorotic mottle virus	*Machlomovirus*
		Sugarcane mosaic virus	*Potyvirus*
Oat	*Avena sativa*	Barley yellow dwarf virus	*Luteovirus*
Rice	*Oryza sativa*	Rice stripe virus	*Tenuivirus*
		Rice hoja blanca virus	*Tenuivirus*
Wheat	*Triticum aestivum*	Barley yellow dwarf virus	*Luteovirus*
		Wheat streak mosaic virus	*Tritimovirus*
Ornamentals			
Chrysanthemum	*Chrysanthemum indicum*	Tomato spotted wilt virus	*Tospovirus*
Dendrobium	*Encyclia cochleata*	Cymbidium mosaic virus	*Potexvirus*
Gladiolus	*Gladiolus* sp.	Bean yellow mosaic virus	*Potyvirus*
Fruits			
Grapefruit	*Citrus paradisi*	Citrus tristeza virus	*Closterovirus*
Grapevine	*Vitis* sp.	Grapevine fanleaf virus	*Nepovirus*
Lime	*Citrus aurantifolia*	Citrus tristeza virus	*Closterovirus*
Melon	*Cucumis melo*	Cucumber mosaic virus	*Cucumovirus*
		Papaya ringspot virus	*Potyvirus*
		Squash mosaic virus	*Comovirus*
		Watermelon mosaic virus	*Potyvirus*
		Zucchini yellow mosaic virus	*Potyvirus*
Papaya[a]	*Carica papaya*	Papaya ringspot virus[a]	*Potyvirus*
Pineapple	*Ananas comosus*	Pineapple wilt-associated virus	*Ampelovirus*
Plum	*Prunus domestica*	Plum pox virus	*Potyvirus*
Raspberry	*Rubus idaeus*	Raspberry bushy dwarf virus	*Idaeovirus*
		Tomato ringspot virus	*Nepovirus*
Strawberry	*Fragaria* sp.	Strawberry mild yellow edge virus	*Potexvirus*
Tamarillo	*Cyphomandra betacea*	Tamarillo mosaic virus	*Potyvirus*
Walnut	*Juglans regia*	Cherry leafroll virus	*Nepovirus*
Watermelon	*Citrullus lanatus*	Cucumber mosaic virus	*Cucumovirus*
		Watermelon mosaic virus	*Potyvirus*
		Zucchini yellow mosaic virus	*Potyvirus*
		Papaya ringspot virus	*Potyvirus*
Forage			
Alfalfa	*Medicago sativa*	Alfalfa mosaic virus	*Alfamovirus*
Grass			
Sugarcane	*Saccharum* sp.	Sugarcane mosaic virus	*Potyvirus*
		Sugarcane yellow leaf virus	*Polerovirus*
		Sorghum mosaic virus	*Potyvirus*
Legumes			
Bean	*Phaseolus vulgaris*	Bean golden mosaic virus	*Begomovirus*
Clover	*Trifolium repens*	Alfalfa mosaic virus	*Alfamovirus*
Groundnut	*Arachis hypogaea*	Peanut clump virus	*Pecluvirus*
		Groundnut rosette virus	*Umbravirus*
Pea	*Pisum sativum*	Alfalfa mosaic virus	*Alfamovirus*
		Bean leafroll virus	*Luteovirus*
		Bean yellow mosaic virus	*Potyvirus*
		Pea enation mosaic virus	*Umbravirus*
		Pea seed-borne mosaic virus	*Potyvirus*
		Pea streak virus	*Carlavirus*
Peanut	*Arachis hypogaea*	Tomato spotted wilt virus	*Tospovirus*
		Groundnut rosette assistor virus	Unassigned
		Peanut stripe virus	*Potyvirus*
Soybean	*Glycine max*	Soybean mosaic virus	*Potyvius*
		Bean pod mottle virus	*Comovirus*
		Southern bean mosaic virus	*Sobemovirus*

Continued

Table 1 Continued

Crop		*Resistance to*	
Category/common name	*Scientific name*	*Virus*	*Genus*
Vegetables			
Cucumber	*Cucumis sativus*	Cucumber mosaic virus	*Cucumovirus*
		Papaya ringspot virus	*Potyvirus*
		Squash mosaic virus	*Comovirus*
		Watermelon mosaic virus	*Potyvirus*
		Zucchini yellow mosaic virus	*Potyvirus*
Lettuce	*Lactuca sativa*	Lettuce mosaic virus	*Potyvirus*
		Lettuce necrotic yellows virus	*Tenuivirus*
Pepper[b]	*Capsicum*	Cucumber mosaic virus[b]	*Cucumovirus*
		Tobacco etch virus	*Potyvirus*
		Potato virus Y	*Potyvirus*
Potato	*Solanum tuberosum*	Potato virus A	*Potyvirus*
		Potato virus X	*Potexvirus*
		Potato virus Y	*Potyvirus*
		Potato leafroll virus	*Polerovirus*
		Tobacco rattle virus	*Tobravirus*
		Tobacco vein mottling virus	*Potyvirus*
Squash[a]	*Cucurbita pepo*	Cucumber mosaic virus[a]	*Cucumovirus*
		Papaya ringspot virus	*Potyvirus*
		Squash mosaic virus	*Comovirus*
		Watermelon mosaic virus[a]	*Potyvirus*
		Zucchini yellow mosaic virus[a]	*Potyvirus*
Sugar beet	*Beta vulgaris*	Beet necrotic yellow vein virus	*Benyvirus*
		Beet western yellows virus	*Polerovirus*
Sweet potato	*Ipomea batatas*	Sweet potato feathery mottle virus	*Potyvirus*
Tomato[b]	*Solanum lycopersicum*	Beet curly top virus	*Curtovirus*
		Cucumber mosaic virus[b]	*Cucumovirus*
		Tobacco mosaic virus	*Tobamovirus*
		Tomato mosaic virus	*Tobamovirus*
		Tomato spotted wilt virus	*Tospovirus*
		Tomato yellow leaf curl virus	*Begomovirus*

[a]Commercially released in the USA.
[b]Commercially released in the People's Republic of China.

However, soon after their release, these potato lines were withdrawn from the market due to food processor rejection. In the People's Republic of China, tomato and pepper resistant to CMV through expression of the virus CP gene have been released.

Benefits of Virus-Resistant Transgenic Plants

Virus-resistant transgenic plants offer numerous benefits to agriculture, particularly in cases where genetic sources of resistance have not been identified or are not easy to transfer into elite cultivars by traditional breeding approaches due to genetic incompatibility or links to undesired traits. This is well illustrated for PRSV-resistant papaya and GFLV-resistant grapevine. In such cases, engineered resistance may be the only viable option to develop virus-resistant cultivars. Engineered resistance may also be the only approach to develop cultivars with multiple sources of resistance by pyramiding several virus-derived gene constructs. This is the case for squash resistant to CMV, ZYMV, and WMV. Commercial releases have demonstrated the stability and durability of the engineered resistance over more than a decade in the case of squash and papaya. Benefits of virus-resistant transgenic plants are also of economic importance because yields are increased and quality of crop products improved. For example, virus-resistant transgenic squash allowed growers to restore their initial yields in the absence of viruses with a net benefit of $19 million in 2004 in the USA. Also, after the release of PRSV-resistant papaya cultivars, papaya production has reached similar level than before PRSV became epidemic in Hawaii, with a $4.3 million net benefit over a 6-year period in Hawaii. Further, benefits are of epidemiological importance with virus-resistant transgenic plants limiting virus infection rates by restricting challenge viruses, reducing their titers, or inhibiting their replication and/or cell-to-cell or systemic movement. Therefore, lower virus levels reduce the frequency of acquisition by vectors and subsequent transmission within and between fields. Consequently, virus epidemics are substantially limited. Recently, it has been shown that transgenic squash resistant to ZYMV and WMV do not serve as virus source for

secondary spread. In addition, benefits of virus-resistant transgenic plants are of environmental importance because chemicals directed to control virus vector populations are reduced or not necessary. Restricting the reliance on chemicals directed to arthropod, fungal, plasmodiophorid, and nematode vectors of plant viruses is important for sustainable agriculture. Finally, benefits of virus-resistant transgenic plants are of social importance. In Hawaii, growing papaya was not viable anymore prior to the release of PRSV-resistant transgenic papaya despite huge efforts to eradicate infected trees in order to limit the propagation of the virus. The impact of PRSV was so severe that some growers abandoned their farms and had to find new jobs. PRSV-resistant transgenic papaya saved the papaya industry and strengthened the social welfare of local communities in Hawaii. Altogether, benefits of virus-resistant transgenic crops are important not only to facilitate their adoption by growers but also to counterbalance real and potential environmental and health risks.

Mechanisms Underlying Resistance

Two types of mechanisms underlying engineered virus resistance have been described, one requiring expression of a transgene protein product (protein-mediated resistance) and the other depending on the expression of transgene transcripts (RNA silencing).

Protein-Mediated Resistance

Resistance to TMV in tobacco plants was initially related to the expression level of the viral CP. Resistance could be overcome by high doses of inoculum and was not very effective against virus RNA inoculation. Interference with an early step in the virus infection cycle, that is, disassembly of TMV particles, has been hypothesized to explain CP-mediated resistance, maybe by inhibition of viral uncoating or reduction of protein–protein interactions between transgene-expressed CP and challenge virus. It would be interesting to analyze whether RNA-mediated resistance is also active in transgenic plants for which the CP has been suspected as an elicitor of the engineered resistance.

RNA Silencing and Post-Transcriptional Gene Silencing

In the early 1990s, the use of untranslatable virus CP transgenes was shown to confer resistance to virus infection, indicating that resistance was RNA rather than protein-mediated. In addition, resistance was correlated to actively transcribed transgenes but low steady-state levels of transgene RNA, indicating a sequence-specific post-transcriptional RNA-degradation system. Remarkably, RNA degradation was shown to target transgene transcripts and viral RNA. This was the first indication of the occurrence of post-transcriptional gene silencing (PTGS) as a manifestation of RNA silencing, a mechanism that inhibits gene expression in a sequence specific manner.

RNA silencing operates through diverse pathways but relies on a set of core reactions that are triggered by dsRNAs, which are processed into RNA duplexes of 21–24 bp in length, called small interfering RNAs (siRNAs), by the enzyme Dicer and its homologs, which have RNAseIII, helicase, dsRNA-binding, Duf283, and PIWI-Argonaute-Zwille (PAZ) domains. One siRNA strand is incorporated into a large multi-subunit ribonucleoprotein complex called the RNA-induced silencing complex (RISC) upon ATP-dependent unwinding and guides the complex to degrade cellular single-stranded RNA molecules that are identical in nucleotide sequence to the siRNA such as challenge viral RNAs.

RNA silencing is a host defense mechanism against viruses that is triggered by dsRNA and targets the destruction of related RNAs. During virus infection, dsRNA intermediates of viral RNA replication, or dsRNA produced from viral RNAs by host polymerases, or partially self-complementary structures of viral genomic RNAs become substrates for the production of siRNAs by Dicer-like (DCL) enzymes. Plants produce at least four different variants of DCLs of which DCL2 and DCL4 provide a hierarchical antiviral defense system.

Viruses have the ability to not only trigger but also suppress RNA silencing as counterdefense mechanism. Indeed, viruses have evolved silencing-suppressor proteins that minimize PTGS. These proteins act in different ways and on different steps of the silencing pathways. Some viral suppressors bind to siRNA duplexes and prevent their incorporation into RISC. Others act directly on the enzymes or cofactors of this defense pathway. It seems that plants have duplicated DCLs as a response to the functional complexity of viral suppressors.

It is anticipated that RNA silencing mechanisms will be devised extensively in the future to create virus-resistant transgenic crop plants. Indeed, resistance can be very strong if transgene transcripts form a hairpin structure containing a substantial base-paired stem, if challenge viruses have extended nucleotide sequence identity with the transgene, and if infection with unrelated viruses carrying silencing-suppressor genes does not interfere with RNA silencing.

Environmental Safety Issues

The insertion and expression of virus-derived genes in plants has raised concerns on their environmental safety. Since the early 1990s, considerable attention has been paid to potential environmental risks associated with the release of virus-resistant transgenic crops. Potential risks relate to the occurrence and outcomes of heterologous

encapsidation, recombination, and gene flow. However, it is important to keep in perspective that these phenomena are known to occur in conventional plants, in particular for heterologous encapsidation and recombination, in the case of co-infection. Therefore, it is critical to determine if they occur in transgenic plants beyond base line events in conventional plants.

Heterologous Encapsidation

Heterologous encapsidation refers to the encapsidation of the genome of a challenge virus by the CP subunits from another virus, upon co-infection or expressed in a transgenic plant containing a viral CP gene. Heterologous encapsidation is a particular case of functional complementation, that is, a transgene might help a challenge virus in another function. Since the CP carries determinants for pathogenicity and vector specificity, among other features, the properties of field viruses may change. For example, it is conceivable that an otherwise vector-nontransmissible virus could become transmissible through heterologous encapsidation in a transgenic plant and infect otherwise nonhost plants. Such changes in vector specificity will be a single generation, not a permanent, event because they will not be perpetuated in the virus genome progeny. Heterologous encapsidation is known to occur in conventional plants subjected to mixed virus infection. Therefore, it is not too surprising that it was documented with herbaceous transgenic plants in the laboratory. In contrast, heterologous encapsidation has not been found to detectable level in transgenic vegetable plants – expressing virus CP gene that were tested extensively in the field over several years at different locations. The only exception was with transgenic squash expressing the CP gene of WMV for which a low rate of transmission of an aphid nontransmissible strain of ZYMV was documented. However, transmission of this ZYMV strain did not reach epidemic proportion. Also, heterologous encapsidation is unlikely to occur in RNA-silenced plants since expression of the transgene does not result in the accumulation of a detectable protein product. Altogether, compelling evidence has shown that heterologous encapsidation in transgenic plants expressing virus CP genes is of limited significance and should be considered negligible in regard to adverse environmental effects.

Recombination

Recombination refers to template switching between viral transgene transcripts and the genome of a challenge virus. Resulting recombinant viruses may have chimerical genomic molecules consisting of a segment from the challenge viral genome and another segment from viral transgene transcripts. It is argued that recombinant viruses may have identical biological properties as their parental lineages or new biological properties such as changes in vector specificity, expanded host range, and increased pathogenicity. Since recombination alters the genome of challenge viruses, new properties of chimera viruses will be stably transmitted to and perpetuated within the virus progeny. Comprehensive studies have documented the occurrence of recombination in transgenic plants expressing viral genes. The stringency of selective pressure applied to the challenge virus has been shown to be a critical factor in the recovery of recombinant viruses. Conditions of high selective pressure enhance the creation of recombinant viruses. In contrast, limited, if any, recombinant viruses are found to detectable level under conditions of low or no selective pressure. It is important to keep in perspective that the latter conditions prevail under field conditions in which plants are infected by functional, not defective, viruses. So far, no recombination event has been found to detectable level in CP gene-expressing transgenic perennial plants that were tested in experimental fields over a decade. Therefore, the significance of recombination in transgenic plants expressing viral genes is very limited in regard to adverse environmental effects. Furthermore, recombination is unlikely to occur in RNA-silenced plants because transgene expression often results in no detectable RNA, especially so when noncoding sequences are used as antiviral constructs.

Gene Flow

Gene flow refers to the pollen-driven movement of *trans*-genes from a virus-resistant transgenic plant into a non-transgenic compatible recipient plant, for example, a wild relative. Hybrids resulting from gene flow can acquire and express virus-derived transgenes, and become resistant to the corresponding viruses. Subsequently, plants acquiring viral resistance traits can have a competitive advantage, exhibit increased fitness, and eventually become more invasive, maybe as noxious weeds. Movement of viral transgene constructs through pollen flow has been documented from virus-resistant transgenic squash into a wild squash relative under experimental field conditions. Hybrids between transgenic and wild squash exhibited increased fitness under conditions of intense disease pressure. In contrast, under conditions of low disease pressure, no difference was observed between hybrids and wild squash in terms of growth and reproductive potential. Since viruses do not limit the size and dynamics of wild squash populations in natural habitats, it is anticipated that gene flow with virus-resistant transgenic squash will be of limited significance. It remains to be seen if increased fitness will provide hybrids with a competitive edge that could eventually lead to enhanced weediness. Altogether, compelling evidence suggest that gene flow with virus-resistant transgenic squash should not be perceived more risky than the equivalent situation with virus-resistant conventional squash.

Gene flow can also occur from a virus-resistant transgenic plant into a compatible conventional plant. Although of negligible biological impact, this phenomenon can be essential for economical reasons such as organic production and export to countries that have not deregulated transgenic crops. Worth noting is the fact that the coexistence of transgenic and conventional papaya in spatiotemporal proximity is a reality in Hawaii.

Real versus Perceived Risks

It is important to discriminate perceived and real risks associated with virus-resistant transgenic plants. Field environmental safety assessment studies have provided strong evidence of limited or no environmental risks, indicating that issues associated with virus-resistant transgenic plants are substantially less significant than initially predicted. To fully grasp the significance of environmental risks, the situation in the absence of transgenic plants needs to be taken into account and considered as base line information. So far, there is no compelling evidence to indicate that transgenic plants expressing viral genes increase the frequency of heterologous encapsidation or recombination beyond background rates. Similarly, there is little evidence, if any, to infer that transgenic plants expressing viral genes alter the properties of existing virus populations or create new viruses that could not arise naturally in conventional plants subjected to multiple virus infection. Also, the consequence of gene flow is perceived identical with transgenic and conventional virus-resistant plants. Therefore, there seems to be increasing scientific evidence that, while initially perceived as major concern, heterologous encapsidation, recombination, and gene flow with transgenic plants expressing viral genes are not deemed hazardous to the environment. Nevertheless, a case-by-case approach is recommended to make sound decisions on the safe release of virus-resistant transgenic plants. Communicating scientific facts on the safety of transgenic plants expressing viral genes is critical to distinguish real and perceived risks, help counterbalance risks and benefits, and assist regulatory authorities in the decision-making process for the release of virus-resistant transgenic crops.

Conclusions

Engineered resistance has expanded the scope of innovative approaches for virus control by providing new tools to develop resistant crop cultivars and increasing opportunities to implement effective and sustainable management strategies. Many advances have been made on the development of virus-resistant transgenic plants over the past two decades. Since its validation with TMV in tobacco plants in 1986, the concept of PDR has been applied successfully against a wide range of viruses in many plant species. Studies on RNA silencing have provided new insights into virus–host interactions. They also shed light on the molecular and cellular mechanisms underlying engineered resistance in plants expressing virus-derived gene constructs. The exploitation of the sequence-specific antiviral pathways of RNA silencing has facilitated the design of transgene constructs for more predictable resistance. Broader spectrum resistance against multiple virus strains should also be achievable more easily by designing virus transgenes in highly conserved sequence regions and applying RNA silencing. It is anticipated that RNA silencing mechanisms will be devised more extensively in the future to create virus-resistant crop plants.

The past two decades have witnessed an explosion in the development of virus-resistant transgenic plants. Further, virus resistance has been extensively evaluated under field conditions. So far, 37 different plant species have been tested successfully in the field for resistance to 58 viruses. Despite remarkable progress, only a limited number of virus-resistant transgenic crops have been released so far for commercial use. Based on their efficacy at controlling virus diseases and high adoption rate by growers, more virus-resistant transgenic crops are likely to reach the market in the future.

There is no doubt that innovative and sustainable control strategies are needed for virus diseases to mitigate their impact on agriculture. Lessons from field experiments with various transgenic crops engineered for virus resistance and the commercial release of virus-resistant squash, papaya, tomato, and pepper have conclusively demonstrated that benefits outweigh by far risks to the environment and human health. A timely release and adoption of new virus-resistant transgenic crops is desirable, in particular in regions where viruses are devastating.

See also: Papaya Ringspot Virus; Plant Antiviral Defense: Gene Silencing Pathway; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes; Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Plant Virus Diseases: Economic Aspects; Recombination; Recombination; *Tobamovirus*; Vector Transmission of Plant Viruses.

Further Reading

Baulcombe D (2004) RNA silencing in plants. *Nature* 431: 356–363.

Bendahmane M and Beachy RN (1999) Control of tobamovirus infections via pathogen-derived resistance. *Advances in Virus Research* 53: 369–386.

Fuchs M, Chirco EM, and Gonsalves D (2004) Movement of coat protein genes from a virus-resistant transgenic squash into a free-living relative. *Environmental Biosafety Research* 3: 5–16.

Fuchs M, Chirco EM, McFerson J, and Gonsalves D (2004) Comparative fitness of a free-living squash species and free-living x virus-resistant

transgenic squash hybrids. *Environmental Biosafety Research* 3: 17–28.
Fuchs M and Gonsalves D (2007) Safety of virus-resistant transgenic plants two decades after their introduction: Lessons from realistic field risk assessment studies. *Annual Review of Phytopathology* 45: 173–220.
Fuchs M, Klas FE, McFerson JR, and Gonsalves D (1998) Transgenic melon and squash expressing coat protein genes of aphid-borne viruses do not assist the spread of an aphid nontransmissible strain of cucumber mosaic virus in the field. *Transgenic Research* 7: 449–462.
Gonsalves C, Lee DR, and Gonsalves D (2004) *Transgenic Virus-Resistant Papaya: The Hawaiian 'Rainbow' was Rapidly Adopted by Farmers and Is of Major Importance in Hawaii Today*.
Gonsalves D (1998) Control of papaya ringspot virus in papaya: A case study. *Annual Review of Phytopathology* 36: 415–437.
Gonsalves D, Gonsalves C, Ferreira S, *et al.* (2004) *Transgenic Virus-Resistant Papaya: From Hope to Reality for Controlling Papaya ringspot virus in Hawaii.* http://www.apsnet.org/online/feature/ringspot.
Lindbo JA and Dougherty WG (2005) Plant pathology and RNAi: A brief history. *Annual Review of Phytopathology* 43: 191–204.
MacDiarmid R (2005) RNA silencing in productive virus infections. *Annual Review of Phytopathology* 43: 523–544.
Powell AP, *et al.* (1986) Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* 232: 738–743.
Qu F and Morris TJ (2005) Suppressors of RNA silencing encoded by plant viruses and their role in viral infections. *FEBS Letters* 579: 5958–5964.
Sanford JC and Johnston SA (1985) The concept of parasite-derived resistance-deriving resistance genes from the parasite's own genome. *Journal of Theoretical Biology* 113: 395–405.
Tepfer M (2002) Risk assessment of virus-resistant transgenic plants. *Annual Review of Phytopathology* 40: 467–491.
Vigne E, Bergdoll M, Guyader S, and Fuchs M (2004) Population structure and genetic diversity within *Grapevine fanleaf virus* isolates from a naturally infected vineyard: Evidence for mixed infection and recombination. *Journal of General Virology* 85: 2435–2445.
Voinnet O (2005) Induction and suppression of RNA silencing: Insights from viral infections. *Nature Reviews Genetics* 6: 206–221.
Waterhouse PM, Wang MB, and Lough T (2001) Gene silencing as an adaptative defense against viruses. *Nature* 411: 834–842.

Plant Resistance to Viruses: Geminiviruses

J K Brown, The University of Arizona, Tucson, AZ, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Bipartite virus A virus having a genome comprising two segments of nucleic acid.
Consensus sequence An identical sequence, usually shared by a group of close virus relatives.
Hemiptera The insect order that contains plant feeding insects such as aphids, leafhoppers, planthoppers, and whiteflies.
Monopartite virus A virus having a genome comprising one segment of nucleic acid.
Reassortment The process by which nonhomologous viral components interact during an infection cycle. Naturally reassorted viruses are thought to be more fit than their homologous counterparts and so arise by natural selection.
Recombination The process by which nucleic acid sequences are exchanged between two or more nucleic acid molecules during replication.
Satellite A segment of nucleic acid that associates with a 'helper' virus and utilizes it for replication and transmission. Satellites typically share little to no sequence homology with the helper virus.
Vector A biological organism that transmits plant viruses from one host to another, usually an arthropod or nematode.

Introduction

Geminiviruses

For perspective, it is important to recognize that the family *Geminiviridae* was established only in 1978. This is an unusual family among plant viruses in that their genomes are circular, single-stranded DNA. When the group was originally established, fewer than 20 possible members were recognized. Even so, among the whitefly-transmitted group, now recognized as the genus *Begomovirus* (originally subgroup III), additional associated diseases had been described, but definitive characterization for most of them was delayed until tools for detecting and identifying them were developed.

Geminiviruses are composed of circular, single-stranded DNA genome that is packaged within a pair of quasi-isometric particles, hence, the name 'geminate', or twinned particles (**Figure 1**). Geminiviruses are divided into four genera based on the genome organization and biological properties. One of the most striking differences between the four genera is their restriction to either monocots or dicots (eudicots), and a highly specific relationship with the respective arthropod vector, treehoppers, leafhoppers, or a whitefly, all of which are classified in the order Hemiptera, suborder Homoptera, and which feed on phloem sap using a slender stylet.

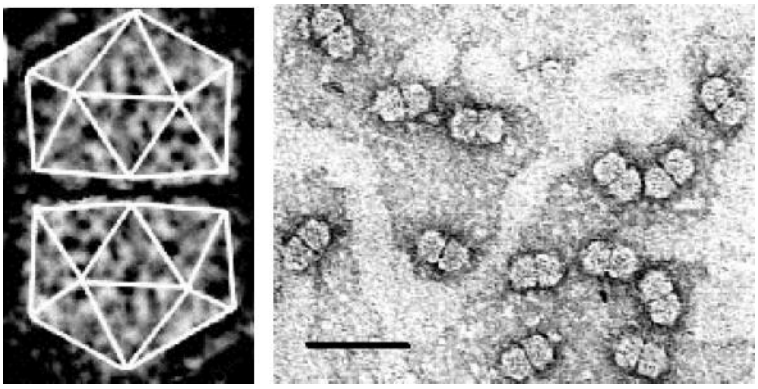


Figure 1 Typical geminate particles (~20 × 32 nm^2) by transmission electron microscopy. Courtesy of C. Fauquet.

The four genera

Geminiviruses with monopartite genomes are transmitted by leafhopper vectors to monocotyledonous plants and are placed in the genus *Mastrevirus*. *Maize streak virus* is the type species. Geminiviruses with monopartite genomes that are transmitted to dicotyledonous plants by leafhopper vectors are classified in the genus *Curtovirus* with *Beet curly top virus* as the type species. The third genus, *Topocuvirus*, was recently established and has a single member, *Tomato pseudo-curly top virus*. Tomato pseudo-curly top virus (TPCTV) is monopartite, infects dicotyledonous plants, and has a treehopper vector. The fourth and largest genus, *Begomovirus*, houses the majority of the species in the family. Begomoviruses are transmitted exclusively by the whitefly *Bemisia tabaci* (Gennadius) complex to dicotyledonous plants, and *Bean golden yellow mosaic virus* is recognized as the type species. They can have either a monopartite or bipartite genome. For bipartite viruses, the two components are referred to as DNA-A and DNA-B. The B component is thought to have arisen from a duplication of DNA-A followed by the loss of all but the two genes involved in cell-to-cell movement and nuclear localization of the virus during replication.

Viral Genome and Protein Functions

Begomoviruses have a circular, single-stranded DNA (ssDNA) genome that replicates through double-stranded DNA (dsDNA) intermediates by a rolling-circle mechanism. The genomes of begomoviruses are approximately 2.6–2.8 kbp in size. Bipartite genomes share a common region (CR) of approximately 200 nt that is highly conserved among cognate components of a viral species, while the analogous region in monopartite viruses is referred to as the large intergenic region (LIR). The CR/LIR contain modular *cis*-acting elements of the origin of replication (*ori*) and promoter elements.

Four to six open reading frames (ORFs) capable of encoding proteins >10 kDa in size are present on the A component or monopartite genome. The viral capsid protein (CP) is encoded by the ORF (AV1), which is the most highly conserved begomovirus gene. The CP of begomoviruses is required for encapsidation of ssDNA and for whitefly-mediated transmission. The CP is also necessary for systemic spread of all monopartite and for some bipartite viruses, depending upon the degree of virus–host adaptation. The AC1 ORF encodes REP, a replication initiation protein, and specificity is mediated through sequence-specific interactions with *cis*-acting elements of the *ori*. The AC2 ORF encodes a transcription factor protein required for rightward gene expression and this protein also functions to suppress gene silencing imposed by the plant host. The AC3 ORF encodes a replication enhancer protein. Movement and systemic infection in bipartite viruses is accomplished by B component ORFs BV1 and BC1, which are responsible for nuclear transport of ssDNA and cell-to-cell movement functions, respectively. For monopartite viruses, several ORFs are involved in movement functions, including the analogous V1 (CP), V2, and the C4 ORF, which exert host-specific effects on symptom severity, virus accumulation, and systemic movement.

Satellite DNAs of Begomoviruses

Certain monopartite begomoviruses are associated with a nonviral circular, ssDNA referred to as a DNA-β type satellite. SatDNAs associate with a 'helper' begomovirus on which they depend for replication, encapsidation, and whitefly-mediated transmission. This type of satDNA comprises all nonviral sequences except for a 9 nt stretch that mimics the sequence of the viral origin of replication; hence, when replication initiates, the DNA-β is recognized as template and is replicated together with the begomoviral genome. In turn, the DNA-β satellite encodes a protein that has experimentally been shown to localize to the nucleus of the cell where it is thought to suppress host-induced gene silencing, an innate defense response that is intended to protect the plant from virus infection.

In the absence of the satellite, the 'helper' virus cannot sustain infection of the host and so if the helper virus becomes separated from its satellite, the virus would quickly become extinct. This relationship seems to imply that the virus itself has lost the capacity to be sufficiently pathogenic and so is dependent upon the satellite for its survival. It is interesting to speculate about the evolutionary significance of this type of interaction, in light of the hypothesis that after a time most pathogens and their hosts 'adjust' to one another, reaching a sort of evolutionary equilibrium. How these 'helper' begomoviruses became disabled and subsequently rescued by such a nonviral sequence is not known. And why they associate only with monopartite genomes, even though they have had ample opportunity for contact with bipartite begomoviruses, which also occur in the Old World, is not understood. It is thought by some that a monopartite, compared to bipartite, genome organization is the more primitive condition. However, it is not clear why satDNAs did not also co-evolve with the most ancient bipartite lineages.

Diversification and Host Shifts

There are over 389 complete geminiviral genome sequences, and at least 200 full-length DNA-β satellite sequences available in public databases, indicating the widespread nature of these viruses, discovered only recently. Geminiviruses appear readily adaptable to new hosts, and have proven remarkably capable of 'host-shifting'. Although the specific coding or noncoding regions of the genome that undergo change during host adaptation have not been identified, geminivirus genomes seem capable of undergoing quite rapid evolutionary change, for example, sometimes in a single growing season. The evidence that such rapid change occurs is taken from the observation that very few virus sequences in endemic species match identically with their closest relatives in a crop species, and many diverge by 10% or more. In only a very few instances have geminiviruses in an endemic species been found in a counterpart cultivated host, and then the virus is known to have emerged only recently as a pathogen of the crop plant. These observations suggest that the development of sustainable, resistant varieties to abate geminivirus diseases may prove difficult if these viruses are likewise capable of diversifying rapidly and overcome resistance genes. Moreover, the members of this virus family are well known for their ability to undergo recombination allowing them to diversify and evolve into more fit species. This phenomenon is best exemplified by the begomoviruses, some of which have been shown to contain sequence fragments that closely match sequences in several extant begomovirus genomes.

Diversification in bipartite viruses also appears to occur through reassortment of the DNA components based on sequence analysis. Under laboratory conditions, mixtures of certain heterologous DNA-A and DNA-B components have been shown to work cooperatively to infect a plant, and some of these 'pseudo-recombinants or reassortants' can infect host species that at least one of the parent viruses cannot. Because geminiviruses apparently do not 'cross-protect' against one another or undermine one another in the event of a mixed infection, it is clear that more than a species can infect the same plant. Experimental evidence has demonstrated that two begomoviruses can occupy the same nucleus in the plant host, a circumstance that would facilitate diversification processes and readily yield new and emerging viral species.

Symptomatology on Respective Major Hosts

Symptoms of infection caused by geminiviruses vary depending on whether the host is a monocot or a dicot, characterized as having distinct arrangements of vascular tissues and parallel or branching leaf vein patterns, respectively (**Figures 2(a)–2(d)**). Even so, geminivirus-infected plants exhibit unique symptom phenotypes that are often readily recognizable to the trained eye.

Monocots infected with mastreviruses often exhibit interveinal chlorosis that takes on the appearance of foliar streaking. Leaves may develop splotchy yellow or white patterns between the veins, and the veins may be distorted and cleared and plant vigor is reduced leading to stunted growth. Commonly, the symptoms develop in leaves closest to the growing tip when infection occurred.

A pattern of symptom development in the newest growth also is typical of begomoviruses, curtoviruses, TPCTV, and several unclassified, monopartite Old World geminiviruses. Symptoms in the leaves of dicots range from mosaic to yellow and bright yellow mosaics or sectored patterns, and leaf curling. Infected plants often develop shortened internodes and become dramatically stunted. Certain viruses have the effect of causing profuse flowering, while at the same time reducing fruit set and fruit size of those that are set. Certain geminiviruses also cause outgrowths on the top or underside of leaves, referred to as enations.

Economic Importance

Viruses of the family *Geminiviridae* are considered emergent pathogens because they have become recently increasingly important in a large number of agriculturally important crops ranging from cereals such as maize and wheat, to species grown for fiber, like cotton and kenaf, and to a number of annual and perennial ornamental plants. Other viruses in this family are primarily yield-limiting

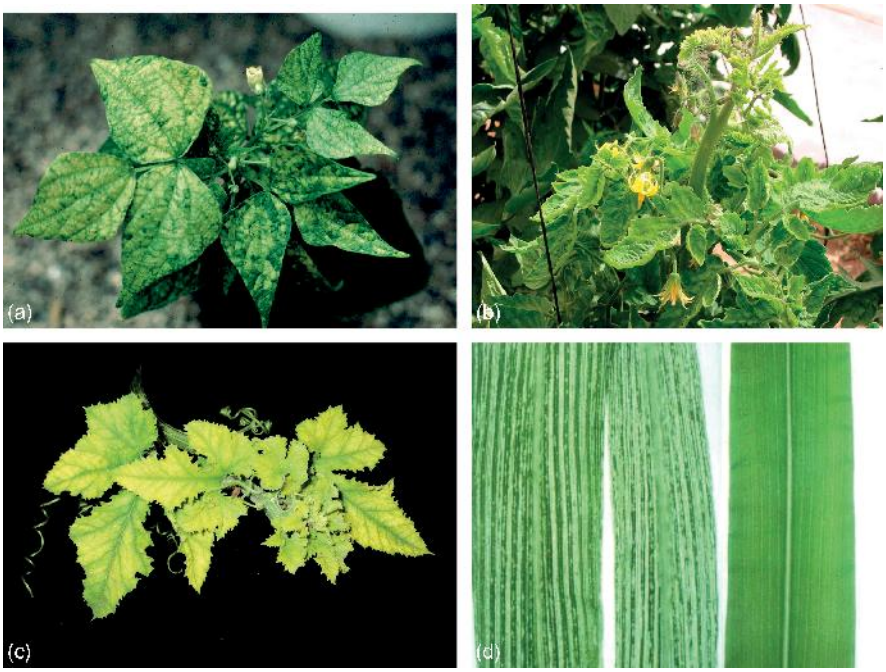


Figure 2 (a) Bean plants infected with the New World, bipartite bean calico mosaic virus showing typical mosaic symptoms; (b) tomato plant infected with the Old World, monopartite tomato yellow leaf curl virus; (c) symptoms of the leafhopper-transmitted beet curly top virus infecting a pumpkin plant; (d) maize leaves showing characteristic symptoms of the Old World monopartite, monocot infected with maize streak virus. (c) Courtesy of R. Larsen. (d) Reproduced by permission of E. Rybicki.

pathogens of vegetable and root crop plants that are important as staples and for vitamins and nutritional variety. Yield losses in infected crops range from 20% to 100%, depending on the virus–host combination and the growth stage of the plant at time of infection. Such extensive reductions in crop productivity often result in conditions of malnutrition and in the loss of precious incomes, particularly in developing countries that have become reliant on exporting their crops.

Geminiviruses are distributed throughout the Tropics, subtropics, and mild climate regions of the temperate zones, occurring on all continents where food, fiber, and ornamental crops are cultivated. Geminiviruses also infect a wide range of endemic plant species, with which they have likely co-evolved, based on the evidence that they appear to cause little damage. This would not be surprising given their predicted longstanding association with uncultivated eudicot species. Thus the endemic species serve as a melting pot for native viruses that find their way into cultivated plants, particularly recently, as practices in many locations have shifted to monoculture production. In the subtropics, it is not uncommon to grow crops year-round. These practices enabled by extensive irrigation projects worldwide have resulted in sustained, high levels of virus inoculum and vector populations in the environment, and more frequent outbreaks of disease, particularly since the 1970s.

Molecular Diagnosis of the Virus and Its Homology with Other Viruses

For most plant viruses, serology has traditionally been the method of choice for identification. However, virus-specific antisera for geminiviruses are not generally available. This is in part because geminiviruses can be difficult to purify. Further, begomoviruses in particular are not good antigens, and a small amount of contaminating plant protein in the purified preparation outcompetes the viral epitopes during immunization. Even when antibodies are available, they are useful primarily for detection, not identification, owing to the high genus-wide conservation of the viral coat proteins. However, the greatest factor underlying the popular use of molecular diagnostics for begomoviruses is that they contain a circular, ssDNA genome that yields a dsDNA intermediate from replication, which is highly amenable to linearization with an endonuclease to yield a dsDNA fragment that can be cloned. Second, shortly after the geminiviruses were discovered, polymerase chain reaction (PCR) was invented and the two clearly went hand in hand.

Commonly, detection is accomplished using PCR primers designed to be broad spectrum (usually degenerate) or virus specific. Because substantial information on begomovirus genome organization and gene function is now available, as are numerous genomic and CP gene sequences, it is possible to scan partial or entire viral

sequences and develop hypotheses regarding the prospective utility of a particular ORF or nontranslated region for inferring phylogenetic relationships. Sequences associated with functional domains of viral polypeptides, nucleotide sequence motifs or regulatory elements that are conserved within the genus or groups of species or strains, or sequences that are highly variable have been explored for this purpose.

Identification can be accomplished by determining the DNA sequence of the viral coat protein (tentative identification) and ultimately the complete DNA-A or monopartite DNA component. More recently the method, rolling-circle amplification, has been introduced as a non-sequence-specific approach for amplifying multimers of a viral genome, which can subsequently be cleaved to a full-length monomer using restriction endonucleases, allowing the sequence to be determined.

Sequence alignments are conveniently presented as cladograms to illustrate predicted relationships and distances can be calculated to estimate divergence. Phylogenetic analysis of the complete genome (monopartite) and A component (bipartite) viral sequences or the CP gene nucleotide sequences indicate strict clustering of begomoviruses by extant geographic origin, and not by monopartite or bipartite genome organization, crop of origin, or host range. Certain Old World viruses may be somewhat more divergent than the majority of New World viruses, perhaps because they have been more isolated by geographic barriers and/or patterns of human travel, suggesting fewer opportunities for interactions between viruses in different regions, while at the same time, great potential for localized diversity. All begomoviruses in the New World have bipartite genomes and many are distributed as overlapping geographic clusters, suggesting a lesser role of geographic barriers, fewer genotypes that have diverged in isolation, and, consequently, a more confounded evolution (**Figure 3**).

The genera in this family are discriminated based on numerical taxonomy in which a percentage nucleotide identity is used to classify them based on relatedness. Within each genus the working cutoff for species versus strains have been determined empirically by comparing the frequencies of percentage divergence across many sequences. For example, for the begomoviruses, a species is presently recognized when it is less than 89% identical to another, a value that has been shown to correlate nicely with biological differences. Species in the genera *Mastrevirus* and *Curtovirus* are classified as separate species when the genome sequence is found to share less than 75% and 89% nt identity, respectively.

All geminiviruses contain a conserved consensus sequence of *c.* 30 nt in length in this region. This sequence contains the T/AC cleavage site at which rolling-circle replication is initiated, and this conserved feature led to early speculation that recombination might occur frequently at this site. In addition, virus-specific, directly repeated sequences that bind the viral replicase-associated protein (REP) during replication initiation are also present in this region and are considered to provide useful clues with respect to the likelihood that the DNA-A and DNA-B components are cognate, or of the same virus. The directly repeated element in bipartite and most monopartite genomes contains two 4–5 nt directly repeated sequences separated by two to four 'spacer' nucleotides which act in a virus-specific manner to bind REP. As a result, these elements constitute informative indicators of viral genealogy useful in defining species and strains when traced through viral lineages. It is not entirely clear how much plasticity in sequence or spacing can be tolerated at this site without interfering with replication. Finally, these repeated sequences in the CR have been explored to make predictions about which bipartite genomes might feasibly interact *in trans* and reassort either with a close relative, or a more distantly related virus that harbors the same or similar iteron sequences and REP binding ability.

Recently Adopted Management Approaches

Management of diseases caused by geminiviruses requires a multifaceted strategy. This involves reduction of vector population levels in the field, typically by insecticide applications and/or encouragement of natural enemies in refuge areas such as predators or parasites of the vector. Sanitation in and around the field in which virus-infected plants, including endemic weeds, are removed after the crop is harvested and before it is planted are common practices. Implementation of a host-free period has proven relatively effective in reducing inoculum levels to a minimum so that when a crop is planted, it achieves near-mature growth before becoming infected with virus. Plants that become infected when they are partially mature usually experience less damage compared to virus infection early after planting. Implementation of a host-free period is accomplished by having a voluntary or an enforced period of time when no virus or vector hosts may be grown for a designated period of time usually ranging from 4 to 8 weeks, across an entire region. This is often an intangible goal given the requirement for complete cooperation. In all of the above examples, a single viruliferous vector can feasibly transmit virus to susceptible young plants, and so the most desirable control strategy is the use of resistant or tolerant crop varieties. For the most part, such varieties are not available. Recently, several resistant cassava, maize, and tomato varieties have been developed as the result of widespread efforts to provide genetically tolerant germplasm. There

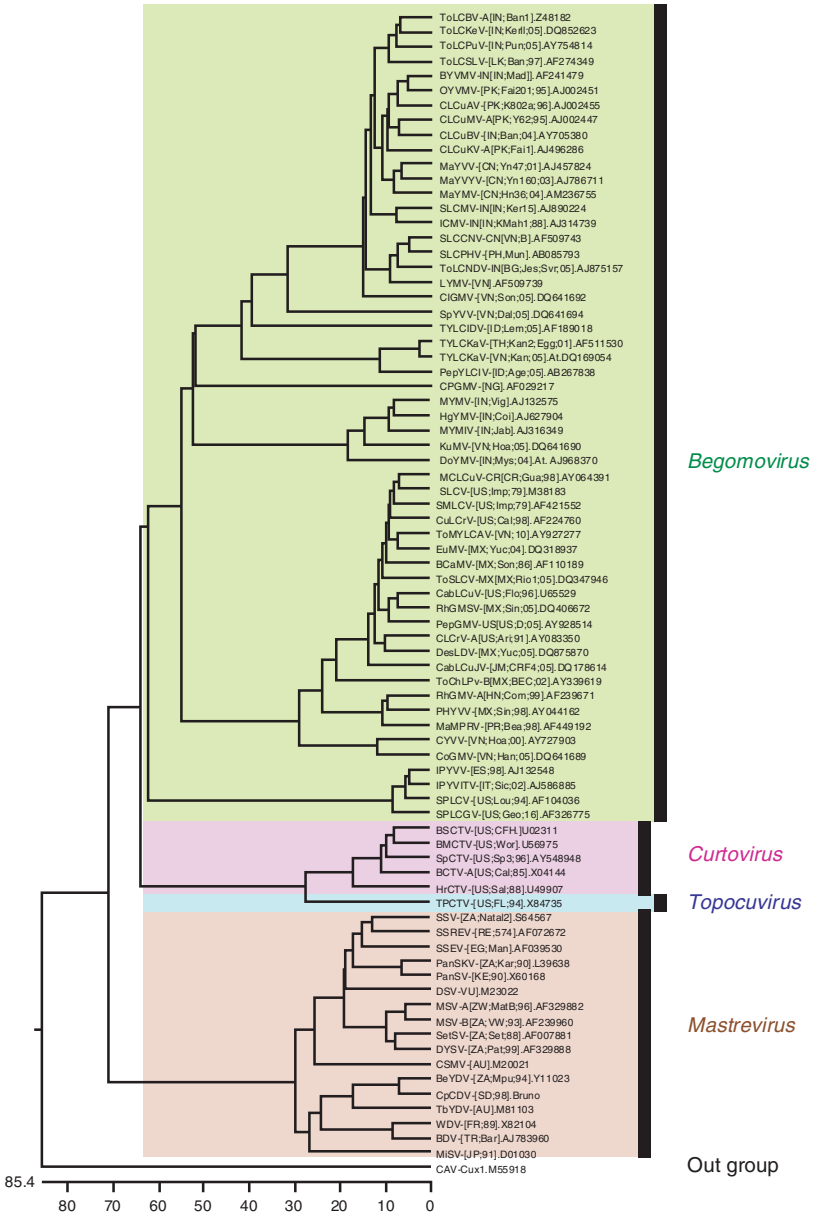


Figure 3 Phylogenetic tree of the family *Geminiviridae* showing the four major virus clades that serve to classify geminiviruses in one of four genera. The viruses are listed in the 8th ICTV Report. Courtesy of C. Fauquet.

also is an interest in developing and employing genetically engineered resistance using virus-derived resistance approaches such as RNA interference; however, the reluctance on the parts of many to accept this strategy has set back progress, which likely would have been brought to fruition for the control of at least certain geminivirus-incited crop diseases.

The recognition that begomoviruses are capable of rapid divergence employing multiple mechanisms underscores the need for accurate molecularly based methods that permit detection and tracking of biologically significant variants. Molecular approaches will continue to be combined to achieve knowledge about viral biology, ecology, and molecular and cellular aspects of the infection cycle. Databases containing genome sequences for geminiviruses now permit comparisons for establishing identity, taxonomic relationships, and interpreting disease patterns. Accurate molecular epidemiological information will assist plant breeding and genetic engineering efforts in developing disease-resistant varieties by enabling the selection of timely and relevant viral species (and variants) for germplasm screening and as sources of the most conserved viral transgene sequences, respectively, for sustainable disease control.

See also: African Cassava Mosaic Disease; Bean Golden Mosaic Virus; Beet Curly Top Virus; Cotton Leaf Curl Disease; Emerging Geminiviruses; Maize Streak Virus; Mungbean Yellow Mosaic Viruses; Tomato Leaf Curl Viruses from India; Tomato Yellow Leaf Curl Virus.

Further Reading

Arguello-Astorga GR, Guevara-Gonzalez RG, Herrera-Estrella LR, and Rivera-Bustamante RF (1994) Geminivirus replication origins have a group-specific organization of iterative elements: A model for replication. *Virology* 203: 90–100.

Bisaro DM (1994) Recombination in geminiviruses: Mechanisms for maintaining genome size and generating genomic diversity. In: Paszkowski J (ed.) *Homologous Recombination and Gene Silencing in Plants*, pp. 39–60. Dordrecht, The Netherlands: Kluwer.

Brown JK (2001) The molecular epidemiology of begomoviruses. In: Khan JA and Dykstra J (eds.) *Trends in Plant Virology*, pp. 279–316. New York: Haworth Press.

Brown JK (2007) *The Bemisia tabaci* complex: Genetic and phenotypic variability drives begomovirus spread and virus diversification. Plant Disease APSNet Feature Article December–January 2006–07. http://www.apsnet.org/online/feature/btabaci/ (accessed February 2008).

Brown JK and Czosnek H (2002) Whitefly transmitted viruses. In: Plumb RT (ed.) *Advances in Botanical Research*, pp. 65–100. New York: Academic Press.

Dry IB, Krake LR, Rigden JE, and Reizan AM (1997) A novel subviral agent associated with a geminivirus: The first report of a DNA satellite. *Proceedings of the National Academy of Sciences, USA* 94: 7088–7093.

Goodman RM (1977) Single-stranded DNA genome in a whitefly-transmitted virus. *Virology* 83: 171–179.

Hanley-Bowdoin L, Settlage SB, Orozco BM, Nagar S, and Robertson D (1999) Geminiviruses: Models for plant DNA replication, transcription, and cell cycle regulation. *Critical Reviews in Plant Sciences* 18: 71–106.

Harrison BD and Robinson DJ (1999) Natural genomic and antigenic variation in whitefly-transmitted geminiviruses (begomoviruses). *Annual Review of Phytopathology* 37: 369–398.

Hou YM and Gilbertson RL (1996) Increased pathogenicity in a pseudorecombinant bipartite geminivirus correlates with intermolecular recombination. *Journal of Virology* 70: 5430–5436.

Idris AM and Brown JK (2005) Evidence for interspecific recombination for three monopartite begomoviral genomes associated with tomato leaf curl disease from central sudan. *Archives of Virology* 150: 1003–1012.

Lazarowitz SG (1992) Geminiviruses: Genome structure and gene function. *Critical Reviews in Plant Sciences* 11: 327–349.

Padidam M, Sawyer S, and Fauquet CM (1999) Possible emergence of new geminiviruses by frequent recombination. *Virology* 265: 218–225.

Sanderfoot AA and Lazarowitz SG (1996) Getting it together in plant virus movement: Cooperative interactions between bipartite geminivirus movement proteins. *Trends in Cell Biology* 6: 353–358.

Saunders K, Norman A, Gucciardo S, and Stanley J (2004) The DNA beta satellite component associated with *Ageratum* yellow vein disease encodes an essential pathogenicity protein (BC1). *Virology* 324: 37–47.

Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes

P Moffett and D F Klessig, Boyce Thompson Institute for Plant Research, Ithaca, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Avirulence gene Any pathogen gene whose protein product induces a resistance response in a plant possessing a matching resistance gene, thereby rendering the pathogen unable to establish an infection.

Gene-for-gene resistance A genetic paradigm whereby the outcome of a plant–pathogen interaction is determined by the genotype of both organisms. Plant disease resistance proteins confer resistance to pathogens possessing a corresponding avirulence gene.

Hypersensitive response A type of programmed cell death induced by a resistant plant in response to pathogen infection. Cell death is normally contained to infected cells.

NB-LRR protein Plant disease resistance proteins containing a central nucleotide-binding domain (NB) and C-terminal leucine-rich repeat (LRR) domain. Also known as NBS-LRR or NB-ARC-LRR proteins.

Resistance gene Plant genes which confer resistance to specific pathogens. Resistance genes confer resistance to pathogens possessing a corresponding avirulence gene.

Salicylic acid A phenolic compound synthesized by plants that plays a critical signaling role for activation and regulation of defense responses to pathogens. Salicylic acid also regulates other plant processes such as thermogenesis.

Virulence gene Any pathogen gene whose function contributes to increased pathogen proliferation or disease symptoms on an infected plant.

Introduction

In many plant–pathogen interactions, host plants possess polymorphic resistance to different strains or races of pathogen which is controlled by a single gene. Disease resistance controlled by recessive genes is often a passive form of resistance wherein the pathogen is unable to utilize host-cell machinery. Dominant resistance (*R*) genes in plants appear to actively recognize the presence of specific pathogens and initiate programmed responses to counteract infection. However, this resistance is dependent on the pathogen possessing a corresponding gene that matches the *R* gene. These pathogen avirulence (*Avr*) genes are also dominant over their cognate virulent (*avr*) alleles. Since the outcome of an attempted infection is dependent on the genotype of both host and pathogen (**Figure 1(a)**), this phenomenon is known as gene-for-gene resistance, also known as race-specific or cultivar resistance. Gene-for-gene resistance has been observed for many plant pathogens including fungi, oomycetes, bacteria, insects, nematodes, and viruses.

R Gene Products

Gene-for-gene resistance was first described over 50 years ago by H. H. Flor who observed that different cultivars of flax possessed dominant *R* genes that were specific to races of flax rust, caused by the fungus *Melampsora lini*, possessing corresponding dominant *Avr* genes. Although viruses and fungi have different genetics, the same concept applies to several virus resistance genes.

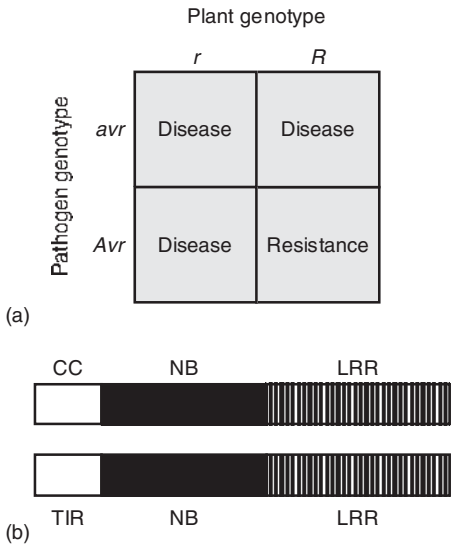


Figure 1 (a) Gene-for-gene resistance. Individual plant genotypes confer susceptibility (*r*) or resistance (*R*) to a given pathogen. This resistance is dependent on whether the pathogen possesses a matching avirulence (*Avr*) gene or not (*avr*). (b) Schematic diagram of the NB-LRR proteins encoded by virus resistance genes. The nucleotide-binding domain (NB) is represented as a black box and the leucine-rich repeat (LRR) domain is represented by a hatched box. These proteins are known as CC-NB-LRR or TIR-NB-LRR proteins depending on whether they possess an N-terminal coiled-coil (CC) or Toll and interleukin receptor (TIR) domain, represented by open boxes.

Genetically, *R* genes have been characterized for many years and have been incorporated into breeding programs of multiple crop plants. Since the cloning of the first *R* gene in 1993, a steady stream of *R* genes have been cloned from a number of plant species. The proteins encoded by these genes were found to belong to a small number of protein classes including transmembrane proteins possessing extracellar leucine-rich repeat (LRR) domains as well as a small number of intracellular serine/threonine kinases. By far, however, the most numerous type of *R* genes encode intracellular proteins containing a central nucleotide-binding (NB) domain as well as a C-terminal LRR domain. These NB-LRR proteins also possess a predicted signaling domain at the N-terminus. NB-LRR proteins can be placed into two classes; those which possess an N-terminal domain with homology to the Toll and interleukin receptors (TIR domain) of animals and those that do not (**Figure 1(b)**). The structure of the N-terminus of the latter class of NB-LRR proteins is not clear; however, many are predicted to form a coiled-coil structure and as such are commonly referred to as CC-NB-LRR proteins. CC-NB-LRR proteins are present in both monocots and dicots whereas TIR-NB-LRRs appear to be present only in dicots. Both types are present in gymnosperms.

As of 2007, over 50 *R* genes encoding NB-LRR proteins of known specificity have been cloned from plants belonging to various species. Sequencing projects have revealed that plant genomes contain large numbers of genes encoding NB-LRR proteins. The *Arabidopsis* genome contains 149 such genes whereas the rice genome encodes between 500 and 600. Many *R* gene loci are polymorphic with different alleles recognizing different pathogen races or even different pathogens. Furthermore, NB-LRR proteins are highly divergent both within and between species such that the *R* gene complement of a given species does not show strong similarity to the *R* gene complement of another unrelated species. Thus, *R* genes as a class of proteins likely possess a great deal of potential for recognition of pathogen diversity. Given the high degree of polymorphism, *R* genes recognizing a specific pathogen can often be identified by testing, for example, a large number of *Arabidopsis* accessions. For crop species, resistance sources are often identified in, and introgressed from, wild relatives. **Table 1** shows an incomplete list of a number of characterized dominant viral resistance genes. Although a number of these genes have not been cloned, they show characteristics typical of NB-LRR-encoding *R* genes such as dominant inheritance and the presentation of a hypersensitive response (HR) (see below).

Table 1 Examples of plant–virus gene-for-gene relationships

R gene	*Encoded protein*	*Plant*	*Origin*	*Virus*	*Avr determinant*	*Resistance response*[a]
Molecularly characterized R genes						
N	TIR-NB-LRR	Tobacco	*N. glutinosa*	Tobamoviruses	Helicase/p50 subunit	HR
Rx	CC-NB-LRR	Potato	*Solanum tuberosum* ssp. *andigena*	PVX	CP	ER
Rx2	CC-NB-LRR	Potato	*Solanum acaule*	PVX	CP	ER
Sw-5	CC-NB-LRR	Tomato	*Solanum peruvianum*[b]	Tospoviruses (TSWV, GRSV, TCSV)	ND	ER, HR
HRT	CC-NB-LRR	*Arabidopsis*	Ecotype Dijon	TCV	CP	HR
RCY-1	CC-NB-LRR	*Arabidopsis*	Ecotype C24	CMV	CP	HR
Tm2	CC-NB-LRR	Tomato	*Solanum peruvianum*[b]	Tobamoviruses	30 kDa MP	ER
Tm2^2	CC-NB-LRR	Tomato	*Solanum peruvianum*[b]	Tobamoviruses	30 kDa MP	ER
Rsv1	CC-NB-LRR[c]	Soybean	*Glycine max*	SMV	P3	ER, HR, SN
Ctv	CC-NB-LRR[c]	Trifoliate orange	*Poncirus trifoliata*	CTV	ND	ER
I	TIR-NB-LRR[b]	Bean	*Phaseolus vulgaris*	BCMV	ND	ER, HR, SN
Genetically characterized R genes						
Nb	ND	Potato	*Solanum tuberosum*	PVX	25 kDa MP	HR
Nx	ND	Potato	*Solanum tuberosum*	PVX	CP	HR
Ry	ND	Potato	*Solanum stoloniferum*	PVY	NIa protease	ER
L1	ND	Pepper	*Capsicum annum*	Tobamoviruses	CP	HR
L2	ND	Pepper	*Capsicum frutescens*	Tobamoviruses	CP	HR
L3	ND	Pepper	*Capsicum chinense*	Tobamoviruses	CP	HR
L4	ND	Pepper	*Capsicum chacoense*	Tobamoviruses	CP	HR
N′	ND	Tobacco	*Nicotiana sylvestris*	Tobamoviruses	CP	HR
Bdm1	ND	Bean	*Phaseolus vulgaris*	BDMV	BV1 protein	HR
TuRB01	ND	Brassica	*Brassica napus*	TuMV	C1 protein	ER
TuRB03	ND	Brassica	*Brassica napus*	TuMV	P3 protein	ER
TurB04	ND	Brassica	*Brassica napus*	TuMV	P3 protein	ER
TuRB05	ND	Brassica	*Brassica napus*	TuMV	C1 protein	HR
Cry	ND	Cowpea	*Vigna unguiculata*	CMV	2a protein	HR
Rym14^{Hb}	ND	Barley	*Hordeum bulbosum*	BaMMV/BaYMV	ND	ER
Rym16^{Hb}	ND	Barley	*Hordeum bulbosum*	BaMMV/BaYMV	ND	ER
Bdv2	ND	Wheat	*Thinopyrum intermedium*	BYDV	ND	ER
Plant–virus interactions resembling gene-for-gene resistance						
ND	ND	Tobacco	*Nicotiana tabacum*	TBSV	P19 protein	HR
ND	ND	*Nicotiana glutinosa*	*Nicotiana glutinosa*	TBSV	P22 MP	HR
ND	ND	*Nicotiana* spp.	*Nicotiana clevelandii*	CaMV	P6 protein	ER, HR, SN
ND	ND	Eggplant	*Solanum melongena*	Tobamoviruses	CP	HR

[a]Certain resistance genes condition more than one type of response depending on viral isolate, genetic background, or environmental conditions.
[b]Formerly known as *Lycopersicon peruvianum*.
[c]The *Rsv1, Ctv*, and *I* genes map to clusters of NB-LRR encoding genes. The individual genes conferring resistance have yet to be definitively assigned.
ND, not determined; CP, coat protein; MP, movement protein; HR, hypersensitive response; ER, extreme resistance; SN, systemic necrosis.
BaMMV/BaYMV, barley mild mosaic virus/barley yellow mosaic virus complex; BCMV, bean common mosaic virus; BDMV, bean dwarf mosaic virus; BYDV, barley yellow dwarf virus; CaMV, cauliflower mosaic virus; CMV, cucumber mosaic virus; CTV, citrus tristeza virus; GRSV, groundnut ringspot virus; PVX, potato virus X; PVY, potato virus Y; SMV, soybean mosaic virus; TBSV, tomato bushy stunt virus; TCSV, tomato chlorotic spot virus; TCV, turnip crinkle virus; TSWV, tomato spotted wilt virus; TuMV, turnip mosaic virus.

Molecular studies have shown that recognition specificity is largely determined by the LRR domain of NB-LRR proteins. For example, in certain cases, the recognition specificities of two similar NB-LRR proteins can be exchanged by swapping their respective LRR domains. Correspondingly, the LRR domain is the most polymorphic region of NB–LRR proteins. Bioinformatics studies have suggested that the LRR domains of *R* genes

are under diversifying selection, suggesting that this class of genes is under selective pressure. There do not appear to be any obvious features that distinguish between those *R* genes that recognize, for example, bacteria versus those that recognize viruses. For example, the *HRT, RCY1*, and *RPP8* genes of *Arabidopsis* are different alleles of the same gene locus but recognize turnip crinkle virus (TCV), cucumber mosaic virus (CMV), and the oomycete *Hyaloperonospora parasitica*, respectively. Similarly, in potato, gene-duplication events have led to highly similar gene paralogues, *Rx* and *Gpa2*, which confer resistance to potato virus X (PVX) and to the nematode, *Globodera pallida*, respectively. Both CC- and TIR-NB-LRR proteins have been shown to specify resistance to viruses as well as other pathogen classes. Additionally, PVX can be engineered to express a bacterial *Avr* gene and a plant expressing the corresponding NB-LRR protein will be resistant to the recombinant virus. Thus, most NB-LRR proteins probably have the potential to evolve specificity to a variety of different pathogen types, allowing for maximum adaptability. As such, the mechanisms of recognition and initiation of disease-resistance responses are likely to be similar between R proteins that recognize viruses and R proteins that recognize other types of pathogens.

Avirulence Genes

Plant R proteins recognize the protein products of pathogen *Avr* genes, the latter being much more diverse in structure and function than the former. Many different types of pathogen proteins are either synthesized in the host cell by intracellular pathogens (viruses) or delivered into the cytoplasm by the various secretion systems of bacteria, fungi, or oomycetes, and many of these are recognized by NB-LRR proteins. In gene-for-gene resistance, an avirulence protein is defined simply as a protein that induces a resistance response in a plant with the appropriate *R* gene. Thus, the pathogen is unable to infect such a plant due to an active defense mechanism on the part of the plant rather than a lack of virulence on the part of the pathogen. In the absence of a corresponding *R* gene, Avr proteins generally act as virulence factors in that they often play a role in protecting the pathogen from host defense responses, actively inhibit host defenses, or are involved in replication of the pathogen. Pathogen *avr* alleles can overcome *R*-gene-mediated resistance either by mutation or by elimination of the appropriate *Avr* gene. However, this often leads to a loss of fitness. Given their small genomes, this is particularly acute for viruses as most viral genes are crucial for viral fitness and often have multiple functions. Thus, mutation may overcome recognition, but can have the side effect of making the viral protein ineffective in one or more of its functions. Viral *Avr* genes may appear to share certain similarities; for example, a number of viral coat proteins (CPs) act as Avr determinants (**Table 1**). However, plant viruses have a limited repertoire of genes. Tobacco mosaic virus (TMV) has only three open reading frames (ORFs) (plus a read-through product) and each of these can act as an Avr determinant in the appropriate plant (see **Table 1**).

R Gene Responses to Viruses

The *N* gene has historically been one of the most studied antiviral resistance genes. Originating from *Nicotiana glutinosa*, the *N* gene was introduced into commercial tobacco cultivars via interspecific crosses as a means of conferring resistance to TMV. The *N* gene was among the first *R* genes to be cloned and encodes a typical TIR-NB-LRR protein. Like a number of TIR-NB-LRR-encoding genes, the *N* gene produces two different transcripts via alternative splicing, one encoding the full protein and a shorter version predicted to encode a protein lacking the LRR domain. The role of these truncated proteins is unclear; plants transgenic for versions of the *N* gene that cannot produce both splice forms are compromised in their resistance to TMV.

In addition to TMV, the *N* gene confers resistance to all other known tobamoviruses with the exception of a virus originally designated TMV-ob, and renamed obuda pepper virus (ObPV). A common strategy for identifying the Avr determinant of plant viruses is to engineer chimeras between virulent and avirulent viral strains in order to narrow down the region responsible for eliciting a resistance response. By exchanging genes between TMV and ObPV, the Avr determinant for the *N* gene was found to reside within the viral replicase protein. Furthermore, transient expression of the 50 kDa helicase domain of the TMV replicase (p50) in *N* plants was shown to be sufficient to induce a HR. The HR is a type of programmed cell death that is often associated with gene-for-gene resistance. In the interaction between TMV and tobacco plants possessing the *N* gene this is manifested as small necrotic lesions at the site of infection (**Figure 2(a)**). In this case the virus is able to replicate to a limited degree, spreading to cells surrounding the initially infected cell before the onset of cell death. As such, a macroscopically visible area undergoes cell death before the virus is fully contained. The HR is associated with *R*-gene-mediated resistance to other types of pathogens as well and its initiation requires only the presence of a matching pair of R and Avr proteins. **Figure 2(b)** shows the HR response resulting from the transient expression of an Avr protein in a plant leaf expressing the appropriate *R* gene.

Virus inoculation on a given plant can often result in an HR even though a strict gene-for-gene relationship has

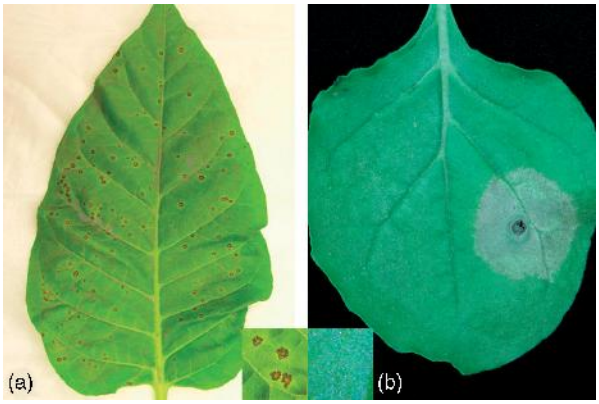


Figure 2 (a) The hypersensitive response (HR). A tobacco leaf expressing the *N* gene has been mechanically inoculated with TMV. Necrotic lesions represent individual infection foci that have subsequently undergone cell death as part of the HR. A higher magnification of necrotic lesions is shown in the inset. (b) Responses of the *Rx* gene. A leaf of *Nicotiana benthamiana,* which is expressing the potato *Rx* gene from a transgene, has been mechanically inoculated with PVX on the left half of the leaf. The resulting resistance is characterized by a lack of viral accumulation or HR response and is known as ER. A higher magnification of the infected leaf is shown in the inset. Note that the only visible signs of infection are the slight leaf damage from the inoculation procedure. On the right side of the leaf, an *Rx*-dependent HR response has been induced by introducing PVX CP via *Agrobacterium*-mediated transient expression. The circle of grayness is caused by dying cells within the area expressing CP.

not been defined due to a lack of variability in either the host or the pathogen. The HR response often indicates that the resistance is due to the presence of an *R* gene. For example, eggplant undergoes a typical HR response when inoculated with tobamoviruses. A compatible interaction has not been seen in this interaction either because the corresponding *R* gene is present in all cultivars of eggplant, or simply because not enough genetic material has been tested. Regardless, this raises the possibility that some cases of nonhost resistance, a phenomenon where all accessions of a plant are resistant to all isolates of a pathogen, may in fact be controlled by the same mechanisms as gene-for-gene resistance.

The second viral resistance gene to be cloned was the *Rx* gene of potato which confers resistance to PVX and encodes a typical CC-NB-LRR-type protein. Using sequence swaps with the single resistance-breaking strain of PVX (strain HB), it was determined that the PVX CP is the Avr determinant for *Rx*. The *Rx* gene was introgressed into potato cultivars from the wild species *Solanum tuberosum* subsp. *andigena*. Additionally, the *Rx2* gene was introgressed from the wild species *Solanum acaule*. Despite their different origins and the fact that *Rx* and *Rx2* are located on separate chromosomes, the predicted gene products are highly similar and condition a similar response to PVX in potato. Unlike the *N* gene, *Rx* and *Rx2* confer what is known as extreme resistance (ER). ER is broadly defined as a lack of visible response to virus infection. In an ER response there is no necrosis and little or no virus accumulation can be detected (**Figure 2(b)**). *Rx*-mediated ER is functional at the single-cell level. When infectious viral RNA is introduced into protoplasts there is no apparent viral accumulation. However, some *R* genes that manifest ER, such as *Tm-2* and *Tm-2*^{2} (see below), do allow virus replication in protoplasts.

Although *Rx*- and *Rx2*-mediated resistance does not normally manifest as an HR, these proteins are capable of inducing an HR. When the Rx or Rx2 proteins are expressed together with the PVX CP an HR is rapidly induced (**Figure 2(b)**). Thus, Rx and Rx2 probably do not induce responses that are qualitatively different from other R proteins but may simply induce a very rapid response, eliminating the source of Avr protein before it can accumulate to levels sufficient to induce an HR.

An example of an *R* gene inducing both ER and HR responses is seen with the *Sw-5* gene of tomato. *Sw-5* encodes a CC-NB-LRR protein conferring resistance to several tospoviruses including tomato spotted wilt virus (TSWV), tomato chlorotic spot virus (TCSV), and groundnut ringspot virus (GRSV). When tomato plants possessing the *Sw-5* gene are mechanically inoculated with tospoviruses, both HR and ER responses can be seen depending on the strain of virus inoculated, suggesting that the HR and ER responses may represent a continuum of responses. These different responses conditioned by the same gene may occur because Sw-5 recognizes the Avr determinant (unknown) of different strains with varying efficiency. The occurrence of an *Sw-5*-induced HR can be affected by environmental conditions as well as genetic background. Cell type can also have an effect on the *Sw-5* response. Although *Sw-5* plants are largely immune to TSWV, infection can still occur from thrips carrying TSWV when they feed on the tomato fruit.

The effect of genetic background has been more extensively studied in the case of the *Arabidopsis HRT* (HR to TCV) gene. The *HRT* gene was discovered by inoculating a number of *Arabidopsis* ecotypes with TCV. The ecotype Dijon (Di) was found to condition an HR-type response to TCV and a line (Di-17) was selected with the most robust resistance. The *HRT* gene encodes a CC-NB-LRR protein and confers recognition of the TCV CP. Inoculation of TCV onto leaves of Di-17 plants results in a typical HR and containment of the virus. However, despite an HR in the initially infected plants, in ~10% of infected plants, the virus is able to move to, and replicate in, systemic tissues. This effect is much more dramatic when Di-17 is crossed with other ecotypes such as Col-0. In this genetic background, HRT still conditions an HR in the infected leaves but nearly all plants allow systemic spread of the virus. Genetic studies suggest that in order to be

fully functional, HRT requires the presence of the recessive Di-17 allele of a gene termed *rrt*. Further studies have suggested that *HRT* requires elevated levels of salicylic acid (SA) in order to be fully functional and *rrt* may condition higher levels of this signaling molecule in Di-17.

The *Arabidopsis* ecotype C24 contains the *RCY1* gene which confers resistance to the Y strain of CMV. The *RCY1* and *HRT* genes are essentially different alleles of the same gene and encode proteins that are 91% identical. *RCY1* is also affected by genetic background and there appears to be a recessive gene present in ecotype Landsberg that abrogates *RCY1*-mediated resistance.

There are a number of other cases where an *R* gene is not able to contain a virus within the inoculated leaf. The *Rsv1* gene of soybean confers ER to all known American isolates of soybean mosaic virus (SMV) except for two. The G7d strain is resistance breaking and produces normal systemic infection in *Rsv1* soybeans. However, the G7 strain of SMV elicits an intermediate response. This strain is able to evade the initial resistance response and spread to systemic tissues. However, the build-up of virus in systemic tissues eventually leads to the initiation of cell death. This systemic necrosis (SN) is not sufficient to contain the virus, causing a trailing HR that eventually leads to the collapse of the plant. It is thought that the SN response is due to a weak recognition of the viral Avr determinant such that the HR is induced only once the virus has accumulated to high levels. Thus, the same responses (i.e., cell death) are induced as in an HR response, but too late to curtail virus spread.

Further evidence for quantitative differences between ER, HR, and SN responses is seen with the *I* locus of bean. The *I* gene has been mapped to a cluster of TIR-NB-LRR genes in bean and confers a defense response to nine different potyviruses, including bean common mosaic virus (BCMV). These responses range from ER to HR to SN depending on the viral isolate. At the same time, variation can be seen even when using the same viral isolate. At 23 °C plants heterozygous for the *I* gene (*I*/*I*) confer ER to BCMV whereas plants with only one copy of the *I* gene (*I*/*i*) confer an HR-type resistance response. At 34 °C, however, *I*/*i* plants undergo a SN response whereas *I*/*I* plants do so at a greatly reduced rate. Thus, the different responses to viruses by resistant plants likely represent a continuum of quantitatively different responses. The degree of response can be modulated by the presumed relative efficiency of recognition of the Avr determinant, gene dosage, temperature, and genetic background.

Mechanisms of Recognition

Given the relatively straightforward genetic relationship between *R* and *Avr* genes, it was originally proposed that this might represent a receptor–ligand interaction. Since LRR domains often act as protein–protein interaction domains, it was proposed that the variable LRR domains of NB–LRR proteins would bind to different Avr proteins and this binding would activate defense responses. However, initial difficulties in demonstrating such interactions led to the formulation of indirect models of recognition. The guard hypothesis states that, since many *Avr* genes are also virulence genes, R proteins would 'guard' the cellular targets of these virulence targets. In this scenario, it is the function of pathogen-encoded virulence factors to modify cellular proteins and this modification is in turn detected by R proteins. Thus, it is not the Avr protein *per se* that is detected, but rather its virulence activity. Several examples of such indirect recognition have been studied. For example, when expressed in *P. syringae*, the Avr proteins AvrB and AvrRpm1 are both recognized by the *Arabidopsis* CC-NB-LRR protein RPM1. RPM1 interacts physically with a cellular protein, RIN4, which in turn can interact with both AvrB and AvrRpm1. This latter interaction is thought to activate the RPM1 protein. The Avr protein AvrRpt2 from *P. syringae* is a cysteine protease and is recognized by the *Arabidopsis* CC-NB-LRR protein RPS2. RPS2 also binds RIN4 and becomes activated when RIN4 is cleaved by AvrRpt2. This activation can be mimicked by genetic ablation of the *RIN4* gene. In this case, RPS2 is constitutively activated in the absence of RIN4, thus the recognition event appears to be the proteolytic removal of a negative regulatory protein. Other probable examples of indirect recognition include recognition of AvrPphB from *P. syringae* by the *Arabidopsis* RPS5 protein through the PBS1 kinase as well as recognition by the tomato NB-LRR protein Prf of AvrPto from *P. syringae* due to the interaction of AvrPto with the Pto kinase.

At the same time, however, there is some indication that certain Avr/R protein pairs may interact directly. Yeast two-hybrid studies have shown Avr proteins interacting with the rice Pi-Ta, *Arabidopsis* RRS1, and the flax L NB-LRR proteins which confer resistance to rice blast fungus, *Ralstonia solanacearum,* and flax rust, respectively. Interactions have also been reported between the viral resistance protein N and its cognate elicitor p50. However, a great deal about these interactions remains unclear, such as whether the Avr proteins interact with the LRR domain, another part of the protein, or a combination thereof. Further studies may also demonstrate examples of indirect modes of recognition by NB-LRR proteins that recognize viruses.

Two traits desired by plant breeders related to recognition are broad-spectrum resistance and durability. Often, *R* genes are deployed that provide good resistance but are quickly overcome by new strains of a pathogen. The durability of an *R* gene may be affected by different aspects of the pathogen such as dispersal rates and

mechanisms, occurrence of sexual reproduction, and variable repertoires of virulence factors. In the latter case, for example, phytopathogenic bacteria have relatively large repertoires of virulence factors which may be partially redundant and dispensable. However, since viruses cannot dispense with any of their limited number of genes, overcoming resistance must occur through sequence changes.

In the field, some viral *R* genes are very durable, such as *N*, *Rx*, and *Ry*. There are no strains of potato virus Y (PVY) that overcome *Ry* and although ObPV can overcome *N*, it is not widespread. Likewise *Rx*-breaking strains of PVX have reduced virulence and are found only in South America. Strains of PVX that overcome the *Nx* and *Nb* genes are more widespread, and TSWV strains that overcome *Sw-5* have been reported in several tomato-growing areas. An interesting case study in durability can be seen with the *Tm-2* and *Tm-2*^{2} genes. These genes were introgressed from different accessions of *Lycopersicum peruvianum* into tomato cultivars as a means of controlling TMV and tomato mosaic virus (ToMV). These genes confer resistance to nearly all strains of tobamoviruses through recognition of the viral movement protein (MP). Molecular cloning has revealed that *Tm-2* and *Tm-2*^{2} are different alleles of the same gene. These genes encode CC-NB-LRR proteins that differ by only four amino acids, two of which are in the LRR domain. Despite this similarity, *Tm-2*^{2} has proven to be more durable than *Tm-2*. Strains of ToMV that overcome *Tm-2* or *Tm-2*^{2} have mutations in different regions of the MP; some mutations can overcome *Tm-2*, but not *Tm-2*^{2}, and vice versa. The *Tm-2*^{2} gene may be more durable because the resistance-breaking mutations have a very negative impact on virus fitness as evidenced by reduced viral load in plants infected with these strains. Furthermore, the *Tm-2*^{2}-breaking strains cause very severe symptoms on *Tm-2*^{2} plants so that infected plants can be easily recognized and eliminated from the field or greenhouse.

Differential recognition by the *N′* gene of tobacco may represent a case where overcoming resistance does not impose a fitness cost. The *N′* gene confers resistance to several tobamoviruses including ToMV, but not TMV. Although *N′* has not been cloned, the Avr determinant has been shown to be the viral CP. Curiously, mutations can be introduced into the TMV CP that result in a gain of recognition by *N′*, but which appear to compromise CP function. It has been hypothesized that the recognition of TMV CP is normally masked from recognition by *N′*, and mutations that interfere with the tertiary or quaternary structure of the CP cause the recognition site to be exposed.

In some cases, viral Avr proteins are able to evade recognition by some resistance genes but not others. For example, the allelic pepper genes *L1–L4* also recognize the CP of tobamoviruses. Certain virus strains are able to overcome *L1*-mediated resistance, some overcome *L1* and *L2*, and some overcome *L1, L2,* and *L3*. However, the *L4* gene is very broad spectrum, controlling all known tobamoviruses.

Signaling Mechanisms

Studies with Rx, HRT, and N have shown that the different domains of NB-LRR proteins undergo physical intramolecular interactions with the LRR and N-terminal domains interacting with the NB domain. These interactions appear to condition an autorepressed state and alterations thereof are associated with activation of the protein. In the presence of PVX CP, the interdomain interactions of Rx are disrupted and the N protein has been shown to undergo self-association in the presence of p50. It is presumed that R proteins induce a signal transduction cascade leading to disease resistance by interacting with signal adaptor molecules. However, such molecules have yet to be identified. A number of proteins required for NB-LRR function have been identified. TIR-NB-LRR proteins, including N, have a general requirement for the EDS1 protein and often also require the EDS1-interacting protein PAD4. Both of these proteins have lipase signature domains although their exact function is not known. Given that the *N* gene is not effective above 32 °C, it is interesting to note that EDS1 expression levels have been shown in *Arabidopsis* to be greatly reduced at higher temperatures. Likewise, the barley MLA NB-LRR proteins accumulate to greatly reduced levels at high temperatures. These phenomena may at least partially explain the temperature sensitivity of some *R* genes.

A number of studies have shown that during a resistance response, kinase cascades are activated and there is a massive transcriptional reprogramming. Studies using virus-induced gene silencing (VIGS) have implicated several protein kinases, including MEK1, MEK2, NTF6, SIPK, WIPK, and NPK1, in *N* gene-mediated resistance. A requirement was also shown for the TGA and WRKY families of transcription factors as well as the individual transcription factors MYB1 and the TGA-interacting protein NPR1. These proteins and protein families have also been implicated in gene-for-gene resistance to other types of pathogens as well. Gene-for-gene resistance may also involve targeted proteolysis since silencing the expression of certain proteins involved in regulated protein degradation, including a subunit of the COP9 signalosome, SKP1 and COI1, also compromises *N*-mediated resistance. To date, the only genes whose silencing has been shown to break *Rx*-mediated resistance are *Hsp90* and *Sgt1*. Along with the Sgt1 binding protein Rar1, these gene products are required for the function of a number of NB-LRR proteins. The HSP90, SGT1, and RAR1 proteins are likely involved in chaperoning proteins in order to allow proper protein folding. Accordingly, these

proteins are required for the accumulation of a number of NB-LRR proteins.

Ethylene plays a role in *R*-gene-mediated antiviral responses. *RCY1* functions at reduced efficiency in *Arabidopsis* containing the ethylene-desensitizing mutations *ein3* and *etr1*. The phytohormone SA also plays a role in *R*-gene-mediated resistance. The involvement of SA is often studied using SA biosynthetic mutants in *Arabidopsis* (such as *eds5*) or by using plants transgenically expressing the bacterial *nahG* gene which encodes a salicylate hydroxylase that degrades SA. The *eds5* mutation and *nahG* transgene both decrease the efficiency of *RCY1*-mediated resistance and completely abrogate *HRT*-mediated resistance.

When tobacco plants expressing *N* and *nahG* are infected with TMV, the resulting HR lesions are larger and the necrosis can spread beyond the infected leaf. Similarly, in *nahG* transgenic tomato, the normally ER response of $Tm\text{-}2^2$ becomes a spreading HR phenotype in the inoculated leaf, although the virus does not spread systemically. The *Rx*-mediated response, however, is not affected by the *nahG* transgene. At the same time, SA has antiviral effects independent of *R*-gene-mediated resistance, such as causing the upregulation of an RNA-dependent RNA polymerase (RdRp) involved in RNAi-based antiviral responses. Thus, SA may enhance *R* gene responses rather than directly targeting virus and its requirement for resistance may depend on the strength of the *R*-gene-mediated response. In agreement with this, SA application results in reduced lesion size in the *N*-mediated response to TMV and an enhancement of *HRT*-mediated resistance. Cell death during the HR induces increased levels of SA. Thus, cell death may indirectly prime and/or augment *R*-gene-mediated antiviral responses. It is clear that cell death itself is not the only mechanism by which virus is eliminated as ER does not involve cell death, and in the *N*–TMV interaction, TMV can be detected outside the area of necrosis. Furthermore, in the interaction between cauliflower mosaic virus (CaMV) and *Nicotiana* spp., resistance is controlled by a single dominant gene. However, whether or not this resistance is accompanied by an HR is controlled by a separate gene. A clear understanding of how viruses are cleared from resistant plant cells will require further study.

See also: Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Plant Antiviral Defense: Gene Silencing Pathway; *Potexvirus*; *Tospovirus*; Cucumber Mosaic Virus; *Tobamovirus*; Virus Induced Gene Silencing (VIGS).

Further Reading

Belkhadir Y, Subramaniam R, and Dangl JL (2004) Plant disease resistance protein signaling: NBS-LRR proteins and their partners. *Current Opinion in Plant Biology* 7: 391–399.

Dangl JL and Jones JD (2001) Plant pathogens and integrated defence responses to infection. *Nature* 411: 826–833.

Harrison BD (2002) Virus variation in relation to resistance-breaking in plants. *Euphytica* 124: 181–192.

Hull R (2002) *Matthew's Plant Virology* 4th edn. San Diego, CA: Academic Press.

Kachroo P, Chandra-Shekara AC, and Klessig DF (2006) Plant signal transduction and defense against viral pathogens. *Advances in Virus Research* 66: 161–191.

Kang BC, Yeam I, and Jahn MM (2005) Genetics of plant virus resistance. *Annual Review of Phytopathology* 43: 581–621.

Martin GB, Bogdanove AJ, and Sessa G (2003) Understanding the functions of plant disease resistance proteins. *Annual Review of Plant Biology* 54: 23–61.

Ritzenthaler C (2005) Resistance to plant viruses: Old issue, news answers? *Current Opinion in Biotechnology* 16: 118–122.

Soosaar JL, Burch-Smith TM, and Dinesh-Kumar SP (2005) Mechanisms of plant resistance to viruses. *Nature Reviews Microbiology* 3: 789–798.

Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes

C Caranta and C Dogimont, INRA, Montfavet, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Allele One of two or more forms that can exist at a single gene locus, distinguished by their differing effects on the phenotype.

Dominant allele An allele that expresses its phenotypic effect even when heterozygous with a recessive allele.

Paralog Paralogs are genes related by duplication within a genome. Orthologs (i.e., genes in different species that evolved from a common ancestral gene) retain the same function in the course of evolution whereas paralogs generally evolve new functions.

Pleiotropic mutation A mutation that has effects on several different characters.

Positional cloning Cloning a gene based simply on knowing its position in the genome without any idea of the function of that gene.
Quantitative trait loci (**QTLs**) QTLs correspond to genomic regions associated with the phenotypic variation of a quantitative trait.
Recessive allele An allele whose phenotypic effect is not expressed in a heterozygote.
TILLING A reverse genetics approach that relies on the ability of a special enzyme to detect mismatches in normal and mutant DNA strands when they are annealed. It can therefore detect single point mutations of the type induced by chemicals conventionally used in mutation breeding programs.
Virulence Genetic ability of a pathogen to overcome genetically determined host resistance and cause a compatible (disease) interaction.

Introduction

A century after the recognition that plant resistance to diseases can be genetically inherited, breeding for naturally occurring resistance genes remains the most effective and sustainable approach to the prevention of diseases caused by plant viruses. Genetic resistance (also called host resistance or cultivar resistance) occurs when some genotypes possess heritable resistance to a particular pathogen whereas other genotypes in the same gene pool, generally at the species level, are susceptible.

According to genetic criteria, resistance can be controlled by one, two, or several genes corresponding to monogenic, digenic, and polygenic resistance, respectively. Usually, mono- and digenic resistances segregate as qualitative traits (for instance, presence vs. absence of viral accumulation in inoculated leaves or presence vs. absence of symptoms), whereas polygenic resistances behave as quantitative traits controlled by several resistance genes named quantitative trait loci (QTLs). Currently, QTL mapping is a standard procedure in quantitative genetics and begins with the collection of genotypic (based on molecular markers) and phenotypic data from a segregating population, followed by statistical analysis to reveal all possible marker loci where allelic variation correlates with the phenotype.

Resistance can show dominant or recessive inheritance and this feature appears closely related to underlying molecular mechanisms. According to the plant phenotype, resistance genes control complete or partial resistance. Partial resistance includes tolerance defined as presence of the virus in all parts of the plant without symptoms and/or yield loss. Anyway, host resistance can occur by complete or partial interruption at any of a number of stages of the virus life cycle including entry into the cell, expression and replication of the viral genome, and cell-to-cell and long-distance movements.

Resistance may be overcome by the appearance of resistance-breaking strains compromising the durability of resistance genes deployed in culture. Genetic analyses comparing resistance-breaking and non-resistance-breaking viral strains have permitted the identification of viral components (i.e., avirulence genes) responsible for this process and produced significant results regarding resistance mechanisms and the molecular basis of compatible interactions between plants and viruses.

This article reviews phenotypic and molecular characteristics related to recessive virus resistance factors, that is, major genes and QTLs identified in the natural diversity of plants.

Survey of Recessive Resistance Genes

Recessive resistance genes are more prevalent for resistance to viruses than for resistance to fungi, bacteria, nematodes, or insects for which resistance is primarily a dominant trait. As described later in this article, this feature appears related to intrinsic properties of viruses. A survey of published recessive resistance against viruses identified in the natural diversity of plants is presented in **Table 1**. Recessive resistance to viruses is especially well documented within the dicots, chiefly Solanaceae, Cucurbitaceae, and Fabaceae, which comprise a number of economically important vegetables. Within monocot species, recessive resistances have been reported in barley, rice, and maize. For such genes, it is also important to keep in mind that the presence of dominant resistance genes that can mask recessive ones (both against the same virus), and/or the highly heterozygous genome structure of species such as *Vitis vinifera*, *Prunus* sp., and cultivated potatoes renders difficult the detection of recessive resistance.

Recessive inheritance of resistance has been described for various viruses which belong to very different viral genera, with RNA as well as DNA genomes (**Table 1**). However, the frequency of recessive resistance can be to some extent related to the virus family or genus. A large majority of recessive resistance genes which have been described to date concern resistance to viruses belonging to the genus *Potyvirus* in a wide range of plant species. *Potyvirus* is the largest genus of plant viruses and includes some of the most common and destructive viruses for a number of cultivated crops worldwide. It is remarkable that approximately 50% of resistance to potyviruses show recessive inheritance, while, for instance, resistance to tobamoviruses, which are also widespread in various crops, is usually monogenic dominant, with a few exceptions such as the *tm* gene for resistance to tobacco mosaic virus in common bean. More generally, ~70% of recessive resistances listed in **Table 1** are efficient against RNA

Table 1 Recessive resistances controlled by major genes or QTLs to plant viruses

Genus (family)	*Virus*	*Host plant*	*Resistance gene(s) or loci*	*Resistance mechanism*
RNA viruses with a 5′ VPg and 3′poly(A) tail				
Bymovirus (*Potyviridae*)	Barley mild mosaic virus	*Hordeum vulgare* (barley)	*rym4, rym5* | about 15 *rym* genes mapped on five chromosomes	Complete resistance From complete to partial resistance
	Barley yellow mosaic virus	*Hordeum vulgare* (barley)	*rym4, rym5* | about 15 *rym* genes mapped on five chromosomes	Complete resistance From complete to partial resistance
	Barley yellow mosaic virus-2	*Hordeum vulgare* (barley)	*rym-5* *rym1, rym11*	Complete resistance
Potyvirus (*Potyviridae*)	Bean common mosaic virus	*Pisum sativum* (pea)	*bcm*	No systemic infection
		Phaseolus vulgaris (bean)	*bc-1, bc-1*^{2}, *bc-2*, *bc-2*^{2}, *bc-3*, *bc-u* (independent locus)	
	Bean yellow mosaic virus	*Phaseolus vulgaris* (bean)	*cyv*	
		Pisum sativum (pea)	*mo*	No systemic infection
		Vicia mungo (mungbean)	2 genes	No symptoms
	Bidens mottle virus	*Lactuca sativa* (lettuce)	*bi*	
	Blackeye cowpea mosaic virus	*Vigna unguiculata* (cowpea)	*bcm*	
	Celery mosaic virus	*Apium graveolens* (celery)	*cmv*	
	Clover yellow vein virus	*Phaseolus vulgaris* (bean)	*desc*	Resistance at a single cell level
		Pisum sativum (pea)	*cyv1* *cyv2* (independent locus)	
	Lettuce mosaic virus	*Lactuca sativa* (lettuce)	*mo1*^{1}, *mo1*^{2}	Reduced accumulation
	Moroccan watermelon mosaic virus	*Cucumis sativus* (cucumber)	*mwm*	No symptoms
	Papaya ringspot virus-W	*Cucumis sativus* (cucumber)	*prsv-1*	Reduced accumulation
		Cucurbita moschata (squash)	*prv*	No symptoms
	Passionfruit woodiness virus	*Pisum sativum* (pea)	*pwv*	Complete resistance
	Pea seedborne mosaic virus	*Lens culinaris* (lentil)	*sbv*	
		Pisum sativum (pea)	*sbm1, sbm3, sbm4* *sbm2* (independent locus)	Resistance at a single cell level
	Peru tomato virus	*Lycopersicon esculentum* (tomato)	Monogenic	
	Pepper mottle virus	*Capsicum annuum* (pepper)	*pvr3*	Systemic movement
		Capsicum chinense (pepper)	*pvr1*	Resistance at a single cell level
	Pepper veinal mottle virus	*Capsicum annuum* (pepper)	*pvr2*^{2} | *pvr6* (digenic)	Complete resistance
	Plum pox virus	*Arabidopsis thaliana*	*rpv1*	Restriction to long-distance movement
	Potato virus A	*Solanum tuberosum* subsp. *andigena*	ra_{adg}	Restriction of vascular movement

Continued

Table 1 Continued

Genus (family)	*Virus*	*Host plant*	*Resistance gene(s) or loci*	*Resistance mechanism*
	Potato virus Y	*Capsicum annuum* (pepper)	*pvr2*^{1}, *pvr2*^{2}	Resistance at a single cell level
			pvr3 (independent locus)	Complete resistance
			Several QTLs	Partial resistance
		Capsicum chinense (pepper)	*pvr1* (allelic to *pvr2*)	Resistance at single cell level
		Lycopersicum hirsutum (tomato)	*pot-1*	Complete resistance
		Nicotiana tabacum (tobacco)	*va*	Cell-to-cell movement
	Tobacco etch virus	*Capsicum annuum* (pepper)	*pvr2*^{2}	Resistance at a single cell level
		Capsicum chinense (pepper)	*pvr1*	Resistance at a single cell level
		Lycopersicum hirsutum (tomato)	*pot-1*	Complete resistance
		Nicotiana tabacum (tobacco)	2 genes linked	Systemic movement
	Tobacco vein mottling virus	*Nicotiana tabacum* (tobacco)	*va*	Cell-to-cell movement
	Turnip mosaic virus	*Brassica campestris* (cabbage)	2 genes	No systemic infection
		Brassica rapa (turnip)	From 1 to 3 genes depending on strains	No systemic infection
	Watermelon mosaic virus	*Cucumis melo* (melon)	monogenic	Reduced accumulation and systemic movement
		Cucumis sativus (cucumber)	*wmv-2*	No symptoms at cotyledon stage
			wmv-3 + *wmv-4* (digenic)	No symptoms at a true leaf stage
		Pisum sativum (pea)	*mo*	No systemic infection
	White lupin mosaic virus	*Pisum sativum* (pea)	*wlv*	
	Zucchini yellow mosaic virus	*Cucumis sativus* (cucumber)	*zym*	Reduced accumulation
	Zucchini yellow fleck virus	*Citrullus lanatus* (watermelon)	monogenic	No systemic infection
		Cucumis sativus (cucumber)	*zyf*	No systemic infection
Comovirus (*Comoviridae*)	Cowpea severe mosaic virus	*Vigna unguiculata* (cowpea)	Oligogenic (3 genes)	Complete resistance

RNA viruses with a 5′ VPg but no 3′poly(A) tail				
Enamovirus (*Luteoviridae*)	Bean leafroll virus	*Pisum sativum* (pea)	*lr*	No symptoms
Polerovirus (*Luteoviridae*)	Beet western yellows virus	*Lactuca sativa* (lettuce)	*bwy*	Reduced accumulation
	Cucurbit aphid-borne yellows virus	*Cucumis melo* (melon)	*cab-1* + *cab-2* (digenic)	Complete resistance
Sobemovirus	Rice yellow mottle virus	*Oryza sativa* (rice)	*rymv1*	Complete resistance
			Several QTLs	Partial resistance
		Oryza glaberrima (rice)	*rymv1*	Complete resistance
RNA viruses with capped 5′ end and non-polyadenylated 3′ end				
Alfamovirus (*Bromoviridae*)	Alfalfa mosaic virus	*Medicago sativa* (alfalfa)	*am-1*	
Bromovirus (*Bromoviridae*)	Cowpea chlorotic mottle virus	*Glycine max* (soybean)	2 genes	Restriction of systemic movement
Cucumovirus (*Bromoviridae*)	Cucumber mosaic virus	*Cucumis melo* (melon)	Polygenic with a major QTL	No symptoms
		Cucurbita pepo (pumpkin)	2 unlinked genes	No systemic symptoms
		Capsicum frutescens (pepper)	Oligogenic (2–3 genes)	No systemic infection
Tobamovirus	Cucumber green mottle mosaic virus	*Cucumis melo* (melon)	Polygenic	No symptoms
Tospovirus (*Bunyaviridae*)	Tomato spotted wilt virus	*Lycopersicon esculentum* (tomato)	sw2, sw3, sw4	Partial resistance
RNA viruses with unmodified 5′ and 3′ ends				
Carmovirus (*Tombusviridae*)	Turnip crinkle virus	*Arabidopsis thaliana*	*rrt* + *HRT* (digenic)	Hypersensitive response
	Melon necrotic spot virus	*Cucumis melo* (melon)	*nsv*	Resistance at a single cell level
Luteovirus (*Luteoviridae*)	Barley yellow dwarf virus	*Hordeum vulgare* (barley)	*ryd1*	Partial resistance
Idaeovirus	Raspberry bushy dwarf virus	*Rubus idaeus*	Monogenic	Complete resistance
Other RNA viruses (not well-characterized genome)				
Umbravirus	Groundnut rosette virus	*Arachis hypogaea* (peanut)	2 genes	Escape to infection/no symptoms
	Rice tungro spherical virus	*Oryza sativa* (rice)	*tsv-1*	No systemic infection
			tsv-2 (independent loci)	
DNA viruses				
Begomovirus (*Geminiviridae*)	Bean golden yellow mosaic virus	*Phaseolus vulgaris* (bean)	*bgm-1*	Reduced symptoms
			bgm-2 (independent loci)	
	Potato yellow mosaic virus	*Lycopersicon pimpinellifolium*	Bigenic	No systemic infection
	Tomato yellow leaf curl virus	*Lycopersicon hirsutum*	Oligogenic	Complete resistance
	Tomato chlorotic mottle virus	*Lycopersicon esculentum* (tomato)	Monogenic	No systemic infection
Curtovirus (*Genimiviridae*)	Beet curly top virus	*Arabidopsis thaliana*	Monogenic, two distinct loci in two ecotypes	Tolerance or no systemic infection
Tungrovirus (*Caulimoviridae*)	Rice tungro bacilliform virus	*Oryza sativa* (rice)	Polygenic	Tolerance, reduced virus accumulation

viruses with a viral protein genome-linked (VPg) bound to the 5′ end of their genome. Other recessive genes are involved in resistance to viruses with different genome organizations and different translation strategies. Resistance to several DNA viruses in the genus *Begomovirus*, viruses transmitted by whiteflies or planthoppers and responsible to very damageable diseases, also present monogenic recessive inheritance.

Resistance controlled by recessive genes involves various phenotypes from complete resistance expressed at the single cell level (as revealed by protoplast infection experiments) or during cell-to-cell or long-distance movements, to different levels of partial resistance, that is, reduced accumulation of the virus, reduced cell-to-cell or long-distance movement, and delayed symptoms or tolerance. Therefore, while most dominant resistances to viruses involve a hypersensitive response or an extreme resistance phenotype, recessive resistances show an important diversity of resistance phenotypes.

There are a number of examples of recessive resistance loci controlling resistance to different pathotypes of the same virus or distinct related viral species. One of the most remarkable examples has been described in pea (*Pisum sativum*), where recessive resistance genes against potyviruses have been mapped to two loci. The first locus includes *bcm* for resistance to bean common mosaic virus (BCMV), *cyv-1* for resistance to clover yellow vein virus (ClYVV), *mo* for resistance to pea mosaic virus, *sbm-2* for resistance to pea seedborne mosaic virus (PSbMV) pathotype L1, *pmv* for resistance to pea mosaic virus (PMV), and *wmv* for resistance to watermelon mosaic virus, while the second locus includes *sbm-1*, *sbm-3*, and *sbm-4*, which govern resistance to three PSbMV pathotypes, *cyv-2* for resistance to CYVV, and *wlv* for resistance to white lupin mosaic virus. Similarly in pepper, alleles at the *pvr2* locus control resistance to potato virus Y (PVY), tobacco etch virus (TEV), and pepper mottle virus (PepMoV). It is noticeable that all these viruses belong to the genus *Potyvirus*. Also in barley, a single recessive locus controls resistance to several bymoviruses, including barley mild mosaic virus, barley yellow mosaic virus, and barley yellow mosaic virus-2. The broad spectrum of these loci can be explained either by the action of one gene with a pleiotopic effect or by a cluster of tightly linked genes. In case of dominant resistance genes, clustering has been widely reported and was shown to correspond to duplicated or evolutionary related sequences of resistance genes with various resistance specificities against distinct pathogen groups (viruses, fungi, bacteria, etc.). Concerning recessive resistance genes against viruses and according to data obtained from the molecular cloning of these genes, broad-spectrum loci appear to result from the pleiotropic effect of a single gene (see next section).

Although most resistances with recessive inheritance are monogenic, there are several interesting examples where resistance alleles at two distinct loci are required to confer resistance. In *Phaseolus vulgaris*, resistance to several strains of BCMV requires the complementary effect of the *bc-u* locus and one at least of the strain-specific genes *bc-1*, *bc-2*, and *bc-3*. In *Capsicum*, resistance to the African potyvirus, pepper veinal mottle virus (PVMV), requires resistance alleles at the *prv2* and *pvr6* resistance loci. In melon, two complementary recessive genes are required to confer complete resistance to cucurbit aphid-borne yellows virus (genus *Polerovirus*). In the tomato-related wild species *Lycopersicon pimpinellifolium*, two recessive genes are also required to confer resistance to potato yellow mosaic virus (genus *Begomovirus*).

Relatively few QTLs for plant viral resistance have been genetically mapped and only in three cases they show a clear recessive inheritance. Several QTLs have been demonstrated to govern recessive resistance to PVY in pepper and to rice yellow mosaic virus (RYMV) in rice. In melon, quantitative resistance to cucumber mosaic virus (CMV) is conferred by a major-effect QTL explaining from 15% to 75% of the phenotypic variation depending on the strain, and also by several minor-effect QTLs. Phenotypically, virus resistance QTLs are usually revealed by a semi-quantitative scoring of symptom severity (that can be next used to calculate the area under the symptom progress curve –AUSPC) and/or a global evaluation of the virus content using enzyme-linked immunosorbent assays. However, these methods are difficult to develop on large-size progenies and do not permit accurate and quantitative resistance evaluations.

Molecular Structure and Mechanisms Underlying Recessive Resistance Genes

Fraser's Hypotheses

In 1990, R. S. S. Fraser proposed two hypotheses to explain the molecular mechanisms underlying recessive resistances to plant viruses. The first one, also called the 'negative model', suggests that resistance might be the consequence of the loss (null allele) or mutation of a specific host factor required by the virus to complete a particular step of its infectious cycle. Such resistance is likely to be passive, that is, it should never induce a hypersensitive nor a defensive response, and the dominant allele encodes a susceptibility factor. This hypothesis is in agreement with the fact that typical plant viruses encode relatively few proteins (between four and ten) and that they need therefore to reroute host cellular components to perform their infectious cycle. The compatibility between a plant and a virus therefore depends on complex interplays between functions encoded by the viral and host genomes. Conversely, the second hypothesis proposes that resistance might be the result of an active mechanism, involving a resistance allele encoding an inhibitor that

interferes with the virus life cycle. In this case, the susceptibility allele encodes a dominant negative regulator of resistance. This hypothesis has been documented in the case of *mlo*, associated in barley with the recessive resistance against powdery mildew, caused by *Erysiphe graminis*.

The Key Role of Translation Initiation Factors in Recessive Resistance

The molecular cloning in 2002 of the first naturally occurring recessive resistance gene against a virus has validated the first hypothesis by demonstrating that mutations in the eukaryotic translation initiation factor 4E (eIF4E) are responsible of *pvr2*-mediated recessive resistance of pepper to PVY and TEV. Since then, although many host factors are required for plant virus interactions, all studies aimed at the characterization of recessive resistance genes from a range of plant species have so far only identified eIF4E and eIF4G proteins from the translation initiation complex.

In eukaryotic cells, translation initiation factor 4E binds to the m^7G cap as the first step in recruiting mRNA into the translation initiation complex. eIF4E is also associated with eIF4G to form the eIF4F complex. eIF4G is a scaffold for the other components of the complex and interacts with several other initiation factors like poly(A)-binding protein (PABP), eIF4A, a helicase for unwinding secondary structures, and eIF3, a multisubunit complex that binds to the 40S ribosomal subunit (**Figure 1**). In plants, two isoforms of eIF4F are present and appear to have complementary roles: eIF4F, containing eIF4F and eIF4G, and eIF(iso)4F, containing eIF(iso)4E and eIF(iso)4G. Although the two complexes are considered equivalent for *in vitro* translation of some mRNAs, they differ in their *in vivo* expression pattern and show some specificity for different capped cellular mRNAs. Plant genes encoding proteins from the eIF4F complex belong to small gene families. For instance, in *Arabidopsis thaliana*, three genes code for proteins of the eIF4E subfamily (i.e., *eIF4E1*, *eIF4E2*, and *eIF4E3*), one gene codes for eIF(iso)4E, one gene codes for eIF4G, and two genes code for proteins of the eIF(iso)4G subfamily (*eIF(iso)4G1* and *eIF(iso)4G2*). Although the structure of these gene families appears less complex in mono- than in dicotyledons, several loci have been identified in all species studied.

Among the eight natural recessive resistance genes against plant viruses which have been cloned in a range of plant species including both mono- and dicotyledons (pepper, lettuce, pea, tomato, barley, rice, melon), six code for eIF4E, one for its paralog eIF(iso)4E, and one for eIF(iso)4G (**Table 2**). All these resistances function against RNA viruses and the majority (five out of eight) against potyvirus infection. eIF4E has also been implicated in barley resistance to a bymovirus (a genus related to potyviruses) and in melon resistance to a carmovirus, and eIF(iso)4G has been implicated in rice resistance to a sobemovirus.

Characteristics of eIF4E-Mediated Resistance

Except for the *pvr6* gene, all resistances result from a small number of amino acid changes in the protein encoded by the recessive resistance allele. Concerning eIF4E-mediated resistance against potyviruses or related

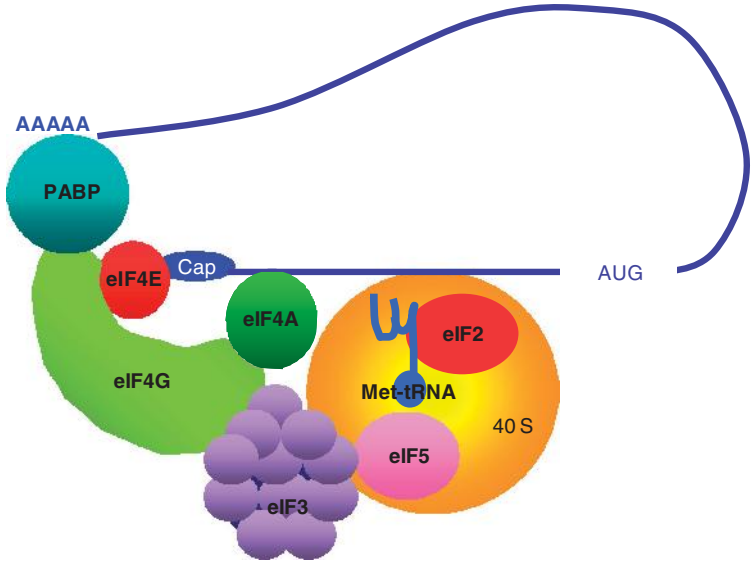


Figure 1 The eukaryotic translation initiation complex. eIF, eukaryotic initiation factor; PABP, poly(A)-binding protein; 40S, 40S ribosomal subunit. Reproduced from Robaglia C and Caranta C (2006) Translation initiation factors: A weak link in plant RNA virus infection. *Trends in Plant Science* 11(1): 40–45, with permission from Elsevier.

Table 2 Characteristics of cloned recessive resistance genes against plant RNA viruses

Genus	*Virus*	*Gene/alleles*	*Species*	*Translation factor*	*Avr gene*
Potyvirus	Potato virus Y	*pvr2*^{1}	*Capsicum annuum*	eIF4E1	VPg
	Potato virus Y and tobacco etch virus	*pvr2*^{2}	*Capsicum annuum*	eIF4E1	VPg
	Potato virus Y and tobacco etch virus and pepper mottle virus	*pvr1*	*Capsicum chinense*	eIF4E1	
Potyvirus	Pepper veinal mottle virus	*pvr6* (+*pvr2*)	*Capsicum annuum*	eIF(iso)4E	
Potyvirus	Lettuce mosaic virus	*mo1*^{1}	*Lactuca sativa*	eIF4E1	CI, VPg, NIa region
	Lettuce mosaic virus	*mo1*^{2}	*Lactuca sativa*	eIF4E1	CI, VPg, NIa region
Potyvirus	Pea seedborne mosaic virus	*sbm1*	*Pisum sativum*	eIF4E1	VPg
Potyvirus	Potato virus Y and tobacco etch virus	*pot-1*	*Lycopersicon hirsutum*	eIF4E1	VPg
Bymovirus	Barley mild mosaic virus and barley yellow mosaic virus	*rym4*	*Hordeum vulgare*	eIF4E1	VPg
	Barley mild mosaic virus and barley yellow mosaic virus and barley yellow mosaic virus-2	*rym5*	*Hordeum vulgare*	eIF4E1	VPg
Sobemovirus	Rice yellow mottle virus	*rymv1*	*Oryza sativa*	eIF(iso)4G	VPg
Carmovirus	Melon necrotic spot virus	*nsv*	*Cucumis melo*	eIF4E1	3′ UTR

bymoviruses in pepper, tomato, lettuce, pea, and barley, most of the resistance-related changes correspond to nonconservative amino acid substitutions and are clustered in two neighboring regions at the surface of the predicted eIF4E three-dimensional structure. In melon, a single amino acid change mapped to the C-terminal part of eIF4E on an accessible region of the protein leads to resistance to MNSV. Amino acid substitutions responsible for resistance of rice to RYMV were also mapped to a surface location of eIF(iso)4G. Collectively these data argue in favor of resistance mechanism(s) mediated by subtle amino acid change(s) on the surface interaction of translation initiation factors.

Resistance genes against potyviruses and bymoviruses control multiple specificities targeted against distinct viruses of the same genus or pathotypes of the same virus (see section above). In pepper, lettuce, and pea, these resistance specificities were explained by the occurrence of small allelic series where distinct alleles were represented as a combination of distinct amino acid substitutions.

An intriguing aspect of eIF4E-mediated resistances is that they cover a diverse range of resistance phenotypes. Although most of them control complete qualitative resistance, they can also be quantitative and/or are components of polygenic resistance. For instance, in pepper, an eIF4E resistance allele at the *pvr2* locus controls a partial resistance to some pathotypes of PVY and was initially mapped as a QTL. Similarly, in lettuce, two eIF4E resistance alleles, *mo1*^{1} and *mo1*^{2}, appear associated with absence of accumulation of lettuce mosaic virus (LMV) at the cellular level, or to reduced accumulation of LMV and lack of symptoms, probably depending on other loci in the host genome. It was proposed that these distinct phenotypes might represent distinct outcomes of the same mechanism playing at quantitatively different intensities.

Among eIF4E-mediated resistances, the *pvr6* resistance gene from pepper is of particular interest. It is involved in resistance to PVMV only when combined with some *pvr2* resistance alleles (demonstrated to code for eIF4E), whereas *pvr6* and *pvr2* do not separately confer any resistance to PVMV. Moreover, *pvr6* alone has no detectable effect on the potyvirus infection process. A recent study showed that the *pvr6* resistance allele encodes a nonfunctional truncated eIF(iso)4E protein and that complete resistance to PVMV results from the combined effect of mutations in translation initiation factors eIF4E and eIF(iso)4E. Therefore, while most potyviruses require one specific eIF4E isoform to achieve infection, PVMV can use both 4E and (iso)4E isoforms.

A further common feature linking eIF4E-mediated resistance against potyviruses and also eIF(iso)4G-mediated resistance against RYMV is that regions of the viral protein named VPg (viral protein genome-linked; a protein that is covalently attached to the 5′ end of the viral RNA) have been mapped as avirulence determinants (i.e., involved in the overcoming of resistance) (**Table 2**). In this regard, resistance in melon to MNSV (lacking a VPg) differs because the viral avirulence determinant corresponding to *eIF4E-nsv* resistance gene was located at the 3′ untranslated region (3′ UTR) of the viral genome.

Natural versus Induced eIF4E-Mediated Resistance

Interestingly, *Arabidopsis thaliana* mutant lines (ethyl methane sulfonate (EMS)-induced mutations or transposon

knockout) of the same genes from the translation initiation complex also exhibit complete or partial resistance phenotypes against RNA viruses, including several potyviruses, and also CMV and turnip crinkle virus (TCV). Similar to naturally occurring resistance alleles, single mutant alleles in *Arabidopsis* confer resistance to diverse related viruses in the genus *Potyvirus*. However, it is striking that a particular virus strain able to infect both *Arabidopsis* and crops appears not to be dependent on the same eIF4E isoforms in different hosts. For instance, TEV and LMV depend on eIF4E1 to infect pepper, tomato, or lettuce, whereas the same viruses depend on eIF(iso)4E to infect *Arabidopsis*.

Another feature linking analysis of natural and induced resistance is the ability of potyviruses to selectively recruit eIF4E isoforms. Indeed, disruption of *eIF(iso)4E* either by point mutations or by T-DNA insertion leads to *Arabidopsis*' lack of susceptibility to four different potyviruses (TEV, LMV, turnip mosaic virus (TuMV), and plum pox virus (PPV)), whereas disruption of *eIF4E1* leads to resistance to a fifth potyvirus, ClYVV. Recently, a crucial role of the eIF4G factors in potyvirus resistance/susceptibility has also been demonstrated by phenotyping *Arabidopsis* T-DNA insertion mutants that are null allele for each of the three *eIF4G* genes: the eIF(iso)4G1 mutant is resistant to PPV and LMV; the eIF4G mutant is resistant to ClYVV; and the double-mutant eIF(iso)4G1 X eIF (iso)4G2 is resistant to TuMV. Altogether these results indicate that eIF4G isoforms are selectively recruited by different potyviruses in a fashion that parallels the selective recruitment of eIF4E isoforms, suggesting the involvement of the whole eIF4F complex in potyvirus infection.

A very striking feature is that despite the key role of components of the eIF4F complex in *Arabidopsis*–RNA virus interactions, eIF4E- or eIF4G-mediated resistance was never identified in the natural diversity of this species. Several studies identified recessive resistance genes against viruses in different ecotypes of *Arabidopsis* (**Table 1**) but, up to now, none encodes eIF4F factors. Similarly, the systematic sequencing of *eIF4E* genes in a core collection of 54 ecotypes of *Arabidopsis* failed to identify signature amino acid substitutions previously demonstrated to be responsible for virus resistance in crops. Collectively, these data point toward a distinct mode of evolution of resistance against RNA viruses in this model species in comparison with crops.

Molecular Mechanism(s) Underlying eIF4E-Mediated Resistance

In link with their high frequency in the natural diversity of plant species, molecular mechanisms underlying eIF4E-mediated resistance have been studied most extensively for potyviruses. A key observation was the ability of eIF4E isoforms to bind the potyviral VPg in yeast two-hybrid and in *in vitro* binding assays. As described previously, the VPg was identified as the virulence determinant for several plant–potyvirus pairs. Moreover, amino acids within the central and C-terminal parts of the PVY VPg have undergone positive selection suggesting that the role of the VPg encoding region in virulence is determined by the protein and not by the viral RNA. A key role for the eIF4E–VPg interaction in the virus cycle is also supported by the demonstration that a mutation in the VPg that abolishes the interaction with eIF4E *in vitro* also prevents viral infection *in planta*. These data, together with signature amino acid substitutions identified at the surface of proteins encoded by resistance eIF4E alleles, argue in favor of a resistance mechanism mediated by disruption or impairing of a direct protein–protein interaction of eIF4E and VPg. This is supported by the recent demonstration that eIF4E proteins encoded by *pvr1* and *pvr2*^{2} resistance alleles from *Capsicum* failed to bind the VPg from TEV strains (by yeast two-hybrid and GST pull-down), whereas eIF4E proteins encoded by the susceptibility alleles interacted strongly. However, the fact that the outcome of the eIF4E–VPg interaction is the determinant of viral infectivity remains uncertain for some other plant–potyvirus pairs. This might be related to the methods used to detect eIF4E/VPg interactions and/or to the occurrence of distinct mechanism(s) not dependent on this sole interaction.

In spite of these data, the precise role of eIF4E and its interaction with the viral VPg has yet to be defined. In eukaryotes, most cellular RNAs contain both a 5′ cap and a 3′ polyA tail that act synergistically to stimulate translation (**Figure 1**). Most positive-sense single-stranded RNA viruses lack either one or both of these structures. For members of the family *Potyviridae*, which have a VPg protein covalently attached to the 5′ end instead of a cap structure, the VPg might function as a cap mimic and recruit the translation initiation complex to the viral RNA. Inconsistent with this hypothesis are the demonstrations that the 5′ UTRs of TEV and TuMV support internal initiation via the recruitment of eIF4G to an internal ribosome entry site (IRES) and that significant translation of potyviral RNA appears possible in the absence of the covalently bound VPg. However, it cannot be completely ruled out that the eIF4E–VPg interaction facilitates and/or enhances cap-independent translation of the viral genome. Several other possible roles for eIF4E during the potyviral life cycle have also been proposed. The eIF4E–VPg interaction might interfere with cap-dependent translation of host mRNA, freeing ribosomes for viral RNA translation. It might also play a role in genome replication in a manner similar to the replication of mammalian picorna-like viruses. In support of this model, potyviral VPg urydylation by the viral polymerase (NIb) has been demonstrated and the existence

of complexes with VPg, NIb, eIF4E, and PABP has also been shown. For *sbm1*-mediated resistance to PsBMV, data also suggested that eIF4E facilitates cell-to-cell movement of the virus. Altogether, these results suggest that distinct mechanisms might be responsible of eIF4E-mediated resistance against potyviruses.

The distinctive features of eIF4E-mediated resistance against MNSV in melon (i.e., resistance controlled by a single amino acid change in the C-terminal part of eIF4E and avirulence determinant located at the 3′ UTR of the viral genome) associated with the fact that RNA of carmoviruses neither possesses a cap or a VPg at the 5′ end, nor a poly(A) tail at the 3′ end, strongly argue in favor of a resistance mechanism distinct from those against potyviruses. The authors of this work speculated that the MNSV avirulence determinant in the 3′ UTR interacts with eIF4E acting as a 5′ cap substitute, bringing the eIF4F complex to the 5′ UTR through a 5′–3′ RNA–RNA interaction. Indeed, they identified in the MNSV 3′ UTR, outside the avirulence determinant, nucleotide stretches with complementarities to nucleotides of a conserved loop in the MNSV 5′ UTR. This feature suggests that MNSV RNA initiation of translation might occur, as for other members of the family *Tombusviridae*, through a 5′–3′ RNA–RNA interaction that circularizes the message and involves eIF4E.

Conclusions

More than one hundred recessive resistance factors against plant viruses were identified in the natural diversity of crops and their wild relatives, among which a large number are currently used to control agricultural losses to viral diseases. The data shown in **Table 1** suggest strong biases toward resistance factors against RNA viruses (~90%) and host responses that show monogenic inheritance (~95%). Recent studies carried out to identify the molecular nature of recessive resistances genes against plant viruses have revealed a remarkable degree of conservation. All genes isolated and characterized to date encode eIF4E or eIF4G factors from the translation initiation complex. Their identification as essential susceptibility factors for RNA virus multiplication has enabled plant virology to make a significant step forward.

In future, the same type of recessive resistance genes is likely to be discovered in other plant families via candidate gene approaches, at least for viral genera for which translation initiation factors were demonstrated to play a key role in plant resistance (**Table 2**). More generally, the candidate gene approach should permit to examine the extent to which eIF4E and eIF4G factors, and also other factors from the translation complex, may serve as candidates for recessive resistance genes. It can also be anticipated that the molecular characterization of recessive resistances using approaches without presupposition on the molecular nature of the gene, such as positional cloning, will lead to the identification of new resistance targets that function as host susceptibility factors. Another promising approach is the identification of host proteins that interact with viral genes or gene products during infection through protein–protein interaction technologies and proteomics. Once the resistance genes are identified, it will be possible to discover novel alleles by surveying natural genetic variants using high-throughput specific marker technology. When such resistance alleles are not available in the natural diversity, it is possible to select plants carrying artificially induced mutations in a target gene through a reverse genetics technology termed TILLING (targeting induced local lesions in genomes). Overall, the characterization of new recessive resistance genes and probably of new susceptibility factors will provide plant biology with novel views on fundamental plant–virus interaction processes. Such knowledge will also significantly contribute to crop improvement for virus resistance through a diversification of genetic targets.

See also: Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes.

Further Reading

Colbert T, Till BJ, Tompa R, *et al.* (2001) High-throughput screening for induced point mutations. *Plant Physiology* 126: 480–484.

Diaz-Pendon JA, Truniger V, Nieto C, *et al.* (2004) Advances in understanding recessive resistance to plant viruses. *Molecular Plant Pathology* 5(3): 223–233.

Dreher TD and Miller AW (2006) Translational control in positive strand RNA plant viruses. *Virology* 344: 185–197.

Fraser RSS (1990) The genetics of resistance to plant viruses. *Annual Review of Phytopathology* 28: 179–200.

Kang B-C, Yeam I, and Jahn MM (2005) Genetics of plant virus resistance. *Annual Review of Phytopathology* 43: 18.1–18.41.

Maule AJ, Caranta C, and Boulton MI (2007) Sources of natural resistance to plant viruses: Status and prospects. *Molecular Plant Pathology* 8: 223–231.

Nieto C, Morales M, Orjeda G, *et al.* (2006) An eIF4E allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. *Plant Journal* 48: 1–11.

Robaglia C and Caranta C (2006) Translation initiation factors: A weak link in plant RNA virus infection. *Trends in Plant Science* 11(1): 40–45.

Thivierge K, Nicaise V, Dufresne PJ, *et al.* (2005) Plant virus RNAs. Coordinated recruitment of conserved host functions by (+)-ssRNA viruses during early infection events. *Plant Physiology* 138: 1822–1827.

Plant Rhabdoviruses

A O Jackson, University of California, Berkeley, CA, USA
R G Dietzgen, The University of Queensland, St. Lucia, QLD, Australia
R-X.Fang, Chinese Academy of Sciences, Beijing, People's Republic of China
M M Goodin, University of Kentucky, Lexington, KY, USA
S A Hogenhout, The John Innes Centre, Norwich, UK
M Deng, University of California, Berkeley, CA, USA
J N Bragg, University of California, Berkeley, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The plant rhabdoviruses have distinctive enveloped bacilliform or bullet-shaped particles and can be distinguished based on whether they replicate and undergo morphogenesis in the cytoplasm or in the nucleus. Consequently, they have been separated into two genera, *Cytorhabdovirus* or *Nucleorhabdovirus*. More than 90 putative plant rhabdoviruses have been described although, in many cases, molecular characterizations necessary for unambiguous classification are incomplete or lacking. Recent analyses indicate that the eight sequenced plant rhabdoviruses have the same general genome organization as other members of the *Rhabdoviridae*, but that each encodes at least six open reading frames (ORFs), one of which probably facilitates cell-to-cell movement of the virus. Thus, plant rhabdoviruses have a number of similarities to members of other rhabdovirus genera, but they differ in several respects from rhabdoviruses infecting vertebrates.

Rhabdoviruses infect plants from a large number of different families, including numerous weed hosts and several major crops. Symptoms of infection vary substantially and range from stunting, vein clearing, mosaic and mottling of leaf tissue, to tissue necrosis. The most serious pathogens include maize mosaic virus (MMV), lettuce necrotic yellows virus (LNYV), rice yellow stunt virus (RYSV), also known as rice transitory yellowing virus (RTYV), eggplant mottled dwarf virus (EMDV), strawberry crinkle virus (SCV), potato yellow dwarf virus (PYDV), and barley yellow striate mosaic virus (BYSMV), which is synonymous with maize sterile stunt virus (MSSV), and wheat chlorotic streak virus (WCSV). A number of other rhabdoviruses also have disease potential that can be affected by agronomic practices, incorporation of genes for disease resistance, and control of insect vectors.

The spread of most plant rhabdoviruses is dependent on specific transmission by phytophagous insects that support replication of the virus, so their prevalence and distribution is influenced to a large extent by the ecology and host preferences of their vectors. Although some rhabdoviruses can be transmitted mechanically by abrasion of leaves, this mode of transmission does not contribute significantly to their natural spread due to the labile nature of the virion. Moreover, seed or pollen transmission of plant rhabdoviruses has not been described; thus, aside from vegetative propagation, direct plant-to-plant transmission is unlikely to be a major factor in the ecology or epidemiology of these pathogens.

This article focuses on recent findings concerning the taxonomy, structure, replication, and vector relationships of plant rhabdoviruses. More extensive aspects of plant rhabdovirus biology, specifically ecology, disease development and control, can be found in earlier reviews.

Taxonomy and Classification

The International Committee on Taxonomy of Viruses (ICTV) has used subcellular distribution patterns to assign plant rhabdoviruses to the genera *Cytorhabdovirus* and *Nucleorhabdovirus* (**Table 1**). Currently the ICTV has assigned eight virus species (BYSMV, *Broccoli necrotic yellows virus* (BNYV), *Festuca leaf streak virus* (FLSV), LNYV, *Northern cereal mosaic virus* (NCMV), *Sonchus virus* (SV), SCV, and *Wheat American striate mosaic virus* (WASMV)) to the genus *Cytorhabdovirus* and seven viruses (*Datura yellow vein virus* (DYVV), *Eggplant mottled dwarf virus* (EMDV), MMV, PYDV, RYSV, *Sonchus yellow net virus* (SYNV), and *Sowthistle yellow vein virus* (SYVV)) to the genus *Nucleorhabdovirus*. However, sufficient new information has been documented to justify provisional inclusion of the recently described maize fine streak virus (MFSV) and taro vein chlorosis virus (TaVCV) in the genus *Nucleorhabdovirus* (**Table 1**). Cereal chlorotic mottle virus (CCMoV) has also been provisionally included in the genus *Nucleorhabdovirus* based on its intracellular distribution and serology. The complete genomic sequences have been determined for three cytorhabdoviruses, LNYV, NCMV, and SCV and for five nucleorhabdoviruses, MFSV, MMV, RYSV, SYNV, and TaVCV. Phylogenetic analyses of these rhabdoviruses have confirmed their taxonomic classification. Most other plant rhabdoviruses have not been investigated in much detail beyond cursory infectivity studies, crude physicochemical analyses of virus particles, and electron microscopic observations of morphogenesis. Consequently, more than 75 putative rhabdoviruses await assignment to a genus (**Table 1**).

Table 1 List of plant rhabdoviruses and their host and vector specificity

Virus	*Host*	*Vector*
Cytorhabdovirus		
Barley yellow striate mosaic virus (BYSMV)	M	P
[Maize sterile stunt virus]		
[Wheat chlorotic streak virus]		
Broccoli necrotic yellows virus (BNYV)	D*	A
Festuca leaf streak virus (FLSV)	M	
Lettuce necrotic yellows virus (LNYV)	D*	A
Northern cereal mosaic virus (NCMV)	M	P
Sonchus virus (SonV)	D*	
Strawberry crinkle virus (SCV)	D*	A
Wheat American striate virus (WASMV)	M	L
[Oat striate mosaic virus]		
Nucleorhabdovirus		
Cereal chlorotic mottle virus (CCMoV)	M	L
Datura yellow vein virus (DYVV)	D	
Eggplant mottled dwarf virus (EMDV)	D*	L
[Pittosporum vein yellowing virus]		
[Tomato vein yellowing virus]		
[Pelargonium vein clearing virus]		
Maize fine streak virus (MFSV)	M	L
Maize mosaic virus (MMV)	M	P
Potato yellow dwarf virus (PYDV)	D*	L
Rice yellow stunt virus (RYSV)	M	L
[Rice transitory yellowing virus]		
Sonchus yellow net virus (SYNV)	D*	A
Sowthistle yellow net virus (SYVV)	D	A
Taro vein chlorosis virus (TaVCV)	M	
Unassigned Plant Rhabdoviruses		
Asclepias virus	D	
Atropa belladonna virus	D	
Beet leaf curl virus	D	LB
Black current virus	D	
Broad bean yellow vein virus	D	
Butterbur virus	D*	
Callistephus chinensis chlorosis virus	D	
Caper vein yellowing virus	D	
Carnation bacilliform virus	D	
Carrot latent virus	D	A
Cassava symptomless virus	D	
Celery virus	D	
Chondrilla juncea stunting virus	D	
Chrysanthemum vein chlorosis virus	D	
Clover enation (mosaic) virus	D	
Colocasia bobone disease virus	D	P
Coriander feathery red vein virus	D*	A
Cow parsnip mosaic virus	D*	
Croton vein yellowing virus	D*	
Cucumber toad skin virus	D	
Cynara virus	D*	
Cynodon chlorotic streak virus	M	P
Daphne mezereum virus	D	
Digitaria striate virus	M	P
Euonymus fasciation virus	D	
Euonymus virus	D	
Finger millet mosaic virus	M	P
Gerbera symptomless virus	D	
Gloriosa fleck virus	D	
Gomphrena virus	D*	
Gynura virus	D	
Holcus lanatus yellowing virus	M	
Iris germanica leaf stripe virus	M	
Ivy vein clearing virus	D*	
Kenaf vein-clearing virus	D	
Laburnum yellow vein virus	D	
Launea arborescens stunt virus	D	
Lemon scented thyme leaf chlorosis virus	D	
Lolium ryegrass virus	M	
Lotus stem necrosis	D	
Lotus streak virus	D	A
Lucerne enation virus	D	A
Lupin yellow vein virus	D	
Maize Iranian mosaic virus	M	P
Maize streak dwarf virus	M	P
Malva sylvestris virus	D	
Meliotus (sweet clover) latent virus	D	
Melon variegation virus	D	
Mentha piperita virus	D	
Nasturtium vein banding virus	D	
Papaya apical necrosis virus	D	
Parsely virus	D*	
Passionfruit virus	D	
Patchouli mottle virus	D	
Peanut veinal chlorosis virus	D	
Pigeon pea proliferation virus	D	L
Pinapple chlorotic leaf streak virus	M	
Pisum virus	D*	
Plantain mottle virus	M	
Poplar vein yellowing virus	D	
Ranunculus repens symptomless virus	D	
Raphanus virus	D*	
Raspberry vein chlorosis virus	D	A
Red clover mosaic virus	D	
Sainpaulia leaf necrosis virus	D	
Sambucus vein clearing virus	D	
Sarracenia purpurea virus	D	
Sorghum stunt mosaic virus	M	L
Soursop yellow blotch virus	D	
Soybean virus	D	
Triticum aestivum chlorotic spot virus	M	
Vigna sinensis mosaic virus	D	
Viola chlorosis virus	D	
Wheat rosette stunt virus	M	P
Winter wheat Russian mosaic virus	M	P

Names in brackets are synonymous to those immediately above. Host: D, dicot; M, monocot. (*) indicates ability to be mechanically transmitted. Vectors: A, aphid, L, leafhopper; LB, lacebug; P, planthopper. Blank spaces indicate that no insect vector has been identified.

Particle Morphology and Composition

Plant rhabdoviruses are normally bacilliform after careful fixation (**Figure 1(a)**) and estimates of their sizes range from 45 to 100 nm in width and 130 to 350 nm in length. The outer layer consists of 5–10 nm surface projections that appear to be composed of G protein trimers that

penetrate a host-derived membrane (**Figure 1(b)**). The nucleocapsid core is composed of the genomic RNA, the nucleocapsid protein (N), the phosphoprotein (P), and the L polymerase protein (**Figures 1(a)** and **1(b)**). Rhabdovirus virions also contain a matrix protein (M) that interacts with the G protein to stabilize the particle. A sixth protein (sc4) is associated with the membrane fractions of SYNV particles but the presence of an sc4 derivative has not been found in virions of other plant rhabdoviruses.

The overall chemical composition (~70 % protein, 2 % RNA, 20–25% lipid, and a small amount of carbohydrate associated with the G protein) of the plant and animal rhabdoviruses is similar. The minus-sense RNA genomes of plant rhabdoviruses, which range in size from ~11 to 14 kb based on sedimentation, gel electrophoretic analyses, and genome sequencing, are slightly larger than those of most described animal rhabdoviruses. The lipids of plant and animal rhabdoviruses consist of fatty acids and sterols that are derived from sites of morphogenesis. The four sterols predominating in SYNV closely approximate sterols in the nuclear envelope, whereas those of NCMV, a cytorhabdovirus, are more typical of cytoplasmic membranes.

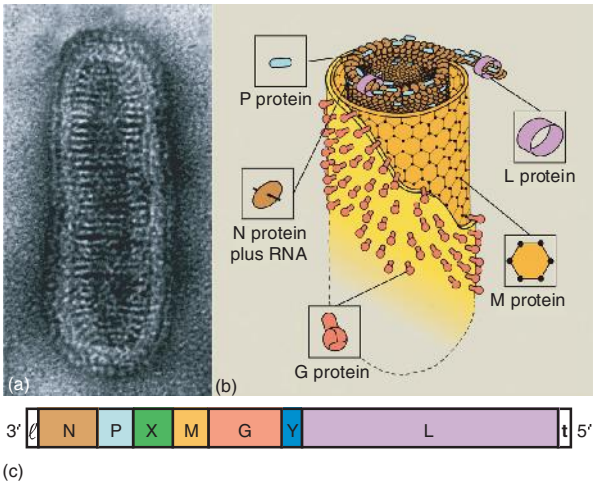


Figure 1 Electron micrograph, diagram, and genome organization of plant rhabdoviruses. (a) Transmission electron micrograph of a negative-stained virus showing the striated inner core, envelope, and glycoprotein spikes. (b) Architecture of the virus particle. The nucleocapsid core is composed of the minus-sense genomic RNA, the nucleocapsid protein (N), the phosphoprotein (P), and the polymerase protein (L). The matrix protein (M) is involved in coiling the nucleocapsid, attachment of the nucleocapsid to the envelope, and associations with the transmembrane glycoprotein (G). (c) Schematic representation of the negative-sense arrangement of genes encoded in the genomes of plant rhabdoviruses. The order of the genes is 3′-ℓ-N-P-X-M-G-Y-L-**t**-5′, where ℓ represents the leader RNA, **t** represents the trailer sequence, X denotes putative movement and undefined plant rhabdovirus genes, and Y shows the location of open reading frames of unknown function in the genomes of several plant and animal rhabdoviruses. Reprinted, with permission, from the Annual Review of Phytopathology, Volume 43, © 2005 by Annual Reviews.

Genomic Structure and Organization

The consensus plant rhabdovirus genome deduced from the eight sequenced viruses is 3-ℓ-N-P-X-M-G-Y-L-**t**-5′ (**Figure 1(c)**). The N, P, M, G, and L genes appear in the same order as in other rhabdoviruses and their encoded proteins are thought to be functionally similar to the five proteins of vesicular stomatitis virus (VSV). A variable number of genes at the X site have been found in each of the sequenced viruses and some of these appear to be involved in movement. The Y sites between the G and L genes encode short ORFs of unknown functions that are present in the nucleorhabdovirus, RYSV, and the cytorhabdoviruses, NCMV and SCV.

Rhabdovirus ORFs are separated by intergenic or 'gene-junction sequences' that provide vital regulatory functions during transcription and replication (**Figure 2(a)**). The gene-junction sequences can be grouped into three elements consisting of (1) a poly (U) tract at the 3′ end of each gene, (2) a variable intergenic element that is not transcribed in the mRNAs, and (3) a short element complementary to the first 5 nt at the 5 start site of each mRNA. In general, the gene-junction sequences of each virus are highly conserved, and those of the plant rhabdoviruses share substantial relatedness and differ mostly at element II. Slightly more limited divergence is noted when comparing the genomes of other families within the order *Mononegavirales*, suggesting that these regulatory sequences have been stringently conserved.

The coding regions are flanked by 3 leader (ℓ) and 5 trailer (**t**) noncoding sequences that represent recognition signals required for nucleocapsid assembly and regulation of genomic and antigenomic RNA replication. These sequences have short complementary termini and small amounts of common sequence relatedness (**Figure 2(b)**). However the plant rhabdovirus ℓ RNAs differ in sequence from the ℓ and **t** sequences of vertebrate rhabdoviruses and are considerably longer than those of VSV. The transcribed ℓ RNA of SYNV is polyadenylated and differs in this respect from the ℓ RNA of VSV and other known rhabdoviruses.

Properties of the Encoded Proteins

The most comprehensive biochemical analyses of the encoded proteins have been carried out with SYNV, LNYV, and RYSV. Overall, the plant rhabdovirus proteins have very little sequence relatedness to analogous proteins of animal rhabdoviruses, with the exception of

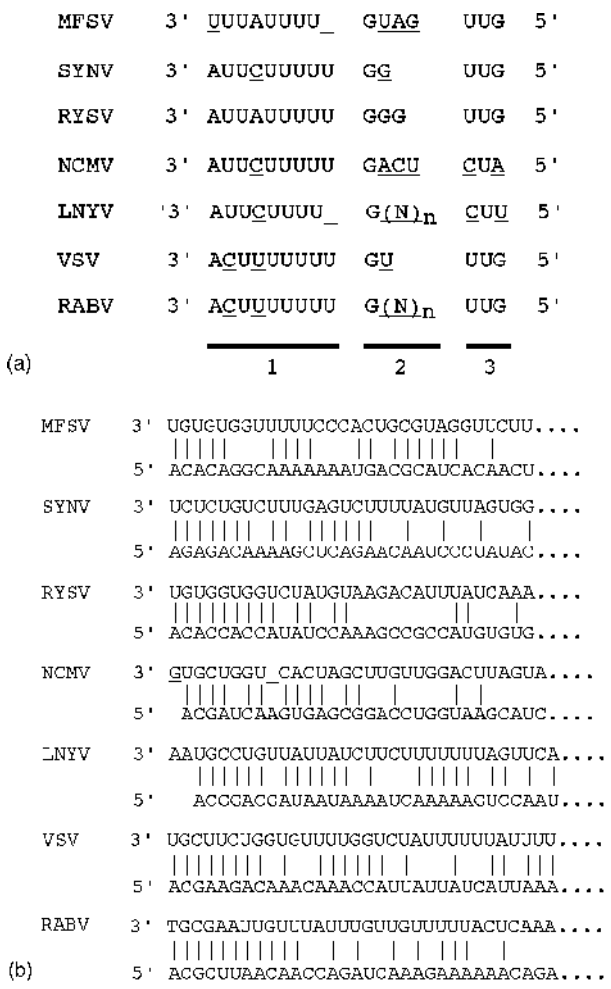


Figure 2 Comparisons of intergenic and terminal noncoding regions of rhabdovirus genomes. (a) Intergenic sequences separating the genes. (b) Complementary sequences at the 3′ and 5′ termini of the genomic RNAs. MFSV (maize fine streak virus), SYNV (sonchus yellow net virus), RYSV (rice yellow stunt virus), NCMV (northern cereal mosaic virus), LNYV (lettuce necrotic yellows virus), VSV (vesicular stomatitis virus), RABV (rabies virus). Modified from figures 2 and 3 of Tsai C-W, Redinbaugh MG, Willie KJ, Reed S, Goodin M, and Hogenhout SA (2005) Complete genome sequence and *in planta* subcellular localization of maize fine streak virus proteins. *Journal of Virology* 79: 5304–5314, with permission from American Society for Microbiology.

the L protein, which has conserved polymerase motifs common to those of most rhabdoviruses. A description of these proteins and their probable functions is outlined below.

The nucleocapsid protein (N)

The N protein functions to encapsidate the viral genomic RNA and is a component of the viroplasms and of the polymerase complex (**Figure 1**). The N genes of the nucleorhabdoviruses, SYNV, MMV, MFSV, TaVCV, and RYSV, and the cytorhabdoviruses, LNYV, SCV, and NCMV have been sequenced. The SYNV, MFSV, MMV, and RYSV nucleorhabdovirus N proteins exhibit short stretches of sequence similarity, suggesting that these four viruses are closely related. These regions of the nucleorhabdovirus N proteins are not significantly related to those of the cytorhabdoviruses and have no extensive relatedness to vertebrate rhabdovirus N proteins.

Experiments conducted in plant and yeast cells have shown that SYNV N protein contains a bipartite nuclear localization signal (NLS) near the carboxy-terminus that is required for nuclear import, and biochemical studies have shown that the protein interacts *in vitro* with importin α homologs. Related nuclear localization sequences are also present in the MFSV and TaVCV N proteins, but this signal is lacking in the N protein of MMV and RYSV. During transient expression in plant cells, the SYNV N protein forms subnuclear foci that resemble the viroplasms found in infected plants, and coexpression of the N and P proteins results in colocalization of both proteins to subnuclear foci (**Figure 3**). These foci require homologous interactions of the N protein that are mediated by a helix-loop-helix motif near the amino-terminus. Interestingly, the SYNV subnuclear foci are distinct from those of nucleolar marker proteins, whereas foci formed during interactions of the MFSV N and P proteins appear to colocalize to the nucleolus.

The phosphoprotein (P)

Direct experiments showing phosphorylation of the P protein are available only for SYNV. The amino-terminal half of the SYNV P protein is negatively charged, as is the case for the other rhabdoviruses. In SYNV, the P protein is phosphorylated *in vivo* at threonine residues and hence differs from the VSV P protein, which is phosphorylated at serine residues. No discernable sequence relatedness is evident between the P protein of SYNV and those of other rhabdoviruses. However, the plant rhabdovirus P proteins have similar hydrophilic cores and the cytorhabdovirus P proteins overall are hydrophilic with similar isoelectric points. In addition, there is nearly 50% sequence identity between the P proteins of MMV and TaVCV. Although these results suggest that the provisional assignments of the P proteins of other plant rhabdoviruses are probably correct, additional data need to be accumulated to obtain a consensus of the functional activities and the biochemical interactions of the putative plant rhabdovirus P proteins.

The P protein is a component of the viral nucleocapsid core and the polymerase complex. The SYNV P protein forms complexes *in vivo* with the N and L proteins that are analogous to N:P and P:L complexes found in VSV-infected cells, and hence the P protein probably functions in SYNV polymerase recycling. Biochemical experiments have shown that the solubility of the SYNV N protein is

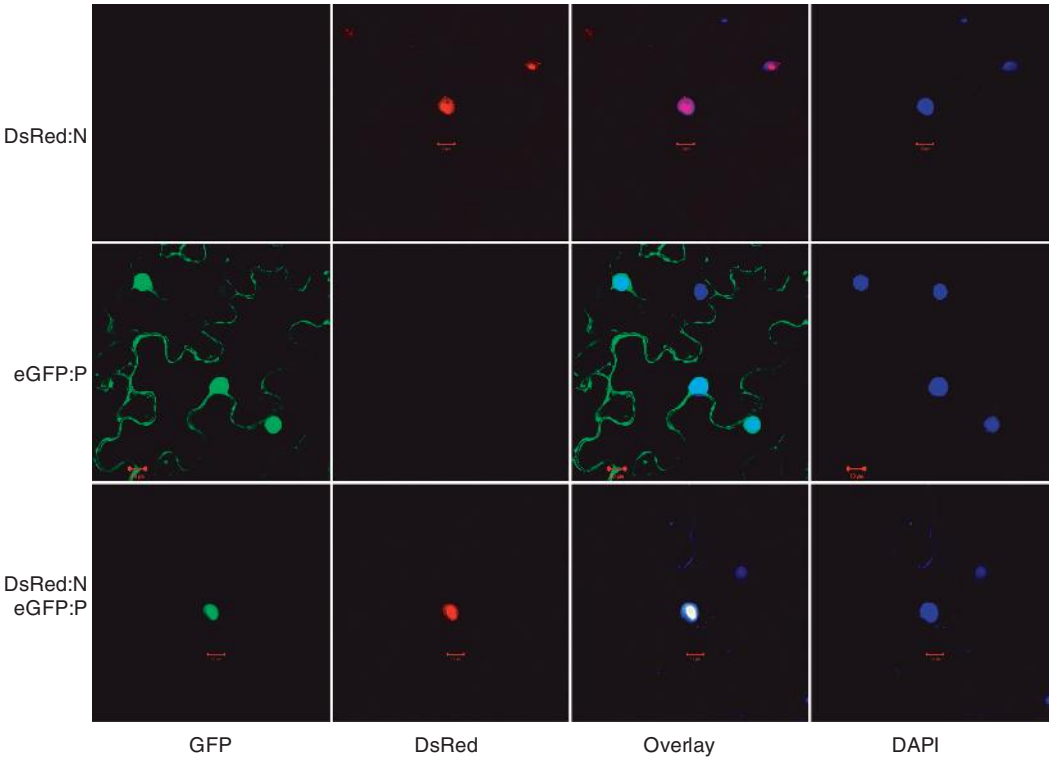


Figure 3 Subcellular localization of the N and P proteins of SYNV. The DsRed:N and eGFP:P fusion proteins were transiently expressed in *Nicotiana benthamiana* leaf tissue via infiltration with *Agrobacterium tumefacians* containing pGD vectors. The confocal micrographs show the subcellular localization of proteins at 3 days after infiltration. The top row depicts the individual expression of the DsRed:N fusion protein. The middle row shows fluroescence in cells expressing eGFP:P alone. The bottom row shows cells coexpressing the DsRed:N and eGFP:P proteins. Nuclei are identified by staining with DAPI (4′-6-diamino-2-phenylindole dihydrochloride). From Deng *et al.*, unpublished.

increased during P protein interactions, so the P protein appears to have chaperone activity. Heterologous N:P protein complexes form by interactions of an internal region of the P protein with the amino-terminal helix-loop-helix region of N that overlaps the N:N protein binding site. The P protein also engages in homologous interactions that are mediated near the amino-terminus of the P protein. Hence, the SYNV P protein has functions similar to those of the P proteins of other well-characterized vertebrate rhabdoviruses.

Reporter gene fusions show that the SYNV P protein, when expressed alone, accumulates in both the nucleus and the cytoplasm (**Figure 3**). The central third of the SYNV P protein is required for nuclear import, but other regions of the protein affect the import efficiency. Recent experiments indicate that the SYNV P protein binds directly to human importin β derivatives *in vitro* and, since the N protein has an NLS site and interacts with importin α, the N and P proteins have different mechanisms for nuclear import. Interestingly, sequence analyses show that the SYNV P protein does not have a bipartite NLS, whereas the P proteins of RYSV and MMV have a bipartite NLS, and both P proteins have a pronounced nuclear localization pattern. Hence, due to these differences between the SYNV and the RYSV and MMV proteins, it is likely that the viruses have diverged in their strategies of nuclear localization. In SYNV and MFSV, coexpression of the P protein with the N protein of the same virus results in colocalization of the complexes to subnuclear foci characteristic of viroplasms. In SYNV, formation of these foci requires interactions of P with the amino-terminus of the N protein. However, the N and P interactions appear to be virus-specific because heterologous combinations of the SYNV and MFSV N and P proteins fail to form subnuclear foci, and the P protein continues to be expressed in both the nucleus and the cytoplasm.

The SYNV P protein also has a leucine-rich nuclear export signal located within the first third of the protein. Reporter proteins fused to the P protein are retained in the nucleus following treatment with Leptomycin B, an inhibitor of nuclear export. Interaction with the host nuclear export receptor XpoI provides further evidence for P protein nuclear export functions. These results and P protein mutagenesis experiments provide strong evidence suggesting that the SYNV P protein is involved in nuclear shuttling activities.

In addition to its role as a structural protein, the SYNV P protein shares many of the hallmarks of

RNA-silencing suppressor proteins. These characteristics include suppression of reporter gene silencing in transgenic plants and the ability to bind small interfering (siRNAs) and single-stranded RNAs *in vitro*. Together, these activities clearly point to key roles of the P protein in nucleocapsid structure, replication, countering innate host defenses, and possibly intercellular movement.

Position X proteins

Like other plant viruses, plant rhabdoviruses must encode proteins to assist in cell-to-cell movement of virus derivatives through the plasmodesmata and their systemic transport through the vascular system. Considerable evidence for a movement function has been accumulated for proteins encoded at position X between the P and M genes (**Figure 1(c)**). The predicted secondary structures of several plant rhabdovirus proteins, including SYNV sc4, LNYV 4b, RYSV P3, MMV P3, and MFSV P4, have a distant relatedness to the TMV 30 K superfamily structural motifs. Additional evidence for a role of the position X-encoded proteins in cell-to-cell movement is their association with host and viral membranes during transient expression. Unpublished evidence indicates that the sc4 protein is phosphorylated, as is also the case with the TMV 30 K movement protein. The movement hypothesis for genes occupying the X position has been reinforced by experiments carried out with RYSV. With this nucleorhabdovirus, the P3 protein is able to *trans*-complement cell-to-cell movement of a movement-defective potato virus X in *Nicotiana benthamiana* leaves. The P3 protein also interacts with the N protein, the major component of nucleocapsids; hence, it could possibly facilitate movement of nucleocapsids through these interactions.

Despite the persuasive evidence for a movement function of genes encoded at position X, considerable diversity appears in the X ORF(s) of several plant rhabdoviruses. For example, although the TaVCV gene X codes for a protein of a size similar to the other X protein genes, the protein has no obvious sequence similarity to proteins in the 30 K superfamily. MFSV encodes two proteins between the putative P and M proteins, and both have different localization patterns from sc4 of SYNV. In addition, four small ORFs reside between the P and M genes of NCMV. Thus, additional studies need to be undertaken to clarify the functional activities of the 'unusual' X proteins.

The matrix protein (M)

The M proteins of plant rhabdoviruses are basic and are thought to function in nucleocapsid binding and coiling, and interactions with the G protein. Sequence alignments of the M proteins of several plant rhabdoviruses have not revealed extensively conserved motifs. Unpublished studies suggest that the SYNV M protein is phosphorylated *in vivo* at both threonine and serine residues. When expressed ectopically, the M proteins of SYNV and MFSV localize in the nucleus. A central hydrophobic region of the M protein is thought to mediate membrane–lipid interactions with the G protein during morphogenesis. In addition to their roles in viral morphogenesis, preliminary experiments indicate that rhabdovirus M proteins have important roles in host–virus interactions because they appear to be able to inhibit host gene expression.

Position Y ORFs

NCMV, RYSV, and SCV contain a short ORF at position Y that separates the genes encoding the G and L proteins (**Figure 1(c)**). Small, nonvirion ORFs preceding the L gene are also found in the genomes of some animal viruses, but the products of these ORFs are either nonstructural or have not been detected in infected cells. The three predicted plant rhabdovirus Y proteins are small (<100 amino acids) and do not share obvious sequence identity with each other or with the nonvirion genes of the animal rhabdoviruses. However, short stretches of the Y ORFs have limited relatedness to other negative-strand RNA virus proteins, suggesting that these regions of the genome may have originated by gene duplication or recombination.

The RYSV P6 protein at position Y is predicted to contain an aspartic protease motif (DTG) and has five potential phosphorylation sites (S/T-X-X-D/E). *In vitro* phosphorylation assays using a GST:P6 fusion protein have shown that P6 is phosphorylated at both serine and threonine residues. Although P6 could not be detected in total protein extracts from infected leaf tissue, immunoblots of purified virus and protein extracts from viruliferous leafhoppers suggest that P6 is associated with virions, so it may have a structural role in infection.

The glycoprotein (G)

The G protein forms the glycoprotein spikes of rhabdovirus virions (**Figure 1**). The plant rhabdovirus G proteins do not have extensive similarity, but they are more closely related to each other than to the G proteins of several vertebrate rhabdoviruses. The plant rhabdovirus G proteins share putative N-terminal signal sequences, a transmembrane anchor domain, and several possible glycosylation sites. In addition, the SYNV G protein contains a putative NLS near the carboxy-terminus that could be involved in transit to the inner nuclear membrane prior to morphogenesis. Several glycosylation inhibitors interfere with N-glycosylation of the SYNV G protein, and tunicamycin treatment blocks SYNV morphogenesis, leading to accumulation of striking arrays of condensed nucleocapsid cores that fail to bud through the inner nuclear membrane. Thus, the G protein has a prominent role in morphogenesis, and the available evidence suggests that glycosylation

is required for interactions of the protein with coiled nucleocapsids.

The polymerase protein (L)

The L proteins of plant rhabdoviruses are present in low abundance within nucleocapsids and in infected cells. The L proteins are the most closely related of the rhabdovirus-encoded proteins and are positively charged with conserved polymerase domains and RNA-binding motifs. The L protein of SYNV is required for polymerase activity, because antibodies directed against the GDNQ (polymerase) motif inhibit transcription. Alignment of the L protein sequence with polymerases of several other nonsegmented negative-strand RNA viruses reveals conservation within 12 motifs. Phylogenetic trees derived from L protein alignments indicate that the nucleorhabdoviruses and cytorhabdoviruses cluster together in two clades separated from the vertebrate rhabdoviruses. This suggests that the plant rhabdoviruses have diverged less from each other than from the vertebrate rhabdoviruses.

Polymerase Activity

A viral RNA-dependent RNA polymerase is activated after treatment of LNYV and BNYV cytorhabdovirus virions with mild nonionic detergents, and this activity cosediments with loosely coiled nucleocapsid filaments that are released from virions. The transcribed products are complementary to the genome, as expected of mRNAs. Thus, the described polymerases of these plant cytorhabdoviruses appear to be similar to the extensively studied polymerases of the vesiculoviruses.

In contrast, no appreciable polymerase activity is evident in dissociated preparations of SYNV or other nucleorhabdovirus virions that have been analyzed. However, an active polymerase can be recovered from the nuclei of plants infected with SYNV. Polymerase activity is associated with a nucleoprotein derivative, consisting of the N, P, and L proteins, that cosediments with SYNV nucleocapsid cores. The polymerase complex can be precipitated with P protein antibodies, but the activity of the complex is not inhibited by these antibodies. However, antibody inhibition experiments demonstrate that the L protein is required for polymerase activity. Kinetic analysis of transcription products also reveals that the complex is capable of sequentially transcribing a polyadenylated plus-sense leader RNA and polyadenylated mRNAs corresponding to each of the six SYNV-encoded proteins. Potential replication intermediates consisting of short incomplete minus-strand products homologous to the genomic RNA are also transcribed. These results thus provide a model whereby nucleorhabdovirus particles require polymerase activation by host components early in infection. In contrast, the polymerases of the cytorhabdoviruses appear to be present in an active form in virions and the released cores are capable of initiating primary transcription immediately upon uncoating *in vitro*.

Cytopathology and Replication

The plant rhabdoviruses vary profoundly in their sites of replication and morphogenesis, and those that replicate in the nucleus differ substantially from vertebrate rhabdoviruses that replicate and assemble in the cytoplasm. In plants, nucleorhabdoviruses replicate in the nucleus, bud in association with the inner nuclear membrane, and accumulate in enlarged perinuclear spaces formed between the inner and outer nuclear envelopes. Similar patterns normally occur in the majority of insect tissues, but MMV also buds in the outer membranes of the salivary glands and nerve cells of its leafhopper vector. Clearly, studies on insect cells need to be emphasized in future work to clarify aspects of insect transmission.

The limited evidence available indicates that the cytorhabdoviruses replicate in the cytoplasm, bud in association with the endoplasmic reticulum (ER), and accumulate in ER-derived vesicles (**Figure 4(a)**). Two slightly different variations in replication of LNYV and BYSMV have been proposed, based on extensive electron microscopic observations of infected cells. Indirect evidence suggests that a nuclear phase may be involved in LNYV replication because the outer nuclear membrane blisters and develops small vesicles that contain some virus particles. However, later in the life cycle, masses of thread-like viroplasms appear in the cytoplasm and these are located close to dense networks of the ER that appear during infection. These proliferated membranes form vesicles that may serve as sites for morphogenesis of the accumulating nucleocapsids. A similar scenario lacking a nuclear phase has been outlined for BYSMV. In this case, membrane-bound viroplasms appear in the cytoplasm and virus particles are found exclusively in association with cytoplasmic membranes that proliferate in close proximity to the viroplasms. Unfortunately, both of the cytorhabdovirus models have been derived solely from ultrastructural observations, and none of these studies has utilized specific antibodies to identify individual virus proteins, viral-specific probes for *in situ* hybridization, or modern techniques of cell biology to probe replication.

No direct information is yet available about the early entry and uncoating events, but a model for nucleorhabdovirus infection predicts that, after entry into the cell during vector feeding or mechanical transmission, rhabdovirus particles associate with the ER to release the nucleocapsid cores into the cytoplasm (**Figure 4(b)**). Released cores may then utilize the host nucleocytoplasmic transport

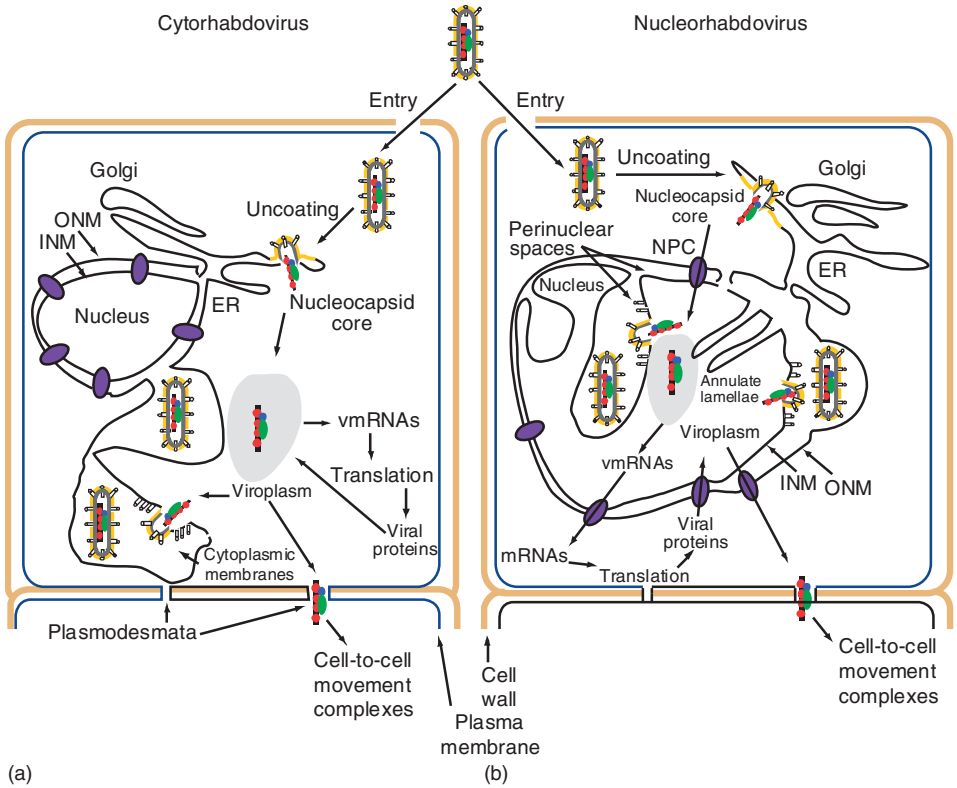


Figure 4 Models for cytorhabdovirus and nucleorhabdovirus replication in plant cells. Most rhabdoviruses are believed to enter plant cells during insect vector feeding and the nucleocapsid is thought to fuse with the endoplasmic reticulum (ER) and be liberated into the cytoplasm. Panels (a) and (b) provide contrasts between the cytorhabdovirus and the nucleorhabdovirus replication strategies, respectively. (a) Cytorhabdovirus replication model. The available information about cytorhabdovirus replication relies almost entirely on ultrastructural observations, and molecular or modern cytological information has not been obtained to extend these observations. After nucleocapsid release, primary and secondary rounds of transcription are followed by nucleocapsid accumulation in viroplasms to form dense masses that are associated with proliferated membrane vesicles. Morphogenesis occurs by budding of nucleocapsids into the ER. During the later stages of replication, large aggregates of bacilliform virions accumulate in pronounced vesicles that are thought to have originated from the ER. (b) Nucleorhabdovirus replication model. The nucleocapsid is thought to be transported into the nucleus through the nuclear pore complex and host components are thought to activate the nucleocapsids to initiate primary rounds of transcription to produce polyadenylated leader RNA and mRNAs for each of the viral proteins. The mRNAs are transported to the cytoplasm and translated, and the N, P and L proteins are transported through the nuclear pore complex into the nucleus. As the N, P, and L proteins increase in abundance, a switch occurs from primary transcription of mRNAs to a mode consisting of intermittent rounds of replication to produce antigenomic and genomic nucleocapsids, followed by secondary rounds of transcription to increase the pool of mRNAs. This phase of replication is regulated by a feedback mechanism that relies on the abundance of the core proteins for encapsidation of nascent leader RNAs. As replication progresses, the nuclei become greatly enlarged, and subnuclear viroplasms appear that consist of large masses of granular material that contain viral RNA and the N, P, and L proteins. Early during replication, some of the newly synthesized nucleocapsids are transported to the cytoplasm where they associate with movement proteins and are transported to other cells through the plasmodesmata. Late in replication, the M protein reaches sufficient concentration to coil the genomic nucleocapsids and mediate interactions with G protein patches at the inner nuclear membrane. During this process, virions undergo morphogenesis by budding through the inner nuclear membrane and accumulate in the perinuclear spaces. Reprinted, with permission, from the Annual Review of Phytopathology, Volume 43, © 2005 by Annual Reviews.

machinery to recognize karyophylic signals present on the N and P proteins to facilitate nucleocapsid entry into the nucleus. During the early stages of infection, the virion-associated polymerase is probably activated by host components to produce an active transcriptase that copies the genomic RNA into capped and polyadenylated mRNAs that are transported to the cytoplasm and translated. The translated N and P proteins are imported into the nucleus by separate mechanisms using host importin α and β proteins, respectively.

After entry into the nucleus, the N, P, and L proteins probably participate in multiple rounds of mRNA transcription, and antigenomic and genomic RNA replication. As replication proceeds, the viroplasms form discrete foci that appear near the periphery of dramatically enlarged nuclei. During the early stages of infection, small amounts

of the nucleocapsids are postulated to be exported to the cytoplasm by interactions of the nuclear export signals on the P protein with host nuclear export receptor proteins. These exported nucleocapsids then interact with movement protein homologs to mediate transport through the plasmodesmata to adjacent cells. As infection progresses, the M protein accumulates in the nucleus and reaches concentrations sufficient to downregulate transcription and participate in coiling of minus-sense RNA nucleocapsid cores. The coiled cores then associate with G protein at sites on the inner nuclear envelope that are located in close proximity to the viroplasms. During budding, numerous enveloped virions accumulate in perinuclear spaces between the inner and outer nuclear envelope where they may be ingested during vector feeding.

A recent discovery that may shed new light on the processes of nucleorhabdovirus replication and maturation has been noted during infection of transgenic *N. benthamiana* plants that express green fluorescent protein (GFP) targeted to the ER. During infections with either SYNV or PYDV, a substantial proportion of the GFP appears to be redistributed to form spherules within the nuclei. In the case of SYNV, the spherules colocalized with foci formed by the N protein. A model to explain this phenomenon is that the spherules contacting the viroplasms in SYNV-infected plants are derived from the ER and become redistributed to the inner nuclear membrane to serve as sites for replication and virion maturation.

Vector Relationships, Distribution, and Evolution

Plant pathogenic rhabdoviruses are highly dependent on arthropod vectors for their distribution between plants. Although some plant rhabdoviruses have no known vector, most well-characterized members are transmitted by insects in which they also multiply, so it is possible that the plant rhabdoviruses radiated from a primitive arthropod. Plant rhabdoviruses are most commonly transmitted by aphids (Aphididae), leafhoppers (Cicadellidae), or planthoppers (Delphaccidae) (**Table 1**). An incompletely characterized putative rhabdovirus, beet leaf curl virus (BLCV), reportedly has a heteropteran beet leaf bug (*Piesma quadratum*) vector, but more extensive molecular and cytological analyses of this virus and other poorly characterized plant rhabdoviruses need to be carried out so their properties and vector relations can be clarified.

Vector–host relationships have profoundly affected plant rhabdovirus distribution and host range. For example, leafhoppers, planthoppers, and aphids are prevalent on both monocots and dicots, but the rhabdoviruses causing diseases of the Gramineae are all transmitted by leafhoppers or planthoppers. Except for PYDV and EMDV, which have leafhopper and planthopper vectors, respectively, dicot-infecting rhabdoviruses whose transmission has been investigated are transmitted by aphids. In all cases of insect transmission that have been carefully examined, rhabdoviruses are persistently transmitted in a propagative fashion, and in many cases, can be transmitted to vector progeny. Long latent periods are required before transmission occurs; insects often remain viruliferous throughout their lives, and transovarial passage has been observed through eggs and nymphs. In addition, strain-specific infection of tissue culture lines and explants combined with serological detection in vector cells provides unequivocal proof that rhabdoviruses replicate with high specificity in leafhopper and aphid vectors.

Several classical studies with PYDV in leafhoppers and SYVV in aphids, as well as recent studies with MMV in its planthopper vector *Peregrinus maidis*, have provided models for tissue-specific events in insect infection (**Figure 5**). After virus acquisition from plants, MMV initially accumulates in epithelial cells of the anterior part of the insect midgut and subsequently in nerve cells. Then, the virus appears in tracheal cells, hemocytes, muscles, and the salivary glands, and finally, in the fat cells, mycetocytes, and epidermal tissues. MMV infection is most extensive in the anterior portion of the gut, nerve cells, tracheal cells, and salivary glands of the planthopper. Based on this order of events, it is postulated that epithelial cells of the midgut are the first virus-entry sites,

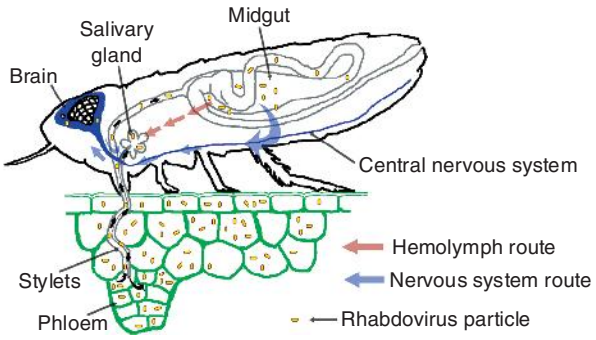


Figure 5 Events occurring during the infection cycle of rhabdoviruses in leafhopper vectors. Viruses are acquired during feeding on plant cells and move from the stylet to the midgut lumen of the digestive tract where they are hypothesized to invade epithelial cells by receptor-mediated endocytosis. From the epithelial cell layers, the virus moves into the nervous system, trachea, and hemolymph and spreads throughout the insect into the salivary glands and reproductive tissues. The salivary glands accumulate high levels of virus particles that are released by exocytosis, transported through the salivary canal, and transmitted to new host plants during subsequent feeding. Reproduced from figure 2a, Hogenhout SA, Redinbaugh MG, and Ammar E (2002) Plant and animal rhabdovirus host range: A bug's eye view. *Trends in Microbiology* 11: 264–271, with permission from Elsevier.

and that the virus quickly moves to the nervous system, trachea, and the hemolymph. From these tissues, MMV can be transmitted systemically to other tissues, including the salivary glands, which support high levels of virus accumulation.

MMV buds through the inner nuclear membrane in plant cells and has a similar pattern of morphogenesis in most cells of *P. maidis* tissues. However, MMV has also been observed to bud frequently from outer membranes in cells of nervous tissue and salivary glands of the planthopper, and similar observations have been made with other virus–vector combinations. Thus, the cellular budding site of MMV, and probably other rhabdoviruses, in insect hosts may be dependent on the cell type. Budding from outer cell membranes in salivary glands may be important because the process could allow release of virions into the saliva and permit introduction into plant cells during feeding.

Genetic experiments with PYDV have shown that highly efficient and inefficient leafhopper vectors can be selected. Continuous passage of PYDV by serial injection of insects can result in isolates that are unable to infect plants. Additional studies have shown that strains that have lost their capacity to be insect-transmitted can be recovered after protracted passage in plants. This phenomenon could provide a mechanism for evolution of vectorless rhabdoviruses, particularly in cases where infections were established in vegetatively propagated hosts.

Rhabdoviruses normally have the capacity to infect a greater range of plant hosts than the narrow range of species colonized by their insect vectors, because experimental host ranges usually can be extended considerably through mechanical transmission. In addition, plant rhabdoviruses have a wider insect host range than their natural insect vector hosts would indicate. For example, the majority of maize-feeding leafhoppers and planthoppers can acquire MFSV, but only one leafhopper, *Graminella nigrifrons*, can transmit this virus to maize. In addition, studies have shown that surrogate nonvectors of SCV injected with infected plant extracts can support replication and transmit virus to dicot hosts that do not support feeding by the native vector. Finally, cowpea protoplast infectivity experiments with the grass rhabdovirus FLSV and with SYNV show that both viruses are able to infect the legume protoplasts, but neither virus is able to infect cowpea plants. Together, these observations indicate that some plant rhabdoviruses have the ability to infect cells of several distantly related hosts, but that the natural host specificity is determined by (1) the insect vector feeding range; (2) the ability of the virus to move through the insect vector into the salivary glands and into the plant; and (3) systemic movement in the infected plant.

During evolution, plant rhabdoviruses faced two major challenges of a fundamentally different nature brought about by the necessity to alternately infect plants and insects. In each host, the virus must utilize different entry methods and accommodate distinct cellular and defense mechanisms. Rhabdovirus acquisition by the vector probably necessitates attachment to specific receptors at the surface of cells in the digestive system, followed by active invasion of the reproductive organs, fat bodies, and salivary glands. Very different barriers must be circumvented to establish systemic infections of plants. In order to establish a primary infection focus, the cell wall must first be breached by mouthparts of the insect, the virus must be introduced into the plant cell, where it uncoats and initiates the replication cycle. To establish systemic infections in plants, the virus must move from cell to cell through very small plasmodesmatal connections, enter the vascular system and spread throughout the plant. Therefore, plant rhabdoviruses are anticipated to have evolved a number of sophisticated mechanisms to circumvent the barriers to infection of their insect and plant hosts.

See also: Fish Rhabdoviruses.

Further Reading

Black LM (1979) Vector cell monolayers and plant viruses. *Advances in Virus Research* 25: 192–271.

Hogenhout SA, Redinbaugh MG, and Ammar E (2002) Plant and animal rhabdovirus host range: A bug's eye view. *Trends in Microbiology* 11: 264–271.

Jackson AO and Wagner JDO (1998) Procedures for plant rhabdovirus purification, polyribosome isolation, and replicase extraction. In: Foster G and Taylor S (eds.) *Plant Virology Protocols: From Virus Isolation to Transgenic Resistance,* vol. 81, ch. 7, pp. 77–97. Totowa, NJ: Humana Press.

Jackson AO, Dietzgen RG, Goodin MM, Bragg JN, and Deng M (2005) Plant Rhabdoviruses. *Annual Review of Phytopathology* 43: 623–660.

Krichevsky A, Kozlovsky SV, Gafney Y, and Citovsky V (2006) Nuclear import and export of plant virus proteins and genomes. *Molecular Plant Pathology* 7: 131–146.

Melcher U (2000) The 30K super family of viral movement proteins. *Journal of General Virology* 81: 257–266.

Sylvester ES and Richardson J (1989) Aphid-borne rhabdoviruses relationships with their vectors. In: Harris KF (ed.) *Advances in Disease Vector Research,* vol. 9, pp. 313–341. New York: Springer.

Tsai C-W, Redinbaugh MG, Willie KJ, Reed S, Goodin M, and Hogenhout SA (2005) Complete genome sequence and *in planta* subcellular localization of maize fine streak virus proteins. *Journal of Virology* 79: 5304–5314.

Tordo N, Benmansour A, Calisher C, *et al.* (2005) Family *Rhabdoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses,* pp. 623–644. San Diego, CA: Elsevier Academic Press.

Plant Virus Diseases: Economic Aspects

G Loebenstein, Agricultural Research Organization, Bet Dagan, Israel

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Estimates on crop losses due to pests and diseases are lacking, with respect to both up-to-date data and accuracy. Data from different countries obtained by a variety of methods should be taken only as a rough guide. Nevertheless, the available information sheds light on the tremendous loss to the food and fiber supply for the ever-growing world population.

A team of German crop scientists, with support of the European Crop Protection Association, published a comprehensive study of pest-induced crop losses on major food and cash crops. In the four principal crops (rice, wheat, barley, and maize) losses due to diseases ranged between 10% and 16% of potential production which translates into approx. US64×10 billion in the years 1988–90. The losses increased to US84×10 billion if four additional crops were included (potatoes, soybeans, cotton, and coffee) that together occupy half the world's cropland, with harvests worth US$300 billion in 1988–90. The study did not cover some important food crops of developing countries, such as cassava, millet, and sorghum, and horticultural crops, which are often heavily affected by virus diseases. The study found that pests and diseases accounted for pre-harvest losses of 42% of the potential value of output during 1988–90, with 15% attributable to insects and 13% each to weeds and pathogens. An additional 10% of the potential yield was lost post-harvest.

Yield losses due to plant virus infections are estimated to range between 10% and 15%, while losses due to fungal pathogens are estimated at 40–60%, the rest being caused by bacterial and phytoplasma pathogens and nematodes. Again, data should be viewed with caution, as many survey data are estimates based on educated guesses. On their face value these data do not agree with the previous data. Apparently, the selection of crops was different and fewer viral diseases were considered.

Yield losses caused by 11 different virus diseases in various crops, including bananas, cassava, cacao, maize, groundnuts varied from 0% up to 100%. In a survey conducted in eight African countries, cassava mosaic disease yield losses were estimated to reach 30–40%.

Economic losses are not only due to reduced vigor and growth resulting in yield losses, but may also affect product quality, as for example deformations in zucchini yellow mosaic virus (ZYMV)-infected squash or plum pox virus-infected peaches. In addition, major economic aspects to be considered are the costs to produce virus-tested propagation material, eradication programs, vector control, and breeding for resistance.

It seems that spread of viruses and subsequent economic losses are more prevalent in tropical and subtropical regions with continuous vegetation throughout the year. The continuous vegetation, annual and perennial, enables persistence of viruses and their vectors. In cooler areas winter temperatures are not favorable for annual vegetation and prevalence of vectors. Similarly, in regions with a dry hot summer, continuous herbaceous vegetation is interrupted which also reduces vectors and subsequent spread of plant viruses.

Economic losses due to viruses are often higher in vegetatively propagated crops such as citrus, cassava, and potato, than in seed-propagated ones. In many plants, viruses are not transmitted through seeds, a mechanism developed through evolution whereby the plant ensures that the next generation starts healthy. In vegetatively propagated crops, if cuttings, seed tubers, bud wood, bulbs, runners, etc. are taken from infected plants the virus will inevitably infect the next generation. This is in addition to spread of the virus by vectors, which may occur in both cases.

During the last decade whitefly transmitted viruses are on the increase in many crops, including vegetables, often becoming a major constraint.

This article is restricted to virus diseases in horticultural and plantation crops where virus diseases cause severe economic losses, many of them in developing countries.

Citrus Tristeza Virus

Citrus tristeza virus (CTV) is probably the most destructive citrus virus in the world. About 30 million trees on sour orange rootstocks were lost in Brazil and Argentina in the years 1940–60, 6.6 million trees in Venezuela in the 1980s, and an estimated 10 million trees in Florida and other Caribbean Basin countries. In these regions CTV was and is still being spread by the brown citrus aphid *Toxoptera citricida*. In Spain CTV killed about 10 million trees and several million more were lost in California, Israel, and other areas. In these areas the melon aphid, *Aphis gossypii*, is spreading CTV. When both vectors are present, as in Florida, spread of CTV is greatly enhanced. Yield losses have been documented in several places, for example, in Jamaica. At two locations totaling 1159 acres, losses over 5 years were more than US$4 million. These costs did not include the costs for removing dead trees and

replanting. However, even though millions of trees in Brazil and Spain were lost it did not affect production of citrus in both countries in the long run. Thus, in Brazil production increased from 1.7 million t in 1960 to more than 20 million t in the 1990s of the previous century. Apparently, replanting new orchards on tolerant rootstocks, together with budwood control, and improved horticultural technologies made it possible to overcome the severe damages by CTV in the old plantations.

Various strains or isolates of CTV are known. Mild strains cause little if any symptoms on most commercial citrus and stem pitting on Mexican lime while severe strains cause decline and death on citrus grafted on sour orange rootstocks; seedling yellow strains cause stunting and yellowing of leaves while orange and grapefruit stem pitting strains will stem-pit sweet orange varieties and grapefruit, respectively. Stem pitting isolates are the most severe strains of CTV and cannot be controlled by the use of tolerant rootstocks.

CTV is a member of the monopartite genus *Closterovirus*, with a positive single-stranded RNA genome encapsidated in a flexuous particle about 2000 nm in length. The virus can be detected by ELISA or by biological indexing on Mexican lime.

The best way to control CTV and other graft-transmissible diseases of citrus is by a mandatory certification program. This program includes a 'clean stock program' for production and maintenance of pathogen-free propagation stock, and 'certification programs' for maintenance and distribution of virus-free material for commercial use. These publicly operated programs are generally self-supportive. Thus, in Florida a fee of US$2 is charged for registration of validated or parent trees.

Cross-protection with mild CTV strains is widely practiced in Brazil, Australia, and South Africa. The control of CTV strains causing stem pitting on sweet orange and grapefruit in Brazil is, by far, the largest and most successful use of cross-protection. By 1980, over 8 million trees of Pera sweet orange were cross-protected. Cross-protection to control CTV in Pera sweet orange in Brazil is still practiced.

An eradication program for tristeza is in operation in California. During 2000–01 the Central California Tristeza Eradication Agency surveyed approximately 31 400 acres and conducted up to 375 000 individual ELISA assays. This program delayed undoubtedly the spread of the disease but is expensive since it also includes the costs of removing infected trees. In Israel a tristeza suppression program was in operation between 1970 and 1977, whereby 300 000 tests were made using indicator plants and electron microscopy and 1700 CTV-infected trees were detected and removed. Between 1979 and 1980, 1.25 million trees were indexed by ELISA and only 0.13% of the tested trees were found to be infected. Apparently the eradication program delayed the outbreak of a tristeza pandemic in Israel by at least 15–20 years.

Cassava Mosaic Disease

Cassava is a low-cost carbohydrate food predominantly grown by resource-poor farmers in developing countries. Virus diseases seriously impede cassava production, especially in Africa, where it is grown in diverse climates, from coastal regions to semiarid zones of the Sahel. Virus diseases are regarded as the major constraint on cassava production.

Several similar but distinct whitefly transmitted geminiviruses cause cassava mosaic disease (CMD) in Africa. They can occur singly or in combination. The most important of them are African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV), ascribed to the genus *Begomovirus*. From 1988 to the present, a major pandemic of an unusually severe form of CMD is spreading throughout East and Central Africa, causing massive losses and affecting food supplies. A distinct strain of EACMV – the Ugandan strain – is the most significant threat to cassava production in Africa.

Yield losses with individual cassava cultivars in different African countries range from 20% to 95%, and continent-wide losses have been estimated at 12–23 million t of fresh tuberous roots per year, worth about US$1200–2300 million. Yield losses in Uganda at the peak of the recent EACMV-pandemic were immense and farmers had to abandon cultivation of cassava in the worst affected areas. It was assumed that each year an area of *c.* 60 000 ha of cassava yielding 600 000 t worth US$60 000 was lost due to CMD.

Symptoms of CMD are mainly green or yellow mosaic. Severe chlorosis is often associated with premature leaf abscission.

CMD is transmitted by the whitefly *Bemisia tabaci*. Virus dissemination between fields and over long distances is mainly through the use of infected stem cuttings as planting material. The disease is caused by several geminiviruses.

CMD can be detected by serological gel-diffusion tests, different ELISA and immunosorbent electron microscopy. Monoclonal antibodies enable cassava mosaic virus isolates to be differentiated though none exists so far for detecting specifically the Ugandan strain of EACMV. Polymerase chain reaction (PCR) with specific nucleotide sequence primers however detects all different cassava mosaic viruses, including EACMV.

For control of CMD, the main emphasis has been on the development of resistant varieties. Phytosanitation, involving the selection of cuttings from healthy plants, rouging of diseased plants, and production of virus-free tissue-cultured planting material are also practiced. Planting date can be adjusted to avoid times when populations of whiteflies are high. Use of insecticides to restrict spread of CMD by controlling the whitefly vector was not effective.

Virus Diseases of Potato

Virus diseases are a major constraint in potato production and often reduce yields by more than 50%. Due to their economic importance much research was devoted to them in the first half of the twentieth century, and new techniques such as serology, meristem cultures, and electron microscopy were developed to control them.

At least 37 viruses naturally infect cultivated potatoes, with potato virus Y (PVY), potato leafroll virus (PLRV), potato virus S (PVS), potato virus M (PVM), potato virus X (PVX), potato aucuba mosaic virus (PAMV), and potato mop-top virus (PMTV) being the most widespread and damaging ones. In addition, potato spindle tuber viroid (PSTV) and phytoplasma diseases are also causing major crop losses in potato.

PLRV is of great economic importance. It is widespread worldwide and in plants grown from infected tubers (secondary infection) yields may be reduced by 33–50%. Tubers from infected plants are small to medium sized. Even greater losses are observed when PLRV occurs together with PVX or PVY, reaching 40–70%. Yield losses due to PVY may reach 10–80%, especially if the virus occurs with PVX. A severe group of PVY strains designated PVY^{NTN} present in Europe, Japan, and the US cause a damaging disease in which tubers develop superficial rings that later are sunken and necrotic. They often become more conspicuous during storage, and can affect 90% of tubers of susceptible cultivars. At present there is no serological or other assay, which can distinguish between PVY^{NTN} and other PVY strains.

Yields of a crop in which all plants are secondarily infected with PVX and PVS will be 5–15% lower than normal. PVS and PVM are common in Eastern European countries. PVM can result in tuber yield losses of 40–75%. PVM occurs in complex with other viruses (especially PVS). PVS isolates may reduce tuber yield by 3–20%. Plants from infected stocks that have been propagated for several generations may suffer more severe losses. Thus, for example, potato yields in Kazakhstan are extremely low, averaging 9 t ha^{-1} during the years 1993–95. In a pilot scheme conducted by the Agricultural Institute of Astana, Kazakhstan, within a USAID project, microtubers obtained from meristems of virus-tested potatoes were used and resulted in a low infection rate in the elite seed tubers. These elite seed gave an increase in yield of about 90% when compared with yields of commercial fields in the vicinity.

Yields in former Soviet Union countries are low, averaging 11.26 t ha^{-1} during 1993–95 as there is no reliable system for providing certified tuber seeds, and farmers use seeds from their own fields. Low yields are to a great extent due to virus diseases carried over in the planting material. Thus, Loshitsky and Belorusky 3 N cultivars developed by the Belarusian Research Institute for Potato Growing (BRIP) yielded 30.0–50.0 t ha^{-1} in experimental fields, while Belarus' average potato yields range between 12.2 and 16.3 t ha^{-1}. Supposing that only 20% of this difference is due to virus infection in the planting material, and extrapolating this over the total area of potato in the former Soviet Union countries of about 6 million ha, producing on an average 70 million t of potato an annual loss of about 18 million t seems to be a conservative estimate.

Costs of seed potato certification schemes should also be considered. The aim of these schemes is to produce true-to-type disease-free (mainly from viruses) potato seed tubers. These schemes nowadays often include *in vitro*-derived plantlets for the initial increase of pathogen-tested clonal selections, including production of minitubers. Prices for certified tuber seeds range between US$350 and 500 per ton while prices for ware potatoes range between US$170 and 180 per ton. If about 3 t seed potato is needed to plant 1 ha, this amounts to an additional expense of about US$450 when certified seeds are used compared to the farmer using his own crop for next year's planting. In fact the difference is presumably higher since the farmer might use the small, not marketable tubers for planting.

Virus Diseases of Sweet Potato

Sweet potato, *Ipomoea batatas*, is the seventh most important food crop in the world in terms of production. They are grown on about 9 million ha, yielding ~140 million t, with an average yield of about 15 t ha^{-1}. They are mainly grown in developing countries, which account for over 95% of world output. Sweet potato is a 'poor man's crop', with most of the production done on a small or subsistence level. Sweet potato produces more biomass and nutrients per hectare than any other food crop in the world. It is well suited to survive in fertile tropical soils and to produce tubers without fertilizers and irrigation and is one of the crops with an important role in famine relief. Thus, for example, across East Africa's semiarid, densely populated plains, thousands of villages depend on sweet potato for food supply. The Japanese used it when typhoons demolished their rice fields. Yields differ greatly in different areas or even fields in the same region. Thus, the average yield in African countries is about 4.7 t ha^{-1}, with yields of 8.9, 4.3, 2.6, and 6.5 t ha^{-1} in Kenya, Uganda, Sierra Leone, and Nigeria, respectively. The yields in Asia are significantly higher, averaging 18.5 t ha^{-1}. China, Japan, Korea, Thailand, and Israel have the highest yields with about 20, 24.7, 20.9, 12, and 33.3 t ha^{-1}, respectively. In South America the average yield is 12.2 t ha^{-1}, with Argentina, Peru, and Uruguay in the lead with 18, 11, and 10 t ha^{-1}, respectively. For comparison, the average yield in the US is 16.3 t ha^{-1}.

These differences in yields are mainly due to variation in quality of the propagation material. Sweet potatoes are vegetatively propagated from vines, root slips (sprouts) or tubers, and farmers in African and other countries often take vines for propagation from their own fields year after year. Thus, if virus diseases are present in the field they will inevitably be transmitted with the propagation material, resulting in a decreased yield. Often these fields are infected with several viruses, thereby compounding the effect on yields. In China, on average, losses of over 20% due to sweet potato virus diseases are observed mainly due to sweet potato feathery mottle virus (SPFMV) and sweet potato latent virus (SwPLV). The infection rate in the Shandong province reaches 5–41%. In countries where care is taken to provide virus-tested planting material, for instance in the USA and Israel, markedly higher yields of 16.3 and 30 t ha ^{1}, respectively, are obtained.

The sweet potato virus diseases (SPVDs) caused by the interaction of SPFMV and sweet potato chlorotic stunt virus (SPCSV) or sweet potato sunken vein virus (SPSVV) (possibly a variant of SPCSV) is the most important virus (complex) disease in East Africa. It can cause losses of 50–80%, especially in Uganda and Kenya, though in another study from Uganda losses were much smaller probably due to relatively high levels of virus resistance in their varieties. In a 3-year field study in Cameroon, SPVD reduced root yields by 56–90% in susceptible varieties. Yield reductions of 78% due to SPVD have been reported from field trials in Nigeria. In Israel in a 2-year field experiment, yield reductions of ~50% were observed in plots planted with SPVD-infected cuttings while infection by SPFMV or SPSVV alone were minor. Cucumber mosaic virus (CMV) can cause a complete failure of the crop when infected sweet potatoes carry SPSVV. However, CMV is unable to infect healthy sweet potato.

At present the best way to control virus diseases in sweet potato is to supply the grower with virus-indexed propagation material. Such programs are operating in Israel and in the Shandong province of China. In Israel, as a result of planting virus-tested material, yields increased at least by 100%, while in China increases ranged between 22% and 92%. The payoff to the farmer has been high and in Israel use of certified material, prepared by special nurseries, is common practice, costing ~3.0 US cents per cutting. Farmers buys about 30% of material needed to plant their fields and fill in the rest from cutting of vines grown from these plants. Yields are high and stable when virus-tested propagation material is used. In African countries such programs are operating only on a limited scale, because sweet potatoes are grown mainly as a food security crop, and not as a commercial one.

Breeding programs might be a future answer and such programs are in operation in Uganda, providing SPVD resistance. It will have to be seen if these improved cultivars will retain their resistance. Thus, several clones obtained from the International Potato Center (CIP) that were claimed to be resistant to SPFMV were found to be susceptible when Israeli and Ugandan virus isolates were tested.

Cacao Swollen Shoot Disease

The disease occurs in West Africa and was first reported in Ghana in 1936. Cacao swollen shoot disease is one of the most economically important viral diseases and has contributed to the drastic decline of cocoa production in Ghana. Since 1936, nearly 200 million cacao trees have been cut out from about 130 000 ha. From 1945 to 1950 yield of beans fell by 86% almost proportional to the killing of trees. In 1953 the 50 million infected trees represented a capital depreciation of £25 million since the discovery of the disease.

Cacao swollen shoot disease virus (CSSV) belongs to the genus *Badnavirus* and has bacilliform virions of 113 nm length and 28 nm width. A range of mealybug species, with *Planococcoides njalensis* being the most important one, transmit CSSV.

Attempts were made to control the disease by use of insecticides and biological control of the mealybugs, breeding for resistant cocoa cultivars, and protection with mild strains, but the main practice is by roguing of infected and their neighboring trees.

The eradication policy of infected trees enforced by the British colonial authorities in the Gold Coast (now Ghana) in the late 1940s met with serious opposition and was a major factor contributing to the rise of the political party led by Kwame Nkruma, who accused the authorities of attempting to destroy farmers' income.

Whitefly-Transmitted Geminiviruses in Tomato

These viruses such as tomato yellow leaf curl virus (TYLCV) have become a limiting factor in tomato production. In the USA whitefly-transmitted geminiviruses (WTGs) appeared in the early 1990s and losses are estimated to reach 20%, while in Cuba, Mexico, Guatemala, Costa Rica, and Brazil yield losses ranged between 30% and 100%. Losses in the Dominican Republic during 1989–95 were estimated at US$50 million.

Chemical control of the whitefly vector is a possibility, but the large whitefly populations make this option inefficient. Physical barriers such as greenhouses protected with fine net (50-mesh) screens are effective while 'floating barriers' of perforated polyethylene sheets stretched over tomato fields prevent the landing of whiteflies. These measures, however, increase the costs of production markedly. The best approach to control WTG is through

the use of resistant or tolerant cultivars. Breeding programs are in progress, but so far no resistant or tolerant cultivars have been obtained.

Plum Pox Virus Sharka Disease of Stone Fruits

Plum pox symptoms were first observed in plums in Bulgaria between 1915 and 1918. Between 1932 and 1960 the disease moved north and east from Bulgaria into Yugoslavia, Hungary, Romania, Albania, Czechoslovakia, Germany, and Russia. The disease was observed mainly in plums and apricots and since the 1980s also in peaches.

Virus infection can cause considerable losses. About 100 million stone fruit trees in Europe are currently infected, and susceptible cultivars can result in 80–100% yield losses. In Eastern and Central Europe, sensitive plum varieties can exhibit premature fruit drop and bark splitting. Some sweet cherry fruits develop chlorotic and necrotic rings and premature fruit drop.

Plum pox does not kill trees but it makes the fruit unmarketable and reduces yield by 20–30%. The fruits drop before maturity and are unfit for use as they are bitter and unsweet. In Bulgaria, losses in 1968 were estimated to be 30 000 t. In the last three decades, the average fruit yield in the Czech Republic dropped by 80%, and the total number of plum trees was reduced from 18 million to 4 million. Emilia-Romagna region of Italy, between 1998 and 2002, 69 000 stone fruit trees were removed due to Sharka infection; and US$450 000 is being spent annually for their removal and replanting. In Spain since 1988, 1.5 million trees have been removed at a cost of US$17 million. In France, plum pox virus (PPV) is mainly present in the southeast part of the country. All trees showing symptoms are eliminated and if infection levels are above 10%, the whole orchard is destroyed. These methods have resulted in the destruction of about 27 000 trees in 1992 (of which 550 were found to be infected) and in 1993 100 ha (mainly peach) were eliminated. From 1973 to 1990, it is estimated that 91 000 trees were destroyed in France.

PPV is a potyvirus transmitted by aphids in a nonpersistent manner. However much of the spread in Europe can be attributed to movement of infected nursery materialfrom the Balkans.

See also: Emerging and Reemerging Virus Diseases of Plants; Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes; Plant Virus Diseases: Fruit Trees and Grapevine; Plant Virus Vectors (Gene Expression Systems); Vector Transmission of Plant Viruses.

Further Reading

Loebenstein G and Thottappilly G (2003) *Virus and Virus-Like Diseases of Major Crops in Developing Countries*. The Netherlands: Kluwer Academic Publishers.

Orke EC, Dehne HW, Schonbeck F, and Weber A (1994) *Crop Production and Crop Protection: Estimated Losses in Major Food and Cash Crops*. Amsterdam: Elsevier.

Van der Zaag DE (1987) Yield reduction in relation to virus infection. In: de Bolas JA and van der Want JPH (eds.) *Viruses of Potatoes and Seed-potato Production*, pp. 146–150. Wageningen, The Netherlands: Pudoc.

Waterworth HE and Hadidi A (1998) Economic losses due to plant diseases. In: Hadidi A, Khetarpal RH, and Koganezawa H (eds.) *Plant Virus Disease Control*, pp. 1–13. St. Paul: American Pythopathological Society Press.

Relevant Website

http://faostat.fao.org – FAOSTAT, Food and Agriculture Organization of the United Nations (FAO).

Plant Virus Diseases: Fruit Trees and Grapevine

G P Martelli, Università degli Studi and Istituto di Virologia vegetale CNR, Bari, Italy
J K Uyemoto, University of California, Davis, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Grapevine (*Vitis* spp.), rosaceous fruit tree (stone and pome fruits), and nut crop (walnut, hazelnut) varieties are propagated by grafting scions onto rootstocks which, in turn, are either clonally propagated or derive from seedlings. Both members (scion and rootstock) of the grafted plant are therefore liable to carry viruses if they come from infected sources, or can be infected in the field. Remarkably, the causal agents of a number of diseases of these different plant species share the same epidemiological behavior and/or taxonomic position as, for example, soil-borne

nepoviruses and pollen-borne ilarviruses and cherry leafroll virus (CLRV).

Virus Diseases of Grapevines

Disease Symptoms and Yield Losses

There are four major virus diseases of grapevines, that is, infectious degeneration-decline, leafroll, rugose wood, and fleck, which differ symptomatologically and in the type of the causal agents.

Infectious degeneration affects European grapes (*Vitis vinifera*) and American rootstocks and is characterized by two distinct syndromes, 'infectious malformations' and 'yellow mosaic' caused by distorting and chromogenic virus strains, respectively. Leaves and shoots of vines infected by distorting virus strains are more or less severely malformed (**Figure 1(a)**), bunches are smaller and fewer than normal, and berries ripen irregularly, are small-sized, and set poorly. Yellow mosaic-affected vines show bright chrome yellow discolorations that may affect leaves (**Figure 1(g)**) shoots, tendrils, and inflorescences. Leaves and shoots show little malformation, but bunches are small and few. Symptoms of 'decline' resemble those of 'infectious malformations' but affected European grape varieties decline and may die. Crop losses due to these diseases can exceed 60–70%.

Leafroll elicits an early discoloration of the interveinal tissues of leaves of infected European vines which turn purple red in red-berried cultivars (**Figure 1(i)**) and yellowish in white-berried cultivars; both interveinal tissue colorations develop against a background of green primary veins. Discolorations are usually accompanied by downward rolling of the leaf margins and thickening of the blade. In white-berried cultivars of *V. vinifera,* the symptoms are similar, but the leaves become chlorotic to yellowish, instead of reddish. Bunches mature late and irregularly. Yield is decreased by 15–20% in average and rooting ability, graft take, and plant vigor are adversely affected as well as the quality of grapes and musts (sugar and protein content, aromatic profile, soluble solids, titratable acidity). Infection of American *Vitis* species and rootstock hybrids is symptomless, except for a variable decrease in vigor.

Rugose wood is a complex disease in which four different syndromes are recognized, that is, Rupestris stem pitting (RSP), Kober stem grooving (KSG), Corky bark (CB), and LN33 stem grooving (LNSG). Infected vines are less vigorous than normal, may show delayed bud opening in spring and a swelling above the bud union, which reflects a marked difference between the relative diameter of scion and rootstock. The bark above the graft union may be exceedingly thick and corky. Some vines decline and die within a few years from planting. The woody cylinder is marked by pits and/or grooves that can



Figure 1 Symptoms induced by: (a) Distorting strain of grapevine fanleaf virus (grapevine). (b) Double infections by apple mosaic virus (yellow banding) and prunus necrotic ringspot virus (shredding) (cherry). (c) American plum line pattern virus (plum). (d) Cherry rasp leaf virus (cherry). (e) Apple stem pitting virus (pear). (f) Apple stem grooving virus (Virginia crab). (g) Chromogenic strain of grapevine fanleaf virus (grapevine). (h) Plum pox virus (apricot). (i) Grapevine leafroll disease (grapevine). (j) Grapevine rugose wood disease (grapevine). (e, f) Courtesy of L. Giunchedi.

show on the scion, the rootstock (**Figure 1(j)**), or both, their severity varying with the scion/stock combination. Under cool and wet climates wood symptoms are milder or absent. No specific symptoms are seen on the foliage, although certain cultivars show rolling, yellowing, or reddening of the leaves similar to those induced by leafroll. Bunches may be fewer and smaller than normal and the crop is reduced on average by 20–30%.

Fleck is a complex consisting of several diseases ('fleck', 'asteroid mosaic', 'rupestris necrosis', and 'rupestris vein feathering') and viruses (grapevine redglobe virus, GRGV) that cause latent or semilatent infections in *V. vinifera* and most American *Vitis* species and rootstock hybrids. Only *Vitis rupestris* reacts to the different diseases with differential symptoms. Although the elusive nature of the complex hinders the assessment of its economic impact, adverse influence on vigor, rooting ability of rootstocks, and on graft take have been reported.

Geographical Distribution

All diseases are ubiquitous. There is not a single viticultural country where surveys were carried out in which one or more of the viruses involved in their etiology have not been found.

Causal Agents and Classification

Several different viruses are involved in the etiology of, or are associated with each single disease.

Infectious degeneration and decline are two diseases caused by nematode-borne viruses with isometric particles *c.* 30 nm in diameter and bipartite RNA genome 2.2–2.8 kb (RNA-1), and 1.5–2.4 kb (RNA-2) in size. Of the 16 viruses recovered from infected grapevines, 15 belong to different species of the genus *Nepovirus* (family *Comoviridae*): (1) subgroup A: *Arabis mosaic virus* (virus: ArMV), *Grapevine deformation virus* (GDefV), *Grapevine fanleaf virus* (GFLV), *Raspberry ringspot virus* (RpRSV), *Tobacco ringspot virus* (TRSV); (2) subgroup B: *Artichoke Italian latent virus* (AILV), *Grapevine Anatolian ringspot virus* (GARSV), *Grapevine chrome mosaic virus* (GCMV), *Tomato black ring virus* (TBRV); (3) subgroup C: *Blueberry leaf mottle virus* (BLMoV), CLRV, *Grapevine Bulgarian latent virus* (GBLV), *Grapevine Tunisian ringspot virus* (GTRV), *Peach rosette mosaic virus* (PRMV), *tomato ringspot virus* (ToRSV). The species *Strawberry latent ringspot virus* (SLRSV) is a member of the genus *Sadwavirus*. Infectious degeneration is caused by European nepoviruses (ArMV, GFLV, GCMV, RpRV, TBRV) and SLRSV, whereas the agents of decline disease are American nepoviruses (BLMoV, PRMV, TRSV, ToRSV).

The agents of leafroll are filamentous viruses with a monopartite RNA genome and a length of 1400–2200 nm, classified in the family *Closteroviridae*. Grapevine leafroll-associated virus 2 (GLRaV-2), the only representative of the genus *Closterovirus*, has a genome 15 528 nt in size. GLRaV-1 (17 647 nt), GLRaV-3 (17 917 nt), and GLRaV-5 are members of the genus *Ampelovirus*. GLRaV-4, GLRaV-6, GLRaV-8, and GLRaV-9 are tentative species in the same genus, whereas GLRaV-7 is unassigned to the family.

Viruses of the rugose wood complex have filamentous particles 730–800 nm long, encapsidating a monopartite RNA genome, and are classified in two genera of the family *Flexiviridae*. The genus *Vitivirus* comprises grapevine virus A (GVA) (7349 nt), the putative agent of 'Kober stem grooving', grapevine virus B (GVB) (7599 nt) and grapevine virus D (GVD); these two viruses may be involved in the etiology of 'Corky bark' rupestris stem pitting-associated virus (RSPaV) (8726 nt), a member of the genus *Foveavirus,* is the putative agent of the homonymous disease.

The family *Tymoviridae* comprises all agents associated with the fleck complex, that is, viruses with isometric particles *c.* 30 nm in diameter showing a prominent surface structure and a monopartite RNA genome. Grapevine fleck virus (GFkV, 7564 nt), the causal agent of fleck, and GRGV are both members of the genus *Maculavirus*, whereas grapevine asteroid mosaic-associated virus (GAMaV) and grapevine vein feathering virus (GVFV) are tentative members of the genus *Marafivirus*.

Transmission

All diseases are graft transmissible and persist in the propagating material which is responsible for their long-distance dissemination. Spread at a site varies with the disease.

Infectious degeneration and decline are two soil-borne diseases. Dorylaimoid nematodes have been identified as vectors of some of their agents. In particular, GFLV is transmitted by *Xiphinema index*; ArMV and SLRSV by *X. diversicaudatum*; ToRSV by *X. americanum sensu stricto*, *X. rivesi,* and *X. californicum*; PRMV by *X. americanum sensu lato* and *Longidorus diadecturus*; TRSV by *X. americanum sensu stricto*; TBRV by *L. attenuatus*; and RpRSV by *Paralongidorus maximus*. Seed transmission has been reported for PRMV, ToRSV, BLMoV, GCMV, and GFLV; however, grapevine is not seed-propagated in commercial viticultural practices.

Some of the viruses of the leafroll and rugose wood complexes are transmitted in a nonspecific semipersistent manner by pseudococcid mealybugs (i.e., *Planococcus ficus* (GVA, GVB, GLRaV-3), *Pl. citri* (GVA, GLRaV-3), *Pseudococcus longispinus* (GVA, GVB, GLRaV-3, GLRaV-5, GLRaV-9), *Ps. affinis* (GVA, GVB, GLRaV-3), *Ps. comstocki* (GVA, GLRaV-3), *Ps. calceolariae* (GLRaV-3), *Ps. viburni* (GLRaV-3), *Ps. maritimus* (GLRaV-3), *Heliococcus bohemicus* (GVA, GLRaV-1, GLRaV-3), *Phenacoccus aceris* (GLRaV-1)) and by soft scale insects (i.e., *Neopulvinaria innumerabilis*

(GVA, GLRaV-1, GLRaV-3), *Pulvinaria vitis* (GLRaV-1, GLRaV-3), and *Parthenolecanium corni* (GLRaV-1)). None of these viruses are seed-borne, contrary to RSPaV, which is pollen-borne and transmitted through seeds. None of the viruses of the fleck complex have known vectors.

Control

Use of clonally selected and sanitized propagative material is the best preventive method currently available for controlling all diseases. Heat therapy, meristem tip culture, and somatic embryogenesis are effective, though to different extents, for the elimination of parenchyma (nepoviruses) and of phloem-restricted (closteroviruses, vitiviruses, foveaviruses, maculaviruses) viruses. Restraining field re-infection of virus-free stocks is, however, difficult because no ultimate control of soil- or airborne vectors is possible. Attempts for introduction of transgenic resistance to some nepoviruses (GFLV, ArMV, GCMV), vitiviruses (GVA and GVB), and closteroviruses (GLRaV-2 and GLRaV-3) are in progress.

Virus Diseases of Stone Fruits

Disease Symptoms and Yield Losses

Stone fruits are affected by several diseases caused by recognized viruses and by unidentified graft-transmissible pathogens which, for brevity sake, are not addressed in this article.

Symptoms of virus infections depend on the pathogen, the host plant, and the environmental conditions. Variants of prunus necrotic ringspot virus (PNRSV) cause different diseases such as calico or bud failure in almond, necrotic ringspot or rugose mosaic in cherry, tatter leaf in cherry (**Figure 1(b)**) and peach, and mule's ear in peach. Variants of prune dwarf virus (PDV) cause yellows in sour cherry, blind wood and narrow leaf in sweet cherry, and gummosis in apricot. Co-infections by PNRSV and PDV cause peach stunt. Peach yields are drastically reduced (30–60%). Other viruses inducing yellow line pattern or vein netting symptoms in plum are Danish plum line pattern, a strain of PNRSV, European plum line pattern, a strain of apple mosaic virus (ApMV), and American plum line pattern virus (APLPV) (**Figure 1(c)**). In Mediterranean countries, mosaic symptoms in almond trees involve PNRSV, PDV, and/or ApMV.

Sharka, induced by plum pox virus (PPV), is characterized by chlorotic/necrotic ring pattern or mottling of the leaves, color breaks of flower petals, distortion of fruit shape, and rings or blotches of their surface (**Figure 1(h)**). Infected trees drop fruit prematurely. Because of this and fruit alterations, the crop can be completely lost. Peach mosaic virus (PcMV) and cherry mottle leaf virus (CMLV) cause delayed bud-break, stunted shoot growth, leaves with chlorotic spots and vein feathering and deformed rough skinned fruits (PcMV) or chlorotic spots over an uneven leaf surface with shredded leaf margins (CMLV). Cherry is resistant to PcMV, while CMLV causes latent infections in peach. Little cherry disease caused by little cherry virus 2 (LChV-2) affects shape and size of the fruits which lack deep color in dark varieties, and are flavorless. Canopy is light green and tree growth reduced. Black Beaut plums (*Prunus salicina*) infected by plum bark necrosis-stem pitting associated virus (PBNSPaV) are short-lived, exhibit gummosis and necrotic bark on branches and trunks, and severe stem pits.

Stem pitting is induced by different ToRSV strains in *Prunus*. In early spring, canopies of affected trees appear light green, progressing to general chlorosis, leaves are drooping and drop prematurely. The basal portion of trunks develops thick, spongy textured bark and pits and grooves on the woody cylinder, and trees decline. ToRSV-infected prune trees with a brown line at the graft union exhibit poor growth, sparse canopy, and in chronic infections develop limb dieback. PRMV causes rosettes of shoots with shortened internodes. Affected trees are dark green, stunted, and fruitless.

Other nematode-borne stone fruit diseases are: apricot bare twig and unfruitfulness caused by SLRSV, decline and death by cherry rasp leaf virus (CRLV), which also elicits the formation of enations on the underside of leaves (**Figure 1(d)**). In Europe, sweet cherry trees with rasp leaf disease are infected by ArMV, CLRV, raspberry ringspot virus (RpRSV), or SLRSV either alone or in combination with PDV. CLRV can also cause rapid decline of infected cherry trees on Colt rootstock. In Germany, TBRV-infected peach trees develop chlorotic spots and distortion of the leaves, dieback of scaffold branches, and tree death. Infections in peach by SLRSV are associated with willow leaf rosette (Italy), court-noué (France), and shoot dwarfing (Germany). In plum, RpRSV infections are associated with mild symptoms, but combined with PPV symptoms are severe and infected trees decline (Poland). Myrobalan latent ringspot virus (MyLRSV) induces stunting, sparse canopy, and decline in Myrobalan B plum rootstocks (France). Recently, a new putative cheravirus named stocky prune virus (StPV) was detected in stunted, low-yielding trees in France.

Apple chlorotic leaf spot virus (ACLSV) causes dark green, sunken mottle in leaves of the peach cv. Okinawa, is associated with a fruit disorder known as pseudopox in apricot and plum, and, in cherry, it induces fruit necrosis or fruit distortion when in combination with PNRSV. Bark split in plum and graft-incompatibility in apricot and peach have also been associated with infections by different ACLSV strains.

Apricot latent virus (ApLV), latent in apricot, elicits symptoms of asteroid spot disease in peach. Cherry green ring mottle virus (CGRMV) severely impacts sour cherry

and Japanese flowering cherry trees, whereas cherry virus A (CVA) is always found in co-infections with other viruses; thus, specific symptoms have yet to be defined.

Geographical Distribution

Due also to symptomless infections in different *Prunus* species, ApMV, PDV, PNRSV, ACLSV, CGRMV, and, presumably, CVA and CLRV have now a worldwide distribution. PPV occurs through most of Europe, the Mediterranean region, and, more recently, became established in Chile, USA (Pennsylvania), Canada (Ontario), and India. PcMV is endemic in southwestern United States and in Northern Mexico. LChV-2 is present in North America, Europe, and Japan. PBNSPaV was identified in USA (California), Italy, Turkey, Morocco, Jordan, and Serbia. ToRSV and CRLV are endemic in North America and PRMV is restricted to Michigan (USA) and neighboring Ontario (Canada).

Causal Agents and Classification

Causal agents of stone fruit virus diseases belong to a wide array of genera and families, the properties of some of which, that is, the genera *Nepovirus* (*Comoviridae*), *Ampelovirus* (*Closteroviridae*), and *Foveavirus* (*Flexiviridae*), have been outlined above. This applies to the nepoviruses ToRSV, PRMV, ArMV, CLRV, RpRSV and TBRV, the ampeloviruses LChV-2 and PBNSPaV, and the foveaviruses ApLV and CGRMV.

ApMV, PNRSV, PDV, and APLPV (genus *Ilarvirus*, family *Bromoviridae*) have quasi-isometric particles 25–35 nm in diameter and a tripartite RNA genome 1.0–1.3 kb (RNA-1), 1.0–1.2 kb (RNA-2), and 0.7–0.9 kb (RNA-3) in size. CRLV, the type species of the recently established genus *Cheravirus*, has isometric particles *c.* 30 nm in diameter and a bipartite RNA genome 7030 nt (RNA-1) and 3315 nt (RNA-2) in size. ACLSV, PcMV, and CMLV (genus *Trichovirus*) have filamentous particles 720–760 nm in length and a 2.2–2.4 kb monopartite RNA genome. The capillovirus CVA has filamentous particles of undetermined length and a monopartite RNA genome 7383 nt in size while PPV, the only potyvirus known to infect stone fruits, has also filamentous particles *c.* 750 nm long, encapsidating a monopartite RNA genome 9741 nt in size.

Transmission

Ilarviruses and CLRV are pollen- and seed-borne, giving rise to infected seedlings. Horizontal spread of PNRSV and PDV requires also the sequential activities of honeybees (*Apis mellifera*) and thrips (*Thrips tabaci* or *Frankliniella occidentalis*). Spread via root grafts between neighboring trees have been reported in sweet cherry orchards in Washington (USA) for CLRV and in nurseries for ApMV. Several aphid species transmit PPV in a nonpersistent manner to account for localized spread, and movement in infected stocks for long-distance spread. The apple mealybug *P. aceris* vectors LChV-2. Similarly, the ampelovirus PBNSPaV is putatively vectored by mealybug species. PcMV is vectored by the bud mite, *Eriophyes insidiosus* and CMLV by *E. inaequalis*. Natural spread by vectors has not been confirmed for ACLSV, CGRMV, and CVA; however, root grafts are known to occur or suspected. ToRSV and CRLV are vectored by the dagger nematode, *Xiphinema americanum sensu stricto* and PRMV by *X. americanum sensu lato* and *Longidorus diadecturus*.

Control

Because infected orchard trees cannot be cured, effective control strategies are production and use of clean stocks and programs for pathogen assays to identify pretested propagation materials. To this aim, rules and regulations have been promulgated in various European and North American countries to enact some form of nursery tree fruit improvement schemes. Although planting clean stocks is an essential first step in prevention and control of virus diseases, in some instances additional steps may be required. With nepoviruses, diseased sites will require soil fumigation, use of resistant rootstocks, and/or broad leaf weed control.

Virus Diseases of Pome Fruits

Disease Symptoms and Yield Losses

Some of the viruses infecting stone fruit trees (ACLSV, ApMV, CRLV, and ToRSV) are also pathogenic to pome fruits. ApMV and tulare apple mosaic virus (TAMV) induce yellow mottling of the leaves which is outstanding on the spring vegetation but tends to fade away when the temperature rises. A yield reduction of 20–40% has been associated with infection by severe ApMV strains. ACLSV is the agent of different syndromes, such as *Malus platicarpa* dwarf, leaf deformation of several ornamental apple species, russet ring of apple fruits, and ring pattern mosaic of the leaves of pear and quince. Sensitive apple and quince cultivars can be heavily damaged; for example, yield of apples can be reduced in excess of 20%.

Apple stem pitting virus (ASPV) infections are latent in the great majority of commercial apple cultivars, whereas, in sensitive rootstocks, this virus elicits stem grooving, epinasty, that is, a marked curling of the leaves caused by necrosis of the main vein, and chlorotic/necrotic mottling of the leaves, followed by decline. Some of its strains are also responsible for diseases that affect the leaves (vein yellowing, necrotic spots) (**Figure 1(e)**) and fruits (stony pit) of pear, and the leaves (sooty ring spot), branches (necrotic grooves and bark necrosis), and fruits (deformation and stony pits) of quince.

Like ASPV, apple stem grooving virus (ASGV) infects latently ungrafted cultivars and rootstocks of apple, pear, and quince. However, a severe disease that leads to decline and death of the plants, known as 'apple junction necrotic pitting' or 'top working', develops when infected scions are used for grafting, especially on Virginia Crab, which reacts. Grafted plants react with stem grooving (**Figure 1(f)**), and necrosis of the tissues at the graft union (brown line) which leads to a rapid decline and death.

A somewhat similar disorder called 'union necrosis and decline' is induced by ToRSV in apples grafted on the rootstock MM.106. 'Flat apple', a disease induced by CRLV, is characterized by deformation of the fruits, reduced growth of lateral branches and upward rolling of the leaves.

Geographical Distribution

Except for TAMV, CRLV, and ToRSV, which are largely confined to the USA, all other pome fruit-infecting viruses (ApMV, ACLSV, ASGV, and ASPV) have a worldwide distribution.

Causal Agents and Classification

Affiliation and properties of ACLSV, ApMV, TAMV, CRLV, and ToRSV are as reported above. ASPV and ASGV are the type species of the genus *Foveavirus* and *Capillovirus*, respectively. Both viruses have filamentous particles *c.* 640 nm (ASGV) or *c.* 800 nm (ASPV) long, and a monopartite RNA genome 6495 nt (ASGV) and 9306 nt (ASPV) in size.

Transmission

ASPV, ASGV, and ACLSV have no known vector. Long-distance dissemination occurs via infected propagative material and nursery productions, whereas spread in apple orchards may take place through root grafts. The epidemiology of ilarviruses (ApMV, TAMV), the nepovirus ToRSV, and the cheravirus CRLV is as reported above.

Control

To control pome fruit viruses the same strategies used for stone fruit viruses can be implemented.

Virus Diseases of Walnut

Disease Symptoms and Yield Losses

Walnut blackline is a trunk union malady in English walnut scions propagated on rootstocks of several *Juglans* species. In California, blackline disease developed in English walnut trees propagated on seedlings of northern California black walnut, the hybrids Paradox and Royal, and Chinese wingnut (*Pierocarya stenoptera*).

In California, blackline diseased trees exhibit poor tree vigor and shoot growth, limb dieback, and yellow, drooped leaves. Rootstocks often produce sucker shoots. The main diagnostic symptoms are comprised of necrotic tissues embedded in bark tissue and into the woody cylinder at the scion-rootstock junction displayed as union blackline in trees of English walnut on black walnut rootstock or tissue necrosis extending downward from the scion-rootstock junction into Paradox rootstock. In Europe, grafted CLRV-infected trees develop a similar decline pattern. In addition, leaves develop chlorotic spots, rings, and yellow line patterns. Such leaf symptoms have not been observed in California.

Geographic Distribution

Walnut blackline disease has been reported in the USA (California and Oregon) and in Europe (Bulgaria, England, France, Hungary, Italy, Romania, and Spain).

Causal Agent and Classification

The incitant of walnut blackline disease is CLRV, genus *Nepovirus*.

Transmission

CLRV spreads by top-working trees with infected scions during cultivar conversion or in nature by pollen infecting healthy walnut trees during the flowering period. The virus is seed-borne and gives rise to infected seedlings.

Control

Use clean sources of scion wood and rootstocks. In orchards requiring supplemental pollination, pollen should be collected from CLRV-free trees.

Virus Disease of Hazelnut

Disease Symptoms and Yield Losses

Hazelnut mosaic diseased trees may develop leaves with chlorotic rings, flecking, and a variety of line patterns. Virus-infected trees may be symptomless also. Nut yields may be halved compared to production on healthy trees.

Geographical Distribution

The disease is reported in commercial orchards in Europe (Bulgaria, Italy, Turkey, Spain, Georgia) and likely occurs in other European countries. In the USA, hazelnut mosaic was detected in breeding lines and clonal germplasm importations, but not in commercial orchards.

Causal Agents and Classification

A complex of ilarviruses (ApMV, PNRSV, or TAMV) has been associated with diseased trees. In the USA, only ApMV has been identified in hazelnut trees.

Transmission

Hazelnut seeds harvested from ApMV-infected trees give rise to infected seedlings. ApMV is not known to be pollen-transmitted or vectored by insects. In contrast, PNRSV is known to be pollen- and seed-borne in several *Prunus* species.

Control

Propagate plants for planting from clean stocks.

See also: Nepovirus; Citrus Tristeza Virus; Flexiviruses; Tymoviruses; *Ilarvirus*; Plum Pox Virus.

Further Reading

Andret-Link P, Laporte C, Valat L, *et al.* (2004) Grapevine fanleaf virus: Still a major threat to the grapevine industry. *Journal of Plant Pathology* 86: 183–195.

Bovey R, Gärtel W, Hewitt WB, Martelli GP, and Vuittenez A (1980) *Virus and Virus-Like Diseases of Grapevines.* Lausanne: Editions Payot.

Desvignes JC, Boyé R, Cornaggia D, and Grasseau N (1999) *Maladies à Virus des Arbres Fruitiers.* Paris: Editions Ctifl.

Jones AL and Aldwinckle HS (eds.) (1990) *Compendium of Apple and Pear Diseases.* St. Paul, MN: APS Press.

Krake LR, Scott NS, Rezaian MA, and Taylor RH (1999) *Graft-Transmissible Diseases of Grapevines.* Collingwood, VIC: CSIRO.

Martelli GP (ed.) (1993) *Detection and Diagnosis of Graft-Transmissible Diseases of Grapevines.* Rome: FAO Publication Division.

Martelli GP and Boudon-Padieu E (2006) Directory of infectious diseases of grapevines. *Options Méditérranéens, Series B* 55: 11–201.

Mink GI (1992) Ilarvirus vectors. *Advances in Disease Vector Research* 9: 261–281.

Nemeth M (1986) *Virus, Mycoplasma, and Rickettsia Diseases of Fruit Trees.* Dordrecht, The Netherlands: Martinus Nijhoff.

Ogawa JM, Zehr EI, Bird GW, Ritchie DF, Uriu K, and Uyemoto JK (eds.) (1995) *Compendium of Stone Fruit and Diseases.* St. Paul, MN: APS Press.

Teviotdale BL, Michailides TJ, and Pscheidt JW (2002) *Compendium of Nut Crop Diseases in Temperate Zones.* St. Paul, MN: APS Press.

Walter B (ed.) (1997) *Sanitary selection of the grapevine. Protocols for Detection of Viruses and Virus-Like Diseases Les Colloques, No. 86.* Paris: INRA Editions.

Walter B, Boudon-Padieu E, and Ridé M (2000) *Maladies à virus, bacteries et phytoplasmes de la vigne.* Bordeaux: Editions Féret.

Wilcox WF, Gubler WD, and Uyemoto JK (eds.) (2007) *Compendium of Grape Diseases.* St. Paul, MN: APS Press.

Plant Virus Diseases: Ornamental Plants

J Engelmann and J Hamacher, INRES, University of Bonn, Bonn, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Chlorotic symptoms Degradation or depletion of chlorophyll leads to light green coloration of plant parts. Characteristic patterns such as ring spots, line patterns, and mosaics occur during virus infections.

Deformations Virus-induced growth abnormalities leading to reduced growth of cells, twisted or curled leaves or a stunted appearance of the whole plant.

Flower breaking Discoloration of the flowers often leading to an uneven distribution or total loss (bleaching) of pigments.

Generative propagation Propagation via pollen and seeds, nonuniform daughter generations are obtained.

Micropropagation Art and science of plant multiplication *in vitro*. The process includes many steps – stock plant care, explant selection, and sterilization, and media manipulation – to obtain proliferation, rooting, acclimation, and growing on of liners.

Necrotic symptoms These symptoms occur when cells die, giving rise to dull grayish, brown, or black coloration of the respective plant tissues. Necrotic lesions are often surrounded by darkly stained cells.

Phylogenetic origin The evolutionary relatedness among groups of organisms (e.g., species and populations). Phylogenetics treats a species as a group of lineage-connected individuals over time.

Serological methods Diagnostic methods based on the specific reaction of antibodies raised in rabbits, mice, or chicken by immunization with plant viruses. Methods are, e.g., ELISA, direct immunoblots, Western blots, or lateral flow tests.

Subgenomic RNA Short copy of a genomic RNA often existing as a single additional translatable RNA for capsid protein within a viral capsid. One of the

translation strategies of RNA-viruses to express their whole genetic information.
Vector transmission Most plant viruses are vectored by arthropods, often hemipteran insects. For persistently transmitted viruses, the virus is ingested, passes through the gut wall into the haemolymph, and then moves to the salivary glands where it can potentially be transmitted to other plants with the saliva. Long acquisition times of more than 1 h, a latency period of several days between uptake and release of the virus, and a long inoculation access of 1 h to several hours is characteristic. Persistently transmitted viruses, have two subclasses termed circulative if there is no multiplication in the insect vector and propagative if there is. For nonpersistently transmitted viruses, the virus is restricted to the tips of the insect stylet, where salivary duct and food canal coalesce. Nonpersistent viruses are efficiently transmitted after relatively brief (<5 min) acquisition and inoculation access periods. Semipersistent transmission is intermediate between nonpersistent and persistent. In this kind of transmission the virus moves to the foregut of the insect. Acquisition and inoculation access periods are longer than with nonpersistent transmission.
Vegetative propagation All kinds of plant propagation where parts of the plant are used to generate new plantlets, aiming uniform daughter generations.

Introduction

Virus diseases are of great economic importance in ornamentals because most of them are propagated vegetatively. This kind of plant propagation, either by tissue culture (micropropagation) or by scions, bulbs, rhizomes, or other tissues, is the most economic method to propagate plants maintaining their uniformity. Unfortunately, by this method viruses are propagated and spread from the mother stock to the next generation as well. After, for example, vegetatively propagated trailing petunias flooded the market in the 1990s, problems with tobamoviruses, mainly tobacco mosaic virus (TMV), arose quickly, compared to seed-propagated petunias.

The risk of virus infections in ornamental cultures is also raised by additional factors:

- introduction and establishment of novel virus vectors (e.g., *Thrips palmi*, *Frankliniella occidentalis*, *Bemisia tabaci*) and/or novel virus species into production areas;
- cross-breeding of novel, potentially virus-infected wild types of plants;
- introduction of novel exotic genera or species to broaden the range of ornamentals;
- cultural practices in closed irrigation and fertilization (nutrient solution) systems;
- worldwide trade with ornamentals; and
- production of plant material in countries representing different standards of production and/or other virus pressure.

As the number of ornamental species and varieties is very high and their phylogenetic as well as geographic origins vary considerably, the number of infecting agents, especially viruses, varies accordingly, giving rise to an immense range of virus–plant pathosystems. Viruses of ornamental plants have very different host ranges, many of them being ubiquitous, such as tomato spotted wilt virus (TSWV), impatiens necrotic spot virus (INSV), or cucumber mosaic virus (CMV). Some of them, however, are specialized and infect only certain species or genera of plants, such as, for example, pelargonium flower break virus (PFBV) or angelonia flower mottle virus (AFMoV). The following section gives an overview of the most common as well as some recently detected viral diseases of economically important ornamentals.

Virus Diseases in Diverse Ornamentals

Virus Diseases of Pelargonium

All pelargonias are often named geraniums. Pelargonias originated from the South African region. The main ornamental forms are obtained by cross-breeding of species, thus representing hybrids. Three forms of pelargonium are of main importance in floriculture: *Pelargonium grandiflorum* hybrids (pot plants), *P. peltatum* hybrids (ivy-leaved hanging pelargonias), and *P. zonale* hybrids (upright growing), the latter two forms being of highest economical importance as bedding or balcony plants. As pelargonias are usually propagated by cuttings or tissue culture, viral diseases endanger production and quality of that crop. In pelargonia, many virus symptoms evolving in late winter and early spring will disappear during summer and may reappear in the next season.

Specific pelargonium viruses

The most prevalent virus in Europe is by far PFBV, a virus assigned to the genus *Carmovirus*, family *Tombusviridae*. This icosahedral virus spreads via draining water, via contaminated pollen carried by thrips vectors, possibly by allowing virus from the surface of infected pollen to enter through feeding wounds, and most efficiently by vegetative propagation of infected plant material. Symptoms vary according to time of the year and cultural practice: pink flowering varieties show flower break (**Figure 1**), leaf

symptoms vary from symptomless to light mottling and to ring spotting (**Figure 2**) as well as line patterns. The virus occurs more often in *P. zonale* than in *P. peltatum* hybrids (**Figure 3**).



Figure 1 Flower breaking on *P. zonale* induced by PFBV. By Joachim Hamacher.



Figure 2 Ring spots on *P. zonale* induced by PFBV. By Joachim Hamacher.

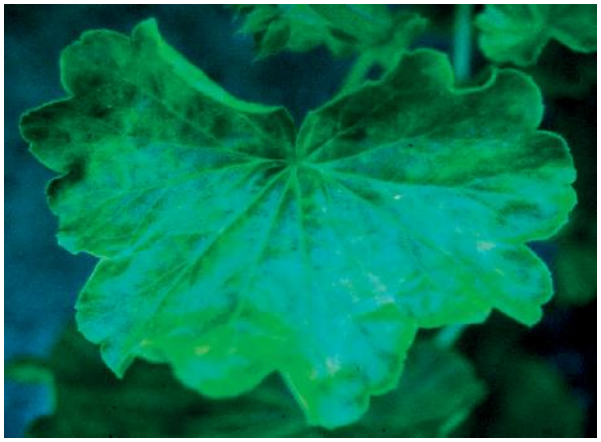


Figure 3 Leaf mottling on *P. peltatum* (PFBV). By Joachim Hamacher.

There has been considerable confusion about some pelargonium viruses causing very similar ring spot and line pattern symptoms. Investigations of these viruses on the molecular level solved the initial disarrangement: four different virus species have been characterized which are proposed to form a new genus of plant viruses named *Pelarspovirus*.

Pelargonium line pattern virus (PLPV) is a spherical virus, 30–32 nm in diameter, belonging to the family *Tombusviridae*, which has many traits of a carmovirus, but is different in one type of protein not expressed by carmoviruses and transcribes only one subgenomic RNA instead of two. It was reported initially in *P. zonale* from Great Britain. Most infections seem to be symptomless, but chlorotic rings and line patterns may develop (**Figure 4**). Investigations from Spain report that PLPV represents the most common symptomless virus disease of pelargonias. It can be transmitted mechanically and by vegetative propagation.

Pelargonium ring spot virus (PelRSV) produces symptoms of pronounced ring and line patterns resembling those induced by PLPV. PelRSV could, however, be clearly distinguished from PLPV by serology and nucleotide sequence as well as by the size of double-stranded RNAs (dsRNAs) produced in *Chenopodium quinoa*.

A further virus belonging to the family *Tombusviridae*, pelargonium chlorotic ring pattern virus (PCRPV) was isolated in Italy as geranium virus isolate 57. It was characterized and proposed to belong to the new virus genus mentioned above. This virus also leads to distinct chlorotic ring pattern symptoms (**Figure 5**).

Symptoms of pelargonium leaf curl virus, assigned to the genus *Tombusvirus*, were first reported in *P. zonale* from Germany as early as 1927. The symptoms are characterized by the appearance of small white-yellowish spots on leaves, which enlarge, becoming round flecks or stellate spots with



Figure 4 Chlorotic ring spots and line patterns induced by PLPV. By Joachim Hamacher.

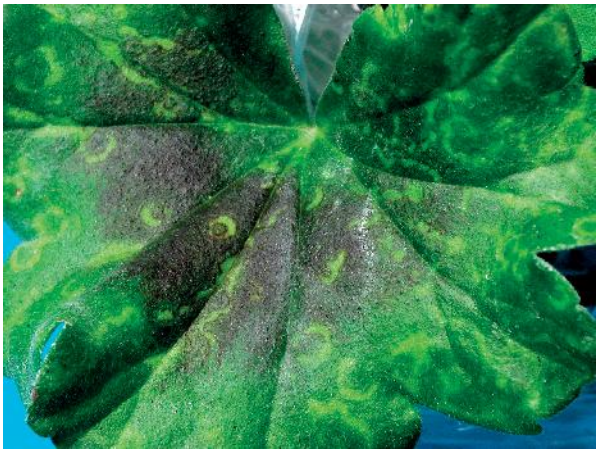


Figure 5 Chlorotic ring patterns on *P. peltazonale* induced by PCRPV. By Joachim Hamacher.



Figure 6 Whitish spots on PLCV-infected *P. zonale*. By Joachim Hamacher.



Figure 7 Leaf crinkling and curling on *P. zonale* caused by PLCV. By Joachim Hamacher.



Figure 8 Yellow net vein symptoms on *P. zonale*, pathogen unknown. By Joachim Hamacher.

necrotic centers (**Figure 6**). In later infection stages leaf crinkling, leaf splitting, and plant degeneration or dwarfing occurs (**Figure 7**). Leaf symptoms will disappear in summer. The virus can be transmitted by water or nutrient solutions in soil-free substrates, mechanically, though with some difficulty, with plant sap, and most efficiently by cuttings from infected symptom-free sources.

Pelargonium zonate spot virus (PZSV), a virus now assigned to the new genus *Anulavirus*, family *Bromoviridae* has been reported from Italy (Apulia). PZSV causes concentric chrome-yellow bands in the leaves of *P. zonale*. Besides mechanical transmission, it can be transmitted by seeds and by pollen in *Nicotiana glutinosa* and *Diplotaxis erucoides*.

Pelargonium vein clearing virus (PelVCV) syn. eggplant mottled dwarf virus (EMDV), assigned to the genus *Cytorhabdovirus*, is reported from southern Italy. It causes mild vein clearing in *P. peltatum* and *P. zonale*. Transmission occurs via mechanical transmission and by vegetative propagation.

An often observed anomaly of pelargonium is named yellow net vein disease. It is graft transmissible and leads to obvious veinal chlorosis (**Figure 8**), persisting throughout the year. A causal virus or other agent has not been isolated or identified up to now.

Pelargonium nonspecific viruses

Some nepoviruses also infect pelargonias, mainly in North American varieties: tomato ringspot virus (ToRSV), tobacco ring spot virus (TRSV), and tomato black ring virus (TBRV) lead to ring spotting and chlorotic flecking and stippling (**Figure 9**). These viruses cause quarantine diseases because of their potential to infect fruit trees and their transmissibility by nematodes, pollen, and seeds.

A considerable threat for floriculturists are two tospoviruses: TSWV and INSV – the first infecting a great number of vegetables and ornamentals, the latter mainly ornamentals. TSWV infects *P. peltatum*, producing ring spots and line patterns (**Figure 10**). INSV infects pelargonias very

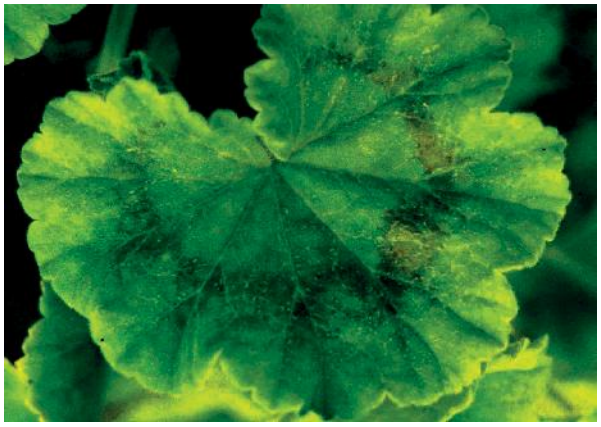


Figure 9 Chlorotic stippling of *P. zonale* induced by TBRV. By Joachim Hamacher.



Figure 11 Chlorotic flecking on *P. peltatum* induced by CMV (holes were punched in the left-hand leaf for analytical purposes). By Dietrich E. Lesemann.



Figure 10 Chlorotic line patterns and rings induced by TSWV on *P. peltatum*. By Joachim Hamacher.

rarely. Both viruses are transmitted by thrips, mainly the western flower thrips (*Frankliniella occidentalis*, Pergande).

CMV is a very ubiquitous virus with the widest known host range of all plant viruses, exceeding 1000 host plant species. It occurs with many different strains, may infect pelargonias and leads to pronounced flecking (**Figure 11**), asymmetry of leaves as well as breaking of the characteristic brown horseshoe zone in *P. zonale*. It can be transmitted by many aphid species in a nonpersistent manner.

Virus Diseases of Solanaceae

Petunia, calibrachoa

When vegetatively propagated trailing petunias (surfinias) came on the market, problems with viruses, unknown in seed-propagated petunias, arose. More than 150 different virus species may infect petunias, but only some of them infect petunias naturally. Petunias are vigorously growing plants requiring a balanced fertilization, water supply, and culture maintenance. If these requirements are not achieved, symptoms suspicious of viral origin may arise very soon. This has to be considered when monitoring the crops.

Specific petunia viruses

A new virus disease of petunias has been identified recently in Brazil. The pathogen has been named petunia vein banding virus (PetVBV) and is assigned to the genus *Tymovirus*. The virus occurred in mixed infections with another spherical virus (possibly PVCV) and produced local chlorotic and necrotic spots and systemic vein banding on petunias when inoculated as single pathogen.

Petunia vein clearing virus (PVCV), genus *Petuvirus*, eludes detection by immunological methods most time of the year, since its dsDNA genome is integrated in the host genome of some Petunia varieties. The genome expression is activated only in late winter/early spring or by stress. During symptom development, few icosahedral virus particles measuring about 45 nm in diameter can be observed by electron microscopy (**Figure 12**). Symptoms are veinal chlorosis with shrunken leaf veins (**Figure 13**) sometimes becoming necrotic.

Viruses nonspecific of petunias

The most frequently detected virus in trailing petunias is TMV. Symptoms vary according to variety, fertilization, and developmental stage of the plant. Veinal chlorosis (**Figure 14**) and necrosis (**Figure 15**), mottling (**Figure 16**), blistering with dark green areas as well as leaf curl and stunting of whole plants may appear. Flowers may also express color deviations as observed with bicolored petunia varieties (**Figure 17**). Tomato mosaic virus (ToMV)-infected plants show similar symptoms, although symptom expression is less severe. Mixed infections of both viruses occur as well. Two other tobamoviruses,

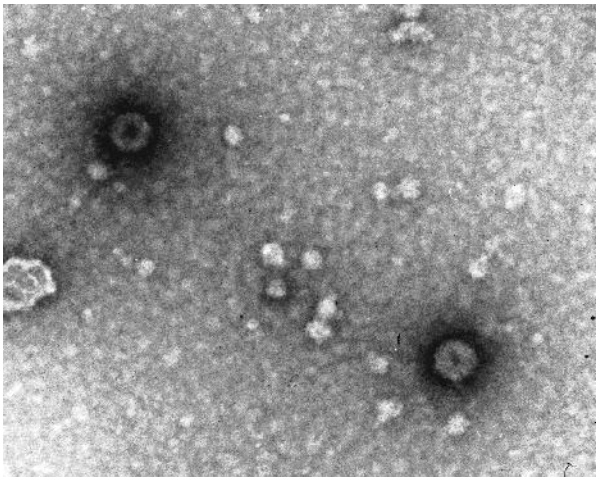


Figure 12 Spherical particles of PVCV from infected petunia showing darkly staining centers. Bar represents 50 nm. By Joachim Hamacher.



Figure 13 Veinal chlorosis of petunia leaves caused by PVCV. By Dietrich E. Lesemann.



Figure 14 Leaf vein chlorosis of young petunia plants induced by TMV. By Joachim Hamacher.

tobacco mild green mosaic virus (TMGMV) and turnip vein clearing virus (TVCV) have been detected in petunias, both leading to severe necrotic symptoms in petunias (**Figure 18**).



Figure 15 Necrotic leaf spots and veins of petunia caused by TMV. By Joachim Hamacher.



Figure 16 Mottling of TMV-infected petunia leaves. By Joachim Hamacher.



Figure 17 Flower breaking in bicolored petunia varieties with TMV infection. By Joachim Hamacher.

Transmission of tobamoviruses with contaminated plant sap is very easy and results in high infection rates, as petunias are vigorously growing plants with soft leaves and many hairs, which break when plants are handled.

Potato virus Y (PVY) is the type virus of the genus *Potyvirus* and often found in petunias. The main symptoms are mottling of leaves and color deviations (mottling) in purple flowering plants, also occurring in mixed infections with CMV (**Figure 19**). Another potyvirus infecting petunias, which induces flower breaking is turnip mosaic virus (TuMV). Potyviruses are aphid transmissible in a nonpersistent manner.

Tospoviruses may infect petunias locally but represent no real danger, as vegetative propagation will not transmit these viruses effectively. Nonflowering petunias, interestingly, may be used to monitor the occurrence of tospoviruliferous thrips, because they develop brown to black local lesions at the edges of thrips-feeding scars when infection has occurred.

Two viruses of the genus *Cucumovirus*, CMV and tomato aspermy virus (TAV) may infect petunias, leading to mosaic, flecking (**Figure 20**) or mottled leaves and TAV to flower deformation. Both viruses are aphid transmissible in a nonpersistent manner.

Alfalfa mosaic virus (AMV) produces marbling of leaves (**Figure 21**) and flower breaking in petunia.

Some nepoviruses (ArMV, TBRV, ToRSV, and TRSV) are reported to infect petunias, producing ring spots, line patterns, or tip necrosis. The symptoms will disappear in the course of infection.

Tobacco necrosis virus (TNV), tobacco rattle virus (TRV), and tobacco streak virus (TSV), were detected to infect petunias, but seem to appear only sporadically.

Some viruses naturally infecting petunias are of minor importance as they do not evoke obvious symptoms. *Calibrachoa mottle virus* (CbMV), assigned as a new virus species mainly infecting *Calibrachoa* sp., does not produce symptoms in petunias. Broad bean wilt virus 1 (BBWV1) is another latent virus of petunia.



Figure 18 Crippling of TMGMV-infected petunia scions (necrosis and deformations). By Joachim Hamacher.

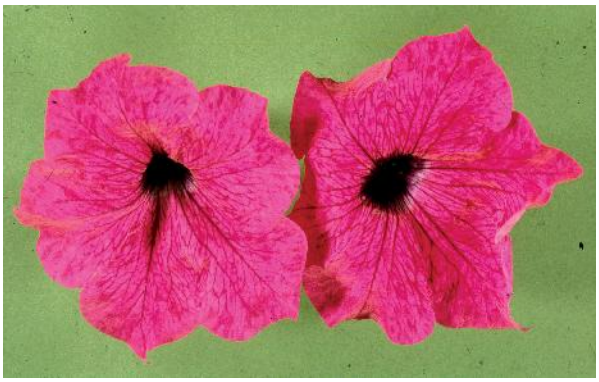


Figure 19 Flower mottle of purple *Surfinia* caused by a mixed infection with PVY and CMV. By Dietrich E. Lesemann.



Figure 20 Bright yellow leaf spots of CMV-infected Surfinia. By Joachim Hamacher.



Figure 21 Marbling of petunia leaves induced by AMV. By Joachim Hamacher.



Figure 22 Chlorotic leaf blotching and mottling of *Calibrachoa* infected by CbMV. By Joachim Hamacher.



Figure 23 Chlorotic line patterns in ToMV-infected *Calibrachoa* leaves. By Joachim Hamacher.

Calibrachoa or 'Million Bells'

The name of calibrachoa goes back to the nineteenth century Mexican botanist and pharmacologist Antonio de Cal y Bracho. Million Bells are solanaceous bedding and balcony plants with growing popularity.

The most frequently detected virus of calibrachoas has been characterized recently, named calibrachoa mottle virus (CbMV) and has been tentatively assigned to the genus *Carmovirus*. Symptoms evolving after infection vary from light mottling to chlorotic blotching (**Figure 22**), but some varieties may remain symptomless.

Tobamoviruses play an important role in calibrachoas. ToMV is of greater importance than TMV, as it leads to pronounced chlorotic ring spots and line patterns (**Figure 23**), whereas TMV remains symptomless or induces only light mottling.

Some other viruses have been diagnosed in calibrachoas such ArMV, TRSV, BBWV1, INSV, PMMoV, RMV, CVB, PVY, and other potyviruses, as well as PNRV and TSV.

Virus Diseases of Balsaminaceae

Impatiens New Guinea hybrids and *I. walleriana* play a prominent role as bedding and balcony plants and may be hosts for quite a lot of virus diseases. The prevalent virus of *Impatiens* spp. is INSV. The symptoms observed after infection with INSV are necrotic spots (**Figure 24**), ring spots or mottling of leaves, and necrotic flecking of stems. On *I. New Guinea* hybrids the discolorations are often accompanied by foliar deformations (**Figure 25**). Besides vegetative propagation, virus transmission of tospoviruses within crops is mainly due to feeding of *Frankliniella occidentalis* (Pergande) the western flower thrips. Other thrips species may transmit as well but are of minor importance (**Table 1**). TSWV occurs by far less frequently on impatiens than INSV, but induces very similar necrotic symptoms.

Tobamoviruses are as well quite common in impatiens. TMGMV and TVCV have been diagnosed in *I. New Guinea* hybrids. The symptoms are very similar and comprise stunting, leaf deformation, or die back of young



Figure 24 Necrotic spots on leaves of INSV-infected *Impatiens New Guinea* hybrid (NGI). By Joachim Hamacher.



Figure 25 Leaf deformation and necrotic spots due to INSV infection of NGI. By Joachim Hamacher.

plants (**Figure 26**), leaf necrosis and deformation as well as foliar reddening on older plants. Pink or orange flowering varieties may exhibit flecking of flowers (**Figure 27**). Black line patterns, resembling symptoms induced by INSV or TSWV, have also been observed on some varieties. The viruses may be symptomless on mature plants. Both viruses may be transmitted by mechanical injury when handling the plants, by contaminated substrate and pots as well as by irrigation.

Leaf narrowing and rugged leaf borders as well as split petals and color breaking are typical symptoms of infections with CMV (**Figures 28** and **29**). Several aphid species may transmit the virus in a nonpersistent manner within seconds.

Further virus species infecting impatiens are TuMV, AMV (aphid transmissible in a nonpersistent manner as well), and TSV, a seed-borne ilarvirus, which probably may be transmitted with contaminated pollen via thrips feeding.

TuMV leads to dark areas at the base or tip of the leaves of some varieties of New Guinea hybrids as well as to asymmetry of the leaves. AMV-infected plants show slight leaf necrosis or cloudy yellowish discoloration of the leaves of green-leaved varieties (**Figure 30**). TSV is reported to occur quite frequently. It induces only slight mosaic on the lower side of the leaves or remains symptomless.

An hitherto not characterized virus on both species of cultivated impatiens occurs rather frequently in Europe and produces chlorotic to yellow concentric ring spots and line patterns (**Figures 31** and **32**). It is graft transmissible between *I. New Guinea* hybrids and *I. walleriana*. In plants exhibiting such symptoms, few spherical virus-like particles, with about 30 nm diameter could be observed by electron microscopy in newly infected tissue (**Figure 33**). A provisional name for the pathogen could be impatiens chlorotic line pattern agent.

Virus Diseases of Scrophulariaceae

Bacopa, Diascia, Nemesia, Angelonia

Bacopa (*Sutera cordata*), *Nemesia* (*N. fruticans* hybrids), *Diascia* (*D. vigilis*), and *Angelonia* (*A. angustifolia*) are genera of the family Scrophulariaceae, continuously gaining popularity.

Sutera cordata (diffusa), also known as *Bacopa,* is often infected by potyviruses. Lettuce mosaic virus (LMV) was detected by electron microscopy as well as by enzyme-linked immunosorbent assay (ELISA). Little and dented leaves, leaf curl, and marginal foliar necroses (**Figure 34**) were observed, but most frequently no symptoms were induced at all. Other filamentous viruses of bacopas are PVY and CVB. BBWV 1 was detected sporadically in bacopa, leading to mottling and flecking of leaves. Tobamoviruses, such as ToMV and TMV, tospoviruses (INSV and TSWV), as well as viruses belonging to the family *Bromoviridae* (AMV and CMV), have also been detected in bacopa.

Nemesia ring necrosis virus (NeRNV) has only recently been described as a new virus and assigned to the genus *Tymovirus*. Before identification as a new species, infections with the respective symptoms have been thought to be induced by scrophularia mottle virus (ScrMV), because of its strong immunological cross-reaction with that virus. NeRNV is widespread, leading to foliar concentric necrotic ring spots and line patterns as well as to sporadical flecking of flowers (**Figures 35** and **36**) in Nemesia, and in Diascia to black discoloration and line patterns, as well as dwarfing of the whole plant or parts of the plant (**Figures 37** and **38**).

Infections of nemesia with tospoviruses lead to necrotic areas and leaf narrowing (**Figure 39**), and in mixed

Table 1 Virus acronyms, species, and genera

Acronym	*Virus/viroid species*	*Virus/viroid genus*	*Virus/viroid family*
AFMoV	*Angelonia flower mottle virus**	*Carmovirus*	*Tombusviridae*
AMV	*Alfalfa mosaic virus*	*Alfamovirus*	*Bromoviridae*
AnFBV	*Angelonia flower break virus*	*Carmovirus*	*Tombusviridae*
ApMV	*Apple mosaic virus*	*Ilarvirus*	*Bromoviridae*
ArMV	*Arabis mosaic virus*	*Nepovirus*	*Comoviridae*
BBWV 1	*Broad bean wilt virus 1*	*Fabavirus*	*Comoviridae*
BCMV	*Bean common mosaic virus*	*Potyvirus*	*Potyviridae*
BWYV	*Beet western yellows virus*	*Polerovirus*	*Luteoviridae*
BYMV	*Bean yellow mosaic virus*	*Potyvirus*	*Potyviridae*
CRSV	*Carnation ringspot virus*	*Dianthovirus*	*Tombusviridae*
CbMV	*Calibrachoa mottle virus**	*Carmovirus*	*Tombusviridae*
ClYMV	*Clover yellow mosaic virus*	*Potexvirus*	*Flexiviridae*
CMV	*Cucumber mosaic virus*	*Cucumovirus*	*Bromoviridae*
CSNV	*Chrysanthemum stem necrosis virus*	*tent. Tospovirus*	*Bunyaviridae*
CSVd	*Chrysanthemum stunt viroid*	*Pospiviroid*	*Pospiviroidae*
CVB	*Chrysanthemum virus B*	*Carlavirus*	*Flexiviridae*
CymMV	*Cymbidium mosaic virus*	*Potexvirus*	*Flexiviridae*
CymRSV	*Cymbidium ringspot virus*	*Tombusvirus*	*Tombusviridae*
CypCSV	*Cypripedium chlorotic streak virus*	tent. *Potyvirus*	*Potyviridae*
CypVY	*Cypripedium virus Y*	*Potyvirus*	*Potyviridae*
EMDV	*Eggplant mottled dwarf virus syn. Pelargonium vein clearing virus*	*Nucleohabdovirus*	*Rhabdoviridae*
DMV	*Dahlia mosaic virus*	*Caulimovirus*	*Caulimoviridae*
DVNV	*Dendrobium vein necrosis virus*	tent. *Closterovirus*	*Closteroviridae*
INSV	*Impatiens necrotic spot virus*	*Tospovirus*	*Bunyaviridae*
LMV	*Lettuce mosaic virus*	*Potyvirus*	*Potyviridae*
LSV	*Lily symptomless virus*	*Carlavirus*	*Flexiviridae*
LVX	*Lily virus X*	*Potexvirus*	*Flexiviridae*
MNSV	*Melon necrotic spot virus*	*Carmovirus*	*Tombusviridae*
NeRNV	*Nemesia ring necrosis virus**	*Tymovirus*	
OFV	*Orchid fleck virus*	*Rhabdovirus*	*Rhabdoviridae*
ORSV	*Odontoglossum ringspot virus*	*Tobamovirus*	
PCRPV	*Pelargonium chlorotic ring pattern virus**	*Pelarspovirus (proposed)*	*Tombusviridae*
PelRSV	*Pelargonium ring spot virus**	*Pelarspovirus (proposed)*	*Tombusviridae*
PelVCV	*Pelargonium vein clearing virus* syn. *Eggplant mottled dwarf virus*	*Cytorhabdovirus*	*Rhabdoviridae*
PetVBV	*Petunia vein banding virus*	*Tymovirus*	
PFBV	*Pelargonium flower break*	*Carmovirus*	*Tombusviridae*
PMMoV	*Pepper mild mottle virus*	*Tobamovirus*	
PLCV	*Pelargonium leaf curl virus*	*Tombusvirus*	*Tombusviridae*
PLPV	*Pelargonium line pattern virus**	*Pelarspovirus (proposed)*	*Tombusviridae*
PNRSV	*Prunus necrotic ringspot virus*	*Ilarvirus*	*Bromoviridae*
PVCV	*Petunia vein clearing virus*	*Petuvirus*	*Caulimoviridae*
PVY	*Potato virus Y*	*Potyvirus*	*Potyviridae*
PZSV	*Pelargonium zonate spot virus*	*Anulavirus (proposed)*	*Bromoviridae*
ReTBV	*Rembrandt tulip breaking virus*	*Potyvirus*	*Potyviridae*
RMV	*Ribgrass mosaic virus*	*Tobamovirus*	
SLRSV	*Strawberry latent ringspot virus*	*Sadwavirus*	*Comoviridae*
SMV	*Soybean mosaic virus*	*Potyvirus*	*Potyviridae*
SrMV	*Scrophularia mottle virus*	*Tymovirus*	
TAV	*Tomato aspermy virus*	*Cucumovirus*	*Bromoviridae*
TBV	*Tulip breaking virus*	*Potyvirus*	*Potyviridae*
TBRV	*Tomato black ring virus*	*Nepovirus*	*Comoviridae*
TBSV	*Tomato bushy stunt virus*	*Tombusvirus*	*Tombusviridae*
TCBV	*Tulip chlorotic blotch virus syn. Turnip mosaic virus*	*Potyvirus*	*Potyviridae*
TEV	*Tobacco etch virus*	*Potyvirus*	*Potyviridae*
TMGMV	*Tobacco mild green mosaic*	*Tobamovirus*	
TMV	*Tobacco mosaic virus*	*Tobamovirus*	
TNV	*Tobacco necrosis virus*	*Necrovirus*	*Tombusviridae*
ToMV	*Tomato mosaic virus*	*Tobamovirus*	
ToRSV	*Tomato ringspot virus*	*Nepovirus*	*Comoviridae*
TRSV	*Tobacco ringspot virus*	*Nepovirus*	*Comoviridae*
TRV	*Tobacco rattle virus*	*Tobravirus*	
TSV	*Tobacco streak virus*	*Ilarvirus*	*Bromoviridae*

Continued

Table 1 Continued

Acronym	*Virus/viroid species*	*Virus/viroid genus*	*Virus/viroid family*
TSWV	*Tomato spotted wilt virus*	*Tospovirus*	*Bunyaviridae*
TulMV	*Tulip mosaic virus*	*Potyvirus*	*Potyviridae*
TuMV	*Turnip mosaic virus*	*Potyvirus*	*Potyviridae*
TVCV	*Turnip vein clearing virus*	*Tobamovirus*	
VanMV	*Vanilla mosaic virus*	*Potyvirus*	*Potyviridae*
VeLV	*Verbena latent virus*	*Carlavirus*	*Flexiviridae*

List of acronyms, virus names, and viral genera.
Names with an asterisk do not appear in the ICTV virus name list.
Adapted from Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International. Committee on Taxonomy of Viruses*. San Diego, CA: Elsevier Academic Press.



Figure 26 Leaf necrosis and distortion of NGI caused by TMGMV. By Joachim Hamacher.



Figure 28 Leaf narrowing and curling of red-leaved NGI infected with CMV. By Joachim Hamacher.



Figure 27 Flower mottle of pink-flowering NGI caused by infection with TMGMV. By Joachim Hamacher.



Figure 29 Flower breaking and deformation of bicolored NGI induced by CMV. By Joachim Hamacher.

infections with NeRNV and TSWV to bleaching of flowers and necrotic flecks and ring spots (**Figures 40** and **41**).

A new virus disease of *Angelonia* sp. has recently been proved to be induced by a new putative member of the genus *Carmovirus*, for which the names AFMoV or angelonia flower break virus (AnFBV) respectively have been proposed. The virus (~30 nm in diameter, **Figure 42**) leads to dark blotching of flowers (**Figure 43**). The virus occurs very frequently in angelonias.



Figure 30 Cloudy yellow mottling of green-leaved NGI infected with AMV. By Joachim Hamacher.



Figure 32 Marbling of *Impatiens walleriana* leaves infected with an unknown virus (impatiens chlorotic line pattern agent) after graft transmission from NGI. By Joachim Hamacher.



Figure 31 Chlorotic line pattern on leaves of NGI infected by an unknown virus (impatiens chlorotic line pattern agent). By Joachim Hamacher.

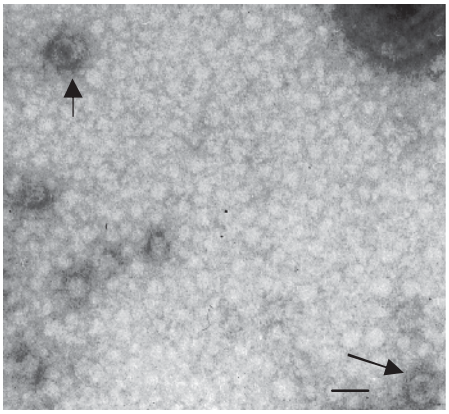


Figure 33 Electron micrograph of impatiens chlorotic line pattern virus-like particles (arrowheads) in plant sap of *I. walleriana* exhibiting symptoms of chlorotic line pattern. Bar represents 35 nm. By Joachim Hamacher.

Virus Diseases of Verbenaceae

Verbenas are traded as upright-, trailing-, and ground-covering forms (e.g., Tapien and Temari). Virus infections of verbenas occur frequently and are induced by a lot of virus species. AFMoV has only recently been detected to occur in varieties of verbenas. It may be latent or leads to distinct flecking of the plants, depending on the respective variety, and to degeneration when co-infected with INSV (**Figures 44** and **45**).

NeRNV has been reported from the UK to induce necrotic and chlorotic flecking, whereas clover yellow mosaic virus (ClYMV) leads to darkly staining and necrotic spots on the base of leaves as well as on stems in trailing verbenas.



Figure 34 LMV-infected bacopa with dented leaves and marginal necrosis. By Joachim Hamacher.



Figure 35 Blotches and ring spots on flowers of nemesia infected with NeRNV. By Joachim Hamacher.



Figure 36 Necrotic concentric ring spots on leaf of nemesia infected with NeRNV. By Joachim Hamacher.

TSWV, INSV, CMV, BBWV1, AMV, and TMV have as well been diagnosed in verbenas. INSV and TSWV cause necrotic flecking, ring spotting, and line patterns (**Figure 46**). CMV and BBWV1 lead to mottling, necrosis, and little leaves (**Figures 47** and **48**). TMV causes yellow mottling, necrotic leaf borders, or die-back of leaves (**Figure 49**).



Figure 37 Black rings and lines on NeRNV-infected diascia leaves. By Joachim Hamacher.

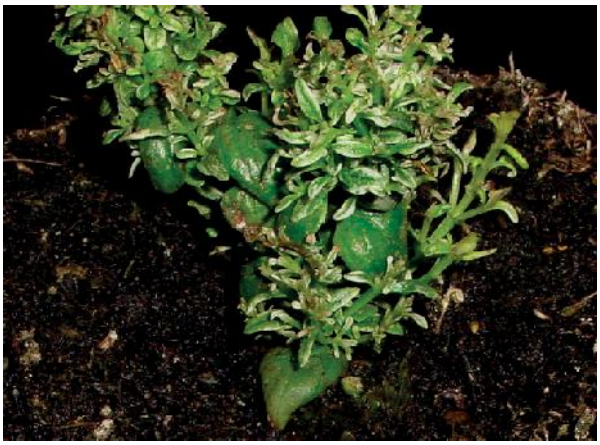


Figure 38 Dwarfed shoots of diascia infected with NeRNV. By Joachim Hamacher.

More viruses have been reported to infect verbenas: ApMV, TSV, ArMV, TRSV, ToRSV, CVB, VeLV, CRSV, MNSV, TEV, and a strain of BYMV, PNRV, RMV, and ToMV (for virus names see **Table 1**).



Figure 39 Necrotic symptoms and leaf narrowing on INSV-infected nemesia. By Joachim Hamacher.



Figure 40 Flower bleaching of nemesia co-infected with NeRNV and TSWV. By Joachim Hamacher.



Figure 41 Necrotic ring spots and flecks on nemesia leaves co-infected with NeRNV and TSWV. By Joachim Hamacher.

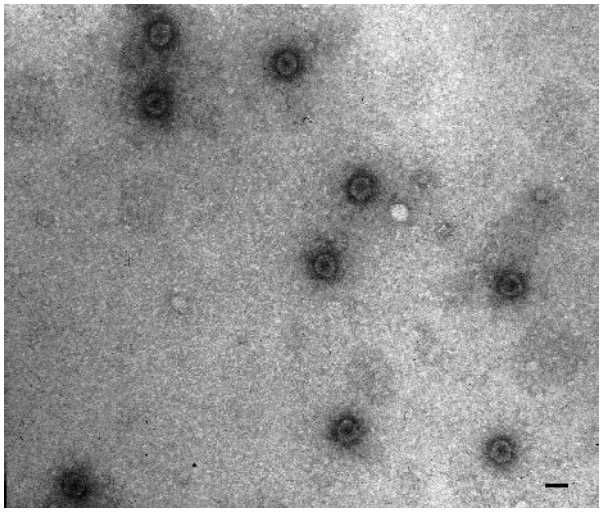


Figure 42 Electron micrograph of particles of AFMoV in plant sap from infected angelonia. Bar represents 30 nm. By Joachim Hamacher.



Figure 43 Darkly colored flecks on flowers (flower mottle) of angelonia infected with AFMoV. By Joachim Hamacher.



Figure 44 Chlorotic spots on leaves of verbena co-infected with AFMoV and INSV. By Joachim Hamacher.



Figure 45 Necrotization of lower leaves of verbena co-infected with AFMoV and INSV. By Joachim Hamacher.



Figure 46 Necrotic lines and spots on TSWV-infected verbena. By Joachim Hamacher.



Figure 47 Necrotic spots and mottling on leaves of BBWV1-infected verbena. By Joachim Hamacher.



Figure 48 Light mottling and little leaves of BBWV1-infected verbena. By Joachim Hamacher.

Virus Diseases of Compositae

Osteospermum and dimorphotheca, dahlia, and chrysanthemum

Compositae or Asteraceae include plant species from all over the world

The South African species *Osteospermum* and *Dimorphotheca*, vernacular name, African daisy or cape daisy, are closely related genera and consist of about 70 (*Osteospermum*) and 2 (*Dimorphotheca*) species of evergreen shrubs, half shrubs, or annual plants. Osteospermums are relatively new to most gardeners. They have gained considerable popularity as summer bedding, balcony, and potted plants in the last decade. The frequently occurring virus disease in both species is caused by LMV which leads to foliar mottling, while some isolates also induce flower breaking. In some cases plants remain symptomless.

The tospoviruses TSWV and INSV may disturb plant growth, leading to a dwarfed appearance, deformed leaves, and chlorosis (**Figures 50** and **51**).

Infections with CMV also lead to deformation of plant parts: leaf narrowing and dwarfing in combination with chlorosis are typical symptoms (**Figure 52**).

Dahlias are native of Mexico and Latin America and belong to a genus with only 30 species. The very important bedding and cut flower forms go back to three species *D. coccinea* Cav., *D. pinnata* Cav., and *D. juarezii* hort. The latter most probably being already a garden form. The modern cultivars result from breeding in West European countries.

Dahlias are frequently infected by dahlia mosaic virus (DMV), a spherical dsDNA virus which mostly induces chlorotic oak leaf patterns (**Figure 53**) and other chlorotic symptoms and may induce stunting of susceptible varieties. The virus is spread via aphids in a nonpersistent or semipersistent way.

TSWV and INSV are among the commonly occurring viruses of dahlias. Symptoms may vary according to virus

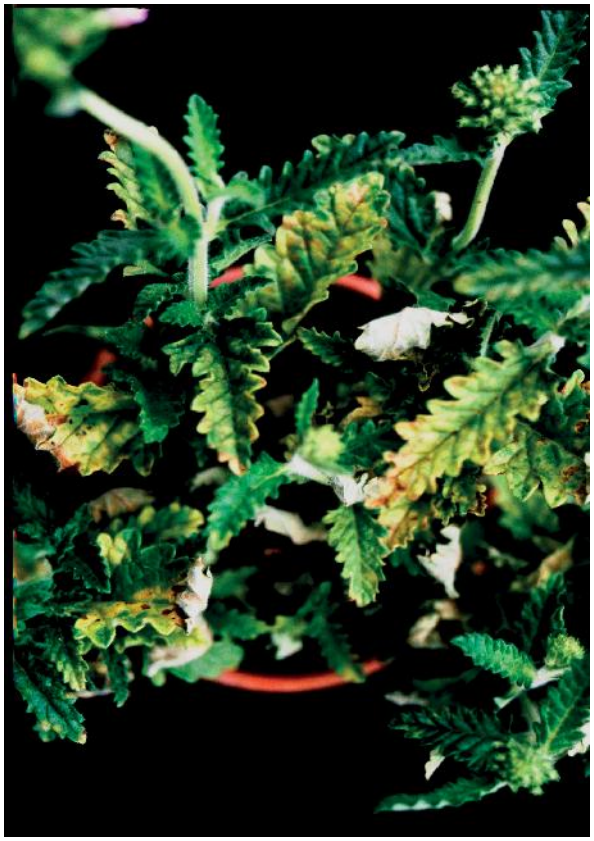


Figure 49 TMV-infected verbena, yellow mottling of leaves, and necrotization of leaf margins. By Joachim Hamacher.



Figure 50 Dwarfing of TSWV-infected osteospermum (left), noninfected control (right). By Joachim Hamacher.

and variety but most often these viruses induce necrotic ring spots becoming concentric (**Figures 54** and **55**). Oak leaf patterns or discrete necrotic lines along the midrib may also appear in young leaves of certain cultivars.



Figure 51 Leaf narrowing, chlorosis, and stunting of TSWV-infected osteospermum plant. By Joachim Hamacher.



Figure 52 CMV infection of osteospermum: leaf narrowing of plant parts. By Joachim Hamacher.



Figure 53 Chlorotic oak leaf pattern on DMV-infected dahlia. By Joachim Hamacher.

Symptoms induced by CMV vary according to the developmental stage of the plant. They comprise light mosaic and typical leaf narrowing, also called 'fern leaf'. In some cultivars oak leaf patterns may prevail (compare **Figure 53**).

TSV appears rather frequently in dahlias. It does not induce typical or severe symptoms, but represents a potential danger for other crops.

The former genus *Chrysanthemum* encompasses about 200 species, originating predominantly from the Mediterranean region and Western Asia but some come from South Africa. *Chrysanthemum indicum* hybrids (*Dendranthema indicum* and *D. x grandiflorum*) originate from China to Japan. The genus *Chrysanthemum* is now differentiated into 14 genera, the economically most important of which are *Argyranthemum* (species *A. frutescens*), traded as potted flowering shrubs) and *Dendranthema* (species *Dendranthema x grandiflorum*), traded as cut plants or potted plants. *Chrysanthemum* is one of the leading ornamentals in the international market.



Figure 54 Chlorotic concentric rings and necrotic lines on TSWV-infected dahlia. By Joachim Hamacher.

Dendranthema hybrids are most frequently infected by chrysanthemum stunt viroid (CSVd). The disease originated in the USA but reached Europe in the 1950s with infected cultivars exported to England. About 70% of the infected plants are systemically stunted with dwarfed leaves (**Figure 56**) and scrubby flowers, exhibiting floral bleaching of red colored cultivars. Color deviations of leaves are not always obvious, but may include pale, upright young leaves. Sometimes, leaf spots or flecks, often associated with leaf distortions ('crinkling'), are also observed. Symptoms are variable and highly dependent on environmental conditions, especially temperature and light. As CSVd is very stable *in vitro*, sap transmission during handling and cutting may occur. Detection of the viroid may be done by visual inspection, grafting onto indicator varieties, bidirectional electrophoresis, molecular hybridization, as well as reverse transcriptase polymerase chain reaction (RT-PCR) and nested RT-PCR, but not by immunological methods or electron microscopy.

A common virus of chrysanthemums is chrysanthemum virus B (CVB), a carlavirus. It leads to very mild leaf mottling or vein clearing in some cultivars; some infected varieties show slight loss of flower quality, and a few varieties sometimes develop brown necrotic streaks on the florets. Many cultivars become entirely infected, often without visible symptoms. CVB occurs in many other ornamental plants with rather long incubation periods and produces rather mild symptoms. Besides vegetative propagation, the virus can be transmitted in a nonpersistent manner by several aphids.



Figure 55 Necrotic ring spots on dahlia infected with TSWV. By Joachim Hamacher.



Figure 56 Dwarfed and stunted chrysanthemum infected with CSVd (left) noninfected control (right). By Joachim Hamacher.



Figure 57 Necrotic flecks of TSWV-infected chrysanthemum leaves. By Rainer Wilke.

Tospoviruses (TSWV and INSV) infect chrysanthemums quite often, inducing irregular chlorotic or necrotic spots (**Figure 57**), mild leaf deformations, primarily on young leaves, as well as browning or wilting of shoots. As tospoviruses are a major threat to growers, elimination of infected plants has to be executed early and rigidly.

TAV is fairly important in chrysanthemums, as it may cause heavy symptoms like breaking, dwarfing, as well as distortion of the flowers, and foliar chlorotic spots or ring spots. Most infected cultivars, however, do not show leaf symptoms.

Other virus diseases induced by AMV, BBWV1, BWYV, LMV, SMV, TMV, ToMV, ToRSV, and TSV have been reported for *Chrysanthemum* spp. but seem to be of minor importance.

Virus Diseases of Liliaceae

Tulipa, Lilium

Taxonomically, Liliaceae are assigned to the order Liliiflorae of the monocotyledonous plants. We concentrate on virus diseases of the genera *Tulipa* and *Lilium*, as they represent large genera with economically very important ornamentals.

Tulips originated from Central Asia and include about 100 different species. Tulips are propagated via bulbs, which develop at the base of the bulb of the previous year.

More than 20 different viruses are reported to infect tulips, the most important of which are TBV, TNV, and TRV.

Tulip breaking virus (TBV) is the most frequently encountered virus in tulips. It is assigned to the genus



Figure 58 Tulips exhibiting flower breaking induced by TBV. By Franz J. Nienhaus.

Potyvirus and can be transmitted by aphids (e.g., *Myzus persicae* and *Aphis fabae*) in a nonpersistent manner. TBV affects color breaking of flowers particularly in late-flowering pink, purple, and red cultivars, while white- and yellow-flowered cultivars are not affected. Breaking symptoms have been described as bars, stripes, streaks, featherings, or flames of different colors on petals (**Figure 58**). The color variation is caused by local fading, intensification, or accumulation of pigments in the upper epidermal layer after development of the normal flower color. Mottling or striping of the leaves also occurs. The infection causes loss of vigor and poor flower production. TBV played an important role in the Dutch 'tulipomania' in the seventeenth century, in that it increased the value of tulips with decorative flower breaking and led to wild speculations with astronomical prices for one variegated tulip bulb. At that time, the undesirable viral cause of the spectacular flower breaking was not yet known.

Tobacco necrosis virus D (TNV-D) causes the so-called Augusta disease, named after the variety in which it was first detected. The virus is transferred to the roots specifically adhering to the zoospores of the chytridiomycete *Olpidium brassicae*. This particularly happens when soil temperatures rise above 9 °C. Infected plants do not necessarily show symptoms, since the virus may be confined to the roots. The disease may suddenly appear when the plants are planted out during frost or when planted early at high temperatures after storage on a standing ground. Stunting and distortion of the shoot and leaves are the most severe symptoms but streaking or angular or elliptical spots are more typical symptoms. Fine necrotic lines in the tepals are of considerable value for differential diagnosis.

TRV (genus *Tobravirus*) is another common virus of tulips with an exceptionally wide host range of cultivated and wild plants. A vector transmission by soil-inhabiting

nematodes of the genera *Trichodorus* and *Paratrichodorus* may spread the virus in the field. Symptoms of the disease include chlorotic flecks, oval lesions and streaks, appearing early in the season. Streaks of darker color may occur on tepals of red flowering varieties, whereas those streaks appear translucent in yellow or white flowers. Plants may become stunted and flowers sometimes deformed.

Tulip virus X (TVX) has been reported in tulips from Great Britain and from Japan. It causes chlorotic or necrotic streaks in leaves and streaks of intensified color mainly at the margins of tepals. The tepals may also become bleached or necrotic, resembling symptoms caused by TNV. TVX is a member of the genus *Potexvirus*. Mechanisms of spread within field or glasshouse plots are unclear but may rely on sap transmission.

Other viruses of tulips are nepoviruses: ArMV, TBRV, TRSV; a cucumovirus: CMV; several potyviruses: LMoV, ReTBV, TCBV, TulMV; a carlavirus: LSV; a tombusvirus: TBSV; and a tobamovirus: TMV (see **Table 1**).

Three viruses are common in lilies, these are lily mottle virus (LMoV), lily virus X (LVX), and lily symptomless virus (LVS).

LMoV is a potyvirus and was formerly thought to be a strain of TBV, but serological, host range, and molecular investigations have set it apart from TBV as an own species. It can be found wherever lilies are grown. Symptoms develop as chlorotic mottle to stripe-mosaic (**Figure 59**) and leaves may be twisted or show narrowing. When young plants are infected, severe yellowing of leaves or browning of veins in stems may occur. As LMoV is a potyvirus its transmission is affected by aphids in a nonpersistent manner. The virus can also infect tulips.

LVS does not induce specific symptoms in many cultivars, but reduced growth, small flowers, and lower bulb yields are recorded. Foliar vein clearing or intercostal light green stripes may develop as well. Other natural hosts are tulip and *Alstroemeria*. The virus can be transmitted nonpersistently by aphids.

LVX is a potexvirus, which normally does not lead to pronounced symptoms in most cultivars, but enhances symptoms of LSV. When symptoms appear, the plants show faint chlorotic spots and sometimes necrotic lesions. The virus can be sap-transmitted by mechanical inoculation onto herbaceous test plants.

Other viruses, reported to naturally infect lilies are ArMV, TRSV, ToRSV, CMV, TRV, TSWV, TMV, and SLRSV.



Figure 59 Intensive streaking of lily flower caused by co-infection of LMoV and LSV. By Martin Bucher.

Virus Diseases of Orchidaceae

The family Orchidaceae contains about 800 genera with more than 25 000 species. Centers of origin are probably the Malaysian region with *c.* 12 000 species followed by the tropics of America with about 10 000 species. Genera with high economic importance are *Cattleya*, *Cymbidium*, *Dendrobium*, *Odontoglossum* hybrids, and *Phalaenopsis*.

Lawson summarizes 27 different viruses of orchids in a detailed review. The most important and often detected



Figure 60 Black line patterns on CymMV infected Cattleya leaves. By Dietrich E. Lesemann.

viruses are cymbidium mosaic virus (CyMV) and odontoglossum ringspot virus (ORSV). Many rhabdoviruses or rhabdovirus-type viruses (nine different viruses) have been reported for orchids as well as potyviruses, such as BYMV, dendrobium mosaic virus syn, bean common mosaic virus (BCMV), cypripedium virus Y (CypVY), cypripedium chlorotic streak virus (CypCSV), TuMV and vanilla mosaic virus (VanMV), tospoviruses TSWV and INSV, a tentative closterovirus: dendrobium vein necrosis virus (DVNV), ToRSV, cymbidium ringspot virus (CyRSV), and CMV.

CyMV is a potexvirus and as such easily sap-transmissible. It infects a wide range of orchids and leads to necrotic flecks or streaks (**Figures 60** and **61**), black necrotic spots or line patterns (**Figure 62**) on *Cymbidium*, *Phalaenopsis*, and in mixed infections together with DVNV in *Dendrobium* (**Figures 63** and **64**). Flower necrosis induced in *Cattleya, Laelia, Cymbidium, Phalaenopsis, Epidendrum,* and *Vanda* has also been shown to be caused by CyMV (**Figure 65**).

ORSV (syn. TMV-O) is a tobamovirus and leads to a range of different symptoms in many orchid species (**Figures 66–68**). Flower breaking in violet *Cattleya* varieties with streaks of intensified pigmentation on tight buds have been observed as well as fine necrotic stripes (**Figure 69**). Symptoms on leaves comprise chlorotic and dark concentric ringspots in *Odontoglossum grande*. The virus is easily sap-transmissible and spread occurs via contaminated cutting tools.

Virus Control in Ornamentals

The ornamental industry is a very important branch of agriculture around the world with high amounts of investment and rising competition among growers. Outbreaks of diseases have shattered the ornamental industry more than once. Especially epidemics of viral or bacterial etiology may be disastrous for the reputation and standing of the enterprise struck by such diseases.

As direct chemical control strategies have proven to be ineffective in plants, only prophylactic measures to prevent or hinder infection and spread of viruses in the respective cultures are effective. Traditional resistance cross-breeding as a reliable measure to combat virus

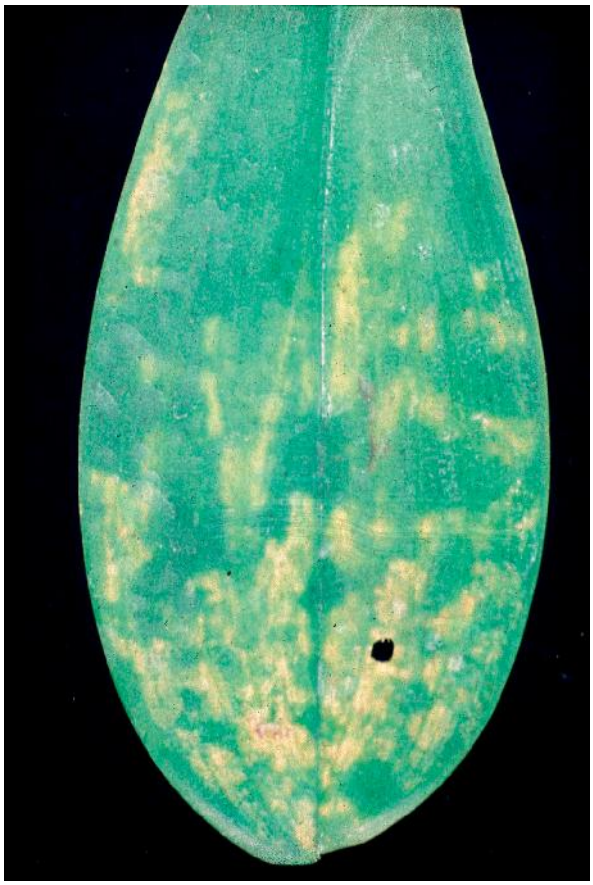


Figure 61 Necrotic flecks on CymMV infected Phalaenopsis leaves. Dietrich E. Lesemann.

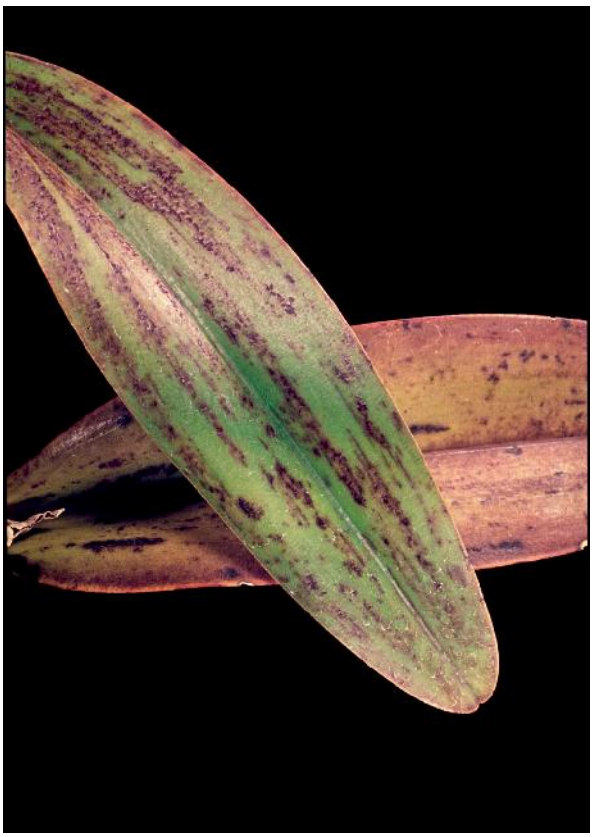


Figure 62 Cattleya leaf with black streaking caused by CymMV. By Dietrich E. Lesemann.



Figure 63 Cattleya flower with black streaking induced by CymMV. By Dietrich E. Lesemann.



Figure 64 Dendrobium leaves with black spots and chlorotic flecks caused by a co-infection of CymMV and DVNV. By Joachim Hamacher.

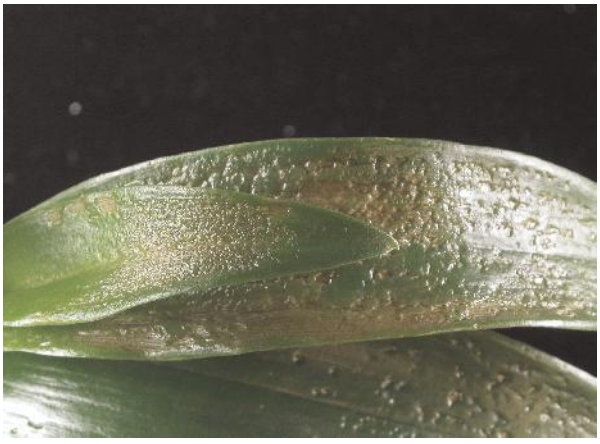


Figure 65 Dendrobium leaves with sunken necrotic spots and necrotic stripes caused by a mixed infection of CymMV and DVNV. By Joachim Hamacher.

diseases, however, is of minor importance, for on one hand cross-breeding of resistance genes is time consuming, and on the other hand the market demands a rapid change or enlargement of the range of varieties. Hygienic measures and, in many cases, meristem culture and thermotherapy are the essential prophylactic strategies to ensure virus-free cultures. The success of transgenic virus-resistant plants as a practicable alternative is strongly dependent on the legislative and the acceptance in the respective countries. To date the most promising strategy for the producer is the establishment of virus-free mother plants and elite stocks as well as continuous monitoring of production stocks. This demands staff skilled in horticultural production, as virus symptoms appear very varied and may be confounded with symptoms provoked by environmental stress or faulty cultural practices. Specialized diagnosis labs have to know the peculiarities of sampling and time of the year, in which virus testing is practicable, because virus concentrations vary considerably during the course of the year in many ornamental species. They tend to be highest in late winter/early spring in countries with moderate climate, and nearly disappear in summer and autumn, thus disabling secure diagnosis. To ensure virus-free production, the European and American plant protection organizations European and Mediterranean Plant Protection Organization (EPPO/OEPP) and US Department of Agriculture-Animal and Plant Health Inspection Service (USDA-APHIS), for example, have established continuously updated guidelines for the production of virus-free ornamentals. This is achieved by quarantine measures and well-regulated production requirements with highest phytosanitary standards. These include regular testing of candidate and elite stocks in specialized diagnostic labs that are either part of the horticultural enterprises or autonomous. Plant clinics and plant protection services contribute to the program as well. Routine testing is done by ELISA or comparable immunological tests. Molecular techniques with higher sensitivity than that of the conventional testing methods are gaining wider fields of application. Upon occurrence



Figure 66 Chlorotic and necrotic ring spots and line patterns on ORSV-infected cymbidium leaves. By Dietrich E. Lesemann.



Figure 68 Black streaking caused by ORSV on cymbidium leaves. By Dietrich E. Lesemann.



Figure 67 Chlorotic striping on cymbidium leaves infected with ORSV. By Dietrich E. Lesemann.



Figure 69 Chlorotic flecking on cattleya flowers infected with ORSV. By Dietrich E. Lesemann.

of symptoms (of unknown cause), electron microscopy may be of great help to clarify the viral etiology of observed symptoms. Regular visual inspections of production stocks (monitoring) ensure high quality and freedom from diseases of the products.

See also: Papaya Ringspot Virus; Plant Antiviral Defense: Gene Silencing Pathway.

Further Reading

Brunt A, Crabtree K, Dallwitz M, Gibbs A, and Watson L (1996) *Viruses of Plants*. In: *Descriptions and Lists from the VIDE Database,* 1484pp. Wallingford, UK: CAB International.

Daughtrey ML and Benson DM (2005) Principles of plant health management for ornamental plants. *Annual Review of Phytopathology* 43: 141–169.

Daughtrey ML, Robert L, Wick RL, and Peterson JL (eds.) (1995) *Compendium of Flowering Potted Plant Diseases.* St. Paul, MN: APS-Press.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.* San Diego, CA: Elsevier Academic Press.

Hammond J (ed.) (2002) ISHS Acta Horticulturae 568: *X International Symposium on Virus Diseases of Ornamental Plants* (CD-ROM format only).

Hull R (ed.) (2001) *Matthews Plant Virology.* San Diego, CA: Academic Press.

Loebenstein G, Hammond J, Gera A, Derks T, and van Zaayen A (eds.) (1996) ISHS Acta Horticulturae 432: IX *International Symposium on Virus Diseases of Ornamental Plants*. (CD-ROM format only).

Loebenstein G, Lawson RH, and Brunt AA (eds.) (1995) *Virus and Virus-Like Diseases of Bulb and Flower Crops,* 543pp. New York: Wiley.

Mokrá V, Brunt AA, Derks T, and van Zaayen A (eds.) (1994) ISHS Acta Horticulturae 377: VIII *International Symposium on Virus Diseases of Ornamental Plants* (CD-ROM format only).

Pirone TP and Perry KL (2002) Aphids: Non-persistent transmission. *Advances in Botanical Research* 36: 1–19.

Ullman DE, Meideros RB, Campbell LR, Whitfield AE, Sherwood JL, and German TL (2002) Thrips as vectors of Tospoviruses. *Advances in Botanical Research* 36: 113–140.

Relevant Websites

http://www.dpvweb.net – Descriptions of plant viruses (Association of Applied Biologists).
http://www.agdia.com – List of ornamental plant samples of Agdia.
http://www.ebi.uidaho.edu – University of Idaho, VIDE Database.

Plant Virus Vectors (Gene Expression Systems)

Y Gleba, S Marillonnet, and V Klimyuk, Icon Genetics GmbH, Weinbergweg, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Magnifection Process for heterologous protein expression in plants that relies on transient amplification of viral vectors delivered to multiple areas of plant body (systemic delivery) by *Agrobacterium*.

Noncompeting vectors Viral vectors capable of co-expressing recombinant proteins in the same cell throughout the plant body.

Introduction

Plant viral vectors have been and are still being developed to take advantage of the unique capability of viruses to extremely rapidly redirect most of the biosynthetic resources of a cell for the expression of a (usually) single nonhost protein – the viral coat protein (CP) (and hopefully, a protein of interest as well). Efficient viral vectors should allow to bypass the limitations of other expression methods, such as the low yield obtained with stable transgenic plants or standard transient expression methods, and the long time necessary for development of stable transgenic lines. Most of the progress achieved so far has been with RNA viruses, and the most advanced vectors have been built using just a handful of plant viruses such as tobacco mosaic virus (TMV), potato virus X (PVX), alfalfa mosaic virus (AMV), and cowpea mosaic virus (CPMV). Tremendous technical progress has been made over the past few years in the development of production processes and industrial plant hosts. In particular, novel developments include the design of vectors that are not just simple carbon copies of wild-type viruses carrying a heterologous coding sequence, but that, instead, have been 'deconstructed' by delegating some rate-limiting functions to agrobacteria or the plant host, thus allowing for a more efficient, versatile, controlled, and safe process.

Viruses have to carry out a number of processes to complete the viral replication cycle. These include host infection, nucleic acid amplification/replication, protein translation, assembly of mature virions, cell-to-cell spread, long-distance spread, reprogramming of the host biosynthetic processes, suppression of host-mediated gene silencing, etc. Viral vectors, in contrast, do not necessarily have to be able to perform all of these functions,

but they have to perform a new function – high-level expression of a heterologous sequence.

Two different strategies can be used to develop a viral vector/host process.

Historically, the first approach that was developed, starting in 1990, consisted of designing vectors that were, in essence, wild-type viruses engineered to express an additional sequence – the gene of interest (approach also known as the 'full-virus' vector strategy). These vectors were expected to perform all of the functions that a virus normally undertakes, from the infection step to the formation of complete infectious viral particles. However, addition of a heterologous sequence reduced the efficiency of many of the normal steps of the replication cycle, such as high-level amplification and systemic movement. Moreover, these vectors did not necessarily provide the maximal level of expression for the protein of interest that one might have expected. Nevertheless, most of the practical results reported in the literature have been obtained with these 'first-generation' viral vectors.

The design of the next-generation viral vectors reflects an approach that admits inherent limitations of the viral replication process applied to heterologous gene expression. Rather than mimic the design of the wild-type virus, the goal is to 'deconstruct' the virus, and eliminate the functions that are either limiting (e.g., too species-specific, or rate-limiting) or undesired (such as the ability to create functional infectious viral particles), and rebuild the process, by either delegating the missing functions to the host (genetically modified to provide those functions in *trans*) or replacing them with analogous functions that are not derived from a virus (the 'deconstructed virus' vector strategy). In these 'second-generation' expression systems, elements of the viral machinery such as RNA/DNA amplification and cell-to-cell movement are integrated along with nonviral processes such as replicon formation via *Agrobacterium*-mediated delivery of T-DNAs encoding the viral vector or via activation from a plant chromosomal DNA encoding a pro-replicon or pro-virus.

First-Generation Virus Vectors

Under the 'full-virus' scenario, delivery of the replicons is provided by infecting the host with a mature viral particle or with a full copy of the viral DNA/RNA. The vector is essentially a functional virus that, in addition to the viral sequence, contains a coding sequence of interest under control of a strong viral promoter such as the CP subgenomic promoter (**Figure 1**(**a**)). Several efficient vectors have been made, primarily based on RNA viruses such as TMV and PVX. Vectors based on TMV were developed in several steps. Earlier versions simply used a duplicated CP subgenomic promoter for expression of the gene of interest, but this led to instability of the vector. Later versions replaced the duplicated subgenomic promoter by sequences derived from a different but phylogenetically related virus. Finally, different 3′ untranslated sequences from a range of related viruses were screened to obtain a vector expressing the gene of interest efficiently.

An alternative expression strategy consists of expressing the protein of interest as a fusion to the CP (**Figure 1**(**a**)). In this case, only an essential part of the protein of interest, such as an immunogenic epitope, is fused to the CP since larger fusions usually eliminate viral particle formation and systemic movement ability.

Large-scale production with either type of vector can be achieved by spraying plants in the field or a greenhouse with a mixture of viral particles and carborundum. Depending on the efficiency of the vector and its ability to move systemically, 2–3 weeks are required for plants to achieve maximal transfection/expression.

During the last few years, improvements have been made to this first-generation vectors. Among the most interesting experiments coming from recent work is the improvement of viral vectors by directed evolution. It was found that cell-to-cell movement of the vectors described above was not as high as with wild-type viruses due to lower expression of the movement protein. The strategy employed was to use DNA shuffling to improve the movement protein gene. This work was very successful and resulted in mutant movement protein genes working better than the native version in the viral vector context, essentially in tobacco, where engineered vectors normally do not work very well. This work suggests that other parts of the viral vectors might be improved by DNA shuffling as well.

In general, first-generation vectors were very successful and were able to provide expression of heterologous protein at the levels close to 10% of total soluble protein or over 1 g recombinant protein kg^{-1} of fresh leaf biomass. Several pharmaceutical proteins made using such vectors have actually reached the stage of clinical trial.

However, despite these successes, first-generation viral vectors have several limitations. The yield that can be obtained with these vectors varies greatly depending on the protein to be expressed. In most cases, only relatively small proteins are expressed efficiently, any proteins larger than 30 kDa are usually poorly expressed. Moreover, yield of heterooligomeric proteins requiring two or more subunits expressed from the same vector are extremely low. With CP fusions, the yield can be as high as 2–4 g kg^{-1} of plant biomass; however, usually only short (up to 25 amino acids) epitopes are tolerated.

Host Improvement

To survive in nature, plants have naturally evolved resistance mechanisms against pathogen attacks. It is therefore expected that plants could be engineered to be more

susceptible to viruses and therefore better hosts for expression of heterologous sequences using viral vectors. A dilemma is that plants susceptible to viral vectors and therefore good hosts for protein expression will also have poor agronomic characteristics.

One important element of plant defense mechanisms against viruses is the ability of plants to induce post-transcriptional silencing. A large amount of work in this area has shown that not only plants are able to defend themselves against viruses, but that viruses have counter-attacked by evolving suppressors of silencing. All these elements uncovered by basic research in virology offer elements that can be incorporated in expression strategies (with or without viral replication) to boost the level of protein expression. Another important field of research consists of approaches that aim at silencing/superactivating some plants genes that are, directly or indirectly, involved in viral replication or spread.

A typical host for viral research is *Nicotiana tabacum*, the host plant of the most-studied plant virus – tobacco mosaic virus. The most commonly used plant in virology is however the wild Australian species, *N. benthamiana*, which is unusually sensitive to a very wide array of viruses. Despite its poor agronomic characteristics, *N. benthamiana* can be grown very well in the greenhouse under controlled conditions and could therefore even be considered for industrial production.

One drawback of using plants as hosts for manufacturing therapeutic proteins is that plant post-translational modifications are not exactly identical to those made by animal/human cells (although, fortunately, not extremely different either). In particular, the enzymatic machinery

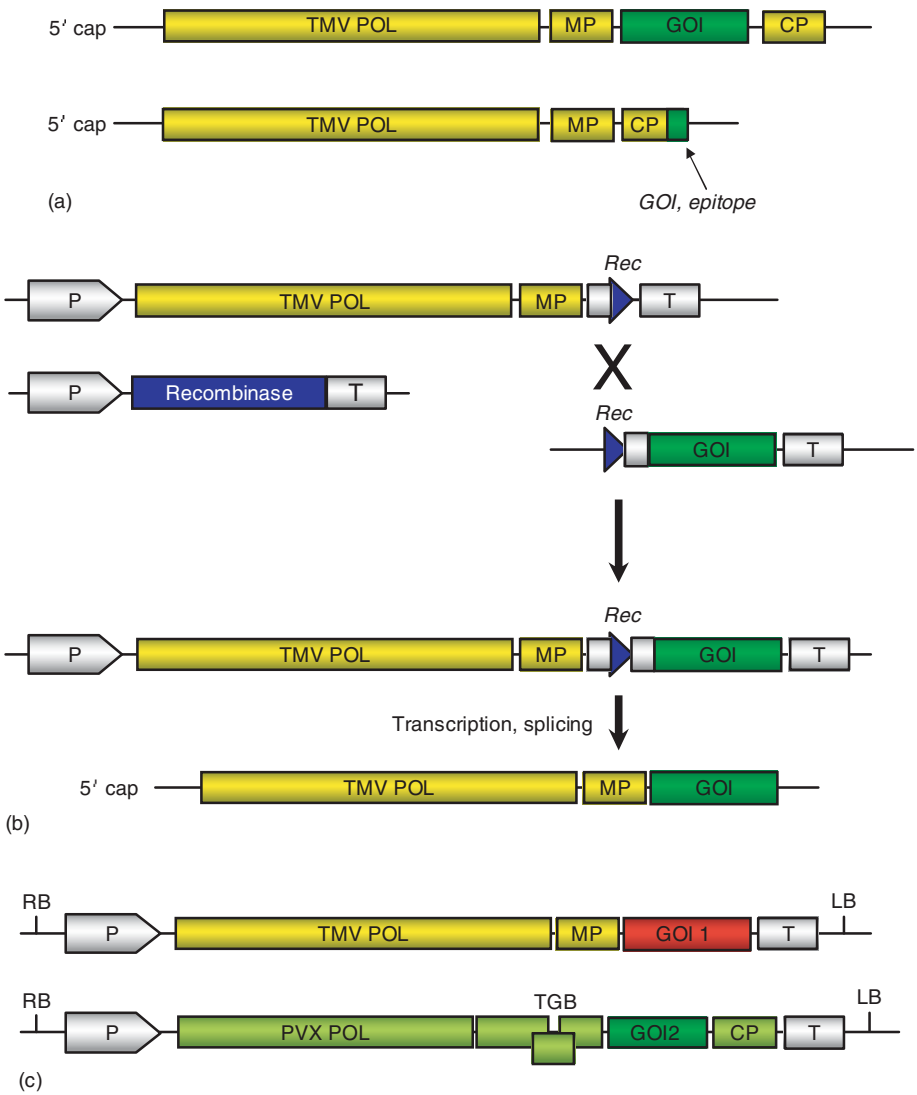


Figure 1 Continued

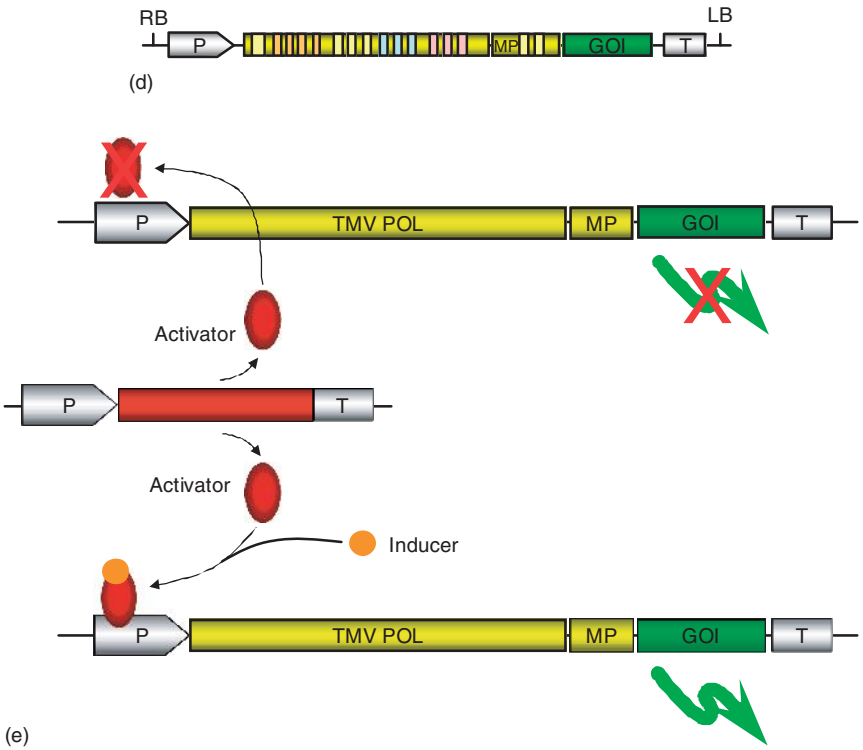


Figure 1 Plant viral vectors currently in use. (a) Typical first-generation RNA expression vectors based on TMV virus. (b) Second-generation vectors. Pro-vector system for rapid *in planta* assembly of viral replicons from T-DNAs delivered by *Agrobacteria*. (c) DNA vectors optimized for *Agrobacterium*-mediated delivery and expression of heterooligomers (IgG antibodies). (d) DNA vectors optimized for *Agrobacterium*-mediated delivery. (e) Systems relying on the regulated release of a replicon from a plant chromosome upon chemical induction. The viral components of the vector backbones are in yellow or in light green and include RNA-dependent RNA polymerase (TMV POL or PVX POL), movement protein (MP), coat protein (CP), triple gene block (TGB); the genes of interest encoding recombinant protein are in green or pink (GOI); the site-specific recombinase (REC) and its recognition sites (*Rec*) are in blue; multiple colored segments on the vector optimized for *Agrobacterium* delivery are plant introns; activator gene and its product are in red; promoter and terminator sequences are in gray.

responsible for N-glycosylation differs in plants and animals. More specifically, plant cells add some specific sugars (core-bound xylose and α-1,3-fucose), which might be immunogenic if protein containing these were administered to humans. In contrast, plant cells do not add other sugars such as terminal β-1,4-galactose residues or sialic acid, which are added to proteins made in animal cells. Fortunately, significant work has been made to engineer plant hosts, including tobacco and *N. benthamiana*, to provide more 'human-like' or even 'designer' post-translational modifications – in particular, low amounts of xylose and α-1,3-fucose as well as galactosyl terminal residues added to the core sugars. Such host plants will be useful for all vector types, whether of the first generation or vectors developed later.

Second-Generation Vectors

This strategy reflects attempts by engineers to part with the inherent limitations of the viral machinery, while keeping the useful viral elements. Some of the elements that can be used outside of the integrated virus system are the 'molecular machines' that provide for infectivity, amplification/replication, cell-to-cell movement, assembly of viral particles, suppression (shutoff) of the synthesis of plant proteins, silencing suppression, systemic spread, etc. Some of these elements are less efficient than others. For example, the ability of the virus to infect the host is low and requires some type of mechanical injury to the plant, or an insect as a vector. Systemic spread is a process that is usually very species-specific, easily impaired as a result of genetic manipulation.Replication/amplification ability, on the other hand, is a central element of viral vectors that is valuable as an 'amplifier'. Fortunately, replication is a relatively robust and species-independent mechanism.

Two basic applications, namely rapid expression in small or large quantities and large-scale industrial production of proteins in plants, each require different design of the expression strategies.

Vectors for Specific Application Areas

Plant Virus Vectors as Research Tools

Many different expression systems have been developed based on the backbones of entirely different viruses, and relying on different modifications of the core viral design. However, since the purpose of using such vectors is often rapid and high-throughput expression, and since only small (usually milligram) amounts of protein are usually required, many existing vectors do not contain the viral component(s) providing systemic movement.

An important and limiting step for the use of viral vectors is infection of the plants with the viral replicons. Use of *Agrobacterium* to deliver a copy of the viral vector encoded on the T-DNA ('agrodelivery') provides an excellent solution since *Agrobacterium* is an extremely efficient vector. All the steps that are necessary for the conversion of the 'agrodelivered' T-DNA into a functional DNA or RNA replicon have been shown to occur in plants. 'Agroinfection' has been used for many years. It is also often much more efficient than mechanical inoculation using viral particles, and is definitely more efficient than using DNA or RNA as infectious molecules. In case of RNA viruses, agroinoculation also represents a very inexpensive alternative to *in vitro* transcription to convert the DNA vector into an infectious RNA.

The use of agrodelivery for inoculation of RNA-viruses vectors also provides entirely new opportunities for vector engineering, since the vector is delivered to the plant cell as a DNA molecule, which can be manipulated prior to conversion into an RNA replicon. In particular, it has been shown that several T-DNAs transiently co-delivered from one or more agrobacteria can be efficiently recombined *in planta* by site-specific recombinases such as Cre or the φc31 integrase. The RNA obtained after transcription of the recombined DNA molecule can then be cleaned of the recombination sites by the nuclear RNA splicing machinery, provided that the recombination sites are engineered by flanking by intron sequences (**Figure 1**(**b**)).

Using such elements, a process has been developed that, in essence, allows simple and inexpensive '*in vivo*' engineering of RNA vectors by simply mixing various combinations of agrobacteria containing different components of a viral vector and co-infiltrating these mixtures into plants. Such an approach allows, for example, to rapidly assemble and express a variety of different protein fusions such as fusions of the protein of interest to targeting or signal peptides, binding domains, various coding sequences, purification domains, or cleavage sites. All of these expression experiments are done by simple mixing of pre-fabricated bacteria strains harboring the desired vector modules and a strain containing the gene-specific module. Depending on the viral vectors and the specific protein studied, milligram quantities of protein can be expressed in various plant compartments or as different fusions, within just a few days.

Vectors for Expression of Heterooligomeric Proteins

The first transgenic plants expressing full-size human antibodies were made around 1990. Despite these early positive results, low yields have prevented the widespread use of this technology for commercial applications. For example, although stably transformed plants express correctly folded and functional antibodies of immunoglobulin of the IgG and IgA classes, yields are generally very low (1–25 mg kg^{-1} of plant biomass). Moreover, the time necessary to generate the first gram(s) of such antibody material is longer than 2 years.

Transient systems, on the other hand, allow production of research quantities of material much faster. However, as for transgenic plants, transfection systems relying on *Agrobacterium*-mediated delivery of standard nonreplicating expresssion cassettes also provide only low yield of expressed proteins. The level of expression of such systems can be increased by using suppressors of silencing, but yields are still inferior to expression using viral vectors and are not readily scalable. Initial attempts to combine the speed of expression of transient systems with the high level of expression of viral vectors failed due to the inability of viral vectors to co-express two genes within one cell at high level; engineering of vectors for expression of two genes from one viral vector led to drastically reduced expression level of both genes; co-infiltration of two vectors expressing a separate gene also failed because competition between vectors resulted in either one or the other protein expressed in individual cells, but not of the two proteins within the same cell (which would be required for expression of a functional IgG antibody).

Recently a solution to this problem was found by cloning the two genes to be expressed in two separate viral vectors that were constructed on the backbone of two different noncompeting viruses such as, for example, TMV and PVX. Unlike vectors that are built on the same backbone, 'noncompeting viral vectors' were shown to be able to efficiently co-infect and replicate within the same cell. Therefore, high level of functional full-size monoclonal antibodies (Mabs) of the IgG class can now be obtained in plants by co-infiltrating two vectors containing the heavy- and the light-chain separately in each vector. This strategy was shown to work with different Mabs of the IgG1 and IgG2 classes; the molecules were found to be fully functional, and the first gram of material could be produced in less than 2 weeks after cloning in the vector (**Figure 1**(**c**)).

Expression Vectors for Industrial-Scale Protein Production: Transient Systems (Magnifection)

Industrial production requires viral amplification and expression in large numbers of plants (in a greenhouse or field) and, to obtain maximal yield, in as many tissues and leaves as possible within each plant. For achieving this goal, a first solution was provided by using vectors capable of systemic spread (the 'full-virus' strategy). Although successful, this strategy had many limitations: large inserts (larger than 1 kbp) could not be efficiently expressed, not only because of less-efficient amplification within each cell, but also because viral vectors containing large inserts are less able to move in systemic tissue without recombining; systemic vectors also never infect all harvestable parts of the plant (e.g., the lower leaves are usually not infected); the process is asynchronous as it invades different leaves in a sequential order. Moreover, for viral vectors designed to express protein fragments as CP fusions, only short epitopes (20 amino acids or less) could be successfully expressed until recently.

A simple technical solution was found to bypass these limitations; a viral vector lacking the ability to move systemically (with a simpler design, and more stable) is delivered to the entire plant using *Agrobacterium*-mediated delivery by vacuum-infiltration of the entire plant. This eclectic technology called 'magnifection' combines advantages of three biological systems: the speed and expression level/yield of a virus, the transfection efficiency and systemic delivery of an *Agrobacterium*, and the post-translational capabilities and low production cost of a plant. Such a process can be inexpensively performed on an industrial scale and does not require genetic modification of plants, and is therefore potentially safer (it also does not generate infectious virions as the full-vector strategy does) and more compatible with the current industrial infrastructure.

For magnifection to become an efficient tool, technical difficulties had however to be addressed. Indeed, initial vectors based on the backbones of plus-sense RNA viruses such as TMV could not be delivered to plant cells efficiently by *Agrobacterium* delivery. The first attempts were made with vectors built on the backbone of TMV and led to a rate of infectivity estimated at one successful transfection event per 10^8 agrobacteria. Later experiments using viral vectors based on different tobamoviral strains (infecting both crucifers and *Nicotiana* plants) were more successful, but clearly not optimal. Analysis of the early infection steps initiated by *Agrobacterium* delivery of T-DNAs encoding RNA viral vectors suggested that a bottleneck for the formation of active replicons may be the low ability of the primary transcript to leave the nucleus. The RNAs of cytoplasmic RNA viruses such as TMV normally never enter the plant cell nucleus at any point during the entire replication cycle and have therefore not evolved to be in contact with the cell RNA-processing machinery and to be exported to the cytosol. *In silico* analysis of the viral genome structure with programs designed to assess whether an unknown sequence is likely to be coding or noncoding (by comparison to expressed nuclear plant genes, using neural networks), showed that the viral RNA is unlikely to be recognized as a correct coding sequence and suggests that it will probably be processed abnormally or even degraded before it is able to reach the cytosol. By changing the codon usage of at least portions of the viral RNA, the viral genome can be modified to look more 'exon-like' (conversion of T-rich sequences to more GC-rich sequences, and removal of putative cryptic splice sites). Such changes have been useful to improve export of functional RNAs to the cytosol, but addition of plant introns improved the process even more significantly. The resultant synthetic T-DNA templates, when delivered as DNA precursors using *Agrobacterium*, provided efficient processing of the DNA information into active amplicons in almost all (>93%) cells of infiltrated tobacco (*Agrobacterium* infiltrated at optical density (OD) of 0.7), a 10^3-fold improvement compared with nonmodified vectors, and an up to 10^7-fold improvement over nonoptimized DNA templates reported in the first publications. Improved vectors lead to one successful infection event per 10–20 infiltrated agrobacteria (**Figure 1**(**d**)).

Using these vectors, a simple fully scalable protocol for heterologous protein expression in plants has been designed that does not require transgenic plants, but instead relies on transient amplification of viral vectors delivered to the entire plant using *Agrobacterium*. Entire plants are infiltrated (although the same procedure can be performed on detached mature leaves) with a highly diluted (up to 10^{-4} dilution) suspension of bacteria carrying a proviral amplicon on the T-DNA. The combination of vacuum infiltration/agroinfection can therefore be considered as replacing the conventional viral functions of primary infection and systemic movement. Viral vector-controlled amplification and cell-to-cell (short distance) spread is performed by the replicon, as is the case with standard vectors. Depending on the vector used, the host organism, and the initial density of bacteria, the magnifection process takes from 4 to 10 days and, for well-expressed proteins, results in level of up to 5 g recombinant protein kg^{-1} of fresh leaf biomass or over 50% of total soluble protein. Furthermore, since the viral vector lacks a CP gene (for tobamoviruses, the CP gene is needed only for plant infection or systemic movement, and is therefore not needed for magnifection), it can express longer genes (up to 2.3 kbp inserts or up to 80 kDa proteins). Infiltration of plants/detached leaves with bacteria can be performed in several ways, one simple process being vacuum infiltration by immersing whole aerial parts of plants in a bacterial suspension and applying a weak vacuum (approximately

minus 0.8–1.0 bar) for 10–30 s. This process can be performed on a large scale and is expected to be commercially viable for production of up to 1 ton of recombinant protein per year. Industrial-scale production will require containment of infiltrated plants which contain agrobacteria (**Figures 2** and **3**).

Expression Vectors for Industrial-Scale Protein Production: Transgenic Systems

A radically different approach to establish an industrial expression system would be to produce a stably transformed plant that would contain an inactive or repressed copy of the viral replicon inserted on one of the host's chromosomes. Induction of viral replication could be activated at the time of the operator's choosing by a specific treatment. Obviously, establishing such a system would slow down the development phase by several months/years, in order to obtain stable transformants and select well-performing lines. However, this delay would be largely compensated by the ease of production that the system would provide and by the resulting low production costs.

Several strategies are possible for the design of an inactive replicon, and specific strategies will of course depend on the type of viral vector used. A viral vector might be encrypted by insertion of additional sequences within the vector, or inversion of part of the vector, and activation could be provided by regulated expression of a recombinase. Alternatively, a replicon could be kept inactive by silencing by the host plant, and activated by regulated expression of a silencing suppressor. Finally, a replicon could be kept in an inactive form by fusion to a promoter that is inactive in the absence of a specific activator or that is repressed in the presence or absence of a particular inducer.

The inducing treatment could be provided in many different ways. A simple strategy would be to induce release of the active replicon by genetic hybridization, which would combine in the same plant both the transgene locus encoding the replicon as well as the transgene(s) that control unencryption (e.g., a silencing suppressor or a recombinase that is developmentally regulated). Alternatively, hybridization could also be used to bring together genomes of multipartite viruses, thus providing for replication of RNAs that carry the transgene of interest but cannot replicate in the absence of the master RNA expressing the necessary RNA-dependent RNA polymerase.

A more versatile solution would be provided by spraying a small chemical inducer on an engineered plant. One technical challenge for establishing such a system is the design of an effective chemical switch that provides a tight control and can be effectively used for an industrial process. One specific issue in dealing with chemically inducible promoters for industrial purpose is the availability of small chemical molecules that are commercially available and safe. Among those available today, one should mention ethanol (used as a part of ethanol-inducible system), the commercial insecticide methoxyfenozide, which acts as an agonist for the ecdysone receptor, and tetracycline antibiotics.

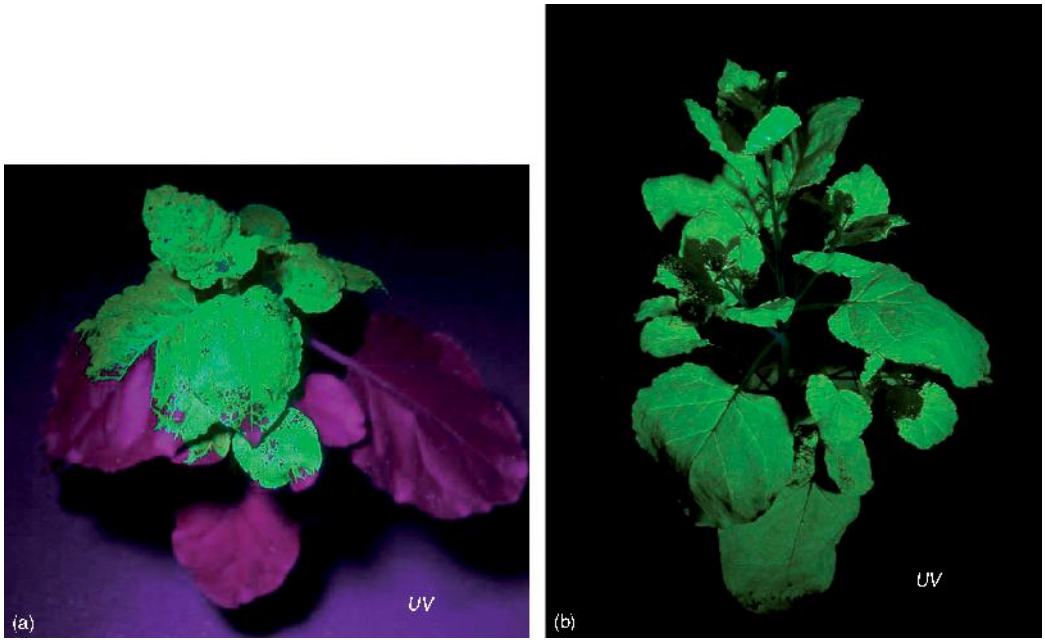


Figure 2 Plant transfection with (a) first- and (b) second- generation vectors; GFP expression in *Nicotiana benthamiana*, photographed under UV light.

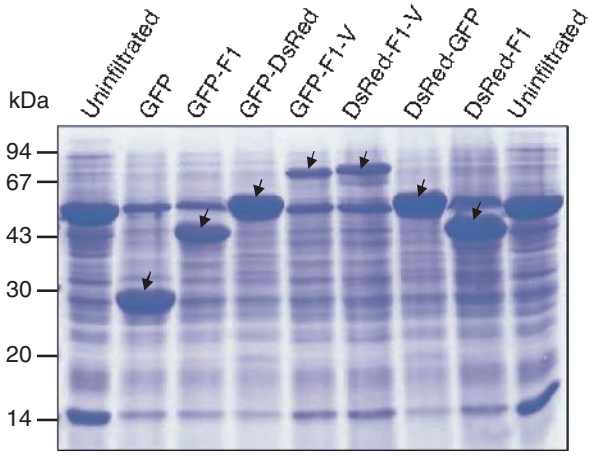


Figure 3 Expression of proteins and protein fusions using second-generation vectors; Coomassie-stained polyacrylamide protein gel showing protein profiles in crude extract from uninfected leaf tissue as well as tissues infected with genes encoding fluorescent protein GFP or protein fusions involving GFP or DsRed as fusion components. The arrow indicates the band with heterologous protein. Molecular weight ladder in kDa is shown on the left side.

Several groups are now working on the development of protocols relying on chemically inducible promoters for induction of both DNA and RNA viral vectors. As one example, an inducible geminivirus viral vector system based on bean yellow dwarf virus has been described. In this system, treatment with ethanol results in expression of the replicase, which induces release and replication of the gene-specific replicon and leads to expression of the gene of interest. Ethanol induction was reported to result in up to 80-fold increase of the mRNA levels and up to 10-fold increase of the translation product. In another example, an inducible system that relies on the estrogen-inducible promoter was shown to work efficiently in tobacco cell suspensions. Upon induction, a modified tomato mosaic virus expressing green fluorescent protein (GFP) instead of its CP amplified and expressed the gene of interest at high levels (10% of total soluble protein). In contrast, neither viral RNA nor GFP were detectable in uninduced cells (**Figure 1**(**e**)).

Despite these positive results, it is clear that the full potential of inducible viral vectors systems has still not been completely reached. Given the progress in this field, it is however expected that fully effective technology processes will be available within the next few years.

Vectors for Manufacturing 'Nanoscale' Materials

Due to their relatively simple macromolecular organization and very high accumulation titers, tobamoviruses provide an extremely cheap source of biopolymers than can be manufactured rapidly and under very simple conditions. TMV, the best-known tobamovirus, is also one of the most extensively studied viruses. It has a positive-sense RNA genome encoded in a single 6.4 kbp RNA molecule. The genome encodes four proteins, including the 17.5 kDa CP, the most abundant viral product and the only component of the TMV capsid. Over 2100 copies of CP fully protect the single-stranded viral RNA, resulting in rigid rod-shaped viral particles with a length of 300 nm and a diameter of 18 nm and a molecular mass of 40 000 kDa. The genomic RNA is packaged inside of a 2 nm-wide canal formed by the assembled CP capsid. Ninety-five percent of the mass of TMV particles consists of the CP. TMV accumulates to levels of up to 10 g kg^{-1} of leaf biomass, and, therefore, the CP represents the most abundant individual protein that can be harvested from plants. The virions can be purified industrially using simple 'low-tech' protocols.

Due to the ability of the CP to polymerize *in vivo* and *in vitro* and the high stability and defined size of the assembled virions, the CP represents a potentially promising biopolymer feedstock for a number of applications in nanobiotechnology. The CP, the basic element of the 'viral polymer' (the viral particle) can be modified in two different ways.

One strategy consists of attaching novel chemical moieties to the viral particles *in vitro* by chemical modification of reactogenic groups exposed on the surface of the virus or in the inner cavity. One such reactogenic group, lysine, allows biotinylation of the capsid. In the case of the TMV CP, which does not contain a lysine, a randomized library was made and screened to introduce this desired amino acid at an externally located position, and this without affecting the formation of viral particles. The protein/peptide fusion component was then expressed independently as a streptavidin fusion, and used to decorate the TMV particles.

Another strategy for modification of the viral core monomer, the CP, consists of engineering the viral vector to express a protein fusion. Since the structure of many viral particles has been determined at atomic resolution, fusion proteins can be designed in such a way that the added sequences are predicted to lie at the surface of the assembled viral particle. For TMV, fusions can theoretically be made either at the N- or the C-terminal ends of the CP since both ends are exposed on the surface of the viral particle. For other viruses, short peptide epitopes can be inserted in surface-exposed loops. However, constraints such as the size or the pI of the inserted sequence are limiting the type of epitopes that can be successfully expressed using this strategy.

So far, extensive work by numerous groups to create new products based on protein fusions has met with limited success. Since the main goal of these studies was

the design of new vaccines by surface display of immunotopes, only short inserts were extensively analyzed. For example, the limited size of peptides that could be fused to the CP of TMV without preventing virion assembly has restricted these systems to expression of 20-amino-acid (aa) or shorter peptide immunogens and of one peptide hormone only. However, it has recently been found that much longer polypeptides of up to 133 aa (for a fully functional fragment of protein A) can be displayed on the surface of TMV as C-terminal fusions to the CP, provided a flexible linker is being used. These nanoparticles coated with protein A allow purification of Mabs with a recovery yield of 50% and higher than 90% purity. The extremely dense packing of protein A on the nanoparticles confers an immunoadsorbent feature with a binding capacity of 2 g Mab per g. This characteristic, combined with the high level of expression of the nanoparticles (more than 3 g kg^{-1} of leaf biomass), provides a very inexpensive self-assembling matrix that could meet industrial criteria for a single-use immunoadsorbent for antibody purification.

The field of nanomaterials built around plant viruses as scaffolds is still *in statu nascendi*, and we believe that it will offer multiple practical solutions not only to specific problems such as mentioned above for downstream processing of antibodies and other proteins, but also to entirely new technology processes that are difficult to imagine today.

	Nuclear transformation	Plastid transformation	*Agrobacterium*, transient	Virus, first generation	Virus, second generation
Yield	++	+++	+	++	+++
Protein yield	++	+++	+	++	+++
R&D speed	+	+	+++	+++	+++
Large genes, complex proteins	+++	+++	+++	+	++
Post-translational processing	+++	+	+++	+++	+++
Scalability	+++	+++	+	+++	+++

Figure 4 Strengths and weaknesses of different expression platforms. The platforms include nuclear transformation, plastid transformation, *Agrobacterium*-mediated transient expression, and the two viral expression platforms. The parameters that are most essential for expression of recombinant proteins, in particular biopharmaceuticals, include yield of recombinant protein, relative yield as a percentage of total soluble protein, speed of research and development supported by the platform, ability to express large genes and manufacture complex proteins, post-translational processing capabilities and scalability. The best performance is assigned three crosses, the worst is assigned one cross.

Conclusions

Although there are currently no products or medicines in the market produced using viral vectors, compared to other expression platforms utilizing plants, this technology has made tremendous progress since its inception approximately 15 years ago (**Figure 4**), and it is simply a matter of time before products made with viral vectors become available. This progress has come as a result of the extensive studies that revealed the structure, functions, and molecular organization of plant viruses. In 1955, fascinated by the elegant simplicity of TMV's morphology, F. Krick supposedly exclaimed: 'Even a child could make a virus'. Today, some 50 years later, we tend to agree with him.

Acknowledgments

The authors wish to thank Dr. Anatoliy Girich, Icon Genetics, Halle, Germany, for the materials used in **Figure 3**.

See also: Virus Particle Structure: Nonenveloped Viruses; Virus Particle Structure: Principles.

Further Reading

Canizares MC, Nicholson L, and Lomonossoff GP (2005) Use of viral vectors for vaccine production in plants. *Immunology and Cell Biology* 83: 263–270.

Donson J, Kearney CM, Hilf ME, and Dawson WO (1991) Systemic expression of a bacterial gene by a tobacco mosaic virus-based vector. *Proceedings of the National Academy of Sciences, USA* 88: 7204–7208.

Gleba Y, Marillonnet S, and Klimyuk V (2004) Engineering viral expression vectors for plants: The 'full virus' and the 'deconstructed virus' strategies. *Current Opinion in Plant Biology* 7: 182–188.

Ma JKC, Drake PMW, and Christou P (2003) The production of recombinant pharmaceutical proteins in plants. *Nature Genetics* 4: 794–805.

Mallory AC, Parks G, Endres VB, *et al.* (2002) The amplicon-plus system for high-level expression of transgenes in plants. *Nature Biotechnology* 20: 622–625.

Marillonnet S, Thoeringer C, Kandzia R, Klimyuk V, and Gleba Y (2005) Systemic *Agrobacterium tumefaciens*-mediated transfection of viral replicons for efficient transient expression in plants. *Nature Biotechnology* 23: 718–723.

Pogue GP, Lindbo JA, Garger SJ, and Fitzmaurice WP (2002) Making an ally from an enemy: Plant virology and the new agriculture. *Annual Review of Phytopathology* 40: 45–74.

Porta C and Lomonosoff GP (2002) Use of viral replicons for the expression of genes in plants. *Biotechnology & Genetic Engineering Reviews* 19: 245–291.

Smith M, Lindbo JA, Dillard-Telm S, *et al.* (2006) Modified tobacco mosaic virus particles as scaffolds for display of protein antigens for vaccine applications. *Virology* 348: 475–488.

Yusibov V, Shivprasad S, Turpen TH, Dawson W, and Koprowski H (1999) Plant viral vectors based on tobamoviruses. *Current Topics in Microbiology and Immunology* 240: 81–94.

Plum Pox Virus

M Glasa, Slovak Academy of Sciences, Bratislava, Slovakia
T Candresse, UMR GDPP, Centre INRA de Bordeaux, Villenave d'Ornon, France

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Plum pox virus (PPV), the agent responsible for the Sharka disease, belongs to the genus *Potyvirus*. The natural host range of this virus is restricted to *Prunus* spp. (stone fruits and ornamental trees). The infection of susceptible genotypes results in characteristic foliar and fruit symptoms and premature fruit drop. The wide geographical distribution of PPV includes most of Europe and the Mediterranean region as well as some countries in Asia and North and South America, although with widely different incidence levels in different countries. PPV is transmitted nonpersistently by more than 20 aphid species, by grafting and vegetative multiplication of infected plants, but is not seed-borne. To date, six strains/groups of PPV have been identified based on biological, serological, and molecular properties (M, D, Rec, C, EA, and W). Although many diagnostic tools are available for the sensitive and/or specific detection of PPV, its uneven distribution in infected woody hosts and its low titer outside of the active growth period significantly complicate its detection. In regions free of PPV, strict quarantine measures are usually enforced. In the quasi-absence of resistant fruit tree varieties, a mix of prophylactic approaches including the use of virus-free propagation material, eradication of diseased trees, and vector control is generally used in an effort to control the virus in regions where it has not reached an endemic status.

Economical Importance

PPV is considered as the most detrimental viral pathogen of stone fruit crops (peach, apricot, plum, Japanese plum). During approximately a century of recognized existence, PPV had a devastating effect on the European stone-fruit industry, mainly in the central and south European countries. Fruit trees infected with PPV cannot be cured and are often eliminated as a consequence of disease eradication or containment efforts. Although the infected trees are usually not stunted and do not die, fruit yields can be severely affected. Besides foliar symptoms, the virus often severely damages fruits, so that they have decreased weight and sugar content, overall lower gustative quality and often become blemished and are frequently unsuitable for consumption or processing. Premature fruit drop can also be observed and may reach 80–100% in susceptible cultivars. Consequently, traditional susceptible cultivars have to be replaced by less susceptible or tolerant cultivars, which are often of lower agricultural or gustatory quality. Infected propagation material and nursery stock (rootstock, budwood, scions) is not marketable and has to be destroyed. Because of its high potential impact on stone fruit crops, plum pox virus is listed as a quarantine pathogen in many parts of the world. In Europe for example, PPV is listed in the EC Plant Health Directive (Annex II of the European Union council directive 2000/29/EEC).

History and Geographical Distribution

The sharka disease was observed for the first time around 1917 on plum cv. Kjustendil in the village of Zemen in Bulgaria, near the Yugoslavian border. The disease is named according to the characteristic symptoms on fruits (sharka means pox in Bulgarian). Initially described on plum, the virus was observed in 1933 on apricot, in the 1960s on peach, and in the 1980s on sour and sweet cherry.

After World War I, the virus progressively spread to a large part of the European continent and Mediterranean basin, probably mostly as a consequence of exchange of infected propagation material. In recent years, the virus has been reported from China, South America (Chile, Argentina), and North America (USA, Canada). However, the prevalence of the disease differs from region to region, from the endemic occurrence observed in the central and eastern European countries, where the virus is well established, to local and more limited incidence observed in other countries where the virus was only introduced recently or where strict phytosanitary control measures have been enforced and have successfully retained the virus under some level of control.

Host Range and Symptomatology

Natural host range is restricted to species of the genus *Prunus*, including cultivated stone fruits – plum (*P. domestica*), Japanese plum (*P. salicina*), apricot (*P. armeniaca*), peach and nectarine (*P. persica*), almond (*P. amygdalus*). Although not infected by the majority of PPV isolates, sweet cherry (*P. avium*) and sour cherry (*P. cerasus*) are also natural hosts for some specific PPV isolates.

Symptoms vary depending on virus isolate, host/cultivar susceptibility, physiological status and age of the host, and environmental conditions, such as temperature.

Depending on the host, the symptoms may affect leaves, flowers, fruits, and stones. In recently infected trees, symptoms tend to be restricted to only some parts of tree but tend to generalize with time. Under field conditions, the symptoms on *Prunus* plants infected with PPV are often masked late in the season or during the warm period of the growing season.

Mixed infection with other viruses such as prunus necrotic ringspot virus (PNRSV) or prune dwarf virus (PDV) may further increase the severity of symptoms.

Foliar symptoms on plum consist generally of pale green chlorotic rings, spots, or patterns. Susceptible cultivars develop shallow ring or arabesque depressions on fruits, sometimes with brown or reddish necrotic flesh and gumming. Tolerant plum cultivars show no symptoms on fruits.

Infected apricots develop chlorotic or pale-green rings and lines on leaves, light-colored depressed rings on fruits, which may be severely deformed. Stones are marked with typical discolored rings.

Symptoms on susceptible peach genotypes are pronounced vein clearing, small chlorotic blotches, and distortions of the leaves. Color-breaking symptoms on the petals are observed in some varieties. Pale rings or diffuse band are visible on the skin of the fruits. In general, the symptoms on peach tend to be less visible in comparison with those on plums and apricots. Infection of almond is often symptomless or with limited foliar symptoms.

Characteristic symptoms on cherries consist of pale-green patterns and rings on leaves. Fruits may be slightly deformed, with chlorotic and necrotic rings and notched marks.

Premature dropping of fruits is frequently observed in the most susceptible varieties of the various hosts, in particular plum and apricot. Depending on host (cultivar) susceptibility, the losses may reach up to 100%.

PPV also infects wild and ornamental trees, such as myrobalan (*P. cerasifera*), Japanese apricot (*P. mume*), nanking cherry (*P. tomentosa*), Canada plum (*P. nigra*), American plum (*P. americana*), dwarf flowering almond (*P. glandulosa*), and blackthorn (*P. spinosa*), which may act as local reservoirs of the virus.

The experimental host range of PPV is large, with over 60 reported host species (such as *Chenopodium foetidum, Nicotiana benthamiana* and *N. clevelandii, Pisum sativum, Ranunculus arvensis, Senecio vulgaris, Stellaria media,* etc.) in eight families. However, no epidemiologically significant contribution of weeds and annual herbaceous plants to the spread of plum pox virus has been reported.

In woody hosts, the virus often shows an uneven distribution within the tree and full systemic invasion of a tree may require several years. Long-distance viral movement is restricted in some resistant (apricot, peach) and hypersensitive (plum) genotypes. The irregular distribution and translocation of the virus in the tree and the low titer outside the active growth period may complicate the detection of the virus when methods of insufficient sensitivity are used.

Virion Structure and Genome Properties

The flexuous filamentous viral particles are approximately 750×15 nm. Viral particles are composed of the single-stranded genomic RNA encapsidated by a single type of capsid protein subunit. The genome is of positive polarity and is 9741–9795 nucleotides in length. It has a polyadenylated 3′ end and a virus-encoded protein (VPg) covalently bound at its 5′ end. The genomic organization is typical of potyviruses, with a single open reading frame encoding a large polyprotein precursor (3125–3143 amino acids, *c.* 355 kDa) that is proteolytically processed by three virus-encoded proteinases (P1, HC-Pro, and NIaPro) to yield as many as ten mature functional proteins.

Strains/Groups

Initially, different strains or groups of isolates have been described on the basis of symptomatology in various experimental hosts. The most developed analysis of this kind relied on symptoms on *C. foetidum,* an experimental local-lesion host. PPV isolates were classified as yellow, intermediate, and necrotic strains depending on the type of local lesions they induced. However, this phenotypic characterization proved to be at least to some extent under environmental control and no relationships could be later established between this biological property and serological/molecular properties.

In the late 1970s, the existence of two serogroups of PPV was demonstrated using agar double diffusion assays employing polyclonal antibodies and a purified, formaldehyde-treated, suspension of undegraded viral particles. These two serogroups were named M (based on the type isolate Marcus from peach in Greece) and D (based on the type isolate Dideron from apricot in southeastern France). Later it was shown that both strains differ also in the coat protein (CP) mobility in denaturing polyacrylamide gel electrophoresis (PAGE) and in the presence or absence of an *Rsa* I restriction site in the region encoding the CP C-terminus.

On the basis of recent molecular and serological analysis, six strains/groups of PPV isolates sharing common biological, serological, and molecular properties are now recognized. This classification has also been validated by comparisons of complete genomic sequences of isolates representative of each of these strains (**Figure 1**).

PPV-M (from the type isolate Marcus). The isolates of this group are present in many European countries, but absent from the Americas and China. They are often

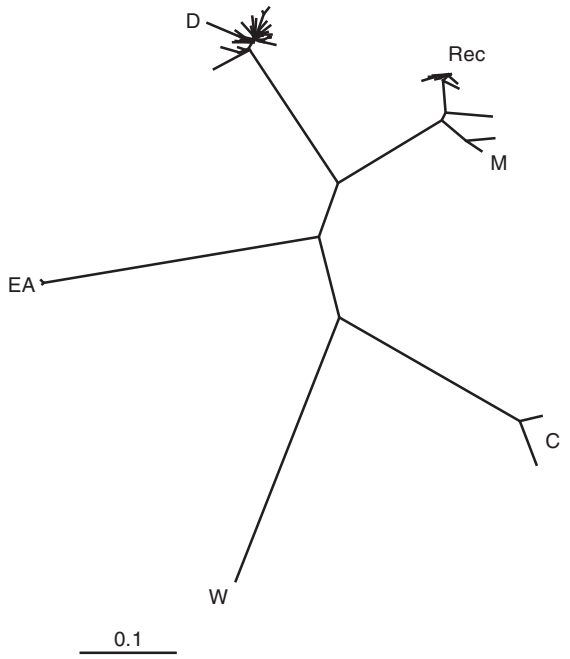


Figure 1 Unrooted phylogenetic tree computed using full-length coat protein gene sequences showing the relationships between isolates representative of all six known PPV strains.

Table 1 Origins and references of plum pox virus isolates for which complete genomic sequences are available

Isolate	*Strain*	*Country of origin*	*Original host*	*Reference*
PS	M	Yugoslavia	Peach	AJ243957
SK-68	M	Hungary	Plum	M92280
Dideron	D	France	Apricot	X16415
NAT	D	Germany	?	NC_001445
SC	D	?	?	X81083
PENN-1	D	USA	Peach	AF401295
PENN-2	D	USA	Plum	AF401296
PENN-3	D	USA	Peach	DQ465242
PENN-4	D	USA	Peach	DQ465243
Fantasia	D	Canada	Nectarine	AY912056
Vulcan	D	Canada	Peach	AY912057
48–922	D	Canada	Peach	AY912058
BOR-3	Rec	Slovakia	Apricot	AY028309
El Amar	EA	Egypt	Apricot	AM157175
El Amar	EA	Egypt	Apricot	DQ431465
SoC	C	Moldova	Sour cherry	Y09851
SwC	C	Italy	Sweet cherry	AY184478
W3174	W	Canada	Plum	AY912055

associated with rapidly spreading epidemics in peach but are less frequently found on plums. Usually, PPV-M isolates are transmitted efficiently by aphids.

PPV-D (from the type isolate Dideron). PPV-D isolates are widespread in all areas where PPV has been reported, including the recent outbreaks in Asia and South and North America. Isolates infect all the susceptible *Prunus* species excluding cherries, but PPV-D isolates are less frequently associated with spreading epidemics in peach.

PPV-Rec (from 'recombinant'). PPV-Rec isolates are derived from a single homologous recombination event between PPV-M and PPV-D isolates, with a cross-over located in the 3′ terminal part of the NIb coding region. This group of isolates was recognized only recently through the use of improved strain-typing methods. It was in fact found that a number of isolates originally described as PPV-M belong in fact to the PPV-Rec group. Isolates belonging to the PPV-Rec strain are widespread in several central and eastern European countries, frequently associated with plums and efficiently transmitted by aphids.

PPV-EA (from the type isolate El Amar) was originally isolated from apricots in the El Amar region of the Nile delta in Egypt in the late 1980s. It has also been observed in peach and in other regions of Egypt but has not been reported from outside this country thus far.

PPV-C (from 'Cherry') was first reported on sour cherry in Moldova and on sweet cherry in Italy. This group of cherry-adapted isolates seems sporadically present in some central and eastern European countries. PPV-C isolates are aphid transmissible and seem to be the only PPV isolates able to systemically infect cherry and *P. mahaleb*. PPV-C isolates are however also able to infect other *Prunus* species under experimental conditions.

PPV-W (from 'Winona') has been recently reported from a few infected plum trees in Canada. The PPV-W genome shows unique features, justifying its classification as a new strain but the epidemiology and other biological properties of this recently characterized strain remain to be determined.

Full-length genomic sequences have been determined for 18 PPV isolates representing each of the six recognized strains (**Table 1**). Moreover, partial sequences, focusing mainly on the 3′ terminal part of genome, are available for a large number of isolates, making PPV one of the genetically best studied potyviruses. The comparison of complete genomic sequences revealed up to 27.7% nucleotide divergence between representative isolates of the PPV-M, D, Rec, EA, C, and W strains (**Table 2**).

Recombination seems to have played a significant role in the evolutionary history of PPV. The PPV-Rec strain derives from a single recombination event involving isolates belonging to the PPV-D and PPV-M strains, with a recombination breakpoint in the C-terminus of the NIb gene. In addition, analysis of complete genomic sequences has recently demonstrated that the PPV-D and PPV-M strains themselves share an ancestrally recombined 5′ part of their genome (5′ noncoding region, P1, HC-Pro, and N-terminus of P3). Other recently

Table 2 Pairwise genetic distances calculated on the complete genomes of representative isolates of all six known PPV strains

	PPV-M	*PPV-D*	*PPV-Rec*	*PPV-EA*	*PPV-C*
PPV-D	0.127				
PPV-Rec	0.106	0.046			
PPV-EA	0.239	0.237	0.233		
PPV-C	0.257	0.266	0.260	0.277	
PPV-W	0.238	0.235	0.233	0.265	0.234

identified recombination events involve PPV-W, which shows a clear recombination signal with PPV-M in the region extending from the P1 coding region to the HC-Pro region and a divergent PPV-M isolate from Turkey (AY677114). It thus appears that at least four of the six recognized strains of PPV (among which the three most prevalent, PPV-D, -M, and -Rec) have recombination events in their evolutionary history. One remarkable consequence is that these four strains all share a similar genomic region corresponding to the C-terminus of their P1 and the N-terminus of their HC-Pro genes (genomic positions ~800–1450). On the other hand, PPV-C and PPV-EA appear to represent independent evolutionary lineages.

Assays that allow the discrimination between the various PPV strains include serological analysis with strain-specific monoclonal antibodies, restriction fragment length polymorphism (RFLP) analysis of polymerase chain reaction (PCR) fragments derived from the various genomic regions (CP, P3–6K1, and CI), PCR with strain specific primers and partial or complete sequence analysis. In addition, proper identification of recombinant isolates may require the use of several techniques targeting different parts of the viral genome or specific primers with binding sites located on both sides of the targeted recombination breakpoint. Strain-specific monoclonal antibodies have so far been obtained for isolates belonging to the PPV-M, PPV-D, PPV-EA, and PPV-C groups, but some isolates may show abnormal typing properties using either monoclonal antibodies or RFLP analysis techniques so that only PCR-based strain-specific assays or sequencing can provide unambiguous PPV strain identification.

Virus Spread

Natural spread of PPV occurs through aphids from neighboring infected reservoirs (cultivated, wild, or ornamental trees), while long-range movement of the virus is linked to international exchange of contaminated propagation material. The recent demonstration that aphids can acquire and transmit the virus from contaminated fruits indicates that fruit shipments could also represent a pathway for international virus movement. The virus is thus usually introduced in a new location by infected propagation material. Once established in the orchard or nursery, the virus is rapidly spread by aphid vectors. PPV is naturally transmitted by over 20 different aphid species in a nonpersistent manner (*Aphis craccivora, A. gossypii, A. hederae, A. spiraecola, Brachycaudus cardui, B. helichrysi, B. persicae, Myzus persicae, M. varians, Phorodon humuli, Rhopalosiphum padi,* etc.). Aphids generally transmit the virus over short distance, that is, next to infected sources, when migrating between plants and making test probes. The virus is transmitted in a nonpersistent manner so that aphids may acquire the virus very rapidly when probing infected plants with their stylets (piercing–sucking mouthpart). However, aphids can only transmit the virus for a short time after virus acquisition as the virus is retained in a viable state in the aphid's mouthparts for a period of only a few minutes to a few hours. The virus appears to be lost by the aphid the first time the aphid probes on the next plant and aphids do not remain infectious after moulting nor do they pass PPV onto their progeny.

The efficiency of transmission depends on the particular aphid species, virus isolate, and host species from which the virus is acquired and to which the virus is transmitted. As for other potyviruses, two viral proteins are known to be involved in the transmission mechanism, HC-Pro (helper component) and CP. The DAG amino acid motif is highly conserved in the N-terminus of the CP protein of aphid-transmissible PPV isolates and loss of transmissibility is correlated with its deletion.

Conflicting results have been reported in the past concerning seed transmission in some *Prunus* species, but recently more thorough studies have failed to demonstrate seed transmission of PPV in any of its woody hosts.

Ecology and Control

In the countries and regions where the virus is not yet present, strict quarantine measures need to be established to prevent the introduction of PPV through legal or illegal importation of infected fruit tree propagation material or fruits. In regions where the disease is present but still under control, a mix of prophylactic approaches is usually implemented, including the use of virus-free propagation material (rootstocks, budwoods), tight aphid vector control, regular inspection of orchards, and prompt eradication of infected plants. If the rate of infected trees in the prospected orchard exceeds a critical level (10–20% in most countries), whole orchards have to be removed.

In regions where eradication is no longer an option and where the spread of the disease is no longer under control (e.g., central and southeastern Europe), the cultivation of less susceptible or tolerant varieties is so far the only option that may allow to continue the production of susceptible fruit species. This practice, however, further contributes to the viral spread.

For PPV control in the long term, most European countries are placing strong emphasis on breeding programs aiming at the development of PPV-resistant varieties. Unfortunately, only a very limited number of natural sources of resistance to PPV have been identified within the *P. armeniaca* and *P. domestica* germplasm, and these are often characterized by low-quality fruits. In peach, despite extensive screening efforts, no resistant varieties are known but resistance has been identified in the wild relative *P. davidiana*. Breeding efforts are ongoing using these various resistance sources to introduce PPV resistance in commercially interesting varieties. Progress is however slowed by the long generation times, the length of the resistance tests, and the often polygenic nature of the resistances involved.

Development of genotypes with a hypersensitive response as an active defense mechanism against PPV is another promising way to produce resistant fruit trees, as was demonstrated for some plum varieties, but no hypersentivity sources are so far known in apricot and peach. In addition, it should be cautioned that in herbaceous crops, hypersensitive resistance to viruses has often proved to be nondurable as a consequence of genetic evolution of the virus.

Early attempts to use cross-protection with attenuated virus isolates, a technique successfully used to control some other potyviruses (zucchini yellow mosaic virus, papaya ringspot virus), have not met with success in the case of PPV.

Transgene-based resistance offers a complementary approach for the development of PPV-resistant stone fruit cultivars. Experimental herbaceous plants transformed with different regions of the PPV genome (i.e., P1, HC-Pro, CI, NIa, Nib, or CP) have been developed and have shown partial or complete resistance to PPV. A transgenic clone of *P. domestica* (C5), containing the PPV capsid protein gene, has been described as highly resistant to PPV in greenhouse and field tests, displaying characteristics typical of post-transcriptional gene silencing (PTGS) based resistance. Although this resistance can be partially overcome by graft inoculation, it provided effective protection against the natural spread of PPV in several field tests, even under conditions of high natural inoculation pressure.

See also: Papaya Ringspot Virus; Potato Virus Y; Potyviruses; Watermelon Mosaic Virus and Zucchini Yellow Mosaic Virus.

Further Reading

EPPO (2004) EPPO Standards PM 7/32 Diagnostic Protocol for Plum pox potyvirus. *EPPO Bulletin* 34: 247–256.

EPPO (2006) A review of plum pox virus. *EPPO Bulletin* 36: 201–349.

Glasa M and Candresse T (2005) Plum pox virus. AAB Description of Plant Viruses. No. 410. http://www.dpvweb.net/dpv/showdpv.php?dpvno=410 (accessed July 2007).

Kegler H, Fuchs E, Gruntzig M, and Schwarz S (1998) Some results of 50 years of research on the resistance to plum pox virus. *Acta Virologica* 42: 200–215.

López-Moya JJ, Fernández-Fernández MR, Cambra M, and García JA (2000) Biotechnological aspects of plum pox virus. *Journal of Biotechnology* 76: 121–136.

Martinez-Gomez P, Dicenta F, and Audergon JM (2000) Behaviour of apricot (*Prunus armeniaca* L.) cultivars in the presence of sharka (*Plum pox potyvirus*): A review. *Agronomie* 20: 407–422.

Poliomyelitis

P D Minor, NIBSC, Potters Bar, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

A funerary stele from about 1300 BC currently in the Carlsberg museum at Copenhagen shows the priest Rom with the withered single limb and down-flexed foot typical of motor neuron destruction caused by poliovirus. It is considered to be the first documentary evidence for an infectious disease of humans. Two highly effective vaccines were developed in the 1950s, and a major program of the World Health Organization (WHO) is underway which may make poliomyelitis the second human disease to be eradicated globally after smallpox.

Pathogenesis and Disease

Poliomyelitis gets its name from the specificity of the virus for the motor neurons that form the gray matter (*polios* and *myelos*, Greek for gray and matter, respectively) of the anterior horn. Despite the obvious physical signs of muscle atrophy and motor neuron degeneration, few cases of poliomyelitis are identifiable in the literature before the end of the nineteenth century, and it is believed that it was extremely rare. However, at this time, the disease began to occur in large epidemics, initially in Scandinavia (particularly Sweden), and then in the USA, most commonly affecting young children (hence the alternative

name of infantile paralysis). The legs are more commonly affected than the arms, and paralysis tends to occur in one limb rather than symmetrically.

Poliovirus occurs in three antigenically distinct serotypes designated 1, 2, and 3, such that infection with one serotype confers solid protection only against other viruses of that serotype. Infections primarily occupy the gut, and most are entirely silent, but in a small number of cases they lead to a systemic infection (the minor disease) 3–7 days post exposure, characterized by fever, rash, or sore throat. Depending on the strain or type of virus, usually less than 1% of all infections lead to the major disease or poliomyelitis which develops on average 7–30 days after infection. Spinal poliomyelitis resulting in lower limb paralysis or bulbar poliomyelitis in which the breathing centers are affected occurs when the lower or upper regions, respectively, of the spinal cord are affected. In the mid-twentieth century, about 10% of cases recovered without sequelae, 80% had permanent residual paralysis, and the remainder died. Encephalitis occurs but is rather rare. Meningitis, also known as abortive or nonparalytic poliomyelitis, occurs at a rate of about 5% of poliomyelitis cases.

Infection is mainly fecal–oral, although the virus can also be transmitted by the respiratory route from the throats of infected individuals. The primary site of infection in the gut is not known but may be the lymphoid tissues, specifically the Peyer's patches and tonsils or the mucosal surfaces in the gut or throat; infectious virus can be found in the local lymph nodes and in more distal mesenteric lymph nodes but it is not clear that the virus necessarily replicates there. The Peyer's patch-associated M-cells are believed by many to be the major infected cells in the gut.

Viremia may occur about 7 days after infection, corresponding roughly to the appearance of the minor disease. It is believed to seed sites including the peripheral and central nervous systems resulting in the major disease. Thus humoral antibodies should prevent poliomyelitis by blocking viremia; the protective effect of passively administered humoral antibodies was shown in the 1950s. This explains the change in the epidemiology of the disease at the start of the twentieth century as hygiene improved, and children were exposed to infection later in life when maternal antibody levels had declined to levels no longer able to confine the infection to the gut.

The Virus Genome

The virus contains a single strand of messenger sense RNA of about 7500 nt. A long highly structured 5′ noncoding region of about 740 nt which serves as an internal ribosomal entry site (IRES) is followed by a single open reading frame and terminates in a 3′ noncoding region of *c.* 70 nt followed by a polyadenylate tract. The 5′ end of the genome is covalently linked to a virus-encoded protein termed VPg. The single open reading frame encodes the structural proteins which make up the capsid (collectively termed the P1 region) followed by the regions encoding the nonstructural proteins P2 and P3. P1 is divided into VP1, VP3, and VP0 (VP2 plus VP4). P2 is cleaved into $2A^{pro}$, 2B, and 2C, and P3 into 3A, 3B (or VPg), $3C^{pro}$, and $3D^{pol}$ by virus-encoded proteases. $3D^{pol}$ is the viral polymerase, but all proteins in the nonstructural part of the genome play a role in RNA replication, which also depends on RNA structural elements and host cell membranes which are extensively rearranged in the course of infection.

The Virus Particle

The infectious virus particle consists of 60 copies each of VP1, VP2, VP3, and the smaller protein VP4; VP2 and VP4 are generated by autocatalytic cleavage of VP0, in the last stage of the maturation of the particle. The proteins are arranged with icosahedral symmetry such that VP1 is found at the pentameric apex of the icosahedron and VP2 and VP3 alternate around the pseudo-sixfold axes of symmetry of the triangular faces of the icosahedron. VP4 is located internally about the pentameric apex and is myristylated. The pentameric apex of the particle is surrounded by a dip or canyon into which the cellular receptor for poliovirus fits. The atomic structure of the virus was solved in 1985.

The Poliovirus Receptor

Although some strains of type 2 poliovirus are able to infect normal mice, only the higher primates and Old World monkeys are susceptible to infection by all serotypes as they possess the specific receptor site required, and this species restriction is one of the factors that makes eradication theoretically possible. The receptor site was identified in 1986 and is a three-domain membrane protein of the immunoglobulin superfamily termed CD155. It is necessary and sufficient for the infection of cells *in vitro*, and transgenic mice carrying the gene for the polio receptor can be infected by all poliovirus types, developing paralysis when infected with wild-type virus. Such mice have been developed as alternatives to monkeys in the safety testing of live polio vaccines.

The tissue distribution of the receptor site does not explain the targets of infection of the virus and it is possible that innate immunity plays a significant role in its highly specific tropism in normal individuals.

Classification

The members of the family *Picornaviridae* to which poliovirus belongs are small nonenveloped viruses of about

30 nm in diameter when hydrated, essentially featureless when examined by electron microscopy and containing a single strand of an RNA molecule of messenger sense. The family is currently split into nine genera on the basis of sequence similarities, as shown in **Table 1**.

The genus *Enterovirus*, of which poliovirus is the archetypal species, is further subdivided into eight species of which *A* to *D* and the *Poliovirus* infect humans. The classification is summarized in **Table 2**, with examples of the viruses within the species.

The sequences of the capsid region of the viruses within a species define a virus type while sequences of other regions of the genome do not. This suggests that viruses within a particular species can exchange genome segments more or less freely. On this basis, polioviruses should be assigned to the species *C enterovirus* but at the time of writing they remain a separate species because of the unique human disease they cause.

The comparison of the sequence of field isolates has proved a very valuable tool in the WHO program to eradicate polio. Viruses from the same geographical region tend to cluster in terms of their sequence largely independent of the year of isolation. Thus it is possible to identify a virus from a case as indigenous to the region where it was isolated or an importation. Similarly, as virus circulation is obstructed by effective vaccination programs, the variety of sequences found declines, indicating progress long before the disease is eradicated. Finally, it is possible to identify strains as wild type or vaccine derived. It has become clear that the rate at which the sequence of a vaccine or wild-type virus lineage drifts in an epidemic or during chronic infection of immunodeficient individuals unable to clear the virus is amazingly constant at about 2–3% silent substitutions per year, or 1% for all substitutions. Thus the comparison of viral sequences can date the common ancestor of two polioviruses accurately and therefore how long they have been circulating.

Table 1 Genera within the family *Picornaviridae*

Genus
Enterovirus
Rhinovirus
Cardiovirus
Aphthovirus
Hepatovirus
Parechovirus
Erbovirus
Kobuvirus
Teschovirus

Table 2 Species within the genus *Enterovirus*

Species	*Serotype*
Poliovirus	1, 2, 3
Human enterovirus A	Coxsackievirus A2, 3, 5, 7, 8, 10, 12, 14, 16 Enterovirus 71
Human enterovirus B	Coxsackievirus B 1–6, coxsackievirus A 9 Human echovirus 1–7, 9, 11–21, 24–27, 29–33 Human enterovirus 69
Human enterovirus C	Coxsackie virus A 1, 11, 13, 15, 17–22, 24
Human enterovirus C	Human enterovirus 68, 70
Bovine enterovirus	Bovine enterovirus 1, 2
Porcine enterovirus A	Porcine enterovirus 8
Porcine enterovirus B	Porcine enterovirus 9, 10

Vaccines

Inactivated polio vaccine (IPV) was developed by Salk. It consists of wild-type virus that has been treated with low concentrations of formalin sufficient to inactivate the virus without affecting its antigenic properties to any great extent. The vaccine was first licensed in 1955 in the USA and reduced the number of cases by over 99%. It has been shown to be capable of eradicating the disease in certain countries, notably Scandinavia and the Netherlands. However, there remained controversy over whether a nonreplicating vaccine could eradicate disease and the virus altogether in less-developed countries where exposure is more intense, and it was possible that live-attenuated vaccines imitating natural infection might give better protection against infection by wild-type viruses. The live-attenuated vaccines given by mouth (oral polio vaccine, OPV) that form the basis of the current eradication campaign were developed by Sabin and introduced in the early 1960s. The Sabin vaccines have been shown to be able to interrupt epidemics and to break transmission if used correctly, resulting in the eradication of the virus in entire regions and possibly eventually the world.

It was also known that the Sabin vaccines altered in the vaccine recipients, becoming more neurovirulent particularly in the case of the type 3 component, and that in rare instances, now estimated at about 1 case per 750 000 first-time vaccinees, the vaccine could cause poliomyelitis in recipients and their immediate contacts. Both IPV and OPV contain a single representative of each of the three serotypes.

Attenuation of the Sabin Vaccine Strains

The genome of poliovirus is of positive (messenger) RNA sense and therefore infectious. Complete genomes of the virulent precursors of the Sabin vaccine strains or isolates

from vaccine-associated cases were cloned, sequenced, and mutations introduced or segments exchanged. Virus recovered from RNA transcribed from the modified plasmids was tested in monkeys or in transgenic mice carrying the poliovirus receptor. It was possible to identify two differences between Leon, the virulent precursor of the Sabin type 3 vaccine strain which would attenuate Leon or would de-attenuate the Sabin strain, one in the 5′ noncoding region, the other in capsid protein VP3. Similarly, there were two major attenuating mutations in the type 2 strain, one in the 5′ noncoding region, the other in capsid protein VP1. The story with type 1 was more complicated. Again there was one mutation in the 5′ noncoding region but several throughout the capsid region. All three of the 5′ noncoding mutations affect the highly structured region shown in **Figure 1**.

All 5′ noncoding mutations have been shown to affect initiation of protein synthesis, all involve allowed but altered base pairing, and all revert or are suppressed in vaccine recipients when sequential isolates are made. Excretion of virus following vaccination with OPV continues on average for 4 or 5 weeks and the reversions observed occur early in the excretion period, usually within 1 or 2 days for the type 3 strain and within a week for the other two types. Other changes also take place, including the reversion or more usually direct or indirect suppression of the VP3 mutation in the type 3 strain.

In addition, the viruses recombine with each other at high frequency. The type 3 strain in particular is usually excreted as a recombinant from about 11 days post immunization with the part of the genome encoding the nonstructural genes from the type 1 or type 2 strains. Complex recombinants with portions from different serotypes are also common, although their selective advantage is not known. The adaptation of the virus to the gut is therefore rapid and subtle and by a variety of mechanisms including reversion, second site suppression, and suppression of the phenotype of poor growth by enhancing fitness by an entirely unrelated route, and may involve mutation or recombination. Once adapted, virus excretion persists typically for several weeks. The drift in the viral sequence over prolonged periods of time referred to above occurs in addition to these selected changes, and appears to be a consequence of a purely stochastic process.

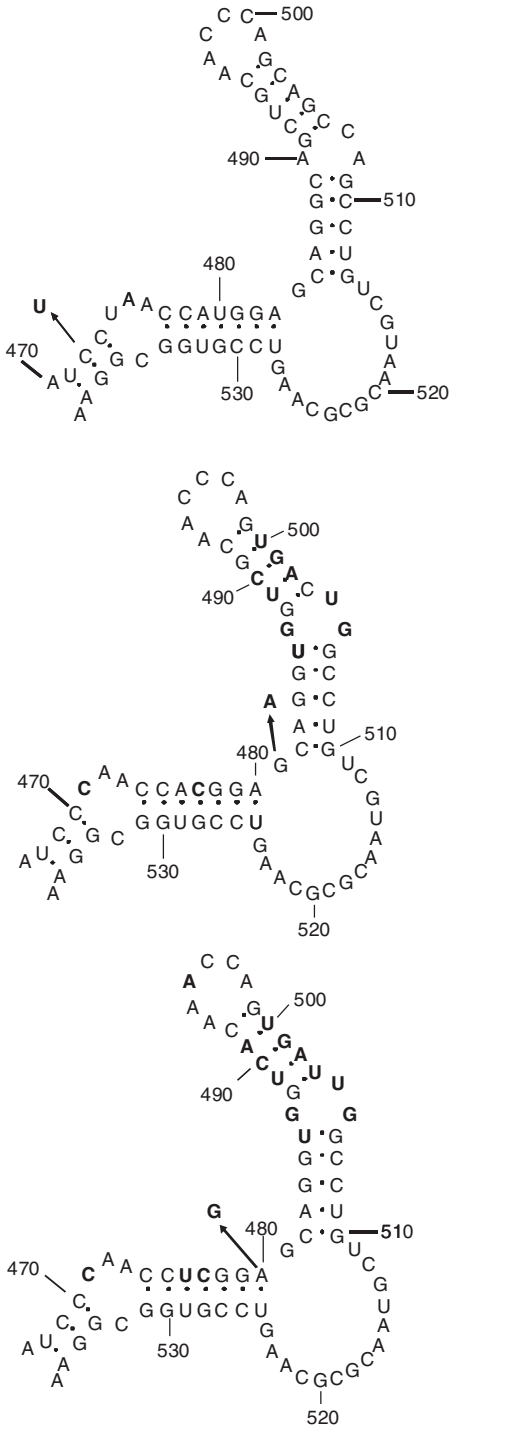


Figure 1 Structure of RNA domain in the 5′ noncoding region of polio involved in attenuating the neurovirulence of the Sabin live-attenuated vaccine strains. Top: type 3; center: type 2; bottom: type 1. Differences from the type 3 sequence are shown in bold, illustrating the general conservation of the base-paired structures. Base changes involved in attenuation are shown by arrows for the three types. Note that for types 1 and 3, the changes result in weaker but allowed base pairing compared to the wild-type sequence.

The Global Polio Eradication Program

For many years, it was thought that OPV could not be effective in tropical countries, and despite large-scale use its impact was in fact minimal. The reasons put forward included interference by other enteric infections, and many other factors, including breast-feeding. The

most plausible explanations are that the vaccine used in routine vaccination programs was probably poorly looked after and therefore inactive, and the epidemiology of the disease was different in tropical and temperate climates. In countries of Northern Europe, poliomyelitis is highly seasonal, occurring essentially only in summer. Thus a routine vaccination campaign in which children are immunized at a set age is able to reduce the number of susceptibles during the winter when the virus is not freely circulating, so that circulation of the wild-type virus in the summer is impaired. Eventually, the wild-type virus dies out. In tropical climates, however, exposure is less seasonal, so that there is no respite during which immune populations can be built up by immunization; it remains a matter of chance whether a child is exposed first to vaccine or wild-type virus, and the impact on disease is correspondingly less. The strategy required is to immunize large proportions of the population at once. This approach was successfully followed in the 1960s in the USA in the southern states, where the climate is more tropical and routine immunization was less effective. However, it was not until the 1980s that the strategy was used in developing countries in South America when vaccine was given in mass campaigns termed National Immunization Days (NIDs). The success of the program led to a resolution in 1988 by WHO to eradicate polio from the world by the year 2000. While this has still not been achieved at the time of writing, there are only four countries from which poliovirus has never been eradicated: India, Pakistan, Afghanistan, and Nigeria. Northern India has proven extremely difficult and some children have contracted poliomyelitis after 10 or more immunizations with OPV. One approach that has been adopted is to use monovalent vaccine instead of the trivalent form containing all three serotypes. The rationale is that in the trivalent vaccine the different serotypes compete so that the most relevant serotype may not infect and immunize, whereas a monovalent type 1 vaccine used in an area where this is the problem serotype will be effective. There is evidence that this gives faster immune responses, and it was first used in Egypt where it eradicated the final type 1 strains. Monovalent vaccines are becoming the major tool in the current stages of eradication.

While there are few countries in which poliomyelitis has not been eradicated, many countries have suffered reintroductions. This is shown in **Figure 2**, which shows cases of the regions where polio has not been completely eliminated.

The first examples of this phenomenon were in central Africa. A decision was taken because of shortage of funds to concentrate immunization efforts on Nigeria which had many cases (**Figure 2**(**a**)). Thus immunization in the surrounding countries suffered, and at the same time resistance to immunization grew in northern Nigeria because of local concerns about supposed contaminants in the vaccine. During the period when immunization ceased, there was a resurgence in polio in Nigeria, which spread to adjacent countries where the immunization activities had been reduced (**Figure 2**(**b**)). As a result, polio spread from Nigeria across much of central Africa, being brought under control eventually by coordinated NIDs across most of the continent, an operation of unprecedented scale. Shortly after this, with polio still endemic in Nigeria, the annual pilgrimage to Mecca resulted in the introduction of polio from northern Nigeria into Yemen and Indonesia (**Figure 2**(**c**)). In 2006, polio got introduced into several African countries from northern India (**Figure 2**(**d**)).

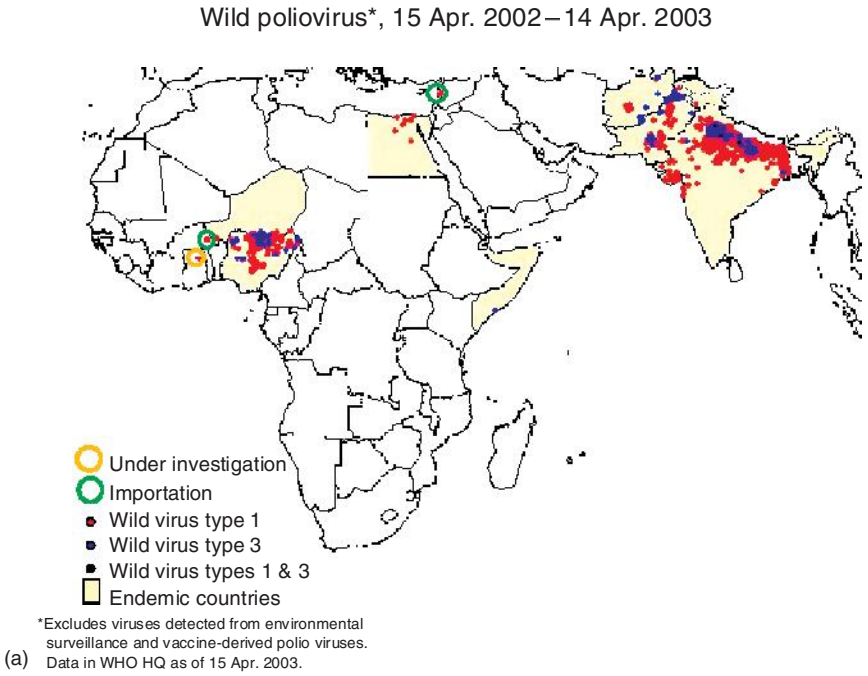
The difficulties of eradication are hard to overestimate, and are compounded by the fact that most infections are entirely silent. It is clear that so long as one country remains a source of the virus the world remains at risk. This complicates the strategies to be followed once there is some confidence that the virus has in fact been eradicated in the wild, which are further complicated by the fact that OPV is a live vaccine derived from wild-type virus that is able to change in the infected vaccinee, from whom virus may be isolated for significant periods.

Vaccine-Derived Poliovirus

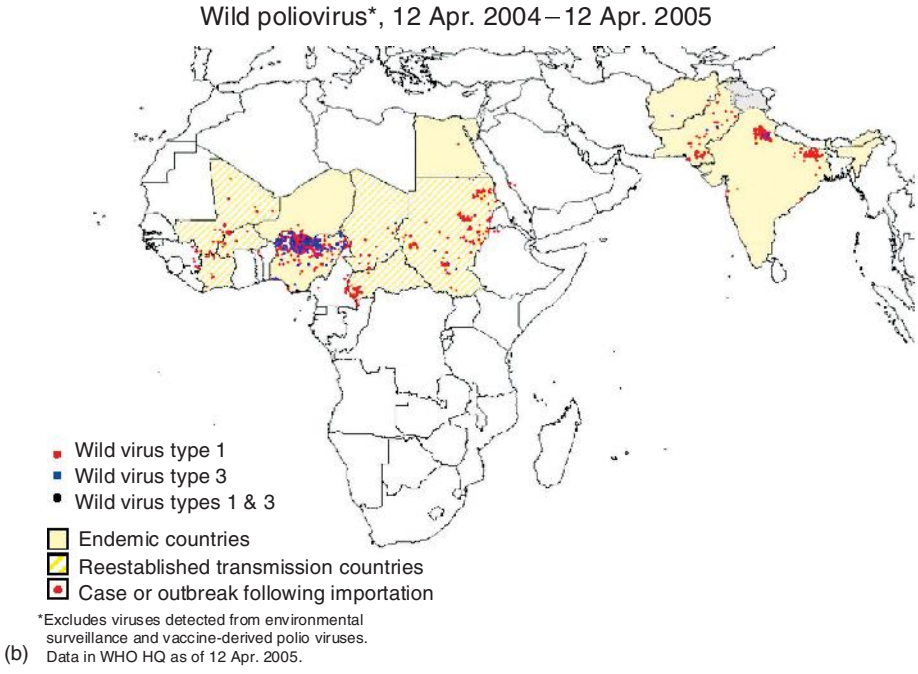
Vaccine-associated paralytic poliomyelitis (VAPP) cases were recognized from the first use of the Sabin vaccine strains, although their unambiguous identification required the molecular methods applied in the 1980s. They occur in primary vaccinees at a rate of about 1 per 750 000 but can also occur in contacts and those previously vaccinated. The occurrence of such cases, while rare, indicates that the vaccine strains can revert to virulence in recipients and that it is possible for the vaccine strains to spread from person to person.

In 2001, it was recognized for the first time that the Sabin vaccine strains could be the cause of outbreaks of poliomyelitis when about 22 cases were identified in Hispaniola, comprising Haiti and the Dominican Republic. Sequencing of the strains showed that they were very closely related to the type 1 Sabin vaccine strains and not to the previous endemic strains of which the last had been isolated 20 years earlier. Moreover, the sequence diversity indicated that the outbreak strains had been circulating unnoticed for *c.* 2 years, as they differed from the vaccine strain by about 2% overall. In addition, they were shown to be recombinant strains with a major portion of the nonstructural regions of the genome from viruses identified as species C enteroviruses unrelated to the vaccine strains. At least three different genomic structures were identified. Subsequently, it was reported that between 1988 and 1993 all supposed wild type 2 strains isolated from poliomyelitis

cases in Egypt were in fact heavily drifted vaccine-related strains. Outbreaks in the Phillipines in 2001, two in Madagascar in 2002 and 2005, one in China in 2004, and one in Indonesia in 2005 have since been recorded. The most likely explanation is that vaccination programs become less vigorous once polio is eliminated from a country and other health issues take priority, so that while administration of OPV continues, coverage is less than 100% giving perfect conditions for the selection of transmissible strains. For reasons that are not understood, all such strains to date have been recombinants with unidentified species C enteroviruses. So far the outbreaks have been limited and easy to control; this was particularly shown in Indonesia where a type 1 outbreak from a



(a)



(b)

Figure 2 Continued

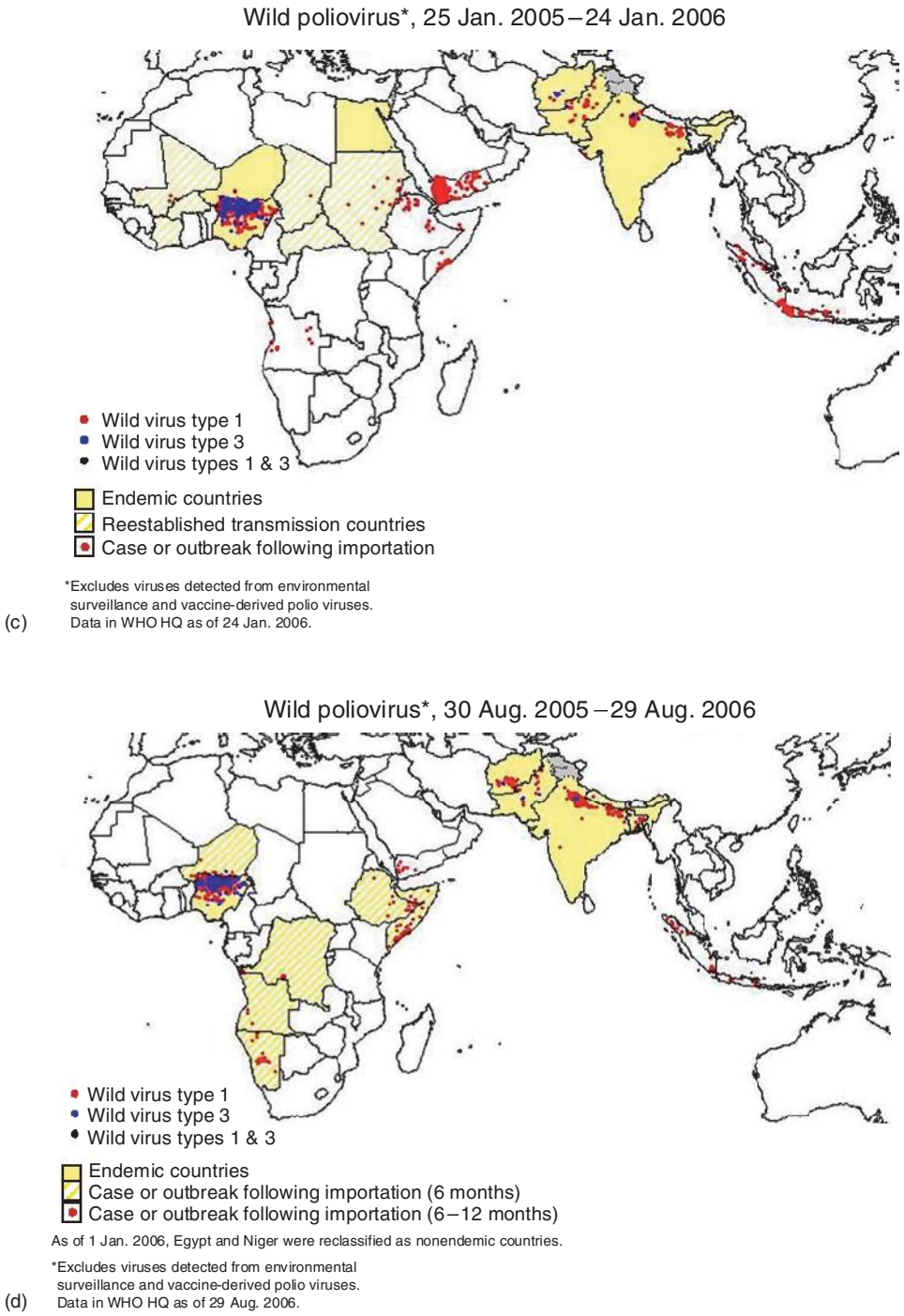


Figure 2 Occurrence of cases of poliomyelitis in remaining areas of infection from 2002 to 2006. Source: World Health Organization (WHO).

virus introduced from Nigeria occurred at the same time as an outbreak of circulating vaccine-derived poliovirus (CVDPV). The wild-type outbreak persisted longer. Virologically, there is no real reason why vaccine-derived viruses should not become as aggressive as wild-type viruses from which they derived, although this does not seem to have happened at the time of writing. The occurrence of such viruses is clearly a problem when cessation of vaccination is considered.

A second issue is the occasional case of long-term excretion of polioviruses by individuals deficient in humoral immunity. The viruses are termed immunodeficient vaccine-derived polioviruses (IVDPVs). Probably only a few percent of these hypogammaglobulinemic individuals

will become long-term excreters of virus even if given OPV, and most stop shedding virus spontaneously after a period, which may be 2 or 3 years. Occasionally virus excretion may continue for much longer; in one instance isolates, were available for 12 years, and the sequence data and medical history of the patient suggest that virus has been shed for well over 20 years. The patient remains entirely healthy, although the virus is highly virulent in animal models. No treatment has been successfully applied to such individuals, although there are claims for success with an antiviral compound in one case. It is of interest that the mechanism by which virus is cleared is still not known. An infected individual can be expected to excrete virus for over 4 weeks and will continue to do so after an immune response is detectable. Immune-deficient patients can stop excreting virus spontaneously with no evidence of an immune response and while termination of excretion correlates in general with a rise in fecal IgA levels, this is neither necessary nor sufficient.

Cessation of Vaccination

OPV is the major tool of the eradication efforts that have been so successful in most of the world. While the remaining pockets of infection seem to be particularly intransigent, it is likely that polio can be eradicated possibly serotype by serotype by the increasing usage of monovalent OPV. In fact, the last known wild type 2 virus was isolated in October 1999, proving that eradication is possible in principle. This leads to the need to stop vaccinating while ensuring that the disease does not re-emerge. So far as CVDPVs are concerned the safest strategy might be to stop vaccinating abruptly after one last mass immunization, so that OPV is not continuously dripped into a population with overall declining immunity. The assumption would be that the virus dies out before susceptibles build up to a sufficient extent to maintain circulation. The strategy would require careful monitoring to ensure that the virus was not returning. IVDPVs are more difficult in that there is no known treatment and the only strategy currently may be to ensure that the populations around the cases have a high level of immunity, which clearly cannot involve the administration of OPV as this would risk starting the cycle all over again.

It is therefore likely that IPV will play a bigger role as time goes by, and many developed countries have already switched to its use, including the USA, Canada, and most of western Europe. IPV is both more expensive and difficult to administer than OPV and currently cannot be easily given in mass campaigns. It tends to be used in countries with higher incomes. However, it is possible that IPV could be combined with other vaccines such as diphtheria and tetanus for use in developing countries.

A remaining problem is that the production of IPV at present involves the growth of very large amounts of wild-type poliovirus. There have been at least two instances of virus escaping from such production facilities, although this may be less likely now that the issue has been recognized and containment at the major production sites greatly improved as a result. IPV production still poses a risk and if IPV were to be the only vaccine against polio production would probably have to increase in scale. Possibilities such as the use of the Sabin vaccine strains to make IPV are being examined as a possibly safer option, but raise complex issues of immunogenicity and yield as well as logistic concerns to do with the clinical trials of novel vaccine formulations which may be necessary.

The most likely current scenario for stopping vaccination seems to be that well-off countries and the better-off in developing countries will continue be immunized, but with IPV rather than OPV, while vaccination for the remainder will simply cease after a major last mass campaign.

See also: Smallpox and Monkeypox Viruses; Vaccine Production in Plants; Vaccine Safety; Vaccine Strategies.

Further Reading

Bodian D (1955) Emerging concept of poliomyelitis infection. *Science* 122: 105–108.

Fine PE and Carneiro IA (1999) Transmissibility and persistence of oral polio vaccine viruses: Implications for the global poliomyelitis eradication initiative. *American Journal of Epidemiology* 150: 1001–1021.

Hammon WD, Coriell LL, Wehrle PF, *et al.* (1953) Evaluation of Red Cross gammaglobulin as a prophylactic agent for poliomyelitis. Part 4: Final report of results based on clinical diagnosis. *JAMA* 151: 1272–1285.

Kew O, Morris-Glasgow V, Landarverde M, *et al.* (2002) Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. *Science* 296: 356–359.

Minor PD (1992) The molecular biology of poliovaccines. *Journal of General Virology* 73: 3065–3077.

Minor PD (1997) Poliovirus. In: Nathanson N, Ahmed R, Gonzalez-Scarano F, *et al.* (eds.) *Viral Pathogenesis*, pp, 555–574. Philadelphia: Lippincott-Raven Publishers.

Minor PD, John A, Ferguson M, and Icenogle JP (1986) Antigenic and molecular evolution of the vaccine strain of type 3 poliovirus during the period of excretion by a primary vaccinee. *Journal of General Virology* 67: 693–706.

Nkowane BU, Wassilak SG, and Orenstein WA (1987) Vaccine associated paralytic poliomyelitis in the United States: 1973 through 1984. *JAMA* 257: 1335–1340.

Paul JR (1971) *A History of Poliomyelitis.* New Haven, CT: Yale University Press.

Sabin AB (1956) Pathogenesis of poliomyelitis: Reappraisal in the light of new data. *Science* 123: 1151–1157.

Sabin AB, Ramos-Alvarez M, Alvarez-Amesquita J, *et al.* (1960) Live orally given poliovirus vaccine: Effects of rapid mass immunization on population under conditions of massive enteric infection with other viruses. *JAMA* 173: 1521–1526.

Relevant Website

http://www.polioeradication.org – The Global Polio Eradication Initiative.

Polydnaviruses: Abrogation of Invertebrate Immune Systems

M R Strand, University of Georgia, Athens, GA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Fat body The main metabolic organ of insects and a primary source of numerous immune effector molecules including antimicrobial peptides.
Hemocoel The body cavity of the insect that contains blood and all internal organs.
Hemocytes Insect blood cells with diverse functions in immunity.
Oviposition The act of laying an egg by an insect.
Parasitoid An insect which is free-living as an adult but which develops as a parasite in or on the body of another invertebrate; parasitoids require only a single host to complete their immature development and almost always kill their hosts; most hosts of parasitoids are other insects and most parasitoids are in the order Hymenoptera (wasps, bees, and ants).

Introduction

The innate immune system of insects consists of both cellular and humoral components that provide defense against a diversity of invading parasites and pathogens. Parasitoid wasps form the largest group of multicellular parasites of insects with many species being of considerable economic importance as biological control agents of insect pests. There are an estimated 200 000 species of parasitoid wasps which may account for 10–20% of all insect species on earth. Almost all insects are attacked by some type of parasitoid but individual parasitoid species are usually specialists that parasitize only one or a few species of hosts. Insects have evolved potent immunological defense responses against parasitoid attack and parasitoids have evolved a diversity of counter-strategies to subvert these defenses. How these defense and counter-defense responses are regulated is not well understood across all insect and parasitoid species. However, it is known that a number of parasitoids rely on different types of microbial symbionts for survival in their hosts. The best characterized of these associations is the large number of parasitoid wasps that carry viruses in the family *Polydnaviridae*.

Polydnaviruses persist as stably integrated proviruses in the genome of their associated parasitoid species and replicate in the ovaries of female wasps in specialized cells that form a structure called the calyx (**Figure 1**). Virions accumulate in the lumen of the lateral oviducts to high density, creating a suspension of virus called calyx fluid. When a wasp lays an egg into a host, she injects a quantity of virus that infects different tissues including hemocytes. Polydnaviruses do not replicate in the wasp's host but expression of viral gene products confers protection from the host's immune system and alters other physiological processes that allow the parasitoid's offspring to successfully develop. Thus, a true mutualism exists between polydnaviruses and wasps as viral transmission depends on parasitoid survival, and parasitoid survival depends on infection of the host by the virus. Another entry in this volume discusses polydnavirus structure and genome organization. This article focuses on the function of polydnaviruses in abrogation of the insect immune response.

Polydnavirus Genera Differ in the Virulence Genes They Encode

Athough polydnaviruses share a similar life cycle, important differences also exist between the parasitoids that carry these viruses and the types of virulence genes different polydnaviruses encode. All members of the *Polydnaviridae* are enveloped and have circular, double-stranded DNA genomes that consist of multiple segments (15 to more than 30) of variable size (3 to >40 kbp) and abundance. However, the family is divided into two genera, *Bracovirus* and *Ichnovirus*, on the basis of their association with seven subfamilies of parasitoid wasps in the family Braconidae (Cardiochilinae, Cheloninae, Dirrhoponae, Mendesellinae, Khoikhoiinae, Miricinae, Microgastrinae; *c.* 17 500 species total) and two subfamilies of wasps in the Ichneumonidae (Campopleginae, Banchinae; *c.* 13 000 species total), respectively (**Figure 2**). Wasps in both families primarily parasitize larval stage Lepidoptera (moths and butterflies).

Comparative studies indicate that each polydnavirus carried by a given parasitoid species is genetically unique. Cumulative genome sizes for polydnaviruses are quite large and range from 180 kbp for microplitis demolitor bracovirus (MdBV), to 268 kbp for campoletis sonorensis ichnovirus (CsIV), and 568 kbp for cotesia congregata bracovirus (CcBV). In contrast, coding densities are very low and tend to be dominated by a small number of genes that have diverged into multiple variants. The majority of these gene family members are expressed in parasitized host insects but polydnaviruses are unable to replicate because their encapsidated genomes lack essential genes

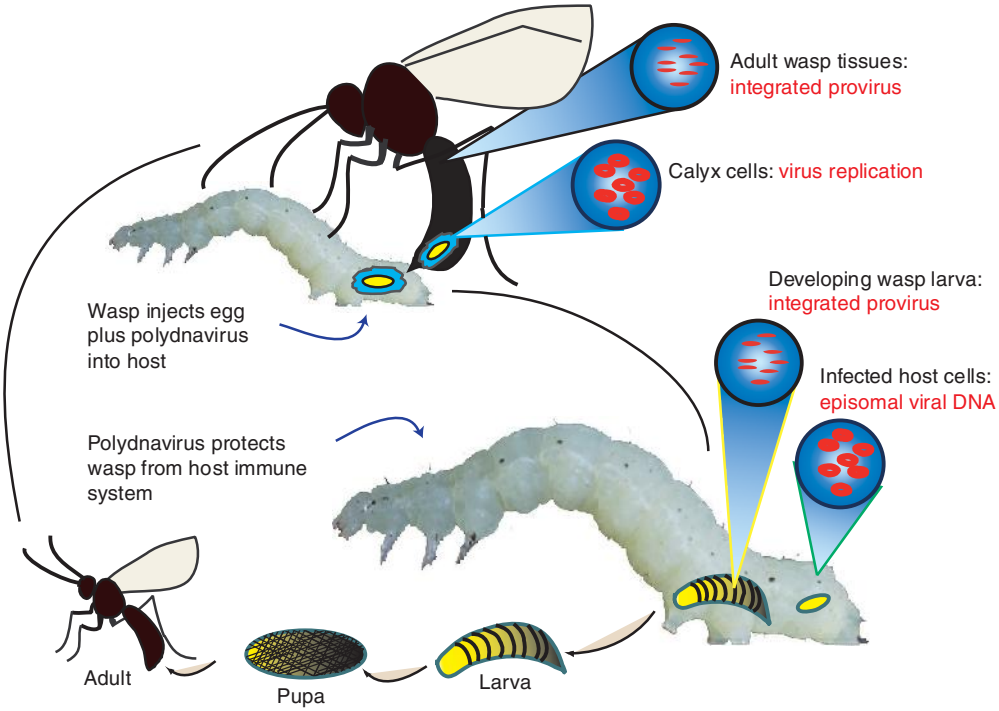


Figure 1 Parasitoid and polydnavirus life cycle. Polydnavirus DNA is integrated into somatic and germ cells of the associated wasp with replication restricted to calyx cells of the ovary. At oviposition, the wasp injects one or more eggs plus virions that infect hemocytes and other host tissues. Viral expression in the absence of replication produces gene products that protect the parasitoid's offspring from the host's immune system. The virus persists in its proviral form in the developing wasp larva. At the completion of wasp larval development the host dies. The wasp larva then pupates, emerges as an adult, and searches for new hosts.

such as polymerases and most structural proteins. Comparison of the fully sequenced MdBV, CsIV, and CcBV genomes to partial genomic sequences from other polydnaviruses identifies only one gene family (ankyrin genes) that is shared between bracoviruses (BVs) and ichnoviruses (IVs) (**Figure 2**). Two gene families are shared among known BVs (ankyrin genes, protein tyrosine phosphatase genes) while six gene families are shared among known IVs (ankyrin genes, innexin genes, cysteine-motif genes, repeat element genes, N-family genes, and polar-residue-rich genes). BVs and IVs from the same wasp genus encode similar gene families, whereas viruses associated with more distantly related wasps share lower levels of sequence similarly and exhibit greater differences in the types of genes and gene families they encode. For example, CcBV encodes cystatin, crv1-like, and C-lectin-related genes for which homologs are known from other cotesia BVs but not BVs from other Microgastrinae like MdBV and other microplitis BVs (**Figure 2**). Individual BVs and IVs also exhibit variation in the number of genome segments produced, the number of variants per gene family, and primary structure of individual genes.

That BVs share more similarities with one another than IVs and vice versa is fully consistent with the phylogeny of their associated wasps under conditions of Mendelian inheritance as proviruses (**Figure 2**). More importantly though, these phylogenetic data indicate that the association of BVs with braconids and IVs with ichneumonids most likely arose independently, and that similarities in the life cycle and genome organization of these viruses reflects convergence driven by their analogous roles in parasitism. Functionally, these patterns further suggest that BVs and IVs have evolved broadly different strategies for abrogating the immune system of host insects. The types of genes or number of variants of the same gene a given BV or IV encodes also likely reflects differences between parasitoids in how they interact with particular hosts or the range of host species they successfully parasitize.

The Insect Immune Response to Parasitoid Attack

A second key factor in understanding polydnavirus-mediated abrogation of the immune response is how insects respond to parasitoid attack in the absence of polydnavirus infection. Like most animals, the innate immune system of insects consists of a diversity of defense responses that are regulated by complex signaling pathways. The primary defense of most insects toward macroparasites, like parasitoids, is a cellular immune response called encapsulation which begins (phase 1) when the host

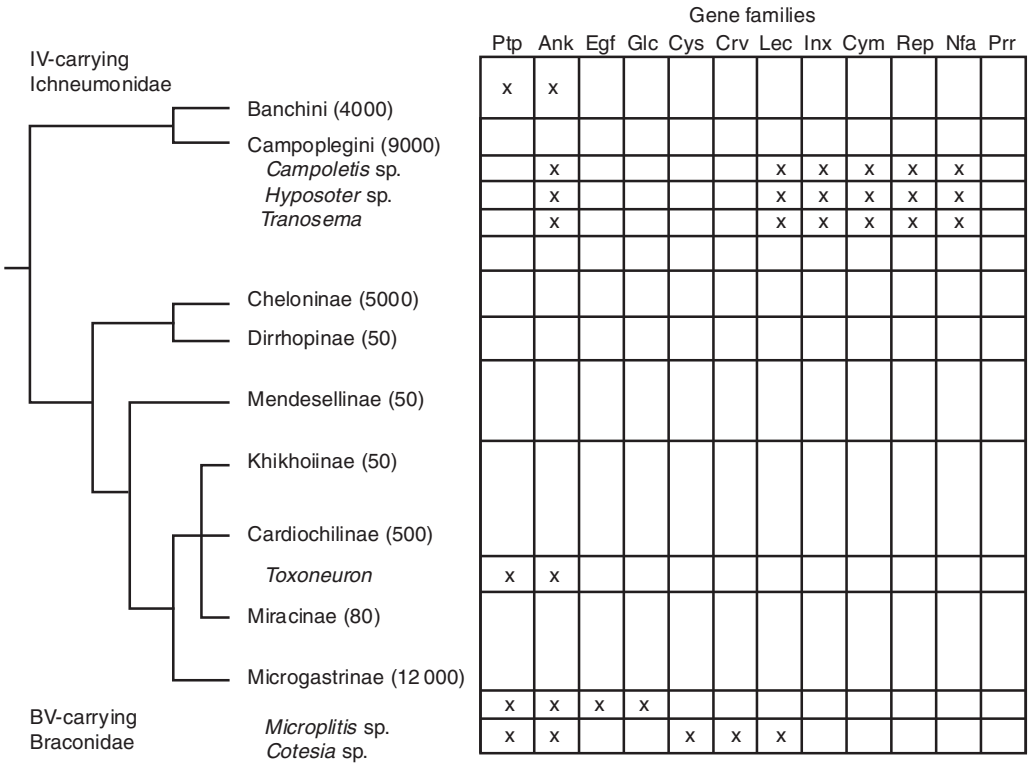


Figure 2 Schematic phylogeny of IV-carrying Ichneumonidae and BV-carrying Braconidae, and comparison of known polydnavirus gene families. The estimated number of species is indicated to the right of each wasp subfamily. Comparison of fully or partially sequenced viral genomes from selected wasp species identifies several shared gene families among IVs and BVs, respectively. Almost no genes, however, are shared between IVs and BVs. Gene family abbreviations: Ptp, protein tyrosine phosphatase; Ank, ankyrin; Egf, Egf motif gene; Glc, glycosylated mucin-like; Cys, cystatin; Crv, secreted Crv-like; Lec, C-lectin like; Inx, viral innexin; Cym, Cys-motif; Rep, repeat domain; Nfa, N-family; Prr, polar residue rich. No sequence data are currently available for IVs and BVs in the other wasp subfamilies presented.

recognizes the parasitoid egg or larva as foreign (**Figure 3**). This results in binding of a small number of hemocytes to the surface of the parasitoid and also stimulates proliferation and differentiation of hemocytes (phase 2). Recruitment of additional hemocytes to the target results in formation of a capsule comprised of overlapping layers of cells that bind to the parasitoid and one another. In phase 3, capsules often melanize due to activation of one or more pro-phenoloxidases (pro-POs). The parasitoid is then killed by asphyxiation and/or narcotizing compounds associated with the melanization pathway. Capsule formation usually begins 2–6 h after parasitism and is completed by 48 h.

Insects produce multiple hemocyte types that are morphologically and functionally distinct. In Lepidoptera, four hemocyte types are recognized (granular cells, plasmatocytes, spherule cells, oenocytoids), with capsules in most species comprised primarily of plasmatocytes. The model insect *Drosophila* in contrast produces three types of mature hemocytes (plasmatocytes, lamellocytes, and crystal cells) with capsules comprised primarily by lamellocytes. The absence of fully sequenced and well-annotated genomes has constrained identification of factors regulating capsule formation in Lepidoptera parasitized by most polydnavirus-carrying wasps. However, whole genome array and experimental studies have identified multiple genes of importance during encapsulation of parasitoids in *Drosophila*. Homologs of these genes are also likely involved in regulating encapsulation in Lepidoptera given the highly conserved nature of this defense response among insects generally.

Over the course of capsule formation in *Drosophila*, significantly different expression profiles are detected for more than 100 genes with functional annotations implicating several of these factors in capsule formation (**Figure 3**). During phase 1 of an encapsulation response, several genes under control of the Toll and JAK/STAT signaling pathways are upregulated that regulate hemocyte proliferation. Selected antimicrobial peptides under control of the Toll and imd pathways as well as proteins in the Tep and Tot families involved in pattern recognition, enzyme regulation, and stress responses are also differentially expressed. During phase 2, factors implicated in hemocyte adhesion include products of the *lectin-24A* and α*PS4 integrin* genes. *Drosophila* encodes several pro-PO genes but

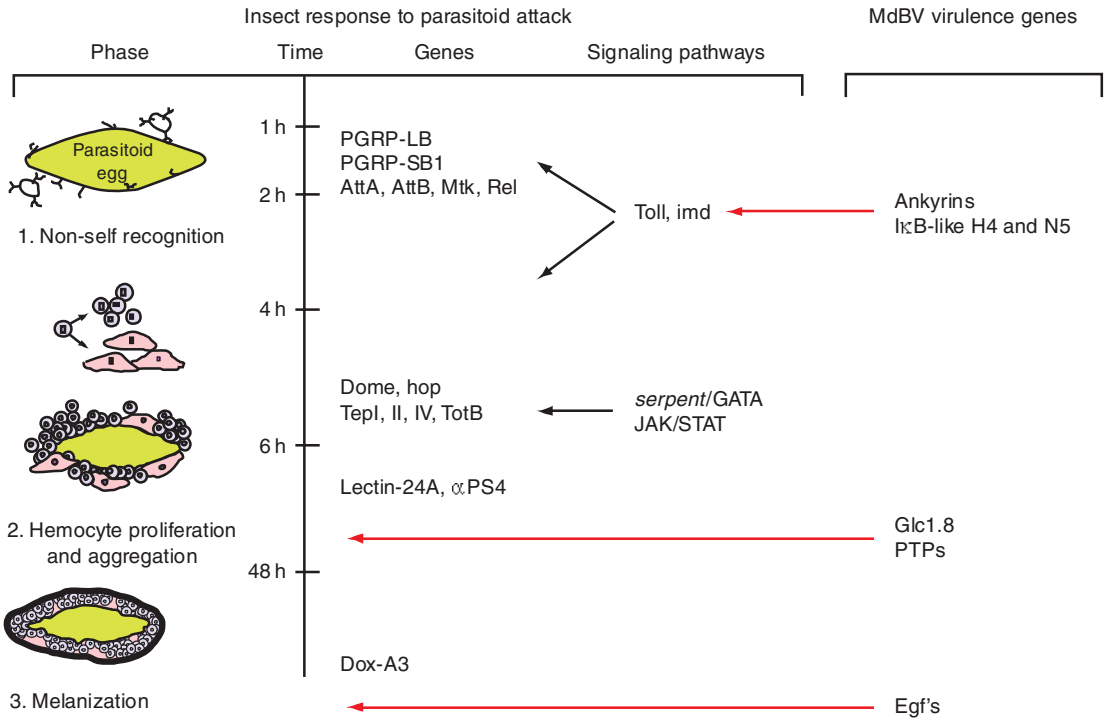


Figure 3 Overview of the host encapsulation response toward parasitoids in the absence of polydnavirus infection, and key virulence genes identified from MdBV involved in abrogation of the insect immune response. The two left-hand columns show the three major phases of capsule formation and the time elapsed since parasitoid attack. Selected insect genes and signaling pathways associated with the major phases of encapsulation are noted to the right of the time line. MdBV genes implicated in immune abrogation and their targets are noted at the far right.

melanization of capsules is associated specifically with upregulation of *proPO59/DoxA3* which is preferentially expressed by lamellocytes.

Polydnavirus Infection of Host Insects

Most polydnavirus-carrying parasitoids oviposit into the hemocoel of their host insect which is followed by the rapid entry of polydnaviruses into host cells and the release of viral DNA within nuclei. BVs and IVs infect several host tissues but host hemocytes are the most heavily infected cells in terms of the amount of detectable viral DNA. Other tissues typically infected by polydnavirues is the fat body which also produces many immune factors with roles in encapsulation and antimicrobial defense. Unlike most other polydnavirus-carrying wasps, chelonine braconids oviposit into newly laid host eggs prior to differentiation of host tissues. Nonetheless, studies of chelonus inantius bracovirus (CiBV) indicate that most viral DNA is still detected in hemocytes with lesser amounts being present in fat body, nervous tissue, and the digestive system. Following infection of host cells, BVs and IVs persist in episomal form until the host is consumed by the developing parasitoid. Viral transcripts are expressed at near-steady-state levels by most polydnaviruses beginning 2–4 h after wasp oviposition and continuing until complete development of the wasp's progeny. However, viral expression is transient in a few systems or in the case of chelonine braconids is delayed until late in the life cycle.

Polydnavirus-Mediated Abrogation of the Host Insect Immune Response

The primary function of BVs and IVs is protection of the developing parasitoid from the host's immune system. This was first reported in 1981 for the ichneumonid *Campoletis sonorensis* and its host *Heliothis virescens*. These studies revealed that *Ca. sonorensis* was always encapsulated when CsIV was absent but was never encapsulated when CsIV was present. The protective effects of CsIV also required expression of one or more viral genes since inactivated virus could not prevent encapsulation of the wasp. Subsequent studies with other BV- and IV-carrying parasitoids produced similar outcomes indicating widespread protection of parasitoids by polydnavirus infection of host insects. Differences also exist among polydnaviruses in how they effect the insect immune response. Some polydnaviruses only impair the ability of the host's

immune system to eliminate the wasp's offspring while others immunosuppress hosts more broadly. A few studies have also identified immune-related activities for polydnaviruses that do not involve gene expression.

Immunosuppressive Effects of BVs

BV-mediated abrogation of the insect immune system is currently best understood for MdBV which is carried by the microgastrine braconid *Microplitis demolitor*. In this system, MdBV broadly immunosuppresses host insects by blocking the ability of hemocytes to bind or phagocytize foreign targets, inducing apoptosis of granular cells, inhibiting inducible expression of antimicrobial peptide genes, and preventing hemolymph from melanizing. All of these effects manifest themselves within 12 h of MdBV infecting a host and also require expression of specific MdBV gene products. Genome-wide screens using RNA interference combined with other functional assays implicate four MdBV gene families in immune abrogation (**Figure 3**). Several of these MdBV genes also have conserved effects in immune cells of both their natural lepidopteran hosts and *Drosophila*. The first characterized anti-encapsulation effector from MdBV is Glc1.8, a cell surface mucin, that is expressed at high levels on the surface of infected host hemocytes. Glc1.8 prevents hemocytes from binding to parasitoid eggs or any other foreign surface. It also inhibits phagocytosis. These effects are due in part to interference with integrin-mediated adhesion but Glc1.8 likely also interacts with other molecules. Some phagocytic and adhesion pathways require tyrosine phosphorylation of several intracellular proteins that serve to link cell surface proteins to the actin cytoskeleton. One member of the MdBV protein tyrosine phosphatase (PTP) gene family dephosphorylates several proteins in insect hemocytes and significantly reduces phagocytosis. In *Drosophila* cells, this virally encoded enzyme also co-localizes to the tyrosine kinase homolog Dfak56, which is a component of focal adhesions associated with integrins that regulate adhesion and phagocytosis. This activity is similar to the bacterial pathogen *Yersinia pestis* which also produces a PTP that blocks phagocytosis by dephosphorylating essential proteins in mammalian macrophages. Encapsulation and phagocytosis by insect hemocytes involve multiple pattern recognition molecules and pathways. The inability of MdBV-infected hemocytes to bind or phagocytize any foreign target suggests that the activity of Glc1.8, a viral PTP, and possibly other factors together block several adhesion and phagocytosis pathways simultaneously.

The Toll and imd signaling pathways regulate expression of a diversity of immune genes following parasitoid attack or infection by other organisms. Both pathways involve specific nuclear factor kappa B (NFκB) transcription factors that are normally regulated by endogenous inhibitor κB (IκB) proteins. The 12-member ankyrin gene family of MdBV shares significant homology with insect IκBs. Two members of the family have also been experimentally shown to disrupt both Toll and imd signaling by binding to specific insect NFκBs and preventing them from translocating to the nucleus and interacting with κB sites in the promoters of target genes (**Figure 3**). This inhibits expression of known antimicrobial peptide genes but also likely suppresses other genes under NFκB regulation that regulate hemocyte proliferation, apoptosis, and other immune functions. An important question is why MdBV and other polydnavirus genes have diversified to form multiple gene variants. Because mammalian NFκB family members have cell-lineage-specific functions, one possibility for the MdBV ankyrin (IκB) family is that variants have functional activities that differ between important immune tissues like hemocytes and the fat body. Another possibility is that sequence variation among family members affects binding preferences for different insect NFκBs or confers binding properties for other host insect proteins.

The fourth MdBV effector belongs to the Egf gene family which encodes predicted proteins related to small serine protease inhibitors previously known only from nematodes. The pro-PO activation cascade of insects is regulated by a complex series of serine proteases that proteolytically activate prophenol oxidase (pro-PO) zymogen to phenol oxidase (PO). PO then hydroxylates monophenols to *o*-diphenols and oxidizes *o*-diphenols to quinones that polymerize to form melanin. The pro-PO cascade has not been fully characterized in any insect but is currently best understood in the lepidopteran *Manduca sexta*. Screening of MdBV Egf family members identified one member of the Egf gene family as a secreted protein that blocks melanization by inhibiting a specific serine protease in the *M. sexta* pro-PO activation cascade (**Figure 3**). Other members of the Egf family are likely also functional serine protease inhibitors but their target substrates are unknown. The picture that collectively emerges is that MdBV broadly immunosuppresses host insects by producing effector proteins that disrupt all three phases of the encapsulation process as well as other components of the insect immune system. These effectors preferentially also target signaling pathways rather than the gene products regulated by these pathways.

Most other studies on BV-mediated immunosuppression involve viruses from wasps in the genus *Cotesia*. Similar to MdBV, severe and permanent inhibition of hemocyte binding to foreign surfaces is reported for CmBV from *Cotesia melanoscela* and CkBV from *Cotesia kariyai*. Infection of *Pieris rapae* by CrBV from *Cotesia rubecula* also results in cytoskeletal alterations in hemocytes but these effects are only transient. Disruption of encapsulation by CcBV from *Cotesia congregata* is associated with both alterations to the cytoskeleton of host hemocytes and formation of aggregations rather than loss of adhesion. CcBV and other *Cotesia*

BVs also reduce melanization. Unlike other BV-carrying braconids, wasps in the genus *Chelonus* are all egg-larval parasitoids. Little is known about the role of chelonine BVs in immune suppression, but studies with CiBV indicate that viral infection of the host protects *Chelonus inanitus* larvae from encapsulation but does not suppress capsule formation of latex beads. The mechanism underlying this parasitoid-specific effect is unknown.

Conservation of the ankyrin and PTP gene families among all known BVs (**Figure 2**) suggests some family members function as inhibitors of NFκB signaling or phagocytosis as found for MdBV. At least some PTP family members from CcBV encode functional tyrosine phosphatases although the substrates and function of these enzymes in insects remain unclear. In contrast, no BVs from *Cotesia* species or other braconids outside the genus *Microplitis* encode homologs of the Glc or Egf gene families, suggesting that disruption of capsule formation or melanization must involve other effectors. Potential candidates include a gene (CrV1) originally identified from CrBV but that is conserved in other *Cotesia* BVs. This secreted protein causes alterations to the actin cytoskeleton of host hemocytes after endocytosis. *Cotesia* BVs also encode C-type lectins and a family of functional cysteine proteases (cystatins). Neither gene family has been experimentally linked to immunosuppression but some C-type lectins function as humoral pattern recognition receptors in insects while certain cystatins have immunomodulatory activities in vertebrates.

Immunosuppressive Effects of IVs

IVs are reported to have broadly similar immunosuppressive effects as BVs that include changes in the adhesive properties of host hemocytes and the number of hemocytes in circulation. In the CsIV system, hemocytes from noninfected hosts exhibit a decreased ability to attach to foreign surfaces when cultured in plasma from virus-infected hosts suggesting that secreted proteins of host or viral origin disrupt capsule formation. Hemocytes from CsIV-infected hosts are also unable to encapsulate eggs or other foreign targets, like glass, indicating that the host encapsulation response is broadly suppressed. In contrast, tranosema rostrale ichnovirus (TrIV) infection blocks encapsulation of *Tranosema rostrale* eggs but has no effect on encapsulation of other foreign targets.

IV infection also affects several elements of the humoral immune response including reduced levels of lysozyme expression in CsIV-infected hosts and suppression of melanization by several IVs. Serine protease inhibitor activity was isolated from the calyx fluid of *Venturia canescens* (=VcVLPs), though direct action on the serine proteases of the PO cascade was not shown. CsIV infection reduces melanization by inhibiting synthesis of enzymes in the melanization pathway at the post-transcriptional level. The resultant changes in enzyme levels alter substrate availability and composition, resulting in a reduced capacity of the host to produce melanin. In nonpermissive hosts of *Ca. sonorensis*, melanization reactions are only temporarily depressed with recovery of melanization occurring in hemolymph as ovarial and/or CsIV protein titers decline.

Few IV genes have been experimentally implicated in mediating any of the immune alterations described above. The CsIV Cys-motif proteins (VHv1.1, VHv1.4) are expressed in all infected tissues but the proteins are secreted and accumulate in the hemolymph where they bind to the surface of hemocytes. Experimental studies also suggest that VHv1.1 protein reduces but does not entirely block encapsulation of *Ca. sonorensis* eggs in hosts infected with a VHv1.1-expressing recombinant baculovirus. Other IV genes are indirectly implicated in immunosuppression because of homology to previously described BV genes or because they are highly expressed in key immune tissues like hemocytes or the fat body. IV-encoded ankyrin genes could potentially function as IκBs but differences in structural motifs between IV and BV ankyrins also suggest that they may have other biological activities. Products of the viral innexin genes from CsIV share high homology with insect innexin proteins that form gap junctions between many types of cells including hemocytes in capsules. CsIV innexin proteins are expressed at high levels in infected host hemocytes and also form functional gap junctions in *Xenopus* oocytes, suggesting they may alter normal gap junction function in a manner that disrupts encapsulation. The P30 ORF from HdIV is likewise predicted to encode a mucin-like protein with weak similarity to Glc1.8 from MdBV (see above) but its function in immunity is unclear.

Immunoevasive Activity of Polydnaviruses

While viral expression is usually required for defense against the host immune response, a few studies indicate that BVs and IVs passively protect parasitoids by coating the surface of the parasitoid egg. Surface features of the virions either prevent the parasitoid from being recognized as foreign by the host's immune system or inhibit binding of hemocytes. This activity also does not require expression of any viral genes. For example, eggs of *Co. kariyai* are coated by CkBV virions as well as other proteins secreted from ovarial cells. Two genes from *Co. kariyai* encode immunoevasive ovarial proteins (IEP1 and -2). These genes are not encoded within the CkBV genome but IEP1 and -2 are detected on the surface of CkBV virions. IEP proteins also confer protection to eggs from encapsulation and elimination of CkBV by hemocytes. Virions do not coat the eggs of *Toxoneuron nigriceps* or *Co. rubecula*, but the eggs of both are coated with ovarial proteins that also inhibit encapsulation. In the case of *Co. rubecula*, antiserum raised against CrBV cross-reacts with one of these

proteins, Crp32, and when this protein is removed eggs are encapsulated by both nonparasitized and CrBV-infected hosts. Among ichneumonids, *V. canescens* produces virus-like particles (VLPs) morphologically similar to the IVs associated with other members of the Campopleginae, but these particles appear to lack any nucleic acid. Hosts parasitized by *V. canescens* also remain capable of mounting an encapsulation response against numerous foreign targets but are unable to encapsulate *V. canescens* because of VLPs and a hemomucin that coats the surface of their eggs. TrIV similarly coats the eggs of *Tranosema rosele* in a manner that also likely confers passive protection from encapsulation.

Future Perspectives

The importance of the insect immune system makes it a critical target for the survival of parasitoids and many other insect pathogens. Polydnaviruses usually abrogate the insect immune system by efficiently introducing immunosuppressive genes into hosts whose products confer protection to the associated parasitoid. The genetic diversity of immunosuppressive and other virulence factors produced by these viruses is also potentially enormous given that more than 30 000 species of parasitoid wasps carry polydnaviruses and that each polydnavirus–parasitoid association is genetically unique. As analysis of polydnaviruses increases, the number of identified gene products will continue to expand. Similarly, as our knowledge of the insect immune system increases, so will the ability to understand the activities of the effector molecules polydnaviruses produce and to use these products as tools for fundamental research or in insect pest management. Additional comparative genomic studies of polydnaviruses will also be essential in establishing the origin and diversification of these unique viruses as immunosuppressive pathogens of insects.

See also: Polydnaviruses: General Features; Viral Pathogenesis.

Further Reading

Brennan CA and Anderson KV (2004) *Drosophila*: The genetics of innate immune recognition and response. *Annual Review of Immunology* 22: 457.
Dupuy C, Huguet E, and Drezen J-M (2006) Unfolding the evolutionary story of polydnaviruses. *Virus Research* 117: 81.
Edson KM, Vinson SB, Stoltz DB, and Summers MD (1981) Virus in a parasitoid wasp: Suppression of the cellular immune response in the parasitoid's host. *Science* 211: 582–583.
Espagne E, Dupuy C, Huguet E, *et al.* (2004) Genome sequence of a polydnavirus: Insights into symbiotic virus evolution. *Science* 306: 286–289.
Gillespie JP, Kanost MR, and Trenczek T (1997) Biological mediators of insect immunity. *Annual Review of Entomology* 42: 611.
Godfray HJC (1994) *Parasitoids: Behavioural and Evolutionary Ecology.* Princeton, NJ: Princeton University Press.
Hoffmann JA (2003) The immune response of *Drosophila*. *Nature* 426: 33.
Lavine MD and Strand MR (2002) Insect hemocytes and their role in cellular immune responses. *Insect Biochemistry and Molecular Biology* 32: 1237.
Webb BA and Strand MR (2005) The biology and genomics of polydnaviruses. In: Gilbert LI, Iatrou K and Gill SS (eds.) *Comprehensive Molecular Insect Science,* vol. 6, 323pp. San Diego, CA: Elsevier.
Webb BA, Strand MR, Deborde SE, *et al.* (2006) Polydnavirus genomes reflect their dual roles as mutualists and pathogens. *Virology* 347: 160.
Wertheim B, Kraaijeveld AR, Schuster E, *et al.* (2005) Genome wide expression in response to parasitoid attack in *Drosophila*. *Genome Biology* 6: R94 (doi:10.1186/gb-2005-2005-6-11-r94).

Polydnaviruses: General Features

A Fath-Goodin and B A Webb, University of Kentucky, Lexington, KY, USA

© 2008 Published by Elsevier Ltd.

Glossary

Bracovirus Polydnavirus associated with braconid wasp.
Calyx cell Specific cell type in the female wasp reproductive tract.
Convergent evolution Organisms that are not closely related independently evolve similar traits.
Ecdysteroid Insect steroid hormone.
Ichnovirus Polydnavirus associated with ichneumonid wasps.
Polydnaviridae Family of DNA insect viruses associated with parasitic wasps.

Taxonomy and Classification

Polydnaviruses (PDVs) are an unusual group of insect viruses that exist in obligate mutualisms with certain parasitic wasps. There are two distinct genera recognized in the family *Polydnaviridae*: the bracoviruses (BVs) of the

genus *Bracovirus* found only in the parasitic wasp family Braconidae and the ichnoviruses (IVs) of the genus *Ichnovirus* associated with the parasitoid family Ichneumonidae.

Virion Structure

Ichnovirus

IV virions are uniform in size (*c.* 85 × 330 nm), have the form of a prolate ellipsoid, and are individually surrounded by two unit-membrane envelopes (**Figure 1**). The inner envelope appears to be assembled *de novo* within the nucleus of infected ovarian calyx cells of the female parasitoid. The IV particles then bud through the plasma membrane of the calyx cells into the oviduct lumen, thereby acquiring the second envelope. IV virions have capsid dimensions large enough to support packaging of the entire genome but there are no data that show whether all or only some of the viral segments are actually present in a single virion. IV virions are complex containing 20–30 structural proteins detected in virions with molecular weights ranging from 10 to 200 kDa. Comparison of electron micrographs of IVs with that of other insect DNA viruses revealed that the IV virion morphology has some resemblance to that of ascoviruses. There are also similarities in their mode of transmission by parasitic wasps that may be indicative of an evolutionary relationship between these two virus groups.

Bracovirus

BV virions consist of enveloped cylindrical nucleocapsids of uniform diameter (30–40 nm) that are variable in length (8–150 nm) (**Figure 1**). BV nucleocapsids may possess long unipolar tail-like appendages of unknown function. The nucleocapsids are surrounded either individually or in groups by a single unit membrane and may be embedded in matrix proteins. There are some data to indicate that BV nucleocapsids encapsidate individual genome segments with the length of the capsid corresponding to the length of the segment. BV membranes are acquired in the nuclei of infected calyx cells of the wasp ovaries apparently by *de novo* membrane synthesis. In contrast to IV particles, mature BV particles are released into the oviduct lumen by lysis of calyx epithelial cells rather than by budding. BV virions are morphologically similar to the insect baculovirus and nudivirus virions but no homology has been detected between the structural genes of these insect viruses.

Genome Organization

PDV genomes are composed of multiple circular double-stranded DNA (dsDNA) segments which vary in size ranging from 2 to over 30 kbp (**Figures 2** and **3**). PDV genomes are unusual in that their genome sizes are comparatively large, ranging from 75 to over 500 kbp, but the gene density is one of the lowest reported for any virus. Analysis of the microplitis demolitor bracovirus (MdBV) and the campoletis sonorensis ichnovirus (CsIV) genomes by agarose gel electrophoresis, quantitative Southern blot hybridization, densitometry, and quantitative polymerase chain reaction (PCR) revealed that segment abundance is variable with some segments being present at a minimum of fivefold higher copy number than others. Furthermore, sequence analysis indicated that hypermolar segments contain large direct repeats that are suggestive of recombination events and consequent gene duplication. These large direct repeats are not present in low-abundance segments. The large repetitive DNA sequences of greater than 50 bp in length in PDV genomes comprise *c.* 15% of the genomic DNA in CsIV and MdBV. Transposable element sequences have been identified in both CsIV and MdBV, most of which are found in the noncoding regions of the genomes. Additional unusual characteristics of the PDV genome are the presence of several genes that harbor introns and some of

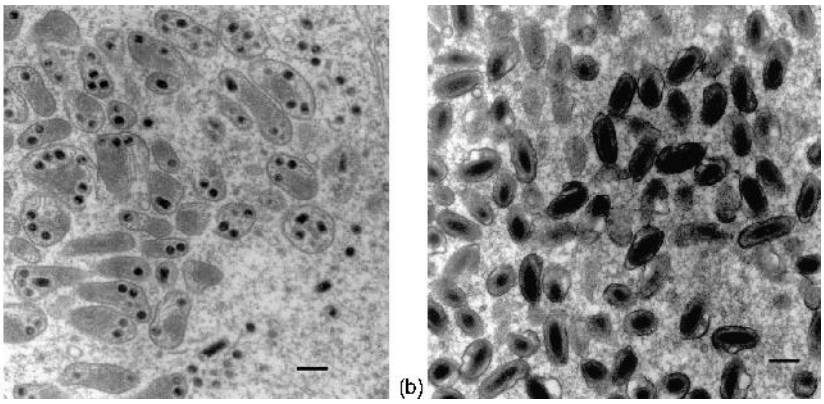


Figure 1 Virion morphology of BVs and IVs. Electron micrographs of the (a) protapanteles paleacritae bracovirus and the (b) hyposoter exiguae ichnovirus illustrate the morphological differences in members of the two PDV genera. Scale = 200 nm. Reprinted from Webb *et al.* (2005) *Polydnaviridae Virus Taxonomy* 8(1): 255–267, with permission from Elsevier.

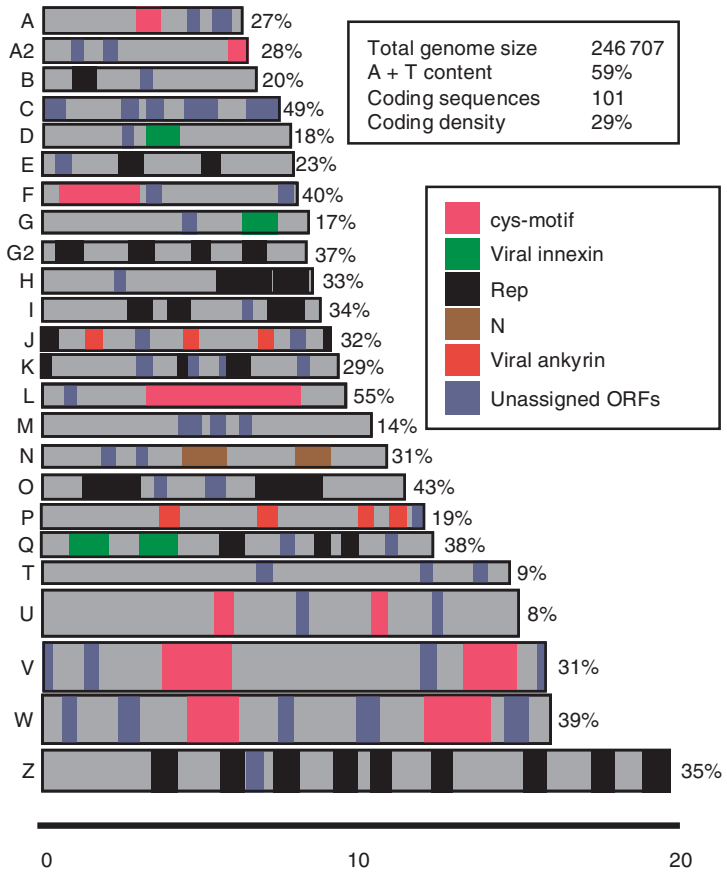


Figure 2 Diagrammatic representation of the CsIV genome. The 24 nonredundant circular CsIV genome segments are represented as linear molecules. To visualize segment size relationship within the genome, individual segments are listed from the smallest (A = 6.1 kbp) to the largest (Z = 19.6 kbp) segment with the segment name to the left. The percentage of coding DNA per segment is identified on the right. Wider bars represent high-copy, repetitive-type segments. ORFs and members of gene families have been annotated by color and located to individual genome segments. Gray regions represent noncoding DNA. Scale bar is in kilobases. Reprinted from Webb BA, Strand MR, Dickey SE, *et al.* (2006) Polydnavirus genomes reflect their dual roles as mutualists and pathogens. *Virology* 347: 160–174, with permission from Elsevier.

the PDV genes are organized into gene families. Furthermore, the encapsidated PDV genome lacks genes required for viral replication and virion assembly, suggesting that these factors reside in the genome of the parasitic wasp.

PDV gene expression has been investigated in most detail in the parasitized insect. Viral genes may be transiently expressed or members of viral gene families may be expressed in a tissue-dependent manner. Genes present on the most abundant segments are typically most highly expressed. Viral genes that are expressed only in the parasitic wasp, only in the parasitized lepidopteran host, or in both hosts have been described.

The genome organization of CsIV and MdBV are described in more detail in the next two sections.

CsIV Genome Organization

The CsIV genome is 246.7 kbp in size and is composed of 24 double-stranded, circular DNA segments ranging from 6.1 kbp (segment A) to 19.6 kbp (segment Z) (**Figure 2**). Some CsIV segments (U, V, W) undergo intramolecular recombination at intrasegmental repeats to generate smaller derivatives of the larger segments, a process referred to as segment nesting. It is believed that segment nesting provides a means for amplifying viral gene copy numbers. Interestingly, segment nesting in CsIV is associated with high-abundance segments and encodes only members of a single gene family, the cys-motif gene family. The coding density of the CsIV genome is 29% whereby individual genome segments have a coding density that ranges from 8% to 40% (**Figure 2**). Analysis of the CsIV genome identified 101 open reading frames (ORFs) whereby a total of 53 CsIV genes can be grouped into five gene families, namely the rep, cys-motif, vankyrin, innexin, and N gene families (**Figure 2**). The rep gene family is the largest gene family in the CsIV genome with 30 rep genes located on ten low-copy segments. The rep genes are characterized by a 540 bp sequence

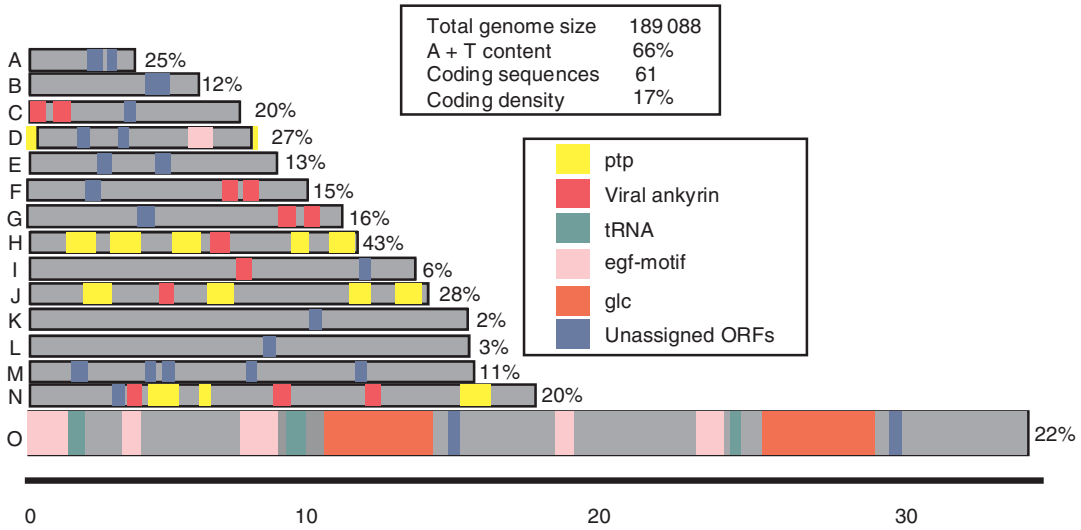


Figure 3 Diagrammatic representation of the MdBV genome. The 15 nonredundant circular MdBV genome segments are represented as linear molecules. To visualize segment size relationship within the genome individual segments are listed from the smallest (A = 3.6 kbp) to the largest (O = 34.3 kbp) segment with the segment name to the left. The percentage of coding DNA per segment is identified on the right. Wider bars represent high-copy, repetitive-type segment. ORFs and members of gene families have been annotated by color and located to individual genome segments. Gray regions represent noncoding DNA. Scale bar is in kilobases. Reprinted from Webb BA, Strand MR, Dickey SE, *et al.* (2006) Polydnavirus genomes reflect their dual roles as mutualists and pathogens. *Virology* 347: 160–174, with permission from Elsevier.

that is present in one to five copies but the function of the rep protein is unknown. The cys-motif gene family consists of ten genes on seven different segments harboring a conserved, cysteine-rich motif which is present in one or more copies in gene family members. Interestingly, all members of the cys-motif gene family contain introns whereas no spliced genes were found in the other CsIV gene families. All members of the cys-motif gene family possess signal peptides and share structural features encoding a novel cystine knot domain. Functional analysis of proteins encoding the cys-motif proteins VHv1.1 and VHv1.4 indicates that these proteins inhibit aspects of host development and cellular immunity. The vankyrin gene family is composed of seven members on segments P and J. Vankyrin genes encode for proteins containing truncated ankyrin repeat domains with sequence homology to the inhibitory domains of nuclear factor kappa B (NFκB) transcription factor inhibitors. Interestingly, the CsIV vankyrin proteins lack the N- and C-terminal regulatory regions required for dissociation and basal degradation of typical IκB (inhibitor of NFκB) suggesting a role for the vankyrin proteins in irreversible binding to modify or inhibit NFκB-mediated signal transduction cascades within the parasitized lepidopteran host. The four members of the vinnexin gene family were identified by sequence homology with invertebrate innexin gap junction proteins involved in cellular communication. CsIV vinnexins may function to alter gap junction proteins in infected host cells, thereby disrupting cell-to-cell communication required for defense responses. The smallest gene family in the CsIV genome is the N gene family with two structurally related members of unknown function located on segment N. The remaining 48 predicted CsIV genes are unique with no detectable homologs in the current protein databases.

Other IV genomes for which genome sequence is available indicate that there is considerable variation both in gene content and genome organization. However, most gene families appear to be present in ichneumonid genomes. The limited data available on banchine IVs indicate that this IV subgroup is more divergent.

MdBV Genome Organization

The MdBV genome is 189 kbp in size and is divided into 15 double-stranded, circular DNA segments that range from 3.6 kbp (segment A) to 34.3 kbp (segment O) (**Figure 3**). The average coding density of the MdBV genome is 17% with the coding density for individual segments ranging from 2% to 43% (**Figure 3**). Analysis of the MdBV genome identified 61 genes; 39 of those are grouped into five gene families, namely the ptp, vankyrin, tRNA, egf-motif, and glc gene families (**Figure 3**). The largest MdBV gene family is the ptp family with 13 members on four different segments encoding for proteins related to protein tyrosine phosphatases (PTPs). PTPs are known to regulate tyrosyl phosphorylation in numerous signal transduction pathways. The 12 vankyrin genes of the MdBV vankyrin

gene family are located on seven different segments. They possess truncated ankyrin repeats and lack the C- and N-terminal control domains similar to the ones found in CsIV. The third gene family consists of seven nearly identical serine tRNAs on segment O. In addition, segment O harbors the two members of the glc gene family and five out of six members of the egf-motif gene family. The egf-motif genes share a cysteine-rich epidermal growth factor-like domain with similarity to the cysteine-rich domain of a unique serine proteinase inhibitor. The glc gene family is composed of two identical glc1.8 genes that encode for a mucin protein. The Glc1.8 protein localizes to the surface of hemocytes of MdBV-infected lepidopteran larvae where it blocks the encapsulation response and phagocytosis. The remaining 21 genes are unique and are predicted to encode for proteins of unkown function, not represented elsewhere in available databases. All members of the glc and egf-motif gene families contain introns while the other gene families do not contain any spliced genes. In contrast to the CsIV genome, segment nesting has not been observed in the MdBV genome.

Only one other BV genome sequence has been reported at this time. The cotesia congregatus bracovirus (CcBV) genome is more than twice as large as the MdBV genome and has a distinctive set of gene families with the ptp, tRNA, and vankyrin gene families present but apparently lacking the glc and mucin gene families. The CcBV genome is also reported to have a much higher number of spliced genes.

PDV Life Cycle and Replication

PDVs have an obligate association with parasitic wasps in the families Braconidae and Ichnomonidae in which the viral genome is stably integrated within the wasp genome. The PDV life cycle has been characterized as having two 'arms', namely the replication/transmission arm, in which PDV replication and transmission occurs in the viruses' wasp host and a pathogenic arm of the life cycle, in which viral gene expression occurs in the lepidopteran host to disrupt host physiological systems (**Figure 4**). Virus replication and assembly begin during the late stage of wasp pupal development and have been linked to changes in the wasp ecdysteroid titers that drive pupal development. In BV, proviral BV segments appear to be tandemly arrayed in the wasp genome and there is some evidence that larger chromosome regions are amplified before excision during viral replication, with individual viral segments then excised from amplified DNA for encapsidation in virions. Replication of the excised, circular form of viral segments was not detected. In IV, replication is thought to involve the excision of viral DNA segments from the wasp genome via site-specific recombination events followed by amplification of circular episomes in a rolling-circle-type mechanism. This model is attractive for the replication mechanism of IVs as their genome segments are not contiguous in the wasp genome. In all PDVs, replication occurs only from proviral DNA in specialized cells of the female oviduct, the calyx cells, and

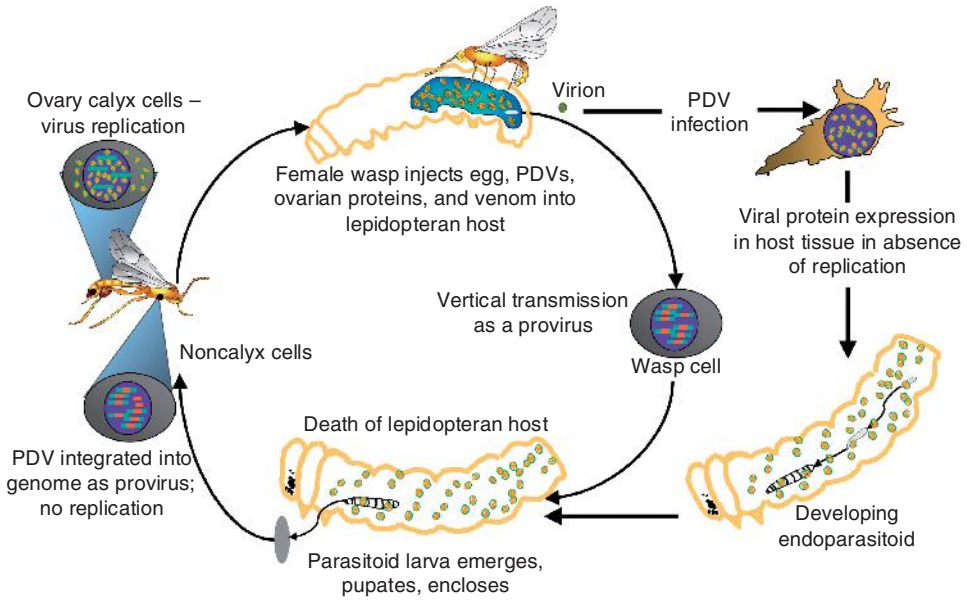


Figure 4 Polydnavirus (PDV) life cycle. The replication and transmission of PDVs and the life cycle of their associated wasp are shown. Viral DNA is transmitted as proviral DNA integrated into the wasp genome (curved arrows) and as circular DNA in virions (straight arrows). In calyx cells of the wasp ovaries and in infected lepidopteran cells, viral DNA is present as circular DNA, whereas in nonreplicative wasp cells the virus exists as a provirus and does not replicate. Reprinted from Webb BA, Strand MR, Dickey SE, *et al.* (2006) Polydnavirus genomes reflect their dual roles as mutualists and pathogens. *Virology* 347: 160–174, with permission from Elsevier.

only for transmission of virus to lepidopteran host insects. PDV virions accumulate to a high density in the oviduct lumen and are transferred to the lepidopteran host with wasp eggs, ovarian proteins, and venom during oviposition. In the lepidopteran host, the virus then infects hemocytes, fat body, and other tissues of the parasitized host. Viral gene expression can be detected as early as 1 h post parasitism and continues through parasitoid larval development. PDV gene expression induces physiological alterations in the lepidopteran host such as disruption of development and suppression of humoral and cellular immunity to permit the parasitoid's survival within host larvae and to mobilize host protein stores to redirect host resources for supporting parasitoid growth. The parasitoid completes its development inside the lepidopteran larva, pupates, and emerges as an adult wasp while the host larva dies. During parasitization, viral DNA is not only present in virions that infect lepidopteran cells but PDVs are also vertically transmitted in the proviral form within the chromosome of the wasp egg to ensure virus transmission to subsequent wasp generations.

Phylogenetic Information

PDVs are associated with two distinct lineages of ichneumonoid wasps. All BV-carrying wasps belong to the monophyletic braconid microgastroid lineage which arose 74 million years ago and contains more than 17 000 species whereas IVs are largely limited to the Campopleginae and Banchinae which consist of approximately 13 000 species. The absence of PDVs in the common ancestor to campoplegines and microgastroids is strongly indicative of an independent origin of IVs and BVs as are their distinctive modes of replication, virion structures, and genomes. Sequence data from different PDV genomes revealed that viruses within a genus have a number of genes in common whereas few genes are shared between IVs and BVs. Interestingly, the vankyrin gene family is the only gene family found to be in common between IVs and BVs but phylogenetic analysis of vankyrins from PDVs and mammalian and *Drosophila* IκBs revealed that IV and BV vankyrins cluster separately. Thus the data provide no clear evidence of shared ancestry between the two viral genera. The divergence in the viral genomes of closely related wasp species within the two PDV genera may be explained by the presence of transposon-derived sequences, large amounts of repetitive DNA, and segment nesting. The suite of unrelated genes in the IV and BV genomes implies that each genus has evolved different functional strategies to immunosuppress and alter the development of the parasitized host. However, similarities in their life cycle and similarities in genome organization between IVs and BVs suggest that common selection pressures among IVs and BVs have driven convergent evolution between the two PDV genera.

See also: Polydnaviruses: Abrogation of Invertebrate Immune Systems.

Further Reading

Beckage NE and Gelman DB (2004) Wasp parasitoid disruption of host development: Implications for new biologically based strategies for insect control. *Annual Review of Entomology* 49: 299–330.

Espagne E, Dupuy C, Huguet E, *et al.* (2004) Genome sequence of a polydnavirus: Insights into symbiotic virus evolution. *Science* 306: 286–289.

Kroemer JA and Webb BA (2004) Polydnavirus genes and genomes: Emerging gene families and new insights into polydnavirus replication. *Annual Review of Entomology* 49: 431–456.

Tumbull M and Webb B (2002) Perspectives on polydnavirus origins and evolution. *Advances in Virus Research* 58: 203–254.

Webb BA and Strand MR (2005) The biology and genomics of polydnaviruses. Comparative physiology and biochemistry. In: Gilbert LI, Iatrou K, and Gill SS (eds.) *Comprehensive Molecular Insect Science,* vol. 5, pp. 260–323. Amsterdam: Elsevier.

Webb BA, Strand MR, Dickey SE, *et al.* (2006) Polydnavirus genomes reflect their dual roles as mutualists and pathogens. *Virology* 347: 160–174.

Whitfield JB (2002) Estimating the age of the polydnavirus/braconid wasp symbiosis. *Proceedings of the National Academy of Sciences, USA* 99: 7508–7513.

Whitfield JB and Asgari S (2003) Virus or not? Phylogenetics of polydnaviruses and their wasp carriers. *Journal of Insect Physiology* 49: 397–405.

Polyomaviruses of Humans

M Safak and K Khalili, Temple University School of Medicine, Philadelphia, PA, USA

© 2008 Elsevier Ltd. All rights reserved.

JC Virus

JC virus (JCV) is classified into species *JC polyomavirus*, genus *Polyomavirus*, family *Polyomaviridae*. The JCV genome contains a small, double-stranded, closed circular DNA of 5130 bp. The capsid exhibits an icosahedral structure, approximately 45 nm in diameter. Structural and antigenic studies have indicated that JCV is closely related to two other polyomaviruses, BK virus (BKV) and simian vacuolating virus 40 (SV40). JCV is the

etiologic agent of a fatal demyelinating disease of the central nervous system (CNS) known as progressive multifocal leukoencephalopathy (PML). Seroepidemiological data indicate that the overwhelming majority of the world's population (70–80%) is infected by JCV early in childhood without apparent clinical symptoms. The virus establishes a persistent infection in the kidneys (latent infection) and reactivates from latency under immunocompromised conditions. As well as the kidneys, hematopoietic progenitor cells, peripheral blood B lymphocytes, and tonsillar stromal cells have been shown to harbor JCV, suggesting that these sites could serve as additional sites for latent infection by JCV.

Recently, two new human polyomaviruses were also discovered. One was isolated from human respiratory tract samples by polymerase chain reaction (PCR) amplification and named KI polyomavirus (KIPyV). The other was isolated from a patient with symptoms of acute respiratory tract infection and named WU virus (WUV). Thus far, follow-up studies have not yielded evidence for associations between infections with KIPyV or WUV and respiratory disease.

JCV was first isolated from the brain tissue of a PML patient in 1971, and this opened new frontiers in polyomavirus research. A piece of brain tissue from a PML patient was used as a source of inoculum to infect primary cultures derived from human fetal brain, and the virus was then successfully cultivated from those long-term cultures. This was the first direct evidence that a neurotropic virus is associated with the development of PML. At the same time, a virus similar in structure was found in the urine of a patient who had undergone renal transplantation. Each virus was named with the initials of the donor, BK virus for the renal transplantation patient and JC virus for the patient with PML. Following the isolation of JCV, the oncogenic potential of the virus was demonstrated both in tissue culture and experimental animals. Particularly, animal model studies showed that JCV induces tumors in tissues of neural origin. In addition, the genome has recently been found to be present in a variety of human tumors.

JCV infects and destroys oligodendrocytes, which are the myelin-producing cells in the CNS, and indirectly causes the death of neurons in the white matter of the brain, because neurons are known to depend for survival on support provided by oligodendrocytes. Subsequently, the destruction of both oligodendrocytes and neurons in the CNS results in PML, a neurodegenerative disease. PML develops mostly in patients with underlying immunosuppressive conditions, including lymphoproliferative diseases, acquired immune deficiency syndrome (AIDS), and Hodgkin's lymphoma, although, in a small number of cases, PML also affects individuals lacking underlying disease. Before the AIDS epidemic, PML used to be a rare complication of middle-aged and elderly patients with lymphoproliferative diseases. However, it is now a commonly encountered disease of the CNS in patients of different age groups, and there is a noticeable increase in the incidence of PML in human immunodeficiency virus (HIV)-infected patients compared to noninfected individuals. The increased incidence of PML in AIDS patients suggests that HIV infection may directly or indirectly influence reactivation of JCV and therefore induction of PML. In support, recent reports indicate that the incidence of PML in HIV-seropositive patients reaches 5%, compared to 0.8% before the AIDS epidemic. Furthermore, reactivation of JCV in patients with multiple sclerosis (MS) or Crohn's disease treated with both interferon $\beta 1\alpha$ and natalizumab, a selective adhesion-molecule blocker, suggests that such an immunosuppressive treatment may be a risk factor in induction of PML in MS and Crohn's disease patients.

Genome Organization: Regulatory and Coding Regions

The JCV genome is composed of bidirectional regulatory elements and coding regions (**Figure 1**). The regulatory region contains the origin of DNA replication (ori) and promoter/enhancer elements, and exhibits hypervariability in its sequence composition; that is, although the regulatory region of a prototype strain, Mad-1, is characterized by the presence of two 98 bp tandem repeats, there are considerable deviations from this in other strains. For example, the archetype strain contains only one copy of the 98 bp repeat with two insertions of 23 bp and 66 bp, and the Mad-4 strain contains two tandem repeats that are only 84 bp in length. The bidirectional nature of the regulatory elements may provide an advantage to the virus to transcribe its genes efficiently, by a mechanism that is currently unknown. The regulatory region of the archetype strain is thought to be more adaptive to a latent infection and, upon reactivation, undergoes a deletion/duplication process within its regulatory region, through which it apparently gains the ability to become a more virulent virus and infect oligodendrocytes. The coding sequences of JCV are divided into two regions, early and late. The early region encodes only regulatory proteins, including small tumor antigen (Smt-Ag), large tumor antigen (LT-Ag), and T′ proteins (T'_{135}, T'_{136}, and T'_{165}). The late region encodes a mixture of structural (VP1, VP2, and VP3) and regulatory (agnoprotein) proteins (**Figure 1**).

JCV is closely related to BKV and SV40 in the coding regions (70–80% identity). However, the regulatory regions are considerably diverged. This feature makes each virus unique with respect to replication in different cell types and tissues. In other words, the regulatory region largely determines the tissue-specific expression of gene products. LT-Ag also contributes to tissue-specific expression. Moreover, *in vivo* and *in vitro* transcription

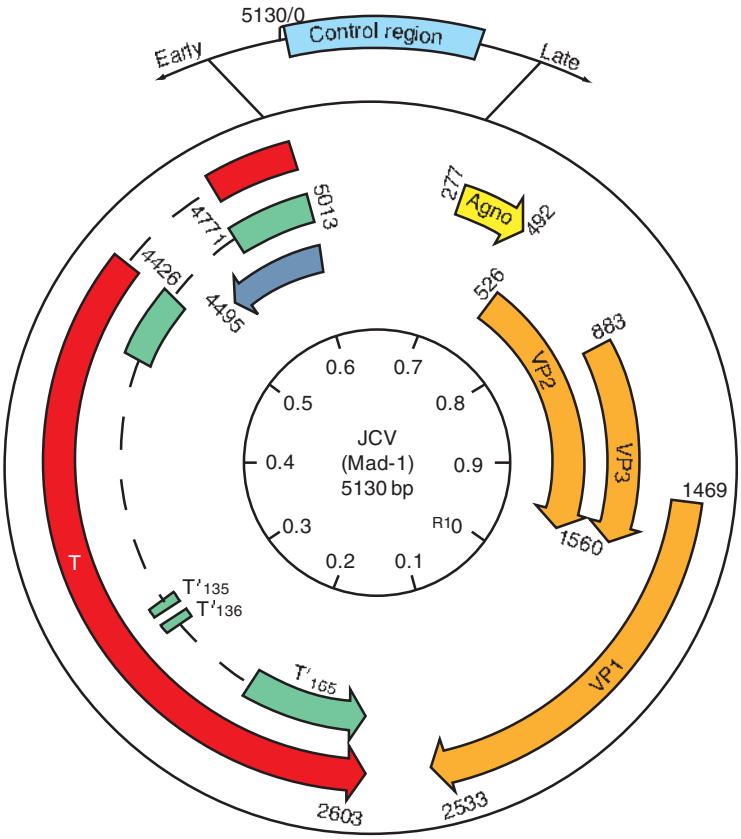


Figure 1 Genomic organization of JCV. The JCV genome is expressed bidirectionally. The early coding region encodes LT-Ag, Sm t-Ag, and T′ proteins (T'_{135}, T'_{136}, and T'_{165}). The late coding region encodes agnoprotein and the three structural proteins VP1, VP2, and VP3. The control (regulatory) region is located between the two coding regions and contains the origin of DNA replication (ori) and promoter/enhancer elements for the early and late promoters.

assays and cell fusion experiments have shown that tissue-specific cellular factors are critical for neurotropic expression of JCV. For instance, the early promoter was shown to be expressed well in glial cells but, when glial cells were fused with fibroblasts to form heterokaryons, early gene expression was significantly downregulated. This suggests that there are positively and negatively acting factors in glial and nonglial cells, respectively.

Regulatory Region

The regulatory region contains ori and multiple *cis*-acting regulatory elements that are involved in transcriptional regulation of the early and late regions. The regulatory region of the Mad-1 strain is shown schematically in **Figure 2**. The only region that shows substantial similarity to the corresponding regions in SV40 and BKV is ori, a 68 bp element located between a nuclear factor kappa B (NFκB) motif and the first 98 bp repeat. Each repeat contains a TATA box located on the early side of the repeat, and the first TATA box with respect to ori is involved in positioning of the transcription start sites for early genes. The second TATA box does not appear to have a similar function for the late genes. The other regulatory elements shown to be critical *cis*-acting elements for expression include Penta, AP-1, and NF-1 motifs that follow the TATA box. These elements serve as binding sites for several transcription factors and contribute to tissue tropism. The major *cis*-acting elements and transcription factors that bind to these regions are illustrated in **Figure 2**.

Cellular Transcription Factors Involved in JCV Gene Expression

Several studies have shown that many cell-specific and ubiquitous factors are involved in expression of the early and late promoters. These factors interact directly or indirectly with the *cis*-acting elements in the regulatory region and control gene expression. The regulatory proteins include LT-Ag and agnoprotein and have also been shown to play critical roles. LT-Ag, a multifunctional phosphoprotein, is not only involved in viral DNA replication but also late gene expression.

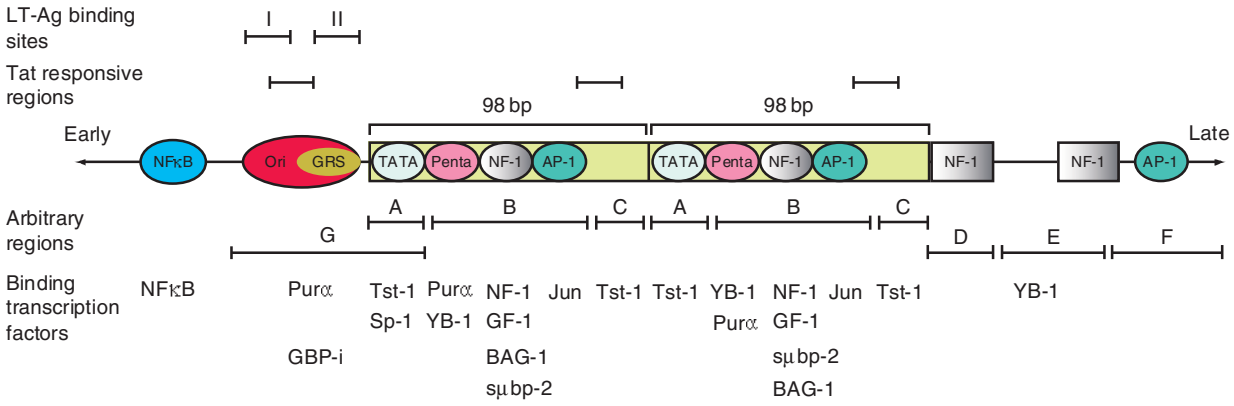


Figure 2 Regulatory region of JCV. The regulatory region contains the origin of DNA replication (ori) and promoter/enhancer elements. The two 98 bp tandem repeats are a characteristic of the Mad-1 strain. Promoter/enhancer elements that serve as binding sites for transcription factors are shown, and include the NFκB-binding element, the pentanucleotide element (Penta), the nuclear factor 1-binding element (NF-1), the GC-rich element (GRS), and the activating protein-1-binding element (AP-1). The transcription factors that have been shown to bind promoter/enhancer elements are shown at the lower part of the Figure. Large T antigen (LT-Ag)-binding sites and Tat-responsive elements are indicated. Arbitrary subregions A to G described in the text are indicated.

Along with cellular factors required for DNA replication, LT-Ag binds to ori, unwinds it, and initiates DNA replication in a bidirectional manner. With respect to the role of LT-Ag role in transcription, studies have shown that, whereas it autoregulates its own transcription from the early promoter, it robustly transactivates the late promoter, resulting in the expression of agnoprotein and capsid proteins.

A close inspection of the regulatory region sequences reveals the presence of multiple *cis*-acting elements to which transcription factors bind and regulate gene expression. These include NFκB, GRS, Penta, NF-1, AP-1, and 'AP-1-like' motifs. In addition, the regulatory region also contains LT-Ag-binding regions and elements responsive to the HIV transactivator, Tat (**Figure 2**). Furthermore, for the sake of simplicity in describing regulatory elements, the regulatory region is divided into arbitrary regions A to G. NFκB, a stress-inducible transcription factor, binds to the NFκB element and modulates gene expression from the early and late promoters. This factor represents a large family of transcription factors that are induced in response to a wide variety of extracellular stimuli, including phorbol ester, cytokines, and viral infection. p50 and p52 are two constitutively expressed subunits of NFκB that activate transcription from region D, whereas subunit p65 regulates transcription through the NFκB motif itself. The NFκB family members p50/p65 also influence transcription indirectly through a 23 bp element present in the regulatory regions of many JCV variants. Another inducible cellular factor, GBP-i, appears to target the GRS sequence present in region G and suppresses late promoter activity. Tst-1 is a member of the well-characterized tissue-specific and developmentally regulated POU family of transcription factors. This factor apparently interacts with distinct binding sites in regions A and C and regulates transcription from the early and late promoters. Interactions of cellular transcription factors with the viral regulatory proteins are also important. In this respect, interaction of Tst-1 with LT-Ag leads to synergistic activation of the early and late promoters. GC-rich sequences in Penta motif target the ubiquitously expressed transcription factor, Sp-1, which stimulates expression from the early promoter. The regulatory region also contains several NF-1-binding sites scattered across regions B, D, and E. NF-1 is important for both viral transcription and replication. Several factors interact with region B, including GF-1, YB-1, Purα, and AP-1. GF-1 is the human homolog of the murine Sμbp-2 protein and transactivates the early and late promoters. Two well-characterized cellular transcription factors, YB-1 and Purα, also interact with this region and regulate transcription and replication through interaction with LT-Ag. Another binding site for YB-1 is present in region E. BAG-1, a novel Bcl-1-interacting protein that is expressed ubiquitously in neuronal and non-neuronal cells, is a novel cellular transcription factor that regulates JCV promoters through the NF-1-binding site. Another transcription factor that interacts with region B is c-Jun, which is a member of the AP-1 family of transcription factors. Members of this family are known as the immediate–early inducible protooncogenes and are critical for the expression of many cellular and viral genes. c-Jun is phosphorylated during the JCV life cycle in an infection-cycle-dependent manner and interacts functionally with LT-Ag. In addition to its own regulatory proteins, the JCV genome is also crossregulated by regulatory proteins encoded by other viruses, including Tat and immediate-early transactivator 2 (IE2) from human cytomegalovirus (HCMV).

Life Cycle

Figure 3 highlights the chain of events during the polyomavirus life cycle. The infection cycle of JCV starts with the attachment of viral particles to cell surface receptors. The serotonin receptor $5HT_{2A}$ as well as α(2-6)-linked sialic acid are critical for the attachment of JCV to the cell surface, and the viral particle is then internalized by a process known as clathrin-dependent endocytosis. Following internalization, the particle is transported to the nucleus by an unknown mechanism. The next step is the uncoating of capsid proteins, which subsequently leads to the expression of early genes in the nucleus. Viral transcripts undergo splicing before they are transported to the cytoplasm and translated. Translation of early transcripts results in the production of early regulatory proteins, including LT-Ag, Sm t-Ag, and T′ proteins (T'_{135}, T'_{136}, and T'_{165}). LT-Ag is then transported back to nucleus to initiate viral DNA replication. In the meantime, LT-Ag transactivates the late promoter for the production of regulatory agnoprotein and the three structural capsid proteins VP1, VP2, and VP3. Capsid proteins are also transported to the nucleus for virus assembly. In the encapsidation process, the capsid proteins are sequentially added to and arranged on the viral genome to package it into capsids. The final step is to release the virions from infected cells. The mechanism for this is not known, but it is thought that it takes place upon lysis of infected cells.

JCV Reactivation and Other Viral Infections

HIV infection and JCV reactivation

It has long been known that impaired cellular immunity is an exclusive predisposing condition for reactivation of JCV and onset of PML. Before the AIDS epidemic, lymphoproliferative disorders used to be the predominant underlying factor for reactivation of JCV from latency. In recent years, however, this trend has changed and a strong association of PML with AIDS has been established. The statistical data in this regard suggest that approximately 5–10% of AIDS patients eventually develop PML. This percentage is predicted to increase

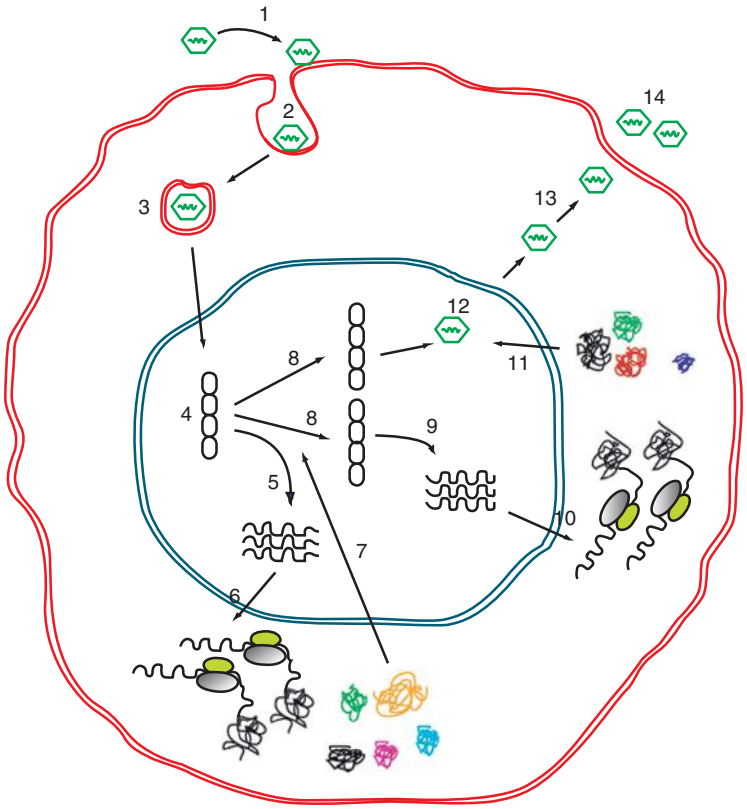


Figure 3 Life cycle of JCV. The steps are indicated by numbers: 1, adsorption of virus particles to cell surface receptors; 2, entry by clathrin-dependent endocytosis; 3, transport to the nucleus; 4, uncoating; 5, transcription of the early region; 6, translation to produce the early regulatory proteins, LT-Ag, Sm t-Ag, and T′ proteins (T'_{135}, T'_{136}, and T'_{165}); 7, nuclear localization of LT-Ag; 8, replication of the viral genome; 9, transcription of the late region; 10, translation of late transcripts to produce agnoprotein and capsid proteins (VP1, VP2, and VP3); 11, nuclear localization of capsid proteins; 12, assembly of virus particles in the nucleus; 13, release of virions by an unknown mechanism; 14, released virions.

as more PML cases are evaluated in AIDS patients. In recent years, application of highly active antiretroviral therapies (HAART) to AIDS patients has had a significant effect on manifestations of AIDS-associated opportunistic infections, including HCMV infection and toxoplasmosis. The effect of HAART on the incidence of PML remains unclear, but there has been an indisputable increase in the frequency of PML since the inception of the AIDS epidemic. One simple explanation for this is that a higher degree and duration of cellular immunosuppression may pertain to HIV infection than to other immunosuppressive conditions. Another explanation is that alterations in the blood–brain barrier caused by HIV infection may render the brain more accessible to JCV infection. Direct activation of the JCV genome by HIV Tat protein or indirect activation by HIV-induced cytokines and chemokines could be other important factors for AIDS-related reactivation of JCV.

The degree and duration of immunosuppression in HIV infection may not be the only factors for the development of PML, because a substantial number of individuals with hematological malignancy, solid tumors undergoing chemotherapy, and/or underlying tuberculosis status post-organ transplantation all have a long period of immunosuppression but do not develop PML as often as HIV-infected individuals. What is special about HIV infection is that many arms of the host defense system are affected, as opposed to the more specific effects seen in other immunosuppressive conditions. It is also notable that PML typically manifests itself as a late complication of HIV infection. Perhaps a substantial loss in the number and function of CD4+ T cells during the late phases of AIDS (as well as other alterations, including deficiencies in chemotaxis, monocyte-dependent T-cell proliferation, Fc-receptor function, C3 receptor-mediated clearance, and oxidative burst response) contributes greatly to suppressive conditions of the immune system in AIDS patients, and this in turn facilitates JCV reactivation and the development of PML. Furthermore, infection of monocytes/macrophages by HIV results in the impairment of antibody-dependent, cell-mediated cytotoxicity, intracellular antimicrobicidal activity, and induction of interferon α secretion. Moreover, the circulating B cells decrease in number as the disease progresses and, in association with HIV infection, B cells secrete disproportional amounts of cytokines. Which of these deficiencies leads to predisposition to the development of PML remains unknown. However, it should be emphasized that on rare occasions PML may develop in the absence of any identifiable underlying immunosuppressive disorder.

The Tat protein is a potent transactivator of the HIV long terminal repeat (LTR). It mediates its transregulatory activity through a specific RNA sequence located in the leader of all HIV-1 RNAs, called the transactivation response (TAR) element. Several critical G residues in the TAR element are required for function. Tat also induces transcription from the JCV late promoter through several TAR elements in the regulatory region. The G residues required for the function of the HIV-TAR element are also conserved in the JCV-TAR element and play an important role in Tat-mediated activation of the late promoter. Thus, Tat may participate directly in activation of the JCV late promoter. It is also thought that Tat may indirectly influence JCV gene regulation through the induction of transcription factors such as NFκB and c-Jun/AP-1, both of which have been shown to interact directly with the JCV promoter. Taken together, the regulatory factors induced by HIV Tat or Tat-induced cytokines may activate JCV promoters directly or indirectly and thereby influence the viral life cycle.

Interaction of JCV with other human viruses

Initial infection by JCV is followed by a persistent infection in the kidney and perhaps in B cells, during which JCV appears to go through several stages: (1) a latent infection state of limited virus production; (2) an activated state, during which the virus causes cell lysis; and (3) a final tissue destruction state, which leads to the onset of PML. Although the precise mechanism(s) of viral reactivation processes is unknown, the immunosuppressed state of the patient is critical for the onset of PML. Moreover, infection of immunosuppressed individuals by other viruses may influence the reactivation process.

In addition to JCV, several human viruses, such as BKV, HCMV, and human herpesvirus 6 (HHV-6), are able to establish persistent infections in various tissues and organs. HCMV is highly prevalent in the human population and can infect a wide range of organs. It is commonly reactivated in bone marrow and renal transplantation patients. Particularly, it is often reactivated after renal transplantation in AIDS patients in the kidney, CNS, lymphoid organs, stromal cells, and CD34+ bone marrow progenitor cells. Recent reports indicate that HCMV infection positively affects the level of JCV DNA replication or possibly transcription. JCV is known to infect only glial cells, but this restricted cell specificity can be overcome by HCMV infection. Thus, fibroblasts, which are generally nonpermissive for the replication of JCV, but can become permissive if HCMV IE2 is provided to JCV-infected cells.

HHV-6 is also a ubiquitous virus, and is detected in close association with JCV in oligodendrocytes within PML lesions. This virus establishes a lifelong infection in various organs, including the brain, urogenital tract, lung, liver, and peripheral blood cells. There appears to be a high correlation between polyomavirus infection and HHV-6 infection. This suggests that polyomaviruses and HHV-6 may have a common host cell not only in the CNS, but also in peripheral organs, and co-infection may have an impact on JCV gene regulation and perhaps on the reactivation process.

BKV has been reported to infect human tissues, such as the urogenital tract, along with JCV. This has been verified by the detection of both viruses in the kidney and the urine. Such infections are commonly encountered after renal and bone marrow transplantation in HIV-infected people, pregnant women, and immunocompetent individuals. However, the influence of double infection on JCV replication remains unclear. Additionally, both viruses have been detected in the peripheral blood cells of healthy and immunocompromised individuals.

Lytic Infection versus Tumor Induction

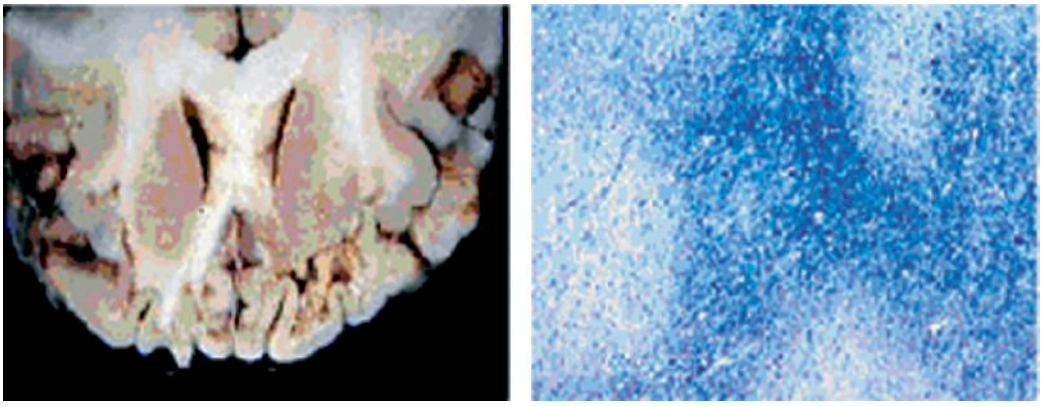
PML

JCV lytically infects oligodendrocytes in the CNS, leading to a white matter disease in humans known as PML (**Figure 4**). Oligodendrocytes are members of the glial cell family in the CNS. The other cell types in the glial family include microglia, astrocytes, and neurons. The primary function of oligodendrocytes is to myelinate the axons that project from the neural cell bodies of the overlying cortex and to protect the neurons. Infection of oligodendrocytes results in the destruction of myelin insulating the neurons, creating sporadic plaques in different parts of the brain. Hence, demyelination occurs as a multifocal (rarely unifocal) process that can develop in any location in the white matter (**Figure 4(b)**). As a result of infection, demyelination can also occur in other regions of the CNS, including the brainstem and cerebellum. Destruction of oligodendrocytes initially leads to the development of microscopic lesions (**Figure 4(b)**), and, as the disease progresses, the demyelinated areas become enlarged and eventually may coalesce, making them visible on gross examination of cut sections (**Figure 4(a)**). In most cases, however, astrocytes have also been found to be abortively infected by JCV, exhibiting enlarged, lobulated nuclear structures. Lipid-laden macrophages frequently migrate to the areas of demyelination, perhaps phagocytizing the myelin breakdown products. In most cases, a large number of HIV-infected macrophages are also found within the necrotic lesions. However, it is not clear how these immune cells infiltrate into areas of demyelination. One possible explanation is that JCV infection may recruit HIV-infected macrophages into demyelinated areas or, alternatively, uninfected macrophages may become infected by HIV after they are recruited into the CNS.

Clinically, the most common signs and symptoms of PML at the time of presentation are visual deficit, which is the most common presenting sign accounting for 35–45% of cases, motor weakness, accounting for 25–33% of cases, and mental deficits (emotional lability, difficulty with memory and dementia), which is seen in approximately one-third of cases. PML usually progresses to death within 4–6 months, although, in occasional cases, clinical signs and symptoms appear to remain stable for a long period of time.

Oncogenic potential of JCV

JCV, BKV, and SV40 are all known to induce a variety of tumors in experimental animals. All also have the ability to induce neoplastic cell transformation in tissue culture. Following its isolation, JCV was demonstrated to induce tumors in experimental animals in tissues of neuronal origin, but the type of the tumor induced by JCV depends



(a) (b)

Figure 4 Histopathological examination of a PML case. (a) Gross appearance of PML in a coronal section of the brain. Multiple areas of cavitation are present in the subcortical white matter of the frontal lobes. (b) Staining for myelin demonstrates several areas of myelin loss in the white matter. Luxol Fast Blue, original magnification ×40. Reproduced by permission from Macmillan Publishers Ltd. *Journal of Clinical Pathology: Molecular Pathology* (Gallia G, Del Valle L, Line C, Curtis M, and Khalili K (2001) Concamitant progressive multifocal leukoencephalopathy and primary central nervous system lymphoma expression JC virus oncoprotein, large T antigen. *Journal of Clinical Pathology: Molecular Pathology* 54(5): 354–359), copyright (2001).

on the type and age of animal and the site of inoculation. For example, when the Mad-1 strain of JCV was inoculated intracerebrally and subcutaneously into newborn Syrian hamsters, more than 80% of animals developed medullablastomas, glioblastomas, or neuroblastomas. An entire biologically active JCV genome was isolated when cells from these tumors were co-cultivated with permissive glial cells. In contrast, when a similar group of animals was inoculated intraocularly with the same strain of JCV, they mostly developed abdominal neuroblastomas in several locations. It was also observed that tumors metastasized to the bone marrow, lymph node, and liver.

Interestingly, JCV is the only polyomavirus that induces tumors in nonhuman primates, such as monkeys. In order to mimic a case resembling PML in humans, owl and squirrel monkeys were inoculated with JCV subcutaneously, intraperitoneally, or intracerebrally. The animals developed tumors at different time intervals. For instance, one owl monkey developed a malignant cerebral tumor similar to astrocytoma in humans after 16 months. Another animal developed a malignant neuroblastoma 25 months after inoculation.

Transgenic mouse models have also been developed to mimic the acute demyelination observed in PML patients. A transgenic mouse was created by using the portion of the JCV genome that contains the promoter and coding regions for LT-Ag. Some of the offspring from this mouse exhibited a mild to severe tremor phenotype. Hypo- and dysmyelination were observed in the CNS but not in the peripheral nervous system (PNS), suggesting that expression of LT-Ag affects myelin formation in the CNS and not in the PNS. Further characterization of myelin formation in transgenic animals revealed that the level of myelin sheath wrapped around the axons was relatively low, although the expression level of proteolipid protein, myelin basic protein, and myelin-associated glycoprotein genes appeared to be normal at the RNA level. In contrast, the respective protein levels appeared to be reduced. The mechanism by which LT-Ag alters the levels of these proteins in transgenic mice remains unknown, but it has been suggested that LT-Ag may influence the rate of translation of mRNA for these genes or other cellular genes that negatively influence the maturation of oligodendrocytes. This may eventually alter myelin formation around the axons.

Our group also described the formation of different tumors in tissues derived from neuronal origin in transgenic mouse models. The LT-Ag coding region under the control of the regulatory region of the archetype strain was utilized to create these transgenic animal models. Histological and histochemical analysis of the tumor masses revealed no sign of hypomyelination in the CNS, which was a feature of this transgenic model. In contrast, cerebellar tumors resembling human medullablastomas were induced.

In addition to the induction of tumors in experimental animals, the JCV genome has also been detected in a variety of human tumors, which raises the possibility of involvement of JCV LT-Ag in tumor formation in humans. Richardson, who first described PML in 1961, reported an incidental case of an oligodendroglioma in a patient with concomitant occurrence of chronic lymphatic leukemia and PML. The association of PML with multiple astrocytomas was also reported in 1983. Similarly, another case in which a patient had a long history of immunodeficiency syndrome with PML was described, and numerous foci of anaplastic astrocytes were observed. The presence of viral particles in both oligodendrocytes and astrocytes, but not in neoplastic astrocytes, in the demyelinating lesions of PML foci was demonstrated by electron microscopy. The presence of a large number of dysplastic or dysmorphic ganglion-like cells, which showed the properties of neurons, was also described recently in the cerebral cortex of a patient with PML. Expression of JCV LT-Ag, but not capsid proteins, was detected in these cells.

In addition to cases of concomitant PML and cerebral neoplasm, JCV has been shown to be associated with human brain tumors in the absence of PML lesions. The detection of JCV DNA in the brain tumors of an immunocompetent patient with a pleomorphic xanthoastrocytoma has been reported. In another investigation, JCV DNA and expression of LT-Ag were detected in tumor tissue from an immunocompetent HIV-negative patient with oligoastrocytoma. These two cases demonstrated the association of JCV with brain tumors in immunocompetent non-PML patients, and further prompted attempts to establish the association of JCV with different types of brain tumors in humans. Analysis of multiple brain tumors for presence of the JCV genome revealed that 57.1% of oligodendrogliomas, 83.3% of ependymomas, 80% of pilocytic astrocytomas, 76.9% of astrocytomas, 62.5% of oligoastrocytomas, and 66% of anaplastic oligodendrogliomas contained JCV early gene sequences. Furthermore, JCV genomic DNA has been detected in tumor tissues of non-neural origin, including the gastrointestinal tract and solid non-neural tumors such as colorectal cancers.

The precise mechanism by which JCV induces tumors is not known, but the tumorogenic protein LT-Ag is known to play a major role in this process. LT-Ag from JCV, as well as from BKV and SV40, has been shown to target major cell cycle regulators, including the tumor suppressor protein p53 and the retinoblastoma (pRb) gene products. This targeting inhibits the functions of these two key regulators of the cell cycle and perhaps others. In fact, protein interaction studies have clearly showed complex formation between LT-Ag and cell cycle regulators including pRb, p53, and p107.

BK Virus

BKV belongs to the same genus as JCV, as species *BK polyomavirus*. Like that of other polyomaviruses, the BKV genome consists of a small, closed circular DNA approximately 5 kbp in size, with the size varying between strains. For example, the genome sizes of the DUN, MM, and AS strains are 5153, 4963, and 5098 bp, respectively.

The structural organization of the BKV genome resembles that of JCV (**Figure 1**) in that it contains bidirectional coding regions (early and late), which are regulated by the regulatory region. The early region encodes only the regulatory tumor antigens, Sm t-Ag and LT-Ag, and the late region is responsible for the capsid proteins (VP1, VP2, and VP3) and a small, basic regulatory protein, agnoprotein. As for JCV and SV40, LT-Ag is essential for viral DNA replication, but the function of Sm t-Ag in BKV regulation is unclear. LT-Ag also potently transactivates the late promoter. The capsid proteins form icosahedral shell structures (39–42 nm in diameter), into which the viral genome is packaged. The capsid proteins are critical for attachment of the virus to cell surface receptors. Although the function of the BKV agnoprotein is unclear, recent evidence from JCV suggests that it plays a role in viral DNA replication, transcription, and cell cycle regulation.

Infection and Associated Disorders

Like JCV, BKV has a worldwide distribution in the human population. Primary infection occurs during early childhood and is subclinical, though occasionally accompanied by mild respiratory illness or urinary tract disease. Little is known about the route of transmission, although induction of upper respiratory disease and detection of latent BKV DNA in tonsils suggest a possible oral or respiratory route. During primary infection, viremia occurs and the virus spreads to various organs, including kidneys, bladder prostate, uterine cervix, lips, and tongue, where it remains in a latent state. Reactivation from the latent state is mostly associated with the immunocompromised state of the individuals. Reactivated virus has been detected in the urine of renal and bone marrow transplant recipients undergoing immunosuppressive therapy and in the urine of pregnant women. Upon reactivation, the virus may cause interstitial nephritis and urethral obstruction in patients receiving renal transplants. BKV has surfaced as a significant pathogen in kidney transplant patients in recent years by association with nephropathy, better known as polyomavirus-associated nephropathy. BKV infection appears to be a serious problem in renal allograft recipients in the first 2 years after transplantation, if not treated properly. In addition, an association between hemorrhagic cystitis and BKV has been shown in bone marrow transplant recipients.

Oncogenicity of the Genome in Experimental Animals

Like JCV, BKV is also oncogenic in experimental animals, including young or newborn mice, rats, and hamsters. The route of inoculation is a significant factor in determining the types of tumors induced. For example, BKV induces tumors in high proportions when inoculated intracerebrally or intravenously, but is weakly oncogenic when inoculated subcutaneously. BKV induces tumors in hamsters in a variety of tissues and organs, including ependymoma, neuroblastoma, pineal gland tumors, fibrosarcoma, esteosarcoma, and tumors of pancreatic islets. Rats inoculated with BKV develop fibrosarcoma, liposarcoma, osteosarcoma, nephroblastoma, and glioma. In a similar setting, however, mice develop only choroid plexus papilloma.

LT-Ag is the primary protein of BKV responsible for tumor induction in experimental animals. The oncogenic ability of LT-Ag has also been tested in transgenic mice models. Like JCV LT-Ag, BKV LT-Ag is known to target and inhibit several key cell cycle regulatory proteins, including p53 and the pRb family members, pRb105, pRb107, and pRb130. BKV also causes renal tumors and hepatocellular carcinoma. In such studies, there appear to be differences among strains of BKV with respect to the ability of LT-Ag to cause tumors. For example, Gardner's strain appears to be more potent in transgenic mice than isolates such as the MM, BKV-IR, or RF strains.

BKV LT-Ag induces cell transformation in tissue culture, although to a lesser extent than SV40 LT-Ag. It has been proposed that a 'hit-and-run' mechanism is most likely to operate during this transformation process; that is, expression of LT-Ag is required for the initiation of a multistage process, but is not required after a certain stage of transformation is reached. For example, in one study it was observed that, although transfection of BKV DNA into human cells resulted in a transformed phenotype, it was absent from most new transformed clones.

Another mechanism by which human polyomavirus LT-Ag may cause transformation is via the induction of chromosomal structural alterations characterized by breaks, gaps, dicentric and ring chromosomes, deletions, duplications, and translocations. While the molecular mechanism of this clastogenic effect of BKV LT-Ag on host DNA is unknown, it is thought that it may reside in the ability of the protein to bind topoisomerase I or in its helicase activity, in which it may induce chromosomal damage while unwinding the strands of cellular DNA. Moreover, since LT-Ag targets and inactivates p53, this may lead to survival of damaged cells, increasing their chance of transformation and immortalization. As a result, the clastogenic and mutagenic activities of the LT-Ag of human polyomaviruses may disturb the crucial function of the genes that are important for the maintenance of

genomic stability, such as oncogenes, tumor suppressor genes, and DNA repair genes.

Association of the Genome with Human Tumors

During late 1970s, BKV DNA was detected in a variety of human tumors and tumor cell lines, which prompted researchers to investigate the possible association of BKV with tumor induction. BKV was found to exhibit a specific oncogenic tropism for ependymal tissue, endocrine pancreas, and osteosarcomas in rodents. This led investigators to focus primarily on the presence of the BKV genome in such tumors. Southern hybridization studies showed that some pancreatic islet tumors, as well as some brain tumors, contain the BKV genome in a free, episomal state. BKV was even rescued from some of the tumors by transfection of human embryonic fibroblasts with tumor DNA.

The BKV genome was also reported in 46% of brain tumors of the most common histotypes, and was found to be integrated into chromosomal DNA in this particular study. Association of human tumors with immunocompromised conditions was also analyzed by Southern blotting, and the BKV genome was associated with Kaposi's sarcoma (KS) at low frequencies (20%).

Recently, normal and neoplastic human tissues, as well as tumor cell lines, were also examined for the presence of the BKV genome by PCR, utilizing primers for the early region. Nucleotide sequence analysis of PCR products revealed the presence of BKV-specific sequences in several brain tumor samples, one osteocarcinoma, two glioblastoma cell lines, one normal brain tissue, and one normal bone tissue specimen. In these studies, expression of the early region was demonstrated in some of the samples by reverse transcription (RT)-PCR. The presence of the BKV genome was also investigated in several different tumors, including urinary tract tumors and carcinomas of the uterine cervix, vulva, lips, and tongue. However, the data obtained from such studies were inconclusive because the proportion of positive samples in neoplastic tissues of the urinary and genital tracts and oral cavity was similar to that detected in corresponding normal tissues. BKV DNA has been shown to be present in a high proportion of KS cases, suggesting that BKV may be an important cofactor in KS.

Conclusion

Since the first cultivation of JCV from a PML patient and BKV from a renal transplant patient in 1971, we have learned much about the biology of both viruses. However, important aspects of the life cycle (viral entry, transport of viral particles to the nucleus, transcription, replication, and assembly and release of virions) remain to be elucidated. The more we investigate the biology of these viruses, the more complex their biology is revealed to be. Understanding the molecular mechanisms underlying the life cycle of these viruses will considerably enhance our view of their complex biology, which should then allow us to design effective therapeutics to intervene in the infection cycle at an early stage, before they cause more advanced disease.

See also: Human Cytomegalovirus: General Features; Human Herpesviruses 6 and 7; Polyomaviruses; Polyomaviruses of Mice; Simian Virus 40.

Further Reading

Berger JR and Concha M (1995) Progressive multifocal leukoencephalopathy: The evolution of a disease once considered rare. *Journal of Neurovirology* 1: 5–18.

Corallini A, Pagnani M, Viadana P, *et al.* (1987) Association of BK virus with human brain tumors and tumors of pancreatic islets. *International Journal of Cancer* 39: 60–67.

Del Valle L, Gordon J, Assimakopoulou M, *et al.* (2001) Detection of JC virus DNA sequences and expression of the viral regulatory protein T-antigen in tumors of the central nervous system. *Cancer Research* 61: 4287–4293.

Dorries K, Loeber G, and Meixensberger (1987) Association of polyomaviruses JC, SV40, and BK with human brain tumors. *Virology* 160: 268–270.

Gallia G, Del Valle L, Line C, Curtis M, and Khalili K (2001) Concamitant progressive multifocal leukoencephalopathy and primary central nervous system lymphoma expression JC virus oncoprotein, large T antigen. *Journal of Clinical Pathology: Molecular Pathology* 54(5): 354–359.

Gordon J, Del Valle L, Otte J, and Khalili K (2000) Pituitary neoplasia induced by expression of human neurotropic polyomavirus, JCV, early genome in transgenic mice. *Oncogene* 19: 4840–4846.

Hirsch HH (2005) BK virus: Opportunity makes a pathogen. *Clinical Infectious Diseases* 41: 354–360.

Kim J, Woolridge S, Biffi R, *et al.* (2003) Members of the AP-1 family, c-Jun and c-Fos, functionally interact with JC virus early regulatory protein large T-antigen. *Journal of Virology* 77: 5241–5252.

Lynch KJ and Frisque RJ (1991) Factors contributing to the restricted DNA replicating activity of JC virus. *Virology* 180: 306–317.

Major EO, Amemiya K, Tornatore CS, Houff SA, and Berger JR (1992) Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. *Clinical Microbiological Reviews* 5: 49–73.

Monaco MC, Jensen PN, Hou J, Durham LC, and Major EO (1998) Detection of JC virus DNA in human tonsil tissue: Evidence for site of initial viral infection. *Journal of Virology* 72: 9918–9923.

Padgett BL, Zu Rhein GM, Walker DL, Echroade R, and Dessel B (1971) Cultivation of papova-like virus from human brain with progressive multifocal leukoencephalopathy. *Lancet* i: 1257–1260.

Raj GV and Khalili K (1995) Transcriptional regulation: Lessons from the human neurotropic polyomavirus, JCV. *Virology* 213: 283–291.

Richardson EP (1961) Progressive multifocal encephalopathy. *New England Journal of Medicine* 265: 815–823.

Sariyer IK, Akan I, Palermo V, Gordon J, Khalili K, and Safak M (2006) Phosphorylation mutants of JC virus agnoprotein are unable to sustain the viral infection cycle. *Journal of Virology* 80: 3893–3903.

Walker DL, Padgett BL, Zu Rhein GM, Albert AE, and Marsh RF (1973) Human papovavirus (JC): Induction of brain tumors in hamsters. *Science* 181: 674–676.

Polyomaviruses of Mice

B Schaffhausen, Tufts University School of Medicine, Boston, MA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Murine polyomavirus, the virus that gives the name to the family *Polyomaviridae* and to the single genus (*Polyomavirus*) in the family, was discovered in the 1950s. Gross and Stewart observed that cell-free extracts from leukemic mice could induce neck tumors as well as leukemia in newborn mice. It soon became apparent that the leukemia-inducing activity could be separated from the activity inducing parotid tumors. Because the viral agent was found to induce a variety of solid tumors, the name 'polyoma' became attached to the virus. Epidemiology showed that the virus was widely disseminated in the mouse population.

Polyomaviruses have been valuable models for basic eukaryotic processes such as splicing and DNA replication. They have been especially important for studying growth regulation. The viruses require the apparatus of cellular DNA synthesis for their own replication. To meet this need they have evolved many different ways to intervene in cellular growth regulation. This intervention can cause tumors. Interestingly, although polyoma can be an extraordinarily potent tumor virus in certain laboratory strains of mice or in immunocompromised animals, the frequency of tumors in natural mouse populations is low despite the broad dissemination of the virus. Much of this resistance reflects the ability of T-cell immunity to prevent viral oncogenesis.

Virus Particles

Two major types of particles are found in lysates of infected cells: virions containing viral DNA and protein, and empty capsids that lack DNA and are noninfectious. Early physical characterization determined a molecular weight for virions of approximately 23 million Da with a sedimentation constant of 240S. The viral capsid is approximately 450 Å in diameter and possesses icosahedral symmetry. The viral coat consists of 72 pentameric capsomeres that are made up of VP1, the major viral structural protein. VP2 and VP3, which consists of the C-terminal 204 amino acid residues of VP2, represent the minor capsid proteins (**Table 1**). They appear to be found at the bottom of the central 'hole' in each capsomere. Although not required to assemble capsids, the infectivity of particles lacking VP2 or VP3 is dramatically reduced. The viral DNA is a closed, circular, supercoiled molecule of approximately 5300 bp. In virions, the DNA is associated with the cellular core histones H2A, H2B, H3, and H4, but not H1. Compared to host chromatin, the histones of the viral particle are much more highly acetylated, a modification known to be associated with gene activity.

Organization of the Viral Genome

The genome (**Figure 1**) is divisible into two almost equal parts. One half is used for the expression of the capsid proteins and the other is used for the expression of the three major early gene products. The early products are called T antigens (large T, middle T, and small T) because

Table 1 Polyomavirus (A2 strain) proteins and sizes (amino acid residues)

Early proteins (no of residues)		*Late proteins*	
Large T antigen	785	VP1	383
Middle T antigen	421	VP2	319
Small T antigen	195	VP3	204

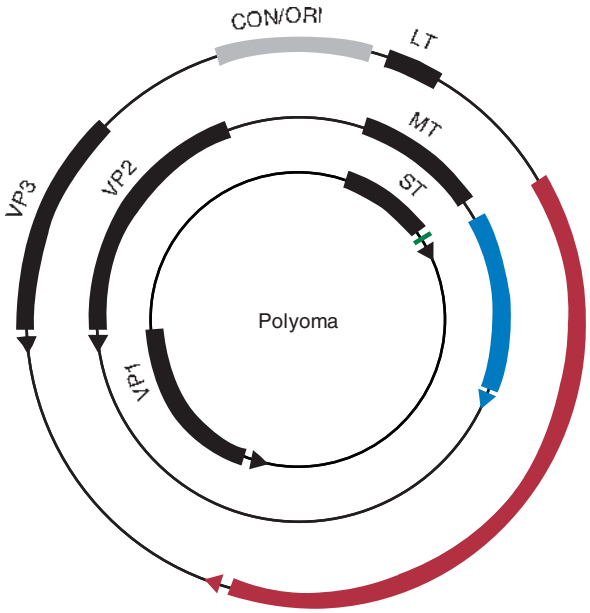


Figure 1 The organization of the polyoma genome. Early transcription of the T antigens (large T, LT; middle T, MT; small T, ST), late transcription of the capsid proteins (VP1, VP2, and VP3), as well as the origin of viral DNA replication (ORI), are regulated by the control (CON) region. The protein-coding sequences are indicated in colors to emphasize reading frame differences for the T antigens.

they were discovered using serum from tumor-bearing animals. Between the initiation codons for the late protein VP2 and the T antigens, there is a region of approximately 470 bp that contains control elements and the initiation sites for transcription and replication (**Figure 2**).

Genetic analysis has defined sequences necessary for viral DNA replication. There is a core origin of approximately 70 bp that includes an A+T-rich region on the late side and a highly purine-rich region (on one strand) on the early side. These regions flank a central 34 bp inverted repeat containing four pentanucleotide large T binding sites arranged as two head-to-head pairs. Replication initiated at this core origin requires a functional enhancer in *cis*.

Viral transcription and replication are regulated by the polyoma enhancer. **Figure 2** shows that the enhancer is located on the late side of the origin region. The enhancer has often been subdivided into two elements alpha (or A) and beta (or B) with overlapping function. It represents the binding sites for a series of cellular proteins that support viral transcription and replication. The most prominent binding sites are for PEA1 (AP1), PEA2 (runx), and PEA3 (ets family). Additional factors include polyomavirus enhancer B binding protein 1 (PEB1), EF-C, PED1, and c/EBP. Within the alpha element is a bipartite PEA1/PEA3 site of particular importance. Not surprisingly, given the number of cellular transcription factors that bind to it, there is redundancy built into the enhancer. The activity of the enhancer varies among cell types. It is clear that the enhancer structure can change in response to cellular environment or viral mutation. Ordinarily, polyoma grows poorly in embryonal carcinoma cell lines such as F9 cells. Viruses selected to grow on these cells were found to have alterations in the enhancer region. Hr-t mutants that lack middle T and small T were found to have enhancer alterations that contribute to their ability to grow. Mutants with deletions in the B enhancer domain grow poorly in neonatal, but almost normally in adult mice.

Not only can alterations in enhancer sequences affect behavior of the virus, but alterations on the early side of the control region can affect the tumor profile as well.

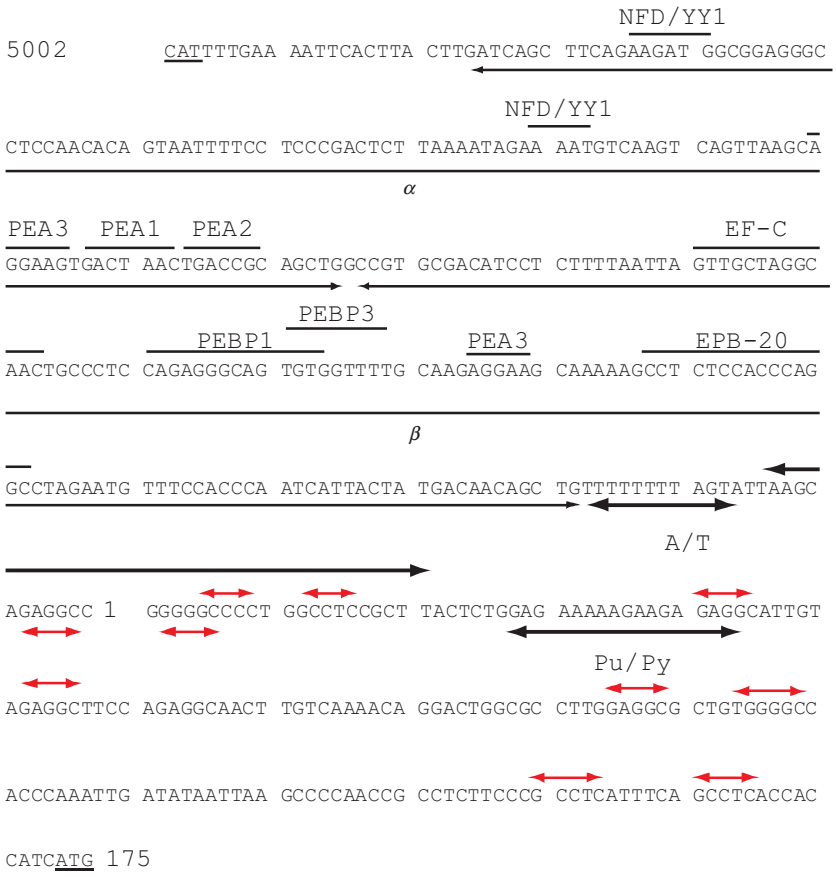


Figure 2 The origin/control region. The sequence between the initation codons for VP2 and large T is shown. The enhancer region is shown by the light arrows indicating the alpha (A) and beta (B) regions. Some transcription factor binding sites are indicated. The A+T-rich, purine-rich (one strand), and central palindrome regions of the core origin are shown by the thick arrows. Large T-binding sequences are indicated by red arrows.

Virus Infection

Infection by polyoma can have three outcomes: productive infection, which results in the production of more virus; 'abortive transformation' in which the infected cell temporarily assumes the transformed phenotype; and stable transformation, which permanently alters the cell to a transformed phenotype. In mouse cells, which are permissive for the virus, productive infection is the predominant response. In nonpermissive rat or hamster cells, abortive transformation is the most common response. Stable transformation is associated with integration of the viral DNA into host chromosomes, although episomal DNA has been demonstrated in tumors. The frequency of stable transformation is generally low and varies with the cell line.

A brief summary may be useful before dealing with the infectious cycle in more detail. The kinetics of infection depends on the multiplicity. In a high-multiplicity infection, early mRNA can be detected by polymerase chain reaction (PCR) as early as 6 h post infection. Large T can be observed by 8 h. Viral DNA replication begins 12–18 h post infection and continues for approximately 20 h. Late transcription is observed following viral DNA replication. Progeny virions begin to appear in the nucleus between 20 and 25 h post infection. Virus production plateaus around 40–48 h with the appearance of cytopathic effects.

Since the virus uses host DNA and RNA polymerases for its replication, it must enter the cell and reach the nucleus. Different polyomaviruses appear to have different entry processes upon infection. It has been known for 40 years that neuraminidase blocks polyoma infection. Polyoma binds sialic acid residues of glycoproteins and GD1a glycolipids with a critical sialic acid-α2,3-Gal structure. Interestingly, differences in the ability of virus strains to recognize different carbohydrate structures, thereby allowing interactions with 'pseudoreceptors', result in changes in virus spread in the mouse. After binding, the virus is internalized by caveolin-dependent and -independent pathways into the endoplasmic reticulum (ER). Successful infection occurs with conformational changes taking place in the ER determined by ER-localized oxidoreductase ERp29 and protein disulfide isomerase. Recent data indicating a role for Derlin2 in infection suggest that the quality control system of the ER is involved in transferring infecting virus to the cytoplasm. Nuclear localization sequences found in the capsid proteins and cellular histones are available to allow transport into the nucleus.

RNA Transcription and Processing

Viral transcription is divided into two types, early and late, that proceed in opposite directions from the viral control region. The early transcription unit that encodes the T antigens is active soon after infection begins. Differential splicing produces the three major early proteins, large T, middle T, and small T; a fourth spliced product, tiny T, has been observed, but its significance is uncertain. Late transcripts encoding the viral structural proteins are made early in infection, but they are not processed efficiently and have a short half-life. Late RNA does not accumulate until viral DNA replication proceeds, and late messages are generated for each of the three capsid proteins VP1, VP2, and VP3. Starting at that time, inefficient polyadenylation and termination generates large, multigenome length, heterogeneous late mRNAs. These RNAs are processed by leader-to-leader splicing so that there are tandem repeats of a 57 bp late leader, which is thought to allow accumulation of stable late message. The accumulation of late message means that there are now sequences present that are antisense to the early transcript. Editing of double-stranded RNA (dsRNA) by the ADAR enzyme converts up to 50% of early RNA adenosines to inosines with a resulting decrease in levels of early protein synthesis. The basis for the switch that allows late leader splicing is not yet fully appreciated, but may well involve the organization of polyadenylation sites and a balance of their editing and cleavage.

Viral Transformation and Tumorigenesis

Investigating polyomavirus transformation has provided repeated insights into normal and abnormal cell behavior. Tyrosine phosphorylation and phosphatidylinositol 3-kinase (PI3-K) are two discoveries that came directly from polyoma middle T studies. The p53 tumor suppressor was discovered using SV40, the monkey polyomavirus. Studies on interactions of large T with the retinoblastoma (Rb) tumor suppressor family (pRb, p107, p130) have led to insights into the E2F family and its regulation. In addition, and in contrast to many oncogenic RNA viruses, polyoma early gene products are also required for productive infection. Large T is required for viral DNA replication. The limited host range of hr-t mutants shows that middle T and small T act to provide a cellular environment that is supportive for viral replication.

Viral transformation is carried out by the early region. As a result of the splicing described above, all four early proteins share a common domain of 79 amino acid residues. This common N-terminus represents a DnaJ domain. Proteins that contain DnaJ domains ordinarily function as cofactors for DnaK proteins that act as molecular chaperones. Small T shares additional 112 amino acid residues with middle T. Each T antigen has unique C-terminal sequences. In the case of small T, the unique C-terminus is only four amino acid residues. The result of this processing is that three proteins, large T, middle T, and small T, have different intracellular localization and

different functions. Each of these proteins has the independent ability to affect cell growth and survival. Viral transformation results from T antigen association with, and regulation of, cellular signal-transducing proteins. Only large T is known to have intrinsic enzymatic activity, but even large T binds cellular proteins to regulate host function. Each of the three major T antigens will be discussed in turn. Because important insights have come from comparison studies with SV40, some mention will be made of SV40 small T and large T as well. SV40 has no direct counterpart to middle T.

Middle T

Middle T is key to polyoma transformation and tumor induction. Middle T is necessary and usually sufficient for transformation in cultured cells. The importance of middle T in tumor induction is clear. Viruses with middle T mutations show decreased tumorigenesis and changes in tumor profile. A number of transgenic models show tumor formation in response to middle T expression in the absence of either small T or large T. A mouse mammary tumor virus (MMTV)-middle T model of breast cancer has been especially well studied. Middle T has been a particularly useful model, because mutations that affect particular associations have usually had clearly identifiable phenotypes. In some instances, both in culture and in animals, middle T requires complementation from other viral oncogenes in order to transform. For example, in primary cells, polyoma middle T needs complementation by large T or nonpolyoma oncogenes for transformation. In REF52 cells, middle T also requires complementation with small T. While the role of middle T in transformation is clear, it is important to remember that middle T also plays an important role in polyoma infection. It can upregulate viral gene expression and viral DNA replication. Middle T has also been shown to regulate phosphorylation of the major capsid protein VP1.

Middle T functions as a kind of adaptor on which cellular signaling proteins are assembled (**Figure 3**). It might be viewed as a kind of constitutively active growth factor receptor. Middle T is associated with membranes; this membrane association is critical for transformation. Middle T binds the major cellular serine/threonine phosphatase PP2A. Cellular PP2A exists as a complex, either as two subunits, A and C, or as three subunits, A, B, and C. The B family is especially diverse, with many members, and appears to be involved in regulating targeting and activity. Both polyoma middle T and small T exist in complexes with the A and C subunits of the enzyme. Interestingly, middle T binds both Aα and Aβ forms of PP2A.

Association with PP2A allows middle Ts to bind and activate some of the src family tyrosine kinases (PTKs). C-src, c-yes, and c-fyn, but not c-hck, are all bound by middle T. Curiously, the PP2A does not have to be catalytically active for the PTK complex to form, suggesting that it may function as some kind of scaffold.

Tyrosine kinase activity is critical for transformation, so middle T mutants lacking associated tyrosine kinase activity are not able to transform. The c-src associated

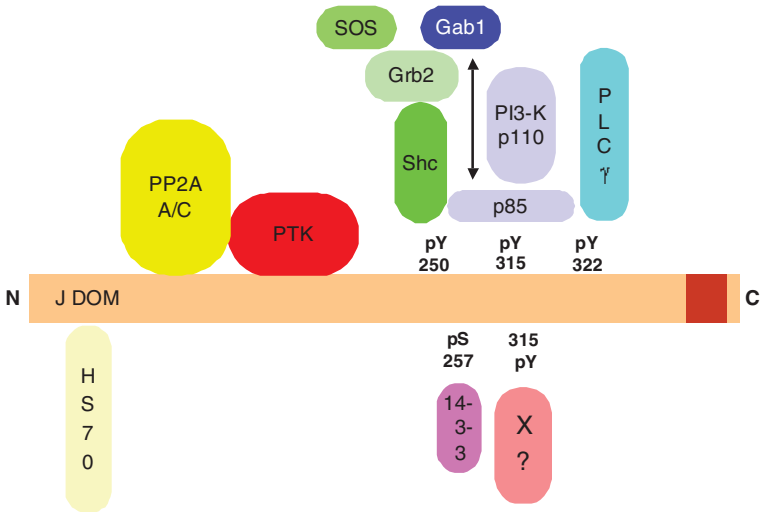


Figure 3 Polyoma middle T. Sites of interaction of middle T with cellular proteins are shown. Each of the signal transducers shown above the middle T sequence is known to be important for transformation. PP2A, protein phosphatase 2A; PTK, src family tyrosine kinase; PI3-K, phosphatidylinositol 3-kinase; X, missing partner at 315 indicated by genetics; PLCγ phospholipase Cγ1. There is also association between Gab1 and PI3-K. The red block represents the hydrophobic membrane attachment site. Small T shares with middle T the N-terminal 191 amino acid residues that include the J domain and PP2A binding site, but not the PTK binding site T and has only four unique residues at the C-terminus.

with middle T is activated, presumably, because it is lacking phosphorylation at Y527. In the complexes with PTK, middle T is phosphorylated on tyrosine residues. The major sites of phosphorylation are at residues 250, 315, and 322. These phosphorylations provide docking sites for signaling molecules. The initial picture of interactions was relatively straightforward. Each of the major sites represents a connection to a signal generator: 315 to PI3-K, 250 to Shc, and 322 to PLCγl. The picture is now more complicated from three points of view. First, there must be phosphorylation at minor tyrosines in the C-terminal half of middle T that can contribute to function. Second, there are clearly multiple connections to PI3-K, for example, mediated by more than one phosphotyrosine. Gab 1 protein, for example, binds middle T through its association with grb2 at 250 and can provide a connection to PI3-K. Third, multiple cellular targets seem to be reached through the single phosphotyrosine at 315. One is PI3-K, but the other remains to be identified.

Genetic analysis has been very useful in illuminating some of the signaling pathways of middle T. Mutation of 322, the PLCγ1 site, has modest effects in some transformation assays, but in low serum there is a substantial effect. Association with PLCγ1 is likely to be the basis for increased levels of IP3 observed in middle-T-transformed cells and for effects on PKC. Mutation of tyrosine 250 affects transforming ability and tumorigenesis, as do mutations just N-terminal to 250 (the NPTY motif). This represents a binding site for the adaptor Shc, and that binding leads to tyrosine phosphorylation of Shc. In turn, Shc binding and tyrosine phosphorylation are responsible for the association of Grb2. This in turn leads to the recruitment of SOS, a ras exchange factor that activates ras. Interestingly, while the 250 phosphorylation site seems to be especially important for transformation either in mouse cells or the transgenic middle T mammary tumor model, virus with a mutation at 250 is relatively efficient at forming tumors. Association with PI3-K, which is abolished by mutation at 315, is profoundly important for transformation in cultured rat fibroblasts and has a dramatic effect on the tumor profile in mice. PI3-Ks are broadly important enzymes that have been strongly linked to cancer. Downstream signaling from PI3-K includes both activation of Akt, a kinase important in preventing apoptosis, and activation of rac1, a G-protein involved in cytoskeletal organization and oxidative signaling.

Small T

In the past, small T has been studied less intensively than large T and middle T. However, increased attention has recently been focused on small T, since SV40 small T can play a role in transformation of human cells. It cooperates with hTert, SV40 large T, and ras in oncogene complementation assays. Transgenic SV40 small T can also contribute to mammary gland tumorigenesis.

Wild-type small T is not sufficient for transformation. Independent expression of small T in fibroblasts enables them to grow to a high cell density. However, small T can complement middle T for tumor induction and transformation. For example, small T can complement middle T for transformation of REF52 cells through its effects on arf-mediated activation of p53. Polyoma small T can resist the effects of p53-induced apoptosis. SV40 small T opposes apoptosis induced by large T. It also opposes Fas-mediated apoptosis of hepatocytes. Reports have suggested that Akt activity is induced by SV40 small T, which might account for these observations. Interestingly, in some cellular contexts both polyoma and SV40 small T also appear to be able to induce apoptosis.

Small T can be found in the nuclear and cytoplasmic compartments. Both SV40 and polyoma small T bind zinc. Like middle T, small T has been shown to bind PP2A. For SV40, the ability to displace specific B subunit family members such as B56γ seems important for its function.

Small T has been connected to a variety of cellular functions. For example, polyoma small T induces the membrane lectin agglutinability that is usually associated with cell transformation. SV40 small T can both disrupt tight junctions and disturb actin structure in epithelial cell monolayers. It has also been reported to perturb centrosome function. Most attention has focused on DNA replication and RNA synthesis. In serum-starved cells, polyoma small T can contribute to S phase induction in conjunction with large T. For example, it regulated the cyclin-cdk inhibitor p27. Small T can also affect viral DNA synthesis. Polyoma small T has also been implicated in virion assembly. For SV40, it has been shown that small T can transactivate or even repress various exogenous promoters. In many instances this has been related to the ability to bind PP2A. For SV40 small T, the interaction with PP2A stimulates the MAP kinase pathway, inducing cell proliferation. To cite just two recent examples of transcriptional activators, SV40 small T activates Sp1 and the FHL2 co-activator in a PP2A-dependent manner.

Large T

Polyomavirus large Ts have a dual role, acting not only directly in viral DNA replication and transcription, but also functioning to alter host-cell signaling. The role of large T in viral DNA replication has been studied extensively. SV40 provided the major model for establishing the mechanisms of cellular DNA replication, and its replication is still the best understood. In a productive infection,

polyoma large T initiates viral DNA replication. It is thought to do this by forming a double hexamer at the two head-to-head pairs of binding sites in the origin region; it then can unwind the origin and recruit factors such as DNA polymerase and RNA polymerase. By analogy to SV40, it is also likely to participate in the elongation phase of DNA synthesis. Its role in replication is important in other contexts as well. In transformation, it is responsible for integration and excision of the viral genome and can also promote recombination. Polyoma large T possesses the biochemical activities that might be anticipated for a protein involved in DNA replication. It binds the polyoma origin region at GAGGC sequences. Like SV40 large T, it possesses helicase and ATPase activities. Large T associates with pol α-primase, and by analogy to SV40 is expected to associate with RNA polymerase and topoisomerase I.

Large Ts are directly involved in cell transformation. Large T is the major transforming protein of SV40. Polyoma large T does not transform by itself, and viruses that make only large T do not cause tumors. Much of the difference in phenotype comes from the obvious interaction of SV40 large T with p53. Although there is a recent indication that polyoma large T may interact with p53 phosphorylated on serine 18, polyoma tumors show no evidence of a p53 block seen in SV40 tumors, and cell lines from tumors retain a normal p53 response to DNA damage. Nonetheless, polyoma large T can cooperate with other oncogenes such as middle T or ras in the transformation of primary cells. Similar complementation can also be seen in tumorigenesis.

Polyoma large T has important effects on cell phenotype, presumably to prepare the cellular environment for viral replication. Large T immortalizes primary cells in a manner dependent on the binding site for the retinoblastoma susceptibility (pRb), p107, and p130 gene products. Large T prevents differentiation, either of myoblasts or preadipocytes. The ability of large T to block withdrawal of myoblasts from the cell cycle and to prevent differentiation is dependent on Rb binding. Large T can induce dramatic apoptosis. These effects also involve interactions with the Rb family.

Given these phenotypes, it is not surprising that large T affects cellular DNA and RNA synthesis. Large T induces cellular DNA synthesis, using both Rb-dependent and -independent mechanisms. In the case of SV40 large T, at least four separate functions that contribute to the induction of cellular DNA synthesis have been identified. These functions have been mapped to the binding domains for p53, pRb/p107, p300, and TEF-1, and at least in the case of pRb requires the J domain as well.

Large T is a transcriptional activator of cellular genes. The first target identified was dihydrofolate reductase (DHFR); since then, many others have been identified as targets, such as the thymidine kinase (TK), human heat shock protein 70 (hsp 70), DNA polymerase alpha (pol α), proliferating-cell nuclear antigen (PCNA), thymidylate synthase (TS), and cyclin A genes. Transactivation of the TK, pol α, PCNA, DHFR, and TS genes requires an intact pRb/p107 binding site on polyoma large T and is mediated via the cellular transcription factor E2F. The ability of large T to activate these E2F-responsive genes depends upon an intact N-terminal J domain that binds hsc 70. As shown most clearly for SV40 large T, the role of this chaperone function is to disrupt E2F-Rb family complexes. While Rb binding is one function, transactivation of cellular and viral promoters by polyoma large T can also occur in the absence of pRb/p107 binding. Similarly the cyclin A promoter can be activated, even when the E2F site is mutant. There are certainly multiple mechanisms that require a more complete understanding. In fact, studies on SV40 large T have suggested that it is a somewhat promiscuous activator. Both the nature of the TATA/Inr element and the upstream sequences can contribute to the activation. This has led to a description of SV40 large T as being a TBP-associated factor (TAF)-like protein. A potentially important mechanism is association with histone acetyltransferases. Large T can associate with p300/CBP-associated factor (PCAF), p300, and CREB-binding protein (CBP).

Figure 4 shows a current view of the anatomy of large T. Large T is a nuclear zinc-containing phosphoprotein of 785 amino acid residues. The zinc-binding motif is a C2H2 element that differs from that in middle T and small T. In large T, it promotes self-association. Large T can be divided into two major domains that exhibit independent function. The N-terminal domain primarily functions to stimulate the host cell, while the C-terminal domain functions primarily in DNA replication. There are additional subdomains, for instance, the DNA-binding domain can function autonomously. Large T functions both in self-association through the zinc-binding region and in association with cellular proteins, including members of the Rb family and the DnaK family of proteins. Large T function can clearly be regulated by its phosphoryla-

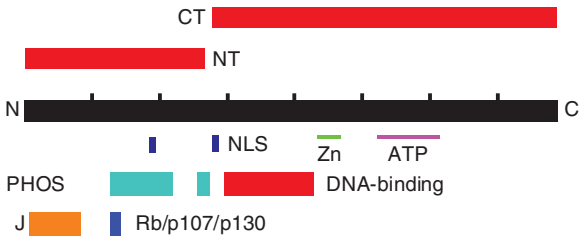


Figure 4 Large T antigen. The positions of the N-terminal (NT) and C-terminal (CT) domains are shown. The positions of the DNA-binding domain, zinc-binding element, ATP-binding domain, J domain, Rb-binding site, and nuclear localization sequences (NLS) are shown as bars. The regions containing phosphorylation sites are also indicated.

tion. Phosphorylation at threonine 278 by cyclin/cdk kinases is required for viral DNA replication.

Host Effects on Transformation and the Transformed Phenotype

Although the discussion of viral transformation and tumorigenesis has focused until now on the viral gene products, there are dramatic differences in the response of different inbred mouse strains to polyoma. Some strains such as C57BL/6J are quite resistant to tumor induction. Others such as C3H/BiDa are quite sensitive as neonates but develop resistance as adults. Much of this variation arises from the immune system. High tumor susceptibility can result, for example, from inheritance of a particular super antigen that affects the T-cell repertoire. Other mouse strains such as Ma/MyJ are resistant for reasons apparently related to spread of virus in the animal rather than immune mechanisms.

Transgenic middle T models point to the effects of host background on tumor behavior. The maternal genotype has a striking effect on tumor latency and on the likelihood of metastasis. Gene expression profiles resulting from middle T expression show obvious differences among strains.

See also: Simian Virus 40; Polyomaviruses of Humans; Polyomaviruses.

Further Reading

Michael JI and Eugene OM (2007) *Polyomaviruses*. In: Knipe D, Howley P, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 2263–2298. Philadelphia, PA: Lippincott Williams and Wilkins.

Stephen MD (2002) Polyoma virus middle T antigen and its role in identifying cancer-related molecules. *Nature Reviews Cancer* 2: 951–956.

Thomas LB (2001) Polyoma virus: Old findings and new challenges. *Virology* 289: 167–173.

Polyomaviruses

M Gravell and E O Major, National Institutes of Health, Bethesda, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Classification

Prior to the Seventh Report of the International Committee on Taxonomy of Viruses, polyomaviruses and papillomaviruses were classified in the family *Papovaviridae*. Commensurate with the release of this report, the genus *Polyomavirus* was removed from this family and elevated to independent status as the family *Polyomaviridae*. Capsid and genome size differences between polyomaviruses and papovaviruses were instrumental in prompting this change. Polyomavirus capsids are 40–45 nm in diameter and their genomes contain about 5000 bp, whereas papillomavirus capsids are 50–55 nm in diameter and their genomes contain 6800–8400 bp. Major differences in the replication cycles of polyomaviruses and papillomaviruses have also been described. The family *Polyomaviridae* currently comprises 13 members: four from monkeys, three from humans, two from mice, and one each from bird, hamster, rabbit, and cow. One virus, athymic rat polyomavirus, has been tentatively assigned to the family.

Morphological, Physicochemical, and Physical Properties

Members of the family *Polyomaviridae* have similar virion structures and the same general genome organization. Virions of polyomaviruses are nonenveloped, icosahedral particles composed of 360 copies of the major structural protein, VP1, and 30–60 copies of the minor structural proteins, VP2 and VP3. These molecules form 72 pentameric capsomers arranged in a skewed ($T = 7$d) lattice. Each capsomer is composed of five copies of VP1 and one copy of VP2 or VP3, each added to the internal cavity formed by association of the 5 VP1 molecules. The C-terminal ends of VP1 molecules extend to anchor neighboring capsomeres together. The VP2 and VP3 molecules are present within, but are not covalently linked to, the virion. In the course of virion assembly, occasional mistakes sometimes occur and aberrant capsid structures (such as empty particles, microcapsids, and tubular structures) are made.

Being nonenveloped, polyomaviruses are ether and acid resistant and relatively heat stable (50 °C, 1 h). However, they are unstable in 1 M $MgCl_2$. Virions have a sedimentation coefficient ($S20_w$) of 240S and an M_r of 2.5×10^7 Da. The buoyant densities of polyomaviruses are 1.2 and 1.34–1.35 g ml^{-1} in sucrose and CsCl gradients, respectively.

When the VP1 DNA sequence of the human polyomaviruses JC virus (JCV) or BK virus (BKV) is inserted into a baculovirus plasmid vector and expressed in insect cells as a recombinant gene, pentamers form resembling virion capsomeres. When these pentameric capsomere-like structures are purified and placed in a solution of physiological pH and ionic strength containing Ca^{2+} ions, directed self-assembly of pentamers into genome-free virions occurs. The capsids formed, which are called virion-like particles (VLPs), have the size, icosahedral symmetry and antigenicity of native virions. Practical use has been made of VLP production. JCV or BKV VLPs have been used in enzyme immunoassays (EIA) to measure titers of JCV- or BKV-specific antibodies elicited by infection with these viruses. Because of the greater sensitivity, specificity, and safety of these genome-free VLPs, their use in EIAs has now largely replaced hemagglutination assays as the preferred method to measure levels of JCV- or BKV-specific antibodies.

Genome

The genome in each polyomavirion contains a single, supercoiled molecule of closed circular, double-stranded DNA of about 5000 bp. It makes up about 10–13% by weight of the virion, the remainder being protein. The genome is composed of three functional regions: the genetically conserved early and late coding regions separated by the hypervariable regulatory region that contains the origin of DNA replication (ORI). Host cell histone proteins H2a, H2b, H3, and H4 associate with the supercoiled genome to form a mini-chromosome-like structure. Polyomavirus DNA synthesis occurs exclusively within the S-phase of the cell cycle. However, although cellular DNA replicates only once during each cellular S-phase, multiple cycles of viral genome replication can occur.

Proteins

Proteins produced during the course of polyomavirus replication are divided into those produced during the early and late stages of infection. The early proteins are nonstructural, and are called the large T and small t proteins (or antigens) based on their sizes; T (in either case) is derived from the word tumor. These T proteins are considered the master regulators because they stimulate cells to produce the enzymes and other factors required for cellular DNA replication, thereby setting up the conditions required for late events during viral DNA replication and virion assembly. The T proteins interfere with aspects of cell cycle regulation and cause cell transformation and sometimes tumorigenesis. They are translated from 2 to 5 mRNAs generated by alternative splicing from a common pre-mRNA.

The T protein genes are transcribed from one of the genomic DNA strands in the counterclockwise direction, and the late genes are transcribed in a clockwise direction from the other genomic strand from the opposite side of ORI. Polyomavirus replication occurs exclusively within the cellular S-phase. Binding of T protein to the hypophosphorylated retinoblastoma susceptibility protein (pRb) permits premature release of the E2F transcription factor, thus stimulating cell entry into S-phase. After recruitment of the host cell DNA polymerase complex to ORI, bidirectional replication is initiated. Activation of the late viral promoter by T protein and association with specific cellular transcription factors results in expression of late virus genes. These include the genes for the virion structural proteins, VP1, VP2, and VP3 and the nonstructural regulatory agnoprotein.

The three structural capsid proteins (VP1, VP2, and VP3) originate from a common precursor mRNA by alternative splicing. The VP2 precursor mRNA contains the coding sequence for the complete VP3 protein, and each protein is translated by virtue of different start codons in the same mRNA. More than 70% of the polyomavirus capsid protein is composed of VP1, the major structural protein. For the polyomaviruses simian virus 40 (SV40), mouse polyomavirus (mPyV), and JCV, the minor capsid proteins (VP2 and VP3) have been reported to be required for proper import of virion proteins to specific nuclear localization sites for assembly. VP2 and VP3 share an identical C-terminal sequence that contains the DNA-binding domain and the VP1-interacting domain. Disulfide bonds and calcium ions are required for maintenance of structural stability of virion capsids. Virion assembly occurs exclusively in the nucleus and has been linked to the presence of a higher calcium ion concentration in the nucleus than in the cytoplasm.

The nonstructural agnoprotein functions in the life cycle of polyomaviruses in several ways. In JCV replication, it has been implicated in regulating transcription and maturation. It has also been shown to interact functionally with YB-1, a cellular transcription factor, and to regulate JCV gene transcription negatively. More recently, it was reported to participate in JCV cell transformation by interfering with the ability of a DNA-dependent protein kinase repair complex, composed of a 470 kDa catalytic subunit and a K70/K80 heterodimer regulatory subunit, to repair breaks in double-stranded cellular DNA, thus inhibiting its role in cellular DNA repair.

Replication Cycle

Nuclear Entry

Major advances have been made in recent years in understanding mechanisms that polyomaviruses use to attach and penetrate cells in order to gain entry into the cytoplasm. All events, both early and late, required for polyomavirus replication occur in the nucleus. Although many steps in this process are now understood, the question of how, where, and in what state the virus minichromosome traverses the nuclear membrane is not fully understood. Intact polyomavirions have been seen inside the nucleus by electron microscopy prior to expression of T proteins, suggesting that the virions that initiate infection enter the nucleus in an undegraded state and are uncoated therein. However, partially uncoated virions have also been seen in the cytoplasm. Whether these partially uncoated intermediates are the entities that initiate virus DNA replication and virion assembly in the nucleus is uncertain. The site of entry and mechanism of virion entry into the nucleus is also not clearly defined. Virions in cytoplasmic vesicles and their fusion with the nuclear membrane have been observed, but whether this is the mechanism of virus genome entry into the nucleus is not clear. Two sites that have been implicated in virion entry into the nucleus are the endoplasmic reticulum and the nuclear pore complex.

Evidence that BKV enters the nucleus via passage through the endoplasmic reticulum follows. To enter the cytoplasm, BKV employs a caveolae-mediated endocytic pathway, which is generally slower than clathrin-mediated endocytosis. It is generally thought that cellular microtubules are involved in the shuttling of newly formed endocytic vesicles to various intracellular locations. Treatment of Vero cells with nocodazole does not prevent the endocytic uptake of BKV into the cytoplasm, but it does cause disassembly of the cellular microtubule network and inhibits movement of endocytic vesicles. BKV replication is inhibited if added to nocodazole-treated cells during the first 8 h of infection, which is the time period generally required for BKV to traverse the cytoplasm to the nucleus where replication begins. Microtubule disassembly prevents directional movement of endocytic vesicles, stopping them from passing through the endoplasmic reticulum to reach the Golgi. Treatment of LNCaP cells with brefeldin A, which blocks transport from the endoplasmic reticulum to the Golgi apparatus, prevents BKV infection of these cells. These results were interpreted to suggest that the route BKV uses for nuclear entry is via the endoplasmic reticulum.

Receptors and Modes of Cytoplasmic Entry

Polyomavirus entry into the cytoplasm has been studied in the greatest detail for mPyV, SV40, JCV, and BKV. The mechanisms of attachment and penetration are described below.

mPyV

This virus was the first polyomavirus for which sialic acids were implicated as an attachment receptor. The receptor studies were initiated in response to finding plaque size variants that produced small or large plaques. Upon sequencing the genomes of these size variants, it was found that the size differences in plaques resulted from a single amino acid change at VP1 position 92, in which glutamic acid was changed to glycine. Studies yielded evidence that the differences between small and large plaques originated from the capability of virus to bind to sialic acids. The small plaque isolates bound well to the straight chain $\alpha(2-3)$-linked sialic acids and the branched moieties containing both $\alpha(2-3)$- and $\alpha(2-6)$-linked sialic acids. In contrast, the large plaque variant bound well to only the straight chain $\alpha(2-3)$-linked structure and poorly to the branched structure. The single amino acid change from glutamic acid to glycine was shown to occur in a site responsible for mPyV hemagglutination of erythrocytes and receptor-binding to permissive cells. Because of this single amino acid change, the small plaque variant gained a receptor attachment advantage over the large plaque variant that translated to production of higher titers of infectious virus and virus having a higher cell transformation efficiency. These results illustrated that even a single amino acid change in a receptor site could produce major changes in virus yield and virulence.

Subsequent to implicating sialic acids as an attachment receptor for mPyV, the protein component of the receptor was found to be $\alpha 4\beta 1$ integrin. Endocytosis of mPyV has been shown to occur by either of two mechanisms: (1) by caveolae-mediated endocytosis, or (2) by an alternative clathrin, caveolin-1, dynamin-1 pathway. The target cell influences the pathway chosen. Recent evidence also has implicated gangliosides as a receptor for mPyV infection. Treatment with ganglioside GD1 of mouse cells lacking a functional receptor for permissive mPyV infection imparted susceptibility to infection to these cells.

SV40

Of the four polyomaviruses described herein, all except SV40 utilize a sialic acid component as receptor. By comparing the crystal structure of the SV40 capsid with that of mPyV, it was shown that the inability of SV40 to bind to sialic acid resided in truncation of an eight amino acid residue segment of an external loop on the capsid essential for binding to sialic acid.

The receptor by which SV40 initiates infection was found to be major histocompatability complex (MHC) class I molecules. Although these molecules mediate binding to cells, they neither envelop virions nor enter cells during the infectious process. Evidence suggests that after SV40 binds to MHC class I molecules, it sends an

intracellular signal that facilitates its entry into cells by caveolae-mediated endocytosis. As with mPyV, gangliosides have been implicated as a receptor for SV40 attachment and cellular entry. GM1 gangliosides are important in initiating SV40 infection.

JCV

Evidence that sialic acid is a receptor for JCV attachment came from initial studies showing that treatment of red blood cells (RBCs) and permissive glial cells with crude neurominodase abrogated hemagglutination of RBCs and infection of permissive glial cells. However, treatment of these cell types with an α(2–3)-specific neuraminidase did not cause inhibition, providing evidence that sialic acid might be a receptor, but not the α(2–3)-linked sialic acid moiety. Results defining the specific sialic acid receptor came from several additional experiments. Binding of high concentrations of JCV to permissive glial cells was shown to block binding of the α(2–6)-linked straight chain specific lectin, *Sambucus nigra* lectin (SNA). Use of specific O-linked and N-linked glycosylation inhibitors further showed that the JCV-specific receptor was an N-linked glycoprotein with a terminal α(2–6)-linked sialic acid.

Chlorpromazine and the related compound clozapine both block clathrin-dependent endocytosis, and also JCV infection. These drugs belong to a class known as serotonin-dopamine inhibitors. Glial cells susceptible to JCV infection coexpress receptors for both serotonin and dopamine. To identify whether serotonin or dopamine is the receptor component required for JCV infection, glial cells permissive to JCV infection were treated with the dopamine antagonists bromocriptine and miniprine, and a dopamine agonist, pergolide. These agents did not block JCV infection and had only minimal influence on serotonin receptors. In contrast, treatment of glial cells with metoclopramide, chlorpromazine, or clozapine, all of which are antagonists of the $5HT_{2A}$ serotoninergic receptor, significantly inhibited JCV infection. Furthermore, monoclonal antibodies specific to $5HT_{2A}$ and $5HT_{2C}$ receptors inhibited JCV infection of glial cells, but monoclonal antibodies to the dopamine receptor did not. The specificity of these antibodies to JCV infection was further illustrated by their failure to inhibit SV40 replication in treated glial cells. This is as expected, since SV40 and JCV utilize different receptors for cell attachment and enter cells by different endocytic pathways.

HeLa cells are mostly refactory to JCV infection, but because they contain the N-terminal α(2–6)-linked sialic acid receptor component on their surface, they bind JCV as well as permissive cells. When transfected with JCV genomic DNA, HeLa cells acquire the capacity to support JCV early gene expression. However, stable or transient transfection of HeLa cells with a $5HT_{2A}$ receptor-containing construct reversed their susceptibility to JCV infection. This illustrates the necessity of having both receptor components, N-terminal α(2–6)-linked sialic acid and a $5HT_{2A}$ protein serotonergic component present on cells for susceptibility to JCV infection. Other than establishing the requirement for both cellular receptors, the mechanism by which $5HT_{2A}$ transfection into HeLa cell alters their susceptibility to JCV infection has not been explained.

After entry into the cell interior, polyomaviruses must enter the nucleus for replication and assembly. For nuclear entry, components of the cellular cytoskeleton have been shown to be important in intracellular movement of virus through the cell cytoplasm, as described above. After virion entry into the cell interior, an intact microtubule network has been reported to be important in the early phase of JCV infection. Furthermore, SVG cells (an astroglial cell line highly susceptible to JCV infection) was shown to require an intact actin cytoskeleton to maintain its susceptibility to JCV infection. It has been suggested that the actin cytoskeleton system does not participate directly in viral movement through the cytoplasm, but may be important in assembling the clathrin machinery necessary for JCV endocytosis. Participation of intermediate filaments is also required for permissive JCV infection of cells.

The etiological link between JCV and progressive multifocal leukoencephalopathy (PML) and the high concentrations of gangliosides in the human brain make them attractive candidates as JCV receptors. The sialic acids of glycoproteins and glycol lipids have been shown to bind to JCV VLPs at their VP1-binding sites. Pretreatment of JCV virions with the ganglioside GT1b also inhibited their ability to infect susceptible glial cells. This evidence was used to infer that GT1b ganglioside bound to JCV VP1 receptor sites, thereby blocking the ability of the virus to initiate infection of the highly JCV susceptible cell lines IMR-32 and SVG and suggesting that both glycoproteins and glycolipids may function as JCV receptors. JCV has been reported to have specificity for N-terminal α(2–6) sialic acids of glycoproteins in order to initiate infection, and has also been reported to bind JCV VLPs at various α(2–3) and α(2–6) structures. These results suggest that both glycoproteins and glycolipids may function as JCV receptors. The question of whether glycolipids are involved in JCV infection of the brain has not been resolved.

BKV

The role of sialic acids as a receptor for BKV entry was reported recently. The cellular receptor that BKV uses to initiate infection is a glycoprotein with an N-linked α(2–3) sialic acid. This was demonstrated by treatment of Vero cells with sialidase S, an enzyme that specifically removes α(2–3)-linked sialic acid from glycoproteins and complex carbohydrates. Reconstitution of the asialo Vero cells by α(2–3)-specific sialyltransferase restored susceptibility of these cells to infection by BKV, but restoration by an α(2–6)-specific sialyltransferase did

not. Evidence that the sialic acid was N-linked was obtained by showing that treatment with tunicamycin, an inhibitor of N-linked glycosylation, reduced BKV infection, whereas treatment with the O-linked glycosylation inhibitor benzylGalNac did not.

The protein component of the BKV receptor has not been determined. However, gangliosides have been implicated in binding to BKV and altering cell susceptiblity to BKV infection. BKV binding to erythrocyte membranes has been investigated by use of sucrose flotation assays. It was shown that binding was to a neuraminidase-sensitive, proteinase K-resistant molecule. It was suggested that the terminal α(2–8)-linked disialic acid motif, present on both GT1b and GD1b gangliosides, was responsible for inhibiting BKV binding to erythrocyte membranes. Furthermore, it was shown that LNCaP cells, which are normally resistant to BKV infection, became susceptible to BKV infection after treatment with gangliosides GT1b and GD1b. Also, it has been reported that recoating cells stripped of sialic acid and galactose with a mixture of all gangliosides derived from Vero cells restored their capacity to be infected by BKV. These results suggest that gangliosides have a role as receptors in BKV attachment and infection. The mechanisms that gangliosides employ in reversing cell susceptibility to BKV infection have not been determined.

In addition to glycolipids, the phospholipid bilayer has also been implicated in BKV hemagglutination and infection. Pre-incubating BKV with phospholipids decreases its ability to infect Vero cells, presumably because BKV binds to the exogenously added lipid, thus blocking its receptor sites from interacting with the host cell. Treating cells with either phospholipase A2 or D cleaves fatty acids, and reduces their permisssiveness to BKV infection and their ability to hemagglutinate RBCs. These parameters can be restored to the native state by adding back various preparations of phospholipids, such as L-α phosphatidylcholine and phospholipids derived from Vero cells. Hemagglutination titers of RBCs treated with phospholipase C are elevated compared to those of untreated controls, although the reason for these results is not completely understood. They point out that gangliosides are important to BKV hemagglutination and infection. Adding various gangliosides to cells has been shown to alter their susceptibility to BKV infection. Currently, four subtypes of BKV have been identified, although no clear-cut link has been established between any of these subtypes and increased BKV virulence. The receptor for BKV attachment has been linked to a region of VP1 between amino acid residues 61 and 83. This sequence aligns with a hydrophilic region that aligns with the the BC loop of the SV40 capsid. It has not been determined whether all of the BKV subtypes share the same amino acid receptor sequence for cellular attachment. As previously explained for mPyV, a single amino acid change in its receptor sequence translated to altered plaque size, hemagglutination activity, virus output, and virulence.

Host Cell Susceptibility

Although the human polyomaviruses JCV and BKV share about 75% DNA sequence homology, they differ widely in many biological properties, including host cell range, cellular receptors, cell entry mechanisms, tissue tropisms, and disease manifestations. A defining feature of these polyomaviruses is the hypervariability of their non-coding regulatory regions. The makeup of these regions affects levels of transcription and influences host cell range and tissue tropism. For an in-depth discussion of JCV variants, based on the nucleotide sequences of their noncoding region, refer to the work by Jensen and Major listed in the 'Further reading' section. The notion that polyomavirus replication is controlled at the intracellular, molecular level came from the similarity in results obtained for host cell susceptibility and virus output when native virions or transfected genomic DNA were used to infect various cell types. Results supporting this conclusion are presented below.

A family of DNA-binding proteins called NF-1, composed of four subtypes called A, B, C, and D (or X) has been linked with site-coding specific transcription of viral genes and JCV replication. Elevated levels of NF-1 class X (NF-1X) mRNA were expressed by brain glial cells that are highly susceptible to JCV infection, but not by nonsusceptible HeLa cells. $CD34^+$ precursor cells of the KG-1 line, when treated with phorbol ester 12-myristate 13-acetate (PMA), differentiated to cells with macrophage-like characteristics and lost susceptibility to JCV infection. To determine whether loss of JCV susceptibility in these cells was linked to reduced levels of NF-1X expression, reverse transcription-polymerase chain reaction (RT-PCR) was used to evaluate levels of mRNA of each of the four NF-1 subtypes found in PMA-treated KG-1 cells. Different levels of specific subtypes of mRNA were observed in the PMA-treated macrophage-like cultures compared with controls. Northern blot hybridization confirmed that the levels of NF-1X expressed by PMA-treated KG-1 cells were lower than those produced by untreated KG-1 cells. Use of gel mobility shift assays later confirmed this finding by showing the induction of specific NF-1-DNA complexes in KG-1 cells undergoing PMA treatment. These results suggested that the binding pattern of NF-1 class members may change in hematopoietic precursor cells, such as KG-1, as they undergo differentiation to macrophage-like cells. Transfection of PMA-treated KG-1 cells with an NF-1X expression vector restored their susceptibility to JCV infection. Transfection of PMA-treated KG-1 cells with NF-1 subtypes A, B, and C vectors failed to restore JCV susceptibility. These data collectively show the importance of NF-1X expression for JCV replication.

The importance of NF-1X expression for JCV replication has also been shown in multipotential human nervous system progenitor cells. By use of selective growth

conditions, these progenitor cells were cultured from fetal brain tissue as undifferentiated, attached cell layers. Selective culture techniques yielded highly purified population of neurons or astrocytes. Infection with JCV virions or with a plasmid encoding the JCV genome demonstrated the susceptibility of astrocytes and, to a lesser degree, their undifferentiated progenitors. However, neurons remained nonpermissive for JCV replication. Expression of the NF-1X transcription factor was much higher in astrocytes than in neurons. Transfection of an NF-1X expression vector into progenitor-derived neuronal cells, prior to JCV infection, yielded JCV protein production. These results indicate that susceptibility to JC infection is regulated at the molecular level. Furthermore, they suggest that differential recognition of viral promoter sequences can predict lineage pathways of multipotential progenitor cells in the human nervous system.

Perspectives

Phylogenetic evidence indicates that polyomaviruses have evolved from a common ancestor. Despite their very similar morphological, physical, and chemical characteristics, events in the life cycle of closely related species can vary considerably. This article contains information of recent vintage on the variety of mechanisms involved in polyomavirus attachment, penetration and movement within the cell to reach the cell nucleus, where replication occurs. Our understanding of the role of sialic acid receptors in polyomavirus replication has been greatly expanded, especially those associated with glycoproteins. Recent work has also implicated various gangliosides as receptor molecules, and much new information should be available shortly on how they contribute to the infectious process.

Information is also presented on how levels of specific cellular transcription factors, such as NF-1X, can influence the infectability of specific cell types by JCV. Much new information has also been published on how the noncoding regulatory region of JCV changes from the archetype arrangement found in most urine isolates to those found in other tissues, such as lymphocytes, tonsils, and in brains of individuals with PML. Modifications to this promoter/enhancer structure can alter cellular host range and may be responsible for switching infection from the latent to lytic states. The importance of immunosuppression in JCV- or BKV-caused disease, and the predominant role of cellular immunity in their prevention, have been firmly established.

The tumorigenicity of JCV and BKV for animals has been widely known for many years. Their nucleic acids have been detected in tumor and surrounding tissue by sensitive PCR techniques. Also, polyomavirus T proteins that impair the cell-cycle regulatory functions of p53 and pRb have been detected by sensitive immunochemical techniques in human colon, gastric, brain, and pancreatic tumors. JCV and BKV persistently infect a high percentage of the human population worldwide. The range of cell types infectable by JCV or BKV has also increased in recent years, establishing the possibility that tumor cells could become infected after tumorigenesis begins.

See also: Polyomaviruses of Humans; Polyomaviruses of Mice; Simian Virus 40.

Further Reading

Cole CN and Conzen SD (2001) *Polyomaviridae*: The viruses and their replication. In: Fields BN, Knipe DM, Howley PM, *et al.* (eds.) *Fields Virology*, 4th edn., vol. 2, pp. 2141–2174. Philadelphia, PA: Lippincott Williams and Wilkins.

Eash S, Manley K, Gasparovic M, Querbes W, and Atwood WJ (2006) The human polyomaviruses. *Cellular and Molecular Life Sciences* 63: 865–876.

Gee GV, Dugan AS, Tsomaia N, Mierke DF, and Atwood WJ (2006) The role of sialic acid in human polyomavirus infections. *Glycoconjugate Journal* 23: 19–26.

Imperiale MJ and Major EO (2007) Polyomaviruses. In: Knipe DM, Howley PM, *et al.* (eds.) *Fields Virology*, 5th edn., vol. 2, pp. 2263–2298. Philadelphia, PA: Lippincott Williams and Wilkins.

Jensen PN and Major EO (2001) A classification scheme for human polyomavirus JCV variants based on the nucleotide sequence of the noncoding regulatory region. *Journal of Neurovirology* 7: 280–287.

Pomovirus

L Torrance, Scottish Crop Research Institute, Invergowrie, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Spore balls Resting spores (cystosori) of *Spongospora subterranea* found in lesions or pustules (powdery scabs) on potato tubers; a single cystosorus comprises 500–1000 resting spores aggregated to form in a ball that is partially hollow, traversed by irregular channels.

Spraing Virus disease symptoms of internal brown lines and arcs in potato tuber flesh.

Introduction

Pomoviruses have tubular rod-shaped particles and tripartite genomes; they are transmitted by soil-borne zoosporic organisms belonging to two genera (*Polymyxa* and *Spongospora*) in the family Plasmodiophoraceae. Pomoviruses have limited host ranges, infecting species in a few families of dicotyledenous plants. Agriculturally important hosts include potato and sugar beet. There are four member species: *Potato mop-top virus*, *Beet soil-borne virus*, *Beet virus Q*, and *Broad bean necrosis virus*. Beet virus Q (BVQ) and beet soil-borne virus (BSBV) can occur in mixed infections with the benyvirus beet necrotic yellow vein virus (BNYVV).

Taxonomy and Classification

The classification of tubular rod-shaped viruses transmitted by soil-borne plasmodiophorid vectors was revised in 1998 to establish four genera: *Furovirus*, *Pomovirus*, *Benyvirus*, and *Pecluvirus*. The revision was prompted by new virus sequence information that revealed major differences in genome properties (number of RNA species, sequence, and genome organization). The genera are not assigned currently to any family. Pomovirus is a siglum from *Potato mop-top virus*, the type species.

Physical Properties of Particles

Pomovirus particles are hollow, helical rods, 18–20 nm in diameter, comprising multiple copies of a single major coat protein (CP; *c.* 19–20 kDa). The CP gene is terminated by a UAG (or UAA in broad bean necrosis virus (BBNV)) stop codon that is thought to be suppressible, readthrough (RT) of which would produce a fusion protein of variable mass (54–104 kDa). One or a few copies of the RT fusion protein are present at the extremity of potato mop-top virus (PMTV) particles thought to contain the 5′ end of the virus RNA. Pomovirus particles are fragile and particle size distribution measurements are variable; PMTV particles have predominant lengths of 125, 137, and 283 nm. PMTV particles sediment as three components with sedimentation coefficients ($S_{20,W}$) of 126, 171, and 236 S.

Genome Properties

Complete genome sequences are available for three member species and an almost complete sequence (without the 5′ and 3′ untranslated regions (UTRs)) is available for BBNV. Pomovirus genomes comprise three species of positive-sense single-stranded RNA of *c.* 5.8–6, 2.8–3.4, and 2.3–3.1 kbp (**Figure 1**). RNA-1 encodes the replicase proteins. It contains a large open reading frame (ORF) that is interrupted by a UGA stop codon (ORF1) (or UAA in BVQ and BSBV); the sequence continues in-phase to encode an RT protein (204–207 kDa). The ORF1 protein (145–149 kDa) contains methyltransferase and helicase motifs while the RT domain contains the GDD RNA-dependent RNA polymerase (RdRp) motif. Phylogenetic analysis reveals that the RdRps of the pomoviruses and soil-borne wheat mosaic furovirus share between 50% and 60% sequence identity.

The 5′ UTR of pomovirus RNAs contains the starting sequence $GU(A)_{1–4}(U)_n$ (except BVQ RNA-1 which begins with AUA). The RNAs are probably capped at the 5′ end since the RNA-1 ORF contains methyltransferase motifs associated with capping activity. The terminal 80 nucleotides of the 3′ UTR can be folded into a tRNA-like structure that contains an anticodon for valine. Both BSBV and PMTV RNAs were shown to be valylated experimentally.

The virus movement proteins are encoded on RNA-2 of PMTV (RNA-3 in BSBV, BVQ, and BBNV). Three

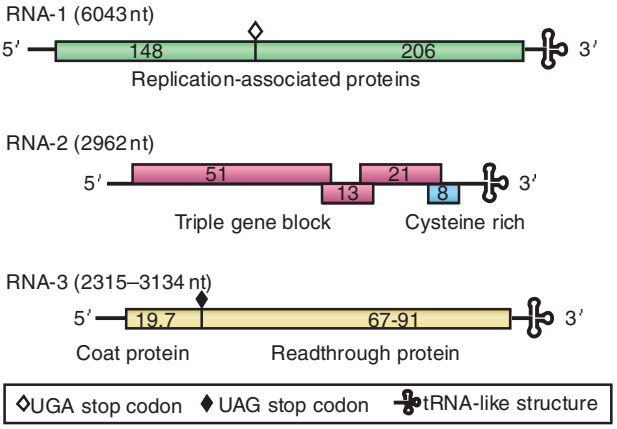


Figure 1 Diagram of the PMTV genome organization; boxes indicate open reading frames with the molecular masses of predicted protein products (kDa) indicated within.

5′ overlapping ORFs encode a conserved module of movement proteins known as the triple gene block (TGB). TGB movement proteins are found in the genomes of other rod-shaped viruses (hordei-, beny-, pecluviruses) and in monopartite filamentous viruses in the genera *Potexvirus* and *Carlavirus*. The TGB proteins (TGB1, TGB2, and TGB3) are named according to their position on the RNA and have molecular masses of 48–53, 13, and 20–22 kDa, respectively. The TGB1 contains a deoxyribonucleotide triphosphate (dNTP) binding site and helicase motifs in the C-terminal half typical of all TGB1 sequences and an extended N-terminal domain found in hordei-like TGB1s that do not share obvious sequence identities. TGB1 binds RNA and is thought to interact with genomic RNAs to facilitate movement. The sequence of the second TGB protein is the most conserved with BSBV, BVQ, and PMTV sharing 63–75% sequence identity and 49% identity with that of BBNV; there is little sequence identity among the TGB3 sequences. Analysis shows that TGB2 and TGB3 proteins contain two hydrophobic regions (predicted transmembrane domains) separated by a hydrophilic domain and these proteins are associated with intracellular membranes in infected plants.

In PMTV and BBNV, a fourth small ORF is predicted that encodes an 8 kDa cysteine-rich or 6 kDa glycine-rich protein, respectively, of unknown function whereas no such ORF is present in BSBV or BVQ. The 8 kDa cysteine-rich protein of PMTV is not needed for virus movement or infection of *Nicotiana benthamiana* and is not thought to function as silencing suppressor. Although a subgenomic RNA (sgRNA) that could encode the 8 kDa protein was detected in infected *N. benthamiana*, the protein is not readily detectable in extracts of infected leaves.

The CP and RT proteins are encoded on RNA-3 of PMTV (RNA-2 in BSBV, BVQ, and BBNV). The PMTV RNA-3 is of variable size, from 2315 to 3134 nt. Analysis of naturally occurring and glasshouse-propagated isolates revealed that deletions of *c*. 500–1000 nucleotides occur in both field and laboratory isolates and that they occur predominantly in the RT domain, particularly in the region toward the C-terminus. Deletions in this region are correlated with loss of transmission by the natural vector *Spongospora subterranea*. The first PMTV sequence to be published contained a shorter form of the CP–RT-encoding RNA and was designated as RNA-3, whereas in BSBV, BVQ, and BBNV the corresponding RNA is RNA-2. The RT domain of BVQ is shorter than the others and the RT coding sequence is followed by two additional ORFs for proteins of predicted mass of 9 and 18 kDa. Amino acid sequence comparisons reveal conserved motifs between these two proteins and domains in the C-terminal portions of BSBV and PMTV RT proteins which may indicate that they have arisen by degeneration of a larger ORF.

The occurrence of encapsidated deleted forms of RNA-2 and RNA-3 in natural and laboratory isolates of PMTV has been described and sequence analysis suggests that these PMTV RNAs contain sites that are susceptible to recombination possibly through a template switching mechanism. Variable base composition is found in natural isolates of BSBV in the sequence at the 3′ end of RNA-3 between the stop codon of the third TGB and the terminal tRNA-like structure.

Virus–Host Interactions and Movement

Cytoplasmic inclusions of enlarged endoplasmic reticulum (ER) and the accumulation of distorted membranes and small virion bundles can be seen by electron microscope examination of thin sections of BSBV- and BVQ-infected leaves. In PMTV-infected potato leaves, abnormal chloroplasts with cytoplasmic invaginations were seen in thin sections as well as tubular structures in the cytoplasm associated with the ER and tonoplast and in the vacuole.

PMTV does not require CP for movement and it is thought that TGB1 interacts with viral RNA forming a movement competent ribonucleoprotein (RNP) complex. Studies of transiently expressed PMTV TGB proteins fused to marker proteins such as green fluorescent protein or monomeric red fluorescent protein in epidermal cells of *N. benthamiana* have helped to elucidate events in intracellular trafficking and indicate that PMTV interacts with the cellular membrane recycling system. PMTV TGB2 and TGB3 were shown to co-localize on the ER (**Figure 2**) and in small motile granules that utilize the actin–ER network to reach the cell periphery and plasmodesmata (PD) and TGB3 contains a putative tyrosine sorting signal (Y-Q-D-L-N), mutation of which inhibits PD localization. TGB2 and TGB3 act together to transport GFP-TGB1 to the PD for movement into neighboring cells and TGB2 and TGB3 have the capacity to gate the PD pore. TGB2 co-localizes in endocytic vesicles with the Rab 5 ortholog Ara 7 (AtRabF2b) that marks the early endosomal compartment. Also, protein-interaction analysis revealed that recombinant TGB2 interacted with a tobacco protein belonging to the highly conserved RME-8 (receptor mediated endocytosis-8) family of J-domain chaperones, essential for endocytic trafficking.

Host Range, Geographical Distribution, and Transmission by Vector

Pomoviruses have a limited host range and are transmitted in soil by zoosporic plasmodiophorid vectors that have been classified as protists (**Figure 3**). Viruses that are transmitted by plasmodiophorid vectors include pomo-, peclu-, furo-, beny-, and bymoviruses and they are thought to be carried

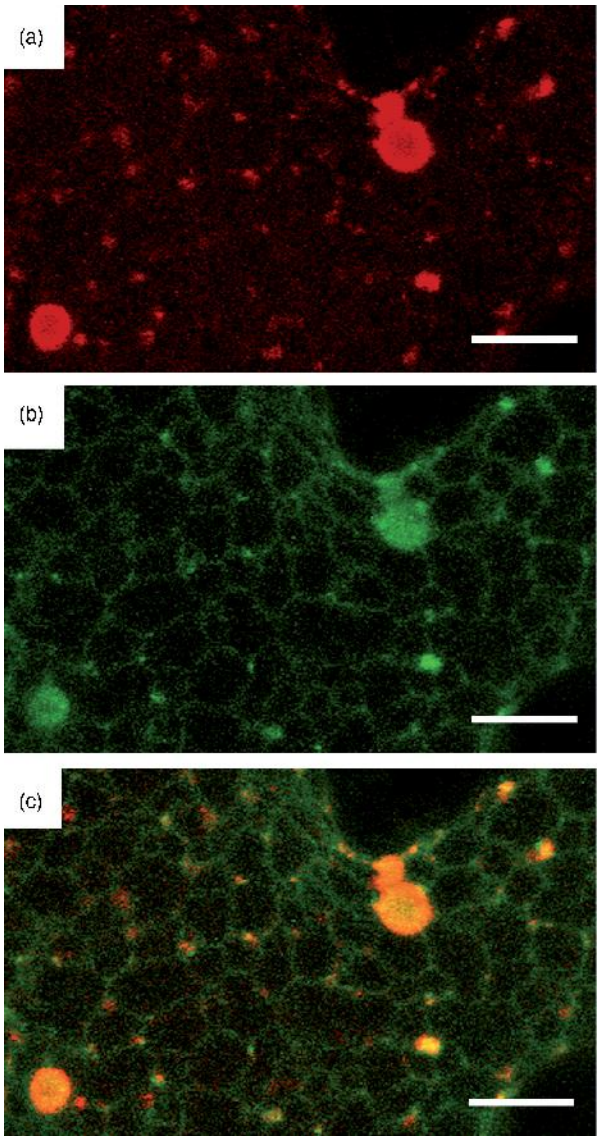


Figure 2 Confocal laser scanning microscope images of PMTV TGB2 and TGB3 fluorescent fusion proteins in epidermal cells of *Nicotiana benthamiana*. Monomeric red fluorescent protein (mRFP) tagged PMTV-TGB2 was transiently expressed with green fluorescent protein (GFP) tagged PMTV-TGB3, the proteins co-localized on membranes of the ER and in motile granules seen moving on the ER network. (a) Expression of mRFP-TGB2 (red channel); (b) expression of GFP-TGB3 (green channel); (c) merged image. Scale = 10 nm.

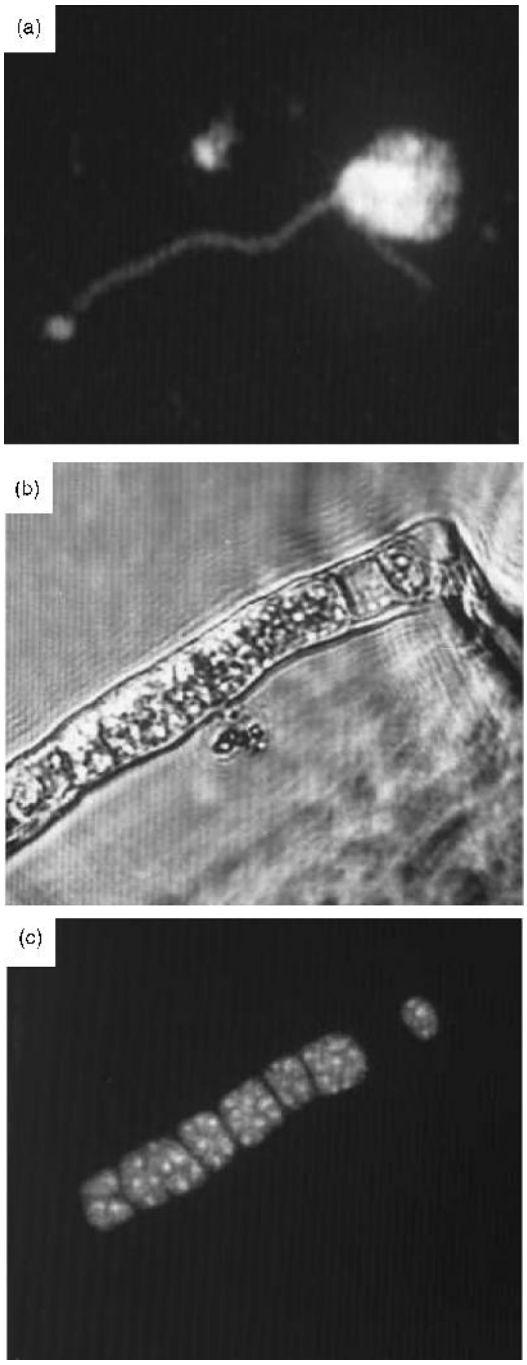


Figure 3 (a) Biflagellate zoopore of *Spongospora subterranea*. (b) Bright field and (c) fluorescence microscope images of zoosporangia in tomato root hair.

within the zoospores. The vector life cycle includes production of environmentally resistant thick-walled resting spores and viruliferous resting spores can survive in soil for many years.

BBNV has been reported only from Japan; it causes necrosis and stunting in broad beans and peas. It is mechanically transmitted by inoculation of sap to a few species including *Vicia faba*, *Pisum sativum*, and *Chenopodium quinoa*.

BVQ and BSBV are often found associated with BNYVV; BVQ is reported only from Europe whereas BSBV is found in sugar-beet-growing areas worldwide. No symptoms have been attributed to BSBV or BVQ alone in sugar beet. The viruses are thought to be transmitted in soil by *Polymyxa betae*. BVQ is mechanically

transmissible only to *C. quinoa* and BSBV to members of the Chenopodiaceae.

PMTV is found in potato-growing regions of Europe, North and South America, and Asia; virus incidence is favored by cool, wet growing conditions. It is transmitted in soil by *S. subterranea,* also a potato pathogen which causes the tuber blemish disease powdery scab. PMTV can be transmitted mechanically to members of the Solanaceae and Chenopodiaceae.

Serological Relationships, Diagnosis, and Control

The viruses are serologically distinct. Distant relationships have been reported between BSBV and BVQ; PMTV and soil-borne wheat mosaic furovirus; and PMTV, BBNV, and tobamoviruses. PMTV, BSBV, and BVQ CPs contain a conserved sequence (SALNVAHQL) that reacts in Western blots with a monoclonal antibody (SCR70) produced against PMTV, but that is not exposed on intact particles. PMTV particles contain an immunodominant epitope at the N-terminus of the CP that is exposed at the surface along the sides of the particles and can be detected by monoclonal antibody SCR69 (**Figure 4**).

The viruses can be detected by serological tests (immunosorbent electron microscopy, enzyme-linked immunosorbent assay) and by assays based on the reverse transcriptase-polymerase chain reaction (RT-PCR) in leaves, roots, or tubers from naturally infected plants. PMTV is known to be erratically distributed in potato leaves and tubers and can move systemically in the absence of CP in potato leaves which raises a risk of false negative diagnosis. In addition, test plants grown in soil that has been previously air-dried can be used as indicators either by observing visual symptoms such as the PMTV-induced necrotic 'thistle-leaf'-shaped line patterns on leaves of *Nicotiana debneyi* or by conducting serological or RT-PCR analysis on the test plant roots or leaves.

PMTV causes an economically important disease affecting the quality of tubers grown for the fresh and processing markets. Tubers of sensitive potato cultivars that are infected from soil during the growing season develop spraing symptoms that include unsightly brown lines, arcs, or marks in the flesh sometimes accompanied by slightly raised external lines and rings (**Figure 5**). Potato plants grown from infected tubers display yellow markings or chevrons on the leaves and may

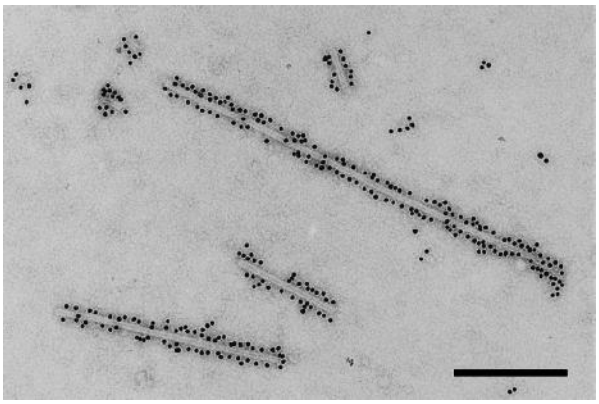


Figure 4 Electron micrograph of PMTV particles labeled with monoclonal antibody SCR69/gold conjugate.



Figure 5 Symptoms of PMTV in potato tubers cv. Nicola.

have shortened internodes (mop-top) producing cracked or malformed tubers; both tuber quality and yield can be affected. However, the virus does not infect all plants grown from infected tubers. Haulm and tuber symptoms vary markedly with cultivar, and some cultivars are symptomlessly infected. Environmental conditions also affect disease incidence and severity, and PMTV incidence was shown to increase with annual rainfall. Soil temperatures of 12–17 °C and high soil moisture at tuber initiation favor powdery scab incidence.

PMTV can be established at new sites by planting infected tubers and once established, PMTV is a persistent problem, as the resting spore balls of the vector *S. subterranea* are long-lived and resistant to drought and agrochemicals. Viruliferous spore balls are spread readily to new sites by farm vehicles, contaminated seed tubers, wind-blown surface soil; motile zoospores can be spread through contaminated irrigation or drainage water. In certain areas, potatoes have become infected by PMTV 18 years after potatoes were last grown (the longest period recorded). There is no effective practicable means to control *S. subterranea* but decreased severity of powdery scab can be achieved by application of fluazinam to soil. In addition, disinfection of tubers with chemicals such as formaldehyde decreases virus incidence, although the efficacy of this treatment depends on the level of soil infestation where the tubers are planted and a combination of tuber and soil treatments may be more effective.

The best prospect for virus disease control is development of resistant cultivars but there are no known sources of PMTV resistance in commercial potato cultivars and most of the commercially grown cultivars are also susceptible to *S. subterranea*. However, plants transformed with virus transgenes (CP and a mutated form of TGB2) have exhibited resistance to PMTV with decreased virus accumulation and incidence in tubers of plants grown in infested soil.

See also: Benyvirus; *Furovirus*; *Hordeivirus*; *Pecluvirus*; *Tobamovirus*.

Further Reading

Arif M, Torrance L, and Reavy B (1995) Acquisition and transmission of potato mop-top furovirus by a culture of *Spongospora subterranea* f. sp. *subterranea* derived from a single cystosorus. *Annals of Applied Biology* 126: 493–503.

Haupt S, Cowan GH, Ziegler A, Roberts AG, Oparka KJ, and Torrance L (2005) Two plant-viral movement proteins traffic in the endocytic recycling pathway. *Plant Cell* 17: 164–181.

Koenig R and Loss S (1997) Beet soil-borne virus RNA1: Genetic analysis enabled by a starting sequence generated with primers to highly conserved helicase-encoding domains. *Journal of General Virology* 78: 3161–3165.

Koenig R, Pleij CWA, Beier C, and Commandeur U (1998) Genome properties of beet virus Q, a new furo-like virus from sugarbeet determined from unpurified virus. *Journal of General Virology* 79: 2027–2036.

Lu X, Yamamoto S, Tanaka M, Hibi T, and Namba S (1998) The genome organization of the broad bean necrosis virus (BBNV). *Archives of Virology* 143: 1335–1348.

Pereira LG, Torrance L, Roberts IM, and Harrison BD (1994) Antigenic structure of the coat protein of potato mop-top furovirus. *Virology* 203: 277–285.

Reavy B, Arif M, Cowan GH, and Torrance L (1998) Association of sequences in the coat protein/read-through domain of potato mop-top virus with transmission by *Spongospora subterranea*. *Journal of General Virology* 79: 2343–2347.

Rochon D'A, Kakani K, Robbins M, and Reade R (2004) Molecular aspects of plant virus transmission by olpidium and plasmodiophorid vectors. *Annual Review of Phytopathology* 42: 211–241.

Sandgren M, Savenkov EI, and Valkonen JPT (2001) The readthrough region of potato mop-top virus (PMTV) coat protein encoding RNA, the second largest RNA of PMTV genome, undergoes structural changes in naturally infected and experimentally inoculated plants. *Archives of Virology* 146: 467–477.

Savenkov EI, Sangren M, and Valkonen JPT (1999) Complete sequence of RNA1 and the presence of tRNA-like structures in all RNAs of potato mop-top virus, genus *Pomovirus*. *Journal of General Virology* 80: 2779–2784.

Zamyatnin AA, Solovyev AG, Savenkov EI, *et al.* (2004) Transient coexpression of individual genes encoded by the triple gene block of potato mop-top virus reveals requirements for TGBp1 trafficking. *Molecular Plant Microbe Interactions* 17: 921–930.

Potato Virus Y

C Kerlan, INRA, UMR1099 BiO3P, Le Rheu, France
B Moury, INRA – Station de Pathologie Végétale, Montfavet, France

© 2008 Elsevier Ltd. All rights reserved.

Brief Description and Significance

Potato virus Y (PVY) was first recognized in 1931 as an aphid-transmitted member within a group of viruses associated with potato degeneration, a disorder known since the eighteenth century. PVY is the type species of the genus *Potyvirus*, one of the six genera in the family *Potyviridae*. PVY is naturally spread by vegetatively propagated material and by aphids in numerous species in a nonpersistent manner. Transmission by contact has also been reported. PVY has a wide host range and is highly variable with some host specificity. Genome sequences and reliable tools for detection and strain differentiation are available. Bioassays and serology have been largely developed.

PVY is one of the most damaging plant pathogens causing significant losses in four main crops around the world: potato, pepper, tomato, and tobacco. In surveys of viruses with worldwide economic importance, PVY was listed in the top-five viruses affecting field-grown vegetables. PVY was also found responsible for damages in petunias in Europe and in eggplant crops in India.

Efficient control strategies depending on the crop have been developed. However none of them seems capable to take into account PVY evolution and to suppress risks of new epidemics.

Viral Particle and Genome

PVY virions are nonenveloped, filamentous, flexuous rods, 730–740 nm long, 11–12 nm in diameter, with an axial canal 2–3 nm in diameter and helical symmetry. Assembly and disruption of PVY particles were studied in detail. Virions contain about 6% nucleic acid and a viral genome linked protein (VPg), but no lipid or other components.

The genome consists of one single-stranded, linear, positive-sense RNA molecule ($3.1–3.2 \times 10^6$ Da) with a polyadenosine sequence at the 3′ terminus. VPg is attached to the 5′ end via a phosphate ester linkage to Tyr^{60}. A three-dimensional model structure of VPg was proposed. Complete genome sequences are available in databases for isolates from potato, tobacco, pepper, tomato, and *Solanum nigrum*. Hundreds of partial sequences of PVY isolates (more than 200 coat protein (CP) sequences) are also available.

PVY RNA is approximately 9700 nt in length excluding the poly(A) tail. As in all members of the genus *Potyvirus*, its single open reading frame is expressed as a large polyprotein (3061–3063 amino acids) autoproteolytically cleaved to yield ten functional proteins: P1 (284 aa), HC-Pro (456 aa), P3 (365 aa), 6K1 (52 aa), CI (634 aa), 6K2 (52 aa), VPg (188 aa), NIaPro (244 aa), NIb (521 aa), and CP (267 aa). There are two distal noncoding regions, 5′NTr (184 nt) and 3′NTr (from 326 to 333 nt) (**Figure 1**).

RNA synthesis is believed to occur in the cytoplasm. The replication complex comprises the proteins NIb, CI and VPg and possibly involves the proteins 6K1 and 6K2. The NIb protein is believed to be the RNA-dependent RNA polymerase.

The CP (29.95 kDa) consists of 267 amino acid residues. It comprises a core (218 aa), highly conserved in members of the genus *Potyvirus*, and two surface-exposed N-terminal (300 aa) and C-terminal (19 aa) regions which are not required for virus assembly and maintenance of infectivity.

The helper component protein (HC-Pro) is supposed to have a biologically active dimeric form (with a subunit molecular mass of 58 kDa). It is indeed capable in the yeast two-hybrid system of self-interaction that can be drastically reduced by mutations in its cysteine-rich region. HC-Pro is involved in PVY accumulation in tobacco and is a suppressor of post-transcriptional gene silencing (PTGS).

Transmission of PVY

PVY is naturally spread by vegetatively propagated plant organs (tubers, cuttings) and by aphids. PVY has also been reported to be spread by plant-to-plant contact,

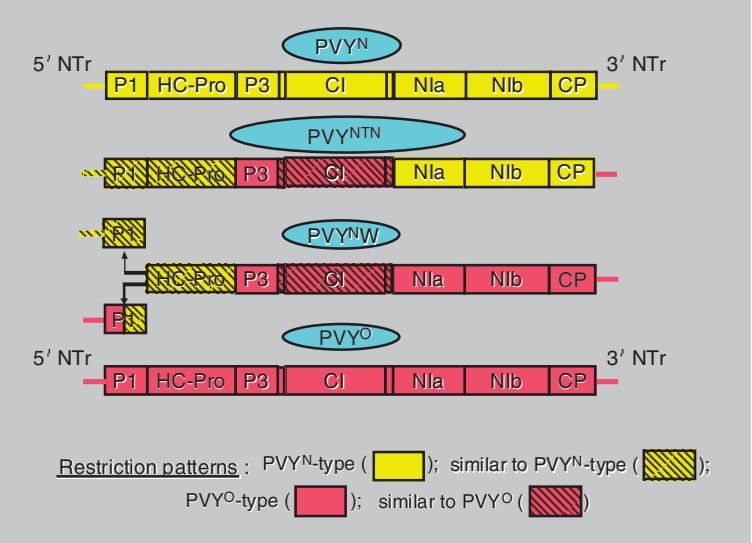


Figure 1 Schematic map of the PVY genome: strains PVY^O and PVY^N, variants PVY^{NTN} and $PVY^{N}W$. Reproduced from Glais L, Tribodet M, and Kerlan C (2002) Genetic variability in Potato Polyvirus Y (PVY): Evidence that PVY^{NW} and $PVYN^{TN}$ variants are single to multiple recombinants between PVY^O and PVY^N isolates. *Archives of Virology* 147: 363–378, with permission from Springer-verlag.

for instance in tobacco and tomato crops in Southern America. It can also be transmitted by contact between sprouts of potato tubers during storage. Transmission by seed has been reported from *S. nigrum* and *Nicandra physaloides*. Transmission by pollen has never been proved for any host plant.

Unlike other potyviruses, PVY displays an unusually large range of aphid vectors. Aphids in 70 species, all in the family Aphidinae, were demonstrated to be able to transmit PVY, most of them with very low efficiencies compared to that of *Myzus persicae*. Apterae and alatae are vectors (**Figure 2**).

PVY is aphid-transmitted in a nonpersistent manner. Acquisition and inoculation periods are brief (a few seconds or minutes). Aphid stylets penetrate into the epidermal cell layer of the plants and puncture plant cell membranes. There is no discernible latent period. PVY does not pass through insect moults and retention of the virus in the aphid usually lasts not more than 1 or 2 h. However longer retention periods (up to 17 h in *Aphis nasturtii*) were reported. Prior starvation of the aphids increases the efficiency of transmission though it does not affect the number of electrically recorded membrane punctures during acquisition periods.

PVY transmissibility is determined by both HC-Pro and CP proteins. All aphid-transmissible PVY isolates contain the 'DAG triplet' (Asp–Ala–Gly) in the CP N-terminal domain. PVY isolates having the sequence DAGE are also aphid-transmissible, unlike those of tobacco vein mottling virus. PVY was the first virus for which it was proved that a virus-induced component of the plant sap is needed for aphid transmission. Effectively, HC-Pro of an efficiently transmitted PVY isolate was shown to mediate the transmission of nonaphid-transmitted PVY isolates and of other potyviruses. Its activity can be blocked by antisera specific to PVY HC-Pro but not by antisera to HC-Pros of other viruses. Monoclonal antibodies (MAbs) to PVY HC-Pro were also produced. Loss of HC-Pro activity correlates with nonretention of virions on aphid stylets. Aphid-transmissible and -nontransmissible isolates differ by one or two amino acid substitutions: Gly^{35} to Asp, Lys^{50} to Glu or Lys^{50} to Asn, Ile^{225} to Val, Ser^{355} to Gly. Lys^{50} is part of a conserved 'KITC motif' (Lys–Ile–Thr–Cys). Changes in or around this motif can result in losses of aphid transmissibility. Conversely the reverse mutation Glu^{50} to Lys restores the helper function of a defective HC-Pro.



Figure 2 Aphid vector of PVY: winged form of *Aphis nasturtii*. Photograph: B. Chaubet, INRA, France.

Host Plants

The natural host range comprises plants in nine families. It includes potato (*S. tuberosum* ssp. *tuberosum*), several species of native potatoes in the Andes namely *S. andigena*, numerous *Solanum* wild species, pepper (*Capsicum* spp.), tobacco (*Nicotiana* spp.), tomato (*Lycopersicon* spp.), eggplant (*S. melongena*), ornementals (*Petunia* spp., *Dahlia* spp.), perennial plants (*Physalis virginiana*, *P. heterophyla*), and a number of annual or perennial self-propagating plants. Many region-specific hosts were recorded such as *Cotula australis* in New Zealand or *Sorbaria tomentosa* in Himalaya.

Weeds in the family Solanaceae are often potential inoculum sources for PVY infections in tomato and pepper crops: *S. nigrum* and *S. dulcamara* in many countries; *S. chacoense* and *P. viscosa* in Southern America; *S. gracile*, *S. aculeatissimum*, *P. angulata*, *P. ciliosa*, and *P. floridana* in Florida. *P. virginiana* and *P. heterophyla* (perennial ground cherries) are overwintering hosts in Northern America. *S. dulcamara* in Western Europe or *Datura* spp. in Mediterranean countries are potential inoculum sources in potato crops as well as volunteer potatoes. Infected pepper and tomato plants, and seed and volunteer potatoes were also identified as potential sources of inoculum in tobacco crops in the USA, Canada, and Italy.

Host specificity clearly stated in the past is currently under reinvestigation. Most PVY isolates appear able to infect common tobacco and tomato cultivars. Conversely most isolates naturally infecting potato are unable to infect pepper cultivars systemically while pepper isolates are usually not detected in potato fields.

PVY is easily sap-transmitted and can be transmitted by stem- and tuber-grafting. Its experimental host range comprises plants in 495 species in 72 genera of 31 families. It includes 287 species in the family Solanaceae (among which 141 *Solanum* species and 70 *Nicotiana* species), 28 species of Amaranthaceae, 25 species of Fabaceae, 20 species of Chenopodiaceae, and 11 species of Asteraceae. A large part of these plants comprises only local lesion hosts. *Datura stramonium*, formerly reported as a host plant, was demonstrated to be totally immune to all strains tested in 1980–90. Some PVY isolates can infect the model plant *Arabidopsis thaliana*.

N. tabacum, *N. benthamiana*, *N. occidentalis* can be used as diagnostic species susceptible to all PVY strains (**Figure 3**). *N. tabacum* plantlets are often used as source and test plants for aphid transmission experiments. *N. tabacum* is a suitable host for virus purification. Yields of purification including a final step of cesium chloride gradient centrifugation usually vary from 10 to 25 mg kg^{-1} of tobacco leaves (**Figure 4**). Leaves of *N. tabacum* are the best virus-infected material to store. Antigenic properties can be retained for 1 year in freeze-dried crude extracts. Infectivity was reported to be preserved in freeze-dried material stored over calcium chloride at 4 °C for 15 years. However from our own experience numerous long-term stored isolates of PVY available in international collections are no longer infectious.

S. demissum Y, *S. demissum* PI 230579, and the hybrid *S. demissum* A6 are local lesion hosts. The 'A6 test' on detached leaves was commonly used in the past as a diagnostic tool for differentiating PVY from potato virus A (PVA), another potyvirus.

Cultivars of *N. tabacum*, potato and pepper, *Chenopodium amaranticolor* (**Figure 5**), *P. floridana*, some accessions of *S. brachycarpum* and *S. sparsipilum* are useful for distinguishing among PVY strains and pathotypes. Potato cultivars such as Bintje or Saco can be used to separate PVY from plants co-infected with PVA or potato virus X (PVX).



Figure 3 Typical mottle induced by a PVY isolate from potato on a leaf of *Nicotiana tabacum* cv *Xanthi* 15 days after mechanical inoculation. Photograph: C. Lacroix, INRA, France.

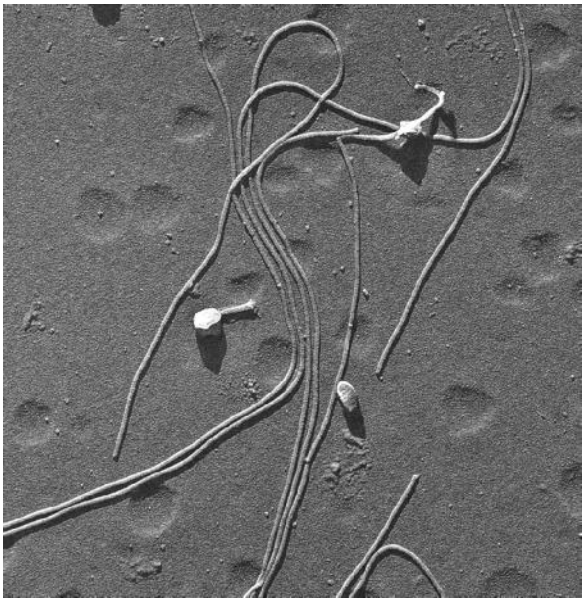


Figure 4 Purified suspension of PVY with a bacteriophage T_4 as internal calibration standard. Magnification 22 000×. Photograph: D. Thomas, CNRS-Rennes 1 University, France.

Serology

PVY is considered to be strongly immunogenic. Antisera with precipitin titers up to 1/4096 and MAbs have been produced in rabbits or mice immunized with purified virus preparations or synthetic peptides, or by using DNA-based immunization, or phage display antibody technology. Numerous sources of antibodies and serological detection kits are available.

ELISA (standard DAS-ELISA and related protocols), dot-blot immunobinding assay, immunosorbent electron microscopy, latex and virobacterial agglutination tests, immunodiffusion in agar gels were intensively studied for use in strain differentiation and virus detection from plant material and from aphids.

Polyclonal antisera do not discriminate among PVY strains. MAbs allow to separate two main serogoups broadly corresponding to potato strains PVY^O and PVY^C from one part and PVY^N from the other part (see below).

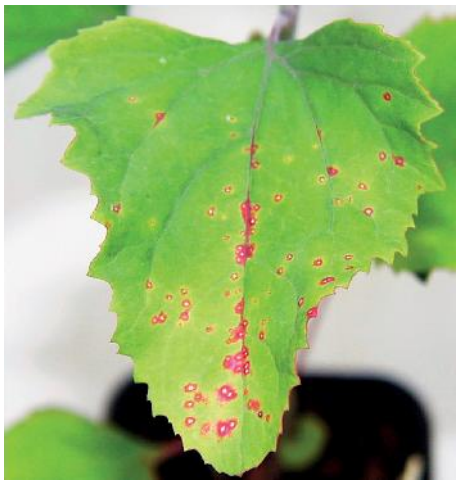


Figure 5 Host plants of PVY: local lesions induced by a PVY isolate from pepper on *Chenopodium amaranticolor* after mechanical inoculation. Photograph. L. Glais, INRA, France.

Specific MAbs to strain PVY^{C} or variants PVY^{NTN} and $PVY^{N}W$ have also been produced, but their reliability still needs confirmation.

Relationships to Other Potyviruses

PVY is distantly serologically related to PVA and potato virus V (PVV), and to 17 other viruses in the genus *Potyvirus* including pepper mottle virus (PepMoV).

PVY, PepMoV, PVV, pepper yellow mosaic virus, pepper severe mosaic virus, wild potato mosaic virus and Peru tomato virus constitute a phylogenetic group distinguishable from other potyviruses including PVA.

Cytopathology

Virions are usually closely associated with inclusions in the cytoplasm of infected cells. They have also been observed within plasmodesmata or aligned on Golgi apparatus, endoplasmic reticulum, and around mitochondria. Most PVY strains induce type-IV inclusions (i.e., pinwheels, scrolls, and short curved laminated aggregates). They consist of a single, nonglycosylated protein (67 kDa), serologically unrelated to the CP protein, or to host proteins. PVY also induces cytoplasmic rod-like amorphous inclusions, but except two isolates from Brazil does not induce large crystalline cytoplasmic and nucleolar inclusions.

Cytopathology in PVY infections has recently been intensively studied in potato and tobacco leaves, notably changes in fresh matter content, photosynthesis, and other metabolic activities. P1 protein was detected in association with cytoplasm inclusions in tobacco cells. Amorphous inclusions appear to be the primary site of HC-Pro accumulation. NIa protein was proved to accumulate in both cytoplasm and nucleus. PVY CP, HC-Pro, and RNA were found within chloroplasts of tobacco leaves suggesting they may alter the chloroplastic function as also proved in transgenic tobacco plants expressing PVY CP.

PVY in Potato

PVY has become in the last decade the most important virus in most growing areas for seed, ware, and processed potatoes. Serious outbreaks were, reported in the 2000s. Tuber quality can be severely affected due to necrosis or defects for processing potatoes. Reduction in size and number of harvested tubers can result in losses up to 80%.

Symptoms consist of mild to severe mottle, often associated with crinkling of the leaves. Yellowing and necrosis (vein necrosis and necrotic spots) frequently occur in the lower leaves. Symptoms also include collapse and dropping of intermediate leaves, which remain clinging to the stem (leaf drop). Secondarily infected plants (when mother tubers are infected) are stunted with crinkled and smaller leaflets (**Figure 6**). Necrosis on and around leaf veins, on petioles, stems, and tubers may occur in numerous cultivars. Some necroses on stems and petioles are called stipple-streak disease. The potato tuber necrotic ringspot disease (PTNRD) is characterized by particular patterns on leaves (sometimes oak-leaf necrosis) (**Figures 7(a)** and **7(b)** and stems, and superficial annular and arched necroses on tubers, first slightly protruding from tuber skin, then becoming dark brown with sometimes crackings of tuber skin (**Figures 7(c)** and **7(d)**).

Potato isolates have historically been divided into three main strain: PVY^{O}, PVY^{N}, and PVY^{C}. PVY^{O} and PVY^{C} are separated on the basis of hypersensitive reactions in potato cultivars bearing the resistance genes Ny_{tbr} and N_c (**Figure 8**). PVY^{N} differs from PVY^{O} and PVY^{C} in causing a veinal necrosis reaction in *N. tabacum* cv *Samsun* or cv *Xanthi* (**Figures 9(a)** and **9(b)**), but does not elicit any hypersensitive response in most potato cultivars. Two amino acids at positions 400 and 419 in the HC-Pro protein are involved in the induction of this necrotic reaction in tobacco. *P. floridana* is a third host for strain differentiation. PVY^{C} induces collapse and premature death of this plant. PVY^{C} was first characterized as non-aphid-transmissible, but many isolates of this strain were proved to be readily aphid-transmissible. Two former PVY^{C} isolates were later recognized as isolates of PVV.

Many unclassified variant isolates were reported in the past, notably isolates classified in a group called PVY^{An}. More recently PVY^{Z} and PVY^{E} were characterized as overcoming the resistance genes Ny_{tbr} and N_c. Two main variants have emerged for two decades: PVY^{NTN}, characterized by its ability to induce tuber necrosis, and $PVY^{N}W$ which differs by its pathogenicity and its serotype O–C instead of serotype N. $PVY^{N:O}$ isolates



Figure 6 PVY on potato. Natural secondary infection by PVY in a potato field: crinkling, yellowing, and growth reduction of the leaflets. Photograph: V. Le Hingrat, FNPPPT, France.

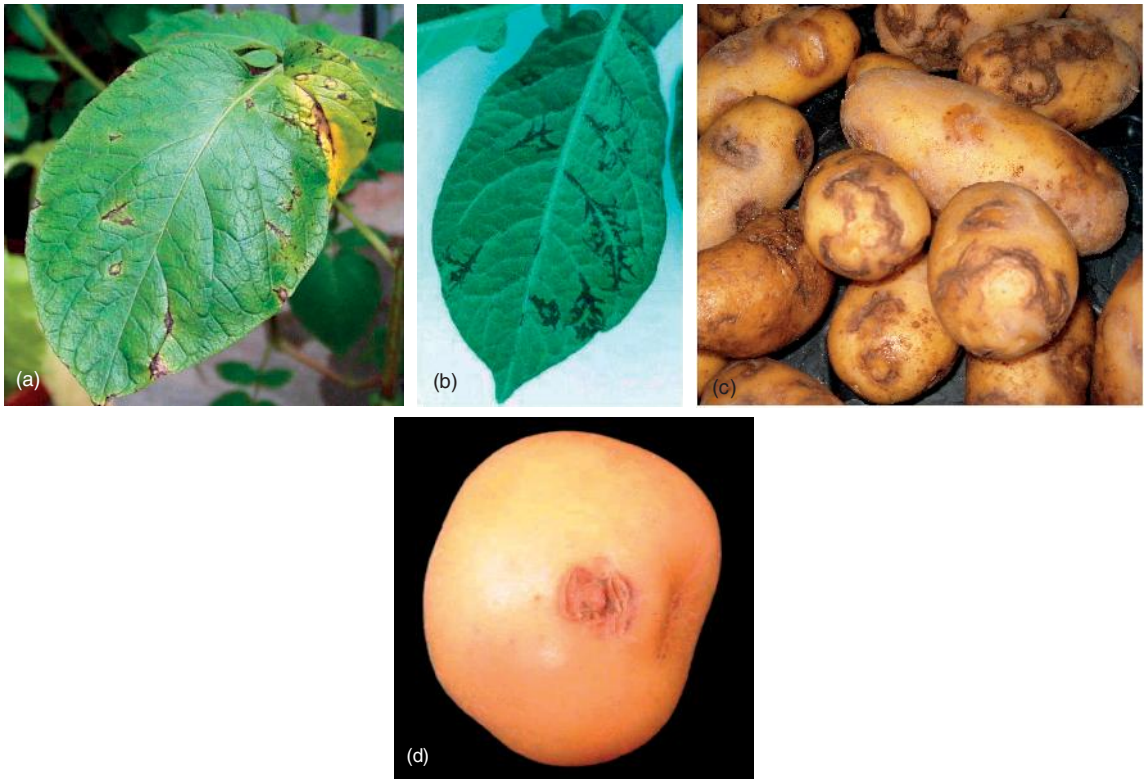


Figure 7 PVY on potato. Symptoms associated with the potato tuber necrotic ringspot disease (PTNRD). (a) Yellowing and necroses on a basal leaf. (b) Necrotic oak-leaf pattern on an intermediate leaf. (c) Typical PTNRD symptoms on tubers of the cv *Monalisa*. (d) Atypical PTNRD induced by a $PVY^{N:O}$ isolate on a tuber of the cv *Yukon Gold*. Photographs: K. Charlet-Ramage, GNIS-INRA, France (a–c) and R. P. Singh Potato Research Centre, NB, Canada (d).

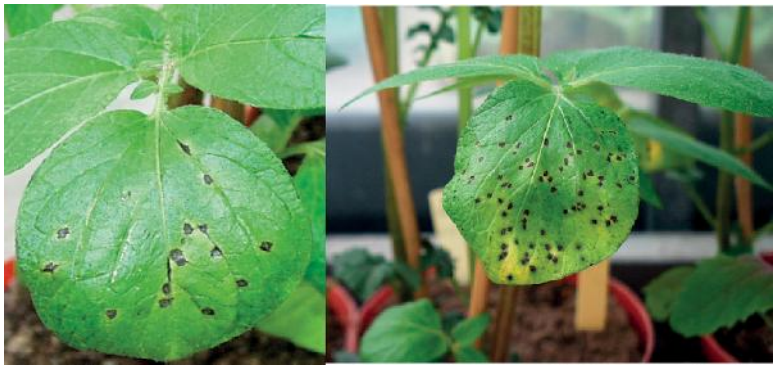


Figure 8 Hypersensitive reactions on potato cultivars 10 days after mechanical inoculation on cv *Desiree* containing the gene Ny_{tbr} inoculated by a PVY^{O} isolate (left) and on cv *Eersteling* containing the gene *Nc* inoculated by a PVY^{C} isolate (right). Photographs: C. Kerlan and J. P. Cohan, INRA, France.

described in the 2000s also share properties with both PVY^{N} and PVY^{O} and some of them induce tuber necrosis. PVY^{O} and PVY^{N} strains are distributed worldwide though PVY^{N} is yet a quarantine pathogen in Canada and the USA. The PVY^{C} strain is less frequent. The PVY^{NTN} variant has been identified in most potato-growing countries including the USA and Peru. The PVY^{N}-W variant has become prevalent in Poland and has been reported in several countries. $PVY^{N:O}$ was reported in Canada and the USA.

PVY potato isolates have been divided into three genetically distinct strains designated PVY^{O}, PVY^{N}, and PVY^{C1}. PVY^{C1} is a part of the genetically defined strain PVY^{C} or PVY^{NP} (nonpotato) which also includes isolates from pepper, tomato, and tobacco. $PVY^{N}W$, $PVY^{N:O}$, and the majority of PVY^{NTN} isolates are recombinants between



Figure 9 PVY indexing on *Nicotiana tabacum* cv *Xanthi* (15 days after mechanical inoculation). (a) Typical vein necrosis and leaf distortion induced by a PVY^N isolate. (b) Typical vein clearing without any leaf distortion induced by a PVYO isolate. Photographs: C. Lacroix, INRA, France.

PVY^N and PVY^O strains, with one to three recombination breakpoints (**Figure 1**). Several tuber necrosis-inducing isolates possess a PVY^N-type genome without any recombination breakpoint. Lastly, North American and European PVY^{NTN} isolates are separated on the basis of polymorphism in the 5′ NTr-P1 region.

Differences in aphid transmission between strains were reported. PVY^N isolates seem better transmitted than other PVY isolates with longer retention periods in aphids. Whatever the strain, *M. persicae* is clearly the most important vector in most potato-growing areas. However despite their low transmission efficiency, other species colonizing potatoes such as *A. nasturtii* (**Figure 2**), or visiting potatoes can also contribute to PVY spread. Some of these species, notably cereal aphids or the pea aphid, were thought to be involved in PVY epidemics in potato crops (in Europe and the USA) due to their high abundance.

Control methods are first based on control of seed potatoes, breeding for resistance and quarantine regulations, especially regarding PVY^N. Sophisticated schemes of seed production include monitoring of aphid vectors, treatments by mineral oils against aphid transmission, eradication of weeds, and post-harvest detection tests using large-scale ELISA. Numerous molecular assays were also developed for PVY detection in leaves, potato tubers, and aphids, including cDNA hybridization, various RT-PCR protocols, and microarray technology. Breeding for potato resistance to PVY takes into account resistance to infection (which includes resistance to virus and to vectors, and is largely used), hypersensitivity resistance (HR) and extreme resistance (ER). HR and ER are based on single dominant genes *Ny* and *Ry*, respectively. *Ry* genes (Ry_{sto}, Ry_{adg}), from *Solanum* wild species, map on chromosomes XI and XII, and confer broad-spectrum resistance, whereas HR genes *Nc*, Ny_{tbr} (which map on chromosome IV) (**Figure 10**) and *Nz* found in old potato cultivars, protect against PVY^C, PVY^O, and PVY^Z, respectively. The NIaPro protein of PVY is involved in the elicitation of *Ry*. The gene *Y-1* involved in the HR reaction has been cloned. More than 20 cultivars containing *Ry* have been produced and this resistance has proved to be quite durable. Marker-assisted selection for resistance to PVY has been developed for genes Ry_{adg}, Ry_{chc}, and Ny_{tbr}. Genetically engineered resistance has been intensively studied and used to generate resistant transgenic plants from commercial potato cultivars. CP-mediated resistance was described as ER with often a broad spectrum.

PVY in Pepper

PVY is the causal agent of major diseases and production losses in pepper crops. In some situations it can affect 100% of the plants and can be the most important disease. PVY affects pepper production worldwide, while other pepper-infecting potyviruses are mostly restricted to particular continents and are not present in Europe. Symptoms are mostly visible on the vegetative parts of the plants but rarely on fruits. Depending on the pepper genotype and on the virus isolate, they consist of mosaic and vein banding on leaves or on necrotic symptoms on leaves, petioles, and stems (**Figure 11**).

Pepper isolates of PVY belong to the same group as the C phylogenetic group of PVY in potato, although they could constitute a separate C subgroup. One recently described exception consists of a O:C recombinant strain inducing veinal necrosis in pepper in Italy. No PVY isolates from the N or O phylogenetic groups have been described as epidemic in pepper. Pepper PVY isolates have been classified according to their pathotype relative to the numerous resistance genes and alleles used to control PVY in pepper crops (see further).

The most widespread and efficient way to control PVY in pepper is through the growing of cultivars carrying resistance genes. The first resistance genes used to control PVY were the $pvr2^1$ and $pvr2^2$ alleles, which control

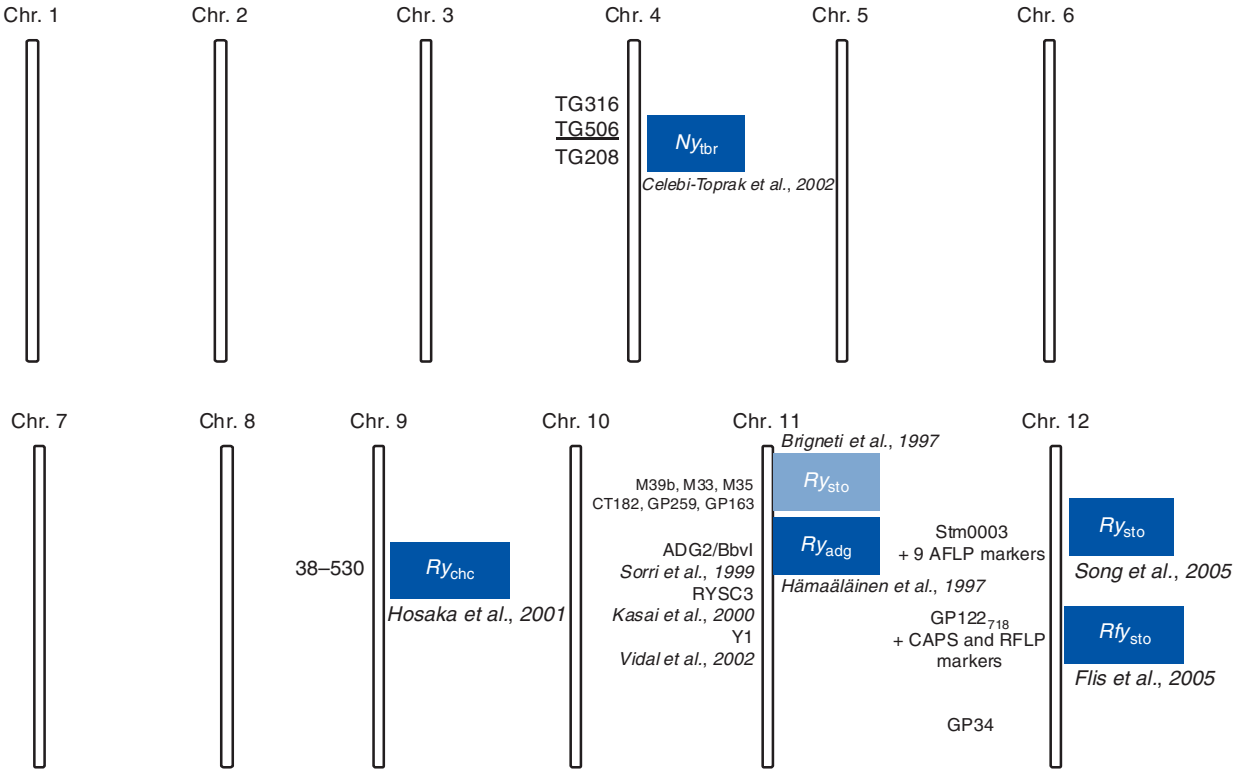


Figure 10 Genetic localization of PVY resistance genes on the potato genome. From S. Marchadour, FNPPPT-INRA, France (unpublished).

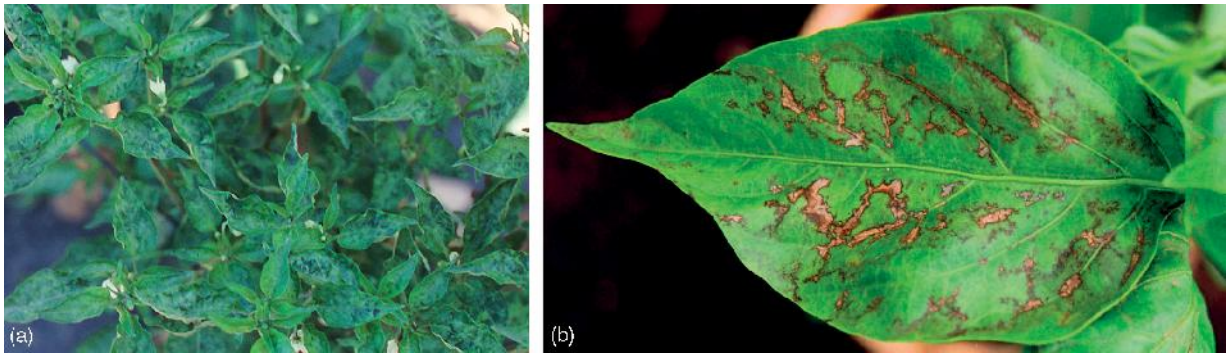


Figure 11 PVY infection in a pepper field: (a) severe crinkling and distortion of the leaves. (b) Necrotic patterns on a leaf. Photographs: P. Gognalons, INRA, France (a) and M. Pepelnjak Slovenia (b).

high-level resistance since no virus can be detected in inoculated organs of the plants. These genes have been exploited for decades in a large number of pepper cultivars and proved to be durable. However, PVY isolates virulent to the $pvr2^1$ allele were occasionally observed, especially in the Mediterranean basin and in tropical regions, while isolates virulent to the $pvr2^2$ allele are exceptional.

The three alleles $pvr2^+$ (susceptibility reference allele), $pvr2^1$ and $pvr2^2$ were used to classify the pepper isolates in three pathotypes: pathotype (0) isolates which can infect only plants with the $pvr2^+$ allele, pathotype (0,1) isolates which only infect plants carrying the $pvr2^+$ or $pvr2^1$ alleles and pathotype (0,1,2) isolates which are virulent toward all three *pvr2* alleles.

It was shown that these alleles correspond to various copies of an isoform of the eukaryotic translation initiation factor 4E (eIF4E), and that amino acid substitutions in the central part of the viral protein genome-linked (VPg) of PVY determined virulence to both $pvr2^1$ and $pvr2^2$.

More recently, the dominant *Pvr4* gene, originating from a *C. annuum* Mexican population, was introgressed into bell-pepper cultivars. This gene present in about 40% of European cultivars confers a high-level resistance to all tested isolates of PVY. This resistance shares

common properties with hypersensitivity-based resistances. No isolate of PVY virulent to *Pvr4* has been described so far, indicating a high durability of the resistance.

Other pepper resistances to PVY have been described (notably polygenic resistances from *C. annuum* and *Pvr7* resistance from *C. chinense*).

Other control measures such as the use of oil sprays or physical barriers (polyethylene sheets and coarse nets) were also used for controlling spread of PVY in pepper crops in Israel and Florida.

PVY in Tomato

PVY can induce severe diseases in tomato crops but has long been considered a pathogen of secondary importance, inducing mild mosaic on the foliage only. Since the 1980s, new strains of PVY have arisen in Mediterranean countries, causing serious yield and quality loss of tomato fruits. These strains induce necrotic lesions on leaves and necrotic streaks on stems in all tomato varieties and frequently affected 100% of the plants, in greenhouses as well as in open fields. Importantly, combination of PVY infections with other viruses such as cucumber mosaic virus can induce very severe diseases in tomato crops.

Data about the genetic diversity of PVY isolates from tomato are scarce. Isolates belonging to the C/pepper group of strains and to the recombinant NTN group have been observed in tomato crops. In laboratory tests, most of the isolates belonging to all phylogenetic groups of PVY induce systemic infections in tomato.

There are few reports about PVY resistance in tomato and related species. Accession PI247087 of *L. hirsutum* was described highly resistant to PVY. The resistance is efficient toward all tested tomato or pepper isolates of PVY, inhibits the multiplication of the virus in the inoculated organs and is controlled by a single recessive gene (named *pot-1*) which maps to a region on chromosome 3 syntenic to the *pvr2* locus in pepper. *Pot-1* was shown to belong to the same family of genes as *pvr2*. Strains of PVY virulent to the *pot-1* resistance can be selected during laboratory tests, a property that is controlled by a single amino acid substitution in the VPg of the virus. The *pot-1* gene was introduced into genotypes of *L. esculentum*, but varieties carrying this gene are not widespread.

PVY in Tobacco

PVY was reported to be the most damaging virus in tobacco crops. It causes height reductions and modifies the chemical composition of cured leaves, especially the nicotine content. Yield losses of 14–59% were reported with incidence of up to 100%. Symptoms on leaves are usually mild mottling but particular chlorotic patterns and necroses, notably the veinal necrosis disease, may also occur (**Figures 12(a)** and **12(b)**).

Three strain groups, M^SM^R, M^SN^R, N^SN^R, have been identified according to their reaction in tobacco cultivars resistant or susceptible to the root-knot nematode (*Meloidogyne incognita*). The NIb protein was found to elicit the hypersensitive response in these resistant tobacco cultivars. VAM-B refers to a resistance-breaking group of isolates overcoming the gene *va* originally found in the genotype Virginia A mutant and characterized by deletions at the Va locus. The VPg protein was suggested to be involved in overcoming resistance conferred by *va*.

Breeding for resistance is the main control method against PVY in tobacco production. Many European and American commercial cultivars display noteworthy levels of recessive resistance. The gene *va* has been extensively



Figure 12 PVY infection on tobacco cv *Burley* in field trials in Southwestern France. (a) Chlorotic oak-leaf and halotic patterns. (b) Severe systemic necrosis. Photograph: D. Blancard, INRA, France.

utilized, though its introgression is often associated with decrease of yield and cured leaf quality. In countries where early infection frequently occurs, additional measures consist of protecting seedbeds by fleece, eradication of weeds, and isolation from potato, tomato and pepper fields. Pathogen-derived resistance has been extensively studied in tobacco, although more for investigating the mechanisms of protection than in order to use this strategy in commercial tobacco cultivars.

Prospects

Strains

Current distinctions within the species *Potato virus Y* is not totally clear. The exact relationship between the necrotic strains from potato and from tobacco (PVY^{N}, $N^{S}N^{R}$, $M^{S}N^{R}$) still must be defined precisely, as the distinction between PVY^{NTN} and other isolates of the PVY^{N} strain. Is there any PVY^{N} isolate unable to induce potato tuber necrosis whatever the conditions? Are $PVY^{N:O}$ isolates different to those previously referred as $PVY^{N}W$ isolates? Molecular classification in most cases correlates only partially with biological classifications since they are based on neutral markers.

Evolution

The large variability and genomic diversity of PVY makes its evolutionary story fascinating. Links between mutation or recombination events and evolution of PVY are thoroughly studied. Current knowledge indicates that PVY was present in pre-Columbian America and might have followed different evolutionary pathways as a result of co-evolution with various solanaceous hosts.

*See also: Vector Transmission of Plant Viruses*Vector Transmission of Plant Viruses; Plant Resistance to Viruses: Engineered Resistance; Plant Virus Diseases: Economic Aspects; Polyomaviruses of Mice; Potato Viruses; Tobacco Viruses.

Further Reading

Blancard D (1998) *Maladies du Tabac.* Paris: INRA.
Blancard D (1998) *Maladies de la Tomate.* Paris: INRA.
de Bokx JA and van der Want JPH (eds.) (1987) *Viruses of Potato and Seed-Potato Production,* Wageningen, The Netherlands: Pudoc.
Edwardson JR and Christie RG (1997) Potyviruses. In: *Florida Agricultural Experiment Station Monograph Series 18-II – Viruses Infecting Pepper and Other Solanaceous Crops*, pp. 424–524. Gainesville, FL: University of Florida.
Flis B, Hennig J, Strelczyk-Zyta D, Gebhardt C, and Marczewski W (2005) The *Ry-f_{sto}* gene from *Solanum stoloniferum* for extreme resistance to potato virus Y maps to chromosome XII and is diagnosed by PCR marker $GP122_{718}$ in PVY resistant potato cultivars. *Molecular Breeding* 15: 95–100.
Glais L, Kerlan C, and Robaglia C (2002) Variability and evolution of *Potato virus Y* (PVY), the type-member of the *Potyvirus* genus. In: Khan JA and Dijkstra J (eds.) *Plant Viruses as Molecular Pathogens*, pp. 225–253. Binghamton, NY: The Haworth Press.
Kasai K, Morikawa Y, Sorri VA, Valkonen JPT, Gebhardt C, and Watanabe KN (2000) Development of SCAR markers to the PVY resistance gene *Ry_{adg}* based on a common feature of plant disease resistance genes. *Genome* 43: 1–8.
Loebenstein G, Berger P, Brunt AA, and Lawson RG (eds.) (2001) *Virus and Virus-Like Diseases of Potatoes and Production of Seed-Potatoes.* Dordrecht, The Netherlands: Kluwer Academic Publishers.
Milne RG (1988) The economic impact of filamentous viruses. In: Milne RG (ed.) *The Plant Viruses, Series 4: The Filamentous Plant Viruses*, pp. 331–407. New York: Plenum.
Moury B, Morel C, Johansen E, and Jacquemond M (2002) Evidence for diversifying selection in potato virus Y and in the coat protein of other potyviruses. *Journal of General Virology* 83: 2563–2573.
Moury B, Morel C, Johansen E, *et al.* (2004) Mutations in *Potato virus Y* genome-linked protein determine virulence toward recessive resistances in *Capsicum annuum* and *Lycopersicon hirsutum*. *Molecular Plant–Microbe Interactions* 17: 322–329.
Ruffel S, Dussault M-H, Palloix A, *et al.* (2002) A natural recessive resistance gene against potato virus Y in pepper corresponds to the eukaryotic initiation factor 4 E (eIF4E). *Plant Journal* 32: 1067–1075.
Ruffel S, Gallois J-L, Lesage M-L, and Caranta C (2005) The recessive potyvirus resistance gene *pot-1* is the tomato orthologue of the pepper *pvr2*-eIF4E gene. *Molecular Genetics and Genomics* 274(4): 346–353.
Shukla DD, Ward CW, and Brunt AA (1994) *The Potyviridae.* Wallingford, UK: CAB International.
Tribodet M, Glais L, Kerlan C, and Jacquot E (2005) Characterization of *Potato virus Y* (PVY) molecular determinants involved in the vein necrosis symptom induced by PVY^{N} isolates in infected *Nicotiana tabacum* cv. Xanthi. *Journal of General Virology* 86: 2101–2105.

Potato Viruses

C Kerlan, Institut National de la Recherche Agronomique (INRA), Le Rheu, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Primary infection (primarily infected plants) Potato plants become infected during the growing season.
Secondary infection Infection results from an infected mother tuber.

Introduction

At least 38 potato viruses have been described, several of them being classified by ICTV as tentative species or possible strains of one species. They can be divided into three groups according to their importance and their distribution in potato-growing areas in the world. They

include well-known viruses, and recently discovered, often poorly characterized viruses. Eight viruses are major pathogens causing severe damages in potato crops (**Table 1**). Many potato viruses occur only in Latin America. None of these viruses is economically important; some of them have been found only once in one cultivar (**Table 2**). The third group comprises viruses that occur in other parts of the world and are either of only local importance or without any significance (**Table 3**).

Potato Viruses: One Century

Viral diseases are thought to have become a threat to potato soon after its introduction in Europe. The phenomenon called potato degeneration was described in the eighteenth century. The leafroll disease was mentioned in the 1750s and the causal agent described in 1916 was one of the first viruses identified. The main species involved in the mosaic complex were identified in 1920s and 1930s, that is, potato virus M, potato virus X, potato virus Y, and potato virus A. Other important potato viruses were only discovered in the 1950s (tobacco rattle virus, potato virus S) or mid-1960s (potato mop-top virus). In the last four decades, an ever-increasing number of viruses were found infecting potato in Latin America, especially in Andean valleys where the potato originates from. Lastly, numerous viruses or strains more common in other host plants were found to infect potato occasionally. Some of them, for instance tomato spotted wilt virus, cause emerging diseases and could become important in the future. We may also point out that a contact-transmitted virus such as pepino mosaic virus, never detected in potato fields so far, was shown to be capable of infecting many important potato cultivars and hence could become a threat in the future.

Since the turn of the twentieth century, potato producers in many countries have recognized that certain potato-growing areas were better than others for the production of seed potatoes. Seed-potato production programs were developed, based during 1940–50 on clonal selection, then on basic seed production involving virus eradication by thermotherapy and meristem culture, and later on specific

Table 1 Major potato viruses

Species *Acronym* *First mention*	*Genus*	*Economical importance*[a]	*Geographical distribution*[a]	*Spread*[a]	*Natural host range* *Other main hosts*	*Variability*[a]
Potato virus Y PVY 1931	*Potyvirus*	Very high	Worldwide	Aphids NP[c], contact?	Wide Tobacco, tomato, pepper	Strains Y^O, Y^C, Y^N Variants Y^{NTN}, Y^NW, $Y^{N:O}$ Serotypes PVY^{O-C}, PVY^N
Potato leafroll virus PLRV 1916	*Polerovirus*	Very high	Worldwide	Aphids P[c]	Narrow *Solanum* spp., tomato, ulluco	Tomato yellow top and *Solanum* yellows strain
Potato virus X PVX 1931	*Potexvirus*	High	Worldwide	Contact	Narrow Tomato, pepper	Strain groups 1, 2, 3, 4 Strains HB, CP Serotypes PVX^O, PVX^A
Potato virus A PVA 1932	*Potyvirus*	Moderate	Worldwide[b]	Aphids NP[c]	Only potato	Four strain groups Three serotypes
Potato virus S PVS 1952	*Carlavirus*	Moderate	Worldwide	Aphids NP[c], contact	Narrow Pepino	Strains PVS^O, PVS^A
Potato virus M PVM 1923	*Carlavirus*	Moderate	Worldwide[b]	Aphids NP[c], contact	Narrow Mainly in Solanaceae	Strain PVM-ID
Tobacco rattle virus TRV 1946	*Tobravirus*	High	Worldwide	Nematodes	Weeds, flower bulbs, beet, tobacco, lettuce, spinach, etc.	Many strains
Potato mop-top virus PMTV 1966	*Pomovirus*	Rather high	Mainly in cooler climates	Fungus	Only potato	PMTV-S, PMTV-T

[a]In potato.
[b]Not found in Andean countries.
[c]Viruses are transmitted by aphids in a nonpersistent (NP), persistent (P) manner.
Species in bold italic letters: the virus (at least one strain) can induce tuber necrosis.

Table 2 Potato viruses only found in Latin America

Species *Acronym* *First report*	*Genus*	*Economical importance*[a]	*Geographical distribution*[a]	*Spread*[a]	*Natural host range* *Other main hosts*	*Variability*[a]
Andean potato latent virus APLV 1966	*Tymovirus*	Very low	Bolivia, Colombia, Ecuador, Peru	Contact, flea beetle, TPS[c]	Only potato and ulluco	Serotypes Hu, CCC, Col-Caj
Andean potato mottle virus APMoV 1977	*Comovirus*	Unknown	Chili, Ecuador, Peru, Brazil	Contact, flea beetle?	Only potato	Strains B, C, H
Arracacha virus B-oca strain AVB-O 1981	*Nepovirus*?	Unknown	Bolivia, Peru	TPS	Only potato and oca	Oca and potato strains
Potato deforming mosaic virus PDMV 1985	*Begomovirus*?	High in one cultivar	Southern Brazil	Whitefly	potato, *S. chacoense*, *S. sisymbrifolium*	Not reported
Potato rough dwarf virus PRDV 1995	*Carlavirus*	Very low	Argentina, Uruguay	Unknown	Only potato	Strain of PVP
Potato virus P PVP 1993	*Carlavirus*	Locally in two cultivars	Brazil	Aphids	Only potato	PRDV strain
Potato virus T PVT 1977	*Trichovirus*	Unknown	Bolivia, Peru	Contact, TPS	Mashua, oca, potato, ulluco	Not reported
Potato virus U PVU 1983	*Nepovirus*	None	Locally in Peru (Comas Valley)	Nematode	Unknown	None

Potato yellow mosaic virus PYMV 1986	*Begomovirus*	Unknown	Venezuela	Whitefly	Narrow Tomato	Not reported
Potato yellow vein virus PYVV 1954	*Crinivirus*	Locally high	Colombia, Ecuador, Peru	Whitefly	Potato and several weeds	Not reported
Potato yellowing virus PYV 1991?	*Alfamovirus*?	Unknown	Peru, Chili, Bolivia	Aphid SP[b], TPS	Only potato	Not reported
Potato 14R virus P14R 1996	*Tobamovirus*?	None	Peru	Unknown	Only potato	None
Solanum apical leaf curl virus SALCV 1983	*Begomovirus*?	Very low	Peru	Unknown	Narrow *Physalis peruviana*, *Solanum nigrum*	Not reported
Sowbane mosaic virus SoMV 1997	*Sobemovirus*	Isolated once from a Mexican line	Unknown (Mexico?)	Unknown	Narrow *Chenopodium* spp.	None
Tobacco ringspot virus TRSV 1977	*Nepovirus*	Low	Peru	Nematode? TPS	Potato, arracacha, oca	TRSV-Ca & PBRSV
Wild potato mosaic virus WPMV 1979	*Potyvirus*	None	Peru	Aphid NP[b]	Narrow Pepino, tomato, wild potato	Not reported

[a]In potato.
[b]Viruses are transmitted by aphids in a nonpersistent (NP), persistent (P), and propagative (Pr) manner.
[c]TPS means true potato seeds.

Table 3 Viruses of limited significance to potato production

Species *Acronym* *First report*	*Genus*	*Economical importance*[a]	*Geographical distribution*[a]	*Spread*[a]	*Natural host range* *Other main hosts*	*Variability*[a]
Alfalfa mosaic virus AMV 1940	*Alfamovirus*	Only locally	Uncommon	Aphids NP[b], pollen, TPS[c]	Wide Clover, tobacco, tomato, pepper	Calico and tuber necrosing strains
Beet curly top virus BCTV 1954	*Curtovirus*	Only locally	Arid and semiarid regions	Leafhoppers	Bean, beet, spinach, tomato, pepper, many cucurbits	Not reported
Cucumber mosaic virus CMV 1958	*Cucumovirus*	Very low	UK, Egypt, India, Saudi Arabia	Aphids NP	Many cucurbits, pepper, tomato, etc.	Serotypes I, II
Eggplant mottled dwarf virus EMDV 1987	*Nucleo-rhabdovirus*	Very low	Iran	Aphids? contact?	Narrow Eggplant, tomato	Not reported
Potato aucuba mosaic virus PAMV 1961	*Potexvirus*	Low	Uncommon	Aphids NPH[b], contact	Narrow Clover, tomato, pepper, tobacco	Mild and severe strains
Potato latent virus PotLV 1996	*Carlavirus*	Unknown	North America	Aphid, whitefly?	Only potato	Not reported
Potato virus V PVV 1986	*Potyvirus*	Low	Bolivia, Peru, North Europe	Aphids NP	Narrow Tomato	Isolates GI, AB, UF

Potato yellow dwarf virus PYDV 1 937	*Nucleo-rhabdovirus*	None	Canada, North USA, Saudi Arabia, South Russia?, Ukraine?	Leafhoppers P[b], Pr	Narrow Ox-eye daisy, periwinkle, clover	Two strains
Tobacco mosaic virus TMV 1977	*Tobamovirus*	None or low	China, India, Saudi Arabia, Andean countries	Contact	Wide Many crops and woody plants	Not reported
Tobacco necrosis virus TNV 1935	*Necrovirus*	Low	Europe, North America	Fungus	Bean, tulip, tobacco, cucumber, pea, lettuce	Strain D
Tobacco streak virus TSV 1981	*Ilarvirus*	Low	Brazil, Peru	Thrips?, TPS? pollen?	Wide	Not reported
Tomato black ring virus TBRV 1950	*Nepovirus*	Rather low	Europe	Nematodes, seeds, TPS	Very wide Grape, fruit trees, vegetables, weeds...	Potato bouquet and pseudo-aucuba strains, two serogroups,
Tomato mosaic virus TMV 1977	*Tobamovirus*	None	Hungary	Contact	Rather wide Pepper, tomato	TMV potato strain
Tomato spotted wilt virus TSWV 1938	*Tospovirus*	Locally high	South America, Australia, India, Europe, South Africa	Thrips	Very wide Tomato, pepper, pea, groundnut, soyabean, tobacco, etc.	Not clearly described

[a]In potato.
[b]Viruses are transmitted by aphids in a nonpersistent (NP), persistent (P), and propagative (Pr) manner; NPH means that aphid transmission requires a helper virus.
[c]TPS means true potato seeds.
Species in bold italic letters: the virus (at least one strain) can induce tuber necrosis.

multiplication systems such as propagation *in vitro* or by stem cuttings or from true potato seeds (TPSs). Planting of certified seed potatoes with low levels of virus infections has now become the main element in the fight against viral diseases. Breeding for virus resistance has been intensively used at least since 1925 for PLRV, and sources of resistance in many wild *Solanum* spp. have been exploited. Three types of resistance were described in the 1950s and reliable knowledge on their genetic background and inheritance was available in the 1970s. Now many resistance genes have been mapped in the potato genome, which has permitted breeding based on molecular markers. Lastly a remarkable catalog of drastic measures has been established in order to avoid the introduction of quarantine viruses. Technical guidelines for safe movement and utilization of potato germplasm have recently been published under authority of relevant international specialized institutions.

Main Traits of the Viral Diseases in Potatoes

Symptomatology and Damages

Secondary infection symptoms are often more severe than those of primary infections, resulting in emergence delays and growth reductions of plants. Combinations of viruses also result in severe symptoms. Common symptoms are mosaic, mottle, or other (bright or pale green) discolorations, crinkling, rolling, or distortion of leaflets, leaf dropping, stunting, and deformation of the whole plant. Various necrotic patterns may occur on leaves, stems, and tubers (both external and internal).

Size, number, aspect, and content of tubers can be affected. Yield losses greatly vary depending on virus, strain, and time of infection. They can reach up to 90%. Some infections can result in defects in processed products such as blackening of tuber flesh. Lastly viruses inducing tuber necrosis (strains of at least 11 species) can make tubers totally unmarketable as ware or processed potatoes.

Spread of Potato Viruses

Potato viruses are efficiently transmitted by aphids, either by species colonizing potatoes or by itinerant species migrating from other crops and weeds. PLRV and PVY are model viruses for studying the mechanisms of persistent and nonpersistent transmission by aphids. PLRV was the first virus of the family *Luteoviridae* for which passage of the virus through the intestine and not the hindgut was demonstrated. Other insects are vectors of less important viruses, notably leafhoppers, thrips, and whiteflies (**Tables 2** and **3**). PVX and some PVS strains are transmitted by contact between foliage or sprouts, between tubers when cutting, and also through farm implements. Several viruses are soil-borne, either transmitted by nematodes (TRV) or by fungi (PMTV, tobacco necrosis virus). Many Andean viruses can be transmitted by TPSs or by pollen. Inoculum sources are in most cases potato itself (volunteer or secondarily infected plants).

Control Methods

Control of seed potatoes

Production of certified seed potatoes nowadays is based on a catalog of measures more or less drastic and sophisticated depending on the country. It is undoubtedly a specialized and costly scheme involving the following main steps: (1) multiplication of virus-free prebasic and basic seeds under conditions where infection risks are minor, for example, first *in vitro* and in vector-proof greenhouses or plastic tunnels, then in isolated fields in areas known for low virus disease pressure; (2) field inspections involving roguing of diseased potatoes and eradication of all sources of inoculum; (3) treatments against vectors, notably insects; (4) specific growing techniques taking into account risks associated to the vector pressure such as early dates of plantation, haulm killing and use of barrier crops; (5) postharvest controls involving large-scale laboratory tests, commonly using ELISA and sometimes molecular techniques. The final aim is the quality grading of the commercial categories of seed potatoes, standards being dependent on each country but usually infection levels should not exceed 10% for all viruses together.

Resistance

Three types of resistance to viruses in potato are commonly distinguished: field resistance or resistance to infection, hypersensitivity (HR), and extreme resistance (ER). Resistance to infection is a polygenically inherited resistance appraised by the number of progeny tubers that are infected. It comprises resistance to virus itself and resistance to vectors. It is stated to be effective against all strains of the virus involved. Scores of resistance levels depend strongly on environmental conditions, time of infection, inoculum, and vector pressure. HR and ER are governed by single dominant genes coded *N* and *R*, respectively (**Figure 1**). ER is an enhanced form of HR, resulting in no obvious symptoms and no systemic infection. Conversely, HR is associated with local necrotic lesions on inoculated leaves but also in most virus–cultivar combinations with systemic necrotic reactions (spots, lines, arches) extending to lower and upper leaves, stems, and tubers. The virus though unevenly distributed can be detected in all these organs. However, except in special cases it is not transmitted to the daughter plants. *N* genes

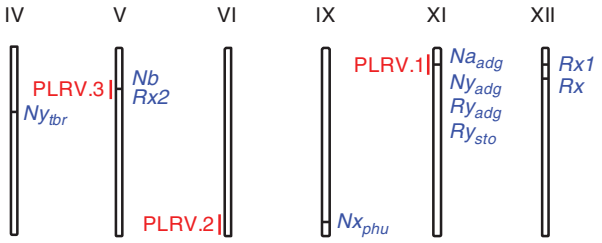


Figure 1 Genomic mapping of resistance genes (in blue) and QTL (in red) to four major viruses (PVY, PLRV, PVX, PVA) in potato. From S. Marchadour, FNPPPT-INRA, France (unpublished).

are strain-specific in contrast to *R* genes, each of which confers resistance to all strains of one or two viruses.

Breeding programs for resistance to infection to the main viruses have been well established in numerous countries producing potatoes. ER is also a frequently employed resistance, notably in countries where the use of certified seeds provides no satisfactory control. *R* genes providing protection against some major viruses (PVX, PVY, PVA, PVM) have been found in several wild *Solanum* species such as *S. stoloniferum*, *S. andigena*, *S. chacoense*, and *S. acaule*. They have been used in breeding programs since the mid-1940s and have appeared to provide sustainable resistance. *N* genes were present in the pool of old potato cultivars and hence have been easily incorporated into new cultivars. Potato is easily transformed by genetic engineering and pathogen-derived resistance against some major viruses (PVY, PLRV, PVX, PMTV) has been intensively studied, though without any significant applied use.

Quarantine measures

Quarantine measures taken against the risk of introducing foreign viruses (species or strains) in one state or continent have demonstrated their full efficiency. Indeed, so far no virus or strain listed as a quarantine pathogen is known to have spread outside its original region. Technical recommendations needed to test any exported or imported material (tubers, cuttings, true potato seeds, pollen, *in vitro* plantlets) have been established. These regulated viruses can be diagnosed by bioassays on selected indicator plants and, for the majority of them, by ELISA. Antibodies to potato virus U and potato yellow vein virus (PYVV) are not available. All potato viruses, except PYVV, potato-deforming mosaic virus and solanum apical leaf curl virus, are sap-transmissible. Molecular techniques or electron microscopy can also be used.

The Major Potato Viruses

Eight viruses are both widespread and damaging and, thus, of great concern in seed potato production (**Table 1**).

Potato Virus Y

PVY has become in the last decade the most important potato virus. It affects most cultivars and causes major yield losses of up to 80% and even more in the case of the potato tuber necrotic ringspot disease (PTNRD) (**Figure 2(a)**).

PVY isolates infecting potato have been divided into three strains (PVY^O, PVY^C, PVY^N), and many pathotypes and variants. Currently, PVY^O and PVY^N are the main widespread strains. PVY^C is less frequent (less than 5% of PVY isolates in France). Variants PVY^{NTN}, PVY^NW, and $PVY^{N:O}$ are also widespread. Most isolates of these variants are recombinants between PVY^O and PVY^N. Strains PVY^O, PVY^C, and PVY^N are differentiated on the basis of their reaction on *Nicotiana tabacum* and on potato cultivars carrying the hypersensitive genes Ny_{tbr} and *Nc*. PVY^NW isolates differ from other PVY^N isolates in their virulence and in belonging to serotype O-C.

Typical symptoms associated with PVY infections are mosaic, crinkle, necrotic patterns, and stunting of the plant in the case of PVY^O secondary infections (**Figure 2(b)**). Infection by PVY^N is often symptomless or associated with a mild mosaic (**Figure 2(c)**), only sometimes with a severe mottle. Yellowing and green halos can be observed on basal leaves (**Figure 2(d)**). Necroses (on or around leaf veins, on petioles, stems, and tubers) are related to hypersensitivity reactions in numerous cultivars (**Figure 2(e)**). Necrosis of basal leaves which remain clinging to the stem (leaf drop) are typical of PVY^O primary infections. PVY^C induces stipple-streaks on stems and brown spots around eyes of tubers. PVY^{NTN}, and some PVY^NW and $PVY^{N:O}$ isolates, can induce PTNRD.

Aphid transmission is undoubtedly the most important means of PVY spread in the fields. However, transmission by plant-to-plant contact has been consistently reported by several authors. Under experimental conditions, 70 aphid species were able to transmit PVY, *Myzus persicae* being the most efficient vector. This species and other aphids colonizing potato fields have long been seen as the main natural vectors. However, due to their abundance in potato fields during many PVY outbreaks, itinerant species multiplying in other crops (cereals, peas, etc.) have also been said to play a significant role in spread of PVY. PVY has a wide host range but besides potato no other host has so far been shown to act as a significant inoculum source, at least in the main potato-growing areas.

Control of PVY in seed fields requires use of mineral oil treatments to prevent aphid transmission. Forecasting and simulation models of PVY spread have been developed in many countries. At least 20 cultivars possess durable immunity to PVY though none of them is economically important. However, Ry_{sto} and Ry_{adg} genes are more and more introgressed into breeding programs.

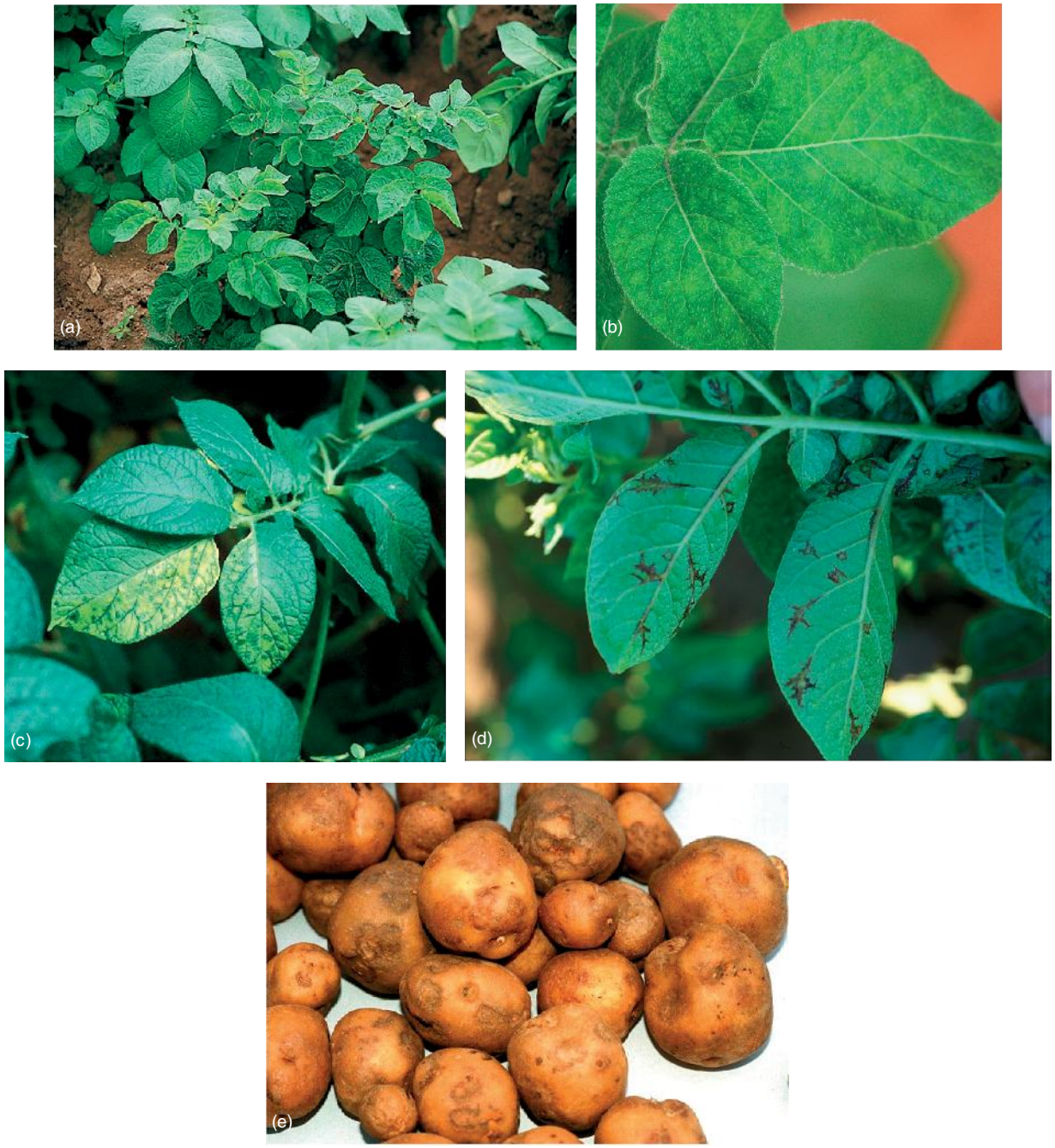


Figure 2 (a) Secondary infection by PVY: mottle and crinkle of the leaflets; stunting of the plant. (b) Primary infection by PVY: mosaic on an apical leaf of cv. Bintje. (c) Primary infection by PVY: typical yellowing with green halos on a basal leaf of cv. Nicola. (d) Oak-leaf patterns and necrotic spots induced by PVY on leaflets of cv. Nicola. (e) PTNRD typical symptoms on tubers of cv. Nicola harvested in a field in Southern France. (a) Reproduced by permission of Y. Le Hingrat, FNPPPT, France. (b) Photograph: Christelle Lacroix, INRA, France. (c–e) Photographs: K. Charlet-Ramage, GNIS-INRA, France.

Potato Leafroll Virus

Although PLRV has been decreasing in incidence, it is yet a serious threat in most potato-growing areas. It can induce heavy yield losses, often more than 50%, sometimes up to 90%. Harvested tubers are small, whereas mother tubers often stay hard and unrotten. Spindling sprouts might occur in old cultivars. In some currently widespread cultivars, tuber quality is reduced due to the occurrence of necrotic spots distributed within the flesh (net necrosis) (**Figure 3**(**a**)). The virus is restricted to





Figure 3 (a) Net-necrosis induced by PLRV in a tuber of cv. Russet Burbank. (b) Secondary infection by PLRV: erect habit, plant stunting and pale yellow foliage. (a) Photograph: J. Martin, LNPV, France. (b) Reproduced by permission of Y. Le Hingrat, FNPPPT, France.

phloem. It induces formation of callose in sieve tubes blocking sugar transport and making the leaflets becoming hard and brittle. Cracking sounds resulting from squeezing such leaflets are a good indicator PLRV infections. It also induces phloem necrosis in petioles and stems. Photosynthesis and chlorophyll content are also reduced. Typical symptoms of secondary infection are an erect habit, plant stunting, and pale yellow foliage (**Figure 3**(**b**)). Leaflets roll upward, whereby lower leaves are dry, brittle, and leathery and may become necrotic. Primary infections are often latent, especially late infections. In most susceptible cultivars, apical leaves are erected and pale yellow (top yellowing) with sometimes purple or red margins.

PLRV is not transmitted by sap inoculation. At least ten aphid species were shown to be capable of transmitting it. PLRV does not multiply in the insect. Natural spread of PLRV in potato crops is mainly due to *M. persicae*, the most efficient vector though with large differences depending on aphid biotype and PLRV isolate. Other aphid species colonizing potatoes are poor vectors. However, *Aulacorthum solani* was shown to play a role in early infections in some PLRV outbreaks in Western Europe in the 1970s. *Capsella bursa-pastoris*, *Datura stramonium*, and *Physalis floridana* can act as virus reservoirs.

PLRV does not show any remarkable variability except that there have been reports on striking differences between isolates in aphid transmission rates. Differences between isolates can be established on the basis of symptom severity (stunting) in *P. floridana*. This host is also the common plant used to test the transmission efficiency of aphids.

In seed-potato producing countries, the use of efficient insecticide treatments has led to a considerable decrease of PLRV incidence. Many cultivars display high levels of field resistance. However, there is no commercial cultivar carrying *R* or *N* genes though ER and HR to PLRV were shown to be present in several pools of *Solanum* wild species. Two genetically engineered cultivars resistant to PLRV were registered in the USA but do not seem to be widespread.

Potato Virus X

PVX was in the past the most common virus in potatoes. It often induces a mild mosaic and mottle, sometimes severe mosaic, rugosity, crinkling of the leaflets, and also tuber necrosis in some cultivars. Yield reduction is usually low, often 10–15% but can be over 50% in the case of infections by severe strains or of mixed infections with PVY and PVA.

PVX has been separated into four strain groups (X^1, X^2, X^3, X^4) that differ in virulence to HR genes *Nx* and *Nb*. PVX^3 is the commonest strain at least in Europe. PVX^4 overcomes both *Nx* and *Nb* but is fortunately rare. PVX_{CP} the common strain in Peru, belongs to group X^2. The strain HB discovered in Bolivia in 1978 overcomes these HR genes and also the *Rx* gene. Amino acid changes in its coat protein are responsible for its virulence. Both PVX_{HB} and PVX_{CP} belong to the serotype PVX^A which is distinct from the serotype PVX^O. Specific monoclonal antibodies and nucleic acid probes have been produced for diagnosis of isolates of each serotype. Reliable indicator cultivars for strain differentiation can be: Desiree or Pentland Crown (*nx*, *nb*) which are susceptible to all strains, King Edward (*Nx*, *nb*) resistant to X^1 and X^3, Bintje (*nx*, *Nb*) resistant to

X^1 and X^2, Maris Piper or Pentland Dell (*Nx*, *Nb*) resistant to X^1, X^2, and X^3. *Gomphrena globosa*, *C. amaranticolor*, and *C. quinoa* are other PVX indicator hosts.

PVX is efficiently controlled by use of certified seeds. Moreover, numerous important European and American cultivars (Ranger Russet, Saturna, Cara) harboring Rx_{adg} or Rx_{acl} are PVX immune. Numerous cultivars also contain *Nx* and *Nb*. PVX_{HB} is not a real threat due to its lack of fitness.

Potato Virus A

PVA is widespread except in some countries where it is rare (Poland) or absent (Andes). It is frequent in susceptible cultivars such as Desiree and Russet Burbank. In the last decade it has become more prevalent in some countries. Yield losses may be negligible or amount to 40%. Infection is often symptomless even in the most susceptible cultivars. Typical symptoms are a very mild mosaic, often transient or only discernible in cloudy weather conditions. Leaflets may be shiny, rough, and slightly rippled. Crinkling occurs in mixed infections with PVX. Combination with PVY also results in a severe disease.

PVA has a limited host range. Other hosts such as tobacco and cherry tomato do not play any significant role in PVA epidemiology. *M. persicae* is the main vector. *M. euphorbiae*, some *Aphis* species, and *Brachycaudus helichrysi* were reported as potential vectors.

PVA isolates have been divided into three serogroups using monoclonal antibodies and into four strain groups based on HR or systemic response in several potato cultivars (notably King Edward). Some isolates were proved to have lost aphid transmissibility. Mutations in proteins 6K2 and VPg were shown to be responsible for particular avirulence in *Nicandra physaloides*.

Virus concentration in infected plants is low and purification of virions is difficult. *N. tabacum* can be used as a propagation host. Virions are weakly immunogenic and specific antisera without any cross-reactivity with PVY are difficult to prepare. Commercial ELISA kits are available, though not always specific. RT-PCR protocols were also reported for the detection in potato leaves and dormant tubers. In the past *Solanum demissum* A6 and *S. demissum* A were frequently used for differentiating PVA from PVY.

A large part of old and current cultivars is either immune or hypersensitively resistant to PVA (notably the widespread cultivars Bintje, Eersteling, Spunta, Kennebec, Charlotte, Monalisa, Katahdin, King Edward).

Potato Virus S and Potato Virus M

PVS and PVM are distantly related to each other and to other potato viruses of the genus *Carlavirus* (PVP, PRDV; recently shown to be strains of the same species). They were widespread in the past infecting all plants of cultivars such as King Edward which were later virus-freed by meristem culture. They are currently quasi-eradicated in Western Europe but are yet widespread in Eastern Europe, notably in Poland, where PVS incidence may reach 100% in some cultivars. Yield losses do not exceed 15%, except in some Polish cultivars infected by PVM. Co-infections by both viruses are frequent. ELISA is routinely used for detection in leaves and tubers. Both viruses are controlled in seed-potato schemes.

PVS

Infections are usually symptomless though leaf mottling may be observed in particular genotypes (**Figure 4**). PVS^O and PVS^A are two strain groups which induce local and systemic reactions on *Chenopodium* spp., respectively. PVS^A can cause severe reactions in potato. Full-length genomes of at least three isolates were sequenced. A broad variability was shown throughout the whole genome. The 'Central European' variant (PVS-CP) which systemically infects *Chenopodium* spp. was genetically closely related to PVS^O but only distantly related to PVS^A.

Both PVS^O and PVS^A can be naturally transmitted by contact and by several aphid species, especially *M. persicae*, *Aphis frangulae*, *A. nasturtii*, *A. fabae*, and *Rhopalosiphum padi*. Natural host range is restricted to several species in the families Solanaceae and Chenopodiaceae. Tomato is immune to PVS^O, but symptomlessly infected by PVS^A. *Nicotiana debneyi* can be used for isolation from plants doubly infected with PVM. Specific anti-PVS^A monoclonal antibodies have been produced. Detection by radioactive and nonradioactive probes as well as by PCR was reported. Many cultivars



Figure 4 Infection by PVS: mottle observed on a plantlet from imported material tested in the Potato Quarantine Station in France. Photograph: J. Martin, LNPV, France.

harbor the Ns_{adg} gene and are efficiently protected. Conversely, resistance in cultivar Saco is polygenic and recessive.

PVM

PVM usually induces very slight mottles and mild abaxial rolling of leaflets (**Figure 5**). Main vectors are aphid species colonizing potato crops. Some isolates that are not transmitted by aphids have been described. PVM-ID is a serologically distinct strain. Potato is the main host naturally infected. Tomato (symptomless infection) and potato cultivar Saco are good hosts for propagation and separation from PVS. The main aphid vectors are *M. persicae*, *A. nasturtii*, and *A. frangulae*. Some strains may also be transmitted by contact. Monogenic dominant resistance was found in *S. gourlayi* (*Gm* gene), *S. megistacrolobum*, and *S. stoloniferum* (HR genes).



Figure 5 Infection by PVM: soft rolling of leaflets and pale green color on an infected plant (right) versus an healthy plant (left). Reproduced by permission of Y. Le Hingrat, FNPPPT, France.

Tobacco Rattle Virus

TRV is a widespread virus infecting numerous weeds and crops. It causes a serious disease in potato, called spraing or stem mottle, mainly in areas with light and peaty soils. Spraing or corky ringspot denotes one type of necrotic arcing in the tuber flesh (**Figure 6(a)**) and on the tuber surface (**Figure 6(b)**). Stem mottle refers to the fact that only one single shoot may be stunted with leaves showing a mosaic and sometimes transient yellow chevrons. Spraing-affected tubers often give rise to virus-free progeny plants, sometimes to infected plants with symptomless daughter tubers. Lastly, some cultivars are symptomless carriers of TRV.

TRV particles are tubular straight rods varying in length depending on the isolate. Normal particle-producing isolates (called M-type) have two genomic RNAs and are readily transmitted by nematodes and by sap inoculation. NM-type isolates have only RNA-1, do not produce particles, are probably not transmitted by nematodes and are not easily sap-transmitted. Some of the best characterized strains were originally obtained from potato: PRN (potato ring necrosis) now used as the type strain although it seems to have lost its transmissibility by nematodes; Oregon strains, which include the variant Oregon Yellow; PSG, and PLB. Monoclonal antibodies to strain PLB have been produced. Complete genomic sequences of many

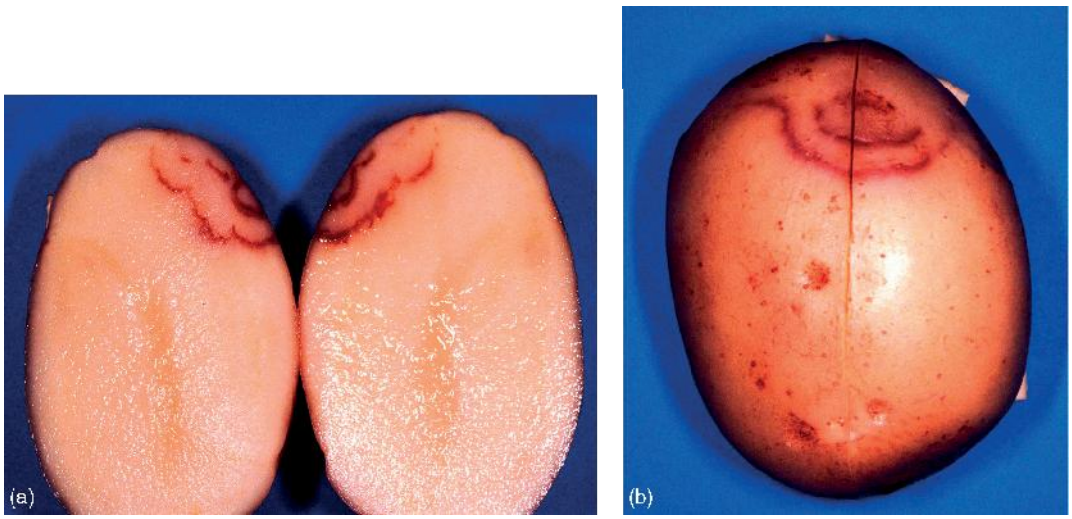


Figure 6 (a) Typical necrotic arches in the tuber flesh (spraing) induced by TRV. (b) Necrotic arches on tubers associated with spraing induced by TRV. (a, b) Photographs: J. Martin, LNPV, France.

potato strains are available in databases, namely for PLB and PSG.

At least 12 nematode species of the genera *Paratrichodorus* and *Trichodorus* are natural vectors, with a high specificity between virus strain and vector species. Adults and juveniles can transmit and retain virions for many months, particles being attached to the esophageal wall. Acquisition and inoculation periods can be short (1 h). TRV does not seem to multiply in its vector and to be transmitted through TPS.

Serological detection of TRV from potato is unreliable. Detection is based on the use of bait and indicator plants (*P. floridana*, *N. tabacum*, *C. quinoa*) but primarily on molecular techniques (RT-PCR and real-time PCR). However, none of these techniques is fully reliable.

Control methods are based on the use of certified seeds and of cultivars more or less resistant to spraing. Tolerant cultivars multiplying the virus without displaying any spraing cannot be recommended since they can contribute to spread of the virus. The use of nematicides for soil treatments is no longer permitted in most countries.

Potato Mop-Top Virus

PMTV can cause a serious disease in potato crops in Northern Europe, especially in cultivars such as Saturna. It is also present in the Andes, Japan, China, and Canada. PMTV induces necroses in potato tubers, both external and internal sinuous, often parallel, lines, also arcs (**Figure 7**). Blotching, skin cracking, and malformation can develop, mainly in cool conditions before harvest or during storage. Typical symptoms on leaflets after secondary infection are yellow or necrotic chevrons and blotches. Mop-top denotes bunched foliage on shortened stems. Virus does not invade all daughter tubers.

PMTV is vectored by the fungus *Spongospora subterranea* f. sp. *subterranea*, the causal agent of potato powdery also. Virus can persist in long-live resting spores of its vector. Strains differing in transmission and virulence have been reported. PMTV-T from Scotland is the type strain.

Potato is usually the only host plant. Many weed species such as *C. quinoa* may be natural hosts in Andean countries. *C. amaranticolor* and *N. debneyi* are local lesion hosts. *N. benthamiana* can be used as a bait plant and a suitable propagation host. ELISA, RT-PCR, and real-time PCR have been successfully used for detection, though results are inconsistent due to erratic distribution of the virus in infected plants. Virions are rod-shaped fragile particles similar to those of TRV. Polyclonal and monoclonal antibodies have been raised though the virus is difficult to purify. Antisera have also been produced using recombinant coat protein as antigen.

Control methods include breeding for tolerance to spraing, but no natural sources of resistance are available so far. Coat protein-derived resistance has been successfully tested in greenhouse conditions.



Figure 7 Necrotic sinuous lines induced by PMTV on a tuber of cv. Kerpondy. Photograph: C. Kerlan, INRA, France.

Viruses with Limited Significance

Many viruses of limited significance in potato may cause spectacular symptoms on potato foliage or tubers



Figure 8 Calico symptoms induced by AMV on potato: bright yellow blotching of leaflets. Photograph: L. F. Salazar, CIP, Peru. Reproduced by permission of C. Jeffries, SASA, UK.



Figure 9 Infection by PAMV: bright yellow spots, flecks and blotches on cv. Ulster Premier. Reproduced by permission of C. Jeffries, SASA, UK.



Figure 10 Infection by TNV: necrotic lesions with crackings of the tuber skin. Reproduced by permission of C. Jeffries, SASA, UK.



Figure 11 External necroses on tubers induced by TSWV. Photograph: J. Martin, LNPV, France.

(**Figures 8–11**). Only TSWV was associated with significant losses in several countries with incidences sometimes reaching up to 90%. Its spread results from invasion of some species of thrips vectors.

See also: Bunyaviruses: General Features; History of Virology: Plant Viruses; *Carlavirus*; *Necrovirus*; *Nepovirus*; *Pomovirus*; *Potexvirus*; *Sobemovirus*; *Tobamovirus*; *Tobravirus*; *Tospovirus*; Vector Transmission of Plant Viruses; Luteoviruses; Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Plant Virus Diseases: Economic Aspects; Potato Virus Y; Potyviruses; Tymoviruses.

Further Reading

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, and Watson L (eds.) (1996) *Viruses of Plants. Descriptions and Lists from the VIDE Database.* Wallingford, UK: CAB International.

de Bokx JA and Van der Want JPH (eds.) (1987) *Viruses of Potato and Seed-Potato Production,* 2nd edn. Wageningen, The Netherlands: Pudoc.

Delleman J, Mulder A, Peeters JMG, Schiper E, and Turkensteen LJ (eds.) (2005) *Potato Diseases.* Den Haag, The Netherlands: NIVAP Holland.

Gebhardt C and Valkonen J (2001) Organisation of genes controlling disease resistance in the potato genome. *Annual Review of Phytopathology* 39: 79–102.

Jeffries CJ (1998) *FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm, No. 19. Potato.* Rome: Food and Agriculture Organization of the United Nations/International Plant Genetic Resources Institute.

Loebenstein G, Berger P, Brunt AA, and Lawson RG (eds.) (2001) *Virus and Virus-Like Diseases of Potatoes and Production of Seed-Potatoes.* Dordrecht, The Netherlands: Kluwer.

Lorenzen JH, Meacham T, Berger PH, *et al.* (2006) Whole genome characterization of *Potato virus Y* isolates collected in the western USA and their comparison to isolates from Europe and Canada. *Archives of Virology* 151: 1055–1074.

Radcliffe EB and Ragsdale EB (2002) Aphid-transmitted potato viruses: The importance of understanding vector biology. *American Journal of Potato Research* 79: 353–386.

Ratke W and Rieckman W (1991) *Maladies et ravageurs de la pomme de terre.* Gelsenkirchen-Buer, Germany: Verlag Th. Mann.

Salazar LF (1966) *Potato Viruses and Their Control.* Lima, Peru: International Potato Center.

Singh RP (1999) Development of the molecular methods for potato virus and viroid detection and prevention. *Genome* 42: 592–604.

Solomon-Blackburn RM and Barker H (2002) A review of host-major gene resistance to potato viruses X, Y, A and V in potato: Genes, genetic and mapped locations. *Heredity* 86: 8–16.

Solomon-Blackburn RM and Barker H (2002) Breeding virus resistant potatoes (*Solanum tuberosum*): A review of traditional and molecular approaches. *Heredity* 86: 17–35.

Valkonen J (1994) Natural genes and mechanisms for resistance to viruses in cultivated and wild potato species (*Solanum* spp.). *Plant Breeding* 112: 1–16.

Valkonen J (1997) Novel resistances to four potyviruses in tuber-bearing potato species, and temperature-sensitive expression of hypersensitive resistance to *Potato virus Y*. *Annals of Applied Biology* 130: 91–104.

Potexvirus

K H Ryu and J S Hong, Seoul Women's University, Seoul, South Korea

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Gene silencing A general term describing epigenetic processes of gene regulation, that is, a gene which would be expressed (turned on) under normal circumstances is switched off by machinery in the cell.
RNA interference (RNAi) A mechanism for RNA-guided regulation of gene expression in which double-stranded RNA inhibits the expression of genes with complementary nucleotide sequences.
Triple gene block (TGB) A specialized evolutionarily conserved gene module of three partially overlapping open reading frames involved in the cell-to-cell and long-distance movement of plant viruses.

Introduction

The genus *Potexvirus* (derived from *Potato virus X*) is one of nine genera in the family *Flexiviridae*. Most potexviruses are found wherever their hosts are grown. The genus *Potexvirus* contains a large number of members or tentative members. Host plants naturally infected with potexviruses may show mosaic, necrosis, ringspot, or dwarf symptoms or may be symptomless. The natural host range of individual viruses is usually restricted to a few plant species, although a few of the viruses can infect a wide range of plant species. Many members of the genus have been identified although only relatively recently. Most potexviruses are not transmitted by vertebrate, invertebrate, or fungal vectors; potato aucuba mosaic virus (PAMV), white clover mosaic virus (WClMV), and a few others are transmitted by aphids. The type species of the genus *Potexvirus* is *Potato virus X*. During the last few decades, potato virus X (PVX) has greatly contributed to our understanding of host resistance and gene silencing mechanisms, in particular because of the use of viral vectors in studying gene expression and RNA silencing in plants.

Taxonomy and Classification

The genus *Potexvirus* is one of nine genera in the family *Flexiviridae*. The type species of the genus is *Potato virus X*, and the name potexvirus was derived from the type species. The genus contains 55 members, of which 32 are definitive species and 23 tentative (**Table 1**). Some members listed as species and many members listed as tentative species have not been sequenced, and their taxonomic status is yet to be verified.

Different degrees of relationship exist among potexviruses, and phylogenetic analysis has revealed the presence of two major branches based on the composition of polymerase, coat protein (CP) and triple gene block (TGB) proteins.

Species demarcation criteria in the genus *Potexvirus* include the following: (1) members of distinct species have less than ~72% nucleotide or ~80% amino acid identity between their CP or replicase genes; (2) members of distinct species are readily differentiated by serology, and some virus strains can be differentiated with monoclonal antibodies; and (3) members of distinct species fail to cross-protect in common host plant species, and they usually have distinguishable experimental host ranges.

Virus Structure and Composition

Virions are flexuous filaments that are not enveloped, and measure about 470–580 nm in length and 13 nm in diameter, have helical symmetry and a pitch of 3.3–3.7 nm. Nucleocapsids are longitudinally striated. Virion M_r is about 3.5×10^6 with 5–7% nucleic acid content. Virions contain a monopartite, linear, single-stranded, positive-sense RNA that has a size range of 5.8–7.5 kbp. The 3′ terminus has a poly(A) tract and the 5′ terminus occasionally has a methylated nucleotide cap. CP subunits are of one type with a size in the range of 22–27 kDa. The genome is encapsidated in 1000–1500 CP subunits. The CPs of some strains of PVX and other viruses are glycosylated.

In some potexviruses such as bamboo mosaic virus (BaMV), a satellite RNA has been reported. The satellite RNA of 836 nt depends on BaMV for its replication and encapsidation. The BaMV satellite RNA (satBaMV) contains a single open reading frame (ORF) encoding a 20 kDa nonstructural protein.

Physicochemical Properties

Purified virion preparations sediment as one component with sedimentation coefficient of 115–130 S. The isoelectric point of the virion is about pH 4.4. The ultraviolet (UV) absorbance spectra of potexviruses have maxima at 258–260 nm and minima at 244–249 nm, with A_{max}/A_{min} ratios of 1.1–1.3, and the A_{260}/A_{280} ratio of purified

Table 1 Virus species in the genus *Potexvirus*

Species	*Virus abbreviation*	*Accession number*[a]
Definitive species		
Alternanthera mosaic virus	AltMV	NC_007731
Asparagus virus 3	AV-3	
Bamboo mosaic virus	BaMV	NC_001642
Cactus virus X	CVX	NC_002815
Caladium virus X	CalVX	AY727533
Cassava common mosaic virus	CsCMV	NC_001658
Cassava virus X	CsVX	
Clover yellow mosaic virus	ClYMV	NC_001753
Commelina virus X	ComVX	
Cymbidium mosaic virus	CYMMV	NC_001812
Daphne virus X	DVX	
Foxtail mosaic virus	FoMV	NC_001483
Hosta virus X	HVX	AY181252
Hydrangea ringspot virus	HdRSV	NC_006943
Lily virus X	LVX	NC_007192
Mint virus X	MVX	NC_006948
Narcissus mosaic virus	NMV	NC_001441
Nerine virus X	NVX	NC_007679
Opuntia virus X	OVX	NC_006060
Papaya mosaic virus	PapMV	NC_001748
Plantago asiatica mosaic virus	PlAMV	NC_003849
Plantago severe mottle virus	PlSMoV	
Plantain virus X	PlVX	
Potato aucuba mosaic virus	PAMV	NC_003632
Potato virus X	PVX	NC_001455
Scallion virus X	ScaVX	NC_003400
Schlumbergera virus X	SVX	AY366207
Strawberry mild yellow edge virus	SMYEV	NC_003794
Tamus red mosaic virus	TRMV	
Tulip virus X	TVX	NC_004322
White clover mosaic virus	WClMV	NC_003820
Zygocactus virus X	ZVX	NC_006059
Tentative species		
Allium virus X	AVX	AY826413
Alstroemeria virus X	AlsVX	NC_007408
Artichoke curly dwarf virus	ACDV	
Barley virus B1	BarV-B1	
Boletus virus X	BolVX	
Centrosema mosaic virus	CenMV	
Chenopodium mosaic virus X	ChMVX	NC_008251
Discorea latent virus	DLV	
Lychnis symptomless virus	LycSLV	
Malva veinal necrosis virus	MVNV	
Nandina mosaic virus	NaMV	
Negro coffee mosaic virus	NeCMV	
Paris polyphylla virus X	PPVX	DQ530433
Parsley virus 5	PaV-5	
Parsnip virus 3	ParV-3	
Parsnip virus 5	ParV-5	
Patchouli virus X	PatVX	
Pepino mosaic virus	PepMV	NC_004067
Rhododendron necrotic ringspot virus	RoNRSV	
Rhubarb virus 1	RV-1	
Smithiantha latent virus	SmiLV	
Viola mottle virus	VMoV	
Zygocactus symptomless virus	ZSLV	

[a]Accession number in GenBank.

preparation as 1.09–1.37. Physical properties of viruses in this genus are: thermal inactivation point 60–80 °C, longevity *in vitro* (several weeks to months), and dilution endpoint 10^{-5}–10^{-6}. Infectivity of sap does not change on treatment with diethyl ether. Infectivity is retained by the virions despite being deproteinized by proteases, phenols, or detergents.

The CPs of potexviruses are partly degraded during the purification and storage of virus preparations. The virions may dissociate by denaturing agents such as sodium dodecyl sulfate (SDS), urea, guanidine hydrochloride, acetic acid, and alkali.

Genome Structure and Gene Expression

The genome is a single-stranded RNA (ssRNA), 5.8–7.5 kbp in size and contains five ORFs, encoding the replicase, three putative protein components of movement proteins (MPs) called TGB, and the CP, from the 5′- to 3′- end in that order (**Figure 1**). Its genome organization resembles that of the genera *Allexivirus*, *Foveavirus*, *Mandarivirus*, and *Carlavirus*, but is distinguished from them by the presence of five ORFs and a small replication protein. The genome RNA is capped at the 5′-end and polyadenylated at the 3′-end. The 146–191 kDa viral replicase ORF is translated from the full-length genomic RNA. For expression of its 3′ proximal viral genes, the virus utilizes at least two subgenomic RNAs. The genome structure of PVX (6435 nt) is represented in **Figure 1**. PVX RNA has 83 nt 5′ untranslated region (UTR) at the 5′-terminus and 76 nt 3′ UTR followed by a poly(A) tail at the 3′-terminus. The 5′ UTR regulates genomic and subgenomic RNA synthesis and encapsidation, and the 3′ UTR controls both plus- and minus-strand RNA synthesis. Potexvirus-infected plants contain double-stranded RNAs (dsRNAs) whose molecular mass corresponds to that of genomic RNA and two subgenomic RNAs. Although potexviruses typically have five ORFs, some potexviruses (cassava common mosaic virus (CsCMV), narcissus mosaic virus (NMV), strawberry mild yellow edge virus (SMYEV), and WClMV) have a sixth smaller ORF located within the ORF5, with no known protein product.

ORF1 encodes a 166 kDa polypeptide that is presumed to be the viral replicase. It contains motifs of methyltransferase, helicase, and RNA-dependent RNA polymerase. ORFs 2, 3, and 4 form the TGB encoding polypeptides of 25, 12, and 8 kDa, respectively, which facilitate the virus cell-to-cell movement. ORF5 encodes the 25 kDa CP. The CP is required for virion assembly and cell-to-cell movement. The 25 kDa TGBp1 protein contains an NTPase-helicase domain but is not involved in RNA replication.

Complete genomic sequences have recently been obtained for many potexviruses (see **Table 1**).

Infectious cDNA clones have been reported for some potexviruses, such as PVX, BaMV, cymbidium mosaic virus (CymMV), zygocactus virus X (ZVX), and WClMV. In particular, PVX has been widely used as a vector for the study of gene silencing and small RNAs as well as for expression of foreign genes in plants.

Viral Transmission

Potexviruses are spread by mechanical contact and horticultural and agricultural equipment. None of the viruses have known invertebrate or fungal vectors. Two potexviruses (PAMV and WClMV) are transmitted by aphids. A few members are not transmitted by mechanical inoculation. Seed transmission may occur with some potexviruses, but is not common.

Cytopathology

The cytoplasm of potexvirus-infected cells contains fibrous, beaded, banded, or irregular aggregates of virus particles. Virus particles, frequently in large aggregates, occur in the cytoplasm and occasionally in the nuclei. The distribution of potexviruses in the infected plant is not tissue specific. Inclusions such as cytoplasmic laminated inclusion components are present in infected cells.

Host Range

The natural host range of individual members is mostly limited, although some species can infect a wide range of experimental hosts. Some of the viruses cause serious damage to their hosts while others cause little damage to infected hosts.

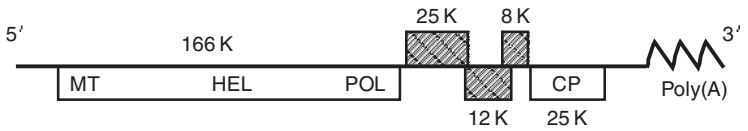


Figure 1 Genome organization of PVX, showing the typical genome structure present in the genus *Potexvirus*, family *Flexiviridae*. The 5′ proximal large ORF encodes an RNA-dependent RNA polymerase (viral replicase), three overlapping ORFs encode the putative MPs (TGBs), and an ORF encodes CP. Motifs in the replicase are methyltransferase (MT), helicase (HEL), and RNA-dependent RNA polymerase (POL).

Symptomatology

Infection by most members of the genus *Potexvirus* is latent or causes only mild mosaic symptoms in the natural host. Symptoms vary cyclically or seasonally and may disappear soon after infection. Some viruses cause necrosis, ringspot, or dwarf symptoms in a wide range of plant species. If symptoms are evident, more severe symptoms such as mosaics appear in the early stages of infection. Some potexviruses such as PVX cause diseases that are of economic importance on their own; however, most of them are associated with more serious diseases when plants are co-infected with other viruses.

Serology

Virions are good immunogens. Most potexviruses are serologically related to several others, with the relationships varying from close to distant.

Geographical Distribution

Potexviruses are found wherever their hosts are grown, and so the geographical distribution of many species is restricted to only certain parts of the world.

Viral Epidemiology and Control

Most potexvirus-associated diseases are usually very mild or symptomless. The need for their control is often perceived as not important. However, crops such as potatoes, certain cactis, and some ornamental crops that may be infected by more damaging potexviruses require suitable control measures. Transgenic potato, tobacco, and orchid plants, which are resistant to infection by PVX and CymMV, have been developed.

See also: Allexivirus; *Capillovirus, Foveavirus, Trichovirus, Vitivirus*; *Carlavirus*; Flexiviruses; Plant Virus Vectors (Gene Expression Systems); Vector Transmission of Plant Viruses.

Further Reading

Adams MJ, Antoniw JF, Bar-Joseph M, *et al.* (2004) The new plant virus family *Flexiviridae* and assessment of molecular criteria for species demarcation. *Archives of Virology* 149: 1045–1060.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (2005) In: *Virus Taxonomy, Classification and Nomenclature of Viruses, Eighth Report of the International Committee on the Taxonomy of Viruses*, 1101pp. New York: Academic Press.

Martelli GP, Adams MJ, Kreuze JF, and Dolja VV (2007) Family *Flexiviridae*: A case study in virion and genome plasticity. *Annual Review of Phytopathology* 45: 73–100 (online published).

Verchot-Lubicz J, Ye CM, and Bamunusinghe D (2007) Molecular biology of potexviruses: Recent advances. *Journal of General Virology* 88: 1643–1655.

Potyviruses

J J López-Moya, Instituto de Biología Molecular de Barcelona (IBMB), CSIC, Barcelona, Spain
J A García, Centro Nacional de Biotecnología (CNB), CSIC, Madrid, Spain

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The existence of potyviruses as a specific group of plant pathogens with numerous members was recognized soon after the onset of virology as a scientific discipline. Historical evidence of symptoms caused in plants by potyviruses includes the color-breaking of infected tulips that was considered fashionable in the seventeenth century and abundantly reproduced in Dutch paintings from that period. Some viruses of the family were among the first plant viruses to be identified due to the importance of the diseases they cause, and relatively soon after potyviruses were grouped according to common characteristics. Electron microscopy provided a clear taxonomic criterion to allocate viruses into the group by showing the consistent presence of pinwheel-shaped cytoplasmic inclusions in plant cells infected by potyviruses. Further contributions to classification were provided by biological and serological studies, followed by deciphering of virus sequences. The finding of biological peculiarities in closely related viruses, with unusual vector organisms or genome composition, led to the current, although still provisional, classification of six genera in the family.

The large number of virus species within the family *Potyviridae* reveals its evolutionary success. Currently almost 200 definitive and tentative species are included in the genus of aphid-transmitted viruses, *Potyvirus*. Members of the family *Potyviridae* are distributed throughout the world, although each particular virus has a specific host range that limits its geographic distribution to the

area potentially occupied by susceptible hosts. Generally speaking, host ranges are constrained to a limited number of natural and experimental hosts, although several potyviruses can infect a considerable number of plant species distributed in many botanical families and some members infect the most economically important crops, including grain, legumes, forages, vegetables, fruits, and ornamentals. The fact that several potyviruses are among the most damaging plant pathogens has fueled both the interest of researchers and the sustained attention of plant breeders and agronomists seeking practical solutions to the losses they cause in many crop species.

This article focuses on current knowledge about viruses in the family *Potyviridae*, with special emphasis on the more recent advances in taxonomy, evolution, diagnostics, functional and structural aspects of viral proteins, as well as host–virus interactions, including characterization of resistance genes and defense responses based on RNA silencing mechanisms. Present and future biotechnological applications of viruses within the family are also considered.

Taxonomy and Classification

The family *Potyviridae* comprises six genera of plant viruses, including the genus *Potyvirus* that is currently the largest genus of plant viruses. Other genera in the family are *Bymovirus*, *Ipomovirus*, *Macluravirus*, *Rymovirus*, and *Tritimovirus*.

Taxonomic standards for classification into the family include: (1) properties of virus particles: long flexuous filamentous (**Figure 1(a)**), (2) cytopathological manifestations: presence of pinwheel or scroll-shaped cylindrical cytoplasmic inclusions in infected plant cells (**Figure 1(b)**), and (3) genome structure and expression strategy: positive-sense ssRNA genomes, with 5′ terminal proteins and 3′ polyA tails, translated as large polyprotein precursors (see the section titled 'Properties of the genome').

Within the family, genera are differentiated according to biological and molecular criteria. Potyviruses are aphid-transmissible and possess a monopartite genome, characteristics that they share with macluraviruses, although particles of the latter are shorter in length. Other monopartite genera include whitefly-transmitted ipomoviruses, and mite-transmitted rymoviruses and tritimoviruses, further distinguished by their vector organisms belonging to the genera *Abacarus* and *Aceria*, respectively. Rymoviruses can also be distinguished by nucleotide similarity, being more closely related to potyviruses than tritimoviruses. Finally, bymoviruses have bipartite genomes that are encapsidated separately, and are transmitted by plasmodiophoraceous fungi of the genus *Polymyxa*. **Table 1** summarizes the family classification.

Phylogenetic analysis based on the alignment of genome regions has established standard thresholds of around

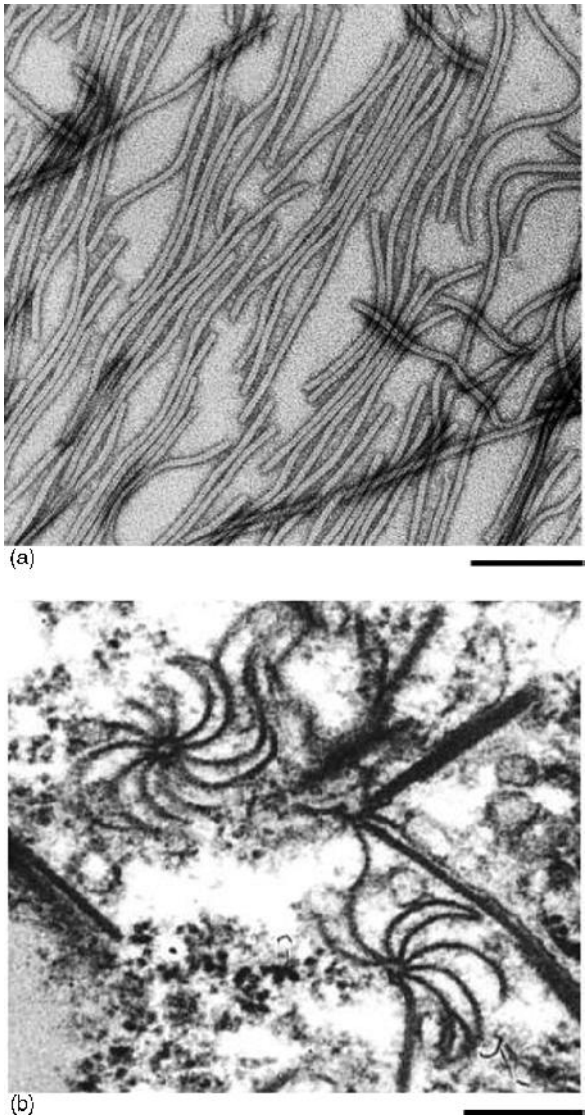


Figure 1 (a) Negative-stain preparation of purified tobacco etch potyvirus particles. (b) Thin section showing the typical pinwheel-shaped cytoplasmic inclusions present in cells of a *Nicotiana benthamiana* plant infected with plum pox potyvirus. Scale = 200 nm. Courtesy of D. López-Abella, CIB, CSIC.

50% and 75% of nucleotide identities (variable depending on the genomic region considered) as demarcation criteria for assigning viruses to genera and species, respectively. This approach has essentially confirmed the classification shown in **Table 1**. An example of phylogenetic analysis is shown in **Figure 2**. Subgrouping viruses, particularly between members of the large genus *Potyvirus*, is also contemplated as a possibility for future refinements in classification, and could follow host range restriction as biological demarcation criteria. Information about taxonomy and sequence of members of the family *Potyviridae* is available at several web-based databases (**Table 2**).

Table 1 Classification of members of the family *Potyviridae* into genera, with indication of type member, number of virus species, transmission vectors, and number of genomic RNAs

		Number of species[a]				
Genus	*Type member*	*Definitive*	*Tentative*	*Genome*	*Vector*[b]	*Particle*[c] *length (nm)*
Bymovirus	Barley yellow mosaic virus (BYMV)	6		Bipartite	Fungi	250–300 500–600
Ipomovirus	Sweet potato mild mottle virus (SPMMV)	2	2	Monopartite	Whiteflies	900
Macluravirus	Maclura mosaic virus (MacMV)	3		Monopartite	Aphids	650–675
Potyvirus	Potato virus Y (PVY)	112	87	Monopartite	Aphids	700–900
Rymovirus	Ryegrass mosaic virus (RyMV)	4	1	Monopartite	Mites	700
Tritimovirus	Wheat streak mosaic virus (WSMV)	2		Monopartite	Mites	700

[a]Number of species according to the International Committee on Taxonomy of Viruses.
[b]Vector organisms responsible for transmission.
[c]Approximate length of virus particles.

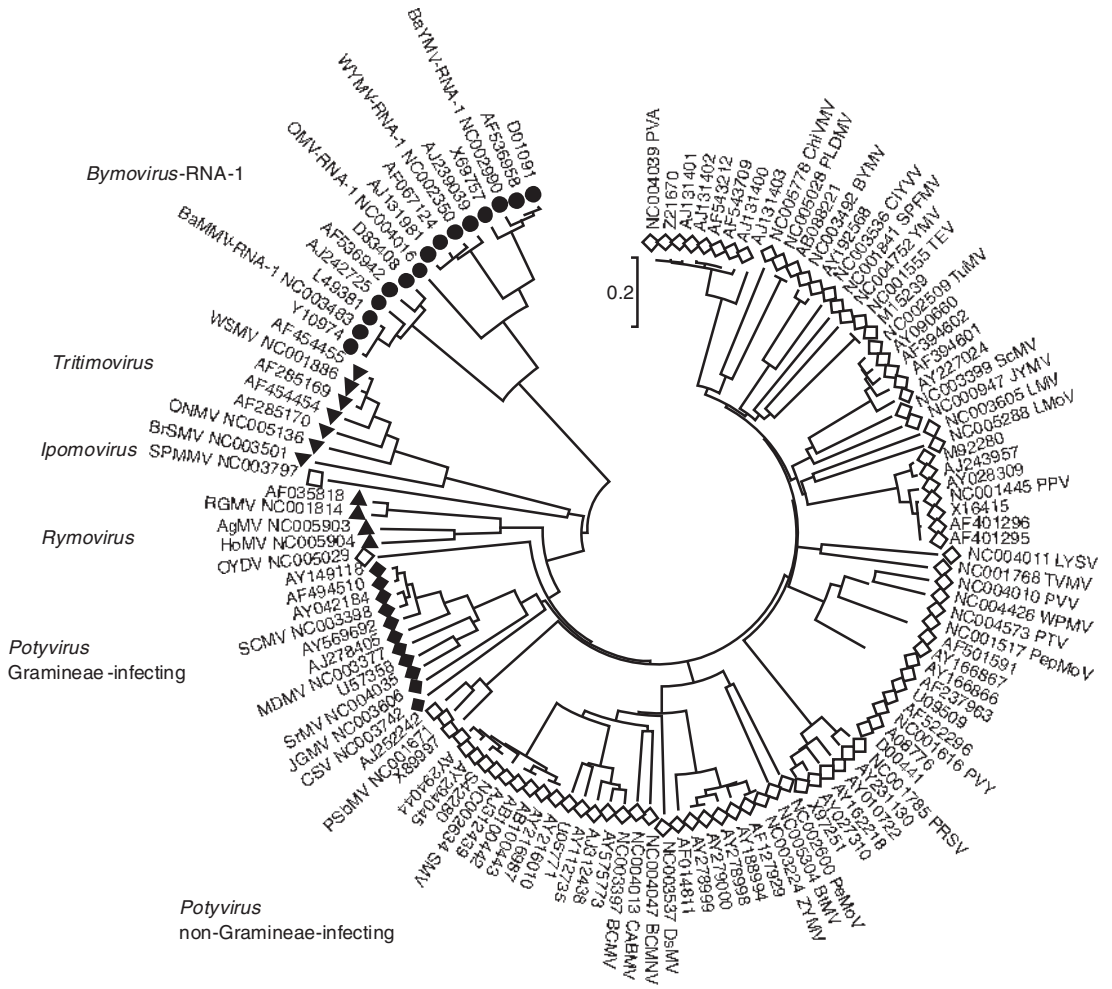


Figure 2 Phylogenetic analysis derived from the comparison of over 120 full-length viral genome sequences of 48 members of the family *Potyviridae*. The figure shows a neighbor-joining tree derived from a ClustalX alignment. Individual viral sequences, indicated by GenBank accession numbers and International Committee on Taxonomy of Viruses (ICTV) abbreviations, are grouped by genera and identified by symbols corresponding to potyviruses (diamonds, open or filled for viruses infecting non-gramineous or gramineous host plants, respectively), rymoviruses (triangles), one ipomovirus (open square), tritimoviruses (inverted triangles), and bymoviruses (circles). For bymoviruses, only the RNA-1 was used for comparison. Reproduced from Wang H, Huang LF, and Cooper JI (2006) Analysis on mutation patterns, detection of population bottlenecks, and suggestion of deleterious-compensatory evolution among members of the genus *Potyvirus*. *Archives of Virology* 151: 1625–1633, with permission from Springer-Verlag.

Table 2 Selection of resources available on the Internet (World Wide Web) for the family *Potyviridae*

Web page address (URL)	*Contents and characteristics*
http://www.ictvdb.rothamsted.ac.uk/Ictv/fs_potyv.htm	ICTV (International Committe on Taxonomy of Viruses): taxonomy structure, list of species
http://image.fs.uidaho.edu/vide/genus039.htm	VIDE (Virus Identification Data Exchange) project: nomenclature, host range, virion properties
http://www.danforthcenter.org/iltab/potyviridae/index.htm	Taxonomy, references, and sequence databases of members of the family
http://www.dpvweb.net/potycleavage/index.html	Analysis of the polyprotein cleavage sites

Virion Properties

Virion particles of viruses belonging to the family *Potyviridae* are flexuous rods constituted of protein (95%) and RNA (5%) (**Figure 1(a)**). Their size is about 11–15 nm wide, with lengths ranging from less than 700 nm in the case of macluraviruses to up to 900 nm in ipomoviruses (**Table 1**). Available data on particle structure suggest an helical assembly of identical coat protein (CP) subunits (about 2000) surrounding the nucleic acid, and a distribution of 7–8 subunits per turn has been suggested for the tritimovirus wheat streak mosaic virus (WSMV).

Molecular weight of CP subunits ranges between 30–40 kDa, with differences mainly due to a variable length of the N-terminal region. A more conserved internal CP core (about 220 amino acids) is probably involved in particle architecture. Both N- and C-termini are exposed at the surface of the particle. Superficially located residues might interact with other proteins during essential processes of the virus life cycle. As an example, the conserved DAG motif near the N-terminus of the CP is required for aphid transmission of tobacco vein mottling virus (TVMV). Recent studies have established the existence of host-dependent post-translational modifications, with probable regulatory functions during the viral cycle, in the CP of several potyviruses, including phosphorylation and glycosylation.

A recently identified peculiarity of potyviral particles is the presence of unusual structures at one of the ends of the long particles, probably associated to the 5′ end of the genomic RNA, in which the VPg and HC-Pro proteins were detected in some particles of purified potato virus Y (PVY) and potato virus A (PVA).

Serology and Diagnostics

Serological tools are the preferred diagnostic system of potyviruses. While the nonstructural viral proteins have limited use in serological diagnosis, the virus particles are usually strongly immunogenic. However, serological relationships among potyviruses are complex, with unexpected and inconsistent cross-reactivities that hindered their application in taxonomy. The cause of this is the presence of common epitopes in the conserved internal CP core, while specific epitopes map in the variable N-terminus, a surface-exposed region prone to degradation. New species-specific antibodies targeted against the immunodominant N-terminal region provided excellent serological detection tools applicable for many viruses.

The easy acquisition of sequence information after reverse transcription-polymerase chain reaction (RT-PCR) amplification is another way to deal with diagnosis. Degenerated primers able to amplify virtually any potyvirus have been described, for instance, targeted against conserved regions flanking the variable CP N-terminus. In combination with specific hybridization techniques or sequencing, the diagnosis is unequivocal. These molecular tools have been extensively used to identify new viruses infecting new hosts. Also, the combination of serological capture of particles with high sensitivity RT-PCR methods has resulted in robust detection systems. The application of modern molecular tools to diagnostics is being continually updated with new, more sensitive and specific approaches.

Properties of the Genome

The genome of monopartite potyviruses is an ssRNA molecule of + (messenger) sense. In all members studied so far, genomic RNA presents a 5′ terminal protein (VPg) and a 3′ polyA tail of variable length. Typical genome sizes range from 9.4 to 10.3 kbp. The genome comprises a single ORF coding for a long polyprotein (340–370 kDa) that generates mature products after ongoing an autoproteolytic processing cascade. The bipartite genome of bymoviruses is divided into two RNAs: a long RNA-1 homologous to the 3′ three-quarters portion of the monopartite potyvirus genome, and a short RNA-2 with partial similarity to portions of the 5′ region of the genome of monopartite potyviruses. Three virus-encoded proteinases are involved in the proteolytic processing of the potyviral polyprotein. The serine proteinase P1 and the cysteine proteinase HC-Pro are responsible for autocatalytic cleavages at their C-ends, and the serine proteinase NIaPro cleaves all other sites. Information on processing cleavage sites for all known viruses of the family is

available on a web-based database (**Table 2**). The dynamics of the process might have regulatory implications for the sequential appearance and accumulation of intermediate and final products (**Figure 3**).

A typical potyvirus genome starts with a 5′ noncoding region (NCR), less than 200 bp long. This leader acts as an enhancer of translation, and although the general mechanism is not fully understood, there is evidence that regulatory elements lie in this region. Studies in plum pox virus (PPV) showed that most of this region is dispensable for infectivity, although it contributes to viral competitiveness and pathogenesis, and that translation takes place by a cap-independent leaky scanning mechanism. In the case of the 5′ leader of tobacco etch virus (TEV), the existence of an internal initiation site has been suggested, with the presence of an RNA pseudoknot domain that conferred cap-independent translation.

Another NCR of about 200 bp is located at the 3′-end of the genome before the polyA tail. Putative RNA structures in this region confer pathogenic properties in TVMV, and work with TEV and clover yellow vein virus (ClYVV) demonstrated the existence of *cis* acting elements necessary for infectivity.

Properties and Functions of Gene Products

The polyprotein coded in the genome of a potyvirus comprises the following gene products (from N-terminus to C-terminus): P1, HC-Pro, P3, 6K1, CI, 6K2, NIaVPg, NIaPro, NIb, and CP. For bymoviruses, RNA-2 encodes two proteins P2-1 and P2-2, while RNA-1 encodes the remaining products starting with P3. Besides the information provided in **Figure 3**, some characteristics of these products are given in this section.

The P1 protein is a serine protease, extremely variable in size (about 30–60 kDa). In TEV, it was shown to act as an accessory factor for genome amplification. This region contains important sequence differences among potyviruses. In the ipomovirus cucumber vein yellowing virus (CVYV) two duplicated P1-like serine proteinases are found, with the second one acting as a suppressor of gene silencing to compensate for the absence of HC-Pro in this virus.

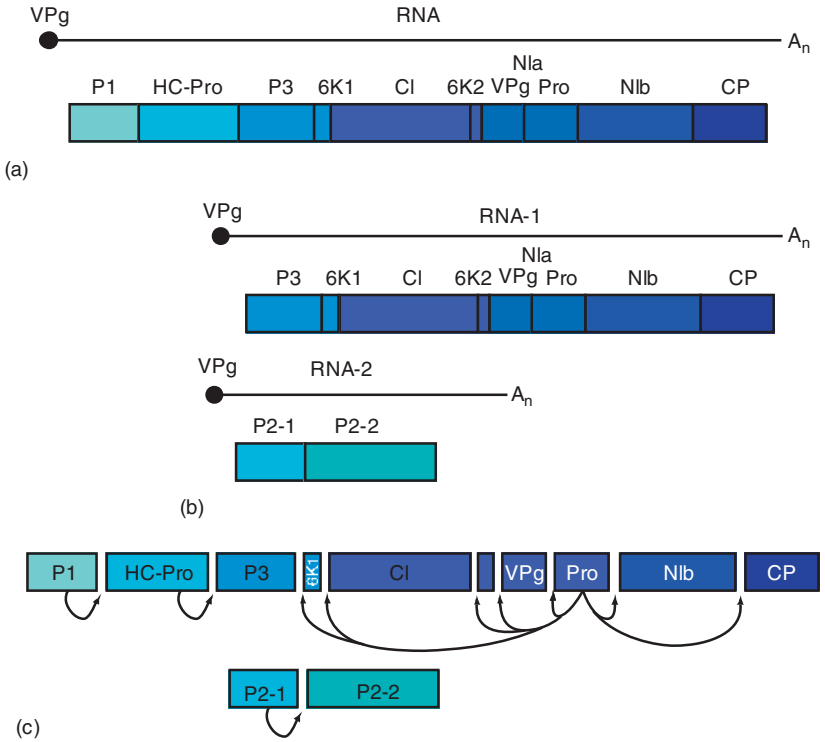
The helper component HC-Pro is a multifunctional protein originally described as a factor required for aphid transmission ('HC' in the name of the protein stands for 'helper component' and refers to this function). Recently it was shown that the HC-Pro of tritimoviruses is also implicated in mite transmission. A modular distribution of functions has been proposed in this product: the N-terminus is essential for vector transmission, the central region is involved in suppression of silencing, and the C-terminal part is a papain-like cysteine proteinase ('Pro' in the name of the protein stands for 'proteinase' and refers to this function). HC-Pro can interact with RNA, and recent data indicate that it binds to double-stranded small interfering RNA (siRNA) molecules, a feature mechanistically coincidental with other silencing suppressors of viral origin. Structural studies with lettuce mosaic virus (LMV) and TEV have demonstrated HC-Pro in pairwise oligomeric forms. Interaction of HC-Pro with several host factors has been described.

In the case of bymoviruses, very limited information regarding roles for P2-1 and P2-2 is available. It has been postulated that P2-2 might play a role in vector transmission, since nontransmissible variants exhibited deletions in this region. However, sequence alignments indicate that P2-1, a putative cysteine proteinase, is more closely related to HC-Pro than P2-2.

Except for P1 and HC-Pro, all gene products are excised from the polyprotein by the action of the proteinase domain of NIa (nuclear inclusion a). The third gene product is P3, a protein of unknown functions, which is followed by a peptide called 6K1. Despite the presence of a typical cleavage sequence, cleavage between P3 and 6K1 of TEV does not occur *in vitro*, and excision does not seem to be required for viability of PPV. Immunology studies found both P3 and the precursor P3-6K1 in infected cells, and the latter is perhaps the functional product, although recently a tagged 6K1 product was also found in infected plants. Products of the P3-6K1 region play roles in host range definition and pathogenicity of several potyviruses.

The CI protein is the largest potyviral gene product. It forms very distinctive pinwheel-shaped cylindrical inclusions in the cytoplasm of infected cells, with high taxonomic value because they are unique to members of the family *Potyviridae* (**Figure 1(b)**). CI exhibits RNA helicase activity, and is supposed to act during RNA replication. Host factors interacting with CI of PPV and TEV have been recently identified. A second small peptide, 6K2, follows CI. This product has been implicated in virus replication, probably through a proposed anchoring capability which may serve to retain the replication complex in virus-induced membrane structures in the cytoplasm. Separation of 6K2 from NIa was also proposed to regulate nuclear targeting, and recent results in PVA reveals roles for 6K2 in movement and symptom induction.

The NIa protein has about 49 kDa, although it is subjected to an internal suboptimal cleavage to originate an N-terminal VPg (21 kDa) and a C-terminal proteinase fragment (28 kDa). VPg is the protein covalently attached to the 5′ end of viral RNA. VPg can be uridylylated *in vitro* by the RNA replicase NIb, suggesting that it can be used as a primer during RNA replication as in the case of picornaviruses. VPg might be associated with host factors being a common pathogenicity determinant that also participates in long-distance movement. In PVA, VPg is translocated as a 'phloem protein' that specifically acts in companion cells to facilitate virus unloading. NIa is a



Protein	Size kDa	Properties and possible function(s)
P1	30–60	Serine proteinase S30. RNA binding. Pathogenicity and suppression of RNA silencing. Host range definition.
HC-Pro	56	Cysteine proteinase C6. RNA and siRNA binding. Vector transmission. Virus movement. Pathogenicity and suppression of RNA silencing.
P3 6K1	40 + 6	Pathogenicity. Host range definition. RNA replication? Membrane anchoring?
CI	70	RNA helicase. RNA binding. Cell-to-cell movement. RNA replication.
	6	Membrane anchoring? RNA replication?
VPg Pro Nla	21 + 28	Covalently linked to RNA. Priming of RNA synthesis. RNA binding. Pathogenicity. Virus movement. Host range definition. Cysteine proteinase C4. Deoxyribonuclease.
Nlb	58	RNA replicase.
CP	30–36	RNA encapsidation. Vector transmission. Virus movement. Symptom determination.
P2-1	25–28	Cysteine proteinase C6.
P2-2	70	Vector transmission?

(d)

Figure 3 Representative genomic maps for viruses in the family *Potyviridae*. A monopartite virus characteristic of a potyvirus is shown in (a), while a bipartite bymovirus is depicted in (b). Genomic structures of ipomo-, maclura-, rymo-, and tritimoviruses are essentially similar to that of potyviruses. ssRNA genomes are shown as solid horizontal lines with VPgs represented by solid circles at each 5′ end, and polyA tails located at each 3′ end. Viral ORFs are depicted below as boxes divided in the different viral products, with names of gene products indicated. The proteolytic processing (rendering functional proteins or regulating their activity) of a typical potyvirus ORF is shown in (c), with indication of the three protease-specific cleavages sites indicated by arrows. Processing of the ORF encoded by the RNA-2 of bymoviruses is also presented. Properties of the different potyviral proteins are shown in (d). Abbreviation names, approximate size (kDa), and some properties and possible function(s) of each protein are indicated.

serine protease with a cysteine at the active site, responsible for the majority of cleavages in the polyprotein, acting in *cis* and *trans* at specific processing sites defined by characteristic heptapeptides. The protease domain of NIa shows nonspecific RNA binding activity, and was shown to exhibit nonspecific double-stranded DNA degradation activity in pepper vein banding virus (PVBV). NIa can form, together with NIb, inclusion bodies in the nucleus (hence the name of these proteins), where it is translocated responding to a bipartite signal sequence. The crystal structure of TEV NIa has recently been solved, providing clues on its mode of action.

NIb (nuclear inclusion b) is the second component of nuclear inclusions, being directed to the nucleus by specific signals in its sequence. NIb is the RNA-dependent RNA polymerase (RdRp) responsible for viral replication. Recruitment for the replication complex is postulated to occur via interaction with NIa. All NIb functions essential for RNA amplification may be provided in *trans* since NIb supplied in transgenic plants complement TEV deletion mutants.

The last product is CP, a protein with many roles in addition to the encapsidation of virus particles, being implicated in aphid transmission through interaction with HC-Pro, and in cell-to-cell and long-distance movement. The importance of maintaining the net charge of the CP N-terminus for infectivity has been shown in TVMV and zucchini yellow mosaic virus (ZYMV).

Replication and Propagation

Potyviruses replicate in the cytoplasm of infected cells, as schematically shown in **Figure 4**. After entering the cell, the viral genomic RNA must first be translated in the cytoplasm. In general, the early events during infection are still poorly understood. Disassembly of particles might

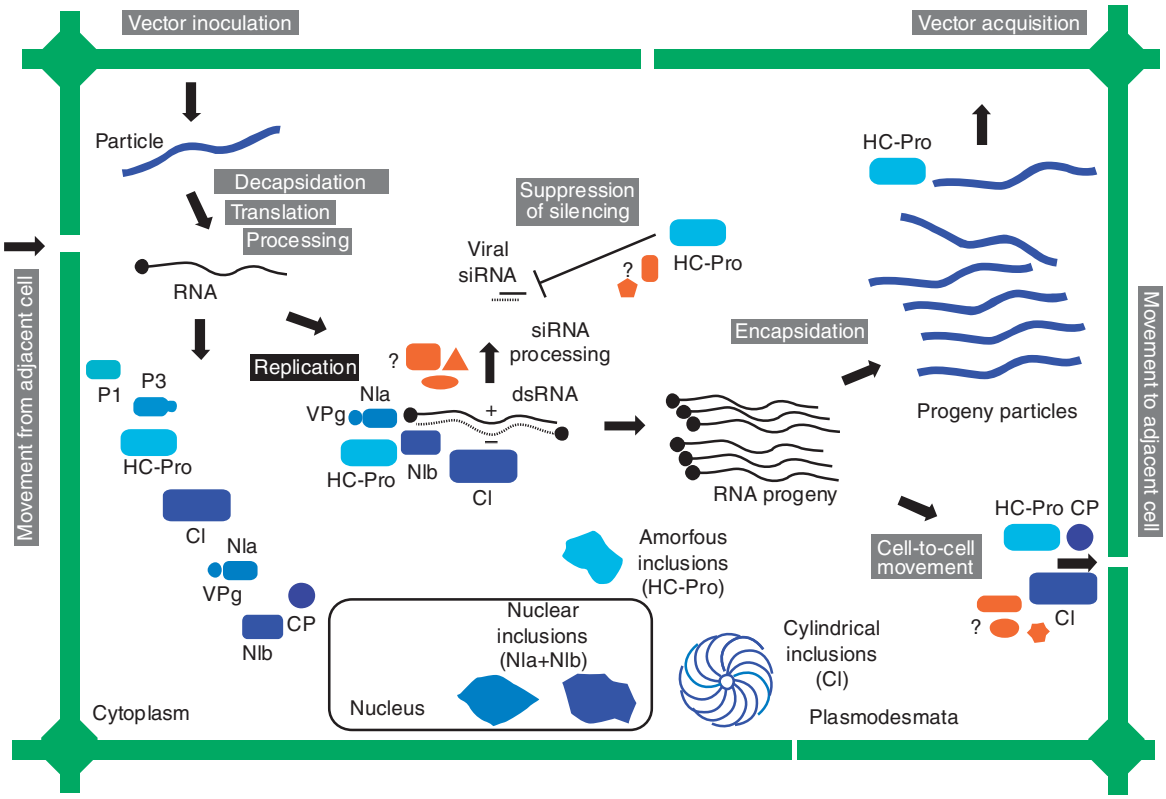


Figure 4 Schematic representation of different events during potyvirus infection of a plant cell. Colors and patterns of gene products match those displayed in **Figure 3**. The possible accumulation of viral proteins as inclusions in different compartments is also indicated. Putative host factors acting during the cycle are depicted as red color objects. The cycle begins (left upper corner) when the viral RNA enters the cell from an adjacent infected cell or a particle is initially inoculated by its vector and the genomic RNA undergoes decapsidation, translation, and processing to originate mature products. The replication complex is assembled with participation of NIb, CI, VPg, 6K2, and NIa, and probably other host-derived factors. The replication complex uses the genomic RNA (+ sense) to generate a complementary chain (sense), which serves as a template for the synthesis of numerous genomic RNAs. Different mechanisms of plant defense might be activated during infection, including activation of the RNA silencing machinery that will produce virus-specific siRNA. A suppressor of RNA silencing, the HC-Pro in potyviruses, allows the virus to overcome this plant defense (indicated by the T-shaped symbol). After replication, the RNA progeny can move to adjacent cells through plasmodesmata, in a form not totally identified with the involvement of HC-Pro, CI, VPg, and CP, or it can be encapsidated and acquired by a vector organism to be transmitted again, in a process requiring HC-Pro.

occur co-translationally, and after translation the replication complex must be formed with participation of several viral products and probably host factors. This complex uses the genomic RNA (+ sense) as template to generate a complementary chain (sense) through a dsRNA intermediate, and proceed with the asymmetric synthesis of numerous genomic RNAs. Virus specific siRNA accumulate during potyvirus infections, revealing the induction of a RNA silencing-mediated antiviral defense. Therefore, the virus needs to counterattack using a silencing suppressor, which in potyviruses was demonstrated to be the HC-Pro protein, although this function might be shared or displaced to other products in other genera. The need for several virus-encoded functions, including replication and silencing suppression, at very early stages of infection, points to the importance of viral RNA translation. Studies performed with pea seed-borne mosaic virus (PSbMV) served to identify a translation shut-off affecting many host proteins during infection. The potyvirus expression strategy through a polyprotein implies production of equimolar amounts of all gene products, giving rise to large excesses of some proteins which might remain soluble, be degraded or secreted, or end up in inclusion bodies. It is interesting that, whereas CI pinwheels are always formed, HC-Pro amorphous or NIa/NIb crystalline inclusions are present in some, but not all, potyviral infections.

Potyvirus RNA replication takes place in membranous structures probably derived mainly from the endoplasmic reticulum. NIb forms the core of the replication machinery, with involvement of CI, VPg, 6K2, and NIa. Other factors encoded by the virus or by the host might also be involved. For instance, NIa of turnip mosaic virus (TuMV) can interact with initiation factors eIF4E and eIF(iso)4E, and also with the PolyA binding protein. The fact that many potyvirus-specific resistance genes encode initiation factors points to the importance of these interactions.

Another essential process for virus infection is transport of the genomic RNA to adjacent cells. All tissues in the plant finally become invaded by the virus, with the probable exception of meristems, although a few seed-transmissible potyviruses might be capable of invading them. CP, but probably not virion formation, is essential for virus movement. In the process of local and systemic spread, again the silencing defense must be confronted by the virus in a race to avoid blockage by the transitivity diffusion of specific silencing signals.

The final step of the replicative cycle is spread of the virus to new plants, which rely on acquisition by the corresponding vector organisms and transmission. Two viral proteins, CP and HC-Pro, are involved in nonpersistent aphid transmission of potyviruses. Available data support a hypothesis in which HC-Pro serves as a reversible bridge to retain virus particles in aphid mouthparts. A conserved PTK domain in the central portion of HC-Pro could participate in binding to the DAG motif of the N-terminal region of CP, while a KITC domain at the N-terminal region of HC-Pro would be involved in binding to unknown structures of the aphid stylet. HC-Pro is also involved in semipersistent transmission of tritimoviruses by eryophid mites. Molecular information about the transmission of macluraviruses by aphids, bymoviruses by plasmodiophorids, rymoviruses by eryophid mites, and ipomoviruses by whiteflies is rather scarce.

Multifunctionality is observed in most potyviral proteins, illustrated, for instance, by HC-Pro. This capacity to participate in multiple processes suggests that potyvirus infection is not a consecutive succession of independent events, but a tightly regulated network of complex interactions between viral and host factors, still to be elucidated.

Pathogenicity

There is little information about how potyviruses cause diseases in their host plants. Several studies have served to identify sequences and/or products in the genome of potyviruses directly implicated in the production of symptoms. As mentioned above, symptom determinants are present in the 5′- and 3′-NCR. In addition, HC-Pro, P3, CI, 6K2, and VPg of different viruses have been described as determinants of pathogenicity, although single products were not always responsible for the different pathogenic responses.

As already mentioned, the P1-HC-Pro region is responsible for the synergistic effect in mixed infections of potyviruses with unrelated viruses, perhaps reflecting the capacity to interfere with RNA silencing-mediated defense. The discovery that HC-Pro is also able to affect miRNA regulated functions in plants provided a molecular explanation to some virus-induced symptoms. However, although it is tempting to regard interference with the metabolism of small RNAs in susceptible plants as a major element of pathogenicity, many other processes may also be affected. For example, specific features of the soybean mosaic virus (SMV) elicitor of the *Rsv1* resistance gene appears to be responsible for induction of either systemic mosaic or lethal systemic hypersensitive response in soybean. The fact that the HC-Pro protein of LMV targets and affects the proteasome might exemplify another way by which potyviruses can cause disease symptoms. Despite the fact that in most cases the mechanisms of pathogenicity remain uncertain, it is reasonable to conclude that the final macroscopic effects caused by potyviruses might be a combination of additive interference with several host functions.

Evolution

Members of the family *Potyviridae* are considered to belong to the picorna-like supergroup, characterized

by the same genome expression strategy, and by a well-conserved set of replication-related proteins present at equivalent positions in the genome, which could be the result of cassette evolution.

Genera in the family show a close relationship, as indicated by the homologies between gene products and their conserved order in the polyprotein. Members of each genus within the family seem to be adapted to specific vector organisms. Moreover, the adaptation to particular host species might have contributed to speciation.

One intriguing issue is the amazingly large number of aphid-transmitted potyviruses. A combination of a very efficient transmission system and easy adaptation to new hosts must have contributed to this large expansion. In particular, vector transmission acting as bottlenecks might lead to speciation events. Together with recombination, switching events, radiation, and host and geographical adaptation are proposed as major traits of evolution. Existence of subpopulations able to differentiate and evolve independently within a single infected perennial plant was observed in PPV, and similar phenomena can happen in epidemics of viruses affecting annual hosts. Experimental evidence in PVA showed that recombination of nearly identical, phenotypically similar virus genomes can give rise to new viral strains with novel virulence and symptom phenotypes. Indications of recombination events, partial duplications, point mutations that apparently confer different host responses, as well as other factors, might help to explain the extraordinary variability observed among potyviruses.

Epidemiology and Control

Strategies currently applicable for potyvirus control are diverse, from cultural practices to the use of genetic resistance. Insecticide treatments against vectors are frequently considered unsatisfactory and have limited use because of the transmission type.

Severity of outbreaks is commonly related to abundance of initial foci of infection, dynamics of vector populations, and other factors, such as the presence of weeds acting as reservoirs of viruses. Human intervention is responsible in many cases for the introduction of emerging diseases into new territories, while vector organisms are mainly involved in propagation within particular regions. In a few potyviruses, seed transmission is also an important means of dissemination. A typical example of well-documented spread of a potyvirus over a territory and over time is the progressive emergence of Sharka disease, caused by PPV, in European countries during the twentieth century, and its recent diffusion to other continents to become nowadays a global pandemic.

The exploitation of pathogen-derived resistance and RNA silencing is yielding promising results for potyvirus control. Transgenic plants incorporating viral sequences were found to be resistant to several potyviruses. An example of success is provided by the engineering of papaya varieties resistant to papaya ringspot virus (PRSV) by expression of a viral CP transgene. Nowadays, RNA silencing is being further exploited for resistance by designing specific transgenes with hairpin structures, or even by direct application of dsRNAs. Cross-protection has also been related to RNA silencing mechanisms. Other approaches which are being explored include expression of ribozymes, plantibodies or inhibitors of proteinases. Stability and biosafety issues are under evaluation in all strategies.

The identification of potyvirus-specific resistance genes is leading to important advances. Characterization of new systems suggested that canonical resistance genes might be operating, for instance TIR-NBS-LRR class in PVY, or NBS-LRR class in SMV. As mentioned, mutations in eukaryotic initiation factors are frequently found as the responsible factor for recessive resistance against potyviruses. The generation of variability in candidate genes, using TILLING or equivalent platforms, could, therefore, be a promising strategy for generation of resistant plants.

Biotechnological Applications

Biotechnological uses of potyviruses are being pursued, both for the expression of foreign sequences in plants, and as a source of genetic elements and products of potential biotechnological utility. In this second aspect, extensive use of the TEV NIa protease to remove affinity tags from fusion proteins is a good example.

Regarding the use of potyviruses as expression vectors, several systems have been tested. Small peptides can be expressed fused to the CP, allowing the chimeras to be used as antigen presentation systems for immunization or diagnosis. Complete foreign genes have been expressed in vectors based on a quite large list of potyviruses which covers an important range of potential hosts. The seminal work with TEV pointed to the P1-HC-Pro junction as an adequate insertion site, but later new sites were exploited, such as the NIb-CP junction, or the P1 region. Recent reports also demonstrated the capacity to use potyviruses as double vectors expressing simultaneously two foreign proteins. The stability of the inserted genes seems to respond to characteristics of the foreign sequence still not completely defined.

The understanding of RNA silencing phenomena and the discovery of viral suppressors can serve as a technological basis to boost expression based on the simultaneous expression of a replicating virus plus a silencing suppressor.

Transgenic plants expressing HC-Pro are being used to improve the expression levels of viral vectors.

Concluding Remarks

Analysis of potyvirus molecular biology has been extraordinarily successful in many aspects, and is likely to continue and provide interesting data. The knowledge currently available about potyvirus replication, movement, and transmission should eventually permit new control strategies to be designed to interfere with these key stages in the virus life cycle. Structure resolution of virus components might also serve to explore new resistance strategies. Diagnostic tools are continually being improved, and future developments will certainly supply specific and sensitive means of virus identification. Current understanding of the selective involvement of host factors in resistance and pathogenesis is fuelling work on virus–plant interactions, a challenging research area. Besides, peculiarities of particular viruses are being unravelled, and will help to understand both generic features and specificities of viruses in the family. Finally, the biotechnological application of potyviruses is also a field in expansion, which will provide in the future more exciting developments.

See also: Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus; Papaya Ringspot Virus; Plum Pox Virus; Potato Virus Y; Watermelon Mosaic Virus and Zucchini Yellow Mosaic Virus; Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Plant Antiviral Defense: Gene Silencing Pathway.

Further Reading

Adams MJ, Antoniw JF, and Fauquet CM (2005) Molecular criteria for genus and species discrimination within the family *Potyviridae*. *Archives of Virology* 150: 459–479.

Aranda M and Maule A (1998) Virus-induced host gene shutoff in animals and plants. *Virology* 243: 261–267.

Berger PH, Barnett OW, Brunt AA, *et al.* (2000) Family *Potyviridae*. In: Fauquet CM, Bishop DHL Carsten EB, *et al.* (eds.) *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, pp. 703–724. San Diego, CA: Academic Press.

Carrington JC, Kasschau KD, and Johansen LK (2001) Activation of suppression of RNA silencing by plant viruses. *Virology* 281: 1–5.

French R and Stenger DC (2003) Evolution of wheat streak mosaic virus: Dynamics of population growth within plants may explain limited variation. *Annual Review of Phytopathology* 41: 199–214.

Kasschau KD, Xie Z, Allen E, *et al.* (2003) P1/HC-Pro, a viral suppressor of RNA silencing, interferes with *Arabidopsis* development and miRNA function. *Developmental Cell* 4: 205–217.

López-Moya JJ, Fernández-Fernández MR, Cambra M, and García JA (2000) Biotechnological aspects of plum pox virus. *Journal of Biotechnology* 76: 121–136.

Ng JCK and Falk BW (2006) Virus–vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. *Annual Review of Phytopathology* 44: 183–212.

Revers F, LeGall O, Candresse T, and Maule AJ (1999) New advances in understanding the molecular biology of plant/potyvirus interactions. *Molecular Plant Microbe Interactions* 12: 367–376.

Riechmann JL, Laín S, and García JA (1992) Highlights and prospects of potyvirus molecular biology. *Journal of General Virology* 73: 1–16.

Robaglia C and Caranta C (2006) Translation initiation factors: A weak link in plant RNA virus infection. *Trends in Plant Science* 11: 40–45.

Shukla DD, Ward CW, and Brunt AA (1994) *The Potyviridae.* Oxon, UK: CABI.

Urcuqui-Inchima S, Haenni A-L, and Bernardi F (2001) Potyvirus proteins: A wealth of functions. *Virus Research* 74: 157–175.

Voinnet O (2005) Induction and suppression of RNA silencing: Insights from viral infections. *Nature Reviews Genetics* 6: 206–220.

Wang H, Huang LF, and Cooper JI (2006) Analysis on mutation patterns, detection of population bottlenecks, and suggestion of deleterious-compensatory evolution among members of the genus *Potyvirus*. *Archives of Virology* 151: 1625–1633.

Poxviruses

G L Smith, P Beard, and M A Skinner, Imperial College London, London, UK

© 2008 Published by Elsevier Ltd.

Introduction

Poxviruses have been isolated from birds, insects, reptiles, marsupials, and mammals. The best known is variola virus (VARV), the cause of smallpox, an extinct disease that claimed millions of victims and influenced human history. All poxviruses have complex, enveloped virions that are large enough to be visible by the light microscope and contain double-stranded DNA (dsDNA) genomes with terminal hairpins linking the two DNA strands into a single polynucleotide chain. Poxvirus genes are transcribed by the virus-encoded RNA polymerase and associated transcriptional enzymes, which are packaged into the virion. Virus morphogenesis and entry have unique features, such as the possession of a thiol-oxidoreductase system to enable disulfide bond formation and morphogenesis in the cytoplasm, and a complex of several proteins for the fusion of infecting virions with the cell membrane. The large genome enables poxviruses to encode many virulence factors that are nonessential for virus replication in cell culture but which influence the outcome of infection *in vivo*. Diseases caused by poxviruses

range from mild infections to devastating plagues, such as smallpox in man, mousepox in the laboratory mouse, and myxomatosis in the European rabbit.

Classification

The family *Poxviridae* comprises two subfamilies, the *Entomopoxvirinae* (**Table 1**) and *Chordopoxvirinae* (**Table 2**), whose members infect insects and chordates, respectively. The subfamily *Entomopoxvirinae* is divided into three genera: *Alphaentomopoxvirus*, *Betaentomopoxvirus*, and *Gammaentomopoxvirus*, which are typified by the species *Melolontha melolontha entomopoxvirus* (the virus name is abbreviated to MMEV), *Amsacta moorei entomopoxvirus 'L'* (AMEV), and *Chironomus luridus entomopoxvirus* (CLEV), respectively (**Table 1**). Alphaentomopoxviruses infect beetles (Coleoptera), betaentomopoxviruses infect butterflies and moths (Lepidoptera), and gammaentomopoxviruses infect mosquitoes and flies (Diptera). Compared to the *Chordopoxvirinae*, relatively few data are available for entomoxpoviruses and only two genomes have been sequenced. Nonetheless, these sequences revealed considerable divergence such that, although both sequenced viruses had originally been assigned to the same genus, *Melanoplus sanguinipes entomopoxvirus* (MSEV) was subsequently removed from this genus and the virus is now an unassigned member of the subfamily. Many features of the replication of entomopoxviruses are likely to be similar to those of chordopoxviruses, as exemplified by the orthopoxvirus *Vaccinia virus* (VACV). However, the morphology of entomopoxviruses differs from that of chordopoxviruses and there are also differences between the three entomopoxvirus genera (see below).

The subfamily *Chordopoxvirinae* is divided into eight genera: *Avipoxvirus*, *Capripoxvirus*, *Leporipoxvirus*, *Orthopoxvirus*, *Parapoxvirus*, *Suipoxvirus*, *Tanapoxvirus*, and *Yatapoxvirus* (**Table 2**). There are also several unassigned poxviruses that might form additional genera once their phylogenetic relationships have been established. For instance, the genome sequences of viruses isolated from Nile crocodiles (*Crocodylus niloticus*) and mule deer (*Odocoileus*

Table 1 Family *Poxviridae,* subfamily *Entomopoxvirinae*

Genus	*Species*[a] *(abbreviation of virus name)*	*Genome accession no.*	*Genome size (bp)*
Alphaentomopoxvirus	*Anomala cuprea entomopoxvirus* (ACEV)		
	Aphodius tasmaniae entomopoxvirus (ATEV)		
	Demodema boranensis entomopoxvirus (DBEV)		
	Dermolepida albohirtum entomopoxvirus (DAEV)		
	Figulus subleavis entomopoxvirus (FSEV)		
	Geotrupes sylvaticus entomopoxvirus (GSEV)		
	Melolontha melolontha entomopoxvirus (MMEV)		
Betaentomopoxvirus	*Acrobasis zelleri entomopoxvirus 'L'* (AZEV)	AF250284	232392
	Amsacta moorei entomopoxvirus 'L' (AMEV)		
	Arphia conspersa entomopoxvirus 'O' (ACOEV)		
	Choristoneura biennis entomopoxvirus 'L' (CBEV)		
	Choristoneura conflicta entomopoxvirus 'L' (CCEV)		
	Choristoneura diversuma entomopoxvirus 'L' (CDEV)		
	Choristoneura fumiferana entomopoxvirus 'L' (CFEV)		
	Chorizagrotis auxiliars entomopoxvirus 'L' (CXEV)		
	Heliothis armigera entomopoxvirus 'L' (HAVE)		
	Locusta migratoria entomopoxvirus 'O' (LMEV)		
	Oedaleus senigalensis entomopoxvirus 'O' (OSEV)		
	Operophtera brumata entomopoxvirus 'L' (OBEV)		
	Schistocera gregaria entomopoxvirus 'O' (SGEV)		
Gammaentomopoxvirus	*Aedes aegypti entomopoxvirus* (AAEV)		
	Camptochironomus tentans entomopoxvirus (CTEV)		
	Chironomus attenuatus entomopoxvirus (CAEV)		
	Chironomus luridus entomopoxvirus (CLEV)		
	Chironomus plumosus entomopoxvirus (CPEV)		
	Goeldichironomus haloprasimus entomopoxvirus (GHEV)		
Unassigned species in the subfamily	*Diachasmimorpha entomopoxvirus* (DIEV)		
Unassigned virus in the subfamily	Melanoplus sanguinipes entomopoxvirus (MSEV)	AF063866	236120

[a]Type species shown in bold.

Data from the International Committe on Taxonomy of Viruses (ICTV) (http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm) and the Poxvirus Bioinformatics Resource Center (http://www.poxvirus.org).

Table 2 Family *Poxviridae*, subfamily *Chordopoxvirinae*

Genus	Species[a] (abbreviation of virus name)	Genome accession no.	Genome size (bp)
Avipoxvirus	*Canarypox virus* (CNPV)	AY318871	359853
	Fowlpox virus (FWPV)	AF198100, AJ581527	288539, 266145
	Juncopox virus (JNPV)		
	Mynahpox virus (MYPV)		
	Pigeonpox virus (PGPV)		
	Psittacinepox virus (PSPV)		
	Quailpox virus (QUPV)		
	Sparrowpox virus (SRPV)		
	Starlingpox virus (SLPV)		
	Turkeypox virus (TKPV)		
	Crowpox virus (CRPV)		
	Peacockpox virus (PKPV)		
	Penguinpox virus (PEPV)		
Capripoxvirus	*Goatpox virus* (GTPV)	AY077836, AY077835	149723, 149599
	Lumpy skin disease virus (LSDV)	AF325528, AF409137	150773, 150793
	Sheeppox virus (SPPV)	AY077833, AY077834, NC_004002	150057, 149662, 149955
Leporipoxvirus	*Hare fibroma virus* (FIBV)		
	Myxoma virus (MYXV)	AF170726	161773
	Rabbit fibroma virus (RFV)	AF170722	159857
	Squirrel fibroma virus (SQFV)		
Molluscipoxvirus	***Molluscum contagiosum virus*** (MOCV)	U60315	190289
Orthopoxvirus	*Camelpox virus* (CMLV)	AY009089, AF438165	202205, 205719
	Cowpox virus (CPXV)	AF482758, X94355, DQ437593	224499, 223666, 228250
	Ectromelia virus (ECTV)	AF012825,	209771, 207620
	Monkeypox virus (MPXV)	AF380138, DQ011156, AY753185, DQ011157	196858, 200256, 199469, 198,780
	Raccoonpox virus (RCNV)		
	Taterapox virus (TATV)	DQ437594	198050
	Vaccinia virus (VACV)	U94848, M35027, AF095689, AY243312	177923, 191737, 189274, 194711
	Variola virus (VARV)	L22579, X69198, Y16780, DQ441447	186103, 185578, 186986, 188251
	Volepox virus (VPXV)		
	Horsepox virus (HSPV)	DQ792504	212633
	Skunkpox virus (SKPV)		
	Uasin Gishu disease virus (UGDV)		
Parapoxvirus	*Bovine papular stomatitis virus* (BPSV)	AY386265	134431
	Orf virus (ORFV)	AY386264, DQ184476	139962, 137,820
	Parapoxvirus of red deer in New Zealand (PVNZ)		
	Pseudocowpox virus (PCPV)		
	Squirrel parapoxvirus		
	Auzduk disease virus		
	Camel contagious ecthyma virus		
	Chamois contagious ecthyma virus		
	Sealpox virus		
Suipoxvirus	***Swinepox virus*** (SWPV)	AF410153	146454
Yatapoxvirus	*Tanapox virus* (TANV)		
	Yaba-like disease virus (YLDV)	AJ293568	144575
	Yaba monkey tumor virus (YMTV)	AY386371	134721
Unassigned viruses in the family	Nile crocodile poxvirus (CRV)	NC_008030	190054
	Mule deer poxvirus (DPV)	AY689436, AY689437	166259, 170560
	California harbor seal poxvirus (SPV)		
	Cotia virus (CPV)		
	Dolphin poxvirus (DOPV)		

Continued

Table 2 Continued

Genus	*Species*[a] *(abbreviation of virus name)*	*Genome accession no.*	*Genome size (bp)*
	Embu virus (ERV)		
	Grey kangaroo poxvirus (KXV)		
	Marmosetpox virus (MPV)		
	Molluscum-like poxvirus (MOV)		
	Quokka poxvirus (QPV)		
	Red kangaroo poxvirus (KPV)		
	Salanga poxvirus (SGV)		
	Spectacled caiman poxvirus (SPV)		
	Yoka poxvirus (YKV)		

[a]Type species shown in bold. Virus names in normal type (not italic) are tentative or unassigned.
Data from the International Committe on Taxonomy of Viruses (ICTV) (http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm) and the Poxvirus Bioinformatics Resource Center (http://www.poxvirus.org).

hemionus) in North America indicate that these will form additional genera. Within each genus there are distinct species and strains thereof. At least one genome sequence is available from each genus and in some cases large numbers of viruses have been sequenced. The orthopoxviruses have been studied most extensively and the poxvirus website lists genome sequences of 48 strains of VARV, 14 strains of VACV, and 9 strains of monkeypox virus (MPXV).

Virion Structure

Poxviruses have large virions with an oval or brick-shaped morphology. The best-studied poxvirus is VACV, which has dimensions of approximately 250 nm × 350 nm. Each infected cell produces two different forms of infectious virion called intracellular mature virus (IMV) and extracellular enveloped virus (EEV). These two forms differ in that EEV is surrounded by an additional membrane containing several virus proteins that are absent from IMV. The EEV form can be produced either by IMV budding through the plasma membrane or by exocytosis of a triple enveloped virus (see 'Morphogenesis', under 'Replication cycle').

The surface of the IMV particle contains surface tubules that have either an irregular appearance (in orthopoxviruses and avipoxviruses) or a basket-weave symmetry (in orf virus (ORFV), a parapoxvirus). The structure of the VACV IMV has been studied most intensively and there are differing views. Some investigators reported that the virus is surrounded by a double lipid membrane, whereas others propose that the outer layer comprises a single membrane surrounded by a protein coat. The evidence now strongly favors the one membrane model. The virus core has a dumbbell shape and there are lateral bodies of unknown function located in the core concavities. The virus core is surrounded by a palisade of protein spikes of 18 nm and the core wall contains pores through which virus mRNAs might extrude during early transcription. The virus genome is packaged in the core together with transcriptional enzymes and capsid proteins.

The structures of entomopoxvirus virions differ from those of chordopoxviruses. Virions of alphaentomopoxviruses have dimensions of approximately 350 nm × 450 nm and have a concave core with a single lateral body. The betaentomopoxvirus virions are a little smaller at about 250 nm × 350 nm with a cylindrical core and a lateral body that has a sleeve-like appearance. The gammaentomopoxvirus virions are 320 nm × 230 nm and are more like chordopoxviruses in having a biconcave core and two lateral bodies.

The general lack of symmetry of poxvirus virions contrasts with the icosahedral capsid of another family of large dsDNA viruses, the herpesviruses. Consequently, the structure of poxviruses is presently refractory to determination by current methods such as cryoelectron microscopy reconstruction and X-ray crystallography.

Genome Structure

Poxvirus genomes are dsDNA molecules that are linked at each terminus by a hairpin loop. The genome length varies from 134.4 kbp for bovine papular stomatitis virus, a parapoxvirus, to 359.9 kbp for canarypox virus, an avipoxvirus. When deproteinized, poxvirus genomes lack infectivity because the virus DNA-dependent RNA polymerase is required for transcription of poxvirus genes. The genomes of chordopoxviruses all show the same basic arrangement. Adjacent to each terminus there is an inverted terminal repeat (ITR) so that the sequence at one end of the genome is repeated at the other end in the opposite orientation. The length of the ITR varies considerably and may be less than 1 kbp and lacking protein coding sequences (e.g., VARV) or >50 kbp and containing numerous genes that are consequently diploid (e.g., some cowpox virus (CPXV) strains). Adjacent to each hairpin

there is a short conserved sequence called the concatemer resolution sequence that is required for DNA replication.

The central region (approximately 100 kbp) of chordopoxvirus genomes encodes proteins that are conserved between chordopoxviruses and (mostly) essential for virus replication. Approximately equal numbers of genes from this region are transcribed leftward and rightward. In contrast, genes within the left and right terminal regions of the genome are transcribed predominantly toward the genome termini. These genes are more variable between viruses in both their type and number, and for the most part are nonessential for virus replication. Instead, these genes encode proteins that affect virus virulence, tropism, and interactions with the host immune system. Within chordopoxviruses the gene order in the central genome region is fairly well conserved, with two exceptions. First, in the avian viruses there are inversions and transpositions of blocks of genes. Second, in both avipoxviruses and crocodilepox virus (CRV) there are groups of inserted genes that are absent from other poxvirus genomes.

The entomopoxvirus genomes contain some of the genes conserved throughout the chordopoxviruses, but differ in that these genes are scattered throughout the genome and are not arranged centrally and in the specific order found in chordopoxviruses.

The nucleotide composition of poxvirus genomes varies considerably. The two sequenced entomopoxviruses are very rich in A + T (82.2% and 81.7%). Several chordopoxviruses are also (A+T)-rich, but less so than the insect viruses. For instance, sheeppox virus (SPPV) and VACV are 75% and 67% A+T, respectively. At the other end of the spectrum, the genome of molluscum contagiosum virus (MOCV) is 36% A+T. Despite these wide variations in base composition, the encoded proteins display considerable similarity.

Gene Content

Comparisons of sequenced chordopoxviruses have identified *c.* 90 genes that are present in every virus. These genes encode proteins that are essential for virus replication, such as capsid proteins and enzymes for transcription and DNA replication. All of these genes are located in the central region of the genome, and are likely to represent the core genome of an ancestral virus from which modern poxviruses have evolved. As poxviruses evolved and adapted to particular hosts, they seem to have acquired additional genes that are located predominantly in the terminal regions of the genome and give each virus its particular host range and virulence. For instance, many of the immunomodulatory proteins expressed by orthopoxviruses are absent from viruses of avian and reptile hosts and also from MOCV, which has a different set of immunomodulators.

If the entomopoxviruses are included in the above comparison, the number of conserved genes falls to fewer than 50. As more poxvirus genomes are sequenced the number of genes conserved in all poxviruses (the minimal gene complement) is likely to be reduced.

Phylogeny

Phylogenetic comparisons of poxviruses indicate that the entomopoxviruses are quite divergent from chordopoxviruses, but the exact relationships of most entomopoxviruses remain unknown owing to lack of sequence data. However, for the chordopoxviruses the situation is clearer. Genome sequences are available for at least one member of each genus, and a phlyogenetic tree (**Figure 1**) shows that the genera *Yatapoxvirus*, *Suipoxvirus*, *Leporipoxvirus*, and *Capripoxvirus* and deerpox virus (DPV)

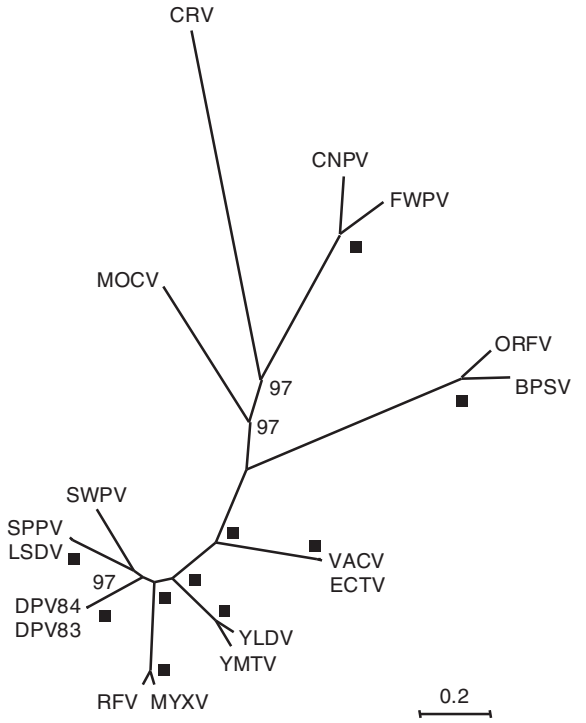


Figure 1 Phylogenetic relationships between chordopoxviruses based on a concatenated amino acid sequence alignment of 83 conserved proteins. Abbreviations: CRV, crocodilepox virus; CNPV, canarypox virus; FWPV, fowlpox virus; ORFV, orf virus; BSPV, bovine papular stomatitis virus; VACV, vaccinia virus; ECTV, ectromelia virus; YLDV, Yaba-like disease virus; YMTV, Yaba monkey tumor virus; MYXV, myxoma virus; RFV, rabbit fibroma virus; DPV, deerpox virus; LSDV, lumpy skin disease virus; SPPV, sheeppox virus; SWPV, swinepox virus; MOCV, molluscum contagiosum virus. Bootstrap values of greater than 70 are indicated at the appropriate nodes and black squares indicate values of 100. Reproduced from Afonso CL, Tulman ER, Delhon G, *et al.* (2006) Genome of crocodilepox virus. *Journal* of *Virology* 80: 4978–4991, with permission from the American Society for Microbiology.

cluster together. The orthopoxviruses are the nearest genus to this cluster, but have slightly larger genomes and other distinguishing features. The remaining genera, *Molluscipoxvirus*, *Parapoxvirus*, and *Avipoxvirus*, as well as the recently sequenced CRV are more divergent from the genera mentioned above. The avian poxviruses and CRV are most divergent and their genomes are distinguished by a lower degree of amino acid similarity, greater numbers of genes that are unique to that genus, and divergent gene order. The next most divergent genus is *Molluscipoxvirus*, which like VARV infects only humans but has very different immunomodulatory proteins compared to orthopoxviruses. Interestingly, there are some genes shared by only MOCV and CRV. Lastly, the parapoxviruses form a quite distinct genus.

Although only eight chordopoxvirus genera have been recognized so far by the International Committee for the Taxonomy of Viruses, the genomes of DPV and CRV are distinct from all other genera and so are likely to represent new genera.

Replication Cycle

Details of the poxvirus replication cycle have been obtained primarily by study of VACV and for greater detail the reader is referred to the article on that virus. Here only an overview is given.

Entry

VACV forms two distinct virion types (IMV and EEV), which are surrounded by different numbers of membranes. These different virions are also produced by viruses from several chordopoxvirus genera, and may be universal for poxviruses. IMV enters by fusion of its membrane with either the plasma membrane or the membrane of an intracellular vesicle following endocytosis. EEV entry is complicated by the second membrane that is shed on contact with the cell by a ligand-dependent nonfusogenic process. This places the IMV from within the EEV envelope against the plasma membrane and thereafter entry takes place as for free IMV. A remarkable feature of VACV fusion is the presence of a complex of nine proteins that are all required for fusion but not binding to the cell. In contrast, the fusion machinery of other enveloped viruses is often only a single protein. The fusion proteins of VACV are conserved in many other poxviruses, suggesting a common mechanism of entry. After a virus core has entered the cell, it is transported on microtubules deeper into the cell (see **Figure 2**(**d**) for an image of a virus core).

Gene Expression

Within minutes of infection the early virus genes are transcribed by the virus-associated DNA-dependent RNA polymerase within the core. The mRNAs are capped and polyadenylated, and are not spliced. They are extruded from the core and translated on host ribosomes. Early genes encode proteins that initiate DNA replication or combat the host response to infection (see 'Immune evasion' under 'Pathogenesis'). Once DNA replication has started, the intermediate class of genes is transcribed and, after expression of intermediate proteins, late transcription starts. Generally, late proteins encode structural proteins, which are expressed at higher levels than the early proteins involved in replication. Unusual features of late mRNAs are that they have a polyA tract just after the 5′ cap structure as well as at the 3′ end, and are heterogeneous in length due to a failure to terminate transcription at specific sites.

DNA Replication

DNA replication occurs in virus factories in the cytoplasm, and is catalyzed by the virus DNA polymerase and associated factors. The terminal hairpins and the adjacent concatemeric resolution sequence are essential for this process. Replication starts with the introduction of a nick near a terminal hairpin, followed by unfolding of the hairpin to enable self-priming from the free 3′-hydroxyl group. After extension to the genome terminus, separation of the parental and daughter strands, and refolding, DNA replication proceeds by strand displacement to form concatemeric molecules in either head-to-head or tail-to-tail configuration. Resolution of the concatemers occurs at the concatemer resolution sequence using the activity of a resolvase. Unit-length monomers are then packaged into virions. For additional details, see the article on VACV.

Morphogenesis

Virus assembly takes place in modified areas of the cytoplasm called factories or virosomes (**Figure 2**). The first structure seen by electron microscopy is crescent shaped, and is composed of host lipid and virus protein. This grows into immature virus (IV) in which the virus genome is packaged together with transcriptional enzymes. Once the virus membrane is sealed, the core condenses, some capsid proteins are cleaved proteolytically, and an IMV is formed. IMV may be released from cells following cell lysis either as free virions or within proteinaceous bodies. The latter are produced by some orthopoxviruses (including ectromelia virus (ECTV), raccoonpox virus, and some strains of CPXV), some avipoxviruses, and entomopoxviruses. These proteinaceous bodies are thought to give the virions extra stability outside the host until taken up by a new susceptible host. Interestingly, the proteinaceous body of entomopoxviruses is disassembled by high pH (as found in the insect gut) whereas the inclusion bodies of CPXV are disaggregated by low pH.

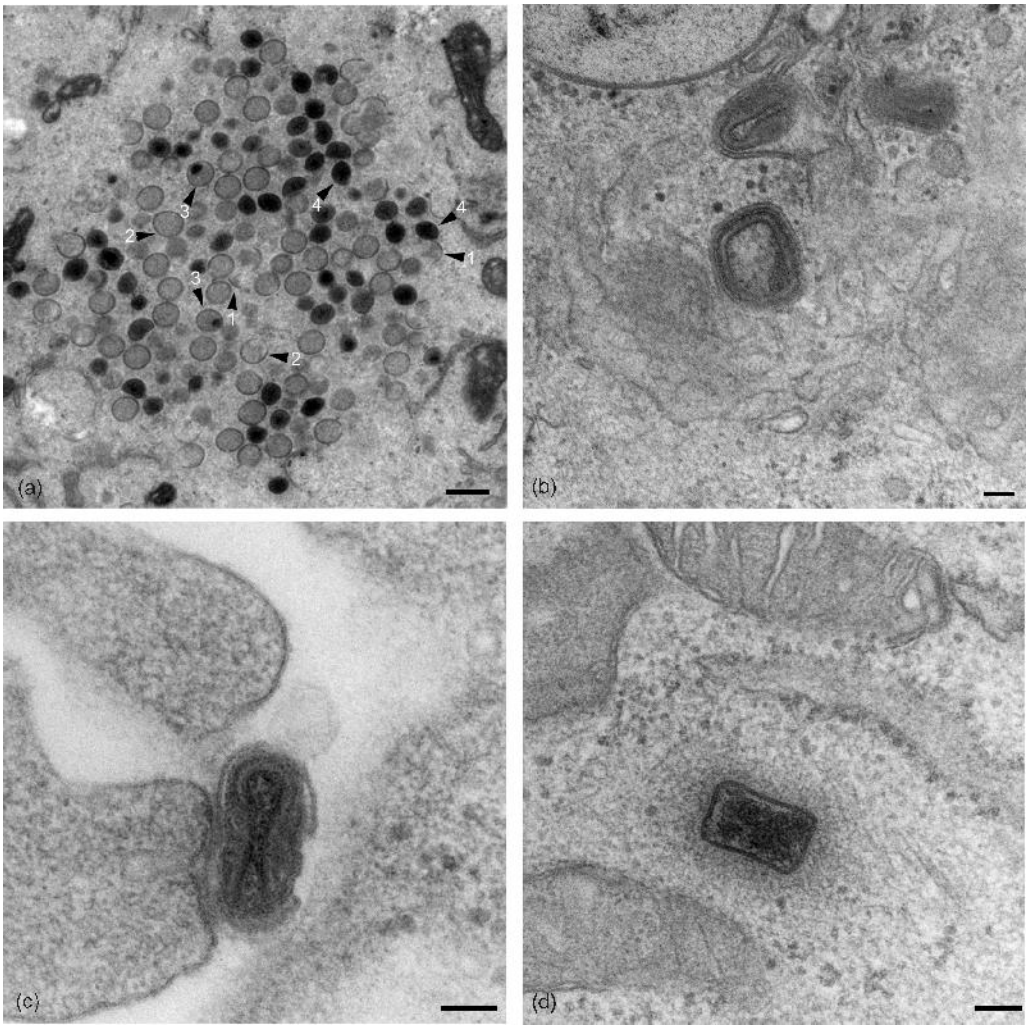


Figure 2 Electron micrographs of VACV. (a) Virus factory showing the different stages of VACV morphogenesis: (1) virus crescent; (2) immature virus, (3) immature virus showing stages of condensation and eccentric nucleoid formation, (4) IMV. (b) Wrapping of IMV to form IEV. The virion at top is partly wrapped while the virion in the center is completely wrapped. (c) CEV on the surface of an actin tail protruding from the cell surface. (d) Virus core within the cytosol shortly after infection. Scale = 500 nm (a), 100 nm (b–d). Reproduced by permission of Michael Hollinshead, Imperial College London.

Not all IMVs remain in the cell until cell lysis, as some are exported from the cell by two distinct pathways that each results in formation of EEV. The first pathway, exemplified by VACV, starts with transport of IMV away from factories on microtubules and the wrapping by a double layer of host membrane derived from the *trans*-Golgi network or early endosomes that has been modified by the insertion of virus proteins (**Figure 2(b)**). The wrapping process produces an intracellular enveloped virus (IEV), which contains three membranes, and this particle is transported to the cell surface on microtubules. At the cell surface, the outer membrane fuses with the plasma membrane to release a virion by exocytosis. The virion is called cell-associated enveloped virus (CEV) if it remains on the cell surface (**Figure 2 (c)**) and EEV if it is released. The majority of EEVs made by VACV are produced in this way. The second pathway, illustrated by fowlpox virus (FWPV), is the budding of an IMV through the plasma membrane to release EEV.

In addition to being released as EEV, CEV can also induce formation of long cellular projections, which are driven by polymerization of actin (**Figure 2(c)**). These are important for efficient cell-to-cell dissemination of virus, because virus mutants that are unable to induce formation of actin tails form small plaques.

Pathogenesis

Diseases caused by poxviruses vary greatly and may be associated with high mortality or cause minor, local, or asymptomatic infections. Examples of systemic diseases

caused by chordopoxviruses include smallpox, monkeypox, mousepox, camelpox, sheeppox, and myxomatosis.

Smallpox was the most serious human disease caused by a poxvirus and produced a systemic infection in which VARV spread sequentially through the body, culminating in the formation of skin lesions through which the virus was released to infect new hosts. A feature of smallpox that distinguished it from chickenpox (caused by varicella-zoster virus, a herpesvirus) was the centrifugal distribution of lesions (abundant on the face and limbs but less so on the trunk). The mortality rate associated with infection of immunologically naive subjects with variola major virus was 30–40%, whereas infection with variola minor virus caused only 1–2% mortality. That VARV was such a dangerous human pathogen may never be fully understood, but VARV contains many immunomodulatory proteins that subvert the host response to infection, especially the innate immune response (see below). Comparisons of the sequences of variola major and variola minor viruses revealed many differences, such that it was not possible to deduce which change(s) was (were) responsible for the large difference in virulence.

Human monkeypox is a systemic infection that is similar to smallpox but, unlike smallpox, the infection spreads very poorly from human to human. The virulence of MPXV depends on the virus strain. Viruses isolated from central Africa (e.g., Zaire) caused up to 10% mortality, whereas those from West Africa are less virulent. For instance, the zoonotic introduction of a West African strain into the USA in 2003 infected at least 37 people but no mortalities were recorded. The genomes of MPXV and VARV are significantly different, such that it is unlikely that MPXV would mutate into a VARV-like virus with efficient human-to-human transmission.

SPPV and goatpox virus (GTPV) are two very closely related members of the genus *Capripoxvirus*, and are of considerable veterinary importance because they are responsible for severe, systemic disease in sheep and goats. The viruses are endemic in parts of Africa, Asia, and the Middle East with a propensity of spreading to neighboring areas, as revealed by recent outbreaks in Vietnam, Mongolia, and Greece. The viruses are transmitted by direct contact, environmental contamination, or biting insects, and cause the most severe disease in young animals where the mortality can reach 100%. The affected animals exhibit typical poxviral disease with discrete, circumscribed cutaneous lesions most numerous in sparsely haired areas. Lesions are also found in the lung, gasterointestinal tract, and mucous membranes.

ECTV causes mousepox in inbred mouse colonies and can have very high mortality rates. For instance, in susceptible mouse strains the lethal dose 50 (LD50) of ECTV is less than one plaque-forming unit. Some features of mousepox have similarity with smallpox and consequently it is being used by some investigators as a model for smallpox. The natural host of ECTV is uncertain but is likely to be wild rodents.

Camelpox virus (CMLV) causes a severe systemic disease in camels that also has some features resembling smallpox. Sequencing of the CMLV genome showed that VARV and CMLV are closely related, although there remain many differences, especially within the terminal regions of the virus genome, so mutation of CMLV into a VARV-like virus is unlikely.

Myxomatosis is a disease caused by myxoma virus (MYXV) in the European rabbit (*Oryctolagus cuniculus*). However, the natural host for MYXV is the jungle rabbit (*Sylviagus brasiliensis*) or brush rabbit (*S. bachmani*), in which the outcome of MYXV infection is mild. MYXV infection in the European rabbit has been used as a model for poxvirus pathogenesis. MYXV virus is also famous as the virus used in Australia in the 1950s to control the European rabbit population, which increased from 13 in 1859 to more than 500 million early in the twentieth century. The spread of MYXV in Australian rabbits is an excellent example of host–pathogen evolution: very soon after release of the highly virulent virus into the rabbit population, the virus strains isolated from infected rabbits were of more modest virulence such that they did not kill all available hosts. Likewise, surviving rabbit populations were more resistant to the effects of virus infection.

Immune Evasion

Poxviruses encode many proteins that block or subvert the host response to infection. The genes encoding these proteins are mostly located within the terminal regions of the genomes and are nonessential for virus replication in cell culture. The proteins are directed predominantly against the innate response to infection, but target a wide range of host molecules both inside and outside the cell. Poxvirus intracellular proteins may block signaling pathways resulting from engagement of Toll-like receptors or receptors for cytokines or interferons. Additionally, intracellular proteins may inhibit the antiviral activity of interferon (IFN)-induced proteins or the pathways leading to induction of apoptosis. Extracellular proteins may inhibit the action of complement, cytokines, chemokines, or IFNs by binding these proteins in solution and preventing them from reaching their natural receptors on cells. Some poxviruses also subvert the cytokine or chemokine systems by encoding cytokines (e.g., interleukin-10, transforming growth factor beta) or chemokine mimics, or by the expression of chemokine receptors on the surface of infected cells. In general, the removal of individual immunomodulatory proteins has caused modest attenuation of virulence in one model or another, but the outcome is uncertain and in some cases an increase in virulence was reported. Very often, the attenuation deriving from loss of an immunomodulatory protein is modest compared to

that caused by deletion of a gene involved in virus intracellular transport or release, but there are exceptional cases where loss of an immunomodulator has caused a several log increase in the virus LD50. The wide variety of immunomodulators expressed by poxviruses makes these viruses excellent models for studying virus–host interactions and provides a logical means by which the immunogenicity of recombinant poxviruses being considered as vaccine vectors can be improved.

Acknowledgments

G. L. Smith is a Wellcome Principal Research Fellow and P. M. Beard holds a Wellcome Trust Intermediate Clinical Fellowship.

See also: Capripoxviruses; Cowpox Virus; Entomopoxviruses; Fowlpox virus and other avipoxviruses; Leporipoviruses and Suipoxviruses; Molluscum Contagiosum Virus; Mousepox and Rabbitpox Viruses; Parapoxviruses; Smallpox and Monkeypox Viruses; Vaccinia Virus; Yatapoxviruses.

Further Reading

Afonso CL, Tulman ER, Delhon G, *et al.* (2006) Genome of crocodilepox virus. *Journal of Virology* 80: 4978–4991.

Buller RM, Arif BM, Black DN, *et al.* (2005) *Poxviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 117–133. San Diego, CA: Elsevier Academic Press.

Fenner F, Henderson DA, Arita I, Jezek Z, and Ladnyi ID (1988) *Smallpox and Its Eradication.* Geneva: World Health Organization.

Fenner F, Wittek R, and Dumbell KR (1989) *The Orthopoxviruses.* London: Academic Press.

Mercer AA, Schmidt A, and Weber O (2007) *Poxviruses.* Berlin: Birkhäuser Verlag.

Moss B (2007) *Poxviridae*: The viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 2905–2946. Philadelphia, PA: Lippincott Williams and Wilkins.

Relevant Website

http://www.poxvirus.org – Poxvirus Bioinformatics Resource Center.

Prions of Vertebrates

J D F Wadsworth and J Collinge, University College London, London, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Codon 129 polymorphism There are two common forms of *PRNP* encoding either methionine or valine at codon 129; a major determinant of genetic susceptibility to and phenotypic expression of prion disease.

Conformational selection model A hypothetical model which explains transmission barriers on the basis of overlap of permissible conformations of PrP^{Sc} (prion strains) between mammalian species.

Molecular strain typing A means of rapidly differentiating prion strains by biochemical differences in PrP^{Sc}.

Prion The infectious agent causing prion diseases.

Prion incubation period The interval between exposure to prions and the development of neurological signs of prion disease; typically months even in rodent models and years to decades in humans.

Prion protein (PrP) A glycoprotein encoded by the host genome and expressed in many tissues but especially on the surface of neurons.

Prion strain Distinct isolates of prions originally identified and defined by biological characteristics which breed true in inbred mouse lines.

PRNP The human prion protein gene; mouse gene is designated *Prnp*.

Protein-only hypothesis The prions that lack a nucleic acid genome, are composed principally or solely of abnormal isomers of PrP (PrP^{Sc}), and replicate by recruitment of host PrP^{C}.

PrP Prion protein.

PrP^{C} The normal *c*ellular isoform of PrP rich in α-helical structure.

PrP^{Sc} The '*sc*rapie' or disease-associated isoform of PrP which differs from PrP^{C} in its conformation and is generally found as insoluble aggregated material rich in β-sheet structure.

Subclinical infection A state where host prion propagation is occurring but which does not produce

clinical disease during normal lifespan; essentially a carrier state of prion infection.

Transmission barrier This describes the observation that transmission of prions from one species to another is generally inefficient when compared to subsequent passage in the same host species

Introduction

The prion diseases are a closely related group of neurodegenerative conditions which affect both humans and animals. They have previously been described as the subacute spongiform encephalopathies, slow virus diseases, and transmissible dementias, and include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, and the human prion diseases, Creutzfeldt–Jakob disease (CJD), variant CJD (vCJD), Gerstmann–Sträussler–Scheinker disease (GSS), fatal familial insomnia (FFI), and kuru. While rare in humans, prion diseases are an area of intense research interest. This is first because of their unique biology, in that the transmissible agent appears to be devoid of nucleic acid and to consist of a post-translationally modified host protein. Secondly, because of the ability of these and related animal diseases to cross from one species to another, sometimes by dietary exposure, there has been widespread concern that the exposure to the epidemic of BSE poses a distinct and conceivably a severe threat to public health in the United Kingdom and other countries. The extremely prolonged and variable incubation periods of these diseases, particularly when crossing a transmission barrier, means that it will be some years before the parameters of any human epidemic can be predicted with confidence. In the meantime, we are faced with the possibility that significant numbers in the population may be incubating this disease and that they might pass it on to others via blood transfusion, blood products, tissue and organ transplantation, and other iatrogenic routes.

Abberant Prion Protein Metabolism Is the Central Feature of Prion Disease

The nature of the transmissible agent in prion disease has been a subject of heated debate for many years. The understandable initial assumption that the causative agent of 'transmissible dementias' must be some form of virus was challenged by the failure to directly demonstrate such a virus (or indeed any immunological response to it) and by the remarkable resistance of the transmissible agent to treatments that inactivate nucleic acids. These findings led to suggestions that the transmissible agent may be devoid of nucleic acid and might be a protein. Subsequently in 1982, Prusiner and co-workers isolated a protease-resistant sialoglycoprotein, designated the prion protein (PrP), that was the major constituent of infective fractions and was found to accumulate in affected brain. The term prion (from *pro*teinaceous *in*fectious particle) was proposed by Prusiner to distinguish the infectious pathogen from viruses or viroids and was defined as "small proteinaceous infectious particles that resist inactivation by procedures which modify nucleic acids."

Initially, PrP was assumed to be encoded by a gene within the putative slow virus thought to be responsible for these diseases; however, amino acid sequencing of part of PrP and the subsequent recovery of cognate cDNA clones using an isocoding mixture of oligonucleotides led to the realization that PrP was encoded by a single-copy chromosomal gene rather than by a putative viral nucleic acid.

Following these seminal discoveries, a wealth of data has now firmly established that the central and unifying hallmark of the prion diseases is the aberrant metabolism of PrP, which exists in at least two conformational states with different physicochemical properties. The normal cellular form of the protein, referred to as PrP^{C}, is a highly conserved cell surface glycosylphosphatidylinositol (GPI)-anchored sialoglycoprotein that is sensitive to protease treatment and soluble in detergents. In contrast, the disease-associated scrapie isoform, designated as PrP^{Sc}, is found only in prion-infected tissue as aggregated material, partially resistant to protease treatment, and insoluble in detergents. Due to its physicochemical properties, the precise atomic structure of the infectious particle or prion is still undetermined but considerable evidence argues that prions are composed largely, if not entirely, of an abnormal isoform of PrP. The essential role of host PrP for prion propagation and pathogenesis is demonstrated by the fact that knockout mice lacking the PrP gene ($Prnp^{o/o}$ mice) are entirely resistant to prion infection, and that reintroduction of PrP transgenes restores susceptibility to prion infection in a species-specific manner.

Human Prion Diseases Are Biologically Unique

Human prion diseases are biologically unique and can be divided etiologically into inherited, sporadic, and acquired forms. Approximately 85% of cases of human prion disease occur sporadically as Creutzfeldt–Jakob disease (sporadic CJD) at a rate of roughly 1 case per million population per year across the world, with an equal incidence in men and women. The etiology of sporadic CJD is unknown, although hypotheses include somatic *PRNP* mutation, or the spontaneous conversion of PrP^{C} into PrP^{Sc} as a rare stochastic event. Polymorphism at residue 129 of human PrP (encoding either methionine (M) or valine (V)) powerfully affects genetic susceptibility

to human prion diseases. About 38% of Europeans are homozygous for the more frequent methionine allele, 51% are heterozygous, and 11% homozygous for valine. Homozygosity at *PRNP* codon 129 predisposes to the development of sporadic and acquired CJD. Most sporadic CJD occurs in individuals homozygous for this polymorphism. This susceptibility factor is also relevant in the acquired forms of CJD, most strikingly in vCJD where all clinical cases studied so far have been homozygous for codon 129 methionine of the PrP gene *PRNP*. Additionally, a *PRNP* susceptibility haplotype has been identified indicating additional genetic susceptibility to sporadic CJD at or near the *PRNP* locus.

Approximately 15% of human prion diseases are associated with autosomal dominant pathogenic mutations in *PRNP*. How pathogenic mutations in *PRNP* cause prion disease is yet to be resolved, however, in most cases the mutation is thought to lead to an increased tendency of PrP^C to form a pathogenic PrP isoform. However experimentally manipulated mutations of the prion gene can lead to spontaneous neurodegeneration without the formation of detectable protease resistant PrP. These findings raise the question of whether all inherited forms of human prion disease invoke disease through the same mechanism, and in this regard it is currently unknown whether all are transmissible by inoculation.

Although the human prion diseases are transmissible diseases, acquired forms have, until recently, been confined to rare and unusual situations. The two most frequent causes of iatrogenic CJD occurring through medical procedures have arisen as a result of implantation of dura mater grafts and treatment with human growth hormone derived from the pituitary glands of human cadavers. Less frequent incidences of human prion disease have resulted from iatrogenic transmission of CJD during corneal transplantation, contaminated electroencephalographic (EEG) electrode implantation, and surgical operations using contaminated instruments or apparatus. The most well-known incidences of acquired prion disease in humans resulting from a dietary origin have been kuru that was caused by cannibalism among the Fore linguistic group of the Eastern Highlands in Papua New Guinea, and more recently the occurrence of variant CJD in the United Kingdom and some other countries that is causally related to human exposure to BSE in cattle. Incubation periods of acquired prion diseases in humans can be extremely prolonged, and it remains to be seen if a substantial epidemic of vCJD will occur within the UK and elsewhere.

PRNP codon 129 genotype has shown a pronounced effect on kuru incubation periods and susceptibility, and most elderly survivors of the kuru epidemic are heterozygotes. The clear survival advantage for codon 129 heterozygotes provides a powerful basis for selection pressure in the Fore. However, an analysis of worldwide haplotype diversity and allele frequency of coding and noncoding polymorphisms of *PRNP* suggests that balancing selection at this locus (in which there is more variation than expected because of heterozygote advantage) is much older and more geographically widespread. Evidence for balancing selection has been shown in only a few human genes. With biochemical and physical evidence of cannibalism on five continents, one explanation is that cannibalism resulted in several prion disease epidemics in human prehistory, thus imposing balancing selection on *PRNP*.

The Protein-Only Hypothesis of Prion Propagation

Despite extensive investigation, no evidence for a specific prion-associated nucleic acid has been found. Instead, a wide body of data now supports the idea that infectious prions consist principally or entirely of an abnormal isoform of PrP. PrP^{Sc} is derived from PrP^C by a post-translational mechanism and neither amino acid sequencing nor systematic study of known covalent post-translational modifications have shown any consistent differences between PrP^C and PrP^{Sc}. The protein-only hypothesis, in its current form, argues that prion propagation occurs through PrP^{Sc} acting to replicate itself with high fidelity by recruiting endogenous PrP^C and that this conversion involves only conformational change. However, the underlying molecular events during infection that lead to the conversion of PrP^C to PrP^{Sc} and how PrP^{Sc} accumulation leads to neurodegeneration remain poorly defined.

The most coherent and general model thus far proposed is that the protein, PrP, fluctuates between a dominant native state, PrP^C, and a series of minor conformations, one or a set of which can self-associate in an ordered manner to produce a stable supramolecular structure, PrP^{Sc}, composed of misfolded PrP monomers. Once a stable 'seed' structure is formed, PrP can then be recruited leading to an explosive, autocatalytic formation of PrP^{Sc}. Such a mechanism could underlie prion propagation and account for the transmitted, sporadic, and inherited etiologies of prion disease. Initiation of a pathogenic self-propagating conversion reaction, with accumulation of aggregated PrP, may be induced by exposure to a 'seed' of aggregated PrP following prion inoculation, or as a rare stochastic conformational change, or as an inevitable consequence of expression of a pathogenic PrP^C mutant which is predisposed to form misfolded PrP. It is now clear that a full understanding of prion propagation will require knowledge of both the structure of PrP^C and PrP^{Sc} and the mechanism of conversion between them.

Structure and Putative Function of PrP^C

PrP is highly conserved among mammals, has been identified in marsupials, birds, amphibians, and fish, and may be present in all vertebrates. It is expressed during early

embryogenesis and is found in most tissues in the adult with the highest levels of expression in the central nervous system, in particular in association with synaptic membranes. PrP is also widely expressed in cells of the immune system. As a GPI-anchored cell surface glycoprotein, it has been speculated that PrP may have a role in cell adhesion or signaling processes, but its precise cellular function has remained obscure.

Mice lacking PrP as a result of gene knockout (*Prnp*$^{o/o}$ mice) show no gross phenotype; however, these mice are completely resistant to prion disease following inoculation and do not replicate prions. *Prnp*$^{o/o}$ mice do however show subtle abnormalities in synaptic physiology and in circadian rhythms and sleep. While the relative normality of *Prnp*$^{o/o}$ mice was thought to result from effective adaptive changes during development, data from *Prnp* conditional knockout mice suggest this is not the case. These mice undergo ablation of neuronal PrP expression at 9 weeks of age and remain healthy without evidence of neurodegeneration or an overt clinical phenotype. Thus, acute loss of neuronal PrP in adulthood is tolerated and the pathophysiology of prion diseases appears to be unrelated to loss of normal PrP function in neurons.

Nuclear magnetic resonance (NMR) measurements and crystallographic determination of PrP from numerous mammalian species, including human PrP, show that they have essentially the same conformation. Following cleavage of an N-terminal signal peptide, and removal of a C-terminal peptide on addition of a GPI anchor, the mature PrP^{C} species consists of an N-terminal region of about 100 amino acids which is unstructured in the isolated molecule in solution and a C-terminal segment, also around 100 amino acids in length. The C-terminal domain is folded into a largely α-helical conformation (three α-helices and a short antiparallel β-sheet) and stabilized by a single disulfide bond linking helices 2 and 3. There are two asparagine-linked glycosylation sites. The N-terminal region contains a segment of five repeats of an eight-amino-acid sequence (the octapeptide-repeat region), expansion of which by insertional mutation leads to inherited prion disease. While unstructured in the isolated molecule, it seems likely that the N-terminal region of PrP may acquire coordinated structure *in vivo* through coordination of either Cu^{2+}or Zn^{2+} ions.

Structural Properties of PrP^{Sc}

PrP^{Sc} is extracted from affected brains as highly aggregated, detergent insoluble material that is not amenable to high-resolution structural techniques. However, Fourier transform infrared spectroscopic methods show that PrP^{Sc}, in sharp contrast to PrP^{C}, has a high β-sheet content. PrP^{Sc} is covalently indistinguishable from PrP^{C} but can be distinguished from PrP^{C} by its partial resistance to proteolysis and its marked insolubility in detergents. Under conditions in which PrP^{C} exists as a detergent-soluble monomer and is completely degraded by the nonspecific protease, proteinase K, PrP^{Sc} exists in an aggregated form with the C-terminal two-thirds of the protein showing marked resistance to proteolytic degradation leading to the generation of N-terminally truncated fragments of di-, mono-, and nonglycosylated PrP (**Figure 1**). While there is no evidence for a specific prion-associated nucleic acid or other protein components, purified prion rods do however contain an inert polysaccharide scaffold.

Defining the precise molecular events that occur during the conversion of PrP^{C} to the infectious isoform of PrP is of paramount importance as this process is a prime target for therapeutic intervention. Direct *in vitro* mixing experiments have been performed in an attempt to produce PrP^{Sc}. In such experiments, PrP^{Sc} is used in excess as a seed to convert PrP^{C} to a protease-resistant form, designated PrP^{Res}. While there are now many examples in the literature of conditions that generate PrP^{Res}, historically such reactions have not been able to demonstrate *de novo* production of prion infectivity. Recently, however, a protein misfolding cyclic amplification system has demonstrated substantial amplification of PrP^{Res} and prion infectivity and may now provide the means to systematically investigate whether the generation of an infectious PrP isoform requires additional, as yet unknown, cofactors for the acquisition of infectivity.

The difficulty in performing structural studies on native PrP^{Sc} has led to attempts to produce soluble β-sheet-rich forms of recombinant PrP which may be amenable to NMR or crystallographic structure determination. Conditions have now been identified in which the PrP polypeptide can be converted between alternative folded conformations representative of PrP^{C} and PrP^{Sc}. At neutral or basic pH PrP adopts an α-helical fold representative of PrP^{C} and this conformation is locked by the presence of the native

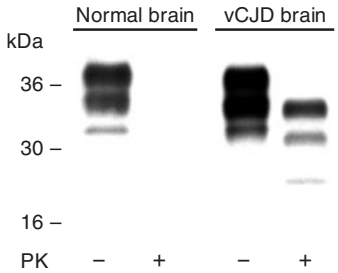


Figure 1 PrP analysis by immunoblotting. Immunoblot analysis of normal human brain and vCJD brain homogenate before and after treatment with proteinase K (PK). PrP^{C} in both normal and vCJD brain is completely degraded by proteinase K, whereas PrP^{Sc} present in vCJD brain shows resistance to proteolytic degradation leading to the generation of N-terminally truncated fragments of di-, mono-, and nonglycosylated PrP.

disulfide bond. Upon reduction of the disulfide bond, PrP^C rearranges to a predominantly β-sheet structure. This alternative conformation, designated β-PrP, is only populated at acidic pH with the PrP^C conformation predominating at neutral pH. Importantly, β-PrP shares overlapping properties with PrP^{Sc}, including partial resistance to proteolysis and a propensity to aggregate into fibrils. Success in producing disease in experimental animals that can be serially propagated following inoculation with PrP^{Sc}-like forms derived from recombinant PrP would not only prove the protein-only hypothesis, but would also provide an essential model by which the mechanism of prion propagation can be understood in molecular detail. In this regard, it has been recently reported that intracerebral injection of a β-sheet-rich fibrillar preparation of N-terminally truncated recombinant mouse PrP (comprising residues 89–230) into mice overexpressing PrP with the same deletion caused neurological disease after about 520 days.

Prion Disease Pathogenesis

Although the pathological consequences of prion infection occur in the central nervous system and experimental transmission of these diseases is most efficiently accomplished by intracerebral inoculation, most natural infections do not occur by these means. Indeed, administration to sites other than the central nervous system is known to be associated with much longer incubation periods, which in humans may extend to 50 years or more. Experimental evidence suggests that this latent period is associated with clinically silent prion replication in lymphoreticular tissue, whereas neuroinvasion takes place later. The M-cells in the intestinal epithelium appear to mediate prion entry from the gastrointestinal lumen into the body, and follicular dendritic cells (FDCs) are thought to be essential for prion replication and for accumulation of disease-associated PrP^{Sc} within secondary lymphoid organs. B-cell-deficient mice are resistant to intraperitoneal inoculation with prions probably because of their involvement with FDC maturation and maintenance. However, neuroinvasion is possible without FDCs, indicating that other peripheral cell types can replicate prions. The interface between FDCs and sympathetic nerves represents a critical site for the transfer of lymphoid prions into the nervous system; however, the mechanism by which this is achieved remains unknown. Distinct forms of prion disease show differences in lymphoreticular involvement that may be related to the etiology of the disease or to divergent properties of distinct prion strains. For example, the tissue distribution of PrP^{Sc} in vCJD differs strikingly from that in classical CJD, with uniform and prominent involvement of lymphoreticular tissues, with the highest amounts (up to 10% of brain concentrations) in tonsil. In contrast, in sporadic CJD, PrP^{Sc} has only been irregularly detected by immunoblotting in noncentral nervous system tissues at very much lower levels. Tonsil biopsy is used for diagnosis of vCJD and to date has shown 100% sensitivity and specificity for diagnosis of vCJD at an early clinical stage; tonsil is the tissue of choice for prospective studies investigating the prevalence of vCJD prion infection within the UK and other populations. The demonstration of extensive lymphoreticular involvement in the peripheral pathogenesis of vCJD raises concerns that iatrogenic transmission of vCJD prions through medical procedures may be a major public health issue. Prions resist many conventional sterilization procedures and surgical stainless steel-bound prions transmit disease with remarkable efficiency when implanted into mice. Disturbingly, cases of transfusion-associated vCJD prion infection have also now been reported. In contrast, there is no epidemiological evidence of transmission of classical CJD via blood transfusion or blood products.

Microscopic examination of the central nervous system of humans or animals with prion disease reveals typical characteristic histopathologic changes, consisting of neuronal vacuolation and degeneration, which gives the cerebral gray matter a microvacuolated or 'spongiform' appearance, and a reactive proliferation of astroglial cells (**Figure 2**). Demonstration of abnormal PrP immunoreactivity, or more specifically biochemical detection of PrP^{Sc} in brain material by immunoblotting techniques, is diagnostic of prion disease (**Figures 1** and **2**) and some forms of prion disease are characterized by deposition of amyloid plaques composed of insoluble aggregates of PrP. The histopathological features of vCJD are remarkably consistent and distinguish it from other human prion diseases with large numbers of PrP-positive amyloid plaques that differ in morphology from the plaques seen in kuru and GSS in that the surrounding tissue takes on a microvacuolated appearance, giving the plaques a florid appearance (**Figure 2**).

Prion Strains

A major problem for the 'protein-only' hypothesis of prion propagation has been to explain the existence of multiple isolates, or strains, of prions. Prion strains are distinguished by their biological properties: they produce distinct incubation periods and patterns of neuropathological targeting (so-called lesion profiles) in defined inbred mouse lines. As they can be serially propagated in inbred mice with the same *Prnp* genotype, they cannot be encoded by differences in PrP primary structure. Usually, distinct strains of conventional pathogen are explained by differences in their nucleic acid genome. However, in the absence of such a scrapie genome, alternative possibilities must be considered.

Support for the contention that prion strain specificity may be encoded by PrP itself was provided by study of two

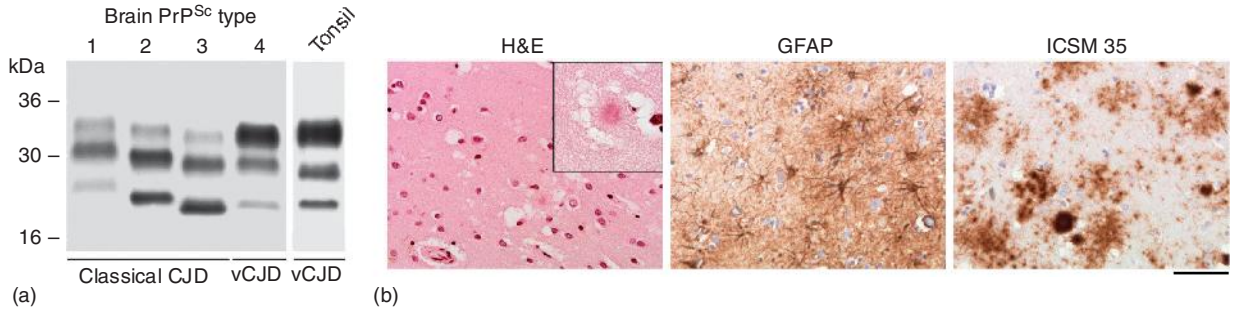


Figure 2 Characterization of disease-related prion protein in human prion disease. (a) Immunoblots of proteinase K-digested tissue homogenate with monoclonal antibody 3F4 showing PrP^{Sc} types 1–4 in human brain and type PrP^{Sc} type 4t in vCJD tonsil. Types 1–3 PrP^{Sc} are seen in the brain of classical forms of CJD (either sporadic or iatrogenic CJD), while type 4 PrP^{Sc} and type 4t PrP^{Sc} are uniquely seen vCJD brain or tonsil, respectively. (b) Brain from a patient with vCJD showing spongiform neurodegeneration following hematoxylin- and eosin staining (H&E), reactive proliferation of astroglial cells following staining with a monoclonal antibody recognizing glial-fibrillary acidic protein (GFAP), and abnormal PrP immunoreactivity following immunohistochemistry using anti-PrP monoclonal antibody ICSM 35 (ICSM 35). Scale (main panels) = 100 μm. Inset, high-power magnification of florid PrP plaques. Courtesy of Professor Sebastian Brandner.

distinct strains of transmissible mink encephalopathy prions which can be serially propagated in hamsters, designated 'hyper' and 'drowsy'. These strains can be distinguished by differing physicochemical properties of the accumulated PrP^{Sc} in the brains of affected hamsters. Following limited proteolysis, strain-specific migration patterns of PrP^{Sc} on Western blots are seen which relate to different N-terminal ends of PrP^{Sc} following protease treatment implying differing conformations of PrP^{Sc}. Distinct PrP^{Sc} conformations are now recognized to be associated with other prion strains and, similarly, different human PrP^{Sc} isoforms have been found to propagate in the brain of patients with phenotypically distinct forms of CJD.

The different fragment sizes seen on Western blots, following treatment with proteinase K, suggests that there are several different human PrP^{Sc} conformations, referred to as molecular strain types. These types can be further classified by the ratio of the three PrP bands seen after protease digestion, corresponding to N-terminally truncated cleavage products generated from di-, mono-, or nonglycosylated PrP^{Sc}. Four types of human PrP^{Sc} have now been commonly identified using molecular strain typing (**Figure 2**), although much greater heterogeneity seems likely. Efforts to produce an unified international classification and nomenclature of human PrP^{Sc} types has been complicated by the fact that the N-terminal conformation of some PrP^{Sc} subtypes seen in sporadic CJD can be altered *in vitro* via changes in metal-ion occupancy or solvent pH. Although agreement is yet to be reached on methodological differences, nomenclature and the biological importance of relatively subtle biochemical differences in PrP^{Sc}, there is strong agreement between laboratories that phenotypic diversity in human prion disease relates to the propagation of disease-related PrP isoforms with distinct physicochemical properties. Polymorphism at *PRNP* residue 129 appears to dictate the propagation of distinct PrP^{Sc} types in humans and it has now become clear that prion strain selection and the propagation of distinct PrP^{Sc} types may also be crucially influenced by other genetic loci of the host genome.

The hypothesis that alternative conformations or assembly states of PrP^{Sc} provide the molecular substrate for clinicopathological heterogeneity seen in human prion diseases (and that this relates to the existence of distinct human prion strains) has been strongly supported by transmission experiments to conventional and transgenic mice. Transgenic mice expressing only human PrP with either valine or methionine at residue 129 have shown that this polymorphism constrains both the propagation of distinct human PrP^{Sc} conformers and the occurrence of associated patterns of neuropathology. Biophysical measurements suggest that this powerful effect of residue 129 on prion strain selection is likely to be mediated via its effect on the conformation of PrP^{Sc} or its precursors or on the kinetics of their formation, as it has no measurable effect on the folding, dynamics, or stability of PrP^{C}. These data are consistent with a conformational selection model of prion transmission barriers and strongly support the 'protein only' hypothesis of infectivity by suggesting that prion strain variation is encoded by a combination of PrP conformation and glycosylation. These findings also provide a molecular basis for *PRNP* codon 129 as a major locus influencing both prion disease susceptibility and phenotype in humans.

The identification of strain-specific PrP^{Sc} structural properties now allows an etiology-based classification of human prion disease by typing of the infectious agent itself. Stratification of all human prion disease cases by PrP^{Sc} type will enable rapid recognition of any change in relative frequencies of particular PrP^{Sc} subtypes in relation to either BSE exposure patterns or iatrogenic sources of vCJD prions. This technique may also be applicable in determining whether BSE has been transmitted to other species thereby posing a threat to human health.

Neuronal Cell Death in Prion Disease

Although various mechanisms have been proposed to explain neuronal death in prion disease, the precise structure of the infectious agent and the cause of neuronal cell death in prion disease remains unclear. While PrP^C is absolutely required for prion propagation and neurotoxicity, knockout of PrP^C in adult brain and in embryonic models has no overt phenotypic effect, effectively excluding loss of of PrP^C function in neurons as a significant mechanism in prion neurodegeneration. Notably, however, there is also considerable evidence that argues that PrP^{Sc} and indeed prions (whether or not they are identical) may not themselves be highly neurotoxic. Consequently, it is now hypothesized that the neurotoxic prion molecule may not be PrP^{Sc} itself, but a toxic intermediate that is produced in the process of conversion of PrP^C to PrP^{Sc}, with PrP^{Sc}, present as highly aggregated material, being a relatively inert end product. The steady-state level of such a toxic monomeric or oligomeric PrP intermediate, designated PrP^L (for lethal), could determine the rate of neurodegeneration. Subclinical prion infection states may generate the toxic intermediate at extremely low levels below the threshold required for neurotoxicity. Recently, direct support for this hypothesis has been demonstrated by depleting endogenous neuronal PrP^C in mice with established neuroinvasive prion infection. This depletion of PrP^C reverses early spongiform change and prevents neuronal loss and progression to clinical prion disease despite the accumulation of extraneuronal PrP^{Sc} to levels seen in terminally ill wild-type mice. These data establish that propagation of non-neuronal PrP^{Sc} is not pathogenic, but arresting the continued conversion of PrP^C to PrP^{Sc} within neurons during scrapie infection prevents prion neurotoxicity. Importantly, this model also validates PrP^C as a key therapeutic target in prion disease.

Future Perspective

The novel pathogenic mechanisms involved in prion propagation are likely to be of wider significance and may be relevant to other neurological and non-neurological illnesses. Indeed, advances in understanding prion neurodegeneration are already casting considerable light on related mechanisms in other, commoner, neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's disease. While the protein-only hypothesis of prion propagation is supported by compelling experimental data and now appears also able to encompass the phenomenon of prion strain diversity, the goal of systematically producing prions *in vitro* remains. Success in producing disease in experimental animals that can be serially propagated following inoculation with PrP^{Sc}-like forms derived from recombinant PrP would provide an essential model by which the mechanism of prion propagation can be understood in molecular detail.

See also: Prions of Yeast and Fungi.

Further Reading

Caughey B and Baron GS (2006) Prions and their partners in crime. *Nature* 443: 803–810.
Collinge J (1999) Variant Creutzfeldt–Jakob disease. *Lancet* 354: 317–323.
Collinge J (2001) Prion diseases of humans and animals: Their causes and molecular basis. *Annual Review of Neuroscience* 24: 519–550.
Collinge J (2005) Molecular neurology of prion disease. *Journal of Neurology, Neurosurgery, and Psychiatry* 76: 906–919.
Collinge J, Sidle KCL, Meads J, Ironside J, and Hill AF (1996) Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. *Nature* 383: 685–690.
Griffith JS (1967) Self replication and scrapie. *Nature* 215: 1043–1044.
Hill AF and Collinge J (2003) Subclinical prion infection. *Trends in Microbiology* 11: 578–584.
Mabbott NA and MacPherson GG (2006) Prions and their lethal journey to the brain. *Nature Reviews Microbiology* 4: 201–211.
Mallucci G and Collinge J (2005) Rational targeting for prion therapeutics. *Nature Reviews Neuroscience* 6: 23–34.
Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. *Science* 216: 136–144.
Prusiner SB (1998) Prions. *Proceedings of the National Academy of Sciences, USA* 95: 13363–13383.
Soto C (2004) Diagnosing prion diseases: Needs, challenges and hopes. *Nature Reviews Microbiology* 2: 809–819.
Weissmann C (2004) The state of the prion. *Nature Reviews Microbiology* 2: 861–871.
Wuthrich K and Riek R (2001) Three-dimensional structures of prion proteins. *Advances in Protein Chemistry* 57: 55–82.

Prions of Yeast and Fungi

R B Wickner, H Edskes, T Nakayashiki, F Shewmaker, L McCann, A Engel, and D Kryndushkin, National Institutes of Health, Bethesda, MD, USA

Published by Elsevier Ltd.

Glossary

Nonchromosomal gene A gene that segregates 4+:0 in meiosis and can be transferred by cytoplasmic mixing, in contrast to chromosomal genes that segregate 2+:2– in meiosis, and are not transferred by cytoplasmic mixing.

Nonsense suppressor tRNA A mutant transfer RNA that recognizes a translational stop codon and inserts an amino acid thus allowing the peptide chain to continue.

Introduction and History

The word prion, meaning 'infectious protein' without need for a nucleic acid, was coined to explain the properties of the agent producing the mammalian transmissible spongiform encephalopathies (TSEs), although 25 years later there remains some debate if the TSEs are indeed caused by prions. The yeast and fungal prions were identified by their unique genetic properties which were unexpected for any nucleic acid replicon, but specifically predicted for an infectious protein.

[PSI] was described by Brian Cox in 1965 as a nonchromosomal genetic element that increased the efficiency of a weak nonsense suppressor transfer RNA (tRNA). [URE3] was described by Francois Lacroute as a nonchromosomal gene that relieved nitrogen catabolite repression, allowing expression of genes needed for utilizing poor nitrogen sources even when a good nitrogen source was available. The [Het-s] prion was described in 1952 by Rizet as a nonchromosomal gene needed for heterokaryon incompatibility in *Podospora anserina*. Each of these elements was later found to be a prion. The [PIN] prion was discovered in 1997 by Derkatch and Liebman in their studies of *de novo* generation of the [PSI] prion.

Genetic Signature of a Prion

Viruses of yeast and fungi generally do not exit one cell and enter another, but spread by cell–cell fusion, as in mating or heterokaryon formation. Infectious proteins (prions) should likewise be nonchromosomal genetic elements. To distinguish prions from nucleic acids, three genetic criteria were proposed (**Figure 1**): (1) If a prion can be cured, it can reappear in the cured strain at some low frequency. (2) Overproduction of the protein capable of being a prion should increase the frequency of the prion arising *de novo*. (3) If the prion produces a phenotype by the simple inactivation of the protein, then this phenotype should resemble the phenotype of mutation of the gene encoding the protein, which gene must be needed for prion propagation.

All three criteria were satisfied by [PSI] and [URE3], strongly indicating, perhaps proving, that they were prions. The [Het-s] prion of *P. anserina* and the [PIN] prion of *Saccharomyces cerevisiae* were likewise proved by application of the same genetic criteria, but because their prion form

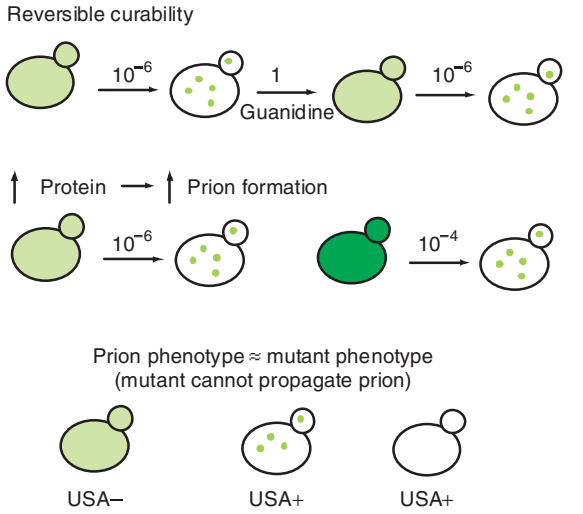


Figure 1 Genetic signature of a prion. Among nonchromosomal genetic elements, those with the three properties shown here must not be nucleic acid replicons and are almost certainly prions. However, only prions for which the prion form of the protein is inactive (such as [URE3] or [PSI]) will have property (3). The [Het-s] and [PIN^{+}] prions are active forms of the HET-s protein and Rnq1 protein, respectively and have properties (1) and (2), but not (3).

produces the phenotype, rather than the absence of the normal form, they do not satisfy criterion (3).

Self-Propagating Amyloid as the Basis for Most Yeast Prions

The finding that Sup35p, Ure2p, and HET-s were protease resistant and aggregated in prion-containing cells, that these proteins (and particularly their prion domains (**Figure 2**)) would form amyloid *in vitro* indicated that a self-propagating amyloid (**Figure 3**) was the basis of these prions. This was confirmed by the finding that the corresponding prions were transmitted by introduction of amyloid formed *in vitro* from the recombinant proteins (see below). However, in some cases, self-modifying enzymes have the potential to become prions (see below).

Chaperones and Prions

Chaperones of the Hsp40, Hsp70, and Hsp104 groups, as well as Hsp90 co-chaperones, have been found to be clearly involved in prion propagation. Millimolar concentrations of guanidine are known to cure each of the amyloid-based prions, and the mechanism of action of guanidine curing has been shown to be specific inhibition of Hsp104. It is believed that at least one function of these chaperones is to break large amyloid filaments into smaller ones which can then be distributed at cell division

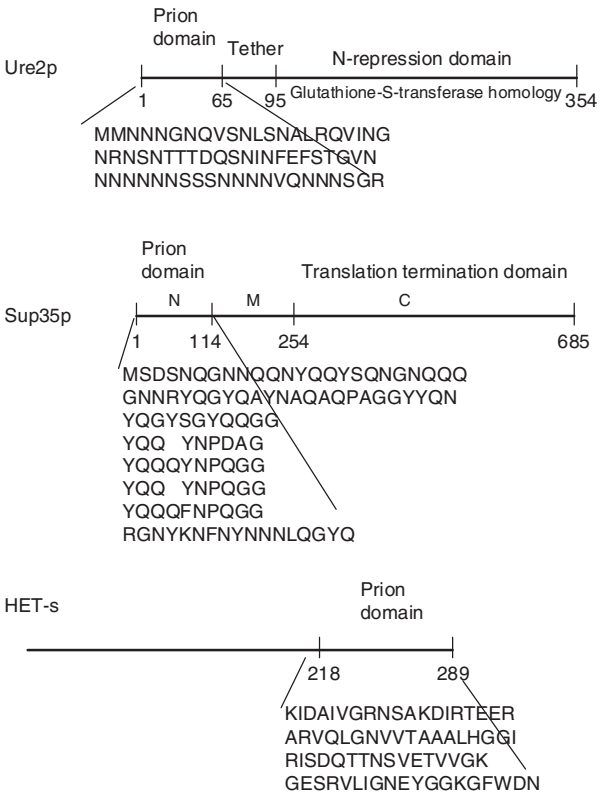


Figure 2 Prion domains. The prion domains of Ure2p, Sup35p, and HET-s are largely unstructured in the native form and in β-sheet in the prion form.

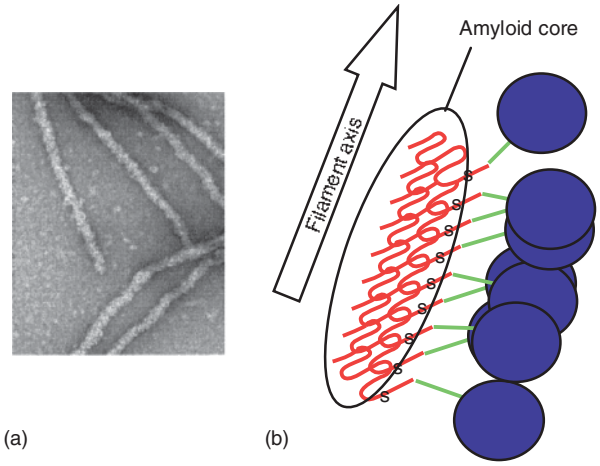


Figure 3 Amyloid. (a) Electron micrograph of amyloid of Ure2p. (b) Model of the structure of Ure2p amyloid. Red, prion domain; green, tether; blue, glutathione-S-transferase-like nitrogen regulation domain. Amyloid of recombinant HET-s, Sup35p, or Ure2p is infectious for yeast transmitting [Het-s], [PSI+], or [URE3], respectively. Amyloid of Ure2p is proposed to have the parallel in-register β-sheet structure shown based on the solid-state NMR studies of a fragment of the Ure2p prion domain, and the fact that the prion domain can be shuffled and still be a prion (see text).

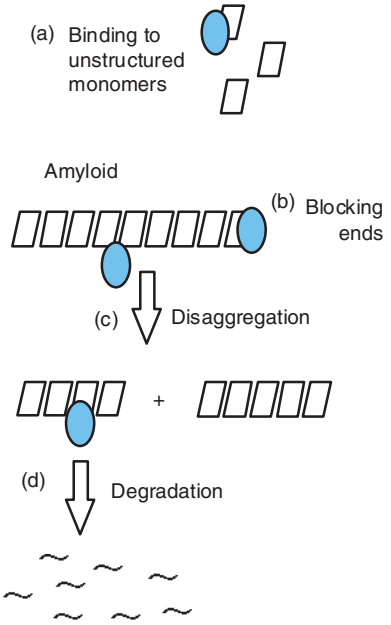


Figure 4 Chaperones and prions. Possible roles of chaperones in prion propagation are diagrammed.

to both daughter cells and insure the inheritance of the prion. Overexpression of some chaperones cure yeast prions, perhaps by solubilizing the filaments or perhaps by binding to the ends of filaments and preventing their elongation with new monomers. There is considerable specificity in which chaperone is needed for which prion and which chaperone can cure which prion by overexpression. The detailed mechanisms of chaperone action on prions (and amyloids in general) remain to be elucidated, but it is clear that they play an important role in these phenomena (**Figure 4**).

The Species Barrier and Prion Variants

Scrapie, a prion disease of sheep, only infects goats after a long incubation period, and subsequent goat-to-goat transmission has a much shorter incubation period. This is called the species barrier, and this barrier can in some cases be absolute, as appears to be the case between sheep and humans. The same phenomenon has now been documented in yeast, where [PSI] prions formed by the Sup35p of one species will not be transmitted to the Sup35p of another species, even though the other species' Sup35p can itself form a prion.

A single protein sequence can form several prions that are distinguishable, in yeast, by the intensity of their phenotype and the stability of their propagation. These are called 'prion variants' and are believed to reflect different amyloid structures. Paradoxically, a similar phenomenon, long documented in the mammalian TSEs, was used as an

argument against the protein-only model. Elucidation of the structure of different prion variants, and the mechanism of their faithful propagation, in some cases across species barriers, remains an important problem.

The bovine spongiform encephalopathy epidemic in the UK has brought the species barrier and prion variant phenomena together. It is clear that the 'height' of the species barrier is a function of the prion variant. Collinge views the species barrier as a reflection of the degree of overlap of possible variants of the prion proteins of the two species. If they have few common amyloid conformers (prion variants) then the barrier will be high. If each sequence can adopt nearly all of the amyloid conformers of the other, there will be little species barrier.

Formation of Prions by Sup35p and Ure2p Homologs

The C-terminal domain of Sup35 is conserved in eukaryotes with a human homolog capable of complementing the *S. cerevisiae* protein. All Sup35 proteins have N-terminal extensions, however, with limited or no sequence homology between species. N-terminal sequences from some species related to *S. cerevisiae* are capable of forming a [PSI+]-like prion. Ure2p is limited to the ascomycete yeasts. As with Sup35p, Ure2 proteins have a conserved C-terminal domain and a variable N-terminal domain that in general is rich in Asn and/or Glu residues. Ure2p homologs of some *Saccharomyces* yeasts can propagate [URE3] in *S. cerevisiae*.

Prion Generation, and [PIN]: A Prion That Gives Rise to Prions

One of the lines of evidence that showed [PSI] was a prion of Sup35p was that overproduction of Sup35p increased the frequency with which [PSI] arises *de novo*. However, it was found that in some strains, overproduction of Sup35p did not yield detectable emergence of [PSI]-carrying clones. Another nonchromosomal genetic element, named [PIN] for [PSI]-inducibility, was found necessary. [PIN] is a self-propagating amyloid form of the Rnq1 (rich in Asn (N) and Gln (Q)) protein, and it promotes *de novo* generation of [URE3] as well as [PSI].

Transfection with Amyloid of Recombinant Proteins

Amyloid filaments formed *in vitro* from recombinant HET-s protein, Sup35p, or Ure2p can efficiently transform cells to the corresponding [Het-s], [PSI], or [URE3] prion. In some cases it was shown that the soluble form or nonspecific aggregates of the protein were ineffective. This argues that the respective amyloids are not by-products or a dead-end stage of these prions, but are themselves the infectious material. All infectious Ure2p amyloids are larger than about 40-mer size. Amyloid formed *in vitro* is capable, for at least [PSI] and [URE3], of transmitting any of several prion variants. This implies that the amyloids can have any of several structures, a fact demonstrated by solid-state nuclear magnetic resonance (NMR) for amyloid of the Alzheimer's disease peptide, Aβ.

Shuffling Prion Domains and Amyloid Structure

The prion domains (**Figure 2**) of Ure2p and Sup35p are quite rich in Asn and Gln residues, and nearly the entire sequence of Rnq1p, the basis of the [PIN] prion, is rich in these amino acids. However, many Q/N-rich proteins are not capable of being prions. Thus, it was assumed that specific sequences in the known prion domains were important for prion formation. The Sup35 prion domain has octapeptide repeats much like those in PrP, and deletion or duplication of these showed substantial effects on prion generation. In addition, single amino acid changes in the prion domain of Sup35p blocked prion propagation.

To critically test whether the Ure2p prion domain had sequences essential for prion development, the entire Q/N-rich region (residues 1–89) was randomly shuffled (without changing the amino acid content) and each of five shuffled sequences were inserted into the chromosome in place of the normal prion domain. Surprisingly, each of these five shuffled sequences could support prion generation and propagation, although one was rather unstable. Each protein with the shuffled sequence could also form amyloid (**Figure 3**) *in vitro*. This showed that it was the amino acid content of the Ure2p prion domain that determines prion formation, and that sequence plays only a minor role.

Similarly, five shuffled versions of the Sup35p prion domain were each inserted in place of the normal sequence. Again, all five shuffled versions allowed formation and propagation of a [PSI]-like prion. It is likely that the effects of deletion or duplication of the octapeptide repeats observed on prion formation or propagation were due to changes in the length or composition of the prion domain. It appears that repeats *per se* are not important for prion generation or propagation.

Shuffleable Prion Domains Suggests Parallel In-Register β-Sheet Structure

Amyloids are β-sheet structures, but there are at least three kinds of β-sheets. Antiparallel β-sheets have adjacent peptide chains oriented in opposite directions: N→C next to C→N. This results in pairing of largely nonidentical residues. A β-helix also involves pairing of largely nonidentical residues, although they are within the

same peptide chain. A parallel β-sheet pairs identical residues if it is in-register, but in principle, one could have an out-of-register parallel β-sheet, in which case nonidentical residues would be bonded to each other.

Prion propagation (and amyloid propagation in general) is a very sequence-specific process. For example, a single amino acid change (at residue 138) can block propagation of scrapie in tissue culture. Humans are polymorphic at PrP residue 129 with roughly equal numbers of alleles encoding M and V. Either M/M or V/V individuals can get Creutzfeldt–Jakob disease (a human prion disease), but M/V heterozygotes cannot. Similarly, a single amino acid change in the prion domain of Sup35p can block propagation of [PSI] from the normal sequence, but the mutant Sup35p can itself become a prion nonetheless. Thus, if a prion amyloid has an antiparallel, parallel out-of-register, or β-helix structure, there must be some form of complementarity between bonded residues. Shuffling such a sequence would be expected to destroy the complementarity. In contrast, shuffling the sequence of a parallel in-register β-sheet would still leave identical residues paired. This suggests that prion domains that can be shuffled without destroying their prion-forming ability are forming parallel in-register β-sheets. Indeed $Ure2p^{10–39}$, a fragment of the prion domain, forms amyloid with a parallel in-register β-sheet structure, as does the Sup35 prion domain.

Biological Roles of Prions: A Help or a Hindrance?

In an attempt to discern whether yeast prions are an advantage or disadvantage to their host organism, cell growth of isogenic [PSI^+] and [psi^-] strains have been carried out under a variety of conditions. To what extent the various growth conditions tested represent the normal yeast habitat seems unknowable, although [psi^-] was an advantage under far more conditions than was [PSI^+].

An alternative approach was to compare the frequency with which [PSI^+] or [URE3] was found in wild strains to those of several 'selfish' RNA and DNA viruses and replicons known in *S. cerevisiae*. In any organism, an infectious element (such as a virus) may be widely distributed in spite of it causing disease in its host because the infection process overcomes and outraces the loss of infected individuals from negative selection. Certainly an infectious element, which is an advantage to its host, will quickly become widespread as selection and infection operate in the same direction. In fact, while the mildly deleterious RNA and DNA viruses and plasmids of yeast are easily found in wild strains neither [URE3] nor [PSI^+] was found in any of the 70 wild strains examined. This indicates that [URE3] and [PSI^+] produce disease in their hosts, and a rather more severe disease than the mild nucleic acid replicons.

The [Het-s] prion of *Podospora* appears to carry out the normal fungal function of heterokaryon incompatibility, thought to be a protection against the sometimes debilitating fungal viruses. Indeed, as one would expect for a prion with a function for the cell, 80% of wild *Podospora* isolates carry [Het-s], confirming its beneficial effects.

Unlike [PSI^+] and [URE3], [PIN^+] is found in wild strains at a frequency similar to that of the parasitic RNA and DNA viruses and plasmids. This suggests that [PIN^+] is at least not as severe a pathogen as are [URE3] and [PSI^+].

Enzyme as Prion

While most of the known prions involve amyloids, the word prion (infectious protein) is more general, requiring only that transmission be by protein alone. If an enzyme is made as an inactive precursor that needs the active form of the same enzyme for its activation, then such a protein can be a prion. The vacuolar protease B (Prb1p) of *S. cerevisiae* can be such a prion in a mutant lacking protease A, which normally activates its precursor. Cells initially carrying only the inactive precursor remain so unless the active enzyme is introduced. Once a cell has some active enzyme, the autoactivation process can continue indefinitely. It is likely that other examples of this type of phenomenon will be found among the many protein kinases, methylases, acetylases, and other modifying enzymes that are known. Indeed, a protein kinase of *P. anserina* appears to be able to become a prion in this manner, producing a nonchromosomal genetic element called 'crippled growth'.

The advent of yeast prions has propelled the prion field forward, and is giving us insight into the broader field of amyloids and how they interact with cellular components. There is already evidence for a number of new prions, mostly in yeasts and fungi, in part because they are so well suited to genetic studies, and in part because their frequent natural mating or heterokaryon formation (in fungi) results in complete mixing and exchange of cellular proteins.

See also: Prions of Vertebrates.

Further Reading

Aigle M and Lacroute F (1975) Genetical aspects of [URE3], a non-Mendelian, cytoplasmically inherited mutation in yeast. *Molecular and General Genetics* 136: 327–335.

Chan JCC, Oyler NA, Yau W-M, and Tycko R (2005) Parallel β-sheets and polar zippers in amyloid fibrils formed by residues 10–39 of the yeast prion protein Ure2p. *Biochemistry* 44: 10669–10680.

Chernoff YO, Lindquist SL, Ono B-I, Inge-Vechtomov SG, and Liebman SW (1995) Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi$^+$]. *Science* 268: 880–884.

Coustou V, Deleu C, Saupe S, and Begueret J (1997) The protein product of the *het-s* heterokaryon incompatibility gene of the fungus *Podospora anserina* behaves as a prion analog. *Proceedings of the National Academy of Sciences, USA* 94: 9773–9778.
Cox BS (1965) PSI, a cytoplasmic suppressor of super-suppressor in yeast. *Heredity* 20: 505–521.
Derkatch IL, Bradley ME, Hong JY, and Liebman SW (2001) Prions affect the appearance of other prions: The story of [*PIN*]. *Cell* 106: 171–182.
Jung G, Jones G, and Masison DC (2002) Amino acid residue 184 of yeast Hsp104 chaperone is critical for prion-curing by guanidine, prion propagation, and thermotolerance. *Proceedings of the National Academy of Sciences, USA* 99: 9936–9941.
King CY and Diaz-Avalos R (2004) Protein-only transmission of three yeast prion strains. *Nature* 428: 319–323.
Maddelein ML, Dos Reis S, Duvezin-Caubet S, Coulary-Salin B, and Saupe SJ (2002) Amyloid aggregates of the HET-s prion protein are infectious. *Proceedings of the National Academy of Sciences, USA* 99: 7402–7407.
Masison DC and Wickner RB (1995) Prion-inducing domain of yeast Ure2p and protease resistance of Ure2p in prion-containing cells. *Science* 270: 93–95.
Paushkin SV, Kushnirov VV, Smirnov VN, and Ter-Avanesyan MD (1997) *In vitro* propagation of the prion-like state of yeast Sup35 protein. *Science* 277: 381–383.
Ross ED, Edskes HK, Terry MJ, and Wickner RB (2005) Primary sequence independence for prion formation. *Proceedings of the National Academy of Sciences, USA* 102: 12825–12830.
Tanaka M, Chien P, Naber N, Cooke R, and Weissman JS (2004) Conformational variations in an infectious protein determine prion strain differences. *Nature* 428: 323–328.
Wickner RB (1994) [URE3] as an altered *URE2* protein: Evidence for a prion analog in *S. cerevisiae*. *Science* 264: 566–569.
Wickner RB, Edskes HK, Ross ED, *et al.* (2004) Prion genetics: New rules for a new kind of gene. *Annual Review of Genetics* 38: 681–707.

Pseudorabies Virus

T C Mettenleiter, Friedrich-Loeffler-Institut, Greifswald-Insel Riems, Germany

© 2008 Elsevier Ltd. All rights reserved.

History

Although its taxonomic species name *Suid herpesvirus 1* testifies that the natural hosts of pseudorabies virus (PrV) are pigs, its symptoms were first described in cattle, and the virus was isolated for the first time from cattle, dogs, and cats. This is due to the fact that PrV infection in swine, particularly in older animals, may produce only innocuous respiratory symptoms or may go unnoticed altogether. However, in other susceptible species productive infection is invariably fatal and characterized by severe central nervous symptoms, a feature that prompted its designation as pseudorabies owing to the rabies-like clinical picture. A typical symptom in these species is extensive pruritus, resulting in the name 'mad itch' to describe the disease in cattle in the USA during the first half of the nineteenth century.

In 1902, the Hungarian physician Aládar Aujeszky reported isolation of the infectious agent from diseased animals (an ox, a dog, and a cat) and differentiated it from rabies. It could be passaged in rabbits, reproducing the typical symptoms. Guinea pigs and mice were also found to be susceptible, whereas chicken and doves were resistant. Thus, the illness has become widely known as Aujeszky's disease (AD). It was not until 1931 that Richard Shope established the identity of the 'mad itch' agent with an infectious agent widely present in domestic pig holdings in the USA. In Germany, Erich Traub was the first to cultivate PrV *in vitro* in organ explants in 1933. One year later, Albert Sabin published his findings of a serological relationship between PrV and herpes simplex virus (HSV), resulting in the inclusion of PrV in the herpesvirus group.

Taxonomy

PrV takes the formal species name *Suid herpesvirus 1* and belongs to the subfamily *Alphaherpesvirinae* of the family *Herpesviridae*. Originally on the basis of serological studies, and later confirmed by molecular biological analyses and comparison of the deduced amino acid sequences of homologous proteins, PrV was shown to be most closely related to bovine herpesvirus 1 (BHV-1) and equine herpesvirus 1 (EHV-1), and also to varicella-zoster virus (VZV). This prompted its assignment to the genus *Varicellovirus* within the *Alphaherpesvirinae*. Alphaherpesviruses are characterized by rapid lytic replication, a pronounced neurotropism with establishment of latency in sensory ganglia, and a broad host range. All these features apply to PrV.

The Virus Particle

PrV particles have a diameter of approximately 180 nm and exhibit the typical herpesvirus morphology. At the center is the genomic DNA enclosed in an icosahedral capsid. The capsid is surrounded by a structure designated the tegument, which is equivalent to the matrix of RNA viruses but significantly more complex.

The envelope, which contains virally encoded glycosylated and nonglycosylated proteins anchored in the lipid bilayer, encloses the capsid and tegument (**Figure 1**).

The Genome

The PrV genome consists of a linear, double-stranded DNA molecule. A complete sequence has recently been assembled from several partial sequences from six different PrV strains. It comprises 143 461 bp with more than 70 protein-coding regions, all of which exhibit homology to genes in related alphaherpesviruses (**Figure 2**). The genome contains a long (U_L) and short (U_S) unique region with the latter bracketed by inverted repeats (IR_S and TR_S), resulting in two isomeric forms of the genome in which U_S is inverted relative to U_L. This arrangement has been designated the class D herpesvirus genome structure. So far, three functional origins of DNA replication have been mapped, two in the inverted repeats and one in the middle of U_L. A fourth candidate located at the left genome end may not be functional. Compared to the genomes of other alphaherpesviruses, which are generally collinear in their gene arrangement, the PrV genome contains an inversion of approximately 40 kbp that encompasses genes homologous to the UL27 to UL44 genes of HSV (**Figure 2**). A similar inversion is also present in the genome of a distantly related avian alphaherpesvirus, infectious laryngotracheitis virus, but its biological significance is unknown.

A list of identified PrV genes and the functions of the encoded proteins is shown in **Table 1**. Wherever possible, PrV genes have been named after their homologs in HSV. However, the UL3.5 gene of PrV, which has homologs in other alphaherpesviruses such as VZV, BHV-1, and EHV-1, is absent from HSV, as are ORF1 and ORF1.2. PrV does not specify homologs of the UL56, UL45, US5, US10, US11, and US12 genes of HSV. Approximately half of the total number of PrV genes are considered 'nonessential', a status indicating that they are individually dispensable for viral replication, at least in cell culture. It is estimated that about half of the total number of viral proteins are located in the virion.

The Capsid

The icosahedral PrV capsid is composed of 162 capsomers. By analogy to the well-analyzed HSV-1 capsid, the capsomers consist of a total of 955 copies of the major capsid protein, the product of the UL19 gene (see below). Homologs of the HSV UL18 and UL38 proteins, which form triplexes connecting and stabilizing the capsomers, as well as the UL35 protein, which is located at the tips of the hexons, have been identified from the genome sequence. Also present are homologs of the UL6 portal protein which forms a dodecameric channel at one vertex for package and release of the viral genome. Several other proteins have been shown to be intimately associated with the capsid, such as the UL17 and UL25 gene products. Whereas the PrV UL17 protein may be present within the capsid shell, the UL25 gene product resides on the outside.

The Tegument

The herpesvirus tegument is a complex structure which, in the case of PrV, contains in excess of 15 viral proteins. It has become clear that tegument formation is an important step in the morphogenesis of the virion, and requires a network of partially redundant protein–protein interactions. It can be divided into capsid-proximal and envelope-proximal parts. The capsid-proximal tegument is composed of the UL36 gene product, the largest protein in PrV (3084 amino acid residues), which physically interacts with the UL37 gene product. From cryoelectron image reconstructions of herpesvirus particles, the UL36 gene product is thought to contact the capsid. The UL46, UL47, UL48, and UL49 gene products are easily stripped from the capsid together with the envelope, consistent

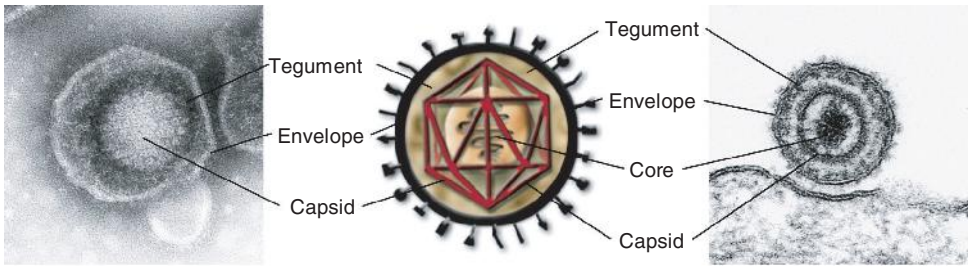


Figure 1 The PrV virion. A schematic diagram of the PrV virion is presented between an electron micrograph of a negatively stained PrV virion (left) and a thin-sectioned virus particle (right). The locations of the virion subcomponents (core, capsid, tegument, and envelope) are indicated. Spikes at the envelope represent viral glycoproteins.

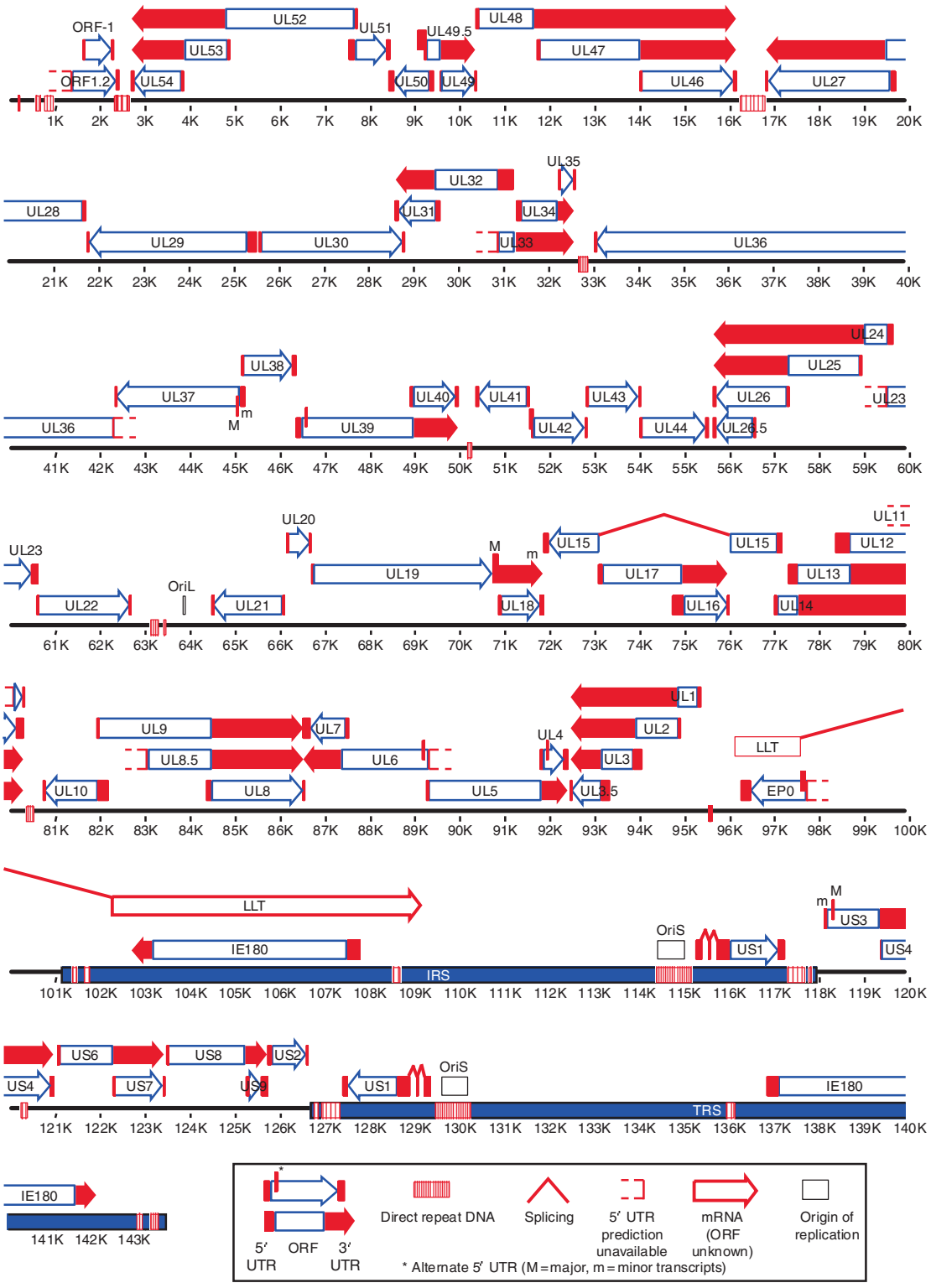


Figure 2 The PrV genome. The transcript and gene organization deduced from the complete genomic sequence is shown. The linear form of the PrV genome comprises the unique long sequence (U_L), the internal inverted repeat (IR_S), the unique short sequence (U_S), and the terminal inverted repeat (TR_S). The predicted locations of protein-coding regions (see **Table 1**), 5′ and 3′ nontranslated regions, DNA repeats, splice sites, and the origins of DNA replication are shown. Reproduced from Klupp BG, Hengartner C, Mettenleiter TC, and Enquist LW (2004) Complete, annotated sequence of the pseudorabies virus genome. *Journal of Virology* 78: 424–440, with permission from the American Society for Microbiology.

Table 1 PrV ORFs

Protein	ORF location[a]	Length (aa)	MW (kDa)	Alias	Function/property[b]	Virion subunit[c]
ORF1.2	1252–2259	335	35.3		Unknown	V (?)
ORF1	1636–2259	207	21.8		Unknown	V (?)
UL54	3815–2730r	361	40.4	ICP27	Gene regulation; early protein	NS
UL53	4833–3895r	312	33.8	gK	Viral egress; glycoprotein K; type III membrane protein	V (E)
UL52	7676–4788r	962	103.3		DNA replication; primase subunit of UL5/UL8/UL52 complex	NS
UL51	7663–8373	236	25.0		Tegument protein	V (T)
UL50	9333–8527r	268	28.6	dUTPase	dUTPase	NS
UL49.5	9257–9553	98	10.1	gN	Glycoprotein N; type I membrane protein; complexed with gM	V (E)
UL49	9591–10340	249	25.9	VP22	Interacts with C-terminal domains of gE and gM; tegument protein	V (T)
UL48	10404–11645	413	45.1	VP16/αTIF	Gene regulation (transactivator); egress (secondary envelopment); tegument protein	V (T)
UL47	11746–13998	750	80.4	VP13/14	Viral egress (secondary envelopment); tegument protein	V (T)
UL46	14017–16098	693	75.5	VP11/12	Unknown; tegument protein	V (T)
UL27	19595–16854r	913	100.2	gB	Viral entry (fusion); cell–cell spread; glycoprotein B; type I membrane protein	V (E)
UL28	21640–19466r	724	78.9	ICP18.5	DNA cleavage/encapsidation (terminase); associated with UL15, UL33, and UL6	pC
UL29	25315–21788r	1175	125.3	ICP8	DNA replication/recombination; binds single-stranded DNA	NS
UL30	25606–28752	1048	115.3		DNA replication; DNA polymerase subunit of UL30/UL42 complex	NS
UL31	29488–28673r	271	30.4		Viral egress (nuclear egress); primary virion tegument protein; interacts with UL34	pV (T)
UL32	30893–29481r	470	51.6		DNA packaging; efficient localization of capsids to replication compartments	pC
UL33	30892–31239	115	12.7		DNA cleavage/encapsidation; associated with UL28 and UL15	NS
UL34	31398–32186	262	28.1		Viral egress (nuclear egress); primary virion envelope protein; tail-anchored type II nuclear membrane protein; interacts with UL31	pV (E)
UL35	32241–32552	103	11.5	VP26	Capsid protein	V (C)
UL36	42314–33060r	3084	324.4	VP1/2	Large tegument protein; interacts with UL37 and UL19	V (T)
UL37	45111–42352r	919	98.2		Tegument protein; interacts with UL36	V (T)
UL38	45168–46274	368	40.0	VP19C	Capsid protein; forms triplexes together with UL18	V (C)
UL39	46470–48977	835	91.1	RR1	Nucleotide synthesis; large subunit of ribonucleotide reductase	V (?)
UL40	48987–49898	303	34.4	RR2	Nucleotide synthesis; small subunit of ribonucleotide reductase	NS
UL41	51498–50401r	365	40.1	VHS	Gene regulation (inhibitor of gene expression); virion host cell shut off factor	V (T)
UL42	51628–52782	384	40.3		DNA replication; polymerase accessory subunit of UL30/UL42 complex	NS
UL43	52842–53963	373	38.1		Unknown; type III membrane protein	V (E)
UL44	54029–55468	479	51.2	gC	Viral entry (virion attachment); glycoprotein C; type I membrane protein; binds to heparan sulfate	V (E)
UL26.5	56535–55699r	278	28.2	VP22a	Scaffold protein; substrate for UL26; required for capsid formation and maturation	pC
UL26	57273–55699r	524	54.6	VP24	Scaffold protein; proteinase; required for capsid formation and maturation	V (C)
UL25	58911–57307r	534	57.4		Capsid associated protein; required for DNA packaging and nuclear egress	V (C)
UL24	59519–59004r	171	19.1		Unknown; possible endonuclease	V (?)
UL23	59512–60474	320	35.0		Nucleotide synthesis; thymidine kinase	NS
UL22	60610–62670	686	71.9	gH	Viral entry (fusion); cell–cell spread; glycoprotein H; type I membrane protein; complexed with gL	V (E)
UL21	66065–64488r	525	55.2		Capsid associated protein; complexed with UL16	V (?)
UL20	66172–66657	161	16.7		Viral egress; type III membrane protein	V (?)
UL19	66744–70736	1330	146.0	VP5	Major capsid protein; forms hexons und pentons	V (C)
UL18	70896–71783	295	31.6	VP23	Capsid protein; forms triplexes together with UL38	V (C)
UL15 (Ex2)	73115–71979r	735	79.1		DNA cleavage/encapsidation; terminase subunit; interacts with UL33, UL28, and UL6	pC

UL15 (Ex1)	77065–75995r					
UL17	73166–74959	597	64.2		DNA cleavage/encapsidation	V (C)
UL16	74986–75972	328	34.8		Tegument protein; complex with UL11 and UL21	V (T)
UL14	77064–77543	159	17.9		Unknown	?
UL13	77513–78709	398	41.1	VP18.8	Protein-serine/threonine kinase	V (T)
UL12	78675–80126	483	51.3		DNA recombination; alkaline exonuclease	?
UL11	80084–80275	63	7.0		Viral egress (secondary envelopment); membrane-associated tegument protein complex with UL16	V (T)
UL10	81935–80754r	393	41.5	gM	Viral egress (secondary envelopment); glycoprotein M; type III membrane protein; C-terminus interacts with UL49; inhibits membrane fusion in transient assays; complexed with gN	V (E)
UL9	81934–84465	843	90.5	OBP	Sequence specific ori-binding protein	NS
UL8.5	83053–84465	470	51.0	OPBC	C-terminal domain of UL9	?
UL8	84462–86513	683	71.2		DNA replication; part of UL5/UL8/UL52 helicase/primase complex	NS
UL7	87479–86679r	266	29.0		Tegument protein; virion formation and egress	V (T)
UL6	89301–87370r	643	70.3		Capsid protein; portal protein; docking site for terminase	V (C)
UL5	89300–91804	834	92.1		DNA replication; part of UL5/UL8/UL52 helicase/primase complex; helicase motif	NS
UL4	91863–92300	145	15.8		Nuclear protein	NS
UL3.5	93150–92476r	224	24.0		Viral egress (secondary envelopment); membrane-associated protein	V (T)
UL3	93860–93147r	237	25.6		Nuclear protein	NS
UL2	94866–93916r	316	33.0	UNG	Uracil-DNA glycosylase	NS
UL1	95314–94844r	156	16.5	gL	Viral entry; cell–cell spread; glycoprotein L; membrane anchored via complex with gH	V (E)
EP0	97713–96481r	410	43.8	ICP0	Gene regulation (transactivator of viral and cellular genes); early protein	?
IE180 (IRS)	107511–103171r	1446	148.6	ICP4	Gene regulation; immediate-early protein	?
IE180 (TRS)	137091–141431					
US1 (IRS)	115995–117089	364	39.6	RSp40/ ICP22	Gene regulation; immediate-early protein	?
US1 (TRS)	128607–127513r					
US3 (minor)	118170–119336	388	42.9	PK	Minor form of protein kinase (53 kDa mobility)	?
US3 (major)	118332–119336	334	36.9	PK	Viral egress (nuclear egress); major form of protein kinase (41 kDa mobility)	V (T)
US4	119396–120892	498	53.7	gG	Glycoprotein G (secreted)	secreted
US6	121075–122277	400	44.3	gD	Viral entry (cellular receptor binding protein); glycoprotein D; type I membrane protein	V (E)
US7	122298–123398	366	38.7	gI	Cell–cell spread; glycoprotein I; type I membrane protein; complexed with gE	V (E)
US8	123502–125235	577	62.4	gE	Cell–cell spread; glycoprotein E; type I membrane protein; complexed with gI; C-terminus interacts with UL49	V (E)
US9	125269–125589	106	11.3	11K	Protein sorting in axons; type II tail-anchored membrane protein	V (E)
US2	125811–126581	256	27.7	28K	Tegument protein; prenylated	V (T)

[a]Numbering starts at 1 on the UL end of the genome. r indicates ORF encoded on reverse strand.
[b]Function/property as demonstrated for the PrV and/or HSV-1 homolog.
[c]V (C): virion capsid component; V (T): virion tegument component; V (E): virion envelope component; V (?): virion component of unknown subviral localization; pV: primary enveloped virion precursor component (not found in mature virion); NS: nonstructural protein; pC: present in intranuclear capsid precursor forms but not found in mature virion; ?: unknown.
Reproduced from Klupp BG, Hengartner C, Mettenleiter TC, and Enquist LW (2004) Complete, annotated sequence of the pseudorabies virus genome. *Journal of Virology* 78: 424–440, with permission from the American Society for Microbiology.

with a location in the envelope-proximal tegument. Correlating with these findings, the PrV UL49 gene product has been shown to interact with the intracytoplasmic C-termini of the gE and gM envelope proteins. Both parts of the tegument may be connected by the UL48 gene product. Tegument proteins enter the cell after fusion of the virion envelope and the cellular plasma membrane during entry, and prime the cell for virus production. The alphaherpesvirus UL48 gene products are strong transactivators of viral immediate-early (IE) gene expression, whereas the UL41 proteins possess endoribonucleolytic activity to degrade preexisting cellular mRNAs. Cellular proteins have also been detected in the PrV tegument, including actin, annexins, and heat shock proteins. Their biological role is unknown.

The Envelope

Receptor-binding proteins, as well as major immunogens, are located in the viral envelope. More than 10 envelope constituents have been identified in PrV. Most are modified by the addition of carbohydrate and thus are glycoproteins. Several type I, type II, and type III PrV glycoproteins have been described (**Table 1**). Since the early nomenclature of PrV glycoproteins was somewhat confusing, it has been agreed to name them after their HSV-1 counterparts. Several of these glycoproteins form complexes, such as homooligomeric glycoprotein B (gB) and heterodimeric gE/gI, gH/gL, and gM/gN. The discovery of gN, as well as the gM/gN complex which is conserved throughout the mammalian and avian herpesviruses, was first made in PrV. Nonglycosylated membrane proteins include the US9, UL20, and UL43 gene products (see **Table 1**). The nonstructural gG is proteolytically cleaved and released from infected cells.

The Replication Cycle

PrV is arguably the most intensively analyzed animal herpesvirus. It has become a major focus of molecular biological research on the basic mechanisms of herpesvirus biology.

In the replication cycle (**Figure 3**), PrV infection of host cells starts with interaction of envelope gC with cell surface heparan sulfate-containing proteoglycans. This interaction is beneficial to, but not essential for, the second step, which involves binding of the essential envelope protein gD to its cellular receptor, nectin. HSV-1 and BHV-1 also use heparan sulfate and nectin for attachment. However, gD-negative and even gC- and gD-negative infectious PrV mutants have been isolated, which indicates that infection can occur by other routes. These mutants harbor additional mutations in gB and gH.

Penetration (fusion of viral envelope and cellular plasma membrane) requires the essential proteins gB and gH/gL. These glycoproteins are conserved throughout the mammalian and avian herpesviruses, indicating a common mechanism for membrane fusion. After penetration, the capsid is transported via microtubules to the nuclear pore, where it docks and releases the viral genome into the cell nucleus through one vertex. Empty capsids may remain bound to the nuclear pore for a considerable time. The entire entry process can be bypassed *in vitro* by transfection of naked viral DNA.

In the nucleus, transcription of viral genes is initiated by expression of the major IE gene, resulting in the translation of a 180 kDa protein (IE180). Although IE180 has long been considered to be the only IE protein of PrV, studies using specific inhibitors have identified that the US1 (RSp40) mRNA is also expressed with IE kinetics. Like other herpesvirus IE proteins, IE180 is a potent transcriptional activator that transinduces the expression of viral early genes. Early genes encode enzymes involved in nucleotide metabolism (e.g., UL23 = thymidine kinase; UL2 = uracil-DNA glycosylase; UL39/UL40 = ribonucleotide reductase; UL50 = deoxyuridine triphosphatase) and DNA replication (UL30/UL42 = DNA polymerase and an associated factor; UL5/UL8/UL52 = helicase-primase complex; UL29 = single-stranded DNA-binding protein; UL9 = origin-binding protein), as well as two protein kinases (US3, UL13).

DNA replication, whether occurring exclusively via a rolling-circle mechanism or involving intra- and intermolecular recombination and branching, results in the formation of head-to-tail fused concatemers of the genome. Finally, late genes encoding primarily virion structural proteins are expressed and their gene products, after translation in the cytosol, are transported into the nucleus for capsid assembly and DNA packaging. Capsid assembly is morphologically similar in all herpesviruses: capsids containing the major capsid protein UL19, triplex proteins UL18 and UL35, hexon-tip protein UL35, and portal protein UL6 assemble autocatalytically around a protein scaffold consisting of the UL26 and UL26.5 gene products. Packaging occurs via the unique portal at one vertex comprising 12 molecules of the UL6 protein. Genome-length molecules are cleaved from concatemeric replication products during packaging, which requires the UL15, UL28, UL32, and UL33 gene products. The UL17 protein may accompany DNA into the capsid. The UL25 gene product is not required for cleavage/packaging but apparently stabilizes the capsid and is essential for triggering primary envelopment. It is located at the outside of the capsid.

Egress of herpesvirus capsids from the nucleus has been, and still is, a matter of debate. However, numerous findings in recent years, to which studies on PrV contributed significantly, have demonstrated that intranuclear capsids gain

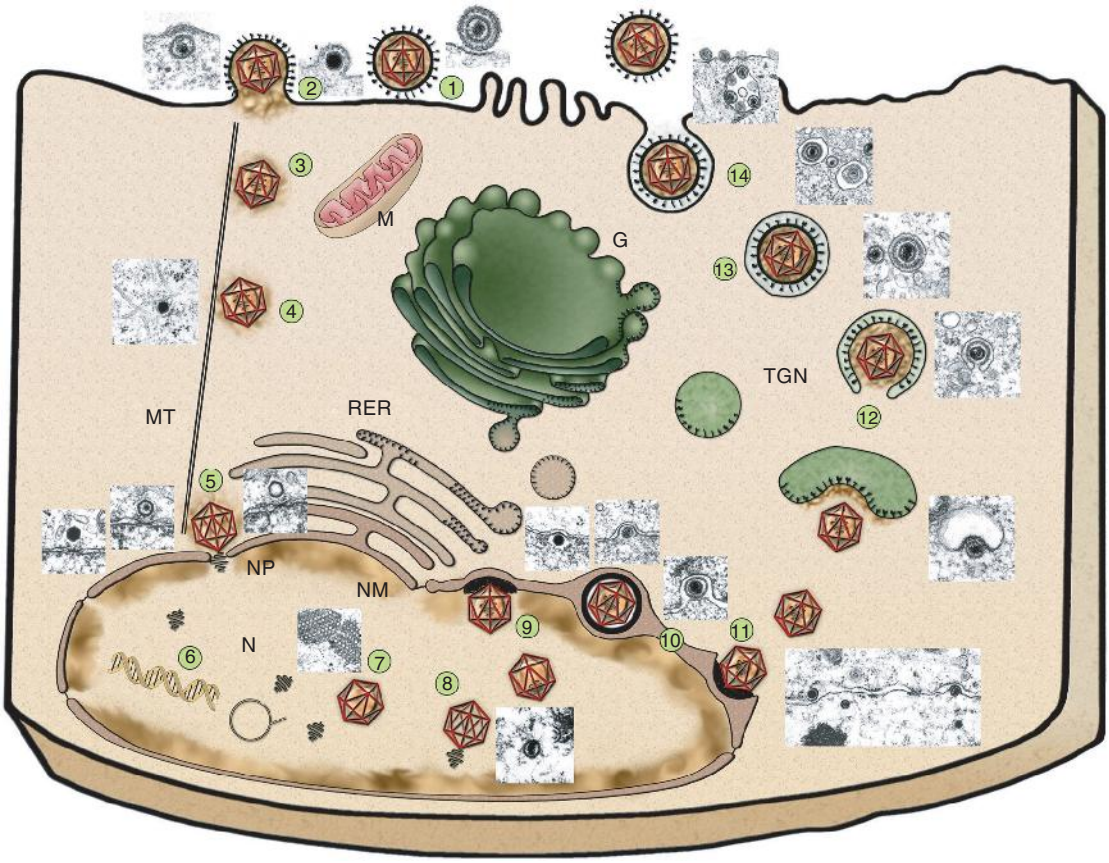


Figure 3 The PrV replication cycle. A diagram of the replication cycle of PrV is shown together with electron micrographs showing the respective stages. After attachment (1) and penetration (2), capsids are transported to the nucleus N (3) via interaction with microtubuli MT (4), docking at the nuclear pore NP (5) where the viral genome is released into the nucleus. Here, transcription of viral genes and viral genome replication occur (6). Concatemeric replicated viral genomes are cleaved to unit-length molecules during encapsidation (8) into preformed capsids (7), which then leave the nucleus by budding at the inner nuclear membrane NM (9) followed by fusion of the envelope of these primary virions located in the perinuclear cleft (10) with the outer nuclear membrane (11). Final maturation then occurs in the cytoplasm by secondary envelopment of intracytosolic capsids via budding into vesicles of the *trans*-Golgi network TGN (12) containing viral glycoproteins (black spikes), resulting in an enveloped virion within a cellular vesicle. After transport to the cell surface (13), vesicle and plasma membranes fuse, releasing a mature, enveloped PrV particle from the cell (14). RER, rough endoplasmic reticulum; M, mitochondrion; G, Golgi apparatus.

access to the cytoplasm by primary envelopment (i.e., budding at the inner leaflet of the nuclear membrane) followed by de-envelopment (fusion) at the outer leaflet. For primary envelopment, the conserved UL31 and UL34 proteins, which form a complex, have been shown to be important, though not always strictly essential, in all three subfamilies of herpesviruses. The UL31/UL34 complex is located in the nuclear membrane and recruits cellular protein kinase C, which phosphorylates and thereby dissociates nuclear lamins, allowing access of nascent capsids to the inner nuclear membrane. Primary enveloped virions in the perinuclear space also contain the UL31/UL34 complex, which constitutes part of the primary envelope (UL34 is a type II membrane protein) and tegument (UL31). The US3 protein kinase is present in primary and mature virions, whereas the UL31 and UL34 proteins are absent from mature virus particles. The mechanisms of de-envelopment are unclear. However, in the absence of the nonconserved and nonessential US3 kinase, primary enveloped virions accumulate in the perinuclear space, demonstrating the participation of this protein in nuclear egress.

Virion morphogenesis is completed in the cytoplasm by tegumentation, final envelopment, and transport of mature virus particles for release at the plasma membrane. Tegumentation apparently starts at two sites: the capsid and the future envelopment site. At the capsid, the conserved tegument proteins UL36 and UL37 interact. At the future envelopment site (i.e., at vesicles derived from the Golgi apparatus), the C-termini of the type I membrane protein gE and the type III membrane protein gM bind the UL49 tegument protein. Presumably, the UL48 protein links the two parts of the tegument, which

drives budding of tegumented virions into the *trans*-Golgi vesicles containing viral glycoproteins to yield mature virions within a vesicle. PrV gM has been shown to relocate other viral and cellular proteins to the *trans*-Golgi and therefore may be involved in assembling the envelope proteins. The conserved UL11 gene product is thought to be involved in directing tegument proteins to the envelopment site. Finally, virion-containing vesicles move to the cell surface, a process in which the UL20 protein is involved, where plasma and vesicle membranes fuse, resulting in release of infectious particles. Apparently, virion gK inhibits an immediate re-fusion of released virions with the cell they just left.

Infectivity can be transmitted via direct cell-to-cell transmission as well as via free virions. Although several of the virion proteins required for penetration (gB, gH/gL) are also required for direct cell-to-cell spread, the mechanism remains enigmatic. In contrast to the situation with other alphaherpesviruses such as HSV, direct cell-to-cell spread of PrV does not require the receptor-binding gD molecule.

Clinical Features of Infection and Pathology

PrV is able to infect most mammals productively, with the exception of humans and other higher primates. However, primate and human cells are infectable in cell culture, and the reason for the natural resistance is not clear. Equids and goats are also rather resistant but may be infected experimentally. In addition, pseudorabies has been reported in many species of wild mammals, including wild boar, feral pigs, coyotes, raccoons, rats, mice, rabbits, deer, badgers, and coatimundi. It is so far not known whether these animals play a role in farm-to-farm transmission of PrV. In susceptible species other than porcines, infection is fatal and animals die from severe neuronal disorders.

After infection of the natural host, the clinical picture varies depending on the age of the animal, the virulence of the virus, and the route of infection. In nature, infection occurs predominantly oronasally, although genital transmission may also take place, especially in feral pigs. After replication in epithelial cells the virus gains access to neurons innervating the facial and oropharyngeal area, in particular the olfactory, trigeminal, and glossopharyngeal nerves. The virus spreads centripetally by fast axonal retrograde transport and reaches the cell bodies of infected neurons where either lytic or latent infection ensues (see below). PrV is disseminated viremically to many organs, where it replicates in epithelia, vascular endothelium, lymphocytes, and macrophages. In nonporcines, PrV is rather strictly neurotropic.

Neonates become prostrate and die quickly, often without nervous signs. In slightly older piglets, severe central nervous system (CNS) disorders are characterized by incoordination, twitching, paddling, tremors, ataxia, convulsions, and/or paralysis, whereas itching is only rarely present (**Figure 4**). Mortality in piglets up to 2–3 weeks of age may be as high as 100%, resulting in severe losses. Piglets at 3–6 weeks of age may still exhibit neurological signs and high morbidity, but mortality is usually reduced. Infection in older pigs induces primarily respiratory symptoms, such as coughing, sneezing, and heavy breathing, resulting from viral replication in, and destruction of, pulmonary epithelium. Despite the absence of overt nervous signs, virus gains access to neurons and remains latently established in the olfactory bulb, trigeminal ganglia, and brain stem or, after venereal transmission, in the sacral ganglia. PrV infection of pregnant sows may result in abortion or delivery of stillborn or mummified fetuses due to endometritis and necrotizing placentitis with infection of trophoblasts. In susceptible species other than swine, PrV infection is invariably fatal, sometimes after a rapid, peracute course without preceding overt clinical signs. Pruritus is a lead symptom of PrV infection in these species which, particularly in rabbits and rodents, may result in violent itching and automutilation. The death of mice, rats, cats, or dogs on farms is often a telltale sign of the presence of PrV prior to the appearance of symptoms in pigs.

Transmission occurs via virus-containing body fluids such as nasal and genital secretions, which gain access to epithelial surfaces within the respiratory or genital tract.

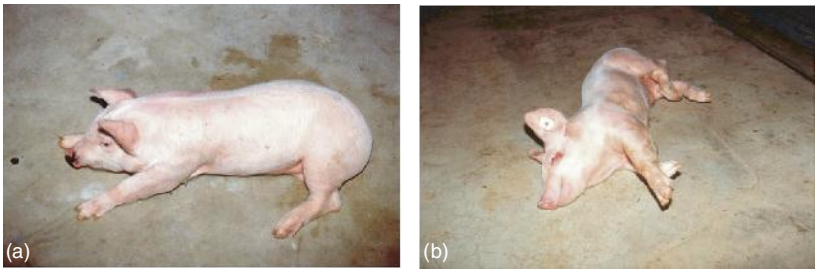


Figure 4 Neurological symptoms of PrV infection in piglets. The animals show ataxia (a), convulsions and paralysis (b) which ultimately lead to death.

Airborne transmission is efficient at short range, but long-range transmission covering several kilometers may also occur. Carnivores become infected by ingesting contaminated meat. After primary replication in epithelial cells, the virus enters the endings of sympathetic, parasympathetic or sensory and motor neurons innervating the area of primary replication. Infection probably occurs by the same mechanism as outlined above for cultured cells. Virus is transported in retrograde fashion to the neuronal cell body, where DNA replication and formation of progeny virions ensues. It is not clear whether complete virions or viral subassemblies are then transported to the synapse, or how transsynaptic transfer occurs. Depending on the virulence of the virus and the age and immune status of the host, infection may not proceed beyond the first neuronal level (i.e., ganglia directly innervating the affected peripheral site). However, virus may also spread to the brain resulting in ganglioneuritis and encephalitis. Lymphocytes can also become infected by PrV and this may help viral spread within the body, playing an important role in infection of the fetus. However, the percentage of infected cells in the blood is rather low, even during acute infection, and difficult to detect. A major target organ for latency in swine is the tonsils, and tonsil biopsies allow reliable detection of virus by molecular biological techniques or virus isolation.

There are no pathognomonic, gross lesions of AD. In piglets, there may be necrotizing tonsillitis, rhinotracheitis, or proximal esophagitis. Other lesions commonly seen include pulmonary edema, necrotizing enteritis, and multifocal necrosis of the spleen, lung, liver, lymph nodes, and adrenal glands. Histologically, PrV causes a nonsuppurative meningoencephalitis and paravertebral ganglioneuritis. The gray matter is especially affected, and infected neurons or astrocytes may present acidophilic intranuclear inclusions. The presence of viral antigen can be visualized by immunostaining and viral genomes can be detected by *in situ* hybridization. PrV infected cells usually show more or less extensive degeneration and necrosis due to lytic viral replication. Whether apoptosis induced by PrV infection also plays a role *in vivo* is unclear. A predominantly T-cell-mediated reaction of the immune system induces ganglioneuritis, polio- or panencephalitis with foci of gliosis contributing to the loss of neuronal function. The described extraneural lesions in pigs and acute myocarditis in carnivores might provide additional explanations for the fatal outcome of infections in which virus cannot be recovered from the brain.

Immunology

Live as well as inactivated vaccines induce efficient protective immunity against AD. Antibodies against a number of viral structural and nonstructural proteins have been detected in infected animals, and virus-neutralizing monoclonal antibodies have been isolated. Antibody responses are primarily directed against the major surface glycoproteins including gB, gC, gD, and gE as well as secreted gG. The most potent complement-independent virus neutralizing antibodies are directed against gC, gD and, to a lesser extent, gB, and subunit vaccines consisting of gB, gC, gD, as well as anti-idiotypic anti-gD antibodies and heterologous vectors expressing gC or gD, elicit protective immunity. In contrast, anti-gE antibodies require complement for neutralization, and anti-gG antibodies have no neutralizing ability at all. Antibodies against whole virus or specific for gB are used in diagnostic assays to detect PrV infection serologically. Major targets for cell-mediated immunity in pigs are primarily gC and, to a lesser extent, gB.

Although the numerous elaborate immune evasion mechanisms of beta- and gammaherpesviruses, including expression of virokines and viroceptors, have not been found in alphaherpesviruses, these viruses still interact with the immune system to evade its activity. Like other alphaherpesvirus gC proteins, PrV gC binds species-specifically to porcine complement component C3, and the gE/gI complex binds the Fc portion of porcine IgG. Secreted gG may bind chemokines, thereby impairing intercellular signaling. Moreover, infection of cells by PrV results in downregulation of major histocompatibility complex class I (MHC-I) antigen presentation, and envelope glycoproteins present at the plasma membrane of infected cells are internalized by as yet unknown factors, resulting in a paucity of antigens presented to the immune system at the cell surface. Recently, the PrV gN protein has been shown to inactivate the transporter which translocates processed peptides for loading onto MHC-I molecules in the endoplasmic reticulum.The combined action of these mechanisms may give the virus an edge over the immune system, facilitating establishment of latency and further virus spread.

Latency

Like other alphaherpesviruses, PrV has the capacity to become latent in neurons. During latency, the genome persists largely quiescently in a presumably circular form. Expression is restricted to one region, the latency-associated transcript (LAT) gene, which encompasses part of the inverted repeat and adjoining U_L region. The LAT gene, which encodes the large latency transcript (LLT), is located antiparallel to the genes encoding IE180 and EP0 (see **Figure 2**), and is transcribed into three different RNAs of 8.4, 8.0, and 2.0 kb. The 8.4 and 2.0 kb species are derived by splicing of a larger precursor. During latency, only the 8.4 kb RNA is produced from a separate promotor that is apparently active only under

latent conditions, whereas the 8.0 and 2.0 kb species are also transcribed during lytic infection.

PrV encodes proteins which are able to suppress apoptotic cell death as a prerequisite for the establishment of latency. The US3 protein kinase has been demonstrated to mediate this function in porcine fetal trigeminal neurons. PrV establishes latent infections predominantly in neuronal tissues such as the trigeminal or sacral ganglia. However, tonsils have also been identified as sites of latency.

Epidemiology and Control

In the twentieth century, PrV has become a pathogen distributed worldwide with the exception of Australia, Canada, and the Scandinavian countries. In major swine-producing areas, PrV infection caused significant economic losses amounting to hundreds of millions of dollars, making it one of the most devastating pig diseases. Control and eradication of PrV infection in pigs relied on two strategies. In areas with a low prevalence of infection, serological screening and consequent elimination of seropositive animals resulted in the eradication of AD from countries such as the UK, Denmark, and East Germany. PrV infection can be diagnosed by detecting either the infectious agent (antigen detection by immunofluorescence or virus isolation) or viral DNA using polymerase chain reaction (PCR). The latter method is also suitable for detecting latent viral genomes. A PrV-specific immune response in live animals can be confirmed using various serological assays (e.g., virus neutralization, latex agglutination, or enzyme-linked immunosorbent assay (ELISA) systems based on complete virus particles or distinct viral antigens such as gB).

To reduce disease prevalence, vaccination with live-attenuated or inactivated vaccines has also been used. However, vaccination does not result in sterile immunity, and vaccinated animals may still be infected with and carry the virus, and these carriers are no longer identifiable by serological analysis. This problem has been solved by the advent of the so-called 'marker' vaccines. This novel concept provided a breakthrough in animal disease control, and serves as a blueprint for control of other infectious diseases. It was based on the finding that several immunogenic envelope glycoproteins of PrV, such as gC, gE, and gG (see above), are not required for productive replication and can be deleted from the viral genome without abolishing virus replication. These gene-deleted strains can be produced easily in conventional cell systems and can be administered as inactivated or modified-live vaccines. In fact, gene-deleted PrV strains were the first genetically engineered live-virus vaccines to be licensed. Thus, PrV has pioneered modern vaccinology. Whereas animals vaccinated with these vaccines do not mount an immune response to the missing gene product, wild-type virus infection results in seroconversion for the differentiating antigen. Serological assays (ELISA) have subsequently been developed that allow easy and sensitive detection of antibodies against these marker proteins, resulting in the identification of animals infected with wild-type virus, regardless of vaccination status (**Figure 5**). Thus, virus circulation can be reduced by vaccination, and infected animals that still harbor field virus can be identified subsequently and eliminated, resulting in cost-efficient eradication.

This breakthrough approach of 'differentiating infected from vaccinated animals' (DIVA) was pioneered in the field with PrV and is now widely accepted and practised

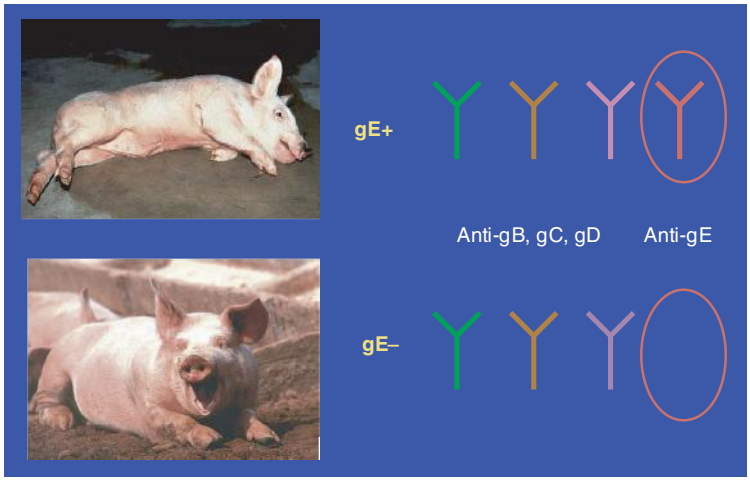


Figure 5 The principle of DIVA or marker vaccination. Whereas antibodies are produced against all immunogenic viral proteins after wild-type infection, antibodies against the missing gene product (circled) will not be formed after vaccination with a gene-deleted virus. The presence or absence of these antibodies is used to differentiate between infected and vaccinated animals. Reprinted with permission from Mettenleiter TC (2005) Veterinary viruses. *Nova Acta Leopoldina NF92* 344: 221–230.

with other infectious diseases, such as BHV-1 infection, classical swine fever, and foot-and-mouth disease. Its application resulted in eradication of PrV from heavily infected West Germany within 10 years, and also recently succeeded in eliminating PrV infection from pig herds in New Zealand and the USA. Although European wild boar and American feral pigs also harbor PrV, there is no epidemiological link between PrV in wild boar and domestic pigs in Europe since clearly different viral strains have been isolated from each. However, in the Southern USA infected feral pigs may represent a source of infection to domestic pig holdings.

PrV as a Tool in Neurobiology

Like other alphaherpesviruses, PrV exhibits a distinct neurotropism, invading the CNS via peripheral nerves. While wild-type strains of PrV may spread within the CNS both laterally and transsynaptically, attenuated PrV mutants have been identified which, under appropriate assay conditions, travel more or less exclusively along nerves and are transported transsynaptically. This property has prompted increasing use of PrV as a transneuronal tracer to label neuronal connections in experimental animal models, and has been useful in elucidating detailed neuroanatomical networks in mice and rats. The virus used most frequently in these studies is the Bartha strain of PrV, a modified-live vaccine strain which had been attenuated by the Hungarian veterinarian Adorján Bartha by multiple passages in embryonated chicken eggs and chicken embryo fibroblasts. Molecular biological analyses demonstrated that this strain carries several lesions compared to wild-type PrV: it lacks the gE, gI, and US9 genes, contains a mutation in the signal sequence for gC, specifies attenuating mutations in the UL21 gene, and expresses a UL10 gene product (gM) that is not glycosylated due to mutation of the N-glycosylation site. The glycoprotein deletion and the UL21 mutation have been shown to be most important for the observed attenuation. Recently, genetically engineered Bartha-derivatives expressing the marker proteins β-galactosidase or green fluorescent protein have been constructed and used in double-tracing studies. Moreover, mutants that express their markers only under specific conditions (e.g., in transgenic cells or animals expressing cre-lox recombinase under control of a tissue-specific promoter) have added further elegant possibilities for tissue-specific labeling.

Future Perspectives

PrV is a fascinating virus with several interesting properties. The availability of conventional and genetically engineered marker vaccines allows effective and cost-efficient disease control campaigns, which have been shown to result in the eradication of virus and disease from animal populations. Although PrV infection is still widespread, in particular in certain areas in Eastern Europe and Asia, concerted efforts could result in the elimination of the disease on a worldwide scale. Beyond its importance as the causative agent of a relevant animal disease, PrV is an ideal tool to study basic mechanisms of herpesvirus (molecular) biology and has the enormous advantage of an experimentally accessible natural virus–host system by infection of pigs. Moreover, its broad host range allows the use of other well-defined animal models for neuroanatomical, immunological, and molecular biological studies. Since PrV replicates exceedingly well in tissue culture, it is also well suited for detailed analysis of the requirements for (alpha) herpesvirus replication. Thus, PrV will remain under intensive scrutiny for sometime to come.

Acknowledgments

The author thanks Harald Granzow and Mandy Jörn for **Figures 1** and **3** and Jens Teifke, Thomas Müller, Hanns-Joachim Rziha, and Barbara Kluppa for helpful comments on the manuscript.

See also: Bovine Herpesviruses; Herpes Simplex Viruses: General Features; Herpes Simplex Viruses: Molecular Biology; Herpesviruses: General Features; Herpesviruses of Birds; Herpesviruses of Horses; Varicella-Zoster Virus: General Features; Varicella-Zoster Virus: Molecular Biology.

Further Reading

Enquist LW, Husak PJ, Banfield BW, and Smith GA (1999) Infection and spread of alphaherpesviruses in the nervous system. *Advances in Virus Research* 51: 237–347.

Granzow H, Weiland F, Jöns B, Klupp B, Karger A, and Mettenleiter TC (1997) Ultrastructural analysis of pseudorabies virus in cell culture: A reassessment. *Journal of Virology* 71: 2071–2082.

Klupp BG, Hengartner C, Mettenleiter TC, and Enquist LW (2004) Complete, annotated sequence of the pseudorabies virus genome. *Journal of Virology* 78: 424–440.

Mettenleiter TC (2000) Aujeszky's disease (pseudorabies) virus: The virus and molecular pathogenesis. *Veterinary Research* 31: 99–115.

Mettenleiter TC (2005) Veterinary viruses. *Nova Acta Leopoldina NF92* 344: 221–230.

Mettenleiter TC, Klupp BG, and Ganzow H (2006) Herpesvirus assembly: A tale of two membranes. *Current Opinion in Microbiology* 9: 423–429.

Pomeranz L, Reynolds AE, and Hengartner CJ (2006) Molecular biology of pseudorabies virus: Impact on neurovirology and veterinary medicine. *Microbiology and Molecular Biology Reviews* 69: 462–500.

Pseudoviruses

D F Voytas, Iowa State University, Ames, IA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

***env*-like gene** A gene found in some sireviruses with similarity to retroviral *env* genes: it occurs downstream of *pol* and often encodes a putative transmembrane protein.
Half-tRNA A tRNA fragment that encompasses the anticodon stem loop and primes reverse transcription of the hemiviruses.
Retrotransposon A mobile genetic element that replicates by reverse transcription but is not infectious.
Virus-like particle The nucleoprotein complex that serves as a replication intermediate for species in the family *Pseudoviridae*.

Introduction

Large-scale DNA sequencing projects have revealed that a significant fraction of most eukaryotic genomes is made up of mobile genetic elements. Among these are the long-terminal repeat or LTR retrotransposons, which share a similar genomic organization and life cycle with members of the family *Retroviridae*. The primary feature that distinguishes the retroviruses from the retrotransposons is that the former are infectious and encode an envelope gene that enables cell-to-cell transmission. The close relationship between the retroviruses and retrotransposons prompted the International Committee on Taxonomy of Viruses to provide a formal taxonomic description for the retrotransposons.

The retrotransposons are divided into the families *Pseudoviridae* (this article) and *Metaviridae* (see elsewhere in this volume). Pseudoviruses are particularly abundant in plant genomes, but are also found in diverse animal, fungal, and protist hosts. Members of the family *Pseudoviridae* are distinguished from those of the families *Metaviridae* and *Retroviridae* by a distinct organization of their *pol* genes: for pseudoviruses, reverse transcriptase (RT) precedes integrase (IN), and this order is reversed for members of related viral families. Phylogenetic analysis of RT amino acid sequences also clearly separates species of the family *Pseudoviridae* from those of the families *Metaviridae* and *Retroviridae*.

The family *Pseudoviridae* is often referred to as Ty1-copia retrotransposons after representative species from the yeast *Saccharomyces cerevisiae* (Ty1) and the insect *Drosophila melanogaster* (copia). For historic reasons, and because they are found in model experimental organisms, Ty1 and copia have been studied extensively at the molecular and biochemical level. Increasingly, research is also being conducted with the Ty5 hemivirus of *S. cerevisiae* and with representative pseudoviruses from plants, such as Tnt1 and Tto1 from tobacco (*Nicotiana tabacum*) and Tos17 from rice (*Oryza sativa*). Because Ty1 is the most intensely studied member of the family *Pseudoviridae*, much of the information in this article on virion properties and replication cycle is derived from research conducted with Ty1.

Taxonomy

The family *Pseudoviridae* is comprised of three genera: *Pseudovirus*, *Hemivirus*, and *Sirevirus* (**Table 1**). The genus *Pseudovirus* has the most species and the broadest host range. Although differences in RT amino acid sequences were originally used to distinguish the hemiviruses and pseudoviruses, as more species have been described, this criterion has proved to be less reliable (see below). Hemiviruses are also distinguished by the primer used for reverse transcription. Pseudoviruses and sirevirus use the 3′-OH of the acceptor stem of a host tRNA to prime DNA synthesis, whereas hemiviruses use a half-tRNA that corresponds to a portion of the tRNA anticodon stem loop. For copia, the tRNA fragment is generated by cleavage to release a 3′-OH that primes reverse transcription; however, the mechanism by which cleavage occurs remains obscure.

To date, the sireviruses are exclusively associated with plant hosts. Phylogenetic analyses indicate that the RT encoded by the sireviruses is distinct from those of the hemiviruses and pseudoviruses. An additional distinctive feature of many sireviruses is the presence of an open reading frame after *pol*. This gene is reminiscent of retroviral *env* genes both in terms of its location within the viral genome and the fact that it is often predicted to encode a transmembrane protein. Whether or not this *env*-like gene encodes a protein that mediates infectivity remains to be determined.

Virion Properties

Members of the family *Pseudoviridiae* are generally thought to replicate through a noninfectious virus-like intermediate referred to as a virus-like particle or VLP. The most thoroughly characterized VLPs are those produced by

Table 1 Viruses in the family *Pseudoviridae*

Genus	*Virus*	*Host species*	*Accession number*
Pseudovirus	Art1	*Arabidopsis thaliana*	Y08010
	AtRE1	*A. thaliana*	AB021265
	Evelknievel	*A. thaliana*	AF039373
	Ta1	*A. thaliana*	X13291
	Melmoth	*Brassica oleracea*	Y12321
	Panzee	*Cajanus cajan*	AJ000893
	Tgmr	*Glycine max*	U96748
	BARE-1	*Hordeum vulgare*	Z17327
	Tnt1	*Nicotiana tabacum*	X13777
	Tto1	*N. tabacum*	D83003
	RIRE1	*Oryza australiensis*	D85597
	Retrofit	*O. longistaminata*	U72726
	Tp1	*Physarum polycephalum*	X53558
	Ty1	*Saccharomyces cerevisiae*	M18706
	Ty2	*S. cerevisiae*	M19542
	Ty4	*S. cerevisiae*	X67284
	Tst1	*Solanum tuberosum*	X52387
	WIS-2	*Triticum aestivum*	X63184; X57168 (LTR)
	Hopscotch	*Zea mays*	U12626
	Sto-4	*Z. mays*	AF082133
Hemivirus	Mosqcopia	*Aedes aegypti*	AF134899
	Tca2	*Candida albicans*	AF050215
	Tca5	*C. albicans*	AF065434
	1731	*Drosophila melanogaster*	X07656
	Copia	*D. melanogaster*	M11240
	Ty5	*S. paradoxus*	U19263
	Lueckenbuesser	*Volvox carteri*	U90320
	Osser	*V. carteri*	X69552
Sirevirus	Endovir	*A. thaliana*	AY016208
	SIRE1	*G. max*	AY205608
	ToRTL1	*Lycopersicon esculentum*	U68072
	Opie-2	*Z. mays*	U68408
	Prem-2	*Z. mays*	U41000

the pseudovirus Ty1 and the copia hemivirus. Although sometimes irregularly shaped, these VLPs are typically round to ovoid and have a mean radius of 30–40 nm. Ty1 VLPs are cytoplasmic, whereas those produced by copia are nuclear. When truncated forms of the Ty1 capsid protein (Gag) are expressed, the VLPs appear more icosohedral, as observed by cryoelectron microscopy (**Figure 1**).

Members of the family *Pseudoviridae* encode a *gag* gene, and Gag proteins are processed by a viral protease to yield mature proteins corresponding to retroviral capsid (CA) and nucleocapsid (NC). Like the retroviruses, many pseudoviruses encode a zinc finger motif in Gag that is part of NC. For Ty1, which lacks a zinc finger signature, the protein encoded by the corresponding region of *gag* appears to function like a retroviral NC.

Gag proteins are the primary structural components of the VLP. Inside the particle are packaged the Pol gene products – RT and IN – along with an LTR to LTR transcript of the viral genome (typically ~5–9 kbp). This RNA is positive sense, capped and polyadenylated, and two RNAs are packaged within the particle (at least for the Ty1). Also found within the VLPs are various DNA intermediates – products of reverse transcription – which include full-length cDNA that represents the unintegrated form of the virus.

Genome Organization and Expression

Most members of the family *Pseudoviridae* encode a single open reading frame with similarity to both retroviral *gag* and *pol* genes (e.g., copia; **Figure 2**). Despite this single translation product, there appears to be a need, as in the retroviruses, to regulate relative amounts of Gag and Pol: for efficient replication, more structural Gag proteins are required to form the VLP relative to the catalytic Pol proteins. Work with the Ty5 hemivirus and the metavirus Tf1 suggests that differential protein stability may determine the proper Gag/Pol ratio in viruses that encode a single polyprotein. That is, at the onset of viral replication, Pol is degraded more quickly relative to Gag to achieve the proper stoichiometry. Some yeast pseudoviruses (e.g., Ty1, Ty2, and Ty4) encode pol in the +1 frame relative

to *gag*, and Gag/Pol stoichiometry is regulated by ribosomal frameshifting (**Figure 2**). Several sireviruses also encode *gag* and *pol* on separate reading frames; however, the location of conserved stop codons suggests that ribosomal frameshifting is not employed, and ribosomal hopping, as described for bacteriophage T4 *gene 60*, has been proposed although not experimentally verified. For the hemivirus copia, *gag* is encoded on a spliced mRNA, and differential splicing determines proper Gag/Pol levels.

Gag amino acid sequences are poorly conserved among members of the family *Pseudoviridae*, although a few weakly conserved motifs have been described in addition to the zinc finger motif that characterizes NC. Gag proteins of sireviruses are considerably larger than those encoded by species in other genera, and the extended region of Gag interacts with components of the dynein microtubular motor, suggesting that it plays a role in transporting the virus throughout the cell.

The coding region for *pol* lies downstream of *gag* and directs the production of three enzymes: protease (PR), IN, and RT. PR lies at the Pol N-terminus and is required for release of the other enzymes from the Pol precursor and for processing Gag. Like the retroviral enzyme, PR is an aspartic protease and undoubtedly functions as a dimer. Downstream of PR lies the coding region for IN. At its N-terminus, IN bears a zinc finger motif followed by a catalytic core domain that is similar to the retroviral enzyme. Adjacent to the IN catalytic domain is a large motif (called GKGY) that is unique to INs encoded by members of the family *Pseudoviridae*. Additionally, the extreme C-terminus of IN is rich in simple sequence

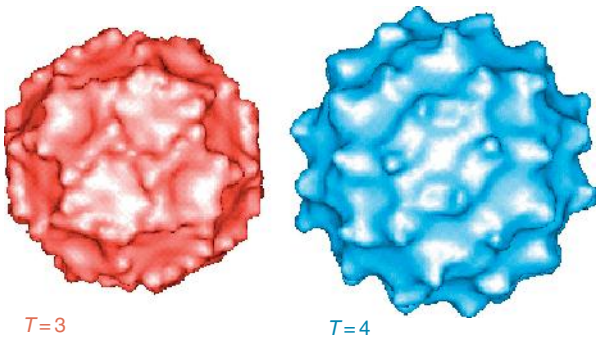


Figure 1 Virus-like particles produced by Ty1 of *S. cerevisiae*. Three-dimensional reconstruction of 1–346 $T=3$ and 1–408 $T=4$ particles calculated to 25 and 35 Å, respectively. Reprinted from Al-Khayat HA, Bhella D, Kenney JM, *et al.* (1999) Yeast Ty1 retrotransposons assemble into virus-like particles whose T-numbers depend on the C-terminal length of the capsid protein. *Journal of Molecular Biology* 292: 65–73, with permission from Elsevier.

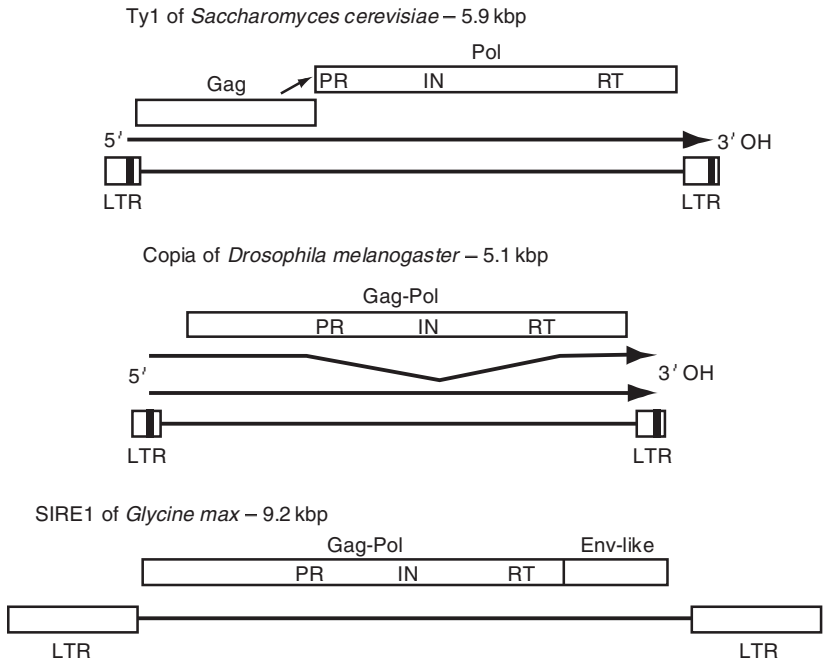


Figure 2 The genomic organization of representative viruses in the family *Pseudoviridae*. Ty1, copia, and SIRE1 are members of the genus *Pseudovirus, Hemivirus,* and *Sirevirus*, respectively. Transcript information is available for Ty1 and copia, and transcripts are depicted by arrows above the viral genomes. Note that the copia transcript is differentially spliced. Boxes on the ends of the viral genomes denote the LTRs. Black boxes within the Ty1 and copia LTRs denote the R region – sequences repeated at the 5′ and 3′ ends of the viral transcript. Encoded proteins are depicted as open boxes. Conserved amino acid sequences are labeled in Pol: protease (PR), integrase (IN), and reverse transcriptase/RNase H (RT). Note that Gag and Pol of Ty1 are on different reading frames, and a Gag-Pol fusion protein is expressed by ribosomal frameshifting. A single stop codon separates *gag-pol* from the *env*-like gene of SIRE1, and a Gag-Pol-Env-like polyprotein is synthesized by stop codon suppression.

motifs and shows considerable size heterogeneity. For the hemivirus Ty5, the IN C-terminus mediates target specificity through interactions with a specific heterochromatin protein (i.e., Sir4). This interaction tethers IN to heterochromatin and determines target site choice. The C-terminus of Pol encodes RT, and like the retroviral enzyme, RT has both RT and RNase H activity.

As described above, many sireviruses encode an additional gene downstream of *pol*. This coding region is often referred to as an *env*-like gene, both because of its location in the viral genome and because it typically encodes a protein with putative transmembrane domains, a hallmark of retroviral Env proteins. The *env*-like genes are often on separate reading frames, and for the SIRE1 virus, the *env*-like gene is separated from *pol* by a stop codon. Expression of the SIRE1 env-like gene is mediated by stop codon suppression, generating a Gag–Pol–Env-like fusion protein. The presence of an *env*-like gene, however, is not a universal feature of sireviruses, and the function of this protein in the life cycle of these elements remains to be determined.

The coding region for members of the family *Pseudoviridae* is flanked by two identical long terminal repeats that can range in size from a few hundred to a few thousand base pairs. The LTRs direct the expression of a genomic mRNA and often bear *cis*-acting sequences that regulate mRNA expression. The full-length genomic transcript has terminal redundancy like the retroviral transcript, and this redundancy is important in the replication life cycle of these elements (i.e., to facilitate strand exchanges during reverse transcription, see below).

Adjacent to the 5′ LTR is a short *cis*-acting sequence called the primer binding site (PBS) that primes reverse transcription. For most members of the family *Pseudoviridae*, the PBS is complementary to a host initiator tRNA methionine. Importantly, and as mentioned above, complementarity of the PBS to the acceptor stem loop of a host tRNA is the distinguishing feature of the hemiviruses. Adjacent to the 3′ LTR is a purine rich sequence (the polypurine tract, PPT) that serves as a primer for second strand DNA synthesis. There is considerable variation in the length of noncoding sequences between the priming sites and the coding regions, particularly for the sireviruses.

Replication Cycle

The mechanism by which viruses in the family *Pseudoviridae* replicate their genomes is principally derived from studies of the Ty1 pseudovirus of *S. cerevisiae*. It is generally assumed that other viruses in the family use a similar replication strategy, which as described below, is very similar to the replication mechanism employed by viruses in the family *Retroviridae*.

The replication cycle begins with the production of a full-length transcript that initiates in the 5′ LTR and terminates in the 3′ LTR. The LTRs are divided into three regions based on sequences present in the transcript: the U3 region includes sequences unique to the 3′ end of the RNA; U5 includes sequences unique to the 5′ end of the RNA, and the R regions are sequences repeated at both ends of the message. The first gene encoded on the full-length mRNA is *gag*, and as mentioned above, *gag* encodes the major CA as well as a small C-terminal peptide that is similar to retroviral NC proteins. CA and NC are derived from the Gag translation product through proteolytic processing by PR. Pol is expressed as a translational fusion with Gag, and in a few species, this occurs through ribosomal frameshifting. Pol is processed by PR to release mature IN and RT.

In addition to serving as a template for translation of Gag and Pol, the viral transcript also serves as a template for the synthesis of DNA through reverse transcription. The full-length mRNA is encapsidated in a precursor VLP consisting of unprocessed Gag and Gag–Pol proteins. Action by PR converts the particle into a mature VLP and likely activates reverse transcription.

The synthesis of viral cDNA from the template mRNA proceeds by a mechanism very similar to that of the retroviruses. The first step is the extension of cDNA from a tRNA primer that anneals to the PBS. The cDNA is extended to the 5′ end of the mRNA generating minus ( )-strand strong-stop DNA (ssDNA). The ( ) ssDNA is transferred to the 3′ end of the full-length RNA where it pairs with the R region and then is extended to generate a nearly full-length ( )-strand cDNA. Plus (+)-strand synthesis begins at the RNase H resistant PPT adjacent to the 3′ LTR. Extension yields a (+) ssDNA that is transferred to the left end of the ( )-strand DNA. Further extension yields a full-length cDNA. The double-stranded cDNA is imported into the nucleus where it is inserted into a chromosomal target site. Integration generates a 5 bp duplication of host target DNA. Some viruses, particularly in yeast, show strong target site preferences. In these cases, the integration machinery recognizes specific protein complexes at the target sites (e.g., transcription complexes, heterochromatin) to mediate target specificity.

Evolutionary Relationships

The taxonomic framework for the family *Pseudoviridae* is based on phylogenetic analyses of RT amino acid sequences encoded by family members (**Figure 3**). Early work indicated that members of the family *Pseudoviridae* form a monophyletic lineage among the diverse mobile genetic elements that encode RTs. The distinctiveness of the pseudovirus RT holds up regardless of the host from

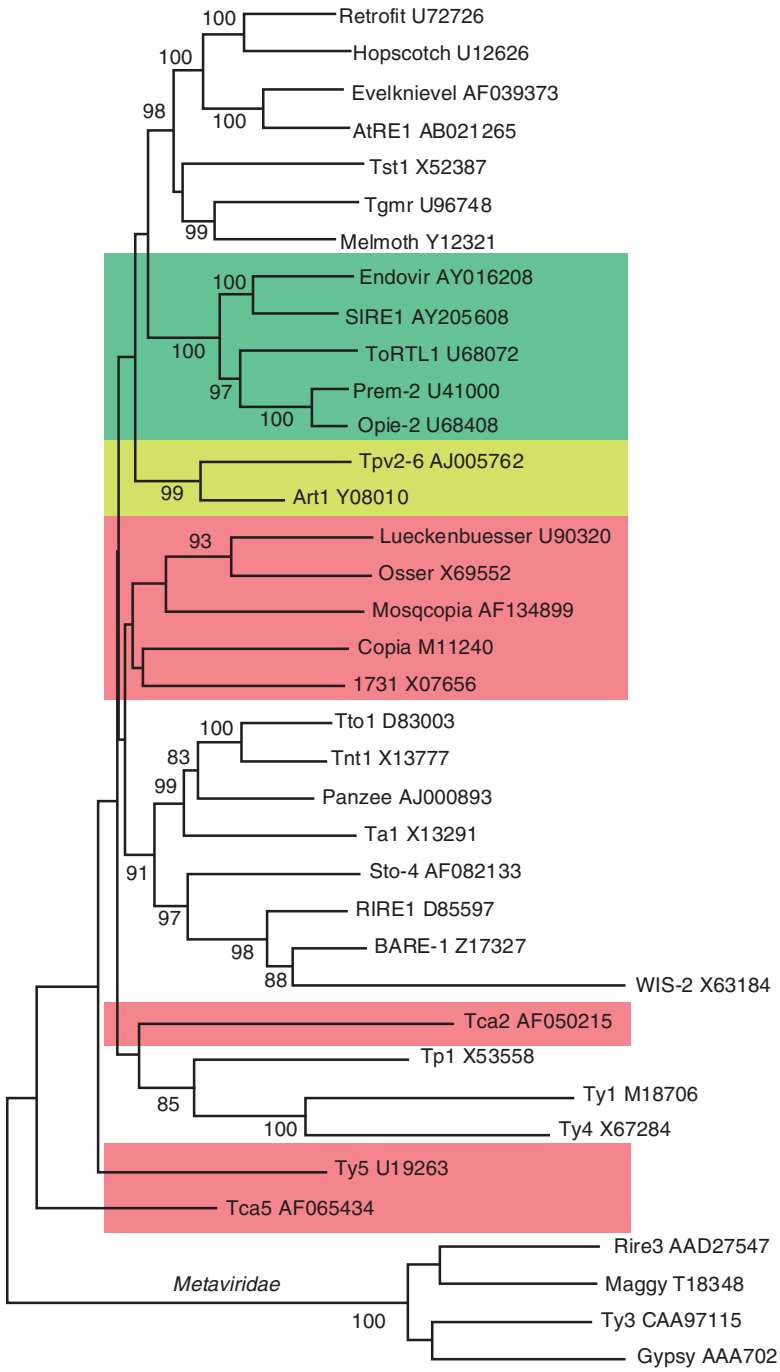


Figure 3 Phylogenetic relationships among members of the family *Pseudoviridae*. The tree is based on RT amino acid sequences and was performed using the neighbor-joining distance algorithm. RTs encoded by species in the family *Metaviridae* were used as an outgroup. The hemiviruses are shaded in pink and the sireviruses in green. Yellow denotes Tpv2-6 and Art1, which have not been formally assigned to the family *Pseudoviridae*.

which the species originate. The organization of *pol*, with IN preceding RT, is also a unique feature that identifies family members.

Designations for the genera *Pseudovirus* and *Hemivirus* were originally based on the primer used for ( ) ssDNA synthesis (i.e., a full- or half-tRNA, respectively). The genera designations were underscored by phylogenetic analyses indicating that RTs encoded by members of these genera were distinctive. Recent genome sequencing efforts, however, have greatly expanded the ranks of the family *Pseudoviridae*. Analysis of the larger dataset indicates that the phylogenetic support for the original genera

designations is weak (**Figure 3**). It may be that derived characters such as the use of particular primers is not an appropriate means for classification, and it is anticipated that the taxonomic structure of the family will need to be reconsidered.

Large-scale genomic sequencing efforts also revealed new lineages within the family *Pseudoviridae*, prompting the designation of the genus *Sirevirus*. The RTs encoded by the sireviruses are phylogenetically distinct from other members of the family, and sireviruses are found only in plant hosts. Although the original sireviruses described encode an additional *env*-like gene, some elements lack this coding sequence, and so distinctiveness of the RT amino acid sequence is the most reliable means of classification.

Genome sequencing efforts have also revealed species with *pol* gene organizations characteristic of the family *Pseudoviridae* and yet RTs that are distinct from the genera described to date (**Figure 3**). Among these are Tpv2 and its relatives, which currently are not officially assigned to the family *Pseudoviridae*. Future taxonomic revisions will have to address their candidacy for assignment to the *Pseudoviridae* and whether or not they define a new genus.

See also: Metaviruses; Retroviruses: General Features.

Further Reading

Al-Khayat HA, Bhella D, Kenney JM, *et al.* (1999) Yeast Ty1 retrotransposons assemble into virus-like particles whose T-numbers depend on the C-terminal length of the capsid protein. *Journal of Molecular Biology* 292: 65–73.

Boeke JD, Eickbush T, Sandmeyer SB, and Voytas DF (2000) Family *Metaviridae*. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, *et al.* (eds.) *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, pp. 358–367. San Diego: Academic Press.

Boeke JD, Eickbush T, Sandmeyer SB, and Voytas DF (2000) Family *Pseudoviridae*. In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, *et al.* (eds.) *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, pp. 349–357. San Diego: Academic Press.

Doolittle RF, Feng DF, Johnson MS, and McClure MA (1989) Origins and evolutionary relationships of retroviruses. *Quarterly Review of Biology* 64: 1–30.

Eichinger DJ and Boeke JD (1988) The DNA intermediate in yeast Ty1 element transposition copurifies with virus-like particles: Cell-free Ty1 transposition. *Cell* 54: 955–966.

Flavell AJ, Dunbar E, Anderson R, Pearce SR, Hartley R, and Kumar A (1992) Ty1-*copia* group retrotransposons are ubiquitous and heterogeneous in higher plants. *Nucleic Acids Research* 20: 3639–3644.

Garfinkel DJ, Boeke JD, and Fink GR (1985) Ty element transposition: Reverse transcriptase and virus-like particles. *Cell* 42: 507–517.

Kikuchi Y, Ando Y, and Shiba T (1986) Unusual priming mechanism of RNA-directed DNA synthesis in *copia* retrovirus-like particles of *Drosophila*. *Nature* 323: 824–826.

Laten HM, Majumdar A, and Gaucher EA (1998) SIRE-1, a *copia*/Ty1-like retroelement from soybean, encodes a retroviral envelope-like protein. *Proceedings of the National Academy of Sciences, USA* 71: 6897–6902.

Mellor J, Malim MH, Gull K, *et al.* (1985) Reverse transcriptase activity and Ty RNA are associated with virus-like particles in yeast. *Nature* 318: 583–586.

Peterson-Burch BD and Voytas DF (2002) Genes of the *Pseudoviridae* (Ty1/*copia* retrotransposons). *Molecular Biology and Evolution* 19: 1832–1845.

Voytas DF and Boeke JD (2002) Ty1 and Ty5 of *Saccharomyces cerevisiae*. In: Craig NL, Craigie R, Gellert M, and Lambowitz AM (eds.) *Mobile DNA II,* 1st edn., 631–662. Washington, DC: American Society for Microbiology Press.

Voytas DF, Cummings MP, Koniczny A, Ausubel FM, and Rodermel SR (1992) *Copia*-like retrotransposons are ubiquitous among plants. *Proceedings of the National Academy of Sciences, USA* 89: 7124–7128.

Xiong Y and Eickbush TH (1990) Origin and evolution of retroelements based upon their reverse transcriptase sequences. *EMBO Journal* 9: 3353–3362.

Zou S, Ke N, Kim JM, and Voytas DF (1996) The *Saccharomyces* retrotransposon Ty5 integrates preferentially into regions of silent chromatin at the telomeres and mating loci. *Genes and Development* 10: 634–645.

Q

Quasispecies

R Sanjuán, Instituto de Biología Molecular y Cellular de Plantas, CSIC-UPV, Valencia, Spain

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Adaptive landscape A graphical representation of fitness as a function of the genotype, first used by the population geneticist S. Wright.
Antagonistic epistasis Genetic interaction that makes mutations have larger effects alone than in combination.
Antagonistic pleiotropy The situation in which a particular mutation that is beneficial in one environment becomes harmful in another environment.
Back-mutation Mutation that regenerates the ancestral genotype.
Biological fitness The number of descendants.
Degenerate quasispecies Quasispecies with more than a single fittest type.
Effective population size The number of individuals of a population that actually contribute to the next generation.
Epistasis Interaction between genetic loci, implying that mutations have nonindependent effects.
Error catastrophe A situation in which the mutation rate is too high to be effectively counteracted by selection and the population becomes a pool of randomly drifting sequences.
Error threshold The critical mutation rate that marks the transition from mutation–selection balance to error catastrophe.
Genetic drift Changes in the genetic composition of finite populations due to random sampling of genotypes between generations.
Master sequence The fittest and most abundant sequence in a nondegenerate quasispecies.
Muller's ratchet Accumulation of deleterious mutations in small populations due to genetic drift that becomes irreversible in the absence of recombination or back-mutations.
Multiplicative landscape An adaptive landscape in which there is no epistasis, that is, mutational effects are independent.
Mutation–selection balance The dynamic equilibrium between the generation of variability through deleterious mutation and its elimination through natural selection.
Mutational robustness Ability to tolerate mutations.
Phase transition A sudden change in the properties of a physical system in response to a change in one or more parameters.
Quasispecies A population at the mutation–selection balance composed by a master sequence and an ensemble of deleterious mutants.
Selection coefficient Denoted *s*, the relative fitness difference between a genotype and the wild type.
Sequence space A discrete space with as many dimensions as the genome length and four values on each dimension corresponding to the four possible nucleotides.
Wild type An arbitrary reference genotype, typically the most abundant one.

The Quasispecies Theory

Historical Context

The word quasispecies, borrowed from chemistry, refers to a population of quasi-identical molecules, by opposition to a molecular species, which is made of fully identical molecules. The term is probably unfortunate in the biological context because first, it could be erroneously thought of as if it made reference to the biological concept of species and second, biological populations are generally variable, making its usage redundant. For these reasons, some virologists have discarded it, while most keep making use of it to refer to a highly variable viral

population. However, this is mainly a matter of semantics. Quasispecies constitute indeed a formal evolutionary theory first introduced in the early 1970s by Manfred Eigen and intended to capture the population dynamics of hypothetical replicons at the origin of life. These replicons were small and displayed a high rate of self-copying error due to the lack of proofreading mechanisms. Pioneer experiments with RNA bacteriophages showed high levels of genetic variability, suggesting error-prone replication and, later on, RNA viruses were confirmed to show high per-base mutation rates and short genomes. The quasispecies theory offered thus a relevant conceptual framework for RNA virus evolution and became a virological paradigm.

Scientific Context

Quasispecies theory is a population genetics theory. Population genetics, which took its first steps in the beginning of the twentieth century, addresses how changes in gene frequencies depend on measurable parameters such as selection coefficients, population sizes, mutation rates, or recombination rates, and provides a well-grounded theory for the study of evolution. Nevertheless, evolution is a very complex process and its mathematical treatment requires the use of simplifying assumptions. Choosing the appropriate assumptions is critical to build accurate models of evolution. This choice, which depends on the specific questions to be addressed, usually makes the difference between evolutionary models.

One central question in population genetics is how much variability can be stably maintained from the interplay between mutation and natural selection. In all populations, the continuous production of deleterious mutations through error-prone replication is counterbalanced by the elimination of these mutations through selection. When a population is sufficiently adapted to its environment, the observed variability is mainly determined by the interplay between the above two opposing factors which, in the long term, reach the so-called mutation–selection balance. Quasispecies theory mainly addresses this issue, though it had already been addressed decades before by the founders of population genetics. Since quasispecies come from the field of physical chemistry, their terminology is somewhat different, but most concepts are fully equivalent to those of population genetics (**Table 1**).

Table 1 Conceptually equivalent terminology in quasispecies and population genetics theories

Quasispecies theory	*Population genetics theory*
Quasispecies	Population at the mutation–selection balance
Stationary state	Mutation–selection balance
Master sequence	Wild type
Superiority of the master	Average selection coefficient
Phase transition	Selective sweep
Excess production	Progeny number
Quality factor	Replication fidelity

The Fundamental Model

The basic quasispecies model describes the population dynamics of macromolecular sequences maintained by error-prone replication. Mutations occurring during the copying process can modify the replication rate of the newly arisen sequences, allowing Darwinian selection to operate. The system can be described by a value matrix $\mathbf{W}$ in which each diagonal element W_{kk} equals the number of nonerroneous copies resulting from exact replication of sequence k and each off-diagonal element W_{kj} equals the number of copies of sequence k resulting from erroneous replication of sequence j. The stationary sequence distribution is called the quasispecies and consists of a master sequence along with a distribution of mutants. Although the slower replicators cannot sustain their abundance level by themselves, they are constantly recreated from other sequences that mutate into them. At the stationary state, the average biological fitness of the population equals the largest eigenvalue of $\mathbf{W}$ and the abundance of each sequence is given by the associated eigenvector.

In practice, given the huge dimension of $\mathbf{W}$ (equal to the genome length), exact solutions of the quasispecies equations cannot be found. If all sequences in a given error class (i.e., all one-error mutants, all two-error mutants, etc.) are assumed to have identical fitness, it becomes possible to estimate the frequency of each sequence as a function of the per-base mutation rate, its number of mutations, its relative fitness, and a recursively defined function that accounts for the fitness of all possible mutant intermediates. However, the fitness of all intermediates is not known in practice. The problem can be solved by assuming that mutations have independent fitness effects, or using population genetics terms, that there is no epistasis. This implies that the fitness of each mutant is simply the product of fitness values associated to each single mutant and thus, that the mean frequency of mutants carrying d mutations relative to that of the master (q_d/q_o), can be simply obtained by summing over all possible combinations of d mutations in a genome of length L. Using the binomial distribution, this can be written as $q_d/q_o = (1 \quad \mu)^{-LBi(d|\mu,L)s^{-d}}$, where μ is the per-base mutation rate.

Assuming that L is large enough to be treated as infinite can be useful to illustrate the equivalence between quasispecies and population genetics models. The binomial distribution can then be substituted by a Poisson distribution of parameter $U = \mu L$, where U is the genomic mutation rate, and $(1 \quad \mu)^{L}$ can be replaced by $e^{\ U}$. Hence, $q_d/q_o = (U/s)^{d/d!}$ and, setting $q_o = e^{-U/s}$, it follows that

q_d is Poisson-distributed with parameter $\lambda = U/s$. Hence, the average number of mutations per genome is U/s and the mean relative fitness of the population is e^{-U}, which are classical population genetics results.

Common Assumptions

Although the quasispecies theory is very general in its basic formulation, some simplifications have to be done for the sake of tractability, going to the detriment of generality. Common assumptions of the original quasispecies models are that: (1) there is a single fittest sequence; (2) mutation rates are constant over time and genotypes; (3) mutants show invariant fitness regardless of the number of mutations they carry; (4) molecules are degraded at a constant rate; (5) the environment is constant; (6) the population size is infinite; and (7) there is no genetic recombination. All of these assumptions were made in most of the analyses originally published by Eigen and co-workers. The situation in which there is a single fittest sequence and all other genotypes have constant fitness can be represented by a two-class single-peak landscape, although real landscapes can be much more complex, with several local adaptive peaks separated by low-fitness regions (**Figure 1**).

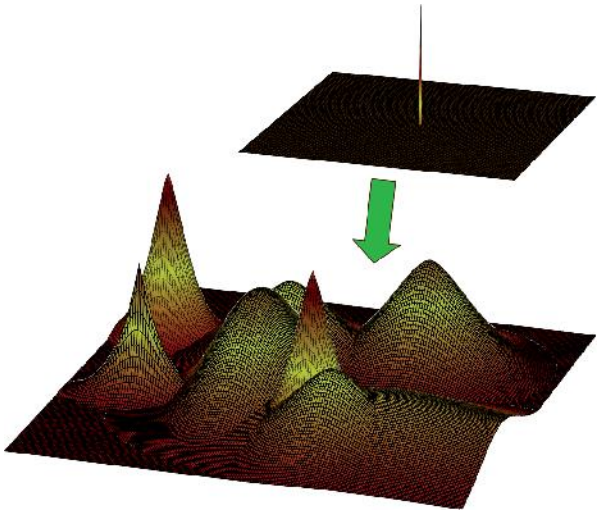


Figure 1 Two hypothetical adaptive landscapes. Sequence spaces are highly dimensional and need to be reduced to only two dimensions for visualization. The third dimension represents fitness, such that peaks in the landscape are local or global fitness maxima, whereas valleys are low-fitness regions. Above, the two-class single-peak landscape originally used by Eigen, in which there is a single fittest sequence and all other types show a lower, constant fitness value. A more complex landscape, with several local peaks, is represented below. Ruggedness appears as consequence of frequent epistasis of variable sign and intensity. The lower example provides a more realistic picture than the above two-class single-peak case, though it might still be much simpler than real landscapes.

Later on, some of the above assumptions were relaxed. For example, Peter Schuster and co-workers studied the case in which there is more than one fittest sequence and called it a degenerate quasispecies. The two master sequences can be located at distant regions of the sequence space separated by low-fitness regions, hence being effectively unconnected. Different generalizations of the two-class single-peak landscape have been studied, including multidimensional landscapes. A simple though useful case is the multiplicative landscape, in which epistasis is assumed to be null and hence fitness decays exponentially with mutation number. Others have also investigated quasispecies in variable environments or with finite population sizes.

Relevant Predictions

Although population genetics and quasispecies are fundamentally equivalent, the assumptions that are typically done in quasispecies models often differ from those made in classical population genetics. Importantly, different assumptions can lead to different predictions. Below, we focus on two relevant examples, concerning the frequency of mutations and the topology of adaptive landscapes.

The evolution of mutational robustness

The Haldane–Muller principle states that the average fitness of the population at the mutation–selection balance does not depend on the fitness effects of mutations or, more specifically, on selection coefficients. The reason is that the more deleterious a mutation is, the more it reduces the average population fitness but, on the other hand, the more its frequency is lowered by selection. These two factors cancel out and the average relative fitness of the population simply writes e^{-U} (see above). This result is based on neglecting back-mutations, which is justified for large genomes replicating at low per-base error rates. However, quasispecies were intended to capture the population dynamics of small replicons with high per-base mutation rates, implying that secondary and back-mutations could not be systematically neglected. The reason is that the probability of occurrence of back-mutations relative to forward mutations increases in short genomes and, at high genomic mutation rates, the evolutionary fate of any given mutant genotype can depend not only on its fitness effect, but also on secondary mutations, or on the influx of mutations regenerating the genotype from neighbors in sequence space.

When secondary and back-mutations are taken into account, the average fitness of the population does not anymore strictly depend on the deleterious mutation rate, but also on the average selection coefficient. In other words, the fitness of a genotype is not only determined by its own replication rate, but also by those of its mutational neighbors. Everything else being equal, the lower

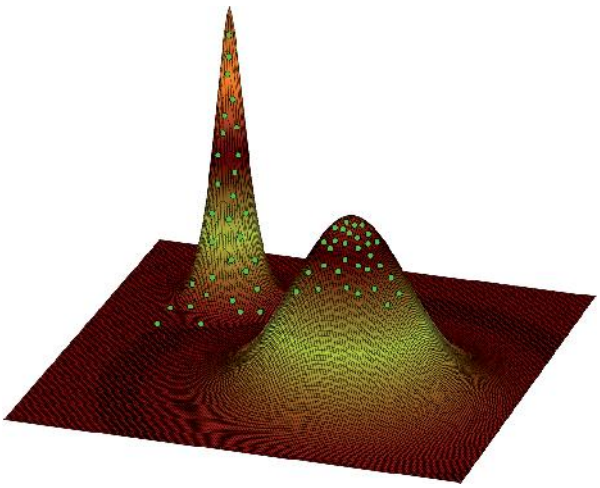


Figure 2 Representation of survival of the flattest in two-peak landscape. One population is located at a higher peak, which should confer it a selective advantage. However, the other population is located at a flatter peak, implying that neighbors in the sequence space have similar fitness and, thus, that the population is more robust to mutation. A prediction of the quasispecies theory is that, at high mutation rates, increased robustness can provide a selective advantage to the latter population.

the selection coefficient the higher the average fitness. A consequence is that, at high mutation rates, a slower replicator can potentially outgrow a faster competitor by virtue of its higher mutational robustness, a phenomenon called the 'survival of the flattest' (**Figure 2**). This effect rapidly vanishes as the mutation rate decreases and the genome length increases, but it can be magnified in small populations, in which genetic drift favors the accumulation of mutations. It should be noted that the statement that the average fitness is not a property of the master but also of the neighboring sequences does not mean that the genotypes constituting the quasispecies establish any kind of cooperation, complementation, or mutualism. Experiments showing cooperative interactions between genotypes should therefore not be considered as supportive of the quasispecies predictions.

The error catastrophe

The existence of an error catastrophe is probably the most widely known prediction of the quasispecies theory. An error threshold is the critical error rate beyond which Darwinian selection cannot further maintain the genetic integrity of the quasispecies. Below the threshold, the quasispecies is stably localized around one or more sequences, but if the mutation rate exceeds the threshold value, the variety of mutants that are in amounts comparable to the fittest types becomes very large and the population is said to enter into error catastrophe. According to the original definition given by Eigen, the threshold satisfies $W_{00} = w_{k\neq 0}$, where W_{00} is the nonmutated progeny of the master and $\bar{w}_{k\neq 0}$ is the average fitness (mutated and nonmutated progeny) of all other sequences present in the population. If $W_{00} < \bar{w}_{k\neq 0}$, the master is overgrown by its own mutational cloud and, in the absence of back-mutation, goes extinct through error catastrophe (although assumptions about back-mutation do not have a bearing on the existence or magnitude of error catastrophes).

The existence of an error threshold, however, is not an obligate prediction of the quasispecies theory. For example, in a multiplicative landscape, the frequency of the master is given by $q_0 = e^{-U/s}$, a quantity that decreases as the mutation rate increases but never reaches zero, hence there is no error threshold. The original quasispecies model produces an error threshold because all mutants are assumed to have a constant fitness regardless of their mutational distance to the master. In this case, $\bar{w}_{k\neq 0}$ is a constant, $\bar{w}_{k\neq 0} = 1 \quad s$, and, therefore, an error threshold deterministically takes place when $L = \log\sigma_0/\mu$, where σ_0 is the superiority of the master over all mutants. It must be noted that, even in this model, though there is an error threshold, no sharp transition in average fitness occurs. Average fitness smoothly decreases with increasing mutation rate until it reaches a plateau value equal to $\bar{w}_{k\neq 0}$. In general, whether there is an error threshold critically depends on the assumed adaptive landscape. Some generalizations of the two-class single-peak landscape predict error catastrophes, but, in general, the outcome mainly depends on the sign of epistasis. Error catastrophes have a greater probability to exist if epistasis is antagonistic, such that mutations tend to have progressively less fitness effects as they accumulate (notice that the two-class single-peak landscape represents an extreme form of antagonistic epistasis). However, recent work has shown that with null epistasis, there can be an error catastrophe if lethal mutations are taken into account. This is a complex theoretical issue which still remains to be fully resolved.

Experimental Evolution of RNA Viruses

RNA viruses are useful tools for experimentally addressing fundamental evolutionary issues while they still allow us to pay attention to the molecular aspects. Based on scientific but also historical reasons, the quasispecies theory is the *de facto* standard for interpreting evolutionary experiments with RNA viruses. RNA viruses are characterized by their error-prone replication and small genomes, which makes the quasispecies an *a priori* relevant theoretical approach. However, even if some predictions are specific to the quasispecies theory, most are equivalent to those made by previously proposed and simpler population genetics models. Furthermore, quasispecies do not provide a theoretical underpinning to some experimental

aspects of viral evolution which are better explained by nondeterministic population genetics models. After three decades of experimental work, some general evolutionary properties of RNA virus populations have been elucidated, most of them being compatible with both quasispecies and classical population genetics. A few data, though, support the need to use the more complex quasispecies formulation.

Key Evolutionary Parameters of RNA Viruses

High per-base mutation rates

In pioneer evolutionary experiments performed in the late 1970s, RNA bacteriophage Qβ populations serially passaged in laboratory conditions were found to be highly variable at the nucleotide level, each viable phage genome differing on average in one to two positions relative to the reference sequence. It was suggested that these populations were in a dynamic equilibrium, with viable mutants arising at a high rate and being strongly selected against, an equilibrium known as the mutation–selection balance. From then on, many evolutionary experiments have been done with different model RNA viruses, including the bacteriophage ϕ6, the vesicular stomatitis virus (VSV), poliovirus-1, or foot-and-mouth disease virus (FMDV). It has been estimated that the mutation rate of RNA viruses is within the range 10^{-6}–10^{-4} substitutions per replication event, which is orders of magnitude higher than that of DNA-based organisms. Biochemical experiments with the avian myeloblastosis virus, VSV, and human immunodeficiency virus type 1 (HIV-1) have established that their polymerases lack 3′ exonuclease activity, providing a basis for error-prone replication. Recent experiments have demonstrated that poliovirus-1 is capable of counteracting the mutagenic effect of base analogs such as ribavirin by evolving specific genotypic changes that confer increased replication fidelity. This suggests that the high mutation rate of RNA viruses is not only a consequence of fundamental biochemical restrictions, but also the product of evolutionary optimization.

Compacted genomes

Another seemingly general property of RNA viruses is their small genome size (e.g., 3569 nt for MS2 phage to 11 162 nt for VSV), with many examples of overlapping reading frames and multifunctional genes. In contrast to multicellular eukaryotes, noncoding regions occupy a small fraction of the genome, hence making silent mutations relatively infrequent. A consequence of genome compactness is that RNA viruses are extremely sensitive to deleterious mutations. For VSV, it has been estimated that single random nucleotide substitutions have average selection coefficients of 0.5 (fitness is decreased by 50%), at least an order of magnitude higher than in DNA-based organisms, and it has been estimated that up to 40% of random mutations inactivate the virus. A related consequence of genome compactness is that epistasis is antagonistic on average, because in such compact genomes, one or few changes are enough to disrupt most of the encoded functions, and hence additional mutations can only produce a comparatively smaller effect.

Rapid growth

RNA viruses critically rely on fast replication for survival. Their rapid infection cycles allow them to reach high titers before the onset of host defense mechanisms. In cellular cultures, phages complete an infection cycle in less than an hour, whereas mammalian viruses do it in a few hours. Each infected cell typically releases hundreds to few thousands of infectious particles and viruses rapidly reach population sizes of several billion particles per milliliter. Intuitively, high particle counts indicate that the number of available genotypic variants is high, which should allow Darwinian selection to operate more efficiently. However, it must be noted that although population sizes are huge, the relevant parameter in evolutionary terms is the effective population size, since it indicates the actual number of particles that contribute to the next generation. Demographic fluctuations, originated by transmission bottlenecks, host defense mechanisms, or antiviral treatments make the effective population considerably smaller than particle counts. For example, in typical passage regimes with mammalian lytic viruses, if 10^4 particles were inoculated into 10^5 cells, effective population sizes would be in the range of 10^4–10^5 particles, whereas particle counts would typically be in the order of 10^9.

Main Evolutionary Properties of RNA Viruses

Rapid adaptation dynamics

Similar to what is found with other microorganisms, a frequent observation in long-term evolution experiments is that RNA viruses show an initially fast adaptation that tends to decelerate over time. Such dynamics indicates that, after being placed in a new environment, populations are evolving from a region of low fitness toward an adaptive peak or plateau. High population sizes make it more likely that beneficial mutations are created and, together with fast replication rates, this explains the observed rapid adaptation. Also, elevated selection coefficients increase the probability that any given beneficial mutation becomes fixed in the population. Finally, high mutation rates produce high levels of genetic variability on which selection can operate, though it must be noted that, since most mutations are deleterious, increasing the mutation rate does not necessarily imply increasing the rate of adaptation. Maximal adaptation rates should indeed be reached at intermediate mutation rates, the optimal mutation value being mainly determined by the selection coefficient against deleterious mutations and the rate of recombination.

Evolutionary parallelisms and convergences

In several serial passage experiments with bacteriophages, plant viruses, and mammalian viruses, sequencing of lineages independently adapted to the same environment has revealed a large amount of evolutionary parallelisms and convergences at the genetic level, both synonymous and nonsynonymous. This pattern indicates that, upon facing identical selective pressures, viruses often find the same adaptive pathways. It is possible that in simple and compacted genomes, such as those of RNA viruses, the number of alternative evolutionary solutions is limited, or that, due to their elevated adaptive potential, RNA viruses are able of systematically finding the highest peak in the adaptive landscape. Both scenarios would indicate that viral evolution at high population sizes has an important deterministic component, as proposed by quasispecies models. However, it is also possible that the common ancestor was located near a local peak surrounded by low-fitness regions and that, since Darwinian selection always pushes populations uphill, these populations would have remained trapped in their local peak.

Fitness tradeoffs

Despite their remarkable adaptability, RNA viruses face some evolutionary constraints. For example, shifts between vertebrate and arthropod hosts impose drastic environmental fluctuations to arboviruses. Several studies have confirmed that adaptation to a novel host often decreases competitive ability in the former host. These tradeoffs can arise by antagonistic pleiotropy or antagonistic epistasis among beneficial mutations. Pleiotropy, epistasis, and hence fitness tradeoffs are expected to be common in compacted genomes with many overlapping functions. A particularly important example of tradeoff is that exerted on mutation rate. Most mutations are deleterious, and hence there is a short-term selective pressure for reducing mutation rates toward whatever limit is imposed by biochemical restrictions, but, on the other hand, mechanisms of replication fidelity should come at a kinetic cost and thus be selected against. Since RNA viruses critically rely on fast replication, the cost of proofreading functions in terms of replication rate might be particularly strong.

Transmission bottlenecks

Considering the fact that RNA viruses show large selection coefficients and high population sizes, it could be concluded that selection is the main factor driving their evolution. However, in nature, viral populations experience strong bottlenecks upon transmission and therefore, effective population sizes are well below particle counts. Low effective population sizes increase the strength of genetic drift, hence favoring the accumulation of deleterious mutations and potentially jeopardizing the survival of the population. This stochastic process is known as Muller's ratchet, named after the population geneticist who first anticipated it in the 1930s. The expected rate of fitness loss equals the product of the mutation rate and the average selection coefficient for deleterious mutations, thus making RNA viruses especially sensitive to Muller's ratchet. This prediction has been experimentally validated by performing serial plaque-to-plaque passages in a variety of RNA viruses, including bacteriophage ϕ6, VSV, FMDV, and HIV-1.

Is the Quasispecies Theory Relevant to the Evolution of RNA Viruses?

There has been some controversy as to whether quasispecies are relevant to RNA virus evolution. Detractors have sometimes argued that the theory overlaps with previous population genetics theory, whereas supporters have claimed that it goes beyond population genetics. Many virologists believe that the elevated genetic variability of RNA viruses proves their quasispecies nature, but the mere observation of genetic variability is a trivial consequence of mutation and hence can hardly be considered supportive of any biological theory. Most of the empirically inferred properties of RNA viruses are already well explained by classical population genetics, as for example the expected high adaptability of large populations, the most likely deleterious effects of mutation, the complex relation between mutation and adaptation rates, the existence and evolutionary implications of fitness tradeoffs and epistasis, or the genetic contamination of small populations throughout Muller's ratchet. To justify the necessity of the extra complexity introduced by the quasispecies theory, evidence for its specific predictions should be provided.

The evolution of robustness

RNA viruses are highly sensitive to mutation compared to more complex microorganisms. Genetic hypersensitivity and large population sizes make selection very efficient at purging deleterious mutations and hence promote the preservation of the wild type. Although this seems to be the predominant survival strategy among RNA viruses, quasispecies theory predicts that at high mutation rates and low population sizes, mutational robustness can be selectively advantageous. The first experimental evidence in favor of this prediction came from work with the bacteriophage ϕ6 showing that the evolution of a genotype can be influenced by the topology of the neighboring regions in the adaptive landscape and that robustness can be a selectable character. A more direct approach was undertaken with viroids, plant pathogens consisting of small, noncoding RNA molecules. Two different viroid species were competed in common host plants; one was characterized by fast population growth and genetic homogeneity, whereas the other showed slow population

Table 2 Differences between lethal mutagenesis and error catastrophe processes

	Lethal mutagenesis	*Error catastrophe*
Nature of the process	Population extinction through mutation accumulation	Delocalization of the quasispecies due to mutation
Name of the threshold	Extinction threshold	Error threshold
Key parameters	Mutation rate, fitness of the wild type, selection coefficient	Mutation rate and selection coefficient
Demography	Population size declines	No changes in population size are specified
Fate of the wild type	Not necessarily extinguished until the end of the process	Extinguished while other variants survive
Dependence on mutation rate	Extinction is more likely and occurs faster at higher mutation rates	Beyond the error threshold, further increases in mutation rate have no effect
Typically assumed effect of mutations on fitness	The fitness of mutant genotypes decays with mutation number	The fitness of mutant genotypes does not decay with mutation number
Mutational signature	No specific changes are required in the consensus sequence	The consensus sequence randomly drifts through time

growth and a high degree of variation. When the mutation rate was artificially increased using UVC radiation, the fitness of the slower replicator increased relative to that of the faster competitor, probably due to its higher mutational robustness, which might support the survival of the flattest hypothesis. Mutagens are especially suited for observing this quasispecies prediction, since they simultaneously increase mutation rates and decrease viral titers. Recent experiments with VSV populations indicate that base analogs such as 5-fluorouracil can provide a selective advantage to robust genotypes.

Error catastrophe and lethal mutagenesis

The consequences of artificially increasing error rates have been explored in cell culture experiments with a variety of RNA viruses, including VSV, HIV-1, poliovirus-1, FMDV, and lymphocytic choriomeningitis virus (LCMV). All of these studies have shown that mutagens are detrimental to viral fitness and, in some cases, lead to extinction. For example, in HIV-1, the addition of the base analog 5-hydroxydeoxycytidine can result in a loss of infectivity after 10–20 serial passages. Virologists, assuming that error catastrophe is a form of lethal mutagenesis, have often interpreted these experimental observations in terms of error catastrophe. However, the two concepts are not equivalent, because lethal mutagenesis implies extinction, whereas the error catastrophe is a shift in sequence space without a bearing on the survival of the population (**Table 2**). The results from lethal mutagenesis experiments are easily explained by classical population genetics, provided fitness is expressed in absolute rather than relative terms. A simple criterion for extinction is $e^{U}R$, where R is the progeny number per infectious particle and e^{U} equals the mean equilibrium fitness of the population (this expression simply states that if the absolute mean fitness is below one, population size will deterministically decline). Therefore, current experimental evidence does not support the error catastrophe prediction. More specific observations would be required (**Table 2**), although it must be noted that the error catastrophe is not a necessary prediction of the quasispecies theory because it critically depends on the assumed adaptive landscape.

See also: Antigenic Variation; Antiviral Agents; Evolution of Viruses; Nature of Viruses; Virus Evolution: Bacterial Viruses; Virus Species.

Further Reading

Bull JJ, Sanjuan R, and Wilke CO (2007) Theory of lethal mutagenesis for viruses. *Journal of Virology* 81: 2930–2939.

Domingo E, Biebricher CK, Eigen M, and Holland JJ (2001) *Quasispecies and RNA Virus Evolution: Principles and Consequences.* Austin, TX: Landes Bioscience.

Domingo E, Sabo D, Taniguchi T, and Weissmann C (1978) Nucleotide sequence heterogeneity of an RNA phage population. *Cell* 13: 735–744.

Eigen M, McCaskill J, and Schuster P (1988) Molecular quasi-species. *Journal of Physical Chemistry* 92: 6881–6891.

Elena SF and Lenski RE (2003) Evolution experiments with microorganisms: The dynamics and genetic bases of adaptation. *Nature Reviews in Genetics* 4: 457–469.

Hartl DL and Clark AG (2006) *Principles of Population Genetics,* 4th edn., Sunderland, MA: Sinauer.

Holmes EC and Moya A (2002) Is the quasispecies concept relevant to RNA viruses? *Journal of Virology* 76: 460–465.

Lenski RE (2000) Evolution, theory and experiments. In: Alexander M, Bloom BR, Hopwood DA, *et al.* (eds.) *Encyclopedia of Microbiology,* 2nd edn., pp. 283–298. New York: Academic Press.

Moya A, Holmes EC, and Gonzalez-Candelas F (2004) The population genetics and evolutionary epidemiology of RNA viruses. *Nature Reviews in Microbiology* 2: 279–288.

Sanjuan R, Cuevas JM, Furio V, Holmes EC, and Moya A (2007) Selection for robustness in mutagenized. *PLoS Genetics* 3(6): e93.

Wagner A (2005) *Robustness and Evolvability in Living Systems.* Princeton, NJ: Princeton University Press.

Wilke CO (2005) Quasispecies theory in the context of population genetics. *BMC Evolutionary Biology* 5: 44.

Wolf JB, Brodie ED III, and Wade MJ (2000) *Epistasis and the Evolutionary Process.* Oxford: Oxford University Press.

R

Rabies Virus

I V Kuzmin and C E Rupprecht, Centers for Disease Control and Prevention, Atlanta, GA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Apoptosis One of the main types of programmed cell death which involves a series of biochemical events leading to morphological changes and death.
Chiropteran Pertaining to members of the order of flying mammals commonly called 'bats'.
Gliosis Proliferation of astrocytes in damaged areas of the central nervous system.
Hematophagous The habit of certain animals of feeding on blood.
Herpestidae A vertebrate family which includes mongooses.
Meninges The system of membranes which envelope the central nervous system. The meninges consist of three layers: the dura mater, the arachnoid mater, and the pia mater.
Neuronophagia Phagocytic destruction of nerve cells.
Paraesthesia A sensation of tingling, pricking, or numbness of a person's skin with no apparent physical effect.

Introduction

Rabies is fatal encephalitis caused by rabies virus and other lyssaviruses. This important zoonosis has been known for over 4000 years, but still causes more than 50 000 human deaths annually besides enormous economic losses, primarily developing countries of Asia and Africa where dog rabies is enzootic. Once symptoms appear, the disease is almost invariably fatal. Since the first successful use of rabies vaccine by Louis Pasteur in 1885, pre-exposure and post-exposure prophylaxis have been improved significantly, and canine rabies has been eliminated from Western Europe and large parts of North America. Nevertheless, the continuing burden of the disease in other parts of the world poses requirements for further development of affordable protective biologicals and treatment regimens for rabies.

Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

Genome and Morphology

Rabies virus is classified as the type species of the genus *Lyssavirus*, family *Rhabdoviridae*, order *Mononegavirales*. The negative-sense single-stranded RNA (ssRNA) genome is *c.* 12 kbp in length (**Figure 1**) and encodes a short leader sequence (*c.* 50 nt), followed by five structural protein genes, separated by nontranscribed intergenic regions. The N gene consists of 1350–1353 nt and codes for the nucleoprotein. Due to its abundance and ease of the detection by fluorescent antibody and RNA-based methods, the nucleoprotein and the N gene are the most common targets of diagnostic tests, and antigenic and phylogenetic typing. The P gene consists of 891–915 nt and codes for the phosphoprotein. This protein is composed of two domains: the NH_2-terminal portion contains an L protein binding site, as well as a weak N protein binding site; the COOH-terminal portion contains a strong N protein binding site. The M gene consists of 606 nt and codes for the matrix protein. This protein is involved in later steps of virion formation, such as tightening of the ribonucleoprotein complex (RNP) into a compressed helical structure and the budding of nucleocapsids at cell membranes to obtain the viral envelope. The G gene consists of 1566–1599 nt and codes for the transmembrane glycoprotein. The glycoprotein interacts with receptors on the cell surface and is responsible for neutralizing antibody production. It is composed of four distinct domains: a signal peptide, ectodomain, transmembrane peptide, and cytoplasmic domain. The glycoprotein, and particularly the ectodomain, play a crucial role in rabies virus (RABV) pathogenicity. For example, one

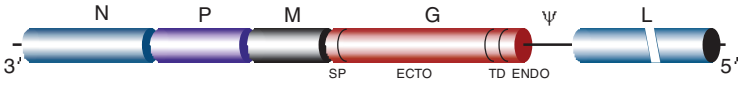


Figure 1 Structure of the rabies virus genome. N, nucleoprotein gene; P, phosphoprotein gene; M, matrix protein gene; G, glycoprotein gene (including SP, signal peptide; ECTO, ectodomain; TD, transmembrane domain; ENDO, endodomain); Ψ, large G-L intergenic region, sometimes referred to as a pseudogene; L, RNA-dependent RNA polymerase gene.

arginine residue at position 333 of the ectodomain is important for peripheral infectivity of RABV. Replacement of this amino acid in vaccine strains significantly attenuates pathogenicity. The L gene is 6381–6426 nt length and codes for the RNA-dependent RNA polymerase. This is a highly conserved polypeptide responsible for replication and transcription of the viral genome.

The RABV virion is bullet-shaped (**Figure 2**), 50–100 nm × 100–430 nm in dimensions, and composed of two structural units: an internal helical RNP, about 50 nm in diameter, and a lipid envelope which is derived from the host cytoplasmic membrane during budding. The RNP is comprised of the RNA genome and N nucleoprotein in tight association. The heavily phosphorylated phosphoprotein and polymerase are also bound to the RNP. The exact position of the matrix protein remains controversial, and may be either contained in the central channel of RNP or embedded into the inner layer of the virion membrane. Knobbed glycoprotein spikes, consisting of three glycosylated ectodomains and serving for binding of the virions to host cell receptors, protrude through the virion membrane.

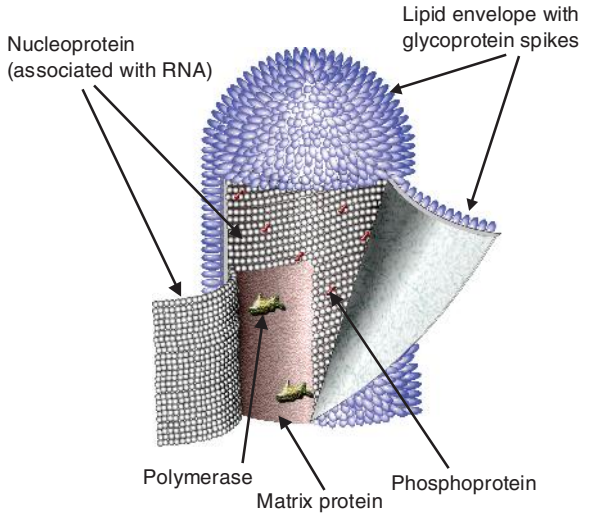


Figure 2 Structure of the rabies virus virion.

Phylogeny and Evolution

RABV is the most broadly distributed member of the genus *Lyssavirus*, circulating in both carnivorous and chiropteran hosts. Phylogenetic lineages of RABV reflect the geographic distribution and association with a particular host species (**Figure 3**). In general, the RABV lineage is split into two large clusters. One includes viruses circulating worldwide among terrestrial carnivores (predominantly canids and mongoose). The second cluster is indigenous to the New World, and includes viruses circulating among raccoons, skunks, and a variety of bats. The first cluster includes the major 'cosmopolitan' canid RABV lineage, believed to have originated in Europe and widely disseminated as a consequence of global colonization during the sixteenth to nineteenth centuries. Presently, these viruses circulate in moderate latitudes of Eurasia, the Middle East, Africa, and the Americas. The majority of RABV vaccine strains, such as PV, ERA (SAD), PM, and HEP, belong to the 'cosmopolitan' RABV lineage. The lineage of Arctic and Arctic-like rabies viruses is monophyletic but, ancestrally, it is linked to the 'cosmopolitan' group. Viruses comprising the Arctic portion of this lineage are distributed in circumpolar regions of Eurasia and North America, whereas distinct groups of Arctic-like rabies viruses circulate in some regions of the Middle East, and southern and eastern Asia. Representatives of some African virus lineages of Canidae and Herpestidae origin are more distantly related. The most divergent representatives of the Old World RABVs circulate in dogs in South Asia. Among the indigenous New World RABV lineages, the majority are tightly associated with particular bat species.

RABV is one of the most slowly evolving negative-stranded RNA viruses, with substitution rates of approximately 2×10^{-4} to 5×10^{-4} per site per year. No recombination has been reported in RABV genomes. Mutations occur due to the lack of proofreading and post-replication error correction by RNA polymerase. However, there is clear evidence of negative selection, as RABV and other lyssaviruses are subjected to strong constraints against amino acid substitutions, probably related to their unique pathobiology. There is some limited evidence that positive selection may have occurred at a few sites in the G gene, such as codons 156, 160, 183, and 370, but the role of these residues is not currently known. Although descriptions of a disease similar to rabies are known from 4000-year-old manuscripts, the origin of current RABV diversity has been estimated at 800–1500 years for separation of the major bat and terrestrial virus lineages, 1200–1800 years for the closest ancestor of

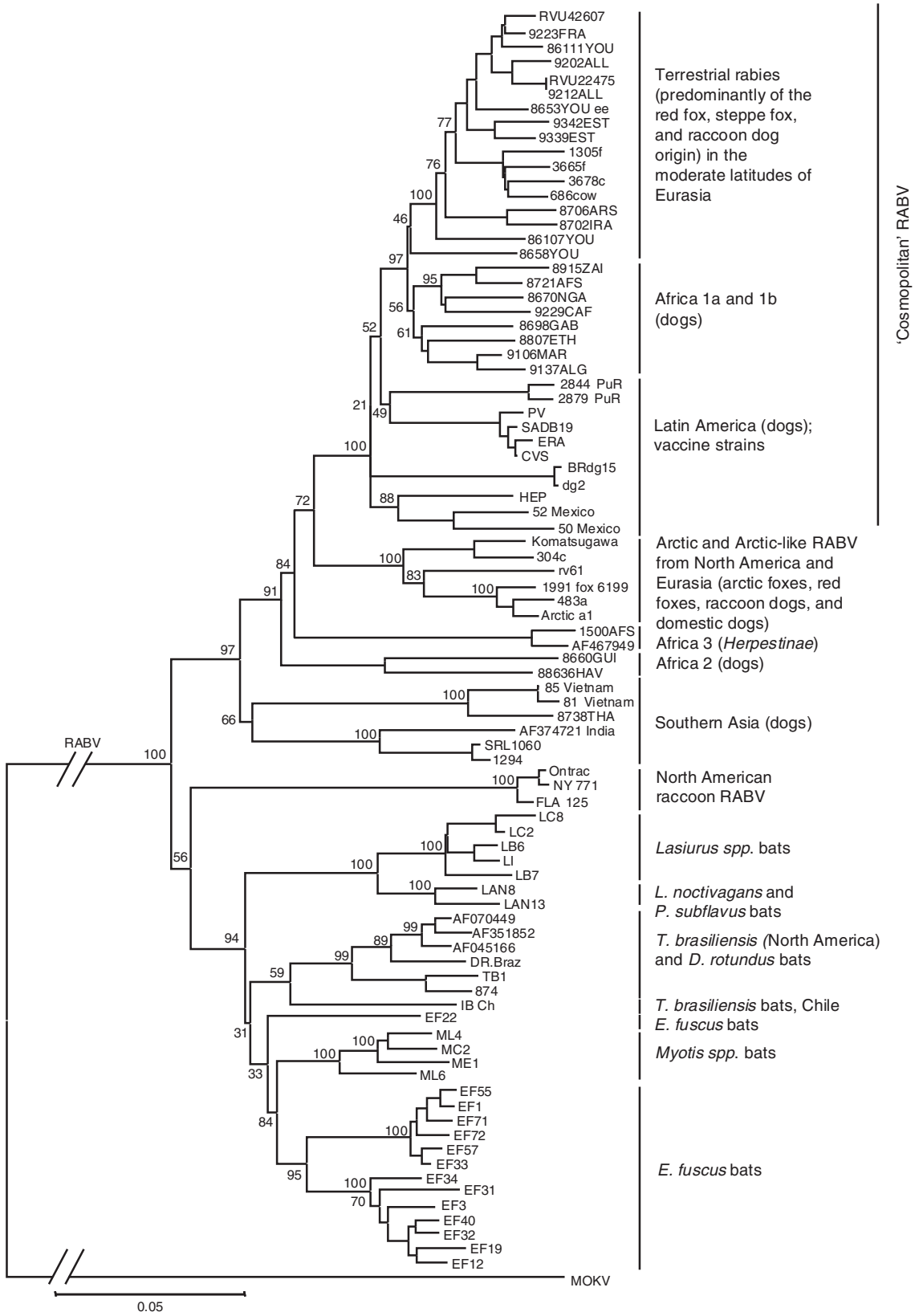


Figure 3 Phylogenetic tree of the major rabies virus lineages based on the sequences of the entire nucleoprotein gene. Bootstrap support values are presented for key nodes, and branch lengths are drawn to scale.

current American bat rabies viruses, and 280–500 years for the 'cosmopolitan' group.

Pathogenesis

After delivery into a wound, RABV can infect several types of cells and replicate at the inoculation site, as has been shown for skeletal muscle cells and fibroblasts. Attachment to cell membrane receptors is mediated by the glycoprotein spikes protruding from the virion membrane. Several types of putative receptors for RABV attachment have been suggested: nicotinic acetylcholine receptor, carbohydrate moieties, phospholipids, and gangliosides. Once bound, the virus enters the cell by endocytosis. Following a decrease in pH in the endosomal vesicle, the viral membrane fuses with the endosomal membrane, and the RNP is released into the cytoplasm. Because RABV RNA has negative polarity, it must be transcribed to produce the complementary positive-sense mRNA. This process is mediated by the viral RNA-dependent RNA polymerase. The RNP serves as a template for transcription and replication, and protects the RNA from nuclease activity. Translation of the viral mRNAs is ensured by the cellular protein synthesis machinery. All processes of transcription, translation, and replication take place in the cytoplasm. The glycoprotein is synthesized at the rough endoplasmic reticulum and delivered to the cytoplasmic membrane. The other viral proteins are expressed in the cytosol by free polyribosomes. At the final stage, transcription and replication are inhibited, the RNP becomes intensively condensed and assembled into mature nucleocapsids, which are subsequently delivered to the cell membrane for the budding of complete virions. During the budding process, virions acquire the lipid envelope and the glycoprotein which is embedded in the cell membrane.

After several replication cycles at the inoculation site, RABV penetrates peripheral nerves and spreads to the central nervous system (CNS) by retrograde axonal transport. Neuronal pathways shield the virus from host immune surveillance, resulting in the absence of an early antibody response. Once delivered to the CNS, the virus disseminates rapidly in the spinal cord, medulla, thalamus, pons, hippocampus, striatum, cerebellum, and cortex. The spread of infection within the CNS occurs from one neuron to another by both axonal and trans-synaptic transport. Neuropathological changes observed in the infected brain are relatively mild histologically and include gliosis, slight neuronophagia, and perivascular infiltration with inflammatory cells, with rare involvement of meninges. Occasionally, more severe brain damage occurs, such as spongiform lesions, extensive neuronal degeneration, and widespread inflammation. Functional alteration of the CNS is much more significant than morphological changes. Apoptosis as a response to RABV infection is an additional prominent factor of neuron damage. Generalized CNS dysfunction leads to a lethal outcome.

Reverse dissemination of virus from the CNS during the clinical period of rabies occurs along peripheral nerves. The RABV RNA may be detected in a variety of organs and tissues at the end of clinical period. However, infectious virus can be isolated from extraneural tissues only occasionally and in low titers. The exception is the salivary glands, where virus passes additional replication cycles and is released into the saliva to enable transmission.

Although a bite is the main method of transmission, non-bite exposures also may occur under unusual circumstances. For instance, two human rabies cases have been attributed to airborne exposure in laboratories, and another two cases have been attributed to airborne exposures in a bat-infested cave in Texas. Aerosol transmission has been demonstrated experimentally, only in very specific conditions with a highly concentrated viral aerosol. Several cases of human-to-human RABV transmission have occurred due to transplantation of cornea, liver, kidneys, and associated tissues obtained from donors who died of rabies. Exposure may occur by direct contact of saliva, nervous or other infected tissues from a rabid animal with mucous membranes, or from scratches. For example, several cases have occurred in trappers who skinned rabid animals without suitable protection.

Various mammalian species exhibit different susceptibilities to the variety of RABV variants, including mutual adaptation of virus and principal host. For example, canids are highly sensitive to homologous RABV variants, and develop the furious form of rabies with high titers of virus in salivary glands. These peculiarities ensure transmission of the infection to a critical number of susceptible individuals, before the death of the rabid animal. A very low level of seroprevalence has been detected in natural populations of foxes, indicating that most RABV contact events lead to a fatal infection. In contrast, a high level of seroprevalence has been detected among bats. These gregarious mammals demonstrate moderate to low susceptibility to RABV. In many cases, bats develop antibody rather than disease when they encounter RABV. In general, RABV evades immunological responses after entering into the nervous system. Development of antibodies could be attributed to peripheral virus activity rather than to CNS infection. There is contradictory evidence as to whether bats or other mammals can survive rabies. Initial observations made at the beginning of the twentieth century on vampire bats suggested that they can be 'asymptomatic carriers' of RABV. More recent reports from Spain have described some cases in which viral RNA was detected in extraneural tissues and oral swabs of naturally infected bats that were negative for rabies

infection in brain tissue. Experimental studies of vampire bats have demonstrated intermittent shedding of RABV in saliva. However, a number of other experiments and field surveillance performed in Europe and the USA provided no evidence in support of a 'carrier' state: bats that developed rabies always died, and virus was detected only in those animals but not in healthy survivors. Some survivors do develop virus-neutralizing antibodies, suggesting a form of abortive infection. Most likely, this abortive infection occurs at the inoculation site. The susceptibility of other mammalian species, that do not serve as principal RABV hosts, is variable. Susceptibility of primates, including humans, is low to moderate, depending on the virus variant.

Indeed, rare cases of survival after the manifestation of clinical signs of rabies are occasionally registered in different animal species. However, these sporadic events cannot be taken in support for a theory of RABV persistence *in vivo*. At least six cases of human recovery after clinical rabies have been published. Five of the patients were vaccinated before clinical onset, and in one case no vaccination was performed. Rabies diagnosis in each case was based on a history of exposure, compatible symptoms, and increasing titers of anti-RABV antibodies in the serum and cerebrospinal fluid (CSF). However, no virus isolates were obtained from these patients.

Clinical Spectrum

The incubation period of rabies can vary from less than 10 days to more than 6 years. The reason for this variability is not yet clear. Some reports attribute long incubation periods to virus replication in the skeletal myocytes before entering the nerves, whereas short incubation periods may be associated with immediate penetration of the nerves and transfer to CNS. Most typical incubation periods after peripheral inoculation of natural hosts and humans vary from 3 to 14 weeks.

Prodromal symptoms are nonspecific: general malaise, fever, chills, sore throat, headache, nausea and vomiting, and sometimes diarrhea, anxiety, and irritability. Humans often suffer from a paresthesia or pain in the inoculation site, and the wound may become slightly inflamed. The prodromal period usually continues for 1–3 days before development of encephalitic symptoms.

Historically, two main clinical forms of rabies have been recognized, based on predominating symptoms: furious (encephalitic) or dumb (paralytic). However, mixed forms occur as well. When the disease is furious, animals or humans become agitated and aggressive. Insomnia, irritability, and anxiety are commonly observed. Other signs, such as pupillary dilation, altered phonation, aimless wandering, drooling of saliva, and muscle tremors and seizures may be noted. Humans often develop hallucinations and delirium. Some symptoms, which have been considered as 'classic' but are observed in 50% or less of patients, are hydrophobia (painful throat seizures at attempts to drink or even due to seeing or hearing running water), aerophobia, photophobia, and phonophobia (seizures in response to airflow, bright lights, and loud sounds, respectively). Subsequently, progressive pareses and paralysis appear. Sick animals or humans become comatose and die, usually due to respiratory failure.

Paralytic rabies is characterized by a greater prevalence of pareses and paralysis from the beginning of disease manifestations, whereas agitation and anxiety are moderate or absent. In part, the form of the disease may depend on the virus variant and animal species. For instance, vampire bat RABVs commonly cause paralytic rabies in cattle and bats. Canine RABV variants from both North America and Eurasia often cause furious rabies. Once symptoms appear, death occurs usually within 1–10 days. Ventilatory support may prolong survival to 3–4 weeks, but no effective treatment exists to date. On occasion, no rabies-specific symptoms can be observed, especially when the paralytic form of disease occurs. Rabies should be considered a possible cause in each case of encephalitis when the etiologic agent is unclear and the probability of lyssavirus exposure cannot be rejected.

Diagnosis

The direct fluorescent antibody test, performed on impressions of infected brain on glass slides, is the gold standard for rabies diagnosis. The brainstem and cerebellum are tissues of choice. Commercially available fluorescein-labeled anti-rabies antibodies, either polyclonal or monoclonal, react with the nucleocapsid of the whole spectrum of RABV variants described to date. The RNP in the infected neurons is condensed into 'inclusions' which are easily observed under the ultraviolet (UV) microscope. The indirect fluorescent antibody test, employing nonlabeled anti-nucleocapsid monoclonal antibodies (N-MAbs), is used commonly for typing of lyssaviruses. Among other methods for RABV antigen capture, some modifications of the enzyme-linked immunosorbent assay (ELISA), including an immuohistochemistry test, have been described and used in research laboratories worldwide.

RABV isolation can be attempted if replication-competent virus is needed for further investigations, or to confirm a negative result obtained by antigen-capturing methods. Isolation can be performed in laboratory mice, aged from suckling to 4 weeks old. Intracerebral inoculation is preferred because the susceptibility of mice to peripheral inoculation is usually 100–10 000 times less than susceptibility to intracerebral inoculation. Incubation periods in mice inoculated intracerebrally usually vary from 3 to 14 days, depending on the dose and particular properties of

the isolate, but may be prolonged up to 6 weeks. Development of typical rabies signs, particularly paralysis and death, is characteristic. The inoculation result must be verified by detection of lyssavirus antigen in the brain of moribund mice. Among available cell cultures, those which derive from mammalian neurons are preferred (e.g., use of mouse neuroblastoma cell culture (MNA) is common in diagnostic laboratories). Susceptibility of MNA cells to RABV is similar to the susceptibility of suckling mice. The result can be detected after 48–72 h of incubation. The disadvantage of cell culture compared to mouse inoculation is related to the quality of the inoculum. Field specimens are often cytotoxic and so can be tested *in vitro* only after filtration or high dilution, reducing sensitivity of the test.

The reverse transcription-polymerase chain reaction (RT-PCR) test is a powerful adjunct diagnostic tool, particularly for antemortem diagnosis of rabies in humans. It is also useful for amplifying small quantities of RNA from infected tissues for use in genetic sequencing and phylogenetic analyses. PCR primer selection depends on the survey aim. For diagnostic purposes, when the virus variant is not known, the choice of primer and PCR regimen is a compromise between specificity and sensitivity. The N gene is targeted most commonly for diagnostic purposes because of its conservation, relative abundance in infected cells, and because it is well studied and represented in the public domain (GenBank). For phylogenetic comparisons, it is useful to amplify a variable region of a gene, but the primers should target flanking conserved regions to ensure specific annealing. For the N gene, the first 400 and the last 320 nucleotides are variable, whereas the space between them (*c.* 600 nt) is conserved. Nested and hemi-nested PCR methods have been developed for RABV. Although they are more sensitive than conventional RT-PCR, nonspecific amplification may still occur, and the result must be verified by molecular hybridization or sequencing of the PCR products. If used properly, and the result is verified, nested PCR is the most sensitive test among PCR-based methods available to date. A real-time PCR test for RABV has also been described. As for other real-time PCR applications, exceptional specificity has been demonstrated for RABV RNA detection. The real-time PCR test can detect highly degraded RNA because very short nucleotide chains ($<$100 nt) are amplified, and allows quantification of the load of viral RNA. A disadvantage of the specificity of the real-time method is the possibility that it could fail to detect genetically divergent viruses. Therefore, real-time PCR is especially suitable for experimental studies when the genetic sequence of the virus is known.

In some cases, particularly for human antemortem diagnosis, the detection of increasing titers of anti-RABV antibodies in serum, and particularly in the CSF, provides a suitable confirmatory diagnosis of rabies.

Epidemiology

RABV is global in distribution with the exception of Australia, Antarctica, and some isolated insular territories that are inaccessible to many viral hosts. Mammals and birds are susceptible to experimental infection, but only the former are relevant in the epidemiology of the disease. In the Old World, RABV circulates among terrestrial mammals (predominantly of the order Carnivora, families Canidae, and Herpestinae). Tropical Asia and Africa are endemic for dog rabies. These territories also encounter the greatest number of human rabies cases (40 000–60 000 annually). Nearly all occur after exposure to dogs. Dogs in Asia and Africa maintain circulation of different RABV lineages but the 'cosmopolitan' variant is distributed most broadly. Dog populations within moderate latitudes of Eurasia are more limited, and implementation of comprehensive vaccination programs has eliminated dog rabies, at least in developed countries. However, RABV still circulates in these countries among wild canids (e.g., red foxes, steppe foxes, raccoon dogs). Developed countries of western Europe are nearing eradication of terrestrial rabies in wildlife because of the implementation of oral rabies vaccination (ORV), but in other territories of the Palearctic, rabies is broadly distributed in wild canids. Some regions, such as conifer forests (taiga), are apparently free of the infection because of extremely low host population densities which are apparently incapable of maintaining RABV. The Arctic variant of RABV, harbored predominantly by Arctic foxes with involvement of other species, such as red foxes, wolves, and dogs, is distributed circumpolarly in the Arctic and subarctic zones.

In the New World, besides the Arctic and 'cosmopolitan' RABV variants introduced from Eurasia and circulating among canids, a number of indigenous RABV lineages are represented. Raccoon rabies emerged in the southeastern US during the 1950s and, to date, has occupied a broad territory along the eastern coast of North America. Skunks maintain circulation of distinct RABV lineages, such as 'south-central skunk', 'north-central skunk', and 'California skunk', and are involved often in the circulation of other lineages, originating from dogs, raccoons, and even bats. For example, a population of striped skunks in Arizona has maintained circulation of an RABV variant which is regularly isolated from big brown bats, indicative of a host shift. At least seven distinct RABV variants have been described to date among hematophagous and insectivorous bats in both North and South America. Migratory species, such as *Lasiurus* spp., *Tadarida brasiliensis*, etc., facilitate virus spread within broad territories along their migration pathways. The number of identified bat-associated RABV lineages will definitely increase with the improvement of surveillance systems, particularly in South America. Bat RABV variants

(predominantly associated with the silver-haired bat, *Lasionycteris noctivagans*, and the eastern pipistrelle bat, *Pipistrellus subflavus*) have been responsible for nearly 90% of domestic human rabies cases reported in the USA during the last 10 years. Many of the bat exposure cases are 'cryptic' in that the circumstances of human exposure to bats frequently are peculiar and little attention is paid to rather small lesions caused by bat bites.

Mongooses also acquire and maintain circulation of distinct RABV variants in some regions. In some instances, they maintain circulation of viruses indistinguishable from those that circulate among dogs (the 'cosmopolitan' variant in the Caribbean and Indian dog variant in southern Asia). In Africa, mongooses harbor a specific RABV variant that circulates exclusively among herpestides of several species.

Prevention and Control

Rabies can be successfully prevented by immunization, not only prior to an exposure, but also after exposure. However, post-exposure prophylaxis (PEP) should commence soon as possible, before virus entry into magistral nerves and CNS, where it becomes largely inaccessible to the immune system due to the blood–brain barrier.

Two primary kinds treatment are used for PEP: vaccines, which lead to the development of active immunity; and anti-rabies immune globulins (RIGs), which provide passive immunity at early stages of immunization when immune response to the vaccine is limited.

Modern commercial human vaccines are pure, potent, safe, and efficacious against all RABV variants described to date. Most human vaccines are derived from the Pasteur virus strain, and are inactivated. Vaccines are manufactured using cell lines or primary cell cultures (such as human diploid cell, avian embryos, Vero, and BHK cells) from which virions are concentrated and partly purified to avoid allergic and adverse reactions. Older generations of nervous-tissue-derived vaccines (sheep or suckling mouse brain) are still used in some developing countries. Due to low potency, they must be administered in significantly higher doses and for longer courses of application. Adverse reactions are more common during vaccination.

Domestic animals are vaccinated against rabies to prevent virus circulation in the population, to eliminate important sources of human exposure, and to avoid economic losses. Most modern veterinary vaccines are inactivated, and potent in the ability to induce appropriate protection for 12–48 months after one dose. Vaccination of dogs and cats is recommended in all territories where contact with RABV is possible. Immunization regulations for other species of domestic and agricultural animals differ depending on the specific epizootic surroundings, geographic and economic circumstances, etc. Quarantine for animals imported from rabies-endemic territories is another measure implemented in some states or districts free of terrestrial rabies.

Outstanding progress in rabies prophylaxis in wildlife has been achieved by implementation of oral vaccination. Oral vaccines contain live-attenuated RABV or recombinant viruses (such as vaccinia virus) expressing the RABV glycoprotein. Wildlife oral vaccination campaigns have been conducted in North America and Europe. As a result, rabies epizootics in wild canids have been significantly reduced or eliminated in most West European countries, Canada, and in parts of the USA. The oral vaccination of raccoon populations, performed in the eastern USA, has prevented further westward spread. Due to very high population density of raccoons, oral vaccination is challenging in this species. To date, oral vaccination programs have not been applied to bats.

Further Reading

Baer GM (ed.) (1991) *The Natural History of Rabies,* 2nd edn. Boca Raton, FL: CRC Press.

Centers for Disease Control and Prevention (CDC) (1999) Human rabies prevention – United States, 1999. Recommendations of the Advisory Committee on Immunization Practices (ACIP). Morbidity and Mortality Weekly Report, vol. 48(no. RR-1), pp. 1–21. Atlanta, GA: CDC.

Jackson AC and Wunner WH (eds.) (2007) *Rabies,* 2nd edn. New York: Academic Press.

Krebs JW, Wilson ML, and Childs JE (1995) Rabies – Epidemiology, prevention, and future research. *Journal of Mammalogy* 76: 681–694.

Meltzer MI and Rupprecht CE (1998) A review of the economics of the prevention and control of rabies. Part 1: Global impact and rabies in humans. *Pharmacoeconomics* 14: 365–383.

Meltzer MI and Rupprecht CE (1998) A review of the economics of the prevention and control of rabies. Part 2: Rabies in dogs, livestock and wildlife. *Pharmacoeconomics* 14: 481–498.

Meslin F-X, Kaplan MM, and Koprowski H (eds.) (1996) *Laboratory Techniques in Rabies,* 4th edn. Geneva: World Health Organization.

Rupprecht CE, Dietzschold B, and Koprowski H (eds.) (1994) *Lyssaviruses.* Berlin: Springer.

Smith JS (1995) Rabies virus. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, and Yolken PH (eds.) *Manual of Clinical Microbiology*, pp. 997–1003. Washington, DC: ASM Press.

Tordo N, Charlton K, and Wandeler A (1998) Rhabdoviruses: Rabies. In: Collier LH (ed.) *Topley and Wilson's Microbiology and Microbial Infections*, pp. 666–692. London: Arnold.

Tsiang H (1993) Pathophysiology of rabies virus infection of the central nervous system. *Advances in Virus Research* 42: 375–412.

World Health Organization (1996) WHO recommendations on rabies post-exposure treatment and the correct technique of intradermal immunization against rabies. *WHO/EMC/ZOO/96.6.* Geneva: World Health Organization.

World Health Organization (2005) WHO expert consultation on rabies. First report. *WHO Technical Report Series*, vol. 931. WHO: Geneva.

Recombination

J J Bujarski, Northern Illinois University, DeKalb, IL, USA and Polish Academy of Sciences, Poznan, Poland

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Genetic recombination of viruses could be defined as the exchange of fragments of genetic material (DNA or RNA) among parental viral genomes. The result of recombination is a novel genetic entity that carries genetic information in nonparental combinations. Biochemically, recombination is a process of combining or substituting portions of nucleic acid molecules. Recombination has been recognized as an important process leading to genetic diversity of viral genomes upon which natural selection can function. Depending on the category of viruses, recombination can occur at the RNA or DNA levels. Since these processes are different for DNA and RNA viruses, they are described separately.

Recombination in DNA Viruses

In many DNA viruses, genetic recombination is believed to occur by means of cellular DNA recombination machinery. Cellular DNA recombination events are of either homologous (general recombination) or nonhomologous types. The nonhomologous recombination events occur relatively rarely and are promoted by special proteins that interact with special DNA signal sequences. In general, homologous recombination events occur much more often and they are most commonly known as genetic crossing-over that happens in every DNA-based organism during meiosis.

The biochemical pathways responsible for DNA crossing-over are well established. General elements involved in general recombination include DNA sequence identity, complementary base-pairing between double-stranded DNA molecules, heteroduplex formation between the two recombining DNA strands, and specialized recombination enzymes. The best-studied recombination system of *Escherichia coli* involves proteins such as recA, and RecBCD, and it has led to a large amount of literature. Interestingly, related DNA recombination proteins have been characterized in eukaryotes, including yeast, insects, mammals, and plants.

Yet certain DNA virus species encode their own recombination proteins, and some of these viruses serve as model system with those to study the recombination processes. One of the best-known systems is of certain bacteriophages that recombine independently from the host mechanisms. These independent pathways are used for repairing damaged phage DNA and for exchanging DNA to increase diversity among the related phages. In Enterobacteria phage M13, high recombination frequency was observed within the origin of phage DNA replication in the *E. coli* host. There, the crossovers have occurred at the nucleotide adjacent to the nick at the replication origin, because of joining to a nucleotide at a remote site in the genome. These results implicated a breakage-and-religation mechanism of such apparently illegitimate cross-overs.

Importantly enough, many of these phage recombination mechanisms are analogous to the pathways operating in the host bacteria. For instance, Rec proteins of phages T4 and T7 are analogous to bacterial RecA, RecG, RuvC, or RecBCD proteins, while RecE pathway in the rac prophage of *E. coli* K-12 or the phage 1 red system influenced the studies of bacterial systems. A correlation of different stages of DNA recombination with transcription and DNA replication during Enterobacteria phage T4 growth cycle is shown in **Figure 1**.

Phage lambda (λ) has a recombination system that can substitute for the RecF pathway components in *E. coli*. The Enterobacteria phage λ moves its viral genome into and out of the bacterial chromosome using site-specific recombination. Based on crystal structures of the reaction intermediates, it is clear how the Enterobacteria phage λ integrase interacts with both core and regulatory DNA elements (**Figure 2**).

Recombination between viral DNA and host genes can lead to acquisition of cellular genes by DNA viruses. For instance, tRNA genes are present in Enterobacteria phage T4. Interestingly, these tRNA sequences contain introns suggesting that Enterobacteria phage T4 must have passed through a eukaryotic host during evolution. Similar viral–host recombination events were observed for retroviruses in eukaryotic cells.

Genetic recombination in DNA viruses is often studied using functional marker mutations. In single-component DNA viruses recombination occurs by exchanging DNA fragments, whereas in segmented DNA viruses, additional events rely on reassortment of the entire genome segments. This complicates the recombination behavior observed among mutants. One method of recombination analysis utilizes so-called conditional-lethal types, where the cells are infected with two variants and the recombinants are selected after application of nonpermissive conditions (two-factor crosses). This allows the mutants to be organized into complementation groups with the relative positions of mutations being placed on a linear map. Another method is called three-factor crosses. Here three

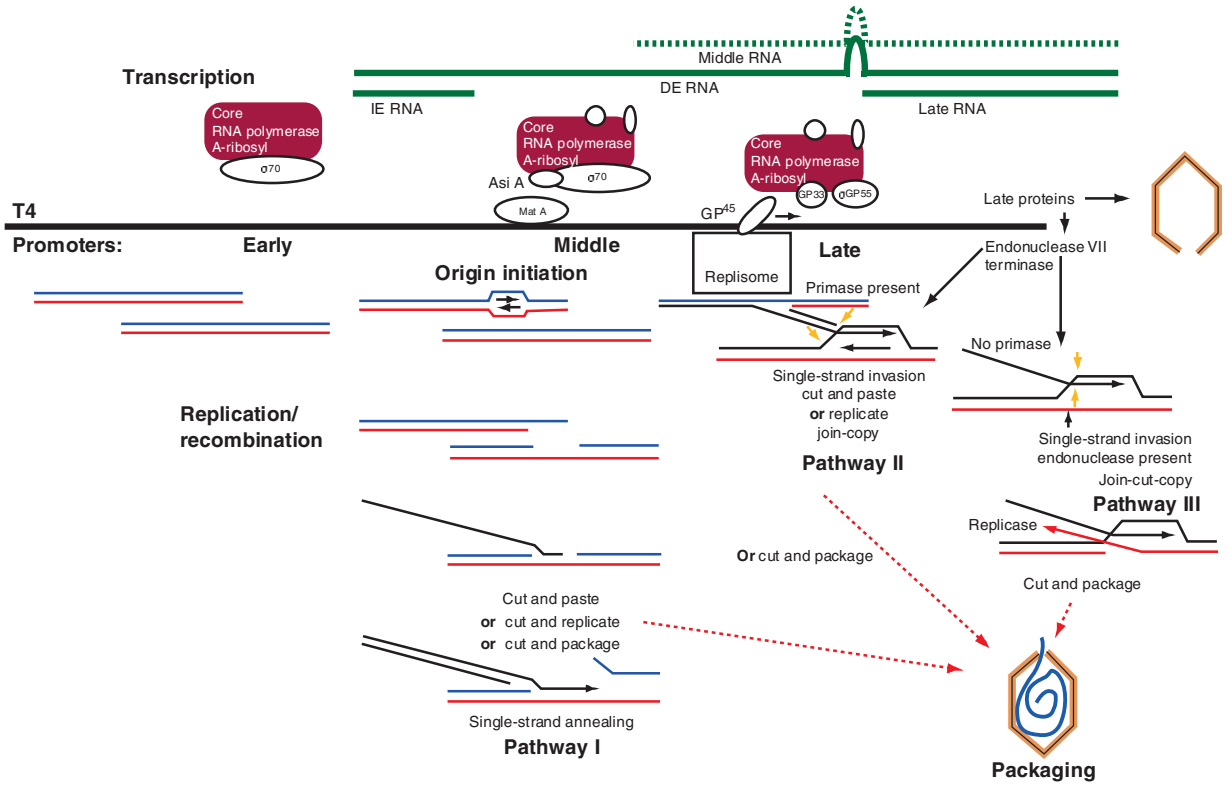


Figure 1 Diagram of the relationship between the Enterobacteria phage T4 transcriptional pattern and the different mechanisms of DNA replication and recombination. (a) Shows the transcripts initiated from early, middle, and late promoters by sequentially modified host RNA polymerase. Hairpins in several early and middle transcripts inhibit the translation of the late genes present on these mRNAs. (b) Depicts the pathways of DNA replication and recombination. Hatched lines represent strands of homologous regions of DNA, and arrows point to positions of endonuclease cuts. Reproduced from Mosig G (1998) Recombination and recombination-dependent DNA replication in enterobacteria phage T4. *Annual Review of Genetics* 32: 379–413, with permission from Annual Reviews.

mutations are employed, with crossing-over occurring between two mutations while the third mutation is not selected. This allows for determination of linkage relationships among mutants and of the order of marker mutations. Due to reassortment, both the two-factor and the three-factor crosses are of less use in segmented DNA viruses.

DNA viruses of eukaryotes also recombine their genomic material. For instance, herpes simplex virus (HSV) was found to support recombination while using pairs of temperature-sensitive mutants (two-factor crossings). In fact, a recombination-dependent mechanism of HSV-1 DNA replication has been described. The recombination frequency was proportional to the distance between mutations which suggested the lack of specific signal sequences responsible for the crossing-over. By using three-factor crossing, the HSV system involved two ts mutants and a syncytial plaque morphology as an unselectable marker. Similarly, in case of adenoviruses, the host range determined by the helper function of two mutations has been used as a third marker between ts mutants. Here, intertypic crosses between ts mutants have been identified based on segregation patterns and the restriction enzyme polymorphism.

Epstein–Barr virus (EBV) is a member of the family *Herpesviridae*, and it carries a long double-stranded genomic DNA, that shows a high-degree variation among strains. These variations include single base changes, restriction site polymorphism, insertions, or deletions. Based on tracking these mutations, it was found that some EBV strains arose due to DNA recombination.

Poxviruses represent the largest DNA viruses known (except those of algae and the mimivius). Homologous recombination was detected in the genome of vaccinia virus (VV), based on the high frequency of intertypic crossovers, the marker rescue, and the sequencing of recombinants. These processes could be both intra- and intermolecular, and they depend on the size of the DNA target. It has been suggested that either viral DNA replication itself or the activity of the viral DNA polymerase might participate in VV DNA recombination. Indeed, some VV proteins with DNA strand transfer activity have been identified.

The DNA genome of Simian virus 40 (SV40, *Papovaviridae*) was found to recombine in somatic cells. The artificially constructed recombinant circular oligomers were used to find high general recombination frequency

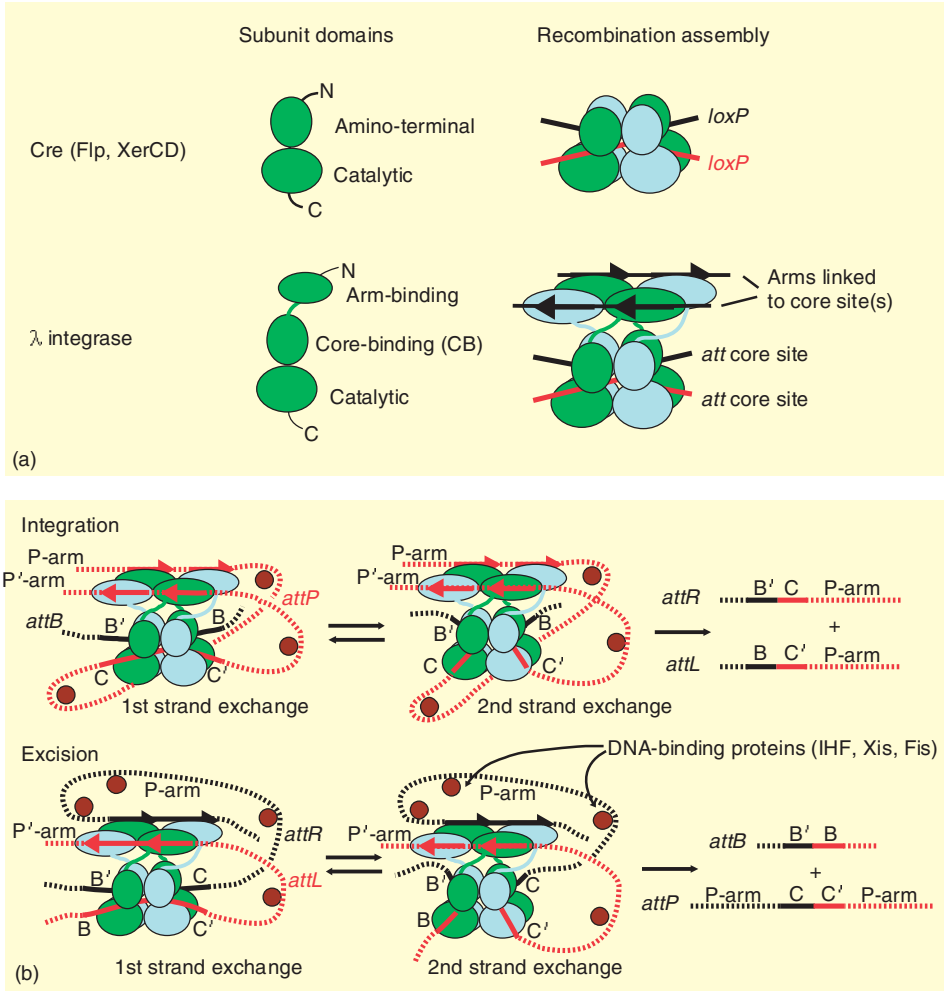


Figure 2 (a) Enterobacteria phage λ integrase compared to the simpler recombinases. Tyrosine recombinases such as Cre have two domains that bind the core recombination sites and carry out recombination on their own. Enterobacteria phage λ integrase has a third, amino-terminal 'arm binding' domain that binds to the arm region of the attachment site. The DNA complex cartoon for Enterobacteria phage λ integrase (lower right) represents the new crystal structures. (b) Integration and excision by Enterobacteria phage λ integrase. The first and second strand exchange cartoons represent the first and second halves of the recombination reaction, respectively. In the first half of integration, for example, Enterobacteria phage λ integrase brings *attP* and *attB* sites together and exchanges the first pair of strands to generate a Holliday junction intermediate. In the second half of the reaction, the Holliday intermediate has isomerized to form a distinct quaternary structure and exchange of the second pair of strands generates recombinant *attL* and *attR* products. Reproduced from Van Duyne GD (2005) Enterobacteria phage λ integrase: Armed for recombination. *Current Biology* 15: R658–R660, with permission from Elsevier.

of SV40 DNA. However, homologous recombination events were rare.

Among plant DNA viruses, genetic recombination was studied in case of geminiviruses and caulimoviruses. The geminiviruses carry a single-stranded DNA genome, composed of either one or two circular DNA molecules. Frequent intermolecular crossing-over events were observed by using mutant combinations. Homologous crossovers were detected to occur intramolecularly between tandem repeats of a geminivirus DNA using agro-infected tobacco plants. The mechanism may involve either homologous crossing-over events or copy-choice processes that rely on template switching by DNA replicase. Moreover, deletions, insertions, and more profound rearrangements have been detected in the geminivirus DNA. These are the illegitimate recombination processes that may involve aberrant breakage-and-religation events or errors in DNA replication, that could occur either inter- or intramolecularly.

Cauliflower mosaic virus (CaMV) belongs to a family of plant double-stranded (ds) DNA pararetroviruses that replicate via reverse transcription. A high recombination rate was observed during CaMV infection *in planta*. These crossovers could occur at the DNA level (thus in the nucleus) or at the RNA level (thus more likely during reverse transcription in cytoplasm). However, features

such as recombinational hot spots and mismatch repair might indicate replicative (i.e., RNA) step, whereas mismatch repair can occur due to the formation of heteroduplex intermediates and thus suggest DNA recombination. These data further suggest that CaMV has the recombination mechanisms available at both steps of its life cycle. Recombination between CaMV variants and the CaMV transgenic mRNAs has been reported and this is believed to represent the RNA–RNA recombination events that happen during reverse transcription.

Recombination in RNA Viruses

RNA is the genetic material in RNA viruses, and a high mutation rate has been observed for the viral RNA genome. This likely occurs during RNA replication by means of action of an RNA-dependent RNA polymerase enzyme due to either replication errors or because of the replicase switching among viral RNA templates. The terms of classic population genetics do not describe RNA viruses. A better description of RNA viral populations is provided with a term 'quasispecies' that has been proposed to address a distribution of RNA variants in the infected tissue.

Many of the RNA viruses limit their life cycle to cytoplasm and thus the observed recombination events among RNAs of plus-stranded RNA viruses must occur outside the nucleus. In general, the RNA crossing-over processes are categorized as being either homologous or nonhomologous, but some earlier authors proposed that there are homologous, aberrant homologous, and nonhomologous RNA recombination types. Aberrant homologous recombination involves crossovers between related RNAs, but the crosses occur at not-corresponding sites leading to sequences insertions or deletions. More recently, mechanistic models were utilized to define the following RNA–RNA recombination classes: (1) The 'similarity-essential' recombination, where substantial sequence similarity between the parental RNAs is required as the major RNA determinant; (2) The 'similarity-nonessential' recombination does not require sequence similarity between the parental RNAs, although such regions may be present; and (3) There is the 'similarity-assisted' recombination where sequence similarity can influence the frequency or the recombination sites but additional RNA determinants are also critical.

Genetic RNA recombination has been described in many RNA virus groups. In particular, sequence data reveal RNA rearrangements reflecting RNA–RNA crossover events during RNA virus evolution. For instance, RNA rearrangements were demonstrated in the genomes of dengue virus-type I, flock house virus, hepatitis D virus, bovine viral diarrhea virus, and equine arthritis virus RNA. For plant RNA viruses, this has been demonstrated in potyviruses such as yam mosaic virus, sugarcane yellow leaf virus, and luteoviruses. Experimentally, RNA recombination has been shown to occur in picornaviruses, coronaviruses, or alphaviruses and in the following plant viruses: plum pox virus, cowpea chlorotic mottle virus, alfalfa mosaic virus, cucumber mosaic virus, tobacco mosaic virus, turnip crinkle virus (TCV), and tomato bushy stunt virus (TBSV). It has also been demonstrated in enterobacteria phage Qbeta, in negative RNA viruses, in double-stranded RNA viruses, and in retroviruses, as well as during formation of defective-interfering (DI) RNAs.

Recombination by reassortment was demonstrated for multisegmental animal RNA viruses, such as influenza virus, and in double-stranded reoviruses and orbiviruses. Specifically, the interpretation of two-factor crosses (using, e.g., ts mutants) in reoviruses turned out to be difficult due to recombination. The mutant sites cannot be ordered on a linear map and often no linkage between mutants could be detected.

Interestingly, there are examples of viral RNA recombination with host-derived sequences. These include the presence of uniquitin-coding region in bovine diarrhea virus, a sequence from 28S rRNA found in the hemagglutinin gene of influenza virus or a tRNA sequence in Sindbis virus RNA. Also, in plant viruses the host-derived sequences were found in potato leaf-roll virus isolates that carry sequences homologous to an exon of tobacco chloroplast. Chloroplast sequences were found in the actively recombining RNAs of brome mosaic virus (BMV). Several plant viruses were also confirmed to recombine with viral RNA fragments expressed in transgenic plants, including cowpea chlorotic mottle virus, red clover necrotic mottle virus, potato virus Y virus, and plum pox virus.

The existence of several RNA virus recombination systems has made possible the studies of the molecular mechanisms of RNA recombination. The majority of RNA recombination models predict copy-choice mechanisms, either due to primer extension (in flaviviruses, carmoviruses), at the subgenomic promoter regions (BMV, poliovirus), or by strand translocation (in nidoviruses). In retroviruses, there are three copy-choice mechanisms: (1) forced (strong stop) strand transfer, (2) pause-driven strand transfer, and (3) pause-independent (RNA structure-driven) strand transfer. However, in enterobacteria phage Qbeta a breakage-and-religation mechanism has been described. Details of some of these systems are discussed below.

The molecular mechanisms of the formation of both nonhomologous and homologous RNA recombinants have been studied using an efficient system of BMV. In order to increase recombination frequency, the BMV RNA3-based constructs were generated where the 3′ noncoding region was extended, while carrying partial deletions. This debilitated the replication of RNA such that the sequence got repaired by recombination with the sequences of other

BMV RNA segments. It appeared that short base-paired regions between the two parental BMV RNA molecules could target efficient nonhomologous crossovers. A proposed model predicted that the formation of local RNA–RNA heteroduplexes could function because they brought together the RNA substrates and because they slowed down the approaching replicase enzyme complexes.

These early studies also analyzed the molecular requirements of homologous recombination by inserting the BMV RNA2-derived sequences into the recombination vector. This revealed the accumulation of both precise and imprecise RNA2–RNA3 recombinants and that the recombination frequencies depended upon the composition of nucleotide sequences within the region of recombination. The crossovers tended to happen at stretches of GC-rich regions alternating with AU-rich sequences suggesting the RNA replicase switching between RNA templates. Elements capable of forming strand-specific, stem–loop structures were inserted at the modified 3′ noncoding regions of BMV RNA3 and RNA2 in either positive or negative orientations, and various combinations of parental RNAs were tested for patterns of the accumulating recombinant RNA3 components. This provided experimental evidence that homologous recombination between BMV RNAs more likely occurred during positive-rather than negative-strand synthesis.

True homologous recombination crossing-over has been observed among the RNA molecules of the same segments during BMV infections. By using nonselective marker mutations at several positions, it was demonstrated that RNA1 and RNA2 segments crossed-over at 5–10% frequency, whereas the intercistronic region in RNA3 supported an unusually high recombination frequency of 70%. The subsequent use of various deletion constructs has revealed that the high-frequency crossing-over mapped to the subgenomic promoter (sgp) region, and in particular to its internal polyA tract. Further studies have shown that sgp-mediated crossing-over has occurred at the minus-strand level (i.e., during plus-strand synthesis), most likely by discontinuous process, where the replicase complex detached from one strand and reinitiated on another strand. This process is most likely primed by a sg RNA3a intermediate, which prematurely terminates on the polyU (in minus strand) tract and re-anneals to this region on another minus RNA3 template (**Figure 3**). Also, it turned out that the frequency of crossing-over and the process of initiation of transcription of sg RNA4 were reversely linked, suggesting a competition between these two reactions.

The role of replicase proteins in BMV RNA recombination has been studied by using well-characterized 1a and 2a protein mutants. A ts mutation in protein 1a 5′ shifted the crossover sites indicating the participation of helicase domain of 1a. Likewise, mutations at several regions of 2a affected the frequency of nonhomologous recombination. The relationship between replication and recombination was studied by using BMV variants that

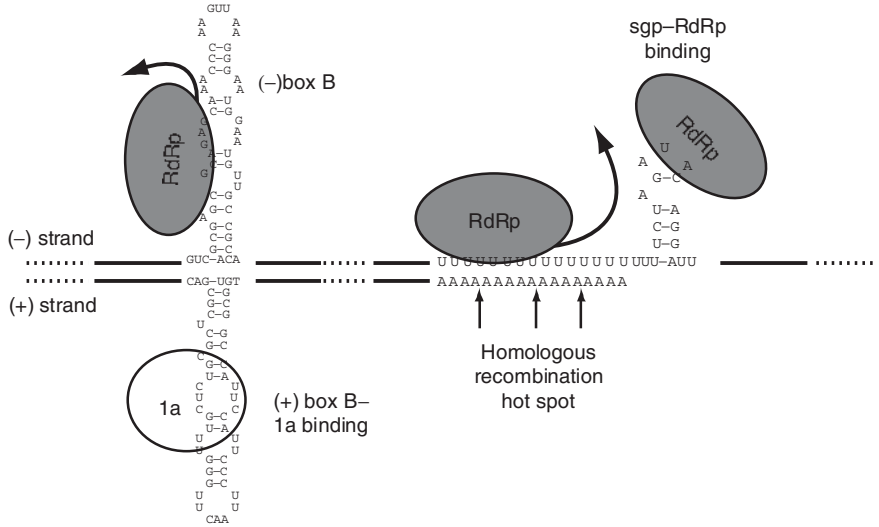


Figure 3 Model illustrating the synthesis of sg RNA3a in view of multiple functions of the intergenic region in ( ) RNA3. The BMV RdRp enzyme complex (represented by gray ovals) migrates alongside the ( )-strand RNA template and pauses (represented by curved arrows) at the secondary structure or, most notably, at the oligoU tract, leading to the formation of subgenomic sgRNA3a. Yet another molecule of the RdRp enzyme binds to the sgp and initiates the *de novo* synthesis of sgRNA4. Also, the rehybridization of the sgRNA3a oligoA tail to the RNA3 ( ) template can resume full-length copying which primes the observed RNA3–RNA3 recombination (5, 69). The (+) and ( ) RNA strands are represented by thick lines and both the oligoU tract in the ( ) strand template and the oligoA 3′ termini are exposed. The stem-and-loop structures adopted by the (+) and the ( ) strands upstream to their oligoU (A) tracts are shown. The binding region to protein 1a via the box B of the stem–loop structure in (+) strands is shown. Reproduced from Wierzchoslawski R, Urbanowicz A, Dzianott A, Figlerowicz M, and Bujarski JJ (2006) Characterization of a novel 5′ subgenomic RNA3a derived from RNA3 of brome mosaic bromovirus. *Journal of Virology* 80: 12357–12366, with permission from American Society for Microbiology.

carried mutations in 1a and 2a genes. This revealed that the 1a helicase and the 2a N-terminal or core domains were functionally linked during both processes *in vivo* and *in vitro*. Also, it was shown that the characteristics of homologous and nonhomologous crossovers could be modified separately by mutations at different protein sites. All these studies confirmed the involvement of replicase proteins in recombination and supported the template-switching model.

More recently, the role of host factors in BMV recombination was addressed by using both yeast and Arabidopsis systems. In yeast, transient co-expression of two derivatives of BMV genomic RNA3 supported intermolecular homologous recombination at the RNA level but only when parental RNAs carried the *cis*-acting replication signals. The results implied that recombination occurred during RNA replication. In *Arabidopsis*, the use of gene-knock-out mutations in the RNA interference pathway revealed that BMV can recombine according to both the copy-choice template-switching and to the breakage-and-religation mechanisms.

The role of replicase proteins in RNA recombination has also been studied in other RNA viruses. For TCV, a small single-stranded RNA virus, a high-frequency recombination was observed between satellite RNA D and a chimeric subviral RNA C. The crossing-over most likely relied on viral replicase enzyme switching templates during plus-strand synthesis of RNA D which reinitiated RNA elongation on the acceptor minus-strand RNA C template. The participation of replicase proteins was demonstrated *in vitro*, where a chimeric RNA template containing the *in vivo* hot-spot region from RNA D joined to the hot-spot region from RNA C. Structural elements such as a priming stem in RNA C and the replicase binding hairpin, also from RNA C, turned to play key roles during recombination, probably reflecting late steps of RNA recombination such as strand transfer and primer elongation. The host factors related to the host-mediated viral RNA turnover have been found to participate in tombusvirus RNA recombination. The screening of essential yeast genes mutants identified host genes that affected the accumulation of TBSV recombinants, including genes for RNA transcription/metabolism, and for protein metabolism/transport. Suppression of TBSV RNA recombination was observed by the yeast Xrn1p 5′–3′ exoribonuclease, likely due to rapid removal of the 5′ truncated RNAs, the substrates of recombination. These 5′ truncated viral RNAs are generated by host endoribonucleases, such as the Ngl2p endoribonuclease.

Coronavirus RNAs were found to recombine between the genomic and DI RNA molecules. It was postulated that recombination has occurred due to the nonprocessive nature of the coronavirus RNA polymerase enzyme (**Figure 4**) and an efficient protocol for targeted recombination has been developed.

Similarly, in nodaviruses, the two-partite RNA viruses, recombination processes were found to occur between RNA segments at a site that potentially could secure base pairing between the nascent strand and the acceptor template. The recombination sites might have been chosen based on factors such as the similarity to the origin of replication or special secondary structures. A postulated model implies the polymerase to interact directly with the acceptor nodavirus RNA template.

A double-stranded RNA Pseudomonas phage Phi6 was hypothesized to recombine its RNA based on a copy-choice template switching mechanism, where the crossovers would have occurred inside the virus capsid structures at regions with almost no sequence similarity. Interestingly, the frequency of recombination was enhanced by conditions that prevented the minus-strand synthesis. Experiments were designed to reveal the effects of drift on existing genetic

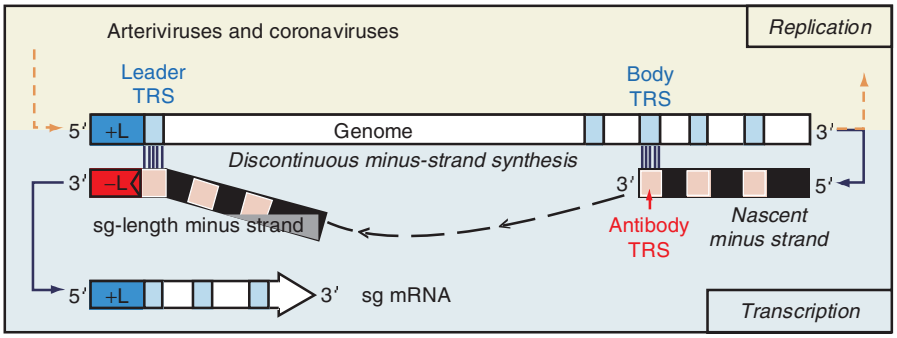


Figure 4 Models for discontinuous transcription from minus-strand sg-length templates in arteriviruses and coronaviruses. These viruses have a common 59 leader sequence on all viral mRNAs. Discontinuous extension of minus-strand RNA synthesis has been proposed as the mechanism to produce sg-length minus-strand templates for transcription. The replicase/transcriptase can attenuate at one of the body TRSs in the 39-proximal part of the genome, after which the nascent minus strand extends with the anti-leader ('L') sequence. Next, the completed sg-length minus strands serve as templates for transcription. Reproduced from Pasternak AO, Spaan WJM, and Snijder EJ (2006) Nidovirus transcription: How to make sense...? *Journal of General Virology* 87: 1403–1421, with permission from Society for General Microbiology.

variation by minimizing the influence of variation on beneficial mutation rate. The segmented genome of the pseudomonas phage Phi6 has allowed to present the first empirical evidence that the advantage of sex during adaptation increases with the intensity of drift.

The enterobacteria phage Qbeta, a small single-stranded RNA virus, could recombine both *in vivo* and *in vitro*. Here, the mechanism of recombination was not based on a template-switching by the replicase, but rather via a replicase-mediated splicing-type religation of RNA fragments. The system produced nonhomologous recombinants, whereas the frequency of homologous crossovers was low. These data suggested an RNA trans-esterification reaction catalyzed by a conformation acquired by enterobacteria phage Qbeta replicase during RNA synthesis. In summary, the results on various plus-strand RNA virus systems demonstrate the availability of a variety of template-switch mechanisms, the mutual-primer-extension on two overlapping RNA strands, the primer-extension on one full-length RNA strand, as well as both replicative and nonreplicative trans-esterification mechanisms where a piece of another RNA is added to the 3′ terminus of an RNA either by viral RdRp or by other enzymes (e.g., RNA ligase), respectively.

The recombination events in retroviruses contribute significantly to genetic variability of these viruses. The crossovers do occur by reverse transcriptase jumpings between the two genomic RNA molecules inside virion capsids. Apparently, the virally encoded reverse transcriptase enzymes have been evolutionarily selected to prone the jumpings between templates during reverse transcription. It turns out that the recombinant jumpings between RNA templates are responsible for both inter- and intramolecular template switchings and also for the formation of defective retroviral genomes. It has been found that the most stable interactions between two copies of retrovirus RNAs were within the 5′ nucleotides 1–754. There is experimental evidence demonstrating that the template 'kissing' interactions effectively promote recombination within the HIV-I 5′ untranslated region. The possibilities of recombination in retroviruses at the DNA level (of the integrated provirus sequences) were discussed earlier in this article.

Defective-Interfering RNAs

There is a variety of subviral RNA molecules that are linked to viral infections. Those derived from the viral genomic RNAs and interfering with the helper virus accumulation or symptom formation are called as DI RNAs. First reports (in 1954) about DI RNAs coexisting with viral infection was provided by Paul von Magnus with influenza virus. Thereafter, numerous both animal and plant viruses were found to generate DI RNAs. Naturally occurring DI RNAs have been identified in coronavirus infections. These molecules appear to arise by a polymerase strand-switching mechanism. The leader sequence of the DI RNAs was found to switch to the helper-virus derived leader sequence, indicating that helper virus-derived leader was efficiently utilized during DI RNA synthesis. Also, the leader switching likely occurred during positive-strand DI RNA synthesis, and the helper-virus positive-strand RNA synthesis tended to recognize double-stranded RNA structures to produce positive-strand DI RNAs. The parts of the coronavirus RNA required for replication and packaging of the defective RNAs were investigated, with both the 5′- and the 3′-terminal sequences being necessary and sufficient. The coronavirus DI RNAs have been utilized to study the mechanism of site-specific RNA recombination. This process relies on the acquisition of a 5′ leader that is normally used for production of numerous coronavirus sg RNAs. Also, these DI RNAs have been used as vehicles for the generation of designed recombinants from the parental coronavirus genome.

In case of plant viruses, tombusviruses and carmoviruses were found to accumulate DI RNAs, which maintain a consistent pattern of rearranged genomic sequences flanked by the 5′ and 3′ unchanged replication signals. In some cases, the base pairing between a partial nascent strand and the acceptor template can lead to the appearance of the rearranged regions in DI RNAs.

In addition to rearranged DI RNAs, some RNA viruses accumulate defective RNAs due to a single internal deletion in the genomic RNA of the helper virus. Such examples include beet necrotic wheat mosaic furovirus, peanut clump furovirus, clover yellow mosaic potexvirus, sonchus yellow net rhabdovirus, and tomato spotted wilt tospovirus. Features such as the ability to translate or the magnitude of the defective RNA seem to affect the selection of the best-fit sizes of DI RNAs during infection.

Another type of single-deletion DI RNAs are produced during broad bean mottle bromovirus (BBMV) infections from the RNA2 segment. A model has been proposed where local complementary regions bring together the remote parts of RNA2 which then facilitates the crossover events. Similar RNA2-derived DI RNAs have been reported to accumulate during the cucumovirus infections.

The closteroviruses, the largest known plant RNA viruses, form multiple species of defective RNAs, including the citrus tristeza virus defective RNAs that arise from the recombination of a subgenomic RNA with distant 5′ portion of the virus genomic RNA (**Figure 5**). Apparently, closteroviruses can utilize sg RNAs for the rearrangement of their genomes.

Negative-strand RNA viruses also form DI RNAs. For instance, in vesicular stomatitis virus (VSV), a rhabdovirus, the *cis*-acting RNA replication terminal elements participate in the formation of the 5′-copy-back DI RNAs, reflecting likely communication between distant portions of the VSV genome.

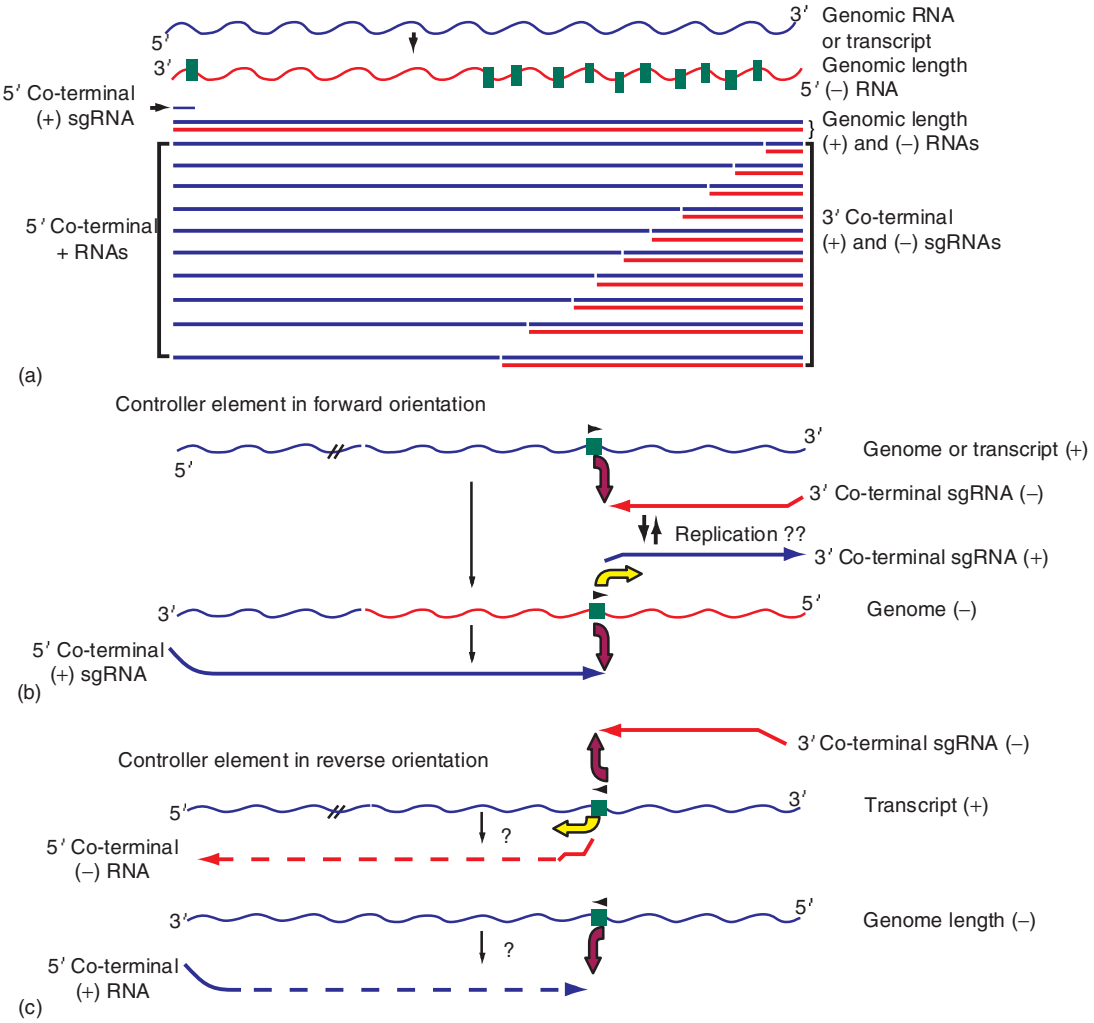


Figure 5 (a) The outline of different species of genomic RNA and 59 and 39 terminal sgRNAs potentially produced in CTV-infected cells. The positive-sense RNAs are shown in blue and the negative-sense RNAs are shown in red. The wavy line represents the genomic RNA or the plus-sense transcript (blue) and the genomic length minus-sense RNA (red) produced from the plus-sense RNA. The solid green boxes on the genomic negative-stranded RNA represent the sgRNA controller elements. The solid lines represent the full array of plus- and minus-stranded genomic and 39- and 59-terminal sgRNAs potentially produced during replication of CTV. (b, c) Models predicting the generation of 59- and 39-terminal positive- and negative-sense sgRNAs with the controller element present in normal and reverse orientation. One control region is shown for clarity. The wavy blue line represents the transcript (blue) containing the control region (green box) in normal and reverse orientation (the direction of the arrowheads above the controller element indicates the orientation of the controller element). The thick curved arrows represent the transcription termination (vertical direction, red) or promotion (horizontal direction, yellow). The solid blue lines with arrowheads represent the positive-sense 39- and 59-terminal sgRNAs and the solid red line with arrowhead indicates the 39-terminal negative-sense sgRNA. The dashed lines with arrowheads indicate the potential 59 terminal positive (blue)- and negative (red)-sense sgRNAs. Reproduced from Gowda S, Satyanarayana T, Ayllon MA, *et al*. (2001) Characterization of the *cis*-acting elements controlling subgenomic mRNAs of *Citrus tristeza virus:* Production of positive- and negative-stranded 39-terminal and positive-stranded 59 terminal RNAs. *Virology* 286: 134–151, with permission from Elsevier.

Summary and Conclusions

Genetic recombination is a common phenomenon among both DNA and RNA viruses. The recombination events have been observed based on natural rearrangements of the sequenced viral genomes. Also, experimental systems demonstrate the occurrence of recombination events that play important roles in securing the genetic diversity during viral infection. Different molecular mechanisms are involved in DNA versus RNA viruses. Many DNA viruses utilize host cellular machinery of general homologous recombination (such as meiotic crossing-over), whereas some encode their own proteins that are responsible for recombination. In addition, certain groups of DNA viruses support site-specific (nonhomologous) recombination events. In general, the virus DNA recombination mechanisms seem to involve post-DNA replication molecular events.

For RNA viruses the majority of known homologous and nonhomologous RNA recombination events appear to be integrally linked to RNA replication machinery. Various types of copy-choice (template-switching) mechanisms were proposed to describe the easy formation of RNA recombinants in numerous RNA virus systems. The roles of both special RNA signal sequences and viral proteins have been elucidated, reflecting the variety of the recombination strategies used by RNA virus groups. The involvement of host cell genes in RNA virus recombination has begun to get elucidated in several RNA viruses. Besides replicational copy-choice mechanisms, some RNA viruses use the breakage-and-religation mechanism where viral RNA gets regenerated by religation from RNA fragments, as shown experimentally for Enterobacteria phage Qbeta. New venues of RNA recombination research just emerge including our better understanding of the involvement of RNA *cis*-acting signals, the role of RNA replication, and the importance of cellular host genes such as RNA ribonucleases or RNA interference.

See also: African Cassava Mosaic Disease; Bean Golden Mosaic Virus; Brome Mosaic Virus; Cotton Leaf Curl Disease; Evolution of Viruses; Tomato Yellow Leaf Curl Virus.

Further Reading

Agol VI (2006) Molecular mechanisms of poliovirus variation and evolution. *Current Topics in Microbiology and Immunology* 299: 211–259.
Briddon RW and Stanley J (2006) Subviral agents associated with plant single-stranded DNA viruses. *Virology* 344: 198–210.
Chetverin AB (2004) Replicable and recombinogenic RNAs. *FEBS Letters* 567: 35–41.
Cromie GA, Connelly JC, and Leach DR (2001) Recombination at double-strand breaks and DNA ends: Conserved mechanisms from phage to humans. *Molecular Cell* 8: 1163–1174.
Figlerowicz M and Bujarski JJ (1998) RNA recombination in brome mosaic virus, a model plus strand RNA virus. *Acta Biochimica Polonica* 45(4): 847–868.
Galetto R and Negroni M (2005) Mechanistic features of recombination in HIV. *AIDS Reviews* 7(2): 92–102.
Gowda S, Satyanarayana T, Ayllon MA, *et al.* (2001) Characterization of the *cis*-acting elements controlling subgenomic mRNAs of *Citrus tristeza virus*: Production of positive- and negative-stranded 39-terminal and positive-stranded 59 terminal RNAs. *Virology* 286: 134–151.
Koonin EV, Senkevich TG, and Dolja VV (2006) The ancient Virus World and evolution of cells. *Biology Direct* 1(29): 1–27.
Masters PS and Rottier PJ (2005) Coronavirus reverse genetics by targeted RNA recombination. *Current Topics in Microbiology and Immunology* 287: 133–159.
Miller ES, Kutter E, Mosig G, Arisaka F, Kunisawa T, and Ruger W (2003) Bacteriophage T4 genome. *Microbiology and Molecular Biology Reviews* 67: 86–156.
Miller WA and Koev G (1998) Getting a handle on RNA virus recombination. *Trends in Microbiology* 6: 421–423.
Mosig G (1998) Recombination and recombination-dependent DNA replication in Enterobacteria phage T4. *Annual Review of Genetics* 32: 379–413.
Nagy PD and Simon AE (1997) New insights into the mechanisms of RNA recombination. *Virology* 235: 1–9.
Noueiry AO and Ahlquist P (2003) Brome mosaic virus RNA replication: Revealing the role of the host in RNA virus replication. *Annual Review of Phytopathology* 41: 77–98.
Pasternak AO, Spaan WJM, and Snijder EJ (2006) Nidovirus transcription: How to make sense...? *Journal of General Virology* 87: 1403–1421.
Poon A and Chao L (2004) Drift increases the advantage of sex in RNA bacteriophage Phi6. *Genetics* 166: 19–24.
Van Duyne GD (2005) Lambda integrase: Armed for recombination. *Current Biology* 15: R658–R660.
Weigel C and Seitz H (2006) Bacteriophage replication modules. *FEMS Microbiology Reviews* 30: 321–381.
White KA and Nagy PD (2004) Advances in the molecular biology of tombusviruses: Gene expression, genome replication, and recombination. *Progress in Nucleic Acid Research and Molecular Biology* 78: 187–226.
Wierzchoslawski R, Urbanowicz A, Dzianott A, Figlerowicz M, and Bujarski JJ (2006) Characterization of a novel 5′ subgenomic RNA3a derived from RNA3 of Brome Mosaic Bromovirus. *Journal of Virology* 80: 12357–12366.
Wilkinson DE and Weller SK (2003) The role of DNA recombination in herpes simplex virus DNA replication. *IUBMB Life* 55(8): 451–458.
Worobey M and Holmes EC (1999) Evolutionary aspects of recombination in RNA viruses. *Journal of General Virology* 80: 2535–2543.

Reoviruses: General Features

P Clarke and K L Tyler, University of Colorado Health Sciences, Denver, CO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Caspase 3−/− mice Mice that do not express caspase 3.
CTL (cytotoxic T lymphocyte) A lymphocyte capable of inducing the death of infected somatic or tumor cells. CTLs express T-cell receptors (TcRs) that can recognize a specific antigenic peptide bound to class I MHC molecules, present on all nucleated cells, and a glycoprotein called CD8.
EHBA (extrahepatic biliary atresia) A progressive congenital disorder that destroys the external bile duct structure of the liver, impairing normal bile flow (cholestasis).
SCID mice Mice with severe combined immunodeficiency, that is, cannot make T or B lymphocytes.

History

In 1959, AB Sabin proposed the designation reovirus (respiratory enteric orphan) for a subgroup of respiratory and enteric viruses not known to be associated with human disease. These viruses had particular distinguishing characteristics including: (1) their size, which at $\leq$75 nm was larger than other known enteric viruses; (2) their capacity to produce cytoplasmic inclusions in monkey kidney cells in tissue culture; (3) their pathogenicity for newborn but not adult mice; and (4) their capacity to hemagglutinate human type O erythrocytes. The first reovirus, isolated from an aboriginal child in 1951 by NF Stanley and colleagues, was named hepatoencephalomyelitis virus. Later, in 1953, M Ramos-Alvarez and AB Sabin isolated the prototype virus for reovirus serotype 1 (reovirus serotype 1 strain Lang, T1L) from the stool of a baby named Lang. In 1955, Ramos-Alverez and Sabin also isolated the prototypes for reovirus serotype 2 from the stool of a child named Jones who had a summer diarrheal illness (reovirus serotype 2 strain Jones, T2J) and for reovirus serotype 3 from the stool of a child named Dearing (reovirus serotype 3 strain Dearing, T3D). A second prototype for reovirus serotype 3 was isolated from an anal swab from a baby named Abney (reovirus serotype 3 strain Abney, T3A) by L Rosen in 1957.

With the isolation and characterization of additional viruses, the acronym 'reo' was retained for the encompassing family of viruses, the *Reoviridae*, all members of which could be called reoviruses. The prefix ortho was added to the names of the initial isolates (orthoreoviruses) and their genus (*Orthoreovirus*) to distinguish them from other members of the family (see below). Despite these formal changes in nomenclature, the orthoreoviruses are still commonly referred to as reoviruses.

Taxonomy and Classification

Currently, the *Reoviridae* is the largest of all the six double-stranded (ds) RNA virus families and its members are also the most diverse in terms of host range (**Table 1**). The genomes of these viruses comprise 10, 11, or 12 segments of dsRNA, each encoding one to three proteins (usually one) on only one of the complementary strands. Their mature virions have characteristic sizes (60–85 nm excluding the extended fiber proteins that project from the surface of some members), no lipid envelope, and proteins arranged in two or three concentric layers that generally reflect icosahedral symmetry. A distinguishing feature of their replication cycles, also found in dsRNA viruses in other families, is the synthesis of viral mRNAs by virally encoded enzymes within the icosahedral particles.

Viruses in the 12 recognized genera of the family *Reoviridae* can be distinguished by differences in relative size, capsid number and structure, genome segment number, nature and number of structural proteins and patterns of reactivity with antisera (**Table 1**). Regions of significant sequence similarity across genus lines are few and of limited length; hence, exchanges of genetic material between genera are unlikely. Viruses in a subset of the genera have a turret-like projection from the innermost capsid layer (**Table 1**). This article focuses on the genus *Orthoreovirus*. The characteristics of other genera of the *Reoviridae* are discussed elsewhere in this encyclopedia.

The genus *Orthoreovirus* currently contains five species including *Mammalian orthoreovirus* (MRV) as species I, *Avian orthoreovirus* (ARV) as species II, *Nelson Bay orthoreovirus* (NBV) as species III, *Baboon orthoreovirus* (BRV) as species IV, and *Reptilian orthoreovirus* (RRV) as species V. All orthoreoviruses, except MRV, are fusogenic and induce syncytia.

Members of the genus *Orthoreovirus* have 10 segments of dsRNA contained in two concentric protein capsids of approximately 85 nm diameter (**Table 1**). Orthoreovirus genomes comprise three large (L1–L3), three medium (M1–M3), and four small (S1–S4) segments (**Table 2**). Most of the genome segments are mono-cistronic, except for S1 which is bi-cistronic in MRV, BRV, and RRV and tri-cistronic in ARV and NBV. Further details on the molecular biology of orthoreoviruses is described elsewhere in this encyclopedia.

Fusogenic Orthoreoviruses

The fusogenic orthoreoviruses include the species ARV, NBV, BRV, and RRV. Avian orthoreoviruses can be isolated from both domestic and wild birds and cause a variety of diseases including tenosynovitis (arthritis), a gastrointestinal maladsorption syndrome, and runting. Although some avian reoviruses can be adapted for growth in mammalian cells, or will grow in mammalian cells under certain conditions, there is little or no evidence for natural infection of mammals. Nelson Bay virus, isolated from an Australian flying fox, has traits intermediate between classical mammalian and avian orthoreoviruses. Although NBV was isolated from a mammal and replicates in mammalian cell cultures, it induces syncytia formation in those cultures and is thus fusogenic. Baboon reovirus, isolated from a colony in Texas, has similar properties to NBV, including replication and syncytia formation in mammalian cultures, but is distinguishable in other respects. Nucleotide sequence analysis indicates that NBV and BRV represent additional phylogenetic groups within the genus *Orthoreovirus*. Fusogenic isolates from snakes represent yet another distinct phylogenetic group within the genus.

Fusogenic reoviruses encode a unique group of small (95–140 amino acids) fusion-associated, small

Table 1 The family *Reoviridae*

Genera	*No. of genome segments*	*No. of capsid layers*	*Hosts*
Turreted[a]			
Orthoreovirus	10	2	Mammals, birds, reptiles
Aquareovirus	11	2	Fish, mollusks
Cypovirus	10	1[b]	Insects
Fijivirus	10	2	Plants, insects[c]
Oryzavirus	10	2	Plants, insects[c]
Myocoreovirus	11/12	2	Fungi
Idnoreovirus	10	2	Insects
Nonturreted			
Rotavirus	11	3	Mammals, birds
Orbivirus	10	3	Mammals, birds, arthropods[c]
Coltivirus	12	3	Mammals, arthropods[c]
Phytoreovirus	12	3	Plants, insects[c]
Seadornavirus	12	3	Mosquitoes[c], mammals, humans

[a]Two groups of genera are designated based on the presence or absence of turrets or spikes situated at the 12 icosahedral vertices of either the virus or core particle.
[b]Most cypovirus particles are characteristically occluded within a matrix of proteinaceous crystals called polyhedra.
[c]Serve as vectors for transmission to other hosts.
Modified from Nibert ML and Schiff LA (2001). Reoviruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology*, 4th edn., pp. 1679–1728. Philadelphia, PA: Lippincott Williams and Wilkins, with permission from Lippincott Williams and Wilkins.

Table 2 List of the dsRNA segments of mammalian orthoreovirus-3De (MRV-3De), their encoded proteins, and selected functions

Gene segment	*Protein*	*Location*	*Function*
L1	λ3 (Pol)	Core	RNA polymerase (Pol)
L2	λ2 (Cap)	Core Spike	Guanylyl transferase, methyl transferase (capping enzyme). 'Turret' protein
L3	λ1 (Hel)	Core	Inner capsid structural protein, binds dsRNA and zinc, helicase (Hel)
M1	μ2	Core	NTPase
M2	μ1	Outer capsid	Multimerizes with σ3. Cleaved to form μ1C and μ1N which assume $T = 13$ symmetry in the outer capsid. μ1C is further cleaved to δ and ϕ during entry
M3	μNS μNSC	Nonstructural (NS)	Binds ssRNA and cytoskeleton. μNSC results from an alternate translational start site and has unknown function.
S1	σ1 σ1s	Outer capsid NS	Viral attachment protein Nonstructural, blocks cell-cycle progression
S2	σ2	Core	Inner capsid structural protein
S3	σNS	NS	ssRNA binding, genome packaging?
S4	σ3	Outer capsid	dsRNA binding, multimerizes with σ1, nuclear and cytoplasmic localization, translational control

Reproduced from Chappell JD, Duncan R, Mertens PPC, and Dermody TS (2005) *Othoreovirus*. In : Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Commitee on Taxonomy of Viruses,* pp. 455–465. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

transmembrane (FAST) proteins. Three distinct members of the FAST protein family have been described; the homologous p10 proteins of ARV and NBV, and the unrelated p14 and p15 proteins of RRV and BRV, respectively. Unlike the well-characterized fusion proteins of enveloped viruses, the FAST proteins are nonstructural viral proteins and are not involved in viral entry into the cell. They appear to mediate cell to cell, rather than virus to cell, membrane fusion.

The fusogenic orthoreoviruses are less well characterized, by far, than the non-fusogenic mammalian reoviruses. The rest of this article focuses on mammalian reovirues.

Mammalian Reoviruses

Serotypes and Strains

The three major MRV serotypes (MRV-1, MRV-2, and MRV-3) represent numerous isolates including the early human reovirus prototype isolates T1L (now MRV-1La), T2J (now MRV-2Jo), and T3D (now MRV-3De). A fourth MRV serotype, Ndelle (MRV-4Nd) contains only one isolate. MRV serotype is determined by the cell attachment protein, σ1 (**Table 2**). The S1 genome segment, which encodes σ1, shows the greatest sequence diversity of all the genome segments (**Figure 1**), with only 26–49% identity between viruses belonging to different serotypes. In contrast, S1 sequence identity between viruses of the

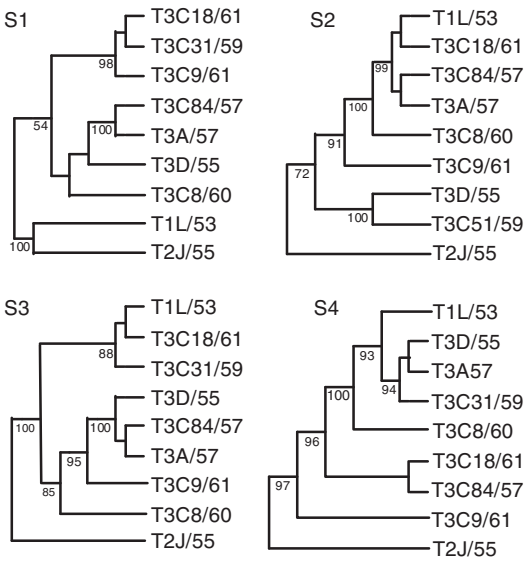


Figure 1 Phylogenetic trees indicating the potential evolutionary relationship and degree of diversity or relatedness between reovirus strains based on the nucleotide sequences of the reovirus S1, S2, S3, and S4 dsRNA segments. Each tree is rooted at the midpoint of its longest branch. Reproduced from Virgin HW, IVth, Tyler KL, and Dermody TS (1997) Reovirus. In: Nathanson N (ed.) *Viral Pathogenesis*, p. 669. Philadelphia, PA: Lippincott-Raven, with permission from Lippincott Williams and Wilkins.

same serotype is 86–99%, which is similar to the sequence identity of other genome segments between viruses belonging to different serotypes (**Figure 1**). This suggests that three versions of the S1 genome segment, corresponding to the three major MRV serotypes, arose from progenitors at different times and have subsequently diverged at a rate similar to that of other segments. Detailed phylogenetic analyses of the evolutionary relationship between a number of MRV strains from different serotypes based on the nucleotide sequence of their S1, S2, S3, and S4 gene segments are shown in **Figure 1**. Interestingly, the phylogenetic trees of individual MRV strains differ depending on the dsRNA segment chosen for comparison, indicating that reassortment of MRV gene segments occurs under natural circumstances.

MRV reassortants can easily be generated by co-infection of susceptible cells or mice with two distinct MRV serotypes. This capacity to exchange genetic material by genome reassortment during mixed infections to produce viable progeny virus strains can be used to identify individual species within the genus *Orthoreovirus*. In contrast to the evidence supporting reassortment of dsRNA segments between reovirus strains within a species, there is an absence of evidence of genetic recombination between either homologous or heterologous dsRNA segments.

Distribution and Host Range

Mammalian orthoreoviruses are ubiquitous in their geographic distribution. Studies of variation in the seasonal pattern of human infection are limited but an increased incidence of childhood illnesses associated with MRV-2 infection in the Northern Hemisphere summer (June–September) has been reported. In addition, many of the initial human reovirus isolates were from infants and children with summer diarrheal illnesses. Evidence of MRV infection has been found in animals of an enormous variety of species including humans, a wide variety of nonhuman primates, swine, horses, cattle, sheep, goats, dogs, cats, rabbits, rats, mice, guinea pigs, voles, bats, and a large number of marsupials.

Epidemiology

The majority of humans develop detectable serum antibodies against all three of the major MRV serotypes by late childhood. In a recent study of 272 serum specimens from young children, rapid loss of maternal antibody was detected between 0 and 6 months of age, seroprevalence was 0% in children 6–12 months of age, and then increased steadily throughout early childhood reaching 50% in children 5–6 years of age. The majority of cases of MRV infection in humans appear to be sporadic in nature, although outbreaks of infection caused by MRV-1 have been described. Age-related susceptibility to MRV infection has also been observed in both natural and experimental infection of animals. Calves, foals, piglets, and neonatal mice thus all appear more susceptible to MRV infection than their adult counterparts. Experimental studies in mice indicate that host immune status is another important factor in determining the nature and outcome of MRV infection. Immunocompetent adult mice develop an immune response but do not generally show clinical or pathological evidence of disease following reovirus infection. By contrast, after MRV infection, SCID mice develop prominent and often lethal hepatic disease. SCID mice and mice with targeted disruptions of the transmembrane exon of IgM (i.e., antibody and B-cell-deficient mice) also show altered patterns of viral clearance following peroral inoculation with MRV.

Transmission

Mammalian orthoreovirus transmission (horizontal spread) under natural circumstances involves respiratory aerosols and secretions, and fecal–oral transmission. In mice, there is an excellent correlation between the capacity of MRVs to grow in the intestine, the amount of virus subsequently shed in the stool, and the efficiency with which an infected animal transmits disease to its uninfected litter mates. The viral L2 gene, which encodes the core spike protein λ2, is the primary determinant of the efficiency of viral transmission following peroral inoculation. Both the L2 (see above) and the S1 gene (which encodes the virus cell attachment protein, σ1, and the small nonstructural

protein, σ1s) influence growth and survival of reovirus in intestinal tissue (**Figure 2**).

Transmission is also influenced by the capacity of the virus to survive the environment after being shed from an infected host. Most MRVs are generally stable below room temperature although, at higher temperatures, strain-specific differences in thermostability become apparent. For example, MRV-1La has a half-life of 19 h at 37 °C, compared to 2.6 h for MRV-3De. MRVs are also stable in aerosols especially in the presence of high relative humidity. Viral outer capsid proteins appear to be the major determinants of virion stability.

Pathogenesis

The basic steps in the pathogenesis of mammalian orthoreovirus infection have been studied extensively in experimental animals, including mice and rats. After peroral or intratracheal inoculation, virions adhere to the surface of epithelial M (microfold) cells, which overlie collections of lymphoid tissue in the small intestine and bronchi that form part of the systems of gut-associated lymphoid tissue (GALT) and bronchus-associated lymphoid tissue (BALT). In the intestinal lumen, virions are partially digested by proteases to generate intermediate subviral particles (ISVPs). It appears that, at least in the intestine, ISVPs are the form of virus particles that bind to M cells. After binding, ISVPs and/or virions are transported across these cells to the underlying intestinal lymphoid tissue. Studies of intestinal infection suggest that replication may occur in macrophages within mucosal lymphoid tissue.

Spread of virus from the site of primary infection to distant tissues and organs, by means of the lymphatic system, blood stream, or by axoplasmic transport within neurons, results in systemic disease. MRV serotypes differ both in their capacity to generate and sustain viremia and the efficiency with which they utilize neuronal transport. Following footpad or intramuscular infection in neonatal mice, reovirus MRV-1La spreads to the central nervous system (CNS) primarily through the blood stream, whereas MRV-3De spreads predominantly through neural pathways. In this model the viral S1 gene determines both the pathway of spread in the infected host and the extent of extra-intestinal spread (**Figure 2**).

Depending on the viral strain, the route of inoculation and host factors such as age and immune status, MRVs can produce injury in a variety of target tissues. Among the most extensively studied targets of viral infection in murine model systems are the CNS, the lung, the heart, the hepatobiliary system, and the gastrointestinal tract (**Figure 2**). The specific pathology induced in these various organ systems is discussed extensively in the references included at the end of this article.

MRV strains often show striking differences in their pattern of organ and tissue tropism. For example, MRV-3De infects neurons and retinal ganglion cells, whereas MRV-1La infects ependymal cells and cells in the anterior lobe of the pituitary gland. Differences in tropism within the brain, the pituitary gland, and the retina are all determined by the viral S1 gene. Studies with monoclonal antibody resistant σ1 variants of MRV-3De indicate that a single amino acid substitution in this gene is sufficient to alter neurovirulence, CNS growth, and pattern of CNS tropism.

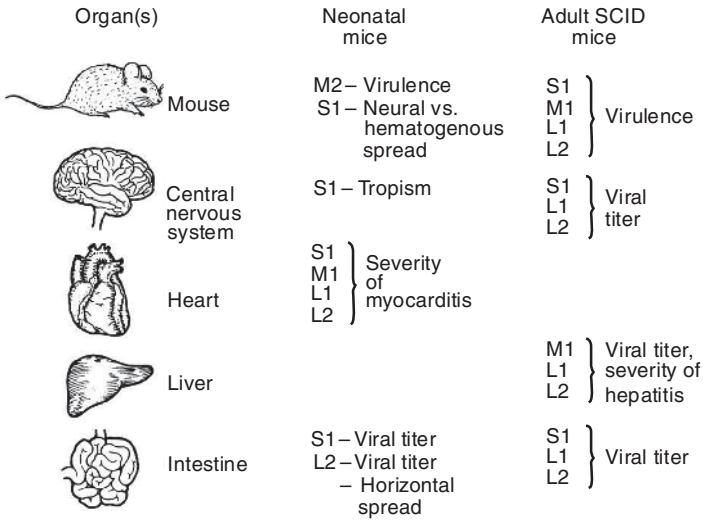


Figure 2 Reovirus dsRNA segments shown to have a role in determining organ-specific virulence in mice. Reproduced from Virgin HW, IVth, Tyler KL, and Dermody TS (1997) *Reovirus*. In: Nathanson N (ed.) *Viral Pathogenesis*, p. 669. Philadelphia, PA: Lippincott-Raven.

Attachment and Penetration

The cell attachment protein, σ1, consists of an elongated fibrous tail that inserts into the virion, and a virion-distal globular head. Four distinct and tandemly arranged morphologic regions within the σ1 tail have been designated (T(i)–T(iv)) based on proximity to the virion surface. A conserved surface at the base of the σ1 head domain of all three of the major MRV serotypes appears to determine virus binding to a serotype-independent receptor junction adhesion molecule-A (JAM-A). The σ1 protein of MRV-3De also contains a receptor binding domain in the T(iii) region of the tail that binds α-linked sialic acid. The relative importance of the JAM-A and sialic acid receptor binding domains of MRV-3De σ1 for efficient attachment and infection of host cells varies between different target cells. MRV-1La also binds a carbohydrate moiety but the nature of the glycosyl ligand remains uncertain. In contrast to MRV-3De σ1, the carbohydrate binding domain of MRV-1La σ1 has been mapped to tail region T(iv). Whereas there seems to be some flexibility on the binding of sialic acid and JAM-A for reovirus growth, both these receptors are required for the ability of reovirus to induce apoptosis in infected cells (see below).

In addition to receptor binding, β-1 integrin has recently been shown to facilitate reovirus internalization suggesting that viral entry occurs by interactions of reovirus virions with independent attachment and entry receptors on the cell surface.

Virus–Cell Interactions

Members of the MRV species induce apoptosis in cultured cells. MRV serotypes differ in this capacity with MRV-1La producing less apoptosis than MRV-3 strains. Serotype-specific differences in the capacity to induce apoptosis are determined by the S1 and M2 genome segments.

As noted above, the S1 genome segment encodes two proteins, the cell attachment protein (σ1) and a nonstructural protein (σ1s), which promotes G_2/M cell-cycle arrest. Several lines of evidence indicate that, at least in some cells in tissue culture, σ1 is the S1-encoded determinant of virus-induced apoptosis. First, apoptosis can be induced by UV-inactivated replication-incompetent virions, which lack σ1s. Second, apoptosis can be induced at nonpermissive temperatures by a variety of reovirus temperature-sensitive (ts)-mutants, which fail to synthesize σ1s in infected cells. Finally, the σ1s null-mutant MA, which fails to induce G2/M arrest in virus infected cells in tissue culture, retains the capacity to induce apoptosis, indicating that σ1s is not required for this process. Recent studies have, however, demonstrated that σ1s is a determinant of the magnitude and extent of reovirus-induced apoptosis *in vivo*, in both the heart and CNS.

The M2 gene encodes the major viral outer capsid protein μ1/μ1c. Apoptosis is inhibited following incubation of infected cells with MAbs directed against μ1 proteins and in cells infected with a temperature-sensitive (ts) membrane-penetration-defective M2 mutant. The μ1 protein is also sufficient to induce apoptosis in transfected cells. These observations support the role of the M2 genome segment in virus-induced apoptosis. In addition, recent studies suggest that binding of σ1 to JAM-A and sialic acid may be dispensible for virus-induced apoptosis and that the M2 gene segment is the only viral determinant of apoptosis when infection is initiated via Fc receptors.

As the M2 gene is a determinant of apoptosis, and as both anti-μ1 and anti-σ3 MAbs (which inhibit the virion-uncoating but not virus-cell attachment) can inhibit apoptosis, early events during virus entry, but subsequent to engagement with cellular receptors, appear to be required for apoptosis. This interpretation has subsequently been supported by experiments indicating that virus-uncoating but not replication is required for apoptosis.

MRV-induced apoptosis is associated with regulation of cellular MAPK signaling pathways, including c-Jun N terminal kinase (JNK) signaling, and transcription factors, including c-Jun and nuclear factor-kappa B (NF-κB). MRV-induced apoptosis also involves both the intrinsic and extrinsic apoptotic signaling pathways. Further details of reovirus-induced apoptotic signaling are provided in the references at the end of the article.

MRVs also induce apoptosis *in vivo* in the CNS and heart where virus infected and apoptotic cells co-localize to regions of viral injury. Virus-induced activation of caspase 3, injury, and viral load are diminished in the presence of chemical inhibitors of apoptosis or in caspase 3−/− mice (**Figure 3**). In addition, MRV-infected caspase 3−/− mice show increased survival compared to wild-type controls (**Figure 3**). These studies indicate that apoptosis is an important mechanism of virus-induced injury in the host and suggest that apoptosis inhibitors may provide useful antiviral therapies.

MRV infection is also associated with other cellular responses that influence virus growth and pathogenesis including: (1) increased expression of inducible NADPH-dependent nitric oxide (NO) synthase (iNOS) in the brains of MRV-infected mice, suggesting that NO may play an antiviral role during reovirus infection; and (2) increased phosphorylation of the eukaryotic initiation factor 2α which facilitates reovirus replication. Global expression analysis using microarrays indicates that, by 24 h following reovirus infection, the expression of 309 cellular genes (2.6% of the total number of genes present on the array) is altered in infected cells. Many of these genes are involved in cell-cycle regulation, apoptosis, and DNA repair. Further analysis of the 5′ upstream sequences of the most differentially expressed genes has revealed highly preserved sequence regions (modules)

and higher-order patterns of modules (supermodules) containing binding sites for multiple transcription factors. This suggests a coordinated mechanism for virus-induced control of the expression of genes involved in similar biological processes.

Clinical Features and Infection

Human orthoreoviruses remain as much human orphan viruses as when they were first described. Human infection occurs during early childhood (see above) and is

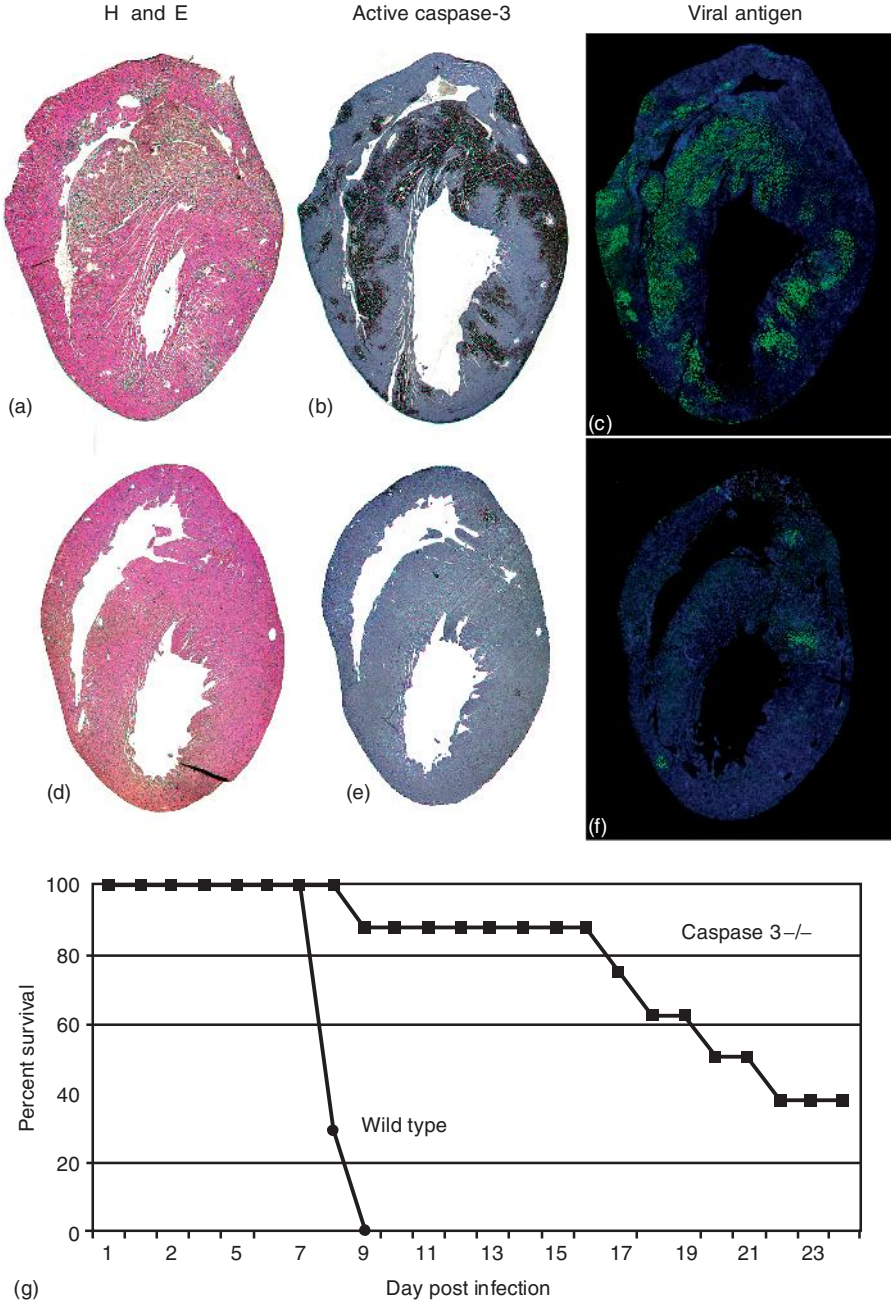


Figure 3 Myocardial injury, apoptosis, and load in MRV-3 strain 8B-infected mice. Two-day-old Swiss-Webster mice were infected with MRV-3 strain 8B followed by intraperitoneal administration of the pharmacologic caspase inhibitor Q-VD-OPH (50 mg kg^{-1} day^{-1}) or its diluent control on days 3–6 post infection. The animals were sacrificed on day 7. Consecutive sections were analyzed for histologic injury (H and E staining), active caspase 3 (brown diaminobenzadine staining), and virus antigen (fluorescent green staining). (a–c) Sections from Q-VD-OPH treated mice or (d–f) controls are shown. (g) Survival curves for MRV-3 strain 8B-infected caspase 3 / animals and wild-type controls are also shown. Reproduced from DeBiasi RL, Robinson BA, Sherry B, *et al.* (2004) Caspase inhibition protects against reovirus-induced myocardial injury *in vitro* and *in vivo*. *Journal of Virology* 78: 11040–11050, with permission from American Society for Microbiology.

either asymptomatic or produces mild symptoms of upper respiratory or intestinal infections or, in some cases, exanthema with fever. The predominant symptoms observed in children during an outbreak of MRV-1La infection included rhinorrhea (81%), pharyngitis (56%), and diarrhea (19%), although the extent to which these were attributable exclusively to the MRV-1La infection itself is unclear. Over half the children shed virus in the stool for at least 1 week and 21% shed virus for at least 2 weeks. The longest reported duration of stool shedding was 5 weeks. Deliberate inoculation of adult human volunteers with MRVs produces similar patterns of infection to those that appear to occur under natural circumstances. Nasal inoculation of seronegative volunteers with MRV-1La, MRV-2Jo, or MRV-3De is associated with seroconversion and shedding of virus in the stool but is typically asymptomatic. Approximately one-third of MRV-1La-inoculated individuals do develop symptomatic infection (fever, headache, coughing, sneezing, rhinorrhea, and generalized malaise) lasting 4–7 days and beginning 24–48 h after viral challenge. Individuals challenged with MRV-3De sometimes develop mild rhinitis. In general, individuals with pre-existing antireovirus antibody prior to challenge with reovirus do not develop signs of clinical disease and do not shed significant amounts of reovirus in stools.

MRV-3De infection of mice produces a disease with clinical and pathological features that resemble human extrahepatoic biliary atresia (EHBA). However, attempts to link reovirus infection to human EHBA have produced conflicting results. Some studies show a higher frequency or higher titers of anti-reovirus antibodies in children with EHBA as compared to controls, whereas other studies do not. Similarly, some studies show that reovirus dsRNA can be amplified from patient tissues with increased frequency compared to controls, whereas other studies do not. Reovirus has not been directly isolated from pathological specimens obtained at biopsy, surgery, or autopsy from patients with EHBA; nor has reovirus been detected in the liver or biliary tissues of patients by immunocytochemistry.

One of the hallmarks of MRV infection in rodents is CNS disease. It is therefore not surprising that several case reports associating reovirus with CNS disease have appeared. Among the most convincing is a case of aseptic meningitis in a previously healthy 3-month-old. The child seroconverted and a serotype 1 MRV was isolated from cerebral spinal fluid (CSF) after inoculation onto green monkey kidney cells. In addition, a serotype 3 MRV strain was isolated from the CSF of a 6.5-week-old child with meningitis. This virus was capable of systemic spread in newborn mice after peroral inoculation and produced lethal encephalitis. Other rare reports of an association between MRV infection and human diseases including encephalitis, keratoconjunctivitis, and pneumonia exist. However, it is important to recognize that reoviruses are responsible for a vanishingly small percentage of the total number of cases of these various illnesses.

Immune Response

As noted above, both SCID mice and antibody and B cell deficient mice show increased susceptibility to MRV infection and diminished capacity to clear the virus. Similar results have been found with immunocompetent neonatal mice depleted of CD4+ and/or CD8+ T cells. This suggests that both B and T cell-mediated immune responses play a critical role in controlling MRV infection.

Following natural or experimental infection, the bulk of both the immunogobulin (Ig)A and IgG antibody response is directed against viral structural proteins and is not serotype-specific, as would be expected by the high degree of homology between proteins of viruses belonging to different serotypes. Serotype-specific antibody responses are directed against $\sigma 1$ which is the least conserved of all the MRV proteins. The nature of the MRV-specific antibody response is influenced by the route of viral inoculation. Following peroral inoculation with MRV-1La, there is an increase in the number of reovirus-specific IgA-producing cells in intestinal Peyers patches and in the spleen. Enteric infection is also associated with the induction of IgG antibody, predominantly of the IgG2a and IgG2b subclasses. Variations in the dominant IgG antibody subclass are influenced both by the route of virus inoculation and the strain of mouse.

T cell responses are also induced during reovirus infection. Following peroral inoculation, MRV-1La-specific MHC-restricted cytotoxic T lymphocytes can be found in Peyer's patches and among the intraepithelial intestinal lymphocyte population. These cells are CD8+, bear the alpha/beta T cell receptor (TCR), are capable of MHC-restricted lysis of virus-infected target cells and increase dramatically after intestinal infection. Perforin, Fas-FasL, and TRAIL pathways are involved in intestinal lymphocyte cytoxicity against MRV-1La. Studies of Vβ TCR usage indicate that MRV infection is associated with oligoclonal expansion of specific TCR subpopulations. Serotype-specific MRV CTL responses are again directed against products of the S1 gene, whereas nonserotype-specific CTL responses are presumably directed against epitopes on other proteins or conserved epitopes within the S1-encoded proteins.

Both antibody and MRV-specific lymphocytes can protect mice against challenge with a variety of MRV strains and from infection by a variety of different routes. Passively transferred MRV-specific immune cells seem more effective than antibody in controlling viral replication at primary sites, whereas antibody may be more effective in controlling growth and spread of virus within certain tissues or organs, including the CNS.

Passive protection can be conferred by monoclonal antibodies specific for each of the viral outer capsid

proteins (σ1, μ1C, σ3) and is associated with inhibited replication at primary sites, reduced viral spread to critical target tissues, and diminished growth and spread of virus within these tissues. Both CD4+ and CD8+ T cells are required for optimal protection following passive transfer of MRV-specific T cells. There is currently no evidence that intestinal intraepithelial γ/δ TCR+ T cells, as opposed to α/β TCR+ T cells, play a significant role in immunity to MRV infection.

In addition to humoral and cellular immune responses, cytokines and other mediators may play a role in modulating MRV infection. MRV-induction of interferon (IFN) depends on both the viral strain and the host cell and differs between mice of different strains. MRV-3De, for example, is a better inducer of IFN in mouse L-cell fibroblasts than MRV-1La. MRV-3De also induces higher levels of chemokine mRNA expression for TNFα and MIP-2 than MRV-1la in pulmonary cells *in vitro* and within the lung following *in vivo* infection.

MRVs are also susceptible to β-IFN, although strains differ strikingly in sensitivity, with MRV-3De being much more sensitive than MRV-1La. Differences in the levels of IFN-induced dsRNA-dependent protein kinase (PKR) may play a role in mediating effects of β-IFN on MRV replication. The σ3 protein inhibits the activation of PKR, by preventing its interaction with dsRNA. Differences in IFN sensitivity of reovirus strains may thus depend, in part, on their σ3 proteins. In cardiac myocyte cultures, reovirus induction of β-IFN is determined by viral core proteins and inversely correlates with the capacity of viruses to induce cytopathic effect *in vitro* and myocarditis *in vivo*. Depletion of β-IFN enhances the myocarditic potential of nonmyocarditic viral strains, suggesting a protective effect for β-IFN. These results contrast with studies of experimental reovirus serotype 2-induced murine diabetes, in which the severity of insulitis in mice correlates with increased expression of γ-IFN, and is ameliorated by administration of anti-γ-IFN antibody. This suggests that IFN induction may play a pathogenetic rather than a protective role in this setting.

See also: African Horse Sickness Viruses; Bluetongue Viruses; Coltiviruses; Insect Reoviruses; Orbiviruses; Plant Reoviruses; Rotaviruses; Seadornaviruses.

Further Reading

Barton ES, Chappell JD, Connolly JL, Forrest JC, and Dermody TS (2001) Reovirus receptors and apoptosis. *Virology* 290: 173–180.

Chappell JD, Duncan R, Mertens PPC, and Dermody TS (2005) *Orthoreovirus*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 455–465. San Diego, CA: Elsevier Academic Press.

Clarke P, Richardson-Burns SM, DeBiasi RL, and Tyler KL (2005) Mechanisms of apoptosis during reovirus infection. In: Griffin DE (ed.) *Role of Apoptosis in Infection. Current Topics in Microbiology and Immunology,* 289: pp. 1–24. Heidelberg: Springer.

Clarke P and Tyler KL (2003) Reovirus-induced apoptosis. *Apoptosis* 8: 141–150.

DeBiasi RL, Robinson BA, Sherry B, *et al.* (2004) Caspase inhibition protects against reovirus-induced myocardial injury *in vitro* and *in vivo*. *Journal of Virology* 78: 11040–11050.

Mertens PPC, Attoui H, Duncan R, and Dermody TS (2004) *Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 447–454. San Diego, CA: Elsevier Academic Press.

Nibert ML and Schiff LA (2001) Reoviruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 1679–1728. Philadelphia, PA: Lippincott Williams and Wilkins.

Tyler KL (2001) Mammalian reoviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 1729–1746. Philadelphia, PA: Lippincott Williams and Wilkins.

Tyler KL and Oldstone MBA (eds.) (1998) *Reoviruses. Current Topics in Microbiology and Immunology,* 223 and 224. Heidelberg: Springer.

Virgin HW, IVth, Tyler KL, and Dermody TS (1997) *Reovirus*. In: Nathanson N (ed.) *Viral Pathogenesis,* p. 669. Philadelphia, PA: Lippincott-Raven.

Reoviruses: Molecular Biology

K M Coombs, University of Manitoba, Winnipeg, MB, Canada

© 2008 Elsevier Ltd. All rights reserved.

Glossary

EOP Efficiency of plating; a mathematical description of the relative capacity of temperature-sensitive virus mutants to replicate at a high, nonpermissive temperature compared to replication at a lower, permissive temperature.

Icosahedral A regular three-dimensional geometric shape consisting of 20 equilateral triangles and 12 vertices; the actual architecture of many 'spherical' viruses.

ISVP Intermediate (or infectious) subviral particle; an intermediate form of reovirus that lacks some proteins, compared to the intact virion, but contains

additional peptides compared to the innermost core; thought to represent the particle that crosses a cell membrane during viral entry.

PFU Plaque-forming unit; the basic unit used to indicate infectious titer for viruses capable of forming 'plaques' (individual localized regions of dead cells in a cell monolayer, each of which is formed by a single infectious virus).

Reassortant A hybrid virus that contains some genes from one parental virus and other genes from another parental virus; arise after co-infection of cells with two different members of the same virus species.

SCID mice Severe combined immunodeficient mice; so named because such animals lack both humoral-mediated and cell-mediated arms of immunity.

Top component A particle that contains all virus proteins but lacks detectable genomic material; so named because, being less dense than intact virions, it is found above virions in density gradients.

Introduction

Viruses comprising the species *Mammalian orthoreovirus* (MRV) are considered the prototype members of the genus *Orthoreovirus* in the virus family *Reoviridae*. Members of this family have a genome of 9–12 segments of double-stranded RNA (dsRNA) surrounded by 2–3 concentric, nonenveloped protein capsids (**Table 1**). Replication is exclusively cytoplasmic, viral uncoating is incomplete, and the inner capsid serves as the enzymatic complex that produces progeny mRNA. The family *Reoviridae* currently contains 12 genera, and inclusion of an additional genus (*Dinovernavirus*) has been proposed (**Table 2**). In addition to the genus *Orthoreovirus*, the family includes rotaviruses, agents responsible for a significant amount of viral gastroenteritis and numerous deaths annually worldwide, the economically important insect-vectored orbiviruses, and a variety of other viruses that infect animals, fungi, and plants. There have been increasing efforts to better understand other members of this genus, including avian orthoreoviruses and fish reoviruses. This review focuses upon MRV.

Table 1 Characteristics of the *Reoviridae*

Structure
About 70–85 nm in diameter
Nonenveloped
Icosahedral
Multiple concentric protein capsids
Innermost capsid serves as transcriptase complex
Outermost capsid serves as gene-delivery system
Genome
Linear double-stranded RNA
9–12 gene segments
Total genome size 18–29 kbp
Segmented genome capable of assortment to produce hybrid reassortant viruses
Most gene segments monocistronic
Replication
Cytoplasmic
Proteolytic processing of intact virion to produce subviral particles
Uncoating is incomplete; innermost core capsid serves to transcribe mRNA

Reovirus Structure

The MRV particle comprises 10 dsRNA genome segments encased by two concentric protein capsids built from multiple, nonequivalent copies of eight different proteins (**Figure 1**). Five proteins make up the inner capsid (called 'core') that surrounds the dsRNA genome and transcribes it to produce progeny mRNA. Three additional proteins make up the outer capsid. In addition to the core and virus, an intermediate form, called the intermediate (or infectious) subviral particle (ISVP) is also naturally found. All three particle forms can be isolated *in vivo* or produced easily in the laboratory, and the protein compositions of each have been determined. The proteins, and their functions, are discussed more fully below. Three MRV serotypes (designated 1, 2, and 3) have been described. Prototype members of each serotype routinely used are type 1 Lang (T1L), type 2 Jones (T2J), and type 3 Dearing (T3D). In addition, a large number of field isolates that represent distinct clones have been identified. Some recent comparative sequence evidence supports establishment of a fourth serotype, represented by Ndelle virus.

Structure of the Genome

The complete nucleotide sequences of all ten genes of all three prototype MRV serotypes have been determined. The genome consists of three large segments (L1, L2, L3) ranging in size from 3854 to 3916 base pairs (bp), three medium segments (M1, M2, M3) ranging in size from 2203 to 2304 bp, and four small segments (S1, S2, S3, S4) ranging in size from 1196 to 1463 bp (**Figure 2** and **Table 3**), for a total aggregate genomic size of 23 606 bp for T1L, 23 578 bp for T2J, and 23 560 bp for T3D. In addition to the dsRNA genome, purified MRV particles contain large amounts (representing nearly 25% of total RNA) of short single-stranded oligoribonucleotides, which are thought to represent abortive gene transcripts. Every MRV dsRNA gene sequenced to date consists of an

Table 2 Members of the *Reoviridae* family

Genus	*Host Range*	*Prototype Virus*
Turreted[a]		
Orthoreovirus	Vertebrates	Mammalian Reovirus
Mammalian	Mammals	*Reovirus 3*
Avian	Birds	*Avian Reovirus S1113*
Baboon	Baboon	*Baboon Reovirus*
Nelson Bay	Flying fox	*Nelson Bay virus*
Unclassified	Reptiles	
Aquareovirus	Vertebrates	*Golden shiner virus*
Cypovirus	Invertebrates	*Bombyx mori cypovirus 1*
Dinovernavirus[b]	Invertebrates	*Aedes pseudoscutellaris reovirus*
Entomoreovirus	Invertebrates	*Hyposoter exiguae reovirus*
Fijivirus	Invertebrates, plants	*Fiji disease virus*
Mycoreovirus	Fungi	*Mycoreovirus 1*
Oryzavirus	Invertebrates, plants	*Rice ragged stunt virus*
Nonturreted		
Coltivirus	Vertebrates, invertebrates	*Colorado tick fever virus*
Orbivirus	Vertebrates, invertebrates	*Bluetongue virus 10*
Phytoreovirus	Invertebrates, plants	*Wound tumour virus*
Rotavirus	Vertebrates	*Simian rotavirus SA11*
Seadornavirus	Vertebrates, invertebrates	*Banna virus*

[a]Turreted; innermost capsid (core) contains prominent spike projections at icosahedral vertices.
[b]Proposed membership within family.

open reading frame of variable length (1095 for S4 to 3867 for L2), bounded by a 5′ nontranslated region (NTR) of variable length (ranging from 12 bases in the S1 gene to 32 nucleotides in the S4 gene) and a 3′ NTR ranging from 35 bases in the L1 gene to 83 nucleotides in the M1 gene. Every gene contains a completely conserved consensus GCUA tetranucleotide at the extreme 5′ end of the gene and a completely conserved consensus UCAUC pentanucleotide (on the plus-sense strand) at the extreme 3′ end of the gene. Although still not known, the current belief is that signals that direct genomic assembly reside in one or both of the NTR. Pairwise comparisons of the T1L, T2J, and T3D NTR show generally high conservation. Most are of gene-specific uniform size (e.g., every L1 gene has a 16-nucleotide (nt) 5′ NTR and 35 nt 3′ NTR and every L3 gene has a 13 nt 5′ NTR and 63 nt 3′ NTR (**Figure 2**, left)) and some NTRs are completely conserved across all three serotypes. For example, the 13 nt L3 5′ NTR is identical in T1L, T2J, and T3D and the 18 nt M3 5′ NTR is 100% conserved across all three serotypes. A few genes show variability in the NTR. For example, the T3D L2 gene 5′ NTR has a 1 nt insertion compared to the T1L and T2J L2 5′ NTR and the T2J M1 3′ NTR has a 1 nt deletion compared to the T1L and T3D M1 3′ NTR.

Pairwise comparisons of gene identity show that, in most cases, T1L and T3D are more closely related to each other than either is to T2J (**Figure 2**, middle). For example, the percent identities of the T1L and T3D L1 genes are ~95% whereas percent identity of the T2J L1 gene compared to either T1L or T3D is <80%. Similar patterns are seen in most other genes, although comparative values range from 78% for T1L:T3D L2 and 76% for T2J:T1L or T3D L2, to ~98% for T1L:T3D M1 and ~70% for T2J: T1L or T3D M1. Only the S1 gene shows a difference, with T1L and T2J being most similar to each other.

The segmented nature of the MRV genome allows genetic mixing if two different reoviruses infect the same cell. Progeny that contain mixtures of parental genome segments (called reassortants) can only arise if the two viruses belong to the same species. Genome segment reassortment may contribute to natural pathogenesis (as seen for rotavirus and influenza virus) and has served as a convenient genetic tool for understanding better reovirus gene/protein structures and functions.

Inner Capsid Structure

The reovirus inner capsid (core) contains the viral genome and is constructed from five proteins, called λ1, λ2, λ3, μ2, and σ2. The core has icosahedral symmetry and a diameter of ~52 nm (excluding the spike 'turrets' which extend outward from each icosahedral fivefold axis (vertex) an additional 5.5 nm) (**Figure 1**). Cores serve as the metabolically active macromolecular 'machines' from which viral mRNA is transcribed, both during replication and *in vitro*. Detailed structural information has been provided from X-ray crystallographic studies of the core, which greatly aid in understanding each protein's structure and/or function.

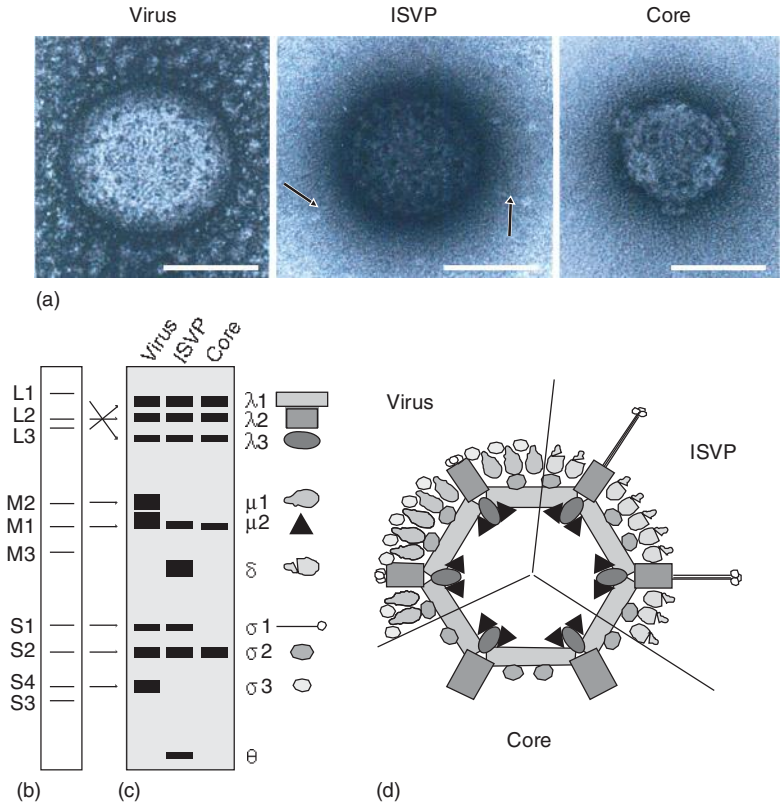


Figure 1 Structure, gene coding, and protein locations within T1L reovirus particle. (a) Electron micrographs of virus, ISVP, and core particles; arrows indicate protruding σ1 molecules on ISVP. (b) Cartoon of T1L dsRNA profile in SDS-PAGE with gene segments L1–S4 labeled on left. (c) Cartoon of protein profiles of virus, ISVP, and core in SDS-PAGE. Each protein is encoded by indicated gene segment in (b) (arrows). (d) Composite cartoon of reovirus virion, ISVP, and core, showing presumptive locations of various structural proteins, and their conversion or removal from each type of particle. Scale = 50 nm (a). Reproduced from Tran AT and Coombs KM (2001) Reoviruses. In: *Encyclopedia of Life Sciences*. New York: Wiley, with permission from John Wiley & Sons Ltd.

Full-length λ1 is a 1275-amino acid, 142 kDa protein encoded in the L3 genome segment. Protein λ1 is present in 120 copies within each reovirus core particle (**Table 3**). It is the major structural protein that forms the inner capsid. There is little serotypic variability in this inner capsid protein, implying fairly rigid structural requirements; pairwise protein identity analyses (**Figure 2**, right) show T1L and T3D λ1 share almost 100% identity (nine changes out of 1275 amino acids) and identity between T2J and either T1L or T3D is ~95%. The 120 λ1 proteins are organized as 60 asymmetric dimers in a $T = 1$ triangulation lattice to form a thin capsid shell (**Figure 1(d)**). The protein has a zinc-finger motif, a separate region that binds dsRNA, and ATPase activity, which probably is involved in transcriptional events.

Full-length λ2 is a 1289 amino acid (1288 aa for T2J), 144 kDa protein encoded in the L2 genome segment. Sixty copies of this protein are organized as distinctive pentameric turrets at each of the particle's 12 icosahedral vertices (**Figure 1**). This is one of the most variable of core proteins; T1L:T3D identity is ~92% and T2J has ~87% identity to T1L and T3D λ2 (**Figure 2**, right). Protein λ2 binds glucose monophosphate (GMP) and *S*-adenosyl-L-methionine, possesses guanylyltransferase and methyltransferase activities, and serves to attach a type-1 ^{7}mG cap structure to nascent mRNA as the mRNA is extruded from transcribing cores. High-resolution structure determinations, coupled with mutagenesis studies, show λ2 to be a multidomain protein.

Full-length λ3 is a 1267 amino acid, 142 kDa protein encoded in the L1 genome segment. Cores contain 12 copies of this minor protein, which serves as the RNA-dependent RNA polymerase (RdRp). Pairwise protein identity values between any two clones is >90% (**Figure 2**, right). Current structural/functional data indicate that a single copy of this protein is located inside the core shell, slightly offset from center, at each of the icosahedral fivefold axes, located directly below the λ1 shell. Recent X-ray crystallographic structure determinations indicate this protein follows the basic 'right-hand' configuration of most nucleic acid polymerases, but also contains extra domains.

Full-length μ2 is a 736 amino acid, 83 kDa protein encoded in the M1 genome segment. This is the least understood of reovirus structural proteins; there is presently

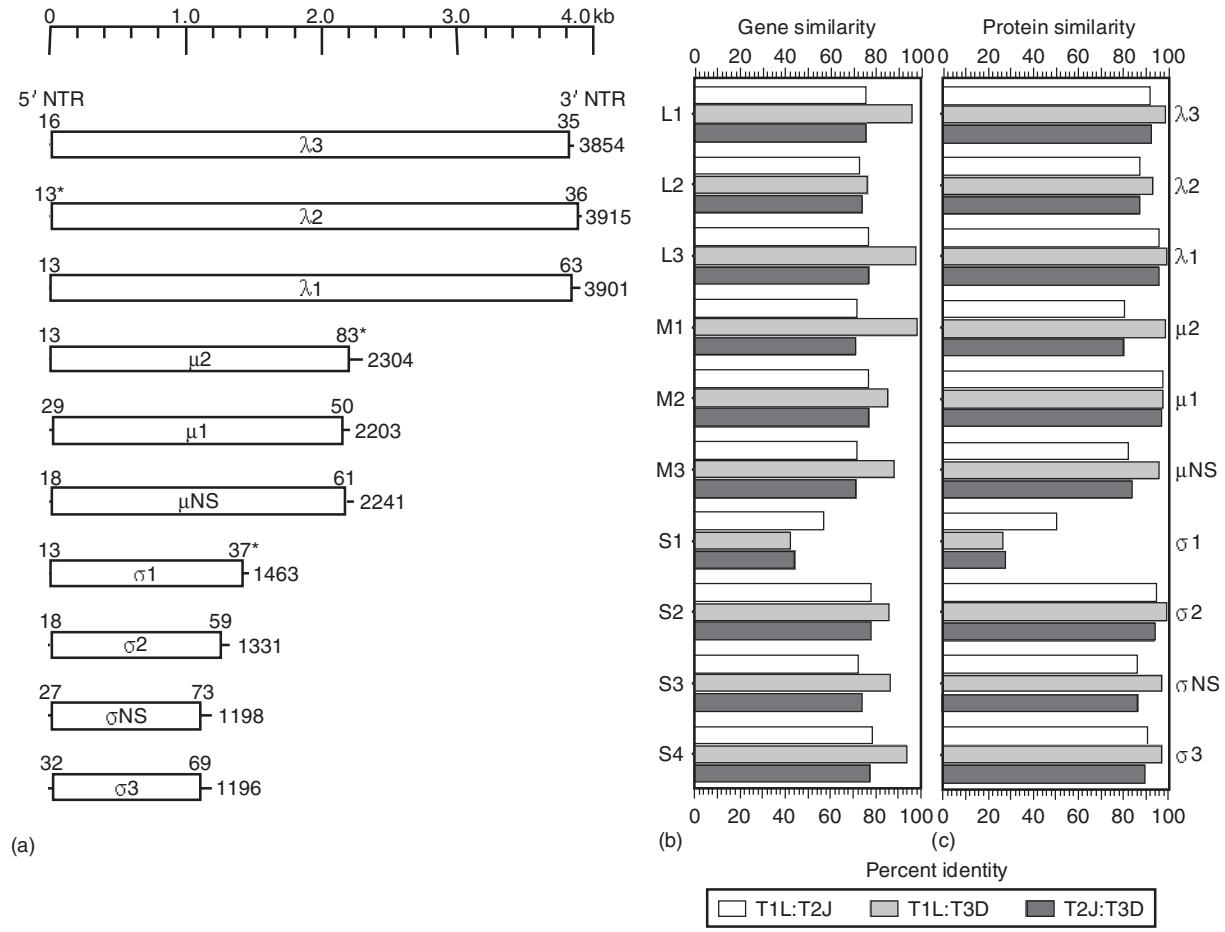


Figure 2 Gene and protein characteristics and similarities. Each of the ten double-stranded RNA genes of mammalian orthoreovirus are shown diagrammatically. (a) Genes, along with kilobase scale bar (top) are indicated. Rectangle corresponds to open reading frame and encoded protein is indicated within box. Total gene length indicated at right. Small numbers above each sequence at extreme left and right correspond to length of nontranslated region (NTR); * indicates variability in NTR length. (b) Pairwise comparisons in nucleotide identity between T1L:T2J, T1L:T3D, and T2J:T3D for each gene; genes indicated between panels A and B. (c) Pairwise comparisons in amino acid identity (indicated at far right) between T1L:T2J, T1L:T3D, and T2J:T3D for each encoded protein.

no high-resolution structure determination, and the precise function(s) remain unknown. The protein may represent an RdRp cofactor and it is present in 20–24 copies, possibly also directly under each of the core vertices. The T1L and T3D μ2 proteins share >98% identity, but the T2J μ2 protein shares only ~80% identity with T1L and T3D μ2. Genetic mapping experiments suggest that μ2 plays a role in determining the severity of cytopathic effect in cultured cells and the level of virus growth in various cells. The protein also is involved in myocarditis, in organ-specific virulence in SCID mice, in *in vitro* transcription of ssRNA, possesses nucleoside triphosphatase activity and binds RNA. It also plays important roles in virus inclusion formation and morphology, a process mediated, in part, by its level of ubiquitination.

Full-length σ2 is a 418 amino acid, 47 kDa protein encoded in the S2 genome segment. One hundred and fifty copies of the σ2 protein decorate the thin λ1 shell (**Figure 1**) and may act as 'clamps' to hold the shell together. Protein σ2 binds RNA and is required for assembly of core capsids. T1L and T3D σ2 share ~99% identity and T2J shows ~94% identity with T1L and T3D σ2.

Outer Capsid Structure

The reovirus outer capsid serves as a 'gene-delivery vehicle'; proteins within this layer are responsible for recognizing host cells and in permitting entry of the viral core into the cellular cytosol. The structures of all outer capsid proteins have been determined by X-ray crystallography. Proteins in the outer capsid are organized in a fenestrated $T = 13(l)$ icosahedral lattice. This lattice is built from only 600 copies of each of two major proteins (μ1, also present as a carboxyl-terminal cleavage product μ1C, and

Table 3 Mammalian orthoreovirus gene and protein characteristics[a]

Gene	*Serotype*	*Size (bp)*[a]	*Protein*	*Copy number*[b]	*Location*	*MW (Da)*[a]	*Size (aa)*[a]	*pI*[a]	*GenBank #*	*Functions*
L1	T1L	3854	λ3	12	Core internal	142 372	1267	8.10	NC004271	RNA-dependent RNA polymerase
	T2J	3854				142 305	1267	8.17	NC004272	
	T3D	3854				142 287	1267	7.92	NC004282	
L2	T1L	3915	λ2	60	Core spike	143 957	1289	5.08	AF378003	Guanylyltransferase;
	T2J	3912				143 166	1288	4.96	AF378005	methyltransferase
	T3D	3916				144 082	1289	5.01	NC004275	
L3	T1L	3901	λ1	120	Core capsid	141 892	1275	5.92	AF129820	RNA binding; NTPase;
	T2J	3901				142 043	1275	6.08	AF129821	RNA triphosphatase;
	T3D	3901				141 847	1275	5.98	AF129822	helicase
M1	T1L	2304	μ2	24	Core internal	83 310	736	6.75	X59945	RdRp cofactor?
	T2J	2303				84 014	736	7.33	NC004254	NTPase; binds RNA
	T3D	2304				83 230	736	6.72	M27261	
M2	T1L	2203	μ1	600	Outer capsid	76 239	708	4.92	AF490617	Cell penetration;
	T2J	2203				76 152	708	4.90	M19355	transcriptase activation
	T3D	2203				76 339	708	4.91	U24260	
M3	T1L	2241	μNS		Nonstructural	80 174	721	5.92	AF174382	Binds RNA;
	T2J	2240				80 512	721	6.02	AF174383	inclusion formation
	T3D	2241				80 195	721	5.69	AF174384	
S1	T1L	1463	σ1	36	Outer capsid	51 481	470	4.97	M35963	Attachment protein;
	T2J	1440				50 482	462	5.18	M35964	hemmaglutinin;
	T3D	1416				49 072	455	5.07	X01161	type-specific antigen
S2	T1L	1331	σ2	150	Core nodule	47 115	418	8.30	L19774	Binds dsRNA
	T2J	1331				47 050	418	8.31	L19775	
	T3D	1331				47 166	418	8.30	L19776	
S3	T1L	1198	σNS		Nonstructural	41 207	366	6.34	M18389	Binds RNA;
	T2J	1198				41 338	366	6.58	M18390	inclusion formation
	T3D	1198				41 061	366	6.34	X01627	
S4	T1L	1196	σ3	600	Outer capsid	41 159	365	6.69	X61586	Binds ssRNA; role in
	T2J	1196				41 230	365	7.18	X60066	assortment and replication
	T3D	1196				41 164	365	6.84	K02739	

[a]From indicated GenBank nucleotide sequence.
[b]Within purified virion particle.

σ3), rather than 780 copies (13 × 60) as seen in nonturreted rotaviruses. This is because vertex positions that would normally contain the 'missing' 180 protein units are occupied by the core λ2 turrets.

Full-length μ1 is a 708 amino acid, N-terminal myristoylated protein encoded in the M2 genome segment. The protein folds into a four-domain structure; three lower predominantly α-helical domains (Domain I, II, and III) plus a fourth jelly roll β barrel head domain (Domain IV). When resolved by standard sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), approximately 95% of virion μ1 appears as an ~4 kDa amino-terminal peptide (called μ1N and not resolved in standard SDS-PAGE) and a 72 kDa carboxyl-terminal portion (called μ1C). Much of this μ1N/μ1C cleavage in purified virions, which takes place between Asn_{42} and Pro_{43}, was recently shown to be an artifact of sample preparation prior to SDS-PAGE. The Asn-Pro amino acid pair is found near the N-terminus in all MRV, avian orthoreovirus, and aquareovirus μ1 (and μ1 homolog) sequences determined to date, which suggests this μ1N/μ1C cleavage plays an important role in the replicative cycle. This protein alone accounts for approximately half of the total virion peptide mass, plays important roles in virion stability, and undergoes several specific cleavages during virus entry (discussed more fully below). Unlike the core proteins (discussed above), the T1L, T2J, and T3D μ1 proteins show approximately the same amount of identity to each other (~97%) (**Figure 2**, right).

Protein σ1 is the cell attachment protein and serotype determinant. It is the most variable of reovirus proteins. The T1L and T2J σ1 proteins are more closely related (but share only ~50% identity) whereas the T1L and T3D, and T2J:T3D σ1 proteins, share only ~27% identity. The protein length varies with particular virus type; full-length T1L σ1 is a 470 amino acid, N-terminally acetylated protein encoded by the S1 genome segment, whereas the T2J σ1 is a 462 amino acid protein and the T3D σ1 is a 455 amino acid protein. The σ1 molecules have an overall stalk/knob structure similar to the adenovirus cell attachment fiber. The virion contains 36 copies of σ1, organized as trimers at each of the vertices.

Full-length σ3 is a 365 amino acid, 41 kDa N-terminally acetylated protein encoded in the S4 genome segment. The protein folds into a two-lobed structure, with the small lobe in contact with other major outer capsid protein μ1 and the large lobe exposed on the virion surface. Like the core proteins (discussed above), the T1L and T3D σ3 proteins share greatest identity (~97%), whereas T2J shares ~90% identity with the σ3 proteins of the other strains. The σ3 protein plays important roles in particle stability, in shutting down host macromolecular synthesis, and in downregulation of interferon-induced dsRNA-activated protein kinase (PKR), possibly by virtue of its intracellular distribution and capacity to interact with μ1C. The σ3 protein contains a zinc-finger motif and binds zinc, which appears important both for correct folding and stability, as well as a separate motif that binds dsRNA. Structural analyses indicate that three copies each of μ1 and σ3 form a heterohexameric $(\mu1/\sigma3)_3$ aggregate. The three copies of μ1 are wound around each other to form a 'base', upon which three σ3 monomers sit. Two hundred such heterohexameric aggregates decorate the viral outer capsid, with the μ1 trimer base contacting the core and the σ3 monomers most distally located in the particle. This protein arrangement generates the ~85 nm diameter fenestrated $T = 13(l)$ lattice. Proteolytic processing of σ3, which initiates in a central specific 'hypersensitive region' (HSR) and then proceeds bidirectionally toward the termini plays critical roles in virus entry and uncoating (discussed more fully below).

Reovirus Replication

Reoviruses infect a wide range of cells, both *in vitro* and *in vivo*. The virus usually infects specialized intestinal epithelial cells (M cells) that overlie Peyer's patches *in vivo*. Virus then migrates between and/or through the M cells into mucosal mononuclear cells in the Peyer's patch, and subsequently into a large number of extraintestinal sites, including heart, liver, and central nervous system. Elucidation of steps in MRV replication has been studied primarily in tissue-cultured mouse L929 fibroblast-like cells, although numerous studies have also been performed in a wide, and growing, range of other cells.

MRV replication is primarily cytoplasmic (**Figure 3**). There seems to be no significant nuclear involvement, although nonstructural virus protein σ1s (which is translated from an alternate S1 reading frame) targets the nucleus. The first step in virus replication is binding of virion to susceptible host cells (**Figure 3**, step 1), a process mediated by viral cell attachment protein σ1. The cellular protein(s) with which σ1 interacts is not completely known, but sialic acid appears to be an important component of the receptor(s). Several proteins have been identified as possible receptors, including junction-adhesion molecule. After initial binding, virus enters cells by either of the two mechanisms. Virus may be taken up by receptor-mediated endocytosis (**Figure 3**, step 2), and then converted into the ISVP by both acidification and proteolysis. A variety of agents that perturb either endosomal acidification (e.g., lysosomotropic agents such as ammonium chloride and chloroquine, or specific acidic protease inhibitors such as E-64), can inhibit infection by intact virions. Additional pharmacologic agents that inhibit a variety of other entry mechanisms (including methyl-β-cyclo-dextrin, which perturbs caveolae and lipid rafts, and chlorpromazine, which inhibits clathrin-mediated endocytosis) also reduce

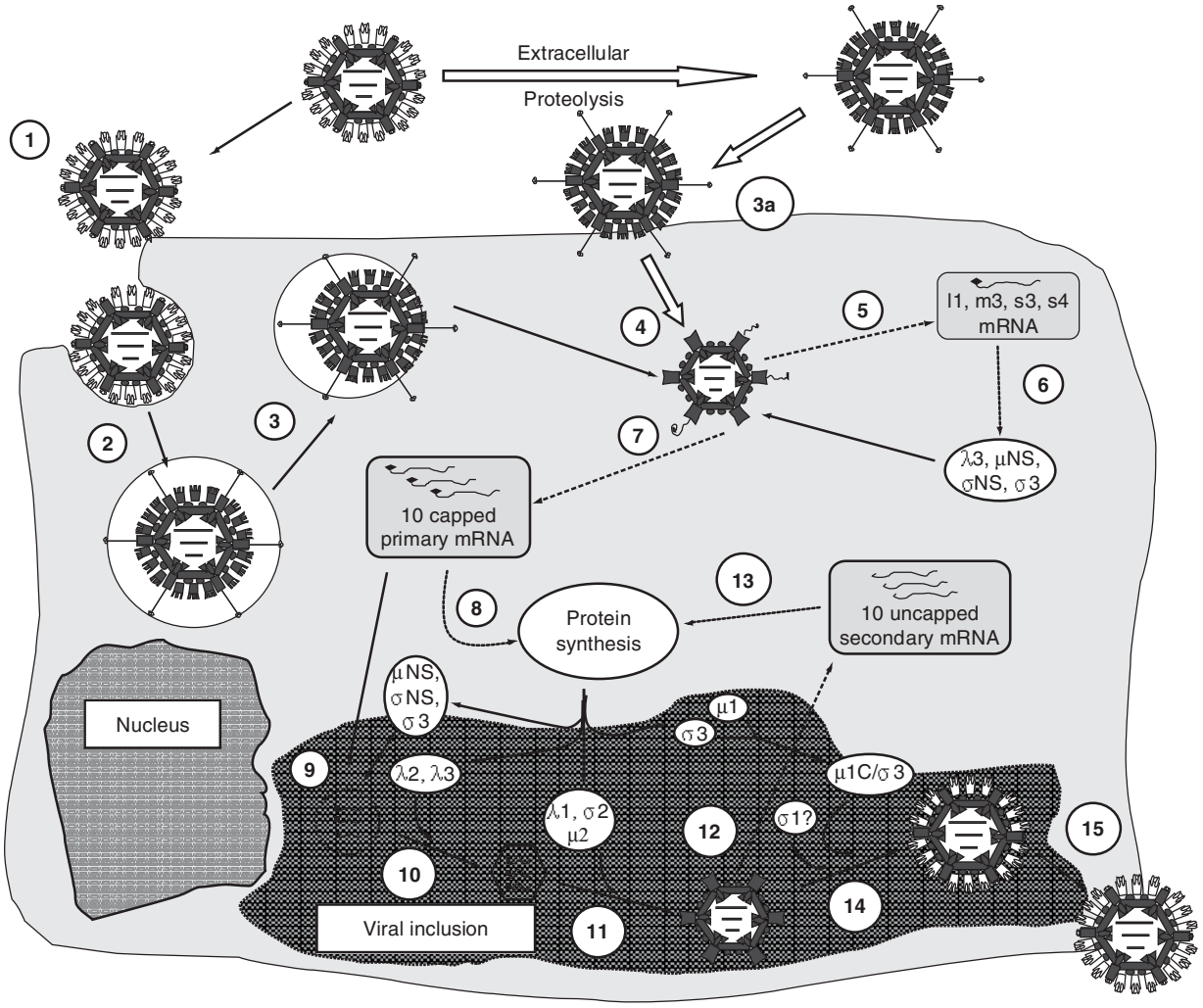


Figure 3 Model of MRV replication cycle. Details are provided in the text. Steps are (1) virus binding (upper left), (2) entry into endosomes where acid-mediated proteolysis occurs to remove outer capsid protein σ3, (3) membrane interaction to allow ISVP to escape endosome, (4) uncoating of ISVP to release transcriptionally active core particle, (5) initial 'pre-early' capped transcription, (6) initial 'pre-early' translation, (7) primary capped transcription, (8) primary translation, (9) assortment of mRNA segments into sets, probably mediated by σ3 and nonstructural proteins, (10) synthesis of negative RNA strands to generate progeny dsRNA (associated with accumulation of core proteins), (11) generation of transcriptase (replicase) complex, (12) secondary uncapped transcription, (13) secondary translation, (14) assembly of outer capsid, which halts transcription, and (15) release (lower right). Core proteins are shown in black and outer capsid proteins are shown in white; ISVP-specific modified proteins are shown in gray. Dashed arrows indicate transcription events leading to production of mRNAs (indicated in gray boxes), dotted arrows indicate translation events leading to production of proteins (indicated in white ovals), and black arrows indicate movement of proteins and viral complexes. An alternate entry mechanism for intermediate subviral particles (ISVPs), that are capable of directly penetrating membranes, is shown with white arrows at the top and in step 3a. Adapted from Tran AT and Coombs KM (2001) Reoviruses. In: *Encyclopedia of Life Sciences*. New York: Wiley, with permission from Wiley.

virus yields in cell culture. Accumulating evidence suggests that outer capsid protein σ3 must be proteolytically clipped, initially within a central HSR that is susceptible to a wide range of acidic, neutral, and basic proteases. Alternatively, the ISVP, which can be generated *in vitro* or extracellularly by a variety of intestinal proteases, appears capable of directly penetrating the cell membrane (**Figure 3**, step 3a). The acidification and protease inhibitors that block infection by intact virions do not block infection by ISVPs, making use of such inhibitors a convenient means for assessing outer capsid protein function. Cleavage, but not complete removal, of outer capsid protein σ3 appears to be a prerequisite for entry into the cytosol. Recent work has begun to generate, and use, a variety of novel particles that appear to represent additional intermediates along the virus → ISVP → core uncoating pathway. Final stages

of membrane permeabilization and cell entry involve cleavage of μ1 to μ1N and μ1C, and release of the myristoylated μ1N peptide from particles. Upon entry into the cytosol, the remaining outer capsid proteins are removed, and the λ2 turrets undergo a dramatic conformational change that opens the λ2 channels further. Both alterations appear necessary for full activation of the viral transcriptase. Resultant core particles (**Figures 1** and **3**, step 4) constitute the final stage of uncoating, and incoming core particles persist throughout the remainder of the replication cycle.

These released cores serve as transcriptionally active RdRp machines that produce viral mRNA. Collectively, the λ2, λ3, and μ2 proteins manifest RdRp, helicase, RNA triphosphatase, methyltransferase, and guanylyltransferase activities to produce the viral mRNA, which is extruded through channels in the modified λ2 spikes. Early work suggested that only four (L1, M3, S3, and S4) of the ten dsRNA genes were initially transcribed to produce mRNA (**Figure 3**, step 5), and that the protein products of these initial transcripts act upon the core by unknown means to promote transcription of all ten genes (**Figure 3**, step 7). However, more recent work questions this 'cascade' interpretation. The ten 'early' transcripts, like parental genomes, contain methylated caps and lack poly(A) tails. The cap structures are provided to nascent mRNAs by the λ2 proteins as the mRNAs are extruded through the λ2 spikes. Viral transcripts are produced in quantities inversely related to transcript length; more 's' transcripts are produced than 'm' transcripts, and more 'm' transcripts are produced than 'l' transcripts. Viral 'early' mRNAs are then translated to produce the full complement of viral proteins (**Figure 3**, step 8). These proteins begin to coalesce and are directed into non-membrane-containing inclusions in the cytoplasm by the σNS, μNS, and/or μ2 proteins (**Figure 3**, step 9). The ten mRNA molecules are also used as templates for progeny minus-strand synthesis (**Figure 3**, step 10), a process most likely mediated by viral proteins. Eventual assembly of a complete progeny virion will require various structural proteins as well as a full complement of the ten dsRNA genes.

The ten different mRNA molecules are believed to be sorted (a process called 'assortment') to ensure that developing viral particles contain correct sets of genes. Viruses, like MRV, that contain multisegmented genomes, are thought to incorporate correct sets of genes by either a 'random' or 'specific' process. Because the random model predicts that only 1 out of every 2755 particles ($10!/10^{10}$) would be infectious, and because particle-to-PFU ratios approaching 2:1 have been described, it seems highly likely that assembly of the reovirus genome uses specific mechanisms. These mechanisms are currently being delineated, and recent evidence indicates regions of a few hundred nucleotides in the gene termini are involved in this process. The assortment process also probably uses nonstructural proteins σNS and μNS, both of which are translated in unusually large amounts and both of which bind single-stranded RNA (ssRNA). The σNS protein appears to perform its functions most efficiently as a multimer and binds nonspecifically most efficiently to ssRNA. Outer capsid protein σ3 also participates in assortment. Immunoprecipitation studies have indicated that proteins λ2 and λ3 are added to these complexes at the same time mRNA is copied into dsRNA. Once the mRNA is copied, the newly transcribed minus strand remains associated with it to generate progeny dsRNA. The plus-sense molecule is no longer available for translation. However, developing particles resemble cores that serve to transcribe additional mRNA, and so presumably also contain other core proteins (**Figure 3**, step 11).

Progeny core-like particles ('transcriptase complexes'), like incoming uncoated cores, are capable of synthesizing viral mRNA (**Figure 3**, step 12). These nascent replicase particles are responsible for the majority of transcription during replication. Transcripts produced during this second wave of transcription are not capped. The switch in translation from cap-dependence to cap-independence remains poorly understood, but is believed to involve intracellular distribution of σ3, its capacity to interact with μ1C, and interactions with several cellular interferon-regulated gene products, including PKR and RNase-L. Assembly of progeny virions requires condensation of the correct numbers of eight different viral proteins with one copy of each of the ten progeny dsRNA genes. *In vitro* 'recoating' studies and studies with assembly-defective, temperature-sensitive reovirus mutants have shed light on pathways by which viral proteins may associate to generate the double-capsid shell that will serve to protect the genome (**Figure 3**, steps 12–15). This is possible because many of the known reovirus temperature-sensitive mutants produce aberrant particles at the nonpermissive temperature (**Table 4**) and the precise genetic lesions (amino acid substitutions fit into available crystal structures) for many of them have been determined. For example, assembly of the core capsid shell requires only major core proteins λ1 and σ2, as indicated by expression studies and analyses of *tsC447*, a mutant defective in σ2. Reversion studies indicated that an $Asn_{383} \rightarrow Asp$ substitution, near the end of a long α-helix that spans residues Thr_{350}–Asn_{386}, is responsible for the inability of *tsC447* to assemble core particles at the nonpermissive temperature. The λ2 proteins then associate with the core shell particles, as implied by generation of 'spike-less' cores by *tsA279*, a double mutant that contains a defective λ2 protein. However, it currently is not known whether λ2 proteins form pentamers before associating with the core shell or whether they associate as monomers and then pentamerize. Addition of σ1 may take place at about this same time because of its intimate association with the λ2 spikes in mature particles. Outer capsid proteins μ1 and σ3 initially associate with each other, as determined both by studies with

Table 4 Characteristics of selected mammalian reovirus temperature-sensitive mutants

Mutant				*Nonpermissive characteristics*[a]			
Group	*Clone*	*Gene*	*Protein*	*EOP*[b]	*dsRNA*	*Protein*	*Phenotype*
Wild-type T3 Dearing				0.2	+	+	Normal
A	*tsA201*	M2	μ1	0.009	+	+	Normal
	tsA279	M2	μ1	0.0002	+		Top component[c]
		and L2	λ2				Spike-less cores
B	*tsB352*	L2	λ2	0.00001	+	+	Cores
C	*tsC447*	S2	σ2	1×10^{-7}			Empty outer shells
D	*tsD357*	L1	λ3	0.00001	+	+	Top component
E	*tsE320*	S3	σNS	0.002			No inclusions
G	*tsG453*	S4	σ3	1×10^{-7}	+	+	Cores
H	*tsH11.2*	M1	μ2	1×10^{-7}			No inclusions
I	*tsI138*	L3	λ1	0.0001			No inclusions

[a]At nonpermissive temperature of 39.5 °C.
[b]EOP; Titer at 39.5 °C : titer at 33.5 °C.
[c]Top component; genome-deficient double-shelled particles.

tsG453, a mutant defective in σ3 protein that forms core-like structures (**Table 4**) rather than ISVP-like structures, and by recoating experiments which have, so far, failed to attach only μ1 (in the absence of σ3) to cores. These proteins form heterohexamers ($(\mu1/3)_3$) that then associate with the nascent core particles to complete the double capsid (**Figure 3**, step 14) and turn off transcription. Virus is released when infected cells lyse (**Figure 3**, step 15). The recent development of a 'reverse genetics system' for the reoviruses should prove extremely beneficial to complement some of the above-described transcapsidation and temperature-sensitive mutant analyses.

See also: African Horse Sickness Viruses; Aquareoviruses; Bluetongue Viruses; Coltiviruses; Enteric Viruses; Orbiviruses; Plant Reoviruses; Reoviruses: General Features; Rotaviruses.

Further Reading

Coombs KM (2006) Reovirus structure and morphogenesis. *Current Topics in Microbiology and Immunology* 309: 117–167.
Joklik WK (1998) Assembly of the reovirus genome. *Current Topics in Microbiology and Immunology* 233(1): 57–68.
Kobayashi T, Antar AAR, Boehme KW, *et al.* (2007) A plasmid-based reverse genetics system for animal double-stranded RNA viruses. *Cell Host and Microbe* 1: 147–157.
Mertens PPC, Attoui H, Duncan R, and Dermody TS (2005) *Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 447–454. San Diego, CA: Elsevier Academic Press.
Nibert ML and Schiff LA (2001) Reoviruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 1679–1728. Philadelphia, PA: Lippincott Williams and Wilkins.
Tran AT and Coombs KM (2001) Reoviruses. In *Encyclopedia of Life Sciences*. New York: Wiley, with permission from John Wiley & Sons Ltd.
Tyler KL (2001) Mammalian reoviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 1729–1745. Philadelphia, PA: Lippincott Williams and Wilkins.

Replication of Bacterial Viruses

M Salas and M de Vega, Universidad Autónoma, Madrid, Spain

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Lagging strand DNA strand synthesized as a series of 5′–3′ DNA fragments that are finally joined together to create an intact DNA strand.
Leading strand DNA strand whose synthesis proceeds continuously in the 5′–3′ direction as the parental duplex is unwound.

Sliding-back mechanism For linear chromosomes containing a terminal protein at their 5′-ends; initiation of DNA replication by which the DNA polymerase uses as template an internal 3′-end nucleotide, with the subsequent sliding of the initiation complex formed to recover the 3′-end terminal nucleotide information.

Okazaki fragment Short fragment of newly replicated DNA (with an RNA primer at the 5′-terminus) produced during discontinuous replication of the lagging strand.
O-some A first-stage initiation complex formed by the binding of lambda protein O to 100 bp of DNA close to the AT rich region of the DNA replication origin.
Processive polymerization Polymerization that takes place without dissociation of the DNA polymerase from the DNA.
Procesivity factor An accessory subunit of DNA polymerases that act to increase the processivity of polymerization.
Replisome The DNA-replicating structure at the replication fork consisting of two replicative DNA polymerases and a primosome.
Primosome A protein complex formed by a primase and a helicase that initiates synthesis of RNA primers on the lagging DNA strand during DNA replication.
Preprimosome For several replication systems, a protein complex assembled at the replication origin before the binding of the primase.
Transpososome The Mu transpososome refers to the Mu transposase protein complex and the three DNA segments bound by this protein complex.
σ replication mode For circular DNA, it refers to replication performed by a rolling circle mechanism.
θ (theta) replication mode For circular DNA, it occurs when two replication forks move in opposite directions from the replication origin, leading to a bidirectional replication mode.

Introduction

The requirement of a DNA/RNA molecule to prime DNA synthesis imposes replication strategies that avoid the loss of genetic information contained at the 5′-end of the lagging strand. Double-stranded (ds) DNA phages have developed different mechanisms to overcome such a problem by yielding head–tail concatemers, dependent on the presence of terminal redundancies, as in phages T4, T7, and SPP1, or on the circularization and further rolling circle replication, as occurs in phage λ. Several phages, such as ϕ29, have evolved to use a protein to prime DNA synthesis from each genome end, the priming protein becoming covalently linked to the 5′-ends of the genome. In other cases, as in phage Mu, replication of dsDNA depends on its capacity to be integrated into the host genome, replicating as a transposable element. The 5′-replication quandary does not exist in circular single-stranded (ss) DNA phages, such as ϕX174, which is replicated by a looped rolling circle to produce circular unit length genomes. In the case of ss- and dsRNA phages no such a problem exists since there is specific recognition by the RNA polymerase of the 3′-end of the template RNA. In addition, in phages λ, SPP1, or ϕX174, the replication of their genomes requires the presence of the host replication machinery, while others such as T4, T7, and ϕ29 use their own replication machinery.

In this article, we summarize the different replication strategies used by some of the well-studied phages.

Replication of dsDNA Phages

DNA Replication of Lamboid Phages

Bacteriophage λ has served as an important model system to elucidate part of the molecular mechanisms underlying eukaryotic and viral DNA replication. The virion particle contains a 48 502 bp linear dsDNA with a single-stranded protruding region, 12 bases long at each 5′-end, complementary to each other. Early after infection of its host *Escherichia coli*, the genome is circularized by means of the complementarity of the above-mentioned 5′-ends, and ligated by a host DNA ligase. Initiation of λ DNA replication requires the products of the early transcribed genes *O* and *P*, in addition to several functions from the host. Four homodimers of protein O bind to four 18 bp long binding sites at the λ replication origin. Through protein–protein interactions, the O dimers are brought together to form the O-some complex, promoting the further melting of an adjacent AT-rich region. The O-some prepares the λ replication origin for binding the host helicase DnaB. DnaB forms a heterodimer with λ protein P, which inhibits DnaB activity bringing it to the replication origin. Host proteins DnaJ, DnaK, and GrpE lead to disassembly of the preprimosome complex, releasing protein P and triggering replication fork movement. At early stages after infection, two DnaB copies unwind the DNA in opposite directions leading to a bidirectional θ (theta) replication mode. Such bidirectionality depends on the transcription activation from promoter P_R (transcriptional activation of *oriλ*), positively controlled by DnaA. At later stages, several DNA molecules start replicating following a σ mode (rolling circle replication) producing long concatemers of λ DNA containing more than 10 genome copies, which are cut at specific sites (*cos* sites) to yield unit length genomes with protruding (cohesive) 5′-ends that will be packaged into the virions. There is uncertainty about the process that governs the switch from θ to σ replication mode, the establishment of the σ replication mode being preceded by a round of unidirectional θ replication. Recent investigations point to the positive control of P_R promoter activity by bacterial DnaA in triggering such a switch. Thus, as the number of genome copies increases, free DnaA is titrated out due to its

interaction with the DnaA-binding sites in λ DNA. It has been proposed that this may lead to inefficient *ori*λ transcriptional activation, leading to unidirectional θ replication followed by σ replication mode.

DNA Replication of Phage SPP1

SPP1 bacteriophage which infects *Bacillus subtilis* is one of the best-studied phages infecting Gram-positive bacteria. Its genome is dsDNA 44 007 bp long, partially circularly permuted with a terminal redundancy of 4%. SPP1 DNA replication depends partly on several replicative proteins from its host, such as the replicative DNA polymerase III and DnaG primase. As in the case of λ phage, SPP1 starts replication following a θ replication mode followed by a σ pathway. SPP1 protein gp38 binds to *ori*L inducing a local unwinding of an adjacent AT-rich sequence. The ssDNA generated is protected by gp36, the SPP1 ssDNA binding protein (SSB). As occurs with λ DNA replication, the helicase gp40 is loaded at the replication bubble by the helicase loader gp39 that inhibits the helicase activity of gp40. gp39 interacts with gp38 forming a heterodimer that is released from the replication origin, ceasing the inactivation of gp40 that now can exert its helicase activity. Later, the primase activity of the host DnaG is required to prime DNA synthesis by the host DNA polymerase III, the replication proceeding in only one direction. The formation of concatemeric molecules requires a switch from the θ mode to σ replication mode. Such a switch is mediated by gp38 which, after one or few rounds of θ-type replication, binds to *ori*L blocking progression of DNA replication. A subsequent nick either in the lagging or the leading strand will provide a 3′ ssDNA tail. In the latter case, a previous processing by gp34 5′–3′ exonuclease is needed. The 3′-OH end can pair with the open *ori*L of other DNA molecule and prime σ replication to generate concatemeric DNA molecules. Further encapsidation is started by cleavage at the *pac* sequence by the terminase small subunit gp1, followed by translocation of the concatemer to the interior of the procapsid.

DNA Replication of T4-Like Phages

The serologically related *E. coli* infecting bacteriophages T2, T4, and T6 are commonly called T-even phages. They have a dsDNA genome 170 kbp long whose termini contain repetitions of 3% of the genome. In addition, the genome of T-even phages contains glucosylated hydroxymethylcytosines that protect DNA from endonucleases and confer double-strand stability. The T-even phages encode for their own replication machinery, which makes them good candidates to study the general mechanism of DNA replication.

At early stages after infection, T4 DNA replication starts from only one replication origin. The T4 helicase/primase complex (gp41/gp61), loaded on to DNA by T4 gp59, moves processively in the 5′–3′ direction in lagging strand synthesis at the same time as the primase activity periodically synthesizes the RNA primers to initiate Okazaki fragment synthesis. Leading strand synthesis is initiated by an RNA molecule synthesized by a host RNA polymerase from early or middle promoters. DNA polymerase (gp43) catalyzes DNA synthesis of both strands assisted by gp45, a trimer that acts as a sliding clamp, holding the DNA polymerase tightly to the DNA. gp44/gp62 complex uses the hydrolysis of ATP to drive the binding of gp45 to DNA. Although T4 DNA replication onset depends on the replication origin, most of T4 DNA replication forks are initiated by using intermediates of recombination as DNA primers at random positions throughout the genome. Once the replication fork reaches the 3′-end, the single-stranded portion of the chain that is templating lagging strand synthesis invades an homology region in other DNA molecules because of the terminal redundancy of its ends, accomplishing a recombination-dependent DNA replication pathway called 'join-copy replication', that depends on genes expressed from early or middle promoters, and that is initiated from the invading 3′ DNA ends. This promotes the appearance of replicating DNA intermediates containing multiple covalently linked copies of the genome. When an endonuclease cuts at either of the invaded DNA strands, 'join-cut-copy recombination' is initiated from the 3′-ends to allow copying of single-stranded segments of an invading DNA. This pathway requires the action of either endo VII or terminase proteins, predominantely synthesized at late infection times, making the join-cut-copy the late pathway for DNA replication, since origin initiation of replication ceases during T4 development (**Figure 1**).

DNA Replication of T7-Like Phages

T7-like phages belong to the family *Podoviridae*. These phages code for a specific RNA polymerase resistant to rifampicine and specific for phage promoters. Phage T7 is the model member of this group of lytic phages. T7 contains a linear dsDNA 39 937 bp long with a terminal direct repetition 160 bp long, used to form concatemers during replication. This terminal repeat is different in both length and sequence in the different T7-like phages. T7 encodes for its own replication machinery and this fact has been used to study protein–protein interactions at the replication fork. Bacteriophage T7 genome is replicated as a linear monomer from replication origins in a bidirectional way at early stages after infection. The primary origin of replication is an AT-rich region (78% A+T) located downstream of ϕ1.1A and ϕ1.1B promoters. These promoters are used by T7 RNA polymerase to initiate synthesis of RNA to prime leading strand synthesis. However, alternative replication origins may exist since

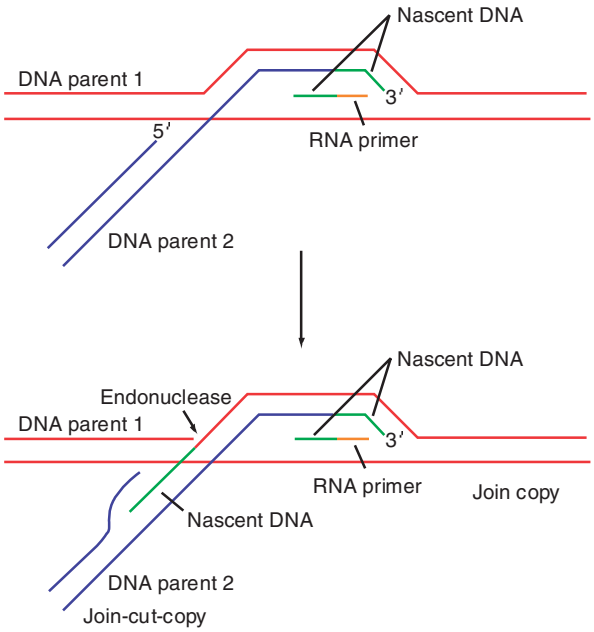


Figure 1 Initiation of DNA replication from intermediates of homologous recombination. Figure shows how ssDNA end of parent 2 invades the homologous dsDNA of parent 1. Reproduced from Mosig G, Gewing J, Luder A, Colowick N, and Vo D (2001) Two recombination-dependent DNA replication pathways of bacteriophage T4, and their roles in mutagenesis and horizontal gene transfer. *Proceedings of the National Academy of Sciences, USA* 98: 8306–8311, copyright (2001) National Academy of Sciences, USA, with permission from National Academy of Sciences.

in the absence of T7 primase, more than 20 RNA–DNA transition sites have been detected, scattered widely downstream from the ϕ1.1 promoter and mostly downstream from the ϕ1.3 promoter. In addition, secondary replication origins have been mapped close to the left genome end by using deletion mutants lacking T7 *ori*. To accomplish T7 genome replication, the phage synthesizes four replication proteins: (1) T7 RNA polymerase (gp1), which synthesizes the RNA primer to the leading strand; (2) T7 helicase/primase (gp4) which performs two activities. On the one hand, its C-terminal half contains a helicase activity that uses the hydrolysis of dTTP to unwind duplex DNA in a 5′–3′ direction with respect to the bound strand. This part of the protein physically interacts with T7 DNA polymerase. On the other hand, the N-terminal half contains a primase activity that catalyzes the synthesis of tetraribonucleotides to function as primers for Okazaki fragments synthesis; (3) T7 DNA polymerase (gp5) that contains both a 5′–3′ synthetic activity and a 3′–5′ exonuclease activity to proofread replication errors. T7 DNA polymerase is a distributive enzyme *per se*; however, *in vivo* it forms a complex with *E. coli* thioredoxin, a protein used by the polymerase as a processivity factor; (4) T7 ssDNA binding protein (gp2.5) that interacts physically with T7 DNA polymerase and primase/helicase. This protein is required for the coordinated synthesis of both leading and lagging strands of the replication fork. This SSB protein coats the ssDNA template of the lagging strand participating in mowing the polymerase from the end of one Okazaki fragment to the initiation site for the next.

Once the replication fork has reached the end of the linear molecule, the 3′-end of the strand used as template for lagging strand synthesis remains uncopied. By means of the terminal repeat sequences, several linear DNA molecules can anneal forming long concatemers containing from 10 to >100 genome equivalents. The concatemer will have a single copy of the terminal repeat between two genomes. Before the encapsidation of unit-length genomes, the terminal repeat sequence has to be duplicated. Many efforts have been done to elucidate the duplication mechanism. Two models have been proposed. One involves the transcription through the terminal repeat of the concatemer, promoting a displaced strand suitable to be cut by an endonuclease and generating a 3′-OH end that can be used to prime DNA synthesis and replicate the terminal repeat. A dsDNA break performed by the T7 terminase complex at the right part of the terminal repeat will provide another 3′-end that will complete duplication of the terminal repeat, allowing the genome to be packaged (**Figure 2(a)**). The presence of a palindromic sequence at the left part of the terminal repeat led to the proposal of an alternative model, invoking the formation of a cruciform structure followed by nicking by an unknown nuclease at a palindromic sequence, creating a hairpin primer for DNA polymerase, that once extended through the terminal repeat and into the genome will provide dsDNA that could be converted into mature left end. Primase initiated synthesis on the displaced strand will proceed through the terminal repeat, generating a mature right end of the next genome to be packaged (**Figure 2(b)**). Probably, both (or more) pathways could coexist to promote T7 DNA encapsidation and the yield of mature viral particles.

Bacteriophages with Terminal Protein at the 5′ DNA Ends

Bacteriophage ϕ29 which is a member of the family *Podoviridae* has a linear dsDNA 19 285 bp long, with a specific protein (product of the viral gene 3) covalently linked to the two 5′-ends called terminal protein (TP). The protein contains 266 amino acids and is linked to the DNA through a phosphoester bond between the OH group of serine 232 in the TP and 5′-dAMP.

Other *B. subtilis* phages related to ϕ29 that also contain linear dsDNA and TP of similar size are classified in three groups: (1) ϕ15, PZA, and PZE that belong to the ϕ29 group; (2) Nf, M2, and B103; and (3) GA-1. The DNA of

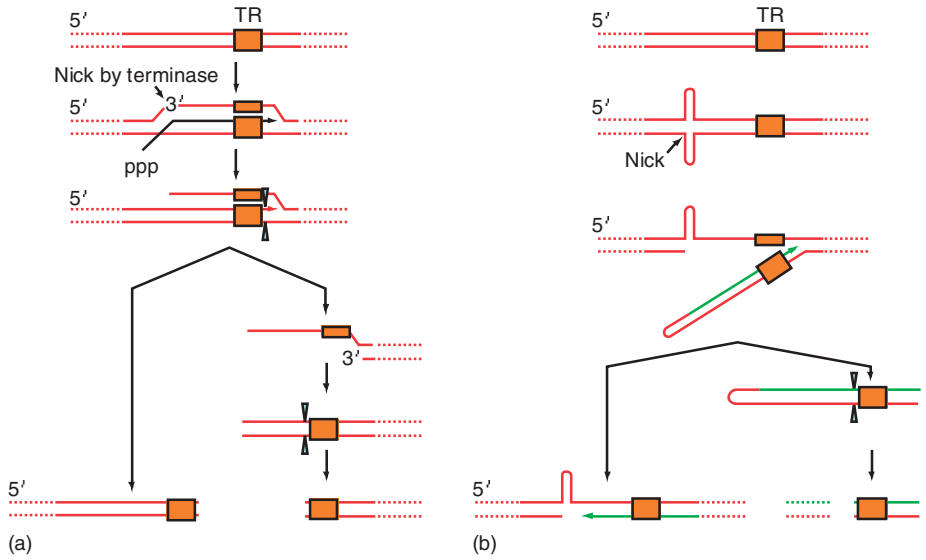


Figure 2 Mechanism for TR duplication. (a) Model involving the transcription through the terminal repeat of the concatemer. (b) The palindromic model. See text for details. (a) Adapted from Fujisawa H and Morita M (1997) Phage DNA packaging. *Genes to Cells 2*: 537–547, with permission from Blackwell Publishing. (b) Reproduced from Chung YB, Nardone C, and Hinkle DC (1990) Bacteriophage T7 DNA packaging III. A ''hairpin'' end formed on T7 concatemers may be an intermediate in the processing reaction. *Journal of Molecular Biology* 216: 939–948, with permission from Elsevier.

all these phages have a short inverted terminal repeat (ITR), six nucleotides long (AAAGTA) for ϕ29, ϕ15, PZA, and B103, eight nucleotides long (AAAGTAAG) for Nf and M2, and seven nucleotides long (AAATAGA) for GA-1, all of them showing a reiteration AAA at their 5′ DNA ends. PRD1, a member of the family *Tectiviridae* of lipid-containing phages infecting *E. coli* and other Gram-negative bacteria, contains a linear dsDNA 14 925 bp long, whose 5′-termini are linked to a 28 kDa TP by a phosphoester bond between tyrosine 190 and 5′-dGMP. The DNA of PRD1 and related phages have a 110 bp long ITR with the reiteration 5′ GGGG at the ends. The *Streptococcus pneumoniae* phage Cp-1 contains a 19 345 bp linear dsDNA with a TP of 28 kDa covalently linked to the 5′ DNA ends by a phosphoester bond between the OH group of threonine and 5′-dAMP. Cp-1 has an ITR of 236 bp with the reiteration 5′ AAA.

In all of the above-mentioned phages, the origins of replication are located at both ends of the linear genome and are specifically recognized by their cognate replication machinery, encoded by the phage, to initiate DNA replication.

Much work has been performed to elucidate the replication mechanism of these phages, the most studied case being bacteriophage ϕ29. DNA polymerase from phage ϕ29 forms, with a free TP, a heterodimer (**Figure 3(a)**) that specifically recognizes both replication origins. The DNA polymerase catalyzes the template-directed formation of a covalent linkage between dAMP and the OH group of serine 232 of the primer TP, giving rise to the TP–dAMP initiation complex, a reaction directed by the penultimate nucleotide of the 3′ reiteration (3′TTT . . . 5′) and stimulated by the ϕ29-encoded protein p6. The TP–dAMP complex slides-back one position to recover the terminal nucleotide, the second 3′-terminal nucleotide acting again as template to direct the incorporation of the second dAMP residue. It has also been shown that phage GA-1 DNA initiates replication at the second nucleotide from the 3′ DNA end. Since the TP–dAMP covalent complex is not a substrate of the 3′–5′ exonuclease proofreading activity of the ϕ29 DNA polymerase, the sliding-back mechanism could provide a way to ensure the fidelity of the initiation reaction. Terminal reiteration also exists in PRD1 and Cp-1 DNAs. Indeed, it was shown that initiation of PRD1 and Cp-1 DNA replication occurs at the fourth and third 3′-terminal nucleotide of the template, respectively. In these cases, a stepwise sliding-back mechanism has been proposed. It is likely that internal initiation and a sliding-back mechanism is a feature of the genomes that initiate replication by protein priming.

The ϕ29 DNA polymerase/primer TP heterodimer does not dissociate after initiation or after sliding back. There is a transition stage in which the DNA polymerase synthesizes a five nucleotide-long DNA molecule while complexed with the primer TP, undergoes some structural changes during incorporation of nucleotides 6–9 (transition), and finally dissociates from the primer TP when nucleotide 10 is incorporated into the nascent DNA chain (elongation mode).

Once dissociated from the heterodimer, the same DNA polymerase molecule starts normal DNA elongation, catalyzing highly processive polymerization coupled to strand

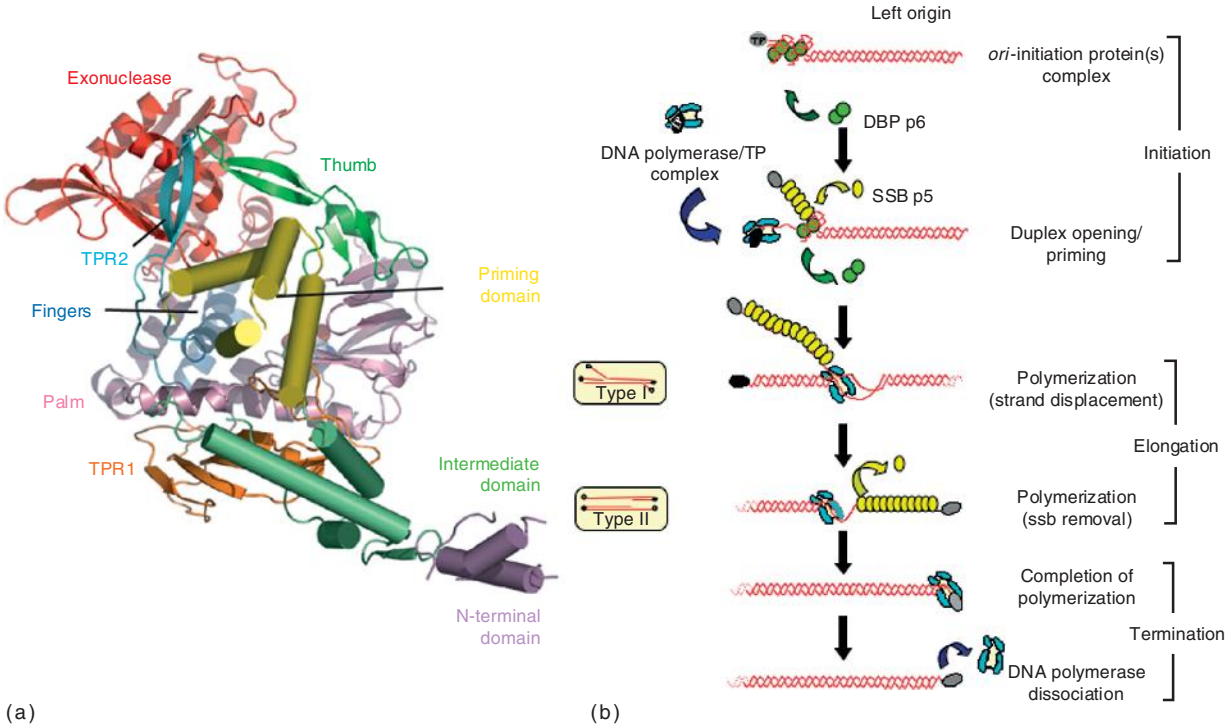


Figure 3 (a) Structure of the ϕ29 DNA polymerase-terminal protein heterodimer. Ribbon representation of DNA polymerase colored by subdomains and terminal protein shown with cylindrical helices. (b) Schematic representation of bacteriophage ϕ29 TP-DNA replication. (a) Adapted from Kamtekar S, Berman AJ, Wang J, *et al*. (2006) The ϕ29 DNA polymerase: protein-primer structure suggests a model for the initiation to elongation transition. *EMBO Journal* 25: 1335–1343, with permission from Nature Publishing Group. (b) With permission, from the Annual Review of Biochemistry, Volume 60 © 1991 by Annual Reviews.

displacement, and, therefore, complete replication of both strands proceeds continuously from each terminal-priming event. As the two replication forks move, DNA synthesis is initially coupled to strand displacement of long stretches of single-stranded ϕ29 DNA, producing type I replication intermediates (**Figure 3** (**b**)). When the two replication forks, moving in opposite directions, meet, a new type of replication intermediate (type II) is found. Electron microscopy analysis of ϕ29 replication intermediates *in vitro* showed that the viral protein p5 binds to the single-stranded portion of both type I and II molecules, thus acting as a SSB during ϕ29 DNA replication. Once replication of both strands is fulfilled, the two DNA polymerase molecules fall off the DNA to start initiation and replication of a new ϕ29 DNA molecule.

ϕ29 DNA replication takes place in close association with the bacterial membrane. Recent studies have identified p1 and p16.7 as membrane-localized, phage-encoded proteins likely to be involved in the membrane association of ϕ29 DNA replication. A multimeric p1 structure would provide an anchoring site for viral replisome through interaction with the primer TP, while p16.7 would recruit the ϕ29 DNA replication intermediates to the membrane by binding both the parental TP and the displaced ssDNA.

Bacteriophage Mu

E. coli phage Mu, a member of the family *Myoviridae*, is of great interest because it is both a bacteriophage and a transposon. The size of its linear dsDNA ranges between 37 and 42 kbp with a mean value of 36.7 kbp. The variability of its length is due to the presence of 50–150 bp and 0.5–3 kbp of host genome flanking the left and right ends of the viral genome, respectively. Once Mu has infected the host cell, the linear DNA is converted into a circular form, induced by the phage protein N that is noncovalently bound to Mu DNA. The early phage protein A (transposase) specifically inserts the viral genome in a random fashion into the host chromosome, through the genomic sequences *att*L and *att*R located at both ends of the viral DNA, following a 'nick-join-process' pathway by which the host sequences, still attached to Mu upon integration, are degraded shortly after, probably by gap repair mechanisms. Bacteriophage Mu replicates as a transposable element. Mu A protein remains bound to the Mu genome once inserted into the host genome, forming an oligomeric transpososome which promotes the transfer of the viral ends to other location of the host DNA, creating replication forks at each end. Host ClpX chaperonin reduces transpososome interaction with the DNA to promote the assembly of prereplisome. Later, primosome

assembly protein PriA binds to the forked DNA structures and recruits PriB, DnaT, DnaB, and DnaC. PriA opens the dsDNA for DnaB binding that leads to the recruitment of DNA polymerase III holoenzyme to complete replisome assembly. In addition, DnaB attracts primase to catalyze lagging strand synthesis. The repetition of this integration process produces more than 100 copies of Mu DNA. Finally, these copies are cut at the *pac* sequence, located at the left end. The DNA is cut in a way that 50–150 bp of bacterial DNA at the left end are encapsidated in the phage particle. The total length of the encapsidated DNA depends on the size of the phage head, in a way that 0.5–3 kbp of host genome will flank the right end.

ϕX174 and Related Phages

ϕX174 and related a3, St1, and G4 bacteriophages are members of the viral family *Microviridiae*. All of them grow on various strains and species of *Enterobacteriaceae*, typically *E. coli*, *Salmonella*, and *Shigella* species. They contain a circular (+) ssDNA genome whose replication has been widely studied in phage ϕX174. The replication cycle can be divided into three stages.

Stage I. Once inside the host cell, the circular ssDNA is covered by the SSB protein, before starting the complementary (–)-strand synthesis. Different members of this group of phages exploit different host enzyme systems for complementary strand synthesis when ssDNA is complexed with SSB. Once covered by SSB, the assembly of preprimosome is carried out. The preprimosome is constituted by proteins PriA, PriB, PriC, DnaT, and DnaB. PriA recognizes the unique sequence *pas*, also called *n′*, that forms a stem–loop structure. PriB and PriC act as stability and specificity factors. DnaT and DnaC load the host helicase DnaB. The preprimosome associates with the host primase DnaG to produce the primosome. Such a complex travels on ssDNA following a 5′- to 3′-direction, with the concomitant synthesis of short RNA molecules by DnaG to prime DNA synthesis by host DNA polymerase III holoenzyme. Host DNA polymerase I removes RNA primers and a DNA ligase ligates the different DNA fragments to produce a circular and supercoiled dsDNA (replicative form I; RFI). The stem loops of other phages such as G4, a3, and St-1 are directly recognized by DnaG primase without the need for auxiliary proteins.

Stage II. Phage protein A nicks between (+)-strand nucleotides 4305 and 4306 at the replication origin (30 bp long), releasing the superhelicity of the DNA molecule to give replicative form II (RFII) DNA molecules. Protein A creates a covalent ester linkage between a tyrosine residue and the 5′-phosphate group of adenylic acid at position 4306 of viral (+)-strand. Host rep protein (helicase) forms a complex with protein A, unwinding the two strands of the duplex. In a coordinated way, DNA polymerase III holoenzyme uses the newly generated (+)-strand 3′-OH to prime the synthesis of a new (+)-strand. The 5′-end of the displaced strand travels with the replication fork in a 'looped rolling circle' way. Once the preprimosome assembly site on the displaced SSB-coated (+)-strand is available, synthesis of a new (–)-strand takes place as in stage I to give more RFI molecules that will be used as templates in further replication cycles. After one round of rolling circle synthesis, protein A cuts the newly generated replication origin, acting as a ligase to give circular (+) ssDNA molecules, protein A being transferred to the newly created 5′-end setting the stage for a new round of replication.

Stage III. GpC protein binds to gpA/rep/ RFII complex, enabling them to serve as template in further RF replication rounds, forcing them to be used as template for the unique generation of (+) ssDNA molecules, that will be encapsidated later.

ss- and dsRNA Phages

The ssRNA coliphages form the family *Leviviridae*, of which the Qβ and MS2 bacteriophages are the best-studied members. Their infection depends on *E. coli* F-pili, normally used for bacterial conjugation. The genome of these phages is an ssRNA molecule with a length ranging from 3500 to 4200 nucleotides, depending on the genera. The viral (+) RNA molecule, once inside the cell, functions as messenger RNA for synthesis of phage proteins, and as template for viral replicase to multiply the viral genome. The high degree of secondary structure shown by the RNA plays a pivotal role in the fine-tuned coordination between translation (which proceeds in the 5′–3′ direction of RNA) and replication (which advances in the opposite direction), which is required to prevent a head to head collision of ribosomes and phage replicase.

To replicate their genome, ssRNA phages make use of a replicase that is composed of four proteins, only one of which coded by the phage (the replicase or β-subunit). The other three are encoded by the host: ribosomal S1 (α-subunit), and translation elongation factors EF-Tu and EF-Ts (γ- and δ-subunits, respectively). The copy of (+) ssRNA into (–) ssRNA requires also the product of the host *hfq* gene, the host factor HF. The 3′-end of (+) RNA is protected against host RNase E and other exonucleases by base pairing. This implies that the terminal nucleotides are also inaccessible to the ribosome and replicase, and suggests that S1 and HF are required. S1 is proposed to anchor the template on the polymerase in a standby complex, waiting for the occasional thermal breathing of the 3′-region, such an opening being assisted by HF. The translation elongation factors bind the RNA to the polymerase. Under such conditions, replicase initiates (–) RNA synthesis at the penultimate 3′-terminal C nucleotide, initially losing the ultimate A. Once replicase reaches the 5′-end of the (+) RNA, the terminal

A nucleotide is recovered by an untemplated addition of an A at the 3′-end of the (−)-RNA. Although both (+)- and (−)-RNA chains are complementary, they do not anneal to form dsRNA. Such annealing is inhibited by the high degree of internal secondary structure formed in each ssRNA molecule. The (−)-ssRNA is used as a template to produce more (+)-ssRNA.

Members of the family *Cystoviridae* contain genomes composed of three dsRNA segments called S, L, and M. From this family, phages ø6, and more recently ø8, have been studied in great detail. Once the host cell is infected by these viruses, a transcriptionally active polymerase complex is released into the bacterial cytoplasm, where transcription of the dsRNA segments takes place. The L segment messenger RNA codes for proteins P1 (main component of the inner shell), P2 (RNA-dependent RNA polymerase), P4 (NTPase), P7 (involved in packaging and replication), and P14. The first four proteins form a polymerase complex which packages the three ssRNA (+)-strands. Such strands have an 18-base consensus sequence at their 5′-ends, and a *pac* sequence 200 bases long. Both the consensus and *pac* sequences are required and sufficient to package plus strands. The ssRNA(+) strands are packaged in a S, M, and L fashion. Once packaged within the viral particle, the polymerase complex synthesizes the minus RNA strand to convert the ssRNA into dsRNA.

See also: History of Virology: Bacteriophages; Icosahedral dsDNA Bacterial Viruses with an Internal Membrane; Icosahedral Enveloped dsRNA Bacterial Viruses; Icosahedral ssDNA Bacterial Viruses; Icosahedral ssRNA Bacterial Viruses; Icosahedral Tailed dsDNA Bacterial Viruses.

Further Reading

Au TK, Agrawal P, and Harshey RM (2006) Chromosomal integration mechanism of infecting Mu virion DNA. *Journal of Bacteriology* 188: 1829–1834.

Bamford DH (1999) Phage ø6. In: Granoff A and Webster RG (eds.) *Encyclopedia of Virology,* 2nd edn., pp. 1205–1208. San Diego, CA: Academic Press.

Barariska S, Gabig M, Wegrzyn A, *et al.* (2001) Regulation of the switch from early to late bacteriophage λ DNA replication. *Microbiology* 147: 535–547.

Chung YB, Nardone C, and Hinkle DC (1990) Bacteriophage T7 DNA packaging III. A ''hairpin'' end formed on T7 concatemers may be an intermediate in the processing reaction. *Journal of Molecular Biology* 216: 939–948.

Fujsawa H and Morita M (1997) Phage DNA packaging. *Genes to cells* 2: 537–547.

Jiang H, Yang J-Y, and Harshey RM (1999) Criss-crossed interactions between the enhancer and the *att* sites of phage Mu during DNA transposition. *EMBO Journal* 18: 3845–3855.

Kamtekar S, Berman AJ, Wang J, *et al.* (2006) The ϕ29 DNA polymerase: Protein-primer stucture suggests a model for the initiation to elongation transition. *EMBO Journal* 25: 1335–1343.

Martínez-Jiménez MI, Alonso JC, and Ayora S (2005) *Bacillus subtilis* bacteriophage SPP1-encoded gene 34.1 product is a recombination-dependent DNA replication protein. *Journal of Molecular Biology* 351: 1007–1019.

Miller ES, Kutter E, Mossig G, Arisaka F, Kunisawa T, and Rüger W (2003) Bacteriophage T4 genome. *Microbiology and Molecular Biology Reviews* 67: 86–156.

Molineux IJ (2006) The T7 goup. In: Calendar R (ed.) *The Bacteriophages,* 2nd edn., pp. 277–301. Oxford: Oxford University Press.

Mosig G, Gewing J, Luder A, Colowick N, and Vo D (2001) Two recombination-dependent DNA replication pathways of bacteriophage T4, and their roles in mutagenesis and horizontal gene transfer. *Proceedings of the National Academy of Sciences, USA* 98: 8306–8311.

Nakai H, Doseeva V, and Jones JM (2001) Handoff from recombinase to replisome: Insights from transposition. *Proceedings of the National Academy of Sciences, USA* 98: 8247–8254.

Ng JY and Marians KJ (1996) The ordered assembly of the ϕX174-type primosome. I. Isolation and identification of intermediate protein-DNA complexes. *Journal of Biological Chemistry* 271: 15642–15648.

Ng JY and Marians KJ (1996) The ordered assembly of the ϕX174-type primosome. II. Preservation of primosome composition from assembly through replication. *Journal of Biological Chemistry* 271: 15649–15655.

Salas M (1991) Protein priming of DNA replication. *Annual Review of Biochemistry* 60: 39–71.

Salas M (1999) Mechanisms of initiation of linear DNA replication in prokaryotes. *Genetic Engineering (New York)* 21: 159–171.

Taylor K and Wegrzyn G (1999) Regulation of bacteriophage λ replication. In: Busby SJW, Thomas CM, and Brown NL (eds.) *Molecular Microbiology*, pp. 81–97. Berlin: Springer.

Van Duin J and Tsareva N (2006) Single-stranded RNA phages. In: Calendar R (ed.) *The Bacteriophages,* 2nd edn., pp. 175–196. Oxford: Oxford University Press.

Replication of Viruses

A J Cann, University of Leicester, Leicester, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

(+)-sense RNA (plus-sense RNA) A virus with a single-stranded RNA genome of the same polarity ('sense') as mRNA.

( )-sense RNA (minus-sense RNA) A virus with a single-stranded RNA genome of the opposite polarity ('sense') as mRNA.

Assembly The stage of replication during which all the structural components come together at one site

in the cell and the basic structure of the virus particle is formed.
Attachment The binding of a virus particle to a specific receptor on the surface of a host cell.
Capsid A protein shell comprising the main structural unit of a virus particle.
Envelope A lipid membrane enveloping a virus particle.
Fusion protein The protein(s) on the surface of a virus particle responsible for fusion of the virus envelope with cellular membranes.
Gene expression An important stage of viral replication at which virus genetic information is expressed: one of the major control points in replication.
Genome replication The stage of viral replication at which the virus genome is copied to form new progeny genomes.
Matrix protein A structural protein of a virus particle which underlies the envelope and links it to the core.
Maturation The stage of viral replication at which a virus particle becomes infectious.
Molecular epidemiology The use of nucleotide sequence information to study the diversity and distribution of virus populations.
mRNA Messenger RNA, translated on ribosomes to produce proteins.
Nucleocapsid The core of a virus particle consisting of the genome plus a complex of proteins.
Penetration The stage of viral replication at which the virus genome enters the cell.
Polyprotein A long polypeptide encoding several mature proteins which are subsequently released by protease cleavage.
Receptor A specific molecule on the surface of a cell which is used by a virus for attachment.
Release The stage of viral replication at which virus particles escape the infected cell.
Tropism The ability of a virus to infect specific cell or tissue types.
Uncoating The stage of viral replication at which structural proteins are lost and the virus genome is exposed to the replication machinery.
Virions Structurally mature, extracellular virus particles.
Virus attachment protein The protein on the surface of a virus particle responsible for binding the receptor.

Unlike cellular organisms, which 'grow' from an increase in the integrated sum of their components and reproduce by division, virus particles are produced from the assembly of preformed components. Once manufactured, virus particles (virions) do not grow or undergo division. This alone makes the process of virus replication distinct from the growth of all other biological agents, and although the term 'grow' is sometimes used in the vernacular to refer to propagation of viruses, it is best to avoid this word when referring to the processes of virus replication.

Although this article will attempt to paint a general picture of the process of virus replication, the type of host cell infected by the virus has a profound effect on the replication process. There are many examples of viruses undergoing different replicative cycles in different cell types. However, the coding capacity of the genome determines the basic replication strategy used by different viruses. This strategy may involve heavy reliance on the host cell, in which case the virus genome can be very compact and need only encode the essential information for a few proteins, for instance, in parvoviruses. Alternatively, large and complex virus genomes, such as those of poxviruses, encode most of the information necessary for replication, and the virus is only reliant on the cell for the provision of energy and the apparatus for macromolecular synthesis, such as ribosomes. Viruses with RNA genomes have no apparent need to enter the nucleus, although during the course of replication, some do. DNA viruses, as might be expected, mostly replicate in the nucleus where host cell DNA is replicated and the biochemical apparatus necessary for this process is located. However, some viruses with DNA genomes (e.g., poxviruses) have evolved to contain sufficient biochemical capacity to be able to replicate in the cytoplasm, with minimal requirement for host cell functions.

Virus replication can be divided into eight stages, as shown in **Figure 1**. It should be emphasized that these are arbitrary divisions, used here for convenience in explaining the replication cycle of a theoretical, 'typical' virus. Regardless of their hosts, all viruses must undergo each of these stages in some form to successfully complete their replication cycle. Not all the steps described here are detectable as distinct stages for all viruses; often they blur together and appear to occur almost simultaneously. Some of the individual stages have been studied in great detail and a considerable amount of information is known about them. Other stages have been much harder to study, and less information is available.

Attachment

The attachment phase of replication comprises specific binding of a virus-attachment protein (or 'antireceptor') to a cellular receptor molecule. Virus receptors on cell surfaces may be proteins (usually glycoproteins) or carbohydrate residues present on glycoproteins or glycolipids. Some complex viruses (e.g., in the *Poxviridae* or *Herpesviridae*) use more than one receptor and therefore have alternative routes of uptake into cells. Most bacteriophage

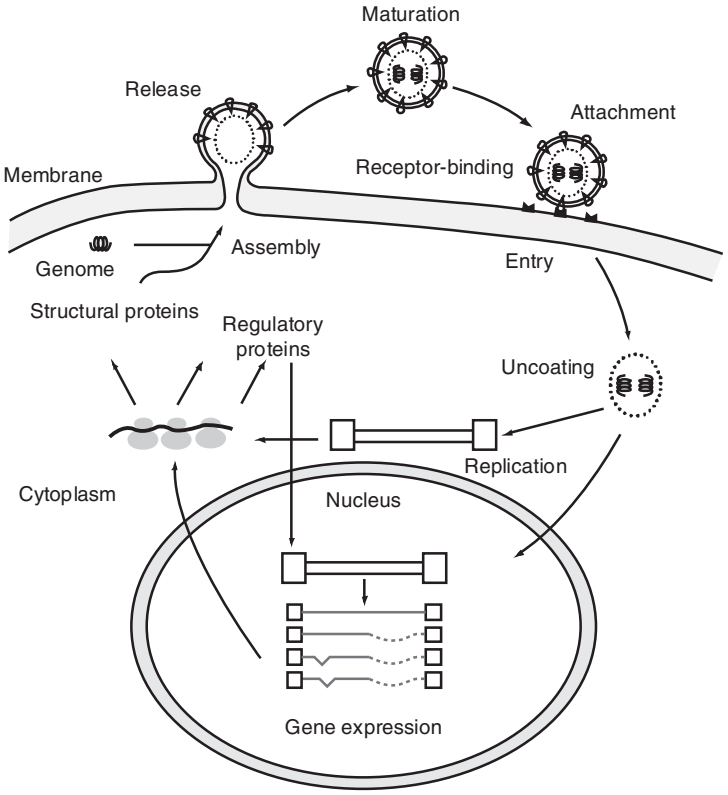


Figure 1 Schematic overview of a generalized scheme of virus replication. Reproduced from Cann AJ (2004) *Principles of Molecular Virology*, 4th edn. Amsterdam: Elsevier, with permission from Elsevier.

receptors are on the bacterial cell wall, although certain phages use cellular appendages (pili, flagella) as primary adsorption sites. Attachment is an automatic docking process and the kinetics of receptor binding are controlled by the chemical and thermodynamic characteristics of the molecules involved, that is, their relative concentrations and availability.

In most cases, the expression (or absence) of receptors on the surface of host cells determines the tropism of a particular virus, that is, the types of cell in which it is able to replicate. The attachment phase of infection therefore has a major influence on viral pathogenesis and in determining the course of a virus infection. Plant viruses must overcome different problems to animal viruses in initiating infection. The outer surfaces of plants are composed of protective layers of waxes and pectin, and each cell is surrounded by a thick wall of cellulose overlying the cytoplasmic membrane. No known plant virus uses a specific cellular receptor of the type that animal and bacterial viruses use to attach to cells and plant viruses must rely on mechanical breaks in the cell wall to directly introduce a virus particle into a cell.

Some virus receptors consist of more than one protein and multiple interactions are required for virus entry. An example of this is human immunodeficiency virus-1 (HIV-1), the primary receptor for which is the T-cell antigen, CD4. The binding site for the HIV-1 attachment protein (antireceptor), gp 120, has been mapped to the first variable region of CD4, although additional amino acids of the second variable domain also contribute toward binding. The sequences important for CD4 binding have also been mapped in gp120. Deletions in this region or site substitutions abolish CD4 binding. In addition to CD4, there is at least one accessory factor which is necessary to form a functional HIV-1 receptor. These factors have now been identified as a family of proteins known as β-chemokine receptors. Multiple members of this class of proteins have been shown to play a role in the entry of HIV-1 into cells, and their distribution in the body is the primary control for the tropism of HIV-1 for different cell types.

Occasionally, the specificity of receptor binding can be subverted by nonspecific interactions between virus particles and host cells. Virus particles may be taken up by cells by pinocytosis or phagocytosis. However, without some form of physical interaction which holds the virus particle in close association with the cell surface, the frequency of these events would be very low. In addition, the fate of viruses absorbed into endocytic vacuoles is usually to be degraded, except in cases where the virus particle enters cells by this route. On occasion, binding of antibody-coated virus particles to Fc receptor molecules on the surface of monocytes and other blood cells can

result in virus uptake. The presence of antiviral antibodies can result in increased virus uptake by cells and increased pathogenicity, rather than virus neutralization, as would normally be expected. The significance of such mechanisms *in vivo* is not known.

Entry

Entry of the virus particle into the host cell normally occurs a short time after attachment of the virus to the receptor. Unlike attachment, cell entry is generally an energy-dependent process, that is, the cell must be metabolically active for this to occur. Three main mechanisms are observed:

1. Translocation of the entire virus particle across the cytoplasmic membrane of the cell. This process is relatively rare among viruses and is poorly understood. It is mediated by proteins in the virus capsid and specific membrane receptors.
2. Endocytosis of the virus into intracellular vacuoles. This is probably the most common mechanism of virus entry into cells. It does not require any specific virus proteins (other than those already utilized for receptor binding) but relies on the normal formation and internalization of coated pits (term to be explained) at the cell membrane. Receptor-mediated endocytosis is an efficient process for taking up and concentrating extracellular macromolecules.
3. Fusion of the virus envelope (where present) with the cell membrane, either directly at the cell surface or following endocytosis in a cytoplasmic vesicle. Fusion requires the presence of a specific fusion protein in the virus envelope, for example, influenza A virus hemagglutinin or the transmembrane glycoproteins of retroviruses. These proteins promote the joining of the cellular and virus membranes which results in the nucleocapsid being deposited directly in the cytoplasm. There are two types of virus-driven membrane fusions: pH-dependent and pH-independent.

The process of endocytosis is almost universal in animal cells and requires the formation of clathrin-coated pits which results in the engulfment of a membrane-bounded vesicle by the cytoplasm of the cell. At this point, any virus contained within these structures is still cut off from the cytoplasm by a lipid bilayer and therefore has not strictly entered the cell. As endosomes fuse with lysosomes, the environment inside these vessels becomes progressively more hostile as they are acidified and the pH falls, while the concentration of degradative enzymes rises. This means that the virus must leave the vesicle and enter the cytoplasm before it is degraded. There are a number of mechanisms by which this occurs, including membrane fusion and rescue by transcytosis. The release of virus particles from endosomes and their passage into the cytoplasm is intimately connected with (and often impossible to separate from) the process of uncoating.

Uncoating

Uncoating describes the events which occur after host cell entry, during which the virus capsid is partially or completely degraded or removed and the virus genome exposed, usually still in the form of a nucleic acid–protein complex. Uncoating occurs simultaneously with or immediately after entry and is thus difficult to study. In bacteriophages which inject their genome directly into the cell, entry and uncoating are the same process.

The removal of a virus envelope during membrane fusion is the initial stage of the uncoating process for enveloped viruses. Uncoating may occur inside endosomes, being triggered by the change in pH as the endosome is acidified, or directly in the cytoplasm. Entry into the endocytic pathway is a hazardous process for viruses because if they remain in the vesicle too long, they will be irreversibly damaged by low pH or lysosomal enzymes. Hence, some viruses have evolved proteins to control this process; for example, the influenza A virus M2 protein is a membrane channel which allows entry of hydrogen ions into the nucleocapsid, facilitating uncoating. The M2 protein is multifunctional, and also has a role in virus uncoating. In the picornaviruses, penetration of the cytoplasm by exit of virus from endosomes is tightly linked to uncoating. The acidic environment of the endosome causes a conformational change in the particle at around pH 5 that reveals hydrophobic domains not present on the surface of mature virus capsids. These hydrophobic patches interact with the endosomal membrane and form pores through which the RNA genome passes into the cytoplasm of the host cell.

The ultimate product of uncoating depends on the structure of the virus genome/nucleocapsid. In some cases, the resulting structure is relatively simple; for example, picornaviruses have only a small basic protein of approximately 23 amino acids covalently attached to the 5′ end of the RNA genome. In other cases, the virus core which remains is highly complex; for example, in the poxviruses uncoating occurs in two stages – removal of the outer membrane as the particle enters the cell and in the cytoplasm, followed by further uncoating as the core passes into the cytoplasm. In this case, the core still contains dozens of proteins and at least 10 distinct enzymes.

The structure and chemistry of the nucleocapsid determines the subsequent steps in replication. Reverse transcription can only occur inside an ordered retrovirus core particle and does not proceed to completion with the virus RNA free in solution. Eukaryotic viruses which replicate in the nucleus, such as members of the

Herpesviridae, *Adenoviridae*, and *Polyomaviridae*, undergo structural changes following penetration, but overall remain largely intact. This is important because these capsids contain nuclear localization sequences responsible for attachment to the cytoskeleton and this interaction allows the transport of the entire capsid to the nucleus. At the nuclear pores, complete uncoating occurs and the nucleocapsid passes into the nucleus.

Transcription and Genome Replication

The replication strategy of a virus depends, in large part, on the structure and composition of its genome. For viruses with RNA genomes in particular, genome replication and transcription are often inextricably linked, and frequently carried out by the same enzymes. Therefore, it makes most sense to consider both of these aspects of virus replication together.

Group I: Double-Stranded DNA

This class can be further subdivided into two as follows:

1. Replication is exclusively nuclear or associated with the nucleoid of prokaryotes. The replication of these viruses is relatively dependent on cellular factors. In some cases, no virus-encoded enzymes are packaged within these virus particles as this is not necessary, whereas in more complex viruses numerous enzymatic activities may be present within the particles.
2. Replication occurs in cytoplasm. These viruses have evolved (or acquired from their hosts) all the necessary factors for transcription and replication of their genomes and are therefore largely independent of the cellular apparatus for DNA replication and transcription. Because of this independence from cellular functions, these viruses have some of the largest and most complex particles known, containing many different enzymes.

Group II: Single-Stranded DNA

The replication of these virus genomes occurs in the nucleus, involving the formation of a double-stranded intermediate which serves as a template for the synthesis of new single-stranded genomes. In general, no virus-encoded enzymes are packaged within the virus particle since most of the functions necessary for replication are provided by the host cell.

Group III: Double-Stranded RNA

These viruses all have segmented genomes, as each segment is transcribed separately to produce individual monocistronic messenger RNAs. Replication occurs in the cytoplasm and is largely independent of cellular machinery, as the particles contain many virus-encoded enzymes essential for RNA replication and transcription since these processes (involving copying RNA to make further RNA molecules) do not normally occur in cellular organisms.

Group IV: Single-Stranded (+)-Sense RNA

These viruses can be subdivided into two groups.

1. *Viruses with polycistronic mRNA such as flaviviruses and picornaviruses.* As with all the viruses in this group, the genome RNA represents mRNA which is translated after infection, resulting in the synthesis of a polyprotein product, which is subsequently cleaved to form the mature proteins.
2. *Viruses with complex transcription such as coronaviruses and togaviruses.* In this subgroup, two rounds of translation are required to produce subgenomic RNAs which serve as mRNAs in addition to the full-length RNA transcript which forms progeny virus genomes. Although the replication of these viruses involves copying RNA from an RNA template, no virus-encoded enzymes are packaged within the genome since the ability to express genetic information directly from the genome without prior transcription allows the virus replicase to be synthesized after infection has occurred.

Group V: Single-Stranded (–)-Sense RNA

The genomes of these viruses can also be divided into two types.

- *Segmented.* The first step in the replication of these viruses (e.g., orthomyxoviruses) is transcription of the (–)-sense RNA genome by the virion RNA-dependent RNA polymerase packaged in virus particles to produce monocistronic mRNAs, which also serve as the template for subsequent genome replication.
- *Nonsegmented.* Monocistronic mRNAs for each of the virus genes are produced by the virus transcriptase in the virus particle from the full-length virus genome. Subsequently, a full-length (+)-sense copy of the genome is synthesized which serves as a template for (–)-sense progeny virus genomes (e.g., paramyxoviruses and rhabdoviruses).

Group VI: Single-Stranded RNA with DNA Intermediate

Retrovirus genomes are composed of (+)-sense RNA but are unique in that they are diploid and do not serve directly as mRNA but as a template for reverse transcription

into DNA. A complete replication cycle involves conversion of the RNA form of the virus genetic material into a DNA form, the provirus, which is integrated into the host cell chromatin. The enzyme reverse transcriptase needs to be packaged into virus particles to achieve this conversion, as virus genes are only expressed from the DNA provirus and not from the RNA genome found in retrovirus particles of retroviruses.

Group VII: Double-Stranded DNA with RNA Intermediate

This group of viruses also relies on reverse transcription, but unlike the retroviruses, this occurs inside the virus particle during maturation. On infection of a new cell, the first event to occur is repair of the gapped genome, followed by transcription. As with group VI viruses, a reverse transcriptase enzyme activity is present inside virus particles, but in this case, the enzyme carries out the conversion of virus RNA into the DNA genome of the virus inside the virus particle. This contrasts with retroviruses where reverse transcription occurs after the RNA genome has been released from the virus particle into the host cell.

Assembly

During assembly, the basic structure of the virus particle is formed as all the components necessary for the formation of the mature virion come together at a particular site in the cell. The site of assembly depends on the pattern of virus replication and the mechanism by which the virus is eventually released from the cell and so varies for different viruses. Although some DNA virus particles form in the nucleus, the cytoplasm is the most common site of particle assembly. In the majority of cases, cellular membranes are used to anchor virus proteins, and this initiates the process of assembly.

For enveloped viruses, the lipid covering is acquired through a process known as budding, where the virus particle is extruded through a cell membrane. Lipid rafts are membrane microdomains enriched in glycosphingolipids (or glycolipids), cholesterol and a specific set of associated proteins. Lipid rafts have been implicated in a variety of cellular functions, such as apical sorting of proteins and signal transduction, but they are also used by viruses as platforms for cell entry (e.g., for HIV-1, SV40, and the rotaviruses), and as sites for particle assembly, budding and release from the cell membrane (e.g., in influenza A virus, HIV, measles virus, and rotaviruses).

As with the earliest stages of replication, it is often not possible to identify the assembly, maturation, and release of virus particles as distinct and separate phases. The site of assembly has a profound influence on all these processes. In general terms, rising intracellular levels of virus proteins and genomes reach a critical concentration and this triggers assembly. Many viruses achieve high levels of newly synthesized structural components by concentrating these into subcellular compartments known as inclusion bodies. These are a common feature of the late stages of infection of cells by many different viruses. Alternatively, local concentrations of virus structural components can be boosted by lateral interactions between membrane-associated proteins. This mechanism is particularly important in enveloped viruses which are released from the cell by budding (see above).

Maturation

Maturation is the stage of the replication cycle at which virus particles become infectious. This often involves structural changes in the newly formed particle resulting from specific cleavages of virus proteins to form the mature products or from conformational changes in proteins which occur during assembly (e.g., hydrophobic interactions). Protein cleavage frequently leads to substantial structural changes in the capsid. Alternatively, internal structural alterations, for example, the condensation of nucleoproteins with the virus genome, often result in changes visible by electron microscopy.

Proteases are frequently involved in maturation, and virus-encoded enzymes, cellular proteases or a mixture of the two may be used. Virus-encoded proteases are usually highly specific for particular amino acid sequences and structures, only cutting a particular peptide bond in a particular protein. Moreover, they are often further controlled by being packaged into virus particles during assembly and only activated when brought into close contact with their target sequence by the conformation of the capsid, for example, by being placed in a local hydrophobic environment, or by changes of pH or cation cofactor concentrations inside the particle as it forms. Retrovirus proteases are good examples of enzymes involved in maturation which are under tight control. The retrovirus core particle is composed of proteins from the *gag* gene and the protease is packaged into the core before its release from the cell on budding. During the budding process, the protease cleaves the gag protein precursors into the mature products – the capsid, nucleocapsid, and matrix proteins of the mature virus particle. Other protease cleavage events involved in maturation are less closely controlled. Influenza A virus hemagglutinin must be cleaved into two fragments (HA_1 and HA_2) to be able to promote membrane fusion during infection. Cellular trypsin-like enzymes are responsible for this process, which occurs in secretory vesicles as the virus buds into them prior to release at the cell surface; however, this process is

controlled by the virus M2 protein, which regulates the pH of intracellular compartments in influenza virus-infected cells.

Release

For lytic viruses (most nonenveloped viruses), release is a simple process – the infected cell breaks open and releases the virus. The reasons for lysis of infected cells are not always clear, but virus-infected cells often disintegrate because viral replication disrupts normal cellular function, for example, the expression of essential genes. Many viruses also encode proteins that stimulate (or in some cases suppress) apoptosis, which can also result in release of virus particles.

Enveloped viruses acquire their lipid membrane as the virus buds out of the cell through the cell membrane, or into an intracellular vesicle prior to subsequent release. Virion envelope proteins are picked up during this process as the virus particle is extruded. This process is known as budding. As mentioned earlier, assembly, maturation, and release are usually simultaneous processes for viruses which are released by budding. The release of mature virus particles from their host cells by budding presents a problem in that these particles are designed to enter, rather than leave, cells. Certain virus envelope proteins are involved in the release phase of replication as well as in receptor binding. The best-known example of this is the neuraminidase protein of influenza virus. In addition to being able to reverse the attachment of virus particles to cells via hemagglutinin, neuraminidase is also believed to be important in preventing the aggregation of influenza A virus particles and may well have a role in virus release. In addition to using specific proteins, viruses which bud have also solved the problem of release by the careful timing of the assembly-maturation-release pathway. Although it may not be possible to separate these stages by means of biochemical analysis, this does not mean that spatial separation of these processes has not evolved as a means to solve this problem.

Further Reading

Cann AJ (2004) *Principles of Molecular Virology,* 4th edn. Amsterdam: Elsevier.

Freed EO (2004) HIV-1 and the host cell: An intimate association. *Trends in Microbiology* 12: 170–177.

Kasamatsu H and Nakanishi A (1998) How do animal DNA viruses get to the nucleus? *Annual Review of Microbiology* 52: 627–686.

Lopez S and Arias CF (2004) Multistep entry of rotavirus into cells: A Versaillesque dance. *Trends in Microbiology* 12: 271–278.

Moore JP, Kitchen SG, Pugach P, and Zack JA (2004) The CCR5 and CXCR4 coreceptors – central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS Research and Human Retroviruses* 20: 111–126.

Rossmann MG, He Y, and Kuhn RJ (2002) Picornavirus–receptor interactions. *Trends in Microbiology* 10: 324–331.

Schneider-Schaulies J (2000) Cellular receptors for viruses: Links to tropism and pathogenesis. *Journal of General Virology* 81: 1413–1429.

Reticuloendotheliosis Viruses

A S Liss and H R Bose Jr., University of Texas at Austin, Austin, TX, USA

© 2008 Elsevier Ltd. All rights reserved.

History

The reticuloendotheliosis viruses are a small group of avian retroviruses that are distinct from the avian leukosis and sarcoma viruses. The founding member of this family, reticuloendotheliosis virus strain-T (REV-T), was isolated in 1958 from lesions of a turkey that died with visceral reticuloendotheliosis. While other members of this family are also pathogenic, REV-T is unique in that it is acutely transforming due to its acquisition of the v-*rel* oncogene. Although periodic outbreaks of reticuloendotheliosis virus infections occur in commercial bird flocks they do not represent a major economic impact. The study of these viruses in the laboratory has provided insight into the mechanism of retroviral replication and has defined a role for Rel/NF-κB proteins in cancer.

Taxonomy and Classification

Reticuloendotheliosis viruses belong to the *Retroviridae* family of viruses. These single-stranded RNA viruses replicate through a DNA intermediate that is integrated into the host genome. The reticuloendotheliosis viruses belong to the subfamily *Orthoretrovirinae* and the genus *Gammaretrovirus*. Members of the reticuloendotheliosis virus family include REV-T, reticuloendotheliosis-associated virus (REV-A), Trager duck spleen necrosis

virus (TDSNV), and chick syncytial virus (CSV). The reticuloendotheliosis viruses belong to a single interference group and can be divided into three distinct antigenic groups (REV-A, TDSNV, and CSV) based on their reactivity with monoclonal antibodies.

Reticuloendotheliosis viruses are more closely related to what have been referred to historically as mammalian type C and simian type D retroviruses than they are to other avian retroviruses. Analysis of their morphology by electron microscopy revealed that they exhibit morphology similar to that of mammalian type C retroviruses. Consistent with this classification, the reverse transcriptase encoded by reticuloendotheliosis viruses exhibits a preference for Mn^{2+} rather than Mg^{2+}. The envelope protein of reticuloendotheliosis viruses share 42% identity with the envelope proteins of simian type D retroviruses and some type C viruses, such as baboon endogenous virus (BaEV). However, the Gag and Pol sequences of reticuloendotheliosis viruses are homologous to those of type C retroviruses but are distinct from type D viruses. In addition, reticuloendotheliosis viruses utilize the same cellular receptor as mammalian type C (RD-114, BaEV, and HERV-W) and simian type D (SRV-1, -2, -3, -4, and -5) retroviruses. Their homology to mammalian retroviruses has led to the hypothesis that the reticuloendotheliosis viruses arose from a common ancestor of mammalian type C viruses that adapted to avian species.

Properties of the Virion

The morphology of reticuloendotheliosis viruses is similar for all members. The viral particles are enveloped, with surface projections that are approximately 6 nm long and an overall diameter of 110 nm. These projections are tapered, with a diameter of 10 nm at the top and 4 nm at the viral membrane. Each particle contains approximately 100 of these projections that are spaced 14 nm apart (center to center). The cores of mature virions are 67 nm in diameter. Reticuloendotheliosis virus particles exhibit a buoyant density of 1.16–1.18 g cm^{-3} in sucrose gradients. The morphology and buoyant density of these viruses are comparable to other gamma retroviruses.

Properties of the Genome

The genomic RNA purified from reticuloendotheliosis virus particles forms a 60–70S complex, which can be dissociated into two identical 35S subunits. The entire genomes of two replication-competent members of this group, REV-A and TDSNV, have been sequenced. Their genomes share 94% identity and are approximately 8.5 kb in length (**Figure 1(a)**). The 5′ and 3′ ends of the genomic RNA contain unique sequences termed U5 and U3, respectively. An additional sequence, R, is also located at both ends and aids in viral replication. The virus contains the *gag*, *pol*, and *env* genes which encode the viral structural proteins, polymerase, and envelope proteins, respectively. In addition, an internal ribosome entry site (IRES) has been identified immediately upstream of *gag*. The replication-defective member, REV-T, was derived from REV-A and contains extensive deletions in the *gag* and *pol* genes (**Figure 2**). In addition, the *env* sequences have almost entirely been replaced with the v-*rel* oncogene in REV-T. These alterations result in a smaller genome of 5.7 kb.

Properties of the Proteins

The three genes encoded by replication-competent reticuloendotheliosis viruses encode polyproteins that comprise the virion structure, as well as those required for viral replication (**Figure 1(b)**). The *gag* gene product, $pr60^{gag}$, is a polyprotein that is subsequently cleaved to form the matrix protein (MA) p12, a protein with an unknown function pp18/pp20, the capsid protein (CA) p30, and the nucleocapsid protein (NC) p10. The Pol polyprotein encodes three products: protease (PR) p15, reverse transcriptase (RT) p84, and integrase (IN) p44. The *env* gene encodes two proteins present in the envelope of the virion: the surface glycoprotein (SU) gp90 and the transmembrane glycoprotein (TM) gp20. The replication-defective REV-T does not encode functional *gag*, *pol*, or *env* gene products. The v-*rel* oncogene expressed by this virus encodes a 59 kDa protein that is a member of the Rel/NF-κB family of transcription factors.

Replication

The replication of reticuloendotheliosis viruses employs a mechanism similar to that of other simple retroviruses. Reticuloendotheliosis viruses utilize a sodium-dependent neutral amino-acid transporter (RDR, SLC1A5, ATB^0) on the surface of susceptible cells as a receptor for infection. The binding of virions to the receptor is mediated by SU. While multiple domains throughout SU are required for this interaction, Asp192 is essential and may form a hydrogen bond with the receptor. TDSNV exhibits a pH-independent mechanism for entry that is likely to involve the direct fusion of the viral and cellular membranes. Once in the cytoplasm of the cell, the RT present in the uncoated viral core begins the process of copying the genomic RNA into a double-stranded cDNA. The first strand synthesis of viral DNA is primed by $tRNA^{Pro}$ binding to sequences 3′ of the U5 region. RT synthesizes a

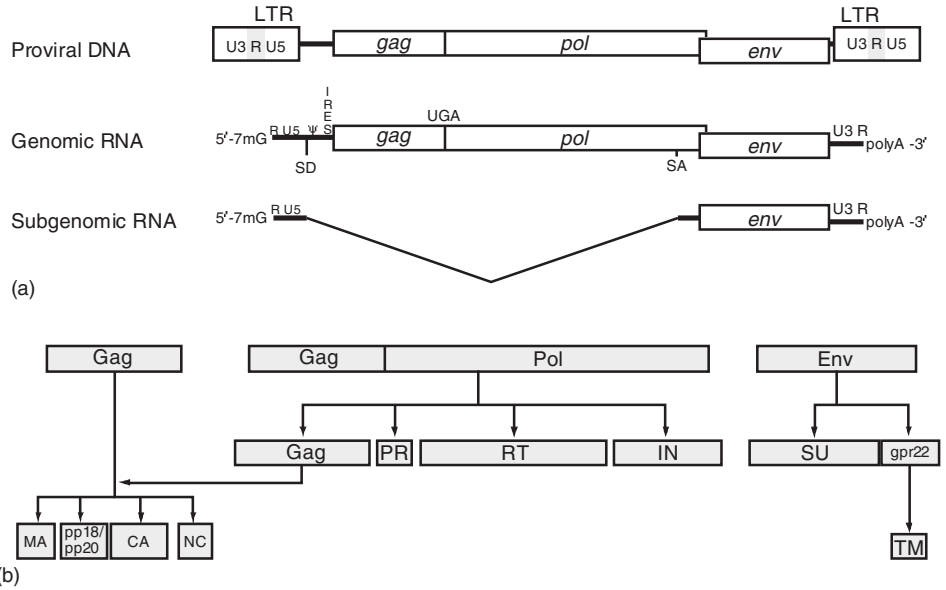


Figure 1 Structure of reticuloendotheliosis virus proviral DNA, RNAs, and proteins. (a) A schematic representation of the proviral DNA of a reticuloendotheliosis virus is shown at the top. The locations of the 5′ and 3′ LTRs are indicated. The relative locations of the U3, R, and U5 sequences of the LTR are also shown. Sequences encoding the *gag*, *pol*, and *env* genes are represented by white boxes. The *env* gene is offset from the *pol* gene to indicate that the coding sequences for these genes overlap. The structure of the viral genomic RNA is indicated in the middle. This RNA is capped at the 5′ end (7mG) and is polyadenylated (polyA) at the 3′ end. The locations of the packaging signal (ψ) and the internal ribosome entry site (IRES) at the 5′ end of the transcript are shown. Regions of the transcript that contain the U5, R, and U3 sequences are noted. The position of the amber stop codon (UGA) that is suppressed to form the gag-pol polyprotein is shown. The positions of the the splice donor (SD) and the splice acceptor (SA) utilized in the formation of the subgenomic RNA are indicated. A schematic of the viral subgenomic RNA is shown at the bottom. This transcript encodes *env* and lacks the sequences for ψ, IRES, *gag* and *pol*. (b) The protein products of the replication-competent reticuloendotheliosis viruses are shown. The Gag and Gag-Pol polyproteins are translated from the viral genomic RNA and Env is translated from the subgenomic RNA. The cleavage products of these polyproteins are indicated.

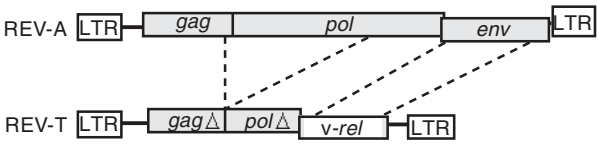


Figure 2 Structural differences between REV-A and REV-T. A schematic representation of the proviral DNA of the replication-competent REV-A is shown. The location of the 5′ and 3′ LTRs as well as the *gag*, *pol*, and *env* genes are indicated. The structural alterations which occurred during the formation of REV-T are indicated below. A nonhomologous recombination event occurred between the *env* sequences of REV-A and turkey c-*rel*, resulting in the formation of the v-*rel* oncogene (white box). The *env* sequences that encode for 11 N-terminal and 18 C-terminal amino acids of v-Rel are indicated by the gray boxes. The 3′ end of *gag* (*gag*Δ) and a large segment of *pol* (*pol*Δ) are deleted in REV-T relative to REV-A. Due to the deletions of *gag*, *pol*, and *env* sequences, REV-T is replication-defective and requires that these proteins be supplied in *trans* by a helper virus, such as REV-A, or a packaging cell line.

cDNA encompassing U5 and R from the 5′ end of the viral genomic RNA. The viral RNA circularizes and the polymerase 'jumps' to the 3′ end of the genomic RNA, a process aided by the complementary R sequences. During synthesis of the cDNA the RNaseH activity of the RT degrades the viral RNA template. A portion of the viral RNA resistant to RNaseH activity serves as a primer for second strand DNA synthesis by RT. The synthesis of viral cDNA results in the duplication of the sequences located at the ends of viral genomic RNA, forming a pair of long terminal repeats (LTRs). Both circular and linear copies of viral DNA can be found in the cytoplasm of recently infected cells. The linear proviral DNAs (**Figure 1(a)**) are transported to the nucleus, where they are randomly integrated into the host genome by the virally encoded IN.

Two RNAs are transcribed from a promoter located in the 5′ LTR of the integrated proviral DNA, an 8.5 kb genomic-length RNA, and a 1.9 kb spliced, subgenomic RNA (**Figure 1(a)**). The genomic RNA encodes Gag and Gag-Pol proteins. Although the 5′ end of the genomic RNA has a 7mG cap, an IRES immediately upstream of *gag* is likely utilized for translation of *gag*. Synthesis of the Pol polyprotein requires the suppression of an amber stop codon at the 3′ end of *gag*. The resulting 200 kDa Gag-Pol protein is proteolytically processed by PR, releasing the Gag and Pol polypeptides.

The subgenomic viral RNA encodes the envelope proteins. These are synthesized as an intracellular precursor

protein, $gPr77^{env}$, which is glycosylated with high mannose carbohydrates. This precursor is also myristylated, directing the envelope proteins to the cellular membrane. This precursor is further glycosylated with more complex carbohydrates to form $gPr115^{env}$. These complex carbohydrates are larger (14–17 kDa) than those found in the Env proteins of MuLV and RSV (3–8 kDa). $gPr115^{env}$ is rapidly cleaved to form the envelope proteins gp90 and gPr22, which are incorporated into the virion.

Viral nucleocapsid assembly occurs in the cytoplasm of cells. This involves the formation of a ribonucleoprotein (RNP) complex composed of viral RNA dimers and 63 kDa Gag precursors. Dimerization of viral genomic RNA is mediated by the dimer linkage structure (DLS), which is located in a 180 nt region between the splice donor (SD) and *gag*. The 5′ end of this sequence forms a double hairpin structure that serves as the packaging signal for the encapsidation of viral RNA into the virion. The presence of this sequence between SD and *gag* ensures that only full-length genomic viral RNA is packaged into virions. The binding of the NC domain of Gag to the DLS facilitates the dimerization of the genomic RNA and is responsible for the specificity of viral RNA packaging. The myristylated MA of the Gag precursor directs the assembled viral nucleocapsids to the cell membrane, where they associate with the viral envelope proteins and eventually are released from the cell by budding.

The virus undergoes a final maturation step at the time of budding or shortly thereafter. The 63 kDa Gag precursor is cleaved by PR, resulting in the appearance of mature CA proteins in the virion. This temporally correlates with the condensation of virion cores from 73 nm during budding to 67 nm shortly after release from the cell. In addition, the transmembrane envelope protein gPr22 is further cleaved to form gp20, which has the fusagenic properties of TM. Release of progeny virus particles can be detected 24 h post infection.

Host Range and Transmission

Outbreaks of reticuloendotheliosis viruses have been reported in geographically diverse regions of the world, including the United States, Australia, and Israel. These viruses infect a wide range of avian species, such as chickens, turkeys, ducks, geese, pheasants, peafowl, prairie chickens, grouse, Japanese quail, and Hungarian partridges. Reticuloendotheliois viruses also infect and replicate in a variety of cultured cell types including fibroblasts and lymphoid cells from avian species and a dog osteosarcoma-derived cell line. The ability of these viruses to infect human cells is controversial; however, the more rigorous studies suggest productive infections do not occur. The failure of these viruses to replicate in certain mammalian cells is not due to an inability to infect these cells, but rather to a restriction in their replication cycle.

Reticuloendotheliosis viruses are most commonly transmitted horizontally within a flock, although vertical and germ line transmission of the virus has been reported. Mosquitoes have also been suggested as a vector for transmission. Reticuloendotheliosis viruses are able to integrate into the genomes of two DNA viruses, Marek's disease virus and fowlpox virus. Some of these integration events retain a full-length infectious provirus, suggesting that DNA viruses containing integrated reticuloendotheliosis provirus may provide a further mechanism for the transmission of reticuloendotheliosis viruses.

Immune Response

The immune response to infection by replication-competent reticuloendotheliosis viruses is dependent on the developmental stage at which the host is exposed to the virus. Infection of embryos induces a tolerant infection in which the bird exhibits a persistent viremia, but lacks antibody production against the virus. Tolerant infections are less common when birds are infected after hatching, and infection of day-old hatchlings generally results in the presence of virus-specific antibody 2–10 weeks post infection. The appearance of virus-specific antibodies in these birds temporally correlates with a decrease in the level of viremia. In addition to this humoral immune response, birds infected with reticuloendotheliosis virus exhibit a cell-mediated response against the infection. A cytotoxic T-lymphocyte (CTL) response mediated by $CD4^{-}/CD8^{+}$ $\alpha\beta$ T cells is observed in reticuloendotheliosis virus-infected birds 7 days after infection and persists for at least 21 days. CTLs mount the primary cell-mediated immune response against a viral infection, and lyse cells presenting viral antigens on their surface in the context of MHC class I molecules.

Pathogenesis

Non-Neoplastic Diseases

Reticuloendotheliosis viruses rapidly induce a severe, but transient immunosuppressed state in infected birds that likely plays an important role in the diseases caused by these viruses. This immunosuppression is characterized by a limited immune response to foreign antigens and a delay in the rejection of allogenic skin grafts in infected birds. Further, splenic lymphocytes from these birds fail to proliferate in response to T-cell mitogens and alloantigens *in vitro*. This immunosuppression does not

appear to affect humoral immunity, since antisera specific to the infecting virus is produced shortly after infection. REV-T- and REV-A-mediated immunosuppression is due to the activation of a suppressor cell population in the spleen of infected birds. This suppressor cell population does not affect the cytotoxic responses of T cells, but rather suppresses their proliferation. The identity of the suppressor cell and the mechanism(s) by which it is activated are unknown.

The reticuloendotheliosis viruses induce a variety of diseases, including a visceral reticuloendotheliosis, spleen necrosis, hepatomegaly, splenomegaly, thymic and bursal atrophy, lymphoid nerve lesions, anemia, runting, and abnormal feather development. The mechanism(s) by which these diseases are induced is largely unknown. Syncytia formation has been observed in cultured cells infected with CSV and may contribute to some of the pathology. The runting syndrome observed in REV-A and REV-T infected chickens is not due to a reduced consumption of food, but rather to an alteration in metabolism. One such metabolic change that may at least partially account for runting is a decrease in phosphoenolpyruvate carboxykinase activity in the liver of infected birds. This enzyme is a key regulator of gluconeogenesis, and its reduced activity may be responsible for depletion of carbohydrate reserves in these birds. Runting and bursal atrophy in chickens have been linked to the structural genes of the virus. Studies performed by domain swapping with REV-A and the less pathogenic CSV demonstrated that both *gag* and *env* of REV-A are required for its full pathogenic effect.

It is possible that Gag proteins play an indirect role in pathogenicity. Infection of cultured cells with reticuloendotheliosis viruses induces a transient cytopathic effect that correlates with the appearance of large amounts of unintegrated and integrated viral DNA. The cells that overcome this cytopathic effect lack unintegrated viral DNA and have a reduced number of integrated proviruses. The transient nature of both the viral DNA accumulation and the cytopathic effect suggests that cell death occurs due to the high levels of this DNA or its encoded proteins. In some cell types, the MA portion of Gag provides a replicative advantage to REV-A relative to viruses containing the MA of less pathogenic reticuloendotheliosis viruses. It is possible that the higher level of virus production provided by the Gag of REV-A is cytotoxic to cells, resulting in some of these syndromes.

Neoplastic Diseases

Replication-competent reticuloendotheliosis viruses can induce both B-cell and T-cell lymphomas. CSV induces B-cell lymphomas after a relatively long latency period (6–10 months) that are indistinguishable from those induced by avian leukosis virus (ALV). Like ALV, these lymphomas are caused by insertional activation of c-*myc*. Proviral insertions are located upstream of the second exon of c-*myc*, with the vast majority lying between the first and second exons. The 5′ LTR of these proviral insertions are either deleted or rendered inactive by deletion of an enhancer sequence located 5′ of the *gag* sequence. T-cell lymphomas in CSV-infected birds have been observed after a relatively short latency period (6 weeks). These lymphomas are derived from thymic T cells and also develop from the insertional activation of c-*myc*. As in the B-cell lymphomas, these proviral integrations reside upstream of exon 2. However, 60% of these integrations are found 5′ of exon 1 and half of the proviruses insert in the opposite transcriptional orientation of c-*myc*. The induction of c-*myc* in many of these tumors appears to be through enhancer activation of a cryptic promoter in exon 1.

Transformation by REV-T

REV-T is the acutely transforming member of the reticuloendotheliosis virus family, inducing fatal lymphomas in experimentally infected birds after just 7–10 days. Large lesions in the spleen and smaller lesions in the liver are indicative of these lymphomas. REV-T transforms cells of various lymphoid lineages, including immature pre-B/pre-T cells, immature and mature B cells, T cells, myeloid cells, and predendritic cells. Although not a target cell for transformation *in vivo*, cultured chicken embryo fibroblasts (CEFs) are also readily transformed by REV-T. REV-T transformed cells are highly malignant and injection of only 100 non-virus-producing cells can induce a fatal lymphoma in young birds. In addition, transformed CEFs injected into the wing web of day-old chicks induces sarcomas at the site of injection. The ability of REV-T to transform cells is due to the presence of the v-*rel* oncogene. However, its transformation potential is not limited to avian cells. Transgenic mice that express v-*rel* under the control of the T-cell-specific *lck* promoter develop multiple lymphomas that are localized in enlarged spleens and livers.

The v-*rel* oncogene is formed through a recombination event between the envelope sequences of REV-A and turkey c-*rel* sequences. c-Rel is a member of the evolutionarily conserved Rel/NF-κB family of transcription factors. All members of this family contain a highly conserved 300 amino acid region located in the N-termini of these proteins termed the Rel homology domain (RHD). Rel/NF-κB proteins are normally sequestered in inactive complexes in the cytoplasm of cells by a family of inhibitory proteins referred to as IκBs. Upon proper extracellular stimulation, IκBs are phosphorylated, ubiquitinated, and ultimately degraded, allowing for the nuclear translocation of active Rel/NF-κB complexes. In the nuclei of cells, homo- and heterodimers of Rel/NF-κB proteins bind to

a 10-bp DNA sequence (κB site) to regulate the expression of genes involved in the immune response, differentiation, proliferation, and stress responses. v-Rel transforms cells by evading the normal regulation of the Rel/NF-κB pathway, leading to the inappropriate activation or suppression of Rel/NF-κB regulated genes.

v-Rel complexes involved in transformation

The transformation potential of v-Rel is dependent on its ability to form dimers, bind DNA, and activate transcription. The κB binding complexes in the nuclei of v-Rel transformed cells consist of v-Rel homodimers and v-Rel heterodimers containing the endogenous Rel/NF-κB proteins c-Rel, NF-κB1 (p50), and NF-κB2 (p52). Analysis of mutants of v-Rel that are defective in homodimer and/or heterodimer formation with specific Rel/NF-κB family members revealed that the ability of v-Rel to dimerize and bind DNA with endogenous Rel/NF-κB proteins is not in itself sufficient for the transformation of chicken lymphocytes. Furthermore, studies from v-*rel* transgenic mice suggested that v-Rel heterodimeric complexes are not needed for transformation. Nuclear κB binding complexes in tumors from these mice consisted of only v-Rel homodimers and v-Rel/NF-κB1 heterodimers. v-Rel was still capable of inducing tumors when heterodimeric complexes were eliminated or reduced by mating v-*rel* transgenic mice with *nfkb1*$^{-/-}$ mice or *ikba* transgenic mice.

The characterization of two v-Rel mutants with an impaired ability to form homodimers demonstrated the importance of v-Rel homodimers in transformation. These mutants weakly transformed chicken splenic lymphocytes, and proviral DNA from cells transformed by these mutants contained multiple secondary mutations that enhanced or restored the ability of these v-Rel mutants to bind DNA as homodimers. Viruses expressing v-Rel mutants containing these secondary mutations transformed cells at levels comparable to or slightly less than viruses expressing wild-type v-Rel. These results suggested that a threshold level of DNA binding by v-Rel homodimers is required for transformation.

Mutations in v-Rel

The transduction of c-*rel* into the *env* sequences of REV-A resulted in the removal of sequences encoding the two N-terminal and 118 C-terminal amino acids of c-Rel. The N-terminus of v-Rel contains 11 N-terminal amino acids encoded by the 5′ end of the *env* gene. The 18 C-terminal amino acids of v-Rel are encoded by out-of-frame *env*-derived sequences. v-Rel has also acquired 14 amino acid substitutions and three deletions relative to turkey c-Rel. The majority of these mutations occur in sequences outside of the RHD. In addition, amino acid substitutions have occurred in the N- and C-terminal *env*-derived

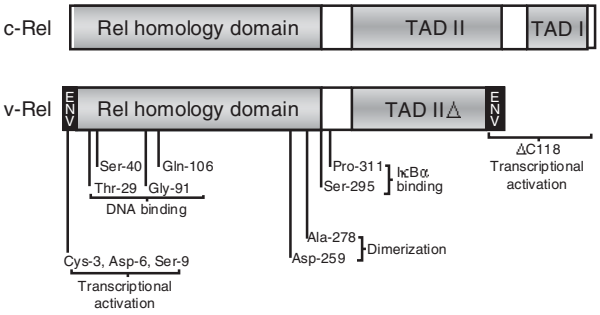


Figure 3 Structural differences between c-Rel and v-Rel. The Rel homology domain is shown as a shaded box. The two transcriptional activation domains of c-Rel (TAD I and TAD II) are similarly indicated. v-Rel is missing TAD I and contains a deletion in TAD II (TAD IIΔ). The 11 N-terminal and 18 C-terminal *env*-derived amino acids (ENV) of v-Rel are indicated by black boxes. Amino acids in v-Rel that differ from c-Rel or the Env of REV-A that alter the biochemical activities of v-Rel are noted. Their influence on DNA binding, transcriptional activation, dimerization, or IκBα binding is also indicated. The serine at amino acid 40 noted in this figure is only found in S2A3 v-Rel, a naturally occurring B-cell tropic variant of v-Rel. Amino acid differences between v-Rel and c-Rel that have no defined effect on the function of v-Rel are not shown.

sequences. The results of extensive studies evaluating the structural and biochemical differences between v-Rel and c-Rel indicate that these mutations modulate the DNA binding, dimerization, IκBα association, and transactivation properties of v-Rel relative to c-Rel (**Figure 3**). By far, the most significant contribution to the transformation potential of v-Rel is the deletion of the 118 C-terminal amino acids of c-Rel, which have transactivation and cytoplasmic retention properties. However, the high transformation potential of v-Rel is not the result of any one mutation or single group of mutations, but rather the functional cooperation of these mutations with one another.

v-Rel target genes

The major focus of v-Rel research in recent years has been the identification and characterization of v-Rel target genes. Although more than 50 genes have been identified with altered expression in cells expressing v-Rel (**Table 1**), few have been analyzed for their role in transformation. What is clear from the study of these genes is that v-Rel transforms cells, at least in part, by efficiently inducing the expression of genes that regulate cell proliferation. One of these, IRF-4, is a member of the interferon regulatory factor family of transcription factors and is elevated in cells expressing v-Rel. Experiments involving the overexpression of IRF-4 with v-Rel and inhibition of IRF-4 expression by antisense technology have identified a key role for IRF-4 in the transformation of both lymphocytes and fibroblasts by v-Rel. IRF-4

Table 1 Genes that exhibit altered expression in cells expressing v-Rel

Functional group	*Genes induced or suppressed by v-Rel*
Rel/NF-κB/IκB family members	c-*rel*, *nf-kb*1, *nf-kb*2, *ikba*
Transcription factors	c-*fos*, c-*jun*, *fra*-2[a], HMG-14b, IRF-1, IRF-3, IRF-4, IRF-8, IRF-10, c-*myb*[a], NAP-1[a], STAT-1
Translation factors	eIF2α[a]
Structural proteins	Actin[a], ARP, β-tubulin[a], CAP-23, δ-crystallin, type I collagen α[a], vimentin
Signal transduction	TC10, *rho*C, *rac*1, *sh3bgrl*[a], *sh3bgrl2*, *sh3bgrl3*
Inhibitors of apoptosis	ch-IAP1, NR13, TERT, TR
Cytokines	IFN1, IL-6[b], IL-8, *mip*-1β, c*tca*, TNF-α[b]
Cell surface proteins	DM-GRASP[c], IFNaR1, IFNaR2, IL-2R, MHC Class I and II, p75, Sca-2
Metabolism	iNOS, mitochondrial cytochrome b, OAS, ODC-antizyme, $p40^{phox}$, $p47^{phox}$
Kinases and phosphotases	JAK1, PP2A
Chaperones	GRP-78

[a]Downregulated in cells expressing v-Rel.
[b]Upregulated in T-cell lymphomas from v-*rel* transgenic mice, not tested in avian cells.
[c]Upregulated in T-cell lymphomas from v-*rel* transgenic mice and avian cells transformed by v-Rel.

regulates the expression of components of the interferon signal transduction pathway, and is likely to contribute to v-Rel-mediated transformation by limiting the induction of this antiproliferative pathway. DM-GRASP, an immunoglobulin superfamily adhesion molecule, is also upregulated in v-Rel transformed fibroblasts and lymphoid cells and functions to promote cell proliferation. This was demonstrated by the ability of a monoclonal antibody specific for DM-GRASP to inhibit the proliferation of v-Rel transformed B-cells.

Although enhanced proliferation is a critical step in the malignant transformation of cells, it is not sufficient. Transformation also requires the suppression of apoptotic pathways, and v-Rel induces the expression of a number of genes with known anti-apoptotic activities, including ch-IAP1 and telomerase. ch-IAP1, a member of the inhibitor-of-apoptosis family, is one of the few known direct transcriptional targets of v-Rel. When temperature-sensitive v-Rel transformed cells were placed at the nonpermissive temperature, the expression of ch-IAP1 was reduced and the cells rapidly underwent apoptosis. Ectopic expression of ch-IAP1 in these cells protected them from apoptosis at the nonpermissive temperature, demonstrating that the induction of ch-IAP1 by v-Rel contributes to the immortalization of cells. In addition, v-Rel activates telomerase during transformation by inducing the expression of two of its components, telomerase RNA (TR) and telomerase reverse transcriptase (TERT). Telomerase has a well-defined role in the maintenance of telomere length, and has been more recently demonstrated to protect cells against apoptosis. v-Rel transformed cells rapidly undergo apoptosis when transfected with siRNAs specific for TERT or treated with a chemical inhibitor of telomerase, defining an important anti-apoptotic role for the elevated telomerase activity in v-Rel transformed cells.

Many of the genes regulated by v-Rel participate in diverse signal transduction pathways in normal cells. Although a number of these genes have been implicated in transformation, the mechanism(s) by which they contribute to transformation remains to be elucidated. Members of the AP-1 family are differentially regulated by v-Rel and the overexpression of *supjun-1*, a dominant negative mutant of AP-1 activity, dramatically decreases the transformation efficiency of v-Rel. The chemokine macrophage inflammatory protein-1β (MIP-1β/CCL4) is strongly induced by v-Rel in a variety of cell types, and its overexpression cooperates with v-Rel and c-Rel in promoting the growth of fibroblasts in soft agar. TC10 is a member of the Rho family of small GTPases and its expression and activity are induced by v-Rel. Both TC10 and a gain-of-function mutant synergistically cooperated with v-Rel to transform fibroblasts. Moreover, co-expression of a dominant-negative mutant of TC10 with v-Rel dramatically decreased the transformation of cells by v-Rel. In contrast to the abundance of genes that are upregulated by v-Rel, few genes have been identified that are downregulated by this oncogene. One gene that is strongly downregulated in v-Rel transformed cells encodes SH3BGRL, a member of the SH3BGR (SH3 domain binding glutamic acid-rich) family of proteins. Overexpression of SH3BGRL in primary splenic lymphocytes dramatically inhibits the ability of v-Rel to transform these cells. Furthermore, an intact SH3-binding domain in SH3BGRL is required for this function. Although the precise functions of SH3BGR family members are unknown, these results indicate that the suppression of an SH3-mediated signaling pathway is important for v-Rel-mediated transformation. SH3BGRL is the only downregulated v-Rel target gene to date with a demonstrated role in transformation.

The v-Rel target genes discussed above invariably exhibit a more dramatic change in expression in response to v-Rel than c-Rel. However, the ability of v-Rel to less efficiently activate the expression of certain genes is also critical for its transformation potential. Both v-Rel and c-Rel directly activate the transcription of *ikba*. However, since v-Rel lacks the most potent transactivation domain of c-Rel, it induces *ikba* expression more slowly and to lower levels than does c-Rel. This prevents the rapid relocalization of v-Rel from the nucleus to the cytoplasm than is observed in cells expressing c-Rel.

The identification of v-Rel target genes has highlighted the importance of this viral oncoprotein as a

model system to study the role of Rel/NF-κB proteins in cancer. Many v-Rel target genes, including those with demonstrated roles in v-Rel-mediated transformation, exhibit altered regulation in human cancers linked to deregulated Rel/NF-κB activity. The recent availability of chicken DNA microarrays will greatly expand our knowledge of the pathways altered by v-Rel during transformation and likely provide additional insight into the mechanisms by which Rel/NF-κB proteins participate in neoplastic disease.

See also: Retroviral Oncogenes; Simian Retrovirus D.

Further Reading

Gilmore TD (1999) Multiple mutations contribute to the oncogenicity of the retroviral oncoprotein v-Rel. *Oncogene* 18: 6925–6937.

Witter RL and Fadly AM (2003) Reticuloendotheliosis. In: Saif YM, Larry R, McDougald LR, Barnes JH, Glisson R, and Swayne D (eds.) *Diseases of Poultry*, 11th edn., pp. 517–535. Ames, IA: Iowa State University Press.

Retrotransposons of Fungi

T J D Goodwin, M I Butler, and R T M Poulter, University of Otago, Dunedin, New Zealand

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Retrotransposition The process by which a retrotransposon replicates.
Retrotransposon A eukaryotic mobile genetic element that can replicate via the reverse transcription of an RNA intermediate and the insertion of the resulting DNA into the host genome.
Reverse transcriptase A DNA polymerase that can use both RNA and DNA as a template.

Introduction

Retrotransposons are mobile genetic elements. They are generally regarded as selfish or parasitic entities and appear as inserts within the genomes of their hosts. They can spread through the host genome by copying their RNA transcripts into DNA and inserting these new DNA copies back into the host chromosomes. As this process, retrotransposition, is replicative it results in an increase in the copy number of the retrotransposons. Over time this can result in retrotransposons reaching very high copy numbers and making up a large proportion of the genome. As an example, there are more than 500 000 copies of the L1 retrotransposon in the human genome and these make up about 17% of human DNA.

Much of what is known about retrotransposons and their interactions with their hosts has been derived from studies with fungi. Fungi are a diverse group of eukaryotes of great ecological, economic, medical, and scientific importance. They range from tiny obligate intracellular pathogens, such as *Encephalitozoon cuniculi*, to large, multicellular organisms that can have a mass in excess of 100 kg, such as *Bridgeoporus nobilissimus*. They include such organisms as the bakers' and brewers' yeast *Saccharomyces cerevisiae*, genetic model organisms such as *Neurospora crassa*, plant pathogens such as the causal agent of rice blast disease *Magnaporthe grisea*, human pathogens such as *Candida albicans* and *Cryptococcus neoformans*, sources of antibiotics such as *Penicillium chrysogenum*, and mycorrhiza-forming fungi such as *Glomus intraradices*. The fungi are classified into six divisions: Ascomycota, Basidiomycota, Chytridiomycota, Glomeromycota, Microsporidia, and Zygomycota. The great majority of well-known fungi, such as *S. cerevisiae*, *C. albicans*, and *N. crassa* are ascomycetes. The next largest group is the basidiomycetes, which includes such organisms as the button mushroom *Agaricus bisporus*, the pathogenic yeast *C. neoformans*, as well as rusts and smuts. The chytrids are unusual fungi that produce flagellated motile spores. This group includes the frog pathogen *Batrachochytrium dendrobatidis*. The zygomycetes are characterized by zygospores formed during sexual reproduction. They include *Rhizopus oryzae*, which is commonly found on decaying vegetable matter and can cause fatal infections in humans. The glomeromycetes include the arbuscular mycorrhizal fungi such as *G. intraradices* and *Gigaspora margarita*. These species form symbiotic associations with the roots of many land plants, the fungus supplying the plant with nutrients obtained from the surrounding soil, in exchange for carbohydrates. The last fungal division, the microsporidia, consists of obligate intracellular parasites of animals. These are atypical fungi in that they lack mitochondria and peroxisomes. They include the human pathogen *Encephalitozoon cuniculi*.

Much of the contribution of fungi to our knowledge of retrotransposons has come from the yeast *S. cerevisiae*. The Ty elements found in this species were among the first retrotransposons to be identified and characterized. Indeed, the term retrotransposon was coined following the demonstration that Ty elements replicate (transpose) through an RNA intermediate, in a series of steps essentially the same as that for retroviruses. The widespread use of *S. cerevisiae* as a model for many features of eukaryotic life, due to its ease of culture and experimental manipulation, rapid growth, readily accessible genetics, and small genome size, meant that many tools were available to assist in the analysis of Ty elements. Numerous features that have made *S. cerevisiae* attractive for the study of retrotransposons are also shared with other fungi. In particular, the general small size of fungal genomes has meant that many complete genome sequences, from an evolutionary diverse array of fungi, have been determined in recent years and many more will become available in the near future. Analyses of these genome sequences are ongoing but have already contributed much to our understanding of retrotransposons. Most importantly, the analysis of a diverse range of fungi has uncovered many types of retrotransposons, and interesting interactions between the retrotransposons and their hosts, that are not observed in *S. cerevisiae*, making it clear that study of a wide range of species will be essential for an in-depth understanding of fungal retrotransposons. Here we will outline many of the features of retrotransposons, with special emphasis on the fungal elements. We also describe the impact of these elements on the fungal genome and the ways in which the interactions between the retrotransposons and their hosts have evolved to permit the long-term survival of the elements while minimizing the deleterious impact on their hosts.

Types of Retrotransposons

Retrotransposons come in a wide variety of forms with diverse sequences and diverse replication mechanisms (**Figures 1** and **2**). The one feature common to all retrotransposons is a sequence encoding reverse transcriptase (RT), the enzyme responsible for copying the RNA transcripts into DNA. The RTs of all known retrotransposons are homologous, indicating that they share a common origin. Phylogenetic analyses based upon alignments of RT sequences (**Figure 2**), together with comparisons of other structural and mechanistic features, have been used to classify retrotransposons into various groups. At the broadest level many retrotransposons can be classified into one of two groups. Members of the first group consist of a protein-coding internal region flanked by generally noncoding long terminal repeats (LTRs) and are known as LTR retrotransposons. The Ty elements of *S. cerevisiae* are all LTR retrotransposons. A typical LTR retrotransposon is 5–6 kbp long and contains two protein-coding open reading frames (ORFs), *gag* and *pol* (**Figure 1**). The *gag* ORF encodes a protein, Gag, which forms the major structural component of a virus-like particle (VLP) within which the reverse transcription reactions take place. The *pol* ORF encodes a polyprotein bearing various enzymatic domains: aspartic protease (PR), the RT, ribonuclease H (RH), and integrase (IN). PR is responsible for processing the initial *gag* and *pol* ORF products (see further ahead) into their functional domains. RT copies the RNA transcripts into DNA, assisted by RH. IN is involved in the insertion of the nascent DNA copy into the host genome. The LTRs flanking the protein-coding regions contain signals regulating transcription of the element, and, due to their redundant nature, they allow a full-length DNA copy of the element to be constructed from somewhat less-than-full-length RNA transcripts (see further ahead). LTR retrotransposons are themselves subdivided into further groups, named the Ty1/copia, Ty3/gypsy, and BEL groups, after founding elements from fungi and insects. The members of these groups can be distinguished from each other by sequence comparisons and the order in which the enzymatic domains appear within the *pol* gene. In Ty1/copia elements the *pol* domain order is PR-IN-RT-RH. In BEL and Ty3/gypsy elements (with a few exceptions) the order is PR-RT-RH-IN.

The second major group of retrotransposons are distinguished from LTR retrotransposons by the absence of LTRs, by differences in the set of encoded proteins, and a completely different replication mechanism (see further ahead). This group of retrotransposons has been given various names, including non-LTR retrotransposons, poly-A retrotransposons, long interspersed nuclear elements (LINEs), and retroposons. However, none of these names is ideal: the 'non-LTR' term refers to a feature all these elements lack, rather than one they all share; the 'poly-A' term refers to a run of A residues found at the 3′ end of many of the first elements of the group to be characterized, but subsequently found to be lacking in many other elements; the LINE terminology is not ideal, as many of the members of this group are not interspersed, but are found only in specific regions of the genome. Finally, the term 'retroposon' has been used in the literature to refer to numerous different groupings of retrotransposons and retrotransposon-like elements, so its usefulness has been lost. It has recently been suggested, however, that the elements of this group be referred to as the target-primed (TP) retrotransposons, after their insertion mechanism (target-primed retrotransposition – see further ahead), which is distinct from that of LTR retrotransposons and is likely to be shared by all members of the group. This is the terminology used in this article.

A typical TP retrotransposon consists of one or two protein-coding regions flanked by 5′ and 3′ untranslated

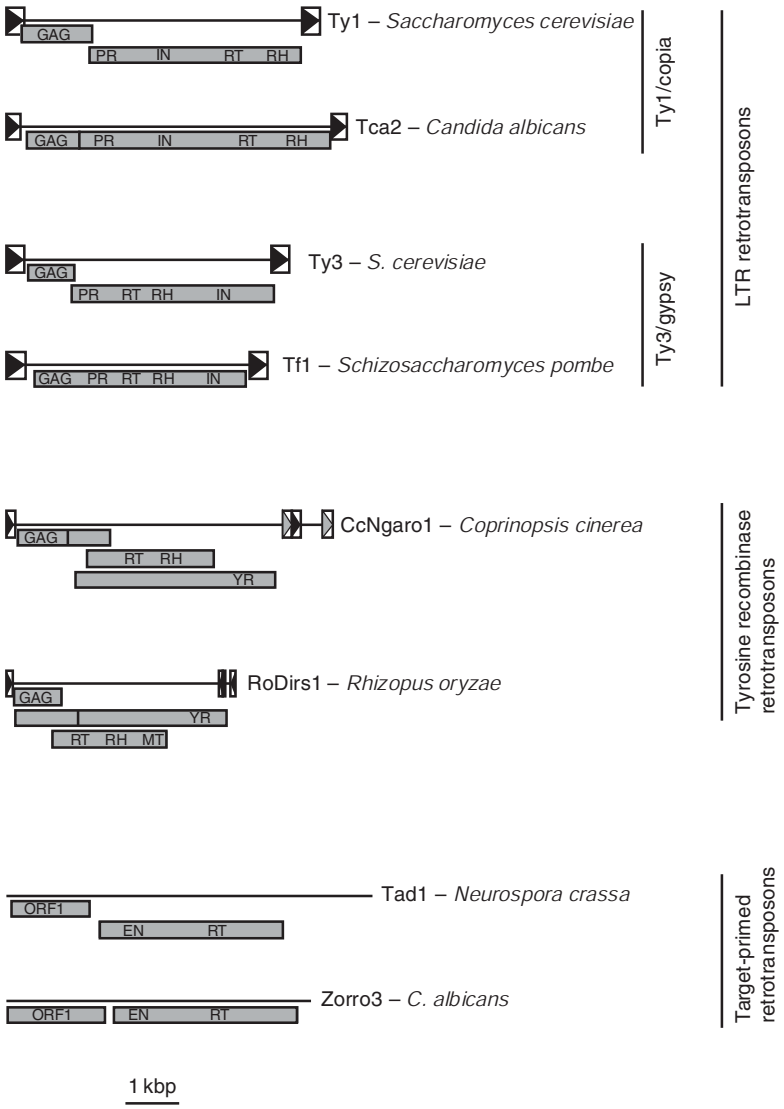


Figure 1 Structures of different types of retrotransposons. The lengths of the elements are indicated by the horizontal lines. Repeat sequences are shown as boxed triangles. Open reading frames are depicted as shaded boxes, with the approximate positions of the various protein-coding domains indicated: EN, endonuclease; IN, integrase; MT, putative methyltransferase; PR, protease; RH, ribonuclease H; RT, reverse transcriptase; YR, tyrosine recombinase. The groups to which the elements belong are shown on the right.

regions (**Figure 1**). All elements encode an RT and an endonuclease (EN). The TP retrotransposons can be divided into 15 or more clades, defined by sequence comparisons and structural distinctions. In members of what are believed to be the older clades, the EN-coding sequence lies downstream of RT and the EN is similar in sequence to certain restriction endonucleases. In the more recently evolved elements, the EN-coding sequence lies upstream of RT and is related to eukaryotic apurinic/apyrimidinic (AP) endonucleases. An RH-coding sequence also makes a sporadic appearance among these latter elements. In the elements with two ORFs, RT and EN are always encoded by the downstream ORF. The upstream ORF, ORF1, is generally poorly conserved. The function of the protein product of ORF1 is unknown, although in some elements ORF1p has been shown to have RNA-binding and nucleic acid-chaperone activities.

In addition to these two major categories of retrotransposons, several other types of retrotransposons have been described. These elements are generally rarer than the LTR and TP retrotransposons and so have not been characterized in as much detail. The first of these additional groups, the tyrosine recombinase (YR) retrotransposons, bear RT- and RH-coding sequences related to

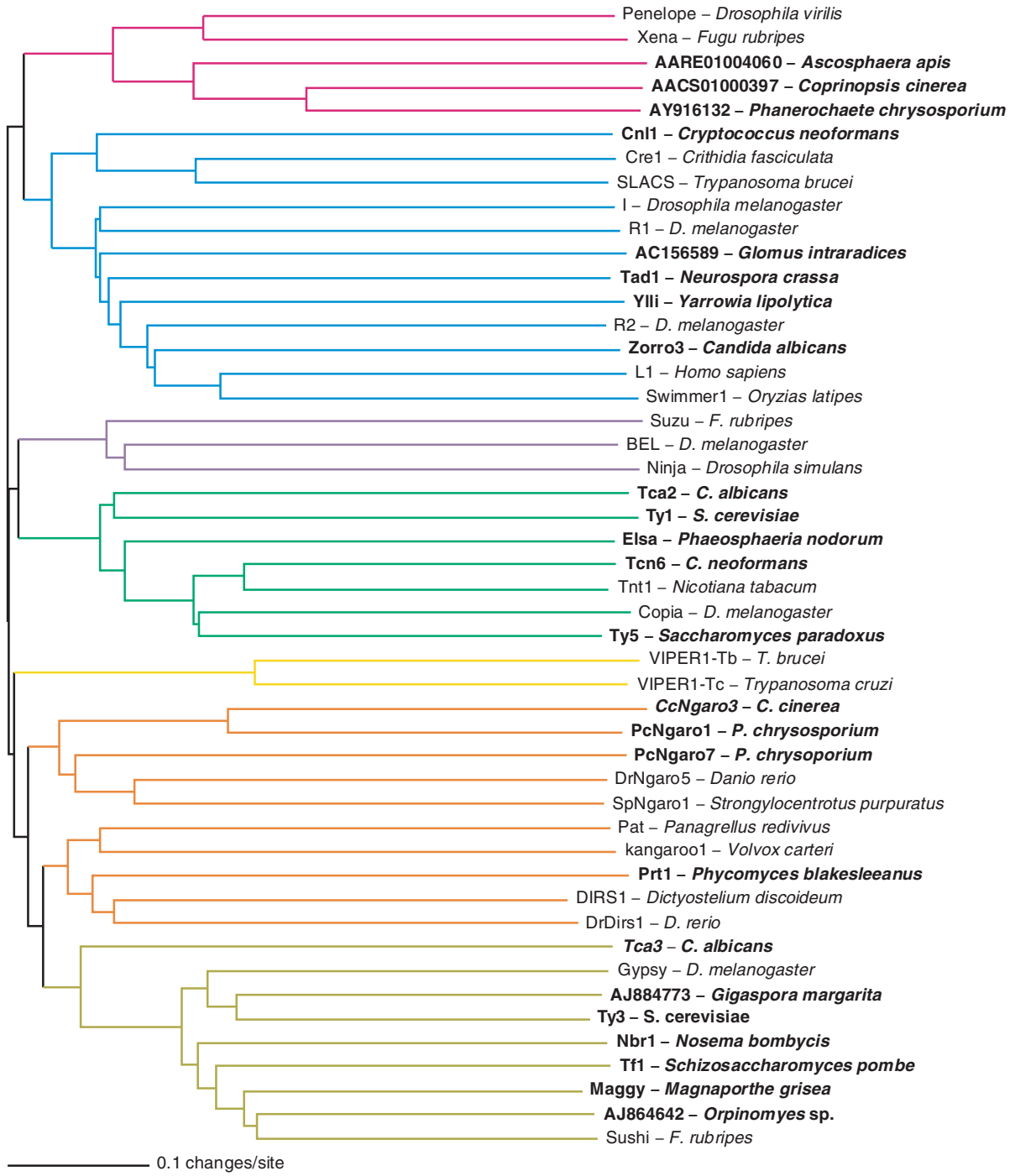


Figure 2 Relationships among retrotransposons. This tree is based on an alignment of RT sequences from a wide variety of retrotransposons. The various groups are indicated in different colors: red, Penelope-like retrotransposons; blue, target-primed retrotransposons; purple, BEL-like LTR retrotransposons; green, Ty1/copia LTR retrotransposons; yellow, VIPER elements; orange, tyrosine recombinase retrotransposons; khaki, Ty3/gypsy LTR retrotransposons. Elements from fungi are in boldface.

those of LTR retrotransposons, but they lack coding sequences for PR and IN. Instead they encode a tyrosine recombinase, related to the recombinase of bacteriophage lambda (**Figure 1**). Like LTR retrotransposons, they also bear flanking repeats, but these are distinct from typical LTRs in that they are either 'split direct repeats' or have a complex inverted-repeat structure. The DIRS1 element from the slime mold *Dictyostelium discoideum* was the first YR retrotransposon to be described. The second group, the Penelope-like

elements (PLEs; named after an element from *Drosophila virilis*), are similar to TP retrotransposons in that they encode an RT and an EN. The EN of PLEs is, however, unrelated to that of TP retrotransposons, but is similar to the Uri endonucleases found in group I introns. PLEs are often found associated with variable terminal repeat sequences in both direct and inverted orientations. Their replication mechanism is not well understood, but may be an unusual form of target-primed retrotransposition. The final category of retrotransposons, the vestigial interposed retroelement (VIPER)-like elements, have been found in only a very small number of species (trypanosomes) to date. They bear RT and RH genes somewhat similar to those of LTR retrotransposons, but otherwise very little is known regarding their conserved features.

Distribution of Retrotransposons

Retrotransposons have only been found in eukaryotic genomes (with the exception of a few rare elements integrated into the genomes of eukaryotic viruses). While RT-encoding elements (group II introns and retrons) do appear in prokaryotes, these elements are not mobile genetic elements in the same sense as the eukaryotic retrotransposons. Among eukaryotes, retrotransposons have been found in almost all genomes that have been examined in detail. However, the diversity of retrotransposons found within particular species varies enormously. For instance, examples of nearly every type of known retrotransposon have been identified in the genome of the zebrafish *Danio rerio*, while only one active retrotransposon, the L1 TP retrotransposon, is known in humans and no retrotransposons of any sort are found in the complete genome sequence of the malaria parasite *Plasmodium falciparum*. Similarly, the distribution of different types of retrotransposons also displays great variation. For instance, members of the Ty3/gypsy class of LTR retrotransposons appear in plants, animals, fungi, and various protists, whereas BEL-like LTR retrotransposons have only been found in animals to date.

Within the fungi, retrotransposons are widespread and abundant (**Table 1**). Most have been found in ascomycetes and basidiomycetes. Only a few have been identified in zygomycetes, glomeromycetes, chytrids, and microsporidia: this is, however, likely to be simply the result of there being less data available for species from these divisions, rather than low numbers of retrotransposons, as those species that have been examined often contain abundant elements. Of the LTR retrotransposons, members of the Ty1/copia group have been identified in a large number of ascomycetes, many of the basidiomycetes that have been examined, and also in one species of chytrid (*Orpinomyces* sp.). None has yet been identified in zygomycetes, glomeromycetes, or microsporidia. Ty3/gypsy elements have been identified in all fungal divisions. They are abundant in many ascomycetes and basidiomycetes and have also been found in microsporidia (*Nosema bombycis* and *Spraguea lophii*), glomeromycetes (*Glomus intraradices* and several species of Gigaspora), chytrids (*Orpinomyces* sp.) and zygomycetes (*R. oryzae*). In many fungi, Ty3/gypsy elements are more abundant than the Ty1/copia elements, suggesting that the former elements have been somewhat more successful in colonizing and persisting in fungal genomes. No BEL-like LTR retrotransposons have been identified, despite the large amounts of sequence data available, suggesting that this group is absent from fungi.

Target-primed retrotransposons are also widespread in fungi with numerous elements again found in ascomycetes and basidiomycetes and examples also identified in zygomycetes (*R. oryzae*), glomeromycetes (*G. intraradices* and some Gigaspora species), and chytrids (*Orpinomyces* sp.). None has yet been identified in microsporidia. Despite their widespread occurrence, TP elements are notably absent from several species for which complete genome sequences are known. In particular, they are absent from *S. cerevisiae* and another model yeast, *Schizosaccharomyces pombe*. This absence from model species has meant that the contribution of fungi to our understanding of the replication mechanisms and other features of TP retrotransposons has been much less than that for LTR retrotransposons.

YR retrotransposons have a more sporadic distribution in fungi than LTR and TP retrotransposons. Examples have been identified in basidiomycetes (*Phanerochaete chrysoporium* and *Coprinopsis cinerea*) and zygomycetes (*Phycomyces blakesleeanus* and *R. oryzae*). None has yet been identified in other divisions. For ascomycetes at least, with the vast amount of sequence data available, this suggests that they are either absent from this division or extremely rare. Sequences related to Penelope retrotransposons are also rare in fungi, but nevertheless, such sequences are evident in at least three fungal divisions: basidiomycetes (*P. chrysosporium* and *C. cinerea*), zygomycetes (*R. oryzae*), and ascomycetes (*Ascosphaera apis*). No elements similar to VIPER have been detected in fungi to date.

Overall, the distribution of the various types of retrotransposons in fungi parallels their distribution throughout eukaryotes in general; that is, TP retrotransposons and members of the Ty1/copia and Ty3/gypsy groups of LTR retrotransposons are common occurrences and found in most species. The types of retrotransposon that are rare in general, such as the YR and Penelope-like retrotransposons, are also rare in fungi. Nevertheless, these latter elements are still widespread, in the sense that they are found in species from several different divisions. Their widespread distribution and a general high level of diversity in their sequences, suggests that these elements have a long

Table 1 Types of retrotransposons identified in various fungal phyla

Phylum	*Type of retrotransposon*	*Examples (host)*[a]	*Accession no.*
Ascomycota	LTR–Ty1/copia	Ty1 (*Saccharomyces cerevisiae*)	M18706
		Tca2 (*Candida albicans*)	AF050215
		Elsa (*Phaeosphaeria nodorum*)	AJ277966
	LTR–Ty3/gypsy	Ty3 (*S. cerevisiae*)	M34549
		Tf1 (*Schizosaccharomyces pombe*)	M38526
		Maggy (*Magnaporthe grisea*)	L35053
	Target–primed	Tad1 (*Neurospora crassa*)	L25662
		Zorro3 (*C. albicans*)	AF254443
	YR	—	
	Penelope	Unnamed (*Ascosphaera apis*)	AARE01004060
Basidiomycota	LTR–Ty1/copia	Tcn6 (*Cryptococcus neoformans*)	Retrobase[b]
	LTR–Ty3/gypsy	MarY1 (*Tricholoma matsutake*)	AB028236
	Target-primed	Cnl1 (*C. neoformans*)	Retrobase[b]
	YR	CcNgaro1 (*Coprinopsis cinerea*)	AACS01000194
	Penelope	Unnamed (*Phanerochaete chrysosporium*)	AY916132
Chytridiomycota	LTR–Ty1/copia	Unnamed (*Orpinomyces* sp.)	AJ864659
	LTR–Ty3/gypsy	Unnamed (*Orpinomyces* sp.)	AJ864642
	Target-primed	Unnamed (*Orpinomyces* sp.)	AJ864661
	YR	—	
	Penelope	—	
Glomeromycota	LTR–Ty1/copia	—	
	LTR–Ty3/gypsy	Unnamed (*Gigaspora margarita*)	AJ884773
	Target-primed	Unnamed (*Glomus intraradices*)	AC156589
	YR	—	
	Penelope	—	
Microsporidia	LTR–Ty1/copia	—	
	LTR–Ty3/gypsy	Nbr1 (*Nosema bombycis*)	DQ444465
	Target-primed	—	
	YR	—	
	Penelope	—	
Zygomycota	LTR–Ty1/copia	—	
	LTR–Ty3/gypsy	Unnamed (*Rhizopus oryzae*)	AACW02000049
	Target-primed	Unnamed (*R. oryzae*)	AACW02000090
	YR	Prt1 (*Phycomyces blakesleeanus*)	Z54337
	Penelope	Unnamed (*R. oryzae*)	AACW01000082

[a]A dash indicates that no element of a particular class has been identified in the phylum to date.
[b]Retrobase (http://biochem.otago.ac.nz).

history in fungi, probably dating back to the last common ancestor of all fungi. In this case, their current sporadic distribution is likely to be the result of the frequent loss of these elements from evolving lineages. It is not clear why these elements should be more frequently lost than TP and LTR retrotransposons. One possibility is that they might not replicate so efficiently and so never reach such high copy numbers and are thus more prone to stochastic loss through deletion or point mutation.

Retrotransposon Replication

LTR Retrotransposons

The replication of LTR retrotransposons is well understood due to its extensive similarities with retroviral replication and the thorough characterization of the Ty elements of *S. cerevisiae*. While specific features may vary from element to element, the general features of Ty1 element replication likely apply to all LTR retrotransposons. The first step in Ty1 replication is transcription of an integrated element. The Ty1 LTRs contain regulatory sequences which cause transcription to begin within the left LTR sequence, proceed all the way through the internal region, and terminate within the right LTR, resulting in a terminally redundant RNA. This RNA is then exported to the cytoplasm where it is translated. In Ty1 (and in all other LTR retrotransposons) the *pol* gene is located downstream of *gag* and is translated at a much lower level. This downregulation of *pol* is achieved by having it positioned in a different translational reading frame from the *gag* ORF, with the 5′ end of *pol* having a small overlap with the 3′ end of *gag*. All translation begins with the *gag* ORF and usually stops at the *gag* termination

codon. The *pol* ORF is translated only when the ribosome undergoes a rare, programmed frameshift near the end of the *gag* ORF. When this occurs, a Gag–Pol fusion protein is produced. Next, the abundant Gag proteins and the rare Gag–Pol fusion proteins assemble into a hollow VLP. The N-terminus of the Gag protein lies on the exterior of the particle and the C-terminus lies on the inside. The fusion of the Pol protein to the C-terminus of Gag in the Gag–Pol fusion ensures that the Pol enzymes are packaged into the interior of the particle. Also packaged inside the particle are the retrotransposon RNA (usually two copies) and a tRNA (initiator Met tRNA) which will act as a primer during the reverse transcription step. The walls of the particle are porous to nucleotides and other small molecules.

The first step in the reverse transcription process is the annealing of the primer tRNA to a complementary sequence in the retrotransposon RNA (the minus-strand primer-binding site (PBS)) that lies just downstream of the left LTR. The 3′ OH group of the tRNA is then used as a primer by the RT to initiate minus-strand DNA synthesis. Minus-strand DNA synthesis then proceeds to the end of the molecule, the 5′ end of the mRNA, where it temporarily halts. The RNA in the RNA/DNA hybrid is then removed by RH. This allows the nascent minus-strand DNA to anneal to the complementary LTR sequence at the 3′ end of the RNA. Minus-strand DNA synthesis then resumes and proceeds to the 5′ end of the template. Next, the RNA of the RNA/DNA hybrid is again degraded by RH. A short purine-rich segment of RNA immediately upstream of the right LTR, known as the polypurine tract, is, however, resistant to removal by RH. This sequence remains bound to the minus-strand DNA and its 3′ OH group is used by RT to prime plus-strand DNA synthesis. This continues as far as the 5′ end of the minus-strand DNA template. The nascent plus-strand is then dissociated from the 5′ end of the minus-strand DNA, possibly by displacement by further plus-strand DNA synthesis initiated from a second poly-purine tract lying in the central region of the element. The displaced plus-strand DNA can then anneal to the 3′ end of the minus-strand DNA at their complementary sequences. A full-length double-stranded DNA copy of the retrotransposon can then be formed by extension of each strand to the end of its template and removal of any RNA nucleotides remaining from the primer sequences. Finally, in a series of steps, which are not so well understood, the new double-stranded DNA molecule associates with IN, exits the VLP, and enters the cell nucleus. Here, the DNA/protein complex associates with the chromosomal DNA and IN mediates the insertion of the retrotransposon into the host genome, completing the replication process.

While most of these steps are likely to be essentially the same in most LTR retrotransposons, many minor variations are known. For instance, in elements such as Tf1 and Tf2 from *S. pombe* (and closely related elements present in other fungi and also in vertebrates) the primer for minus-strand DNA synthesis is not a tRNA, but rather the 5′ end of the elements own mRNA which is complementary to the PBS. The two sequences anneal to each other and the resulting structure is recognized by the element's RH and cleaved to produce a free 3′ OH which is then used as a primer. As another example, in the element Tca2 from *C. albicans*, the *gag* and *pol* ORFs are in the same phase and separated by a stop codon, rather than being separated by a frameshift (**Figure 1**). Expression of the Gag–Pol fusion protein in this element presumably occurs via the occasional suppression of the *gag* stop codon. In other elements, for example, Tf1 and Tf2 (**Figure 1**), the *gag* and *pol* ORFs are fused into a single ORF and translated together. The correct stoichiometry of the Gag and Pol proteins is subsequently obtained by the preferential degradation of Pol.

Target-Primed Retrotransposons

The replication of TP retrotransposons is not as well understood as that of LTR retrotransposons, at least partly due to the lack of a suitable model system in an experimentally tractable microorganism. Nevertheless, much has been learned from the study of TP elements in insect and mammalian systems. Replication begins with transcription initiated from an internal promoter in the 5′ untranslated region. Transcription proceeds to the 3′ end of the element to produce a full-length RNA. The RNA is exported to the cytoplasm where it is translated. In the case of elements with two ORFs, the first ORF is translated much more efficiently than the second ORF. Following translation, the proteins remain associated with the RNA to form a RNA/protein complex. The complex moves to the nucleus and associates with the chromosomal DNA. The EN produces a single-stranded nick in the host chromosome and the resulting 3′ OH group is used as a primer by RT to synthesize minus-strand DNA directly into the insertion site. The following steps are not well understood but presumably include the nicking of the second strand of the target site by EN, the use of the resulting 3′ OH group to prime plus-strand synthesis, the completion of both strands of DNA synthesis, and the sealing of the ends of the retrotransposon to the host chromosomes. Although fungal elements have, as yet, contributed little to our understanding of TP retrotransposition, several TP elements have recently been identified in yeasts that may be developed into useful experimental systems for analyzing the process in more detail. These elements include Zorro3 from *C. albicans* and Ylli from *Yarrowia lipolytica*.

Other Retrotransposons

Very little is known about the replication of the other types of retrotransposons and most of this is based upon analyis of sequence data rather than direct experimental evidence. Briefly, replication of the YR retrotransposons is thought to proceed via an RNA which is copied into a circular, double-stranded DNA by the actions of RT and RH. The circular DNA is then integrated into the host chromosome by recombination mediated by the tyrosine recombinase. It is likely that PLEs integrate via a mechanism related to target-primed retrotransposition. Nothing is known about the replication of the VIPER-type of retrotransposon. The recent identification of YR and Penelope-like retrotransposons in fungi, and the likelihood that more will be identified in the near future as more fungal genome sequence data are obtained, may soon permit the development of systems to characterize these elements in more detail.

Retrotransposons and Fungal Genomes

Retrotransposons can have many and varied effects on the genomes of their hosts. They can make up a significant proportion of the host genome. In humans, the L1 TP retrotransposon alone makes up about 17% of the genome. In some plants, up to as much as 90% of the genome is made up by retrotransposons. Obviously, such an abundance of retrotransposons will have a profound effect on the functioning of the genetic material. In the fungi that have been analyzed in depth, the proportion of the genome made up by retrotransposons is generally lower than the figures quoted above. Figures for fungi include 3.1% for *S. cerevisiae*, 1.3% for *C. albicans*, 1.1% for *S. pombe*, 5.4% for *M. grisea* (all ascomycetes), and 7.6% for *Microbotyrum violaceum* (a basidiomycete). It should be noted that many of the fungi that have been analyzed in depth have been chosen in part because of their small genome size (10–50 Mbp). However, fungi with much larger genomes exist. For instance, among the glomeromycetes the genome size of *Gigaspora margarita* has been estimated at ~740 Mbp and that of *Scutellospora gregaria* at >1000 Mbp. It is possible that fungi with such large genomes might contain very large numbers of retrotransposons. On the other hand, some fungi have much lower numbers of retrotransposons. For instance, the microsporidian *E. cuniculi* has a very small genome (~2.9 Mbp) which contains no identifiable retrotransposons at all. Likewise, the yeast *Pichia farinosa* appears to contain no retrotransposons, despite these elements being abundant in closely related species.

In addition to making up a significant fraction of many fungal genomes, retrotransposons can act as powerful mutagens. For instance, they can insert into or adjacent to genes thus altering gene structure and/or regulation. They can create new copies of genes via the occasional accidental copying of gene transcripts into DNA and the insertion of these back into the genome. The resulting sequences will often be nonfunctional (retropseudogenes) but occasionally a new copy will be inserted in an intact form, adjacent to a promoter sequence where it will be expressed and perform some useful function. Retrotransposons can move their flanking sequences around the genome, potentially creating new arrangements of exons in genes and/or altering promoter sequences. The retrotransposon sequences themselves may occasionally be adopted to perform functions useful for the host. In addition, by providing regions of sequence similarity dispersed throughout the genome, retrotransposons can promote recombination events between otherwise nonhomologous regions, leading to chromosomal rearrangements. For example, the breakpoints of many chromosomal translocations in *S. cerevisiae* have been found to coincide with Ty elements. While the majority of mutational events associated with retrotransposons are likely to be neutral or deleterious to the hosts, occasional events will result in beneficial rearrangements. This suggests that the relationship between retrotransposons and their hosts should not be considered as strictly the same as that of parasite and host, but more as one with mutual benefits. In this regard, it is also of interest to note that, since the long-term survival of a retrotransposon is dependent on the long-term success of its host, many retrotransposons have developed strategies that act to minimize the damage they cause. For instance, some retrotransposons direct their integration to specific areas of the genome where they are least likely to cause deleterious mutations. As an example, the integration of Ty3 occurs very precisely 1–4 bp upstream of tRNA genes, areas where the insertion of the retrotransposon is unlikely to have a negative impact. Likewise, Ty5 integration is directed to areas of silent chromatin.

Not only can retrotransposons have profound effects on their hosts, but the hosts can also have profound effects on their retrotransposons. This is usually in the form of mechanisms to eliminate active retrotransposons, thus minimizing their potential mutagenic effects. Active retrotransposons can be eliminated in various ways. For instance, in addition to random point mutations, LTR retrotransposons can be inactivated by recombination between the two LTRs of a single element. This results in the internal region being excised and just a single LTR remaining at the original insertion site. In some species retrotransposons can be silenced by RNA interference or through methylation of repeat sequences. One of the most interesting mechanisms for eliminating active retrotransposons to have been identified in fungi is a process called

RIP (repeat-induced point mutation). This was first found in *N. crassa* and similar processes have since been identified in a variety of other filamentous ascomycetes. RIP acts during the sexual cycle and efficiently identifies and mutates repeat sequences that are greater than about 400 bp in length and share more than about 80% sequence identity. In *N. crassa* RIP produces a large number of C-to-T and G-to-A mutations in both copies of the repeated sequence. RIP progressively mutates the repeated sequences over successive sexual cycles until they no longer are sufficiently similar to be recognized (i.e., <80% identity). This process very effectively inactivates retrotransposons. Its efficiency is demonstrated by the finding that in the strain of *N. crassa* whose genome was sequenced, numerous retrotransposon relics were identified but not a single active element remains. RIP in *N. crassa* is one of the more extreme examples of the extents to which fungi may go to eliminate active transposable elements. Its actions not only eliminate active retrotransposons but also appear to prevent gene duplication of any sort, thus eliminating this pathway as a means for evolving new gene functions. This is illustrated by the finding that in *N. crassa* the most closely related paralogous genes were duplicated >200 million years ago, likely predating the origins of RIP. Although RIP very effectively inactivates retrotransposons, an active element, the Tad1 TP retrotransposon, has nevertheless been identified in some strains of *N. crassa*. It is thought that active copies of this element have evaded RIP by persisting in strains that have not recently gone through a sexual cycle and by occasionally spreading via anastomoses between different asexual lineages. Analyses of these active Tad elements may have revealed a previously unsuspected importance of the asexual phase in the biology of *N. crassa*.

One of the most interesting features that becomes apparent when the sets of retrotransposons in different fungal genomes are compared is the great variation. Different species vary in the proportion of the genome made up by retrotransposons (as outlined above), in the diversity of retrotransposons that they harbor (e.g., in *S. pombe* the only known retrotransposons are two very closely related members of the Ty3/gypsy group of LTR retrotransposons, whereas species such as *C. albicans* or *C. neoformans* harbor a much wider range of elements), in the distribution of elements along the chromosomes (e.g., in *S. pombe* the elements seem to be fairly evenly distributed along the chromosomes, whereas in *C. neoformans* elements were found to be greatly concentrated in putative centromeric regions), and in whether or not there are many active elements (e.g., *S. cerevisiae* contains numerous intact elements, whereas nearly all elements in *N. crassa* are heavily mutated). In most cases the reasons for the variation have not been determined. No doubt some of the variation is simply due to chance, but the remainder likely reflects fundamental differences between the host species and the elements that they contain. Determining the reasons behind the variation will lead to important insights into the evolution of fungal genomes.

Uses of Fungal Retrotransposons

Fungal retrotransposons have been put to numerous uses. For instance, Ty elements have been employed as insertional mutagens and gene-tagging systems in *S. cerevisiae*. As another example, retrotransposons have been employed in population genetics studies of various species, such as the ascomycete *M. grisea* and the basidiomycete *Chondrostereum purpureum*. Probably the most important uses of fungal retrotransposons, however, are as models for elements from other species. Fungi are eukaryotes and contain many of the same cellular features as higher organisms, including man. However, the rapid growth, simple lifecycle, and ease of experimental manipulation of many fungi mean that it is possible to use them for studying aspects of eukaryotic cells that would be difficult or impossible in other organisms. The fact that fungi contain retrotransposons closely related to elements of profound importance, such as retroviruses and TP retrotransposons, makes them ideal for studying many aspects of retrotransposons and the retrotransposition process.

See also: Metaviruses; Polyomaviruses of Mice; Retrotransposons of Plants; Retrotransposons of Vertebrates; Retroviruses: General Features.

Further Reading

Daboussi M-J and Capy P (2003) Transposable elements in filamentous fungi. *Annual Review of Microbiology* 57: 275–299.

Galagan JE and Selker EU (2004) RIP: The evolutionary cost of genome defense. *Trends in Genetics* 20: 417–423.

Goodwin TJD and Poulter RTM (2000) Multiple LTR-retrotransposon families in the asexual yeast *Candida albicans*. *Genome Research* 10: 174–191.

Goodwin TJD and Poulter RTM (2001) The diversity of retrotransposons in the yeast *Cryptococcus neoformans*. *Yeast* 18: 865–880.

Kim JM, Vanguri S, Boeke JD, Gabriel A, and Voytas DF (1998) Transposable elements and genome organization: A comprehensive survey of retrotransposons revealed by the complete *Saccharomyces cerevisiae* genome sequence. *Genome Research* 8: 464–478.

Roth J-F (2000) The yeast Ty virus-like particles. *Yeast* 16: 785–795.

Retrotransposons of Plants

M-A Grandbastien, INRA, Versailles, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Env Envelope protein of retroviruses and errantiviruses, containing a transmembrane domain and a host receptor-binding domain, and mediating formation of infectious virions and targeting of host receptor cells.

Env-like Coding domains possessing some features similar to those of the env domains of retroviruses (such as a transmembrane domain), but for which no similar function has been shown as yet; env-like domains are found in addition to the gag-pol domain of many plant LTR retrotransposons.

Gag (group-specific antigen) Nucleic acid-binding protein forming the structural core of the retrovirus virion core and of the cytoplasmic VLP of retrotransposons.

Int Integrase function ensuring the insertion of the double-stranded DNA daughter copy in the genome; also sometimes referred to as endonuclease (endo).

LTR (long terminal repeat) Long sequence found repeated in the same orientation at both ends of retroviruses and LTR retrotransposons and containing promoter and regulatory sequences involved in transcription, that starts in the 5′ LTR and terminates in the 3′ LTR.

PBS (primer binding site) Short sequence found downstream to the 5′ LTR, and involved in the priming of the ( )-DNA strand synthesis during reverse transcription.

PPT (polypurine tract) Short AG-rich sequence found upstream to the 3′ LTR, and involved in the priming of the (+)-DNA strand synthesis during reverse transcription.

Prot Protease protein involved in the cleavage of the functional proteins units from the (gag) pol polyprotein produced by the retrovirus or the retrotransposon.

RT Reverse transcriptase protein ensuring the synthesis of the double-stranded daughter DNA copy from the RNA template produced by the integrated provirus or retrotransposon; an associate ribonucleaseH (RNaseH) function ensures the concomitant degradation of the RNA template.

VLP (virus-like particle) Cytoplasmic intermediate analogous to the virion core of retroviruses; the VLP is constituted by structural gag proteins associated to two copies of the RNA template, together with enzymatic proteins (RT, int); the reverse transcription of the RNA template is thought to occur within the VLP.

Introduction and General Classification

Transposable elements are ubiquitous mobile DNA sequences found in both prokaryotic and eukaryotic genomes. For a long time, they were considered as 'parasite' DNA, but have now been shown to be major components of plant genomes, where they can represent up to 80% of the bulk of large cereal genomes. Transposable elements, with a few notable exceptions, encode the functions involved in their mobility and are classified into two main classes with radically different transposition mechanisms. Class I elements or retrotransposons transpose via an RNA intermediate that is reverse-transcribed into a daughter copy DNA and re-inserted into the genome, while class II elements or DNA transposons move directly from DNA to DNA. Retrotransposons thus belong to the large class of reverse-transcribing elements (or retroelements) that multiply by transferring their genetic information from RNA to DNA, and are close relatives to viral entities such as animal retroviruses or pararetroviruses infecting both animal and plant hosts. However, in contrast to true infectious viruses, which propagate only functional genomes, retrotransposons are vertically transmitted intrinsic components of host genomes. Their integrated genomes are thus submitted to evolutionary drift, with various mutations and restructurations destroying their functionality, and defective copies can still be amplified, sometimes at surprisingly high levels, via functional related copies. Plant retrotransposons are thus found in a tremendous variety of structural variants, including highly defective and deleted versions, making their classification a difficult task. In addition, retrotransposons are composed of modules that frequently show incongruent phylogenies, and this intrinsic chimerical nature often obscures classifications based on phylogenies.

There are two main categories of retrotransposons: LTR retrotransposons that contain two long terminal repeats (LTRs), and non-LTR retrotransposons often referred to as retroposons. All functional copies encode as basic modules the structural and functional proteins required for the retrotransposition cycle (summarized in **Figure 1**). These include a structural RNA-binding protein (gag or gag-like),

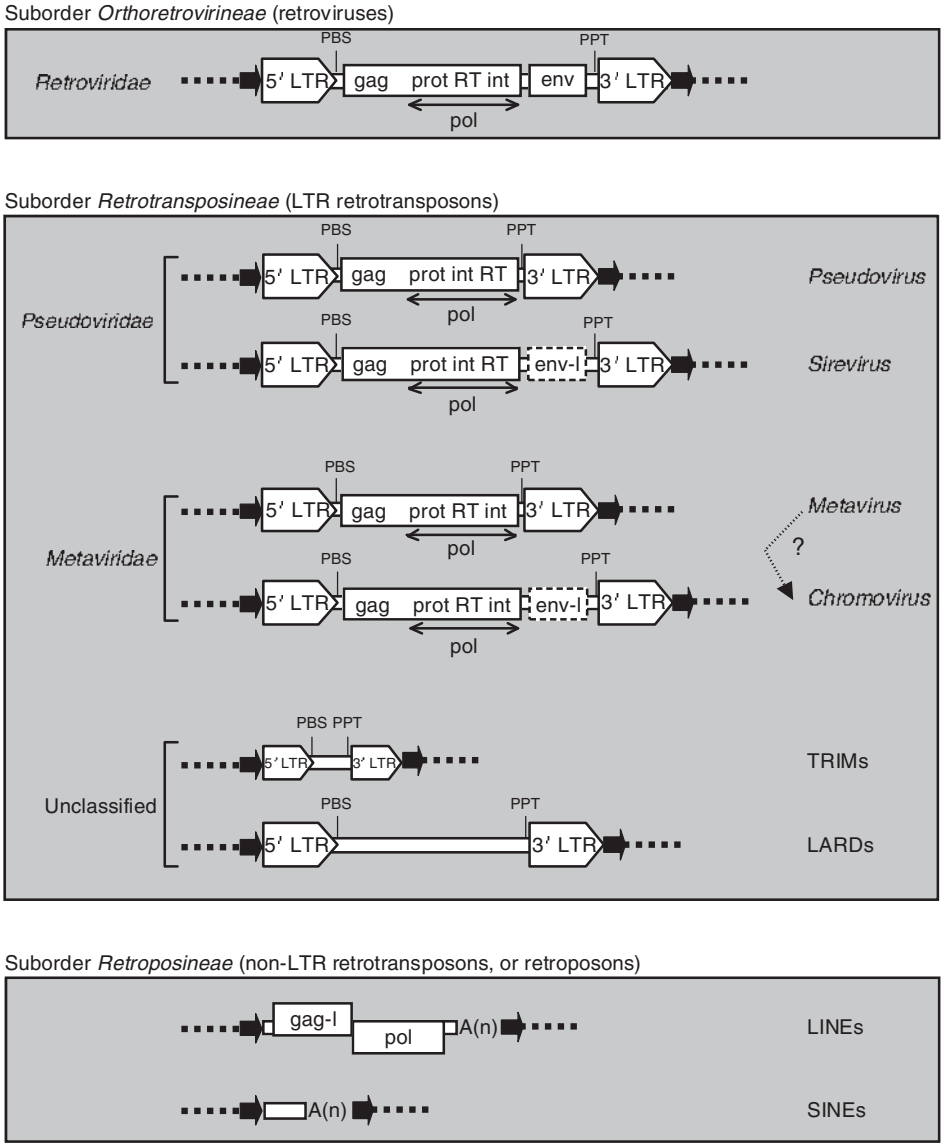


Figure 1 Structural diversity of retrotransposons found in plant genomes. The structure of a retrovirus has been provided for reference. The gag and pol domains are encoded in one or two frames, depending on the element, and have been represented here as a single ORF for simplification. Family names are indicated on the left and genus names on the right. LTR, long terminal repeat; gag, nucleic acid-binding protein forming the structural core of the retrovirus virion core and of the cytoplasmic VLP of retrotransposons; prot, protease protein involved in the cleavage of the functional proteins units from the (gag) pol polyprotein; int, integrase function ensuring the insertion of the double-stranded DNA copy in the genome; RT, reverse transcriptase ensuring the synthesis of the double-stranded DNA copy from the RNA template; env, envelope protein ensuring infectiosity of the retrovirus; env-l, env-like coding domain possessing structural features similar to env, but for which no similar function has been shown yet (a dotted box framing the env-like domain indicates that elements within the genus may or may not contain the domain); PBS, primer binding site; PPT, polypurine tract. Arrows flanking elements represent the target site duplication. The question mark linking the genera *Metavirus* and *Chromovirus* indicates that the definition of *Chromovirus* as an additional genus within the family *Metaviridae* has not been included in the official classification, with the result that many chromoviruses are still considered to be metaviruses.

and a pol domain encoding the reverse transcriptase (RT) ensuring the synthesis of the double-stranded daughter DNA copy from the RNA template, as well as in most cases a protein ensuring insertion of the double-stranded DNA daughter copy in the genome (referred to as integrase for most elements). A number of plant LTR retrotransposons contain an additional coding domain currently termed env-like, although, as discussed below, functional and structural analogies to retroviral env proteins remain to be demonstrated.

LTR retrotransposons have been further classified in Ty1/*copia*-type elements and Ty3/*gypsy*-type depending

on the order of the coding domains. Non-LTR retrotransposons include LINEs (long interspersed nuclear elements), which are elements carrying coding sequences, and SINEs (short interspersed nuclear elements), small noncoding elements of a few hundred base pairs that exploit the transposition machinery of LINEs to ensure their amplification. LTR retrotransposons are by far the most abundant transposable elements in plant genomes, while LINEs appearsomewhat less represented, in contrast to mammalian genomes. SINEs are also abundant in plant genomes, although their small size prevents them from playing a large structural role.

The continuous discovery of new structural variants of these major categories has made it necessary to revise and refine previous classifications. Retrotransposons have been included in a recent classification proposed for all reverse-transcribing elements, and based on viral nomenclature with type species forms defined for some genera (**Table 1**). All retroelements have been defined as belonging to class *Retroelementopsida*, presently divided in two orders, order *Retrovirales* that encompasses retroviruses, pararetroviruses, and LTR retrotransposons, and order *Retrales* that contains non-LTR retrotransposons and bacterial retrons. While order *Retrales* has not been further organized at the present time, order *Retrovirales* has been divided in several suborders, families, and genera.

Suborder *Orthoretrovirinae* contains the family *Retroviridae*, or true retroviruses, of which no example is known in plants at the present time.

Suborder *Pararetroviridae* contains two families of pararetroviruses, one of which (*Caulimoviridae*) is represented in plants. Pararetroviruses represent sensibly different lineages of retroelements, and are thought to derive from preexisting viruses having acquired a reverse transcription function, presumably from retrotransposons of the family *Metaviridae* . Many plant genomes also contain vertically transmitted integrated endogenous pararetroviral sequences (EPRVs), which represent integrated derivatives of pararetroviruses, although the infectious counterpart has not always been characterized. Plant pararetroviruses are not discussed here.

Suborder *Retrotransposineae* contains LTR retrotransposons, and has been divided in family *Pseudoviridae* (Ty1/*copia* retrotransposons) and family *Metaviridae* (Ty3/*gypsy* retrotransposons), based on the organization of the pol domain.

The Life Cycle of LTR Retrotransposons

High Similarities with Intracellular Steps of the Retroviral Cycle

LTR retrotransposons represent by far the most abundant plant retrotransposons. They share striking similarities with animal retroviruses, both in their structure (**Figure 1**) and in their replication cycle (**Figure 2**). Like integrated retroviral forms (termed proviruses), LTR retrotransposons are terminated by two LTR sequences. As the reverse transcription process generates two identical LTRs, with mutations accumulating subsequently, the rate of divergences between LTRs of a given copy is often used as a molecular clock to date its insertion. The gag and pol coding domains are found between the LTRs. Other typical features include short sequences known as PBS (primer binding site) and PPT (polypurine tract). The PBS is found immediately downstream the 5′ LTR, and is involved in priming of the ( )-DNA strand synthesis during reverse transcription. It is generally complementary to the 3′ end of a tRNA recruited for the priming of DNA synthesis ($tRNA^{met}$ for most *Pseudoviridae*, more variable for *Metaviridae*). The PPT is a short AG-rich sequence found immediately upstream to the 3′ LTR, and involved in the priming of the (+)-DNA strand synthesis. Inserted copies are typically bounded by short direct repeat of the target host site, generated upon insertion, and usually 5 bp long for plant LTR retrotransposons.

Transcription begins in the 5′ LTR, ends in the 3′ LTR, and produces both the RNA template used for reverse transcription and the mRNA(s) that will be translated into proteins, after cleavage of the gag-pol polyprotein by the protease (prot) function. The gag protein binds to the RNA template and forms a cytoplasmic virus-like particle (VLP) analogous to the retroviral virion core. Together with the RNA template, VLPs encapsulate the RT that will produce the double-stranded DNA copy, and the integrase (int) that will be involved in transfer of the linear DNA copy to the nucleus and its insertion in the genome. This amplification cycle includes multiple steps of control, with transcription being absolutely crucial for retrotransposition. As a consequence, the mode of amplification of a particular element will be conditioned by its transcriptional regulation.

The amplification cycle of LTR retrotransposons is thus closely related to the intracellular steps of the retroviral cycle, with strong similarities in the reverse transcription and integration processes, as well as similar involvement of a cytoplasmic encapsulated intermediate. It has to be noticed that the amplification of non-LTR retrotransposons is sensibly different, with reverse transcription occurring at the integration site. The reality of the retroviral-type cycle of LTR retrotransposons has been demonstrated in yeast, but not yet in plants. However, VLPs have been detected for the barley BARE1 element. In addition, it has been demonstrated very recently that plant elements of the genus *Sirevirus* (see below) encode unusually large Gag domains extended in their C-terminus, and that this Gag extension binds to LC8/LC6 dynein proteins. Although the biological significance of this interaction remains to be determined, it is very similar to the binding of some human retroviruses'

Table 1 Classification of retroelements

	Type species	*A few examples in Viridiplantae*[a]
Order *Retrovirales*		
Suborder *Orthoretrovirineae*		
Family *Retroviridae* (retrovirus)		Not yet
Suborder *Pararetrovirineae* (pararetrovirus)		
Family *Hepadnaviridae*		Not yet
Family *Caulimoviridae*		CaMV, BSV, integrated EPRVs (banana BSV; petunia PVCV, tobacco TVCV, *Nicotiana* NtoEPRV, and Ns EPRV)
Suborder *Retrotransposineae* (LTR retrotransposons)		
Family *Pseudoviridae* (Ty1/*copia* retrotransposons)		
Genus *Pseudovirus*	Ty1	Ta1 (*Arabidopsis* X1329), Tnt1 (tobacco X13777), Tst1 (potato X52387), Tto1 (tobacco D83003), WIS2 (wheat X63184), BARE1 (barley Z17327), Hopscotch (maize U12626), Tto1 (tobacco D83003), Tos17 (rice D85876), RIRE1 (rice D85597), Art1 (arabidopsis Y08010), Retrofit (rice U72726), Melmoth (kale Y12321), Tgmr (soybean U96748), Stonor (maize AF082134), Panzee (pigeon pea AJ000893), Tpv2 (bean AJ005762), LERE1 (tomato AF275345), Retrolyc1 (*S. peruvianum* AF228701), AtRE1 (*Arabidopsis* AB021265), TLC1 (*S. chilense* AF279585), Toto1 (tomato AF220602), Angela (wheat DQ666286)
Genus *Hemivirus*	Copia	Osser (*Volvox* X69552)
Genus *Sirevirus*	SIRE1	SIRE1 (soybean AY205608), ToRTL1 (tomato U68072), Endovir (*Arabidopsis* AY016208) Opie-2 (maize U68408), PREM2 (maize U41000), Osr7 (rice AP002538), Osr8 (rice AC021891)
Family *Metaviridae* (Ty3/*gypsy* retrotransposons)		
Genus *Errantivirus*	gypsy	Not yet
Genus *Metavirus*	Ty3	Athila (*Arabidopsis* X81801), Cyclops (pea AJ000640), RIRE8 (rice AB014740), RIRE2 (rice AB030283), BAGY2 (barley AJ279072), Cinful (maize AF049110), Grande1 (maize X97604), Piggy1 (pea AY299398), Ogre (pea AY299398), Diaspora (soybean AF095730)
		Genus *Chromovirus*?: del1 (lilium X13886), IFG7 (pine AJ004945), Reina (maize U69258), RIRE3 (rice AB014738), RIRE7 (rice AB033235), Galadriel (tomato AF119040), tekay (maize AF448416), Beetle1 (beet AJ539424)
Genus *Semotivirus*	BEL	Not yet
Genus *?*	DIRS	Not yet
Unclassified		LARDs: Sukkula (barley AY054378), Squiq (rice AY355293), Spip (rice AY355292), Dasheng (rice)
		TRIMs: (potato AJ276865), Katydid (*Arabidopsis*), mini-Toto1 (tomato X58273)
Order *Retrales*		
Suborder *Retroposineae* (non-LTR retrotransposons)		
	L1	Cin4 (maize Y00086), del2 (lilium Z17425), Isabelle (maize AF326781), Karma (rice AB081316), Bali1A (rapeseed AF525305)
	Alu	TS (tobacco D17453), S1 (rapeseed L76840), Au (*Aegilops* AB046134)
Suborder *Retronineae* (bacterial retrons)		

[a]Elements have been classified according to year of publication or sequence submission to Genbank databases. Note that in several cases, only partial sequences are available. Extensive data on various plant elements can be found at: Repbase, a database of repetitive DNA elements of all organisms, the TIGR Plant Repeat Database and the Triticeae-specific TREP database. Retroelements were classified according to Hull R (2001) Classifying reverse transcribing elements: A proposal and challenge to the ICTV. *Archives of Virology* 146: 2255–2261; and Boeke JD, Eickbush T, Sandmeyer SB, and Voytas DF (2004) *Pseudoviridae/ Metaviridae* In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 397–420. San Diego, CA: Elsevier Academic Press.

Gag protein to LC8 proteins to ensure intracellular movement of virion cores along microtubules. Such observations reinforce the strong similarities between the life cycle of retroviruses and LTR retrotransposons.

The major difference between retroviruses and LTR retrotransposons resides in the infectious potentialities of the former, mediated by the env function that allows them to produce extracellular virions and infect host

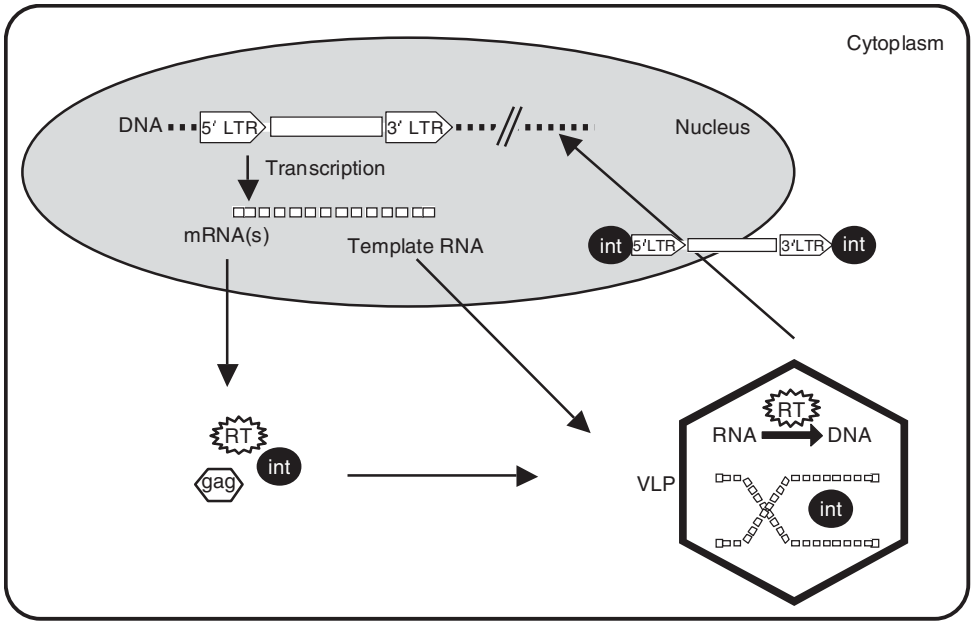


Figure 2 The amplification cycle of LTR retrotransposons. Transcription begins in the 5′ LTR and ends in the 3′ LTR, and produces both the RNA template used for reverse transcription and mRNAs translated into proteins. The gag protein binds to the RNA template and forms a cytoplasmic virus-like particle (VLP). Together with the RNA template, VLPs encapsulate the RT that will produce the double-stranded DNA copy, and the integrase (int) involved in transfer of the linear DNA copy to the nucleus and its insertion in the genome.

receptor cells. *Bona fide* retrotransposons, on the other hand, are not infectious and their amplification cycle is exclusively intracellular. However, we shall see later that this assumption should be treated with care and that the delimitation between retroviruses and retrotransposons is increasingly fuzzy, possibly even in plants.

It is now considered as certain that retroviruses are derivatives of some LTR retrotransposon *Metaviridae* lineages. LTR retrotransposons are themselves derived, with LINE-like ancestors, from the assembly of various modules together with an RT-like function derived from ancestral cellular functions used for the transition of genetic information from RNA to DNA at the dawn of life. Retroviruses have evolved from LTR retrotransposons through transduction of env domains ensuring their ability to exit the cell. Although the process can be reversed (e.g., with vertebrate endogenous retroviruses having lost their env functionality and reversed to a restricted intracellular life cycle), retroviruses can be seen as the ultimate evolutionary step in freeing of cellular genetic information outside of the cell, and they basically represent LTR retrotransposons that have succeeded in life. It is not clear whether this process has occurred only once during the evolution of *Retroviridae*; however, it is now increasingly clear that a number of their LTR retrotransposons' left-behind cousins have repeatedly attempted to join them in acquiring an extracellular life and that some of them have truly succeeded, albeit not yet in plants.

Evolution as Quasispecies-Like Populations

Other similarities of retrotransposons with retroviral characteristics include their high level of sequence variability. This has been best illustrated for elements related to the tobacco Tnt1 element, which are found in many species of the family Solanaceae, and evolve via a high variability of the LTR region carrying regulatory features (U3 region). Species of the genus *Nicotiana* contain Tnt1 elements that are classified into several groups (termed subfamilies) which are closely related, except in their U3 regions that differ strongly. Members of each subfamily are detected within each species, and their U3 divergence thus predates the *Nicotiana* radiation. However, the respective proportions of each subfamily differ in each species, indicating that each host species has preferentially amplified specific subfamilies. Similar evolutionary patterns have been detected in other Solanaceae species, such as tomato, pepper, or aubergine, which contain elements closely related to *Nicotiana* elements, except in the U3 region, and which can also be further subdivided in subfamilies based on the nature of the U3 region only. Interestingly, the different tobacco Tnt1 subfamilies are each expressed in response to different stimuli, within the same host genome. The very ancient Tnt1 element has thus evolved in Solanaceae a large range of highly related progeny populations that have gained new regulatory sequences and new expression patterns.

This strategy is highly similar to the evolutionary patterns of RNA viruses and retroviruses, generating

populations of closely related but different genomes referred to as quasispecies. This variability has been attributed to the high-error-prone process of reverse transcription, due the lack of proofreading repair activity of RNA polymerases and reverse transcriptases. This allows viral populations to evolve very rapidly when environmental conditions change and endow retroviruses with high adaptative capacity. Thus, LTR retrotransposons similarly evolve continuums of closely related sequences, which can be defined as quasispecies-like. This evolutionary pattern allows them to vary their amplification conditions, as regulatory controls in the U3 will determine the conditions in which the element will be transcribed and amplified. The preferential amplification of specific subfamilies in each host species suggests that optimal patterns of amplification may have been selected and maintained in each host genome, possibly depending on its own evolutionary, reproductive, and environmental history.

The Different Genera of LTR Retrotransposons

The Family *Pseudoviridae* (Ty1/*copia* Retrotransposons)

Pseudoviridae are differentiated from all other LTR retrotransposons and from retroviruses by an unusual organization of the pol domain, with the int domain placed before the RT domain (**Figure 1**). This confirms that the different LTR retrotransposon families have evolved by independent acquisition of modular functions. Historically, Ty1/*copia* retrotransposons have been the first LTR retrotransposons characterized in higher plants, and the best-known plant LTR retrotransposons belong to this family, including the tiny handful of elements for which mobility has been demonstrated. *Pseudoviridae* have been classified in the genera *Pseudovirus* (type species Ty1 of yeast), *Hemivirus* (type species *copia* of Drosophila), and the recent genus *Sirevirus* (type species, SIRE1 of soybean).

Pseudoviruses and hemiviruses differ by the fact that the former use a full $tRNA^{met}i$ to prime synthesis of the ( )-DNA strand, while the latter use a half $tRNA^{met}i$. So far, no hemivirus has been referenced in plants, and all LTR retrotransposons usually – and incorrectly – referred to as *copia*-type retrotransposons in fact classify in the genus *Pseudovirus*. Sireviruses have so far been identified only in plants. They are phylogenetically well separated from the two other genera, and display some very interesting characteristics. In particular, several members of the genus have acquired additional ORFs carrying additional functions that present some similarities to the env domain of retroviruses, such as a transmembrane domain (TMD). This is, for instance, the case with SIRE1, ToRTL1, and Endovir (**Table 1**). A number of other elements classified as sireviruses, such as Opie-2, PREM-2, Osr7, or Osr8 (**Table 1**), do not carry additional ORFs with typical env-like features. However, all of them carry a significant amount of noncoding DNA after the end of the pol domain, from a few hundred base pairs to over 1 kbp, while members of the genera *Pseudovirus* and *Hemivirus*, such as Tnt1, Ty1, or *copia*, separate the pol domain from the 3′ LTR by a few dozen nucleotides or less. This suggests that the env-less sireviruses may also have contained similar additional ORFs that have decayed over evolutionary times.

The Family *Metaviridae* (Ty3/*gypsy* Retrotransposons)

The family *Metaviridae* is characterized by an organization of the pol domain identical to that of retroviruses, with the int protein encoded at the end of pol, after the RT domain (**Figure 1**). Metaviruses appear to be basal to both retroviruses and pseudoviruses, and the family *Metaviridae* therefore encompasses a large variety of members, whose classification is probably not at its final stage yet. Although metaviruses have been characterized in plants more recently than pseudoviruses, they have been found to be more abundant in several plant genomes.

The family *Metaviridae* comprises the genera *Errantivirus* (type species *gypsy* of drosophila), *Metavirus* (type species Ty3 of yeast), and *Semotivirus* (type species BEL of drosophila) (**Table 1**). To these three genera are often added the DIRS retrotransposons, originally described from *Dictyostelium discoidum,* that show differences with other metavirus elements. DIRS do not carry typical LTRs, that is, direct repeats in the same orientation, and do not encode an int. Instead, they are bounded by inverted long repeats and integration in the host genome is ensured by a tyrosine-recombinase. In spite of these differences, they are more related to metaviruses, both in terms of RT sequence and organization of the pol domain, with the tyrosine-recombinase placed downstream to the RT domain. They could represent chimerical elements having acquired an RT domain from some metaviruses, and are placed by several authors as a putative additional genus (still unnamed) in this family. No DIRS, semotivirus, or errantivirus has yet been characterized in plants, with the totality of so-called *gypsy*-like retrotransposons of plants classified in the genus *Metavirus* (**Table 1**).

Errantiviruses are grouped together on the basis of the presence of an additional env-like domain, which ensures a real envelope function for the two infectious *gypsy* and ZAM elements of drosophila. *Gypsy* and ZAM are the first examples of true retroviruses outside of the vertebrate subphylum. However, their phylogenies place them quite apart from retroviruses, and they are more closely related to other LTR retrotransposons of the family *Metaviridae*. They do presumably represent recent progresses of LTR retrotransposons toward an extracellular life cycle.

The separation of errantiviruses and metaviruses has been interpreted by many authors as based on the lack of an env or env-like domain in metaviruses. However, the situation is more complex. Many plant elements presently classified in the genus metavirus indeed do not encode for additional ORFs after the pol domain (such as del1, IFG7, Reina, RIRE3, RIRE7, Galadriel, tekay, and Beetle-1; **Table 1**). In contrast to env-less sireviruses mentioned above, these elements do not show significant stretches of additional sequences between the pol domain and the 3′ LTR (only a few dozens at the most, often very few), indicating that they may never have contained any additional ORF. However, a large number of plant elements presently classified in the genus *Metavirus* do contain an additional ORF with typical env-like features (such as Athila, Cyclops, RIRE8, RIRE2, BAGY2, Cinful, Grande1, Piggy1, or Ogre; **Table 1**). The two groups of elements cluster separately in most phylogenetic studies, indicating that they could be considered as two different genera of the family *Metaviridae*. An additional genus, *Chromovirus,* has recently been proposed within the family *Metaviridae* , based on the presence of a chromodomain in the integrase . However, it has not been included in the current official classification. Interestingly, all plant metaviruses devoid of additional coding domains seem to be chromoviruses. It is therefore likely that the classification of *Metaviridae* will be reconsidered in future, to separate the two groups of metaviruses and recognize the existence of the chromodomain. Interestingly, the soybean Diaspora element is devoid of any additional DNA between pol and the 3′ LTR; however it clusters with env-containing elements, suggesting that its original env-like domain has been deleted. Therefore, the presence/absence of an env-like domain would not be a relevant criterion in the redefinition of genera within the family *Metaviridae*.

Unclassified LTR Retrotransposons

A number of LTR retrotransposons have recently been discovered that cannot be classified into any of the above genera due to their lack of coding sequences. The TRIMs (terminal repeat retrotransposons in miniature) consist of small LTRs framing a short central domain of noncoding sequences, while the LARDs (large retrotransposon derivatives) are larger defective derivatives that contain a large internal domain without significant similarities to retrotransposon coding domains. Both TRIMs and LARDs share many of the structural characteristics of LTR retrotransposons, such as RT priming sites PBS and PPT, and are highly amplified, suggesting efficient transactivation by functional retrotransposons. For several LARDs, sequence identities have been found with LTRs of canonical LTR retrotransposons (e.g., Dasheng with RIRE2, Squip with RIRE8, Spip with RIRE3), suggesting that they have been derived from these elements, and may eventually be classified together. Similarly, some TRIM-like small elements are derived from canonical LTR retrotransposons, such as the tomato mini-Toto1 element, derived from the Toto1 pseudovirus. In addition, a number of intermediate situations are frequently found, such as TRIM derivatives containing larger additional unknown DNA, a possible intermediate situation between TRIMs and LARDs. The existence of TRIMs and LARDs and various intermediates indicates that plant genomes contain a large range of retrotransposons with structural features much more diverse and complex than previously thought. As long as the features required in *cis* for amplification are conserved (e.g., LTRs, PBS, and PPT, as well as a few internal sequences involved in packaging), and as long as the LTR promoter remains transcriptionally active to ensure the production of an RNA template, these defective or atypical elements can be amplified to high levels using the structural and enzymatic retroviral functions provided by their original functional parent, or possibly by other related LTR retrotransposons.

Env-Like Functions in Plants?

Although plant retrotransposons with additional ORFs of the env type have been detected, the potential role of such a function in infectivity remains very hypothetical in plants. Retroviral env glycoproteins carry a number of typical structures, such as a TMD mediating formation of infectious virions and a host receptor-binding domain, mediating targeting of host receptor cells. No significant homology to any retroviral env gene has been shown for any plant element; however, such identification is highly unlikely due to the intrinsic high variability shown by animal retroviral env proteins. As the definition of env-like functions in plant retrotransposons is mostly based on the presence of TMDS, the issue of the naming of these additional ORFs has been hotly debated. TMDs can also be found in various proteins, and the env denomination has a specific meaning related to anchoring of the protein in the membrane envelope of extracellular virion particles and to recognition of receptors on target cells to ensure viral infection after membrane fusion. Quite likely, such mechanisms are rarely used by plant viruses for host-to-host transmission, due to the cell wall barrier. Plant retrotransposons carrying additional ORFs related to functions used by most plant viruses to ensure host-to-host transmission, for example, via vector insects, have not yet been found. However, examples of enveloped viruses do exist in plants, and retrotransposon-encoded env-like proteins could be involved in anchoring of VLPs to cellular membranes, playing a role in cell-to-cell transmission via plasmodesmata and possibly in transmission by insects.

Furthermore, conservation of env-like domains has been observed between elements from different plant

genera within both the *Metaviridae* and the *Pseudoviridae* (SIRE1 of soybean and Endovir of *Arabidopsis*). In addition, conserved splice acceptor sequences are detected 5′ of env-like domains of several plant metaviruses, and production of an env-like spliced subgenomic RNA has been demonstrated for the barley BAGY-2 element, a mechanism similar to those used by retroviruses to ensure production of the env protein. In contrast, sireviruses such as SIRE1 use stop codon suppression to express env-like protein. The existence of such specific expression mechanisms strongly suggests that env-like domains are not mere incidental transduction of cellular functions, as shown for the maize Bs1 element, and that acquisition of additional coding domains with TMDs, whether in a quest or not for extracellular life, may play an important role in the plant LTR retrotransposon life cycle. In the search of a better definition, and in spite of lack of evidence for any retroviral env-type function, it has been proposed to maintain the env denomination due to its vernacular long-term use: for example, the nucleocapsid protein used to build up the VLP core particle is commonly termed gag in all retrotransposons, by analogy to the gag 'group-specific antigen' region of retroviruses, although such functional denomination does not make sense in plants.

A striking observation is that LTR retrotransposons generally have been very efficient in acquiring env-like domains, and have done so many times. For instance, additional ORFs with env-like features have been observed both for Ty1/*copia* and Ty3/*gypsy* retrotransposons, suggesting that they have been acquired independently during evolution. In addition, the *Metaviridae* contain clear examples of acquisition of env function from different sources, for example, from baculoviruses for gypsy of drosophila, and from phleboviruses and herpesviruses for the semotiviruses Cer of *Caenorhabditis elegans* and Tas of *Ascaris lumbrocoides*, respectively. This indicates that LTR retrotransposons have repeatedly been able to hijack viral functions leading to extracellular autonomy, and there is no conceivable reason for plants to be an exception.

The Nomenclature Issue

Rules for naming individual elements also continue to be hotly debated. Earlier retrotransposons have been named according to authors' personal fancies (e.g., Athila, Cyclops, Opie, Ogre) or based on the plant species they were isolated from, but following no defined rule (e.g., Tnt1 for Transposon of Nicotiana Tabacum, BARE-1 for Barley Retrotransposon, WIS-2 for Wheat Insertion Sequence). An official nomenclature has recently been proposed for all retroelements, with three letters used for the species, up to three (exceptionally four) for the element and 'V' for virus. In this nomenclature, the yeast Ty1 and drosophila *copia* elements have been renamed SceTy1V and DmeCopV, respectively, and Tnt1 and BARE1 have been renamed NtaTnt1V and HvuBV, respectively. However, this nomenclature has so far not been implemented by the plant scientific community, most likely because it does not solve the major problem arising from the presence of highly related elements in different plant species or genera, which is a general rule in plants.

In addition, most transposable element populations, and especially retrotransposons, are generally composed of populations of closely related sequences. Defining whether a particular copy is related to an already known element, or is different, is thus often a difficult task. A consensus position has been to consider that copies that show above 75% identity over most of their length (not taking into account deletions or insertions of unrelated material) are variants of the same element. However, many elements have been discovered and named independently. For instance, the wheat WIS2 and Angela elements are quite similar to the barley BARE1 element, with internal nucleotidic sequences 75–80% identical between BARE1 and WIS2 and over 80% identical between BARE1 and Angela. Similarly, *Solanum* subsection *Lycopersicon* counterparts of the tobacco Tnt1 element have been named Retrolyc1 (first identified in *S. peruvianum*) and TLC1 (identified in *S. chilense*), although Retrolyc1 and Tnt1 nucleotide sequences are over 85% identical and TLC1 and Retrolyc1 are 93% identical. This highlights the difficulties inherent to a nomenclature based on host species names.

A proposition was made to replace species-based names by first names, a trend that seems to be developing at present, at least in the plant field. More specifically, it has been proposed that female first names should be used for retroelements and male first names for class II DNA transposons, although this particular proposal is not really implemented. Such type of nomenclature should alleviate the problem of the presence of related elements in different hosts, provided authors make sure their favorite first name has not been used yet. Attempts to unify the nomenclature (as well as the general classification) of transposable elements of Triticeae are currently coordinated by Dr. Thomas Wicker, who curates the TREP database for Triticeae repeats. This should lead to the redefinition of consensus guidelines that could be fruitfully followed by the entire scientific community for transposable elements of all plant genera.

See also: Caulimoviruses: General Features; Caulimoviruses: Molecular Biology; Endogenous Retroviruses; Metaviruses; Movement of Viruses in Plants; Pseudoviruses; Reoviruses: General Features; Retrotransposons of Fungi; Retrotransposons of Vertebrates; Retroviruses: General Features; Vector Transmission of Plant Viruses.

Further Reading

Boeke JD, Eickbush T, Sandmeyer SB, and Voytas DF (2004) *Pseudoviridae/Metaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 397–420. San Diego, CA: Elsevier Academic Press.

Casacuberta JM, Vernhettes S, Audéon C, and Grandbastien M-A (1997) Quasispecies in retrotransposons: A role for sequence variability in Tnt1 evolution. *Genetica* 100: 109–117.

Grandbastien M-A, Audeon C, Bonnivard E, *et al.* (2005) Stress activation and genomic impact of Tnt1 retrotransposons in Solanaceae. *Special Issue: Retrotransposable Elements and Genome Evolution. Cytogenetic and Genome Research* 110: 229–241.

Havecker ER, Gao X, and Voyas DF (2004) The diversity of LTR retrotransposons. *Genome Biology* 5: 225.

Havecker ER, Gao X, and Voytas DF (2005) The sireviruses, a plant-specific lineage of the Ty1/*copia* retrotransposons, interact with a family of proteins related to dynein light chain 8. *Plant Physiology* 139: 857–868.

Hull R (2001) Classifying reverse transcribing elements: A proposal and challenge to the ICTV. *Archives of Virology* 146: 2255–2261.

Kalendar R, Vicient CM, Peleg O, Anamthawat-Jonsson K, Bolshoy A, and Schulman AH (2004) Large retrotransposon derivatives: Abundant, conserved but nonautonomous retroelements of barley and related genomes. *Genetics* 166: 1437–1450.

Kordis D (2005) A genomic perspective on the chromodomain-containing retrotransposons: Chromoviruses. *Gene* 247: 161–173.

Lucas H, Yot P, Lockhart BEL, and Capy P (2004) Taxa of viruses, virus-like and subviral agents infecting Poaceae, class '*Retroelementopsida*'. In: Lapierre H and Signoret PA (eds.) *Virus and Virus Diseases of Poaceae (Graminae)*, pp. 279–303. Paris: INRA Editions.

Malik HS, Henikoff S, and Eickbush TH (2000) Poised for contagion: Evolutionary origins of the infectious ability of invertebrate retroviruses. *Genome Research* 10: 1307–1318.

Peterson-Burch BD, Wright DA, Laten HL, and Voytas DF (2000) Retroviruses in plants? *Trends in Genetics* 16: 151–152.

Vicient CM, Kalendar R, and Schulman AH (2001) Envelope-class retrovirus-like elements are widespread, transcribed and spliced, and insertionally polymorphic in plants. *Genome Research* 11: 2041–2049.

Wicker T, Sabot F, Hua-Van A, *et al.* (2007) A unified classification system for eukaryotic transposable elements. *Nature Previews Genetics* 8: 973–982.

Witte CP, Le QH, Bureau T, and Kumar A (2001) Terminal-repeat retrotransposons in miniature (TRIM) are involved in restructuring plant genomes. *Proceedings of the National Academy of Sciences, USA* 98: 13778–13783.

Wright DA, Daniel F, and Voytas DF (2001) *Athila4* of *Arabidopsis* and *Calypso* of soybean define a lineage of endogenous plant retroviruses. *Genome Research* 12: 122–131.

Relevant Websites

http://www.girinst.org – Genetic Information Research Institute (GIRI).
http://www.tigr.org – The TIGR Plant Repeat Databases, J. Craig Venter Institute (JCVI).
http://wheat.pw.usda.gov – The Triticeae Repeat Sequence Database (TREP), GrainGenes, Agricultural Research Service, USDA.

Retrotransposons of Vertebrates

A E Peaston, The Jackson Laboratory, Bar Harbor, ME, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ancestral retrotransposon A retrotransposon present in the genome of a common ancestor of two or more host groups. Also referred to as ancestral repeat.

Apurinic/apyrimidinic Endonuclease enzyme that catalyzes the cleavage of a phosphodiester bond in a DNA molecule.

Autonomous retrotransposon A retrotransposon encoding proteins required for its reverse transcription and transposition.

Clade A group of organisms consisting of a single common ancestor and all its descendents.

DDE transposases A class of transposase enzymes containing a highly conserved amino acid motif, aspartate–aspartate–glutamate (DDE), required for metal ion coordination in catalyzing integration of retrotransposon cDNA into the host DNA, thus also known as integrase.

Exaptation In broad terms, a feature conferring evolutionary fitness on an organism but which was originally nonfunctional or designed for some other function in the organism. Thus, the genes of a retrotransposon newly inserted in the host genome maybe, over evolutionary time, co-opted for function in the host; in this new role they are called exaptations.

Homoplasy A structure arising in two or more species as the result of a convergent evolution, and not as a result of common descent which indicates that the feature in one species is homologous to that in the other.

Lineage-specific retrotransposon A retrotransposon introduced into the genome of one but not another host grouping after their evolutionary divergence.

Nucleotide substitution rate The rate at which single nucleotide mutations occur within regions of a genome not subject to selection. One way to

estimate this is by comparison of the consensus sequences of ancestral repeats with their remnant sequences in different host genomes.

Y-transposase, also known as tyrosine recombinase A class of transposase enzymes which can use a conserved tyrosine to cut and rejoin its DNA substrates by a 3′ phosphotyrosine linkage; used by some retrotransposons to integrate their cDNA into the host DNA, and thus sometimes called integrase.

History

Transposable elements are defined segments of DNA which replicate and move to other loci within the genome by a variety of mechanisms. The vast majority of these mobile elements in vertebrates are retrotransposons, which replicate by means of an RNA intermediate that is reverse-transcribed into DNA and inserted in a new location within the genome. Retrotransposons are found in all vertebrate genomes, but have been intensively studied in relatively few.

Retrotransposons were initially detected as discrete fragments of genomic DNA that rapidly reannealed after denaturation, and were recognized to be repetitive sequence elements interspersed within genomic DNA. The advent of DNA analysis by restriction enzyme fragmentation led to the identification of discrete families of repetitive elements whose members shared particular sets of internal restriction sites. The most highly repeated long and short sequences were named long interspersed repetitive elements (LINEs) and short interspersed repetitive elements (SINEs), respectively.

The LTR elements, a third general type of retrotransposon resembling the integrated form of proviruses, was similarly discovered. These endogenous retroviruses and retrovirus-like elements are generally restricted to an intracellular life cycle and vertical transmission through the germline and, unlike 'true' retroviruses, are not infectious. However, notable exceptions blur this distinction.

The advent of whole genome sequencing and sequence analysis is rapidly providing detailed pictures of vertebrate retrotransposon landscapes, such as those of a pufferfish (*Takifugu rubripes*), the chicken (*Gallus gallus*), the laboratory mouse (*Mus musculus*), human (*Homo sapiens*), dog (*Canis lupus familiarus*), and others. New lineage-specific retrotransposons of vertebrates such as primate-specific SVA (SINE-R, VNTR, Alu), are coming to light, as well as the discovery within selected vertebrate lineages of ancient retrotransposons, such as the Penelope-like elements (PLEs) that are present in the genomes of metazoans, fungi, and amoebozoans. Analysis of retroelement phylogenies can assist in disentangling evolutionary relationships of their hosts.

Nomenclature

Different structural and functional classification schemes for retrotransposable elements have been proposed. No scheme has been generally accepted by both the virology and the retroelement communities, and at the level of individual elements, nomenclature is frequently based on historical tradition and can be confusing. A simple classification scheme based primarily on genetic structure is used here, with acknowledgment that, with the exception of reverse transcriptase, coding domains are not uniformly retained across different groups. Detailed discussion of classification and individual elements is outside the scope of this article, but the subject can be pursued through references listed in Further Reading.

Retrotransposon Structural and Functional Features

Retrotransposons are usually subdivided into two structural superfamilies based on the presence or absence of terminal directly repeated sequences of several hundred nucleotides, the LTRs. The LTR-retrotransposon superfamily includes elements resembling integrated or endogenous proviruses, the non-LTR superfamily includes LINEs, SINEs, and SVA elements. In each superfamily, there are autonomous elements, which encode reverse transcriptase (RT) as well as a variable set of other proteins necessary for replication and transposition of the element. The superfamilies also include nonautonomous elements whose open reading frames (ORFs) usually encode no proteins, or putative proteins lacking homology to other transposable element proteins and lacking known function. Nonautonomous elements always have an autonomous partner to provide the necessary proteins *in trans*. A third group of retrotransposable elements, the PLEs, were originally described in invertebrates but have more recently also been reported in vertebrates. PLEs have distinct genomic and transcript structural features which preclude a neat fit in either the LTR or non-LTR superfamilies; consequently, this group will be treated independently in this article.

Fundamental structural features of non-LTR and LTR retrotransposons are illustrated schematically in **Figure 1**. A useful repository of consensus sequences of retrotransposons from different animal species, and their classification is curated by the Genetic Information Research Institute.

LTR Superfamily

Four major groups of LTR retrotransposons have been identified in vertebrates to date: Ty3/*gypsy*; BEL; the vertebrate retrovirus group; and Ty1/*copia*. The hepadnaviruses, a fifth group encoding RT similar to LTR retrotransposons but which are circular and lack LTRs, are discussed elsewhere in this encyclopedia. The LTR retrotransposon groups are distinguished from one another by amino acid sequence comparison of their homologous enzymatic domains, primarily RT, and by features specific to one or two groups. Nonautonomous retrotransposon families are distinguished from one another by DNA sequence comparisons of their internal domain and of their LTRs.

The DIRS1 (Dictyostelium repeat sequence 1) subclass LTRs are either inverted repeats, or split direct repeats. In addition, these elements lack the typical integrase and protease coding domains, containing instead a domain encoding a bacteriophage lambda recombinase-like protein also known as tyrosine-recombinase or Y-transposase. These features of DIRS1 make its inclusion in the LTR superfamily problematic, although its RT has homology to the more typical elements of the superfamily.

Structure

LTR elements are typically constructed as two direct LTRs flanking an internal sequence of variable coding content (**Figure 1**). The LTRs are necessary and sufficient for promoter activity and transcription of the retroelement. Functional features common to LTRs include: 5′ TG and 3′ CA dinucleotides necessary for integration into the host genome; Pol II promoter elements and a transcription start site; and a polyadenylation and cleavage signal.

In typical autonomous LTR retrotransposons, the internal sequence contains a variable but small number of ORFs. Most elements contain an ORF including Gag (group-specific antigen) and Pol (polymerase) domains, and some additionally contain an ORF for Env (envelope). Gag encodes a structural polyprotein integral to the formation of cytoplasmic virus-like particles in which reverse transcription takes place. Pol encodes several enzymatic activities: (1) protease required to cleave the translated products of the gag–pol transcript into their functional forms; (2) RT for transcribing the retrotransposon RNA into double-stranded cDNA; (3) ribonuclease H (RNase H) for processing the RNA template prior to plus strand cDNA synthesis; and (4) integrase incorporating an aspartate (D), aspartate, glutamate (E) (DDE)-type

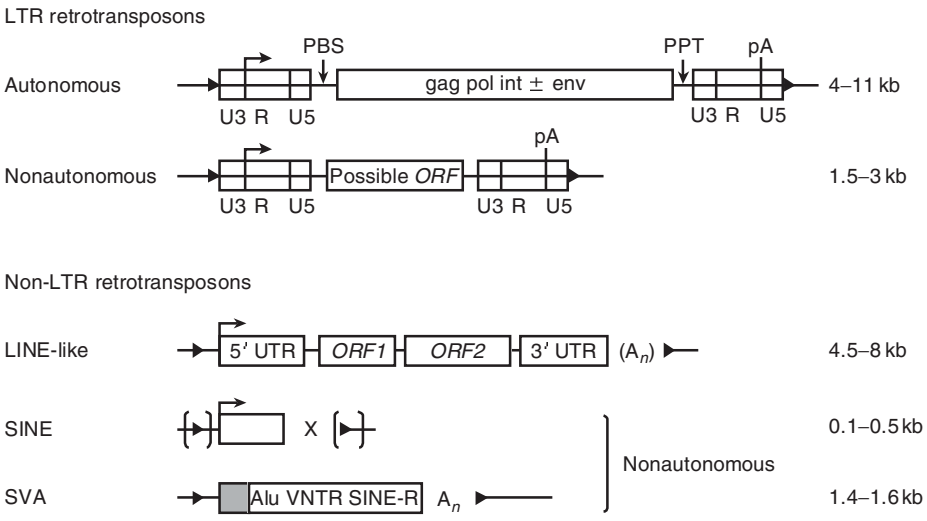


Figure 1 General structure of selected retrotransposons. A typical LTR retrotransposon is flanked by target site duplications (black arrowheads) and its LTR domains (hatched) contain a pol II promoter, transcription start site and a polyadenylation and cleavage signal. The internal domain of autonomous elements contains ORFs encoding homologs of retroviral gag and pol genes, and occasionally an additional env gene homolog. The short ORF of nonautonomous elements does not encode these proteins, if any. LINE-like elements terminate in family-specific 3′ UTRs with or without poly (A) tails, the poly (A) tail (A_n) indicated here is typical of L1. Flanking target site duplications typical of the L1 clade may or may not be present in other LINE-like clades. The autonomous LINE-like elements contain an internal pol II promoter and ORFs encoding structural and reverse transcriptase (RT) genes required for retrotransposition. SINEs contain an internal pol III promoter but lack an ORF, and may or may not be flanked by target site duplications (in parentheses), depending on their specific LINE-like partner. SVA consists of a 5′ variable number hexameric repeat (gray box), followed by an Alu-like element, a VNTR domain, the SINE-R element derived from the human endogenous retrovirus HERVK10, and a poly (A) tail. SVA elements are thought to be mobilized *in trans* by L1 proteins, and are flanked by target site duplications typical of L1. Right angled arrow, transcription start site; pA, polyadenylation and cleavage signal; PBS, primer binding site for initiating minus strand cDNA synthesis; PPT, polypurine tract used for initiation of plus strand cDNA synthesis. Element sizes are approximate ranges. The diagrams are not to scale.

transposase activity. The integrase sequence of the chromovirus genus of the Ty3/*gypsy* group additionally encodes a chromodomain, a domain found in chromatin complex structural proteins or chromatin remodeling proteins. Further information regarding retroviral genes and structure can be found elsewhere in the encyclopedia. The Pol domain order is usually protease, RT, RNase H, integrase, except for the Ty1/Copia group and the Gmr1-like elements of the Ty3/Gypsy group in which integrase is upstream of RT.

In addition to coding sequence, the internal sequence contains a 5′ primer binding site (PBS) for first-strand DNA synthesis and a 3′ polypurine tract which serves as the primer binding site for second-strand DNA synthesis. These two features are conserved in the internal sequence region of nonautonomous LTR retrotransposons.

Only a few LTR retrotransposons in any genome are intact, a majority of them being inactivated by different mutations. These may be due to intrinsically error-prone, reverse transcription by RT, to mutation associated with the nucleotide substitution rate of the specific genome, to mutation associated with methylation of CpG dinucleotides of the element, to insertional mutation by other transposable elements, or to recombination and deletion. Solitary LTRs usually far outnumber full-length elements in the genome and are understood to be the result of homologous recombination between the 5′ and 3′ LTRs of a full-length element, with excision and loss of the intervening sequence. Over time, older transpositionally active elements of a retrotransposon lineage are inactivated by mutation, becoming functionally extinct. Younger functionally intact members of the lineage continue the retrotransposition activity, or if none exist then that lineage becomes extinct. Transposition-incompetent elements in the genome can be viewed as molecular fossils of past transposition activity.

Transposition mechanism

Most LTR-retrotransposons are believed to replicate and transpose using a complex process very similar to that of infectious retroviruses, as covered elsewhere in this encyclopedia. In the DIRS1 group, the presence of a Y-transposase and lack of target site duplications suggests a different mechanism, involving RT-mediated synthesis of a closed circular cDNA, and insertion into the target using Y-mediated recombination.

Non-LTR Superfamily

Retrotransposons included in this superfamily are the autonomous LINEs and nonautonomous SINEs and SVA elements. One clade of LINEs is the LINE-1 clade (L1), and to minimize confusion, the general category of 'LINE' retrotransposons will be referred to hereafter as LINE-like.

LINE-like retrotranposon structure

Multiple clades of LINE-like retrotransposons have so far been recognized on the basis of RT and other protein sequence comparisons. At least nine clades are represented in vertebrate genomes. Common structural features of LINE-like retrotransposons include a 5′ untranslated region (UTR) containing a (G+C)-rich promoter region, followed by two nonoverlapping open reading frames (ORF1 and ORF2), and a 3′ UTR.

However, different clades are distinguished by many structural differences. A brief description of the 3′ UTR and ORF differences between the first three identified vertebrate LINE-like clades (LINE-1 (L1), CR1, and RTE) is given below to convey a sense of the structural features distinguishing LINE-like retrotransposons.

Variable-sized target-site duplications flank full-length elements of the L1 and RTE lineages, but not the CR1 lineage. The 3′ UTR of L1 characteristically contains an AATAAA polyadenylation signal and terminates in a 3′ poly (A) tail. In contrast, the 3′ UTR of CRI elements contains no A or AT-rich regions and terminates in a $[(CATTCTRT)\ (GATTCTRT)_{1\text{-}3}]$ motif, while the very short 3′ UTRs of the RTE clade are dominated by A/T-rich trimer, tetramer, and/or pentamer repeats.

In the L1 clade, ORF1 encodes a single-stranded, nucleic acid-binding protein with nucleic acid-chaperone activity. A similar activity is proposed for the ORF1 product of CR1, while the 43-amino-acid ORF1 of RTE is thought to be too short to encode these components. In most LINE-like clades, ORF2 encodes a large protein with an N-terminal endonuclease and a C-terminal RT domain. As in most LINE-like clades, the endonuclease is of the apurinic/apyrimidinic type (AP) in L1, CR1, and RTE elements. A site-specific endonuclease typifies a minority of clades. A third domain in the ORF2-encoded protein, a COOH-terminal cysteine-rich domain, is conserved in mammalian L1 elements.

As with LTR retrotransposons, most LINE-like retrotransposons in a genome are transpositionally disabled. For example, of the half million or so L1 copies in the human genome, less than 1% are intact full-length elements. In all LINE-like lineages, 5′ truncation is an exceptionally common mutation, likely reflecting the rarity of successful full-length element reverse transcription and insertion. An interesting speculation is that, by providing opportunity for L1 to acquire novel 5′ ends, 5′ truncation may be an evolutionary strategy for L1 to evade suppression of transcription by the host.

LINE-like retrotransposon transposition

LINE-like retrotransposons are thought to all use a target-primed mechanism of transposition, reverse transcribing their RNA directly on the new chromosomal integration site, although the details likely vary between the different clades and are not well understood for all. The human L1 is

perhaps the best-studied vertebrate LINE-like retrotransposon and is used here to outline the process. Genetic evidence and recent cell culture experiments suggest the following model (**Figure 2**). Following transcription, nuclear export, and translation of the L1 mRNA in the cytoplasm, L1 proteins preferentially associate with their encoding transcript ('*cis* preference') forming a cytoplasmic ribonucleoprotein particle. Upon access of the particle to the nucleus, L1 endonuclease nicks the host DNA at a loose consensus sequence (3′-AA/TTTT-5′) in the minus strand. The L1 transcript poly (A) tail base pairs with the nicked target and the target thus primes first strand synthesis using L1 RT with L1 mRNA as template. A nick in the target plus strand, offset from the minus-strand nick, is made by an unknown enzyme, possibly L1 endonuclease. Second strand synthesis proceeds using the first strand as template, perhaps primed by microhomology-mediated priming between the first strand and the 3′ end of the plus strand. Host enzymes are thought to be involved in completion of synthesis of both new strands and DNA repair linking them to the host DNA, creating the flanking, perfect target-site duplications typical of L1. L1 uses host ribonuclease H to degrade L1 RNA template, but other clades of LINE-like retrotransposons encode their own RNase H.

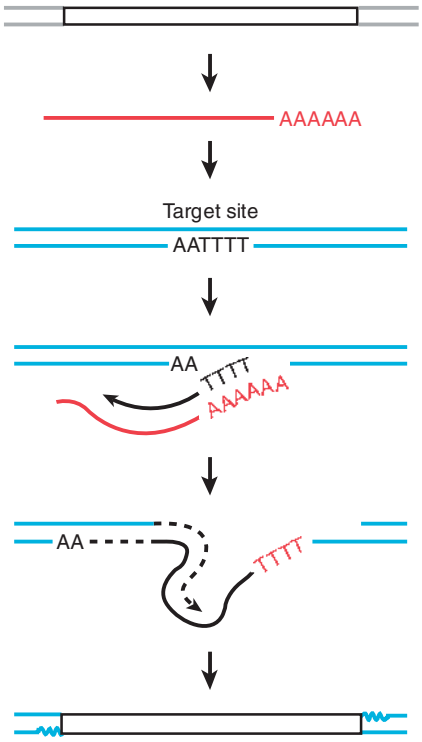


Figure 2 Retrotransposition mechanism of L1 elements. Polyadenylated L1 transcripts (red line) are transported to the cytoplasm, packaged in their own translation products, and then transported back to the nucleus. In the nucleus, L1 endonuclease nicks the minus strand of the new insertion site (blue line) at the target consensus sequence 5′ TTTT/AA 3′. The T-rich 3′-OH minus strand primes reverse transcription and first strand synthesis (black line). A nick in the plus strand, staggered in position to the minus strand nick, exposes 3′ plus strand target DNA which is thought to prime L1 second strand transcription (black broken line) using the first strand as template, after RNA degradation by RNase H and strand switching by RT. Finally, host enzymes are thought to participate in repairing the gaps in the host DNA, creating target-site duplications flanking the L1 (zig-zag lines indicate duplicated host DNA).

SINE structure

SINEs are small nonautonomous retrotransposons, usually derived from different cellular structural RNAs, such as 7SLRNA or 5S rRNA; however, most eukaryotic SINE families are derived from tRNAs. A SINE generally consists of a 5′ structural RNA-like region containing an internal polymerase III promoter, a region unrelated to structural RNA, and a 3′ end derived from a LINE-like retrotransposon in the same genome. An exception is Alu elements, whose 3′ ends are not shared with L1, their autonomous partner, unless one counts the poly (A) tail. SINEs are usually flanked by target site duplications typical of the element from which their 3′ end is derived.

SVA structure

SVA elements, exclusively found in hominoid primates, are chimeric elements named for their principal components, SINE-R, VNTR, and Alu. A 5′ (CCCTCT) hexamer repeat is followed by an antisense Alu sequence and then, multiple copies of a variable number tandem repeat (VNTR). The 3′ portion consists of the primate-specific SINE-R, derived from an LTR-retrotransposon thought to be human endogenous retrovirus K-10 (HERVK-10), and finally a polyadenylation and cleavage signal and poly (A) tail.

Transposition of SINE and SVA

Experimental evidence from several vertebrate species indicates that, as long suspected, the 3′ end of SINE transcripts is recognized *in trans* by the cognate LINE-like machinery for transposition. For example, transcripts of Alu elements, and the mouse SINEs, B1, B2, and ID, are recognized and transposed by L1 machinery, whereas UnaSINE1, an eel SINE, is retrotransposed by UnaL2. Similarly, the characteristics of SVA insertions strongly suggest they use the L1 machinery for retrotransposition.

Penelope-Like Elements

Structure

In a few species of vertebrates, a limited number of elements with intact ORFs resembling the Penelope element of *Drosophila virilis* have been described. The elements are flanked by short target site duplications, and usually consist of LTRs flanking anINT. The LTR sequences do not resemble those of LTR retrotransposons, and are thought

to represent tandem arrangement of two copies of the element with variable 5′ truncation of the upstream copy. The upstream LTR may be preceded by an inverted LTR fragment. The single ORF includes an N-terminal domain containing a conserved DKG amino acid motif, followed by the RT domain, a variable length linker sequence thought to contain a nuclear localization signal, and an endonuclease domain. The endonuclease is of the GIY-YIG type, otherwise unreported in eukaryotes.

Transposition

It is not clear how PLEs are transposed. The presence of introns in genomic copies of some PLEs found in invertebrates, and their absence from cDNA of the element, argues against an L1-like '*cis* preference' action of PLE proteins. Other possibilities include unconventional transposition of full-length unspliced mRNA, or use of a DNA template for transposition.

Evolutionary Features

Origin

Phylogenetic analyses of autonomous retrotransposable elements have historically relied on amino acid sequence alignments of RT, the only protein coding domain common to all elements. The evidence indicates that LINE-like retrotransposons are divided into 17 or more clades, many of which have wide distribution in eukaryotes. These analyses, and abundant representation of LINE-like retrotransposons in basal eukaryote genomes, support the origin of ancestral LINE-like retrotransposons close to the emergence of the eukaryotic crown group in the Proterozoic eon. Combined analyses of RT, RNaseH, and endonuclease domains suggest that ancestral LINE-like retrotransposons evolved from group II introns in genomes of eubacteria, and fungal and plant organelles, and originally possessed a single ORF for RT and a site-specific endonuclease. Early branching lineages such as L1 acquired a second ORF (ORF1), and the site-specific endonuclease was replaced by a relatively non-site-specific AP endonuclease probably acquired from the host DNA repair machinery. Later branching lineages also acquired an RNaseH domain, most likely from their eukaryotic host, but elements within these lineages have not all retained this domain. Most evidence suggests that LINE-like retrotransposon transmission is strictly vertical through the germline. Evidence supporting horizontal transmission of RTE from snakes to ruminants has been reported, although the mechanism is unknown, and the topic remains controversial.

Phylogenetic studies of LTR elements, together with their absence from some basal eukaryotic genomes, suggest they arose more recently than LINE-like retrotransposons, although this is not a settled topic. It has been speculated that LTR retrotransposons arose as a chimera between a non-LTR retrotransposon carrying RT and RNaseH and a DNA transposon carrying an integrase domain. The acquisition of additional ORFs distinguishes many clades. Evidence suggests the vertebrate retrovirus group acquired a second RNaseH domain, and the primary sequence of the first degenerated, maintaining some structural information but not the catalytic site. The acquisition of Env, enabling infectious transfer is a striking feature of some lineages within the vertebrate retroviral group, although the origin, potentially ancient, of Env in this group is obscure. Strong phylogenetic evidence from invertebrate genomes indicates that other LTR element groups independently acquired different Env-like genes from infectious viruses, but vertebrate representatives of these are yet to be described. The evolutionary success of the Chromovirus clade of the Ty3/*gypsy* group, found in plants and fungi as well as vertebrates, has been attributed to its acquisition of the chromodomain. As with LINE-like retrotransposons, LTR elements have had varying success in colonizing vertebrate genomes, although all major LTR groups are represented by an active element in at least one species of vertebrates (**Table 1**).

New autonomous endogenous LTR elements are acquired through horizontal transfer of exogenous elements, which can then invade the genome and be transmitted through the germline, blurring the distinction between exogenous and endogenous elements. An example of current interest is the ongoing infectious epidemic and endogenization of the Koala retrovirus. However, some genera of the vertebrate retrovirus group, such as the lentiviruses, appear incapable of generating endogenous elements. This appears to be the result of Env mutations that disable their ability to infect germ cells. New nonautonomous elements arise through recombination, and all are transmitted in the germline. In general, LTR elements seem to be active within a genome over short evolutionary scales relative to LINE-like lineages.

As previously mentioned, SINEs appear to have arisen from structural RNA sequences. The primate Alu, mouse B1, and related families were derived from 7SL RNA. However, almost all other SINEs are derived from tRNA, and are placed as an evolutionarily older family than the 7SL-derived group. A new SINE-like family of diverse low-copy-number species- or lineage-specific retrotransposons derived from small nucleolar RNA was recently described in vertebrates.

Construction of a RT-based PLE polygeny is problematic since PLE RT differs from all of other retroelement RTs and more closely resembles telomerase. This, together with their distinct structure, has led to general agreement that PLEs form a separate group from the LTR and non-LTR retrotransposons. A relatively early origin for PLEs is supported by grouping of PLEs found in many eukaryotic genomes. Degenerate, and full-length PLEs

Table 1 Retrotransposon clades identified in some vertebrate genomes

Type of retrotransposon	*Cartilaginous fish*[a,b]	*Teleost fish*[c] *(Tn, Tr, Dr)*	*Mammals*[d] *(Hs, Mm, Cf)*	*Birds (chicken)*[e]
Non-LTR retrotransposons[f]	2.15	0.78, 1.32, 0.39	20.42, 19.2, 16.49	6.5
Restriction-enzyme like				
NeSL	n.a.	0.08, 0.01, <0.01	n.d., n.d., n.d.	n.d.
R2	n.a.	n.d., n.d., <0.01	n.d., n.d., n.d.	n.d.
R4	n.a.	Fossils,[e] 0.09, n.d.	n.d., n.d., n.d.	n.d.
Apurinic/apyrimidinic				
R1	*	n.d., n.d., n.d.	n.d., n.d., n.d.	n.d.
L1/TX1	*	0.03, 0.06, 0.02	16.9, 18.8, 14.5	n.d.
RTE/Rex3[g]	n.a.	0.18, 0.39, 0.2	n.d., n.d., n.d.	n.d.
L2/Maui	***	0.04, 0.53, 0.11	3.22, 0.38, 1.84	0.1
L3/CR1[h]	***	n.d., n.d., n.d.	0.31, 0.05, 0.15	6.4
Rex1/Babar	***	0.45, 0.25, 0.05	n.d., n.d., n.d.	n.d.
I/Bgr	n.a.	0.01, fossils, 0.01	n.d., 0.01, n.d.	n.d.
Jockey	*	n.d., n.d., n.d.	n.d., n.d., n.d.	n.d.
LOA	*	n.d., n.d., n.d.	n.d., n.d., n.d.	n.d.
LTR retrotransposons	0.1	0.12, 0.30, 0.40	8.29, 9.87, 3.25	
Vertebrate retroviruses	*	0.03, 0.09, <0.01	8.29, 9.87, 3.25	1.3
TY1/Copia	n.a.	0.02, 0.01, <0.01	n.d., n.d., n.d.	n.d.
TY3/Gypsy[j]	*	0.06, 0.17, 0.13	n.d., n.d., n.d.	n.d.
BEL	*	Fossils, 0.02, 0.01	n.d., n.d., n.d.	n.a.
DIRS1[h]	n.a.	0.02, 0.01, 0.25	n.d., n.d., n.d.	n.a.
Penelope-like elements	n.a.	0.06, 0.09, <0.01	n.d., n.d., n.d.	n.a.
SINEs[h]	1.36		13.14, 8.22, 9.12	Fossils

[a]The estimated percentage of the genome occupied by the respective elements is shown. Where numeric estimates are unavailable, *indicates detected at low frequency, ***indicates detected at high frequency. There are some differences in calculation of the estimates as indicated below.

[b]*Callorhincus milii.* The estimates are based on analysis of 18 Mb of random sequence from this fish; copy number is uncertain and additional retrotransposon clades may be identified when the complete sequence is available. From Venkatesh B, Tay A, Dandona N *et al*. (2005) A compact cartilaginous fish model genome. *Current Biology* 15: R82–R83.

[c]Teleost fish considered here include *T. nigroviridis*, *T. rubripes*, and *D. rerio* (Tn, Tr, Dr respectively); the percentage is the percentage of RT gene-containing sequence from whole genome shotgun sequences. From Volff JN, Bouneau L, Ozouf-Costas C, and Fischer C (2003). Diversity of retrotransposable elements in compact pufferfish genomes. *Trends in Genetics* 19: 674–678.

[d]Mammals here include human, mouse and domestic dog (Hs, Mm, Cf, respectively) From Waterston RH, Lindblad-Toh K, Birney E, *et al.* (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature* 420: 520–562; Lander ES, Linton LM, Birren B, *et al.* (2001) Initial sequencing and analysis of the human genome. *Nature* 409: 860–921; Kirkness EF, Bafna V, Halpern AL, *et al.* (2003) The dog genome: Survey sequencing and comparative analysis. *Science* 301: 1898–1903.

[e]From Hillier LW, Miller W, Birney E, *et al*. (2004) Sequence and comparative analysis of chicken genome provide unique perspectives on vertebrate evolution. *Nature* 432: 695–716.

[f]Non-LTR retrotransposons are subdivided into a phylogenetically older group encoding a restriction enzyme-like endonuclease, and a younger group with an apurinic/apyrimidinic endonuclease.

[g]Members of this clade detected in reptiles.

[h]Members of this clade detected in reptiles and amphibians.

[i]Fossils of this clade detected in reptiles, potentially active elements detected in amphibians.

n.a. indicates data not known. n.d. indicates the element was not detected after searching. Fossils are elements deemed to be extinct, having lost the means of retrotransposition or lacking evidence of recent retrotransposition.

have been reported from fungi and a wide range of invertebrates. In vertebrates, they have so far been found in teleost fish, sharks, and amphibia, not always as degenerate molecular fossils. It is possible that some are still active in the fish *Tetraodon nigroviridis* and *Danio rerio*.

Retrotransposons as Phylogenetic Markers

At any point in time, relatively few copies of a retrotransposon are capable of replication and retrotransposition. As these copies accumulate mutations, the mutations are inherited by subsequent members of the retroelement lineage in the host genome. Once the host lineage splits, new insertions will occur independently in each descendant lineage. Since stably integrated elements are identical by descent, and the probability of parallel independent insertions into a genome is low, retrotransposons can be considered to be homoplasy-free characters, offering unique utility as markers to study evolution of host species. Retrotransposons have been used in several species of fish, mammals, birds, and reptiles, to clarify phylogenetic relationships. As suggested above, these analyses rest on the assumption that, in general, retrotransposons integrate randomly into genomes, with an

exceptionally low probability that an element would independently insert in orthologous positions in two species. Another assumption is that, unless removed by segmental deletion, an insertion remains in its locus once fixed in the genome, eventually becoming unrecognizable as a result of accumulated mutations. Evidence that these assumptions do not always hold indicates that, as with analyses based on other genome elements, care is required in the conduct and interpretation of phylogenetic analyses based on retroelements.

Retrotransposon Effects on the Genome

Transposable elements have profoundly affected the structure and function of vertebrate genomes in many different ways.

Retrotransposon Content

Vertebrate genomes differ markedly in the total quantity and diversity of retrotransposons they contain, and the evolutionary trajectory of different elements. Genome size has been correlated to the quantity of transposable elements in the genome. Retrotransposons occupy approximately 35–50% or more of mouse, human, and domestic dog genomes. The chicken genome is approximately 39% of the size of mouse and human genomes, but only about 8% of the genome is recognizable retrotransposable elements. In the very compact genomes of smooth pufferfish *T. nigroviridis* and *Takifugu rubripes*, roughly 12% the size of mouse and human genomes, retrotransposable elements occupy less than 5% of the DNA. The enormous retrotransposon copy number accumulation in species with large genomes suggests that these species lack some constraint on retrotransposon activity that is present in animals with small genomes. It has been suggested that retrotransposons physically organize the genome through higher order chromatin structuring, provision of dispersed regulatory units, and other means. Thus, differences in genome retrotransposon content could significantly affect the operation of different genomes.

The pufferfish genomes, considering their small size, contain a remarkable diversity of retrotransposons in comparison with mammals. In mammalian genomes, 4 LINE-like clades have been identified (L1, L2, RTE, and CR1) with L1 the major currently active element, while in pufferfish genomes seven clades have been identified (NeSL, R4, L1, RTE, I, L2, Rex1) most of which have been recently active. Within the L1 clade alone, a single lineage has dominated L1 activity in mouse and human genomes since the mammalian radiation and comprises about 20% of the genome, whereas multiple L1 lineages predating the mammalian L1 emergence are active in several fish species, although present in very low copy numbers. All the major groups of LTR retrotransposons are represented in pufferfish genomes, as are PLEs. In contrast, only the vertebrate retrovirus group (endogenous retroviruses) and a few molecular fossils of the Ty3/Gypsy and DIRS1 groups are present in the mouse and human genomes, and there is no evidence for the presence of PLEs. The zebrafish, *D. rerio*, with a larger genome than the pufferfish, also hosts a great variety of retrotransposons. Whether these obvious differences in retrotransposon evolutionary biology between the three fish genomes and the mouse/human genomes represent the general case between teleosts and mammals is as yet unknown. Birds, as exemplified by the chicken, are different again. A single LINE-like retrotransposon, CR1, comprises about 90% of all identified chicken retrotransposons, L2/MIRs and endogenous retroviruses equally comprise the remainder. Curiously, the chicken genome lacks SINEs although it contains faint remnants of ancient SINEs pre-dating the bird-mammal split. A variety of retrotransposons have been reported from reptile genomes, some revealing lineage-specific retrotransposons such as the Sauria SINE derived from a LINE-like element of the RTE clade.

Mammalian genomes *per se* can differ markedly from one another in their retrotransposon content and activity, reflecting the evolutionary trajectory of different elements. For example, LINE, SINE, and LTR elements occupy similar percentages of mouse and human genomes. However, endogenous retroviruses are almost extinct in humans, while multiple families of endogenous retroviruses are active in rodents. The L1 lineage appears to be still active in most mammalian genomes, but recent evidence indicates its extinction in several tribes of sigmodontine rodents at or after their divergence from the earliest extant genus. As would be predicted, L1 extinction was linked to extinction of B1, a rodent SINE thought to be transposed by L1. Unexpectedly, vigorous expansion of an endogenous retrovirus, MysTR, to very high copy numbers unprecedented in any other endogenous retrovirus group, was also linked to L1 extinction. Whether there is any relationship between L1 activity and endogenous retrovirus activity is unknown.

Genomic Distribution of Retrotransposons

Diverse patterns of retrotransposon distribution in genomes are dependent on the type of element and the host genome. In the mouse and human, retrotransposons are generally dispersed widely through the genome. In contrast, retroelements strongly cluster in heterochromatic gene-poor regions in *T. nigroviridis*, similar to the distribution in *Drosophila*. Whether this extremely uneven distribution is specific to small genomes, or teleosts, is unknown, and how and why it might arise is the subject for some speculation.

At a smaller scale, much variation is evident within genomes. The density of retrotransposons varies among different chromosomes in individual vertebrate species, and in mammals is usually highest on the X and Y chromosomes. A higher density of L1 on X chromosomes than autosomes is also observed in the Ryuku spiny rat, *Tokudaia osimensis*, in which both males and females have an XO karyotype, arguing against the hypothesis that evolutionary selection of a high density of L1 on X is due to involvement of L1 in X-inactivation. Although LTR elements are distributed more or less uniformly in mouse and human genomes, L1 occurs at much higher density in gene-poor, AT-rich regions and the human SINE, Alu, occurs at high density in GC-rich regions. Closer analysis demonstrates young Alus preferentially occur in AT-rich regions, whereas in older Alus a stronger bias toward GC-rich regions emerges. One of several possible explanations for this skewed distribution of LINEs and Alus is selective targeting of L1 and Alu to AT-rich regions, and subsequent positive selection for Alus in gene-rich GC-rich regions. A significant antisense bias observed for many older intact LTR elements and solitary LTRs located in mammalian introns, is thought to arise from negative selection of sense-oriented elements bearing strong splice acceptor or donor sites, or strong transcriptional regulatory function.

Effect on Genes

Through their specific exaptation for use by the host, or through incorporation within genes, retrotransposons significantly contribute to gene evolution, and some examples follow. Sequences from LTR elements alone occupy about 1.5% of mouse and 0.8% of human genes, and genes containing these elements tend to be newly evolved genes. Indeed, LTRs drive developmentally regulated expression of cellular genes in early mouse embryos, perhaps an evolving example fitting with the hypothesis that randomly distributed retrotransposons provide a means to set up, over evolutionary time, co-ordinated transcriptional regulatory circuits. An interesting example of exaptation is the independent selection by sheep, primate, and rodent lineages of Env expression from lineage-specific endogenous retroviruses for function in placental syncytiotrophoblast morphogenesis. At least one ultraconserved sequence in mammalian genomes has proved to be an ancient SINE whose multiple insertions, predating the divergence of amniotes and amphibians, have been exapted for use in transcriptional regulation or as a conserved exon in multiple unrelated genes.

New insertions can also disrupt normal gene function through interference with transcriptional regulation, through physical alteration of transcripts by aberrant splicing or premature termination, and through exon shuffling by inadvertent transduction of non-retrotransposon sequences. While L1 generates processed pseudogenes in the human genome, the paucity of processed pseudogenes in the chicken genome indicates that CR1 does not, suggesting that its retrotransposition machinery does not recognize mRNA. Thus, different vertebrate LINE-like retrotransposons may differ in their effects on different genomes.

Retrotransposons also act as substrates for recombination, fostering genomic instability, and are involved in both duplications and deletions within genomes, and other structural rearrangements. For example, in the human genome, SVA elements are reported to be associated with about 53 kbp of genomic duplications in the human genome, including duplication of entire genes and the creation of new genes. Recently, recombination hot spots in mouse and human genomes were linked with a sequence, CCTCCCT, found in an ancient nonautonomous LTR element (THE1) in humans. The LTR element itself is not recognizable in mouse, likely having been mutated beyond recognition by the higher nucleotide substitution rate in this species. Finally, illegitimate recombination between Alu elements in primate genomes has been linked with occasional genomic deletions. However, the effect of different retrotransposons on genome integrity may vary according to the retrotransposon type and host species. For example, high karyotypic variation in sigmodontine rodent species with extinct L1 compared with those maintaining active L1 supports the notion that L1 proteins may be important for DNA break–repair in these animals. Interestingly, a single L1 element, L1_MM, was enriched in regions of low recombination activity in the mouse, and L1 is underrepresented in human recombination hot-spots.

Host Responses to Retrotransposons

The inherent tendency of retrotransposons to amplify their copy number creates mutagenic insertions potentially harmful to the host, leading to the view of retrotransposons as genomic parasites. An adaptive response by the host would selectively encourage the evolution of repressive mechanisms directed against retroelements, and this in turn would exert selective pressure on retroelements to resist repression. It has been suggested that evolution of the APOBEC3 family of cytidine deaminases, cellular inhibitors of retrotransposition of LINE-like, SINE and LTR retrotransposons, may have been driven in part as a genome response to invasion of mammalian genomes by retrotransposons. In addition to random mutation of active elements within the genome, other strategies to inhibit retrotransposon proliferation include transcriptional silencing through epigenetic modifications to chromatin and DNA, and post-transcriptional silencing through RNA interference. From time to time, retroelements

escape repression and undergo expansion in the affected genome. Thus, continued activity of a retroelement in the genome, such as of endogenous retroviruses in the mouse, may indicate that a host is lagging in the evolutionary race to control the genome invader. Some retrotransposons have been astoundingly successful in certain genomes, for example, there are over 1×10^6 Alu copies in the human genome, consisting of one family with about 20 subfamilies. This, together with the low frequency of pathogenic effects for many retroelements, and the adoption by hosts of many elements for their own biology, suggest the controversial idea that many retrotransposons have formed or are forming a symbiotic rather than parasitic relationship with their hosts.

Acknowledgments

This work was supported in part by VSPHS NIH (R01HD037102).

See also: Retroviruses: General Features.

Further Reading

Aparicio S, Chapman J, Stupka E, *et al.* (2002) Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes*. *Science* 297: 1301–1310.
Craig NL, Craigie R, Gellert M, and Lambowitz AM (eds.) (2002) *Mobile DNA II*. Herndon, VA: ASM Press.
Curcio MJ and Derbyshire KM (2003) The outs and ins of transposition: From mu to kangaroo. *Nature Reviews Molecular Cell Biology* 4: 865–877.
Eickbush TH and Furano AV (2002) Fruit flies and humans respond differently to retrotransposons. *Current Opinion in Genetics and Development* 12: 669–674.
Furano AV, Duvernell DD, and Boissinot S (2004) L1 (LINE-1) retrotransposon diversity differs dramatically between mammals and fish. *Trends in Genetics* 20: 9–14.
Goodwin TJ and Poulter RT (2001) The DIRS1 group of retrotransposons. *Molecular Biology and Evolution* 18: 2067–2082.
Hedges DJ and Deininger PL (2006) Inviting instability: Transposable elements, double-strand breaks, and the maintenance of genome integrity. *Mutation Research: Fundamental Mechanisms of Mutagenesis* doi:10.1016/j.mrfmmm.2006.11.021.
Hillier LW, Miller W, Birney E, *et al.* (2004) Sequence and Comparative analysis of chicken genome provide unique perspectives on vertebrate evolution. *Nature* 432: 695–716.
Kazazian HH, Jr. (2004) Mobile elements: Drivers of genome evolution. *Science* 303: 1626–1632.
Kordis D (2005) A genomic perspective on the chromodomain-containing retrotransposons: Chromoviruses. *Gene* 347: 161–173.
Kirkness EF, Bafna V, Halpern AL, *et al.* (2003) The dog genome: Survey sequencing and comparative analysis. *Science* 301: 1898–1903.
Lander ES, Linton LM, Birren B, *et al.* (2001) Initial sequencing and analysis of the human genome. *Nature* 409: 860–921.
Malik HS and Eickbush TH (2001) Phylogenetic analysis of ribonuclease H domains suggests a late, chimeric origin of LTR retrotransposable elements and retroviruses. *Genome Research* 11: 1187–1197.
Ostertag EM and Kazazian HH, Jr. (2001) Biology of mammalian L1 retrotransposons. *Annual Review of Genetics* 35: 501–538.
Piskurek O, Austin CC, and Okada N (2006) Sauria SINEs: Novel short interspersed retroposable elements that are widespread in reptile genomes. *Journal of Molecular Evolution* 62: 630–644.
Roy-Engel AM, Carroll ML, El-Sawy M, *et al.* (2002) Non-traditional Alu evolution and primate genomic diversity. *Journal of Molecular Biology* 316: 1033–1040.
van de Lagemaat LN, Medstrand P, and Mager DL (2006) Multiple effects govern endogenous retrovirus survival patterns in human gene introns. *Genome Biology* 7: R86.
Venkatesh B, Tay A, Dandona N, *et al.* (2005) A compact cartilaginous fish model genome. *Current Biology* 15: R82–R83.
Volff JN (ed.) (2005) *Cytogenetic and Genome Research, Vol. 110: Retrotransposable Elements and Genome Evolution*. Basel: S Karger AG.
Voff JN, Bouneau L, Ozouf-Costas C, and Fischer C (2003) Diversity of retrotransposable elements in compact Pufferfish genomes. *Trends in Genetics* 19: 674–678.
Waterston RH, Lindblad-Toh K, Birney E, *et al.* (2002) Initial sequencing and comparative analysis of the mouse genome. *Nature* 420: 520–562.
Weber MJ (2006) Mammalian small nucleolar RNAs are mobile genetic elements. *Public Library of Science Genetics* 2: e205doi:10.1371/journal.pgen.0020205.

Relevant Website

http://www.girinst.org – Genetic Information Research Institute, Mountain View.

Retroviral Oncogenes

P K Vogt and A G Bader, The Scripps Research Institute, La Jolla, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Chimeric transcript An mRNA that encodes a fusion protein derived from two individual and originally separate genes.

Subtractive hybridization A method to identify differentially expressed mRNAs; the method is based on hybridizing a 'tester' mRNA population with a 'driver' mRNA population and selectively eliminating tester–driver hybrids.

Historical Perspective

Retroviruses that carry an oncogene induce neoplastic transformation of cells in culture and rapidly cause tumors in the animal. Early studies with Rous sarcoma virus in chicken embryo fibroblasts showed a controlling influence of the retroviral genome on the properties of the transformed cell and suggested that the virus carries oncogenic information. The isolation of temperature-sensitive mutants of Rous sarcoma virus firmly established a dominant role of viral genetic information in the process of virus-induced carcinogenesis. These mutants of Rous sarcoma virus encode an unstable oncoprotein and are able to transform chicken embryo fibroblasts at a low, permissive temperature but fail to induce oncogenic changes at an elevated, nonpermissive temperature. Yet the virus is able to propagate under both permissive and nonpermissive conditions, demonstrating that virus viability and replication are not affected by the mutation and that oncogenicity is governed by genetic information that is distinct from viral replicative genes. Cells transformed by temperature-sensitive Rous sarcoma virus under permissive conditions become normal in morphology and growth behavior if the cell cultures are switched to the nonpermissive temperature. Thus, viral genetic information is required for the initiation as well as for the maintenance of the transformed cellular phenotype. A physical correlate to these genetic experiments was found in studies with transformation-defective Rous sarcoma virus. These spontaneously emerging variants of the virus fail to induce transformation in cell culture but are able to generate viable progeny virus that remains transformation defective. The transformation-defective viruses contain genomes that are about 20% smaller than the genome of oncogenic Rous sarcoma virus. The missing genetic information is of obvious importance for oncogenicity. Subtractive hybridization of DNA transcripts from transformation-defective and transformation-competent viral genomes generates a specific cDNA probe for the oncogenic sequences in Rous sarcoma virus. This probe hybridizes to cellular DNA, revealing the presence of sequences that are homologous to the transforming information of Rous sarcoma virus in the genome of all vertebrate cells. Retroviral oncogenes are therefore derived from the cell genome. The cellular versions of retroviral oncogenes are also referred to as proto-oncogenes or, more commonly, as c-*onc* genes; the corresponding viral versions are called v-*onc* genes. The discovery of oncogenes owes much to the exceptional genetic structure of Rous sarcoma virus. This virus is unique among oncogene-carrying retroviruses in that it is replication competent, containing a full set of viral genes plus the oncogene. Loss of the oncogene in transformation-defective variants still leaves a viable virus, greatly facilitating molecular and genetic analyses.

Taxonomy of Oncogene-Carrying Retroviruses

Retroviruses are broadly divided into two categories, viruses with simple genomes and viruses with complex genomes. Simple retroviral genomes contain four coding regions with information for virion proteins. These regions are referred to as *gag*, which directs the synthesis of matrix, capsid, and nucleoprotein structures; *pro*, generating the virion protease; *pol*, containing the information for the reverse transcriptase and integrase enzymes; and *env*, which encodes the surface and transmembrane components of the envelope protein. Complex retroviral genomes contain additional information for regulatory nonvirion proteins that are translated from multiply spliced mRNAs.

In taxonomic terms, retroviruses form a family that consists of the subfamilies *Orthoretrovirinae* and *Spumaretrovirinae*. Oncogenes are carried by only two genera of the orthoretroviruses. These are the alpharetroviruses, representing the avian leukosis complex of viruses, and the gammaretroviruses, encompassing murine, feline, and primate leukemia and sarcoma viruses. This restriction of oncogenes to two viral genera probably reflects a set of conditions that have to be met for the acquisition of cellular sequences by a retroviral genome. These conditions include absence of cytotoxicity and efficient viral integration and replication. Complex retroviruses may not tolerate the insertion of cellular sequences into the viral genome because some functions of the regulatory nonvirion proteins that would be displaced by a cellular insert can probably not be complemented in *trans*. These are all hypothetical reasons for the occurrence of oncogenes in just a few retroviruses; mechanistic explanations of these restrictions are currently not available.

Acquisition of Cellular Sequences by Retroviral Genomes

The acquisition of a cellular oncogene by a retrovirus is a rare event that occurs during viral passage in an animal but is seldom seen in cell culture. Because *de novo* acquisition of cellular sequences cannot be reproduced with significant frequency in cultured cells, the molecular mechanism of acquisition has to be reconstructed using information derived from the structure of viral genomes and from the virus life cycle. The life cycle of retroviruses contains two steps that leads to genetic recombination. (1) Retroviruses are diploid and thus can form heterozygous viral particles. Recombination between distinct but related viral genomes encased in the same particle occurs very frequently and probably results from copy-choice events that occur during reverse transcription. (2) Integration of the provirus into the cellular

genome produces a recombinant between virus and cell (**Figure 1**). These two recombinational activities of retroviruses can explain the incorporation of cellular sequences into the viral genome. The first step in this acquisition consists of the integration of a provirus containing a single 5′ (left-hand) long terminal repeat (LTR) into the oncogene proper or into the immediate upstream vicinity of a cellular oncogene. This provirus can then produce a chimeric RNA transcript that starts in the viral LTR, continues by read-through into the cellular gene, and terminates with the poly A stretch of the cellular gene. Alternatively, such a chimeric transcript can be generated by a splicing event that uses a viral splice donor and a cellular splice acceptor in joining upstream viral to downstream cellular sequences. Chimeric RNAs of this type would be incorporated into virions. In the subsequent cycle of infection, reverse transcription could effect a second recombination event, generating a junction between the 3′ region of the cellular sequences and a part of the viral genome carrying the 3′ terminal repeat sequences that are essential for the efficient production of proviral DNA. In this model, the first step of recombination in acquiring cellular sequences is the integration of the provirus, which is DNA-based recombination. The second step occurs during reverse transcription of a heterozygote particle and is RNA-based recombination. It is possible, however, to envisage a second recombination step that is also DNA based. It requires the integration of another provirus immediately downstream of the cellular oncogene to serve as donor of the necessary 3′ (right-hand) LTR for the new provirus that now carries an insertion of cellular sequences. Currently available experimental evidence can be adduced to support either model for the second recombination step in the acquisition of a cellular oncogene. Specific oncogene acquisitions may in fact occur by either mechanism.

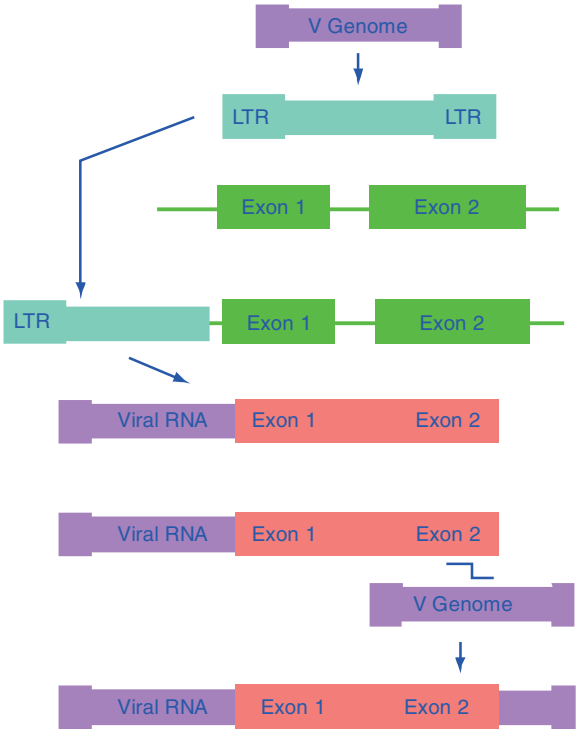


Figure 1 A hypothetical mechanism for the acquisition of a cellular oncogene by a retroviral genome. The genome of a retrovirus without oncogene is transcribed into DNA and, in a first recombinational event, is integrated upstream of a cellular oncogene containing two exons. The right-hand long terminal repeat (LTR) and adjacent sequences of the viral genome are lost during the integration process. Transcription from the integrated proviral LTR generates a chimeric mRNA by read-through or aberrant splicing. This mRNA contains cell-derived oncogene and viral information. The chimeric mRNA is packaged into viral particles together with wild-type viral genomes. In the next round of infection the second recombinational event occurs during reverse transcription and adds 3′ viral terminal sequences to the DNA transcript, facilitating the production and integration of a functional provirus that contains a cell-derived oncogene. Green: DNA, red and purple: RNA.

Retroviruses Carrying an Oncogene Are Replication Defective

The incorporation of cellular sequences into the retroviral genome occurs at the expense of viral sequences that are displaced in the process. With one notable exception, Rous sarcoma virus, transducing retroviruses lack one or several essential viral genes. Typical deletions extend from within the viral *gag* gene into the *env* gene, eliminating the 3′ portion of *gag*, all of the *pol* gene, and part of *env*. Such defective viruses can infect cells, integrating into the cellular genome, producing the oncoprotein, and inducing neoplastic transformation, but they are unable to synthesize infectious progeny virus. For infectious virus production, they require co-infection of the same cell with a closely related helper retrovirus that in *trans* provides those replicative functions that are missing from the defective transforming virus. Helper retroviruses contain a complete set of viral genes but have not incorporated any cellular oncogenic sequences. They do not induce oncogenic transformation when replicating in cultured cells. In animal infections, they can cause tumors by insertional mutagenesis after extended latent periods. These tumors result from transcriptional upregulation of a cellular oncogene by promoter activities of a provirus integrated nearby.

Oncoproteins Are Often Fusion Proteins

As a consequence of the initial integration event that leads to oncogene acquisition, viral oncogenes usually code for fusion proteins, consisting of an N-terminal portion

derived from the virus and a C-terminal component representing the oncoprotein proper. The viral sequences generally include the N-terminus of the Gag polyprotein. The initial recombination event also often eliminates short lengths from the N-terminus of the cellular oncoprotein, except when viral sequences are spliced onto the cellular gene. Fusion of the cellular oncoproteins to viral *gag* sequences can have important consequences that contribute to the gain of function associated with the viral oncogene. The efficiency of protein translation and the stability of the fusion protein are often increased. Gag sequences also provide an affinity for the plasma membrane which can be critical in activating the oncogenic potential of the protein.

Functional Classes of Oncogenes

Retroviral oncogenes code for components of cellular growth-regulatory signals (**Table 1**). The major functional categories include growth factors, receptor and nonreceptor tyrosine kinases, serine-threonine and lipid kinases, adaptor proteins, hormone receptors, and a variety of transcriptional regulators. Cellular growth signals are propagated from the cell periphery to the nucleus. Therefore, the nuclear oncoproteins coding for transcriptional regulators are the ultimate effectors of oncogenicity, converting the signal into a pattern of gene expression that is the basis of the oncogenic phenotype of the cell. Because of this pivotal role of nuclear oncoproteins in specifying neoplastic properties, oncogenic transformation can be viewed as a case of aberrant transcription. However, there is also abundant evidence for a critical role of protein translation in oncogenesis. Oncogenesis induced by the PI3K pathway in particular depends on differential translation of specific growth-promoting proteins.

Retroviral Transduction of a Cellular Oncogene Results in Gain of Function

Compared to their cellular counterparts, retroviral oncoproteins show an increase in activity. Some of this gain of function is purely quantitative: the viral promoter assures highly efficient transcription of the oncogene. There are several oncogenes for which mere overexpression is sufficient to turn them into effective agents of neoplastic transformation. These code for wild-type oncoproteins that deregulate cellular growth by their virus-mediated abundance. However, many oncoproteins carry specific mutations that are responsible for the gain of function. These mutations remove or inactivate domains of the oncoprotein that effect negative regulation or may enhance specific enzymatic activities of the oncoprotein by other mechanisms including conformational change, improved substrate affinity, or change in cellular localization. They may stabilize the protein or alter the spectrum of downstream targets. Thus both quantitative and qualitative changes resulting from viral transduction and the associated mutation of the oncogene contribute to the gain of function.

Cooperation of Oncogenes

A few retroviruses carry two oncogenes. These oncogenes are derived from distinct cellular loci situated at distant positions within the host genome. Retroviruses with two oncogenes include Avian erythroblastosis virus R (AEV-R), carrying v-*erbA* and v-*erbB*, Avian myeloblastosis–erythroblastosis virus E26, encoding *myb* and *ets* as a single Gag-myb-ets protein product, and Avian myelocytoma virus MH2, which contains the v-*myc* and v-*mil* (raf) genes. Each of these single oncoproteins can induce neoplastic transformation on its own. However, viruses with two oncogenes are more potent in transformation, indicating that the two oncoproteins cooperate. Whereas primary avian cells can be readily transformed by viruses carrying a single oncogene, in mammalian cells oncogenic transformation generally requires the cooperation of two or more oncogenes. The reasons for this difference in cellular susceptibility to oncogene action are not known.

Coda and Outlook

There are still significant gaps in our knowledge of retroviral oncogenes. We do not know and cannot reproduce the exact mechanism of oncogene acquisition by a retroviral genome. We have only tentative explanations for the failure of some groups of retroviruses to capture cellular sequences. We have no idea why certain growth-promoting cellular genes have not shown up as retroviral oncogenes. Have not enough retroviruses been studied or is there an active mechanism that excludes certain genes? Does some cryptic homology between provirus and oncogene determine the spectrum of genes that can be incorporated? On a more basic level, there is evidence that oncogenes can induce tumors by activating the transcription of specific micro-RNAs. Are there also rapidly oncogenic retroviruses that carry a micro-RNA gene as an oncogene?

Despite these puzzling questions, at a fundamental level the nature and workings of retroviral oncogenes are well understood and have provided important insights into the mechanisms of virus-induced carcinogenesis. The discovery that all retroviral oncogenes are derived from cellular information has greatly expanded the significance of these genes. Originally seen as viral

Table 1 Classes of retroviral oncogenes

Functional groups and oncogenes	*Identity and function of cellular homolog*	*Retrovirus*
Growth factor		
sis	Platelet–derived growth factor (PDGF)	Simian sarcoma virus
Receptor tyrosine kinases		
erbB	Receptor of epithelial growth factor (EGF)	Avian erythroblastosis virus
fms	Receptor of colony-stimulating factor 1 (CSF-1)	McDonough feline sarcoma virus
sea	Receptor of macrophage-stimulating protein (MSP)	Avian erythroblastosis virus S13
kit	Hematopoietic receptor of stem cell factor (SCF)	Hardy–Zuckerman 4 feline sarcoma virus
ros	Orphan receptor tyrosine kinase	Avian sarcoma virus UR2
mpl	Hematopoietic receptor of thrombopoietin	Mouse myeloproliferative leukemia virus
eyk	Closest homolog of mammalian c-*mer*; c-Mer ligands include anticoagulation factor protein S and the growth arrest-specific gene product Gas6	Avian retrovirus RPL30
Hormone receptor		
erbA	Thyroid hormone receptor	Avian erythroblastosis virus
G proteins		
H-*ras*	GTPase; MAPK signal transduction	Harvey murine sarcoma virus
K-*ras*	GTPase; MAPK signal transduction	Kirsten murine sarcoma virus
Adaptor protein		
crk	Adaptor protein containing SH2 and SH3 domains; PI3K/Akt signal transduction	Avian sarcoma virus CT10
Nonreceptor tyrosine kinases		
src	MAPK and PI3K/Akt signal transduction	Rous sarcoma virus
yes	Src family kinase; signal transduction	Avian sarcoma virus Y73
fps	Cytokine receptor signaling; the *fps* and *fes* oncogenes are derived from the same cellular gene	Fujinami poultry sarcoma virus
fes	Cytokine receptor signaling; the *fps* and *fes* oncogenes are derived from the same cellular gene	Gardner–Arnstein feline sarcoma virus
fgr	Src family kinase; signal transduction	Gardner–Rasheed feline sarcoma virus
abl	Cytoskeletal signaling and cell cycle regulated transcription	Abelson murine leukemia virus
Serine/threonine kinases		
mos	Regulator of cell cycle progression; required for germ cell maturation; activates MAPKs	Moloney murine sarcoma virus
raf	MAPKKK, MAPK signal transduction	Murine sarcoma virus 3611
akt	PI3K/Akt signal transduction	Murine retrovirus AKT8
Lipid kinase		
p3k	PI 3-kinase; PI3K/Akt signal transduction	Avian sarcoma virus 16
Transcriptional regulators		
jun	bZIP protein of AP-1 complex; homo- and heterodimer with AP-1 family members; cell cycle progression	Avian sarcoma virus 17
fos	bZIP protein of AP-1 complex; heterodimer with AP-1 family members; cell cycle progression	FBJ murine osteogenic sarcoma virus
myc	bHLH-ZIP protein; heterodimer with Max; cell cycle progression	Avian myelocytoma virus MC29
myb	HTH protein; development of hematopoietic system	Avian myeloblastosis virus

Continued

Table 1 Continued

Functional groups and oncogenes	*Identity and function of cellular homolog*	*Retrovirus*
ets	HTH protein; myeloid and eosinophil differentiation	Avian myeloblastosis–erythroblastosis virus E26
rel	p65 NF-κB subunit; survival pathways	Avian reticuloendotheliosis virus
maf	bZIP protein; homo- and heterodimers with various bZIP proteins; differentiation of various tissues	Avian musculoaponeurotic fibrosarcoma virus
ski	Adaptor protein for various transcription factors; chromatin-dependent transcriptional regulation; muscle differentiation	Avian Sloan-Kettering retrovirus
qin	Avian homolog of mammalian brain factor 1 (BF-1/FoxG1); forkhead/winged helix (FOX) protein; monomer; neuronal differentiation	Avian sarcoma virus 31

Abbreviations: Gas6, growth arrest-specific gene 6; MAPK, mitogen-activated protein kinase; MAPKKK, mitogen-activated protein kinase kinase kinase; SH2, Src homology domain 2; SH3, Src homology domain 3; PI3K, phosphoinositide 3-kinase; AP-1, activator protein 1; bZIP, basic region leucine zipper; bHLH-ZIP, basic region helix–loop–helix leucine zipper; HTH, helix–turn–helix; NF-κB, nuclear factor kappa B; FBJ, Finkel–Biskis–Jinkins.

pathogenicity genes, they have become universal effectors of oncogenicity. Viruses have been demoted to just one of several instruments that can activate these genes; mutation, overexpression, and amplification are some of the others. The study of oncogenes, initially a somewhat esoteric part of virology, has grown to determine the course of cancer research during the past three decades and has contributed immensely to our understanding of cancer in general. As targets of specific inhibitors, oncoproteins are now revolutionizing cancer treatment. Gleevec, directed at the BCR-ABL oncoprotein in chronic myelogenous leukemia, and Iressa, inhibiting mutants of the epithelial growth factor receptor in non-small cell lung cancer, have dramatically proven the promise of therapy targeted to oncoproteins.

Acknowledgments

This work was supported by grants from the National Cancer Institute. This is manuscript number 18 394 of The Scripps Research Institute.

See also: Feline Leukemia and Sarcoma Viruses.

Further Reading

Bister K and Jansen HW (1986) Oncogenes in retroviruses and cells: Biochemistry and molecular genetics. *Advances in Cancer Research* 47: 99–188.

Duesberg PH and Vogt PK (1970) Differences between the ribonucleic acids of transforming and nontransforming avian tumor viruses. *Proceedings of the National Academy of Sciences, USA* 67: 1673–1680.

Hughes SH (1983) Synthesis, integration, and transcription of the retroviral provirus. *Current Topics in Microbiology and Immunology* 103: 23–49.

Land H, Parada LF, and Weinberg RA (1983) Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. *Nature* 304: 596–602.

Martin GS (1970) Rous sarcoma virus: A function required for the maintenance of the transformed state. *Nature* 227: 1021–1023.

Schwartz JR, Duesberg S, and Duesberg PH (1995) DNA recombination is sufficient for retroviral transduction. *Proceedings of the National Academy of Sciences, USA* 92: 2460–2464.

Stehelin D, Guntaka RV, Varmus HE, and Bishop JM (1976) Purification of DNA complementary to nucleotide sequences required for neoplastic transformation of fibroblasts by avian sarcoma viruses. *Journal of Molecular Biology* 101: 349–365.

Stehelin D, Varmus HE, Bishop JM, and Vogt PK (1976) DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. *Nature* 260: 170–173.

Swanstrom R, Parker RC, Varmus HE, and Bishop JM (1983) Transduction of a cellular oncogene: The genesis of Rous sarcoma virus. *Proceedings of the National Academy of Sciences, USA* 80: 2519–2523.

Tam W, Hughes SH, Hayward WS, and Besmer P (2002) Avian bic, a gene isolated from a common retroviral site in avian leukosis virus-induced lymphomas that encodes a noncoding RNA, cooperates with c-myc in lymphomagenesis and erythroleukemogenesis. *Journal of Virology* 76: 4275–4286.

Temin HM (1960) The control of cellular morphology in embryonic cells infected with Rous sarcoma virus *in vitro*. *Virology* 10: 182–197.

Toyoshima K and Vogt PK (1969) Temperature sensitive mutants of an avian sarcoma virus. *Virology* 39: 930–931.

Varmus HE (1982) Form and function of retroviral proviruses. *Science* 216: 812–820.

Varmus HE (2006) The new era in cancer research. *Science* 312: 1162–1165.

Vogt PK (1971) Genetically stable reassortment of markers during mixed infection with avian tumor viruses. *Virology* 46: 947–952.

Vogt PK (1971) Spontaneous segregation of nontransforming viruses from cloned sarcoma viruses. *Virology* 46: 939–946.

Wang LH (1987) The mechanism of transduction of proto-oncogene c-src by avian retroviruses. *Mutation Research* 186: 135–147.

Relevant Website

http://www.ncbi.nlm.nih.gov – Virus Databases Online (ICTVdB Index of Viruses), National Center for Biotechnology Information.

Retroviruses of Insects

G F Rohrmann, Oregon State University, Corvallis, OR, USA

© 2008 Published by Elsevier Ltd.

Glossary

Baculovirus F protein The envelope fusion protein encoded by many baculoviruses that is related to the env protein of insect retroviruses.
Errantivirus A genus of insect retroviruses of the family *Metaviridae*.
Semotivirus A genus of the family *Metaviridae* containing retrotransposons and retrovirus-like elements encoding an env-like ORF.
TniTedV A virus of *Trichoplusia ni* called Ted.

Introduction to Retroelements

Retroelements are a diverse group of genetic sequences that have the capacity to be transcribed into RNA, reverse-transcribed into DNA, and inserted into a new site in a genome. A major category of these elements has long terminal repeat (LTR) sequences that are directly repeated at the 5′ and 3′ termini of the complete cDNA. These structures contain signals essential for RNA transcription and cDNA synthesis. LTR-retrotransposons are also distinguished from all other retroelements by sequence features within the reverse transcriptase region (see below) and by the nature of primers employed by this enzyme. Reverse transcriptases of LTR retroelements use the 3′ end of an annealed tRNA to prime reverse transcription. In contrast, non-LTR retrotransposons use the 3′ end of DNA to prime reverse transcription.

Major Divisions of LTR Retroelements

There are two major lineages of LTR-containing retrotransposons that have been differentiated by the domain order in their reverse transcriptases (**Figure 1**). They are the *Pseudoviridae* which have a domain order of proteinase, integrase, reverse transcriptase-RNase H (PR, IN, RT-RH), and the *Metaviridae* (PR, RT-RH, IN). The *Metaviridae* (from Greek *metathesis*: transposition) includes three genera the semotiviruses (from Latin *semoti*: distant, removed) which refers to their distant relationship to other genera of the *Metaviridae*, and the metaviruses and errantiviruses (from Latin *errans*: to wander). Three other lineages including the family *Retroviridae*, the *Caulimoviridae* (a family of DNA plant viruses with an RNA intermediate), and DIRS retrotransposons are also thought to have been derived from the family *Metaviridae* based on the relatedness of the reverse transcriptase sequences.

The Origin of Insect Retroviruses: 'Capture' of an Envelope Fusion Protein from the *Baculoviridae*

Individual members of all three genera of the family *Metaviridae* may contain an env-like open reading frame (ORF); however, both the errantiviruses and a lineage within the semotiviruses are specific to insects and appear to have originated by recombination events in which their progenitors incorporated an *env* gene from a baculovirus. The phylogenetic relationship between these different lineages of envelope proteins is shown in **Figure 2**. The two major groups of insect retroviruses are the *gypsy*-like errantivirus lineage and the *Kanga* and *Roo* semotivirus lineages. Although errantivirus and the semotivirus lineages resemble retroviruses, they have not been included within the family *Retroviridae,* because they are distinct lineages, and evidence that they are infectious is limited (see below).

Errantivirus Infectivity

The most intensively studied errantivirus is the *gypsy* element from *Drosophila melanogaster* (designated drosophila melanogaster Gypsy virus (DmGypV)) and most research has been focused on this virus. The reasons for questions regarding the infectivity of errantiviruses stem from the inability to manipulate them by employing commonly used virological techniques. Although they produce virus-like particles (vlps), the virus has not been titered, plaqued, or neutralized with antibodies, and no evidence of cytopathogenic effects on cells exposed to the virus have been documented. However, indirect evidence has been presented suggesting that DmGypV is infectious for *Drosophila*. These data were obtained by feeding a strain of *Drosophila* that lacks active DmGypV transposition with either purified vlps from insects with tranpositionally active DmGypV, or extracts derived from such insects and then documenting increased levels of transposition in the recipient insects. Similarly, it was observed that the DmGypV could be transmitted between cells in culture.

The Role of env in Errantivirus Infectivity

The evidence implicating env in errantivirus infectivity is varied. In one study, it was found that a preparation of two monoclonal antibodies against DmGypV env mixed with the vlp fraction reduced the number of insertion events in insect feeding experiments. In addition, evidence suggests that an integrated Moloney leukemia virus-luciferase construct pseudotyped with a DmGypV envelope is infectious for *Drosophila* cells. This suggested that DmGypV env is capable of mediating infection of *Drosophila* cells. Although DmGypV may be infectious, their infectivity appears to be very limited. It has been suggested that since they are adapted for integration into the cell genome, they no longer require propagation via infection. However, this does not explain why they have retained, conserved, and continue to express an *env* gene. Since envelope proteins often play major roles in the virulence of other viruses, the errantivirus *env* proteins may allow the viruses to be infectious and spread between different organisms, but this infectivity may be restricted by features of their env proteins. It may also facilitate spread between cells or tissues within an organism, although evidence has been presented that in some instances, at least, env is not required for viral movement between cells within organisms or between cultured cells.

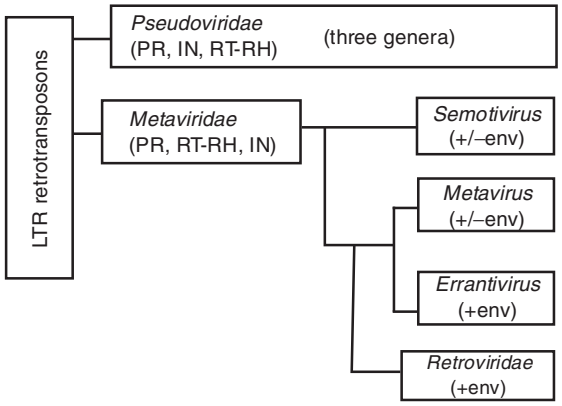


Figure 1 Schematic relationship of families of LTR retroelements based on the phylogeny of their reverse transcriptase domain. The genera of the *Pseudoviridae* are not shown. Two categories of the *Metaviridae*, the *Caulimoviridae* and DIRS retrotransposons are not included. Insect retroviruses comprise the genus *Errantivirus* and are also found in the genus *Semotivirus*. Abbreviations: PR, proteinase; IN, integrase; RT, reverse transcriptase; RH, RNase H. Not to scale.

Errantivirus Distribution

Five different categories of errantiviruses have been found in the sequenced genome of *D. melanogaster* and

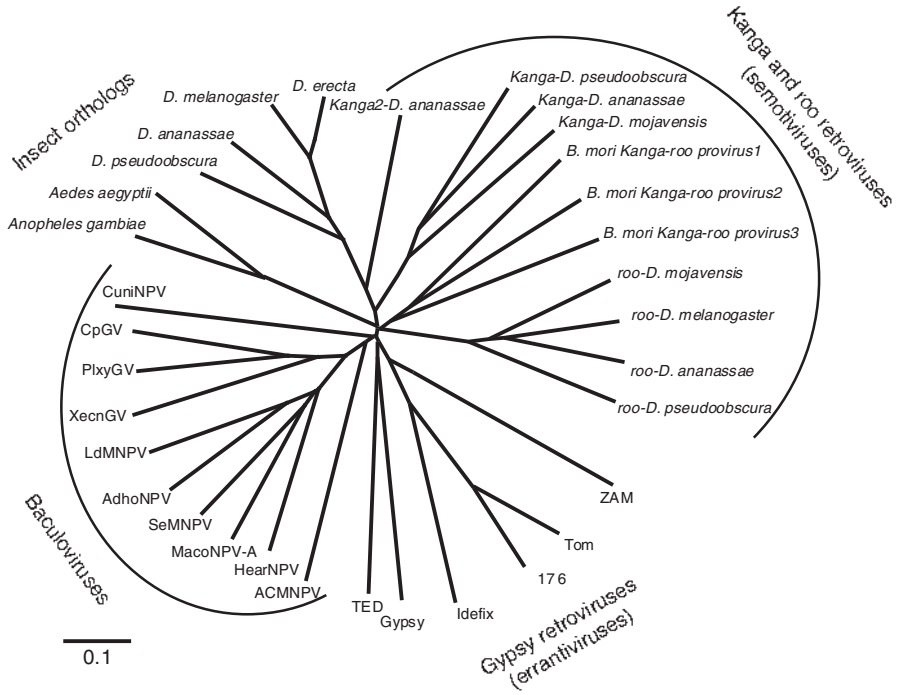


Figure 2 Neighbor-joining phylogenetic tree of baculovirus envelope fusion proteins, insect retrovirus env proteins, and orthologous proteins from insects. The tree was derived from an alignment of central domains of the proteins. Adapted from Malik HS and Henikoff S (2005) Positive selection of iris, a retroviral envelope-derived host gene in *Drosophila melanogaster*. *PLoS Genetics* 1: 429–443, with permission from HS Malik.

they range from a single full-length copy of DmeGypV, to 29 partial and 18 full-length copies of element Dme297V. DmeZamV was not found in this sequence indicating the variability of errantivirus distribution between *D. melanogaster* strains. Errantiviruses have also been identified in a number of other insect species. They are divided into two phylogenetic groups based on a primer binding site of either $tRNA^{Ser}$ or $tRNA^{Lys}$, which are predicted to be used for the initiation of cDNA synthesis.

Semotivirus Distribution

Although several retrovirus-like lineages of the semotiviruses have been reported, they are distinct from the errantiviruses (**Figure 2**). An apparent insect specific semotivirus lineage with an env ORF related to the baculovirus F gene became evident when the genomes of *Drosophila* species other than *D. melanogaster* became available. The additional species include *D. ananassae*, *pseudoobscura*, *mojavensis*, and *erecta*.

Additional Relationships of Errantiviruses to Baculoviruses

The relatedness of the errantivirus env and the baculovirus *F* proteins may reflect more than a fortuitous recombination event between these two viruses. The errantivirus TniTedV is a mid-repetitive element (about 50 copies per genome) in *Trichoplusia ni* and is capable of transposition from the insect into the baculovirus genome. It encodes a full complement of retrovirus ORFs including an *env* gene. A key feature of the relationship that may have led to the capture of a baculovirus F gene by a pre-errantivirus retrotransposon involves the ability of baculoviruses to express genes at very high levels. This feature appears to be due at least in part to the fact that they encode an RNA polymerase capable of high levels of transcription. This polymerase recognizes a novel promoter sequence (DTAAG) that is found in the TniTedV LTR as a palindrome. When TnTedV is integrated into the viral genome, mRNA is expressed from the LTR promoters at high levels late in the baculovirus infection cycle. Therefore, integration into a baculovirus genome may reflect a strategy to exploit baculovirus late gene expression to express the integrated retrotransposon/retrovirus genome at high levels. This could result in the production of a mixture of retrovirus particles and occluded baculoviruses containing integrated retroviruses and could provide two methods of escape from an insect with a fatal baculovirus infection – they could survive either by integration into baculovirus genomes, or as infectious virus particles. The evolution of this relationship between a baculovirus and a primordial LTR-type retrotransposon provides a clear pathway, via DNA recombination, for the transposable element to incorporate the baculovirus F homolog into its genome, thereby converting it into an infectious retrovirus.

Features of Baculovirus F and Errantivirus env Proteins

The baculovirus F and errantivirus env proteins appear to be members of a group of envelope fusion proteins common to many vertebrate viruses (**Figure 3**). Although these proteins, with the exception of baculovirus F and insect retrovirus env protein, lack convincing sequence relatedness, it has long been suggested that a number of envelope fusion proteins from a variety of disparate viruses are related. This is based on their features including that cleavage is often required for their activation and the fact that the fusion protein complex is membrane associated via a transmembrane domain. In addition, the membrane-associated peptide contains a hydrophobic fusion peptide sequence downstream of the cleavage site followed by predicted coiled-coil domains that are involved in forming hairpin-like structures that are important in virus–cell fusion. Such structures have been characterized in fusion proteins from viruses as diverse as filoviruses, retroviruses, orthomyxoviruses, and paramyxoviruses. Evidence suggests that baculovirus F proteins are members of this group, and it has been demonstrated that they require cleavage for activation. Errantivirus env proteins also have similar features consistent with their being members of this group.

Cleavage of Baculovirus F and Errantivirus env Proteins

One of the most striking features of the comparison of the baculovirus F and errantivirus env proteins is the conservation of the region which includes the polybasic furin-like cleavage site and the sequence immediately downstream (**Figure 3**). This region is conserved in all baculovirus F proteins except those that use GP64 as their fusion protein. Cleavage at this site is required for the baculovirus F proteins to carry out cell fusion. Similarly, the homologous region in DmGypV env has been shown to be required for cleavage. DmGypV env has also been shown to localize to cell membranes which is a prerequisite for many envelope proteins. Therefore, the *errantivirus env* genes encode many of the motifs associated with envelope fusion proteins including the furin-like cleavage site, which is critical for activation of other fusion proteins.

Just downstream of the furin cleavage motif is a 21-amino-acid hydrophobic sequence that is the most

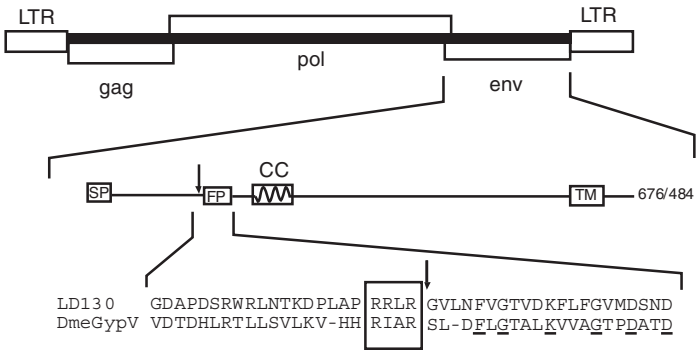


Figure 3 Comparison of predicted envelope fusion protein of a baculovirus to a predicted env protein of an errantivirus. Two representative proteins are shown: LD130 from the *Lymantria dispar* nucleopolyhedrovirus and DmeGypV env from the gypsy errantivirus of *D. melanogaster*. The diagram shows the predicted signal peptide (SP), cleavage site (arrow), fusion peptide (FP), coiled-coil region (CC), and transmembrane domain (TM). The numbers at the end are the lengths of LD130 and DmeGypV env, respectively. Beneath the diagram is the sequence surrounding the cleavage site. The boxed sequence is the furin cleavage signal. The underlined amino acids in the fusion peptide region are conserved between many baculovirus envelope fusion (F-type) proteins and errantivirus env proteins.

conserved region shared between baculovirus F and errantivirus env proteins and has the features of a fusion peptide.

A Cellular Homolog of the Insect Retrovirus *env* and *Baculovirus F* Genes

A cellular homolog (called cg4715 in *D. melanogaster*) of the insect retrovirus *env* gene has been identified in the *Sophophora* subgenus of *Drosophila* (**Figure 2**). It is preserved in the same location relative to adjacent genes in the different *Drosophila* species and its phylogeny corresponds to that of the host insects indicating that it is inherited in a strictly vertical manner rather than through horizontal transfer. The cg4715 lineage is most closely related to the *env* gene from a branch of insect retroviruses and phylogenic analyses suggest that it was incorporated by at least two independent events; once into a member of the *Drosophila* and once into a mosquito lineage (**Figure 2**). In addition, in some *Drosophila* there are two adjacent cg4715 genes indicating that a gene duplication event occurred. The second gene represents a distinct lineage that is characterized by the presence of a single predicted intron suggesting that it is spliced. In contrast to the cg4715 homologs found in the *Drosophila* lineage, those present in mosquitoes appear to contain a functional furin cleavage site. It has been suggested that insect recruitment of cg4715 occurred because its endogenous expression might protect insects from infection by retroviruses and baculoviruses that share a homologous env protein. This may result from its interacting with and blocking retrovirus receptors on the cell surface or by acting as dominant negative inhibitors. In the latter scenario, cg4715 would interact with and form multimers with the env protein of invading retroviruses and prevent infection because of their inability to be cleaved. Therefore, the retrovirus env proteins would be trapped in defective multimeric complexes.

See also: Ascoviruses; Baculoviruses: Molecular Biology of Granuloviruses; Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Baculoviruses: Molecular Biology of Sawfly Baculoviruses; Metaviruses; Oryctes Rhinocerous Virus; Ustilago Maydis Viruses.

Further Reading

Boeke JD, Eickbush T, Sandmeyer SB, and Voytas DF (2004) *Metaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 359–367. San Diego, CA: Elsevier Academic Press.

Boeke JD, Eickbusch T, Sandmeyer SB, and Voytas DF (2004) *Pseudoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 663–672. San Diego, CA: Elsevier Academic Press.

Eickbush TH and Malik HS (2002) Origins and evolution of retrotransposons. In: Craig NL, Craigie R, Gellert M and Lambowitz AM (eds.) *Mobile DNA II*, pp. 1111–1144. Washington, DC: ASM Press.

Kaminker J, Bergman C, Kronmiller B, *et al.* (2002) The transposable elements of the *Drosophila melanogaster* euchromatin: A genomics perspective. *Genome Biology* 3(12), doi: 10.1186/gb-2002-3-12-research0084.

Malik HS and Henikoff S (2005) Positive selection of iris, a retroviral envelope-derived host gene in *Drosophila melanogaster*. *PLoS Genetics* 1: 429–443.

Malik HS, Henikoff S, and Eickbush TH (2000) Poised for contagion: Evolutionary origins of the infectious abilities of invertebrate retroviruses. *Genome Research* 10: 1307–1318.

Pearson MN and Rohrmann GF (2002) Transfer, incorporation, and substitution of envelope fusion proteins among members of the *Baculoviridae, Orthomyxoviridae,* and *Metaviridae* (insect retrovirus) families. *Journal of Virology* 76: 5301–5304.

Terzian C, Pélisson A, and Bucheton A (2001) Evolution and phylogeny of insect endogenous retroviruses. *BMC Evolutionary Biology* 1(3), doi:10.1186/1471-2148-1-3.

Retroviruses of Birds

K L Beemon, Johns Hopkins University, Baltimore, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Avian retroviruses have been studied for 100 years. Avian leukosis virus (ALV) was discovered in 1908 by Ellermann and Bang, and the related Rous sarcoma virus (RSV) was isolated by Peyton Rous in 1911. Numerous isolates of ALV and of transforming viruses, which cause sarcomas and a variety of hematopoietic neoplasms, were reported in the decades that followed. Progress in understanding their nature was very slow until the development of cell culture assays in the late 1950s and the use of genetic and cell biological approaches to study replication and transformation. The discovery of reverse transcriptase (RT) in 1970 and of the origin and mechanism of action of viral oncogenes in the decade following led to an explosion of research activity. In addition to the genetic information of ALV needed for replication, RSV has a transduced oncogene, *src*, that enables it to induce sarcomas rapidly *in vivo* and to transform cells in culture. Src was the first retroviral oncogene to be characterized and the first tyrosine kinase to be identified. Currently, avian retroviruses provide a robust system for studies of retroviral gene expression and of virion assembly *in vitro*, as well as for studies of oncogenesis.

Taxonomy and Classification

The avian leukosis viruses comprise a single genus *Alpharetrovirus*, of the family *Retroviridae* and the subfamily *Orthoretrovirinae*. Although they share structural and biological characteristics with the mammalian C-type gammaretroviruses (such as murine leukemia virus), these two groups are not closely related. All ALVs are closely related to one another sharing considerable sequence and antigenic identity. Isolates are differentiated by subgroup (i.e., receptor utilization) and the presence or absence of oncogenes.

Distribution, Host Range, and Propagation

ALVs are endemic in flocks of domestic chickens (*Gallus gallus*) worldwide, and natural infections seem limited to this species, within which they are of some economic importance. Related endogenous viruses are found in ring-necked and golden pheasant (but not other related birds, such as turkeys), but exogenous viruses have not been isolated from other species. ALV will replicate efficiently in species closely related to the chicken such as quail, turkeys, and pheasants, but less so in more distant species such as ducks. RSV of some subgroups can transform mammalian cells and induce tumors in mammals, but with greatly reduced efficiency, and virus replication in mammalian cells cannot be reproducibly observed. The restriction of the virus to avian species is due to a lack of suitable receptors for most subgroups as well as to blocks to viral gene expression. The rare transformants that arise in RSV-infected mammalian cells often display rearrangements in proviral DNA that relieve this block.

A variety of cell types from gallinaceous birds (including chickens, turkeys, and quail) can be used to propagate ALVs and their relatives. Primary and secondary fibroblast cultures or cell lines (DF-1) are most commonly used, as well as lines derived from quail tumors (QT6). To avoid problems associated with frequent recombination, it is advisable to use cells that do not contain related endogenous proviruses, such as cells from species other than chickens or from chickens bred to contain no such proviruses.

Properties of the Virion

Like all retroviruses, ALVs are transmitted as enveloped virions of about 100 nm diameter, derived by budding from the host cell membrane. Lipids derived from the host plasma membrane are present in the viral envelope and make up 35% of the virion weight. Within the retrovirus family, they are defined as having a C-type morphology. Small, dispersed spikes project from the surface of the virion; these consist of trimers of the two *env*-encoded proteins, SU (surface) and TM (transmembrane). The internal core of the virion is of uncertain symmetry in mature virions but appears in electron micrographs as a centrally located, roughly spherical structure about 30 nm in diameter. Immature virions seen during or shortly after budding have a more open, spherical core structure, substantially larger in diameter than the processed one. The core comprises about 1500 copies each of the four gag-encoded proteins (as well as protease) and about 100 copies each of RT and integrase.

Properties of the Genome

The ALV genome consists of a homodimer of positive-sense, single-stranded (ss) RNA about 7500 bp in length. Transforming viruses, in which an oncogene has been

inserted, have genomes varying in length from about 3.2 kbp (for UR2 virus) to about 9.3 kbp (for nondefective Rous sarcoma virus). In most of these viruses, the oncogene has replaced some of the normal genome, leading to genetically defective virus, which requires co-infection with helper ALV for replication. The genome is modified and processed by cell machinery. It contains a *5′*m7GpppGm capping group and a *3′* poly (A) sequence, as well as some internal m6A residues. In *in vitro* translation systems, it is capable of serving as mRNA for the *gag-pro* and *gag-pro-pol* gene products. As with all retroviruses, the order of genes is *5′-gag-pro-pol-env-3′*.

Important noncoding regions found near the end of the genome are necessary to provide signals for virus replication. These include an 18–21 base sequence (R) repeated at each end as well as unique sequences U3 (*c.* 250 bp) near the *3′* end and U5 (*c.* 80 bp) near the *5′* end which are duplicated in the long terminal repeat (LTR) during reverse transcription. The LTR contains sequences controlling transcription initiation and polyadenylation. Adjacent to these are the sites for initiation of reverse transcription: the primer binding (PB) sequence next to U5. Between PB and the beginning of *gag* is an approximately 300 bp leader region, which contains signals important for the dimerization and packaging of the genome into virions. The direct repeat (DR) sequences flanking the *src* gene in RSV are necessary for cytoplasmic accumulation and packaging of full-length viral RNA. ALV has one copy of the DR sequence. Other regulatory sequences are within coding sequences. The negative regulator of splicing (NRS) within the *gag* gene suppresses splicing and also promotes polyadenylation. ALVs with mutations in the NRS can lead to rapid-onset lymphomas *in vivo*, characterized by readthrough and splicing from the viral genome into downstream oncogenes or onco-miRs.

Properties of the Proteins

The virion contains nine proteins, the products of four coding regions. The Gag proteins constitute the major structural components and are sufficient to form recognizable virions if expressed alone. The Gag-Pro precursor is processed during release of virus into four Gag proteins: MA (matrix, about 19 kDa) which interacts with the cell membrane; p10, a 10 kDa protein of unknown function and location; CA (capsid, about 27 kDa) which forms the core shell structure; and NC (nucleocapsid, about 12 kDa), an RNA-binding protein necessary for specific encapsidation of genome RNA. The Gag-Pro precursor also contains the 15 kDa PR (protease) peptide necessary for processing all internal virion proteins. The Pol reading frame is expressed as a fusion protein with Gag and Pro, processed to yield RT (usually present as a heterodimer of 98 and 66 kDa reflecting partial processing by PR), and integrase (IN, about 32 kDa); these are the two enzymatic activators necessary for synthesis and integration of the DNA provirus. The *env* gene encodes the Env precursor (Pr95) which is processed as a membrane protein and cleaved by host cell proteases to yield the SU glycoprotein, which has an apparent molecular weight of about 85 kDa, about half of which is due to the provision of *c.* 14 N-linked carbohydrate side chains, and the TM glycoprotein, which has an apparent molecular weight of about 37 kDa. The SU and TM products remain as a disulfide-bonded heterodimer with SU containing the activity necessary for receptor binding and TM-mediating fusion with the cellular membrane.

Physical Properties

Virions of ALV have an equilibrium density in sucrose solutions of about 1.16–1.18 g ml^{-1} and a sedimentation coefficient of about 600S. They are quite labile and are readily inactivated by extremes of pH, as well as by heat or mild detergent treatments. They are somewhat radiation resistant, perhaps reflecting the recombinational repair capability provided by the dimeric genome.

Replication

Replication of ALV is like that of other retroviruses, and this group of viruses provided some of the important early models for studying the process. Entry of the virion follows interaction with a specific receptor on the cell surface. Genetically, at least ten subgroups (A–J) have been identified on the basis of distinct receptor recognition. The presence of receptors for specific subgroups is polymorphic among birds. Three unlinked genetic loci (*Tv-a*, *Tv-b*, and *Tv-c*) for ALV receptors have been genetically identified in chickens. The dominance of susceptibility over resistance alleles at each of these loci implies that they encode the receptor directly. The *Tv-b* locus has several alleles, controlling susceptibility to subgroups B, D, and E. Receptors for ASLV subgroups A, B, D, and E have been cloned. The Tv-a receptor resembles a portion of the receptor for low-density lipoprotein and is unrelated to other known retroviral receptors. The receptors for B, D, and E are all in the tumor necrosis factor receptor family. Chickens have two alleles that can act as receptors for these viruses. Entry of the virion core into the cell is by fusion of viral and cellular membranes, perhaps following endocytosis.

Once within the cytoplasm of the infected cell, the process of reverse transcription within the poorly defined core structure copies the ssRNA genome into a molecule of double-stranded (ds) DNA. This process – which varies little from that of other retroviruses – includes a series of

'jumps' from one end of the template to the other. The product is a dsDNA molecule, which differs from the genomic RNA by the presence at either end of the LTR. The LTR contains sequences necessary for DNA integration and for synthesis and processing of viral RNA.

Integration of viral DNA into more or less random sites in the cell genome is accomplished by the IN protein which has entered the cell with the virion and remains with the DNA in an ill-defined structure. The process of integration leads to the insertion of the viral DNA into cell DNA in the same general organization as both genome and unintegrated DNA. Integrated ALV DNA is characterized by the loss of two bases from each end of the viral sequence and the duplication of six bases of cell DNA at the integration site.

Transcription of the provirus into genomic and mRNA is mediated by cellular RNA polymerase II directed to the correct initiation site by promoter and enhancer sequences in the LTR. The strength of the enhancer elements is a major factor distinguishing pathogenic from nonpathogenic ALV isolates. Unlike some other retroviruses, there is no apparent role of virus-encoded proteins in regulating the transcription process. Processing of the viral transcripts includes addition of poly(A) following a canonical signal (AAUAAA) in the RNA derived from the 3′ LTR and splicing of the fraction of the transcripts destined to become mRNA for the *env* gene. The splicing removes most of the *gag*, *pro*, and *pol* sequences, leaving the beginning of *gag* fused to *env*.

Translation of the full-length RNA leads to two products: The Gag-Pro precursor of about 76 kDa and the Gag-Pro-Pol precursor of about 180 kDa. Synthesis of the latter molecule is made possible by a 1 translational frameshift about 5% of the time, bypassing the termination codon at the end of Pro. Assembly of the precursors is at the cell surface, and is coincident with budding, implying a simultaneous association of the precursors with the genome, with the cell membrane, and with one another. Release of the immature particle (characterized by a hollow, symmetrical core which almost fills the virion) is rapidly followed by cleavage of the Gag-Pro and Gag-Pro-Pol precursors to yield the finished proteins. This cleavage is accompanied by condensation of the core into its mature form. Since the PR protein embedded in the Gag-Pro precursor contains only one-half of the active site, dimerization of this domain is necessary for cleavage to occur. This requirement probably helps to delay cleavage until the appropriate time.

Once infected, the host cell is usually not killed by virus replication. A strong superinfection resistance due to blockage or loss of viral receptors develops soon after infection and prevents accumulation of proviruses by reinfection. In some cases, weak or slow development of superinfection resistance is associated with a cytopathic interaction of the virus with its host cell.

Transformation

A unique characteristic of ALV and a few other retroviruses is their ability to incorporate certain host sequences into their genome and alter the function of these proto-oncogenes to generate oncogenes. The presence of an oncogene renders the virus capable of inducing malignant transformation of cells in culture and one of a variety of malignant and rapidly fatal diseases in birds. At least 20 distinct cell sequences have been incorporated by ALV into a very large number of distinct isolates. RSV, which contains *src*, is the prototype oncogene-containing virus. Other notable oncogene-containing ALV variants include avian myeloblastosis virus (AMV; containing *myb*); avian myelocytomatosis virus-29 (MC-29; *myc*); avian erythroblastosis virus (AEV; *erb*-A and *erb*-B); Fujinami sarcoma virus (FSV; fps); and University of Rochester sarcoma virus-2 (RU-2; *ros*). Study of the genetic alterations that distinguish these oncogenes from proto-oncogenes, and the enzymatic and physiological function of the proteins they encode has been a keystone of modern cancer research. Incorporation of oncogenes into the virus genome is usually at the expense of some viral genes and co-infection of a cell with a wild-type (helper) ALV is thus necessary to provide viral proteins for replication of oncogene-containing viruses. RSV, which is a replication-competent transforming virus, is the exception.

Endogenous Viruses

Another unique feature shared by ALV and a few other retrovirus groups is their ability to become established in the germline and inherited stably as endogenous proviruses. Naturally occurring endogenous proviruses form a distinct lineage of ALVs, showing a specific host range (subgroup E) for which many domestic chickens lack receptors (a phenomenon known as xenotropism), and a reduced replication capacity and pathogenicity relative to exogenous viruses. Endogenous viruses are usually expressed at a very low rate, due largely to methylation of CpG residues in the proviral DNA, and are often (but not always) defective in sequence.

Genetics

Strain differences among ALV isolates are primarily in host range and are encoded by differences within the central portion of SU; other parts of the genome, with the exception of the U3 end of the LTR, are quite highly conserved. Like other retroviruses, ALVs exhibit very high rates of homologous recombination – a consequence of the diploid genome and the 'jumping' mechanism of reverse transcription. The latter also permits relatively

high rates of nonhomologous recombination, leading to frequent (but not usually lethal) rearrangements of the genome as well as the occasional acquisition of foreign sequences such as oncogenes.

Evolution

Amino acid sequence relationships reveal a common origin of all retroviruses, but the ALV group forms a divergent branch, with its closest relative being the mouse mammary tumor virus. Whereas the recent spread of viruses among chickens is probably due largely to human intervention, the virus group is of considerable antiquity, since distantly related endogenous viruses are widespread in the genomes of avian and even mammalian species. The closely related endogenous viruses seem to be recent introductions derived by germline infection with exogenous virus since they are found only in *Gallus gallus*, and not in other species of *Gallus*, although they do appear in more distantly related pheasants.

Transmission and Tissue Tropism

Transmission of virus is principally vertical by infection of the offspring through virus secreted into the egg. Indeed, high titers of ALV are often detectable in commercial hen's eggs. Horizontal spread of virus is naturally much more rare, requiring close contact, but virus can be readily spread from infected birds via contaminated needles during vaccination or through vaccines prepared from infected eggs or cell cultures.

All isolates of ALV replicate efficiently in fibroblast cultures and in the bursa. Tropism for other tissues varies among isolates and is determined by both *env* and LTR sequences.

Pathogenicity

ALVs induce a wide spectrum of disease in naturally or experimentally infected animals. The prototypic disease induced by ALV is a B-cell lymphoma arising in the bursa of Fabricius starting a few months after infection and spreading to the liver and other organs during its course. Other malignancies, including erythroleukemia, sarcoma, and others, are not uncommon depending on the strain of virus and bird and the time and route of inoculation. The malignant diseases induced by viruses, which do not contain oncogenes, are the consequence of insertional activation of cellular proto-oncogenes (such as *c-myc* in the case of lymphoma, *c-erb*-B in erythroleukemia, and others). In addition to malignancies, these viruses also induce hemangiomas, osteopetrosis, and wasting diseases. In some cases, an immune response against infected cells may be important; in others, cytopathic effects of the sort noted above may play a significant role.

Acquisition of oncogenes by ALVs greatly alters the nature and course of the disease. Infection of newly hatched chicks with AMV, for example, can lead to their death from myeloblastic leukemia in as few as 5 days. Moribund animals display enormously elevated myeloblast counts and a level of viremia sufficient to render the plasma noticeably turbid. Similarly, birds inoculated with RSV develop rapidly growing, usually fatal, sarcomas at the site of injection in a few weeks. It should be noted that the oncogene-containing viruses are not efficiently transmitted from one animal to another due to their rapid pathogenicity. In most cases, they have probably arisen in the animal from which they were isolated and would have died out if not brought into the laboratory.

Not all members of this group are highly pathogenic. RAV-0 (an endogenous virus) can infect susceptible chickens and induce viremia, but disease is rare and occurs only after a long latent period. The reduced virulence is probably an important feature of viruses inherited in the germline.

Immune Response

In infected birds, the only significant immune response is the appearance of type-specific neutralizing antibodies, which apparently recognize the regions of Env involved in receptor recognition. Group specific responses against Env or other proteins are not usually observed in infected chickens, although inoculation of virus into mammals induces antibodies capable of recognizing all virion proteins in the absence of subgroup-specific reactivity. The limited immune response observed in infected chickens has been attributed to the presence of endogenous proviruses whose expression (even at a low level) can induce tolerance to antigens in common with infecting virus. Indeed, it has been suggested that induction of tolerance might be a desirable feature for the animal, since it could prevent or limit immunopathological sequelae of infection. Postinfection immune response seems to be of little consequence in preventing subsequent malignant disease, since the cells, which will eventually form the tumor, are probably infected quite soon after infection, and the long latency reflects the necessity for subsequent rare events (such as mutations in other genes) rather than a continuing period of virus replication.

Prevention and Control

ALV-induced disease is a cause of some economic loss to the poultry industry in the United States, and occasional more serious epizootics (such as a recent outbreak of

hemangioma in Israel) due to ALV have occurred. Control of infection is generally by detection and culling of infected individuals. No useful vaccination strategy has been developed. In principle, it should be possible to virtually eliminate the disease by breeding the appropriate *Tv-a* and *Tv-b* alleles into commercial strains; in practice, this has not been done very often. A more recent strategy is to introduce defective proviruses encoding envelope protein into the germline of birds; these can block infection by inducing superinfection resistance.

Future

Although of economic importance to the poultry industry, the value of ALV and the related oncogene-containing viruses to science has been far greater. The study of these viruses as models will continue to illuminate fundamental aspects of retrovirus biology. Continued searches for new transforming viruses and selected retroviral integration sites are likely to yield novel and important oncogenes. The goal of eradication of ALV disease from commercial chickens is attainable with present technology; its realization is largely a matter of economic considerations.

See also: Endogenous Retroviruses; Retroviruses: General Features.

Further Reading

Coffin JM, Hughes SH, and Varmus HE (1997) *Retroviruses*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Flint SJ, Enquist LW, Racaniello VR, and Skalka AM (2004) *Principles of Virology, Molecular Biology, Pathogenesis, and Control of Animal Viruses,* 2nd edn. Washington, DC: ASM Press.

Goff SP (2006) *Retroviridae*: The retroviruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al*. (eds.) *Fields Virology,* 5th edn., pp. 1871–1940. Philadelphia, PA: Lippincott Williams and Wilkins.

Retroviruses: General Features

E Hunter, Emory University Vaccine Center, Atlanta, GA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Lenti From Latin *lentus*, 'slow'; refers to the slow development of pathology associated with lentivirus infections.

Retro From Latin *retro*, 'backward'; refers to the activity of reverse transcriptase and the transfer of genetic information from RNA to DNA.

Spuma From Latin *spuma*, 'foam'; refers to the vacuolated morphology of spumavirus infected cells.

Introduction

The family *Retroviridae* contains a large and diverse group of viruses that infect vertebrates. They are enveloped viruses that undergo a unique replication cycle, which clearly distinguishes them from other viruses. Virions generally contain a single-stranded RNA genome that upon introduction into the target cell is converted to a double-stranded DNA (dsDNA) copy by a process termed reverse transcription. This DNA version of the viral genome is then integrated into the chromosomal DNA of the cell, allowing the virus to persist and produce progeny for as long as the cell lives and providing a mechanism for lifelong infection in the vertebrate host.

The retrovirus family includes two human pathogens, human immunodeficiency virus (HIV), the causative agent of acquired immune deficiency syndrome (AIDS), and human T-lymphotropic virus 1 (HTLV-1), which induces T-cell lymphomas and degenerative nervous system disease in man. The family also includes important pathogens of horses, cows, sheep, cats, and rodents, where members induce cancers, anemias, arthritis, immunodeficiencies, and degenerative disease.

Retroviruses are an ancient group of viruses with archival evidence in the form of endogenous genomes pointing to infections that date back tens of millions of years. They have provided new tools and insights into molecular biology, have yielded clues to the basis of cancer, and continue to impact mankind through their pathogenic potential.

History

Although in 1904 equine infectious anemia was the first retroviral disease to be described, it was not recognized

until much later that this was the result of viral infection. Four years later, however, Ellerman and Bang showed that chicken leukosis, a form of leukemia, was caused by a virus, and 3 years after that (1911) Peyton Rous reported cell-free transmission of sarcomas in the chicken. This virus, Rous sarcoma virus (RSV), named after its discoverer, together with the avian leukosis viruses (ALVs), is representative of the genus *Alpharetrovirus*. In part because these observations were made in birds, the scientific community did not immediately appreciate the importance of these oncogenic virus discoveries and it was 25 years before John Bittner identified the first mammalian retrovirus. He demonstrated that mouse mammary tumors were caused by a milk-transmitted, filterable agent. Then in 1957, Ludwik Gross described the development and serial cell-free passage of a highly potent strain of mouse leukemia virus. This was followed over the next two decades by the discovery and isolation of many oncogenic retroviruses from mice, cats, cows, and nonhuman primates.

It was also in the 1950s (1954) that Sigurdsson described visna, a neurological disease in sheep that slowly and progressively induced paralytic symptoms in its host. This led the authors to put forward the concept of slow viral infections and to the nomenclature that now describes members of the genus *Lentivirus* (derived from Latin: *lentus*, slow).

Spumaviruses are unusual in that they were not isolated from a specific disease state but rather from cell cultures derived from a healthy monkey contaminated by a simian member of this genus in 1954. In cell culture, the viruses induce a characteristic foamy appearance in the cytoplasm of the cell and this led to the nomenclature of the genus (derived from Latin: *spuma*, foam).

The first human retrovirus to be described in 1980 was HTLV-1, a member of the genus *Deltaretrovirus*, which induces a cutaneous T-cell lymphoma in a small number of individuals infected years earlier with the virus. It was only 3 years later that human immunodeficiency virus type-1 (HIV-1 – initially called lymphadenopathy-associated virus (LAV)) was isolated from patients with early manifestations of AIDS by Montagnier and his coworkers, and in 1984 that the link between this lentivirus (initially termed HTLV-III) and AIDS was conclusively established by Gallo and his colleagues. Since then numerous nonhuman primate, feline, and bovine members of the genus *Lentivirus* have been described, and they provide powerful animal models for the study of HIV/AIDS.

The archival evidence of previous retroviral infections in the form of endogenous proviruses that are found in high copy number in the genome of several mammalian species, including human, argues for multiple instances of widespread retroviral infections in mammals. Endogenous proviruses can vary from intact genomes capable of producing complete virus (modern endogenous viruses) to highly mutated variants that have evolved with the host and date back tens of millions of years (ancient endogenous retroviruses).

Taxonomy and Classification

The family *Retroviridae* contains two subfamilies: the *Orthoretrovirinae* and the *Spumaretrovirinae*. Differentiation between subfamilies is based on morphological characterization, replication differences, and variation in the expression and function of viral proteins. Six genera are present in the *Orthoretrovirinae*: *Alpharetrovirus*, which includes the avian leukosis and sarcoma viruses; *Betaretrovirus*, which includes mouse mammary tumor virus (MMTV) and Mason–Pfizer monkey virus (M-PMV); *Gammaretrovirus*, which contains the mammalian 'C-type' retroviruses, including rodent and primate leukemia and sarcoma viruses and the avian reticuloendotheliosis virus (REV); *Deltaretrovirus*, which includes bovine leukemia virus (BLV) an HTLV-1; *Epsilonretrovirus*, comprising the fish retroviruses, including the walleye dermal sarcoma virus (WDSV); and *Lentivirus*, which include HIV-1 and HIV-2, as well as viruses from ungulates such as visna-maedi and equine infectious anemia virus (EIAV). The subfamily *Spumaretrovirinae* is comprised of a single genus, *Spumavirus*, which contains the prototype foamy virus (PFV; originally named human foamy virus but more recently shown to be of chimpanzee origin). Members of this genus have been isolated from cats, cows, horses, and a variety of nonhuman primates but not from humans. Spumaviruses have replication characteristics intermediate between the orthoretroviruses and members of the *Hepadnaviridae*. Their genomic organization is similar to other retroviruses and reverse transcription of the viral genome is a prerequisite for infection, assembly and budding occur on intracellular membranes, DNA transcription from the RNA genome occurs prior to virus release from the cell, and the viral (reverse) transcriptase is translated independently from a spliced mRNA and requires the viral genome for incorporation (**Figure 1**).

Genome Structure and Organization

The viral genome is genetically diploid, consisting of two linear, positive-sense, single-stranded RNAs that range in size from 7 to 11 kbp depending on the species in question. The RNA monomers are held together in a (70S) dimer through hydrogen bonds in a 5′-located dimer linkage structure. Each monomer of RNA is polyadenylated at the 3′ end and has a cap structure (type 1) at the 5′ end. The purified virion RNA is not infectious and must be converted into a dsDNA provirus via a process of reverse transcription in order for the virus life cycle to proceed.

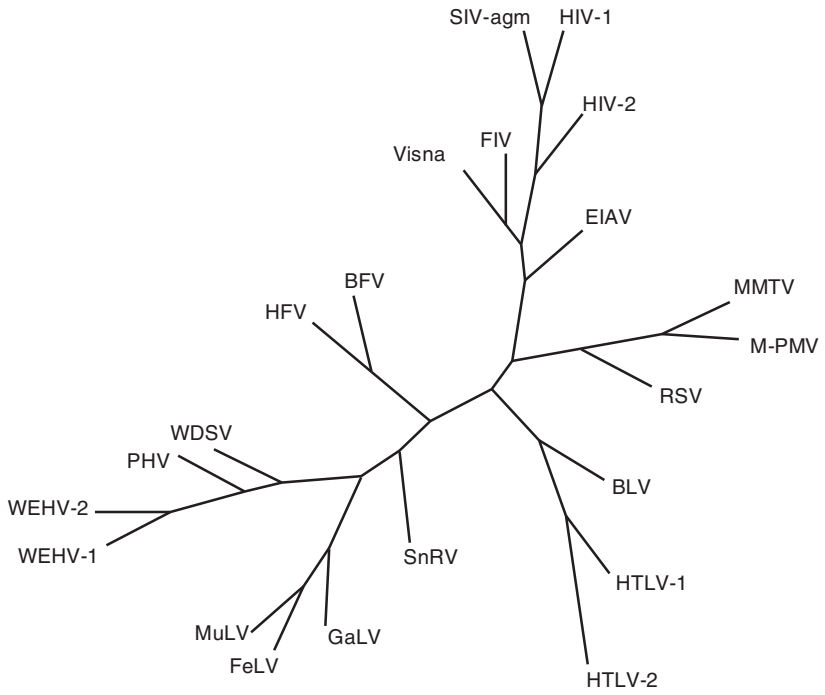


Figure 1 Phylogenetic relationships: phylogenetic analysis of conserved regions of the pol genes of retroviruses. An unrooted neighbor-joining phylogenetic tree was constructed based on an alignment of the amino acid residues of reverse transcriptase genes of several retroviruses. Courtesy of Quackenbush S and Casey J. Reproduced from *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, Elsevier/Academic Press, with permission.

Each RNA monomer has a specific tRNA molecule base-paired to a region (termed the primer binding site) near the 5′ end of the genomic RNA. This tRNA acts as the primer for DNA synthesis on the RNA template.

Infectious viruses have a minimum of four genes that encode the virion structural and replication proteins in the order: 5′-*gag–pro–pol–env*-3′. The *gag* gene encodes the viral structural proteins that are translated initially as a gag polyprotein precursor; *pro* encodes the viral aspartyl proteinase (PR), which mediates polyprotein cleavage during virus maturation; *pol* codes for the reverse transcriptase enzyme (RT) and the viral integrase (IN). RT is responsible for converting the viral RNA genome into a dsDNA provirus, which must then be integrated into the chromosomal DNA of the target cell by the IN protein (**Figure 2**).

Members of the genera *Deltaretrovirus*, *Epsilonretrovirus*, *Lentivirus*, and *Spumavirus* encode additional genes encoding nonstructural proteins important for the regulation of gene expression and for virus replication. The primate lentiviruses appear to have the most complex set of six accessory genes that include *tat*, which encodes a 'transactivator of transcription', and '*rev*', which codes for a protein (Rev) that transports unspliced and partially spliced viral RNA transcripts out of the nucleus. Genes encoding proteins with similar transcription enhancing and RNA transport functions are found in the deltaretroviruses (*tax* and *rex*) and spumaviruses (*tas*).

Some members of the alpharetroviruses and the gammaretroviruses carry cell-derived sequences that are important in pathogenesis. These cellular sequences are either inserted in a complete retrovirus genome (e.g., some strains of RSV), or in the form of substitutions for deleted viral sequences (e.g., murine sarcoma virus). Such deletions render the virus replication defective and dependent on replication-competent helper viruses for production of infectious progeny. In many cases, the cell-derived sequences form a fused gene with a viral structural gene that is then translated into one chimeric protein (e.g., gag-onc protein).

Virus Morphology

The virions are spherical, enveloped particles 80–100 nm in diameter, that for the most part derive their lipid bilayer from the plasma membrane of an infected cell. Inserted into the viral envelope are glycoprotein surface projections, which are about 8 nm in length and have the appearance of knobbed spikes. An internal core encapsidates the viral ribonucleoprotein and associated replicative enzymes. This structure has a spherical appearance in

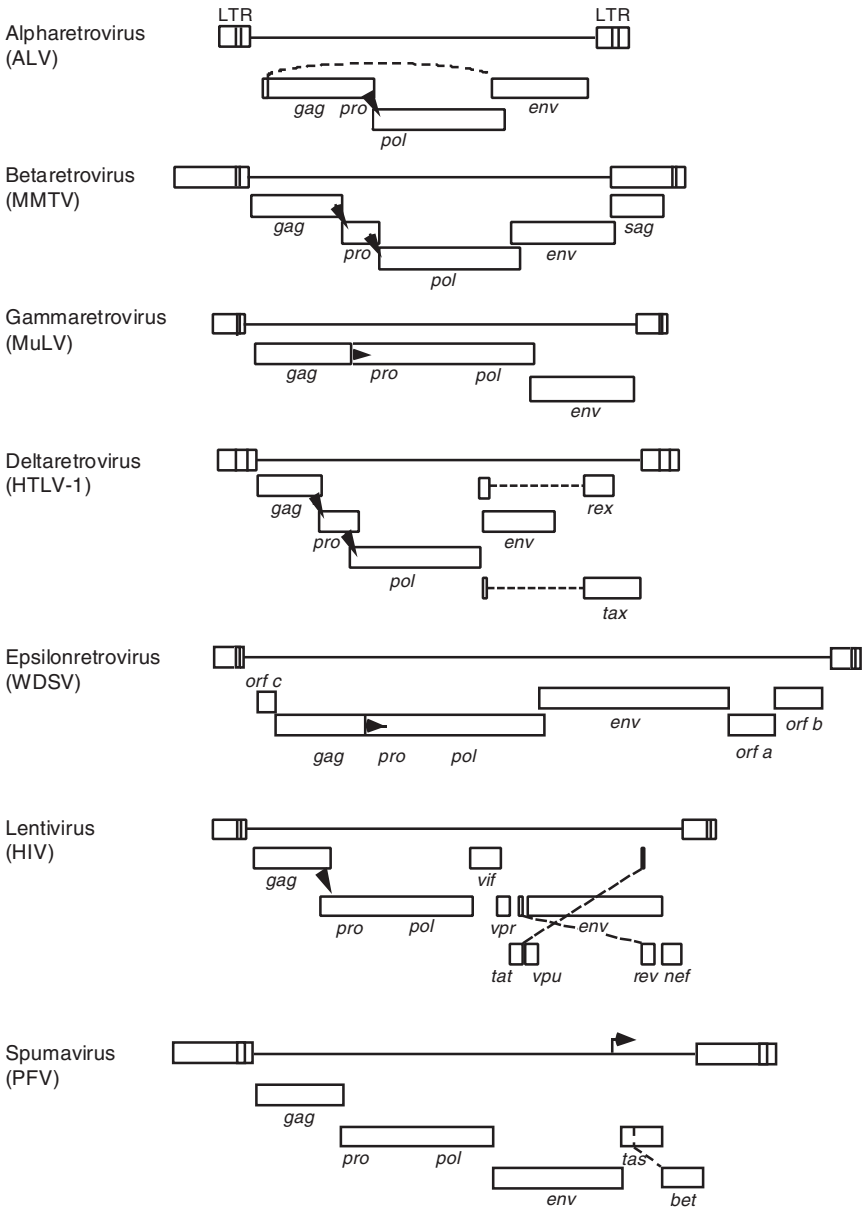


Figure 2 Genomic organization of retroviruses: the different prototypical provirus genomes for each genus are shown indicating the positions of the LTRs and encoded structural genes (*gag*, *pro*, *pol*, *env*) and certain other nonstructural genes (e.g., *tax* and *rex* in the deltaretroviruses) as well as their reading frames (ribosomal frameshift or ribosomal readthrough sites: arrowheads). LTR, long terminal repeat.

members of the genera *Alpharetrovirus*, *Gammaretrovirus*, and *Deltaretrovirus* of the subfamily *Orthoretrovirinae* and in members of the subfamily *Spumaretrovirinae*. It is spherical or rod shaped for members of the genus *Betaretrovirus*, and has a truncated cone shape in virions from the genus *Lentivirus*. Retroviruses have a characteristic buoyant density of 1.16–1.18 g cm^{-3} and a sedimentation coefficient (S_{20w}) of approximately 600S in sucrose (**Figure 3**).

Two distinct morphogenic pathways exist. Members of the genera *Alpharetrovirus* and *Gammaretrovirus*, which assemble their immature capsids at the plasma membrane, were historically classified as C-type viruses based on electron microscopy, and members of the genus *Lentivirus* are also assembled via this pathway. In contrast, members of the genus *Betaretrovirus* assemble immature capsids (previously termed A-type particles) in the cytoplasm, which are then enveloped at the plasma membrane with either a B-type (MMTV) or D-type (M-PMV) morphology. Members of the subfamily *Spumaretrovirinae* also assemble immature capsids in the cytoplasm, but these are enveloped primarily at intracellular membranes.

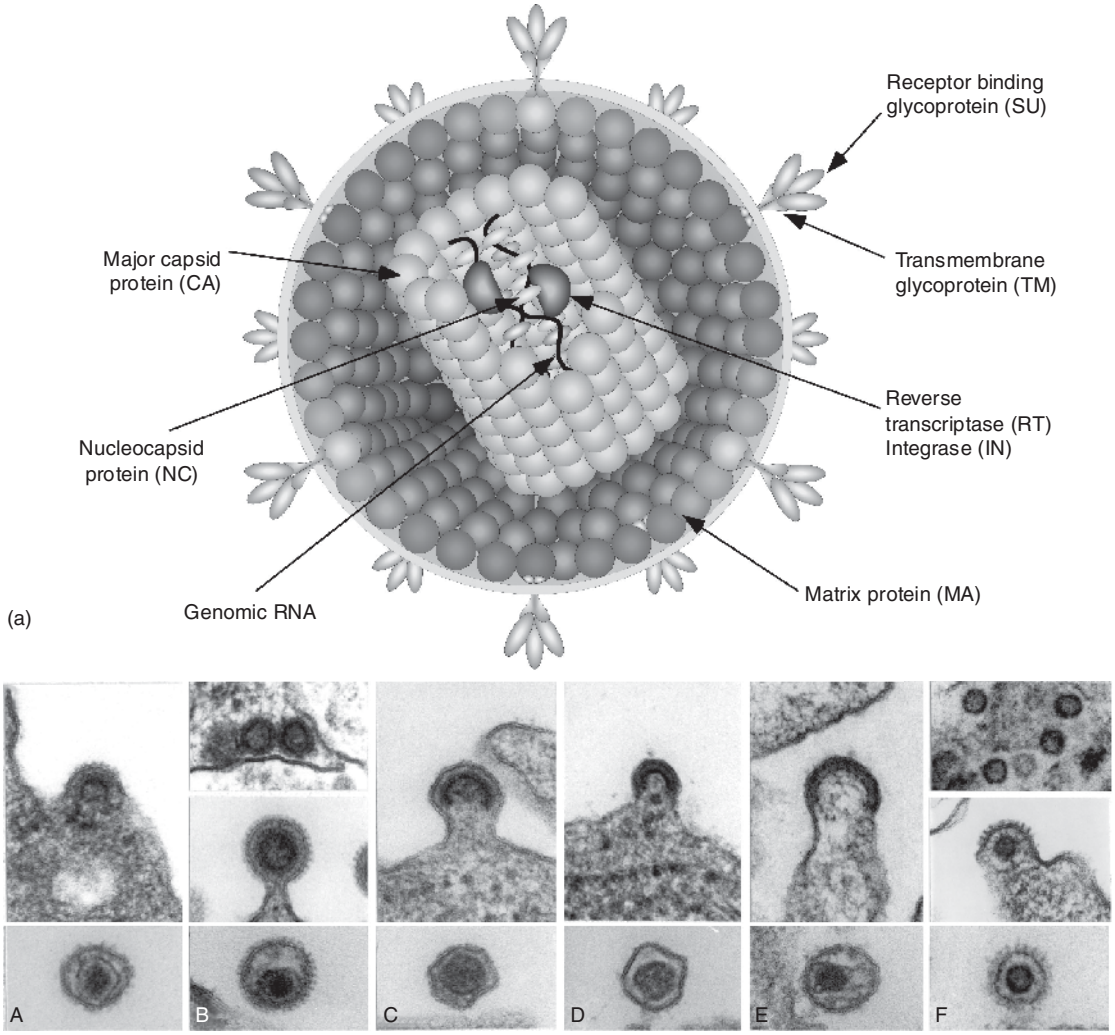


Figure 3 Virion structure and morphology. (a) Schematic cartoon (not to scale) shows the inferred locations of the various structures and proteins. (b) A, *Alpharetrovirus*: avian leukosis virus (ALV), type 'C' morphology; B, *Betaretrovirus*: mouse mammary tumor virus (MMTV), type 'B' morphology; C, *Gammaretrovirus*: murine leukemia virus (MLV); D, *Deltaretrovirus*: bovine leukemia virus (BLV); E, *Lentivirus*: human immunodeficiency virus 1 (HIV-1); F, *Spumavirus*: human foamy virus (HFV). The bar represents 100 nm. Reproduced from *Virus Taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses*, Elsevier/Academic Press, with permission.

Viral Proteins

The surface of the virion is studded by envelope glycoprotein (Env) spikes comprised of three copies each of two envelope proteins: SU (surface) and TM (transmembrane). These individual components are synthesized as part of a single Env precursor, which is encoded by the viral env gene and is proteolytically cleaved during intracellular transport and prior to assembly into the virus. The number of Env trimers/virion can vary from an estimated low of 7–14 for HIV to a high of more than 150 for primate foamy viruses. SU acts as the receptor-binding component and TM as a membrane-spanning anchor that mediates virus and cell membrane fusion during virus entry.

For members of the *Orthoretrovirinae*, virions contain 3–6 internal, nonglycosylated structural proteins (encoded by the *gag* gene). These are, in order from the N-terminus, (1) MA (matrix protein), (2) a protein, frequently phosphorylated, that in some viruses plays a role in viral budding (3) CA (capsid protein), (4) NC (nucleocapsid protein), and (5) a small C-terminal protein, found in some viruses, that can play a role in assembly and/or budding. These proteins are translated as a single gag polyprotein precursor, which is cleaved by a virus-encoded aspartyl proteinase to the mature products. The MA is often modified with a myristic

acid moiety that is covalently linked to the N-terminal glycine. This modification combined with basic residues in the MA domain form a bipartite signal that facilitates intracellular transport of the gag precursor and its association with the plasma membrane. Viruses of the genera *Alpha-*, *Beta-*, and *Gammaretrovirus* encode a protein between MA and CA, which contains one or two motifs that recruit host factors (ESCRT proteins) necessary for successful release of the budding virus from the cell. In primate lentiviruses, these motifs are encoded in a protein at the C-terminus of gag. The CA domain of the Gag precursor plays a key role in immature capsid assembly as well as in the formation of the mature viral core, which encapsidates the viral genome and replicative enzymes. Coating of the viral RNAs by NC appears to facilitate the compact packing of the viral genome into the core.

For members of the subfamily *Spumaretrovirinae,* the Gag protein is only cleaved once near the C-terminus and there are no mature cleavage products analogous to MA or NC of the *Orthoretrovirinae*. Gag also lacks features of other retroviral Gag proteins such as the addition of myristic acid at the N-terminus, the Cys-His boxes of NC, and the major homology region (MHR) of CA. Instead there are three glycine–arginine-rich (GR) boxes near the C-terminus, which are likely involved in assembly and/or RNA binding.

In all retroviruses three nonstructural, enzymatic proteins are incorporated into virions. These are the aspartyl protease (PR, encoded by the *pro* gene), the reverse transcriptase (RT, encoded by the *pol* gene), and integrase (IN, encoded by the *pol* gene). PR is required for cleavage of the Gag precursor, an obligate step in maturation and for virus infectivity. In some viruses a dUTPase (DU, role unknown) is also present. Proteins constitute about 60% of the virion dry weight.

Retrovirus Replication Cycle

Attachment and Penetration

Entry into the host cell is mediated by an interaction between the virion glycoproteins and specific protein receptors at the surface of the host cell. This interaction, sometimes aided by low pH in endosomal vesicles, induces a conformational change in the Env trimer that allows the fusion peptides of the TM proteins to be inserted into the target cell membrane and additional rearrangements of TM, which bring the viral and cell membranes together. Fusion of the viral envelope with the plasma membrane then occurs. Retroviral receptors are cell-surface proteins. For HIV, both the CD4 protein, which is an immunoglobulin-like molecule with a single transmembrane region, and a chemokine receptor (CCR5 or CXCR4), which span the membrane seven times, are required for membrane fusion. The receptors for ecotropic murine leukemia virus (MLV), amphotropic MLV, and gibbon ape leukemia virus (GALV) as well as M-PMV are involved in the transport of small molecules. These transporters have a complex structure with multiple transmembrane domains. For the ALVs two receptors have been identified: that for subgroup A viruses is a small molecule with a single transmembrane domain, distantly related to a cell receptor for low-density lipoprotein while that for subgroup B viruses is related to the TNF-receptor family of proteins.

Once the viral membrane has fused with that of the target cell, the viral core is exposed to the intracellular environment of the cytoplasm. It is not clear whether a specific uncoating event occurs or whether initiation of nucleic acid transcription is sufficient to partially disassemble the viral core. Subsequent early reverse transcription events are carried out in the cytoplasm in the context of a nucleoprotein complex derived from the mature capsid.

Reverse Transcription

Replication of the viral genome starts with reverse transcription by RT of virion genomic RNA into cDNA. This process was so termed because at the time of its discovery, it reversed the accepted flow of genetic information in the cell. The 3′ end of the genome-associated tRNA acts as a primer for synthesis of a negative-sense cDNA transcript (**Figure 4(b)**). Because the primer binding site is located near the 5′ end of the genome, the initial short product (minus-strand strong stop) must transfer to the 3′ end of the genome through duplicated sequences (R) that are present at each end of the viral RNA. The transferred transcript can now prime continued cDNA synthesis (**Figure 4(c)**).

The RT enzyme is structured such that the DNA synthetic active site is located in close proximity to an RNase H active site that specifically digests the RNA template strand present in the newly formed RNA–DNA hybrid. Reverse transcription thus involves the concomitant synthesis of DNA and digestion of the viral RNA. Short undigested (purine-rich) RNA products of this process act to prime virus-sense cDNA synthesis on the negative-sense DNA transcripts (**Figure 4(d)**). One of these polypurine primers (PP) located near the 3′ end of the genome initiates a second (plus-strand strong stop) strand transfer, this time using homologies in the tRNA primer-binding site (PB, **Figure 4(e)**). Both strand transfers result in duplication of 5′ and 3′ genomic RNA sequences respectively, so that in its final form the single linear dsDNA transcript derived from the diploid viral genome contains long terminal repeats (LTRs) composed of sequences from the 3′ (U3) and 5′ (U5) ends of the viral RNA that now flank the R sequence (**Figure 4(f)**). A high frequency of recombination is observed during the process of reverse transcription when, following co-infection of a cell, two genetically distinct RNAs are packaged into

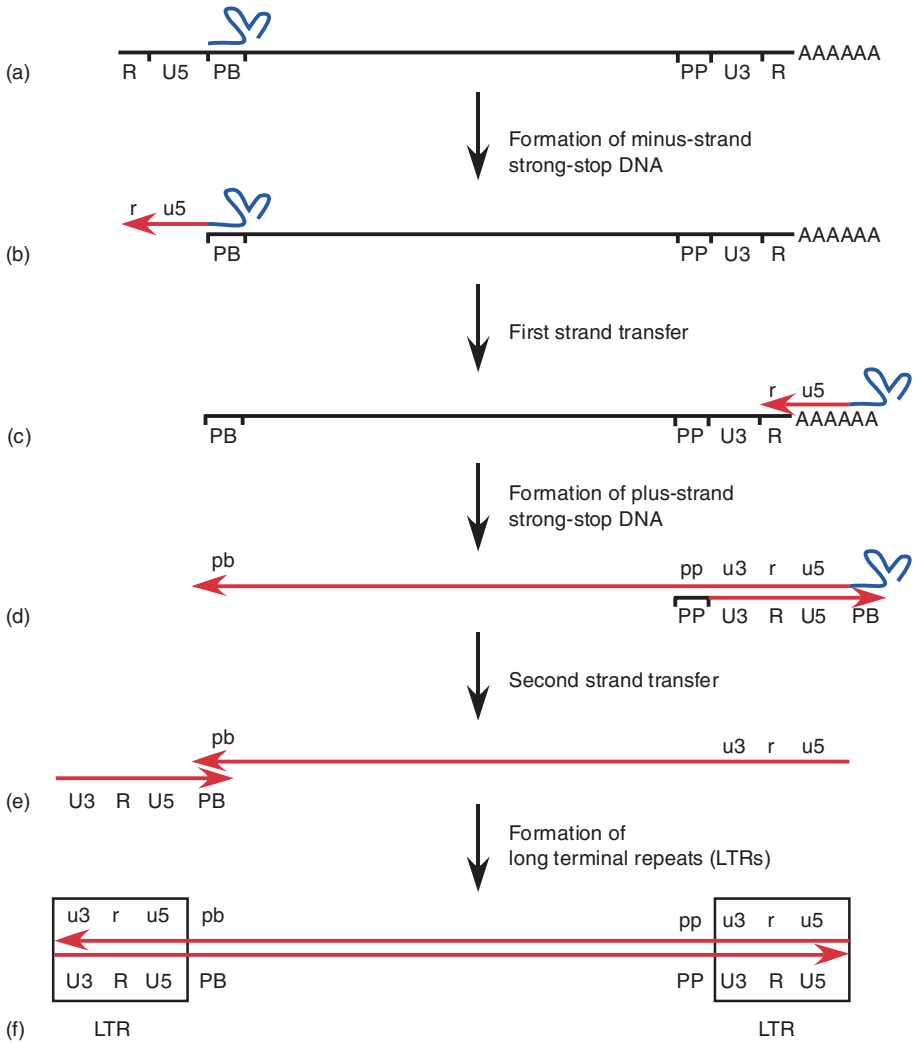


Figure 4 Reverse transcription: process of reverse transcription of the retroviral genome. RNA (black lines), DNA (red lines).

a retrovirus. This appears to reflect the frequent transfer of the elongating RT from one template RNA to the other, and implies that the RNAs are packaged in the core in a fashion that facilitates this.

Integration

The linear, double-stranded, greater-than-genome-length DNA product of reverse transcription complexed with the viral integrase is called the pre-integration complex (PIC). It must be transported into the nucleus in order for the viral DNA to be integrated into the chromosomal DNA of the host to form an integrated provirus. For most retroviruses, nuclear membrane breakdown during cell division is required for entry of the PIC, but the lentivirus PIC can be transported into the nucleus via nuclear pores, allowing proviral integration in nondividing cells. Integration is mediated by the viral IN protein. The ends of the virus DNA are joined to cell DNA, following the removal of two nucleotides from the ends of the linear viral DNA. Integration generates a short duplication of cell sequences at the integration site, the length of which is virus specific. Proviral DNA can integrate randomly at many sites in the cellular genome with no specific sequence being targeted, although there does appear to be a preference for integration in or near actively transcribed genes. Once integrated, a sequence is generally incapable of further transposition within the same cell. The map of the integrated provirus is collinear with that of unintegrated viral DNA. Integration appears to be a prerequisite for virus replication.

Genomic and mRNA Transcription

The integrated provirus is transcribed by cellular RNA polymerase II into virion RNA and mRNA species in response to transcriptional signals in the U3 region of the viral LTRs. In some genera, virus-encoded transactivating

proteins also regulate transcription. Transcription starts at the beginning of the 5′ R region and proceeds to the end of the 3′ R′ sequence. Signals in the U5 region of the 3′ LTR promote cleavage and polyadenylation of the RNA at this site. There are several classes of mRNA depending on the virus and the genetic organization of the retrovirus. With the exception of the spumaviruses, an mRNA comprising the whole retroviral genome serves for the translation of the *gag*, *pro*, and *pol* genes. This results in the formation of polyprotein precursors, which are the source of the structural proteins, protease, RT, and IN, respectively. A smaller mRNA consisting of the 5′ end of the genome spliced to sequences from the 3′ end of the genome that include the *env* gene and the U3 and R regions, is translated into the precursor of the envelope proteins. In viruses that contain additional genes, various additional forms of spliced mRNA are also made; however, all these spliced mRNAs share a common sequence at their 5′ ends. Spumaviruses are unique in that they make use of an internal promoter (IP) located in the *env* gene upstream of the accessory protein reading frames. Most primary translational products in retrovirus infections are polyproteins, which require proteolytic cleavage before becoming functional. The *gag*, *pro,* and *pol* products are generally produced from a nested set of primary translation products. For *pro* and *pol*, translation involves bypassing translational termination signals by ribosomal frameshifting or by readthrough at the *gag-pro* and/or the *pro-pol* boundaries (**Figure 1**).

Assembly and Release of Virions

Immature capsids assemble either at the plasma membrane (a majority of the genera) or at intracytoplasmic particles (*Betaretrovirus* and *Spumavirus*). Little is known about the intracellular targeting of Gag precursor proteins within the cell, although the myristic acid modification at the N-terminus of Gag and positively charged amino acids in the MA domain appear to provide a bipartite signal to initiate budding at the plasma membrane. For the betaretroviruses, a short (cytoplasmic targeting/retention) signal (CTRS) in the MA domain of Gag interacts with components of the dynein motor so that translating polysomes are transported to the pericentriolar region (microtubule organizing center) of the cell, where immature capsid assembly occurs. Mutations in the CTRS result in plasma membrane assembly of immature capsids. A similar mechanism for intracellular targeting and assembly of Gag appears to be utilized by the spumaviruses.

Most retroviruses are released from the cell by a process of budding from a region of the plasma membrane where viral glycoproteins must also be targeted. There is evidence in several genera that interaction of Gag and Env components occurs at an intracellular location prior to co-localization at the budding site. This process of budding, however, does not appear to require the Env proteins since expression of Gag alone is sufficient for release of virus particles. The final pinching-off and release of virus requires the complex cellular machinery (ESCRT) normally involved in multivesicular body formation, which is recruited to the site of budding by sequence motifs in Gag. Polyprotein processing of the internal proteins occurs concomitant with or just subsequent to release of virus from the cell and is accompanied by maturation of the virion. This includes morphological changes that include condensation of the viral RNA into an electron-dense ribonucleoprotein core and the acquisition of infectivity.

Pathogenesis

Members of the family *Retroviridae* establish persistent lifelong infections in their hosts – a reflection of their replication cycle and their ability to insert a copy of the proviral genome into the chromosome of a target cell. Because retroviruses are in general noncytopathic to their host cells and function as effective parasites that siphon off only a small percentage of the macromolecular machinery, continued production of progeny viruses over the lifespan of the cell is thus the norm. Moreover, in the context of the vertebrate host, this means that curing infection is effectively impossible, since a single retrovirus-infected cell can be the source of systemic infection.

Viruses from several retroviral genera are capable of inducing tumor formation in their natural hosts. It was the 'acute transforming' retroviruses, exemplified by RSV, that provided the key to our initial understanding of how retroviruses induce cancer in their hosts. These viruses, members of the genera *Alpha-* and *Gammaretrovirus*, have transduced cellular genes (now known as proto-oncogenes) that function in the signal transduction pathways involved in growth factor upregulation of cell proliferation. With the exception of certain RSV isolates, these acutely ransforming retroviruses are generally replication defective, since the inserted oncogene replaces replicative genes, and require a helper virus to provide the missing replicative functions. Nevertheless, they are capable of rapidly inducing a variety of cancers in their hosts. The replication-competent WDSV, a member of the genus *Epsilonretrovirus*, also induces cell transformation through expression of a cell-derived gene, although in this case it is related to the cyclin family of regulators.

For the so-called 'chronic transforming' members of the genera *Alpha-*, *Beta-*, and *Gammaretrovirus*, persistent infection with the high numbers of associated viral integration events eventually results in the insertional activation of cellular oncogenes. This was first defined for ALV where, in birds with this form of leukemia, frequent integrations just upstream of the *c-myc* oncogene

in malignantly transformed cells were found to result in its unregulated expression. Related mechanisms that deregulate cellular oncogene expression have been described for MMTV, MuLV, and other vertebrate 'leukemia' viruses. The human pathogen HTLV-1 induces T-cell-derived tumors in a fraction of infected individuals but this appears in part to be the result of *trans*-activation of cellular genes such as Il-2 and Il-2 receptors as well as inactivation of cell-cycle regulators such as p53 by Tax, which leads to unregulated proliferation of T-cells.

Members of the genus *Lentivirus*, including visna-maedi virus, EIAV, caprine arthritis encephalitis virus (CAEV), and HIV-1, generally establish persistent infections that progressively impose a defined pathology on their host. It was the progressive central nervous system degeneration in sheep induced by visna virus that led to the term 'slow infections' and to the nomenclature for this genus. The human pathogen HIV-1 is typical of members of the genus; although it is cytopathic in CD4+ T-lymphocytes, it establishes a persistent disease through constant cycles of infection of lymphocytes and macrophages in lymphoid tissues. Generally, it takes several years for depletion of the immune system to progress to a level where opportunistic infections and cancers, which are the hallmark of AIDS, develop. Interestingly, the simian immunodeficiency viruses (SIVs) do not appear to cause disease in their natural hosts, where presumably there has been co-evolution of virus and host to reach a nonpathogenic equilibrium.

See also: AIDS: Disease Manifestation; AIDS: Global Epidemiology; AIDS: Vaccine Development; Endogenous Retroviruses; Retroviral Oncogenes.

Further Reading

Desrosiers R (2007) Nonhuman lentiviruses. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 2215–2241. Philadelphia, PA: Lippincott Williams and Wilkins.

Freed EO and Martin MA (2007) HIVs and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 2107–2185. Philadelphia, PA: Lippincott Williams and Wilkins.

Goff SP (2007) *Retroviridae*: The retroviruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 1999–2069. Philadelphia, PA: Lippincott Williams and Wilkins.

Kuritzkes DR and Walker B (2007) HIV-1: Pathogenesis, clinical manifestations, and treatment. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 2187–2214. Philadelphia, PA: Lippincott Williams and Wilkins.

Lairmore MD and Franchini G (2007) Human T-cell leukemia viruses types 1 and 2. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 2070–2105. Philadelphia, PA: Lippincott Williams and Wilkins.

Linial M (2007) Foamy viruses. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn., pp. 2245–2262. Philadelphia, PA: Lippincott Williams and Wilkins.

Linial ML, Fan H, Hahn B, *et al.* (2004) *Retroviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses,* pp. 421–440. San Diego, CA: Elsevier Academic Press.

Rhinoviruses

N W Bartlett and S L Johnston, Imperial College London, London, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Afebrile Without a fever.
Bronchopulmonary dysplasia Chronic lung disease of infancy that follows mechanical ventilation and oxygen therapy for acute respiratory distress after birth in premature newborns.
Ciliated Cells with hair-like structures (cilia) on the surface.
Cytopathology Cell damage.
Endocytosis Uptake of material into the cell by membrane-bound vesicles.
Endosome Membrane-bound vesicle formed during endocytosis.
Heterotypic Of different types.
Nasal mucosa Mucous membrane lining the nasal cavity.
Nasopharynx The portion of the pharynx extending from the posterior nares to the level of the soft palate.
Nim Neutralizing immunogenic site.
Otitus media Infection and inflammation of the middle ear space and ear drum.
Peak expiratory flow The maximum flow at the outset of forced expiration.
Rhinorrhoea The free discharge of a thin nasal mucus.
Sinusitis Inflammation of the sinus.
TBP TATA-binding protein, part of RNA polymerase II transcription system.

History

Rhinoviruses are the most common infectious agents of humans and most frequent cause of the common cold (acute nasopharyngitis), a mild disease of the upper respiratory tract. More recently, their role in acute exacerbations of asthma and other airway disease has been highlighted, implicating the virus in illnesses significantly more severe than the common cold. Hieroglyphs representing the cough and common cold date back to ancient Egypt. In the fifth century BC Hippocrates gave a description of the disease and in the first century Pliny the Elder suggested 'kissing the hairy muzzle of a mule' as therapy for colds. The common cold was also known among the ancient American Indian, Aztec, and Maya civilizations. It was not until first Walter Kruse and then Alphonse Donchez in the early part of the twentieth century demonstrated that viruses (filtered material from the nasal secretions from a cold sufferer) caused the common cold. The first isolations of human rhinovirus (HRV) were reported by two laboratories in the late 1950s: Price in 1956 and Pelon and co-workers in 1957. With advances in tissue culture techniques that matched temperature and pH conditions to those found in the nose, identification of serologically distinct rhinoviruses increased rapidly such that by 1987 100 serotypes had been identified (**Table 1**). Efforts in the 1960s and 1970s to develop vaccines based on inactivated or attenuated viruses were unsuccessful due to the large number of rhinovirus serotypes and ineffective mucosal immunization. Lack of a practical small animal model for rhinovirus infection has meant that experimental human challenge models have been used to study virus pathogenesis. Although it has been long recognized that rhinovirus infections are the most frequent cause of the common cold, recent epidemiologic studies using sensitive polymerase chain reaction (PCR) techniques and data from human studies has highlighted the importance of rhinoviruses as precipitants of serious respiratory illnesses. This is especially evident in the context of persons suffering chronic airway diseases such as asthma and chronic obstructive pulmonary disease (COPD).

Taxonomy and Classification

The family *Picornaviridae* (pico = small + RNA) contains three genera of human pathogens that are structurally and genetically closely related: enterovirus, parechovirus, and rhinovirus. Rhinoviruses have been isolated from humans and cattle, with HRVs comprising by far the largest group. The HRV genus currently consists of 102 serotypes (HRV-1A to HRV-1B and HRV-2 to HRV-100, Hanks). Designation of serotypes is based on antibody neutralization, with absence of cross-reactivity of polyclonal antisera with defined serotypes constituting designation of a distinct serotype. (It should be noted that extensive serotyping has not been performed since the 1980s and several rhinovirus isolates await classification and may represent new serotypes.) The three bovine rhinoviruses (BRV-1–3) also await classification. All serotypes (except HRV-87) segregate into two genetic clusters or species based on nucleotide sequence across the VP4/VP2 interval: human rhinovirus A (HRV-A; 76 serotypes) and human rhinovirus B (HRV-B; 25 serotypes). Analysis of nucleotide sequences from HRV87 demonstrated that it is an enterovirus, most closely related to human enterovirus 68 (species D). The rhinovirus genus can also be classified into two groups according to receptor tropism, with approximately 90% of HRV (major receptor group) exploiting the intercellular adhesion molecule 1 (ICAM-1, CD54) for cell binding. The remainder (minor receptor group) use members of the low-density lipoprotein (LDL) receptor family. HRV-87 uses an as yet unknown sialoprotein for cell-surface attachment. The rhinovirus genus can also be divided into two groups according to the variability in susceptibility to capsid-binding drugs. The pattern of susceptibility has served as an alternative method of classification of HRV serotypes into two groups, A and B.

Virion Structure and Physical Properties

Rhinoviruses, typical of the family *Picornaviridae*, are small (approximately 30 nm, molecular mass of 8.5×10^6 kDa) icosahedral particles (**Figure 1**) composed of 60 copies of each of the four capsid proteins VP1–VP4 with molecular masses of 32, 29, 26, and 7 kDa, respectively. The capsid proteins are symmetrically arranged into protomers which contain one copy of VP1, VP3, and a precursor (VP0) in which VP2 and VP4 are covalently linked. Protomers, arranged around a fivefold axis, form pentamers and 12 pentamers form the icosahedral capsid shell. Capsid precursors encapsidate viral genomic RNA

Table 1 Species[a] and receptor binding[b] grouping of human rhinovirus serotypes (excluding HRV-87)

Human rhinovirus A	*Human rhinovirus B*
1A, **1B**, **2**, 7, 8, 9, 10, 11, 12, 13, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 28, **29**, **30**, **31**, 32, 33, 34, 36, 38, 39, 40, 41,43, **44**, 45, 46, **47**, **49**, 50, 51, 53, 54, 55, 56, 57, 58, 59, 60, 61, **62**, 63, 64, 65, 66, 67, 68, 71, 73, 74, 75, 76, 77, 78, 80, 81, 82, 85, 88, 89, 90, 94, 95, 96, 98, 100	3, 4, 5, 6, 14, 17, 26, 27, 35, 37, 42, 48, 52, 69, 70, 72, 79, 83, 84, 86, 91, 92, 93, 97, 99

[a]Species classification is based on genetic clustering of capsid (VP4/VP2) gene nucleotide sequences.
[b]Viruses shown in bold type bind to the LDL receptor (minor group); the remainder bind to ICAM-1 (major group).

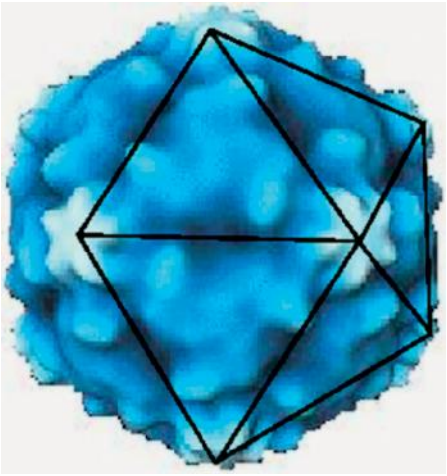


Figure 1 Human rhinovirus 14 solved by cryoelectron microscopy and image reconstruction. The fivefold axis of symmetry is superimposed on the image. Courtesy of The Big Picture Book of Viruses.

to form 150S provirions. Maturation cleavage of VP0 to VP2 and VP4 is the final step of assembly and yields infectious mature virions. VP1, VP2, and VP3 structurally constitute most of the capsid. They are similar in structure and have eight-stranded, antiparallel β-barrel motifs differing mainly in the loops and elaborations which join or project from the β-strands. VP1 is the most external and dominant structural protein and contains most of the motifs known to interact with cellular receptors and neutralize monoclonal antibodies. VP4 is smaller, has an extended structure, and lies at the RNA–capsid interface in close association with the RNA core and functions as an anchor to the virus capsid. A deep cleft, called the canyon, surrounds the fivefold axes of icosahedral symmetry and encloses the ICAM-1 binding site in the major receptor group viruses. Amino acids at the base of the canyon are more conserved than those on the protruding rim of the canyon and surface of the virion, which are more prone to substitutions and contain the binding sites for neutralizing antibody. Beneath this canyon, within VP1, lies a pore that leads to a hydrophobic pocket occupied by a pocket factor that is likely to be a fatty acid. The minor group LDL receptor binds to the star-shaped dome on the fivefold axis of the virion.

Rhinoviruses have a buoyant density in CsCl of 1.38–1.42. The property that distinguishes them from the closely related enteroviruses is their acid lability with inactivation occurring below pH 6. In contrast most rhinoviruses are thermostable, surviving for days at 20–37 °C. The ability to survive for extended periods of time in the environment is likely to be an important factor in their spread. Several serotypes (3–12, 15, 18, and 19) are relatively stable at 50 °C for 1 h. The lack of a lipid membrane enables rhinoviruses to resist 20% ether, 5% chloroform, and sodium deoxycholate solutions. Alcohol/phenol disinfectants are effective virucidal agents.

Properties of the Rhinovirus Genome

The nonenveloped virion encapsidates a genome composed of a single-stranded positive-(messenger) sense RNA molecule of 7100–7400 nucleotides. This RNA molecule functions directly as a message, encoding a single open reading frame containing approximately 2150 codons flanked at both termini by untranslated regions (UTRs) (**Figure 2**). At the 5′ terminus the 600-nucleotide-long UTR is covalently linked to a small, virus-encoded protein, VPg which initiates viral RNA synthesis. The UTRs have important secondary and tertiary structures; the first 100 nucleotides of the 5′-UTR forms a clover leaf structure that binds viral and host proteins forming a nucleoprotein complex required for viral RNA replication. The RNA molecule is directly translated from a type I internal ribosome entry site (IRES) also located in the 5′-UTR which directs recruitment of host-cell ribosomes and cap-independent translation of viral proteins. Downstream of the 5′-UTR is the capsid-coding region (P1), followed by the nonstructural protein encoding regions P2 and P3. The 3′ terminus of the genome contains a short (approximately 40 nucleotides) UTR and terminates with a poly-A tail. The single ~250 kDa polyprotein encoded by the rhinovirus genome is processed both during and after translation into mature viral proteins by a sequence of cleavages executed by virus-encoded proteases.

Properties of Rhinovirus Proteins

The rhinovirus proteins are numbered 1A(VP4), 1B(VP2), 1C(VP3), 1D(VP1), 2A, 2B, 2C, 3A, 3B, 3C, 3D, according to their physical location in the unprocessed polyprotein. The polyprotein is proteolytically cleaved to yield four capsid (structural) proteins and ten nonstructural proteins: seven mature proteins and three intermediate proteins with functions distinct from their cleavage products (2BC, 3AB, and $3CD^{pro}$). Three of the products of polyprotein processing function as proteases (^{pro}) while $3D^{pol}$ is the virally encoded RNA-dependent RNA polymerase. All of the nonstructural proteins are required for replication of the viral RNA. In addition, some nonstructural proteins alter host-cell function. The VPg protein (3B) acts as a primer for the initiation of RNA synthesis. Proteins $2A^{pro}$ and $3C^{pro}$ are cysteine proteases involved in processing the viral polyprotein. The first cleavage is catalyzed by $2A^{pro}$ which cleaves only at tyrosine–glycine bonds and performs the primary cleavage between the capsid and nonstructural precursors at the junction of the C terminus of VP1 and its own N terminus releasing

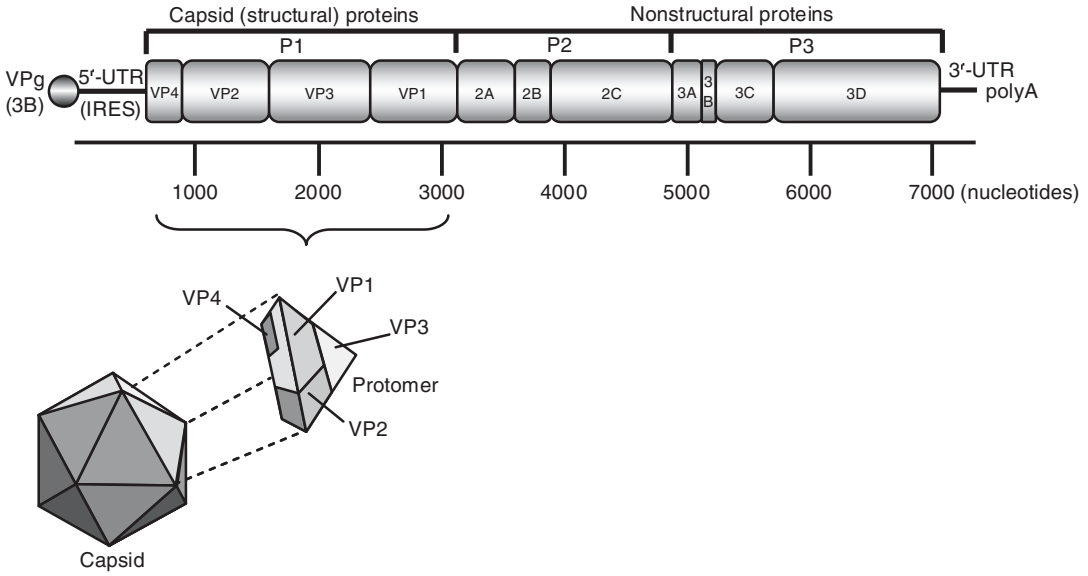


Figure 2 The rhinovirus single-stranded positive-sense RNA genome. The small VPg protein (3B) is attached to the 5′-UTR which encodes a type I internal ribosome entry site (IRES). The genome is polyadenylated (polyA) at the 3′ terminus. The single polyprotein encoded by the genome is cleaved by proteases to yield individual capsid and nonstructural proteins.

P1. The $2A^{pro}$ also mediates shutdown of host-cell translation by cleaving cellular eIF-4G, a key factor in cap-dependent translation. The $3C^{pro}$ catalyzes most of the subsequent cleavage events on the picornaviral polyprotein and along with the $3CD^{pro}$ cleaves the polyprotein at glutamine–glycine bonds. Rhinovirus $3C^{pro}$ may also play a role in virus-induced shutoff of host-cell transcription (by RNA polymerase II) by cleavage of the transcription factor TBP. It is thought that 3C enters the nucleus via a nuclear localization signal within the 3CD precursor. Experiments with the 2B protein of enteroviruses indicate that this viral protein is a 'viroporin' increasing plasma membrane permeability and inhibiting secretory pathways. The 3A protein may also modulate the secretory functions of cells affecting surface expression of host major histocompatibility complex (MHC) class I.

Life Cycle

Entry, Replication, and Assembly

The major group HRVs utilize ICAM-1 to attach to cells. ICAM-1 is a cell-surface glycoprotein and a member of the immunoglobulin (Ig) protein superfamily. Once bound to the receptor the viral particle is internalized by receptor-mediated endocytosis. Replication of rhinoviruses takes place in the cytoplasm of host cells. Uncoating and release of the viral RNA into the cytoplasm occurs after acidification of the late endosome. Acidification triggers conformational changes in the capsid centered on the fivefold axis, where a channel through the capsid and the endosomal membrane opens allowing RNA release. The positive-(message) sense RNA genome is translated into a single large polyprotein from the IRES located in the 5′-UTR of the rhinovirus genome. The IRES forms a complex secondary structure which can direct ribosomes to the polyprotein start AUG, thus initiating cap-independent translation. This allows the synthesis of viral proteins while cap-dependent translation of cellular proteins is shut off. The polyprotein is processed into functionally active proteins through a sequence of cleavages performed by virus-encoded proteases (described in the previous section). The cloverleaf secondary structure of the 5′-UTR in the viral genome binds viral (3C or 3CD precursor) and host (poly (A)-binding protein) proteins to form a nucleoprotein complex required for RNA replication, which is catalyzed by newly synthesized $3D^{pol}$ (viral RNA-dependent RNA polymerase), the most highly conserved polypeptide among members of the family *Picornaviridae*. Initially primed by the terminally bound VPg protein, the viral RNA polymerase uses the genomic positive-sense RNA as a template for synthesizing negative-sense copies which in turn act as templates for positive-sense RNA synthesis. Some newly synthesized positive-sense RNA copies act as messages, whereas others are packaged into virus particles. The viral polymerase has a mutation rate of one every 2200 bases (approximately four mutations per transcript) and is therefore at the threshold for genetic maintenance. This characteristic in conjunction with the ability to undergo viral recombination is probably a significant reason why rhinoviruses are such efficient pathogens of humans.

There is still much not known about the assembly of virus particles. Capsid assembly is via the precursor 5S protomers and 14S pentamers already described and takes

place in association with membranes. RNA is thought to be packaged into pre-formed 80S capsids although the molecular mechanism by which this process occurs is not well understood. Maturation to yield infectious virus relies on cleavage of VP0 into VP2 and VP4. Productive infection induces apoptosis (programmed cell death) and cell lysis facilitating release of virus from the cytoplasm.

Host Range, Propagation, and Detection

HRVs exhibit a high degree of species specificity due to the inability to bind nonhuman ICAM-1 on the cell surface. In addition, there appears to be a block to viral replication in nonpermissive cells. As a result efficient growth occurs only in human and some primate cells. An attempt to adapt a serotype for a mouse infection model had limited success and currently no small-animal HRV infection model has been reported. Nonhuman cells can be made permissive for infection if manipulated to express human ICAM-1 on the cell surface. Primary cells such as human embryonic kidney, bronchial epithelial, tonsil, and continuous human cell lines such as HeLa, H292, and HEP-2 can support growth of HRVs. The most commonly used cells for rhinovirus growth are the WI-38 strain and the MRC-5 strain of diploid fibroblasts, foetal tonsil cells, and HRV-sensitive HeLa (e.g., Ohio HeLa). Growth of virus in cell monolayers is usually detected by the appearance of cytopathic effect (CPE), which initially appears as foci of rounded up cells. HRVs can be plaqued on a number of cell lines with most techniques employing a semisolid overlay. Many HRV serotypes, particularly group B members, do not grow well in tissue culture. Nevertheless virus culture in susceptible cell lines has been the 'gold standard' for laboratory diagnosis of respiratory virus infections. However tissue culture techniques are generally laborious, time consuming, and insensitive when compared with more recent PCR-based assays. For this RNA is extracted from potentially infected samples and copied to cDNA by reverse transcription. PCR using HRV-specific primers targeting conserved viral sequences, such as the 5′-UTR, is then performed to determine the presence of viral genetic material in the original sample. PCR techniques have advanced further with the development of quantitative real-time PCR (qRT-PCR). This technique is more sensitive than conventional PCR and enables the number of viral RNA copies in a sample to be measured. In addition to direct detection of HRV by culture or PCR, antiviral antibodies can be measured by serological methods such as enzyme-linked immunosorbent assay (ELISA). Serological assays are diagnostically useful only when the HRV serotype is already known (i.e., for research purposes such as experimental human infections).

Serological Relationship and Antigenic Variability

With over 100 immunologically distinct serotypes currently recognized HRVs exhibit remarkable antigenic variability. The existence of a large number of HRV serotypes is in contrast to two other medically important picornaviruses, the polioviruses (three serotypes) and hepatitis A virus (a single serotype). While the development of virus-specific neutralizing antibody correlates with protection from disease, anti-HRV antibodies are highly serotype specific, rarely exhibiting cross-serotype neutralizing activity. Several serotypes can be grouped on the basis of low-level immunological cross-reactivity with hyperimmune rabbit serum (HRV-36, -58, and -89, for example), but it is not known whether this plays any role in cross-serotype protection. Mutagenesis studies have identified four neutralizing immunogenic sites along the edge of the capsid canyon. Nim-1a and Nim-1b on VP1 are located above the canyon, and Nim-2 on VP2 and Nim-3 on VP3 are positioned below. Structural analyses of neutralizing antibodies complexed with virus indicate that effective neutralizing activity depends on bivalent antibody binding that both blocks receptor interaction and stabilizes the virus capsid. Typically the most effective neutralizing antibodies bind with high affinity and span the canyon receptor site. In contrast to antibody responses, T-cell responses may be cross-reactive against numerous HRV serotypes, with the major T-cell epitopes buried within the viral capsid where amino acid sequences are more conserved.

Epidemiology

The common cold is almost certainly the commonest illness affecting mankind, occurring in all populations and ages throughout the year. The illness that results from infection is a major cause of morbidity and in turn lost productivity through absenteeism from work or school. Infection rates are highest among infants and children who may be infected up to 12 times a year. Infection rates decrease with age with adults infected on average 2 to 5 times per year. Infections exhibit a seasonal pattern and are more prevalent in autumn and in late spring. It is clear that school attendance is a major contributing factor to seasonality of infection. HRVs cause between 35% and 60% of common colds. While cold exposure does not seem to be a contributing factor to susceptibility to HRV infection, factors such as age and family structure are clearly important. Infections increase significantly from the second year of life, peak around the age of 6, and decline thereafter. The family unit is a major site for spread of HRV in modern societies. Most often an infected child introduces the

infection into the family with other siblings and the mother most at risk of secondary infection, presumably because of increased exposure. Schools and day-care centers are also sites of high transmission due to overcrowding, low immunity, and the unhygienic habits of children. Rhinoviruses have also been shown to spread among university students and boarding school residents. HRVs also cause a significant number of afebrile respiratory illnesses in military populations. All populations are affected and observations indicate that multiple HRV serotypes circulate within a population at any given time, and prevalent serotypes change from year to year.

Pathogenesis of Rhinovirus-Induced Disease

Transmission

Two routes are likely to be important for person to person spread of rhinovirus infection via virus-contaminated respiratory secretions: direct hand–surface–hand contact and aerosol inhalation. Rhinoviruses are able to survive on environmental surfaces for several hours at ambient temperature. HRVs were recovered from up to 90% of hands of persons with a cold and from a range of environmental objects such as door knobs, dolls, cups, and glasses. Transfer of virus through touching such objects occurs in seconds. Subsequent rubbing of the nose or eyes with infected hands can result in direct inoculation. Despite compelling evidence supporting direct transmission, demonstrating this in natural circumstances has been less convincing. In contrast there is good evidence suggesting inhalation of aerosols is the major route of HRV infection.

The incubation period preceding virus shedding is 1–4 days, usually 2–3 days. Virus in nasal secretions peaks 2–4 days post infection and remains high for 7–10 days, although low levels may persist for as long as several weeks. Atopic asthmatic subjects may have impaired virus clearance and increased virus load, factors that correlate with increased severity of illness. High virus titers in nasal secretions, increased symptoms, time spent in contact, and social factors such as crowding and poor hygiene contribute to increased transmission of virus.

Pathogenesis and Clinical Features

The nose is the main portal for HRV entry into the body and the primary site of replication is the epithelial surface of nasal mucosa. The virus is then transported to the posterior nasopharynx by ciliated epithelial cells. Clinical signs of illness are generally limited to common cold-like symptoms. HRV replication may also occur in the lower airways and this may actually be a common occurrence following infection of the upper airways. Unlike influenza or adenovirus infections HRV infection produces little, if any cytopathology of the nasal mucous membrane. The absence of histopathology in infected nasal mucosa initially led to the suggestion that mediators of inflammation produced by infected cells in the airway are responsible for cold symptoms. HRV infection *in vitro* and *in vivo* induces production of numerous pro-inflammatory and immune mediators including interferons (IFN-α/β, IFN-λ), chemokines that attract neutrophils (CXCL8/IL-8, CXCL5/ENA-78, CXCL1/GROα), esoinophils (Eotaxin/CCL10, CCL5/RANTES) or lymphocytes (CCL5/ RANTES, CXCL10/IP-10), pro-inflammatory cytokines (IL-1β, IL-2, IL-4, IL-11, IL-12, IL-13, IL-16, IFN-γ, and TNFα), growth and differentiation factors (IL-6, G-CSF, GM-CSF, IL-11), cell adhesion molecules ICAM-1 and vascular cell adhesion molecule (VCAM), and respiratory mucins. The presence of an array of pro-inflammatory mediators is supportive of an immune rather than cytopathic mechanism of rhinovirus-induced illness (**Figure 3**).

Rhinovirus infections are usually relatively trivial, producing symptoms of the common cold including rhinorrhoea, sneezing, nasal discharge and obstruction, coughing, and sore throat. Fever and malaise are less commonly seen than in other respiratory virus infections. Sleep patterns are often disrupted affecting mood and mental functioning. Symptoms appear after a 24–48 h incubation period, peak 2–3 days later, and last for 5–7 days in total, but may persist for up to 2–4 weeks. Severity of symptoms resulting from HRV infections is highly variable ranging from a barely apparent illness to a non-influenza flu-like disease. The most common complications associated with infection of the upper respiratory tract in children are acute otitis media and sinusitis. Rhinoviruses can also infect the lower respiratory tract and thereby account for lower respiratory symptoms and can cause serious and debilitating disease, particularly in young children, the elderly, the immunocompromised, and patients with chronic disorders such as cystic fibrosis and bronchopulmonary dysplasia. In patients hospitalized with respiratory problems rhinovirus infection has been associated with pneumonia in infants, wheezing in asthmatic children, exacerbations of chronic bronchitis, asthma and COPD, and congestive heart failure in older adults. HRV infection is the most frequent causative agent of asthma exacerbations. Recent studies have demonstrated that 80–85% of asthma exacerbations in children are associated with respiratory virus infections, with rhinoviruses accounting for two-thirds of these infections or 50% of all exacerbations (**Figure 4**).

Immune Response and Immunity

Serum antibody specific to the infecting HRV develops between 7 and 14 days after inoculation, but initially antibody-free volunteers may take up to 21 days to be

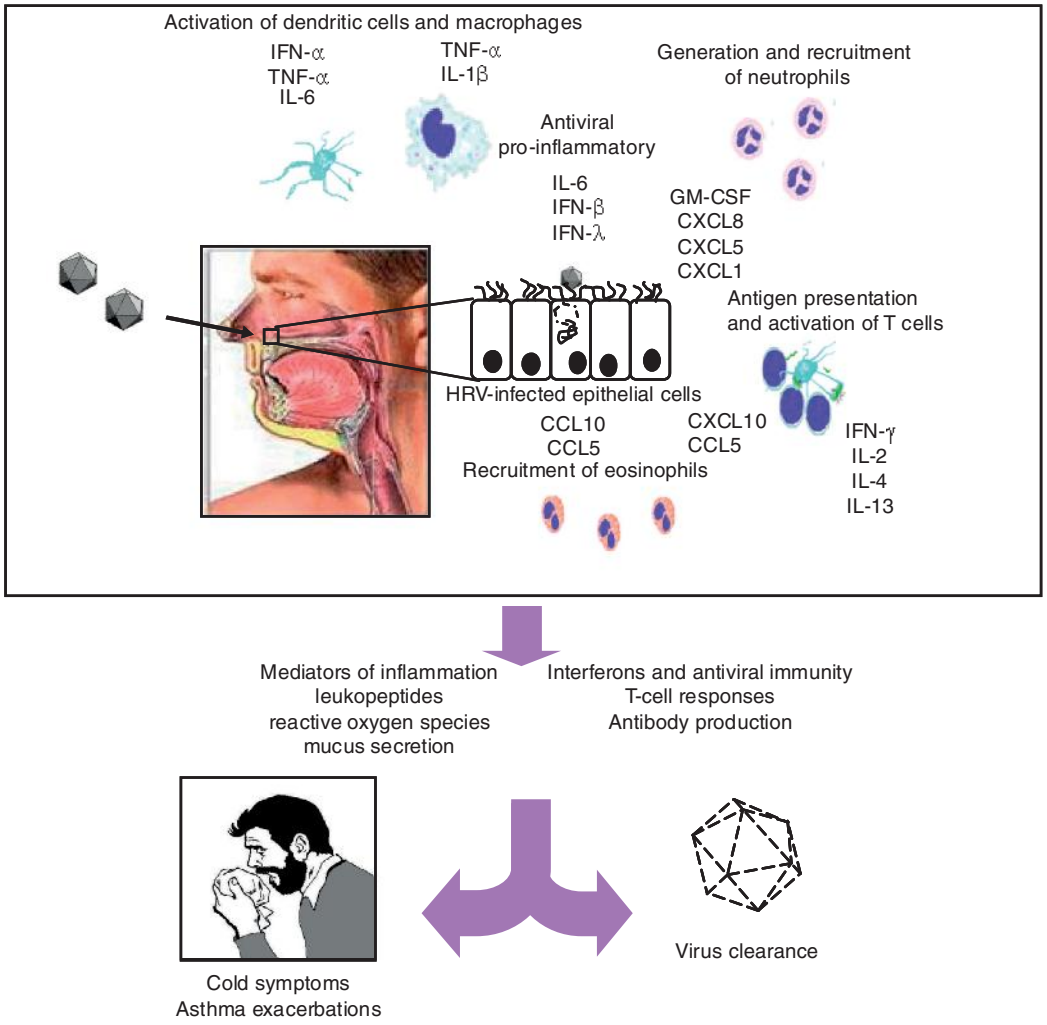


Figure 3 Mechanism of rhinovirus-induced illness. Infection of nasal epithelium stimulates production of a range of pro-inflammatory mediators causing immunopathology usually associated with cold symptoms, or exacerbations of asthma in susceptible individuals.

detected. Virus-specific IgG and IgA antibodies remain low for the first week post infection, peaking approximately a week later. The dominant serum antibody response to infection is IgG1, followed by IgG3, IgG4, and IgG2; IgA1 is the dominant IgA subclass. IgG antibodies stay at high levels for at least a year, while IgA levels decline slowly, but remain detectable for the same period. IgA is the dominant immunoglobulin in nasal secretions, becoming detectable 2 weeks post inoculation, peaking 1 week later. Serum and secretory antibody persist for several years after infection, although levels decline. Given that HRV-specific antibodies appear relatively late in the infection, humoral immunity is likely to be not essential for recovery from viral illness but may be involved in final viral clearance. Preexisting antibodies are likely to be important in protection from reinfection with the same serotype. Mechanisms of antibody-mediated virus inactivation might include virus aggregation and complement activation, blocking receptor binding and stabilization of capsid to prevent uncoating.

Cellular immunity is also activated following HRV infection. In contrast to the highly serotype-specific humoral response, virus-specific lymphocytes may be activated by several serotypes indicating shared viral epitopes, but their role in subsequent protection is not known.

Prevention and Control

Currently there are no effective strategies for prevention or treatment of rhinovirus common colds. The large number of serotypes and lack of heterotypic humoral immunity is a major issue precluding the use of conventional vaccine approaches. Vaccine enhancement of virus-specific cell-mediated immunity may be a more promising approach since T-cell epitopes are more conserved.

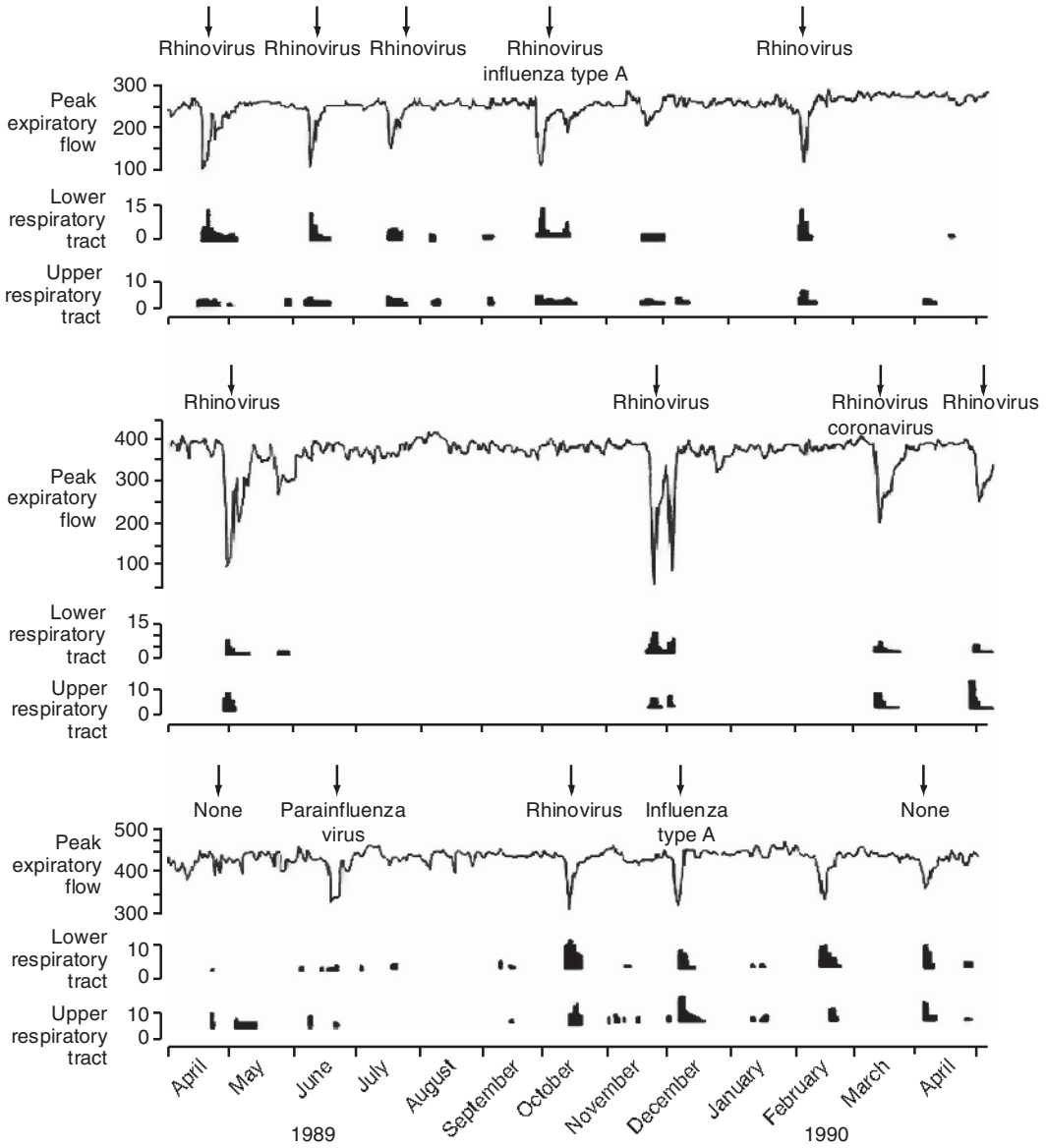


Figure 4 Association of marked reductions in lung function (peak expiratory flow) and exacerbations of lower respiratory tract symptoms with rhinovirus infection for three asthmatic children. Reproduced from SL Johnston, PK Pattemore, G Sanderson, *et al.* (1995) Community study of role of viral infections in exacerbations of asthma in 9–11-year-old children. *British Medical Journal* 310(6989): 1225–1229, with permission from BMJ Publishing Group.

The lack of a vaccine or specific therapies and the commonness of the illness have resulted in the emergence of numerous nonspecific therapies for the common cold. These include ascorbic acid, zinc gluconate, echinacea, and inhalation of hot humidified air. The efficacy of any nonspecific measure in prevention or treatment of the cold is yet to be generally accepted. Most work has been invested into the development of chemotherapeutic approaches. These include pharmacological antiviral agents that indirectly inhibit virus replication such as IFN-α. Studies demonstrated that IFN-α was effective in preventing the onset of cold symptoms when administered prophylactically. However it had little to no effect when given after infection and was often associated with various side effects. A large number of compounds designed to inhibit virus uncoating and/or cell binding and entry have been studied. So far an effective drug free of side effects is yet to emerge from this research. One such drug, Pleconaril, was submitted for approval to market as a treatment for the common cold in adults. Evidence showed that Pleconaril reduced the duration of cold symptoms if taken within the first 24 h of a cold. Unfortunately, the drug was not approved due to interactions in test subjects with the oral contraceptive and associated side effects.

Another approach has involved blocking virus binding to cells using a soluble form of the receptor. A number of reports have demonstrated that soluble ICAM-1 inactivates the virus and is effective at inhibiting viral entry. Despite encouraging results with soluble ICAM-1 no development is currently ongoing for these agents due to problems with formulation and delivery.

See also: Common Cold Viruses; Picornaviruses: Molecular Biology.

Further Reading

Contoli M, Message SD, Laza-Stanca V, *et al.* (2006) Role of deficient type III interferon-lambda production in asthma exacerbations. *Nature Medicine* 12(9): 1023–1026.

Couch RB (2001) Rhinoviruses. In: Knipe DM, Howely PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 4th edn., pp. 777–797. Philadelphia, PA: Lippincott Williams and Wilkins.

Edwards ME, Kebadze T, Johnson MW, and Johnston SL (2006) New treatment regimes for virus-induced exacerbations of asthma. *Pulmonary Pharmacology and Therapeutics* 19: 320–334.

Heikkinen T and Jarvinen A (2003) The common cold. *Lancet* 361(9351): 51–59.

Johnston SL, Patterson PK, Sanderson G, *et al.* (1995) Community study of role of viral infections in exacerbations of asthma in 9–11-year-old children. *British Medical Journal* 310(6989): 1225–1229.

Papadopoulos NG and Johnston SL (2004) Rhinoviruses. In: Zuckerman AJ, Bantavala JR, Griffiths PD, and Schoub BD (eds.) *Principles and Practice of Clinical Virology,* 5th edn., pp. 361–377. West Sussex, England: Wiley.

Wark PA, Johnston SL, Bucchieri F, *et al.* (2005) Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *Journal of Experimental Medicine* 201(6): 937–947.

Ribozymes

E Westhof and A Lescoute, Université Louis Pasteur, Strasbourg, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Nucleolytic Qualifies ribozymes which undergo cleavage and ligation following a nucleophilic attack of the 2′ hydroxyl group on the adjacent 3′ phosphate group.
Transesterification Transfer of a phosphoryl group.
Transpeptidation Formation of a peptide bond.
Concatemer Multiple copies of a DNA sequence arranged end to end in tandem.

Introduction

The discovery in the 1980s of RNA molecules with catalytic activity has revolutionized molecular biology. Ribozymes, molecules able to catalyze various chemical reactions, are widespread in biology. Large- and small-size ribozymes can be distinguished. The large ribozymes comprise the group I introns, the group II introns, and ribonuclease P (RNaseP). A step of maturation of pre-mRNAs in some bacteria or in nuclear eukaryotic rRNAs implicates the self-splicing of group I introns which use a guanosine molecule as a cofactor to catalyze a two-step transesterification reaction. The crystal structures of group I introns show that the catalytic core is conserved and stabilized by long-range interactions that involve different peripheral elements. At the same time the structures of the specificity domain of two RNaseP RNAs, responsible for the maturation of pre-tRNA, have been solved and show that the global architecture of the molecule is conserved partly due to the long-range interactions. Most importantly, it was established that the rRNA 23S is responsible for catalyzing the transpeptidation reaction during protein synthesis. Among the simplest catalytic RNA molecules are the small nucleolytic RNA species. The small ribozymes include the Varkud satellite ribozyme (VS ribozyme), the hairpin ribozyme, the hepatitis delta virus (HDV), and hammerhead ribozymes. They carry out reversible cleavage and ligation reactions in the presence of physiological concentrations of magnesium ions. Ribozyme activity is necessary during the replication of some plant pathogen RNA genomes.

The Chemical Reactions

The reaction catalyzed by small nucleolytic ribozymes takes place at a specific phosphodiester bond by a transesterification reaction involving the 2′-hydroxyl group for cleavage and the 5′-hydroxyl group for ligation. The cleavage reaction proceeds by activation of the 2′-hydroxyl adjacent to the scissile phosphate. The 2′-hydroxyl group performs a nucleophilic attack on the adjacent phosphorus atom, which leads to a pentacoordinate transition state. After capturing a proton, the 5′-oxygen atom leaves leading to the formation of a cyclic 2′,3′-phosphate group (**Figure 1**). Ligation is the reverse reaction of cleavage and involves: (1) activation of the 5′-hydroxyl

group by a base; (2) attack of the 2′,3′-cyclic phosphate; and (3) liberation and protonation of the 2′-hydroxyl group. Magnesium ions are generally necessary for the native folding of RNA molecules. Whether magnesium ions participate actively or indirectly in the catalysis reaction is still a matter of debate. During recent years, it has become clear, however, that the nucleic acid bases themselves can play an active role in acid–base catalysis. Nowadays, the crucial roles for efficient catalysis of tertiary contacts between peripheral elements, even in small ribozymes, have been firmly established.

The Ribozymes of Plant Viroids and Virusoids

Replication by the Rolling-Circle Mechanism

Two of the autocatalytic RNAs, the hammerhead and hairpin ribozymes, were discovered first in some viroids and virusoids responsible for several economically important infectious diseases of plants. These pathogens are small circular single-stranded RNAs, without a capsid, possess no open reading frame (ORF) and the RNAs are classified as noncoding RNAs (**Figure 2**). Their replication takes place (1) in the cytoplasm with a helper virus in the case of virusoids; (2) in an autonomous way (without helper virus) in chloroplasts, or in the nucleus in the case of viroids. The replication of these circular genomes is made according to a 'rolling-circle' mechanism, which involves the copying of the circular genome positive (+) strand by an RNA polymerase of the host or helper virus to give a multimeric negative-strand RNA (–) (concatemer) (**Figure 3**). In the case of the viroid family *Pospiviroidae*, the synthesized linear concatemers of the (–) strands are used as matrix for the synthesis of (+) concatemers; the circularization of those which form unit genomes seems to require an RNA ligase as indicated by the presence in some instances of a 2′ phosphomonoester and a 3′,5′ phosphodiester bond.

In the family *Avsunviroidae*, the (–) concatemer undergoes an autocleavage reaction involving a hammerhead ribozyme. The mechanism of circularization of the (–) strand to be used as a matrix is still controversial. Indeed, two hypotheses have been proposed: either the intervention of an RNA ligase or the ribozyme catalyzes the opposite reaction and ligates the RNA. In the end, the (–) strand is used as matrix for the synthesis of (+) concatemers by the mechanism of rolling circle. After cleavage by the hammerhead ribozyme, the (+) strands are circularized. Thus, only the RNA intermediaries are implicated in this mechanism of replication since the viroid genome does not code for proteins. Thus, the various biological properties of the viroids, such as the ability to identify the host, depend exclusively on the sequence and the structure of their RNAs.

The hammerhead ribozyme plays a fundamental role in the replication of these pathogenic RNA genomes. Yet, its role is not limited to this function as it has been identified in genomes of the triton (*Notophtalamus viridescens*), in parasitic schistosomes, and in cave crickets (*Dolichopoda* sp.). Two hammerhead ribozymes have been found also in the *Arabidopsis thaliana* genome.

The Hairpin Ribozyme

The hairpin ribozyme is involved in the processing of RNA molecules generated in the replication cycles of various species. The tobacco ringspot virus satellite RNA (sTRSV), for example, carries a hammerhead ribozyme on the (+) strand and a hairpin ribozyme on the (–) strand. The crystal structure of the hairpin ribozyme shows that it is comprised of two important structural elements: a four-way helical junction which is necessary for the optimal function of the ribozyme and two internal loops within the interacting helices (**Figure 4**). The minimal ribozyme (i.e., without the other two arms) catalyzes the cleavage reaction but requires 3 orders of magnitude higher concentration of magnesium cations (Mg^{2+}) than the natural form. Single-molecule studies show that the natural ribozyme exists in three states: an undocked form (where helices do not contact each other), an intermediate state (with helices docked but without contact between the internal loops), and a docked form (with intimate contacts between the loops). Although the junction is not essential to the hairpin ribozyme activity, it greatly enhances the rate of folding of the ribozyme in an active form at physiological concentration of Mg^{2+} ions. These auxiliary elements play the same role as in the hammerhead ribozyme and appear to act as 'folding enhancers'. However, there is no evidence for the direct implication of metal ions in the chemistry of the hairpin ribozyme.

The Hammerhead Ribozyme

Classification

The natural hammerhead ribozymes can be classified into two main types according to the helix numbering which carries the RNA 3′ and 5′ ends. In type 1, the 5′ end is carried by helix I which always presents an internal loop. Helix II presents in some instances an internal loop before the terminal loop. Helix III does not present a terminal loop but possibly an internal loop. In type 3, the 5′ end is carried by helix III. Helices I and II present sometimes an internal loop preceding the terminal loop. Ribozymes where the 5′ end is carried by helix II have, so far, never been observed in any genomes.

Structure and folding

The structure of the catalytic core of the hammerhead ribozyme was determined by crystallography using an RNA/DNA hybrid as well as in the 'all RNA' form with a methyl group on the 2′-hydroxyl of the site of cleavage

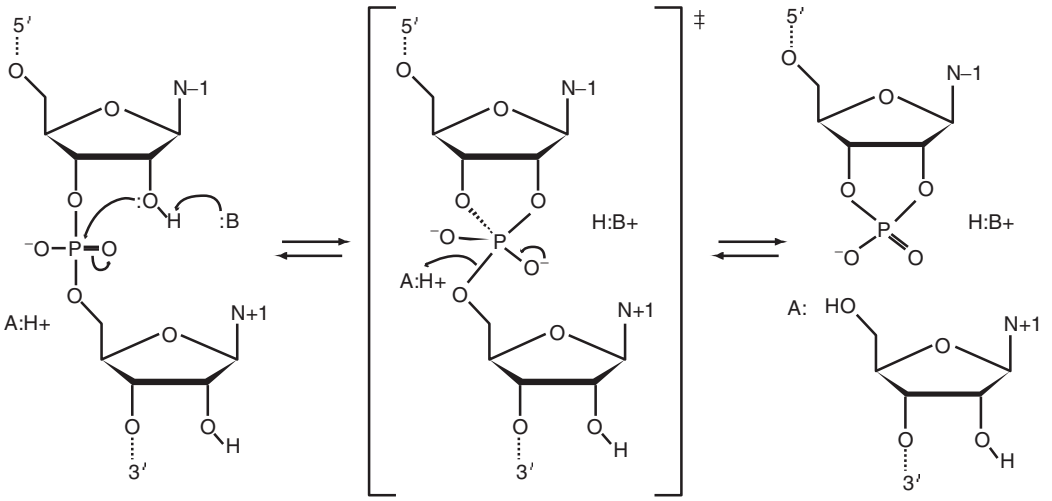


Figure 1 Reactions occurring during self-cleavage of the nucleolytic ribozymes like the hammerhead, the hairpin, the Varkud, or the Hepatitis delta virus ribozymes. The adjacent 2′-hydroxyl group is activated for nucleophilic attack. In a concomitant way, a proton is given to the leaving 5′-oxygen group.

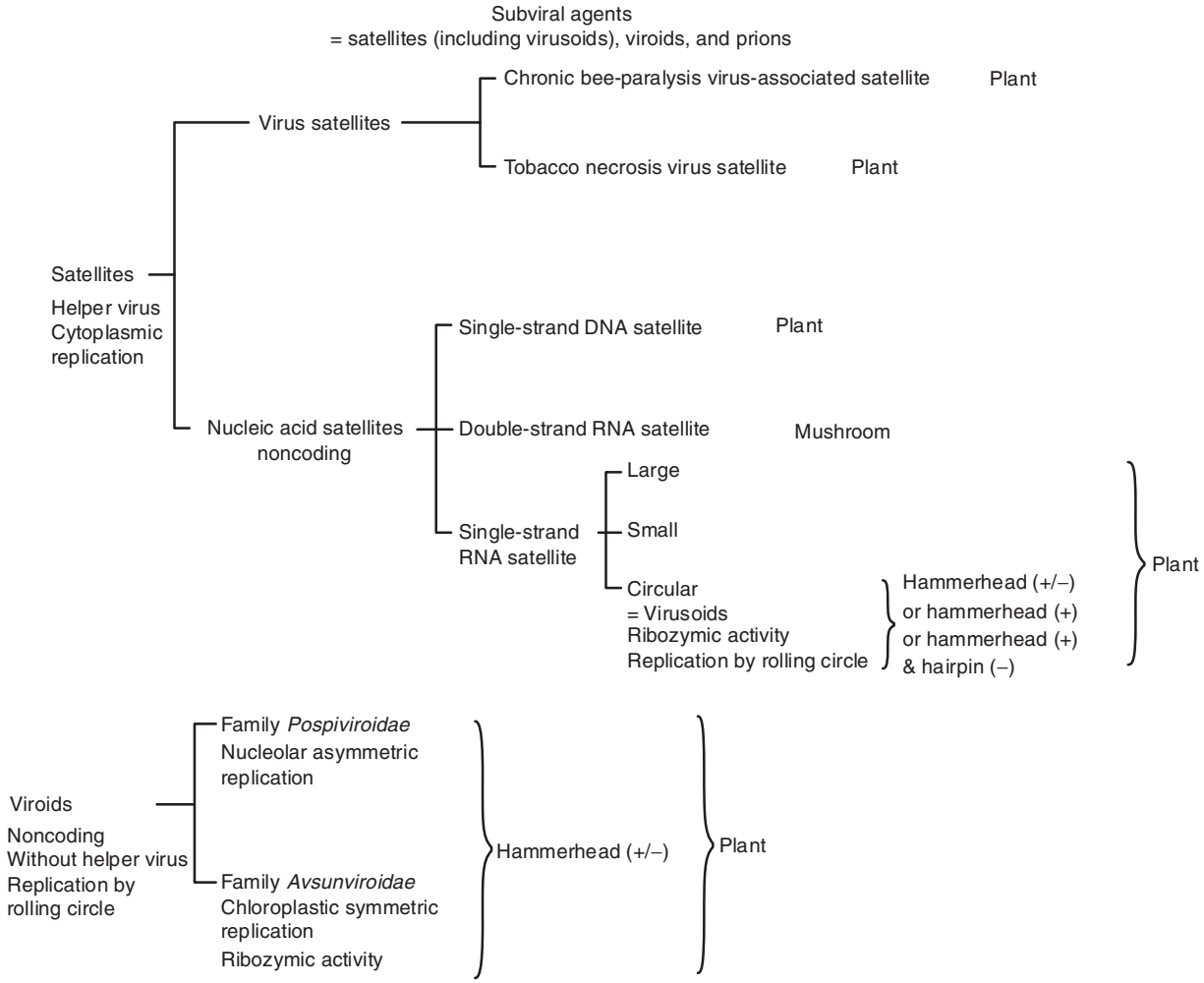


Figure 2 Classification of the satellites and viroids. The viroids proliferate and are copied in an autonomous way (without helper virus). Their circular RNA genomes do not have an open reading frame. The virusoids are satellite RNAs with circular single-stranded genomes. They do not code for protein, contrary to the satellite genomes of viruses which code for capsid proteins.

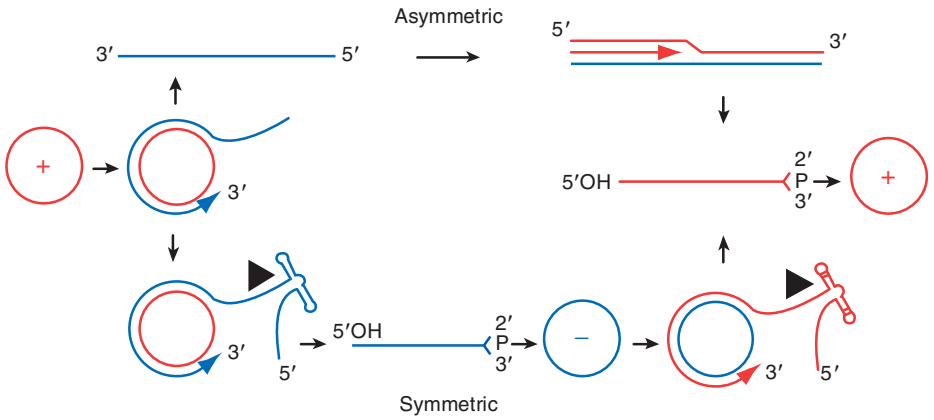


Figure 3 Mechanism of replication using the rolling-circle mechanism.There are two main ways for replication: an asymmetrical way (top) which requires only one rolling circle as seen in the family *Pospiviroidae* and a symmetrical way which requires two rolling circles specific to the family *Avsunviroidae*. The (+) strands are drawn in red and the (–) strands in blue. The hammerhead ribozymes in the cleavage of the concatemers utilize only the symmetrical way.

which prevents the cleavage reaction. The two practically identical structures show a catalytic core composed of 11 nucleotides essential for catalysis, organized in a three-way junction from which the helices I, II, and III leave. Helix II is stacked on helix III, while helix I is parallel to helix II. Helix II, composed of Watson–Crick base pairs, is stacked on three non-Watson–Crick base pairs.

Many structural studies of the ribozyme in solution have shown the importance of Mg^{2+} ions for folding. Indeed, in absence of divalent ions, the core of the ribozyme is not structured and the helices are equidistant from each other, revealing a disorganization of stacking, whereas in the presence of divalent ions the ribozyme folds in two stages to an active structure. At a low concentration, helices II and III are stacked but the angle between helices I and II is very large and the catalytic core is not structured; the ribozyme is inactive. At higher concentration, helix I reorients to a position near helix II and the ribozyme is then in an active conformation. However, the majority of the studies on hammerhead ribozyme folding were carried out on minimal ribozymes presenting the conserved and presumably essential elements, that is, 13 nucleotides of the catalytic core of which 11 cannot be mutated without causing loss of activity, and three helices I, II, and III with variable length and termination. These minimal ribozymes are active in an optimal way at high concentrations of Mg^{2+} ions (>10 mM) but almost inactive at physiological concentration of Mg^{2+} (0.1–0.3 mM).

However, recent studies showed that the tertiary contacts between the terminal loops of helices I and II, peripheral to the catalytic core and nonessential for catalysis, are essential for the activity of hammerhead ribozymes in physiological conditions (low concentration of Mg^{2+} ions). The role of the peripheral structures and their interactions in the catalytic activity of the hammerhead ribozymes is expected from previous observations

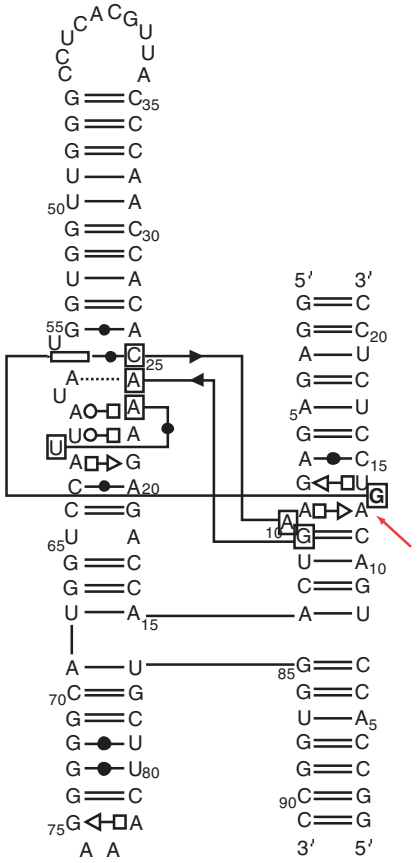


Figure 4 Schematic diagram of the interactions present in the three-dimensional crystal structure of the hairpin ribozyme (PDB ID 1HP6). The red arrow indicates the cleavage site.

on longer ribozymes such as group I introns. The progressive deletion of the peripheral area of group I introns results in increasing requirements of salts and ultimately in loss of activity. The various categories of group I intron

all have the same catalytic core, although the outlying areas and the tertiary interactions differ and they all contribute to the stability of the catalytic core. This suggests that during evolution, several folding solutions were found which ensured the optimal catalytic activity of the ribozyme. In the same manner, the peripheral area of the natural hammerhead ribozymes evolved so as to optimize the folding and the catalytic activity for biological needs under natural conditions of Mg^{2+} ion levels. The tertiary interactions between these regions reduce the conformational space accessible to the molecule and facilitate the folding of the ribozyme, thereby increasing its catalytic activity. It is clear that the catalytic core of the ribozyme had to undergo structural changes, which explains the increase in its catalytic efficiency although the precise nature of the changes is unknown. The structure of the full-length *Schistosoma mansoni* hammerhead ribozyme reveals the structural basis for the effectiveness of natural ribozymes and explains most of the apparent inconsistencies between the structure of the minimalist ribozymes and the biochemical data.

Role of Peripheral Elements in the Mechanism of Ligation

Like any enzyme, the hammerhead and hairpin ribozymes are able to carry out the reaction opposite to cleavage, that is, ligation. However, in majority of conditions, cleavage is favored at least 100-fold compared to ligation. The various stages for ligation are (1) activation of the 5′-hydroxyl by a base, (2) attack of the 2′,3′-cyclic phosphate, and (3) release and protonation of 2′-hydroxyl, restoring the 3′,5′-phosphodiester bond.

The Hairpin Ribozyme

The studies of the dynamics of the hairpin ribozyme made it possible to determine the conformational kinetic properties of the reactions of ligation and cleavage. They show that after cleavage the four-way junction of the ribozyme moves into an undocked state. The reaction of cleavage is definitely slower than the reaction of ligation, but ligation is slower than helical unstacking. The product is thus normally released before ligation takes place. However, if the product of cleavage remains bound to the enzyme, the ribozyme alternates between docked and undocked state before ligation takes place. These experiments also show that the nature of the product is a factor influencing the speed of ribozyme unfolding. Indeed, the presence of a 2′,3′-cyclic phosphate increases the unfolding of the ribozyme compared to a product having either a 3′-phosphate or 3′-hydroxyl. Therefore, the product has important effects on structural dynamics of the ribozyme, and the loss of covalent continuity in the backbone is not the only factor increasing the undocking of the ribozyme. The undocking promoted by the presence of cyclic phosphate, involves detachment of the product. This is in agreement with the biology of the ribozyme in its natural environment where it cleaves the RNA concatemer (produced by rolling circle) and releases a monomeric 2′,3′-cyclic phosphate product. In the case of the hairpin ribozyme (–) strand of the satellite RNA 'sTRSV', the helix formed by the product and the enzyme, with a length of six base pairs, has a weak dissociation constant. Single-molecule spectroscopy shows that docking–undocking events can take place several times before the ligation does take place. The equilibrium constant of cleavage and ligation shows a significant bias toward ligation with an internal equilibrium constant of $K_{int} = k_L/K_C = 34$. This preference for ligation ensures that during virus replication a certain number of circular RNAs will be maintained which will be used as matrix for the synthesis of the concatemer. On the other hand, the rate of undocking is greater than the rate of ligation which allows cleavage of concatemers because of the rapid undocking that follows cleavage.

The Hammerhead Ribozyme

For the hammerhead ribozyme, it has been shown that the kinetic constant of ligation ($k_{-2} = 0.008$ min ^{1}) is 100 times lower than the constant of cleavage ($k_2 = 1\ min^{-1}$); the equilibrium constant $K_{eq} = k_2/k_{-2}$ thus has a value of 125 (**Figure 5**). This is explained by the more favorable thermodynamic state of the enzyme/product complex than of the complex enzyme/substrate which favors the formation of the latter by driving the reaction toward cleavage. The tertiary interactions, by limiting the accessible conformational space (i.e., by stabilizing the folding of the complex enzyme/product), increase the ligation activity of the ribozyme. The same phenomena are observed for the hairpin ribozyme, since the presence of the native four-way junction with four helices moves the internal equilibrium toward binding and increases the rate of folding.

The Mechanism of Monomer Circularization after Replication

The peripheral elements together with the catalytic core are not only involved in the reaction of cleavage but also in the reaction of ligation. Each monomeric copy of the genome, product of the replication by rolling circle and cleavage by the hammerhead ribozyme, must be circularized to be used in turn as a replication matrix. Little is known about the *in vivo* circularization of the monomer. The viroid RNAs belonging to the family *Pospiviroidae* are probably ligated by an RNA ligase as indicated by the presence of a 2′-phosphomonoester and a 3′,5′-phosphodiester bond at the site of ligation. On the other hand,

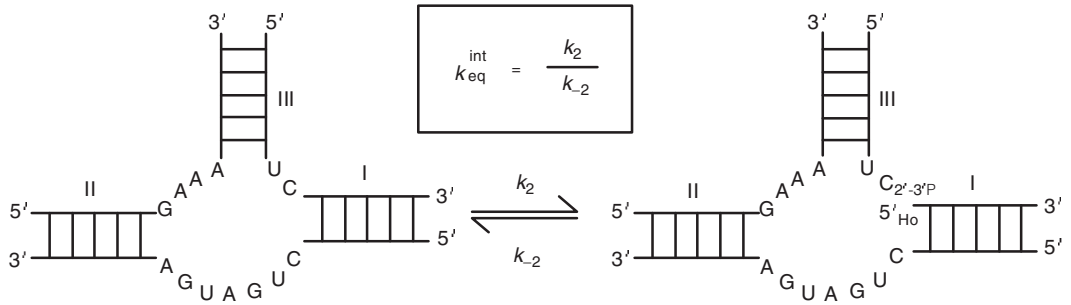


Figure 5 Secondary structure and reaction of the catalytic core of a minimal hammerhead ribozyme. The reaction of cleavage is defined by the constant of cleavage k_2 and the reaction of ligation is defined by the constant of ligation k_{-2}. The definition of the equilibrium constant is framed.

information on the ligation of viroid RNAs in the family *Avsunviroidae* is limited. In the case of the circularization of the genome of peach latent mosaic viroid (PLMVd), a 2′,5′-phosphodiester bond has been observed *in vitro* and *in vivo*. Such a bond would prevent cleavage of the ribozyme and would ensure the maintenance of an essential circular RNA matrix by the rolling-circle mechanism.

Yeast and Human Satellite Viruses

The *Neurospora crassa* versus Ribozyme

The VS RNA is transcribed from the mitochondrial DNA of *Neurospora crassa*. The VS RNA contains a ribozyme of around 150 nt in length acting in the processing of replication intermediates. There is only limited information available on its structure. The VS ribozyme comprises five helices organized in two three-way junctions (2–3–6 and 3–4–5 junctions) to form a Y shape. A pseudoknot is formed between the terminal loop of the substrate helix and the helix V of the ribozyme (**Figure 6**). The scissile phosphate is within an internal loop of the substrate stem–loop. The catalyzed reaction requires divalent Mg^{2+}cations.

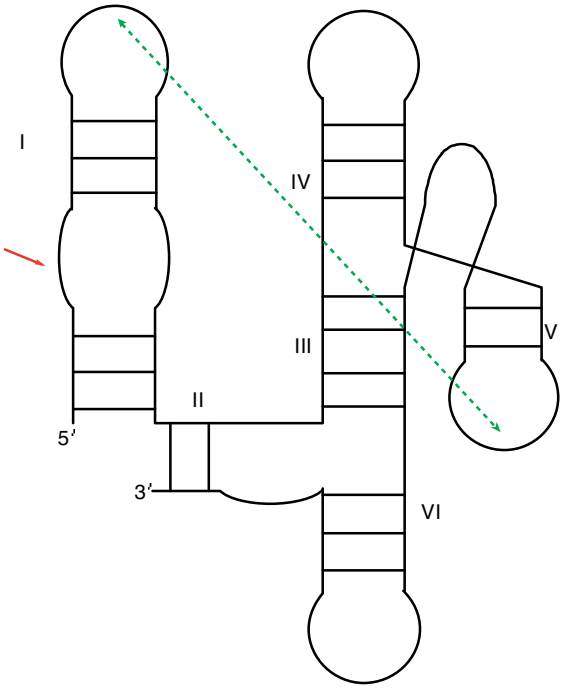


Figure 6 Schematic representation of the secondary structure of the VS ribozyme. The pseudoknot is indicated by the green arrow. The red arrow indicates the cleavage site.

The Human HDV

The HDV is a satellite virus of hepatitis B virus (HBV) on which it is dependent for its replication cycle. The genome of HDV is a 1700-nt-long circular single-stranded RNA. Its replication is RNA directed without DNA intermediates. Like certain pathogenic subviral RNAs that infect plants, HDV RNA replicates by a rolling-circle mechanism. Both genomic (+) and complementary (−) strands of RNA contain a single ribozyme of about 85 nt. The HDV ribozyme folds into a compact structure comprising a double pseudoknot and its disruption results in a marked loss of activity (**Figure 7**). The X-ray structures of the two states of the ribozyme (pre- and post-cleaved states) reveal a significant conformational change in the RNA after cleavage and point to the role of a divalent metal ion in the cleavage mechanism.

Conclusions

The hammerhead and hairpin ribozymes were initially studied in their minimalist form, which resulted in weak activities due to the presence of numerous conformations in dynamic exchange, most of them inactive, which required high concentrations of divalent ions for activity. However, the peripheral elements are essential for optimal activity in native conditions. These peripheral regions, by interacting with each other, facilitate and stabilize folding into a single active structure. They are, therefore, necessary

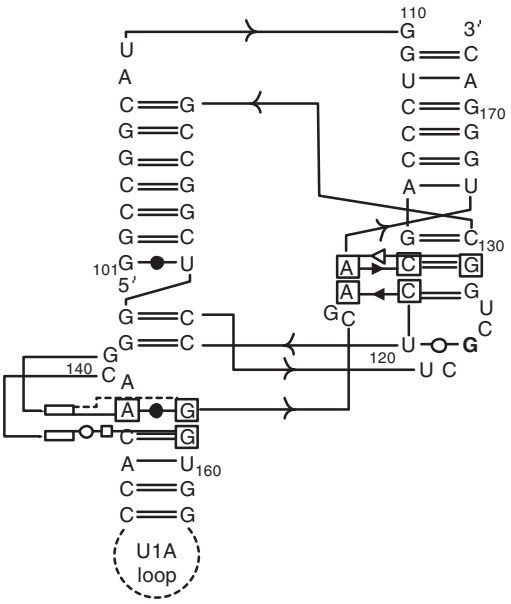


Figure 7 Schematic diagram of the interactions present in the three-dimensional crystal structure of HDV ribozyme after self-cleavage (PDB ID 1DRZ).

in physiological conditions although they are not directly implied in the catalysis. These results put into question the conclusions drawn previously from data obtained with molecular systems that had been simplified and reduced to the extreme. Full understanding of the chemical and biological actions of ribozymes is certainly not yet achieved, however, and surprises are still lurking. Recently, a ribozyme structurally derived from group I introns was shown to catalyze the formation of a 2′,5′-linkage leading to the formation of a lariat structure mimicking the cap of mRNA. Our present knowledge underlines the fundamental links existing between the folding pathway, the selection and stabilization of a single native state by tertiary interactions between peripheral elements and the catalytic activity of ribozymes.

See also: Icosahedral Enveloped dsRNA Bacterial Viruses; Icosahedral ssRNA Bacterial Viruses; Interfering RNAs; Satellite Nucleic Acids and Viruses.

Further Reading

Blount KF and Uhlenbeck OC (2005) The structure–function dilemma of the hammerhead ribozyme. *Annual Review of Biophysics and Biomolecular Structure* 34: 415–440.
Daros JA, Elena SF, and Flores R (2006) Viroids: An Ariadne's thread into the RNA labyrinth. *EMBO Reports* 7: 593–598.
Doudna JA and Cech TR (2002) The chemical repertoire of natural ribozymes. *Nature* 418: 222–228.
Gesteland RF, Cech TR, and Atkins JF (eds.) (2006) *The RNA World.* New York: Cold Spring Harbor Laboratory Press.
Khvorova A, Lescoute A, Westhof E, and Jayasena SD (2003) Sequence elements outside the hammerhead ribozyme catalytic core enable intracellular activity. *Nature Structural and Molecular Biology* 10: 708–712.
Lafontaine DA, Norman DG, and Lilley DM (2002) The global structure of the VS ribozyme. *EMBO Journal* 21: 2461–2471.
Leontis NB and Westhof E (2001) Geometric nomenclature and classification of RNA base pairs. *RNA* 7: 499–512.
Lilley DM (2004) The Varkud satellite ribozyme. *RNA* 10: 151–158.
Lilley DM (2005) Structure, folding and mechanisms of ribozymes. *Current Opinion in Structural Biology* 15: 313–323.
Martick M and Scott WG (2006) Tertiary contacts distant from the active site prime a ribozyme for catalysis. *Cell* 126: 309–320.
McKay DB (1996) Structure and function of the hammerhead ribozyme: An unfinished story. *RNA* 2: 395–403.
Westhof E (2007) A tale in molecular recognition: The hammerhead ribozyme. *Journal of Molecular Recognition* 20: 1–3.

Rice Tungro Disease

R Hull, John Innes Centre, Colney, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Agroinoculation Infection of a plant by injecting *Agrobacterium* containing a modified Ti plasmid into which an infectious copy of the viral genome has been inserted. In the case of pararetroviruses, this infectious copy comprises the sequence that can express the more-than-full-length RNA transcript required for the reverse transcription stage of viral replication.
Pararetrovirus A virus that replicates by reverse transcription but differs from retroviruses in that it encapsidates the DNA phase of replication and that virus replication does not require integration of the viral genome into the host chromosome.
Semipersistent transmission Virus–vector interaction in which the virus is acquired rapidly on feeding on an infectious plant, and then is transmitted by relatively short feeds on a healthy host plant. The virus interacts with specific sites in the vectors' mouthparts or foregut.

Rice Tungro Disease

The characteristic symptoms of rice tungro disease had been reported from several Southeast Asian countries for many years before they were recognized in 1963 to be of viral etiology. During and since the 1960s, there have been several severe outbreaks of the disease mainly associated with the major agronomic improvements in rice cultivation resulting from the 'green revolution'. The disease is found in all countries of South and Southeast Asia and in the south and southeast of China (**Figure 1**). The importance and distribution of the disease is evidenced by the variety of local names for it: *accep na pula* (Philippines), *mentek* (Indonesia), *penyakit merah* (Malaysia), yellow orange leaf (Thailand), leaf yellowing (India). In the early 1990s, it was estimated that tungro caused annual losses in excess of $1.5 billion without taking account of the cost of insecticide to control the vector. In parts of India, the disease causes losses of up to 50% of the expected crop yield.

In many rice cultivars, tungro infection is evidenced by a marked stunting of plants, discoloration of the leaves, and reduced tiller number. The leaves of tungro-infected plants are yellow or orange (**Figure 2**) with the discoloration starting at the tip and extending down toward the base; infected leaves are slightly rolled inward. Tolerant rice cultivars usually show only partial discoloration which may not be apparent on younger leaves. The disease is transmitted in the semipersistent manner by several leafhopper species with the rice green leafhopper, *Nephotettix virescens* (**Figure 3**), being the most important vector. It is also transmitted by *Nephotettix cincticeps*, *Nephotettix nigropictus*, *Nephotettix malayanus*, and *Recilia dorsalis*; these other vectors may be of relative importance in certain situations. The distribution of the disease is circumscribed by the distribution of *N. virescens* (**Figure 1**).

It was not until 1978 that it was recognized that tungro is caused by a complex of two viruses, rice tungro bacilliform virus (RTBV) and rice tungro spherical virus (RTSV) (**Figure 4**). When these viruses are separated, RTBV causes moderate symptoms in rice but is not leafhopper transmitted. RTSV, on the other hand, is leafhopper transmitted but causes few, if any, symptoms; in some Japanese rice cultivars, it caused a disease termed 'waika'. In the disease complex, both viruses are leafhopper transmitted, RTSV effecting the transmission of RTBV as well as itself. RTSV enhances the symptoms of RTBV in infected plants giving rise to the severe symptoms.

Most host range studies on the tungro viruses are unreliable as the association of the two viruses was not then recognized. Host range studies are also constrained by the ability of the vector species to transmit the virus to the plant species tested. RTSV appears to have a restricted host range limited to members of the Poaceae and Cyperaceae (*Echinochloa crus-galli*, *Echinochloa glabrescens*, *E. colona*, *Leptochloa chinensis*, *Leersia hexandra*, *Oryza sativa*, *Panicum repens*, *Cyperus rotundus*); RTSV causes few, if any, symptoms in these hosts.

The severity of the disease has encouraged extensive molecular studies on both tungro viruses even though they have been difficult to work with as they are in very low concentration in the plant and are not mechanically transmissible. RTBV is transmissible by agroinoculation.

Rice Tungro Bacilliform Virus

RTBV is a pararetrovirus belonging to a monospecific genus, *Tungrovirus*, in the family *Caulimoviridae*. It has bacilliform particles of about 130 nm length (though there can be longer ones in some isolates) and 30 nm diameter (**Figure 5**). The particle structure is based on a $T = 3$ icosahedron cut across its threefold axes, the tubular portion being made up of rings of hexamer subunits with a repeat distance of about 10 nm.

The viral coat protein comprises one major species of M_r 37 000; this may be processed to give a product of M_r about 33 000 and possibly others. The molecular mass of the coat protein has been measured by mass spectrometry and the N- and C-terminal sequences have been determined. This protein contains two specific amino acid sequence motifs, $CXCX_2CX_4HX_4C$ characteristic of reverse transcribing virus *gag* proteins and $CX_2CX_{11}CX_2CX_4CX_2C$ unique to 'badnaviruses'.

The particles contain a circular double-stranded DNA genome of about 8 kbp which has one discontinuity at a specific position in each strand. The genomes of several isolates have been sequenced and the virus has been shown to have four open reading frames (ORFs), all on one strand (**Figure 6**). The start codon of ORF1 is ATT but the function of its product of M_r 24 000 is unknown. ORF2 encodes a product of M_r 12 000 which interacts with coat protein and is possibly involved in virus assembly. The polyprotein encoded by ORF3 is processed by an aspartate protease to give: N-terminal protein(s) thought possibly to be cell-to-cell movement protein(s), viral coat protein, aspartate protease, and the replicase comprising reverse transcriptase and RNaseH activities. The ORF4 product of M_r of 46 000 has no known function.

As with all plant pararetroviruses, the genome is transcribed asymmetrically to give a more-than-genome-length RNA, which is the template for reverse transcription and is also the template for the translation of the first three ORFs. The fourth ORF is translated from an mRNA spliced from the more-than-genome-length transcript (**Figure 6**).

There are two major strains of RTBV, one from Southeast Asia and the other from the Indian subcontinent. These strains have about 70% sequence similarity and the Indian strain can be distinguished from the Asian



Figure 1 Distribution of rice tungro disease; the circle shows the distribution of the main leafhopper vector.



Figure 2 Field symptoms of rice tungro disease.



Figure 3 The rice green leafhopper, *Nephotettix virescens*.

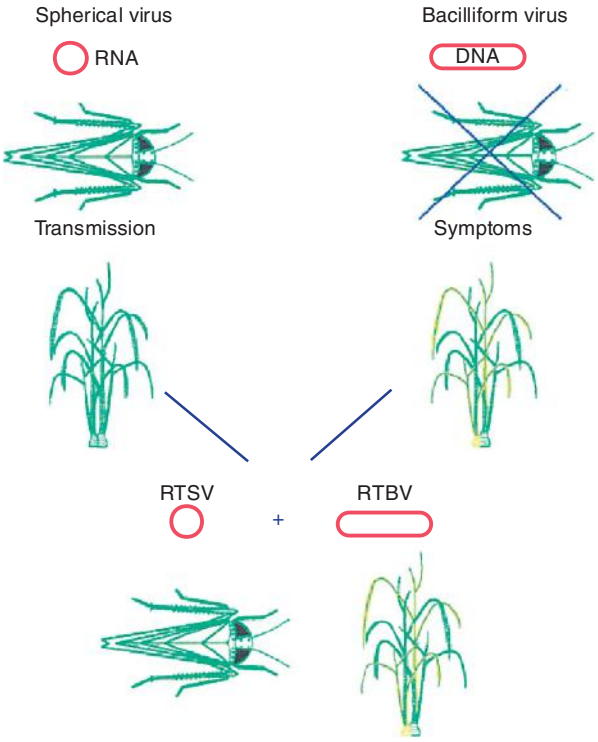


Figure 4 Involvement of RTBV and RTSV in tungro disease. Reprinted, with permission, from the *Annual Review of Phytopathology*, Volume 34 © 1996 by Annual Reviews.

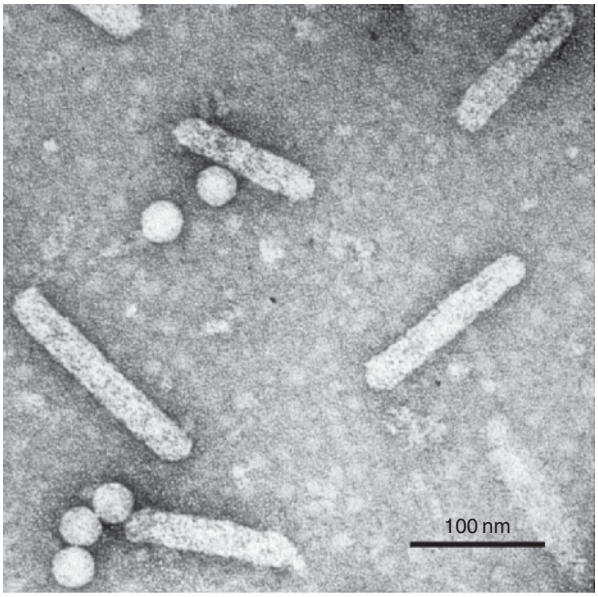


Figure 5 Particles of RTBV and RTSV negatively stained in uranyl acetate.

strain by a deletion of *c.* 65 nt in a noncoding region. Within field isolates, there is microvariation which might be expected from a replication mechanism which does not involve proof reading.

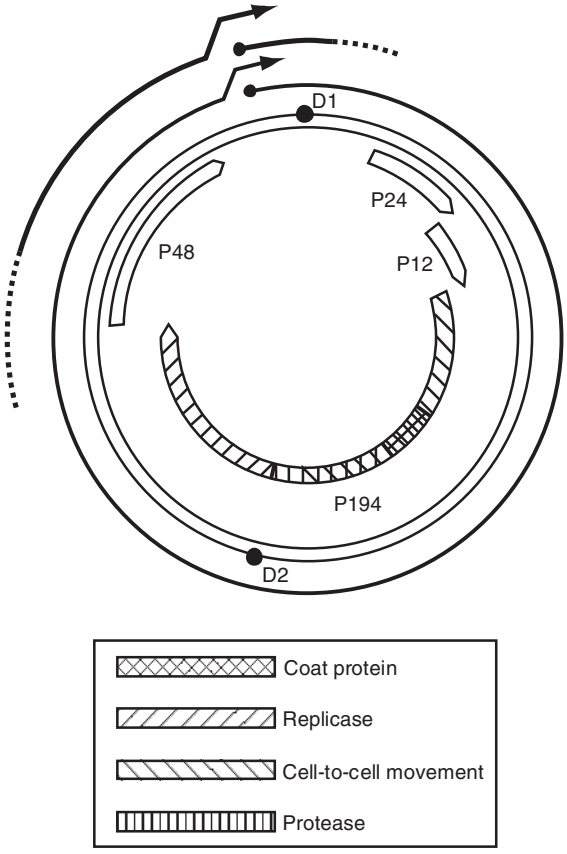


Figure 6 Genome organization of RTBV. The double complete circles represent the dsDNA genome, the positions of the discontinuities (D1 and D2) being shown. Inside the double circles, the arced boxes represent the ORFs, the size of the product being indicated in kDa. Outside the double circles, the positions of the 35S RNA transcript and the spliced mRNA for ORF4 are shown, the arrowheads marking the 3′ ends and the dotted lines the splice. The infills of the boxes represent the functions of the ORF products. Reproduced from Hull R (2001) *Matthews' Plant Virology*, 4th edn., figure 6.4. London: Academic Press, with permission from Elsevier.

Rice Tungro Spherical Virus

RTSV belongs to the genus *Sequivirus* in the family *Sequiviridae* and has isometric particles of about 30 nm diameter (**Figure 5**). RTSV is limited to vascular tissues where it is restricted to the phloem cells. Virus particles are scattered in the cytoplasm or are embedded in nonenveloped electron-dense granular inclusions. Particles also occur as crystalline aggregates often in vacuoles. Small vesicles containing fibers are found in the cytoplasm, usually along the wall of infected cells.

The capsid is made up of three coat protein species (CP1, CP2, and CP3) that are apparently in equimolar amounts, and is thought to have a similar $T = 1$ icosahedral architecture to that of picornaviruses. Identification of cleavage sites by N-terminal sequencing shows that



Figure 7 Genome organization of RTSV. The single line represents the (+)-strand RNA genome. The boxes show the coding regions with the final gene products after processing identified for those that are known. The infills represent the function of the gene products – see **Figure 6** for details of these. Reproduced from Hull R (2001) *Matthews' Plant Virology*, 4th edn., figure 6.4. London: Academic Press, with permission from Elsevier.

CP1 is 22.9 kDa and CP2 is 22.3 kDa; the C-terminus of CP3 has not been identified. In Western blots of crude extracts from infected plants, an antiserum to CP3 identifies a band at 33 kDa and several in the range 40–42 kDa; the nature of these larger bands is unknown.

Each RTSV particle contains one molecule of single-stranded (+)-strand RNA of about 12.2 kbp. The RNA encodes a large polyprotein of about 393 kDa and has two short ORFs at the 3′ end (**Figure 7**). The polyprotein is processed to give various products including the three coat protein species, a cysteine or 3C-type protease, and an RNA-dependent RNA polymerase at its 3′ end. Functions of other products have not yet been determined. No functions have been ascribed to the products of the 3′ short ORFs and there is no evidence that these are expressed.

RTSV strain Vt6 overcomes the resistance to the type strain in rice cultivar TKM6. Several serological variants of RTSV have been reported. Molecular techniques reveal much microvariation in the sequences encoding CP1 and CP2 but there is no evidence for major geographic strains that reflect those of RTBV.

Control

There are three major approaches to controlling tungro: by the manipulation of various cultural practices such as planting time, having a crop-free period, and seedbed protection; invasion of young susceptible rice plants by viruliferous leafhoppers can be avoided. The leafhoppers can be controlled by spraying insecticide but this has not proved to be particularly effective. There have been various programs to breed resistance to tungro but most have proved of limited success. The International Rice Research Institute has screened more than 40 000 accessions of rice for resistance to tungro viruses and have found two basic types of resistance, that to the vector and that to the virus. Resistance to the vector has proved to be not very durable and has broken down in less than 1 year in some places. Resistance to each of the viruses is primarily tolerance reducing virus replication and/or reducing symptoms and transmission efficiency. As noted above, resistance to RTSV has been overcome by a new strain of the virus. However, these forms of resistance are being incorporated into breeding programs as resistance to RTSV can reduce the spread of the disease and resistance to RTBV can mitigate losses due to reduced symptoms. There are also attempts to use transgenic approaches to conferring protection against these two viruses but so far nothing has proved to be fully effective.

See also: Cereal Viruses: Rice.

Further Reading

Anjaneyulu A, Satapathy MK, and Shukla VD (1994) *Rice Tungro.* New Delhi: Oxford and IBH Publishing Co. Pvt. Ltd.
Hull R (1996) Molecular biology of rice tungro viruses. *Annual Review of Phytopathology* 34: 275–297.
Hull R (2001) *Matthews' Plant Virology,* 4th edn., London: Academic Press.

Rice Yellow Mottle Virus

E Hébrard and D Fargette, IRD, Montpellier, France
G Konaté, INERA, Ouagadougou, Burkina Faso

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Rice yellow mottle virus (RYMV) is a member of the genus *Sobemovirus*. RYMV is present only on the African continent. It was first reported in Kenya in 1966 and in Côte d'Ivoire in 1974 in the irrigated rice fields. Since the early 1990s, RYMV is present everywhere in sub-Saharan Africa and in Madagascar where rice is grown. It affected all types of rice cultivations, including lowland, upland, rain-fed, floating, and mangrove rice. RYMV regularly induces severe yield losses to rice production ranging from 25% to 100%. In some regions, when epidemics

are recurrently very severe, farmers abandoned their fields and eradicated the tropical forests in order to plant new rice fields. Highly susceptible cultivars have been eliminated by the disease. RYMV is now ranked as the main biotic threat to rice production in Africa.

Virion Properties

RYMV has icosahedral particles of 25 nm in diameter. The virions contain a single coat protein (CP) of 29 kDa, a genomic RNA (gRNA), and one subgenomic RNA (sgRNA) molecule. The capsid contains 180 copies of the CP subunit arranged with $T = 3$ symmetry (**Figure 1**). The structure of RYMV was determined by X-ray crystallography at 2.8 Å resolution and compared to the structure of southern cowpea mosaic virus (SCPMV) and sesbania mosaic virus (SeMV). Sobemovirus CP subunits are chemically identical but structurally not equivalent. Three types of CP subunits termed A, B, C are related by quasi-threefold axes of symmetry and are involved in different inter-subunit contacts. In the C-type subunits, a longer part of the N-terminus is ordered (residues 27–49), forming an additional β-strand named βA arm. The analysis of molecular determinants involved in the $T = 3$ assembly of SeMV demonstrated the major role of βA arm. Sobemovirus particles are stabilized by divalent cations, pH-dependent protein–protein interactions, and salt bridges between protein and RNA. Upon alkali treatment in presence of chelators, the capsid shell swells and becomes sensitive to enzymes and denaturants. In these conditions, RYMV is more stable than SCPMV. This property is likely due to the 3D swapping of the RYMV βA arm around the distal quasi-sixfold axes as found with SeMV whereas the SCPMV βA arm makes a U-turn and is localized around the nearby quasi-sixfold axes. Another reason for the better stability of RYMV is likely to be the strong RNA–protein interactions resulting from the presence of ordered RNA within the capsid shell. RYMV particles exist in three forms with different stability (1) an unstable swollen form dependent on basic pH but lacking Ca^{2+}, (2) a transitional compact form dependent on acidic pH, but also lacking Ca^{2+}, and (3) a stable compact form that is pH independent and contains Ca^{2+}. The compact form is highly infectious and probably required for virus movement and transport by vector.

Recently, molecular diversity of the CP was analyzed in relation to the capsid three-dimensional structure in order to identify which amino acids are involved in the differential recognition by certain monoclonal antibodies. The residues in position 178 and 180, despite their internal localization in the capsid, can modify the antigenic reactivity, with Mabs G and E allowing serotypes Sr3 and Sr5 to be differentiated.

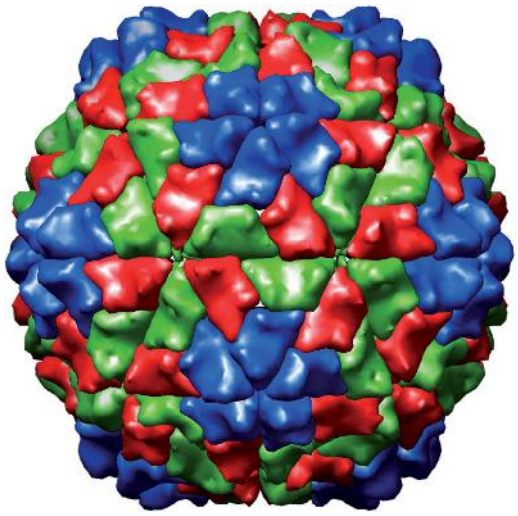


Figure 1 RYMV capsid structure. The capsid comprised 180 copies of one single type of polypeptide arranged in $T = 3$ quasi-equivalent symmetry. The icosahedral asymmetric unit contains three subunits: A (in blue), B (in red), and C (in green). Each subunit is involved in different inter-subunit contacts. This image was automatically generated from ViPER virus capsid PDB file 1F2N using the MultiScale extension to the Chimera interactive molecular graphics package. Reproduced from Rice yellow mottle virus In: *Characterization, Diagnosis and Management of Plant Viruses*, Vol. 4, Ch. 2, pp. 31–50, 2008. Houston, TX: Studium Press, with permission from Studium Press.

Organization of the Genome

The genomic RNA is one single-stranded messenger-sense molecule, 4450 nucleotides in size. The 5′ terminus of the RNAs has a genome-linked protein (VPg), and lacks a poly(A) tail. RYMV often encapsidates, in addition to its genomic RNA, a viroid-like satellite RNA (satRNA) that is dependent on a helper virus for replication. RYMV satRNA has 220 nt and is the smallest naturally occurring viroid-like RNA known today. The RYMV satellite RNA is not involved in pathogenicity.

Like other sobemoviruses, the RYMV genome is organized in 4 overlapping open reading frames (ORFs). Two noncoding regions at the RNA extremities contain 80 and 289 nt, respectively. The ORF1 and the ORF4 are localized at the 5′ and 3′ extremities, respectively. An intergenic region of 54 nt separates the first two ORFs. The two coding regions ORF2a and ORF2b overlapped, ORF2b being situated in the −1 reading frame within ORF2a (**Figure 2**).

The ORF1 encodes a protein P1 which is dispensable for replication but is required for infectivity in plants. RYMV P1 exists in two forms of 18 and 19 kDa which probably result from degradation or post-translational modifications. P1 is required for cell-to-cell movement of the virus. Moreover, RYMV and cockfoot mottle virus

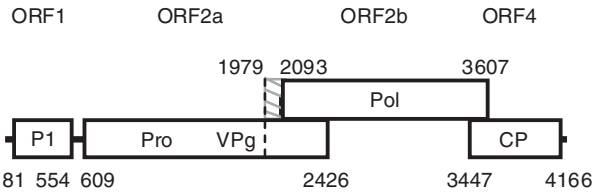


Figure 2 RYMV genomic organization. Positions of the ORFs are indicated in nucleotides. P1, proteinase (Pro), VPg, RNA-dependent RNA polymerase (Pol), and CP are labeled. The dotted line at nucleotide 1979 represents the frameshifting signal. The fusion point of the polyprotein P2a+b is unknown, and an AUG codon present at the beginning of the ORF2b (nucleotide 2093) is indicated by the vertical line. Reproduced from Rice yellow mottle virus In: *Characterization, Diagnosis and Management of Plant Viruses*, Vol. 4, Ch. 2, pp. 31–50, 2008. Houston, TX: Studium Press, with permission from Studium Press.

(CoMV) P1 are suppressors of post-translational gene silencing in the nonhost plant *Nicotiana benthamiana*. The two P1 are able to suppress the initiation and the maintenance of silencing but the suppression of systemic silencing is stronger with RYMV P1 than with CoMV P1.

The polyproteins encoded by the RYMV ORF2 are presumably translated from leaky translation. The start codon of ORF2 appeared to be in a more favorable context than ORF1. The ORF2b is translated as a polyprotein fused with ORF2a after a-1 programmed ribosomal frameshifting mechanism. This phenomenon happened with *in vitro* efficiency from 26 to 29% for the CoMV. The presence of a heptanucleotide slippery sequence UUUAAAC and a predicted stem–loop structure are necessary. The ORF2a contains sequence motifs for the proteinase and the VPg and the ORF2b encodes the RNA-dependent RNA polymerase (RdRp). RYMV proteinase contain the consensus sequence $H(X_{32-35})[D/E](X_{61-62})TXXGXSG$ characteristic of serine proteinases, they cleave between E–T and E–S amino acid residues.

RYMV VPg is not absolutely required for infectivity but *in vitro* transcripts required capping to be infectious. The putative and known sobemovirus VPgs are characterized by the presence of a conserved W[A/G]D sequence followed by a D- and E-rich region. The position of sobemovirus VPg, like polerovirus VPg, differs from the genome arrangement VPg proteinase–polymerase characteristic of many RNA virus species including members of the families *Picornaviridae* and *Comoviridae*. The localization of the polymerase was predicted from the presence of the GDD motif and surrounding conserved motifs characteristics of RdRp. Little is known about the replication signals needed for initiation of plus and minus-strand synthesis in sobemoviruses.

The ORF4 is translated from the subgenomic RNA and encodes the CP (which appeared as a doublet of 28–29 kDa). RYMV CP is dispensable for replication but required for movement to long distance and perhaps from cell to cell. Encapsidation is likely to be essential for long distance movement. This movement was suggested to occur during the cell differentiation to vessels. The N-terminal sequence contains a putative bipartite nuclear localization signal (NLS). This highly basic region is thought to interact with viral RNA and to stabilize the virion.

Relationships of the Species with Other Taxa

The species *Rice yellow mottle virus* belongs to the genus *Sobemovirus* which is not assigned to any family. The genus contains 13 definitive species including *Southern bean mosaic virus*, the type species. The sobemovirus CPs are related to those of necroviruses (*Tombusviridae*), whereas the proteinase, VPg, and polymerase are related to those of poleroviruses and enamoviruses (*Luteoviridae*).

Biological Properties

The natural host range of RYMV is restricted to a few members of the Poaceae family, principally *Eragrostidae* and *Oryzae* sp. The most commonly cited species are *Oryza sativa*, *O. longistaminata,* and *Echinocloa colona*. A few additional Poaceae plants have been infected experimentally.

RYMV particles are present in all plant parts including roots and seeds. Particles are present in large amount in mesophyll cells, and in vascular tissues mainly in xylem and associated parenchyma cells. Particles were found individually, as aggregates, or as crystalline forms in the cytoplasm and the vacuoles of infected cells. In addition, the cytoplasm of infected cells contains fibrillar material located either in vesicles or distributed in diffused patches. Virus particles are also found in mature xylem, as well as inside the primary wall.

Cytological changes were found in the chloroplasts of infected mesophyll cells where the starch grains decreased in size and number. The chloroplasts form invaginations containing mitochondria, peroxysomes, and virus particles. Electron-dense materials were observed in the nuclei and associated with fibrillar elements in cytoplasmic vesicles. The most dramatic changes induced by RYMV occurred in the cell walls of parenchyma and mature xylem cells causing disorganization of the middle lamellae wall. From infected cells, a viral ribonucleoprotein complex moves from cell to cell to reach the vascular bundle sheath. Viral replication, encapsidation, and storage in vacuoles occur mainly in vascular parenchyma cells. The calcium linkage from pit membranes to virus particles during xylem differentiation could contribute to disruption of these membranes and facilitate systemic virus transport. In the

upper leaves, virions are suspected to cross the pit membrane to infect new vascular cells and virions spread out by cell-to-cell movement through plasmodesmata. At this stage of infection, most replication occurs in mesophyll and vascular cells, and most of the virions accumulate in large crystalline patches.

Vector transmission is mainly due to chrysomelidae vectors, but other biting insects including the grasshopper *Conocephalus* have been reported to occasionally transmit the virus. Transmission is not persistent. The virus is present within the seed, although RYMV is not seed-transmissible. Abiotic transmissions through plant residues, irrigation water, gutation water, direct contact between plants, and contamination by infected agricultural tools have been observed. Experimentally, mechanical transmission is easily achieved.

Diagnostic and Identification

RYMV diagnosis was first based on symptoms. However, many factors such as mineral deficiencies can induce symptoms of yellow mottle on rice. Therefore, several tools for specific detection of RYMV were developed. First, polyclonal antibodies (PAbs) directed against different isolates were used in double-diffusion tests and in direct antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). No cross-reactions with other sobemoviruses was observed, demonstrating the high specificity of these antibodies. Western-blot analysis can be used to detect RYMV with PAbs. These antibodies were also used in immunolocalization techniques. Immunoprinting tests derived from the direct tissue blotting technique were developed to analyze the distribution of the virus in entire leaves. Moreover, cytological detection of RYMV CP in rice tissues can be performed by immunofluorescence microscopy after glutaraldehyde/paraformaldehyde fixation and labeling with secondary antibodies linked to fluorescein. Polyclonal antibodies directed against P1 produced in *E. coli* are also available, and were used in Western-blot analyses. Monoclonal antibodies were also developed to study RYMV diversity. Indirect triple antibody ELISA (TAS-ELISA) with strain-specific MAbs allows the major serotypes to be differenciated.

In parallel to the serological methods, molecular tools were developed. The first full-length sequence was obtained after RNA extraction from purified virus. Several specific primers were defined to perform RT-PCR amplification and to produce infectious transcripts. Northern and Southern blot methods are available using a DNA probe obtained by PCR amplification of the CP gene (ORF4) from the infectious clone CIa. *In situ* hybridization tests of the RNA in rice tissues were also performed. In this case, the probe was DNA, obtained from the full-length genome of the clone CIa labeled randomly with digoxigenin-UTP. RT-PCR amplification of the CP gene followed by direct DNA sequencing progressively has replaced other molecular methods of analyses.

Epidemiological Aspects

Typical symptoms of RYMV are a mottle and a yellowing of the leaves. However, streaks, necrosis, and whitening are sometimes observed with some cultivars and under specific growing conditions. Infection resulted in stunting of the plant, reduced tillering, poor panicle exertion, and sterility. Death of the plants of susceptible cultivars occurs after early infection. Severe yield losses ranging from 20% to 100% after RYMV infection have been reported in several countries. Most cultivars currently grown are susceptible to RYMV. Heavy infection is associated with reduced fertility.

RYMV is an emergent disease. It is thought that the virus was originally harbored in wild Poaceae and transferred only recently to cultivated rice. The rapid and intense spread of the virus was associated to the change of agricultural practices in response to increasing food demand. Very productive but susceptible *O. sativa* cultivars have been introduced from Asia which allows two crop cycles a year resulting in the maintenance of the inoculum all over the year. RYMV propagation probably occurs as follows: (1) a virus source made of volunteers, of wild Poaceae or plant residues act as primary inoculum; (2) a direct contamination of the new rice fields occur after mechanical transmission by man or biological infection by insects; (3) rice beds can also be infected by insect vector; (4) the new rice field originating from these infected seed-beds are contaminated by man during replanting; (5) further propagation of the virus is done by man, animals, wind, and irrigation water (**Figure 3**).

The epidemiology has been little studied and there are even uncertainties about the respective importance of biotic and abiotic transmission. Under these conditions, precise forecasting of RYMV infection is not possible, although growing intensification of rice cultivation in Africa will no doubt favor RYMV spread, unless durable resistant cultivars are introduced. Phytosanitary measures are sometimes advised although they are often economically not practicable and their impact to reduce virus spread is unknown. They include protection of seedbeds by nets, disinfection of tools used at replanting, destruction of volunteers and rice residues. Chemical control of the vectors is not economically feasible, is ecologically dangerous, and is most unlikely to be successful considering the large number of species involved in transmission.

Several breeding programs have been conducted in order to select and breed resistant cultivars. The genotype and the phenotype of two kinds of natural resistance

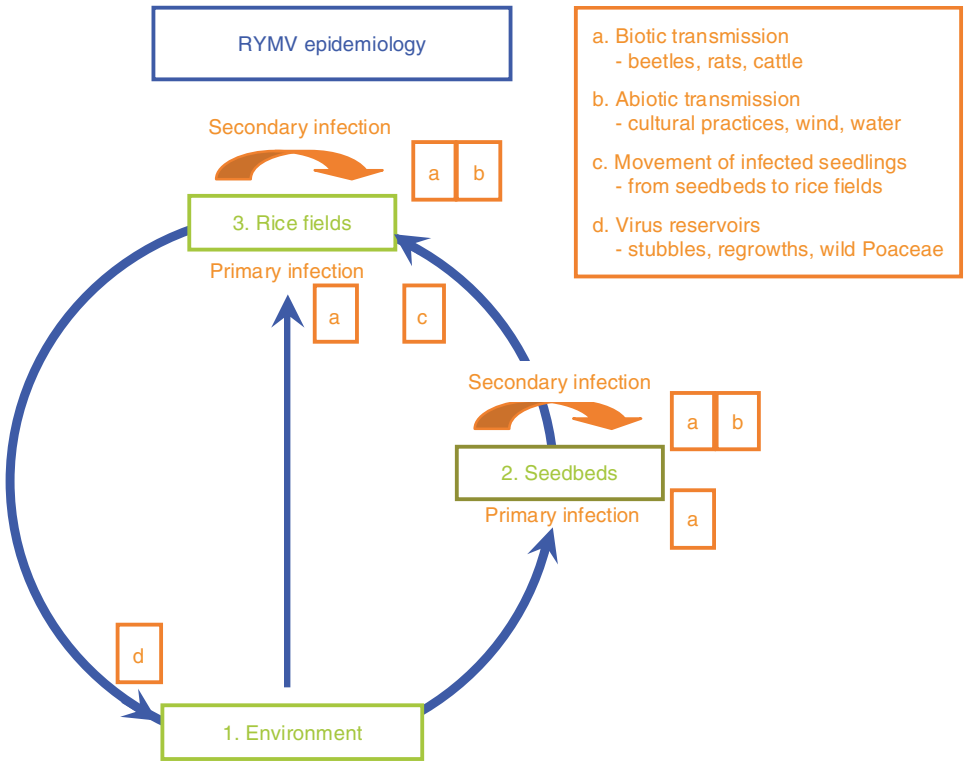


Figure 3 Descriptive model of RYMV epidemiology. 1. Environment. RYMV is present in the environment in rice stubble and regrowths and in wild Poaceae. 2. Rice seedbeds. Primary infection occurred via biotic transmission (beetles, rats, cows) whereas cultural practices contribute to secondary infection in seedbeds 3. Rice fields. Primary infection occurred via biotic transmission from the environment, and also when transplanting infected seedlings from seedbeds. Both biotic and abiotic transmissions contribute to secondary spread in rice fields. After harvesting, RYMV is perpetuated in contra-season in the environment through rice stubbles and regrowths and transmitted to wild Poaceae.

have been characterized. Partial resistance is encountered in cultivars of *O. japonica* species with a delayed virus multiplication and symptom expression. Partial resistance is polygenic and a major quantitative trait locus has been identified on chromosome 12. High resistance has been identified in a limited number of accessions of *O. glaberrima* and *O. sativa indica* species. No symptoms are apparent and virus content is most often undetectable. This resistance is monogenic and recessive. The high resistance is controlled by the recessive gene *Rymv1* that maps on chromosome 4 and encodes the translation initiation factor eIF(iso)4G. Different alleles were identified in the sativa-resistant varieties and the *O. glaberrima* accessions. Compared to susceptible varieties, they are characterized by a point mutation or a small deletion in the conserved domain of the gene. The high resistance was efficient against representative isolates of the main virus strains. However, high resistance was overcome experimentally. The VPg was identified as the virulence factor. A single point mutation was sufficient to break the resistance. Transgenic plants with portions of the ORF2 expressing partial resistance have also been produced. This transgenic resistance is thought to involve a gene silencing mechanism. However, the transgenic lines showed a less effective, partial, and temporary resistance compared to natural resistances.

Diversity and Evolution

The diversity of RYMV was assessed by studying isolates from several countries where the disease has been observed. Isolates were serologically typed with monoclonal antibodies. Their CP was sequenced. Several isolates representative of the geographic and molecular diversity were fully sequenced. RYMV is a highly variable virus and analyses of the geographic distribution of the genetic diversity elucidated the process of evolution and of dispersal of the virus. RYMV showed a high level of population structure marked at the continental scale with three subdivisions: East Africa, Central Africa, and West Africa. The highest diversity was observed in East Africa, with a pronounced peak in Eastern Tanzania, and a decrease from the east to the west of the continent. This pattern

suggests a westward expansion with a succession of founder effects and subsequent diversification phases. Accordingly, genetic diversity would be adversely affected by recurrent bottlenecks occurring along the route of colonization, resulting in the lowest diversity in the extreme west. This, together with accumulation of *de novo* mutations postdating population separation, provides an explanation for the genetic differences among strains across Africa.

See also: Cereal Viruses: Rice; Emerging and Reemerging Virus Diseases of Plants; *Sobemovirus*.

Further Reading

Abubakar Z, Ali F, Pinel A, *et al.* (2003) Phylogeography of rice yellow mottle virus in Africa. *Journal of General Virology* 84: 733–743.

Albar L, Bangratz-Reyser M, Hébrard E, *et al.* (2006) Mutations in the eIF (iso)4G translation initiation factor confer high resistance of rice to rice yellow mottle virus. *Plant Journal* 47: 417–426.

Bonneau C, Brugidou C, Chen L, *et al.* (1998) Expression of the rice yellow mottle virus P1 protein *in vitro* and *in vivo* and its involvement in virus spread. *Virology* 244: 79–86.

Brugidou C, Holt C, Yassi A, *et al.* (1995) Synthesis of an infectious full-length cDNA clone of rice yellow mottle virus and mutagenesis of the coat protein. *Virology* 206: 108–115.

Fargette D, Konate G, Fauquet C, *et al.* (2006) Molecular ecology and emergence of tropical plant viruses. *Annual Review of Phytopathology* 44: 235–260.

Fargette D, Pinel A, Abubakar Z, *et al.* (2004) Inferring the evolutionary history of rice yellow mottle virus from genomic, phylogenetic and phylogeographic studies. *Journal of Virology* 78: 3252–3261.

Fargette D, Pinel A, Halimi H, *et al.* (2002) Comparison of molecular and immunological typing of isolates of rice yellow mottle virus. *Archives of Virology* 147: 583–596.

Hébrard E, Pinel-Galzi A, Bersoult A, *et al.* (2006) Emergence of a resistance-breaking isolate of rice yellow mottle virus during serial inoculations is due to a single substitution in the genome-linked viral protein VPg. *Journal of General Virology* 87: 1369–1373.

Hébrard E, Pinel-Galzi A, Catherinot V, *et al.* (2005) Internal point mutations of the capsid modify the serotype of rice yellow mottle sobemovirus. *Journal of Virology* 79: 4407–4414.

Konaté G, Traoré O, and Coulibaly M (1997) Characterization of rice yellow mottle virus isolates in Sudano-Sahelian areas. *Archives of Virology* 142: 1117–1124.

Pinel A, Traoré O, Abubakar Z, Konaté G, and Fargette D (2003) Molecular epidemiology of the RNA satellite of rice yellow mottle virus in Africa. *Archives of Virology* 148: 1721–1733.

Qu C, Liljas L, Opalka N, *et al.* (2000) 3 D domain swapping modulates the stability of members of an icosahedral virus group. *Structure* 8: 1095–1103.

Sorho F, Pinel A, Traoré O, *et al.* (2005) Durability of natural and transgenic resistances in rice to rice yellow mottle virus. *European Journal of Plant Pathology* 112: 349–359.

Traoré O, Pinel A, Hébrard E, *et al.* (2006) Occurrence of resistance-breaking isolates of rice yellow mottle virus in West and Central Africa. *Plant Disease* 90: 256–263.

Traoré O, Traoré M, Fargette D, and Konaté G (2006) Rice seedbed as a source of primary infection by rice yellow mottle virus. *European Journal of Plant Pathology* 115: 181–186.

Rift Valley Fever and Other Phleboviruses

L Nicoletti and M G Ciufolini, Istituto Superiore di Sanità, Rome, Italy

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arbovirus Any virus of vertebrates biologically transmitted by infected hematophagous arthropods.
Aseptic meningitis Inflammation of the covering of the brain (meninges) caused by a virus.
Epizootic A disease affecting a large number of animals at the same time within a particular region or geographic area.
Phlebotomus fever viruses or sandfly fever viruses Viruses that cause disease of brief duration characterized by sudden onset fever, headache, pain in the eyes, malaise, and leukopenia and are transmitted by the bite of infected sandflies.
Phlebovirus A genus in the family *Bunyaviridae* containing many viruses, of which the best known are Rift Valley fever virus and sandfly fever viruses.
Sandfly Any of various small biting two-winged flies of the families Psychodidae, Simuliidae, and Ceratopogonidae.
Transovarial transmission Vertical transmission of a virus from mother to offspring.
Zoonosis A disease of animals that can be transmitted to humans.

Introduction

The genus *Phlebovirus* is one of the five genera of the family *Bunyaviridae*, in which are included more than 300 virus species. The five genera (*Orthobunyavirus, Hantavirus, Nairovirus, Phlebovirus, Tospovirus*) are grouped in one family, primarily because they share structural characteristics,

all bear a tripartite RNA genome of negative polarity, and all have a roughly similar protein-coding pattern within each genome segment. It has been demonstrated that Uukuniemi virus, the prototype virus of a group of tick-borne viruses, and its relatives have the same ambisense coding strategy as do phleboviruses. In addition, a high degree of similarity occurs in proteins of Uukuniemi virus and in those of some phleboviruses. As a consequence, all viruses previously included in the genus *Uukuvirus* are now classified in the genus *Phlebovirus*. Therefore, in the genus *Phlebovirus*, viruses previously known as phlebotomus fever viruses and uukuviruses are now placed.

The first description of what was most likely phlebotomus fever occurred at the time of the Napoleonic wars, when a similar disease was reported as 'Mediterranean fever'. The disease was first described with considerable accuracy by Pick in 1886, who termed it 'dog fever' (*hundsfieber*), probably due to the marked signs and symptoms of conjunctivitis, which resembled the eyes of a bloodhound. At the same time in Italy, the disease was already known as pappataci fever, suggesting a possible link with sand flies. In Yugoslavia, epidemics of the disease occurred each summer among newly arrived Austrian troops stationed along the Adriatic coast.

Rift Valley fever virus (RVFV) was first isolated in 1930 during investigation of an epidemic with high mortality rates among sheep on a farm in the Rift Valley in East Africa. In retrospect, epizootics due to this virus had been identified as early as 1912. Epizootics were reported to have occurred in many sub-Saharan countries after 1940 and the vector-borne origin of the disease was definitively proved in 1948.

RVFV is one of the most important viral zoonoses in Africa. Transmission of the virus to humans occurs via arthropod vectors, aerosols of blood or amniotic fluid of infected livestock, or by direct contact with infected animals. RVFV in humans is manifested by a broad spectrum of infections, from asymptomatic infection to a benign febrile illness, to a severe illness (approximately 1–3% of cases) that can include retinitis, encephalitis, and hemorrhagic fever. In addition to the human illness, disability, and suffering, RVFV outbreaks can result in devastating economic losses when livestock in an agricultural society are affected.

RVFV was first confirmed outside Africa in September 2000. An outbreak occurred in southwestern coastal Saudi Arabia and neighboring coastal areas of Yemen. RVFV isolated from the floodwater mosquito *Aedes vexans arabiensis* during the outbreak was closely related to strains from Madagascar (1991) and Kenya (1997), suggesting that the virus was imported through infected mosquitoes or livestock from East Africa.

From blood samples taken during an epidemic occurring in Italy among Allied troops during World War II, Sabin isolated two serologically distinct agents, sandfly fever Sicilian virus and sandfly fever Naples virus. Since then, many distinct viruses have been isolated from sandflies or humans in both the Old World and the New World and, on the basis of serological relationships, classified in the phlebotomus fever group (**Table 1**).

In 1971, in Italy, Toscana virus (TOSV) was isolated from the sandfly, *Phlebotomus perniciosus*. The virus was shown to be antigenically related to sandfly fever Naples virus, and antibody to TOSV was shown to occur at a relatively high prevalence of antibodies in healthy humans. TOSV has since been shown to cause human disease and has been associated with acute neurologic disease.

Morphology, Structure, and Strategy of Replication

The prototype phlebovirus is RVFV. Similar to other bunyaviruses, the virion is spherical or pleiomorphic, depending on the method used for fixation, with a diameter of 90–100 nm. The virion consists of a core containing the genome and its associated proteins, which are in turn surrounded by an envelope composed of a lipid bilayer containing equivalent numbers of two glycoproteins.

The genome consists of three single-stranded RNA segments: large (L, 6.5–8.5 kbp), medium (M, 3.2–4.3 kbp), and small (S, 1.7–1.9 kbp). Sequence studies demonstrate that the three segments are identical on the 3′-end (UGUGUUUC) and a complementary 5′-end. The S RNA produces many copies of the nucleocapsid protein (20–30 kDa) and the L RNA produces a few copies of the large (L) protein (150–250 kDa), which is a transcriptase.

Table 1 Viruses of the genus *Phlebovirus*

Virus species	*Viruses*
Bujaru virus	Bujaru, Munguba
Candiru virus	Candiru, Alenquer, Itaituba, Nique, Oriximina, Turuna
Chilibre virus	Chilibre, Cacao
Frijoles virus	Frijoles, Joa
Punta Toro virus	Punta Toro, Buenaventura
Rift Valley fever virus	Rift Valley fever, Belterra, Icoaraci
Salehebad virus	Salehebad, Arbia
Sandfly fever Naples virus	Sandfly fever Naples, Karimabad, Tehran, Toscana
Uukuniemi virus	Uukuniemi, EgAN 1825–61, Fin V 707, Grand Arbaud, Manawa, Murre, Oceanside, Ponteves, Precarious Point, RML 105355, St. Abbs Head, Tunis, Zaliv Terpeniya
Unassigned	Aguacate, Anhanga, Arboledas, Arumowot, Caimito, Chagres, Corfou, Gabek Forest, Gordil, Itaporanga, Odrenisrou, Pacui, Rio Grande, sandfly fever Sicilian, Saint-Floris, Urucuri

The glycoproteins, together with a nonstructural protein (NS_M), are translated from an mRNA complementary to the M segment as a precursor which is post-translational to G_N and G_C proteins. The S segment codes for two proteins. The N protein is read from a subgenomic mRNA complementary to the 3′-end segment of the viral RNA; the second, a nonstructural protein (NS_S, 29–37 kDa), is read from a subgenomic virus-sense mRNA species corresponding to the 5′-half of the viral RNA (**Figure 1**). This strategy of replication is called 'ambisense', and it is utilized also by members of the genus *Tospovirus* as well, and of viruses of the family *Arenaviridae*.

The maturation of phleboviruses occurs in intracellular smooth membranes, principally in the Golgi complex. Exceptionally, RVFV matures on the cell surface of infected rat hepatocytes. The glycoproteins accumulate in the Golgi complex, causing vacuolization, but the Golgi complex remains functional. Virions bud into Golgi vesicles, which are then transported to the cell surface where the particles are released by exocytosis.

The Agents

Rift Valley Fever

RVF was first recognized as a viral zoonosis in Kenya in 1930. Since then, several massive epizootics affecting domestic livestock have been reported in widely separated parts of Africa. In 1977–78, RVFV appeared for the first time in Egypt in an epizootic epidemic of unprecedented size. It was estimated that in some areas along the Nile, 25–50% of sheep and cattle were infected, and that there were as many as 200 000 human cases and at least 600 deaths. Several types of severe and sometimes fatal illnesses were observed in humans, including hepatitis with hemorrhagic manifestations, meningoencephalitis, and retinitis. During this epidemic, RVFV was isolated from *Culex pipiens* mosquitoes; however, arthropods of many species have also been shown to be potential vectors.

During epizootics of RVFV, disease occurs first in animals, then in humans. RVFV produces severe disease in domestic animals, sheep being more susceptible than cattle, goats least susceptible. A greater than 90% rate of mortality is observed among infected lambs, and 20–60% among adult sheep. Pregnant ewes (90–100%) abort within a few days after infection.

RVFV can be transmitted to humans by contact with tissues or blood of infected animals during slaughter, autopsy, or disposal of infected animals. The possibility of transmission of the virus via such an airborne route among abattoir or laboratory workers has been recognized. Vector-borne transmission, particularly at the beginning of epizootic phase, is important.

The most common form of illness associated with RVFV infection in humans is the acute febrile disease (see **Table 2**). The febrile period lasts 4–7 days; patients then recover completely within 2 weeks. During the epidemic of RVFV in 1977–78 in Egypt, 5–10% of patients experienced a typical febrile illness, followed by decreased visual acuity after 7–20 days. Hemorrhagic RVFV, which occurs in about 1% of cases, was not documented until a 1975 epizootic in South Africa. Patients infected during this epidemic experienced the sudden onset of a febrile illness, which was followed 2–4 days later by jaundice and hemorrhagic manifestations. Hematemesis, melena, gingival bleeding, and petechial and purpuric skin lesions were common. In fatal cases, death occurred within a week of the onset of jaundice. Shock and hepatic insufficiency represented the most probable cause of death. Mortality rates range from 0.2% to 14%. The exact incidence of each of the severe clinical syndromes complicating RVFV during the epidemics has not been established. These rates could be

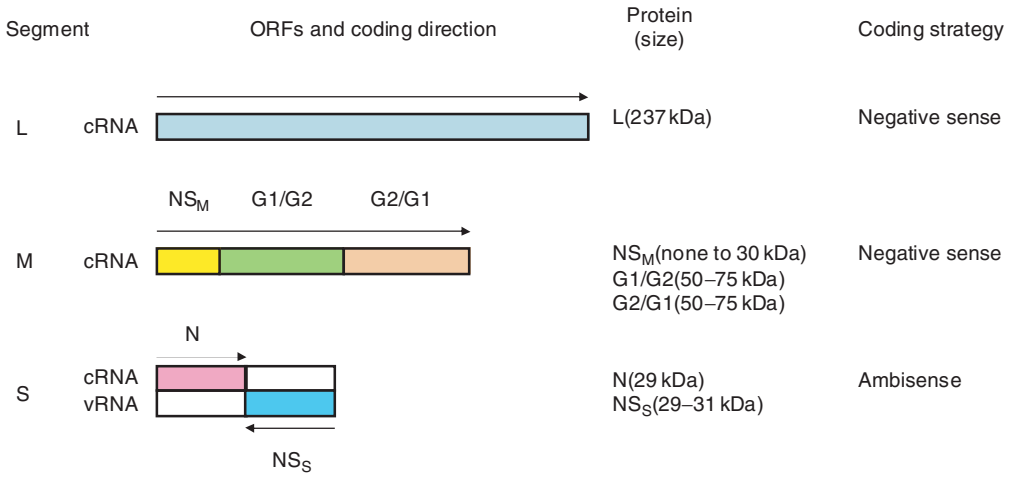


Figure 1 Genome organization and coding strategy of phleboviruses.

Table 2 Phleboviruses infecting humans

Virus	*Evidence of infection*	*Isolation from humans*	*Geographic distribution*	*Arthropod association*
Alenquer	Febrile illness	Blood	Brazil	Unknown
Arboledas	Antibody		Colombia	*Lutzomyia* spp.
Arumowot	Antibody		Africa	*Culex antennatus, Culex rubinotus, Mansonia uniformis*
Bujaru	Antibody		Brazil	Unknown
Cacao	Antibody		Panama	*Lutzomyia trapidoi*
Candiru	Febrile illness	Blood	Brazil	Unknown
Chagres	Febrile illness	Blood	Panama, Colombia	*Lutzomyia trapidoi, Lutzomyia ylephiletor*
Corfou	Antibody		Greece	*Phlebotomus major*
Gabek Forest	Antibody		Africa	Unknown
Gordil	Antibody		Africa	Unknown
Karimabad	Antibody		Iran	*Phlebotomus* spp.
Punta Toro	Febrile illness	Blood	Panama	*Lutzomyia trapidoi, L. ylephiletor*
Rift Valley fever	Febrile illness Hemorrhagic fever	Blood, CSF	Africa, Arabian peninsula	*Aedes caballus, Aedes circumluteolus, Aedes lineatopennis, Aedes vexans arabiensis, Culex theileri, Culicoides* spp., *Eretmapodites chrysogaster, Mansonia africana*
Saint-Floris	Antibody		Africa	Unknown
Salehabad	Antibody		Iran	*Phlebotomus* spp.
Sandfly fever Sicilian	Febrile illness	Blood	North Africa, South Europe, Central Asia	*Phlebotomus* spp.
Sandfly fever Naples	Febrile illness	Blood	North Africa, South Europe, Central Asia	*P. papatasi, P. perfiliewi*
Toscana	Febrile illness Meningitis	CSF	Italy, Spain, Portugal, Cyprus, France, Greece	*P. perniciosus, P. perfiliewi*

influenced by susceptibility of the human population, the virulence of various RVFV strains, and population dynamics of the vector.

Sandfly Fever Viruses

Classical phlebotomus fevers caused by sandfly fever Naples and sandfly fever Sicilian viruses are mild, self-resolving, flu-like illnesses characterized by sudden onset, fever, frontal headache, low back pain, generalized myalgia, retro-orbital pain, conjunctival injection, photophobia and malaise, and, in some cases, nausea, vomiting, dizziness, and neck stiffness (see **Table 2**). Fever is always present, ranging from 38 to 41 °C. The incubation period is 2–6 days, and the duration of disease varies from a few hours to four days (phlebotomus fever is also known as 'a 3-day fever'). Patients completely recover within 1–2 weeks. No deaths associated with phlebotomus fever have been reported but the large numbers of cases that can occur during epidemics, particularly during wars, make these illnesses potentially incapacitating to entire populations, and therefore are important.

Toscana Virus

TOSV was initially isolated from *P. perniciosus* in central Italy in 1971. The virus was subsequently isolated from *Phlebotomus perfiliewi* (but never from *Phlebotomus papatasi*) in other areas of Italy and from the brains of Kuhl's Pipistrelle bats (*Pipistrellus kuhlii*) captured in areas where the insect vectors were present. The virus also has been isolated from humans. Transovarial transmission has been demonstrated experimentally by viral isolation from male *Phlebotomus* spp. Venereal transmission from infected males to uninfected females has also been demonstrated. For many years, the known distribution of TOSV was limited to Italy and Portugal. More recently, the geographic distribution of the virus has been extended to France, Spain, Slovenia, Greece, Cyprus, and Turkey, according to results from viral isolation and serologic surveys.

TOSV is neurovirulent, a characteristic it shares with RVFV. Clinical cases with signs and symptoms ranging from aseptic meningitis to meningoencephalitis caused by TOSV are observed annually during the summers (see **Table 2**). All studies reported that the highest risk of acquiring TOSV is in August, which corresponds with peak sandfly activity.

Seroprevalence studies suggest that a substantial proportion of infections likely result in asymptomatic infections or cause only mild illnesses. Only severe cases, those involving CNS disease, require hospitalization.

After an incubation period ranging from a few days to 2 weeks, disease onset is intense, with headache, fever, nausea, vomiting, and myalgias. Physical examination may show neck rigidity Kernig signs, poor levels of consciousness, tremors, paresis, and nystagmus.

In most cases, cerebrospinal fluid (CSF) contains more than 5–10 cells with normal content of sugar and protein in it, or leukopenia. The mean duration of the disease is 7 days, and the outcome is usually favorable. However, a small number of severe cases with unusual symptoms have been reported. No sequelae have been described.

A study including subjects of a high-risk, professionally exposed population reported a seroprevalence of 70%, but without neurological symptoms. These data confirmed that TOSV infection can occur with either mild or no symptoms and suggest the frequent presence of TOSV infection in regions where the vector occurs.

Diagnostic Procedures

Diagnosis of phlebovirus infections has been classically attempted by isolating the agent or demonstrating seroconversion in paired acute- and convalescent-phase serum samples. Several molecular techniques have been developed and used to demonstrate the presence of the virus or viral RNA in patient samples.

Virus Recovery and Detection

The recovery of phleboviruses from acutely ill phlebotomus fever patients is rare, given that viremias associated with disease are transient (24–36 h) and most patients do not seek medical care within this period.

The most common method of phlebovirus isolation is intracranial inoculation of suckling mice. However, several studies have demonstrated that Vero cells (from kidney tissue of an African green monkey) are more sensitive than are newborn mice for isolation of phleboviruses from wild-caught sandflies. Similar results have been obtained with RVFV.

Identification of isolates can be made with serological methods, such as immunofluorescence, neutralization in mice, or by plaque reduction (PRNT) using hyperimmune sera. Identification can also be performed by complement fixation (CF) or hemagglutination-inhibition (HI); however, care should be taken in areas where more than one phlebovirus circulates, as these latter tests do not discriminate between certain antigenically related viruses. For example, sandfly fever Naples, Toscana, and Tehran viruses are indistinguishable by CF, yet easily identified by PRNT.

Notable diagnostic success in TOSV infections was achieved through molecular reverse transcription-polymerase chain reaction (RT-PCR) techniques. This approach has shown the occurrence of different TOSV variants.

Serologic Tests

Serologic diagnosis can be performed with many different methods. Most of the work in the past has been done with HI. PRNT provides more clear-cut results, but this test is time consuming, and is not more useful for diagnosis than are other tests when a single serum sample is available. However, the PRNT is the most common assay for assessing antibody titer.

Enzyme-linked immunosorbent assays for IgG and IgM antibodies have been established in the case of many phleboviruses; diagnosis is obtained in most cases by detection of specific IgM antibody. The use of immunoenzymatic techniques has been very useful in the developing tests in which the recombinant nucleoprotein expressed in *Escherichia coli* is used as the specific antigen.

Epidemiology

Epizootics of RVFV have been reported in many African countries. Prior to 1977, it was believed that the infection was confined to sub-Saharan Africa. However, the epizootic/epidemic in Egypt in 1977–78 occurred in areas outside the recognized range of the virus. Retrospective studies indicated that RVFV had not been endemic in Egypt prior to the epidemic. The epidemic occurred after the progressive implementation of the Aswan dam project, which regulated the irrigation of the Nile delta. The dam resulted in an increase in the number of breeding places of mosquitoes and also facilitated the transport of animals from Sudan to northern Egypt. From 1973 to 1976, an epizootic outbreak of RVFV occurred in Sudan, which extended from south to north and possibly reached Egypt in 1977.

RVFV was firstly detected outside Africa in September 2000. In southwestern coastal Saudi Arabia and neighboring coastal areas of Yemen, an epizootic occurred with >120 human deaths and major losses in livestock populations from disease and required slaughter. Most RVF activity was associated with flooded wadi agricultural systems; no cases were reported in the mountains or in dry sandy regions, where surface water does not accumulate long enough to sustain mosquito breeding.

Representative phleboviruses have been isolated in Southern Europe, Africa, Central Asia, and the Americas.

Many phleboviruses occur in tropical and subtropical regions, but some important ones are active in temperate areas (e.g., sandfly fever Naples virus, sandfly fever Sicilian virus, and TOSV in Southern Europe and in the Middle East). Most phleboviruses (66%) have been isolated in Central and Southern America and are associated with sandflies of the genus *Lutzomyia*. On the contrary, the majority of sandfly fever viruses isolated in the Old World always are associated with *Phlebotomus* spp. sandflies.

In general, each serotype has a unique distribution, with limited geographical overlap. However, there are indications of simultaneous circulation of different phleboviruses in the same sandfly population. Sandfly fever Naples and sandfly fever Sicilian viruses have the largest geographical distributions, paralleling that of their vector *P. papatasi*. Reported isolations as well as results of serologic studies indicate that sandfly fever Naples and sandfly fever Sicilian viruses are present in the Mediterranean coastal regions of Europe and North Africa, the Nile valley, most of southwest Asia, areas adjacent to the Black and Caspian Seas, and Central Asia as far as Bangladesh.

Despite the preponderance of phleboviruses in the New World, the incidence of phlebotomus fever, and in general of human infections, in the Americas is relatively low. On the contrary, the Old World phleboviruses, such as sandfly fever Naples virus, sandfly fever Sicilian virus, and TOSV, are relatively widely distributed and are well-known causes of epidemics of human infections. In some rural human communities in endemic areas of the Old World, the prevalence of phlebovirus infection is quite high among the indigenous population. For example, results of serosurveys aimed at defining the extent and prevalence of infection with different serotypes among indigenous human populations indicated prevalences as high as 62% for Karimabad virus in some provinces of Iran and 59% for sandfly fever Sicilian and 56% for sandfly fever Naples viruses in Egypt.

A similar serosurvey for the presence of antibodies to sandfly fever Sicilian virus, sandfly fever Naples virus, and TOSV was done in 1977 in Italy. These seroepidemiological studies indicated that both sandfly fever Sicilian and sandfly fever Naples viral infections decreased after the 1940s, probably as the result of insecticide spraying during malaria eradication campaigns. On the contrary, age-specific antibody rates suggested that TOSV, which is transmitted by *P. perniciosus* and *P. perfiliewi*, is endemic in Italy. A high infection rate (24.8%) was observed among residents of the region where the virus was first isolated. Similar results have been found in other Mediterranean countries (Spain, France, Greece, Cyprus).

Because illnesses due to phlebotomus fevers have been of considerable military and historical interest, much of the early research on phlebotomus fevers was performed by military physicians and epidemiologists. In 1984, cases of sandfly fever Sicilian virus infections were documented in Cyprus among Swedish United Nations soldiers. A follow-up study revealed that 11 of 298 soldiers seroconverted to sandfly fever viruses (mostly Sicilian) during a 6 month stay in Cyprus in the summer of 1985. Infection due to sandfly fever viruses (mostly sandfly fever Sicilian virus) was diagnosed in Swedish tourists contracting a febrile illness associated with their travel during 1986–88 to the Mediterranean region. In contrast, a serological survey among Cypriots revealed a higher antibody prevalence to the sandfly fever Naples virus than to sandfly fever Sicilian virus and TOSV.

Clinical cases with signs and symptoms ranging from aseptic meningitis to meningoencephalitis caused by TOSV are observed annually in central Italy during the summer. The incidence of cases of meningitis due to TOSV is directly related to yearly differences in the density of the vector sandflies, which is greatly influenced by variations of climatic conditions. Most cases have been reported in residents or travellers in central Italy or Spain, and sporadically from other Mediterranean countries, such as Portugal, Cyprus, France, and Greece.

In Spain, TOSV is one of the three leading causes of meningitis. A large study conducted in different regions of Spain showed the presence of IgG antibodies to TOSV (26.2%), sandfly fever Naples virus (2.2%), and sandfly fever Sicilian virus (11.9%).

Studies of people living on the Ionian Islands and on the western mainland of Greece showed a seroprevalence for TOSV of 60% and 35%, respectively. It is also reported that in Cyprus 20% of the healthy population had IgG antibody to TOSV.

Therapy

The mild nature of phlebotomus fevers has not encouraged studies on the possibility of treating the disease with specific antiviral compounds. Rather, treatment of phlebotomus fever patients is symptomatic. Due to the severity of the disease, several studies have been done both *in vitro* and *in vivo* in an effort to devise possible treatments of RVFV infections. Two different approaches have been evaluated: the use of an antiviral compound such as ribavirin or its derivatives, or the use of interferon or interferon inducers.

High doses of ribavirin have been used successfully to treat experimental infections with RVFV in several strains of mice. However, at low drug doses, treatment failures occur, resulting in death due to either hepatitis or subsequent encephalitis. Better results have been obtained when animals were treated with liposome-encapsulated ribavirin.

Experimental data and epidemiological experience suggest that the major utility of interferon in RVFV infections could most likely be in early postexposure prophylaxis (e.g., laboratory accidents, high-risk exposures). The success of interferon treatment initiated after

the appearance of clinical symptoms has not been assessed. However, if maximal levels of circulating interferon in humans are obtained before early viremia, as is seen in rhesus monkeys, it is possible that interferon treatment begun at the first appearance of symptoms could affect the course of illness by limiting the incidence of future complications, particularly hemorrhagic fever. In addition, laboratory data and anecdotal information suggest that ribavirin and convalescent plasma may be beneficial in treating hemorrhagic fevers due to RVFV.

Prevention and Control

Vaccines

To date, there are no vaccines available against phlebotomus fever viruses. Given the large number of serotypes that can infect humans, and the lack of cross-immunity between them, to be practical a phlebotomus fever vaccine would have to be polyvalent. Moreover, in view of the relatively benign nature of the disease, vaccination against phlebotomus fever is not cost-effective or advisable as a general public health policy.

As RVFV is primarily of veterinary importance and human epidemics follow livestock epizootics, the immunization of susceptible animals is the most effective means of controlling RVFV infection.

Formalin-inactivated RVFV vaccines have been used for many years in South Africa, Egypt, and Israel to immunize sheep and cattle but they require multiple inoculations to ensure lasting protection. This type of vaccine is recommended for nonendemic areas as well as for livestock to be exported from enzootic areas.

An inactivated RVFV vaccine, developed for human use, is recommended for use to immunize at-risk laboratory and field researchers, or other people at high risk of infection. No clinical cases have been reported after vaccination; however, a few subclinical infections have been detected.

Two live RVFV vaccine candidates have been developed. One is a minute plaque variant and the other is an extensively mutagenized strain, MP-12, passaged in the diploid human lung cell line, MRC-5. Experimental studies in animals have demonstrated that a single dose of MP-12 RVFV vaccine is immunogenic and nonabortogenic in pregnant ewes. It can, therefore, be used safely to protect pregnant ewes and newborn lambs during an RVFV outbreak without increasing mortality due to vaccination.

RVFV MP12 vaccine was developed in cells certified for human vaccine production, and this vaccine may be employed for protecting both humans and livestock against epizootic RVFV infection. Because it is attenuated, this vaccine may be used to prevent an impending RVFV outbreak in an area where the virus is nonendemic, without the risk of spread to the environment.

Prevention of Transmission

During the Egyptian epidemic of RVFV in 1977–78, insecticides were used both inside and outside human and animal shelters to reduce the adult populations of mosquito vectors. However, it was not demonstrated that the use of these chemicals actually decreased the incidence of the disease. In sub-Saharan Africa, where RVFV outbreaks are thought to occur following flooding of vector mosquito breeding habitats, the possibility of using encapsulated formulations of insecticides to control the larval stages has been considered.

Measurements of green leaf vegetation dynamics recorded by advanced very high resolution radiometer instruments on polar-orbiting, meteorological satellites were used to derive ground moisture and rainfall patterns in Kenya and monitor resulting flooding of mosquito larval habitats likely to support RVFV vector mosquitoes (*Aedes* spp. and *Culex* spp.). These data could be used by local authorities for implementations of specific mosquito control measures to prevent transmission of RVFV to susceptible vertebrate hosts.

At present, the only method effective in controlling phlebotomus fevers is to reduce human contact with the vectors that carry their etiologic agents. Insecticides are extremely effective in the control of peridomestic sandfly species, but are of little value for sylvan species. Mechanical means (e.g., protective clothing, bed nets and screening of windows) and use of repellents, such as diethyltoluamide, can also be used to prevent human–vector contact.

See also: Bunyaviruses: General Features; Replication of Viruses.

Further Reading

Baldelli F, Ciufolini MG, Francisci D, *et al.* (2004) Unusual presentation of life-threatening TOSV meningoencephalitis. *Clinical Infectious Diseases* 38: 515–520.

Balkhy HH and Memish ZA (2003) Rift Valley fever: An uninvited zoonosis in the Arabian peninsula. *International Journal of Antimicrobial Agents* 21: 153–157.

Braito A, Corbisiero R, Corradini S, *et al.* (1997) Evidence of Toscana virus infections without central nervous system involvement: A serological study. *European Journal of Epidemiology* 13: 761–764.

Charrel RN, Gallian P, Navarro-Mari JM, *et al.* (2005) Emergence of Toscana virus in Europe. *Emerging Infectious Diseases* 11(11): 1657–1663.

Ciufolini MG, Maroli M, Guandalini E, Marchi A, and Verani P (1989) Experimental studies on the maintenance of Toscana and Arbia viruses (*Bunyaviridae: Phlebovirus*). *American Journal of Tropical Medicine and Hygiene* 40: 669–675.

Flick R and Bouloy M (2005) Rift Valley fever virus. *Current Molecular Medicine* 5: 827–834.
Geisbert TW and Jahrling PB (2004) Exotic emerging viral diseases: Progress and challenges. *Nature Medicine* 10: S110–S121.
Nicoletti L, Verani P, Caciolli S, *et al.* (1991) Central nervous system involvement during infection by the phlebovirus Toscana of residents in natural foci in central Italy (1977–1988). *American Journal of Tropical Medicine and Hygiene* 45: 429–434.
Nicoletti L, Ciufolini MG, and Verani P (1996) Sandfly fever viruses in Italy. *Archives of Virology Supplement* 11: 41–47.
Sánchez Seco MP, Echevarría JM, Hernández L, Estévez D, Navarro-Marí JM, and Tenorio A (2003) Detection and identification of Toscana and other phleboviruses by RT-PCR assays with degenerated primers. *Journal of Medical Virology* 71: 140–149.
Torres-Velez F and Brown C (2004) Emerging infections in animals – potential new zoonoses? *Clinics in Laboratory Medicine* 24: 825–838.
Weaver SC (2005) Host range, amplification and arboviral disease emergence. *Archives of Virology Supplement* 19: 33–44.

Relevant Website

http://www.cdc.gov – CDC Special Pathogens Branch, Centers for Disease Control and Prevention.

Rinderpest and Distemper Viruses

T Barrett, Institute for Animal Health, Pirbright, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cytopathic effect Alterations in the microscopic appearance of cultured cells following virus infection.
Enzootic A disease constantly present in an animal community in a defined geographic region.
Epizootiology The study of disease epidemics in animal populations.
Hemagglutinin Any substance that causes red blood cells to agglutinate; many viruses possess a hemagglutinin protein in the outer envelope.

History

Rinderpest and its related viruses form a distinct group of paramyxoviruses, the genus *Morbillivirus*. In addition to measles virus (MV), the type virus of the genus, they include important animal pathogens: rinderpest virus (RPV), which infects many species of large ruminant; peste des petits ruminants virus (PPRV), a similar disease of small ruminants; canine distemper virus (CDV), found mainly in carnivores; phocid distemper virus (PDV), in seals; and the cetacean morbillivirus (CeMV), which is found in whales, dolphins, and porpoises. Of the animal viruses, rinderpest is the most important economically. It was also known as cattle plague in the past because of the high mortality associated with infections and the speed with which it spreads in naive cattle populations, which made it an easily recognizable disease. It is one of the oldest documented plagues of domestic livestock. Aristotle (384–322 BC) described a disease in cattle, *struma*, that had all the characteristics of rinderpest and other descriptions date to the fourth century when invasion by Huns into Europe resulted in outbreak of highly contagious disease which had all the characteristics of rinderpest. Steppe cattle of Central Asia are thought to be the original source of rinderpest and 'steepe murrain' was the old English name for the disease. Panzootics of rinderpest were also brought to western Europe with the Mongol invasions in the thirteenth century. In 1709, the disease again entered Europe through Venetian trade with the east and by 1714 had spread as far west as Britain. The economic and social consequences which followed in the wake of these plagues led to the establishment of the first veterinary schools – the first at Lyon, France, in 1762. Other European countries followed France's example and set up their own veterinary schools. Subsequent brief reintroductions into Europe in the early twentieth century, and into South America and Australia through the import of infected cattle led to the establishment of the Office Internationale des Epizooties (OIE), a body that functions as the World Organisation for Animal Health, to deal specifically with animal diseases in relation to international trade.

Rinderpest was introduced into Africa with disastrous consequences in the late 1880s with cattle imported from India to feed Italian troops in fighting a colonial war in Abyssinia (now Ethiopia). The subsequent panzootic spread to nearly all parts of the African continent within a period of 10 years, reaching South Africa by 1897. The devastation that followed its path as it swept across the African continent wiped out 90% of the domestic cattle and wild buffalo (*Syncerus caffer*). A number of other large wild ruminant species were also highly susceptible to the virus and died in large numbers during this so-called 'Great African Pandemic'.

A similar plague of small ruminants, peste des petits ruminants (PPR), was first scientifically documented in West Africa in the early 1940s and is also known as *kata* in that region. At first, it was thought that PPRV was a variant of RPV adapted to small ruminants but it was subsequently shown to be a genetically distinct virus with an independent epizootiology in areas where both viruses co-circulated.

Canine distemper, which infects many terrestrial carnivore species, is also a disease with a long history. In the eighteenth century, Edward Jenner studied its neurological effects following infection in dogs. In 1988, a distinct but closely related virus, phocine distemper virus (PDV), was isolated from seals found dying in large numbers along the beaches of Northern Europe and which showed clinical signs similar to distemper in dogs. Both harbor (*Phoca vitulina*) and gray (*Halichoerus grypus*) seals were affected and the epizootic resulted in the deaths of more than 20 000 seals. In 1990, large numbers of striped dolphins (*Stenella coeruleoalba*) in the Mediterranean Sea were found dying from a similar infection and a virus was isolated from sick animals. This virus has since been associated with mass die-offs among whales, porpoises, and dolphins (order *Cetacea*) and a fatal epizootic in bottlenose dolphins (*Tursiops truncatus*) from the northwestern Atlantic Ocean in the 1980s. A morbillivirus was also isolated from diseased porpoises (*Phocoena phocoena*) found along the coasts of Ireland and the Netherlands between 1988 and 1990 during the epizootic of PDV in seals. This virus was later found to be very closely related to the dolphin virus and genetically quite distinct from all other morbilliviruses. The dolphin and porpoise virus isolates are now commonly referred to as CeMV, and these virus infections have the potential to cause severe disease and threaten the ecology of many marine mammal species.

Taxonomy and Classification

As indicated above, these antigenically closely related viruses are classified in the genus *Morbillivirus* within order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Paramyxovirinae*. The name is derived from the diminutive (*morbilli*) of the Latin word *morbus* meaning 'little plague', to distinguish measles from deadlier diseases such as smallpox. The paramyxoviruses are large enveloped pleimorphic particles which bud from the surface of infected cells and the different species are indistinguishable in the electron microscope. They vary in diameter from 300 to 500 nm. The lipid envelope is derived from the host cell membrane as the virions bud from the cell and it encloses a ribonucleoprotein (RNP) core which contains the genome encapsidated by the nucleocapsid protein giving it a characteristic 'herring bone' appearance. Unlike other paramyxoviruses, the morbilliviruses generally lack neuraminidase but a highly substrate-specific neuraminidase activity has been reported for PPRV and RPV. Only MV and PPRV viruses can hemagglutinate red blood cells reproducibly.

Geographic Distribution

Until the mid-1980s, rinderpest was found in much of tropical Africa and in western and southern Asia. Control and eradication program were established in the mid-1980s and this effort, based on mass vaccination campaigns in the endemic regions, has succeeded in eliminating the disease from Asia and the Middle East; the virus is now almost certainly confined to a defined region of eastern Africa known as the southern Somali ecosystem, a region in eastern Africa comprising southeast Ethiopia, southern Somalia, and northeast Kenya.

PPR is enzootic in West Africa and on the Indian subcontinent and is now spreading from Afghanistan into Central Asia (**Figure 1**). It first appeared as a recognized disease in India in 1988 and has subsequently been found as far east as Bangladesh and north as far as Nepal. Epizootics regularly occur in the Middle East and Turkey through import of infected animals. Turkey's proximity to Southern Europe poses a threat to small ruminants in that region. The widespread distribution of PPRV in southwest Asia suggests that the virus had been present on the continent for some considerable time before it was identified in India. Its presence was most likely masked by rinderpest since all morbillivirus-like diseases in small ruminants in India before 1988 were considered to be caused by rinderpest infections. With the subsequent elimination of rinderpest from Asia and the development of more accurate diagnostic techniques it has now become easier to chart the spread of PPRV in the region.

CDV is found in all but the hottest and most arid regions of the globe. The development of attenuated live vaccines in the late 1950s greatly reduced the incidence of the disease in domestic dogs in developed countries. However, many wildlife species are susceptible to CDV infection and can act as reservoirs for the virus. CDV has also been found in Lake Baikal seals (*Phoca sibirica*) and Caspian Sea seals (*Phoca caspica*) and it poses a threat to the survival of the latter species which is highly endangered. There is also serological evidence for infection in Antarctic seals and, as there are no native carnivores on that continent, unvaccinated sledge dogs are blamed for introducing the virus.

The geographic distribution of PDV is not fully defined but, based on serological evidence, it appears to be an endemic infection in some species of seal in the northern Atlantic and Arctic oceans. These are considered to be the most likely source of PDV for the European

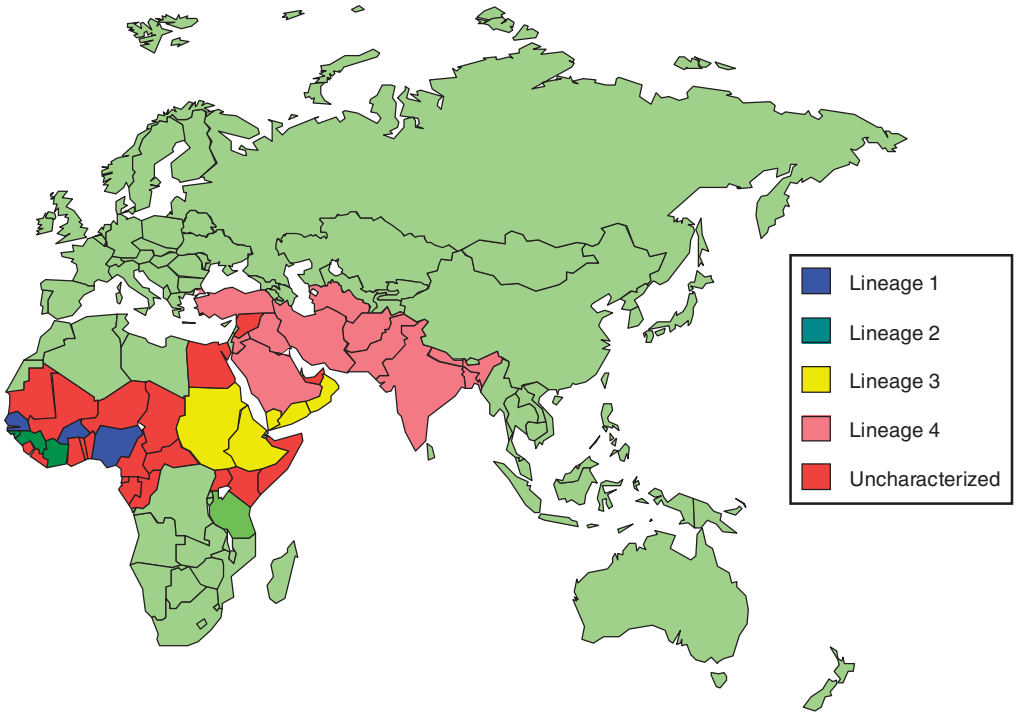


Figure 1 The distribution of PPRV. Where known, the different lineages are color-coded. There are only serological or capture enzyme-linked immunosorbent assay (ELISA) data available for countries shown in red.

seal epizootics. The CeMV appears to have a worldwide distribution and there is serological evidence for the presence of this virus in diverse species from all of the world's major oceans.

Host Range Virus

Each morbillivirus is generally able to cause serious disease only in one order of mammals, the exception being CDV. All cloven-hoofed animals (Artiodactyla) are thought to be susceptible to infection with RPV but the disease is not manifest in all. In the case of cattle, Indian and African breeds (*Bos indicus*, zebu) are more resistant than European (*Bos taurus*). The virus can also infect a range of wild ungulates but disease progression depends on the innate resistance of the species concerned. Some, such as kudu (*Tragelaphus imberbis*), eland (*Taurotragus* spp.), giraffe (*Giraffa* spp.), and wildebeest (*Connochaetes* spp.), are highly susceptible to the virus and died in large numbers during the first African pandemic. Others, mainly small antelope species, proved to be more resistant (**Table 1**).

The full host range of PPRV is unknown but, in addition to sheep and goats, several species of antelope have been fatally infected by contact with infected sheep. Outbreaks of PPRV have been reported in game reserves and zoos where the mortality was 100% in some species.

Table 1 Susceptibility of wildlife to rinderpest

Very high	Buffalo, eland, kudu, wart hog
High	Giraffe, bushbuck, bush pig, sitatunga, Uganda cob, bongo, wildebeest
Moderate	Reedbuck, topi, gemsbok, blesbok, bontbok, oribi, impala, springbok
Low	Waterbuck, dukier, orynx, Grant's gazelle, dikdik, hartebeest
Very low	Thomson's gazelle, hippopotamus, gerenuk

Based on data from Plowright W (1982) The effects of rinderpest and rinderpest control on wildlife in Africa. In: Edwards MA and McDonald U (eds.) *Symposia of the Zoological Society of London, No. 50: Animal Diseases in Relation to Animal Conservation*, p. 1. London: Academic Press.

Goats are generally considered to be more sensitive to PPRV infection than sheep. Indian buffaloes (*Bulbalus bulbalis*) have also been reported to have died from PPRV infections. Cattle have been found in West Africa which were seropositive for PPRV, with up to 80% prevalence in some herds, but there is no evidence that it can cause disease in cattle.

CDV can infect most carnivores but in some it may result in only a mild or subclinical infection, for example, in domestic cats. It causes severe disease in all members of the Canidae (dog, wolf, fox), Mustelidae (ferret, weasel, mink), Procyonidae (raccoon, panda), as well as in collared peccaries (*Tayassu tajacu*, order Artiodactyla). More recently, CDV has been shown to be responsible

for high mortalities in both wild and captive big cats and in hyenas (*Crocuta crocuta*). Failure to recognise the disease earlier in these species may have been due to a lack of awareness of a possible viral etiology and/or the availability of diagnostic tools to detect the virus. Outbreaks of CDV in Siberian and Caspian seals have extended its host range to include these species. PDV is known to infect many species of seal in the North Atlantic and Arctic oceans but its full host range is unknown. There is also serological evidence that terrestrial carnivores in Canada, including polar bear (*Ursus maritimus*), lynx (*Fellis lynx*), and wolves (*Canis lupus*), have been infected with the virus and also with CeMV. CeMV infections have been described in a variety of cetaceans and there are serological data indicating infection in many more.

Virus Propagation

All known morbilliviruses can be propagated on Vero cells, which lack the ability to produce interferon, but it generally requires several blind passages to adapt the virus to these cells. This adaptation can alter the receptor-binding characteristics of the virus and often attenuates it for the natural host. Clinical isolates of rinderpest that retain their pathogenicity can best be grown on primary bovine kidney cells or transformed lymphoid cell lines such as B95a cells. PPRV is normally grown on lamb kidney cells and primary mitogen-stimulated dog or ferret macrophages are the most suitable cells for the isolation of CDV. Alternatively, Madin–Darby canine kidney (MDCK) cells have been shown to be useful for CDV isolation from infected tissues. Primary seal kidney cells were initially used to isolate PDV. Virus is most readily obtained from tissues such as mucosal lesions, lymph nodes, or by co-cultivation of washed buffy coat from infected animals with suitable tissue culture cells. Lung tissue is also a good source of virus for PPRV, CDV, and PDV isolation. Typical cytopathic effects such as cell elongation, cell rounding, the formation of stellate cells, and syncytia can be observed 3–12 days post infection of the cell cultures although several blind passages may sometimes be necessary before cytopathic changes are observed.

Host range is to some extent, but not completely, determined by the expression of a suitable receptor on the host cell. The CD150 molecule, also known as signal lymphocyte activating molecule or SLAM, is thought to be the main cellular receptor for wild-type RPV, CDV, and MV isolates. SLAMs specific to their respective host species may be general morbillivirus receptors. CDV replicates better and causes extensive cell fusion in Vero cells expressing dog SLAM (Vero DST) compared to normal Vero cells and these are a better cell line for propagation and titration of CDV isolates. The expression of SLAM on cells of lymphoid origin, activated lymphocytes, immature thymocytes, macrophages, and mature dendritic cells explains the strong lymphotropism of these viruses. Tissue culture-adapted viruses can use other receptors such as CD46. Alterations in receptor recognition could possibly be a means whereby these viruses could widen their host range in future.

Properties of the Genome

The morbillivirus genomes consist of a single strand of negative-sense RNA just under 16 kbp in length. They are organized into six contiguous, nonoverlapping, transcription units which encode the six structural proteins, namely the nucleocapsid (N), the phospho (P), the matrix (M), the fusion (F), the hemagglutinin (H), and the large (L) proteins, the latter being the viral RNA-dependent RNA polymerase. They have highly conserved sequences at their 3′ (leader) and 5′ (trailer) terminal extremities that act as promoters for transcription and replication (**Figure 2**). The complete promoter elements include sequences that extend into the untranslated region at the start of the N gene open reading frame (ORF) and the untranslated region at the end of the L gene ORF. These regions contain all the *cis*-acting signals necessary for primary transcription as well as for the production of a full-length positive-sense RNA genome copy required for the production of new genome RNA. There are semi-conserved start–stop sequence motifs at the start and end

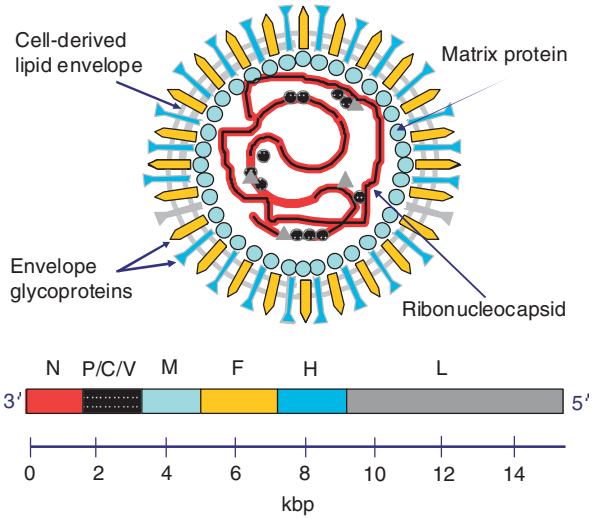


Figure 2 Cartoon showing the structure of morbillivirus virions. The outer envelope is shown with two projecting glycoproteins (H and F) and the inner helical nucleocapsid containing the genome RNA encapsidated with the N protein and the P and L proteins associated with it. The matrix protein is shown as a ring of circles underneath the virus envelope. The linear order of genes in the genome RNA from 3′ to 5′ is also shown.

of each mRNA transcription unit: (UCCU/C) at each transcription start; and a sequence rich in U residues, which signals the polyadenylation of the mRNAs, at the end. Between the end of one transcription unit and the start of the next is an intergenic triplet (usually GAA) which is not translated into mRNA. Downstream mRNA synthesis depends on termination of the upstream mRNA. The N, P, and L proteins, along with the genome RNA, constitute the transcription/replication unit of the virus, the RNP core. The F and H glycoproteins are embedded in a lipid envelope which is derived from the host cell during the budding process. The nonglycosylated M interacts with cytoplasmic domains of the membrane-associated F and H proteins and also with the nucleocapsid RNPs formed in the cytoplasm during replication and brings together the two components that make up the budded virion. The M protein is essential for efficient virus budding to occur.

Morbilliviruses also produce two nonstructural proteins (C and V) encoded in the P gene transcription unit. The first of these, the C protein, is translated by ribosomes that scan past the first AUG codon and start at the second which is located about 20 nt downstream. This protein is in a different reading frame to the P protein and bears no antigenic relationship to it. The second nonstructural protein, the V protein, in derived by alternative transcription of an mRNA from the P transcription unit by which a nontemplated G residue added to approximately 50% of the P mRNAs. The extra Gs are inserted at a specific, highly conserved, sequence (5′ UUAAAAAGGG[G]CACAG), known as the editing site, positioned about halfway along the P protein ORF. This so-called editing process is a property of the virus polymerase as it does not occur in artificial transcription systems. Translation of this mRNA produces the V protein which is a chimeric protein consisting of the N-terminus of the P protein with a new, shorter C-terminus rich in cysteine residues derived from template sequence in the third reading frame. The V mRNA is also capable of translating the C protein as its coding region is located before the editing site in P. The nonstructural proteins have functions in controlling transcription and replication and also are involved in virus evasion of the host's innate immune responses.

Morbilliviruses, like some other paramyxoviruses, have a strict genome length requirement in that they should be divisible by 6 (the 'rule of six'). This requirement can be explained by the fact that each N protein monomer associates with exactly 6 nt and that efficient transcription and/or replication can only occur if the RNA genome is encapsidated by the N protein in its entirety. Reverse genetics systems have been established for MV, RPV, and CDV. This enables virus to be 'rescued' from a DNA copy of its genome and this copy can be manipulated to make virus mutants which can then be used to determine the functions of various proteins and sequence motifs and to study the molecular basis of host range and pathogenicity.

Evolution

The close antigenic relationship and sequence similarity of morbilliviruses indicate that they all evolved from a common ancestor; however, the details of their evolutionary history are unclear. The phylogenetic relationship between the different morbilliviruses, based on the sequence of the P proteins, is shown in **Figure 3**. RPV, PPRV, CDV, and CeMV are equidistant from each other. It appears, therefore, that no existing morbillivirus represents the ancestral virus. MV is genetically more closely related to RPV and PDV to CDV, suggesting that they evolved more recently from their respective common ancestors. A factor that must be considered in their evolutionary history is that animals which have recovered from morbillivirus infections are immune for life and so fairly large populations are required to ensure a constant supply of naive hosts needed to maintain these viruses in circulation. The minimum population size that satisfies this requirement has been estimated to be about 300 000 for MV, and so this virus could not have existed before settled human populations became large enough. Herds

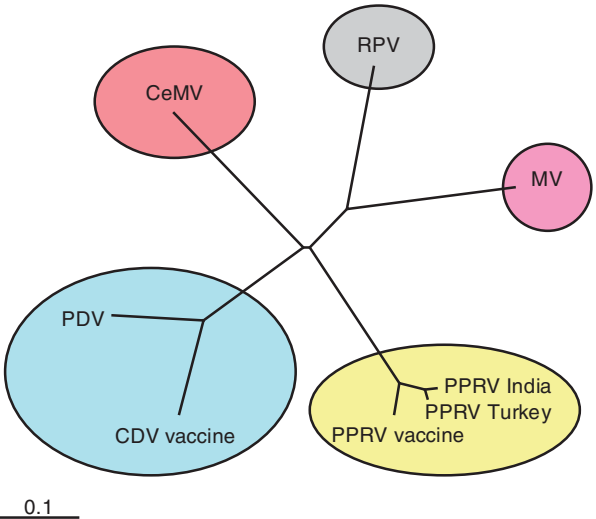


Figure 3 Radial tree showing the morbillivirus phylogenetic relationships based on their P protein sequences. The P proteins were aligned using ClustalW (BLOSUM) matrix program. A neighbor-joining radial tree was then generated from this alignment using ClustalX v1.83 and TreeView v1.6.6. Three different PPRV sequences were included to show the extent of variation within each morbillivirus type. The PPRV vaccine and PPRV Turkey are the most distant viruses in the group. Access numbers: RPV (X98291); PPRV (AY560591, X74443, AJ849636); CDV (AF305419); PDV (D10371); DMV (AJ608288); MV (AB012948).

of ruminants roaming the steppes of Central Asia, the historic source of rinderpest, would, however, have been able to maintain a morbillivirus in circulation. It is probable that when human communities became large enough, rinderpest, or a rinderpest-like infection, was passed to them from their domesticated cattle. RPV and PPRV are equidistant from the putative ancestral virus and so PPRV did not evolve directly from RPV and must have a long independent evolutionary history in small ruminants.

Carnivores preying on infected ruminants may similarly have become infected with the progenitor morbillivirus which subsequently evolved in these species to become CDV. CDV infects a wide variety of carnivores and so there is potentially a large reservoir of virus in susceptible wildlife species. PDV most probably evolved quite recently from CDV. Seals have many opportunities to become infected with CDV by contact with terrestrial carnivores such as wolves, foxes, dogs, and polar bears. Arctic seals populations, unlike European seal populations, are large enough to enable a morbillivirus to be maintained and subsequent evolution would select a virus more adapted to replicate efficiently in seals.

The two CeMVs that have been isolated from dolphins and porpoises are very closely related to each other, as related as different lineages of either RPV or PPRV, and can be considered to constitute one virus species. CeMV is antigenically closest to PPRV but is equidistant for the putative ancestral virus by sequence analysis and so this virus also has had a long independent evolutional history.

Serological Relationships and Variability

All the morbilliviruses are antigenically related, the F, M, and N proteins being the most highly conserved across the group. The two virus-coded glycoproteins, the H and F, are embedded in the virus envelope and neutralizing antibody responses are generated to both of these proteins. The H protein, responsible for attachment of the virions to the host cell surface receptor molecule and is therefore a determinant of host range, is the least cross-reactive of the morbillivirus proteins. While it is possible to differentiate strains of each morbillivirus by monoclonal antibody and sequence analysis, these variations do not result in different serotypes and for vaccination purposes each morbillivirus has only one serotype.

Epizootiology

Rinderpest and PPR are normally introduced into new regions by importation of live animals. Transmission through infected meat and meat products is considered to be a very low risk due to the highly labile nature of the viruses. Historically, rinderpest outbreaks have been associated with wars and civil conflict when there is uncontrolled movement of people and troops, often bringing live animals from enzootic regions as food. Sheep and goats, and possibly other ruminants, may show only mild disease or subclinical infection with RPV but nevertheless they can then pass the infection to in-contact cattle. Trade in live animals is also another factor that has been responsible for long-distance spread of rinderpest.

Epizootics of rinderpest in naive populations are generally very severe and mortality rates can exceed 90% and affect all age groups, the classic characteristics of cattle plague. The last outbreak of this nature occurred in Pakistan in 1994. In endemic areas, disease is less severe and most often affects animals less than 2 years of age as older animals are generally immune due to previous exposure or vaccination. Newborns are protected for up to 9 months by maternal antibody. The rinderpest virus strain in circulation in the last enzootic focus in Africa belongs to African lineage 2 (**Figure 4(a)**) and is unusual in that it causes only mild disease in cattle. It becomes clinically apparent when it spreads to highly susceptible wildlife species such as buffalo, eland, and kudu. These have suffered very high mortalities when infected with this strain of virus during outbreaks in Kenyan national parks and because of this wildlife are considered excellent sentinels for incursions of rinderpest. They are closely monitored for evidence of reintroduction of the virus from its only known enzootic focus in the southern Somali ecosystem.

PPRV shows a similar epizootiology and can also cause very high mortalities in naive populations of sheep and goats. Introductions into disease-free areas are usually traceable to movement of infected animals from enzootic regions. Phylogenetic analysis on PPRV strains reveals that there are four distinct lineages in circulation (**Figure 4(b)**). PPRV infections in wildlife have not been studied in any detail but the severity of disease when it occurs in captive small ruminants infected by contact with domestic livestock makes it unlikely that there is a significant wildlife reservoir of the virus.

CDV causes periodic epizootics in domesticated dogs and wild carnivores and it remains a problem in poor urban areas where there are many stray dogs and vaccination is not carried out rigorously. In enzootic areas, the density of the susceptible population is an important factor in determining the frequency of epizootics and can also affect its maintenance and spread. Again, disease is most often seen in young animals after maternal antibody has waned. A variety of wild carnivore species are highly susceptible to CDV infections and presence of the virus can be a high-risk factor for some endangered species.

The source of PDV in the two epizootics that occurred in European seals is thought to have been either Canadian harp seals (*Phoca groenlandica*) or Arctic ringed seals (*Phoca hispida*). Retrospective analysis of seal sera from Canada

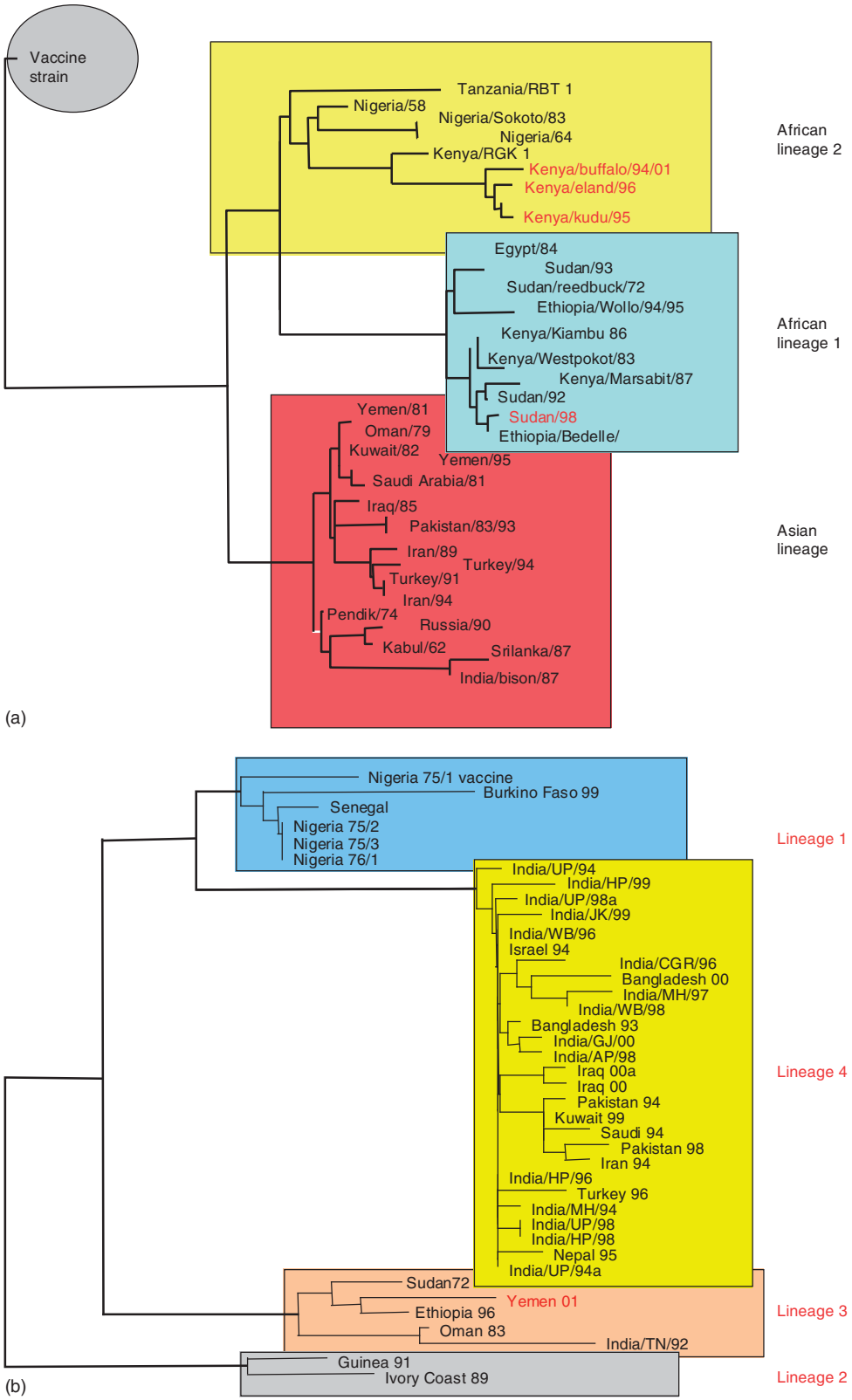


Figure 4 A computer-generated tree showing the relationship between (a) the African and Asian lineages of rinderpest virus and (b) the currently circulating PPRV lineages. The trees were generated using the PHYLIP program using partial sequence data from the F protein gene. Only RPV African lineage 2 remains extant and the outbreaks in Kenya since 1994 are highlighted in red. The vaccine strain of rinderpest dates to the early part of the twentieth century and was used as the outgroup in the rinderpest and PPRV analysis.

dating back to the early 1980s showed that they were positive for virus-specific antibodies. The two European PDV epizootics, in 1988 and 2002, were remarkably similar and this may be explained by the susceptibility, migratory patterns, and/or breeding habits of the seals. The gray seal is the most likely candidate to act as the vector in the transmission of PDV between Arctic and European seals and between colonies of harbor seals as they are known to move much greater distances between haulout sites. Another important factor is the relative resistance of gray seals to the disease, possibly enabling PDV to circulate in that population without necessarily causing high mortality.

The pilot whale (*Globicephala* spp.) is considered to be the most likely endemic source of CeMV and also the vector for its transmission to other species. Pilot whale populations have the characteristics required for both; they move in large groups (pods), have a widespread pelagic distribution, and are known to associate with many different cetacean species. A high proportion of pilot whales sampled in the mid-1990s showed evidence of infection and over 90% of pilot whales that were involved in mass strandings between 1982 and 1993 were seropositive for morbillivirus.

Transmission

All morbilliviruses are extremely labile in the environment and are inactivated by heat, ultraviolet (UV) light, and chemicals that alter pH or destroy their lipid envelopes. Therefore, although highly contagious, they require close contact between infected and susceptible animals for their transmission which normally occurs via infected air droplets. All secretions and excretions potentially harbor virus and, along with fomites, can also be a source of infection. In the case of seals, contact at haulout sites allows the proximity required for aerosol transmission.

Clinical Features of Infection

Morbillivirus infections begin in the upper respiratory tract and, after a variable incubation period spread from the local draining lymph nodes via the lymph and blood to other lymphatic tissues and then to the upper and lower respiratory tracts, gastrointestinal mucosa and in some cases the brain. With virulent strains, there is a marked leucopenia leading to a deficiency in the immune system. The incubation period ranges from 3 to 6 days in natural infections or following experimental parenteral inoculation. With less virulent strains of RPV, the incubation period can extend to 15 days and in many cases there may be no clinical signs but the animals seroconvert. The cell (epithelial and lymphoid) tropisms explain the pathological signs most associated with morbillivirus-induced disease: mucocutaneous lesions, severe infection in the gastrointestinal tract; and destruction of the lymphoid organs and consequent immunosuppression. In some cases, skin lesions can be associated with RPV infection but this is not a common finding. Hyperkeratosis of the cornea leading to blindness is a striking feature seen in kudu. Pneumonia is less marked in RPV than in PPRV and other morbillivirus infections.

In CDV infections, the incubation period can range from 1 to 6 weeks. Animals then show an initial febrile response and develop clinical signs commonly seen in other morbillivirus infections: mucopurulent nasal and ocular discharges, vomiting, diarrhoea, and pneumonia. Neurological signs such as convulsions, tremors, and seizures or behavioral changes are seen and they can develop acutely or weeks or months later or they may follow a subclinical infection. Recovered dogs frequently show persistent nervous tics or involuntary movements of one or more legs. In some cases, a hyperkeratosis (hard pad) develops on the foot pads.

Seals infected with either CDV or PDV also show the usual signs associated with a morbillivirus infection, fever, serous or mucopurulent oculonasal discharge, conjunctivitis, dyspnea, diarrhea, lethargy, and abortion in pregnant females. Bronchopneumonia is the most marked pathological feature in aquatic and marine mammal infections (CDV, PDV, and CeMV) which severely affects the animal's ability to dive and forage for food and quickly results in loss of body condition and a reduced blubber thickness. During the epizootic in the Mediterranean in 1990–91, many striped dolphins (*Stenella coeruleoalba*) were also found to be in poor body condition and loss of fat stores led to decreased buoyancy. Skin lesions, necrosis of the buccal mucosa, lymphodepletion, and hemoconcentration were commonly observed. Neurological signs similar to those found in CDV infections are a feature of both PDV and CeMV infections.

Pathology and Histopathology

The severity of gross pathological lesions observed in RPV and PPRV infections, like the clinical signs observed, is related to the virulence of the virus strain involved. Erosions and ulcerations are found in the upper respiratory, urinogenital, and digestive tracts. In the small intestine there is necrosis and destruction of Peyer's patches. Destruction of the epithelial lining of the gut is responsible for the severe bloody diarrhea seen in acute cases and the packed cell volume can be increased by 40–65%. In the cecum, colon, and rectum of animals infected with RPV and PPRV, so-called 'zebra' or 'tiger' stripes are often found and result from the distension of blood vessels

packed with erythrocytes. Dehydration also causes changes in hematology and blood chemistry. A nonsuppurative encephalitis with central nervous system (CNS) degeneration occurs during infections with CDV, PDV, and CeMV, but no CNS involvement has ever been reported in ruminants infected with either RPV or PPRV. Immunosuppression associated with morbillivirus infections can lead secondary bacterial infections which may complicate both the clinical and pathological findings and latent or concurrent infections can be activated.

Histologically, the morbilliviruses show a strong tropism for epithelial and lymphoid cells and all lymphoid organs are affected with damage to the mesenteric lymph nodes, the gut-associated lymphoid tissue, the lymphoid follicles of tonsils, lymph nodes, spleen, and mucosa-associated lymphoid tissues, where severe destruction of the B- and T-cell areas is seen in infections with virulent strains. Mild strains induce less extensive lymphoid destruction and mucosal lesions and in these animals tissue samples show unremarkable histopathological changes in the gastrointestinal tract and the lymphoid tissues and this may account for the reduced ability for these strains to transmit by contact.

Immune Response

There is a strong cell-mediated component in the response to morbillivirus infection and immunosuppressed individuals are known to be at extreme risk from MV. Neutralizing antibodies are generated only in response to the H and F glycoproteins and vaccines containing purified H or F proteins are effective only if administered with a strong cytotoxic T-cell-stimulating adjuvant, for example, Quil A (ISCOM vaccines). Inactivated whole virus vaccines give only a poor and short-lived protection. Both the H or F proteins can confer immunity to disease as poxvirus recombinant vaccines expressing either of these glycoproteins can confer fairly long-term (up to 3 years) immunity to clinical disease. In addition, RPV recombinants can also confer cross-immunity to PPR disease in the absence of cross-neutralizing antibodies. All morbillivirus infections appear to give lifelong protection against disease but it is not clear if subclinical reinfection can occur.

Prevention and Control

In 1711, during a prolonged epizootic of rinderpest in Italy, the Pope's physician, Giovani Lancisi, promulgated rules to deal with the disease in cattle. He insisted on movement controls on all animals in the areas affected, the slaughter of diseased and in-contact cattle, and their burial in lime. He also introduced the idea of quarantine and his policies were backed by strong legal enforcement with severe punishments for transgressors, principles which are still applied today to control animal diseases. This approach, along with import restrictions on cattle from the East, succeeded in controlling the disease in Europe and by the beginning of the twentieth century western Europe was free of enzootic rinderpest. However, these conditions are not always easy to impose in developing countries.

The existence of only one serotype for each of the morbilliviruses, the absence of persistence of infectious virus, and lifelong immunity after recovery from the initial acute infection suggest that outbreaks of these viruses should be easy to control. In addition, the morbilliviruses need close contact for infection to occur, are labile and do not survive long in the environment, and disinfection of infected premises is fairly straightforward. These characteristics of the virus, and the availability of a safe and effective vaccine, were the main drivers for the decision to try to control rinderpest following its resurgence in the early 1980s. International rinderpest control campaigns were begun in the late 1980s, and in 1992 the United Nations Food and Agriculture Organisation (FAO) recommended the global eradication of rinderpest as an internationally coordinated program. In 1994, this became the Global Rinderpest Eradication Programme (GREP), a time-bound program to eliminate rinderpest from the world by the year 2010.

All this effort has succeeded in eradicating the virus from Asia and has eliminated it from most of Africa. The last outbreak of rinderpest occurred in India in 1995 and in Pakistan in 2000. In Africa, the last confirmed case caused by African lineage 1 virus was in southern Sudan in 1998 and so this has probably been eliminated in the field, while the last confirmed case of African lineage 2 was in buffalo in Meru National Park in 2001. Meru is in northeastern Kenya, within the southern Somalia ecosystem, and the major obstacle to completing the GREP by the projected date of 2010 is the continuation of conflict in Somalia.

It was feared that even if rinderpest were eliminated from the domestic cattle populations wild ruminants might act as a reservoir of infection, but history shows that when the disease is eliminated from cattle it disappears from surrounding wildlife, as evidenced by its disappearance from Tanzania, South Africa, and southern Kenya, areas of the continent with many wildlife species. Nevertheless, during outbreaks, rinderpest-infected wildlife can help spread the disease over large distances.

In contrast, only limited resources have been directed to solving the problem of PPRV although a very effective live-attenuated vaccine is also available. From an economic and social perspective, PPR is now considered to be of great importance as it threatens small-ruminant production, the mainstay of many subsistence farmers in much of the developing world.

CDV vaccines have been very effective in controlling infections in domestic dogs; however, not much can be done to prevent infections in wild carnivores, seals, dolphins, and whales. Even if good vaccines were available, vaccination of wild animal populations is logistically very difficult. There are also ethical issues to take into account, such as the potential for uncontrolled spread of vaccine, which may not be attenuated for all species, and the disturbance caused to the animals which may also be harmful.

Diagnosis

Rapid and accurate diagnosis is the key to success in controlling morbillivirus outbreaks in domestic livestock. Clinical signs, however, are not always clear enough to make a confirmatory diagnosis, even when severe clinical signs are evident, as other viruses can mimic those commonly seen in morbillivirus infections. For example, rinderpest and bovine viral diarrhea viruses are often confused and PPRV can be mistaken for pasteurellosis or other microbial pneumonias. Confirmatory laboratory diagnosis is therefore essential.

Simple and rapid diagnostic tests such as capture enzyme-linked immunosorbent assay (ELISA) and reverse-transcriptase polymerase chain reaction (RT-PCR) have been developed for RPV and PPRV in recent years, and, since these are easy to use for analyzing large numbers of samples, these are now favored for virus detection. With RT-PCR, the DNA product can be sequenced and used for phylogenetic analysis that can be used to identify the strain of virus involved and the potential source of virus entering a new region.

For serological detection of morbillivirus antibodies, the ELISA format is favored and the success of the rinderpest eradication campaign depended to a large extent on the ability to seromonitor large herds following vaccination. The test is based on the use of a highly specific monoclonal antibody directed against the H protein of RPV in a competitive ELISA format. A companion test for the detection of PPRV antibodies is also available.

Vaccination

The first attenuated rinderpest vaccines were produced in the 1930s when the virus was adapted to replicate in goats (caprinized) and rabbits (lapinized). In the 1940s, a chick embryo-(avianized) adapted vaccine was produced. These vaccines were widely used but they had drawbacks in that they were not fully attenuated and could cause disease in more susceptible breeds. In the early1960s, a tissue culture-adapted strain of RPV, the 'Plowright vaccine', was developed by multiple passage of the virus in primary bovine kidney cells. The vaccine is relatively easy to produce, safe for use in all cattle breeds, and does not spread by contact. It is highly effective in preventing disease and the immunity induced in vaccinated cattle proved to be lifelong. This vaccine has been used successfully since the early 1960s for the control of rinderpest. A similar vaccine to control PPR was produced in the late 1980s by multiple passages of the virus on Vero cells and this is now being used to control the disease in parts of Africa and Asia.

Egg-adapted (Onderstepoort vaccine) and canine tissue culture-attenuated (Rockborn) vaccines for CDV were produced in the 1950s and are still widely used to vaccinate dogs against distemper. Immunity lasts for several years following vaccination of dogs with either vaccine. The distemper vaccines are not attenuated for all carnivores; for example, some species of ferret are extremely susceptible to CDV and develop disease on vaccination.

All morbillivirus vaccines, like their wild-type progenitors, are extremely fragile and heat-labile and so it is expensive and logistically difficult to store and use them in hot climates. The establishment of an effective cold chain for the delivery of vaccine was an essential feature of the success of the rinderpest eradication campaign as was follow-up seromonitoring studies to determine the level of herd immunity and the effectiveness of vaccination teams.

Future Prospects

Rinderpest is currently on the verge of global eradication and if successful it will be the first veterinary virus disease to have been eradicated globally and the second after smallpox. This goal must not be forgotten and the mild strain of the disease which may still persist in Somalia is of great concern. The worst case scenario is that such a clinically mild strain could move unnoticed into other regions of Africa where, since mass vaccination has ceased, there is a vast naive cattle and wildlife population which could become reinfected. This would provide an excellent opportunity for the virus to evolve to become more pathogenic for cattle. There is a high probability that this could happen as the virus most closely related to this strain was isolated from a giraffe in Kenya in 1962 and is highly pathogenic in cattle.

There is also a major gap in our understanding of the ecology of morbillivirus disease in wild animals, especially in marine and aquatic mammals, and this is an area of research which should be encouraged.

Many questions also remain concerning the host range determinants, biology, molecular biology, and pathogenesis of the morbilliviruses which hopefully can be addressed using reverse genetics.

See also: Border Disease Virus; Bovine Viral Diarrhea Virus; Epidemiology of Human and Animal Viral Diseases;

Measles Virus; Paramyxoviruses of Animals; Viral Pathogenesis.

Further Reading

Appel M (1987) Canine distemper virus. In: Apple MJ (ed.) *Virus Infections of Carnivores,* p. 133. Amsterdam: Elsevier.

Barrett T (2001) Morbilliviruses: Dangers old and new. In: Smith GL, McCauley JW, and Rowlands DJ (eds.) *Society for General Microbiology, Symposium No. 60: New Challenges to Health – Threat of Virus Infection*, 155pp. Cambridge: Cambridge University Press.

Barrett T, Pastoret P-P, and Taylor WP (eds.) (2005) *Biology of Animal Infections: Rinderpest and Peste des Petits Ruminants Virus,* 341pp. London: Elsevier.

Barrett T and Rima BK (2002) Molecular biology of morbillivirus diseases of marine mammals. In: Pfeiffer CJ (ed.) *Molecular and Cell Biology of Marine Mammals*, pp. 161–172. Melbourne, FL: Krieger Publishing Company.

Kock RA, Wambua JM, Mwanzia J, *et al.* (1999) Rinderpest epidemic in wild ruminants in Kenya 1993–97. *Veterinary Record* 145: 275–283.

Plowright W (1982) The effects of rinderpest and rinderpest control on wildlife in Africa. In: Edwards MA and McDonald U (eds.) *Symposia of the Zoological society of London, No. 50: Animal Diseases in Relation to Animal Conservation*, 1pp. London: Academic Press.

Rotaviruses

J Angel and M A Franco, Pontificia Universidad Javeriana, Bogota, Republic of Colombia
H B Greenberg, Stanford University School of Medicine and Veterans Affairs Palo Alto Health Care System, Palo Alto, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Antigenemia Presence of viral antigen in blood.
CFTR Cystic fibrosis transmembrane conductance regulator, a chloride channel localized in the apical membrane of epithelial cells implicated in secretory diarrhea.
Genotype Specific genetic makeup of one or more viral genes determined by sequence comparison.
Intussusception Pathological event in which the intestine acutely invaginates upon itself and becomes obstructed, followed by local necrosis of gut tissue.
Serotype Significant differences in the antigenic composition of the neutralizing antigens, VP4 and VP7 in the case of rotavirus.
Transcytosis Active transport by which polymeric IgA and IgM antibodies are transported from the basolateral to the lumen of the intestine by the polymeric Ig receptor.

Introduction

Using electron microscopy, rotaviruses were discovered as the etiological agents of epizootic diarrhea of infant mice (EDIM) in 1963 and of calf scours in 1969. Using this same technique, Ruth Bishop identified a rotavirus (RV) in intestinal biopsies of children with diarrhea in 1973. Since then, rotaviruses have been recognized as the most important cause of severe gastroenteritis in children worldwide and an important pathogen of the young of many animals.

Notwithstanding that the overall global mortality from childhood diarrhea decreased in the last 20 years, recent studies suggest that the proportion of hospitalizations attributable to RV-induced diarrhea may have increased during the same time period. For this reason, the burden of RV-related deaths was recently revised, and it is estimated that RV causes around 611 000 childhood deaths every year. More than 80% of these deaths occur in developing countries of sub-Saharan Africa, and South Asia. Worldwide, it is estimated that nearly every child under 5 years of age will have an episode of RV diarrhea, one in five will require medical attention, one in 65 will be hospitalized, and approximately one in 293 will die from RV disease. Although deaths due to RV are rare in developed countries, the incidence of viral infection is the same as in developing countries and health costs associated with RV disease are considerable. In the United States, for example, it has been estimated that 58 000–70 000 rotavirus-associated hospitalizations occur each year and cost-effectiveness studies clearly justify the use of RV vaccines in that country.

Rotaviruses are very well adapted to their host: they replicate very efficiently, sterilizing immunity is not developed and, despite an important host range restriction, many animal hosts exist (**Table 1**). These characteristics help to explain the high viral prevalence, and suggest that the prevention of severe disease is an appropriate goal for vaccination.

Morphology

Rotaviruses were given their name because, when examined by classical electron microscopy, they appear as wheel (*rota*)-shaped, 70 nm particles. However, by cryoelectron microscopy (a method that permits visualization of the viral spikes), the viral diameter is 100 nm (**Figure 1**). Using this method, the virus particle has been shown to be formed by three concentric layers of proteins: the core comprises viral structural protein 2 (VP2), the RNA-dependent RNA polymerase (VP1), guanyl tranferase (VP3), and the viral genome (**Figure 1**), the intermediate layer is formed by structural protein VP6, the most abundant and most antigenic viral protein, and the external layer comprises 780 copies of glycoprotein VP7 and 60 viral spikes formed by VP4. The surface of the virion has three types of pores that penetrate into the interior of the capsid. These channels seem to be important during viral replication, allowing exchange of compounds in aqueous solution to the inside of the capsid and the export of nascent RNA transcripts.

Several RV structural proteins and nonstructural proteins (NSPs) have been crystallized, permitting the initiation of detailed molecular studies of viral physiology. By this method, the viral spikes have been shown to consist of VP4 trimers, the structure of which rearranges upon trypsin cleavage (a process that enhances viral infection) and probably again on entry into the cell. These changes resemble the conformational transitions of membrane

Table 1 Virus-associated factors that contribute to high viral prevalence and reinfections

Characteristic	*Comments*
Natural infection does not generate sterilizing immunity	Goals of vaccination are to decrease severe disease but not to prevent infection
Multiple animal hosts exist	Eradication does not seem feasible
Short incubation period (1–2 days)	Does not allow time for the recall of high levels of immune effector mechanisms
The entry cell is the same as the cell used for viral replication	Does not allow time for the recall of high levels of immune effector mechanisms
Virus is excreted in high quantities (up to 10^{11} pfu g^{-1} of feces)	High levels of viral dissemination in the environment
Up to 30% of children excrete antigen up to 57 days after onset of diarrhea	High level of viral dissemination
High rate of viral mutation and gene reassortment	May permit the virus to evade the immune system. Currently unproven
Over 50% of infections are asymptomatic	RV well adapted to the human host

Reprinted from Franco MA, Angel J, and Greenberg HB (2006) Immunity and correlates of protection for rotavirus vaccines. *Vaccine*, 24: 2718–2731, with permission from Elsevier.

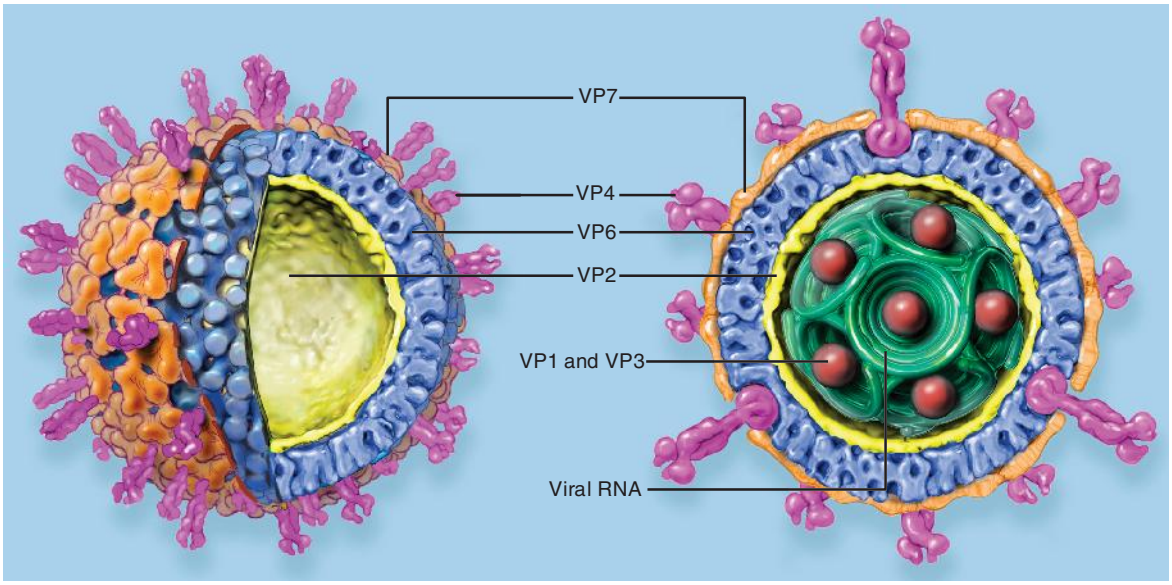


Figure 1 An artist's reconstruction based on cryoelectron microscopy studies of an RV particle. Shown are the seven structural proteins, and the viral RNA. Reproduced with permission from Andrew Swift, Swift Illustration.

fusion proteins of enveloped viruses. The crystal structure of the viral hemaglutinin VP8 (VP4 is cleaved by trypsin into VP8 and VP5) that contains several virus-neutralizing epitopes has also been determined. Details of the characteristics and function of the viral proteins are described elsewhere in this encyclopedia.

Classification and Epidemiology

Rotaviruses are classified in the genus *Rotavirus* of the family *Reoviridae* that comprises icosahedral, nonenveloped viruses with segmented, double-stranded RNA (dsRNA) genomes. Based primarily on epitopes in VP6, rotaviruses are classified serologically into seven groups (A–G). These serologically distinct groups are also very distinct genetically and genome segment reassortment does not occur between serogroups. According to current taxonomic classification, serogroups A–E each correspond to a different RV species. Serogroups F and G are currently regarded as tentative species. Most human pathogens fall into groups A, B, and C. The information presented in this chapter is mostly limited to group A rotaviruses which are, by far, the most common cause of severe diarrhea in humans. Among group A rotaviruses, antigenic differences in VP6 have also been identified and used to establish viral subgroups, primarily for epidemiological studies. Serotypes within each serogroup are defined by epitopes in VP7 (glycoprotein, G types) and VP4 (protease, P types) that induce neutralizing antibodies. As the genes encoding these two proteins segregate separately during genome segment reassortment, a binary serotyping system has been developed to identify isolates.

Genotypes, determined by nucleic acid sequence similarity of genes encoding VP7 and VP4, and serotypes, determined by antigenic similarity in VP4 or VP7, as tested by neutralization assays, are generally equivalent for VP7. Currently, 15 G types have been described. G1, G2, G3, G4, and G9 constitute more than 92% of all G serotypes of humans detected globally and appear to be equally virulent. For VP4, there is no direct relationship between genotypes and serotypes and, therefore, a dual system P classification is in use (P genotype numbers are denoted in brackets and P serotypes without brackets). At least 23 P genotypes P[1]–P[23] and 14 serotypes have been described. More than 91% of circulating human RV strains express the P[8] and P[4] genotypes. These genotypes correspond to two subtypes of P1 serotype (P1A and P1B, respectively) that share some cross-reactive epitopes.

Overall, strain variability is less than would be expected from a random association of G and P genotypes since human RV strains belonging to G1, G3, and G4 serotypes are preferentially associated with P[8], while G2 serotype strains are most frequently associated with P[4] genotypes. Importantly, cross-hybridization studies using labeled viral RNA have shown that, in general, viruses that express P[8] form a different genogroup than viruses that express P[4].

In addition to these more prevalent strains, rotaviruses with unusual serotypes currently circulate and can arise sporadically in developed and particularly in developing countries. Several serotypes may coexist within a community, but, in temperate climates especially, each season is usually dominated by a single serotype that may change from season to season. The global distribution of rotaviruses varies over continents: G1P[8] represents over 70% of RV infections in North America, Europe, and Australia, but only about 30% of the infections in South America and Asia, and 23% in Africa. These differences in geographic distribution can probably be explained by variations in sanitary and climatic conditions and/or closer contact of individuals with animal rotaviruses in areas with more RV diversity.

Animal and human group A rotaviruses can undergo genome segment reassortment *in vitro* and *in vivo*. Reassortment originates in cells simultaneously infected with two different rotaviruses and results in progeny viruses that have a combination of genes from each parental strain. In several cases, human rotaviruses have been found that have genes of animal RV origin, adding a further dimension to strain diversity. However, rotaviruses have an important host range restriction in that, in general, humans are infected only with human rotaviruses. This restriction has been exploited for the development of several viral vaccines.

In the temperate zones of the world (mostly developed countries), RV infection occurs primarily during epidemic peaks in the cooler months of the year. A yearly wave of rotaviral illness spreads over the United States; it begins in the southwest in November and terminates in the northeast in March. A similar phenomenon has recently been reported to occur in Europe. This pattern is not seen in countries within 10° of latitude from the Equator (mostly developing countries) where epidemics occur year-round. No clear explanation is available for these temporal patterns of viral prevalence.

Rotaviruses are highly contagious and spread easily but, unlike certain human bacterial infections that occur disproportionately in developing countries, the prevalence of RV infection is the same in developed and developing countries, implying that sanitation and hygiene are not effective measures for disease control.

The Viral Genome and Viral Replication

The RV genome comprises 11 dsRNA segments that code for six structural proteins and five or six nonstructural proteins. The size of segments varies from 0.6 to 3.3 kbp. Each segment contains a single long open reading frame

(ORF), with the exception of segments 9 and 11, each of which may contain two ORFs. The 5′ and 3′ ends of the RNA segments have noncoding regions that differ between rotaviruses from groups A, B, and C. These sequences are important in transcription, replication, and reassortment of the virus genome.

The viral RNA itself is not infectious. For this reason, the engineering of recombinant rotaviruses has been very difficult, and this has impeded functional analysis of the viral RNA noncoding sequences and of the viral proteins. This problem has been partially solved by the development of a cell-free system that supports the synthesis of dsRNA from exogenous mRNA, and by gene silencing using small interfering RNA (siRNA) to specifically inhibit translation of viral proteins. In addition, a reverse genetics system for introduction of site-specific mutations into the dsRNA genome of infectious RV has been recently developed (see *recommend readings*). Evaluation of the utility of this method is eagerly awaited by scientists in the field.

Rotaviruses are highly variable. Three mechanisms for generating genetic diversity have been identified: point mutation, genome segment reassortment, and recombination. Rotaviruses have high rate of mutation and it has been estimated that, on average, at least one mutation occurs during each genome replication. Reassortment of genome segments also occurs at high frequency during mixed infections with two or more rotaviruses, both *in vitro* and *in vivo*. Natural reassortment *in vivo* appears to influence the serotypic diversity in humans, especially in less developed countries (see below). Recombination and related rearrangements of viral RNA segments (e.g., partial gene duplication or deletions) probably play a minor role in generating viral diversity but may be important in longer-term viral evolution.

RV entry into host cells is a multistep process and several molecules have been identified as RV receptors or co-receptors. However , the process has been shown to vary in different viral strains. For example, for several animal RV strains, the first binding step involves the interaction of VP8 with sialic acid, while some human RV strains appear to bind initially to GM1 ganglioside. As a second step, RV binds to the integrin $\alpha 2\beta 1$ in an interaction mediated by the integrin-binding motif DGE in VP5. In addition to these two interactions, integrins $\alpha v\beta 3$ and $\alpha x\beta 2$, and the heat shock protein hsp70, have also been shown to be involved at a later step of rotavirus cell entry. It seems that the association of some of these molecules with rafts is important for viral entry.

Although it was initially proposed that rotaviruses enter by direct penetration, current models favor the hypothesis that virus entry is by endocytosis. Inside the cell, VP5 seems to induce a size-selective membrane permeabilization of the putative viral endosome that facilitates the transit of Ca^{2+} from the vesicle. When Ca^{2+} concentrations in the endosome are lowered, a disassembly of VP4 and VP7 is postulated to occur. This event permeabilizes the membrane of the endosome and the viral transciptase (viral particles without VP4 and VP7) gains access to the cytoplasm and begins to synthesize viral mRNAs. The mRNAs produced by the transcriptase are exact copies of each genome segment, with a 5′-terminal type 1 methylated cap structure and without a 3′-terminal poly(A) sequences.

The viral mRNAs are translated, giving rise to the structural and nonstructural proteins necessary to complete the viral replication cycle. NSP3 is reported to shut off the synthesis of cellular proteins and induces the preferential translation of viral proteins. These proteins accumulate in the cytoplasm in an electron dense region called the viroplasm, where the viral genome is replicated and the assembly of progeny double-layered particles takes place. The mechanism by which one viral particle assembles with each and only one of the 11 RNA segments is unknown. Synthesis of the dsRNAs occurs following the packaging of viral mRNAs into intermediate precursors of double-layered particles.

Assembled double-layered particles interact with NSP4, that has been synthesized by ribosomes associated with the endoplasmic reticulum (ER), and bud into the ER lumen. In this organelle, the double-layered particles acquire a transient lipid membrane. Then, in a very poorly understood mechanism (probably related to the high Ca^{2+} levels of the ER), the viral particles acquire VP7 and lose the transient enveloping membrane, giving rise to the triple-layered particle. Recent experiments suggest that VP4 is acquired by the viral particle in a compartment outside the ER.

The physiological mechanism of exit of the mature triple layer viral particle from the cell is unknown. In polarized Caco-2 cells (intestinal epithelial cells derived from a human colon adenocarcinoma), RV is released before cell death using a vesicle-associated vectorial transport system to the apical pole. However, rotaviruses are lytic viruses, and could also exit the cell after cell lysis. The mechanism of cell death induced by RV is not completely understood. Results in polarized Caco-2 cells and *in vivo* studies in mice suggest that it is by viral induced cell apoptosis.

Pathogenesis

Important viral antigenemia and some level of viremia are observed in the initial phase of RV-induced diarrhea in children and animals. In mice, extraintestinal viral replication occurs commonly during homologous and some

heterologous infections. Also in this model, the level and location of extraintestinal replication varies between RV strains and replication can occur in several leukocytes subsets. However, the clinical relevance of these findings is still unclear and, in children and in animals, the bulk of RV replication most likely occurs in the mature villus tip cells of the small bowel.

RV-induced diarrhea probably occurs by multiple mechanisms that vary, depending on the animal species analyzed (**Table 2**). Pathological findings in the small intestine of children with RV diarrhea include: shortening and atrophy of the intestinal villi, enterocyte vacuolization with distended cisternae of the endoplasmic reticulum, and mononuclear infiltration in the intestinal lamina propia. However, in children a direct relationship between the extent of histopathology and disease has not been demonstrated. This finding suggests that RV diarrhea can occur without important enterocyte death.

In pigs, RV infection induces an intestinal lactase deficiency and increased lactose in feces induces an osmotic diarrhea. Elevated levels of lactose are also observed in the feces of some RV infected children. Lactase deficiency could be due to RV-induced enterocyte destruction or to alteration in the synthesis or metabolism of disaccharidases. In mice, two mechanisms of diarrhea, which do not involve enterocyte destruction, have been identified. NSP4, and a derived peptide of NSP4, induce diarrhea in mouse pups but not adult animals, making it the first viral enterotoxin described. However, its role in RV-induced diarrhea in other species has not been characterized. Also in mice, RV has been shown to activate the intestinal autonomous neural system and increase the secretion of water and electrolytes, as well as the intestinal motility. Racecadotril, an inhibitor of the enteric nervous system, is somewhat efficacious in treating RV-induced diarrhea in children, suggesting that this mechanism plays at least some role in human diarrhea.

It is possible that the three mechanisms (malabsorption, NSP4 enterotoxicity, and enteric neural system stimulation) may play a major or minor role in the pathophysiology of RV-induced diarrhea, depending on the species and the time point after the onset of disease.

Diagnosis, Clinical Characteristics, and Treatment

Initial efforts to isolate wild-type rotaviruses in tissue culture were not very successful. For this reason, early clinical studies characterized rotaviruses by isolating viral RNA from feces and then analyzing the RNA by electrophoresis in polyacrylamide gels. Using this method, rotaviruses were classified depending on the pattern of migration of their RNA segments (electropherotypes). At present, this method seems of limited value because a relationship between electropherotypes and virulence or serotypes is not generally apparent. Currently, diagnosis is commonly conducted using commercial ELISAs that detect viral antigen (mostly VP6) in the feces. Rotavirus is shed in very large amounts making the ELISA a highly effective and accurate diagnostic assay. For epidemiological studies, human RV strains present in feces can be grown in cell culture in MA104 (African green monkey kidney) and Caco-2 cells using trypsin for enhancement of viral growth during the culture. Trypsin cleaves VP4 and increases infectivity in culture. Genotype characterization of RV strains is mainly by reverse transcription-polymerase chain reaction (RT-PCR). RV detection by electron microscopy is only conducted in research laboratories, especially when other viral pathogens are being investigated.

Rotaviruses are highly contagious since approximately 10^{11} particles per gram of feces are excreted, and they are very resistant to ambient conditions. In addition, viral excretion in most children lasts for up to 10 days and, in some children, may extend for 2 months after onset of infection. Rotaviruses are mainly transmitted by an oral–fecal route although, in some cases, a respiratory route has been suggested.

In developing countries, the peak incidence of RV disease occurs in children between 6 and 11 months of

Table 2 Mechanisms of RV-induced diarrhea (may vary according to the animal species studied)

Mechanism	*Comments*
Action of NSP4 as a toxin induces a secretory diarrhea	Only demonstrated in rodents. Non-CFTR mediated. Occurs early in infection prior to cell death
RV stimulates the enteric nervous system (ENS) inducing a secretory diarrhea and increased intestinal motility	Drugs that inhibit the ENS are useful to treat RV diarrhea. Occurs early in infection prior to cell death
Altered metabolism of disaccharidases and other enterocyte membrane proteins induces malabsorptive/osmotic diarrhea	Occurs early in infection prior to cell death
Enterocyte death contributes to malabsorptive/osmotic diarrhea	Late mechanism. In polarized intestinal epithelial cell lines and *in vivo* in murine enterocytes, RV infected cells seem to die by apoptosis

Reprinted from Franco MA, Angel J, and Greenberg HB (2006) Immunity an correlates of protection for rotavirus vaccines. *Vaccine*, 24: 2718–2731, with permission from Elsevier.

age. In contrast, in developed countries, the highest incidence is observed in older children (2 years old). This difference is probably related to differences in sanitation in the different settings. Notwithstanding this variation, RV incidence is similar in both developing and developed countries but mortality is mainly observed in developing countries, presumably due to limited access to appropriate health care. The relative protection of infants younger than 2 months of age, which occurs worldwide, could be related to the presence of protective maternal antibodies. Up to 50% of adults caring for children with RV diarrhea can become infected and, of these, 50% develop disease which is generally mild.

The primary clinical syndrome caused by RV infection is acute gastroenteritis. After a short incubation period of 48 h or less, children frequently present with vomiting that lasts for 1–2 days. The vomiting is often accompanied with fever (37.9 °C or greater). Subsequently, or at the same time, a watery diarrhea appears and, if it is not treated, frequently induces dehydration. It is estimated that up to 50% of RV infections in children are asymptomatic but some of these may represent second or third exposures.

Children attending day care institutions are at high risk of developing RV-induced diarrhea. Although RV infections in neonatal care units have been classically described as asymptomatic, probably due to the presence of maternal antibodies, severe symptomatic outbreaks have also been described. RV strains that induce nosocomial infections in neonatal nurseries are generally different from those circulating in the community. RV infection can cause severe and prolonged disease in children with primary immunodeficiencies, some of whom develop a systemically disseminated infection. Acquired immunodeficiency also predisposes to severe RV disease in bone marrow- and liver-transplanted children. The role of RV-induced disease in immunosuppressed adults with HIV seems, at present, less important.

RV diarrhea is self-limited and treatment is aimed at reducing symptoms until the immune response resolves the infection. Children with mild diarrhea are treated by oral rehydration. Those presenting moderate to severe dehydration may require intravenous rehydration. In cases of severe disease, treatment with probiotics, preparations that contain antibodies against RV, Racecadotril (an inhibitor of the enteric nervous system), and Nitasoxanide (a drug of unknown mechanism of action) have been shown to accelerate resolution of the disease. However, it is not yet clear whether these interventions truly provide significant advantages to ill children.

Immune Response

Immunity to RV is incompletely understood and animal models have been important in the acquisition of our currently available knowledge. Pepsin and gastric acid seem to be important host defense factors against RV infection, since these factors inactivate rotaviruses in adult mice but not in suckling mice. In addition, the innate immune response and interferons, in particular, seem to mediate an antiviral effect, and viral mechanisms for evading this response involving NSP1 have recently been suggested. However, mice lacking T and B cells become chronically infected with RV, suggesting that the adaptive immune system is essential for viral elimination. Also, children lacking T and B cells, or only B cells have been shown to shed virus chronically.

Antibodies seem to be the principal mechanism that mediates protection against viral reinfection. In agreement with the fact that most viral replication occurs in the intestine, the localization of virus specific B cells in the intestine seems important for their capacity to mediate protection. It is postulated that local IgA antibodies can mediate expulsion of rotaviruses inside the enterocytes and exclusion (to avoid *de novo* infection of enterocytes) of rotaviruses in the gut lumen. Neutralizing antibodies to VP4 and/or VP7 can block enterocyte infection directly when present in the gut lumen (exclusion). In mice, anti-VP6 non-neutralizing polymeric IgA may bind virus VP6 during transcytosis from the basolateral membrane of enterocytes to the gut lumen and 'expulse it'. These antibodies may also inhibit RV transcription intracellularly. In addition, antibodies to NSP4 may block diarrhea, but not infection, by blocking the enterotoxic property of the molecule. However, the antiviral effects of antibodies against VP6 and NSP4 have only been shown to be protective in mice. In piglets, a model that is probably closer to humans than mice, the presence of antibodies against VP4 and/or VP7 seems necessary for protection.

Although local antibodies appear to be the principal mechanism that protects against viral infection, T cells also directly mediate antiviral immunity, at least in mice. CD4+ T cells are also essential for the development of more than 90% of the RV-specific intestinal IgA, and thus their presence seems critical for the establishment of protective long-term memory responses. Moreover, murine RV-specific CD8+ T cells are involved in the timely resolution of primary RV infection and can mediate short-term partial protection against reinfection.

In children, primary RV infections are generally the most severe, with severity decreasing as the number of reinfections increases. Complete protection against moderate-to-severe illness is achieved after two natural RV infections, whether symptomatic or asymptomatic. In agreement with the results of animal models mentioned previously, total serum RV IgA, induced by a primary infection, seems to be the best but imperfect correlate of protection against subsequent reinfections. Primary RV

infection induces homotypic neutralizing antibodies to VP7 and VP4 and some level of heterotypic immunity, but the role in protection of these antibodies is still controversial. In this respect, it is noteworthy that an attenuated G1P[8] human RV vaccine has been shown to induce excellent protection against severe diarrhea caused by P[8] rotaviruses, independently of their G type. This vaccine can also induce protection against G2P[4] rotaviruses, but this protection is somewhat lower than against the P[8] viruses. Altogether, these findings suggest a role for neutralizing antibodies directed against VP4 in protection. However, since, as mentioned previously, P[4] viruses belong to a separate genogroup from most P[8] viruses, differences in immunity to other nonneutralizing viral proteins could also be the explanation for the relatively lower protection rate induced by the vaccine against P[4] viruses.

The lack of understanding of RV immunity limits our capacity to develop new RV vaccines. Recent studies have tried to characterize RV-specific lymphocytes in humans to better understand human immunity. Due to the relative inaccessibility for study of the human intestinal immune tissue, cells involved in the RV immune response have been studied in peripheral blood. It is hypothesized that at least a fraction of these lymphocytes are recirculating to and from the intestine, and thus may reflect intestinal immunity. In blood of children with acute RV infection, plasmablasts and plasma cells (secreting mainly RV specific IgM) are found, and these express homing receptors, that allow them to return to the intestine. In the convalescence phase, RV-specific B cells are mainly memory cells, some of which express intestinal homing receptors. In addition, RV-specific CD4+ and CD8+ T cells have been characterized. In children and adults with acute infection, both types of T cells that secrete gamma interferon circulate, but in unexpectedly low frequencies. It is speculated that the limited induction of RV-specific T cells can be related to the occurrence of delayed viral clearance and symptomatic reinfections in a subset of individuals.

Viral Vaccines

The first commercially available RV vaccine was a simian-human reassortant tetravalent vaccine (RotaShield). Although this vaccine was highly effective, it was withdrawn from the market 1 year after its introduction due to its temporal association with low levels of intussusception (1 in 10 000 children). Two new RV vaccines have been recently licensed for use in many countries worldwide. These vaccines appear to be safe and not cause intussusception. The vaccine produced by Merck (RotaTeq^{TM}) contains mono-reassortants of a bovine virus with G1, G2, G3, G4, and P1A[8] human RV genes. The vaccine produced by GlaxoSmithKline (GSK, Rotarix) is a human-attenuated G1P1A[8] virus. In trials that involved over 60 000 infants, both of these vaccines have been shown to be safe and to provide over 70% protection against any RV diarrhea and over 98% protection against severe RV infection. Importantly, both vaccines reduced the rates of all gastroenteritis-related hospitalizations of any cause by over 40%. Despite these encouraging results, it is still undetermined if these vaccines will work in very poor developing countries in Africa and Asia in which children can be malnourished and in which atypical RV strains may circulate frequently. Moreover, the intussusception associated with RotaShield was predominantly seen in children older than 2 months, and the children studied in the safety evaluation of the two new vaccines were mostly 2 months of age. Thus, the safety of the new RV vaccines in children of more than 2 months of age has not been fully established and the development of second-generation vaccines may be desirable. A promising strategy to drastically reduce the risk of intussusception is to administer the vaccine to neonates, in whom intussusception almost never occurs. Current studies in animal models are focused on the development of second generation nonreplicating RV vaccines, such as DNA vaccines, and recombinant RV proteins or virus-like particles.

Further Reading

Franco MA, Angel J, and Greenberg HB (2006) Immunity and correlates of protection for rotavirus vaccines. *Vaccine* 24(5): 2718–2731.

Franco MA and Greenberg HB (2002) Rotaviruses. In: Richman DD, Hayden FG, and Whitley RJ (eds.) *Clinical Virology,* 2nd edn., pp. 743–762. Washington, DC: ASM Press.

Komoto S, Sasaki J, and Taniguchi K (2006) Reverse genetics system for introduction of site-specific mutations into the double-stranded RNA genome of infectious rotavirus. *Proceedings of the National Academy of Sciences, USA* 103(12): 4646–4651.

Rothman KJ, Young-Xu Y, and Arellano F (2006) Age dependence of the relation between reassortant rotavirus vaccine (RotaShield) and intussusception. *Journal of Infectious Diseases* 193(6): 898.

Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, *et al.* (2006) Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *New England Journal of Medicine* 354(1): 11–22.

Santos N and Hoshino Y (2005) Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Reviews in Medical Virology* 15: 29–56.

Vesikari T, Matson DO, Dennehy P, *et al.* (2006) Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *New England Journal of Medicine* 354(1): 23–33.

Rubella Virus

T K Frey, Georgia State University, Atlanta, GA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Birth defects Malformations or abnormalities developed during gestation that are apparent at, or soon after, delivery.
Congenital infection Infection of the fetus during gestation as a result of virus passage through the placenta.
Elimination program Vaccination program designed to eliminate 'indigenous' sources of a pathogen from a geographic region and thus restrict sources of the pathogen to imports from other regions.
Enveloped virion Virus particle with a lipid bilayer membrane, or envelope, surrounding the capsid or core. The envelope is usually derived from host cell membranes and contains virus-specified glycoproteins decorating its outer surface.
Icosahedral capsid In the virus particle, the proteinaceous shell surrounding the virus genome – in this case, with symmetry of an icosahedron (appears quasi-spherical or round in electron micrographs).
Live, attenuated vaccine A vaccine formulation using an infectious variant of the virus that has been attenuated or weakened by repeated passage in cell culture or alternate hosts. Such vaccines recapitulate the infection, including induction of a complete adaptive immune response, but do not cause disease.
Persistent infection Infection characterized by continued presence of the virus, often despite the induction of an adaptive immune response.
Plus-strand RNA virus Virus with a single-stranded RNA genome that is of messenger RNA sense and can be directly translated to produce virus-specified proteins.
Serodiagnosis Detection or diagnosis of infection through immune status, usually the presence of IgM antibodies or a rise in IgG antibodies in paired sera collected at the time of symptoms and convalescence (post-recovery).
Systemic infection Infection that spreads from local site entry via the lymphatic system and blood stream to one or more target internal organs or tissues.

Introduction

Rubella virus (RUBV) infection causes a benign disease known as rubella or German measles that can result in profound birth defects if contracted *in utero*. Norman Gregg, an Australian ophthalmologist, first reported the association of congenital cataracts as a consequence of gestational rubella in 1941, establishing RUBV as a major teratogen. The last major epidemic of rubella to impact the United States occurred in 1964–65, resulting in 20 000 congenital rubella syndrome (CRS) cases, and subsequently live, attenuated vaccines were developed and applied in the US, Canada, Japan, and several European countries by 1969. These programs have been successful in greatly reducing the incidence of both rubella and CRS in these countries, particularly the US, in which indigenous rubella was declared eliminated in 2004. Currently, ~50% of countries have national rubella vaccination programs, but the majority of the world's population is not covered and rubella thus remains a worldwide challenge.

Classification

RUBV is a member of the family *Togaviridae* and the only member of the genus *Rubivirus*. The other togavirus genus, *Alphavirus*, contains 26 members, all of which are arthropod-borne. In common with the alphaviruses, the rubella virion consists of a single-stranded, plus-sense genomic RNA contained within a quasi-spherical core or capsid, composed of a single virus-specified protein, C, which is surrounded by a lipid envelope in which are embedded two virus-specified glycoproteins, E1 and E2. Unlike alphaviruses, RUBV has no invertebrate vector and the only known natural reservoir is humans. RUBV also is antigenically distinct from the alphaviruses. Phylogenetic analysis of E1 gene nucleotide sequence of worldwide RUBV isolates has led the World Health Organization to propose a standardized taxonomy for RUBVs which consists of two clades, 1 and 2, and genotypes within each clade. Clade 1 contains seven genotypes and is distributed worldwide. Clade 2 contains three clades and appears to be restricted to Asia.

Host Range and Virus Propagation

RUBV has no known natural host other than humans. No reliable animal model exists for the study of RUBV

pathogenesis. RUBV replicates in a number of laboratory cell culture lines; however, in most of these no cytopathic effects (CPEs) are routinely observed. Continuous cell lines commonly used to propagate RUBV include Vero (African green monkey kidney), RK-13 (rabbit kidney), and BHK-21 (baby hamster kidney). In all cell lines in which RUBV replicates, persistent infections are readily established and maintained, whether or not the cell line exhibits a functional interferon system, and persistent RUBV infection in cell culture cannot be cured by the inclusion of neutralizing antibodies in the culture medium because virus budding occurs at intracytoplasmic locations. Thus, RUBV is highly adapted for persisting infection.

Properties of the Virion

Rubella virions are 60–70 nm spherical particles composed of an electron-dense core separated from the lipid envelope by an electron-lucent zone. Rubella virions exhibit a marked degree of pleomorphism (**Figure 1**). The virion has a density of 1.18–1.19 g ml^{-1}, whereas isolated capsids have a density of 1.44 g ml^{-1}. The C protein (~34 kDa), which comprises the capsid, is present as disulfide-linked homodimers. Although presumed to be icosahedral, the symmetry of the RUBV capsid has not been solved.

The virion spikes are formed by two virion glycoproteins, E1 and E2. E1 has a molecular weight of 59 kDa whereas E2 is a heterogeneous species ranging from 44 to 50 kDa due to differential glycosylation. Both E1 and E2 appear to be primarily in the form of heterodimers which are easily disrupted by routine preparation techniques. The higher-order architecture of the virion spikes is entirely unclear. E1 is more exposed on the virion surface than is E2, contains both the viral hemagglutinin and receptor site, and is also immunodominant in terms of the humoral response.

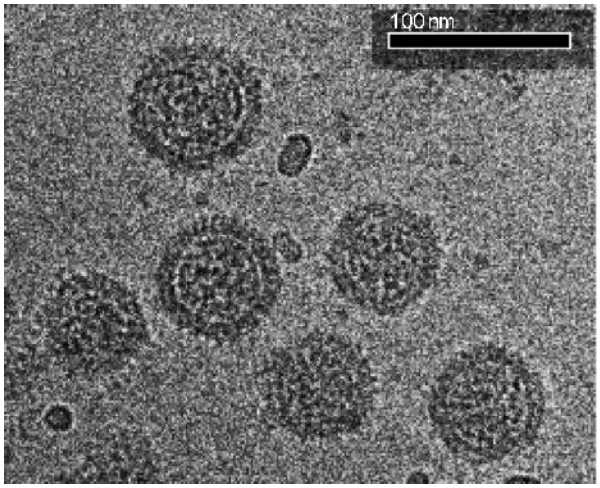


Figure 1 Cryoelectron micrograph of rubella virions. Unlike conventional negative staining, the virions have a uniform structure when visualized by this technique; however, pleomorphism of particle diameter and the gap between the core and envelope remains apparent. Courtesy of Tao Sun, Yumei Zhou, Michael Rossmann, and Teryl Frey (unpublished data).

Rubella virions are stable at physiological pH values and can be frozen at temperatures below –20 °C for years, without loss of infectivity. Live, attenuated vaccine virus is stored in lyophilized form. Rubella virions are susceptible to most commonly used inactivating agents, such as formaldehyde, UV light, and lipid solvents.

Genomic Organization

The RUBV genomic RNA is 9762 nucleotides in length and contains a 5′ terminal cap structure and a 3′ terminal poly(A) tract. A distinctive feature of the genomic RNA is that it contains 30% guanine residues and 39% cytosine residues, the highest G+C content of all RNA viruses. The genome contains two long, nonoverlapping open-reading frames (ORFs) plus untranslated regions (UTRs) at the 5′ and 3′ ends and between the ORF's (**Figure 2**). The 5′ proximal ORF, or nonstructural proteins ORF (NS-ORF), encodes a 2116-amino-acid product that is proteolytically cleaved into two products, 150 kDa (P150) and 90 kDa (P90), which are at the N- and C-termini of the ORF, respectively. The cleavage is mediated by a papain-like cysteine protease located at the C-terminus of P150. P150 and P90 are responsible for virus RNA replication. By computer-assisted comparisons with other viruses, P150 contains a domain predicted to have methyl/guanylyltransferase activity (responsible for forming the cap structure at the 5′ end of the genomic and subgenomic RNAs) in addition to the protease, whereas P90 contains both a helicase domain and an RNA-dependent RNA polymerase (or replicase) domain. The 3′ proximal ORF, or structural protein ORF (S-ORF), encodes a 1063-amino-acid product that is proteolytically processed into the virion proteins by a cell protease, signal endopeptidase. The order of the virion protein genes within the ORF is 5′ C-E2-E1 3′. The S-ORF is translated from a subgenomic RNA synthesized in infected cells and containing the sequences from the start site through the 3′ end of the genome. An infectious clone for RUBV has been developed. An infectious clone is a cDNA copy of the viral RNA contained in a plasmid in which it is placed adjacent to an RNA polymerase promoter. Since RUBV has a plus-sense genome, *in vitro* transcripts from the plasmid will initiate virus replication following transfection into susceptible cells. The infectious clone allows for studies of the effects of site-directed mutagenesis on the RUBV genome.

Intracellular Replication Cycle

The receptor for RUBV on the surface of susceptible cells has not been identified. Following attachment to the receptor, the virus is taken into the cell by receptor-mediated endocytosis. In the reduced pH environment of the endocytic vesicle, fusion between the viral envelope and the vesicular membrane occurs, releasing the capsid and genomic RNA into the cytoplasm of the cell.

The genomic RNA is translated to produce the NS protein precursor which is cleaved into P150 and P90 (**Figure 3**). These proteins then use the genomic RNA

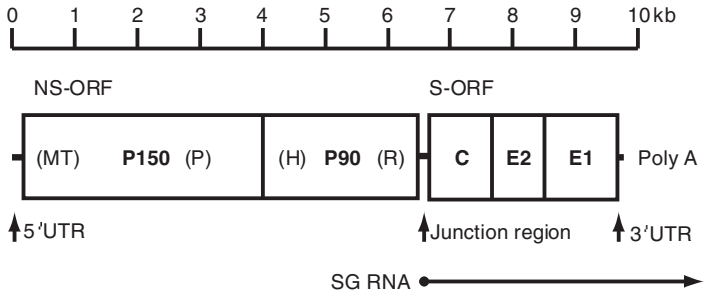


Figure 2 Coding strategy of the RUBV genome. Shown is a schematic representation of the RUBV genomic RNA with untranslated regions (UTRs) drawn as solid black lines and coding regions (ORFs) as open boxes (NS-ORF, nonstructural protein ORF; S-ORF, structural protein ORF). Within each ORF, the coding sequences for the proteins processed from the translation product of the ORF are delineated and, in addition, within the NS-ORF, the locations of motifs associated with the following activities are indicated: methyl/guanylyltransferase (MT), protease (P), helicase (H), and RNA-dependent RNA polymerase (R). The sequences encompassed by the subgenomic RNA (SG RNA) are also shown. The scale at the top of the diagram is in kilobases.

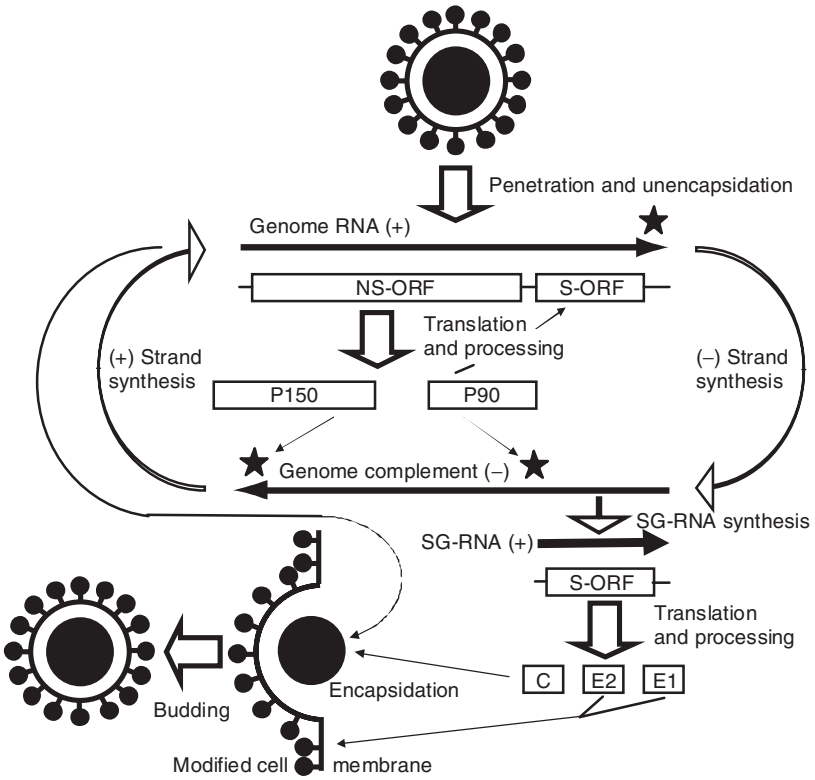


Figure 3 Replication strategy of RUBV. The plus-sense genome and subgenomic RNA are represented by solid black arrows indicating plus polarity; beneath each, the ORFs that they contain are shown as open boxes. The minus-sense genome RNA complement, represented as a dotted arrow, is used solely as a template for the two plus-sense RNA species. Putative *cis*-acting sequences on each RNA, which are recognized by the virus RNA-dependent RNA polymerase to initiate synthesis of complementary RNAs, are marked with stars. The general functions of the virus proteins are indicated by arrows (e.g., P150 and P90 functioning as the RNA-dependent RNA polymerase by interacting with *cis*-acting sequences on the viral RNA species and synthesizing complementary strands).

as a template for synthesis of a genome-length, minus-sense RNA. The genome-length, minus-sense RNA is then used as the template for synthesis of both the genomic RNA and the subgenomic RNA. Synthesis of the subgenomic RNA is initiated by internal recognition of sequences on the genome-length, minus-sense RNA template. Host-cell proteins are likely involved in the replication process and, interestingly, recent evidence indicates that the C protein is as well. RNA synthesis is asymmetric in infected cells in that more of the plus-sense species than the minus-sense RNA is produced. The uncleaved NS protein precursor is active in minus-strand RNA synthesis, while the cleaved P150/P90 complex appears to be active in only plus-strand RNA synthesis. Thus the activity of the NS protease through mediating this cleavage is important in regulating plus- and minus-sense RNA synthesis. RUBV RNA synthesis occurs in cytopathic vacuoles of lysosomal origin, in infected cells.

In the structural protein ORF, E2 and E1 are immediately preceded by hydrophobic signal sequences that function to direct translation of secreted and membrane-associated proteins into the lumen of the endoplasmic reticulum (ER). Therefore, following translation of the C sequences within the ORF, the E2 signal sequence mediates association of the translation complex with the ER. C–E2 cleavage is mediated by signal endopeptidase, or signalase, which functions in the lumen of the ER to remove signal sequences from secreted and membrane-associated proteins; unlike the proteins of the alphaviruses, RUBV C protein does not have autocatalytic protease activity. Following cleavage, the E2 signal sequence remains associated with C. Similarly, the E1 signal sequence maintains the association of the translational complex with the ER, signalase mediates the E2–E1 cleavage, and the E1 signal sequence remains attached to E2. Soon after synthesis, heterodimerization of E2 and E1 occurs in the lumen of the ER. The three-dimesional folding of E1 appears to be a complicated process requiring intramolecular disulfide-bond formation by all 20-Cys residues in the ectodomain of the protein. Both E1 and E2 acquire high-mannose glycans in the ER; E1 contains three potential glycosylation sites and E2 contains four and all appear to be utilized. The sites of O-glycosylation of the E2, which accounts for the size heterogeneity of E2, is not known. E1 contains an ER retention signal that is only overridden once conformational folding is complete, after which the E1–E2 heterodimer migrates to the Golgi. In the Golgi the N-glycans of both E2 and E1 are modified to complex form, although modification is not complete and the extent of modification on both proteins is heterogeneous. Modifications of the O-glycans on E2 also occur and E2 contains a Golgi retention signal, indicating that the Golgi is the preferred site of viral budding in infected cells. However, late in infection E1 and E2 migrate to the cell surface and budding can also occur at this site in some cell lines.

RUBV capsid morphogenesis occurs in association with cell membranes. The association of RUBV capsid protein with membranes is probably mediated by the E2 signal sequence, which is retained at the COOH terminus of C. In fact, C may associate with the E2–E1 heterodimer and migrate as a passenger on the cytoplasmic side of vesicles transporting E2–E1 from the ER to the Golgi and among the Golgi stacks. Unlike the alphaviruses, whose capsids accumulate in infected cells, RUBV capsids only become visible in association with deformed, thickened membranes that appear to be in the early process of budding. A putative encapsidation signal has been localized near the 5′ terminus of the genomic RNA. The C protein is phosphorylated and phosphorylation/dephosphorylation by cell enzymes is proposed as the regulator of the process by which the genome is unencapsidated following entry (phosphorylation) and encapsidated later in infection following replication (dephosphorylation). Interestingly, in cells in which the complete S-ORF is expressed in the absence of genomic RNA, virus-like particles form and are secreted and these have the same morphology and isopycnic density as do virions.

RUBV replicates in the cytoplasm of the infected cell. None of the virus proteins exhibit any involvement with the nucleus during infection and RUBV infection does not appear to inhibit cell macromolecular synthesis in any grossly detectable manner; however, perturbations of specific macromoleclar products and induction of specific genes may occur. Microsopically, RUBV-infected cells appear similar to uninfected cells; however, rearrangements of cellular cytoskeletal elements and organelles such as mitochondria have been reported. RUBV reportedly inhibits growth in primary human cell cultures in part due to an inhibition of mitosis; however, the virus has no reproducible effect on the growth of stable cell lines. In those cell lines which exhibit CPE (Vero and RK-13 cells), cell death is due to apoptosis.

Genetics and Evolution

The RUBV genome is extremely stable. Currently, the genomes of 19 independent strains of RUBV from eight genotypes, five in clade 1 and three in clade 2, have been sequenced in their entirety and all are nearly identical in terms of the size of the genome as well as the coding and noncoding regions within the genome. The only exceptions are 1–2 nucleotide deletions occasionally encountered in the junction region. Maximal observed distance at the nucleotide level is 5% among clade 1 viruses, 7.5% among clade 2 viruses, and 9% between viruses in the two clades. Across the 19 genomic sequences, 78% of the nucleotides are conserved, explaining the low level of variability. A unique feature of RUBV evolution is that

changes to G and C are selected for, indicating an adaptive advantage of the high G+C content of the genome.

Because there are no known close relatives of RUBV, the origin of the virus prior to its introduction into the human population is unknown. Except for short stretches at the 5′ end of the genome and at the subgenomic promoter site, RUBV and the alphaviruses share no nucleotide homology. Thus these two genera are only distantly related. RUBV and the alphaviruses belong to the 'alphavirus-like superfamily' of plus-sense RNA viruses, which includes a large number of plant viruses as well as human hepatitis E virus, the sole member of the genus *Hepevirus*. Within this superfamily, computer-assisted phylogenetic analysis of the NS proteins indicates that RUBV is more closely related to hepatitis E virus than to the alphaviruses and this dissimilarity is borne out by differences in the order of motifs in the NS-ORF. Thus, it is hypothesized that the evolution of the togaviruses may have been more complicated than simple divergence from a common ancestor and probably involved recombination between progenitors of the current alphaviruses, RUBV, hepatitis E virus, and, possibly, certain plant viruses.

Serologic Relationships and Variability

RUBV is monotypic and immunological characterization of diverse strains, including both clade 1 and 2 viruses, has only revealed subtle antigenic differences which map to C or E2. As might be anticipated from the lack of serologic cross-reaction with the alphaviruses, there is no homology at the amino acid level between RUBV and alphaviruses within the virion proteins.

Epidemiology

Historically, RUBV was endemic worldwide. Over the past 35 years, vaccination programs have curtailed this distribution, as discussed below. Before the advent of vaccination programs, rubella was considered a disease of middle childhood in temperate zones, with seasonal peak occurrence in the spring and epidemics at 5–9-year intervals. However, in tropical zones the highest infection rates were in children under 5 years of age. RUBV is not as transmissible as is measles virus and even during epidemics, susceptibles are spared. Thus, infection of adolescents and young adults, the population at risk for CRS, in endemic areas is not uncommon.

Transmission and Tissue Tropism

RUBV is transmitted between individuals by aerosol. The epithelium of the buccal mucosa provides the initial site for virus replication and the mucosa of the upper respiratory tract and nasopharyngeal lymphoid tissue serve as portals of virus entry. The virus is then spread by local lymphatics, which seed regional lymph nodes where further virus replication occurs. After an incubation period of 7–9 days, virus appears in the blood. Viremia ceases with the onset of detectable rubella-specific antibody, shortly after the rash appears 2–3 weeks post infection. Patients are most infectious immediately preceeding and during the rash phase; virus generally disappears from nasopharyngeal secretions within 4 days of appearance of the rash. Congenitally infected infants shed virus for 3–6 months following birth and are a source of transmission during that period. Reinfection with RUBV occurs, usually without clinical illness or virus shedding. There are a small number of cases in which RUBV reinfection of pregnant women with well-documented immunity has resulted in CRS.

During pregnancy, placental tissues are very susceptible to infection. Placental infection results in scattered foci of necrotic syncytiotrophoblast and cytotrophoblast cells and evidence of damage to vascular endothelium. Following placental infection, virus can spread to the fetus but this does not always occur and RUBV is more often recovered from placental tissue than from fetal products of conception. Once fetal infection occurs, virus spreads throughout the fetus and almost any organ may be infected. Severe fetal damage is only associated with infection during the first trimester of pregnancy; the rate of CRS is >50%, 25%, and 10% when infection occurs during the first, second, and third months, respectively. This is due to a combination of an apparent decline in the efficiency of placental transfer after the first trimester and a reduction in the ability of the virus to inflict fetal damage after this time of gestational development.

Clinical Features of Infection

Rubella acquired in childhood or early adulthood is usually mild and it is estimated that up to 50% of rubella infections are clinically inapparent. Symptomatic rubella encompasses combinations of maculopapular rash, lymphadenopathy, low-grade fever, conjunctivitis, sore throat, and arthralgia. The rash is the most prominent feature and appears following an incubation period of 16–20 days. The rash begins as distinct pink maculopapules on the face that then spread over the trunk and distally onto the extremities. The maculopapules coalesce and the rash fades over several days. An associated posterior cervical and suboccipital lymphadenopathy is also characteristic. Infrequent complications include thrombocytopenia and post-infectious encephalitis. Acute polyarthralgia and arthritis following natural RUBV infections of adults are common and occur more frequently and with greater severity in women than in men. Joint involvement is

usually transient; however, chronic arthritis, persisting or recurring over several years, has been reported.

The clinical manifestations of CRS apparent at birth vary widely, most frequently including thrombocytopenic purpura ('blueberry muffin syndrome'), intrauterine growth retardation, congenital heart disease (patent ductus arteriosus or pulmonary artery or valvular stenosis), psychomotor retardation, eye defects (cataract, glaucoma, retinopathy), hearing loss, and hepatomegaly and/or splenomegaly. Nearly 80% of CRS children show some type of neural involvement, particularly neurosensory hearing loss.

Most clinical manifestations of congenital rubella are evident at or shortly following birth and some are transient. However, recognition of retinopathy, hearing loss, and mental retardation may be delayed for several years in some cases. Progressive consequences of congenital rubella have become increasingly appreciated as CRS children from the 1964 epidemic have been followed longitudinally. These predominantly involve endocrine dysfunction (diabetes mellitus, which ultimately affects 40% of CRS patients, and thyroid dysfunction). A rare, fatal neurodegenerative disease, progressive rubella panencephalitis (PRP), described in CRS patients, bears superficial resemblance to subacute sclerosing panencephalitis associated with measles virus. Subsequently, PRP cases have been reported in individuals infected postnatally.

Pathogenesis, Pathology, and Histopathology

There is limited information on the pathogenesis of uncomplicated rubella because of the benign nature of the illness. With respect to the complications that can accompany acute rubella, the postinfectious encephalitis is thought to be autoimmune in nature since RUBV cannot be isolated from cerebrospinal fluid or brain at autopsy. Interestingly, however, extensive inflammation and demyelination are not observed. In a few cases of rubella arthritis, the presence of RUBV in synovial fluid and/or cells has been demonstrated and therefore it is assumed that virus persistence is involved. However, considering the age and sex factors in the incidence of arthritis, it seems likely that immunopathological mechanisms also play a role. In one study, human leukocyte antigen (HLA) class II haplotypes predisposing adult women to arthritis and arthralgia following rubella vaccination were found to also correlate with predisposition to arthritis and arthralgia regardless of vaccination status.

Following fetal infection, virus can be isolated from practically every organ of abortuses or infants who die soon after birth. However, only 1 in 10^3 to 1 in 10^5 cells are infected and it is not known how such a low infection rate leads to the profound birth defects exhibited in CRS. Affected organs are routinely small for gestational age and contain reduced numbers of cells. Considering the inhibitory effect of RUBV on primary cells, it is thought that virus infection early in organogenesis inhibits cell division, leading to both retardation and alteration in organ development. Virus persistence continues after birth, as evidenced by virus shedding, which generally ceases within 6 months of age. Whether virus persistence continues beyond cessation of shedding and plays a role in the delayed and progressive manifestations of CRS is not known.

Histologically, affected organs of CRS patients show a limited number of well-recognized malformations, with noninflammatory histopathology predominating. Particularly apparent are vascular lesions and focal destruction in tissue bordering these lesions. These lesions are likely to be due to virus replication in the vascular endothelium and damage to neighboring tissue may play a role in the pathogenesis of CRS. The neuropathology of CRS is of interest not only because of the defects manifest shortly after birth, but also because some CRS patients develop schizophrenia-like symptoms later in life. CRS brains are generally free of gross morphological malformations, with a common tendency toward microcephaly. Vascular damage, leptomeningitis, decreased number of oligodendroglial cells, and alteration of white matter are observed. Recently, magnetic resonance imaging of a group of CRS adults with schizophrenia-like symptoms revealed specifically reduced cortical gray matter and enlargement of the ventricles, which were not previously observed aspects of CRS-induced neuropathology. Interestingly, the comparative finding that non-CRS schizophrenia patients exhibit a pattern of brain dysmorphosis similar to that found in CRS patients with schizophrenia-like symptoms supports the hypothesis that schizophrenia is developmental in nature (there is some evidence for a viral trigger to schizophrenia).

Immune Response and Serodiagnosis

Following acute infection, anti-RUBV IgM antibodies are detectable at the time of rash onset and for a month or two afterwards, so that serodiagnostic testing for the presence of IgM is the current primary means for diagnosis of acute RUBV infection; in the succeeding weeks anti-RUBV antibodies in all immunoglobulin classes appear. The dominant early and persistent IgG response is in the IgG_1 subclass and antibodies of this class persist indefinitely after natural infection of otherwise healthy individuals. The majority of the antibody response is directed to the E1 glycoprotein, with proportionally lesser amounts of the response directed at E2 or C. Although neutralizing and complement-fixing antibodies are induced as well, the

classical assay for the presence of anti-RUBV antibodies has been hemagglutination inhibition (HAI) and the current standard level of 10 IU ml ^{1} is based on an HAI titer of roughly 8. Because of the importance of serodiagnostic testing for rubella, a worldwide commercial market for rubella tests exists and a number of commercial laboratories offer such kits, most of which are based on latex agglutination or enzyme immunoassay.

RUBV-specific cellular immune responses are measurable within 1–2 weeks of onset of illness. Major histocompatibility complex (MHC)-restricted CD4+ epitopes have been mapped to all three of the virus structural proteins; however, CD8+ epitopes have thus far only been mapped to the C protein. HLA class I and II alleles both positively and negatively associated with antibody and lymphoproliferative responses following rubella vaccination have recently been determined.

Following fetal infection, the fetus produces IgM antibody, detectable at 18–20 weeks of gestation, and maternal IgG antibody crosses the placenta. Both types of antibody exhibit virus-neutralizing activity *in vitro*; however, neither is sufficient to resolve virus infection during gestation. As discussed above, the intracellular maturation of virus probably shields it from antibody. After birth, the presence of IgM or a lack of decline of IgG titer are both considered diagnostic of fetal infection. CRS infants exhibit various degrees of impairment in the cellular immune response to RUBV and it is thus a deficiency in this arm of the immune response that allows the virus to persist. Considering the cellular immune deficiency in CRS infants, it is curious that detectable virus persistence ends relatively shortly after birth. The means by which virus persistence is cleared under these conditions is not understood.

Diagnosis

The common symptoms of acute rubella, lymphadenopathy, erythematous rash, and low-grade fever, are nonspecific and easily confused with illnesses caused by other common pathogens. A definitive diagnosis of rubella requires detection of the presence of IgM antibodies or a rise in IgG antibodies in paired acute-phase and convalescent-phase serum samples. Virus is also readily isolated from saliva or nasopharyngeal washings at the time of rash onset. Reverse transcription-polymerase chain reaction (RT-PCR) assays have been developed to detect virus in saliva, blood, or urine. Generally, virus isolation is only done to collect specimens for molecular epidemiological analysis. Diagnosis of acute rubella is of utmost importance during early pregnancy. There is no intervention for congenital rubella other than abortion; however, as discussed above, maternal infection does not always lead to CRS, resulting in an extremely difficult decision. RT-PCR can be used to detect virus RNA in amniotic fluid or chorionic villi, but is rarely employed. Diagnosis of CRS in a newborn is initially made on the basis of symptoms and confirmed in the laboratory by serological testing for the presence of IgM antibodies, by lack of decrease of IgG antibodies in serum specimens, or by detection of virus or virus RNA. As stated above, CRS infants shed virus and therefore are a source of contagion.

In countries with vaccination programs, the need for detection of acute rubella is rare. However, serodiagnosis for determination of immune status is routine for pre-pregnancy planning, employment in medical facilities and, in some states in the US, to obtain a marriage license. Serodiagnosis can also be used in lieu of proof of vaccination, which is required for school enrollment. Individuals found to be seronegative are subsequently vaccinated, which is done postpartum in the case of a woman who is pregnant at the time of testing.

Prevention and Control of Rubella

As discussed above live, attenuated vaccines were developed and placed in use by 1970. The vaccine used in most countries is the RA 27/3 vaccine. In Japan, five live, attenuated vaccine strains were developed and are currently in use. Additionally, at least one Chinese vaccine strain is currently in use in China.

Attenuated RUBV vaccines cause subclinical infection with transient viremia in susceptible patients. Natural transmission of vaccine virus has not been reported. The RA27/3 vaccine strain produces seroconversion in greater than 95% of recipients. Vaccine-induced titers are lower than those induced by natural infection but appear to last indefinitely. The RUBV vaccine is generally administered to children in trivalent form with the measles- and mumps-attenuated vaccines (MMR) but inclusion of the recently licensed varicella vaccine in a tetravalent vaccine is being strongly considered.

The RUBV vaccines have been among the most successful in terms of induction of immunity with an absence of side effects. However, two issues have arisen concerning rubella vaccination. The first is that the vaccine virus can cross the placenta and infect the fetus. However, US and Israel registries of deliveries to women inadvertently vaccinated during early pregnancy revealed no reported congenital abnormalities. Similar findings were reported in subsequent studies conducted in other countries in conjunction with mass vaccination campaigns. Nevertheless, vaccination during pregnancy is contraindicated and

is deferred until postpartum. Second, is the occurrence of arthralgia and arthritis following vaccination. Joint complications are nonexistent in children with the currently used rubella vaccines; however, transient arthralgia and arthritis is reasonably common among adult female vaccinees. There have also been reports of chronic arthritis and related neurological involvement following vaccination of adult women. Although these complications are consistent with complications that can accompany natural rubella in adult females, recent studies have shown that the incidence of such vaccine-related complications is low and cannot be statistically differentiated from the incidence of similar symptoms in control, unvaccinated populations.

Since the inception of rubella vaccination in 1969, the US has employed a strategy of universal vaccination at 15 months of age augmented with vaccination of seronegative 'at-risk' individuals (women planning pregnancies, healthcare workers) which was successful in bringing the incidence of rubella and CRS to low levels by 1988. A resurgence of rubella and CRS that occurred between 1989 and 1991 among foci of unvaccinated individuals led, in part, to administration of a second dose of MMR vaccine when the recipients were between 5 and 10 years of age. After 2000, the incidence of rubella fell to fewer than 25 cases per year and in 2004 and it was concluded that indigenous rubella had been eliminated from the US. Vaccination programs have also been in place in Europe and Japan since the development of live, attenuated vaccines; however, developing countries were slower to institute rubella vaccination programs. However, efforts among developing countries have accelerated since 2000, particularly in conjunction with ongoing measles elimination efforts, and currently roughly 50% of countries worldwide have national rubella vaccine programs. The World Health Organization Regions of the Americas and Europe have set goals for elimination of rubella and CRS by 2010.

Despite the recent increase in intensity of rubella vaccination efforts worldwide, most of Asia and Africa are not currently included and thus well over half of the world's population is not covered. The cost of the vaccine, the general mildness of the disease, and the nature of national public health infrastructures are deterrents to vaccination programs in developing countries. Moreover, estimates of CRS incidence are difficult to obtain because the three hallmark symptoms of CRS, deafness, blindness, and mental retardation, are not uniformly present and are not readily diagnosable in newborns. Surveys in developing countries show that the rate of CRS in developing countries is the same as that in developed countries prior to vaccination and a recent economic analysis concluded that the cost/benefit ratio for rubella vaccination was similar to that for hepatitis B vaccination, a pathogen that is universally recognized to impart a societal load. Nevertheless, the success of worldwide rubella vaccination appears dependent on piggy-backing on global measles vaccination programs. In addition to its efforts to initiate rubella vaccination programs in developing regions, the WHO established a global Measles–Rubella Surveillance Network in 2003. Interestingly, rubella vaccination and the concomitant reduction in rubella simplifies measles surveillance which requires diagnosis of rubella because of the similarity of symptoms of the two diseases. A major modification in measles/rubella vaccination may be forthcoming in the form of aerosolized vaccines. Aerosol administration mimics the natural route by which these two viruses are contracted and preliminary studies show that aerosolized vaccine uptake and efficacy is as good as or better than the current injection route. Use of aerosolized vaccines would reduce vaccination costs by eliminating needles and the concomitant need for trained personnel for administration. Finally, it is emphasized that rubella reduction in developing countries imparts benefit to developed countries, in which most rubella outbreaks are due to importation, by decreasing such outbreaks and thus easing control efforts. However, discontinuation of rubella vaccination in developed countries would require eradication.

Future

Because of its clinical similarity to any of a diverse group of clinical diseases, RUBV will remain a fascinating pathogen. As an example, the incidence of diabetes in CRS patients is the best statistically direct association between a specific human virus and a specific autoimmune disease. The mechanism of viral involvement, if any, in each of these diseases is not fully understood. Elucidating the mechanism of RUBV-induced birth defects could also provide understanding into teratogenesis by other infectious agents. Unfortunately, our present understanding of disease mechanisms in the RUBV-related syndromes is hindered by the lack of a suitable animal model system that fully mimics the infection seen in humans, making development of an animal model a research priority. RUBV is taxonomically unique and appears to have evolved as a recombinational hybrid of distantly related ancestor viruses. Thus, investigation of the molecular biology of RUBV likely will reveal novel replication strategies and yield insight into virus evolution. The greatest challenge concerning RUBV is the current and forthcoming elimination efforts in most regions of the world.

See also: Central Nervous System Viral Diseases; Evolution of Viruses; Hepatitis E Virus; Measles Virus; Togaviruses: Alphaviruses; Togaviruses: General Features.

Further Reading

Best JM, Castillo-Solorzano C, Spika JS, *et al.* (2005) Reducing the global burden of congenital rubella syndrome: Report of the World Health Organization steering committee on research related to measles and rubella vaccines and vaccination, June 2004. *International Journal of Infectious Diseases* 192: 1890–1897.

Frey TK (1994) Molecular biology of RUBV. *Advances in Virus Research* 44: 69–160.

Hobman TC and Chantler JK (2007) RUBV. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 5th edn., pp. 1069–1100. Philadelphia: Lippincott Williams and Wilkins.

Law LJ, Ilkow CS, Tzeng WP, *et al.* (2006) Analyses of phosphorylation events in the RUBV capsid protein: Role in early replication events. *Journal of Virology* 80: 6917–6925.

Ovsyannikova IG, Jacobson RM, Vierkant RA, Jacobsen SJ, Pankratz VS, and Poland GA (2005) Human leukocyte antigen class II alleles and rubella-specific humoral and cell-mediated immunity following measles–mumps–rubella-II vaccination. *International Journal of Infectious Diseases* 191: 515–519.

Plotkin SA (2006) The history of rubella and rubella vaccination leading to elimination. *Clinical Infectious Diseases* 43(supplement 3): S164–S168.

Reef SE, Frey TK, Theall K, *et al.* (2002) The changing epidemiology of rubella in the 1990s: On the verge of elimination and new challenges for control and prevention. *JAMA* 28(7): 464–472.

Tzeng WP, Matthews JD, and Frey TK (2006) Analysis of RUBV capsid protein-mediated enhancement of replicon replication and mutant rescue. *Journal of Virology* 80: 3966–3974.

Zhou Y, Ushijima H, and Frey TK (2007) Genomic analysis of diverse RUBV isolates. *Journal of General Virology* 88: 932–941.

S

Sadwavirus

T Iwanami, National Institute of Fruit Tree Science, Tsukuba, Japan

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Natsudaidai A popular sour citrus grown in some parts of Japan.
Satsuma A major mandarin-type citrus grown in Japan and some other Asian countries.

Introduction

The genus *Nepovirus* includes viruses which are transmitted by nematodes with polyhedral particles. Typical nepoviruses have a broad host range, a single coat protein, and a bipartite single-stranded RNA genome and are transmissible through seed. Sadwaviruses have been previously considered atypical and tentative members of the genus *Nepovirus*. Like typical nepoviruses, sadwaviruses have polydedral virus particles which contain two species of genomic RNA, but can be distinguished on the basis of the genome organization, sequence homologies, and the number of coat proteins. Some sadwaviruses are transmitted by aphids.

The derivation of the name comes from the virus species, *Satsuma dwarf virus*, the type member of the genus *Sadwavirus*. The major disease caused by satsuma dwarf virus (SDV) is stunting, accompanied by the presence of boat-shaped leaves, in Satsuma mandarin (*Citrus unshiu* Marc.).

Taxonomy and Classification

Satsuma dwarf virus, *Strawberry latent ringspot virus*, and *Strawberry mottle virus* are the definite members of the genus *Sadwavirus*. Lucerne Australian symptomless virus (LASV), Rubus Chinese seed-borne virus (RCSV), and Black raspberry necrosis virus (BRNV) are tentative members (**Table 1**).

Virus species are demarcated on the basis of type of biological vector, if known, host range, absence of serological cross-reaction, absence of cross-protection, and difference in amino acid sequence (less than 75% in the large coat protein and the proteinase-polymerase region).

The genus *Sadwavirus* has not been assigned to any virus family.

Strains and Synonyms

Citrus mosaic virus (CiMV), natsudaidai dwarf virus (NDV), and navel orange infectious mottling virus (NIMV) are distantly related strains of *Satsuma dwarf virus*. Typical symptoms of CiMV-infected Satsuma mandarin are spotting and blotching of the fruit rind. NDV induces vein clearing and mottling on new leaves of nastudaidai (*Citrus natsudaidai*). NIMV produces persistent chlorotic spots on leaves of sweet orange. The strains of *Satsuma dwarf virus* share around 80% sequence homology at the amino acid level.

Strawberry latent ringspot virus has a few synonyms, including rhubarb virus 5. Some isolates from olive, peach, raspberry, and grapevine were serologically distinguishable from the type strain, but sequence diversity among these isolates and strains has not been studied.

Geographic Distribution

SDV occurs mostly in Japan, but has also been found in China and Turkey. Strawberry latent ringspot virus (SLRSV) is distributed throughout Europe and the USA, as well as Australia and New Zealand. Strawberry mottle virus (SMoV) is probably spread worldwide, as well as BRNV. Occurrence of LASV and RCSV is limited to Australasia and China, respectively.

Virion Properties

Particles of sadwaviruses are icosahedral, about 26–30 nm in diameter (**Figure 1**). Some particles are penetrated

Table 1 Species in the genus *Sadwavirus*[a]

Species and strains	*Abbreviation*	*RNA-1 accession number*	*RNA-2 accession number*
Satsuma dwarf virus			
Citrus mosaic virus	CiMV		D64079 (partial)
Natsudaidai dwarf virus	NDV		AB032750 (partial)
Navel orange infectious mottling virus	NIMV		AB000282 (partial)
Satsuma dwarf virus	SDV	AB009958	AB009959
Strawberry latent ringspot virus			
Strawberry latent ringspot virus	SLRSV	NC 006964	NC 006965
Strawberry mottle virus			
Strawberry mottle virus	SMoV	AJ311875	AJ311876
Tentative species in the genus			
Black raspberry necrosis virus	BRNV	NC 008182	NC 008183
Lucerne Australian symptomless virus	LASV		
Rubus Chinese seed-borne virus[a]	RCSV		

[a]Species names are given in italic script; strain names and tentative species names are in Roman script.

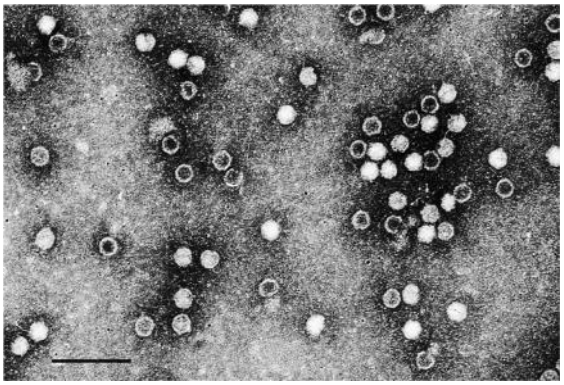


Figure 1 Electron micrograph of negatively stained virus particles of SDV. Scale = 100 nm.

by negative stain in the electron microscope. Typically, particles of sadwaviruses are of three types, named according to the relative rates of sedimentation of purified preparations. Top component (T) particles are empty shells. Middle component (M) and bottom component (B) particles contain genomic RNA. The buoyant densities in CsCl are about 1.43 and 1.46 g cm^{-3}, respectively. SLRSV has only B particles with buoyant density about 1.46 g cm^{-3}. Sadwavirus particles contain two sizes of RNA molecules. SDV has M and B particles that contain either of the different RNAs. In contrast, SLRSV has only B particles. Some particles contain one larger RNA, others contain two molecules of smaller RNA.

Genome Organization

The genome of sadwaviruses consists of two molecules of positive-sense single-stranded RNAs called RNA-1 and RNA-2. The sizes of RNA-1 and RNA-2 are about 7 and 4–5.5 kb, respectively. The 3′ termini of both RNAs are polyadenylated. In the case of SLRSV, both RNAs are linked at the 5′ termini to a small protein (VPg). VPgs of other sadwaviruses are unknown.

Both RNA-1 and RNA-2 have a single large open reading frame (**Figure 2**). The general genome organization is similar to that of como- and nepoviruses. The polyprotein encoded by RNA-1 contains domains for helicase, protease, and RNA-dependent RNA polymerase, whereas the polyprotein encoded by RNA-2 has movement protein and coat proteins. The amino acid sequences of the N-termini of polyproteins encoded by RNA-1 and RNA-2 of SDV are highly conserved. The feature is observed in the polyproteins of tomato ringspot virus in the genus *Nepovirus*. The function of these regions is unknown.

Sequence Comparisons

Comparison among the sequences of sadwaviruses of different species at nucleotide and amino acid levels shows few marked similarities. Some regions show about 20–40% sequence identities, and other parts are less similar.

Phylogeny

Complete genomic nucleotide sequences of SDV, BRNV, SLRSV, and SMoV have been determined. Partial sequences of CiMV, NDV, and NIMV are available (**Table 1**). There is little information on the nucleotide sequences of other sadwaviruses. To reveal relationships, the RNA-dependent RNA polymerase region, which is the most conserved among sadwa-, como-, nepo-, and other plant picorna-like viruses, has been used for phylogenetic comparisons. Alignment of the most conserved regions of the RNA-dependent RNA polymerase region shows that all plant picorna-like viruses are separated to

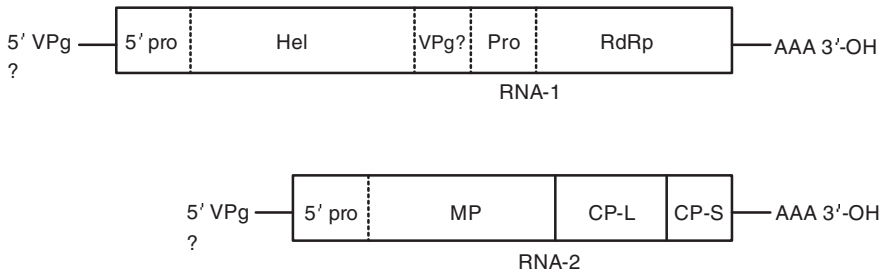


Figure 2 Genome organization of SDV. The boxes represent polyproteins. The vertical solid lines within the box show sites where cleavages occur in the polyprotein and the dashed lines indicate sites where cleavages are presumed to occur. Abbreviations: VPg, genome-linked viral protein; 5′ pro, 5′ protein; Hel, helicase; Pro, protease; RdRp, RNA-dependent RNA polymerase; MP, movement protein; CP-L and CP-S, large and small components of coat protein; AAA, poly(A).

three distinct groups: the first and second groups include comoviruses and nepoviruses, respectively, whereas the third group consists of SDV (and closely related CiMV, NDV, NIMV), SMoV, apple latent spherical virus (ALSV, which is a member of a newly established genus *Cheravirus*), and viruses of the family *Sequiviridae*, which have monopartite RNA geneome.

Satellites

SLRSV has strains that contain satellite RNAs. The SLRSV satellite encodes a polyprotein, which shows little homology with other known proteins. There is no evidence as to the function of the polyprotein. Other sadwaviruses do not contain a satellite RNA.

Host Range and Virus Propagation

Natural host of SDV, CiMV, NDV, and NIMV is limited to woody plants, whereas SMoV, LASV, and RCSV are confined to herbaceous plants. BRNV infects *Rubus* spp. SLRSV has a wide natural host range that includes both woody and herbaceous plants. All sadwaviruses are readily transmitted by mechanical inoculation to herbaceous plants of several families, including Chenopodiaceae, Fabaceae, and Solanaceae. Common experimental hosts used for the propagation of sadwaviruses are *Chenopodium quinoa*, *Cucumis sativus*, and *Physalis floridana*. SMoV propagates very poorly in any of the experimental herbaceous hosts, and purification of virus particles is difficult.

Epidemiology

SLRSV is transmitted by the soil nematode, *Xiphinema diversicaudatum*, and the transmission from plants to plants is slow, reflecting the restricted mobility of the nematode. SDV, CiMV, NDV, and NIMV are disseminated in a similar manner, and soil transmission is suspected, although vectors have not yet been identified. SMoV and BRNV are transmitted by aphids. All sadwaviruses but SMoV are seed-borne, and dissemination in seeds is important for long-distance movement of some sadwaviruses. Indeed, RCSV was first found in the UK from imported seeds from China.

Hosts of SDV, CiMV, NDV, and NIMV (citrus), BRNV (raspberry), SMoV (strawberry), SLRSV (strawberry and fruit trees) are vegetatively propagated, and movement of contaminated budwoods and tubers plays an important role in the long-distance dissemination. For example, occurrence of SDV in China and Turkey is obviously related to unchecked budwoods from Japan.

Cytopathology

Tubules which contain virus particles arranged in a single file are formed in the cytoplasm of infected cells. A file of virus particles is sometimes observed in a plasmodesma between two cells. Virus particles have also been detected in the vacuoles of infected cells in crystalline forms. These structures are also commonly observed in cell infected with comoviruses and nepoviruses.

Prevention and Control

Control measures consist of establishment of healthy stocks, chemical controls of vectors (nematode for SLRSV, aphids for BRNV and SMoV), and introduction of tolerant cultivars when available. Distributions of virus-free materials are important for vegetatively propagated crops (citrus for SDV, strawberry for SMoV and SLRSV, fruit trees for SLRSV). Several diagnostic tools including enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase-polymerase chain reaction (RT-PCR) are available for most of the sadwaviruses. Chemical fumigation of citrus field to prevent soil transmission of SDV is partially effective at best. Many strawberry cultivars are symptomless hosts for either SMoV or SLRSV. Mixed infection with other

strawberry viruses induces apparent foliar symptoms and decline of tubers.

See also: Nepovirus; Sequiviruses.

Further Reading

Halgren A, Tzanetakis IE, and Martin RR (2007) Identification, characterization, and detection of black raspberry necrosis virus. *Phytopathology* 97: 44–50.

Iwanami T, Kondo Y, and Karasev AV (1999) Nucleotide sequences and taxonomy of satsuma dwarf virus. *Journal of General Virology* 80: 793–797.

Le Gall O, Iwanami T, Karasev A, *et al.* (2005) Genus *Sadwavirus*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 799–802. San Diego, CA: Elsevier Academic Press.

Mayo MA and Robinson DJ (1996) Nepoviruses: Molecular biology and replication. In: Harrison BD and Murant AF (eds.) *The Plant Viruses,* vol. 5, pp. 139–185. New York: Plenum.

Murant AF, Jones AT, Martelli GP, and Stace-Smith R (1996) Nepoviruses: General properties, diseases, and virus identification. In: Harrison BD and Murant AF (eds.) *The Plant Viruses,* vol. 5, pp. 99–137. New York: Plenum.

Thompson JR, Leone G, Lindner JL, Jelkmann W, and Schoen CD (2002) Characterization and complete nucleotide sequence of strawberry mottle virus: A tentative member of a new family of bipartite plant picorna-like viruses. *Journal of General Virology* 83: 229–239.

Satellite Nucleic Acids and Viruses

P Palukaitis, Scottish Crop Research Institute, Invergowrie, UK
A Rezaian, University of Adelaide, Adelaide, SA, Australia
F García-Arenal, Universidad Politécnica de Madrid, Madrid, Spain

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Satellite-like RNA A subviral genome dependent upon another virus for replication and encapsidation, but is required for vector transmission of the helper virus.
Satellite RNA or satellite DNA A subviral genome dependent on a helper virus for both replication and encapsidation.
Satellite virus A subviral genome dependent on a helper virus for its replication, but encoding its own capsid protein.
Virus-associated nucleic acid A subviral genome that depends on another virus for encapsidation and transmission, but not for its replication.

Introduction

Satellites of viruses constitute a heterogeneous collection of subviral agents. Satellites can be differentiated from other subviral nucleic acids, such as subgenomic (sg) RNAs, defective (D) or defective interfering (DI) RNAs, and viroids, by their molecular, biological, and genetic nature. Unlike sg RNAs, D RNAs, and DI RNAs, satellites and viroids have little or no sequence similarity to any known virus. Whereas viroids are replicated by host polymerases, RNA satellites are replicated by the polymerase of a virus, referred to as the helper virus (HV). Satellites have been found associated with DNA and RNA viruses, and in the latter case, with both ssRNA and dsRNA viral genomes, the satellites being of the same nucleic acid type as the HV. While, in general, the HV can exist independent of the satellite, there are exceptions where a satellite contributes to the transmission of the HV and thus is referred to as being satellite-like. In addition, some viral RNAs are found in association with other viral genomes on which they depend for their encapsidation and transmission, but not their replication. These viral RNAs are referred to here as virus-associated RNAs. Satellites are divided basically into two main groups; those that encode their own capsid protein (CP) are called satellite viruses, while those that require their HV for both replication and encapsidation are referred to as satellite RNAs or DNAs. There are also satellites of satellites, in which some satellite RNAs are replicated by the HV, but are encapsidated by the CP of a satellite virus. In addition, in the case of the carmovirus turnip crinkle virus (TCV), which replicates both satellite RNAs and DI RNAs, it also produces a chimeric RNA (referred to as satC) that is in part a DI RNA and in part a satellite RNA (satD). While the vast majority of satellites are found in association with plant viruses, a few have been found in association with animal or fungal viral genomes. Most plant viruses do not contain satellites associated with them, but as some satellites can significantly affect the disease induced by the HV, the presence of satellites has important consequences for viral-induced diseases.

History

The use of the term satellite as a subviral agent was first conceptualized in 1962 by Kassanis, to describe the relationship of a 17-nm diameter viral particle found in association with some isolates of the 26-nm diameter necrovirus tobacco necrosis virus (TNV). The smaller particle was dependent on TNV for its accumulation, but was serologically unrelated to TNV. It became known as the satellite virus of TNV (now known as tobacco necrosis satellite virus, TNSV). A few other satellite viruses have been described since then, but, in general, they are rare. By contrast, satellite RNAs are more common. The first satellite RNA was described by Schneider in 1969, in association with the nepovirus tobacco ringspot virus (TRSV), the satellite RNA being encapsidated by the CP of the HV. The symptoms induced by TRSV were attenuated dramatically by the presence of the satellite RNA. This is not always the case, with some satellite RNAs having no effect on either the HV accumulation or disease induced by the HV, and a few satellite RNAs exacerbating the HV-induced disease. Most of these satellite RNAs contain ssRNA genomes, while a few contain genomes of dsRNA. Some of the satellite RNAs with ssRNA genomes are translated to produce proteins, which may or may not be required for their replication, depending on the particular satellite RNA. Satellite DNAs were first described in 1997 by Dry and colleagues, in association with the geminivirus tomato leaf curl virus (TLCV). It was isolated from field-infected tomato plants in northern Australia. Satellite DNAs also are encapsidated by the CP of their HV. Since then, satellite DNAs referred to as satellite DNA β have been described in association with many geminiviruses, principally from Asia.

Geographical Distribution

Since satellite RNAs are only found associated with their specific HVs, they are limited to the distribution range of the HV and the vectors of the HV. On the other hand, not all isolates of the HV have satellites associated with them, further delimiting the distribution of satellites. Nevertheless, as many of the HVs are distributed worldwide, so are many of the satellites. The distribution of satellite DNA β has so far been linked to the geminiviruses of the Old World, an exception being DNA β in honeysuckle that appears to have been distributed through vegetative plant material.

Classification

There is no correlation between the taxonomy of their HV and the presence of satellites. Satellites are associated with viruses belonging to at least 17 genera, and with only a limited number of species within those genera. Satellites do not constitute a homogeneous group in terms of their nucleic acid type, size, sequence, structure, or translatability. Most satellites are composed of RNA, but some consist of DNA. Most satellites are linear molecules, but some RNA satellites and all of the DNA satellites are circular in structure. Some satellites may encode proteins, while many do not. Thus, satellites are classified primarily into categories based on the features of the above properties, vis-à-vis their nucleic acid form and their genetic capacity. All satellites are grouped into the following categories, first differentiated on the basis of whether they are satellite viruses (**Table 1**) or satellite nucleic acids (**Table 2**), but

Table 1 Satellite viruses

Helper virus/satellite virus	*Particle size (nm)*	*CP*[a]	*Satellite RNA size (nt)*	*Accession no.*
Subgroup 1				
Chronic bee-paralysis virus (CBPV)/ CBPV-associated satellite virus (CBPVA)	17	NR[b]	~1100 (three species)	
Subgroup 2				
Necrovirus				
Tobacco necrosis virus (TNV)/satellite TNV (STNV)	17	~21 600	1239	J02399
Sobemovirus				
Panicum mosaic virus (PMV)/satellite PMV (SPMV)	16	~17 500	824–826	M17182
Tobamovirus				
Tobacco mosaic virus (TMV)/satellite TMV (STMV)	17	~17 500	1059	M24782
Nodavirus				
Macrobranchium rosenbergii nodavirus (MrNV)/extra small virus (XSV)	15	~17 000	796	AY247793
Unassigned				
Maize white line mosaic virus (MWLMV)/ satellite MWLMV (SMWLMV)	17	23 961	1168	M55012

[a]CP, capsid protein MW.
[b]NR, not reported.

Table 2 Satellite nucleic acids

Helper virus/satellite	*Satellite size*	*Encoded protein (aa/kDa)*	*Accession no.*
ssDNA satellites (circular)			
Ageratum yellow vein virus (AYVV)/AYVV satellite DNA β	1347	118/13.7	AJ252072
Bhendi yellow vein mosaic virus (BYVMV)/BYVMV satellite DNA β	1353	140/	AJ308425
Cotton leaf curl Bangalore virus (CLCuBV)/CLCuBV satellite DNA β	1355	118/	AY705381
Cotton leaf curl Gezira virus (CLCGV)/CLCGV satellite DNA β	1348	117/13.6	AY077797
Cotton leaf curl Multan virus (CLCMV)/CLCMV satellite DNA β	1349	118/13.7	AJ298903
Eupatorium yellow vein virus (EuYVV)/EuYVV satellite DNAβ	1356	116/13.5	AJ438938
Honeysuckle yellow vein virus (HYVV)/HYYV satellite DNA β	1344	116/13.5	AJ316040
Malvastrum yellow vein virus (MYVV)/MYVV satellite DNA β	1350	118/	AJ786712
Tobacco curly shoot virus (TCSV)/TCSV satellite DNA β	1354	118/	AJ421484
Tomato yellow leaf curl China virus (TYLCCV) /TYLCCV satellite DNA β	1336	118/	AJ421621
Tomato leaf curl virus (TLCV)/TLCV satellite DNA	682	None	U74627
dsRNA satellites (linear)			
L-A ds RNA virus of *Saccharomyces cerevisiae*/M satellite RNAs	1801 bp 0.5–1.8 kb	NR NR	U78817
Trichomonas vaginalis T1 virus (TVTV)/TVTV satellite RNA	497 bp ∼700 bp ∼1700 bp	NR NR NR	U15991 NR NR
Ophiostoma novo-ulmi mitovirus 3a (OnuMV3a)/OnuMV3a S-dsRNA	738–767 bp	4 small ORFs	AY486119
Large ssRNAs satellites			
Benyvirus			
Beet necrotic yellow vein virus (BNYVV)/BNYVV RNA 5	1342-1447 nt	∼26 kDa	U78292
Nepovirus			
Arabis mosaic virus (ArMV)/ArMV large satellite RNA	1104 nt	∼39 kDa	D00664
Chicory yellow mottle virus (CYMV)/CYMV large satellite RNA	1145 nt	∼39 kDa	D00686
Grapevine Bulgarian latent virus (GBLV)/GBLV satellite RNA	∼1500 nt	NR	NR
Grapevine fanleaf virus (GFLV)/GFLV satellite RNA	1114 nt	∼37 kDa	D00442
Myrobalan latent ringspot virus (MLRV)/MLRV satellite RNA	∼1400 nt	∼45 kDa	NR
Strawberry latent ringspot virus (SLRV)/SLRV satellite RNA	1118 nt	∼36 kDa	X69826
Tomato black ring virus (TBRV)/TBRV satellite RNA	1372-1375 nt	∼48 kDa	X05689
Potexvirus			
Bamboo mosaic virus (BaMV)/BaMV satellite RNA	836 nt	∼20 kDa	L22762
Small, linear ssRNAs satellites			
Carmovirus			
Turnip crinkle virus (TCV)/TCV satellite RNA	194 nt, 230 nt, 356 nt		X12749
Cucumovirus			
Cucumber mosaic virus (CMV)/CMV satellite RNA	333–405 nt		M18872
Peanut stunt virus (PSV)/PSV satellite RNA	393 nt		Z98198
Necrovirus			
Beet black scorch virus (BBSV)/BBSV satellite RNA	615 nt		AY394497
Tobacco necrosis virus (TNV)/TNV small satellite RNA	620 nt		NR
Nepovirus			
Chicory yellow mottle virus (CYMV)/CYMV small satellite RNA	457 nt		NC006453
Sobemovirus			
Panicum mosaic virus (PMV)/PMV satellite RNA	350 nt		NR
Tombusvirus			
Artichoke mottled crinkle virus (AMCV)/AMCV satellite RNA	∼700 nt		NR
Cymbidium ringspot virus (CymRSV)/CymRSV satellite RNA	621 nt		D00720
Carnation Italian ringspot virus (CIRV)/CIRV satellite RNA	∼700 nt		NR
Pelargonium leaf curl virus (PLCV)/PLCV satellite RNA	∼700 nt		NR
Petunia asteroid mosaic virus (PAMV)/PAMV satellite RNA	∼700 nt		NR
Tomato bushy stunt virus (TBSV)/TBSV satellite RNA	612 nt, 822 nt		AF022788
Umbravirus			
Pea enation mosaic virus (PEMV)/PEMV satellite RNA	717 nt		U03564
Circular ssRNAs satellites			
Polerovirus			

Continued

Table 2 Continued

Helper virus/satellite	*Satellite size*	*Encoded protein (aa/kDa)*	*Accession no.*
Cereal yellow dwarf virus (CYDV-RPV)/CYDV-RPV satellite RNA	322 nt		M63666
Nepovirus			
Arabis mosaic virus (ArMV)/ArMV satellite RNA	300 nt		NC001546
Tobacco ringspot virus (TRSV)/TRSV satellite RNA	359 nt		M14879
Sobemovirus			
Lucerne transient streak virus (LTSV)/LTSV satellite RNA	324 nt		X01984
Rice yellow mottle virus (RYMV)/RYMV satellite RNA	220 nt		NC003380
Solanum nodiflorum mottle virus (SNMV)/SNMV satellite RNA	377 nt		J02386
Subterranean clover mottle virus (SCMV)/SCMV satellite RNA	332 nt, 388 nt		M33000
Velvet tobacco mottle virus (VTMoV)/VTMoV satellite RNA	365–366 nt		J02439

Table 3 Satellite-like and virus-associated nucleic acids

Helper virus/satellite-like/virus-associated nucleic acid	*Size of nucleic acid*	*Encoded protein*
Satellite-like ssRNAs[a]		
Benyvirus		
Beet necrotic yellow vein virus (BNYVV)/BNYYV RNA 3	1754 nt	~25 kDa
BNYVV/BNYVV RNA 4	1467 nt	~31 kDa
Umbravirus		
Groundnut rosette virus (GRV)/GRV satellite RNA	895–903 nt	
Virus-associated nucleic acids[b]		
Hepadnavirus		
Hepatitis B virus (HBV)/hepatitis delta virus (HDV)	1979 nt	~22 kDa (δAg-S) ~24 kDa (δAg-L)
Luteovirus		
Beet western yellows virus (BWYV) /	2843 nt	~85 kDa, plus others
BWYV-associated RNA (BWYVaRNA)		
Carrot red leaf virus (CRLV) /		
CRLV-associated RNA (CLRVaRNA)	2835 nt	~85 kDa, plus others
Begomovirus		
Cotton leaf curl virus (CLCuV)/		
CLCuV-associated DNA 1 (CLCuVaDNA 1)	1376 nt	~33 kDa
Nanovirus		
Banana bunchy top virus (BBTV)/BBTV-associated DNAs S1, S2, and S3	~1100	
Faba bean necrotic yellows virus (FBNYV)/FBNYV-associated DNAs C1, C7, C9, and C11	~1000	~33 kDa
Milk vetch dwarf virus (MDV)/MDV-associated DNAs C1, C2, C3, and C10	~1000	~33 kDa
Subterranean clover stunt virus (SCSV)/SCSV-associated DNAs C2 and C6	~1000	~33 kDa

[a]Dependent on a helper virus for replication and encapsidation, but is essential for vector transmission of the helper virus.
[b]Dependent on a helper virus for encapsidation, but not for replication.

having in common the fact that they are not required for the replication of the HV.

1. Satellite viruses:
   - subgroup 1 – chronic bee-paralysis virus-associated satellite virus
   - subgroup 2 – satellites that resemble tobacco necrosis virus
2. Satellite nucleic acids:
   - ssDNA satellites
   - dsRNA satellites
   - ssRNA satellites: (1) subgroup 1 – large, ssRNA satellites; (2) subgroup 2 – small, linear, ssRNA satellites; (3) subgroup 3 – circular, ssRNA satellites.

Some RNAs previously described as satellite RNAs contribute to the natural means of transmission of the HV and thus are considered satellite-like RNAs, rather than true satellites (**Table 3**). In other cases, there are nucleic acids that are dependent on the HV for encapsidation, but

not replication. These subviral agents are considered here as virus-associated nucleic acids (**Table 3**) and will be described and differentiated below.

General Properties and Effects of Satellites

Satellite Viruses

Best characterized of the satellite viruses (**Table 1**) are the satellites that resemble TNSV, all of them depending, for their replication, on plant viruses with ssRNA genomes. All satellite viruses in this subgroup contain an ssRNA of 800–1200 nt. Particles are isometric, 17 nm in diameter, with a $T=1$ symmetry, built of 60 protein subunits. The particle structure has been determined at high resolution by X-ray diffraction for TNSV, tobacco mosaic satellite virus (TMSV), and panicum mosaic satellite virus (PMSV), with ~80% of the encapsidated RNA in stem–loop structures. Particle structure differs from that of their HV. In spite of the different structure of CP and virus particle, the particles of both the satellite and HV may share important properties; for example, the particles of TNSV and TNV are able to bind specifically to the zoospores of the vector fungus *Olpidium brassicae*. The 1000–1200 nt RNA of TNSV does not have a methylated cap structure or a genome-linked protein (VPg) in its 5′ end. Unlike most plant virus RNAs it has a phosphorylated 5′ end. The 3′ termini may share a structure with that of the HV genomic RNA (gRNA); for instance, in both tobacco mosaic virus and TMSV, the 3′ termini form tRNA-like structures and are aminoacylable with histidine. *Cis*-acting sequences necessary for RNA amplification have been mapped at the 5′ and 3′ nontranslated regions (NTRs) of PMSV RNA as well as within the CP open reading frame (ORF), and are conserved in a D RNA, which is maintained by PMSV. A translational enhancer domain has been mapped in the 3′ NTR of TNSV. In addition to the ORF encoding the CP, some satellite viruses contain other ORFs; it remains unclear if the encoded products have any role *in vivo*.

Interference with the accumulation of the HV has been described for TNSV. Satellite viruses can also modify the symptoms induced by the HV, as is the case with PMSV, which in co-infection with panicum mosaic virus (PMV) enhances the mild symptoms of PMV to cause a severe mosaic and chlorosis. Symptom induction is due to the PMSV CP, and a chlorosis-inducing domain has been mapped. In addition to its structural and symptom-inducing functions, the CP of PMSV is involved in systemic movement, binds PMV particles, and counters the effects of post-transcriptional gene silencing suppressors.

The particles of TNSV may contain a noncoding satellite RNA of about 620 nt, which depends on TNV for replication and on TNSV for encapsidation. A satellite RNA with similar dependence relationships has been described for PMV and PMSV. These systems are good examples of the complexity of dependence relationships in satellitism.

The other subgroup of satellite viruses contains satellites found associated with chronic bee-paralysis virus (CBPV). The satellites consist of three RNA species of about 1.1 kbp, which can be encapsidated either in 17-nm isometric particles by the satellite-encoded CP, or by the CP of the HV, CBPV. The satellites interfere with CBPV replication.

Satellite Nucleic Acids

ssDNA Satellites

All the DNA satellites reported to date are associated with a single virus genus, *Begomovirus*, in the family *Geminiviridae* (**Table 2**). This group of over 100 viruses is transmitted by whiteflies and their geminate particles encapsidate either one or two ssDNA species, each of about 2700 nt, as well as satellite DNAs that may occur. The first satellite DNA was found in association with TLCV and is a single-stranded (ss), circular DNA molecule of 682 nt, which can be supported for replication by a number of begomovirus species. It lacks any significant ORF or identifiable promoter element and does not contribute to the infection of TLCV. More recently, a large group of ss, satellite DNAs termed DNA β has been isolated. They are related to TLCV satellite DNA but are about twice the size of the TLCV satellite DNA and encode a protein known as βC1. The C1 protein is expressed from a single complementary-sense transcript with conserved regulatory elements.

The search for the causal agents of Ageratum yellow vein disease led to the discovery of the first DNA β satellite because it was required for disease symptom expression. Subsequently, a similar role was established for cotton leaf curl virus (CLCuV) DNA β, which is part of a disease complex causing major crop losses in Pakistan. Some geminivirus satellite DNAs reported as DNA β are of similar size to TLCV satellite DNA and lack a βC1 gene. Geminivirus satellite DNAs therefore can be considered a single group in which some species are defective for encoding a protein.

Geminivirus satellite DNAs can exert a drastic effect on the symptoms produced by their HV. The pathogenesis is mediated by the βC1 protein. The mechanism of βC1-mediated pathogenesis is not clear; however, the protein causes a drastic disease-like phenotype when expressed transgenically. This severe effect on host plants suggests changes to growth pattern and is accompanied by vein thickening, enations, and development of leaf-like structures. The protein has been demonstrated to be a suppressor of gene silencing, raising the possibility that it may control host functions by microRNA regulation.

Geminivirus satellite DNAs do not show a strict affinity for HV. TLCV satellite DNA is supported for

replication by viruses as diverse as tomato yellow leaf curl virus, African cassava mosaic virus, and even beet curly top virus, which belongs to a different genus. In another case, the DNA β associated with Ageratum yellow vein disease was found to be maintained in experimental plants infected with Sri Lankan cassava mosaic virus (SLCMV), which has a bipartite genome. This interaction altered the host range of SLCMV to include Ageratum. Interestingly, the satellite could substitute for DNA B component implying a functional similarity. Other evidence supporting the role of geminivirus satellite DNAs in movement has also been obtained. The DNA A component of tomato leaf curl New Delhi virus (TLCNDV) is not capable of systemic infection in the absence of DNA B and remains confined to the sites of inoculation where it is capable of replication; however, CLCuV DNA β can substitute DNA B of TLCNDV to restore systemic infection in tomato. Surprisingly, systemic infection of DNA A component alone, accompanied by symptoms, could also be mediated by transient expression of the βC1 protein.

dsRNA Satellites

A number of examples of dsRNA satellites have been described, but only two are recognized officially at this time (**Table 2**): one group is associated with the yeast *Saccharomyces cerevisiae* and is designated the M satellites of L-A dsRNA virus; the other group of three dsRNAs is associated with the Trichomonas vaginalis T1 virus (TVTV) of the eponymous protozoan. Both HVs are in the family *Totiviridae*. In the case of the three TVTV satellite RNAs of 497, ~700, and ~1700 bp, the ds satellite RNAs are maintained only by some isolates of TVTV, which both replicate and encapsidate these dsRNAs. The L-A dsRNA virus M satellites consist of several dsRNAs varying from 1.0 to 1.8 kbp in size. These dsRNA satellites are dependent on genes of the HV for both replication and encapsidation. The M1 dsRNA satellite encodes a toxin which kills other yeasts not harboring this dsRNA. In its prototoxin form, this protein provides immunity to the yeast secreting the toxin. Other L-A dsRNA viruses have different M satellites associated with them, all of them also encoding toxin and immunity systems.

All of the putative dsRNA satellites were found with fungi, in association with dsRNA viruses in the families *Hypoviridae*, *Narnaviridae*, *Partitiviridae*, and *Totiviridae*. One of these dsRNA satellites, from Ophiostoma novo-ulmi mitovirus 3a (OnuMV3a) in the genus *Mitovirus*, of the family *Narnaviridae* consists of a group of dsRNAs of 738–767 bp, designated OnuMV3a S-dsRNA (**Table 2**). These dsRNA satellites did not affect the hypovirulence associated with their HV in the fungus *Sclerotinia homeocarpa*. Their nucleotide sequences indicate that these OnuMV3a S-dsRNAs did not have the coding capacity for their own replicase, and thus presumably depend on the replicase of the HV.

ssRNA Satellites

Subgroup 1: Large ssRNA satellites

Subgroup I satellites have messenger RNA properties. These satellite RNAs are between 0.8 and 1.5 kb in size and encode nonstructural proteins that are expressed *in vivo* (**Table 2**). Most satellites in this subgroup are associated with nepoviruses. Large ssRNA satellites of nepoviruses share the 5′ and 3′ structural features of the gRNAs of the HV: a 3′-terminal poly(A) sequence and a 5′-terminal VPg that, in the analyzed instances, is indistinguishable from that on the HV genome and thus, is encoded by it. The encoded nonstructural protein of different satellite RNAs are basic proteins of 36–48 kDa. For the satellite RNAs of tomato black ring virus (TBRV), grapevine fanleaf virus (GFLV), and arabis mosaic virus (ArMV), it has been shown that the encoded proteins are needed for the replication of the satellite RNA. Most large satellite RNAs of nepoviruses do not seem to have an effect on the accumulation or pathogenicity of the HV. However, this may depend on the experimental system, as the large satellite RNA of ArMV was shown to modulate the symptoms of the HV depending on the species of host plant.

Large satellite RNAs (1342–1347 nt) have been found to be associated with many isolates of the benyvirus beet necrotic yellow vein virus (BNYVV). These satellite RNAs, designated BNYVV RNA 5, encode a protein of 26 kDa, which is responsible for intensification of the rhizomania disease induced by infection of BNYVV RNAs 1 and 2, plus its two satellite-like RNAs 3 and 4.

Another large satellite RNA has been found to be associated with the potexvirus bamboo mosaic virus (BaMV), the only satellite RNA encapsidated into rod-shaped particles. BaMV satellite RNA encodes a 20-kDa protein that is expressed *in vivo* but is not needed for satellite RNA replication. Interestingly, this protein shares significant sequence similarity with the structural protein of the PMSV, and binds cooperatively to RNA, with a preference for the satellite RNA. The presence of BaMV satellite RNA significantly reduces the accumulation of BaMV RNA. *Cis*-acting sequences required for BaMV satellite RNA replication have been mapped at the 5′ NTR, and comprise a stem–loop structure that is conserved among BaMV satellite RNA variants.

Subgroup 2: Small, linear ssRNA satellites

This subgroup contains ss satellite RNAs associated with seven genera of plant viruses (**Table 2**), although many of the members are associated with nepoviruses. There are no circular forms of these satellite RNAs present in infected cells, although dimer or higher multimer forms may be detected in virions and/or in infected cells. These satellite RNAs vary in size from ~200 to ~800 nt and do not appear to encode proteins, although isolates of some satellite RNAs contain nonconserved ORFs. These RNAs

appear to have highly ordered secondary structures, which is probably responsible for the high stability and infectivity of these RNAs, as well as their biological properties. Most of the small ss satellite RNAs either have no effect on or reduce the accumulation of their HV. This reduction in HV accumulation often, but not always, results in a decrease in the disease symptoms induced by the HV. Moreover, in a few cases, the presence of the satellite RNA reduced or had no effect on the HV accumulation, but intensified the disease induced by the HV. One of the latter, a tomato necrosis-inducing satellite RNA of the cucumovirus cucumber mosaic virus (CMV), could even induce necrosis in tomato independent of the HV, if expressed in the complementary-sense orientation within the genome of virus vector but not when expressed as a transgene; however, in the case of other satellite RNAs of CMV, different strains of CMV and the related HV tomato aspermy virus (TAV) have been used to show that the HV makes a contribution to the disease syndrome associated with the satellite RNA. The basis for satellite RNA-mediated attenuation of disease symptoms is not clear, but may not be due simply to reduction of the HV titer, since CMV satellite RNAs supported by TAV show an attenuation of symptoms, but no effect on the accumulation of the HV. Similarly, the reduction of HV level may not be due simply to competition between the HV and the satellite RNA for the same replicase, since data from TCV and CMV satellite RNAs indicate that satellite RNAs may affect the suppression of RNA silencing function of their associated HV, leading to a reduction in the titer of the virus due to RNA silencing. A general model for satellite- and viroid-mediated intensification of disease symptoms has been proposed, caused by silencing inducing RNAs generated from such subviral RNAs acting on specific host mRNAs.

Subgroup 3: Small, circular ssRNA satellites

This subgroup of satellite RNAs contains members that vary in size from 220 to 388 nt, and are associated with three genera of plant viruses (**Table 2**). The satellite RNAs exist in circular as well as linear forms in infected plant cells, but only the circular satellite RNA form is encapsidated by the helper sobemovirus, while only the linear satellite RNA form is encapsidated by the helper polerovirus and nepoviruses. Multimeric, linear forms of these satellites are produced in infected cells and, in some cases, these are also packaged into virus particles. The multimeric forms are generated via a rolling-replication mechanism and the unit-length linear as well as circular forms are generated via ribozymes. There is no mRNA activity associated with these various satellite RNAs, and they all have a high degree of secondary structure. The satellite RNAs either have no effect on (sobemovirus satellites) or reduce (nepovirus and polerovirus satellites) the accumulation of their HV. In addition, the presence of satellite RNAs either reduces (nepovirus and polerovirus satellites) or exacerbates (sobemovirus satellites) the symptoms induced by the helper virus. The molecular basis for symptom attenuation or intensification is unknown.

Satellite-Related Nucleic Acids

A number of subviral nucleic acids have been described that have in some instances been referred to as satellite RNAs or DNAs. However, as these nucleic acids either contribute to the infection cycle of the HV, or are not dependent upon an HV for their replication, they are better described as satellite-like or virus-associated nucleic acids, respectively (**Table 3**).

Satellite-like ssRNAs

This group of subviral RNAs includes the satellite RNAs of groundnut rosette virus (GRV) and RNAs 3 and 4 of BNYVV. In the case of satellite RNAs of GRV, they are required for transmission of GRV by the luteovirus groundnut assistor virus. How these satellite RNAs of 895–903 nt affect transmission is unknown, since they do not code for any functional proteins. Some of the satellite RNAs of GRV also affect accumulation of the HV and the symptoms induced by the HV, causing either attenuation due to severely reduced replication of the HV, or exacerbation, producing unique symptoms. In the case of BNYVV, where only RNAs 1 and 2 are essential for replication, movement within leaves and particle assembly, RNA 3 (1774 nt) is required for spread of the virus into roots and RNA 4 (1467 nt) is essential for virus transmission by the soil-fungus vector of BNYYV. Thus, BNYYV RNAs 3 and 4 are considered satellite-like, in that they contribute to the natural infection cycle of the virus. By contrast, BNYYV RNA 5 is a true satellite, in that it is not required for any phase of the infection cycle of BNYYV, although its encoded 26 kDa protein in combination with the 25 kDa protein encoded on RNA 3 intensifies symptom expression of the disease rhizomania.

Virus-associated nucleic acids

This group of subviral agents does not fit the above definition of satellites, but as they are peripheral to but dependent on an HV for their encapsidation, they have sometimes been referred to as satellite-like RNAs or DNAs. However, as a functional distinction exists between these subviral nucleic acids and those of the satellite-like agents described above, which are dependent on an HV for their replication, these subviral agents are collectively referred to as (virus-) associated nucleic acids.

Hepatitis delta virus

Hepatitis delta virus (HDV) is a subviral agent of the human pathogen, hepatitis B virus (HBV). HDV is replicated in the nucleus by the host DNA-dependent RNA

polymerase II, but is dependent upon the envelope proteins of HBV for its encapsidation and transmission, and is thus not a satellite. The HDV (1679 nt) RNA folds into an unbranched rod-like structure similar to that of viroids, and in common with the circular ssRNA satellites, contains ribozymes that are involved in the processing and maturation of this RNA during replication. The HDV RNA encodes two forms of a protein designated the delta antigen (δAg). The smaller, 22 kDa form (δAg-S), which is a nuclear phosphoprotein, is required for replication of HDV RNA, while the 24 kDa larger form (δAg-L), which contains an additional 19 aa at the C-terminus and is produced late in infection, is required along with δAg-S for assembly of the HDV RNA into HBV envelope particles. δAg-L also acts as an inhibitor of HDV replication. HDV intensifies the severity of liver disease caused by HBV.

Luteovirus-associated RNAs

Some isolates of two luteoviruses, beet western yellows virus and carrot red leaf virus (CRLV) were found to have associated with them additional RNAs of size 2.8 kb. These associated RNAs encoded their own viral replicases, but required the respective luteoviruses for their movement, encapsidation, and transmission. These RNAs, designated BWYVaRNA (2843 nt) and CRLVaRNA (2835 nt), have similar genome organizations, are about 50% similar in sequence, and both intensify the disease caused by their HV. CLRVaRNA together with CLRV and the umbravirus carrot mottle virus induced carrot motley dwarf disease.

Begomovirus- and nanovirus-associated DNAs

Circular ssDNA species of about 1300 nt, unrelated to geminivirus genomes, have been isolated from plants infected with some Old World begomoviruses. These molecules, named DNA 1, resemble a DNA component of nanoviruses. CLCuV-associated DNA1 is an example (**Table 3**). They encode their own replicase-associated protein (Rep) and replicate independent of the HV. In the absence of a nanovirus CP which allows aphid transmission, the virus-associated DNAs are encapsidated in helper geminiviruses and are transmitted by their whitefly vector. Nanoviruses themselves may also contain virus-associated DNAs. These are about 1.0 or 1.1 kbp in size and capable of autonomous replication but require the respective nanovirus for their movement, encapsidation, and transmission by aphids.

Replication and Structure

Satellite DNAs

The replication of geminivirus satellite DNAs mimics that of the HV. Unlike RNA viruses, geminiviruses do not encode a polymerase and depend on host DNA polymerases for replication. The only geminivirus-encoded protein essential for replication is Rep. Geminivirus satellite DNAs utilize the HV Rep and contain the conserved sequence for DNA nicking by the Rep. Replication takes place by a combination of rolling circle and recombination dependent mechanisms and the DNA is encapsidated in the HV virion. The replication is independent of βC1 expression.

Sequence comparisons at the nucleotide and amino acid levels reveal a number of conserved structural features, despite a significant level of sequence divergence. These include an A-rich region, a sequence of about 80 nt referred to as the satellite conserved region, and putative stem–loop structure containing a mononucleotide motif, also present in geminivirus genomes.

Satellite RNAs

A general property of satellite RNAs is that their replication depends on the replication machinery of the HV, which involves virus- and host-encoded factors. Hence, replication of satellite RNAs depends on interactions with both the HV and the host plant. Satellite RNAs may or may not share structural features at their 5′ and 3′ termini in common with the HV RNA. For instance, the large satellite RNAs of the nepoviruses have an HV-encoded VPg at the 5′ end and a poly(A) tail at the 3′ end, as do their HV RNAs, but the satellite RNAs of CMV have a 5′ cap structure, as do the CMV RNAs, but unlike the HV, the satellite RNAs do not have a tRNA-like structure that can be valylated at the 3′ end. Structural differences and, often, differences in the replication process between the satellite RNA and the HV RNA, indicate that the replication machinery of the HV may need to be adapted to replicate the satellite RNA, in ways which are not completely understood. The HV replication complex could be modified by satellite-encoded factors, as has been hypothesized for the large satellite RNAs of nepoviruses, or by unidentified host factors. It should be pointed out that in the best characterized systems, the efficiency of replication depends on the host plant. For CMV satellite RNAs, replication efficiency also depends on the strain of HV. Similarly, while the expression of the HV RNA-dependent RNA polymerase was enough for the replication of cereal yellow dwarf virus (CYDV) satellite RNA in the homologous host, this was not the case for cymbidium mosaic virus satellite RNA in a heterologous host system, in which, however, a DI RNA was amplified.

Replication has been studied best in the small noncoding satellite RNAs. For the small linear satellite RNAs of TCV and CMV, multimeric forms of both positive (arbitrarily defined as the encapsidated sense) and negative sense are found in infected tissues. The junction between monomers can be perfect or have deletions. Circular forms are not found, and replication does not proceed through

a rolling-circle mechanism. Regulatory sequences for RNA replication have been mapped in detail in the satellite RNAs of TCV. In the hybrid satC molecule, a hairpin structure is a replication enhancer and has a role in depressing the accumulation of the HV. Also, structural motifs favoring recombination have been analyzed extensively in satD and satC of TCV.

The replication of small, circular satellite RNAs is by a rolling-circle mechanism; upon, infection, linear, multimeric forms of the negative strand are synthesized. The multimeric plus strand is then synthesized on this template or on negative strand circular monomers, depending on the satellite RNA. The positive strand multimer is cleaved autocatalytically and ligation occurs for those satellite RNAs that are encapsidated as a circular form. Hammerhead and hairpin ribozyme structures are found in these satellite RNAs monomers or dimers, which catalyze hydrolysis or hydrolysis and ligation, respectively. Other structures required for replication and encapsidation have been mapped in CYDV satellite RNA.

For one circular satellite RNA, a DNA counterpart has been shown to occur in the host genome, as a retroid element. HDV, which depends on HBV for encapsidation, shares structural features and replication mechanisms with the small, circular satellite RNAs.

Structural analyses have been done mostly with small satellite RNAs. Models for the *in vitro* secondary structure have been proposed for several satellite RNAs (e.g., CMV satellite RNA, TCV satellite RNAs, TRSV satellite RNA, and CYDV satellite RNA) based on nuclease sensitivity and chemical modification of bases data. For CMV satellite RNA, *in vivo* models have also been proposed. All analyzed satellite RNAs have a high degree of secondary structure, with more than 50% and up to 70% of bases involved in pairing. The high degree of secondary structure could explain the high stability of these molecules as well as the very high infectivity reported for some of them, such as CMV satellite RNA and TCV satellite RNA. The high degree of secondary structure may also be related to their biological activity, which for these noncoding molecules must depend on structural features. As has been detailed in other sections, sequences, structural domains, and tertiary interactions involved in pathogenicity and replication have been well characterized for some small satellite RNAs.

Sequence Variation and Evolution

Sequence variation and evolution has been analyzed most for CMV satellite RNA. Early experiments under controlled conditions showed a high variability and genetic plasticity. Populations started from cDNA clones rapidly evolved to a swarm of sequences whose master sequence changed upon passage on different hosts. Analysis of genetic variants from natural populations showed again a high diversity for CMV satellite RNA. In natural populations, satellite RNA diversification and evolution proceeded by mutation accumulation and by recombination. Constraints to genetic variation were analyzed, and were related to the maintenance of base pairing in secondary structure elements. Population diversity of CMV satellite RNA was higher than for the HV, and the population structures of CMV and the satellite RNA were not correlated. These analyses also showed that CMV satellite RNA behaved in nature as a molecular hyperparasite spreading epidemically on the CMV populations. The incidence of CMV satellite RNA in CMV populations has been shown to be low, except during episodes of epidemics of tomato necrosis. High diversity of natural populations has also been shown for PMSV RNA and for BaMV satellite RNA. For BaMV satellite RNA, the diversity was highest in the NTRs. Conversely, the population of rice yellow mottle virus satellite RNAs over sub-Saharan Africa showed little sequence diversity. The incidence of these three satellite RNAs in the analyzed populations of the respective HV was, in all cases, high.

Phylogenetic analyses divide geminivirus satellites broadly into two categories, those isolated from host plants of the family Malvaceae and the rest isolated from other plants, mostly in the Solanaceae. Compared to satellite RNAs, satellite DNAs do not exhibit a wide diversity. They are structurally similar, and lack strict specificity for HV species. Geminivirus satellite DNA sequences analyzed exhibit a minimum overall similarity of about 47% and 37%, at the nucleotide level and amino acid level, respectively. To date, over 120 DNA β sequences have been reported in data banks; however, many of these are members of the same species because their overall sequence similarity is well above 90%. Some examples of distinct geminivirus satellites that have been adequately characterized are listed in **Table 2**.

There has been much speculation on the origin of satellite RNAs. For CMV satellite RNA and TCV satellite RNA, it has been suggested that they could have been generated out of small sequences synthesized upon the HV RNA as a template by the HV polymerase. This hypothesis is no longer sustained by those that proposed it. Whatever the mechanism of generation of the satellite RNAs, it seems that the satellitism as a phenomenon, that is, the dependence on a certain virus for replication, has evolved independently several times. This is suggested by the lack of correlation between satellite and HV taxonomy, and by the existence of subviral nucleic acids with different degrees of dependence on an HV. For instance, an evolutionary line from nondependent viruses to satellites such as BaMV satellite RNA through satellite viruses could have proceeded by size and information content reduction. A phylogenetic relationship between viroids and small circular satellite RNAs has also been proposed.

Expression of Foreign Sequences from Satellite Vectors

The satellite RNAs of BaMV and BNYVV, as well as the satellite DNA of tomato yellow leaf curl China virus isolate Y10 (TYLCCNV-Y10), all of which express proteins not required for the replication or spread of the satellite or HV, have been used as expression vectors of foreign sequences. In the case of the satellite RNA of BaMV, the expression of such sequences and the accumulation of the satellite RNA were reduced considerably in systemically infected leaves. Nevertheless, this satellite expression system has been useful for studying *cis*- and *trans*-acting replication signals. The 26 kDa protein encoded by RNA 5 of BNYYV has been replaced with the sequence encoding the green fluorescent protein, which was expressed in both the inoculated leaves and systemically infected leaves. The small size of the satellite DNA of TYLCCNV-Y10 precludes use of this system for expression of most genes, but the system has been used to express plant gene segments inducing RNA silencing in several plants species. The TMSV system also has been used to express plant gene sequences, in place of its CP, resulting in RNA silencing of those plant genes, but not of the satellite virus or its HV. The modified TMSV expression vector was able to spread through the plant in the absence of its CP.

Satellite-Mediated Control of Viruses

The ability of some satellite RNAs to attenuate the symptoms induced by their HV has been utilized to develop strategies for disease control. In one strategy, the satellite RNA of CMV has been used in combination with mild strains of CMV as a 'vaccine', to pre-inoculate tomato plants and ameliorate symptoms induced by the replicating challenge virus (through the attenuating ability of the vaccine satellite RNA), in fields and greenhouses. Moreover, the vaccine could also prevent or reduce the frequency of infection of both the HV (through cross-protection by the HV of the vaccine strain) and of satellite RNAs that exacerbate the disease caused by the HV (through cross-protection by the vaccine satellite RNA). In a second strategy, the satellite RNAs of CMV, TRSV, and GRV have been expressed in transgenic plants, and provided either resistance to infection by the respective HV, or tolerance to the disease induced by the HV and/or related pathogenic satellites.

See also: Beta ssDNA Satellites.

Further Reading

Briddon RW and Stanley J (2006) Subviral agents associated with plant single-stranded DNA viruses. *Virology* 344: 198–210.

Dry IB, Krake LR, Rigden J, and Rezaian MA (1997) A novel subviral agent associated with a geminivirus: The first report of a DNA satellite. *Proceedings of the National Academy of Sciences, USA* 94: 7088–7093.

Gossele V, Fache I, Meulewaeter F, Cornelissen M, and Metzlaff M (2002) SVISS – A novel transient gene silencing system for gene function discovery and validation in tobacco plants. *Plant Journal* 32: 859–566.

Palukaitis P and García-Arenal F (2003) Cucumoviruses. *Advances in Virus Research* 62: 241–323.

Simon AE, Roossinck MJ, and Havelda Z (2004) Plant virus satellite and defective interfering RNAs: New paradigms. *Annual Reviews in Phytopathology* 42: 415–437.

Tao X and Zhou X (2004) A modified satellite that suppresses gene expression in plants. *Plant Journal* 38: 850–860.

Vogt PK and Jackson AO (eds.) (1999) *Satellites and Defective Viral RNAs*. Heidelberg: Springer.

Seadornaviruses

H Attoui, Université de la Méditerranée, Marseille, France
P P C Mertens, Pirbright Laboratory, Woking, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arthralgia Joint pain.
Cerebral palsy Disability resulting from damage of the brain before, during, or shortly after birth and manifested as muscular and speech disturbances.
Hemiplegia Total or partial paralysis of one side of the body resulting from an affliction of the motor centers in the brain.
Myalgia Muscle pain.
Viremia The presence of virus in the blood.

Introduction

The family *Reoviridae* includes 12 recognized genera and 3 proposed genera (**Table 1**). The genera *Coltivirus*, *Seadornavirus,* and *Orbivirus* include arboviruses that can cause disease in animals (including humans).

In 1991, the International Committee for the Taxonomy of Viruses (ICTV) formally recognized the genus *Coltivirus* (sigla from Colorado tick virus) containing the 12-segmented double-stranded RNA (dsRNA) animal viruses that were previously classified within the genus *Orbivirus*. *Colorado tick fever virus* is the prototype species of the genus *Coltivirus* and includes strains of several tick-borne viruses (including isolates of Colorado tick fever virus (CTFV) from humans and ticks; California hare coltivirus (CTFV-Ca) from a hare in northern California; and Salmon River virus (SRV) from humans in Idaho). The genus *Coltivirus* also contains a second distinct species: *Eyach virus* (EYAV) isolated from ticks in Europe.

The seadornaviruses are 12-segmented mosquito-borne dsRNA viruses that were initially considered as tentative species within the genus *Coltivirus*. However, based on their antigenic properties and nucleotide sequence comparisons to the coltiviruses and other members of the *Reoviridae,* their taxonomic status was reassessed. They are now formally recognized as members of the genus *Seadornavirus* (sigla: from *South East Asian Dodeca RNA virus*; genus recognized in 2000), which includes the type species *Banna virus* (BAV), isolated from humans; two other species, *Kadipiro virus* (KAD) and *Liao ning virus* (LNV); and several unclassified isolates from mosquitoes. The seadornaviruses have been implicated in a variety of pathological manifestations in humans, including flu-like illness and neurological disorders, as described below.

Historical Overview of Seadornaviruses

The first seadornaviruses were reported in 1980 and 1981, when numerous isolates of 12-segmented dsRNA viruses were made from pooled homogenates of mosquitoes in Indonesia and were initially designated as JKT-6423, JKT-6969, JKT-7043, and JKT-7075. These original viruses were recently renamed as Banna virus (BAV) and Kadipiro virus (KDV). Shortly after the initial identification of BAV in Indonesia, a similar virus was isolated from the serum and cerebrospinal fluid (CSF) (2 isolates) and sera (25 isolates) of human patients with encephalitis in Yunnan province in the south of China and BAV was reported as a causative agent of human viral encephalitis. Seadornaviruses have subsequently been isolated on several occasions from humans, cattle, pigs, and mosquitoes and are vectored by *Anopheles*, *Culex,* and *Aedes* species. BAV is now regarded as endemic in southeast Asia, particularly in Indonesia and China.

Table 1 Twelve recognized genera and the three proposed genera of family *Reoviridae*

	Type species			*No. of segments*	*Hosts of the member viruses*
Genus					
1. *Idnoreovirus*	*Diadromus pulchellus reovirus*			10	Insects
2. *Cypovirus*	*Cypovirus type 1*			10	Insects
3. *Oryzavirus*	*Rice ragged stunt virus*			10	Plants
4. *Fijivirus*	*Fiji disease virus*			10	Plants
5. *Orthoreovirus*	*Mammalian orthoreovirus*			10	Primates, ruminants, bats, birds, reptiles
6. *Orbivirus*	*Bluetongue virus*			10	Ruminants, equids, rodents, bats, marsupials, birds, humans
7. *Rotavirus*	*Rotavirus A*			11	Mammals, birds
8. *Aquareovirus*	*Aquareovirus A*			11	Aquatic animals
9. *Mycoreovirus*	*Mycoreovirus-1*			11 or 12	Fungi
10. *Phytoreovirus*	*Wound tumor virus*			12	Plants
11. *Coltivirus*	*Colorado tick fever virus*			12	Mammals including man
12. *Seadornavirus*	*Banna virus*	Isolates:	BAV-Ch BAV-In6423 BAV-In6969 BAV-In7043	12	Humans, porcine, cattle
	Kadipiro virus	Isolates:	KDV-Ja7075		
	Liao ning virus	Isolates:	LNV-NE9712 LNV-NE9731		
Proposed genera					
1. Dinovernavirus	Aedes pseudoscutellaris reovirus			9	Insects
2. Mimoreovirus	Micromonas pusilla reovirus			11	Algae
3. Cardoreovirus	Eriocheir sinensis reovirus			12	Crabs



Figure 1 The known world distribution of seadornaviruses (shaded areas): BAV and LNV in China and BAV and KDV in Indonesia.

Distribution and Epidemiology

BAV was initially isolated from mosquitoes and mammalian species in 1980–81, between latitudes of 39° N and 38° S, in southeast Asia (**Figure 1**). BAV was subsequently isolated from humans in the Yunnan province of southern China (Xishuangbanna prefecture in 1987). A virus identified as BAV was also isolated in 1987 from patients with fever and flu-like manifestation, in Xinjiang province of western China province. In 1992, two 12-segmented dsRNA viruses were isolated from serum of patients with an unknown fever in Mengding county of Yunnan province. Eight further isolates of antigenically Banna-like viruses were made from the sera of 24% of patients with an unidentified fever and viral encephalitis in Xinjiang province of northwest China in 1992. Twelve-segmented dsRNA viruses that are described as antigenically related to BAV have also been isolated from humans in various other provinces in China, including Beijing, Gansu, Hainan, Henan, and Shanshi and consequently BAV is now classified as a BSL3 arboviral agent.

Four viruses initially identified as JKT-6423, JKT-6969, JKT-7043, and JKT-7075, were isolated from *Culex* and *Anopheles* mosquitoes in central Java in Indonesia. The first three isolates were isolates of BAV, while JKT-7075 represents the only known strain of the *Kadipiro virus* species.

Recently, isolates of two distinct serotypes of *Liao ning virus* (LNV) were obtained from *Aedes dorsalis* mosquito in the Liaoning province of north eastern China.

Vectors, Host Range, and Transmission

BAV is the only seadornavirus that has been isolated from humans. Experimentally, seadornaviruses have also been shown to infect and replicate in adult mice and can be detected in the infected mouse blood from 3 to 5 days post infection. LNV replicates readily in several mammalian cell lines with massive lytic effect and kills adult mice. KDV and LNV have only been isolated only from mosquitoes. Indeed seadornaviruses are thought to be transmitted by mosquitoes and have been isolated from *Culex vishnui, Culex fuscocephalus, Anopheles vagus, Anopheles aconitus, Anopheles subpictus*, and *Aedes dorsalis*.

BAV, KDV, and LNV all occur in tropical and subtropical regions, where other mosquito-borne viral diseases, including Japanese encephalitis (JEV) and dengue, are endemic. This suggests that conclusive diagnosis and differentiation of these diseases may be difficult, based on clinical signs alone. Indeed several cases were diagnosed as JE in China (Henan province, Naijing city, and Fujian province in summer–autumn season of 1994), despite the absence of detectable JEV, or JEV-specific antibodies. However, a proportion of these patients (7/98) did show a 4- to16-fold rise in anti-BAV immunoglobulin G (IgG) by enzyme-linked immunosorbent assay (ELISA), indicating a BAV infection. A further 130 sera (from 1141 patients diagnosed with JE or viral encephalitis) from Chinese health institutes were also positive for BAV-specific immunoglobulin M (IgM).

In the summer of 1987, 16 BAV-like isolates were recovered from sera of 53 cattle (30% of the tested animals) and 4 isolates recovered from sera of 13 pigs (30% of the tested animals) collected in a slaughterhouse in the Dai nationality autonomous prefecture of the Xishuangbanna area of Yunnan province, where BAV has been previously isolated from humans.

BAV is regarded as 'a common cause of viral encephalitis and fever in humans during summer–autumn in

China. Its high isolation rate and wide geographical distribution make BAV an important public health problem in China. It is likely that seadornaviruses are also endemic in other countries within Southeast Asia.

Virion Properties, Genome, and Replication

Seadornavirus particles, like those of many of the 'unturreted' reoviruses, are approximately spherical in appearance with icosahedral symmetry. The intact virion is 60–70 nm in diameter and has three concentric capsid layers, containing a total of seven structural proteins. The double-layered core-particle contains five proteins (VP1, VP2, VP3, VP8, and VP10) and is approximately 50 nm in diameter, with a central cavity that contains the 12 segments of the viral genome.

VP1 (the polymerase) and VP3 (the capping enzyme) form the transcriptase complex that is present within the core as a dimer of VP1 and a monomer of VP3. This complex is located at each of the 12 fivefold axes of the viral particle. The subcore layer is composed of 120 copies of VP2 and represents the $T = 2$ layer. The outer layer of the core is made of 780 copies of VP8 and represents the $T = 13$ layer. Based on sequence comparison to other viruses of the *Reoviridae* and in particular the rotaviruses, the seadornavirus VP10 proteins are thought to be anchored at the core surface. The outer capsid layer, which is composed of only two proteins (VP4 and VP9) (**Figure 2**), completely surrounds the core particle.

The BAV genome encodes five nonstructural proteins which are the VP5, VP6, VP7, VP11, and VP12. VP7 contains sequences which are found in various protein kinases. VP12 contains a dsRNA-binding domain and might play a role in circumventing cell defense mechanisms, possibly acting as an anti-PKR component.

Negative staining and electron microscopy have shown that the surface of the virus particles has a well-defined capsomeric structure and icosahedral symmetry. The outer surface also has spikes that are similar to those of the rotaviruses (**Figure 3(a)**). The virus core has a smooth outline that is typical of the nonturreted members of the family *Reoviridae* (**Figure 3(b)**).

Like the orbiviruses, the seadornaviruses are unstable in CsCl and readily lose their outer coat proteins. However, intact virus particles have been purified from infected tissue culture supernatant by ultracentrifugation on colloidal silica gradients (Percoll). Purified virus particles are stable at 4 °C and approximately pH 7.0 for up to 3–4 months. However, increased acidity decreases their infectivity, which is abolished at pH 3.0. The virus is more stable as an unpurified cell culture lysate at 4 °C, which is a convenient way for medium-term storage. For longer periods of storage, viruses are stable at –80 °C, and infectivity can be further conserved by addition of 50% fetal calf serum.

The infectivity of seadornaviruses is decreased considerably by heating to 55 °C. Treatment with sodium dodecyl sulfate (SDS) disrupts the virus particle and consequently abolishes infectivity completely. Infectivity is very significantly reduced by treatment with butanol,

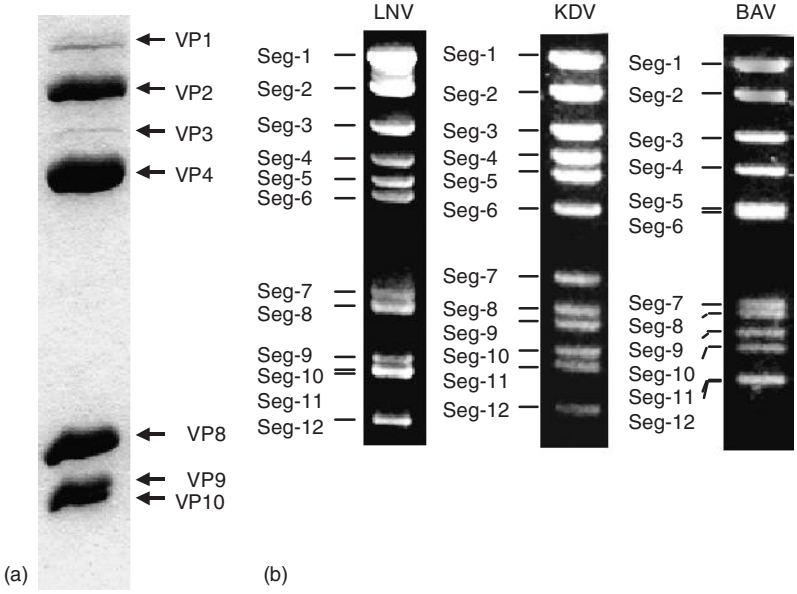


Figure 2 The PAGE profile of the structural proteins of Banna virus and the AGE profile of the genomes of seadornaviruses. (a) The PAGE profile of the structural proteins of Banna virus separated on a 10% polyacrylamide gel: VP4 and VP9 are the outer coat proteins. (b) The AGE profile of the genomes of Liao ning virus (LNV), Kadipiro virus (KDV), and Banna virus (BAV) run in a 1% agarose gel. The distinct electropherotypes of the three species allow to distinguish them.

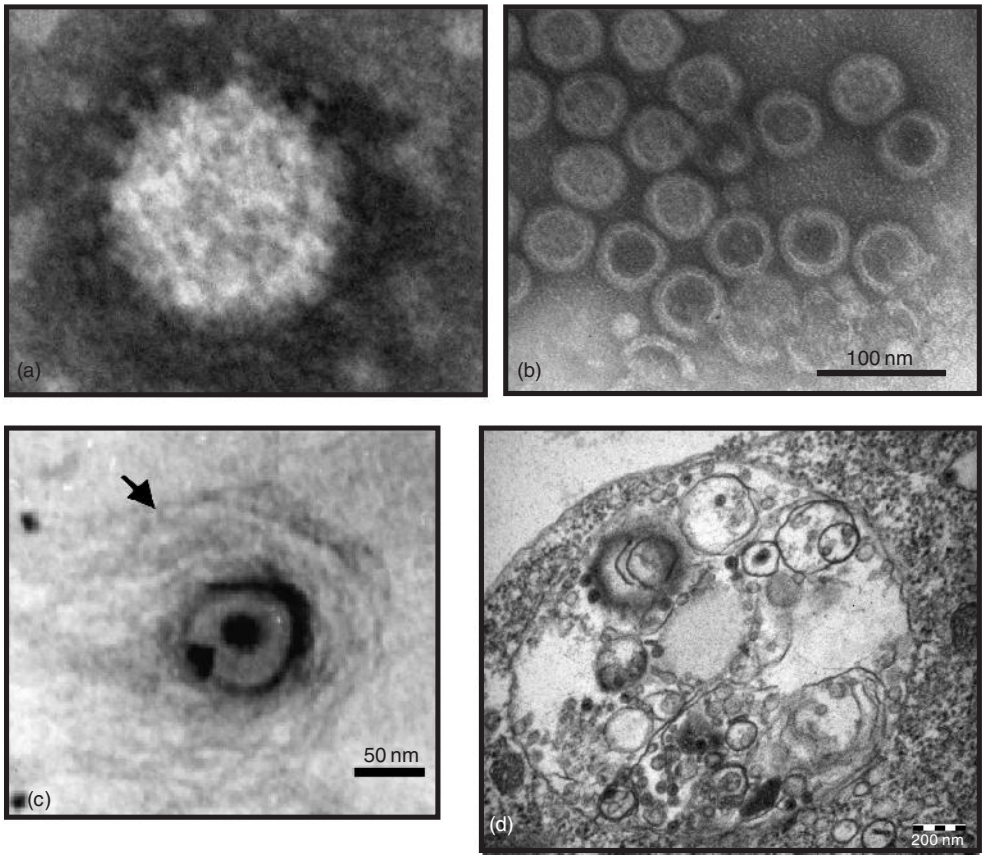


Figure 3 Electron micrographs of Banna virus particles. (a) A whole particle with spike proteins at the surface; (b) double-layered purified cores with smooth outlines; (c) particles surrounded by temporary envelope structure; and (d) thin section of infected C6/36 cells with virus particles seen within vacuole structures.

although organic solvents such as Freon 113 or Vetrel XF do not affect virion structure or infectivity and have been used successfully during purification of virus particles from cell lysates.

During replication, core-like particles are found within vacuole-like structures in the infected cell cytoplasm (**Figure 3(c)**), and are thought to be involved in virion morphogenesis. These structures appear similar to those found in cells infected with rotaviruses, where progeny particles bud into the endoplasmic reticulum, acquiring the outer coat components and generating mature virus particles.

Seadornaviruses replicate in a number of mosquito cell lines, including C6/36 and AA23 (both from *Aedes albopictus*), A20 (*Aedes aegypti*), and Aw-albus (*Aedes w albus*). Over 40% of the progeny virus particles are liberated in the culture medium (as determined by titration), prior to cell death and massive cytopathic effect (CPE) (fusiform cells), which (with BAV, LNV and KDV) is evident at 48–72 h post infection. Infected cells do not lyse initially and the virus is released by budding, acquiring a temporary membrane envelope in the process (**Figure 3(d)**). However, cell lysis does occur late in infection, as a result of cell death. Intracellular radiolabeling of viral polypeptides has shown that label is incorporated predominantly into viral polypeptides, even in absence of inhibitors of DNA replication such as actinomycin D, demonstrating 'shut off' of host-cell protein synthesis.

Like the other members of the genus, LNV can replicate in several mosquito cell lines, but it is the only seadornavirus that readily replicates in a variety of transformed or primary mammalian cells, including BHK-21, Vero, BGM, Hep-2, and MRC-5, leading to massive cell lysis after 48 h post infection.

The seadornavirus genome consists of 12 segments of dsRNA that are identified as Seg-1 to Seg-12 in the order of decreasing molecular weight and order of migration during agarose gel electrophoresis (AGE). The full-length genome sequence of BAV, KDV, and LNV has been determined. The genome comprises approximately 21 000 bp and the segment length ranges between 3747 and 862 bp. The genomic RNA of BAV and LNV show 6–6 electrophoretic profiles in 1% agarose gel electrophoresis (**Figure 2**). The genome of KDV migrates in 6–5-1 profile. Each genome segment encodes a single protein with an open reading frame spanning almost the whole length of the segment (**Figure 4**).

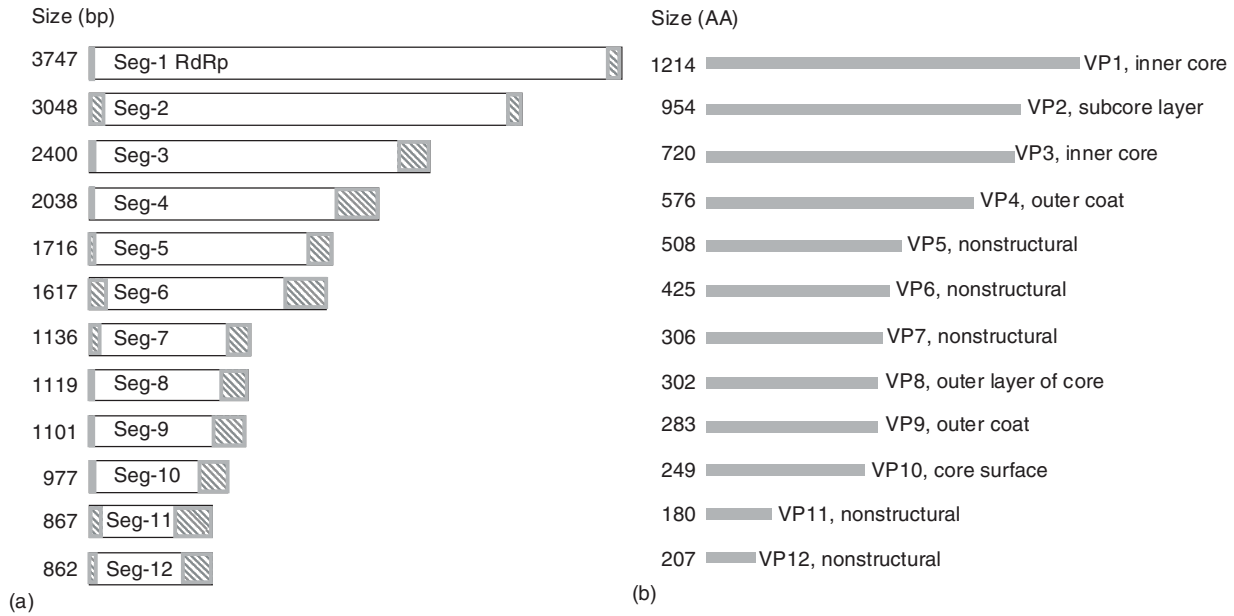


Figure 4 (a) Organization of the genome segments of BAV and (b) their putative encoded proteins. Shaded areas in (a): 5′ and 3′ NCRs. See www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/Bannavirus-Proteins.htm.

Table 2 Correspondence between seadornavirus genes (relative to the type species Banna virus), putative functions, and protein copy numbers per intact virus particle

BAV	*LNV (%AA identity with BAV)*	*KDV (%AA identity with BAV)*	*Function (location)*	*Protein copy number per particle*
Seg-1 (VP1, Pol)	Seg-1 (VP1, Pol) (41)	Seg-1 (VP1, Pol) (42)	RNA-dependent RNA polymerase (core)	24
Seg-2 (VP2, T2)	Seg-2 (VP2, T2) (27)	Seg-2 (VP2, T2) (33)	$T=2$ protein, nucleotide binding (core)	120
Seg-3 (VP3, Cap)	Seg-3 (VP3, Cap) (37)	Seg-3 (VP3, Cap) (38)	Capping enzyme (core)	12
Seg-4 (VP4)	Seg-4 (VP4) (32)	Seg-4 (VP4) (32)	(Outer coat)	~330
Seg-5 (VP5)	Seg-6 (VP6) (26)	Seg-6 (VP6) (27)	(Nonstructural)	0
Seg-6 (VP6)	Seg-5 (VP5) (26)	Seg-5 (VP5) (26)	NTPase (nonstructural)	0
Seg-7 (VP7)	Seg-7 (VP7) (27)	Seg-7 (VP7) (29)	Protein kinase (nonstructural)	0
Seg-8 (VP8, T13)	Seg-8 (VP8, T13) (23)	Seg-9 (VP9, T13) (21)	(Core)	780
Seg-9 (VP9)	Seg-10 (VP10) (18)	Seg-11 (VP11) (23)	Cell attachment (outer coat)	~310
Seg-10 (VP10)	Seg-9 (VP9) (24)	Seg-10 (VP10) (24)	(Core)	~260
Seg-11 (VP11)	Seg-12 (VP12) (37)	Seg-12 (VP12) (34)	(Nonstructural)	0
Seg-12 (VP12)	Seg-11 (VP12) (35)	Seg-8 (VP8) (26)	dsRNA-binding (nonstructural)	0

BAV, Banna virus, KDV, Kadipiro virus, and LNV, Liao ning virus. The copy number was determined by radiolabeling of the proteins using ^{35}S methionine. The encoded putative proteins are indicated between brackets followed by the nomenclature that is used in the *Eighth Report of the ICTV* and presented on the website www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/protein-comparison.htm.

Antigenic and Phylogenetic Relationships between Seadornaviruses

The earliest data that was generated concerning the relationships between different seadornaviruses were obtained by RNA cross-hybridization analyses, which identified BAV and KDV as members of distinct genogroups. Antigenic relationships between different seadornaviruses were also investigated using polyclonal mouse immune-sera. BAV from southern China and Indonesia, LNV from northeast of China, and KDV from Indonesia, which are classified as distinct species, show no cross-reaction in neutralization tests. Subsequent comparisons of nucleotide and amino acid sequences confirmed that BAV, KDV, and LNV represent three distinct virus species, with AA identities between homologous proteins of 24–42% (**Table 2**). A neighbor-joining tree based on an alignment of the most highly conserved seadornavirus protein, VP1 (the viral

polymerase), shows BAV, KDV, and LNV as three distinct phylogenetic groups, confirming their status as distinct species (**Figure 5(a)**).

Antigenic variation between different BAV strains was also investigated using immune-sera, and identified two distinct serotypes, that did not cross-neutralize. These have been named as BAV serotypes A and B. A seroneutralization epitope was identified on the outer coat protein VP9. Sequence analysis of Seg-9 showed only 40% amino acid identity between BAV serotypes A and B, identifying two genotypes: genotype A (represented by isolates BAV-Ch (China) and BAV-In6423 (Indonesia)); and genotype B (represented by isolates BAV-In6969 and BAV-In7043 (Indonesia)) (which match with virus serotypes A and B).

Seg-7, which codes for nonstructural protein VP7, also shows a significant variation, between the genotypes/serotypes, with only 70% amino-acid-sequence identity between types A and B. The amino-acid-sequence identity (VP1–VP12) between different BAV isolates within a single serotype ranged from 83% to 100%, while sequence identity between different serotypes ranged from 73% to 95% (excluding VP9). This level of genetic divergence is comparable to that observed between serotypes in other insect-transmitted arboviruses of family *Reoviridae*, in particular the orbiviruses. The two identified serotypes of LNV have nucleotide sequence identities ranging between 81% and 90%, while amino acid identity ranging between 80% and 96%. The lowest AA identity (80%) was found in the proteins of the outer capsid VP10 (the homolog of BAV VP9), which defines virus serotype in the seadornaviruses. A neighbor-joining tree, comparing the sequences of cell-attachment outer capsid proteins of

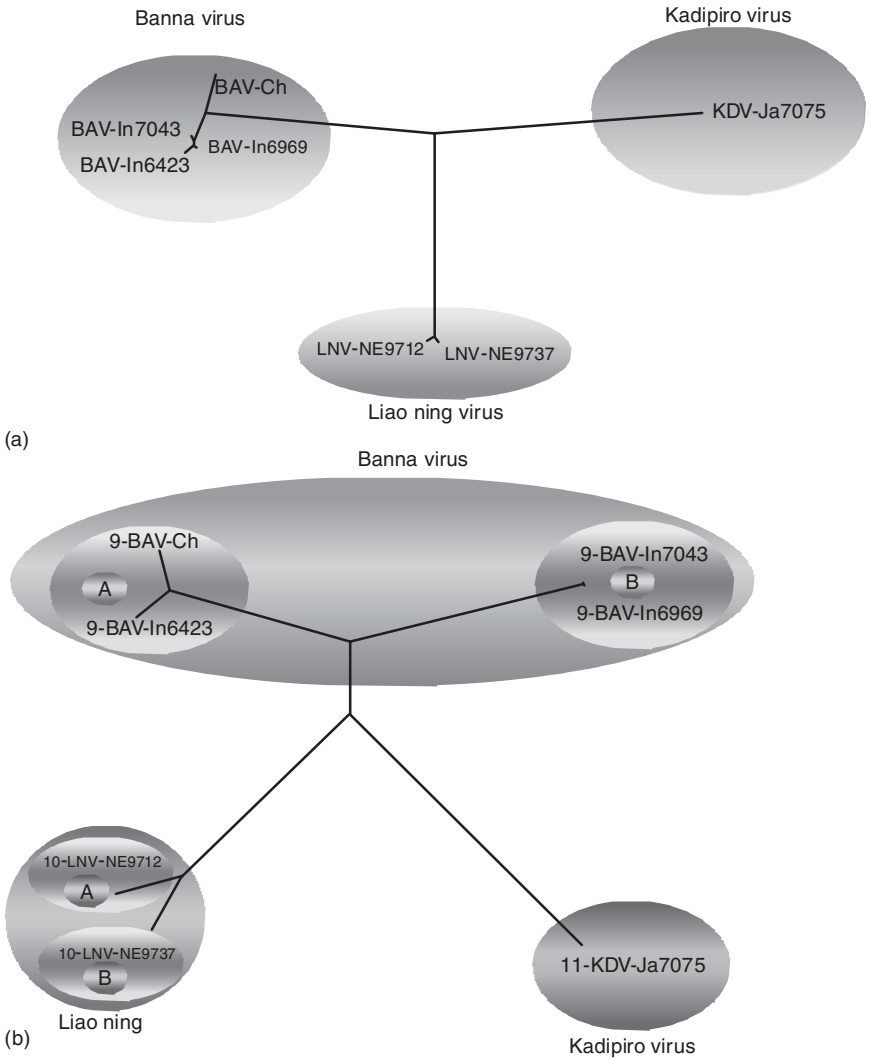


Figure 5 Phylogenetic tree for seadornaviruses based on (a) the polymerase gene and (b) on the cell-attachment (and seroneutralization) protein. The phylogenetic comparison based on the cell-attachment (and seroneutralization) protein distinguishes the the two genotypes (serotypes) of BAV and also those of LNV.

BAV (VP9), KDV (VP11), and LNV (VP10) clearly identifies the two serotypes of BAV (A and B) and distinguishes the A and B serotypes of LNV (**Figure 5(b)**).

All 12 segments of BAV, KDV, and LNV have conserved sequences, located at their 3′ and 5′ termini. The motifs 5′-GUAU$^{A}/_{U}{}^{A}/_{U}$A$^{A}/_{U}{}^{A}/_{U}{}^{A}/_{U}$-3′ and 5′-C$^{C}/_{U}$GAC-3′ (+ve strand) were found in the 5′ noncoding region (NCR) and the 3′ NCR of BAV, respectively, while in KDV, these motifs were 5′-GUAGAA$^{A}/_{U}{}^{A}/_{U}{}^{A}/_{U}{}^{A}/_{U}$-3′and 5′-$^{C}/_{U}$GAC-3′ (+ve strand) and in LNV they were 5′-GUUAU$^{A}/_{U}{}^{A}/_{U}{}^{A}/_{U}$-3′ and 5′-$^{C}/_{U}{}^{C}/_{U}$GAC-3′ (+ve strand), respectively. For the BAV and KDV viruses, the 5′ and 3′ terminal trinucleotides of all segments are inverted complements. LNV has a difference in position 3 of the 5′ ends and therefore only the 5′ and 3′ terminal dinucleotides of all segments are inverted complements.

The genetic relation between BAV, LNV, and KDV is reflected in their morphological characteristics. Electron microscopic analysis showed that the three viruses are morphologically identical.

Within the family *Reoviridae*, the most conserved gene between different genera encodes the RNA-dependent RNA polymerase (RdRp). Values for amino acid identity of lower than 30% can be used to distinguish members of distinct genera. A tree constructed from the alignment of polymerase sequences of representative members of the family *Reoviridae* (**Table 3**) is shown in **Figure 6**. Calculations of amino-acid-identity values based on this alignment show that the coltivirus polymerases exhibit a maximum of 15% identity with those of the seadornaviruses, confirming the status of *Coltivirus* and *Seadornavirus* as a distinct genera. In contrast amino acid identities as high as 28% were detected between the seadornaviruses and the rotaviruses (members of a distinct genus of 11-segmented viruses also within the family *Reoviridae*).

Evolutionary Relationships between BAV and Rotaviruses

A sequence comparison of the structural proteins of BAV (VP1, VP2, VP3, VP4, VP8, VP9, and VP10) to those of other members of the *Reoviridae*, have shown similarities between VP9 and VP10 of BAV and the VP8* and VP5* subunits of the outer coat protein VP4 of rotavirus A, with amino acid identities of 21% and 26%, respectively. This suggests that an evolutionary jump has occurred between the two genera, involving the expression of two BAV proteins (VP9 and VP10) from two separate genome segments rather than the generation of VP8* and VP5* by the proteolytic cleavage of a single gene product (as seen in the cleavage of rotavirus VP4). VP3 of BAV, which is the guanylyltransferase of the virus also exhibits significant identity (21%, AA identity) with the VP3 (guanylyltransferase) of rotavirus.

Structural Features and Relation to Rotaviruses

Electron micrographs of purified, intact BAV particles show striking similarities to those of the rotaviruses. Numerous protein spikes were observed on the surface of BAV, reminiscent of those of rotaviruses. This morphological resemblance has not previously been reported between rotaviruses and other members of family *Reoviridae*. The evolutionary relationship between the seadornaviruses and rotaviruses was further confirmed when the atomic structure of BAV VP9 was determined by X-ray crystallography. VP9 is a trimeric molecule that is held together by an N-terminal helical bundle (**Figure 7**). The N-terminal tail of the BAV VP9 monomer is reminiscent of the coiled-coils structures of the HIV gp41 protein, and the carboxy terminal of the VP5* of rotavirus (**Figure 7**). In contrast the monomer of BAV VP9 has a head domain made mainly of β-sheets (**Figure 7**), which shows significant structural similarities to rotavirus VP8*. However, VP8* does have a sialic acid-binding domain, which is absent from the head domain of the VP9 of BAV. This might explain why rotavirus infectivity can be decreased when cells are pretreated with sialidases although BAV infectivity, to C6/36 mosquito cells, is not altered by a similar treatment.

Functional Studies

Expressed BAV VP9 was used in competition assays with intact virus particles, in an attempt to decrease their infectivity, as demonstrated with the outer capsid proteins of some other reoviruses (e.g., the avian orthoreoviruses). Surprisingly, however, pretreatment of cells with VP9 increased the infectivity of BAV by 10–100 times. VP9 is thought to act as a membrane fusion protein and the N-terminal coiled-coils may be responsible for such an activity. This may explain why soluble trimeric VP9 can increase viral infectivity. VP9 trimers not only bind receptor but also initiate endocytosis, perhaps by receptor oligomerization. The initiation of endocytosis facilitates penetration by virus particles present at or near the cell surface and thereby increases their infectivity.

BAV VP9 also carries the virus seroneutralization epitopes. Anti-VP9 antibodies are highly neutralizing and define the two serotypes A and B of BAV. Animals immunized with VP9 failed to replicate the virus when challenged with the homologous type. Animals immunized with VP9 of the heterologous serotype showed unaltered virus replication and viremia. Similar results were obtained in cell cultures. VP9 could therefore be used as a vaccine subunit for immunization. Sequence analysis of BAV VP3 suggests that it is the viral capping enzyme (CaP).

This has been confirmed experimentally by incubating virus particles with α-^{32}P GTP. VP3 was covalently

Table 3 Sequences of RdRp used in phylogenetic analysis (**Figure 6**) of various members of family *Reoviridae*

Species	*Isolate*	*Abbreviation*	*Accession number*
Genus *Seadornavirus* (12 segments)			
Banna virus	Ch	BAV-Ch	AF168005
Kadipiro virus	Java-7075	KDV-Ja7075	AF133429
Liao ning viurus	LNV-NE9712	LNV-NE9712	AY701339
Genus *Coltivirus* (12 segments)			
Colorado tick fever virus	Florio	CTFV-Fl	AF134529
Eyach virus	Fr578	EYAV-Fr578	AF282467
Genus *Orthoreovirus* (10 segments)			
Mammalian orthoreovirus	Lang strain	MRV-1	M24734
	Jones strain	MRV-2	M31057
	Dearing strain	MRV-3	M31058
Genus *Orbivirus* (10 segments)			
African horse sickness virus	Serotype 9	AHSV-9	U94887
Bluetongue virus	Serotype 2	BTV-2	L20508
	Serotype 10	BTV-10	X12819
	Serotype 11	BTV-11	L20445
	Serotype 13	BTV-13	L20446
	Serotype 17	BTV-17	L20447
Palyam virus	Chuzan	CHUV	Baa76549
St. Croix river virus	SCRV	SCRV	AF133431
Genus *Rotavirus* (11 segments)			
Rotavirus A	Bovine strain UK	BoRV-A/UK	X55444
	Simian strain SA11	SiRV-A/SA11	AF015955
Rotavirus B	Human/murine strain IDIR	Hu/MuRV-B/IDIR	M97203
Rotavirus C	Porcine Cowden strain	PoRV-C/Co	M74216
Genus *Aquareovirus* (11 segments)			
Golden shiner reovirus	GSRV	GSRV	AF403399
Grass Carp reovirus	GCRV-873	GCRV	AF260511
Chum salmon reovirus	CSRV	CSRV	AF418295
Striped bass reovirus	SBRV	SBRV	AF450318
Genus *Fijivirus* (10 segments)			
Nilaparvata lugens reovirus	Izumo strain	NLRV-Iz	D49693
Genus *Phytoreovirus* (10 segments)			
Rice dwarf virus	Isolate China	RDV-Ch	U73201
	Isolate H	RDV-H	D10222
	Isolate A	RDV-A	D90198
Genus *Oryzavirus* (10 segments)			
Rice ragged stunt virus	Thai strain	RRSV-Th	U66714
Genus *Cypovirus* (10 segments)			
Bombyx mori cytoplasmic polyhedrosis virus 1	Strain I	BmCPV-1	AF323782
Dendrlymus punctatus cytoplasmic polyhedrosis 1	DsCPV-1	DsCPV-1	AAN46860
Lymantria dispar cytoplasmic polyhedrosis 14	LdCPV-14	LdCPV-114	AAK73087
Genus *Mycoreovirus* (11 or 12 segments)			
Rosellinia anti-rot virus	W370	RaRV	AB102674
Cryphonectria parasitica reovirus	9B21	CPRV	AY277888
Genus Mimoreovirus (11 segments)			
Micromonas pusilla reovirus	MPRV	MPRV	DQ126102
Genus Dinovernavirus (9 segments)			
Aedes pseudoscutellaris reovirus	ApRV	ApRV	DQ087277
Genus Cardoreovirus (12 segments)			
Eriocheir sinensis reovirus	Isolate 905	EsRV	AY542965

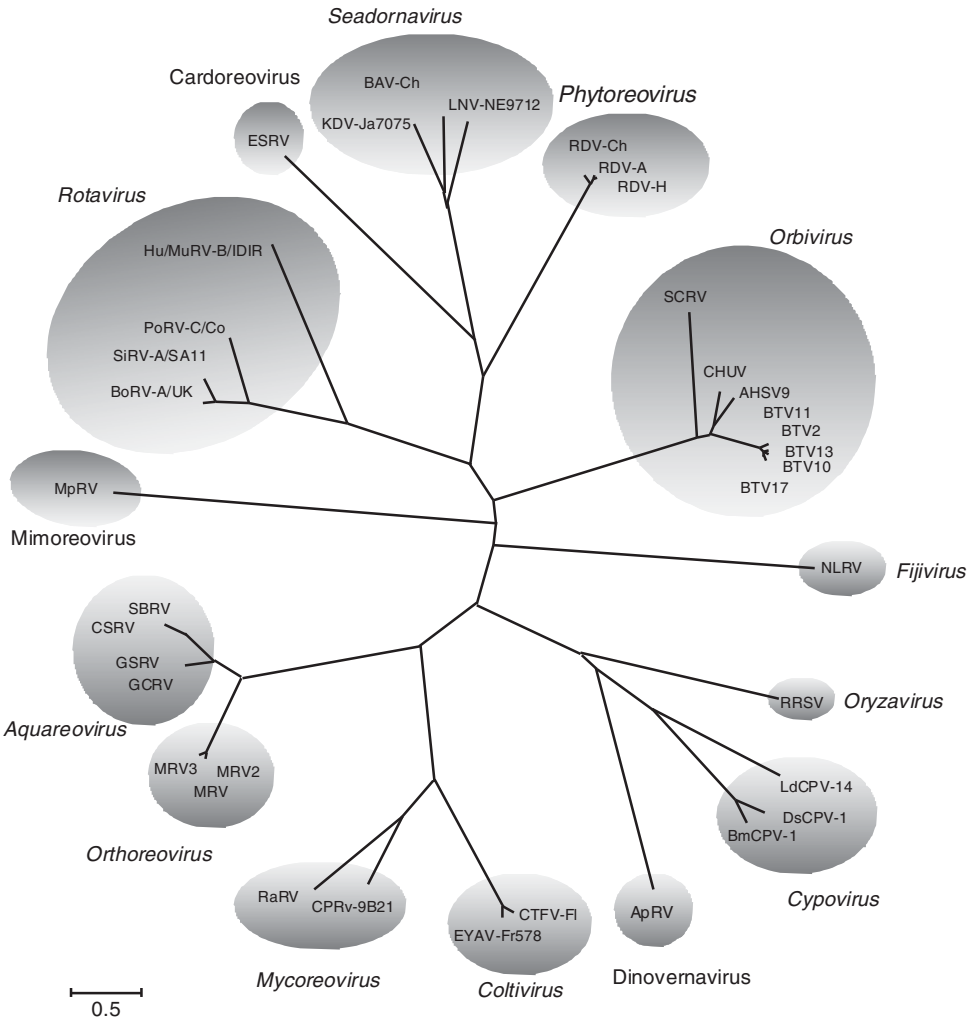


Figure 6 Phylogenetic relations among members of the *Reoviridae* based on the sequences of their putative RNA-dependent RNA polymerases (RdRps). Neighbor-joining phylogenetic tree built with available polymerase sequences (using the Poisson correction or gamma-distribution algorithms) for representative members of 12 recognized and 3 proposed genera of family *Reoviridae*. The abbreviations and accession numbers are those provided in **Table 3**.

labeled with α-^{32}P derived from GTP. The reaction was found to be dependent on the presence of divalent ions such as magnesium. The recombinant VP3 also covalently bound α-^{32}P GTP and exhibited an inorganic pyrophosphatase activity, possibly as a detoxifying mechanism which removes the inorganic pyrophosphate accumulated during RNA and Cap structure synthesis by the virus-core-associated enzymes. Similar properties were described for the capping enzyme VP4 of Bluetongue virus (genus *Orbivirus*).

Clinical Features and Diagnostic Assay for Seadornaviruses

As mentioned earlier, the only seadornavirus that has been isolated from humans and associated with human disease (to date) is BAV. Humans infected with BAV develop flu-like manifestation, myalgia, arthralgia, fever, and encephalitis. Reports of children born to infected mothers (as revealed by serological assays) show various manifestations including cerebral palsy, hemiplegia, delay of development, and viral encephalitis.

A diagnostic serological assay was developed for different serotype of BAV, based on outer coat protein VP9 (which is responsible for cell-attachment and sero-neutralization). Molecular diagnostic assays have also been developed for BAV and KDV. These are RT-PCR-based assays, which have been validated using an infected murine model in which viral RNA could be detected in blood as early as 3 days post infection. These RT-PCR assays can also be used to distinguish genotypes A and B of BAV, based on distinct amplicons of different lengths, that are obtained by specific primers at different locations in

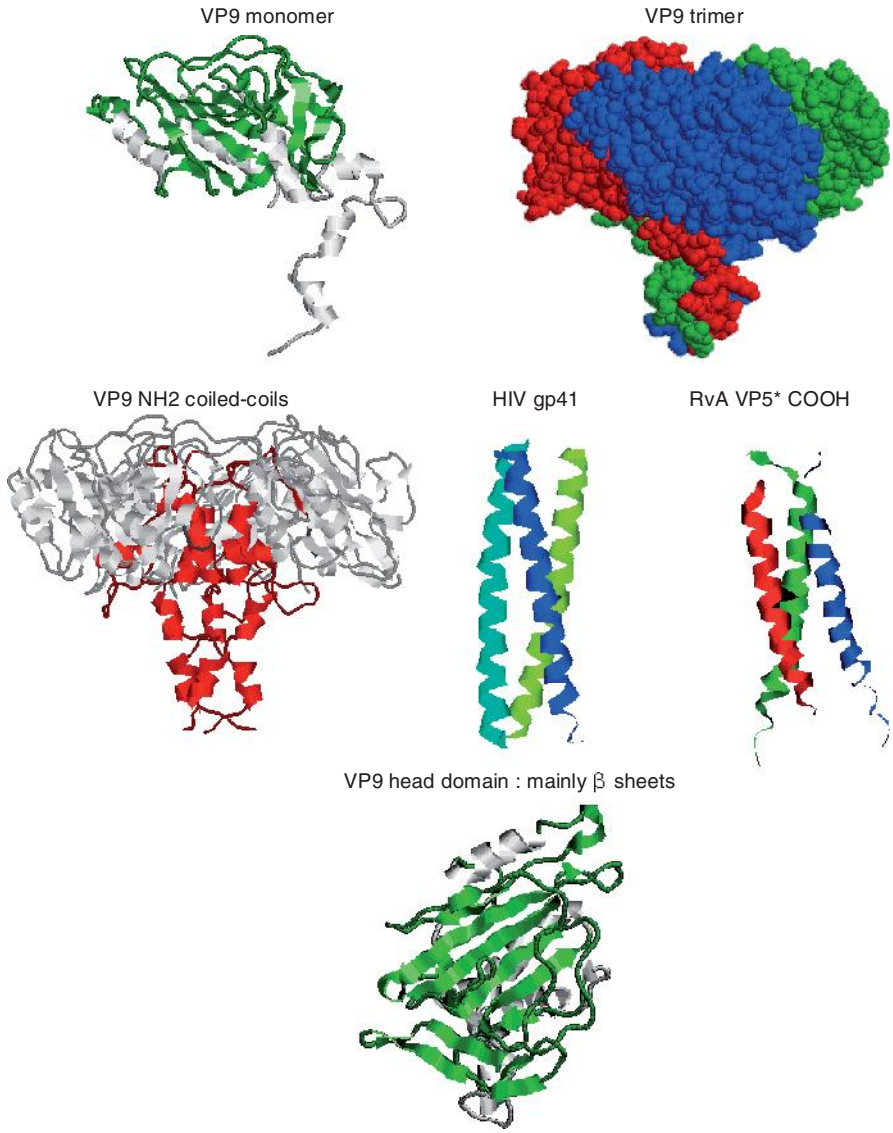


Figure 7 The structure of the VP9 outer capsid protein of Banna virus determined by X-ray crystallography at a resolution of 2.6 angstrom. The NH2 terminal of the BAV VP9 is organized in the form of coiled-coils that are similar to those found in fusion active proteins such as HIV gp41 and VP5* of rotavirus. RvA, Rotavirus A.

Seg-9. A quantitative PCR assay has also been developed for BAV based on the sequence of Seg-10. A standard RT-PCR assay was recently developed for LNV based on the sequence of Seg-12, which can be used to detect viral RNA in infected mouse blood.

Severity of Infection in Animal Model and Diversification of Genome Sequence after Replication in Permissive Animals

Banna, Kadipiro, and Liao ning viruses all replicate in mice after intraperitoneal injection. The viral genome can be detected in mouse blood 3 days until 5–7 days post injection. Clearance of the viremia is accompanied by the appearance of antibodies. When animals were injected on a second occasion with the same virus, BAV and KDV failed to replicate, demonstrating the immune status of the mice. However, LNV replicated in the immunized mice causing death with a severe hemorrhagic syndrome. An analysis of the PCR amplicons from the infected mice blood showed very few changes in the sequences of the BAV and KDV genomes. However, there was considerable diversification in the genome of LNV, as demonstrated by analyses of the sequence of Seg-12, leading to formation of a diverse quasispecies. This might explain why LNV can continue to replicate in the immunized mice, as diversification of the sequence might help the virus to 'escape' the immune system.

Treatment and Immunity

There is no specific treatment for BAV infection. Patients infected with BAV have shown a fourfold rise in the anti-BAV antibody titers in paired sera tested by ELISA, showing that an immune response is developed to the virus infection. Mice experimentally infected with BAV developed viremia. The clearance of the virus from the blood circulation occurred concomitantly with the appearance of anti-BAV antibodies. Based on the mouse model, immunization with a recombinant expressed VP9 is likely to provide a significant level of protection against an initial infection with the homologous virus type. Immunization with VP9 could therefore provide a significant level of protection in high-risk areas and it might form the basis for an effective subunit vaccine.

See also: Human T-Cell Leukemia Viruses: Human Disease; Reoviruses: General Features.

Further Reading

Attoui H, Billoir F, Biagini P, *et al.* (2000) Complete sequence determination and genetic analysis of Banna virus and Kadipiro virus: Proposal for assignment to a new genus (*Seadornavirus*) within the family *Reoviridae*. *Journal of General Virology* 81: 1507–1515.

Attoui H, Charrel R, Billoir F, *et al.* (1998) Comparative sequence analysis of American, European and Asian isolates of viruses in the genus *Coltivirus*. *Journal of General Virology* 79: 2481–2489.

Attoui H, de Lamballerie X, and Mertens PPC (2005) *Coltivirus, Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 497–503. San Diego, CA: Elsevier Academic Press.

Attoui H, de Lamballerie X, and Mertens PPC (2005) *Seadornavirus, Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 504–510. San Diego, CA: Elsevier Academic Press.

Billoir F, Attoui H, Simon S, *et al.* (1999) Molecular diagnosis of group B coltiviruses infections. *Journal of Virological Methods* 81: 39–45.

Brown SE, Gorman M, Tesh RB, and Knudson DL (1993) Coltiviruses isolated from mosquitoes collected in Indonesia. *Virology* 196: 363–367.

Chen BQ and Tao SJ (1996) Arbovirus survey in China in recent ten years. *Chinese Medical Journal (Engl)* 109: 13–15.

Mertens PPC, Attoui H, and Bamford DH (eds.) (2002) Identification of comparable proteins of the dsRNA viruses. In: *The RNAs and Proteins of dsRNA Viruses*. www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/protein-comparison.htm (accessed May 2007).

Mertens PPC, Duncan R, Attoui H, and Dermody TS (2005) *Reoviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 447–454. San Diego, CA: Elsevier Academic Press.

Mohd Jaafar F, Attoui H, Bahar MW, *et al.* (2005) The structure and function of the outer coat protein VP9 of Banna virus. *Structure* 13: 17–29.

Mohd Jaafar F, Attoui H, Gallian P, *et al.* (2004) Recombinant VP9-based enzyme-linked immunosorbent assay for detection of immunoglobulin G antibodies to Banna virus (genus *Seadornavirus*). *Journal of Virological Methods* 116: 55–61.

Mohd Jaafar F, Attoui H, Mertens PPC, *et al.* (2005) Structural organisation of a human encephalitic isolate of Banna virus (genus *Seadornavirus*, family *Reoviridae*). *Journal of General Virology* 86: 1141–1146.

Tao SJ and Chen BQ (2005) Studies of coltivirus in China. *Chinese Medical Journal (Engl)* 118: 581–586.

Relevant Website

http://www.iah.bbsrc.ac.uk – The RNAs and Proteins of dsRNA Viruses.

Sequiviruses

I-R Choi, International Rice Research Institute, Los Baños, The Philippines

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Helper component A viral-encoded protein that mediates the transmission of viruses by vectors.
Semipersistent transmission A mode of vector-mediated transmission of plant viruses in which viruses are usually acquired within few minutes, and retained up to several days by vectors. Viruses do not multiply in vectors and often require a helper component during semipersistent transmission.

Introduction

Sequiviridae is a relatively newly recognized family of viruses, although the viruses classified into this family had been reported already in the early 1970s. Viruses belonging to the family *Sequiviridae* were often referred to as 'plant picorna-like viruses' due to their similarities to picornaviruses in virion morphology and genome structure. They have a monopartite single-stranded RNA (ssRNA) genome encapsidated in isometric particles. They infect plants and are usually transmitted by insect

vectors in nature. Special attention has been given to two of the members, maize chlorotic dwarf virus (MCDV) and rice tungro spherical virus (RTSV), since they are involved in major diseases of important cereal crops. Chlorotic dwarf disease is considered to be the second most important viral disease of maize in the USA, and tungro disease is the most serious threat to rice production in South and Southeast Asia. One of the unique biological features found in these viruses is that most of them act as or require a helper virus for transmission by insect vectors. The name of the family comes from the Latin word *sequi* which means to follow or accompany, in reference to the dependent insect transmission of parsnip yellow fleck virus (PYFV), one of the type members in this family.

Taxonomy

The family *Sequiviridae* is divided into two genera, *Sequivirus* and *Waikavirus*. At present the genus *Sequivirus* has two species *Parsnip yellow fleck virus* (type species) and *Dandelion yellow mosaic virus*. Three species, *Rice tungro spherical virus* (type species), *Maize chlorotic dwarf virus*, and *Anthriscus yellows virus* are classified as members of the genus *Waikavirus* (**Table 1**). In addition, lettuce mottle virus is considered as a tentative member of the genus *Sequivirus*.

Viruses belonging to the genus *Sequivirus* infect mesophyll and epidermal cells and are able to be transmitted by mechanical inoculation and insect vectors, while those belonging to the genus *Waikavirus* are usually limited in phloem tissue and transmitted only by insect vectors. Sequiviruses are dependent on a helper virus for their transmission by insect vectors. Waikaviruses are independently transmitted by insect vectors and presumably encode a helper component in their genomes. The viruses in the family *Sequiviridae* are primarily classified on the basis of their biological and physical characteristics, but conspicuous differences are also found in their genome features. The genomes of MCDV and RTSV are approximately12 kb in length and polyadenylated at the 3′ end, whereas that of PYFV is about 10 kb and devoid of the 3′ poly(A) region.

Properties of Virions

Viruses belonging to *Sequiviridae* have nonenveloped isometric particles of approximately 30 nm in diameter (**Figure 1**). The sedimentation coefficients of the virions are 153–159S for sequiviruses and 175–183S for waikaviruses. The buoyant density of PYFV virions in CsCl is 1.49 g ml^{-1}, and that of waikaviruses is 1.51–1.55 g ml^{-1}. Genome sequences and immunodetection of the viruses belonging to the family *Sequiviridae* indicate that the virions consist of three capsid proteins (CPs). The sizes of CPs range from 22 to 35 kDa, depending on virus species and isolates. The virion of RTSV (Philippine-type strain A) consists of three proteins of 22.5 (CP1), 22 (CP2), and 33

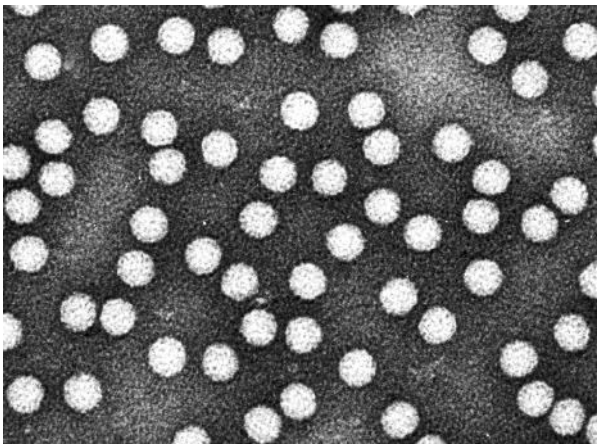


Figure 1 Virions of RTSV. The estimated size of particles is approximately 30 nm.

Table 1 Virus members in the family *Sequiviridae*

Genus/virus	*Genome size (kb)*	*Geographical distribution*	*Major natural host*	*Transmission vector*
Sequivirus				
Parsnip yellow fleck virus	9.9	Europe	Parsnip, hogweed, cow parsley	Aphids (*Cavariella aegopodii, C. pastinacae*)
Dandelion yellow mosaic virus	10.0[a]	Europe	Lettuce, dandelion	Aphids (*Acyrthosiphon solani, Myzus ornatus, M. ascalonicus, M. persicae*)
Waikavirus				
Rice tungro spherical virus	12.2	Asia	Rice, *Oryza* species	Green leafhoppers (*Nephotettix virescens* and four other species)
Maize chlorotic dwarf virus	11.8	USA	Maize, Johnson grass	Deltocephaline leafhopper (*Graminella nigrifrons*)
Anthriscus yellow virus	10.6[a]	Eurasia, UK	Cow parsley	Aphid (*Cavariella aegopodii*)

[a]Estimated size (nucleotide sequence not determined).

(CP3) kDa. The predicted molecular masses for the CP of MCDV (Tennessee (TN) isolate) are 22, 23, and 31 kDa, and those of PYFV (P-121 isolate) are 22.5, 26, and 31 kDa.

Genome Structure

The genomes of viruses belonging to the family *Sequiviridae* are positive-sense, monopartite ssRNA. The length of genomes varies significantly, ranging from approximately 10 kb for sequiviruses to 12 kb for waikaviruses. The genomes contain a large open reading frame (ORF) encoding a polyprotein presumably proteolytically processed by viral-encoded protease(s) during translation (**Figure 2**). The large ORF in the genome of PYFV (P-121 isolate) putatively encodes a polyprotein consisting of 3027 amino acid residues with the predicted molecular mass of about 336 kDa. The large ORF in the genomes of waikaviruses encodes a polyprotein of approximately 400 kDa, which has about 3440–3470 amino acid residues. In addition to the ORF for the polyprotein, short ORFs were also identified near the 3′ and the 5′ ends of MCDV and RTSV genomes. Two RNA species which seemingly correspond to subgenomic transcripts from the short ORFs locating near the 5′ end of the RTSV genome (strain A) were detected from infected plants. However, the lengths and locations of the short ORF in the genomes of waikaviruses vary considerably among viruses and isolates, and the presence of products translated from these ORFs was not confirmed in plants. The 5′-untranslated region (UTR) in the genome of PYFV (P-121 isolate) is about 0.28 kb in length, while those of waikaviruses are longer, about 0.43 kb in MCDV and 0.52 kb in RTSV. The 5′-UTR in the genomes of MCDV and RTSV has several AUG sequences upstream of the putative polyprotein start site, and appeared to form extensive secondary structures. As observed in the genome of picornaviruses, such secondary structures may serve as the internal ribosomal entry site to avoid the interference on translation by upstream AUG sequences. No stable secondary structures are recognized in the 5′-UTR of the PYFV genome; however, several short stretches of pyrimidines (UCUCUY) are present in the region. The 3′-UTR in the genomes of waikaviruses are polyadenylated and unusually long. The length of 3′-UTR in the genomes of MCDV and RTSV are approximately 1.0 and 1.2 kb, respectively, provided that the short ORFs near the 3′ end of genomes are not translated. The 3′-UTR in the genome of PYFV is approximately 0.5 kb in length. Unlike the genomes of waikaviruses, that of PYFV is not polyadenylated, but a part of 3′-UTR is likely to form a stem–loop structure which is similar to that found in the genomes of flaviviruses.

Properties of Viral Proteins

Based on the protein sequences predicted from the large ORF, and the actual sequences of the N-termini of CPs, it was predicted that the polyproteins of viruses belonging to the family *Sequiviridae* are cleaved into at least seven proteins through proteolytic maturation. It appears that the N-terminal half of the polyprotein contains regions for a leader protein and three CPs. Protein regions showing similarities to helicase, protease, and RNA-dependent RNA polymerase (RdRp) of picornaviruses and comoviruses are recognized in the central to carboxyl (C)-terminal regions of the polyproteins. The arrangement of functional domains in the polyproteins of the viruses belonging to *Sequiviridae* shows significant similarity to that of picornaviruses, indicating that the strategies of genome replication and expression for the viruses of *Sequiviridae* might be analogous to those of picornaviruses.

Comparative sequence analysis indicated that the polyproteins of the viruses belonging to the family *Sequiviridae*

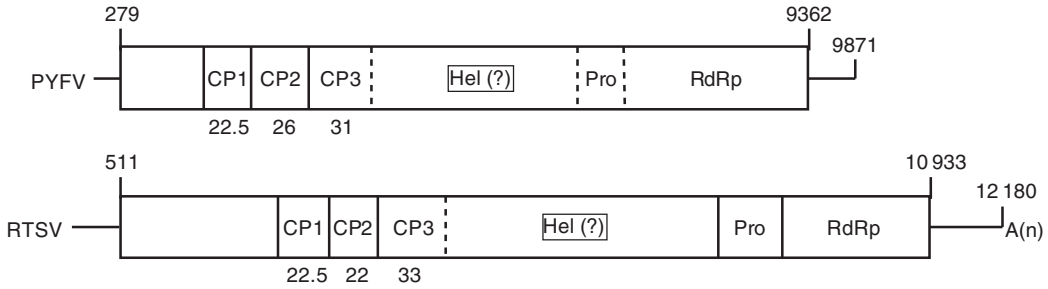


Figure 2 Genome structure of PYFV (P-121 isolate) and RTSV (strain A). The ORFs for polyprotein are indicated with rectangles. Horizontal lines at both ends of the ORFs represent the 5′- and 3′-UTR of the genome. Solid vertical lines dividing the ORF correspond to the positions of proteolytic cleavage sites in the polyproteins when translated, while the positions corresponding to predicted cleavage sites are indicated with dotted lines. Positions of translation start and stop codons for the polyproteins and the length of the entire genomes are indicated with the numbers above the genomes. Numbers below the regions encoding CP1–CP3 are the predicted molecular weight (kDa) for the respective CP. Shadowed areas represent the approximate region encoding NTP-binding domain. The regions in the ORF are shown with Hel (?) for putative NTPase/helicase, Pro for 3C-like cysteine proteinase, and RdRp for RNA-dependent RNA polymerase. A(n) indicates the polyadenylation at the 3′ end of the RTSV genome.

contain a protease region similar to the 3C cysteine protease of picornaviruses and other viral proteases resembling the 3C protease such as the 24 kDa protease of cowpea mosaic virus (CPMV) and the NIa protease of tobacco etch virus. For example, considerable similarity was found between the region delimited by the amino acid residues 2643 and 2853 in the polyprotein of RTSV (strain A) and a region in the 24 kDa protease of CPMV. Immunodetection using an antiserum raised against a protein containing the putative protease region of RTSV indicated that the size of mature protease in infected plants is approximately 35 kDa. The sequence context of cleavage site for the 3C cysteine protease and the results from *in vitro* translation from partial genomic RNA templates defined the region of the protease in the RTSV polyprotein to be from amino acid residues 2527–2852. The RTSV protease presumably acts *in cis* to cleave itself from the adjacent protein regions, but *trans*-cleavage by the RTSV protease was also observed to occur between the protease and the putative helicase regions *in vitro*. Based on the sequence alignment among the 3C-type proteases, amino acid residues such as His^{2680}, Glu^{2717}, Cys^{2811}, and His^{2830} in the RTSV polyprotein were predicted to constitute the catalytic triad or substrate-binding pocket of the cysteine protease. Substitutions of these amino acid residues abolished or drastically reduced the proteolytic activity, substantiating their critical roles in the cysteine protease. Conserved amino acid residues which may constitute the catalytic triad were also identified in the protease regions of MCDV and PYFV, although the involvement of the individual residues in the protease activity has not been experimentally demonstrated.

The C-terminal region in the polyproteins of viruses belonging to the family *Sequiviridae* shows extensive similarity in amino acid sequence to RdRp of picornaviruses and comoviruses. The RdRp region of RTSV (strain A) was defined in the region between amino acid residues 2853 and 3473 with the conserved YGDD motif at amino acid residues 3270–3273. The RdRp region of MCDV (isolate TN) has the conserved YGDD motif at amino acid residues 3238–3241. In addition, motifs such as DYSXFDG (amino acid residues 3129–3135 in the MCDV polyprotein) and $PSGX_3TX_3NS$ (amino acid residues 3189–3200) were identified to be conserved among the RdRp regions of MCDV, CPMV, and tomato black ring virus (TBRV). Multiple alignment of the RdRp region of PYFV (isolate P-121) with those of CPMV, TBRV, and poliovirus showed that they share several conserved motifs including sequences YGDD (amino acid residues 2629–2632 in the PYFV polyprotein), $PSGX_3TX_3NS$ (amino acid residues 2580–2591), and FLKR (amino acid residues 2684–2687).

The central region flanked by the CP and the protease regions in the polyproteins of viruses belonging to the family *Sequiviridae* contains sequence motifs GX_4GKS and DD, which are conserved among proteins with NTP-binding domain. The NTP-binding domain of the central polyprotein regions shows extensive similarity to the corresponding domains in the 58 kDa protein of CPMV and the 2C proteins of picornaviruses. The 58 kDa protein and the 2C protein presumably function as a nucleoside triphosphatase (NTPase)/helicase required for the initiation of negative-strand RNA synthesis. Antibodies specific to protein segments from the central region of the MCDV polyprotein reacted with three protein species in extracts from infected plants. The sizes of the proteins detected were smaller than predicted from the intact central region, indicating that the protein in the central region might be processed into smaller proteins.

It appeared that the polyproteins of viruses belonging to *Sequiviridae* have a region for putative leader protein(s) at the N-terminal region. Although the predicted sizes of the putative leader proteins are about 40 kDa in PYFV, and 70–78 kDa in waikaviruses, the sizes of proteins detected with the antisera specific to the putative leader proteins are significantly smaller than predicted. An antiserum specific to the putative RTSV leader protein detected a protein of about 32 kDa in extracts from infected plants. Meanwhile, proteins with apparent sizes of 50, 35, and 25 kDa in the extracts from plants infected with MCDV (severe (S) strain) reacted with the antibody specific to the putative MCDV leader protein. These results suggest that the putative leader proteins of waikaviruses may be processed post-translationally in plants. The function associated with the leader protein region is still unclear. However, such leader protein found in the polyprotein of aphthoviruses in the family *Picornaviridae* has a protease activity which cleaves itself autocatalytically from the polyprotein. It was proposed that the 35 kDa protein of MCDV is generated from the leader protein region through autoproteolysis at the putative cleavage site ALVRLFHGSAE (amino acid residues 150–160), while the 25 kDa protein results from the cleavage at Q^{445}/S^{446} and Q^{686}/S^{687} by a cysteine protease. The 25 kDa protein may function as the helper component in the insect transmission of MCDV since the protein was observed to accumulate in vector insects after feeding on plants infected with MCDV. This observation is consistent with the result from a serological blocking experiment showing that the helper component is not the virion or CP.

Three consecutive regions of CPs are present in the N-terminal half of the polyproteins of the viruses belonging to the family *Sequiviridae*. The proteolytic cleavage sites for the RTSV CPs determined by N-terminal amino acid sequencing were mapped to Q^{644}/A^{645}, Q^{852}/S^{853}, and Q^{1055}/D^{1056} for the junctions between leader protein/CP1, CP1/CP2, and CP2/CP3, respectively. The context of these cleavage sites suggests that the CPs are processed *in trans* by a cysteine protease. However, the cleavage at these sites with the RTSV protease was not detected *in vitro*. The difference in the reactivity among the CPs of

RTSV with the antibody raised against the virus particles indicated that the 33 kDa CP (CP3) is the major antigenic determinant on the surface of particles, although the structural details of virus particles have not been elucidated yet. The size of CP3 of RTSV (Philippine isolate) in the crude extract from infected plants detected by the antibody specific to CP3 appeared to be 40–42 kDa, markedly larger than that detected from purified virus preparation or that expected from the genome sequence. It is likely that the size of CP3 in infected cells is larger due to post-translational modification, but the modified moiety appeared to be cleaved off probably by the treatment with cellulolytic enzymes during virion purification.

Phylogenetic Relationships

Comparison of amino acid sequences revealed that the sequence similarities of the MCDV (isolate TN) polyprotein to those of RTSV (strain A) and PYFV (isolate P-121) were 51% and 35%, respectively. Overall similarity in genome organization and the presence of several conserved motifs in the polyproteins indicate that the viruses of the family *Sequiviridae* are closely related to picornaviruses and comoviruses. The NTP-binding domain appeared to be the most conserved region in the polyproteins among the viruses belonging to the family *Sequiviridae*, picornaviruses and comoviruses. The NTP-binding domain in the polyprotein of MCDV (TN isolate) showed significant sequence similarity to those of viruses such as RTSV (strain A, 79%), PYFV (isolate P-121, 55%), CPMV (46%), hepatitis A virus (HAV, 47%), and poliovirus (45%). The regions of RdRp also show significant similarities to one another among members of the family *Sequiviridae*, picornaviruses and comoviruses. For instance, the sequence similarities in the RdRp region of MCDV (TN isolate) to those of related viruses are 75% for RTSV, 50% for PYFV, 48% for CPMV, 44% for TBRV, and 40% for HAV. Phylogenetic analysis based on the sequences of NTP-binding domains indicates that PYFV and RTSV are not noticeably similar to each other compared to their relatedness to picornaviruses and comoviruses. However, phylogenetic analysis based on the regions of RdRp suggests that PYFV and RTSV are more closely related to each other than to picornaviruses and comoviruses. Unlike the region of RdRp and NTP-binding domain, the CPs of viruses belonging to the family *Sequiviridae* show no sequence similarities to those of comoviruses, and only limited similarities to those of some picornaviruses. The 26 kDa CP of PYFV and the 22.5 kDa CP of RTSV contain amino acid sequences resembling those in VP3 of encephalomyocarditis virus and human rhinovirus 14. The closer relatedness of nonstructural protein regions in members of the family *Sequiviridae* to those of comoviruses, and the similarity in the CP sequences among PYFV, RTSV, and some picornaviruses imply that the family *Sequiviridae* may fit taxonomically between *Comoviridae* and *Picornaviridae*.

Variation of Isolates and Strains

Isolates of PYFV are largely divided into two groups. One is the parsnip serotype which includes isolates from parsnip, celery, and hogweed, while those from carrot and cow parsley belong to the other group, the *Anthriscus* serotype. The two groups of isolates are distinguishable by reciprocal immunodiffusion tests with antisera raised against isolates belonging to either group. In addition to the difference in natural hosts, the artificial inoculation of test plants with the respective isolates showed evident difference in host ranges between the two groups of isolates, although minor differences in host range and symptoms on certain plants were also observed among the isolates within each serotype.

Examination of nucleotide sequences of the RTSV CP revealed broad genotypic variation among and within geographic isolates, although the relationship of genotypic variation with the pathogenicity is not understood yet. Isolates of RTSV from the Philippines and Malaysia show about 95% sequence similarity, while those from Bangladesh and India differ from the Philippine isolate by about 15%. The CP3 of the Indian isolate was distinguishable from that of the Philippine isolate in electrophoretic mobility and the response to cellulolytic enzyme. Genotypic survey on the CP sequences of RTSV field isolates collected from various sites in the Philippines and Indonesia indicated that a high degree of genetic diversity exists among the field isolates, and that infections with mixed genotypes in single sites are not uncommon. Phylogenetic analysis based on the CP sequences of the RTSV field isolate suggested that the clustering of genotypes found in the Philippines sites was significantly different from that found in the Indonesia sites, indicating geographic isolation of RTSV populations. Strain Vt6 of RTSV was found to possess enhanced virulence, showing infectivity to some rice cultivars which the type strain A may not be able to infect. Amino acid sequence of strain Vt6 was approximately 95% identical to that of strain A, with greater dissimilarity in the leader protein region and the putative small ORF found near the 3′ end.

Few isolates of MCDV with distinctive biological and genotypic characteristics have been reported. The S isolate of MCDV was observed to produce more pronounced symptoms than the type (T) isolate. The mild (M1) isolate usually exhibits mild symptoms by itself, but it develops severe symptoms by synergistically interacting with other MCDV isolates. The deduced amino acid sequences of isolates S and T show 99.5% identity, while that of isolate M1 has only 61% identity to that of isolate T. In fact, antisera raised against isolate T react strongly with isolate

S, but not with isolate M1. Isolate TN of MCDV is also significantly divergent from isolate T, showing only 60% of amino acid sequence identity. The low levels of amino acid sequence identity among the isolates of MCDV raised the possibility that they may represent distinct virus species.

Interactions between Viruses

Viruses belonging to the family *Sequiviridae* are often detected in plants infected with other viruses, for instance, cow parsley infected with PYFV and anthriscus yellows virus (AYV), and rice plants infected with rice tungro bacilliform tungrovirus (RTBV) and RTSV. Such mixed infections appear to be the outcome of the dependent insect transmission of one virus on the other. Incidences of mixed infection in lettuce with dandelion yellow mosaic virus and lettuce mosaic virus were also reported, but their relationships in aphid-mediated transmission are still unclear.

PYFV is transmitted by mechanical inoculation, but AYV is not. Both viruses are also transmitted by aphids in a semipersistent manner. However, the aphid-mediated transmission of PYFV is dependent on AYV. PYFV is transmitted by aphids from plants infected with both viruses or by aphids previously fed on plants infected with AYV. Aphids seem to retain PYFV and AYV for up to 4 days. Even though AYV cannot infect parsnip, PYFV can be transmitted to parsnip by aphids fed on other plants infected with AYV and PYFV. Therefore, AYV apparently acts only as the helper virus for aphids to acquire PYFV, and is not necessary for the infection process of PYFV.

Rice tungro disease is caused by the interaction between RTSV and RTBV. Both viruses are transmitted by green leafhoppers (GLHs) in a semipersistent manner. GLHs transmit RTSV and RTBV simultaneously or singly from source plants infected with both viruses. RTSV is independently transmitted by GLHs, but RTBV can be transmitted by GLHs which feed on plants infected with RTSV. Although GLHs retain RTSV for only 3–4 days, their ability to acquire and transmit RTBV may persist for 7 days. Neutralization of RTSV-viruliferous GLHs with anti-RTSV immunoglobulin markedly reduced the ability to transmit RTSV but still retained the ability to acquire and transmit RTBV. These observations indicate that RTSV is the helper virus for the GLH-mediated transmission of RTBV, but the helper function is associated with factors other than RTSV virions or CP. Rice plants infected with RTBV alone exhibit symptoms such as stunted growth, yellow to yellow-orange discoloration of leaves, and reduced tillering. The symptoms become more severe when plants are simultaneously infected with RTBV and RTSV, despite the fact that RTSV alone does not cause conspicuous symptoms except occasional slight stunted growth (**Figure 3**). Such synergistic effects of RTSV on symptom development are also observed when RTSV co-infects plants with viruses such as rice grassy stunt virus and rice ragged stunt virus.

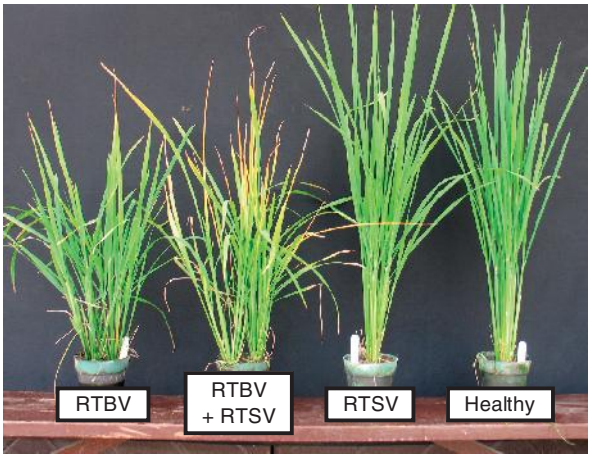


Figure 3 Synergistic effects between RTSV and RTBV on the symptom development in rice. Rice plants shown (left to right) were infected with RTBV alone (RTBV), both RTBV and RTSV (RTBV + RTSV), RTSV alone (RTSV), or not infected (healthy). Plants infected with both RTBV and RTSV were stunted and showed severe discoloration of leaves.

See also: Cereal Viruses: Maize/Corn; Cereal Viruses: Rice; Picornaviruses: Molecular Biology; Rice Tungro Disease; Vector Transmission of Plant Viruses.

Further Reading

Azzam O, Yambao MLM, Muhsin M, McNally KL, and Umadhay KML (2000) Genetic diversity of rice tungro spherical virus in tungro-endemic provinces of the Philippines and Indonesia. *Archives of Virology* 145: 1183–1197.

Chaouch-Hamada R, Redinbaugh MG, Gingery RE, Willie K, and Hogenhout SA (2004) Accumulation of maize chlorotic dwarf virus proteins in its plant host and leafhopper vector. *Virology* 325: 379–388.

Elnagar S and Murant AF (1976) Relations of the semi-persistent viruses, parsnip yellow fleck and anthriscus yellows, with their vector, *Cavariella aegopodii*. *Annals of Applied Biology* 84: 153–167.

Hemida SK and Murant AF (1989) Host ranges and serological properties of eight isolates of parsnip yellow fleck virus belonging to the two major serotypes. *Annals of Applied Biology* 114: 101–109.

Isogai M, Cabauatan PQ, Masuta C, Uyeda I, and Azzam O (2000) Complete nucleotide sequence of the rice tungro spherical virus genome of the highly virulent strain Vt6. *Virus Genes* 20: 79–85.

Reddick BB, Habera LF, and Law MD (1997) Nucleotide sequence and taxonomy of maize chlorotic dwarf virus within the family *Sequiviridae*. *Journal of General Virology* 78: 1165–1174.

Shen P, Kaniewska M, Smith C, and Beachy RN (1993) Nucleotide sequence and genomic organization of rice tungro spherical virus. *Virology* 193: 621–630.

Turnbull-Ross AD, Mayo MA, Reavy B, and Murant AF (1993) Sequence analysis of the parsnip yellow fleck virus polyprotein: Evidence of affinities with picornaviruses. *Journal of General Virology* 74: 555–561.

Severe Acute Respiratory Syndrome (SARS)

J S M Peiris and L L M Poon, The University of Hong Kong, Hong Kong, People's Republic of China

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Bioavailability A measurement of the proportion of the orally administered dose of a therapeutically active drug that reaches the systemic circulation and is available at the site of pathology.
Coryza A runny nose.
Desquamation Shedding of epithelialium.
Lymphopenia Reduction of lymphocytes in the circulating blood below the normal range for age.
Myalgia Muscle pain.
Pathognomonic Characteristic and diagnostic of a particular disease.
PEGylated An adjective for describing molecules conjugated with polyethylene glycol (PEG).
Radiological abnormalities Atypical findings observed by medical imaging procedures (e.g., chest X-ray).

History

From November 2002 to January 2003, cases of an unusually severe atypical pneumonia were being observed in Guangdong Province, China. The disease was characterized by the lack of response to conventional antibiotic therapy and the occurrence of clusters of cases within a family or healthcare setting. In retrospect, these were the first known cases of the disease that was later to be called severe acute respiratory syndrome (SARS). Through January, the numbers of cases of this unusual 'atypical pneumonia' continued to increase with examples of 'super-spreading incidents' that were to punctuate the course of the subsequent SARS epidemic. Between 16 November and 9 February, 305 cases were identified, one-third of them in healthcare workers (**Table 1**).

On 21 February 2003, a 65-year-old doctor working in a hospital in the city of Guangzhou, the provincial capital of Guangdong, arrived in Hong Kong and checked into Hotel M. He had treated patients with 'atypical pneumonia' in Guangzhou and had been ill himself since 15 February. His 1-day stay on the ninth floor at this hotel led to the infection of at least 17 other guests or visitors, some of whom traveled on to Hanoi, Toronto, Vancouver, Singapore, USA, Philippines, Guangzhou, and Australia. Five of these secondary cases initiated clusters of infection in Hanoi, Singapore, Toronto and two clusters of infection within Hong Kong. This was the most significant single event in the global spread of SARS, and arguably the most dramatic known event in the global spread of any infectious disease. However, because the secondary cases had largely dispersed outside of Hong Kong, this cluster of cases remained 'invisible' until the epidemiological linkages were reconstructed in mid-March.

Between 26 February and 10 March, disease outbreaks were recognized in the Hanoi-French Hospital in Vietnam and in Prince of Wales Hospital in Hong Kong. Dr. Carlo Urbani, a World Health Organization (WHO) communicable diseases expert stationed in Vietnam, examined the first cases of the disease outbreak in Hanoi and provided WHO with the first case descriptions of this new disease. Later, Dr. Urbani was himself one of the victims who succumbed to this disease. On 12 March, the WHO issued a Global Health Alert regarding an atypical pneumonia that was a particular risk to healthcare workers. Subsequently, Singapore and Toronto also reported clusters of cases. On 15 March, the WHO issued a Travel Advisory. The new disease was named SARS and a preliminary case definition was provided. The WHO set up virtual networks of virologists, clinicians, and epidemiologists to rapidly collate, evaluate, and disseminate information about the new disease.

Within weeks, SARS had spread to affect 8096 patients in 29 countries across five continents with 744 fatalities, an overall case–fatality rate of 9.6%. Healthcare facilities served as a major amplifier of infection, constituting 21% of all reported cases.

By 21–24 March, the etiological agent of SARS was identified to be a novel coronavirus, subsequently termed SARS coronavirus (SARS CoV). Serological tests demonstrated that the human population had no prior evidence of infection with SARS CoV, indicating that this virus had newly emerged in humans and implying a likely zoonotic origin.

Early case detection and isolation of infected individuals reduced and interrupted SARS CoV transmission across the world. By 5 July 2003, the WHO announced that all chains of human transmission of SARS were broken and the outbreak was at an end. This was indeed a historic triumph for global public health. Although SARS was subsequently to re-emerge to cause limited human disease (and in one instance, limited human-to-human transmission) as a result of laboratory escapes and zoonotic transmission from the live game animal markets of Guangdong in December 2003–January 2004 (**Table 1**), the human outbreak of SARS had been controlled.

Table 1 A chronology of events in the emergence of SARS

Date	*Key events*
16 November 2002	45-year-old man in Foshan city, Guangdong Province, mainland China becomes ill with fever and respiratory symptoms and transmits the disease to four other relatives.
10 December 2002	35-year-old restaurant chef working in Shenzhen is admitted to Heyuan City People's Hospital. Transmits disease to eight healthcare workers.
January 2003	Pneumonia outbreaks in Guangzhou (capital city of Guangdong Province). These include number of healthcare workers infected through the care of patients with the disease.
11 February 2003	Guangdong health authorities report an outbreak of respiratory disease in Guangdong with 305 cases and five deaths, one-third of the cases being in healthcare workers caring for patients with the disease. Cases were reported from Foshan, Heyuan, Zhongshan, Jiangmen, Guangzhou, and Shenzhen municipalities of Guangdong Province.
21 February 2003	A 65-year-old doctor from Guangdong arrives and checks in at Hotel M in Hong Kong. His stay of 1 day at this hotel leads to the infection of at least 17 other guests or hotel visitors who initiate clusters of infection within Hong Kong, Vietnam, Singapore, and Toronto.
26 February 2003	A 48-year-old 'Hotel M contact' is admitted to Hanoi-French Hospital in Vietnam and is the source of an outbreak there. Seven healthcare workers were ill by 5 March.
1 March 2003	A 22-year-old 'Hotel M contact' is admitted to Tan Tock Seng Hospital, Singapore. She will pass on infection to 22 close contacts.
4 March 2003	A 26-year-old Hotel M contact admitted to Prince of Wales Hospital, Hong Kong. His illness is relatively mild and is not categorized as severe pneumonia. He transmits infection to 143 persons including 4 members of his family, 67 healthcare workers or medical students, and 30 other patients.
5 March 2003	A 78-year-old 'Hotel M contact' dies at home in Toronto, Canada. Four family members are infected. They are the source for the subsequent Toronto outbreak.
5–10 March 2003	Outbreaks are recognized in Hanoi and Hong Kong.
12 March 2003	WHO issues a Global Alert about atypical pneumonia in Guangdong, Hong Kong, and Vietnam that appears to place healthcare workers at high risk.
13–14 March 2003	Singapore and Toronto report clusters of atypical pneumonia. In retrospect, both groups have an epidemiological link to Hotel M. One of the doctors who had treated develops symptoms while traveling and is quarantined on arrival in Germany on 15 March.
15 March 2003	WHO has received reports of over 150 cases of this new disease, now named severe acute respiratory syndrome (SARS). An initial case definition is provided. Travel advisory issued.
17 March 2003	WHO multicenter laboratory network on SARS etiology and diagnosis is established.
21–24 March 2003	A novel coronavirus is identified in patients with SARS.
12 May 2003	The genome sequence of the SARS coronavirus is completed.
June 2003	A virus related to SARS CoV is detected in civets and other small mammals in live game-animal markets in Guangdong.
5 July 2003	Lack of further transmission in Taiwan, the last region to have SARS transmission, signals the end of the human SARS outbreak.
September 2003	Laboratory-acquired SARS coronavirus infection in Singapore.
December 2003–January 2004	Re-emergence of SARS infecting humans from animal markets in Guangdong. Laboratory-acquired SARS coronavirus infections in Taiwan.
February 2004	Laboratory-acquired SARS leads to community transmission in Beijing and Anhui in China.

Adapted from Peiris JSM, Guan Y, Poon LLM, Cheng VCC, Nicholls JM, and Yuen KY (2007) Severe acute respiratory syndrome (SARS). In: Scheld WM, Hooper DC, and Hughes JM (eds.) *Emerging Infections* 7, p. 23. Washington, DC: ASM Press, with permission from ASM Press.

Virology

SARS Virus

SARS coronavirus is a member of the genus *Coronavirus* within the family *Coronaviridae* and the order *Nidovirales*. Coronaviruses are classified on genetic and antigenic characteristics into three groups and SARS CoV is presently regarded as a group 2b coronavirus. It is an enveloped, positive-sense, single-stranded RNA virus with a genome size of approx 29.7 kbp. The virus particle is approximately 100–160 nm in diameter with a distinctive corona of petal-shaped spikes on the surface which is comprised of the spike glycoprotein (S). The S protein is in a trimeric form on the viral surface. It has an N-terminal variable subdomain (S1) which contains the motifs responsible for receptor binding. A more conserved subdomain (S2), which contains heptad repeats and a coiled-coil structure, is important in the membrane fusion process. The S1–S2 subdomains remain in a noncleaved form in the intact SARS CoV virion and cleavage is believed to occur within the endocytic vesicle during the viral entry process. The envelope also contains a transmembrane glycoprotein M and in much smaller amounts, an envelope (E) protein. The M protein is a

triple-spanning membrane protein and has a key role in coronavirus assembly. The hemagglutinin-esterase (HE) glycoprotein, found in some group 2 coronaviruses, is absent in SARS CoV. The nucleocapsid protein (N) interacts with the viral genomic RNA to form the viral nucleocapsid. Viral replication complexes are believed to be localized within double-membraned vesicles or autophagosomes.

SARS CoV Genome

The genome of SARS CoV is that of a typical coronavirus. The viral genomic RNA has at least 14 open reading frames (ORFs) (**Figure 1**). The genome codes for 16 nonstructural proteins (nsp1–16), 5 structural proteins, and 7 accessory proteins. The genomic RNA encoding the replicase gene functions as mRNA to generate polyproteins 1a and 1ab. The translation of ORF1b is directed by a –1 ribosomal frameshift (RFS) signal that contains a nucleotide slippery sequence (5′-UUUAAAC-3′) and an RNA pseudoknot. By contrast, the structural and accessory proteins are products derived from subgenomic RNA (sgRNA 2–9) which are synthesized by discontinuous RNA transcription. Translated products from ORF2 (S), ORF4 (E), ORF5 (M), and ORF9a (N) are viral structural proteins as described above. Recently, it was reported that the protein encoded by the ORF3a, which is able to interact with S and contains ion channel activity, is also a structural protein. However, the full function of this protein is yet to be determined.

The polyproteins 1a and 1ab generated from the replicase gene are cleaved by a papain-like proteinase (part of nsp3) and a 3C-like proteinase (nsp5) to generate 16 nonstructural proteins (**Figure 1(b)**). Nsp12 is a primer-dependent RNA-dependent RNA polymerase (RdRp), whereas nsp8 is a noncanonical RdRp (nsp8) synthesizing primers utilized by nsp12. In addition, eight nsp7 and eight nsp8 subunits are able to form a hexadecamer with a hollow, cylinder-like structure. RNA-binding studies and the overall architecture of this nsp7–nsp8 complex suggest that it might encircle RNA and confer processivity of nsp12. The nsp9 is a single-stranded RNA-binding protein and is able to interact with nsp8. The nsp13 is a helicase and unwinds duplex RNA (and DNA) in a 5′-to-3′ direction. The nsp3, nsp14, nsp15, and nsp16 have been shown to have ADP-ribose 1′-phosphatase, 5′-to-3′ exonuclease, endoribonuclease, and 2′-*O*-ribose methyltransferase activities, respectively. These four proteins are distantly related to cellular enzymes involved in RNA metabolism. These observations may be relevant to viral RNA processing. The nsp10 contains two zinc finger motifs and is suggested to be a regulator of vRNA synthesis. The biological functions of nsp1, nsp2, nsp4, nsp6, and nsp11 are largely unknown. The nsp1 is reported to induce chemokine dysregulation and host mRNA

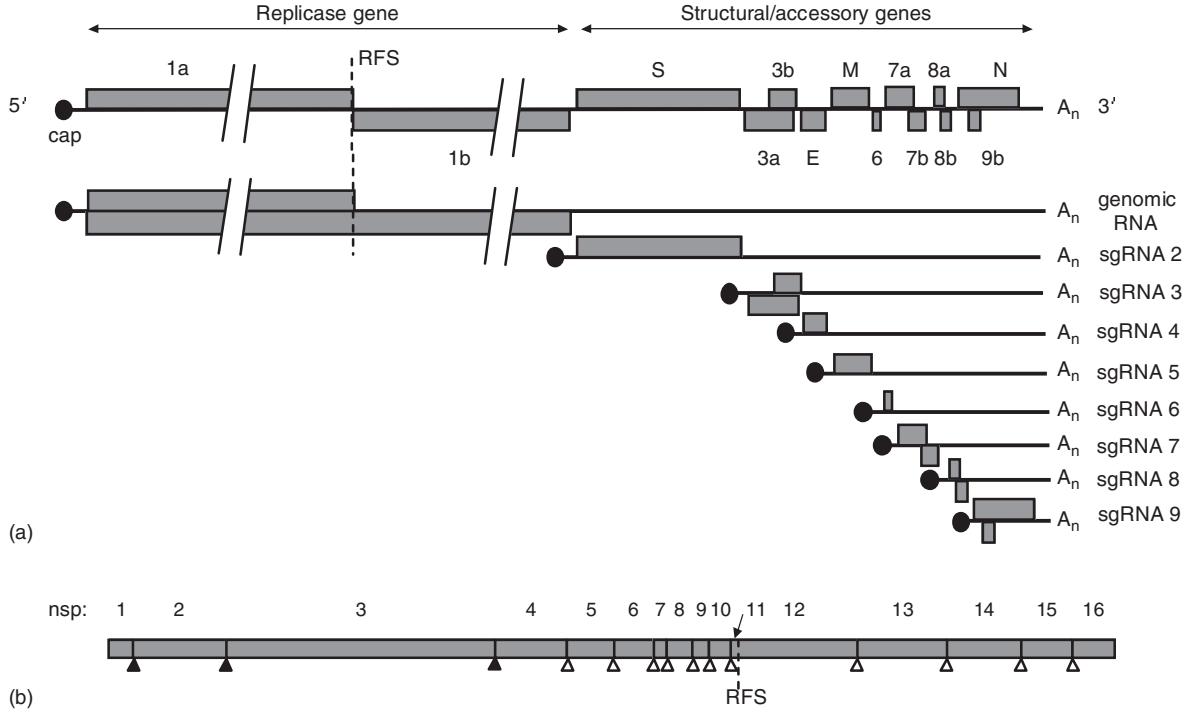


Figure 1 SARS CoV genome. (a) Genomic organization of SARS CoV. The 14 ORFs are expressed from the genome RNA and a nested set of subgenomic mRNA (sgRNA 2–9) that all have a common leader sequence derived from the 5′ end of the genome. The genomic RNA and all sgRNA contain a 5′ cap and a polyadenylated tail at the 3′ end. (b) Domain organization of the proteins for ORF1ab. Black and white arrow heads represent the sites cleaved by papain-like and 3C-like proteinases, respectively. The ribosomal frameshift (RFS) site is highlighted by a broken line.

degradation. The nsp2 is dispensable for virus replication. The nsp4 and nsp6 each contain a putative transmembrane domain.

Apart from the ORFs encoding the replicase and structural proteins, the viral genome contains additional ORFs that code for accessory proteins (3b, 6, 7a, 7b, 8a, 8b, and 9b). Genetically modified recombinant viruses without these accessory ORFs have been shown to be replication competent in cell cultures, indicating that the accessory ORFs may not be essential for virus replication *in vitro*. However, recombinant viruses with deletions in these regions are attenuated, suggesting that these proteins might have functions that are important for viral replication and pathogenesis *in vivo*. The accessory proteins from ORF3b and ORF7a induce apoptosis in transfected cells. There is also evidence suggesting that the 7a protein is incorporated into virions. The protein encoded in ORF6 has been shown to inhibit the nuclear import of STAT-1 and function as an interferon antagonist in infected cells. These properties might relate to virus virulence. Interestingly, comparative sequence analysis of SARS CoV isolated from palm civets (see below) and humans showed that all animal isolates contained a 29-nucleotide (nt) sequence which is absent from most human isolates obtained in the later phase of the SARS outbreak. As a result, the ORF8 in these human SARS CoVs encodes 8a and 8b proteins, whereas the corresponding ORF in the animal isolates encodes a single protein, known as the 8ab protein. These proteins from the animal and human ORF8 have differential binding affinities to various SARS CoV structural proteins. Furthermore, the expression of E can be downregulated by 8b but not 8a or 8ab in infected cells. These observations may suggest that the 29-nt deletion might modulate the replication or pathogenesis of the human SARS CoV. The crystal structure of the 9b protein suggests that it might be a lipid binding protein but its function is yet to be identified. Overall, these accessory proteins may play roles in viral replication and pathogenesis.

Ecology and Animal Reservoir

Until the end of January 2003, 39% of patients with SARS in Guangdong had handled, killed, or sold wild animals or prepared and served them as food. However, such risk factors were found in only 2–10% of cases from February to April 2003 when the virus had adapted to efficient human-to-human transmission. Thus, the early epidemiological evidence pointed to the live game animal trade as a potential source of the SARS CoV. SARS-like coronaviruses were identified in a number of small mammalian species sold in the live game animal markets in Guangdong, including the palm civet (*Paguma larvata*), raccoon dog (*Nyctereutes procyonides*), and the Chinese ferret badger (*Melogale moschata*). A high proportion of individuals working in these markets were observed to have developed antibodies to SARS CoV, although none of them had a history of the disease. Viruses isolated from the re-emergent SARS cases in Guangdong in December 2003–January 2004 were more similar to those found in civets in these markets, rather than to viruses causing the global outbreak in early 2003. These observations strongly implicated the live game animal trade as the interface for interspecies transmission of a precursor animal SARS-like coronavirus to humans.

SARS CoV can be shed for weeks in experimentally infected palm civets but many of the other species appear to clear the virus rapidly. While civets in live animal markets were often observed to be positive for SARS-like coronavirus RNA, civets tested in the farms that supply these markets and those caught in the wild rarely have evidence of infection. Thus, palm civets were believed not likely to be the natural reservoir of the precursor SARS CoV (see below). More recently, group 2b coronaviruses related to SARS CoV have been identified in *Rhinolophus* bats in Hong Kong and mainland China. Such bats are also sold live in these game animal markets. It is now believed that these or related bat coronaviruses may be the precursor from which SARS CoV originated (see below).

Phylogeny

SARS CoV and the SARS-like civet and bat coronaviruses form a distinct phylogenetic subgroup (2b) within the group 2 coronaviruses (**Figure 2**). Genetic and phylogenetic analysis indicates that the viruses associated with the early phase of the human SARS outbreak are more closely related to the viruses found in palm civets and other small mammals in the live game animal markets in Guangdong. The genomes of viruses in the early phase of the human outbreak in 2003 were observed to be under strong positive selective pressure, suggesting that the virus was rapidly adapting in a new host. Furthermore, virus in civets was also found to be under strong positive selective pressure, supporting the view that civets were not the natural host of the precursor SARS-like coronavirus. The search for the precursor of SARS CoV led to the discovery of a number of novel coronaviruses in bats which are related to group 1 and group 2 coronaviruses. Some of these bat coronaviruses are genetically related to SARS CoV (group 2b) and are likely to be the direct or indirect precursor of SARS CoV (see below).

Interestingly, considered overall, the recently discovered group 1 and group 2 (including SARS CoV-like) bat coronaviruses appear to be in evolutionary stasis while many other mammalian coronaviruses still appear to be under evolutionary selection pressure, raising the intriguing possibility that bats may in fact be the precursors, not only of SARS CoV, but also of most other mammalian coronaviruses.

Virus Receptors

The functional receptor for SARS CoV on human cells is the angiotensin-converting enzyme 2 (ACE-2) which

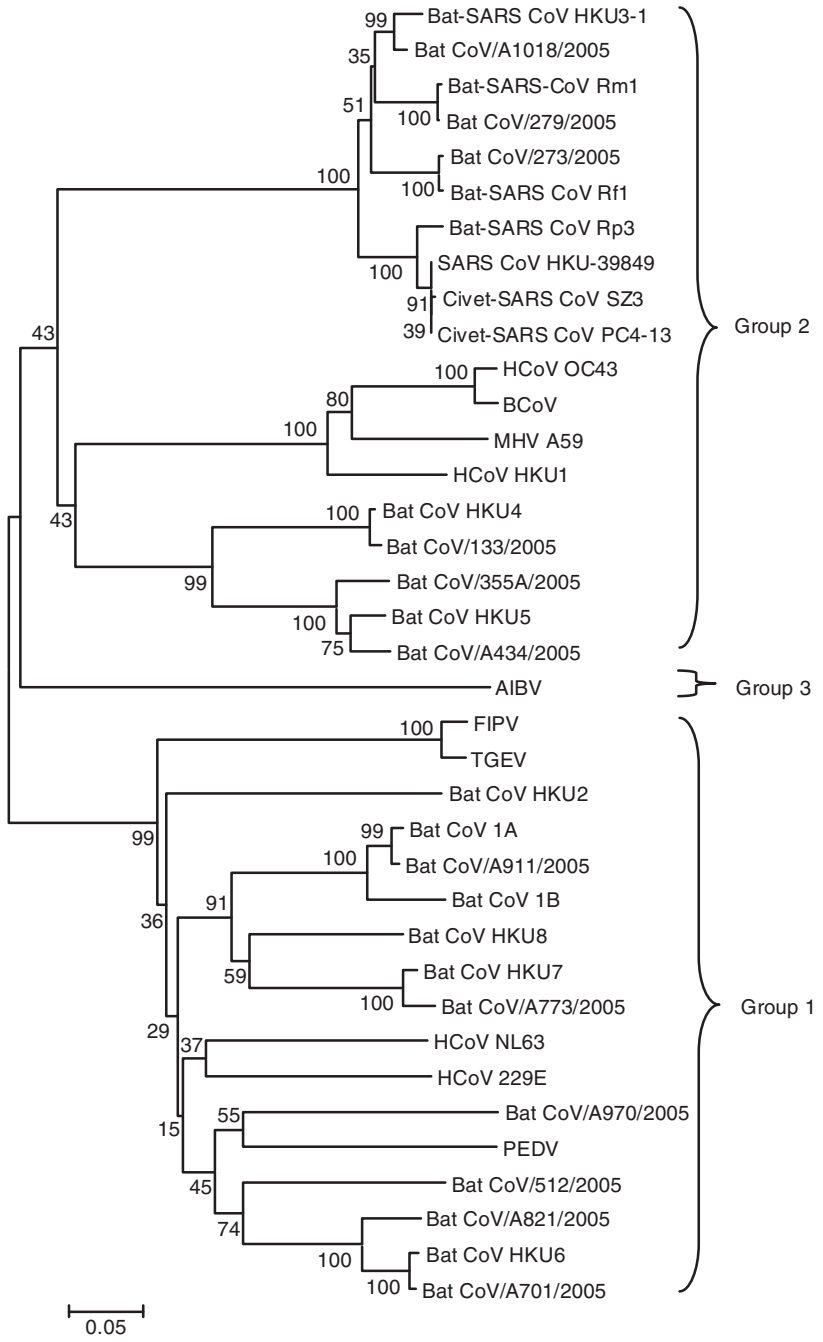


Figure 2 Phylogenetic analysis of RNA sequences coding for the RNA-dependent RNA polymerase (partial sequence). The phylogenetic tree was constructed by the neighbor-joining method and bootstrap values were determined with 1000 replicates. Human SARS CoV (GenBank accession AY278491.2), SARS CoVs isolated from palm civets in 2003 (AY304486.1) and 2004 (AY613948.1) and bat CoVs [Bat CoV 1A (DQ666337.1), Bat CoV 1B (DQ666338.1), Bat CoV HKU2 (DQ249235.1), Bat-SARS CoV HKU3–1 (DQ022305), Bat CoV HKU4 (DQ249214.1), Bat CoV HKU5 (DQ249217.1), Bat CoV HKU6 (DQ249224.1), Bat CoV HKU7 (DQ249226.1), Bat CoV HKU8 (DQ249228.1), Bat-SARS CoV Rp3 (DQ071615), Bat-SARS CoV Rm1 (DQ412043), Bat-SARS CoV Rf1 (DQ412042), Bat CoV/A434/2005 (DQ648819.1), Bat CoV/A701/2005 (DQ648833.1), Bat CoV/A773/2005 (DQ648835.1) Bat CoV/A821/2005 (DQ648837.1), Bat CoV/A970/2005 (DQ648854.1) Bat CoV/A911/2005 (DQ648850.1), Bat CoV/A1018/2005 (DQ648795.1), Bat CoV/133/2005 (NC_008315), Bat CoV/273/2005 (DQ648856), Bat CoV/279/2005 (DQ648857), Bat CoV/355A/2005 (DQ648809.1), Bat CoV/512/2005 (DQ648858)] were aligned with references sequences as indicated. Reference sequences are: transmissible gastroenteritis virus, TGEV (DQ811789); HCoV 229E (AF304460); HCoV NL63 (AY567487); HCoV-OC43 (AY391777); HCoV-HKU1 (DQ415903 HKU1); porcine epidemic diarrhea virus, PEDV (AF353511); avian infectious bronchitis virus, AIBV (AY646283); mouse hepatitis virus, MHV (AY700211); bovine coronavirus, BCoV (AF220295); feline infectious peritonitis virus, FIPV (AY994055).

binds the receptor-binding motif (amino acid residues 424 to 494) of the SARS CoV spike (S) protein. While the human SARS CoV S protein binds efficiently to both human and civet ACE-2, the civet-like SARS CoV S protein binds efficiently to ACE-2 from civets but poorly to human ACE-2. The spike protein of the bat SARS-like coronavirus lacks the ACE-2 receptor-binding motif and is therefore unlikely to bind to human ACE-2.

These findings explain the increased human transmissibility of SARS CoV in the later stages of the SARS outbreak, the observation that human SARS CoV efficiently infects civets under experimental conditions, and the failure of civet SARS CoV or bat SARS-like CoV to replicate productively in primate (Vero-E6, FRhK4) cells that support replication of human SARS CoV. This finding also explains the poor virulence and transmissibility of re-emergent SARS in December 2003–January 2004 when humans are believed to have been infected with a civet-like SARS CoV.

Other cell-surface molecules such as L-SIGN, DC-SIGNR, DC-SIGN (CD209), and L-SECtin may serve as binding receptors but do not appear to be functional viral receptors in the absence of ACE-2. They may, however, promote cell-mediated transfer of the virus to other susceptible target cells. On the other hand, binding to L-SIGN appears to lead to proteasome-dependent viral degradation and it may function as a scavenger receptor (see below).

Human Disease

Transmission

Respiratory droplets are the major source of infectious virus for transmission of SARS. However, aerosol exposure has probably contributed to disease transmission, at least in some defined instances where aerosol-generating procedures (e.g., nebulizers, high-flow oxygen therapy, intubation) have been used. The unusual stability of SARS CoV also suggests that contaminated surfaces and fomites may contribute to disease spread. As SARS CoV is present in feces and urine (and possibly other body secretions), these body fluids may also play a part in disease transmission. The largest single outbreak of SARS at the Amoy Gardens apartment block in Hong Kong, where over 300 individuals were infected from a single index case, is believed to have been caused by aerosols generated from infected body secretions (e.g., feces).

The estimated incubation period for SARS is 2–14 days. During the 2003 outbreak, the majority of cases did not transmit disease at all and only a few patients accounted for a disproportionately large number of secondary cases. Host factors may have played a role in these super-spreading events but, in many cases, there was a unique combination of host factors and environmental circumstances that facilitated transmission. In contrast to the high transmission rates in these super-spreading events and within hospitals, there was less evidence of secondary transmission within the family or within households (e.g., 15% in Hong Kong). Notwithstanding the 'super-spreading phenomenon' that has characterized SARS, the basic reproduction number (Ro) of SARS is estimated to range from 2 to 4.

Seroepidemiological studies of contacts of SARS patients (both adults and children) have revealed that asymptomatic infection was uncommon. The absence of large numbers of asymptomatic transmitters and the paucity of transmission during the first 5 days of illness explain the success of the public health measures of aggressive case detection and isolation in interrupting transmission of human-adapted SARS CoV and the control of the global disease outbreak. These features of SARS have been attributed to the observation that, unlike many other acute viral respiratory infections, SARS transmission has mostly occurred only after the fifth day of illness. This is, in turn, probably related to the low viral load in the upper respiratory tract during the early phase of the illness (see below).

Clinical Features

As the clinical features of SARS are not pathognomonic, a contact history and virological evidence of infection are important for confirmatory diagnosis. SARS typically starts with myalgia and loose stools around the time of onset of fever without coryza or sore throat (seen in 70% of patients). The upper respiratory manifestations are less commonly observed. Radiological abnormalities have been observed in >60% of cases at initial presentation and preceded lower respiratory tract symptoms in approximately 41% of patients.

Children have had much milder illness than adults and mortality rates progressively increase with age. Some patients, particularly those with progressive lower respiratory tract involvement have had a watery diarrhea. Other extrapulmonary manifestations included hepatic dysfunction and a marked lymphopenia involving both B, T (CD4 and CD8 subsets), and natural killer (NK) cells. High serum levels of chemokines (interleukin 8 (IL-8), CCL2, and CCl10) and pro-inflammatory cytokines (IL-1, IL-6, IL-12) have been observed.

The overall case–fatality rate was 9.6% and the terminal events were severe respiratory failure associated with acute respiratory distress syndrome (ARDS) and multiple organ failure. Age, presence of co-morbidities, and viral load in the nasopharynx and serum during the first 5 days of illness correlated with an adverse prognosis.

Autopsy findings of those who died in the first 10 days of illness were diffuse alveolar damage, desquamation of pneumocytes, and hyaline membrane formation. Viral

RNA was detected by quantitative polymerase chain reaction (PCR) at high copy number in the lung, intestine and lymph nodes, and at lower levels in spleen, liver, and kidney. In lung biopsy tissue or in autopsy tissue of patients dying in the first 10 days after disease onset, viral antigen and viral nucleic acid were demonstrated by immunohistochemistry and *in situ* hybridization methods respectively, in alveolar epithelial cells and to lesser extent in macrophages. A few unconfirmed studies have also reported the detection of virus particles or viral RNA in multiple organs but these findings require independent confirmation.

Laboratory Diagnosis

Highly sensitive and specific real-time PCR assays for detection of viral RNA remain the best choice for early SARS diagnosis. Viral RNA has been detected in respiratory specimens, feces, serum, and urine. Specimens from the lower respiratory tract such as endotracheal aspirates have higher viral load than those from the upper respiratory tract and are better diagnostic clinical specimens. As viral load is low during the first 5 days of disease, a negative PCR result from specimens collected at this time does not exclude the diagnosis. Testing multiple specimens improves the detection rate of SARS. Virus culture on Vero E6 or FRhK-4 cells and viral antigen detection tests are much less sensitive than reverse transcriptase PCR (RT-PCR) for detecting the virus. While viral RNA remains detectable in the respiratory secretions and feces for many weeks after the onset of illness, specimens rarely yield a virus isolate after the third week of illness.

Sero-conversion by immunofluoresence or neutralization occurs during the second week of illness and can provide reliable retrospective diagnosis. Enzyme-linked immunoassays using inactivated whole virus or recombinant antigens are convenient alternatives for serological screening, but any positive results must be confirmed by the more specific immunofluoresence or neutralization tests.

Pathogenesis

The primary mechanism of lung damage appears to be due to infection of type 1 and type 2 pneumocytes which are key target cells of the virus. Type 2 pneumocytes are important in the repair of lung injury and infection of these cells can potentially impair the regenerative responses of the lung and aggravate the respiratory impairment.

Whereas mice deficient in NK, T or B lymphocytes display similar kinetics of viral replication to normal mice, infection of mice with defects in the STAT1 signaling pathway results in more prolonged viral replication and more severe disease. These findings indicate the importance of innate immune responses in the control of infection, at least in the mouse. Infection of epithelial cells, macrophages, and myeloid dendritic cells fails to induce a type 1 interferon response although other interferon response genes are activated. Viral proteins expressed from ORF3b, ORF6, and the N gene have interferon antagonist effects *in vitro*. In contrast, macrophages and dendritic cells respond to infection *in vitro* with strong chemokine responses, including those (e.g., CCL10) that are elevated in the serum of SARS patients, and macrophage-chemoattractant chemokines (CCL2). This may explain the predominantly macrophage infiltrate in the lung.

There is evidence of viral replication within intestinal epithelial cells but there is minimal cellular infiltrate or disruption of intestinal architecture and the pathogenesis of diarrhoea in SARS remains unclear.

Treatment

As SARS emerged as a disease of unknown etiology, empirical therapeutic options were initially tested including broad spectrum antivirals and immunomodulators such as ribavirin, intravenous immune globulin, type 1 interferon, SARS convalescent plasma, and corticosteroids. However, in the absence of controlled clinical trials, no conclusions can be drawn on the efficacy of these interventions.

Anti-SARS CoV activity *in vitro* has been demonstrated for several therapeutics already in clinical use for other conditions, including lopinavir–nelfinavir, glycyrrhizin, baicalin, reserpine, and niclosamide. There are contradictory reports on the *in vitro* activity of ribavirin, interferon beta, and interferon alpha. In summary, taking into account bio-availability of these compounds and *in vitro* data, interferon alpha *n*1/*n*3, leukocytic interferon alpha, interferon beta and nelfinavir appear to be worthy of animal studies and randomized placebo-controlled clinical trials if SARS was to return.

A clinical trial of lopinavir 400 mg with ritonavir 100 mg orally every 12 h (added to an existing regimen of ribavirin and corticosteroid therapy) appeared to provide clinical benefit compared to historical controls. However, the lack of concurrent controls makes it difficult to draw conclusions. Similarly, a limited clinical trial of 13 patients using interferon alfacon-1 treatment showed a trend toward improved radiological and clinical outcomes, but without achieving statistical significance.

Studies in primate models have demonstrated prophylactic or therapeutic benefit from PEGylated recombinant interferon alpha-2b and from small interfering RNA therapy. More recently, screening of combinatorial chemical libraries *in vitro* has identified potential inhibitors of the viral protease, helicase, and spike protein-mediated entry.

Animal Models

Experimental SARS CoV infection leads to virus replication in a number of animal species including nonhuman primates (e.g., cynomolgous and rhesus macaques, African

green monkeys, and marmoset monkeys), mice (BALB/c, C57/BL6), Golden Syrian hamsters, ferrets, and cats. Only some of these develop pathological lesions in the lungs (cynomolgous macaques, ferrets, hamsters, marmosets, aged BALB/C mice).

Interestingly, whereas SARS CoV replicates in the lung of both young and aged (12–14 months) BALB/c mice, only aged mice manifest clinical symptoms and histological evidence of lung pathology. This is reminiscent of disease in humans in which children have mild illness (see above). Furthermore, few animal models reproduce the gastrointestinal manifestations of the illness.

While the ideal animal model for understanding SARS pathogenesis is lacking, those that support viral replication (with or without clinical disease) are adequate for evaluating the efficacy of vaccines.

Vaccines and Immunity

A wide range of strategies have been explored for development of SARS vaccines. These have included: inactivated whole virus vaccines; subunit vaccines including baculovirus expressed S1 subdomain or the complete trimeric spike protein of the virus expressed in mammalian cells; DNA vaccines expressing S (full-length and fragments), N, M, or E proteins; and vectored vaccines based on modified vaccinia Ankara (MVA) virus, vesicular stomatitis virus, adenoviral vectors carrying S, M, or N proteins, and attenuated parainfluenza virus type 3 vectored vaccines carrying S, E, M, and N proteins. Neutralizing antibody responses and, where appropriate, cell-mediated immune responses have been measured as correlates of immunity. Some of these vaccines have been evaluated in experimental models by challenging with infectious SARS CoV.

Trials in hamsters of attenuated parainfluenza virus type 3-vectored vaccines individually expressing SARS CoV S, E, M, and N proteins have indicated that only the S protein construct elicits neutralizing antibody and protects against experimental challenge. Furthermore, passive transfer of serum containing S protein neutralizing antibody has been shown to be sufficient to induce protective immunity in mice. It is concluded that neutralizing antibody to the S protein is an important correlate of protection. The receptor-binding determinant of the S1 subdomain is an immuno-dominant epitope and a critical determinant for virus neutralization.

As antibody can enhance rather than protect against the coronavirus disease feline infectious peritonitis, antibody-dependent enhancement has been a concern for SARS-Co vaccine development. However, no evidence of vaccine-enhanced disease has been observed to date, with two possible exceptions. There is a report that a modified vaccinia Ankara virus S protein vaccine has led to hepatitis in vaccinated ferrets but this has not been independently confirmed. There is also a report that S protein antibody elicited by a subunit vaccine enhances entry of pseudo-particles carrying S spike into lymphoblastoic cell lines which lack ACE-2 and are not normally permissive to infection. However, in the challenge experiments in hamsters, the vaccine did not induce protection and there is no evidence of disease enhancement.

Passive immunization with human monoclonal antibodies to the S protein has been successful at protecting mice and ferrets from experimental challenge by reducing viral load in the lung but not in the nasopharynx.

Most of these active and passive immunization studies have evaluated protection from challenge using the homologous human-adapted SARS CoV. However, a newly emergent SARS outbreak will probably arise from the animal reservoir and it is therefore important to investigate cross-protection against animal SARS-like CoV. As none of the civet or bat SARS CoV has yet been successfully grown *in vitro*, the cross-reactive neutralizing antibody response has been studied using lentiviruses pseudotyped with CoV S protein from a civet virus (SZ3), a civet-like virus causing re-emergent SARS in humans in December 2003 (GD03), and from a human SARS CoV (Urbani-strain) isolated from the major human SARS outbreak in 2003. The viruses pseudotyped with human Urbani virus S protein were neutralized by antibodies to the civet SARS-like virus but pseudotypes with the civet-like S protein were not neutralized by antibodies to the human SARS CoV (Urbani). On the contrary, antibody to the Urbani virus appeared to enhance the infectivity of the GD03 and SZ3 pseudotyped viruses. These findings appear to reflect receptor usage of these viruses as it has been shown that GD03 and SZ3 bind poorly to human ACE-2 (see above). The development of vaccines that can prevent re-emergence of SARV CoV from its zoonotic reservoir remains a challenge.

Conclusion: Will SARS Return?

Like many recent emerging infectious diseases that threaten human health, SARS was a zoonosis. The SARS CoV that was responsible for the global outbreak in 2003 was well adapted to bind to human ACE-2 and was efficiently transmitted human-to-human. Laboratories remain a potential source of infection from such viruses and, as occurred in February 2004, laboratory escape can lead to a community outbreak.

The SARS-like coronavirus found in civets (and other mammals) in live game-animal markets is very closely related to SARS CoV, but it binds inefficiently to the human ACE-2 receptor (see above). Consequently, when human infection with the civet SARS-like CoV occurred in December 2003–January 2004, there was no human-to-human transmission and clinical disease was mild. While SARS-like coronaviruses have been found in bats, they are

genetically distinct to SARS CoV and the bat SARS-like CoV S protein appears unable to bind to human or civet ACE-2. Thus, it is likely that re-emergence of a virus capable of causing human disease from this source probably requires extensive adaptation in an intermediate host (e.g., small mammals such as civets). While it is difficult to assess the likelihood of SARS re-emergence, this possibility cannot be excluded.

The rapid expansion of the live game-animal trade and the development of large markets in southern China which house a diversity of wild and domestic animal species were probably important in facilitating the emergence of SARS CoV. It is therefore possible that, like Ebola, SARS may re-emerge at intervals in the future. However, a number of epidemiological characteristics of SARS (see above) should allow it to be contained by public health interventions, once the disease is diagnosed. Indeed, the chain of community transmission arising from a laboratory escape of SARS CoV in February 2004 was contained by such public health measures and community transmission was aborted. However, if the dynamics of transmission of a re-emergent virus are different, and particularly if transmission occurs earlier in the illness and there are more asymptomatic infections, the options for control and the ultimate consequences may be very different. It remains important, therefore, to understand better the ecological and viral factors that predispose to interspecies transmission and the emergence of animal viruses with efficient competence for transmission in humans. Attention should be directed toward the adaptation strategies and the ecological factors that are important in determining interspecies transmission, rather than focus on the disease itself (i.e., SARS). Efforts to understand better the molecular basis for interspecies transmission that led to the genesis of SARS CoV will help us to prepare better for the next emerging infectious disease challenge; whether this comes from SARS CoV, avian influenza H5N1, or a yet unknown virus.

See also: Coronaviruses: General Features; Coronaviruses: Molecular Biology.

Further Reading

Chan JCK and Taam Wong VCW (2006) *Challenges of Severe Acute Respiratory Syndrome*. Singapore: Elsevier.

Cinatl J, Michaelis M, Hoever G, Preiser W, and Doerr HW (2005) Development of antiviral therapy for severe acute respiratory syndrome. *Antiviral Research* 66: 81.

de Haan CAM and Rottier PJM (2006) Hosting the severe acute respiratory syndrome coronavirus: Specific cell factors required for infection. *Cellular Microbiology* 8: 1211.

Gillim-Ross L and Subbarao K (2006) Emerging respiratory viruses: Challenges and vaccine strategies. *Clinical Microbiology Reviews* 19: 614.

Gorbalenya AE, Enjuanes L, Ziebuhr J, and Snijder EJ (2006) Nidovirales: Evolving the largest RNA virus genome. *Virus Research* 117: 17.

Li W, Wong SK, Li F, *et al.* (2006) Animal origins of the severe acute respiratory syndrome coronavirus: Insight from ACE-2 protein interactions. *Journal of Virology* 80: 4211.

May RM, McLean AR, Pattison J, and Weiss RA (2004) Emerging infections: What have we leant from SARS? *Philosophical Transactions of the Royal Society of London, Series B* 359: 1045.

Peiris JSM, Guan Y, Poon LLM, Cheng VCC, Nicholls JM, and Yuen KY (2007) Severe acute respiratory syndrome (SARS). In: Scheld WM, Hooper DC, and Hughes JM (eds.) *Emerging Infections 7*, p. 23. Washington, DC: ASM Press.

Peiris JSM, Yuen KY, Osterhaus ADME, and Stohr K (2003) The severe acute respiratory syndrome. *New England Journal of Medicine* 349: 2431.

Peiris M, Anderson L, Osterhaus A, Stohr K, and Yuen KY (2005) *Severe Acute Respiratory Syndrome*. Oxford: Blackwell.

Perlman S and Holmes KV (eds.) (2006) The *Nidoviruses*: Towards control of SARS and other nidovirus diseases. *Advances in Experimental Medicine and Biology*, vol. 581. 617pp. New York: Springer.

Stockman LJ, Bellamy R, and Garner P (2006) SARS: Systematic review of treatment effects. *PLoS Medicine* 3: e343.

World Health Organization(2003) A multicentre collaboration to investigate the cause of severe acute respiratory syndrome. *Lancet* 361: 1730.

World Health Organization, Western Pacific Region (2006) *SARS. How a Global Epidemic was Stopped*. Geneva: WHO Press.

Zhong NS and Zeng GQ (2003) Our strategies for fighting severe acute respiratory syndrome (SARS). *American Journal of Respiratory and Critical Care Medicine* 168: 7.

Shellfish Viruses

T Renault, IFREMER, La Tremblade, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Aquaculture Cultivation of aquatic animals or plants.
Bivalve Marine or freshwater mollusks having a soft body with plate-like gills enclosed within two shells hinged together.
Gills Respiratory organ of aquatic animals that breathe oxygen dissolved in water.
Hatchery A place where eggs are hatched under artificial conditions.
Hemocyte Any blood cell especially in invertebrates.

Larva The immature free-living form of most invertebrates which on hatching from the egg is fundamentally unlike its parent and must metamorphose.
Mantle A protective layer of epidermis in mollusks that secretes a substance forming the shell.
Mollusk Invertebrates having a soft unsegmented body usually enclosed in a shell.
Nursery A place for the cultivation of juveniles under controlled conditions.
Shellfish Aquatic invertebrates belonging to the crustacean or mollusk families.
Velum Membrane of mollusk larvae that allows swimming activity.

Introduction

A natural abundance of shellfish was common in many areas of the world until the early twentieth century. However, industrial and urban development and population growth in coastal areas, coupled with extreme harvest pressure, appear to have contributed to a steady decline in natural shellfish populations. This decline in wild harvests, together with a greater demand for seafood from an increasing world population, have driven the development of technology for the intensive management and cultivation of shellfish. As a result, global shellfish production, the greatest proportion of which is bivalves, was estimated to be 10 732 000 metric tons in the year 2000. However, as husbandry practices have developed, the significant impact of infectious diseases on productivity and product quality has been increasingly recognized. Numerous examples worldwide have demonstrated that entire shellfish industries in coastal areas are susceptible to diseases and that the production of healthy shellfish is a key to the economic viability of mollusk farming.

The study of shellfish diseases is a relatively young science and the discovery of viruses in marine mollusks is a fairly recent event. Viral diseases have seriously affected the aquaculture industry during the last decades. Viral pathogens are often highly infectious and easily transmissible, and are commonly associated with mass mortalities. Viruses interpreted as members of the families *Iridoviridae*, *Herpesviridae*, *Papovaviridae*, *Reoviridae*, *Birnaviridae*, and *Picornaviridae* have been reported as associated with disease outbreaks and causing mortality in various mollusks. However, there is currently a lack of information concerning the occurrence of mollusk viruses worldwide, and the basic method for identification and examination of suspect samples is still predominantly histopathology. This technique enables the identification of cellular changes associated with infection but does not provide conclusive identification of mollusk viruses unless completed by other methods such as transmission electron microscopy. Moreover, as there is a lack of marine mollusk cell lines and, since invertebrates lack antibody-producing cells, the direct detection of viral agents remains the only possible approach to diagnosis.

As filter feeders, bivalves may also bioaccumulate viruses from humans and other vertebrates, acting as a transient reservoir. The consumption of raw or undercooked shellfish can result in human disease and contamination of shellfish cultivated in coastal marine waters by microorganisms that are pathogenic to humans is a public health concern worldwide. The association of shellfish-transmitted infectious diseases with sewage pollution has been well documented since the late nineteenth and early twentieth centuries. Human enteric viruses, including rotaviruses, enteroviruses, and hepatitis A virus, are the most common etiological agents transmitted by shellfish. These enteric viruses are associated with several human diseases ranging from ocular and respiratory infections to gastroenteritis, hepatitis, myocarditis, and aseptic meningitis. Many of these viruses are transmitted by the fecal–oral route and are highly prevalent in locations with poor sanitation. There is considerable literature on the implications for human health. However, this is not the subject of the present article.

Irido-Like Viruses

Hosts and Locations

Infections by irido-like viruses have been reported in oysters in France and in the USA. Two distinctive conditions have been associated with mass mortalities in adult Portuguese oyster, *Crassostrea angulata*, along French coasts: gill necrosis virus disease and hemocytic infection virus disease. A viral infection similar to the latter was reported in the Pacific oyster *Crassostrea gigas* during summer mortalities in the Bay of Arcachon (Atlantic coast, France) long after the disappearance of the Portuguese oyster. A third type of irido-like virus, the oyster velar virus (OVV), has been reported from hatchery-reared larval Pacific oysters on the west coast of North America (Washington State, USA).

Disease Manifestations and Epizootiology

Gill necrosis virus disease is regarded as the primary cause of disease outbreaks and mortalities that occurred in the late 1960s among Portuguese oysters on the Atlantic coast of France. The disease appears to have affected up to 70% of oyster populations with maximum losses reported in 1967. Losses subsequently declined and survivors recovered from the disease. The first gross signs were small perforations in the center of yellowish discolored

zones of tissue on gills and labial palps. Further development and extension of the lesions resulted in larger and deeper ulcerations. In advanced stages, total destruction of affected gill filaments was observed. Yellow or green pustules also developed on the adductor muscle and mantle.

In 1970, high mortality rates were again reported in *C. angulata* oysters in France. Mortality was first observed in the basin of Marennes Oleron (Altantic coast) and in Brittany. The high mortality rates that occurred during this epizootic led to almost total extinction of French *C. angulata* by 1973. The disease affected adult oysters. No distinctive clinical signs were noted (e.g., no gill lesions). Histological observations included an acute cellular infiltration consisting of atypical virus-infected hemocytes. Pacific oysters seemed to be resistant to the vius and subsequently replaced *C. angulata* in France. However, a morphologically similar virus was reported from Pacific oysters during an outbreak of summer mortality in the Bay of Arcachon (Atlantic coast, France) in 1977. Although affected Pacific oysters exhibited virtually no gross signs, the presence of atypical cells interpreted as infected hemocytes and degeneration of connective tissues were reported in infected animals.

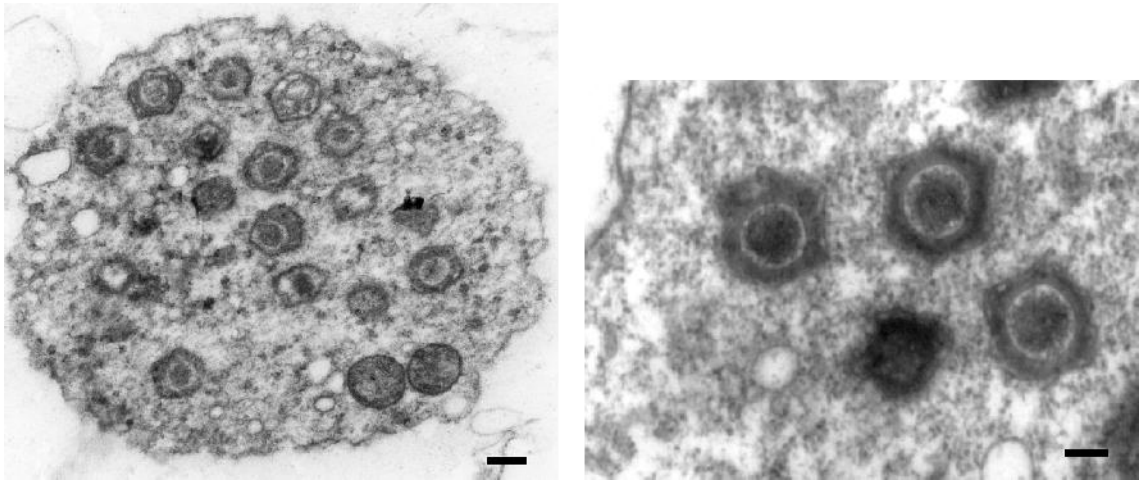
Oyster velar virus disease (OVVD) of the Pacific oyster occurred in the USA from mid-March through mid-June each year from 1976 to 1984, suggesting that the expression of the disease may be related to particular environmental conditions. When cultured at 25–30 °C, mortalities in oyster larvae greater than 170 μm in shell length typically begin at about 10 days of age. The infection results in the sloughing of ciliated velar epithelial cells and detachment of infected cells from the velum. Other cells lose cilia and infected larvae become unable to move normally.

Descriptive Histopathology

Histologically, gill necrosis virus disease is characterized by tissue necrosis with massive hemocytic infiltration around the lesions. The most distinctive lesion is the occurrence of giant polymorphic cells which may be up to 30 μm in size and contain large fuchsinophilic granules in the cytoplasm. In some giant cells, a voluminous basophilic inclusion (5–15 μm) occupies the greatest part of the cytoplasm in which finer basophilic granules (0.4–0.5 μm) are also present. Electron microscopy indicates that the inclusions are the viroplasm, and the fine granules are large viral particles. The most characteristic histological lesion of hemocytic infection virus disease is an acute cellular infiltration with the presence of atypical hemocytes in the connective tissues. Basophilic intracytoplasmic inclusion bodies are found in atypical blood cells in which irido-like virus particles can be observed in ultrathin sections. OVVD disease manifests histologically by the presence of intracytoplasmic inclusion bodies, 1.2–4 μm in diameter, located most commonly in the ciliated velar epithelium. The presence of DNA in the inclusion bodies is suggested by a positive Feulgen and Rosenbeck reaction.

Viruses

Mature icosahedral virions (380 nm diameter) are scattered throughout the cytoplasm of infected cells (**Figure 1(a)**). The outer shells of virions appear to consist of two trilaminar layers. The electron-opaque core (250 nm diameter) is limited by a three-layered fringe of definite width and surrounded by a layer of dense material



(a) (b)

Figure 1 Transmission electron micrographs of irido-like particles infecting *Crassostrea angulata* oysters. (a) Intracytoplasmic irido-like virus particles in an infected *C. angulata* cell (gill necrosis virus disease). Scale = 200 nm. (b) Irido-like virus particles from *C. angulata*. Virions are icosahedral in shape with a central electron-dense core, surrounded by an electron-lucent zone followed by another dense layer. Two unit membranes separated by a clear zone enclose the particle. Scale = 100 nm.

(**Figure 1**(**b**)). Morphogenesis takes place in the cytoplasm. Oyster irido-like viruses have not been isolated from infected tissue and have not been characterized biochemically. However, the presence of viral DNA was demonstrated by histochemical techniques including acridine orange staining and the Feulgen and Rosenbeck reaction. The characteristic morphology and cytoplasmic localization of these large DNA viruses suggest that they may eventually be classified as members of the family *Iridoviridae*. However, no molecular characterization has yet been conducted and there remains a need for definitive demonstration of viral etiology for the reported diseases.

Herpesviruses

Hosts and Locations

Herpes-like virus infections have been identified in various marine mollusk species throughout the world, including the USA, Mexico, France, Spain, the UK, New Zealand, Australia, and Taiwan. The first description of a virus morphologically similar to members of the family *Herpesviridae* in a bivalve mollusk was reported in 1972 in the eastern oyster, *Crassostrea virginica*. Since then, a wide host range has been reported for herpes and herpes-like viruses infecting bivalve species, including the Pacific oyster *C. gigas*, the European oyster *Ostrea edulis*, the Antipodean flat oyster *Ostrea angasi*, the Chilean oyster *Tiostrea chilensis*, the Manila clam *Ruditapes philippinarum*, the carpet shell clam *Ruditapes decussatus*, the Portuguese oyster *C. angulata*, the Suminoe oyster *C. ariakensis*, and the French scallop *Pecten maximus*. It is noteworthy that recently a herpes-like virus has also been observed by transmission electron microscopy in the gastropod mollusk *Haliotis diversicolor supertexta* in Taiwan associated with high mortality rates.

Disease Manifestations and Epizootiology

Herpesvirus and herpes-like virus infections have been associated with high mortalities of hatchery-reared larvae and juveniles stages of several bivalve mollusk species. Observations by transmission electron microscopy indicate that larvae exhibit generalized infections, whereas focal infections usually occur in juveniles. Although viral infections have also been observed in adult bivalves, they are apparently less sensitive than younger stages. Infected larvae exhibit velar and mantle lesions. They swim weakly in circles and shortly before death settle at the bottom of the tanks. Infected juveniles exhibit sudden high mortalities in a short period of time (less than 1 week) often during the summer. Histologically, lesions are confined to connective tissues. Fibroblast-like cells exhibit abnormal cytoplasmic basophilia and enlarged nuclei with marginated chromatin. Other cell types including hemocytes and myocytes show extensive chromatin condensation. Peculiar patterns of chromatin, ring-shaped or crescent-shaped, are also observed suggesting that apoptosis may occur. Viral DNA and proteins have been detected in asymptomatic adult oysters. Like other herpesviruses, the *C. gigas* herpesvirus seems to be capable of long-term persistence in the infected host. The pathogenicity of the virus for the larval stages of *C. gigas* has been demonstrated by experimental transmission to axenic larvae. Attempts to reproduce symptoms experimentally in juveniles and adult oysters have so far been inconclusive.

Virus Ultrastructure

Based primarily on virion morphology and aspects of morphogenesis and genome organization, Ostreid herpesvirus 1 (OsHV-1) is currently classified as an unassigned member of the family *Herpesviridae*. Particles present in the nucleus are circular or polygonal in shape. Empty particles are presumed to be capsids; others containing an electron-dense toroidal or brick-shaped core are interpreted as nucleocapsids (**Figure 2**(**a**)). Capsids and nucleocapsids are scattered throughout the nucleus in infected cells (**Figure 2**(**b**)). An electron–lucent gap of approximately 5 nm with fine fibrils is observed between core and capsid. Digital reconstruction of the OsHV-1 capsid based on cryoelectron microscopic images indicates an icosahedral structure with a triangulation number of $T = 16$, which is an architecture unique to herpesviruses. Prominent external protrusions at the hexon sites, and a relatively flat and featureless appearance of the inner surface, reported for OsHV-1 capsids, are also characteristic features of herpesviruses. Extracellular particles are usually enveloped with a trilaminar unit-membrane and measure 100–180 nm in diameter (**Figure 2**(**c**)). Tegument between the outer membrane and the capsid shell of enveloped particles is either absent or minimal (**Figure 2**(**c**)).

Genome Structure and Organization

Virus particles have been purified from fresh infected *C. gigas* larvae and the entire OsHV-1 genome has been cloned and sequenced (GenBank accession number AY509253). The total genome size is 207 439 bp. The overall genome organization is TR_L-U_L-IR_L-X-IR_S-U_S-TR_S (**Figure 3**) in which TR_L and IR_L (7584 bp) are inverted repeats flanking a unique region (U_L, 167 843 bp), TR_S and IR_S (9774 bp) are inverted repeats flanking a unique region (U_S, 3370 bp), and X (1510 bp) is located between IR_L and IR_S. A somewhat similar genome structure has been reported for certain vertebrate herpesviruses (e.g., herpes simplex virus and human cytomegalovirus). A small proportion of OsHV-1 genomes either lacks the X-sequence or contains an additional X-sequence at the left terminus.

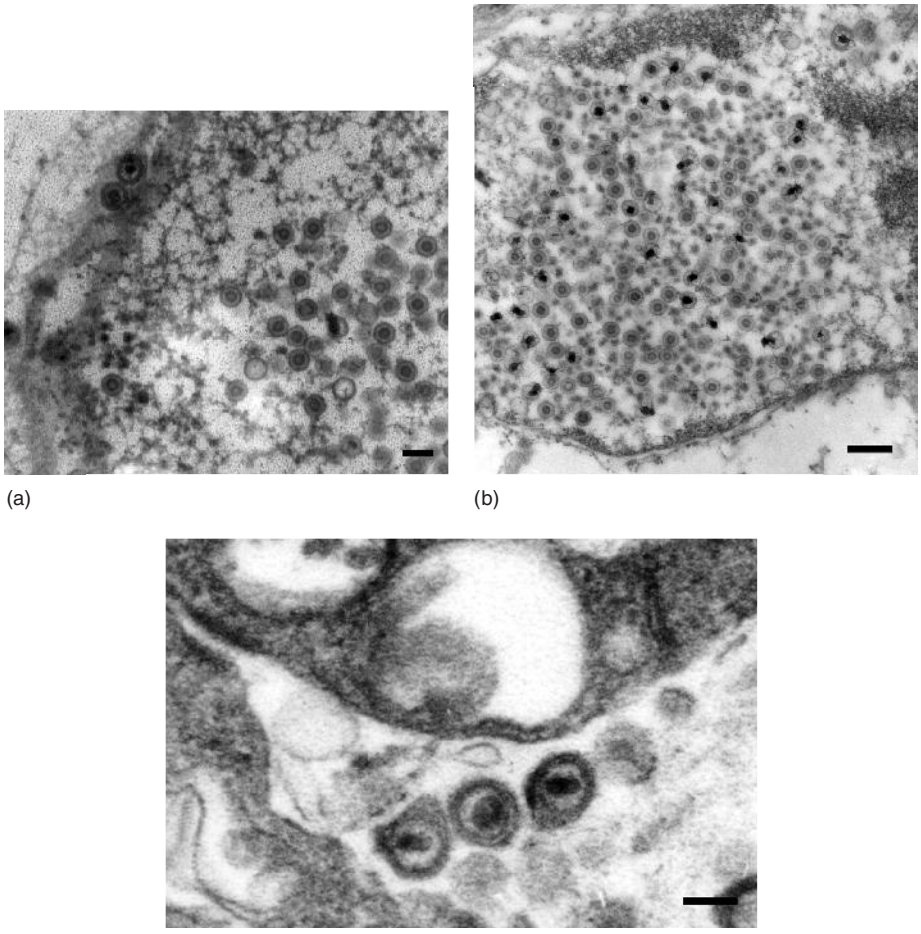


Figure 2 Transmission electron micrographs of ostreid herpesvirus 1 (OsHV-1) infecting Pacific oyster larvae. (a) Intranuclear spherical or polygonal virus particles; some particles appear empty and other contain an electron-dense core. Scale = 100 nm. (b) Nucleus of an infected interstial cell containing empty capsids and nucleocapsids. Scale = 200 nm. (c) High magnification of extracellular enveloped particles. Scale = 100 nm.

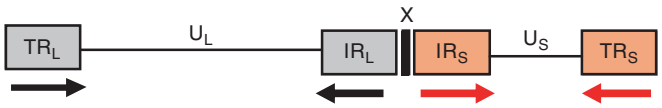


Figure 3 General genome organization of ostreid herpesvirus 1 (OsHV-1). TR_L and IR_L are inverted repeats flanking the unique region U_L. TR_S and IR_S are inverted repeats flanking the unique region U_S.

Since herpesvirus genomes are packaged into capsids from head-to-tail concatemers, this minor genome form may result from rare cleavage of concatemers at X–TR_S rather than at IR_L–IR_S. Moreover, approximately 20–25% of genomes contain a 4.8 kbp region of U_L in inverse orientation. The two orientations of U_L and U_S are present in approximately equimolar amounts in viral DNA, giving rise to four genomic isomers. This is also a feature of the vertebrate herpesvirus genomes with similar structures and results from recombination between inverted repeats during DNA replication. The genome termini are not unique but a predominant form is apparent for each. The IR_L–IR_S junction is also not unique, but the predominant form corresponds to a fusion of the two termini if each possesses two unpaired nucleotides at the 3′-end. Unpaired nucleotides are characteristic of herpesvirus genome termini.

Detailed analysis of the OsHV-1 genome sequence indicates that there are 124 unique open reading frames (ORFs). Owing to the presence of inverted repeats, 12 ORFs are duplicated resulting in a total of 136 genes in the viral genome. These numbers include several fragmented genes, each of which is counted as a single ORF. It is not yet known if splicing contributes to further elaboration of gene expression. A total of 38 genes shares

sequence similarities with other genes of the virus, defining 12 families of related genes. These include one gene family encoding proteins containing helicase motifs, one family encoding product related to inhibitors of apoptosis (IAPs), one family derived from a deoxyuridine triphosphatase gene, three families predicted to encode membrane-associated proteins, two families encoding RING-finger proteins, two families whose products are predicted to be secreted, and two other families. Gene families are also present in all other sequenced herpesviruses. The observation that IAPs are also encoded by baculoviruses and entomopoxviruses (both of which have insect hosts) underscores the importance of the apoptotic responses of invertebrates against viral infections. Vertebrate herpesviruses and poxviruses do not encode IAPs, and subvert the battery of host defenses by other pathways. Amino acid sequence comparisons have provided functional information on 25 genes that are not members of families. Seven genes encode enzymes. These include the catalytic subunit of DNA polymerase, two subunits of ribonucleotide reductase, a helicase, a putative primase, and the ATPase subunit of terminase. Two additional genes encode RING-like proteins. One protein is related to a eukaryotic protein of unknown function which is brain-specific in vertebrates. The ORF30 protein is related in an N-terminal cysteine-rich domain to a protein of unknown function in mammalian herpesviruses. A total of 15 genes encodes proteins which have predicted signal or transmembrane sequences and therefore may be associated with membranes, one specifying a putative ion channel protein.

Evolution and Taxonomy

Even though OsHV-1 shares a similar capsid architecture, amino acid sequence comparisons have failed to identify a single protein that has homologs in proteins unique to herpesviruses, with the exception of ORF30 which contains a domain found to date only in a subset of mammalian herpesviruses. Several OsHV-1 proteins have homologs that are distributed widely in nature (e.g., DNA polymerase), but these are no more closely related to homologs in other herpesviruses or to homologs in other organisms. This finding is also characteristic of comparisons between herpesviruses that infect fish or amphibians and those that infect mammals or birds. The strongest genetic indication of a common origin resides with the ATPase subunit of the terminase, which is involved in packaging DNA into the capsid. Homologs of this gene are present in all herpesviruses, and T4 and related bacteriophages. The T4 and OsHV-1 genes are unspliced, whereas those in herpesviruses of mammals and birds contain one intron and those in herpesviruses of fish and amphibians contain two introns. The available data support the view that herpesviruses of mammals and birds, of fish and amphibians, and of invertebrates form three major lineages of the herpesviruses. The invertebrate herperviruses would have evolved as a separate lineage about a billion years ago, and the fish viruses about 400 million years ago. OsHV-1 is currently the single representative of what may be a large number of invertebrate herpesviruses. Recent data show that OsHV-1 can infect several bivalve species. This contrasts with vertebrate herpesviruses which are generally confined to a single species in nature. Consequently, the true host of OsHV-1 is unknown. The apparent loss of several gene functions in OsHV-1 prompts the speculation that this may have promoted interspecies transmission in the context of introduction of non-native bivalve species and use of modern aquaculture techniques.

Diagnosis and Epidemiological Surveys

Light microscopy remains the preferred method for diagnosis of herpes-like virus infections in suspect samples. However, this method is poorly suited to diagnosis of viral diseases and should be supported by other techniques such as transmission electron microscopy. Even so, microscopic techniques are time consuming and unsuitable for epidemiological surveys. The lack of bivalve cell lines precludes *in vitro* culture and the observation of virus cytopathic effects. The purification of OsHV-1 from fresh infected larval *C. gigas* has served as a platform for the generation of molecular biological reagents for diagnosis. Procedures to detect herpesviruses in oysters using polymerase chain reaction (PCR) and *in situ* hybridization (ISH) have been developed and are suitable for epidemiological surveys of field samples, such as are currently being performed on oyster spat and larvae from commercial hatcheries and shellfish farms in France.

Picorna-Like Viruses and Other Small Virus-Like Particles

Virus-like particles of 27 nm in diameter have been reported in *Mytilus edulis* mussels from Denmark. These particles were enclosed in vesicles and arranged singly or in paracrystaline arrays. Acute cellular infiltrations were associated with virion detection and were interpreted as granulocytomas. Electron-dense, unenveloped virus-like particles (25–45 nm) have also been detected in farmed *Perna canaliculus* and *Mytilus galloprovincialis* mussels suffering mortalities in New Zeland. Extensive hemocytosis and necrosis of interstitial cells, basal cells, and digestive tubule epithelial cells were observed. Small DNA-negative virus-like particles (22–30 nm) were also reported in digestive and secretory cells of scallops, *Pecten novaezelandiae,* and toheroa, *Paphies ventricosum,* from New Zealand. Mass mortalities in Japanese pearl oysters, *Pinctada fucata martensii*, which have occurred in Japan since

1994, have been associated with a nonenveloped virus (25–33 nm) called Akoya virus. The disease was characterized by necosis and degeneration of muscle fibers. A morphologically similar virus was detected in the pearl oyster, *Pinctada margaritifera*, from French Polynesia associated with granulomas and focal necrosis within the adductor muscle. These lesions were similar to those reported in the mussel *M. edulis* suffering from granulocytomas. Paraspherical or polygonal-shaped virus-like particles (40 nm) consisted of a membrane-like envelope coating a central 35 nm electron-dense core. Icosahedrical–spherical (27–35 nm), nonenveloped virus particles have also been detected in the cytoplasm of connective tissue cells from cultured carpet shell clams, *R. decussatus*, suffering mortalities in Galicia (Spain). More recently, nonenveloped, icosahedral (19–21 nm) virus-like particles have been associated with large foci of massive hemocytic infiltration in cockles, *Cerastoderma edule*, from the same area (Galicia, Spain). The detection of red deposits on histological sections after methyl green pyronin staining suggested that this is an RNA virus.

All these virus-like particles are similar to those described first in mussels from Denmark. Due to their size, morphology, and the formation of paracrystalline arrays, they have been assumed to belong to the family *Picornaviridae*. However, no molecular information is available on these viruses to date.

Papova-Like Viruses

Intranuclear, nonenveloped virus-like particles (50–55 nm) with icosahedral symmetry were first reported from the gonadal epithelia of eastern oysters, *C. virginica*, in the USA in 1976 and in Canada in 1994. The virions were interpreted as papova-like viruses. A papova-like virus has also been detected in gonadal tissues during a health survey of cultured Pacific oyster, *C. gigas*, from the southern coast of Korea. More recently, histological examination of *C. gigas* oysters in France has revealed several cases of abnormally large basophilic cells in gonadal tissues. Electron microscopy examination has revealed nonenveloped icosahedral particles 50 nm in diameter.

Reo-Like Viruses and Birnaviruses

A virus tentatively assigned the family *Reoviridae* has been isolated from juvenile eastern oysters, *C. virginica,* using a fish cell line. Virions have been described as slightly oval particles (79 nm in diameter) containing a distinct inner core and clear spike-like projections on the outer capsid. Birnaviruses have been isolated from different bivalve species in Europe and Taiwan. A virus tentatively named 'marine birnavirus' (MABV) has also been isolated during a high mortality episode from oysters cultured in the Uma Sea (Japan). MABVs have been defined as a group within the genus *Aquabirnavirus*. Although the pathogenicity of certain MABV strains appears to be weak in shellfish, it has been observed in some mollusk species (the clam *Meretrix lusoria*, the Agemaki or jack knife clam *Sinovacura constricta*, and the Japanese pearl oyster, *P. fucata*) that stressors such as changes in temperature, spawning, and exposure to heavy metals can result in mortality by increasing host suceptibility. MABVs may thus be opportunistic pathogens that persistently infect marine organisms and become pathogenic under stressful conditions. Birnavirus-like particles were also isolated from the thin telling, *Tellina tenuis*, and the flat oyster, *Ostrea edulis*, from the coast of Britain and east coast of Canada. MABVs isolated from shellfish appear to be pathogenic to fish. Moreover, based on serological and genomic properties, strains isolated from shellfish and fish seem similar. This may indicate that the host range of MABV may be broad. Assays to reproduce experimentally the infection using the reovirus-like and birnavirus-like particles isolated on a fish cell line have shown inconsistent results, and a firm conclusion on the significance of these viruses for shellfish is still unknown.

Conclusion

In some cases, mollusk viruses have been detected only as inconsequential infections in animals that are suffering from another known disease or from an environmental stress such as pollution. However, several mass mortality outbreaks in mollusks have been attributed to viral infections. The almost total extermination of the Portuguese oyster, *C. angulata*, in French and European Atlantic waters in 1973 has been associated with irido-like virus infections. Viruses morphologically similar to members of the *Herpesviridae* have also been associated with high mortality rates in various marine mollusk species around the world.

The production of healthy shellfish from hatcheries and nurseries is a critical aspect of the conservation and management of natural populations and extensive farming areas. Selective breeding of hatchery stock will also be important for aquaculture development. This may lead to a substantial international trade in bivalve gametes and larvae to allow distribution of genetically improved seed stock. However, significant production problems including the elimination of viral diseases must be solved before hatcheries can become a major supplier for the industry. High-density production systems including commercial hatcheries and nurseries are an important source of viral diseases in aquaculture and the movement of stock must be considered as one of the major risks of disease spread. The risk of viral disease in invertebrate aquaculture

species is accentuated by the lack of specific chemotherapies and vaccines. Improved knowledge and understanding of shellfish viruses is needed to develop new tools for disease control.

See also: Iridoviruses of Invertebrates.

Further Reading

Arzul I, Renault T, Lipart C, and Davison AJ (2001) Evidence for inter species transmission of oyster herpesvirus in marine bivalves. *Journal of General Virology* 82: 865–870.

Barbosa-Solomieu V, Dégremont L, Vazquez-Juarez R, *et al.* (2005) Ostreid herpesvirus 1 detection among three successive generations of Pacific oysters (*Crassostrea gigas*). *Virus Research* 107: 47–56.

Chang PH, Kuo ST, Lai SH, *et al.* (2005) Herpes-like virus infection causing mortality of cultured abalone *Haliotis diversicolor supertexta* in Taiwan. *Diseases of Aquatic Organisms* 65: 23–27.

Chou HY, Chang SJ, Lee HY, and Chiou YC (1998) Preliminary evidence for the effect of heavy metal cations on the susceptibility of hard clam *(Meretrix lusoria)* to clam birnavirus infection. *Fish Pathology* 33: 213–219.

Comps M and Duthoit JL (1979) Infections virales chez les huîtres *Crassostrea angulata* (Lmk) et *C. gigas* (Th.). *Haliotis* 8: 301–308.

Davison AJ, Trus BL, Cheng N, *et al.* (2005) A noval class of herpesvirus with bivalve hosts. *Journal of General Virology* 86: 41–43.

Farley CA, Banfield WG, Kasnic JRG, and Foster WS (1972) Oyster herpes-type virus. *Science* 178: 759–760.

Le Deuff R-M and Renault T (1999) Purification and partial genome characterization of a herpes-like virus infecting the Japanese oyster, *Crassostrea gigas*. *Journal of General Virology* 80: 1317–1322.

Lees D (2000) Viruses and bivalve shellfish. *International Journal of Food Microbiology* 59: 81–116.

Lipart C and Renault T (2002) Herpes-like virus detection in *Crassostrea gigas* spat using DIG-labelled probes. *Journal of Virological Methods* 101: 1–10.

McGeoch DJ, Rixon FJ, and Davison AJ (2006) Topics in herpesvirus genomics and evolution. *Virus Research* 117: 90–104.

Meyers TR and Hirai K (1980) Morphology of a reo-like virus isolated from juvenile American oysters (*Crassostrea virginica*). *Journal of General Virology* 46: 249–253.

Rasmussen LPD (1986) Virus-associated granulocytomas in the marine mussel, *Mytilus edulis*, from three sites in Denmark. *Journal of Invertebrate Pathology* 48: 117–123.

Renault T and Novoa B (2004) Viruses infecting bivalve molluscs. *Aquatic Living Resources* 17: 397–409.

Renault T, Le Deuff R-M, Lipart C, and Delsert C (2000) Development of a PCR procedure for the detection of a herpes-like virus infecting oysters in France. *Journal of Virological Methods* 88: 41–50.

Shrimp Viruses

J-R Bonami, CNRS, Montpellier, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cephalothorax The shrimp head, containing the main organs, hepatopancreas, stomach, foregut and midgut, gonads, heart, gills.

Epizootic An epidemic in animal populations. Rapid spreading of a disease.

Hepatopancreas Organ of the digestive tract with secretion–absorption functions and located in the cephalothorax (head) of shrimp. Also called the digestive gland.

Postlarvae Stage of shrimp development, after the larval stages.

Introduction

To date, more than 20 viral diseases have been reported in shrimp and prawns. Most of the described viruses are related, but often only on the basis of morphological characteristics to known virus families. Two of the most important pathogens of shrimp have been sufficiently characterized to be accepted by the International Committee on Taxonomy of Viruses (ICTV) as members of new virus families within the classification and nomenclature of viruses. Yellow head virus (YHV), together with closely related gill-associated virus (GAV), have been classified as members of the new genus *Okavirus* in the new family *Roniviridae*. White spot syndrome virus (WSSV) has been classified as the only known member of the new genus *Whispovirus* in the new family *Nimaviridae*. A third major pathogen, Taura syndrome virus (TSV), has been accepted for classification in the family *Dicistroviridae*. Although not yet officially accepted by the ICTV, six other shrimp viruses have been sufficiently well characterized to be considered as possible members of four existing virus families: *Totiviridae* (infectious myonecrosis virus, IMNV), *Nodaviridae* (macrobrachium rosenbergeii nodavirus, MrNV), *Parvoviridae* (infectious hypodermal and hematopoietic necrosis virus, IHHNV, and hepatopancreatic parvovirus, HPV) and *Baculoviridae* (BP-type and MBV-type viruses).

History

Three periods can be defined in the recent history of shrimp virus discovery: the first was during the 1970s, in which observations of viral agents were made by 'chance'; the second period commenced in the 1980s, in which

evidence of viral pathogenic agents was obtained as a result of disease observations or mortalities occurring in shrimp farms; and finally, since the early 1990s with the development of molecular biology, more structured and intensive approaches to the descriptions of the pathogens and the associated diseases were undertaken.

The first virus of Crustacea was reported in 1966 by Vago in the Mediterranean crab *Portunus depurator*. The virus appeared to be related to members of the *Reoviridae*. In shrimp, occluded bacilliform particles were reported in 1973 by Couch during investigations on the effect of pollutants in pink shrimp (*Penaeus duorarum*). The agent was reported to be closely related to members of the *Baculoviridae*.

In 1981, Sano and colleagues reported the detection of a 'nonoccluded baculovirus' in kuruma shrimp (*Penaeus japonicus*) during mortalities in hatchery-reared shrimp in two prefectures in Japan. Two years later (1983), Lightner reported a transmissible disease named infectious hypodermal and hematopoietic necrosis (IHHN) as the Pacific blue shrimp (*Penaeus stylirostris*) as the source of mortalities in ponds. While at first thought to be related to members of the *Picornaviridae*, IHHNV was later assigned to the *Parvoviridae* after investigations on the structure of the genome. Since that time, reports of new viruses have increased in parallel with the rapid development of shrimp farming globally and, to date, more than 20 viruses have been reported in shrimp (**Table 1**).

Table 1 Known shrimp viruses

Genome	*Virus family*[a]	*Acronym*	*Virus name*	*Host*	*Genomic data*
ssRNA	*Dicistroviridae*	TSV	Taura syndrome virus	*P. vannamei*	Complete
	Roniviridae	YHV	Yellow head virus	*P. monodon*	Complete
		GAV	Gill-associated virus		Complete
	Bunyaviridae	MoV	Mourilyan virus	*P. monodon*	Partial
				P. japonicus	
	Totiviridae	IMNV	Infectious myonecrosis virus	*P. vannamei*	Complete
	Rhabdoviridae	RPS		*P. stylirostris*	n.d.
				P. vannamei	
	Togaviridae	LOVV	Lymphoid organ vacuolisation virus	*P. vannamei*	n.d.
	Nodaviridae	MrNV/XSV	Macrobrachium rosenbergii nodavirus/extra small virus	*M. rosenbergii*	Complete
		LSNV		*P. monodon*	n.d.
dsRNA	*Birnaviridae*	IPN-like virus	Infectious pancreatic necrosis-like virus	*P. japonicus*	n.d.
	Reoviridae	Reo-Pj		*P. japonicus*	n.d.
		Reo-Pm		*P. monodon*	n.d.
		Reo-Pv		P. vannamei	n.d.
		PBRV		*Palaemon* sp.	n.d.
ssDNA	*Parvoviridae*	IHHNV	Infectious hemocytic and hematopoietic virus	*P. stylirostris*	Complete
		HPV	Hepatopancreatic parvovirus	*P. semisulcatus*	Complete
		HPV-like		*P. merguiensis*	n.d.
				M. rosenbergii	n.d.
					n.d.
		SMV		*P. monodon*	n.d.
		LPV		*P. monodon*	n.d.
				F. merguiensis	n.d.
				P. esculentus	n.d.
dsDNA	*Iridoviridae*			*Protrachypene precipua*	n.d.
	Nonoccluded bacilliform virus	BMNV or (PjNOB)	Baculoviral midgut gland necrosis virus	*P. japonicus*	Partial
		PHRV			n.d.
		CcBV		*Crangon crangon*	n.d.
	Baculoviridae	PvNPV or (BP-type)	Single-nucleocapsid polyedrosis virus	*P. duorarum*	n.d.
		PmNPV or (MBV-type)	Single-nucleocapsid polyedrosis virus	*P. vannamei*	Partial
		MbNPV	Single-nucleocapsid polyedrosis virus	*P. monodon*	Partial
				Metapenaeus bennettae	n.d.
	Nimaviridae	WSSV	White spot syndrome virus	Penaeids and numerous other crustaceans	Complete

[a]Only TSV, GAV, YHV, IHHNV, and WSSV have been formally classified to date.

The major developments in shrimp virus research commenced in the early 1990s following the development and application of molecular tools. Cloning and sequencing of genome fragments allowed construction of specific and sensitive tools for detection and diagnosis. These DNA-based methods, such as the polymerase chain reaction (PCR) and dot-blot hybridization, have led to the use of commercial diagnostic kits for selection of healthy shrimp and the production of specific pathogen-free (SPF) breeding stock. SPF shrimp have proven to be particularly useful, not only in limiting or preventing production losses due to disease on farms, but also as experimental animals for studies on shrimp viruses.

Impact of Viral Diseases on Farmed Shrimp Production

The rapid progress in shrimp virology during the past two decades is clearly related to the high commercial value of these farmed crustaceans. This has significantly increased our understanding of viruses infecting only invertebrates which previously had been confined primarily to insect viruses. However, in contrast to most insect viruses in which investigations were oriented primarily toward biological control of insect pests and their potential use as gene expression vectors, shrimp virus research has focused on disease prevention.

It is difficult to know the impact of viral diseases on shrimp populations in the natural environment. Diseased shrimp are rarely found, essentially due to predation and cannibalism when animals appear weakened. However, under farming conditions, where the density of the shrimp population can easily exceed 300 000 postlarvae per hectare, the consequence of a disease outbreak and associated mass mortalities can be disastrous. In 2003, total world production from shrimp farming was more than 1.6 million metric tons, representing a value of almost US$ 9000 million and accounting for about 25% of marketable shrimp production (capture and farming). Viral disease has been a major problem for this large and expanding industry and the economic and socioeconomic impacts have been severe. For example, a report commissioned by the World Bank in 1996 estimated the annual global cost of disease (primarily viral) to shrimp farming was US$ 3000 million or 40% of production capacity at that time. In China and Thailand alone, annual production losses due to WSSV have reached US$ 500–1000 million. In Ecuador, the emergence of WSSV in 1999 resulted in a 75% drop in annual production and 130 000 industry workers were reported to have lost their jobs. The production records of these major shrimp-producing countries exhibit depressions coinciding with the sequential emergence of major viral pathogens. In contrast, in Brazil, which was free of the major diseases until 2003, shrimp production constantly increased. Nevertheless, Brazil has also experienced significant production losses since that time with the emergence of infectious myonecrosis caused by a new toti-like virus.

Factors Responsible for Viral Disease Emergence in Shrimp

Disease emergence in shrimp aquaculture can be attributed to three primary factors: (1) the common practice in the shrimp farming industry of introducing each season fresh wild broodstock collected from the natural environment as the source of seed (postlarval shrimp) for farms; (2) national and international trade in broodstock and commercial seed (postlarvae) to be transferred to ponds until they reach the marketable size; and (3) the culture of shrimp in earthern ponds in high densities.

The use of wild brooders in hatcheries commonly occurs without adequate knowledge of their health status or adequate quarantine. This often results in the introduction of pathogens into the farming system and it is a practice that persists even though SPF shrimp, bred in biosecure facilities, are now readily available in many countries.

Trade in broodstock and seed also commonly occurs without checking for at least the known pathogens. This practice was responsible for the rapid worldwide spread of the IHHNV, the introduction of TSV to the Eastern Hemisphere, and the rapid spread of WSSV from the Eastern to the Western Hemisphere. The practice of farming in brackish water earthern ponds creates an environment in which new pathogens may be encountered and disease spread rapidly once outbreaks occur. As a result, short explosive epizootics may, in few days, wipe out the whole shrimp population of a farm.

Environmental and host factors also play an important role in the emergence of shrimp disease. Poor-quality pond conditions (e.g., high salinity, pH, or nitrogen) can cause physiological stress in shrimp leading to increased susceptibility to disease. The shrimp culture medium (brackish water) allows very efficient transfer of free viruses, either as free infectious agents or in pathogen vectors. As ectotherms, shrimps are susceptible to water temperature shifts that can stimulate the replication of viruses that commonly persist in shrimp as unapparent infections. Shrimp also lack the sophisticated adaptive immune mechanisms (antibodies, cytokines, T-cells) deployed by vertebrates to combat viral infections and so have less capacity to survive disease.

Characteristics of Some Shrimp Viruses

Shrimp Baculoviruses

Two distinct groups of viruses, sharing numerous characters with members of the family *Baculoviridae*, have been

described in shrimp. They have been improperly called BP-type viruses (BP, baculovirus penaei) and MBV-type viruses (MBV, monodon baculovirus) on the basis of the morphology of their occlusion bodies (OBs). The BP-type group exhibits tetrahedral OBs; the MBV group exhibits rounded OBs. It is classified as a tentative species (*Penaeus monodon* NPV, PemoNPV) in the genus *Nucleopolyhedrovirus* of the family *Baculoviridae*.

General characteristics

All baculoviruses detected in shrimp develop in the nuclei of digestive epithelial cells (hepatopancreas and mid-gut) with the release of OBs within feces. They are bacilliform in shape and comprise a trilaminar envelope surrounding a rod-shaped nucleocapsid. Virions may be occluded in paracrystalline occlusion bodies. Only small fragments of the double-stranded DNA (dsDNA) genome have been cloned and sequenced for a few shrimp baculoviruses. For the BP-type virus, Penaeus duorarum single nuclear polyhedrosis virus (PdSNPV), the genome has been shown by electron microscopy to be a circular structure with an estimated size of 75×10^6 kDa (113–115 kbp). There appear to be different morphotypes of BP-type baculoviruses which were distinguished based on differences in particle size; two different BP genotypes have been reported in the same shrimp tissue section by *in situ* hybridization.

Polyhedra

In contrast to insect baculoviruses, shrimp baculovirus OBs are unenveloped and comprise large subunits (SuOBs) of polyhedrin which are icosahedral in shape (**Figure 1**). The SuOBs are always associated in triplets. They are organized in two different crystalline forms: the BP-type viruses form parallel rows in all three dimensions (**Figure 2(a)**); the MBV-type viruses assemble four triplets to form a 'rosette' (hollow sphere) as the building block of the OB (**Figure 2(b)**). MBV-type OBs are more sensitive to thaw–freezing but each OB type disaggregates spontaneously in CsCl gradients. Compared to insect baculovirus polyhedrin subunits, both BP- and MBV-type SuOBs are larger in dimensions (15–22 nm compared to 6.5–9 nm diameter) and molecular mass (52–58 kDa compared to ~30 kDa).

There is evidence of antigenic cross-reactivity between PdSNPV and MBV SuOBs, but this requires confirmation as, unexpectedly, PdSNPV SuOBs were also found to cross-react with both polyhedrin and granulin from the insect baculoviruses Autographa californica nuclear polyhedrosis virus and and Trichoplusia ni granulosis virus, respectively.

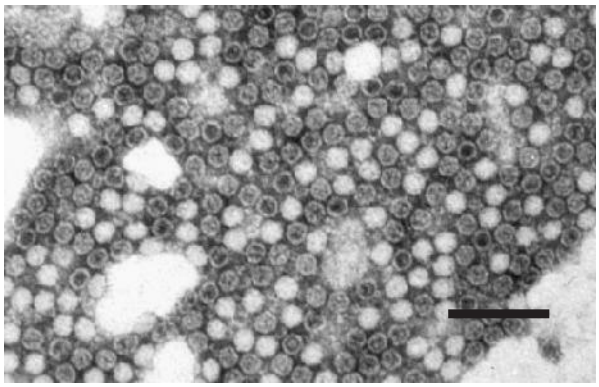


Figure 1 Purified SuOBs (polyhedrin) from PmSNPV (MBV-type virus). Note the full and empty virus-like particles. The particles were negatively stained with 2% phosphotungstic acid (PTA). Scale = 100 nm. Reproduced from Bonami: JB, Aubert H, Mari J, Poulos BT, and Lightner DV (1997) The polyhedra of the occluded baculoviruses of marine decapod crustacea: A unique structure, crystal organization, and proposed model. *Journal of Structural Biology* 120: 134–145, with permission from Elsevier.

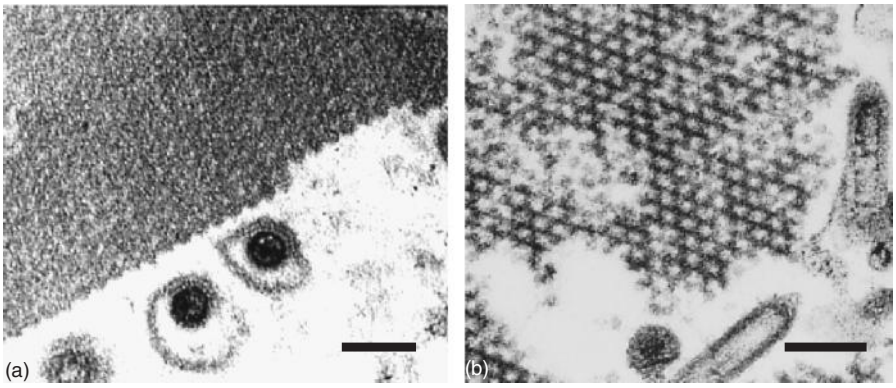


Figure 2 Ultrathin sections in occlusion bodies of shrimp baculoviruses; (a) PvSNPV (BP-type virus) and (b) PmSNPV (MBV-type virus). The two types of crystal (bullet-like arrangement and rosette formation) are evident. Scale = 100 nm (a, b). Reproduced from Bonami JB, Aubert H, Mari J, Poulos BT, and Lightner DV (1997) The polyhedra of the occluded baculoviruses of marine decapod crustacea: A unique structure, crystal organization, and proposed model. *Journal of Structural Biology* 120: 134–145, with permission from Elsevier.

Viral infection cycle

All life stages of the pink shrimp (*P. duorarum*), white shrimp (*Penaeus setiferus*), and the brown shrimp (*Penaeus aztecus*) are susceptible to BP-type baculovirus, while only larval and postlarval stages of the Pacific white shrimp (*Penaeus vannamei*) and Pacific blue shrimp (*P. stylirostris*) have been observed to be infected. In the black tiger shrimp (*Penaeus monodon*), postlarvae, juveniles, and adults have been observed to be infected with MBV.

For PdSNPV in *P. duorarum*, the major steps in the infection cycle have been described including the appearance of naked nucleocapsids in the cytoplasm of epithelial digestive cells, nucleoprotein release at nuclear pores, followed by all morphogenesis steps in infected nuclei and the development of tetrahedral OBs. Cellular pathological changes include nuclear hypertrophy and perinuclear membranous proliferation. Nuclear hypertrophy associated with increasing size of OBs leads to the release of the nuclear contents into the digestive lumen of hepatopancreatic tubules and gut, and the excretion of OBs in the feces. The infection cycle is completed by oral ingestion and OB dissolution in the digestive tract of the new host. A similar infection cycle is hypothesized for MBV-type baculoviruses. In addition to horizontal transmission, vertical transmission is suspected, based on the presence of OBs in very young postlarvae a few days after hatching.

Geographic distribution and host range

BP-type virus infection is widespread in farmed and wild penaeid shrimp in the Americas. The known distribution is from the Gulf of Mexico in the north through the Caribbean, the east coast of South America as far south as the State of Bahia in Central Brazil. On the Pacific coast, the distribution ranges from Mexico to Peru. BP-type virus has also been observed in wild shrimp from Hawaii. Several penaeid shrimp species have been reported to be infected, both in aquaculture facilities and in the wild environment. These include *P. duorarum*, *P. aztecus*, *P. vannamei*, *P. setiferus*, *P. stylirostris*, *P. penicillatus*, *P. schmitti*, *P. paulensis*, *P. subtilis*, *Trachypenaeus similis*, and *Protrachypene precipua*.

MBV-type virus infections are widely distributed in penaeid shrimp of the Eastern Hemisphere, particularly in the Indo-Pacific countries of China, India, Indonesia, Philippines, Malaysia, Thailand, Sri Lanka, and Australia where the virus is enzootic in wild stocks. The virus has also been reported in Kuwait, Oman, Israel, Italy, and West Africa (Kenya and Gambia). It has been observed in imported penaeid shrimp in Tahiti and Hawaii, Mexico, Ecuador, Brazil, Puerto Rico, and some states of the USA. The known host range includes *P. monodon*, *P. merguiensis*, *P. semisulcatus*, *P. indicus*, *P. penicillatus*, *P. esculentus*, and *P. vannamei*.

Shrimp Parvoviruses

Two viruses sharing the primary characteristics of members of the family *Parvoviridae* have been reported in penaeid shrimp. IHHNV causes a systemic infection of multiple organs of ectodermal and mesodermal origin as and is described in the Pacific blue shrimp (*P. stylirostris*). It is associated with runt and deformity syndrome (RDS) in Pacific white shrimp (*P. vannamei*) and occurs commonly as a low-level persistent infection in apparently healthy black tiger shrimp (*P. monodon*). HPV causes an infection of the digestive system (epithelial cells of hepatopancreas and mid-gut) in black tiger shrimp *P. monodon* and in the fleshy prawn (*P. chinensis*). HPV isolates from *P. monodon* (HPVmon) and *P. chinensis* (HPVchin) represent two distinct genetic lineages. A third parvo-like virus, spawner-isolated mortality virus (SMV) infects *P. monodon* but is less well characterized.

IHHNV and HPV are each nonenveloped icosahedral viruses, 22 nm in diameter and containing a linear single-stranded DNA (ssDNA) genome. IHHNV virions have been reported to contain four polypeptides as detected by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and silver staining (74, 47, 39, and 37.5 kDa). For HPVchin, only one virion structural protein has been observed (54 kDa) but analysis of HPVmon has revealed a doublet protein band (57 kDa major band and 54 kDa minor band). In addition to differences in tissue tropism, the viruses display differences in cytopathology. During HPV infection, cellular lesions correspond to typical densonucleosis in insects with the formation of enlarged, densely stained, and Feulgen-positive nuclei. In IHHNV infections, lesions are discrete, often difficult to detect, and display characteristic eosinophilic intranuclear Cowdry type A inclusion bodies.

Genomic organization

The IHHNV genome comprises 4075 nucleotides (according to a recent GenBank submission) and contains three long open reading frames (ORFs) on the complementary (positive) strand (**Figure 3**). The three ORFs are referred to as left, mid, and right. The left ORF is in a different reading frame (+1) to mid- and right ORFs. As for other parvoviruses, noncoding termini at each end of the genome contain palindromic sequences. The left ORF encodes a 666 amino acid protein with high homology with putative major nonstructural protein (NS-1) of mosquito brevidensoviruses. The mid-ORF overlaps the left ORF and encodes a 363 aa protein of unknown function (possibly an NS-2 protein). The right ORF overlaps the left ORF by 62 nt and encode a 329 aa protein. By analogy to other parvoviruses, it has been speculated that the right ORF encodes the capsid proteins but this is not consistent with the four structural proteins reported in purified virions. The negative strand also contains an ORF with a

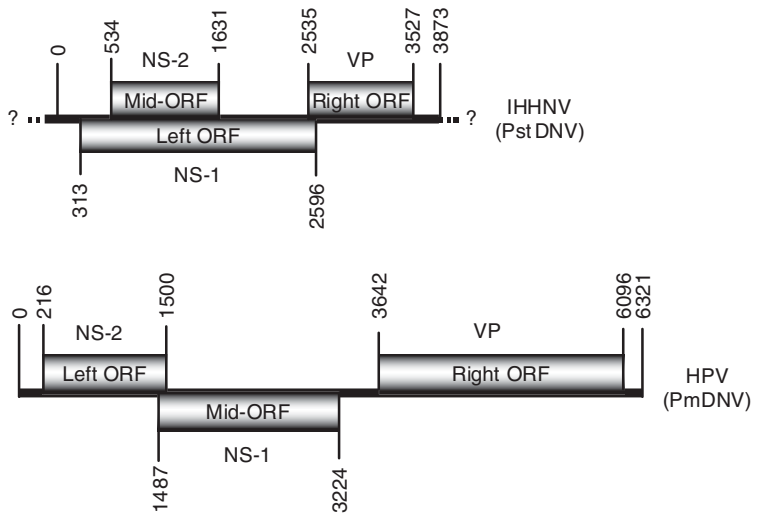


Figure 3 Genome organization of IHHNV and HPV. IHHNV (PstDNV) is according to Shike H, Dhar AK, Burns JC, *et al*. (2000) Infections hypodermal and haematopoietic necrosis virus of shrimp is related to mosquito brevidensoviruses. *Virology* 277: 167–177.

coding capacity of 134 amino acids. Further work is required to define the IHHNV expression strategy.

The HPV genome comprises 6321 nucleotides containing three long ORFs in the complementary (positive) strand and palindromic noncoding termini that form hairpin-like structures, as found in other parvoviruses (**Figure 3**). ORF1 (left ORF) encodes a 428 amino acid putative nonstructural protein-2 (NS-2) of unknown function. ORF2 (mid-ORF), in −1 frame relative to ORF1 encodes a 579-amino-acid polypeptide that contains conserved replication initiator motifs, NTP-binding, and helicase domains similar to NS-1 of other parvoviruses. The right ORF (ORF3), which is in the same frame as ORF1, encodes the 818-amino-acid capsid protein (VP protein).

Taxonomic position

In terms of morphology and genome structure and organization, both IHHNV and HPV share similarities with members of the family *Parvoviridae*. Genomic analysis has clearly indicated that IHHNV is closely related to mosquito densoviruses and it has recently been classified as a tentative species (*Penaeus stylirostris densovirus*), in the genus *Brevidensovirus*. Similarly, HPV was recently proposed as new member of subfamily *Densovirinae* to be designated as the species *Penaeus monodon densovirus*.

Geographic distribution and host range

HPV was first observed in wild and farmed penaeid shrimp in Australia, China, Korea, Philippines, Indonesia, Malaysia, Kenya, Kuwait, and Israel. It has also been reported subsequently from several locations in North and South America (Pacific coast of Mexico and El Salvador). Natural infections have been reported in *P. merguiensis*, *P. semisulcatus*, *P. chinensis*, *P. esculentus*, *P. monodon*, *P. japonicus*, *P. penicillatus*, *P. indicus*, *P. vannamei*, and *P. stylirostris*.

IHHNV is widely distributed in the Americas (Brazil, Ecuador, Central America, Mexico, Peru, southeast USA), the Central Pacific (Guam, Hawaii, Tahiti, and New Caledonia), and Asia and the Indo-Pacific area (Indonesia, Malaysia, Philippines, Thailand, and Australia). Natural infections have been reported in *P. stylirostris*, *P. vannamei*, *P. occidentalis*, *P. californiensis*, *P. monodon*, *P. semisulcatus*, and *P. japonicus*. It is likely that infection with IHHNV or a similar virus also occurs in other penaeid shrimp species.

Recent investigations have revealed evidence of the integration of IHHNV-related sequences within the genomes of *P. monodon* populations from Africa (Madagascar, Tanzania, Mozambique) and Australia. The integrated sequences vary by up to 14% from epidemic IHHNV and do not appear to be associated with the formation of complete or infections virions.

Infectious Myonecrosis Virus

In 2002, a new disease associated with low mortalities was reported in the Pacific white shrimp *Penaeus vannamei* from northeastern Brazil. Clinical signs included necrotic areas in muscles, resulting sometimes in a whitish and opaque appearance to the tail. The causative agent was named infectious myonecrosis virus (IMNV) on the basis of the clinical signs.

General properties

In infected animals, virions accumulate in the cytoplasm of target cells (muscular cells, hemocytes, and connective tissue cells) forming basophilic inclusions. IMNV has a density of 1.366 g ml^{-1} in cesium chloride. It is a nonenveloped icosahedral virus, 40 nm in diameter (**Figure 4**) with a genome consisting of a single segment of double-stranded RNA (dsRNA), 7560 bp in length. The capsid contains a single major polypeptide of 106 kDa.

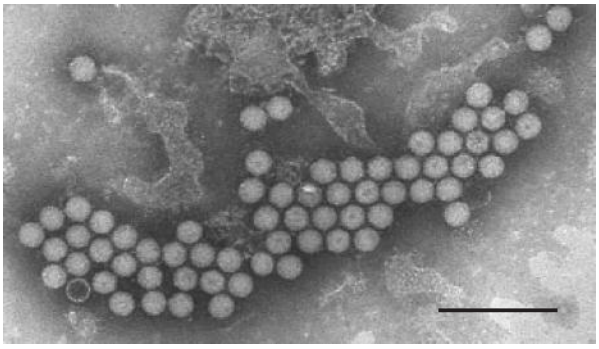


Figure 4 Purified IMNV virions. The particles were negatively stained with 2% phosphotungstic acid (PTA). Scale = 200 nm. From Poulos BT, Tang KFJ, Pantoja CR, Bonami JR, and Lightner DV (2006) Purification and characterization of infectious myonecrosis virus of penaeid shrimp. *Journal of General Virology* 87: 987–996.

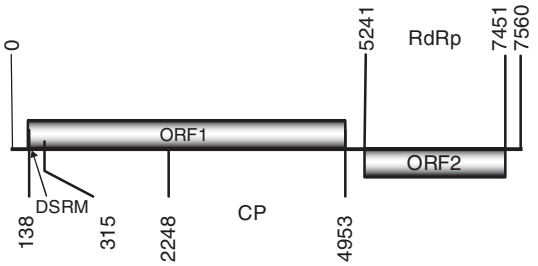


Figure 5 Genome organization of IMNV.

Genomic organization

The IMNV genome organization is illustrated in **Figure 5**. Two large nonoverlapping ORFs in different reading frames (ORF1 and ORF2) are flanked by 3′ untranslated region (UTR) and 5′ UTR of 109 and 135 nt, respectively, and are separated by a noncoding region of 287 nt. ORF1 contains a dsRNA-binding motif (DSRM) of 60 amino acids in the N-terminal region. The translated sequence of 901 amino acids corresponds approximately to the size determined in SDS-PAGE for the major capsid protein (106 kDa). ORF2 encodes an RNA-dependent RNA polymerase (RdRp) with significant sequence similarity to members of the family *Totiviridae*.

Taxonomic position

Phylogenetic analysis of IMNV RdRp clusters this virus within members of the genus *Giardiavirus* in the family *Totiviridae*. The genome structure and organization are also consistent with classification in the *Totiviridae*. However, as known giardiviruses infect only flagellated protozoan parasites (*Giardia lamblia* and *Trichomonas vaginalis*), further consideration of the properties of IMNV will be required before formal classification can be obtained.

Geographic distribution and host range

The disease was, until recently, restricted to farmed *P. vannamei* in northeastern Brazil. However, recently the virus and the disease have been reported at several sites in Indonesia where infected *P. vannamei* postlarvae have been introduced from Brazil. *Penaeus stylirostris* and *P. monodon* shrimps are susceptible to experimental infection with IMNV.

MrNV/XSV Complex

White tail disease occurs in farmed giant freshwater prawns (*Macrobrachium rosenbergii*) and is named after the obvious clinical signs in diseased postlarvae. Two viruses, Macrobrachium rosenbergii nodavirus (MrNV) and a very small virus-like particle named XSV (extra small virus), are each found in diseased prawns. The disease was first observed in a hatchery in Guadeloupe Island in French West Indies where abnormal and sudden mortalities had been recorded since 1994. Losses were variable in intensity (5–90% cumulative mortality), depending on the broodstock used to generate the postlarvae. The first gross sign of the disease is the presence of whitish postlarvae 2–3 days after emergence. The prevalence of opaque and milky postlarvae increases dramatically 1–2 days later and changes are particularly obvious in abdomen (tail). The mortality rate reaches a maximum 5 days after the first observation of gross signs.

In diseased prawns, the most affected tissues are the striated muscles in the abdomen and cephalothorax and the connective tissues of all organs. The cytoplasm of infected cells contains discrete, pale to darkly basophilic inclusions, ranging from <1 to 40 μm in diameter. Muscles exhibit multifocal areas of hyaline necrosis of the fibers, with moderate edema.

General properties of isolated particles

Two types of particles are observed in the cytoplasm of infected cells (**Figure 6**). The larger particles are nonenveloped, icosahedral in shape, and 26–27 nm in diameter, with a density in CsCl of 1.27–1.28 g ml^{-1}. The genome consists of two segments of linear single-stranded RNA (ssRNA) of 3202 (RNA1) and 1250 nt (RNA2). The capsid contains a single major protein of 43 kDa (CP43). The properties of this virus are similar to those of nodaviruses and it has been named Machrobrachium rosenbergii nodavirus (MrNV).

The smaller particles are nonenveloped, icosahedral, and 15 nm in diameter, and have a density in CsCl of 1.325 g ml^{-1}. The genome is composed of a single segment of linear ssRNA 796 nt in length. The capsid comprises coat proteins of 16 kDa (CP16) and 17 kDa (CP17). Because the particles are very small, this agent has been called extra small virus (XSV).

Genomic organization

The organization of the MrNV genome is shown in **Figure 7**. RNA1 and RNA2 each encodes a long ORF

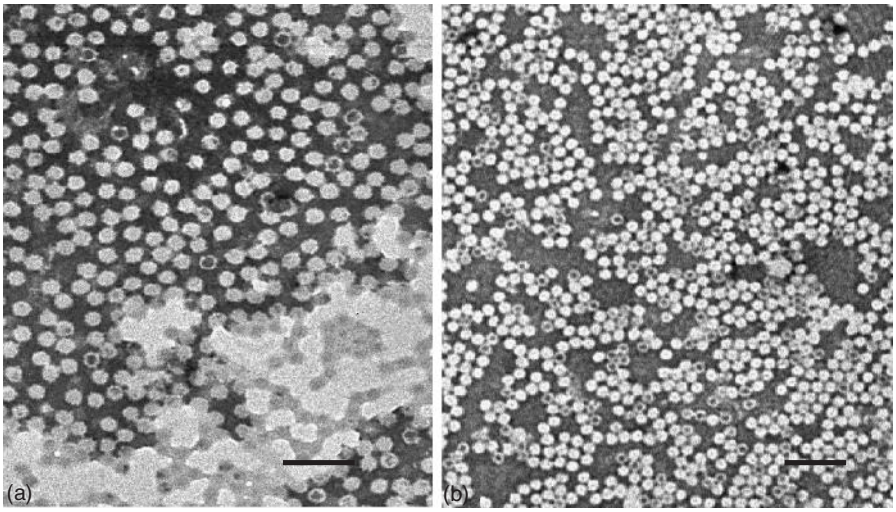


Figure 6 Purified MrNV and XSV particles. The particles were negatively stained with 2% phosphotungstic acid (PTA). Scale = 50 nm. (a, b) Reproduced from Bonami JR, Shi Z, Qian D, and Sri Widada J (2005) White tail disease (WTD) associated virions and characterization of MrNV as a new type on nodavirus. *Journal of Fish Diseases* 28: 23–31, with permission from Blackwell Publishing.

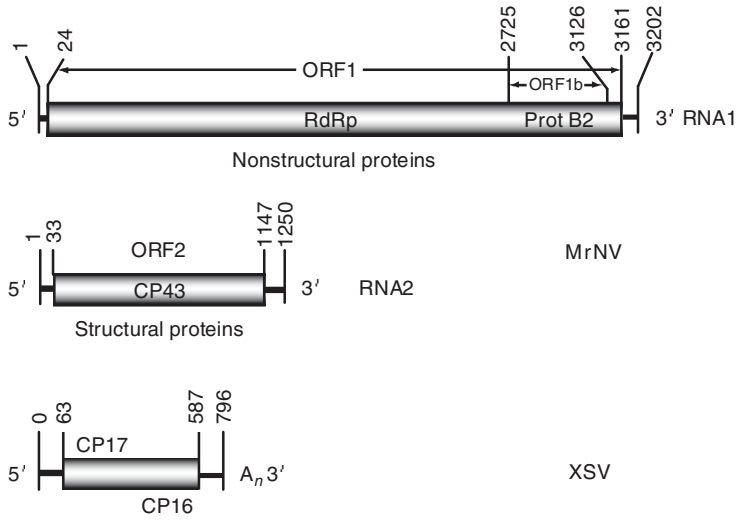


Figure 7 Genome organizations of MrNV and XSV.

flanked by terminal noncoding regions, and lacks a 3′ poly(A) tail. The coding capacity is 1046 and 371 amino acids, equivalent to 122 and 43.5 kDa, for ORF1 and ORF2, respectively. Similar to other nodaviruses, RNA1 contains two long ORFs encoding nonstructural proteins: ORF1 encodes the 1046-amino-acid (~122 kDa) A protein which contains motifs characteristic of the RdRp; and ORF1b, located at the 3′ end of the segment, encodes the 134-amino-acid (~13 kDa) B protein. ORF2 encodes the 371-amino-acid (~43.5 kDa) capsid protein (CP43). The coding assignment of RNA2 has been demonstrated by N-terminal amino acid sequencing of CP43. As for all other nodaviruses, a nonencapsidated RNA3 of 453 nucleotides has also been detected. The MrNV RNA3 is longer than that of any other nodavirus RNA3 (~380–400 nt) and is likely to be a subgenomic mRNA encoding ORF1b.

The 796 nt XSV genome contains a single ORF located between nucleotides 63 and 587, and a short poly(A) tail. A potential polyadenylation signal (AAUAAA) is located 6 nt upstream of the poly(A). The ORF encodes both capsid proteins (CP16 and CP17). Amino acid sequence analysis indicates that CP16 is synthesized by internal initiation at a second methionine residue 11 amino acids downstream of the first initiation codon. The two forms of the capsid protein are synthesized in an equimolar ratio.

Taxonomic position

No formal taxonomic classification of MrNV has yet been assigned. The particle morphology and genome organization are similar to those of members of the family *Nodaviridae*. Amino acid sequence alignments of the RdRp indicate highest homology with members of the genera *Alphanodavirus* (insect nodaviruses) and *Betanodavirus* (fish nodaviruses). Sequence similarities are higher with alphanodaviruses than with betanodaviruses. However, coat protein sequence alignments indicate that the distance between MrNV and alphanodaviruses is similar to those separating alphanodaviruses and betanodavirues. This suggests that MrNV may represent a possible third genus within the family.

The appropriate taxonomic classification of XSV is currently unclear. Sequence similarity searches have not indicated any significant homology with known virus genomes. As the monocistronic genome encodes only the capsid proteins, it is possible that the RdRp required for replication and transcription may be provided by MrNV. This agent meets the primary criteria of satellite viruses and appears more similar to the known satellite viruses infecting plants (e.g., tobacco necrosis satellite virus-like) than those infecting insects (e.g., chronic bee paralysis-associated satellite virus).

MrNV/XSV relationships

MrNV and XSV are each always found associated in white tail disease infections in *M. rosenbergii* in all hatcheries and farms where disease is reported. Analysis by quantitative (real-time) polymerase chain reaction (PCR) has indicated variable proportions of MrNV RNA and XSV RNA in different isolates. However, nothing is known to date about their respective roles in the disease or its severity.

Geographic distribution and host range

White tail disease was first recognized in a hatchery in Guadeloupe Island and was then reported in Martinique Island and in the Dominican Republic. It is suspected that infected postlarvae were introduced to Puerto Rico.

In China, the disease was recorded in Zhejiang, Jiangsu, Shanghai, Guangxi, and Guangdong Provinces and, more recently, it has been reported in Taiwan. In India, the disease has been reported in Andhra Pradesh and Tamil Nadu on the east coast and in Kerala on the west coast where *M. rosenbergii* farming was developed to replace marine (*P. monodon*) culture following the dramatic effects of the WSSV epidemic.

To date, white tail disease has only been reported in the giant freshwater prawn *M. rosenbergii*. However, marine shrimp (*P. monodon*, *P. indicus*, and *P. japonicus*) are susceptible to experimental infection, suggesting a possible role as reservoir. No disease has been reported in marine shrimp species.

Control of Viral Diseases in Shrimp

As invertebrates, marine shrimp and freshwater prawns lack the acquired immune systems that exist in vertebrates. Consequently, a disease control based on vaccination cannot be used. Some antiviral defense reactions (innate immunity) have been reported in Crustacea but the mechanisms by which these occur are largely unknown and prevention appears as the most useful method for control. For this reason, specific and sensitive DNA-based tools for early detection are now available for most of the viral diseases. These tools allow selection of healthy broodstock and a good knowledge of the health status of postlarval seed introduced into ponds. The availability of these tools has also led to the establishment of biosecure breeding programs for commercial production of specific pathogen-free (SPF) broodstock. The more widespread use of SPF stock will significantly reduce the risk of future emergence of devastating viral diseases in shrimp aquaculture.

See also: Baculoviruses: General Features; Giardiaviruses; Insect Viruses: Nonoccluded; Nodaviruses; Parvoviruses of Arthropods; Satellite Nucleic Acids and Viruses; Taura Syndrome Virus; Totiviruses; White Spot Syndrome Virus; Yellow Head Virus.

Further Reading

Bonami JR, Aubert H, Mari J, Poulos BT, and Lightner DV (1997) The polyhedra of the occluded baculoviruses of marine decapod crustacea: A unique structure, crystal organization, and proposed model. *Journal of Structural Biology* 120: 134–145.

Bonami JR, Mari J, Poulos BT, and Lightner DV (1995) Characterization of the HPV, a second unusual parvovirus pathogenic for penaeid shrimp. *Journal of General Virology* 76: 813–817.

Bonami JR, Shi Z, Qian D, and Sri Widada J (2005) White tail disease of the giant freshwater prawn, *Macrobrachium rosenbergii*: Separation of the associated virions and charaterization of MrNV as a new type of nodavirus. *Journal of Fish Diseases* 28: 23–31.

Bonami JR, Trumper B, Mari J, Brehelin M, and Lightner DV (1990) Purification and characterization of the infectious hypodermal and haematopoietic necrosis virus penaeid shrimps. *Journal of General Virology* 71: 2657–2664.

Couch JA (1991) *Baculoviridae*. Nuclear polyhedrosis viruses. Part 2: Nuclear polyhedrosis viruses of invertebrates other than insects. In: Adam JR and Bonami JR (eds.) *Atlas of Invertebrate Viruses*, pp. 205–226. Boca Raton, FL: CRC Press.

Lightner DV (1988) Diseases of cultured penaeid shrimp and prawns. In: Sindermann CJ and Lightner DV (eds.) *Diseases Diagnosis and Control in North American Marine Aquaculture*, pp. 8–127. Amsterdam: Elsevier.

Lightner DV (ed.) (1996) *A Handbook of Shrimp Pathology and Diagnostic Procedures for Diseases of Cultured Penaeid Shrimp*, 304pp. Baton Rouge, LA: World Aquaculture Society.

Mari J, Bonami JR, and Lightner DV (1993) Partial cloning of the genome of the IHHNV, an unusual parvovirus pathogenic from penaeid shrimp. Diagnosis of the disease using a specific probe. *Journal of General Virology* 74: 2637–2643.

Pillai D, Bonami JR, and Sri Widada J (2006) Rapid detection of macrobrachium rosenbergii nodavirus (MrNV) and extra small virus (XSV), the pathogenic agents of white tail disease of *Macrobrachium*

rosenbergii (De Man), by loop-mediated isothermal amplification. *Journal of Fish Diseases* 29: 275–283.
Poulos BT, Tang KFJ, Pantoja CR, Bonami JR, and Lightner DV (2006) Purification and characterization of infectious myonecrosis virus of penaeid shrimp. *Journal of General Virology* 87: 987–996.
Sahul Hameed AS, Yoganandhan K, Sri Widada J, and Bonami JR (2004) Studies on the occurrence of macrobrachium rosenbergii nodavirus and extra small virus-like particles associated with white tail disease of *M. rosenbergii* in India by RT-PCR detection. *Aquaculture* 238: 127–133.
Shike H, Dhar AK, Burns JC, *et al.* (2000) Infectious hypodermal and haematopoietic necrosis virus of shrimp is related to mosquito brevidensoviruses. *Virology* 277: 167–177.
Sri Widada J, Richard V, and Bonami JR (2004) Characteristics of the monocistronic genome of extra small virus (XSV), a virus-like particle associated with macrobrachium rosenbergii nodavirus (*Mr*NV): Possible candidate for a new species of satellite virus. *Journal of General Virology* 85: 643–646.
Sukhumsirichart W, Attasart P, Boonsaeng V, and Panyim S (2006) Complete nucleotide sequence and genomic organization of hepatopancreatic parvovirus (HPV) of *Penaeus monodon*. *Virology* 346: 266–277.
Zhang H, Wang J, Yuan J, *et al.* (2006) Quantitative relationship of two viruses (MrNV and XSV) in white tail disease of *Macrobrachium rosenbergii* de Man. *Diseases of Aquatic Organisms* 71: 11–17.

Sigma Rhabdoviruses

D Contamine and S Gaumer, Université Versailles St-Quentin, CNRS, Versailles, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Permissive allele A host allele which allows or participates in viral propagation.
Restrictive allele A host allele which opposes viral propagation.
Stabilized lineage for a virus A female or male host lineage, with infected primordial germinal cells, which transmits the virus to its progeny.

Introduction

Sigma virus (SIGV) is a rhabdovirus that naturally infects fruit flies (*Drosophila* spp.). SIGV infection causes increased sensitivity to carbon dioxide (CO_2) gas, which is anesthetic for flies. Whereas uninfected flies recover rapidly from the effects of CO_2 exposure upon return to a normal atmosphere, flies infected with SIGV remain irreversibly paralyzed. This effect has been observed among both wild flies and laboratory strains of as many as 13 *Drosophila* species and in three of these (*D. melanogaster*, *D. affinis*, and *D. athabasca*) it has been shown to be the consequence of infection.

The discovery of SIGV originates from 1937 when a study by P. L'Héritier and G. Tessier described a CO_2-sensitive strain of *D. melanogaster*. While this gas is commonly used as an anesthetic in fly genetics, these flies were irreversibly paralyzed when exposed to a CO_2-rich atmosphere. The paralysis was specific to CO_2, and dependent on the gas concentration and temperature during exposure. For example, irreversible paralysis appears at 10 °C with CO_2 concentrations higher than 50% while CO_2 concentrations must reach 75% to induce irreversible paralysis at 16 °C.

Transmission of this characterstic appeared to be heritable but could not be linked to any chromosome. The agent was considered to be cytoplasmic and was named Sigma. Its infectivity was demonstrated by inoculation of resistant flies with sensitive-fly extracts. Inactivation by X-ray irradiation indicated the sensitive volume diameter to be 42 nm and filtration through a 180-nm membrane eliminated 99% of infectivity. In 1965, Sigma was finally described as a particle of 70×140 nm similar to vesicular stomatitis virus (VSV) or rabies virions. Thereafter, studies on SIGV focused on its hereditary transmission among flies. More recently, publications on SIGV have been centered on the population genetics of the SIGV–*Drosophila* linkage and descriptions of the defense mechanisms developed by *D. melanogaster*.

Classification

SIGV is currently classified as an unassigned member of the family *Rhabdoviridae*. The virion morphology, genome organization, and sequence relationships of several structural proteins are clearly consistent with its assignment as a rhabdovirus. However, it is not sufficiently closely related to other rhabdoviruses to be classified in any existing genus. It is closer to vesiculoviruses than to other rhabdoviruses according to phylogenetic studies (**Figure 1**) and biological properties (i.e., the CO_2 symptom induced by both vesiculoviruses and SIGV).

Virion and Genome Structure

The SIGV virion is a spiked and enveloped bullet-shaped particle of approximately 75×140–200 nm containing a helical nucleocapsid (**Figure 2**). The genome is a

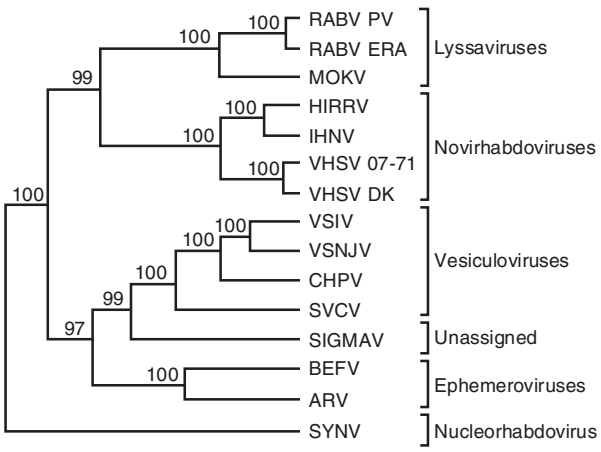


Figure 1 Phylogenetic tree of 15 rhabdovirus glycoproteins. The values adjacent to the branches indicate the boostrapping confidence limits. SIGV is not assigned to any genus of the *Rhabdoviridae* but the most conserved proteins (including the G protein) are most closely related to those of the vesiculoviruses. Reproduced from Björklund HV, Higman KH, and Kurath G (1996) The glycoprotein genes and gene junctions of the fish rhabdoviruses spring viremia of carp virus and hirame rhabdovirus: Analysis of relationships with other rhabdoviruses. *Virus Research* 42: 65–80, with permission from Elsevier.

negative-sense single-stranded RNA. It contains five genes arranged in the same order as in other rhabdoviruses (3′-*N*-*P*-*M*-*G*-*L*-5′) and encodes proteins with significant levels of sequence identity to the corresponding proteins of rhabdoviruses. For example, the P protein of SIGV is acidic and the distribution of charges is similar to other rhabdovirus P proteins. The charge and size of the M protein of SIGV are also similar to the M proteins of vesiculoviruses and the primary domains (basic domain, proline-rich domain, hydrophobic domain) are found in other rhabdovirus M proteins. The SIGV genome also contains an additional gene (*X*) between *P* and *M* genes (**Figure 3**). The *X* gene encodes a putative protein of 298 amino acids that is of unknown function, but which contains three conserved domains found in reverse transcriptases. Another unusual feature of the sigma genome is the 33 nt overlap of the *M* and *G* genes. The putative transcription initiation sequence CAACANG (+ sense) sequence is found at the beginning of the *P*, *X*, *M*, *G*, and *L* genes. The putative transcription termination/polyadenylation sequence $CAUG(A)_7$ (+ sense) terminates the *N*, *P*, *X*, *M*, and *G* genes. Phylogenetic analyses based on the N and G proteins indicate that SIGV clusters with vesiculoviruses (**Figure 1**).

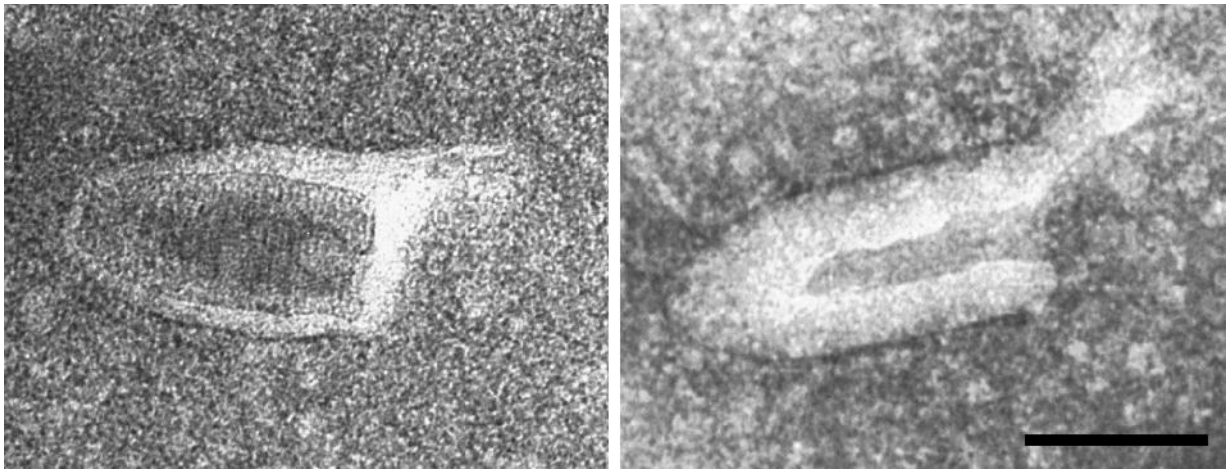


Figure 2 SIGV virions as observed by negative-contrast electron microscopy. The membrane fragment evident on the top right-hand side of the virions in each illustration is frequently but not always observed. Scale = 100 nm.

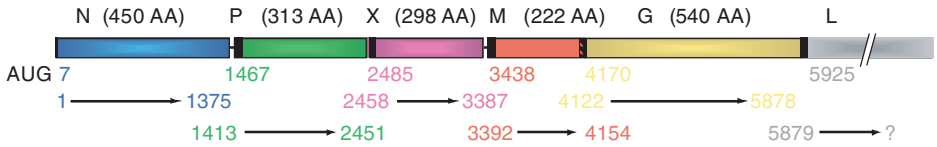


Figure 3 Genome organization of SIGV. The single-strand RNA genome encodes six proteins (N, P, X, M, G, and L) for which the size are indicated. The X protein is also named PP3 (protein product 3). The genome sequence has been obtained for all regions except the L gene which has only been partially sequenced. By comparison with other rhabdoviruses, the SIGV L gene is estimated to be approximately 6000 nt in length, resulting in a complete genome sequence of approximately 12 000 nt. The locations of mRNA transcripts and the position of putative initiation codons (AUG) are indicated. The arrows indicate the direction of transcription and the numbers indicate the transcription initiation and termination points. The M and G transcripts overlap from nucleotides 4122–4154.

CO_2 Sensitivity

The molecular basis of CO_2 sensitivity is unknown. However, the CO_2-induced paralysis of wings and legs correlates with viral invasion of thoracic ganglia that are involved in the nervous control of locomotion. Following abdominal inoculation of flies, several cycles of replication are necessary for SIGV to reach the thoracic ganglia and induce CO_2 sensitivity. Following injection of one infectious dose at 25 °C, an incubation period of 20 days is required for expression of the symptom and, as the virus dose is increased, the mean incubation period decreases. Moreover, a dose of virus that sensitizes flies to CO_2 in 10 days when injected into the abdomen, leads to CO_2-induced paralysis in only 3 days if injected into the thoracic ganglia.

The paralysis symptom is specific to CO_2 as other gases (e.g., N_2, H_2, CO, propane, and volatile acids) do not induce similar effects. This characteristic distinguishes SIGV from other viruses infecting *Drosophila* such as the entomobirnavirus drosophila X virus which induces sensitivity to both CO_2 and N_2 in what appears to be a general anoxia. There is evidence, however, that several vesiculoviruses, although not naturally infecting drosophila, induce CO_2-specific sensitivity in flies similar to that induced by SIGV. Each vesiculovirus tested to date, including vesicular stomatitis Indiana virus (VSIV), Cocal virus, pike fry rhabdovirus, and spring viremia of carp virus, is capable of replication in *D. melanogaster* and induces sensitivity to CO_2 but not to N_2 or propane. In contrast, the lyssavirus rabies virus (CVS strain) and two novirhabdoviruses of fish (infectious hematopoeitic nectosis virus and vesicular hemorrhagic septacaemia virus) do not replicate and do not induce CO_2 symptoms in *D. melanogaster*. The specificity of CO_2-induced paralysis in flies infected with SIGV or vesiculoviruses suggests that the underlying molecular mechanism responsible for the symptom is likely to be similar. Nevertheless, CO_2 symptoms vary for different vesiculoviruses. For example, Cocal virus induces CO_2-dependent paralysis when the virus titer in the nervous system reaches the maximum. Later in infection, although the virus titer in the entire fly remains constant, the titer decreases in the thoracic ganglia and CO_2 sensitivity disappears. It has also been observed that, following infection of flies with VSIV, a proportion of the exposed population displays a delayed CO_2 symptom while the remainder of the CO_2-sensitive flies are immediately paralyzed. The delayed sensitivity correlates with a more rapid invasion of the cephalic ganglia, than the thoracic ganglia and results in death 2–3 days after exposure, presumably due to paralysis of the mouthparts. Direct injection of SIGV into the cephalic ganglia also leads to this delayed sensitivity and lethality.

Host Range

SIGV has been reported to trigger CO_2 sensitivity in 15 of the 16 *Drosophila* species tested to date, the exception being *D. repleta*. However, some *D. melanogaster* genotypes are also resistant to SIGV replication, and so CO_2 sensitivity, and the observed resistance of *D. repleta* may not be valid for all genotypes.

One of the criteria used to demonstrate virus replication is the recovery of a virus yield that is in excess of the original infecting dose. This criterion is very stringent. Indeed, the virus yield from infected flies decreases soon after infection due to the entry of the nucleocapsids into the cytoplasm of infected cells, and there is a period of latency before replication generates new progeny virus. Therefore, a less-stringent criterion for SIGV replication is the observation of a higher virus yield than can be recovered during the latent period. Using these criteria, of 13 *Diptera* species (*Phormia terranovae*, *Ceratitis capitata*, *Musca domestica*, *Calliphora erythrocephala*, *Sarcophaga argyrostoma*, *Glossina mortisans*, *Aedes albopictus*, *Aedes aegypti*, *Aedes detritus*, *Anopheles stephensi*, *Toxorhynchites amboinensis*, *Culex pipiens*, *Culex quinquefasciatus*) that have been tested, only the fleshfly (*Phormia terranovae*) and the mosquito (*Aedes albopictus*) appeared incapable of supporting SIGV replication. As for *D. repleta*, there may be genotypic variation in the susceptibility of these insects and it is possible that susceptibility can be increased by SIGV strain adaptation. This is supported by the observation that SIGV can be adapted to replication in *A. albopictus* cell cultures by passage in the mosquito *Toxorynchites amboinensis*. The selected variants multiply and induce CO_2 sensitivity in *A. albopictus*. Adaptation of SIGV to replication in restrictive genotypes of *D. melanogaster* has also been observed. SIGV has not been observed to replicate in tests conducted in nine insect species representing five orders (*Blattaria*, *Orthoptera*, *Lepidoptera*, *Hymenoptera*, *Hemiptera*) other than *Diptera* or in cultured vertebrate cells.

Virus Transmission

SIGV is not transmitted between insects by casual contact and no vector of horizontal transmission has been identified. In nature, SIGV transmission appears to be exclusively vertical and, although 100% prevalence of infection can be achieved in laboratory strains, only a proportion of natural populations of flies are infected.

There is no evidence of integration of the SIGV genome into host chromosomes and the virus appears to remain exclusively in the cytoplasm. SIGV establishes a stable infection in oogonia, and between 10 and 40 viral genomes can be detected per oogonium according to the virus strain. Thereafter, there is an accumulation of

genomes as oocyte volume increases. This balance reflects an autoregulation of viral infection in which cellular proteins may play a role and allows virus transmission during cell division. The nature of the process also implies low cytopathogenicity of SIGV in oocytes. Furthermore, if viral genomes segregate stochastically following cell division, relatively rare events of completely asymmetrical distribution would lead to the presence of uninfected individuals in the progeny of an infected female.

In a lineage stabilized for SIGV infection, some females will not transmit the virus to 100% of their progeny and infected females are less fertile. This should lead to a rapid elimination of the virus if the males were not able to transmit the infection. The efficiency of viral transmission by males can be as high as 90% in the progeny of a cross between uninfected females and males infected with a recently isolated strain of wild virus. In *D. melanogaster*, the male progeny of such a cross does not transmit the virus to the next generation. However, the female progeny can transmit SIGV to the next generation and some of their daughters are the source of stabilized lineages. These transmission rules are the roots of the invasive character of the virus and allow the virus to maintain itself in nature.

In *D. affinis* and *D. athabasca*, SIGV transmission follows similar rules with the exception that the male progeny of an uninfected female crossed with an infected male can transmit the virus to the next generation.

Virus Replication Cycle

The replication cycle of SIGV has been studied *in vivo* following injection of a viral extract into the abdomen of flies. There is a rapid reduction in recoverable infectivity following inoculation such that after 1 h, only 1% of the infecting dose can be recovered and new viral production commences between 24 and 48 h post infection. Studies of the viral replication cycle have been conducted using both wild-type viruses and temperature-sensitive (*ts*) mutants. Heat–shock experiments have identified three groups of *ts* mutants corresponding to three phases in the replication cycle. The first group of mutants identified a transiently thermolabile complex comprising the viral genome and viral proteins. The half-life of thermosensitivity is 4 h with a maximum span of 9 h post infection. The molecular basis of thermoresistance has not been determined. However, similar studies in vesiculoviruses suggest that this phase could include the steps that precede replication of the viral genome such as the release of the ribonucleoprotein complex from the endosome and primary transcription and protein synthesis.

A second group of *ts* mutants is affected in a replication phase that has a half-life of 9 h, commencing 7 h post infection and terminating at 14 h post infection. This phase corresponds to genome replication since hereditary transmission of these mutants is interrupted at the restrictive temperature. Late functions are also altered, as have been observed in molecular studies of vesiculoviruses which have shown that the N, P, and L proteins are essential for genome replication and secondary transcription.

A third group of *ts* mutants is affected in the late phase of the viral cycle. At the restrictive temperature, these mutants can be hereditarily transmitted but no infectious particles are produced and CO_2-induced paralysis of the host is not observed. The proteins modified in these mutants could be the G, M, or X proteins. Since the N, P, and L proteins are involved in viral replication and the X protein is absent in vesiculoviruses, CO_2 sensitivity must be due to G, M, or both G and M proteins.

At 20 °C, the viral replication cycle can vary in length between 25 and 90 h post infection in different individuals with an average of 60 h. The cycle length varies according to temperature and is much faster at 25 °C, as is the metabolism of flies. The host genotype also influences the cycle length. In more permissive genotypes, the replication cycle is faster and in the most permissive genotypes, the longest cycle is only 48 h in duration.

Host Immunity

A reduced fertility that has been observed in female flies infected experimentally with SIGV should be a strong selective pressure in nature. Surprisingly, poor fertility is not evident in natural populations. A loss of fertility is observed in crosses between infected females from a natural population and uninfected males from laboratory strains. These results suggest that the viral infection cycle is controlled by the host genome. Indeed, seven such genes are known: *ref(1)H*, *ref(2)M*, *ref(2)P*, *ref(3)G*, *ref(3)O*, *ref(3)D,* and *ref(3)V*. Each is polymorphic with alleles segregating into two categories. Permissive alleles allow the viral infection cycle to proceed while restrictive alleles restrict virus cycling. The only exception is the *ref(3)V* gene for which the restrictive allele blocks hereditary transmission of SIGV from stably infected males.

The most intensively studied of the *ref* genes is *ref(2)P*. Restrictive *ref(2)P* alleles modify the rules for hereditary transmission of SIGV. The frequency of uninfected flies in the progeny of infected parents increases with the number of restrictive alleles in the genome of the female. Stabilized mothers that are homozygous for a restrictive allele do not display CO_2 sensitivity even though they remain capable of virus transmission. Their progeny are transmission defective but may be CO_2 sensitive, depending on the genotype. Moreover, when stabilized males are crossed with uninfected females, the proportion of progeny that is infected is twofold lower when the

mother is heterozygous for *ref(2)P* (permissive/restrictive) and zero when the mother is homozygous restrictive for *ref(2)P*. These restrictive alleles are often encountered in natural populations and do not appear to be counter-selected in uninfected drosophila. When the populations are infected with currently observed virus strains, which are sensitive to this defense system, *ref(2)P* restrictive alleles are favored until their frequency reaches 0.3. At this frequency, the sensitive strain of virus is eliminated. Thereafter, the lack of counter-selection of restrictive alleles maintains their frequency around 0.3, which protects the population from any new invasion by a sensitive virus.

Three functional domains have been identified in both the Ref(2)P protein and its mammalian homolog, p62/sequestosome-1. Two protein–protein interaction motifs are found: an amino-terminal PB1 (Phox and Bem 1) domain and a more central ZZ zinc finger. At the other end of both proteins there is a carboxy-terminal UBA (ubiquitin-binding area) domain. The Ref(2)P PB1 domain mediates the interaction with the drosophila atypical protein kinase C (DaPKC) and p62 binds mammalian aPKCs. The physiological function of Ref(2)P remains unknown even though it is essential for male fertility in some specific genotypes.

A comparison of susceptibility to the SIGV between flies that are homozygous for permissive alleles of *ref(2)P* and flies *ref(2)P*$^{-/-}$ has shown that permissive Ref(2)P protein is required for the virus to multiply at highest efficiency. For SIGV strains that are the most sensitive to *ref(2)P* alleles, a 16-fold higher dose is required for infection of a *ref(2)P*$^{-/-}$ genotype and a 10 000-fold higher dose is required for infection of a homozygous restrictive genotype than to infect homozygous permissive flies.

A comparison of the 15 sequenced alleles of *D. melanogaster ref(2)P*, among which three are restrictive, and the reference sequence of *D. simulans ref(2)P* indicate that the ancestral gene was permissive. Three mutations affecting the PB1 domain of the protein are necessary and sufficient to convert a permissive *ref(2)P* allele into a restrictive allele. Both permissive and restrictive alleles with sequence variations in the PB1 domain have been shown to form a monophyletic group that shows less internal variability than the group of ancestral permissive alleles. This suggests that the three variations in the PB1 domain that affect susceptibility to SIGV infection have emerged relatively recently. The high frequency of these variants (up to 50% of the observed alleles) indicates that their existence provides a selective advantage in drosophila populations.

The interaction between SIGV and the *ref(2)P* gene is highly specific. None of the other viruses that replicate in *D. melanogaster*, such as drosophila X virus and vesiculoviruses, is sensitive to restrictive *ref(2)P* alleles. A unique amino acid change in the PB1 domain is sufficient to suppress the restrictive character of an allele. In a viral population, genotypes with a capacity to replicate efficiently in a restrictive environment exist, even if the virus has never been exposed to restrictive alleles. For this reason, the elimination of sensitive viruses in a natural fly population by restrictive *ref(2)P* alleles is often associated with a new infection of the population by adapted viruses. Therefore, one could doubt that restrictive alleles provide a real defense system against SIGV but *ref(2)P* is not the only *ref* gene.

Each of the *ref* genes impacts independently on the viral replication cycle. For example, if a particular viral strain requires a tenfold higher dose to successfully infect flies that are restrictive at the *ref(2)P* locus, and a tenfold higher dose to infect flies restrictive at the *ref(2)M* locus, a 100-fold higher dose is required to overcome the flies that are restrictive for both *ref* genes. Adapted viruses can be isolated from a clone sensitive to *ref(2)P* restrictive alleles and studied for their sensitivity to *ref(2)M* restrictive alleles. As shown in **Figure 4**, the viral mutants

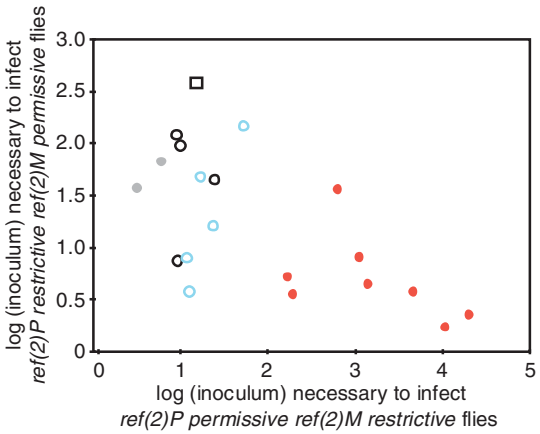


Figure 4 Virus clone distribution according to the inoculum size necessary to infect different fly genotypes. The reference *Drosophila melanogaster* strain was homozygous permissive for both *ref(2)P* and *ref(2)M*. The size of inoculum necessary to infect these flies was arbitrarily defined as 1.00. The *y*-axis values are the logarithm of the smallest inoculum capable of infecting flies homozygous for both a permissive *ref(2)M* allele and homozygous for a restrictive *ref(2)P* allele. The *x*-axis values are the logarithm of the smallest inoculum capable of infecting flies homozygous for both a permissive *ref(2)P* allele and a restrictive *ref(2)M* allele. The square represents the parental virus while circles represent viral mutants adapted to *ref(2)P* restrictive alleles. Open circles symbolize mutants that are not significantly different from the parental virus when assayed in *ref(2)M* restrictive flies. Blue circles depict the results observed for thermosensitive mutants, while gray and red circles show the observation made for mutants that are slightly adapted to both *ref* genes and mutants that are more sensitive to *ref(2)M* restrictive alleles than the parental clone, respectively. Among the mutants that are at least 20 times more adapted to *ref(2)P* than the parental clone, three are thermosensitive and thus would not survive in nature. The majority (seven out of eight) of the other 'adapted mutants' are more sensitive to *ref(2)M* restrictive alleles.

most adapted to *ref(2)P* restrictive alleles become more sensitive to restrictive alleles of *ref(2)M*. Increasing the frequency of *ref(2)M* restrictive alleles can also contribute to the elimination of viral mutants, only allowing viruses adapted to both *ref(2)P* and *ref(2)M* restrictive alleles to invade a fly population. It appears that the various *ref* genes cooperate to defend the fly population against the virus. For example, if the frequency of *ref(2)P* restrictive alleles was around 0.3, the frequency of *ref(2)M* restrictive alleles could rise while viruses sensitive to *ref(2)M* would disappear.

There are still SIGV strains in nature which are sensitive to *ref(2)P* restrictive alleles. In addition, a virus adapted to *ref(2)P* with an increased sensitivity to *ref(2)M* (i.e., equivalent to the red circles of **Figure 4**) is yet to be found in nature. Moreover, analysis of *ref(2)P* allele genealogy suggests that, if all the viruses present in a region were adapted to *ref(2)P* restrictive alleles, a new mutation in the PB1 domain of the protein would be selected to generate a new restrictive allele. Therefore, Ref(2)P remains as an active defensive mechanism against natural SIGV infections. The role of the other Ref proteins in host immunity remains unknown.

Concluding Remarks

In earlier studies, the detailed rules of vertical transmission of SIGVs in *D. melanogaster* populations illustrated how vector insects such as mosquitoes or sandflies can become the reservoirs of a virus. Currently, the two major interests in SIGV research are the description of the host–virus interactions in natural populations together with the development of an improved understanding of defensive mechanisms mediated by the *ref* genes. This immunity has certain similarities to innate immunity since its components are hereditary, but they differ because of the high specificity of protection and the memory effect that is generated in host populations. Strictly speaking, Ref(2)P may be part of the innate immune response through its interaction with DaPKC and a more extensive study could reveal that other *ref* genes also form part of a complex innate host immune system in flies.

See also: Chandipura Virus; Vesicular Stomatitis Virus; Fish Rhabdoviruses.

Further Reading

Björklund HV, Higman KH, and Kurath G (1996) The glycoprotein genes and gene junctions of the fish rhabdoviruses spring viremia of carp virus and hirame rhabdovirus: Analysis of relationships with other rhabdoviruses. *Virus Research* 42: 65–80.

Carpenter JA, Obbard DJ, and Maside X (2007) The recent spread of a vertically transmitted virus through populations of *Drosophila melanogaster*. *Molecular Ecology* 16: 3947–3954.

Carré-Mlouka A, Gaumer S, Gay P, *et al.* (2007) Control of sigma virus multiplication by the *ref(2)P* gene of *Drosophila melanogaster*. An *in vivo* study of the PB1 domain of Ref(2)P. *Genetics* 176: 409–419.

Fleuriet A and Periquet G (1993) Evolution of the *Drosophila melanogaster*–sigma virus system in natural populations from Languedoc (southern France). *Archives of Virology* 129: 131–143.

Landès-Devauchelle C, Bras F, Dezélée S, and Teninges D (1995) Gene 2 of the sigma rhabdovirus genome encodes the P protein and gene 3 encodes a protein related to the reverse transcriptase of retroelements. *Virology* 213: 300–312.

Teninges D, Bras F, and Dezélée S (1993) Genome organization of the sigma rhabdovirus: Six genes and a gene overlap. *Virology* 193: 1018–1023.

Teninges D and Bras-Herreng F (1987) Rhabdovirus sigma, the hereditary CO_2 sensitivity agent of *Drosophila*: Nucleotide sequence of a cDNA clone encoding the glycoprotein. *Journal of General Virology* 68: 2625–2638.

Wang Y, Cowley JA, and Walker PJ (1995) Adelaide River virus nucleoprotein gene: Analysis of phylogenetic relationships of ephemeroviruses and other rhabdoviruses. *Journal of General Virology* 76: 995–999.

Wayne ML, Contamine D, and Kreitman M (1996) Molecular population genetics of *ref(2)P*, a locus which confers viral resistance in *Drosophila*. *Molecular Biology and Evolution* 13: 191–199.

Simian Alphaherpesviruses

J Hilliard, Georgia State University, Atlanta, GA, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Members of the subfamily *Alphaherpesvirinae* in the family *Herpesviridae* have been identified in Old World monkeys and apes and in New World monkeys. It appears that nearly all host species have co-evolved with one or more alphaherpesviruses, and so it comes as no surprise that new agents in this group continue to be discovered. Indeed, the increased use of nonhuman primates in biomedical research has facilitated the discovery and characterization of such viruses. As assays improve, more virus isolates from nonhuman primates have been identified, and subsequently have been differentiated from closely related viruses by molecular and immunological means. The

benefit of enhanced techniques for virus differentiation is exemplified by the early reports in the 1970s that simian agent 8 (SA8; *Cercopithecine herpesvirus 2*) was not only found in vervets but also in baboons. It was not until nearly two decades later that the virus isolated from baboons in a captive colony was recognized to be herpesvirus papio 2 (HVP-2; *Cercopithecine herpesvirus 16*). Although improved technology enables differentiation of closely related herpesviruses, it remains a time-consuming task to find species-specific viruses, characterize the associated pathogeneses, and verify the natural hosts. Like human alphaherpesviruses, nonhuman primate alphaherpesviruses fall into the genera *Simplexvirus* and *Varicellovirus*.

Simplexviruses are readily isolated because they replicate rapidly in cell cultures from a variety of mammals. They produce a distinct cytopathology as quickly as 24–48 h following infection. Examples of this cytopathology and some of the differences among simplexviruses are shown in **Figure 1**. The property of rapid growth is one of the initial criteria for establishing that an isolate is an alphaherpesvirus. These viruses are most frequently isolated from mucosal sites or from necropsy samples, and occasionally from fomites. With sequence analysis readily available, they can be differentiated easily from close counterparts endemic in related species of Old and New World monkeys, apes, and humans. The zoonotic potential of these viruses is of particular interest, because at least one, B virus (*Cercopithecine herpesvirus 1*), can be rapidly lethal in humans when antiviral therapy is initiated too late.

Frequently, the identity of the natural host of a virus isolate has been established by the use of seroepidemiological assays. The natural host often shows 80–100% seroprevalence in the wild by the time the animals reach sexual maturity. Most data in this regard have been summarized from studies of vervets, baboons, and macaques in the wild, although there are excellent studies that have focused on apes and New World monkeys. The assays, however, upon which seroprevalence are based are not always uniform, and may utilize antigens that are not necessarily unique to the specific virus for which antibody is reactive, resulting in difficulty identifying the natural host of a particular virus. Nonetheless, the plethora of assays has afforded investigators the opportunity to establish candidates for the natural host, and, as assays improve, the identity of the natural host can be narrowed.

Owing to the close evolutionary relationships between monkeys, most notably within Old World groups and New World groups, the viruses that have co-evolved in each host group share significant similarities genotypically and phenotypically. Although genes are arranged mostly in a collinear layout in viruses from both groups of nonhuman primates, viruses from Old World and New World monkeys differ from each other more than viruses from within each monkey group. This point is best illustrated by studies evaluating immunological cross-reactivity among nonhuman primate alphaherpesviruses between groups and within groups. Within either of the Old World and New World groups, extensive genetic conservation renders classical serological assays using virus or infected cell lysates of relatively limited value in identifying the natural host, due to significant cross-reactivity of immunogenic epitopes. An assay for detection of HVP-2 will cross-react sufficiently strongly with mangabey or langur herpesviruses, such that differentiation of the antibody specificity will be nearly impossible using a virus- or infected

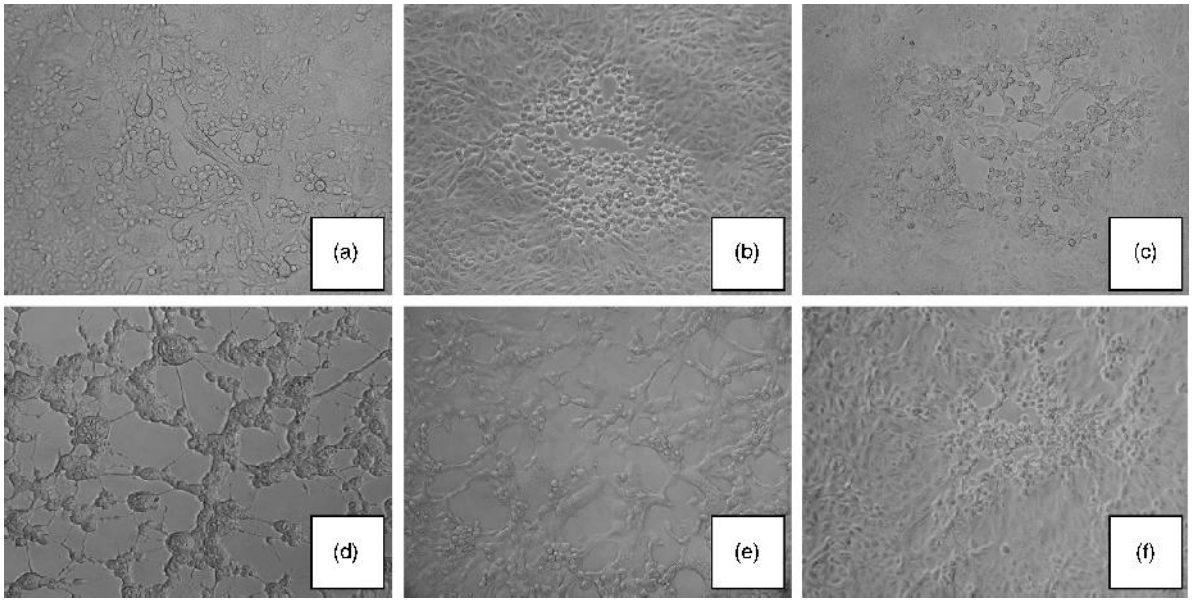


Figure 1 Vero cells in culture (ATCC-081) 24 h after infection with (a) B virus (E2490), (b) HSV-1 (KOS), (c) HVP-2 (SWF), (d) mangabey herpesvirus (EM-GS), (e) SA8 (Hull), or (f) langur herpesvirus (BZ).

cell-based immunoassay. With the advent of recombinant viral proteins, differentiation assays for human alphaherpesviruses have improved, but conservation of the major antigenic proteins can still cloud identification of endemic populations in nonhuman primates. The strategies that differentiate alphaherpesviruses in humans have not proved to be entirely successful when extrapolated to identifying alphaherpesviruses in nonhuman primates. The best example of this is the confusion caused by the finding that SA8 was endemic in baboons as well as vervets, which was still manifest decades after the first publication citing the identification of this agent in baboons. It is now appreciated that the viruses infecting vervets and baboons are distinct alphaherpesviruses, SA8 and HVP-2, respectively. Identification of the natural host of simian varicella virus (SVV) provides a similar example, since both macaques (Asian) and vervets (African) host similar, if not identical, varicelloviruses.

Classification and Evolution

The current list of nonhuman primate alphaherpesviruses is shown in **Table 1**. Nonhuman primate alphaherpesviruses belong to genus *Simplexvirus* (with human herpes simplex virus (HSV) types 1 (HSV-1) and 2 (HSV-2)), with the exception of SVV (*Cercopithecine herpesvirus 9*), which belongs to genus *Varicellovirus* (with human varicella-zoster virus, VZV). The marked genetic similarities between the nonhuman primate viruses and their counterparts in humans strongly support a co-evolutionary history.

The genomes of the Old World monkey simplexviruses have been shown to exist in four isomeric forms, like those of HSV (type E genome structure). The DNA sequences of B virus, SA8, mangabey herpesvirus, langur herpesvirus, and HVP-2 are similar in size (151–157 kbp) to those of their human counterparts, with somewhat greater G+C contents. The genetic contents of these viruses are very similar to that of HSV-1 and HSV-2, except that they lack the gene encoding the neurovirulence protein ICP34.5. The varicelloviruses have a type D genome structure, and the genetic content of SVV is very similar to that of VZV, differing in only a couple of genes at the left genome terminus.

In accord with the relationships among their hosts, simplexviruses of New World primates are more distantly related to those of Old World primates, and the genomes are smaller in size with relatively less complex isomeric organization. The evolutionary distances are apparent from the fact that antisera prepared against the major HSV proteins cross-react with homologous proteins from Old World, but not New World, simplexviruses. Thus far, no varicelloviruses have been found to be endemic in New World monkeys.

Virion Structure

Nonhuman primate alphaherpesviruses are similar in structure to the other members of the subfamily *Alphaherpesvirinae*. By electron microscopy, virus particles consist of capsids sheathed by a tegument and surrounded by an envelope. The icosahedral capsids are assembled in the infected cell nucleus and finally enveloped in the cytoplasm. Unenveloped capsids can be found in both the cytoplasm and nucleus, and are most abundant in the latter. Temporal studies of the kinetics of virus replication suggest that the envelope is acquired in a two-step process, similar to that of HSV. The envelope is rich in virus glycoproteins, which, as judged from antibodies induced during infection, are highly immunogenic. Sequencing and immunological cross-reactivity studies indicate that glycoprotein B is the most highly conserved glycoprotein.

Table 1 Nonhuman primate alphaherpesviruses

Host	*Common virus name*	*Official species name*
Old World		
Vervet (African green monkey)	Simian agent 8 (SA8)	*Cercopithecine herpesvirus 2*
Baboon	Herpesvirus papio 2 (HVP-2)	*Cercopithecine herpesvirus 16*
Macaque	B virus	*Cercopithecine herpesvirus 1*
Macaque	Simian varicella virus (SVV) (also known as Patas delta, Liverpool vervet and Medical Lake macaque herpesvirus)	*Cercopithecine herpesvirus 9*
Langur	Langur herpesvirus	Currently unnamed
Mangabey	Mangabey herpesvirus	Currently unnamed
New World		
Spider monkey	Spider monkey herpesvirus (also known as herpesvirus ateles)	*Ateline herpesvirus 1*
Marmoset	Marmoset herpesvirus (also known as herpesvirus saimiri and, in other platyrrhine monkeys, herpesvirus tamarinus)	*Saimiriine herpesvirus1*

Physical Properties and Epidemiology

Each of the nonhuman primate alphaherpesviruses has features in common with its human counterparts with respect to host range, replication kinetics, transmission, tissue tropism, pathogenecity, histopathology, and induced immune responses. The exception is B virus, which can infect humans, often resulting in a fatal zoonotic infection when left untreated. Each virus is predominantly found in one type of monkey, and recent experience indicates that antibody cross-reactivity with isolates from other species should be evaluated carefully in order to avoid deducing wrongly that a virus is endemic in multiple monkey types. As mentioned above, this is particularly apparent in the case of SA8 and HVP-2, but also features in case studies of alphaherpesviruses from Asian and African monkeys, as well as greater and lesser apes. Although apes are likely to have co-evolved with their own respective alphaherpesviruses, investigators have not yet found herpesviruses other than HSV-1 and HSV-2 in these animals.

Transmission of nonhuman primate alphaherpesviruses from animal to animal occurs as a result of biting, scratching, and splashing activities that contaminate susceptible mucosal epithelial cells. Seroprevalence of virus-induced antibodies increases with the onset of sexual maturity, but infected infants have also been identified, albeit infrequently. Infection is associated with few or no apparent symptoms, whether the virus is latent or replicating actively. Periodic reactivations from latency have been associated with virus shedding from mucus membranes, and this is the time period during which transmission is greatest. Except for B virus, none of the nonhuman primate alphaherpesviruses appears to be transmissible to humans under natural circumstances. Evidence for transmission of human viruses to apes has been substantiated in certain circumstances, as mentioned above, but nonhuman primates are not usually found to be infected with human alphaherpesviruses. When transmission of Old World simplex- or varicelloviruses to New World primates occurs, infection is readily apparent with high morbidity and frequent mortality.

Virus Replication

Nonhuman primate alphaherpesviruses appear to follow the same pattern of biosynthetic activities leading to virus replication and assembly as do their human counterparts, with some exceptions noted. The nonhuman primate simplexviruses replicate in a manner similar to HSV, with a replication cycle of approximately 18 h. Cytopathic effects in cultured cells are also similar, with the exceptions of B virus and mangabey herpesvirus, which induce cell fusion between infected cells and also with neighboring uninfected cells. **Figure 1** shows representative cytopathic effects of B virus versus other alphaherpesviruses in nonhuman primate cells. SVV replicates with kinetics similar to VZV over an interval of 48–72 h in cell culture and, like VZV, remains mostly cell associated.

Following adsorption of a nonhuman primate alphaherpesvirus to a susceptible cell, de-enveloped capsids are released into the cytoplasm and proceed to the nuclear membrane by mechanisms that are poorly understood, but which probably involve the cell's cytoskeleton components. Microarray studies have revealed that host gene remodeling in B virus-infected cells begins within the first hour post infection, and within 3 h post infection the events are clearly distinguishable from those transpiring in HSV-infected cells. Nonetheless, the outcome at the level of the infected cell in culture is the same for both human and nonhuman primate alphaherpesviruses – productive virus replication. The temporal cascade of protein synthesis is conserved, with immediate early, early, and late expression. The major difference between human and nonhuman primate alphaherpesviruses is that the US11 protein is produced as an immediate early protein in the nonhuman primate viruses and as an early protein in HSV-1 and HSV-2. This protein in nonhuman primate viruses prevents phosphorylation of protein kinase R, which in turn prevents phosphorylation of eIF-2α, thus blocking apoptosis in the infected cell and enabling virus replication.

Immune Response to Infection

Nonhuman primates infected by their respective alphaherpesviruses induce humoral antibodies generally within 7–14 days following the onset of acute replication. Antibody titers, however, are not apparent in all animals within this period. Titers can also wax and wane depending on the intervals between reactivated infections. Nonetheless, intermittent virus shedding makes reliance on virus isolation or polymerase chain reaction (PCR) impractical; thus, detection of antibodies is used as the current indicator of whether an animal is infected. Antibodies induced in Old and New World monkeys are cross-reactive with the alphaherpesviruses infecting animals within each group, but there is no apparent cross-reactivity between the two groups. There is little evidence that Old World monkeys can be infected by New World monkey viruses, but New World monkeys generally succumb to viruses from Old World monkeys or humans. Cross-species housing is generally avoided.

The antibodies most commonly produced are against the major glycoprotein (glycoprotein B). There is no clear-cut evolution of the humoral immune response, but the antibodies most observed include those reactive with glycoprotein B, glycoprotein C, glycoprotein D,

glycoprotein G, and the major capsid protein. Having said this, antibody profiles from different animals are quite distinct, as in the case of human antibody profiles induced against HSV.

Future Perspectives

Identification and characterization of simplex- and varicelloviruses from nonhuman primates afford the opportunity to learn more about the origins, evolutionary processes, and pathogenic properties of these viruses. Thus, study of a particular member provides insights on the virus as an individual species and as a representative of a lineage of related agents. Perhaps even more important is the development of understanding on how to approach novel viruses, whether newly discovered or emerging. Knowledge of these viruses in relation to their natural hosts often cannot be extrapolated to their effects in foreign hosts, and the responses of a non-natural host can influence pathogenesis in unexpected ways. This is illustrated by B virus infection of humans, the most dramatic example of cross-species transmission among the herpesviruses. Current attention focused worldwide on emerging viruses and viruses that invade foreign hosts places an emphasis on primate alphaherpesviruses that will teach investigators for decades to come.

See also: Herpes Simplex Viruses: General Features; Herpes Simplex Viruses: Molecular Biology; Herpesviruses: General Features; Simian Gammaherpesviruses; Varicella-Zoster Virus: General Features; Varicella-Zoster Virus: Molecular Biology.

Further Reading

Cohen JI, Davenport DS, Stewart JA, *et al.* (2002) Recommendations for prevention of and therapy for exposure to B virus (*Cercopithecine herpesvirus 1*). *Clinical Infectious Diseases* 35: 1191–1203.

Davison AJ and Clements JB (1997) Herpesviruses: General properties. In: Mahy BWJ and Collier LH (eds.) *Topley and Wilson's Microbiology and Microbial Infections,* 9th edn., pp. 309–323. London: Arnold.

Eberle R and Hilliard J (1995) The simian herpesviruses. *Infectious Agents Disease – Reviews Issues and Commentary* 4: 55–70.

Gray WL, Gusick NJ, Ek-Kommonen C, Kempson SE, and Fletcher TM, III (1995) The inverted repeat regions of the simian varicella virus and varicella-zoster virus genomes have a similar genetic organization. *Virus Research* 39: 181–193.

Weigler J (1992) Biology of B virus in macaque and human hosts: A review. *Clinical Infectious Diseases* 14: 555–567.

Whitley RJ and Hilliard J (2007) Cercopithecine herpesvirus 1 (B Virus). In: Knipe DM and Howley PM (eds.) *Fields Virology,* 5th edn., vol. 2, p. 2889. Philadelphia: Lippincott Williams and Wilkins.

Simian Gammaherpesviruses

A Ensser, Virologisches Institut, Universitätsklinikum, Erlangen, Germany

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The *Gammaherpesvirinae* is a large subfamily of the family *Herpesviridae*. Although gammaherpesviruses usually cause limited disease upon primary infection of their natural hosts, several are relevant tumor viruses of the hematopoietic system and form an important chapter of viral oncology. The first clearly identified human herpesvirus, Epstein–Barr virus (EBV; species *Human herpesvirus 4*), is the prototype of genus *Lymphocryptovirus* (whose members are referred to as lymphocryptoviruses or γ1-herpesviruses) and the cause of infectious mononucleosis. Homologs of EBV had been recognized for decades in various Old World primates, and have been found recently in several species of American monkeys. They may serve to develop models for pathogenesis or treatment of human lymphoproliferative diseases and cancers that are caused by EBV, such as B-cell lymphomas and other lymphoproliferative syndromes, nasopharyngeal carcinomas, and, possibly, gastric cancer.

The second genus of gammaherpesviruses, *Rhadinovirus* (whose members are referred to as rhadinoviruses or γ2-herpesviruses), is distinct biologically and molecularly. The prototypic member of this group, herpesvirus saimiri (HVS; species *Saimiriine herpesvirus 2*), and herpesvirus ateles (HVA; species *Ateline herpesvirus 2* and ateline herpesvirus 3) were detected as T-lymphotropic viruses in neotropical primates and raised primary interest from the fact that they cause fulminant T-cell lymphomas in numerous primate species as well as in rabbits. The related animal pathogens alcelaphine herpesvirus 1 and ovine herpesvirus 2 cause malignant catarrhal fever, a T lymphoproliferative disease of ruminants. Although no exact correlates of these T-cell tumors exist in human pathology, HVS strains of subgroup C are capable of transforming human and simian T lymphocytes to continuous growth in cell culture. This provided for the first time a reliable means of immortalizing human T lymphocytes in cell culture, a useful tool for T-cell immunology. These viruses have been used as expression

vectors for gene transfer in T lymphocytes and have facilitated study of the mechanisms of episomal persistence in the T-cell system. Further interest in the rhadinoviruses arose when the first human member of this genus was recognized. This virus was found to be strongly associated with all forms of Kaposi's sarcoma (KS), as well as with multicentric Castleman's disease and primary effusion lymphoma (PEL). Since DNA from this virus is regularly found in all KS forms, specifically in the spindle cells of KS, it was also termed KS-associated herpesvirus (KSHV; species *Human herpesvirus 8*). Viral membrane-associated oncoproteins Stp and Tip, which act on T-lymphocyte signaling, were defined in HVS, though it is far less clear which of several candidate genes encode the relevant oncoproteins of KSHV.

For many years, research on lymphotropic simian herpesviruses focused on the tumorigenic T-lymphotropic rhadinoviruses, especially HVS. Then the discovery of KSHV prompted research on B-lymphotropic agents and led to the description of rhesus rhadinovirus (RRV; species *Cercopithecine herpesvirus 17*) and several closely related rhadinoviruses in various Old World primates, although these are only loosely associated with pathogenicity or tumor induction. New World primate EBV-like viruses were discovered recently, and an increasing number of DNA sequences from additional, new gammaherpesviruses are being amplified from diverse host species using degenerate polymerase chain reaction (PCR) techniques that target strongly conserved herpesvirus genes, such as that encoding DNA polymerase. A provisional compilation of the better defined primate gammaherpesviruses is represented in **Table 1**, and more extensive information is available in the website of the International Committee on Taxonomy of Viruses (ICTV).

Herpesvirus Saimiri and Herpesvirus Ateles

This section focuses on the basic biology, gene content, and viral mechanisms of oncogenic transformation of HVS and HVA, and their possible applications as T-cell vectors and in cell-based immunotherapy. These gammaherpesviruses must not be confused with two alphaherpesviruses isolated from the same host species, designated as species *Saimiriine herpesvirus 1* and *Ateline herpesvirus 1*, respectively.

History, Host Range, Transmission, and Pathology

HVS was originally isolated by Melendez and others from captive monkeys of various species, but it soon became clear that this virus is found regularly only in squirrel monkeys (*Saimiri sciureus*), whose natural habitat is South American rainforests. Squirrel monkeys are usually infected via saliva within the first two years of life. The virus does not cause disease or tumors, and establishes lifelong persistence. In other New World primate species such as tamarins (*Saguinus* spp.), common marmosets (*Callithrix jacchus*), or owl monkeys (*Aotus trivirgatus*), infection with HVS causes acute peripheral T-cell lymphoma within less than 2 months after experimental intramuscular or intravenous infection. Intramuscular injection of purified virion DNA can also cause disease in susceptible primates.

HVS strains are classified into three subgroups (A, B, and C) depending on pathogenic properties and on sequence divergence in the left-terminal nonrepetitive region of the genome (**Figure 1**). The major representative strains are the prototypic A11 for subgroup A; B S295C and B-SMHI for subgroup B; and C488 and C484 for subgroup C.

Viruses of HVS subgroups B and C are considered to be the least and most oncogenic, respectively. Tamarins are susceptible to viruses of all subgroups, whereas subgroup B viruses are not able to cause disease in adult common marmosets. Strain C488 causes acute peripheral T-cell lymphoma within only a few weeks in common marmosets or cottontop tamarins (*S. oedipus*). A similar fulminant disease is induced in Old World rhesus and cynomolgus monkeys (*Macaca mulatta* and *M. fascicularis*, respectively) by large intravenous doses of C488. Similar to the situation in New World primates, the disease in cynomolgus monkeys is designated as a pleomorphic, peripheral T-cell lymphoma or a pleomorphic, T-lymphoproliferative disorder. A high-titer infection in New Zealand white rabbits results in tumor induction, but pathogenicity has not been reported in rodents. HVS can be isolated from the peripheral blood cells of persistently infected squirrel monkeys or diseased tamarins, presumably from infected T cells, by co-cultivation with permissive owl monkey kidney (OMK) cells. HVS replicates productively in, and induces cell lysis of, OMK cells and some primary mesenchymal cultures established from marmosets, and less efficient replication is possible in Vero (African green monkey) cells.

HVA can be isolated at a high frequency from spider monkeys (*Ateles* spp.). Strain 810 from *A. geoffroyii* is a member of species *Ateline herpesvirus 2*, whereas strain 73 and related strains (87, 93, and 94) from *A. paniscus* are isolates of ateline herpesvirus 3. HVA replicates in OMK cells, but remains mostly cell-associated with syncytia formation. As a result, supernatants of such cultures have low, unstable virus titers.

Like HVS, HVA is not pathogenic in its natural host, but causes acute T-cell lymphomas in various New World primate species, including cottontop tamarins and owl monkeys. The pathological changes are similar to those observed after HVS infection. In addition, HVA transforms

Table 1 Primate gammaherpesviruses

Species	*Common name(s) and abbreviation(s)*	*Host*	*Associated pathogenicity*
Genus *Rhadinovirus*			
Human herpesvirus 8	Kaposi's sarcoma-associated herpesvirus (KSHV)	Human (*Homo sapiens*)	Kaposi's sarcoma, multicentric Castleman's disease, primary effusion lymphoma
NA[a]	Chimpanzee rhadinovirus	Chimpanzee (*Pan troglodytes*)	Unknown
NA	Gorilla rhadinovirus	Gorilla (*Gorilla gorilla*)	Unknown
Cercopithecine herpesvirus 17	Rhesus rhadinovirus (RRV), *Macaca mulatta* rhadinovirus	Rhesus macaque (*M. mullata*)	B-cell hyperplasia?
NA	Retroperitoneal fibromatosis-associated herpesvirus (RFHV, RFHVMn, RFHVMm)	Southern pig-tailed macaque (*M. nemestrina*), rhesus macaque (*M. mullatta*)	Retroperitoneal fibromatosis?
NA	*Macaca nemestrina* rhadinovirus 2 (MnRRV)	Southern pig-tailed macaque (*M. nemestrina*)	Unknown
Saimiriine herpesvirus 2[b]	Herpesvirus saimiri (HVS)	Squirrel monkey (*Saimiri sciureus*)	T-cell lymphoma in other neotropical monkey species
Ateline herpesvirus 2	Herpesvirus ateles (HVA)	Spider monkey (*Ateles paniscus*)	T-cell lymphoma in other neotropical monkey species
NA	Herpesvirus ateles strain 73 (HVA), ateline herpesvirus 3	Spider monkey (*A. paniscus*)	T-cell lymphoma in other neotropical monkey species
Genus *Lymphocryptovirus*			
Human herpesvirus 4[b]	Epstein–Barr virus (EBV)	Human (*Homo sapiens*)	B-cell lymphoma, nasopharyngeal lymphoma, Hodgkin's disease
Pongine herpesvirus 1	Herpesvirus pan, chimpanzee lymphocryptovirus	Chimpanzee (*Pan* sp.)	Unknown
Pongine herpesvirus 2	Orangutan herpesvirus	Orangutan (*Pongo* sp.)	Unknown
Pongine herpesvirus 3	Gorilla herpesvirus	Gorilla (*Gorilla* sp.)	Unknown
Cercopithecine herpesvirus 12	Baboon herpesvirus, herpesvirus papio	Baboon (*Papio* sp.)	Spontaneous B-cell lymphoma (and in immunosuppressed animals)
Cercopithecine herpesvirus 14	African green monkey EBV-like virus	African green monkey (*Chlorocebus aethiops*)	Unknown
Cercopithecine herpesvirus 15	Rhesus EBV-like herpesvirus, rhesus lymphocryptovirus	Rhesus macaque (*M. mullatta*)	Spontaneous B-cell lymphoma (and in immunosuppressed animals)
NA	Cynomolgus EBV-like virus, *Macaca fascicularis* gammaherpesvirus (herpesvirus MF1, A4, TsB-B6, Si-IIA-EBV)	Cynomolgus monkey (*M. fascicularis*)	Spontaneous B-cell lymphoma (and in immunosuppressed animals)
Callitrichine herpesvirus 3	Marmoset lymphocryptovirus	Common marmoset (*Callithrix jacchus*)	Spontaneous B-cell lymphoma
NA	Gold-handed tamarin lymphocryptovirus (SmiLHV1)	Gold-handed tamarin (*Saguinus midas*)	Unknown
NA	Squirrel monkey lymphocryptovirus (SscLHV1)	Squirrel monkey (*S. sciureus*)	Unknown
NA	White-faced saki lymphocryptovirus (PpiLHV1)	White-faced saki (*Pithecia pithecia*)	Unknown

[a]NA, species not assigned by ICTV.
[b]Type species of the genus.

T cells of certain New World monkey species (such as cottontop tamarin) in culture, yielding cytotoxic T-cell lines. Human T cells are not susceptible to transformation with various HVA strains, but could be transformed by a recombinant HVS C strain in which the HVS oncogenes were replaced by the HVA oncogene Tio.

Transformed T-cell lines have been derived from HVA-infected tamarins and cultivated continuously for several years. Whereas in most cases virus particles were found initially, virus production was frequently lost after prolonged culture. The episomal DNA is heavily methylated in such nonproductive cell lines, and rearrangements or

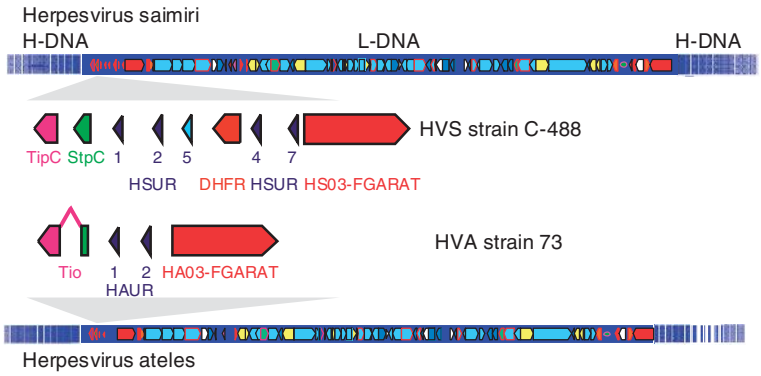


Figure 1 Gene arrangements at the left genome end of HVS and HVA. The oncoproteins Stp, Tip, or Tio are encoded at the variable left terminal region of the coding L-DNA. Stp, saimiri transformation-associated protein of the respective subgroup A, B, or C; Tio, two-in-one-protein of HVA; Tip, tyrosine kinase-interacting protein of HVS subgroup C; HSUR or HAUR, HVS or HVA-encoded URNA; HS03-/HA03-FGARAT, formylglycineamide ribotide amidotransferase ORF3.

large deletions are evident in the viral genomes. Marmoset and tamarin T cells can be transformed by HVS to stable T-cell lines *in vitro* and are designated as semipermissive, since virus particles are released, although to lower titers than from OMK cells.

Genome Properties, Replication, and Gene Content

The term 'rhadino' viruses was coined from the ancient Greek word ραδινοσ for fragile, because the viral genomic DNA breaks upon isopyknic centrifugation in CsCl gradients into two classes of highly differing densities. The L DNA (low density, low G + C content) contains the viral protein-coding genes, and the repetitive H DNA (high density, high G + C content) from the genome termini is noncoding. The intact viral (M) genome has intermediate density. Two strains of HVS, strain A11 and the highly oncogenic subgroup C strain C488, have been sequenced. The A11 H DNA consists of multiple tandem repeats of 1444 bp (70.8% G + C), and the unique L DNA comprises 112 930 bp (34.5% G + C). The size of the M DNA genome is variable owing to different numbers of H DNA repeats attached to both ends of the linear virion genome. In strain C488, the L DNA comprises 113 027 bp, and it is flanked by arrays of two distinct repeat unit types of 1318 and 1458 bp, the shorter representing the longer with 140 bp deleted. The packaged M genome of C488 is approximately 155 kbp in size, with a range of 130–160 kbp owing to variable numbers of terminal H DNA repeats. The HVS L DNA contains at least 76–77 protein-coding open reading frames (ORFs) and encodes 5–7 U RNAs (termed HSURs) (**Figure 1**).

HVA strain 73 has a similar genome structure to HVS, with a slightly shorter L DNA of 108 409 bp (36.6% G + C), and H DNA consisting of multiple tandem repeats of 1582 bp (77.1% G + C). The HVA L DNA contains 73 ORFs and only two genes for U RNA-like transcripts (termed HAURs). HVA does not encode ORF12, vIL17, vCD59, or vFLIP homologs, but the genes encoding superantigen (SAG), cyclin, and G-protein-coupled receptor (GPCR) are conserved. Thus, HVA may be an ancient variant of HVS that has either collected a smaller set of cell-homologous genes or has secondarily lost several genes.

In all gammaherpesviruses, the genes that are conserved among the herpesvirus subfamilies are arranged in blocks. Flanking or interspersed among the blocks are other genes, most of which do not occur in the other subfamilies. Among these are transforming oncogenes and viral homologs of cellular genes, which are described below. Most genes are well conserved between different HVS strains, but there is pronounced sequence variation near the left end of the HVS L DNA and in the region of the R transactivator gene (*orf50*) and the adjacent glycoprotein gene (*orf51*), a region that is also highly variable among other rhadinoviruses.

The replication mechanism of rhadinoviruses has not been investigated in much detail, and is generally considered to follow that of other herpesviruses. The lytic origin of DNA replication (OriLyt) in HVS strain A11 has been mapped to an untranslated region upstream of the thymidylate synthase gene. A putative latent origin of DNA replication (OriP) in the left-terminal region of the L DNA in strain C484 was reported to mediate episomal maintenance, but is not conserved between different HVS strains and is not required for viral replication or episomal persistence. Thus, although HVS persists in transformed human T cells as stable nonintegrated episomes at high copy number, OriP and the viral factors involved remain unidentified. Histone modification of the HVS C488 episome in human T cells has been analyzed, and bears similarities to that of KSHV episomes in B cells.

Infection of tissue culture cells by HVS is asynchronous, and hence the assignment of HVS genes to the immediate early (IE) phase of infection is based mostly

on experiments using cycloheximide to inhibit protein synthesis. The IE57 post-transcriptional regulator encoded by *orf57* appears to be the sole regulatory viral IE gene. It codes for a nuclear phosphoprotein of 52 kDa with structural and functional homology to herpes simplex virus ICP27 and EBV BMLF1. IE57 stimulates the expression of unspliced, and represses the expression of spliced, transcripts, has been shown to redistribute nuclear components of the splicing machinery, and is involved in nuclear RNA export. A strong viral transactivator function was mapped to the delayed early gene *orf50*, the homolog of the EBV R transactivator gene. Owing to differential splicing and promoter usage, this gene codes for a full-length protein (ORF50A) and a smaller, C-terminal variant (ORF50B). The transactivation domain resides in the C-terminal region of these proteins and binds to the TATA-binding protein in the basal transcription complex. Although IE57 is highly conserved between subgroups A and C, the *orf50* region is divergent. Neither HVS nor HVA encodes a homolog of bZip/Zta of EBV or KSHV.

The HVS ORF73 protein of strains A11 and C488 localizes to the host cell nucleus, and, like the latent nuclear antigen (LANA) of KSHV, can associate with host cell chromosomal DNA. The A11 ORF73 protein can associate with cellular p32 and binds to GSK-3β. Although not detectable by northern blotting of RNA from C488-transformed human T cells, *orf73* transcripts are detectable by reverse transcription-polymerase chain reaction (RT-PCR). The C488 ORF73 protein can downregulate the *orf50A* and *orf50B* promoters, and this prevents ORF50-mediated activation of viral replication gene promoters. This suggests that the HVS ORF73 protein, and its homologs in the other rhadinoviruses, can block initiation of the lytic replication cascade, thereby controlling the transition between latency and lytic replication.

Sequestered Cellular Genes

Rhadinoviruses such as HVS and KSHV contain several intronless genes that are homologous to cellular genes; in this context, a role for reverse transcription during putative capture of these genes might be speculated upon. A few of these cellular gene homologs are unique to specific viruses, and some are common to several rhadinoviruses (and to lymphocryptoviruses, including EBV). This suggests that successful uptake of cellular genes is a rather infrequent event during herpesvirus evolution. Most of these cellular homologs can be categorized into two major groups: (1) genes related to cellular growth control or nucleotide metabolism, and (2) genes that modulate innate or adaptive immune functions, including apoptosis. For example, HVS *orf72* codes for a functional viral cyclin D, and homologs related to nucleotide metabolism include a dihydrofolate reductase (DHFR; *orf2*) and a functional thymidylate synthase (TS; *orf70*). Both *orf3* and *orf75* encode large tegument proteins that share similarity with formylglycineamide ribotide amidotransferase (FGARAT). It is thought that these enzymes may possibly augment the free nucleotide pools and could thus facilitate DNA synthesis and virus replication.

Oncogenic Signaling and Transformation

The Stp Oncoproteins

The HVS oncogenes required for induction of T-cell leukemia and T-cell transformation *in vitro* reside in the variable region at the left end of the L DNA (**Figure 1**). Subgroup A and B strains have a single gene termed *stpA* or *stpB* (saimiri transformation-associated protein of subgroup A or B strains), and subgroup C strains carry *stpC* (*stp* of subgroup C strains) and *tip* (tyrosine kinase-interacting protein). The proteins StpA and StpB share limited sequence homology with StpC, but are structurally unrelated to Tip. Although *stpA* and *stpC/tip* are not required for viral replication, deletion of either *stpA, stpC*, or *tip* abolishes transformation by HVS *in vitro* and pathogenicity *in vivo*. *stpA*- or *stpC*-transfected rodent fibroblasts form foci *in vitro* and induced tumors in nude mice. *stpA*-transgenic mice develop polyclonal peripheral T-cell lymphomas, and an *stpC* transgene induces epithelial tumors.

stpC and *tip* are transcribed into a single bicistronic mRNA from a common promoter directed toward the left end of the L DNA, with *tip* situated downstream from *stpC*. Transcription of *stpC/tip* is regulated similarly to IE genes in human T cells, and no obvious viral factors seem to be involved. The *stpC/tip* promoter carries euchromatic histone modifications in C488-transformed human T cells.

The 102-residue StpC phosphoprotein has an N terminus of 17 mostly charged residues, and the C terminus contains a hydrophobic region that probably serves as an anchor to perinuclear membranes. In between are 18 collagen tripeptide repeats of the form $(GPX)_n$, which may mediate multimerization of the protein. StpA and the less efficiently transforming StpB bind to, and are phosphorylated by, the nonreceptor tyrosine kinase Src. StpC interacts with the small G-protein Ras and stimulates mitogen-activated protein (MAP) kinase activity. Both StpA and StpC interact with tumor necrosis factor receptor-associated factors (TRAFs), leading to nuclear factor kappa B (NFκB) activation.

The Tip Oncoprotein

The subgroup C-specific 40 kDa Tip phosphoprotein has been shown to co-precipitate with the T-cell-specific nonreceptor Src family tyrosine kinase p56/Lck in C488-transformed T cells. Tip-transgenic mice develop T-cell proliferations. Tip has an N-terminal glutamate-rich

region, duplicated in some strains, followed by one or two serine-rich regions, a bipartite kinase-interacting domain, and a C-terminal hydrophobic domain that anchors the molecule at the inside of the plasma membrane. The kinase-interacting domain consists of nine residues with homology to the C-terminal regulatory regions of various Src kinases (CSKH), and a proline-rich SH3-domain-binding sequence (SH3B). Several tyrosine residues, three of which are conserved between all strains investigated, are substrates for Lck. Tyrosine residue 127 (Y127) is the major tyrosine phosphorylation site of Tip (strain C488), but this modification does not enhance Lck binding in T cells. Recombinant viruses expressing mutations in Tip show that the strong Lck binding mediated by cooperation of the SH3B and CSKH motifs is essential for transformation of human T cells by C488, whereas Tip Y127 is required for transformation in the absence of exogenous interleukin-2, suggesting its involvement in cytokine signaling pathways.

Tip binding to Lck modulates the kinase activity and could result in an altered substrate specificity, contributing to the abrogation of ZAP70 phosphorylation. This dysregulation may further link Tip-bound Lck to alternative downstream effectors. In addition, the implication of Tip Y114 with constitutively active signal transducers and activators of transcription (STATs), especially STAT3, and the role of STATs in growth regulation and oncogenesis in multiple cell types, suggest a central role for Tip-induced STAT activity in viral T-cell transformation. However, recombinant HVS C488 expressing Tip with a tyrosine-to-phenylalanine mutation at residue 114 was able to transform primary human T lymphocytes in the absence of STAT1 or STAT3 activation. Tip is further associated with lipid rafts, and this is essential for the T-cell receptor (TCR) and CD4 downregulation but not for inhibition of TCR signal transduction and activation of STAT3 transcription factor. The activation of Lck and the inhibition of T-cell signaling by Tip may represent two different aspects of the same function, since the activation of Lck by Tip might trigger negative feedback mechanisms, such as apoptosis, in stably transfected Jurkat cells expressing high levels of Tip.

The Tio Oncoprotein

A spliced gene with two exons is located at the junction between H DNA and the left-terminal L DNA in HVA strain 73. The encoded protein shares local similarity with StpC and Tip of HVS subgroup C strains, and was therefore termed 'two in one' (Tio). Tio is expressed in HVA-transformed simian T cells, and is bound to, and phosphorylated by, the Src family tyrosine kinases Lck or Src. Phosphorylation of Tio at Y136 is required for successful transformation of human T cells. These cells are also transformed by recombinant HVS C488 in which *stpC* and *tip* has been replaced by a *tio* cDNA transcribed from a heterologous promoter. Furthermore, Tio induces NFκB signaling via direct interaction with TRAF6.

Growth Transformation of Human T Cells by Rhadinoviruses

Human T-cell growth transformation by HVS subgroup C strains has provided a reproducible technique for generating T-cell lines, and has opened up a new research direction linking T-cell biology, signal transduction pathways, and viral transforming functions. Infection of cord or peripheral blood mononuclear cells, thymocytes, or established human T-cell clones by C488 results in T-cell lines that grow continuously without restimulation by antigen or mitogen and do not require the presence of feeder or antigen-presenting cells. Many HVS subgroup C strains are able to transform human T cells, though to a varying extent; C488 is often preferred, as it achieves dependable growth transformation. Recombinant HVS C488 in which *stpC* and *tip* have been replaced by HVA *tio* can offer increased efficiency of human T-cell transformation along with a decreased requirement for IL-2. HVS C488 carrying mutations in Tip is being investigated for an expanding range of T-cell phenotypes.

The resulting polyclonal T-cell lines display the irregular morphology of T blasts. They carry nonintegrated HVS genomes in high copy numbers, have a normal karyotype, and are not tumorigenic in nude or severe combined immune-deficient (SCID) mice. The phenotype of HVS-transformed T cells is remarkably stable for many months in culture. It corresponds to that of mature, activated CD4+ CD8– or CD4– CD8+ T cells, usually with αβ-type (less frequently γδ-type) T-cell receptors. Transformed lines derived from established T-cell clones show the phenotype and human leukocyte antigen-restricted of the parental T cells. Cellular responses after CD3, CD4, or IL-2 receptor stimulation or antigen contact can be measured by signal transduction parameters, by proliferation, or, most reliably, by interferon-γ production. Transformation of cytotoxic T lymphocytes (CTLs) is rather inefficient, but may be increased by optimized protocols for prestimulation and culture of CTLs.

Transformation by HVS C488 has, in many cases, been the only way to cultivate and amplify T cells from patients with primary human immune deficiencies, including genetic T-cell defects involving the CD3γ chain, IL-2Rγ chain, CD95/Fas, IL-12R, major histocompatibility complex class II, Wiskott–Aldrich syndrome, or CD18/LFA-1. HVS-transformed human CD4+ T cells provide a productive system for T-lymphotropic viruses such as human herpesvirus 6 and human immunodeficiency virus (HIV) types 1 and 2, including primary clinical and macrophage-tropic HIV isolates.

Although most HVS-transformed New World monkey T lymphocytes produce infectious viral particles, HVS-transformed human T-cell lines maintain an intact viral genome but do not shed infectious virus. Production of infectious particles is also not induced by specific or nonspecific stimulation of the cells, using phorbol esters, nucleoside analogues, or other drugs that can reactivate viruses such as EBV or KSHV. Many macaque T-cell lines have been shown to shed very low amounts of virus particles, in contrast to their human counterparts, and the infusion of HVS-transformed autologous T cells into donor macaques did not cause disease. The reinfused T cells persisted for extended periods and the animals were protected against challenge with HVS C488. This is a relevant observation, since macaques are a common model for the situation in humans, and HVS-transformed simian T lymphocytes are similar to their human counterparts in many characteristics, including retained antigen specificity and presentation.

Alterations

StpC and Tip are the only viral proteins that have been demonstrated regularly in HVS-transformed human T cells, and yet their expression alone or together in a lentiviral background is not sufficient to transform primate T cells. The HSURs are expressed abundantly in a similar way to the small, noncoding RNAs (EBERs) of EBV, but deletion of all the HSUR genes does not influence virus replication or T-cell transformation. Viral transcription other than that of the bicistronic *stpC/tip* genes is rarely detected in human T cells; it is restricted to *ie14/vsag*, and few others at extremely low abundance (*orf57*/IE57, *orf50*/RTA, *orf70*/TS, *orf71*/vFLIP, *orf72*/vCyclin, and *orf73*/LANA). Some of these increased after stimulation with phorbol ester. Many other viral genes, such as the weakly transcribed *orf71* and *orf72*, have been shown by deletion analysis not to be required for T-cell transformation.

Compared to parental, untransformed T cells, a few cellular and biochemical alterations have been detected consistently in HVS-transformed T-cell lines: CD2 and its ligand CD58 are both expressed at high densities on the cell surface and there is hyper-responsiveness to CD2 ligation. Since withdrawal by limiting dilution halts the growth of HVS-infected human T cells, IL-2 induction by CD2–CD58 contact likely contributes to the transformed phenotype of HVS-transformed human T cells. Furthermore, subcloning of HVS-transformed cells is not possible. The Src family protein tyrosine kinase $p53/56^{Lyn}$ is usually expressed in B cells. Lyn is also found in HVS-transformed T-cell lines, similar to HTLV-1-immortalized T cells, but is not activated by HVS Tip. HVS-transformed T cells secrete high amounts of the Th1 cytokine interferon gamma (IFN-γ), and Th2-skewed T cells or Th2 clones shift toward a Th1 or Th0 profile. Many transformed clones also secrete large amounts of chemokines, such as MIP-1α and MIP-1β, and CCL1/I-309, which may protect HVS-transformed T cells from apoptosis via CCR8. IL-26, a new IL-10 cytokine family member, was discovered due to its over-expression in HVS-transformed T cells. IL-26 may influence T-cell interaction with epithelial cells *in vivo* but seemingly does not contribute to HVS-mediated T-cell transformation.

Gene Transfer

HVS vectors are attractive for gene transfer into T cells, since the functional phenotype of transformed T lymphocytes is maintained and the T cells can be simultaneously expanded by transformation. They may even be considered for therapeutic redirection of human T-cell antigen specificity, as tools for experimental cancer therapy applications. However, replication-deficient vector variants are necessary, and a number of biosafety aspects remain to be clarified. Remarkably, ganciclovir administration does not prevent pathogenesis by HVS expressing a TK suicide gene, and tumor induction is even more rapid than with a wild-type HVS control.

Genetic alteration of HVS-transformed cells can be achieved by transduction with retroviral or lentiviral vectors, or by using recombinant HVS. Efficient infection and occasionally limited productive replication of HVS have been observed in various human cell types, including human bone marrow stroma cells, primary fibroblasts, and hematopoietic precursors. Although foreign genes were first inserted into the genome of HVS more than two decades ago, the reconstitution of virus from overlapping cosmids and engineering of bacterial artificial chromosomes has greatly facilitated mutational analysis of the HVS genome and expression cloning in HVS. This includes attenuated, nononcogenic vectors deleted in the transformation-associated left-terminal region of L DNA that harbors the HVS oncogenes. Episomally persisting herpesvirus vectors, based either on replication defective viruses or on amplicons, are currently regarded as a promising alternative that can avoid side effects of integration, which is now a major concern in the field of gene transfer.

Rhesus Rhadinovirus and Related Old World Primate Rhadinoviruses

Natural Occurrence and Pathology

The discovery of KSHV as the first human rhadinovirus in 1994 greatly stimulated the search for rhadinoviruses in other Old World primates. Serological studies using KSHV-derived antigens indicated that a related herpesvirus may exist in rhesus monkeys. This led to the isolation

of RRV by co-cultivation of lymphocytes from seropositive rhesus monkeys with rhesus fibroblasts by Desrosiers and colleagues in 1997. RRV seems to be very widespread in captive monkeys and, in contrast to KSHV, can be propagated efficiently in cell culture. Although there exists no clear disease association for RRV in infected healthy macaques, there is one report concerning rhesus macaques that were immunosuppressed by previous infection with simian immunodeficiency virus (SIV). In this case, RRV infection resulted in a multifocal lymphoproliferative disease resembling multicentric Castleman's disease. However, this had not been noticed previously in numerous studies of SIV-infected macaques of unknown, but presumably mostly positive, RRV infection status.

Using degenerate PCR of the DNA polymerase gene, DNA fragments of rhadinovirus origin have been identified in various Old World primates, including African green monkey, chimpanzee, gorilla, and mandrill. Phylogenetic analysis of short sequences has revealed that Old World primate rhadinoviruses probably segregate into two groups: one that is more closely related to KSHV and another that is more closely related to RRV.

Genome Structure and Replication

Analysis of the genome sequences of two independent strains has revealed that RRV is indeed more closely related to KSHV than to the prototypic rhadinovirus HVS. The RRV genome organization is essentially collinear with that of KSHV. It has (at least) 79 genes, 67 of which are homologous to genes found in both KSHV and HVS. Of the remaining 12 genes, 8 are similar to KSHV genes (see below). Interestingly, *orf2*/DHFR is in the same position as in HVS, and different from that in KSHV. The RRV OriLyt is located in the same region as in HVS and KSHV, between *orf69* and *orf71*/vFLIP. The functions of viral transactivators and regulatory proteins resemble those of KSHV. The gene content of RRV is similar to that of KSHV, but contains only one *vMIP* gene and lacks *K3* and *K5*. The genes encoding CCPH and vIL-6 are conserved in RRV, and eight genes are present with homology to the family of viral interferon regulatory factors (*vIRF-1* through *vIRF-8*). Several large DNA viruses have been shown to encode micro-RNAs (miRNAs), including EBV and the rhadinoviruses RRV and KSHV, and miRNA genes are evolutionary conserved in at least the lymphocryptoviruses. However, the role of miRNA and RNA interference in the viral context is controversial. Given the specificity of RNA interference, it remains to be determined whether this can have a role in herpesviral transformation of foreign hosts. An interesting speculation is that herpesviral miRNAs may act as specificity factors that initiate heterochromatin assembly of the latent viral genome.

Rhadinoviruses from Retroperitoneal Fibrosis

In an approach using a degenerate PCR technique, Rose and co-workers identified fragments of a herpesvirus DNA polymerase gene in tissue specimens from retroperitoneal fibromatosis (RF) from macaque species, *M. nemestrina* (six cases) and *M. mulatta* (one case). RF is a rare disease occurring in immunesuppressed macaques that consists of aggressively proliferating fibrous tissue with a high degree of vascularization; thus, it somewhat resembles KS. Earlier transmission studies indicated that an infectious agent may be involved in RF pathogenesis. Sequence comparisons indicate that the DNA polymerase and adjacent genes of these two potentially novel rhadinoviruses, tentatively termed RFHVMm and RFHVMn, are related more closely to KSHV than RRV. Attempts to isolate the viruses on cultured cells have so far been unsuccessful. The possible coexistence of two different rhadinoviruses in the same host animal is also indicated, since in one study all RF-diseased macaques harbored RRV DNA (and all were coinfected with simian retrovirus 2 and/or SIV). In these animals, RFHV DNA was present at significantly higher copy numbers in the RF tumors.

Lymphocryptoviruses of Old and New World Primates

Gammaherpesviruses closely related to EBV have been recognized in several species of Old World primates from the mid-1970s. The genome of rhesus lymphocryptovirus (abbreviated to rhesus LCV; species *Cercopithecine herpesvirus 15*) has been sequenced. Until recently, the paradigm was that the lymphocryptoviruses are restricted to Old World primates, including humans. However, a virus related to EBV was isolated from common marmosets, both from healthy animals and animals with spontaneous B-cell lymphomas. Related lymphocryptoviruses have also been detected in several other New World primate species (**Table 1**). The new lymphocryptovirus (marmoset LCV; species *Callitrichine herpesvirus 3*) has an EBV-like genome structure, and determination of the genome sequence has shown that several Old World primate lymphocryptovirus-specific genes are absent. Specifically, homologs of EBV BCRF1/vIL10, BARF1/CSF-1R, BARF0, the EBERs, and several other genes of unknown function have not been detected, and marked divergences exist in LMP-1, LMP-2, EBNA-LP, EBNA-2, and the EBNA-3 family. Also, the organization of the putative marmoset LCV OriP-region is clearly distinct from that in Old World primate lymphocryptoviruses.

In vitro transformation of B cells by human and simian Old World primate lymphocryptoviruses seems to be mostly restricted to the natural host or closely related

species. However, experimental T-cell tumors can be induced in rabbits following infection by cynomolgus LCV or baboon LCV.

Conclusion and Perspective

Viruses of the gammaherpesvirus genera *Lymphocryptovirus* and *Rhadinovirus* can be found in New World and Old World primates, including humans. Although several members of both genera are closely associated with viral oncogenesis, the simian viruses do not generally provide a straightforward model for multifaceted human diseases. The direct transforming action of viral oncogenes, as well as a chronic inflammatory reaction that may be affected by KSHV-encoded or KSHV-induced cytokines or angiogenic factors, may contribute to the genesis of KS. The KSHV-related simian rhadinoviruses do not provide a corresponding animal model as yet. Historically, interest in the rhadinoviruses has focused on the long-established prototype, HVS. Although a comparable virus-associated, acute, peripheral, pleomorphic T-cell lymphoma is not known yet in humans, this disease, which is induced reproducibly by HVS within weeks, can serve as an experimental model for general tumor development. The ability of certain HVS strains to transform human T lymphocytes to stable proliferation in culture provides a valuable tool for laboratory studies of T-cell immunology, including inherited and acquired immunodeficiency. In addition to their use as an immunological and biochemical T-cell model, HVS-transformed T cells can provide a source for the purification of specifically overexpressed cytokines or chemokines from culture supernatants. A detailed analysis of differential gene expression will lead to identification of signaling pathways that lead to lymphocyte transformation by herpesviral oncoproteins. Those involving STAT, nuclear factor of activated T cells (NFAT), and NFκB may be particularly important, perhaps providing hints to the roles of the respective pathways in human (nonviral) T-cell malignancy. Furthermore, rhadinovirus-transformed T-cell lines can be valuable tools in screening for specific drugs that target these pathways.

The side effects of retroviral integration have shown the requirement for efficient nonintegrating vectors. Recombinant rhadinoviruses can deliver foreign genes into primary human mesenchymal cells and T lymphocytes; this may prepare the ground for future therapeutic applications of persisting rhadinoviral vectors in adoptive immunotherapy. Safety considerations will prevent the use of unconditionally transforming rhadinovirus vectors, and require the development of novel rhadinovirus-based T-lymphotropic episomes, including conditional/attenuated or amplicon vector systems. Analysis of viral genome episomal modification in host T cells and the detection of genomic insulating regions can provide markers for the selection of regions suitable for the insertion of transgenes into the viral backbone.

Acknowledgments

Original work included in this review article was supported by the Deutsche Forschungsgemeinschaft (SFB643 TP A2, EN423/2-1), the Wilhelm Sander Stiftung, Bavarian International Graduate School of Science (BIGS), and the Interdisciplinary Center for Clinical Research (IZKF) at the University of Erlangen-Nuremberg.

See also: Epstein–Barr Virus: General Features; Epstein–Barr Virus: Molecular Biology; Herpesviruses: Discovery; Herpesviruses: General Features; Immune Response to viruses: Antibody-Mediated Immunity; Kaposi's Sarcoma-Associated Herpesvirus: General Features; Kaposi's Sarcoma-Associated Herpesvirus: Molecular Biology; Murine Gammaherpesvirus 68; Simian Alphaherpesviruses.

Further Reading

Alberter B and Ensser A (2007) Histone modification pattern of the T cellular *Herpesvirus saimiri* genome in latency. *Journal of Virology* 81: 2524–2530.

Bruce AG, Bakke AM, Bielefeldt-Ohmann H, *et al.* (2006) High levels of retroperitoneal fibromatosis (RF)-associated herpesvirus in RF lesions in macaques are associated with ORF73 LANA expression in spindleoid tumour cells. *Journal of General Virology* 87: 3529–3538.

Cai X, Schäfer A, Lu S, *et al.* (2006) Epstein–Barr virus microRNAs are evolutionarily conserved and differentially expressed. *PLoS Pathogens* 2: e23.

Cho NH, Feng P, Lee SH, *et al.* (2004) Inhibition of T-cell receptor signal transduction by tyrosine kinase-interacting protein of herpesvirus saimiri. *Journal of Experimental Medicine* 200: 681–687.

Cullen BR (2006) Viruses and microRNAs. *Nature Genetics* 38: S25–S30.

Damania B (2004) Oncogenic gamma-herpesviruses: Comparison of viral proteins involved in tumorigenesis. *Nature Reviews Microbiology* 2: 656–668.

Ensser A (2006) Transformation by herpesviruses: Focus on T-cells. *Future Virology* 1: 109–121.

Ensser A and Fleckenstein B (2004) Herpesvirus saimiri transformation of human T lymphocytes. In: Coligan JE, Bierer BE, Margulies DH, *et al.* (eds.) *Current Protocols in Immunology*, pp. 7.21.1–7.21.10. New York: Wiley.

Ensser A and Fleckenstein B (2005) T-cell transformation and oncogenesis by γ2-herpesviruses. *Advances in Cancer Research* 93: 91–128.

Ensser A, Thurau M, Wittmann S, *et al.* (2003) The genome of herpesvirus saimiri C488 which is capable of transforming human T cells. *Virology* 314: 471–487.

Heck E, Friedrich U, Gack MU, *et al.* (2006) Growth transformation of human T-cells by herpesvirus saimiri requires multiple Tip–Lck interaction motifs. *Journal of Virology* 80: 9934–9942.

Li HW and Ding SW (2005) Antiviral silencing in animals. *FEBS Letters* 579: 5965–5973.

Pfeffer S, Zavolan M, Grasser FA, *et al.* (2004) Identification of virus-encoded microRNAs. *Science* 304: 734–736.

Searles RP, Bergquam EP, Axthelm MK, *et al.* (1999) Sequence and genomic analysis of a rhesus macaque rhadinovirus with similarity to

Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8. *Journal of Virology* 73: 3040–3053.
Schäfer A, Lengenfelder D, Grillhösl C, *et al.* (2003) The latency-associated nuclear antigen homolog of herpesvirus saimiri inhibits lytic virus replication. *Journal of Virology* 77: 5911–5925.

Relevant Websites

http://en.wikipedia.org – Cebidae; Herpesviridae; Rhadinovirus.
http://www.ncbi.nlm.nih.gov – International Committee on Taxonomy of Viruses, ICTVdb.

Simian Immunodeficiency Virus: Animal Models of Disease

C J Miller and M Marthas, University of California, Davis, Davis, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Dysplasia Abnormal growth of tissues, organs, or cells.
Endemic Prevalent in a particular group of animals or people.
Epitope The part of a macromolecule that is recognized by the immune system.
Hypergammaglobulinemia Increased blood levels of gamma globulin (IgG).
Hyperplasia An abnormal increase in the number of cells in an organ or a tissue with consequent enlargement.
MHC-I (major histocompatibility complex I) T-cell epitopes are presented on the surface of an antigen-presenting cell, where they are bound to MHC molecules. T-cell epitopes presented by MHC class I molecules are typically peptides between 8 and 11 amino acids in length, while MHC class II molecules present longer peptides, and nonclassical MHC molecules.

Taxonomy and Discovery

Simian immunodeficiency viruses (SIVs) are members of the genus *Lentivirus* and the family *Retroviridae*. The genus *Lentivirus* includes viruses that infect ungulates (maedi-visna virus of sheep; caprine arthritis-encephalitis virus, CAEV), horses (equine infectious anemia virus, EIAV), cows (bovine immunodeficiency virus, BIV), wild and domesticated cats (feline immunodeficiency virus, FIV), nonhuman primates (NHPs; SIV), and humans (human immunodeficiency virus, HIV). All retroviruses are spherical, 80–100 nm in diameter, and have a diploid, single-stranded RNA genome and viral enzymes inside a viral protein case, or core, which is enveloped by a host cell membrane studded with viral glycoproteins. The diploid genome of single-stranded RNA is linked noncovalently near the 5′ end of the molecules. The 5′ end of the viral RNA is capped and the 3′ end is polyadenylated. DNA synthesis by the viral encoded reverse transcriptase (RT) is primed by host tRNA that is base-paired to the viral RNA. The double-stranded DNA provirus is integrated into the host chromosomes by a viral encoded integrase and the remaining events in transcription, translation, and assembly are host cell dependent. Depending on the cell type infected, particles assemble and bud through the plasma membrane or into membrane-lined intracytoplasmic vesicles. The morphological feature that distinguishes lentiviruses from other retroviruses is the cone or rod shape of the viral core protein in a mature virion.

SIV_{mac} was the first member of the group to be identified following isolation of a retrovirus from a captive rhesus macaque housed at a US primate center. Apparently, the virus was introduced into captive macaque populations in the US during experiments into the transmission of kuru and leprosy, at which time material derived from tissue of SIV-infected African monkeys was deliberately introduced into Asian macaques. SIV has been eliminated from captive populations of macaques in the US through a rigorous testing program. Fortunately, serology provides an effective and economical tool for screening for infection. Extended quarantine periods and regular serologic testing programs are rigorously applied to maintain the SIV-free status of captive macaque colonies.

Nomenclature and Classification of Primate Lentiviruses

Primate lentiviruses are classified as originating in NHPs (SIVs) or humans (HIVs). SIVs are given specific names based on the NHP species from which they are isolated, for example, SIV_{cpz} was isolated from chimpanzees and SIV_{deb} from De Brazza's monkeys.

Recently, a hierarchical nomenclature has been developed to describe the high level of genetic diversity found among the many HIV isolates. HIV is first divided into two

broad types, designated HIV-1 and HIV-2. Both HIV types are subdivided into groups consisting of phylogenetically similar viruses that resulted from different nonhuman-primate–human SIV transmission events (**Figures 1** and **2**). Within a group, phylogenetic clusters of viruses are termed subtypes. There is a movement toward a unified, standard nomenclature to describe genetic diversity within and among SIVs in which a three-letter abbreviation of the vernacular name of the NHP species is used (**Figure 3**). For example, the SIV from chimpanzees is termed SIV_{cpz} and subspecies designation is provided a three-letter abbreviation – thus $SIV_{cpz.Ptt}$ is derived from *Pan troglodytes troglodytes*. SIVs of African green monkeys (SIV_{agm}) are divided into four subtypes, each named for the African green monkey subspecies from which it was isolated (**Figure 3**).

Genome Organization

Like all retroviruses, lentivirus genomes contain long terminal repeats (LTRs) at each end and genes encoding three virion structural components: core (*gag*), polymerase (*pol*), and envelope (*env*). In addition, all primate lentivirus genomes also contain five accessory genes: *vif*, *vpr*, *rev*, *tat*, and *nef*. The genomes of a subset of primate lentiviruses

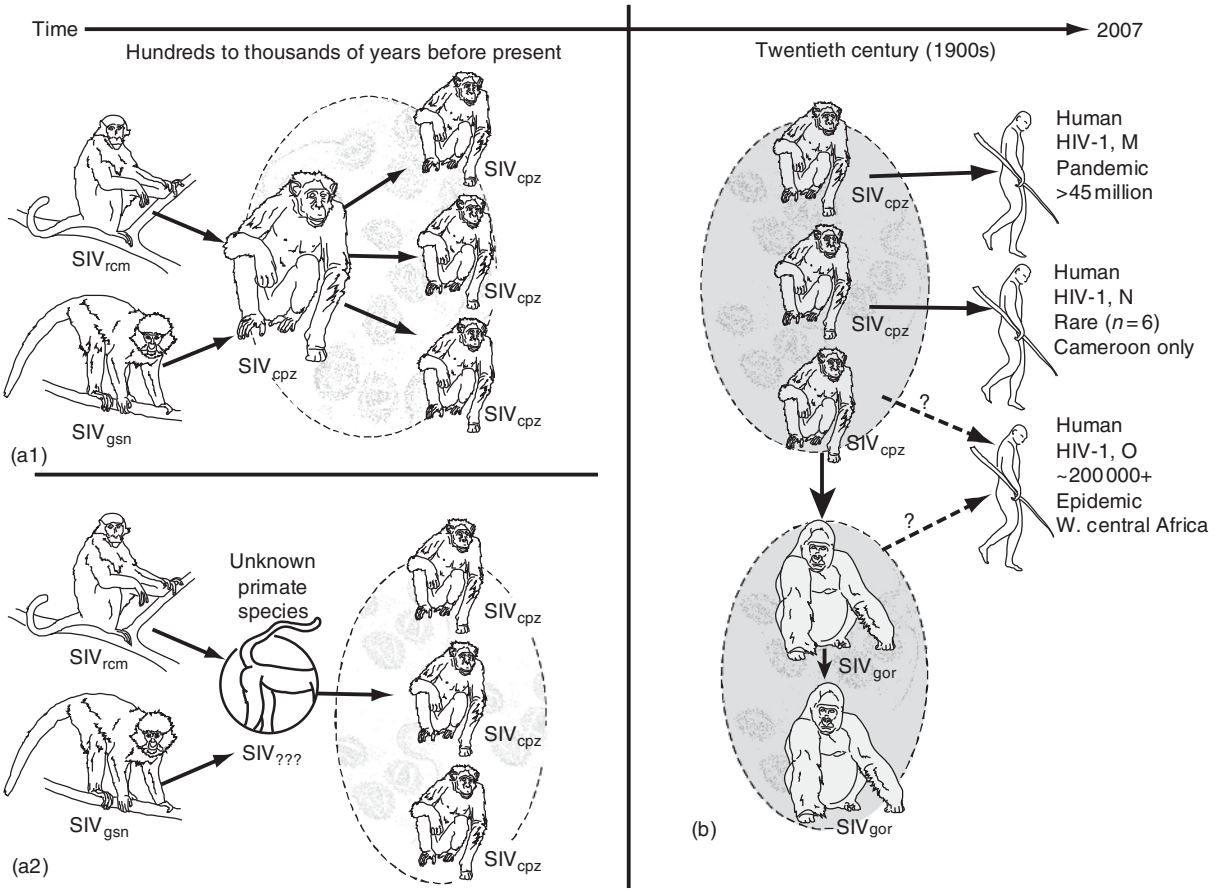


Figure 1 Origin of HIV-1 inferred from phylogenetic analyses of SIV and HIV-1 genomes. (a) The earliest event is transmission of SIVs from African monkeys (red-capped mangabey (RCM) infected with SIV (SIV_{rcm}) and greater spot nose monkey (GSN) infected with SIV (SIV_{gsn})) to chimpanzees followed by virus recombination that resulted in SIV_{cpz}. Two scenarios are consistent with the data: (a1) SIV_{rcm} and SIV_{gsn} initially infected a chimpanzee; or (a2) SIV_{rcm} and SIV_{gsn} first infected an unknown primate species where virus recombination occurred – in either case, a recombinant SIV was transmitted among chimpanzees producing SIV_{cpz}. (b) Three separate transmissions of SIVs to humans occurred from distinct populations of chimpanzee (SIV_{cpz}) or gorilla (SIV_{gor}) in the 1900s producing three HIV-1 groups, M (main), N, and O (outlier). Recent, but limited, phylogenetic data show that HIV-1 group O is most closely related to SIV_{gor}. The most likely scenario suggested by the data is that chimpanzees transmitted SIV_{cpz} to gorillas which resulted in SIV_{gor}; it is unknown whether HIV-1 group O was transmitted to humans from gorillas or from an as yet unidentified chimpanzee reservoir infected with SIV_{cpz} more closely related to HIV-1 O. Only HIV-1 groups M and O have established efficient human-to-human transmission. Group M HIV-1 is pandemic, infecting over 45 million persons; group O HIV-1 is epidemic having infected an estimated several thousands of persons in Africa. In contrast, only six individuals from Cameroon are known to be infected with HIV-1 group N. Dotted circles indicate distinct populations of a primate species; shading indicates SIV isolated from individuals of this species; solid arrows show inferred transmissions of SIV; dashed arrows indicate where SIV transmission is hypothesized, but there are insufficient data to confirm.

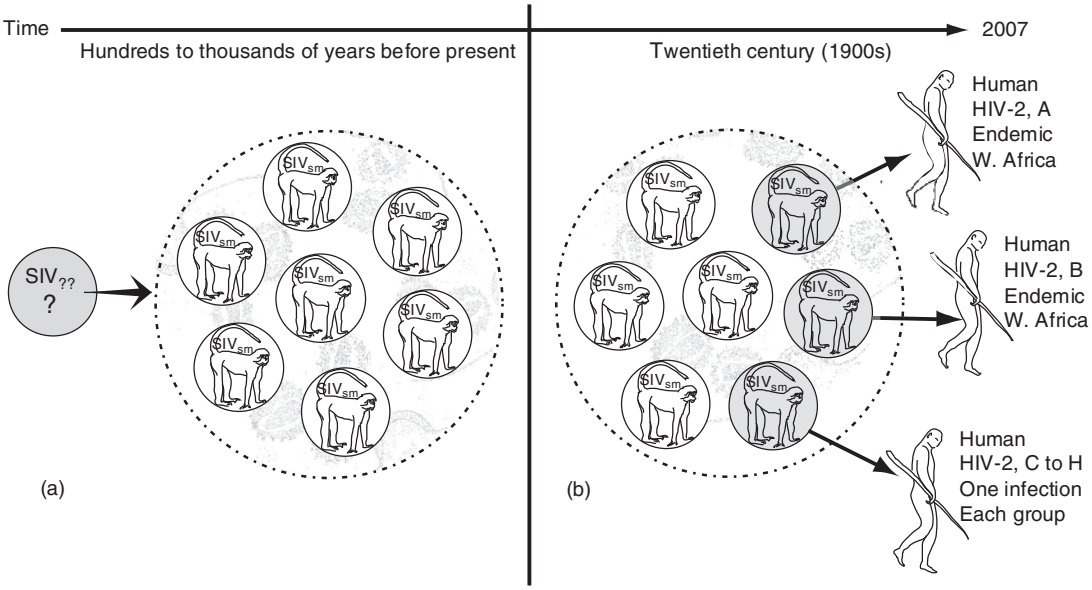


Figure 2 Origin of HIV-2 inferred from phylogenetic analyses of SIV and HIV-2 genomes. (a) SIV probably infected sooty mangabeys (SMs) thousands of years ago. Multiple separate transmissions of SIV_{sm} from distinct populations of SMs to humans occurred during the 1900s giving rise to eight HIV-2 groups, A–H (b). Only HIV-2 groups A and B have resulted in efficient human transmission and established epidemics; a single person is known to be infected with each of the HIV-2 groups C–H. Dotted circles indicate distinct populations of SMs; shading indicates SIV_{sm} isolated from individual SM in a population; arrows show inferred transmissions of SIVs.

have one of two unique genes: *vpr* or *vpu*. Three types of genomic structure are observed for primate lentiviruses (**Figure 4**). Group A includes SIVs only from African NHPs; these SIVs cause no disease in the host species from which the SIV was isolated: SIV_{agm}, SIV_{syk}, SIV_{lhoest}, $SIV_{mnd\text{-}1}$, SIV_{sun}, and SIV_{col} (A, **Figure 4**). SIVs in group A all have the basic primate lentivirus genome structure, which contains only five accessory genes. Group B includes HIV-2 and SIVs most closely related to HIV-2 (SIV_{sm}, SIV_{mac}, SIV_{rcm}, and $SIV_{mnd\text{-}2}$); each group B virus has a *vpx* gene which is absent in the Group A viruses (B, **Figure 4**). Group C viruses include HIV-1, SIV_{cpz}, and other SIVs with a *vpu* gene, but lacking *vpx* (SIV_{gsn}, SIV_{mon}, and SIV_{mus}; C, **Figure 4**).

As retroviruses, all lentiviruses also have two complete RNA genomes in a single virion. Therefore, when a host cell is infected with two or more genetically different primate lentiviruses, two different genomic viral RNAs can be packaged into the same virion, generating recombinant viral genomes in the next viral replication cycle. *vpx* shares sequence similarity with *vpr* and is thought to have originated by recombination among SIV genomes. Thus, viral recombination has been a dominant force in the evolution of primate lentiviruses as suggested by the diagrams of primate lentivirus genomes in **Figure 4**. As SIVs are isolated from more primate species and their full genome sequences compared, it was found that some primates harbored SIV with apparently 'mosaic' genomes which included portions of structural genes derived from SIVs of different primate species. Thus, recombination among SIVs has resulted in novel viruses; for example, the current SIV_{cpz} evolved from a recombinant between two SIVs – SIV_{rcm} from red-capped mangabeys and SIV_{gsn} from greater spot nose monkeys (**Figure 1**).

Evolution

All primate lentiviruses are more closely related to each other than to lentiviruses from nonprimates. This genetic similarity suggests primate lentiviruses co-evolved with their host species and that primates have not been infected by lentiviruses from other mammals, such as ungulates or felines. SIVs have been isolated from many wild African NHP species, but no SIVs have been found to infect any wild Asian or New World NHPs. This suggests that lentiviruses became established in primates sometime after the divergence of Old and New World primate species (~35–40 million years ago). Although they lack an endemic SIV, Asian macaques are susceptible to experimental infection with a variety of SIV isolates and develop acquired immune deficiency syndrome (AIDS), similar to HIV-infected humans. The estimated origin of the macaque genus is ~6 million years ago and emigration of macaques from Africa to Eurasia began ~5 million years ago. Thus, it is probable that lentiviruses were not widespread among African primates 5 million years ago.

Lentiviral replication generates high genetic diversity in two ways: mutation caused by the highly error-prone retroviral polymerase (RT) and recombination between

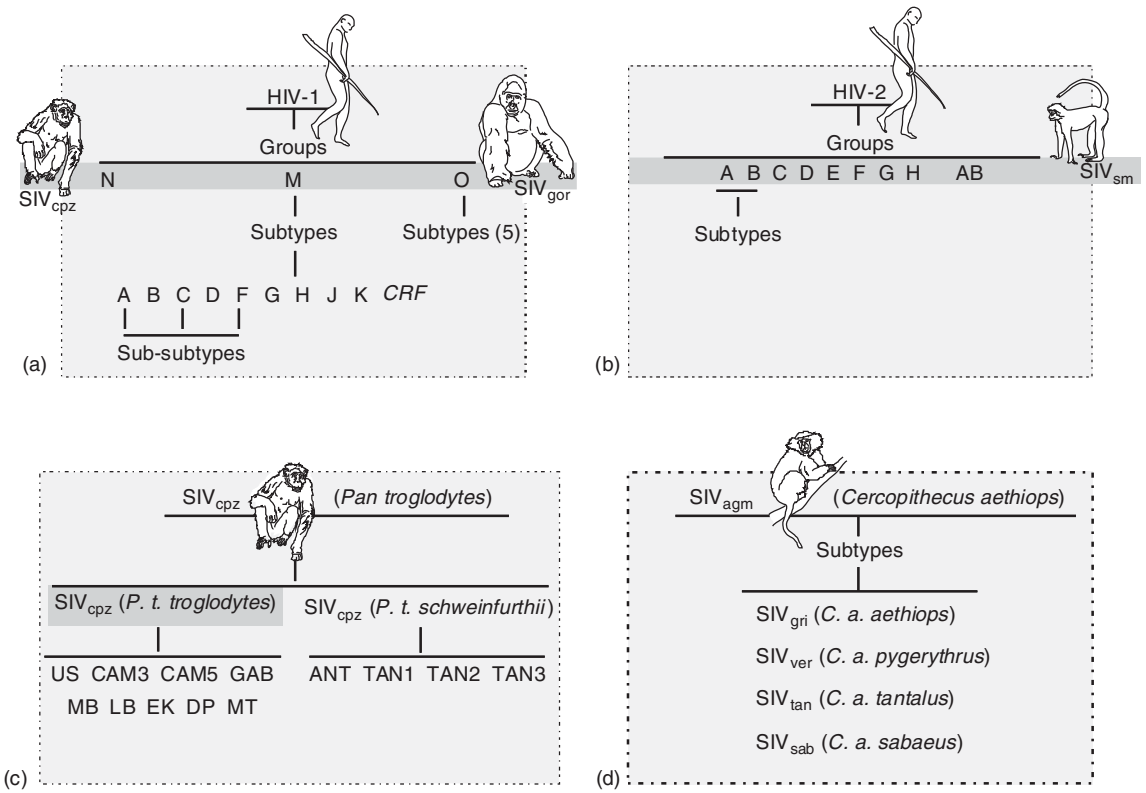


Figure 3 Classification and phylogenetic relationships of primate lentiviruses. (a) HIV-1 lineage. HIV-1 is divided into three groups: M (main), N (non-M/non-O or 'new'), and O (outlier); based on phylogenetic clustering with SIVs, each group represents a separate chimpanzee- or gorilla-to-human transmission event (see also **Figure 1**). Subtype diversification occurred in humans after each cross-species transmission; CRF indicates inter-subtype recombinants. Clusters of diversity within a subtype are called sub-subtypes. The shaded bar indicates the SIVs which gave rise to the HIV-1 groups. (b) HIV-2 lineage. HIV-2 is divided into eight groups (A–H); based on phylogenetic clustering with SIVs, each group represents a separate sooty mangabey-to-human transmission event (see also **Figure 2**); AB indicates recombinant virus. The shaded bar indicates SIV_{sm} giving rise to the HIV-2 groups. (c) SIV_{cpz} lineage. SIV_{cpz} is designated as one subtype although SIV_{cpz}'s have been isolated from two subspecies of chimpanzees, *Pan troglodytes troglodytes* and *Pan troglodytes schweinfurthii*; multiple SIV_{cpz}'s from each of these two subspecies have been isolated and sequenced (abbreviations are listed). SIV_{cpz} from *P. t. troglodytes* (shaded bar) is most closely related to HIV-1. (d) SIV_{agm} lineage. There are four subtypes of SIV_{agm}, one for each of the four subspecies of African green monkey, *Cercopithecus aethiops*: *C. a. aethiops* (grivet), *C. a. pygerythrus* (vervet), *C. a. tantalus* (tantalus), and *C. a. sabaeus* (sabaeus).

two different viral genomic RNA molecules packaged in a virion. The majority of African NHP species have genetically distinct SIVs. The isolation of more than one distinct SIV from a primate species provides evidence for regular interspecies SIV transmission (**Figures 1** and **3**). A notable example is isolation of two SIVs from mandrills (SIV_{mnd-1} and SIV_{mnd-2}; **Figure 4**) that are as genetically different from each other as are HIV-1 and HIV-2.

Thus, genetic diversity of primate lentiviruses has evolved in two ways: (1) within a single host species, and (2) by transmission of SIV from one primate host species to another (cross-species transmission). Cross-species SIV transmission has occurred in both wild and captive primates. The most likely modes of SIV transmission between wild primate species are thought to be fighting among different primates or hunting and eating of one primate species by another; for example, wild chimpanzees are known to kill and eat a variety of monkeys that share the same geographic range. When a cross-species SIV transmission event occurs in an individual already SIV infected, there is the opportunity for viral recombination and, thus, the generation of SIV variants with novel phenotypes, including increased pathogenicity. Such a dual SIV infection is proposed to have been the origin of SIV_{cpz} (**Figure 1**). Dual (or multiple) SIV infection of individuals can also occur when a primate species has genetically distinct SIV variants in one or different populations of animals. Thus, intraspecific SIV recombination increases the genetic diversity within an SIV lineage (**Figure 3**).

Virology

The life cycle of SIV is similar to all retroviruses; once uncoated from the capsid, the RNA genome is reverse-transcribed into a full-length DNA genome that

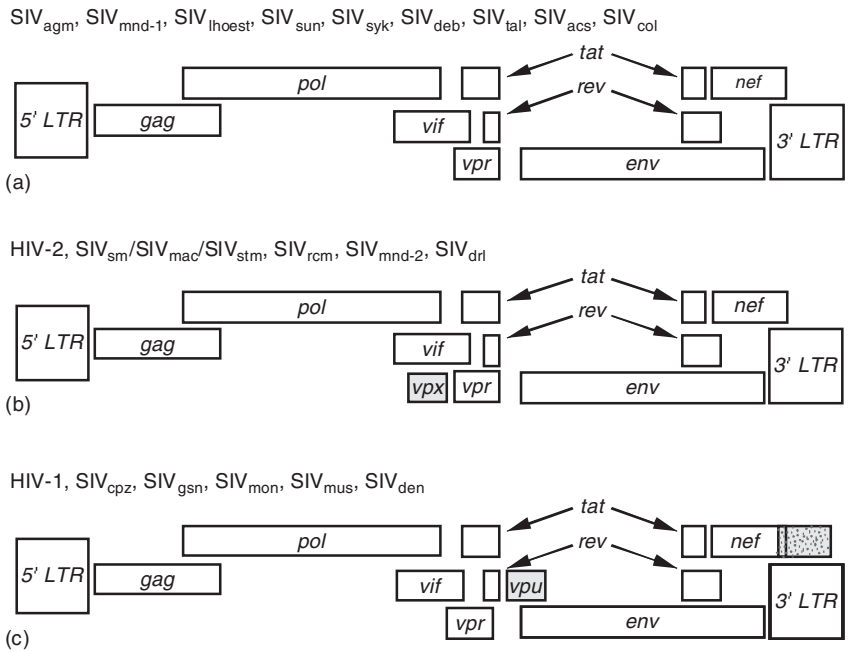


Figure 4 Genomic structure of primate lentiviruses. Viral genes are represented by rectangles with names inside and arrows show genes with two separate coding regions. Group A includes SIVs only from African NHPs (agm, African green monkey; mnd, mandrill; lhoest, l'Hoest monkey; sun, sun-tailed monkey; syk, Sykes monkey; deb, de Brazza's monkey; tal, talapoin; acs, *ascanius*; col, colobus). Group B includes HIV-2 and SIVs most closely related to HIV-2; each group B virus has a *vpx* gene (shaded box), that is absent in the Group A viruses (sm, sooty mangabey; mac, macaque; stm, stump-tailed macaque; rcm, red-capped mangabey; mnd, mandrill; drl, drill). Group C viruses include HIV-1, SIV_{cpz}, and other SIVs with a *vpu* gene, but lacking *vpx* (cpz, chimpanzee; gsn, greater spot nose monkey; mon, mona monkey; mus, mustached guenon; den, Dent's mona monkey). For HIV-1 and SIV_{cpz} genomes only, the *nef* gene (stippled) does not overlap the *env* gene.

is transported to the nucleus and integrated into host chromosomes. Once integrated, the provirus produces a variety of RNA species that can be spliced to produce all the proteins required for virion assembly and egress. In T cells the immature virions bud from the plasma membrane into the extracellular space, but in macrophages virions often bud into intracellular vesicles that can eventually fuse with the plasma membrane of the cell releasing the progeny virions.

SIV can be propagated in many human T-cell and macrophage cell lines derived from tumors, mitogen-stimulated peripheral blood mononuclear cells (PBMCs), monocyte-derived macrophages, etc., although the range of permissive cell types varies from isolate to isolate. In PBMC cultures and many cell lines, viral infection results in obvious cytopathic effects including ballooning degeneration and syncytia formation due to fusion of cell membranes. Syncytium formation is mediated by the envelope glycoprotein and allows direct cell-to-cell spread of the infection.

The CD4 molecule is the primary cellular receptor for SIV. As with HIV, infection is primarily mediated by CD4 but interaction with CD4 alone is not sufficient to allow viral entry into the cell. A number of chemokine receptors are required co-receptors for SIV and HIV infection. The SIV envelope glycoprotein (gp120) binds to CD4 resulting in a conformational shift in the glycoprotein that exposes a co-receptor binding site. This secondary binding site interacts with a chemokine receptor on the cell surface, and fusion of cell and virion membranes results. Although various SIV strains can use a variety of chemokine receptors (CCR5, BOB, Bonzo) as a co-receptor *in vitro*, CCR5 is the most important co-receptor for SIV *in vivo* as it widely expressed by target cells (T cells, macrophages, dendritic cells (DCs)) in the body. Chimeric viruses have been constructed using SIV as a backbone for inserting HIV genes. In HIV infection, differential co-receptor usage by HIV variants has been implicated as a determinant in virulence and pathogenesis. Some of the SIV/SHIV (SHIV) viruses have been constructed using the *env* from HIV variants known to preferentially use CxCR4 or CCR5 as co-receptors. Although differential co-receptor usage can be demonstrated by the chimeric viruses *in vitro*, the extent to which altered replication capacity independent of co-receptor usage contributes to observed differences in pathogenesis has been difficult to determine.

Host cellular factors have recently been identified that confer resistance or susceptibility to productive SIV infection. In host cells resistant to another species' lentivirus, the host protein APOBEC3G is incorporated into the virion during particle formation. During reverse transcription, virion-incorporated APOBEC3G deaminates

the minus strand of viral DNA and inactivates/degrades the viral genome. In permissive cells, the *vif* gene permits SIV to replicate by targeting APOBEC3G for degradation in the proteosome and preventing its incorporation into the virion. However, the activity of *vif* is species specific. Thus human APOBEC3G is inhibited by HIV-1 Vif but not by SIV_{agm} Vif, and AGM APOBEC3G is inhibited by SIV_{agm} Vif, but not by HIV-1 Vif. TRIM5alpha, a member of the poorly understood tripartite motif (TRIM) family of proteins, is another host-restriction factor. TRIM5alpha restricts HIV replication in rhesus macaques, by interacting with the virion capsid and blocking the uncoating of HIV-1 after entry but before reverse transcription.

Transmission and Dissemination

When experimentally inoculated onto intact mucosal surfaces, SIV rapidly infects local DCs, macrophages, and T cells. Although the number of SIV-infected cells is generally higher in the lamina propria adjacent to regions of epithelial damage, SIV-infected DCs and T cells can be found within the intact epithelium of the genital tract, indicating that the virus can cross the intact genital mucosa. Within 24 h of infection, SIV-infected cells can be detected in the lymph nodes that drain a mucosal inoculation site. Initially, there is limited viral replication in local tissues prior to widely disseminated infection in peripheral lymphoid tissues, but by 1 week, post inoculation (PI), viral replication is explosive with dramatic increases in viral RNA levels in all lymphoid tissues. At 10–14 days PI, the peak of viral replication and plasma vRNA levels occurs, and adaptive immune responses develop. By day 28–56 PI, plasma vRNA levels decline to moderate levels, where they remain for a variable period of time. Eventually viral replication increases, CD4+ T-cell levels decline further and AIDS develops. Examples of plasma viral levels in rhesus macaques inoculated with two pathogenic SIV variants are provided in **Figure 5**.

Disease Pathogenesis

While the endemic SIV infections of African monkeys and apes seem to be minimally pathogenic, SIV infections of Asian macaques cause simian AIDS. This disease results from the profound destruction of CD4+ T cells in lymphoid organs throughout the body. To an even greater degree than HIV infection of people, SIV replicates to very high levels in the infected host with as many as 10^9 vRNA copies per ml of plasma during peak infection. This high replicative capacity coupled with the error-prone viral RT produces viral quasi-species of tremendous genetic diversity in infected individuals. This viral quasi-species makes the virus very adaptable to changing conditions in the host as antiviral immune responses develop or successive waves of target cells are destroyed. As in HIV infection, the level of SIV RNA in plasma is an excellent indicator of clinical prognosis in Asian macaques.

The central feature of AIDS is the destruction of CD4+ T cells and the crippling effect this has on the host's ability to control opportunistic, or latent, infections. The majority of the CD4+ T cells in the body reside in the gastrointestinal (GI) tract and other mucosal sites, and these cells are activated memory and $CCR5^+$ T cells. Thus the GI tract is a major site for the destruction of CD4+ T cells. As viral replication occurs, the level of immune activation and lymphocyte proliferation dramatically

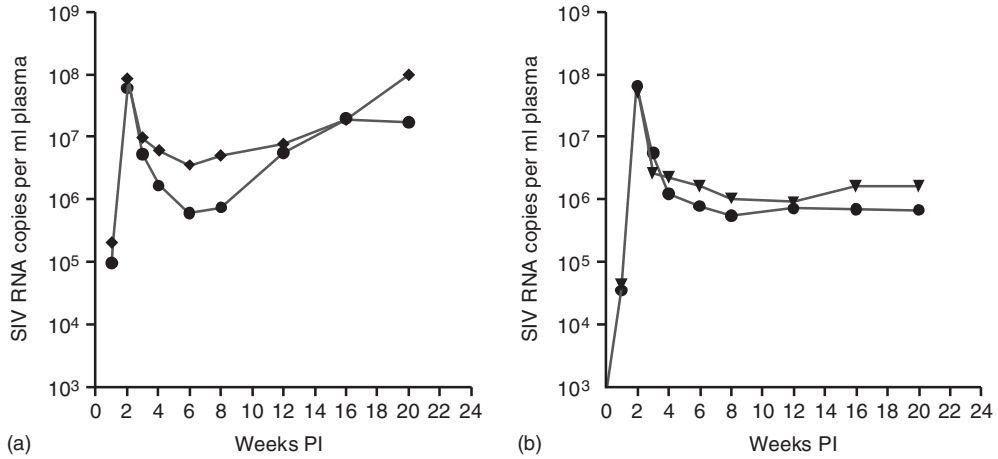


Figure 5 Plasma SIV RNA levels in rhesus macaques following intravenous inoculation of pathogenic SIV. (a) Plasma vRNA levels in two macaques inoculated with SIVmac251. (b) Plasma vRNA levels in 2 macaques inoculated with SIVmac239. Note in all four animals there was a peak in plasma vRNA at 2 weeks PI followed by a variable period of declining or stable plasma vRNA. While setpoint vRNA levels remained stable in both SIVmac239 inoculated animals over the period of observation, plasma vRNA had begun increasing by 24 weeks PI in both SIVmac251-infected animals, suggesting that these animals would soon develop clinical AIDS.

increases as the host attempts to mount an immune response. Many of the transcriptional signals involved in the host's innate and adaptive immune responses are controlled by nuclear factor-kappa B (NF-κB) transcriptional factors. NF-κB is also a key promoter for SIV replication as the viral LTR contains binding sites for NF-κB transcription factors. Thus the host immune response to SIV generates activated T cells with high levels of critical molecules (dNTPs, transcriptional factors) and, paradoxically, these are the cellular targets needed for optimal viral replication.

In addition to the effects of CD4+ T-cell depletion on the host defenses, SIV can directly produce disease. Thus SIV crosses the blood–brain barrier, infecting resident and transient cells of the macrophage lineage, which can result in viral encephalitis. In addition, the high levels of viral antigen and strong antibody responses can produce immune complex glomerulonephritis, while SIV infection of bone marrow macrophages is presumed to be the cause of the anemia and other hematologic abnormalities that SIV infection produces. The above features are also common in HIV infection, but SIV infection of macrophages can produce histiocytic inflammation with syncytial cell formation in the lung, lymphoid tissues, and GI tract.

The above discussion describes the pathogenesis of AIDS in SIV-infected Asian macaques, but in the African primates that are natural hosts of SIV there is little clinical effect of infection. A key difference lies in the response of natural hosts and Asian macaques to SIV infection. Thus SIV-infected African primates mount a complete adaptive immune response but there is little inflammation and no chronic immune activation associated with these immune responses despite continuous high-level viral replication. In marked contrast, SIV infection of macaques, as with HIV infection of humans, produces chronic immune activation and inflammation that is associated with depletion of central memory T-cell pools and loss of effector memory T cells. Thus despite levels of viral replication that are similar to Asian macaques, SIV infection rarely produces AIDS in natural hosts indicating that inflammation and immune activation are necessary for AIDS pathogenesis.

Specific strains of SIV have altered pathogenesis with a number of molecular clones and biologic isolates being attenuated for replication in Asian macaques. Although these variants do not produce disease in the typical time course of viruses with higher replicative capacity, some eventually produce disease in adults or infant macaques, while other attenuated SIV variants (SIVmac1A11) have failed to produce disease in infected macaques after more than 15 years of observation. In addition, some strains of SIV have specific tropism for monocyte/macrophages that are mediated by post-entry events. Infections with these macrophage-tropic SIV strains are more commonly associated with SIV-induced meningoencephalitis than other SIV strains.

Immune Response to SIV Infection

As SIV infection is lifelong, the host immune response cannot clear the infection but, in some cases, it can exert considerable control on the level to which SIV replicates. Before day 5 after mucosal SIV inoculation, there is little evidence of innate immune responses with only modest increases in type 1 interferon levels and interferon-stimulated gene levels at the site of inoculation. At days 6–7 PI, there is a dramatic and simultaneous increase in innate antiviral immune responses in all lymphoid tissues that coincides with the dramatic explosion of viral replication in these tissues. By days 10–14 PI, SIV-specific CD8+ T-cell responses are present in blood and in the mucosal sites of inoculation, with strong antiviral T-cell responses widespread by day 28 PI. This rapid increase in antiviral effector T cells occurs as viral replication and plasma vRNA levels decline from a peak at 14 days PI. The temporal relationship between the decline in plasma viremia and the appearance of antiviral T cells has been interpreted to be evidence that CD8+ T cells are critical in the control of HIV replication. Further evidence for the control of SIV replication by CD8+ lymphocytes has been obtained by using monoclonal antibodies to the α-chain of the CD8 complex to transiently deplete T cells and natural killer (NK) cells. In chronically infected animals, plasma vRNA levels dramatically increase during the period of CD8+ lymphocyte depletion and then rapidly fall as the lymphocyte population is replenished. Finally, as in HIV infection, detailed studies of well-characterized MHC-I-restricted T-cell epitopes in infected macaques have shown that as the host T-cell response targets a specific peptide sequence, viral variants with proteins that contain a variant of the targeted epitope become increasingly common. The effect of these mutated epitopes on viral replication is variable. In some cases, these variants are relatively unfit and the virus gains little advantage; however, in other cases, these immune escape variants are relatively fit and viral replication increases dramatically as the escape variants appear in the viral population. Finally, as in humans, specific MHC-I alleles of macaques (i.e., Mamu-A*01) are associated with particularly strong CD8+ T-cell responses, enhanced immune control of viral replication, and increased disease-free survival times after infection. Taken together, these observations argue that CD8+ T-cell responses can play a significant role in controlling viral replication. B-cell responses also have a role in controlling HIV and SIV replication. Thus it has been shown that passive transfer of high-titer SIV-specific gammaglobulin or neutralizing anti-HIV antibodies inhibits SIV and SHIV replication and retards the pace of disease progression. Further, if macaques are prevented from developing anti-SIV IgG antibodies by B-cell depletion prior to infection with highly pathogenic SIV, they rapidly develop uncontrolled viral replication, and

progress to AIDS in a few months PI, while most SIV-infected macaques make strong antibody responses and develop AIDS at 10–24 months PI.

Clinical Features, Pathology, and Histopathology of SIV Infection in Asian Macaques

End-stage disease in SIV infection is indistinguishable from human AIDS and can be divided into four broad categories: (1) opportunistic infections, (2) SIV-mediated inflammatory diseases, (3) neoplastic diseases, and (4) diseases of unknown etiology. The clinical course of SIV varies with the strain involved but rhesus macaques infected with common pathogenic SIV strains develop AIDS within 6–24 months PI. In the first few weeks of infection, all animals initially develop lymphocytosis consisting largely of CD8+ T cells, which resolves in 4–6 weeks. Lymphadenopathy and splenomegaly are apparent by 2–4 weeks PI and these conditions remain manifest until a very late stage of the disease when lymphoid collapse can occur. As in HIV infection, hypergammaglobulinemia due to polyclonal B-cell activation is often a feature of the disease. Anemia can also be a feature of the clinical disease. Weight loss and diarrhea are very common in SIV-infected animals and can be the result of opportunistic infections (*Mycobacterium avium* complex, cytomegalovirus, adenovirus, *Cryptosporidium* sp., *Ameoba* sp., *Balantidium coli*) or an unknown etiology. Often these enteric conditions do not respond to antibiotic therapy. Lymphomas are the only neoplastic condition of significance in SIV-infected macaques. In fact, a B-cell lymphoma arising in a 19-year-old sooty mangabey infected with SIV may represent the best candidate for the case of an SIV-related fatal disease in a naturally infected African primate.

Lymphoid tissues, including the mucosal-associated lymphoid tissues of the GI, reproductive, and respiratory tracts, are the targets of SIV infection, and a range of histopathologic changes occur in these tissues from follicular hyperplasia to dysplasia, followed by follicular collapse and expansion of the paracortex, ultimately ending in lymphoid depletion, collapse, and fibrosis. These changes occur independent of any opportunistic infections. The same pattern of histologic changes occurs in lymphoid tissues of HIV-1-infected humans and these histologic changes accurately reflect the clinical stage of the infection in both SIV and HIV.

SIV Infections as Animal Models of AIDS

The HIV-1 pandemic continues unabated and developing effective vaccines and therapies is the greatest current public health need. Samples from HIV-infected individuals have provided key insights into AIDS pathogenesis. However, direct experimental testing of specific hypothesis arising from these studies cannot be undertaken in humans for ethical reasons and HIV-1 infection of chimpanzees does not produce AIDS. Thus, SIV infection of Asian macaques is the most widely accepted animal model of HIV pathogenesis. The biology of the macaque immune system, and the key organ systems (gut, lymphoid tissue, and reproductive tract) involved in AIDS pathogenesis and HIV transmission are very similar to humans, and SIV and HIV are closely related phylogenetically. Thus, SIV infection of macaques closely mimics the pathogenesis, virology, immunology, and pathology of HIV infection in the human. The model has been used to show that infection with a molecular clone of SIV is sufficient to cause AIDS, that the GI tract is a major site of $CD4^+$ T-cell depletion, and that some vaccine strategies elicit immune responses that can provide considerable control of viral replication. The macaque monkey model of AIDS has been used to define the molecular determinants of viral pathogenesis, the basis for nonpathogenic infections in natural hosts. These animal models have been particularly valuable for defining the mechanisms of HIV transmission and events in acute infection that are very difficult to study in humans.

Sexual HIV transmission can occur through oral, anal, or vaginal intercourse. Allowing SIV-discordant macaques to mate normally would seem to be most similar to sexual HIV infection. However, sexual SIV transmission occurs at a low or variable rate after natural mating, and, in addition, there is significant biting behavior during mating and thus significant potential for blood-borne SIV transmission. When SIV-discordant juvenile rhesus macaques were housed in groups, SIV transmission most commonly occurred when uninfected, dominant animals bit their SIV-infected, subdominant cagemates as part of social interactions. Thus, once deposited in the mouth of an uninfected macaque SIV-infected blood-transmitted infection therefore biting during mating would confound transmission studies.

A more controlled experimental approach to reliably transmit SIV across the genital mucosa is to intravaginally inoculate female macaques with a known quantity of well-characterized SIV stock. Most studies have used suspensions of cell-free SIV virions, but infection can be transmitted by intravaginal inoculation with SIV-infected cells. In order to reliably transmit SIV to monkeys by a single intravaginal inoculation, relatively high doses of cell-free virus (2–3 log 10 more virus) are used compared to intravenous inoculation. In addition, exogenous progestins have been used to ensure reliable intravaginal SIV transmission, as these hormones thin up the genital mucosa, lower the barrier to transmission, and enhance SIV transmission. Finally, intravaginally inoculating

animals with relatively low doses of virus, approximately the same dose needed for IV transmission, repeatedly over the course for several months eventually produced infection in all exposed animals, although the number of exposures needed to acquire infection is variable; this strategy probably best models the level of virus exposure that occurs during HIV sexual transmission.

HIV transmission by sexual, intravenous, and perinatal routes is often associated with the acquisition of a limited distribution of genetic variant. In many instances, the transmitted variant represents a minor variant in the donor's virus population. Given the extent of genetic diversity between HIV isolates, these findings have been interpreted to suggest that HIV transmission may involve selective entry or selective amplification of specific viral variants. However, the inherent limitations of all studies using human samples include small sample sizes and uncertainty as to the genetic identity and the extent of genetic diversity of the virus population in the donor at the time of transmission. Thus, the mechanisms that underlie the sexual transmission of HIV variants are unclear, and are difficult to assess because of the difficulties in establishing the precise time of infection. The SIV macaque model for HIV transmission is particularly valuable for evaluating the role of viral selection during transmission. The model allows access to information that is usually unattainable in human studies, such as: the genotypic and phenotypic properties of the infecting virus; knowledge of the exact time of virus exposure; and the characteristics of viral variants in the infected host immediately after transmission. Both IV and IVAG SIV inoculation transmit genetically diverse populations of SIV env V1–V2 variants to macaques. However, compared to the complex SIV populations in the IV inoculated animals, most IVAG inoculated animals are infected with SIV populations that have relatively low genetic diversity in the env gene. The finding that genetically diverse SIV populations are transmitted to some IVAG inoculated monkeys is consistent with the observation that a more genetically diverse population of viral variants is sexually transmitted from HIV-infected men to women. The model has also been used to show that recombination can occur readily *in vivo* after mucosal SIV exposure and thus viral recombination contributes to the generation of viral genetic diversity and enhancement of viral fitness in the peracute stages of infection. The model has also been used to show that the mucosal barrier of the female genital tract greatly limits the infection of cervicovaginal tissues after intravaginal SIV inoculation, and thus the initial founder populations of infected cells are small. Despite limited foothold, SIV rapidly disseminates to distal sites, and continuous seeding from an infection in the genital tract is likely critical for the later establishment of a productive disseminated systemic infection.

Perinatal HIV transmission can occur at any time during gestation, delivery, or breast-feeding. Allowing female macaques infected with virulent SIV before or during pregnancy is most similar to perinatal HIV infection, because SIV/SHIV transmission can occur any time during gestation, delivery, or breast-feeding. However, mother–infant SIV transmission occurs at a low or variable rate and the timing of virus transmission is unknown. Alternatively, female macaques can be infected with SIV after delivery and the infant allowed to breast-feed normally. This model best mimics natural HIV breast milk transmission by eliminating fetal exposure to virus and transplacental transfer of maternal virus-specific antibodies to the infant. Thus, this approach controls more variables and transmission rates are high; however, a major limitation is that the time at which breast-feeding infants become SIV infected varies substantially (i.e., a few weeks to several months). Finally, direct oral inoculation of infant macaques with SIV or SHIV can be performed without infecting their dams. This approach controls most of the important variables related to the viral inoculum (dose, number, timing, and duration of virus exposure), maternal host immune response (level and quality of anti-HIV-specific maternal/passively transferred antibodies), and infant rearing (by uninfected dams or in a primate nursery). This system has been used to show that after oral inoculation of infant rhesus macaques with virulent SIVmac251, virus disseminates to distal lymphoid tissues faster than after oral inoculation of juvenile macaques or vaginal inoculation of adult macaques with the same virus.

In addition to helping define critical steps in AIDS pathogenesis, these SIV mucosal transmission models are ideal for testing vaccines and microbicide strategies designed to prevent HIV transmission. The demonstrated utility of the SIV model for vaccine testing contrasts sharply with the inability of models using macaques infected with CxCR4 SHIVs (SHIV 89.6P) to meaningfully segregate HIV-1 vaccine candidates by relative efficacy. Thus the SIVs will continue to be the most valuable model of HIV infection and a critical tool in AIDS research.

See also: Bovine and Feline Immunodeficiency Viruses; Human Immunodeficiency Viruses: Antiretroviral agents; Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Origin; Human Immunodeficiency Viruses: Pathogenesis; Simian Immunodeficiency Virus: General Features; Simian Immunodeficiency Virus: Natural Infection.

Further Reading

Butler IF, Pandrea I, Marx PA, and Apetrei C (2007) HIV genetic diversity: Biological and public health consequences. *Current HIV Research* 5: 23–45.

Gardner MB (2003) Simian AIDS: An historical perspective. *Journal of Medical Primatology* 32: 180–186.

Gordon S, Pandrea I, Dunham R, Apetrei C, and Silvestri G (2005) The call of the wild: What can be learned from studies of SIV infection of natural hosts? In: Leitner T, Foley B, Hahn B, *et al.* (eds.) *HIV Sequence Compendium 2005, LA-UR 06–0680*, pp 2–29. Los Alamos, NM: Los Alamos National Laboratory.

Greenier JL, Miller CJ, Lu D, *et al.* (2001) Route of simian immunodeficiency virus inoculation determines the complexity but not the identity of viral variant populations that infect rhesus macaques. *Journal of Virology* 75: 3753–3765.
Jayaraman P and Haigwood NL (2006) Animal models for perinatal transmission of HIV-1. *Frontiers in Biosciences* 11: 2828–2844.
Kestler H, Kodama T, Ringler D, *et al.* (1990) Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. *Science* 248: 1109–1112.
Kim EY, Busch M, Abel K, *et al.* (2005) Retroviral recombination *in vivo*: Viral replication patterns and genetic structure of simian immunodeficiency virus (SIV) populations in rhesus macaques after simultaneous or sequential intravaginal inoculation with SIVmac239Deltavpx/Deltavpr and SIVmac239Deltanef. *Journal of Virology* 79: 4886–4895.
Lifson JD and Martin MA (2002) One step forwards, one step back. *Nature* 415: 272–273.
Long EM, Martin HL Jr., Kreiss JK, *et al.* (2000) Gender differences in HIV-1 diversity at time of infection. *Nature Medicine* 6: 71–75.
Marthas ML and Miller CJ (2007) Developing a neonatal HIV vaccine: Insights from macaque models of pediatric HIV/AIDS. *Current Opinion in HIV and AIDS* 2(5): 367–374.
Miller CJ (1994) Mucosal transmission of SIV. In: Desrosiers RC and Letvin NL (eds.) *Current Topics in Microbiology and Immunology*, pp. 107–122. Berlin: Springer.
Miller CJ, Alexander NJ, Sutjipto S, *et al.* (1989) Genital mucosal transmission of simian immunodeficiency virus: Animal model for heterosexual transmission of human immunodeficiency virus. *Journal of Virology* 63: 4277–4284.
Miller CJ, Li Q, Abel K, *et al.* (2005) Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. *Journal of Virology* 79: 9217–9227.
Veazey RS and Lackner AA (2004) Getting to the guts of HIV pathogenesis. *Journal of Experimental Medicine* 200(6): 697–700.

Relevant Website

http://www.hiv.lanl.gov – HIV Sequence Database: Nomenclature Overview (modified 26 April 2007), HIV Databases by Los Alamos National Laboratory.

Simian Immunodeficiency Virus: General Features

M E Laird and R C Desrosiers, New England Primate Research Center, Southborough, MA, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Simian immunodeficiency virus (SIV) was first isolated in 1984 from captive rhesus macaques (*Macaca mulatta*) at the New England Primate Research Center (NEPRC). This virus was originally called STLV-III because it displayed similar morphology, growth characteristics, and antigenic properties to the newly described immunosuppressive virus HTLV-III of humans. When HTLV-III was renamed human immunodeficiency virus (HIV), the name STLV-III was also changed to SIV. Retrospective studies have shown that SIV was introduced to the NEPRC when a group of rhesus macaques with immuosuppressive disease was delivered from another primate center 15 years prior to the initial SIV isolation. The original cohort of rhesus monkeys was most likely accidentally infected with SIV from wild-caught sooty mangabey monkeys at the same institution. SIV has been subsequently isolated from other captive macaque species (*M. fascicularis*, *M. nemestrina, and M. arctoides*) that were dying of immunosuppression-associated diseases, and from many species of feral asymptomatic African nonhuman primates (**Table 1**).

Taxonomy and Classification

SIVs belong to the genus *Lentivirus* of the family *Retroviridae*. Related lentiviruses have been isolated from sheep, goats, horses, cattle, cats, and humans. Based on host species and genetic analysis, 14 discrete evolutionary groupings of primate lentiviruses are now recognized (**Figure 1**). Even within a single grouping, discrete subgroupings are defined based on host subspecies, geography, and genetic distance. Within a specific subgroup whose host range covers an extensive geographical area, discrete genetic sub-subgroups are further defined that correlate with monkey subspecies and precise natural geographic habitat.

The lentiviruses have a common morphogenesis and morphology that distinguish them from other retrovirus subgroups. Lentivirus particles are 80–100 nm in diameter and consist of an RNA genome and viral enzymes enclosed in viral protein core that is encased by a cell-derived membrane spiked with viral envelope glycoproteins. In lymphocytes, immature lentiviruses bud from the plasma membrane without a preformed nucleoid; mature particles contain a characteristic conical or rod-shaped nucleoid. Classification of lentiviruses by morphology alone is consistent with classification by phylogenetic analysis of polymerase (*pol*) gene sequences. The *pol* gene exhibits the greatest degree of sequence conservation and viruses classified as lentiviruses have *pol* gene sequences more closely related to one another than to other retroviruses.

Lentiviruses also share similarities in certain biological properties and genome organization. All lentiviruses have a propensity to replicate in macrophages and produce long-term, persistent infections in susceptible hosts.

Table 1 Detailed listing of primate lentiviruses[a]

Virus designation	*Primate Lentivirus grouping*	*Species (common)*	*Species (formal)*	*Subspecies isolates*
HIV-1	HIV–1/SIVcpz	Humans	*Homo sapiens*	
SIVcpz	HIV–1/SIVcpz	Chimpanzees	*Pan troglodytes*	*P. t. troglodytes* *P. t. schweinfurthi*
SIVsm	SIVmac/SIVsm/HIV-2	Sooty mangabeys	*Cercocebus atys*	
SIVmac	SIVmac/SIVsm/HIV-2	Macaques	*Macaca mulatta*	*M. arctoides* *M. nemestrina* *M. fascicularis*
HIV-2	SIVmac/SIVsm/HIV-2	Humans	*Homo sapiens*	
SIVagm	SIVagm	African green monkeys	*Chlorocebus aethiops*	*C. a. grivet* *C. a. tantalus* *C. a. sabeus* *C. a. alboqularis* *C. a. nictitans*
SIVsyk	SIVsyk	Sykes' monkeys	*Cercopithecus mitis*	
SIVgsn	SIVgsn/SIVmon/SIVmus	greater spot-nosed monkey	*Cercopithicus mitis*	
SIVmon	SIVgsn/SIVmon/SIVmus	mona monkey	*Cercopithicus mona*	
SIVmus	SIVgsn/SIVmon/SIVmus	mustached monkey	*Cercopithicus cephus*	
SIVlhoesti	SIVsun/SIVlhoesti	L'hoest monkey	*Cercopithicus lhoesti*	*C. l. lhoesti*
SIVsun	SIVsun/SIVlhoesti	Sun-tailed monkey	*Cercopithicus lhoesti*	*C. l. solatus*
SIVdeb	SIVdeb	DeBrazza monkey	*Cercopithicus neglectus*	
SIVden	SIVdeb	Dent's mona monkey	*Cercopithicus mona denti*	*C. m. denti*
SIVrcm	SIVrcm	Red-capped mangabey	*Cercocebus torguatus*	*C. t. torguatus*
SIVmnd	SIVmnd 1	Mandrill	*Mandrillus sphinx*	
SIVmnd	SIVmnd 2	Mandrill	*Mandrillus sphinx*	
SIVdrl	SIVmnd 2	Drill	*Mandrillus leucophaeus*	
SIVcol	SIVcol	Querza colobus	*Colobus querza*	
SIVolc	SIVolc	Olive colobus	*ProColobus badius*	
SIVwrc	SIVwrc	Western red colobus	*Pilocolobus badius*	
SIVtal	SIVtal	Angolia-talapoin monkey	*Miopithicus talapoin*	
SIVtal	SIVtal	Gabon talapoin monkey	*Miopithicus ogouensis*	

[a]Partial pol sequences have also been obtained from a black mangabey (*Loplcocebus aterrimus*) and from a Schmidt's guenon (*Cercopithicus ascanius schmidti*). In addition to the primate lentiviruses listed, serologic surveys for the detection of antibodies to SIV have suggested SIV infection of a variety of other species.

SIVs use CD4 as the first of two receptors used sequentially for viral entry into cells. The chronic disease induced by SIV includes immunodeficiency, undoubtedly because of the targeting of CD4+ lymphocytes for infection through the use of CD4 as the primary receptor. In addition to the *gag*, *pol*, and *env* genes that are found in all simpler retroviruses, lentiviruses have a number of auxiliary genes (**Figure 2** and **Tables 2** and **3**).

The SIVs are named according to the primate species of origin, for example, SIVmac from macaques or SIVsmm from sooty mangabey monkeys. Widespread availability of DNA sequencing has allowed an in-depth understanding of phylogenetic relationships among SIVs. However, to date, only 39 of the 69 recognized species of nonhuman primates that inhabit sub-Saharan Africa have been surveyed; additional distinct SIV groupings will likely be identified.

Geographic Distribution and Host Range

Many different species of African nonhuman primates are known to be infected with SIV in their natural habitats. However, few studies have investigated the distribution or extent of natural SIV infection. There has recently been an effort to overcome this shortfall of information by identifying and assessing the extent of SIV infection in wild primate populations. In 2002, Peeters *et al.*, collected and screened 788 blood samples from wild-caught monkeys for the prevalence of SIV infection in 13 different monkey species in Cameroon. The study examined the rates of SIV infection in monkeys hunted for bushmeat and those captured as pets as possible routes of zoonotic transmission. It was reported that 18.4% of bushmeat samples and 11.6% of pets tested positive for SIV infection. These results identified four species of monkeys not previously known to harbor SIV (*Cercocebus agilis*, *Lophocebus albigena*, *C. pogonias*, and *Papio anubis*) and likely underestimate the extent of SIV prevalence as not all native primate species were screened.

The origins of both HIV-2 and HIV-1 in humans are believed to have occurred through cross-species transmission events from SIV-infected simians relatively recently in history. SIVsmm is closely related to HIV-2 with the same genome organization and both viruses group together phylogenetically apart from the other 13 groups

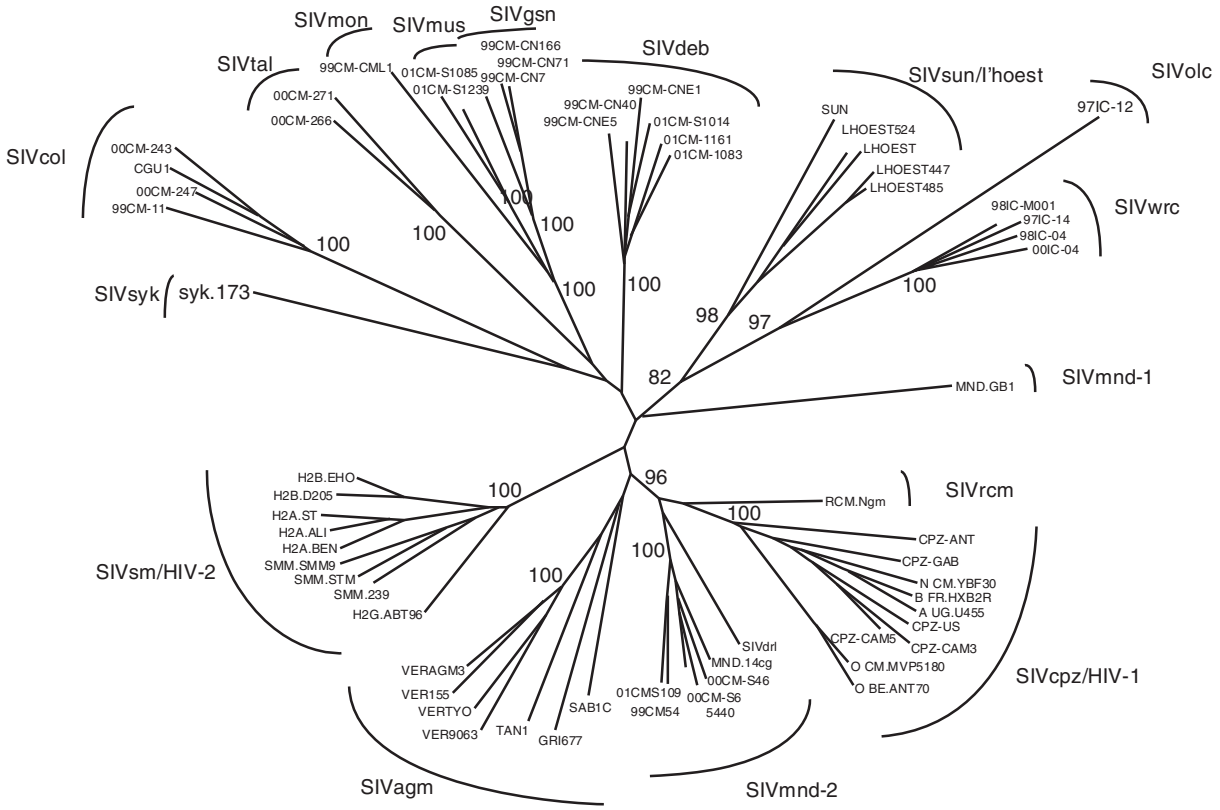


Figure 1 Phylogeny of primate lentiviruses. The 14 groupings of primate lentiviruses are shown. Please see **Table 1** for species abbreviations. Adapted from Courgnaud V, Formenty P, Koffi CA, *et al*. (2003) Partial molecular characterization of two simian immunodeficiency viruses (SIV) from African colobids: SIVwrc from Western Red Colobus (*Piliocolobus badius*) and SIVolc from Olive Colobus (*Procolobus verus*). *Journal of Virology* 77(1): 744–748, with permission from American Society for Microbiology.

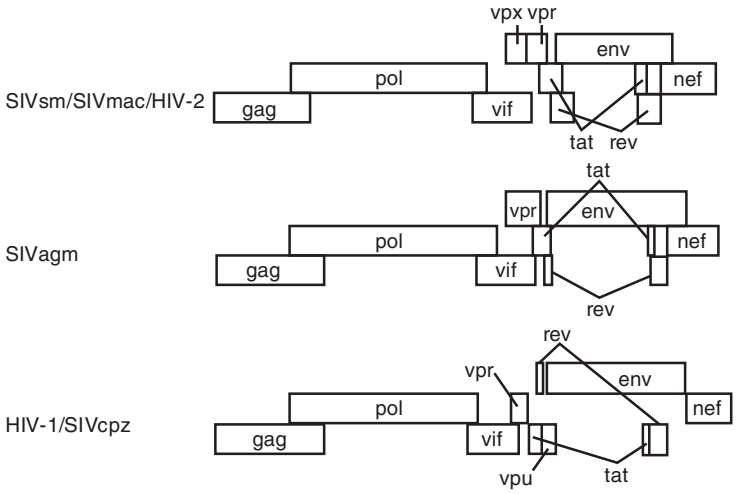


Figure 2 Genome organizations of representative primate lentiviruses.

of primate lentiviruses. The sooty mangabey monkey is native to the coastal forest regions of western Africa, where HIV-2 emerged and is now endemic in the human population. Thus, strong circumstantial evidence involving both viral sequences and geographic distribution link the monkey SIVsmm and HIV-2 in western Africa. In 2006, Keele and co-workers examined the wild chimpanzee (*Pan troglodytes troglodytes*) populations of Cameroon to determine the extent of natural SIVcpz infection and to investigate which infected populations may be responsible

Table 2 Presence of auxiliary genes in SIV species

	SIVsm/SIVmac/HIV- 2	*SIVagm*	*SIVsyk*	*SIVsun/SIVl'hoesti*	*HIV-1/SIVcpz*	*SIVgsn/SIVmon/SIVmus*	*SIVrcm*
vif	+	+	+	+	+	+	+
vpu					+	+	
vpr	+	+	+	+	+	+	+
vpx	+						+
tat	+	+	+	+	+	+	+
rev	+	+	+	+	+	+	+
nef	+	+	+	+	+	+	+

Different SIV species may vary with respect to the auxiliary genes that they carry. The presence or absence of these auxiliary genes do not always associate with phylogenetic clustering. For example, SIVmnd2 has a *vpx* gene, but SIVmnd1 does not. SIVden from a pet Dent's Mona monkey (*Cercopithecus mona denti*) has a *vpu* gene, although it clusters more closely to the SIV from DeBrazza monkeys, SIVdeb. SIVdeb has a *vpr* gene, but no *vpx* or *vpu* genes.

Table 3 Auxiliary gene function in SIV

Auxiliary gene product	*Essential for replication?*	*Early gene product?*	*Function*
tat	Yes	Yes	Potent activator of viral gene expression; enhances LTR-driven transcription
rev	Yes	Yes	Required for efficient transport of unspliced and singly spliced viral RNAs into the cytoplasm
nef	No	Yes	Functional activities include CD4 downregulation, MHC downregulation, infectivity enhancement and lymphocyte activation
vif	Yes/no[a]	No	Blocks restricting activity of innate cellular proteins, APOBEC-3G and APOBEC-3F
vpr	No	No	Involved in G_2/M phase cell-cycle arrest; mediates apoptosis of CD4+ T cells
vpx	No	No	Facilitates nuclear import of the preintegration complex in nondividing cells

[a]SIV strains containing a deletion in *vif* (SIVΔvif) can be grown in a *vif*-complementing cell line; when inoculated into animals no virus or PCR-amplifiable sequences could be recovered from PBMCs; however, monkeys still developed lowlevel antibody titers suggesting highly attenuated infection.

for the cross-species transmission events that initially introduced HIV-1 into the human population. By measuring the presence of anti-HIV cross-reactive antibodies and the ability to amplify viral gene sequences it was determined that SIV infection was widespread but uneven among the chimpanzee populations, with the prevalence of infection ranging from 23% to 35% in some isolated groups to a 4–5% infection rate in others, while still others had a complete absence of SIV infection. Sequence and phylogenetic analyses of the newly identified wild SIVcpz strains supported that distinct geographical chimpanzee groups acted as the sources of HIV-1 groups M and N in the human population.

Although SIVs naturally infect a variety of African nonhuman primates, a single example of natural infection of Asian Old World monkeys is yet to be reported. SIVsmm and SIVagm, when used to infect macaque monkeys (Asian Old World primates), can persist and cause an AIDS-like disease. Accidental introduction of SIVsmm into macaque monkeys in captive United States colonies occurred and was spread unknowingly into other macaques for more than a decade before it was identified and eliminated. At least one clear case of laboratory-acquired infection of a human with SIVmac has been documented.

Virus Propagation and Receptor Use

SIVs can be propagated in mitogen-stimulated primary peripheral blood mononuclear cells (PBMCs), in monocytes/macrophages from the primate host, and in many cultured cell lines including human tumor-derived CD4+ T-lymphocyte cell lines. The types of cells that can be infected by different strains of SIV correlate with the receptor(s) that are expressed on the cell surface. Some acutely pathogenic strains are unusual in their ability to replicate in lymphocytes of resting PBMC cultures without prior stimulation. In PBMC cultures and many cell lines, viral infection results in the fusion of cellular membranes producing large syncytial cells. Syncytium formation, which is mediated by *env*, allows the virus to spread directly from cell to cell in addition to direct infection. Several isolates also grow well in cultured macrophages derived from lung, blood, or bone marrow.

As with HIV-1, the SIVs use both CD4 and a chemokine receptor for viral entry. The SIVsmm/SIVmac/HIV-2 and SIVagm groups of viruses are known to use CCR5 as their principal co-receptor. However, a variety of other chemokine receptors, CCR2b, CCR3, STRL33 (Bonzo), GPR15 (Bob), and GPR1, also can be used as the co-receptor,

depending on the individual virus isolate. A larger percentage of isolates from the SIVsmm/SIVmac/HIV-2 group of viruses show less dependence on CD4 for entry than do HIV-1 variants. Isolates of SIVsmm/SIVmac appear to use CXCR4 as the principal co-receptor much less frequently than HIV-1 isolates. SIVs from red-capped mangabeys (*Cercocebus torquatus torquatus*) predominantly use CCR2b as the principal co-receptor.

Genetics

SIV, like other retroviruses, replicates its genome through a proviral DNA intermediate. From the 5′ cap to the 3′ polyadenylation site, the SIV genome is approximately 9.6 kbp in length. The viral particle contains a diploid genome of single-stranded RNA that is linked noncovalently near the 5′ end of the molecules. The 5′ end of the viral genome is capped and the 3′ end is polyadenylated. DNA synthesis by the viral-encoded reverse transcriptase is primed by host tRNA that is base-paired to viral RNA. The double-stranded proviral DNA is integrated into the host cell chromosome by a viral-encoded integrase and further replication events of transcription, translation, and particle assembly depend on cellular components. Particles then assemble at and bud through the plasma membrane. Because of this replication strategy, cloned DNA representing the entire proviral genome can yield infectious virus.

All retroviruses contain certain standard features in their genomic organization (**Figure 2**). Sequences regulating DNA synthesis, integration, transcription, and other functions are contained in the long terminal repeat (LTR) region at each end of the provirus. Open reading frames (ORFs) encoding the major structural and nonstructural proteins lie between the LTRs. Genes are encoded in any of three possible ORFs; overlaps between ORFs are common. All retroviruses contain three standard genes called *gag* (group-specific antigen), which encodes the core proteins; *pol* (polymerase), which encodes the viral reverse transcriptase, protease, and integrase; and *env* (envelope), which encodes the envelope glycoproteins.

Env is essential for virus replication. The envelope glycoproteins are responsible for binding the receptor and co-receptor on the cell surface and mediating viral entry. The *env* products are the main targets of antibodies that can neutralize infection. In addition, determinants of cell and tissue tropism often map to the *env* gene. Derivatives in which the SIVmac *env* has been replaced by envelope of HIV-1 are replication competent in macaque cells and are capable of infecting rhesus monkeys. These recombinant viruses are known as simian–human immunodeficiency viruses (SHIVs). Serial passage of several SHIV strains has resulted in second-generation SHIVs that are consistently pathogenic in macaques.

In addition to *gag*, *pol*, and *env*, all lentiviruses, including SIV, encode additional accessory genes not found in other simple retroviruses (**Tables 2** and **3**). Both SIV and HIV encode *tat* (transactivator protein), *rev* (regulator of gene expression), *vif* (viral infectivity factor), *nef* (originally termed negative factor), and *vpr* (viral protein 'r'). SIVagm and SIVsmm/HIV-2/SIVmac encode an additional gene, *vpx* (viral protein 'x'), thought to be a duplicated homolog of *vpr*. The ORF for *vpu*, found in HIV-1/SIVcpz and SIVgsn/SIVmon/SIVmus/SIVden, is not contained in HIV-2 or in other SIVs. These auxiliary genes likely contribute to the complex life cycle of lentiviruses, including persistent viral replication and immune evasion.

Some of the accessory proteins found in SIV can be deleted without abrogating the ability of the virus to replicate *in vivo* and *in vitro*, specifically *nef*, *vpr*, and *vpx*. However, the presence and the conservation of these genes in several different subgroups of lentiviruses suggest that they contribute to the virus' ability to replicate and persist *in vivo*. Cloned proviral DNAs containing deletions in these auxiliary ORFs have been used to study the contributions to replication and functional activity of the auxiliary genes in the context of experimental animal infection. Auxiliary gene functional data are summarized in **Table 3**.

Evolution

Comparison of genetic sequences among human and simian immunodeficiency suggests that there are at least 14 discrete groups of primate lentiviruses in existence: HIV-1/SIVcpz; SIVmnd-2; SIVagm; SIVsmm/HIV-2; SIVsyk; SIVcol; SIVtal; SIVmon/SIVmus/SIVgsn; SIVdeb/den; SIVsun/l'hoest; SIVolc; SIVwrc; SIVmnd-1; and SIVrcm (**Figure 1**). It is thought that HIV-1 and HIV-2 evolved from simian viruses that entered the human population through cross-species transmission events relatively recently in human history. Cross-species transmission among nonhuman primates occurring in nature may have generated further pathogenic variants; however, it is likely that most of the primate immunodeficiency viruses have long been present in the natural host but not recognized until recently. The SIVs and HIVs are more closely related to each other than to any other nonprimate retrovirus, suggesting that they are inherently primate viruses, not derived from nonprimate viruses that were introduced via other cross-species transmission events.

Serologic Relationships and Genetic Diversity

The *pol* gene generally contains the greatest degree of sequence conservation and therefore is most often used

for the comparison of lentiviruses from different groups or subgroups to assess relatedness. *Pol* sequences from one subgroup of SIV (e.g., SIVsmm) will generally contain only a 55–60% amino acid identity when compared to another SIV subgroup (e.g., SIVagm). When different lentivirus groups are compared (e.g., EIAV with SIV) the amino acid identity in *pol* is often 35% or less, and the sequence homology found in other genes is even less. Antiserum to the Gag protein is generally cross-reactive to different strains within a group, whereas antiserum to the envelope is not and can be used to distinguish between isolates within a group.

Epidemiology

SIV has been found in many species of nonhuman primates throughout sub-Saharan Africa, but in most cases infection does not seem to cause an AIDS-like disease in the natural host. There are only a few examples of immunodeficiency in monkey species naturally infected with SIV. In contrast, though infection does not appear in nature, SIV infection of Asian macaques in captivity induces an AIDS-like disease similar to that observed in HIV-infected humans.

Transmission

There is little information regarding natural modes of SIV transmission. A study of wild grivet monkeys in Awash National Park in Ethiopia analyzed SIVagm serologic status as compared to age, sex, and risk. Infection was found overwhelmingly in females of reproductive age and was nearly absent among younger female animals. In the male population, infection was only observed in monkeys that were fully adult. These data support a predominantly sexual mode of transmission among the grivet population. SIV transmission through contact with infected blood from aggressive contacts (e.g., bite and scratch wounds) may also be a prominent mode by which SIV may be spread. In addition, maternal–infant transmission of SIV has been observed in captive animals.

Experimental infection of laboratory animals has most commonly been performed by direct needle inoculation. However, mucosal exposure is being used more frequently, especially in vaccine studies, as a model for the most common routes of HIV infection.

Features of Infection

While SIV infection of the natural host is usually not associated with any disease progression, SIV infection of macaques induces both acute and chronic disease symptoms that are similar to that which HIV-1 causes in human patients. SIV infection of rhesus macaques is generally thought to be the closest model of AIDS in humans.

The main sites of pathogenic SIV replication shortly after infection have been localized to the gastrointestinal (GI) tract, thymus, spleen, and other lymphoid tissues. SIV has been detected at early time points within periarteriolar lymphoid sheaths in the spleen, paracortex of lymph nodes, and medulla of the thymus. SIV infection of rhesus monkeys results in a dramatic and selective depletion of CD4+ T cells in the GI tract within days of infection, before depletion is evident in the peripheral lymphoid tissues. Coincident with the loss of CD4+ T cells in the GI tract is the productive infection of large quantities of mononuclear cells at this site. It is now clear that SIV replicates principally in the CD4+ CCR5+ cells of the memory T-cell phenotype. These cells predominate in the gut and other mucosal sites and are found at much lower levels in peripheral lymphoid tissues. Therefore, the GI tract appears to be a major site of SIV replication and CD4+ memory T-cell depletion early in the course of infection. Within the thymus, marked depletion of thymic progenitor cells has been observed 21 days post infection with pathogenic SIV; this cell depletion is followed by increased cell proliferation in the thymus and a marked increase in thymocyte progenitors. SIV can also be found in the central nervous system (CNS) at early time points following infection. The cells that are targeted in the brain, either early in infection or in late-stage SIV-induced encephalitis, are primarily cells of the monocyte/macrophage lineage.

Viral loads in infected animals decrease with the onset of immune responses, and this decrease correlates with CD8+ T-cell lymphocytosis and the rise of SIV-specific antibodies. Following immune system activation, animals enter an asymptomatic period of infection of variable duration. Viral replication persists during this period, inducing immune abnormalities, including gradual declines in CD4+ T-cell count, CD4/CD8 ratio, and the ability to respond to mitogens. Infected animals can also exhibit chronic diarrhea and wasting resulting in up to a 60% loss of the original body weight. Over the course of disease, dramatic changes, including hyperplasia and/or atrophy, take place in most of the lymphoid tissues. Terminal stages of SIV infection are characterized by a range of diseases that can be grouped into four broad categories: SIV-related inflammatory disease, opportunistic infections associated with SIV-induced immunosuppression, neoplastic diseases, and diseases of unknown pathogenesis. The tropism of SIV strains for monocytes/macrophages correlates with dramatic inflammatory and degenerative changes seen in the CNS, lung, digestive tract, and other organs that are separate from the pathogenesis of opportunistic infections. The characteristics and frequency of inflammatory lesions in SIV-infected macaques closely resembles those seen in HIV-infected

patients. SIV-induced encephalitis is frequent, although the appearance of brain lesions is dependent on the infecting SIV strain. At necropsy, 30–50% of SIVmac-infected animals have characteristic multinucleate giant cell encephalitis that closely resembles that seen in HIV-associated encephalitis. The opportunistic infections seen in infected monkeys are also similar to those observed in HIV-infected individuals and include *Pneumocystis carinii*, *Mycobacterium avium*, *Crytposporidia* sp., *Toxoplasma gondii*, rhesus Epstein–Barr virus (rhesus lymphocryptovirus), cytomegalovirus, polyomavirus (SV40), and adenovirus. Neoplastic diseases during SIV infection are primarily limited to lymphomas, the frequency of which varies from study to study. Lymphoma induction in SIV-infected macaques has additionally been associated with the co-infection of rhesus Epstein–Barr virus. Diseases of unknown pathogenesis include generalized lymphoproliferative syndrome, arteritis, and arteriopathy.

Acutely pathogenic strains exist, such as SIVsmPBj14, which can be acutely lethal in infected macaques. SIVsm PBj14 infection causes death in infected monkeys within 14 days. These animals have very high viral loads, severe GI disease, cytokine disregulation, lymphoproliferative disease, and organ system failure. The increased pathogenicity of this virus has been attributed to the creation of an ITAM motif by a tyrosine at residue 17 of the nef protein. This change allows the virus to induce lymphocyte activation and replicate to high titers in PBMC cultures without prior stimulation. However, the disease induced by the majority of SIV infections is typically chronic and manifests itself over the course of 1–3 years post infection.

Several independent research groups have constructed recombinant forms of SIVmac with HIV-1 *env*, *tat*, *rev,* and, in some cases, *nef* and/or *vpu* genes renamed 'SHIV' (for simian–human immunodeficiency virus) that have been passaged in macaques to establish pathogenic virus strains. Although most of the HIV-1 envelopes that were used to construct these SHIVs were dual-tropic, in that they can use either CCR5 or CXCR4 as a co-receptor, the large majority of SHIV viruses appear to target primarily CXCR4-expressing cells when used to infect rhesus macaques. The pathogenic SHIVs consistently, rapidly, and irreversibly deplete CD4+ T cells from the periphery and can be acutely lethal.

Immune Response and Persistence

SIV-infected macaques typically produce high levels of antiviral antibodies and high-frequency cytotoxic T-lymphocyte (CTL) responses to the infecting virus. These immune responses persist for the lifetime of the infected host in both natural and experimental infection. SIV deletion mutants that are progressively more attenuated based on viral load measurements generate progressively weaker anti-SIV antibody responses. Anti-SIV CTL responses have been demonstrated as being major histocompatibility complex (MHC) restricted. Detailed investigation of CTL responses has been impeded by a lack of information regarding MHC types in different monkey species. However, a considerable amount of new information regarding MHC alleles and their cognate peptides is emerging for rhesus macaques.

The importance of CD8+ lymphocytes in limiting the extent of SIV or SHIV replication has been definitively shown using CD8+ T-cell depletion. Extensive depletion of CD8+ cells was accomplished by intravenous administration of large doses of anti-CD8 monoclonal antibodies. When CD8+ T cells were depleted during primary infection, viral replication continued unabated after the usual peak of viral loads, 10–14 days post infection; this was in stark contrast to undepleted animals, in which intact immune responses typically act to decrease viral loads after 14 days post infection. During chronic infection, elimination of CD8+ lymphocytes through depletion resulted in a rapid and dramatic increase in viremia, which was again suppressed upon removal of anti-CD8 antibody and the return of the SIV-specific CD8+ lymphocytes. Depletion of CD8+ T cells in animals infected with SHIV viruses has facilitated the appearance of the more highly pathogenic, passaged variants.

In macaques developing SIV-induced disease from wild-type strains of SIV, viral-specific, proliferative responses of CD4+ T cells are typically weak or absent all together. However, infection by attenuated SIV mutants containing a deletion of the *nef* gene produces strong, SIV-specific, CD4+ helper cell proliferative responses. This situation recalls that of HIV infection in humans, in which HIV-specific CD4+ proliferative responses in progressing patients are usually weak or absent, but are often very strong in nonprogressors that are able to control their infection. It seems that as CD4+ helper T cells try to respond to SIV at sites of infection, they arrive in the location where they are the ideal target cells for the invading virus. In pathogenic infections, the virus wins the battle between it and the responding CD4+ T cells.

All lentiviruses persist in the infected host through chronic active viral replication. Over the course of the months and years of chronic infection, macaques infected with SIV are producing and turning over millions of viral particles and infected cells every day. Although active replication persists throughout the course of infection, there are some cells that are most likely infected in a quiescent or latent fashion. The extent of chronic active replication may also differ depending on the infecting virus strain and the host. Consistent with prolonged antigen expression and chronic replication is the long-term persistence of a high level of circulating antibody and viral-specific CTLs. Nonpathogenic SIV derivatives also continue to replicate at low levels over long periods, as

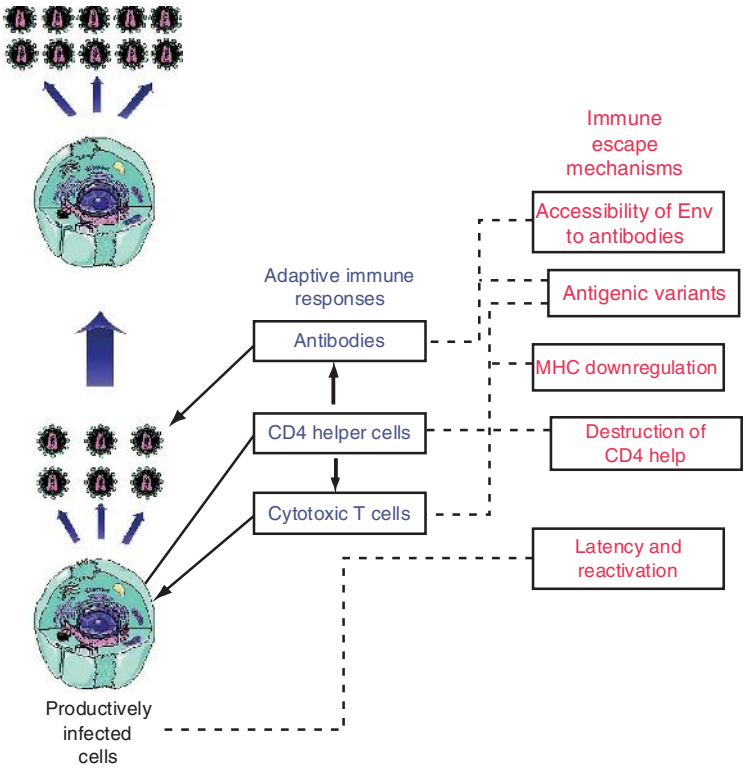


Figure 3 Simian immunodeficiency virus uses a variety of strategies to avoid recognition and clearance by both the humoral and cellular arms of the immune system.

demonstrated by accumulated sequence changes in these viral genomes and persistent antibody titers.

The dilemma of all lentiviruses is how to replicate persistently in the face of an apparently strong immune response. The levels of antiviral antibody and the frequency of CTLs in the infected individual have been measured and appear to be consistently high. Several strategies are used by SIV and other lentiviruses to allow persistent replication and evade the immune response. These are summarized in **Figure 3**.

Prevention and Control

Extensive testing and removal programs have essentially eliminated SIV from captive macaque colonies. However, continued vigilance is required to minimize the chance that breeding colonies may again become accidentally exposed to the virus. Animals can be easily and conveniently tested serologically for infection. Cases of SIV infection in humans are extremely rare and laboratory personnel that work with SIV follow the same precautions when working with SIV as working with HIV-1. Disposable gloves and surgical gowns are used, all work with live virus is performed in a biosafety hood, procedures creating aerosols are avoided, and any use of glass or needles in conjunction with live virus is minimized.

Future Perspectives

The development of a safe, effective, affordable vaccine for HIV/AIDS is one of the greatest challenges of our time. SIV will inevitably play an important role in instructing what is needed for protective immunity and how best to make a vaccine. Because SIVs are the closest known relatives of HIVs, the induction of AIDS in macaques by infectious molecular clones of SIV represents the best existing animal model for AIDS. SHIV infections are also extensively used in vaccine development because it allows for the analysis of HIV-1 envelope-containing vaccine products in an established system. Vaccine studies in animal models are vitally important as they provide useful information in several different ways. Head-to-head comparisons of different vaccine approaches can be performed to investigate which approach is more effective, at least within defined experimental conditions. Further, more in-depth analyses of specific vaccine approaches may also provide fundamental insights into what is needed to establish persistent, protective immunity to SIV and/or HIV infection. The worldwide crisis of HIV infection and AIDS has brought attention to the SIV system as a source of information that will shed light on the human condition. SIV as an animal model can contribute to further understanding of the most critical issues for future progress, including better understanding of pathogenesis,

improvements in therapy and, most importantly, the development of a safe, effective, and affordable vaccine.

See also: Human Immunodeficiency Viruses: Antiretroviral agents; Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Pathogenesis.

Further Reading

Campbell RSF and Robinson WF (1998) The comparative pathology of lentiviruses. *Journal of Comparative Pathology* 119: 333–395.

Courgnaud V, Formenty P, Akoua-Koffi C, *et al.* (2003) Partial molecular characterization of two simian immunodeficiency viruses (SIV) from African colobids: SIVwrc from Western Red Colobus (*Piliocolobus badius*) and SIVolc from Olive Colobus (*Procolobus verus*). *Journal of Virology* 77: 744–748.

Daniel MD, Letvin NL, King NW, *et al.* (1985) Isolation of T-cell tropic HTLV-III-like retrovirus from macaques. *Science* 228: 1201–1204.

Desrosiers RC (2001) Nonhuman Lentiviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 2095–2122. Philadelphia, PA: Lippincott Williams and Wilkins.

Desrosiers RC (2004) Prospects for an AIDS vaccine. *Nature Medicine* 5: 723–725.

Johnson WE and Desrosiers RC (2002) Viral persistence: HIV's strategies of immune system evasion. *Annual Review of Medicine* 53: 499–518.

Keele BF, Van Heuverswyn F, Li Y, *et al.* (2006) Chimpanzee reservoirs of pandemic and nonpandemic HIV-1. *Science* 313: 523–526.

Kestler H, Kodama T, Ringler D, *et al.* (1990) Induction of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. *Science* 248: 1109–1112.

Koff WC, Johnson PR, Watkins DI, *et al.* (2006) HIV vaccine design: Insights from live attenuated SIV vaccines. *Nature Immunology* 7: 19–23.

Peeters M, Courgnaud V, Abela B, *et al.* (2002) Risk to human health from a plethora of simian immunodeficiency viruses in primate bushmeat. *Emerging Infectious Disease* 8: 451–457.

Veazey RS, DeMaria M, Chalifoux LV, *et al.* (1998) Gastrointestinal tract as a major site of CD4+ T cell depletion and viral replication in SIV infection. *Science* 280: 427–431.

Simian Immunodeficiency Virus: Natural Infection

I Pandrea, Tulane National Primate Research Center, Covington, LA, USA
G Silvestri, University of Pennsylvania, Philadelphia, PA, USA
C Apetrei, Tulane National Primate Research Center, Covington, LA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

APOBEC Human protein superfamily that interferes with the HIV/SIV replication. This family of proteins has cytidine deaminase activity and has been suggested to play an important role in innate antiviral immunity.
Catarrhines Relating to or being any of a division of primates (*Catarrhina*) comprising the Old World monkeys, great apes, and hominids that have nostrils close together and directed downward, 32 teeth, and a tail, when present, which is never prehensile.
Chemokine receptors Family of approximately 20 different G-protein-coupled receptors that have seven transmembrane segment polypeptides, and which cause cell activation. Each receptor subtype is capable of binding multiple chemokines within the same family.
Endemic (1) Natural to or characteristic of a specific place; native; indigenous. (2) A disease which persists in a given population or locality.
Giant cell disease Pathologic condition specific for nonhuman primates with severe immunodeficiency characterized by infiltration with syncytial cells in multiple tissues.
Guenon An Old World monkey native to sub-Saharan Africa; possesses a round head with beard, and 'whiskers' at side of face; slender, with long hind legs and tail; some species with colorful coats.
Sympatric Of two or more population or taxa, inhabiting the same geographic area.

Introduction

More than 40 different types of simian immunodeficiency viruses (SIVs) naturally infect different species of monkeys and apes. Two of these, SIVcpz, which naturally infects chimpanzees, and SIVsmm, which naturally infects sooty mangabeys (SMs), are the ancestors of HIV-1 and HIV-2, respectively. Inadvertent cross-species transmission of SIVsmm from naturally infected SMs to different species of macaques resulted in severe immunodeficiency and subsequent development of the animal models for AIDS. Unlike HIV/SIV infection of humans and macaques, which normally progresses to AIDS, natural SIV infection is generally nonpathogenic in African nonhuman primates (NHPs). The mechanisms behind this lack of disease progression are currently under investigation.

History

The history of SIVs began two decades prior to virus discovery, when two outbreaks of opportunistic infections and lymphoma occurred in rhesus macaques (RMs) (1968) and in stump-tailed macaques (1973) at the California National Primate Research Center (CNPRC). SIVmac was discovered in 1985, during another outbreak of lymphomas in RMs at the New England Primate Research Center from monkeys transferred from CNPRC. However, occurrence of SIV infection in macaques was perplexing since tests carried out on RMs in Asia failed to reveal any evidence of SIV circulation in the wild. In 1986, at the Tulane National Primate Research Center, attempts to transmit leprosy from SMs to RMs resulted in a new AIDS outbreak in RMs, establishing the link between pathogenic SIVs in macaques and an African monkey species. During the following 20 years, more than 40 SIVs were identified in different African NHP species.

SIVsmm is also the ancestor of HIV-2. At least eight cross-species transmissions in West Africa resulted in the emergence of the eight HIV-2 groups (AH). In 1989, the discovery of SIVcpz in chimpanzees from Gabon identified the ancestor of HIV-1. At least three cross-species transmissions were at the origin of HIV-1 groups M, N, and O. Groups M and N resulted from cross-species transmission of SIVcpz from *Pan troglodytes troglodytes* in West-Central Africa. Group O is more closely related to the recently discovered SIVgor from *Gorilla gorilla*.

The arguments to support cross-species transmission from NHPs as the origin of HIVs are: (1) similarities in viral genome organization; (2) phylogenetic relatedness; (3) prevalence in the natural host; (4) geographic coincidence; and (5) plausible routes of transmission. All these criteria are fulfilled by both SIVsmm/HIV-2 and SIVcpz+SIVgor/HIV-1. Therefore, the discovery of SIVs in African species identified the origin of HIVs. However, the events behind HIV emergence are still under debate. Some authors consider that human exposure to SIVs through bush meat consumption is the original source of AIDS ('cut-hunter theory'). Others believe that simian exposure is necessary but insufficient for HIV emergence as a human pathogen, which requires adaptation to the new human host. Reuse of needles and syringes or transfusions may have played a role in triggering SIV adaptation to humans. It is probable that deforestation, political unrest, increase in urbanization and travel in the second half of the twentieth century also acted as cofactors of HIV emergence. Altogether, the action of these factors explain why HIVs only emerged in the second half of the twentieth century while people in sub-Saharan Africa were exposed to SIVs for millennia.

Virology

Currently, there are 47 fully sequenced SIV genomes from 21 NHP species. Partial genomic sequences are available for 13 additional SIVs, and serological evidence only of SIV infection has been obtained for seven primates (**Table 1**). Asian species of Old World monkeys (colobine and macaques), as well as some African species (such as baboons) do not carry a species-specific SIV, suggesting that the last common ancestor of the catarrhines (Old World monkeys and apes) was not SIV-infected 25 million years ago and that SIV emerged after species radiation, from a nonprimate source.

Classification and Taxonomy

In most instances, the infected NHP species represents the reservoir of that virus type, which is designated by a three-letter abbreviation of the vernacular name of the host (**Table 1**). When related NHP subspecies are infected, the subspecies name is included in virus designation. Thus, for chimpanzee subspecies infected by SIVs, the two SIVcpz are identified as SIVcpz.Ptt (from *Pan troglodytes troglodytes*) and SIVcpz.Pts (from *P. t. schwenfurthii*). For individual isolates, nomenclature includes SIV type and the country of origin: SIVrcmGB1 is a red-capped magabey virus isolated from Gabon. The year of sample can also be included: SIVsmmSL92 is an SM virus isolated from Sierra Leone in 1992. This feature is useful in tracking the origin and evolution of viruses.

Phylogenetic Relationships

SIVs have a starburst phylogenetic pattern, suggesting the evolution from a single ancestor, and a high genetic divergence, forming six SIV lineages with genetic distances of up to 40% in Pol proteins (**Table 2** and **Figure 1**). Each SIV lineage is represented by two or more strains. The l'hoesti lineage is unique in being formed by SIVs circulating in distantly related species. The relationship between SIV lineages and newly characterized SIVs is complicated by sequence diversity and recombination that results in different clustering patterns when different genomic regions are analyzed.

To better understand SIV phylogenetic relationships, a brief presentation of primate species radiation follows. African NHPs belong to two different groups: Old World monkeys and anthropoid primates (apes). Two ape genera are endemic in Africa: *Pan* (formed by the four subspecies of chimpanzees and bonobo) and *Gorilla*. The Old World monkeys (family *Cercopithecidae*) are divided into two subfamilies (*Cercopithecinae* and *Colobinae*), separated 11 million years ago (**Figure 1**). *Cercopithecinae*

Table 1 African apes and monkeys infected with SIV

Species/ subspecies	*Virus type*	*Geographic location*[a]	*Seroprevalence*	*Pathogenicity*	*Cross-species transmission*
Common chimp (*Pan troglodytes troglodytes*)	SIVcpz.Ptt	Central Africa (Cameroon, Gabon, Congo)	<10%	Not reported	Humans, HIV-1 groups M and N*P. t. velerosus*
Eastern chimp (*Pan troglodytes schweinfurthii*)	SIVcpz.Pts	East Africa (Tanzania, Democratic Republic of Congo-DRC)	<10%	Thrombocytopenia	Not reported
Pan troglodytes velerosus	SIVcpz.Ptt[b]	Zoo in Cameroon		Not reported	
Gorilla gorilla	SIVgor	West-Central Africa (Cameroon)	?	Not reported	Humans, HIV-1 group O
Sooty mangabey (*Cercocebus atys*)	SIVsmm	West Africa (Sierra Leone, Liberia, Ivory Coast)	20–58%	AIDS	Humans, HIV-2 Experimentally to *M. mulatta* (SIVmac), *M. nemestrina* (SIVptm), *M. fascicularis* and *M. arctoides* (SIVstm): AIDS. Accidentally to *L. aterrimus* (AIDS)
Red-capped mangabey (*Cercocebus torquatus*)	SIVrcm	West-Central Africa (Gabon, Cameroon, Nigeria)	10–20%	Not reported	Agile mangabey Experimentally, to *Macaca mulatta* and *M. fascicularis* (no AIDS)
Agile mangabey (*Cercocebus agilis*)	SIVagi	West-Central Africa (Cameroon)	0–10%	Not reported	Not reported
White-crowned mangabey (*Cercocebus lunulatus*)	SIVagm.ver[b]	Zoo in Tanzania		Not reported	
Gray-crested mangabey (*Lophocebus albigena*)	?	Central Africa	?	?	?
Black mangabey (*Lophocebus aterrimus*)	SIVbkm	Central Africa (DRC)	Not known	Not reported	Not reported
Mandrill (*Mandrillus sphinx*)	SIVmnd-1	Central Africa (Gabon)	50%	AIDS in captivity	Not reported
	SIVmnd-2	West-Central Africa (Cameroon, Gabon)	50%	AIDS in captivity	Experimentally, to *M. mulatta* (transient infection)
Drill (*Mandrillus leucophaeus*)	SIVdrl	West-Central Africa (Nigeria, Cameroon, Gabon, Bioko)	Not known	Not reported	Not reported
Yellow baboon (*Papio cynocephalus*)	SIVagm.ver[c]	Tanzania	Not known	Not reported	Not reported
Chacma baboon (*Papio ursinus*)	SIVagm.ver[c]	South Africa	Not known	Not reported	Not reported
Allen's monkey (*Allenopithecus nigroviridis*)	?	Central Africa	?	?	?
Talapoin (*Miopithecus talapoin, M. ougouensis*)	SIVtal	Central Africa (Gabon, Angola, Cameroon)	11%	Not reported	Transient infection in Rh upon experimental transmission

Continued

Table 1 Continued

Species/ subspecies	*Virus type*	*Geographic location*[a]	*Seroprevalence*	*Pathogenicity*	*Cross-species transmission*
Patas (*Erythrocebus patas*)	SIVagm.ver[c]	West Africa	Not known	Not reported	Not reported
Grivet (*Chlorocebus aethiops*)	SIVagm.gri	East Africa	>50%	Not reported	Not reported
Vervet (*Chlorocebus pygerythrus*)	SIVagm.ver	East and South Africa	>50%	AIDS in a monkey co-infected with STLV	Naturally, to baboons in the wild and white-crowned mangabeys in captivity; Experimentally, to *M. nemestrina* (AIDS) and *M. mulatta* (transient infection)
Tantalus (*Chlorocebus tantalus*)	SIVagm.tan	Central Africa	>50%	Not reported	Not reported
Sabaeus (*Chlorocebus sabaeus*)	SIVagm.sab	West Africa	>60%	Not reported	Naturally transmitted to patas (No AIDS); experimentally transmitted to Rh (no AIDS)
Diana (*Cercopithecus diana*)	?	West-Central Africa	?	?	?
Greater spot-nosed monkey (*Cercopithecus nictitans*)	SIVgsn	Central Africa	4–20%	Not reported	Potential source virus for SIVcpz
Blue monkey (*Cercopithecus mitis*)	SIVblu	Central-East Africa	>60%	Not reported	Not reported
Syke's monkey (*Cercopithecus albogularis*)	SIVsyk	East Africa	30–60%	Not reported	Experimentally, to *M. mulatta* (transient infection)
Mona (*Cercopithecus mona*)	SIVmon	West-Central Africa (Cameroon, Nigeria)	Not known	Not reported	Not reported
Dent's mona (*Cercopithecus denti*)	SIVden	Central Africa	10%		
Crested mona (*Cercopithecus pogonias*)	?	West Africa	?	?	?
Campbell's mona (*Cercopithecus campbelli*)	?	West Africa	?	?	?
Lowe's mona (*Cercopithecus lowei*)	?	West Africa	?	?	?
Mustached monkey (*Cercopithecus cephus*)	SIVmus	Central Africa	3%	Not reported	Potential source virus for SIVcpz
Red-tailed monkey (*Cercopithecus ascanius*)	SIVasc/ SIVschm	Central Africa (DRC)	Not known	Not reported	Not reported
Red-eared monkeys (*Cercopithecus erythrotis*)	SIVery	Central Africa (Bioko)	Not known	Not reported	Not reported

Continued

Table 1 Continued

Species/ subspecies	*Virus type*	*Geographic location*[a]	*Seroprevalence*	*Pathogenicity*	*Cross-species transmission*
De Brazza's monkey (*Cercopithecus neglectus*)	SIVdeb	West-Central and Central Africa	40%	Not reported	Not reported
Owl-faced monkey (*Cercopithecus hamlyni*)	?	Central Africa	?	?	?
L'Hoest's monkey (*Cercopithecus lhoesti*)	SIVlhoest/ SIVlho	East Africa	50%	Not reported	Experimentally, to *M. nemestrina* (AIDS)
Sun-tailed monkey (*Cercopithecus solatus*)	SIVsun	Central Africa	Not known	Not reported	Source virus for SIVmnd-1; Experimentally, to *M. nemestrina* (AIDS); Experimentally, to *M. fascicularis* (transient infection);
Preuss's monkey (*Cercopithecus preussi*)	SIVpre	Central Africa (Bioko)	Not known	Not reported	Not reported
Mantled colobus (*Colobus guereza*)	SIVcol	Central Africa	28%	Not reported	Not reported
Western Red colobus (*Piliocolobus badius*)	SIVwrc	West Africa	40%	Not reported	Not reported
Olive colobus (*Procolobus verus*)	SIVolc	West Africa	40%	Not reported	Not reported

[a]Countries listed correspond to reported evidences of SIV circulation in that NHP species and not to species distribution.
[b]Cross-species transmission in captivity.
[c]Cross-species transmission in the wild.

Table 2 SIV clusters based upon phylogenetic relationships

Cluster	*Species*	*SIV strain*	*Comments*
1	Arboreal guenons (*Cercopithecus*)	SIVsyk, SIVblu, SIVgsn, SIVdeb, SIVmon, SIVden, SIVmus, SIVasc, SIVtal, SIVery	Ancestral source of SIVcpz/SIVgor/HIV-1 (SIVgsn, SIVmon, SIVmus, and SIVden harbor a *vpu* gene); lineage formed by all arboreal guenons; partial sequences from SIVbkm from the black mangabey cluster in this lineage
2	Sooty mangabey	SIVsmm	Ancestral virus of SIVmac/HIV-2; SMs from Ivory Coast harbor SIVsmm strains related to the epidemic HIV-2 groups A and B; those from Sierra Leone are the sources of HIV-2 groups C-H
3	African Green Monkey	SIVagm (SIVagm.ver, SIVagm.tan, SIVrcm. gri, SIVagm.sab)	Four different SIV subtypes described for each species in the genus *Chlorocebus*, suggesting host-dependent evolution; SIVagm.sab is a recombinant between an SIVagm ancestor and a SIVrcm-like virus
4	L'Hoest supergroup, mandrill	SIVlhoest, SIVsun, SIVmnd-1, SIVpre	Host-dependent evolution for monkeys in the C.l'hoesti supergroup; cross-species transmission from solatus guenon to mandrills
5	Red-capped Mangabeys	SIVrcm, SIVagi	Originally considered recombinants, now appear to be 'pure' viruses; SIVagi is cross-species transmitted from RCMs
6	Mantled colobus	SIVcol	First virus isolated from *Colbinae*; other viruses from Western colobus species do not cluster with SIVcol

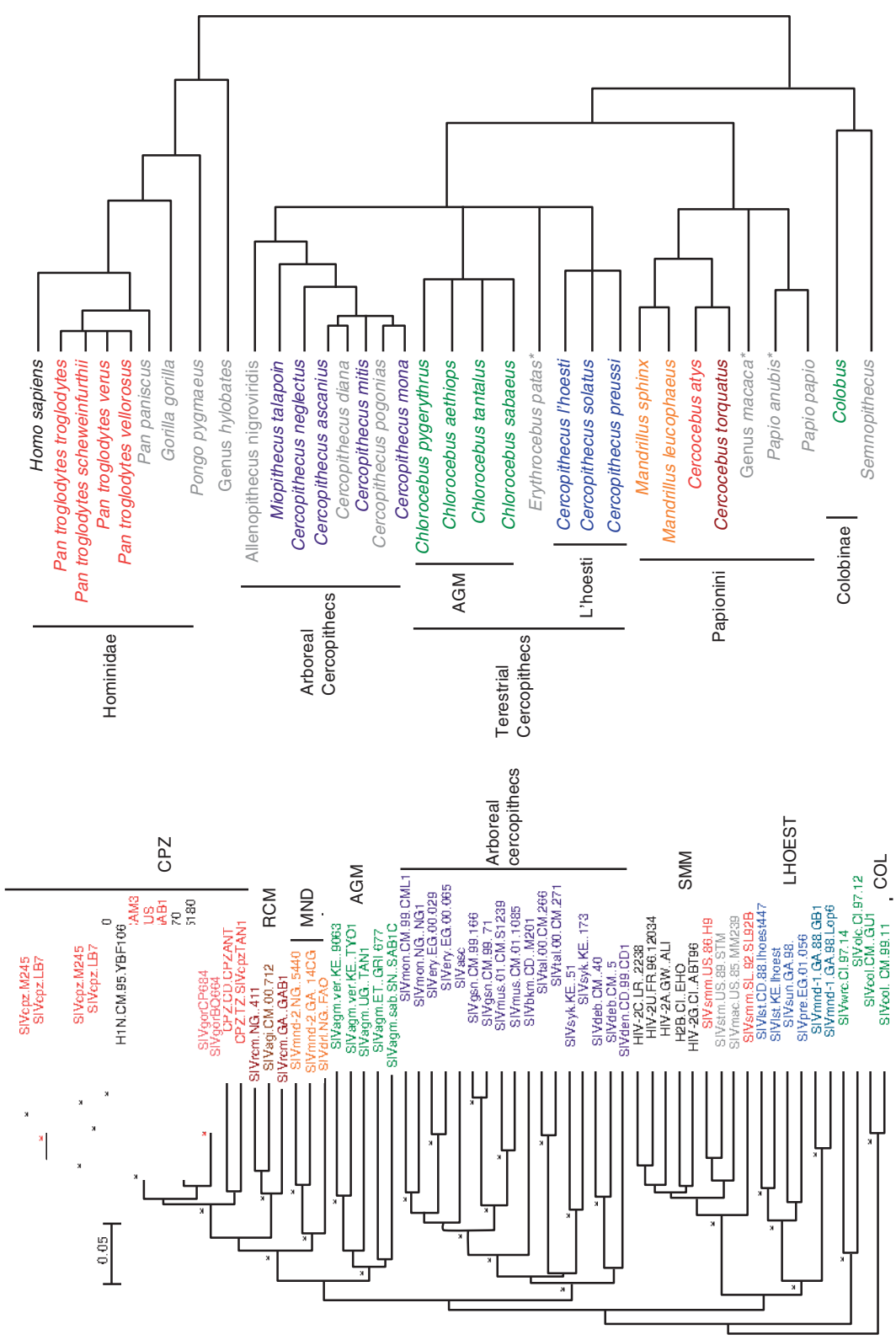


Figure 1 Comparison between SIV phylogeny (a) and primate phylogeny (b). Neighbor-joining tree constructed from available SIV sequences (a); primate phylogeny is a schematic using relationships cited in the text (b). While general alignment of host vs. virus can be observed, cross-species transmissions and viral recombination events make this correlation less than absolute. Asterisks indicate significant bootstrap values. Adapted from VandeWoude S and Apetrei C (2006) Going wild: Lessons from T-lymphotropic naturally occurring lentiviruses. *Reviews in Clinical Microbiology Reviews* 19: 728–762, with permission from American Society for Microbiology.

are divided into two tribes: Papionini (mangabeys (*Cercocebus* and *Lophocebus*), baboons (*Papio*), mandrills and drills (*Mandrillus*), gelada (*Theropithecus*), and the Asian genus, *Macaca*) and Cercopithecini (25 species in 3 arboreal genera: *Allenopithecus*, *Miopithecus*, and *Cercopithecus*, and three terrestrial genera: *Erythrocebus*, *Chlorocebus*, and *Cercopithecus lhoesti* supergroup).

The approximate equidistance among the major SIV lineages does not always match the relationships among their hosts (**Figure 1**). Thus, terrestrial monkeys form a single clade indicating that the evolutionary transition between arboreality and terrestriality has occurred only once among the extinct lineages. However, each of the terrestrial genera are infected with specific viral lineages. Arboreal guenons are infected with a cluster of viruses sharing biological properties and structural features. Papionini monkeys are infected with related viruses, though a higher proportion of recombinant viruses can be observed in these monkeys.

Genome Organization and Composition

SIVs have a complex genomic structure with three structural genes – *gag* (group antigen gene), *pol* (polymerase), and *env* (envelope) – and several accessory genes whose number varies in different SIVs. The accessory genes *vif* (virus infectivity factor), *tat* (transcriptional *trans*-activator), and *rev* are facilitators of viral transcription and activation; *tat* and *rev* each consists of two exons; *nef* induces CD4 and class I downregulation. Three accessory genes are specific for primate lentiviruses: *vpr*, *vpx*, and *vpu*. All primate lentiviruses harbor *vif*, *rev*, *tat*, *vpr*, and *nef*. The presence of *vpx* and *vpu* is variable and defines three patterns of genomic organization (**Figure 2**): (1) SIVsyk, SIVasc, SIVdeb, SIVblu, SIVtal, SIVagm, SIVmnd-1, SIVlhoest, SIVsun, and SIVcol contain no *vpx* or *vpu*; (2) Papionini viruses, SIVsmm, SIVmac, SIVstm, SIVrcm, SIVmnd-2, SIVdrl together with HIV-2 harbor a *vpx* gene, acquired following a nonhomologous recombination and duplication of the *vpr*; and (3) SIVcpz, SIVgor, SIVgsn, SIVmus, SIVmon, SIVden together with HIV-1 encode a *vpu* gene. *vpu* first appeared in cercopithecines, which appear to be the reservoir for viruses in the SIVcpz/HIV-1 lineage. SIVblu, SIVolc, SIVwrc, SIVbkm, SIVery, and SIVagi have not yet been completely sequenced; therefore, their classifications are pending.

SIV Recombination

Virus substrain recombination is a hallmark of SIVs and represents an important intrinsic mechanism other than rapidly accumulating point mutations for developing strains adapted to evade host defense mechanisms or for cross-species transmission. The most critical recombination of SIVs appears to be that involving SIVgsn/mon/mus and SIVrcm which resulted in the origin of the chimpanzee SIVcpz. Subsequent cross-species transmission from chimpanzees to humans is the root cause of the HIV/AIDS

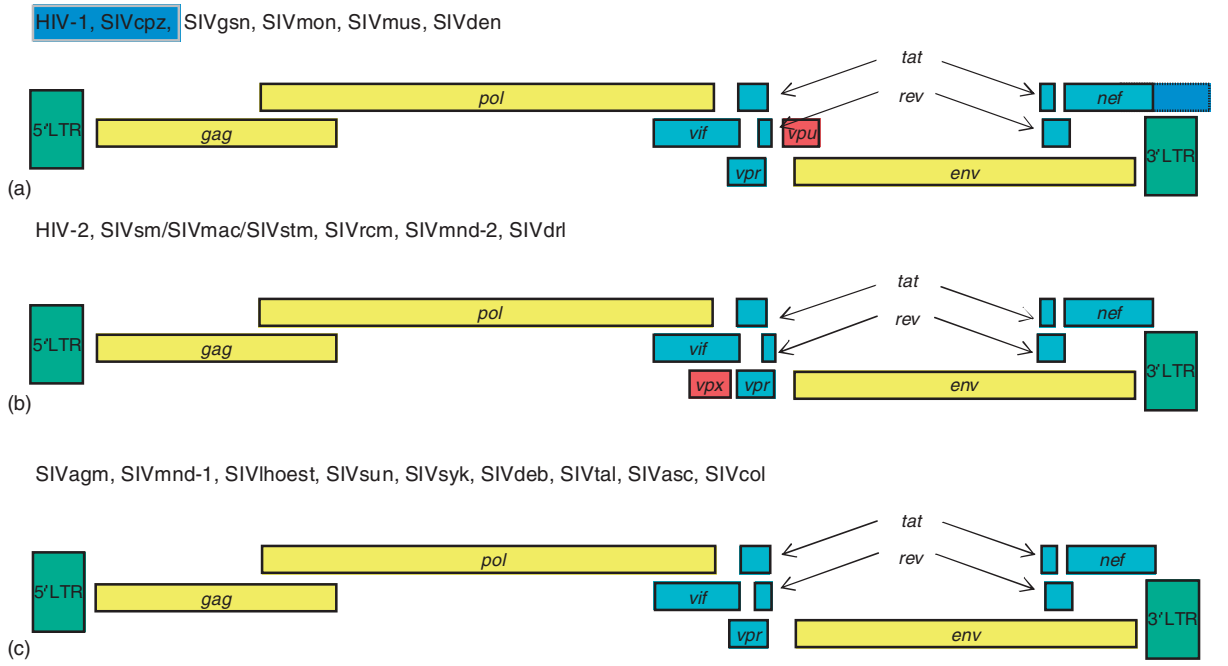


Figure 2 Genomic organization of the SIV strains belonging to different genomic types. SIV classification based on genomic structures is not superimposable on phylogenetic relationships. For references, see **Table 1**. Adapted from VandeWoude S and Apetrei C (2006) Going wild: Lessons from T-lymphotropic naturally occurring lentiviruses. *Reviews in Clinical Microbiolology Reviews* 19: 728–762, with permission from American Society for Microbiology.

pandemic. Other recombinant SIVs include SIVagm.sab (containing SIVrcm-like fragments) and SIVmnd-2/SIVdrl (mosaic between SIVrcm and SIVmnd-1).

Host Range

Typically, SIVs are restricted to their host species (species-specific). However, cross-species transmissions can occur as rare events, the most notable of which were those of SIVrcm and SIVgsn/mon/mus to chimpanzees to generate SIVcpz and those of SIVcpz and SIVsmm to humans to generate HIV-1 and HIV-2, respectively. Macaque exposure to SIVsmm, SIVagm, SIVsun, and SIVlho resulted in persistent infection and induced AIDS. In the wild, SIVagm has been isolated from a yellow baboon (*Papio cynocephalus*), a chacma baboon (*P. ursinus*), and patas monkey (*Erythrocebus patas*). In captivity, in Kenya, SIVagm.ver was transmitted to a white-crowned mangabey (*Cercocebus lunulatus*). None of these recipient species have been reported to date to carry a specific SIV, which may explain the higher susceptibility to cross-species transmitted infection. It is not known if SIVagm is endemic or pathogenic in these species. SIVmnd-1 also resulted from cross-species transmission of SIVsun from sympatric *Cercopithecus lhoesti solatus*. The reasons for success of cross-species transmission infections, and the factors necessary for infection to result in pathogenicity, have not been clearly delineated.

Coreceptor Usage

Similar to HIV-1, SIVs use CD4 as the binding receptor, and chemokine co-receptors such as CCR5 and CXCR4. Most of the SIVs naturally infecting African NHPs use CCR5 as the main co-receptor. Different from HIV-1, for which a switch in viral tropism from R5 ('macrophage' tropic) to X4 ('lymphocyte' tropic) occurs with disease progression, no correlation between co-receptor usage and pathogenesis *in vivo* can be established for SIVs. SIVmnd-1, SIVagm.sab and some strains of SIVsmm use CXCR4, with no pathologic consequence. Experimental infection of sabeaus AGMs with SIVagm.sab (an X4/R5 virus) did not show a particular pattern of viral replication or disease progression. SIVrcm uses the CCR2b co-receptor for viral entry as a consequence of a 24-bp deletion in the CCR5 gene. As such, this example illustrates selected viral evolution, similar to CXCR4 infection of humans who possess the delta-32 mutation in the CCR5 gene.

Diagnosis

Antibody Detection

Serology is the gold standard for studying the prevalence of SIVs in NHPs. Commercial HIV-1/HIV-2 enzyme-linked immunosorbent assay (ELISA) and Western blot assays can be used for anti-SIV antibody screening in NHPs due to cross-reactivity with other lentiviral lineages. For a more sensitive detection of SIVs, two strategies are available: use of a highly sensitive line assay (INNO-LIA HIV, Innogenetics) as a screening test; more than 10 different new SIV types have been identified using this strategy. Alternatively, the use of SIV-specific synthetic peptides allows for increased sensitivity (Gp41/36 peptide) and specificity (V3 peptides); several SIVs have been discovered using this technique.

Propagation and Assay in Cell Culture

The efficiency of *in vitro* isolation of SIVs varies widely. The ability to replicate in human PBMC or T-cell lines has been documented for SIVcpz, SIVsm/SIVmac, SIVagm, SIVlhoest, SIVmnd-1, SIVrcm, SIVmnd-2, and SIVdrl and constitutes the major argument for the threat that these viruses may pose for humans (**Table 3**). SIVs' ability to infect human macrophages has also been reported for SIVsmm, SIVagm, and SIVmnd. SIVsun and SIVsyk cannot replicate in human peripheral blood mononuclear cells (PBMCs) or macrophages. SIVagm replication in human PBMCs is strain specific. SIVmnd-1 was reported to replicate in human PBMCs but not in macrophages. Some SIVs might require special culture conditions.

Epidemiology

Prevalence in the Wild

Due to their number, genetic diversity, and large distribution in sub-Saharan Africa, guenons (tribe Cercopithecini) are the largest reservoir species for SIV. SIV prevalence is high (50–60%) in some monkey species (AGMs, SMs, mandrills, l'Hoest and Syke's monkeys) and significantly lower (4–5%) in others (greater spotted nose monkeys, mustached monkeys, or agile mangabeys). Only two chimpanzee subspecies and one of gorilla were reported to carry SIVs at low prevalence levels. Geographical foci of SIVcpz infection were defined within the endemic area. The highest SIVcpz prevalence was observed in Cameroon (10%), in agreement with HIV-1 emergence in that area. The SIVgor strains also originated from Cameroon. No evidence of SIV infection was thus far reported for some African species, most notably baboons and some species of mangabeys.

Modes of Transmission

Epidemiologic patterns of SIV seroconversion in natural hosts showed the most efficient virus transmission during adult contact, similar to HIV-1, which is spread by sexual contact via primarily mucosal exposure. Horizontal

Table 3 Host range of *in vitro* replication SIV in different blood subsets and human T-cell lines

Growth support	*Cell description*	*SIVcpz*	*SIVsm*	*SIVmac*	*SIVagm*	*SIVlhoest*	*SIVsun*	*SIVmnd-1*	*SIVsyk*	*SIVtal*	*SIVrcm*	*SIVmnd-2*	*SIVdrl*
human PBMC[a]		+	+	+	±	+	−	+	−	−	+	+	+
human MDM[b]		−	+	+	±	+	−	−	−		±		
macaque PBMC			±	+					−	−		+	+
chimpanzee PBMC		+	−			+		+	−	−	−	−	−
MT2	T-cell line		±		+	−	−			−	−	−	−
C8166	T-cell line				+	+	+			−		−	−
H9	Cloned from Hut78		+	+	−	−	−				+	−	−
MT4	T-cell line			+	+	+	+				−	−	+
U937	Promonocytic cell line			−	−	−	−		−		−	−	−
SupT1	T-cell line		+	−	+	+	+		+	−	−	+	+
PM1			±		−	−	−				+	−	−
Hut78	T-cell line		+	+	−	−	−					−	−
Molt 4 Clone 8	T-cell line	+	−	−	+	+	+		−	−	−	+	+
CEMss	T-cell line	+			+	+	+				−	−	+
CEMx174	T-cell–B-cell hybrid line		+	+	−	+	−		++	+	−	+	+

[a]PBMC-peripheral blood mononuclear cells.
[b]MDM-monocyte-derived macrophages.

transmission also occurs by biting or aggressive contact for dominance. While maternal to offspring transmission has been reported, it is relatively rare compared to horizontal transmissions.

Pathogenesis and Pathology

Pathogenicity of Natural SIV Infection

For 20 years, it was believed that natural SIV infections were nonpathogenic. This was a major paradox given the context of an active viral replication and high prevalence levels. However, occasionally, natural SIV infection of mandrills, AGMs, and SMs may eventually lead to the development of immunodeficiency. Cases of progression to AIDS in African NHP hosts are rare, possibly because host↔virus adaptation has occurred, resulting in a long-term persistent infection with an incubation period that exceeds the normal life span of the naturally infected animal.

AIDS was also reported to develop in African NHPs after infection with heterologous viruses (an SIVsmm-infected black mangabey, HIV-2-infected baboons, and a subset of HIV-1-infected chimpanzees). In these cases, disease progression occurred earlier than in naturally infected African NHP hosts, and the outcome of cross-species transmitted SIV infections varied widely, with some animals clearing the cross-species transmitted SIV, others being persistently infected (albeit without disease progression) and the rest progressing to AIDS.

Cell and Tissue Tropism

Upon infection, SIVs are disseminated to tissues by the blood. The target cells are CD4+ T lymphocytes and macrophages, with lymphocytes vastly predominating in terms of infected cells. The major sites of SIV replication are the gastrointestinal tract, lymph nodes (LNs), spleen, and other lymphoid tissues. Natural hosts for SIV infection (SMs, AGMs, mandrills, and chimpanzees) express lower levels of CCR5 on CD4+ T cells in blood and mucosal tissues, compared to immunodeficiency-susceptible hosts (macaques, baboons, and humans). Moreover, chimpanzees, which are more recent hosts of SIV, show an intermediate level of CD4+ CCR5+ T cells. As CCR5 is the main co-receptor for SIVs, African species with endemic naturally occurring SIVs may be less susceptible to pathogenic disease because they have fewer receptor-expressing targets for infection, leading to an evolutionary mechanism of 'passive co-existence' between SIV replication and natural host immune system function.

Virus Replication *In Vivo*

The lack of disease in African NHPs is not associated with effective host containment of viral replication, this is in contrast to HIV/SIV pathogenic infections for which levels of plasma viral loads (VLs) are the best predictor of the disease progression. Experimental SIV infections in natural hosts (SMs, AGMs, and mandrills) showed a consistent pattern of SIV replication with a peak of viremia (10^6–10^9 copies per ml of plasma) occurring around days 9–11 post infection, followed by a sharp decline (1–2 logs) and attainment of a stable level of VL (set point), which is maintained at high levels (10^5–10^6 copies per ml of plasma) during chronic infection (**Figure 3**). Experimental data were confirmed in naturally SIV-infected African NHPs, where chronic VLs were higher than in HIV-1 chronically infected asymptomatic patients and remained relatively constant over years. Some species-specific differences in viral replication between different African NHP species can occur without significant pathogenic consequences: SIV VLs are generally lower in AGMs than in other African NHP species. SIV proviral loads in the LNs are also 100-fold lower in AGMs than in naturally infected SMs or MNDs.

Immune Response and Persistence

Acute SIV infection in the natural host induces massive mucosal CD4+ T-cell depletion, of the same magnitude as in pathogenic HIV/SIV infection. However, during chronic infection, in spite of persistent high viral replication, there is a partial immune restoration of CD4+ T-cells in natural hosts, probably as a consequence of the preservation of a mucosal immunologic barrier and normal levels of immune activation. In marked contrast to HIV infection, in which a failure of the lymphoid regenerative capacity is an important factor in the pathogenesis of the immunodeficiency, the regenerative capacity of the CD4+ T-cell compartment is fully preserved in natural hosts and may play a key role in determining the lack of disease progression. Interleukin-7 (IL-7) has a critical role in preserving T-cell regeneration and in avoiding CD4+ T-cell depletion and disease progression in natural infections.

The high level of viral replication during chronic SIV infection in natural hosts is associated with low level of immunologic pressure, T-cell activation and proliferation, and apoptosis, resulting in limited bystander pathology. Thus, natural hosts of SIV are not confronted with the massive tissue destruction observed in HIV-1/SIVmac infection. This equilibrium is probably disrupted after cross-species transmission of viruses, when virus penetrates a new ecological niche, inducing a different immune response.

In natural SIV infections, *de novo* immune responses are muted compared to pathogenic HIV/SIVmac infections that induce robust neutralizing and cellular immune responses and continuous immune escape. Therefore, it is believed that natural SIV infections are characterized by tolerance to the virus or to specific antigens or epitopes,

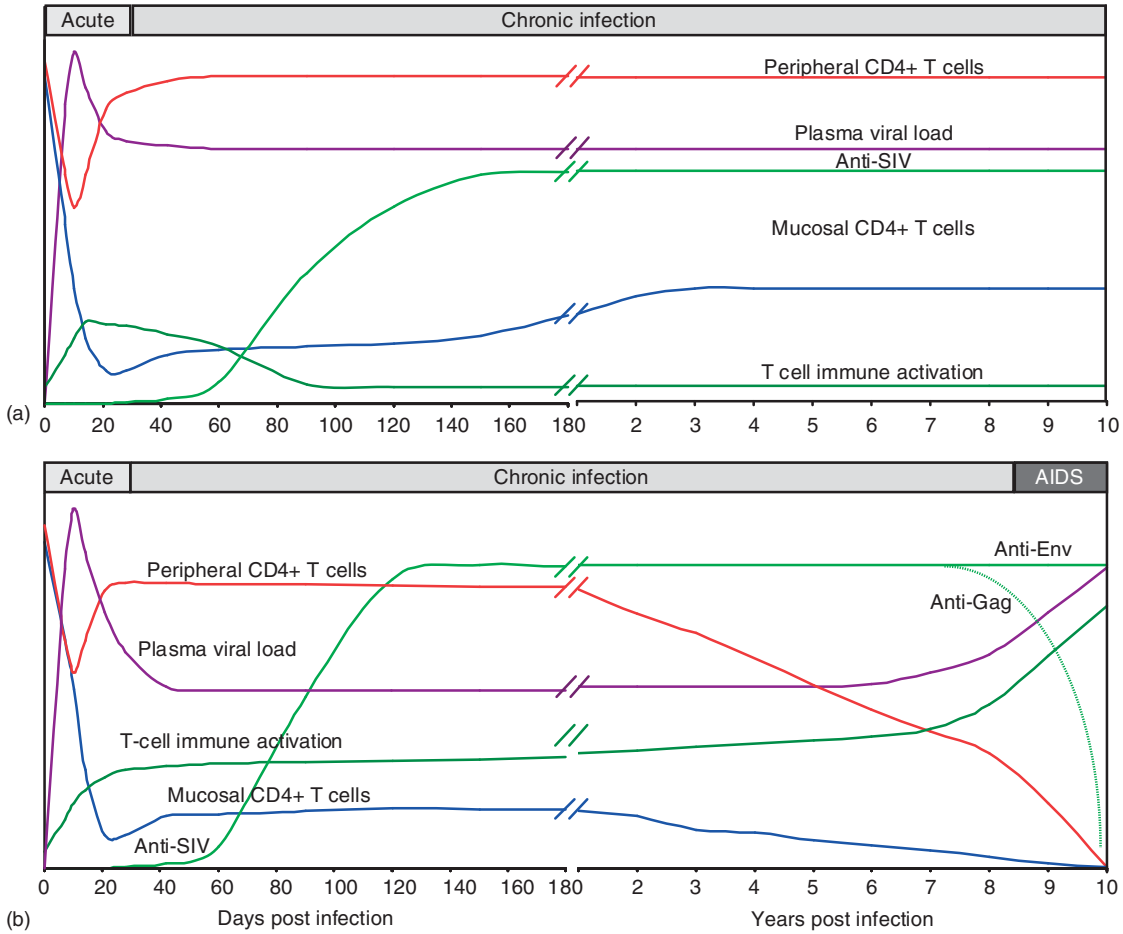


Figure 3 Pathogenesis of SIV infection in natural hosts (a) compared to the pathogenesis of HIV-1 infection (b). No significant difference can be observed between the two models during acute infection. During chronic SIV infection, natural hosts harbor higher viral loads, lower antibody titers, lower levels of T-cell immune activation. CD4+ T-cell levels are maintained at near pre-infection levels in natural hosts. Mucosal CD4+ T cells are partially restored during chronic infection, despite high levels of viral replication. Progression to AIDS is absent in most cases, being a rare outcome of SIV natural infection.

or by immune responses that differ qualitatively and quantitatively from those observed in pathogenic infections. Antibody responses are observed in natural SIV infection, but mainly directed to Env rather than Gag, as in SIVmac infection. The intensity of the antibody response is lower in natural hosts: for equivalent VL, antibody titers in SMs are about one log lower than in RMs.

Neutralizing antibodies are rarely detected in SIV-infected SMs, while SIVagm is susceptible to neutralization depending on the cell line used in the assay. In contrast to HIV-1, SIVagm infectivity is enhanced by the addition of soluble CD4 and this enhanced infectivity can be abrogated by SIVagm-specific antibodies. However, very high amounts of passively transferred specific immunoglobulins failed to prevent SIVagm infection suggesting that humoral immune response in AGMs is largely ineffective.

SIV-infected SMs and AGMs develop cytotoxic T-lymphocyte responses that are functional to some degree in controlling viral replication. Moreover, there is evidence of transient but massive expansion of CD8+ T cells in infected AGMs, SMs, and mandrills during acute infection, showing that the immune system of natural hosts is influenced by SIV infection. It is not yet clear if CD8+ T-cell expansion results from active stimulation of the specific immune response or from a nonspecific stimulation of the immune system in general.

SIV-specific T-cell responses can be detected in the majority of naturally SIV-infected NHPs. However, their magnitude is generally lower than in HIV-infected patients. In addition, no correlation was found between breadth or magnitude of SIV-specific T-cell responses and either VLs or CD4+ T-cell counts. Moreover, the magnitude of the SIV-specific cellular responses did not appear to determine the level of T-cell activation and proliferation in SMs and AGMs. Therefore, the presence of a strong and broadly reactive T-cell response to SIV antigens is not a requirement for the lack of disease progression in natural infections; conversely, the complete

suppression of SIV-specific T-cell responses (i.e., immunologic tolerance and/or ignorance) is not required for the low levels of T-cell activation that are likely instrumental in avoiding AIDS.

Both acute and chronic natural SIV infections (SMs, AGMs, and mandrills) are associated with lower levels of T-cell activation, pro-inflammatory responses, immunopathology, and bystander apoptosis than pathogenic HIV/SIV infection. In addition, natural hosts of SIV maintain T-cell regenerative capacity, with normal bone marrow morphology and function, normal levels of T-cell receptor excision circle (TREC)-expressing T cells and preserved LN architecture. This downregulation of the immune response favors preservation of CD4+ T-cell homeostasis and is completely different from pathogenic HIV/SIV infections for which chronic immune activation and proliferation drive excessive activation-induced T-cell apoptosis, and ultimately result in the collapse of the immune system and progession to AIDS. Altogether, this is consistent with the hypothesis that chronic immune activation is a major determinant of disease progression during HIV infection.

In AGMs, low immune activation levels are due to a strong anti-inflammatory response (with induction of TGF-β1 and FOXP3, and a significant increase in IL-10 expression), which occurs early in the SIVagm infection. Together with an early increase in the levels of CD4+ CD25+ T cells, this results in the rapid establishment of an anti-inflammatory environment which may prevent damages to the mucosal immunologic barrier, microbial translocation, and thus aberrant chronic T-cell hyperactivation that is correlated with progression to AIDS during HIV-1/SIVmac infection.

Virulence

Viral factors may be related to a lack of virulence in natural SIV infections. In RMs, SIVmac *nef* gene deletion mutants were reported to replicate poorly *in vivo* and to be nonpathogenic, which corroborates the description of *nef* gene mutations of HIV-1-infected long-term progressors. Nef downregulates CD4, CD28, and the class I major histocompatibility complex, resulting in virus immune evasion. Nef may also enhance the responsiveness of T cells to activation, but this effect is not uniformly observed among SIVs. All SIVs from African monkeys have open reading frames corresponding to a functional Nef, and therefore the *nef* structure cannot account entirely for differences in pathogenicity. Nef proteins from the great majority of primate lentiviruses, including HIV-2, down-modulate T-cell receptor-CD3, which subsequently blocks T-cell activation. In contrast, Nef proteins derived from HIV-1 and closely related SIVs do not induce CD3 downregulation, which may have predisposed the simian precursor of HIV-1 to greater pathogenicity in humans. However, simian counterparts of HIV-1 (SIVcpz and SIVgsn/SIVmon/SIVmus), which do not induce CD3 downregulation, do not typically induce AIDS in their natural hosts. Further, SIVmac, which induces CD3 downregulation, is even more pathogenic in RMs than HIV-1 is in humans, suggesting that this accessory gene does not solely account for virulence.

Clinical and Pathologic Features

In contrast to macaques in which SIV infection constantly leads to an AIDS-like disease characterized by opportunistic infections and cancers, SIV infection in natural hosts generally does not show any clinical or pathological abnormalities. During progression to AIDS, SIV-infected RMs initially develop lymphadenopathy with confluent follicular hyperplasia, followed by LN atrophy due to lymphoid depletion and fibrosis. In contrast, SIV-infected natural hosts display normal LN morphology without evidence of either hyperplasia or depletion. No follicular trapping or CD8+ T-cell infiltration of the germinal centers and no replacement of the normal LN architecture with connective tissue is observed in chronic SIV infection of natural hosts. Also, no thymic disinvolution, nodular lymphocytic infiltrates, or giant cell disease are seen in SIV-infected natural hosts. The few AIDS cases reported in African NHP hosts presented with the entire range of diseases and pathologic lesions of AIDS.

Host Genetic Resistance

SIV species specificity has typically been ascribed to factors such as virus–host receptor compatibility and cellular machinery needed to direct viral replication. Specific host factors also prevent SIV cross-species infections *in vitro*.

Cytidine deaminase and Vif

Vif is involved in species specificity of SIVs. Its cellular target is a member of the cytidine deaminase APOBEC family. The cellular deaminase is incorporated into the virion during the reverse transcription to direct the deamination of cytidine to uridine on the minus strand of viral DNA. Deamination results in catastrophic G-to-A mutations followed by inactivation and/or degradation of the viral genome. At least two primate APOBEC family members (APOBEC3G and APOBEC3F) play a central role in antagonizing viral replication because they are expressed in natural targets of SIVs, including lymphocytes and macrophages. Lentiviruses are able to successfully infect and replicate in host target cells containing APOBEC when host-adapted Vif interferes with this mechanism. Vif activity is species specific: human APOBEC3G is inhibited by HIV-1 Vif but not by SIVagm Vif, whereas AGM APOBEC3G is inhibited by SIVagm Vif, but not by HIV-1 Vif. This specificity relies on a single amino acid change that can alter the ability of *vif* to interfere with APOBEC activity.

TRIM5-α

The cytoplasmic body component TRIM5-α, previously referred to as REF-1 and LV-1, restricts HIV-1 infection of monkey cells. TRIM5-α interferes with the viral uncoating step that is required to liberate viral nucleic acids into the cytoplasm upon viral binding and fusion with the target cell. Sensitivity to TRIM5-α restriction is dictated by a small region in the viral capsid gene, previously shown to be involved in cyclophilin A binding. Subtle amino acid differences in this region influence the strength of binding to TRIM5-α and, hence, relative sensitivity to its restriction. HIV-2, but not closely related SIVmac, is highly susceptible to RM TRIM5-α. Furthermore, HIV-2 is weakly restricted by human TRIM5-α, which may contribute to the lower pathogenic potential of the HIV-2 vs. HIV-1 in humans. Strategies to exploit TRIM5-α restriction for intervention of HIV-1 replication are under development.

See also: Host Resistance to Retroviruses; Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Origin; Human Immunodeficiency Viruses: Pathogenesis; Retroviruses: General Features; Simian Immunodeficiency Virus: Animal Models of Disease; Simian Immunodeficiency Virus: General Features.

Further Reading

Beer BE, Bailes E, Sharp PM, and Hirsch VM (1999) Diversity and evolution of primate lentiviruses. In: Kuiken CL, Foley B, Hahn B, *et al.* (eds.) *Human Retroviruses and AIDS 1999*, pp. 460–474. Los Alamos, NM: Theoretical Biology and Biophysics Group and Los Alamos National Laboratory.

Chakrabarti LA (2004) The paradox of simian immunodeficiency virus infection in sooty mangabeys: Active viral replication without disease progression. *Frontiers in Biosciences* 9: 521–539.

Drucker E, Alcabes PG, and Marx PA (2001) The injection century: Massive unsterile injections and the emergence of human pathogens. *Lancet* 358(9297): 1989–1992.

Gordon S, Pandrea I, Dunham R, Apetrei C, and Silvestri G (2005) The call of the wild: What can be learned from studies of SIV infection of natural hosts? In: Leitner T, Foley B, Hahn B, *et al.* (eds.) *HIV Sequence Compendium 2004*, pp. 2–29. Los Alamos, NM: Theoretical Biology and Biophysics Group and Los Alamos National Laboratory.

Hirsch VM (2004) What can natural infection of African monkeys with simian immunodeficiency virus tell us about the pathogenesis of AIDS? *AIDS Reviews* 6(1): 40–53.

Muller MC and Barre-Sinoussi F (2003) SIVagm: Genetic and biological features associated with replication. *Frontiers in Biosciences* 8: D1170–D1185.

Norley S and Kurth R (2004) The role of the immune response during SIVagm infection of the African green monkey natural host. *Frontiers in Biosciences* 9: 550–564.

Sharp PM, Shaw GM, and Hahn BH (2005) Simian immunodeficiency virus infection of chimpanzees. *Journal of Virology* 79(7): 3891–3902.

VandeWoude S and Apetrei C (2006) Going wild: Lessons from T-lymphotropic naturally occurring lentiviruses. *Clinical Microbiology Reviews* 19: 728–762.

Simian Retrovirus D

P A Marx, Tulane University, Covington, LA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Codon The basic unit of the genetic code consisting of three consecutive nucleotides in DNA that specify the order of amino acids in a protein.

Monocistronic mRNA Messenger RNA that codes for a single protein.

Monoclonal antibody Antibody made *in vitro* that reacts with a single protein.

Pneumocystis carinii A fungus that causes pneumonia, especially in persons or animals with suppressed immune systems.

Polyprotein A precursor protein that is cut after its synthesis into smaller functional proteins.

Ribosomal frameshifting A change in the translational reading frame that allows the synthesis of different proteins from a single region of overlapping genes. For example, if the genetic code begins on the first of the three nucleotides of a codon, the out-of-frame code would begin on the second nucleotide. A second frameshift beginning on the third nucleotide is also possible. Frameshifting is an economical way to code for proteins.

Syncytium Cell-derived structure in culture or *in vivo* giant cell consisting of fused cells having more than one nucleus.

Virion Infectious viral particle containing the genome of the virus and a full complement of viral proteins required for growth and replication.

Western blot Nitrocellulose blotting paper containing viral polypeptides sorted by molecular size. The blot is soaked in diluted serum suspected of containing antibodies. A positive test requires binding to polypeptides from at least two different viral genes. More weight is often given to env polypeptide reactions. False results against a single *gag* coded protein commonly occur and are reported as indeterminant.

Zoonosis Microbial infection (virus, bacteria, or parasite) acquired directly through animal contact that progresses to a clinical disease.
Zoonotic infection Microbial infection (virus, bacteria, or parasite) acquired directly through animal contact that may or may not progress to disease.

Introduction

Simian type D retroviruses (SRVs) and Mason–Pfizer monkey viruses (MPMVs) are members of the family *Retroviridae* and comprise one species belonging to the genus *Betaretrovirus*. These viruses naturally infect members of the nonhuman primate genus *Macaca*, commonly known as macaque monkeys. SRV appears to be highly species specific, although the virus will replicate in the cells of other primate species, including tissue culture cells of human origin. Betaretroviruses are characterized by their simple genomic structure consisting of 4 genes, *gag*, *pro*, *pol*, and *env* (**Figure 1**).

MPMVs and SRVs are exogenous retroviruses, meaning that the virus is acquired from another infected macaque monkey. This transmission mechanism distinguishes SRVs from endogenous D retroviruses that are inherited and are a part of the genome of the host species. Endogenous D retroviruses occur in both New and Old World monkeys, including African baboons as well as Asian langur and South American squirrel monkeys.

Seven distinct isolates are known and each can be distinguished by serum neutralization tests or competitive binding tests between homologous p27 *gag* proteins. They are named SRV serotypes 1–7. Serotype 3 is also known as MPMV. A nomenclature has been devised that is similar to that used for influenza viruses and is an aid for tracking isolates and their origins. For example, D1/rhe/CA/84 represented the D retrovirus that was isolated at the California Primate Center Davis, CA from a rhesus macaque with an acquired immune deficiency syndrome (AIDS)-like disease and was reported in 1984. D2/Cel/OR/85 was a 1985 SRV-2 isolate from a Celebes black macaque with retroperitoneal fibromatosis (RF) at the Oregon Primate Center. MPMV is the prototype of this viral species and retains its original name for historical reasons.

History

In 1971, the first D retrovirus isolate was reported. The virus was recovered from a rhesus monkey with a

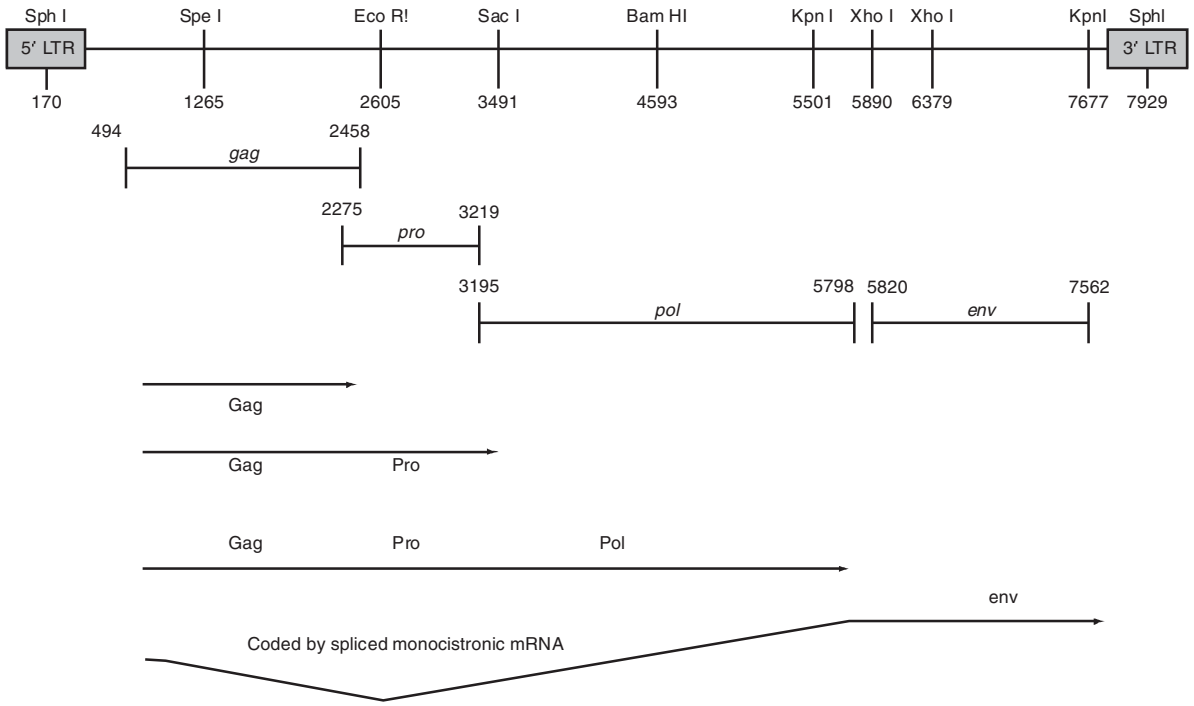


Figure 1 Map of the genome of primate D retroviruses. Top is the provirus that is 8105 nt and codes for four genes in three separate reading frames, *gag*, *pro*, *pol*, and *env*. The messenger RNAs consist of two forms, a genomic-length mRNA that is translated into the polyprotein Gag. After frameshifting(s), Gag-Pro and Gag-Pro-Pol precursors are made. Polyproteins are cleaved into smaller functional virion proteins. The second mRNA form is a spliced mRNA that encodes the Env proteins, SU (also named gp70) and TM (also named gp20). Reprinted from Marracci GH, Avery NA, Shiigi SM, *et al*. (1999) Molecular cloning and cell-specific growth characterization of polymorphic variants of type D serogroup 2 simian retroviruses. *Virology* 261: 43–58, with permission from Elsevier.

mammary carcinoma. The virus was named after the Mason Institute in Worcester, Massachusetts, where the macaque had been housed. The Pfizer Pharmaceutical Company had supported research using this female monkey, hence the name Mason–Pfizer monkey virus (MPMV). The impact of MPMV was greatly enhanced because the virus was isolated directly from cancerous breast tissue. Because of this association with neoplasia, MPMV was classified as an oncornavirus (Onco RNA for cancer-causing RNA virus). This classification persisted for many years.

The finding of MPMV in monkey breast cancer tissue prompted numerous reports in the 1970s of D retroviruses in association with human breast cancer. With the development of sensitive detection techniques along with complete sequencing of the MPMV and SRV genomes, it became apparent that nonhuman primate D retroviral sequences were not causally associated with human tumors. A 1975 report on immune impairment in neonatal monkeys infected with MPMV marked the beginning of our understanding of primate D retroviruses as a causative agent of simian AIDS. The modern era of type D retrovirus research deals with its immunosuppressive properties.

AIDS, the disease, was discovered in 1981 in persons displaying symptoms of a severe and fatal immunodeficiency. The first detailed report consisted of four men with *Pneumocystis carinii* pneumonia, evidence of cytomegalovirus infection (CMV), and Kaposi sarcoma, a skin cancer not commonly seen in young men. Early theories on the cause of AIDS implicated drug-induced impairment of the immune system. Drugs were known to suppress the immune system, but the idea that severe and fatal immune deficiency could be caused by a virus was not yet appreciated. A breeding group of rhesus monkeys that was housed outdoors at the California National Primate Research Center in Davis, CA, played a significant role in changing that perception. This breeding group had a higher mortality rate than monkeys in other outdoor groups only a short distance away. The affected animals had diarrhea, cytomegalovirus infections, and fibrosarcoma, a tumor that resembled Kaposi sarcoma upon microscopic inspection. A link was made between AIDS in monkeys and AIDS in humans. The study of this group of monkeys resulted in the isolation and characterization of a virus related to MPMV. This distinct D retrovirus was named simian retrovirus (SRV) and was used to prove the retroviral etiology of this fatal immune deficiency disease in monkeys. A similar virus was isolated from rhesus macaques at the New England Primate Research Center.

Replication

The synthesis of new progeny virus begins with the DNA provirus that is integrated into a host DNA chromosome (**Figure 1**). The provirus is a double-stranded (ds) DNA copy of the virus genome. The provirus is flanked by two repeated sequences called the long terminal repeat (LTR). The order of the coding and noncoding regions are the LTR, *gag* (for group antigen), *pol* (polymerase), *env* (envelope), and a second copy of the LTR. The viral genome is synthesized from the minus DNA strand of the provirus by host cell enzymes. The newly made viral genome is the same sense as messenger RNA (+-stranded) and is exported from the nucleus without being spliced. The cellular requirement for splicing is bypassed by the interaction of the constitutive transport element (CTE) with cellular proteins. This genomic RNA is translated in the cytoplasm into Gag and Pol viral proteins in a process that involves several steps.

The *gag* gene codes for 654 amino acids that are synthesized as a 78 kDa polyprotein precursor of smaller functional virion proteins. The *gag*-encoded polyprotein is cleaved by a protease enzyme, which in turn is coded by the *pro* gene. This Gag precursor is cleaved into six mature virion proteins, the p10 matrix protein (polypeptide 10 000 Da molecular weight (MW)), pp24 (phosphoprotein), p12 (assembly scaffold domain), p24 major capsid protein, p14 nucleocapsid protein, and p4 (chaperonin binding domain). The major capsid protein, p24 (in M-PMV, p27), is the most abundant protein in the mature virion (**Figures 2(b)** and **3**). The p10 protein is the matrix protein and resides just under the external envelope of the virion (**Figure 3**). The nucleocapsid protein p14 forms a tightly associated complex with the RNA genome. Unlike most other retroviruses, D-type retroviruses form immature capsids that are preassembled in the cytoplasm (**Figure 2** – see arrows). This is facilitated by a cytoplasmic targeting and retention signal in the matrix domain of Gag, which binds to the microtubule motor dynein and targets translating polysomes to the pericentriolar region of the cell where assembly occurs. The p12 region of Gag acts to facilitate this intracytoplasmic assembly process.

The protease (*pro*) gene overlaps the *gag* region by 61 codons (183 nt). The protease is an enzyme that cleaves protein and is highly conserved across the genus with 82.8% and 83.8% identity between SRV-1 and MPMV, respectively. The protease itself is translated from the genome-length mRNA (**Figure 1**) as two polyproteins (Gag-Pro) and Gag-Pro-Pol. Because the *pro* gene is out of frame with respect to *gag*, the polyprotein that contains the protease results from a ribosomal frameshifting mechanism during translation of genome-length mRNA. The final protease protein is produced by two autocatalytic cuts, one at the N-terminus producing a 17 kDa product and a second cut producing the final 13 kDa protease enzyme. The protease is incorporated into the immature capsids in the cytoplasm (**Figure 2** – see arrows). It functions to cleave the *gag*-coded polyprotein into smaller proteins that make up the internal capsid structure of

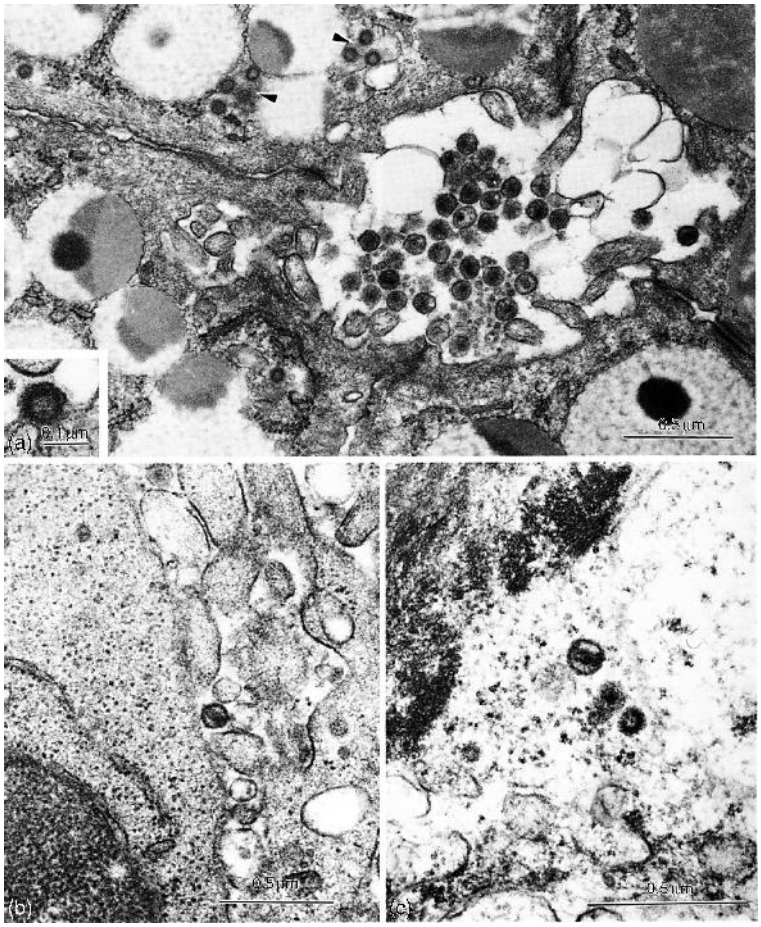


Figure 2 Electron microscopic demonstration of type D retroviral particles in (a) salivary gland, (b) germinal center of a lymph node, and (c) spleen of rhesus monkeys with AIDS. In the salivary gland (a), numerous mature extracellular particles in a small acinar lumen, as well as immature intracytoplasmic A particles (arrows) and a budding particle (inset) are seen. In the germinal center (b), a single mature virion particle adjacent to a lymphoid cell and numerous cellular processes possibly belonging to follicular dendritic cells are seen. (c) In the spleen, a single mature particle is seen in the extracellular space. Modified from Lackner AA, Rodriguez MH, Bush CE, *et al.* (1988) Distribution of a macaque immunosuppressive type D retrovirus in neural, lymphoid, and salivary tissues. *Journal of Virology* 62: 2134–2142, with permission from American Society for Microbiology.

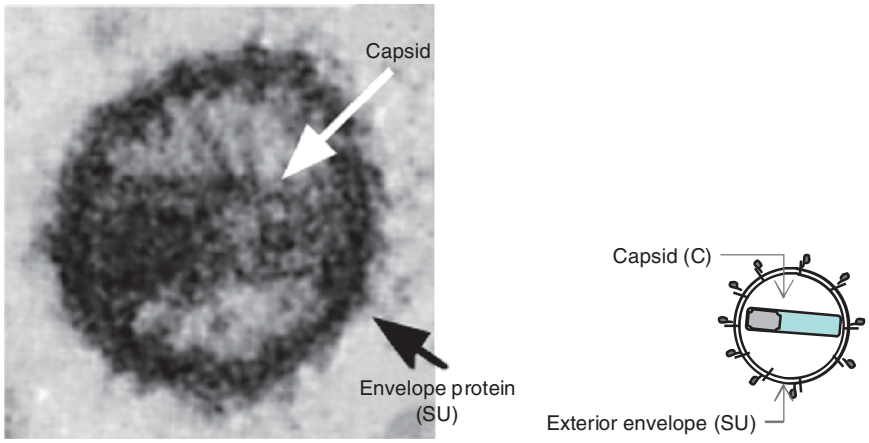


Figure 3 Mature D retrovirus virion showing the external envelope and the major capsid core. Modified from Marx PA, Maul DH, Osborn KG, *et al.* (1984) Simian AIDS: Isolation of a type D retrovirus and transmission of the disease. *Science* 223: 1083–1086, with permission from AAAS.

the virion. The three-dimensional (3-D) structure of the protease has been resolved and this 3-D structure is well conserved across the retrovirus family.

A second ribosomal frameshift within the *pro-pol* overlap region generates a larger polyprotein precursor containing the pol proteins (**Figure 1**). The *pol* gene encodes the reverse transcriptase (RT)-endonuclease and integrase proteins. The gene encoding the integrase of MPMV is located at the 3′ end of the *pol* open reading frame and the RT is encoded by the 5′ end of the gene. The RT, integrase, and endonuclease enzymes function after the progeny virus infects a new cell, and are incorporated into the immature capsids as Gag-Pro and Gag-Pro-Pol precursors. These polyproteins undergo post-translational cleavage during virion maturation to produce separate RT and endonuclease/integrase proteins. They are required to initiate the early steps of replication after the virus enters a host cell to begin a new cycle of replication.

The *env* gene is expressed from the 3′ end of the genome (**Figure 1**). *env* encodes two proteins, the external envelope spike protein (SU for surface) and the transmembrane protein (TM). The mRNA coding of the Env proteins is spliced in the nucleus to remove *gag* and *pol* coding regions. The mRNA is exported to the cytoplasm and is handled like a monocistronic mRNA. The *env* gene product is cleaved into the SU or gp70 (glycoprotein 70 000 Da) (**Figure 1**) and the TM (or gp20). Both the SU gp70 and TM gp20 proteins have sugar molecules bound to some of the amino acids that make up these two proteins. The SU glycoprotein can potentially contain 22 sugar residues. The TM is a membrane-spanning protein that anchors the complex in the virion membrane. An immunosuppressive motif is present within the TM protein and this protein motif may play a role in immune suppression. A monoclonal antibody is available against the TM-gp20 and this antibody is useful for diagnostic testing. The external glycoprotein is the least conserved among the macaque D retroviruses and is reflected in different serotypes. Neutralizing antibodies are defined by binding to the external glycoprotein. Therefore the major biological differences between SRV-1, SRV-2, MPMV, SRV-4, and SRV-5 are in fact differences in the amino acid sequence of Env.

Immature capsids (see arrows in **Figure 2**) are transported to the plasma membrane where they bud through the membrane (**Figure 2** inset) and acquire the Env proteins. The morphology of the completed D-type virion consists of an envelope and a cylindrical or rod-shaped core (**Figure 3**; a virion grown in tissue culture (**Figure 2**), and virions in the extracellular tissue space of an infected rhesus monkey).

The extracellular infectious virion initiates a new round of replication by attaching itself to a susceptible cell via a specific receptor on the surface membrane of the host cell. The receptor molecule for SRV and MPMV is an amino acid transport protein (ATBo) that is present in human tissues and cell lines, including white blood cells.

After entry in the cell, the RT synthesizes a minus DNA strand from the +-sense incoming SRV RNA genome. The RT next catalyzes the synthesis of a complementary plus sense DNA strand, making a dsDNA copy of the D retrovirus RNA genome that contains two LTRs. The LTRs flank the viral genes and, through their interaction with the viral integrase, ensure that the genome will integrate in the correct orientation for expression of the viral genes. Usually DNA codes for RNA, but the RT reverses the process by making DNA from an RNA template, hence its name reverse transcriptase. The integrase then enzymatically integrates the linear dsDNA copy of the original RNA genome into host cell DNA. This dsDNA copy of the virus genome is called a provirus. With transcription of the provirus to form full-length genomic mRNA and spliced env mRNA, the replication cycle is completed and begins a new round.

Natural Hosts

The natural host of SRV and MPMV is the Asian monkey genus *Macaca* or macaques. The known hosts are primarily common laboratory monkeys, such as the rhesus monkey (*M. mulatta*) of northern India, southern China, and geographical areas in between. SRV infection of rhesus macaques in China has been reported. Other macaque species housed at Primate Research Centers are also commonly infected. They include the cynomolgus macaque (*M. fascicularis*), also known as the long-tailed macaque and crab-eating macaque of Southeast (SE) Asia; *M. nemistrina*, the pig-tailed macaque of SE Asia; *M. nigra*, the Sulawesi or Celebes black macaque; and the bonnet macaque, *M. radiata*, of southern India. Feral cynomolgus macaques are infected, showing a natural Asian origin of this virus. Type D retroviruses are a significant problem for laboratory research, since infected animals may develop an AIDS-like disease. The most common SRV isolate in primate centers is SRV-2, followed by SRV-1, SRV-5, and MPMV. SRV-4 is only known from a single outbreak in cynomolgus macaques at a public health laboratory in Berkeley, California.

Transmission

SRV is present in blood, urine, saliva, lymphoid, and nonlymphoid tissues of infected macaques (**Figure 2**). With the possible exception of brain tissue, infectious SRV is found throughout the body. Inoculation of any of these fluids or tissues to rhesus monkeys will transmit the

infection and disease. Although infection is easily transmitted under laboratory conditions, a classic experiment on SRV infections transmitted in outdoor enclosures strongly pointed to natural transmission by bite from infected saliva. In these experiments at the California Primate Center, it was first shown that uninfected monkeys must be in physical contact with infected monkeys for transmission to occur. The need for this contact was proven by keeping uninfected monkeys in the same enclosure with infected monkeys, but separated by two fences creating a 10-foot barrier. The barrier prevented contact between infected and uninfected groups, but allowed birds, rodents, and insects to move freely between the enclosures as well as allowing rainwater to wash back and forth. The result was that only monkeys in physical contact with infected monkeys became SRV infected even after 5 years of testing.

SRV transmission to healthy monkeys was linked to healthy carriers, in particular to female healthy carriers that occupied socially dominant roles in the enclosure. Healthy carriers were infected for as long as 10 years without developing the AIDS-like disease that is characteristic of SRV infections. Repeated testing of the saliva of healthy carriers showed >1 million infectious units of SRV per ml of saliva, hence the likely link between saliva and transmission. Healthy carriers were a major problem for laboratory colonies because they could spread the SRV immune deficiency and themselves remain undetected, compromising the usefulness of these research colonies. The problem is now largely controlled by screening for SRV infection by antibody enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) testing. Infected animals are removed from contact with the rest of the laboratory colony. This test and removal program is a highly successful approach to developing specific pathogen-free colonies for research.

Broad *In Vitro* and *In Vivo* Cell Tropism

SRV has a broad cellular tropism and infects lymphoid, monocytes, and epithelial cells *in vitro*. SRV readily infects human T-cell lines, HuT-78, CEM-SS, MT-4, and SubT-1. The human B Raji cell line is also infected and SRV induces syncytia formation in this cell line. Adherent mononuclear cells from rhesus macaques were also infected *in vitro*. Peripheral blood mononuclear cells collected from infected animals and separated into CD4+ and CD8+ T-lymphocytes were also infected.

In vivo studies carried out on SRV-1-infected rhesus macaques showed infection of epithelial and lymphoid cells in the gut and elsewhere (**Figure 2**). The oral cavity and salivary glands have also been examined. Mucosal epithelia cells were heavily infected as early as 1 month post inoculation. Rarely, Langerhans cells were also shown to be infected using immunohistochemical techniques. Southern blot analyses showed salivary glands and lymphoid tissue to be more heavily infected than brain tissue. The infection of epithelia cells *in vivo* is striking in its widespread nature.

Pathogenesis

The pathogenesis of SRV, MPMV, and SRV-2 is well described. Inoculation of any of these three grown in rhesus monkey tissue culture will induce an AIDS-like disease in most of the infected macaques. Pathogenesis studies are best carried out using virus grown in cells of the original host. Cultivation of the virus in human cells, especially Raji B cells, may attenuate the virus. The clinical course for SRV-1 is typically one-third developing disease in less than 6 months, one-third developing disease in 6 months to 2 years, and a third recover but remain antibody positive for SRV. Recovered animals may develop AIDS after a long clinically latent normal period. The disease is very similar to AIDS in human beings and includes opportunistic infections such as generalized cytomegalovirus disease and its associated pneumonia, wasting, chronic diarrhea unresponsive to therapy, and severe anemia. Infecting with a molecular clone, ruling out adventitious agents, proved the pathogenesis of SRV-1.

The disease induced by SRV is AIDS, but SRV should not be confused with simian immunodeficiency virus (SIV), a lentivirus related to human immunodeficiency virus 2 (HIV-2) that also causes AIDS in rhesus macaques. Macaques are not the natural hosts of SIV, since SIV naturally occurs in only sub-Saharan African cercopithecine monkeys and apes. The species naturally infected with SRV are all Asian macaque species. These two infections can be easily distinguished by specific Western blot. AIDS caused by SRV and SIV are both severe immunodeficiency syndromes, but differ in that SIV-induced AIDS is frequently associated with pneumocystis pneumonia, atypical tuberculosis, and B-cell lymphomas. In contrast, Kaposi sarcoma-like neoplasias are seen exclusively in type D SRV-induced AIDS.

Simian RF

A tumor that occurs in the space behind the abdominal cavity (retroperitoneum) is associated with SRV-2 infections and the retroperitoneal fibromatosis herpesvirus (RFHV). RFHV is the macaque homolog of the human rhadinovirus that is associated with the Kaposi's sarcoma herpesvirus (KSHV). This monkey tumor is therefore an excellent model for Kaposi's sarcoma, one of the tumors that commonly occur in AIDS patients. DNA sequence data identified KSHV related herpes viruses in the RF

tissue of pig-tailed and rhesus macaques. The basic fibroblast growth factor was found to be associated with RF tissues in SRV-2-infected macaques. The fibrosarcoma associated with SRV-1-induced AIDS is also a Kaposi-like tumor.

Vaccines

Two types of vaccines have been successfully tested in the SRV AIDS model. The first used a killed-virus formulation. The virus was inactivated with formalin and injected intramuscularly to induce antibody. The SRV challenge virus used to test the efficacy of the vaccine was prepared in isogenic rhesus monkey kidney cells, therefore ruling out induction of anticellular antibody as a protective mechanism. Protection was associated with neutralizing antibody. This was the first vaccine shown to be effective against a primate retrovirus. The second vaccine used a recombinant vaccinia virus vector. The vaccinia virus vector expressed the envelope proteins (gp70 and gp20) of SRV-1 and MPMV. Upon challenge with live SRV-1 by the intravenous route, both MPMV- and SRV-1-immunized animals were protected. The vaccine therefore conferred cross-protection for SRV types 1 and MPMV, demonstrating *in vivo* their close relationship. The neutralizing antibody did not cross-react with the more distant SRV-2.

Human Infections with SRV and Related Viruses

D-type retroviruses easily infect tissue culture cells derived from humans. Consequentially, several research groups have reported contamination of tissue cell lines. Therefore, there is a strong risk of contaminating cell cultures with this virus in the laboratories. Evidence for human infection that is based only on isolation of a D-type retrovirus from cultured human cells must be viewed with caution.

Human infections with SRV or MPMV have been reported in widely different diseases such as cancer and schizophrenia. Evidence of D-type virus has been reported in children with Burkett's lymphoma and in adult humans with breast cancer. However, direct evidence of SRV as an etiologic agent of human disease is thus far lacking.

An MPMV serological survey of European and African blood donors in Guinea-Bissau revealed 1 of 61 to be weakly positive for the MPMV p27 Gag polypeptide using Western blots. Squirrel monkey retrovirus-specific Western blots also revealed a few additional positive samples. Reaction in a blood donor consisting of only a single polypeptide is usually reported as an indeterminant result and is a false positive reaction. Nevertheless, D virus is widespread in nature and sequences related to the SRVs are found in the genomes of several primate species. Therefore, exposure to the virus is clearly possible. Nevertheless, extensive studies on over 1000 persons with various diseases have failed to find evidence of SRV infections in humans. Diseases tested included lymphoproliferative disease patients, HIV-1 infected persons, persons with unexplained low CD4 lymphocyte counts, blood donors, and intravenous drug users from the USA and Thailand. Serum samples were screened for antibodies against SRV by an ELISA, and reactive samples were re-tested by Western blot. None of the samples were seropositive.

The most convincing evidence for nonpathogenic SRV infections in humans comes from a serosurvey of workers occupationally exposed to macaque D-type retroviruses at primate centers in the USA. Occupational exposure could come from an accidental bite from infected monkeys during routine care such as cage cleaning or accidental exposure by a contaminated needle stick. Two of 231 persons tested were strongly positive by Western blot. Each person displayed antibody to more than one gene of SRV (**Figure 4**). Lanes 1 and 2 showing a reactive band at the 70, 31, 24, and 20 Da markers indicated reaction with the *pol*, *env*, and *gag* gene products. One individual had neutralizing antibody to SRV-2, providing convincing evidence of an

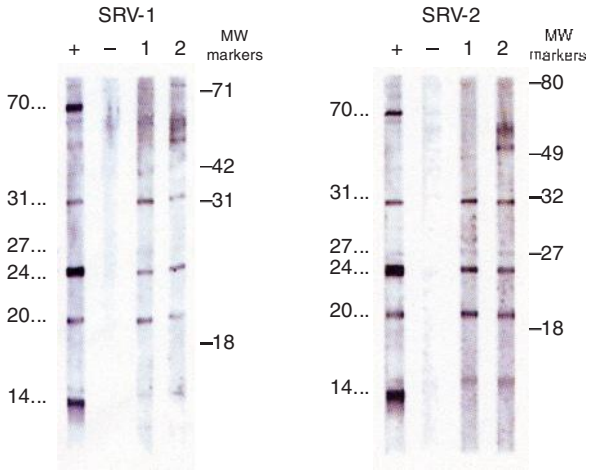


Figure 4 WB reactivity against SRV-1 and SRV-2 on initial screening in sera from two persons occupationally exposed to nonhuman primates. Lanes: (+)-lane is known SRV-positive serum from a rhesus monkey; (–)-lane is SRV-negative serum from a known negative rhesus monkey; lane 1, subject 1, lane 2, subject 2. MW, molecular weight (weights are in thousands of daltons). gp70 is the surface spike of SRV. Reproduced from Lerche NW, Switzer WM, Yee JL, *et al.* (2001) Evidence of infection with simian type D retrovirus in persons occupationally exposed to nonhuman primates. *Journal of Virology* 75(4): 1783–1789, with permission from American Society for Microbiology.

active SRV infection in the past. The most important tests to document active infections were negative. Repeated attempts to amplify the genomic DNA specific for SRV from specimens, as well as attempts to recover infectious virus, were not successful.

These findings are nevertheless important for understanding zoonotic infections and the related but more dangerous outcome of zoonosis. Zoonotic infections are fairly common under the right exposure conditions, but a zoonosis is a rare outcome. Even though SRV may infect human blood cells *in vivo* and grow temporary in the new host, the immune system of the non-natural human host is capable of eliminating the zoonotic infection. The past infection will be evident from trace amounts of specific antibody that was induced during the transient infection. Infection of humans with simian D and other simian retroviruses is well documented, but evidence of zoonosis has not been found.

See also: Mouse Mammary Tumor Virus; Replication of Viruses; Simian Immunodeficiency Virus: General Features.

Further Reading

Colcher D, Spiegelman S, and Schlom J (1974) Sequence homology between the RNA of Mason–Pfizer monkey virus and the RNA of human malignant breast tumors. *Proceedings of National Academy of Sciences, USA* 71(12): 4975–4979.

Daniel MD, King NW, Letvin NL, Hunt RD, Sehgal PK, and Desrosiers RC (1984) A new type D retrovirus isolated from macaques with an immunodeficiency syndrome. *Science* 223: 602–605.

Gardner MB and Marx PA (1985) Simian acquired immunodeficiency syndrome. In: Klein G (ed.) *Advances in Viral Oncology,* vol. 5, pp. 57–81. New York: Raven Press.

Heidecker G, Lerche NW, Lowenstine LJ, *et al.* (1987) Induction of simian acquired immune deficiency syndrome (SAIDS) with a molecular clone of a type D SAIDS retrovirus. *Journal of Virology* 61(10): 3066–3071.

Lackner AA, Rodriguez MH, Bush CE, *et al.* (1988) Distribution of a macaque immunosuppressive type D retrovirus in neural, lymphoid, and salivary tissues. *Journal of Virology* 62: 2134–2142.

Lerche NW, Marx PA, Osborn KG, *et al.* (1987) Natural history of endemic type D retrovirus infection and acquired immune deficiency syndrome in group-housed rhesus monkeys. *Journal of the National Cancer Institute* 79(4): 847–854.

Lerche NW, Switzer WM, Yee JL, *et al.* (2001) Evidence of infection with simian type D retrovirus in persons occupationally exposed to nonhuman primates. *Journal of Virology* 75(4): 1783–1789.

Linial M and Weiss R (2001) Other human and primate retroviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 2123–2139. Philadelphia: Lippincott Williams and Wilkins.

Marracci GH, Avery N, Shiigi S, *et al.* (1999) Molecular cloning and cell-specific growth characterization of polymorphic variants of type D serogroup 2 simian retroviruses. *Virology* 261: 43–58.

Marx PA, Maul DH, Osborn KG, *et al.* (1984) Simian AIDS: Isolation of a type D retrovirus and transmission of the disease. *Science* 223: 1083–1086.

Marx PA, Pedersen NC, Lerche NW, *et al.* (1986) Prevention of simian acquired immune deficiency syndrome with a formalin-inactivated type D retrovirus vaccine. *Journal of Virology* 60(2): 431–435.

Rose TM, Strand KB, Schultz ER, *et al.* (1997) Identification of two homologs of the Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8) in retroperitoneal fibromatosis of different macaque species. *Journal of Virology* 71(5): 4138–4144.

Sonigo P, Barker C, Hunter E, and Wain-Hobson S (1986) Nucleotide sequence of Mason–Pfizer monkey virus: An immunosuppressive D-type retrovirus. *Cell* 45(3): 375–385.

Stromberg K, Benveniste RE, Arthur LO, *et al.* (1984) Characterization of exogenous type D retrovirus from a fibroma of a macaque with simian AIDS and fibromatosis. *Science* 224(4646): 289–292.

Wolfheim JH (ed.) (1983) *Primates of the World.* Washington: University of Washington Press.

Simian Virus 40

A L McNees and J S Butel, Baylor College of Medicine, Houston, TX, USA

© 2008 Elsevier Ltd. All rights reserved.

History

Simian virus 40 (SV40) was discovered in 1960 as a contaminant of poliovaccines. Hundreds of millions of people worldwide were inadvertently exposed to infectious SV40 in the late 1950s and early 1960s when they were administered contaminated virus vaccines prepared in rhesus macaque kidney cells. SV40 had unknowingly contaminated batches of both the inactivated and live attenuated forms of the poliovaccine and preparations of some other viral vaccines. Although primary cultures of monkey cells were known to be commonly contaminated with indigenous viruses and safety testing was carried out, SV40 had escaped detection in part because it failed to induce cytopathic effects in rhesus cells. However, when it was inoculated into African green monkey kidney cells, a prominent cytoplasmic vacuolization developed. Originally christened as 'vacuolating virus', the name was later changed to SV40 to conform to a numerical system of designating simian virus isolates.

Concern about the vaccine contaminations heightened considerably when it was found in 1962 that SV40 was tumorigenic in newborn hamsters and could transform many types of cells in culture. Subsequently, manufacturers

treated poliovirus vaccine seed stocks to remove infectious SV40, and screening methods were implemented to increase detection of infectious SV40 in vaccine lots.

Because of the potential risk to public health posed by the previous distribution of contaminated poliovaccines, SV40 became the focus of intensive investigation. For scientists, SV40 has turned out to be an invaluable tool for dissecting molecular details of eukaryotic cell processes. Numerous techniques now commonly used in molecular biology were pioneered in the SV40 system. It continues to serve as a model for basic studies of viral carcinogenesis.

Taxonomy and Classification

Viral species *Simian virus 40* is classified in the genus *Polyomavirus* in the family *Polyomaviridae* (**Table 1**). It was previously classified as a member of the *Papovaviridae* family. The International Committee on Taxonomy of Viruses has designated SV40 as the type species of the 13 members of the family *Polyomaviridae*, which includes murine polyomavirus, human polyomaviruses BK virus (BKV) and JC virus (JCV), as well as isolates from hamsters, rabbits, cattle, birds, and baboons. The human and animal polyomaviruses are antigenically distinct, and in most cases there is only one recognized serotype for each virus.

Properties of the Virion

SV40 particles are small and spherical, with a diameter of approximately 45 nm. Infectious virions have a sedimentation coefficient of 240S in sucrose and band at a density of 1.34 g ml ^{1} in CsCl; empty capsids have a density of 1.29 g ml^{-1}. The molecular mass of the SV40 virion has been estimated at 270 kDa. The DNA content is 12.5% (w/w). The major capsid protein (VP1) accounts for 75% of the total virion protein. VP2 and VP3 are minor capsid proteins. Cellular histones (H2A, H2B, H3, H4) are used to condense the viral DNA for packaging and are present in the core of the particle. There is no lipid envelope. SV40 does not agglutinate erythrocytes.

Table 1 Properties of SV40

Classification	Family *Polyomaviridae*
Strain variation	Genetically stable; one serotype, multiple strains
Virion	Icosahedral, 45 nm in diameter, no envelope
Genome	Circular covalently closed dsDNA, 5200 bp
Proteins	Three structural proteins, VP1, VP2, VP3; cellular histones condense DNA in virion; nonstructural replication protein, T-antigen, is potent oncoprotein
Replication	In certain primate kidney cells; nucleus; stimulates cell DNA synthesis; long growth cycle
Natural host	Asian macaques, especially the rhesus monkey
Diseases	Asymptomatic persistent infections in natural hosts; tumors in experimentally infected rodents; associated with human tumors and with kidney disease
Historical note	Contaminant in early poliovaccines administered to millions of people

SV40 particles exhibit icosahedral symmetry. The virion is composed of 72 pentameric capsomeres composed of the VP1 protein arranged on a $T = 7$d icosahedral surface lattice. This structure (hexavalent capsomeres having pentameric substructure) demands nonequivalent contacts between pentamers. This seems to be accomplished by the C-termini of the VP1 polypeptides, which extend as arms from one pentamer and fit into binding sites on adjacent pentamers, providing the necessary flexibility to build a capsid. The N-terminal arm of VP1 is completely internal in the virus particle. Minor capsid proteins VP2 and VP3 are predominantly internal as well and do not contribute to the basic structure of the virus outer shell.

The virus particles are very resistant to heat inactivation but are relatively labile when heated in the presence of divalent cations. Whereas SV40 is stable at 50 °C for hours, incubation in the presence of 1 M $MgCl_2$ at 50 °C for 1 h will effectively inactivate the virus if it is monodispersed. At a higher temperature (60 °C), ~99% of infectious virus is inactivated within 30 min in the absence of divalent cations. Purified virions can be disrupted by strong alkaline conditions (pH 10.5), by lower pH (9.2) plus a reducing agent, or by detergent treatment. Virus particles are resistant to acid treatment (pH 3.0). Intact virus particles are not affected by nucleases, but in the presence of a reducing agent nuclease can enter the virion and cleave the viral DNA. SV40 is efficiently inactivated by UV light irradiation, following single-hit kinetics.

Properties of the Viral Genome

The SV40 genome is a circular, covalently closed, double-stranded (ds) DNA molecule of about 5 kbp (**Figure 1**). The native DNA assumes a superhelical configuration (form I) that sediments at 21S in a neutral sucrose gradient. A single-stranded (ss) nick generates relaxed circular dsDNA molecules (form II) that sediment at 16S, whereas a ds break produces linear dsDNA (form III, 14S). Alkaline denaturation of form I DNA produces dense cyclic coils that sediment at 53S. Form II DNA is converted to ss circular (18S) and ss linear (16S) molecules by denaturation. The supercoiled (form I) molecules can be separated from relaxed circular and linear forms by centrifugation of a DNA preparation in CsCl gradients with ethidium bromide. The form I molecules will band in a lower

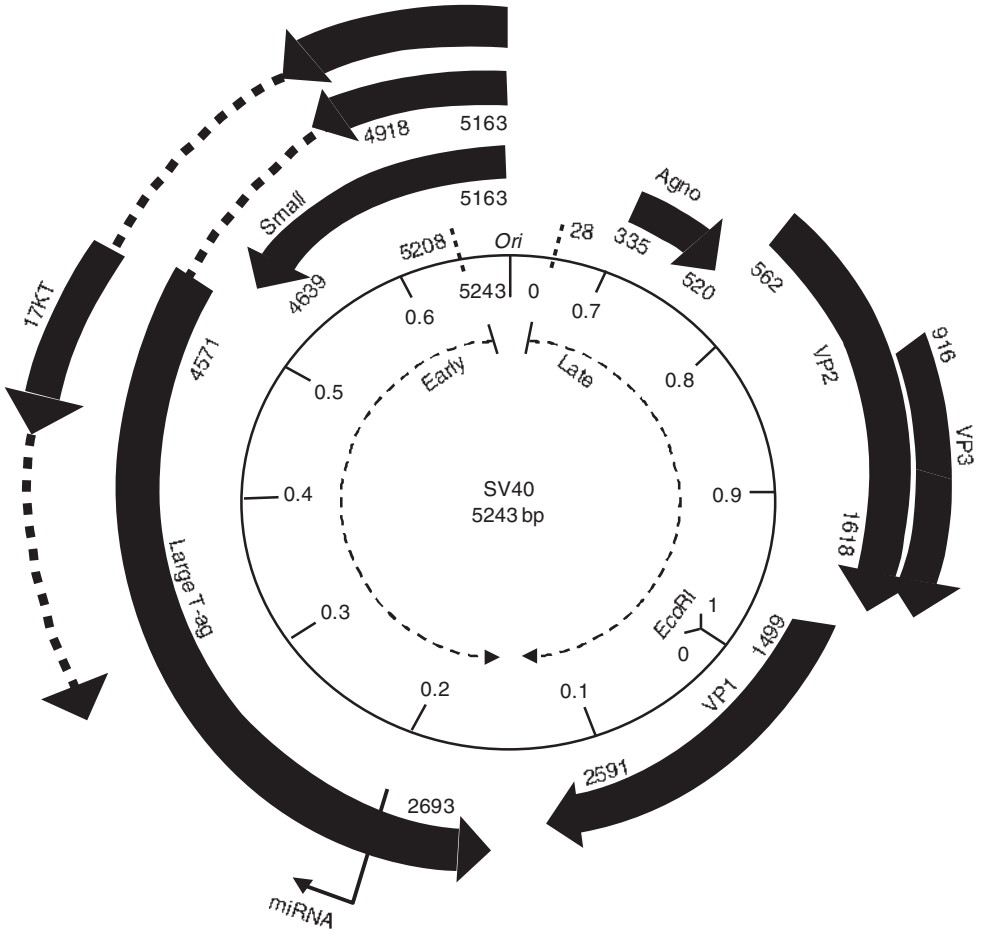


Figure 1 Genetic map of SV40. The circle represents the circular SV40 DNA genome. The unique *Eco*RI site is shown at map unit 0/1. Nucleotide numbers begin and end at the origin (*Ori*) of viral DNA replication (0/5243). Boxed arrows indicate the open reading frames that encode the viral proteins. Arrowheads point in the direction of transcription; the beginning and end of each open reading frame is indicated by nucleotide numbers. Note that T-ag is coded by two noncontiguous segments on the genome. The genome is divided into 'early' and 'late' regions that are expressed before and after the onset of viral DNA replication, respectively. Only the early region is expressed in transformed cells.

position in the gradient. The DNA forms also separate during electrophoresis in a neutral agarose gel; the supercoiled molecules migrate the fastest, the linear forms move at an intermediate speed, and the relaxed circles migrate the slowest.

The viral DNA both in virions and infected cells is associated with cellular histones H2A, H2B, H3, and H4. The histones are assembled in 24–26 nucleosomes on the viral DNA. The nucleosome structure and histone composition of the viral minichromosome mimic the chromatin structure of cellular DNA.

SV40 DNA was the first eukaryotic viral genome to be physically mapped by restriction endonuclease analysis (1971) and to be completely sequenced (1978). The DNA of reference strain 776 with a duplicated enhancer region contains 5243 bp for a calculated molecular weight of 3.5×10^6 Da. The genome is numbered in a clockwise direction from 1 to 5243, the central nucleotide of the unique *Bgl*I recognition site being assigned as 0/5243. Numbering continues through the late region in the 'sense orientation' and the early region in the 'antisense orientation'. The numbering system begins and ends (0/5243) in the middle of the functional origin of DNA replication. The unique *Eco*RI site at nucleotide 1782 was arbitrarily chosen as a point of reference and assigned a value of 0/1.0 on the circular map. Laboratory-adapted strains of SV40 contain a duplication or rearrangements of the 72 bp element in the enhancer region, whereas most natural isolates do not.

The SV40 genome has compact regulatory sequences and overlapping genes. The single origin of replication (core *Ori* = 64 bp in size) is embedded within a nontranslated regulatory region. These elements control transcription and replication and span about 400 bp. The coding regions are expressed early or late in infection, and represent the 'early' nonstructural genes and the 'late'

structural genes, respectively, and are transcribed off opposite strands of the viral DNA.

There is a variable domain at the 3′ end of the T-ag gene, encompassing about 270 bp. Nucleotide changes within this region can be used to distinguish strains of SV40. Although genetic variation among SV40 strains is minimal, three different genogroups have been distinguished. Sequence variations in this region are detected in human-tumor-associated SV40 sequences. Possible *in vivo* biological differences among SV40 strains are unknown but variations in oncogenic potential in rodent models have been observed.

Properties of Viral Proteins

SV40 encodes seven gene products: three 'early' nonstructural proteins (large T-antigen (T-ag), small t-antigen (t-ag), 17K T-ag (17KT)), three 'late' structural proteins (VP1, VP2, VP3), and a maturation protein (LP1 or agnoprotein).

The nonstructural proteins are expressed early in infection, before the onset of viral DNA synthesis. The coding regions of the two T-ags and 17KT overlap; alternative splicing of viral transcripts determines each protein sequence. Large T-ag of strain 776 (**Table 2**) contains 708 amino acids (~90 kDa), and small t-ag contains 174 residues (~20 kDa). The large and small T-ags share 82 N-terminal amino acids, whereas the remainder of each protein is unique. The T-ag/t-ag common exon contains a 'J-domain', believed to modulate hsc70 activity in the assembly and disassembly of multiprotein complexes.

Large T-ag is an essential replication protein required for initiation of viral DNA synthesis. It stimulates host cells to enter S-phase and undergo DNA synthesis and is the SV40 transforming protein. Large T-ag contains a nuclear transport signal (126-Pro-Lys-Lys-Lys-Arg-Lys-Val-132) that targets the protein into the nucleus. However, about 10% of the T-ag in the cell is found in the cytoplasm and the plasma membrane. The biology of small t-ag is enigmatic. It is a cytoplasmic protein that is not essential for viral replication in cultured cells. It associates with the regulatory and catalytic subunits (36 and 63 kDa) of protein phosphatase 2A and is believed to cause cellular growth stimulation. It is required for transformation of some human cells by SV40. Its role during natural infections by SV40 remains to be elucidated. The function of 17KT is unknown.

The functions of large T-ag in SV40 DNA replication are regulated by phosphorylation (**Figure 2**). The sites of phosphorylation are clustered near the ends of the molecule, one region lying between residues 106 and 124 and the other between residues 639 and 701. The majority of the phosphorylated residues are serines, although two threonine residues also become phosphorylated. Unlike many oncoproteins, T-ag is not phosphorylated at tyrosine residues.

T-ag is a DNA-binding protein that recognizes multiple copies of the sequence GAGGC in three T-ag-binding sites in the viral *Ori*. The minimal origin-specific DNA-binding domain of T-ag lies between residues 131 and 259. T-ag is predicted to have a zinc finger motif, typical of DNA-binding proteins, between amino acids 302 and 320. T-ag-specific ATPase and helicase activities are required in addition to DNA-binding activity in order for T-ag to function in initiation of DNA replication. The ATP-binding domain of T-ag is similar in structure to other ATP-binding proteins and is located between residues 418 and 627.

Large T-ag forms complexes with several cellular proteins. Such interactions are involved in T-ag functions in viral DNA replication, induction of cellular DNA synthesis, and cell-cycle progression. Target cellular proteins found in heterooligomeric structures with T-ag include transcriptional coactivators (CBP, p300, p400), tumor suppressor proteins (p53, pRb, p107, p130), DNA polymerase α, the molecular chaperone heat-shock protein hsc70, cell-cycle regulatory proteins cdc-2 and cyclin, and tubulin. The

Table 2 Properties and functions of SV40 T-ag

Structural properties	
Size[a]	708 amino acid residues, 82 N-terminal residues shared with t-ag, 81 632 Da, M_r 90 000–100 000 Da
Modifications	Phosphorylation, N-terminal acetylation, *O*-glycosylation, poly-ADP-ribosylation, palmitylation, adenylation
Supramolecular structure	Zinc finger, nuclear localization signal, J domain, monomers, dimers, higher homooligomers; heterooligomers with transcriptional coactivators (CBP, p300, p400); heterooligomers with DNA polymerase α; hsc70; cdc-2, cyclin, tubulin, Bub1, TEF-1, Cul7, Nbs1, Fbw7; tumor suppressor proteins (p53, pRb, p107, p130)
Subcellular distribution	Predominantly nuclear
Functions	
Replication and transactivation	Specific DNA binding (viral origin of replication), initiation of viral DNA replication; ATPase activity, helicase activity; autoregulation of viral early transcription, induction of viral late transcription
Host cell effects and transformation	Entry of cells into S phase and initiation of cellular DNA replication, complex formation with cellular proteins p53, pRb, p107, p130; adenovirus helper function; effect on host range, initiation and maintenance of cellular transformation, induction of immunity to SV40 tumor cells, target for cytotoxic T cells

[a]SV40 strain 776.

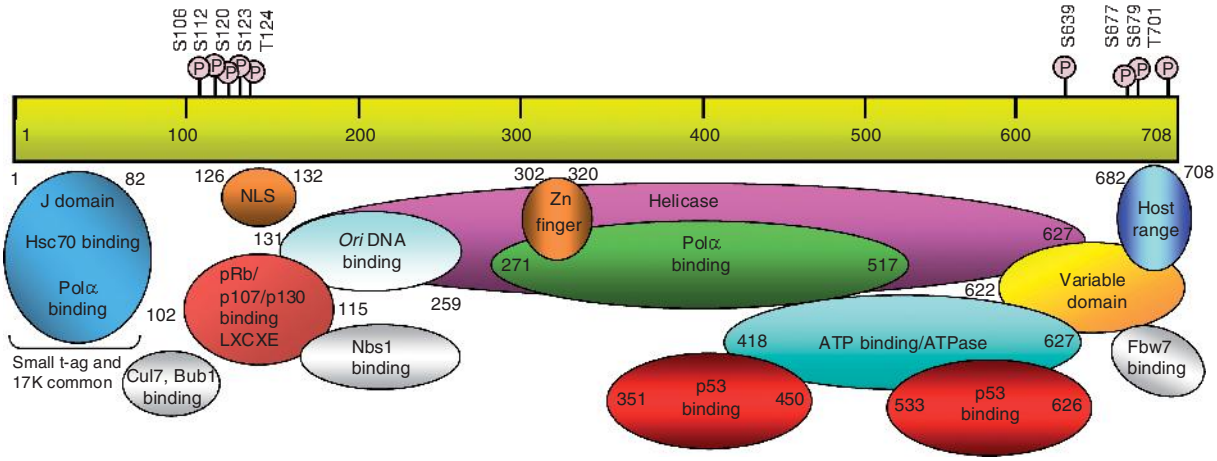


Figure 2 Functional domains of SV40 large T-ag. The numbers given are the amino acid residues using the numbering system for SV40-776. Regions are indicated as follows. Small t-ag common: region of large T-ag encoded in the first exon. The amino acid sequence in this region is common to both large T-ag and small t-ag. Polα binding: regions required for binding to polymerase α-primase. Hsc70 binding: region required for binding the heat shock protein hsc70. Cul7 binding: region required for binding of Cul7. pRb/p107/p130 binding: region required for binding of the Rb tumor suppressor protein, and the Rb-related proteins p107 and p130. NLS: contains the nuclear localization signal. *Ori* DNA binding: minimal region required for binding to SV40 *Ori* DNA. Nbs1 binding: region required for binding of Nbs1. Helicase: region required for full helicase activity. Zn finger: region which binds zinc ions. p53 binding: regions required for binding the p53 tumor suppressor protein. ATP binding/ATPase: region containing the ATP binding site and ATPase catalytic activity. Host range: region defined as containing the host range and Ad helper functions. Variable domain: region containing amino acid differences among viral strains. Fbw7 binding: region required for Fbw7 binding. The circles containing a P indicate sites of phosphorylation found on large T-ag expressed in mammalian cells. S indicates a serine and T indicates a threonine residue.

indicated cellular proteins are not all found in the same T-ag-associated complex; many subpopulations of T-ag exist in a cell.

The variable domain at the C-terminus of T-ag encompasses the host range–adenovirus helper function exhibited by SV40 (and mapped to T-ag) in some monkey kidney cell lines. The significance of the variable domain to natural infections by the virus is unknown.

The structural (capsid) proteins are expressed late in infection, after the onset of DNA replication. They are synthesized in much greater abundance than the early proteins. The major capsid protein, VP1, contains 362 amino acids (~45 kDa). The minor structural proteins are VP2 (352 residues, ~38 kDa) and VP3 (234 residues, ~27 kDa). The coding regions for VP2 and VP3 overlap, and they are translated in the same reading frame, so the sequence of VP3 is identical to the C-terminal two-thirds of VP2. VP3 is synthesized by independent initiation of translation via a leaky scanning mechanism, not proteolytic cleavage of VP2. The N-terminal portion of VP1 is derived from sequences that encode the C-termini of VP2 and VP3. However, VP1 is translated in a different reading frame from a different spliced transcript, so it shares no sequences with VP2 and VP3. VP1 is modified by phosphorylation and acetylation.

The late proteins are required only for the assembly of progeny virions during lytic infection. They are not involved in the early phases of viral replication. They are synthesized in the cytoplasm and move into the nucleus where particle morphogenesis occurs. The minor capsid proteins contain nuclear transport signals. The VP2/3 signal is Gly-Pro-Asn-Lys-Lys-Lys-Arg-Lys-Leu (VP2, residues 316–324; VP3, residues 198–206). For VP1, two clusters of basic residues within the N-terminal 19 residues are independently important for nuclear targeting. Mutations in VP1 affect capsid assembly and/or virion stability. Mutations in VP2 and VP3 affect the uncoating process when virions penetrate new host cells.

The agnoprotein LP1 is synthesized late in infection but is not found in virus particles. It is a small (62 residue, ~8 kDa) basic protein involved in particle assembly. It is believed that LP1 interacts with VP1 molecules to inhibit self-polymerization until they interact with viral minichromosomes in the nucleus to form virions.

Replication

Overview of SV40 Replication Cycle

The SV40 replication cycle is cleanly divided into early and late events, with the onset of viral DNA replication being the dividing landmark. SV40 virions attach to receptors on the cell surface, become internalized, and are transported to the cell nucleus where the viral DNA is uncoated. After uncoating, the half of the genome that contains the early region is transcribed ('early' mRNAs).

Viral early proteins (T-ags) are synthesized, cellular genes are expressed, and the cells enter S-phase. Viral DNA replication then begins. 'Late' mRNAs are transcribed from the other half of the viral genome (the opposite strand), and viral structural proteins are synthesized. Virus particles are assembled and SV40 is released from the cell surface in a manner dependent on intracellular vesicular transport. The SV40 multiplication cycle can take 24–72 h to complete. New particles are detected by 24 h. The time course of the virus growth cycle is dependent upon the virus strain and host tissue, the viral multiplicity of infection, and the growth state of the host cell at the time of infection.

Strategy of Replication of Nucleic Acid

SV40 DNA is replicated in the cell nucleus as a free unintegrated minichromosome. The only viral components required are the viral origin of replication on the DNA and the T-ag protein; all other factors are provided by the host cell replication machinery. T-ag is required for the initiation of DNA replication. The specific T-ag functions required are its DNA-binding ability and its ATPase/helicase activities. The relative simplicity of the SV40 system has allowed the development of cell-free replication systems and the identification of factors involved in mammalian DNA replication.

T-ag binds to the viral *Ori*, a 64 bp segment that contains binding site II for T-ag. In an ATP-dependent process, T-ag causes localized unwinding of the *Ori* region; cellular ss binding protein is required to stabilize the unwound single strands. The cellular DNA polymerase α-primase complex initiates DNA replication, and replication proceeds bidirectionally, with the two forks advancing at equal rates. Elongation involves DNA polymerase α, DNA polymerase δ, and proliferating cell nuclear antigen. Termination occurs 180° away from the viral *Ori*; topoisomerase II segregates the newly synthesized daughter molecules. Cellular histones are added to the new strands during the process of DNA replication.

Replication of SV40 DNA occurs in certain cell types of humans, monkeys, and possibly hamsters, and this permissiveness seems to depend in part on the nature of the DNA polymerase α-primase complex.

Characterization of Transcription

Transcription of the viral DNA is carried out by the cellular RNA polymerase II. In the noncoding region of SV40 DNA near the origin of replication are early and late promoter structures and enhancer elements. Early transcription begins at about nucleotide 5237, proceeds in the counterclockwise direction, and ends at the polyadenylation site at nucleotide 2694. The early promoter contains a TATA box about 30 bp upstream of the early RNA initiation site. (This start site is about 70 nucleotides upstream of the initiation codon shared by the early proteins.) There are three G + C-rich regions, the '21 bp repeats', located 40–103 nucleotides upstream, which are binding sites for the Sp1 cellular factor. Even farther upstream are the SV40 enhancer elements, the 72 bp elements, which contain binding sites for other cellular factors that regulate transcription. The primary early transcripts are differentially spliced to generate the mRNAs that code for the three T-ags.

There is no requirement for virus-encoded proteins, but early transcription is regulated by T-ag. T-ag regulates its own synthesis as it first binds to site I and then to sites II and III on the viral DNA. The presence of T-ag at site II blocks the binding of RNA polymerase.

Late transcription begins after viral DNA synthesis is underway. The abundance of late transcripts is much greater than the early transcripts because progeny DNA molecules are utilized as templates. A heterogeneous collection of late mRNAs is made, with late transcription beginning at multiple sites between nucleotides 120 and 482 and proceeding in the clockwise direction, ending at a polyadenylation site at nucleotide 2674. Both the 21 bp repeats and the 72 bp elements have positive effects on late transcription. The late transcripts are alternatively spliced into two size classes (19S, 16S). VP1 is synthesized from 16S RNA, and both VP2 and VP3 are translated from the 19S species. The agnoprotein is synthesized predominantly from the most abundant species of 16S RNA.

SV40 microRNAs (miRNAs) have been described that accumulate during the late phase of the infection cycle and are complimentary to early viral mRNAs. The SV40 miRNAs function to target the early mRNAs for cleavage, which effectively reduces the expression of T-ag.

Post-Translational Processing

No post-translational cleavages are involved in the production of SV40 proteins. As noted above, T-ag and VP1 are modified in various ways, including phosphorylation.

Uptake and Release of Virions

The attachment of SV40 particles to the cell surface is mediated by VP1. The major histocompatibility class I molecules and the ganglioside GM1 molecules function as receptors. Attached particles are internalized by caveolae-mediated endocytosis into vesicles that contain caveolin-1. These vesicles fuse with caveosomes and the virus particles are transferred to the endoplasmic reticulum, from which they exit and then enter the nucleus. Conformational changes are thought to occur that expose the nuclear localization signals on capsid proteins and allow the virions to squeeze through the nuclear pore

complex. The capsid disassembles in the nucleus, releasing the viral DNA.

Maturation of progeny virions occurs in the nucleus, where the viral nucleic acid is replicated. Viral proteins are synthesized in the cytoplasm off viral transcripts exported from the nucleus, and the proteins are then transported back into the nucleus. The structural proteins condense around the viral minichromosomes. There is a packaging signal on SV40 DNA that includes the *Ori* and part of the enhancer element. During the maturation process, the agnoprotein is released and is not retained as a component of mature virions. There are size constraints for packaging DNA – molecules ranging from 3.5 to 5.7 kbp can be encapsidated into SV40 particles.

Some progeny virions are released from the surface of infected cells via a mechanism dependent on intracellular vesicular transport, but the majority stay associated with the cell until cell death. The release of virus from ruptured and fragmenting cells may also be a mechanism of virus exit from infected cells. SV40 infections are not lytic and host cells are killed as the result of a variety of effects, including the release of lysosomal enzymes into the cytoplasm and damage to the cell mitochondria. Late in infection, monkey kidney cells develop a characteristic cytopathic effect, cytoplasmic vacuolization. As many as 10^4 virus particles can be produced by an infected cell, although some cells produce fewer particles.

Geographic and Seasonal Distribution

The geographic distribution of SV40 can only be inferred, as no comprehensive surveys have been conducted. SV40 is found naturally in wild populations of certain Asian macaque species, and its geographic distribution in the wild presumably reflects its narrow host range. Infections in humans are more widespread geographically, possibly because contaminated poliovaccines were broadly distributed. Nothing is known about seasonal effects on natural infections by SV40.

Host Range and Virus Propagation

Polyomaviruses, in general, have a narrow host range, with each virus infecting only one or a few closely related species. Based on antibody surveys of wild populations of primates, the natural hosts for SV40 appear to be a few species of Asian macaque monkeys, especially the rhesus (*Macacca mulatta*). In captivity, several related species are easily infected, including the cynomolgus macaque (*M. fascicularis*) and the African green monkey, which belongs to the same family as macaques (Cercopithecidae). The virus grows poorly in more distantly related primates. SV40 can infect humans.

SV40 is propagated in tissue culture in established cell lines derived from kidneys of African green monkeys. Characteristic vacuolated cells appear in response to viral replication. The virus grows in rhesus kidney cell lines in which it establishes a persistent infection but produces no cytopathic effects.

SV40 typically does not cause tumors in its natural hosts. To demonstrate its tumorigenic potential, the virus must be inoculated into experimental animals (newborn hamsters are most susceptible). Many types of cells can be transformed in culture, including those of rodent, monkey, and human origin.

Genetics

SV40 is genetically stable, although rearrangements in the regulatory region can occur *in vivo*. Sequence variations exist at the 3′ end of the T-ag gene among different isolates, which are stable on passage *in vivo* and *in vitro*. Adaptation of natural isolates to tissue culture often involves the selection of viruses with duplications or rearrangements in the viral regulatory region. The origins of several SV40 strains are listed in **Table 3**.

Serologic Relationships and Variability

Only one serotype of SV40 is known. The virus does not undergo noticeable antigenic variation. Perhaps restrictions imposed by the symmetry of the capsid permit only minimal deviation in amino acid sequence of the structural proteins, making most changes lethal for the virus.

There is a genus-specific antigenic determinant on the major capsid protein, VP1, that is shared by all animal and human polyomaviruses. It is expressed in infected cells and is internal in the virion. Antibodies are elicited against it by immunization with disrupted capsids or with purified VP1 protein. Antibodies against the shared determinant are not neutralizing, as the site is not exposed on the surface of virus particles. The structural proteins of SV40 and the two human polyomaviruses (BKV and JCV) are antigenically distinct, but display some cross-reactive determinants in enzyme-linked immunosorbent assay (ELISA) tests. The T-ags of SV40, BKV and JCV show antigenic cross-reactivity.

Epidemiology

Most adults of the Asian macaque species believed to be natural hosts for SV40 have neutralizing antibodies to the virus. Few of the juvenile animals of those species, in the wild, have antibodies. However, in captivity the young

Table 3 Origin of SV40 strains

Virus strain	*Year isolated*	*Source*
SV40-776[a]	1960	Adenovirus type 1 seed stock prepared in monkey kidney cells
VA45-54	1960	Uninoculated rhesus kidney cells
Baylor	1961	Type 2 Sabin oral poliovaccine prepared in 1956 in monkey kidney cells
A2895	*c*. 1961	Tumor from hamster injected with rhesus monkey kidney cells
777	1962	Inactivated poliovaccine
Rh911	1962	Uninoculated rhesus monkey kidney cells
N-128	1965	Uninoculated rhesus monkey kidney cells (Russia)
SVPML-1	1970	Cultured human brain cells from patient with progressive multifocal leukoencephalopathy
SVMEN[b]	1984	Human meningioma (cloned directly, Germany)
SVCPC[b]	1995	Human choroid plexus carcinoma
SV40-K661	1998	Brain from rhesus monkey coinfected with simian immunodeficiency virus (SIV)
SV40-H328[a]	1998	Brain from rhesus monkey coinfected with SIV
SV40-T302	1998	Brain from rhesus monkey coinfected with SIV
SV40 –I508	1998	Brain from rhesus monkey coinfected with SIV

[a]SV40-776 and SV40-H328 are identical except for differences in the viral regulatory region.
[b]SVMEN and SVCPC are identical.
Data taken from Forsman ZH, Lednicky JA, Fox GE, *et al.* (2004) Phylogenetic analysis of polyomavirus simian virus 40 from monkeys and humans reveals genetic variation. *Journal of Virology* 78: 9306–9316.

animals are readily infected if they have contact with a virus-positive animal.

Serologic surveys have detected SV40 neutralizing antibodies in humans with prevalences ranging from 2% to 10%. However, whether SV40 infection typically induces a detectable and sustained humoral or cellular immune response in humans has not been determined. SV40 DNA has been detected in human tumors, but epidemiological studies based on SV40-reactive serum antibodies in ELISA tests do not support an association of SV40 exposure with human cancers. The results of such epidemiological studies should be interpreted with caution, however, due to an inability to differentiate with certainty those who were exposed to an SV40-contaminated vaccine and those who were not. The working stocks in use between 1961 and 1978 by a major eastern European manufacturer of poliovirus vaccines have been shown to contain infectious SV40. This finding raises questions as to whether all poliovirus vaccines used worldwide after 1963 were free from SV40 contamination.

Transmission and Tissue Tropism

SV40 establishes persistent infections in the kidneys, and possibly lymphocytes, of susceptible hosts. The level of persistent virus present may be very low. Modes of transmission are not known, but transmission probably occurs due to virus shed in the urine or stool. Experiments have established that susceptible animals can be infected by the oral, respiratory, or subcutaneous routes. Both viremia and viruria occur in infected animals. SV40 may cause neurologic disease in immunocompromised hosts.

The major known source of human exposure to SV40 was via the administration of contaminated viral vaccines before SV40 was recognized. Human exposure could also occur by contact with infected monkeys, a situation limited to small numbers of animal handlers. Transmission between human hosts is hypothesized to occur but has not been documented. It is presumed that patterns of tissue tropism and transmission similar to those described in monkeys would be observed in humans infected by SV40.

Pathogenicity and Pathology

SV40 infections in normal monkeys appear to be asymptomatic and harmless. However, SV40 has been associated with a fatal case of pulmonary and renal disease, as well as with cases of progressive multifocal leukoencephalopathy, in unhealthy rhesus monkeys. SV40 can cause widespread infections in monkeys suffering from simian acquired immune deficiency syndrome and has been found in a brain tumor; no tumors have been found in immunocompetent, natural hosts. Transgenic mice carrying wild-type SV40 DNA develop choroid plexus papillomas and die rapidly because of the physiological importance of the tumor site. When foreign tissue-specific regulatory sequences are substituted for the native promoter-enhancer of the virus, SV40 expression can be directed to other tissues in transgenic animals and lethal tumors usually appear. Intraperitoneal inoculation of SV40 into weanling hamsters produces mainly mesotheliomas, intravenous inoculation of SV40 leads to leukemia, reticulum cell sarcoma, and osteogenic sarcoma, and subcutaneous inoculation induces undifferentiated carcinomas or sarcomas. SV40 DNA has been detected in several types of human cancers, including brain tumors (especially those from children in the first decade of life), mesotheliomas,

osteosarcomas, and non-Hodgkin's lymphomas. SV40 DNA is sometimes found in tumors arising in persons too young to have been exposed to the contaminated vaccines in use between 1955 and 1963. The role SV40 may have played in the induction of those tumors is under investigation.

Immune Response

SV40, like other members of the genus *Polyomavirus*, induces an asymptomatic, persistent infection in natural hosts. An antibody response to capsid antigen is elicited that can be detected in neutralization assays. It is well documented with the human viruses BKV and JCV that impaired cell-mediated immunity is associated with virus re-activation, showing that viral replication is under the influence of the immune system of the host; the same presumably applies to SV40.

Little is known about the immune response of humans to infection by SV40. Small numbers of individuals exposed to contaminated vaccines were analyzed for neutralizing antibody responses to SV40. Humoral responses were detected in some vaccinees and were variable and dependent on the size of inoculum and route of inoculation. Recent serological surveys have detected SV40 neutralizing antibody in 2–10% of persons not exposed to SV40-contaminated viral vaccines. Antibodies to SV40 were most often detected in people with some type of immune suppression. ELISA-based assays detected some cross-reactive antibodies against SV40, BKV, and JCV. SV40 T-ag specific cellular immune response has been detected in some patients with SV40 DNA-positive tumors.

Experimental studies have shown that animals with active infections by SV40 may produce humoral antibodies against the replication oncoprotein, T-ag. It should be noted that a T-ag antibody response could not be used to monitor SV40 infections in humans because of the cross-reactivity among the T-ags of SV40, BKV, and JCV.

In vitro and *in vivo* studies demonstrate that SV40 tumor-bearing rodent animals develop a strong immune response to T-ag. Both humoral and cell-mediated responses occur and are sufficient to prevent tumor growth in some cases. In studies using the Syrian golden hamster model, inoculated animals frequently produced virus-neutralizing antibody and T-ag antibody responses, and the titers of both tended to be higher in tumor-bearing animals. In murine models, cytotoxic T cells directed against T-ag determinants at the cell membrane limit tumor progression.

Interferon is induced only weakly by the polyomaviruses and is not thought to be an important component of the host response to SV40.

Prevention and Control

No control measures are available to prevent SV40 infection.

Future Perspectives

The reports of antibodies to SV40 in humans and the infrequent association of SV40 markers with human tumors suggest that SV40 may be present in the human population. It is important to determine the natural history of SV40 in humans, including modes of transmission and factors affecting susceptibility to infection. Because of its small genetic content and dependence on host cell functions, SV40 will continue to be a useful model system for discerning mechanisms of cellular processes, such as mammalian cell DNA replication, cell cycle progression, and growth control processes altered in neoplasia.

See also: Polyomaviruses of Humans; Polyomaviruses of Mice.

Further Reading

Ahuja D, Sáenz-Robles MT, and Pipas JM (2005) SV40 large T antigen targets multiple cellular pathways to elicit cellular transformation. *Oncogene* 24: 7729–7745.

Cole CN and Conzen SD (2001) *Polyomaviridae*: The viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 4th edn., pp. 2141–2174. Philadelphia: Lippincott.

Cutrone R, Lednicky J, Dunn G, *et al.* (2005) Some oral polio vaccines were contaminated with infectious SV40 after 1961. *Cancer Research* 65: 10273–10279.

Dang-Tan T, Mahmud SM, Puntoni R, and Franco EL (2004) Polio vaccines, simian virus 40, and human cancer: The epidemiologic evidence for a causal association. *Oncogene* 23: 6535–6540.

Forsman ZH, Lednicky JA, Fox GE, *et al.* (2004) Phylogenetic analysis of polyomavirus simian virus 40 from monkeys and humans reveals genetic variation. *Journal of Virology* 78: 9306–9316.

Gazdar AF, Butel JS, and Carbone M (2002) SV40 and human tumours: Myth, association or causality? *Nature Reviews Cancer* 2: 957–964.

Hahn WC, Dessain SK, Brooks MW, *et al.* (2002) Enumeration of the simian virus 40 early region elements necessary for human cell transformation. *Molecular and Cellular Biology* 22: 2111–2123.

Liddington RC, Yan Y, Moulai J, *et al.* (1991) Structure of simian virus 40 at 3.8-Å resolution. *Nature* 354: 278–284.

Schell TD and Tevethia SS (2001) Control of advanced choroid plexus tumors in SV40 T antigen transgenic mice following priming of donor $CD8^+$ T lymphocytes by the endogenous tumor antigen. *Journal of Immunology* 167: 6947–6956.

Shah K and Nathanson N (1976) Human exposure to SV40: Review and comment. *American Journal of Epidemiology* 103: 1–12.

Stewart AR, Lednicky JA, and Butel JS (1998) Sequence analyses of human tumor-associated SV40 DNAs and SV40 viral isolates from monkeys and humans. *Journal of Neurovirology* 4: 182–193.

Vilchez RA and Butel JS (2004) Emergent human pathogen simian virus 40 and its role in cancer. *Clinical Microbiology Reviews* 17: 495–508.

Smallpox and Monkeypox Viruses

S Parker, Saint Louis University School of Medicine, St. Louis, MO, USA
D A Schultz, Johns Hopkins University School of Medicine, Baltimore, MD, USA
H Meyer, Bundeswehr Institute of Microbiology, Munich, Germany
R M Buller, Saint Louis University School of Medicine, St. Louis, MO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Enanthem Eruptive lesion of mucous membranes.
Exanthem Eruptive lesion of skin.
Papule A small, solid, elevated lesion of the skin that is often inflammatory.
Pock A pustule on the body caused by an eruptive disease.
Pustule A small elevated pus-containing lesion of skin.
Vesicle A small cavity within the epidermis containing serous fluid.

Variola Virus

Smallpox is caused by variola major virus (VARV), which is a member of family *Poxviridae*, subfamily *Chordopoxviriniae*, genus *Orthopoxvirus*. VARV is a 200–250 nm brick-shaped enveloped virus with a double-stranded DNA genome of approximately 186 kbp. Compared with other orthopoxviruses, VARV exhibits gene conservation toward the center of the genome, with genetic variation increasing toward the termini. The genes in the terminal regions appear to encode virulence factors that differ among orthopoxviruses. Virus replication occurs in the cytoplasm of the host cell where intracellular mature virions (IMVs) and extracellular enveloped virions (EEVs) are produced. Humans are the only natural host of VARV, although monkeys can be infected when exposed to artificially high doses and baby mice and rabbits can briefly propagate the virus. For additional information regarding the replication and architecture of VARV, the reader is refered to the article on vaccinia virus in this encyclopedia. Vaccinia virus is an extensively studied orthopoxvirus that has many biological similarities to VARV.

History

Smallpox, so named to differentiate it from great-pox (*syphilis*), was described by Edward Jenner as "the most dreadful scourge of the human species." Although the exact number of deaths during Jenner's time is unknown, it is estimated to have been approximately 400 million in the twentieth century alone. Historically, smallpox has had a close association with humans. The origin of VARV remains unknown, but the dubious accolade probably goes to Egypt or India. Unmistakable descriptions of smallpox were documented in fourth-century China, seventh-century India, and tenth-century Mediterranean and southwestern Asia. Moreover, Egyptian mummies buried over 3000 years ago have skin lesions that are consistent with smallpox. Before the fifteenth century, smallpox was generally confined to the Eurasian landmass. However, European colonists introduced smallpox to the Americas, central and southern Africa, and Australia between the fifteenth and eighteenth centuries with devastating consequences, as indigenous populations were decimated with case–fatality rates approaching 90%. Smallpox enabled a handful of conquistadors such as Cortez and Pizarro to subjugate large parts of central and South America to Spanish rule, thereby permanently altering the future of these regions. This was not an isolated pattern.

By the end of the nineteenth century a milder and less lethal form of smallpox, named variola minor, became apparent. This virus was first documented in South Africa during 1904, but had been clinically apparent in the USA since 1896. Originally described as Amass (alastrim in South America), this virus eventually became recognized in Brazil during the 1960s and in Botswana, Ethiopia, and Somalia during the 1970s. The variola minor derivatives of variola major (classical smallpox) are believed to have originated in several places throughout the globe as the virus adapted to humans. The case–fatality rates for variola major were 16–30% and 1% for variola minor.

Clinical Features

Clinically, smallpox in an unvaccinated person has a 7–19 day incubation period from the time infection is established within the respiratory tract until the first symptoms of fever, malaise, headache, and backache occur, culminating in the start of the characteristic rash. The rash starts with papules, which sequentially transform into vesicles and then pustules; a majority of these lesions are located on the head and limbs (often confluent) compared to the trunk. The rash is typically centrifugal (head and limbs),

but centripetal (trunk) rashes have been reported. Lesions are 0.5–1 cm in diameter and can spread over the entire body. Once pustules have dried, scabs will form which eventually desquamate during the following 2–3 week period. The resultant feature of these cutaneous lesions is the formation of the classic pock scar that is apparent on the skin of surviving patients.

Two clinical variations of smallpox have been identified. Flat-type smallpox is a rare form of the disease (about 6% in unvaccinated people), and is characterized by lesions that remain level with the skin. It was more frequently observed in children and usually resulted in death. Another variation of the disease is hemorrhagic smallpox (<2% in unvaccinated people), which occurred mainly in adults. Although this was a rare form of the disease, it also had a high mortality rate and is characterized by hemorrhages into the skin and/or mucous membranes early in the course of illness. Subconjuctival hemorrhages were most common as well as bleeding from the gums and other parts of the body.

Although smallpox is typically spread by respiratory droplets over a short distance, some examples of long-distance transmission are evident. One such case occurred in 1978 at the University of Birmingham, UK, where a woman who was vaccinated 12 years earlier died of smallpox. She is identified as the last human fatality of the disease. It is widely believed that VARV traveled up through an air duct that connected a smallpox virology laboratory to her work station. Other theories suggest that this woman was exposed by using the laboratory telephone or simply from laboratory personnel. Another case occurred in a hospital at Meschede, Germany in 1970. In this case, a recent returnee from Pakistan is believed to have initiated 19 other cases of smallpox on all levels of a large general hospital, despite being isolated for the 5 days of his stay. Factors that enabled VARV to travel long distances in the hospital were likely: a building design that facilitated strong rising air currents when it was heated, the patient's severe cough, and the humidity level in the hospital.

Variolation and Vaccination

Historically, it was understood that humans who survived an initial smallpox infection never developed the disease again. Furthermore, persons infected with VARV by cutaneous scratches suffered a less severe form of the disease. For these reasons, the practice of inoculating naive persons with pustular fluid collected from smallpox victims became a common practice; this type of inoculation was called variolation. Variolation usually induced a milder form of the disease, which was typified by a severe, local, cutaneous lesion at the site of variolation with smaller satellite cutaneous lesions; however, variolation sometimes led to generalized rashes with associated deaths. Variolation was likely developed in both India and China and was subsequently introduced to Egypt and the rest of Africa in the seventeenth century and Europe and its colonies in the eighteenth century.

By the end of the eighteenth century, variolation had been widely accepted throughout the world as a means to prevent smallpox. The widespread use of variolation in Great Britain and her North American colonies widely reduced the impact of the virus in the upper classes but not in the general population as a whole. Despite its successes, the mortality rate and the frequent development of classical smallpox in many patients, with associated disease transmission, meant that variolation was less than ideal. Fortunately, a solution emerged from the common observation that milkmaids were rarely susceptible to smallpox. This lack of susceptibility was widely attributed to a zoonotic disease, cowpox. Based on these observations, Edward Jenner inoculated a boy with cowpox virus and observed the child's resistance to smallpox. Over time, more children were inoculated, exposed, and subsequently resisted smallpox. By the beginning of the nineteenth century this method of vaccination (*vacca,* Latin for cow) had become accepted, as it afforded the same level of protection as variolation without the associated risks of mortality and transmissibility. In some countries (France in particular), vaccination was substituted with orthopoxviruses causing horsepox (a method called equination). Moreover, in a recent study it was found that the causative agent of horsepox is closely related to the vaccinia virus strains derived from the historic smallpox vaccine, supporting the hypothesis that horsepox, or close relatives, replaced cowpox virus as the preferred virus used for worldwide vaccination at some point in the mid-nineteenth century. By the 1950s, endemic smallpox had been eliminated from most industrial nations.

All smallpox vaccines used during the eradication campaign were prepared from vaccinia virus; however, this vaccine was not without complications. In some cases, atypically severe lesions developed, coupled with severe symptoms and occasionally death. The most common complications of vaccination were noted in persons with eczema, where the eczematous region became rapidly inflamed and necrotic with frequent spread of the virus to healthy tissue. Immunocompromised individuals also presented with complications because the vaccination site failed to heal and secondary lesions appeared and spread; this complication was typically fatal.

Smallpox Eradication

In May 1959 the World Health Assembly tasked the World Health Organization (WHO) with the goal of eradicating smallpox globally. Although this was not the first eradication program, it was the only successful one.

Approximately 20 years later, in 1980, the WHO declared that smallpox had been eradicated from the world. Despite plans to destroy all stocks of the virus by the end of the twentieth century, none have been realized. Their preservation is due to the stated need (among others) to develop and evaluate antiviral agents, as VARV is a potential bioweapon. The virus which once dominated the health of mankind is categorized as a bio-safety level (BSL)-4 agent, and virus collections are still kept officially in two BSL-4 WHO reference laboratories in the USA and Russia (as of 2007). Any work with live VARV has to be approved by a WHO advisory committee. The genome sequences of 45 VARV strains are freely available online (see the 'Relevant website' section).

Monkeypox Virus

Monkeypox virus (MPXV) is also a member of the genus *Orthopoxvirus*. The MPXV virion is consistent in structure with other orthopoxviruses, that is, a 200–250 nm brick-shaped, enveloped virus with characteristic surface tubules and a dumbbell-shaped core (**Figure 1(b)**). Its genome is approximately 199 kbp of double-stranded DNA. Orthopoxviruses have host specificities ranging from narrow (e.g., VARV and ectromelia virus) to broad (e.g., cowpox and vaccinia viruses), and the capability of MPXV to infect rodents, nonhuman primates, and humans places it in the latter group. MPXV and VARV cause similar diseases in humans, although they are distinct viruses.

History

MPXV was first isolated in 1958 from the vesiculo-pustular lesions found on infected cynomolgus macaques imported to the State Serum Institute of Copenhagen, Denmark. During the next few years, similar outbreaks were reported in monkey colonies in the USA and in a zoo in Rotterdam, The Netherlands. In the latter case, the first animals affected were giant anteaters from South America, but the disease spread to various species of apes and monkeys. The viruses isolated from these animals were found to be similar to each other and to represent a species of orthopoxvirus that had not been described previously. Currently, MPXV is classified as a BSL-3 agent for animal studies.

Human Monkeypox

MPXV remained primarily of academic interest throughout most of the 1960s. Attitudes changed radically when it was realized that MPXV could infect humans in known smallpox-free locales. This gave rise to concerns that MPXV could fill the niche vacated by VARV. However, a WHO-driven campaign suggested that this was unlikely. It was generally assumed that MPXV infections in humans had been occurring before VARV was expunged, but that they were masked under the guise of smallpox.

The most severe human MPXV infections have been reported in the Congo Basin area of Africa, whereas attenuated human infections have generally occurred in West African countries. Human infections usually result from handling MPXV-infected animals (bush-meat); however, cases of human-to-human transmission have been reported. In 2003, an MPXV outbreak occurred when MPXV-infected West African rodents were imported into the USA and thereafter infected native prairie dogs destined for sale in the pet industry. Human infections were initiated by several routes, which appeared to affect the clinical manifestations of the disease. No fatalities occurred, but the virus was of the less aggressive West African type (see below). Nevertheless, this incident demonstrated the ease with which MPXV can penetrate the interspecies barrier.

Clinical Features

MPXV-infected humans develop a skin rash and follow a disease course similar to that observed in smallpox victims (see above). However, some differences exist between smallpox and human monkeypox. First, humans infected with MPXV frequently present with severely swollen lymph nodes (lymphadenopathy of the neck, inguinal and axillary regions), which is not clinically apparent with smallpox victims. Second, a hemorrhagic form of monkeypox has not been reported (however, it has been reported in some laboratory-housed African dormice infected with MPXV). Interestingly, humans infected with MPXV typically present with a rash similar to that observed in less severe cases of smallpox; to quantify this somewhat, approximately 58% of smallpox patients and 11% of human monkeypox (Congo Basin strain) cases had >100 pocks, respectively.

Epidemiology of Human Monkeypox

In humans, MPXV is a zoonotic infection that has limited capacity to transmit within the population. Between 1970 and 1979, only 47 cases of human monkeypox were reported in five African countries. Most (81%) of the cases were in the Democratic Republic of the Congo (DRC), and mathematical modeling experiments concluded that MPXV could not sustain itself in the unvaccinated human population without zoonotic amplification. Conversely, between 1970 and 2005, 2131 cases were reported in 12 countries. Most (94%) of the cases were in the DRC, and many were not confirmed in the laboratory. The majority of cases were reported in villages within, or close to, the tropical rainforest. African children were the most affected, with a 10% case–fatality rate. The reason for the upsurge, particularly in Africa, is unknown, but it has been suggested that a number of reported cases were

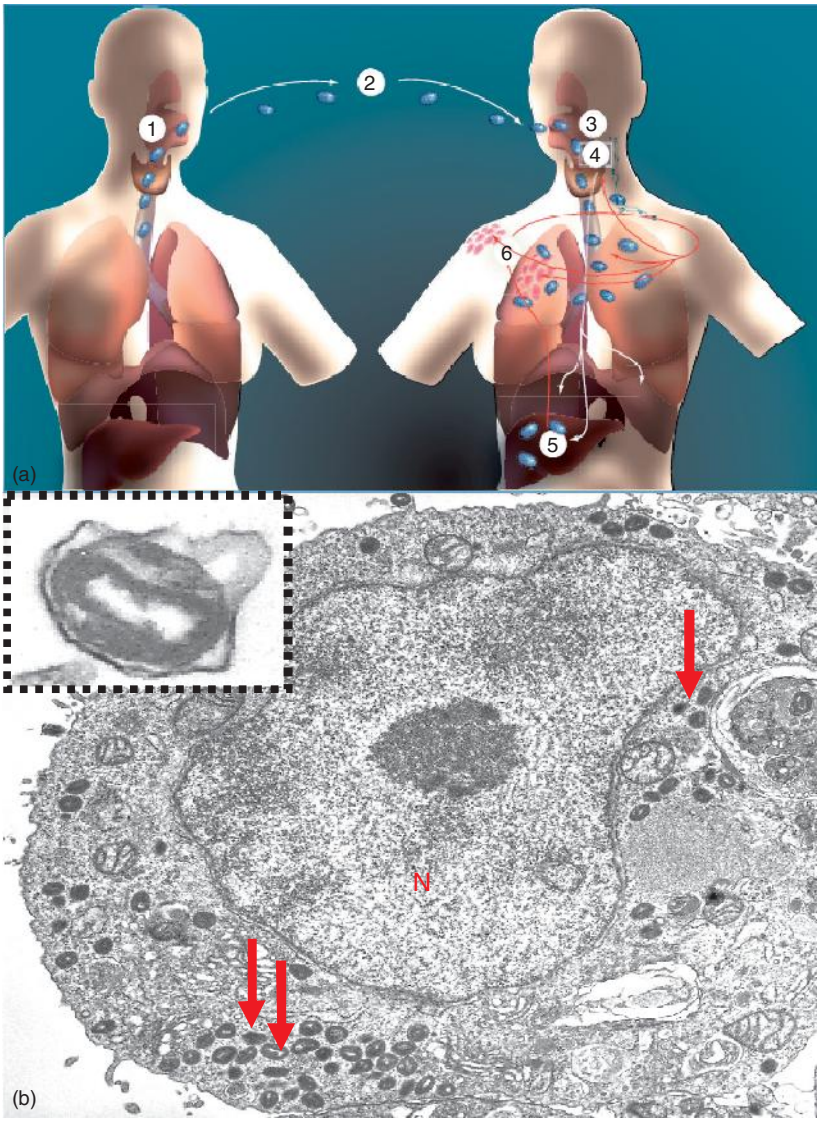


Figure 1 (a) Schematic of the natural life cycle of MPXV. (1) Release of the virus from the original host via the oropharyngeal mucosa. (2) Aerial dispersion of virus particles. (3) Seeding of the respiratory mucosa in a new host. (4) Initiation of replication and neutralization of the host's immune response. (5) Primary viremia and infection of internal organs and lymphatic system (white arrows). (6) Secondary viremia and development of exanthem and enanthem (red arrows). (b) A transmission electron micrograph (approximately ×10 000) of a BSC-1 cell infected with MPXV (red arrows). Virus replication occurs in the cytoplasm (not the nucleus, N). The inset image is an MPXV EEV particle (approximately ×165 000). Note the biconcave core and loose-fitting outer membrane. Adapted from Parker S, Nuara A, Buller RML, and Schultz DA (2007) Human monkeypox: An emerging zoonotic disease. *Future Microbiology* 2: 17–34, with permission from Future Medicine Ltd.

actually cases of chickenpox, caused by varicella-zoster virus (a herpesvirus). A likely contributing factor is the cessation of smallpox vaccination by the WHO *c.* 1980, because recent vaccination with vaccinia virus is 85% effective at protecting against severe MPXV-induced disease. The broad host range of MPXV is also likely to permit additional species to become reservoirs or incidental hosts, thus increasing the exposure risk for humans.

Studies between 1981 and 1986 revealed that most human monkeypox cases occurred as single sporadic infections after contact with animals. The first-generation secondary attack rate in nonvaccinated household contacts was approximately 9% (compared to 58% with smallpox). The attack rate decreased over the second and third generations, and fourth generation attacks were very rare. That said, it has recently been reported that the transmissibility of MPXV is increasing in human hosts. Genetic changes that improve transmissibility to similar levels as those seen in VARV would be required for MPXV to infect humans in an endemic fashion.

Genetics

Although MPXV and VARV present similar disease profiles in humans, neither of the viruses is believed to have given rise to the other. Rather, both are considered to have arisen from a progenitor virus(es) similar to the cowpox virus lineage. MPXV isolates from West Africa are less virulent and less transmissible in human populations than isolates from the Congo Basin. Consistent with other orthopoxviruses, MPXV strains exhibit gene conservation toward the center of the genome, with variation increasing in frequency toward the termini, which encode for specific virulence genes. Genomic differences between strains have been mapped with restriction fragment length polymorphism studies and DNA sequencing techniques. Sequence analyses of strains from West Africa and the Congo Basin have revealed that isolates are approximately 95% identical to each other and approximately 96% identical to VARV. This value increases to 99% when comparing isolates from only West African or only the Congo Basin regions, allowing a separation into two groups or clades. The genomic sequences of eight MPXV strains are available online (see the 'Relevant website' section).

Ecology

The broad host range of MPXV and seroprevalence studies suggest that several animal species, rather than a single species, may act as reservoirs for MPXV in nature. In the latter part of the twentieth century, several field studies were conducted in the lowland tropical forests of the Congo Basin and West Africa; these studies revealed that MPXV can infect many animal species, including squirrels (*Funisciurius* spp. and *Heliosciurus* spp.) and nonhuman primates (such as *Cercopithecus* spp.). Species that are seropositive for MPXV antibodies have some similar and some dissimilar traits in relation to diet and habitat preference; approximately 40% are arboreal, 40% are semiterrestrial, and 20% are terrestrial. Therefore, MPXV infects species that inhabit all levels of the lowland tropical rainforest in the Congo Basin and West Africa. For a thorough discussion of this topic, the reader is directed to the article by Parker *et al.* listed in the 'Further reading' section.

New Hosts and Geographic Expansion

The interaction of MPXV with reservoir and incidental hosts is still poorly understood, as is the potential for virus transmission to humans within and outside its geographical range. Until the outbreak in the USA in 2003, MPXV had remained fairly localized to a handful of countries in central and West Africa, with the majority of cases detected in the DRC. The USA outbreak added to the breadth of host species capable of supporting MPXV replication, and demonstrated the potential of the virus to expand its geographical range. No human infections were attributed to the shipment of animals that entered the USA from Africa; rather, most patients had direct contact with infected native prairie dogs that had been housed with the imported African rodents. Subsequent to the USA outbreak, prairie dogs that were experimentally infected with MPXV were found to have ulcerative lesions on their lips, tongues, and buccal mucosa. High titer MPXV could be cultured from the nasal discharge and oropharynx of these animals for up to 22 days post infection, indicating that transmission from prairie dogs was likely via the respiratory and mucocutaneous routes. The USA outbreak was caused by the less virulent West African strain, which probably made it easier to bring under control.

A more recent example (2005) of MPXV expanding its environs is found in 19 human monkeypox infections discovered in the previously MPXV-free country of Sudan. This outbreak occurred some 300 miles northeast of the edge of the tropical rainforest – the traditional home of the Congo Basin strain of MPXV. From experience with vaccinia virus, it would not be surprising if MPXV continues to adapt to new species. Such adaptation has already occurred with prairie dogs in the USA and is possibly the reason for the outbreak in Sudan.

Person-to-Person Transmission

MPXV and VARV transmission are likely similar. Human-to-human transmission can be separated into six steps, as demonstrated in **Figure 1**. Step 1: release of virions from lesions in the oropharyngeal mucosa and their aerosolization into the new host's breathing space. Step 2: virus particles, most likely in the EEV form, are transmitted by aerosols. Step 3: seeding of the new host's respiratory mucosa is initiated. Step 4: MPXV replication creates foci of infection and production of specific proteins to neutralize the immune response. Step 5: the primary viremia denotes successful virus replication and spread from the initial site(s) of infection to lymphoid tissues and internal organs. Step 6: the secondary viremia occurs when the virus moves from the infected lymphoid tissues and internal organs to the cornified and mucosal epithelium to cause the exanthem and enanthem, respectively. Transmissibility is dependent on the number of lesions in the host oropharyngeal mucosa, virus survivability in the face of the host immune response, and the ability of the virus to produce infectious virions for exhalation from the respiratory tract. As an explanation of the increased transmissibility of VARV over MPXV, it is likely that VARV produces more infectious virions in the respiratory mucosa than MPXV.

Treatment

Currently, the public health importance of human monkeypox is minor compared to that of VARV before the 1980s. However, MPXV is becoming a more common infection in

central Africa, where there seems to be a rise in the number of transmission generations observed during outbreaks. MPXV could be controlled in the human population by vaccination against smallpox. However, considering the current poor transmissibility in human populations and zoonotic nature of MPXV, this would need to be weighed carefully against the adverse reactions expected from vaccination. As of 2007, two antiviral drugs with activity against orthopoxviruses are being evaluated in animal models.

Diagnostics

Historically, biological properties have been used to identify and differentiate orthopoxviruses. Growth characteristics in embryonated chick eggs were particularly useful during the smallpox eradication campaign. However, this approach is labor and time consuming and requires a high level of skill. Even today, electron microscopy is a first-line technique, but does not allow for differentiation between orthopoxvirus species. Real-time polymerase chain reaction (PCR) is now regarded as the technique of choice for species differentiation, and several protocols are available specifically to identify and differentiate VACV and MPXV from other poxviruses.

Future Perspectives

VARV has been exterminated from the world's human population and could only be reintroduced by artificial release of clandestinely stored stocks. Such an incident would have devastating ramifications. MPXV is of minor public health significance when compared to VARV, but human MPXV infections are increasing. Elimination of MPXV is not possible because, unlike VARV, the virus is likely to have several animal reservoirs. Lastly, the possibility that terrorist groups or rogue nations might bioengineer VARV or MPXV to enhance virulence and transmissibility is a potential threat. Since the molecular biology of both viruses (and other orthopoxviruses) is fairly well understood, genetic tinkering aimed at enhancing virulence is a possibility, although techniques to increase transmissibility are less well developed.

See also: Cowpox Virus; Mousepox and Rabbitpox Viruses; Poxviruses; Vaccinia Virus; Varicella-Zoster Virus: General Features.

Further Reading

Fenner F, Henderson DA, Arita I, Jezek Z, and Ladnyi ID (eds.) (1988) *Smallpox and Its Eradication.* Geneva, Switzerland: World Health Organization.

Jezek Z and Fenner F (1988) Human monkeypox. In: Melnick JL (ed.) *Monographs in Virology,* vol. 17. Basel, Switzerland: Karger.

Parker S, Nuara A, Buller RML, and Schultz DA (2007) Human monkeypox: An emerging zoonotic disease. *Future Microbiology* 2: 17–34.

Tulman ER, Delhon G, Afonso CL, *et al.* (2006) Genome of horsepox virus. *Journal of Virology* 80: 9244–9258.

Relevant Website

http://www.biovirus.org – Viral Bioinformatics Resource Center (VBRC).

Sobemovirus

M Meier, A Olspert, C Sarmiento, and E Truve, Tallinn University of Technology, Tallinn, Estonia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

–1 Ribosomal frameshifting Event occurring during translation elongation when the ribosome shifts its frame for reading the mRNA exactly one position in the upstream direction.

Icosahedral particle Spherical viral particle that is a polyhedron having 20 faces.

Leaky scanning mechanism Mechanism during translation initiation for escaping the first start codon, which occurs when the first AUG resides in a very poor context and therefore only some ribosomes initiate translation at that point.

Polycistronic RNA Contains the genetic information to translate more than one protein.

Satellite RNA (satRNA) Subviral agent consisting of RNA that becomes packaged in protein shells made from coat protein of the helper virus and whose replication is dependent on that virus.

***T* = 3 particle** Icosahedral virus particle that contains three chemically identical coat protein monomers in the icosahedral asymmetric unit; *T* = 3 particle contains 180 coat protein molecules.

VPg Protein that is attached to the 5′ end of viral genomic RNA.

Introduction

It was proposed in 1969 that single-component-RNA beetle-transmitted viruses be placed into a southern bean mosaic virus group. Since 1995, this group has been recognized by the International Committee on Taxonomy of Viruses (ICTV) as the genus *Sobemovirus* (sigla from *so*uthern *be*an *mo*saic *virus*) unassigned to any family. The establishment of this virus group was based on similarities in particle morphology, capsid stabilization, sedimentation coefficients, sizes of protein subunits and genomic RNA, features in mode of vector transmission, and distribution of the particles within the cell. In the *Eighth Report of the International Committee on Taxonomy of Viruses*, 13 virus species were accepted as definite species of the genus *Sobemovirus* and four tentative species were proposed (**Table 1**).

In addition, two viruses presently not recognized by the ICTV are closely related to the sobemoviruses. Nucleotide sequence comparison of the polymerase, VPg, and coat protein (CP) genes of papaya lethal yellowing virus (PLYV) shows high homology to sobemoviruses (about 41–51% with lucerne transient streak virus (LTSV), southern bean mosaic virus (SBMV), southern cowpea mosaic virus (SCPMV), and cocksfoot mottle virus (CfMV)). Snake melon asteroid mosaic virus (SMAMV) RNA-dependent RNA polymerase (RdRp) fragment possesses 71% amino acid sequence similarity to rice yellow mottle virus (RYMV) RdRp.

The host range of sobemoviruses is usually narrow; individual viruses can naturally infect plants from one family only. The exception is sowbane mosaic virus (SoMV) that infects plants from the families Chenopodiaceae, Vitaceae, and Rosaceae. Some sobemoviruses (SBMV, SCPMV, SoMV) are distributed throughout the world; others are limited to one continent or even to one country (**Table 1**).

Sobemoviruses are readily transmitted mechanically. RYMV, for example, is efficiently transferred from plant to plant by farming operations, donkeys, cows, grass rats, wind-mediated leaf contacts, soil, etc. In addition, sobemoviruses are transmitted by vectors. The most common vectors are different species of beetles that transmit sobemoviruses in a semipersistent manner. However, blueberry shoestring virus (BSSV) and SoMV are transmitted by aphids, SoMV also by leafminers and leafhoppers, and velvet tobacco mottle virus (VTMoV) by mirids. Several viruses in the genus are seed-transmissible (**Table 1**).

Genome Organization and Replication

The full-length genomic nucleotide sequences have been determined for members of nine sobemovirus species. Their genome sizes vary from 4.0 to 4.5 kb. The genomic (as well as subgenomic) RNA has a viral genome-linked protein (VPg) covalently bound to its 5′ end. The 3′ terminus of the genomic RNA is nonpolyadenylated.

All the sequenced sobemoviruses have a polycistronic positive-sense single-stranded RNA (ssRNA) genome that consists of four open reading frames (ORFs) (**Figure 1**). The genome is compact, as for most viruses all ORFs overlap. ORFs 1, 2a, and 2b are all translated from the genomic RNA. The initiation of translation from the genomic RNA is facilitated at least in case of CfMV by the translational enhancer in the 5′ untranslated region (UTR) of the genome. Translation of ORF1 and ORF2a occurs via a leaky scanning mechanism. ORF2b is expressed as a fusion protein with ORF2a through a 1 ribosomal frameshift mechanism. Previously, it was reported that some sobemoviruses express the RdRp from a single in-frame polyprotein, not via a –1 translational frameshift. Recently, it has been demonstrated, however, that the difference between the two kinds of genomic organization resulted from a single erroneous extra nucleotide in the genomic sequences of these sobemoviruses that RdRp was thought to be expressed without the –1 ribosomal frameshifting mechanism. Thus, all sequenced sobemovirus species possess similar genomic organization.

A genome 3′-proximal ORF is translated from the subgenomic RNA (sgRNA). The sgRNA has been detected in sobemovirus-infected tissues as well as in virus particles. In addition to the genomic and sgRNA, some sobemoviruses (LTSV, RYMV, subterranean clover mottle virus (SCMoV), Solanum nodiflorum mottle virus (SNMoV), VTMoV) encapsidate a circular viroid-like satellite RNA (satRNA). The sizes of these circular satRNAs range from 220 to 390 nt, the 220 nt RYMV satRNA being the smallest known naturally occurring circular RNAs.

The replication of sobemoviruses uses is poorly understood. The genomic RNA of incoming virus particles is probably uncoated by the co-translational disassembly mechanism that is followed by RNA replication. Little is known about the signals needed for the replication. The 5′ terminal nucleotides of the genomic RNA are ACAAAA for SCPMV, ACAA for RYMV, ACAAA for LTSV and ryegrass mottle virus (RGMoV), ACAAAA for SCMoV, CACAAAA for Sesbania mosaic virus (SeMV) and SBMV, and CAAAA for turnip rosette virus (TRoV). For all these viruses, the 5′ ACAAA or ACAAAA sequence is also present upstream from the CP translation initiation codon, indicating the possible 5′ terminus of sgRNA. This sequence is also characteristic of 5′ termini of polero-, diantho-, and barnaviruses. Due to the conservation of this sequence, it or its complementary sequence in ( )-strand has been predicted to function in viral RNA replication by promoting or enhancing the binding of viral RdRp. Different from other sobemoviruses, such motif is present neither at the 5′ end nor upstream from the CP translation initiation codon in CfMV genome. In addition, all sobemoviruses contain a polypurine tract 5′-aAGgAAA near the beginning of their genomic RNA. Nearly nothing is known about signals at the 3′ end of the genomic RNA essential for the initiation of the synthesis of the genomic

Table 1 Viruses of the genus *Sobemovirus* and their biological properties

Virus	*Abbr.*	*Distribution*	*Natural host*	*Insect vector*	*Transmission*	
					Mechanical	*Seed*
Definitive species						
Blueberry shoestring virus	BSSV	USA (Maine, Michigan, New Jersey, Virginia), Canada (New Brunswick, Ontario, Quebec)	*Vaccinium corymbosum, V. angustifolium*	*Masonaphis pepperi* (aphid)	Yes	No
Cocksfoot mottle virus	CfMV	Europe (France, Germany, Norway, Russia, UK), New Zealand, Japan	*Dactylis glomerata, Triticum aestivum*	*Lema melanopus, L. lichensis* (beetles)	Yes	No
Lucerne transient streak virus	LTSV	Australia (Victoria, Tasmania), New Zealand, Canada	*Medicago sativa*	ND	Yes	No
Rice yellow mottle virus	RYMV	Africa (Benin, Burkina Faso, Cameroon, Chad, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea Bissau, Kenya, Liberia, Madagascar, Mali, Malawi, Mauritania, Mozambique, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Tanzania, Togo, Uganda)	*Oryza sativa, O. longistaminata*	*Chaetocnema pulla, Sesselia pusilla, Trichispa sericea* (beetles)	Yes	No
Ryegrass mottle virus	RGMoV	Japan, Germany	*Lolium multiflorum, Dactylis glomerata*	ND	Yes	ND
Sesbania mosaic virus	SeMV	India (Andra Pradesh)	*Sesbania grandiflora*	ND	Yes	ND
Solanum nodiflorum mottle virus	SNMoV	Australia (Queensland, New South Wales)	*Solanum nodiflorum, S. nitidibaccatum, S. nigrum*	*Epilachna sparsa, E. doryca australica,*		
			Yes	No		
			E. guttatopustulata, Psylliodes sp. (beetles), *Cyrtopeltis nicotianae* (mirid)			
Southern bean mosaic virus	SBMV	USA (Arkansas, California, Lousiana), North and South America (Brazil, Colombia, Mexico), Africa (Côte d'Ivoire, Morocco), Europe (France, Spain), Iran	*Phaseolus vulgaris*	*Ceratoma trifurcata, Epilachna variestis* (beetles)	Yes	Yes
Southern cowpea mosaic virus	SCPMV	USA (Wisconsin), Africa (Botswana, Ghana, Kenya, Nigeria, Senegal), Asia (India, Pakistan)	*Vigna unguiculata*	*Ootheca mutabilis* (beetle)	Yes	Yes
Sowbane mosaic virus	SoMV	USA, Canada, Central and South America, Europe (Bulgaria, Czech Republic/ Slovakia, Croatia, France, Hungary, Italy, Moldova), Japan, Australia (Queensland, New South Wales, Victoria, Tasmania)	*Chenopodium* spp., *Atriplex subrecta, Spinacia oleracea, Vitis* sp., *Prunus domestica, Alisma plantago-aquatica, Danae racemosa*	*Myzus persicae* (aphid), *Liriomyza langei* (leafminer), *Circulifer tenellus* (leafhopper), *Halticus citri* (fleahopper)	Yes	Yes
Subterranean clover mottle virus	SCMoV	Australia (New South Wales, South Australia, Tasmania, Victoria, Western Australia)	*Trifolium subterraneum*	ND	Yes	Yes
Turnip rosette virus	TRoV	UK	*Brassica campestris, B. nigra*	*Phyllotreta nemorium* (beetle)	Yes	ND

Continued

Table 1 Continued

Virus	Abbr.	Distribution	Natural host	Insect vector	Transmission	
					Mechanical	Seed
Velvet tobacco mottle virus	VTMoV	Australia (Northern Territory, Queensland, South Australia)	*Nicotiana velutina*	*Cyrtopeltis nicotianae* (mirid), *Epilachna* spp. (beetle)	Yes	No
Tentative species						
Cocksfoot mild mosaic virus	CMMV	Europe (Czech Republic/ Slovakia, Denmark, France, Germany, Norway, UK), Canada (Ontario)	*Phleum pratense, Dactylis glomerata, Agrostis stolonifera, Bromus mollis, Festuca pratensis, Poa trivialis, Triticum aestivum*	*Myzus persicae* (aphid), *Lema melanopus* (beetle)	Yes	No
Cynosurus mottle virus	CnMoV	Europe (Germany, UK, Ireland), New Zealand	*Cynosurus cristatus, Agrostis tenuis, A. stolonifera, Lolium perenne* X *L. multiflorum*	*Lema melanopus* (beetle), *Rhopalosiphum padi* (aphid)	Yes	ND
Ginger chlorotic fleck virus	GCFV	India, Malaysia, Mauritius, Thailand	*Zingiber officinale*	ND	Yes	ND
Rottboellia yellow mottle virus	RoMoV	Nigeria	*Rottboellia cochinchinensis*	ND	Yes	ND

minus strand. A potential tRNA-like structure has been attributed to the 3′ end of some sobemoviruses, but no experimental data are available on that.

Mutation and recombination rates associated with the replication by sobemoviral RdRps are largely uncharacterized. No intra- or interspecies recombinant sobemoviruses have been described so far. Based on the phylogenetic analysis of RYMV sequences, it has been concluded that RYMV has evolved in the absence of recombination events. No recombinants were also detected between CfMV and RGMoV in doubly infected plants. However, several defective interfering (DI) RNA molecules have been cloned from CfMV-infected plants containing the 5′ end of the genomic RNA linked to 850–950 nt of the 3′ terminus. SatRNAs have several interesting interactions with the replication of the helper sobemoviruses. For example, LTSV supports the replication of satRNA of SNMoV but SNMoV does not replicate LTSV satRNA. At the same time, LTSV satRNA replication is supported by CfMV, SBMV, SoMV, and TRoV, whereas this support is host dependent.

Gene Products and Their Functions

P1

P1 is encoded by the 5′ terminal ORF of the viral genomic RNA and its translation occurs with poor efficiency, as the translation initiation context is suboptimal. The molecular masses of different P1s range between 11.7 and 24.3 kDa and, surprisingly, there is no similarity between the P1 amino acid sequences, making this region the most variable one in the genome of sobemoviruses.

The most-studied P1 proteins are the ones of RYMV, SCPMV, and CfMV. All these proteins are required for systemic infection and are dispensable for viral replication. The P1 of SCPMV has also been shown to be nonessential for viral assembly. In addition, RYMV P1 is described as an important pathogenicity determinant. Both CfMV P1 and RYMV P1 act as suppressors of RNA silencing in *Nicotiana benthamiana*, a nonhost species. P1 of CfMV binds ssRNA in a sequence-independent manner and it does not bind double-stranded small interfering RNAs (siRNAs).

The transient gene expression of ORF1 from CfMV and from SCMoV demonstrated that P1 – as a green fluorescent protein (GFP) fusion protein – is involved in movement. However, the cell-to-cell movement of P1, independent of other viral components, was very limited.

Little is known about the subcellular localization of P1. In the case of CfMV, the P1 is coupled with cellular membranes and/or heavily aggregated when overexpressed in insect cells.

It is worth mentioning that the 5′ terminal half of the genomes of sobemoviruses and poleroviruses is similar in organization. Moreover, poleroviral P0, encoded by the 5′ terminal ORF, shares many features with sobemoviral P1: it acts as suppressor of RNA silencing, it is the

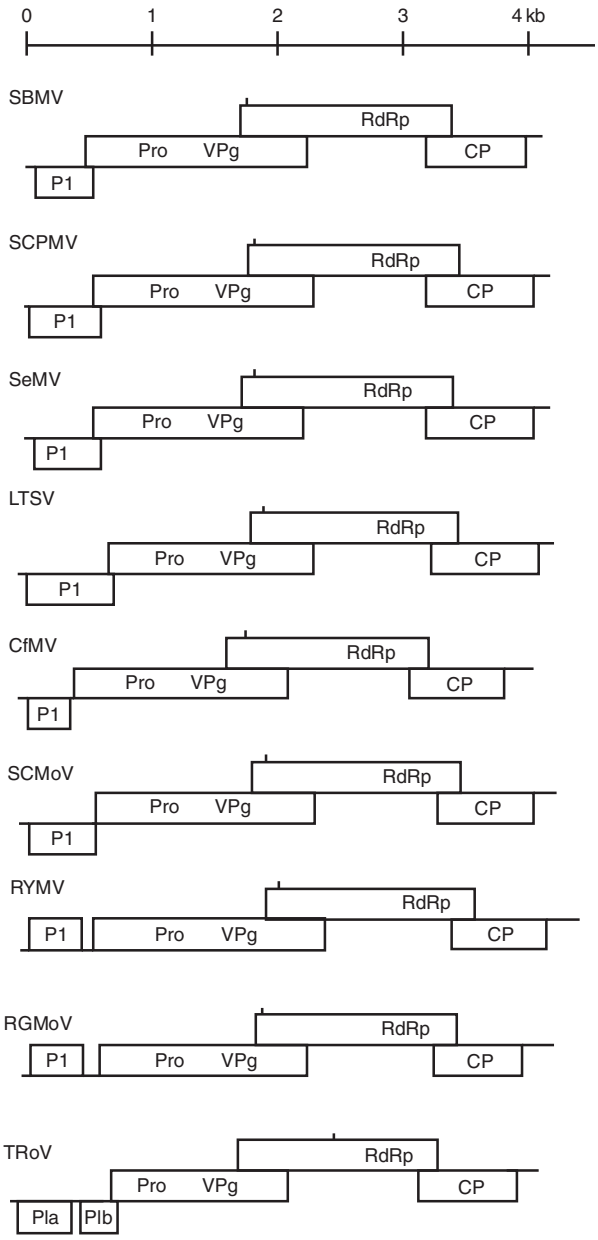


Figure 1 Genome organization of sobemoviruses.

most divergent protein of the viral genome, and it has a poor translation initiation context.

Polyprotein

Translation of the P2a or P2a2b polyprotein takes place from sobemoviral genomic RNA via a leaky scanning mechanism. The comparison of the sequences surrounding the initiation codons for ORF1 and ORF2a/ORF2a2b of sobemoviruses with the consensus sequences established for plant mRNAs shows that the sequence surrounding the ORF2a initiation codon is always in a more favorable context for translation by plant ribosomes.

In vitro translation of sobemoviral RNAs as identified a protein with a molecular mass of about 100–105 kDa. This protein is encoded by partially overlapping ORF2a and ORF2b due to the signal for −1 ribosomal frameshifting. The −1 ribosomal frameshift is needed to regulate the production of sobemoviral RdRp which is encoded by ORF2b. Although no sobemoviral RdRp has been molecularly characterized yet, the presence of the highly conserved GDD motif with its surrounding (characterized as SGSYCTSSTNX$_{19–35}$GDD) feature for RdRps of positive-strand ssRNA viruses has been identified by computer-based sequence analysis of sobemovirus genomes. In the case of CfMV, the consensus signal for −1 ribosomal frameshift event has been shown to consist of a slippery sequence UUUAAAC and a stem–loop structure located 7 nt downstream of it. The slippery sequence UUUAAAC, followed by a simple stem–loop structure, is absolutely conserved for all sequenced sobemoviruses. In a wheat germ extract, CfMV-derived 1 ribosomal frameshifting takes place with an efficiency of *c.* 10%. It has been proposed that the C-terminal processing products of ORF2a-encoded protein regulate the efficiency of the frameshifting.

ORF2a encodes the N-terminal part of the polyprotein that contains at least a viral serine protease and a VPg. The position of the VPg in between the viral protease and replicase (Pro-VPg-RdRp) is unique to sobemo-, polero-, enamo-, and barnaviruses. It is proposed that sobemoviral VPgs exist in a 'natively unfolded state' lacking both secondary and tertiary structures. The only conserved amino acid sequence element observed among sobemoviral VPgs is a WAD or WGD motif followed by a D- or E-rich region.

The proposed consensus amino acid sequence for the catalytic triad of the serine proteases of sobemo-, polero-, enamo-, and barnaviruses is H(X$_{32–35}$)[D/E](X$_{61–62}$)TXXGXSG. The glycine and histidine residues downstream from the catalytic residues (H181, D216, S284 for SeMV) are suggested to be the site for substrate binding. The crystal structure of the protease domain has been determined for SeMV at 2.4 Å resolution. The structure exhibits the characteristic features of trypsin fold.

Mutations of active site residues of H181A, D216A, or S284A render the SeMV protease inactive. The SeMV serine protease domain lacking the N-terminal 70 amino acids is inactive *in trans*. According to *in silico* analysis, the N-termini of the sobemoviral polyprotein sequences (except LTSV that lacks the domain respective to N-termini of other sobemoviral polyproteins) show the presence of high-propensity membrane helices. Interestingly, the presence of the VPg domain at the C-terminus of the SeMV protease domain lacking the N-terminal 70 amino acids restores the protease activity both in *cis* and in *trans*. Furthermore, the substitution of conserved W43 in SeMV VPg to phenylalanine or the deletion of the entire VPg domain abolishes the proteolytic processing of the viral polyprotein.

The N-terminal sequencing of SBMV, CfMV, and SeMV VPgs attached to viral genomes as well as other approaches have indicated that the polyprotein is processed at E/T or E/N sites between the protease, VPg, and RdRp domains. Additionally, A/V cleavage site was identified at the N-terminus of SeMV polyprotein by mass spectrometric analysis. As the alanine and valine residues are small enough to be accommodated in the active site without steric hindrance, it is suggested that the specificity of sobemoviral proteases depends not only on the sequence but also on the conformation of the polypeptide.

Virion Structure and Coat Protein

The virions of members of the genus have an icosahedral capsid roughly the size of 30 nm, which is assembled according to $T = 3$ symmetry. The capsid contains 180 molecules of a single ~30 kDa CP, which is translated from an sgRNA. The single-stranded genomic RNA and sgRNA together with VPg are packaged inside the virion. The three-dimensional structures of SCPMV, SeMV, RYMV, RGMoV, and CfMV virions have been determined utilizing X-ray crystallography. Each icosahedral unit of the virion comprises of three quasi-equivalent subunits A, B, and C, which have minor differences in conformation (**Figure 2(a)**). The A subunits group at fivefold axes while pairs of B and C subunits meet at threefold axes. In addition to protein–protein and protein–RNA interactions each icosahedral unit is stabilized by three calcium-binding sites located between subunits AB, BC, and CA.

The CP is divided into two domains: C-terminal S (shell) domain, which has an eight-strand jellyroll β-sandwich topology, common to nonenveloped icosahedral viruses, and N-terminal R (random) domain, which is disordered in subunits A and B, but is partially structured in subunit C. The S domain is the building block of the virion, whereas the R domain is involved in the regulation of the capsid structure. The primary sequences of CPs among the members of the genus are quite different. However, the three-dimensional structures are nearly identical, for example root mean square difference between RYMV and SCPMV is 1.4 Å. Regardless of that, two different structures of the R domain in C subunit have been found (**Figure 2(b)**). In SCPMV and SeMV the N-terminus of subunit C, makes a U-turn and extends toward the threefold axis nearest to C, where it makes a β-structure together with R domains from analogous C subunits. In RYMV and CfMV there is no U-turn, instead the N-terminal arm of subunit C extends toward subunit B and makes a similar structure at the distal threefold axes closest to subunit B. When the R domain of SCPMV or SeMV CP is removed, only $T = 1$ particles are formed, indicating the importance of the region in $T = 3$ particle formation.

Due to its complex nature, very little is known about the mechanism of capsid formation. Yet, there is evidence suggesting that the virion assembly could be nucleated by AB dimers at icosahedral fivefold axes since pseudo $T = 2$ SeMV particles comprise of groups of A and B subunits. Studies with SCPMV, SeMV, and RYMV particles demonstrate that the stability of the virions depends greatly on pH and the availability of calcium ions. Upon alkaline pH or removal of the cations the virus particles swell and become less stable. Mutation analysis of SeMV CP calcium-binding sites demonstrates that cation-mediated interactions are mainly needed for particle stability. The R domain of all sobemovirus CPs is rich in basic amino acid residues and contains an arginine-rich motif (ARM). Studies with SCPMV and SeMV CP demonstrate that ARM is essential for RNA encapsidation but not for particle formation. However, the presence of RNA enhances the overall stability of capsids. The N-terminus of SCPMV CP withholds a potential to form an α-helix and can interact with membranes *in vitro*. It is proposed that the R domain of all sobemoviral CPs contains a nuclear localization signal.

The functions of sobemoviral CPs in viral life cycle besides capsid formation are not fully understood. Studies with full-length clones of SCPMV and RYMV display that the CP is dispensable for virus replication but systemic virus movement is completely abolished in the absence of CP. Sobemoviral CPs have also been reported to complement the long-distance movement of taxonomically distinct plant viruses.

Subcellular Localization, Short- and Long-Distance Movement

Sobemoviral particles are found mainly in mesophyll and vascular tissues, but also in epidermal, bundle sheath, and guard cells. The quantity of particles present is in correlation with the severity of symptoms. In vascular tissues there are reports of virus particles in both xylem and phloem. CfMV, SCPMV, and SBMV virus particles have been found in phloem companion cells, whereas RYMV particles have been detected predominantly in xylem. RYMV particles accumulated in xylem parenchyma cells and vessels; additionally association with intervascular pit membranes was observed. For RYMV, the common belief is that the virus is transported between xylem cells through pit membranes.

Subcellularly virus particles are found at least in cytoplasm, vacuoles, and nuclei. Virus particles in cytoplasm or vacuoles are known to form crystalline structures, sometimes particles are found in vesicles. No particles have been found in mitochondria and chloroplasts, but the latter are noted to form finger-like extrusions in infected cells. Studies with RYMV suggest that vacuoles of xylem parenchyma cells become the storage compartments for virions in late phase of infection. It is proposed that swollen and less compact virions coexist in

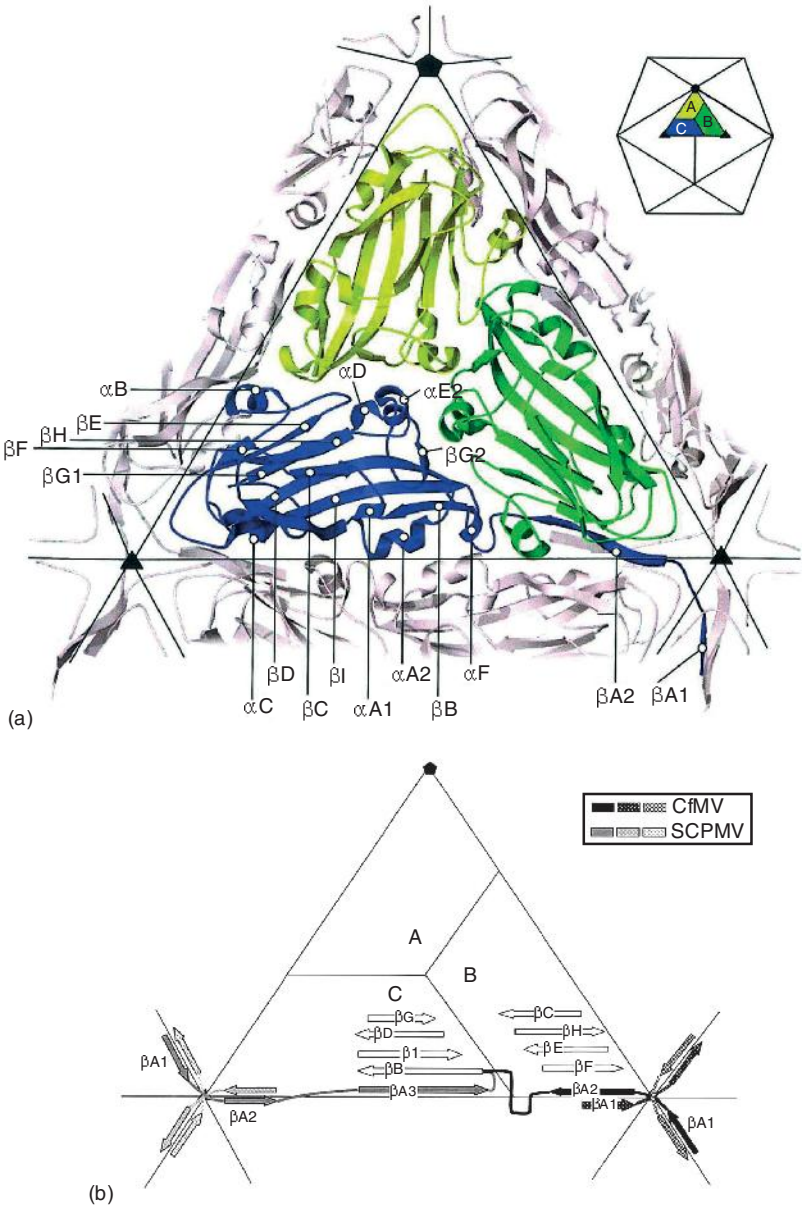


Figure 2 (a) Arrangement of CP molecules in CfMV capsid. (b) A schematic representation of the N-terminal arm in sobemoviruses. Reproduced from Tars K, Zeltins A, and Liljas L (2003) The three-dimensional structure of cocksfoot mottle virus at 2.7 Å resolution. *Virology* 310: 288–289, with permission from Elsevier.

the cytoplasm, whereas vacuoles with their lower pH and higher Ca^{2+} concentration contain compact virions.

Studies with SCPMV, SBMV, and RYMV emphasize that cell-to-cell and vascular movement of sobemoviruses are two distinct processes, whereas the long-distance movement is dependent on the correct capsid formation.

Pathology, Economic Importance, Resistance

Sobemoviral infections can cause a variety of disease symptoms: mild or severe chlorosis and mottling, stunting, necrotic lesions, vein clearing, sterility. Subcellularly, sobemoviruses form crystalline arrays and tubules in the cytoplasm, some of which are enveloped in endoplasmic reticulum-derived vesicles. It has been observed that nucleolus of the RYMV-infected cell enlarges. Probably the most dramatic change induced by RYMV occurs in the cell walls of parenchyma and mature xylem cells, where middle lamellae of the wall are disorganized. RGMoV has been reported to induce apoptotic cell death in oat leaves. The outcomes of these histopathological changes range from symptomless infections of sobemoviruses to severe diseases and death of plants. RYMV infection also causes important changes in the abundance of many host proteins.

For instance, the expression levels of several defense- and stress-related proteins like superoxide dismutase and different heat shock proteins increase several times.

Several sobemoviruses are economically important pathogens. RYMV causes one of the most damaging and rapidly spreading diseases of rice in Africa. Yield losses fluctuate between 10% and 100%, depending on plant age prior to infection, susceptibility of the rice variety, and environmental factors. PLYV, causing serious chlorosis, is responsible for an important disease of papaya in northeast Brazil. SCMoV decreases seed and herbage production in Australia. Over time, SCMoV-infected pastures become weedy and unproductive. SBMV infections in common bean leads to the mosaic and distortion of pods and reduced size and number of seeds.

Natural resistance to sobemoviruses has been detected at least for CfMV in cocksfoot, for CnMoV in *Cynosurus cristatus*, for RYMV in rice and *Oryza glaberrima*, for SBMV in beans, for SCMoV in subterranean clover, and for SCPMV in cowpea. The molecular mechanisms conferring resistance have only been described for RYMV in *Oryza* species. Namely, the recessive resistance gene *Rymv-1* encodes the eukaryotic translation initiation factor eIF(iso)4G whose interaction with viral VPg is responsible for the high-resistance trait. In parallel, a quantitative trait locus (QTL) is described conferring partial resistance against

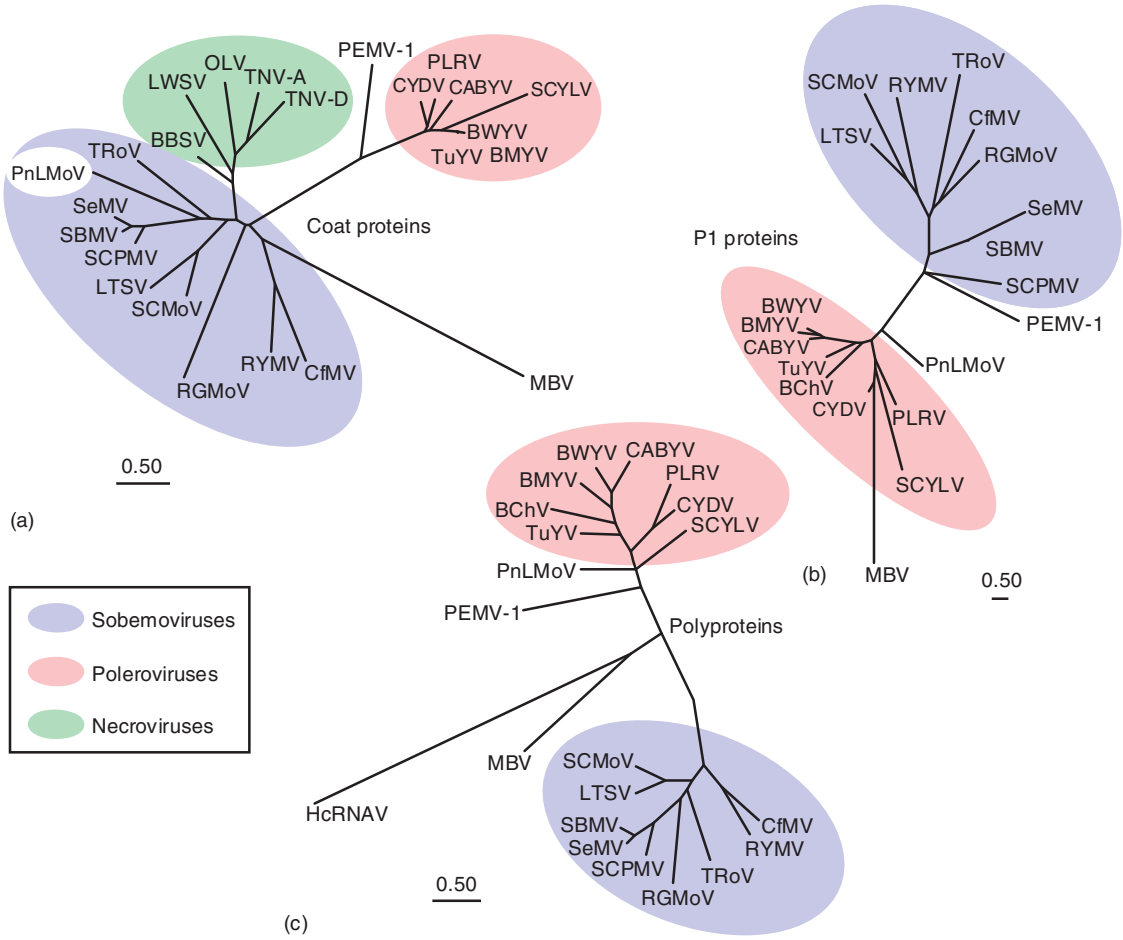


Figure 3 Unrooted phylogram of sobemoviral proteins and the respective proteins of related viruses using maximum-likelihood method. (a) Coat proteins, (b) P1 proteins, and (c) polyproteins. The bar shows the scale of branch length. The protein sequences were aligned with ClustalW and the phylogenetic trees were constructed using PHYLIP PROML 3.6.1 and visualized using Treetool. Viruses (with GenBank accession number) involved in the analyses presented in this figure were the following. Sobemoviruses: CfMV, cocksfoot mottle virus (DQ680848); LTSV, lucerne transient streak virus (U31286); RYMV, rice yellow mottle virus (AJ608206); RGMoV, ryegrass mottle virus (AB040446); SeMV, Sesbania mosaic virus (AY004291); SBMV, southern bean mosaic virus (AF0558871); SCPMV, southern cowpea mosaic virus (M23021); SCMoV, subterranean clover mottle virus (AF208001); TRoV, turnip rosette virus (AY177608). Poleroviruses: BChV, beet chlorosis virus (AF352024); BMYV, beet mild yellowing virus (X83110); BWYV, beet western yellows virus (AF473561); CYDV, cereal yellow dwarf virus (Y07496); CABYV, cucurbit aphid-borne yellows virus (X76931); PLRV, potato leafroll virus (D00530); ScYLV, sugarcane yellow leaf virus (AF157029); TuYV, turnip yellows virus (X13063). Enamovirus: PEMV-1, pea enation mosaic virus 1 (L04573). Barnavirus: MBV, mushroom bacilliform virus (U07551). Necroviruses: BBSV, beet black scorch virus (AF452884); LWSV, leek white stripe virus (X94560); OLV-1, olive latent virus 1 (X85989); TNV-A, tobacco necrosis virus A (M33002); TNV-D, tobacco necrosis virus D (D00942). Unclassified viruses: PnLMoV, poinsettia latent mottle virus (AJ867490); HcRNAV, Heterocapsa circularisquama RNA virus (AB218609).

several RYMV isolates, but the molecular mechanisms responsible for that trait are unknown. Pathogen-derived transgenic resistance against RYMV has been achieved by transforming plants with constructs expressing either RdRp or CP sequences of the virus.

Phylogenetic Relationships

Within the genus *Sobemovirus*, phylogenetic analysis of different proteins indicates that three species – SeMV, SBMV, and SCPMV – are very closely related to each other. Also LTSV and SCMoV as well as CfMV and RYMV cluster into two corresponding subgroups within the genus (**Figure 3**).

When the sobemoviral sequences are compared to all other sequences available, it is evident that the 5′ terminus of the sobemoviral genomes together with ORF1 are unrelated to any other known viral genera (**Figure 3**). The middle part of the genomes (encoding for the successive domains of Pro-VPg-RdRp), however, is similar to those of genera *Polerovirus* and *Enamovirus* from the family *Luteoviridae*. In contrast, the 3′ part of the sobemoviral genomes encoding for the CP is more closely related to CP genes of the genus *Necrovirus* from the family *Tombusviridae* (**Figure 3**). These similarities indicate that possibly early recombination events have played an important role during the evolution of sobemoviruses, luteoviruses, and tombusviruses. This possibility is further supported by the existence of poinsettia latent mottle virus (PnLMoV) whose sequence (AJ867490) shows a close relationship to poleroviruses within the first three quarters of its genome (encoding P1 and polyprotein), but rather to sobemoviruses in the last quarter (encoding CP gene) (**Figure 3**).

A virus belonging to the single species *Mushroom bacilliform virus* of the family *Barnaviridae* (genus *Barnavirus*) has a genomic organization similar to sobemoviruses (except that it lacks ORF1 of sobemoviruses) and its Pro-VPg-RdRp and CP genes are related to the same sequences of different sobemoviruses. In addition, putative protease and RdRp of a positive-sense ssRNA virus infecting a marine dinoflagellate *Heterocapsa circularisquama*, HcRNAV (Heterocapsa circularisquama RNA virus, AB218609), are similar to those of the sobemo- and poleroviruses as well as of PnLMoV and MBV.

See also: Barnaviruses; *Necrovirus*; Rice Yellow Mottle Virus.

Further Reading

Albar L, Bangratz-Reyser M, Hébrard E, Ndjiondjop M-N, Jones M, and Ghesquière A (2006) Mutations in the eIF(iso)4G translation initiation factor confer high resistance of rice to rice yellow mottle virus. *Plant Journal* 47: 417–426.

Aus dem Siepen M, Pohl JO, Koo BJ, Wege C, and Jeske H (2005) Poinsettia latent mottle virus is not a cryptic virus, but a natural polerovirus–sobemovirus hybrid. *Virology* 336: 240–250.

Gayathri P, Sateshkumar PS, Prasad K, Nair S, Savithri HS, and Murthy MRN (2006) Crystal structure of the serine protease domain of Sesbania mosaic virus polyprotein and mutational analysis of residues forming the S1-binding pocket. *Virology* 346: 440–451.

Hull R and Fargette D (2005) Genus *Sobemovirus*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 885–890. San Diego, CA: Elsevier Academic Press.

Kouassi NK, N'Guessan P, Albar L, Fauquet CM, and Brugidou C (2005) Distribution and characterization of rice yellow mottle virus: A threat to African farmers. *Plant Disease* 89: 124–133.

Mäkelainen K and Mäkinen K (2005) Factors affecting translation at the programmed 1 ribosomal frame-shifting site of cocksfoot mottle virus RNA *in vivo*. *Nucleic Acids Research* 33: 2239–2247.

Meier M and Truve E (2007) Sobemoviruses possess a common CfMV-like genomic organization. *Archives of Virology* 152: 635–640.

Qu C, Liljas L, and Opalka N (2000) 3D domain swapping modulates the stability of members of an icosahedral virus group. *Structure* 8: 1095–1103.

Tamm T and Truve E (2000) Sobemoviruses. *Journal of Virology* 74: 6231–6241.

Tars K, Zeltins A, and Liljas L (2003) The three-dimensional structure of cocksfoot mottle virus at 2.7 Å resolution. *Virology* 310: 288–289.

St. Louis Encephalitis

W K Reisen, University of California, Davis, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Bridge vector Vector responsible for carrying virus from the primary cycle to tangential hosts such as humans.
Diapause Insect hibernation.
Gonotrophic cycle Recurrent cycle of blood feeding and egg laying by female mosquitoes.
Maintenance vector Vector responsible for transmission of virus among primary vertebrate host species.

Neuroinvasive Ability of virus to invade the central nervous system.
Vector competence Ability of an insect to become infected with and transmit a pathogen.
Viremia Concentration of virus within peripheral blood.
Viremogenic Ability to elicit an elevated viremia response.

History

St. Louis encephalitis virus (SLEV) probably has been present in the New World within its enzootic cycle for thousands of years. The arrival of European settlers in the 1600s and the extensive agricultural development that followed greatly altered the landscape by clearing and irrigating vast areas of North America and establishing extensive urban centers. These changes probably increased the abundance of peridomestic *Culex* mosquito species and avian hosts such as house finches and mourning doves, introduced new avian hosts such as house sparrows, intensified human–vector mosquito contact, and probably increased the incidence of human infection. However, diagnosis of diseases caused by arbovirus infections such as SLEV assuredly was confounded with other infections causing fever and central nervous system (CNS) disease during summer.

During the summer of 1933, a major encephalitis epidemic with more than 1000 clinical cases occurred in St. Louis, Missouri. These cases occurred during the middle of an exceptionally hot, dry summer and were concentrated within areas of the city adjacent to open storm water and sewage channels that produced a high abundance of *Culex* mosquitoes. A virus, later named St. Louis encephalitis virus, was isolated at autopsy from human brain specimens. Mouse protection assays using convalescent human sera demonstrated that SLEV differed from other viruses causing seasonal CNS disease, such as the equine encephalitides, poliomyelitis, and vesicular stomatitis. The epidemiological features of this epidemic included the late summer occurrence of cases (especially in persons over 50 years of age), exceptionally warm temperatures, and elevated *Culex* mosquito abundance associated with a poorly draining wastewater system. These features remain the hallmark of SLEV epidemics to date.

A multidisciplinary team of entomologists, vertebrate ecologists, epidemiologists, and microbiologists from the University of California subsequently investigated an SLEV epidemic in the Yakima Valley of Washington State during 1941 and 1942 and established the components of the summer transmission cycle, including wild birds as primary vertebrate hosts and *Culex* mosquitoes as vectors. The isolations of SLEV from *Culex tarsalis* and *Culex pipiens* mosquitoes were among the first isolations of any virus from mosquitoes and stimulated the redirection of mosquito control in North America from *Anopheles* malaria vectors and pestiferous *Aedes* to *Culex* encephalitis vectors.

Understanding the basic transmission cycle, an appreciation of the wide range of clinical symptoms, and the development of laboratory diagnostic procedures provided an expanding view of the public health significance of SLEV, with epidemics or clusters of cases recognized annually throughout the US. Wide geographic distribution and consistent annual transmission since 1933 has resulted in >1000 deaths, >10 000 cases of severe illness, and >1 000 000 mild or subclinical infections. The largest documented SLEV epidemic occurred during 1975 in the Ohio River drainage, with >2000 human cases documented. Other substantial human epidemics involving hundreds of cases have occurred in Missouri (1933, 1937), Texas (1954, 1956, 1964, 1966), Mississippi (1975), and Florida (1977, 1990). Smaller outbreaks have been recognized in California (1952), New Jersey (1962), and several other states plus Ontario (1975), Canada. Cases reported annually to the Centers for Disease Control and Prevention (CDC) since 1964 are shown in **Figure 1**.

Distribution

SLEV is distributed from southern Canada south through Argentina and from the west to the east coasts of North America and into the Caribbean Islands. Historically, human cases have been detected in Ontario and Manitoba, Canada, all of the continental US (except the New England States and South Carolina, **Figure 2**), Mexico, Panama, Brazil, Argentina, and Trinidad. The low number of human cases in Canada probably reflects the warm temperature requirements for SLEV replication in the mosquito host, whereas the low numbers of cases from tropical America may reflect inadequate laboratory diagnosis, the circulation of attenuated virus strains, and/or enzootic cycles involving mosquitoes that feed infrequently on humans. Support for this geographic distribution comes from laboratory-confirmed human cases, SLEV isolations from birds, mammals, and mosquitoes, and serological surveys of mammal and avian populations.

Classification

Taxonomically, SLEV is classified within the Japanese encephalitis virus (JEV) complex in the genus *Flavivirus* of the family *Flaviviridae*. Related viruses within this group include Japanese encephalitis, Murray Valley encephalitis, West Nile, and Usutu. SLEV consists of a positive-sense, single-stranded RNA enclosed within a

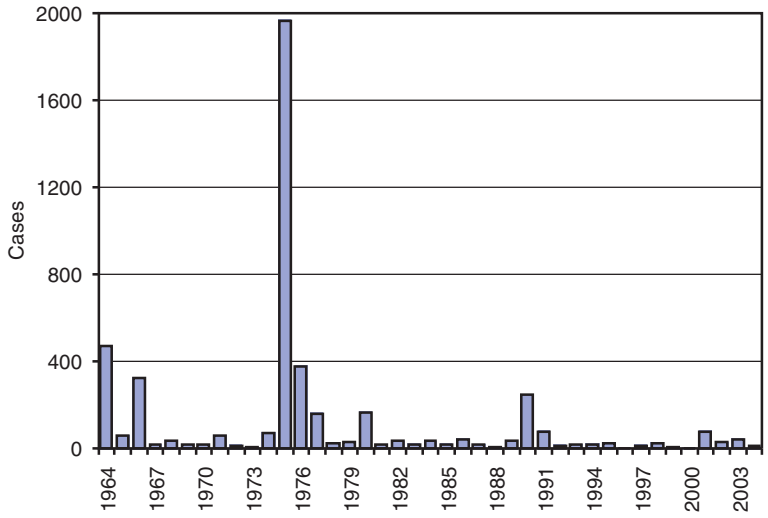


Figure 1 Number of clinical cases of St. Louis encephalitis reported to the US CDC, 1964–2004. Data provided by ArboNet, Center for Disease Control and Prevention, Ft. Collins, CO, USA.

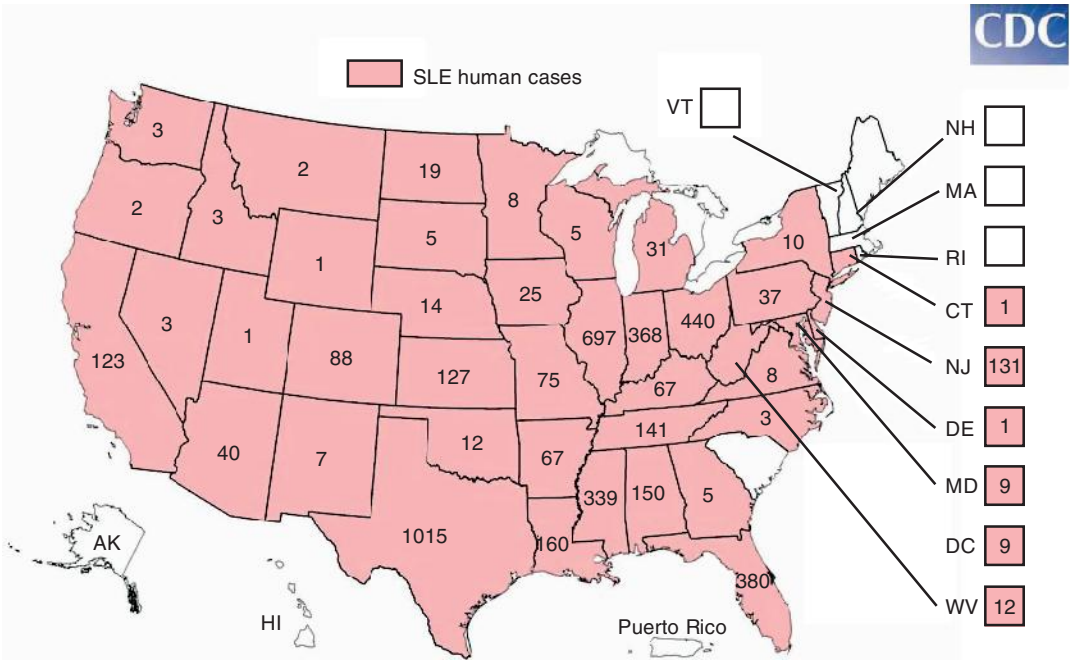


Figure 2 Distribution of human St. Louis encephalitis cases in the US, 1964–2004. Map provided by ArboNet, Center for Disease Control and Prevention, Ft. Collins, CO, USA.

capsid composed of a single polypeptide (C) and surrounded by an envelope containing one glycosylated (E) and one nonglycosylated (M) protein. Marked differences in the severity of SLEV epidemics stimulated interest in possible differences among isolates made over time and space. Detailed studies by the CDC during the 1980s clearly demonstrated geographic variation among 43 different SLEV isolates using oligonucleotide finger printing and virulence in model vertebrate hosts. These strains are grouped into six clusters: (1) east central and Atlantic USA, (2) Florida epidemic, (3) Florida enzootic, (4) eastern USA, (5) Central and South America with mixed virulence, and (6) South America with low virulence. Changes in virulence were attributed, in part, to differences in mosquito vector competence and were supported by the historical presence or absence of human cases. Subsequent genetic sequencing studies extended the understanding of SLEV genetics and provided further insight into patterns of geographical variation. Sequences of the envelope gene from SLEV strains isolated in California

from 1952 to 1995 varied temporally and spatially, but indicated regional persistence in the Central Valley for at least 25 years as well as sporadic introduction and extinction. Studies in Texas using a single-strand conformation polymorphism technique showed that multiple SLEV strains circulate concurrently and remain highly focal, whereas other strains amplify and disseminate aggressively during some summers, but then disappear. Further analyses of sequences from 62 isolates made throughout the known geographical range of SLEV indicated that there have been seven lineages that overlapped somewhat with the six groups the CDC defined previously using oligonucleotide fingerprinting: (1) western USA, (2) central and eastern USA and three isolates from Mexico and Central America, (3) one mosquito isolate from Argentina, (4) five isolates from Panama mosquitoes, (5) South American strains plus an isolate from Trinidad, (6) one Panama isolate from a chicken, and (7) two isolates from Argentina rodents. Collectively, these data indicated that SLEV strains vary markedly in virulence and that the frequency and intensity of epidemics in the US may be related to genetic selection by different host systems. Interestingly, transmission within the Neotropics appears to have given rise and/or allowed the persistence of less-virulent strains that rarely amplify to produce epidemic-level transmission, a scenario duplicated by West Nile virus in the Americas.

Host Range

Arthropods

Although a wide variety of mosquitoes occasionally have been found infected in nature, three avian-feeding species within the genus *Culex* appear to be the most frequently infected and important arthropod hosts: *C. pipiens* (including the subspecies *C. pipiens quinquefasciatus* at southern latitudes, *C. p. pipiens* at northern latitudes, and intergrades) in urban and periurban environments throughout North and South America, *C. tarsalis* in irrigated agricultural settings in western North America including northern Mexico, and *Culex nigripalpus* in the southeastern US, the Carribean, and parts of the Neotropics. Although these species feed predominantly on birds, they also feed on mammals including humans, and therefore function as both maintenance and bridge vectors. Other *Culex* species such as *stigmatosoma* in the west, *restuans* and *salinarius* in the east, and perhaps species in the subgenus *Melanoconion* in the Neotropics also may be important in local transmission. Ticks have been found naturally infected, but their role in virus epidemiology most likely is minimal.

Wild Birds

The importance of avian host species appears to be related to vector *Culex* host-selection patterns as well as to avian susceptibility to the virus. Species can be separated into those frequently, sporadically, and never found infected in nature, and these groupings are related directly to their nocturnal roosting/nesting behavior and the questing behavior of *Culex* vectors. Wild birds do not develop apparent illness following experimental infection, but their viremia response varies markedly, depending upon virus strain, bird species, and bird age. Titers sufficient to infect mosquitoes typically are limited to 1–5 days post-infection. Based on serological surveys during or after epidemics, peridomestic passerifoms (including house finches, house sparrows, cardinals, and blue jays) and columbiforms (including mourning oves and rock doves or domestic pigeons) seem to be infected most frequently. In house sparrows, SLEV strains isolated from *C. pipiens* complex mosquitoes from the central and eastern USA produced elevated viremias, whereas strains isolated from *C. tarsalis* from the western USA were weakly viremogenic. Although host competence studies have been limited, the adults of few bird species seem to develop elevated viremias. However, nestling house finches, house sparrows, and mourning doves produce high viremias that readily infect mosquitoes. Therefore, the nesting period of multibrooded species may be critical for virus amplification. Regardless of their viremia response, most experimentally infected birds produce antibody and, although titers typically decay rapidly, these birds remain protected for life.

Humans

Humans are incidental hosts and do not produce viremias sufficient to infect mosquitoes. Like most arboviruses that cause CNS disease, infection with SLEV does not result in a clear clinical picture in humans and most infections remain unrecognized, unless associated with an epidemic. When presented with such diverse symptoms, few physicians initially suspect SLEV, even in endemic areas. Most SLEV infections, especially in young or middle age groups, fail to produce clinical disease, and infected individuals rarely experience more than a mild malaise of short duration with spontaneous recovery.

Domestic Animals

Although frequently antibody positive during serosurveys, SLEV infection does not produce elevated viremias or cause clinical illness in domestic animals, including equines, porcines, bovines, or felines. In a single experiment, dogs (purebred beagles) produced a low-level viremia, with only two of eight dogs developing clinical illness. Similar to wild birds, immature fowl <1 month old (including chickens and ducks) consistently developed sufficient viremia to infect mosquitoes, but did not develop clinical illness. Adult chickens (>22 weeks old)

usually failed to develop a detectable viremia, and along with immature birds, developed long-lasting antibodies.

Wild Mammals

The response of wild mammals to natural or experimental infection varies. Serosurveys occasionally have shown higher SLEV prevalence in mammals than in birds, but these data could be confounded because mammalian hosts typically live longer than avian hosts and therefore have a longer history of exposure. Rodents in the genera *Ammospermophilus* and *Dipodomys* were susceptible to infection after subcutaneous (s.c.) inoculation, whereas *Spermophilus*, *Rattus*, *Sigmodon*, and *Peromyscus* were refractory. Similarly varied were lagomorphs: *Lepus* was susceptible, whereas four species within *Sylvilagus* ranged from refractory to susceptible. Raccoons and skunks were refractory, whereas opossums and woodchucks were susceptible. Like birds, susceptible mammals produced an immediate viremic response that generally persisted for <1 week, and all species produced detectable antibodies regardless of their viremia response. SLEV frequently has been isolated from bats (*Tadarida*, *Myotis*, etc.), and many populations exhibit a high prevalence of neutralizing antibody. Overall, the role of mammalian infection in SLEV epidemiology is complex and difficult to interpret. All reputed *Culex* vectors feed most frequently on avian hosts, occasionally on large mammals and lagomorphs, rarely on rodents, and almost never on bats.

Pathogenicity

In humans, clinical disease due to SLEV infection may be divided into three syndromes in increasing order of severity: (1) 'Febrile headache' with fever, headache possibly associated with nausea or vomiting, and no CNS illness; (2) 'Aseptic meningitis' with high fever and stiff neck; and (3) 'Encephalitis' (including meningoencephalitis and encephalomyelitis) with high fever, altered consciousness, and/or neurological dysfunction. The onset of illness may be sudden (<4 days after infection) and acute, leading rapidly to encephalitis, or insidious, progressing gradually through all three syndromes. Symptoms may resolve spontaneously during any stage of the illness, with full recovery. Acute illness may be followed by 'convalescent fatigue syndrome' in <50% of patients, with complaints of general weakness, depression, and the inability to concentrate that generally resolve within 3 years. Other sequelae include headache, disturbances in gait, and memory loss.

Pathogensis in SLEV follows a course similar to other flaviviruses in the JEV complex. The extent of illness usually is dependent upon viremia level and duration. Virus replication occurs within the lymphatic system soon after infection, and resulting viremias reflect the balance between virus production and release by the lymphatic system and clearance mediated by phagocytes of the liver and spleen. The probability of CNS involvement is directly correlated with the extent and duration of the viremia, although the mechanism of neuroinvasion remains unclear. Movement from peripheral to central nervous tissue most likely is by passive transport through neuron cytoplasm and then by transport across associated membranes after cell lysis. CNS pathology consists of necrosis of neurons and glia cells and inflammatory changes. Inflammatory changes typically are most important in slowly progressing or sublethal CNS disease and sequelae. Viral clearance is dependent upon a functional immune system and the rapid production of neutralizing antibody, which usually appears within 7 days after infection.

Epidemiology

Transmission of SLEV is complex and requires that the virus replicate in and avoid the immune responses of alternating insect and vertebrate hosts under temperatures ranging from below 0 °C in diapausing mosquitoes to more than 40 °C in febrile avian hosts. Annual transmission activity may be divided into overwintering, vernal and/or summer amplification, and autumnal subsidence periods.

Overwintering

Three possible mechanisms may explain the persistence of SLEV at temperate latitudes; however, few supportive field data are available.

Persistence in mosquito populations

Three mechanisms may explain SLEV overwintering within vector mosquito populations. First, low-level vertical passage of SLEV from infected females to F1 progeny has been demonstrated repeatedly in laboratory experiments. Although not detected for SLEV in nature, vertical transmission has been documented for other viruses in the JEV complex, including JEV and West Nile virus (WNV). Second, *C. p. pipiens* females destined for diapause have been shown to take small blood meals during late summer and early fall without ovarian development. Two isolations of SLEV made from diapausing *C. p. pipiens* females collected resting during winter in Maryland were considered to have been infected by this mechanism, although infection by vertical transmission also was possible. Third, *Culex p. quinquefasciatus* and *C. nigripalpus* do not enter reproductive diapause, remain reproductively active throughout winter at southern latitudes and, depending upon ambient temperature, could maintain SLEV by continued, infrequent transmission among resident birds. Experimentally infected, reproductively active *C. p. quinquefasciatus* females have been shown

to survive winter as gravid females and to then transmit SLEV to recipient birds throughout the following spring.

Persistence in vertebrate populations

SLEVs may also persist over winter within vertebrate host populations. Passeriform birds infrequently develop chronic infections that persist as long as a year following experimental infection. However, attempts to demonstrate natural relapse or to trigger relapse experimentally have not been successful. Flaviviruses, including SLEV, have also been isolated repeatedly from bats, and experimental infections in bats destined for hibernation have been maintained for 20 days at 10 °C. When returned to room temperature, SLEV was detected in the brown fat and at low levels in the blood. These data indicated that bats could function as an overwintering host. However, studies of mosquito host-selection patterns indicated that bats rarely, if ever, were fed upon by host-seeking mosquitoes.

Reintroduction of virus

An alternative hypothesis to local persistence involves annual or periodic reintroduction of virus into northern latitudes from southern refugia. Long-distance movement of SLEV has been indicated indirectly from genetic evidence as well as by the reappearance of SLEV after years of absence. Two possible hypotheses address reintroduction, but neither is well supported by field evidence. Many species of birds and some bats have long-distance annual migrations that could allow the transport of virus from foci active during winter in southern latitudes or south of the equator to receptive areas north of the equator during spring. These vertebrate migrations typically are very consistent in their summer and winter destinations, and this would allow the same or similar genetic strains to reappear each summer at the same locality. However, molecular genetic studies of North, Central, and South American isolates indicate that they are relatively distinct, thereby implying infrequent genetic exchange. In addition, migratory birds do not seem to be frequently involved in transmission because they infrequently are found positive for virus or antibody.

Amplification

Regardless of the persistence mechanism, summer enzootic amplification transmission in North America involves *Culex* mosquitoes and primarily birds in the orders Passeriformes and Columbiformes. Humans become infected tangentially to the primary cycle, do not develop viremias sufficient to infect mosquitoes, and are considered to be 'dead end' hosts (**Figure 3**). Transmission appears to be initiated after the *Culex* vectors resume blood-feeding and reproductive activity, and ambient temperatures warm sufficiently to allow the replication of virus in the mosquito host. Infection is acquired when a female *Culex* blood feeds on a viremic avian host. Virus imbibed within infectious blood meals taken in early in spring when ambient temperatures average <17 °C may lay dormant until warm conditions or changes in mosquito physiology stimulate replication. Under warm temperatures, virus replicates rapidly, disseminates within the mosquito during the ensuing extrinsic incubation period, and then may be transmitted by bite after the female oviposits and attempts to imbibe a subsequent blood

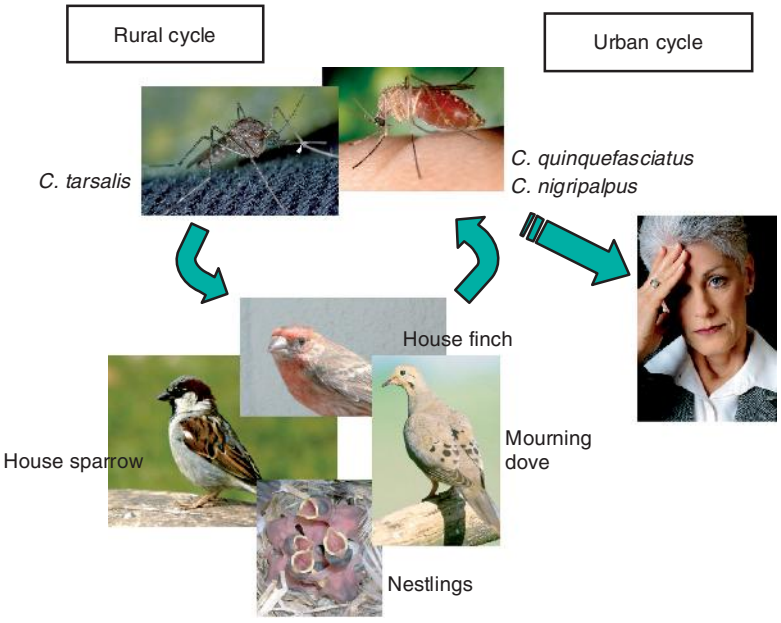


Figure 3 Amplification transmission cycle of St. Louis encephalitis virus in North America.

meal. The duration of the extrinsic incubation period is temperature dependent and requires >10 days and perhaps two mosquito gonotrophic cycles when temperatures average 22 °C. In contrast, the viremia response in susceptible avian hosts typically is of short duration, lasting 2–4 days.

Four distinct transmission cycles of SLEV are defined by differences in the biology of the primary vector mosquito species and their distribution, and include: (1) rural North America, west of the Mississippi River transmitted by *C. tarsalis*; (2) rural and urban central and eastern North America transmitted by members of the *C. pipiens* complex; (3) Florida, Caribbean, and parts of Central America transmitted by *C. nigripalpus*; and (4) urban and rural South America transmitted by *C. pipiens* complex and mosquitoes of other taxa.

Subsidence

Intensity of enzootic transmission and occurrence of new human cases always subsides rapidly during autumn. Cool evening temperatures slow the replication of SLEV within infected mosquito hosts, decreasing the efficiency of transmission and, concurrently, the combination of cool water temperature and shortening days during larval development initiates reproductive diapause (*C. tarsalis, C. p. pipiens*) or quiescence (*C. p. quinquefasciatus*, *C. nigripalpus*) in vector females emerging during fall. The fall mosquito population declines in abundance and divides into newly emerged females that do not routinely bloodfeed and survive the winter, and remnants of the summer population that continue reproductive activity, but fail to survive winter. The critical day length that triggers the onset of diapause in *C. p. pipiens* may occur in late summer at northern latitudes, markedly shortening the SLEV transmission season. During warm days, however, females may become infected when taking partial blood meals from viremic birds, survive winter, and then transmit the virus after diapause is terminated by warm spring temperatures. *Culex p. quinquefasciatus* does not undergo diapause, so that reproductive activity may continue through winter, albeit at a rate slowed by winter temperatures. Populations exploiting underground storm water systems for resting or for larval development may be exposed to relatively warm temperatures throughout winter.

Risk Factors for Human Infection

Five factors have been associated with human risk of SLEV infection.

Residence

Clearly, place of residence markedly affects the risk of infection, with geographic regions in the southern USA having the greatest numbers of human cases and greatest incidence of disease (**Figure 2**). Based on experimental infection patterns in laboratory mice, virus strains from this geographical area also exhibit greater neurovirulence than strains from the western USA or South America. Because of mosquito abundance relative to humans and host-selection patterns, urban residents seem to be at greater risk for SLEV infection than rural residents. However, these conclusions may be confounded by protective immunity acquired early in life that may be greater among rural residents and by low apparent: inapparent case ratios that require a substantially large population to produce recognizable clusters of human cases.

Age

In the absence of acquired immunity, clinical illness and fatality rates, but not necessarily infection rates, increase dramatically with age. Infection seems to occur equally among different age classes as indicated by the increase in antibody as a function of age in endemic areas and by cohort seroconversion rates determined after epidemics in previously unexposed populations. For example, using data following the 1964 Houston (Texas) epidemic, seroprevalence rates remained similar among cohorts, whereas the case–incidence rates increased from 8.2 per 100 000 for the 0–9-year-old cohort to 13.5–27.6 for the 10–59-year-old cohorts and to 78.0 for the >60-year-old group; apparent to inapparent ratios decreased concomitantly from 1:806 to 1:490–1:239 and to 1:85, respectively. Case–fatality rates among 2288 cases reported to the CDC from 1971–83 increased from <6.7% for 0–64-year-old age classes to 9.5% for the 65–74-year-old class to 18% for the >75-year-old class.

Occupation

In the West, where SLE historically was a rural disease, infection risk was greatest among male agricultural workers who frequently lived in suboptimum housing and worked at night. However, infection patterns during recent urban outbreaks indicated that attack rates were highest among elderly women. These data indicated that there may be differences in risk related to vector species, with elderly women infected most readily during urban outbreaks associated with the *C. pipiens* complex, and men working outdoors at greatest risk during rural outbreaks associated with *C. tarsalis*.

Socioeconomic status

Historically, socioeconomic status has been related closely to the distribution of cases during urban epidemics. Homes and municipal drainage systems frequently were not well maintained in low-income neighborhoods, and this was related to the distribution of human cases, but not necessarily the occurrence of virus within the enzootic transmission cycle. TV and air conditioning ownership that brought people indoors during the evening *Culex* host-seeking period was found to reduce risk.

Weather

Climate variability affects temperature and precipitation patterns, mosquito abundance and survival, and therefore SLEV transmission. Annual temperature changes based on the El Niño/southern oscillation in the Pacific alter precipitation and temperature patterns over the Americas and cycle with varying intensity at 3–5 year intervals. These cycles alter storm tracks that affect mosquito and avian abundance, the intensity and frequency of rainfall events, and groundwater depth, all related to SLEV risk. Above-normal temperatures have been especially necessary for northern latitude SLEV epidemics, because elevated temperatures are required for effective SLEV replication within the mosquito host.

Prevention and Control

Effective vector control remains the only approach available to suppress summer virus amplification and prevent human infections. Best results are achieved using an integrated management approach that focuses on mosquito vector population suppression through habitat inspection and larviciding. Failure of larval management can be followed by emergency adult control focusing on reducing the force of transmission and preventing human infection. Protection of the human population by vaccination does not seem cost-effective or prudent, because there is no human-to-human transmission, few human infections produce disease, and infection rates remain relatively low, even during epidemics. However, if regional infection rates were to become high, thereby placing selected cohorts at high risk for disease, then selective vaccination may be warranted. There currently is no approved commercial vaccine for SLEV, although vaccination against other flaviviruses such as JEV may impart some protection. Control of avian hosts such as house sparrows and pigeons in urban situations could be done, but this approach is not generally acceptable to the public. Notification of the public of infection risk through the media and the wide scale use of personal protection through changes in behavior (staying indoors after sunset) and/or repellent application were credited with reducing the number of infections during the 1990 epidemic in Florida.

See also: Japanese Encephalitis Virus; Tick-Borne Encephalitis Viruses.

Further Reading

Day JF (2001) Predicting St. Louis encephalitis virus epidemics: Lessons from recent, and not so recent, outbreaks. *Annual Review of Entomology* 46: 111–138.

Kramer LD and Chandler LJ (2001) Phylogenetic analysis of the envelope gene of St. Louis encephalitis virus. *Archives of Virology* 146: 2341–2355.

Monath TP (1980) In: *St. Louis Encephalitis*, 680pp. Washington, DC: American Public Health Association.

Monath TP and Tsai TF (1987) St. Louis encephalitis: Lessons from the last decade. *American Journal of Tropical Medicine and Hygiene* 37: 40s–59s.

Reeves WC, Asman SM, Hardy JL, Milby MM, and Reisen WK (eds.) (1990) In: *Epidemiology and Control of Mosquito-Borne Arboviruses in California, 1943–1987*, 508pp. Sacramento, CA: California Mosquito and Vector Control Association.

Reisen WK (2003) Epidemiology of St. Louis encephalitis virus. In: Chambers TJ and Monath TP (eds.) *The Flaviviruses: Detection, Diagnosis and Vaccine Development*, pp. 139–183. San Diego, CA: Elsevier.

Sweetpotato Viruses

J Kreuze and S Fuentes, International Potato Center (CIP), Lima, Peru

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cultivar decline A vegetatively propagated cultivar suffering from reduced vigor as a result of a chronic (but often symptomless) disease.

Differential hosts Special species of plants varying in susceptibility to a given disease agent, such that their distinctive symptoms facilitate a presumptive identification of the causal agent.

Indexing Any procedure for demonstrating the presence of a pathogen(s) in susceptible plants. The virus indexing combines information on viruses with methodologies for their detection to assure effective safe movement of sweetpotato germplasm.

Introduction

Sweetpotato

Sweetpotato (*Ipomoea batatas* (L.) Lam.) is a dicotyledonous, perennial plant, producing edible tuberous roots. It belongs to the family Convolvulaceae, the morning glory.

This family contains about 55 genera. The genus *Ipomoea* is thought to contain over 500 species with ploidy levels ranging from $2x$ to $6x$. Sweetpotato is the only *Ipomoea* species of economic importance as a food crop, and has both $4x$ and $6x$ forms ($2n = 4x = 60$ or $2n = 6x = 90$). Thousands of cultivars of sweetpotato are grown throughout the tropics and subtropics. With an annual production of more than 133 million tons globally, sweetpotato currently ranks as the seventh most important food crop on a fresh-weight basis in the world, and fifth in developing countries after rice, wheat, maize, and cassava. The production is concentrated in East Asia, the Caribbean, and tropical Africa, with the bulk of the crop (88%) being grown in China (**Figure 1**). Sweetpotato performs well in relatively poor soils, with few inputs, and has a short growing period. Among the major starch staple crops, it has the largest rates of production per unit area per unit time: in some areas up to three harvests per year can be achieved. Sweetpotato roots are rich in vitamin C and essential mineral salts. Due to the high beta-carotene content of yellow and orange-fleshed storage roots, they are being promoted to alleviate vitamin A deficiency in East Africa and Eastern India.

Viruses of Sweetpotato

Until recently, viruses of sweetpotato have been relatively poorly studied as compared to viruses of other crops. Still, more than 20 different viruses have been described infecting sweetpotato worldwide, but only 15 of these are currently recognized by the International Committee on Taxonomy of Viruses (ICTV; **Table 1**). This number, however, will most likely increase by additional surveys (**Figure 2**) and by indexing germplasm collection (**Figure 3**).

Vegetative propagation, usually by taking cuttings from a previous crop, increases the risk of a buildup of viruses. The importance of virus diseases and their buildup in farmers' planting materials has been shown convincingly in China, where sweetpotato cultivars planted using pathogen-tested materials yielded 30–40% more, on average, than those grown from farm-derived planting materials. Next to weevils, virus diseases form the most important biotic production constraint in sweetpotato. Most sweetpotato-infecting viruses, however, show only mild or no symptoms when in single infection and the damages caused by sweetpotato viruses are mostly through synergistic mixed infections. Viruses of the families *Potyviridae* and *Geminiviridae* as well as sweetpotato chlorotic stunt virus (SPCSV) are particularly significant in relation to sweetpotato cultivar decline.

Due to low virus titers and absence of symptoms from single infections in sweetpotato by most viruses, grafting and in some cases sap transmission onto indicator plants is often required to increase virus concentration and detect viruses reliably (**Figure 3**). Commonly used indicator plants are *Ipomoea setosa*, *I. nil*, *I. purpurea*, *I. aquatica*, and in some cases *Nicotiana benthamiana* and *N. clevelandii*.

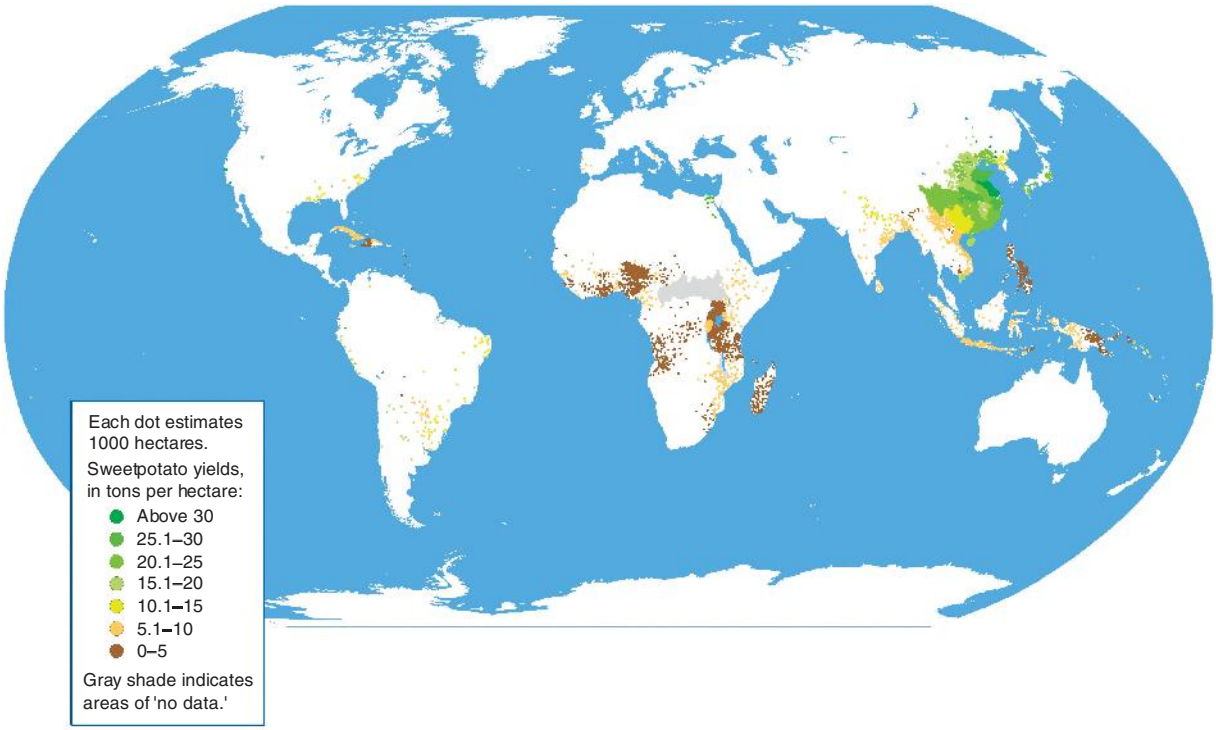


Figure 1 Sweetpotato cultivation: production areas and average yields.

Table 1 List of viruses that have been reported infecting sweetpotato

Genus/family	*Species/virus*	*Reported distribution*[a]	*Vector*	*Detection methods*[b]	*Strains/ serotypes*	*Component of synergistic disease*[c]	*Sequence from GenBank*[d]
Begomovirus	*Ipomoea yellow vein virus* (IYVV)	Spain, Italy	Whiteflies?	PCR	None reported		AJ132548
Begomovirus	*Sweetpotato leaf curl Georgia virus* (SPLCGV)	USA, Puerto Rico	Whiteflies	NA hybridization, PCR	None reported		AF326775
Begomovirus	*Sweetpotato leaf curl virus* (SPLCV)	Far East, USA, China, Taiwan, Japan, Korea, Europe, Africa?, Peru	Whiteflies	NA hybridization, PCR, qPCR	None reported	Unnamed (with SPFMV)	AF104036
Carlavirus	Sweetpotato chlorotic fleck virus (SPCFV)	Africa, South America, Asia, Cuba, Panama, China, Taiwan, Japan, Korea, New Zealand	Unknown	Serology	East Africa type, Asia type	Camote kulot, unnamed (with SPCSV)	NC_006550
Carlavirus?	C-6 virus	USA, Peru, Cuba, Dom, Rep., Indonesia, Philippines, P. Rico, Egypt, Uganda?, Kenya, South Africa, New Zealand	Unknown	Serology	None reported	Camote kulot, unnamed (with SPCSV)	None available
Caulimovirus?	Sweetpotato caulimo-like virus (SPCaLV)	South Pacific Region, Madeira, China, Egypt, Puerto Rico, Uganda, Kenya?, Nigeria	Unknown	Serology, PCR	None reported		None available
Crinivirus	*Sweetpotato chlorotic stunt virus* (SPCSV)	Worldwide	Whiteflies	Serology, NA hybridization, RT-PCR, qRT-PCR	EA, WA	SPVD, SPCD, Camote Kulot, SPSMD	RNA1: AJ428554, RNA2: AJ428555
Cucumovirus	*Cucumber mosaic virus* (CMV)	Israel, Egypt, Kenya, Uganda?, Japan, South Africa, New Zealand	Aphids	Serology	None reported	Unnamed (with SPCSV)	RNA1: D00356, RNA2: D00355, RNA3: D10538
Geminiviridae	Ipomoea crinkle leaf curl virus (ICLCV)	Israel	Whiteflies	NA hybridization	None reported		None available
Ilarvirus	*Tobacco streak virus* (TSV)	Guatemala	Thrips	Serology	None reported		RNA1: U80934, RNA2: U75538, RNA3: X00435
Ipomovirus	*Sweetpotato mild mottle virus* (SPMMV)	Africa, Indonesia, China, Philippines, Papua New Guinea, India, Egypt, New Zealand	Whiteflies?	Serology, RT-PCR	Different strains	Camote Kulot, SPSMD	Z73124
Ipomovirus	Sweetpotato yellow dwarf virus (SPYDV)	Taiwan, Far East	Whiteflies	Serology	None reported		None available
Luteoviridae	*Sweetpotato leaf speckling virus* (SPLSV)	Peru, Cuba	Aphids	NA hybridization, RT-PCR	None reported		DQ655700

Continued

Table 1 Continued

Genus/family	*Species/virus*	*Reported distribution*[a]	*Vector*	*Detection methods*[b]	*Strains/ serotypes*	*Component of synergistic disease*[c]	*Sequence from GenBank*[d]
Nepovirus	Sweetpotato ringspot virus (SPRSV)	Papua New Guinea, Kenya?	Unknown	Serology	None reported		None available
Potyvirus	*Sweetpotato feathery mottle virus* (SPFMV)	Worldwide	Aphids	Serology, NA hybridization, RT-PCR, qRT-PCR	EA, RC, O, C	SPVD, SPCD, Camote Kulot	D86371
Potyvirus	*Sweetpotato latent virus* (SPLV)	Africa, Taiwan, China, Japan, India, Philippines, Indonesia, Egypt	Aphids	Serology, NA hybridization	None reported	Camote Kulot	X84011*, X84012*
Potyvirus	Sweetpotato mild speckling virus (SPMSV)	Argentina, Peru, Indonesia, Philippines, China, Egypt, Uganda?, Kenya?, South Africa, Nigeria, New Zealand	Aphids	Serology	None reported	SPCD, Camote Kulot	U61228*
Potyvirus	Sweetpotato vein mosaic virus (SPVMV)	Argentina	Aphids	No available	None reported		None available
Potyvirus	*Sweet potato virus G* (SPVG)	China, Japan, USA, Egypt, Ethiopia, Nigeria, Barbados, Peru	Aphids	Serology, RT-PCR, qRT-PCR	None reported		Z83314*, AJ515380*
Potyvirus	Sweetpotato virus 2	Taiwan, USA, China, South Africa, Portugal, Australia, Barbados	Aphids	Serology, RT-PCR, qRT-PCR	Geographical/ genetical lineages	Unnamed (with SPCSV)	AY178992*, AY232437*
Tobamovirus	*Tobacco mosaic virus* (TMV)	USA	None	Serology	None reported		X02144, AJ132845

[a]? signifies unconfirmed.
[b]NA, nucleic acid; RT-PCR, reverse transcription PCR; qPCR, quantative real-time PCR; qRT-PCR, quantative real-time reverse transcription PCR.
[c]SPVD, sweetpotato virus disease (Africa, Peru); SPCD, sweetpotato chlorotic dwarf (Argentina); SPSMD, sweetpotato severe mosaic disease (Africa); Camote kulot (Philippines).
[d]Complete genome sequence except those marked with (*).

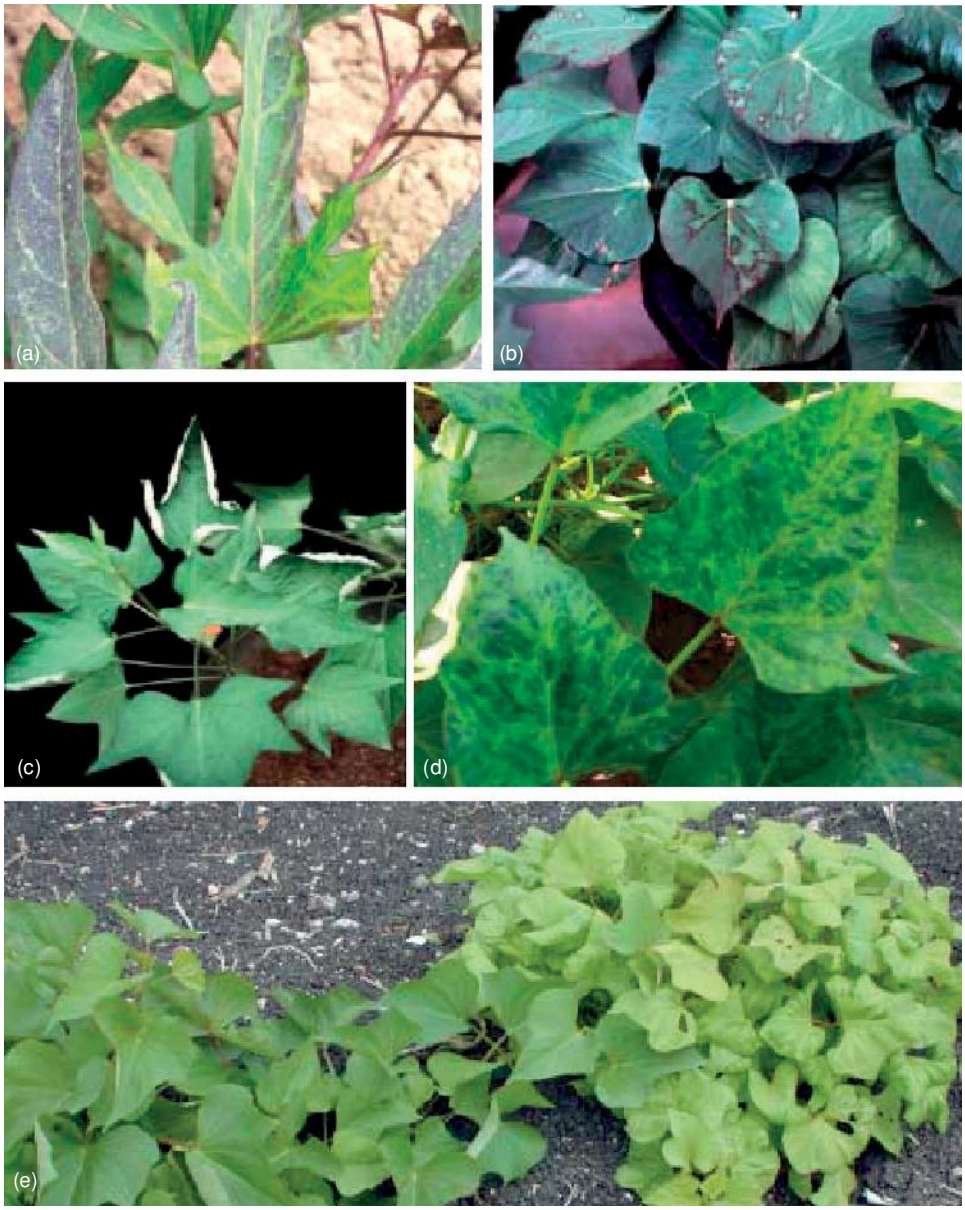


Figure 2 Sweetpotato plants showing symptoms of vein clearing (a and b), leaf curling (c), mosaic (d), and chlorosis, stunting, and leaf deformation. Some times the vein clearing is surrounded by purple pigmentation.

Potyviruses

Several potyviruses infect sweetpotato and usually only cause transient, mild, or no symptoms when infecting sweetpotato by themselves. The most widespread of these, and the one studied in most detail is sweetpotato feathery mottle virus (SPFMV, genus *Potyvirus*, family *Potyviridae*) that occurs wherever sweetpotato is grown. In many cases infection of sweetpotato plants with SPFMV causes mild or no symptoms, although certain strains can cause qualitative damage due to internal cork or cracking of the tuberous roots. However, quantitative losses due to reduced plant vigor associated with chronic infection with SPFMV have also been experienced. Yet, it is as a component of complex virus diseases that SPFMV probably causes the greatest damage.

Sweetpotato Feathery Mottle Virus

SPFMV has flexuous filamentous particles measuring 830–850 nm in length. They contain a single-stranded, positive-sense RNA genome of about 10.6 kb, which is

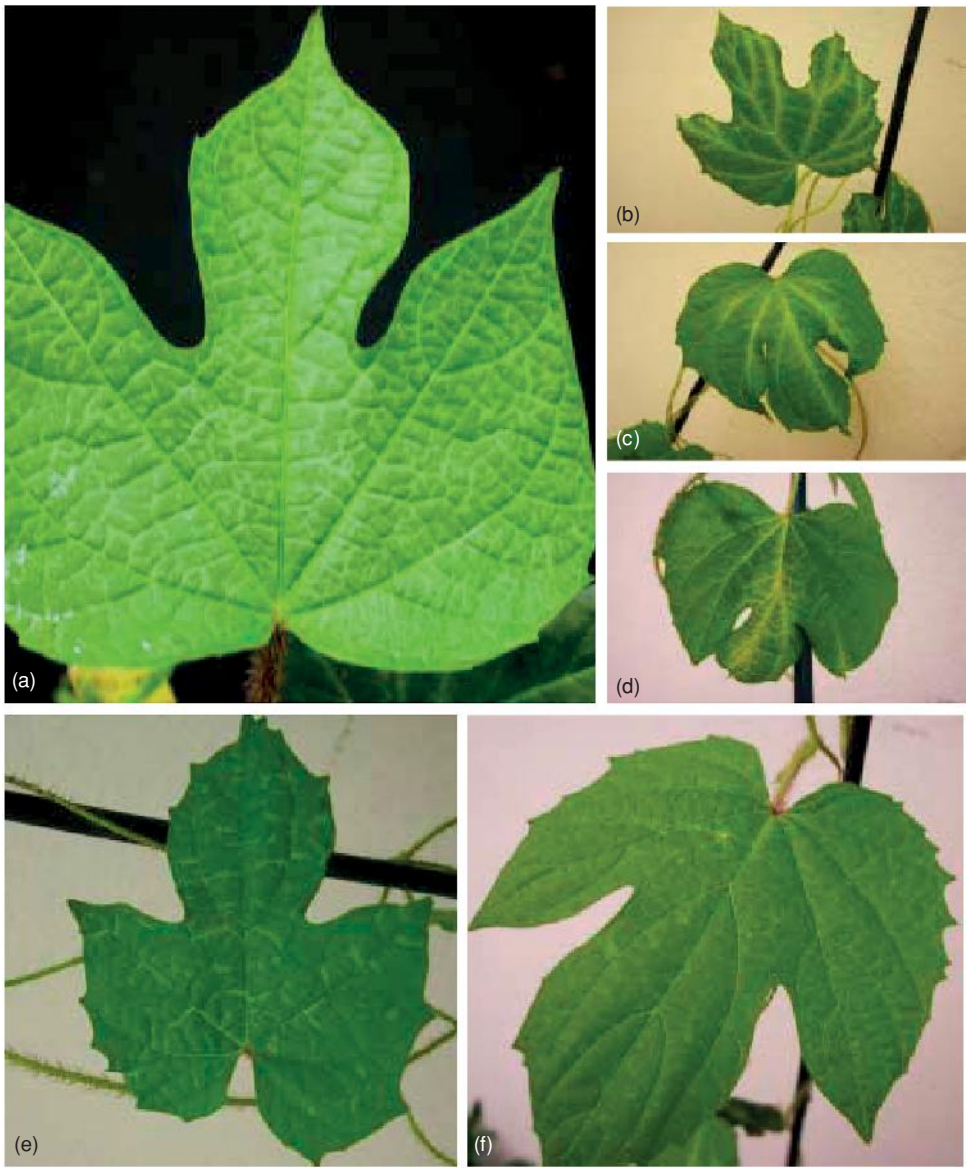


Figure 3 *Ipomoea setosa* graft-inoculated with scions from sweetpotato plants collected in farmer's fields in Peru. Infected with sweetpotato feathery mottle virus (a–d) and sweetpotato virus G (e). Faint chlorotic spots (f) induced by an unknown virus. The causal virus tested negatively to available antisera in NCM-ELISA.

larger than the average size (9.7 kb) of a potyvirus genome. The coat protein (CP) of SPFMV is also exceptionally large (38 kDa) as compared to other potyviruses, which is largely due to the insertion of a contiguous sequence at the 5′-end of the CP cistron. SPFMV is transmitted by several aphid species (i.e., *Aphis gossypii*, *A. craccivora*, *Lipaphis erysimi*, *Myzus persicae*) in a non-persistent manner. However, these aphids rarely colonize sweetpotato under field conditions and therefore itinerant alate aphids are probably the most efficient vectors of SPFMV. The experimental host range of SPFMV is narrow and mostly limited to plants of the family Convolvulaceae, and especially to the genus *Ipomoea*, although some strains have been reported to infect *N. benthamiana* and *Chenopodium* spp. Symptoms, host range, and serology have been used to group SPFMV isolates into two strains, the common strain (C) and the more severe russet crack (RC) strain. However, based on phylogenetic analysis of 3′ nt sequences of an extensive number of isolates it is now clear that SPFMV can be distinguished in four phylogenetic lineages RC, O (ordinary), EA (East Africa), and C (**Figure 4**). Based on molecular data the C strain is rather distantly related to the remaining strains and may in the future be classified as a separate virus.

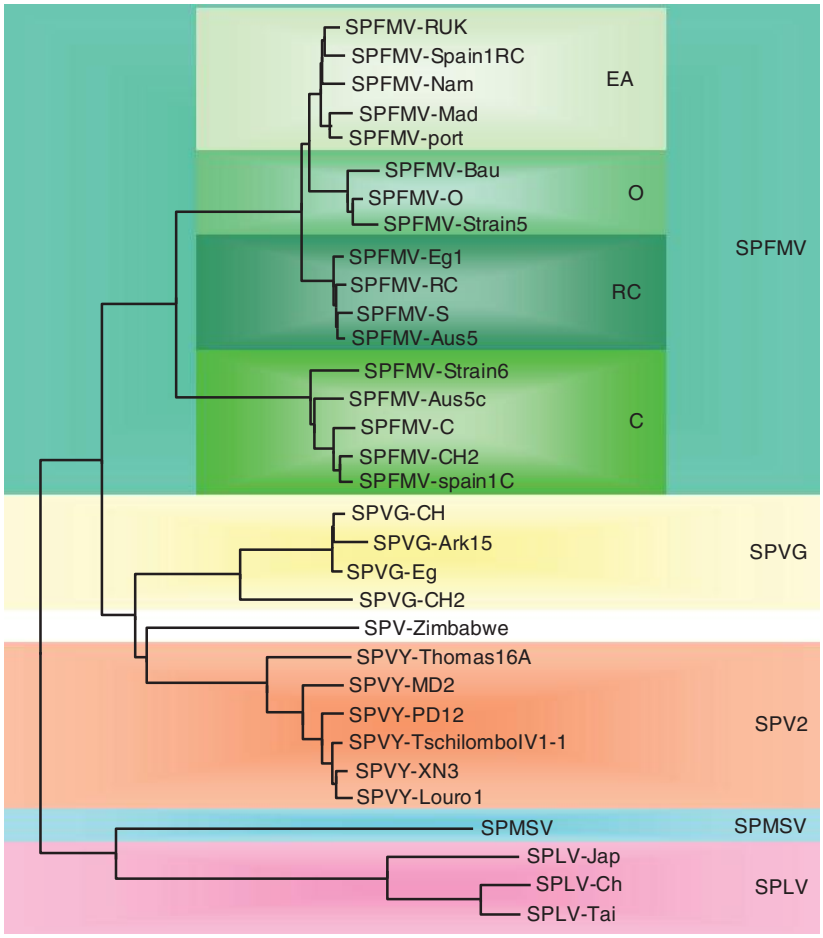


Figure 4 Phylogeny of representative isolates of sweetpotato-infecting potyviruses based on complete CP sequences. Clades corresponding to described virus species are shaded in different colors. The four recognized strain groups of SPFMV are also indicated and shaded in different colors of green. One sequence, originally described as SPFMV from sweetpotato in Zimbabwe, is distinct from all other named species.

Other Potyviruses

Several other potyviruses are known to infect sweetpotato, some of which are distributed widely. They have in common that they are mostly symptomless and in low titers when infecting sweetpotato by themselves, but can be distinguished by their symptoms induced in indicator plants such as *I. nil* and *I. setosa* (**Figure 3**), or by symptoms induced through synergism with SPCSV. With the exception of sweetpotato vein mosaic virus (SPVMV) sequence information is available for these viruses and their phylogenetic relationships are depicted in **Figure 4**.

Sweetpotato virus G (SPVG) was first reported from China, but is now known to occur also in the Americas and Africa. Another virus, originally identified as sweetpotato virus 2 in Taiwan, was recently further characterized simultaneously by two groups suggesting the species names sweetpotato virus Y and ipomoea vein mosaic virus, respectively, and has been reported from Taiwan, China, USA, South Africa, Australia, and Barbados. The name SPV2 is currently being considered for this virus by the ICTV. Sweetpotato latent virus (SPLV) was also first reported from China, but is now known to occur in most major sweetpotato-growing areas in Asia. The two remaining potyviruses reported were first described from Argentina and are SPVMV and sweetpotato mild speckling virus (SPMSV). Whereas SPMSV has also been detected in Peru and Indonesia, neither antibodies nor sequence information are available for SPVMV, and the virus has not been reported from elsewhere. The number of sweetpotato-infecting potyvirus species will certainly still increase through future surveys, for example, a potyvirus distinct from any of the above mentioned was isolated from infected sweetpotato in Zimbabwe and remains to be characterized in more detail (**Figure 4**).

Sweetpotato mild mottle virus (SPMMV, genus *Ipomovirus*) is transmitted by whiteflies in a nonpersistent

manner. It should be noted, however, that since the initial report, it has not been possible to confirm independently the whitefly transmissibility of SPMMV. A study of the variability of SPMMV in Uganda using 3′ nt sequences showed the virus consisted of a population of distinct sequence variants (>85.9% nt identity in the CP), which however did not show any particular clustering. A distinct feature of SPMMV as compared to other sweetpotato-infecting viruses is its exceptionally broad host range including species in 14 families. SPMMV has serologically been detected throughout Africa, Indonesia, China, Philippines, Papua New Guinea, India, Egypt, and New Zealand. Another whitefly-transmitted potyvirus with properties distinct from SPMMV was described in Taiwan and named sweetpotato yellow dwarf virus (SPYDV). The relationship between these two viruses is however unclear as no sequence information is available for SPYDV.

Begomoviruses

Sweetpotato leaf curl diseases typical of geminivirus infection (**Figure 2(c)**) have been reported from Peru, Japan, Taiwan, Korea, China, Puerto Rico, Costa Rica, Spain, United States, Africa, and other countries. The host range of the *Ipomoea*-infecting begomoviruses (family *Geminiviridae*) is narrow and mostly restricted to species in the family Convolvulaceae (especially to the genus *Ipomoea*), but *N. benthamiana* (Solanaceae) can also be infected. They cause leaf curl on some hosts, and yellow vein or leaf distortion and chlorosis symptoms on others. Some sweetpotato cultivars are symptomless. As they induce mild, transient symptoms in the standard virus indicator, *I. setosa,* a universally reliable biological indicator is lacking. Some *Ipomoea* species (*I. aquatica, I. cordatotriloba, I. purpurea*) can however be used as differential hosts to distinguish *Ipomoea*-infecting begomoviruses.

Only three sweetpotato-infecting begomovirus species have been officially recognized by the ICTV (**Table 1**): *Sweetpotato leaf curl virus* (SPLCV), *Sweetpotato leaf curl Georgia virus* (SPLCGV), and *Ipomoea yellow vein virus* (IYVV). DNA B components have not been identified for any of these viruses indicating they are monopartite begomoviruses. Sequences of AC1 gene fragments from isolates obtained from infected *Ipomoea* spp. from USA, China, Taiwan, Korea, Puerto Rico, Spain, and Italy all fall into two groups corresponding to SPLCV/IYVV and SPLCGV. A third phylogenetically distinct group of begomoviruses was identified from infected sweetpotato in Spain, representing a putative fourth species. Similarly the sequence of a SPLCV isolate from China shows <83% identity to other published sequences indicating yet another virus species. Another virus, proposed to be named ipomoea crinkle leaf curl virus (ICLCV) and reported from Israel, differed in host range from SPLCV but its exact relationship to other identified viruses remains unclear.

From a taxonomic point of view, the *Ipomoea*-infecting begomoviruses are a curiosity; phylogenetic analysis of IYVV and several strains of SPLCV and SPLCGV revealed that these viruses form a separate unique cluster within the genus *Begomovirus*, dissimilar to both the New World and Old World begomoviruses. Additionally, the relatively poor transmission rates of the *Ipomoea*-infecting begomovirus by *Bemisia tabaci* may be a reflection of the low CP amino acid sequence identity (46%) between them and the other begomoviruses.

Sweetpotato Leaf Curl Virus

The virus has geminate particles of *c.* 18×30 nm with a genome of 2828 nt. Its genomic DNA and organization is similar to that of monopartite begomoviruses. SPLCV virus was first reported from Japan and Taiwan but now it is known to occur in several countries on different continents. SPLCV can cause up to 30% reductions in yield of storage roots. Various *Ipomoea* species are susceptible to SPLCV, such as *I. purpurea* causing leaf curl and stunt, *I. aquatica* causing yellow vein symptoms, *I. nil*, *I. setosa,* and *N. benthamiana* causing leaf curl symptoms. Co-infections of SPFMV and SPLCV in *I. setosa* and *I. nil* induce severe leaf distortion, general chlorosis, and stunting.

Ipomoea Yellow Vein Virus

IYVV was first found in Spain infecting *I. indica* plants showing yellow vein symptoms, but has since then been found infecting cultivated sweetpotato as well. Its properties, including typical geminivirus symptoms, detection of geminate particles by electron microscopy, and complete nucleotide sequence confirmed its begomovirus nature. Based on nucleotide sequence similarity of >89% over the entire genome, IYVV should be considered a strain of SPLCV, and will probably be revised accordingly in the future. However, contrary to SPLCV which is transmitted by *B. tabaci* biotype B, IYVV-[Spain] was not transmitted by biotypes Q, S, or B of *B. tabaci*.

Sweetpotato Leaf Curl Georgia Virus

SPLCGV, previously called ipomoea leaf curl virus, has a genome of 2773 nt with an organization typical of other Old World monopartite begomoviruses. The complete nucleotide sequence of SPLCGV is 82–85% identical to those of SPLCV and IYVV, which is below the species threshold of 89% nucleotide sequence identity for begomoviruses. Like SPLCV, SPLCGV is transmitted by *B. tabaci* biotype

B and induces similar symptoms in most *Ipomoea* species (various degrees of leaf curling). The means to distinguish SPLCGV from SPLCV are the use of *I. aquatica* and *I. cordatrotiloba* as differential hosts and a combination of polymerase chain reaction (PCR) and restriction enzyme digestion.

Sweetpotato Chlorotic Stunt Virus

Due to its ability to mediate severe synergistic viral diseases with several other sweetpotato-infecting viruses including potyviruses, cucumoviruses, and carlaviruses, SPCSV is probably the most devastating virus of sweetpotato worldwide. In single infection, this virus causes no symptoms at all or usually only mild stunting combined with slight yellowing or purpling of older leaves, symptoms which are easily confused with nutritional deficiencies.

SPCSV belongs to the genus *Crinivirus* of the family *Closteroviridae*. The particles of SPCSV are 850–950 nm in length and 12 nm in diameter. The size of the major coat protein is 33 kDa, which is similar to other criniviruses. SPCSV is transmitted by whiteflies (e.g., *B. tabaci*, *B. afer*, and *Trialeurodes abutilonea*) in a semipersistent, noncirculative manner, and is not mechanically transmissible. Similar to most other sweetpotato-infecting viruses, the host range of SPCSV is limited mainly to the family Convolvulaceae and the genus *Ipomoea*, although *Nicotiana* spp. and *Amaranthus palmeri* are reportedly susceptible. SPCSV has also been detected in the ornamental species Lisianthus (*Eustoma grandiflorum*). SPCSV can be serologically divided into two major serotypes. One of the serotypes (EA for East Africa) was first identified in East Africa, and also occurs in Peru, while the other serotype (WA for West Africa) was first identified in West Africa and occurs additionally in the Americas, and the Mediterranean, but not in East Africa. The two serotypes correlate to two genetically distantly related strain groups based on coat protein as well as Hsp70h gene similarities.

The genome of an SPCSV isolate from Uganda has been entirely sequenced and consists of two RNA segments of 9407 and 8223 nt. The SPCSV genome encodes two unique proteins, p22 and RNase3, not known to occur in any other RNA viruses. mRNAs corresponding to these proteins are transcribed early during infection and they are cooperatively able to suppress RNA silencing, a process that requires the RNA binding and double-stranded RNase activity of the RNase3 protein.

Other Viruses

Some viruses recognized by ICTV that affect other crops (i.e., cucumber mosaic virus, tobacco mosaic virus, and tobacco streak virus), are sporadically found infecting sweetpotato. Information on these viruses is extensively available. Other sweetpotato-specific viruses have not yet being assigned to a genus (i.e., sweetpotato leaf speckling virus – SPLSV) or recognized as a species (i.e., sweetpotato chlorotic fleck virus – SPCFV; C-6 virus; sweetpotato caulimo-like virus – SPCaLV) by the ICTV (**Table 1**).

Sweetpotato Leaf Speckling Virus

SPLSV has isometric particles *c.* 30 nm in diameter. The virus is transmitted by *Macrosiphon euphorbiae* in a persistent manner. It has a restricted geographical distribution. The sequence of the CP and 17K encoding region has been determined and is characteristic of luteo- and poleroviruses sequences, with highest similarity to potato leafroll virus (PLRV). Although these two viruses can be detected with heterologous CP probes, they are not serologically related.

Sweetpotato Chlorotic Fleck Virus

SPCFV has filamentous particles measuring about 800 nm in length consisting of its genome encapsidated by polypeptide subunits of 33.5 kDa. The virus appears to have a wide geographic distribution in sweetpotato crops in South America, Africa, and Asia. SPCFV has a narrow host range in the families Convolvulaceae and Chenopodiaceae, but some strains/isolates infect *N. occidentalis*. SPCFV is mostly symptomless in its natural host; hence, it was also referred to as sweetpotato symptomless virus in Japan.

Sequence analysis of the entire genome of SPCFV (9104 nt) provides unambiguous evidence for the assignment of SPCFV as a distinct species in the genus *Carlavirus*. The RNA of SPCFV is larger than that of the other carlaviruses due to its considerably larger replicase (238 vs. 200–223 kDa) and a long untranslated region of 236 nt between ORF4 and ORF5. Phylogenetic analysis of CP sequences suggests that there is some geographically associated variation among SPCFV isolates.

Complex Virus Diseases of Sweetpotato

Multiple virus infections are common in sweetpotato and synergistic interactions are often involved. The most common of these disease complexes, known as sweetpotato virus disease (SPVD), is caused by simultaneous infection with SPFMV and SPCSV (**Figures 5** and **6**). This disease is characterized by chlorosis, small, deformed leaves, and severe stunting, and can reduce yields of infected plants by up to 99%. Despite the apparent broad meaning of the name SPVD, the symptoms are so characteristic that the name has become restricted to the disease

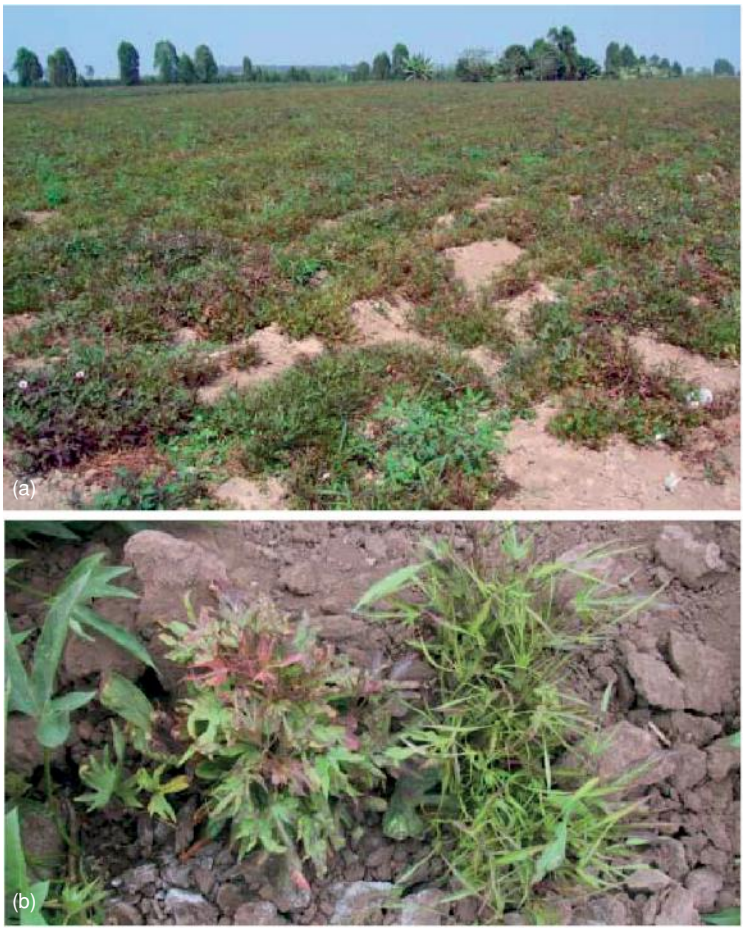


Figure 5 Sweetpotato plants affected by viruses in Peru. Sweetpotato field with a large number of plants affected by sweetpotato virus disease complex (a) and a close up of an affected plant showing stunting, mosaic, and leaf deformation (b).

with these symptoms and caused by these viruses. SPVD is the most serious disease of sweetpotato in Africa and Peru, and may be the most important virus disease of sweetpotato globally.

Other viral disease complexes have also been described, which invariably seem to involve SPCSV. In Israel and Egypt cucumber mosaic virus (CMV, genus *Cucumovirus*, family *Bromoviridae*) is found infecting sweetpotato together with SPCSV and usually also SPFMV, producing symptoms similar to SPVD and causing up to 80% reduction in yield. It was shown that CMV could only infect sweetpotato if the plants were first infected with SPCSV. Interestingly, this seems not to be the case for CMV in Egypt, where it is found infecting sweetpotato with or without SPCSV. In Argentina, a disease locally known as chlorotic dwarf is caused by infection with SPCSV and SPFMV and/or SPMSV, and is the most important disease of sweetpotato in the country. Once again, the symptoms resemble those of SPVD and are most severe when all three viruses infect sweetpotato simultaneously. In the Philippines SPCSV together with several other viruses causes a disease locally known as Camote Kulot.

In all the mentioned disease complexes, infection with each virus separately causes only mild or no symptoms in sweetpotato. They are thus caused by a synergistic interaction between the viruses. As both SPFMV and SPCSV are involved in all these diseases, the variation in the strains of these viruses should be important factors determining the disease severity.

Experimentally SPCSV can induce synergism with all tested potyviruses (including SPMMV), CMV as well as carlaviruses, and is always associated with an increase in the titers of the co-infecting virus and reduced yield of storage roots. For instance, the dual infection of SPCSV and SPMMV has been named 'sweetpotato severe mosaic disease (SPSMD)'. Yet there are reports indicating that strains of SPFMV and SPV2 may differ in their ability to cause synergism with SPCSV. Triple infections involving SPCSV, SPFMV, and an additional virus are even more severe, leading to further increase of the SPFMV

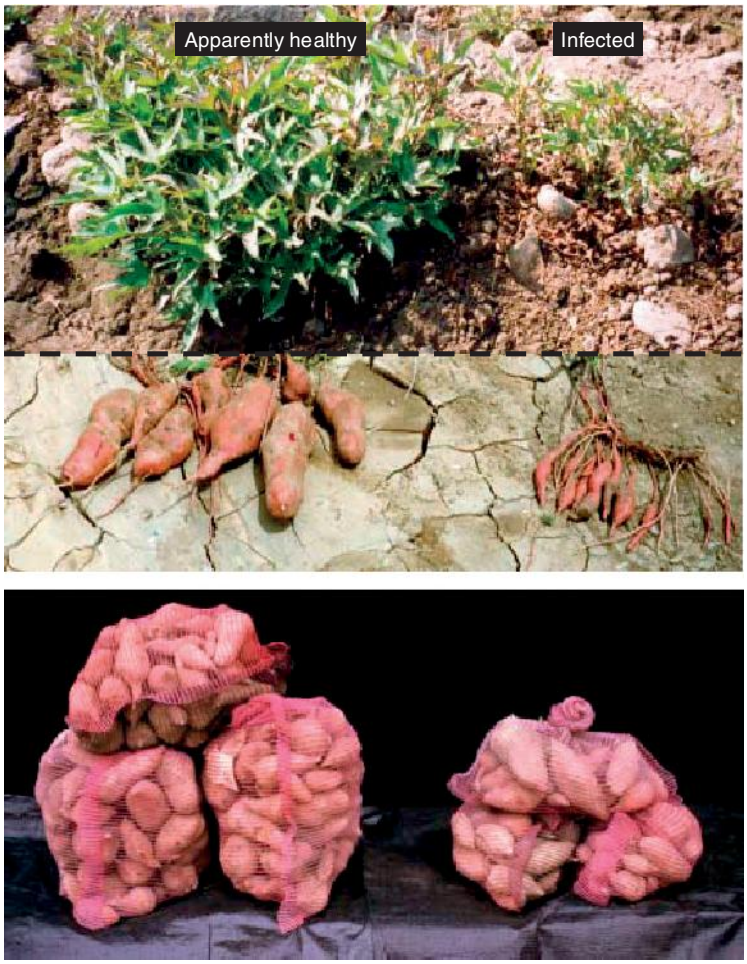


Figure 6 Effect of viruses on sweetpotato plants (top) and yield of storage roots (bottom). Observe yield reduction on sweetpotato variety Costanero (30 plants each treatment).

titers, whereas the titers of the third virus may either increase or decrease. It is thought the synergistic effects of SPCSV on other viruses may be due to interference with RNA silencing, because they are associated with substantially increased accumulation of co-infecting viruses.

See also: Carlavirus; Citrus Tristeza Virus; *Ilarvirus*; Luteoviruses; Plant Antiviral Defense: Gene Silencing Pathway; Plant Resistance to Viruses: Geminiviruses; Potyviruses; Viral Suppressors of Gene Silencing.

Further Reading

Ames T (ed.) (2002) *Acta Horticulturae. 583, ISHS: Proceeding of the First International Conference on Sweetpotato. Food and Health for the Future.* Brugge, Belgium: ISHS.

Clark CA and Moyer JW (1988) *Compendium of Sweetpotato Diseases*, 74pp. Minnesota: The American Phytopathological Society.

Kreuze J (2002) *Acta Universitatis agriculturae Sueciae. Agraria, vol. 335: Molecular studies on the Sweet Potato Virus Disease and Its Two Causal Agents*. Doctoral Dissertation, Dept. of Plant Biology, SLU (ISBN 91–576–6180–4; ISSN 1401–6249).

Loebenstein G, Fuentes S, Cohen J, and Salazar LF (2003) Sweet potato. In: Loebenstein G and Thottappilly G (eds.) *Virus and Virus-Like Diseases of Major Crops in Developing Countries*, pp. 223–248. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Nakasawa Y and Ishiguro K (eds.) (2000) *Proceedings of International Workshop on Sweetpotato Cultivar Decline Study.* Kyushu National Agricultural Experimental Station (KNAES), 8–9 September 2000, Miyakonojo, Japan. http://www.knaes.affrc.go.jp/acre/Old/Workshop/WS2000/workshop/index_workshop.html (accessed July 2007).

Tairo F, Mukasa SB, Jones RAC, *et al.* (2005) Unravelling the genetic diversity of the three main viruses involved in sweet potato virus disease (SPVD), and its practical implications. *Molecular Plant Pathology* 6: 199–211.

Taura Syndrome Virus

A K Dhar, Advanced BioNutrition Corp, Columbia, MD, USA
F C Thomas Allnutt, National Science Foundation, Arlington, VA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Internal ribosomal entry site (IRES) The sequence in a RNA transcript that allows internal entry of the ribosomal assembly for translation in a cap-independent manner.
Quasispecies Due to the error-prone replication of RNA, a specific RNA virus exists as a steady state or equilibrium mixture of mutant sequences clustered around a master sequence or sequences.
Specific pathogen free (SPF) Shrimp lines that have been bred in captivity and certified to be free of a specific disease or diseases.

Introduction

Taura syndrome disease (TSD) was first described as a shrimp disease in 1992 from Pacific white shrimp (*Penaeus vannamei*) farms located along the mouth of the Taura River basin in Guayas Province, Ecuador. Initially the disease was incorrectly attributed to the toxic effects of fungicides used in a near by banana plantation. The etiologic agent was later proven to be of viral origin. The virus was named Taura syndrome virus (TSV) based on the location where the disease was first recorded. Movement of TSV-contaminated shrimp stocks led to the rapid spread of the disease throughout the Americas between 1992 and 1996. Since the initial discovery of TSD, the economic losses to the shrimp farming industry in the Western Hemisphere have been enormous. From the Americas, the disease spread to Taiwan in 1999, and subsequently TSV epizootics have been recorded in China, Thailand, Korea, and Indonesia.

TSV particles are nonenveloped and icosahedral, measuring 31–32 nm in diameter (**Figure 1**), and have a buoyant density of 1.338 g ml^{-1}. The viral capsid contains three major (VP1, VP2, and VP3) and one minor (VP0) polypeptide. TSV genome is a linear, positive-sense, single-stranded RNA of approximately 10.2 kb. Nascent viral RNA synthesis occurs in intracellular vesicle-like membranes within the cytosol of the host cells. In infected cells, mature virions are also seen in close association with proliferating membranes. Currently, TSV has been classified as an unassigned member of the family *Dicistroviridae*.

History and Classification

TSD, caused by the TSV, is listed as a reportable disease by the Office Internationale des Epizooties (OIE), the international organization responsible for ensuring worldwide information transparency by dissemination of the current status of important animal diseases. TSD etiology was initially very controversial causing uncertainty that contributed to TSV spread through the Western Hemisphere between 1992 and 1996. The resulting epizootic cost the shrimp farming industry in the Americas an estimated US$1.2–2 billion. In 1999, TSD was reported from Taiwan and since then, the disease has spread to China, Thailand, Korea, and Indonesia. The disease has already caused considerable economic losses in Asia.

Based on clinical and histological data, researchers from Ecuador recognized a new syndrome in *P. vannamei* shrimp farms located along the mouth of the Taura River basin (Guayas Province, Ecuador) during the summer of 1992. The cause of TSD was initially attributed to toxicity by two systemic fungicides, Tilt (Propiconazole, Ciba-Geigy) and Calixin (Tridemorph, BASF) that were being sprayed on local banana plantations to control black leaf wilt disease or Singatoka negra. It was hypothesized that fungicide-contaminated runoff resulted in mortalities in shrimp farms. During the 2 years following its discovery, TSD spread west through Ecuador's most concentrated

shrimp-farming region, southward into Northern Peru, and northward to the Pacific Coast of Colombia. These latter regions had neither banana plantations nor application of the suspected fungicides. Meanwhile, attempts to induce TSD experimentally by waterborne, oral, or injection delivery of Tilt, Calixin, or Benlate O.D. (Benomyl, DuPont, a widely used banana fungicide in Ecuador) failed. During May 1994, two separate outbreaks of TSD occurred among cultured *P. vannamei* in Oahu (Hawaii, USA), representing the first TSD incursion into the United States. Subsequently, the induction of TSD clinical signs and histological lesions was demonstrated by experimental challenge of healthy shrimp *per os*, and the viral etiology was established. The virus was named Taura syndrome virus.

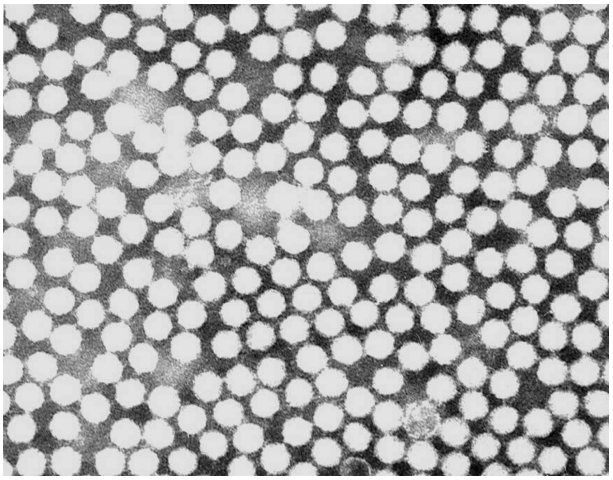


Figure 1 Transmission electron micrograph of CsCl gradient-purified and negatively stained (with 2% PTA) TSV particles. The virus particles are nonenveloped and icosahedral, measuring 31–32 nm in diameter.

Based on morphology and biophysical properties, TSV was then presumed to be a member of the family *Picornaviridae*. Availability of TSV genome sequence data and phylogenetic analysis using the capsid gene sequence of TSV and picornavirus superfamily viruses, revealed two major clusters (**Figure 2**). One cluster contains mammalian picornaviruses and picornavirus superfamily viruses infecting insects while the other cluster contains the plant picornaviruses. Within the first cluster, there were two subclusters. First subcluster contains insect picornavirus superfamily viruses with a dicistronic genome. The second subcluster contains two groups: one group includes sacbrood virus (SBV) of honeybee and infectious flacherie virus (IFV) of silkworm that share more similarities with mammalian than insect picornaviruses and the

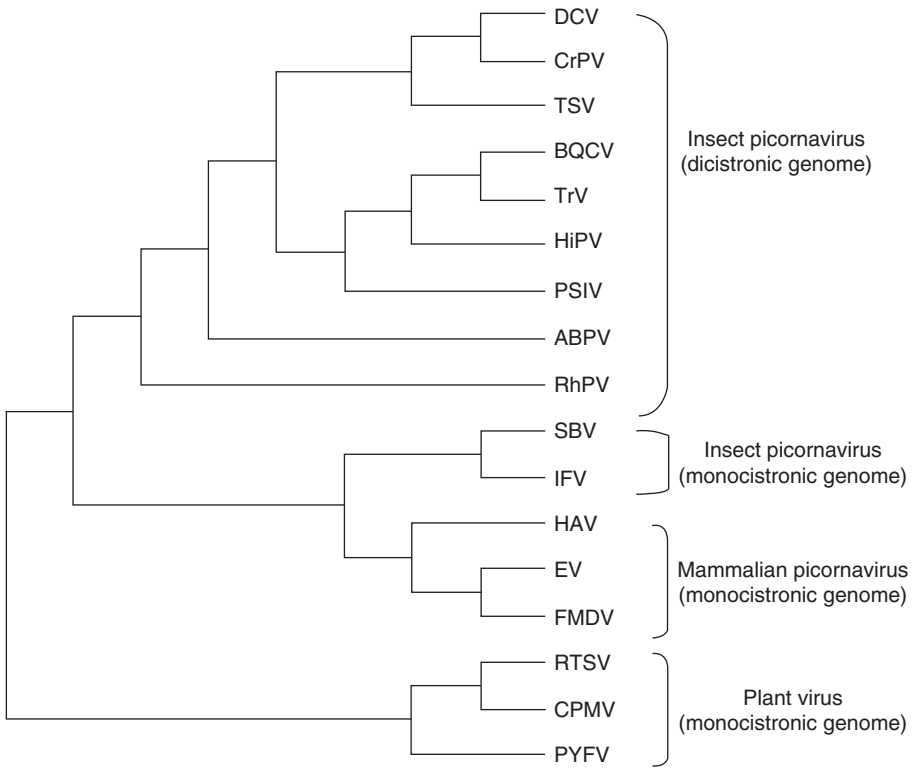


Figure 2 Phylogenetic analysis of TSV and other RNA viruses infecting insects, plants, and mammalian hosts. Reproduced from Dhar AK, Cowley JA, Hasson KW, and Walker PJ (2004) Genome organization, biology and diagnosis of Taura syndrome virus and yellowhead virus of penaeid shrimp. *Advances in Virus Research* 63: 353–419, with permission from Elsevier.

other group is the mammalian picornaviruses (**Figure 2**). The picornavirus superfamily viruses with dicistronic genomes were subsequently classified under a new family, *Dicistroviridae*, of which TSV is a new but unassigned member.

To date, the genomes of RNA viruses infecting a wide range of insect species have been sequenced. These include cricket paralysis virus (CrPV, AF218039), drosophila C virus (DCV, AF014388), acute bee paralysis virus (ABPV, NC002548), black queen cell virus (BQCV, AF183905), sacbrood virus (SBV, AF092924), rhopalosiphum padi virus (RhPV, AF022937), plautia stali intestine virus (PSIV, AB006531), triatoma virus (TrV, AF178440), himetobi P virus, (HiPV, AB017037), infectious falcherie virus (IFV, AB000906), homalodisca coagulata virus-*1* (HoCV-1, DQ288865), aphid lethal paralysis virus (ALPV, AF536531), Kashmir bee virus (KBV, AY275710), solenopsis invicta virus-1 (SINV-1, AY634314), and TSV of shrimp (AF277675). The genome organization of IFV and SBV is similar to mammalian picornaviruses. They contain a single open reading frame (ORF) with the capsid genes located at the 5′ end and nonstructural genes at the 3′ end. In contrast, the genomes of CrPV, DCV, ABPV, BQCV, RhPV, PSIV, TrV, HiPV, HoCv-1, ALPV, KBV, SINV-1, and TSV contain two ORFs (ORF1 and ORF2) separated by an intergenic region (IGR). At the 5′ end, ORF1 encodes the nonstructural genes, and at the 3′ end ORF2 encodes the capsid proteins (as seen for TSV in **Figure 5**). All show greater sequence similarity to each other than to mammalian picornaviruses. To date, eight of these viruses (ALPV, BQCV, CrPV, DCV, HiPV, PSIV, RhPV, and TrV) have been assigned into the newly created family *Dicistroviridae*, genus *Cripavirus,* with CrPV as the type species. SINV-1 is tentatively assigned to this genus, while APBL and TSV are net assigned to any genus but placed in the *Dicistroviridae* family.

Host Range and Clinical Signs

Species of penaeid shrimp differ in their susceptibility to TSV. *Penaeus vannamei*, the principal penaeid species cultivated in the Americas, and *P. schmitti* are highly susceptible to TSV. TSV causes serious disease in postlarval (PL), juvenile, and adult *P. vannamei*, but has not been reported in PL smaller than the PL11 stage (11 day old). Other American penaeid species, such as *P. stylirostris*, *P. setiferus*, *P. duorarum,* and *P. aztecus*, are generally less susceptible to TSV. Among the Eastern Hemisphere penaeid species, TSV infection has been reported in *P. monodon*, *P. japonicus*, and *P. chinensis*.

To combat catastrophic losses caused by TSV in *P. vannamei*, TSV-resistant lines of specific pathogen-free (SPF) *P. vannamei* have been developed by selective breeding. However, due to the ability of the virus to mutate rapidly, less susceptible or resistant species and selected breeding lines may succumb to TSD as more virulent strains emerge. This was observed during TSV epizootics in Mexico, in 1999, when TSV-tolerant *P. stylirostris* showed high mortality due to emergence of a virulent strain of TSV.

The clinical signs of acute-phase TSV infection in *P. vannamei* include lethargy, anorexia, opaque musculature, ataxic swimming behavior, flaccid bodies, soft cuticles, and chromatophore expansion resulting in reddening of the uropods, appendages, and body. These shrimp are typically in late pre-molt or early post-molt stage. Cumulative mortalities during acute-phase infection may reach 95%. In a pond where a TSV outbreak occurs, dead or dying shrimp float on the watersurface, attracting predatory birds (seagulls, cormorants, terns, etc.). Individuals that survive acute-phase infection develop grossly visible, multifocal, melanized lesions on the cephalothorax, tail, and appendages (**Figure 3**). These lesions are characteristic of the transition phase of infection. Chronically

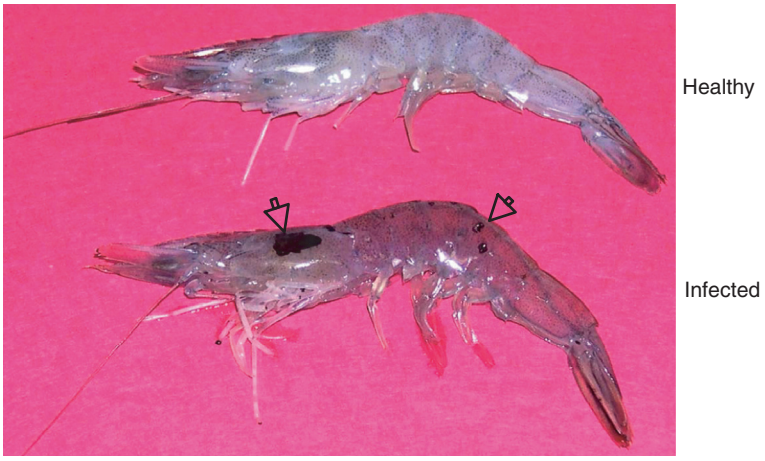


Figure 3 *Penaeus vannamei* shrimp displaying typical clinical signs of Taura syndrome disease at the end of the acute phase. Multifocal, melanized lesions on the thorax and tail are visible (indicated by arrows) in TSV-infected shrimp.

infected shrimp shed the cuticle with melanized foci and display normal behavior but can remain asymptomatic carriers of TSV.

Pathogenesis

TSD pathogenesis consists of three overlapping yet clinically and histologically distinct phases: a peracute to acute phase, a short transition phase, and a persistent chronic phase.

Acute Phase

The acute phase begins approximately 24 h post exposure and persists for approximately 7 days. The acute phase is characterized by the rapid development of severe, multifocal cuticular epithelial necrosis and high mortalities (**Figure 4**(**b**)). Target tissues of TSV infection include the cuticular epithelium of the foregut, gills, appendages, hindgut, and general body cuticle. Lesions sometimes extend into underlying subcuticular connective tissue and striated muscle. In severe cases, the antennal gland, hematopoietic tissue, testes, and ovaries may also be infected. Acutely infected epithelial cells detach from the underlying stroma, assume a spherical shape, and this is followed by cell lysis and release of virions into the circulatory system. Infected cells display highly basophilic pyknotic and karyorrhectic nuclei and marked cytoplasmic eosinophilia, with variably staining and sized cytoplasmic inclusion bodies. These histological lesions are collectively called 'peppered' or 'buckshot-laden' lesions, and are considered pathodiagnostic for acute phase of TSD (**Figure 4**(**b**)).

Transition Phase

Shrimp surviving acute-phase infection enter a brief transitional phase for approximately 5 days before entering into chronic phase. Appearance of multifocal melanized lesions on the cephalothorax and tail, with declining mortalities, are characteristic of the transition phase (**Figure 3**). Histologically, transition-phase animals present infrequent, scattered, epithelial lesions, normally appearing lymphoid organ (LO) arterioles (tubules) that display a TSV-positive signal by *in situ* hybridization (ISH), and the onset of spheroid development within the LO. Transition-phase animals are lethargic, anorexic, start molting, and shed melanized exoskeleton.

Chronic Phase

During the chronic phase, mortality ceases and animals resume normal behavior. The chronic phase may last for 8–12 months in experimentally infected *P. vannamei*. The histological hallmark of chronic phase infection is the appearance of LO spheroids (**Figure 4**(**d**)). Occasionally, ectopic spheroids are also found associated with tegmental glands located within connective tissues of the cephalothorax and appendages. Spheroids consist of phagocytic semigranular and granular hemocytes undergoing apoptosis.

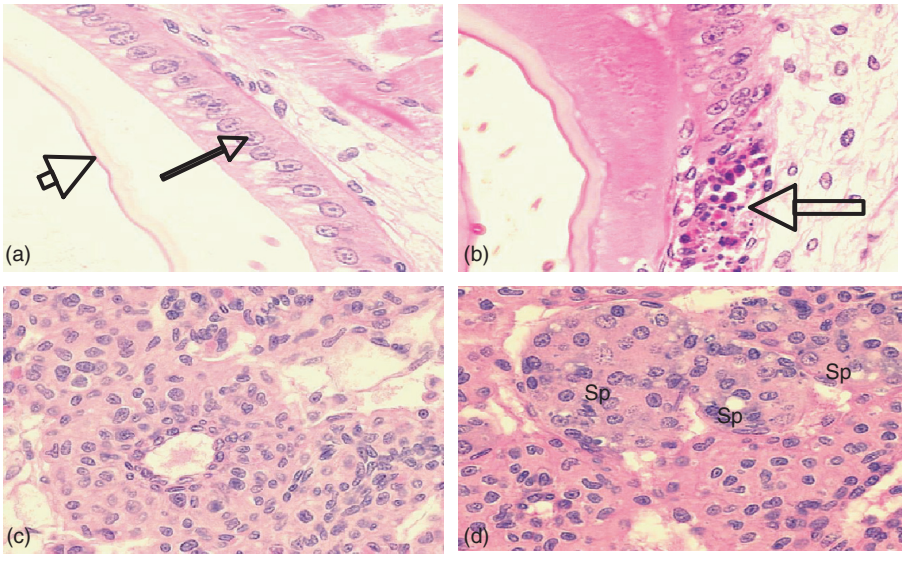


Figure 4 Histopathology by H&E staining of juvenile *Penaeus vannamei* infected with Taura syndrome virus. (a) Normal stomach cuticular epithelium (thin arrow) and cuticle (thick arrow); (b) acute-phase TSV infection evident within the stomach cuticular epithelium (arrow) with nuclear pyknosis, karyorrhexis, and cytoplasmic eosinophilia clearly visible; (c) normal lymphoid organ (LO) showing a prominent arteriole with centralized lumen; and (d) chronic TSV infection visible inside the LO, displays prominent spheroids (Sp). All panels are at ×600 magnification.

Genetics

Genome Organization and Gene Expression

The TSV genome consists of a linear, positive-sense, single-stranded, polyadenylated RNA of 10 205 nucleotides (nt). It has a 417 nt 5′ untranslated region and a 224 nt 3′ untranslated region. There are two ORFs. ORF1 encodes nonstructural proteins, while ORF2 encodes structural proteins (**Figure 5**). ORF1 is 6324 nt long, encoding a 2107 amino acid (aa) polyprotein with a molecular mass of 234 kDa. ORF2 is 3036 nt long, encoding a 1011 aa long polypeptide with a molecular mass of 112 kDa. Nonstructural ORF1 proteins contain conserved helicase (NTP-binding protein), protease, and RNA-dependent RNA polymerase (RdRp) motifs. The RNA helicase consensus sequence (Gx_4GK) is at aa 752–758 and has similarity with the cognate domains of other *Dicistroviridae* and insect picornaviruses. The protease domain in the TSV ORF1-encoded polypeptide, located between aa 1380 and 1570, shows similarity with the 3C proteases of the *Dicistroviridae*, *Picornaviridae*, *Sequiviridae,* and *Comoviridae*. The TSV protease motif is partially conserved, with glycine replaced by cysteine. Similar to picornaviruses, the His-Asp-Cys catalytic triad in the protease domain is conserved in TSV. The RdRp domain, located at the 3′ end of ORF1, contains eight conserved motifs analogous to those in picornaviruses of insects, mammals, and plants.

The N-terminal region of the ORF1-encoded polypeptide contains a stretch of 65 aa (166–230) that shows similarity to the 'inhibition of apoptosis' proteins (IAP) found in mammals, yeast, insects, and some DNA viruses. No other RNA viruses are known to encode polypeptides containing such a motif. It is unknown if this IAP motif of TSV plays a role during the long-term chronic-phase infection in evading the host immune system or facilitates virus replication.

There is an IGR of 207 nt between the two ORFs in the TSV genome. The IGR region contains an internal ribosomal entry site (IRES) that allows cap-independent translation of the ORF2 capsid proteins. Functional IRES elements have also been identified in the IGRs of CrPV and PSIV. In mammalian cells infected with picornaviruses, structural and nonstructural proteins are produced in equimolar amounts. However, in insect viruses, like DCV, structural proteins are produced in excess. This is due to IRES-mediated translation of DCV coat proteins. Translation of the two distinct polyproteins in DCV (ORF1 and ORF2) appears to be independently controlled. This is in contrast to picornaviruses encoding a single ORF in which a single polyprotein is processed post-translationally to generate both the structural and nonstructural proteins. The cap-independent translation in PSIV ORF2 has been demonstrated *in vitro* using a rabbit reticulocyte lysate system. In CrPV, the initiation codon for IRES-mediated translation has been identified as CCU, whereas, the initiation codon in PSIV and RhPV is CUU. The CCU/CUU triplets have been identified as part of the inverted repeat sequence of the IRES elements that form RNA pseudoknot structures essential for IRES activity. In TSV, there is an in-frame methionine upstream of ORF2 although N-terminal sequencing of the VP2 capsid protein has revealed alanine at the terminal position (ANPVEIDNFDTT). The alanine codon is preceded by proline (CCU) and methionine (AUG) codons (MPANPVE). Site-specific mutation in TSV has demonstrated that conversion of the AUG to UUG has no effect on capsid protein expression. Additionally, cell-free expression in the absence of initiator methionine tRNA or in the presence of ribosome inhibitor is possible with TSV. These results indicate that the IRES element in TSV IGR is functional and that the TSV intergenic AUG codon does not act as a translation initiation site. TSV has an unusual side stem–loop structure in its IGR-IRES region that is involved in this cap-independent translation. The presence of a methionine codon in the IGR-IRES has been shown to be unimportant to translation; that is, TSV translation of ORF2 does not require initiator tRNA and relies instead on the IRES element for translation initiation.

The TSV genome is transcribed as a single ~10 kb transcript and does not produce subgenomic RNA for translation of the capsid genes. This distinguishes TSV from many positive-strand RNA viruses (e.g., *Calciviridae* and *Togaviridae*) in which capsid proteins encoded in the 3′ end of the genome are generally translated from subgenomic RNA.

Genetic Diversity

The isolate that caused the first TSV epizootics in the USA (Hawaii) was subsequently characterized using biological, serological, and molecular data as Serotype A. In 1999, significant mortalities in a TSV-tolerant strain of the Western blue shrimp (*P. stylirostris*) occurred at shrimp farms in México and there were concerns that a new TSV

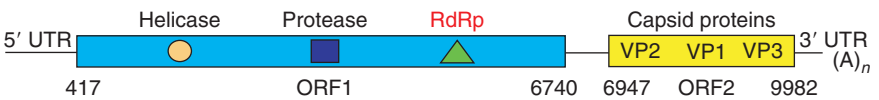


Figure 5 Genome organization of Taura syndrome virus (TSV). The conserved helicase, protease, and the RNA-dependent RNA polymerase (RdRp) domains are indicated in open reading frame 1 (ORF1). IGR, intergenic region; UTR, untranslated region.

strain might have emerged. These shrimp showed acute- or chronic-phase TSV infections by staining with H&E, and were virus-positive by reverse transcriptase polymerase chain reaction analysis (RT-PCR) and by ISH. However, these animals were TSV-negative by immunohistochemical (IHC) analysis using a TSV-specific monoclonal antibody (mAb) specific to the VP1 capsid gene.

Nucleotide sequences of capsid genes (VP1 and VP2) of the Mexican isolates revealed nonconservative amino acid replacements (such as S→A, polar uncharged to non-polar hydrophobic in VP1, and Q→K, polar uncharged to positively charged in VP2) compared to the Hawaiian isolate. Phylogenetic analysis showed that the Mexican isolate falls into a different clade. The Mexican isolate was classified as TSV serotype B.

In 2001, significant mortalities of *P. vannamei* occurred at shrimp farms in Belize, resulting from TSV epizootics. Using OIE diagnostic methods and sequence analysis, the Belize isolate was shown to represent a new serotype, TSV serotype C (**Table 1**).

In 2004, a TSV epizootic occurred in some farms in Texas, USA, near shrimp-processing plants that process frozen shrimp from Southeast (SE) Asia. Phylogenetic analysis using the VP1 capsid gene sequence revealed that TSV Texas isolate groups with the isolates from SE Asia (Thailand, Taiwan, and China, **Figure 6**). The spread of TSV to Asia most likely resulted from the introduction of TSV-infected *P. vannamei* from South America. The first TSV epizootic in Asia occurred in Taiwan in 1998, and subsequently spread to other Asian countries. It is possible that the Texas isolate was introduced through imported shrimp from SE Asia. Based on the phylogenetic analysis, the Asian isolates have been grouped tentatively as serotype D (**Figure 6**). Further characterization (biological and serological) might reveal that this group represents more than one serotype.

Overall, the TSV isolates show very high similarity (~95%) in nucleotide sequence of the VP1 gene among the isolates and most of the variations are located in the C-terminal end of the VP1 capsid gene. In CrPV, the type species of the genus *Cripavirus*, family *Dicistroviridae*, the crystal structure of the capsid gene has revealed that the C-terminal end of the VP3 gene (homologous to TSV VP1) is a helical extension on the virion surface. Therefore, this region is less constrained by structural requirements allowing mutations to accumulate. These mutations have serious implications for antibody-driven diagnosis and potentially modulate the virulence of the newly evolved strain(s).

Evolution and Spread

RNA viruses exist as 'quasispecies' in nature due to the high mutation frequency caused by the lack of effective proofreading activity of RdRp. The presence of a large array of related genotypes in the population allows an RNA virus to evolve and adapt to changes in the host and the environment. The spread and evolution of TSV appears to provide an example of this process. In México, until about the mid-1990s, *P. vannamei* was the principal species for shrimp farming. When TSV epizootics in México reached a peak in 1996, farmers started switching from TSV-susceptible *P. vannamei* to TSV-tolerant *P. stylirostris*. This resulted in the decline of TSV epizootics and, by 1998, shrimp production in México stabilized. In 1999, a new strain of TSV evolved in México that caused significant mortalities in previously tolerant *P. stylirostris*. The replacement of *P. vannamei* with *P. stylirostris* in shrimp farms in México might have contributed to the development of a virulent strain of TSV as the virus adapted to a new host species.

The transportation of live postlarval and adult shrimp (*P. vannamei*) from South America to Asia facilitated TSV dissemination in Asia and in 1998, the first TSV outbreak was reported in Taiwan. From Taiwan, TSV spread to other Asian countries. In Thailand, *P. vannamei* cultivation commenced in late 1990. Due to the ban on cultivation of native *P. monodon* species in freshwater areas in Thailand in 2000 and widespread slow growth in cultivated *P. monodon* in 2001 and 2002, farmers adopted *P. vannamei* as an alternative species. During this time, almost 70% of the broodstock in Thailand was imported from China. In 2003, the first TSV outbreak was confirmed in Thailand. In 2004, TSV epizootics occurred in farms in Texas, USA, located proximal to shrimp-processing plants that import frozen and bait shrimp from SE Asia. Phylogenetic analysis, using nucleotide sequence data from the TSV capsid gene VP1, showed that the isolates from Thailand, China, Taiwan, and Texas formed a single clade (**Figure 6**). As naïve shrimp populations are exposed to TSV, virus/host selection results in the emergence of more virulent strain(s) with devastating consequences. The

Table 1 List of TSV serotypes identified based on biological, genomic, and serological properties

Serotype	*Place of origin*	*Year*	*Species of origin*
TSV serotype A	Hawaii, USA	1994	*Penaeus vannamei*
TSV serotype B	Sinaloa, México	1998	*Penaeus stylirostris*
TSV serotype C	Belize	2001	*Penaeus vannamei*
TSV serotype D	Asia	1999–2005	*Penaeus vannamei, Penaeus monodon, Metapenaeus ensis*

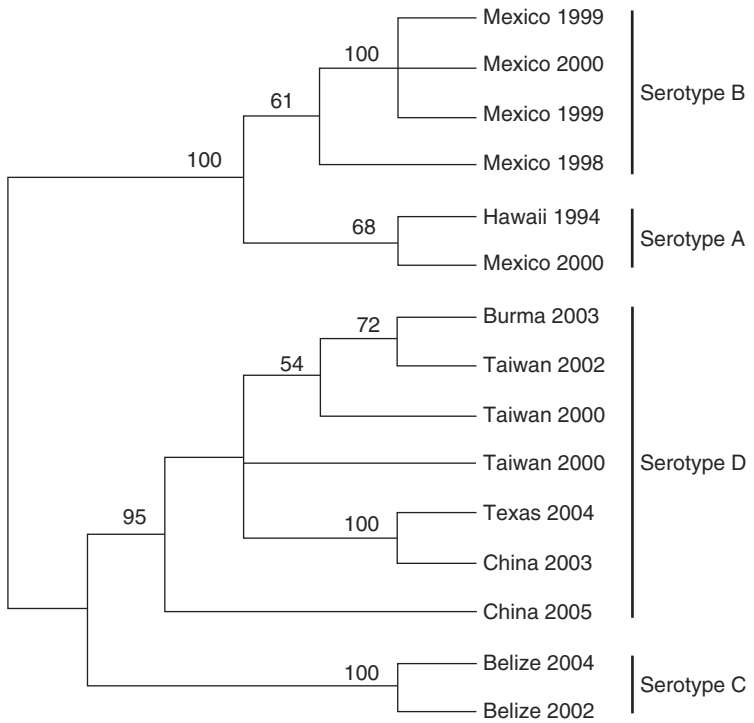


Figure 6 Maximum likelihood phylogenetic tree of TSV isolates collected from different geographical areas. The phylogenetic tree was constructed using the phylogenetic analysis using parsimony (PAUP) method, and numbers on the branches represent bootstrap values. The origin and the year of collection of these isolates are indicated.

availability of TSV genomic information has provided valuable insight into the origin, evolution, and spread of TSV worldwide.

Dissemination

TSV is transmitted by cannibalism of infected moribund or dead shrimp by healthy shrimp in a pond or in an experimental tank. The virus can remain infectious in decaying shrimp carcasses for up to 3 weeks and these may serve as a source of infection. In addition, chronically infected shrimp harbor infectious TSV within the LO and hemolymph for 8–12 months post infection, representing a source for renewed outbreaks in ponds, canals, or adjacent estuaries. In experimentally induced TSV epizootics, waterborne transmission has been reported to occur up to 48 h following peak mortality.

Mechanical transmission of TSV can occur via an aquatic insect (water boatmen, *Trichocorixa reticulate*) living in ponds and via seagulls (*Larus atricilla*). Infective TSV has been demonstrated in the seagull feces collected from the banks of ponds where TSV outbreaks have occurred.

Since the initial outbreak of Taura syndrome in Ecuador, the geographic range of the disease has spread and TSV is now considered endemic along the mid-American Pacific Coast, ranging from northern Peru through to México. Wild postlarvae and broodstock showing acute and/or chronic disease have been collected in Ecuador, Honduras, El Salvador, and Southern México. Therefore, wild postlarvae and broodstock are potential sources of dissemination for TSV. The movement of TSV-infected live postlarvae and adult shrimp has been the principal means by which TSV spread into shrimp farming nations within the Western Hemisphere and Asia. Export of frozen commodity shrimp and bait shrimp from infected areas also may have contributed to the spread of TSV across the continents.

Diagnosis

Taura syndrome diagnosis and the detection of TSV can be conducted by observation of clinical signs, bioassay, histopathology, enzyme-linked immunosorbent assay (ELISA), ISH using nonradiolabeled probes, and reverse transcription-polymerase chain reaction (RT-PCR).

As no immortalized shrimp cell lines are currently available, detection of infectious virus relies on *in vivo* bioassays. The bioassay protocol involves exposing SPF juvenile *P. vannamei* to TSV-suspect shrimp either by *per os* challenge or by injection of a tissue extract.

TSV-positive shrimp are identified by gross signs and histopathology.

A standard diagnostic tool for identifying TSV-induced pathology involves H&E histology of Davidson's alcohol formalin acetic acid fixative (AFA)-preserved shrimp. The presence of acute-phase lesions in cuticular epithelia is considered to be a definitive diagnosis of TSV infection (**Figure 4**).

Digoxigenin-labeled cDNA probes have been developed for TSV detection by ISH. Positive ISH reactions are identified by the presence of a blue-black precipitate within the cytoplasm of TSV-infected cells. ISH has greater sensitivity than H&E staining, as TSV can be detected in shrimp with mild acute infections and in asymptomatic carriers.

ELISA-based dot blot detection of TSV capsid protein using a TSV-specific monoclonal antibody is available and has been modified for application to IHC detection of TSV in histological sections. IHC has advantages over ISH in that it is a more economical and rapid assay with similar sensitivity. However, IHC using monoclonal antibodies can fail to detect some TSV strains due to epitope drift, as discussed above.

An RT-PCR method has been described for TSV detection in hemolymph, providing a nonlethal diagnostic method that is rapid and has a greater sensitivity than other methods. Recently, real-time RT-PCR methods using SYBR Green dye and TaqMan probes have been developed for rapid detection and quantification of TSV. The real-time RT-PCR is highly sensitive, specific, detects subclinical infections, and has a high throughput potential. The drawback with the real-time RT-PCR is its high cost and the need for significant technical expertise. However, combining real-time PCR with conventional diagnosis might be acceptable for large-scale shrimp farm operations to screen broodstock and field samples for TSV.

Control/Prevention

The current TSV control methods emphasize biosecurity at the farm level and the utilization of SPF, TSV-resistant shrimp for stocking. This approach is preventative and has considerable success in the control of TSD on carefully managed farms. Since the immune system of shrimp is relatively primitive, the development of a traditional vaccination procedure for control of TSV is not likely. Experimentation with RNA interference and the expression of antisense RNA against the TSV capsid gene in transgenic shrimp show promise in the control of the disease but currently have only been tested at the laboratory level. Also, disease protection by nonspecific dsRNA indicates that shrimp have a fairly strong innate immune response that could be exploited.

Acknowledgment

The authors thank Dr. Kenneth W. Hasson, Texas Veterinary Medical Diagnostic Laboratory (TVMDL), College Station, Texas for providing the histopathology and electron microscopy photographs.

See also: Picornaviruses: Molecular Biology; White Spot Syndrome Virus; Yellow Head Virus.

Further Reading

Bonami JR, Hasson KW, Mari J, Poulos BT, and Lightner DV (1997) Taura syndrome of marine penaeid shrimp: Characterization of the viral agent. *Journal of General Virology* 78: 313–319.

Cevallos RC and Sarnow P (2005) Factor-independent assembly of elongation-competent ribosomes by an internal ribosome entry site located in an RNA virus that infect penaeid shrimp. *Journal of Virology* 79: 677–683.

Dhar AK, Cowley JA, Hasson KW, and Walker PJ (2004) Genome organization, biology and diagnosis of Taura syndrome virus and yellowhead virus of penaeid shrimp. *Advances in Virus Research* 63: 353–419.

Dhar AK, Roux MM, and Klimpel KR (2002) Quantitative assay for measuring the Taura syndrome virus (TSV) and yellow head virus (YHV) load in shrimp by real-time RT-PCR using SYBR Green chemistry. *Journal of Virological Methods* 104: 69–82.

Hasson KW, Lightner DV, Mohney LL, Redman RM, Poulos BT, and White BL (1999) Taura syndrome virus (TSV) lesion development and the disease cycle in the Pacific white shrimp *Penaeus vannamei*. *Diseases of Aquatic Organisms* 36: 81–93.

Hasson KW, Lightner DV, Poulos BT, *et al.* (1995) Taura syndrome in *Penaeus vannamei*: Demonstration of a viral etiology. *Diseases of Aquatic Organisms* 23: 115–126.

Hatakeyama Y, Shibuya N, Nishiyama T, and Nakashima N (2004) Structural variant of the intergenic internal ribosomal entry site elements in dicistroviruses and computational search for their counterparts. *RNA* 10: 779–786.

Mari J, Poulos BT, Lightner DV, and Bonami JR (2002) Shrimp Taura syndrome virus: Genomic characterization and similarity with members of the genus *Cricket paralysis-like viruses*. *Journal of General Virology* 83: 915–926.

Nielsen L, Sang-oum W, Cheevadhanarak S, and Flegel TW (2005) Taura syndrome virus (TSV) in Thailand and its relationship to TSV in China and the Americas. *Diseases of Aquatic Organisms* 63: 101–106.

Office International des Epizooties (OIE) Fish Disease Commission (2003) Manual of diagnostic manual for aquatic animal diseases. ch 4. 1. 1, Taura Syndrome. http://www.oie.int/eng/normes/fmanual/A_summry.htm (accessed August. 2007).

Robalino J, Browdy CL, Prior S, *et al.* (2004) Induction of antiviral immunity by double-stranded RNA in a marine invertebrate. *Journal of Virology* 78: 10442–10448.

Robles-Sikisaka R, Hasson KW, Garcia DK, *et al.* (2002) Genetic variation and immunohistochemical differences among geographic isolates of Taura syndrome virus of penaeid shrimp. *Journal of General Virology* 83: 3123–3130.

Srisuvan T, Pantoja CR, Redman CR, and Lightner DV (2006) Ultrastructure of the replication site in Taura syndrome virus (TSV)-infected cells. *Diseases of Aquatic Organisms* 73: 89–101.

Tang KFJ and Lightner DV (2005) Phylogenetic analysis of Taura syndrome virus isolates collected between 1993 and 2004 and virulence comparison between two isolates representing different genetic variants. *Virus Research* 11: 69–76.

Yu CI and Somg YL (2002) Outbreak of Taura syndrome in Pacific white shrimp *Penaeus vannamei* cultured in Taiwan. *Fish Pathology* 35: 21–24.

Taxonomy, Classification and Nomenclature of Viruses

C M Fauquet, Danforth Plant Science Center, St. Louis, MO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Classification The categorization of organisms into defined groups on the basis of identified characteristics.
Family A category in the virus taxonomic classification of related organisms, comprising one or more genera.
Genus A category in the virus taxonomic classification of related organisms, comprising one or more species.
Nomenclature The assigning of names to organisms in a scientific classification system.
Order A category in the virus taxonomic classification of related organisms, comprising one or more families.
Species A virus species is a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche.
Taxonomy The science of classifying plants, animals, and microorganisms into increasingly broader categories based on shared features. The practice or principles of classification.

Introduction

Virus taxonomy is a very important but controversial field of science. It was ranked as the first constraint for the modern development of virus databases, and the exponential increase in virus sequencing is worsening the situation. However, substantial progress has been made particularly in the last 20 years, both on the conceptual framework and practical implication of virus taxonomy. The International Committee on Taxonomy of Viruses (ICTV) is the only committee of the Virology Division, of the International Union of Microbiological Societies (IUMS), in charge of that task since 1966, for the international virology community. Virus Taxonomy Reports have been published regularly by ICTV and they became the reference in virus taxonomy and nomenclature. This article aims at providing some historical information about the establishment and changes in virus taxonomy and describes the current status of virus classification, nomenclature, and orthography.

There is no such thing as a 'natural' or a 'biological' classification; by essence any classification is an arbitrary human invention and viruses are no exception. The question really is: "How can we classify viruses in such way that it makes sense and is useful to many scientists?" The need for virus classification is not only supported by the common human need for organization, but also as a scientific tool to compare viruses and extrapolate useful information from one virus to another and from one family to another. Crucial biological information can be extrapolated directly from human viruses like picornaviruses to plant comoviruses and vice versa, when the classification indicates many structural and genomic common characteristics. When a newly discovered virus is assigned to a particular taxon, this virus can immediately be granted a number of *a priori* properties that only need confirmation and that have an immediate impact on specific virological studies. Furthermore, although it is not clearly stated by ICTV that the current classification is thought to reflect virus evolution, it is accepted that virus taxonomy is aiming at this objective and could become a tool by itself to study and evaluate virus evolution.

Virus nomenclature cannot be dissociated from classification. There must be a coherent system for naming viruses accompanied with a system for classifying viruses. Furthermore, using correct orthography and typography of virus taxa is not simply an excercise meant to complicate the task of virologists but is based on rules that help scientists to extract useful information from what is written down. It is therefore important to establish and follow guidelines for orthography, nomenclature, and classification of viruses. For all these reasons, virus classification and nomenclature have always been very controversial and have led to passionate discussions over the past four decades.

Historical Background of Virus Taxonomy

The first evidence for the existence of viruses was shown by Beijerinck in 1898, but it was not until the 1920s that virologists began to classify viruses. The first system referred to pathogenic properties of animal and human viruses and to symptoms for plant viruses. For example, viruses sharing the pathogenic properties causing hepatitis (e.g., hepatitis A virus, hepatitis B virus, yellow fever virus, Rift Valley fever virus) were grouped together as 'the hepatitis viruses'. In 1939, Holmes published a classification of plant viruses dependent on host reactions and differential host species using a binomial–trinomial nomenclature based on the name of the infected plant. It was only in the 1950s, with the utilization of the electron microscope, that

the first real virus classification was established. Naturally the shape and size of virus particles became major criteria for virus classification. Because of that powerful and rapid technology the number of newly discovered viruses increased rapidly and several hundreds of new viruses were listed in a short period of time.

In 1966 in Moscow, at the International Congress for Microbiology, 43 virologists created the International Committee on Nomenclature of Viruses (ICNV) with the aim of developing a worldwide recognized taxonomy and nomenclature system for all viruses. The name of the ICNV was changed in 1974 to the more appropriate ICTV. The ICTV, which is the only committee of the Virology Division of the International Union of Microbiological Societies, is now recognized as the official international body that decides on all matters related to taxonomy and nomenclature of viruses.

Since 1966, virologists have agreed that all viruses isolated from different organisms should be classified together in a unique system, separate from that of microorganisms such as fungi, bacteria, and mycoplasma. However, there has been much controversy on how to achieve this aim. Lwoff, Horne, and Tournier in 1962 proposed the adoption of a system classifying viruses into subphyla, classes, orders, suborders, and families. It was also proposed that the hierarchical classification would be based on the type of nucleic acid (DNA or RNA), the strandedness (single = ss or double = ds), the presence or absence of an envelope, the capsid symmetry, the type of replication cycle (with or without an RNA intermediate for DNA viruses), and the number of genome segments. This hierarchical classification system has never been recognized by the ICTV, but most of the criteria used to demarcate the major classes of viruses formed the basis of the universal taxonomy system now in place, and all published ICTV reports have used this scheme with only minor changes.

It is only in the last 15 years that a hierarchical classification level higher than the family was proposed and accepted. A first order, *Mononegavirales*, was accepted in 1990, and the orders *Caudovirales* and *Nidovirales* were adopted in 1996. In 2005, ICTV considered introducing four new orders namely *Picornavirales, Herpesvirales, Reovirales,* and *Retrovirales*, and these may become accepted in the near future.

It is important to note that the species category for viruses was only adopted in 1991. From then onwards viruses were assigned to species or tentative species. In addition, a list of species demarcation criteria has been established for each family. It is anticipated that by 2010 the ICTV will have introduced species criteria for all viruses and that some level of homogeneity will have been reached, although it is perfectly acceptable to have different sets of criteria for different families of viruses.

Since the establishment of the ICTV, a total of eight virus taxonomic reports have been published. At the first meeting of the Committee in Mexico City in 1970, two families with corresponding two genera and 24 floating genera were adopted to begin grouping the vertebrate, invertebrate, and bacterial viruses together, and in addition, 16 plant virus 'groups' were introduced. Although virologists working with vertebrate viruses had assigned viruses to genera and families for many years, plant virologists until 1993 used the term 'group' to designate viruses with similar properties. It was only in 1995 that the ICTV adopted a uniform system for all viruses, encompassing 2644 assigned viruses. The *Eighth ICTV Report on Virus Taxonomy*, published in 2005 describes a Universal Virus Classification that comprises 3 orders, 73 families, 9 subfamilies, 287 genera, and 5450 viruses belonging to 1950 species (**Table 1**).

Over the past four decades the number of classified viruses, as well as the number of each type of taxa, has increased exponentially and continues to grow (**Figure 1**). Because DNA sequencing has become a routine technique, it seems likely that the number of recognizes viruses and viral taxa will continue to grow exponentially. Furthermore, virus genome sequences provide qualitative and quantitative criteria for defining the molecular variability of viruses that are useful for classification purposes. Sequencing will also permit identification and classification of many viruses that are difficult to isolate and characterize by other methods.

Organization and Structure of ICTV

The ICTV is the only Committee of the Virology Division of the International Union of Microbiological Societies. It is a non-profit organization composed of volunteered virologists from many countries who make decisions on virus names and taxa through a democratic process. The ICTV operates through subcommittees and study groups consisting of more than 500 virologists with expertise in human, animal, insect, protozoa, archaea, bacteria, mycoplasma, fungi, algae, and plant viruses.

Taxonomic proposals are initiated and formulated by study groups or by single individuals. The proposals are examined, offered to public scrutiny, accepted by the relevant subcommittee and presented for approval by the Executive Committee of the ICTV. All decisions are ratified by postal vote, where all members of the ICTV and more than 50 national microbiological societies are represented. Presently, there are 75 study groups working in concert with six subcommittees: one each for the vertebrate, invertebrate, plant, bacterial, and fungal viruses and one for the virus ICTV DataBase (ICTVdB). The ICTV does not impose taxa but ensures that all propositions are compatible with the International Code for Virus Classification and Nomenclature for accuracy, homogeneity, and consistency. The ICTV regularly

Table 1 The order of presentation of viruses in the eighth ICTV report

Order	*Family*	*Subfamily*	*Genus*	*Type species*	*Host*
The DNA viruses					
The dsDNA viruses					
Caudovirales					
	Myoviridae		''T4-like viruses''	*Enterobacteria phage T4*	B
			''P-like viruses''	*Enterobacteria phage P*	B
			''P-like viruses''	*Enterobacteria phage P*	B
			''Mu-like viruses''	*Enterobacteria phage Mu*	B
			''SP1-like viruses''	*Bacillus phage SP1*	B
			''ϕH-like viruses''	*Halobacterium phage ϕH*	Ar
	Siphoviridae		''λ-like viruses''	*Enterobacteria phageλ*	B
			''T1-like viruses''	*Enterobacteria phage T1*	B
			''T5-like viruses''	*Enterobacteria phage T5*	B
			''L5-like viruses''	*Mycobacterium phage L5*	B
			''c2-like viruses''	*Lactococcus phage c2*	B
			''ψM1-like viruses''	*Methanobacterium phage ψM1*	Ar
			''ϕC31-like viruses''	*Streptomyces phage ϕC31*	B
			''N15-like viruses''	*Enterobacteria phage N15*	B
	Podoviridae		''T7-like viruses''	*Enterobacteria phage T7*	B
			''P2-like viruses''	*Enterobacteria phage P2*	B
			''ϕ29-like viruses''	*Bacillus phage ϕ29*	B
			''N4-like viruses''	*Enterobacteria phage N4*	B
	Tectiviridae		*Tectivirus*	*Enterobacteria phage PRD1*	B
	Corticoviridae		*Corticovirus*	*Pseudoalteromonas phage PM2*	B
	Plasmaviridae		*Plasmavirus*	*Acholeplasma phage L2*	B
	Lipothrixviridae		*Alphalipothrixvirus*	*Thermoproteus tenax virus 1*	Ar
			Betalipothrixvirus	*Sulfolobus islandicus filamentous virus*	Ar
			Gammalipothrixvirus	*Acidianus filamentous virus 1*	Ar
	Rudiviridae		*Rudivirus*	*Sulfolobus islandicus rod-shaped virus 2*	Ar
	Fuselloviridae		*Fusellovirus*	*Sulfolobus spindle-shaped virus 1*	Ar
			Salterprovirus	*His1 virus*	Ar
	Guttaviridae		*Guttavirus*	*Sulfolobus newzealandicus droplet-shaped virus*	Ar
	Poxviridae	*Chordopoxvirinae*			
			Orthopoxvirus	*Vaccinia virus*	V
			Parapoxvirus	*Orf virus*	V
			Avipoxvirus	*Fowlpox virus*	V
			Capripoxvirus	*Sheeppox virus*	V
			Leporipoxvirus	*Myxoma virus*	V
			Suipoxvirus	*Swinepox virus*	V
			Molluscipoxvirus	*Molluscum contagiosum virus*	V
			Yatapoxvirus	*Yaba monkey tumor virus*	V
		Entomopoxvirinae			

Continued

Table 1 Continued

Order	Family	Subfamily	Genus	Type species	Host
			Alphaentomopoxvirus	*Melolontha melolontha entomopoxvirus*	I
			Betaentomopoxvirus	*Amsacta moorei entomopoxvirus 'L'*	I
			Gammaentomopoxvirus	*Chironomus luridus entomopoxvirus*	I
	Asfarviridae		*Asfivirus*	*African swine fever virus*	V, I
	Iridoviridae		*Iridovirus*	*Invertebrate iridescent virus 6*	I
			Chloriridovirus	*Invertebrate iridescent virus 3*	I
			Ranavirus	*Frog virus 3*	V
			Lymphocystivirus	*Lymphocystis disease virus 1*	V
			Megalocytivirus	*Infectious spleen and kidney necrosis virus*	V
	Phycodnaviridae		*Chlorovirus*	*Paramecium bursaria Chlorella virus 1*	AI
			Coccolithovirus	*Emiliania huxleyi virus* 86	AI
			Prasinovirus	*Micromonas pusilla virus SP*	AI
			Prymnesiovirus	*Chrysochromulina brevifilum virus PW1*	AI
			Phaeovirus	*Ectocarpus siliculosus virus 1*	AI
			Raphidovirus	*Heterosigma akashiwo virus 01*	AI
	Baculoviridae		*Nucleopolyhedrovirus*	*Autographa californica multiple nucleopolyhedrovirus*	I
			Granulovirus	*Cydia pomonella granulovirus*	I
	Nimaviridae		*Whispovirus*	*White spot syndrome virus 1*	I
	Herpesviridae	*Alphaherpesvirinae*			
			Simplexvirus	*Human herpesvirus 1*	V
			Varicellovirus	*Human herpesvirus 3*	V
			Mardivirus	*Gallid herpesvirus 2*	V
			Iltovirus	*Gallid herpesvirus 1*	V
		Betaherpesvirinae			
			Cytomegalovirus	*Human herpesvirus 5*	V
			Muromegalovirus	*Murid herpesvirus 1*	V
			Roseolovirus	*Human herpesvirus 6*	V
		Gammaherpesvirinae			
			Lymphocryptovirus	*Human herpesvirus 4*	V
			Rhadinovirus	*Saimiriine herpesvirus 2*	V
			Ictalurivirus	*Ictalurid herpesvirus 1*	V
	Adenoviridae		*Mastadenovirus*	*Human adenovirus C*	V
			Aviadenovirus	*Fowl adenovirus A*	V
			Atadenovirus	*Ovine adenovirus D*	V
			Siadenovirus	*Frog adenovirus*	V
			Rhizidiovirus	*Rhizidiomyces virus*	F
	Polyomaviridae		*Polyomavirus*	*Simian virus 40*	V
	Papillomaviridae		*Alphapapillomavirus*	*Human papillomavirus 32*	V
			Betapapillomavirus	*Human papillomavirus 5*	V
			Gammapapillomavirus	*Human papillomavirus 4*	V
			Deltapapillomavirus	*European elk papillomavirus*	V

			Epsilonpapillomavirus	*Bovine papillomavirus 5*	V
			Zetapapillomavirus	*Equine papillomavirus 1*	V
			Etapapillomavirus	*Fringilla coelebs papillomavirus*	V
			Thetapapillomavirus	*Psittacus erithacus timneh papillomavirus*	V
			Iotapapillomavirus	*Mastomys natalensis papillomavirus*	V
			Kappapapillomavirus	*Cottontail rabbit papillomavirus*	V
			Lambdapapillomavirus	*Canine oral papillomavirus*	V
			Mupapillomavirus	*Human papillomavirus 1*	V
			Nupapillomavirus	*Human papillomavirus 41*	V
			Xipapillomavirus	*Bovine papillomavirus 3*	V
			Omikronpapillomavirus	*Phocoena spinipinnis papillomavirus*	V
			Pipapillomavirus	*Hamster oral papillomavirus*	V
	Polydnaviridae		*Bracovirus*	*Cotesia melanoscela bracovirus*	I
			Ichnovirus	*Campoletis sonorensis ichnovirus*	I
	Ascoviridae		*Ascovirus*	*Spodoptera frugiperda ascovirus 1a*	I
	Unassigned		*Mimivirus*	*Acanthamoeba polyphaga mimivirus*	Pr, V
The ssDNA viruses					
	Inoviridae		*Inovirus*	*Enterobacteria phage M13*	B
			Plectrovirus	*Acholeplasma phage L51*	B
	Microviridae		*Microvirus*	*Enterobacteria phage ϕX174*	B
			Chlamydiamicrovirus	*Chlamydia phage 1*	B
			Bdellomicrovirus	*Bdellovibrio phage MAC1*	B
			Spiromicrovirus	*Spiroplasma phage 4*	B
	Geminiviridae		*Mastrevirus*	*Maize streak virus*	P
			Curtovirus	*Beet curly top virus*	P
			Topocuvirus	*Tomato pseudo-curly top virus*	P
			Begomovirus	*Bean golden yellow mosaic virus*	P
	Circoviridae		*Circovirus*	*Porcine circovirus-1*	V
			Gyrovirus	*Chicken anemia virus*	V
	Unassigned		*Anellovirus*	*Torque teno virus*	V
	Nanoviridae		*Nanovirus*	*Subterranean clover stunt virus*	P
			Babuvirus	*Banana bunchy top virus*	P
	Parvoviridae	*Parvovirinae*			
			Parvovirus	*Minute virus of mice*	V
			Erythrovirus	*Human parvovirus B9*	V
			Dependovirus	*Adeno-associated virus 2*	V
			Amdovirus	*Aleutian mink disease virus*	V
			Bocavirus	*Bovine parvovirus*	V
		Densovirinae			
			Densovirus	*Junonia coenia densovirus*	I
			Iteravirus	*Bombyx mori densovirus*	I
			Brevidensovirus	*Aedes aegypti densovirus*	I
			Pefudensovirus	*Periplaneta fuliginosa densovirus*	I

Continued

Table 1 Continued

Order	Family	Subfamily	Genus	Type species	Host
The DNA and RNA reverse transcribing viruses					
	Hepadnaviridae		*Orthohepadnavirus*	*Hepatitis B virus*	V
			Avihepadnavirus	*Duck hepatitis B virus*	V
	Caulimoviridae		*Caulimovirus*	*Cauliflower mosaic virus*	P
			Petuvirus	*Petunia vein clearing virus*	P
			Soymovirus	*Soybean chlorotic mottle virus*	P
			Cavemovirus	*Cassava vein mosaic virus*	P
			Badnavirus	*Commelina yellow mottle virus*	P
			Tungrovirus	*Rice tungro bacilliform virus*	P
	Pseudoviridae		*Pseudovirus*	*Saccharomyces cerevisiae Ty1 virus*	F, P
			Hemivirus	*Drosophila melanogaster copia virus*	F, I
			Sirevirus	*Glycine max SIRE1 virus*	P
	Metaviridae		*Metavirus*	*Saccharomyces cerevisiae Ty3 virus*	F, P, I
			Errantivirus	*Drosophila melanogaster Gypsy virus*	I
			Semotivirus	*Ascaris lumbricoides Tas virus*	I
	Retroviridae	*Orthoretrovirinae*			
			Alpharetrovirus	*Avian leukosis virus*	V
			Betaretrovirus	*Mouse mammary tumor virus*	V
			Gammaretrovirus	*Murine leukemia virus*	V
			Deltaretrovirus	*Bovine leukemia virus*	V
			Epsilonretrovirus	*Walleye dermal sarcoma virus*	V
			Lentivirus	*Human immunodeficiency virus 1*	V
		Spumaretrovirinae			
			Spumavirus	*Simian foamy virus*	V
The RNA viruses					
The dsRNA viruses					
	Cystoviridae		*Cystovirus*	*Pseudomonas phageϕ6*	B
	Reoviridae		*Orthoreovirus*	*Mammalian orthoreovirus*	V
			Orbivirus	*Bluetongue virus*	V, I
			Rotavirus	*Rotavirus A*	V
			Coltivirus	*Colorado tick fever virus*	V, I
			Seadornavirus	*Banna virus*	V
			Aquareovirus	*Aquareovirus A*	V
			Idnoreovirus	*Idnoreovirus 1*	I
			Cypovirus	*Cypovirus 1*	I
			Fijivirus	*Fiji disease virus*	P, I
			Phytoreovirus	*Wound tumor virus*	P, I
			Oryzavirus	*Rice ragged stunt virus*	P, I
			Mycoreovirus	*Mycoreovirus 1*	F
	Birnaviridae		*Aquabirnavirus*	*Infectious pancreatic necrosis virus*	V
			Avibirnavirus	*Infectious bursal disease virus*	V

			Entomobirnavirus	*Drosophila X virus*	I
	Totiviridae		*Totivirus*	*Saccharomyces cerevisiae virus L-A*	F
			Giardiavirus	*Giardia lamblia virus*	Pr
			Leishmaniavirus	*Leishmania RNA virus 1–1*	Pr
	Partitiviridae		*Partitivirus*	*Atkinsonella hypoxylon virus*	F
			Alphacryptovirus	*White clover cryptic virus 1*	P
			Betacryptovirus	*White clover cryptic virus 2*	P
	Chrysoviridae		*Chrysovirus*	*Penicillium chrysogenum virus*	F
	Hypoviridae		*Hypovirus*	*Cryphonectria hypovirus 1*	F
	Unassigned		*Endornavirus*	*Vicia faba endornavirus*	P
The negative-stranded ssRNA viruses					
Mononegavirales					
	Bornaviridae		*Bornavirus*	*Borna disease virus*	V
	Rhabdoviridae		*Vesiculovirus*	*Vesicular stomatitis Indiana virus*	V, I
			Lyssavirus	*Rabies virus*	V
			Ephemerovirus	*Bovine ephemeral fever virus*	V, I
			Novirhabdovirus	*Infectious hematopoietic necrosis virus*	V
			Cytorhabdovirus	*Lettuce necrotic yellows virus*	P, I
			Nucleorhabdovirus	*Potato yellow dwarf virus*	P, I
	Filoviridae		*Marburgvirus*	*Lake Victoria marburgvirus*	V
			Ebolavirus	*Zaire ebolavirus*	V
	Paramyxoviridae	*Paramyxovirinae*			
			Rubulavirus	*Mumps virus*	V
			Avulavirus	*Newcastle disease virus*	V
			Respirovirus	*Sendai virus*	V
			Henipavirus	*Hendra virus*	V
			Morbillivirus	*Measles virus*	V
		Pneumovirinae			
			Pneumovirus	*Human respiratory syncytial virus*	V
			Metapneumovirus	*Avian metapneumovirus*	V
	Unassigned		*Varicosavirus*	*Lettuce big-vein associated virus*	P
			Ophiovirus	*Citrus psorosis virus*	P
	Orthomyxoviridae		*Influenzavirus A*	*Influenza A virus*	V
			Influenzavirus B	*Influenza B virus*	V
			Influenzavirus C	*Influenza C virus*	V
			Thogotovirus	*Thogoto virus*	V, I
			Isavirus	*Infectious salmon anemia virus*	V
	Bunyaviridae		*Orthobunyavirus*	*Bunyamwera virus*	V, I
			Hantavirus	*Hantaan virus*	V
			Nairovirus	*Dugbe virus*	V, I
			Phlebovirus	*Rift Valley fever virus*	V, I
			Tospovirus	*Tomato spotted wilt virus*	P, I
	Unassigned		*Tenuivirus*	*Rice stripe virus*	P, I
	Arenaviridae		*Arenavirus*	*Lymphocytic choriomeningitis virus*	V

Continued

Table 1 Continued

Order	Family	Subfamily	Genus	Type species	Host
			Deltavirus	*Hepatitis delta virus*	V
The positive-stranded ssRNA viruses					
	Leviviridae		*Levivirus*	*Enterobacteria phage MS2*	B
			Allolevivirus	*Enterobacteria phage Qβ*	B
	Narnaviridae		*Narnavirus*	*Saccharomyces 20S narnavirus*	F
			Mitovirus	*Cryphonectria mitovirus 1*	F
	Picornaviridae		*Enterovirus*	*Poliovirus*	V
			Rhinovirus	*Human rhinovirus A*	V
			Cardiovirus	*Encephalomyocarditis virus*	V
			Aphthovirus	*Foot-and-mouth disease virus*	V
			Hepatovirus	*Hepatitis A virus*	V
			Parechovirus	*Human parechovirus*	V
			Erbovirus	*Equine rhinitis B virus*	V
			Kobuvirus	*Aichi virus*	V
			Teschovirus	*Porcine teschovirus*	V
	Unassigned		*Iflavirus*	*Infectious flacherie virus*	I
	Dicistroviridae		*Cripavirus*	*Cricket paralysis virus*	I
	Marnaviridae		*Marnavirus*	*Heterosigma akashiwo RNA virus*	F
	Sequiviridae		*Sequivirus*	*Parsnip yellow fleck virus*	P
			Waikavirus	*Rice tungro spherical virus*	P
	Unassigned		*Sadwavirus*	*Satsuma dwarf virus*	P
			Cheravirus	*Cherry rasp leaf virus*	P
	Comoviridae		*Comovirus*	*Cowpea mosaic virus*	P
			Fabavirus	*Broad bean wilt virus 1*	P
			Nepovirus	*Tobacco ringspot virus*	P
	Potyviridae		*Potyvirus*	*Potato virus Y*	P
			Ipomovirus	*Sweet potato mild mottle virus*	P
			Macluravirus	*Maclura mosaic virus*	P
			Rymovirus	*Ryegrass mosaic virus*	P
			Tritimovirus	*Wheat streak mosaic virus*	P
			Bymovirus	*Barley yellow mosaic virus*	P
	Caliciviridae		*Lagovirus*	*Rabbit hemorrhagic disease virus*	V
			Norovirus	*Norwalk virus*	V
			Sapovirus	*Sapporo virus*	V
			Vesivirus	*Vesicular exanthema of swine virus*	V
	Unassigned		*Hepevirus*	*Hepatitis E virus*	V
	Astroviridae		*Avastrovirus*	*Turkey astrovirus*	V
			Mamastrovirus	*Human astrovirus*	V
	Nodaviridae		*Alphanodavirus*	*Nodamura virus*	I
			Betanodavirus	*Striped jack nervous necrosis virus*	V
	Tetraviridae		*Betatetravirus*	*Nudaurelia capensis β virus*	I

		Omegatetravirus	*Nudaurelia capensis ω virus*	I
	Unassigned	*Sobemovirus*	*Southern bean mosaic virus*	P
	Luteoviridae	*Luteovirus*	*Barley yellow dwarf virus-PAV*	P
		Polerovirus	*Potato leafroll virus*	P
		Enamovirus	*Pea enation mosaic virus-1*	P
	Unassigned	*Umbravirus*	*Carrot mottle virus*	P
	Tombusviridae	*Dianthovirus*	*Carnation ringspot virus*	P
		Tombusvirus	*Tomato bushy stunt virus*	P
		Aureusvirus	*Pothos latent virus*	P
		Avenavirus	*Oat chlorotic stunt virus*	P
		Carmovirus	*Carnation mottle virus*	P
		Necrovirus	*Tobacco necrosis virus A*	P
		Panicovirus	*Panicum mosaic virus*	P
		Machlomovirus	*Maize chlorotic mottle virus*	P
Nidovirales				
	Coronaviridae	*Coronavirus*	*Infectious bronchitis virus*	V
		Torovirus	*Equine torovirus*	V
	Arteriviridae	*Arterivirus*	*Equine arteritis virus*	V
	Roniviridae	*Okavirus*	*Gill-associated virus*	I
	Flaviviridae	*Flavivirus*	*Yellow fever virus*	V, I
		Pestivirus	*Bovine viral diarrhea virus 1*	V
		Hepacivirus	*Hepatitis C virus*	V
	Togaviridae	*Alphavirus*	*Sindbis virus*	V, I
		Rubivirus	*Rubella virus*	V
	Unassigned	*Tobamovirus*	*Tobacco mosaic virus*	P
		Tobravirus	*Tobacco rattle virus*	P
		Hordeivirus	*Barley stripe mosaic virus*	P
		Furovirus	*Soil-borne wheat mosaic virus*	P
		Pomovirus	*Potato mop-top virus*	P
		Pecluvirus	*Peanut clump virus*	P
		Benyvirus	*Beet necrotic yellow vein virus*	P
	Bromoviridae	*Alfamovirus*	*Alfalfa mosaic virus*	P
		Bromovirus	*Brome mosaic virus*	P
		Cucumovirus	*Cucumber mosaic virus*	P
		Ilarvirus	*Tobacco streak virus*	P
		Oleavirus	*Olive latent virus 2*	P
	Unassigned	*Ourmiavirus*	*Ourmia melon virus*	P
		Idaeovirus	*Rasberry bushy dwarf virus*	P
	Tymoviridae	*Tymovirus*	*Turnip yellow mosaic virus*	P
		Marafivirus	*Maize rayado fino virus*	P, I
		Maculavirus	*Grapevine fleck virus*	P
	Closteroviridae	*Closterovirus*	*Beet yellows virus*	P
		Ampelovirus	*Grapevine leafroll-associated virus 3*	P
		Crinivirus	*Lettuce infectious yellows virus*	P

Continued

Table 1 Continued

Order	*Family*	*Subfamily*	*Genus*	*Type species*	*Host*
	Flexiviridae		*Potexvirus*	*Potato virus X*	P
			Mandarivirus	*Indian citrus ringspot virus*	P
			Allexivirus	*Shallot virus X*	P
			Carlavirus	*Carnation latent virus*	P
			Foveavirus	*Apple stem pitting virus*	P
			Capillovirus	*Apple stem grooving virus*	P
			Vitivirus	*Grapevine virus A*	P
			Trichovirus	*Apple chlorotic leaf spot virus*	P
	Barnaviridae		*Barnavirus*	*Mushroom bacilliform virus*	F
Unassigned viruses					
Unassigned Vertebrate Viruses					V
Unassigned Invertebrate Viruses					I
Unassigned Prokaryote Viruses					B
Unassigned Fungus Viruses					F
Unassigned Plant Viruses					P
The subviral agents: Viroids, satellites and agents of spongiform encephalopathies (prions)					
Viroids					
	Pospiviroidae		*Pospiviroid*	*Potato spindle tuber viroid*	P
			Hostuviroid	*Hop stunt viroid*	P
			Cocadviroid	*Coconut cadang-cadang viroid*	P
			Apscaviroid	*Apple scar skin viroid*	P
			Coleviroid	*Coleus blumei viroid 1*	P
	Avsunviroidae				5
			Avsunviroid	*Avocado sunblotch viroid*	P
			Pelamoviroid	*Peach latent mosaic virus*	P
Satellites					
Vertebrate Prions					V
Fungi prions					F

Virus hosts: Al, Algae; Ar, Archaea; B, Bacteria; F, Fungi; I, Invertebrates; P, Plants; Pr, Protozoa; V, Vertebrates.

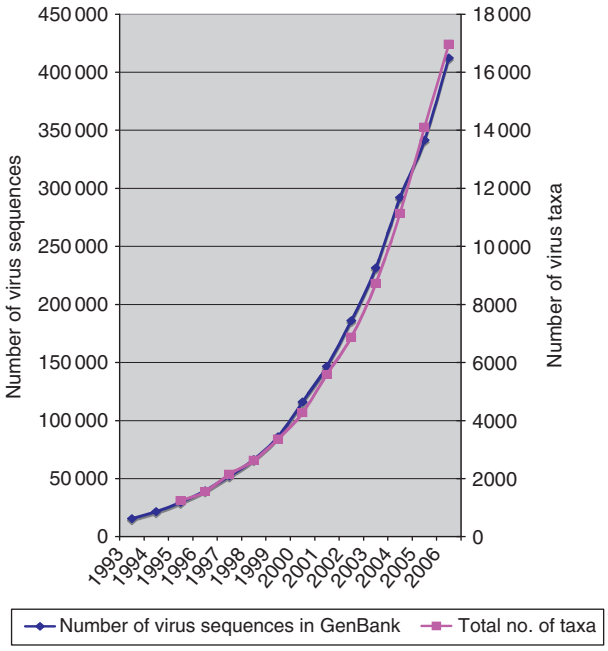


Figure 1 Number of virus taxa (including isolates) and virus sequences stored at GenBank since 1993.

publishes reports describing all existing virus taxa and containing a complete list of classified viruses with their abbreviations. The ICTV published its Eighth Report in 2005. An internet website is also maintained where all new taxonomic proposals are loaded and where the most important information relative to virus taxonomy is made available and updated regularly. The increasing number of virus species and virus strains being identified, along with the explosion of data on many descriptive aspects of viruses and viral diseases, particularly sequence data, has led the ICTV to launch an international virus database project (ICTVdB) and a Taxonomic Proposal Management System specifically to handle taxonomic proposals.

Polythetic Classification and Demarcation Criteria

There are currently two systems in use for classifying organisms: the Linnean and the Adansonian systems. The Linnean system is the monothetic hierarchical classification applied by Linnaeus to plants and animals, while the Adansonian is a polythetic hierarchical system. Although convenient to use, the Linnean system has shortcomings when applied to the classification of viruses because there is no obvious reason to privilege one criterion over another. The Adansonian system considers all available criteria at once and makes several classifications, taking the criteria successively into consideration. Criteria leading to the same classifications are considered correlated and are therefore not discriminatory. Subsequently, a subset of criteria is considered, and the process is repeated until all criteria can be ranked to provide the best discrimination of the species. Furthermore, qualitative and quantitative data can be simultaneously considered when building such a classification. In the case of viruses, the method is not used on a systematic basis, although it has been shown that at least 60 characters are needed for a complete virus description (**Table 2**).

The increasing number of reported viral nucleic acid sequences allows the construction of phylogenetic trees based on a single gene or a group of genes. Sequence comparisons by themselves have not satisfactorily provided a clear classification of all viruses together but are widely used at the order, family, and genus levels. Recently the National Center for Biotechnology Information (NCBI) in Washington developed a system of pairwise sequence comparisons (the so-called PASC system) between viral sequences which allows a new virus to be assigned to known taxa. It seems probable that, in future, virus classification will make increasing use of sequence data.

For more than 40 years, the ICTV has been classifying viruses essentially at the family and genus levels using a nonsystematic polythetic approach. Viruses are first clustered in genera and then in families. A subset of characters including physicochemical, structural, genomic, and biological criteria is then used to compare and group viruses. This subset of characters may change from one family to another according to the availability of the data and depending on the importance of a particular character for a particular family. Obviously, there is no homogeneity in this respect in the current virus classification system, and virologists weigh the criteria in a subjective process. Nevertheless, over time, there has been a great stability of the current classification at the genus and family levels. It is also clear that hierarchical classification above the family level will encounter conflicts between phenotypic and genotypic criteria and that virologists may have to reconsider the entire classification process in order to progress at this level.

Virus Taxa Descriptions

Virus classification continues to evolve with the technologies available for describing viruses. The first wave of descriptions, those before 1940, took into account mostly the visual symptoms of viral diseases along with modes of viral transmission. A second wave, between 1940 and

Table 2 Virus family descriptors used in virus taxonomy

I. Virion properties
  A. Morphology properties of virions
    1. Virion size
    2. Virion shape
    3. Presence or absence of an envelope and peplomers
    4. Capsomeric symmetry and structure
  B. Physical properties of virons
    1. Molecular mass of virions
    2. Buoyant density of virions
    3. Sedimentation coefficient
    4. pH stability
    5. Thermal stability
    6. Cation (Mg^{++}, Mn^{++}) stability
    7. Solvent stability
    8. Detergent stability
    9. Radiation stability
  C. Properties of genome
    1. Type of nucleic acid – DNA or RNA
    2. Strandedness – single stranded or double stranded
    3. Linear or circular
    4. Sense – positive, negative, or ambisense
    5. Number of segments
    6. Size of genome or genome segments
    7. Presence or absence and type of 5′-terminal cap
    8. Presence or absence of 5′-terminal covalently linked polypeptide
    9. Presence or absence of 3′-terminal poly(A) tract (or other specific tract)
    10. Nucleotide sequence comparisons
  D. Properties of proteins
    1. Number of proteins
    2. Size of proteins
    3. Functional activities of proteins (especially virion transcriptase, virion reverse transcriptase, virion hemagglutinin, virion neuraminidase, virion fusion protein)
    4. Amino-acid-sequence comparisons
  E. Lipids
    1. Presence or absence of lipids
    2. Nature of lipids
  F. Carbohydrates
    1. Presence or absence of carbohydrates
    2. Nature of carbohydrates

II. Genome organization and replication
    1. Genome organization
    2. Strategy of replication of nucleic acid
    3. Characteristics of transcription
    4. Characteristics of translation and post-translational processing
    5. Site of accumulation of virion proteins, site of assembly, site of maturation and release
    6. Cytopathology, inclusion body formation

III. Antigenic properties
    1. Serological relationships
    2. Mapping epitopes

IV. Biological properties
    1. Host range, natural and experimental
    2. Pathogenicity, association with disease
    3. Tissue tropisms, pathology, histopathology
    4. Mode of transmission in nature
    5. Vector relationships
    6. Geographic distribution

1970, brought together an enormous amount of information from studies of virion morphology (electron microscopy, structural data), biology (serology and virus properties), and physicochemical properties of viruses (nature and size of the genome, number and size of viral proteins). The impact of descriptions on virus classification has been particularly influenced by electron microscopy and the negative-staining technique for virions in the 1960s and 1970s. With this technique, viruses could be identified from poorly purified preparations of all tissue types and information about size, shape, structure, and symmetry could be quickly provided. As a result, virology progressed simultaneously for all viruses infecting animals, insects, plants, and bacteria. Since 1970, the virus descriptors list has included genome and replication information (sequence of genes, sequence of proteins), as well as molecular relationships with virus hosts.

The most recent wave of information used to classify viruses is virus genome sequences. Genome sequence comparisons are becoming more and more prevalent in virus taxonomy as exemplified by the presence of a significant number of phylogenetic trees in the *Eighth ICTV Report.* Some scientists promote the concept of quantitative taxonomy, aimed at demonstrating that virus genome sequences contain all the coding information required for all the biological properties of the viruses. This is in complete agreement with the polythetic concept of virus species definition if one considers that the unique sequence of a genome contains in fact all the information of the virus to perform all the steps of its replication cycle with structural and nonstructural genes and all of its biological functions. A good example of quantitative taxonomy is the re-classification of flaviviruses from the genus *Flavivirus* in the family *Togaviridae* into the new family *Flaviviridae* based upon sequencing of the yellow fever virus genome and comparisons with the gene sequence arrangement of members of the genus *Alphavirus* in the family *Togaviridae.* Another recent example is the merging of the genera *Rhinovirus* and *Enterovirus* in the family *Picornaviridae,* based on the fact that phylogenetic trees and pairwise comparisons did not support the continued distinction between the two genera.

There is a correlative modification of the list of virus descriptors, and **Table 2** lists the family and genus descriptors which are used in the current ICTV report. **Table 2** lists 45 different types of properties where each property (e.g., morphology) can take on different individual states (e.g., filamentous, icosahedral, etc.). A universal lists of virus descriptors has been established which is used by the ICTVdB. It contains a common set of descriptors for all viruses and subsets for specific viruses in relation to their specific hosts (human, animal, insect, plant, and bacterial).

The Order of Presentation of the Virus Classification

Currently, and for practical reasons only, virus classification is structured according to the 'Order of Presentation of Viruses' indicated in **Table 1**. The presentation of virus orders, families, and genera in this particular order reflects convenience rather than any hierarchical or phylogenetic consideration. The Order of Presentation of Viruses follows four criteria: (1) the nature of the viral nucleic acid, (2) the strandedness of the nucleic acid (single stranded (ss) or double stranded (ds)), (3) the use of a reverse transcription process (DNA or RNA), and (4) the sense of gene coding on the encapsidated genome (positive, negative, or ambisense). These four criteria give rise to six clusters comprising the 86 families and unassigned genera (genera without a designated family). Within each cluster, families and unassigned genera have been listed according to their possible affinities. For example, the families *Picornaviridae, Dicistroviridae, Sequiviridae, Comoviridae,* and *Potyviridae* are listed one after another because they share a number of similarities in their genome organization and sequence relatedness and they may form the basis for a proposed order in the future.

Table 3 List of criteria demarcating different virus taxa

I. Order
Common properties between several families including:
Biochemical composition
Virus replication strategy
Particle structure (to some extent)
General genome organization
II. Family
Common properties between several genera including:
Biochemical composition
Virus replication strategy
Nature of the particle structure
Genome organization
III. Genus
Common properties within a genus including:
Virus replication strategy
Genome size, organization, and/or number of segments
Sequence homologies (hybridization properties)
Vector transmission
IV. Species
Common properties within a species including:
Genome arrangement
Sequence homologies (hybridization properties)
Serological relationships
Vector transmission
Host range
Pathogenicity
Tissue tropism
Geographical distribution

A New Virus Taxon: The Virus Species

For many years, virologists debated the existence of virus species which was a very controversial issue and a series of definitions surfaced at regular intervals but none was adopted. However, in 1991, the ICTV Executive Committee accepted the species concept and the adopted definition is "A virus species is a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche." This simple definition has already and will continue to have a profound effect on virus classification. In the *Eighth Report of the ICTV*, the 'List of Species' and the 'List of Tentative Species' are accompanied by a 'List of Species Demarcating Criteria' provided for each genus. Naturally, this list of criteria should follow the polythetic nature of the species definition, and more than one criterion should be used to determine a new species. It is obvious that most criteria are shared among the different genera, within and across families. These shared criteria include host range, serological relationships, vector transmission type, tissue tropism, genome rearrangement, and sequence homology (**Table 3**). However, while the nature of the criteria is similar, the levels of demarcation clearly differ from one family to another. This may reflect differences in appreciation from one family to another, but most likely reflects the differential ranking of a particular criterion in different families. The huge differences in sequence homologies (up to 30%) among lentivirus nucleoprotein sequences may not have the same biological significance as small differences for potyvirus capsid protein sequences (0–10%), and therefore universal levels of sequence identity for similar genes may not exist for viruses. However, it is important to note that the nature of the demarcating criteria at the genus level will probably not change since they have passed the test of years. Despite the fact that they were mostly established using biochemical and structural criteria, most of them have remained valid when correlated with genome organization and sequence data.

A Uniform Nomenclature for All Virus Taxa

Nomenclature is tightly associated with classification, in the sense that the taxonomic names indicate, to some extent, the nature of the taxa. Similarly for viruses, the ICTV has set rules for virus nomenclature and the orthography of taxonomic names that are regularly revised and improved. The international virus species names end in 'virus', international genus names in

'...virus', international subfamily names in '...virinae', international family names in '...viridae', and international order names in '...virales'. In formal taxonomic usage, the virus order, family, subfamily, genus, and species names are printed in italics (or underlined) and the first letter is capitalized. For all taxa except species, new names are created following ICTV guidelines. Because of the difficulty in creating new official international names for virus species, it has been decided in 1998 by the ICTV to use the existing English vernacular virus names. However, to differentiate virus species names from virus names it has also been decided that their typography would be different, that is, the species names would be italicized, and the first letter of the name capitalized while the virus names would not. In addition ICTV had created an additional category called 'Tentative Species Names' to accommodate viruses that seemed to belong to a new species, but did not have enough data to support this decision; it was also a way to 'reserve' a name already used in literature. In 2005, ICTV decided to replace this category by 'Unassigned Viruses' in the genus.

Latinized binomials for virus names have been supported by animal and human virologists of the ICTV for many years but have never been implemented. Their recommendation was in fact withdrawn from ICTV nomenclature rules in 1990, and consequently, such names as *Herpesvirus varicella* or *Polyomavirus hominis* should not be used. For several years, plant virologists adopted a different nomenclature, using the vernacular name of a virus but replacing the word 'virus' by the genus name: for example, *Cucumber mosaic cucumovirus* and *Tobacco mosaic tobamovirus*. This system is called 'the non-latinized binomial system', although the binomial order is the opposite of the typical latinized binomial system where the genus name ends with the virus name. Though this usage is favored by many scientists, and examples of such a practice can be found for human, animal, and insect viruses (e.g., *Human rhinovirus*, *Canine calicivirus*, and *Acheta densovirus*), it has not yet been adopted as a universal system by the ICTV; however, it has been decided that each study group would decide what is best for the viruses they deal with and the new names would have to be ratified through a formal taxonomic proposal by the ICTV.

In formal usage, the name of the taxon precedes the name of the taxonomic unit: for example, "the family *Picornaviridae*' or "the genus *Rhinovirus*'. In informal vernacular usage, virus order, family, subfamily, genus, and species names are written in lower case roman script; they are not capitalized or italicized (or underlined) – for example 'animal reoviruses'. To avoid ambiguous identifications, it has been recommended to journal editors that published virological papers follow ICTV guidelines for proper virus identification and nomenclature and that viruses should be cited with their full taxonomic terminology when they are first mentioned in an article, for example, order *Mononegavirales*, family *Paramyxoviridae*, subfamily *Pneumovirinae*, genus *Pneumovirus*, species *Human respiratory syncytial virus*.

A Universal Classification System

The present universal system of virus taxonomy is set arbitrarily at the hierarchical levels of order, family, subfamily, genus, and species. Lower hierarchical levels, such as suborder, subgenera, and subspecies, may be considered in the future if need arises. Hierarchical levels under the species level such as strains, serotypes, variants, and pathotypes are established by international specialty groups and/or by culture collections, but not by the ICTV.

Species

The species taxon is always regarded as the most important taxonomic level in classification but has proved difficult to apply to viruses. In 1991, the ICTV accepted the definition of species, stated above, proposed by Marc van Regenmortel. The major advantage of this definition is that it can accommodate the inherent variability of viruses and is not dependent on the existence of a unique set of characteristics. Members of a polythetic class are defined by more than one property and no single property is absolutely indispensable. Thus, in each family, it might be possible to determine the set of properties of the class 'species' and thus to verify if the family members are representatives of the class 'species' or if they belong to a different taxonomic level.

Many practical matters are related to the definition of a virus species. These include (1) homogeneity of the different taxa across the classification, (2) diagnostic-related matters, (3) virus collections, (4) evolution studies, (5) biotechnology, (6) sequence database projects, (7) virus database projects, (8) publication matters, and also (9) intellectual property rights.

Genera

There is no formal ICTV definition for a genus, but it is commonly considered as "a population of virus species that share common characteristics and are different from other populations of species." Although this definition is somewhat elusive, this level of classification seems stable and useful. Some genera have been moved from one

family to another (or from one family to an unassigned genus status such as the genus *Hepevirus*) over the years, but the composition and description of these genera has remained very stable. The characters defining a genus are different from one family to another. The use of subgenera has been abandoned in current virus classification.

Families

Genera are usually clustered in families, and most of the time, when a new genus, obviously not belonging to any existing family, is created, virologists also create a new family. Even after the creation of the ICTV, plant virologists have continued to classify plant viruses in 'groups', refusing to place them in genera and families. This position was mostly caused by a refusal to accept a binomial nomenclature. However, because of obvious similarities, plant reoviruses and rhabdoviruses had been integrated into the families *Reoviridae* and *Rhabdoviridae* (**Table 1**). Plant virologists subsequently accepted in 1995 the placing of plant viruses into species, genera, and families. The number of unassigned genera is regularly decreasing with time; the most recent clustering is the creation of the family *Flexiviridae* with the genera *Potexvirus*, *Carlavirus*, *Mandarivirus*, *Foveavirus*, *Capillovirus*, *Allexivirus*, *Vitivirus,* and *Trichovirus*. However, there are still 22 unassigned genera that do not belong to any family. Their presence originates mostly from the preference of plant virologists for accumulating data on virus species and genera before clustering genera in families. The unassigned genus status is now being used by animal virologists as a convenient temporary classification status. Examples are the unassigned genera *Anellovirus*, that is close to the family *Circoviridae* but different enough to be separated, the previously unassigned genus *Cripavirus* that has been upgraded to full family status (*Dicistrovirida*), and another unassigned genus *Iflavirus* that has been created to accommodate new picorna-like viruses that are not typical picornaviruses.

Orders

As mentioned above, the higher hierarchical levels for virus classification are extremely difficult to establish. To date only three orders have been accepted: *Caudovirales*, *Mononegavirales*, and *Nidovirales*. The first order, *Mononegavirales*, was established in 1990 and comprises the nonsegmented ssRNA negative-sense viruses, namely, the families *Bornaviridae*, *Filoviridae*, *Paramyxoviridae*, and *Rhabdoviridae*. This order was formed because of the great similarity between these families over many criteria, including their replication strategies. A second order, *Caudovirales*, contains all families of dsDNA phages possessing a tail, including the *Myoviridae*, *Podoviridae*, and *Siphoviridae*. The order *Nidovirales* comprises the families *Coronaviridae*, *Arteriviridae,* and *Roniviridae* and was created because it was clear that the viruses belonging to these families share many properties and yet are so different that they cannot be placed together in the same family. Many members of the ICTV advocate the creation of many more orders, and as a matter of fact four new orders encompassing the families *Herpesviridae, Picornaviridae, Reoviridae, and Retroviridae* have been proposed and provisionally named but it has been decided to proceed cautiously in this area so as to avoid creation of short-lived orders. The creation of formal taxa higher than the orders – for example kingdoms, classes, and subclasses – has not been considered by the ICTV.

See also: Nature of Viruses; Phylogeny of Viruses; Virus Classification by Pairwise Sequence Comparison (PASC); Virus Databases; Virus Species.

Further Reading

Adams MJ, Antoniw JF, Bar-Joseph M, *et al.* (2004) The new plant virus family *Flexiviridae* and assessment of molecular criteria for species demarcation. *Archives of Virology* 149: 1045–1060.

Dolja VV, Boyko VP, Agranovsky AA, and Koonin EV (1991) Phylogeny of capsid proteins of rod-shaped and filamentous RNA plant viruses: Two families with distinct patterns of sequence and probably structure conservation. *Virology* 184: 79–86.

Dolja VV and Koonin EV (1991) Phylogeny of capsid proteins of small icosahedral RNA plant viruses. *Journal of General Virology* 72: 1481–1486.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2004) *Virus Taxonomy: Eigth Report of the International Committee on Taxonomy of Viruses,* p.1258. San Diego, CA: Elsevier Academic Press.

Francki RIB, Milne RG, and Hatta T (1985) *Atlas of Plant Viruses.* Boca Raton, FL: CRC Press.

Harrison BD, Finch JT, Gibbs AJ, *et al.* (1971) Sixteen groups of plant viruses. *Virology* 45: 356–363.

Koonin EV (1991) The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA viruses. *Journal of General Virology* 72: 2197–2206.

Mayo MA and Horzinek MC (1998) A revised version of the International Code of Virus Classification and Nomenclature. *Archives in Virology* 143: 1645–1654.

Van Regenmortel MHV (1990) Virus species, a much overlooked but essential concept in virus classification. *Intervirology* 31: 241–254.

Van Regenmortel MHV, Bishop DHL, Fauquet CM, Mayo MA, Maniloff J, and Calisher CH (1997) Guidelines to the demarcation of virus species. *Archives of Virology* 142: 1505–1518.

Relevant Website

http://www.ictv.ird.fr – ICTV; Taxonomic Proposal Management System.

Tenuivirus

B C Ramirez, CNRS, Paris, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

RNA silencing Evolutionarily conserved mechanism in many eukaryotes to target and degrade aberrant RNA molecules. It constitutes an antiviral defense in plants and insects.

Introduction

The tenuiviruses were first described in the Fifth Report of the International Committee on Taxonomy of Viruses in 1982 as nonenveloped plant viruses possibly possessing a negative single-stranded (ss) RNA genome. Their name comes from the Latin 'tenuis', (thin, fine, weak) which refers to the structure of the viral particle as seen by electron microscopy (**Figure 1**). Epidemics of rice stripe virus (RSV) and rice hoja blanca virus (RHBV) cause important yield losses in rice-growing areas of Asia and the former USSR and of tropical America, respectively. Tenuiviruses exhibit unique properties that make them different from other plant viruses. Some properties of tenuiviruses are the following:

1. The peculiar flexuous viral particles have a thread-like morphology and can adopt circular forms (**Figure 1**(**a**)).
2. The viruses are persistently transmitted by a particular species of planthopper in a circulative, propagative manner. For some of the members of the genus, it has been demonstrated that the virus multiplies both in the host plant and in the insect vector. Multiplication of the virus in the vector may have deleterious effects on the insect. The viruses can be transmitted transovarially by viruliferous female planthoppers to their offspring, and through sperm from viruliferous males.
3. The genome of tenuiviruses is multisegmented and composed of ssRNAs that have either negative or ambisense polarity (**Figure 2**).
4. An RNA-dependent RNA polymerase (RdRp) is associated with the viral particle.
5. A nonstructural protein of 16–22 kDa accumulates in large amounts in infected plants, forming large inclusions (**Figure 1**(**b**)).
6. It has been observed for some tenuiviruses that the mRNAs are synthesized via cap-snatching (**Figure 3**).
7. Tenuiviruses infect plants of the family Poaceae.

Type Species and Other Species in the Genus

Rice stripe virus (RSV) is the type species of the genus *Tenuivirus*. Other species in the genus are *Echinochloa hoja blanca virus* (EHBV), *Maize stripe virus* (MSpV), *Rice grassy stunt virus* (RGSV), *Rice hoja blanca virus* (RHBV), and *Urochloa hoja blanca virus* (UHBV). Tentative species are Brazilian wheat spike virus (BWSpV), European wheat striate mosaic virus (EWSMV), Iranian wheat stripe virus (IWSV), rice wilted stunt virus, and winter wheat mosaic virus.

Virion Properties

Morphology

The particles also referred as ribonucleoproteins (RNPs) are thin filaments that may appear circular or spiral shaped (**Figure 1**(**a**)). The RNPs are 3–10 nm in diameter, with lengths proportional to the sizes of the RNAs they contain. No envelope has been observed.

Physical and Physicochemical Properties

RNP preparations can be separated into four or five components by sucrose density gradient centrifugation. The buoyant density of the RNP in CsCl when centrifuged to equilibrium is 1.282–1.288 g cm^{-3}. RNA constitutes 5–12% of the particle weight.

Components

The tenuivirus genome is composed of 4–6 noncapped ssRNA segments; the approximate size of the genome of the type member RSV is 17 kbp (**Figure 2**). The largest segment (RNA1, ~9 kbp) of RSV, MSpV, and RHBV is of negative polarity and encodes the RdRp. Segments 2 (RNA2, 3.3–3.6 kbp), 3 (RNA3, 2.2–2.5 kbp), and 4 (RNA4, 1.9–2.2 kb) of RSV, MSpV, and RHBV are ambisense. Segment 5 (1.3 kbp) detected in virions of MSpV and of EHBV is of negative polarity. A fifth RNA segment has also been reported for some isolates of RSV. RGSV RNA1, 2, 5, and 6 are homologous to RNA1, 2, 3, and 4, respectively, of other tenuiviruses, whereas RNA3 (3.1 kbp) and 4 (2.9 kbp) are ambisense and unique to RGSV. Subgenomic RNAs (sgRNAs) of different sizes and of either polarity are detected; they serve as mRNA for the synthesis of the viral proteins (**Figure 3**).

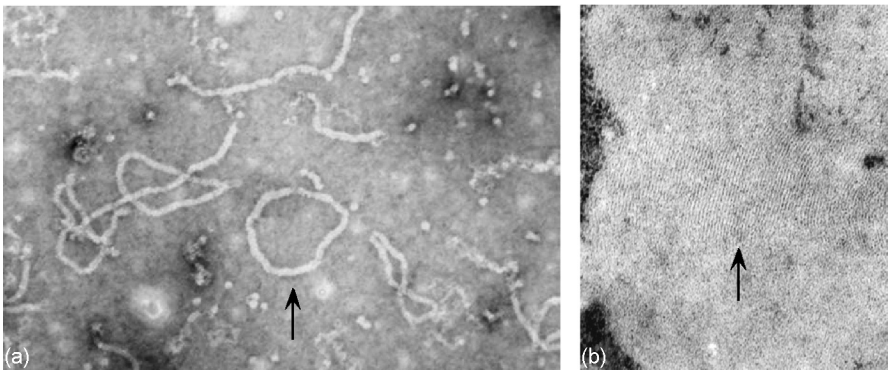


Figure 1 (a) Micrograph of purified ribonucleoproteins (RNPs) of rice hoja blanca virus (RHBV): An RNP is indicated by the arrow. (b) Ultrathin section of a rice leaf cell infected with RHBV. The arrow indicates viral inclusion bodies inside the nucleus with cytopathic effects.

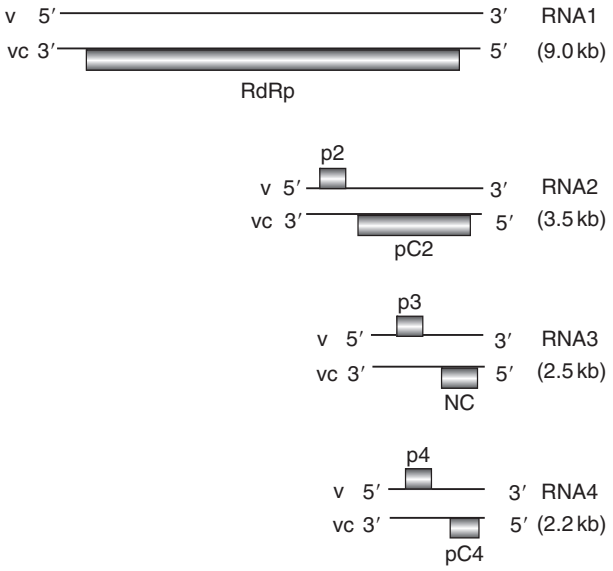


Figure 2 Schematic representation of the genome organization of RSV. RdRp, RNA-dependent RNA polymerase; NC, nucleocapsid protein; p4, nonstructural protein, which accumulates in viral inclusion bodies; v, viral sense RNA; vc, viral complementary RNA.

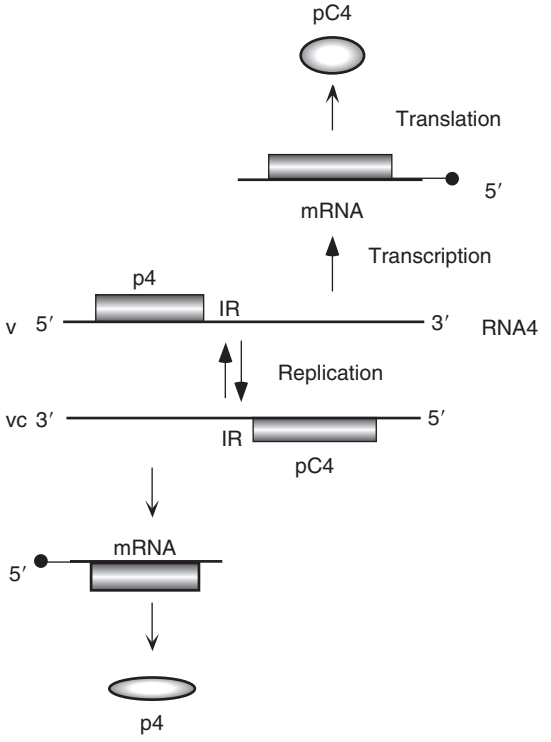


Figure 3 Schematic representation of the replication, transcription, and translation strategies of the ambisense RNA segments of tenuiviruses. Cap and nonviral nucleotides of host origin used as primers by the RdRp as a result of cap-snatching are represented by '—●'. IR, intergenic region.

The RNPs contain a nucleocapsid (NC) protein of 34–35 kDa, and small amounts of a 230 kDa protein which is co-purified with RNPs of RSV, RHBV and RGSV. This protein is the RdRp, associated with filamentous RNPs. The RNA polymerase activity of RHBV is capable of replicating and transcribing the RNA segments *in vitro*.

Genome Organization, Encoded Proteins, Replication and Transcription

The 5′ and 3′ terminal sequences (for about 20 nt) are complementary to each other: they can base-pair and give rise to circular RNPs. The terminal 8 nt (5′ ACACAAAG) and their complement are conserved between tenuiviruses and viruses of the genus *Phlebovirus* of the family *Bunyaviridae*. Several RNA segments encode two proteins in an ambisense arrangement (**Figure 2**). The RdRp and NC protein (32 Kda) are encoded by the viral complementary (vc)RNA1 and vcRNA3, respectively. The p3 (24 Kda) encoded by the viral (v)RNA3 is a suppressor of RNA silencing as demonstrated for RHBV in both plant and insect hosts. A major nonstructural protein (p4; 22 Kda) that accumulates in infected plants

is encoded by vRNA4. The sizes of other RSV proteins shown in **Figure 2** are 23 kDa (p2), 94 kDa (pC2), 24 kDa (p3), and 32 kDa (pC4).

For MSpV, RHBV, and RSV, the mRNAs are synthesized via cap-snatching (**Figure 3**). The 5′ end of the mRNAs contains 10–17 nonviral nucleotides and are capped, these extra sequences are derived from host cell mRNAs that are taken or snatched by the RdRp and used as primers to initiate mRNA synthesis. Intergenic noncoding regions located between the open reading frames (ORFs) can in certain cases adopt hairpin structures. The cap-snatching mechanism has been observed for mRNA synthesis of influenza viruses and viruses of the families *Bunyaviridae* and *Arenaviridae*.

Antigenic Properties

The NC proteins of RSV and MSpV are serologically related, as are the NC and p4 proteins of RSV and RGSV. Likewise, the NC proteins of RHBV, EHBV, and UHBV are serologically related. The NC protein of RSV reacts weakly with antibodies made to virion preparations of RGSV or RHBV.

Biological Properties

Host Range

Plant hosts of tenuiviruses all belong to the family Poaceae.

Cytopathology

In infected plants large inclusion bodies (**Figure 1(b)**) are observed which contain the major nonstructural protein p4.

Symptoms

Infected plants exhibit chlorotic stripes and yellow stippling on the leaf blade. Rice plants infected with RHBV at an early stage of development are stunted and may develop necrosis.

Insect Vector and Kind of Transmission

Each virus species is transmitted by a particular species of planthopper in a circulative, propagative manner and can be transmitted transovarially by viruliferous female planthoppers to their offspring, and through sperm from viruliferous males. The principal vectors of the species are *Laodelphax striatellus* for RSV, *Tagosodes cubanus* for EHBV, *Peregrinus maidis* for MSpV, *Nilaparvata lugens* for RGSV, *Tagosodes orizicolus* for RHBV, and *Caenodelphax teapae* for UHBV. Known vectors of the tentative species are *Sogatella kolophon* for BWSpV, *Javesella pellucida* for EWSMV, and *Ukanoes tanasijevici* for IWSV.

Criteria for Species Demarcation and Phylogenetic Relationships between Species in the Genus

The criteria for species demarcation are given as follows:

1. Vector specificity: transmission by different species of vector.
2. Different sizes and/or numbers of RNA segments.
3. Host range: abilities to infect different key plant species.
4. Amino acid (aa) sequence identity of less than 85% between corresponding gene products.
5. Nucleotide sequence identity of less than 60% between corresponding noncoding intergenic regions.

An example of species discrimination is that between RSV and MSpV. RSV is transmitted by *L. striatellus* and infects 37 graminaceous species including rice and wheat. MSpV is transmitted by *P. maidis* and infects maize, occasionally sorghum, and a few other graminaceous plants but not rice or wheat. RSV isolates have genomes of four RNA segments and the MSpV genome consists of five segments.

An example of difficult species demarcation is the group of the hoja blanca viruses (RHBV, EHBV, and UHBV). They have different vectors, different sizes, and numbers of RNA segments, different hosts, and the nucleotide sequence identity of their intergenic regions is less than 60%. However, the aa sequences of the four proteins on RNAs 3 and 4 are about 90% identical between RHBV, EHBV, and UHBV. Since four of five criteria are met, these viruses could be considered as distinct species that possibly separated recently and are now diverging with little field contact between them.

Phylogenetic analysis of the sequence data from RNA3 and RNA4 of the tenuiviruses shows that RHBV, EHBV, and UHBV are related and form a group distinct from RSV and MSpV (**Figure 4**).

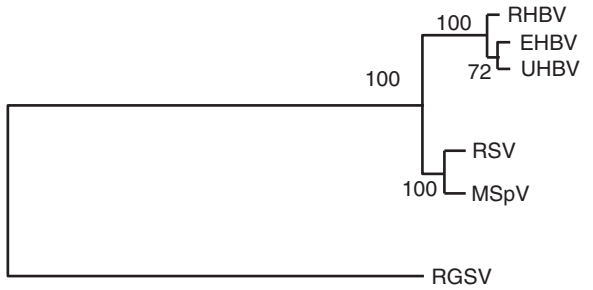


Figure 4 Phylogenetic tree showing the relationships between tenuiviruses on the basis of the nucleotide sequences of the ORFs on RNAs 3 and 4 (RNAs 5 and 6 for RGSV). Shown is the percentage bootstrap support at each node. RHBV, *Rice hoja blanca virus;* EHBV, *Echinochloa hoja blanca virus*; UHBV, *Urochloa hoja blanca virus*; RSV, *Rice stripe virus*; MSpV, *Maize stripe virus*; RGSV, *Rice grassy stunt virus*.

Relation to Other Taxa

Viruses of the genus *Tenuivirus* share several properties with those of the genus *Phlebovirus* of the family *Bunyaviridae*. The multisegmented genomes of tenui- and phleboviruses contain negative-sense and ambisense components. The 5′ and 3′ terminal complementary sequences of viruses of either genus can base-pair and could give rise to circular RNPs. The terminal 8 nt (5′ ACACAAAG) and their complement are conserved between tenui- and phleboviruses. Synthesis of mRNA by the viral RdRp follows a cap-snatching mechanism for viruses of the two genera. Tenuiviruses and most genera in the family *Bunyaviridae* infect their insect vectors as well as their plant hosts. The different number of genome components and the apparent lack of an enveloped viral particle distinguish tenuiviruses from viruses in the family *Bunyaviridae*.

Acknowledgments

The author is grateful to F. Morales for the electron microscope pictures and to Anne-Lise Haenni for useful discussions and critical reading of the manuscript.

See also: Bunyaviruses: General Features.

Further Reading

de Miranda JR, Muñoz M, Wu R, and Espinoza AM (2001) Phylogenetic placement of a novel tenuivirus from the grass *Urochloa plantaginea*. *Virus Genes* 22: 329–333.

de Miranda JR, Muñoz M, Wu R, Hull R, and Espinoza AM (1996) Sequence of rice hoja blanca tenuivirus RNA-2. *Virus Genes* 12: 231–237.

de Miranda JR, Ramirez BC, Muñoz M, *et al.* (1997) Comparison of Colombian and Costa Rican strains of rice hoja blanca tenuivirus. *Virus Genes* 15: 191–193.

Estabrook EM, Suyenaga K, Tsai JH, and Falk BW (1996) Maize stripe tenuivirus RNA 2 transcripts in plant and insect hosts and analysis of pvc2, a protein similar to the Phlebovirus virion membrane glycoproteins. *Virus Genes* 12: 239–247.

Falk BW and Tsai JH (1998) Biology and molecular biology of viruses in the genus Tenuivirus. *Annual Review of Phytopathology* 36: 139–163.

Haenni A-L, de Miranda JR, Falk BW, *et al.* (2005) *Tenuivirus*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberg U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Virus*, pp. 717–723. San Diego, CA: Elsevier Academic Press.

Huiet L, Feldstein PA, Tsai JH, and Falk BW (1993) The maize stripe virus major non-capsid protein messenger RNA transcripts contain heterogeneous leader sequences at their 5′ termini. *Virology* 197: 808–812.

Nguyen M, Ramirez BC, Golbach R, and Haenni A-L (1997) Characterization of the *in vitro* activity of the RNA-dependent RNA polymerase associated with the ribonucleoproteins of rice hoja blanca tenuivirus. *Journal of Virology* 71: 2621–2627.

Ramirez BC, Garcin D, Calvert LA, Kolakofsky D, and Haenni A-L (1995) Capped non-viral sequences at the 5′ end of the mRNA of rice hoja blanca virus RNA4. *Journal of Virology* 69: 1951–1954.

Ramirez BC and Haenni A-L (1994) Molecular biology of tenuiviruses, a remarkable group of plant viruses. *Journal of General Virology* 75: 467–475.

Ramirez BC, Lozano I, Constantino L-M, Haenni A-L, and Calvert LA (1993) Complete nucleotide sequence and coding strategy of rice hoja blanca virus RNA4. *Journal of General Virology* 74: 2463–2468.

Ramirez BC, Macaya G, Calvert LA, and Haenni A-L (1992) Rice hoja blanca virus genome characterization and expression *in vitro*. *Journal of General Virology* 73: 1457–1464.

Shimizu T, Toriyama S, Takahashi M, Akutsu K, and Yoneyama K (1996) Non-viral sequences at the 5′-termini of mRNAs derived from virus-sense and virus-complementary sequences of the ambisense RNA segments of rice stripe tenuivirus. *Journal of General Virology* 77: 541–546.

Toriyama S, Kimishima T, and Takahashi M (1997) The proteins encoded by rice grassy stunt virus RNA5 and RNA6 are only distantly related to the corresponding proteins of other members of the genus *Tenuivirus*. *Journal of General Virology* 78: 2355–2363.

Toriyama S, Kimishima T, Takahashi M, Shimizu T, Minaka N, and Akutsu K (1998) The complete nucleotide sequence of the rice grassy stunt virus genome and genomic comparisons of the genus *Tenuivirus*. *Journal of General Virology* 79: 2051–2058.

Tetraviruses

J A Speir and J E Johnson, The Scripps Research Institute, La Jolla, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The *Tetraviridae* are a family of viruses with nonenveloped $T=4$ particles that package single-stranded, positive-sense RNA genomes and infect only a single order of insects, the Lepidoptera (moths and butterflies). This family stands out from the rest of the known viruses based on these properties alone. It is the only RNA virus family with a host range restricted to insects and the only family with nonenveloped icosahedral $T=4$ particles. The unusual symmetry of their capsids has become the basis for their family name (from the Greek *tettares*, four).

The first report of a larvae disease later to be attributed to a tetravirus was from South Africa in 1941. Researchers Tooke and Hubbard described a devastating annual mortality of the emperor pine moth, *Nudaurelia cytherea capensis*, such that the dead larvae formed a carpet beneath the pine trees (*Pinus radiata*). The outbreaks have now

reduced the larvae population on the pine plantations to a great extent. Five different viruses were found in the infected larvae that were named nudaurelia α, β, γ, δ, and ε, with β being the most abundant. The nudaurelia β virus (NβV) was extensively studied and became the type member of a new virus group called the nudaurelia β-like viruses (betatetraviruses) that was officially recognized in 1982. These studies showed NβV had a monopartite genome and the first known $T=4$ icosahedral capsid. The most recently characterized betatetravirus is from the USA, named Providence virus, which was discovered by Pringle, Ball, and colleagues in 2003. Importantly, this is the first tetravirus that replicates in cell culture, greatly aiding the study of tetravirus molecular biology (see below).

While the betatetraviruses were the first tetraviruses discovered and form the majority of the family, Hendry and colleagues isolated another virus from *Nudaurelia* larvae in 1985, the nudaurelia ω virus (NωV), which was physically similar to NβV but encapsidated a second genomic RNA. NωV became the type member for a second genus within the *Tetraviridae*, the ω-like genus (omegatetraviruses). A second omegatetravirus was isolated by Hanzlik and colleagues in 1993 from Australia, the helicoverpa armigera stunt virus (HaSV), and a third was discovered in 2005 by Yi and colleagues from the Yunnan Province of China, the dendrolimus punctatus tetravirus (DpTV). Both HaSV and DpTV are closely similar to NωV (see below). Currently, the family consists of three omegatetraviruses, and nine betatetraviruses, as well as eight other possible members (**Table 1**). The tetravirus family has grown little in recent years, with only a few new members identified in the last decade. This is due to their limited host range, the difficult field collections of the insect hosts due to the locations where they thrive, and the difficulty or impossibility of rearing the host insects in the laboratory. Also, several aspects of their life cycle remain poorly characterized due to their inability to grow in cell culture despite extensive efforts covering a wide variety of cell types. In spite of these drawbacks, studies of the tetraviruses have expanded to a great extent. It is possible to raise HaSV from laboratory-grown insects, Providence virus is the first tetravirus found to replicate in cell culture, and virus-like particles (VLPs) can be produced in a baculovirus expression system for NωV, HaSV, and TaV. These systems have allowed for advances in detailed biophysical descriptions of these capsids, including a crystal structure of NωV, and new data on replication, pathology, and the biotechnological potential of this unique virus family.

Table 1 Members of the *Tetraviridae*

Virus	*Acronym*	*Family of Lepidoptera host*	*Geographic location*
Betatetraviruses			
Nudaurelia capensis β virus[a]	NβV	Saturniidae	South Africa
Antheraea eucalypti virus[b]	AeV	Saturniidae	Australia
Dama trima virus	DtV	Limacodidae	Malaysia
Dasychira pudibunda virus[c]	DpV	Lymantriidae	UK
Philosamia cynthia X ricini virus	PxV	Saturniidae	UK
Providence virus	PrV	Noctuidae	USA
Pseudoplusia includens virus	PiV	Noctuidae	USA
Thosea asigna virus[d]	TaV	Limacodidae	Malaysia
Trichoplusia ni virus	TnV	Noctuidae	USA
Omegatetraviruses			
Nudaurelia capensis ω virus[a]	NwV	Saturniidae	South Africa
Dendrolimus punctatus tetravirus	DpTV	Lasiocampidae	China
Helicoverpa armigera stunt virus	HaSV	Noctuidae	Australia
Unassigned possible members[e]			
Acherontia atropas virus	AaV	Sphingidae	Canary Islands
Agraulis vanillae virus	AvV	Nymphalidae	Argentina
Callimorpha quadripuntata virus	CqV	Arctiidae	UK
Eucocytis meeki virus	EmV	Cocytiidae	Papua New Ginea
Euploea corea virus	EcV	Danadidae	Australia/Germany
Hypocritae jacobeae virus	HjV	Arctiidae	UK
Lymantria ninayi virus	LnV	Lymantriidae	Papua New Ginea
Nudaurelia ε virus[f]	NεV	Saturniidae	South Africa

[a]Type virus for genus.
[b]Serological evidence shows identity to NβV.
[c]Host renamed *Calliteara pudibunda.*
[d]Host renamed *Setothosea asigna*.
[e]Viruses showing serological relationship to a known betatetravirus (excluding NεV), but otherwise uncharacterized.
[f]NεV resembles the tetraviruses but is serologically unrelated to any known member.
Reproduced by permission of Elsevier.

Genome Organization, Replication, and Capsid Assembly

General Characteristics of Tetravirus Genomic RNAs

The main characteristic separating the betatetraviruses and omegatetraviruses is the RNA encapsidated by the particles in each genus. All tetraviruses analyzed for RNA content share the presence of a large genomic RNA strand of about 5.3–6.5 kbp in length and a smaller genomic or subgenomic RNA strand of about 2.5 kbp. In the monopartite β-like viruses, both the replicase and capsid protein genes are on the large genomic RNA strand (~6.5 kbp). In the bipartite ω-like viruses, the replicase and capsid protein genes are split between the large (~5.3 kbp) and small (~2.5 kbp) genomic RNA strands, respectively.

Although the betatetraviruses are monopartite, the capsids often package two RNA species. A subgenomic mRNA of 2.5 kbp coding for the capsid protein is also encapsidated with the genomic RNA strand in NβV, PrV, TaV, and TnV. Thus, virus particles from both genera package two RNA molecules totaling ~7.8–9.0 kbp. Experimental evidence also suggests that the ω-like viruses package both genomic RNAs in a single particle, as is the case for the nodaviruses, the only other small spherical RNA animal viruses with a bipartite genome. This contrasts with plant viruses having multipartite genomes, which package single genomic RNA strands in different particles.

Analysis of the HaSV genomic RNAs revealed 5′ cap structures and distinctive tRNA-like structures at the 3′ termini, which are not polyadenylated. A tRNA-like structure also exists at the 3′ terminal of the NβV RNA, and no β-like virus genome has been found to be polyadenylated. However, both the HaSV and NβV RNAs could be polyadenylated, indicating no terminal blockage. Therefore, all tetravirus genomic RNAs are likely to have unblocked, tRNA-like 3′ termini and 5′ caps, although the existence of the cap has not been experimentally tested with any β-like virus.

β-Like Genome Organization and Replication Model

The complete nucleotide sequence of the NβV genome (6625 nt) was elucidated in 1998, and partial cDNA clones of the TaV and PrV genomes have been analyzed more recently (2001 and 2003, respectively). All three show nearly identical organization. The single genomic RNAs are dicistronic and have overlapping open reading frames (ORFs) (**Figure 1**). The 5′ ends encode the replicase and the 3′ ends encode the capsid protein genes, which have extensive overlap with the replicase. The NβV replicase ORF contains 5778 nt encoding 1925 amino acids with a calculated molecular weight of 215 kDa. It contains the three domains characteristic of viruses in the α-like RNA virus superfamily (the N-terminal methyltransferase domain, the nucleotide-binding or helicase domain, and the RNA-dependent RNA polymerase (RdRp) domain), but lacks the motifs for the papainlike protease and proline-rich hinge domains that are implicated in autoprocessing of the replicase proteins of some +RNA viruses of vertebrates. Finally, the genomic RNA appears to be the mRNA for the replicase, since its initiation codon is the first located at the 5′ end of the genomic RNA. Translation studies of other β-like virus genomic RNAs have also produced large polypeptides, supporting this scenario.

The sequence of the putative TaV replicase shows a novel permutation among RNA viruses, and together with birnaviruses and an unassigned possible tetravirus (Euprosterna elaeasa virus) defines a unique, ancient lineage of RdRp's. None of the other sequenced tetravirus replicases have a permutation. The TaV replicase lacks both the methyltransferase and helicase domains and the RdRp has little homology with other RNA virus replicases, which as a group have a universal sequence conservation. In 2002, Gorbalenya and colleagues discovered that the catalytic palm subdomain of the TaV RdRp had permuted the conserved 'A–B–C' motif to 'C–A–B' by moving the C motif, which has two Asp residues critical for activity, 110 residues upstream in the sequence. Strikingly, structural modeling demonstrated that this permutation can be accommodated in the conserved RdRp structures by simply rearranging the connectivity of three loops, all of which are positioned away from the active site. Thus, the TaV RdRp is likely to have a conserved fold but with unique connectivity, which might be a major event in the evolution of polymerases.

The 3′ proximal ORF of NβV has 1836 nt and encodes the 66 kDa capsid protein precursor (named α). The capsid protein ORF overlaps the replicase ORF by more than 99% and is in the +1 reading frame relative to the replicase. The capsid protein gene is apparently translated through the production of the previously mentioned 2.5 kbp subgenomic RNA (**Figure 1**), yet replication experiments designed to detect double-stranded RNA (dsRNA) intermediates do not show evidence for a separate complex for the β-like subgenomic RNA indicating that both genomic and subgenomic RNAs are produced from a single full length (−)RNA template. One copy of the genomic (+)RNA, one copy of the subgenomic (+)RNA, and 240 copies of the expressed capsid protein precursor assemble to form an ~400 Å diameter icosahedral $T = 4$ virion (**Figure 2**). The capsid protein is labeled a precursor at this stage because each copy executes an autocatalytic cleavage to produce a large protein (β, ~60 kDa) and a small protein (γ, ~6 kDa), but only after the capsid is assembled (**Table 2**). Both the β and γ polypeptides remain part of the virion after cleavage.

The capsid protein precursors in TaV and PrV may be significantly larger than that of NβV. An additional codon

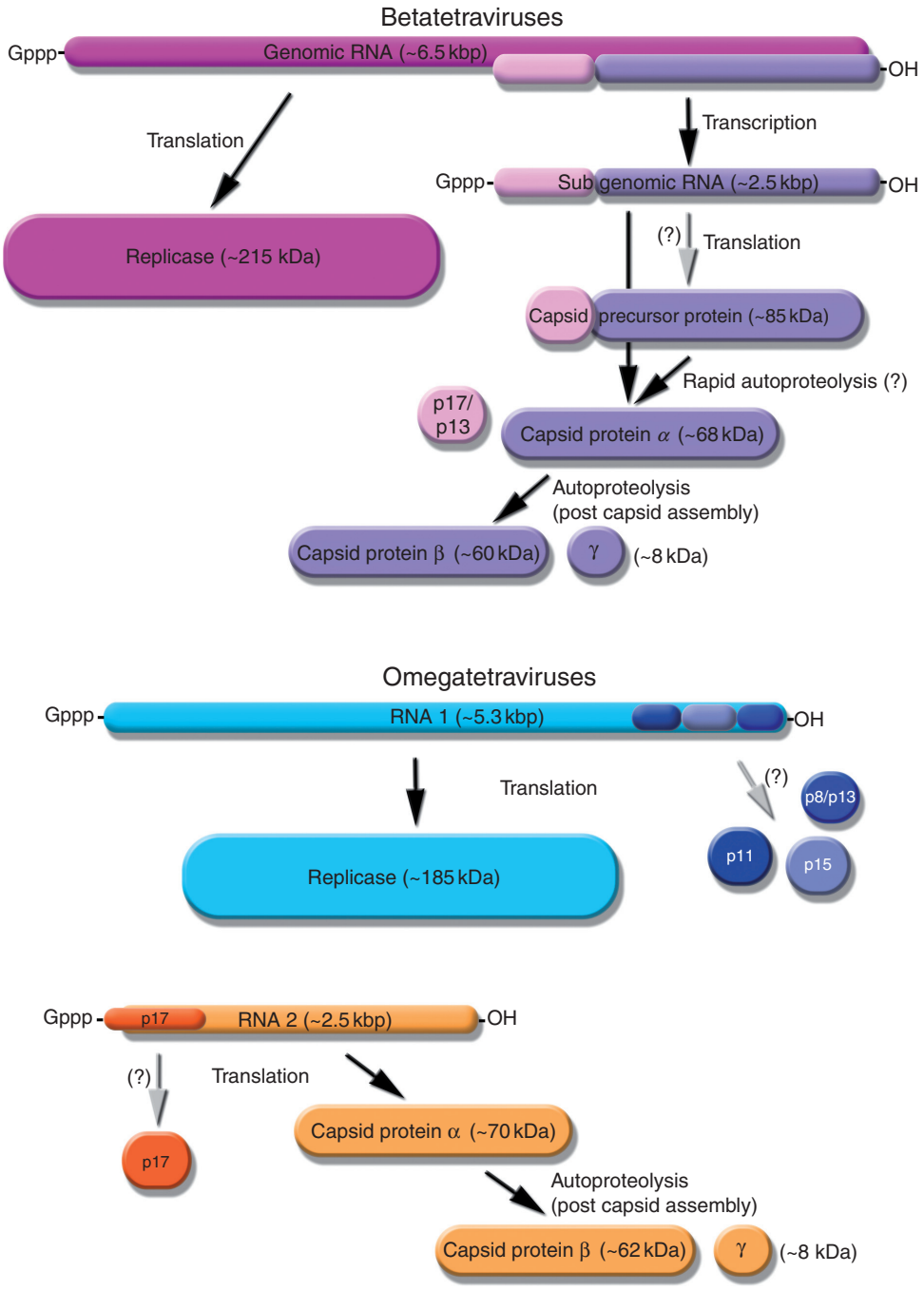


Figure 1 Genome organization and replication strategy of the tetraviruses. Each diagram represents the consensus of available data, but some group members may have slightly different organizations and steps from those shown. The individual events and gene products are discussed in the text. The gray arrows with question marks represent little, if any, translation of those gene products or an unverified event. While autocatalytic processing of the 68–70 kDa capsid protein is nearly identical, significant differences exist between the two groups in their pathways and their potential products. The β-like group utilizes a subgenomic RNA for translation of the capsid precursor protein, which may have two autoproteolytic processing events. The ω-like group has additional ORFs that overlap the end of the replicase gene with unknown functions. Both groups encode 13–17 kDa proteins on the small RNAs in addition to the capsid protein, but it is an additional ORF that would be translated as part of a polyprotein in the β-like group and an overlapping ORF that would be translated separately in the ω-like group.

exists upstream from the known N-terminus of the capsid proteins found in the TaV and PrV virions, yet the extra codon is transcribed to their subgenomic RNAs (**Figure 1**). The additional codons would add approximately 17 and 13 kDa to the TaV and PrV capsid protein precursors, respectively. Since the assembled capsids do not have a protein of this size, these precursors possibly undergo two post-assembly, autocatalytic cleavages, with the

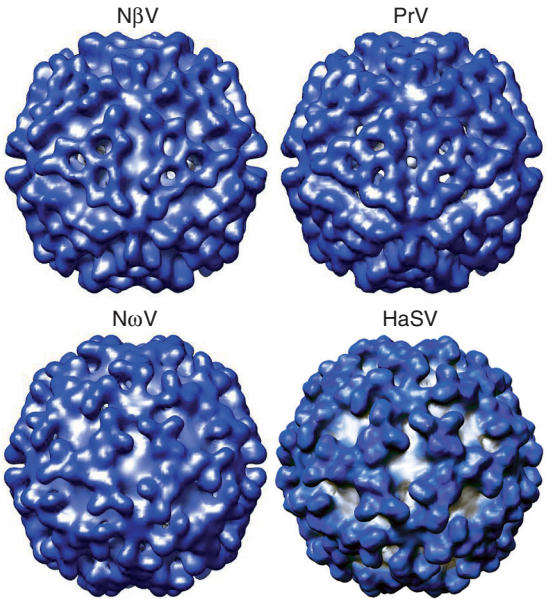


Figure 2 Cryoelectron microscopy reconstructions of the icosahedral tetravirus virions. All four structures are at ~25–30 Å resolution and are viewed down their icosahedral twofold axes. The capsids are ~400 Å in diameter and have $T = 4$ quasi-icosahedral symmetry. The capsid morphologies within each group are closely similar (β-like at top, ω-like at bottom), but differ between the groups. Most notably, within the triangular facets, the β-like viruses have a pitted surface where the ω-like surface is filled in. TaV also displays the same pitted surface in negative-stain EM photographs. The recent crystal structure determination of PrV showed this is mainly due to different rotations of the Ig-like domains relative to the β-barrels when compared to the subunits in the NωV crystal structure (see **Figure 3**). This supports the suggested role of the Ig-like domain in determining the restrictive host cell specificity of these viruses by displaying distinctly different surfaces to cellular receptors.

additional N-terminal cleavage producing polypeptides corresponding to the extra codons (p17, p13). Evidence for this came from expressing the entire capsid protein precursor of TaV in a recombinant baculovirus system as a single polypeptide (p17 + α). TaV VLPs were produced and contained only polypeptides of 58 and 7 kDa, suggesting that the additional 17 kDa had been previously removed by auto-proteolysis. However, it has not yet been shown that the extra codons are translated *in vivo* as part of the capsid protein precursor, and neither p17 nor p13 has been found in assembled particles (authentic or VLPs) of either virus.

ω-Like Genome Organization and Replication Model

The complete sequences of both RNA1 and RNA2 from HaSV and DpTV were determined in 1995 and 2005, respectively, and the complete RNA2 sequence from NωV was initially determined in 1992 and amended in 2005. Partial data for the RNA1 sequence of NωV are also available (**Table 2**). Like the β-like group, the RNAs of the ω-like group have near-identical organization. The length of RNA1 is 5312 nt in HaSV and 5492 in DpTV. Both RNAs contain a single large ORF covering over 90% of their total length, and three smaller ORFs located within the 3′ portion of the large ORF (**Figure 1**). While the three smaller ORFs are in the same reading frame, they are out of frame with the large ORF.

The large ORFs encode the viral replicases with calculated molecular weights of 187 and 180 kDa for HaSV and DpTV, respectively. Like the replicases of the β-like viruses, they also contain the N-terminal methyltransferase domain, the nucleotide-binding or helicase domain, and the RdRp domain, but lack the motifs for the papain-like protease and proline-rich hinge domains. The domains share from 63% to 76% identity between the HaSV and DpTV replicases, and about 36% identity with the same domains from the β-like replicases. The HaSV and NωV replicases are more closely related, sharing close to 90% identity. RNA1 self-replication has been detected before RNA2 replication has been observed, and protein expressed from HaSV RNA1 in a baculovirus system is able to replicate RNA2 transcripts at very low levels.

The three small ORFs encode proteins of 11, 15, and 8 kDa in HaSV and 11, 15, and 13 kDa in DpTV. In both genomes, the stop codon for p11 is located immediately to the 5′ side of the p15 ORF, allowing these two ORFs to form a single 26 kDa protein if the stop codon was suppressed. Expression of these proteins would almost certainly require a subgenomic RNA due to the long 5′ leading sequence; however, the existence of this RNA has not been confirmed. The amino acid sequences of all three ORFs show no discernible relationship to other proteins; therefore, no putative functions can be assigned.

The RNA2 lengths in NωV, HaSV, and DpTV are 2445, 2478, and 2490 nt, respectively. Each has a long (~280 nt) 5′ lead sequence followed by two overlapping genes starting at the first two AUG codons. The first ORF encodes a 17 kDa protein (p17) and overlaps the larger second ORF that encodes the 70–71 kDa capsid protein precursor (**Figure 1**). The initiation codon for p17 is in a poor context, suggesting that it will be translated inefficiently by scanning ribosomes. However, *in vitro* translation studies of both NωV and HaSV RNA2's revealed both gene products were produced. The function of p17, if any, remains unclear, but preliminary experimental observations have supported a regulatory role in genomic RNA replication, and/or a function analogous to that of the movement proteins of plant viruses.

The close relationship between the ω-like viruses is seen in their capsid protein precursors (α). The amino acid lengths of the precursors are 644, 647, and 643 for NωV, HaSV, and DpTV, respectively, and they share

Table 2 Properties of tetravirus capsid proteins, genomic (g), and subgenomic (sg) RNAs[a]

	Beta (Da)	*Gamma (Da)*			*GenBank accession number(s)*
β-Like virus			*gRNA (bases)*	*sgRNA (bases)*	
NβV	58 448[b]	7975[b]	6625[b]	2656[b]	AF102884
PrV	60 649[b]	7343[b]	6400	2500	AF548354
TaV	58 327[b]	6781[b]	6500	2500	AF282930, AF062037
AeV			Present		
TnV	67 000–68 000		5865[c]		
DtV	62 000–66 000		Present		
PxV	62 400		Present		
DpV	66 000		5555[c]		
PiV	55 000		5865[c]		
ω-Like virus			*gRNA1 (bases)*	*gRNA2 (bases)*	
NωV	62 019[b]	7817[b]	5300	2445[b]	S43937[d]
HaSV	63 378[b]	7309[b]	5312[b]	2478[b]	U18246, L37299
DpTV	62 107[b]	7636[b]	5492[b]	2490[b]	AY594352, AY594353
Unassigned possible members			*gRNA or RNA1*	*sgRNA or RNA2*	
NεV	61 000				

[a]Blank fields represent undetermined or unavailable data.
[b]Data derived from sequence analysis.
[c]Converted from RNA molecular weight using average of 324 Da/nt.
[d]Sequence was amended in 2005 by GenBank accession numbers DQ054382 and DQ054403 to determine the full 5′ terminal sequence of RNA2.
Reproduced by permission of Elsevier.

Table 3 Percentage of sequence identity and similarity between the capsid proteins of six tetraviruses (three ω-like: HaSV, NωV, DpTV; three β-like: PrV, NβV, TaV). The similarity score is in parentheses

	NωV	*HaSV*	*DpTV*	*PrV*	*NβV*	*TaV*
NωV		66 (72)	87 (93)	37 (46)	29 (35)	27 (29)
HaSV	66 (72)		66 (78)	38 (47)	29 (36)	27 (33)
DpTV	87 (93)	66 (78)		37 (55)	25 (39)	26 (41)
PrV	37 (46)	38 (47)	37 (55)		27 (35)	28 (35)
NβV	29 (35)	29 (36)	25 (39)	27 (35)		35 (43)
TaV	27 (29)	27 (33)	26 (41)	28 (35)	35 (43)	

between 66% and 86% sequence identity (**Table 3**). One copy of the genomic (+)RNA1, one copy of the genomic (+)RNA2, and 240 copies of the expressed capsid protein precursor assemble to form an ~400 Å diameter icosahedral $T = 4$ virion (**Figure 2**). As in the β-like viruses, each copy of α undergoes an autocatalytic cleavage to produce a large protein (β, ~62–63 kDa) and a small protein (γ, ~7–8 kDa) after the capsid is assembled (**Table 2**), and both the β and γ polypeptides remain part of the virion after cleavage. Both genomic RNAs are likely to be replicated from separate (−)RNA templates based on larvae infected with NωV having separate dsRNAs corresponding to each of the genomic RNAs. RNAs 1 and 2 represent the complete genomic information required for virus growth based on studies of HaSV assembly and replication in plant and insect cells.

Providence Virus Replication in a Fat Body Cell Line

PrV is currently the only tetravirus shown to replicate in cell culture. The virus was discovered in 2003 as a persistent infection of a midgut cell line (MG8) derived from the corn earworm (*Helicoverpa zea*). In addition to the *H. zea* midgut, PrV was also shown to infect the *H. zea* fat body cell line (FB33) at low doses. A time course study of the PrV infection cycle was conducted using the FB33 cells. Input virus genomic and subgenomic (+)RNA is detected inside the cells at 6 h post infection (h.p.i.). Viral RNA synthesis is first detected at 18 h.p.i. and significantly increases between 18 and 24 h.p.i. At 36 h.p.i., total viral RNA accumulation reaches its maximum level, and (−)RNA also reaches its peak level, which is about 10-fold lower than that of corresponding (+)RNA.

The PrV β capsid protein (~60 kDa) appears at 6 h.p.i. along with the genomic and subgenomic RNAs. The β protein begins increasing in copy number after about 24 h.p.i., and after 36 h the uncleaved capsid protein precursor (α) also accumulates, slightly lagging behind the appearance of the subgenomic RNA that is thought to drive its translation. Although the α protein accumulated in infected cells, it is not detected in purified virions. In contrast, p13 translation was not detected during the time course experiment and it is not found in purified virions. However, proteolytic processing and degradation of this precursor could be extremely rapid as observed with the baculovirus-expressed TaV precursor.

To determine if assembled virions could be seen in infected cells, both MG8 and FB33 cells infected with PrV for 3 days were sectioned and examined by TEM. Both cell lines contained virus particles that were ~400 Å in diameter. The particles were seen only in the cytoplasm and often associated with one or more unidentified cytoplasmic vesicles that appear to be induced by the infection. In a few percent of the FB33 cells with very high virus accumulation, large crystalline particle arrays appeared within the cytoplamic structures.

Capsid Assembly

PrV particles are seen only in the cytoplasm of sectioned fat body cells at all time points measured; thus, it is highly probable they assemble in the cytoplasm and the cytoplasmic structures produced by the infection may aid in the process. In lieu of cell culture systems, assembly of VLPs has been achieved using the capsid proteins from NωV, HaSV, and TaV (the only β-like virus for which successful VLP production has been reported). The VLPs will assemble upon expression of only the capsid protein precursor in heterologous expression systems, namely yeast, plant protoplasts, and baculoviruses. Considerable yields of VLPs can be purified from the baculovirus expression systems and this has enabled a number of biophysical and biochemical studies of tetravirus capsids, particularly those of NωV and HaSV (see below). Like native virions, the VLP capsid protein subunits undergo autocatalytic cleavage from α to β and γ peptides, can specifically package viral RNA, and can bind specifically to a larval midgut cell receptor.

Capsid Structure and Dynamics

High-Resolution Capsid Structure

Both X-ray crystallography and cryoelectron microscopy image reconstruction (cryoEM) have been used to examine the different structural states of tetravirus capsids. The crystal structure of authentic NωV was determined in 1996 to 2.8 Å resolution, and is currently the only tetravirus crystal structure that has been published. Recently, the authors' laboratory has also determined the crystal structure of purified HaSV VLPs, and found it closely similar to that of NωV. The NωV crystal structure showed that the capsid is icosahedral, 420 Å in diameter, and composed of 240 capsid proteins arranged with $T = 4$ quasi-symmetry. Thus, the 60 icosahedral asymmetric units each have four subunits, named A–D, in unique chemical environments (**Figure 3**(**a**)). The 644 amino acid capsid subunit has three domains: an exterior immunoglobulin (Ig)-like fold, a central β-barrel in tangential orientation, and an interior helix bundle (**Figure 3**(**b**)). The helix bundle is created by the N- and C-termini of the polypeptide before and after the β-barrel fold, and the Ig-like domain is an insert between the E and F β-strands of the barrel. The exterior Ig domain is unique in nonenveloped viruses and is expected to be involved in cell binding and virus tropism, and has the least conserved sequence among tetravirus subunits. The interior helix domain is anticipated to interact with the RNA genome and may have a role in capsid polymorphism (see below). All 240 of the NωV capsid subunits have undergone autoproteolysis between residues N570 and F571 after assembly, leaving the cleaved portion of the C-terminus, called the γ peptide, associated with the capsid. The quasi-symmetry is controlled by a molecular switch (**Figures 3**(**a**) and **3**(**b**)). In NωV, a segment of the γ peptide (residues 608–641) is only ordered in the C and D subunits, and functions as a wedge between the ABC and DDD morphological units to prevent curvature and create a flat contact. The interface between two ABC units is bent partly due to the lack of ordered γ peptide. Importantly, the subunit structure and auto-proteolysis revealed a strong relationship between tetraviruses and the $T = 3$ insect nodaviruses. Indeed, the β-barrel folds and autocatalytic sites superimpose with little variation, and the γ peptide in the nodaviruses is also involved in quasi-equivalent switches.

The crystal structure of PrV, the first high-resolution structure of a β-like virus, was recently determined in the authors' laboratory (data not shown). The PrV subunit retains the three domains and a superimposable autoproteolysis site (all four subunits show a clear break at the cleavage site), but the Ig domain and the first and last ordered residues of the termini in the helical domain have different positions and structure compared to NωV. The different Ig domain position was first observed in the cryoelectron microscopy reconstruction of NβV as a distinctly different surface structure compared to the ω-like particle surfaces (**Figure 2**). The use of subunit termini in PrV is more like that seen in the nodaviruses. The quasi-symmetry switch in PrV has swapped elements compared to the omegatetraviruses, and represents a new and unique mixture of structural features among the insect viruses.

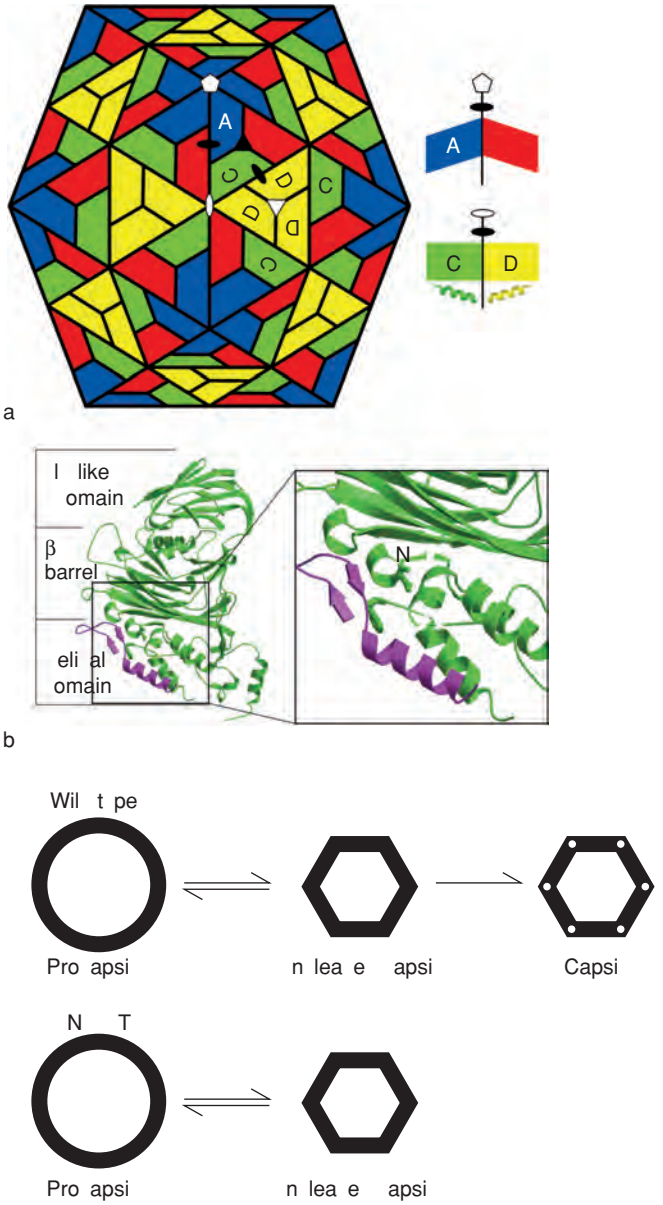


Figure 3 Structure and dynamics of the NωV capsid. (a) The subunit organization in the NωV $T = 4$ capsid crystal structure. There are four subunits in the asymmetric unit (A in blue, B in red, C in green, and D in yellow). Icosahedral symmetry axes are marked with white symbols, quasi-symmetry axes are marked with black symbols. Quasi-twofold dimer interfaces occur with the bent A–B or flat C–D conformations (right). The well-ordered C-terminal helices in the C–D subunits comprise a molecular switch that stabilizes the flat interface. (b) Ribbon diagram of the C subunit illustrates the three domain tertiary structure present in all 240 subunits (exterior of capsid on top, interior on bottom). Maturation cleavage occurs within the helical domain between residues Asn570 and Phe571. The molecular switch, present only in the C or D subunits, is highlighted in magenta. (c) In the wild-type virus, the procapsid converts to uncleaved capsid with the drop of pH, followed by a maturation cleavage, which locks the particle in capsid conformation after ~10% of the subunits are cleaved. In the cleavage-defective mutant (N570T), particle conformational change becomes reversible.

Capsid Maturation, Auto-Proteolysis, and Large-Scale Transitions

NωV and HaSV coat proteins expressed in the baculovirus system assemble into round and porous intermediate VLPs that are 480–490 Å in diameter, called procapsids, when purified at neutral pH (pH 7.6). The procapsid coat proteins remain in the uncleaved α precursor form until a reduction in pH. When exposed to acidic conditions (pH 5.0), procapsids underwent a large-scale structural rearrangement (maturation) to the

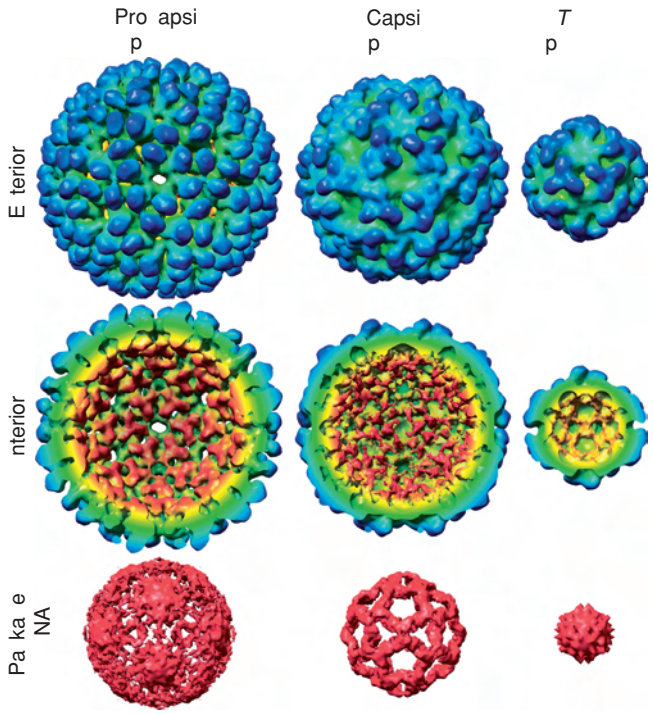


Figure 4 CryoEM reconstructions between 23 and 27 Å resolution of three HaSV polymorphic capsid forms. The polymorphic form and its associated pH are given at the top. Three views of each form are shown with radial coloring from red on the interior to blue on the exterior. The procapsid and capsid diameters are similar to those of NωV (490 and 430 Å, respectively). Note the well-ordered dodecahedral RNA structure in the mature capsid, which has been observed in several insect viruses. The $T = 1$ form has so far been found only in HaSV purifications as an extra peak in the sucrose gradients, and only appears after the acid maturation step (i.e., not found at pH 7.6). The diameter of the $T = 1$ particles is 260Å and the protein shell is composed only of a truncated version of the β subunits (55 kDa).

smaller, angular mature capsid, where α begins to cleave into the β and γ polypeptides (**Figures 3(c)** and **4**). Small-angle X-ray scattering (SAXS) demonstrated that the pH-induced conformational change occurs in less than 100 ms in NωV, while the autocatalytic cleavage event had a half-life of hours. The relationship of the conformational change and cleavage was also examined using SAXS, which suggested that the conformational change was initially reversible until approximately 10% of the subunits had cleaved, and then the particles were 'locked' in the capsid conformation (**Figure 3(c)**). Finer pH titrations of NωV revealed intermediate capsid states at pH 5.8 and 5.5 (data not shown). Presumably similar intermediates also exist for HaSV.

Both ANS (1-anilino-8-naphthalene sulfonate) and the thiol-reactive fluorophore, maleimide-ANS (MIANS), have been bound to procapsids to further characterize the conformational transition to capsid. Absorption of the covalently attached MIANS (forms covalent thiol-ether linkage with cysteines) situated at subunit interfaces can report on environment changes as it is buried during maturation. Unexpectedly, acidification of the labeled particles trapped them at an intermediate stage of maturation and the autocatalytic cleavage was inhibited. The labeled VLPs showed no cleavage after 4 h of incubation at pH 5.0 buffer while wild-type particles were fully cleaved. A cryoEM image reconstruction of the MIANS-labeled VLPs at pH 5.0 revealed the particles are 440 Å in diameter, having only progressed about halfway along the trajectory between procapsid and capsid. This demonstrates that cleavage depends on the conformational change and is not triggered by just lowering the pH, and provides support for the strategy of targeting subunit interfaces with antiviral compounds as a means of disrupting the viral life cycle.

Cleave Site Mutation Allows Reversible Transition and Procapsid Structure Analysis

Mutagenesis studies have shown the asparagine at the scissile bond (N570) in NωV is required for cleavage. Substituting threonine for asparagine (N570T) results in assembly of less stable particles, but they do not cleave and continue to undergo the conformational change reversibly as a function of pH (**Figure 3(c)**). CryoEM structures at 8 Å resolution of cleaved and N570T VLPs showed the role of cleavage in stabilizing the capsid form. There are more extensive inter-subunit contacts between the C-termini and the neighboring N-termini in cleaved

particles that results in more ordered C-terminal helices, which accounts for the added stability.

The N570T mutant was also used to study the structure of the uncleaved procapsid state. An 11 Å resolution cryoEM structure of N570T procapsids displays a morphology indistinguishable from procapsids formed by wild-type subunits. The higher resolution showed, at pH 7.6, that portions of the C-terminal regions of the γ polypeptides of the A and B subunits, which are not ordered in the capsid form, are ordered and contribute to a second molecular 'wedge' that prevents hinging between the A and B subunits. It makes the quasi-twofold interactions at the A–B and C–D interfaces closely similar in the procapsid, producing its round morphology and consistent with its role as an assembly intermediate during NωV particle maturation.

Pathobiology

Host Range

Lepidopteran insects are the only known hosts for tetraviruses. No replication in other animals has been detected. The hosts are confined largely to the Noctuidae, Saturniidae, and Limacodidae families within the Lepidoptera order. The range of species that they are able to infect differs between the viruses. HaSV appears unable to infect species outside the Heliothinae subfamily, while TnV and DpV are able to infect insects outside the family of their nominal host.

Transmission and Symptoms

Horizontal transmission via ingestion by larvae has been demonstrated for several tetraviruses with a widely varying range of symptoms. Only slight growth retardation is observed using high doses of TnV, but NβV infection of larvae is lethal, causing cessation of feeding, and moribund, discolored, and flaccid appearance at 7–9 days post infection. A dependence upon the larval life stage has also been seen. HaSV is highly active against neonate larvae in the first three instars of development, needing as little as 5000 particles to cause them to stunt and cease feeding within 24 h and to die within 4 days. In contrast, no detectable symptoms occur in later larval development, signifying a high degree of developmental resistance. It has not been demonstrated whether adults or pupae are capable of being infected by tetraviruses. Vertical transmission of tetraviruses is also believed to occur, but the evidence remains undefinitive.

Histopathology and Tropism

Natural infection by tetraviruses appears to be exclusively in the larval midgut, although PrV has been cultured in fat body cells. In a definitive experiment, Northern blots of RNA extracted from HaSV-infected larvae showed viral RNA only in the midgut tissue, even after virus was injected into the hemocoel.

In a quantitative study of HaSV infection of its host, the African or cotton bollworm, supplying the virus to larvae *per os* during the first three instars of development resulted in an infection restricted to three of the four midgut cell types. The virus initiated infection in closely situated foci that expanded and converged with others over time. In response, the midgut cells increased their rate of sloughing and apoptosis to an extent that incapacitated the midgut due to loss of all but a few cells. Infection of older larvae results in only sparsely situated foci, which fail to expand and eventually disappear presumably due to cell sloughing. These data indicate that cell sloughing is an immune response existing throughout development, but older larvae have an additional mechanism for increased resistance to infection.

Biotechnology

Application as Biopesticides

Small RNA viruses offer attractive properties as biological control agents for pests: high specificity, high efficacy, and potentially self-replicating. A notable drawback is the difficulty in producing large quantities of infectious virions, but the simplicity of the tetraviral genomes makes production of virions in nonhost cells feasible. Agrobacterium-mediated transgenesis has been used to place the genes required for HaSV assembly into tobacco plants, which resulted in stunted, HaSV-infected larvae when they fed on the modified plants. Expanding on this result, Hanzlik and colleagues conducted the first field trial of HaSV as a control agent in 2005 on a sorghum crop in Queensland, Australia. The semipurified HaSV preparation reduced the larval population by 50% at both 3 and 6 days post application (dpa), which was equivalent to a commercial baculovirus preparation. Notably, the amount of HaSV on the sorghum heads increased at 5.5 dpa, probably reflecting dispersal of newly produced virus from cadavers and frass. These results indicate that HaSV could be used effectively as a biopesticide.

Application as Chemical Platforms

A study in 2006 demonstrated that the Ig domains of NωV particles could be modified to possibly alter its tropism and/or to carry specific chemical tags. A $(His)_6$ tag was inserted into the GH loop (between A378 and G379) of a capsid precursor construct that was then expressed in a baculovirus system. The His-tagged p70 self-assembled into VLPs that had similar morphological and RNA encapsidation properties as wild-type VLPs. Two assays

using paramagnetic nickel beads confirmed that multiple affinity tags were present on the surface of the modified VLPs and could bind the beads. Thus, the GH loop of NωV appears to be a suitable modification site for further biotechnology applications.

See also: Cryo-Electron Microscopy; Nodaviruses; Virus Particle Structure: Nonenveloped Viruses; Virus Particle Structure: Principles.

Further Reading

Canady MA, Tsuruta H, and Johnson JE (2001) Analysis of rapid, large-scale protein quaternary structural changes: Time resolved X-ray solution scattering of nudaurelia capensis ω virus. *Journal of Molecular Biology* 311: 803–814.

Christian PD, Murray D, Powell R, Hopkinson J, Gibb NN, and Hanzlik TN (2005) Effective control of a field population of *Helicoverpa armigera* by using a small RNA virus Helicoverpa armigera stunt virus (*Tetraviridae, Omegatetravirus*). *Journal of Economic Entomology* 98: 1839–1847.

Gorbalenya AE, Pringle FM, Zeddam J-L, *et al.* (2002) The palm subdomain-based active site is internally permuted in viral RNA-dependent RNA polymerases of an ancient lineage. *Journal of Molecular Biology* 324: 47–62.

Hanzlik TN and Gordon KHJ (1997) The *Tetraviridae*. *Advances in Virus Research* 48: 101–168.

Helgstrand C, Munshi S, Johnson JE, and Liljas L (2004) The refined structure of nudaurelia capensis ω virus reveals control elements for a *T = 4* capsid maturation. *Virology* 318: 192–203.

Johnson JE and Reddy V (1998) Structural studies of nodaviruses and tetraviruses. In: Miller LK and Ball LA (eds.) *The Insect Viruses*, pp. 171–223. New York: Plenum.

Lee KL, Tang J, Taylor D, Bothner B, and Johnson JE (2004) Small compounds targeted to subunit interfaces arrest maturation in a nonenveloped, icosahedral animal virus. *Journal of Virology* 78: 7208–7216.

Maree HJ, Van der Walt E, Tiedt FA, Hanzlik TN, and Appel M (2006) Surface display of an internal His-tag on virus-like particles of nudaurelia capensis omega virus (NωV) produced in a baculovirus expression system. *Journal of Virological Methods* 136: 283–288.

Pringle FM, Johnson KN, Goodmann CL, McIntosh AH, and Ball LA (2003) Providence virus: A new member of the *Tetraviridae* that infects cultured insect cells. *Virology* 306: 359–370.

Taylor DJ, Krishna NK, Canady MA, Schneemann A, and Johnson JE (2002) Large-scale, pH-dependent, quaternary structure changes in an RNA virus capsid are reversible in the absence of subunit autoproteolysis. *Journal of Virology* 76: 9972–9980.

Theiler's Virus

H L Lipton and S Hertzler, University of Illinois at Chicago, Chicago, IL, USA
N Knowles, Institute for Animal Health, Pirbright, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cardiovirus Genus in the family *Picornaviridae*.
Demyelination Pathological process of damage to the proteolipid coat around nerve fibers (axons).
L* Virus-encoded protein that is translated in alternative reading frame to that of the polyprotein.
Theilovirus Species within the genus *Cardiovirus*.
Theiler's murine encephalomyelitis viruses (TMEVs) Abbreviation for mouse Theiler's viruses.

History, Geographic Distribution, and Host Range

The mouse encephalomyelitis viruses are enteric pathogens of mice. Discovered by Max Theiler in the early 1930s and originally called murine polioviruses, these agents are commonly referred to as Theiler's murine encephalomyelitis viruses (TMEVs). Theiler initially recovered isolates from mice with spontaneous paralysis housed at the time in the animal colony at the Rockefeller Institute; subsequently, TMEVs were found in virtually all nonbarrier mouse colonies, where they caused asymptomatic intestinal infections. While TMEVs are widely distributed in the world, their host range is narrow, including mice and rats. Serological evidence indicates that *Mus musculus*, the feral house mouse, is the natural host, but several other species of voles and possibly rats may also serve as hosts. As is the case for other picornaviruses, following peripheral routes of infection, TMEV spreads to the central nervous system (CNS) producing encephalitis or poliomyelitis (spontaneous paralysis). In the older literature, the incidence of spontaneous paralysis was reported to be approximately one paralyzed animal per 1000–5000 colony-bred mice. Since TMEVs may go undetected unless appropriate serological tests are performed, these agents are a potential hazard for investigators using mice in biomedical research.

In recent years, this group of viruses has assumed additional importance, because TMEV infection in mice provides one of the few available experimental animal models for multiple sclerosis. TMEV-induced demyelinating

disease in mice is a relevant model for multiple sclerosis because: (1) chronic pathological involvement is virtually limited to the CNS white matter; (2) myelin breakdown is accompanied by mononuclear cell inflammation; (3) demyelination results in clinical disease, for example, spasticity, from involvement of upper motor neuron pathways; (4) myelin breakdown is in part immune-mediated; and (5) the disease is under multigenic control with a strong linkage to the major histocompatibility complex (MHC) gene H2D.

Classification and Serologic Relationships

Based on the complete nucleotide sequence and genome organization, TMEVs have been classified in the genus *Cardiovirus* of the family *Picornaviridae* along with encephalomyocarditis virus (EMCV). EMCV and TMEV (or Theilovirus) constitute separate species within the genus *Cardiovirus*. Polyclonal antisera show no cross-neutralization between TMEV and EMCV; however, since the coat proteins of the two *Cardiovirus* species share ~50% identity of their amino acid sequences, cross-reactions are observed with antibody binding assays such as enzyme-linked immunosorbent assay (ELISA) and complement fixation. Phylogenetic analysis of the complete P1 capsid-coding region has revealed three major clades: TMEV, Vilyuisk human encephalomyelitis virus (VHEV), and the Theiler's-like virus (TLV) of rats (**Figure 1**). These three clades presumably represent three distinct serotypes of the species *Theilovirus*; however, comprehensive serological studies remain to be performed.

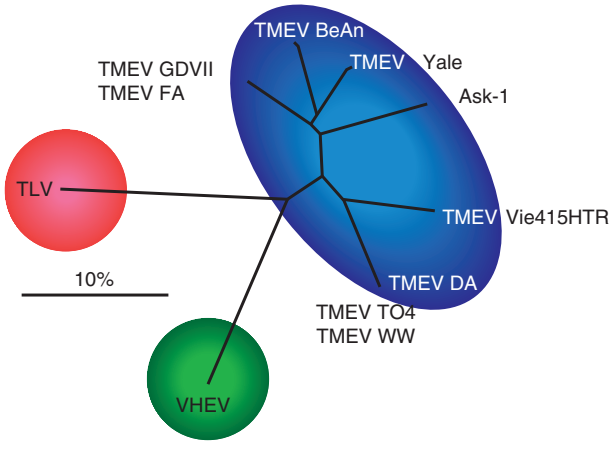


Figure 1 Phylogenetic analysis of the P1 capsid-coding region of the Theiloviruses showing three clades, presumably representing three distinct serogroups: TMEV, Vilyuisk human encephalomyelitis virus (VHEV), and Theiler's-like virus of rats (TLV).

Physical Properties

Because the TMEVs do not have an envelope they are insensitive to chloroform, ether, nonionic detergents, such as deoxycholate, NP40 and Tween-80, and the ionic detergent sodium dodecyl sulfate, but are inactivated by 0.3% formaldehyde and HCl 0.1 mol l^{-1}. TMEVs are rapidly destroyed at temperatures over 50 °C and lose some infectivity upon lyophilization. Purified virions can be stored for long periods of time at −70 °C without loss of infectivity, but slowly lose infectivity on storage at −20 °C. Enteroviruses require stability at low pH to pass through the acidic conditions of the stomach. TMEVs are stable over the entire pH range from 3 to 9.5, but in contrast to the other cardioviruses, such as EMCV, they are not highly thermolabile in the presence of 0.1 mol l^{-1} chloride or bromide in the pH range 5–7.

Virion Structure

Picornavirions have a relative molecular mass of ~8.5 × 10^6 Da, of which 30% is RNA and 70% protein. Theiler's virions are 30 nm in diameter and their structure reveals only a spherical virion by negative staining (**Figure 2(a)**). Virions have a sedimentation coefficient of 150 S by velocity centrifugation in sucrose and a buoyant density of 1.34 g ml^{-1} by isopycnic centrifugation in cesium salts.

The three-dimensional structures of the GDVII, BeAn, and DA strains have been determined at ~3 Å resolution by X-ray crystallography (**Figures 2(b)** and **2(c)**). The overall architecture is quite similar to that of other picornaviruses and closely resembles that of Mengo virus, another member of the EMCV species. Each virion is composed of 12 pentamers, with each pentamer made up of five protomers, each containing a single copy of the four capsid proteins. Each of the three major capsid proteins VP1, VP2, and VP3 consist of a wedge-shaped eight-stranded antiparallel β-barrel. The N-termini of the capsid proteins form an extensive, intertwined network on the inner surface of the protein shell. The loops connecting the β-strands form the outer surface features of the protein shell and provide surface differences with Mengo virus. A 25 Å depression on the virion surface at the junction between VP1 and VP2 along the twofold axis, termed the pit, is believed to be the viral receptor and is a broad depression. The TMEV surface structures are differentiated from that of Mengo virus in having: (1) a larger VP1 CD double loop, with loop I containing an extra five residues, that is shifted more toward the VP2 EF puff at the twofold axis, while loop II is directed more toward the fivefold axis; (2) an 11-residue insertion in the VP2 EF puff forming a double loop in which the inserted loop interacts with

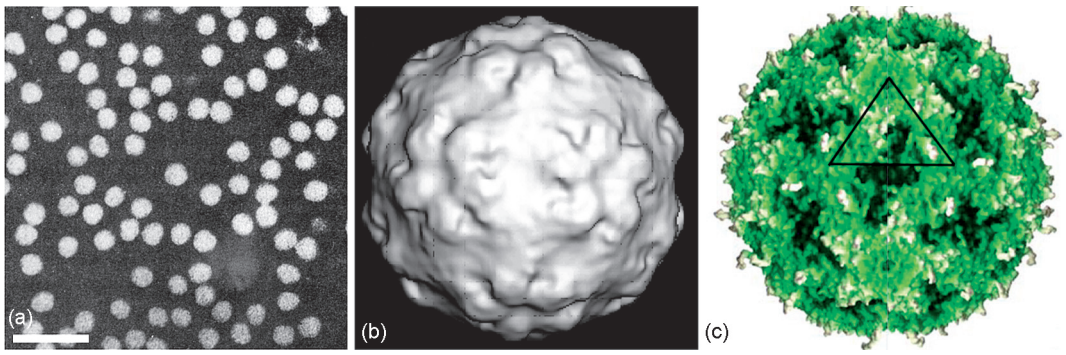


Figure 2 Theiler's virions. (a) Negative staining of purified BeAn virus particles showing the morphology of featureless virus profiles due to the impermeability of the capsid to the electron dense stain. Scale = 100 μm. (b) Cyroelectron micrograph of a GDVII virion at 20 Å resolution showing the star-shaped profile of the plateau around the fivefold axes. (c) GRASP image of a BeAn virion showing a topographical relief map of the VP1 and VP2 surface loops in white. The superimposed triangle represents a protomer with its apex pointing at the fivefold axis. The darkened area within the outlined iscosahedral unit is a surface depression of the pit or receptor binding site. This depression extends across the twofold axis.

the VP1 FMDV GH loop; and (3) the tip of the VP3 knob (a loop inserted in βE) points directly outward on the rim of the pit.

Properties of the Genome

The genetic component of the TMEV virion is a single-stranded, positive-polarity RNA molecule 8100 nt in size that has a sedimentation coefficient of 35 S. The virion RNA has a virally encoded 20 amino acid protein, VPg, covalently linked to the 5′ uridine, and a poly(A) tract on the 3′ end. The complete genomes of the GDVII, DA, BeAn strains, as well as TLV have been sequenced. With the notable absence of a poly(C) tract in the 5′ untranslated region, the organization and sequence of the TMEV genome is remarkably similar to that of EMCV. The polyprotein of the prototypic BeAn strain initiates at the AUG codon at nucleotide 1065 and extends for 6909 nt (or 2303 codons), ending at the single UGA termination triplet at base 7972 (**Figure 3**). The polyprotein-coding region is flanked by 5′ and 3′ untranslated sequences of 1064 and 125 nt, respectively. In BeAn the 5′ untranslated region contains a stretch of 11 pyrimidines interrupted by a single purine before the AUG at nucleotide 1065. In picornaviruses, the 5′ untranslated region mediates cap-independent translation and serves as an internal ribosome entry site (IRES). The TMEV 5′ untranslated sequences have been predicted to form stable secondary structures, which in the 500 nt upstream of the authentic AUG (at 1065) are nearly identical to those predicted for EMCV and foot-and-mouth disease virus. In BeAn, eight AUGs precede the initiator AUG, but none of them has an optimum Kozak context sequence. Hence, it could be argued that selection of the authentic initiator AUG after binding of ribosomes to TMEV RNA does not involve internal ribosome binding. However, BeAn nucleotides ~500–1065 determine a structure that serves as an IRES in bicistronic mRNAs both *in vitro* (BHK-21 cells). A poly(A) tail of indeterminate length is present on the 3′ end of the viral genome.

Translation: Polyprotein Processing and Final Gene Products

The 12 TMEV gene products are the result of post-translational processing of a 2303 amino acid polyprotein (mol. weight of 256 kDa) (**Figure 3**). The polyprotein of GDVII virus contains no insertions or deletions; however, two VP1 amino acids are deleted in DA, TO4, and WW viruses. The processing scheme follows a standard L-4-3-4 picornavirus polypeptide arrangement, that is, the leader protein (L), four capsid proteins in part one (P1) of the genome, three proteins in P2, and four proteins in P3 (**Figure 3**). The coding limits of individual viral proteins have been predicted by analogy with those of EMCV since the only confirmation to date of a deduced sequence is that of the N terminus of 1D. The eight amino acids flanking the putative cleavage sites are highly conserved for the two viruses. All of the cleavage sites in the polyprotein except for two, 1A/1B and 2A/2B, are processed by the viral protease 3C. The TMEV 3C protease therefore processes Q-C, as well as Q-S and Q-A, dipeptides and, in addition, the E-N dipeptide at the 1D/2A cleavage. However, only 6 of 8 Q-G, 2 of 13 Q-S, and 1 of 7 Q-A dipeptides in the polyprotein are cleaved by 3C, indicating that involvement of secondary, tertiary, or both types of structure is also important for recognition of these particular dipeptides. The tetrapeptide NP/GP at 2A/2B which is the primary cleavage site in the cardiovirus polyprotein is cleaved autocatalytically, as it is in EMCV.

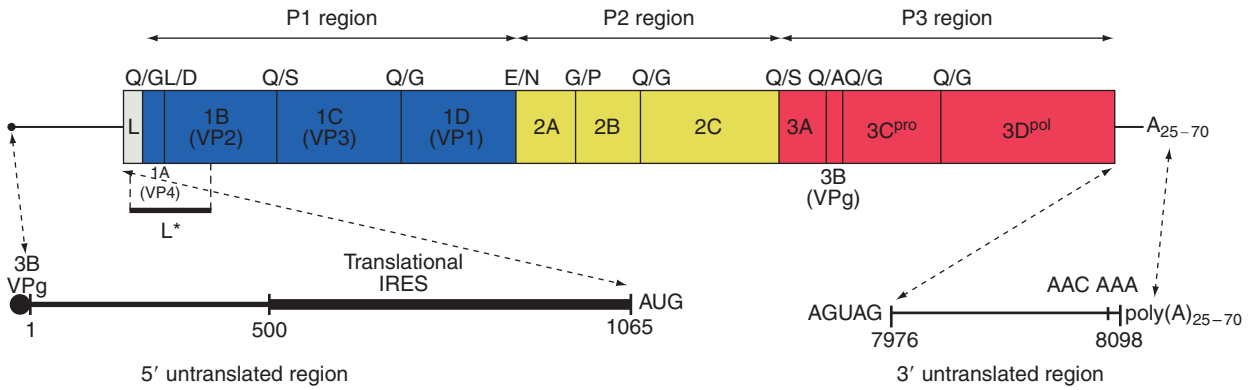


Figure 3 TMEV 8 kbp genome showing 5′ and 3′ untranslated regions (enlarged) and the polyprotein which is superimposed over the 7 kbp nucleotide open reading frame (ORF) and contains the final 12 viral gene products. The 5′ end of the viral RNA is not capped but has protein 3B (VPg) covalently attached to the first nucleotide. A ~500 base internal ribosome entry site (IRES) is present in the 5′ untranslated region immediately upstream of the authentic AUG initiation codon for the polyprotein. The 3′ untranslated region ends in a poly (A) tail. The ORF is divided into L, P1 (blue) encoding the four capsid proteins, P2 (yellow) three nonstructural proteins, and P3 (red) four nonstructural proteins, including the 3C cysteine protease which makes all but two of the cleavages in the polyprotein, and the 3D, the RNA-dependent RNA polymerase. Cleavage dipeptides are shown above viral protein boundaries. Finally, the location of the L* protein that is translated out-of-frame is shown near the N-terminus of the polyprotein.

Table 1 Functional activities of the TMEV nonstructural proteins

Nonstructural protein	*Activities*
Leader (L)	Binds to Ran, inhibiting import and export of proteins to and from the nucleus, including those involved in the innate interferon response
L*	Out-of-frame 19-kDa protein initiated by AUG or ACG codons immediately downstream of the authentic AUG for polyprotein translation; has a putative role in TMEV persistence
2A	A C-terminal tetrapeptide NP/GP responsible for autocatalytic cleavage of 2A/2B, the primary cleavage in the cardiovirus polyprotein
2B	Possible role in cell membrane permeability
2C	A multifunctional protein involved in viral RNA replication, including ATPase, GTPase, membrane binding, and RNA binding activities
3A	Inhibition of ER-to-Golgi transport that results in reduction of host cell cytokine secretion
3B	Covalently linked to the 5′-terminal uridine of the single-stranded RNA and primer for the viral RNA-dependent RNA polymerase
3C	Cysteine-reactive protease responsible for all of the monomolecular and bimolecular cleavages in the polyprotein except for the assembly-dependent maturation cleavage of VP0 (1A/1B) and autocatalytic cleavage at 2A/2B
3D	RNA-dependent RNA polymerase responsible for viral RNA replication

Cleavage of the polyprotein gives rise to three primary products, the first of which (116 kDa) contains the leader protein (8 kDa), the P1 capsid proteins, and the first P2 protein 2A (15 kDa). Thus, the initial precursor released from the polyprotein is like that of the other cardioviruses but differs from that of other groups of picornaviruses. The capsid proteins are arranged in the following order: 1A (VP4; 7 kDa), 1B (VP2; 29 kDa), 1C (VP3; 25 kDa), and 1D (VP1; 30 kDa). The second processing precursor (2BC) is 51 kDa and gives rise to 2B (14 kDa) and 2C (37 kDa). The third or C-terminal precursor protein is 88 kDa and is processed into the four mature proteins 3A (10 kDa), 3B (2 kDa), 3C (24 kDa), and 3D (52 kDa). The functions of the nonstructural proteins are shown in **Table 1**. Another protein, termed L*, translated out-of-frame, is initiated 22 nt downstream of the authentic AUG in the polyprotein, giving rise to an 18 kDa protein that was originally shown to be involved in the TMEV persistent infection.

Viral RNA Replication

Cardiovirus replication is similar to that of other picornaviruses. Following entry, VPg is removed from the 5′ end of the RNA, and the RNA is directly translated using

cellular factors. The viral genomic RNA (plus-sense), is then transcribed into negative-strand copies, and each one is used as template for reiterative synthesis of ~25–50 plus-sense RNAs identical to the genome of the virus. An RNA structure located within the VP2 sequence of TMEV is necessary for RNA replication, and is referred to as the *cis*-acting replicative element (CRE). The CRE functions as a template for uridylylation of viral protein 3B (VPg) forming $VPgpUpU_{OH}$ which primes positive-strand RNA synthesis. Viral protein 3D is the RNA-dependent RNA polymerase which catalyzes both of these processes.

Viral Genomic Determinants in Pathogenesis

The existence of two naturally occurring TMEV neurovirulence groups provides a useful system for investigating molecular pathogenesis. Moreover, the difference in neurovirulence in terms of mean 50% lethal dose between high and low neurovirulence strains is of the order of 10^5 plaque forming units. With the advent of reverse genetics the generation of full-length cDNA clones of high neurovirulence GDVII and low virulence DA and BeAn viruses was feasible. Viral RNAs transcribed from cDNAs are infectious upon transfection of mammalian cells. To identify pathogenetic determinants, for example, those of neurovirulence and persistence, recombinant viruses between parental cDNAs have been assembled and point mutations introduced into wild-type strains for analysis in cell culture and mice.

Neurovirulence and persistence have been mapped to the L-P1 regions encoding the L and L* proteins and the capsid proteins. The results suggest that the mechanism for the pathogenetic properties of neurovirulence and persistence involve several determinants, including an exclusive role for the capsid in neurovirulence and the out-of-frame L* protein and capsid (exterior surface of the virion) in persistence. The latter observation suggested that receptor-mediated events may be involved in persistence; it was demonstrated that persistence required the ability of low neurovirulence strains to bind to its sialic acid co-receptor. The L* protein was initially recognized because only the low neurovirulence strains have an out-of-frame AUG start codon. However, the ACG codon in high neurovirulence strains was then shown to initiate L* translation, and chimeric TMEVs that contain a high neurovirulence L protein in a low neurovirulence background were shown to persist in the mouse CNS as well as the low neurovirulence parental virus. The role of the L* protein in persistence will require further study.

Transmission and Tissue Tropism

TMEVs are transmitted by the fecal–oral route and are separable into two biological groups based on neurovirulence (**Table 2**). The first group, consisting of three isolates, GDVII, FA, and ASK-I, possesses high neurovirulence, causing a rapidly fatal encephalitis in mice. Some 10–15 isolates, including viruses recovered from the CNS of spontaneously paralyzed mice and feces of asymptomatic mice, form a second, low neurovirulence group. Experimentally, the low neurovirulence strains produce poliomyelitis (early disease) followed by demyelinating disease (late disease). When mice are inoculated intracerebrally with cell culture-adapted low neurovirulence strains, the poliomyelitis phase is attenuated (subclinical); whereas, brain-derived stocks of these viruses produce both disease phases.

Pathogenesis

Very little information is available about the pathogenesis of TMEV infection following peripheral routes of infection, including the oral route. In general, isolates from either of the neurovirulence groups do not readily produce CNS disease following peripheral routes of inoculation, with the exception of TO(B15), a mutant selected for its invasiveness from the intestinal tract. When mice are inoculated intracerebrally with the high neurovirulence strains, virus replicates widely in the brain and spinal cord, causing encephalitis or encephalomyelitis. Thus,

Table 2 Two TMEV neurovirulence groups

Characteristic	*High neurovirulence*	*Low neurovirulence*
Isolates (strains)	GDVII, FA, ASK-1	DA, BeAn 8386, Yale, WW, TO4, TO(B15), Vie415HTR
Disease	Encephalomyelitis	Poliomyelitis/demyelination
Incubation period	1–10 days	7–20 days/>30 days
CNS target cell	Cortical and motor neurons	Motor neurons/macrophages and oligodendrocytes
LD_{50}	1–10 PFU	$>10^5$ PFU
Persistent infection	No	Yes
Temperature sensitive	No	Yes
Carbohydrate co-receptor	Heparan sulfate	α2,3-linked sialic acid
Differences in virion structure	Only in the Cα chain of the VP2 puff B	

neurons as well as glial cells (astrocytes and oligodendrocytes) become infected in the cerebral cortex, hippocampus, basal ganglia, thalamus, brainstem, and spinal cord. Affected mice develop hunched posture and hind-limb paralysis. A rapid demise is the result of widespread cytolytic infection. The following sections focus on the pathogenesis of the biphasic disease produced by the low neurovirulence strains, which provides an experimental model system for human demyelinating disease, multiple sclerosis.

Clinical Features of Infection

The intracerebral route of inoculation maximizes the incidence of neurological disease. Following intracerebral inoculation, the low neurovirulence strains produce a distinct biphasic CNS process in susceptible strains of mice, which is characterized by poliomyelitis during the first 2 weeks post infection, followed by chronic, inflammatory demyelination that begins during the second or third week post infection and becomes clinically manifest between 1 and 3 months post infection. Some investigators who inoculated lower amounts of virus observed an even later onset of demyelinating disease. Mice with poliomyelitis develop flaccid paralysis, usually of the hind limbs; only one limb may be affected or paralysis may involve both hind limbs and spread to involve the forelimbs, occasionally leading to death. In contrast to the fatal outcome of paralysis produced by the Lansing strain of human poliovirus type 2, complete recovery from TMEV-induced poliomyelitis is usually seen. Residual limb deformities may be seen as the result of extensive anterior horn cell infection and severe paralysis (early disease).

Gait spasticity is the clinical hallmark of the demyelinating (late) disease. Late disease is first manifest by slightly unkempt fur and decreased activity, followed by an unstable, waddling gait. Subsequently, generalized tremulousness and ataxia develop, and the waddling gait evolves into overt paralysis. Incontinence of urine and priapism are seen. As the disease advances, prolonged extensor spasms of the limbs occur spontaneously followed by difficulty in righting. Extensor spasms can be induced by turning an animal over on its back by quickly rotating the tail. Weight loss occurs during late disease. The clinical manifestations of late disease are progressive and lead to an animal's demise in several to many months post infection. A functional motor assay using a rotorod apparatus has been used to quantitate motor function in this disease.

Pathogenesis and Histopathology

Motor neurons in the brainstem and spinal cord are the main targets of infection during poliomyelitis, but sensory neurons and astrocytes are also infected. TMEVs do not replicate in endothelial and ependymal cells. A brisk microglial reaction is elicited, with the appearance of numerous microglial nodules, particularly in the anterior gray matter of the spinal cord. Examples of neuronophagia are quite frequent at this time, but little lymphocytic response is seen. The poliomyelitis phase lasts 1–4 weeks, after which time little residual gray matter involvement is inapparent other than for astrocytosis.

As early as 2 weeks post infection, inflammation of the spinal leptomeninges begins to appear, followed by involvement of the white matter. Initially, the inflammatory infiltrates are almost exclusively composed of lymphocytes, but at later times plasma cells and macrophages become numerous (**Figure 4**(**a**)). The influx of macrophages is in close temporal and anatomic relationship with myelin breakdown. Both light microscopic and ultrastructural studies show that myelin breakdown is related to the presence of macrophages (**Figure 4**), which either actively strip myelin lamellae from otherwise normal-appearing axons or are found in contact with myelin sheaths undergoing vesicular disruption. Foci of inflammation and myelin destruction extend from the perivascular spaces into the surrounding white matter, leading to sharply demarcated plaques of demyelination (**Figure 4**(**b**)). The ultrastructure studies during the initial phase of myelin breakdown have not shown alterations in oligodendroglia, which are in close apposition with naked but otherwise normal axons; however, oligodendrocytopathology has been observed later although it appears to be a minor contributor to demyelination.

Sites of TMEV Persistence

TMEV persistence clearly involves active virus replication, since infectious virus can be readily isolated from the CNS of infected mice and high viral genome copy numbers are present. *In situ* hybridization has revealed two populations of CNS cells differing in the number of viral genomes. Virus replication in the majority of these cells ($>90\%$) appears to be restricted, as they contain <500 viral genomes. A small percentage of CNS cells contain >1500 genomes, possibly as many as 10^4–10^5, and are probably productively infected. The absolute number of viral genomes as determined by *in situ* hybridization is probably only an approximation. Restricted virus production has been demonstrated in macrophages isolated from the CNS of diseased mice; therefore, macrophages appear to be the primary target for persisting virus. It is also possible that some of the cells with restricted infection are astrocytes. The kinetics of virus replication in the CNS cells with restricted infection remains to be elucidated – the length of the replicative cycle and whether the cells are lysed or continue to produce infectious virus for longer times is not known. TMEV infection *in vitro*

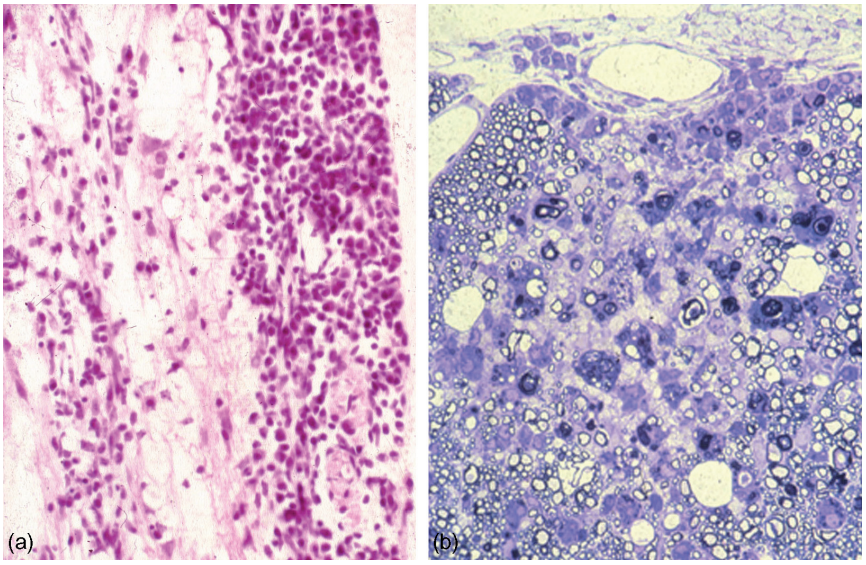


Figure 4 Histopathology of TMEV-induced demyelinating disease (late disease) in an SJL mouse. (a) H&E-stained longitudinal section (6 μm) of the spinal cord showing intense mononuclear inflammatory cell infiltrates in the leptomeninges (right) and parenchyma where many vacuolated macrophages are observed. (b) Toluidine blue-stained, Epon-embedded coronal section (1 μm) of a spinal cord showing a discrete plaque-like area of demyelination (many naked axons present) and lipid-laden macrophages at the cord margin and surrounded by normal myelinated axons. Magnification ×400 (a); ×300 (b).

only occurs in monocytes once they have differentiated into macrophages. Virus production in infected macrophage cell lines is restricted but viral translation, polyprotein processing, and assembly of virion intermediates (protomers and pentamers) as well as virions appear to be normal. Ultimately infected macrophages undergo apoptosis, which is caspase-9 and -3 dependent, consistent with activation of the mitochondrial pathway. In contrast, oligodendrocytes appear to be productively infected, since ultrastructural analysis has shown crystalline arrays of virions in oligodendrocytes in demyelinating lesions in mice. Oligodendrocytes may correspond to the CNS cells containing large numbers of viral genomes by *in situ* hybridization. These data suggest that a cytolytic infection of oligodendrocytes contributes to demyelination along with immune-mediated mechanisms of myelin damage.

Immune Response

During the first week, TMEV-infected mice mount a virus-specific humoral immune response that reaches a peak by 1–2 months post infection and is sustained for the life of the host. Neutralizing and other virus-specific antibodies have been measured. The majority of the antiviral IgG response in persistently infected, susceptible mice is IgG2a subclass, with little antiviral IgM detected by day 14 post infection, whereas IgG1 antiviral antibodies appear to predominate in resistant as well as in immunized mice. Murine CD4+ T cells of the Th1 subset mediate delayed-type hypersensitivity (DTH) and regulate IgG2a production via interferon-γ production, whereas CD4+ Th2 cells regulate IgG1 and IgE production via interleukin 4. Thus, the predominant IgG2a antiviral response in susceptible mice may be an *in vivo* measure of preferential stimulation of a Th1-like pattern of cytokine synthesis. Virus-specific CD8+ cytolytic T-cell responses have also been shown to help in virus clearance during the acute phase of the infection, and may be responsible for the resistance of C57BL/6 mice to the demyelinating disease.

Infected, susceptible strains of mice also produce substantial levels of virus-specific CD4+ T-cell responses. T-cell proliferation and DTH appear by 2 weeks post infection and remain elevated for at least 6 months. Both DTH and T-cell proliferation have been shown to be specific for TMEV and mediated by CD4+ class II restricted T cells. A temporal correlation has also been found between the onset of demyelination and the appearance of virus-specific CD4+ T-cell responses, as well as for high levels of virus-specific DTH. High levels of the Th1 cytokines interferon-γ and TNF-α also correlate temporally with the evolution of demeylination. DTH and CD4+ T-cell proliferative responses in infected and immunized susceptible SJL mice are directed toward immunodominant regions (peptides) in each of the three major coat proteins. T-cell responses to these epitopes in VP1 and VP2 are believed to participate in the immunopathology.

Although mice mount virus-specific humoral and cellular immune responses early in the infection and peak CNS virus titers fall by 100 000-fold, TMEVs

somehow evade immune clearance to persist at low infectious levels indefinitely. Extraneural persistence has not been observed. Current dogma holds that humoral immunity is more important than cellular immunity in clearing infections by nonenveloped viruses, such as picornaviruses. Evidence has been presented for a role for both neutralizing antibodies and cytolytic CD8+ T cells in TMEV clearance. The precise mechanism by which TMEVs evade immune surveillance is not known but does not appear to involve antigenic variation. Although complement and virus-antibody deposition in the CNS parenchyma has detected extracellular transport of virus as infectious virus antibody complexes, viral aggregates, or as virus contained within cellular membranes, are a possible means whereby virus could be protected from TMEV-specific immune responses and continue to infect other cells. This is an area for further study to enable a better understanding of how TMEV evade immune surveillance.

Immune-Mediated Mechanism of Demyelination

Appropriately timed immunosuppression can prevent the clinical signs and pathological changes of TMEV-induced demyelinating disease, indicating that the immune response participates in myelin breakdown. A number of different immunosuppressive modalities have proved to be effective, including cyclophosphamide, antilymphocyte serum, antitumor necrosis factor antibodies, and monoclonal anti-IA, CD4+, and CD8+ antibodies. If given too early in the course of early disease, the infection in neurons is potentiated and results in encephalitis and a high mortality rate. Thus, immunosuppression may be most effective when administered after the first week of infection. The incidence of demyelinating disease is increased in SJL mice infected with a dose of virus that normally produces a low incidence of disease and adoptively immunized with TMEV VP2-specific T-cell line. This observation supports a role for CD4+ T cells in mediating TMEV-induced demyelinating disease.

The effector mechanism by which a nonbudding virus, such as TMEV, might lead to immune-mediated tissue injury is unknown. Because TMEV antigens have been primarily found in macrophages, it has been proposed that myelin breakdown results from an interaction between virus-specific T cells trafficking into infected areas of the CNS and the virus. Thus, myelinated axons may be nonspecifically damaged as a consequence of a virus-specific immune response, that is, an 'innocent bystander' response. In this circumstance, cytokines produced by MHC class II-restricted, TMEV-specific T_{DTH} cells primed by interaction with infected macrophages lead to the recruitment and activation of additional macrophages in the CNS, resulting in nonspecific macrophage-mediated demyelination. This hypothesis is consistent with the CNS pathology observed in mice exhibiting TMEV-induced demyelinating disease and the fact that antigen-specific T cells and T-cell lines have been shown to cause bystander CNS damage via macrophage activation in other model systems. Alternatively, in the case of extensive infection of oligodendrocytes, demyelination might result from immune injury to these myelin-maintaining cells expressing TMEV antigens in conjunction with H-2 class I determinants. CD8+ T cells would then be the likely T cells to kill infected oligodendrocytes; however, widespread degeneration of oligodendrocytes has not been observed.

Acknowledgments

The authors thank Jan-Yve Sgro for providing the GRASP image of a BeAn virion and Paul Chipman for the cryoelectron micrograph of a GDVII virion.

See also: Cardioviruses.

Further Reading

Adami C, Pritchard AE, Knauf T, Luo M, and Lipton HL (1998) Mapping a determinant for central nervous system persistence in the capsid of Theiler's murine encephalomyelitis virus (TMEV) with recombinant viruses. *Journal of Virology* 71: 1662–1665.

Borson NO, Paul C, Lin X, *et al.* (1997) Brain-infiltrating cytolytic T lymphocytes specific for Theiler's virus recognize $H2D^b$ molecules complexed with a viral VP2 peptide lacking a consensus anchor residue. *Journal of Virology* 71: 5244–5250.

Dethlefs S, Brahic M, and Larsson-Sciard EL (1997) An early, abundant cytotoxic T-lymphocyte response against Theiler's virus is critical for preventing viral persistence. *Journal of Virology* 71: 8875–8878.

Grant RA, Filman OJ, Fujinami RS, Icenogle JP, and Hogle JM (1992) Three-dimensional structure of Theiler virus. *Proceedings of the National Academy of Sciences, USA* 89: 2061–2065.

Jelachich ML, Bramlage C, and Lipton HL (1999) Differentiation of M1 myeloid precursor cells into macrophages results in binding and infection by Theiler's murine encephalomyelitis virus (TMEV) and apoptosis. *Journal of Virology* 73: 3227–3235.

Kumar ASM, Reddi HV, Kung A, and Lipton HL (2004) Virus persistence and disease in a mouse model of multiple sclerosis requires binding to the sialic acid co-receptor. *Journal of Virology* 78: 8860–8867.

Lobert PE, Escriou N, Ruelle J, and Michiels T (1999) A coding RNA sequence acts as a replication signal in cardioviruses. *Proceedings of the National Academy of Sciences, USA* 96: 11560–11565.

Luo M, He C, Toth KS, Zhang CX, and Lipton HL (1992) Three-dimensional structure of Theiler's murine encephalomyelitis virus (BeAn strain). *Proceedings of the National Academy of Sciences, USA* 89: 2409–2413.

Paul S and Michiels T (2006) Cardiovirus leader proteins are functionally interchangeable and have evolved to adapt to virus replication fitness. *Journal of General Virology* 87: 1237–1246.

Penna-Rossi C, Delcroix M, Huitinga I, *et al.* (1997) Role of macrophages during Theiler's virus infection. *Journal of Virology* 71: 3336–3340.

Peterson JO, Waltenbaugh C, and Miller SD (1992) IgG subclass responses to Theiler's murine encephalomyelitis virus infection and immunization suggest a dominant role for Th1 cells in susceptible mouse strains. *Immunology* 75: 652–658.
Roussarie J-P, Ruffie C, and Brahic M (2007) The role of myelin in Theiler's virus persistence in the central nervous system. *PLoS Pathogens* 3(2): e23(doi:10.1371/journal.ppat.0030023).
Simas JP and Fazakerley JK (1996) The course of disease and persistence of virus in the central nervous system varies between individual CBA mice infected with the BeAn strain of Theiler's murine encephalomyelitis virus. *Journal of General Virology* 77: 2701–2711.
Trottier M, Wang W, and Lipton HL (2001) High numbers of viral RNA copies in the central nervous system during persistent infection with Theiler's virus. *Journal of Virology* 75: 7420–7428.
Yauch RL, Palma JP, Yahikozawa H, Chang-Sung K, and Kim BS (1998) Role of individual T-cell epitopes of Theiler's virus in the pathogenesis of demyelination correlates with the ability to induce a Th1 response. *Journal of Virology* 72: 6169–6174.

Tick-Borne Encephalitis Viruses

T S Gritsun and E A Gould, University of Reading, Reading, UK

© 2008 Elsevier Ltd. All rights reserved.

Taxonomy, Nomenclature, and Phylogenetic Relationships

'Tick-borne encephalitis antigenic complex' was the original term for viruses now classified as the mammalian tick-borne flaviviruses. Together with the seabird-associated tick-borne flaviviruses they comprise one ecological group in the genus *Flavivirus,* family *Flaviviridae*. The genus contains two other groups, namely, the mosquito-borne flaviviruses and the flaviviruses with no known arthropod vectors. Other flaviviruses are referred to as nonclassified flaviviruses. The prototype TBEV is a human and animal pathogen. The earliest TBEV isolate, known as Russian Spring and Summer Encephalitis virus, was isolated in 1937 in far-East Asia. Currently, the TBEV (currently classified as virus species in the group of mammalian tick-borne flaviviruses) is subdivided into three subtypes, far-Eastern, Siberian, and West European reflecting their antigenic, phylogenetic, and geographic relationships. Other antigenically related but distinct tick-borne flaviviruses include Omsk hemorrhagic fever virus, Powassan virus (POWV), Langat virus, Kyasanur Forest disease virus, Alkhurma virus, Louping ill virus, Spanish sheep encephalomyelitis virus, Turkish sheep encephalomyelitis virus, and Greek goat encephalomyelitis virus. Each of them may cause encephalitis and/or hemorrhagic disease in humans, farmed, domestic, and wild animals. Other related mammalian tick-borne flaviviruses, not known to cause human or animal disease, include Royal Farm virus, Karshi virus, Gadgets Gully virus, and Kadam virus. The viruses associated primarily with seabirds and their ticks are Saumarez Reef virus, Meaban virus, and Tyuleniy (Three Arch) virus. They are not recognized pathogens but they show interesting geographic dispersion that reflects the flight patterns of the birds with which they are associated. The phylogenetic relationships between the TBEVs are illustrated in **Figure 1**.

Virions

Infectious (mature) virions are spherical particles (50 nm) with a relatively smooth surface and no distinct projections. They have an electron-dense core (30 nm) surrounded by a lipid membrane. The core consists of positive-polarity genomic RNA (~11 kbp) and capsid protein C (12K). The lipid membrane incorporates an envelope glycoprotein (E, 53K) and a membrane glycoprotein (M, 8K). The immature (intracellular) virions contain a precursor membrane protein (prM, 18K), the cleavage of which occurs in the secretory pathway during egress of virions from infected cells.

Virions sediment at about 200S and their buoyant density is 1.19 g ml^{-1}, although there is significant heterogeneity among them. Electron micrographs frequently revealed virions in association with cellular membranes, probably explaining this heterogeneity. They are most stable at pH 8.0, although TBEV virions remain infectious in normal human gastric juice at pH 1.49–1.80. As with all enveloped viruses, infectivity of TBEVs decays rapidly at temperatures above 40 °C. Most flaviviruses agglutinate erythrocytes but a few nonagglutinating strains of TBEVs have been described. Most of the physical characteristics of the TBEVs were established using virus isolated from mammalian cells. However, after adaptation to ticks, they become less cytopathic for cultured cells and laboratory animals. These virions do not move toward the cathode in rocket immunoelectrophoresis and they have reduced hemagglutinating activity. These phenotypic characteristics may be reversed following re-adaptation to mammalian cells and host-selection of virions with a shifted net surface charge due to a single amino acid substitution in the E glycoprotein.

The E glycoprotein mediates virus binding to cellular receptors and thereby directly affects virus host range, virulence, and immunological properties by inducing

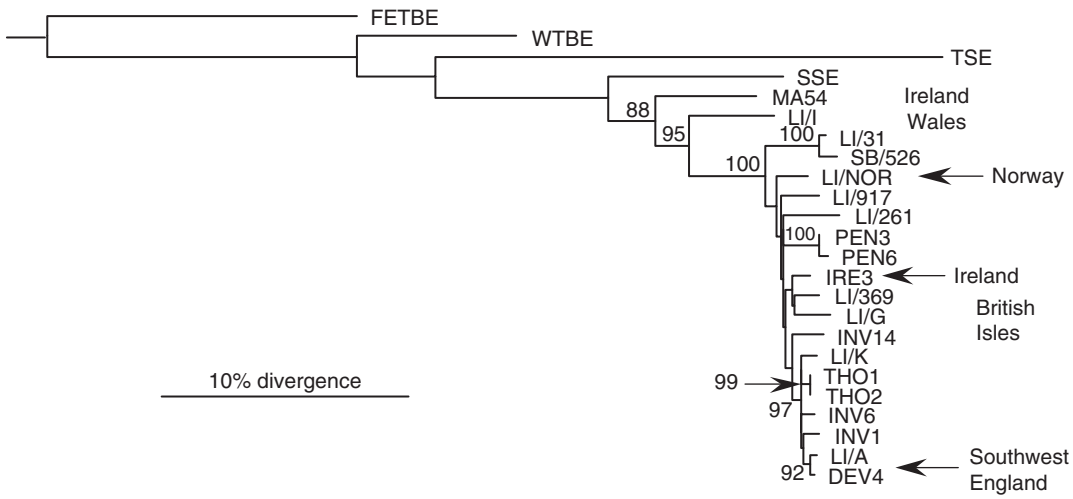


Figure 1 Maximum likelihood phylogenetic tree of the E gene from 24 tick-borne flaviviruses. Branch lengths are drawn to scale and all nodes supported by more than 75% bootstrap support are indicated. The tree is rooted with the sequence from FETBE virus, Sofjin (RSSE) strain. The three main populations of virus in the British Isles are indicated, along with those viruses secondarily introduced into Ireland and Norway, and the viruses found in the south-west of England. Reproduced from McGuire K, Holmes EC, Gao GF, Reid HW, and Gould EA (1998) Tracing the origins of Louping ill virus by molecular phylogenetic analysis. *Journal of General Virology* 79: 981–988.

protective antibodies. X-ray crystallography has revealed the E-protein ectodomain (N-terminal 395 amino acids) as homodimers folded in a 'head-to-tail' manner (**Figure 2**(**a**)) and orientated parallel to the membrane surface. It contains three structural domains, each based on β-sheets: the central domain I, the dimerization (fusion) domain II, and receptor domain III (dI, dII, and dIII in **Figure 2**). The C-terminal 101 residues of the E protein form a stem-anchor region consisting of two stem α-helices and two transmembrane α-helices that anchor the E protein into the lipid bilayer (**Figure 3**). Domain II contains a hydrophobic fusion peptide consisting of 13 residues that is highly conserved between all flaviviruses. It is located on the tip of domain II and plays a central role in fusion of the virion membrane to cellular endosomal membranes resulting in release of virion RNA into the cytoplasm (**Figure 2**).

A three-dimensional image reconstruction of the virion surface reveals a protein shell composed of 90 E-dimers organized into a 'herringbone' configuration; three quasi-parallel E-dimer molecules make up the main structural asymmetric unit of the shell (**Figures 4**(**b**) and **4**(**c**)). The fivefold symmetry axes that appear as holes are generated by appropriate positioning of five domain IIIs and their lateral surface is accessible to cellular receptors and neutralizing antibodies. The M protein protrudes through holes formed between dimerization domains of E molecules. The transmembrane regions of the M and E proteins traverse the external but not the internal layer of the lipid membrane and there is no direct contact with the nucleocapsid (**Figure 3**).

In contrast with those of many other enveloped viruses, the flavivirus capsid is poorly organized and appears to be positioned randomly in the core. In solution, the capsid protein appears as dimer molecules with four helices (**Figure 5**). Two C-terminal helices fold into the positively charged interface interacting with viral RNA; the residues on the opposite side of these helices are hydrophobic and support dimerization. Two of the internal α-helices form capsid-dimers, are hydrophobic, and are required for contact with the virion membrane.

TBEV Life Cycle

Virus Entry into the Cells

Cellular receptors for tick-borne flaviviruses

The virus life cycle commences with the attachment of virions to specific receptors on clathrin-coated pits on the cell surface. Flaviviruses infect a wide variety of primary and continuous cells, derived from mammalian, avian, and arthropod tissues. It is not yet clear if the different flaviviruses use similar or different cell surface receptors. Cellular heparan sulfate molecules are involved in TBEV attachment to cells. Nevertheless, cells that lack heparan sulfate are still sensitive to virus infection. For TBEV, a number of different proteins have been associated with cell receptor activity, including the high-affinity laminin receptor and α1β3-integrin that also recognizes laminin as a natural ligand.

The E protein mediates attachment and entry of virions into cells

The immunoglobulin-like folding of the E-protein domain III implies that it is involved in cell attachment,

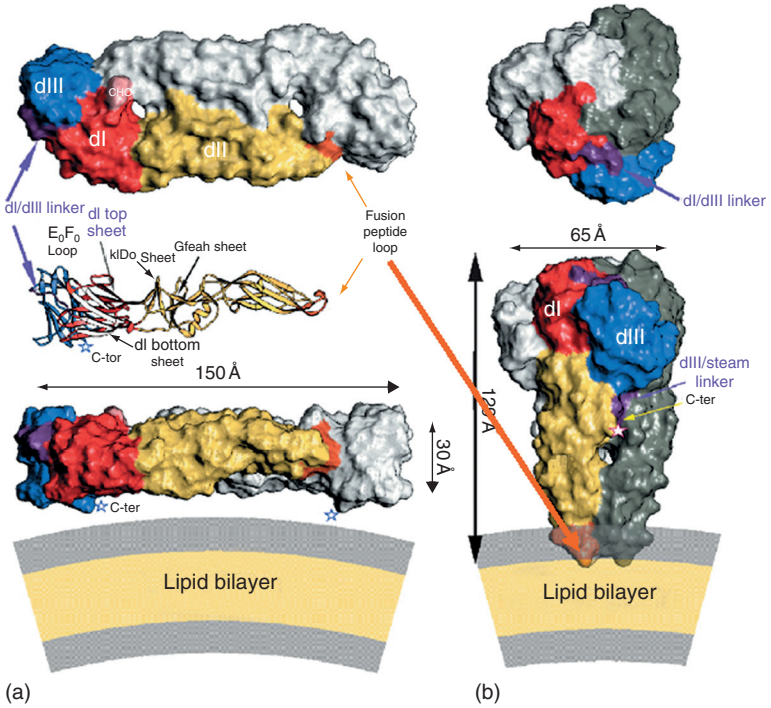


Figure 2 Three-dimensional analysis of TBEV envelope (E) glycoprotein (a) at neutral pH (in mature virion) and (b) acidic pH (postfusion conformation). The view from above and lateral view correspond to the top and bottom rows. Protein domains dI, dII, and dIII are colored in red, yellow, and blue, respectively. Ribbon diagram corresponds to the lateral view of the E protein. Reproduced from Bressanelli S, Stiasny K, Allison SL, *et al*. (2004) Structure of a flavivirus envelope glycoprotein in its low-pH-induced membrane fusion conformation. *EMBO Journal* 23: 728–738.

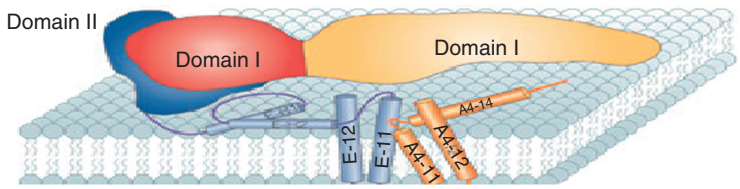


Figure 3 Schematic presentation of flavivirus structural glycoproteins E and M on the membrane surface. The coloring of the domains I, II, and III is as on **Figure 2**. Two α-helices of the stem-anchor region and two α-helices of the transmembrane domain of the E protein are depicted as blue- and colored cylinders. One α-helix of the stem region and two α-helices of the transmembrane domains of the M protein are depicted as orange-colored cylinders. Reproduced from Mukhopadhyay S, Kuhn RJ, and Rossmann MG (2005) A structural perspective of the flavivirus life cycle. *Nature Reviews Microbiology* 3: 13–22, with permission from Macmillan Magazines Limited.

mediated by high-affinity cellular receptors. The receptors on the surface of vertebrate and invertebrate cells are different and probably recognized by different residues on domain III. However, domains I and II on the surface of the E protein also appear to play a role in virus–cell interactions. Domain I carries N-linked carbohydrates, which are recognized by cell surface lectins. Domain II is enriched with patches of basic positively charged surface residues that have been shown to mediate virion attachment to the cellular, negatively charged, heparan sulfate; domains I and III might also contribute to this type of interaction.

Virus attachment to the cell receptors initiates receptor-mediated endocytosis, followed by fusion of virion and endosomal membranes (**Figures 4(c)–4(e)**). Exposure of the attached virus to the endosomal acid pH converts the herringbone configuration into a fusogenic structure resulting in particle expansion and eventually in the re-assembly of the E dimers into vertical trimers (**Figures 2** and **4**). It is believed that during this process domains I and II flex relative to each other, allowing domain II and the stem region to move toward the endosomal membrane facilitating trimerization of domain II; the fusion tripeptide is embedded into the target membrane followed by formation of a pre-fusion intermediate (**Figure 6**). Trimerization spreads from fusion peptides to domain I; meanwhile domain III rotates relative to domain II pushing the stem region back toward the fusion peptide. This refolding

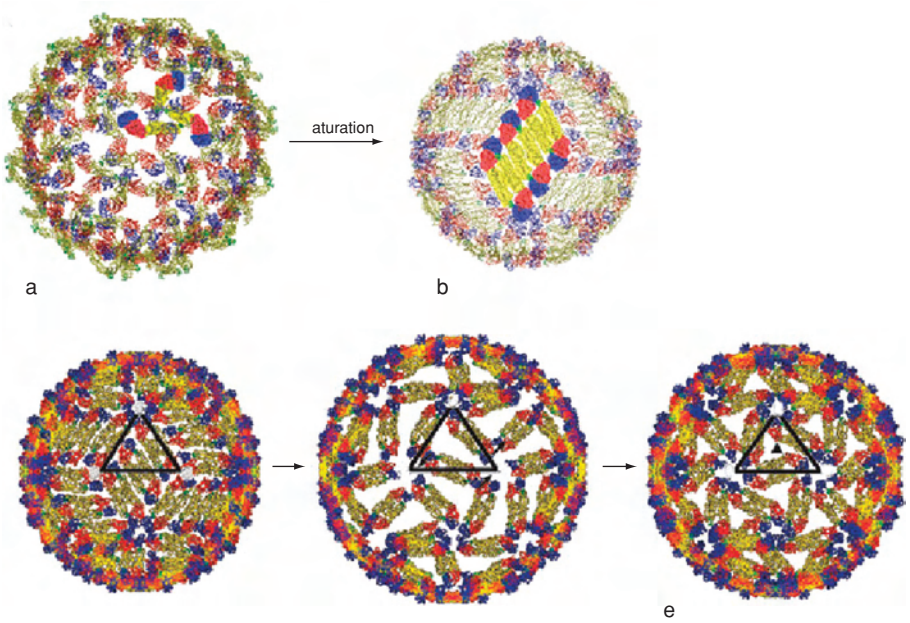


Figure 4 Different conformations of E protein during the transition from immature (a) into the mature (b) particles and during pH-dependent fusion of mature particles (c), through the putative intermediates T = 3 (d and e). Domains I, II, and III are depicted with the same colors as those on the **Figure 2**. Reproduced from Ma L, Jones CT, Groesch TD, Kuhn RJ, and Post CB (2004) Solution structure of Dengue virus capsid protein reveals another fold. *Proceedings of the National Academy of Sciences, USA* 101: 3414–3419, with permission from National Academy of Sciences USA.

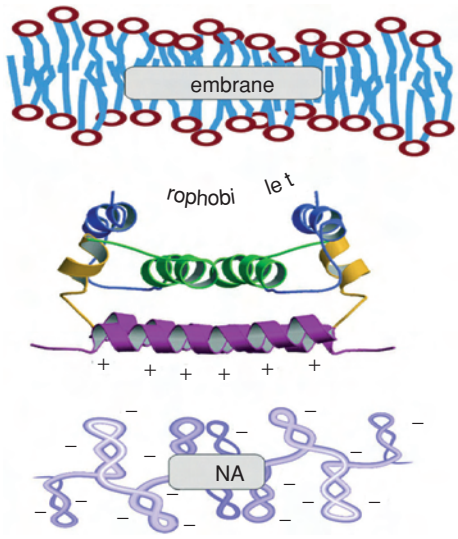


Figure 5 Model for the molecular interactions of flavivirus capsid dimers with genome RNA and virion membrane. Reproduced from Ma L, Jones CT, Groesch TD, Kuhn RJ, and Post CB (2004) Solution structure of Dengue virus capsid protein reveals another fold. *Proceedings of the National Academy of Sciences, USA* 101: 3414–3419, with permission from National Academy of Sciences USA.

brings two membranes together, resulting in the formation of the 'hemifusion stalk' with only the proximal lipid leaflets fused. The 'zipping' up of stems followed by the migration of transmembrane domains eventually leads to fusion of the distal lipid outlets and formation of a fusion pore. In the final low-pH conformation, domains I and III appear to move to the tip of the vertical trimer whereas the fusion peptide becomes embedded in the membrane eventually being juxtaposed to the transmembrane domains (**Figures 2(b)** and **6**). Five trimers form the fusion pore; the stem region appears to play an essential role in promoting and stabilizing trimer assembly. Although the E-protein domains shift and rotate during exposure to acidic pH, each retains the neutral-pH conformation, the essential feature of class II fusion molecules that do not require major molecular rearrangements at acidic pH.

Strategy of the TBEV Genome

Translation and processing of virus proteins

After uncoating, the RNA is translated into a polyprotein of ~3400 residues from one open reading frame (ORF) that is co-translationally translocated and anchored in the endoplasmic reticulum (**Figure 7**). It is then processed by cellular signalases and viral serine protease producing three structural (capsid, prM, and E) and seven nonstructural (NS1 through NS5) proteins. The gene order, protein molecular masses, and their membrane localization are shown in **Figure 7**. Viral protease activity is provided by the N-terminal domain (~180 residues) of the NS3 protein in association with cofactor activity of the NS2B protein that probably anchors the NS3 into

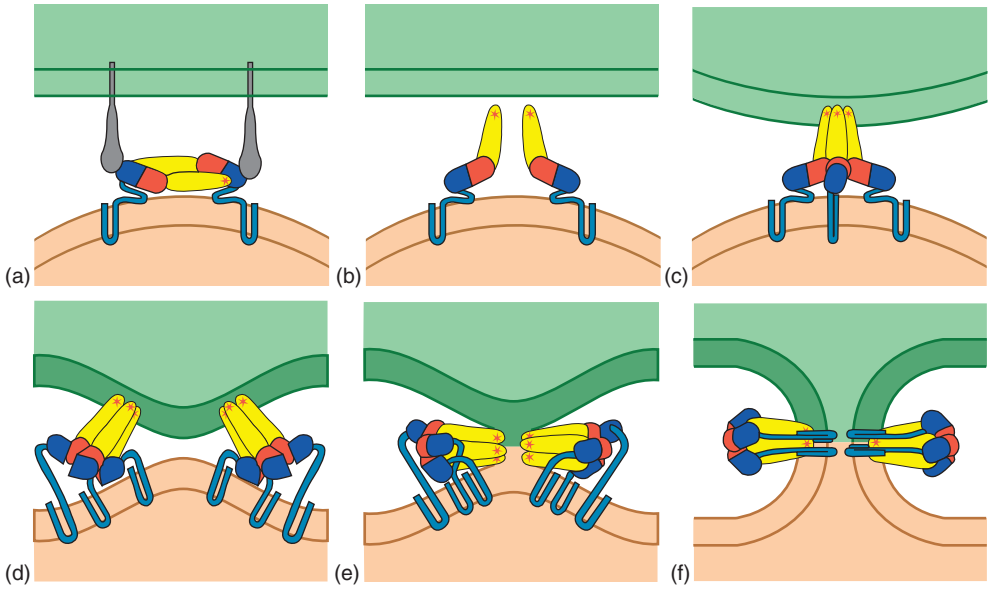


Figure 6 Tentative mechanism for the fusion of viral (brown) and endosomal (green) membranes mediated by the flavivirus E protein following interaction with cellular receptor (gray) (a). Acidic pH triggers the movement of domain II (with fusion peptide on the top) toward the endosomal membrane (b) with its subsequent trimerization and insertion into the endosomal membrane (c). Trimerization spread toward domains I and III (d) causing the C-terminal part of E protein to fold backwards toward the fusion peptide. The trimerization between stem-anchor and domain II (e) results in partial fusion (e) and eventually in the formation of a fusion pore (f). Reproduced with permission from Modis Y, Ogata S, Clements D, and Harrison SC (2005) Variable surface epitopes in the crystal structure of Dengue virus type 3 envelope glycoprotein. *Journal of Virology* 79: 1223–1231.

the membrane. The ORF is flanked by 5′- and 3′-untranslated regions (UTRs; ~ 130 and 700 bp, respectively) that contain signals essential to initiate translation and replication. The 5′-UTR preceding the ORF is capped by $m^7GpppAmpN_2$ and initiates virus translation *in vitro* although an interactive effect from the 3′-UTR has also been demonstrated. Computer-assisted prediction has demonstrated folding of the 5′-UTR into a Y-shaped structure, conserved between all flaviviruses; mutations in this structure appear to impact on virus translation and replication. The translation of flavivirus proteins is probably carried out in specialized smooth membrane structures, called convoluted membranes, derived from proliferation of the rough endoplasmic reticulum in response to flavivirus infections.

Replication of viral RNA

The initiation of viral replication (synthesis of negative strand on positive strand from the 3′-UTR) requires direct interaction between the 5′- and 3′-UTRs resulting in the formation of a double-stranded RNA (dsRNA) stem and circularization of the virus genome. A terminal 3′ long stable hairpin (3′ LSH) structure has been revealed in the 3′-UTR that, together with the 5′-Y-shaped-structure and dsRNA circularization stem, forms a complex promoter required to initiate virus RNA replication. Additional predicted conserved secondary RNA structures in the 3′-UTR might function as replication enhancers, essential for efficient RNA synthesis and virus transmission. The synthesis of genome-size dsRNA (replicative form, RF) accomplishes the first stage of viral replication.

Flavivirus RNA replication is semiconservative and asymmetric, with an average of one nascent positive-sense single-stranded RNA (ssRNA) molecule (genomic) per negative-sense template, with no free minus-strands and with 10–100 times excess of positive-strand relative to negative-strand RNA synthesis. The replicative intermediate presents a partially double-stranded RNA with nascent, and displaced, plus-sense ssRNA molecules undergoing elongation. Viral RNA capping of the 5′-UTR occurs on the displaced plus-strand RNA; the trifunctional NS3 and NS5 proteins provide nucleoside triphosphatase and guanylyl/methyltransferase activities, respectively.

Formation of the replication complex involves nonstructural proteins (**Figure 8**) most of which are bi- and even trifunctional. The C-terminal domain of NS5 protein acts as a viral RNA polymerase and the C-terminal domain of NS3 as a helicase; both NS5 and NS3 proteins interact with 3′-LSH to initiate virus replication. The functions of other nonstructural proteins are less precisely identified. The NS1 glycoprotein forms membrane-associated hexamers that dissociate into dimers. This protein is translocated in the endoplasmic reticulum and secreted together with virions in mammalian but not in mosquito cells; its anchoring into the membranes is mediated by

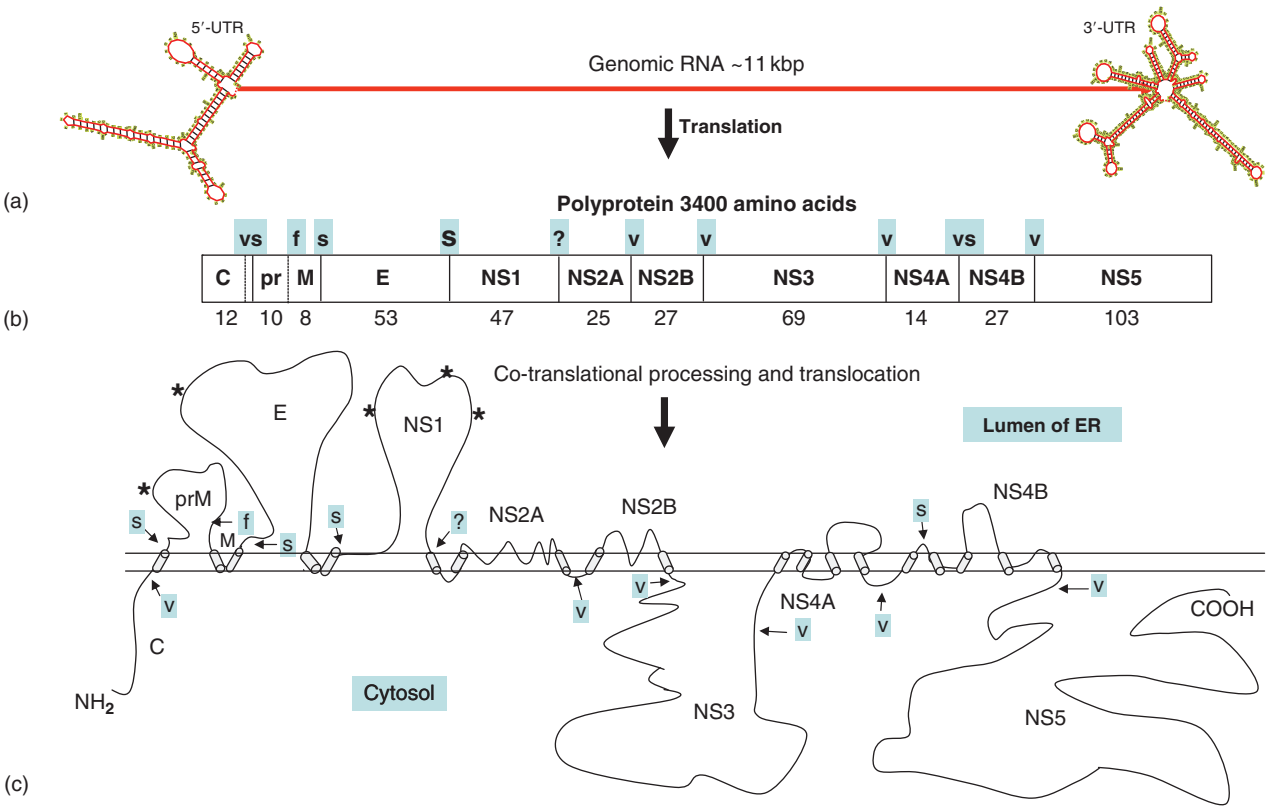


Figure 7 Genome strategy of TBEV. (a) Genomic RNA is presented as a solid line on the top; the 5′- and 3′-UTR are depicted in the predicted conformations according to Gritsun TS, Venugopal K, Zanotto PM, *et al*. (1997) Complete sequence of two tick-borne flaviviruses isolated from Siberia and the UK: Analysis and significance of the 5′- and 3′-UTRs. *Virus Research* 49: 27–39. (b) Translation and co-translational processing of flavivirus polyprotein. The flavivirus polyprotein is depicted as a bar, with specified individual proteins and their molecular masses (numbers below the bar). (c) Membrane topology of viral proteins in relation to the lumen of the ER and cytoplasm after the completion of co-translational processing and translocation. Adapted from Westaway EG, Mackenzie JM, and Khromykh AA (2003) Kunjin RNA replication and applications of Kunjin replicons. *Advances in Virus Research* 59: 99–140. Transmembrane domains are shown as cylinders. Glycosylation is indicated as (*). The polyprotein is processed by ER signalases (s) and viral-specific protease NS2B-NS3 (V). The cleavage of M from prM is carried out by the Golgi protease, furin (f) and cleavage between NS1 and NS2A is by an unknown (?) ER protease.

glycosyl-phosphatidylinositol. The NS1 protein induces the production of protective antibodies that also participate in complement-mediated lysis of infected cells, implying a role in immunopathology. The NS2A protein is found in association with the NS3 helicase domain, the NS5 protein, and the 3′-LSH. It may be involved in trafficking of viral RNA between the translation, replication, and virion assembly sites. The NS2B protein is a membrane-anchored cofactor of the serine proteinase, NS3. It also has membrane-permeability modulating activity possibly related to replication. The hydrophobic NS4A protein in conjunction with the NS1 protein probably anchors the polymerase complex to cell membranes and is involved in virus-induced membrane rearrangements that compartmentalize virus translation, replication, and assembly processes (**Figure 8**).

Replication of flavivirus RNA is associated with host membrane 75–100 nm vesicular packets enclosed in the second outer membrane and connected to convoluted membranes, the sites of virus translation. The vesicular packets proliferate in the perinuclear region in response to virus infection and are probably derived from the *trans*-Golgi network. The architecture of the replication complex in relation to vesicular packets and convoluted membranes is illustrated in **Figure 9**. The replicative forms and replication complexes are enclosed within vesicular packets, whereas nascent ssRNA is externally located. In addition, a variety of cellular proteins associates with the 5′-UTR, 3′-UTR, and the RNA polymerase; they probably facilitate assembly of the replication complex and trafficking of the viral RNA/polymerase into the appropriate cellular compartment for replication.

Assembly, Maturation, and Release of Virions

The sequence of molecular events during virion morphogenesis is not completely understood. On completion of post-translational processing, the prM and E proteins rapidly form heterodimeric complexes; the

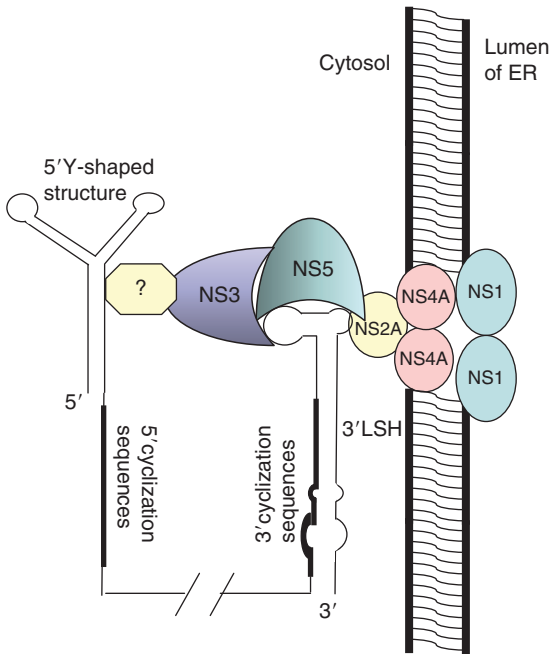


Figure 8 Representation of the assembly of the membrane-anchored polymerase complex. The conformations of 3′LSH and 5′ Y-shaped structure are shown. This stage probably precedes the cyclization of the virus genome mediated by direct interaction between the inverted complementary 5′- and 3′-cyclization sequences (thick lines); this is followed by the initiation of minus-strand RNA synthesis. Adapted from Westaway EG, Mackenzie JM, and Khromykh AA (2002) Replication and gene function in Kunjin virus. *Current Topics in Microbiology and Immunology* 267: 323–351.

chaperone-like function of the prM protein is essential for correct folding of E protein. Heterodimers are rapidly assembled into immature particles or, in the absence of capsid, into virus-like particles that also accumulate during infection. Virion packaging is coupled with RNA replication; only actively replicating RNA is encapsidated. The orientation of structural proteins in the lipid membrane suggests that assembly is mediated by capsid budding through the endoplasmic reticulum. However, assembled virus capsids have never been visualized, neither have budding particles nor specific release mechanisms been identified.

Assembly of structural proteins in the viral lipid membrane initially results in the formation of immature (intracellular) virions that are noninfectious, resistant to acidic pH, and unable to be structurally rearranged prior to fusion. Image reconstruction has defined the different organization of E/prM proteins compared with E/M proteins on the surface of immature and mature virions, respectively (**Figure 4**). Sixty projections were identified on the surface of immature virions making them look larger (60 nm) in comparison with smooth 50 nm mature virus particles. Each spike is formed by three heterodimeric E/M molecules. In immature particles the E protein points away from the membrane with the fusion peptide at its extremity. Virion maturation is accompanied by cleavage of prM to M, mediated by the cellular protease furin in the *trans*-Golgi network at acidic pH, during the transportation of virions to the cell surface. PrM-to-M cleavage results in the dissociation of E/M trimeric spikes with the formation of E/E homodimers, probably following shift of the stem-transmembrane domain relative to the ectodomain. In mature particles, the E ectodomain lies on the virus membrane, with the fusion peptide embedded in the cavity between domains I and III of the neighboring dimer. Formation of the herringbone building unit and release of the glycosylated pre-peptide then follows. Eventually, *trans*-Golgi-derived vesicles packed with mature virions follow the host secretory pathway, fuse with the plasma membrane, and release mature virions.

Pathogenesis of TBEV

Incidence of Tick-Borne Encephalitis

Classical tick-borne encephalitis (TBE) is contracted by humans when they are fed upon by infected *Ixodes* spp. ticks. Forested areas across the Northern Hemisphere, with thick moist undergrowth and abundance of small wild animals, provide ideal habitats for ticks and for TBEV. Long-term survival of the virus occurs mainly in ticks, which remain infected throughout their 2–5-year life cycle. Efficient virus transmission between infected nymphs and noninfected larvae occurs on the forest animals when these ticks co-feed. There is no requirement for viremia, since the virus can be transmitted to the larvae in animals that are not susceptible to the virus. Moreover, nonviremic transmission between the ticks can occur even on immune animals. Cells that migrate between the skin surface and local lymph nodes (e.g., dendritic cells) become infected and are then imbibed by the feeding noninfected larvae. Nonviremic transmission provides an efficient mechanism for long-term virus survival in the absence of overt disease. At the same time, susceptible animals such as sheep, goats, horses, pigs, dogs, grouse, and even humans may inadvertently become infected by ticks either in the forests or by ticks carried away from the forests. Under these circumstances the vertebrate host may become viremic and the virus may then be transmitted to a noninfected tick that subsequently transmits the virus to another vertebrate host when the infected tick feeds for a second time. Such situations arise on the sheep-rearing moorlands in the British Isles, Norway, Spain, Turkey, Greece, and other parts of Europe. In some situations sheep/goat and grouse populations may be severely affected.

The incidence of human TBE varies from year to year in different areas. The Urals and Siberia annually

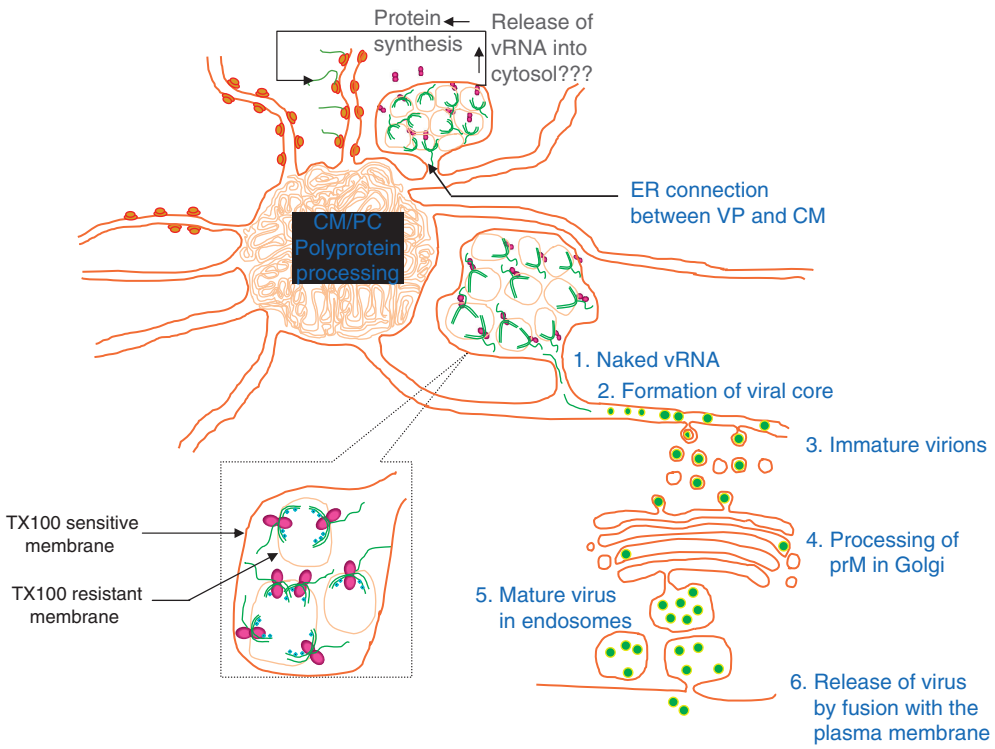


Figure 9 Proposed model of flavivirus replication in connection with cellular membranous structures. Translation of viral proteins is associated with CMs whereas replication is carried out within VPs. The dsRNA of RF in association with proteins of the polymerase complex (RC) is anchored to the internal leaflet of VP membrane whereas newly synthesized plus ssRNA is released from the RC. Reproduced with permission from Uchil PD and Satchidanandam V (2003) Architecture of the flaviviral replication complex. Protease, nuclease, and detergents reveal encasement within double-layered membrane compartments. *Journal of Biological Chemistry* 278: 24388–24398.

record the highest number of hospitalized cases. These numbers have risen from 700 to 1200 cases per year in the 1950s and 1960s to greater than 11 000 per year in the 1990s, following *Perestroika* and initial breakdown of the infrastructure. The incidence in Europe is lower but nonetheless significant, with about 3000 cases per year. Although it can affect people of all ages, the highest incidence occurs among the most active groups, that is, 17–40 year olds.

The incidence of clinical disease in endemic regions is dependent on the frequency of forest visits, the density of ticks in different years, the concentration of virus in ticks, and the virulence of circulating strains. Taking all these factors into account, it was estimated that one clinical case must occur for every 100 people bitten by ticks, and this correlates with the observation that in regions where more than 60% of ticks carry virus, about 1.4% of people develop TBE after being bitten.

Variety of Clinical Manifestations

TBEV is recognized as a dangerous human pathogen, causing a variety of clinical manifestations, although asymptomatic infections constitute about 70–95% of all TBEV infections. Symptoms include (1) mild or moderate fever with complete recovery of patients (about 90% of all clinical manifestations), (2) subacute encephalitis with nearly complete recovery or residual symptoms that may or may not disappear with time, (3) severe encephalitis associated with irreversible damage to the central nervous system (CNS), resulting in disability or death (about 4–8%), and (4) slow (months to decades) progressive or chronic encephalitis (1–2%).

Initially in the 1940–50s it was believed that the same virus produced encephalitis over the whole of Europe and Asia. However, with the improvement of serological diagnosis and the advent of phylogenetic analysis, three subtypes of TBEV were identified.

Human infections with far-Eastern TBEV subtype viruses produce the most severe form of CNS disorder, with a tendency for the patient to develop focal meningoencephalitis or polyencephalitis, and case–fatality rates between 20% and 40%. Siberian strains are often associated with high prevalence of the nonparalytic febrile form, with case–fatality rates rarely exceeding 6–8%. There is a tendency for some patients to develop chronic TBE, predominantly in association with Siberian TBEV strains. The disease produced by West European TBEV strains is biphasic, with fever during the first phase and neurological disorders of differing severity during the

second phase, which occurs in 20–30% of patients, with a case–fatality rate of 1–2%. While Louping ill virus and the related Spanish, and Turkish/Greek viruses produce fatal encephalitis in animals, they are only rarely associated with disease in humans, probably primarily because of the lower (in comparison with TBEV) rates of human exposure to infected ticks.

Biphasic milk fever is an unusual form of TBE originally observed in western Russia mainly associated with the consumption of goat's milk. West European strains of TBEV were isolated from unpasteurized goat's milk and from patients. Later, similar outbreaks of biphasic milk fever were reported in central Europe and Siberia. The apparent difference in clinical manifestations of TBE contracted by tick-bite or by the alimentary route may be explained by differences in the type of immunological response.

Human Disease Produced by Other Tick-Borne Flaviviruses

POWV also produces encephalitic infections in humans although not on an epidemic scale. POWV circulates in Russia, the USA, and Canada, where it may cause human encephalitis with a high incidence of neurological sequelae and up to a 60% case–fatality rate. In North America POWV has diverged to produce a closely related deer tick virus (DTV) that has a predilection for different rodent species. Langat virus has been isolated in Malaysia and Thailand; there are no registered cases of human disease associated with this virus. However, when Langat virus was used as a live, attenuated vaccine in human trials in Russia, one in 10 000 patients developed encephalitis.

Three tick-borne flaviviruses cause hemorrhagic disease rather than encephalitis in humans, namely Omsk hemorrhagic fever virus, Kyasanur Forest disease virus, and Alkhurma virus. As yet there is no explanation for these differences in virulence characteristics. Epidemic foci of Omsk hemorrhagic fever virus in the Omsk and Novosibirsk regions of western Siberia usually follow epizootics in muskrats (*Ondatra zibethica*) from which local hunters become infected by the virus when they handle infected animals. The most marked pathological signs of the disease are focal visceral hemorrhages in the mucus of the nose, gums, uterus, and lungs. Convalescence is usually uneventful without residual effects; fatal cases have been recorded, but rarely (0.5–3%).

Kyasanur Forest disease virus was first recognized in 1957 in India where it caused hemorrhagic disease among monkeys and humans, frequently with fatal outcome. It is believed that perturbation of regions of the forest for land development led to increased exposure of monkeys and humans to ticks carrying the virus. Subsequently, it has become evident that this virus circulates throughout western India and this may explain the close antigenic and genetic link with Alkhurma virus, which was isolated from fatal human cases of hemorrhagic fever in Saudi Arabia. Recent evidence has demonstrated the presence of Alkhurma virus in ticks associated with camels. Whether or not this or a closely related virus circulates in Africa remains to be determined.

Prevention and Control of TBE

Vaccination is the most efficient method available for preventing TBE in enzootic regions. Currently two inactivated vaccines are commercially available. The European vaccine has significantly reduced the annual incidence of TBE in Austria and Germany. A similar vaccine, based on a far-Eastern strain of TBEV, has been used successfully in Russia to immunize at-risk laboratory personnel. Other preventive measures routinely employed in TBE endemic areas are (1) education of residents in methods of avoiding tick-bites (when visiting tick-infested areas, wearing appropriate clothing, regularly inspect for feeding ticks, report tick-bite to medical authorities); (2) treat cats and dogs with acaricides; (3) clear thick, moist vegetation areas from around houses; and (4) spray acaricides in forested areas, close to habitation.

Human trials of a live, attenuated vaccine for Langat virus produced an unacceptably high incidence of TBE (1/10 000). However, these trials demonstrated the higher protection efficiency of live, attenuated vaccines in comparison with inactivated vaccines. Currently safer strategies to produce live, attenuated vaccine are being developed such as (1) RNA- or DNA-based vaccines; (2) engineering TBEV mutants with multiple attenuating mutations or large deletions within their genomes, resulting in the loss of neuroinvasiveness; and (3) engineering chimeric yellow fever virus vaccine containing substituted TBEV E and M proteins.

Currently there are no safe, effective antivirals for TBEV infections, but there is promising progress in tests with small-interfering RNAs (siRNAs). These short molecules, ~21 nt long, bind to homologous regions of viral mRNA, thus interfering with the replication cycle. Another antiviral strategy with which rapid progress is being made is based on the design of molecules that can target specific regions within viral replicative enzymes. If the twentieth century was significant for its development of effective vaccines, the twenty-first century might be recognized for its development of effective antivirals.

See also: Japanese Encephalitis Virus; West Nile Virus.

Further Reading

Aberle JH, Aberle SW, Kofler RM, and Mandl CW (2005) Humoral and cellular immune response to RNA immunization with flavivirus replicons derived from tick-borne encephalitis virus. *Journal of Virology* 79: 15107–15113.

Anderson R (2003) Manipulation of cell surface macromolecules by flaviviruses. *Advances in Virus Research* 59: 229–274.
Bressanelli S, Stiasny K, Allison SL, *et al.* (2004) Structure of a flavivirus envelope glycoprotein in its low-pH-induced membrane fusion conformation. *EMBO Journal* 23: 728–738.
Gelpi E, Preusser M, Garzuly F, Holzmann H, Heinz FX, and Budka H (2005) Visualization of Central European tick-borne encephalitis infection in fatal human cases. *Journal of Neuropathology and Experimental Neurology* 64: 506–512.
Gritsun TS, Lashkevich VA, and Gould EA (2003) Tick-borne encephalitis. *Antiviral Research* 57: 129–146.
Gritsun TS, Nuttall PA, and Gould EA (2003) Tick-borne flaviviruses. *Advances in Virus Research* 61: 317–371.
Gritsun TS, Tuplin AK, and Gould EA (2006) Origin, evolution and function of flavivirus RNA in untranslated and coding regions: Implications for virus transmission. In: Kalitzky M and Borowski P (eds.) *Flaviviridae: Pathogenesis, Molecular Biology and Genetics*, pp. 47–99. Norwich, UK: Horizon Scientific Press.
Heinz FX and Allison SL (2003) Flavivirus structure and membrane fusion. *Advances in Virus Research* 59: 63–97.
Heinz FX, Stiasny K, and Allison SL (2004) The entry machinery of flaviviruses. *Archives of Virology Supplement* 18: 133–137.
Lindenbach BD and Rice CM (2001) *Flaviviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., vol. 1, pp. 991–1042. London: Lippincott Williams and Wilkins.
Markoff L (2003) 5′- and 3′-noncoding regions in flavivirus RNA. *Advances in Virus Research* 59: 177–228.
Mukhopadhyay S, Kuhn RJ, and Rossmann MG (2005) A structural perspective of the flavivirus life cycle. *Nature Reviews Microbiology* 3: 13–22.
Rey FA, Heinz FX, Mandl C, Kunz C, and Harrison SC (1995) The envelope glycoprotein from tick-borne encephalitis virus at 2 Å resolution. *Nature* 375: 291–398.
Uchil PD and Sachidanandam V (2003) Architecture of the flaviviral replication complex. Protease, nuclease, and detergents reveal encasement within double-layered membrane compartments. *Journal of Biological Chemistry* 278: 24388–24398.
Westaway EG, Mackenzie JM, and Khromykh AA (2003) Kunjin RNA replication and applications of Kunjin replicons. *Advances in Virus Research* 59: 99–140.

Tobacco Mosaic Virus

M H V Van Regenmortel, CNRS, Illkirch, France

© 2008 Elsevier Ltd. All rights reserved.

The Beginnings of Virology

Tobacco mosaic virus occupies a unique place in the history of virology and was in the forefront of virus research since the end of the nineteenth century. It was the German Adolf Mayer, working in the Netherlands, who in 1882 first described an important disease of tobacco which he called tobacco mosaic disease. He showed that the disease was infectious and could be transmitted to healthy tobacco plants by inoculation with capillary glass tubes containing sap from diseased plants. Although Mayer could not isolate a germ as the cause of the disease, he did not question the then prevailing view that all infectious diseases were caused by microbes and he remained convinced that he was dealing with a bacterial disease.

About the same time, in St. Petersburg, Dmitri Ivanovsky was studying the same disease and he reported in 1892 that when sap from a diseased tobacco plant was passed through a bacteria-retaining Chamberland filter, the filtrate remained infectious and could be used to infect healthy tobacco plants. Ivanovsky was the first person to show that the agent causing the tobacco mosaic disease passed through a sterilizing filter and this gave rise to the subsequent characterization of viruses as filterable agents. A virology conference was held in 1992 in St. Petersburg to celebrate the centenary of this discovery. Although Ivanovsky is often considered one of the fathers of virology, the significance of his work for the development of virology remains somewhat controversial because all his publications show that he did not really grasp the significance of his filtration experiments. He remained convinced that he was dealing with either a small bacterium or with bacterial spores and never appreciated that he had discovered a new type of infectious agent.

Following in the footsteps of Mayer, Beijerinck in Delft, Holland, again showed in 1898 that sap from tobacco plants infected with the mosaic disease was still infectious after filtration through porcelain filters. He also demonstrated that the causative agent was able to diffuse through several millimeters of an agar gel and he concluded that the infection was not caused by a microbe.

Beijerinck called the agent causing the tobacco mosaic disease a *contagium vivum fluidum* (a contagious living liquid), in opposition to a *contagium vivum fixum.* In those days the term *contagium* was used to refer to any contagious, disease-causing agent, while the term *fixum* meant that the agent was a solid particle or a cellular microbe. On the basis of his filtration and agar diffusion experiments, Beyerinck was convinced that the agent causing tobacco mosaic was neither a microbe nor a small particle or corpuscle (meaning a small body or particle from the Latin *corpus* for body). He proposed instead that the disease-causing agent,which he called a virus, was a living liquid containing a dissolved, nonparticular and noncorpuscular entity.

Lute Bos has claimed that Beijerinck's introduction in 1898 of the unorthodox and rather odd concept of a *contagium vivum fluidum* marked the historic moment when virology was conceived conceptually. However, it is clear that his definition of a virus as a soluble, living agent not consisting of particles certainly does not correspond to our modern view of what a virus is. It is Beijerinck's insistence that a virus is not a microbe and his willingness to challenge the then widely held view that all infectious diseases are caused by germs that make many people regard his contribution to the development of virology as more important than that of Ivanovsky. In 1998, a meeting held in the Netherlands commemorated the centenary of the work of Beijerinck on TMV. About the same time a meeting was held in Germany to honor the contribution of Loeffler and Frosch who, also in 1898, had shown that the agent causing the foot-and-mouth disease of cattle was able to pass through a Chamberland-type filter, in the same way as TMV. In addition the German workers also established that their disease-causing agent was not able to go through a finer grain Kitasato filter, from which they concluded that the agent, which was multiplying within the host, was corpuscular and not soluble as claimed by Beijerinck. Loeffler, however, continued to believe that his pathogen was a very small germ or spore, invisible in the light microscope, that was unable to cross the small pores of a Kitasato filter.

There is no doubt that none of these 'fathers' of virology realized the nature of the filterable pathogenic agents they were investigating and that the term virus which they used did not have the meaning it has today. It took another 30 years before chemical analysis eventually revealed what viruses are actually made of.

Physical and Chemical Properties of TMV

A major change in our perception of what viruses are occurred in 1935 when Stanley, working at the Rockefeller Institute in Princeton, obtained needle-like crystals of TMV that were infectious and consisted of protein. He used methods that were being developed at the time for purifying enzymes and was greatly helped by a bioassay developed by Holmes in 1929 that made it possible to quantify the amount of TMV present in plant sap. In this assay, extracts containing the virus are rubbed on the leaves of *Nicotiana glutinosa* tobacco plants which leads to a number of necrotic lesions proportional to the amount of virus present.

Stanley's demonstration that TMV was a crystallizable chemical substance rather than a microorganism, a discovery that earned him the Nobel Prize in 1946, had a profound impact on the thinking of biologists because it suggested that viruses were actually living molecules able to reproduce themselves. For a while, it seemed that viruses closed the gap between chemistry and biology and might even hold the key to the origin of life.

Stanley had originally described TMV as a pure protein, but in 1936 Bawden and Pirie showed that the virus contained phosphorus and carbohydrate and actually consisted of 95% protein and 5% RNA. TMV thus became the first virus to be purified and shown to be a complex of protein and nucleic acid. Following the development of ultracentrifuges in the 1940s, ultracentrifugation became the standard method for purifying TMV and many other viruses.

In 1939, TMV became the first virus to be visualized in the electron microscope and in 1941, TMV particles were shown to be rods about 280 nm long and 15 nm wide. Subsequently, X-ray analysis established that the rods were hollow tubes consisting of a helical array of 2130 identical protein subunits with 16(1/3) subunits per turn and containing one molecule of RNA deeply embedded in the protein subunits at a radius of 4 nm (**Figure 1**). The length of the TMV particle is controlled by the length of the RNA molecule which becomes fully coated with protein and is thereby protected from nuclease attack.

The viral protein subunits can also aggregate on their own, without incorporating RNA, to form rods very similar to TMV particles but in this case the rods are of variable length (**Figure 2**). It is possible to degrade the virus particles with acetic acid or weak alkali and to obtain in this way dissociated protein subunits and viral RNA, the latter being rapidly degraded by nucleases. In 1956 Gierer and Schramm in Tübingen, Germany, and Fraenkel-Conrat in Berkeley, California, showed that if the viral RNA was obtained by degrading the particles with phenol or detergent, intact RNA molecules were obtained that were infectious and could produce the same disease as intact virus. This was the first demonstration that the nucleic acid component of a virus was the carrier of viral infectivity and that it possessed the genetic information that coded for the viral coat protein.

In 1960 the TMV coat protein was the first viral protein to have its primary structure elucidated when its sequence was determined simultaneously in Tübingen and in Berkeley and found to consist of 158 amino acids. Subsequently, the coat protein sequence of numerous TMV mutants obtained by treating the virus with the mutagenic agent nitrous acid was also determined and these sequence data helped to establish the validity of the genetic code that was being elucidated in the early 1960s in Nirenberg's and Ochoa's laboratories. One of the changes induced by the action of nitrous acid on viral RNA is the nucleotide conversion of cytosine (C) to uracil (U). This changes the codon CCC for the amino acid proline to the codons UCC or CUC for serine and leucine, respectively. In addition, these two codons can be further converted to UUU which codes for phenylalanine. When the coat protein sequences of various TMV mutants were determined, it was found that

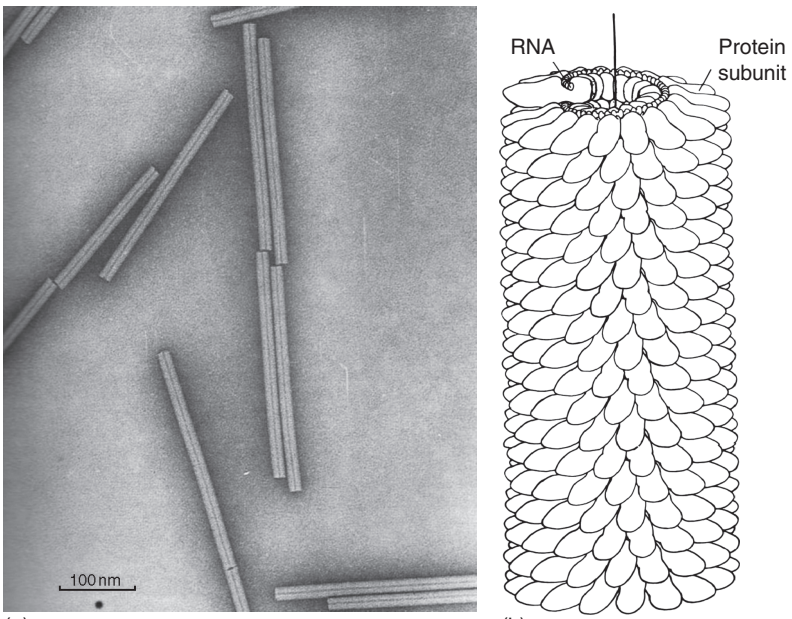


Figure 1 (a) Electron micrograph of negatively stained TMV particles. (b) Diagram of a TMV particle showing about one-sixth of the length of a complete particle. The protein subunits form a helical array with 16(1/3) units per turn and the RNA is packed at a radius of about 4 nm from the helix axis. From Klug A (1999) The tobacco mosaic virus particle: Structure and assembly. *Philosophical Transactions of the Royal Society*: *Biological Sciences* 354: 531–535.

most of the exchanged amino acids could be attributed to C→U nucleotide conversions, which confirmed the validity and universality of the proposed genetic code.

A particularly interesting mutation present in mutant Ni 1927 was the exchange proline to leucine at position 156 in the sequence which was found to greatly decrease the chemical stability of the virus. It had been known for many years that the virus was resistant to degradation by the enzyme carboxypeptidase and that this enzyme released only about 2000 threonine residues from each virus particle. The C-terminal residue in the coat protein is in fact threonine and the enzyme degradation data were interpreted at the time to mean that the virus particle contained 2000 subunits. However, when the proline at position 156 was replaced by leucine in the mutated protein, the enzyme was able to degrade the protein far beyond the C-terminal residue. The location of a proline near the C-terminus together with the presence of an acetyl group on the N-terminus are actually responsible for the remarkable resistance of the virus to exopeptidase degradation. The same exchange at position 156 in mutant Ni 1927 also led to an interesting discovery in immunology (see below).

Self-Assembly of TMV Particles

Dissociated coat protein molecules of TMV are able to assemble into different types of aggregates depending on the pH and ionic strength (**Figure 2**). The disk aggregate is a two-layer cylindrical structure with a sedimentation coefficient of 20 S, where each layer consists of a ring of 17 subunits compared with the 16(1/3) molecules present in each turn of the assembled helix. The disks are able to form stacks which can be either polar or nonpolar, depending on the relative orientation of adjacent disks (**Figure 2**). Short stacks of disks were shown to be nonpolar by immunoelectron microscopy, using monoclonal antibodies that reacted with only one end of the viral helix but were able to bind to both ends of the stacked disks. Whereas short polar stacks of disks can be transformed via lock-washer intermediates into helices (**Figure 2**), this is not possible for nonpolar stacks which cannot be incorporated as such during the elongation process that leads to the formation of RNA-containing virus particles.

It is generally accepted that initiation of TMV assembly from its RNA and protein components requires a 20 S protein aggregate, which could be either a disk or a short, lock-washer type proto-helix. The surface of this aggregate constitutes a template which is recognized by a specific viral RNA sequence. The nucleation of the assembly reaction was found to occur by a rather complex process involving a hairpin structure on the viral RNA, known as the origin of assembly and located about 1000 nucleotides from the 3′ end, which is inserted through the central hole of the 20 S aggregate. The nucleotide sequence of the stem–loop hairpin is responsible for the preferential incorporation of viral RNA rather than foreign RNA. Elongation occurs by addition of more protein subunits which pull up more RNA through the central hole. This

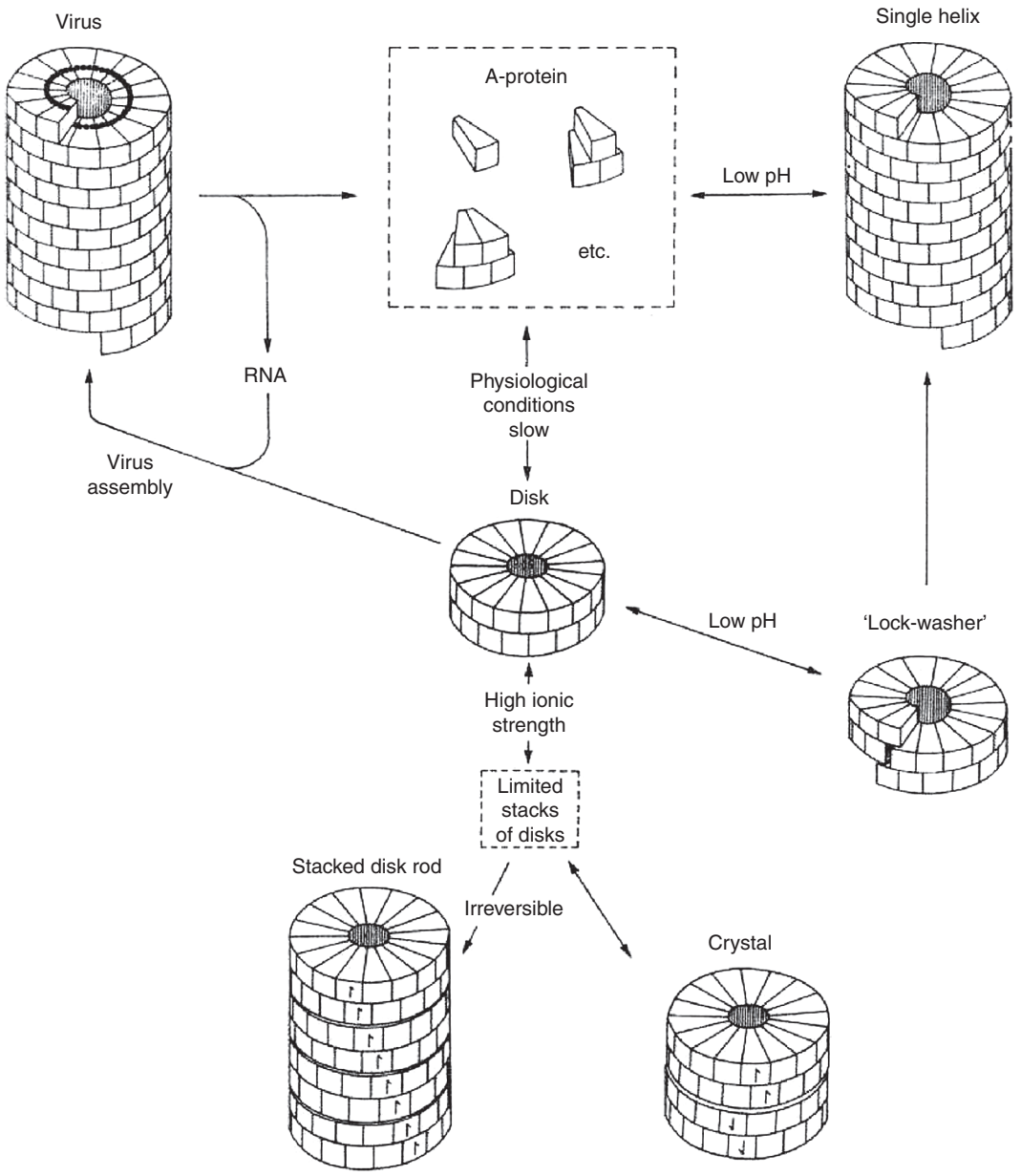


Figure 2 Diagram showing the different polymorphic aggregates of TMV coat protein obtained at different pH values and ionic strengths. Note that both polar and nonpolar stacked disks can be obtained. The 'lock-washer' is not well defined and represents a metastable transitory state. Adapted from Durham ACH and Klug A (1972) Structures and roles of the polymorphic forms of tobacco mosaic virus protein. *Journal of Molecular Biology* 67: 315–332, with permission from Elsevier.

process requires that the 5′ tail of the RNA loops back down the central hole and is slowly 'swallowed up' while the 3′ tail continues to protrude freely from the other end. This unexpected mechanism was visualized by electron micrographs taken in Hirth's laboratory in Strasbourg which revealed how particles were growing with both 3′ and 5′ RNA tails protruding initially from the same end of the particle. The elongation is thus bidirectional and occurs faster along the longer 5′ tail by incorporation of 20 S aggregates and more slowly toward the 3′ end through incorporation of 4 S protein. The elucidation of the assembly process was greatly facilitated by the sequencing of the viral RNA which was completed in 1982. TMV RNA consists of 6395 nucleotides and was the first genome of a plant virus to be sequenced completely.

Virus Disassembly

Since TMV particles are extremely stable *in vitro*, it was not at all obvious how the RNA managed to be released from the particles in order to start the virus

replication cycle. Using a cell-free translation system, Wilson discovered that the disassembly of TMV particles is initiated when the end of the particles containing the 5′ terminus of the RNA becomes associated with ribosomes. This leads to viral subunits becoming dislodged from the particles while the 5′ terminal open reading frame in the RNA is being translated by the ribosomes, a process known as co-translational disassembly. This mechanism allows the coat protein subunits to protect the RNA from enzymatic degradation until the particle has reached a site in the infected cell where translation can be initiated.

Antigenicity of TMV

The antigenic properties of TMV have been studied extensively for more than 60 years and these studies have given us much information on how antibodies recognize proteins and viruses. TMV is an excellent immunogen and antibodies to the virus are readily obtained by immunization of experimental animals. When the sequence of TMV coat protein became available, it was possible for the first time to locate the antigenic sites or epitopes of a viral protein at the molecular level. Initial studies focused on two antigenic regions of the coat protein, the C-terminal region (residues 153–158) situated at the surface of virus particles and the disordered loop region (residues 103–112) located in the central hole of the particles and accessible to antibodies only in dissociated protein subunits (**Figure 3**). The C-terminal hexapeptide coupled to bovine serum albumin was used by Anderer in Tübingen to raise antibodies and the resulting antiserum was found to precipitate the virus and neutralize its infectivity. Since both natural peptide fragments and synthetic peptides were used in this work, Anderer and his colleagues should be credited with the discovery that synthetic peptides can elicit antibodies that neutralize the infectivity of a virus. Only when similar results were obtained with animal viruses 15 years later, did the potential of peptides for developing synthetic vaccines become clear.

It has been known since the 1950s that intact TMV particles and dissociated coat protein subunits harbor different types of epitopes recognized by specific antibodies. Certain epitopes present only on virions are constituted by residues from neighboring subunits that are recognized as a single entity by certain antibodies; other epitopes arise from conformational changes in the protein that result from intersubunit bonds. Both these types of epitopes which depend on the protein quaternary structure and are absent in dissociated protein subunits have been called neotopes. Another type of epitope known as cryptotope occurs on the portion of the protein surface that is buried in the polymerized rod and becomes accessible to antibodies only in the dissociated subunits.

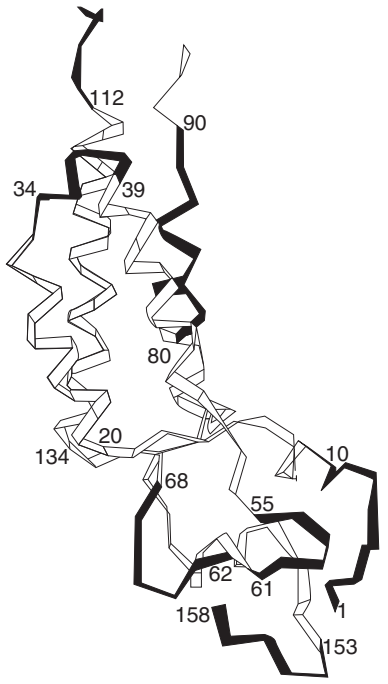


Figure 3 Backbone of the TMV coat protein subunit based on crystallographic data. Residues 94–106 have been omitted because this region, located on the inside of the particle, is disordered. The N and C termini of the protein are located on the outer surface of TMV particles. The position of seven continuous epitopes (residues 1–10, 34–39, 55–61, 62–68, 80–90, 108–112, and 153–158) is indicated by solid lines. Reproduced from Al Moudallal Z, Briand JP, and Van Regenmortel MHV (1985) A major part of the polypeptide chain of tobacco mosaic virus is antigenic. *EMBO Journal* 4: 1231–1235, with permission from Nature Publishing Group.

The mapping of neotopes and cryptotopes on the surface of TMV coat protein was greatly simplified once monoclonal antibodies (Mabs) became available. Many continuous epitopes were identified at the surface of dissociated coat protein subunits by measuring the ability of peptides to react with Mabs or with antisera (**Figure 3**). These epitopes were found to correspond to regions of the protein shown by X-ray crystallography to possess a high segmental mobility. This correlation between antigenicity and mobility along the peptide chain was also found to exist in other proteins and was used to develop algorithms for predicting the location of epitopes in proteins from their primary structure.

The surface of the protein subunits accessible at either end of the virus particle is different and the one located near the 5′ terminus end of the RNA harbors the two helices corresponding to residues 73–89 and 115–135 (**Figure 3**). Many Mabs specific for this surface have been obtained and in addition to reacting with both ends of nonpolar stacked disks, they were found to block TMV disassembly by sterically preventing the interaction between RNA and

ribosomes. It has been suggested that if such antibodies could be expressed in plants, they might be able to control viral infection.

Another interesting immunological phenomenon was discovered when TMV antibodies were analyzed for their ability to react with certain TMV mutants. It was found that all rabbits immunized with TMV induced the formation of heterospecific antibodies, that is, antibodies that were unable to react with the TMV immunogen but recognized the mutant Ni 1927 quite well. When all the antibodies in a TMV antiserum capable of reacting with TMV were removed by cross-absorption with the virus, it was found that the depleted antiserum still reacted strongly with this mutant which had a single proline→leucine exchange. Apparently the removal of the proline at position 156 exposes binding sites for both peptidases and antibodies that are normally out of reach in the wild-type protein structure. The induction of heterospecific antibodies by immunization with TMV is another illustration of the difference between the antigenicity and immunogenicity of proteins. TMV particles possess the immunogenic capacity of eliciting heterospecific antibodies that react with the Ni 1927 mutant but do not have the antigenic capacity of reacting with these antibodies. The reverse situation where an antigenic peptide or protein is able to react with a particular antibody but is unable to induce the same type of antibody when used as immunogen is a more commonly observed phenomenon which greatly hampers the development of synthetic peptide vaccines.

Many of the viruses that are currently classified in the genus *Tobamovirus* were initially considered to be strains of TMV on the basis of similar particle morphology and ability to cross-react with TMV antibodies. Antigenic relationships between different tobamoviruses were quantified using a parameter known as the serological differentiation index (SDI) which is the average number of twofold dilution steps separating homologous from heterologous antiserum titers. A close correlation exists between the antigenic distance of two viruses expressed as SDI values and the degree of sequence difference in their coat proteins. In general, when two viruses differ antigenically by an SDI value larger than 4, they are considered to belong to separate species. This is valid only if relatedness is measured with polyclonal antisera containing antibodies to a range of different epitopes since comparisons made with Mabs specific for a single epitope will emphasize antigenic similarities or differences depending on which particular Mab is used.

Relationships between individual tobamoviruses are nowadays usually assessed by comparisons between viral genomes. Phylogenetic studies have shown that tobamoviruses are very ancient and co-evolved with their angiosperm hosts, which means that they are at least 120 million years old.

Replication and Cell-to-Cell Movement of TMV

TMV-infected tissues contain four viral proteins: the 126 kDa and 183 kDa proteins of the replicase complex, the 30 kDa movement protein, and the 17.6 kDa coat protein. TMV replication is initiated by the translation of the viral RNA to produce the replicase proteins and this leads to the synthesis of minus-sense and plus-sense copies of the RNA. Translation of the viral coat protein gene then occurs leading to the assembly of progeny virus particles from full-length genomic RNA and coat protein. The translation of the coat protein and movement protein is controlled by the production of two separate subgenomic mRNAs, a mechanism later found to occur in many other viruses but first demonstrated with TMV.

The mechanism that allows plant viruses to move from cell to cell in their hosts has remained a mystery for many years. The rigid cellulose-rich walls of plant cells impede intercellular communication which occurs only through tubular connections known as plasmodesmata. However, plasmodesmata are too narrow to allow the passage of virus particles. Studies with the 30 kDa TMV movement protein showed that this protein changed the size exclusion limit of the plasmodesmata, allowing the virus to move through them in the form of a thin, less than 2 nm wide, ribonucleoprotein complex composed of the genomic RNA and the movement protein. Movement proteins have subsequently been found in many other plant viruses and shown to possess RNA-binding properties.

Studies with TMV also revealed how plant viruses encode a suppressor to combat the post-transcriptional gene silencing reaction that plants use to fight virus infection.

Biotechnology Applications of TMV

TMV-resistant plants have been obtained by transforming them with a DNA copy of the TMV coat protein gene. This coat protein-mediated resistance is due to the ability of transgenically expressed coat protein to interfere in transgenic cells with the disassembly of TMV particles. Coat protein-mediated resistance has subsequently been obtained with many positive-sense RNA plant viruses.

A portion of the TMV RNA leader sequence, called omega, was shown to enhance the translation of foreign genes introduced into transgenic plants. This translational enhancer has been incorporated successfully in many gene vectors for a variety of applications.

In another type of application, TMV particles have been used as surface carriers of foreign peptide epitopes for constructing recombinant vaccines and producing them in plants. It was found that the N- and C-termini of the TMV coat protein as well as the surface loop area corresponding to residues 59–65 were able to accept foreign peptide

fusions without impairing the ability of the resulting chimeric virus to infect plants systemically. Several experimental vaccines against viral and parasitic infections that are based on genetically engineered TMV particles produced in tobacco are currently under development.

In conclusion, it is clear that a series of historical accidents together with the fact that large amounts of remarkably stable TMV particles could be obtained from infected plants, allowed this virus to play a major role in the development of virology. In addition, studies of TMV also contributed significantly to the advancement of molecular biology and to our understanding of the physicochemical and antigenic properties of macromolecules.

See also: Antigenicity and Immunogenicity of Viral Proteins; Nature of Viruses; Plant Virus Vectors (Gene Expression Systems); *Tobamovirus*; Vaccine Production in Plants; Viral Suppressors of Gene Silencing.

Further Reading

Al Moudallal Z, Briand JP, and Van Regenmortel MHV (1985) A major part of the polypeptide chain of tobacco mosaic virus is antigenic. *EMBO Journal* 4: 1231–1235.

Bos L (1999) Beijerinck's work on tobacco mosaic virus: Historical context and legacy. *Philosophical Transactions of the Royal Society, London, Series B* 534: 675–685.

Calisher CH and Horzinek MC (eds.) *100 Years of Virology. The Birth and Growth of a Discipline*, pp. 1–220. Vienna: Springer.

Creager ANH (ed.) (2002) *The Life of a Virus. Tobacco Mosaic Virus as an Experimental Model*, pp. 1–398. Chicago, IL: University of Chicago Press.

Durham ACH and Klug A (1972) Structures and roles of the polymorphic forms of tobacco mosaic virus protein. *Journal of Molecular Biology* 67: 315–332.

Harrison BD and Wilson TMA (1999) *Tobacco Mosaic Virus: Pioneering Research for a Century*. *Philosophical Transactions of the Royal Society, London, Series B* 354: 517–685.

Hirth L and Richards KE (1981) Tobacco mosaic virus: Model for structure and function of a simple virus. *Advances in Virus Research* 26: 145–199.

Mahy BWJ and Lvov DK (eds.) (1993) *Concepts in Virology. From Ivanovsky to the Present*, pp. 1–438. Langhorne, PA: Harwood Academic Publishers.

Scholthof K-BG (2004) Tobacco mosaic virus: A model system for plant biology. *Annual Review of Phytopathology* 42: 13–34.

Scholthof K-BG, Shaw JG, and Zaitlin M (1999) *Tobacco Mosaic Virus: One Hundred Years of Contributions to Virology*, pp. 1–256. St. Paul, MN: American Phytopathologial Society Press.

Van Helvoort T (1991) What is a virus? The case of tobacco mosaic disease. *Studies in History and Philosophy of Science* 22: 557–588.

Van Regenmortel MHV (1999) The antigenicity of TMV. *Philosophical Transactions of the Royal Society, London, Series B* 354: 559–568.

Van Regenmortel MHV and Fraenkel-Conrat H (eds.) (1986) *The Plant Viruses. Vol. 2: The Rod-Shaped Plant Viruses,* pp. 1–180. New York, NY: Plenum.

Tobacco Viruses

S A Tolin, Virginia Polytechnic Institute and State University, Blacksburg, VA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Transient virus vector The use of a virus, such as tobacco mosaic virus, for the expression of a foreign gene following insertion of the sequence at a site in the viral genome.

Virus vector A biological organism, such as an aphid, leafhopper, thrips, whitefly, or fungus, that is capable of specific horizontal transmission of a virus from one plant to another.

Introduction

Tobacco has played a role in agricultural history and in the history of virology. Twenty plant virus names begin with tobacco, indicating that tobacco was a host of great agricultural and economic importance, was susceptible to a large number of viruses, and was the object of early virus research. Early in the last century, when viruses were first discovered and named, research programs emphasized controlling diseases of tobacco, many of which were caused by viruses. This article discusses the role of the tobacco in virology, viruses as causes of tobacco diseases, and some of the major milestones in virology achieved with tobacco viruses.

Tobacco is in the genus *Nicotiana* (family Solanaceae), created in 1565 and named after a French promoter of tobacco, Jean Nicot von Villemain. Although the genus is quite diverse and contains about 100 species, only two species have been extensively cultivated as commercial crops. *Nicotiana rustica* was originally grown by Native Americans in the eastern United States, and was the first tobacco species introduced to England and Portugal. *Nicotiana tabacum* was grown in Mexico and South America, and was introduced to the early Spanish explorers. With the cultivation of the species by the Virginia colony in Jamestown, *N. tabacum* became the preferred tobacco type, and is the predominant species today.

Tobacco is the most widely grown nonfood crop in the world, and is thought to have been used by people in the Americas for smoking and chewing since 1000 BC. It played a major role in the colonization of the Americas by Europeans, and was an economic driver for nearly four centuries. Although always a controversial and exotic commodity, tobacco became established in Europe before coffee, tea, sugar, and chocolate. In spite of the human health risks of tobacco products, tobacco continues to be grown throughout the world.

Three major types of the *N. tabacum* predominate, each with different characteristics depending on the final use of the tobacco product. The most common type is Virginia or flue-cured tobacco, which is used for cigarette, pipe, and chewing tobacco. It is grown worldwide, with the USA and China as major producers. Burley tobacco, the second-most popular type, is air-cured and used in chewing tobacco, and is flavored and blended for American-type cigarettes and pipe tobacco. Burley tobacco is also widely grown, but in not as many countries as is Virginia tobacco. A third type, Oriental or Turkish tobacco, is a small-leafed tobacco and is sun-cured and used in English blends. The name is derived from the eastern Mediterranean area where it is grown. Oriental cultivars Samson from Turkey and Xanthium from Greece have been widely used in virus research.

Viruses as Causal Agents of Tobacco Diseases

There are 20 viruses that were either first isolated from tobacco or contain tobacco as the host in their name (TEV, TWV) (**Table 1**). Chronologically these reports span over a century. Seven viruses naturally infect tobacco, but were first isolated from other crops and named from them (**Table 2**). The viruses are discussed individually, but some are synergistic in mixed infections and cause symptoms more severe than either virus alone. Satellites viruses and RNAs associated with tobacco viruses were the first to be recognized as subviral entities (**Table 3**). The greatest recent new activity with tobacco viral diseases has been in understanding the complexity of the viruses with similarities to tobacco leaf curl virus (family *Geminiviridae*) and their worldwide distribution.

Tobacco in Virology History

Tobacco was used as a host plant for virology research leading to many fundamental discoveries, only a few of which are highlighted here. *Nicotiana* species widely used for virus propagation and host range tests are *N. clevelandii*, *N. tabacum*, *N. glutinosa*, *N. megalosiphon*, *N. sylvestris*, and, more recently, *N. benthamiana*. These species are reported as being experimentally susceptible to 267, 231, 201, 93, 57, and 141 viruses, and insusceptible to 80, >200, 175, 35, 35, and 40 viruses, respectively. Responses of tobacco species and cultivars to mechanical inoculation are used for virus and strain identification. Cytopathic effects and inclusion bodies induced by plant viruses were first observed by light microscopy with X-bodies of TMV and nuclear and cytoplasmic inclusions of TEV and PVY. Details of plant virus replication processes were advanced by studies using tobacco leaf protoplasts. Tobacco was also the first plant to be genetically transformed by recombinant DNA techniques, and the first in which pathogen-derived resistance was demonstrated by the insertion of the coat protein gene from TMV. Tobacco transformed with noncoding viral sequences of TEV led to the discovery of RNAi and viral-induced gene silencing (VIGS).

Viruses Causing Economically Important Diseases of Tobacco

Tobamoviruses

The causal agent of a mosaic disease of tobacco, tobacco mosaic virus (TMV, genus *Tobamovirus*) became the first virus to be purified, crystallized, and characterized. Milestones in virology history that have been accomplished with TMV have been well documented, and will not be repeated here. The virus has a rigid, rod-shaped particle constructed of a single capsid protein arranged helically around a single strand of RNA encoding four proteins, functions of which are well characterized in viral replication and movement. Transient vectors can be constructed from TMV, permitting insertion and expression of foreign genes for new products, or for labeling virus for pathogenicity and cellular biology experiments.

The mosaic disease occurs worldwide wherever tobacco is grown. The stability of the virus in tobacco products, its highly infectious nature, and efficient inoculation by mechanical abrasion contributes to the difficulty tobacco farmers have had in controlling this virus. In certain cultivars of burley and ornamental tobaccos, the *N* gene for resistance has provided durable resistance for nearly 75 years, considered remarkable for an RNA virus, as resistance-breaking strains have not developed. Use of the *N* gene in Virginia or flue-cured tobacco, however, has not been successful because of poor-quality traits associated with linked genes. Efforts to resolve this using hybrids have only been moderately successful because systemic necrosis can develop in field-infected plants and cause more serious loss than that caused by mosaic.

Other tobamoviruses infecting tobacco are tomato mosaic virus (ToMV) and tobacco mild green mosaic virus (TMGMV). ToMV and TMV are seed-borne on tomato seed, but reportedly not on tobacco seed. However, production of tobacco seedlings in float trays in

Table 1 Chronology of discovery and naming of tobacco viruses

Virus name	*Virus acronym*	*Genus/family*	*Year described*	*Crop of origin*	*Country*	*Investigator*	*Vector taxon*
Tobacco mosaic virus	TMV	*Tobamovirus*	1892, 1896 1914	Tobacco Tobacco	USSR USA	Iwanowski Allard	None
Tobacco etch virus	TEV	*Potyvirus/Potyviridae*	1921	*Datura* spp.	USA	Blakeslee	Aphid
Tobacco ringspot virus	TRSV	*Nepovirus* / *Comoviridae*	1927	Tobacco	USA (VA)	Fromme	Nematode
Tobacco mild green mosaic virus	TMGMV	*Tobamovirus*	1929	*N. glauca*	Canary Islands	McKinney	None
Tobacco rattle virus	TRV	*Tobravirus*	1931	Tobacco	Germany	Boning	Nematode
Tobacco leaf curl virus	TLCV	*Begomovirus* / *Geminiviridae*	1931	Tobacco	Tanzania	Storey	Whitefly
Tobacco necrosis virus	TNV	*Necrovirus* *Tombusviridae*	1935	Tobacco	UK	Smith & Bald	Fungus
Tobacco streak virus	TSV	*Ilarvirus* /*Bromoviridae*	1936	Tobacco	USA (WI)	Johnson	Thrips
Tobacco yellow dwarf virus	TYDV	*Mastrevirus*/ *Geminiviridae*	1937	Tobacco	Australia	Hill	Leafhopper
Tobacco mottle virus + Tobacco vein distorting virus	TMoV + TVDV	*Umbravirus* + *Luteovirus*	1946	Tobacco	Zimbabwe	Smith	Aphid
Tobacco stunt virus	TStV	*Varicosavirus*	1950	Tobacco	Japan	Hidaka	*Olpidium brassicae*
Tobacco wilt virus	TWV	*Potyvirus/Potyviridae*	1959	*Solanum jasminoides*	India		Aphid
Tobacco bushy top virus	TBTV	*Umbravirus*	1962	Tobacco	Rhodesia		Aphid
Tobacco yellow vein virus + Tobacco yellow vein assistor virus	TYVV + TYVAV	*Umbravirus* + *Luteoviridae*	1972	Tobacco	Malawi	Adams & Hull	Aphid
Tobacco vein mottling virus	TVMV	*Potyvirus/Potyviridae*	1972	Tobacco	USA (NC)	Gooding	Aphid
Tobacco necrotic dwarf virus	TNDV	*Luteoviridae*	1977	Tobacco	Japan	Kubo	Aphid
Tobacco apical stunt virus	TASV	*Begomovirus*/ *Geminiviridae*	1999	Tobacco	Mexico	Brown	Whitefly
Tobacco curly shoot virus	TCSV	*Begomovirus*/ *Geminiviridae*	2002	Tobacco	China (Yunnan)	Zhou	Whitefly

Table 2 Additional viruses reported to naturally occur in tobacco

Virus name	*Virus acronym*	*Genus/family*	*Year described*	*Crop of origin*	*Country*	*Investigator*	*Vector taxon*
Cucumber mosaic virus	CMV	*Cucumovirus*/ *Bromoviridae*	1916	Cucumber	USA	Doolittle Jagger	Aphid
Tomato mosaic virus	ToMV	*Tobamovirus*	1919	Tomato	USA	Clinton	None
Tomato spotted wilt virus	TSWV	*Tospovirus*/ *Bunyaviridae*	1930	Tomato	India	Samuel	Thrips
Alfalfa mosaic virus	AMV	*Alfamovirus*/ *Bromoviridae*	1931	Alfalfa	USA	Weime	Aphid
Potato virus Y	PVY	*Potyvirus*/*Potyviridae*	1931	Potato	USA	Smith	Aphid
Eggplant mottled dwarf virus	EMDV	*Nucleorhabdovirus*/ *Rhabdoviridae*	1969	Eggplant	Italy	Martelli	Cicadellid leafhopper
Eggplant mosaic virus – tobacco strain	EMV-T	*Tymovirus*/ *Tymoviridae*	1996	Tobacco	Brazil	Ribiero	Chrosomelid beetle

Table 3 Satellite viruses and nucleic acids associated with tobacco viruses

Satellite name	*Helper virus*	*Year described*	*Crop of origin*	*Investigator – country*	*Characteristics*
Tobacco necrosis satellite virus	TNV	1960	Tobacco	Kassanis – UK	18 nm, $T=1$
Tobacco mosaic satellite virus	TMV	1986	*N. glauca*	Dodds – US	17 nm, $T=1$; 1059 nt
Tobacco bushy top virus satellite RNA	TBTV	2001	Tobacco	China	Linear, 650–800 nt
Tobacco ringspot virus satellite RNA	TRSV	1972	Bean	Schneider	Circular, 359 nt, viroid-like
Cucumber mosaic virus satellite RNA	CMV	1976	Tobacco	Kaper – Italy	Linear, 330–390 nt
Tobacco leaf curl virus DNA β	TLCV	2003	Tobacco	Zhou – China	Circular, 1300–1350 nt
Tobacco curly shoot virus DNA β	TCSV	2005	Tobacco	Li – China	Circular

greenhouses suggests anecdotally that TMV-infested seed may provide inoculum source for seedling infections. Instances of spread of ToMV from tomato seedlings to tobacco seedlings have been observed. ToMV causes more severe symptoms than does TMV. TMGMV naturally infects *N. glauca*, and is often associated with an icosahedral satellite virus, satTMV (**Table 3**).

Bromoviruses

Certain viruses of tobacco are the type members of three genera of the family *Bromoviridae*, namely cucumber mosaic virus (CMV, genus *Cucumovirus*), alfalfa mosaic virus (AMV, genus *Alfamovirus*), and tobacco streak virus (TSV, genus *Ilarvirus*). These viruses continue to occur in tobacco worldwide. Disease outbreaks are sporadic, but are of economic significance in fields infected at a high incidence. All three viruses have very wide host ranges, are known to infect a number of weeds and native plants naturally, and are seed transmitted in many of their host plants. CMV and AMV are transmitted in a stylet-borne, nonpersistent manner by many species of aphids. TSV, however, is spread by contact and through pollen carried by thrips.

Common properties include a tripartite, single-stranded RNA (ssRNA) genome, and encapsidation of a fourth subgenomic RNA encoding the capsid protein. Particles have icosahedral symmetry, but AMV forms multiple bacilliform particles and TSV forms quasi-icosahedrons. CMV capsids are of $T=3$ symmetry.

Cucumber mosaic and alfalfa mosaic viruses

Symptoms caused by CMV are similar to those of TMV. CMV is an important tobacco pathogen in Asia and in certain European countries. In recent surveys in Greece, burley, Virginia, and oriental types all had a high incidence of CMV, even up to 100% infected plants in some fields. Symptoms of AMV on tobacco are a bright yellowing of leaf areas, broad rings, and mosaic on young leaves. AMV incidence was lower, but was highest in tobacco growing near alfalfa fields, a source of the virus.

Many fundamental discoveries of genome structure, organization, and encapsidation occurred with AMV and CMV in the 1960s and 1970s using tobacco as the main test

species. Sucrose density-gradient centrifugation enabled separation of the multiple particles of AMV, leading to experiments to demonstrate the requirement of RNA1, RNA2, and RNA3 plus either RNA4 or free coat protein for infectivity. Genetic experiments were conducted by mixed inoculations with RNA species from biologically distinct strains. Similar experiments were conducted with CMV, which required separation on cesium chloride gradients based on density to demonstrate separate encapsidation of RNA1, RNA2, and RNA3 + RNA4 in a third capsid. Infectivity required RNAs 1, 2, and 3, but not RNA4 or coat protein. A fifth, small RNA species, initially termed RNA5 or CARNA5 (CMV-associated RNA), was later shown to be the first satellite RNA species. These satellite RNAs can either ameliorate or exacerbate symptoms in crops. Severe lethal necrosis symptoms are associated with CMV satellite RNA in tomato in Italy and tobacco in India.

Tobacco streak virus

TSV is rarely reported on tobacco, but is an important pathogen of many annual and vegetatively propagated crops and wild species worldwide. In tobacco, TSV causes necrotic lines along veins of leaves as virus moves systemically from the point of inoculation. Symptoms develop on a few leaves, and then plants recover. Incidence of infected tobacco plants is usually low. Thrips are recognized as a vector of TSV. TSV belongs to subgroup 1 of the genus *Ilarvirus*, which is divided into six subgroups based on serological relatedness.

Potyviruses

Three viruses of the genus *Potyvirus* (family *Potyviridae*), tobacco etch virus (TEV), potato virus Y (PVY), and tobacco vein mottling virus (TVMV), are among the most important viral pathogens of tobacco. All are aphid transmitted, with flexuous filamentous particles *c.* 13 × 750 nm containing an ssRNA genome of about 10 kb. Successful aphid transmission depends upon a DAG sequence in the N-terminus of the coat protein and a viral sequence encoding a helper component (HC). The genome is expressed as a polyprotein, which is cleaved by viral-encoded proteases (Pro) into a single structural and several nonstructural proteins. Both TEV and PVY occur nearly worldwide and have wide host ranges. Diseases caused by potyviruses are managed in part through resistant cultivars such as TN86, a burley tobacco resistant to TVMV, TEV, and PVY. Several burley varieties have partial or high resistance to one or more viruses, as well as resistance to fungi and nematodes.

Tobacco etch virus

TEV has a rather narrow host range, but is found in solanaceous weeds including *Datura* sp., from which it was first isolated in the 1920s. The virus is common only in North and South America. Isolates vary in symptom severity and ability to cause necrosis or severe versus mild etch in tobacco. TEV is also known to cause severe symptoms in pepper and tomato crops in the USA, Central America, and the Caribbean. The few isolates that have been sequenced show very little diversity, and are indistinguishable serologically.

TEV has also been a model virus in the elucidation of many host–virus interactions and viral genome expression. The 5′ leader of TEV genomic RNA is used in many constructs for genetic engineering, as it contains an internal ribosome entry site and directs efficient translation of uncapped mRNA. TEV was the first virus demonstrated to encode proteases, including the autocatalytic small nuclear inclusion proteinase and a protease linked to helper component (HC-Pro).

Transgenic resistance to TEV was the first to be due to RNA interference and gene silencing, as nontranslatable sequences of the capsid protein-encoding region of the viral genome were more effective than the capsid protein translatable sequences. This observation led to the discovery RNA interference as well as the silencing suppression function by the HC-Pro region of the genome.

Potato virus Y

PVY, first isolated from potato in the 1930s as one of a complex of viruses associated with the century-old potato degeneration, is one of the most studied plant viruses and is the type member of the genus *Potyvirus*. PVY remains an important pathogen of potato and also of tobacco. Severe epidemics of PVY in tobacco have been reported recently in China. In Greece, PVY is one of the most prevalent viruses isolated from seedbeds and fields. Symptoms associated with PVY are vein clearing and/or top necrosis in oriental tobacco, and yellowing with veinal necrosis in Virginia and burley tobacco. PVY has a wide host range among the Solanaceae, including 70 species of *Nicotiana* and is also found in annual and perennial weeds in 15 or more plant families. A number of strains have been described both on potato and on tobacco, distinguishable by inoculation to differential hosts and by sequence analysis.

Other potyviruses

TVMV was not described until the 1970s, when it emerged as a new virus in the burley tobacco-growing regions of the USA, namely Kentucky, North Carolina, Tennessee, and Virginia. Severe losses were recorded until resistance to this virus, and partial resistance to PVY and TEV, was incorporated into burley tobacco cultivars. A virus isolated from tobacco in Nigeria was identified as being closely related to the potyvirus pepper veinal mottle virus, and both reacted with TVMV antibody in electron microscope serology, but not enzyme-linked

immunosorbent assay (ELISA). However, this reaction alone is not sufficient to prove virus identity. Only one additional potyvirus, tobacco wilt virus (TWV), has been reported from tobacco, and it is only known in India.

Tospoviruses

Tomato spotted wilt virus (TSWV), a member of the genus *Tospovirus* (family *Bunyaviridae*), is a serious pathogen on tobacco in many regions of the world. This thrips-transmitted virus has a very wide host range infecting over 800 species in 90 or more plant families, both monocots and dicots, and causes significant yield losses in a number of economically important crops. The virus has spherical, membrane-bound particles, 80–120 nm, with a three-segment, ambisense genome encapsided in the same particle. Symptoms in tobacco infected early are severe leaf necrosis and stunting. Several management approaches have involved weed reservoir and vector management. In Georgia in the southeastern US, successful management has been accomplished with compounds to induce resistance combined with imidochloprid insecticide applications in seedling trays and at transplanting. Yield losses were reduced significantly.

Geminiviruses (Family *Geminiviridae*)

Mastreviruses

Tobacco yellow dwarf virus (TYDV) was described in Australia in 1937, and later determined to be one of only three dicot-infecting members of the genus *Mastrevirus* (family *Geminiviridae*). It is yet to be recorded outside of Australia where it causes severe dwarfing and downward leaf curl in tobacco, and a lethal, necrotic disease in bean (syn. bean summer death virus). The virus is transmitted persistently by the brown leafhopper *Orosius argentatus*, but not by other leafhoppers nor mechanically. TYDV also infects several summer annual and autumn–spring growing plants, providing a continuous succession of host plants for the virus, and a source of virus for tobacco. Like other mastreviruses, TYDV has a single-stranded DNA genome, single component, and has coding regions in both virion sense and complementary sense sequences.

Begomoviruses

The tobacco leaf curl disease was first described in Tanzania in the early 1930s, in which infected tobacco plants were severely crinkled, curled, and dwarfed. The disease was successfully transmitted by the whitefly *Bemisia tabaci*, but not mechanically, suggesting that a virus was the causal agent. Similar disease symptoms in tobacco were noted as early as 1902 in South Africa, in 1912 in the Netherlands East Indies, in the 1930s in India, and in Brazil and Venezuela prior to 1950. Positive association of the tobacco leaf curl disease with a begomovirus was not demonstrated until DNA analyses and polymerase chain reaction (PCR) became available. The name tobacco leaf curl virus (TLCV, genus *Begomovirus*, family *Geminiviridae*) is now accepted for this virus.

However, new viruses are being described worldwide in association with severely diseased tobacco. There are numerous recent reports of TLCV-like viruses from Asia, including Japan, China, and India, as well as from Dominican Republic and Cuba. Those viruses are now accepted as members of the genus *Begomovirus*: tobacco leaf curl Japan virus (TbLCJV), tobacco leaf curl Kochi virus (TbLCKoV), tobacco leaf curl Yunnan virus (TbLCYNV), tobacco leaf curl Zimbabwe virus (TbLCZV), and tobacco curly shoot virus (=tobacco leaf curl – China, TbCSV). Sequences have also been reported for tobacco leaf curl virus – Karnataka 1 and 2 from India. Tentative begomovirus members include tobacco apical stunt virus, tobacco leaf curl India virus, and tobacco leaf rugose virus – Cuba.

All TLCV-like viruses have a single-stranded, monopartite DNA genome, and have only the DNA-A component. TbLCYNV was the first virus to be shown to have a DNAβ component, a type of satellite DNA widely associated with the virus in the Yunnan Province of China and with TbCSV (**Table 3**). Like other satellites, DNA β molecules have no sequences in common with their helper virus, DNA-A, which replicates independently of DNA β. With TbCSV, DNA β affects pathogenicity, with increasing symptom severity in the presence of specific molecules.

Viruses Causing Diseases of Tobacco of Lesser or Minor Importance

Nepoviruses

Tobacco ringspot virus (TRSV) caused a severe disease of tobacco in Virginia in the 1920s. Symptoms initially were severe necrotic rings with green centers, and a pattern of necrotic lines described as an 'oak-leaf' pattern. Tobacco plants expressed symptoms on three to four leaves developing following inoculation, but not on later-developing leaves. This apparent recovery was the first phenomenon to be described as acquired immunity. In recent research, recovery from symptoms induced by TRSV was the first to be attributed to plant defenses and to involve RNAi. The ssRNA genome is bipartite and encapsidated in separate icosahedral particles, typical of members of the family *Comoviridae*. Additional small RNAs found in the 1970s to also be encapsidated in TRSV particles were the first satellite RNAs to be discovered (**Table 3**) and later shown to have hammerhead, ribozyme-like structures capable of self-cleavage. These structures are also found in certain viroids.

TRSV is the type species of the genus *Nepovirus*. It is classified in subgroup A of this genus based on RNA2 size and sequence, and on serological relationships. The genus is named from the property of being nematode transmitted (by *Xiphenema* spp.) and having polyhedral particles. The virus is spread naturally in North America by these nematodes as well as by possible arthropod vectors. It has a very wide host range and is found naturally in many weeds and woody species. Additionally, TRSV has been disseminated worldwide in vegetative propagules of perennial crops and ornamentals, but does not appear to spread in the absence of the nematode vector.

Artichoke yellow ringspot virus (AYRSV) is known to infect tobacco naturally, causing chlorotic blotches, rings, and lines, and to spread naturally in Greece, Italy, and Eurasia. It is reported to spread via pollen to seeds or seed parent plants of *N. clevelandii*, *N. glutinosa*, and *N. tabacum*. Serological evidence indicates that AYRSV is a distinct species, and RNA2 properties place it in the subgroup C of nepoviruses, which includes tomato ringspot and cherry leaf roll viruses.

Tobraviruses

Tobacco rattle virus (TRV, genus *Tobravirus*), first described on tobacco in the 1930s, is now seldom reported on tobacco crops. Symptoms of rattle on tobacco are systemic necrotic flecks and line patterns, and death or stunting of shoots. It was known as *Mauche*, *ratel*, *Streifen und Kräuselkrankheit* in Germany, its geographic origin. Distribution today is limited to Europe, Japan, New Zealand, and North America. The host range of TRV is very wide, as it infects over 400 species of both dicots and monocots, many of which are weeds and other wild plants. TRV causes important diseases of potato, pepper, and various ornamentals.

Biological vectors of TRV are nematodes in the genera *Paratrichodorus* and *Trichodorus* (Trichodoridae). *Nicotiana clevelandii* is recommended as a propagative host for TRV, and as a trap plant for testing nematode transmission characteristics. Transmissibility is mediated by read-through proteins encoded on RNA-2 of the bipartite, ssRNA genome which extend from the surface of rigid rod-shaped particles of various sizes. This region of RNA-2 has been exploited for expression of introduced genes, and the virus is now widely used as a virus-induced gene silencing (VIGS) vector for genetic experiments.

Necroviruses

Tobacco necrosis virus (TNV) is no longer associated with diseases of tobacco, but rather with bean stipple streak and tulip necrosis diseases. TNV is classified in the genus *Necrovirus* (family *Tombusviridae*). It is one of the few viruses transmitted by the fungus *Olpidium brassicae* and often occurs in irrigated soils and in greenhouses. Virus particles adsorb to the surface of resting spores, on which they do not survive for long periods. Symptoms on tobacco are localized necrosis, with very little systemic movement within infected plants. Electron microscopy of purified TNV revealed 26–28 nm isometric particles of $T = 3$ symmetry. Some cultures also had 17 nm particles, which were a satellite virus (satTNV) encoding a capsid protein that assembled into a $T = 1$ particle. satTNV is found in Europe and North America in association with bean, but not with tobacco.

Varicosaviruses

Tobacco stunt virus (TStV) is reported only from Japan, where it was found in the 1950s to cause severe stunting and necrosis in tobacco. This virus was among the first to be shown to be transmitted by a fungus, *O. brassicae* (order Chytridiales), in which it is borne internally in resting spores and persists for 20 years or more. Once soil is infested with spores containing virus, tobacco planted into the soil will become infected and show symptoms within 2 weeks. The RNA genome of TStV is encapsided as two segments in straight, rod-shaped, rather labile, particles. The genome is now classified as being negative sense and single-stranded, with genome organization like that of rhabdoviruses. Taxonomically, TStV is in the genus *Varicosavirus*, whose name comes from the enlarged (varicose) veins induced in lettuce by the virus now known as lettuce big-vein associated virus. Recent molecular analyses suggest that TStV is a strain of this virus, rather than a distinct species.

Luteoviruses and Umbraviruses

Diseases caused by luteoviruses alone

Tobacco necrotic dwarf virus (TNDV) of the genus *Enamovirus* (family *Luteoviridae*) causes severe disease symptoms in some tobacco fields in Japan. It is transmitted by the aphid *Myzus persicae* in a persistent manner, but not mechanically. Early infection causes stunting and premature yellowing or death of young leaves of tobacco, but distribution is apparently limited.

Tobacco rosette and other diseases with dependent transmission

An aphid-transmitted disease known as tobacco rosette was widespread in Zimbabwe in sub-Saharan Africa in the 1930s. Smith in 1946 discovered that the sap-transmissible tobacco mottle virus (TMoV) could only be transmitted by aphids if plants were co-infected with tobacco vein distorting virus (TVDV), making tobacco rosette the first disease complex associated with dependent transmission. TVDV, a member of the genus *Enamovirus*, is the assistor virus for TMoV, a presumed member of the genus

Umbravirus based on sap transmissibility and other biological properties. Sequence data of one of the two recognized strains of TMoV confirmed this assignment in 2001.

Two other diseases of tobacco have complex etiology presumed to involve an umbravirus–luteovirus complex. Tobacco yellow vein virus (TYVV), a tentative species of the genus *Umbravirus*, and tobacco yellow vein assistor virus, a tentative member of the family *Luteoviridae*, were described as causing a tobacco disease with chlorotic vein-banding and leaf malformation symptoms in Malawi. Tobacco bushy top disease occurring in Rhodesia since the early 1960s is known to be caused by TVDV and a different umbravirus, tobacco bushy top virus (TBTV), and is characterized by pale green, mottling, and extreme shoot proliferation. TBTV and TVDV have recently been detected in China in tobacco showing severe witches' broom symptoms, the etiology of which had been presumed to be phytoplasma. Sequence data from these viruses confirm their classification. The significance of this observation is that this is the first incidence of an umbravirus–luteovirus complex reported in China, and first outside of sub-Saharan Africa.

Tymoviruses

A virus was isolated from naturally infected tobacco in Brazil in 1996, and described as eggplant mosaic virus-tobacco strain (EMV-T), the first report of a virus of the genus *Tymovirus* (family *Tymoviridae*) from tobacco. The virus caused mosaic symptoms, but did not reduce plant productivity. It was experimentally transmitted by sap and by the chrysomelid beetle, *Diabrotica speciosa*. As this is the only report of its incidence in tobacco, EMV-T is of only minor importance in tobacco in a limited geographic region. EMV is a pathogen of eggplant and other solanaceous vegetables, and was limited in distribution to Trinidad and Tobago. It is closely related to Andean potato latent virus, but is considered a distinct species. Genomic relationships between EMV and EMV-T have not been reported.

Nucleorhabdoviruses

Eggplant mottled dwarf virus (EMDV, genus *Nucleorhabdovirus*, family *Rhabdoviridae*) was found in tobacco plants in Greece. Plants showed severe stunting, leaf deformation, and vein clearing, and were not infected by other common tobacco viruses. Incidence was at less than 0.01%, mainly in plants near the edges of the field suggesting a weed reservoir for the virus, which has a cicadellid leafhopper vector, *Agallia vorobjevi*. EMDV has been known in the Mediterranean area since 1969 and was reported from tobacco in Italy in 1996 as tobacco vein yellowing virus, now a synonym of EMDV. EMDV infects other solanaceous crops, and appears to be spreading to a wider geographical range from Spain to the Middle East.

See also: Alfalfa Mosaic Virus; Cucumber Mosaic Virus; Emerging and Reemerging Virus Diseases of Plants; History of Virology: Plant Viruses; *Ilarvirus*; Plant Resistance to Viruses: Engineered Resistance; Plant Virus Diseases: Economic Aspects; Potato Virus Y; Ribozymes; Satellite Nucleic Acids and Viruses; Tobacco Mosaic Virus; Tomato Spotted Wilt Virus; Vector Transmission of Plant Viruses; Viral Suppressors of Gene Silencing; Virus Induced Gene Silencing (VIGS).

Further Reading

Adams MJ and Antoniw JF (2005) DPVweb: An open access Internet resource on plant viruses and virus diseases. *Outlooks on Pest Management* 16: 268–270.

Adams MJ, Antoniw JF, and Fauquet CM (2005) Molecular criteria for genus and species discrimination within the family. *Potyviridae Archives for Virology* 150: 459–479.

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, and Zurcher EJ (eds.) (1996 onward) Plant viruses online: Descriptions and lists from the VIDE Database. Version: 20 Aug. 1996. http://image.fs.uidaho.edu/vide/ (accessed May 2007).

Chatzivassiliou IK, Efthimiou K, Drossos E, Papadopoulou A, Poimenidis G, and Katis NI (2004) A survey of tobacco viruses in tobacco crops and native flora in Greece. *European Journal of Plant Pathology* 110: 1011–1023.

Cui XF, Xie Y, and Zhou XP (2004) Molecular characterization of DNAβ molecules associated with tobacco leaf curl Yunnan virus. *Journal of Phytopathology* 152: 647–650.

Harrison BD and Wilson TMA (1999) Milestones in the research on tobacco mosaic virus. *Philosophical Transactions of the Royal Society of London, Section B* 345: 521–529.

Lindbo JA and Dougherty WG (2005) Plant pathology and RNAi: A brief history. *Annual Review of Phytopathology* 43: 191–204.

Mansoor S, Briddon RW, Zafir Y, and Stanley J (2003) Geminivirus disease complexes: An emerging threat. *Trends in Plant Science* 8: 128–134.

Paximadis M, Idris AM, Torres-Jerez I, Villareal A, Rey MEC, and Brown JK (1999) Characterization of tobacco geminiviruses in the Old and New World. *Archives of Virology* 144: 703–717.

Ratcliff F, Martin-Hernandez AM, and Baulcombe DC (2001) Tobacco rattle virus as a vector for analysis of gene function by silencing. *Plant Journal* 25: 237–245.

Sasaya T, Ishikawa K, Kuwata S, and Koganezawa H (2005) Molecular analysis of coat protein coding region of tobacco stunt virus shows that it is a strain of *Lettuce big-vein virus* in the genus Varicosavirus. *Archives of Virolology* 150: 1013–1021.

Shew HD and Lucas GB (eds.) (1991) *Compendium of Tobacco Diseases,* 96pp. St. Paul, MN: APS Press.

Simon AE, Roossinck MJ, and Havelda Z (2004) Plant virus satellite and defective interfering RNAs: New paradigms for a new century. *Annual Review of Phytopathology* 42: 415–437.

Syller J (2003) Molecular and biological features of umbravirus, the unusual plant viruses lacking genetic information for a capsid protein. *Physiological and Molecular Plant Pathology* 63: 35–46.

Xie Y, Jiang T, and Zhou X (2006) Agroinoculation shows tobacco leaf curl Yunnan virus is a monopartitie begomovirus. *European Journal of Plant Pathology* 115: 369–375.

Relevant Website

http://www.dpvweb.net – DPVWeb Home Page.

Tobamovirus

D J Lewandowski, The Ohio State University, Columbus, OH, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Origin of assembly Stem–loop structure that is the site of initiation of virion assembly.
Pseudoknot An RNA structure with base pairing between a loop and other regions of the RNA.

Introduction

Early research in the late 1800s on the causal agent of the mosaic disease of tobacco led to the discovery of viruses as new infectious agents. Thus tobacco mosaic virus (TMV), the type species of the genus *Tobamovirus*, became the first virus to be discovered, and subsequently has had a significant role in many fundamental discoveries in virology. The first quantitative biological assay for plant viruses was the use of *Nicotiana glutinosa* plants, which produce necrotic local lesions when inoculated with TMV and many tobamoviruses. The resistance gene *N* that confers this hypersensitive response-type resistance to TMV was the first resistance gene against a plant virus to be cloned and characterized. TMV was the first virus to be purified and crystallized, which led to the discovery of the nucleoprotein nature of viruses and determination of the atomic structure of the coat protein and the virion. TMV was the first virus to be visualized in the electron microscope, confirming the predicted rigid rod-shaped virions. The genetic material of TMV was shown to be RNA, a property previously thought to be restricted to DNA. The first viral protein for which an amino acid sequence was determined was the coat protein of TMV. TMV was the first virus to be mutagenized and the subsequent determination of coat protein sequences from a number of strains and mutants helped to establish the universality of the genetic code. Methods of infecting plant protoplasts with viruses were developed with the tobacco–TMV system, creating a synchronous system to study events in the infection cycle. The TMV 30 kDa protein was the first viral protein shown to be required for virus movement.

Taxonomy and Classification

The genus *Tobamovirus* has not been assigned to a family. Currently, there are 23 recognized species within the genus *Tobamovirus* (**Table 1**). Several recently sequenced viruses are tentative members of new species (**Table 1**). Although tobamoviruses comprise one of the more intensively studied plant virus genera, taxonomy has often been confusing. Historically, plant viruses with rigid virions of approximately $18 \times 300\,\text{nm}^2$ and causing various diseases were all designated strains of TMV. Thus, many viruses originally referred to as TMV strains are now recognized as belonging to separate species. For example, the tobamovirus that was referred to as the tomato strain of TMV, and is approximately 80% identical to TMV at the nucleotide sequence level, is actually tomato mosaic virus. One criterion for distinguishing the members of separate tobamovirus species is a nucleotide sequence difference of at least 10%.

Virus Structure and Composition

Tobamovirus virions are straight tubes of approximately $18 \times 300\,\text{nm}^2$ with a central hollow core 4 nm in diameter. Virion composition is approximately 95% protein and 5% RNA. For TMV, approximately 2100 subunits of a single coat protein are arranged in a right-handed helix around a single genomic RNA molecule, with each subunit associated with three adjacent nucleotides. Protein–protein associations are the essential first event of virion assembly. Coat protein subunits assemble into several types of aggregates. Coat protein monomers and small heterogeneous aggregates of a few subunits are collectively referred to as 'A-protein'. The equilibrium between A-protein and larger aggregates is primarily dependent upon pH and ionic strength. Purified coat protein and viral RNA can assemble into infectious particles *in vitro*. Larger aggregates are disks composed of two individual stacked rings of coat protein subunits, and protohelices. Protohelices contain approximately 40 coat protein subunits arranged in a spiral around a central hollow core, similar to the arrangement within the virion. A sequence-specific stem–loop structure in the RNA, the origin of assembly (OAS), initiates encapsidation and prevents defective packaging that could result from multiple independent initiation events on a single RNA molecule. Virion assembly initiates as the primary loop of the OAS is threaded through a coat protein disk or protohelix with both ends of the RNA trailing from one side. The conformation of the coat protein protohelix changes as the RNA becomes embedded within the groove between the two layers of subunits. Elongation is

Table 1 Definitive and tentative members of the genus *Tobamovirus*

Cucumber fruit mottle mosaic virus	CFMMV
Cucumber green mottle mosaic virus	CGMMV
Frangipani mosaic virus	FrMV
Hibiscus latent Fort Pierce virus	HLFPV
Hibiscus latent Singapore virus	HLSV
Kyuri green mottle mosaic virus	KGMMV
Maracuja mosaic virus	MaMV
Obuda pepper virus	ObPV
Odontoglossum ringspot virus	ORSV
Paprika mild mottle virus	PaMMV
Pepper mild mottle virus	PMMoV
Ribgrass mosaic virus	RMV
Sunn-hemp mosaic virus	SHMV
Sammons' Opuntia virus	SOV
Tobacco latent virus	TLV
Tobacco mild green mosaic virus	TMGMV
Tobacco mosaic virus	TMV
Tomato mosaic virus	ToMV
Turnip vein-clearing virus	TVCV
Ullucus mild mottle virus	UMMV
Wasabi mottle virus	WMoV
Youcai mosaic virus	YoMV
Zucchini green mottle virus	ZGMMV
Tentative members	
Brugmansia mild mottle virus	
Cucumber mottle virus	
Cactus mild mottle virus	
Streptocarpus flower break virus	
Tropical soda apple mosaic virus	

bidirectional, proceeding rapidly toward the 5′ end of the RNA as the RNA loop is extruded through the elongating virion and additional coat protein disks are added. There is disagreement about the mechanism of elongation toward the 3′ terminus of the RNA, but it appears that this slower process involves the addition of smaller protein aggregates.

Subgenomic mRNAs containing the OAS are encapsidated into shorter virions that are not required for infectivity. The OAS is located within the open reading frame (ORF) for the movement protein of most tobamoviruses. The level of accumulation of a particular subgenomic mRNA containing the OAS determines the relative proportion of that particular virion species. Thus, all tobamovirus virion populations contain a small percentage of movement protein subgenomic mRNAs. In some tobamoviruses, including cucumber green mottle mosaic virus, hibiscus latent Singapore virus (HLSV), kyuri green mottle mosaic virus, maracuja mosaic virus, sunn-hemp mosaic virus, zucchini green mottle mosaic virus, and cactus mild mottle virus, the OAS is located within the coat protein ORF. Thus, these so-called subgroup 2 tobamoviruses produce a significant proportion of small virions that contain coat protein subgenomic mRNA. Hybrid nonviral RNAs containing an OAS will also assemble with coat protein into virus-like particles of length proportional to that of the RNA.

Genome Organization

The genome of tobamoviruses consists of one single-stranded positive-sense RNA of approximately 6300–6800 nt (**Figure 1**(**a**)). There is a methylguanosine cap at the 5′ terminus, followed by an AU-rich leader 55–75 nt in length. The 3′ nontranslated end of the RNA consists of sequences that can be folded into a series of pseudoknot structures, followed by a tRNA-like terminus. The hibiscus-infecting tobamoviruses HLSV and hibiscus latent Fort Pierce virus contain a polyA stretch between the 3′ end of the coat protein ORF and the tRNA-like structure. The tRNA-like terminus can be aminoacylated *in vitro*, and in most cases specifically accepts histidine. The exception is the 3′ terminus of SHMV, which accepts valine and appears to have arisen by a recombination event between a tobamovirus and a tymovirus.

Four ORFs that are contained within all tobamovirus genomes (**Figure 1**(**a**)) correspond to the proteins found in infected tissue. Two overlapping ORFs begin at the 5′ proximal start codon. Termination at the first in-frame stop codon produces a 125–130 kDa protein. A 180–190 kDa protein is produced by readthrough of this leaky termination codon approximately 5–10% of the time. The remaining proteins are expressed from individual 3′ co-terminal subgenomic mRNAs, from which only the 5′ proximal ORF is expressed (**Figures 1**(**c**) **and 2**(**a**)). The next ORF encodes the 28–34 kDa movement protein, which has RNA-binding activity and is required for cell-to-cell movement. The 3′ proximal ORF encodes a 17–18 kDa coat protein. A subgenomic mRNA containing an ORF for a 54 kDa protein that encompasses the readthrough domain of the 180–190 kDa ORF has been isolated from infected tissue, although no protein has been detected.

Within the protein-coding regions of the genome, there are nucleotide sequences that also function as *cis*-acting elements for subgenomic mRNA synthesis, virion assembly, and replication. Gene expression from subgenomic mRNAs is regulated both temporally and quantitatively. The movement protein is produced early and accumulates to low levels, whereas the coat protein is produced late and accumulates to high levels. The regulatory elements for subgenomic mRNA synthesis are located on the genome-length complementary RNA overlapping the upstream ORF (**Figure 1**(**a**)). There is limited (40%) sequence identity between the TMV movement protein and coat protein subgenomic promoters. The TMV movement protein subgenomic promoter is located upstream of the movement protein ORF, flanking the transcription initiation site. Unlike the movement protein subgenomic promoter, full activity of the coat protein subgenomic promoter requires sequences within the coat protein ORF.

Viral Proteins

The tobamovirus 125–130/180–190 kDa proteins are involved in viral replication, gene expression, and movement. Both are contained in crude replicase preparations, and temperature-sensitive replication-deficient mutants map to these ORFs. The 125–130/180–190 kDa proteins contain two functional domains common to replicase proteins of many positive-stranded RNA plant and animal viruses (**Figure 1** (**b**)). The N-terminal domain has methyltransferase and guanylyltransferase activities associated with capping of viral RNA. The second common domain is a proposed helicase, based upon conserved sequence motifs. The readthrough domain of the 180–190 kDa protein has sequence motifs characteristic of RNA-dependent RNA polymerases. Both proteins are necessary for efficient replication, although the TMV 126 kDa protein is dispensable for replication and gene expression in protoplasts. The 125–130 kDa protein (or sequences within this region) of the 180–190 kDa protein are required for cell-to-cell movement. Additionally, these multifunctional proteins are symptom determinants, as mutations in mild strains map to these ORFs.

The 28–34 kDa movement protein has a plasmodesmatal binding function associated with its C-terminus and a single-stranded nucleic acid-binding domain associated with the N-terminus. The movement protein–host interaction determines whether the virus can systemically infect some plant species. Although principally a structural protein, the coat protein is also involved in other host interactions. Coat protein is required for efficient long-distance movement of the virus. Coat protein is also a symptom determinant in some susceptible plant species and an elicitor of plant defense mechanisms in other plant species.

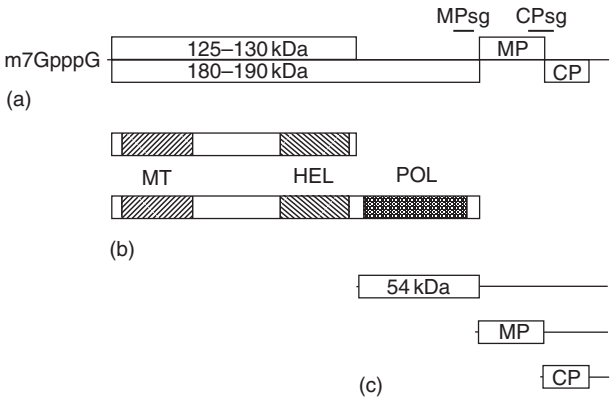


Figure 1 Tobamovirus genome organization and gene expression strategy. (a) Tobamovirus genome organization. ORFs designated as open boxes. Nontranslated sequences designated as lines; positions of subgenomic promoters are marked. (b) Nonstructural proteins involved in tobamovirus replication. Functional domains shared with other viruses within the 'alphavirus supergroup' are designated as hatched boxes. (c) Subgenomic mRNAs with 5′ proximal ORF labeled. MP, movement protein; CP, coat protein; MT, methyltransferase; HEL, helicase; POL, polymerase; MPsg, MP subgenomic mRNA; CPsg, CP subgenomic mRNA.

Interactions between Viral and Host Proteins

Available evidence suggests that the interactions of viral proteins with host factors are important determinants of viral movement and host ranges. Amino acid substitutions in the movement protein and 125–130/180–190 kDa

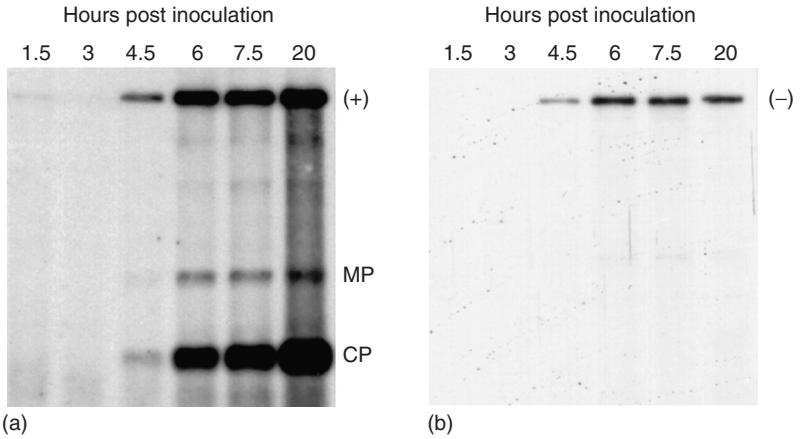


Figure 2 Time course of accumulation of TMV positive-stranded (a) and negative-stranded (b) RNAs in tobacco protoplasts. Total RNA was extracted from tobacco suspension cell protoplasts transfected with TMV *in vitro* transcripts at the time points indicated and analyzed by Northern blot hybridization. (+), TMV genomic RNA; MP, movement protein subgenomic RNA; CP, coat protein subgenomic RNA; (–), negative-stranded complement to the genomic RNA.

proteins can alter the movement function in different hosts. Some viruses, including tobamoviruses, can assist movement of other viruses that are incapable of movement in a particular plant species. These interactions suggest that there are more precise associations of viral proteins with host factor(s) than with viral RNA. Additionally, precise coat protein–plant interactions are required for movement to distal positions within the plant. The helicase domain of the 130–190 kDa proteins elicits the *N* gene-mediated resistance in *N. glutinosa.*

Virus Replication

Virions or free viral RNA will infect plants or protoplasts. Because tobamoviruses have a genome consisting of messenger-sense RNA that is infectious, one of the first events is translation of the 5′-proximal ORFs to produce the proteins required for replication of the genomic RNA and transcription of subgenomic mRNAs. When virions are the infecting agent, the first event is thought to be co-translational disassembly, in which the coat protein subunits at the end of the virion surrounding the 5′ end of the RNA loosen, making the RNA available for translation. Ribosomes then associate with the RNA, and translation of the 126/183 kDa ORFs is thought to displace coat protein subunits from the viral RNA. After the formation of an active replicase complex, a complementary negative-strand RNA is synthesized from the genomic positive-strand RNA template. Negative-strand RNA serves as template for both genomic and subgenomic mRNAs. Negative-strand RNA synthesis ceases early in infection, while positive-strand RNA synthesis continues. This results in an asymmetric positive- to negative-strand RNA ratio. Early in infection, genomic RNA functions as template for negative-strand RNA synthesis and as mRNA for production of the 126/183 kDa proteins. Later in the infection cycle, most of the newly synthesized genomic RNA is encapsidated into virions. Subgenomic mRNAs transcribed during infection function as mRNA for the 3′ ORFs. Within cells of an infected leaf, replication proceeds rapidly between approximately 16 and 96 h post infection within a cell, then ceases. Even though the infected cells become packed with virions, these cells remain metabolically active for long periods. During the early stages of infection of an individual cell, the infection spreads through plasmodesmatal connections to adjacent cells. This event requires the viral movement protein that modifies plasmodesmata to accommodate larger molecules and the 126/183 kDa proteins. Movement through plasmodesmata does not require the coat protein. A second function of the movement protein appears to be binding to the viral RNA to assist its movement through the small plasmodesmatal openings. The movement protein also appears to associate with the cytoskeleton. As the virus spreads from cell to cell throughout a leaf, it enters the phloem for rapid long-distance movement to other leaves and organs of the plant, a complex process that requires the coat protein.

cis-Acting Sequences

The 5′ nontranslated region contains sequences that are required for replication. This region is an efficient translational leader. The 3′ nontranslated region contains *cis*-acting sequences that are involved in replication. Certain deletions within the pseudoknots are not lethal, but result in reduced levels of replication. Exchange of 3′ nontranslated elements between cloned tobamovirus species has resulted in some lethal and nonlethal hybrids, suggesting a requirement for sequence specificity and/or secondary structure. The 3′ nontranslated region appears to be a translational enhancer, both in the viral genome and when fused to heterologous reporter mRNAs. Sequences encoding the internal ORFs for the movement and coat proteins are dispensable for replication. Duplication of the subgenomic promoters results in transcription of an additional new subgenomic mRNA. Heterologous tobamovirus subgenomic promoters inserted into the viral genome are recognized by the replicase complex and transcribed. Foreign sequences inserted behind tobamovirus subgenomic mRNA promoters have been expressed to high levels in plants and protoplasts.

Satellite Tobacco Mosaic Virus

Satellite tobacco mosaic virus (STMV), a tobamovirus-dependent satellite virus, was isolated from *Nicotiana glauca* plants infected with tobacco mild green mosaic virus (TMGMV). The STMV genome consists of one single-stranded positive-sense RNA of 1059 nt. The 240 3′ nucleotides share approximately 65% sequence identity with TMGMV and TMV, contain pseudoknot structures, and have a tRNA-like terminus. No sequence similarity with any tobamovirus exists over the remainder of the genome. Two overlapping ORFs that are expressed in *in vitro* translation reactions are present in the genomic RNA of most STMV isolates. The 5′-proximal ORF encodes a 6.8 kDa protein that has not been detected *in vivo* and is not present in all STMV isolates. The second ORF encodes a 17.5 kDa coat protein that is not serologically related to any tobamovirus coat protein. The 17 nm icosahedral virions are composed of a single STMV genomic RNA encapsidated within 60 STMV coat protein subunits. Replication of natural populations of STMV is supported by other tobamoviruses, but at lower levels than with the natural helper virus, TMGMV. The host range of STMV parallels that of the helper virus.

See also: Plant Virus Diseases: Economic Aspects; Satellite Nucleic Acids and Viruses; Tobacco Mosaic Virus.

Further Reading

Gibbs AJ (1977) Tobamovirus group. CMI/AAB Descriptions of Plant Viruses, No. 184. http://www.dpvweb.net/dpv/showadpv.php?dpvno=184 (accessed July 2007).

Hull R (2002) *Matthews' Plant Virology,* 4th edn., New York: Academic Press.

Lewandowski DJ (2005) *Tobamovirus* genus. In: Fauquet CM, Mayo MA, Maniloff J, Desselburger U, and Ball LA (eds.) *Virus Taxonomy, Classification and Nomenclature of Viruses: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1009–1014. San Diego, CA: Elsevier Academic Press.

Pogue GP, Lindbo JA, Garger SJ, and Fitzmaurice WP (2002) Making an ally from an enemy: Plant virology and the new agriculture. *Annual Review of Phytopathology* 40: 45–74.

Scholthof KB (2005) Tobacco mosaic virus, a model system for plant virology. *Annual Review of Phytopathology* 42: 13–34.

Scholthof KBG, Shaw JG, and Zaitlin M (eds.) (1999) *Tobacco Mosaic Virus: One Hundred Years of Contributions to Virology.* St. Paul, MN: APS Press.

Van Regenmortel MHV and Fraenkel-Conrat H (eds.) (1986) *The Plant Viruses, Vol. 2*: *The Rod-Shaped Plant Viruses*. New York: Plenum.

Tobravirus

S A MacFarlane, Scottish Crop Research Institute, Dundee, UK

© 2008 Elsevier Ltd. All rights reserved.

Taxonomy and Characteristics

The genus *Tobravirus* is comprised of three species, the type species *Tobacco rattle virus* (TRV) together with *Pea early-browning virus* (PEBV) and *Pepper ringspot virus* (PepRSV), which was previously referred to as the CAM strain of TRV. The genus has not been assigned to a virus family. Tobraviruses have a genome of two, positive-sense, single-strand RNAs that are packaged separately into rod-shaped particles. In some situations, the larger genomic RNA (RNA1) can cause a systemic infection in the absence of the second, smaller RNA (RNA2) and without the formation of virus particles. Tobraviruses are transmitted between plants by root-feeding nematodes of the genera *Trichodorus* and *Paratrichodorus*, and in some plant species are also seed transmitted.

Virus Particle Production and Structure

Tobravirus RNA1 is encapsidated into the L (long) particle with a length of 180–215 nm, depending on virus species, and RNA2 is encapsidated into S (short) particles which range in length from 46 to 115 nm, depending on virus isolate (**Figure 1**). Both L and S particles have an apparent diameter of 20–23 nm, depending on the technique used to examine them. *In vitro* translation experiments using RNA extracted from purified virus preparations, as well as studies with the TRV SYM isolate, which has an unusual genome structure, showed that some, if not all, tobravirus subgenomic (sg)RNAs are also encapsidated, in particles of various lengths. The tobraviruses encode a single coat protein (CP), molecules of which assemble in a helical arrangement around a central cavity with a diameter of 4–5 nm, and with a distance of 2.5 nm between successive turns of the helix. *In vitro* reconstitution experiments suggested that virus particle formation initiates at the 5′ end of the viral RNA, although the encapsidated sgRNAs do not carry the 5′ terminal part of the virus genomic RNAs. Peptide mapping showed that the major antigenic regions of the CP are, in descending order of strength, the C-terminal 20 amino acids (aa), 5 aa in the central region of the protein and 5 aa at the N-terminus. This and other spectroscopic analyses suggest that the N- and C-termini are exposed on the outer surface of the particle, while the central region is exposed in the central canal (where interactions of the CP with the viral RNA take place). The C-terminal domain appears to be unstructured and

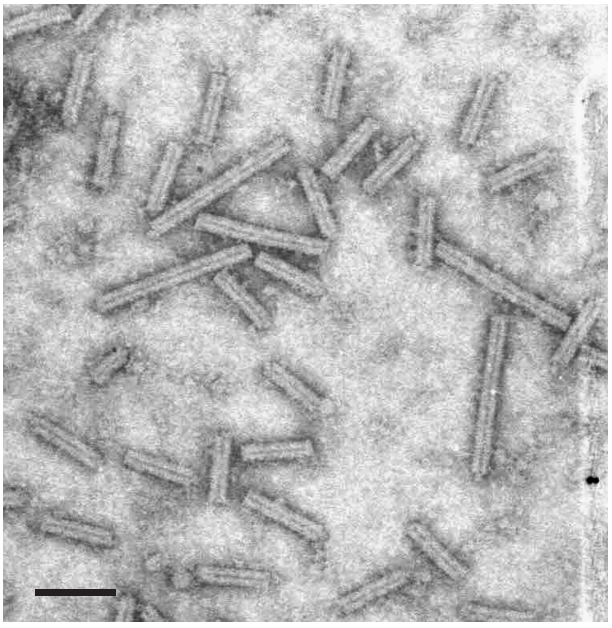


Figure 1 Electron micrograph of long and short particles of TRV isolate RH. Scale = 100 nm.

plays a role in interactions with other virus proteins that are involved in nematode transmission of the virus. Particularly with TRV, there is significant amino acid sequence difference between the CP of different virus isolates, resulting in many different serotypes of this virus.

M-Type and NM-Type Infections

In early studies with tobraviruses (usually with TRV), the infectivity of fractionated and purified L and S particles was examined, showing that L particles were infectious (producing local and systemic symptoms on particular hosts) but that S particles were not. However, plants infected with L particles did not contain virus particles, whereas plants infected with both L and S particles did contain (both) virus particles. This was explained by sequencing, which revealed that RNA1 encodes proteins for RNA replication and movement, whereas RNA2 encodes the CP. Because infections with RNA1 do not produce virus particles, they were very difficult to maintain by repeat inoculation of sap extracts, and were referred to as nonmultiplying (NM-type) infections. Infections derived from both RNA1 and RNA2 and producing virus particles were easily passaged and were referred to as multiplying (M-type) infections. Subsequently, it was found that extraction with phenol of RNA from NM-infected plants in fact produced highly infectious preparations.

Early observations also suggested that NM-type infections caused more severe symptoms than M-type infections and moved only slowly up the plant, giving rise to the theory that unencapsidated RNA1 could spread systemically only from cell to cell via plasmodesmata. However, experiments with TRV and PEBV carrying defined mutations in the CP gene demonstrated unequivocally that unencapsidated virus RNAs moved very rapidly via the vascular system in both *Nicotiana benthamiana* and *Nicotiana clevelandii* plants. In addition, with wild-type (encapsidated) virus, systemic infection always included both RNA1 and RNA2 but with the (unencapsidated) CP mutants RNA2 occasionally became separated from RNA1 and was not detected in some systemic leaves. One PEBV mutant carried only a small (28 aa) deletion at the C-terminus of the CP but did not produce virus particles or indeed any detectable CP in infected plants. Nevertheless, this mutant moved systemically as quickly as did wild-type virus and in this case RNA2 did not become separate from RNA1. This suggests, perhaps, that some form of CP but not incorporated into particles, which can be present at very low levels, ensures the coordinated transport of RNA1 and RNA2 through the phloem.

In another study, plants were infected with TRV RNA1 and two types of RNA2, one wild type and the second encoding a CP in which the 15 aa at the C-terminus were replaced with three nonviral residues. Both RNA2 species were encapsidated by the CP that they encoded but neither appeared to be encapsidated by the CP from the other RNA2. Apparently, although RNA1 is encapsidated *in trans*, RNA2 is only encapsidated *in cis*. The mechanism for this is not known but may be linked to the role of CP in the coordinated systemic movement of RNA1 and RNA2.

Genome Structure and Expression

The viral RNAs have a 5′ methylated guanosine cap. The 3′ end of the RNAs is not polyadenylated but folds into a tRNA-like pseudoknot structure that, in contrast to some other virus RNAs, cannot be aminoacylated. RNA1 is from 6.8 to 7 kb in size, and RNA2 varies between isolates from 1.8 to ~4 kb (**Figure 2**). Complete sequences have been obtained for RNA1 from four isolates of TRV, two isolates of PEBV, and one isolate of PepRSV. In addition, complete sequences have been obtained for RNA2 from 15 isolates of TRV, three isolates of PEBV, and one isolate of PepRSV. (Information on RNA sequences can be obtained from the eighth ICTV report.) Other than the sequencing, almost no further molecular studies have been carried out on PepRSV.

RNA Sequences

The larger RNA (RNA1) is highly conserved in nucleotide sequence between different isolates of the same virus (e.g., 99% identity between RNA1 of TRV isolates SYM and ORY), but there is much less identity between isolates of the different tobravirus species (e.g., 62% identity between RNA1 of TRV isolate SYM and PEBV isolate SP5). The smaller RNA (RNA2) varies considerably both in terms of overall nucleotide sequence identity as well as protein-coding capacity between isolates of the same

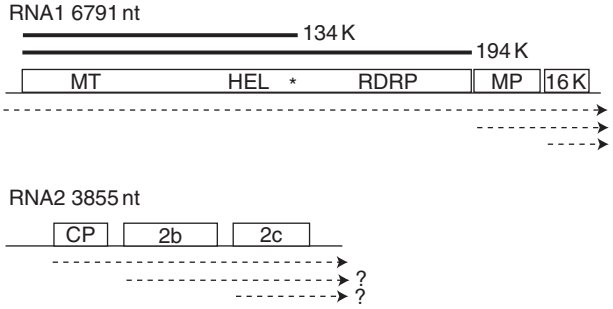


Figure 2 Genome diagram and expression strategy of TRV isolate PpK20. Open boxes denote virus genes. The solid lines above the RNA1 genes shows the location of the two, overlapping replicase genes. The 134K protein contains methyltransferase (MT) and helicase (HEL) motifs. The C-terminal part of the 194K protein contains an RNA-dependent RNA polymerase (RDRP) motif. The asterisk denotes the 134K gene termination codon where readthrough translation to produce the 194K protein occurs. The movement protein (MP) gene is also known as the 29K or 1a gene. The cysteine-rich 16K gene is also known as the 1b gene. For RNA2, CP denotes the coat protein gene. The dashed lines beneath each RNA denote the 3′ co-terminal subgenomic RNAs that are known or suspected to exist.

virus (TRV or PEBV), although phylogenetic analysis shows that the CPs of the different tobraviruses, which are encoded by RNA2, are more closely related to each other than to CPs from viruses in other genera. The tobraviruses are most closely related to tobamoviruses, in terms of particle structure, overall gene organization, and viral protein sequence homologies.

RNA1 has a 5′ noncoding region (NCR) of between 126 and 202 nt, and a 3′ NCR of 459–255 nt. RNA2 has a much larger 5′ NCR of 470–710 nt, and a 3′ NCR of 780–392 nt. There is almost no sequence homology between the 5′ NCRs of the tobraviruses; however, the 25 nt at the 3′ terminus of the TRV and PEBV RNAs are identical and there is 70% identity over the next 140 nt. Consequently, when recombinants were made between the RNA2 of TRV PpK20 and PEBV SP5, the replicase complex of both viruses could replicate RNA2 molecules carrying the 3′ NCR from either virus. However, RNA2 carrying the TRV 5′ NCR could only be replicated by a TRV replicase complex, and RNA2 carrying the PEBV 5′ NCR could only be replicated by a PEBV replicase complex.

Expression of Virus Genes

The 5′ proximal gene of RNA1, encoding viral replicase proteins, is expressed by direct translation of the genomic RNA. The two other RNA1-encoded genes (1a and 1b) are expressed from sgRNAs that start with the sequence AUA (within a conserved motif GCAUA) and that are co-terminal with the 3′ end of the genomic RNA.

The 5′ proximal gene of RNA2 of almost all tobravirus isolates encodes the CP. However, the CP gene is located at least 470 nt downstream of the 5′ terminus of RNA2, and this region contains numerous (at least six) AUG codons upstream of the translation initiation codon of the CP gene, that are inhibitory to CP expression. Experiments to delete parts of the 5′ NCR of PEBV RNA2 showed that only a very small amount of CP is translated *in vitro* from full-length (genomic) RNA2 but that translation is increased 25-fold when the 5′ NCR is removed. Thus, even though the CP gene is the 5′ proximal gene on RNA2, it (and the other genes on RNA2) is expressed from an sgRNA. Uniquely, the CP gene of TRV SYM is located near to the 3′ end of RNA2, downstream of another gene. The sgRNA for this CP is much shorter than genomic RNA2 and is encapsidated into a clearly identifiable VS (very short) virus particle.

A stem–loop sequence is found upstream of tobravirus sgRNA start sites, and is particularly conserved for the CP sgRNA. The introduction of mutations into this structure (8 bp stem, 4 nt loop) upstream of the PEBV CP gene showed that the stem–loop forms an essential part of the sgRNA promoter, and that the structure of the stem rather than its actual sequence is important for sgRNA synthesis *in planta*. The promoter regions for the TRV and PEBV CP genes could be used as a cassette and moved to other locations in RNA2 to express nonviral genes.

Recombination in Tobravirus RNAs

The large variation in the overall length of RNA2 from different tobravirus isolates reflects the fact that most are recombinant molecules, where the 3′ part of RNA2 has been replaced by sequences from the 3′ part of RNA1. The recombination junction can occur at any position in RNA2 that is downstream of the CP gene, and the recombinant may retain none, some, or even both of the other genes (2b and 2c) that might be considered RNA2 specific. The region of RNA1 that is transferred to RNA2 always includes the 3′ NCR, often includes the 1b gene immediately upstream of the 3′ NCR, and may occasionally include part of the 1a gene that is located upstream of the 1b gene. This means that many of these recombinant isolates carry two, probably both functional, copies of the 1b gene, one on RNA1 and the second on RNA2. The mechanism for recombination is not known but is speculated to be caused by template switching by viral replicase from RNA1 to RNA2 during minus-strand RNA synthesis. Sequences that resemble the 5′ terminus of tobravirus genomic RNAs (rich in A and U residues) are often found at or near the recombination junction, and conceivably could facilitate the recombination event. It is also not completely clear when tobravirus recombination occurs, though the feeling is that repeated passage of virus by mechanical inoculation in glasshouse-grown plants, bypassing the nematode transmission process, either encourages recombination or reduces selection against the survival of recombinants. Nevertheless, at least one known recombinant isolate, TRV PaY4, which was cloned after only a very limited period of multiplication in the glasshouse, did retain its ability to be nematode transmitted.

A different recombination process also occurs in which the CP and/or 2b gene in RNA2 of some TRV isolates appears to have been derived from PEBV. For this reason, serological analysis is not always successful in discriminating between the different tobraviruses. Attempts to reproduce this recombination by co-inoculating TRV and PEBV to plants in the glasshouse were not successful, suggesting that this may be only a rare occurrence, or may be stimulated by particular environmental conditions. Nevertheless, one report showed that 30% of the TRV isolates recovered from fields in the coastal bulb-growing region in the Netherlands were TRV/PEBV recombinants.

Viral Proteins

RNA1

RNA1 encodes proteins for virus RNA replication and intraplant movement (local and systemic). The 5′ half of RNA1 encodes a large (134–141 kDa) protein that contains methytransferase and helicase domains and is expected to be part of the viral replicase complex.

Readthrough translation of the stop codon of this protein produces a larger protein (194–201 kDa) that contains motifs associated with RNA-dependent RNA polymerase proteins. *In vitro* translation experiments showed that the opal (UGA) translation termination codon of the TRV 134 kDa protein is suppressed by tRNAs that incorporate tryptophan or cysteine at this position. Downstream of the replicase genes is the 1a gene encoding a 29–30 kDa cell-to-cell movement protein. Disruption of this gene prevents accumulation of TRV in inoculated leaves but can be overcome by co-inoculation with tobacco mosaic virus (TMV) or to transgenic plants expressing the TMV 30K movement protein. The C-terminal 1b gene encodes a 12–16 kDa cysteine-rich protein that is involved in seed transmission of PEBV in pea, pathogenicity of TRV and PEBV, and in cultured *Drosophila* cells suppresses RNA silencing. Deletion of the 1b gene prevents systemic movement of TRV in *N. benthamiana*, which can be recovered by co-expression, from TRV RNA2, of other cysteine-rich proteins from PEBV, soil-borne wheat mosaic virus, and barley stripe mosaic virus, as well as by the 2b silencing suppressor protein of cucumber mosaic virus.

RNA2

RNA2 encodes proteins for virus particle formation and transmission by nematodes. Several studies have identified the CP as being involved in the nematode transmission process, and deletion of part of the C-terminal domain of the CP prevented the transmission of PEBV without affecting virus particle formation. Infectious, nematode-transmissible clones of three TRV isolates and one PEBV isolate have been sequenced. In addition to the CP, these RNAs all carry two other genes encoding the 2b and 2c proteins, both of which (for PEBV) have been detected by Western blotting in extracts of virus-infected plants. Mutation studies showed that the 2b gene is necessary for transmission of TRV and PEBV, whereas the 2c protein was only required for transmission of PEBV. This difference may reflect the different species of nematode that transmitted the particular virus isolates used in these studies, rather than a clear mechanistic difference between TRV and PEBV transmission. A further, small open reading frame, encoding a putative 9 kDa protein immediately downstream of and in-frame with the CP gene, is present in PEBV-TpA56 and TRV TpO1. For PEBV, mutation of this gene greatly reduced nematode transmission frequency, although the protein was not detected by Western blotting.

The 2b proteins of the different tobravirus isolates share some amino acid sequence homology with each other, ranging from only 11% identity (TRV Umt1 vs. TRV PaY4) to 99% identity (TRV Umt1 vs. TRV OR2). They also range in size from 238 aa (TRV PaY4) to 354 aa (TRV PpK20). The TRV PaY4 2b protein has been show to act *in trans*, with nematode transmission of a 2b-mutant virus being complemented by co-infection with wild-type virus. However, this appears to be isolate specific, as the TRV PaY4 2b mutant was complemented only by wild-type TRV PaY4 and not by wild-type TRV PpK20.

The 2b protein may influence nematode transmission by more than one mechanism. In a microscopy study of the roots of *N. benthamiana* plants infected by PEBV, large numbers of particles of the wild-type virus were found in all regions of the root tip, which is where the vector nematodes preferentially feed, whereas virus particles of a mutant deleted for the 2b and 2c genes were present in roots only in much lower numbers. A similar enhancement was found in the efficiency of invasion of roots in *N. benthamiana*, as well as in leaves of *Arabidopsis thaliana*, when TRV carrying the 2b gene was compared with that of TRV lacking the 2b gene.

The 2b protein also physically interacts with the TRV CP (as examined by yeast two-hybrid (Y2H) analysis) and the virus particle (as examined by immunoelectron microscopy). One suggestion is that the 2b protein acts as a bridge to trap virus particles to specific sites of retention on the nematode esophageal cuticle. Aggregates of virus particles have been observed using the electron microscope to collect in this part of the nematode but the co-location of the 2b protein has not yet been demonstrated. It appears that interaction between the CP and 2b protein stabilizes the 2b protein, as in plants infected with wild-type TRV PaY4, carrying both PaY4 CP and PaY42b genes, the 2b protein was detected by Western blotting. However, with a recombinant TRV carrying the TRV PpK20 CP gene and the TRV PaY4 2b gene, the 2b protein could not be detected, possibly because these proteins from two different isolates cannot interact with one another. This is reinforced by results from the Y2H study, where the TRV PpK20 CP and PpK20 2b proteins were found to interact. Similarly, the PaY4 CP and PaY4 2b proteins interacted; however, the PpK20 CP did not interact with the PaY4 2b protein and the PaY4 CP did not interact with the PpK20 2b protein.

Although the 2c protein is involved in transmission of PEBV, little else is known about this protein. Amino acid sequence homologies between the 2c proteins of different TRV isolates range from almost none to over 95% identity. In a Y2H assay, the TRV PpK20 2c protein interacted with the PpK20CP, and removal of the CP C-terminal flexible domain did not affect the interaction.

Tobraviruses as Gene Expression/ Silencing Vectors

As RNA1 encodes all the proteins necessary for tobravirus replication and movement, the RNA2 can be modified to carry nonviral sequences without greatly affecting virus infection. Expression vectors have been constructed from all three tobraviruses, in which a duplicate CP

promoter sequence is inserted downstream of the native CP gene followed by restriction sites to allow the cloning of other sequences. Together, the tobraviruses can infect a wide range of plant species, often without causing particularly severe symptoms, features which increase their utility as expression vectors.

Plants infected with tobraviruses often undergo a rapid recovery in which infection symptoms and virus levels fall dramatically, although the plants do not become free of virus, most particularly in the meristem regions. However, these plants have developed a strong resistance to further infection by the same virus, most likely by an RNA silencing-based mechanism. Although not well understood, it seems that tobraviruses are potent triggers of RNA silencing but do not encode a strong silencing suppressor protein to counteract this host defense activity. A consequence of this is that when a host plant sequence is inserted into the virus genome, very strong silencing is initiated that targets expression of the host gene itself, a process known as virus-induced gene silencing (VIGS). TRV has become one of the most widely used VIGS vectors for studies of plant gene function, and recent work has shown that PEBV is also a very effective VIGS vector for studies in pea.

Diseases Caused by Tobraviruses

TRV is found in many regions (in Europe, North America, Japan, and Brazil) and has a particularly wide host range, infecting more than 100 species in nature and more than 400 species when tested in the glasshouse, although not all of these infections are systemic. As tobraviruses are transmitted by soil-inhabiting nematodes, infection may be limited in the field to the roots. Weeds may play an important role in the maintenance and spread of tobraviruses, with *Capsella bursa-pastoris*, *Senecio vulgaris*, *Stellaria media*, and *Viola arvensis* being the most commonly found weed hosts of TRV. Virus transmission in seed of these plants was also reported. Many crop plants are infected by TRV, the major diseases being those of potato and ornamental bulbs (narcissus, gladiolus, tulip, lily, and crocus). The symptoms of TRV infection in potato are the formation of arcs and flecks of brown corky tissue in the tuber which is referred to as spraing, and which can make the tuber unfit for sale. Both M-type and NM-type infections can produce spraing symptoms, the biochemical basis for which is not known. It was thought that potato cultivars that did not show spraing symptoms were resistant to TRV; however, recent work has shown that some infections can be symptomless. Nevertheless, even these symptomless infections lead to significant reductions in tuber yield and tuber quality. Inclusion of tubers carrying symptomless infection could have major consequences for the production and distribution of seed potatoes.

PEBV has been reported in several European countries (UK, Netherlands, Italy, Belgium, Sweden) as well as Algeria and Morocco. In the field, it infects mainly legumes, including pea, faba bean, French bean, lupin, and alfalfa. Several weeds and other crop plants may be infected, although often only in the roots.

PepRSV has only been reported from Brazil, where it infects pepper, tomato, and artichoke, as well as local weed species.

See also: Hordeivirus; *Nepovirus*; *Tobamovirus*; Viral Suppressors of Gene Silencing.

Further Reading

Harrison BD and Robinson DJ (1978) The Tobraviruses. *Advances in Virus Research* 23: 25–77.

MacFarlane SA (1999) Molecular biology of the tobraviruses. *Journal of General Virology* 80: 2799–2807.

MacFarlane SA and Robinson DJR (2004) Transmission of plant viruses by nematodes. In: Gillespie SH, Smith GL, and Osbourne A (eds.) *SGM Symposium 63: Microbe–Vector Interactions in Vector-Borne Diseases*, pp. 263–285. Cambridge: Cambridge University Press.

Robinson DJ (2005) Tobravirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1015–1019. San Diego, CA: Elsevier Academic Press.

Visser PB, Mathis A, and Linthorst HJM (1999) Tobraviruses. In: Granoff A and Webster R (eds.) *Encyclopedia of Virology,* 2nd edn., pp. 1784–1789. London: Elsevier.

Togaviruses Causing Encephalitis

S Paessler, University of Texas Medical Branch, Galveston, TX, USA
M Pfeffer, Bundeswehr Institute of Microbiology, Munich, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arbovirus A virus transmitted to vertebrates by hematophagous (blood-feeding) arthropods.

Cimicid A hemipteran bug of the family *Cimicidae*.
Encephalitis Acute inflammation of the brain.
Enzootic Infection or illness affecting or peculiar to animals of a specific geographic area.

Epidemic disease An illness that spreads rapidly and widely and affects many individuals in an area or a population at the same time.
Epizoodemic An extensive outbreak of a virus in both humans and other vertebrates.
Epizootic Infection or illness in a large number of animals at the same time within a particular region or geographic area, the number of affected animals being in clear excess of that which would be expected for the specific region and period of time.
Etiology The study of causes of diseases or pathologies.
Togavirus A virus of the family *Togaviridae*.

Introduction

In the 1930s, previously unrecognized viruses were isolated from diseased horses in California, Virginia, and New Jersey, and from an infected child in Caracas, Venezuela. These subsequently were named Western equine encephalitis virus (WEEV), Eastern equine encephalitis virus (EEEV), and Venezuelan equine encephalitis virus (VEEV), respectively. Since then, numerous isolations of these viruses have been obtained from infected mosquitoes and from horses, humans, and other vertebrates, mainly birds and rodents. In nature, these viruses are transmitted by hematophagous arthropods.

The virus family *Togaviridae* comprises two genera: *Alphavirus* and *Rubivirus*. Rubiviruses do not usually cause encephalitis and will not be discussed further in this article. Encephalitic alphaviruses are neuroinvasive and cause mild-to-severe neurological symptoms. The alphaviruses WEEV, EEEV, VEEV, and, more rarely, Ross River virus, Chikungunya virus, and Highlands J virus (HJV) can cause encephalitis in equines (see **Figure 1**) or humans.

The prototype alphavirus, Sindbis virus, does not typically cause encephalitis in humans and is encephalitic only under experimental conditions in mice. Therefore, this virus will also be discussed elsewhere. Among the naturally occurring alphaviruses, WEEV, EEEV, and VEEV, have widespread distributions in North, Central, and South America (see **Figure 2**). WEEV is distributed in the US from the mid-Western states of Michigan and Illinois to the West coast and in western Canada, and clinical cases have been reported in 21 states, whereas EEEV is distributed from Texas to Florida along the Gulf coast and from Georgia to New Hampshire along the Atlantic Coast and in eastern Canada, with cases reported in 19 states, including the mid-Western states of Wisconsin, Illinois, and Michigan. Transmission of VEEV occurs predominantly in Central and South America, with the exception of Everglades virus (formerly VEEV subtype II), which is found in Florida, and Bijou Bridge virus (formerly VEEV subtype IIIB), found in cimicid cliff swallow bugs (*Oeciacus vicarius*) in western North America.

Western Equine Encephalitis Virus

This virus was first isolated in 1930 from the brain of an encephalitic horse in California. According to the US Centers for Disease Control and Prevention, 639 confirmed human cases of WEE have occurred in the US from 1964 to 2005. Likely due to changes in irrigation practices and to successful mosquito control programs in the western US, the annual number of cases has been declining, with fewer than ten cases per year since 1988. Infections with WEEV are generally asymptomatic or result in mild disease after an incubation period of 2–7 days with nonspecific symptoms, for example, sudden onset of fever, headache, nausea, vomiting, anorexia, and malaise, although these can be followed by altered mental status, weakness, and signs of meningeal irritation, evidence of more serious infections. Encephalitis occurs in a minority of infected individuals and may lead to neck stiffness, confusion, tonic–clonic seizures, somnolence, coma, and death. Mild-to-severe neurological sequelae can be found in survivors of encephalitis, especially in those <1 year old, at rates between 5% and 30%. In addition, serological studies indicated that the relation of inapparent to apparent infections changes with age. In children <1 year old, the relation is about 1:1, between 1 and 4 years of age it is about 58:1, and in children >14 years old it is >1000:1. The overall case–fatality rate is about 3%. Encephalitis due to WEEV is characterized by vasculitis and focal hemorrhages mainly affecting the basal ganglia and the nucleus of the thalamus. Because small hemorrhages can occur in the white and gray substance throughout the central nervous system (CNS), they can be mistaken for resolved infarcts in elderly patients.

WEEV is maintained in an enzootic cycle between its natural vertebrate hosts, passerine birds, and its most common mosquito vector, *Culex tarsalis*, a species associated with irrigated agriculture and stream drainages in the western US. Transmission to horses and humans is mediated by so-called bridging mosquito vectors, including *Ochlerotatus melanimon* in California, *Aedes dorsalis* in Utah and New Mexico, and *A. campestris* in New Mexico (see **Figure 3**). Depending on the climate, the natural transmission cycle may be maintained throughout the year. In more moderate climate areas, WEEV may persist in yet-unrecognized hosts, or may be reintroduced annually by migratory birds that move great distances. Isolations of WEEV have been documented in the Veracruz region of Mexico and in South America. Genetic analyses of WEEV isolated in South America (Brazil and northern Argentina) revealed a level of nucleotide

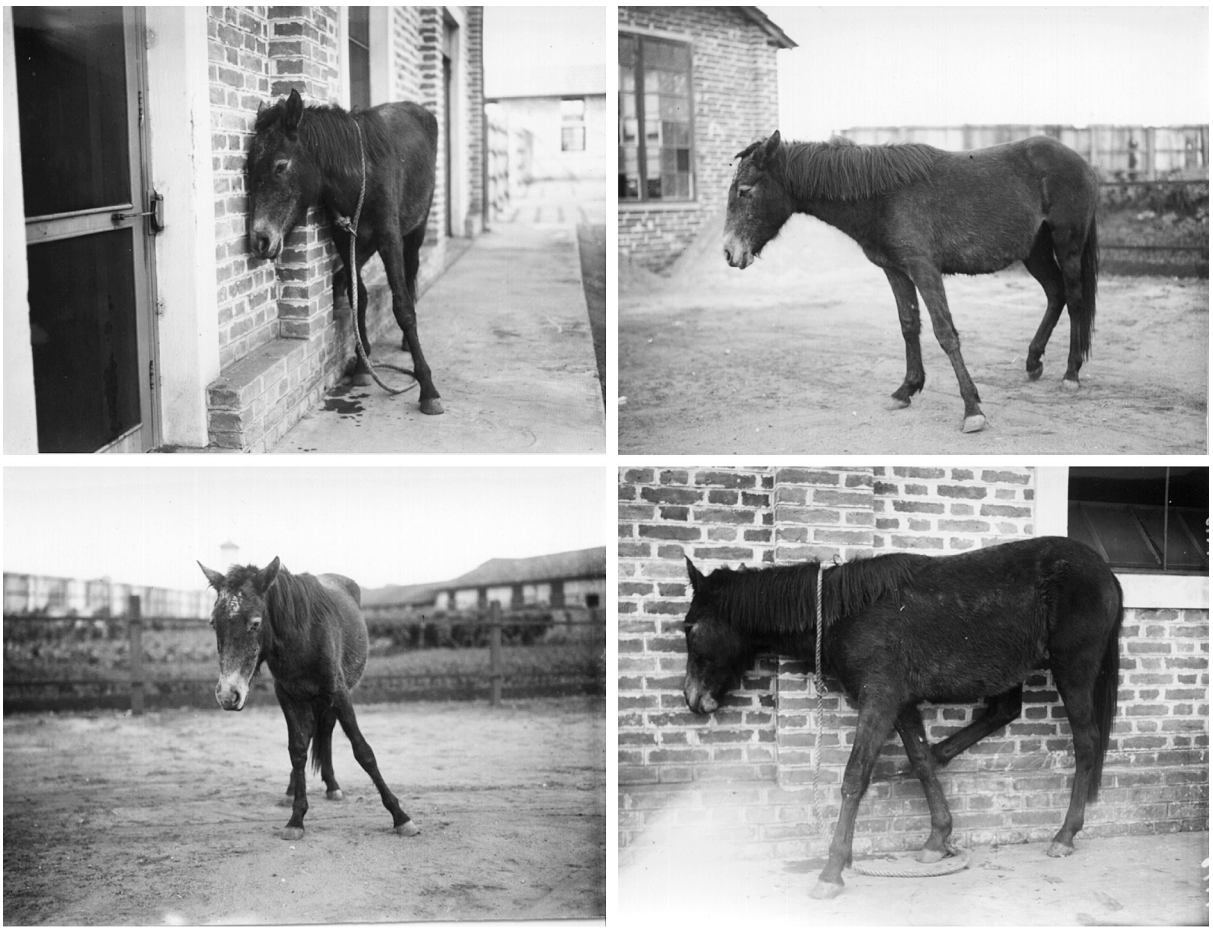


Figure 1 Western equine encephalitis in equines.

identity >90% in the E2/6K/E1 coding region when compared to isolates from California, Texas, and as far north as Montana, suggesting a monophyletic nature of the WEEV lineage, with an overall slow evolution. Hahn and colleagues showed that WEEV is a natural recombinant virus, with its capsid amino acid sequence similar to that of EEEV, while the remaining part of the structural polyprotein was more similar to that of Sindbis virus. Further phylogenetic analyses, including the nonstructural genes as well, confirmed that WEEV and the closely related Highlands J, Fort Morgan, and Buggy Creek viruses are recombinant viruses derived from an ancestor formed by an EEEV-like (5′ two-thirds of the genome) and Sindbis virus-like (3′ one-third of the genome) parental virus.

Eastern Equine Encephalitis Virus

The first isolates of EEEV were obtained in 1933 from infected horses in Virginia and New Jersey. The main EEEV transmission cycle occurs between birds and mosquitoes via *Culiseta melanura* mosquitoes. However, the principal arthropod vector for transmission of EEEV to humans or to horses are *Aedes*, *Coquillettidia*, and *Culex* mosquitoes which, unlike *Culiseta melanura*, tend to feed on both birds and mammals. Virus transmission occurs most commonly in and around freshwater hardwood swamps in the Atlantic and Gulf Coast states and in the Great Lakes region. Most cases of EEE have been reported from Florida, Georgia, Massachusetts, and New Jersey. In horses, in some bird species, and in dogs, EEEV can cause severe disease and experimental data suggest that equines can develop viremia upon experimental infection; however, it is believed that horses do not serve as amplifying hosts during epidemics.

According to the US Centers for Disease Control and Prevention, 220 confirmed cases of EEE have occurred in the US from 1964 to 2004. This virus is probably the most virulent of the encephalitic alphaviruses, with a case–fatality rate estimated at >33%. After a 4–10-day incubation period symptoms begin with sudden onset of fever, general muscle pains, and headache of increasing severity. In human cases of encephalitis, fever, headache, vomiting, respiratory symptoms, leucocytosis, hematuria, seizures, and coma may occur. Clinical studies of serologically confirmed and human EEEV infections using

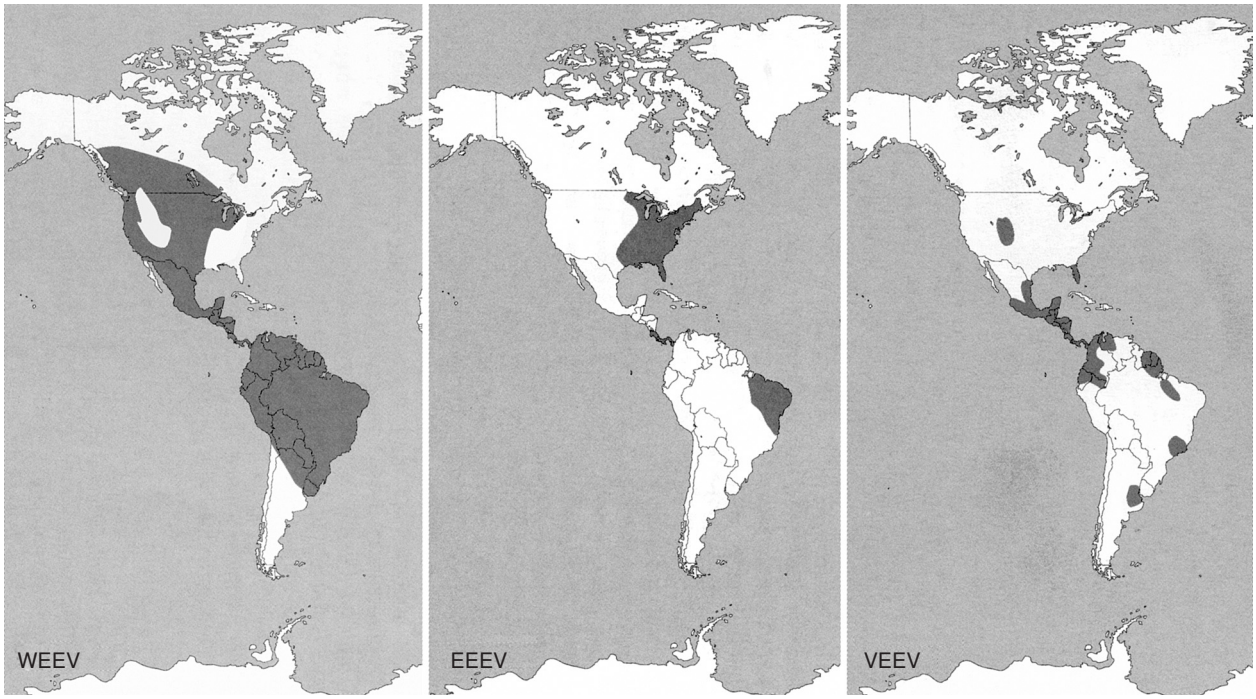


Figure 2 Geographic distribution of equine encephalitic togaviruses.

magnetic resonance imaging and computed tomography have shown changes in the basal ganglia and thalami, suggesting brain edema, ischemia, and hypoperfusion in the early stage of disease. Investigations of gross pathology of fatal human cases revealed brain edema with necrosis, facial or generalized edema, vascular congestion, and hemorrhage in the brain and visceral organs. The predominant micropathological manifestations in the brain include vasculitis, hemorrhage, and encephalitis.

The pathogenesis of EEEV is poorly understood; however, data from experimental infections of mice, guinea pigs, and rhesus monkeys, and from histopathological studies of equine and porcine cases are available. The mouse encephalitis model is well established for several alphaviruses, although it generally lacks the ability to reproduce the vascular component of the disease that is typical for EEE in humans. A hamster model of EEE, used to study acute vasculitis and encephalitis, was described recently. The virus penetrates the brain quickly in animal models and the neuronal phase of the disease develops rapidly. The virus is capable of establishing productive infection in all parts of the mouse or hamster brain. The experimental studies have suggested early infection of periventricular and perivascular neuronal cells in the basal ganglia and hippocampus. It is difficult to explain the early involvement of the basal ganglia and brainstem in experimental infections and to our knowledge no specific anatomic characteristics of these brain regions have been described that might help explain their susceptibility to early infection. In contrast to VEEV (see below), EEEV appears to rapidly invade the brain of infected animals via blood, and the first antigen-positive neuronal cells are located in the basal ganglia and brainstem in the hamster model. Certain findings in animal models, such as the hamster, are similar to early clinical manifestations of EEEV infections in humans. The inflammatory response in the brain is prominent in cases in which animals had survived for at least 5 days, and is produced by macrophages, lymphocytes, and neutrophils. Some of the animal models also display the peripheral pathological changes described in fatal human EEE cases, including congestion and numerous microhemorrhages in the liver, spleen, and lung.

Venezuelan Equine Encephalitis Virus

In 1938, the first isolation of VEEV was obtained from the brain of an encephalitic animal in Venezuela. Like WEEV and EEEV, VEEV is a zoonotic pathogen, transmitted between vector mosquitoes and vertebrate hosts, namely rodents and humans in enzootic cycles, and horses and humans in epidemic or epizootic cycles. Although during epizoodemics essentially any mosquito can be found infected with VEEV, *Ochlerotatus taeniorhynchus* is one of the principal vectors believed to be responsible for the transmission of VEEV during outbreaks, whereas *Culex* (*Melanoconion*) species mosquitoes transmit enzootic strains of VEEV. These viruses are also highly infectious via the aerosol route, and have been responsible for numerous laboratory accidents (>150 cases without an associated perforating injury) and have been developed as a biological weapon in the US and in the former Soviet

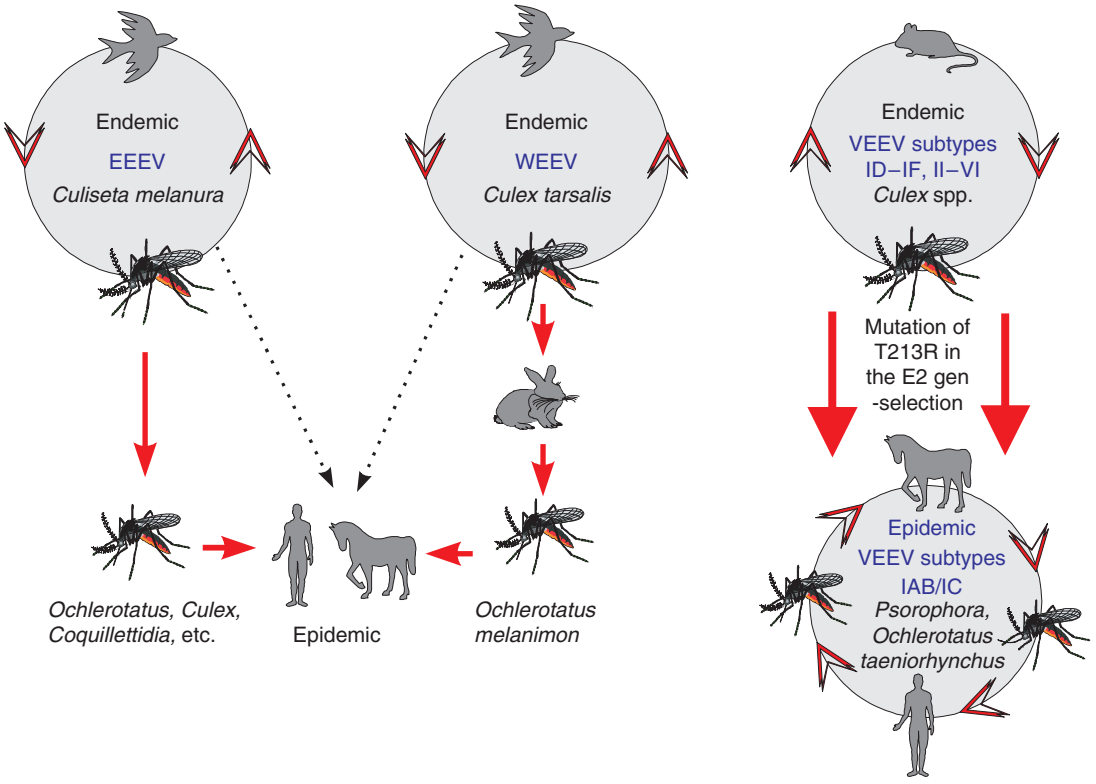


Figure 3 Epidemiology of equine encephalitic togaviruses. Ecological alterations lead to infections of horses and humans with Eastern equine encephalitis virus and Western equine encephalitis virus, but genetic shifts from subtype ID to IC Venezuelan equine encephalitis viruses trigger high viremia in horses, causing them to be virus amplifiers and sustaining epidemics/epizootics.

Union. VEEV infection has also been associated with abortion and fetal death in humans. Equids develop high-titer viremias, which serve as sources of infection for subsequently feeding mosquitoes. In recent years, spillover to humans during equine epizootics has resulted in epidemics of VEEV.

The VEEV antigenic complex, of which VEE virus is the prototype member, is divided into six distinct antigenic subtypes (I–VI). These subtypes correlate with human and equine pathogenicity but major human epidemics and equine epizootics have been associated almost exclusively with subtypes IAB and IC. Enzootic transmission is generally associated with subtypes ID and IE, which are less virulent for horses. In contrast, epizootic subtypes IAB and IC are highly pathogenic for horses, with case–fatality rates of up to 83% reported. The most recent major outbreak occurred in 1995 in Venezuela and Colombia in which 75 000–100 000 human cases occurred, and more than 300 fatal encephalitis cases were recorded. This epidemic was associated with VEEV subtype IC. In 1993, equine disease was associated with VEEV-IE in Mexico and in the period between 1993 and 1995, human cases of VEEV-ID-associated disease occurred in Peru.

Interferons (IFNs) have long been recognized as essential components of the innate immune response to various viral and bacterial infections. It is well established that IFN-α and IFN-β can protect against alphaviral disease and that the virulence correlates with the resistance to IFN-α and IFN-β. Previous studies with VEEV suggested that the IFN-α and IFN-β resistance or sensitivity phenotype correlated with epizootic potential and equine virulence, although others observed little or no difference.

Generally, severe encephalitis in VEEV infection is less common than with EEEV and WEEV infections, although VEEV-associated encephalitis is a more common outcome in children. In adults, VEEV infection usually results in flu-like symptoms and encephalitis is rare. In humans, while the case–fatality rate is low (<1%), neurological diseases, including disorientation, ataxia, mental depression, and convulsions, can be detected in up to 14% of infected individuals, especially children. Neurological sequelae in humans also are common. The predominant pathological findings in fatal human VEE cases include (1) in the CNS: edema, congestion, hemorrhages, vasculitis, meningitis, and encephalitis; (2) in the lungs: interstitial pneumonia, alveolar hemorrhage, congestion, and edema; (3) in lymphoid tissue: follicular necrosis and lymphocyte depletion; and (4) in the liver: diffuse hepatocellular degeneration.

A murine model for VEEV-induced encephalitis and lymphotropism has been established. Experimental studies have demonstrated that the murine model is

characterized by biphasic disease, which starts with productive infection of lymphoid tissue and ends in the destruction of the CNS by aggressive viral replication and a 'toxic' neuroinflammatory response, which is uniformly lethal. By the time encephalitis has developed in an infected mouse, infectious virus is usually absent from peripheral organs and blood. However, virus replicates to high titers in the brain and encephalitic mice die 5–7 days after infection. While the classical mouse model is useful to study lethal encephalitis in mice, it does not enable scientists to study VEEV clearance from the brain because naive mice uniformly succumb to CNS infection. To overcome that barrier, and to study the specific anti-VEEV immune response, as well as virus clearance from the brain, researchers use pre-immunized mice that are susceptible to VEEV infection of the CNS but survive the infection. In general, T cells have been shown to play essential roles in the hosts' defense against alphavirus infection, survival, encephalitis, and also in repair of neural damage and homeostasis in the brain. The brain has been proposed to be a distinct immune regulatory tissue, given its status as an immune-privileged site and that T-lymphocyte entry into the CNS under normal homeostatic conditions is thought to be restricted to memory T cells. Upon viral infection in the periphery and the onset of lymphopenic conditions (characteristic of VEEV infection) followed by entry into the brain, a combination of naive T cells and virus-specific memory T cells may migrate to sites of CNS infection. $CD4^+$ T cell effector functions in encephalitis may include a combination of Th1, Th2, and regulatory T cell ($CD25^+$, forkhead family transcription factor, $Foxp3^+$) activities.

$CD8^+$ T cells contribute to both recovery and pathology of encephalitic flavivirus infections, but their role in VEEV-induced encephalitis, immunopathology, and protection is unclear. In general, it has been hypothesized that cell-mediated cytotoxicity is less critical for control of cytopathic viruses such as VEEV, as compared to control of noncytopathic viruses. This general paradigm can be extended to the CNS where elimination of virus from neurons is thought to be via nonlytic mechanisms, due to the poor regenerative capacity of this cell type.

Once the blood–brain barrier is breached by the virus, local antibody production by B cells in the brain plays a role in prevention of viral entry into cells of the CNS and facilitates virus clearance via Fc receptors. Immunohistochemistry studies have shown that B cells are present in the brains of mice that are vaccinated and which survive for 1 month after challenge with VEEV via the intranasal route.

It is believed that VEEV pathogenesis, particularly the development of clinical signs of encephalitis, such as paralysis, depends both on the virus-mediated and immune-mediated neuronal cell death, and that the development of these encephalitic signs depends on two factors, direct damage of neurons and specific characteristics of the neuroinflammatory response. In addition, some studies have indicated that the early neuroinflammatory response in vaccinated animals (rapid adaptive response to infection) is incapable of controlling the initial replication of the challenge VEEV in the brain, which occurs at levels comparable to those measured in naive animals on the first 3 days after intranasal infection. Production of infectious VEEV in the brain of a vaccinated mouse is reduced 7 days after infection and is undetectable 1 month after infection in immunocompetent mice. It is likely that the reduction of VEEV replication in the brain of vaccinated animals on day 7 might impact survival. However, recent studies on genetically modified mice with preexisting anti-VEEV immunity have shown that a high level of replication in the brain over a period of 28 days is not lethal.

Protection from lethal encephalitis in mice appears to be dependent on the presence of αβ T-cell receptor (TCR)-bearing cells but not on γδ TCR-bearing cells. The relative degree of immune-mediated inflammation in various TCR-deficient mice does not fully correlate with viral clearance. Remarkably, vaccinated γδ TCR-deficient mice are protected from lethal viral challenge by intranasal inoculation but VEEV can persist up to 28 days post inoculation. Virus clearance is not affected in vaccinated, immunocompetent mice, or in surviving animals lacking functional IFN-γ receptor. Most notably, γδ T-cell-deficient mice tolerate (1) high levels of viral replication in the first 7 days after infection (acute phase), which is similar to levels observed in unvaccinated, immunocompetent mice that become paralyzed and succumb to infection; and (2) lower-level virus persistence for at least 28 days that is not observed in immunocompetent mice (asymptomatic, persistent phase). The virus can persist in the brain despite a moderate level of inflammation and cellular infiltration at the site of infection. Such viral persistence has not been observed in other animal models. Observations described in recent studies may provide opportunity in the future to dissect potential intervention points and, perhaps, to add prognostic markers that correlate with protection against VEEV encephalitis in vaccinated humans.

Highlands J Virus

As pointed out earlier, HJV is a member of the recombinant WEEV lineage. Therefore, it is not surprising that this virus initially was listed as a subtype of WEEV but later raised to the taxonomic level of 'virus' based on oligonucleotide and antigenic mapping. HJV is similar epidemiologically to EEEV in that (1) it is transmitted by the same freshwater swamp mosquitoes, *Culiseta melanura*; (2) it shares the same geographical distribution in the eastern parts of North America and as far west as Texas; and (3) its primary vertebrate hosts are passerine

birds. Curiously, however, with one recorded exception (an encephalitic horse from Florida in 1964), it has not been linked to equine or human diseases. HJV may cause severe disease in chickens (up to 7% mortality) and young turkeys, and it is responsible for severe losses in egg production in adult turkeys, but has not been detected in the brains of these diseased birds. Thus, the horse case may have had a co-morbidity that went unnoticed. In comparison of VEEV, EEEV, and WEEV, HJV has to be mentioned, but it is not considered an encephalitic alphavirus.

Diagnosis and Treatment

WEEV

IgM antibodies to WEEV usually are detectable at time of onset of the disease in either horses or humans, using 'IgM antibody-capture enzyme-linked immunosorbent assay' (MAC-ELISA) and within a few days after onset by hemagglutination-inhibition and neutralization assays. A fourfold or greater rise or fall in IgG antibody to WEEV in paired acute- and convalescent-phase samples taken at least 10 days apart also indicates infection with WEEV. Depending on the geographical origin of the suspected case or the particular travel history, a neutralization assay may be required to sort out cross-reacting antibodies against VEEV, EEEV, HJV, or other alphaviruses. If direct virus detection is required, virus can be isolated in a variety of vertebrate and mosquito cell lines; however, Vero cells (African Green Monkey kidney cells) are preferentially used. 'Reverse transcription-polymerase chain reaction' (RT-PCR) and real-time RT-PCR protocols for specific amplification of viral RNA are available. Immunohistochemical detection of viral antigen can be performed on brain tissues from dead horses. Clinical parameters usually are within the normal range, but cerebrospinal fluid (CSF) displays elevated levels of protein (90–110 mg dl^{-1}) and a leukocytosis (up to 500 cm^{-3}, mainly lymphocytes).

EEEV

EEEV infection is diagnosed using serological assays, especially by detection of IgM in serum and CSF in the presence of CNS or febrile disease, and neutralizing antibody testing of acute- and convalescent-phase samples. Virus isolation and detection of viral nucleic acid is possible, as described above for WEEV.

VEEV

Currently, VEEV infection is diagnosed principally by direct detection, for example, nucleic acid or virus isolation from acute-phase serum or spinal fluid or by serological assay, such as detection of VEEV-specific IgM in the CSF using MAC-ELISA or monoclonal antibody-based antigen-capture ELISA. The plaque-reduction neutralization test (PRNT), which like MAC-ELISA is useful in distinguishing VEEV infections from infections with other alphaviruses (see above), cannot be used to identify the serotype. Recently, a VEEV-specific blocking ELISA was described that also identifies serotype-specific antibodies against VEEV in sera of humans, equids, or rodents.

No effective antiviral treatment exists for these encephalitic arboviruses and treatment remains symptomatic.

See also: Rubella Virus; Togaviruses: Equine Encephalitic Viruses.

Further Reading

Bastian FO, Wende RD, Singer DB, and Zeller RS (1975) Eastern equine encephalomyelitis. Histopathologic and ultrastructural changes with isolation of the virus in a human case. *American Journal of Clinical Pathology* 64: 10–13.

Beck CE and Wyckoff RWG (1938) Venezuelan equine encephalomyelitis. *Science* 88: 530.

de la Monte S, Castro F, Bonilla NJ, Gaskin de Urdaneta A, and Hutchins GM (1985) The systemic pathology of Venezuelan equine encephalitis virus infection in humans. *American Journal of Tropical Medicine and Hygiene* 34: 194–202.

Deresiewicz RL, Thaler SJ, Hsu L, and Zamani AA (1997) Clinical and neuroradiographic manifestations of eastern equine encephalitis. *New England Journal of Medicine* 336: 1867–1874.

Ehrenkranz NJ and Ventura AK (1974) Venezuelan equine encephalitis virus infection in man. *Annual Review of Medicine* 25: 9–14.

Femster R (1957) Equine encephalitis in Massachusetts. *New England Journal of Medicine* 257: 701–704.

Garcia-Tamayo J, Carreno G, and Esparza J (1979) Central nervous system alterations as sequelae of Venezuelan equine encephalitis virus infection in rat. *Journal of Pathology* 128: 87–91.

Getting V (1941) Equine encephalomyelitis in Massachusetts. *New England Journal of Medicine* 224: 999–1006.

Griffin DE (2001) Alphaviruses. In: Knipe DM and Howley PM (eds.) *Fields' Virology,* 4th edn., pp. 917–962. New York: Lippincott.

Griffin DE (2003) Immune responses to RNA-virus infections of the CNS. *Nature Reviews Immunology* 3: 493–502.

Paessler S, Aguilar P, Anishchenko M, *et al.* (2004) The hamster as an animal model for eastern equine encephalitis and its use in studies of virus entrance into the brain. *Journal of Infectious Diseases* 189: 2072–2076.

Paessler S, Ni H, Petrakova O, *et al.* (2006) Replication and clearance of Venezuelan equine encephalitis virus from the brains of animals vaccinated with chimeric SIN/VEE viruses. *Journal of Virology* 80: 2784–2796.

Philpotts RJ, Brooks TJ, and Cox CS (1980) Laboratory safety for arboviruses and certain other viruses of vertebrates. The subcommittee on arbovirus laboratory safety of the american committee on arthropod-borne viruses. *American Journal of Tropical Medicine and Hygiene* 29: 1359–1381.

Ryzhikov AB, Ryabchikova EI, Sergeev AN, and Tkacheva NV (1995) Spread of Venezuelan equine encephalitis virus in mice olfactory tract. *Archives of Virology* 140: 2243–2254.

Walton TE and Grayson MA (1998) Venezuelan equine encephalomyelitis. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. IV, pp. 203–231. Boca Raton, FL: CRC Press.

Weaver SC, Salas R, Rico-Hesse R, *et al.* (1996) Re-emergence of epidemic Venezuelan equine encephalomyelitis in South America. *Lancet* 348: 436–440.

Togaviruses Causing Rash and Fever

D W Smith, PathWest Laboratory Medicine WA, Nedlands, WA, Australia
J S Mackenzie, Curtin University of Technology, Shenton Park, WA, Australia
M D A Lindsay, Western Australian Department of Health, Mount Claremont, WA, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Analgesics Medications to relieve pain.
Arbovirus A virus transmitted to vertebrates by hematophagous (blood-feeding) insects and which replicates in the insect, as opposed to 'mechanical' transmission.
Arthritis Acute or chronic inflammation of one or more joints, usually accompanied by pain and stiffness, and sometimes swelling of the joint.
Fascia/fasciitis Fascia are bands or sheaths of connective tissues that support or connect parts of the body. Fasciitis is inflammation of these structures, most commonly the fascia of the sole of the foot and the wrist.
Maculopapular (referring to a rash) A mixture of flat red areas (macules) and small red raised spots (papules).
Nonsteroidal anti-inflammatory agents Medications used to relieve inflammation that are not corticosteroids, aspirin, or acetaminophen. Common ones include ibuprofen, naproxen, and indomethacin.
Sylvatic Involving wild animals.
Synovial Pertaining to the synovium, a thin membrane that lines joints and tendons and secretes synovial fluid.
Tenosynovitis Inflammation of tendons and the synovial membrane surrounding the tendon.
Teratogenic Causing malformations of an embryo or fetus.
Urticarial Characterized by pale or reddened irregular, elevated patches, and severe itching. An urticarial rash is also called 'hives'.

Introduction

Viruses of the family *Togaviridae* (togaviruses) are enveloped, positive-stranded RNA viruses of 60–70 nm diameter, and include viruses of two genera, *Alphavirus* and *Rubivirus*. The latter contains a single species, Rubella virus, a major cause of fever and rash internationally; it is discussed elsewhere in this series. Alphaviruses are distributed worldwide and cause illness in many hundreds of thousands of people each year. Clinical illness ranges from mild febrile illnesses to severe illnesses, such as encephalitis and hemorrhagic fever. One of the most common clinical manifestations of alphavirus infections is fever accompanied by rash and/or arthritis. The alphaviruses causing these illnesses are all closely related and belong to two major and one minor antigenic groups: the Semliki Forest virus (SFV) antigenic group (**Figure 1**), which includes SFV, Chikungunya virus (CHIKV), O'nyong-nyong virus (ONNV), and Ross River virus (RRV); and the Sindbis virus (SINV) virus group (**Figure 2**), comprising SINV and Mayaro virus (MAYV). Barmah Forest virus (BFV) is in its own antigenic complex but is genetically linked to the SFV complex viruses. The diseases they cause are usually named after the virus, though an antigenic subtype of SINV is the cause of Ockelbo disease, Pogosta disease, and Karelian fever, while the disease due to RRV has been called epidemic polyarthritis in the past. All are mosquito-borne viruses, but with differences in their ecology and epidemiology. However, there are significant similarities in the clinical aspects of the diseases caused by these viruses, likely reflecting common pathogenic features.

Clinical Manifestations

Many infected individuals will develop clinical illness, and symptomatic to asymptomatic infection ratios have been estimated at 2:1 to 4:1 for CHIKV, ONNV, and MAYV disease, from 1:80 to 3:1 or higher for RRV infections in Australia, and 1:20 to 1:40 for Ockelbo and Pogosta diseases (**Table 1**).

Infections occur at all ages and in both sexes. The male to female ratio varies in different studies but there are no major gender effects on clinical illness. Infection of children in endemic areas or during epidemics occurs commonly, but clinical disease is less common and usually milder than in adults, and arthritis is rare in children.

Acute Infection

Clinical illness presents mainly as joint pains and muscle pains, accompanied by fever and/or rash in a varying proportion of cases.

Patients typically present with joint pains and muscle pains that are usually preceded by a few days of fever, at least in those who become febrile. Sore throat and

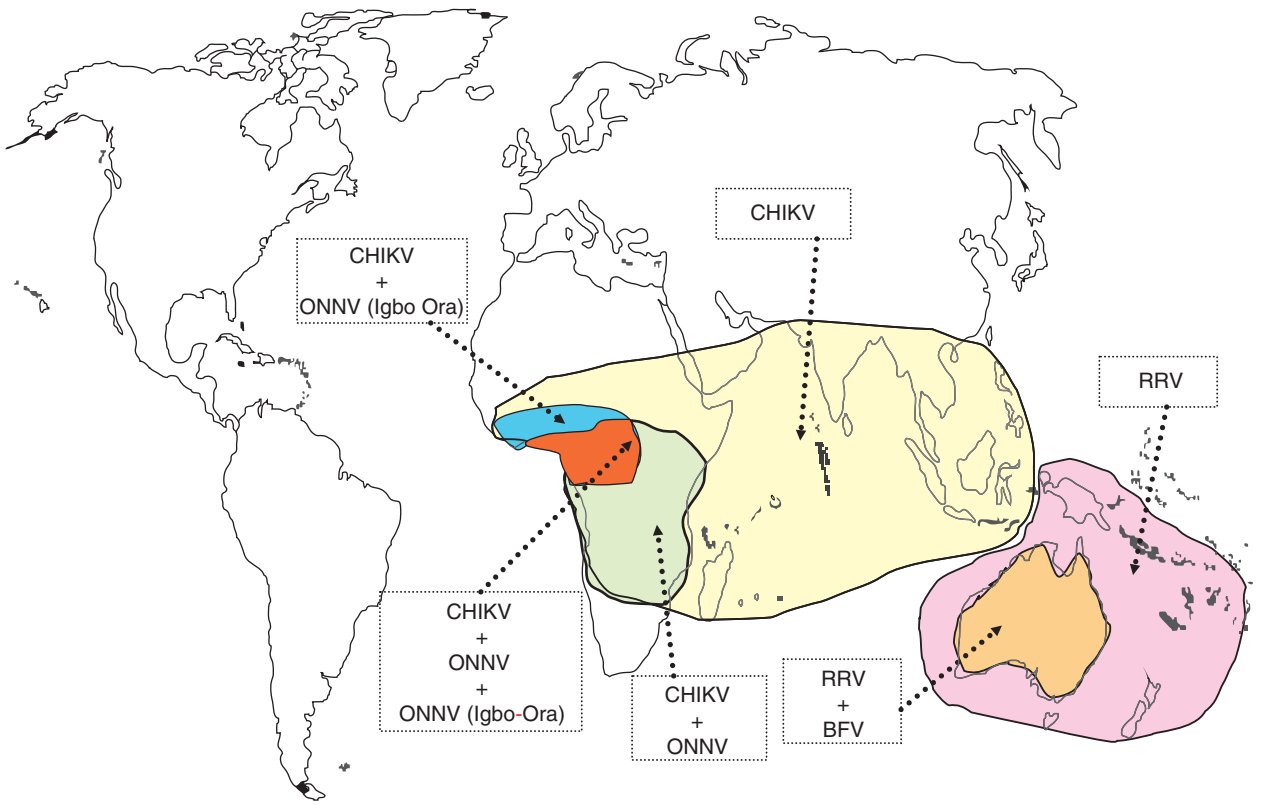


Figure 1 Geographical distribution of the major alphaviruses of the Semliki Forest virus antigenic complex that cause fever, rash, and arthritis in humans.

headache are also commonly reported, and conjunctivitis occurs occasionally. Rash generally follows the other manifestations by a few days, but can sometimes precede them or appear simultaneously. With illness due to RRV and BFV, fatigue is also a prominent clinical feature, and is probably a feature of the other alphaviruses as well. Lymphadenopathy is prominent with some of the alphaviruses, CHIKV causing generalized lymphadenopathy and ONNV causing enlarged posterior cervical lymph nodes.

The pattern of joint involvement is consistent across this group of alphaviruses, commonly involving the ankles, knees, fingers, wrists, elbows, and shoulders. Several joints are involved and the joint involvement is usually symmetric. Other joints including the jaw and the spine may also be affected and back pain is a common complaint. Pain is the most common feature, but most patients also have stiffness of the joints and, less frequently, swelling. The swelling can be due to effusion and/or synovial and soft tissue swelling. During recovery there is a gradual decline in both the number and severity of joint involvement. Inflammation of the fascia of the sole of the foot and the wrist is often reported with RRV and CHIKV infection. Pressure on nerves due to swelling of the fascia may cause tingling in the extremities. This is common with RRV, CHIKV, ONNV, and SINV disease. Muscle pain and tenderness is a prominent part of the clinical illness, usually involving the limbs and shoulders. This can sometimes be more troublesome than the joint pains and it is important to determine whether limb pain is arising from the joints or the muscles.

When rash occurs it is usually maculopapular and most florid on the face, trunk, and upper part of the limbs. However, it can involve the whole body surface, including the palms of the hands, the soles of the feet, and the scalp. Urticarial or vesicular rash occurs in some patients with SINV or BFV disease, but is rare in patients infected with the other viruses. Itchy rash is common with Pogosta disease and ONNV infection. Evidence from RRV and SINV studies suggests that the rashes probably result from presence of virus in the skin and the resulting local immune responses. The RRV rash shows a monocytic infiltrate with presence of RRV antigen within epidermal cells, while the rare purpuric rashes are associated with cytotoxic T-cell responses and capillary leakage.

Gastrointestinal symptoms such as vomiting, abdominal pain, and diarrhea have been reported with RRV and CHIKV illness, particularly in children. Severe illness is rare, other than hemorrhagic disease resembling dengue that can occur following CHIKV infection.

In summary, the majority of infections with the arthritogenic alphaviruses are benign but temporarily debilitating. Fever and rash, if present, usually last less than a week and most patients recover fully within 4 weeks. Joint pains, muscle pains, and lethargy are the slowest to resolve.

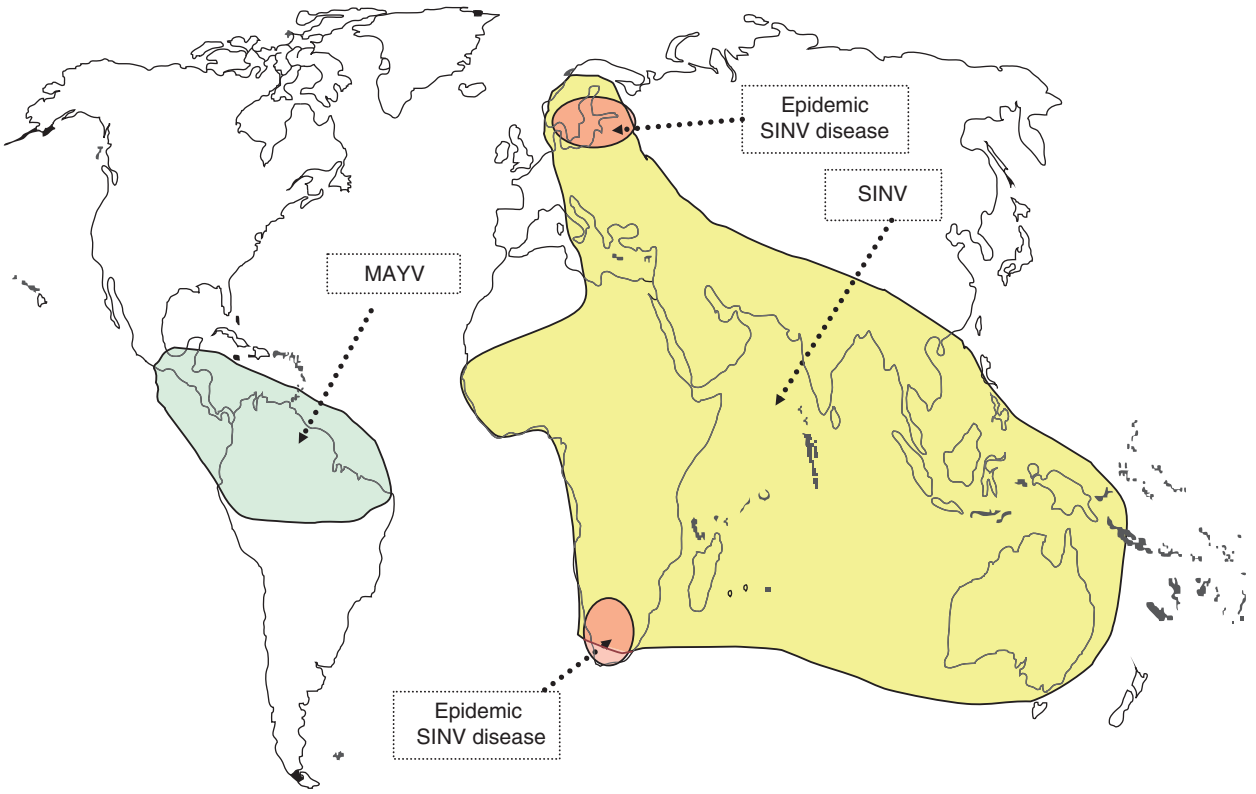


Figure 2 Geographical distribution of the major alphaviruses of the Sindbis virus antigenic complex that cause fever, rash, and arthritis in humans.

Chronic Illness Following Infection

One of the features of alphavirus arthritis is the frequency of prolonged illness, especially persisting joint pains. For SINV, joint pain lasting more than 2 years has been reported for 50% of cases in Finland (i.e., Pogosta disease) and for 25% of cases in Sweden (i.e., Ockelbo disease). Persisting joint pain is also common in Russian cases (i.e., Karelian fever). Similarly, joint pains commonly persist for many months following acute CHIKV infection, and have been reported to follow MAYV infection. Most RRV patients return to full physical activity within 3–6 months, but some suffer from persisting disability due to joint pains, muscle pains, fatigue, and depression. One study found that joint pain persisted for more than 3 months in about 70% of patients. A separate prospective case-controlled study found that at 12 months after onset 90% of patients still had joint pain, 80% had tiredness, 75% had joint stiffness, and 50% had muscle and/or tendon pain.

Infection in Pregnancy

Generally, infection in pregnancy has no special implications for the mother. RRV causes fetal and neonatal death in mice, but there is no evidence of this in humans. During outbreaks in the Pacific Islands possible congenital infection occurred in a small percentage of women, but there was no effect on length of gestation or fetal outcome, and no evidence of congenital malformation. In contrast, during the 2005 Indian Ocean outbreak of CHIKV, there was a 12% rate of transmission to the fetus, and most of these babies had either meningoencephalitis or a coagulation disorder.

Pathogenesis of Alphavirus Arthritis

The virus is introduced subcutaneously by a blood-feeding infected mosquito (**Table 2**) and initial replication probably occurs within subcutaneous tissue and local skeletal muscle. Little is known about the pathogenesis of the systemic manifestations of alphavirus infection, and interest has focused on the joint disease. RRV arthritis induces a predominantly monocytic inflammatory response in synovial fluid in humans. T-cell-derived interferon-γ has been found in joint fluid of RRV-infected humans, and is secreted by RRV-specific T cells of humans and mice. In animal models or *in vitro* a number of alphaviruses including RRV, BFV, CHIKV, and SINV infect synovial monocytes/macrophages and, in the case of RRV, can be found in synovial cells. A combination of release of inflammatory mediators from the infected monocytes/macrophages and the cytotoxic T-cell responses to viral antigens are the likely explanations for the synovial swelling, effusion, and joint pain experienced in acute alphavirus infection.

Table 1 The clinical features of the major alphaviruses causing fever, rash, and arthritis in humans

	Chikungunya virus	*O'nyong-nyong virus*	*Ross River virus*	*Barmah Forest virus*	*Sindbis virus (Pogosta)*	*Sindbis virus (Ockelbo)*	*Mayaro virus*
Incubation period	3–12 days, usually 2–7 days	>8 days	3–21 days, usually 7–9 days	7–9 days	<7 days	<7 days	up to 12 days, usually 6–12 days
Fever	100%	Similar to CHIK	45–55%	50%	45%	40%	100%
Joint pains	80–100%	Similar to CHIK	95–100%	70–85%	95%	95%	50%
Arthritis (Joint stiffness/swelling)	Usually, soft tissue	Similar to CHIK	80–90%	30%	45%	60%	NI[a]
Fatigue	NI[a]	NI[a]	70–80%	80%	NI[a]	NI[a]	NI[a]
Rash	30–50% Maculopapular	30–50% Maculopapular, itchy; some vesicular	50–60% Maculopapular	50–100% Maculopapular; 10% vesicular, some urticarial	90%	95%	30%
Muscle pains	50%	Similar to CHIK	60–90%	70–80%	50%	NI[a]	75%
Tenderness of palms and soles, and/or fasciitis	20–30%	Similar to CHIK	50–60%	NI[a]	NI[a]	NI[a]	NI[a]
Backache	50%	Similar to CHIK	30–60%	NI[a]	NI[a]	NI[a]	NI[a]
Headache	50%	Similar to CHIK	Common	NI[a]	40%	NI[a]	100%
Lymphadenopathy	Generalized, common and prominent	Cervical, common and prominent	Common; generalized in 1–20%	Common; generalized in 5–10%	Not prominent	NI[a]	NI[a]
Severe illness/ fatalities	Hemorrhagic disease, encephalitis, death	None reported	Possible meningitis/ encephalitis	None reported	None reported	None reported	Hemorrhagic disease
Other	Conjunctivitis reported as common in some outbreaks. Severe congenital infections	Conjunctivitis common	Glomerulonephritis, asymptomatic congenital infections	None reported	None reported	None reported	None reported

[a]NI, no information.

Table 2 Summary of the major ecological characteristics of the arthritogenic alphaviruses

Virus	*Reservoir*	*Major vectors*	*Geographic distribution*
Chikungunya virus	Nonhuman primates, possibly rodents	Africa (rural): *Aedes africanus, Ae. furcifer, Ae. luteocephalus, Ae. taylori*; Asia: *Ae. aegypti, Ae. albopictus*	Africa, Saudi Arabia, SE Asia, Philippines
O'nyong-nyong virus	Unknown	*Anopheles funestus, An. gambiae* (and related species)	Uganda, Kenya, Tanzania, Zaire, Malawi, Mozambique, Senegal, Zambia, Cameroon, and the Central African Republic; Igbo Ora variant in Nigeria, the Central African Republic, Côte d'Ivoire.
Ross River virus	Marsupials, especially macropods	*Culex annulirostris, Ae. vigilax, Ae. camptorhynchus, Ae. notoscriptus, Ae. sagax*	Australia, New Guinea, Irian Jaya, Pacific Islands
Barmah Forest virus	Marsupials, especially macropods	*Cx. annulirostris, Ae. vigilax, Ae. camptorhynchus*	Australia
Mayaro virus	Possibly wild vertebrates	*Haemagogus* spp.	Central America, northern South America, the Amazon Basin, Trinidad
Sindbis virus	Range of wild and domestic birds	Northern Europe: *Ae.* spp., *Culiseta* spp., *Culex* spp.; Africa: *Culex* spp., *Aedes* spp., *Mansonia* spp., *Cx. univittatus* (in South Africa); Australia: *Cx. annulirostris*	Southern and northeastern Africa, Scandinavia, Finland, Russia, Central and Eastern Europe, Asia, Southeast Asia, and Australia

Attempts to isolate RRV from the joint fluid or tissues of patients with persisting joint disease following RRV infection have been unsuccessful, but RRV antigens have been detected within synovial fluid mononuclear cells. RRV RNA has been found within synovial tissue by polymerase chain reaction (PCR) and RRV can persist within macrophage cultures *in vitro* even in the presence of neutralizing antibodies. All this suggests that the chronic arthritis may result from a persistent, nonreplicative infection that induces an ongoing immune response to viral antigens.

There is also evidence that host factors are likely to influence outcomes. RRV joint disease in humans has been associated with HLA-DR7 positivity, which has a possible role in reducing the cytotoxic T-cell responses to the virus.

Diagnosis of Infection

A number of the alphaviruses produce a viremia detectable by culture or nucleic acid amplification during the early stages of infection, though most patients are not seen until after this period. Alphaviruses will grow in mosquito cells such as C6/36, AP-61, or TRA-284 incubated for 3–4 days at 28 °C, followed by blind passage to indicator cells, such as Vero, baby hamster kidney (BHK), or chick embryo cells that are incubated at 37 °C for a few days. The virus can be identified using monoclonal antibodies, by neutralization tests, or by amplification and characterization of viral nucleic acid using virus-specific primers, probes, or product sequencing.

Detection of viral RNA is significantly more sensitive than is culturing and has been used for detection of CHIKV, RRV, and SINV in blood, and occasionally from other clinical material. A variety of assays directed at NSP1 or E2 viral protein sequences have been used either in uniplex or multiplex formats and, more recently, DNA microarrays have been used for virus identification and characterization.

However, the culture and PCR-based methods are expensive, difficult to access, and negative in most cases. Therefore, diagnosis is almost exclusively done using serological tests. Most diagnostic testing uses the enzyme immunoassays (EIAs) or hemagglutination inhibition (HI) tests, with some use of indirect immunofluorescence antibody (IFA) assays. EIAs and IFA tests can be used to specifically detect either immunoglobulin G (IgG) or immunoglobulin M (IgM). HI will detect both IgG and IgM but is relatively insensitive for IgM detection. Neutralizing antibody titers are regarded as the most specific of the tests, but are confined to specialized laboratories.

IgM antibodies nearly always appear within 1 week of onset of illness, followed by a rise in IgG titer. It is characteristic of alphavirus infections that IgM persists for long periods of time. RRV IgM and BFV IgM usually persist for several months and sometimes years, independent of whether the patient has ongoing symptoms. Persisting IgM has also been documented for SINV and CHIKV, and is likely to be common to all the arthritogenic alphaviruses. Therefore detection of IgM does not, by itself, prove recent infection. That is best diagnosed either by seroconversion from IgG negative to IgG positive or by a fourfold or greater rise in rise in IgG titer between acute and convalescent samples tested in parallel.

False-positive IgM results may also occur occasionally and appear to be a problem particularly with EIA tests,

but can occur with other tests as well. They are due to either a nonspecific reaction of the assay or to cross-reaction with IgM produced in response to infection with a related virus. For example, false-positive BFV IgM is occasionally seen in patients with genuine RRV IgM, incorrectly suggesting a dual infection. Cross-reacting antibodies are more likely to occur between closely related viruses, such as ONNV and CHIKV, or MAYV and SFV, but also occasionally occur with rubella or with infectious and noninfectious conditions that cause polyclonal B-cell activation.

Therefore, the proper interpretation of alphavirus serology requires a good clinical history of the illness and knowledge of the viruses to which the patient may have been exposed. Nevertheless, standard serological tests performed in the correct clinical context have proved to be very reliable.

Treatment of Alphavirus Infections

There is a very limited literature on the treatment of alphavirus arthritis. Rest and gentle exercise assist many patients, and nonsteroidal anti-inflammatory agents and simple analgesics are commonly used and provide substantial benefit for the musculoskeletal manifestations.

There are currently no specific antiviral agents available for the treatment of alphavirus infections, nor are there likely to be any in the near future. Drugs used for treatment of rheumatoid arthritis help some patients. Hydroxychloroquine has shown a benefit for patients with post-CHIKV arthritis in one small trial, and corticosteroids have been used for acute RRV arthritis, but most practitioners remain reluctant to use these agents until there are more data about long-term benefits and safety.

Barmah Forest Virus

BFV was first isolated from *Culex annulirostris* in southeast Australia in 1974, then from mosquitoes in other areas of eastern and northern Australia. Human cases were not reported until the 1980s in southeastern Australia, Queensland, the far north of Western Australia, and in the Northern Territory. The virus was subsequently found to be endemic in the tropical areas of northern Australia. Since then there has been appearance of human disease in new areas both within the tropical north and in the more southerly temperate areas where RRV is also active. BFV activity in new areas has been marked by human epidemics of varying size, after which it settles into an endemic pattern that is similar to, but not always coincident with, RRV activity. Human infections have now occurred throughout mainland Australia, with seasonality and incidence varying between regions.

BFV has been isolated from many of the same mosquito species that carry RRV. It is assumed that the same marsupial hosts are important in maintenance and amplification of the virus, but the full range of vertebrate hosts remains to be determined. BFV shows genetic homogeneity across Australia, suggesting that the same strain of BFV spreads widely across the country.

BFV disease has been reported in people aged 5–73 years old, although most cases of both BFV disease and RRV disease occur between the ages of 20 and 60 years. Clinically it is very similar to RRV disease, though joint involvement is less common and less severe, while rash is more common and more likely to be vesicular or urticarial.

Chronic illness occurs following BFV arthritis, but there are few data regarding this. Chronicity is less common after BFV infection than following RRV infection and probably occurs in about 10% of patients.

Chikungunya Virus

CHIKV disease was first recognized following an outbreak of arthritis in Tanzania in 1952, and shortly after was isolated from human serum as well as from *Aedes* spp. and *Culex* spp. mosquitoes. The name is a Swahili word meaning 'that which contorts (or bends up)' and refers to the severe joint pains and stiffness associated with this illness.

The virus is widespread across sub-Saharan Africa, Saudi Arabia, the Indian subcontinent, and Southeast Asia, especially the Philippines. Two major genetic lineages exist, of which one is restricted to West Africa. The other is further subdivided into Asian and East African sublineages. While there are some biological differences between these lineages, it is not known whether these have any specific pathogenic implications. There are data to suggest that genetic diversity may be seen among CHIKV strains responsible for individual outbreaks in Africa and in adjacent areas. For example, during the recent Indian Ocean outbreak, the virus was from the East African sublineage, but showed evolution during the course of the outbreak. The Asian sublineage shows a much greater genetic conservation.

In Africa, the virus shows low-level endemic activity in rural areas, where it is believed to be maintained in a cycle involving *Aedes* spp. mosquitoes, nonhuman primates, and, possibly, rodents. The role of nonhuman mammalian hosts in Asia is not known, though seropositive primates have been described. Occurrence of epidemic disease in Africa and Asia is associated with the rainy season and increases in numbers of *Aedes aegypti* and, in Asia, *Ae. albopictus*. Urban outbreaks may continue over 2 or more years, but cases then typically disappear for several years. *Mansonia* spp. and *Culex* spp. have also been implicated as potential vectors in laboratory studies.

Epidemics occur either due to reemergence of the virus in an area of prior activity once sufficient time has passed for a susceptible population to reestablish, or due to movement of the virus into areas without previous activity. They may involve up to half the population. There have been regular occurrences of epidemics in Africa, with the most recent being in Kinshasa in 1999/2000. In Asia a number of outbreaks occurred in the 1960s, followed by a relatively quiescent period in the 1970s, reemergence in Indonesia in 1982, further spread within Malaysia in 1988–89, reemergence in Thailand in 1995, and in Indonesia in 2001–03. The most recent outbreak began in late 2004 in southwestern Indian Ocean islands and subsequently spread over the next couple of years to affect several hundred thousand people in that region, with the largest number of cases on Réunion, the Seychelles, and in several areas of India. Subsequently, small numbers of cases were detected worldwide in travelers returning from epidemic areas.

CHIKV acute illness is more severe than that seen with the other alphaviruses, with fever that is more frequent and higher, worse joint pain, a greater incidence of severe disease, and occasional deaths. While most patients recover within a few days or weeks, chronic joint pains lasting months to years occur in about 10% of patients.

Hemorrhagic manifestations, including petechiae and gingival bleeding due to CHIKV infection, are well described but rare and can lead to a misdiagnosis of dengue. This is complicated by the fact that these two viruses may co-circulate and patients may be simultaneously infected by both. Fatalities in the past have been extremely rare and were reported to occur in the very young or in those with severe hemorrhagic disease, though it is not certain that the fatalities were due to CHIKV. In the recent Indian Ocean outbreak, a number of cases of CHIKV encephalitis and deaths have been reported. The latter have occurred in the elderly or those with co-morbidities.

Mayaro Virus

MAYV was first isolated from a forest worker in Trinidad in 1954, and is now known to be widely distributed in Central America, northern South America, and the Amazon Basin. Transmission is primarily by forest-dwelling *Haemagogus* spp. mosquitoes, though *Mansonia venezuelensis* and *Ae. aegypti* are possible vectors. The virus is probably maintained in a sylvatic cycle between mosquitoes and wild vertebrates. There are two genotypes, one being widespread and the other found only in Brazil.

Occasional outbreaks and sporadic cases occur within the endemic areas following human contact with the forest environment. The illness is very similar to that caused by CHIKV, including hemorrhagic disease in some cases.

Una virus was initially classified as subtype of MAYV, but is now regarded as a separate species and has not been shown to cause human disease.

O'nyong-nyong Virus (Including Igbo-Ora Variant)

ONNV is closely related to CHIKV both virologically and clinically. The disease was first described in Uganda in 1959 and the name is a tribal word for painful joints. It was subsequently isolated from human serum and from mosquitoes. That epidemic eventually involved more than 2 million people in Uganda, Kenya, Tanzania, Zaire, Malawi, Mozambique, Senegal, and Zambia. Spread was thought to have occurred by movement of viremic humans. This virus has also been found in Cameroon and the Central African Republic. A variant called Igbo-Ora was later found in West Africa, in Nigeria, Central African Republic, and Côte d'Ivoire. ONNV disappeared for 35 years before reappearing in Uganda in 1996/1997, with subsequent cases in Kenya. Infections due to the major strain (i.e., not Igbo-Ora) have recently been reported from Côte d'Ivoire and Chad.

Transmission is by *Anopheles funestus* and *An. gambiae*. It is presumed that there is a nonhuman mammalian host to account for maintenance of the virus between epidemics, but it has not yet been identified.

The clinical illness is very similar to CHIKV disease, though fever is usually milder and there is prominent cervical lymphadenopathy. Joint pain persisting for several months has been noted, but the frequency is not known.

Ross River Virus

RRV was named after an area near Townsville in northeastern Australia, where it was first isolated from *Ae. vigilax* in 1966, from mosquitoes collected in 1959. It was isolated from the blood of a febrile child in 1973, then from a case of epidemic polyarthritis in 1979. It has since been found in Papua New Guinea, Irian Jaya, and the Solomon Islands. In 1979–80 an epidemic occurred in various Pacific Islands involving over 50 000 cases, probably originating from a viremic air traveler from Australia.

Three genotypes of RRV have so far been described. Genotype 1 was present in Queensland in northeastern Australia until the mid-1970s, after which it disappeared. Genotype 2 has always been the major type on the east coast of Australia and is now the major current circulating type throughout Australia. Genotype 3 was the dominant type in the southwest of Australia until 1996, since which it has largely been replaced by genotype 2 virus. Pacific Islands isolates belong to genotype 2.

RRV is primarily maintained in a cycle between mosquitoes and vertebrate hosts. Two salt-marsh mosquitoes, *Ae. vigilax* and *Ae. camptorhynchus,* are important in northern and southern coastal areas of Australia, respectively. In some coastal and inland areas, transmission to humans occurs from several freshwater breeding species, especially *Cx. annulirostris* but also *Ae. sagax* and *Ae. normanensis*, as well as *Ae. camptorhynchus* breeding in brackish and freshwater. Other species, such as *Coquillettidia linealis* and *Ae. notoscriptus*, may play significant roles in urban environments.

The major vertebrate hosts for both maintenance and amplification of RRV are the macropod marsupials (kangaroos, wallabies, and euros). Horses and small marsupials, such as possums, may be important in periurban and urban areas. There is circumstantial evidence that RRV may survive for years in desiccation-resistant eggs of some *Aedes* spp.

RRV disease is reported every year in Australia, with between 2000 and 8000 notified cases per annum. In the tropical northern parts of the country most activity is seen during the wet season from December to May. Further south in temperate regions, human infections occur predominantly in late spring, summer, or autumn. In those seasons, rainfall or tidal inundation of coastal breeding sites coupled with warm temperatures leads to an increase in vector numbers. Major outbreaks in these areas occur every 2–4 years. In the arid inland regions of Australia occasional outbreaks occur following heavy rainfall with flooding.

RRV disease has been reported in people ranging in age from less than 1 year old to 88 years old, although most cases occur between the ages of 20 and 60 years. Clinical illness appears to be uncommon in children. The clinical illness is typical of these alphaviruses, though tenosynovitis and fasciitis are more common. Joint pains, muscle pains, and fatigue persist for months or years in 70–90% of patients.

Semliki Forest Virus

SFV was first isolated in 1942 from mosquitoes in Uganda and is distributed across sub-Saharan Africa. Serological surveys show that human infection is common in endemic areas, but it has only been associated with a single reported human outbreak of fever, headache, and joint pains that occurred in the Central African Republic in 1987.

Sindbis Virus (Including Ockelbo Virus)

SINV was first isolated from *Culex univittatus* at Sindbis in Egypt, and subsequently from a wide range of species of mosquitoes and vertebrates in Europe, the Middle East, Africa, India, Asia, the Philippines, and Australia. It was not until 1961 that it was isolated from the blood of a febrile human in Uganda, and then linked to human disease in South Africa in 1963.

SINV is the most widely distributed arbovirus causing human disease. It is found throughout southern and northeastern Africa, Northern, Central, and Eastern Europe, Asia, Southeast Asia, and Australia, but clinical disease is largely restricted to Sweden (Ockelbo disease), Norway (Ockelbo disease), Finland (Pogosta disease), Russia (Karelian fever), and South Africa. Clinical disease has only rarely been reported in Asia. Only isolated cases of illness in humans have been reported from Australia, although serological studies have indicated that human infections occur regularly.

Many species of birds and of other vertebrates have been shown to be capable of being infected with SINV, but disease has been identified only in humans. Mosquitoes feeding on birds appear to be the primary source of human infection. In Sweden the virus has been found primarily in the fieldfare (*Turdus pilaris*), redwing (*T. iliacu*), and song thrush (*T. philomelos*), and in Norway game birds also carry the virus. Transmission to humans is via *Aedes*, *Culiseta*, and *Culex* spp. in Sweden, Norway, and Finland. In Africa *Culex*, *Aedes*, and *Mansonia* spp. are important, with *Cx. univittatus* being the primary human vector in South Africa. In Australia, *Cx. annulirostris* is the dominant vector species, but it is also been isolated from *Aedes* spp. No maintenance cycles involving nonhuman vertebrates have been identified as yet in any country.

Two major lineages circulate: the Paleoarctic/Ethiopian and the Oriental/Australian. It is believed that migratory birds are responsible for the spread of the two lineages, though within each there is a genetic heterogeneity consistent with local adaptation and evolution of the virus. Studies in Australia have shown that there is also temporal evolution of the virus within a lineage, presumed to be due to periodic introduction of new variants by migratory birds. A third unique lineage has been identified in the southwest of that country.

The restricted distribution of human epidemics suggests that the Paleoarctic/Ethiopian lineage has a greater ability to cause disease than does the Oriental/Australian lineage. However, the ecology of SINV and the pathogenesis of disease are still poorly understood, so other factors may account for the unusual disease patterns.

Epidemics of Ockelbo disease and Pogosta disease occur during summer and autumn in northern Europe, coincident with the peak mosquito breeding period. Ockelbo occurs each year, while Pogosta disease has shown a consistent periodicity of 7 years since it was first recognized in 1974, the reasons for which are uncertain. Summer–autumn SINV epidemics have also been described in South Africa. Patients usually recover within a few weeks but chronic joint pains are common.

The Future

Alphavirus infections impose a substantial human health and economic burden, without any immediate prospect of antiviral therapy or vaccines. Further research is needed to understand the ecology and epidemiology of these viruses and the pathogenesis of disease.

See also: Arboviruses; Rubella Virus; Togaviruses: Alphaviruses; Togaviruses Causing Encephaitis; Togaviruses: General Features; Togaviruses: Molecular Biology; Togaviruses Not Associated with Human Disease; Vector Transmission of Animal Viruses; West Nile Virus.

Further Reading

Griffin DE (2001) Alphaviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., pp. 917–962. Philadelphia, PA: Lippincott Williams and Wilkins.

Harley D, Sleigh A, and Ritchie S (2001) Ross River virus transmission, infection, and disease: A cross-disciplinary review. *Reviews in Clinical Microbiology* 14: 909–932.

Johnson BK (1989) O'nyong-nyong virus disease. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. III, pp. 217–223. Boca Raton, FL: CRC Press.

Jupp PG and McIntosh BM (1989) Chikungunya virus disease. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. II, pp. 137–157. Boca Raton, FL: CRC Press.

Kurkela S, Manni T, Myllynen J, Vaheri A, and Vapalahti O (2005) Clinical and laboratory manifestations of Sindbis virus infection: Prospective study, Finland, 2002–2003. *Journal of Infectious Diseases* 191: 1820–1829.

Laine M, Luukkainen R, and Toivanen A (2004) Sindbis virus and other alphaviruses as a cause of human arthritic disease. *Journal of Internal Medicine* 256: 457–471.

Mackenzie JS, Broom AK, Hall RA, *et al.* (1998) Arboviruses in the Australian region, 1990 to 1998. *Communicable Diseases Intelligence* 22: 93–100.

Niklasson B (1989) Sindbis and Sindbis-like viruses. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. III, pp. 167–176. Boca Raton, FL: CRC Press.

Pinheiro FP and LeDuc JW (1989) Mayaro virus disease. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. III, pp. 137–150. Boca Raton, FL: CRC Press.

Powers AM, Aguilar PV, Chandler LJ, *et al.* (2006) Genetic relationships among Mayaro and Una viruses suggest distinct patterns of transmission. *American Journal of Tropical Medicine and Hygiene* 75: 461–469.

Powers AM, Brault AC, Tesh RB, and Weaver SC (2000) Re-emergence of Chikungunya and O'nyong-nyong viruses: Evidence for distinct genetic lineages and distant evolutionary relationships. *Journal of General Virology* 81: 471–479.

Rulli NE, Suhrbier A, Hueston L, *et al.* (2005) Ross River virus: Molecular and cellular aspects of disease pathogenesis. *Pharmacology and Therapeutics* 107: 329–342.

Russell RC (2002) Ross River virus: Ecology and distribution. *Annual Reviews in Entomology* 47: 1–31.

Suhrbier A and Linn ML (2004) Clinical and pathological aspects of arthritis due to Ross River virus and other alphaviruses. *Current Opinions in Rheumatology* 16: 374–379.

Togaviruses Not Associated with Human Disease

L L Coffey, Institut Pasteur, Paris, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Cohabitation experiment Fish needle-inoculated with wild-type salmonid alphaviruses (SAVs) are placed in the same tank with naive fish to determine whether SAV can be transmitted from infected to uninfected fish via cohabitation. Alternatively, fish immunized with inactivated SAVs are placed in the same tank as naive fish and then fish experimentally inoculated with wild-type SAVs are introduced to determine whether immunized fish can passively transfer SAV immunity to naive individuals in the same water.

Lice Any of numerous small, flat-bodied, wingless biting or sucking insects of the orders Mallophaga or Anoplura, many of which are external parasites of vertebrates.

Smolt A young salmon at the stage intermediate between the parr and the grilse, when it becomes covered with silvery scales and first migrates from freshwater to the sea.

Introduction

The virus family *Togaviridae* comprises two genera, *Alphavirus* and *Rubivirus*. The latter includes only Rubella virus and will be discussed elsewhere in this encyclopedia. The alphavirus-type species is Sindbis virus which, as do many other alphaviruses, causes fever with rash. There are many medically important alphaviruses, including Eastern equine encephalitis virus, Western equine encephalitis virus, and

Venezuelan equine encephalitis virus; these also will be discussed elsewhere in this encyclopedia.

Most alphaviruses are transmitted by hematophagous arthropods to susceptible terrestrial mammals and, as mentioned, can cause diseases in them ranging from simple fevers to fatal encephalitides. The exceptions are alphaviruses that have not been associated with human rash and fever or encephalitis, or occur only in fish. These and certain other viruses are outlined below and summarized in **Table 1**.

Humans in South America have been shown to have antibody to the mosquito-borne alphaviruses Una virus (UNAV, Semliki Forest antigenic complex) and Trocara virus (not placed in an antigenic complex); these viruses have not been associated with human disease. However, UNAV is closely related to Mayaro virus, a known human pathogen. Antibody to Ndumu virus (NDUV) was found in humans in South Africa in the 1950s and NDUV isolates from *Mansonia* and *Aedes* mosquitoes cause encephalitis in mice; NDUV has not been associated with human disease. Cabassou virus (CABV), a member of the Venezuelan equine encephalitis virus complex, has been isolated from mosquitoes, marsupials, and birds in French Guiana; human disease caused by CABV has not been observed. Whataroa virus (WHAV; Western equine encephalitis virus complex) has been isolated from mosquitoes, birds, and marsupials in New Zealand and from mosquitoes in Australia. Neutralizing antibody to WHAV has been detected in humans in those countries as well; human disease caused by WHAV has not been recorded. Bebaru virus (BEBV), a Semliki Forest complex virus, has been isolated from *Culex* (*Lophoceraomyia*) spp. and *Aedes butleri* mosquitoes in Malaysia and Australia. Neutralizing antibody to BEBV has been detected in Malaysians. Relatively little is known about these six viruses. They have not often been isolated from mosquitoes and their transmission cycles are not defined.

Neutralizing antibodies to Middelburg virus (MIDV), a member of the Middelburg virus complex, have been detected in humans in South Africa, Mozambique, and Angola; no human disease has been associated with MIDV. Neutralizing antibodies to MIDV have been detected in cattle, goats, sheep, and horses in South Africa. However, it is unknown whether livestock participate in the natural transmission of MIDV. Numerous MIDV isolates have been made from *Aedes* and *Mansonia* mosquitoes and virus transmission by *Aedes caballus* has been documented. Experimentally infected *Ae. caballus* successfully transmitted MIDV to lambs starting 4 days post-infection, and the lambs developed viremia and a stiff gait. Despite this, MIDV has not been associated with disease in livestock.

Getah virus (GETV), a member of the Semliki Forest virus complex, was first isolated from mosquitoes in Malaysia and subsequently from mosquitoes in other Asian countries, Australia, and eastern Russia. The first recognition of disease caused by GETV was in racehorses in Japan in 1978. Antibody to this virus has been detected not only in horses but also in humans, pigs, and other mammals in Asia and Australasia. Pathogenicity of GETV is relatively mild and appears to be limited to horses and pigs. Several outbreaks of GETV disease in horses have been reported in Japan and India. Disease is principally characterized by fever, rash, and limb edema and also includes (India) depression, anorexia, and lymphocytopenia. The rash may be caused by immune complex-mediated hypersensitivity. Horses recover completely. Pigs are highly susceptible to GETV infection, with occasional deaths of newborn piglets or fetuses occurring by transplacental infection; adult pigs are less likely to be symptomatic. Because Japanese encephalitis virus, a flavivirus, also causes deaths of fetal pigs, laboratory diagnosis is required to distinguish between infections caused by these two viruses.

Fort Morgan virus (FMV), a Western equine encephalitis complex virus, was first isolated in 1973 from *Oeciacus vicarius* (Hemiptera: Cimicidae) bugs in a nest of cliff swallows occupied by house sparrows (*Passer domesticus*). Subsequently, numerous FMV isolates, plus isolates of a closely related subtype, Buggy Creek virus, were made from *O. vicarius* bugs, house sparrows, and cliff swallows (*Petrochelidon pyrrhonota*); no human disease caused by FMV has been observed. Experimentally infected *O. vicarious* transmitted FMV to nestling sparrows 1 month after infection and for up to 312 days after being held at temperatures simulating hibernation. Mosquitoes of certain *Culex* and *Culiseta* species were refractory to FMV infection, suggesting that transmission is likely mediated only by *O. vicarius*.

Until recently, knowledge of alphaviruses was limited to viruses affecting terrestrial mammals and birds. The recent discovery of alphaviruses in lice parasitizing elephant seals (family Phocidae, genus *Mirounga*) and in fish indicates that alphaviruses occur in both terrestrial and marine environments. It is unknown whether louse-borne Southern elephant seal virus (SESV) causes disease in elephant seals, but outbreaks of sleeping disease and pancreas disease (PD), both caused by salmonid alphaviruses (SAVs), are widespread in farmed fish throughout Western Europe and North America and cause major economic losses to aquaculture industries.

Southern Elephant Seal Virus

SESV was first isolated in 2001 from seal lice, *Lepidophthirus macrorhini*, collected from southern elephant seals (*Mirounga leonina*) on Macquarie Island, 2000 km south of the Australian mainland. Whereas the vector competence of *L. macrorhini* has not been evaluated, Macquarie Island has no mosquitoes and ticks are rare

Table 1 Alphaviruses not associated with human disease or that occur only in fish or have been associated with marine mammals

Virus (abbreviation)	*Antigenic complex*[a]	*Origin*	*Original source*	*Distribution*	*Reservoir*	*Vector*
Aura virus (AURAV)	WEE	Brazil (1959)	*Culex* (*Melanoconion*) spp.	Northern South America	Terrestrial mammals?	Mosquitoes
Bebaru virus (BEBV)	SF	Malaysia (1956)	*Culex* (*Lophoceraomyia*) spp.	Malaysia, Australia	Not known	Mosquitoes
Cabassou virus (CABV)	VEE	French Guiana (1968)	*Culex (Melanoconion) portesi*	South and Central America	Not known	Mosquitoes
Fort Morgan virus (FMV)	WEE	Colorado (1973)	*Oeciacus vicarius*	Western North America	Birds	*Oeciacus vicarius* (bugs)
Buggy Creek virus (subtype of FMV)	WEE	Oklahoma (1981)	*Oeciacus vicarius*	Western North America	Birds	*Oeciacus vicarius* (bugs)
Getah virus (GETV)	SF	Malaysia (1955)	*Culex gelidus*	Eurasia, Australia	Terrestrial mammals	Mosquitoes
Middelburg virus (MIDV)	Middelburg	South Africa (1957)	*Aedes caballus*	Africa	Terrestrial mammals?	Mosquitoes
Ndumu virus (NDUV)	Ndumu	South Africa (1959)	*Aedes circumluteolus*	Africa	Terrestrial mammals	Mosquitoes
Norwegian salmonid alphavirus (NSAV)	Unclassified	Norway (2005)	*Salmo salar*	Norway	Salmon	Lice?
Salmon pancreatic disease virus (SPDV)	Unclassified	Western Europe (1976)	*Salmo salar*	Western Europe, United States	Salmon	Lice?
Sleeping disease virus (SDV)	Unclassified	Western Europe (1985)	*Oncorhynchus mykiss*	Western Europe	Trout	Lice?
Southern elephant seal virus (SESV)	Unclassified	Macquarie Island (2001)	*Lepidophthirus macrorhini*	Australia	Not known	*Lepidophthirus macrorhini* (lice)?
Trocara virus (TROV)	Unclassified	Brazil (1984)	*Aedes serratus*	South America	Not known	Mosquitoes
Una virus (UNAV)	SF	Colombia (1964)	*Psorophora ferox*	South America, Panama	Terrestrial mammals	Mosquitoes
Whataroa virus (WHAV)	WEE	New Zealand (1962)	*Culex pervigilans*	New Zealand	Birds, marsupials	Mosquitoes

[a]Antigenic complex abbreviations: SF: Semliki Forest, VEE: Venezuelan equine encephalitis, WEE: Western equine encephalitis.
A question mark indicates a postulated host.

on elephant seals, further suggesting that lice are vectors of this virus. SESV has not been isolated from elephant seals, but the prevalence of neutralizing SESV antibody is high in a population of elephant seals on Macquarie Island and is higher in older animals.

Genetic comparisons have revealed that SESV is most closely related to Australasian alphaviruses in the Semliki Forest complex, but antigenic studies showed that SESV is only very distantly related to other members of the Semliki Forest complex. That alphaviruses may be vectored by lice on migrating elephant seals traveling long distances, including between Australasia and South America via Antarctica, suggests that dispersal of some alphaviruses may be by such mechanisms, although seal populations other than those on Macquarie Island have not been sampled for evidence of SESV infection.

Salmonid Alphaviruses

Alphaviruses enzootic in fish include salmon pancreas disease virus (SPDV), sleeping disease virus (SDV), and Norwegian salmonid alphavirus (NSAV). These viruses cause morbidity and mortality in farmed salmon and trout. The SAV shares only ~33% amino acid identity with other alphaviruses, the basis for considering them as quite distinct from other alphaviruses.

Salmon Pancreas Disease Virus

PD has been reported in farmed Atlantic salmon, *Salmo salar*, in Western Europe and the United States since 1976 and causes up to 50% mortality in salmon smolts. Affected smolts develop necrosis of the exocrine pancreas, cardiomyopathy, skeletal myopathy that causes infected fish to swim sluggishly, and inappetance, all of which lead to stunted growth and, sometimes, death. Infected salmon smolts experience a triphasic disease including: (1) a preacute stage characterized by pancreatic exocrine necrosis and basophilia of pancreatic acinar cells; (2) an acute phase marked by hemorrhagic pancreatic necrosis and occasional liver and kidney lesions as well as inappetance and inactivity; and (3) a post-acute phase in which the pancreas recovers histopathologically, although survival and weight are lower than in uninfected fish of the same age.

For many years the causative agent of this disease was not known, and attempts to isolate a virus from moribund fish were unsuccessful. The strongest evidence that PD was transmissible came from experimental studies in which PD developed in naive fish injected with spleen homogenates from diseased salmon and from experiments in which naive fish that cohabited in tanks with PD-afflicted fish developed the disease. The etiological agent of PD was identified in 1999 as the alphavirus SPDV on the basis of sequence analysis of viral RNA extracted from tissues of infected farmed salmon. Ectoparasitic sea lice of salmon, *Lepeophtheirus salmonis*, have been postulated as vectors but SPDV-infected lice have not been reported and experimental vector competence studies are lacking. Since PD has been reported only in salmon smolts, natural reservoirs of SPDV presumably are fish. Cohabitation experiments suggest that transmission can occur via direct horizontal spread among salmon in the absence of arthropod vectors, although whether lice were eliminated from experimentally infected salmon is unclear. Further, the stability of SPDV in seawater of various temperatures or evidence of contact transmission among fish or via their excreta has not been evaluated.

As with other alphaviruses, SPDV contains a single-stranded positive-sense RNA genome of approximately 12 kbp. SPDV shares fundamental biological properties with other alphaviruses, although it replicates more slowly in cell cultures than do other alphaviruses and only infects select fish cells. Salmon smolts experimentally infected with SPDV develop hemorrhagic pancreatic necrosis consistent with lesions observed in wild fish. Smolts immunized with inactivated cultured SPDV are protected against cohabitation challenge with wild-type SPDV, indicating that tissue-culture adapted SPDV may have potential as a vaccine candidate to protect against PD.

Sleeping Disease Virus

Outbreaks of SDV have been recognized since 1985 in farmed freshwater rainbow trout, *Oncorhynchus mykiss*, in France, Great Britain, and Norway. Diseased trout lay on their sides at the bottom of their tanks, reminiscent of a sleeping state. Lesions of diseased fish include the sequential development of pancreatic atrophy, cardiac necrosis, and, ultimately, skeletal muscle degeneration. Sleeping disease has been estimated to affect 30% of fish farms in northern France. However, estimates are based on behavioral observations and scant histological studies of moribund fish, and it is difficult to discriminate between pancreatic lesions induced by SDV and those occurring in the similar disease caused by the salmonid birnavirus infectious pancreatic necrosis virus. As with SPDV, mechanisms of SDV transmission are not well understood. One SDV isolate was made from rainbow trout farmed in seawater in close proximity to diseased salmon in Norway, suggesting water-borne transmission. Another possibility is vector-borne transmission via an arthropod or another unrecognized organism that associates with both trout and salmon.

Until 2000, efforts to isolate and characterize SDV proved unsuccessful because the virus does not produce cytopathic effects (CPEs) in most fish cell lines. Isolation of SDV typically requires incubating SDV-infected fish organ homogenates with rainbow trout or Chinook salmon embryo cells at 10 °C for 7–11 days, with CPE generally only becoming evident after several passages. Rainbow trout inoculated intraperitoneally with SDV have detectable viremias within 1 week after inoculation and develop neutralizing antibody to the virus.

Although it exhibits unique ecological characteristics, SDV is presently classified as a strain of SPDV. Genetic analyses of isolates revealed that SDV resembles mammalian alphaviruses with respect to genome organization, conserved nucleotide sequence elements, and amino acid motifs. However, the SDV E3 protein lacks glycosylation. The SDV E1 and E2 glycoproteins diverge genetically from other alphaviruses by 66–70%. This genetic diversity between SDV (and the other SAV) and all other alphaviruses is greater than the degree of diversity between any two mammalian alphaviruses, indicating that the SAVs are the most genetically divergent alphavirus species known. Both SDV and SPDV share only 26% envelope 2 (E2) gene amino acid identity with SESV, a level similar to the identities that SDV and SPDV share with respect to E2 proteins of other alphaviruses. This lack of sequence similarity between marine alphaviruses is evidence against a common aquatic origin of alphaviruses. Comprehensive sequence comparisons using all available SAV sequences with members of other alphavirus complexes are needed if we are to clarify the phylogenetic relationships between SAVs and other alphaviruses, including SESVs.

Sequence comparisons of SDV isolated from geographically distant areas in Europe revealed that strains fall into three genotypes according to location of isolation, but these genetic distinctions are not accompanied by detectable antigenic differences. Even with newly developed monoclonal antibody-based immunoperoxidase detection assays, SDV isolates cannot be differentiated antigenically from one another or from SPDV. Due to difficulties in distinguishing SDV isolates from each other and from SPDV, and to the similar clinical presentations of the three SAVs and infectious pancreatic necrosis virus, definitive diagnosis of SAV is achieved by comparisons of sequenced strains.

Comparative studies revealed that SDV and SPDV share 91% amino acid identity and that SPDV has a 24 nt insertion at the C-terminus of the nsP3 gene that encodes a protein involved in viral RNA synthesis; the phenotypic relevance of this insertion is unknown. Given the rapid and artificial evolution of alphaviruses in cell culture, genomic analyses of SAV that are customarily recovered after extensive fish cell passage should be interpreted with caution. For example, the first published SPDV sequence, derived from virus subjected to repeated passage, contained cell-culture-adapted variant sequences that were not representative of sequences generated from low or unpassaged SPDV isolates.

Norwegian Salmonid Alphaviruses

In 2005, a genetically distinct SAV, NSAV, was identified in diseased salmon and rainbow trout in Norway. NSAV differs genetically from SDV by ~7% and from SPDV by ~9%. Fish infected with NSAV experience disease similar to PD and SD. Unlike certain terrestrial alphaviruses that use small rodents as reservoirs, isolates of NSAV collected from geographically dispersed Norwegian farms raising saltwater fish do not segregate phylogenetically according to physical proximity, suggesting either extensive gene flow within the fish reservoir or a common source of virus. Given that farmed smolts do not migrate in open water, movement of fish by humans for aquaculture is one possible means of NSAV dispersal.

See also: Togaviruses Causing Encephaitis; Togaviruses: Equine Encephalitic Viruses.

Further Reading

Büchen-Osmond C (2003) Taxonomy and classification of viruses. In: Murray PR, Baron EJ, Jorgenson JH, Pfaller MA, and Yolken RH (eds.) *Manual of Clinical Microbiology,* 8th edn., vol. 2, pp. 1217–1226. Washington, DC: ASM Press.

Desvignes L, Quentel C, Lamour F, and Le Ven A (2002) Pathogenesis and immune response in Atlantic salmon (*Salmo salar* L.) parr experimentally infected with salmon pancreas disease virus (SPDV). *Fish and Shellfish Immunology* 12: 77–95.

Fukunaga Y, Kumanomido T, and Kamada M (2000) Getah virus as an equine pathogen. *Veterinary Clinics of North America: Equine Practice* 16: 605–617.

Hodneland K, Bratland A, Christie KE, Endresen C, and Nylund A (2005) New subtype of salmonid alphavirus (SAV), *Togaviridae*, from Atlantic salmon *Salmo salar* and rainbow trout *Oncorhynchus mykiss* in Norway. *Diseases of Aquatic Organisms* 66: 113–120.

Karlsen M, Hodneland K, Endresen C, and Nylund A (2005) Genetic stability within the Norwegian subtype of salmonid alphavirus (family *Togaviridae*). *Archives of Virology* 151: 861–874.

Kokernot RH, McIntosh BM, and Worth CB (1961) Ndumu virus, a hitherto unknown agent, isolated from culicine mosquitoes collected in northern Natal, Union of South Africa. *American Journal of Tropical Medicine and Hygiene* 10: 383–386.

Kumanomido T (2001) Getah virus. In: Service MW (ed.) *The Encyclopedia of Arthropod-Transmitted Infections*, pp. 194–197. Oxon, UK: CABI Publishing.

La Linn M, Gardner J, Warrilow D, *et al.* (2001) Arbovirus of marine mammals: A new alphavirus isolated from the elephant seal louse, *Lepidophthirus macrorhini*. *Journal of Virology* 75: 4103–4109.

López-Dóriga MV, Smail DA, Smith RJ, *et al.* (2001) Isolation of salmon pancreas disease virus (SPDV) in cell culture and its ability to protect against infection by the 'wild-type' agent. *Fish and Shellfish Immunology* 11: 505–522.

Munro ALS, Ellis AE, McVicar AH, Mc Lay HA, and Needham EA (1984) An exocrine pancreas disease of farmed Atlantic salmon in Scotland. *Helgoländer Meeresunters* 37: 571–586.

Powers AM, Brault AC, Shirako Y, *et al.* (2001) Evolutionary relationships and systematics of the alphaviruses. *Journal of Virology* 75: 10118–10131.
Tomori O (2001) Middelburg virus. In: Service MW (ed.) *The Encyclopedia of Arthropod-Transmitted Infections*, pp. 339–340. Oxon, UK: CABI Publishing.
Travassos Da Rosa APA, Turell MJ, Watts DM, *et al.* (2001) Trocara virus: A newly recognized alphavirus (*Togaviridae*) isolated from mosquitoes in the Amazon Basin. *American Journal of Tropical Medicine and Hygiene* 64: 93–97.
Villoing S, Béarzotti M, Chilmonczyk S, Castric J, and Brémont M (2000) Rainbow trout sleeping disease virus is an atypical alphavirus. *Journal of Virology* 74: 173–183.
Weston JH, Villoing S, Brémont M, *et al.* (2002) Comparison of two aquatic alphaviruses, salmon pancreas disease virus and sleeping disease virus, by using genome sequence analysis, monoclonal reactivity, and cross-infection. *Journal of Virology* 76: 6155–6163.

Togaviruses: Alphaviruses

A M Powers, Centers for Disease Control and Prevention, Fort Collins, CO, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Alphavirus A virus in the genus *Alphavirus*.
Clade All of the descendants of a common ancestor (also known as a monophyletic group).
Genotype Genetic composition of individuals; used to delineate relatedness of a group of individuals.

Introduction

The alphaviruses are a group of antigenically related arthropod-borne viruses (arboviruses) that were first isolated in the 1930s. The arboviruses were originally divided into one of three serological groups (A, B, or C) based upon results of hemagglutination inhibition (HI) tests as performed by Casals and Brown. Later characterization of the viruses based upon genetic and virion properties led to the inclusion of those viruses clustered in group A in the genus *Alphavirus*.

Alphaviruses can be broadly categorized into three distinct groups based upon the type of illness they produce in humans and/or animals. Disease patterns include: (1) febrile illness associated with a severe and prolonged arthralgia, (2) encephalitis, or (3) no apparent or unknown clinical illness. Any particular alphavirus will fit into only one of the above profiles but additional symptoms such as demyelination may also be present. Additionally, some of the viruses can induce abortion or stillbirth in animals, decreased egg production in avians, or mortality in vertebrates. Further, a small number of alphaviruses, those strains belonging to the Salmon pancreas disease virus (SPDV) species, infect and cause illness only in fish. The alphaviruses have a wide geographic distribution and are present virtually everywhere on the earth. However, patterns of illness do not correlate with geography as viruses causing any given disease syndrome are found under diverse climatic and ecological conditions. Rather, distribution of a particular virus is tied to vertebrate reservoir and invertebrate vector availability.

Taxonomy and Classification

The genus *Alphavirus* is one of two genera within the family *Togaviridae*. While the second genus, *Rubivirus*, contains only a single species (*Rubella virus*), there are 29 recognized species in the genus *Alphavirus* currently divided into at least eight antigenic complexes (**Table 1**).

Evolutionary Relationships

Numerous phylogenetic studies have been performed on members of the genus *Alphavirus* since the advent of sequencing and genetic analyses. Overall, these derived evolutionary relationships closely agree with the antigenic relationships associated with the surface glycoproteins that have been established for members of this group based upon serological techniques. Evolutionary relationships have not been found to vary with the gene(s) or sequences examined with the exception of the members of the western equine encephalitis (WEE) antigenic complex. When viruses in the WEE complex are examined, they are found to be associated with the WEEV genotype when comparing E2 or E1 glycoproteins, while genetic comparisons of other gene regions result in alignment with viruses in the eastern equine encephalitis virus (EEEV) genotype. This unique feature is a result of the fact that most New World WEE complex viruses

Table 1 Alphaviruses[a]

Antigenic complex	*Species*	*Abbreviation*	*Distribution*	*GenBank acc. no.*
BF	*Barmah Forest virus*	BFV	Australia	U73745
EEE	*Eastern equine encephalitis virus*	EEEV	N. America, Mexico, Caribbean, C. America, S. America	U01034
MID	*Middelburg virus*	MIDV	Africa	AF398374
NDU	*Ndumu virus*	NDUV	Africa	AF398375
SF	*Bebaru virus*	BEBV	Malaysia	AF398376
	Chikungunya virus	CHIKV	Africa, Asia	AF192905
	Getah virus	GETV	Asia, Oceania	AF398377
	Mayaro virus	MAYV	S. America, Trinidad	AF398378
	O'nyong nyong virus	ONNV	Africa	M33999
	Ross River virus	RRV	Oceania, Australia	M20162
	Semliki Forest virus	SFV	Africa; Vietnam	X04129
	Una virus	UNAV	S. America, Panama	AF398381
VEE	*Venezuelan equine encephalitis virus*	VEEV	C., N., S. America	L01442
	Mosso das Pedras virus	MDPV	S. America	AF398382
	Everglades virus	EVEV	Florida (USA)	AF075251
	Mucambo virus	MUCV	S. America, Trinidad	AF398383
	Tonate virus	TONV	Fr. Guiana	AF398384
	Pixuna virus	PIXV	S. America	AF075256
	Cabassou virus	CABV	French Guiana	AF398387
	Rio Negro virus	RNV	Argentina	AF398388
WEE (Sindbis like)	*Aura virus*	AURAV	S. America	AF126284
	Sindbis virus	SINV	Africa, Eurasia, Australia	V00073
	Whataroa virus	WHAV	New Zealand	AF398394
WEE (recombinants)	*Fort Morgan virus*	FMV	Western N. America	AF398389
	Highlands J virus	HJV	Eastern N. America	U52586
	Western equine encephalitis virus	WEEV	S. and N. America	J03854
Unclassified	*Trocara virus*	TROV	S. America	AF252264
	Salmon pancreas disease virus	SPDV	Europe, N. America	AJ012631 AJ238578
	Southern elephant seal virus	SESV	Australia	EMBL DS44746

[a]Classification according to the most recent report of the ICTV; antigenic complexes according to SIRACA.

are descendants of an ancestor that arose by recombination of an EEEV with a Sindbis (SIN)-like virus. This recombination event was a double crossover event that combined the E2/E1 genes of the SINV-like ancestor with the other genes and noncoding regions of an EEEV ancestor.

Phylogenetic analyses have been most useful in the study of the Old World viruses which have been studied in far less detail than the encephalitic alphaviruses that only exist in the western hemisphere. Partial genome sequence analyses have produced conflicting topologies, particularly in the placement of Middelburg virus (MIDV), which has been not been extensively characterized. However, the use of longer sequences in phylogenetic comparisons and evaluations of tree topologies using likelihood tests provides the best estimates of evolutionary relationships given the data available. A phylogeny based upon distance matrix analysis of the E1 gene of representatives of each virus is presented in **Figure 1** demonstrating the monophyletic clustering of viruses within a given antigenic complex. Significantly, the clades generated by all phylogenetic analyses accurately reflect associations of viruses that share medically important characteristics. For example, the encephalitic alphaviruses cluster together and are more closely related to each other than those viruses that express other disease patterns providing evidence of the ancient relationships that exist among the alphaviruses.

Phylogenetic relationships cannot conclusively verify the geographic origin of the alphaviruses as New World and Old World origins are equally parsimonious with each requiring three transoceanic introduction events. Internal branch lengths of distance matrix trees also suggests that the most basal alphaviruses are those that infect

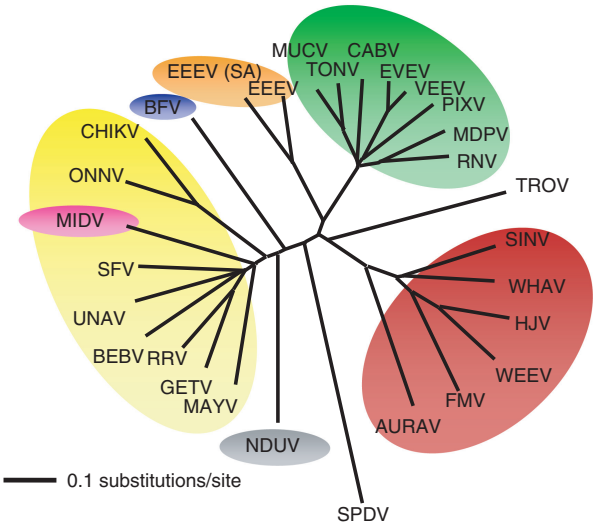


Figure 1 Unrooted phylogenetic tree of all *Alphavirus* species generated from the E1 nucleotide sequences using the F84 algorithm of the neighbor-joining program (SESV is not included because no homologous sequence for this region was available). Virus abbreviations are from **Table 1**. Antigenic complexes are indicated by colored circles.

fish indicating that an ancestral alphavirus adapted to infect fish. Other lineages of alphaviruses evolved as insect-borne viruses as most alphaviral species are recognized to be. Even the southern elephant seal virus (SESV), which has been found to infect lice, supports this hypothesis.

Virion Structure

Alphavirus virions are small (60–70 nm in diameter), icosahedral particles exhibiting $T=4$ symmetry. The single-stranded RNA genome is contained within a capsid core comprised of the virally encoded C protein. This nucleocapsid is then encased in a lipid envelop acquired by budding from the host cell plasma membrane making the virions sensitive to ether and detergents. The envelop membrane also contains the viral E1 and E2 glycoprotein heterodimers that are arranged as trimers on the surface. Binding to cellular receptors occurs via the E2 glycoprotein after which conformational changes in the glycoprotein trimers result following endocytosis.

Genome Organization and Replication

The alphaviruses have a genome consisting of a linear, positive-sense, single-stranded RNA molecule of approximately 11.2–11.8 kbp in size depending upon the species (**Figure 2**). The nonstructural proteins, required for viral replication, are encoded in the 5′ two thirds of the genome, while the structural genes are collinear with the 3′ one third.

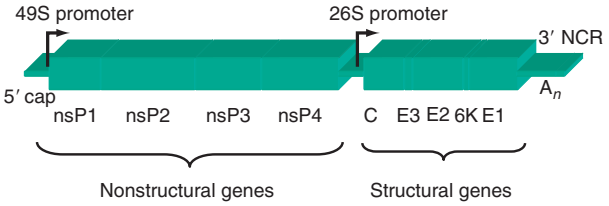


Figure 2 Organization of the alphavirus genome showing genes, promoter elements, and noncoding regions (NCRs).

The 5′ end of the genome has a 7-methylguanosine cap, while on the 3′ end a polyadenylation tail is present. Just upstream of the poly A tract is a 3′ noncoding region of varying length depending upon the virus. This region contains specific repeat elements that are distinctly associated with each of the different viruses. There are secondary structures associated with this noncoding region that may be involved in replication, host specificity, or virulence patterns.

There are four nonstructural genes, designated nsP1–4, which are necessary for viral replication; each has specific functions during the replication process. The nsP1 protein is required for initiation of synthesis of minus-strand RNA and also functions as a methyltransferase to cap the genomic and subgenomic RNAs during transcription. The second gene, nsP2, encodes a protein that has RNA helicase activity in its N-terminus while the C-terminal domain functions as a proteinase for the alphavirus nonstructural polyprotein. Cleavage of the nonstructural polyprotein by both nsP2 and polyproteins containing nsP2 occurs at three distinct sites and depends upon the composition of the polyprotein. This differential cleavage serves as a regulatory action leading to alternative processing pathways at different times during the infectious process to modulate the rate of replication. For example, early during infection, P123 and P1234 (made by readthrough translation of a stop codon present between the nsP3 and nsP4 genes) are produced by translation of the genomic mRNA. As the infection proceeds, P123 cleaves between nsP1 and nsP2 to produce nsP1 and P23. The P23 polyprotein is autoproteolytic and cleaves itself to generate the individual nsP2 and nsP3 proteins (**Figure 3**). The nsP3 gene encodes a protein consisting of two domains, a widely conserved N-terminal domain and a hypervariable C-terminus, whose functions are not fully understood. The C-terminal region has been shown to tolerate numerous mutations, including large deletions or insertions, and still produce viable viral particles in vertebrate cells. It is possible that some of these nsP3 mutations may play a role in defining cell type and invertebrate vector specificity. The final nonstructural protein, nsP4, encodes the viral RNA-dependent RNA polymerase (RdRp) and contains the characteristic GDD motif associated with this highly conserved element.

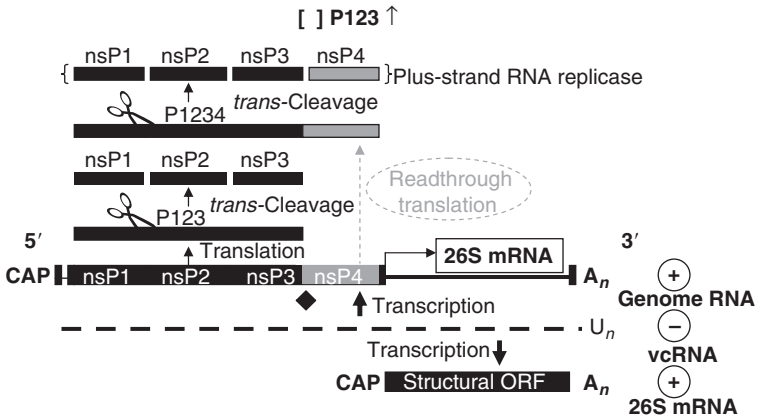


Figure 3 Model for the processing of the alphavirus nonstructural polyprotein during replication. When low levels of P123 are present, *cis*-cleavage of P1234 generates the minus-strand RNA replicase of the virus. This results in primarily negative-sense RNA being generated from the incoming genomic RNA of the virus (upper panel). As the level of the *trans*-acting protease P123 rises in the infected cell, cleavage in *trans* generates other RNA replicase complexes. This results in a shift by the virus from the production of primarily negative-sense RNA to primarily positive-sense RNA. Eventually, replicase complexes capable of producing negative-sense RNA will no longer be present in the infected cell resulting in the complete cessation of negative-sense RNA synthesis (lower panel). The presence of a leaky opal termination codon (depicted as a black diamond) in the virus genome is believed to lead to a more rapid buildup of P123 in the infected cell, and thus a more rapid conversion to the production of positive sense RNA by the virus. Courtesy of Dr. Kevin Myles.

A second polyprotein is generated by translation of a subgenomic mRNA that is generated from an internal promoter immediately downstream of the nonstructural open reading frame. This polyprotein encodes the structural proteins and is processed to produce a capsid protein, the two major envelope surface glycoproteins (E1 and E2) and two small peptides (E3 and 6K) that are not associated with intact virions. E1 and E2 are post-transcriptionally modified in the endoplasmic reticulum and Golgi apparatus before being transported to the plasma membrane where they maintain a close association with each other forming a dimeric spike structure. As virion formation proceeds, the nucleocapsids present in the cytoplasm are moved to the cell membrane. Here, they bind to the surface glycoproteins before budding from the cell. In terms of function, the E2 protein has been found to be an important determinant of antigenicity and cell receptor binding in both the vertebrate host and the insect vector for other alphaviruses. The other major structural protein, E1, has been found to contain domains associated with membrane fusion.

Transmission

The alphaviruses as a whole are transmitted to vertebrate hosts by a broad range of mosquito species. There are some alphaviruses that have non-mosquito invertebrate vectors; Fort Morgan virus (FMV) is transmitted by a cimicid bug (*Oeciacus vicarious*) and SESV is postulated to be transmitted by lice (*Lepidophthirus macrorhini*). Further, the fish alphavirus, salmon pancreas disease virus (SPDV), is not known to have an invertebrate vector. However, for the mosquito-borne alphaviruses, any given virus tends to be transmitted enzootically by only a single or small number of invertebrate species; these very specific host–virus interactions are related to geography and ecological dynamics associated with the mosquito vectors. In contrast to enzootic maintenance, epidemic or epizootic outbreaks may utilize numerous species of mosquitoes but transmission by these species rarely continues once the outbreak subsides. A few of the virus–vector–host interactions have been extensively studied and are described below.

The most well studied alphaviral transmission cycles are those of the equine encephalitides: EEEV, Venezuelan equine encephalitis virus (VEEV), and WEEV. Curiously, all of these viruses, even though they cause encephalitis, exist only in the New World, and are more closely related to each other than other members of the genus *Alphavirus*, utilize extremely different invertebrate vectors in their transmission cycles. EEEV is maintained in North America in a transmission cycle including *Culiseta melanura* mosquitoes and avians. However, this species of mosquito rarely feeds on mammals so epidemic or epizootic transmission to humans or equines typically involves multiple other species of mosquitoes in several genera. The South American cycle of EEEV transmission is not well characterized but isolates of virus have been obtained from *Culex* (*Melanoconion*) mosquitoes implicating them as the likely enzootic vectors. The majority of the viruses within the VEE complex circulate in continuous enzootic habitats between small vertebrate rodent hosts and mosquitoes of the subgenus *Culex* (*Melanoconion*). However, outbreaks of VEEV are a result of

transmission by a number of different mammalophilic mosquitoes in the genera *Aedes* and *Psorophora*. Finally, like North American EEEV, WEEV is transmitted in cycles involving avian hosts; however, the predominant mosquito species that vectors this virus is *Culex tarsalis* in North America. WEEV virus may be vertically transmitted by *Aedes* species mosquitoes in temperate climates where year-round maintenance of the virus does not occur.

Of the arthralgic-causing alphaviruses, only a few have well-characterized transmission cycles. Chikungunya virus (CHIKV) (prevalent throughout Africa as well as Southeast Asia and parts of Oceania) appears to be maintained in sylvatic cycles involving wild nonhuman primates and forest-dwelling *Aedes* spp. mosquitoes including *Aedes furcifer*, *Aedes taylori*, *Aedes luteocephalus*, *Aedes africanus*, and *Aedes neoafricanus* in western and central Africa. However, while forest-dwelling mosquito species are primary vectors in west and central Africa, the urban mosquito *Aedes aegypti* has been found to be the most significant vector in Asia with virtually all Asian mosquito isolates coming from this species. Urban or large outbreaks in east Africa are also likely associated with the presence of *Ae. aegypti* mosquitoes. Other common peridomestic species (*Aedes albopictus*, *Aedes vittatus*, and *Anopheles stephensi*) that have been found in abundance in CHIKV endemic areas have also been found to be competent vectors in the laboratory. Both humans and wild nonhuman primates are likely to be the vertebrate reservoirs for CHIKV. The most closely related virus to CHIKV, o'nyong nyong virus (ONNV), is unique among the alphaviruses in that the primary epidemic mosquito vectors are members of the genus *Anopheles*. The virus was isolated from collections of *Anopheles funestus* and *Anopheles gambiae* obtained from epidemic areas and entomological studies have further implicated *An. funestus* as a principal outbreak vector because it was the most abundant species from which isolates of ONNV were obtained during one outbreak. The identity of the vector of ONNV during interepidemic periods remains unknown as does any vertebrate reservoir. Another arthritis-causing alphavirus, Ross River virus (RRV), has been isolated from over 30 different species of mosquitoes; however, the most likely vectors of public health concern are *Aedes vigilax*, *Aedes camptorhynchus*, and *Culex annulirostris* due to their abundance during outbreaks, the number of isolates of virus obtained from these species, and their temporal and spatial association with outbreaks of RRV disease. The vertebrate hosts of RRV are not defined with certainty. Based upon serosurveys, virus isolations, and experimental infection studies, marsupials, macropods, livestock, domestic pets, birds, and humans have all been postulated to play a role either in maintenance or amplification of the virus. Further studies correlating human disease with reservoir status would prove useful in clarifying the role of each of these vertebrates in RRV transmission.

Epidemiology

The epidemiological patterns of the alphaviruses are as diverse as their geography and ecological characteristics. Generally, outbreaks of human or animal illness due to alphavirus infections coincide with peak mosquito seasons in temperate zones while they occur year-round in tropical climates. The magnitude of each outbreak can vary dramatically depending upon whether the outbreak was localized to urban or rural settings. However, in both ecologies, the attack rates can be significant if a virulent alphavirus is the etiological agent. For example, morbidity rates between 30% and 70% are typical during CHIKV epidemics and mortality rates for EEEV outbreaks can reach as high as 70%. The seropositivity rates often correlate with the vector infectivity and transmissibility rates as well as host preference of the mosquito. In general, disease is often more severe in young children and elderly patients and rates of immunity are lower in young children. This indicates that many alphaviruses produce long-lived antibodies that are presumably protective for life. Occupational exposure differences to mosquitoes are likely the cause of any higher incidence rates that may be associated with males compared with females.

See also: Parvoviruses of Arthropods; Togaviruses Causing Encephaitis; Togaviruses Causing Rash and Fever.

Further Reading

Griffin D (2001) Alphaviruses. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., vol. 1, pp. 917–962. Philadelphia: Lippincott Williams and Wilkins.

Harley D, Sleigh A, and Ritchie S (2001) Ross River virus transmission, infection, and disease: A cross-disciplinary review. *Clinical Microbiology Reviews* 14: 909–932.

Monath TP and Trent DW (1981) *Togaviral Diseases of Domestic Animals, Comparative Diagnosis of Viral Diseases,* vol. IV, 331–440. New York: Academic Press.

Powers AM, Brault AC, Shirako Y, *et al.* (2001) Evolutionary relationships and systematics of the alphaviruses. *Journal of Virology* 75: 10118–10131.

Russell RC (2002) Ross River virus: Ecology and distribution. *Annual Reviews of Entomology* 47: 1–31.

Schlesinger S and Schlesinger MJ (2001) *Togaviridae*: The viruses and their replication. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 4th edn., vol. 1, pp. 895–916. Philadelphia: Lippincott Williams and Wilkins.

Strauss JH and Strauss EG (1994) The alphaviruses: Gene expression, replication, and evolution. *Microbiology Reviews* 58: 491–562.

Weaver SC, Frey TK, Huang HV, *et al.* (2005) *Togaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 999–1008. San Diego, CA: Elsevier Academic Press.

Weaver SC, Powers AM, Brault AC, and Barrett AD (1999) Molecular epidemiological studies of veterinary arboviral encephalitides. *Veterinary Journal* 157: 123–138.

Weston J, Villoing S, Bremont M, *et al.* (2002) Comparison of two aquatic alphaviruses, salmon pancreas disease virus and sleeping disease virus, by using genome sequence analysis, monoclonal reactivity, and cross-infection. *Journal of Virology* 76: 6155–6163.

Togaviruses: Equine Encephalitic Viruses

D E Griffin, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Arbovirus Virus maintained in an infection cycle involving hematophagous arthropods and vertebrate hosts.

Encephalitis Inflammation of the brain associated with cognitive changes.

History

Nomenclature of the encephalitic alphaviruses recognizes the characteristic geographic distribution of each and the initial identification of the viruses as causes of encephalitis in horses. The first clear record of epidemic equine encephalitis is from 1831, when an outbreak in Massachusetts, USA, resulted in the deaths of 75 horses. Over the next 100 years, several local epidemics of encephalitis in horses occurred along the Atlantic seaboard. The virus of this eastern encephalitis (EEEV) was first isolated from the brain of an affected horse in 1933 by Tenbroek and Merrill. However, 2 years earlier, Meyer, Haring, and Howitt had isolated a virus from central nervous system (CNS) tissues of two horses involved in an epidemic of equine encephalitis in the San Joaquin Valley of California. The EEEV and the western equine encephalitis virus (WEEV) virus were shown to be antigenically distinct. Both diseases occurred in summer epidemics, and, in 1933, Kelsor showed that WEEV could be transmitted by mosquitoes; shortly thereafter, mosquito transmission of EEEV was shown. In 1938, both EEEV and WEEV were isolated from humans with encephalitis occurring in the same regions as the equine cases. In 1936, an epidemic of equine encephalitis occurred in the Goajira region of Venezuela and the virus causing this epidemic, isolated independently by Beck and Wyckoff and Kubes and Rios, was not neutralized by sera from animals immunized with EEEV or WEEV and was designated Venezuelan equine encephalitis virus (VEEV).

Taxonomy and Classification

In 1954, three serologic groups (A, B, and C) of arthropod-borne viruses had been distinguished by Casals and Brown on the basis of cross-reactivity in hemagglutination inhibition (HI) tests. EEEV, WEEV, and VEEV constituted the original group A arboviruses. A second cross-reacting set, including dengue, St. Louis encephalitis, and yellow fever viruses, constituted the group B arboviruses, and the other viruses, mostly from Brazil, were designated group C viruses. As viruses became classified on the fundamental properties of the virion, the group A viruses became the alphaviruses (genus *Alphavirus*) within the family *Togaviridae* of enveloped RNA viruses. HI was used to classify alphaviruses into six broad antigenic complexes; EEE, WEE, and VEE form three of these complexes. The EEE and VEE complexes contain only EEEV and VEEV. The WEE complex includes several viruses in addition to WEEV (e.g., Whataroa, Kyzylagach, Highlands J, Fort Morgan, Aura, and Sindbis), distinguished from each other by complement fixation, neutralization, or other tests.

Viruses within each complex have been subtyped using reactivity with monoclonal antibodies, kinetic HI (EEEV, VEEV), or neutralization (WEEV, VEEV) assays. EEEV and WEEV viruses each have North American and South American subtypes. WEEV and VEEV also can be subdivided into epizootic and enzootic strains. The VEE viruses are differentiated by HI and neutralization tests into subtypes I (VEEV), II (Everglades), III (Mucambo), IV (Pixuna), V (Cabassou), and VI (Rio Negro). Epizootic strains of VEEV are equine virulent and fall within subtype I, which is further subdivided antigenically into IAB and IC (epizootic strains) and ID, IE, and IF (enzootic strains). Sequence information has confirmed the validity of most of these distinctions.

Virion and Genome Structure

Properties of the Virion

Alphavirus virions are 60–65 nm in diameter. The RNA is enclosed in a capsid formed by a single protein arranged as an icosahedron. The nucleocapsid is enclosed in a lipid envelope derived from the host cell plasma membrane and which contains the viral-encoded glycoproteins, E1 and E2. These proteins form heterodimers that are grouped as trimers to form knobs on the virion surface that are arranged with a $T=4$ symmetry. Glycoproteins are arranged with 240 copies of each protein interacting with 240 copies of capsid protein. The virion is sensitive to ether and detergent. Infectivity can be inactivated by heat or acid. The viruses are stable at −70 °C for long periods.

Properties of the Genome

The 49S genome is a single-stranded, nonsegmented, capped, and polyadenylated message-sense RNA that is

infectious. Complete sequence information is available for representative members of all three virus complexes. The genomes contain approximately 11 700 nucleotides and have the nonstructural proteins at the 5′-end and the structural proteins at the 3′-end. Highly conserved regions of genome are the 19 nucleotides at the 3′ terminus, important for negative-strand synthesis, and 21 nucleotides at the junction between the nonstructural and structural genes, important for synthesis of the subgenomic RNA. Analysis of codon usage shows an underutilization of the dinucleotide CpG.

Properties of the Viral Proteins

Five potential structural proteins (C, E3, E2, 6K, and E1) are encoded in the 3′-end of the genome. The capsid protein is 259 (EEEV, WEEV) to 275 (VEEV) amino acids long. The N-terminal portion is conserved, basic, and binds the genomic RNA, and the C-terminal portion interacts with the cytoplasmic tail of E2 and with other capsid proteins to form the nucleocapsid. The E3 protein is 59 (VEEV), 60 (WEEV three serologic groups, A, B, and C), or 63 (EEEV) amino acids long, serves as a signal sequence for E2, and, *in vitro*, is shed into the supernatant fluid after cleavage by a furin-like protease in the *trans*-Golgi network. The E2 glycoprotein is a transmembrane protein that is 420 (EEEV) to 423 (WEEV, VEEV) amino acids long and has two (EEEV) or three (WEEV, VEEV) N-linked glycosylation sites. The intracytoplasmic portion has a second stretch of hydrophobic amino acids. The 6K protein is 55 (WEEV, VEEV) or 56 (EEEV) amino acids long and serves as a signal peptide for E1. By analogy to Sindbis virus, small amounts are probably incorporated into the virion. The E1 protein is 439 (WEEV), 441 (EEEV), or 442 (VEEV) amino acids long and has one or two N-linked glycosylation sites. E1 has a short (one or two residue) intracytoplasmic tail and a positionally conserved internal hydrophobic stretch of amino acids in the N-terminal portion of the protein that serves as the fusion peptide for virion entry into the cell. The 5′-end of the genome encodes four nonstructural proteins that function in replication of viral RNA and in production of the genomic and subgenomic RNA.

Replication

For the most part, knowledge of the replication of EEEV, WEEV, and VEEV is based on the extensive studies of Sindbis and Semliki forest viruses. Initial attachment to the cell may involve interaction of the E1 glycoprotein, which has hemagglutination activity, with phospholipids on the cell surface. Efficient subsequent entry involves binding of E2 to cellular proteins followed by receptor-mediated endocytosis. Within the acidified endosomal compartment, the glycoprotein spikes undergo a conformational change that results in fusion with the endosomal membrane and release of the nucleocapsid into the cytoplasm. Acidified endosomes may not be essential for infection of mosquito cells. Once released from the nucleocapsid, the virion RNA serves as mRNA and translation of the nonstructural proteins is initiated. Cytopathic vacuoles form and negative-strand 49S RNA, positive-strand 49S RNA, and positive-strand 26S subgenomic RNA are transcribed. The 26S RNA represents the 3′-portion of the genome from which the structural proteins are translated as a polyprotein. After translation of the capsid protein is complete, it is autocatalytically cleaved from the nascent chain. E2 and E1 are transported into the endoplasmic reticulum, cleaved by cellular proteases, and processed through the Golgi. At the plasma membrane, nucleocapsids align with regions containing the E1–E2 heterodimers and bud from the cell surface. The process may be different in mosquito cells, where intracytoplasmic compartments containing mature virions are seen *in vitro*. However, these structures have not been seen during ultrastructural studies of alphavirus infection in mosquitoes.

Geographic and Seasonal Distribution

The encephalitic alphaviruses are geographically restricted. With few exceptions, alphaviruses are transmitted primarily by mosquitoes and, therefore, transmission is restricted to warm months of the year. EEEV is endemic in eastern Canada and from New Hampshire along the Atlantic seaboard and Gulf Coast to Texas in the United States, in the Caribbean, Central America, and along the north and east coasts of South America. Inland foci exist around the Great Lakes. In the northern part of the range, cases occur between July and October, while in the southern region cases can occur throughout the year. Viruses of the WEE complex are widely distributed throughout the Americas as three closely related but antigenically distinct viruses (WEEV, Highlands J virus, and Fort Morgan virus). Highlands J virus is endemic on the East Coast of the United States in the same areas as EEEV. WEEV is widely distributed in the western plains and valleys of the United States and Canada and is found in South America. Enzootic VEE viruses are perennially active in subtropical and tropical areas of the Americas (subtype II in Florida; ID and IE in Central America; IF and III–VI in South America). Epizootics have appeared in Venezuela, Colombia, Peru, and Ecuador at approximately 10 year intervals.

Host Range and Virus Propagation

The life cycle of the encephalitic alphaviruses involves replication in invertebrate vectors, primarily mosquitoes,

and in reservoir vertebrate hosts, primarily birds (EEEV and WEEV) or small mammals (VEEV). In epidemic periods, additional hosts, such as horses and humans, are infected. EEEV causes encephalitis in humans, horses, pigeons, and pheasants. Many birds are susceptible to infection but remain asymptomatic, despite prolonged viremia. The amplifying species for EEEV in North America are wading birds and migratory song birds. In South America, forest-dwelling rodents and marsupials become infected and may provide additional reservoirs. Infection has also been reported in turtles and snakes. In invertebrates, virus is most consistently recovered from *Culiseta melanura* in North America, *Culex taeniopus* in the Caribbean, and *Culex* (*Melanconion*) spp. in South America. Mosquitoes of many species can serve as bridge vectors to mammals.

WEEV may cause encephalitis in horses and humans. The enzootic cycle in North America involves domestic and wild birds and *Culex tarsalis* for WEEV in the western United States and songbirds and *Culiseta melanura* for Highlands J virus in the eastern United States. Serosurveys and virus isolations have demonstrated evidence of natural infection in chickens and other domestic birds, passerine birds, pheasants, rodents, rabbits, ungulates, and snakes.

Epizootic strains of VEEV can cause disease in horses and in humans. The enzootic strains of VEEV can infect horses but these infections are asymptomatic and may protectively immunize horses against epizootic strains. Enzootic strains produce mild disease in humans. A variety of wild birds are susceptible to infection, but small mammals serve as an important reservoir, with efficient transmission of infection by *Culex* (*Melanconion*) spp. mosquitoes.

In addition to the native hosts, a number of laboratory animals are susceptible to infection. All three viruses cause encephalitis in monkeys, mice, rats, guinea pigs, and hamsters. Disease is generally age dependent, so that young animals more often develop fatal infections than do adult animals. Primary isolates of these viruses are often made in newborn mice.

In vitro, the viruses are routinely propagated in cultures of chick embryo fibroblasts, BHK-21 cells, or Vero cells. Most strains will form plaques in these cells and plaque assays provide the usual basis for virus quantitation. Mosquito cell lines support replication, often without cytopathic effect. Many other cell lines (e.g., L-cells, HeLa cells) support replication as well.

Genetics

Alphaviruses show genetic changes by accumulation of point mutations in the genomic RNA, but this occurs at a rate that is much slower than that predicted for other RNA viruses. Recombination is infrequent, but can be demonstrated *in vitro* and occurs at least occasionally in nature because sequence analysis has shown that WEEV is the result of recombination between EEEV and Sindbis-like viruses.

Evolution

The alphaviruses derive from a single unknown protoalphavirus and are part of the alphavirus superfamily of viruses. Viruses in this superfamily have a similar genetic organization and include many RNA plant viruses. In general, amino acids important in secondary structure (e.g., cysteine) have been conserved for the structural glycoproteins E1 and E2, suggesting that the three-dimensional structure is similar for all alphaviruses. Sequence analyses suggest that the encephalitic alphaviruses evolve slowly in nature. The capsid protein and E1 glycoprotein are the most conserved of the structural proteins, whereas the E2 glycoprotein is more divergent. EEEV has evolved independently in North and South America over the last 1000 years and there is currently one group in North America and the Caribbean and three groups in South America, one in the Amazon River basin, one on the coasts of South and Central America and the third in Brazil. North American EEEV isolates are highly conserved, varying by less than 2% over 63 years. South American isolates are evolving more rapidly, perhaps locally within different vector–host relationships. Rates of divergence of WEEV and Highlands J virus of 0.1–0.2% per year have been estimated. It is hypothesized that short transmission seasons and limited host mobility constrain genetic diversity in a geographic region.

Epidemiology

EEEV causes localized outbreaks of equine, pheasant, and human encephalitis in the summers. Cases of equine encephalitis are usually the first indicators of an outbreak. In North America, the primary enzootic cycle is maintained in shaded freshwater swamps where the vector is the ornithophilic swamp mosquito *Culiseta melanura* and the reservoir hosts are migratory passerine songbirds. Young birds are probably most important for virus amplification because they are more susceptible to infection, have a prolonged viremia, and are less defensive toward mosquitoes. Human and equine cases usually occur within 5 mi (8.045 km) of the swamp, with virus being transmitted by epizootic vectors such as *Coquillittidia perturbans*, *Aedes sollicitans*, and, potentially, *Aedes albopictus*. The enzootic vector in the Caribbean is probably *Culex taeniopus* and in South America *Culex (Melanoconion)* spp. Epizootics appear approximately every 5–10 years and are usually associated with heavy rainfall that increases the populations

of enzootic and epizootic mosquito vectors. The mechanism of overwintering in northern areas is not known. There is no evidence for overwintering in mosquitoes. Human infections are unusual, with an average of 5–10 cases per year in the United States (**Figure 1**). Serological surveys in the northeastern United States suggest that there are approximately 23 inapparent infections for every case of encephalitis, but children are more susceptible and this declines to only 8:1 for children under 4 years of age.

WEEV is maintained in an endemic cycle involving domestic and passerine birds and *Culex tarsalis*, a mosquito particularly adapted to irrigated agricultural areas. Interseasonal persistence occurs in saltwater marshes, where vertical transmission of WEEV in *Culex tarsalis* has been demonstrated. Occasional isolations have been made from *Culex stigmatosoma, Aedes melanimon*, and *Aedes dorsalis*, also competent vectors. Transmission from this enzootic cycle has resulted in small numbers of cases of encephalitis in humans. However, periodically, there have been widespread summer epidemics of equine encephalitis in North America with significant extension into humans. The estimated case to infection ratio is 1:58 in children under 5 years and 1:1150 in adults. Recently, for reasons that are not completely clear, cases of equine and human cases have declined (**Figure 2**). In the eastern United States, Highlands J virus has been isolated from ornithophilic mosquitoes (*Culiseta melanura*, the enzootic vector for EEEV) in freshwater swamp habitats along the Atlantic coast, as well as from birds. Ecologic restriction and limited pathogenicity may explain the paucity of human and equine disease caused by Highlands J virus. The lack of significant human disease during equine outbreaks of WEE in South America may be related to the feeding habits of the vector or to a difference in virulence for humans of the South American strains.

VEEVs are maintained in enzootic cycles by *Culex (Melanoconion)* spp. mosquitoes that live in tropical and subtropical swamps throughout the Americas and breed near aquatic plants. They feed at dawn and dusk on a wide variety of rodents, birds, and other vertebrates. Humans living in these areas have a high prevalence of antibody, but little-recognized disease. Epizootic strains of VEEV arise by mutation from enzootic strains and are isolated only during outbreaks. Epizootics have occurred primarily in Latin America in cattle ranching areas during the rainy season. Formalin-inactivated vaccines containing residual live virus are suspected to be responsible for initiating the 1969–72 outbreak in Central America and which extended to Texas by 1971. During epizootics, horses are an important amplifying species. Virus has been isolated from several species of mosquitoes including *Aedes taeniorhynchus, Aedes aegypti, Mansonia dubitans*, and *Psorophora confinnis*. The incidence of encephalitis in clinically ill humans is generally less than 5% and the overall mortality less than 1%.

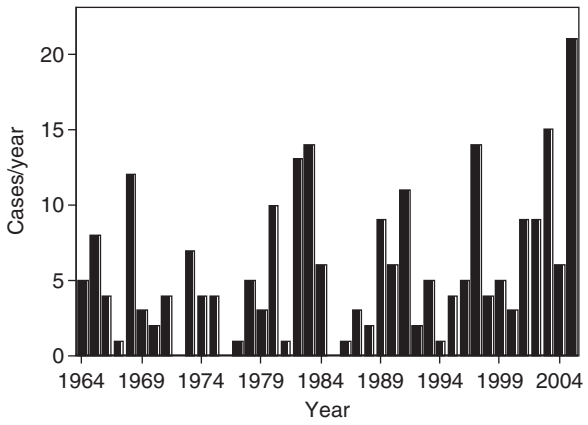


Figure 1 Numbers of human cases of eastern equine encephalitis reported in the United States from 1964 to 2005. Data are from the Centers for Disease Control and Prevention.

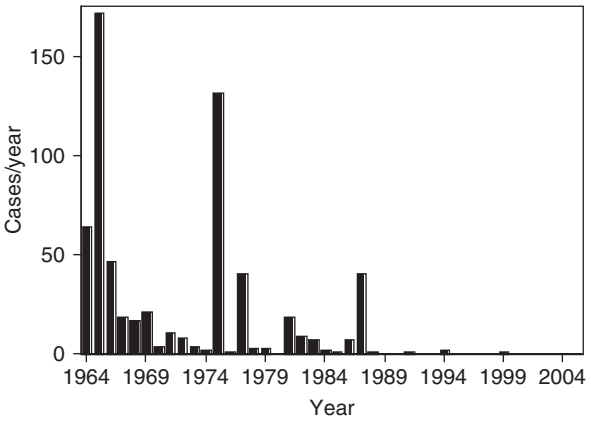


Figure 2 Numbers of human cases of western equine encephalitis reported in the United States from 1964 to 2005. Data are from the Centers for Disease Control and Prevention.

Transmission and Tissue Tropism

The primary mode of alphavirus transmission to birds and mammals is through the bite of an infected mosquito which inoculates virus extravascularly. Mosquitoes become infected by feeding on a viremic host, are able to transmit the virus 4–10 days later (extrinsic incubation), and remain persistently infected. Maintenance of this cycle requires an amplifying host that develops a viremia of sufficient magnitude to infect mosquitoes feeding on it. Other modes of transmission are of occasional importance. EEEV persists in the feather follicles of infected pheasants and secondary transmission among penned pheasants can occur through feather picking and cannibalism. VEEV can be transmitted by the respiratory route between infected horses and to humans in the laboratory. WEEV and VEEV can be transmitted transplacentally.

In mammals, EEEV replicates primarily in muscle and in neurons and glial cells of the CNS, with occasional involvement of liver and lymphatic tissue. Skeletal and myocardial muscle and the CNS of infected birds contain virus. In mosquitoes, there is infection of the midgut, muscle, and salivary glands without involving the nervous system. WEEV replicates primarily in skeletal and cardiac muscle, brown fat, and the choroid plexus, ependyma, and neurons in the CNS of mammals. Little is known of the tissue tropism of WEEV in birds. Epizootic strains of VEEV infect the upper respiratory tract, lymphatic and myeloid tissue, pancreas, liver, and CNS to varying degrees in different mammals. Mosquito infection is initiated in the midgut epithelial cells and spreads through the hemolymph to the salivary glands and flight muscles.

Pathogenicity

EEEV strains vary in their pathogenicity for horses (and presumably for humans), with South American strains being less virulent than North American strains. The molecular basis of these differences have not yet been defined. The North American strains of EEEV are among the most virulent of the alphaviruses, causing severe encephalitis in humans, horses, dogs, pigs, pigeons, emus, quail, and pheasants. The case–fatality rate in humans is 30–50%, up to 90% in horses and 50–70% in pheasants. Laboratory studies indicate a similar neurovirulence of EEEV for monkeys, mice, and hamsters. Hamsters also develop hepatitis and lymphatic organ infection. At 3–4 weeks of age, mice become relatively resistant to peripheral, but not intracranial, inoculation. Birds vary in their susceptibility, with birds of some species developing disease, while birds of many other species show no morbidity or mortality, despite a prolonged viremia. Pheasant deaths are caused by encephalitis while young chickens develop myocarditis. Mosquitoes develop persistent infection of the fat body and salivary glands.

WEEV in the western United States causes epidemics of encephalitis in humans, horses, and emus, but the case–fatality rate of 10% for humans, 20–40% for horses, and 10% for emus is lower than for EEEV in the eastern US. Highlands J virus has been reported to cause rare fatal disease in horses and turkeys. South American strains of WEEV cause fatal encephalitis in horses, but little disease in humans. Epizootic strains are neurovirulent and neuroinvasive in adult mice, whereas enzootic strains are not. Highlands J virus is intermediate in virulence between North and South American strains of WEEV. With increasing age mice become relatively resistant to fatal infection, whereas hamsters remain susceptible to Highlands J virus.

Infection of horses with epizootic strains of VEEV frequently is fatal. Disease is associated with leukopenia and a high-titered, prolonged viremia. In contrast to EEE and WEE, encephalitis is not always apparent and virus is shed in nasal, eye, and mouth secretions as well as in urine and milk. Experimental infection of laboratory animals produces a variety of disease patterns. In guinea pigs and rabbits, VEEV produces necrosis in lymph nodes, spleen, thymus, intestinal, and conjunctival lymphoid tissue, liver, and bone marrow. Hamsters develop encephalitis and pancreatitis in addition to widespread involvement of myeloid and lymphoid tissue. In rats and mice, there is more limited destruction of lymphoid and myeloid tissue and death is usually due to encephalitis. Virus enters the CNS through the olfactory tract and causes neuronal infection and apoptosis. Neonatal mice show extensive replication in many tissues, including brain, myocardium, and pancreas. Comparative studies of the virulent TRD and avirulent TC-83 strains of IA serotype VEEV and construction of recombinant viruses have led to identification of the 5′-noncoding region and the E2 envelope glycoprotein as important determinants of virulence for mice, whereas changes in nsP3 and E2 determine virulence for horses.

Clinical Features of Infection

EEEV is the most virulent of the encephalitic alphaviruses, causing a high mortality due to encephalitis. Prodromal symptoms of fever, headache, and myalgias are common. The onset of encephalitis tends to be fulminant and is associated with continued fever, increased headache, meningismus, obtundation, and seizures. The overall case–fatality rate is 30% in recent studies, with higher rates in children and the elderly. Recovery is more likely in those individuals that have a long (5–7 day) prodrome and do not develop coma. Sequelae are common, with more than 80% of survivors having significant neurological residua, including paralysis, seizures, and mental retardation. The diagnosis is usually made by detection of antibody in serum or cerebrospinal fluid.

WEEV can cause encephalitis with signs and symptoms similar to those caused by EEEV. There is a 3–5 day prodrome of fever and headache that may progress to irritability, nuchal rigidity, photophobia, and altered mental status. Severe disease, seizures, fatal encephalitis, and significant sequelae are more likely to occur in infants and in young children.

Infection with epizootic strains of VEEV usually causes relatively mild disease in humans. Illness in adults usually is manifested by fever, headache, myalgias, and pharyngitis 2–5 days after exposure. Severe diseases, including fulminant reticuloendothelial infection and encephalitis, may occur in young children. Children recovering from encephalitis may be left with neurological deficits. Fetal abnormalities and spontaneous abortions may occur in infections during pregnancy.

The cerebrospinal fluid during alphavirus encephalitis usually shows a moderate (up to 4000 cells mm^{-3}) pleocytosis with predominance of either polymorphonuclear or mononuclear cells. Protein is usually elevated and the glucose is normal. The electroencephalogram and magnetic resonance imaging scans are usually abnormal, but computed tomographic scans may be normal or indicative only of edema.

Pathology and Histopathology

Initial CNS infection with EEEV in experimental animals is of the capillary endothelial or choroid plexus epithelial cells and spread within the CNS can be cell to cell or through cerebrospinal fluid. The targeted cell within the CNS is the neuron and damage to this cell may be severe and irreversible. Histopathology demonstrates a diffuse meningoencephalitis with widespread neuronal destruction, neuronophagia, gliosis, and perivascular inflammation, with polymorphonuclear and mononuclear leukocytes early, and vasculitis and vessel occlusion late. Hamsters also exhibit necrosis of hepatocytes and lymphatic tissue accompanied by local infiltration of mononuclear leukocytes. Initial infection of mosquitoes is of midgut epithelial cells. Infection is facilitated when virus in the serum is concentrated next to these cells as the infected blood meal clots. Infected midgut epithelial cells subsequently degenerate and slough, and this process may facilitate penetration of the virus into the hemocoel and rapid dissemination of the infection.

Pathological examination of brains from fatal human cases of WEE demonstrates early perivascular extravasation of blood followed by endothelial hyperplasia, perivascular mononuclear and polymorphonuclear inflammation, and parenchymal necrosis. Areas of neuronal degeneration, glial nodules, and demyelination are found. Neonatal mice develop acute inflammation and necrosis in skeletal and smooth muscle, cartilage, and bone marrow. In vertebrate animals with encephalitis, the brain shows multifocal areas of necrosis and widespread lymphocytic infiltration of the leptomeninges and perivascular regions of the brain parenchyma. The heart shows a necrotizing, inflammatory myocarditis. Infiltration of mononuclear leukocytes into areas of lung, liver, and brown fat also occurs.

The pathology of VEE in horses includes cellular depletion of bone marrow, spleen, and lymph nodes, pancreatic necrosis, and, in cases with encephalitis, swelling of vascular endothelial cells, edema, and mononuclear cell cuffing of cerebral vessels and meningitis followed by the later appearance of demyelinating lesions. Small mammals with widespread involvement of the reticuloendothelial system may develop ileal necrosis.

Both innate and adaptive immune responses are induced by infection. Interferon-α/β (IFN-α/β) is produced quickly after infection. Virus-specific IgM is often detectable by enzyme immunoassay or by virus neutralization very early after onset of the disease in both serum and cerebrospinal fluid and provides a means for rapid diagnosis of infection. IgG antibody appears 10–14 days after onset and can be measured by enzyme immunoassay, HI, or neutralization. Many lines of evidence suggest that recovery from infection is primarily dependent on the antibody response. Extensive experimental studies to define the antibody specificity and the mechanisms of recovery and protection have been done using VEEV and Sindbis virus, a member of the WEE complex. Neutralizing and non-neutralizing antibodies are protective. Multiple epitopes on the E1 and E2 glycoproteins induce neutralizing, HI, and protective antibody. Sequencing of monoclonal antibody escape mutants has localized one neutralization domain to E2 residues 180–210 in VEEV. Immunization with peptides has shown that an antibody response to residues 1–25 of the VEEV E2 glycoprotein is also protective.

Cellular immunity has received more limited study, but virus-specific lymphoproliferative and cytotoxic responses have been documented and a mononuclear inflammatory response is common. The importance of cellular immune responses for recovery or for contribution to fatal disease has not been established but IFN-γ can contribute to virus clearance from the CNS. Prior infection with VEEV increases subsequent antibody responses to unrelated antigens. Antithymocyte globulin extends time to death in mice infected with VEEV, suggesting a T-cell-mediated immunopathogenic component to fatal disease.

Prevention and Control

Prevention of infection relies on efforts to control mosquito populations by spraying and reduction of breeding places. Individual use of protective measures such as mosquito repellents and protective clothing are important. Vaccines against EEEV, WEEV, and VEEV are available for horses and against EEEV for birds. Experimental human vaccines against EEEV, WEEV, and VEEV also are available for laboratory workers exposed to these agents. Most of these vaccines consist of formalin-inactivated virus, but TC-83 is a successful live attenuated vaccine against VEEV for use in horses. TC-83 is also used as a vaccine for humans, although side effects are common. No antiviral agents are of proven usefulness in treatment of these infections.

Future

Construction of full-length cDNA alphavirus clones that can be transcribed into infectious RNA provides tremendous potential for understanding the functions of

various genes and their importance for replication and virulence in the multiple hosts necessary for maintenance of these viruses in their natural cycles. An understanding of the folding and three-dimensional structures of each of the structural and nonstructural proteins is needed for interpretation of much of the sequence and virulence data that has been acquired. Further information on virulent and avirulent strains and functional analysis of the nonstructural proteins are likely to provide the next level of understanding of virus–host relationships and the conditions that lead to outbreaks.

See also: Central Nervous System Viral Diseases.

Further Reading

Calisher CH (1994) Medically important arboviruses of the United States and Canada. *Clinical Microbiology Reviews* 7: 89–116.
Griffin DE (2007) Alphaviruses. In: Knipe DM and Howley PM (eds.) *Field's Virology,* 5th edn., pp. 1024–1067. Philadelphia: Lippincott Williams and Wilkins.
Weaver SC and Barrett AD (2004) Transmission cycles, host range, evolution, and emergence of arboviral disease. *Nature Reviews Microbiology* 2: 789–801.
Weaver SC, Ferro C, Barrera R, *et al.* (2004) Venezuelan equine encephalitis. *Annual Review of Entomology* 49: 141–174.

Togaviruses: General Features

S C Weaver, University of Texas Medical Branch, Galveston, TX, USA
W B Klimstra and K D Ryman, Louisiana State University Health Sciences Center, Shreveport, LA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Alphavirus A virus in the genus *Alphavirus*.
Arbovirus A virus transmitted to vertebrates by hematophagous (blood-feeding) insects.
Endemic A disease constantly present in a community in a defined geographic region.
Enzootic A disease constantly present in an animal community in a defined geographic region.
Epidemic Outbreak of human disease above the normal (endemic) incidence.
Epizootic Outbreak of disease in animals above the normal (enzootic) incidence.
Vector An arthropod (mosquito in the case of most alphaviruses) that transmits an arbovirus.
Zoonotic A human disease whose causative agent is maintained in populations of wild animals.

Introduction and History

The *Togaviridae* is a family of enveloped, single-stranded, plus-strand RNA viruses that occur nearly worldwide. The family includes the genus *Alphavirus*, a group of zoonotic viruses maintained primarily in rodents, primates, and birds by mosquito vectors. Human disease occurs when people intrude on enzootic transmission habitats and are bitten by infected mosquitoes, or when the virus emerges to cause epizootics and epidemics. The other genus in the family *Togaviridae* is *Rubivirus*, with Rubella virus, the etiologic agent of 3-day measles (German measles), as its only member.

The first togaviruses to be isolated were the alphaviruses Western equine encephalitis virus (WEEV) and Eastern equine encephalitis virus (EEEV) in 1930 (although EEEV was not described until 1933), followed by Venezuelan equine encephalitis virus (VEEV) in 1938. Serologic tests using hemagglutination inhibition (HI) later indicated that these three viruses were antigenically related, and in 1954 Casals and Brown designated two groups of arboviruses: 'Group A' included WEEV, EEEV, and VEEV, Semliki Forest virus (SFV), and Sindbis virus (SINV), while 'Group B' is now known to include scores of flaviviruses. The HI test also indicated degrees of relatedness within viruses of Group A, with cross-reactions between EEEV and VEEV stronger than reactions between these viruses and WEEV, SFV, or SINV. These interrelationships, which formed the basis for the definitions of the antigenic complexes of alphaviruses, have generally stood the test of time, and are reflected in the phylogenetic relationships described below. Other assays including complement fixation (CF) and neutralization (NT) tests were used in some cases to define relationships on a finer scale. The introduction of the kinetic HI test later allowed further discrimination of certain alphaviruses, such as the antigenic varieties within the VEE complex and between North American and South American EEEV strains. The serological interrelationships among the alphaviruses were later determined to reflect the envelope glycoproteins in the

case of HI and NT, and a mixture of structural and nonstructural proteins in the case of CF.

For some time, the *Togaviridae* included four genera: *Alphavirus, Rubivirus, Flavivirus,* and *Pestivirus*. Not until the morphology, RNA genome organizations and coding strategies, as well as the protein and nucleotide sequences were determined for representatives was it recognized that the former two genera differ dramatically from the latter two. The flaviviruses and pestiviruses were then placed into a distinct family, the *Flaviviridae* (now with three genera), and the *Alphavirus* and *Rubivirus* genera were retained in the family *Togaviridae*.

Structure, Systematics, and Evolution

The basis for grouping the alphaviruses and Rubella virus into the *Togaviridae* is that they have very similar genome organizations and coding strategies, as well as primary sequence homology that can be detected in short motifs in the nonstructural protein genes (**Figure 1**). Alphavirus virions have been visualized at high resolution using cryoelectron microscopy. The E1 envelope glycoprotein domain that lies outside of the lipid envelope has been crystallized and its structure solved to atomic resolution. The folds of the alphavirus E1 protein are very similar to those of the flavivirus envelope protein, indicating that these proteins are homologous (share a common ancestral gene) and that the togaviruses and flaviviruses share a distant, common ancestor, at least for the E1/E protein genes which, due to their extensive divergence, cannot be detected from the nucleotide or amino acid sequences.

Alphavirus virions are about 70 nm in diameter; the nucleocapsid, which forms in the cytoplasm of infected cells, includes one molecule of genomic RNA and 240 copies of the capsid protein arranged in hexons and pentons in a $T = 4$ lattice. This icosahedral structure dictates the arrangement of the envelope proteins in the virion. Outside of the plasma membrane-derived lipid envelope, trimeric envelope spikes on the surface of the virion are composed of E1/E2 heterodimers, with the E2 protein most exposed and believed to interact with cellular receptors. The Rubella virion appears as a spherical particle, 60–70 nm in diameter, in electron micrographs. Rubella virus differs from alphaviruses in that the electron translucent zone between the nucleocapsid and envelope proteins is wider and the rubiviruses have a $T = 3$ icosahedral symmetry.

The alphaviruses and rubiviruses are quite distantly related and both share sequence homology with several taxa of plant viruses, some of which are transmitted by insects. These relationships suggest that the ancestor of these viruses was an insect virus that underwent a process called modular evolution that led to several genome rearrangements, including segmentation.

Before the era of molecular genetics, the alphaviruses were grouped into several antigenic complexes based on cross-reactivity in serologic assays including HI and CF (but generally not plaque reduction neutralization; PRNT) (**Table 1**). Within some of these complexes, the VEE complex, for example, differentiation of antigenic subtypes and varieties, some of which have fundamental and critical epidemiological and virulence differences, was accomplished using specialized forms of HI and, in some cases, PRNT. Direct genetic evidence of homology

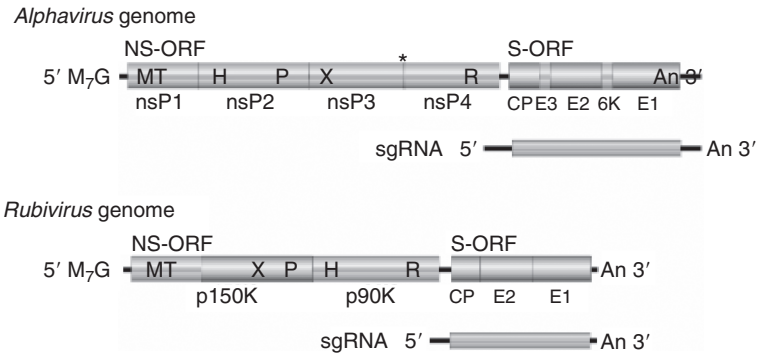


Figure 1 Togavirus genomic coding strategies. Shown are comparative schematic representations of the alphavirus and rubivirus genomic RNAs with untranslated regions represented as solid black lines and open reading frames (ORFs) as open boxes (NS-ORF, nonstructural protein ORF; S-ORF, structural protein ORF). Within each ORF, the coding sequences for the proteins processed from the translation product of the ORF are delineated. The asterisk between nsP3 and nsP4 in the alphavirus NS-ORF indicates the stop codon present in some alphaviruses that must be translationally read through to produce a precursor containing nsP4. Additionally, within the NS-ORFs, the locations of motifs associated with the following activities are indicated: MT, methyltransferase; P, protease; H, helicase; X, unknown function; and R, replicase. The sequences encompassed by the subgenomic RNA (sgRNA) are also shown. Reproduced from Weaver SC, Frey TK, Huang HV, *et al.* (2005) *Togaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 999–1008. San Diego, CA: Elsevier Academic Press, with permission from Elsevier.

Table 1 Members of the genus *Alphavirus*

Antigenic complex	*Virus*	*Antigenic subtype*	*Antigenic variety*	*Clinical syndrome in humans*	*Distribution*
Barmah Forest	Barmah Forest virus (BFV)			Febrile illness, rash, arthritis	Australia
Eastern equine encephalitis (EEE)	Eastern equine encephalitis virus (EEEV)	I–IV		Febrile illness, encephalitis (none recognized in Latin America)	North, Central, South America
Middelburg	Middelburg virus (MIDV)			None recognized	Africa
Ndumu	Ndumu virus (NDUV)			None recognized	Africa
Semliki Forest	Semliki Forest virus (SFV)			Febrile illness	Africa
	Chikungunya virus (CHIKV)			Febrile illness, rash, arthritis	Africa
	O'nyong-nyong virus (ONNV)			Febrile illness, rash, arthritis	Africa
	Getah virus (GETV)			None recognized	Asia
	Bebaru virus (BEBV)			None recognized	Malaysia
	Ross River virus (RRV)	Sagiyama		Febrile illness, rash, arthritis	Australia, Oceania
	Mayaro virus (MAYV)			Febrile illness, rash, arthritis	South and Central America, Trinidad
	Una virus (UNAV)			None recognized	South America
Venezuelan equine encephalitis (VEE)	Venezuelan equine encephalitis virus (VEEV)	I	AB	Febrile illness, encephalitis	North, Central, South America
			C	Febrile illness, encephalitis	South America
			D	Febrile illness, encephalitis	South America, Panama
			E	Febrile illness, encephalitis	Central America, Mexico
	Mosso das Pedras virus (MDPV)		F		
	Everglades virus (EVEV)			Febrile illness, encephalitis	Florida (USA)
	Mucambo virus (MUCV)	III	A	Febrile illness, myalgia	South America, Trinidad
			C	Unknown	Peru
			D	Febrile illness	Peru
	Tonate virus (TONV)	III	B	Febrile illness, encephalitis	Brazil, Colorado (USA)
	Pixuna virus (PIXV)			Febrile illness, myalgia	Brazil
	Cabassou virus (CABV)			None recognized	French Guiana
	Rio Negro virus (RNV)			Febrile illness, myalgia	Argentina
Western equine encephalitis	Sindbis virus (SINV)			Febrile illness, rash, arthritis	Africa, Europe, Asia, Australia
		Babanki		Febrile illness, rash, arthritis	Africa
		Ockelbo		Febrile illness, rash, arthritis	Europe
		Kyzylagach		None recognized	Azerbaijan, China
	Whataroa virus (WHAV)			None recognized	New Zealand
	Aura virus (AURAV)			None recognized	South America
	Western equine encephalitis virus (WEEV)	Several		Febrile illness, encephalitis	Western North, South America
	Highlands J virus (HJV)				Eastern North America
	Fort Morgan virus (FMV)	Buggy Creek		None recognized	Western North America
Trocara	Trocara virus (TROV)				South America
Salmon pancreas disease	Salmon pancreas disease virus (SPDV)			Pancreatic disease (salmon)	Atlantic Ocean and tributaries
	Sleeping disease virus (SDV)			Sleeping disease (trout)	Worldwide
Southern elephant seal virus	Southern elephant seal virus (SESV)			None recognized	Australia

among the alphaviruses, and later between the alphaviruses, rubella virus, and some plant viruses, came first from direct protein sequencing, followed by nucleotide sequencing. Eventually, nucleotide sequencing largely replaced the serological tests for determining relationships among the alphaviruses, although the latter remain useful from an epidemiological standpoint.

Nearly complete phylogenetic trees can now be constructed from homologous E1 envelope glycoprotein gene nucleotide or deduced amino acid sequences (**Figure 2**). These trees largely agree with the original antigenic groupings, with the exception of Middelburg virus, which genetically belongs within the SF complex. The relationships of the New World members of the WEE antigenic complex have more complex relationships when the nonstructural protein genes are analyzed; these viruses group more closely with EEEV than with the Old World SINV-like members of the WEE complex or with the New World member, Aura virus. These relationships reflect an ancient recombination event between a SINV-like ancestor and a virus closely related to an ancestor of EEEV (**Figure 2**). The recombinant ancestor later gave rise to the WEE, Highlands J, and Fort Morgan virus ancestors.

Most estimates for the rate of RNA sequence evolution among alphaviruses are on the order of 10^4 substitutions per nucleotide per year, similar to those of other arboviruses and lower than those of most vertebrate viruses that do not use arthropods as vectors. These low rates may reflect constraints imposed by alternate replication on disparate hosts, as well as by persistent infection of mosquito vectors. However, the timescale for evolution of the togaviruses and alphaviruses is difficult to determine from sequence data because the ability of phylogenetic

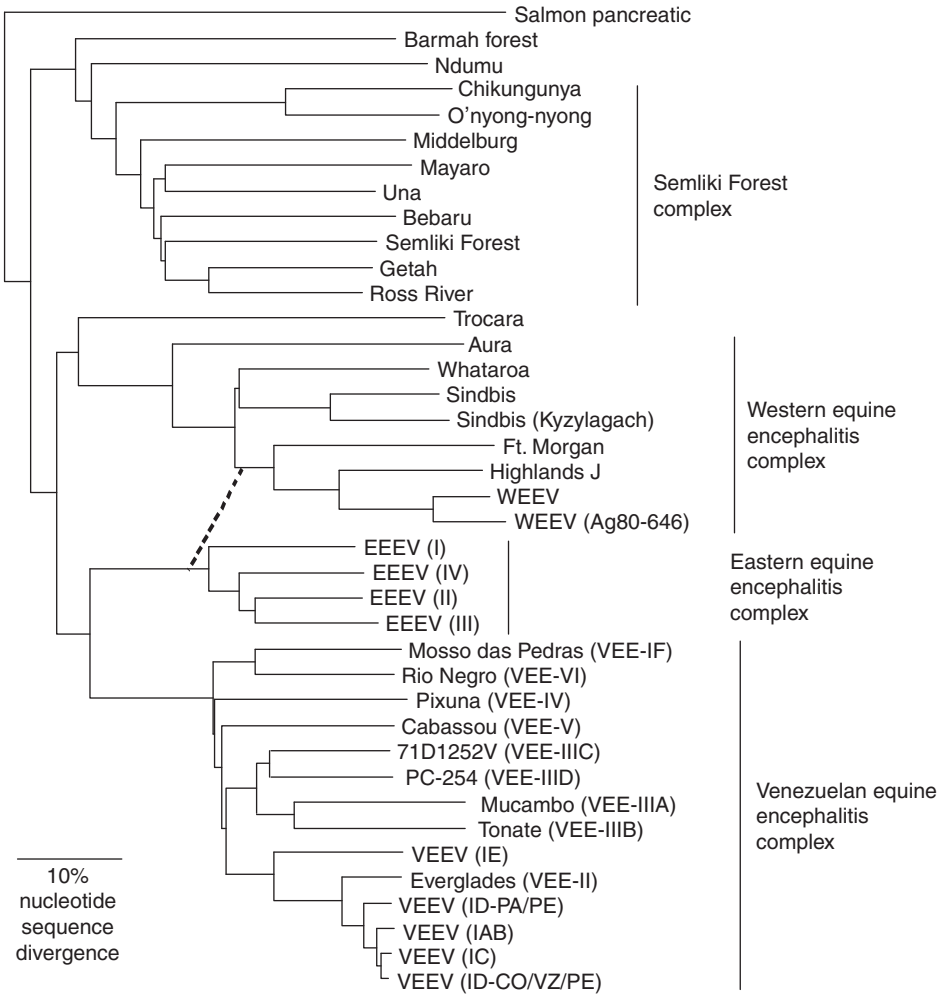


Figure 2 Phylogenetic tree of all alphavirus species except southern elephant seal virus (the homologous sequence region is not available), and selected subtypes and variants, generated from partial E1 envelope glycoprotein gene sequences using the neighbor joining program with the HKY distance formula. Antigenic complexes are shown on the right. The dashed line indicates the ancestral recombination event that led to Highlands J, Fort Morgan, and Western equine encephalitis viruses. Rubella virus cannot be included in this analysis because there is no detectable primary sequence homology with alphavirus structural protein sequences.

methods to compensate for sequential substitutions of the same nucleotides or amino acids is unknown.

Phylogenetic studies of alphaviruses generally point to purifying selection as dominating their evolution, presumably because most mutations are deleterious, especially when efficient replication in widely divergent hosts (mosquitoes and vertebrates) is required. Experimental evolutionary studies employing cell culture model systems also indicate that most adaptive mutations are host cell specific, supporting the hypothesis that the alternating host replication cycle constrains adaptation to new hosts by alphaviruses. The mobility of reservoir hosts appears to influence patterns of alphavirus evolution and their phylogeography. Viruses that use avian hosts, such as EEEV, WEEV, SINV, and Highlands J virus, tend to evolve within a relatively small number of sustained lineages that have very wide geographic distributions. For example, some lineages of WEEV have been sampled from California to Argentina. The efficient dispersal of these viruses by their highly mobile reservoir hosts presumably allows competition among different sympatric lineages to limit virus diversity. In contrast, alphaviruses that use hosts with more limited mobility, such as VEE complex viruses that rely on rodents and other small mammals, tend to exhibit more genetic diversity and lineages confined to smaller, nonoverlapping geographic regions.

Transovarial transmission from infected adult females to their offspring has been documented for a few alphaviruses, but it is not known whether this is important for maintenance of these viruses in nature. Persistent infection of vertebrate hosts also appears to be rare and is not known to be involved in long-term maintenance of alphaviruses. However, mechanisms for the overwintering of alphaviruses in temperate regions, which has been suggested by several genetic studies, remain enigmatic and could include transovarial transmission and/or persistent infection of reservoir hosts.

Compared to the alphaviruses, rubella virus is relatively conserved genetically, with only one serotype described. However, phylogenetic analyses indicate the existence of at least two genotypes, with genotype I isolates predominant in Europe, Japan, and the Western Hemisphere, and the more diverse genotype II isolated in Asia and Europe. There is considerable geographic overlap between these genotypes and subgenotypes, presumably reflecting the efficient dispersal of Rubella virus by infected people.

Diseases

Most of the alphaviruses cause febrile disease in humans and/or domestic animals. However, a few alphaviruses have not been associated with any disease, including Aura, Trocara, Middelburg, Ndumu, Ft. Morgan, and Una viruses. The most severe alphaviral feature, encephalitis, is caused by the New World viruses EEEV, VEEV, and WEEV. In humans, apparent:inapparent infection rates range from very low for EEEV (*c.* 1:23) to *c.* 10:1 for VEEV. However, mortality rates in apparent human infections are inversely correlated with these ratios, with *c.* 50% for EEEV and less than 1% for VEEV. In equids, most infections are apparent, with high mortality rates (*c.* $\geq$50%) for all three viruses. Any of these viruses can also cause disease in a wide range of other domesticated and wild animals, with EEEV documented as the etiologic agent of severe disease in pigs, deer, emus, turkeys, pheasants, cranes, and other birds. Everglades virus is also known to cause neurologic disease in humans, and Highlands J virus has caused documented disease in domestic birds and in a horse.

Several of the Old World alphaviruses including Chikungunya (CHIKV), O'nyong nyong (ONNV), Ross River, and Barmah Forest viruses cause an arthralgic disease syndrome often accompanied by rash. Mayaro virus (MAYV), another member of the SF complex that was probably introduced into South America from the Old World, is the exception to the relationship between hemispheric distributions and disease syndromes. The arthralgic disease caused by these alphaviruses can be highly incapacitating and chronic, with affected persons unable to work or function normally for several months or longer.

Rubella virus is the etiologic agent of rubella, also known as German or 3-day measles, a generally benign infection. Occasionally, more serious complications such as arthritis, thrombocytopenia purpura, and encephalitis can occur. Congenital transmission to the fetus during the first trimester of pregnancy can lead to serious birth defects that comprise congenital rubella syndrome. Congenitally infected children suffer a variety of autoimmune and psychiatric disorders in later life, including a fatal neurodegenerative disease known as progressive rubella panencephalitis. Rubella has been largely controlled through vaccination in most developed countries, although it remains endemic worldwide.

Transmission Cycles

All alphaviruses are zoonotic, and the vast majority have transmission cycles involving mosquito vectors and avian or mammalian reservoir and/or amplification hosts. Exceptions include the fish viruses, sleeping disease virus, and salmon pancreatic disease virus; southern elephant seal virus was isolated from lice and may use these insects as mechanical, rather than biological, vectors, although transmission has not been documented. The mosquito-borne alphaviruses often have relatively narrow vector ranges, relying on one or a few principal mosquito vectors

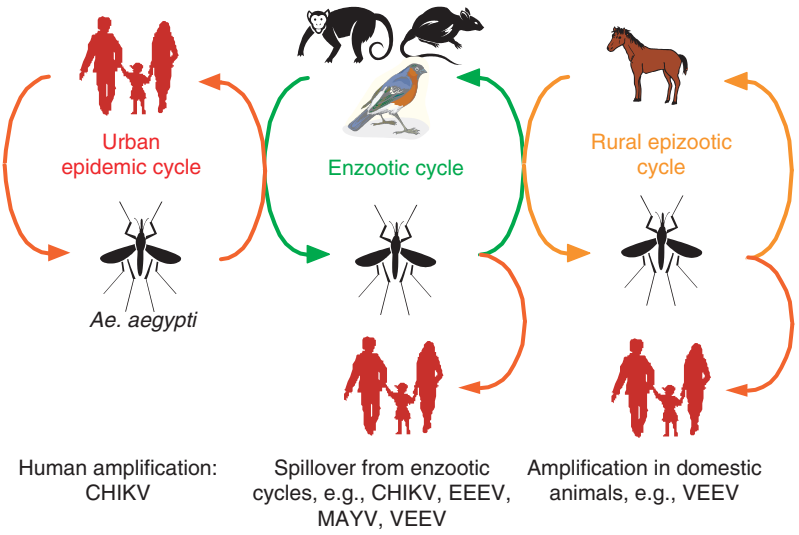


Figure 3 Cartoon showing mechanisms of human infection by alphaviruses. At the center is an enzootic cycle, typically involving avian, rodent, or nonhuman primates as amplification and/or reservoir hosts and mosquito vectors. Humans become infected via direct spillover when they enter enzootic habitats and/or when amplification results in high levels of circulation. Transmission to humans may involve the enzootic vector or bridge vectors with broader host preferences. Right panel: Secondary amplification involving domestic animals can increase circulation around humans, increasing their chance of infection via spillover. In the case of VEEV, mutations that enhance equine viremia are needed for secondary equine amplification. Left panel: CHIKV can use humans for amplification, resulting in urban epidemic cycles and massive outbreaks. Expanded form of virus abbreviations is provided in **Table 1**. Reproduced from Weaver SC (2005) Host range, amplification and arboviral disease emergence. *Archives of Virology* 19(supplement): 33–44, with permission from Springer-Verlag.

(**Figure 3**). Spillover to humans and domesticated animals results in epidemics and epizootics, with the affected animals (including humans) usually not generating sufficient viremia to participate in the transmission cycle (i.e., acting only as dead-end hosts). VEEV temporarily adapts via mutation to utilize equids as highly efficient amplification hosts (with high viremias), resulting in hundreds to several thousands of horses, donkeys, and mules affected during epizootics, and increased spillover to humans that results in epidemics of similar proportions. CHIKV and ONNV cause major epidemics by using humans as amplification hosts and, as vectors, mosquitoes that live in close association with humans, such as *Aedes aegypti, Ae. albopictus* (CHIKV), and *Anopheles* spp. (ONNV). Some alphaviruses, including EEEV, probably use secondary ('bridge') vectors during epidemics and epizootics because the primary mosquito vector (e.g., *Culiseta melanura* for EEEV) has a very narrow host range limited mainly to the animals serving as the reservoir hosts (**Figure 3**).

The infection of mosquito vectors by alphaviruses begins with the ingestion of a blood meal from a viremic vertebrate host. Susceptibility and minimum infectious doses for alphavirus infection of mosquitoes vary dramatically. Following the passage of ingested blood into the posterior midgut of the alimentary tract during feeding, infection of epithelial digestive cells occurs, presumably before the deposition of the peritrophic matrix around the blood meal, which begins with hours of feeding. Recent studies indicate that typical VEEV infections begin with fewer than 100 of the approximately 10 000 epithelial cells becoming infected, and thus the initial midgut infection may represent a bottleneck in alphavirus populations. After replication in the midgut epithelial cells, alphaviruses bud principally from the basal plasma membrane and somehow traverse the basal lamina to enter the hemocoel, or open body cavity of the mosquito. Then, transported by the hemolymph, the virus has access to many internal organs including the salivary glands, where replication leads to virus shedding into the saliva. Upon a subsequent blood meal from a naive host, transmission can occur via saliva deposited extravascularly during probing to locate a blood vessel, or intravascularly during engorgement. The amount of virus transmitted during blood feeding also varies widely, but rarely exceeds 100 infectious units of VEEV, again representing a bottleneck for virus populations. The extrinsic incubation period from an infectious blood meal to transmission by bite can occur in as little as 3 days, depending on the virus, mosquito, and temperature of incubation. However, mosquitoes generally do not re-feed until egg development and oviposition has occurred, which can require a week or more.

The sites of initial alphavirus replication in the vertebrate host are generally known only from studies using subcutaneous or intradermal needle inoculations, rather

than by mosquito feeding, and can include Langerhans cells, dendritic cells, dermal macrophages, and fibroblasts in the skin. Because virus is also deposited intravascularly during mosquito feeding, other sites could be important in natural infections. Secondary replication and amplification sites also vary, including lymph nodes, spleen, myocytes, chondrocytes, osteoblasts, and neurons.

Rubella virus is transmitted via the respiratory route among infected humans, with no vector involvement. Transplacental transmission can result in severe complications and sequelae.

Alphaviruses of Greatest Medical and Veterinary Importance

The majority of alphaviruses can cause at least mild disease in humans, and most exceptions are lesser-known viruses that have not been well studied epidemiologically. Recent studies of 'dengue-like' illness in several locations have revealed that several alphaviruses such as VEEV, MAYV, and CHIKV account for a significant number of cases diagnosed clinically as dengue. Proper serological testing or virus detection is needed to accurately determine the cause of many tropical fevers that present with nonspecific 'flu-like' signs and symptoms. Brief descriptions of the most important alphaviruses follow.

Chikungunya Virus

CHIKV is probably the most important alphaviral pathogen worldwide, although it rarely causes fatal disease. CHIKV, named after a local Makonde word meaning 'that which bends up', refers to the characteristic posture assumed by patients suffering severe joint pains. The virus probably occurs in most of sub-Saharan Africa, as well as in India, Southeast Asia, Indonesia, and the Philippines. In Africa, a sylvan transmission cycle between wild primates and arboreal *Aedes* spp. mosquitoes has been characterized, and the virus probably occupies a niche similar to that of the flavivirus, yellow fever virus. In Asia and nearby islands, and recently on islands off the eastern coast of Africa in the Indian Ocean, urban and suburban CHIKV epidemics usually have been associated with *Ae. aegypti* and/or *Ae. albopictus* transmission among human amplification hosts. Explosive epidemics have affected hundreds of thousands to millions of people, and many cases are probably unrecognized because CHIKV infection is difficult to distinguish clinically from dengue. Because CHIKV infection of humans is sporadic, and no reservoir hosts or sylvatic vectors are known from Asia, it is not known whether the virus is zoonotic outside of Africa, where it is believed to have originated. Although CHIKV historically has been regarded as a nonfatal human pathogen, recent fatal cases on islands off the eastern coast of Africa in the Indian Ocean indicate a possible increase in virulence, although improved surveillance is an alternative explanation.

Eastern Equine Encephalitis Virus

In human cases, North American strains of EEEV are the most virulent of the alphaviruses with case–fatality rates generally exceeding 50%. Fortunately, only 220 confirmed cases occurred in the US from 1964 to 2004. A wide variety of domesticated mammals and birds also suffer fatal infections in North America. In South and Central America, EEEV is widespread and equine outbreaks are common, but human EEE is rare, probably due to lower human virulence in the genetically distinct strains that occur there. Attack rates in North America tend to be highest in young children and the elderly.

Transmission cycles of EEEV in North America involve *Culiseta melanura* as the principal enzootic mosquito vector, and passerine birds as reservoir hosts in hardwood swamp habitats. Bridge vectors in the genera *Aedes*, *Coquillettidia*, and *Culex* may be responsible for most transmission to humans and domestic animals. Foci occur in swamps along the eastern seaboard and the coast of the Gulf of Mexico, but have also been documented as far west as Texas and the Dakotas. In South and Central America, the ecology and epidemiology of EEEV are poorly understood. Isolates from mosquitoes suggest that the most important enzootic vectors are members of the subgenus *Culex* (*Melanoconion*), which are also enzootic vectors of most of the VEE complex viruses. Reservoir hosts are not well understood, but may include mammals or birds.

Mayaro Virus

Mayaro virus has been isolated from persons suffering from an arthralgia with rash in Surinam, French Guiana, Colombia, Panama, Brazil, Peru, and Bolivia, and probably occurs in lowland tropical forests throughout much of South America. As are infections with many other alphaviruses, clinical diagnosis of MAYV infection is unlikely and many cases are misdiagnosed as dengue. Sporadic and focal infections usually occur in people living near or working in tropical forests, and forest-dwelling mosquitoes in the genus *Haemagogus* are probably the principal vectors. Wild primates probably serve as reservoir hosts. Mayaro virus activity is often detected during investigations of outbreaks of sylvan yellow fever, probably because both viruses share the same mosquito vectors.

Mayaro virus infection is characterized by a sudden onset of fever, headache, myalgia, chills, and arthralgia, sometimes accompanied by dizziness, photophobia, retro-orbital pain, nausea, vomiting, and diarrhea. Though not known to be fatal, the arthralgia may be severe, may

persist for up to 2 months, and usually affects the wrists, ankles, and toes, and less commonly the elbows and knees. Approximately two-thirds of patients develop a fine maculopapular rash on the trunk and extremities.

O'nyong-nyong Virus

ONNV, a close relative of CHIKV, derives its name from the description by the Acholi tribe, meaning 'joint breaker'. The virus was first isolated and characterized in 1959 during an epidemic involving *c.* 2 million people in Uganda, Kenya, Tanzania, Mozambique, Malawi, and Senegal. Since that epidemic ended in 1962, only a single major epidemic (1996, in the Rakai, Mbarara, and Masaka districts of southwestern Uganda and bordering Bukoba district of northern Tanzania) and a few sporadic reports of virus isolation have been reported. ONNV causes an arthralgia-rash syndrome that can persist for months, and all age groups are affected. Epidemic transmission involves *Anopheles funestus* and *An. gambiae* mosquitoes, which also transmit malaria parasites in Africa. ONNV is the only known alphavirus with *Anopheles* spp. vectors.

Ross River Virus

Ross River virus (RRV) infection has been recognized in Australia since 1928 as associated with epidemic polyarthritis. The disease there may involve up to thousands of people annually, and occurs mainly during the summer and autumn as sporadic cases and small outbreaks, usually among vacationers and other persons living or traveling in rural areas. RRV is maintained in Australia primarily among marsupials and other wild vertebrates, with *Culex annulirostris* and *Aedes vigilax* and other mosquitoes serving as vectors. Human cases and explosive epidemics also have been documented in islands of the South Pacific. Epidemiologic studies implicated *Ae. polynesiensis* as a vector and suggested that humans may have served as amplification hosts.

Following 3–21 days of incubation, disease begins suddenly with headache, malaise, myalgia, and joint pain. Joints may be swollen and tender. Multiple joints are involved; the most commonly affected are the ankles, fingers, knees, and wrists. Pain and loss of function usually last for several weeks, but some patients have persistent or recurrent arthralgia and arthritis for up to a year. About one-half of epidemic polyarthritis patients develop a maculopapular rash, usually lasting 5–10 days; 30–50% also have low-grade fever. Persons 20–50 years of age are most commonly affected, and the incidence is higher in females. Viremia is transient and may precede the onset of arthritis. The skin rash and joint swelling may be due to a local cell-mediated immune response rather than to immune complexes or complement-mediated reaction. The synovial exudate contains no detectable virus and consists almost entirely of mononuclear leukocytes.

Venezuelan Equine Encephalitis Virus

VEE was recognized first in Venezuela in 1936. The etiologic agent, VEEV, has caused explosive equine epizootics and epidemics in many regions of the Americas. The last major epidemic in northern Venezuela and Colombia in 1995 involved approximately 100 000 persons. VEEV strains use two distinct transmission cycles: enzootic, and epidemic or epizootic. The enzootic cycles generally rely on small rodent reservoir hosts and mosquito vectors in the subgenus *Culex* (*Melanoconion*), and occur in tropical forest and swamp habitats from Florida to Argentina. These viruses and subtypes are generally avirulent for equids, but are pathogenic for humans and can cause fatal disease. In contrast, epidemic or epizootic VEEV (subtypes IAB, IC) are virulent for both equids and humans, but have only temporary, unstable transmission cycles involving *Aedes* and *Psorophora* spp. mosquitoes, which transmit the virus among equids circulating high levels of viremia (**Figure 3**). People generally become infected by mosquitoes that previously engorged on viremic equids. VEE outbreaks are believed to occur following mutations of enzootic subtype ID viruses, which result in the transformation to an equid-virulent, IAB or IC subtype.

Following an incubation period of 1–4 days, symptoms of human VEE include fever, lethargy, headache, chills, dizziness, body aches, nausea, vomiting, and/or prostration. Symptoms usually subside after several days, but may recrudesce. Disease occurs in all age groups and both sexes, but severe neurologic signs, including convulsions and seizures, occur primarily in children (4–14% of pediatric cases) and usually late in the illness. Case–fatality rates are estimated at 0.5%.

Western Equine Encephalitis Virus

WEEV was first isolated from a horse brain in the Central Valley of California during a major epizootic in 1930. Like EEEV, WEEV was not established as an etiological agent of human disease until 1938. Later, WEEV was identified nearly throughout the Americas including the eastern US, but more detailed antigenic and genetic studies eventually elucidated other members of the WEE antigenic complex including Highlands J virus in eastern North America and Fort Morgan virus in the central United States. The distribution of WEEV is now believed to include western North America through Central and South America to Argentina. Virus strains from this wide range are remarkably conserved genetically, suggesting efficient transport by birds during migrations.

Antigenically and genetically distinct WEEV subtypes with more limited distributions in South America have also been identified, and some are less virulent in murine models. All of these WEEV-related New World viruses are believed to have descended from an ancient, recombinant alphavirus that obtained its nonstructural and capsid protein genes from an EEEV-like ancestor and its envelope glycoprotein genes from an ancestor of SINV.

In the western US, WEEV is transmitted primarily in agricultural habitats by *Culex tarsalis* mosquitoes among passerine birds, principally sparrows and house finches. A secondary cycle involving *Aedes* spp. mosquitoes and lagomorphs has also been described in central California, and *Aedes* mosquitoes have been implicated as equine epizootic vectors in Argentina. Human infections with WEEV range from inapparent (the vast majority) to a flu-like syndrome to life-threatening encephalitis and meningitis. Symptomatic infection typically includes a sudden onset with fever, headache, nausea, vomiting, anorexia, and malaise, followed by cognitive symptoms, weakness, and meningeal involvement. Young children tend to be affected more severely than adults, and 5–30% suffer permanent neurologic sequelae. The overall human case–fatality rate is about 3%. WEEV also causes encephalitis in equids, with case–fatality rates of 10–50%. Despite a history of producing epidemics with thousands of human cases, WEEV has apparently declined as a human pathogen since the 1970s, probably due in part to irrigation, mosquito control, and culturally reduced human exposure to mosquitoes.

Alphavirus Pathogenesis

Detailed pathogenesis studies of alphavirus infections have been performed for only a few members of the genus. Although they do not normally cause encephalitis in humans or domestic animals, SINV and SFV have served as models for other alphaviruses because they can cause encephalitis in young mice yet require lower levels of biocontainment than the human-virulent members of the genus. In the experimental encephalitis model these viruses cause apoptotic death of neurons after intracerebral inoculation. Studies with these and other aphaviruses described below indicate that the type 1 interferon response is critical for the initial control of infection, and that humoral immunity is most important for clearance of primary infection and for protection against subsequent infections. Cell-mediated immunity has been regarded as less important, and elimination of alphaviruses from the central nervous system is thought to occur via noncytolytic mechanisms.

Among the highly human-pathogenic alphaviruses, VEEV pathogenesis has received the most attention. The murine model produces uniformly fatal encephalitis following all routes of infection. Following subcutaneous infection, dendritic cells are believed to be infected in the dermis, followed by migration to the draining lymph node where high levels of replication result in viremia and lymphodepletion. Also associated with infection is a vigorous innate immune response that may contribute to neuroinvasion and/or febrile illness. VEEV typically invades the central nervous system via olfactory neurons. Encephalitis develops by day 5–7, when the virus has been cleared from the periphery by the adaptive immune response. However, VEEV continues to replicate to high titers in the brain and paralysis is quickly followed by death. Clinical encephalitis probably results from both virus- and immune-mediated neuronal cell death.

The pathogenesis of EEEV is poorly understood, but appears to differ fundamentally from that of VEEV in the murine model; EEEV also produces a biphasic disease, but the initial phase involves replication in fibroblasts, osteoblasts, and skeletal muscle myocytes. Invasion of the central nervous system probably occurs by a vascular route. Hamsters appear to more accurately reproduce the vascular component of EEE, which is prominent in human infections.

Among the alphaviruses that cause rash/arthralgia syndromes, only RRV has received much study as an etiological agent. In the murine model, initial targets of subcutaneous infection are similar to those of EEEV, including bone, joint, and skeletal muscle, with severe associated inflammation involving macrophages, natural killer cells, and CD4+ and CD8+ T lymphocytes. Recent studies with mice deficient in T lymphocytes indicate that the adaptive immune response does not play a critical role in the development of arthritic disease.

Diagnosis and Treatment

Diagnosis of alphavirus infections is rare because clinical signs and symptoms are similar to those caused by many other viral pathogens, including dengue and influenza viruses. Definitive diagnosis generally requires virus isolation from blood taken during the first 2–4 days of illness, or serologic confirmation as described below. Most alphaviruses can be isolated by intracerebral inoculation of newborn mice (usually fatal), and cause cytopathic effects in cells of a variety of mammalian and avian lines. Serodiagnosis can be made by detecting a fourfold or greater change in antibody titers in acute-phase and convalescent-phase serum samples drawn 1–3 weeks apart, using an enzyme-linked immunosorbent assay (ELISA), immunofluorescence, hemagglutination inhibition, CF, or NT. Detection of antiviral immunoglobulin M (IgM) using ELISA has more recently been applied for this purpose and is now the standard assay.

Treatment of most alphavirus infections is symptomatic and supportive. Anticonvulsive therapy may be effective in severe cases of encephalitis, especially in children. Lymphoid depletion caused by VEEV may lead to bacterial infection of the gastrointestinal tract, and antibiotic therapy should be considered in severe cases. Pneumonia secondary to VEE and EEE also is common. Neurologic sequelae occur following severe EEE, VEE, and WEE, including headache, amnesia, anxiety, and motor impairment.

Control of Disease

As is the case for many other arboviruses, control of alphaviral diseases relies upon interruption of transmission by mosquito vectors, and sometimes on vaccination of amplification hosts such as equids in the case of VEE. Mosquito vector control during epidemics and epizootics usually relies upon aerial application of insecticides such as malathion, optimally applied soon after floodwater species emerge from the aquatic immature stages, prior to dispersal, extrinsic infection, and transmission. People present during epidemics should avoid exposure to mosquitoes through limitation of outdoor activities and the use of repellents containing the active ingredient meta-*N*,*N*-diethyl toluamide (DEET) or picaridin. Persons entering sylvatic or swamp habitats where enzootic EEEV or VEEV circulate in the Americas, as well as forests of Africa enzootic for CHIKV, should also take these precautions. The diel periodicity of mosquito vectors should also be considered when planning outdoor activities and control measures. For example *Haemagogus* mosquitoes, the principal vectors of MAYV, feed during the daytime in forested neotropical habitats, and bed nets and window screens therefore do not effectively protect people. *Aedes aegypti*, an important urban vector of CHIKV, readily enters and rests in homes, and outdoor applications of adulticides are largely ineffective in controlling epidemics.

See also: Japanese Encephalitis Virus; Rubella Virus; Tick-Borne Encephalitis Viruses; Togaviruses: Alphaviruses; Togaviruses: Equine Encephalitic Viruses; Togaviruses: Molecular Biology; Togaviruses Not Associated with Human Disease.

Further Reading

Calisher CH and Karabatsos N (1988) Arbovirus serogroups: Definition and geographic distribution. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. 1, pp. 19–57. Boca Raton, FL: CRC Press.

Casals J and Brown LV (1954) Hemagglutination with arthropod-borne viruses. *Journal of Experimental Medicine* 99(5): 429–449.

Griffin DE (2001) Alphaviruses. In: Knipe DM and Howley (eds.) *Fields Virology,* 4th edn., pp. 917–962. New York: Lippincott Williams and Wilkins.

Powers AM, Brault AC, Shirako Y, *et al.* (2001) Evolutionary relationships and systematics of the alphaviruses. *Journal of Virology* 75(21): 10118–10131.

Strauss JH and Strauss EG (1994) The alphaviruses: Gene expression, replication, and evolution. *Microbiology Reviews* 58(3): 491–562.

Weaver SC (2005) Host range, amplification and arboviral disease emergence. *Archives of Virology* 19(supplement): 33–44.

Weaver SC and Barrett AD (2004) Transmission cycles, host range, evolution and emergence of arboviral disease. *Nature Reviews Microbiology* 2(10): 789–801.

Weaver SC, Frey TK, Huang HV, *et al.* (2005) *Togaviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 999–1008. San Diego, CA: Elsevier Academic Press.

Togaviruses: Molecular Biology

K D Ryman and W B Klimstra, Louisiana State University Health Sciences Center at Shreveport, Shreveport, LA, USA
S C Weaver, University of Texas Medical Branch, Galveston, TX, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Apoptosis A cascade of cellular responses to a stimulus (e.g., virus infection) resulting in cell death.
Cytopathic effect Destructive changes in cell morphology, structure, or metabolic processes resulting from virus infection.

Full-length cDNA clone A copy of an RNA virus genome that has been reverse-transcribed into DNA and placed into a subcloning vector. With positive-sense RNA viruses, infectious genomic RNA is generally directly synthesized from bacteriophage transcription promoters located upstream of the virus sequences.

Host cell shut-off Interruption or cessation of host transcription, translation, or other processes that occurs within virus-infected cells.
Interferon A pro-inflammatory cytokine that is produced after virus infection and stimulates antiviral activities in cells expressing the IFNAR receptor complex.
Interferon-stimulated gene (ISG) A gene whose transcription is increased after interferon signaling. Some ISGs are directly antiviral and can inhibit virus transcription or translation.
Positive-sense RNA genome The genomic material of the virus resembles a cellular messenger RNA and the open reading frame can be translated on host ribosomes.
Receptor Structures (e.g., protein, sulfated polysaccharide, phospholipid) found upon the surface of host cells that interact with virus attachment proteins and mediate attachment of virus particles to cell surfaces. These structures may also mediate subsequent events in cell entry such as viral protein rearrangements leading to membrane fusion.
Replicon system A replication-competent but propagation-incompetent form of a cloned virus. In many replicon systems, the replicon genome can be packaged into a virus-like particle by *trans*-expression of structural proteins.
RNA-dependent RNA polymerase (RdRp) The primary RNA synthesis enzyme for RNA viruses which uses viral genomic RNA as a template for transcription/replication.

Introduction

The family *Togaviridae* includes the genera *Alphavirus* and *Rubivirus*. The genus *Alphavirus* is comprised of 29 virus species segregated into five antigenic complexes, most of which are transmitted between vertebrate hosts by mosquito vectors and are capable of replicating in a wide range of hosts including mammals, birds, amphibians, reptiles, and arthropods. In contrast, the sole member of the genus *Rubivirus* is Rubella virus, which is limited to human hosts and is primarily transmitted by respiratory, congenital, or perinatal routes. Together, the viruses are a significant worldwide cause of human and livestock disease. Alphavirus disease ranges from mild to severe febrile illness, to arthritis/arthralgia and fatal encephalitis, while Rubella virus is an important cause of congenital abnormalities and febrile illness frequently accompanied by arthralgia/arthritis in the developing world. At the molecular level, the togaviruses are relatively uncomplicated, small, enveloped virions with single-stranded, positive-sense RNA genomes encoding two polyproteins, one translated from the genomic RNA and the other from a subgenomic mRNA transcribed during replication. However, their replication is very tightly regulated and intimately associated with host cell metabolic processes. Currently, an important aspect of togavirus research is designed to exploit their molecular biology for therapeutic and gene therapy applications. Here, we summarize major aspects of togavirus structure, genome organization, replicative cycle, and interactions with host cells.

Virion Structure and Genome Organization

Togavirus virions are small and enveloped, comprising an icosahedral nucleocapsid composed of 240 capsid (C) protein monomers, cloaked in a lipid envelope studded with membrane-anchored glycoprotein components, E1 and E2. Their tightly packed, regular structure has been revealed by high-resolution reconstruction of the particles by cryoelectron microscopy (**Figure 1**). The envelope glycoproteins of alphaviruses are similarly arranged on the outer surface of particles. E1 and E2 form a stable heterodimer, and three heterodimers interact to form 'spikes' distributed on the virion surface in an icosahedral lattice that mirrors the symmetry of the nucleocapsid. The regularity of the spike distribution is determined by the direct hydrophobic interaction of the E2 glycoprotein tail with a pocket on the surface of the C-protein. E2 projects outward from the virion to form the spikes that interact with host-cell attachment receptors, while the E1 glycoprotein, which mediates fusion with host cell membranes, appears to lie parallel to the lipid envelope. The E2 and E1 proteins are glycosylated at one to four positions, depending on the virus strain, during transit through the host-cell secretory apparatus.

The encapsidated alphavirus genome consists of a nonsegmented, single-stranded, positive-sense RNA molecule of approximately 11–12 kb with a 5′-terminal methylguanylate cap and 3′ polyadenylation, resembling cellular mRNAs (**Figure 2**). The genome is divided into two major regions flanked by the 5′ and 3′ nontranslated regions (NTRs) and divided by an internal NTR: (1) the 5′-terminal two-thirds of the genome encode the four nonstructural proteins (nsPs 1–4); and (2) the 3′ one-third of the genome encodes the three structural proteins (C, precursor E2 [PE2], and precursor E1 [6K/E1]). Overall, the capped and polyadenylated 9.5 kb Rubella virus genome is similarly organized (**Figure 2**), but the 5′-proximal open reading frame (ORF) encodes only two nsPs (P150 and P90) and three structural proteins are encoded by the 3′ ORF (C, E1, and E2).

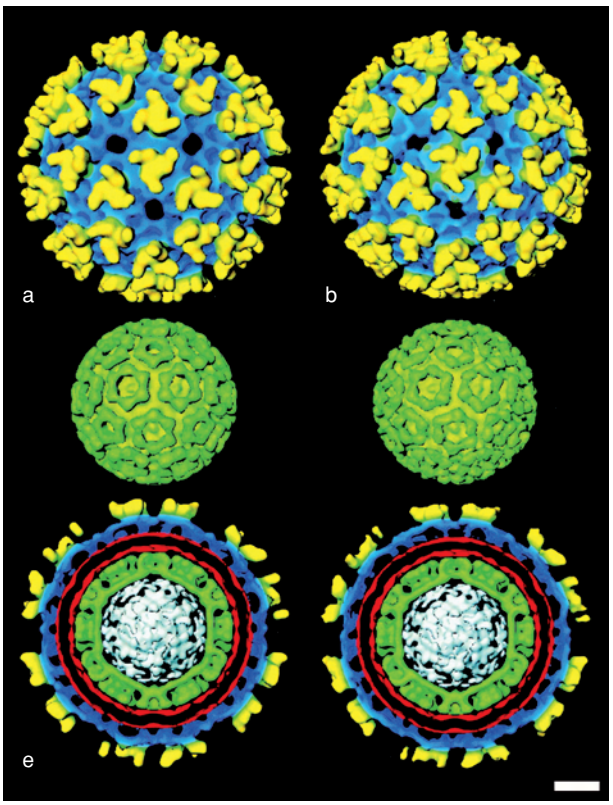


Figure 1 Structure of New World (VEEV) and Old World (SINV) alphaviruses determined by image reconstructions of electron micrographs. Isosurface view along a threefold axis of VEEV (a) and SINV (b) reconstructions showing outer spike trimers (yellow) and envelope skirt region (blue). Isosurface representations of VEEV (c) and SINV (d) nucleocapsids viewed along a threefold-symmetry axis. Cross-sections through VEEV (e) and SINV (f) perpendicular to the threefold axis and in plane with a vertical fivefold axis showing trimers (yellow), skirt region (blue), virus membrane (red), nucleocapsid (green), and RNA genome (white). Scale = 100 Å. Reproduced from Paredes A, Alwell-Warda K, Weaver SC, Chiu W, and Watowich SJ (2007) Venezuelan equine encephalomyelitis virus structure and its divergence from old world alphaviruses. *Journal of Virology* 75: 9532–9537, with permission from American Society for Microbiology.

Infection and Replication Cycle

The togavirus genome encodes very few but, therefore, necessarily multifunctional proteins. The replication of these viruses is very tightly regulated and as little as a single nucleotide change can alter susceptibility to cellular defense mechanisms. The following section outlines molecular aspects of togavirus infection and replication pertinent to the virus–cell interaction. Replication processes of viruses of the family *Togaviridae* have been most extensively studied for the alphaviruses, Sindbis virus (SINV) and Semliki Forest virus (SFV), and extrapolated to the other family members.

Virus Attachment, Entry, and Uncoating

The entry pathways of Rubella virus have undergone only limited study, but there is some evidence that a glycolipid molecule serves as an attachment receptor on some cell types. The nature of the receptor for naturally circulating, 'wild-type' alphaviruses has been examined more extensively, but remains controversial, as is the case with many arthropod-borne viruses. Typically, arthropod-borne viruses are thought to interact either with a single, evolutionarily conserved molecule expressed on most cells, or to utilize different receptors in different hosts and with cells derived from different tissues. The 67 kDa high-affinity laminin receptor (HALR) has been identified as an initial attachment receptor for cell culture-adapted, laboratory strains of SINV and an antigenically related molecule has similarly been identified for Venezuelan equine encephalitis virus (VEEV). In addition, receptor activity for particular cell types has been attributed to several other, as yet uncharacterized, proteins. However, more recent studies have indicated that efficient cell binding by many laboratory strains of alphaviruses is due to interaction with heparan sulfate (HS), a sulfated glycosaminoglycan molecule, and that this phenotype is conferred by positively charged amino acid substitutions in E2 that accompany adaptation to cultured cells. Currently, it is unclear whether or not the 67 kDa HALR or other identified receptor proteins act in concert with HS to bind cell culture-adapted alphavirus strains or if any of these molecules function in the natural replication cycle of wild-type alphaviruses. Moreover, it has become clear that many noncell culture-adapted alphavirus strains bind only weakly to commonly used cell lines, such as those derived from fibroblasts. Recently, DC-SIGN and L-SIGN, C-type lectins, were found to bind carbohydrate modifications on E1 and/or E2 proteins of both HS-binding and non-HS-binding strains of SINV and VEEV, promoting infection. The interaction of the lectins with virion carbohydrates is greatly enhanced by replication of viruses in mosquito cells, most likely due to the retention of high mannose carbohydrate structures, which are processed to complex carbohydrates in mammalian cells. Therefore, C-type lectins may be important attachment receptors for alphaviruses when transmitted by mosquitoes, and receptor utilization could be determined, at least in part, by the cell type in which alphaviruses replicate. Since DC-SIGN and L-SIGN receptors are expressed by a subset of alphavirus-permissive cells, these results further suggest that infection by alphaviruses may be mediated by different receptors on different cell types.

Following receptor interactions, which may initiate uncoating-related conformational changes in E1 and E2, alphavirus and Rubella virus virions enter acidified endosomes via a dynamin-dependent process. The decreasing pH in the endosome results in more dramatic

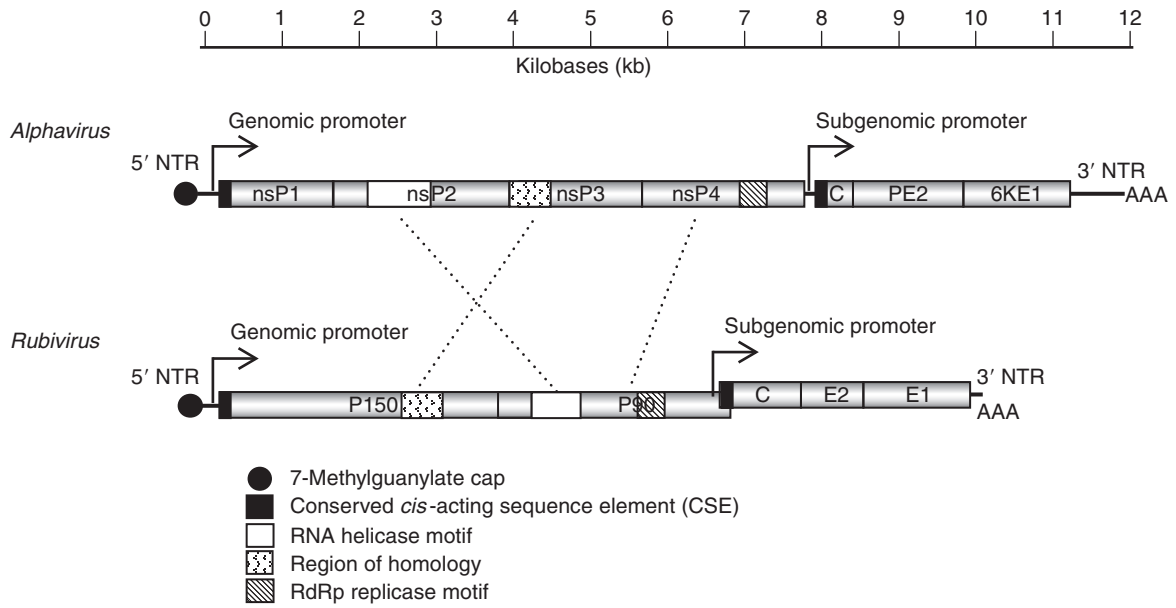


Figure 2 Comparison of *Alphavirus* (SINV) and *Rubivirus* (RUB) genome organization. Regions of nucleotide homology and regions encoding homologous amino acid sequence within the nonstructural protein ORF are shown. ORFs are denoted by boxes and nontranslated regions (NTRs) by lines. Note that for SINV the two ORFs are separated by a NTR region, whereas for RUB the two ORFs overlap in different translational frames. Reproduced from Dominguez G, Wang C-T, and Frey TK (1990) Sequence of the genome RNA of rubella virus: Evidence for genetic rearrangement during togavirus evolution. *Virology* 177: 225–238 with permission from Elsevier.

conformational changes (threshold of ~pH 6.2 with SFV) in the E2/E1 spike leading to formation of E1 homotrimers and exposure of a putative class II fusogenic domain in E1 that is thought to interact with the host cell membrane, promoting fusion of host and virion lipid bilayers. In mammalian cells, SFV membrane fusion is cholesterol-dependent and sphingolipid-dependent, and inhibitors of endosomal acidification block infection. Endosomal fusion leads to a poorly characterized entry of nucleocapsids into the cytoplasm and to their association with ribosomes, most likely directing final uncoating of the genomic RNA and translation initiation.

Genome Translation, Transcription, and Replication

Togavirus genome transcription and replication occur by a strictly positive-sense strategy and are entirely cytoplasmic (**Figure 3**). The alphavirus replicase complex comprises four nsPs encoded in the 5′-terminal ORF, all of which are essential for viral transcription and replication: (1) nsP1 is a guanine-7-methyltransferase and guanylyl-transferase; (2) nsP2 has NTPase, RNA helicase, and RNA triphosphatase activities in its amino-terminal domain, while the carboxy-terminus has highly specific thiol protease activity; (3) nsP3 is a phosphoprotein with unknown function; and (4) nsP4 is the catalytic RNA-dependent RNA polymerase (RdRp). In addition to the final products, some of the processing intermediates have distinct and indispensable functions during the replication process. The 5′-proximal ORF of the Rubella virus genome is also translated as a polyprotein (p200) and cleaved in *cis* by the Rubella virus protease (NS-pro) to form the replication complex comprising P150 and P90. However, the order of its conserved motifs differs from that of the alphavirus nsP polypeptide (**Figure 2**): P150 contains the putative methyltransferase (N-terminal) and protease sequences (C-terminal), while P90 contains the predicted helicase (N-terminal) and RNA polymerase (C-terminal).

Once the nsPs have been translated from the infecting, positive-sense RNA genome, full-length RNAs complementary to the genomic sequence (negative-sense) are synthesized, creating partially double-stranded (ds) RNA replicative intermediates. The RdRp complex then switches to preferential synthesis of positive-sense RNAs, which continues throughout the remainder of the infection replication cycle, while negative-sense RNA transcription ceases entirely. Two positive-sense, capped, and polyadenylated RNAs are synthesized on the negative-sense template: full-length progeny genomes that are packaged into virions and subgenomic (26S) RNAs that are collinear with the 3′ one-third of the genome and encode the structural protein ORF. The 26S RNA accumulates in the cells to 5–20-fold molar excess over genomic RNA and is efficiently translated into a polyprotein that is co-translationally processed to produce the structural proteins.

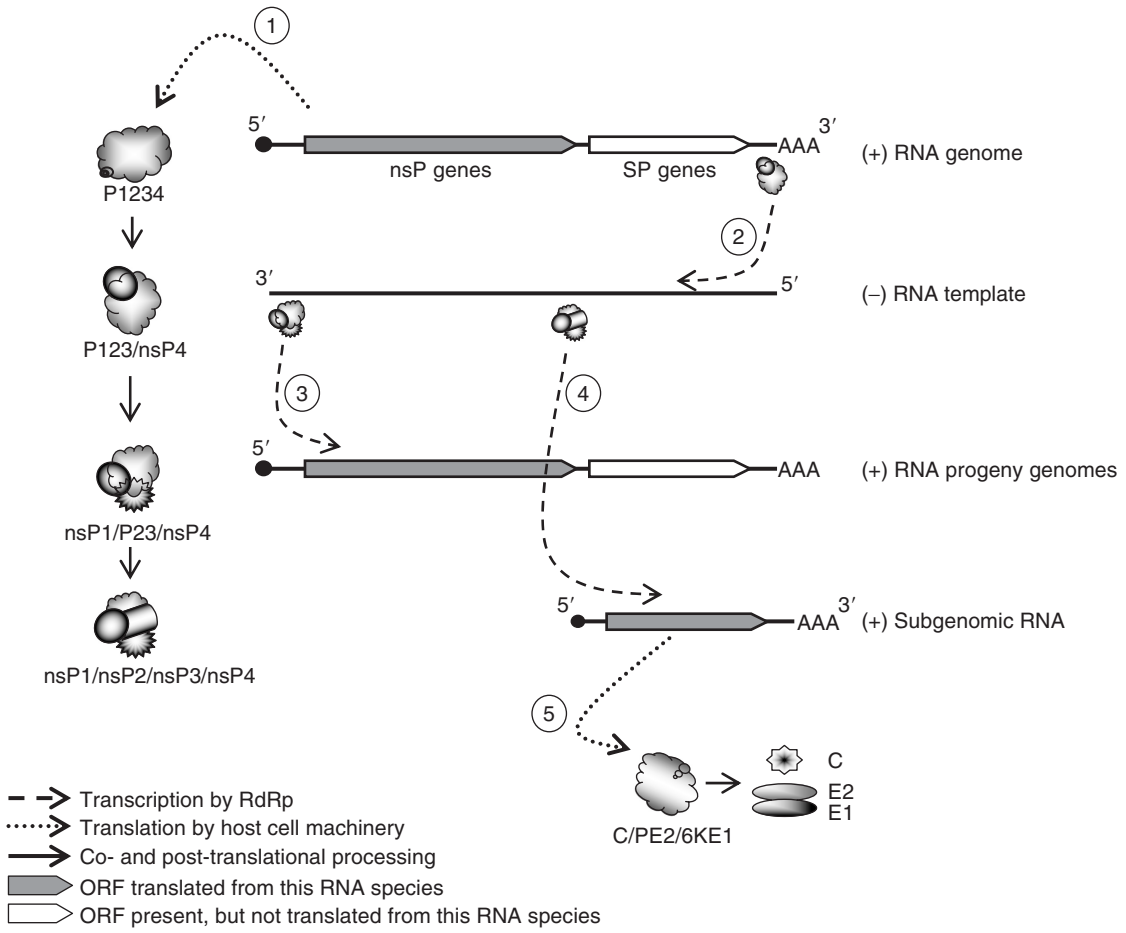


Figure 3 Diagrammatic representation of *Alphavirus* replication in a permissive cell. The replication cycle is depicted as a series of temporally regulated steps: ① Translation and processing of nonstructural polyprotein P1234 or P123; ② Synthesis of complementary, negative-sense RNA by P123/nsP4; ③ Synthesis of progeny genomes by nsP1/P23/nsP4; ④ Synthesis of subgenomic RNAs by nsP1/nsP2/nsP3/nsP4; ⑤Translation and processing of structural polyprotein C/PE2/6KE1. Progeny genomes are then packaged into nucleocapsids and bud at the plasma membrane to release progeny virions.

Alphavirus genome replication and transcription are tightly regulated temporally by sequential processing of the nsPs, which alters replicase composition and RdRp activity. For some alphaviruses (e.g., SFV), the nsPs are translated as P1234, while others (e.g., SINV) translate the nsPs as either P1234 or P123 polyproteins, depending upon read-through of an opal termination codon. The nascent P1234 polyprotein is partially processed by autocatalytic nsP2 protease-mediated cleavage at the 3/4 junction and assembled into the primary P123/nsP4 RdRp complex. This short-lived P123/nsP4 primarily synthesizes genomic, negative-sense RNA replicative intermediates. Membrane-bound P123 is cleaved relatively slowly in *cis* at the 1/2 junction, perhaps to enable formation of protein–protein interactions prior to proteolytic processing, and to form the nsP1–P23–nsP4 replicase that preferentially synthesizes positive-sense RNA. After the aminoterminus of nsP2 has been released, rapid autocatalytic cleavage of P23 produces a mature, stable RdRp complex (nsP1–nsP2–nsP3–nsP4), which transcribes only positive-sense RNA. Evidence suggests that nsP1–P23–nsP4 and the mature replicase may be biased, respectively, toward synthesis of genome-length or subgenomic mRNA, providing further temporal regulation of the replication cycle. Late in infection, when free nsP2 concentrations are high, cleavage at the 2/3 junctions is favored. P1234 polyproteins are rapidly processed into short-lived P12 and P34 precursors, thereby precluding assembly of new replication complexes and terminating negative-strand synthesis. *cis*-Cleavage of the P12 precursor gives rise to nsP1, which targets the plasma membrane, and nsP2. Interestingly, much of nsP2 is transported to the nucleus, suggesting a function beyond the replication and transcription of viral RNAs, as discussed below. P34 is cleaved in *trans* by free nsP2, yielding nsP3, which aggregates in the cytoplasm, and nsP4, which is rapidly degraded by the ubiquitin pathway.

Specificity of togavirus RNA replication by the RdRp replicase is achieved via differential recognition of *cis*-acting conserved sequence elements (CSEs) in the

termini of the viral genome and negative-sense genome template. These CSEs are conserved in all alphaviruses, as well as in Rubella virus. A 19-nucleotide (nt) CSE in the 3′ NTR immediately upstream of the poly(A) tail, is the core promoter for synthesis of genome-length, negative-sense RNA replicative intermediates, and probably interacts with the 5′ NTR via translation initiation factors to initiate replication and/or translation. The complement of the 5′ NTR in the minus-strand RNA serves as a promoter for the synthesis of positive-sense genomes. A third *cis*-acting element that is essential for replication, the 51-nt CSE, is also found near the 5′ end of the genomic RNA within the nsP1 gene. The primary sequence and two-stem–loop secondary structure of this CSE are highly conserved among alphaviruses and serve as replication and translation enhancers. Finally, a 24-nt CSE is found upstream of and including the start of the subgenomic RNA, the complement of which in the negative-strand forms the 26S subgenomic promoter and translation enhancer element.

Togavirus RNA replication occurs on cytoplasmic surfaces of endosome-derived vesicles or cytopathic vacuoles (CPVs). Expression of the nsP polyprotein (particularly nsP1) actively modifies intracellular membranes to create these compartments and to mediate membrane association of the replicase via membrane phosphatidylserine and other anionic phospholipids. The surfaces of CPVs have small invaginations or 'spherules', in which the nsPs and nascent RNA are sequestered, creating a microenvironment for RNA replication, synthesis of structural proteins, and assembly of nucleocapsids. The formation of CPVs in togavirus-infected cells may be linked to stress-induced autophagic mechanisms intended for the targeted degradation of cellular proteins and organelles, but exploited by the virus.

Structural Protein Maturation, Genome Packaging, and Egress of Virions

The subgenomic mRNAs of alphaviruses and of Rubella virus are translated as polyproteins; however, processing of the polyprotein differs between the two. With alphaviruses, the capsid protein is cleaved autoproteolytically from the E2 precursor (known as PE2 or P62) in the cytoplasm and the remaining polyprotein is translocated into the ER lumen, where N-linked oligosaccharide addition occurs. The 6K hydrophobic protein, which is primarily located in the ER membrane, is then cleaved from the polyprotein by the host signalase. With Rubella virus, the capsid-E2 and E2–E1 cleavages are all completed by signalase. Furthermore, Rubella virus lacks the 6K protein and mature E2 is produced in the ER. Alphavirus PE2 and E1 proteins form heterodimers in the ER that are anchored by their transmembrane domains and involve intra- and intermolecular disulfide linkages and associations with host chaperone proteins such as Bip and calnexin/calreticulin. The PE2/E1 heterodimers are routed to the cytoplasmic membrane and PE2 is cleaved into mature E2 by a host furin-like protease as a late event, in a structure between the *trans* Golgi network and cell surface. The cleaved E3 fragment is lost from some (e.g., SINV), but not all (e.g., SFV) virus particles. PE2-containing virions appear to bud normally from vertebrate cells, but are often defective in conformational rearrangements associated with cell fusion, supporting the hypothesis that the presence of PE2 stabilizes the glycoprotein heterodimer during low pH exposure in the secretory pathway.

Budding of particles occurs through an interaction between C and the cytoplasmic tail of E2; however, it is unclear at which point in the secretory pathway this interaction occurs and whether the interaction is between C monomers, oligomers, or RNA-containing preformed nucleocapsids. With alphaviruses, RNA packaging is directed by an RNA secondary structure in either the nsP1 or nsP2 genes, depending upon the virus, leading to selective packaging of the genome over the subgenome, which is in molar excess. In the accepted model, final budding occurs at the cytoplasmic membrane at sites enriched in E2/E1 heterodimers and is driven by E2 tail-C interactions that force an extrusion of the host lipid bilayer, envelopment of the particle, and release.

Infectious Clone Technology

Knowledge of the genome organization and replication cycle of the togaviruses have enabled the construction of full genome-length cDNA clones from which infectious RNA molecules can be transcribed *in vitro* and transfected into permissive cells to initiate productive virus replication. This procedure allows easy introduction of specific mutations into the virus genome followed by generations of genetically homogeneous virus populations encoding the mutation(s). Indeed, much of the work described in this article was performed using virus mutants generated from cDNA clones. In addition, 'double-subgenomic promoter viruses' (DP-virus) have been created in which the subgenomic promoter is reiterated immediately upstream of the authentic subgenomic promoter or at the 3′ end of the E1 coding region to drive expression of genes used as reporters of infection or vaccine vectors (e.g., GFP, luciferase, or immunogens from infectious microorganisms) or to test the effect of protein/RNA expression in the context of a togaviral infection (e.g., antiviral or apoptosis-inhibiting proteins, or interfering RNAs). Propagation-defective virus-like particles or 'replicon' systems have also been developed in which the structural protein genes are deleted and replaced with a heterologous protein gene. With alphaviruses, replicon genomes can be packaged to form replicon particles by expressing the structural proteins in *trans*. Typically, the replicon genome RNA is co-electroporated with two

'helper' RNAs, which are replicated and transcribed by the nsPs provided by the replicon genome and separately encode the capsid and PE2/6K/E1 proteins. Use of these bipartite helper systems minimizes the potential for generation of propagation-competent progeny. In contrast, Rubella virus exhibits a dependence of expression on the nsP ORF from the same nucleic acid as the structural proteins, limiting this approach. Replicon particles can infect a single cell and express the heterologous protein at high levels from the subgenomic promoter but, in the absence of structural protein expression, no progeny virions are produced. Therefore, these vectors allow detailed examination of the interaction of the virus with single cells and can also be used as vaccines with little possibility that adverse effects associated with propagating vial vectors will occur in vaccinated hosts. The results of numerous studies have indicated that togavirus DP-viruses and replicons will likely become important vector systems for the delivery of heterologous gene products to cells *in vitro* and *in vivo*.

Togavirus–Host Cell Interactions

The togavirus–cell interaction can be viewed as a conflict between the cell and the virus: the virus must replicate to propagate itself, generally leading to cytopathic effects (CPEs), while the host cell attempts to suppress virus replication in order to avoid CPE or to save the greater organism from death (in which case the individual cell may be sacrificed). In the following section, these interactions are addressed as a continuum from poorly restrained virus replication leading to rapid CPE on one extreme, to host-cell-circumscribed virus replication and prolonged cell survival on the other.

Cell Viability and CPE

Historically, alphaviruses (e.g., prototypic SINV) have been considered to be strongly cytopathic, causing rapid killing of cultured vertebrate cells that are highly permissive of their replication. In contrast, alphavirus replication in mosquito cells is most often associated with persistent infection and limited cell death. Rubella virus is also cytopathic for vertebrate cells at high infection multiplicities, but can develop persistent infection at low multiplicity and does not infect mosquito cells. Only recently has the complex interaction between the virus infection cycle and cell death begun to be unraveled. Infection of many vertebrate cell lines with alphaviruses or Rubella virus results in morphological changes (e.g., chromatin condensation, nuclear fragmentation, and formation of membrane-enclosed apoptotic bodies), as well as molecular changes (e.g., caspase activation and DNA fragmentation), associated with programmed cell death/apoptosis. Expression of SINV structural glycoproteins, particularly the transmembrane domains, can cause apoptosis in rat AT-3 prostatic adenocarcinoma cells. Furthermore, mutation from glutamine to histidine at position 55 of the SINV E2 glycoprotein has been associated with increased apoptosis in cultured cells and neurons in mice. However, although accumulation of viral structural proteins accelerates CPE development, apoptosis also occurs in replicon-infected cells in the absence of structural protein synthesis, linked to replication and nsP2 activities. Complicating this picture further is that interaction of high concentrations of UV-inactivated SINV particles with cell surfaces can initiate cell death pathways in cells of some lines, suggesting that viral replication/gene expression is not always required for killing. Finally, evidence also exists for alphavirus-induced necrotic cell death (i.e., not involving active cellular processes) in subpopulations of infected neurons *in vivo*. Therefore, the stimulus for CPE caused by togaviruses may depend upon the virus genotype, dose, the infected cell type, and/or the infection context. At the molecular level, multiple pathways, including mitochondrial cytochrome *c* release, TNF-α death receptor signaling, sphingomyelinase activation and production of ceramide, and redox-stress and inflammatory-stress pathway activation have been associated with togavirus-induced cell death, further supporting the idea that induction of particular mechanisms may be dependent upon multiple virus and host cell factors.

Host-Protein Synthesis Shut-Off

Togavirus replication in highly permissive vertebrate cells devastates cellular macromolecular synthesis, arresting both cellular transcription and translation by independent mechanisms. Transcriptional downregulation of cellular mRNAs and rRNAs can be directly mediated by the mature SINV nsP2 protein even in the absence of viral replication and is critically involved in the production of CPE. This activity is determined by integrity of the nsP2 carboxy-terminal domain, not by helicase or protease activity and can be greatly reduced by point mutations in this region. Moreover, the degree of nsP2-mediated pro-apoptotic stimulus appears to vary considerably between different togaviruses. Nonstructural protein-mediated inhibition of cellular transcription in highly permissive cells, such as fibroblast cell lines, dramatically suppresses the cell's ability to generate an antiviral stress response and is likely beneficial for togavirus replication and dissemination. However, infection with the noncytopathic nsP2 mutants described above restores the cell's response. In contrast, infection of dendritic cells and macrophages, which may be less permissive to infection than fibroblasts or may express a different ensemble of antiviral response mediators, produces a vigorous stress response after virus exposure and large quantities of new mRNAs are synthesized.

Translation of cellular mRNAs is also dramatically inhibited within hours of togavirus infection in highly permissive cells. This process of host-protein synthesis 'shut-off' occurs independent of transcriptional arrest. The degree to which translational shut-off occurs is inextricably linked with the extent of viral RNA replication, such that incomplete translation inhibition is associated with permissivity and mutations in viral replicase complex proteins, particularly nsP2. As a cellular response, translational inhibition is a stress-induced defense mechanism, able to recognize viral infection, amino acid starvation, iron deficiency, and accumulation of misfolded proteins in the endoplasmic reticulum (ER) via the activation of stress kinases. The activity of four distinct stress kinases converges to phosphorylate eukaryotic translation initiation factor (eIF) 2α, inhibiting GTP–eIF2–tRNAi-Met ternary complex formation and globally suppressing translation initiation. However, the expression of specific stress-inducible cellular proteins continues under conditions of phosphorylated eIF2α and generalized shut-off. If the stress response fails to clear the virus and restore homeostasis, the effects of prolonged shut-off become detrimental to the infected cell, leading to CPE and apoptotic cell death. Phosphorylation of eIF2α during togavirus infection is triggered by the presence of dsRNA primarily through activation of the dsRNA-dependent protein kinase (PKR). In some situations, eIF2α phosphorylation appears to be largely responsible for translation inhibition, since the overexpression of a nonphosphorylatable eIF2α mutant abrogates shut-off despite efficient viral replication. However, the arrest of host protein synthesis has also been shown to occur independently of PKR activity and in the apparent absence of eIF2α phosphorylation, by an unknown mechanism.

In response to this cellular defense mechanism, togaviruses have evolved degrees of tolerance to translational shut-off, enabling them to circumvent this block and redirect the cell's inoperative translational apparatus to the synthesis of viral proteins. Indeed, once shut-off has occurred, virtually the only proteins synthesized by the cell are virus-encoded structural proteins, expressed from the subgenomic mRNA. This implies that host protein synthesis shut-off confers an advantage to the virus, allowing usurpation of cellular translation machinery and potentially dampening the host cell's antiviral stress response. Translation enhancer elements located in the 5′ termini of SINV and SFV 26S (and, very likely, Rubella virus) mRNAs, particularly the highly stable RNA hairpin downstream of the AUG start codon, facilitate the continued expression of alphavirus structural proteins during the translation inhibition imposed by eIF2α phosphorylation. SFV infection has been shown to induce the transient formation of stress granules containing cellular TIA-1/R proteins, which sequester cellular mRNAs, but disassemble in proximity to the viral replicase. The temporal correlation between eIF2α phosphorylation, stress granule assembly and localized disassembly, and the transition from cellular to viral protein synthesis suggest that these may be important processes in generalized shut-off of protein synthesis and avoidance of the shut-off by the 26S mRNA. It is likely that the togaviruses also evade and/or antagonize PKR/phospho-eIF2α-independent translation arrest. Notably, the 26S mRNA also has a low requirement for the translation complex scaffolding protein, eIF4G, although integrity of eIF4G is not known to be affected by togavirus replication.

The Interferon-Mediated Antiviral Response

Antiviral activity of interferon-alpha/beta (IFN-α/β) is a critical determinant of the outcome of togavirus infection both *in vitro* and *in vivo*. In mice, the absence of this response results in greatly increased susceptibility to alphavirus infection and disease. In cell culture, IFN-α/β pretreatment profoundly blocks virus replication and protects cells from CPE induced by all togaviruses tested. However, some alphaviruses (e.g., VEEV) appear to be more resistant to the effects of exogenously added IFN-α/β than others (e.g., SINV). All togaviruses tested also stimulate the production of IFN-α/β from cultured cells to varying degrees. Presumably, IFN-α/β induction results from triggering of cellular pattern recognition receptors (PRRs), such as toll-like receptors (TLRs), PKR and/or cytoplasmic RNA helicases such as RIG-I or MDA-5 by 'pathogen-associated molecular patterns' (PAMPs). In addition to IFN-α/β induction, these receptors stimulate a general inflammatory response in infected cells by activating the NF-κB pathway (which is activated after SINV infection). Historically, cytoplasmic dsRNA, produced as a component of the togavirus replicative cycle has been considered the primary PAMP; however, UV-inactivated preparations of some alphaviruses (e.g., SFV) can elicit inflammatory responses in certain cultured cells suggesting multiple pathways of pathogen detection and response. Secreted IFN-α/β signals through its cognate receptor on infected and uninfected cells to upregulate expression of many IFN-stimulated genes (ISGs), producing antiviral proteins, some of which directly inhibit togavirus replicative processes. In less permissive vertebrate cells, induced IFN-α/β can act upon the infected cell to suppress togavirus replication, prevent overt CPE, and promote a persistent infection.

Several studies have evaluated the individual and combined contribution of the two best-characterized IFN-α/β-inducible antiviral pathways, the PKR pathway (described above), and the coupled 2–5A synthetase/RNase L pathway, to the control of alphavirus replication. The latter pathway is composed of the interferon-inducible 2′–5′ oligoadenylate synthetase (OAS) family of dsRNA-dependent enzymes and dormant, cytosolic RNase L. dsRNA-activated 2′–5′ OAS synthesizes 2′–5′-linked

oligoadenylates that specifically bind and activate RNase L which then cleaves diverse RNA substrates, thus inhibiting cellular and viral protein synthesis. As described above, constitutively expressed and IFN-α/β-inducible PKR is activated by dsRNA binding to phosphorylate eIF2α, causing a decline in cap-dependent translation of viral and cellular mRNAs. Infection of mice with targeted disruption of the PKR gene, the RNase L gene or both, revealed that PKR, but not RNase L, is involved in the early control of SINV replication *in vivo* and in primary cell cultures. Surprisingly, IFN-α/β-mediated antiviral responses against SINV are largely intact in the absence of PKR and/or RNase L, confirming the existence of 'alternative' IFN-α/β-induced pathway(s) capable of curtailing togavirus replication. Gene transcription and *de novo* protein synthesis are required for this activity. Although the proteins involved are yet to be characterized, one such pathway involves a novel inhibition of cap-dependent translation of infecting viral genomes in the absence of eIF2α phosphorylation. In addition, the interferon-inducible proteins MxA, ISG15, and the zinc-finger antiviral protein (Zap) have some inhibitory effects upon alphavirus replication. Considering the potent inhibitory effects of IFN-α/β toward numerous togaviruses, the expression of additional mechanisms to evade or antagonize the IFN-α/β response is likely critical for togavirus virulence, although convincing evidence of their existence is yet to be presented.

Conclusions and Perspectives

Research into the molecular biology of togaviruses has two primary goals: (1) to understand the relationship of virus interactions with single cells to replication and disease pathogenesis in natural hosts and, subsequently, to identify targets for therapeutic intervention; and (2) to manipulate virus–cell interactions to capitalize upon the potential for use of these viruses as tools for gene therapy and vaccination. In the examples described above, the dissection of virus attachment, entry, translation, replication, effects upon host cells, and host cell responses to infection all have identified vulnerabilities through which virus infection may be curtailed and many laboratories are currently developing antiviral and/or disease-ameliorating strategies. Furthermore, research into virus stimulation of CPE or stress responses has led to an improved understanding of how to, for example, maximize CPE (important for the development of tumor-destroying vectors) or minimize host antiviral activity (important for expression of immunogens by vaccine vectors). Some important issues that remain to be addressed include the full elucidation of the complex relationship of virus replication to development of CPE, the nature of antiviral stress response mediators capable of blocking virus replication, and the mechanisms through which togaviruses antagonize and/or evade these responses.

See also: Togaviruses: Alphaviruses; Togaviruses: General Features; Togaviruses Not Associated with Human Disease.

Further Reading

Dominguez G, Wang C-T, and Frey TK (1990) Sequence of the genome RNA of rubella virus: Evidence for genetic rearrangement during togavirus evolution. *Virology* 177: 225–238.

Griffin DE (2001) In: Fields BN, Knipe DM, Howley PM, *et al.* (eds.) *Fields Virology,* 4th edn., 917pp. Philadelphia: Lippincott.

Li ML and Stollar V (2004) Alphaviruses and apoptosis. *International Reviews of Immunology* 23: 7.

Paredes A, Alwell-Warda K, Weaver SC, Chiu W, and Watowich SJ (2007) Venezuelan equine encephalomyelitis virus structure and its divergence from old world alphaviruses. *Journal of Virology* 75: 9532–9537.

Schlesinger S and Schlesinger MJ (2001) *Togaviridae*: The viruses and their replication. In: Fields BN, Knipe DM, Howley PM, *et al.* (eds.) *Fields Virology,* 4th edn., 895pp. Philadelphia: Lippincott.

Strauss JH and Strauss EG (1994) The alphaviruses: Gene expression, replication, and evolution. *Microbiology Reviews* 58: 491.

Tomato Leaf Curl Viruses from India

S Chakraborty, Jawaharlal Nehru University, New Delhi, India

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Pseudorecombinants New strains of a virus that result from the reassortment of genome nucleic acids during the replication of viruses with divided genomes in mixed infections.

Recombinant A new strain/species of a virus that occurs as a result of the breakage and renewal of covalent links in a nucleic acid chain for rearrangement of nucleic acids in the chain.

Synergism The association of two or more viruses acting at the same time, which enhances symptom severity.

Introduction

Whitefly-transmitted geminiviruses cause epidemics in vegetable, staple, and fiber crops. The diseases are generally associated with local or regional whitefly (*Bemisia tabaci* Gennadius) infestations. They cause enormous economic losses in several crops in the Tropics, which provide ideal conditions for the perpetuation of viruses and the insect vector. Intensive agricultural practices necessitated by the ever-increasing demands of a rapidly growing population and the introduction of new genotypes, cropping pattern, and crops have further aggravated the situation. For example, the continuous cultivation of crops (such as cotton, tomato, pepper, beans, soybean, and melon which are susceptible to the viruses and are attractive hosts for the whiteflies) certainly account for some of the increase in the severity and the vast spread of diseases caused by geminiviruses.

Tomato leaf curl disease (ToLCD) is the most devastating disease of tomato, affecting a large area under cultivation; it can be on the scale of an epidemic. Incidence of this disease was first reported from northern India in 1948. During the 1950s, incidence was reported from central India, followed by occurrence of the disease in the main tomato-growing regions of southern India. Since then, this disease has emerged as a major threat to tomato cultivation, and incidence has increased after introduction of high-yielding tomato varieties during the late 1960s. The disease is ubiquitous with the crop and has been observed in all the tomato-producing areas of the country. Leaf curl disease of tomato is so serious that the ability of small farmers to cultivate tomato in several major production areas, especially during the peak of whitefly infestation season, has been eliminated.

Yield loss varied with the age of plants at the time of infection and varieties being tested. The virus affects all stages of the growth causing 17.6–99.7% loss in yield, depending on the stage of the crop at the time of infection. When plants get infected early, within 20 days after transplanting, they remain stunted and produce few or no fruits and the yield loss may reach up to 100%. However, if the plants are infected at 35 and 50 days after transplanting, the yield loss is reduced to 74.1% and 28.9%, respectively. This indicates that earlier the infection, higher is the yield loss.

Symptoms

Leaf curl disease of tomato is characterized by severe stunting of the plants with upward and downward rolling and crinkling of the leaves. Infected plants exhibit intervenal yellowing, vein clearing, and crinkling and puckering of the leaves, sometimes accompanied by inward rolling of the leaf margins. The disease induces severe stunting, bushy growth, and partial or complete sterility, depending on the stage at which infection has taken place (**Figure 1**).



Figure 1 (a–g) Variation of symptoms induced by begomoviruses infecting tomatoes in India.

Transmission

Under natural conditions, whiteflies transmit the tomato leaf curl viruses (ToLCVs) from infected to healthy plants. Even a single whitefly can transmit the virus. Minimum acquisition access period and inoculation access period of 30 min each is required for successful transmission to occur. Pre-acquisition and pre-inoculation starving of the vector results in higher levels of transmission. ToLCV can persist up to 10 days after acquisition in a single adult whitefly. Females are more efficient transmitters than the males. The virus is also transmitted by dodder (*Cuscuta reflexa* Roxb.). Under artificial conditions, grafting can transmit the disease. It is also known that some of the isolates of ToLCVs are also sap transmissible under laboratory conditions.

Epidemiology

Like other vector-borne diseases affecting crop species, the factors contributing to ToLCD buildup are: (1) availability of virus inoculum and the vector around the fields;

(2) the movement of viruliferous vectors into the freshly sown field; (3) the susceptibility of the variety that builds up vector population and allows establishment of the virus; (4) weather parameters favoring vector population buildup; and (5) vector biotype which can effectively transmit the disease. Interaction of these factors leads to epidemic outbreak. The most important factors responsible for the epidemic are source of inoculum and vector. In India, wherever tomato is grown continuously, there is increase in leaf curl disease incidence. Virus inoculum in the weeds and continuous cropping contribute to rapid disease buildup. Weather parameters, both at macro- and micro-level, affecting the developmental stages of the plant and the life cycle of the vector are important. Tomato is grown in different agroclimatic zones in India, which makes prediction of the outbreak of epidemics more difficult. However, there is definitely a correlation between vector population and disease incidence.

Maximum temperature and rainfall play an important role for spread of the disease in southern India, while minimum temperature and minimum relative humidity influence the whitefly population in the north. ToLCD incidence depends on weather conditions (humidity, rainfall, temperature). It was observed that the disease progresses from February to June, when the dry and hot season with low humidity prevail. The incidence may even reach up to 100% during these months. These types of weather conditions favor whitefly multiplication and disease spread. The whitefly population and ToLCV incidence remain comparatively less during winter and rainy seasons. The tropical climate in southern India allows year-round tomato cropping, which, together with the presence of perennial host plants for both ToLCVs and *B. tabaci*, enables an easy carry-over of ToLCD between growing seasons. Whitefly biotype B can effectively transmit the virus. Overall, factors like long persistence of the virus in the vector, efficient transmission by the biotype, cultivation of tomato throughout the year, and abundance of weed hosts are the contributing factors for the high incidence of the disease under natural conditions.

Host Range

ToLCV strains/species are easily transmitted by grafting and through vector to a wide range of weeds and cultivated crops. Molecular detection using ToLCV-specific primers has led to identification of a range of plant species that can harbor ToLCVs in India. ToLCVs can infect crops such as *Lycopersicon esculentum*, *L. peruvianum*, *L. hirsutum*, *L glandulosum*, *L. pimpinellifolium*, *Capsicum annuum*, *Nicotiana tabacum*, *Vigna unguiculata*, and *Luffa cylindrica*. The viruses perpetuate on many weed hosts, viz. *Acanthospermum hispidum*, *Ageratum conyzoides*, *Blainvella rhomboids*, *Euphorbia hirta*, *Fraveria hirta*, *Parthenium hysterophorus*, *Malvastrum coromandalinum*, and *Croton bonplandianum*.

Genome Organization

Full-length genome of isolates of ToLCVs have been cloned and sequenced. Three types of genomic DNA (DNA-A, DNA-B, and DNA-β) have been found to be associated with begomoviruses causing ToLCD in India.

Apparently, the isolates from southern India have a monopartite genome (DNA-A) associated with DNA-β while both monopartite (DNA-A with DNA-β) and bipartite (DNA-A and DNA-B) begomoviruses have been found to infect tomatoes in northern India. The genome organization of DNA-A (ranges between 2739 and 2759 bp) resembles other begomoviruses having two open reading frames (ORFs) on the viral strand (AV1 and AV2) and four on the complementary strand (AC1, AC2, AC3, and AC4). Genome organization of DNA-B (ranges between 2656 and 2686 bp) and DNA-β (genome size between 1344 and 1376 bp) also resembles other begomoviruses having two ORFs (one on the viral strand, BV1, and the other on the complementary strand, BC1) and one on the complementary strand (βC1), respectively.

Diversity of Tomato-Infecting Begomoviruses

ToLCD appears to be caused by a complex of several viruses based on symptom variations on different indicator hosts. During the 1980s, based on symptoms produced in a particular tomato cultivar, ToLCVs were divided into five groups: (1) severe leaf curl with thickening of veins, (2) severe symptom with enation, (3) screw pattern of leaf arrangement, (4) vein purpling and leaf curl, and (5) exclusively downward curling of leaves. Variability was subsequently also found in the epitope profiles of ToLCVs collected from Karnataka, with groupings suggesting that the tomato crop and some neighboring weed species were hosts to the same ToLCV strains/species. Species status for begomoviruses, however, cannot be conferred based on symptom type or epitope profile.

In accordance with the ICTV *Geminiviridae* study group guidelines, nucleotide sequence identity of DNA-A, and genome organization (see **Table 1**), the following five Indian ToLCV species are to be demarcated: *Tomato leaf curl Bangalore virus*, *Tomato leaf curl Gujarat virus*, *Tomato leaf curl Karnataka virus*, *Tomato leaf curl New Delhi virus*, and *Tomato leaf curl Pune virus*. Except for *Tomato leaf curl Pune virus*, four species have been characterized in detail based on their biological and molecular properties (**Figure 2**).

Table 1 GenBank accession numbers of selected begomoviruses DNA-A, DNA-B and DNA-β sequences used for analysis

		Accession numbers		
Species	*Virus name*	*DNA-A*	*DNA-B*	*Abbreviation*
DNA-A and DNA-B				
Tomato leaf curl Bangalore virus	Tomato leaf curl Bangalore virus-[Ban1]	Z48182		ToLCBV-[Ban1]
	Tomato leaf curl Bangalore virus-[Kerala]	DQ887537		ToLCBV-[Ker]
	Tomato leaf curl Bangalore virus-[Kolar]	AF428255		ToLCBV-[Kol]
	Tomato leaf curl Bangalore virus-[Ban5]	AF295401		ToLCBV-[Ban5]
	Tomato leaf curl Bangalore virus-[Ban4]	AF165098		ToLCBV-[Ban4]
	Tomato leaf curl Bangalore virus-[Ban AVT1]	AY428770		ToLCBV-[Ban AVT1]
Tomato leaf curl Bangladesh virus	Tomato leaf curl Bangladesh virus	AF188481		ToLCBDV
Tomato leaf curl Gujarat virus	Tomato leaf curl Gujarat virus-[Mirzapur]	AF449999		ToLCGV-[Mir]
	Tomato leaf curl Gujarat virus-[Vadodara]	AF413671		ToLCGV-[Vad]
	Tomato leaf curl Gujarat virus-[Varanasi]	AY190290	AY190291	ToLCGV-[Var]
	Tomato leaf curl Gujarat virus-[Nepal]	AY234383		ToLCGV-[Nepal]
Tomato leaf curl Joydebpur virus	Tomato leaf curl Joydebpur virus	DQ673859		ToLCJoV
Tomato leaf curl Karnataka virus	Tomato leaf curl Karnataka virus-[Bangalore]	U38239		ToLCKV-[Ban]
	Tomato leaf curl Karnataka virus-[Janti]	AY754812		ToLCKV-[Janti]
Tomato leaf curl New Delhi virus	Tomato leaf curl New Delhi virus-[Lucknow]	Y16421	X89653	ToLCNDV-[Luc]
	Tomato leaf curl New Delhi virus-[Mild]	U15016		ToLCNDV-[Mild]
	Tomato leaf curl New Delhi virus-[Severe]	U15015	U15017	ToLCNDV-[Svr]
	Tomato leaf curl New Delhi virus-[Pakistan]	AF448058	AY150305	ToLCNDV-[PK]
	Tomato leaf curl New Delhi virus-[Pakistan-Islamabad]	AF448059	AY150304	ToLCNDV-[PK-IS]
Tomato leaf curl Malaysia virus	Tomato leaf curl Malaysia virus-[India]	DQ629102		ToLCMYV-[IN]
Tomato leaf curl Pune virus	Tomato leaf curl Pune virus	AY754814		ToLCPV
Tomato leaf curl Sri Lanka virus	Tomato leaf curl Sri Lanka virus	AF274349		ToLCSLV
DNA-β				
Tomato leaf curl beta-[Aurangabad]		EF095958		ToLCB-[Aur]
Tomato leaf curl beta-[Bangalore]		AY428768		ToLCB-[Ban]
Tomato leaf curl beta-[Chinthapalli]		AY43855		ToLCB-[Chi]
Tomato leaf curl beta-[Coimbatore]		AY438560		ToLCB-[Coi]
Tomato leaf curl beta-[Jabalpur]		AY230138		ToLCB-[Jab]
Tomato leaf curl beta-[New Delhi]		AJ542490		ToLCNDB
Tomato leaf curl beta-[Pune]		AY838894		ToLCB-[Pune]
Tomato leaf curl beta-[Rajasthan]		AY438558		ToLCB-[Raj]
Tomato leaf curl beta-[Varanasi]		AY438559		ToLCB-[Var]

Tomato Leaf Curl New Delhi Virus

During the mid-1990s, two isolates of this virus were reported from New Delhi and Lucknow. In addition to the severe isolate, a mild isolate was also described from New Delhi. It has a bipartite genome with DNA-A and DNA-B. However, occurrence of DNA-β has also been observed. Infectivity of the cloned DNAs has been demonstrated.

Tomato Leaf Curl Gujarat Virus

Three isolates from Varanasi, Vadodara, and Mirzapur belong to this species. Among them, Varanasi strain has been characterized in detail. DNA-A alone is infectious but DNA-B increases symptom severity. Association of DNA-β has also been observed with this mono-bipartite species under natural conditions. Tomato leaf curl Gujarat virus (ToLCGV) is also sap transmissible to tomato, pepper, *N. benthamiana*, and *N. tabacum*. Unexpectedly, ToLCGV-Var DNA-A (AY190290) and DNA-B (AY190291) share a common region (CR) of 155 bp that is only 60% identical which were cognate pair of components that cause severe disease of tomato under field conditions.

Tomato Leaf Curl Bangalore Virus

This virus was reported for the first time from Bangalore, southern India. Several isolates referred to as tomato leaf curl virus Ban1, Ban3, Ban4, Ban5, and Kolar belong

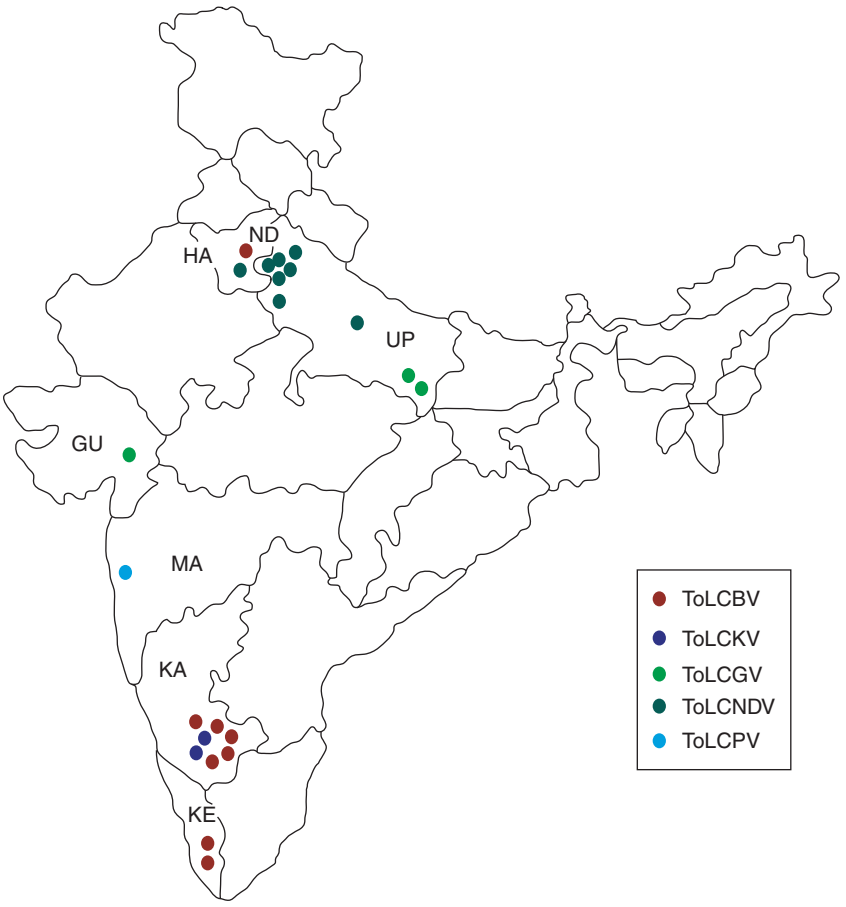


Figure 2 Distribution of five species of tomato-infecting begomoviruses in India. Presence of ToLCVs was located based on availability of full-length DNA-A sequences in GenBank.

to this species. It contains a monopartite DNA-A genome, and a satellite DNA-β molecule was observed to be present with tomato leaf curl Bangalore virus (ToLCBV) infection. Pathogenicity of cloned DNAs has not been demonstrated.

Tomato Leaf Curl Karnataka Virus

This sap-transmissible virus has been isolated from Bangalore. DNA-A alone is infectious and produces typical leaf curl symptoms on tomato. Association of DNA-β has also been observed.

Tomato Leaf Curl Pune Virus

This is the most recently reported new virus from India, whose only DNA sequences are available in GenBank. Tomato leaf curl Pune virus (ToLCPV) contains a DNA-β molecule, which is quite distinct from all other known ToLCVs.

In addition, viruses such as tomato leaf curl Bangladesh virus, tomato leaf curl Malayasia virus, and tomato leaf curl Joydebpur virus are also known to infect tomatoes in India.

Molecular Relationships among ToLCVs

Relationships among the type member of the five species (viz. ToLCBV, ToLCGV, tomato leaf curl Karnataka virus (ToLCKV), tomato leaf curl New Delhi virus (ToLCNDV), and ToLCPV) revealed that there is a great degree of diversity among the Indian tomato-infecting begomoviruses. To examine the diversity of the sequences, phylogenetic trees were generated of the 18 full-length DNA A sequences together with representative sequences present in GenBank (**Figure 3**). The trees constructed using either neighbor joining or most parsimonious methods for full-length sequences were all similar. Members of four different species are closely related and form a well-knitted cluster with the exception of ToLCMYV-[IN]

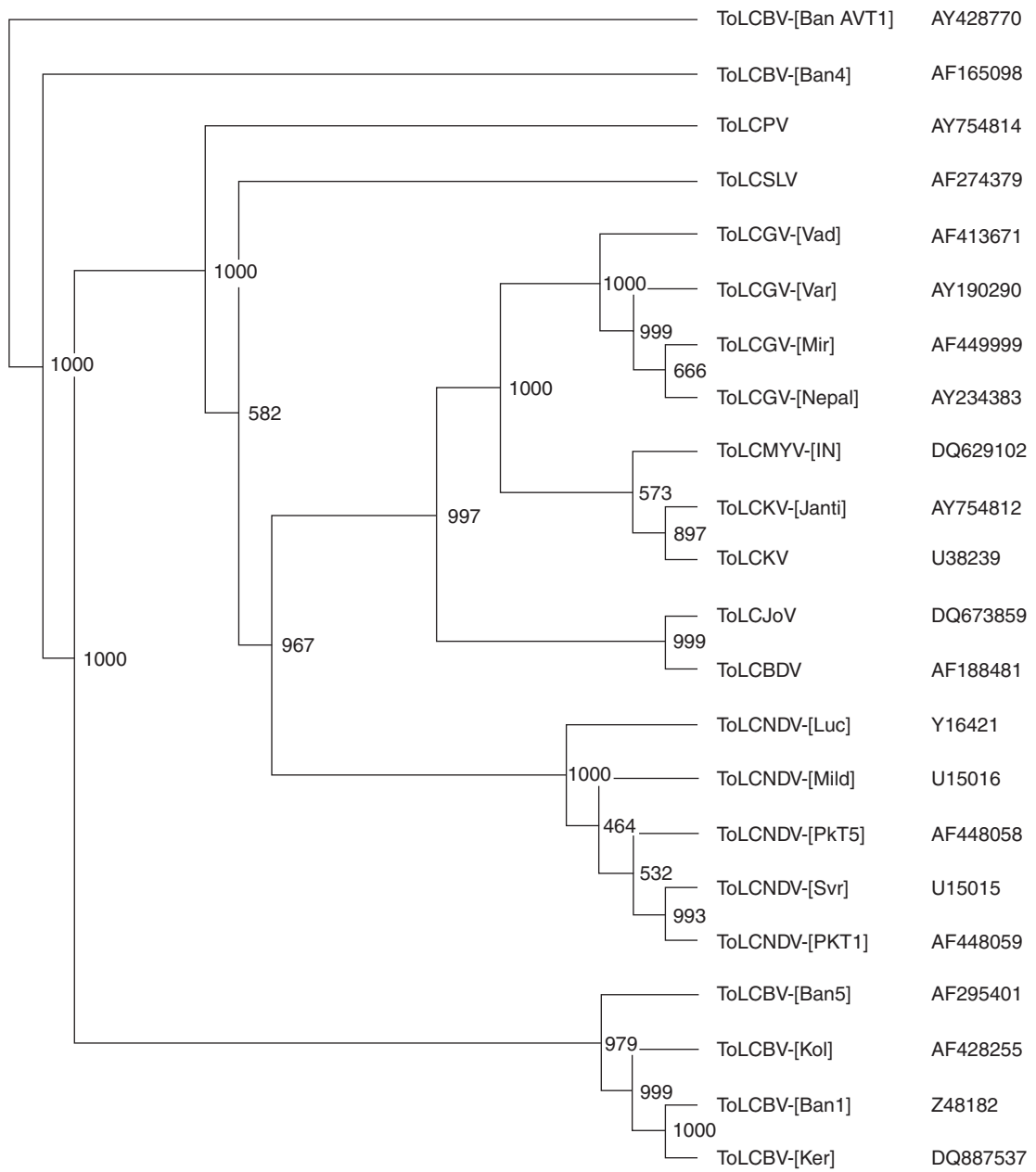


Figure 3 Phylogenetic analyses of begomoviruses causing ToLCD in India with other GenBank-published begomovirus sequences. Numbers at nodes indicate the bootstrap value out of 1000 replicates. The tree was generated with the PHYLIP programs using full-length DNA-A sequence data of Indian ToLCVs and other selected ToLCVs of the SE Asia. The full names of the viruses can be found in **Table 1**.

which was grouped with ToLCKV-Janti and ToLCKV. Full-length DNA-A sequences of Indian ToLCVs formed five different clusters, which generally had 72–82% nucleotide identity between clusters. However, one cluster formed by ToLCKV, belonging to a species known to have arisen through recombination, shared up to 88.3% with ToLCBVs.

Four out of the five sequences typed as ToLCBV shared 94.2–99.5% nucleotide identity with each other. ToLCPV also fell into the ToLCBV group, but had only 88% nucleotide identity to previously published ToLCBVs. ToLCBDVs originated from Bangladesh are distinct and form a different cluster while tomato leaf curl Sri Lanka virus (ToLCSLV) is close to ToLCPV and ToLCBV.

Comparison of DNA-A Sequence

Representatives of five species share varied degree of identity among each other (66–88%). Minimum nucleotide identity (66%) was observed with ToLCGV and

ToLCNDV while maximum identity of 88% was observed between ToLCBV and ToLCPV. Among the five species, ToLCKVs share more than 75% homology with all the species (**Table 2**(**a**)).

Comparison of DNA-B Sequence

GenBank database revealed the presence of four full-length DNA-B sequences associated with ToLCD in India. DNA-B of ToLCVs is highly conserved among each other in comparison to DNA-A. All the DNA-Bs are closely related (80–97%) (**Table 2**(**b**)). ToLCNDV, ToLCNDV-[Luc], and ToLCGV-[Var] have the same pairwise comparison profile all along their genomes.

Comparison of DNA-β Sequence

DNA-β molecules associated with ToLCVs share a less degree of homology as compared to DNA-A and DNA-B (**Table 2**(**c**)), indicating their uniqueness. So far nine full-length sequences have been reported with ToLCD in India and a wide range of 63–96% identity was observed among them. ToLCB-Varanasi share 96% with ToLCB-Aurangabad, followed by ToLCB-Jabalpur having 94% identity with ToLCB-Chinthapalli and by ToLCB-Coimbatore having 93% with ToLCBB. Two DNA-β sequences available from ToLCD-infected samples from Pune share 96% identity with each other.

Comparison of Common Regions

The replication of the B-component by the Rep protein of the A-component is possible because of the existence of the so-called CR, a short stretch of ±200 nt that usually is highly conserved between the two molecules. The CRs of the DNA-A (CRAs) of A-components of several isolates of a particular species are very closely related, with 89–99% identity. The CRAs of different species share 52–77% identity with each other. Sequence identities between the CRs of ToLCGV-[Var] DNA-B and genomic components of ToLCNDV are very high: it is 83% identical to ToLCNDV-[Svr] DNA-A CR and 86% identical to ToLCNDV DNA-B CR, indicating that these sequences could pertain to isolates of the same species. However, the CRs of DNA-A and DNA-B of ToLCGV-[Var] are only 61% identical. The lowest sequence identity (52%) was

Table 2 Percent identity (nucleotide) among begomoviruses causing TLCD in India

(a) DNA-A

	ToLCBV	*ToLCGV-Var*	*ToLCKV*	*ToLCNDV-Svr*	*ToLCPV*
ToLCBV	–	72	82	72	88
ToLCGV-Var		–	84	66	79
ToLCKV			–	76	83
ToLCNDV-Svr				–	72
ToLCPV					–

(b) DNA-B

	ToLCGV-Var	*ToLCNDV-Luc*	*ToLCNDV 1*	*ToLCNDV 2*
ToLCGV-Var	–	84	85	85
ToLCNDV-Luc		–	80	80
ToLCNDV 1			–	97
ToLCNDV 2				–

(c) DNA-β

	ToLCB-Var	*ToLCB-Aur*	*ToLCB-Chi*	*ToLCB-Jab*	*ToLCB-Ban*	*ToLCB-Coi*	*ToLCB-Pun*	*ToLCB-Raj*	*ToLCB-Nd*
ToLCB-Var	–	96	64	65	64	63	63	65	64
ToLCB-Aur		–	64	64	64	63	63	64	65
ToLCB-Chi			–	94	77	77	79	71	70
ToLCB-Jab				–	77	77	78	69	69
ToLCB-Ban					–	93	78	68	68
ToLCB-Coi						–	78	67	68
ToLCB-Pun							–	69	69
ToLCB-Raj								–	80
ToLCB-Nd									–

observed between the CR of ToLCGV-[Var] DNA-A and the CR of ToLCNDV DNA-B. A multiple alignment of the CRs and inter-cistronic regions of the five species causing ToLCD in India revealed that all the isolates of ToLCNDV, ToLCGV, and ToLCKV have similar or very related iteron sequences (GGTGT-XX/X-GGAGT) while ToLCBV and ToLCPV have similar iteron sequences (GGTGG-XX/X-GGTGG) (**Table 3**). The exception is the ToLCNDV-Mld and ToLCNDV-Luffa isolates whose iteron sequence is GGCGT-CT-GGCGT. The major difference between the CRs of the DNA-A among these viruses is found in the spacer sequence between the two iterons that varies between 2 and 3 nt. It was also observed that a third identical iteron is present at the 5′ end of the CRs. The CRs of DNA-A of ToLCGV-[Var], compared with DNA-B of ToLCGV-[Var], DNA-B of ToLCNDV, and DNA-A of ToLCNDV-Svr, share more than 85% identity in the first 80 nt of their CRs, whereas the region between the TATA box and the hairpin loop is below 50%.

Recombination

Tomato-infecting begomoviruses from India appear to have a great capacity to recombine. The amount of material that viruses may exchange ranges from small fragments of a few nucleotides to very large fragments of 2000 nt or more. When DNA-A was compared, no recombination was observed among ToLCGV-[Var] with other two isolates of the same species, ToLCGV-[Vad] or ToLCGV-[Mir]. Remarkably, a short possible recombination (150 nt) was observed between ToLCGV-[Var] DNA-A and ToLCNDV at the 3′ end of AC1. With ToLCKV, ToLCGV share high nucleotide sequence identity for their IR (approximately the first 100 nt), the 5′ end of AV1 (200 nt), and a long stretch of 1350 nt from the 5′ end of AC3 to the 5′ end of the IR (**Figure 4**). This indicates that, at these sites, recombination events possibly took place between these two tomato-infecting viruses or with a third unknown virus. Putative recombination sites among isolates of ToLCBV have been identified as AV1, AV2, AC1, and IR of the viral that may account of variability in strains/species.

Table 3 Rep binding sequences of ToLCVs from India

Virus species	*DNA-A*	*DNA-B*
ToLCBV	GGTGG-AAT-GGTGG	
ToLCGV	GGTGT-ATT-GAGT	GGTGT-CT-GGTGT
ToLCKV	GGTGT-ACT-GGAGT	
ToLCNDV-Svr	GGTGT-CT-GGAGT	GGTGT-CT-GGTGT
ToLCNDV-Mild	GGCGT-CT-GGCGT	
ToLCPV	GGTGG-AAC-GGTGG	

Pseudo-Recombination and Synergism

Under natural conditions, mixed virus infections in a single plant possess biological and epidemiological implications. For the first time, synergism between two distinct species of begomoviruses infecting tomatoes in India was observed that results in an increase in viral DNA and symptom severity. Also, the occurrence of a more virulent pseudo-recombination between two distinct species may explain the sudden breakdown of resistant tomato cultivars and the development of epidemics in tomato-growing areas in India. Recently, the association of both ToLCGV-[Var] and ToLCNDV-Svr components in a single severely infected tomato plant under natural conditions has been detected. Also, based on coat protein (CP) gene sequences, associations of ToLCGV and ToLCBV, ToLCBV and ToLCKV, and ToLCBV and ToLCNDV have been observed in a single plant. The synergistic role of ToLCGV DNA-A and ToLCNDV-Svr DNA-A, resulting in a much higher level of ToLCNDV-Svr DNA-A in turn, helped to a more efficient replication of the B-components, and particularly the ToLCGV

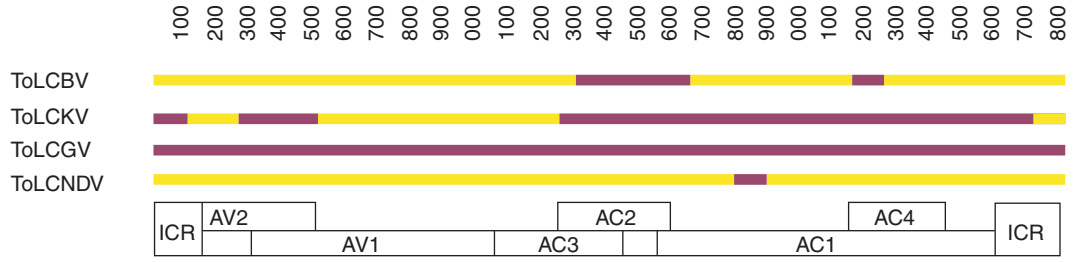


Figure 4 Diagrammatic representation of recombinant fragments between tomato leaf curl Gujarat virus-[Varanasi] and another three biologically characterized ToLCVs originating from India. Each line represents a linearized begomovirus genome (in the sense orientation starting from the origin of replication, in the CR) and each presence of red segments indicates homologous stretches of sequences to ToLCGV-[Var] at the strain level (>89%) within the genome of other Asian geminiviruses. Positions of the regions of the genome are represented at the top of the figure and a representation of a linearized geminivirus genome is represented at the bottom.

DNA-B component, resulting onto greater DNA accumulation in the systemically infected leaves, and consequently more severe symptoms. Since ToLCNDV-Svr and ToLCGV-[Var] belong to the same genus, infect the same hosts, and are transmitted by the same whitefly vector, they are therefore more likely to co-infect the same plants. The synergism between the two viruses will increase the amount of both the viruses in the systemically infected leaves and increase chances of transmission, if need be. As a consequence, doubly infected plants have a considerable potential as sources of inoculum for both viruses, and whiteflies feeding on such plants would, therefore, more easily acquire and transmit both viruses to virus-free plants. This information provides another source of geminivirus biodiversity.

Exchange of genomic components of the members of two distinct species of begomoviruses causing ToLCD in India can form infectious pseudo-recombinants. Transcomplementation between ToLCNDV-Svr DNA-A and ToLCGV DNA-B resulted in a more severe symptom phenotype as compared to wild type. ToLCGV DNA-B can also transcomplement ToLCNDV-Mld (mild isolate) and ToLCKV. The viable nature of these pseudo-recombinants was attributed to the highly conserved nature of AC1 and CR and also to identical iteron sequences. A highly specific interaction between the *Rep* protein of ToLCNDV (mild and severe strains) with their cognate iteron sequences also demonstrated the intimate relationship between these elements and the consequence in terms of DNA accumulation and symptoms. This led to the concept that matching N-Rep and iterons is required for efficient replication and consequently a severe symptomatology, and it also was supportive of the species concept where most of the time, with the exceptions of the recombinant in these regions, these two elements are different between species. Although the perfect match between the N-Rep sequence and the iteron sequences is probably vital in most of the cases, it may not be fatal in that particular one. This suggests that there must be other cooperative factors that are shared between the Rep of ToLCNDV-Mild and ToLCGV-[Var] B component CR, to the point that they compensate a nonmatching iteron interaction.

Replication and Pathogenesis

A highly specific interaction between the *Rep* protein of ToLCNDV (mild and severe strains) and their cognate iteron sequences also demonstrated the intimate relationship between these elements and the consequence in terms of DNA accumulation and symptoms. Two strains of ToLCNDV, viz. severe and mild, share 94% sequence identity on the basis of symptoms on tomato and tobacco. The studies demonstrated that the amino acid at position 10 in Rep protein coupled with a change in the binding site sequence may determine the replication of viral DNA. Change of Asp10 to Asn in Rep protein of the mild strain accompanied by exchange of the 13-mer binding site (making it identical to the severe strain) altered its replication, leading to increased accumulation of viral DNA. In addition, the modified mild strain could replicate heterologous strain DNA-B, indicating that the interaction of Rep protein with its binding site may be essential for replication of viral DNA.

Mutational analysis of ToLCNDV-Svr virion-sense ORFs has been carried out to assign the function. Plants inoculated with infectious DNA which contained deletions in AV2 developed very mild symptoms and accumulated only low levels of both single-stranded (ss-) and double-stranded (ds-) viral DNA, whereas inoculated protoplasts accumulated both ss- and dsDNA to wild-type levels, showing that AV2 is required for efficient viral movement. Mutations in the CP caused a marked decrease in ssDNA accumulation in plants and protoplasts while increasing dsDNA accumulation in protoplasts. The results demonstrated that multiple functions provided by AV2, BV1, and BC1 are essential for viral movement, and that changes in A-component virion-sense mRNA structure or translation affect viral replication.

The role of the movement protein (MP) and nuclear shuttle protein (NSP) in the pathogenicity of ToLCNDV, a bipartite begomovirus, has been elucidated using either potato virus X (PVX) expression vector or by stable transformation of gene constructs under the control of the 35S promoter in *N. tabacum*. No phenotypic changes were observed in any of the three species when the MP was expressed from the PVX vector or constitutively expressed in transgenic plants. Expression of the ToLCNDV NSP from the PVX vector in *N. benthamiana* resulted in leaf curling that is typical of the disease symptoms caused by ToLCNDV in this species. However, expression of NSP from PVX in *N. tabacum* and *L. esculentum* resulted in a hypersensitive response (HR), suggesting that the ToLCVDV NSP is a target of host defense responses in these hosts. The NSP, when expressed as a transgene under the control of the 35S promoter, resulted in necrotic lesions in expanded leaves that initiated from a point and then spread across the leaf. The necrotic response was systemic in all the transgenic plants. N-terminus of NSP is required for the HR. These findings demonstrate that the ToLCNDV NSP is a pathogenicity determinant as well as a target of host defense responses. The necrosis in transgenic tobacco plants is systemic, as it starts from a point on the fully emerged leaf and spreads over the lamina and to other leaves. Thus, ToLCNDV NSP is an avirulence determinant that interacts with the product of a resistance gene encoded by a host defense system, possibly an R gene product, triggering a host defense response involving an HR in *N. tabacum* and tomato.

Management

Until the late 1990s, the main control method employed against ToLCD was intensive use of insecticides targeted at viruliferous immigrant adult *B. tabaci* that spread ToLCVs into and within tomato crops. Identification of sources of resistance is difficult as the leaf curl disease syndrome is caused by different tomato begomoviruses. In the recent past, however, three high-yielding ToLCD-resistant tomato varieties have been developed that can be grown successfully with minimal insecticide use. Artificial screening through whiteflies has been carried out to identify resistant/tolerant varieties from different *Lycopersicon* spp., viz., *L. peruvianum*, *L. peruvianum* f. *glandulosum*, *L. peruvianum* f. *regulare*, *L. esculentum*, *L. chilense*, *L. hirsutum* f. *glabratum*, and *L. pimpinellifolium*. Gene governing resistance has also been mapped in tomato cultivar, H-24 (a derivative from *L. hirsutum* f. *glabratum*) on chromosome 11 against TYLCV and ToLCBV. In order to manage the deployment of this valuable resource, improve the efficacy with which further ToLCV-resistant material is screened, and investigate resistant-genotype/virus interactions, an improved understanding of the diversity and distribution of ToLCVs present will help in developing strategies for ecofriendly management of ToLCD. Agrobacterium-mediated inoculation of cloned DNAs will certainly provide better tool for identification of R genes in tomatoes in the future. Pathogen-derived resistance needs more attention in order to develop broad-spectrum resistance against Indian ToLCVs.

See also: Beta ssDNA Satellites; Potato Viruses; Satellite Nucleic Acids and Viruses; Tomato Yellow Leaf Curl Virus.

Further Reading

Chakraborty S, Pandey PK, Banerjee MK, Kalloo G, and Fauquet CM (2003) *Tomato leaf curl Gujarat virus*, a new begomovirus species causing a severe leaf curl disease of tomato in Varanasi, India. *Phytopathology* 93: 1485–1495.

Chatchawankanphanich O and Maxwell DP (2002) Tomato leaf curl Karnataka virus from Bangalore, India, appears to be recombinant begomovirus. *Phytopathology* 92: 637–645.

Chatterji A, Chatterji U, Beachy RN, and Fauquet CM (2000) Sequence parameters that determine specificity of binding of the replication-associated protein to its cognate in two strains of *Tomato leaf curl virus-New Delhi*. *Virology* 273: 341–350.

Chowda Reddy RV, Colvin J, Muniyappa V, and Seal S (2003) Diversity and distribution of begomoviruses infecting tomatoes in India. *Archives of Virology* 150: 845–867.

Green SK and Kalloo G (1994) Leaf curl and yellowing viruses of pepper and tomato: An overview. *Technical Bulletin No. 21.* Tainan, Republic of China: Asian Vegetable Research and Development Center.

Muniyappa V, Venkatesh HM, Ramappa HK, *et al.* (2000) Tomato leaf curl virus from Bangalore (ToLCV-Ban4): Sequence comparison with Indian ToLCV isolates, detection in plants and insects, and vector relationships. *Archives of Virology* 145: 1583–1598.

Padidam M, Beachy RN, and Fauquet CM (1995) Tomato leaf curl from India has a bipartite genome and coat protein is not essential for infectivity. *Journal of General Virology* 76: 25–35.

Vasudeva RS and Sam Raj J (1948) A leaf curl disease of tomato. *Phytopathology* 38: 364–369.

Tomato Spotted Wilt Virus

H R Pappu, Washington State University, Pullman, WA, USA

© 2008 Elsevier Ltd. All rights reserved.

Taxonomy

The species *Tomato spotted wilt virus* (TSWV) belongs to the genus *Tospovirus* in the family *Bunyaviridae*. Of the more than 300 species of primarily arthropod-borne viruses described in the family *Bunyaviridae*, a small proportion infects plants. Members of other genera in this family are important pathogens of humans and animals. TSWV is considered as the type member of the genus and hence its name formed the basis for coining the genus name. Discovery of a second tospovirus, *Impatiens necrotic spot virus* (INSV), was followed by description of more tospoviruses from several parts of the world. To date, there are more than 14 distinct tospoviruses described. Descriptors for classification of new viruses as tospoviruses include genome organization (**Figure 1**), thrips transmission, host range, and serological and molecular relationships of the nucleoprotein (N) gene. Excellent reviews on various aspects of tospoviruses have been published in the last few years. This review focuses on summarizing recent advances in our understanding of the tospovirus biology, molecular biology, epidemiology, and control.

Biology

TSWV was first described in 1915. The virus has a wide host range; it infects more than 900 plant species that include numerous crops and weeds. The virus is

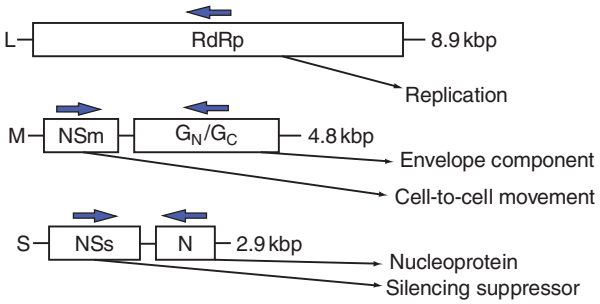


Figure 1 Genome organization of TSWV. Functions of each gene are noted on the right side.



Figure 2 Biological variability of TSWV isolates. Response of *Nicotiana tabacum* to a severe strain (top) and a mild strain (bottom).

mechanically transmissible and is not seed transmitted. In nature, TSWV is transmitted by several species of thrips. Crops that are affected by TSWV include bean, lettuce, peanut, pepper, potato, tobacco, and tomato. Biologically distinct isolates of TSWV exist in nature. Isolates that differ in thrips transmissibility, symptomatology, and symptom severity have been described (**Figure 2**). Variability in the N gene sequence of TSWV isolates suggested geographic delineation in natural virus populations which may be useful for attribution: that is, tracing the potential source or origin of a particular isolate. TSWV causes systemic infection in most of the crops it infects. Infection at early stages of the plant growth causes the most damage that may include severe stunting of the entire plant which often results in death. TSWV epidemics in peanut, pepper, tobacco, and tomato in southeastern United States caused major economic losses and forced shifts in production practices. Losses due to TSWV outbreaks in peanut were estimated at more than US $100 million in Georgia alone in the USA.

Molecular Biology

The morphology and genome structure and organization of tospoviruses share several features with members of other genera in the family *Bunyaviridae*. Particles are pleiomorphic, and 80–120 nm in diameter. The genome of tospoviruses includes three RNAs referred to as large (L), medium (M), and small (S). 5′ and 3′ terminal sequences of the RNAs are conserved. L RNA is in negative sense while M and S RNAs are ambisense in their genome organization. L RNA codes for the RNA-dependent RNA polymerase (RdRp). M RNA encodes precursors for two structural glycoproteins, G_N and G_C, and a nonstructural protein, NSm. The S RNA codes for the nucleocapsid protein (N) and another nonstructural protein, NSs. The three genomic RNAs are tightly linked with the N protein forming ribonucleoproteins (RNPs). These RNPs are encased within a lipid envelope consisting of two virus-encoded glycoproteins, and a host-derived membrane. The genome organization, gene products, and their roles are shown in **Figure 1**. Due to the negative strandedness of the genome, virions contain several molecules of the RdRp to initiate initial rounds of replication of the virion RNAs. Genome expression is facilitated through synthesis of subgenomic RNAs.

Considerable progress has been made in our understanding of the functional roles of various TSWV genes. The 331 kDa RdRp coded by the L RNA serves as a multifunctional, replication-associated protein and is believed to function cooperatively with host-encoded factors. Screening a cDNA library of *Frankliniella occidentalis* using fragments of TSWV RdRp, a putative transcription factor that binds to TSWV RdRp was isolated which was shown to bind to TSWV RNA and enhance TSWV replication. Mammalian cells expressing this putative transcription factor supported TSWV replication. Since TSWV is transmitted by several thrips species it remains to be seen if similar transcription factors exist in other thrips vectors.

Assignment of functions to various TSWV gene products was done using indirect approaches since a reverse genetics system based on an infectious cDNA clone is not yet possible for a negative-sense RNA virus such as TSWV. Using indirect approaches, the functions of the M RNA-encoded glycoproteins and NSm were recently deciphered. The absence of a gene similar to NSm in other genera of the family *Bunyaviridae* suggests that NSm serves a function that facilitates TSWV infection of plants. Virus movement in plants is mediated by a

virus-encoded movement protein and a similar function was suggested for NSm and subsequent experimental evidence supported this hypothesis. Recently, the role of NSm in TSWV life cycle was investigated *in vitro* and *in planta*. Virus-encoded movement proteins tend to bind to viral RNAs and facilitate virus movement through plasmodesmata. *In vitro*-expressed NSm interacted with N protein and bound ssRNA in a sequence-nonspecific manner. Members of DnaJ family were found to bind NSm in a yeast-two-hybrid system. Transgenic *Nicotiana tabacum* plants expressing NSm produced symptoms suggestive of TSWV infection. Biochemical analyses of this plant response showed the accumulation of callose, an indicator of the triggering of plant defense response. Constitutive expression of the NSm and its subsequent interference with the plasmodesmata's transport functions could have resulted in abnormal growth pattern seen in these transgenic plants. More direct evidence of the role of NSm in virus movement was obtained by heterologous complementation studies using an infectious cDNA clone of tobacco mosaic virus (TMV) and replacing TMV genes with the NSm gene of TSWV. The NSm gene complemented a movement-deficient mutant of TMV in tobacco plants and facilitated the long-distance movement of the TMV–TSWV NSm hybrid. The NSm protein, when expressed *in planta*, mediated tubule formation in infected protoplasts.

TSWV mutants lacking the envelope were not thrips transmissible, indicating that the determinants for thrips transmission are localized on the glycoprotein-containing envelope. Using two distinct isolates of TSWV that differ in thrips transmission, genetic reassortants (=pseudo-recombinants) were generated by coinoculating plants with both isolates. The resulting reassortants were evaluated for their ability to be thrips transmitted and the source of the genomic components of each of these reassortants was determined. Only those reassortants that had the M RNA derived from the thrips-transmissible isolate retained thrips transmissibility confirming the functional contribution of M RNA to thrips transmission (**Figure 3**). Sequence comparisons of the glycoprotein genes of these two isolates showed that a point mutation played a critical role in determining the thrips transmissibility. The roles of G_N and G_C in TSWV–thrips vector interaction are beginning to be elucidated and are discussed under 'Transmission and epidemiology'. Moreover, the M RNA was shown to carry determinants for host adaptation and overcoming host plant resistance as well as pathogen-derived resistance in tomato and tobacco. In the absence of a reverse genetics system, complementation studies

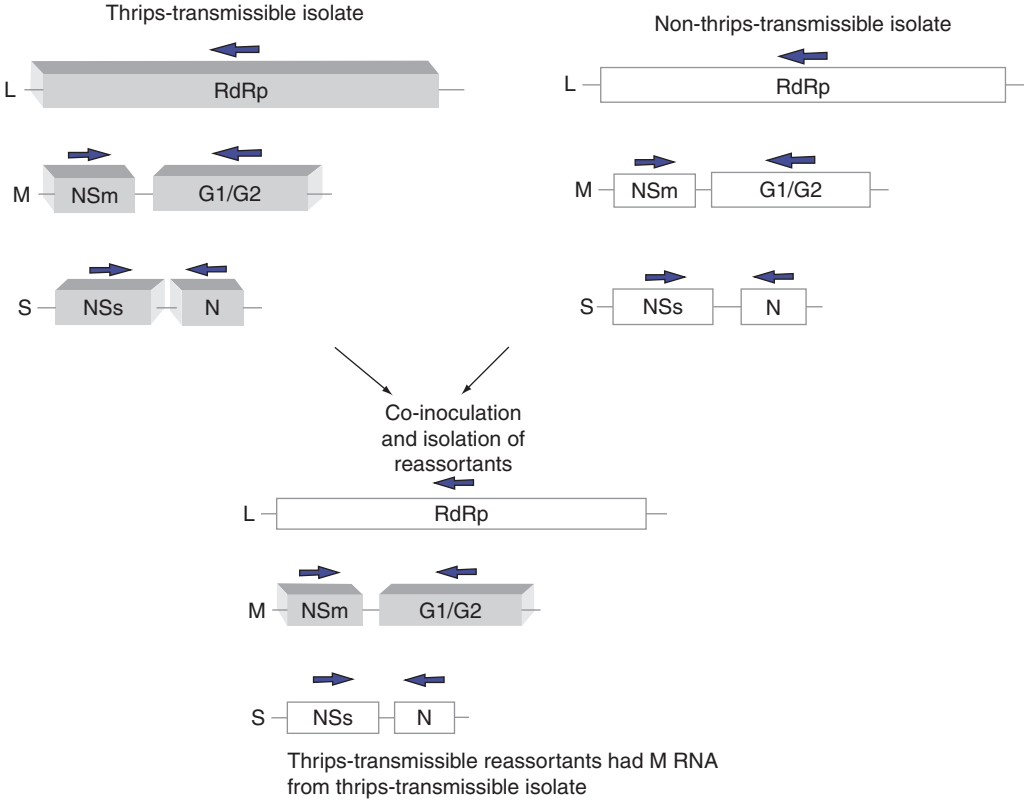


Figure 3 Genetic reassortment as a tool for the functional analysis of tospovirus genes. Localization of thrips transmission determinants on the M RNA of TSWV.

and genetic reassortment tudies such as those mentioned above offer alternate approaches for studying structure–function relationships of various TSWV genes.

The S RNA-encoded proteins, N and NSs, play important roles in the TSWV infection cycle. The N protein, as part of the RNP, serves as the structural protein and may also have some regulatory role in modulating the transcription and replication. Additionally, the involvement of the N protein in particle assembly was suggested based on its interaction with one of the two viral glycoproteins. When co-expressed with G_N and G_C in mammalian BHK21 cells, N protein displayed selective interaction with G_C and the subsequent localization of the N and glycoprotein complex in the Golgi apparatus. The nonstructural protein, NSs, encoded by the S RNA was shown to be a suppressor of RNA silencing.

Diagnosis

TSWV produces a wide range of foliar symptoms. Symptomatology varies depending on the strain, host species, and genotype, and is also influenced by environmental factors such as temperature. In most cases, accurate diagnosis is facilitated by serological or molecular techniques. Purified TSWV is a good immunogen and virus-specific antisera and enzyme-linked immunosorbent assay (ELISA) kits are commercially available. Molecular techniques based on reverse transcription-polymerase chain reaction (RT-PCR) have been developed that can detect the virus in plants and thrips vectors. Sensitive, rapid real-time PCR methods are also available. An NSs-specific monoclonal antibody was produced which was shown to be effective in identifying adults that are capable of transmitting the virus since detection of NSs in thrips is an indication that the virus had multiplied in the vector.

Transmission and Epidemiology

TSWV is transmitted by several species of thrips in a circulative and propagative manner. These include *F. occidentalis, Frankliniella schultzei, Frankliniella intonosa, Frankliniella bispinosa, Frankliniella fusca, Thrips setosus*, and *Thrips tabaci*. The virus is not known to be seed transmitted. Hence, susceptible crops with overlapping production seasons and prevalence of weed hosts and thrips vectors constitute the most important factors for TSWV epidemics. TSWV has to be introduced into a crop by viruliferous thrips and patterns of disease spread mostly suggests primary spread with little secondary spread within the crop. Considerable progress has been made in our understanding of thrips–TSWV interactions, especially in the case of TSWV–*F. occidentalis* association. For the adult thrips to become a transmitter, the larva has to acquire the virus by feeding on an infected plant. First and second instar larvae are capable of acquiring the virus though the former is more efficient. The virus starts replicating in the larva and survives through the developmental stages. The emerging adult transmits the virus and continues so for life. There is no evidence of transovarial transmission. Thus, the virus has to be acquired by each generation of thrips. Therefore, tospoviruses are capable of replicating in both their host plants and thrips vectors. This intimate biological association between tospoviruses and their thrips vectors had created possibilities for evolution and shifting preferences and specificities between individual tospoviruses and thrips species. New vector species of TSWV began to emerge in the past decade. For example, *F. bispinosa* was reported as a vector of TSWV. For TSWV epidemics to occur, thrips vectors should complete a life cycle on virus-infected host plants. Infected plants that do not support thrips lifecyle can be considered as a dead end in disease epidemiology. The economic impact of TSWV on a number of important crops combined with the fact that thrips-borne inoculum is the most important contributory factor to virus outbreaks resulted in extensive research efforts to understand the basis of TSWV–thrips interactions and the virus and insect factors that contribute to the specificity of virus–vector relations. Of more than 5000 thrips species described, only seven of them are known to transmit TSWV, suggesting complex interactions of recognition, acquisition, replication, and movement at the virus–vector association level. Much is now known about the interaction between TSWV and *F. occidentalis*.

The virus upon acquisition was shown to move through the midgut and subsequently reaches the salivary glands. It is hypothesized that the close proximity of midgut and salivary glands in the thrips larval stage facilitates the virus movement whereas the virus fails to do so as the thrips reaches adult stage. This may explain the inability of adult thrips to transmit the virus if the virus is acquired for the first time in its adult life. The specificity of TSWV and thrips vectors may be due to the presence of a receptor in the vector species which may be absent in nonvector species. A 50 kDa protein and a 94 kDa protein were identified as potential thrips proteins involved in interaction with TSWV. However, cloning the gene for the putative receptor remains to be accomplished. A soluble form of *in vitro*-expressed TSWV G_N protein was used to study its role in recognition by thrips vectors. When thrips were fed with the purified G_N, the protein could be detected in the midgut epithelial cells of the larvae which subsequently resulted in the prevention of TSWV acquisition, suggesting that G_N may be involved in virus recognition by the thrips vector. While G_N potentially mediates the specific recognition between TSWV and its thrips vector, sequence comparisons

and biochemical analysis of G_C protein indicated that it facilitates the fusion and subsequent uptake of the virion into vector cells.

Management

TSWV has been an economically important constraint for several crops over the past several decades in several parts of the world. Due to the nonpredictive nature of the outbreaks combined with the lack of forecasting, adoption of preventive measures has not always been practical. The disease cycle has proven to be extremely difficult to break because of the wide and often overlapping host range of both the virus and the thrips vectors.

However, multidisciplinary research in the past decade has led to identification of factors that contribute to TSWV epidemics and development of practical control recommendations which resulted in reduced impact of TSWV in several crops. An integrated management approach must be taken as no single control tactic was found to be effective by itself. The crucial element in the management program is growing virus-resistant or virus-tolerant cultivars. TSWV-resistant cultivars with desirable agronomic traits are now available for peanut, pepper, and tomato. Resistance-breaking strains of TSWV continue to pose a threat, potentially limiting the durability of resistant cultivars. Resistance governed by multigenes should be used where available to make the resistance more durable. Production practices such as growing on plastic mulch or reflective mulch significantly reduced the disease incidence in certain crops. Novel strategies utilizing chemicals such as acibenzolar-*S*-methyl that induce systemic acquired resistance are found to be effective in reducing the incidence of TSWV (**Figure 4**). Thrips management based on selective use of insecticides during the early part of the cropping season combined with resistant cultivars resulted in reduced incidence and increased yield in tomato. Ability to forecast the epidemics by making use of information such as the seasonal dynamics of TSWV transmitters in the vector populations will help growers make appropriate management decisions. Pathogen-mediated resistance due to post-transcriptional gene silencing was shown to be effective in several crops. Expression of single-chain antibodies, TSWV N gene, or various modifications of the N gene were found to be effective in conferring resistance. Genetic engineering can be very useful in situations where natural sources of host plant resistance are not available or difficult to transfer to existing agronomically desirable cultivars. Consumer acceptance and other market-related issues have hampered the efforts to commercialize this technology.



Figure 4 Protective effect of acibenzolar-*S*-methyl against TSWV. Both leaves were mechanically inoculated with TSWV. Leaf on the right was treated with acibenzolar-*S*-methyl prior to inoculation with TSWV. Reproduced from Mandal B, Pappu HR, and Csinos AS (2003) Suppressive effect of Actigard on tomato spotted wilt virus. APSnet Image of the Week, with permission of the American Phytopathological Society.

See also: Tospovirus; Vector Transmission of Plant Viruses.

Further Reading

Adkins S (2000) Tomato spotted wilt virus – positive steps towards negative success. *Molecular Plant Pathology* 1: 151–157.

Bucher E, Sijen T, De Haan P, Goldbach R, and Prins M (2003) Negative-strand tospoviruses and tenuiviruses carry a gene for a suppressor of gene silencing at analogous genomic positions. *Journal of Virology* 77: 1329–1336.

Culbreath AK, Todd JW, and Brown SL (2003) Epidemiology and management of tomato spotted wilt in peanut. *Annual Review of Phytopathology* 41: 53–75.

Csinos AS, Pappu HR, McPherson RM, and Stephenson MG (2001) Management of *Tomato spotted wilt tospovirus* in flue-cured tobacco with acibenzolar-*S*-methyl and imidacloprid. *Plant Disease* 85: 292–296.

de Medeiros RB, Figueiredo J, Resende RO, and de Avila AC (2005) Expression of a viral polymerase-bound host factor turns human cell lines permissive to a plant- and insect-infecting virus. *Proceedings of the National Academy of Sciences, USA* 102: 1175–1180.

Lewandowski DJ and Adkins S (2005) The tubule-forming NSm protein from tomato spotted wilt virus complements cell-to-cell and long-distance movement of tobacco mosaic virus hybrids. *Virology* 342: 26–37.

Mandal B, Pappu HR, and Csinos AS (2003) Suppressive effect of Actigard on tomato spotted wilt virus. APSnet Image of the week. http://www.aspnet.org/online/Archive/2003/W000022.asp.

Mandal B, Pappu HR, Csinos AS, and Culbreath AK (2005) Response of peanut, pepper, tobacco, and tomato cultivars to two biologically distinct isolates of *Tomato spotted wilt virus*. *Plant Disease* 90: 1150–1155.

Moyer JW (2000) Tospoviruses. In: Hull R (ed.) *Encyclopedia of Microbiology,* vol. 4, pp. 592–597. New York: Academic Press.

Riley DG and Pappu HR (2004) Tactics for management of thrips (Thysanoptera: Thripidae) and tomato spotted wilt virus in tomato. *Journal of Economic Entomology* 97: 1648–1658.

Rinne PL, van den Boogaard R, Mensink MG, *et al.* (2005) Tobacco plants respond to the constitutive expression of the tospovirus

movement protein NS(M) with a heat-reversible sealing of plasmodesmata that impairs development. *Plant Journal* 43: 688–707.
Sherwood JL, German TL, Moyer JW, Ullman DE, and Whitfield AE (2000) Tomato spotted wilt. In: Maloy OC and Murray TD (eds.) *Encyclopedia of Plant Pathology*, pp. 1030–1031. New York: Wiley.
Sin SH, McNulty BC, Kennedy GG, and Moyer JW (2005) Viral genetic determinants for thrips transmission of tomato spotted wilt virus. *Proceedings of the National Academy of Sciences, USA* 102: 5168–5173.
Soellick T-R, Uhrig JF, Bucher GL, Kellmann J-W, and Schreier PH (2000) The movement protein NSm of tomato spotted wilt tospovirus (TSWV): RNA binding, interaction with TSWV N protein, and identification of interacting plant proteins. *Proceedings of the National Academy of Sciences, USA* 97: 2373–2378.
Takeda A, Sugiyama K, Nagano H, *et al.* (2002) Identification of a novel RNA silencing suppressor, NSs protein of tomato spotted wilt virus. *FEBS Letters* 532: 75–79.
Whitfield AE, Ullman DE, and German TL (2005) Tospovirus–thrips interactions. *Annual Review of Phytopathology* 43: 459–489.

Tomato Yellow Leaf Curl Virus

H Czosnek, The Hebrew University of Jerusalem, Rehovot, Israel

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Agroinoculation An alternative route for viral infection; the viral genome is cloned, usually as a head-to-tail dimer, in the T-DNA of *Agrobacterium tumefaciens* and is delivered to plants by inoculation.
Introgression Incorporation of chromosomal segments of wild tomato species in the domesticated tomato by crosses and selection; introgression lines are used to localize genes to the tomato chromosomes.
Rolling circle Mechanism of replication followed by many viral DNA and by begomoviruses in particular.
Viruliferous whitefly Insect that has acquired virus from an infected plant and is ready to infect other host plants.
Whitefly Insect that belongs to the order *Homoptera*; they cause damage to plants by feeding and by vectoring plant viruses.

Introduction

In the late 1950s the tomato cultures in the Jordan valley of Israel were unexpectedly affected by a disease of unknown etiology. The disease was accompanied by large populations of whiteflies. The suspicion that the whiteflies were the vector of a viral disease was confirmed following controlled transmission experiments in the laboratory. The virus was named tomato yellow leaf curl virus (TYLCV). The virus was isolated and its genome sequenced in the late 1980s.

TYLCV is a member of the genus *Begomovirus* of the family *Geminiviridae*, which includes viruses transmitted by the whitefly *Bemisia tabaci*. Begomoviruses have a genome either split between two circular single-stranded (ss) DNA - molecules of approximately 2700 nt each named DNA A and DNA B (bipartite) or with a single genomic DNA A-like molecule (monopartite). TYLCV is monopartite. The relationships between the virus, the vector, and the host tomato plant have been the object of many studies.

From the early 1960s tomato cultures have been under the constant threat of TYLCV-like begomoviruses worldwide. TYLCV has quickly spread to the Middle East, Central Asia, North and West Africa, Southeast Europe, the Caribbean islands, Southeast USA, and Mexico. TYLCV-related begomoviruses have been identified in Italy, the Maghreb and Western Africa, and the Arabian Peninsula. Breeding programs for resistance have started in the mid-1970s and several commercial varieties with adequate resistance have been released. Several loci tightly linked to TYLCV resistance have been assigned to the small arm of tomato chromosome 6. A variety of strategies have been devised based on the pathogen-derived resistance concept, which involves the expression of functional as well as dysfunctional viral genes. RNA-mediated virus resistance based on antisense RNA and post-translational gene silencing was efficient but was highly sequence dependent.

Virus Structure

Like all geminiviruses, TYLCV has a characteristic particle of twinned morphology of approximately 20×30 nm in size (**Figure 1(a)**). The virus capsid (total m.w. 3330 kDa) consists of two joined, incomplete icosahedra, with a $T = 1$ surface lattice containing a total of 22 capsomeres each containing five units of a 260-amino-acid coat protein (30.3 kDa). TYLCV has a single 2787 nt (total m.w. 980 kDa) covalently closed-genomic circular ssDNA.



Figure 1 Virions, host tomato plants and whitefly vector. (a) Viral particles with twinned morphology of approximately 20 × 30 nm in size. (b) Left – infected tomato plant with typical symptoms; right – noninfected tomato plant. (c) Adult *B. tabaci* whiteflies, approximately 2 mm in size.

Host Range

The domesticated tomato *Solanum esculentum* (formerly *Lycopersicon esculentum*) is the primary host of TYLCV. Most of the wild tomato species such as *S. chilense*, *S. habrochaites* (formerly *L. hirsutum*), *S. peruvianum*, and *S. pimpinellifolium* include accessions that are symptomless carriers and are used as genitors in breeding programs for TYLCV resistance. Several cultivated plants (bean (*Phaseolus vulgaris*), petunia (*Petunia hybrida*), and lisianthus (*Eustoma grandiflorum*)) are hosts of TYLCV and present severe symptoms upon whitefly mediated inoculation. Weeds, such as *Datura stramonium* and *Cynanchum acutum*, present distinct symptoms, whereas others, such as *Malva parviflora*, are symptomless carriers. Plants used to rear whiteflies, such as cotton (*Gossypium hirsutum*) and eggplant (*Solanum melongena*), are immune to the virus. Experimental hosts of the virus include jimsonweed (*Datura stramonium*). Some plants recalcitrant to whitefly mediated inoculation, such as *Nicotiana benthamiana* and *N. tabacum*, may be infected by TYLCV DNA clones using agroinoculation.

Transmission to Tomato

In nature TYLCV is transmitted exclusively by the whitefly *B. tabaci* in a persistent manner (**Figure 1**). Symptoms included growth arrest leaflets cupped inward with yellow margins, and barely produced fruits (**Figure 1**). *B. tabaci* thrives in commercial fields of cotton, tomato, eggplant, and pepper, in tropical and subtropical countries. It also infests the greenhouses in these regions as well as those in temperate countries. Whiteflies may cause dramatic damages due to feeding and the transmission of begomoviruses. *B. tabaci* comprises many types (or biotypes) that can be distinguished by their plant host range, fertility, as well as with molecular markers (especially from the mitochondrial genome). Among the many biotypes, B and Q are extremely efficient vectors of TYLCV.

Transmission of TYLCV to tomato by *B. tabaci* B biotype has been studied thoroughly. The virus is transmitted to tomato plants after vector-feeding on infected tomato plants or alternative hosts. The incidence of the disease is directly correlated with the pressure of the whitefly population. One to three viruliferous insects are able to infect a tomato plant. The efficient acquisition access period (AAP) is 15–30 min, the latent period is 8–24 h, and the efficient inoculation access period (IAP) is at least 15 min. Female *B. tabaci* are more efficient vectors than males. The ability of the whiteflies to transmit TYLCV to tomato test plants steadily decreases with age, from 100% to 10–20% during their adult life time.

Symptoms develop on inoculated seedlings 2–3 weeks after insect first inoculation feeding. In the field inoculation can occur immediately after transplantation. Infected seedlings remain stunted and do not yield fruits. Infection at a later stage affects vegetative growth and fruit setting. Disease incidence increases rapidly and in severely affected regions results in yield reduction of up to 100%.

The viral DNA replicates in the nuclei of infected cells and is mostly phloem limited. Apart from whiteflies, TYLCV can be transmitted by grafting and agroinoculation. It is not mechanically transmitted and it is not propagated by seeds.d

Genome Organization and Expression

A schematic drawing of the TYLCV genome is shown in **Figure 2**. It replicates according to a rolling circle mechanism. The viral genome encodes two large open reading frames (ORFs) on the viral strand (V1 and V2), and four on the complementary strand (C1–C4). A 313 nt long intergenic region (IR) contains a 29 nt long stem–loop structure with the conserved nanonucleotide TAATATTAC which is the origin of replication (Ori) of the virus. The IR also contains the promoters of the V1, V2, C1, and C4 genes.

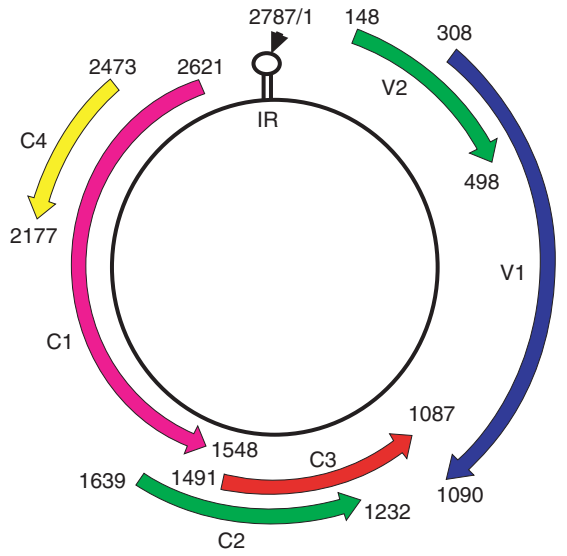


Figure 2 Genome organization of tomato yellow leaf curl virus. The single-stranded virion DNA comprises 2787 nt. Open reading frames (ORFs) of virion-sense and complementary-sense strand polarity are designated (V) and (C), respectively. ORFs are represented by an arrow; numbers indicate first and last nucleotide of each ORF. V1 encodes the capsid protein (CP), V2 a movement protein, C1 the replication initiator protein (Rep), C2 a transcriptional activator protein (TrAP), C3 a replication enhancer protein (REn), and C4 a symptom and movement determinant. IR: intergenic region. The conserved inverted repeat flanking the conserved nanonucleotide sequence TAATATTAC is symbolized by a stem–loop; an arrow head indicates the cleaving position of Rep in the TAATATT/AC loop; A at the cutting site (/) is nucleotide number one, by definition.

- V1. The V1 ORF encodes the capsid protein (CP). The CP is a multifunctional protein: The N- and C-termini of CP monomers interact to form the capsid (one viral genomic molecule is encapsidated in each geminate particle). The CP has a nuclear localization signal (NLS) and it is able to shuttle the viral genomic DNA in and out of the nucleus. It is essential for infectivity, and it is the only viral protein recognized by the insect vector.
- V2. The V2 ORF has properties analogous to those of a movement protein and in some cases mutants affect symptom expression.
- C1. The C1 ORF encodes the replication initiator protein (Rep). It is the only viral protein necessary for replication. The functional protein is an oligomer. The Rep protein recognizes the Ori located in the IR and specifically cleaves the nanonucleotide TAATATTAC between nucleotides 7 and 8. Together with plant host polymerase(s) it initiates viral DNA replication according to the rolling-circle model. Short inverted repeats located in the IR upstream the stem–loop structure are recognized by the Rep protein and also involved in the replication process. Rep also interacts with another viral protein, the viral replication enhancer protein (REn), encoded by ORF C3 and with a variety of cellular proteins such as a retinoblastoma-like protein, an interaction that may enhance plant cell replication and thereby viral multiplication.
- C2. The ORF C2 encodes a transcriptional activator protein (TrAp). Both ORFs C2 and C3 are transcribed from a promoter located in the 3′ end of the C1 ORF. TrAp enhances transcription of the viral strand promoter. It is able to bind to viral genomic DNA and possesses an NLS. In addition, the C2 gene product acts as silencing suppressors.
- C3. The ORF C3 encodes the replication enhancer protein (REn). This protein interacts with itself to form oligomers. REn interacts with Rep and with cell-cycle associated host proteins to increase the amount viral DNA (genomic and double-stranded) in the infected plant.
- C4. The ORF C4 encodes a protein not essential for infectivity but contributes to the spread of the virus in the plant and induction of symptoms. The protein may also act as a silencing suppressor, but this has not been definitively proved.

Geographical Distribution

The name TYLCV was coined in the early 1960s to describe a virus transmitted by the whitefly *B. tabaci* that affected tomato cultures in Israel. Early diagnosis of TYLCV was essentially based on symptom observation, although symptoms vary greatly as function of soil, growth conditions, and climate. Serology has been of limited use because whitefly transmitted geminiviruses share many epitopes. The analysis of DNA sequences has become the tool of choice, allowing one to accurately identify the virus and to evaluate its relationship with other TYLCV isolates. TYLCV has been reported in the mid- and late 1970s in Cyprus, Jordan, and Lebanon. It has been identified in Egypt and Turkey in the early 1980s. The virus has spread to Turkey, Iran and the Asian republics of the former USSR, and to Saudi Arabia and Yemen during the mid–late 1990s. Two TYLCV isolates closely related to the Middle Eastern virus have been described in Japan in the late 1990s. In China, TYLCV isolate TYLCVs has been identified in the southwest province of Guangxi. In the early 1990s two virus isolates belonging to a new species related to the Middle Eastern TYLCV, named tomato yellow leaf curl Sardinia virus, have been identified in Sardinia (TYLCSV-Sar) and in Sicily (TYLCSV-Sic), Italy. The Sardinian isolate TYLCSV-Sar has been discovered in Spain in the early 1990s. By he mid-1990s the Middle Eastern TYLCV was found in Portugal and in Spain; in the latter country, it tended to displace the previously established Italian virus.

Recombinants between the two viruses have been found. TYLCV has invaded North Africa, possibly from Spain and Italy. In Morocco, both the Italian and the Middle Eastern strain were discovered in the early 2000s. In Tunisia the Italian virus was identified in the early 2000s. TYLCV appeared in Southern France in 1999. In 2000, the Middle Eastern strain of TYLCV was identified in Crete, Attiki, and Southern Peloponnesus in Greece. In East Africa, TYLCV was present in Sudan as early as the late 1970s. In Tanzania a virus distinct from the Mediterranean isolates has been sequenced. In the Réunion Island the virus was detected in the late 1990s. The Middle Eastern strain of TYLCV has appeared in the Western Hemisphere in the mid-1990s in the Caribbean Islands, first in the Dominican Republic, then Cuba, Jamaica, Puerto Rico, and the Bahamas. From there, the virus has reached the USA, identified first in Virginia in the late 1990s, then in Florida, Georgia, Louisiana, North Carolina, and Mississippi. It seems that TYLCV is now found in several regions of Mexico.

Hence during the last decade TYLCV has spread extremely fast and constitutes a major limiting factor to tomato cultivation, worldwide. In many regions, the invading Mediterranean strain of TYLCV coexists with local TYLCV strains, but in several cases it has almost replaced them, as in Southern Spain. The Sardinian isolate of TYLCSV has been recently detected in Jordan and in Israel, indicating that the expansion of TYLCSV is not unidirectional, from the Eastern Mediterranean region to other parts of the world as thought not long ago.

Taxonomy and Classification

TYLCV is the name of the virus originally isolated in Israel. Sequence comparisons of TYLCV-like viruses analyzed worldwide have revealed that the name TYLCV was used mistakenly to name a complex of closely as well as distantly related begomoviruses affecting tomato. Viruses with nucleotide sequence homology of more than 89% have been considered to be strains of the same species while viruses with homologies of less than 89% have been considered as belonging to different virus species. Accordingly, the begomoviruses affecting tomato have been classified into several species: all the TYLCV isolates known today have a monopartite genome.

The members of the TYLCV species, as well as members of species closely related to TYLCV updated as of December 2006, are listed in **Table 1**; the acronym and the GenBank accession number of the DNA sequence are provided.

This classification is rendered even more complicated by the recent discovery that recombination between members of different species of geminiviruses happens relatively frequently. For example, a naturally occurring recombination has been recently found in the Almeria region, Spain, between TYLCV and TYLCSV. The recombinant virus, coined tomato yellow leaf curl Malaga virus (TYLCMalV-[ES], AF271234), probably occurred because the two virus species have coexisted in the tomato plants grown in the greenhouse.

Additional begomoviruses that infect tomato cultures have not been assigned to the TYLCV and are discussed elsewhere in this encyclopedia. These viruses clearly differ from the various TYLCV isolates in the symptoms they induce on tomato, in their host range, and in their nucleotide sequence.

Virus–Vector Relationship

Path of the Virus in the Insect

Like all begomoviruses TYLCV follows a definite path in its *B. tabaci* vector. Whiteflies feed when their stylets reach the virus-rich phloem of infected leaves. TYLCV can be detected in the insect head approximately 10 min after feeding started. From the esophagus the virus reaches the midgut where it can be detected approximately 30 min after it was first found in the head. At this point, some of the viruses might be excreted through the hindgut. The crossing of TYLCV from the midgut to the hemolymph is surprisingly fast. The virus reaches the hemolymph 30 min after it was first detected in the midgut, 90 min after the beginning of the AAP. The crossing of the gut is likely to be an active process involving specialized unknown receptors. To avoid degradation in the hemolymph, geminiviruses interact with a GroEL-like chaperonin produced by the insect endosymbiotic bacteria and excreted in the hemolymph. TYLCV can be detected in the salivary glands approximately 5.5 h after it was first detected in the haemolymph, 7 h after the beginning of the AAP, approximately 1 h before the insects are able to infect tomato plants. Once the virus reaches the salivary gland, crossing several cell walls that may constitute selective receptor-mediated barriers, it is almost immediately excreted into the salivary pump and from there into the plant together with the saliva. The capsid (and the coat protein) is the only begomoviral structure recognized by the would-be receptors and by the endosymbiotic chaperonin.

Retention of TYLCV in the Insect Vector

The virus can be detected in every stage of vector development. Following a 48 h AAP on TYLCV-infected tomato, viral DNA remains associated with *B. tabaci* for the entire adult life of the insect while infectivity decreases with time, but not entirely. Various TYLCV isolates present sometimes different parameters of interactions with their whitefly host. For example, the maximum retention period

Table 1 List of 59 virus isolates (written in black) used in this article belonging to 15 different strains (written in red) and six different species of the *Tomato yellow leaf curl virus* cluster (written in green). The accession numbers of the complete A component sequence is indicated in the second column and the abbreviation of the name of the virus isolates and strains is indicated in the third column

Tomato leaf curl Sudan virus		
Tomato leaf curl Sudan virus – Gezira		ToLCSDV-Gez
Tomato leaf curl Sudan virus – Gezira [Sudan:Gezira:1996]	AY044137	ToLCSDV-Gez[SD:Gez:96]
Tomato leaf curl Sudan virus – Shambat		ToLCSDV-Sha
Tomato leaf curl Sudan virus – Shambat [Sudan:Shambat:1996]	AY044139	ToLCSDV-Sha[SD:Sha:96]
Tomato leaf curl Sudan virus – Yemen		ToLCSDV-YE
Tomato leaf curl Sudan virus – Yemen [Yemen:Tihamah:2006]	EF110890	ToLCSDV-YE[YE:Tih:06]
Tomato yellow leaf curl Axarquia virus		
Tomato yellow leaf curl Axarquia virus – [Spain:Algarrobo:2000]	AY227892	TYLCAxV-[ES:Alg:00]
Tomato yellow leaf curl Malaga virus		
Tomato yellow leaf curl Malaga virus – [Spain:421:1999]	AF271234	TYLCMalV-[ES:421:99]
Tomato yellow leaf curl Mali virus		
Tomato yellow leaf curl Mali virus – Ethiopia		TYLCMLV-ET
Tomato yellow leaf curl Mali virus – Ethiopia [Ethiopia:Melkassa:2005]	DQ358913	TYLCMLV-ET[ET:Mel:05]
Tomato yellow leaf curl Mali virus – Mali		TYLCMLV-ML
Tomato yellow leaf curl Mali virus – Mali [Mali]	AY502934	TYLCMLV-ML[ML]
Tomato yellow leaf curl Sardinia virus		
Tomato yellow leaf curl Sardinia virus – Italy		TYLCSV-IT
Tomato yellow leaf curl Sardinia virus – Italy [Italy:Sardinia:1988]	X61153	TYLCSV-IT[IT:Sar:88]
Tomato yellow leaf curl Sardinia virus – Sicily		TYLCSV-Sic
Tomato yellow leaf curl Sardinia virus – Sicily [Israel:Henryk:2005]	DQ845787	TYLCSV-Sic[IL:Hen:05]
Tomato yellow leaf curl Sardinia virus – Sicily [Italy:Sicily]	Z28390	TYLCSV-Sic[IT:Sic]
Tomato yellow leaf curl Sardinia virus – Sicily [Tunisia:Bkalta 3:2002]	AY736854	TYLCSV-Sic[TN:Bk3:02]
Tomato yellow leaf curl Sardinia virus – Spain		TYLCSV-ES
Tomato yellow leaf curl Sardinia virus – Spain [Spain:Almeria 2:1992]	L27708	TYLCSV-ES[ES:Alm2:92]
Tomato yellow leaf curl Sardinia virus – Spain [Spain:Canary]	AJ519675	TYLCSV-ES[ES:Can]
Tomato yellow leaf curl Sardinia virus – Spain [Spain:Murcia 1:1992]	Z25751	TYLCSV-ES[ES:Mur1:92]
Tomato yellow leaf curl Sardinia virus – Spain[Morocco:Agadir:2002]	AY702650	TYLCSV-ES[MA:Aga:02]
Tomato yellow leaf curl virus		
Tomato yellow leaf curl virus – Gezira		TYLCV-Gez
Tomato yellow leaf curl virus – Gezira [Sudan:1996]	AY044138	TYLCV-Gez[SD:96]
Tomato yellow leaf curl virus – Iran		TYLCV-IR
Tomato yellow leaf curl virus – Iran [Iran]	AJ132711	TYLCV-IR[IR]
Tomato yellow leaf curl virus – Israel		TYLCV-IL
Tomato yellow leaf curl virus – Israel [Australia:Brisbane:2006]	1934[a]	TYLCV-IL[AU:Bri:06]
Tomato yellow leaf curl virus – Israel [China:Shangai 2:2005]	AM282874	TYLCV-IL[CN:SH2:05]
Tomato yellow leaf curl virus – Israel [Cuba]	AJ223505	TYLCV-IL[CU]
Tomato yellow leaf curl virus – Israel [Dominican Republic]	AF024715	TYLCV-IL[DO]
Tomato yellow leaf curl virus – Israel [Egypt:Ismaelia]	AY594174	TYLCV-IL[EG:Ism]
Tomato yellow leaf curl virus – Israel [Egypt:Nobaria:1991]	EF107520	TYLCV-IL[EG:Nob:91]
Tomato yellow leaf curl virus – Israel [Israel:Rehovot:1986]	X15656	TYLCV-IL[IL:Reo:86]
Tomato yellow leaf curl virus – Israel [Italy:Sicily:2004]	DQ144621	TYLCV-IL[IT:Sic:04]
Tomato yellow leaf curl virus – Israel [Japan:Haruno:2005]	AB192966	TYLCV-IL[JR:TosH:05]
Tomato yellow leaf curl virus – Israel [Japan:Misumi:Stellaria]	AB116631	TYLCV-IL[JR:Mis:Ste]
Tomato yellow leaf curl virus – Israel [Japan:Miyazaki]	AB116629	TYLCV-IL[JR:Miy]
Tomato yellow leaf curl virus – Israel [Japan:Omura:Eustoma]	AB116630	TYLCV-IL[JR:Omu:Eus]
Tomato yellow leaf curl virus – Israel [Japan:Omura]	AB110217	TYLCV-IL[JR:Omu]
Tomato yellow leaf curl virus – Israel [Japan:Tosa:2005]	AB192965	TYLCV-IL[JR:Tos:05]
Tomato yellow leaf curl virus – Israel [Jordan:Tomato:2005]	EF054893	TYLCV-IL[JO:Tom:05]
Tomato yellow leaf curl virus – Israel [Lebanon:Tomato:2005]	EF051116	TYLCV-IL[LB:Tom:05]
Tomato yellow leaf curl virus – Israel [Mexico:Culiacan:2005]	DQ631892	TYLCV-IL[MX:Cul:05]
Tomato yellow leaf curl virus – Israel [Morocco:Berkane:2005]	EF060196	TYLCV-IL[MO:Ber:05]
Tomato yellow leaf curl virus – Israel [Puerto Rico:2001]	AY134494	TYLCV-IL[PR:01]
Tomato yellow leaf curl virus – Israel [Spain:Almeria:Pepper:1999]	AJ489258	TYLCV-IL[ES:Alm:Pep:99]
Tomato yellow leaf curl virus – Israel [Tunisia:2005]	EF101929	TYLCV-IL[TN:05]
Tomato yellow leaf curl virus – Israel [Turkey:Mersin:2004]	AK812277	TYLCV-IL[TR:Mer:04]
Tomato yellow leaf curl virus – Israel [US:Florida]	AY530931	TYLCV-IL[US:Flo]
Tomato yellow leaf curl virus – Mild		TYLCV-Mld
Tomato yellow leaf curl virus – Mild [Israel;1993]	X76319	TYLCV-Mld[IL;93]
Tomato yellow leaf curl virus – Mild [Japan:Aichi]	AB014347	TYLCV-Mld[JR:Aic]

Continued

Table 1 Continued

Tomato yellow leaf curl virus – Mild [Japan:Atumi]	AB116633	TYLCV-Mld[JR:Atu]
Tomato yellow leaf curl virus – Mild [Japan:Daito]	AB116635	TYLCV-Mld[JR:Dai]
Tomato yellow leaf curl virus – Mild [Japan:Kisozaki]	AB116634	TYLCV-Mld[JR:Kis]
Tomato yellow leaf curl virus – Mild [Japan:Osuka]	AB116636	TYLCV-Mld[JR:Osu]
Tomato yellow leaf curl virus – Mild [Japan:Shimizu]	AB110218	TYLCV-Mld[JR:Shi]
Tomato yellow leaf curl virus – Mild [Japan:Shizuoka]	AB014346	TYLCV-Mld[JR:Shz]
Tomato yellow leaf curl virus – Mild [Japan:Yaizu]	AB116632	TYLCV-Mld[JR:Yai]
Tomato yellow leaf curl virus – Mild [Jordan:Cucumber:2005]	EF158044	TYLCV-Mld[JO:Cuc:03]
Tomato yellow leaf curl virus – Mild [Jordan:Homra:2003]	AY594175	TYLCV-Mld[JO:Hom03]
Tomato yellow leaf curl virus – Mild [Jordan:Tomato:2005]	EF054894	TYLCV-Mld[JO:Tom:03]
Tomato yellow leaf curl virus – Mild [Lebanon;LBA44:05]	EF185318	TYLCV-Mld[ILB;LBA44:05]
Tomato yellow leaf curl virus – Mild [Portugal:2:1995]	AF105975	TYLCV-Mld[PT:2:95]
Tomato yellow leaf curl virus – Mild [Reunion:2002]	AJ865337	TYLCV-Mld[RE:02]
Tomato yellow leaf curl virus – Mild [Spain:72:1997]	AF071228	TYLCV-Mld[ES:72:97]
Tomato yellow leaf curl virus – Mild [Spain:Almeria:1999]	AJ519441	TYLCV-Mld[ES:Alm:99]

[a]Australian accession.
Species names are in italic fonts and strain and isolate names are in roman fonts.

of isolates of TYLCSV (TYLCSV-[IT:Sar]) was 8 days from the end of the AAP compared to 5 weeks for TYLCV.

Both TYLCV and TYLCSV can be detected in the eggs and in adult progeny of viruliferous whiteflies in low incidence. However while the TYLCV progeny was able to transmit the virus to tomato plants, the TYLCSV progeny did not. Although the question of the possible replication of TYLCV in its insect vector remains controversial, transcripts of viral genes V1, V2, and C3 accumulated in *B. tabaci* long after transfer of viruliferous whiteflies on nonhost cotton plants. In contrast to TYLCV, transcripts of the bipartite begomovirus ToMoV did not accumulate in the whiteflies.

Deleterious Effects of TYLCV on Life Expectancy and Fertility of the Whitefly Vector

Three-day-old insects raised on eggplants (a TYLCV nonhost) following a 48 h AAP on TYLCV-infected tomato plants showed a reduction of 17–23% in their life expectancy compared to insects that have not acquired the virus, and to a 40–50% decrease in the mean number of eggs laid. These deleterious effects, in addition to the invasion of the reproductive system, suggest that TYLCV has some features reminiscent of an insect pathogen.

Sexual Transmission

TYLCV can be transmitted among whiteflies in a sex-dependent manner, in the absence of any other source of virus. TYLCV was transmitted from viruliferous males to females and from viruliferous females to males, but not among insects of the same sex. The recipient insects were able to efficiently inoculate tomato test plants. In the recipient insects TYLCV was first detected in the hemolymph, but not in the head as in the case of acquisition from infected plants. Therefore the virus follows, at least in part, the circulative pathway associated with acquisition from infected plants. Insect to insect virus transmission was instrumental in increasing the number of whiteflies capable of infecting tomato test plants in a whitefly population. Accordingly, a plant virus can be sexually transmitted among its insect vector.

Breeding Tomato for Resistance to TYLCV

Incorporation of Resistance Trait from Wild Tomato Species

The domesticated tomato *S. esculentum* is extremely susceptible to TYLCV. Breeding tomato for resistance started in the early 1960s and is going on since then. It has been initially observed that some accessions of wild tomato species exhibited resistance to the virus and could be crossed with the domesticated tomato. The first successful breeding program was initiated with *S. peruvianum.* Although this type of resistance was controlled by three to five recessive genes, a first hybrid with acceptable resistance was released in 1988. Advanced breeding lines and commercial varieties containing this type of resistance were released subsequently. Resistance genes from several different accessions of the wild tomato species *S. chilense* have been incorporated into breeding programs in the early 1990s. For each *S. chilense* accession, resistance is determined by a different semidominant gene and several minor genes. The wild tomato species *S. habrochaites* has been used in the late 1990s to produce breeding lines in which resistance is under the control of a major dominant locus.

After more than 25 years of effort, the best resistant cultivars and breeding lines give far higher yields upon

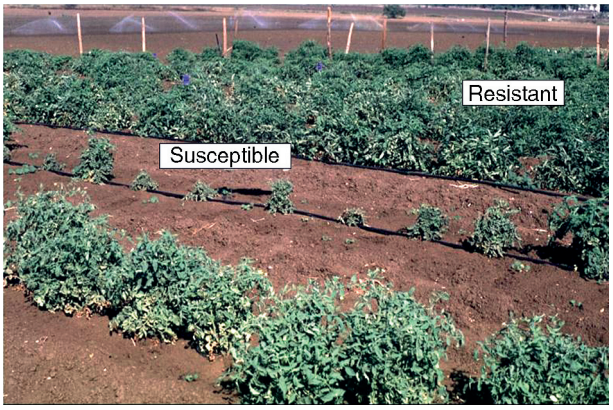


Figure 3 TYLCV-infected tomato fields planted with susceptible and resistant tomato genotypes. Note that the susceptible plants are stunted, and they will not produce fruits; for comparison, the resistant plants will remain symptomless, or have mild symptoms, and will yield.

infection than those of susceptible cultivars; disease symptoms are absent or mild but all of them contain various amounts of virus (**Figure 3**). It is interesting to note that in many cases (especially when the resistance originates from certain accessions of *S. chilense* and of *S. habrochaites*), the tomato lines are resistant to TYLCV and also to a number of begomoviruses in very different parts of the world, some of them monopartite and others bipartite. For example, line Lh902 that originated from *S. habrochaites* is resistant to TYLCV in the Mediterranean region as well as to a wide range of bipartite geminiviruses in Central America.

Genetically Engineered Tomato for Resistance

A variety of strategies have been devised based on the expression of functional as well as dysfunctional viral genes such as coat protein and Rep protein. A tomato interspecific hybrid expressing the TYLCV coat protein gene under the control of the 35S CaMV promoter responded upon whitefly mediated TYLCV inoculation showed expression of delayed disease symptoms and recovery from disease. Tomato inbred lines have been transformed with a 3′ truncated Rep gene (two-fifths of the gene) and the virus intergenic region from the Florida isolate of TYLCV (TYLCV-[US:Flo]). Resistance was achieved upon whitefly mediated inoculation in field conditions. However, these plants were susceptible to the bipartite begomovirus ToMoV from Florida. Similarly, tomato plants transformed with the Rep gene of TYLCSV (TYLCSV-[IT-Sar]) truncated in its 3′ end (leaving 210 amino acids out of 257) exhibited resistance to TYLCSV-[IT:Sar] but were susceptible to the related strain from Spain TYLCSV-ES[ES]. The viral Rep gene altered in the NTP-binding site crucial for virus replication has been used as *trans*-dominant proteins to block virus replication.

RNA-mediated virus resistance was highly sequence dependent. Expression of antisense sequence of the TYLCV-[IT-Sar] Rep gene in transgenic *N. benthamiana* resulted in repression of nearly all virus replication. The incorporation of a ribozyme structure into the antisense construct did not increase the efficiency of the system. Resistant tomato plants have been developed which exploit the mechanism of post-transcriptional gene silencing via double-stranded RNA sequences. Noncoding conserved regions from the genomes of TYLCV, TYLCV-mild (TYLCV-Mld[IL]), and from the TYLCV strains from Egypt (TYLCV-[EG:Ism]), and TYLCSV from Sardinia (TYLCSV-[IT:Sar]) and Spain (-TYLCSV-ES[ES]) were used to design a construct that can trigger broad resistance against the different TYLCVs. A high level of resistance was obtained when plants were inoculated with TYLCV-[EG:Ism], TYLCV-Mld[IL], and TYLCSV-ES[ES]. A positive correlation between resistance and the accumulation of virus-specific siRNAs was observed in silenced plants.

Localization of Loci Linked to Resistance to TYLCV in Tomato for Possible Use in Marker-Assisted Breeding

Mapping genes in tomato has been facilitated by the development of saturated maps based on DNA polymorphism (RFLP, AFLP, SSR, SCAR, etc.). A TYLCV-resistance gene originating from *S. chilense* accession LA 1969, *Ty-1*, has been mapped to tomato chromosome 6 using RFLP markers. Three additional loci linked to resistance against TYLCV have been mapped to chromosome 6 using resistant tomato inbreds derived from *S. chilense* accessions LA1932, LA2779, and LA1938. Another resistant gene originating from *S. pimpinellifolium* has been mapped, using RAPD markers, also to chromosome 6 but to a locus different from *Ty-1*. It can be noted that a locus conferring resistance to a nonrelated TYLCV isolate belonging to the species tomato leaf curl Taiwan virus from Taiwan, originating from *L. hirsutum* LA1777, has been located in chromosome 11 using RFLP markers.

See also: African Cassava Mosaic Disease; Bean Golden Mosaic Virus; Beet Curly Top Virus; Emerging Geminiviruses; Tomato Leaf Curl Viruses from India; Satellite Nucleic Acids and Viruses.

Further Reading

Czosnek H (ed.) (2007) *Tomato Yellow Leaf Curl Virus Disease: Management, Molecular Biology, Breeding for Resistance.* Dordrecht: Springer.

Lapidot M and Friedmann M (2002) Breeding for resistance to whitefly-transmitted geminiviruses. *Annals of Applied Biology* 140: 109–127.
Nakhla MK and Maxwell DP (1998) Epidemiology and management of tomato yellow leaf curl disease. In: Hadidi A, Khetarpal RK, and Koganezawa H (eds.) *Plant Virus Disease Control*, pp. 565–583. St. Paul, MN: APS Press.
Picó B, Diez MJ, and Nuez F (1996) Viral diseases causing the greatest economic losses to tomato crop. Part II: The tomato yellow leaf curl virus – A review. *Scientia Horticulturae* 67: 151–196.

Tombusviruses

S A Lommel and T L Sit, North Carolina State University, Raleigh, NC, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Modular evolution Evolution of viral genomes involving the combination of common genes/gene families.
Movement protein A plant viral protein that potentiates viral cell-to-cell movement through plasmodesmata.
Origin of assembly Unique RNA sequence and structure that specifically binds CP to initiate capsid assembly.
Polycistronic The characteristic of a given RNA containing more than one gene (open reading frame).
Quasiequivalence Capsid protein subunits arranged so that they are in somewhat equivalent environments with respect to their adjacent subunits.
(Silencing) suppressor Virally encoded product that inhibits a stage in the host RNA silencing pathway.
Subgenomic RNAs Less-than-full-length RNAs that are produced during replication, usually to express internal open reading frames.

Introduction

The *Tombusviridae* is a relatively large and diverse family of single-stranded, positive-sense, RNA plant viruses with common morphological, structural, molecular, and genetic features. Due to their small size and extremely high virus titer in experimental hosts, viruses in the family are particularly well characterized in terms of virion structure, replication, gene expression strategies, local and systemic movement, suppression of host gene silencing and associated satellite viruses, and defective interfering RNAs.

The family is constituted based on a unifying phylogenetic and biological feature. The RNA-dependent RNA polymerase of tombusviruses is highly conserved in terms of sequence identity, genomic structure, and gene expression and function. Biologically, tombusviruses share the property of being primarily soil transmitted, often without a biological vector, and accumulate to high levels in the roots of infected plants. Beyond these constants the family is remarkably diverse in biology, pathology, host range, and genome organization.

Many of the viruses now comprising the family *Tombusviridae* have been studied for a number of decades. Viruses like tomato bushy stunt virus (TBSV), carnation ringspot virus (CRSV), carnation mottle virus (CarMV), and cymbidium ringspot virus (CymRSV) were first described and virions purified and characterized in the 1940s. The taxonomic and phylogenetic relatedness of many of the viruses now comprising this family was not resolved until the mid-1980s, when genomes of these viruses were first cloned and sequenced.

Taxonomy, Phylogeny, and Evolution

The family *Tombusviridae* of plant viruses is composed of the genera *Aureusvirus*, *Avenavirus*, *Carmovirus*, *Dianthovirus*, *Machlomovirus*, *Necrovirus*, *Panicovirus, and Tombusvirus*, with more than 43 species and 15 tentative species recognized. Several genera are represented by many species whereas three genera are monotypic. The type species of the genus *Aureusvirus* is *Pothos latent virus*. This genus is quite similar to the genus *Tombusvirus* but is distinguished by having significantly different sized movement and silencing suppressor proteins. The type species of the monotypic genus *Avenavirus* is *Oat chlorotic stunt virus*. This species constitutes a separate genus because the genome organization is intermediate between those of the genera *Carmovirus* and *Tombusvirus*. Furthermore its capsid protein (CP) is significantly larger than those found in other genera whose CPs have a protruding (P) domain. *Carnation mottle virus* is the type species of the genus *Carmovirus*. This genus is distinguished by having two small proteins associated with virus movement and a CP with a P domain. The genus *Dianthovirus*, of which

Carnation ringspot virus is the type species, has the most dramatic taxonomic distinction: its genome is split into two segments. *Maize chlorotic mottle virus* is the type and monotypic species of the genus *Machlomovirus*. This genus is structurally quite similar to the genus *Panicovirus* but contains an additional open reading frame (ORF) at the 5′ end of its genome which nearly completely overlaps the polymerase. The genus *Necrovirus* is represented by *Tobacco necrosis virus A*. The genome organization and expression strategy are quite similar to those in the carmoviruses, but the necroviruses have the phylogenetically distinct CPs lacking a P domain. The genus *Panicovirus* is also represented by a single species, *Panicum mosaic virus*. This virus is distinguished by having carmovirus-like movement proteins (MPs), a CP without a P domain, as well as several accessory genes (**Figure 1**).

This family serves as an excellent example supporting the concept of modular evolution of viruses. The polymerase is the sole shared module binding the family. Within the limitations of the viral polymerase the family has taken great liberties with the arrangement and expression of the various genes on its polycistronic RNA. While it is true that all members of the family have a $T = 3$ icosahedral virion, different genera achieve this structure using two phylogenetically distinct CP modules. At this time, it appears that this family has acquired at least three phylogenetically different cell-to-cell movement modules. The sources of the virus suppressor of host gene silencing appear to be equally diverse. In addition, various species within a particular genus have acquired additional unique accessory modules, possibly for transmission by fungal vectors.

The minimal viral polymerases of the *Tombusviridae* lack any identifiable helicase motif, and do not have a nucleotide triphosphate binding motif. They do, however, have the canonical glycine–aspartate–aspartate (GDD) motif found in most RNA polymerases. All *Tombusviridae* polymerases are similarly expressed from an interrupted ORF by one of two translational regulatory mechanisms: terminator readthrough or −1 ribosomal frameshifting (**Figure 1**). The *Tombusviridae* polymerase is also phylogenetically related to that in the genus *Luteovirus* and, to a lesser extent, *Enamovirus* but not with the genus *Polerovirus* in the family *Luteoviridae*. Polymerases of tombusviruses belong to the Sindbis-like superfamily of RNA polymerases, but they are often categorized in a separate subgroup because of their reduced size and missing motifs.

The virions of tombusviruses are morphologically similar. They all form icosahedral $T = 3$ symmetry particles approximately 30–38 nm in diameter. Virions are formed from 180 copies of a single CP subunit ranging from 25 to 48 kDa in size. Virions parse into two morphological subclasses the first of which have rough or granular surfaces and the second smooth surfaces. The smooth particles, such as those found in the genera *Machlomovirus*, *Necrovirus*, and *Panicovirus*, have smaller CPs and lack the P domain found in the larger CPs (**Figure 2**).

There are also common elements in the viral genome structures of tombusviruses. They are all small, single-stranded, positive-sense RNA viruses, encoding four to six ORFs on a single RNA molecule, with the exception of the dianthoviruses, which have a split or bipartite genome. In addition, members of the *Tombusviridae* all rely on the generation of one or more subgenomic RNAs (sgRNAs) to express genes from the polycistronic viral genome.

Virion Structure

Although the underlying size and structure of the virions are similar, the members can be broadly subdivided into two groups based on the presence or absence of a P domain at the c-terminus of the CP. Genera containing P domains (*Aureusvirus*, *Avenavirus*, *Carmovirus*, *Dianthovirus*, and *Tombusvirus*) produce virions that are 32–38 nm in diameter and display a granular surface. These CP subunits range in size from 37 to 48 kDa. The X-ray crystal structures of TBSV and turnip crinkle virus (TCV; *Carmovirus*) revealed the discrete organization of each CP subunit. Generally, the CP subunit can be divided into four domains: the RNA-binding (R) domain is located at the N-terminus followed by the arm (a), shell (S), and P domains (66, 35, 67, and 110 amino acids, respectively, in TBSV). The R domain contains many basic residues and is found in the interior of the virion. Cryoelectron microscopy reconstructions have further revealed the presence of internal ordered cages of RNA intertwined with CP residues from the R domains beneath the virion surface. This internal scaffold may play a role in directing specific packaging of viral RNA and formation of the icosahedral virions. The virion structure is primarily formed by the globular S domains (stabilized by a pair of Ca^{2+} per subunit) which are composed of two sets of four-stranded antiparallel β-sheets. The S domain is also the most highly conserved region of the CP subunit. There is a flexible hinge of five residues between the S and P domains that allows the CP subunit to adopt different configurations by varying the angle between the domains. This feature of the CP subunit overcomes the structural constraints imposed by the icosahedral morphology. P domains (containing antiparallel β-sheet structures in a jellyroll conformation, with one six-stranded β-sheet and one four-stranded β-sheet) of adjacent CP subunits dimerize to produce 90 projections leading to the granular surface texture. Genera lacking the P domain (*Machlomovirus*, *Necrovirus*, and *Panicovirus*) produce virions that are 30–32 nm in diameter with a smooth surface similar to viruses in the genus *Sobemovirus*. These shorter CP subunits range from 25 to 29 kDa in size.



Figure 1 Genomic organization of the type species for each genus in the family *Tombusviridae*. The genomes are aligned vertically at the site of the polymerase readthrough event. Boxes represent known and predicted ORFs with the sizes of the respective proteins (or readthrough products) indicated, for example, p27 for 27 kDa protein. Similarly, colored boxes represent proteins with extensive sequence conservation and function. RT, translational readthrough of termination codon; –1 FS, -1 ribosomal frameshifting event; CP, capsid protein; CRSV, carnation ringspot virus (genus *Dianthovirus*); TBSV, tomato bushy stunt virus (genus *Tombusvirus*); PoLV, pothos latent virus (genus *Aureusvirus*); OCSV, oat chlorotic stunt virus (genus *Avenavirus*); CarMV, carnation mottle virus (genus *Carmovirus*); TNV-A, tobacco necrosis virus A (genus *Necrovirus*); PMV, panicum mosaic virus (genus *Panicavirus*); MCMV, maize chlorotic mottle virus (genus *Machlomovirus*).

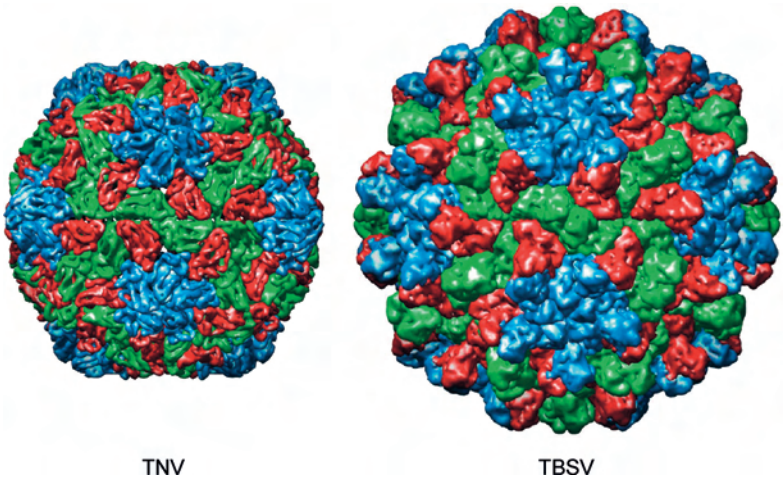


Figure 2 Virion images of tombusviruses based on X-ray crystal structures. Left image is tobacco necrosis virus (TNV) at 2.25 Å. The capsid protein of this virus does not contain a protruding domain and is representative of species in the genera *Machlomovirus*, *Panicovirus*, and *Necrovirus*. Right image is TBSV at 2.9 Å. The capsid protein of this virus contains a protruding domain and is representative of species in the genera *Avenavirus*, *Aureusvirus*, *Carmovirus*, *Dianthovirus*, and *Tombusvirus*. For both images, the individual subunits are colored according to their various conformations: A (blue), B (red), and C (green). From Shepherd CM, Borelli IA, Lander G, *et al.* (2006) VIPERdb: A relational database for structural virology. *Nucleic Acids Research* 34 (Database Issue): D386–D389, VIPERdb.

Due to the quasi-equivalent nature of the CP subunits when arranged in an icosahedron, each subunit can take on one of three distinct conformations, termed A, B, and C (respectively, blue, red, and green in **Figure 2**). The A and B conformations differ in the angle between the S and P domains and their arrangement on the virion surface (A: fivefold axes, B: threefold axes). The C conformation differs from A and B in that their R/a domains intertwine to form an ordered internal structure around threefold axes of symmetry termed the β-annulus. The three different CP subunit conformations pack together as either AB or CC dimers in the virion particle. For TCV, virion assembly initiates with three CP dimers and viral RNA followed by formation of the virion shell according to structural constraints imposed by CP/RNA interactions. In TCV, the origin of assembly RNA sequence (which specifically initiates the interaction with CP subunits) is contained within a 186 nt region at the 3′ end of the CP ORF. Assembly studies with TCV also showed that only RNAs equal to or smaller than 4.35 kbp in size could be packaged, suggesting very strict size limitations for icosahedral virions.

Virions of tombusviruses have an M_r of ~8.2–8.9×10^6 and produce a single, well-defined band upon centrifugation with sedimentation coefficients ranging from 118 to 140$S_{20,w}$. Virion densities range from 1.34 to 1.36 g cm^{-3} in CsCl gradients. Virions are stable at acidic pH, but expand above pH 7 and in the presence of ethylenediaminetetraacetic acid (EDTA). Virions are resistant to elevated temperatures although thermal inactivation usually occurs above 80 °C. Due to the lack of a lipid membrane, virions are insensitive to organic solvents and nonionic detergents.

Most genera produce virions containing a single molecule of positive-sense, linear single-stranded RNA (ssRNA) ranging in size from 3.7 to 4.8 kb depending on the genus. The genomic RNA constitutes about 17% of the particle weight. The exception is the genus *Dianthovirus* where virions contain two genomic RNAs: a large RNA-1 of ~3.9 kb and a smaller RNA-2 of 1.5 kb. The genomic RNAs lack a 5′ cap structure and the 3′ ends are not polyadenylated nor do they form tRNA-like structures. Satellite RNAs (satRNAs) of nonviral origin are associated with several genera. Satellite viruses (which code for their own CP) are also associated with several genera but these are readily distinguishable due to their significantly smaller dimensions. Both small RNAs modify the symptoms of their respective helper viruses.

Genome Organization and Replication Strategy

The dimensions of the icosahedral virions constrain the genome sizes of the various members of the family *Tombusviridae*. This forces the members to adopt several strategies for expression of their genetically compact, polycistronic genomes. Their unifying genomic feature is the presence of an interrupted ORF for the virally encoded RNA-dependent RNA polymerase at the 5′ end of the genome. The interruption is manifested as either an amber termination codon which may be read through (most members) or a −1 ribosomal frameshifting signal (genus *Dianthovirus* only). In either case, this leads to the direct translation of two ORFs with identical amino

termini from the genomic RNA (**Figure 1**). These polymerase subunits share a high degree of sequence similarity within the family. Notably, they do not contain a helicase-type motif and the genomes do not encode a helicase-like ORF. ORFs downstream of the polymerase are expressed via 3′ co-terminal sgRNAs with the resident ORFs produced with an assortment of translational strategies such as ribosomal scanning and frameshifting. Aside from the single CP ORF mentioned previously, the genomes of *Tombusviridae* also encode one or more MPs that are involved in cell-to-cell spread of the virus. These range in size from the 34–35 kDa version found in the genus *Dianthovirus* to the 22 kDa product found in the genus *Tombusvirus* and down to the 6–9 kDa pair found in the genus *Carmovirus*. In most cases, the MPs can potentiate the local movement of unencapsidated viral RNA but systemic infection generally requires encapsidation. The exception is for members of the genus *Tombusvirus* which can infect systemically without CP albeit at a reduced rate. The other prominent protein product with a clearly defined function is the genus *Tombusvirus* p19 protein which is a potent suppressor of post-transcriptional gene silencing (PTGS). p19 binds double-stranded short interfering RNAs (siRNAs) that are generated by a Dicer-like RNase as part of the host RNA-silencing response to viral infection. This binding prevents incorporation of the siRNAs into the RNA-induced silencing complex (RISC) to prevent further cleavage of viral RNAs. Although other members of the family may not encode a unique suppressor protein, it is known that other proteins can have suppressor activity aside from their primary functions. This is the case for TCV where the CP is the viral suppressor of PTGS. Various additional ORFs are also encoded with undefined functions. One other method utilized by members of the family *Tombusviridae* for gene expression is genome segmentation which is only employed by the genus *Dianthovirus* where RNA-2 is monocistronic and encodes the MP.

Since the genomic RNAs are positive sense, they are directly infectious as unencapsidated RNAs. However, the lack of a 5′ cap structure and a 3′ poly(A) tail precludes the normal circularization procedure employed by mRNAs for translation. Instead, a cap-independent mechanism involving a long-distance RNA–RNA interaction between sequence elements in the 5′ and 3′ noncoding regions has been demonstrated in TBSV and tobacco necrosis virus-A (TNV-A) and proposed in the case of red clover necrotic mosaic virus (RCNMV; genus *Dianthovirus*) RNA-1. The direct translation readthrough strategy ensures that the longer ORF (containing the catalytic site of the polymerase) is present in only 5–10% of the translation products since readthrough is inefficient. Translation of the virally encoded polymerase subunits is followed by localization to and proliferation of cellular membranes. The location of viral replication varies dependent on the particular virus and is specified by the pre-readthrough portion of the polymerase. In all cases studied to date, the polymerase anchors on membranes, causing proliferations. The advent of yeast-based replication systems for TBSV, cucumber necrosis virus (CNV; genus *Tombusvirus*), and CymRSV has provided valuable insights into the host components utilized by the viral replication complex.

Once the polymerase has been assembled, it binds to the 3′ terminus to initiate minus-strand synthesis. If full-length copies are generated, these serve as templates for synthesis of progeny genomic RNAs. This synthesis is highly asymmetric with positive-stranded RNA accumulating at much higher levels than negative-stranded RNA. Occasionally, positive-strand synthesis will be terminated prematurely which leads to the formation of templates for plus-strand sgRNA synthesis. This premature termination is controlled by long-distance RNA–RNA interactions which occur in *cis* (for instance, in TBSV) or in *trans* (for instance, RCNMV). Generally, sgRNAs are produced later in the infection cycle and behave as monocistronic mRNAs. However, the sgRNAs also demonstrate various translational strategies such as ribosomal scanning in the case of p19 expression which is nested within the p22 ORF in TBSV. In this case, the p22 start codon is suboptimal and occasionally read through by ribosomes before translation at the optimal p19 start codon.

During replication, defective interfering (DI) RNAs (which are not packaged into virions) arise in viruses belonging to some genera. These DI RNAs are derived from the viral genome and are replicated to very high titers which lead to their interfering with viral genomic replication levels. DI RNAs have been used extensively to delimit critical sequence elements required for RNA replication.

Transmission, Host Range, and Epidemiology

The natural host range of a given species is relatively narrow; however, the experimental host range tends to be broad. Members can infect either monocotyledonous or dicotyledonous plants, but no species can infect both. Natural infections can be limited to or are often concentrated in the root system. Many species induce a necrosis symptom in the foliar parts of the plant. All species are readily transmitted by mechanical inoculation and by host vegetative propagation and some may be transmitted by contact and through seeds. A number of species are readily detected in the soil, surface waters, rivers, lakes, and even the ocean. Transmission by the chytrid fungi in the genus *Olpidium* and beetles have also been reported for members of several genera. Most, if not all, members can be transmitted through the soil either dependent on, or

independent of, a biological vector. This rather unusual soil and water mode of transmission is unique to this plant virus family and is based on the unusually robust constitution of the virion.

Geographical distribution of particular species varies from wide to restricted. Most species occur in temperate regions although legume-infecting carmoviruses and a tentative member of the genus *Dianthovirus* have been recorded from tropical areas.

In nature, viruses in the family *Tombusviridae* can be spread in a variety of ways, including seed and pollen transmission, mechanical transmission, vegetative propagation and grafting, growth in infected soil, and by vectors such as fungi, thrips, and beetles. As mentioned above, one hallmark of members of the family *Tombusviridae* is the amount of virus present in the roots of infected plant tissue, and the ease of transmission through the soil, whether that transmission is dependent on a biological vector or not. For viruses that can be transmitted by fungi, there is a considerable amount of specificity required between the virus and the fungal vector. For example, CNV (*Tombusvirus*), melon necrotic spot virus (*Carmovirus*), and cucumber leaf spot virus (*Aureusvirus*) are transmitted by the root-inhabiting chytrid fungus *Olpidium bornovanus*, whereas TNV (*Necrovirus*) is transmitted exclusively by *Olpidium brassicae*.

Pathogenesis

For the species in which it has been studied, the virus titers and pathogenic effects are at least as high, if not higher, in the root system of the host as in the other parts of the plant. This is consistent with the robust nature of the virions and that viruses in the family are transmitted through the soil.

Many tombusviruses generate DI RNAs that can affect pathogenesis. DI RNAs are essentially deletion mutants of the viral genome which are generated by errors in replication such as rearrangement or recombination. Several species such as TBSV and TCV produce DI RNAs during viral infection. For TBSV the presence of DI RNAs results in attenuation of symptoms, whereas for TCV the presence of DI RNAs intensifies symptoms. Many species, including both TBSV and TCV, are also known to harbor satRNAs. These small RNAs are generally not derived from viral sequences, but they depend on the helper virus for replication and packaging. As with DI RNAs, they can attenuate TBSV or intensify symptoms (TCV).

The origin of nonhomologous satRNAs is unclear at present, but like DI RNAs, satRNAs can be generated from recombination events with other satRNAs, the viral genome, DI RNAs, or host sequences. Both DI RNAs and satRNAs have been useful molecular tools for studying viral replication and recombination. Since both satRNAs and DI RNAs are dependent on the host or parent virus for replication, they must have retained any sequence or structural signals required for recognition by the viral polymerase, and therefore can help determine exactly what those signals are.

Recombination can facilitate viral evolution as well as repair of viral genomes, and so it is an important factor in the virus life cycle and it can have profound effects on pathogenesis. In the family *Tombusviridae*, recombination in the genera *Carmovirus* and *Tombusvirus* has been shown to repair damaged or deleted 3′ ends of virus-associated RNAs, as well as generate new satRNAs or DI RNAs. Recombination can occur between both homologous and nonhomologous sequences, probably by a copy-choice mechanism in which the viral replicase jumps from one template to another during the replication process.

Plants infected with tombusviruses display a distinctive cytopathology, observed by electron microscopy as dense-staining features. These features are known as multivesicular bodies, and are believed to be sites of viral replication as well as accumulation of exceedingly high concentrations of progeny virions. To date, cell biology studies have shown that species can replicate on, remodel, and proliferate membranes of peroxisomes, mitochondria, and the cortical ER. There appears to be no correlation between a specific organelle membrane used for replication and taxa.

See also: Assembly of Viruses: Enveloped Particles; Assembly of Viruses: Nonenveloped Particles; *Carmovirus*; Defective-Interfering Viruses; Luteoviruses; Machlomovirus; *Necrovirus*; Satellite Nucleic Acids and Viruses; Togaviruses: Alphaviruses; Vector Transmission of Plant Viruses; Viral Suppressors of Gene Silencing.

Further Reading

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.* San Diego, CA: Elsevier Academic Press.

Kneller EL, Rakotondrafara AM, and Miller WA (2006) Cap-independent translation of plant viral RNAs. *Virus Research* 119: 63–75.

Martelli GP, Gallitelli D, and Russo M (1988) Tombusviruses. In: Koenig R (ed.) *The Plant Viruses, Polyhedral Virions with Monopartite RNA Genomes,* vol. 3, pp. 13–72. New York: Plenum.

Miller WA and White KA (2006) Long-distance RNA–RNA interactions in plant virus gene expression and replication. *Annual Review of Phytopathology* 44: 447–467.

Morris TJ and Carrington JC (1988) Carnation mottle virus and viruses with similar properties. In: Koenig R (ed.) *The Plant Viruses, Polyhedral Virions with Monopartite RNA Genomes,* vol. 3, pp. 73–112. New York: Plenum.

Nagy PD and Pogany J (2006) Yeast as a model host to dissect functions of viral and host factors in tombusvirus replication. *Virology* 344: 211–220.

Nagy PD and Simon AE (1997) New insights into the mechanisms of RNA recombination. *Virology* 235: 1–9.

Robbins MA, Reade RD, and Rochon DM (1997) A cucumber necrosis virus variant deficient in fungal transmissibility contains an altered shell protein domain. *Virology* 234: 138–146.

Rochon D, Kakani K, Robbins M, and Reade R (2004) Molecular aspects of plant virus transmission by olpidium and plasmodiophorid vectors. *Annual Review of Phytopathology* 42: 211–241.
Rossman MG and Johnson JE (1989) Icosahedral RNA virus structure. *Annual Review of Biochemistry* 58: 533–573.
Russo M, Burgyan J, and Martelli GP (1994) Molecular biology of *Tombusviridae*. *Advances in Virus Research* 44: 381–428.
Scholthof HB (2006) The tombusvirus-encoded P19: From irrelevance to elegance. *Nature Reviews Microbiology* 4: 405–411.
Shepherd CM, Borelli IA, Lander G, *et al.* (2006) VIPERdb: A relational database for structural virology. *Nucleic Acids Research* 34 (Database Issue): D386–D389.
White KA and Nagy PD (2004) Advances in the molecular biology of tombusviruses: Gene expression, genome replication, and recombination. *Progress in Nucleic Acid Research and Molecular Biology* 78: 187–226.

Torovirus

A E Hoet, The Ohio State University, Columbus, OH, USA
M C Horzinek, Utrecht University, Utrecht, The Netherlands

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Colostrum-deprived (CD) calves Conventional calves that did not receive antibody-rich first milk or colostrum during the first 6–12 h of life, therefore lacking of passive immunity from the mother (calves are hypogammaglobulinemic individuals when they born).
Gnotobiotic (Gn) calves Animals that are obtained and maintained in germ-free environments, in which the composition of any associated microbial flora, if present, is fully defined.

History

Equine torovirus (EToV), originally referred to as Berne virus, was accidentally isolated in equine kidney cells in 1972 from a rectal swab taken from a horse with diarrhea. EToV is the only torovirus that has been propagated in cell culture, in lines of equine dermis or embryonic mule skin cells, where it causes a cytopathic effect that results in cell lysis. While Berne virus was not neutralized by antisera against known equine viruses, serologic cross-reactions were observed in neutralization tests and enzyme-linked immunosorbent assay (ELISA) using sera from calves that had been experimentally infected with morphologically similar particles, then named 'Breda' viruses.

The first Breda virus, now called bovine torovirus (BToV), was discovered in 1979 during an investigation in a dairy herd in Breda (Iowa), in which severe neonatal calf diarrhea had been a problem for three consecutive years. After this initial report, other strains of BToV were identified in beef calves from Ohio, and in a colostrum-deprived (CD) calf from Iowa. Despite repeated attempts, BToV cannot be adapted to grow in cell or tissue cultures and must be passaged in gnotobiotic (Gn) calves, which has hampered its biochemical, biophysical, and molecular characterization. Most of the studies on the pathogenesis and pathology of torovirus infections have been done in BToV-infected Gn and CD calves, as well as in limited field studies; in contrast, most of the biochemistry and morphogenesis data on toroviruses are based on EToV studies.

In 1984, torovirus-like (TVL) particles were detected in the feces from human patients with gastroenteritis by using electron microscopy (EM). Since then, reports of human toroviruses (HToVs) in children and adults with acute diarrhea have appeared in several countries. TVL particles have also been detected in fecal samples from pigs, and named porcine torovirus (PToV). Proof that the observed structures were not artifacts was obtained when toroviral RNA sequences were found in the feces of piglets and in stools from humans with diarrhea. In recent years, TVL particles have also been detected in turkeys and have been associated with a 'stunting syndrome'.

Taxonomy and Classification

Toroviruses are single-stranded, positive-polarity RNA viruses with a peplomer-bearing envelope. The term *torus* (Latin) refers to the circular convex molding in the form of a doughnut that some columns or pilaster have at their bases; indeed, it was the unique biconcave disk and C-shape of the virion in the extracellular environment that suggested this naming. Since 1992, the genus *Torovirus* has been included with the genus *Coronavirus* and the newly recognized genus *Bafinivirus* in the family *Coronaviridae*, based on similarities in genomic organization and replication strategies. Toro- and coronaviruses are also

ancestrally related: their polymerase and envelope genes diverged from those of a common predecessor. Because of their inclusion in the family *Coronaviridae,* the nomenclature for coronavirus genes, mRNAs, and structural proteins have also been applied to toroviruses. However, the lack of sequence homology in the structural genes and the absence of antigenic relatedness with coronaviruses justify their taxonomic position as a separate genus. The International Committee on Taxonomy of Viruses (ICTV) presently recognizes four species in the genus *Torovirus*: *Equine torovirus*, *Bovine torovirus*, *Porcine torovirus*, and *Human torovirus.*

The families *Coronaviridae* and *Arteriviridae*, as well as the new family *Roniviridae*, are the constituents of the order *Nidovirales*, the second order in animal virology (after the order *Mononegavirales*). This assignment is based on their similar basic genomic organization and common replication strategy: the synthesis of a 3′ co-terminal nested set of subgenomic mRNAs, and the possession of two open reading frames (ORFs) connected by a frameshift site to express a replicase directly from the genomic RNA. This nested set of mRNAs was the foundation for the name of the order *Nidovirales* (from Latin *nidus*, 'the nest').

Virion Properties

Torovirus particles possess a nucleocapsid with helical symmetry coiled into a hollow tube (diameter 23 nm, average length 104 nm, periodicity 4.5 nm). Extracellular, negatively stained torovirus virions are generally observed as kidney- or C-shaped particles (105–140 nm × 12–40 nm). They can also be seen as spherical or oval particles (89 ± 7 nm × 75 ± 9 nm) or rod-shaped virions (35 nm × 170 nm), depending on the different orientations of the virions with respect to the electron beam. A graphic representation of a torovirion is shown in **Figure 1**. A tightly fitting envelope, 11 nm thick, surrounds the virion structure bearing prominent drumstick-shaped peplomers (17–24 nm), and a fringe of shorter spikes (8–10 nm), which represent the spike and the hemagglutinin-esterase (HE) proteins, respectively. Intracellularly, toroviruses are observed as elongated tubules with rounded ends (rod-shaped virions, 35–42 nm × 80–105 nm), located in the cytoplasm of infected intestinal cells.

Genome

The torovirus genome consists of a single-stranded, polyadenylated RNA of positive (messenger) polarity, which is about 28.5 kbp in length. Recently, the BToV genome has been completely sequenced, and it has been shown to comprise 28 475 nt and contain six ORFs (see **Figure 1**), each of which encodes a known protein. ORF1a and ORF1b are the most 5′ proximal reading frames and constitute the replicase (RNA-dependent RNA polymerase) gene, which is expressed as a large precursor protein directly from the genomic RNA by a ribosomal frameshift mechanism, similar to other nidoviruses. The large product of these ORFs is apparently involved in the synthesis of a negative-strand RNA and the onset of genomic and subgenomic RNA synthesis. The other four ORFs correspond to structural protein genes and are expressed by the production of a 3′

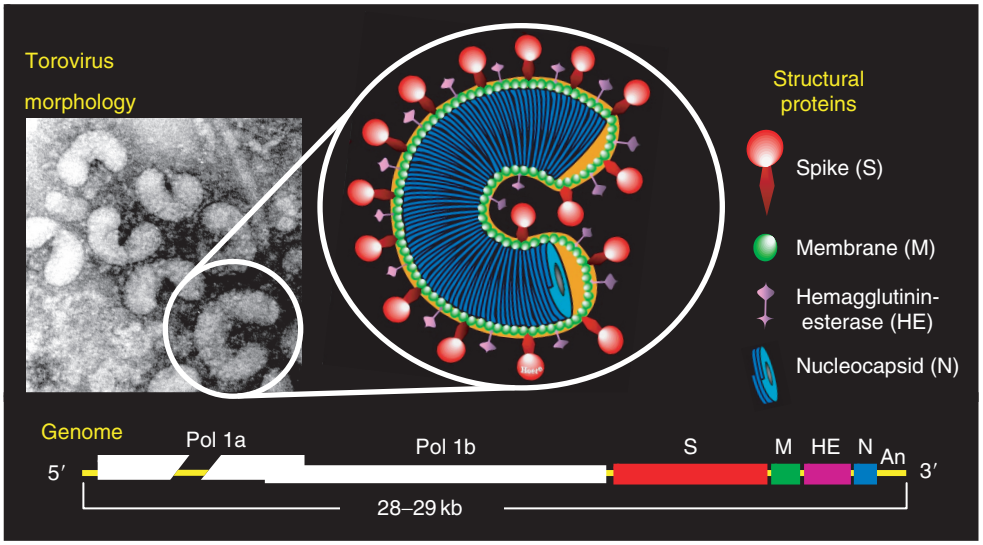


Figure 1 Typical extracellular morphology of a torovirus as visualized by immune electron microscopy (IEM), with a graphic representation of torovirus virion and its structural proteins and genome. Modified with permission from Hoet AE (in press) Torovirus pathogenesis and immune responses. In: Perlman S, Snijder EJ, and Gallagher TM (eds.) *The Nidoviruses*, copyright 2008 by the ASM Press.

co-terminal nested set of four mRNAs. The BToV ORF2 (4752 nt), ORF3 (702 nt), ORF4 (1248–1251 nt), and ORF5 (504 nt) encode the spike (S), membrane (M), HE, and nucleocapsid (N) proteins, respectively.

Proteins

Proteins with molecular weights of 20, 22, 37, and 80–100 kDa have been identified in EToV virions. Detergent treatment of virions releases the 22, 37, and 80–100 kDa proteins, which indicates their association with the envelope. Similar polypeptides of 20, 37, 85, and 105 kDa have been identified in purified BToV by means of surface radioiodination. The nucleocapsid (N) protein (167 aa) is the most abundant polypeptide found in the virion (80–84%) with a predicted molecular weight of 18.3–19.2 kDa. Blotting experiments performed with EToV have identified this internal protein as the only RNA-binding polypeptide in the virion. The M glycoprotein (233 aa, 26.5 kDa) is the second most abundant protein (~13%) and is probably associated with the envelope. Computer analysis has revealed characteristics of a class III membrane protein lacking a cleaved signal sequence, but containing three successive transmembrane α-helices in the N-terminal half. The M protein accumulates in intracellular membranes, predominantly those of the endoplasmic reticulum and is believed to play a role in assembly, maturation, and nucleocapsid recognition during the budding process.

The heterogeneous, N-glycosylated, 80–100 kDa protein is recognized by both neutralizing and hemagglutination-inhibiting monoclonal antibodies and is therefore identified as the spike (S) protein projecting from the virion surface. The spike (S) gene encodes an apoprotein (1581 aa) with a molecular weight of about 178 kDa. The deduced amino acid sequence contains domains typical of type I membrane glycoproteins: an N-terminal signal sequence, a putative C-terminal transmembrane anchor, and a cytoplasmic tail. The HE is a class I membrane N-glycosylated protein (416–417 aa) of 65 kDa that is also located in the BToV envelope. It has an N-terminal signal sequence, a C-terminal transmembrane domain, several N-glycosylation sites, and a putative 'FGDS' motif which displays acetylesterase activity specific for *N*-acetyl-9-*O*-acetylneuraminic acid. The HE protein could be an additional receptor-binding protein (next to the spike protein) but without a function in viral entry. EToV virions lack an HE protein, only possessing a partial sequence in ORF4.

Physical Properties

Buoyant densities of 1.16, 1.17, 1.18, and 1.14 g ml^{-1} have been reported for the virions of EToV, BToV serotype 1, BToV serotype 2, and HToV, respectively. EToV is remarkably stable in the environment, and relatively resistant to phospholipase C, trypsin, chymotrypsin, and even deoxycholate; however, Triton X-100 and organic solvents destroy its infectivity. EToV is stable within a wide range of pH, being inactivated only below pH 2.5 or above pH 10.3. BToV1 appears to be less stable than both EToV and BToV2 as changes in its sedimentation behavior and density have been observed after prolonged storage at −70 °C. The infectivity of a fecal preparation containing BToV1 has been reported to be lost completely after 3 weeks at 4 °C, whereas EToV in cell-free supernatant remained stable for 92 days. Storage of toroviruses at −20 to −70 °C helps to preserve infectivity. However, even at these temperatures the viruses will deteriorate, though at a slower rate. Repeated cycles of freezing and thawing of purified BToV2 results in loss of peplomers and disintegration of virions.

Replication and Morphogenesis

Because it can be grown in cell culture, the morphogenesis of EToV has been most extensively studied. Ultrastructural and immunofluorescence (IF) studies on intestinal cells from BToV-infected calves have shown similarities with EToV morphogenesis.

Attachment, Entry, and Uncoating

Attachment to the apical surface of enterocytes is apparently mediated through the spike proteins, but the HE proteins may be involved as well. Entry or penetration of BToV into enterocytes is apparently by receptor-mediated endocytosis. Lysosomal degradation in vesicles containing the virus is probably responsible for uncoating and subsequent release of the BToV RNA. The site at which this occurs has not yet been determined.

EToV replication occurs in the cytoplasm. Preformed tubular capsids bud through membranes of the Golgi stack and of the endoplasmic reticulum. A host cell nuclear function seems to be required since ultraviolet (UV) preirradiation of cells, actinomycin D, and α-amanitin have been reported to reduce virus yields. The replication cycle takes around 10–12 h to complete.

Transcription

In EToV-infected cells, five virus-specific, polyadenylated mRNAs are found with sizes of >20.0, 7.5, 2.1, 1.4, and 0.8 kbp. Northern (RNA) blot hybridizations with restriction fragments from cDNA clones have shown that the five EToV mRNAs form a 3′ co-terminal nested set. Sequence analysis has revealed the presence of four complete ORFs with initiation codons coinciding with the

5′ ends of EToV RNAs 2–5, respectively; RNA 5 is contiguous on the consensus sequence. EToV RNAs 1–3 are transcribed independently, as has been shown by UV transcription mapping. The genes for M, HE, and N are preceded by short noncoding 'intergenic' regions, containing a transcription-regulating element (TRE) conforming to the consensus sequence 5′ (C)ACN$_{3–4}$CUUUAGA 3′. A copy of this sequence is also present at the extreme 5′ terminus of the genome. In contrast, the S gene overlaps with the replicase gene and the N-terminal 28 residues of S are in fact encoded by an internal (–1) reading frame within ORF1b; moreover, there is no TRE. The production of this 3′ co-terminal nested set of mRNAs characterizes the toroviruses and justifies their inclusion into the order *Nidovirales*.

Translation

No RNA-dependent RNA polymerase is found in torovirus virions. The torovirus replicase is probably translated as soon as the RNA is liberated. Translation yields two large polyproteins, from which by proteolytic cleavage the various subunits of the viral replicase/transcriptase are derived, as well as accessory proteins of as yet unknown function. Downstream of ORF1b, there are the genes for the structural proteins S, M, HE, and N (as ordered from 5′ to 3′); these are translated from four subgenomic mRNAs, numbered 2–5 (with the genomic RNA as RNA1).

Post-Translational Processing

The N-glycosylated S protein is derived from the processing of a 200 kDa precursor present in infected cells, but not in virions. Eighteen potential N-glycosylation sites, two heptad repeat domains, and a possible 'trypsin-like' cleavage site exist in the spike protein amino acid sequence. The mature S protein consists of two subunits and their electrophoretic mobility upon endoglycosidase F treatment suggests that the predicted cleavage site is functional *in vivo*. The heptad repeat domains are probably involved in the generation of an intrachain coiled-coil secondary structure; similar interchain interactions can play a role in the formation of the observed S protein dimers. The intra- and interchain coiled-coil interactions may stabilize the stalk of the torovirus peplomers.

Assembly, Budding, Egress, and Maturation

About 10 h after infection, EToV particles are observed within parts of the unaltered Golgi apparatus, and extracellularly. At that time, tubular structures of variable length, diameter, and electron density appear in the cytoplasm, and also in the nucleus of infected cells, probably representing preformed nucleocapsids. It is unknown whether the accumulation of nucleocapsids in the nucleus reflects a nuclear phase in the replication of EToV or some sort of defective assembly. Viruses predominantly bud into the lumen of Golgi cisternae. The preformed nucleocapsid tubules approach the Golgi membrane with one of the rounded ends oriented toward the membrane and attach to it laterally. During budding, the nucleocapsid is apparently stabilized, leading to a higher electron density and a constant diameter (23 nm). Release into the intestinal lumen is probably through reverse pinocytosis. Virus maturation apparently occurs intracellularly during the egress process, where the virus nucleocapsid appearance changes from a straight rod (intracellular) into a torus shape (extracellular). The characteristic torus morphology of BToV is only observed in extracellular viral particles or in vacuoles near the cell surface, and never in the cytoplasm.

Geographic Distribution

In cattle, toroviruses have been detected by ELISA, reverse-transcriptase polymerase chain reaction (RT-PCR), and/or immune electron microscopy (IEM) in Austria, Belgium, Canada, Costa Rica, France, Germany, Great Britain, Hungary, Japan, Netherlands, New Zealand, South Africa, and the USA. BToV-seropositive cattle have also been reported in Belgium, France, Germany, India, Switzerland, United Kingdom, and the USA, with seroprevalence ranging between 55% and 94.6%. Most adult horses tested in Switzerland possess neutralizing antibodies to EToV. HToV appears to occur in Canada, Brazil, France, Great Britain, India, the Netherlands, and the USA.

Host Range

Neutralizing antibodies to EToV have been found in sera from horses, cattle, goats, sheep, pigs, rabbits, and feral mice, but not in humans or in carnivores. The host range of BToV appears to be restricted to cattle; however, seropositive reactions to BToV have also been detected in several ungulate species.

TVL particles have been observed in the feces of children and adults with diarrhea. Interestingly, these can be aggregated after the addition of anti-BToV calf sera, can be detected by using a BToV antigen capture ELISA, and the hemagglutination of rat erythrocytes by TVL particles can be inhibited by BToV antisera. These observations indicate antigenic cross-reactivity between HToV and BToV, and may point to a zoonotic connection. TLVs have also been seen in fecal samples of cats and dogs but neither serologic nor molecular identification has been obtained.

Genetic Relationships

There is limited information available on genetic variation amongst toroviruses. The complete genome sequence has been reported only for BToV. However, partial genome sequences have been obtained and used to chart the genetic diversity. In general, there is little divergence (20–40%) among known genotypes of EToV, BToV, PToV, and HToV in the (S, M, HE, and N) genes. Phylogenetic analyses have shown that all BToV strains are closely related, whether they are of European or American origin. Also, all PToV variants form a distinct genetic cluster. However, BToV and PToV sequences are sufficiently different to be assigned as different genotypes. HToVs show a high degree of similarity to New World BToVs (83%), and less with European strains of BToV (73%) and PToV (56%).

Antigenic Properties

In addition to the typical torovirus morphology, BToV, EToV, HToV, PToV, and Lyon-4 virus (Breda-like BToV detected in France) share common antigens. Currently, only one strain of EToV has been isolated, and all attempts to obtain a second equine isolate have been fruitless. Two strains of BToV have been reported in addition to the original isolate described by Gerald Woode and colleagues. One of the strains was detected in feces from a 5-month-old diarrheal calf in Ohio; the second (Iowa strain) was recovered from a 2-day-old experimental animal. On the basis of their cross-reactivity in ELISA, IEM, and hemagglutination/hemagglutination inhibition (HA/HI) assays using rat or mouse erythrocytes, the three isolates were assigned to two serotypes: BToV1, represented by the Iowa 1 isolate, and BToV2, comprising the Ohio and the second Iowa isolate.

Antigenic cross-reactivity has been demonstrated by ELISA, IEM, HI, and immunoblotting between BToV and HToV, indicating a close relationship. Several authors reported that HToV particles detected in humans with persistent diarrhea, and morphologically similar to BToV, could be agglutinated by BToV antiserum; stronger reactions are observed when BToV-2 antisera are used. Hyperimmune sera to BToV have also been used to detect toroviruses in humans by ELISA and IEM.

Epidemiology

Several epidemiological studies have demonstrated a high seroprevalence of BToV antibodies in several different groups of cattle, indicating that the virus may circulate with high frequency in these populations. BToV have also been detected by ELISA and/or RT-PCR in cases of gastroenteritis in cattle. Up to 44% of the BToV-positive samples from these cases did not contain other major enteric pathogens. Calves up to 4 months of age are highly susceptible to diarrhea induced by BToV, especially those below 3 weeks of age. The virus has also been recognized in 5–6-month-old beef calves arriving from sales barns. Intermittent BToV shedding can occur in young calves during the first 10 months of life. Older calves and adult animals can also shed BToV at different ages, perhaps by intermittent subclinical infections or by contracting new BToV infections. Levels of maternal BToV-specific antibodies circulating in the calf influence the clinical outcome of the infection; a seronegative neonatal calf is about seven times more likely to develop diarrhea than a seropositive calf.

Transmission and Tissue Tropism

It has been suggested that the transmission of BToV is via the oral/nasal route by direct contact with contaminated feces or nasopharyngeal secretions. Oral inoculation of calves with BToV has been shown to induce diarrhea with virus shed in feces under experimental conditions. The nasal route is another possible pathway for entry as BToV antigen and viral RNA have been detected in the nasal secretions of feedlot calves. Additionally, diarrhea has been induced after intranasal inoculation in Gn and CD calves. For bovine coronavirus (BCoV), respiratory tract infections have been reported to occur prior to enteric infections, indicating the possible importance of this route of transmission in the spread and pathogenesis of this distantly related group of enteric nidoviruses. It is possible that BToV, like BCoV, could initially replicate in nasal epithelial cells, and thereby amplify before being swallowed and infecting the intestinal tract. This hypothesis for BToV pathogenesis should be further studied.

BToV has a tissue tropism for enterocytes located from the lower half of the villi extending into the crypts, affecting the caudal portion of the small intestine (midjejunum through ileum) and the large intestine. Infection of other types of cells and organs by BToV has not been reported.

Pathogenesis and Clinical Features of Infection

All BToV strains are pathogenic, causing mild to profuse diarrhea in experimentally and naturally infected young calves. Twenty-four to seventy-two hours post exposure, the first clinical signs are observed (mild fever, depression, weakness, and anorexia), followed by a greenish-yellow to bright yellow watery diarrhea that lasts for 3–5 days. Calves may develop severe dehydration and die. Fecal virus shedding begins 24–72 h post infection, coinciding with the onset of diarrhea, and lasts for 2–6 days. Shedding

peaks around 3–4 days post infection. Mixed infections with other enteric viruses, such as rotaviruses or astroviruses, result in a more severe watery diarrhea than is induced by either virus alone. In CD calves with a normal intestinal flora, diarrhea is generally more severe than in Gn calves. Sporadic and recurrent shedding of BToV can last for up to 4 months. BToV has also been detected in nasal samples. Further studies are needed to analyze BToV replication and shedding in the respiratory tract as well as its role in respiratory pathologies.

EToV seroconversion occurs in horses between 10 and 12 months of age, without the appearance of symptoms. Experimentally infected animals (intravenous route) have been reported to seroconvert without clinical signs. Oral infection experiments in horses have not been reported to date.

Several studies have shown an association of HToV infection with diarrhea in children. In one study, 35% of children with enteritis shed HToVs in their feces, but only 14.5% of the asymptomatic controls shed the virus (statistically significant difference – odds ratio 3.1). Affected children showed watery diarrhea, vomiting three to four times daily, and dehydration as a consequence; neither fever, nor the presence of other enteric pathogens, was recorded. Recently, fecal excretion of HToV has been associated with nosocomial infections in infants with necrotizing enterocolitis (NEC). Immunocompromised children appear to be highly susceptible to disease following HToV infection.

Pathology and Histopathology

The target organs of BToV in calves are the lower half or two-thirds of the small intestine and the entire large intestine, particularly the spiral colon. There is little macroscopic evidence of the infection. Histological examination shows villous atrophy and epithelial desquamation in randomly scattered areas from the mid-jejunum to the lower small intestine, as well as areas of necrosis in the large intestine. As shown by IF, both crypt and villus epithelial cells contain antigen. The watery diarrhea is probably a result of the loss of reabsorptive capacity of the colonic mucosa, combined with malabsorption in the small intestine. The germinal centers of Peyer's patches are depleted of lymphocytes and may occasionally show fresh hemorrhage. The dome epithelial cells, including the M-cells, display the same cytopathic changes as seen in the absorptive cells of the villi. Virions are found in cells of both the small and large intestine, and between enterocytes at the basal and lateral plasma membranes. In macrophages of the lamina propria, virions in various stages of degradation are found.

Antigen is detected as early as 48 h after infection in epithelial cells of the lower half of the villi and crypts of the affected areas, as well as in dome epithelium. Fluorescence is cytoplasmic (although a few nuclei may be faintly stained) and generally most pronounced in the intestines with the least tissue damage. The mid-jejunum is infected first and the infection eventually reaches the large intestine. Diagnosis by IF should be performed preferentially on sections of the large intestine from calves killed after the onset of diarrhea (i.e., several days after infection of the epithelium).

Immune Response

Up to the age of 4 months, all calves in a sentinel experiment regularly excreted BToV in their feces. They showed early serum IgM responses despite the presence of IgG1 isotype maternal antibodies, but no IgA seroconversion. Antibody titers then decreased below detection; persistent IgG1 titers developed in only a few animals. After introduction into the dairy herd at 10 months of age, all calves developed diarrhea and shed virus. Seroconversion for all antibody isotypes was observed at this stage, indicating lack of mucosal memory. In contrast, coronavirus infection in the presence of maternal antibodies leads to isotype switch and a memory response.

Prevention and Control

There are no specific preventive measures for this virus; however, general hygiene, biosecurity practices, and the intake of adequate, protective amounts of colostrum can be used to prevent BToV infections. There are no reports on the effects of disinfection or heat sterilization on toroviruses.

Acknowledgments

The authors would like to thank R. de Groot, Utrecht, The Netherlands, for the use of unpublished information from his torovirus review article in press.

See also: Coronaviruses: General Features; Coronaviruses: Molecular Biology.

Further Reading

Cavanagh D and Horzinek MC (1993) Genus *Torovirus* assigned to the *Coronaviridae*. *Archives of Virology* 128: 395.

De Groot RJ (in press) Molecular biology and evolution of toroviruses.

Draker R, Roper RL, Petric M, and Tellier R (2006) The complete sequence of the bovine torovirus genome. *Virus Research* 115: 56–68.

Hoet AE (in press) Torovirus pathogenesis and immune responses. In: Perlman S, Snijder EJ, and Gallagher TM (eds.) *The Nidoviruses.* Washington, DC: ASM Press.

Hoet AE, Cho KO, Chang KO, *et al.* (2002) Enteric and nasal shedding of bovine torovirus (Breda virus) in feedlot cattle. *American Journal of Veterinary Research* 63: 342–348.
Hoet AE and Saif LJ (2004) Bovine torovirus (Breda virus) revisited. *Animal Health Research Reviews* 5: 157–171.
Hoet AE, Smiley J, Thomas C, *et al.* (2003) Association of enteric shedding of bovine torovirus (Breda virus) and other enteropathogens with diarrhea in veal calves. *American Journal of Veterinary Research* 64: 485–490.
Horzinek MC (1999) Molecular evolution of corona- and toroviruses. *Advances in Experimental Medicine and Biology* 473: 61–72.
Koopmans M and Horzinek MC (1994) Toroviruses of animals and humans: A review. *Advance Virus Research* 43: 233.
Lodha A, de Silva N, Petric M, and Moore AM (2005) Human torovirus: A new virus associated with neonatal necrotizing enterocolitis. *Acta Paediatrica* 94: 1085–1088.
Smits SL, Lavazza A, Matiz K, *et al.* (2003) Phylogenetic and evolutionary relationships among torovirus field variants: Evidence for multiple intertypic recombination events. *Journal of Virology* 77: 9567–9577.
Snijder E and Horzinek MC (1995) The molecular biology of toroviruses. In: Siddell S and ter Meulen V (eds.) *Coronaviruses*, pp. 219–238. New York: Plenum Press.
Vries AAF, de Horzinek MC, Rottier PJM, *et al.* (1997) The genome organization of the *Nidovirales*: Similarities and differences between arteri-, toro-, and coronaviruses. *Seminars in Virology* 8: 33.

Tospovirus

M Tsompana and J W Moyer, North Carolina State University, Raleigh, NC, USA

© 2008 Published by Elsevier Ltd.

Glossary

Ambisense genome Viral RNA genome with open reading frames in both the viral- and viral complementary (vc) sense on the same genome segment.
Envelope Membrane-like structure that packages genome segments.
IGR The intergenic region is the untranslated, A-U rich region found between the two open reading frames on the S and M RNA segments.
Negative sense genome Viral RNA genome that codes for proteins in the vc sense. Transcription of vc mRNA is required for translation of viral proteins.
Nucleocapsid Viral RNA encapsidated in the nucleoprotein.
Protoplast Plant cell lacking its cell wall.
RNP Ribonucleoprotein complex consisting of the viral RNA genome segment, nucleoprotein, and a small number of polymerase molecules.
Virion Quasispherical structure containing the viral genome and bounded by a membrane-like envelope.

History

Diseases now known to be caused by tomato spotted wilt virus (TSWV) were first reported in 1915 and were shown to be of viral etiology by 1930. This taxon of plant viruses was categorized as a monotypic virus group consisting of a single virus (TSWV) until the report of impatiens necrotic spot virus (INSV) in 1991. Thus, most of the characteristics which define the genus *Tospovirus* were obtained through investigation of TSWV even after the discovery of additional viruses in the genus (**Table 1**). Biological investigations beginning in the 1940s revealed a virus that had an unusually large host range and occurred in nature as a complex mixture of phenotypic isolates. However, it was one of the least stable viruses and most difficult plant viruses to mechanically transmit. Although the enveloped virions were observed in the 1960s, molecular characterization and elucidation of the genome organization were not completed until the early 1990s. The virus was shown to be vectored by thrips in the 1930s and later transmitted in a persistent manner. Thrips were demonstrated to be a host for replication of the virus and that replication was required for transmission in the early 1990s. Later it was recognized that limited, localized replication may occur in thrips that does not result in the thrips becoming viruliferous. Advances in gene function and cellular biology have been limited due to the absence of a robust *in vitro* plant or thrips cell culture system, and lack of an efficient reverse genetics system. However, limited progress has been made utilizing gene expression systems and classical viral genetics.

Taxonomy and Classification

Tospoviruses constitute the only genus of plant-infecting viruses in the family *Bunyaviridae*, however, these viruses share many molecular characteristics typical of other members of this virus family. They have an enveloped virion containing the viral genome which is distributed among three RNA segments that replicate in a manner consistent with that of other negative strand viruses. All three segments have highly conserved, complementary

Table 1 List of *Tospovirus* species[a, b, c]

Tospovirus species	*Abbreviation*
Groundnut bud necrosis virus (Peanut bud necrosis virus)	GBNV
Groundnut ringspot virus	GRSV
Groundnut yellow spot virus (Peanut yellow spot virus)	GYSV
Impatiens necrotic spot virus	INSV
Tomato chlorotic spot virus	TCSV
Tomato spotted wilt virus	TSWV
Watermelon silver mottle virus	WSMoV
Zucchini lethal chlorosis virus	ZLCV
Tentative *Tospovirus* species	
Capsicum chlorosis virus (Gloxinia tospovirus) (Thailand tomato tospovirus)	CACV
Chrysanthemum stem necrosis virus	CSNV
Iris yellow spot virus	IYSV
Groundnut chlorotic fan-spot virus	GCFSV
Physalis severe mottle virus	PhySMV
Watermelon bud necrosis virus	WBNV

[a]http://www.ncbi.nlm.nih.gov/ICTVdb.
[b]Whitfield AE, Ullman DE, and German TL (2005) Tospovirus–thrips interactions. *Annual Review of Phytopathology* 43: 459–489.
[c]Synonyms are indicated inside parentheses.

termini resulting in a pan-handle structure and genes with functions similar to those of viruses in other genera are located in similar locations on the genome. However, the genome organization is distinct from the other genera. The small (S) and middle (M) segments each encode two genes in opposite or ambisense polarity.

Classification of a *Tospovirus* population as a distinct species (virus) is based upon the similarity of sequence between the nucleocapsid genes of the respective viruses. This is in contrast to the system used to differentiate viruses in other genera which traditionally relied on serological neutralization of infectivity or other biological properties (hemagluttination) mediated by the glycoproteins. Tospovirus isolates with greater than 90% nucleotide similarity in the nucleocapsid gene are classified as isolates of the same species (virus). Serologically related isolates with 80–90% sequence identity are subjectively classified as strains or as distinct species depending on other criteria. Isolates with less than 80% identity are classified as distinct species.

Geographic Distribution

TSWV, the type member of the tospoviruses is found worldwide in temperate regions in association with its thrips vector. The wide host-range of TSWV and its thrips vector is consistent with the geographic distribution. Other tospoviruses have more well-defined distribution. For example, GBNV, WBNV, and WSMoV, that are transmitted by *Thrips palmi*, a thrips species found only in the subtropics are only known to occur in Southeast Asia. Another anomaly is INSV. While INSV is reported to occur around the world, it is almost entirely limited to greenhouse-grown floral crops.

Host-Range and Virus Propagation

TSWV has one of the most diverse host-ranges of any plant-infecting virus. The virus infects over 925 plant species belonging to 70 botanical families, both monocots and dicots. In addition, TSWV infects approximately ten thrips species. Important economic plants susceptible to TSWV include tomato, potato, tobacco, peanut, pepper, lettuce, papaya, and chrysanthemum. Other tospoviruses (e.g., IYSV) have much narrower host-ranges and thus the broad host-range of TSWV is not characteristic of the genus. These viruses can be transmitted mechanically or by their thrips vector, but are not transmitted transovarially, by plant seeds or pollen. Purified RNA preparations are not infectious. There are no robust plant or insect culture systems for tospoviruses. However, plant and insect protoplasts have been successfully inoculated.

Virion Properties

Tospovirus virions are quasispherical, enveloped particles 80–120 nm in diameter (**Figure 1**). Two viral coded glycoproteins, G_N and G_C, are embedded in the viral envelope and form surface projections 5–10 nm long. Ribonucleoprotein (RNP) particles consisting of the viral RNA encapsidated in the nucleoprotein (nucleocapsid), and a small number of polymerase molecules are contained within the envelope. Nucleocapsids are pseudocircular due to noncovalent bonding of the complementary RNA termini. Intact virions as well as carefully prepared RNPs retrieved from sucrose or $CsSO_4$ gradients are infectious. There are several reports that TSWV and INSV isolates, while infectious, are defective for virion formation.

Genome Properties

Tospoviruses have a single-stranded, tripartite RNA genome with segments designated as L, M, and S in order of decreasing size (**Figure 2**). The termini of each of the RNA segments consist of an eight nucleotide sequence (5′ AGAGCAAU 3′) that is strictly conserved among all tospoviruses. The remaining untranslated region at the termini also has a high degree of complementarity. Base pairing at the termini between the inverted complementary sequences supports a pan-handle structure that most likely serves as a promoter for replication.

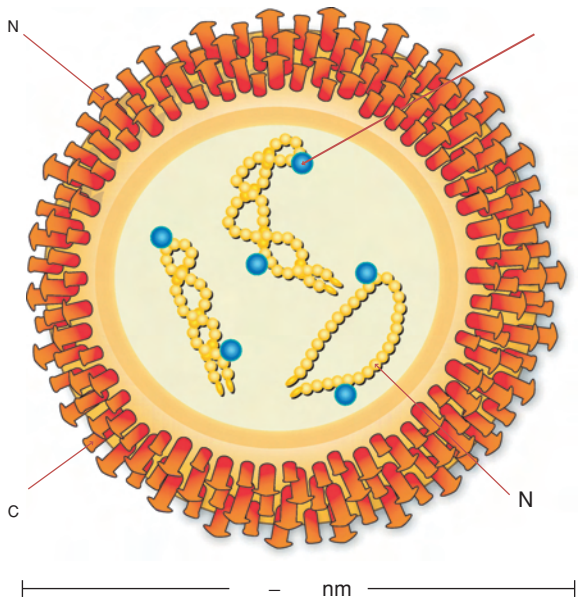


Figure 1 Tospovirus quasispherical virion particles. The S, M, and L RNA genomic segments are encapsidated by the nucleoprotein, are in association with L protein molecules, and form pan-handle structures due to the complementarity of their 5′ and 3′ ends. The glycoproteins G_N and G_C are embedded within the viral envelope.

The L RNA is 8.9 kbp and codes for the L or RdRp protein in the viral complementary (vc) sense (**Figure 2**). The M and S RNAs are in ambisense orientation. The M RNA is 4.8 kbp and codes in the viral sense for the nonstructural protein NSm and for the G_N/G_C precursor glycoprotein in the vc sense. The S RNA is 2.9 kbp and codes in the viral sense for the nonstructural protein NSs and the nucleocapsid protein in the vc sense (**Figure 2**).

TSWV M and S RNA IGRs have variable lengths, are A–U rich, and are the most hypervariable regions of the genome. The 5′ and 3′ ends of the IGRs are conserved, separated by variable sequences, deletions, and insertions. In addition, highly conserved sequences are embedded within the S RNA IGR. A 33 nucleotide (nt) duplication occurring in the S RNA IGR of some isolates has been correlated with loss of competitiveness in mixed infections of isolates with and without the duplication. A 31nt conserved sequence, with significantly higher GC-content compared to the remaining S RNA IGR, has also been found in some TSWV isolates. The IGRs of the M and S segments have high inclination for base pairing thought to be involved in initiation and termination of transcription. There is speculation that the termination of transcription is dictated by a conserved nucleotide sequence (CAACUUUGG) in the center of the S and M RNA IGR or that it is due to a secondary structure highly stabilized in the 31nt region referred to above.

Full length molecules of the M and S RNAs are found in infected tissue and purified virions in both the viral and vc sense (approximate ratio of 10:1), consistent with ambisense segments from other viruses. Defective interfering RNAs (DIs) associated with attenuated symptom expression and increased replication rate are also frequently observed. DIs from the L ORF in TSWV infected tissue are the result of and associated with attenuated infectivity. Deletions, frameshift, and nonsense mutations in the G_N/G_C ORF have been shown to interfere with thrips transmissibility and virion assembly. Recently, frameshift and nonsense mutations with unknown effect have also been identified in the N ORF. The formation of DIs is favored by repeated mechanical passage in certain plant hosts, high inoculum concentration, and low temperatures. Available evidence supports the hypothesis that secondary structure rather than sequence is the primary determinant of the site of deletion. There is also a high frequency of DIs that maintain the original reading frame resulting in translation of truncated proteins whose existence was confirmed in nucleocapsid preparations.

Protein Properties

The 331.5 kDa L protein encoded by the L RNA has been identified as the putative RdRp, through sequence homology with other members of the *Bunyaviridae* and identification of sequence motifs characteristic of polymerases. RdRp activity has been associated with detergent-disrupted TSWV virion preparations. The 33.6 kDa NSm protein encoded by the M RNA has been shown to induce tubule structures in plant protoplasts and insect (*Spodoptera* and *Trichoplusia*) cells. Induction of tubules in plants, ability to change the size exclusion limit of plasmodesmata, an early expression profile and complementation of cell-to-cell and systemic movement in a movement-defective tobacco mosaic virus vector is evidence that NSm is the TSWV movement protein and that it supports long-distance movement of viral RNAs. In thrips, NSm does not aggregate into tubules, indicating that this protein might not have any function in the vector's life cycle. It is also known that NSm specifically interacts with the N protein, the At-4/1 intra- and intercellular trafficking plant protein and binds single-stranded RNA in a sequence-nonspecific manner. An NSm homolog is absent in the animal infecting *Bunyaviridae*. The 127.4 kDa G_N/G_C precursor glycoprotein also coded by the M RNA contains a signal sequence that allows its translation on the endoplasmic reticulum. Proteolytic cleavage of the polyprotein does not require other viral proteins. The M_rs of G_N and G_C is 78 kDa and 54 kDa respectively. Evidence for the involvement of the glycoproteins in thrips transmission is provided by: (1) their interaction with proteins of the thrips vector, (2) their association with the insect midgut during acquisition, (3) the loss of thrips transmissibility of envelope-deficient mutants, (4) the presence of a

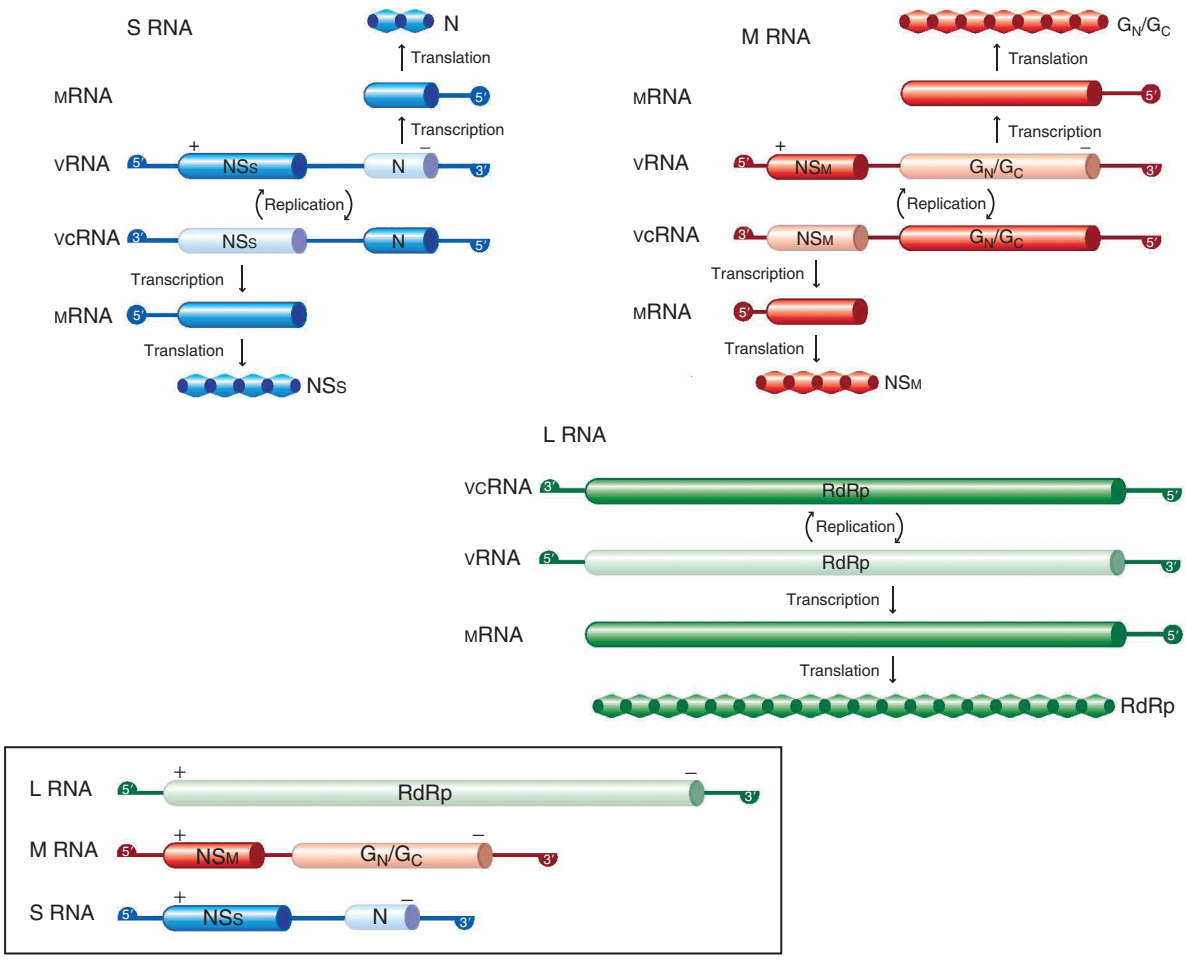


Figure 2 Tospovirus ambisense genome organization (inset in figure) and expression strategy. Positive (+) and negative (–) sense ORFs are dark and light shaded tubes respectively. Proteins from the S and M segments are translated from subgenomic mRNAs which are capped with 10–20 nt of non-viral orgain at the 5′ end.

glycoprotein sequence motif that is characteristic for cellular attachment domains and (5) the observation that only reassortants with the M RNA of a thrips-transmissible isolate rescue thrips transmissibility. Specifically, G_N is involved in virus binding and/or entry in thrips midgut cells, whereas G_C is a possible fusion protein playing a significant role in pH-dependent virus entry. It is also believed that these proteins are implicated in virion assembly. The NSs protein encoded on the S RNA is 52.4 kDa and accumulates to high levels as loose aggregates or paracrystalline arrays of filaments. NSs has RNA silencing suppressor activity, affects symptom expression in TSWV-infected plants, and is not present in the mature virus particle. The N protein, also encoded by the S RNA, ranges in size from 29 to 3 kDa depending on the virus. This protein encapsidates the viral RNA segments, is highly abundant, and is the predominant protein detected in serological assays. A 'head-to-tail' interaction of the nucleoprotein N terminus (aa 1–39) with the C terminus (aa 233–248) results in multimerization.

Replication

Replication of viral RNA and assembly of virions occurs in the cytoplasm of both plant and insect cells. Tospovirus replication, however, has mainly been described based on plant infection. Upon entry into the plant cell, the virus loses its membrane and releases infectious nucleocapsids into the cytoplasm. In thrips cells, infection by tospoviruses is accommodated by binding of the viral surface glycoproteins to a host cell receptor(s) (possibly a 50 kDa and/or a 94 kDa protein). This is followed by release of infectious nucleocapsids into the cytoplasm, through fusion between the viral and thrips membranes possibly initiated by low pH. Depending on the concentration of N protein, the viral RNA is either transcribed or replicated. At low N concentrations, the polymerase transcribes mRNAs that are translated into the virus proteins. Translation of proteins from the S and M ambisense RNAs occurs from subgenomic mRNAs (**Figure 2**). The S and M subgenomic mRNAs are capped

at the 5′ terminus with 10–20 nucleotides of nonviral origin indicating that tospoviruses utilize a cap-snatching mechanism to regulate transcription. Leader sequences of alfalfa mosaic virus have also been detected as caps of TSWV mRNA in mixed infections of the two viruses. The TSWV transcriptase has a reported preference for caps with multiple base complementarity with the viral template. Upon increase of N protein concentration, the polymerase switches its mode to replication with the viral RNA serving as the template. Replicated viral RNAs form RNPs that can presumably associate with the NSm protein for movement through plasmodesmata to adjacent plant cells through tubular structures. Alternatively, RNPs form new virions by associating with the glycoproteins and budding through the Golgi membranes. Virions are initially double-membraned, but soon coalesce and form groups of virions with a single membrane surrounded by another membrane.

Pathogenicity and Cytopathology

Tospoviruses are noted for the severity of the diseases they cause in plants. Symptoms are highly variable, depending on the virus, the virus isolate, the host plant, time of the year, and environment, and are thus of little diagnostic value. Chlorosis, necrosis, ring or line patterns, mottling, silvering, and stunting often appear on inoculated and systemically infected leaves. Systemic invasion of plants is frequently nonuniform. Stems and petioles may exhibit necrotic lesions. Observed symptoms often mimic disease and injury caused by other biotic and abiotic stresses. Infection of younger plants results in severe stunting and high mortality rates. TSWV has been shown to affect more severely *Datura*, *Nicotiana,* and *Physalis* plants under a specific temperature regime (daytime, $29 + 2\,^{\circ}C$, nighttime, $24 + 3\,^{\circ}C$). The effect of tospovirus infection on thrips has been controversial, due to the confounded effects of plant host, virus, and environment on the insect vector and the genetic variability of thrips and virus populations. TSWV infection of *F. occidentalis* provided evidence that thrips exhibit an immune response to the virus. Recent work with TSWV-infected *F. fusca* reared on infected foliage indicated a direct effect of the virus on thrips resulting in reduced fitness. The same study showed that the plant infection status and the TSWV isolate have also an effect on the insect, explaining the variable results obtained from independent studies of virus pathogenicity on the insect vector.

Tospoviruses induce characteristic cytopathic structures that are host and virus-isolate dependent. In addition to virions, inclusions of viroplasms consisting of the NSs or N protein may be abundant in the cytoplasm. NSs may aggregate in loose bundles (e.g., TSWV) or in highly ordered paracrystalline arrays (e.g., INSV). Excess N protein occurs in granular electron dense masses. NSs and nucleocapsid protein inclusions have been observed in infected plant and insect cells. NSm protein induces tubule structures in plant protoplasts and insect cells.

Transmission and Epidemiology

Tospoviruses are transmitted from plant to plant by at least ten thrips species in the genera *Frankliniella*, *Scirtothrips*, and *Thrips*. Among the more common vectors are *Frankliniella occidentalis*, *F. fusca*, *F. schultzei*, *F. intonsa*, *F. bispinosa*, *Thrips palmi*, *T. setosus*, and *T. tabaci*. Thrips feed on the cytoplasm of plant cells. The contents of infected cells are ingested and the virus is transported along the lumen of the digestive tract to the midgut, the primary binding and entry site into the insect cells. The brush border of the midgut lumen is the first membrane barrier that the virus encounters. The virus replicates in the midgut and crosses the basement membrane into the visceral muscle cells. The virus subsequently enters the primary salivary glands. It has been hypothesized that the virus moves from the midgut to the salivary glands through infection of ligament-like structures, or when there is direct contact between membranes of the visceral muscles and the primary salivary glands during the larval stages of development. A less plausible hypothesis is that the virus infects the salivary glands after entry and circulation in the hemocoel. Viral inoculum is introduced into plants in the insect saliva coincident with feeding on the plant by adult thrips.

The process of successful acquisition occurs only by larvae and acquisition rates decrease as larvae develop, affecting adult vector competency. Vector competency is also determined by the thrips' feeding preference on a particular host, the uniformity of distribution of virus in plant cells, the rate of virus replication in the midgut, and the extent of virus migration from the midgut to the visceral muscle cells and the salivary glands. In some instances the virus can be acquired by adult thrips and infects midgut cells, but is unable to spread further possibly due to the formation of an age-dependent midgut barrier (e.g., basal lamina). Research has shown the existence of thrips transmitters with detectable levels of virus, nontransmitters with detectable virus, and nontransmitters with no detectable virus, supporting multiple sites for vector specificity between tospoviruses and thrips. Evidence for replication of the virus in the insect vector is based on the accumulation of NSs and the visualization of viral inclusions in midgut epithelial cells, muscle cells, and the salivary glands. Although the virus is maintained transtadially throughout the life of the insect, there is no evidence for transovarial transmission. Thus, each generation of thrips must acquire the virus during the larval stages.

The primary dispersal of tospoviruses is by adult thrips and dissemination of infected somatic tissue in vegetatively propagated crops. These viruses are thought to move long distances in thrips carried by wind currents. They may also survive in commercial agricultural systems in weeds that serve as a bridge between crops. Infected summer weeds (e.g., in NC *I. purpurea*, *I. hederacea*, *M. verticillata*, *A. palmeri*, *C. obtusifolia*, *R. scabra*, *Ambrosia artemisiifolia* L., *Polygonum pensylvanicum* L., *and Chenopodium album* L.) are the principal source for spread of TSWV to winter annual weeds, from which the virus is spread to susceptible crops in spring. Secondary spread within a crop can only occur in crops that concomittantly support virus infection and reproduction of the vector as only the larval stage can acquire the virus for transmission. Transmission through plant seed and pollen has not been conclusively demonstrated. The emergence of these viruses as serious pathogens in crops has been attributed to the increased prevalence of *F. occidentalis* as an agricultural pest on a worldwide basis.

Genetics and Evolution

The knowledge base for genetics and evolution of tospoviruses has been derived almost exclusively from TSWV. TSWV has a characteristic ability to adapt to new or resistant hosts and to lose phenotypic characters following repeated passage in experimental hosts, especially *Nicotiana benthamiana*. The virus occurs in plants as a heterogeneous mutant population with one or two predominant haplotypes and 9–21 rare haplotypes. Recent research shows that natural TSWV variants evolve in nature through recombination, random genetic drift, and mutation. Intergenomic recombination is important for the genesis and evolution of ancestral TSWV lineages. Genetic drift during thrips transmission and mutation concurrent with virus population growth, shape the genetic architecture of the most recently evolved lineages. The existence of single viral strains as mutant populations and recombination in ancestral viral lineages arm TSWV with a unique genetic reservoir for causing disease and spreading in epidemic proportions in nature. Additional research at the species level supports a distinct TSWV geographical structure and the occurrence of species-wide population expansions. TSWV is also known to use reassortment of genome segments to adapt to resistant hosts under specific laboratory conditions. The determinants of adaptation to resistance in tomato and pepper have been mapped to the M and S RNAs respectively. Little is known about the thrips–tospovirus coevolution and the genetic diversity of the thrips vector itself. The altered status of *Thrips tabaci* as a TSWV vector is one of the very few likely examples of coevolution between tospoviruses and their insect vector.

Detection and Diagnosis

Tospoviruses have certain unique biological properties that are useful for diagnosis. These viruses can be mechanically transmitted by gently rubbing inoculum on plants dusted with carborundum. *Nicotiana glutinosa* L., *Chenopodium quinoa* Wild., and garden petunia give characteristic lesions that progress as spots or concentric zones, and sometimes as lethal necrosis. Tospoviruses can also be identified by electron microscopy of leaf-dip preparations on thin sections of infected plants. Additional techniques for identification are based on the enzyme-linked immunosorbent assay (ELISA) using polyclonal and monoclonal antibodies, and detection of viral-specific nucleic acids using ribo- and cDNA-probes. The reverse transcription-polymerase chain reaction (RT-PCR) is the most powerful and commonly used technique for detecting small amounts of tospovirus RNA. Real time RT-PCR has been successfully used to detect and quantify TSWV in leaf soak and total RNA extracts from infected plants and thrips. RT-PCR with degenerate primers can detect five distinct tospovirus species. Tissue selection and sampling strategy are critical factors in TSWV diagnosis and detection regardless of the technique. Because, TSWV titer varies throughout the plant and does not spread uniformly throughout plants that are 'systemic' hosts, sampling strategies should be validated in each situation.

Prevention and Control

Tospoviruses cause significant economic losses annually, due to suppressed growth, yield, and reduced quality. These viruses can be partially managed in well-defined cropping systems such as glasshouses by obtaining uninfected plant propagules, implementing a preventative thrips control program in high risk areas, together with constant monitoring of production areas for thrips and infected plants. However, these strategies are costly and require intensive management. Control in field crops is problematic due to the array of external sources of inoculum. Vector control is generally ineffective against the introduction of virus from external sources, due to thrips' high fecundity, ability to develop insecticide resistance, and to infest many TSWV-susceptible crops. Some measure of control can be achieved using thrips-proof mesh tunnels in the field and reflective mulches. Cultural practices such as utilization of virus-tested planting stock, careful selection of planting dates, removal of cull piles and weeds, rotation with nonsusceptible crops, prevention of planting TSWV-susceptible crops adjacent to each other, reduced in-field cultivation to avoid movement of thrips from infected sources, can reduce the spread of tospoviruses. In peanuts, higher plant density, planting from early until late May and application of selected

insecticides have reduced the incidence of TSWV. In flue-cured tobacco, early-season treatment with activators of plant defenses and insecticides have also significantly reduced TSWV incidence.

Deployment of resistant cultivars has provided benefits in only three of the crops infected by TSWV. Although little is known about the benefits of host resistance against most of the tospoviruses, TSWV defeated nearly every resistance gene deployed against it in many crops. Single-gene resistance is available for TSWV in a limited number of tomato (*Sw-5*) and pepper (*tsw*) cultivars. Naturally occurring, resistance-breaking isolates of TSWV have been recovered from pepper and tomato cultivars containing their respective resistance genes. A co-dominant cleaved amplified polymorphic sequence (CAPS) marker has been developed for TSWV marker-assisted selection in pepper. 'Field' resistance has been reported for some peanut varieties. Progress has been made in understanding the genetic basis of the ability of TSWV to overcome single gene resistance by mapping determinants to specific segments of the TSWV genome and characterizing the selection process. Pathogen-derived resistance utilizing the N and NSm genes has been effective in some greenhouse and field tests; however, isolates have been obtained that overcome nucleocapsid mediated resistance. Best suppression of TSWV epidemics has been achieved with the integrated use of moderately resistant cultivars, chemical, and cultural practices. The impact of these viruses on agricultural production is in large part due to the absence of durable forms of resistance in the affected crops or other highly effective control measures.

Future Perspectives

The last decade has been characterized by exciting developments in our understanding of tospovirus molecular biology, evolution, and virus–host relationships. Further progress in understanding replication and gene function requires the development of efficient reverse genetics, plant and insect culture systems for tospoviruses. In addition, effective management of these viruses will depend on a deeper understanding of thrips' genetic diversity, virus–thrips coevolution and the changes in viral and thrips population dynamics upon exertion of specific selective forces. Such understanding can be acquired only through integrated research at the interface of virology, entomology, and ecology.

See also: Tomato Spotted Wilt Virus.

Further Reading

Adkins S (2000) Tomato spotted wilt virus-positive steps towards negative success. *Molecular Plant Pathology* 1: 151–157.

Best RJ (1968) Tomato spotted wilt virus. In: Smith KM and Lauffer MA (eds.) *Advances in Virus Research*, pp. 65–145. New York: Academic Press.

de Avila AC, de Haan P, Kormelink R, *et al.* (1993) Classification of tospoviruses based on phylogeny of nucleoprotein gene sequences. *Journal of General Virology* 74: 153–159.

German TL, Ullman DE, and Moyer JW (1992) Tospoviruses: Diagnosis, molecular biology, phylogeny, and vector relationships. *Annual Review of Phytopathology* 30: 315–348.

Goldbach R and Peters D (1994) Possible causes of the emergence of tospovirus diseases. *Seminars in Virology* 5: 113–120.

Prins M and Goldbach R (1998) The emerging problem of tospovirus infection and nonconventional methods of control. *Trends in Microbiology* 6: 31–35.

Sin SH, McNulty BC, Kennedy GG, and Moyer JW (2005) Viral genetic determinants for thrips transmission of tomato spotted wilt virus. *Proceedings of the National Academy of Sciences, USA* 102: 5168–5173.

Tsompana M, Abad J, Purugganan M, and Moyer JW (2005) The molecular population genetics of the tomato spotted wilt virus (TSWV) genome. *Molecular Ecology* 14: 53–66.

Ullman DE, Meideros R, Campbell LR, Whitfield AE, and Sherwood JL (2002) Thrips as vectors of tospoviruses. In: Plumb R (ed.) *Advances in Botanical Research*, pp. 113–140. London: Elsevier.

Ullman DE, Sherwood JL, and German TL (1997) Thrips as vectors of plant pathogens. In: Lewis TL (ed.) *Thrips as Crop Pests*, pp. 539–565. London: CAB International.

Whitfield AE, Ullman DE, and German TL (2005) Tospovirus–thrips interactions. *Annual Review of Phytopathology* 43: 459–489.

Totiviruses

S A Ghabrial, University of Kentucky, Lexington, KY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Mycoviruses Viruses that infect and multiply in fungi.

Pseudoknot A secondary structure in viral mRNA that slows movement of the ribosome and may cause a frameshift that allows entry to an alternative reading frame during translation.

Ribosomal frameshifting Ribosomes switching reading frame on an mRNA, in response to the presence of a slippery site and/or a pseudoknot, to synthesize a protein or a polyprotein from two overlapping reading frames.

Introduction

The discovery of the killer phenomenon in the 1960s in yeast (*Saccharomyces cerevisiae*) and in the smut fungus (*Ustilago maydis*) eventually led to the discovery of the isometric double-stranded (ds) RNA mycoviruses with nonsegmented genomes, currently classified in the genus *Totivirus* (family *Totiviridae*). Killer strains of yeast or smut secrete a protein toxin to which they are immune, but which is lethal to sensitive cells. The precursor to the killer toxin is encoded by a satellite dsRNA, which is dependent on a helper virus with nonsegmented dsRNA genome (totivirus) for encapsidation and replication. Unlike the helper totiviruses associated with the yeast and smut killer systems, other members of the family *Totiviridae*, including the viruses that infect filamentous fungi (members of the newly proposed genus *Victorivirus*) and those that infect parasitic protozoa (members of the genera *Giardiavirus* and *Leishmaniavirus*), are not known to be associated with killer phenotypes. Purified preparations of some of these viruses, however, can contain dsRNA species suspected of being satellite or defective dsRNAs.

The isometric dsRNA totiviruses that infect fungi and protozoa are unique among dsRNA viruses in that their genomes are undivided, whereas the genomes of all other dsRNA viruses are segmented. The yeast and smut totiviruses and associated killer systems, as well as the totiviruses infecting parasitic protozoa, are discussed elsewhere in this encyclopedia. In addition to examining the similarities and differences among members of the family *Totiviridae*, this article will focus on the totiviruses that infect filamentous fungi, a group of viruses that are phylogenetically more closely related to each other than to other totiviruses and utilize a different strategy to express their genomes.

Taxonomy and Classification

The family *Totiviridae* encompasses a broad range of viruses characterized by isometric particles, ~40 nm in diameter, that contain a monosegmented dsRNA genome coding for a capsid protein (CP) and an RNA-dependent RNA polymerase (RdRp). At present, three genera are recognized: *Totivirus*, *Giardiavirus*, and *Leishmaniavirus*. Viruses currently placed in the genus *Totivirus* infect yeast, smut fungi, or filamentous fungi, whereas those in the latter two genera infect parasitic protozoa. Two distinct RdRp expression strategies have been reported for species in the family *Totiviridae*: those that express RdRp as a fusion protein (CP–RdRp) by ribosomal frameshifting, such as saccharomyces cerevisiae virus L-A and the viruses that infect parasitic protozoa; and those that synthesize RdRp as a separate nonfused protein by an internal initiation mechanism, as shown for Hv190SV and proposed for all of the other totiviruses that infect filamentous fungi. Although *Helminthosporium victoriae 190S virus* (Hv190SV) is the only species recognized by the International Committee on Taxonomy of Viruses (ICTV) as a member of the genus *Totivirus*, the complete nucleotide sequences of several tentative totiviruses that infect filamentous fungi have recently been reported (**Table 1**). Sequence and phylogenetic analyses have demonstrated that these viruses have many properties in common with Hv190SV and that they are more closely related to each other than to the viruses infecting the yeast and smut fungi. A new genus (genus *Victorivirus*) was recently proposed to accommodate Hv190SV and the related viruses infecting filamentous fungi, with Hv190SV as the type species. The name 'Victorivirus' is derived from the specific epithet of *H. victoriae*, the host of the proposed type species.

In addition to fungal and protozoal hosts, totiviruses may also have crustacean hosts. A nonsegmented dsRNA virus with isometric particles, designated penaeid shrimp infectious myonecrosis virus (IMNV), was recently isolated from diseased penaeid shrimp and tentatively assigned to the family *Totiviridae*. IMNV is the causal agent of the shrimp myonecrosis disease characterized by necrosis of skeletal muscle, particularly in the distal abdominal segments and tail fan. Phylogenetic analysis based on the RdRp region of viruses in the totivirus family suggests that giardia lamblia virus (GLV; genus *Giardiavirus*) is the closest relative to IMNV. Interestingly, both IMNV and GLV, unlike all other members of the family *Totiviridae*, are infectious as purified virions. IMNV is presently unclassified and it has yet to be determined whether it is a member of a novel genus in the family *Totiviridae* or whether IMNV is a member of a novel family of dsRNA viruses that infect invertebrate hosts. The complete nucleotide sequences of several members and tentative members of the family *Totiviridae* have been published and the GenBank accession numbers are listed in **Table 1**.

Virion Properties

The buoyant densities in CsCl of virions of members of the totivirus family range from 1.36 to 1.43 g cm^{-3}, and the sedimentation coefficients of these virions range from

Table 1 List of virus members and tentative members in the family *Totiviridae*, length of their genomes, size of encoded gene products and GenBank accession numbers

Virus[a]	*Abbreviation*	*Genome length (nt)*	*Size (kDa)*		*GenBank accession no.*
			CP	*RdRp/CP–RdRp*[b]	
Genus: *Totivirus*					
Saccharomyces cerevisiae virus L-A	ScV-L-A	4579	76	171[b]	NC_003745
Saccharomyces cerevisiae virus L-BC	ScV-L-BC	4615	78	176[b]	NC_001641
Ustilago maydis virus H1	UmV-H1	6099	81	201[b]	NC_003823
Genus: *Victorivirus*					
Helminthosporium victoriae 190S virus	Hv190SV	5179	81	91	NC_003607
Chalara elegans RNA virus 1	CeRV1	5310	81	96	NC_005883
Coniothyrium minitans RNA virus	CmRV	4975	81	93	NC_007523
Epichloe festucae virus 1	EfV-1	5109	80	90	AM261427
Gremmeniella abietina RNA virus L	GaRV-L	5133	81	90	NC_003876
Helicobasidium mompa totivirus 1-17	HmTV-1-17	5207	83	93	NC_005074
Magnaporthe grisea virus 1	MgV-1	5359	77	91	NC_006367
Sphaeropsis sapinea RNA virus 1	SsRV-1	5163	89	92	NC_001963
Sphaeropsis sapinea RNA virus 2	SsRV-2	5202	83	91	NC_001964
Genus: *Giadiavirus*					
Giardia lamblia virus	GLV	6277	98	210[b]	NC_003555
Trichomonas vaginalis virus 1	TVV-1	4648	74	160[b]	U57898
Trichomonas vaginalis virus 2	TVV-2	4674	79	162[b]	NC_003873
Trichomonas vaginalis virus 3	TVV-3	4844	79	156[b]	NC_004034
Genus: *Leishmaniavirus*					
Leishmania RNA virus 1–1	LRV1-1	5284	82	98	NC_002063
Leishmania RNA virus 1–4	LRV1-4	5283	83	99	NC_003601
Leishmania RNA virus 2–1	LRV2-1	5241	78	88	NC_002064
Unclassified					
Penaeid shrimp myonecrosis virus	IMNV	7560	99	196[b]	NC_007915
Eimeria brunetti RNA virus 1	EbRV	5358	83	98	NC_002701

[a]The names of the ICTV-recognized virus species are written in italics.
[b]RdRp is expressed or proposed to be expressed as a CP–RdRp fusion protein.

160S to 190S (S_{20w}, in Svedberg units). Particles lacking nucleic acid sediment with apparent sedimentation coefficients of 90–105S. Isolates of ScV-L-A and UmV-H1 may have additional components, containing satellite or defective dsRNAs, with different sedimentation coefficients and buoyant densities. Virion-associated RdRp activity can be detected in all totiviruses examined to date. Protein kinase activity is associated with Hv190SV virions; capsids contain phosphorylated forms of CP.

Virion Structure and Composition

The totiviruses have isometric particles, approximately 40 nm in diameter, with icosahedral symmetry (**Figure 1**). The capsids are single-shelled and encompass a single major polypeptide. The capsids consist of 120 CP subunits of molecular mass in the range of 76–98 kDa. The capsid structures of three members of the family *Totiviridae* have been determined, at least one (the yeast L-A virus) at near atomic resolution using X-ray crystallography, and the other two (UmV-H1 and Hv190SV) at moderate resolutions (~1.4 nm) using cryo-transmission electron microscopy combined with three-dimensional image reconstruction. In all cases, the capsids of the fungal totiviruses are made up of 60 asymmetric CP dimers arranged in a '$T = 2$' layer. Compared to the yeast L-A capsid, the Hv190SV capsid shows relatively smoother outer surfaces. The quaternary organization of the Hv190SV particle, however, is remarkably similar to the yeast L-A and the cores of the larger dsRNA viruses of plants and animals: the A-subunits cluster around the fivefold axis and B-subunits around the threefold axis.

The ScV-L-A CP removes the 5′ cap structure of host mRNA and covalently attaches it to the histidine residue at position 154. The decapping activity is required for efficient translation of viral RNA. The published yeast L-A capsid structure reveals a trench at the active site of decapping. The decapping activity has yet to be demonstrated for any other totivirus CPs.

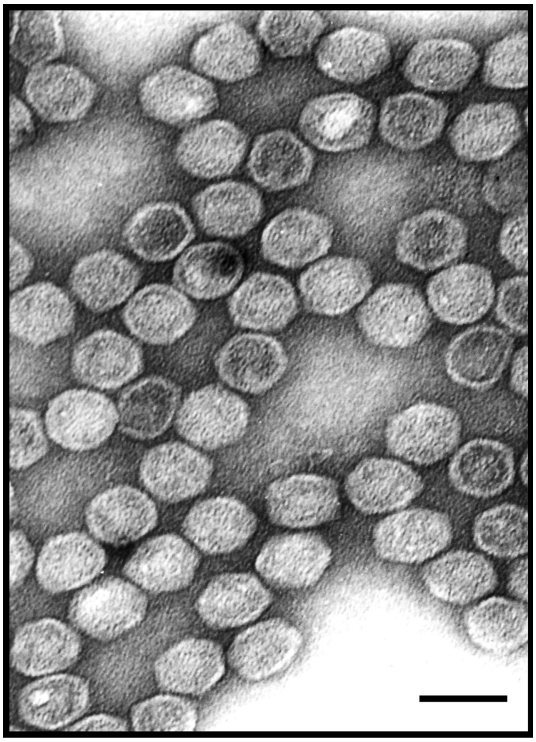


Figure 1 Negative contrast electron micrograph of particles of an isolate of *Helminthosporium victioriae 190SV*, the type species of the newly proposed genus *Victorivirus*. Scale = 50 nm.

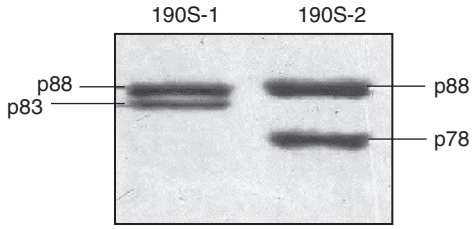


Figure 2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of Hv190SV sedimenting components 190S-1 and 190S-2. Purified preparations of Hv190SV contain two types of particles, 190S-1 and 190S-2, which differ slightly in sedimentation rates. The 190S-1 capsids contain p88 and p83, occurring in approximately equimolar amounts, and the 190S-2 capsids comprise similar amounts of p88 and p78. The capsid proteins p88 and p83 are phosphorylated, whereas p78 is nonphosphorylated.

Although a single gene encodes the capsid of Hv190SV, like other totiviruses, the Hv190SV capsid comprises two closely related major CPs, either p88 and p83 or p88 and p78. The capsids of all other totiviruses so far characterized appear to contain only a single major CP. Interestingly, HmTV-1-17, a totivirus infecting a filamentous fungus, has similar capsid heterogeneity to that of Hv190SV. It would be of interest to determine whether capsid heterogeneity and post-translational modification (phosphorylation and proteolytic processing) of the primary CP is a common feature of totiviruses infecting filamentous fungi (members of the tentative genus *Victorivirus*). Purified Hv190S virion preparations contain two types of particles, 190S-1 and 190S-2, which differ slightly in sedimentation rates (190S-1 is resolved as a shoulder on the slightly faster sedimenting component 190S-2) and capsid composition. The 190S-1 and 190S-2 virions are believed to represent different stages in the virus life cycle. The 190S-1 capsids contain p88 and p83, occurring in approximately equimolar amounts, and the 190S-2 capsids comprise similar amounts of p88 and p78. p88 and p83 are phosphoproteins, whereas p78 is nonphosphorylated (**Figure 2**). Totivirus virions encapsidate a single molecule of dsRNA, 4.6–6.3 kbp in size. Some totiviruses may additionally contain satellite dsRNAs or defective dsRNAs, which are encapsidated separately in capsids encoded by the totivirus genome.

Genome Organization and Expression

In general, the genome organization of the totiviruses infecting fungi and protozoa are similar: each virus genome contains two large open reading frames (ORFs); the 5′ proximal ORF encodes a CP and the 3′ ORF encodes an RdRp (see the genome organization of Hv190SV as an example; **Figure 3**). Except for LRV2-1 and UmV-H1, the RdRp ORF overlaps the CP ORF and is in the −1 frame (ScV-L-A, ScV-L-BC, Hv190SV, TVV-2, and GLV) or in the +1 frame (LRV1-1 and LRV1-4) with respect to the CP ORF. The RdRp ORF of LRV2-1 does not overlap the CP ORF, and is separated from it by a stop codon. The Umv-H1 genome contains only a single ORF that encodes a polyprotein that is predicted to be autocatalytically processed by a viral papain-like protease to generate the CP and RdRp proteins.

The totiviruses express their RdRps either as CP–RdRp (gag-pol-like) fusion proteins or as separate nonfused proteins. Expression of RdRp as a CP–RdRp fusion protein via −1 ribosomal frameshifting has been well documented for ScV-L-A. Virion-associated CP–RdRp has been detected as a minor protein in the capsids of ScV-L-A, ScV-L-BC, TVV, and GLV. Although CP–RdRp fusion proteins were neither detected *in vivo* nor associated with virions of LRV1-1, LRV1-4, or LRV2-1, expression of RdRp as a fusion protein by +1 ribosomal frameshifting or ribosomal hopping (LRV2-1) has been proposed.

The overlap region between ORF1 and ORF2 of ScV-L-A (130 nt), LRV1-1 and LRV1-4 (71 nt), and GLV (122 nt) contains the structures necessary for ribosomal frameshifting including a slippery site and a pseudoknot structure to promote fusion of ORF1 and ORF2 *in vivo*. Although the overlap region in TVV is short (14 nt), it contains a potential ribosomal slippage heptamer.

The overlap regions in the dsRNA genomes of Hv190SV and Hv190S-like viruses (members of the

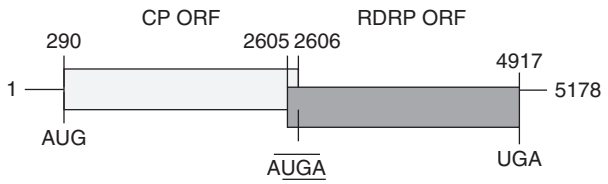


Figure 3 Genome organization of *Helminthosporium victoriae 190S virus*, the type species of the newly proposed genus *Victorivirus*. The dsRNA genome encompasses two large overlapping open reading frames (ORFs) with the 5′ ORF encoding a capsid protein (CP) and the 3′ ORF encoding an RNA-dependent RNA polymerase (RdRp). Note that the termination codon of the CP ORF overlaps the initiation codon of the RdRp ORF in the tetranucleotide sequence AUGA. Adapted from Ghabrial SA and Patterson JL (1999) *Encyclopedia of Virology*, 2nd edn., pp. 1808–1812. New York: Academic Press, with permission from Elsevier.

newly proposed genus *Victorivirus*), on the other hand, are of the AUGA type where the initiation codon of the RdRp ORF overlaps the termination codon of the CP ORF (**Figure 3**), suggesting that expression of RdRp occurs by a mechanism different from translational frameshifting. For Hv190SV, the stop codon of the CP ORF (nucleotide position 2606-UGA-2608) overlaps with the start codon (nucleotide position 2605-AUG-2607) for the RdRp ORF in the sequence AUGA (**Figure 3**). The complete nucleotide sequences of Hv190SV and eight other putative members of the family *Totiviridae* infecting filamentous fungi (members of the genus *Victorivirus*) have been reported and the sequences deposited in the GenBank, with Hv190SV being biochemically and molecularly best characterized of these (**Table 1**).

The 5′ end of the plus strand of all totiviruses dsRNAs examined to date is uncapped and the 3′ end is not polyadenylated. The 5′ end of Hv190SV dsRNA is uncapped and highly structured and contains a relatively long (289 nucleotides) 5′ leader with two minicistrons. These structural features of the 5′ untranslated region (UTR) of Hv190SV dsRNA suggest that the CP-encoding ORF1 (with its AUG present in suboptimal context according to the Kozak criteria) is translated via a cap-independent mechanism. The 5′-UTR of the leishmaniavirus LRV-1-1 functions as an internal ribosome entry site (IRES). Translation of the uncapped giardiavirus GLV mRNA in *Giardia lamblia* is initiated on a unique IRES element that contains sequences from a part of the 5′-UTR and a portion of the capsid coding region.

The UGA codon at position 2606–2608 of the Hv190SV genomic plus strand was verified by site-directed mutagenesis to be the authentic stop codon for ORF1 (**Figure 3**). The RdRp-encoding downstream ORF2 of Hv190SV is in a −1 frame with respect to ORF1 (**Figure 3**) and is expressed via an internal initiation mechanism (a coupled termination–initiation mechanism is proposed). The Hv190SV RdRp is detectable as a separate, virion-associated component, consistent with its independent translation from ORF2. The tetranucleotide AUGA overlap region, or a very similar structure, is characteristic of the overlap region of all putative members of the totivirus family that infect filamentous fungi (victoriviruses).

The initial report on the molecular characterization of IMNV, a monosegmented dsRNA virus infecting penaeid shrimp and tentatively assigned to the family *Totiviridae*, concluded that the viral genome encompasses two non-overlapping ORFs with ORF1 encoding a polyprotein comprised of a putative RNA-binding protein and a CP. The coding region of the RNA-binding protein was located in the first half of ORF1 and contained a dsRNA-binding motif in the first 60 amino acids. The second half of ORF1 encoded a CP, as determined by amino acid sequencing, with a molecular mass of 99 kDa. ORF2 encoded a putative RdRp with the eight conserved motifs characteristic of totiviruses. Phylogenetic analysis based on the RdRp clustered IMNV with GLV, the type species of the genus *Giardiavirus* in the family *Totiviridae*. Important novel features of the genome organization of IMNV, however, were most recently uncovered that has significant bearing on how the viral proteins are expressed. These features include two encoded '2A-like' motifs, which are likely involved in ORF1 polyprotein 'cleavage', a 199 nt overlap between ORF1 and ORF2, and the presence a 'slippery heptamer' motif and predicted RNA pseudoknot in the region of ORF1–ORF2 overlap. The latter features probably allow ORF2 to be translated as a fusion with ORF1 by '−1' ribosomal frameshifting. Although the generation of CP as a polyprotein and the potential involvement of encoded '2A-like' peptides (GDVESNPGP and GDVEENPGP) in processing of the polyprotein to release the major CP represent novel features not shared by totiviruses, the potential expression of RdRp as a CP–RdRp fusion protein via ribosomal frameshifting is a common strategy utilized by totiviruses for expressing their RdRps. Experimental evidence for the presence of the putative CP–RdRp fusion protein in purified virions or in infected tissues, however, is lacking.

Virus Replication Cycle

Limited information is available on the replication cycle of totiviruses and has mainly been derived from *in vitro* studies of virion-associated RNA polymerase activity and the isolation of particles representing various stages in the replication cycle. In *in vitro* reactions, the RNA polymerase activity associated with virions of the fungal totiviruses ScV-L-A, UmV-H1, and Hv190SV,

isolated from lag-phase cultures, catalyzes end-to-end transcription of dsRNA, by a conservative mechanism, to produce mRNA for CP, which is released from the particles. Purified ScV-L-A virions, isolated from log-phase cells, contain a less-dense class of particles, which package only plus-strand RNA. In *in vitro* reactions, these particles exhibit a replicase activity that catalyzes the synthesis of minus-strand RNA to form dsRNA. The resultant mature particles, which attain the same density as that of the dsRNA-containing virions isolated from the cells, are capable of synthesizing and releasing plus-strand RNA.

A proposed life cycle of Hv190SV is depicted in **Figure 4**. Host-encoded protein kinase and protease have been shown to be involved in post-translational modification of CP. Phosphorylation and proteolytic processing are proposed to play a role in the virus life cycle; phosphorylation of CP may be necessary for its interaction with viral nucleic acid and/or phosphorylation may regulate dsRNA transcription/replication. Proteolytic processing

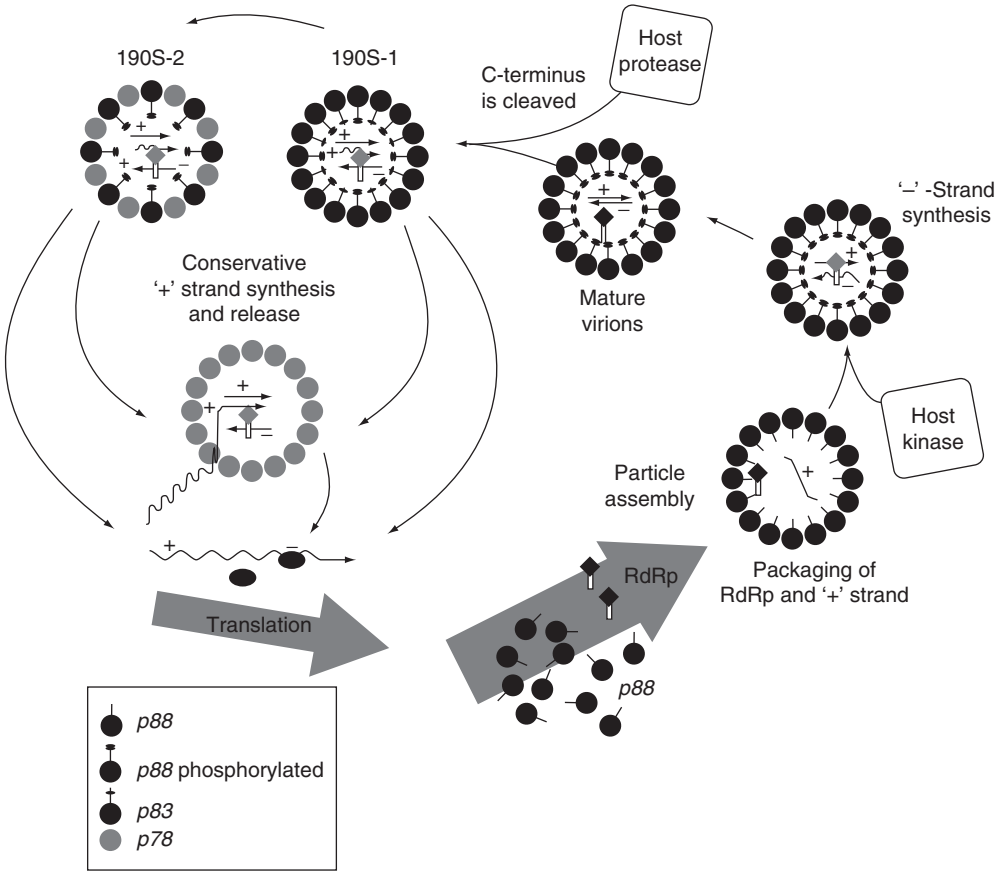


Figure 4 Life cycle of Hv190SV. Mature virions contain a single dsRNA molecule and their capsids are composed entirely or primarily of the capsid protein (CP) p88. Virions representing different stages of the virus life cycle can be purified from the infected fungal host *Helminthosporium victoriae* including the well-characterized 190S-1 and 190S-2 virions. These two types of virions differ in sedimentation rate, phosphorylation state, and CP composition; 190S-1 capsids contain p88 and p83, whereas the 190S-2 capsids contain p88 and p78 (p88 is the primary translation product of the CP gene; p83 and p78 represent post-translational proteolytic processing products of p88 at its C-terminus). p88 and p83 are phosphorylated, whereas p78 is nonphosphorylated. The virions with phosphorylated CPs (p88+p83) have significantly higher transcriptase activity *in vitro* than those containing the nonphosphorylated p78. Transcription occurs conservatively and the newly synthesized plus-strand RNA is released from the virions. Phosphorylation of CP is catalyzed by a host kinase, and is proposed to play a regulatory role in transcription/replication. A host-encoded protease catalyzes the proteolytic processing of phosphorylated p88; this occurs in two steps, leading first to p83 (the generation of the 190S-1 virions) and then to p78 (190S-2 virions). The conversion of p88-p83-p78 is proposed to play a role in the release of the plus-strand RNA transcripts from virions. The released plus-strand RNA is the RNA that is translated into CP and RNA-dependent RNA polymerase (RdRp) and packaged in capsids assembled from the primary translation product p88. It is not known whether p88 is phosphorylated before or after assembly. Synthesis of minus-strand RNA occurs on the plus-strand RNA template inside the virion; phosphorylation may be involved in turning on the replicase activity. Adapted from Ghabrial SA and Patterson JL (1999) *Encyclopedia of Virology*, 2nd edn., pp. 1808–1812. New York: Academic Press, with permission from Elsevier.

and cleavage of a C-terminal peptide, which leads to dephosphorylation and the conversion of p88 to p78, may play a role in the release of the plus-strand RNA transcripts from virions (**Figure 4**).

Biological Properties

There are no known natural vectors for the transmission of the fungal totiviruses. They are transmitted intracellularly during cell division, sporogenesis, and cell fusion. Although the yeast totiviruses are effectively transmitted via ascospores, the totiviruses infecting the ascomycetous filamentous fungi are essentially eliminated during ascospore formation. The leishmaniaviruses are not infectious as purified virions and are propagated during cell division. The giardiavirus GLV and the unclassified IMNV, on the other hand, are infectious as purified virions. Successful transfection of the protozoa *Giardia lamblia* has also been accomplished via electroporation with plus-strand RNA transcribed *in vitro* from GLV dsRNA. Whereas IMNV causes a myonecrosis disease in its crustacean host, GLV is associated with latent infection of its flagellated protozoan human parasite *G. lamblia*. GLV is released into the medium without lysing the host cells and the extruded virus can infect many virus-free isolates of the protozoan host.

There are no known experimental host ranges for the fungal totiviruses because of the lack of suitable infectivity assays. As a consequence of their intracellular modes of transmission, the natural host ranges of fungal totiviruses are limited to individuals within the same or closely related vegetative compatibility groups. Furthermore, mixed infections with two or more unrelated viruses are common, probably as a consequence of the ways by which fungal viruses are transmitted in nature. Dual infection of yeast with ScV-L-A and ScV-L-BC and the filamentous fungus *Sphaeropsis sapinea* with SsRV-1 and SsRV-2 are examples of mixed infections involving totiviruses. Dual infection of *H. victoriae* with Hv190SV and the chrysovirus Hv145SV is an example of a mixed infection involving two unrelated viruses belonging to two different families, a totivirus and a chrysovirus.

With the exception of IMNV, an unclassified virus tentatively assigned to the family *Totiviridae*, which causes a myonecrosis disease in shrimp, totiviruses are generally associated with symptomless infections of their hosts. A possible exception to this rule is Hv190SV, since mixed infections with Hv190SV and Hv145SV are associated with a debilitating disease of the fungal host. The disease phenotype and the two viruses are transmitted via hyphal anastomosis and diseased isolates are characterized by reduced growth, excessive sectoring, aerial mycelial collapse, and generalized lysis (**Figure 5**). The disease phenotype of *H. victoriae*, the causal agent of Victoria blight of oats, is of special interest not only because diseased isolates are hypovirulent, but also because of the probable viral etiology. The disease phenotype was also transmitted by incubating fusing protoplasts from virus-free fungal isolates with purified virions containing both the Hv190S and 145S viruses. The frequency of infection and stability of the newly diseased colonies, however, were very low, and verification of transmission was based on virus detection by immune electron microscopy. A systematic molecular approach based on DNA-mediated transformation of a susceptible virus-free *H. victoriae* isolate with full-length cDNA clones of viral dsRNAs is currently being pursued to verify the viral etiology of the disease phenotype and elucidate the roles of the individual viruses in disease development.

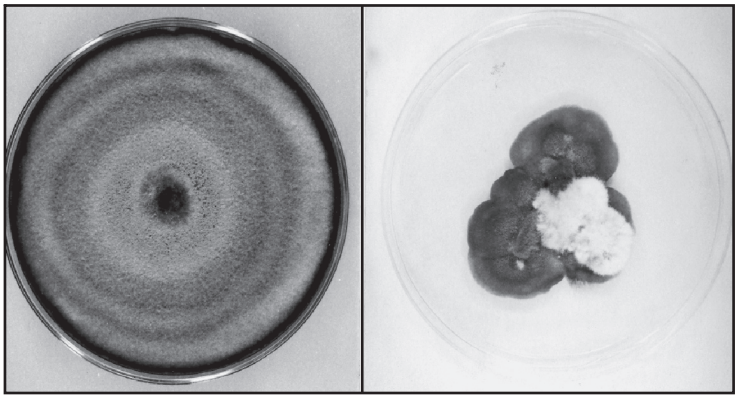


Figure 5 Colony morphology of virus-free (healthy), virus-infected (diseased) isolates of *Helminthosporium victoriae*. The colonies were grown for a week on potato dextrose agar medium at room temperature. The healthy colony shows uniform mycelial growth that extends to the entire plate, whereas the diseased colony is stunted and shows excessive sectoring.

A transformation vector for *H. victoriae* based on the hygromycin B resistance marker was constructed and used to transform an *H. victoriae* virus-free isolate with a full-length cDNA clone of Hv190SV dsRNA. The Hv190SV cDNA was inserted downstream of a *Cochliobolus heterostrophus GPD1* promoter and upstream of an *Aspergillus nidulans trpC* terminator signal. The hygromycin-resistant transformants expressed the Hv190SV CP, as determined by Western-blotting analysis. Transformation of a normal virus-free fungal isolate with a full-length cDNA of Hv190SV dsRNA conferred a disease phenotype. Symptom severity varied among the transformants from symptomless to severely stunted and highly sectored (**Figure 6**). Symptom severity correlated well with the level of viral capsid accumulation. Integration of the viral genome in the host DNA was verified by Southern blot hybridization analysis and expression of the viral dicistronic mRNA was demonstrated by northern analysis. Like natural infection, the primary translation product of the *CP* gene (p88) was phosphorylated and proteolytically processed to generate p83 and p78. The CP assembled into virus-like particles indistinguishable in appearance from empty capsids normally associated with virus infection. The empty capsids accumulated to significantly higher levels than in natural infections. Although the RdRp was expressed and packaged, no dsRNA was detected inside the virus-like particles or in the total RNA isolated from mycelium. Despite inability to launch dsRNA replication from the integrated viral cDNA, the demonstration that the ectopic 190SV cDNA copies were transcribed and translated and that the resultant transformants exhibited a disease phenotype provides convincing evidence for a viral etiology for the disease of *H. victoriae*. The role of the Hv145SV in disease development, however, has yet to be elucidated.

Virus–Host Relationships

The yeast killer system, comprised of a helper totivirus (ScV-L-A) and associated satellite dsRNA (M-dsRNA), is one of the very few known examples where virus infection is beneficial to the host. The ability to produce killer toxins by immune yeast strains confers an ecological advantage over sensitive strains. The use of killer strains in the brewing industry provides protection against contamination with adventitious sensitive strains. Totiviruses maintain only the genes that are essential for their survival (RdRp and CP), but make efficient use of host proteins. The host cells have evolved to support only a defined level of virus replication, beyond which virus infection may become pathogenic. Because of amenability to genetic studies, the yeast–virus system has provided significant information on the host genes required to prevent viral cytopathology. A system of six chromosomal genes, designated superkiller (or SKI) SKI2, SKI3, SKI4, SKI6, SKI7, and SKI8, negatively control the copy number of the totivirus ScV-L-A and its satellite M-dsRNAs. The only crucial function of these genes is to block virus multiplication. Mutations in any of these

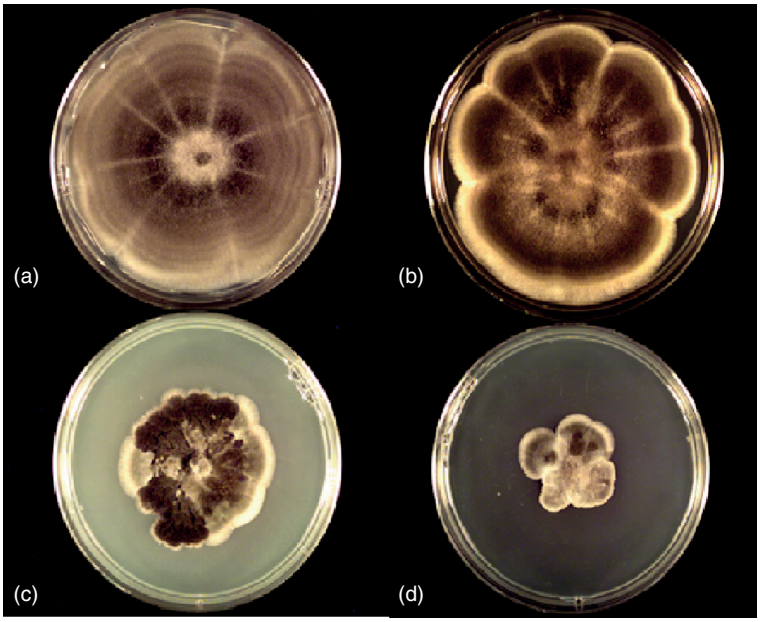


Figure 6 Colony morphology of a virus-free *Helminthosporium victoriae* isolate transformed with a full-length cDNA clone of the totivirus Hv190SV dsRNA. Colonies transformed with vector alone (a) or with recombinant vector containing full-length cDNA to Hv190SV dsRNA (b–d) were grown for a week at room temperature. Note that colonies (b)–(d) show increasingly more severe symptoms (reduced growth and sectoring).

SKI genes lead to the development of the superkiller phenotype as a consequence of the increased copy number of M-dsRNA. The SKI genes affect primarily the initiation of translation rather than the stability of mRNA, and are thus part of a cellular system that specifically blocks translation of nonpolyadenylated mRNAs (like the plus-strand transcripts of totiviruses). About 30 chromosomal genes, termed MAK genes (for maintenance of killer), are required for stable replication of the satellite M-dsRNA. Only three of these MAK genes are necessary for the helper virus (ScV-L-A) multiplication. Mutants defective in any of 20 MAK genes show a decreased level of free 60S ribosomal subunits. Since the *mak* mutations affecting 60S subunit levels are suppressed by *ski* mutations, and since the latter act by blocking translation of nonpolyadenylated mRNAs, the level of 60S ribosomal subunits is critical for translation of nonpolyadenylated mRNAs.

The Hv190S totivirus that infects the plant pathogenic fungus *H. victoriae* utilizes host-encoded proteins (a protein kinase and a protease) for post-translational modification of its CP. Phosphorylation and proteolytic processing of CP may play a role in regulating transcription and the release of plus-strand transcripts from virions (**Figure 4**).

The *H. victoriae*-virus system is well characterized and provides a useful model system for studies on virus–host interactions in a plant pathogenic fungus. A major attribute of this system is the fact that the virus-infected *H. victoriae* isolates exhibit a disease phenotype (**Figure 5**), which is rare among fungal viruses. Modulation of fungal gene expression and alteration of phenotypic traits as a consequence of mycovirus infection are little understood, with the exception of the chestnut blight fungus–hypovirus system. It was previously demonstrated that the fungal gene *Hv-p68* is upregulated as a result of virus infection and proposed that upregulation of this gene might play a role in virus pathogenesis (**Figure 7**). Hv-p68, a novel alcohol oxidase/RNA-binding protein, belongs to the large family of FAD-dependent GMC oxidoreductases with 67–70% sequence identity to the alcohol oxidases of methylotrophic yeasts. Hv-p68, however, shows only limited methanol-oxidizing activity and its expression is not induced in cultures supplemented with methanol as the sole carbon source (**Figure 7**). The natural substrate for Hv-p68 is not known, but the structurally similar alcohol oxidases are known to oxidize primary alcohols irreversibly to toxic aldehydes. Overexpression of Hv-p68 and putative accumulation of toxic intermediates was proposed as a possible mechanism underlying the disease phenotype of virus-infected *H. victoriae* isolates. Overexpression of Hv-p68 in virus-free fungal isolates, however, resulted in a significant increase in colony growth and did not induce a disease phenotype. Thus, overexpression of Hv-p68 *per se* is not sufficient to induce the disease phenotype in the absence of virus infection. If the function of *Hv-p68* is similar to that of the homologous alcohol oxidases of methylotrophic yeasts or other filamentous fungi, that is, irreversible oxidation of primary alcohols into aldehydes, then overexpression of Hv-p68 could lead to an accumulation of toxic aldehydes and development of disease phenotype. The finding that colonies overexpressing the Hv-p68 protein did not exhibit the disease phenotype and grew more rapidly than the nontransformed wild-type suggests that accumulation of toxic aldehydes did not occur, and that such aldehydes were probably assimilated into carbohydrates via the xylulose monophosphate pathway.

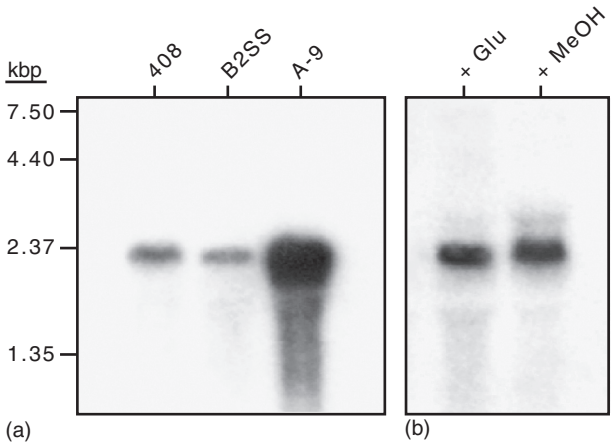


Figure 7 Northern analysis of Hv-p68 mRNA transcript levels. (a) Total RNA (25 μg) isolated from the virus-free isolates 408 and B-2ss and the virus-infected isolate A-9 were electrophoresed on a formaldehyde/ agarose gel, blotted, and hybridized under high stringency conditions with a radiolabeled probe for Hv-p68. Hv-p68 mRNA, 2.25 kbp in size, was detected in RNA samples from all three *H. victoriae* isolates. The level of Hv-p68 transcript in cultures of the virus-infected isolate A-9, however, was at least 15-fold higher than that for the virus-free isolates. (b) Total RNA (30 μg) isolated from cultures of the virus-free isolate B-2ss grown for 3 days in minimal medium supplemented with either glucose (Glu) or methanol (MeOH). Similar amounts of Hv-p68 mRNA were detected in total RNA isolated from fungal cultures supplemented with either glucose or methanol, as a carbon source. Reproduced from Soldevila AI and Ghabrial SA (2001) A novel alcohol oxidase/RNA-binding protein with affinity for mycovirus double-stranded RNA from the filamentous fungus *Helminthosporium* (*Cochliobolus*) *victoriae*. *Journal of Biological Chemistry* 276: 4652–4661, with permission from the American Society for Biochemistry and Molecular Biology, Inc.

Hv-p68, which co-purifies with viral dsRNA (mainly that of Hv145SV), is a multifunctional protein with alcohol oxidase, protein kinase, and RNA-binding activities. Hv-p68 is detectable as a minor component of Hv190SV capsid. Recent evidence strongly suggests that Hvp68 is the cellular protein kinase responsible for phosphorylation of the capsid proteins. The RNA-binding activity of Hv-p68 has been demonstrated by gel mobility shift and northwestern blot analysis. Furthermore, the RNA-binding domain of Hv-p68 was mapped to

the N-terminal region that contains the ADP-binding domain. Because dsRNA-binding proteins are known to sequester dsRNA and suppress antiviral host defense mechanisms, it is feasible that overexpression of the dsRNA-binding protein Hv-p68 may lead to the induction of the disease phenotype by suppressing host defense. This idea is consistent with the finding that overexpression of Hv-p68 led to enhancement in the accumulation of Hv145S dsRNA. The role of Hv145SV in the development of the disease phenotype, however, is not yet clear. Furthermore, it is curious that Hvp68 co-purifies with Hv145S dsRNA since the latter is predicted to be confined to the viral capsids where virus replication takes place. Recent results, however, suggest that a significant proportion of the Hv145V dsRNA does not appear to be encapsidated. Considering the multifunctional nature of the Hv-p68 protein, additional studies are needed to determine whether or not Hv-p68 upregulation has a role in viral pathogenesis.

Evolutionary Relationships among Totiviruses

Sequence comparison analysis of the predicted amino acid sequences of totivirus RdRps indicated that they share significant sequence similarity and characteristically contain eight conserved motifs. This sequence similarity was common to all totiviruses so far characterized including the totiviruses that infect the yeast, smut, and filamentous fungi, as well as those infecting parasitic protozoa. As indicated earlier, the viruses infecting filamentous fungi (members of the newly proposed genus *Victorivirus*) express their RdRp separate from the CP by an internal initiation mechanism,

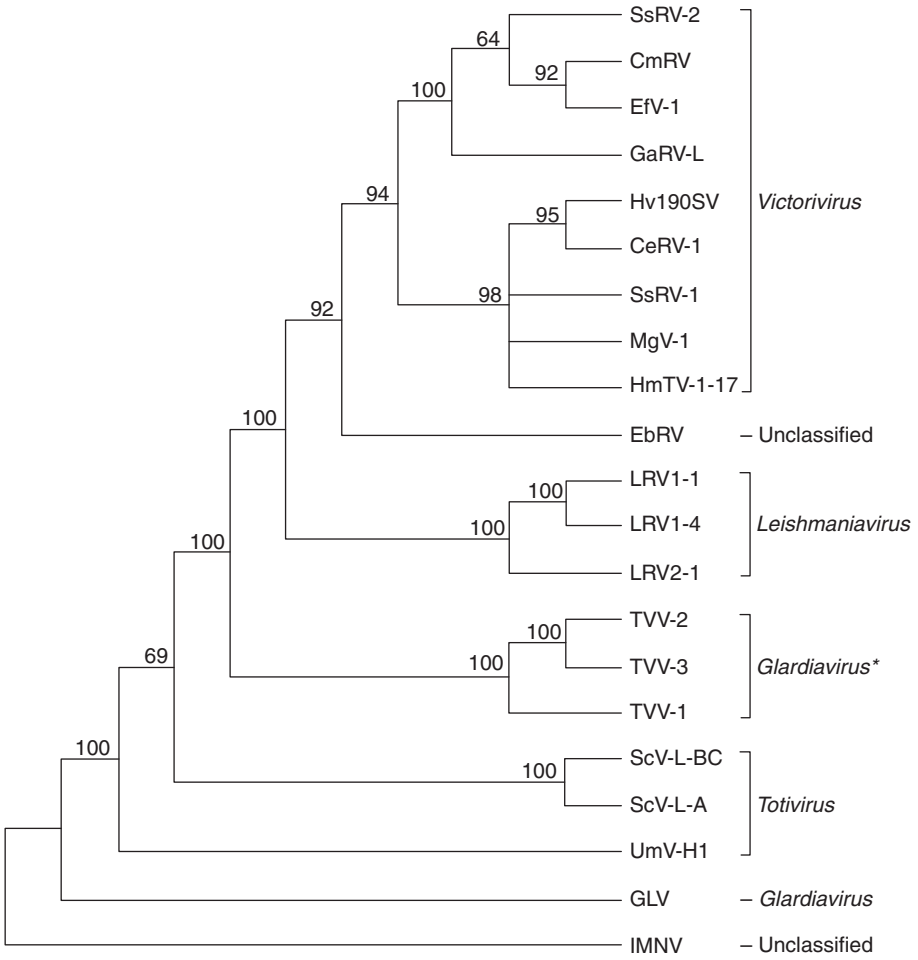


Figure 8 Neighbor-joining phylogenetic tree constructed based on the RdRp conserved motifs and flanking sequences. The RdRp sequences were derived from aligned deduced amino acid sequences of members of the family *Totiviridae* using the program CLUSTAL X. Motifs 1–8 and the sequences between the motifs, as previously designated by Ghabrial SA in 1998, were used. See **Table 1** for virus name abbreviations and GenBank accession numbers. The phylogenetic tree was generated using the program PAUP*. Bootstrap numbers out of 1000 replicates are indicated at the nodes. The tree was rooted with the RdRp of the penaeid shrimp infectious myonecrosis virus (IMNV), an unclassified virus tentatively assigned to the family *Totiviridae*, which was included as an outgroup.

whereas the other members of the family *Totiviridae* express their RdRps as fusion proteins (CP–RdRp), mainly via a ribosomal frameshifting mechanism. Phylogenetic analysis based on multiple alignments of amino acid sequences of totivirus RdRp conserved motifs (**Figure 8**) reflects these differences as the viruses infecting filamentous fungi (victoriviruses) are most closely related to each other and form a distinct large well-supported cluster (bootstrap value of 94%). Likewise, phylogenetic trees based on the CP sequences showed similar topology to those based on RdRp sequences (**Figure 9**). The fact that independent alignments of CP and RdRp sequences give similar phylogenetic relationships (**Figures 8** and **9**) further supports the creation of a new genus to accommodate the viruses infecting filamentous fungi.

The leishmaniaviruses LRV1, LRV2, and LRV4 (percent sequence identities of 46%) are most closely related to each other and form a discrete cluster with 100% bootstrap value. This is also true for the two yeast viruses ScV-L-A and ScV-LBC (RdRp identity of 32%), members of the genus *Totivirus*. UmV-H1, the third member in the genus *Totivirus* forms a phylogenetic clade distinct from the cluster of the two yeast viruses. This may reflect the difference in RdRp expression strategy between these viruses since UmV-H1 expresses its RdRp via the generation of a polyprotein followed by proteolytic processing to release the viral protein, whereas the yeast viruses express their RdRps as CP–RdRp fusion proteins via a –1 ribosomal frameshifting. It is of interest that Hv190SV and related viruses (victoriviruses) are phylogenetically more closely to the leishmaniaviruses than to the yeast viruses (**Figures 8** and **9**). It was previously hypothesized that leishmaniaviruses and the fungal totiviruses are of old origin having existed in a single-cell-type progenitor

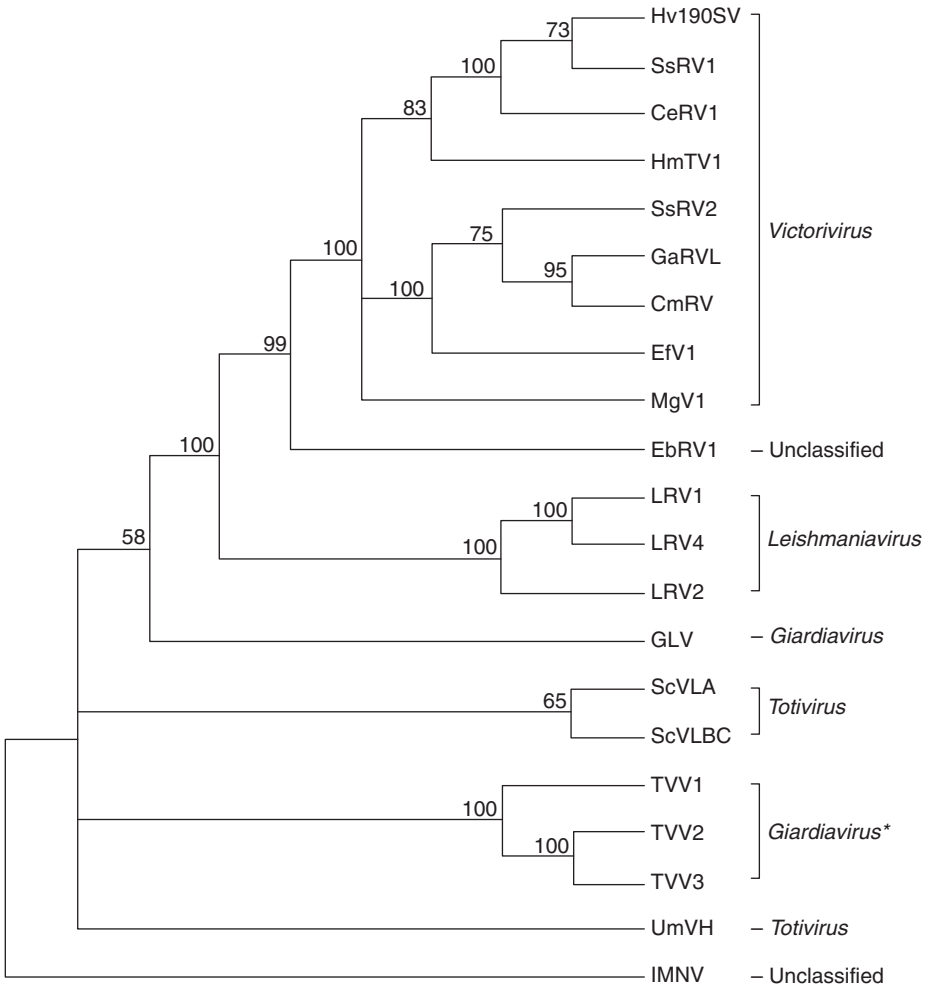


Figure 9 Neighbor-joining phylogenetic tree constructed based on the complete amino acid sequences of the capsid proteins of viruses in the family *Totiviridae*. The CP multiple sequence alignment was performed with the program CLUSTAL X and the phylogenetic tree was generated using the program PAUP*. See **Table 1** for virus name abbreviations and GenBank accessions numbers. Bootstrap numbers out of 1000 replicates are indicated at the nodes. The tree was rooted with the CP of the penaeid shrimp infectious myonecrosis virus (IMNV), an unclassified virus tentatively assigned to the family *Totiviridae*, which was included as an outgroup.

prior to the divergence of fungi and protozoa. The finding that the unclassified Eimeria brunetti RNA virus 1 (EbRV-1), which infects an apicomplexan parasitic protozoa, is more closely related to members of the new genus *Victorivirus* than to protozoal viruses is consistent with this idea. EbRV-1 may represent an intermediate progenitor in the evolution of 'victoriviruses'.

It is worth noting that the classification of TVV1, TVV2, and TVV4 as tentative members of the genus *Giardiavirus* is not supported by phylogenetic analyses based on the totivirus CPs or RdRps since they do not cluster with GLV, the type species of the genus *Giardiavirus*. The trichomonas vaginalis viruses are most closely related to each other as they form a distinct phylogenetic cluster with 100% bootstrap support (**Figures 8** and **9**). It would probably be justifiable to create a new genus in the family totiviridae to accommodate these viruses.

Taxonomic considerations of totiviruses may benefit greatly from elucidating the capsid structure of representatives of the genera *Leishmaniavirus* and *Giardiavirus* (including the tentative members TVVs). Furthermore, resolving the capsid structure of the unclassified viruses EbRV-1 and IMNV may prove useful for their taxonomic placement. Information on the genome organization and RdRp expression strategy of EbRV-1 is lacking and such information would be crucial in determining whether a novel genus in the family *Totiviridae* should be created for EbRV-1. IMNV has some features that distinguishes it from totiviruses including that it expresses its CP as a polyprotein that undergoes autoproteolytic processing. It is likely that IMNV would be classified as member of a novel family of dsRNA viruses that infect invertebrate hosts.

See also: Fungal Viruses; Giardiaviruses; Leishmaniaviruses; Ustilago Maydis Viruses; Viral Killer Toxins; Yeast L-A Virus.

Further Reading

Castón JR, Luque D, Trus BI, *et al.* (2006) Three-dimensional structure and stoichiometry of *Helminthosporium victoriae190S* totivirus. *Virology* 347: 323–332.

Cheng J, Jiang D, Fu Y, Li G, Peng Y, and Ghabrial SA (2003) Molecular characterization of a dsRNA totivirus infecting the sclerotial parasite *Coniothyrium minitans*. *Virus Research* 93: 41–50.

Ghabrial SA (1998) Origin, adaptation and evolutionary pathways of fungal viruses. *Virus Genes* 16: 119–131.

Ghabrial SA and Patterson JL (1999) *Encyclopedia of Virology*, 2nd edn., pp. 1808–1812. New York: Academic Press.

Ghabrial SA, Soldevila AI, and Havens WM (2002) Molecular genetics of the viruses infecting the plant pathogenic fungus *Helminthosporium victoriae*. In: Tavantzis S (ed.) *Molecular Biology of Double-Stranded RNA: Concepts and Applications in Agriculture, Forestry and Medicine*, pp. 213–236. Boca Raton, FL: CRC Press.

Huang S and Ghabrial SA (1996) Organization and expression of the double-stranded RNA genome of *Helminthosporium victoriae* 190S virus, a totivirus infecting a plant pathogenic filamentous fungus. *Proceedings of the National Academy of Sciences, USA* 93: 12541–12546.

Icho T and Wickner RB (1989) The double-stranded RNA genome of yeast virus L-A encodes its own putative RNA polymerase by fusing two open reading frames. *Journal of Biological Chemistry* 264: 6716–6723.

Nibert ML (2007) '2A-like' and 'shifty heptamer' motifs in penaeid shrimp infectious myonecrosis virus, a monosegmented double-stranded RNA virus. *Journal of General Virology* 88: 1315–1318.

Nomura K, Osaki H, Iwanami T, Matsumoto N, and Ohtsu Y (2003) Cloning and characterization of a totivirus double-stranded RNA from the plant pathogenic fungus, *Helicobasidium mompa* Tanaka. *Virus Genes* 23: 219–226.

Park Y, James D, and Punja ZK (2005) Co-infection by two distinct totivirus-like double-stranded RNA elements in *Chalara elegans* (*Thielaviopsis basicola*). *Virus Research* 109: 71–85.

Preisig O, Wingfield BD, and Wingfield MJ (1998) Coinfection of a fungal pathogen by two distinct double-stranded RNA viruses. *Virology* 252: 399–406.

Romo M, Leuchtmann A, and Zabalgogeazcoa I (2007) A totivirus infecting the mutualistic fungal endophyte *Epichloë festucae*. *Virus Research* 124: 38–43.

Soldevila A and Ghabrial SA (2000) Expression of the totivirus Helminthosporium victoriae190S virus RNA-dependent RNA polymerase from its downstream open reading frame in dicistronic constructs. *Journal of Virolology* 74: 997–1003.

Soldevila AI and Ghabrial SA (2001) A novel alcohol oxidase/RNA-binding protein with affinity for mycovirus double-stranded RNA from the filamentous fungus *Helminthosporium* (*Cochliobolus*) *victoriae*. *Journal of Biological Chemistry* 276: 4652–4661.

Tuomivirta TT and Hantula J (2005) Three unrelated viruses occur in a single isolate of *Gremmeniella abietina* var. *abietina* type A. *Virus Research* 110: 31–39.

Wickner RB, Wang CC, and Patterson JL (2005) Totiviridae. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 581–590. San Diego, CA: Elsevier Academic Press.

Transcriptional Regulation in Bacteriophage

R A Weisberg, D M Hinton, and S Adhya, National Institutes of Health, Bethesda, MD, USA

Published by Elsevier Ltd.

Introduction

Most bacteriophages regulate their own gene expression, many regulate host gene expression, and a few even regulate genes of other phages. Regulation enables temperate phages to establish and maintain lysogeny; it allows both temperate and virulent phages to carry out developmental programs in which groups of genes are expressed in an ordered, temporal sequence as their products are needed during the lytic growth cycle, and it

helps phages to commandeer the transcriptional resources of their hosts. In this article we describe the biological roles and mechanisms of regulation employed by several extensively studied model phages of *Escherichia coli*: temperate phage λ and virulent phages T4, T7, and N4. We emphasize transcriptional regulation, although we present examples of regulated RNA and protein stability as well. Investigations of these regulatory strategies have not only elucidated much about phage biology, but have also yielded many insights into regulatory mechanisms that are used by the host. We also describe several other strategies found in some less extensively studied phages.

Transcript Initiation and Elongation by Host RNA Polymerase

When a phage chromosome enters a cell, it encounters a host RNA polymerase (RNAP) that has specific requirements for promoter recognition. Prokaryotic cellular RNAPs consist of a large, multisubunit core, consisting of two copies of the α subunit, and one each of the β, β′, and ω subunits. Core has the ability to synthesize RNA, while a sixth subunit, σ-factor, is needed to identify and bind to promoters, and to initiate RNA synthesis at specific start sites. Primary σ-factors are used during exponential growth for the expression of housekeeping genes while alternate σ factors are needed to transcribe bacterial genes required under certain growth conditions or at times of stress. Many phages direct the synthesis of transcription factors that interact with, modify, or replace host σ. Another class of phage-encoded factors targets core subunits, and these factors can regulate either transcript initiation or transcript elongation.

Hundreds of prokaryotic σ factors have been identified, and they share up to four regions of similar sequence and function. Primary σ factors, like σ^{70} of *E. coli*, have three well-characterized regions with the potential to recognize and bind promoters. Residues in region 2 interact with a −10 DNA element (positions −12 to −7), residues in region 3 contact an extended −10 (TGn) motif (positions −15 to −13), and residues in region 4 contact a −35 element (positions −35 to −29) (**Figure 1**; the negative numbers indicate the number of base pairs upstream of the transcription start point). Only two of these three contacts are necessary for good promoter activity, and the majority of host promoters are −10/−35 promoters that use the DNA binding of regions 2 and 4. The −10/−35 promoters also require an interaction between residues in region 4 and a structure in core, called the β-flap, which is required to position region 4 correctly for its contact with the DNA.

RNAP also contacts host promoters through interactions between the C-terminal domain (CTD) of the α-subunits of polymerase core and A–T-rich sequences located between −40 and −60 (UP elements). Although RNAP contains two α subunits, they are not equivalent; one is bound to β and the other is bound to β′. Each α-CTD can interact specifically with a promoter proximal UP element, centered at position −41, or a promoter distal UP element, centered at position −52. In addition, the α-CTD domains can interact nonspecifically with DNA located in the −40 to −60 region.

Initiation of transcription from a −10/−35 promoter by host RNAP is a multistep process. After binding to the promoter through the interactions of σ^{70} with the −10 and −35 elements, the enzyme distorts a region around the −10 element, a step called isomerization, and becomes

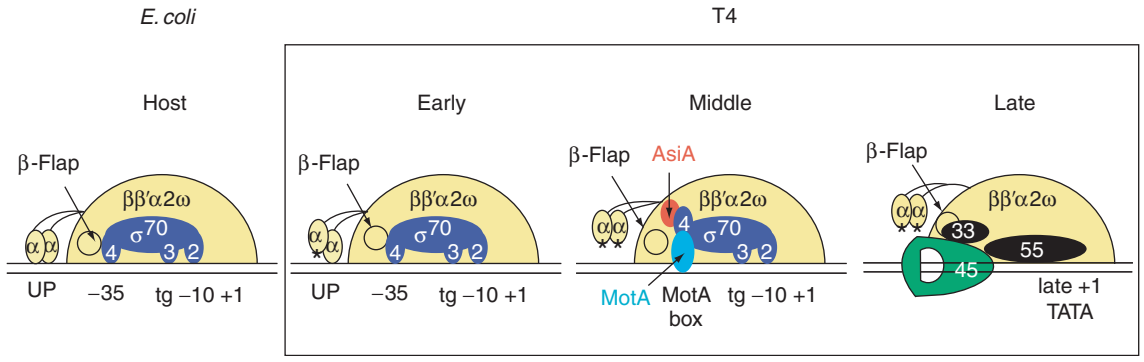


Figure 1 Changes to (*E. coli*) RNAP during T4 infection. Panels from left to right present cartoons of RNAP at host, T4 early, T4 middle, and T4 late promoters. Core polymerase (β, β′, α_2, and ω) is in tan. CTDs of α-subunits, which can interact with upstream promoter DNA (UP), and the β-flap are indicated. Region 2, 3, and 4 of σ^{70}, which recognize a −10 element, TGn, and a −35 element, respectively, are in dark blue. T4-catalyzed ADP-ribosylation of an α-CTD, which prevents the interaction of α-CTD with DNA, is denoted with a star. Recognition of a T4 middle promoter, which contains a σ^{70} −10 element and a MotA box element, requires σ^{70}-containing RNAP, the T4 activator, MotA, and the T4 coactivator, AsiA. Recognition of a T4 late promoter, which has a TATA element at −10, requires core polymerase, a T4 α-factor composed of gp33 and gp55, and the T4 activator gp45. See text for details.

competent to initiate synthesis of an RNA chain that is complementary to the template strand of the DNA and that grows in a 5′ to 3′ direction. As the chain grows, its association with the template and enzyme is stabilized, principally through the formation of 8–9 bp of RNA:DNA hybrid adjacent to the 3′-end. Upstream of this point, the nascent transcript dissociates from the template strand of the DNA, and an additional 5–6 nt fill an exit channel before the chain emerges from the enzyme. During this process, the newborn elongation complex (EC) releases its grip on the promoter and begins translocating along the template at a rate of about 50 bp $^{-1}$s. Occupancy of the exit channel by RNA weakens binding of σ^{70} to the core and facilitates σ^{70} release from the EC. However, the kinetics of σ^{70} release are controversial, and there is evidence that it can remain associated with the EC even after considerable translocation.

Transcript elongation stops when the EC reaches a sequence that encodes a transcription terminator, at which point RNAP dissociates from the template and the RNA. Two classes of terminators are known in *E. coli*: intrinsic and Rho-dependent. Intrinsic terminators are nascent transcripts that can form a stable, base-paired stem–loop or hairpin that is followed by a U-rich stretch. Termination occurs within the U-rich stretch, typically 6–7 nt from the base of the hairpin. Although the nascent terminator transcript is sufficient for termination, the efficiency can be altered by proteins. Rho protein, which is necessary for termination at the second class of terminator, binds to nascent RNA upstream of the termination point. Once bound, it can use ATP to translocate in a 5′ to 3′ direction toward the EC. It is believed that termination occurs when Rho catches up to a paused EC.

Phage T4

T4 is the best-characterized member of the T4-type phages of the family *Myoviridae*, consisting of phages distinguished by a contractile tail. T4 has a 169 kbp linear chromosome, which is terminally redundant and circularly permuted. Consequently, the T4 genome is shown as a circle (**Figure 2**). Injection of the DNA into the host initiates a pattern of T4 gene expression that results in a burst of phage about 20 min after infection. The production of phage proteins is regulated primarily at the level of temporally controlled transcription through the synthesis of early, middle, and late RNAs.

T4 Early Transcription

Productive T4 infection requires exponentially growing cells. Consequently, polymerase containing σ^{70} is the major species present when T4 infects. A top priority for T4 is to rapidly commit the host polymerase to its agenda.

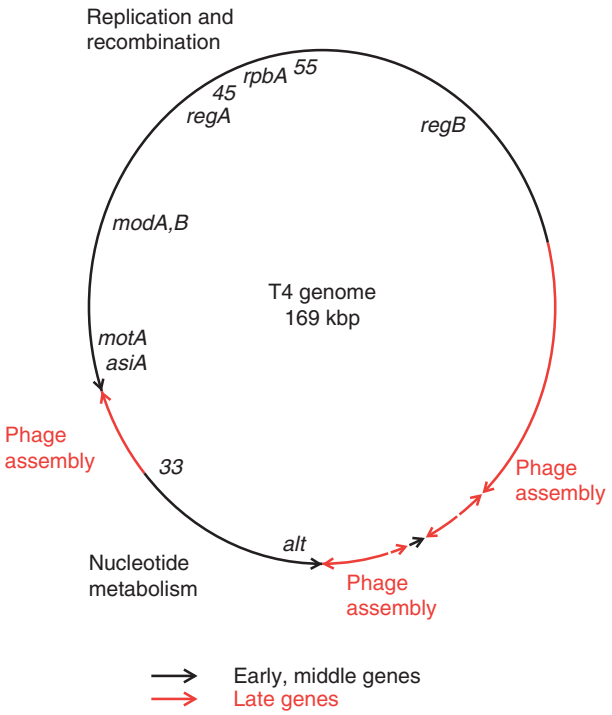


Figure 2 Map of bacteriophage T4. The 169 kbp linear T4 DNA is represented as a circle because it is circularly permuted and terminally redundant. Black regions contain early/middle genes, which include genes whose products are needed for DNA replication and recombination and nucleotide metabolism. Red regions contain late genes, whose products are primarily involved in phage assembly. The positions of specific genes referred to in the text are also shown.

It accomplishes this task through multiple strategies. Immediately after infection, T4 programs strong transcription from its early promoters, which are recognized by σ^{70} containing host RNAP. These promoters are needed for expressing early genes, many of which encode proteins that are involved in quickly moving the host resources to the phage, such as nucleases that degrade the host DNA and proteins that will be needed for middle gene expression. T4 early promoters have the recognition features of the σ^{70}-dependent −10/−35 promoters, but these promoters also have the extended −10 sequence for σ^{70} region 3 and UP elements for α-CTD contact. Consequently, the 40 or so early promoters are extremely strong, and they compete very efficiently with the more than 600 host promoters for productive transcription initiation by the available polymerase.

To increase further the advantage of early promoters over host promoters, T4 encodes a protein, Alt, that chemically modifies host RNAP by ADP-ribosylation. This modification specifically increases the activity of T4 early promoters relative to those of the host. Alt is actually present within the phage head, and it is injected

into the host along with phage DNA. Thus, the Alt modification begins immediately after infection. Although Alt-dependent ADP-ribosylation affects a minor fraction of many *E. coli* proteins, it primarily modifies a specific residue (Arg265) on one of the α-subunits. It is thought that this modification occurs on the same α on every polymerase, but which α-subunit is unknown. Arg265 is located within the CTD of the α-protein, and normally this residue directly contacts UP element DNA. However, ADP-ribosylation of Arg265 eliminates this DNA interaction. Sequence differences between T4 early promoters and host promoters in the −40 to −60 region (the α-CTD binding sites) may explain how this asymmetric ADP-ribosylation favors T4 early promoters.

T4 transcription gains another advantage over the host early after infection because T4 DNA itself is modified, containing glucosylated, hydroxymethyl cytosines. This modification protects the phage genome from multiple nucleases that are encoded by T4 early genes and digest unmodified host DNA within the first few minutes of infection. In addition, T4 encodes an early protein (Alc) that specifically terminates transcription on unmodified host DNA.

T4 Middle Transcription

T4 middle gene expression begins about 1 min after infection. Middle genes include genes that encode T4 replication proteins, transfer RNAs, and proteins that will be needed later to switch from middle to late promoter recognition. At the start of middle transcription, two early gene products, the ADP-ribosylating enzymes ModA and ModB, complete the ADP-ribosylation of Arg 265, resulting in the modification of both α-subunits. This prevents any contact between the α-CTD domains and the −40 to −60 sequences, thereby further decreasing initiation from host promoters. In addition, ADP-ribosylation of the second α-subunit now lessens the activity of T4 early promoters. Thus, the action of ModA/ModB should contribute to the switch from T4 early to T4 middle transcription. Early gene expression is also decreased by the action of another early gene product, RegB endoribonuclease, which cleaves within the sequence GGAG present in the ribosome binding site of many early transcripts. Although RegB is present even after late gene expression has begun, and some middle and late T4 transcripts contain the RegB recognition sequence, only cleavage of early RNA is observed. In particular, the mRNA of the T4 MotA protein, which is required for middle promoter activation (see below), is an RegB substrate. Evidence suggests that the secondary structure of the mRNA is important for RegB recognition, but the exact nature of RegB specificity is not yet understood.

T4 middle RNA is synthesized using two separate strategies. First, early and middle genes are located together with almost all middle genes downstream of early genes (**Figure 2**). Consequently, transcription from T4 early promoters produces middle RNA as these early transcripts extend into middle genes. Although there has been speculation that this extension involves an active T4 antitermination process, perhaps like that of λ (below), definitive evidence for such a mechanism has not been found and the control of this extension is not yet understood.

Second, T4 activates the initiation of transcription at more than 30 middle promoters. These promoters contain the σ^{70} −10 element and are dependent on RNAP containing σ^{70} for transcription. However, they lack the σ^{70} −35 element and have instead a different consensus sequence, the 'MotA box', centered at −30 (**Figure 1**). Activation of middle promoters occurs by a process called σ appropriation and requires two T4-encoded proteins, MotA and AsiA. MotA is a transcription activator that interacts both with the MotA box and with the far C-terminus of σ^{70}, a region that contains some of the residues that normally interact with the β-flap. AsiA is a small protein that binds tightly to σ^{70} region 4. When MotA is present, AsiA coactivates transcription from T4 middle promoters. By itself AsiA also inhibits transcription from σ^{70}-dependent promoters that require an interaction with the −35 element.

AsiA binds to multiple residues in σ^{70} region 4 and dramatically changes its conformation. Some of these residues normally interact with the −35 element, and some with the β-flap, but the structure that results from AsiA binding no longer interacts with either. Thus, AsiA binding inhibits transcription from σ^{70}-dependent promoters that require these interactions. Furthermore, by removing the interaction of σ^{70} with the −35 sequences, AsiA helps MotA interact with the MotA box, centered at −30. This is because the 9 bp MotA box includes sequences that would normally interact at least indirectly with σ^{70} region 4. In addition, AsiA frees the far C-terminus of σ^{70} to interact with MotA, since the structural changes prevent this portion of σ^{70} from interacting with the β-flap.

Interestingly, AsiA binds rapidly to free σ^{70}, but poorly, if at all, to σ^{70} that is present in polymerase. Because there is an excess of core relative to σ^{70} in *E. coli*, very little free σ^{70} is available at any given time. Consequently, the binding of AsiA to σ^{70} must occur when σ^{70} is released from core, which occurs in this case after initiation at early promoters. Thus, transcription from early promoters directly promotes the switch to middle transcription, helping to coordinate the start of middle transcription with the vitality of early transcription.

T4 Late Transcription

Late genes primarily encode proteins needed to form virions and to process replicated T4 DNA. The switch

from middle to late transcription begins about 6 min after infection. The synthesis of some early and middle proteins decreases because translation of their mRNA is repressed by the T4-encoded middle protein, RegA, which competes with ribosomes for binding to the translational initiation sites of some middle mRNAs. RegA binds to an AU-rich motif but the precise requirements for binding are unknown. In addition, middle RNA synthesis decreases because the previous ADP-ribosylation of α-CTD and the binding of a T4 middle protein, RpbA, to core polymerase favor transcription from late promoters, described below, over transcription from middle promoters.

Transcription from T4 late promoters is accomplished by host core polymerase in combination with three phage-encoded middle proteins: gp33, gp55, and gp45 (**Figure 1**). Gp55 shares sequence similarity with σ region 2, and like region 2, gp55 recognizes and contacts a specific DNA element, in this case TATAAATA, centered at position −10 (**Figure 1**). Gp55 interacts with gp45 and the β′-subunit of RNAP. Gp33 interacts with gp45 and the β-flap of RNAP. Gp45 forms a ring that encircles T4 DNA and can move along it in either direction. Interaction of RNAP-bound gp33 with DNA-bound gp45 connects RNAP to DNA upstream of the gp55 DNA contact. Thus, gp33 and gp55 together can be considered a T4 σ-factor for late promoters. Interestingly, gp45 is also the DNA polymerase clamp protein, a processivity factor that is needed to keep T4 DNA polymerase from dissociating from DNA during replication. As a consequence, transcription from late promoters requires active DNA replication, because gp45 is loaded onto the DNA through its role as the DNA polymerase clamp. This connection of late transcription to the replication of phage DNA serves to coordinate the expression of late genes, whose functions are primarily DNA packaging and capsid assembly, with the amount of phage DNA. T4 late transcription continues until 20 min after infection, when the fully formed phage with the packaged phage genomic DNA are released through lysis of the cell.

Phages T7 and T3

T7 and T3 belong to the family *Podoviridae*, comprising phages with short tails. Their virions respectively contain about 39.9 and 38.7 kbp of double-stranded nonpermuted DNA. A short segment is directly repeated at each end ('terminal redundancy'). The chromosomal arrangement of genes with identical functions is similar in the two phages, and to several other sequenced phages of the family, in accord with the modular evolution of phages. We will describe the transcriptional regulation of T7, because it is the best-studied member of the entire group, mentioning T3 when appropriate. The genetic map and the transcription pattern of T7 are shown in **Figure 3**. T7 encodes 56 open reading frames (ORFs) most of whose functions are known. The ORFs include three cases of programmed translational frameshifting.

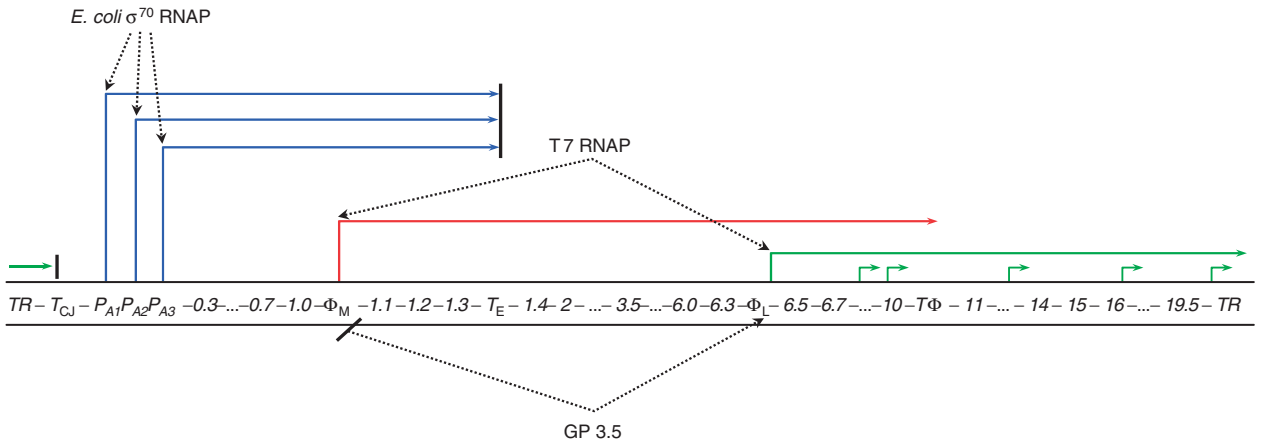


Figure 3 Phage T7 promoters, transcripts, repressors, and activators. Only genes and sites mentioned in the text are shown, and the diagram shows the gene order as it appears in packaged DNA. *Escherichia coli* RNAP transcribes the early promoters, P_{A1}, P_{A2}, and P_{A3} (blue arrows), and T7 RNAP transcribes the middle and late promoters, Φ_M and Φ_L (red and green arrows, respectively). Five other T7 RNAP-dependent promoters located downstream of Φ_L are also shown. T_E, T_Φ, and T_{CJ} are the terminators discussed in the text. TR means terminal repeat. The transcript from Φ_L (green arrow) continues through the left terminal repeat and ends at T_{CJ} because T7 chromosome ends are joined by a single copy of the terminal repeat during DNA replication (see text). Activation and repression of Φ_L and Φ_M by Gp3.5 are indicated by dotted lines ending in an arrow and bar, respectively. A bar across the transcript arrow heads signify terminated transcripts.

Temporal Expression of Genes

Studies of T7 protein synthesis after infection of the host show three temporal stages of gene expression. Early or class I genes (genes *0.3* to *1.3*), middle or class II genes (*1.4* to *6.3*) and late or class III genes (*6.5* to *19.5*) with some overlap, as described later. All T7 genes are transcribed from the same strand, from left to right. The genes in each class are physically contiguous, and the three groups, I, II and III, are also positioned from left to right in the phage chromosome in that order.

Early genes are transcribed by host σ^{70}-RNAP, and their products modify the intracellular environment so as to facilitate phage development. Only gene *1.0* in this group is essential for phage growth; it encodes a single polypeptide RNA polymerase (T7 RNAP) that specifically transcribes the class II and III genes. The class II gene products are involved in phage-specific DNA metabolic reactions, whereas the class III genes encode DNA packaging, virion assembly, and host cell lysis proteins.

DNA Entry and Coupled Transcription

Transcription of T7 and T3 phage DNA, unlike that of some other phages, starts before the complete genome is injected into the host. After adsorption of the phage, the products of phage genes *14*, *15*, and *16* are released from the virion and form a tunnel from the tail tip across the outer and inner membranes of the host for DNA entry into the cytosol. However, only 850 bp from the left end of the phage chromosome (**Figure 3**), which include the major early promoters P_{A1}, P_{A2}, and P_{A3}, enter the host. Transcription from these promoters by host RNAP must begin before additional DNA can enter. The reason for the barrier to further DNA entry is not known, but it is DNA sequence independent. Mutations that allow DNA entry in the absence of transcription map in the middle of gene *16*, suggest that the middle segment of Gp16 is at least partly responsible for the blockade. Although transcription from the three early promoters in the 850 bp region is sufficient, T7 RNAP-mediated transcription takes over in completing the DNA-entry process in normal infection.

Early Transcription

As mentioned earlier, T7 transcription begins immediately after infection at the strong adjacent P_{A1}, P_{A2}, and P_{A3} promoters, although several weak promoters, as well as some RNAP binding sites, which do not initiate transcription, are distributed in the class I region (not shown in **Figure 3**). The importance, if any, of these other promoters and the RNAP binding sites is not known. Transcription from the early promoters continues after complete genome entry and terminates at different sites with incomplete efficiency within the ~8000 bp long polycistronic transcription unit or at a Rho-independent terminator (T_E) located at the end of the transcription unit encompassing genes *0.3–1.3*. T7 early transcription terminates at T_E both *in vivo* and *in vitro*, but T3 early transcription terminates at T_E only *in vivo*, suggesting the involvement of a termination factor in the latter phage.

Middle Transcription

Transcription of the class II genes (from *1.4* to *6.3*) by T7 RNAP begins about 4–6 min and continues until about 15 min after infection. These genes are transcribed from several T7 RNAP specific promoters, of which the major one, Φ_M, is shown in **Figure 3**. Note that three class I genes – *1.1*, *1.2*, and *1.3* – are also transcribed by T7 RNAP because they are located downstream of the middle promoter Φ_M but upstream of the early terminator T_E (**Figure 3**). T7 but not host RNAP ignores the T_E terminator when transcribing genes *1.1* to *1.3*. Transcription from Φ_M terminates weakly at the T7 RNAP terminator, T_Φ, which is located between genes *10* and *11*. Specific transcription from Φ_M can be demonstrated *in vitro*, but only on templates that lack the class III promoters, indicating that a mechanism exists for high level transcription from Φ_M *in vivo*. It has been suggested that Φ_M has much-reduced affinity for T7 RNAP compared to that of class III promoters, but the middle promoter is stimulated *in vivo* by an unusually high local concentration of T7 RNAP, which is encoded by an adjacent gene.

Late Transcription

There are several T7 RNAP promoters that direct expression of late genes (**Figure 3**). The first late promoter, Φ_L, directs transcription of all genes located to the right of gene *6.3*, from gene *6.5* to the last gene, *19.5*. The late genes are transcribed from 8 min after infection until cell lysis occurs. Compared to the strength of Φ_M, Φ_L is intrinsically strong. Note that class III genes *6.5* through *10* are transcribed from both Φ_M and Φ_L, presumably because these gene products are needed in larger amounts. Although T7 RNAP initiating at Φ_L also terminates at T_Φ with reduced efficiency, all late transcription terminates at site T_{CJ}. T_{CJ} is downstream of Φ_L in phage DNA that is actively replicating and transcribed within infected cells, but the two sites are separated by the ends of the packaged T7 chromosome (**Figure 3**). This is because replication produces concatemers – repeating units of nonredundant T7 sequence that are separated by the terminal repeat – and packaging produces the chromosome ends from the concatemers. T_{CJ} has the sequence 5′-ATCTGTT with no secondary structure potential. It acts as a transcription pause site *in vitro*.

T7 RNA Polymerase

T7 RNAP is a single subunit enzyme of 98 000 Da. It recognizes a 19 bp sequence (consensus: TAATACGACTCACTATAGG) centered on position −8 from the start point of transcription (+1), which is the first G of the final GG sequence. There are 17 identified middle and late promoters that are used exclusively by T7 and not by host RNAP. T7 RNAP does not recognize host promoters and many of the host terminators, whether the latter are Rho-dependent or Rho-independent. T7 RNAP can utilize both T7 and T3 promoters but T3 RNAP is somewhat specific. The open complexes made by T7 RNAP distort at least 10 bp of double-stranded promoter DNA and are unstable until short RNA oligomers are made. Both T3 and T7 RNAP initiate transcription with 5′-GG. Significantly, the T7 enzyme catalyzes RNA synthesis 5–10 times faster than the host RNAP. As mentioned, besides Φ_M and Φ_L, there are several other promoters distributed among class II and III genes that are transcribed by T7 RNAP, and transcription from these promoters increases the products of certain genes. The 3′-ends of the RNA made by the phage enzyme without the aid of any other factors at T_Φ are also different from that by the host enzyme, $G(U)_6G\text{-}3'$ for T7 versus $G/C(U)_{6\text{-}7}\text{-}3'$ for the host. As mentioned below, the termination efficiency is modulated by the phage protein Gp3.5. T3, but not T7, RNAP needs the *E. coli* protein DnaB helicase for initiation, elongation, and termination of replication *in vivo*. DnaB is essential for both phage DNA replication and RNA synthesis in high salt *in vitro*. The significance of the sharing of a protein between transcription and replication is not known.

Regulation of Transcription

T3 and T7 regulate gene expression mainly at the level of transcription. We mentioned that the gene products made from early transcripts create a favorable environment for phage growth and produce T7 RNAP. At this point, transcription of class II genes begins. As soon as the synthesis of middle proteins reaches an optimal level, both early and middle transcription are turned off, and the transcription machinery is directed exclusively to expression of late genes. The temporal control and relative amounts of transcription of different genes are achieved in several ways. First, temporal control is facilitated by positioning of the three groups of genes, I, II, and III, in the order from left to right; and second, mechanisms exist to switch from early to middle and from middle to late transcription (below). In addition, some gene products are needed in large amounts for a sustained period. The corresponding genes are located in areas of overlapping transcription and possess additional, strategically located promoters in the phage chromosome. For example, as noted earlier, genes *1.1* through *1.3* are transcribed as part of both early and middle transcription units. Next, differential levels of transcription of some class I genes are achieved by 'polarity', that is, expression of promoter proximal genes in higher amounts than promoter distal ones. Polarity is caused by Rho-dependent transcription termination signals in the early transcription unit. Finally, the mode of entry of T7 DNA into the host is also an important element in temporal control of transcription.

There are three components of the early to middle switch, and all are due to the products of early transcription. First, gene *0.7* product inactivates host RNAP. Gp0.7 is a seryl–threonyl protein kinase that phosphorylates the β- and/or β′-subunits of host RNAP. Phosphorylation decreases early transcription to about 25%. The kinase inactivates itself in a timely fashion after it is no longer needed. Second, T7 RNAP, the gene *1.0* product, accumulates. This enzyme recognizes the middle and late promoters. The host RNAP activity is also inhibited by the third factor, the middle gene *2.0* product. Gp2.0 binds to the host RNAP in a 1:1 stoichiometry. Although Gp2.0 is essential for phage growth, Gp0.7 is not. Much needs to be learned about Gp2.0 action.

The switch from middle to late transcription requires the product of middle gene *3.5*. This protein binds to T7 RNAP, forming a complex that inhibits transcription strongly from Φ_M but weakly from the late promoter, Φ_L. Biochemical experiments have shown that Gp3.5 inhibits the transition from the initiating to the elongating complex. The Φ_M–T7 RNAP complexes are much weaker than the corresponding late promoter complexes. Gp3.5 destabilizes the former complexes more readily than the latter ones. Interestingly, Gp3.5 has three additional functions. It is a lysozyme, which is required for release of progeny phage after infection, it stimulates DNA replication, and, indirectly, it enhances termination at T_{CJ}. Ordinarily, T_{CJ} is a pause site for T7 RNAP, and Gp3.5 increases pausing at this site.

RNA Processing

The long polycistronic RNA molecules made at early, middle, and late times after phage infection are cut at specific sites by the host enzyme, RNase III, to yield discrete, relatively stable mRNAs. The processing sites in early T7 RNA have been studied in more detail. RNase III cleaves at specific sites within hairpin structures that are located between coding sequences. Although some of the RNA molecules that result from RNase III cleavage are translated more efficiently than the uncut RNA, RNase III cleavage is not essential for phage growth.

Phage N4

The virulent phage N4 uses transcription strategies that differ significantly from those of T7 or T4. Early genes are located at the left end of the N4 chromosome, in the first 10 kbp of the ~70 kbp, linear, double-stranded DNA. Transcription of the early genes requires a phage-coded, virion-encapsulated polymerase, vRNAP, which is injected into the host along with the phage DNA. This polymerase is distantly related to the T7 family of RNA polymerases (above). N4 early promoter sequences have a conserved motif and a sequence that can form a hairpin with a stem of 5–7 nt and a loop of 3 nt in single-stranded DNA. *In vivo* transcription by vRNAP requires the activity *E. coli* gyrase and *E. coli* SSB (*Eco*SSB), the host single-stranded DNA binding protein that is normally a member of the host DNA replication machinery. It is thought that gyrase-catalyzed, negative supercoiling of the DNA leads to local melting and extrusion of the hairpin, which then provides the features needed for polymerase promoter recognition. *Eco*SSB melts the complementary strand hairpin while the template strand hairpin, which is resistant to SSB melting, remains available for vRNAP recognition. In addition, SSB prevents annealing of the RNA product to the template DNA. Thus, *Eco*SSB acts as an architectural transcription factor as well as a recycling factor that makes the template DNA available for multiple rounds of early transcription.

Middle N4 genes encode the N4 replication proteins and are located within the left half of the N4 genome. Middle genes are transcribed by a second phage-encoded RNA polymerase, N4 RNAPII, composed of the N4 early products p4 and p7. This polymerase is also a member of the T7 polymerase family. N4 middle promoters contain an AT-rich sequence at the transcription start site. By itself, N4 RNAPII is inactive on double-stranded DNA and has very limited activity on single-stranded DNA. Active transcription requires another N4 protein, gp2, which interacts specifically with single-stranded DNA and with N4 RNAP II. It is thought that an as yet unidentified protein binds to the promoter element while gp2 stabilizes a single-stranded region at the promoter start and brings N4 RNAPII to the transcription start site. This system differs significantly from the other well-characterized phage transcription systems and may provide a good model system for investigating mitochondrial transcription, which is also carried out by a member of the T7 polymerase family.

Transcription of N4 late genes, which are located in the right half of the phage genome, requires *E. coli* RNA polymerase. Late promoters have regions of limited similarity to σ^{70} DNA elements and when present in linear DNA, they are weak promoters for σ^{70} polymerase. Transcription *in vivo* and with linear templates *in vitro* requires the N4 SSB protein. Surprisingly, the single-stranded DNA-binding function of SSB is not needed for this activation. Rather a transactivating domain of SSB that interacts with the β'-subunit of *E. coli* RNA polymerase core is required. The mechanism of this activation is not known, but is thought to occur at a step after initial binding of RNA polymerase to the promoter. Thus, this late N4 system should also provide insight into a mode of transcriptional activation that has not been previously characterized.

Phage λ and the Regulation of Lysogeny and Lysis

Lambda is the founding member of a large family of temperate bacteriophages that are related by common gene organization and limited sequence similarity. Although λ belongs to the *family Siphoviridae* by virtue of its long, noncontractile tail, several close relatives are not members of this group. Lambda chromosomes isolated from virions consist of about 49 kbp of linear, nonpermuted, mostly double-stranded DNA. Complementary single-stranded extensions of 12 bases at each end enable the chromosome to circularize by end-joining after it is transferred from the virion into an infected cell. Transcription of all phage genes, both early and late, is catalyzed by host RNAP. Infection with λ, unlike infection with virulent phages, does not always lead to cell lysis and liberation of progeny phage. Instead, infected cells frequently survive and give rise to lysogens – cells that contain a quiescent, heritable copy of the λ chromosome called prophage (**Figure 4**). The prophage expresses few genes and replicates in synchrony with the host, so that each daughter cell also contains a prophage. The 'decision' between lytic growth and lysogen formation after infection is controlled at many levels (below). Indeed, λ, like other temperate species, has evolved sophisticated mechanisms to ensure that a substantial fraction but not all infected cells survive and become lysogenic, and that intermediate responses, for example, survival without inheritance of a prophage (abortive lysogeny), are rare.

Once a lysogen has formed, loss of the prophage, or 'curing', is infrequent. Lambda and its relatives insert their chromosomes into specific sites in the bacterial chromosome during the establishment of lysogeny and remain there during subsequent cell division. Although site-specific prophage insertion is widespread, it is not a general feature of lysogeny; some temperate phages that are not closely related to λ use other strategies that also ensure prophage retention. For example, phage Mu, inserts its chromosome nonspecifically, while phage P1 does not insert at all, but instead exists in lysogens as a single-copy plasmid whose replication is coupled to that of the host chromosome. Lysogeny is not a dead end; the prophage can switch into an

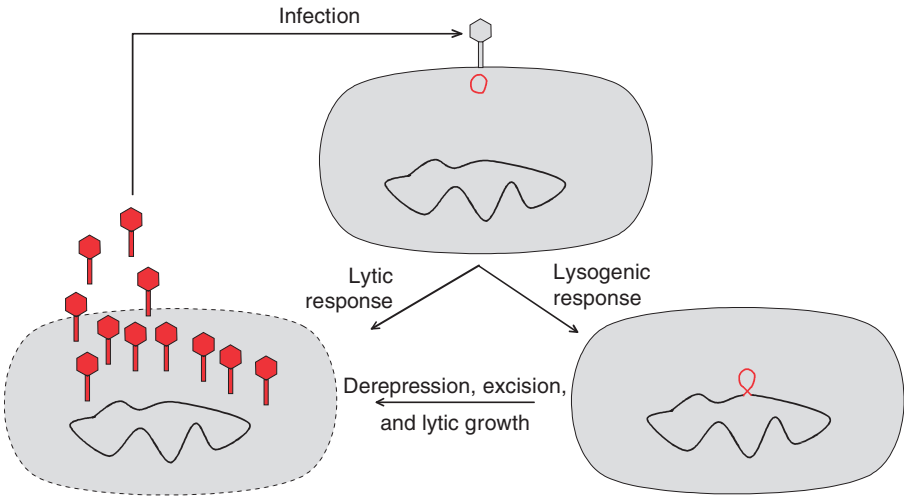


Figure 4 The λ life cycle. After infection, the linear phage chromosome enters the cell and the single-stranded ends cohere to form a ring (in red, top panel). When the infected cell enters the lytic cycle, early and late phage genes are fully expressed, phage DNA replication ensues, the resulting replicas are packaged into phage heads, and the cell lyses, releasing infectious particles into the environment (left bottom panel). When the infected cell enters the lysogenic cycle, the phage expresses mainly early genes to a limited extent. Among these genes are *cI* and *int*. CI prevents further phage gene expression, and Int catalyzes insertion of the phage into the bacterial chromosome (lower right panel). The resulting lysogen passes a copy of the inserted, repressed prophage to its descendents. When repression fails, the prophage excises and enters the lytic cycle.

active state in which many virus genes are expressed, the prophage is excised from the bacterial chromosome, and normal lytic growth ensues (**Figure 4**). This transition, known as the genetic (or, more correctly, epigenetic) switch, is normally infrequent but can be induced experimentally by treatment of cells with DNA damaging agents. Such treatment causes a signal cascade that leads to autoproteolytic cleavage of repressor, a phage protein that prevents most phage gene expression (below). Experimental induction of lysogens by DNA damage is not universal; lysogens of some temperate phages, such as P2, are not inducible.

The Establishment and Maintenance of Lysogeny

Immediately after infection, host RNAP initiates transcription at the λ early promoters, P_L and P_R (**Figure 5**). P_R directs the transcription of the *cII* gene, whose product is the central effector of the lysogenic response. CII protein promotes lysogeny by activating the coordinate initiation of transcription at three λ promoters: P_{RE}, P_{Int}, and P_{aQ} (**Figure 5**). P_{RE}, the promoter for repressor establishment, directs the transcription of the *cI* gene, and P_{RE} activation thus provides a burst of CI protein synthesis shortly after infection. CI, or repressor, prevents transcription of nearly all λ genes in an established lysogen, and, indeed, is required for the maintenance of lysogeny. CI blocks the initiation of early transcripts by binding to operator sites that overlap P_L and P_R (below). It blocks late gene transcription indirectly, by preventing the

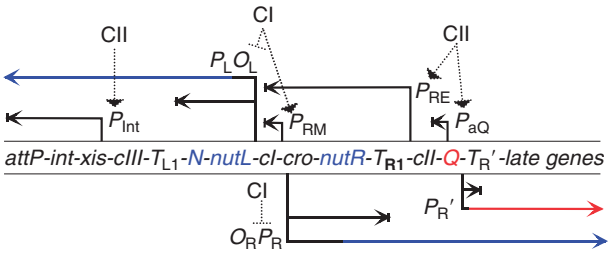


Figure 5 Phage λ promoters, transcripts, repressors, activators, and antiterminators. Only genes and sites mentioned in the text are shown. The top, light, horizontal line represents a DNA strand written with 5′ to 3′ chemical polarity, and the parallel bottom line represents the complementary strand written 3′ to 5′. Activation or repression of individual promoters by CI and CII proteins is indicated by dotted lines terminated by an arrowhead or bar, respectively. Transcripts and their orientation are indicated by heavy solid lines with arrowheads, with each transcript adjacent to the transcribed strand. A bar across an arrowhead indicates a terminated transcript. T_{L1} and T_{R1} are the first of several transcription terminators in the P_L and P_R operons, respectively, and T_R' is the first terminator in the P_R', or late operon. N suppresses terminators by binding to ECs after they transcribe the *nutL* and *nutR* sites (see text). This is indicated by a color change from black to blue in the transcript. Q suppresses termination at T_R' and downstream terminators by binding to the EC shortly after initiation at P_R' (see text). This is indicated by a color change from black to red in the transcript.

synthesis of Q, an early protein that activates transcription of late genes (**Figure 5**). CI is not only a repressor, it is also an activator. It activates the initiation of transcription at P_{RM}, the promoter for repressor maintenance,

a second promoter for *cI* transcription. Thus, once *cI* transcription has initiated from P_{RE}, it is self-sustaining. This is essential to maintain lysogeny because CI prevents continued transcription of *cII*.

P_{Int}, the second CII-activated promoter, directs the transcription of the *int* gene. Int protein or integrase is a topoisomerase that binds to specific sequences within the phage and host 'attachment sites'. These two sites differ from each other in the number, type, and disposition of Int binding sites. The phage attachment site also contains sequences that specifically bind several molecules of IHF, a host-encoded DNA bending protein that promotes the formation of a recombinogenic structure that can capture the bacterial attachment site. Int then uses its topoisomerase activity to catalyze breakage, exchange, and rejoining of the four DNA strands at specific positions within the two sites. This inserts the prophage into the bacterial chromosome and ensures its inheritance by the progeny of the infected cell. Reversal of insertion, or excision, is rare as long as CI continues to repress transcription (below).

Finally, P_{aQ}, the third CII-activated promoter, directs the synthesis of an antisense RNA that inhibits the production of Q, a phage protein that promotes transcription of late genes. Inhibition of Q synthesis increases the frequency of lysogeny after infection by delaying the production of late phage proteins that would kill the cell. Once lysogeny is established, repressor prevents further transcription of Q, as noted earlier.

The rate of CII accumulation after infection is controlled by the action of several phage and host gene products, a few of which are described in more detail below. Initially, λ Cro protein and, eventually, CI inhibit initiation of the *cII* transcript at P_R. Lambda N protein promotes transcript elongation through *cII* by suppressing T_{R1}, a rho-dependent transcription terminator (**Figure 5**). Host RNase III in concert with the antisense OOP RNA (not shown in **Figure 5**) cleaves the *cII* transcript. Finally, host FtsH protease degrades CII, but λ CIII protein inhibits its degradation. The net effect of these multiple layers of control is probably to make the lysis–lysogeny decision responsive to internal and environmental cues. A complete quantitative description of the kinetic details of the response is cherished goal of systems biologists.

Dimers of CII and dimers, tetramers, and octamers of CI bind to specific sites that overlap the promoters they regulate. Both use a helix–turn–helix DNA binding motif to recognize their sites. CII binds specifically to sequences that straddle the −35 element of the relevant promoters, and binding is thought to activate transcription initiation through contacts between the tetramer and σ^{70} RNAP. These contacts enhance binding of RNAP to the promoter and isomerization of the bound complex. CI dimers bind to repeated tandem operator sites that flank and interpenetrate each promoter: O_{L1}, O_{L2}, and O_{L3} for P_L, and O_{R1}, O_{R2}, and O_{R3} for P_R. One dimer binds to each operator, and neighboring dimers can interact cooperatively with each other as well as with dimers bound near the other promoter as shown in **Figure 6**. Cooperative interactions strengthen operator binding and make it more sensitive to changes in CI concentration, thus sharpening the transition between full repression and full derepression and decreasing the probability of biologically unproductive intermediate states. Repressor dimers bound to O_{L1} and O_{L2} occlude binding of RNAP to P_L, and, similarly, binding to O_{R1} and O_{R2} occludes P_R. Repressor bound at O_{R2} has an additional function; it contacts the σ-subunit of RNAP bound to P_{RM}, promoting isomerization of the bound enzyme and subsequent *cI* transcription. O_{R3} binds repressor relatively weakly and is not required for repression

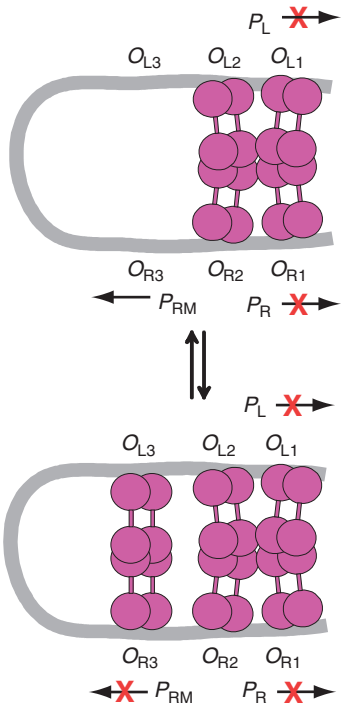


Figure 6 Binding of repressor. The pairs of purple dumbbells represent repressor dimers bound to subsites of O_L and O_R. One end of each dumbbell represents the N-terminal portion of the protein, which contains a DNA-binding domain. The other end represents the C-terminal portion of the protein, which contains regions that can participate in cooperative interactions with similar regions in neighboring bound dimers to form tetramers, and with a tetramer bound to a distant operator to form an octamer. At low concentrations of repressor, the cooperative interactions and the intrinsic affinities of the binding sites lead to occupancy of subsites 1 and 2 of each operator. In this configuration, promoters P_L and P_R are repressed and promoter P_{RM} is activated (top of figure). At higher concentrations of repressor, all six subsites are occupied and all three promoters are repressed (bottom of figure). Repression is indicated by a red 'X' over the arrows representing transcripts.

of P_R. This operator is occupied only at relatively high repressor concentration, and occupancy prevents a further increase in concentration by repressing P_{RM}. Thus, *cI* transcription from P_{RM} is both positively and negatively autoregulated.

Prophage Excision

If the level of repressor in a lysogen drops below the level needed to inhibit transcription, the prophage expresses genes needed for excision and lytic growth. Two λ proteins, Int and Xis, are needed for prophage excision. Int, as noted earlier, catalyzes breakage and rejoining of DNA strands. Xis is a directionality factor; it is required for excision and inhibits insertion. Analogous directionality factors are produced by many other temperate phages. Xis molecules bind specifically and cooperatively to sequences within the attachment sites and help to recruit Int to an adjacent binding sequence. This favors recombination between the two attachment sites that flank the prophage and inhibits recombination between the phage and host attachment sites. Interestingly, when Xis concentration is limiting, the host-encoded Fis protein can alleviate the deficiency. It is likely that Fis and IHF, the other host-encoded protein needed for recombination, help to couple the efficiencies of insertion and excision to the physiological state of the host.

Although Int and Xis are encoded by adjacent genes, the protein levels are differentially controlled so that the efficiency and direction of recombination are coupled to the appropriate stages of the phage life cycle. In infected cells destined for lysogeny, *int* but not *xis* is transcribed from CII-activated P_{Int} (**Figure 5**). Xis, which is made from the P_L transcript, is unstable, so it inhibits insertion only briefly when transcription from P_L is repressed after infection. After prophage induction, both *int* and *xis* are transcribed from P_L, and their products promote rapid prophage excision. Excision is usually followed by phage DNA replication and the expression of late genes. Although excision is not a prerequisite for the subsequent events, it is necessary for synthesis of concatemers of λ DNA, a substrate for packaging phage chromosomes into virions.

Lytic Growth

Lambda lytic growth is broadly similar to that of virulent phages, although the regulatory strategies differ considerably. The Cro, N, and Q proteins regulate transcription mainly during lytic growth. None of them is produced in lysogens because CI directly represses their syntheses. Cro is a transcriptional repressor, similar to CI in binding specificity and structure. However, subtle differences in binding specificity and the absence of cooperative interactions weaken Cro repression of P_L and P_R, and adapt it to serve as a negative regulator of early gene transcription during lytic growth. Cro is essential for lytic growth because it represses transcription of *cII*, thus enabling a fraction of infected cells to enter the lytic pathway. Both N and Q are 'antiterminators'; they act during transcript elongation to suppress termination. N enhances expression of early, and Q of late genes (**Figure 5**). Nearly all λ genes are located downstream of terminators and therefore require antitermination for full expression. *Cro* and *N* are exceptions, and, indeed, they are normally expressed before any other λ proteins are produced.

What is the role of terminators in λ biology? Their role in the expression of early genes is unclear, since termination is rapidly and efficiently suppressed as N accumulates after infection. Possibly the delay in early gene expression until the concentration of N reaches an effective level influences the lysis–lysogeny decision. Indeed, it has been shown that the efficiency of translation of the N message is increased by RNase III cleavage, and that the intracellular concentration of RNase III varies with the growth phase of the culture. Alternatively, or in addition, termination adds to the effect of repressor in silencing early gene expression in lysogens. In any event, early gene terminators are likely to confer some evolutionary advantage because several are conserved in phages related to λ. By contrast, the role of termination in controlling late gene expression is clear because P_R', the promoter for these genes, is constitutive. Thus, termination prevents transcription of late genes in lysogens and premature transcription of late genes during lytic growth.

Antitermination by N and Q is limited to λ transcription because both proteins bind specifically to phage nucleic acid before they act. N binds nascent transcripts of the *nutL* and *nutR* sites, which are located downstream of P_L and P_R, respectively (**Figure 5**). RNA binding alters N structure and facilitates its transfer to the nearby elongation complex, where it acts to suppress termination. Several host proteins enhance N-dependent antitermination: NusA, NusB, NusE/RpsL, and NusG. Some of these proteins bind *nut* RNA, some bind the elongation complex, and one, NusA, binds N. All of these proteins have roles in host biology that are independent of N, and their abundance and activity might link the efficiency of antitermination to as yet unknown aspects of host physiology. Nevertheless, N is clearly the central actor since some level of antitermination has been observed *in vitro* in the absence of all of the Nus factors and the *nut* site when N is present in excess. Despite considerable effort, the location of the N binding site on the EC and the nature of the antiterminating modification are still unknown. The modification is stable *in vitro* in the presence of NusA and persists *in vivo* for considerable time and distance after it has been established. Persistence is important for λ physiology since important terminators, notably those preceding the *Q* and *xis* genes, are located more than 5 kbp from the *nut* sites.

Q binds to a site in the nontranscribed region of the λ late promoter, P_R' (**Figure 5**). It is transferred from this site to the EC after synthesis of a 16 or 17 nt transcript. At these points the EC pauses because the σ^{70}-subunit, which has not yet been released, binds to a transcribed DNA sequence that resembles the extended −10 regions of certain genuine *E. coli* promoters. Sigma-70 interacts with Q after its transfer to the EC, but the locations of any additional contacts are unknown. Q modification of the EC decreases the duration of the 16/17 pause and suppresses downstream terminators.

Q and N suppress nearly all terminators that have been tested, both intrinsic and rho-dependent, and also increase the average rate of elongation. The mechanisms of suppression and, indeed, of termination are controversial. It is widely accepted that formation of the hairpin stem of intrinsic terminators disrupts the upstream (5′) segment of the RNA:DNA hybrid, which consists mainly of relatively weak rU:dA base pairs. It is not clear how hairpin formation is linked to hybrid disruption, and what the subsequent steps in termination consist of. Among the mechanisms that have been considered to explain N and Q action are inhibition of hairpin formation, strengthening of the weak RNA:DNA hybrid, and increase of elongation rate so that the EC escapes from the termination zone.

It is interesting that the termination/antitermination mode of gene control is not general among temperate phage. For example, it has not been found in phages Mu or P2, which regulate transcription exclusively through activators and repressors of initiation. However, several interesting variants of the termination/antitermination strategy have been discovered in other phages. In lysogens of the mycobacterial phages L5 and Bxb1, the repressor arrests the progress of the EC by binding to numerous 'stoperator' sites in the phage chromosome and physically blocking further translocation. The repressor of coliphage P4 also prevents transcript elongation, but by a quite different mechanism. P4 repressor is an RNA that activates termination by pairing with phage transcripts upstream of terminators. It has been suggested that the resulting RNA:RNA duplexes alter the secondary structure of the transcripts in such a way as to favor termination. Finally, HK022, an *E. coli* phage related to λ, uses antitermination to express its early genes but lacks a protein analogous to N. Instead, the HK022 P_L and P_R operons encode short, structured RNA segments that bind to the EC and suppress downstream terminator sites as efficiently as N does for λ. These RNAs act exclusively *in cis*, thus limiting antitermination to phage transcripts.

Conclusions

Both temperate and virulent phages alter the transcriptional and translational machinery of their hosts to suit their own ends. We have described phage-encoded mechanisms that change promoter utilization, transcript elongation rate, and transcript termination efficiency. These changes can be modest or drastic, ranging from chemical modification of a host RNAP subunit to synthesis of a new and structurally dissimilar polymerase. The selective advantage conferred by some of these changes is poorly understood and may be an adaptation to evolutionary pressures that are not apparent in laboratory conditions. An example that we have not previously described is the existence of phage-encoded transfer RNAs that duplicate or augment existing host activities. Among the differences between the regulatory strategies used by temperate and by virulent phages is the lethal character of some mechanisms used by the latter group. Most striking among these is degradation of host DNA, which can be seen soon after infection by T4 and T7. Another is inactivation of host RNAP. Temperate phages can also kill their hosts, but this typically occurs after the phage enters the lytic growth pathway. The essence of the temperate lifestyle is peaceful coexistence of the prophage and the host. A prophage that conferred a selective disadvantage on its lysogenic host would probably not last long in nature. If there is a consistent difference between the regulatory strategies of the two phage types, it is the ability of temperate species to prevent the expression of genes that harm the host. In contrast, the essence of being a lytic phage is to invade, take over, and destroy as efficiently as possible. Nevertheless, the extent of this difference should not be exaggerated. Some virulent phages have close relatives that are known only as prophage-like components of bacterial chromosomes, and some temperate phages have close relatives that have lost elements required for repressing gene expression and are, at first view, indistinguishable from virulent species. In any event, the tactics of both the temperate and virulent phages have taught researchers many elegant mechanisms for gene expression and control.

Acknowledgment

The authors are grateful to R. Bonocora for designing Figure 1 and to Rodney King and Bill Studier for comments. This work was supported by the Intramural Research Program of the NIH, National Institute of Child Health and Human Development, National Institute of Diabetes and Digestive and Kidney Diseases, and the National Cancer Institute.

See also: Genome Packaging in Bacterial Viruses; History of Virology: Bacteriophages; Icosahedral ssDNA Bacterial Viruses.

Further Reading

Calendar R (2006) *The Bacteriophages.* New York: Oxford University Press.
Greive SJ and von Hippel PH (2005) Thinking quantitatively about transcriptional regulation. *Nature Reviews Molecular Cell Biology* 6(3): 221–232.
Miller ES, Kutter E, Mosig G, Arisaka F, Kunisawa T, and Ruger W (2003) Bacteriophage T4 genome. *Microbiology and Molecular Biology Reviews* 67(1): 86–156.
Oppenheim AB, Kobiler O, Stavans J, Court DL, and Adhya S (2005) Switches in bacteriophage lambda development. *Annual Review of Genetics* 39: 409–429.
Ptashne M (2004) *A Genetic Switch. Phage Lambda Revisited.* Cold Spring Harbor, NY: Cold Spring Harbor Press.

Transmissible Spongiform Encephalopathies

E D Belay and L B Schonberger, Centers for Disease Control and Prevention, Atlanta, GA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Alleles Mutually exclusive forms of the same gene on homologous chromosomes.
Codon A sequence of three nucleotide bases in a gene that specifies an amino acid to be incorporated into a protein during its synthesis.
Encephalopathy Brain disease resulting from infectious agents such as prions.
Genetic polymorphism The regular occurrence in a population of two or more components of a gene with greater frequency than can be explained by recurrent mutation.
Mutation A permanent inheritable change in the genetic material of a host.
Prion Small, proteinaceous infectious particles that resist inactivation by procedures that affect nucleic acids; they are believed to be the causative agents of scrapie and other spongiform encephalopathies of animals and humans.
Prion protein A normal cellular version of the infectious prions encoded by the prion protein gene; its cellular function is not well understood.

Introduction

Prion diseases, also known as transmissible spongiform encephalopathies, constitute a group of fatal, human, and animal subacute neurodenerative diseases caused by an unconventional agent. Strong evidence indicates that their etiology and pathogenesis involve modification of a host-encoded normal cellular protein known as the prion protein (PrP^{C}). A unique hallmark of this group of diseases includes their sporadic occurrence without any apparent environmental source of infection or genetic association with prion protein gene mutations or as a result of disease transmission from infected humans and animals. Neuropathologically, most prion diseases manifest with widespread neuronal loss, spongiform lesions, and astrogliosis. Characteristically, no signs of inflammation are detected in pathologic samples. However, the presence of an abnormal, pathogenic prion protein, often called a scrapie prion protein (PrP^{Sc}) after the first recognized prion disease, scrapie, is demonstrable in the brain and often in other tissues of humans and animals affected by prion diseases. The incubation period of acquired forms of prion diseases is usually measured in years and sometimes in decades.

Etiologic Agent of Prion Diseases

Studies that characterized the causative agents of prion diseases initially focused on understanding the agents responsible for scrapie in sheep. This disease is the most common prion disease of animals and was first reported in the 1730s in England. Before the 1980s, prion diseases were widely believed to be caused by 'slow viruses' despite the fact that no viral particles or disease-specific nucleic acids were identified in association with scrapie transmission in laboratory animals. The scrapie agent could not be grown in cell culture, a feature that hampered research to characterize the agent. The successful transmission of scrapie to laboratory mice in 1961 contributed to overcoming this hurdle and greatly facilitated efforts to understand the nature of the agent. Quantitation of infectivitiy in a sample, however, still was tedious and required a year to complete.

Two distinctive properties of the scrapie agent led to the suspicion that the agent was devoid of nucleic acids and, thus, may not be a virus but primarily composed of a protein. These properties included (1) resistance of the scrapie agent to procedures, such as treatment with ultraviolet light and ionizing radiation, that normally

inactivate other microorganisms, including viruses and (2) the reduction of the infectivity of the scrapie agent by procedures that denature or degrade proteins. The concept that the scrapie agent might replicate in the absence of nucleic acids or might just be a protein was postulated as early as the 1960s by Alper and colleagues, Pattison and Jones, and Griffith. In 1982, Prusiner and colleagues described the successful purification of a hydrophobic protein, the presence of which was required for scrapie transmission in laboratory animals. Prusiner introduced the term 'prion' to describe this protein by borrowing and mixing the first few letters from the descriptive phrase 'proteinaceous infectious' particle. Since then, additional evidence has accumulated indicating that prions may be acting alone in causing prion diseases. However, the critical steps in the production, propagation, and pathogenesis of this infectious protein remain unclear. As a result, the study of prions has become an important, relatively new area of biomedical investigation. Some critics of the prion hypothesis still believe that nucleic acids undetected by current methods may play a crucial role in the pathogenesis of prion diseases.

Prions appear to be composed largely or entirely of the abnormal protein designated as PrP^{Sc}. This protein is an abnormal conformer of a host-encoded cellular protein, PrP^{C}, which in humans is encoded by the prion protein gene located on the short arm of chromosome 20. PrP^{C} is a structural component of cell membranes of neurons and other tissues. Its normal function is poorly understood. However, it appears to be involved in supporting neuronal synaptic activity, copper binding, and neuroprotective functions by interacting with other cell-surface proteins.

The underlying pathophysiologic mechanism in the occurrence of prion diseases involves the biochemical conversion of PrP^{C} into the pathogenic PrP^{Sc}. This conversion occurs by a poorly defined post-translational autocatalytic process, possibly requiring the aid of cofactors such as proteins or nucleic acids. The initial instigating PrP^{Sc} molecule may originate from exogenous sources or within the brain from somatic or germline prion protein gene mutations. Knockout mice devoid of the prion protein gene are resistant to scrapie infection, indicating that the production of PrP^{C} is required for the generation and propagation of PrP^{Sc}. During its conversion, PrP^{Sc} acquires more β-sheet structure that renders it resistant to proteolytic enzymes, conventional disinfectants, and standard sterilization methods. A higher proportion of the tertiary structure of PrP^{C}, on the other hand, is composed of α-helices which make it more sensitive to denaturation by proteinase-K treatment. Removal of the neuroprotective functions of PrP^{C} as more of it becomes converted to the pathogenic PrP^{Sc} and accumulation of PrP^{Sc} in neurons have been suggested as major contributory factors in the underlying pathogenesis of prion diseases and widespread neuronal death.

Prion Diseases of Animals

Prion diseases of animals include scrapie in sheep, goats, and moufflon; bovine spongiform encephalopathy (BSE) in cattle; feline spongiform encephalopathy in domestic and zoo cats; ungulate spongiform encephalopathy in exotic zoo ruminants; chronic wasting disease (CWD) in deer, elk, and moose; and transmissible mink encephalopathy (TME) in mink (**Table 1**). Epidemiologic evidence indicates that feline and ungulate spongiform encephalopathies were caused by the BSE agent possibly transmitted via consumption of BSE-contaminated feed. Although strong evidence is lacking, speculations have persisted that scrapie in sheep may have been the original source of prion diseases in other animals, such as BSE and CWD.

TME occurred in outbreaks among ranched mink primarily in the US but also in Canada, Finland, Germany, and Russia. The last known outbreak of TME occurred in 1985 in Wisconsin. Nonepizootic cases of TME have not been reported.

Scrapie

Although scrapie was recognized as a distinct clinical entity of sheep over 250 years ago, many aspects of the disease including its natural origin in flocks and the precise means by which it usually spreads remain uncertain. Experimentally, the disease was first transmitted by intraocular inoculation of scrapie brain extracts. In the 1940s, more than 1500 sheep developed scrapie from receipt of a vaccine against louping ill virus that contained scrapie-contaminated lymphoid tissue.

Scrapie transmission may occur by different postulated mechanisms. A commonly cited source of transmission, for example, is the placenta and amniotic fluid of scrapie-infected ewes. These tissues are known to harbor the infectious agent and can cause scrapie when fed to sheep. They may contaminate pastures and barns that, in turn, may remain potentially infectious for years. Another possible source of spread is feces because prion replication occurs in gut lymphoid tissues after oral inoculation in sheep and goats. The importance of oral transmission is supported by experimental studies that detected prions in sheep tonsils examined early during the incubation period. Other poorly defined scrapie transmission mechanisms include (1) the vertical transfer of the scrapie agent and (2) the possible chance occurrence of scrapie caused by hypothesized rare, spontaneous changes in the animal's cellular prion protein.

Scrapie occurs endemically in many countries, including Europe and North America. Australia and New Zealand have sizable sheep populations but are generally recognized as free of the disease. To protect their 'scrapie free' status, these countries have established extensive

Table 1 Animal and human prion diseases

Type of prion disease	*Affected host*	*Year first described or identified*
Animal prion diseases		
Scrapie	Sheep and goats	1730s
Bovine spongiform encephalopathy	Cattle	1986
Chronic wasting disease	Deer, elk, and moose	1967
Transmissible mink encephalopathy[a]	Mink	1947
Feline spongiform encephalopathy[b]	Domestic and wild cats	1990
Ungulate spongiform encepahlopathy[b]	Exotic ruminants (e.g., kudu, nyala)	1986
Human prion diseases		
Kuru		1950s
Sporadic CJD		1920s
Iatrogenic CJD		1974[c]
Variant CJD		1996
Familial CJD		1924
Gerstmann–Sträussler–Scheinker syndrome		1936
Fatal familial insomnia		1986

[a]The last known outbreak of transmissible mink encephalopathy occurred in 1981 in Wisconsin.
[b]The known feline and ungulate spongiform encephalopathies are believed to have resulted from BSE transmission.
[c]The first report of iatrogenic CJD was in a recipient of cornea obtained from a CJD decedent; human pituitary growth hormone-associated CJD was first reported in 1985 and dura mater graft-associated CJD in 1987.
CJD, Creutzfeldt–Jakob disease.

safeguards to prevent the introduction of scrapie into their herds from imported animals.

The breed of sheep and polymorphisms of the prion protein gene can greatly influence susceptibility to scrapie. Experimental transmissions with scrapie-infected tissues, for example, have confirmed the differing susceptibility to scrapie of different breeds of sheep. Other studies of Suffolk sheep in the US indicated that susceptibility to scrapie was highly correlated with a polymorphism in the prion protein gene at codon 171 (glycine or arginine); the presence of arginine confers resistance to the disease.

Chronic Wasting Disease

Mule deer, white-tailed deer, and Rocky Mountain elk are the major known natural hosts for CWD. In 2005, a hunter-killed moose was confirmed with CWD in Colorado, suggesting that this member of the deer family too is a natural host. The disease was first identified as a fatal wasting syndrome of captive mule deer in the late 1960s in research facilities in Colorado. Subsequently, it was identified in mule deer in a research facility in Wyoming and then in captive elk in facilities in both Colorado and Wyoming. In 1977, CWD was first recognized as a spongiform encephalopathy.

In the early 1980s, the disease was recognized in free-ranging elk. By the mid-1990s, CWD was regarded as endemic in deer and elk in a contiguous area in northeastern Colorado and southeastern Wyoming. The known geographic extent of CWD has increased dramatically in the US since 1996. As of mid-2006, CWD in free-ranging cervids had been reported in 74 counties of 11 states, including Colorado, Kansas, Illinois, Nebraska, New Mexico, New York, South Dakota, Utah, West Virginia, Wisconsin, and Wyoming. In Canada, since 2001, CWD has been identified in wild deer and elk in Saskatchewan and since 2005 in several mule deer in Alberta.

Among cervids, CWD is most likely transmitted by direct animal contact or indirectly by exposure to a contaminated environment. Unlike other prion diseases, CWD is highly communicable in its natural host, particularly among captive cervids. In addition to becoming emaciated, animals with CWD characteristically develop polydipsia, polyuria, increased salivation, and difficulty in swallowing. These characteristics probably contribute to residual contamination of the environment that appears to be a very important source of transmission.

CWD does not appear to occur naturally outside the cervid family. It has been transmitted experimentally by intracerebral injection to laboratory mice, ferrets, mink, squirrel monkeys, goats, and cattle. However, experimental studies have not demonstrated transmission of CWD to cattle after oral challenge or after having cattle reside with infected deer herds.

No human cases of prion disease with strong evidence of a link with CWD have been identified despite several epidemiologic investigations of suspected cases. In addition, transgenic mice experiments indicate the existence of a significant species barrier against CWD transmission to humans. Nevertheless, concerns remain that this species barrier may not provide complete protection to humans against CWD and that the level and frequency of human exposures to CWD may increase with the spread of CWD in North America.

As a precaution, efforts to reduce human exposure to the CWD agent are generally recommended. Meat from depopulated CWD-infected captive cervids has not been allowed to enter the human food or animal feed supply. To minimize their risk of CWD exposure, hunters are encouraged to consult with their state wildlife agencies to identify areas where CWD occurs and to continue to follow advice provided by public health and wildlife agencies.

Bovine Spongiform Encephalopathy

Prion diseases became a focus of worldwide attention after a large outbreak of BSE in cattle emerged in the UK and spread to other countries. This attention increased dramatically when evidence accumulated indicating that the BSE agent was responsible for the occurrence of variant Creutzfeldt–Jakob disease (CJD) in humans. By far the largest number of BSE cases was reported from the UK, followed by other European countries.

Although BSE was first recognized in the UK in 1986; earlier cases probably occurred since the early 1980s. The number of UK BSE cases increased rapidly in the second half of the 1980s and early 1990s, peaked in 1992 with 37 280 confirmed cases, and has markedly declined since then (**Figure 1**). In 2005, the number of confirmed UK BSE cases had dropped to 225.

Clinically, the signs of BSE include neurologic dysfunction, including unsteady gait with falling and abnormal responses to touch and sound. In some animals, the onset of BSE can be insidious and subtle and may be difficult to recognize. During the early phase of the UK BSE outbreak, the public media introduced the popular term 'mad cow' disease to describe the strange disease causing fearful and aggressive behavior in some of the cattle infected with BSE.

Although the original source of the BSE outbreak is unknown, the two most accepted hypotheses are cross-species transmission of scrapie from sheep and the spontaneous occurrence of BSE in cattle. Data on the latter hypothesis may become available with increased detection and monitoring rates of atypical, as well as typical cases of BSE. According to the first hypothesis, transmission of scrapie to cattle occurred because of the practice of feeding cattle protein derived from rendered animal carcasses including those of scrapie-infected sheep. In the past, cattle feed rendering in the UK involved several treatment steps, including exposure of the feed to prolonged heating in the presence of a hydrocarbon solvent. Some researchers have suggested that omission of these steps in the late 1970s and early 1980s in the UK contributed to the emergence of BSE by allowing scrapie infectivity to survive the rendering process. Regardless of the origin of BSE, the epidemiologic evidence indicates that feeding cattle rendered BSE-infected carcasses greatly amplified the BSE outbreak. Several other factors may have contributed to the emergence of BSE in the UK, including a relatively high rate of endemic scrapie, a high population ratio of sheep to cattle, and the inclusion of rendered meat and bone meal at high rates in cattle feed.

Since the BSE outbreak was first detected, an estimated >2 million cattle have been infected with BSE in the UK. Approximately half of these BSE-infected cattle would have been slaughtered for human consumption, potentially exposing millions of UK residents. Beginning in 1988, UK animal and public health authorities implemented several protective measures to prevent further exposure of animals and humans to BSE-infected cattle products. The implementation of these measures, particularly animal feed bans, led to the dramatic decline in the UK BSE outbreak.

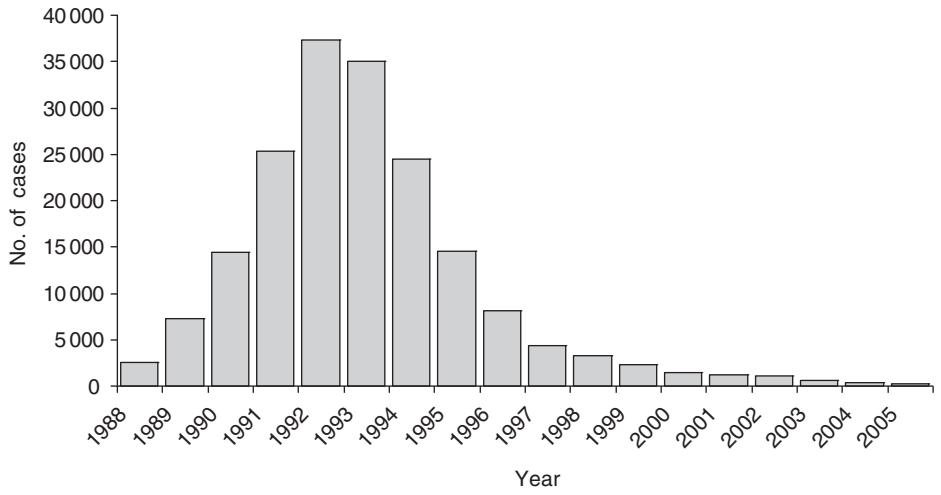


Figure 1 Bovine spongiform encephalopathy cases reported in the UK. BSE cases shown are by year of restriction: 442 BSE cases identified before 1988 are not included.

BSE was reported for the first time outside the UK in Ireland in 1989 and in Portugal and Switzerland in 1990. By August 2006, the number of countries that reported one or more BSE cases in native cattle increased to 25, including 21 countries in Europe. The four countries outside Europe that reported BSE cases are Canada, Israel, Japan, and the US. The BSE outbreak appears to be declining in most European countries, although some cases continue to occur.

In North America, BSE was first detected in 1993 in a cow that had been imported into Canada from the UK. Rendered cohorts of this cow may have been responsible for the 11 BSE cases subsequently identified during 2003–06 among cattle born in Canada; one of these cases was identified in Washington State but was later traced to a farm in Canada. At least six of these 11 BSE cases were born after the 1997 ruminant feed ban which was instituted to prevent BSE transmission among cattle. Because of the continued occurrence of new BSE infections after the 1997 ruminant feed ban, a specified risk material ban was recently instituted in Canada to further reduce cattle exposure to BSE by excluding potentially infectious nervous tissues from all animal feed. In 2005 and 2006, respectively, BSE was confirmed in an approximately 12-year-old cow born and raised in Texas and 10-year-old cow from Alabama. The source of BSE infection for these two cows remains unknown.

Because cattle carcasses were included in the production of animal feed, potential transmission of BSE to other animals was considered during the early phase of the BSE outbreak in the UK. BSE-like diseases were identified in zoo animals (ungulate spongiform encephalopathy) beginning in the late 1980s and in domestic cats (feline spongiform encephalopathy) beginning in 1990, indicating the potential for the BSE agent to cross the species barrier and spread to other animals. This development led to the establishment of enhanced CJD surveillance in the UK to monitor the possible transmission of BSE to humans.

Prion Diseases of Humans

Prion diseases of humans include kuru, CJD, variant CJD, Gerstmann–Sträussler–Scheinker syndrome (GSS), and fatal familial insomnia (FFI) (**Table 1**). Kuru is a fatal ataxic disease that was first described in the 1950s among the Fore tribe of the highlands of Papua New Guinea. In 1959, W. J. Hadlow described the similarity of the neuropathology of this disease with that of scrapie. In 1963, Gajdusek and colleagues successfully transmitted kuru by inoculating kuru brain tissue intracerebrally into chimpanzees, making this disease the first human prion disease to be successfully transmitted to experimental animals.

Kuru is the first epidemic human prion disease to be investigated. Since its investigation began in 1956, over 2700 cases have been documented. Strong epidemiologic evidence suggests that kuru spread among the Fore people by ritualistic endocannibalism. After this practice ended in the late 1950s, no children born after 1959 developed the disease and the number of new cases dramatically declined. Likely incubation periods of seven male cases recently reported by Collinge and colleagues ranged from 39 to 56 years and the incubation periods may have been up to 7 years longer. These male cases and four female cases had kuru from July 1996 to June 2004. The majority of these 11 kuru patients were reported to be heterozygous at the polymorphic codon 129 of the prion protein gene, a genotype associated with extended incubation periods and resistance to prion disease.

Creutzfeldt–Jakob Disease

CJD is the most common form of prion disease in humans. It was first recognized in the 1920s and bears the name of two German neurologists, Creutzfeldt and Jakob, who separately reported patients with rapidly progressive neurodegenerative diseases. Two of the patients initially reported by Jakob had the typical neuropathological features that we now recognize as CJD.

CJD is usually characterized by the onset of dementia, ataxia, or behavioral abnormalities. Later in the course of the illness, CJD patients commonly develop dysarthria, movement disorders such as myoclonus and tremors, and akinetic mutism. The presence of a characteristic electroencephalogram (EEG) finding of triphasic, periodic sharp wave complexes can be demonstrated with multiple testing in about 75% of patients. Elevated levels of 14-3-3 proteins in the cerebrospinal fluid (CSF) can also be demonstrated in most CJD patients. Elevated CSF 14-3-3 is a marker for rapid neuronal death and in the appropriate clinical context can often help in making a premortem diagnosis of CJD. This test is nonspecific, however, and may be elevated in other neurologic conditions that result in rapid neuronal death. More recently, magnetic resonant imaging (MRI) findings showing high intensity in the basal ganglia and cortical regions of the brain have been correlated with a CJD diagnosis. The median age of CJD patients at the time of death is 68 years with approximately 70% of cases occurring between 55 and 75 years of age. Typically, the disease progresses rapidly over a period of several weeks. Over 50% of the patients die within 6 months and 80% within 1 year of disease onset. A definitive diagnosis of CJD can only be made by histopathologic or immunodiagnostic testing of brain tissues obtained at autopsy or biopsy. Histopathologic examination of brain tissue demonstrates the hallmark triad of spongiform lesions, neuronal loss, and astrogliosis. Immunodiagnostic assays, such as immunohistochemistry and

Western blot testing, that show the presence of PrP^{Sc} confirm the CJD diagnosis.

Historically, three different forms of CJD have been reported: sporadic, iatrogenic, and familial CJD. Sporadic CJD occurs in the absence of outbreaks with no known environmental source of infection. Decades of research has not identified a specific source of infection for sporadic CJD that accounts for about 85% of patients. Spontaneous generation of the pathogenic prions was hypothesized as a cause for sporadic CJD, possibly resulting from random somatic mutations or errors during prion protein gene expression. Iatrogenic CJD, on the other hand, is associated with transmission of the CJD agent via medical interventions such as administration of contaminated human pituitary hormones and the use of contaminated dura mater grafts and neurosurgical equipment. Familial CJD has been associated with the presence of inheritable prion protein gene mutations. Beginning in the mid-1990s, the emergence of a variant form of CJD, linked with BSE transmission to humans, was reported. This newly emerged disease differs from other forms of CJD by the young age of affected patients and its clinical and pathologic features (**Table 2**).

Sporadic CJD is a heterogeneous disorder that can be further subdivided into five different subtypes based on the Western blot characteristics of protease-resistant fragment of PrP^{Sc} and the polymorphism at codon 129 of the host prion protein gene. These different subtypes correlate with characteristic clinical and neuropathologic phenotypes. The most common subtype is associated with a 21 kDa PrP^{Sc} fragment, designated type 1, and the presence of methionine at the polymorphic codon 129 of the prion protein gene.

Variant Creutzfeldt–Jakob Disease

In 1996, the identification of a cluster of young patients with a prion disease was reported in the UK as part of the CJD surveillance system that was established in response to concerns about the potential spread of BSE to humans. Because the patients' age and their clinical progression and neuropathologic profile were different from other endemic CJD patients, the term 'new variant CJD' was initially used to describe this emerging prion disease in humans. This term was later shortened to variant CJD. Since 1996, the number of variant CJD cases increased and strong scientific evidence indicated that variant CJD resulted from BSE transmission to humans. As of June 2007, a total of 202 variant CJD patients was reported worldwide, including 165 patients from the UK, 21 from France, four from Ireland, three from the US, two each from Netherlands and Portugal, and one each from Canada, Italy, Japan, Saudi Arabia, and Spain. At least seven of the non-UK variant CJD patients (two each from the US and Ireland, and one each from Canada, France, and Japan) were believed to have acquired variant CJD during their past residence or visit in the UK.

Variant CJD can be distinguished from the more common classic CJD by the clinical and laboratory findings (**Table 2**). The median age at death of variant CJD patients is 40 years younger than sporadic CJD patients (28 and 68 years, respectively), but their median illness duration is longer (14 and <6 months, respectively). Unlike classic CJD patients, variant CJD patients predominantly have psychiatric manifestations at onset with delayed appearance of frank neurologic signs and the typical 'pulvinar sign' on the MRI. The diagnostic EEG

Table 2 Clinical and pathologic characteristics distinguishing variant Creutzfeldt-Jakob disease (variant CJD) from classic CJD

Characteristic	*Variant CJD*	*Classic CJD*
Median age (range) at death (years)	28 (14–74)	68 (23–97)[a]
Median duration of illness (months)	13–14	4–5
Clinical presentation	Prominent psychiatric/behavioral symptoms, painful sensory symptoms, delayed neurologic signs	Dementia, early neurologic signs
Periodic sharp waves on electroencephalogram	Almost always absent	Often present
'Pulvinar sign' on magnetic resonance imaging[b]	Present in >75% of cases	Very rare or absent
Presence of 'florid plaques' on neuropathologic Sample	Present in great numbers	Rare or absent
Immunohistochemical analysis of brain tissue	Marked accumulation of PrP-res[c]	Variable accumulation
Presence of agent in lymphoid tissue	Readily detected	Not readily detected
Increased glycoform ratio on Western-blot analysis of PrP-res	Present	Not present
Genotype at codon 129 of prion protein	Methionine/valine[d]	Polymorphic

[a]US CJD surveillance data 1979–2001.
[b]Symmetrical high signal in the posterior thalamus relative to that of other deep and cortical gray matter.
[c]Protease-resistant prion protein.
[d]A patient with preclinical vCJD related to blood-borne transmission was heterozygous for methionine and valine.

finding that is common in classic CJD patients is very rare in patients with variant CJD. For unknown reasons, all variant CJD patients tested to date had methionine homozygosity in the prion protein gene at codon 129, which is polymorphic for methionine or valine. This homozygosity is present in approximately 35–40% of the general UK population. Similar to classic CJD, a definitive diagnosis of variant CJD requires laboratory testing of brain tissues. In addition to the spongiform lesion, neuronal loss, and astrogliosis typical of most prion diseases, the neuropathology in variant CJD patients is characterized by the presence of numerous 'florid plaques', consisting of amyloid deposits surrounded by a halo of spongiform lesions.

Studies in the UK have indicated the probable secondary person-to-person transmission of the variant CJD agent in three patients by blood collected up to 3.5 years before variant CJD onset in the donors. Because a large proportion of the UK population has potentially been exposed to the BSE agent, concerns still exist about additional secondary spread of the agent via blood products and possibly via contaminated surgical instruments.

Prion Diseases of Humans Associated with Genetic Mutations

One of the intriguing properties of prion diseases in humans is the fact that they can be both infectious and inheritable. The inherited or genetic forms of prion diseases are associated with insertion, deletion, or point mutations of the open reading frame of the prion protein gene. At least 24 different point mutations of the prion protein gene have been described in association with human prion diseases. These genetic prion diseases have widely varying clinical and neuropathologic manifestations. Historically, genetic forms of prion diseases, in part based on their phenotypical expression, were classified as familial CJD, GSS, and FFI. Beginning in 1989, many types of insertion mutations associated with markedly heterogeneous phenotypes have been reported in familial clusters.

In addition to influencing susceptibility to variant CJD, the polymorphism at codon 129 markedly influences the clinicopathologic phenotype of several inherited prion diseases. The most striking example of this influence is the phenotype associated with codon 178 mutation that substitutes aspartic acid with asparagine. Patients who have this mutation in combination with methionine on the mutant allele at codon 129 present with the FFI phenotype, whereas patients who have valine at codon 129 of the mutant allele present with the familial CJD phenotype. The codon 129 polymorphism may also influence the age at onset and duration of illness in some prion diseases.

Familial CJD

Patients with familial CJD generally have clinicopathologic phenotype similar to nongenetic forms of CJD. The disease has a dominant inheritance pattern and over half of affected family members carrying the mutation eventually die of CJD. Familial CJD has been reported among many family clusters from Canada, Europe, Japan, Israel, the US, and several Latin American countries. It is most frequently associated with a mutation substituting glutamic acid with lysine at codon 200 of the prion protein gene. Arguably, familial CJD associated with codon 200 mutation is the most common inheritable form of prion disease in humans. The largest familial cluster was reported among Jews of Libyan and Tunisian origin. About 14 other less-frequent mutations associated with familial CJD have been reported from many countries.

Gerstmann–Sträussler–Scheinker syndrome

The term GSS is used to describe a heterogeneous group of inherited human prion diseases that are characterized by a long duration of illness (median: ~5 years) and the presence of numerous PrP-amyloid plaques, primarily in the cerebellum. GSS carries the name of three physicians who in 1936 reported the disorder among patients spanning many generations of an Austrian family. The disease in this family was later shown to be associated with a mutation at codon 102 of the prion protein gene and may have been first identified as early as 1912.

At least 13 different types of prion protein gene mutations or a combination of mutations in at least 56 kindred or families have been reported in association with the GSS phenotype. Familial clusters with the GSS phenotype have been reported from Canada, Europe, Japan, Israel, Mexico, and the US. Many of the GSS mutations are associated with a greater degree of variability in the disease phenotype than other inherited forms of prion diseases. The most frequent GSS mutation results in leucine for proline substitution at codon 102 and is coupled with methionine at the polymorphic codon 129 of the mutant allele. Patients with this mutation commonly manifest with ataxia, dysarthria, movement disorders, and possibly dementia and akinetic mutism. The illness can last for up to 6 years in some patients with the GSS 102 mutation. In other forms of GSS, an illness duration exceeding 20 years has been reported.

Fatal familial insomnia

FFI is a human prion disease with predominant involvement of the thalamus, resulting in a clinical phenotype characterized often by intractable insomnia and autonomic nervous system dysfunction, including abnormalities in temperature regulation, increased heart rate, and hypertension. The neuropathologic lesions are more severe in the thalamus than other regions of the brain. FFI is primarily associated with a mutation at codon 178

of the prion protein gene resulting in a substitution of aspartic acid with asparagine in combination with methionine at the polymorphic codon 129 of the mutant allele. Occasionally, sporadic FFI cases with no apparent mutation in the prion protein gene have been reported. FFI has been identified in Australia, Canada, Japan, the US, and several European countries.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the funding agency.

See also: Prions of Yeast and Fungi.

Further Reading

Belay ED, Maddox RA, Williams ES, Miller MW, Gambetti P, and Schonberger L (2004) Chronic wasting disease and potential transmission to humans. *Emerging Infectious Diseases* 10: 977–984.

Belay ED and Schonberger LB (2005) The public health impact of prion diseases. *Annual Review of Public Health* 26: 191–212.

Prusiner SB (1982) Novel proteinaceous infectious particles cause scrapie. *Science* 216: 136–144.

Prusiner SB (2003) *Prion Biology and Diseases,* 2nd edn., New York: Cold Spring Harbor Laboratory Press.

Tumor Viruses: Human

R Grassmann and B Fleckenstein, University of Erlangen – Nürnberg, Erlangen, Germany
H Pfister, University of Köln, Cologne, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

RDA (representational difference analysis) PCR-based technique for the identification and cloning of DNA sequences (e.g., virus DNA) present in a particular cell but not in a matched reference cell.

Introduction

Viruses are responsible for about 15–20% of all cancers worldwide. Human tumor viruses constitute a heterogeneous group of viruses, which are causally linked to the development of malignant diseases in humans. Conventionally, the term is confined to those viruses that are likely to cause cancer by malignant conversion of infected cells. Accordingly, the human tumor viruses include the Epstein–Barr virus (EBV), the Kaposi's sarcoma-associated herpesvirus (KSHV), the high-risk human papillomaviruses (HPV), the hepatitis B virus (HBV), the hepatitis C virus (HCV), and the human T-cell leukemia virus (HTLV-1). Other viruses, such as HIV, support tumor development and growth indirectly, for example, by inhibiting the immune response to the tumor but do not infect the progenitors of malignant cells. Besides their systematic differences, most tumor viruses share several of the following features:

- establishment of chronic or long-term persistent infection;
- presence of viral gene functions, which interfere with cellular growth control, apoptosis control, DNA repair, or genomic stability; and
- capacity to transform cells in culture and/or to be oncogenic in experimental animal systems.

These features are not unique to tumor viruses but also shared by other human viruses, which are not yet clearly demonstrated but were suspected to cause human malignancies. These include some subtypes of human adenoviruses, the human polyomaviruses (BK, JC), many subtypes of papillomaviruses, and HTLV-2.

Virally induced tumors contain mostly viral genomes or parts of viral genomes, frequently integrated into the cellular genomes. As a hallmark of their infection prior to malignant conversion, many virally induced tumors are clonal in respect to integrated viral sequences. All human malignancies caused by tumor viruses are rare consequences of the infection and develop after long-term viral persistence. This indicates the necessity of rare secondary events, which are crucial to viral oncogenesis. Among them could be accumulation of genetic damage including cellular mutations induced by viral or nonviral factors, including physical and chemical carcinogens.

EBV-Associated Lymphoproliferative Malignancies

EBV, a member of the *Herpesviridae*, was the first virus to be linked to the oncogenesis of a human malignant disease. In 1964 it was identified by Epstein, Achong, and Barr in a B-cell line derived from an African Burkitt's lymphoma. Besides Burkitt's lymphoma, there are two other histologically and clinically distinct types of EBV-associated B-cell lymphoma, Hodgkin's disease and the lymphomas of immunosuppressed individuals. These three EBV-associated lymphoid malignancies differ in the patterns of the viral latent-gene expression and seem to be derived from cells at different positions in the B-cell differentiation pathway. Besides B-lymphoid malignancies, the virus was also found associated with some rare types of T-cell and natural killer (NK) cell lymphomas.

Lymphocyte Transformation *In Vitro*

EBV is capable of immortalizing human primary B lymphocytes, which resemble phenotypically activated B lymphocytes and are capable of proliferating permanently in culture. Cell lines usually contain EBV genomes as nonintegrated covalently closed circular double-stranded DNA in various copy numbers. Due to a block in structural gene expression, the transformed cells usually do not synthesize progeny viruses; instead, they express a series of latency-associated genes, which are also active in various combinations in human malignancies associated with EBV. These genes include five EBV-associated nuclear antigens (EBNA1, 2, 3A–C), two latent membrane proteins (LMP1, 2), and several noncoding RNAs. Most of these genes have important functions in the viral latent persistence. EBNA2 and LMP1 are essential for the *in vitro* transformation of B cells, as has been confirmed by using recombinant forms of EBV that lack individual latent genes. These studies have also highlighted a crucial role for EBNA1, EBNA-LP, EBNA3A, and EBNA3C in the transformation process. The main transforming protein of EBV is LMP1. It fulfills the criteria of a classic oncogene (e.g., rodent-fibroblast transformation). Although it lacks any homology to cellular proteins, LMP1 functionally mimics co-stimulatory receptors of the tumor necrosis factor (TNF) superfamily. Independent of a TNFR ligand, it exerts its pleiotropic effects, including the induction of cell-surface adhesion molecules and activation antigens, and the upregulation of anti-apoptotic proteins. These signals account for both the growth- and survival-stimulating functions of LMP1.

Lymphomas in Immunosuppressed Individuals

Individuals who are compromised in their cellular immunity (T-cell immune reaction) are at risk for the development of EBV-positive B-cell lymphoproliferative diseases. These include the immunoblastic lymphomas in patients with genetic immunodeficiencies, in AIDS patients, and the post-transplantation lymphomas (PTLs) in patients under immunosuppressive therapy after organ transplantation. PTLs are polyclonal or monoclonal lesions, which mostly arise within the first year of allografting, when immunosuppression is most severe. Almost all of these early onset tumors are EBV positive. The growth-stimulating EBV latent genes including EBNA2 and LMP1 are expressed; it suggests that the PTL consists of virus-transformed cells, which closely resemble *in vitro*-transformed lymphocytes that grow out in the absence of effective T-cell surveillance.

Hodgkin's Lymphoma

EBV-induced infectious mononucleosis was recognized as a risk factor for the development of Hodgkin's lymphoma. This malignant tumor is characterized by the predominance of a nonmalignant infiltrate which vastly outnumbers the malignant cells (Hodgkin cells, Reed–Sternberg cells: HRS cells). All HRS cells within a tumor are part of the same clone and are probably derived from crippled germinal-center cells that have been rescued from the germinal-center reaction.

The infiltrate distinguishes the subtypes, the nodular sclerozing (NS), mixed cellularity (MC), and rarer lymphocyte-depleted (LD), which are to different extents associated with EBV. Approximately 40% of Hodgkin's lymphoma in the developed world is associated with EBV and between 50% and 90% of MC and LD subtypes. The EBV genome in the tumor is clonal and present in every HRS cell which is evidence for a causal role in the pathogenesis of Hodgkin's lymphoma. The HRS cells express a particular subset of latent-cycle proteins – EBNA1, LMP1, and LMP2. A plausible pathogenetic role for the virus could be the prevention of apoptosis in cells that have undergone a defective germinal center reaction. In particular, LMP1 by stimulating co-stimulatory (TNFR) pathways is capable of replacing a T-cell signal that prevents apoptosis. Whether EBV continues to contribute to the malignant phenotype at the time of tumor presentation is not known to date.

Burkitt's Lymphoma

Burkitts lymphoma is a worldwide distributed B-cell lymphoma with defined subtypes. Whereas only 15–25% of the sporadic form, which prevails in Europe and Northern America, is EBV associated, the endemic form is nearly to 100% positive for EBV. Endemic Burkitts lymphoma is a frequent childhood tumor in the humid lowlands of eastern and central Africa. It presents in children around 8 years of age as a unilateral swelling of the jaw. Due to early metastasis the disease is usually multifocal at diagnosis.

Burkitt's lymphoma and derived cell lines (from both EBV-positive and EBV-negative cases) are now confirmed to be of germinal-centroblast origin. In this stage of B-cell development, the germinal-center reaction of the B cell (including somatic hypermutations of the V chains and isotype switching) is almost finished and cells are starting to enter the memory compartment. Common denominators of all BL forms are chromosomal translocations, which result in increased expression of the cMYC protein. The contribution of EBV to the pathogenesis of Burkitt's lymphoma remains still unclear. The most compelling evidence of EBV's involvement in endemic BL is the high frequency (98%) of tumors carrying viral DNA and the presence of clonal EBV in all of the tumor cells. The viral gene expression is limited to mainly EBNA1. The transforming LMP1 protein is absent and even seems to be incompatible with the growth of BL cells *in vivo*. Thus, the virus might have an initiating role in which growth-transforming B-cell infections establish a pool of target cells that are at risk of a subsequent *MYC* translocation. Epidemiological evidence indicates that co-infection with malaria and HIV is an important co-factor, which increases tumor incidence, possibly by chronic stimulation of the B-cell system.

Lymphoid Malignancies with T-Cell or NK-Cell Characteristics

EBV is also associated with three types of T-cell malignancies. In particular, the nasal T-cell lymphoma has a high rate of association. It is an extranodal lymphoma of the angiocentric type, which primarily develops in the nosal caveola. The tumor is relatively frequently found in Southeast Asia. Frequently nasal EBV-associated lymphomas resemble the phenotype of NK cells (CD3−/CD56+). The EBV-gene expression includes EBNA1, LMP1, and LMP2 in various extents.

EBV-Associated Carcinomas

The infection of epithelia by EBV eventually results in malignant transformation and the development of carcinomas. Such EBV-associated cancers are the anaplastic nasopharyngeal carcinoma (NPC), a subset of gastric adenocarcinomas and certain salivary gland carcinomas.

Nasopharyngeal Carcinoma

NPCs are highly malignant neoplasias, which mostly occur in adults between the ages of 20 and 50 years. The prognosis is poor; most frequently, NPC presents with early metastasis into cervical lymph nodes and the skull. The anaplastic (undifferentiated) form of NPC (aNPC) shows the most consistent worldwide association with EBV (virtually 100% worldwide). The anaplastic type of the NPC is recognized as a separate clinical entity. It differs from other types of NPC by its low grade of differentiation of the tumor cells and a characteristic tendency to extended lymphocyte infiltrations. The aNPC is particularly common in areas of China and Southeast Asia, reaching a peak incidence of around 20–30 cases per 100 000. An etiologic role of the virus in tumor development is supported by following characteristic features of the tumor cells: (1) virtually all aNPC contain EBV-DNA, and (2) the viral episomes in the tumors are monoclonal. From this observation one can deduce that the infection event has occurred prior to tumorigenesis. This assumption is supported by the presence of monoclonal EBV-genomes in the noninvasive progenitor lesions, the *in-situ*-NPC. (3) Besides EBNA1, the tumors express LMP2 and partly (40%) the viral oncoprotein LMP1. The lack of LMP1 expression in some tumors however seems to be a secondary late event since it has been reported that the premalignant lesions of NPC all express LMP1. Thus, the presence of the growth-signal-mediating LMP1 and LMP2 is additional evidence for the causal role of the virus in tumorigenesis.

Other Carcinomas

EBV is present in a high proportion (>90%) of lymphoepithelioma-like gastric carcinomas, which morphologically closely resemble NPC. About 5–25% of gastric adenocarcinomas are also associated with EBV. These tumors display a restricted pattern of EBV latent-gene expression, including EBNA1 and LMP2A. A possible role of EBV in the pathogenesis of gastric carcinomas seems to be confined to late tumorigenesis, which is suggested by the absence of EBV infection in premalignant gastric lesions. A subset of salivary gland carcinomas have also been found to be EBV-positive including the expression of latent genes like EBNA1 and LMP1 and 2 in parts of the tumors. Recently, EBV has also been detected in some carcinomas of the breast and liver.

Malignant Diseases Related to the KSHV

By using a polymerase chain reaction (PCR)-based technique for the selective amplification of unknown sequences ('representational difference analysis') Chang and co-workers identified in 1994 a new human herpesvirus in the tissue of Kaposi's sarcoma (KS). The new virus was designated as human herpesvirus type 8 (HHV8) or KSHV. It is highly associated with KS but also found in several rare lymphoproliferative conditions, including Castleman's disease and body cavity-based lymphoma (synonymous: primary effusion lymphoma).

Kaposi's Sarcoma

The tumor was first described in 1872 by Moritz Kaposi as 'multiple idiopathic pigmented sarcoma of the skin'. It is a

multifocal, proliferative lesion of spindle-shaped cells with slit-like vascular spaces in skin and mucous membranes of the oral cavity, gastrointestinal tract, and pleura. The tumor cells, termed KS spindle cells, are likely of endothelial origin. KSs can be grouped into four clinical subtypes: (1) classic, (2) endemic or African, (3) transplantation-associated or immunosuppressive therapy-associated, and (4) epidemic or HIV/AIDS-associated. Based on the following evidence, a causal role of KSHV in the pathogenesis of KS is now widely accepted: (1) KSHV genomes are regularly detected in all subtypes of KS; (2) KSHV is present in the endothelial and spindle cells, the neoplastic component of the tumor; and (3) epidemiological and prospective cohort studies in HIV-infected individuals show high correlation of KSHV infections with a later development of KS. Viral gene expression in the latently infected KS cells is likely to support the growth of tumor cells. Among the viral proteins synthesized are a viral homolog of a D-type cyclin (vCYC), which is capable of stimulating the cell cycle in the G1 phase, and a viral homolog of FLICE-inhibitory protein (vFLIP), which is a potent suppressor of extrinsic apoptosis. Furthermore, latently infected KS spindle cells produce the 'latency-associated nuclear antigen' (LANA-1) that is required for the replication of circular viral episomes and the activation of a wide range of cellular genes.

B-Lymphoid Malignancies

'Multicentric Castleman's disease' (MCD) is a lymphoproliferative condition, characterized by enhanced B-cell proliferation and vascular proliferation in expanded germinal centers. In these lesions KSHV can be detected. In a proportion of B cells surrounding the follicular centers of MCD, several viral proteins were found to be expressed. These include LANA-1, the viral interleukin-6 (vIL-6), and the several proteins with homology to interferon regulatory factors (vIRF-1/K9, K10.5/LANA-2, K10). The signal transduction cascades induced by these viral proteins might be relevant for the pathogenesis of this disease. The second B-lymphoid malignancy associated with KSHV is the 'primary effusion lymphoma' (synonymous: body cavity-based lymphoma). This rare lymphoma develops mostly in AIDS patients in the spaces of pleura, pericard, or peritoneum.

Papillomaviruses as Major Cause of Human Cancers

There is nowadays sufficient evidence that certain, so-called 'high-risk' types of HPV are carcinogenic to humans in the cervix (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 66). In developing countries, carcinoma of the cervix uteri is the most frequent type of female cancer. Even in industrialized countries, in spite of extensive preventive screening, this cancer is among the most frequent female malignancies. HPV DNA can be found in virtually all cervical cancers, and the early genes E6 and E7 are usually expressed. The most prevalent type in epidermoid carcinomas is HPV16. HPV18 may be preferentially associated with adenocarcinomas. Other high-risk types have been detected in a few cases of squamous cell carcinoma each. HPV DNA was also demonstrated in more than 50% of the less-prevalent carcinomas of the vulva and penis (basaloid and warty tumors), the vagina, and the anus; HPV16 is again the most frequent type, followed by HPV18. HPV6 and HPV11 with a lower carcinogenic potential were detected in verrucous carcinomas of vulva, penis, and anus.

HPV16 and other members of the high-risk papillomavirus group immortalize primary human keratinocytes and induce resistance to differentiation stimuli. Histological abnormalities can be observed in stratifying keratinocyte cultures that resemble those in precancerous, intraepithelial lesions *in vivo*. The cells are not tumorigenic in nude mice initially, but quickly change to an aneuploid karyotype, which is in keeping with frequently occurring abnormal mitoses in HPV16-positive lesions. At higher passage level, malignant clones reproducibly arise, which indicates that HPV infection is sufficient to induce cancer cells in combination with additional spontaneous or virus-induced modifications. The viral genes E6 and E7 are required to trigger these effects. They encode proteins that inactivate tumor suppressors and modulate cell-cycle regulation, DNA repair, and apoptotic processes, for example, by interacting with the cellular proteins p53, p105-RB (the retinoblastoma protein), p21, p27, Bak, and PDZ domain proteins. E6 activates the catalytic subunit of the telomerase as an important step in cell immortalization. E6 and E7 induce chromosomal instability, mitotic defects, and aneuploidy, which will finally contribute to tumor progression. The E6 and E7 proteins of low-risk viruses display much lower affinities to the cellular proteins, in parallel with a lower or non-detectable transforming potential *in vitro*.

Much attention has been paid to the possible role of viral DNA integration in tumor progression. HPV18 DNA appears integrated into the cellular genome in almost all cervical cancers, and HPV16 DNA in about two-thirds of the cases. This is in contrast with benign and premalignant lesions, where the viral DNA usually persists extrachromosomally. There is no evidence for a specific integration site, but HPV DNA has been repeatedly detected in the vicinity of the *myc* proto-oncogene in combination with an overexpression of the cellular gene. Probably, more important is the upregulation of viral oncogene expression following critical integration events, which result in a clonal selection of the affected cells.

The persistence of viral DNA and the continual expression of transforming genes in advanced cancers suggest that HPV functions are also involved in the maintenance of the malignant phenotype. An experimental suppression of E6 and E7 expression inhibited the proliferation of HPV-positive cervical cancer cell lines and reduced the cloning efficiency in semisolid medium, thus indicating that the viral proteins are still modulating the growth of malignant cells.

The genital tract HPVs are also responsible for many HPV infections at extragenital mucosal sites such as the oral cavity, the oropharynx, and most notably the larynx. However, cancers arising in this field harbor HPV DNA less frequently than genital tumors (oral cavity and larynx on average about 25%, oropharynx 35%, preferentially carcinoma of the tonsil). The reason for the striking difference between the genital and aerodigestive tracts is not known. Either the etiology of many oral and laryngeal cancers is unrelated to HPV, or the relevant HPV types are not yet characterized or the viral DNA is no longer necessary for cancer cells and is finally lost.

Different HPV types induce various proliferative skin lesions that are benign, like plantar, common and flat warts. An association between HPV and skin cancer becomes obvious in epidermodysplasia verruciformis (EV). EV patients are infected with a subgroup of HPVs, which induce characteristic persisting macular lesions disseminated over the body. Many EV patients develop squamous cell skin carcinomas, mainly at sun-exposed sites, which suggest a co-carcinogenic effect of ultraviolet light. The DNA of HPV5 or 8 persists extrachromosomally in high copy number in more than 90% of the cancers. HPV14, 17, 20, or 47 were occasionally detected. The prevalence of specific HPVs is in striking contrast with the plurality of HPV in benign lesions and has been interpreted as reflecting a higher oncogenic potential of these types.

The carcinogenic potential of HPV8 could be clearly demonstrated in transgenic mice with the early genes of HPV8 under control of the keratin 14 promoter, which regularly develops papillomas with moderate and severe dysplasia and carcinomas in 6% of animals without any further treatment with physical or chemical carcinogens. E6 turned out to be the major oncogene in the mouse skin, necessary and sufficient for carcinogenesis. In contrast with genital HPV, no complex formation could be detected between HPV8 E6 and the cellular p53 protein, which suggests different strategies of transformation. Human keratinocytes transduced with recombinant retroviruses expressing HPV8 E7 invaded the dermis when tested in organotypic skin cultures.

A high prevalence of HPV DNA in squamous and basal cell carcinomas of the skin, particularly of immunosuppressed but also of immunocompetent patients, has been demonstrated by highly sensitive PCRs. Evidence is accumulating for many novel HPV types related to EV HPVs and cutaneous types. That there is a strong association between genital HPV16 and rare squamous cell neoplasms from the finger is remarkable. Individual skin tumors were frequently noted to be infected by several HPVs. No single HPV type predominates in skin cancers of non-EV patients, so far as is known. HPV DNA persists at very low concentrations in many skin cancers, usually at less than one genome copy per cancer cell. The relevance of these findings to the pathogenesis of cutaneous cancer remains to be determined. The possibilities discussed above for carcinomas of the aerodigestive tract are also valid for skin carcinomas.

Chronic Hepatitis B and C in Hepatocarcinogenesis

Hepatocellular carcinoma (HCC) is among the most common fatal malignancies in humans worldwide. An association with HBV from the Hepadnavirus family was suggested by the geographical coincidence of a high incidence of HCC in Southeast Asia and equatorial Africa with high rates of chronic HBV infections, frequently contracted congenitally. Prospective studies demonstrated about a 100-fold increased risk of hepatoma among carriers of the HBV surface antigen (HBsAg). Integrated HBV DNA can be detected in a large proportion of the tumors from high-risk areas and in hepatoma-derived cell lines. HBV is the first human tumor virus against which vaccination programs have been initiated on a broad basis. First signs of a decrease in the incidence of hepatoma in populations vaccinated in the 1970s substantiate the viral role in cancer development.

Liver cancer usually develops only after several decades of chronic HBV-induced hepatitis and may thus be triggered by accumulating genetic damage due to inflammation and continuous cell regeneration. A specific contribution of HBV might be expected from *cis* effects following integration of viral DNA, but except for a few case reports no consistent evidence has been obtained for the activation of particular proto-oncogenes. A transactivation of transcription may be more relevant; this can be achieved by the viral X protein, the large surface protein, and a truncated $preS_2/S$ protein. The viral $preS_2/S$ gene, which normally encodes a surface protein, appears frequently disrupted in cancers as a consequence of DNA integration and then gives rise to the transactivator. All HBV transactivators exert pleiotropic effects via the protein kinase C/raf-controlled signal pathway, finally activating transcription factors such as AP-1 and NF-κB and proliferation. The analysis of viral integration patterns and functional assays suggest that at least one transactivator may function in most hepatomas.

Mutations in the p53 tumor suppressor gene occur in about 30% of human hepatomas. They are observed more often in countries with dietary contamination by mutagenic aflatoxin and seem to be a late event in liver carcinogenesis. The X protein was shown to interact with elements of the DNA repair system, which may increase the mutation rate of p53.

Two types of large HBsAg with deletions at the $preS_1$ and $preS_2$ regions were detected in 60% of HCC patients. The preS mutant proteins can initiate endoplasmic reticulum stress to induce oxidative DNA damage and genomic instability. Liver cancer arose not only in mice transgenic for the X gene but also in mice transgenic for preS mutants.

More recently, seroepidemiological evidence was obtained for a correlation between HCV infections and hepatoma. Antibodies against HCV were detected in between 13% and over 80% of liver cancer patients around the world. Over 60% of acute hepatitis C becomes chronic and may progress to cirrhosis and HCC. Latency periods between primary infection and cancer are usually measured in decades, but in some cases the intervals are rather short (5–10 years). The cumulative prevalence of hepatoma in cirrhotic HCV-infected patients is over 50%, indicating that HCV substantially increases the risk of HCC. HCV is related to flaviviruses and pestiviruses and is the first human tumor-related virus with an RNA genome and no DNA intermediate during replication. The role of the virus in carcinogenesis is not yet clear. Liver injury during chronic hepatitis may be responsible for malignant conversion, but there is also some evidence that HCV is more directly involved. HCV appears to persist and replicate in hepatocytes during malignant transformation. Viral proteins interact with many host-cell factors and affect cell signaling, transcription, translation, proliferation, and apoptosis. The HCV capsid core protein and the nonstructural proteins NS3 (a serine proteinase), NS4B, and NS5A revealed transformation potential in tissue culture. Both HCV core and NS5A target the Wnt-β-catenin pathway, which appears crucial in human HCC. As discussed for HBV, HCV also induces endoplasmic reticulum and oxidative stress.

The Role of HTLV-1 in the Oncogenesis of Adult T-Cell Leukemia/Lymphoma

In 1977 adult T-cell leukemia/lymphoma, a malignancy of CD4-positive T lymphocytes, was first recognized as a distinct clinical entity by Uchiyama, Takatsuki, and colleagues. Three years later, in 1980, Poeisz, Gallo, and co-workers isolated the first human retrovirus, HTLV-1 from the lymphoma-subtype of ATLL. HTLV-1, but not the closely related HTLV-2 is highly associated with ATLL. Besides ATLL, HTLV also is linked to the pathogenesis of a chronic neurodegenerative disorder, called HTLV-associated myelopathy/tropical spastic paraparesis.

Transformation of T Cells

HTLV-1-infected T lymphocytes derived from leukemic and nonleukemic patients, in contrast to normal T cells, regularly give rise to permanent cultures, which are capable of expressing all viral proteins. The virus *in vitro* has immortalizing capacity: primary human T lymphocytes can be transformed to permanent growth in tissue culture, which phenotypically resembles ATLL cells. A nonstructural regulatory protein of the virus, Tax, is capable of mediating the transformation. Besides regulating viral transcription, this multifunctional protein interferes with cellular control of survival, proliferation, and genomic stability. It combines many features characteristic of oncogenes, among them the ability to immortalize primary human T cells to permanent growth. These cells closely resemble HTLV-1-transformed and patient-derived T cells. Tax is also capable of inducing malignant growth in animal models. In HTLV-1 Tax transgenic mice it induces leukemia which is similar to the clinical pattern of ATLL.

Adult T-Cell Leukemia/Lymphoma

About 1–3% of all HTLV-infected individuals develop ATLL, mostly after several decades of asymptomatic viral persistence. Typically, the disease manifests at the age of 40–60 years. Frequent symptoms are cutaneous lesions, pulmonary complications, hepatosplenomegaly, and hypercalcemia, which at least in part can be attributed to the capacity of the malignant cells to infiltrate organs. Generally, ATLL is categorized into four forms: (1) acute, (2) chronic, (3) smoldering, and (4) lymphoma-type, which are all associated with HTLV-1. The smoldering and chronic ATLL are less-severe forms of the disease, which may convert to the aggressive acute ATLL. The lymphoma-type is characterized by the presence of extensive lymphadenopathy in the absence of blood or bone marrow involvement. Acute ATLL is the most frequent form (more than 55%) and until now barely curable and mostly fatal. Leukemia cells exhibit an unusual and characteristic morphology with lobulated nuclei. Regarding the surface phenotype they resemble T helper cells, from which they are probably derived (CD4), expressing high amounts of the interleukin 2 receptor (IL2Rα). Viral gene expression including the Tax gene is rather low and the role of the protein for the maintenance of the malignant proliferation unclear. A causative role of HTLV-1 in ATLL is now generally accepted because of the following evidence: (1) the geographic correspondence of ATLL and HTLV-1; (2) the almost 100% association of HTLV-1 and ATLL; (3) the clonal integration of the provirus in

ATLL cells, which indicates infection prior to malignant transformation; and (4) the oncogenic capacity of HTLV-1 and its oncoprotein Tax in animal models.

General Conclusions and Future Perspectives

In most cases, long latency periods of many years or several decades elapse between primary infection by tumor viruses and first symptoms of cancer. All human tumor viruses are widespread in the world population. They contribute to malignant disease, mainly by initiation of oncogenesis, but regular infection does not immediately result in cancer. Thus, all human tumor viruses are important or necessary risk factors for particular cancers, but require additional events to induce malignant disease. This implies that in principle, all virally induced cancers can be prevented by protective immunization. As examples, vaccination against HBV and recently, against high-risk HPV are powerful prophylactic means to fight the associated cancers. The low manifestation rates of virus-induced malignancies also imply that the mere proof of an infection with a tumor virus is of limited value for the management of patients and cancer prevention. Specific diagnostic tests have to be designed that evaluate parameters of the viral infection more closely related to malignant conversion. In many but not all cases, continuous viral expression is detectable in malignant tumors, which raises the prospect of virus-specific pharmacological interference for adjuvant cancer therapy or cancer immunotherapy. For instance, the neoplastic phenotype of HPV-positive genital carcinoma cells seems to be affected by viral functions and may thus be a promising therapeutical target. Even in cases, in which primary infection and initial growth transformation apparently lead, through tumor progression, to a constitutive form of proliferation where viral gene products are not necessary for growth, virus may be used to target the tumor. For instance, stimulating specifically the immune response against viral antigens may be an appropriate future strategy.

See also: Epstein–Barr Virus: General Features; Epstein–Barr Virus: Molecular Biology; Hepatitis B Virus: General Features; Hepatitis B Virus: Molecular Biology; Hepatitis C Virus; Human Immunodeficiency Viruses: Antiretroviral agents; Human Immunodeficiency Viruses: Molecular Biology; Papillomaviruses: Molecular Biology of Human Viruses; Retroviral Oncogenes.

Further Reading

Cougot D, Neuveut C, and Buendia MA (2005) HBV-induced carcinogenesis. *Journal of Clinical Virology* 34(supplement 1): S75.
Grassmann R, Aboud M, and Jeang KT (2005) Molecular mechanisms of cellular transformation by HTLV-1 Tax. *Oncogene* 24(39): 5976–5985.
IARC Working Group (2007) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 90, Human papillomaviruses.* Lyon, France: International Agency for Research on Cancer.
Levrero M (2006) Viral hepatitis and liver cancer: The case of hepatitis C. *Oncogene* 25: 3834–3847.
Neipel F and Fleckenstein B (2005) Human herpesvirus-8. In: ter Meulen V and Mahy B (eds.) *Topley and Wilsons, Microbiology and Microbial Infections: Virology*, pp. 541–558. London: Arnold.
Pfister H (2003) Human papillomavirus and skin cancer. *Journal of the National Cancer Institute* (Monograph) 31: 52.
Proietti FA, Carneiro-Proietti AB, Catalan-Soares BC, and Murphy EL (2005) Global epidemiology of HTLV-I infection and associated diseases. *Oncogene* 24(39): 6058–6068.
Schulz TF (2006) The pleiotropic effects of Kaposi's sarcoma herpesvirus. *Journal of Pathology* 208(2): 187–198.
Thorley-Lawson DA (2005) EBV the prototypical human tumor virus – just how bad is it? *Journal of Allergy and Clinical Immunology* 116(2): 251–261.
Young LS and Rickinson AB (2004) Epstein–Barr virus: 40 years on. *Nature Reviews Cancer* 10: 757–768.
zur Hausen H (2006) *Infections Causing Human Cancer.* Weinheim, Germany: Wiley-VCH Verlag.

Tymoviruses

A-L Haenni, Institut Jacques Monod, Paris, France
T W Dreher, Oregon State University, Corvallis, OR, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Icosahedron A solid having 20 faces and 12 vertices.
Open reading frame Region of a genome that can be translated into a protein.
Phylogeny The complete evolutionary history of group of animals, plants, bacteria, viruses, etc.
Pseudoknot RNA structure formed by base-pairing between nucleotides within the loop subtending a stem and nucleotides outside of this loop.

sgRNA Subgenomic RNA, derived from a viral RNA genome during replication, serves as mRNA for the expression of genes that are translationally silent in the genomic RNA.
Vein-clearing Yellowing along the leaf veins.

The Family and Its Distinguishing Features

The members of the family *Tymoviridae* are presented in **Table 1**. Like the family itself, the genus *Tymovirus* derives its name from the type species, *Turnip yellow mosaic virus*. Turnip yellow mosaic virus (TYMV) was first isolated in 1946 and is by far the most intensively studied member of the family. Indeed, TYMV is one of the best-characterized plant viruses. The family was recently created in recognition of the close relationships between the genera *Tymovirus*, *Marafivirus*, and the founding member of newly created genus *Maculavirus*, grapevine fleck virus (GFkV). Poinsettia mosaic virus (PnMV) is currently a family member unassigned to a genus. Complete genome sequences are available for the type species of each of the three genera of the *Tymoviridae* and for PnMV.

The members of the family *Tymoviridae* are characterized by their icosahedral, nonenveloped, ~29 nm virions, that can readily be visualized by negative-staining electron microscopy (EM). Infections produce a characteristic

Table 1 Members of the family *Tymoviridae*

Species name	*Virus abbreviation*	*Complete sequence accession no.*
Genus *Tymovirus*		
Andean potato latent virus	APLV	
Belladonna mottle virus	BeMV	
Cacao yellow mosaic virus	CYMV	
Calopogonium yellow vein virus	CalYVV	
Chayote mosaic virus	ChMV	AF195000
Clitoria yellow vein virus	CYVV	
Desmodium yellow mottle virus	DYMoV	
Dulcamara mottle virus	DuMV	AY789137
Eggplant mosaic virus	EMV	J04374
Erysimum latent virus	ErLV	AF098523
Kennedya yellow mosaic virus	KYMV	D00637
Melon rugose mosaic virus	MRMV	
Okra mosaic virus	OkMV	EF554577
Ononis yellow mosaic virus	OYMV	J04375
Passion fruit yellow mosaic virus	PFYMV	
Peanut yellow mosaic virus	PeYMV	
Petunia vein banding virus	PetVBV	
Physalis mottle virus	PhyMV	Y16104
Plantago mottle virus	PlMoV	AY751779
Scrophularia mottle virus	SrMV	AY751777
Turnip yellow mosaic virus	TYMV	J04373, X16378, X07441
Voandzeia necrotic mosaic virus	VNMV	
Wild cucumber mosaic virus	WCMV	
Tentative members		
Anagyris vein yellowing virus	AVYV	AY751780
Nemesia ring necrosis virus	NeRNV	AY751778
Genus *Marafivirus*		
Bermuda grass etched-line virus	BELV	
Maize rayado fino virus	MRFV	AF265566
Oat blue dwarf virus	OBDV	U87832
Citrus sudden death-associated virus	CSDaV	AY884005, DQ185573
Tentative members		
Grapevine asteroid mosaic-associated virus	GAMaV	
Grapevine rupestris vein feathering virus	GRVFV	AY706994
Genus *Maculavirus*		
Grapevine fleck virus	GFkV	AJ309022
Tentative member		
Grapevine red globe virus	GRGV	
Unassigned virus		
Poinsettia mosaic virus	PnMV	AJ271595

mixture of filled, infectious virions and empty or near-empty capsids. Regular surface features, representing prominent peaks formed by pentamers and hexamers of coat protein (CP) molecules, are evident by EM (**Figure 1**).

The genomes of members of the *Tymoviridae* are composed of a single positive-stranded RNA generally 6.0–6.5 kb long, although the genome of GFkV is 7.5 kb long. Subgenomic (sg) RNAs of 1 kb or less are associated with CP expression. All *Tymoviridae* genomes have a distinctive skewed nucleotide composition that is rich in C residues (32–50%). The unifying characteristic of the genome design of all *Tymoviridae* members is the presence of a long open reading frame (ORF) that covers most of the genome and encodes the replication polyprotein with identifiable domains: methyltransferase, papain-like proteinase, helicase, and RNA-dependent RNA polymerase (RdRp) in order N- to C-terminal (**Figure 2**). The CP ORF is situated downstream of the polyprotein ORF, to which it is fused in the marafivirus and PnMV genomes. Close familial relationships are easily discerned from alignments of the sequences of the RdRp and CP genes.

Most viruses in the family have narrow host ranges. Many of the tymoviruses have been isolated from noncrop hosts and have thus far not presented major disease threats to crops. Marafiviruses are associated with significant crop losses, perhaps resulting from their more effective transmission by flying insects.

Tymoviruses

Properties and Distinguishing Characteristics

Although virtually all information about tymoviruses has been derived from studies on TYMV, it is considered to be generally applicable to the other members of the genus. Virions of tymoviruses contain genomic RNAs 6.0–6.7 kb long and produce a single 3′ collinear sgRNA less than 1 kb in length encoding the ~20 kDa CP. Their genomes include a distinctive 16-nt-long 'tymobox' sequence, and the genomes of most species possess a 3′ tRNA-like structure (TLS) that can be efficiently esterified with valine. The genomes encode three ORFs (**Figure 2**), two of which almost completely overlap: the overlapping protein (OP) and replication protein (RP) ORFs, which begin at AUG codons separated by only 4 nt. Tymoviruses replicate in all major tissues of their host plants, and accumulate to high levels (more than 0.1 mg g^{-1} leaf tissue). Both filled and empty or near-empty particles accumulate. They are readily transmitted mechanically under laboratory conditions, and are spread over limited distances by beetle vectors in nature. Infection produces mosaic symptoms and a distinctive cytopathy that is evident upon EM observation by the appearance of small vesicles on the surface of chloroplasts, together with vacuolation and clumping of chloroplasts.

Capsid Structure

Virions of tymoviruses are highly stable ~29 nm, $T = 3$ icosahedra formed by 180 copies of the single CP, arranged as 12 pentamers and 20 hexamers. These groupings form the vertices with fivefold and sixfold symmetry that constitute the surface peaks visible upon high-resolution EM observation (**Figure 1**). Intersubunit stabilization is provided primarily by hydrophobic protein–protein contacts, allowing the formation of stable shells that appear to be devoid of RNA. These empty or near-empty capsids can account for about one-third of the particles present in infected tissues and are readily identifiable by internal staining in negative-contrast EM (**Figure 1**). They

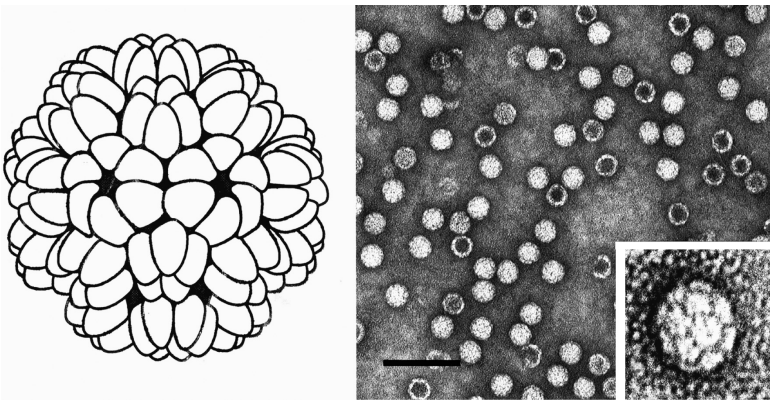


Figure 1 Tymovirus particle structure. A cartoon of the arrangement of subunits into pentamers and hexamers to build the $T = 3$ icosahedron is shown at left. A negative-contrast electron micrograph of virions and 'empty' particles of belladonna mosaic tymovirus is shown at right, with the high-magnification inset showing the prominent surface structure. Scale = 100 nm. Image courtesy Dr. D.-E. Lesemann. Reproduced from Mayo MA, Dreher TW, and Haenni A-L (2000) Genus *Tymovirus* In: van Regenmortal MHV, Fauquet CM, Bishop DHL, *et al*. (eds.) *Seventh Report of the International Committee on Taxonomy of Viruses.* New York: Academic Press, with permission from Elsevier.

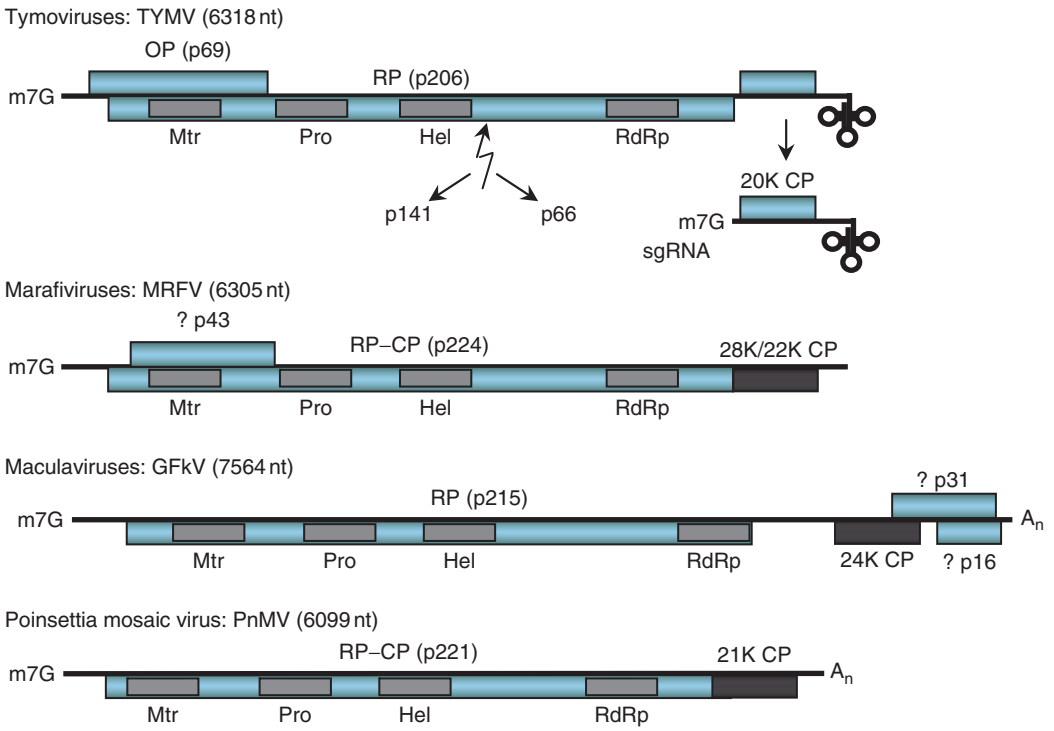


Figure 2 Genomes of the type members of the genera of the family *Tymoviridae* and of PnMV. All genomes have a 5′-m7GpppG cap, but the 3′ terminal structures vary. TYMV has a valine-specific tRNA-like structure (cloverleaf), GFkV and PnMV have a poly(A) tail, while MRFV has neither. The known or predicted (?) expressed ORFs are indicated with the molecular weight (K, kDa) of the predicted protein. The RPs all possess methyltransferase (Mtr), papain-like proteinase (Pro), helicase (Hel), and RdRp domains. The TYMV RP is cleaved as indicated, and similar cleavages are expected for the other viruses. The TYMV OP is the viral movement/RNAi suppressor protein. The TYMV CP is expressed from a sgRNA, and all other CPs are probably also expressed from sgRNAs (not shown). The 28 kDa MRFV CP (whose true size is likely to be closer to 25 kDa) may be produced by proteolytic cleavage from the RP–CP fusion protein, and an analogous event may occur with PnMV. Expression of the MRFV p43 and GFkV p31 and p16 has not yet been validated.

sediment as the 'top component' at 45–55S in CsCl density gradients, and readily separate from the 'bottom component', the infectious 110–120S virions that contain the genomic RNA. Minor components of intermediate density contain a range of subgenomic-size RNAs that can be translated to yield CP. Roughly equimolar amounts of genomic and sgRNAs are encapsidated, but the precise disposition of sgRNA in the various particles has not been determined. Like many other viral CPs, tymoviral CPs fold into eight-stranded, β-barrel, jelly-roll structures. However, unlike some other CPs, there is no positively charged domain at the N terminus for interaction with RNA and charge neutralization during packaging. Charge neutralization is thought to be provided by polyamine molecules (mostly spermidine) associated with the RNA. The crystal structures of empty and infectious particles have been determined for TYMV and physalis mottle virus. Consistent with the dominance of protein–protein interactions in particle stabilization, empty and infectious particles have very similar structures.

Packaging signals in tymoviral RNAs have yet to be identified, though RNA recruitment may involve conserved hairpins in the 5′ untranslated region (UTR) and interaction of C-rich segments of the RNA with CP at low pH. Localized areas of low pH (5–6) have been postulated to arise at the surfaces of photosynthesizing chloroplasts. Tymoviral replication occurs in characteristic vesicles that form at the chloroplast surfaces (see below), and capsid formation is most active near these vesicles, suggesting a possible coupling between replication and encapsidation during infection. Despite much effort, there has been no success in developing a cell-free packaging system. These efforts have focused on the possible role of 'artificial top component' (ATC) capsids as decapsidation and encapsidation intermediates. ATCs are protein shells devoid of RNA that can be made from infectious virions by treatments such as freeze–thawing and exposure to high pH and pressure. They are similar in structure to infectious virions, but lack a capsomere of six CP molecules that is ejected during the treatment, allowing RNA escape.

Genome Organization

Tymoviral genomic RNAs possess a 5′-m7GpppG cap and terminate at the 3′ end in -CC(A) except for

dulcamara mottle virus (DuMV) that is in most cases part of a TLS. The typical tymoviral TLS is just over 80 nt long, has a distinctive pseudoknot close to the 3′ terminus, and is a close structural mimic of cellular $tRNA^{Val}$. A valine-specific anticodon is present, and the 3′ terminus can be specifically aminoacylated with valine. The valylated RNAs of some, though not all tymoviruses, can form a tight complex *in vitro* with the GTP-bound form of translation elongation factor eEF1A. Most molecules of encapsidated RNA lack the terminal A residue in the -CC(A) end, which is thought to be added by the host tRNA-specific CCA-nucleotidyltransferase at the beginning of infection. The main roles of the TYMV TLS are thought to be (1) as a 3′ enhancer of translation initiation, (2) as a regulator of the onset of RNA replication by modulating access by the polymerase to the 3′ end, and (3) in maintaining an intact-CCA 3′ end. A minority of tymoviral genomic RNAs have 3′ UTRs that lack the typical valine-specific TLS (DuMV, erysimum latent virus, and nemesia ring necrosis virus, NeRNV).

Tymoviral RNAs act as cap-dependent messenger RNAs. Of the three ORFs, two (OP and RP) are expressed directly from the genomic RNA, whereas the third (CP) is expressed from the 5′-capped sgRNA (**Figure 2**). Although the sequence contexts around the OP and RP initiation codons vary, 4 nt always separates these closely spaced AUGs. The RP ORF covers most of the viral genome, and encodes the ~200 kDa RP, the only viral protein essential for supporting viral RNA replication in protoplasts. This ORF encodes discrete domains (**Figure 2**) that are discernable by virtue of sequence relationships to similar domains encoded by a wide variety of positive-stranded RNA viral genomes. Particularly the RdRp domain is well conserved and appropriate for phylogenetic comparisons. Closely related, yet distinct, tymoviral genomes have RdRp domains with about 70% nucleotide sequence identity. The RP is translated as a precursor protein that subsequently undergoes maturation cleavage catalyzed by the papain-like proteinase domain. The single known cleavage separates the RdRp domain from the remainder of the RP; thus, for TYMV, the precursor p206 is processed to yield p141 and p66.

The OP ORF almost entirely overlaps the RP ORF. Its length and the sequence of the encoded protein are highly variable. OPs range between 49 and 82 kDa, and have only 25–40% amino acid sequence identity between the most closely related pairs of viruses. OP expression is needed for establishment of infection and for spread of the virus in plants. The OP is a suppressor of the host RNA silencing antiviral response and is also believed to be the viral movement protein. It is a substrate for ubiquitin-dependent degradation by the proteasome. Long-distance movement of the virus in plants requires expression of the CP.

The CP ORF initiates close to the end of the RP ORF, sometimes even a little upstream. CP sequences are variably conserved among tymoviruses, with 36–86% amino acid sequence identity between the most closely related pairs of viruses.

Tymoviral genomes possess a highly conserved sequence, the 16 nt tymobox (–GAGUCUGAAUUGCUUC–) with small variations in some viruses, especially wild cucumber mosaic virus, just upstream of the CP ORF. The tymobox and its associated 'initiation box' sequence CAA(U/G) positioned 8 or 9 nt downstream of the tymobox are believed to serve as the core elements of the sgRNA promoter. The tymobox overlaps with the 3′ end of the RP ORF, resulting in the presence of the tripeptide – ELL – near the C terminus of all RPs.

Replication Cycle

The ultrastructural changes that reflect viral replication activity and that result in the distinctive pathology of the chloroplasts have been particularly well described for TYMV. As is typical of positive-stranded RNA viruses, the replication cycle is completed entirely in the cytoplasm, and RNA replication occurs in association with membranes, specifically the chloroplast outer membrane. Vesicles 50–80 nm in diameter form as invaginations of the two outer membranes of chloroplasts. They form before the appearance of virions, which are initially found close to vesicle clusters and later throughout the cytoplasm; in some cases, empty capsids accumulate in nuclei. The RdRp has been localized to zones on chloroplasts that are rich in vesicles, and this localization depends on protein–protein interaction between the proteinase and RdRp domains of the mature RPs p141 and p66, respectively.

RNA replication occurs via the production of full-length minus strands, whose synthesis in TYMV is directed by the 3′ terminal –CCA serving as promoter and initiation site. No other minus-strand promoter elements have been identified in the 3′ UTR. As mentioned above, the tymobox appears to function with the initiation box as the core of the promoter directing sgRNA synthesis by internal initiation on the minus strand. The sgRNA of TYMV has a m7GpppA 5′ terminus.

Studies on the functions of viral proteins and of *cis*-acting sequences in the genomic RNA have been greatly facilitated by the use of 'infectious clones', that is, molecularly cloned cDNA versions of the genome from which infectious RNA can be derived.

Infection and Transmission

Tymoviruses cause chlorotic mosaic, vein-clearing, and mottling symptoms, generally without strong stunting. Host ranges are mostly narrow, and to date, no tymoviruses infecting monocot plants have been isolated. Tymoviruses

are transmitted over limited distances by chrysomelid beetles, and some are weakly seed-transmissible. They can also be transmitted mechanically.

Phylogenetic Relationships and Species Demarcation

In the past, serology using antisera raised against intact virus was the main criterion in classifying tymoviruses and in distinguishing between species. This is no longer the most convenient approach, however, and it has in fact been shown that distinct viruses whose genomic and CP sequences have identities less than 80% and 90%, respectively, can appear to be serologically identical. This seems to be due to similar or identical dominant epitopes within otherwise distinct CPs.

The study of relationships among tymoviruses and to other viruses will undoubtedly in the future rely on the interpretation of sequences derived from the genomic RNA. The phylogenetic trees based on the sequences of the CP and the RdRp (**Figure 3**) show similar relationships, both among the tymoviruses and to the other members of the family *Tymoviridae*. This suggests that recombination between these coding regions has not been a strong evolutionary force among the *Tymoviridae*. However, the genome of NeRNV, which has a tobamoviral-type TLS capable of aminoacylation with histidine, indicates that recombination can occur and shape tymoviral genome evolution. The complete genome sequences are available for 13 tymoviruses or tentative tymoviruses (**Table 1**).

Alignments based on the RP protein sequences indicate that the capilloviruses, carlaviruses, trichoviruses, and potexviruses, all members of the *Flexiviridae*, are the next most closely related virus groups. More distant sequence relationships indicate that members of the family *Tymoviridae* belong to the alpha-like virus group.

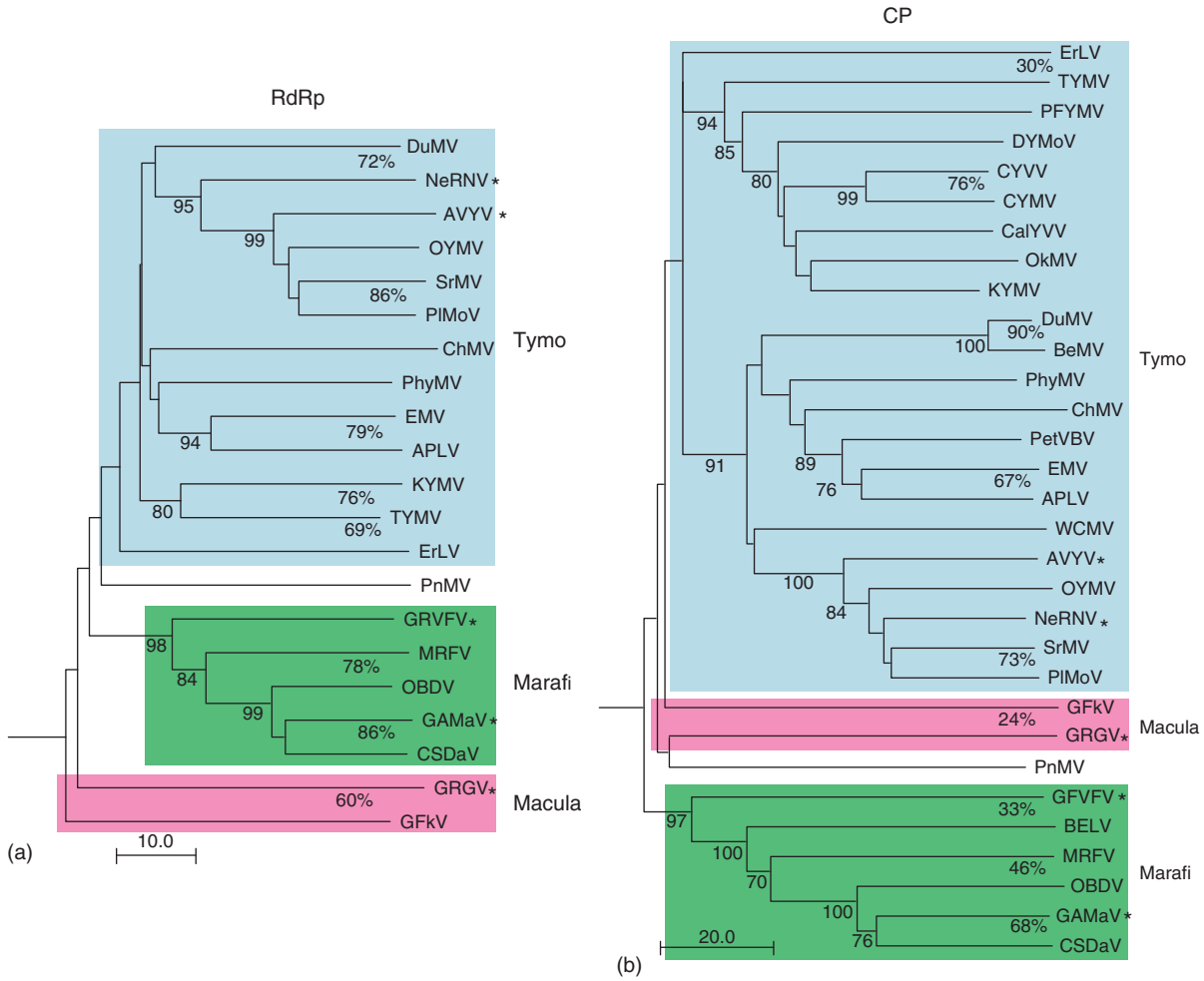


Figure 3 Phylogenetic trees showing the relationships between members of the family *Tymoviridae* based on neighbor-joining amino acid alignments of (a) the core RdRp domain and (b) the CP sequences. Bootstrap values (%) are indicated at well-supported nodes, and percent identities taken from pairwise alignments are indicated between selected sequences. Branch lengths reflect the number of amino acid differences. Viruses from each of the three genera are separately shaded as marked. Viruses with tentative taxonomic status are marked with an asterisk.

Marafiviruses

Properties and Distinguishing Characteristics

Three current members of the genus *Marafivirus* are viruses principally infecting monocots (grasses), while another infects citrus and the two tentative marafiviruses were isolated from grapevine. Plant host ranges are narrow. The genomes are 6.3–6.8 kb long, with a single ORF encoding an RP/CP fusion protein covering most of the genome. Two forms of the same CP (~25 and ~21 kDa) are found in virions. A single 3′-collinear sgRNA (<1 kb) encoding the smaller form of the CP is produced during infection. A slightly modified form of the tymobox sequence, termed 'marafibox', is present in the genome near the junction between the RdRp- and capsid-coding sequences. Marafiviruses are phloem-limited and not mechanically transmissible. The grass-infecting marafiviruses are transmitted by cicadellid leafhoppers in a persistent-propagative manner, involving virus replication in the insect.

A distinctive feature of the marafiviruses is the presence of two CPs in the virions, a major CP ~21 kDa form and a minor N-terminally extended 22–28 kDa form. The latter is thought to arise by proteolytic release from the polyprotein, whereas the former is expressed from the sgRNA. The production of this sgRNA is probably under the control of the marafibox, a 16 nt sequence (–CA(A/G)GGUGA AUUGCUUC–) very closely related to the tymobox, and the –CA(A/U)-initiation box located 8 nt downstream. The roles of the two forms of CP are unclear.

Virion Structure, Genome Organization, Replication Cycle, and Phylogenetic Relationships

Marafiviruses produce virions and empty capsids that are similar to those produced by tymoviruses when observed by negative-staining EM. However, due to the phloem-limited replication of marafiviruses, the yield of virus is low, limiting the usefulness of EM for viral identification. Complete genome sequences are available for four confirmed or tentative marafiviruses (**Table 1**).

Marafivirus genomic RNAs appear to be 5′-capped but they lack the 3′-TLS that is distinctive of tymoviruses. Apart from maize rayado fino virus (MRFV), marafivirus genomes possess a 3′ poly(A) tail. Almost the entire genome of marafiviruses is devoted to encoding a large 224–240 kDa polyprotein, which includes replication-associated and CP domains (**Figure 2**). The relationships between the CP and RdRp domains of the marafiviruses and other members of the family *Tymoviridae* are indicated in **Figure 3**. The polyprotein is believed to be proteolytically processed by the viral proteinase at two sites, between the helicase and RdRp domains as in tymoviruses, and between the RdRp and CP domains. Although the only experimentally mapped polyprotein cleavage site in a member of the family *Tymoviridae* is that occurring in TYMV, amino acid sequence alignments have discerned probable cleavage sites occurring immediately downstream of Gly-Gly or Gly-Ala dipeptides at the helicase–RdRp and RdRp–CP junctions in marafivirus polyproteins.

At the present state of relative paucity in marafivirus genome sequences, the taxonomic implications of evident variation in genome organization are uncertain. In addition to lacking a poly(A) tail, MRFV is the only marafivirus possessing a significant overlapping ORF, encoding a putative 43 kDa protein, whose expression *in vivo* has not been verified. Related but strongly interrupted coding sequences have been discerned overlapping the methyltransferase domains of other marafiviral genomes and, based on sequence similarities to tymoviral OP, it has been postulated that these sequences represent variously degenerate versions of the tymoviral OP. The loss of OP during evolution may explain the phloem limitation of marafiviruses. Citrus sudden death-associated virus (CSDaV) is the only marafivirus with an additional ORF near the 3′ end of the genome. This ORF almost completely overlaps the CP ORF and could encode a 16 kDa protein.

The three grass-infecting marafiviruses, MRFV, OBDV, and Bermuda grass etched-line virus, are serologically related, but are not serologically cross-reactive with tymoviruses or more distantly related viruses. Despite the indications of genome variability mentioned above, the marafiviruses do form a phylogenetic group with interspecies relationships similarly close to those between the various tymoviruses (**Figure 3**). Sequence relationships among the RP and CP ORFs clearly link the marafiviruses to the other members of the *Tymoviridae*. Like the tymoviral CPs, marafivirus CPs lack a cluster of basic amino acids at the N terminus.

Infection and Transmission

The best-studied marafiviruses are MRFV and OBDV. They cause stunting and chlorotic leaf spots or streaks, general leaf discoloration, or enations on leaf and stem veins. Cytological symptoms are restricted to the phloem and adjacent parenchyma cells, with clearly discernible hyperplasia and hypertrophy. These viruses are not mechanically transmissible or transmissible through seed, but are vectored in nature by leafhoppers. Significantly, MRFV and OBDV are maintained for long periods and through moults in leafhopper vectors, and transmission can occur from viruliferous vectors over a period of several weeks. OBDV replicates in the aster leafhopper (*Macrosteles fascifrons*), and the transmission behavior of MRFV is also consistent with replication in the cicadellid leafhopper (*Dalbulus maidis*). MRFV is commonly associated and co-transmitted with maize stunt spiroplasma or maize bushy stunt mycoplasma in causing economically significant outbreaks of corn stunt syndrome. OBDV can also be co-transmitted with a

mycoplasma. The presence of virus in the leafhopper vectors is not associated with detectable symptoms or loss of reproductive fitness.

The most economically significant marafivirus is probably CSDaV. This virus is believed to be the causative agent of the citrus sudden death syndrome, which has had devastating effects on orange trees in Brazil. Preliminary evidence suggests transmission by aphids. The grapevine-infecting tentative marafiviruses, grapevine asteroid mosaic-associated virus and grapevine rupestris vein-feathering virus, have no known insect vector.

Maculaviruses

Properties and Distinguishing Characteristics

Recent sequencing of the complete genome of GFkV supported the creation of the new genus *Maculavirus*. The distinguishing characteristics were the length of the GFkV genome, the absence of a tymobox/marafibox, the lack of a fused RP/CP ORF as present in marafivirus genomes, and the lack of phylogenetic clustering with either tymoviruses or marafiviruses. Grapevine red globe virus (GRGV) is a tentative member of the genus. Both viruses infect only *Vitis* species (grapevine and relatives). In common with marafiviruses, these maculaviruses are restricted to the phloem and are not sap transmissible. No insect vectors have been identified. As for all other members of the family *Tymoviridae*, maculaviruses produce ~29 nm particles with prominent surface features observable by EM. Top component and infectious bottom component particles are produced. GFkV infections are cytologically distinct from marafivirus infections through the presence of severely modified mitochondria ('multivesiculate bodies').

The genome of GFkV (7564 nt) is the longest among the members of the family *Tymoviridae*, and has the highest cytosine content (49.8%). The arrangement of RP and CP ORFs is similar to that of tymoviruses (**Figure 2**). The RP ORF encodes a 215 kDa protein with replication-associated domains that present sequence relatedness to the tymoviral and marafiviral RPs. The amino acid sequence of the RdRp domain shows the closest relationship to sister genera (59% and 67% identities with the RdRps of a tymovirus and marafivirus, respectively).

The 24.5 kDa GFkV CP has 23–31% sequence identity with CPs of tymoviruses and marafiviruses, and like other CPs of members of the family *Tymoviridae* it lacks a cluster of basic amino acids at the N terminus. The GFkV genome encodes two additional proteins from ORFs that partially overlap the CP ORF in the 3′ region of the genome. No functions for these putative proteins are predicted from sequence comparisons. Close relationship between GFkV and GRGV is rather weakly supported by sequence comparisons (**Figure 3**).

The GFkV genome is probably capped at the 5′ end and has a poly(A) tail at the 3′ end, in common with most marafiviruses and PnMV. It is the only known member of the *Tymoviridae* to lack a tymobox- or marafibox-like sequence. Nevertheless, two or more sgRNAs, ~1.3 and ~1.0 kb in length, are present in infected tissues. These RNAs appear to be packaged in both top- and bottom-component particles, in the latter case apparently together with the genomic RNA.

Poinsettia Mosaic Virus

Properties and Distinguishing Characteristics

PnMV possesses properties characteristic of both the tymoviruses and marafiviruses. The ~28 nm virus particles have the EM structure typical of the *Tymoviridae* and separate into typical top components containing the sgRNA and infectious bottom components. Like tymoviruses, PnMV is produced in high yield during infection, is not phloem-limited, and can be mechanically transmitted in the laboratory. Replication is associated with chloroplast cytopathy, though not with the appearance of the replication-associated vesicles typical of tymovirus infection. The properties and coding arrangement of the 6.1 kb RNA are more closely aligned with those of the marafiviruses (**Figure 2**). The genomic RNA is polyadenylated, contains a marafibox-related putative sgRNA promoter, and possesses a single long ORF that encodes an RP/CP fusion protein (p221). No OP ORF is present. The 21–24 kDa PnMV CP is presumably expressed from the 0.65 kbp sgRNA; the involvement of potential cleavage of the RP/CP fusion protein to produce a second CP variant is not known. Although PnMV sequences are more closely related to the marafiviruses than tymoviruses, the relationships appear to be insufficiently close to warrant inclusion in the genus *Marafivirus* (**Figure 3**).

In commercial poinsettia cultivation, PnMV is transmitted by vegetative propagation; the virus is not transmissible via seed or pollen. The natural host range is restricted to *Euphorbia* sp., especially poinsettia, *E. pulcherrima*. Symptoms appear seasonally, varying from inapparent to light mottling. PnMV is often associated with poinsettia cryptic virus (family *Partitiviridae*) and with a phytoplasma that is responsible for the desirable free-branching phenotype.

See also: Flexiviruses.

Further Reading

Bradel BG, Preil W, and Jeske H (2000) Sequence analysis and genome organisation of poinsettia mosaic virus (PnMV) reveal closer relationship to marafiviruses than to tymoviruses. *Virology* 271: 289–297.

Canady MA, Larson SB, Day J, and McPherson A (1996) Crystal structure of turnip yellow mosaic virus. *Nature Structure Biology* 3: 771–781.

Ding SW, Howe J, Keese P, *et al.* (1990) The tymobox, a sequence shared by most tymoviruses: Its use in molecular studies of tymoviruses. *Nucleic Acids Research* 18: 1181–1187.

Dreher TW (2004) *Turnip yellow mosaic virus*: Transfer RNA mimicry, chloroplasts and a C-rich genome. *Molecular Plant Pathology* 5: 367–375.

Edwards MC, Zhang Z, and Weiland JJ (1997) Oat blue dwarf marafivirus resembles the tymoviruses in sequence, genome organization, and expression strategy. *Virology* 232: 217–229.

Francki RIB, Milne RG, and Hatta T (eds.) (1985) *Atlas of Plant Viruses, Vol. I.* Boca Raton, FL: CRC Press.

Hirth L and Givord L (1988) Tymoviruses. In: Koenig R (ed.) *The Plant Viruses*, pp. 163–212. New York: Plenum.

Jakubiec A, Notaise J, Tournier V, *et al.* (2004) Assembly of *Turnip yellow mosaic virus* replication complexes: Interaction between the proteinase and polymerase domains of the replication proteins. *Journal of Virology* 78: 7945–7957.

Kadaré G, Rozanov M, and Haenni A-L (1995) Expression of the turnip yellow mosaic virus proteinase in *Escherichia coli* and determination of the cleavage site within the 206 kDa protein. *Journal of General Virology* 76: 2853–2857.

Koenig R, Pleij CW, Lesemann DE, Loss S, and Vetten HJ (2005) Molecular characterization of isolates of anagyris vein yellowing virus, plantago mottle virus and scrophularia mottle virus: Comparison of various approaches for tymovirus classification. *Archives of Virology* 150: 2325–2338.

Maccheroni W, Alegria MC, Greggio CC, *et al.* (2005) Identification and genomic characterization of a new virus (*Tymoviridae* family) associated with citrus sudden death disease. *Journal of Virology* 79: 3028–3037.

Martelli GP, Sabanadzovic S, Abou-Ghanem Sabanadzovic N, Edwards MC, and Dreher T (2002) The family *Tymoviridae*. *Archives of Virology* 147: 1837–1846.

Mayo MA, Dreher TW, and Haenni A-L (2000) Genus *Tymovirus* In: van Regenmortal MHV, Fauquet CM, Bishop DHL, *et al*. (eds.) *Seventh Report of the International Committee on Taxonomy of Viruses.* New York: Academic Press

Morch MD, Boyer JC, and Haenni A-L (1988) Overlapping open reading frames revealed by complete nucleotide sequencing of turnip yellow mosaic virus genomic RNA. *Nucleic Acids Research* 16: 6157–6173.

Sabanadzovic S, Ghanem-Sabanadzovic NA, Saldarelli P, and Martelli GP (2001) Complete nucleotide sequence and genome organization of grapevine fleck virus. *Journal of General Virology* 82: 2009–2015.

Weiland JJ and Dreher TW (1989) Infectious TYMV RNA from cloned cDNA: Effects *in vitro* and *in vivo* of point substitutions in the initiation codons of two extensively overlapping ORFs. *Nucleic Acids Research* 17: 4675–4687.

U

Umbravirus

M Taliansky, Scottish Crop Research Institute, Dundee, UK
E Ryabov, University of Warwick, Warwick, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Fibrillarin An abundant nucleolar protein that participates in the processing and modification of rRNAs and a methyltransferase.
Nucleolus A prominent subnuclear domain and the site of transcription of ribosomal RNA (rRNA), processing of the pre-rRNAs, and biogenesis of pre-ribosomal particles, and also participates in other aspects of RNA processing and cell function.
Phloem Plant vascular system used for rapid long-distance transport of assimilates and macromolecules.
Plasmodesmata Plant-unique intercellular membranous channels that span plant cell walls linking the cytoplasm of adjacent cells.

Introduction

Umbraviruses differ from other plant viruses in that they do not encode a coat protein (CP) and, thus no conventional virus particles are formed in infected plants. The name of the genus *Umbravirus* is derived from the Latin *umbra*, which means a shadow or an uninvited guest who comes with an invited one. This name reflects the way in which umbraviruses depend for survival in nature on an assistor virus, which is always a member of the family *Luteoviridae*. For transmission between plants, the CP of the luteovirus forms aphid-transmissible hybrid virus particles encapsidating umbraviral RNA. In nature, each umbravirus is associated with one particular luteovirus, although in experiments transcapsidation is not needed for umbravirus accumulation within infected plants because functions such as protection and movement of the virus RNA do not require the presence of the luteovirus or its CP. Moreover, under experimental conditions, mechanical transmission of umbraviruses can take place without the aid of an assistor virus. This implies that umbraviruses encode some product(s) that functionally compensate for the lack of a CP (see below).

Currently the genus *Umbravirus* includes seven distinct virus species: *Carrot mottle virus* (CMoV), *Carrot mottle mimic virus* (CMoMV), *Groundnut rosette virus* (GRV), *Lettuce speckles mottle virus* (LSMV), *Pea enation mosaic virus-2* (PEMV-2), *Tobacco mottle virus* (TMoV), and *Tobacco bushy top virus* (TBTV). These viruses together with the tentative members of the genus *Umbravirus* are listed in **Table 1**. Some of these viruses have been known since the early days of plant virology. The first to be described was TMoV, reported from Zimbabwe and Malawi in 1945. The most economically important umbravirus is GRV, which is endemic throughout sub-Saharan Africa. Sporadic, unpredictable outbreaks of groundnut rosette disease (actually caused by a satellite RNA of GRV) cause severe crop losses. Pea enation mosaic disease outbreaks are also sporadic and localized, but losses of nearly 90% in peas and up to 50% in field beans have been reported. One or both of the carrot-infecting umbraviruses probably occurs worldwide wherever carrots are grown, but they are uncommon in commercial crops because the insecticides used to control carrot fly also control the aphid vectors of CMoV and CMoMV. LSMV is reported only from California, USA.

Virus Properties

Although no virions are formed in plants infected with umbraviruses unaccompanied by the assistor virus, the infectivity of CMoV and GRV in buffer extracts of infected leaves is surprisingly stable: infectivity remains for several hours at room temperature and is resistant to treatment with ribonuclease. Infectivity is, however, sensitive to a treatment with organic solvents suggesting that lipid-containing structures are involved in the protection of umbravirus RNA.

Table 1 Virus members of the genus *Umbravirus*

Virus	*Abbreviation*	*Sequence*	*Accession no.*	*Assistor virus*
Carrot mottle mimic virus	(CMoMV)	Complete genomic RNA	U57305	Carrot red leaf virus (CaRLV)
Carrot mottle virus	(CMoV)			Carrot red leaf virus (CaRLV)
Groundnut rosette virus	(GRV)	Complete genomic RNA	Z69910	Groundnut rosette assistor virus (GRAV)
		Satellite RNAs	Z29702–Z29711	
Lettuce speckles mottle virus	(LSMV)			Beet western yellows virus (BWYV)
Pea enation mosaic virus-2	(PEMV-2)	Complete genomic RNA	U03563	Pea enation mosaic virus-1 (PEMV-1)
		Satellite RNA	U03564	
Tobacco bushy top virus	(TBTV)	Complete genomic RNA	AF402620	Unknown
Tobacco mottle virus	(TMoV)	Partial genomic RNA	AY007231	Tobacco vein distorting virus (TVDV)
Tentative members in the genus				
Sunflower crinkle virus	(SuCV)			Unknown
Sunflower yellow blotch virus	(SuYBV)			Unknown
Tobacco yellow vein virus	(TYVV)			Tobacco yellow vein assistor virus (TYVAV)

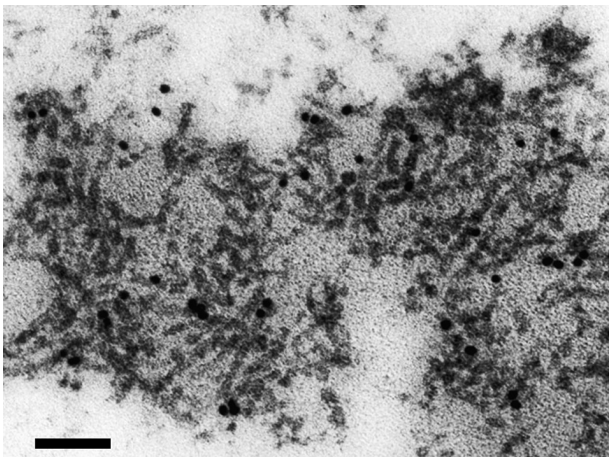


Figure 1 Electron microphotograph of a section of a *Nicotiana benthamiana* cell expressing GRV ORF3 protein. The section shows a complex of filamentous RNP particles embedded in an electron-dense matrix. The section was labeled by *in situ* hybridization with an RNA probe specific for viral RNA. Scale = 100 μm.

Indeed, in plants infected with a number of umbraviruses, including CMoV, enveloped structures ~50 nm in diameter were observed. However, an infective fraction from GRV-infected tissue contained complexes with a buoyant density of 1.34–1.45 g cm^{-3} consisting of filamentous ribonucleoprotein (RNP) particles, composed of the umbraviral ORF3 protein (see below) and virus RNA, embedded in a matrix (**Figure 1**). The relationship between the enveloped and filamentous structures is unclear.

Genome Organization and Expression

The genome of umbraviruses consists of a single linear segment of positive-sense, single-stranded RNA (ssRNA) (**Figure 2**). The complete genomic RNA sequences of CMoMV, GRV, PEMV-2, and TBTV are comprised of 4201, 4019, 4253, and 4152 nt, respectively. There is no polyadenylation at their 3′ termini and there is no information about modifications of their 5′ termini. At the 5′ end, a very short noncoding sequence precedes ORF1, which encodes a putative 31–37 kDa protein. ORF2, which overlaps the 3′ end of ORF1, potentially encodes a 63–65 kDa protein, but lacks an AUG initiation codon. It is likely that ORF1 and ORF2 are translated as a single 94–98 kDa protein by a −1 translational frameshifting mechanism; the sequence associated with frameshifting events in several animal and plant viruses is found in the region at the 3′ end of the ORF1. The predicted amino acid sequence of the ORF2-encoded product has similarities with the sequences of RNA-dependent RNA polymerases (RdRps) of viruses in the families *Tombusviridae* and *Luteoviridae* and contains all eight conserved motifs of RdRp of positive-strand RNA viruses. A short untranslated stretch of nucleotides separates ORF2 from ORF3 and ORF4, both of which almost completely overlap in different reading frames and encode 26–29 kDa products. The ORF4 contains motifs characteristic of the cell-to-cell movement proteins (MPs) of plant viruses, in particular those of cucumoviruses. The ORF3 products from different umbraviruses possess up to 50% homology to each other but show no

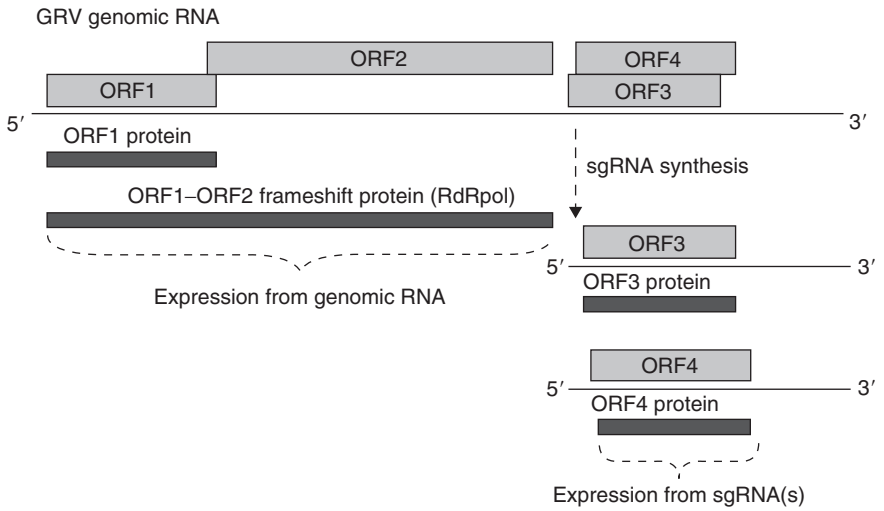


Figure 2 Organization and expression strategy of the GRV genome. Translation of the ORF1 and the ORF2 with a frameshift event results in production of the ORF1–ORF2 frameshift protein; subgenomic RNA(s) (sgRNA(s)) synthesis is required for expression of the ORF3 and ORF4. Solid lines represent RNA molecules; gray boxes represent open reading frames (ORFs); black boxes represent translation products.

significant similarity to any other viral or nonviral product. The ORF3 and OFR4 products are likely to be translated from subgenomic RNA(s) – 3′ terminal RNAs of the appropriate size were detected in GRV-infected plant tissue. It should be pointed out that the sequenced genomes of umbraviruses do not encode a potential CP(s). The essential role of the ORF3–ORF4 block in movement of umbraviruses within plants has been experimentally demonstrated and is discussed below.

Replication

Replication of umbravirus RNA presumably involves the ORF1/ORF2-encoded RdRp. Leaves of plants infected with umbraviruses contain abundant double-stranded RNA (dsRNA) including a major species of about 4.4–4.8 kbp corresponding in size to that expected for a double-stranded form of the viral genomic RNA which may be an umbravirus RNA replication intermediate. No other details of the replication mechanism have been elucidated.

Satellite RNA

Satellite RNAs are associated with some umbraviruses. In the case of GRV, satellite RNA is found in all naturally occurring isolates, and is primarily responsible for the symptoms of groundnut rosette disease. GRV satellite RNA is an ssRNA of about 900 nt which relies on GRV for its replication and, more unusually, is also required for the groundnut rosette assistor virus (GRAV)-dependent aphid transmission of GRV. Thus, unlike most virus satellite RNAs, it is essential for the biological survival (though not the replication) of its helper virus. The role of the satellite RNA in the transmission process is to mediate transcapsidation of GRV RNA by GRAV protein to form stable aphid-transmissible hybrid virus particles. Although different GRV satellite RNA variants contain up to five potential ORFs, none of the ORFs is essential for any of the functions and biological properties that have been ascribed to GRV satellites. In contrast, the satellite RNA that is associated with some isolates of PEMV-2 is not required for transcapsidation of PEMV-2 RNA by the CP of its assistor virus PEMV-1 or for aphid transmission of the hybrid particles, and other umbraviruses, such as CMoV, do not have satellite RNAs, yet are transcapsidated by their assistors and thus transmitted by aphids. The reasons for these differences have not been explained.

Cell-to-Cell Movement Function

The highly conserved ORF4-encoded proteins of umbraviruses exhibit significant sequence similarity with cell-to-cell MPs of other plant viruses, in particular the 3a proteins of cucumoviruses. Therefore, it has been suggested that this protein is involved in cell-to-cell trafficking of umbravirus RNA through plasmodesmata. This suggestion has been confirmed by a number of genetic, cytological, and biochemical approaches. By using the gene replacement strategy, it was demonstrated that that the GRV ORF4 protein could functionally replace the MPs of unrelated viruses, Potato virus X (PVX) (all the products encoded by the triple gene block and the CP) and cucumber mosaic virus (CMV) (the 3a MP and the CP). Localization of the GRV ORF4 protein has much in common with localization of MPs of other plant virus groups. The green fluorescent protein (GFP)-tagged GRV ORF4 protein targeted to

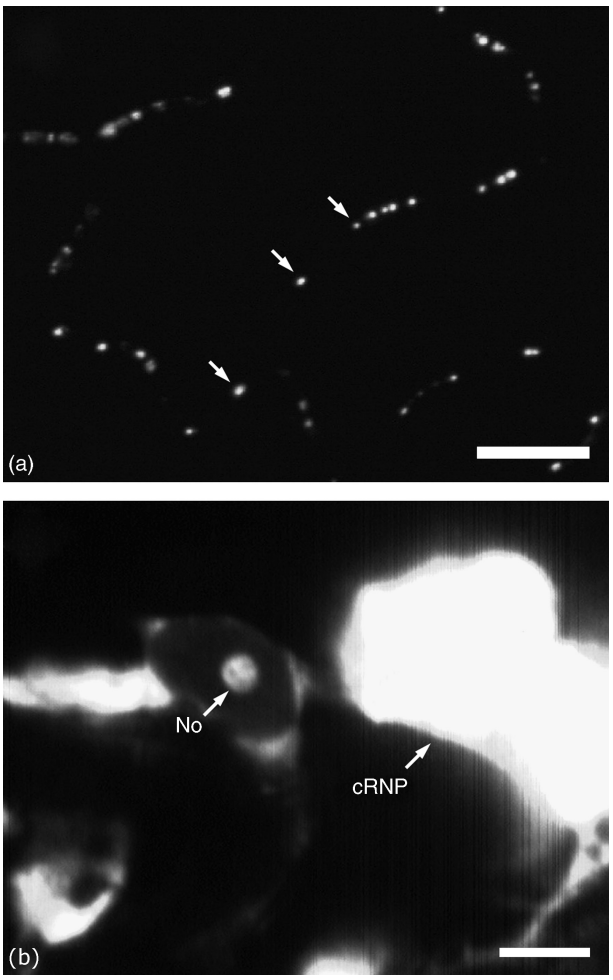


Figure 3 Confocal laser scanning images showing the localization of (a) GRV ORF4 protein fused to GFP to plasmodesmata (shown by arrows) and (b) GRV ORF3 protein fused to GFP to the nucleolus (No) and cytoplasmic inclusions containing ORF3 protein RNP particles (cRNP). Scale = 25 μm (a), 10 μm (b).

plasmodesmata (**Figure 3**(**a**)). Also, this protein formed extended tubular structures on the surface of protoplasts infected either with GRV or with the heterologous virus expressing the GFP-tagged GRV ORF4. The GRV ORF4 protein binds to RNA *in vitro* in a noncooperative manner which may make the viral RNA of the RNP complex accessible for translation and replication.

Phloem-Dependent Long-Distance Movement Function

One of the striking features of umbraviruses is their ability to move long distance within the plant without having a conventional CP. Involvement of CPs in long-distance movement of viral infection has been shown for a number of plant viruses, including tobacco mosaic virus (TMV) which utilizes the CP exclusively for its long-distance but not cell-to-cell movement function. By using the gene replacement strategy it was demonstrated that the ORF3 proteins of GRV, PEMV-2, and ToMV were able to functionally substitute for the TMV CP in the long-distance movement process. The hybrid TMV mutants expressing the umbraviral ORF3 proteins were able to move rapidly through the phloem. It was also shown that specific mutations in the ORF3 protein of PEMV-2 abolish the ability of this virus to move long distance without affecting its cell-to-cell spread within inoculated leaves.

It should be noted that the mechanisms of the long-distance movement facilitated by umbraviral ORF3 proteins is different from those mediated by suppressors of RNA silencing such as the 2b protein of CMV and the HC-Pro protein of potyviruses. Rather than supporting virus infection by suppressing the host RNA-mediated response, the ORF3 protein seems to protect RNA by binding to viral RNA. It was shown that the ORF3 proteins of GRV, PEMV-2, and ToMV increase the stability of viral RNA and protect it from RNases. Immunogold-labeling and *in situ* hybridization experiments showed that the GRV ORF3 protein accumulated in cytoplasmic granules consisting of filamentous RNP particles composed of the ORF3 protein and viral RNA (**Figure 1**). It has been suggested that these particles may be a form in which viral RNA moves long-distance through the phloem. Also the ORF3-containing RNP complex may protect viral RNA from nucleases and the RNA silencing machinery.

Involvement of the Nucleolus in Umbravirus Systemic Infection

In addition to cytoplasmic granules, the GRV ORF3 protein was also found in nuclei, predominantly targeting nucleoli (**Figure 3**(**b**)). Sequence analysis of the ORF3 proteins of umbraviruses revealed the presence of both a nuclear localization signal (NLS) and a nuclear export signal (NES), the functional roles of which in nuclear import and export of the GRV ORF3 has been confirmed experimentally by genetic analysis. Functional analysis of ORF3 protein mutants revealed a correlation between the ORF3 protein nucleolar localization and its ability to form RNP particles and transport viral RNA long distances. It was also shown that the ORF3 protein interacts with a nucleolar protein, fibrillarin, redistributing it from the nucleolus to the cytoplasm, and that such an interaction is absolutely essential for umbravirus long-distance movement through the phloem.

Interaction with Assistor Virus

Although umbraviruses accumulate and spread very efficiently within infected plants, they depend on the

assistance of luteoviruses for their survival in nature, as they require encapsidation by luteoviral CP for horizontal transmission by aphids. In turn, umbraviruses facilitate the movement of phloem-limited luteoviruses to and from the phloem, as well as the cell-to-cell movement of luteoviruses between mesophyll and epidermal cells. It was shown that the ability to promote cell-to-cell movement of luteoviruses is a unique feature of the umbraviral ORF4 MP. Moreover, the ORF4 MP of GRV can facilitate cell-to-cell movement of *Potato leafroll virus* even when it is expressed from heterologous PVX or CMV genomes. In most instances, the luteovirus partner does not depend on the umbravirus infection for its survival in nature. A complex consisting of PEMV-1 (the genus *Enamovirus*, family *Luteoviridae*) and PEMV-2 is a notable exception. Unlike other members of the family *Luteoviridae*, PEMV-1 on its own lacks the ability to move, even through the phloem; both long-distance and cell-to-cell movement functions of PEMV-1 are provided by the umbraviral component of the complex, PEMV-2. Such a strong mutual dependence and adaptation between umbraviruses and luteoviruses suggest a long co-evolution, which has resulted in establishing a range of interactions from facultative coexistence (GRV/GRAV) to complete dependence (PEMV-1/PEMV-2).

Similarity with Other Taxa

Amino acid sequence comparisons showed that the putative RdRp encoded by CMoMV, GRV, PEMV-2, and TBTV belong to the so-called supergroup 2 of RNA polymerases, as do those of viruses in the genera *Carmovirus, Necrovirus, Machlomovirus,* and *Tombusvirus*. Since these enzymes are the only universally conserved proteins of positive-strand RNA viruses, the genus *Umbravirus* might be considered to be in or close to the family *Tombusviridae*.

Host Range

Individual umbraviruses are confined in nature to one or two host plant species. For example, groundnut is the only known natural host of GRV and the entire rosette disease complex (GRV, its satellite RNA and GRAV). Experimental host ranges of umbraviruses are broader but still restricted. They usually induce a leaf mottle and/or mosaic in infected plants.

Transmission

In nature, umbraviruses are transmitted by aphids, but only from plants that are infected also with an assistor luteovirus. The mechanism of the dependent transmission is the encapsidation of the umbraviral RNA by the CP provided by the assistor virus. Hence, the transmission of the dependent umbravirus occurs in the same persistent (circulative, nonpropagative) manner as that of the assistor-luteovirus.

Prevention and Control

For the avoidance of pea enation mosaic disease, it is recommended that pea or faba bean crops should be sited away from alfalfa and clover fields. Early sowing and close spacing can reduce groundnut rosette disease incidence, probably by inhibiting the landing response of the vector. This approach is also effective against tobacco rosette disease.

However, the best control approach is to use resistant cultivars if they are available. Resistance to GRV controlled by two independent recessive genes has been found and groundnut lines possessing this resistance have been developed.

A possibility for the future is the deployment of transgenic forms of resistance. Strategies for engineering transgenic resistance against umbraviruses include the transformation of plants with translatable or nontranslatable sequences from the umbraviruses themselves or their satellite RNAs.

See also: Luteoviruses; Plant Virus Vectors (Gene Expression Systems).

Further Reading

Demler SA, Borkhsenious ON, Rucker DG, and de Zoeten GA (1994) Assessment of the autonomy of replicative and structural functions encoded by the luteo-phase of pea enation mosaic virus. *Journal of General Virology* 75: 997–1007.

Kim SH, Ryabov EV, Brown JW, and Taliansky M (2004) Involvement of the nucleolus in plant virus systemic infection. *Biochemical Society Transactions* 32: 557–560.

Nurkiyanova KN, Ryabov EV, Kalinina NO, *et al.* (2001) Umbravirus-encoded movement protein induces tubule formation on the surface of protoplasts and binds RNA incompletely and noncooperative. *Journal of General Virology* 82: 2579–2588.

Ryabov EV, Robinson DJ, and Taliansky ME (1999) A plant virus-encoded protein facilitates long distance movement of heterologous viral RNA. *Proceedings of the National Academy of Sciences, USA* 96: 1212–1217.

Ryabov EV, Robinson DJ, and Taliansky M (2001) Umbravirus-encoded proteins that both stabilize heterologous viral RNA *in vivo* and mediate its systemic movement in some plant species. *Virology* 288: 391–400.

Taliansky M, Roberts IM, Kalinina N, *et al.* (2003) An umbraviral protein, involved in long-distance RNA movement, binds viral RNA and forms unique, protective ribonucleoprotein complexes. *Journal of Virology* 77: 3031–3040.

Taliansky ME and Robinson DJ (2003) Molecular biology of umbraviruses: Phantom warriors. *Journal of General Virology* 84: 1951–1960.

Ustilago Maydis Viruses

J Bruenn, State University of New York, Buffalo, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Kex2p. Kexin, furin A membrane-bound proteinase in the Golgi that processes some secreted proteins in many eukaryotes.

Ustilago maydis Smut fungus. A plant pathogen infecting maize, wheat, oats, and barley that can adopt either yeast or mycelial form.

Introduction

The *Ustilago maydis* viruses belong to the genus *Totivirus* within the family *Totiviridae*. They are a related group of viruses that, like many fungal and protozoan viruses, exist as permanent passengers in their host cells, to which they are not deleterious. These are double-stranded RNA (dsRNA) viruses with similar structure and life cycle, derived from a very ancient ancestor but still possessing recognizable sequence similarities in their common protein, the viral RNA-dependent RNA polymerase (RdRp). They are not naturally infectious and are passed from cell to cell by meiosis or mitosis, although they will, inefficiently, infect protoplasts. In some cases, they provide a selective advantage to the host, encoding cellular toxins that kill cells of the same or related species that lack the virus. These killer toxins are apparently derived from cellular genes that have been co-opted by resident viruses. In some cases (as discussed below) cellular ancestors of these toxins still exist. When multiple species of genomic and satellite dsRNA exist in the fungal and protozoan dsRNA viruses, they are separately encapsidated. This is no disadvantage to viruses that do not depend on an infectious cycle for propagation.

Taxonomy

The fungal and protozoan dsRNA viruses generally fall into three groups: the totiviruses, the chrysoviruses, and the partitiviruses, all of which are closely related as judged by the sequence of their RdRps (**Figure 1**). In this scheme, only the giardiaviruses (which are presently classified as a separate genus within the family *Totiviridae*) are, rather inappropriately, marooned between the chrysoviruses and the partitiviruses. However, the *Ustilago maydis* viruses are placed closest to the *Saccharomyces* viruses, with which they have much in common. The three different *Ustilago maydis* viruses (UmVP1H1, UmVP6H1, and UmVP1H2) for which sequence information exists are more closely related to each other than to any other fungal viruses. This is now a common phenomenon among the fungal viruses, in which several similar dsRNA viruses may share the same host cell: ScV-L1 and ScV-La (*Saccharomyces cerevisiae*), DdV1 and DdV2 (*Discula destructiva*), and GaV-L1 and GaV-L2 (*Gremmeniella abietina*). However, there are cases in which two unrelated dsRNA viruses occupy the same cell (HvV145S and HvV190S; SsV1 and SsV2). Aside from the similarities in RdRp sequence, the *Ustilago maydis* and *Saccharomyces* viruses share a number of other characteristics.

Genome Structure

The totiviruses are dsRNA viruses with a single defining property: a single segment of dsRNA encodes all necessary

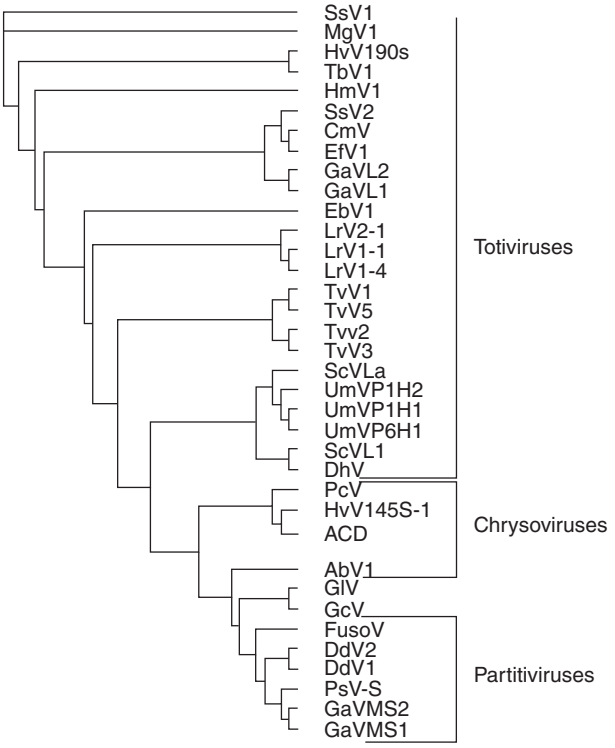


Figure 1 Cladogram of fungal and protozoan dsRNA virus RdRps. For clarity, statistics have been omitted, but each branch is supported by at least 500/1000 bootstraps. This is a Treeview rendering of a Clustal X alignment of complete RdRp sequences. Only a few of the known partitiviruses are shown due to space limitations.

viral functions. All RNA viruses of eukaryotes have the same problem of expression: eukaryotic ribosomes generally initiate only in one place on an mRNA. Encoding more than one protein on a single RNA requires some method of obviating this limitation. The totiviruses have adopted a number of strategies for this purpose: programmed ribosomal frameshifting (ScV, GLV, LRV, TVV), stop and go translation, using overlapping termination and initiation codons (HvV190S, CmRV or CmV), internal initiation (SsV or SsRV), and proteolytic processing (UmV).

The UmV dsRNAs fall into three categories: H (heavy), M (medium), and L (light). The H segments that have been partially or completely sequenced code for viral capsid and RdRp polypeptides. The M segments encode secreted polypeptides, some of which are toxins that kill susceptible cells (killer toxins). The L segments have no significant open reading frames, and are exact copies of the 3′ portions of M plus strands.

The one UmV H segment entirely sequenced (P1H1) has a single open reading frame encoding a polypeptide of 1820 amino acids with an N-terminal capsid and a C-terminal RdRp sequence. There is a putative papain-like protease between the capsid and RdRp domains, so that generation of the capsid polypeptide appears to occur by endoproteolytic cleavage. The proportion of cap and cap-pol fusion protein in ScV is quite critical for capsid assembly; too much of either results in loss of the virus. If this is the case in UmV, the proportions must be controlled by the efficiency of endoproteolytic cleavage; with some low but finite probability, the protease must fail, generating a fusion protein containing the RdRp domain. Alternatively, the RdRp domain might be packaged as a single domain, as it must be in the partitiviruses. In either case, this is a rather sloppy way of doing things, since a large proportion of the C-terminal domain must be discarded as useless. However, it is no more wasteful than the processing of the poliovirus polyprotein.

There are three main isolates of *Ustilago maydis* whose viral dsRNAs have been characterized – P1, P4, and P6, named after the killer toxins that some viral segments encode (see further ahead). Some isolates have only three viral dsRNAs (H1, M1, L1), while others may have as many as seven segments. A summary of the known coding regions of these dsRNAs is shown in **Table 1**. In all cases, only the viral plus strand has an open reading frame, and the predicted protein products have been demonstrated by *in vitro* protein synthesis, and/or by isolation *in vivo*. Presumably all totivirus mRNAs, like their genomic plus strands, are uncapped and lack polyA. This has only been directly demonstrated for ScV.

The *Ustilago maydis* Killer Toxins

The KP1, KP4, and KP6 toxins are encoded by P1M2, P4M2, and P6M1. The KP4 toxin is the best characterized of these. The KP4 toxin is synthesized as a 127-amino-acid preprotoxin from which the amino-terminal 22 amino acids are removed by signal peptidase during secretion. The resulting toxin is an unglycosylated peptide of 105 amino acids and 11.1 kDa. The KP4 toxin is the only killer toxin known to be not processed by Kex2p. Its three-dimensional structure is known (**Figure 2**). It is a monomer with five disulfides stabilizing a structure with seven beta-strands and three alpha-helices, consisting of a single beta-sheet with five antiparallel strands and two antiparallel alpha-helices at about a 45° angle to the beta-strands. Its structure is somewhat similar to that of the SMK toxin, even though the latter consists of a heterodimer. The SMK toxin is encoded by the nuclear genome of *Pichia farinosa*, rather than by a viral dsRNA, and it has essentially no primary structure similarity to the KP4 toxin. SMK is synthesized as a preprotoxin which is proteolytically processed to produce the heterodimer, so we can view the KP4 toxin as a preprotoxin that has lost

Table 1 Protein products of the UmV dsRNAs

dsRNA	*Length (bp)*	*Product*	*Length (aa)*	*Cleavage*	*Function*
P1H1	6099	cap-pol	1820	None	RdRp
		cap	?	Endoprotease	capsid
P1M1	1504	prepropp	256		prepropp
		P1M1 alpha	103	sp, Kex2p	?
		P1M1 beta	83	Kex2p	?
P1M2	1034	KP1 pptoxin	291		pptoxin
		KP1 alpha	120	sp, Kex2p	?
		KP1 beta	117	Kex2p	KP1 toxin
P4M2	1006	KP4 pptoxin	128		pptoxin
		KP4 toxin	105	sp	KP4 toxin
P6M2	1234	KP6 pptoxin	219		pptoxin
		KP6 alpha	79	sp, Kex2p	KP6 alpha
		KP6 beta	81	Kex2p	KP6 beta

RdRp, RNA-dependent RNA polymerase; Prepropp, prepropolypeptide; sp, signal peptidase; Kex2p, Kex2p secretory proteinase; pptoxin, preprotoxin; ?, unknown.

its Kex2p sites (or SMK as a preprotoxin that has gained Kex2p sites).

The latter interpretation seems more likely, because there are other fungal proteins encoded by nuclear genomes that are highly similar to the KP4 toxin (**Figure 3**). The two proteins shown aligned with the KP4 toxin are from *Aspergillus oryzae* (BAE57860) and from *Gibberella zeae* (XP_380238). These are both predicted proteins without known function. However, both are predicted (by the SignalP 3.0 Server) to be secreted proteins, and their mature peptides are predicted to begin with the same amino-terminal amino acid sequence as the KP4 toxin, LGINCR, or with an extra amino-terminal K (in the *A. oryzae* peptide). None of these proteins has predicted Kex2p cleavage sites (xyKR, xyRR, or xyPR, where x is hydrophobic and y is anything), and the KP4 toxin is known to be processed only by signal peptidase, alone among fungal killer toxins. Five of the ten cysteines in the KP4 toxin are conserved in both of these proteins, which share 25% and 36% sequence identity with the KP4 toxin, and a sixth cysteine may also be shared. The *A. oryzae* peptide has eight total cysteines and the *G. zeae* peptide 9. Hence each would have to be missing at least one of the five disulfides present in the KP4 toxin, and if they shared the KP4 structure would have to be stabilized by other interstrand interactions. K42 is known to be critical for KP4 toxin function, but this residue is not in a region conserved in the three proteins (**Figure 3**). The SMK toxin, with a structure somewhat similar to that of KP4, has only four disulfides. The striking similarity of these three proteins, all secreted, all lacking any N-linked sites for glycosylation, all processed only by signal peptidase, implies that the KP4 toxin was captured by the virus from a cellular transcript, since the *G. zeae* peptide, the *A. oryzae* peptide, and SMK are all encoded by nuclear genes. This evolutionary process is more obvious with the ScV k1 and k2 toxins, whose viral genes preserve the polyA they derived from the 3′ end of cellular messengers as internal portions of the viral plus strands.

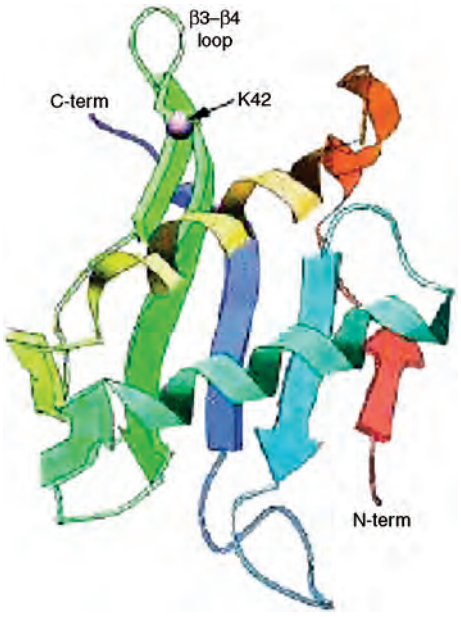


Figure 2 Structure of the KP4 toxin shown as a ribbon rendering. Figure courtesy of Tom Smith.

The KP4 toxin is the only one of the UmV killer toxins about which we have some knowledge of mode of action. The KP4 toxin interferes with the function of calcium channels both in susceptible fungal cells and in mammalian cells. Calcium transport and signaling in fungi are now known to be necessary for many cellular processes and to involve the products of dozens of genes. The fungal calcium channel consists of a typical alpha-subunit (encoded by CCH1 in *S. cerevisiae*) and at least one more polypeptide (encoded by MID1 in *S. cerevisiae*). Presumably, the target for KP4 is located in the cell wall or cell membrane, since its effects are reversible, but it need not be the calcium channel itself. The SMK toxin is known to target a cell membrane ATPase in *S. cerevisiae*, which is susceptible to the SMK toxin but not to KP4. KP4 seems to act by interfering with calcium-regulated signal transduction pathways, thereby preventing cell growth and division. Resistance to KP4, which is encoded by a nuclear gene, might involve an alteration of any component of these signal transduction pathways.

The KP6 toxin is composed of two polypeptides, KP6-alpha and-beta, which are processed from a single precursor protein (a prepropolypeptide) of 219 amino acids encoded on P6M2. The preprotoxin is cleaved by

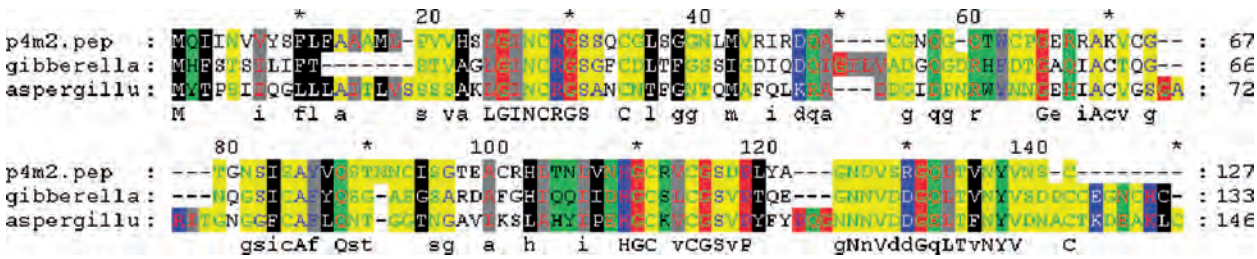


Figure 3 Comparison of KP4 (p4m2.pep) with cellular homologs. This is a Gendoc rendering of a Clustal X alignment showing physical–chemical similarities (color) and similarity (small letters in consensus line) or identity (capital letters in consensus line).

signal peptidase after residue 19; by Kex2p cleavages after residues 27, 108, and 138; and by Kex1p cleavage after residue 137 to yield two polypeptides, alpha of 79 amino acids (8.6 kDa) and beta of 81 amino acids (9.1 kDa). Alpha and beta are not necessarily found as a multimer and can be added separately to susceptible cells to reconstitute killer activity; both are necessary for activity. Unlike the KP4 toxin, KP6 has irreversible effects on susceptible cells, actually killing cells rather than inhibiting growth. The structure of KP6-alpha is known (**Figure 4**). The monomer is a four-stranded antiparallel beta-sheet with two alpha-helices on one side, a small alpha-helix at the N-terminus, and a separate beta-strand, with four disulfide bonds, involving all eight cysteines in the polypeptide. In the crystal, the polypeptide forms trimers held together by salt bridges, and the trimers sit on each other forming an hour-glass shape, suggestive of an ion pore. However, expression of the KP6-beta peptide alone in susceptible cells is lethal, suggesting a mode of action more like that of colicins or diphtheria toxin, in which one of the toxin polypeptides is introduced into the cell by the action of one or more other toxin polypeptides and acts lethally within the cell. KP6-beta also has six cysteines, suggesting a structure with three disulfides. The two monomer nature of the KP6 toxin is reminiscent of the *S. cerevisiae* k1 toxin, which also has two monomers derived from a single prepropolypeptide by signal peptidase, Kex2p, and Kex1p processing. However, in the k1 toxin, the alpha and beta monomers are covalently bound by intermolecular disulfides, which are absent in KP6. The toxin is properly processed and secreted from *S. cerevisiae*, although there are some differences; none of the *Ustilago maydis* killer toxins (or other secreted proteins, apparently) are glycosylated, and processing of mutant precursors is different in yeast and *Ustilago maydis*. The KP6-alpha polypeptide has one possible site for N-linked glycosylation, which is used in yeast but not in *Ustilago maydis*. Unlike the KP4 toxin, the KP6 toxin polypeptides have no significant similarity to any proteins currently in the databases. Resistance to the KP6 toxin maps to the nuclear genome, as with KP4.

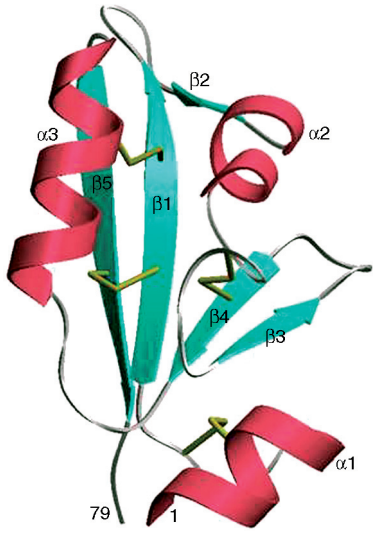


Figure 4 Structure of KP6-alpha shown as a ribbon rendering. Courtesy of the American Society for Biochemistry and Molecular Biology.

The KP1 toxin is encoded by P1M2 and translated as a preprotoxin of 291 amino acids, subsequently cleaved by signal peptidase and Kex2p to produce mature polypeptides alpha (120 amino acids) and beta (117 amino acids). Only beta is required for toxin activity. KP1-beta, like the other *Ustilago maydis* killer polypeptides, has an unusual number of cysteines (six) which, if all were involved in disulfides as in KP6-alpha and in KP4, would indicate three disulfides. P1M2 is different from the toxin encoding dsRNAs in UmVP4 and UmVP6 (P4M2 and P6M1). The 3′ noncoding regions of the plus strands of P4M2 and P6M1 are entirely homologous to the L segments in UmVP4 and UmVP6, respectively, while the L segment in UmVP1 is derived from P4M1. P4M1 does encode a prepropolypeptide, whose processed polypeptides are secreted but have no detectable relation to the KP1 toxin (**Table 1**). Nothing is presently known of the mechanism of action of the KP1 toxin, and none of the UmVP1M1 or UmvP1M2 polypeptides have any homologs in the databases. Resistance to the KP1 toxin may be nuclear or cytoplasmic, hinting that some immunity may result from expression of one or more viral dsRNAs, as with the *S. cerevisiae* k1 toxin.

Replication and Transcription

Like all dsRNA viruses, UmV has a capsid-associated transcriptase that makes the viral plus strands. However, little is known of the nature of the transcripts or the mechanism of transcription. One peculiarity of this system is the presence of dsRNAs (L, 354–355 bp) entirely homologous to the 3′ ends of the plus strands of their cognate dsRNAs (M). Presumably, like all viral dsRNAs, these are the result of replication of plus strands within nascent viral particles. It is not clear if the source of the L plus strands is internal transcription initiation or processing of M plus strands. Normal-sized L plus strands (with exactly the predicted 5′ ends) are produced either by the viral transcriptase or by RNAP II transcription of cDNA clones in *Ustilago maydis* or heterologous systems, implying that a cleavage event must be responsible for production of the L segments.

This cleavage must be either an inherent property of the cognate M plus strand or cleavage by a ubiquitous nuclease activity recognizing a universal feature of the M plus strands. No ribozyme activity can be demonstrated in the cognate M plus strands *in vitro*. However,

all the cognate M plus strands have a peculiar predicted secondary structure, in which the predicted L plus strand includes a very long predicted hairpin stem (23 perfect base pairs in P4M2) immediately following the predicted cleavage site (which is highly conserved). These should make ideal substrates for the recently described cellular machinery (dicer) responsible for siRNA production, which is ubiquitous in eukaryotes.

The function of the L segments is obscure: production of toxin proceeds perfectly well from cDNA constructs lacking the L encoding region, in either homologous or heterologous systems, and the P1M2 segment (encoding the KP1 toxin) has no cognate L segment. The L segments must include a packaging signal, but it is not clear what prevents its inclusion in an M plus strand sequence lacking the 3′ L sequence, a situation that does exist with P1M2. In the closely related virus ScV, a sequence as small as 18 bases serves as the packaging signal.

Transcription in dsRNA viruses may be either semiconservative (resulting in displacement of the parental plus strand) or conservative (*de novo* synthesis of plus strands). Transcription in reovirus and ScV is conservative and in bacteriophage phi6 is semiconservative. An interesting difference in the structure of the viral RdRp may be responsible for this. The phi6 RdRp has an insertion of some 20 amino acids within the N-terminal F motif, an insertion that is absent in the reovirus RdRp (and absent in all the other known RdRp and reverse transcriptase structures). None of the totivirus, chrysovirus, or partitivirus RdRps has a phi6-like insertion; most may therefore be conservative transcriptases. However, UmVP1M2 has been reported to be semiconservatively transcribed.

Viral Structure

Like other fungal viruses, UmV has a small icosohedral capsid of about 41 nm in diameter, with a sedimentation coefficient of 172S and a buoyant density in CsCl of $1.42\,g\,ml^{-1}$. All dsRNA viruses so far characterized in detail (bluetongue virus, or BTV; reovirus, or Reo; and ScV, the *S. cerevisiae* virus) are icosohedral with an inner (or complete) capsid structure of 120 copies of a capsid protein. This is a highly unusual arrangement in viruses, which generally behave according to the Caspar and Klug rules for assembly of icosohedral viruses, which call for the simplest capsids to consist of 60 copies of a single polypeptide ($T = 1$) or 180 copies of a single polypeptide ($T = 3$). However, the dsRNA viruses do not really violate this stricture, since they have 60 copies of a capsid polypeptide in one configuration and 60 copies in another, so that, in effect, they have 60 copies of an asymmetric dimer, in which the two monomers share portions with identical shape and portions with different shape. The *Ustilago maydis* viruses obey this generalization, although, like all the fungal and protozoan viruses, they have a single capsid shell, not two, as in BTV and Reo. One variation of this arrangement exists in the *Penicillium chrysogenum* virus (PcV), in which the two monomers are actually part of a single polypeptide chain, the two halves of which probably share regions of identical structure.

This unique capsid structure must play an important role in properly arranging the viral polymerase within the particle to allow transcription and replication of the genomic dsRNA. In ScV and UmV, this is accomplished by making the polymerase as the carboxy-terminal portion of a fusion protein in which the N-terminal is the capsid polypeptide. In ScV, two copies of this fusion protein are incorporated into each viral particle. Each viral particle has one copy of a single species of viral dsRNA. In UmV and ScV (as well as the other totiviruses), this single species encodes both capsid polypeptide and RdRp. When additional dsRNAs are present (e.g., toxin-encoding species), these are also separately encapsidated, and are considered satellite dsRNAs: they are completely dependent on the capsid/RdRp-encoding dsRNA for their transcription, replication, and packaging. Satellite dsRNAs may be present in more than one copy per virion. This is a considerably simpler strategy than pursued by the reoviruses, orbiviruses, and cystoviruses, all of which package multiple species of dsRNA in each viral particle and as many as 12 copies of the viral RdRp in each.

Genetic Engineering

The production of killer toxins by a plant pathogen (*Ustilago maydis*) has excited some interest in using the toxin to genetically engineer resistance to the fungus in its host crop plants. Since it is possible to produce both KP6 and KP4 toxins in heterologous systems using cDNA expression vectors, transgenic maize (KP6 and KP4) and wheat (KP4) plants have been constructed that express the toxins. These plants do secrete active killer toxins and exhibit some resistance to toxin-sensitive species of *Ustilago*. Since the toxins are harmless to animals when eaten, this may provide a new method of preventing the periodic smut infestations that occasionally decimate susceptible grain crops.

See also: Viral Killer Toxins; Yeast L-A Virus.

Further Reading

Bruenn J (2002) The double-stranded RNA viruses of *Ustilago maydis* and their killer toxins. In: Tavantzis SM (ed.) *dsRNA Genetic Elements. Concepts and Applications in Agriculture, Forestry, and Medicine*, pp. 109–124. Boca Raton, FL: CRC Press.

Bruenn J (2003) A structural and primary sequence comparison of the viral RNA dependent RNA polymerases. *Nucleic Acids Research* 31: 1821–1829.

Bruenn J (2004) The *Ustilago maydis* killer toxins. In: Schmitt MJ and Schaffrath R (eds.) *Microbial Protein Toxins*, pp. 157–174. Heidelberg: Springer.

Cheng RH, Caston JR, Wang G-J, *et al.* (1994) Fungal virus capsids: Cytoplasmic compartments for the replication of double-stranded RNA formed as icosahedral shells of asymmetric Gag dimers. *Journal of Molecular Biology* 244: 255–258.

Gage MJ, Rane SG, Hockerman GH, and Smith TJ (2002) The virally encoded fungal toxin KP4 specifically blocks L-type voltage-gated calcium channels. *Molecular Pharmacology* 61: 936–944.

Gu F, Khimani A, Rane S, Flurkey WH, Bozarth RF, and Smith TJ (1995) Structure and function of a virally encoded fungal toxin from *Ustilago maydis*: A fungal and mammalian Ca^{2+} channel inhibitor. *Structure* 3: 805–814.

Jiang D and Ghabrial SA (2004) Molecular characterization of Penicillium chrysogenum virus: reconsideration of the taxonomy of the genus Chrysovirus. *Journal of General Virology* 85: 2111–2121.

Kang J, Wu J, Bruenn JA, and Park C (2001) The H1 double-stranded RNA genome of *Ustilago maydis* virus-H1 encodes a polyprotein that contains structural motifs for capsid polypeptide, papain-like protease, and RNA-dependent RNA polymerase. *Virus Research* 76: 183–189.

Li N, Erman M, Pangborn W, Duax WL, *et al.* (1999) Structure of *Ustilago maydis* killer toxin KP6 α-subunit: A multimeric assembly with a central pore. *Journal of Biological Chemistry* 274: 20425–20431.

Naitow H, Tang J, Canady M, Wickner RB, and Johnson JE (2002) L-A virus at 3.4 A resolution reveals particle architecture and mRNA decapping mechanism. *Nature, Structural Biology* 9: 725–728.

Soldevila AI and Ghabrial SA (2000) Expression of the Totivirus Helminthosporium victoriae 190S virus RNA-dependent RNA polymerase from its downstream open reading frame in dicistronic constructs. *Journal of Virology* 74: 997–1003.

Wickner RB (1996) Double-stranded RNA viruses of *Saccharomyces cerevisiae*. *Microbiological Reviews* 60: 250–265.

V

Vaccine Production in Plants

E P Rybicki, University of Cape Town, Cape Town, South Africa

© 2008 Published by Elsevier Ltd.

Glossary

Immunogenicity Ability of a substance to stimulate the acquired immune response in a recipient.
Pharming The production of pharmaceutically relevant substances in plants.
Vaccine Substance which elicits an immune reaction which protects against infection by a natural pathogen.

Introduction

The concept of plant-produced vaccines, and in particular viral vaccines for animals and humans, is both recent – the first vaccine-relevant protein was made in plants only in 1989 – and controversial, with dire misgivings still being expressed concerning a number of issues. The central planks of the justification for plant-produced pharmaceuticals in general, and for the production of vaccines in particular, have been that their production in plants is both safe and potentially very cheap. A strong additional argument has been that the use of food plants will allow edible vaccines to be locally produced where they are needed most – in the developing world. Thus, the kind of argument that has developed is that plant production of especially viral vaccines allows the possibility of producing large amounts of high-grade vaccines for very low cost (1) for diseases where there is a high burden of preventable disease, (2) where existing vaccines are expensive, (3) where there are no vaccines for 'orphan diseases', and (4) where therapeutic vaccination could be very effective in treating disease.

The need for low-cost vaccines is borne out by the fact that at least 3 million people die every year of vaccine-preventable diseases, yet it takes many years on average for any new vaccine's price to come down to levels (<US$ 1 per dose) where it can be incorporated into the extended programme of immunization (EPI) bundle.

As with any maturing technology, however, problems have come to light along with proofs of concept, and the current view of the field is very different to the early one. Thus, concerns over genetic 'contamination' of food crops have largely blunted the prospect of edible viral vaccines delivered via unprocessed food plants; regulatory concerns – both for production and registration – have tempered the optimism of early years concerning widespread and rapid adoption of the technology; production via transgenic plants has, on the whole, proved to be less reliable and lower-yielding than was hoped; immunogenicity of model antigens delivered orally has often proved to be disappointingly low; and, most importantly, it has been realised that costs of production are far less important in determining the final price of a vaccine than was generally assumed.

However, there have been notable successes as well: a number of animal model systems have been thoroughly tested, and protection against disease has been obtained; production systems have to some extent been worked out, and the first products have been licenced; transient expression systems are proving to have great potential for even large-scale production; and, most significantly, large pharmaceutical companies are increasingly interested in the prospects of making high-value products in plants.

The increasing exposure of the concept to public view has also brought its share of problems: it was after all the public outcry over 'contamination' of food plants with vaccine protein genes that led to the involvement of regulatory agencies in the issue, and a moratorium on the use of food crops for vaccine production. Moreover, public acceptance of human vaccines made in plants is by no means assured, and the anti-genetically modified (anti-GM) lobby has already made an issue of the means of production. Together with these sobering developments has come the realization that high-value products not meant for use in humans are probably the first best use of the advantages of plant production of biopharmaceuticals.

For example, it is probably a far better idea to use plants to produce proteins and other macromolecules as reagents or components of diagnostic kits than it is to produce far more regulated vaccine components – as well as being a much quicker route to financial gain.

While the initially determinedly rosy picture of very cheap, edible vaccines against orphan diseases and developing country scourges has not been realized, there is now an altogether more realistic consensus view on how to proceed with the application of this still very promising technology to human and animal health. This article covers the historical development of the concept of plant-produced viral vaccines and relevant expression systems, as well as central issues affecting production, purification, and application of such vaccines.

History

The first publication on the production of vaccine-relevant proteins in plants was in 1989, when A Hiatt and colleagues at the Scripps Institute in La Jolla produced mouse-derived monoclonal antibodies (MAbs) in transgenic tobacco (see **Table 1**). This was soon followed in 1992 by the first of a series of papers – in this case involving hepatitis B virus (HBV) surface antigen (HBsAg) – by the group of Charles J. Arntzen and Hugh Mason on the production of human viral vaccine candidate antigens in transgenic plants. While they did not at the time hold out the promise of edible vaccines, they did conclude that plants did produce antigens that were equivalent to those produced in more conventional systems, and they held promise as low-cost vaccine production systems. Concurrent with these advances was a rapid and parallel improvement in the understanding of mucosal immunity and the fact that the mucosa-associated lymphoid tissue (MALT) – and, in particular, the gut-associated lymphoid tissue (GALT) – was probably the premier 'organ' of the entire adaptive immune response. It was perhaps inevitable that these two should come together in the concept of 'edible vaccines', where vaccine antigens would be introduced into people and animals in food, given that most human and animal viruses gain entry to their hosts via mucosal surfaces.

The first proof of concept for edible plant-produced vaccines was performed in the mid-1990s using a bacterial protein – *Escherichia coli* heat-labile enterotoxin (LT-B) – but this was soon followed by work on HBsAg, secretory antibodies, Norwalk virus capsid protein, and a *Vibrio cholerae* enterotoxin subunit vaccine (CTB). These studies laid important ground in terms of demonstrating conclusively that plant-produced vaccine antigens were effectively homologous to those produced by more conventional routes, and that oral dosing or gavage of experimental animals elicited significant immune responses, in the absence of replication of the agent. However, the first demonstration that a plant-produced viral antigen was protective against disease came from an animal model system and the production of the foot-and-mouth disease virus (FMDV) structural protein VP1: mice that were parenterally immunized with a crude extract of transgenic *Arabidopsis thaliana* plants were protected against viral challenge. Another development along these lines in 1999 was the proof that injection of rabbits with rabbit hemorrhagic

Table 1 Landmarks in plant-produced vaccine research

Year	*Landmark*
1989	Expression of immunoglobulin genes in plants
1992	Expression of hepatitis B surface antigen in transgenic plants
1995	Oral immunization with a plant-produced recombinant bacterial antigen
1996	Plant expression of Norwalk virus capsid protein and its oral immunogenicity
1998	Use of a recombinant plant monoclonal secretory antibody in humans
1998	LT-B gene in potatoes protects mice against LT
1999	Oral immunization with potato-produced VP60 protein protects against hemorrhagic disease virus
1999	Protection against FMDV by vaccination with foliar extracts of rTMV-infected plants expressing FMDV VP1
2000	Human responses to Norwalk virus vaccine delivered in transgenic potatoes
2001	Oral immunogenicity of oral HPV L1 co-administered with *E. coli* enterotoxin mutant R192G or CpG DNA
2002	Plant-derived HPV 16 E7 oncoprotein induces immune response and specific tumor protection in mice
2002	Boosting of a DNA measles immunization with an oral plant-derived measles virus vaccine
2003	Production of HPV-16 VLPs in transgenic potato and tobacco and oral immunogenicity
2003	Expression of HPV-16 VLPs in transgenic tobacco and parenteral immunogenicity
2003	Oral immunogenicity of HPV-11 VLPs expressed in potato
2003	Oral immunization with rotavirus VP7 in transgenic potatoes induces high titers of mucosal neutralizing IgA
2004	Conformational analysis of hepatitis B surface antigen fusions in an *Agrobacterium*-mediated transient expression system
2004	Oral TGEV vaccine in maize seed boosts lactogenic immunity in swine
2004	Mucosally targeted HIV gp41-derived fusion protein with CTB elicits transcytosis-blocking Abs
2005	Magnifection – TMV-based transient agroinfection platform
2005	Immunogenicity as a boost in humans of potatoes containing HBsAg
2005	Development of a plant-based vaccine for measles
2006	Proof of concept of a plant-produced papillomavirus vaccine in a rabbit model
2006	A plant-derived edible rotavirus subunit vaccine is stable for over 50 generations in transgenic plants

disease virus (RHDV) VP60, produced in transgenic plants, could protect them against virus challenge.

Another important development in the mid-1990s was the application of transient plant virus-based expression systems to the expression of vaccines. The first experimental vaccines produced were chimeras of tobacco mosaic virus (TMV) capsid protein fused to malarial peptides; by the late 1990s, a HIV-1 gp41 peptide fused to cowpea mosaic virus capsids had been tested in mice both parenterally and orally. However, it was again an animal model which provided the best proof of concept, and again with FMDV VP1: production of whole VP1 in plants infected with recombinant TMV and parenteral vaccination of mice with leaf extracts allowed successful protection against live challenge with FMDV. This work provided some of the first evidence that full-length foreign proteins could successfully be produced using a plant virus, in amounts that were sufficient to allow immunization using only crude extracts. However, the technology was limited in that the choice of appropriate vectors was limited, as was the choice of host plants, and vectors were often unstable and did not express large proteins very well.

By the late 1990s, it was apparent that many of the early fears voiced by vaccinologists and immunologists about oral delivery of vaccines 'tolerizing' the recipients to the vaccine – and thereby abolishing an immune response to it – were essentially groundless. The basis for a good response to an orally delivered vaccine was that it should be particulate, and that some form of adjuvant may be required. This was borne out by work with human papillomavirus (HPV) virus-like particles produced in a baculovirus expression system, where the number of particles necessary for a strong systemic humoral and mucosal response to orally introduced VLPs in mice could be reduced by an order of magnitude by co-administration with CpG DNA or mutant *Escherichia coli* LT-B (LT R192G). This work, together with reports on oral immunization with respiratory syncytial virus (RSV) F protein and HBsAg, showed that strong Th1-type responses could be elicited by such treatment. The state of the art in mucosal immunity at the time can be summarized as follows: (1) the mucosal immune system consisted of different compartments (such as gut, respiratory, genital mucosa) which intercommunicated, so that, for example, intranasal immunization gave significant immunity at the vaginal mucosa; (2) oral vaccination elicited antigen-specific mucosal secretory IgA as well as systemic immunity, and CD8+ T-cell responses could be elicited. While co-administration of adjuvants such as LT-B, CTB, CpG DNA, and saponins considerably improved mucosal responses and allowed lower dosages, fusion of nonpolymerizing polypeptides to self-assembling adjuvant entities such as LT-B and CTB also allowed significant improvements in immunogenicity.

By 2000, the first human trial results of Norwalk virus capsid protein made in potato had shown that the vaccine was both tolerated and immunogenic, with almost all volunteers developing a significant immune response. Shortly afterward, the Arntzen group showed that a plant material containing HBsAg given orally was an effective prime for a parenteral boost with HBsAg. In 2002, the first successful combination of DNA prime–plant-produced protein boost vaccination in mice with a measles envelope glycoprotein vaccine showed that plant-produced vaccines could be used in very effective combination with DNA vaccines. However, yields of virus vaccine proteins in transgenic plants are low, and oral immunization even with concentrated extracts is not very successful. In contrast, production of antibodies in plants has been more successful and many different kinds of immunoglobulins with various specificities have been produced.

Plant tissues used for production of viral vaccines include leaf and stem tissues of tobaccos of various varieties, *Arabidopsis*, alfalfa, spinach, and potatoes; of aquatic weeds such as *Lemna* spp. (duckweed); seeds of tobaccos, beans, and maize; fruits like tomatoes and strawberries; carrots; single-cell cultures of *Chlorella* and *Chlamydomonas*; suspension cell cultures of tobacco and other plants; hairy root cultures derived from various plants via *Agrobacterium rhizogenes*; and transformed chloroplasts of a variety of plant species. Experience gained from these various systems showed that there was no way of predicting whether or not a given DNA sequence would express protein at a reasonable level, or that the protein would be stable. Although chloroplast expression sometimes gave high yields, this system was not suitable for glycosylated proteins. Single-cell cultures offered few advantages over conventional fermentation/or cell culture techniques, and it seemed that accumulation of proteins in seeds was preferable to expression in green tissue, because of easier purification and higher accumulation levels.

A major setback to the development of plant-produced vaccines occurred in 2002 when soybean and maize harvests in two states in the USA were found to contain maize seeds from 'volunteer' plants engineered to express transmissible gastroenteritis virus (TGEV) capsid protein. The company involved – ProdiGene – had proprietary technology allowing high levels of accumulation of foreign proteins in especially maize seeds, and had potential oral vaccine products including a hepatitis B vaccine, an LT-B vaccine to treat *E. coli* infections in humans, as well as other products under development with large commercial partners. ProdiGene was fined and forced to clean up the seed by the US Department of Agriculture, which has since issued guidelines to prevent a recurrence. The fallout from these incidents has led to an effective moratorium on the development of 'pharmed' products in food crops including vaccine pharming. Another limitation

became obvious in 2005 when the Mason–Arntzen group at Arizona State University released results of a small human trial of a potato-based HBsAg vaccine. In this trial, volunteers previously immunized parenterally with a conventional vaccine ate doses of potato containing ~850 μg of antigen and 63% of them developed increased serum anti-HBsAg titers. However, the immunogenicity was still disappointingly low, despite the ingestion of relatively large doses of antigen.

Growing public unease over the use of transgenic plants for biopharmaceutical production, with the attendant risks of 'contamination' of crop plant gene pools, has prompted the development of other technologies for producing such materials in plants. One such technology is the *Agrobacterium tumefaciens*-based transient expression system: this allows very high levels of expression without the uncertainties inherent in the regeneration and propagation of transgenic plants, and was being used very successfully to produce antibody molecules by 2004. Its main advantage is that the simultaneous expression of a large number of constructs can very rapidly be investigated. Indeed, the Mason group used transient 'agroexpression' very productively in 2004 to explore the conformation and chimeric fusion properties of HBsAg.

By 2005, another major advance in expression technology was announced, which combined the advantages of agroinfection and of viral vectors: this was the 'magnifection' technology of ICON Genetics, which was based on the introduction of recombinant DNA into plants by agroinfection and the subsequent amplification of gene expression by the recombinational reconstitution of a self-replicating TMV-based RNA genome.

Recently, serious reservations have been expressed by researchers and companies about the regulatory acceptance of plant-produced vaccines and other therapeutics, for human and even for animal use. The observed reluctance of large companies to buy into the technology, or to fund it, is probably due to the lack of appropriate regulatory structures and some conservatism in regulatory agencies concerning plant products. However, there is no reason to believe that existing frameworks and regulations could not be adapted to handle plant-produced vaccines: this was demonstrated in 2006 when Dow AgroSciences registered a plant-produced vaccine against Newcastle disease virus (NDV), which affects poultry. This was the first plant-based vaccine registered in the US, and the first to be licenced by the USDA's Animal and Plant Health Inspection Service. The HN envelope glycoprotein of NDV was produced in suspension-cultured tobacco cells, thus avoiding all the production and regulatory problems associated with whole plant production. In the same year, the Centre for Genetic Engineering and Biotechnology (CIGB) in Cuba registered a tobacco-produced MAb for use in purification of their commercially produced HBV vaccine: this development obviates the need for MAb production in ascitic fluid from mice, and considerably reduces the cost of manufacture of a vaccine which is distributed worldwide.

The introduction in mid-2006 of Merck's yeast-produced Gardasil anti-human papillomavirus (HPV) L1 major capsid protein VLP vaccine was hailed as a major development in cancer prevention, and the vaccine is predicted to be a 'blockbuster'. However, at a cost of US$ 360 per course of three doses, it will not make inroads into limiting cervical cancer incidence in developing countries. In 2003, three groups published simultaneous accounts of plant production of HPV L1 proteins. While the antigens were correctly folded and assembled, the yields were low and oral and parenteral immunogenicities were also low: however, these problems were circumvented for HPV-16 L1 in early 2007 by a combination of codon optimization and intracellular targeting, and excellent parenteral immunogenicity was achieved.

Obstacles to Vaccine Pharming

The following obstacles impede the development and potential application of plant-based vaccines, especially in developing countries:

1. increasingly stringent regulatory barriers in developed countries which deter commercial development of new vaccines generally, and vaccines depending on new technology in particular;
2. declining commercial interest in producing vaccines due to negligible return on vaccines needed in developing countries (people who do need vaccines cannot afford them and have to rely on governments and/or aid agencies for making them available);
3. earlier expectations from the technology have not been fulfilled;
4. many developing countries have little or no infrastructure for the approval of experimental drugs and vaccines, or for GM crops;
5. there is widespread and profound suspicion in developing countries of 'GM products', usually fueled by developed country activists;
6. the 'guinea pig' argument: why should developing country populations be used to test products not used in developed countries, where existing but expensive 'best practice' products are already available? and
7. production of vaccine antigens in plants may not significantly decrease the cost of the final vaccines, given that downstream processing and other costs remain the same.

Most of these obstacles could possibly be overcome: countries could pass relevant legislation; regulatory bodies could adopt suitable guidelines; publicity/and information campaigns could change public perceptions, both in the developed as well as in the developing world.

The 'guinea pig' argument could be countered by testing products in developed countries at the same time as in developing countries.

However, a more serious obstacle is that conventional pharma industry is not engaging with plant production technology primarily because it is seen as technology in search of a product, when the technology already available is adequate to the task. Moreover, it is a serious misconception that using plants to produce vaccines would lower prices significantly, as cost of materials and processing is only a minor component of total retail vaccine cost, that is, about 24%. Thus, even a significantly lower cost of vaccine material would not change the cost of the product much, especially as processing costs would remain the same however cheaply one could produce raw vaccine ingredients.

The Way Forward

Vaccine pharming has moved on toward maturity in an encouraging way in recent years, as the dogma of 'cheap oral vaccines' has to some extent shifted to an acceptance that processed and possibly injectable products may be preferred to raw or crudely processed oral vaccines. Indeed, as new needle-free developments in vaccine delivery such as oral and nasal and transdermal delivery are becoming increasingly popular, plant-made vaccines may find a niche, especially in products that need less processing than those intended for parenteral delivery.

It is possible that practitioners will come to accept that success will not be achieved by trying to bring cheap vaccines and therapies for poor people, but by producing generics. For example, it may be a better idea to concentrate on HPV vaccines, which currently are very expensive and have only a few remaining years of patent protection, rather than on HBV vaccines, which are out of patent, are produced conventionally by several high-volume producers, and currently sell for low enough prices to be included in EPI packages.

Another important area to focus on is animal vaccines: these are necessarily very low cost for meat animals such as poultry, sheep, and cattle, and animal model systems have shown very satisfactorily that viral and other plant-produced vaccines could be very useful. The development times are shorter and regulatory hurdles are lower than for similar human vaccines, and edible or minimally processed oral vaccines are probably far more feasible for animals than humans. Major targets could be the already-tested FMDV vaccine for cattle, sheep, pigs, and even wild animals; Newcastle disease and other chicken and poultry viruses; H5N1 and other influenza viruses in domestic and wild fowl; and African horsesickness virus which is currently threatening to emerge into Europe.

It is possible that expression of viral antigens in, for example, maize seed could allow very cheap production of orally dosable vaccines which could be stored for years in a dried form for very little expense, to be pulled out when and if the need for emergency barrier immunization occurred.

It is important for the development of plant-made vaccines that several large companies are interested in the technology, and have done a lot of thorough investigation of its potential. It is especially significant that the first product – Dow's Newcastle disease vaccine – has run the regulatory course and was accepted in 2006.

What is probably needed for the successful future application of pharmed products, especially in developing countries, is a highly effective set of plant-derived products – vaccines and therapeutics – which have been approved by regulatory bodies such as the US Food and Drug Administration (FDA) and European Medicines Agency (EMEA). This will lead to public acceptance and a more welcoming stance by industry, both of which are essential if the technology is to survive.

See also: Plant Virus Vectors (Gene Expression Systems); Vaccine Strategies; Vector Transmission of Plant Viruses.

Further Reading

Daniell H (2006) Production of biopharmaceuticals and vaccines in plants via the chloroplast genome. *Biotechnology Journal* 1: 1071–1079.

Gerber S, Lane C, Brown DM, *et al.* (2001) Human papillomavirus virus-like particles are efficient oral immunogens when coadministered with *Escherichia coli* heat-labile enterotoxin mutant R192G or CpG DNA. *Journal of Virology* 75: 4752–4760.

Gleba Y, Klimyuk V, and Marillonnet S (2005) Magnifection – a new platform for expressing recombinant vaccines in plants. *Vaccine* 23: 2042–2048.

Kirk DD and Webb SR (2005) The next 15 years: Taking plant-made vaccines beyond proof of concept. *Immunology and Cell Biology* 83: 248–256.

Mason HS, Lam DM, and Arntzen CJ (1992) Expression of hepatitis B surface antigen in transgenic plants. *Proceedings of the National Academy of Sciences, USA* 89: 11745–11749.

Thanavala Y, Huang Z, and Mason HS (2006) Plant-derived vaccines: A look back at the highlights and a view to the challenges on the road ahead. *Expert Review of Vaccines* 5: 249–260.

Voinnet O, Rivas S, Mestre P, and Baulcombe D (2003) An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. *Plant Journal* 33: 949–956.

Vaccine Safety

C J Clements, The Macfarlane Burnet Institute for Medical Research and Public Health Ltd., Melbourne, VIC, Australia
G Lawrence, The Children's Hospital at Westmead, Westmead, NSW, Australia and University of Sydney, Westmead, NSW, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Adjuvant A chemical additive to vaccines that improves the presentation of the antigen to the immune system of the recipient, thereby increasing the effectiveness of the vaccine. The commonest adjuvant is an aluminum salt.
Adventitious agent A contaminating virus or other infectious organism.
Adverse event following immunization Any medical incident that follows immunization and is believed to be caused by the immunization.
Anaphylaxis A hypersensitivity reaction characterized by sudden onset and rapid progression of signs and symptoms in at least two organ systems. It results in release of histamine from mast cells and may follow administration of drugs and vaccines. The severity of the reaction varies from mild symptoms of flushing to severe bronchospasm, collapse, and death. It is treatable with adrenaline.
Auto-disable syringe A form of disposable syringe that automatically locks after one use, preventing its reuse. It is the syringe design of choice for immunization.
Causality assessment A scientific method for assessing the relationship between an adverse event and the receipt of a dose of vaccine.
Clinical trial A research activity that involves the administration of a test regime to humans to evaluate its efficacy and safety. There are classically four phases in clinical trials assessing vaccines.
Guillain–Barré syndrome A polyradiculoneuritis resulting in various degrees of motor and sensory disturbance. The condition frequently follows an acute infection or administration of vaccine.
High-titer measles vaccine A measles vaccine that contains at least one log greater ($>$log 10^4) numbers of live viral particles than a standard vaccine preparation (log 10^3).
Hypotonic hyporesponsive episode (**HHE**) The sudden onset of limpness, reduced responsiveness, and change in skin color in an infant following the administration of a vaccine. Typically (though not exclusively), this may follow administration of pertussis-containing vaccines.
Immunization The process of presenting an antigen to the immune system with the purpose of inducing an immune response. Immunization is synonymous with vaccination.
Injection equipment Any material needed to introduce a vaccine into the body of the recipient. Classically refers to needles and syringes, but new technologies for administering vaccines will widen its usage.
Neurovirulence test One of several tests that is required of live viral vaccines before release to ensure that the vaccine virus does not negatively affect the central nervous system.
Presentation How a vaccine is presented for use (e.g., as a liquid, freeze-dried powder, one-dose vial, 10-dose vial, etc.).
Preservative A chemical (most frequently thiomersal/thimerosal) that is included in a liquid vaccine preparation that inhibits the growth of organisms that inadvertently contaminate the vaccine.
Program error A mistake made by vaccination staff that occurs during administration of a vaccine.
Protective immunity Immunity that protects against actual disease, not simply evoking an immune response.
Regulatory authority An institution/laboratory that takes responsibility for quality control of vaccines used in a country.
Safety box A tough cardboard box into which can be placed used injection equipment. It is generally then transported to an incinerator or can be burnt on the spot, thus safely disposing of medical waste that might otherwise be a hazard.
Stabilizer Proteins or chemicals that are added to a liquid vaccine to maintain it in a stable or unchanging state.
SV40 Simian virus 40 is a virus found in certain monkeys and cell cultures derived from monkey tissues.
Vaccine diluent A liquid (usually sterile water or saline) that is used to reconstitute freeze-dried vaccine.

Vaccine safety The discipline of ensuring that vaccines are manufactured, transported, and administered with minimum risk to the recipient, vaccinator, or general public.

Introduction

Vaccines are arguably the most effective weapon in the modern fight against infectious diseases. They avert millions of deaths and cases of disease every year. But there is no such thing as a 'perfect' vaccine that protects everyone who receives it and is entirely safe for everyone. The scientist attempts to create a vaccine that protects nearly everyone, is extremely effective, and is as safe as humanly possible. That still leaves some vaccine recipients who are not fully protected; and worse, a very small number who suffer negative consequences from the vaccine itself.

There are many aspects that can be considered in the design phase which influence the ultimate safety and public acceptability of a vaccine (**Figure 1**). Vaccine safety can be categorized into aspects related to the manufacture of the vaccine, delivery of the vaccine to the recipient through an immunization programme, and individual and population responses to the vaccine. This article describes the efforts taken to ensure that a vaccine is manufactured, tested, and administered as safely as possible, and identifies how vaccine safety is measured and monitored after a vaccine is licensed for use in the population.

Vaccines are designed to evoke a protective response from the immune system as if it were the actual infectious disease. While a vaccine is designed to maximize the body's reaction and minimize any pathological effects, effective vaccines (i.e., capable of inducing protective immunity) may also produce some undesirable side effects. Clinical trials determine whether side effects are tolerable or not, and detect certain adverse events before the vaccine reaches general distribution. Most adverse reactions are mild and clear up quickly.

Design factors can help minimize programmatic errors in the administration of the vaccine by reducing the chance of human error. The most common types of programmatic errors are unsafe or contaminated injections. It is sobering to realize that up to one-third of injections (not just for vaccination) are not sterile. New technologies have been introduced in the last decade that make injections much safer, including an auto-disable syringe that can only be used once and must be discarded because the plunger mechanism locks after use. This device helps minimize transmission of diseases that result from the reuse of contaminated injection equipment.

Vaccine adverse reactions can be due to the vaccine itself, or to immunological factors in the individual such

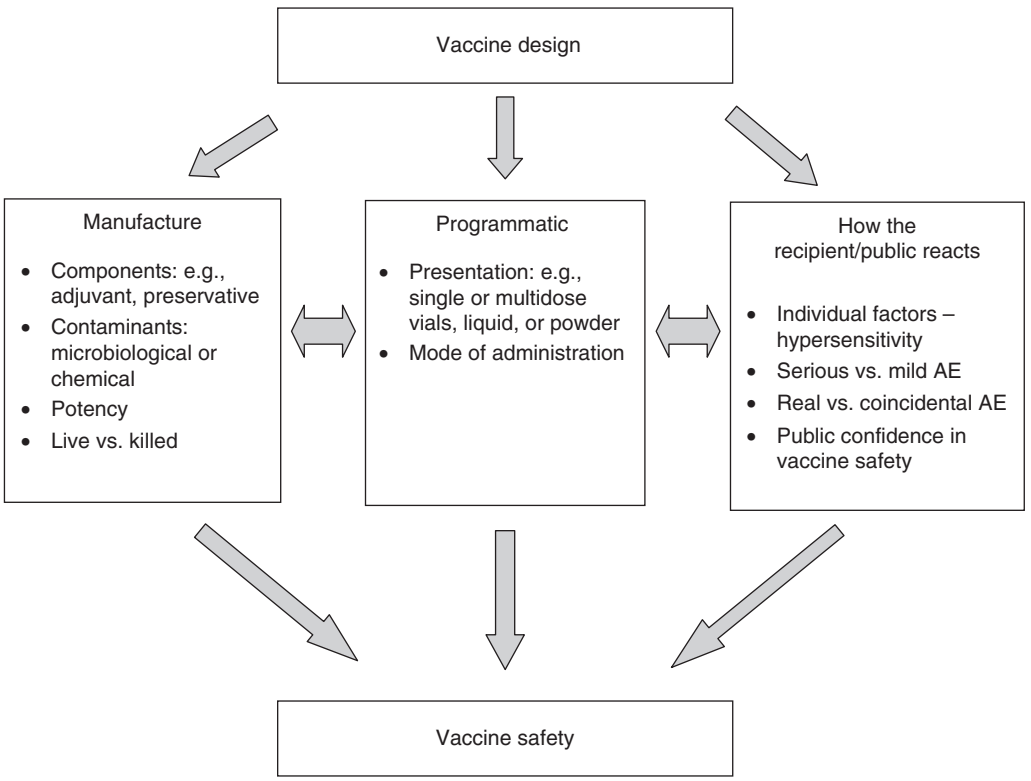


Figure 1 Components of vaccine safety.

as anaphylaxis or hypersensitivity reactions. However, the majority of adverse events thought to be related to the administration of a vaccine are actually not due to the vaccine itself – many are simply coincidental events. For example, many children have a cough around the time of vaccination, but this is independent of the vaccine, not caused by it. An appropriate causality assessment, using internationally consistent methods, is necessary to fully determine whether a particular vaccine causes a specific reaction. Even when scientists have been able to show that a particular event is unrelated to vaccine administration, the public may still perceive there to be a link.

Vaccine Design

Manufacture

A vaccine consists of many parts, only one of which is the antigen by which it is known. Other components may include an adjuvant, a preservative, antibiotics, stabilizers, and certain ingredients that remain from the manufacturing process. There may be components not stated on the information sheet that are classified as proprietary that the manufacturers are not obliged to declare. The safety of the vaccine can be affected by each and all of its components.

How a vaccine is manufactured will also determine its content. For instance, thiomersal may be used in the manufacture of pertussis vaccines to maintain sterility during the manufacturing process. While the bulk of the chemical is removed, the final product will still contain minute amounts of thiomersal. Influenza vaccines are grown on chicken eggs – trace amounts of egg proteins not removed in the purification process can cause severe allergic reactions in susceptible individuals.

Safeguards in vaccine manufacture now make it highly unlikely that currently available vaccines are contaminated with infectious agents that cause disease. Manufacturers supplying the international market are required to submit vaccines to extensive tests to detect known potential pathogens. As well, they must test cell lines used in the production of vaccines for the presence of a variety of infectious agents. Once manufactured, vaccines must undergo rigorous clinical trials for safety and effectiveness before being licensed by regulatory authorities.

Procedures designed to detect and prevent the presence of adventitious agents in vaccines include characterization of the cell substrates used for vaccine production as well as testing the products by specified methods. For example, manufacturers are required to test their cell lines used for the production of poliovirus vaccines (generally monkey kidney cells) for a variety of infectious agents such as tuberculosis, SV40, herpes viruses (including simian cytomegalovirus), Coxsackie virus, and lymphocytic choriomeningitis virus. Cell tissue culture tests must show an absence of cytopathogenic effects. Adult and suckling animals are also used to screen for viable microbial agents. A neurovirulence safety test is conducted for the live oral poliovirus vaccine.

The advent of bovine spongiform encephalitis (BSE) in the 1990s generated concern that the etiological agent might be contaminating vaccines through the use of calf serum during manufacture. Industry has taken steps to ensure that the risk to vaccines from BSE is virtually zero.

New technologies can also give rise to new concerns during manufacture. For instance, some modern vaccines are grown on chick embryo fibroblasts. These vaccines may test positive by a polymerase chain reaction assay for avian leukocytosis virus, a retrovirus not unlike the HIV/AIDS virus. Because the assay is so sensitive, it detects nonreplicating fragments of the virus that have no pathological significance. The cell substrates used for measles/mumps/rubella (MMR) vaccine are derived from flocks free of avian leukosis virus. MMR vaccine is also tested extensively for adventitious viral activity. As technology improves, regulatory authorities are likely to require the application of new methods to screen for infectious agents in vaccines.

Potency

The potency (or viral titer) of a vaccine can influence both its safety and its ability to induce protective immunity. When developing new rotavirus vaccines, the safety and efficacy of low-, medium-, and high-viral-titer formulations of human–bovine reassortant vaccines were assessed in randomized controlled clinical trials of infants. The medium-potency formulation was selected for use in phase III trials as it was found to be more efficacious than the high- and low-titer formulations and had a slightly lower, but not clinically significant, incidence of fever. In another example, a high-titer formulation of measles vaccine was used in some African countries in 1989–92 to protect infants from as early as 6 months of age. Unexpected results showed that the survival of infants who received the high-titer vaccine before 9 months of age was substantially lower than those who received the standard titer vaccine at 9 months of age. The high-titer vaccine was withdrawn from use.

Live or killed vaccine

There are three main types of viral vaccines currently in widespread use: live attenuated virus (e.g., MMR, yellow fever, varicella vaccines), killed virus (e.g., influenza vaccine), and recombinant protein (e.g., hepatitis B vaccine). Safety issues differ to some extent for the three types of vaccine. For all, the risk of an adverse event is highest in the immediate post-vaccination period due to reactions to vaccine components and to programmatic errors. Live-attenuated viral vaccines must undergo viral replication in the recipient to produce sufficient

antigen to generate a protective immune response. The vaccine recipient may experience a mild form of the disease, particularly at the time of peak viral load. Febrile seizure in young children 5–14 days after receiving MMR vaccine is a known adverse event. There is also the potential risk of reversion to virulence if the vaccine strain of virus undergoes genetic mutations either *in vitro* during manufacture or in the vaccinee, that can cause significant illness. Killed vaccines usually contain trace amounts of the inactivating agent (often formaldehyde) that can cause reactions in some individuals. There is also the potential risk of incomplete inactivation with the effect that the vaccine could contain trace amounts of live virus capable of replication in the host to cause disease. An atypical variant of measles infection has been reported in some recipients of killed measles vaccine who were at risk of developing severe delayed hypersensitivity reaction following exposure to wild measles virus. As well as containing no viral genetic material, recombinant protein vaccines have the advantage of presenting fewer antigens to the immune system, thus minimizing the risk of hypersensitivity reaction. However, they are often produced in yeast cells, traces of which may remain after purification and cause allergic reactions in some individuals.

Programmatic Factors, Presentation, and Administration

A 'programmatic error' is caused by an error or errors in the handling or administration of a vaccine. The error is usually the fault of a person rather than the fault of the vaccine or other technology. It can generally be prevented through staff training and an adequate supply of safe injection equipment although vaccine design factors can help minimize the chance of human error.

A programme error may lead to a cluster of events. Improper immunization practice can result in abscesses or other blood-borne infections. Freeze-dried vaccines (such as measles-containing vaccines and yellow fever) are presented as a dry powder in a vial that needs reconstituting with liquid. Errors can occur in reconstituting the powder, such as the use of inappropriate or contaminated liquid. Once a vaccine is reconstituted, it is open to the environment and makes an ideal culture medium for organisms such as *Staphylococcus*. Fatal toxic shock syndrome has been reported following administration of a multidose vial of reconstituted measles vaccine that had become contaminated. In such circumstances, a number of infants immunized from the same vial may die within a short time of injection. To avoid this catastrophe, field staff are required to discard reconstituted vaccine after 6 h, or at the end of the vaccination session, whichever is sooner.

There are options for the way a vaccine is presented that can affect its safety in the field. To reduce cost, vaccines are often presented in multidose vials. It is common in developing countries for diphtheria–tetanus–pertussis (DTP) and measles vaccines to be in 10-dose vials, while BCG is often in 20-dose vials. The downside of multidose vials is that they have the potential for contamination (see above). In contrast, single-dose presentations are unlikely to become contaminated as the vial is discarded once the single dose has been used. Prepackaged mono-dose vials reduce the need for human handling (and hence reduce the risk of contamination) but are more costly per unit. Industrialized countries tend to favor mono-dose presentations because of their increased safety and convenience.

There are a number of different modes of administration of vaccines, and these affect safety in different ways. Aerosol presentations are attractive because they do not need a needle or syringe, thus reducing the risk of contamination. However, administering an inhaled vaccine to a 9-month-old infant is not without its practical problems. Nasal delivery bypasses the intestinal barrier, but recipients with purulent rhinitis (a common situation in developing countries) may interfere with successful seroconversion. Edible vaccines and vaccines presented using skin patches are also attractive for their apparent freedom from these safety concerns.

The injectable vaccine is by far the commonest presentation in the world today. Injections are one of the most common healthcare procedures. Five to ten percent of some 16 billion injections administered worldwide each year are given for immunization. Unsafe injections or unsafe practices in relation to immunization are not only responsible for cases of hepatitis B and C, HIV, and other serious and potentially lethal side effects suffered by vaccine recipients, but may also pose an occupational hazard to health providers and an environmental hazard to communities. Furthermore, unsafe injection practices can seriously impede the progress made by immunization programmes, and have a substantial effect on global immunization coverage. Fortunately, a number of technologies are at hand that help make injections safer.

The reuse of standard single-use disposable syringes and needles becomes unsafe when they are not disposed of after use but are reused multiple times. The auto-disable syringe, which is now widely available at low cost, presents the lowest risk of person-to-person transmission of blood-borne pathogens (such as hepatitis B or HIV) because it cannot be reused. The auto-disable syringe is the equipment of choice for administering vaccines, both in routine immunization and mass campaigns. In 1999, the World Health Organization (WHO) and other United Nations organizations issued a joint statement promoting the use of auto-disable syringes in immunization services. 'Safety boxes', puncture-proof containers – for the collection and disposal of used disposable and auto-disable syringes, needles, and other injection materials – reduce the risk from contaminated needles and syringes. The safe

disposal of used needles and syringes is a critical component of any vaccination program if infection is to be prevented. Poor management of injection-related waste exposes patients, healthcare workers, waste handlers, and, indeed, the wider community, to infections, toxic effects, and injuries.

Program errors may occur when a new vaccine is integrated into an existing immunization programme that has important (and sometimes subtle) differences in its presentation, preparation, or administration. Errors such as the omission of a component, injection of oral vaccines, and the use of incorrect diluent to reconstitute freeze-dried vaccines are examples of this type of errors. While thermostabilty of a vaccine is not strictly an issue of vaccine safety, failure to maintain the cold chain is a significant component of programmatic error. This can lead to vaccine failure and the need to revaccinate populations, thus re-exposing them to the risk, albeit small, of vaccine adverse reactions.

How the Recipient/Public Reacts

While the recipient of a dose of vaccine will hopefully react by developing immunity to the challenging antigen, it is common for there to be various other less desirable reactions following vaccine administration. The WHO describes an adverse vaccine reaction as either 'common' or 'rare'. Common reactions are generally mild, settle without treatment, and have no long-term consequences. More serious reactions are very rare – usually of a fairly predictable (albeit extremely low) frequency.

Rates of common, mild vaccine reactions

The purpose of a vaccine is to induce immunity by causing the recipient's immune system to react to the vaccine. It is not surprising that vaccination commonly results in certain mild side effects (**Table 1**). Local reaction, fever, and systemic symptoms can result as part of the normal immune response. In addition, some vaccine components (e.g., aluminum adjuvant, antibiotics) can lead to reactions. A successful vaccine reduces these reactions to a minimum while inducing maximum immunity.

Rates of rare, more severe vaccine reactions

Most of the rare vaccine reactions (e.g., seizures, thrombocytopenia, hypotonic hyporesponsive episodes, persistent inconsolable screaming) are self-limiting and do not lead to long-term problems (**Table 2**). Anaphylaxis, while potentially fatal, is treatable without leaving any long-term effects.

Public perceptions of vaccine safety

While vaccine manufacture and administration have been getting progressively safer over the last 30 years, the perception of the public has been changing. Certain reports of adverse events following immunization (AEFIs) published in the medical literature over the past few years have resulted in controversy. While generating provocative hypotheses, the studies on which these reports are based have generally not fulfilled the criteria needed to draw conclusions about vaccine safety with any degree of certainty. Yet these reports have had a major influence on public debate and opinion making. Sadly, health professionals have not always handled public announcements about such matters very well and the debate has often spilt over into the political arena and policymaking. At times, mishandling of public information has resulted in a reduced public acceptance of a vaccine. Even when scientific evaluation has been correct, the public has often been swayed by other factors. This again underlines the importance of a correct assessment of causality.

Until very recently, communication with the public by health professionals and vaccine manufacturers has often been on a paternalistic basis: "We know what is best for you." The level of education of the general public has risen, helped by the Internet; freedom-of-information laws in many countries have allowed the public to access what was previously privileged information. As a result, members of the public are often well informed on a given public health issue such as vaccine safety.

Table 1 Summary of common minor viral vaccine reactions

Vaccine	*Local reaction (pain, swelling, redness)*	*Fever*	*Irritability, malaise, and nonspecific symptoms*
Hepatitis B	Adults up to 30% Children up to 5%	1–6%	
Measles/MMR	Up to 10%	Up to 5%	Up to 5%
Oral polio (OPV)	None	Less than 1%	Less than 1%[a]
Influenza	>10%	Up to 10%	Up to 10%

[a]Diarrhoea, headache, and/or muscle pains.

Note: the rates due to the vaccine administration will be lower as these symptoms occur independently as part of normal childhood.

Source: Supplementary information on vaccine safety. Part 2: Background rates of adverse events following immunization. WHO/V&B/00.36. http://www.who.int/vaccines-documents/DocsPDF00/www562.pdf.

Table 2 Summary of rare, serious viral vaccine reactions, onset interval, and rates

Vaccine	*Reaction*	*Onset interval*	*Rate per million doses*
Hepatitis B recombinant vaccine	Anaphylaxis	0–1 h	1–2
	Guillain–Barré syndrome (plasma derived)	1–6 weeks	5
Measles/MMR[a]	Febrile seizures	5–12 days	333
	Thrombocytopenia (low platelets)	15–35 days	33
	Anaphylaxis	0–1 h	1–50
Oral polio (OPV)	Vaccine-associated paralytic poliomyelitis (VAPP)	4–30 days	1.4–3.4[b]
Japanese encephalitis	Serious allergic reaction		10–1000
	Neurological event		1–2.3
Yellow fever	Post-vaccination encephalitis	7–21 days	500–4000 in infants less than 6 months[c]
	Allergic reaction/anaphylaxis	0–1 h	5–20
	Catastrophic system collapse resembling naturally acquired infection	2–7 days	2–32 (higher rates in the elderly or history of thymoma)[d]

[a]Reactions (except anaphylaxis) do not occur if already immune (~90% of those receiving a second dose); children over 6 years unlikely to have febrile seizures.
[b]VAPP risk is higher for first dose (1 per 1.4–3.4 million doses) compared to 1 per 5.9 million for subsequent doses and 1 in 6.7 million doses for contacts.
[c]Isolated cases with no denominator make it difficult to assess the rate in older children and adults, but it is extremely rare (less than four cases per 8 million doses).
[d]Studies have reported >10-fold higher risk of this rare adverse event among elderly vaccine recipients. There is a suggestion that reduced thymus gland activity (through age, immunosuppression, or surgical removal) increases the risk.
Source: Supplementary information on vaccine safety. Part 2: Background rates of adverse events following immunization. WHO/V&B/00.36. http://www.who.int/vaccines-documents/DocsPDF00/www562.pdf.

Discussions about vaccine safety now must contend with this new era. It is no longer sufficient for professionals to be factually correct – they must present information in ways that are perceived to be correct. A key component of this is representing themselves in ways that generate the confidence and trust of the public. In Jordan, a vaccine scare followed hard on the heels of a scare about water contamination, with major consequences. The public lost confidence in the truthfulness of government spokespersons, even though their claims that the vaccines in question were safe later proved to be correct. A similar situation occurred in the United Kingdom when there was a large decline in MMR immunization rates after publication of a now-disproved hypothesis that MMR vaccine caused autism. One of the factors attributed to this was loss of public confidence in the government and health spokespersons because of the way the BSE situation had been handled some years earlier.

Monitoring and Assessment of Vaccine Safety

The clearest and most reliable way to determine whether an adverse event is causally related to vaccination is by comparing rates of the event in a vaccinated and nonvaccinated group in a randomized clinical trial. Such trials, however, are unlikely to be large enough to assess very rare events, and postmarketing surveillance systems are required to identify events potentially related to vaccination. Postmarketing surveillance capability is improving; more countries now have monitoring systems, and more importance is attached to the reporting of suspected links between vaccination and adverse events. These systems have been successful in bringing to light serious adverse events after vaccines have been approved for use by regulatory authorities. A recent example is intussusception after administration of reassortant rhesus rotavirus vaccine.

Evaluating Adverse Events – Causality Assessment

Clinical events such as anaphylaxis or rash may occur subsequent to vaccination. But are they 'caused' by vaccination? It would be highly likely that collapse within minutes of receiving a dose of vaccine was due to the vaccine. But a rash 3 weeks later may not be – more likely it was coincidental. It is the area between these two scenarios that needs clarification.

Assessment of whether a given vaccine causes a particular adverse reaction varies from the casual observation to the carefully controlled study. The public frequently forms a decision about a vaccine's safety based on the information available to them – often a report based on unscientific observations or analyses that fail to stand the scrutiny of rigorous scientific investigation. Submitting a

study to a scientific process rather than to partially informed opinion is crucial in determining whether a vaccine actually causes a given reaction. If undertaken carelessly or without scientific rigor, the study results will be inconclusive at best, may result in the inappropriate withdrawal of a valuable vaccine from use, or at worst may result in the exposure of a population to a dangerous vaccine.

When the Global Advisory Committee on Vaccine Safety (GACVS) undertakes causality assessment, the criteria it looks for in arriving at conclusions include consistency of observations, strength of the association, specificity, temporal relation, and biological plausibility. Clearly, not all these criteria need to be present, and neither does each carry equal weight for a causal relationship between an adverse event and the vaccine to be determined. Biological plausibility is a less robust criterion than the others described.

An association between vaccine administration and an adverse event is most likely to be considered strong when the evidence is based on well-conducted human studies that demonstrate a clear association in a study specifically designed to test the hypothesis of such association. Such studies may be randomized controlled clinical trials, cohort studies, case-control studies, or controlled case-series analyses. Case reports by themselves, however numerous and complete, do not fulfill the requirements for testing hypotheses.

Surveillance of AEFIs

Vaccines are generally administered to healthy individuals, so any report of vaccine administration causing harm needs to be taken seriously. Although the vaccines in general used in national immunization programmes are extremely safe and effective, adverse events can occur following vaccine administration and no vaccine is perfectly safe. In addition to the vaccines themselves, the process of immunization is a potential source of adverse events. An AEFI is any adverse event that follows immunization that is believed to be caused by the immunization. Using this terminology allows description and analysis of the event without prejudging causality. Immunization will tend to be blamed for any event that happens after immunization. AEFIs can be classified into five categories:

- vaccine reaction: a reaction due to the inherent properties of the vaccine;
- programme error: an error in the immunization process;
- coincidental event: unrelated to the immunization, but has a temporal association;
- anxiety-related: a reaction can arise from the pain of the injection rather than the vaccine; and
- unknown: in some cases the cause of the AEFI remains unknown.

It is important to investigate a report of an AEFI to determine into which of the above categories it fits. The response to an AEFI will vary depending on the cause. However, many countries have ineffective reporting systems or no system at all. Currently, only 35% of 192 countries, and only 25% of 165 nonindustrialized countries, have an adequately functioning system for monitoring adverse events following immunization. The benefits of good surveillance of AEFIs are shown in **Table 3**.

Table 3 Benefits of monitoring AEFIs

Improves vaccine safety by creating awareness among health professionals of the risks of vaccination
Identifies urgent problems that need investigation and action
Improves capacity to respond to AEFI reports
Improves the quality of the vaccination programme by monitoring performance and increasing staff confidence
Stimulates other aspects of programme monitoring, e.g., case reporting
Public perceives that health authorities understand and monitor vaccine safety, building public confidence in immunization
Detects signals for potential follow-up and research
Estimates rates for serious AEFIs
For comparison between products
To determine risks and benefits of immunization
To validate prelicensure data
Identifies programmatic errors and batch problems

Benefits of monitoring AEFIs

As vaccine-preventable infectious diseases continue to decline, people have become increasingly concerned about the risks associated with vaccines. Furthermore, technological advances and continuously increasing knowledge about vaccines have led to investigations focused on the safety of existing vaccines which have sometimes created a climate of concern. Allegations regarding vaccine-related adverse events that are not rapidly and effectively dealt with can undermine confidence in a vaccine and ultimately have dramatic consequences for immunization coverage and disease incidence.

Not only is public awareness of vaccine safety issues rising, there are increasing opportunities for AEFIs to occur due to various factors. For instance, an increasing number of vaccine doses is now given in campaigns than ever before (**Table 4**). Polio virus is targeted for eradication and measles elimination is being implemented in many parts of the world. As a result, vast numbers of doses of vaccine are being given over short time intervals. Thus, even if the AEFI rates stay the same, the actual number of cases is likely to be much higher than would be expected over the same time period, simply because so many doses have been administered.

Classical Safety Events

SV40

Between 1959 and 1963, both inactivated and live attenuated poliovirus vaccines were inadvertently contaminated

Table 4 Possible effect of immunization campaigns on vaccine safety

A real rise in program errors due to pressure on staff
Increased workload on staff can increase human error
Extra or new staff may not be fully trained in administration technique
Because large numbers of doses are administered there may be a rise in the absolute number of AEFIs even though rates may not change
Campaigns can generate popular opposition and rumors about vaccine safety
Rate of AEFIs prior to campaign may be unknown, so that any report of an AEFI appears to be due to the campaign

with simian virus 40 (SV40), a monkey virus known to be oncogenic for newborn hamsters. SV40 was found to be present in monkey kidney tissue used for propagation of the poliovirus during manufacture. From 1961, manufacturers were required by control authorities to test for SV40 using infectivity assays and different cell cultures, and lots positive for SV40 were not released. A few years later, the source of monkeys used for production was changed to species that do not harbor SV40. There is no evidence that polio vaccine administered since the early 1960s has contained SV40, and large epidemiological studies have not identified an elevated cancer risk in persons who received SV40-contaminated vaccines, but fragments of SV40 DNA have recently been identified in certain human tumors.

Swine flu

Influenza vaccine has been the source of a number of issues related to safety over the years. Mass immunization against swine-like influenza was carried out in 1976–77 using the A/New Jersey swine-influenza vaccine. Initially it appeared that this vaccine generated an increased risk of acquiring vaccine-related Guillain–Barré syndrome (GBS). In contrast, the 1978–79 influenza vaccine was not associated with a statistically significant excess risk of GBS. Although the original Centers for Disease Control study of the relation between A/New Jersey/8/76 (swine flu) vaccine and GBS demonstrated a statistical association and suggested a causal relation between the two events, controversy has persisted. At least one recent review of the data seemed to confirm the link. Another review suggested that there was no increased risk of acquiring GBS associated with the influenza vaccines administered during these seasons and that the causative 'trigger agent' in the A/New Jersey (swine) influenza vaccine administered in 1976 has not been present in subsequent influenza vaccine preparations.

There was no increase in the risk of vaccine-associated GBS from 1992–93 to 1993–94. A study by Lasky suggested only slightly more than one additional case of GBS per million persons vaccinated against influenza. More recently, an oculorespiratory syndrome has been described following influenza vaccine.

Recent Safety Events

A number of events in recent years has provoked concern that certain vaccines might be unsafe. Most of these concerns have proved to be unfounded, and some remain unproven or incompletely understood.

Thiomersal

Thiomersal is a mercury-containing preservative used in certain liquid vaccines such as DTP and hepatitis B vaccines. It came into the public gaze in 1999 as the result of a study being undertaken to estimate how much mercury was being administered to children in North America through vaccines. It transpired that the amount exceeded the allowed maximum according to environmental health permitted limits. Scientists assumed that ethyl mercury in thiomersal would have the same toxicology as methyl mercury, a chemical about which a great deal was known. Over the ensuing years, evidence was accumulated that showed it actually had a different metabolic pathway in the human body compared with methyl mercury. Both were metabolized in the liver and excreted in the gut, but methyl mercury was reabsorbed and accumulated in the body. Ethyl mercury, on the other hand, was found to be passed in the stool and lost from the body, thus avoiding a cumulative effect. Overwhelming scientific evidence indicates no clear link between neurodevelopmental disorders or autism and the receipt of vaccines containing thiomersal. One set of authors clearly flies in the face of the weight of scientific evidence, yet support a widely held public belief that thiomersal (in the quantities present in vaccines) is bad for children. Live viral and live bacterial vaccines do not contain thiomersal, largely because the preservative would kill the vaccine organism, rendering the vaccine useless.

Hepatitis B vaccine and multiple sclerosis

Reports of multiple sclerosis developing after hepatitis B vaccination have led to the concern that this vaccine might be a cause of multiple sclerosis in previously healthy subjects. A number of reliable studies have been consistent in showing that there is no association between hepatitis B vaccination and the development of multiple sclerosis.

MMR autism and bowel disease

Reports have proposed a link between the administration of the MMR vaccine and juvenile autism, Crohn's disease, and other forms of inflammatory bowel disease. The weight of scientific evidence strongly favors the conclusion that there is no direct association between measles virus, measles vaccine, autism, or inflammatory bowel disease.

Intranasal influenza vaccine and Bell's palsy

After the introduction of an inactivated intranasal influenza vaccine that was used only in Switzerland, 46 cases of Bell's palsy were reported. The relative risk of Bell's palsy in one study was found to be 19 times the risk in the controls, corresponding to 13 excess cases per 10 000 vaccinees within 1–91 days after vaccination. As a result, this vaccine is no longer in clinical use.

Yellow fever vaccine

Until recently, yellow fever vaccine was considered to be very safe. It has been given to many millions of people over more than 60 years to protect against yellow fever virus infection, which can be fatal in 5% or more of cases. From 1996 onward, a previously unrecognized adverse event of multiorgan failure and death was detected through passive surveillance in the United States, Australia, and other countries. Subsequent studies found that the risk of this rare adverse event was approximately 3 per 1 million doses, and vaccine recipients aged 65 years and older had a 10-fold higher risk than younger vaccinees, particularly following the first dose of the vaccine.

HIV Infection

The era of the HIV epidemic has had an impact on vaccine safety in a number of ways.

- It may be transmitted by injection (not only during vaccination). It could thus be transmitted by incorrect injection practices during routine immunization to a small number of infants attending a clinic at the same time. And it could theoretically be transmitted during mass campaigns where many more children might be at potential risk.
- Staff may be infected because of needle-stick injury or other improper handling of infected body fluids.
- It may alter the immune response of the recipient, either making him/her vulnerable to some negative effect of the vaccine, or diminishing the effect the vaccine has on protecting the infant. Some vaccines are not as effective in more immune-suppressed individuals, and severely immune-depressed infants must avoid vaccines such as BCG as they are at risk of disseminated BCG, which may be lethal.
- The course of the disease might be altered. The activation of CD4+ T lymphocytes following immunization could potentially augment HIV replication and result in accelerated progression to disease. Several, but not all, investigators have described increased HIV RNA plasma levels lasting several days following immunization.

On the positive side, the HIV epidemic has alerted health workers to the potential dangers of contamination during vaccine administration and has resulted in the safer use of syringes and proper disposal of injection equipment.

Conclusions

The design and manufacture of vaccines are of such high standards today that they have become extremely safe. But it will never be possible to say that everything is known about the subject and that vaccines are totally safe. Nor can the unpredictable human element in administration ever be totally excluded from the equation. But new methods of safety testing will emerge as each new threat is discovered. And new technologies will complement existing safety measures. However, communicating to vaccine recipients or their parents that the vaccine they are about to receive is neither totally effective nor totally safe makes health professionals uncomfortable. But the reality is that vaccines are many orders of magnitude safer than contracting the disease. Through constant vigilance, we can anticipate that vaccines will remain extremely safe – as safe as humanly possible. Any lesser alternative is unacceptable.

See also: AIDS: Vaccine Development; Antigenicity and Immunogenicity of Viral Proteins; DNA Vaccines; Immune Response to viruses: Antibody-Mediated Immunity; Neutralization of Infectivity; Vaccine Strategies; Yellow Fever Virus.

Further Reading

Afzal MA, Minor PD, and Schild GC (2000) Clinical safety of measles, mumps and rubella vaccine. *Bulletin of the World Health Organization* 78(2): 199–204.

Bellaby P (2003) Communication and miscommunication of risk: Understanding UK parents' attitudes to combined MMR vaccination. *British Medical Journal* 327: 725–728.

Causality assessment of adverse events following immunization(2001) *Weekly Epidemiology Record, 23 March, 12,* 76: 85–92. http://www.who.int/vaccine_safety/reports/wer7612.pdf(accessed February 2007).

Clements CJ and McIntyre PB (2006) When science is not enough – A risk/benefit profile of thiomersal-containing vaccines. *Expert Opinion on Drug Safety* 5(1): 17–29.

Cutts FT, Clements CJ, and Bennett JV (1997) Alternative routes of measles immunization: A review. *Biologicals* 25: 323–338.

Dellepiane N, Griffiths E, and Milstein JB (2000) New challenges in assuring vaccine quality. *Bulletin of the World Health Organization* 78: 2155–2162.

Halsey N (2003) Vaccine safety: Real and perceived issues. In: Bloom BR and Lambert P-H (eds.) *The Vaccine Book*, pp. 371–385. Amsterdam: Academic Press.

Kharabsheh S, Al Otoum H, Clements CJ, *et al.* (2001) Mass psychogenic illness following Td vaccine in Jordan. *Bulletin of the World Health Organization* 79(8): 764–770.

Madsen KM, Lauritsen MB, Pedersen CB, *et al.* (2003) Thimerosal and the occurrence of autism: Negative ecological evidence from Danish population-based data. *Pediatrics* 112(3, Part 1): 604–606.

Moss WJ, Clements CJ, and Halsey NA (2003) Immunization of children at risk for infection with human immunodeficiency virus. *Bulletin of the World Health Organization* 81(1): 61–70.

Safranek TJ, Lawrence DN, Kurland LT, *et al.* (1991) Reassessment of the association between Guillain–Barré syndrome and receipt of swine influenza vaccine in 1976–1977: Results of a two-state study. Expert Neurology Group. *American Journal of Epidemiology* 133(9): 940–951.

Shah K and Nathanson N (1976) Human exposure to SV40: Review and comment. *American Journal of Epidemiology* 103: 1–12.
Simonsen L, Kane A, Lloyd J, Zaffran M, and Kane M (1999) Unsafe injections in the developing world and transmission of bloodborne pathogens: A review. *Bulletin of the World Health Organization* 77(10): 789–800.
Supplementary information on vaccine safety. Part 2: Background rates of adverse rates of adverse events following immunization. WHO/V&B/00.36. http://www.who.int/vaccines-documents/DocsPDF00/www562.pdf(accessed February 2007).
Thompson NP, Montgomery SM, Pounder RE, and Wakefield AJ (1995) Is measles vaccination a risk factor for inflammatory bowel disease? *Lancet* 345: 1071–1074.

Relevant Websites

http://www.cioms.ch – Council for International Organizations of Medical Sciences (CIOMS) – WHO Working Group on Vaccine Pharmacovigilance.
http://www.brightoncollaboration.org – The Brighton Collaboration.
http://www.who.int – The WHO Expert Committee on Biological Standardization; WHO Programme for International Drug Monitoring; WHO's Global Advisory Committee on Vaccine Safety (GACVS).
http://www.who-umc.org – Uppsala Monitoring Centre (UMC), A WHO Collaborating Center.

Vaccine Strategies

I Kusters and J W Almond, sanofi pasteur, Lyon, France

© 2008 Elsevier Ltd. All rights reserved.

Introduction

To develop a new viral vaccine, get it licensed, and bring it to market is a lengthy, complex, and very expensive task. It requires a detailed knowledge of all aspects of the virus, especially its structure, epidemiology, pathology, and immunobiology, and demands a close collaboration between fundamental scientists, regulatory authorities, and industrial scientists and engineers. Completely new vaccines against human viruses appear infrequently on the market and the cost and complexity of their development has escalated with time, mainly due to the increased regulatory pressures to have highly defined products and to ensure complete clinical safety and high efficacy. Over the past 20 years or so, six new human virus vaccines have been developed for licensure in major markets; these are hepatitis B, Japanese encephalitis, hepatitis A, varicella (recently with a zoster formulation), and, over the past year, rotavirus, and human papilloma virus (HPV). The introduction of HPV vaccines is a technological triumph that offers to dramatically reduce the incidence of cervical cancer worldwide in the long term. The newest rotavirus vaccines seem, so far, to be free from the complications that led to the withdrawal of the earlier Rotashield vaccine in 1999 and promise to be very effective in reducing the burden of rotavirus diarrhea worldwide. In spite of this impressive progress, however, virus vaccine development has not accelerated over recent decades, and there remains a significant list of human virus diseases of widespread prevalence for which there are no vaccines available. These include human immunodeficiency virus (HIV), hepatitis C, hepatitis E, Eptein–Barr virus, herpes simplex virus (HSV), cytomegalovirus (CMV), respiratory syncytial virus (RSV), and parainfluenza viruses (PIV) (see **Table 1**).

In the veterinary world, the list of successes is significantly more impressive, and both the time cost of developing a new vaccine can be substantially reduced by the ability to reach proof of concept through direct challenge experiments, often with the wild-type virus. In this arena, the requirements are often very different to those for human vaccines, especially for vaccines of production animals where ease of administration and cost per dose are paramount. Recognizing that many approaches and outstanding fundamental challenges are similar for human and veterinary virus vaccines, this article focuses principally on the former with occasional reference to veterinary vaccines where they illustrate particular concepts.

Fueled by the new technologies of genomics, proteomics, and molecular immunology, the past 20 years have seen an impressive increase in our knowledge of all aspects of virology, providing insights to guide new vaccine concepts. The biological properties of viruses influencing choice of strategy include pathogenesis, serotype diversity,

Table 1 Human viruses causing disease with important medical need for which no vaccines are available

Adenoviruses	Human immunodeficiency virus (HIV)
Chikungunya	Human metapneumovirus (hMPV)
Cytomegalovirus (CMV)	
Dengue	Norwalk virus
Enterovirus 71 (EV71)	Parainfluenza virus (PIV)
Epstein–Barr virus (EBV)	Parvovirus B19
Hantavirus	Respiratory syncytial virus (RSV)
Hepatitis C (HCV)	Rhinovirus
Hepatitis E (HEV)	SARS
Herpes simplex virus (HSV)	West Nile virus

antigenic variation, immune evasion mechanisms, latency, and route of transmission. New vaccine candidates have been described for a good number of the viruses listed in **Table 1**, and although many of these are in an early, pre-proof-of-concept stage, some are substantially developed and offer realistic prospects of licensure over the next decade. The most promising of these, based on pre-clinical and clinical results obtained so far, are dengue, hepatitis E, and HSV and CMV. Significant challenges remain however, and really promising candidate vaccines against pathogens such as HIV, hepatitis C, and infant RSV remain elusive. This article focuses on the strategies available to develop new viral vaccines and discusses some of the challenges posed by the more difficult targets.

Types of Vaccines

There are multiple possible approaches to the development of a viral vaccine that can be generally described as follows:

1. Killed whole or split virus vaccines. This approach requires that the virus can be grown to high titer in cell culture or other scalable medium such as hens eggs; that the virus can be successfully and completely inactivated using an agent such as formaldehyde or B-propiolactone without destroying immunogenicity; that, from an industrialization perspective, the immunogenic dose is low to modest with respect to virus yield (in the 10 μg range) and that the killed whole or split particle elicits protective immunity. This approach has had excellent successes in the form of vaccines such as inactivated polio vaccine (IPV), hepatitis A (HAV) vaccine, and influenza.
2. Subunits or single proteins prepared by recombinant DNA methods and fermentation processes in cell culture. This approach may work well when a single protein can provide immunity and where the expression system allows appropriate folding and processing of the viral protein.
3. Live-attenuated vaccines. There are several approaches possible.
   a. Use of a closely related animal virus that is not well adapted for efficient and widespread replication in humans and therefore does not cause disease, but nevertheless provokes an immune response that protects against the corresponding human virus. The best known example of this is the use of vaccinia virus to vaccinate against smallpox, but a similar approach has been used for rotaviruses, with genetic reassortment to confer appropriate antigenicity.
   b. Development of an empirically attenuated human virus by multiple passages in tissue culture, typically of nonhuman origin, and/or passage in animals. Attenuation is usually achieved by the accumulation of a number of mutations that affect the efficient functioning at normal body temperature of various genes or gene products, thereby reducing virulence. Replication competence, however, is maintained at a sufficient level to stimulate a protective immune response. Although there are some drawbacks to this approach from a safety perspective (e.g., a risk of reversion), this method has provided a bedrock of vaccinology over many decades and has worked well for viruses such as polio – oral polio vaccine (OPV), mumps, measles, rubella, and yellow fever.
   c. Live-attenuated vaccines prepared by knowledge-based manipulation of the viral genome. There are several examples of candidate vaccines in this category including HSV and influenza.
4. Vectored or chimeric virus approaches. This is where an existing virus vaccine can be modified genetically to carry genes encoding antigens from a foreign virus. The chimeric vaccine should retain the attenuation and growth characteristics of the parent vaccine strain but stimulate immunity against the foreign virus.
5. Naked DNA. This is where a DNA encoding viral antigens plus appropriate expression control sequences is administered directly to the recipient. Expression of the DNA leads to an immune response against the antigens encoded.

Principles of Vaccine Development and Examples

When developing a new vaccine, the choice of approach is made very much on a case-by-case basis, and for a given virus is driven by knowledge of its biology, structure, antigenic diversity, and pathogenesis. High importance should be given to what type of immunity arises as a result of natural infection and whether the pathogen can cause persistent and/or repeated infections in a single host. Experiments in animal models may also allow the dissection of the immune response to identify correlates of protection. The use of primates in particular can be useful if the disease produced is similar to that observed in humans. However, many viruses are highly host specific and may have evolved strategies to evade immune responses that may also be host specific (such as recruitment of downregulators of complement fixation). Care must therefore be taken as results in animal models may not be entirely reproducible in the natural host.

Killed Vaccines

Evidence that circulating antibodies are sufficient to provide immunity may come, for example, from the observation that the disease is modified or exacerbated in

immune deficiencies such as hypo-gammaglobulinaemia and/or that passive immune globulin can protect against infection and disease. The latter observation was made for several viruses prior to vaccine development, including hepatitis A, suggesting that the key to vaccine development in these cases would be the stimulation of a strong humoral immune response. Such responses can often be adequately provided by killed vaccines, and so this was an obvious choice of approach. The strategy will also be influenced by the successes and failures with closely related viruses (either human or animal) that have similarities in epidemiology, pathogenesis, and mode of transmission. Thus, for HAV, the successful paradigm of the inactivated polio virus vaccine from the same virus family (*Picornaviridae*) provided further confidence that a killed whole virus particle approach would be effective. Indeed, inactivated HAV vaccines were developed successfully on this basis by several companies in the early to mid-1990s. Current HAV vaccines are prepared by propagating the virus in an approved cell substrate, human fibroblasts or human diploid cell culture MRC-5, purification, inactivation using formalin, and adjuvanted with aluminum hydroxide. The vaccines are given parenterally, as a two-dose series, 6–18 months apart.

A further example of a killed vaccine is rabies vaccine. Several WHO-recommended inactivated rabies vaccines are available currently. They are all similar in being whole virus, used after inactivation by β-propiolactone, and purification and concentration by ultracentrifugation and/or ultrafiltration. The vaccines are required to have a protective potency defined as >2.5 international units (IU). The potency is determined by the National Institute of Health (NIH) potency test which is based on assays using intra-cerebral challenge of previously immunized mice. The main differences between the rabies vaccines currently available lies in the cell culture used for production. The cell cultures used for the WHO-recommended vaccines are MRC-5 cells for purified human diploid cell vaccine (HDCV), Vero cells for purified Vero cell culture rabies vaccine (PVRV), and primary duck or chicken embryo fibroblasts for purified duck embryo vaccine (PDEV) and purified chick embryo culture vaccine (PCECV), respectively.

When a new virus emerges that poses a severe threat to human health such as the human coronavirus which caused severe acute respiratory syndrome (SARS) in 2002, it is necessary to start working immediately to develop a vaccine. The huge challenge in such a scenario is time. Generally, to develop a vaccine from basic research through animal studies, clinical lot development, analytical test development, clinical trials, industrial scaleup, and licensure takes 8–12 years. These timelines can be compressed in case of extreme urgency, but this compression is not unlimited. Although experience on existing vaccines can be exploited, all processes and procedures need to be evaluated, validated, and implemented. Working with a BMBL Section III Laboratory Biosafety Level 3 (BSL 3) agent, such as human SARS coronavirus, is not exceptional for vaccine manufacturers, but many precautions need to be taken in regards to equipment and laboratory practices, as little was known about the SARS virus in the early stages. The choice of the vaccine approach was indeed influenced by the time factor. If the virus grows well in cell culture, an inactivated viral vaccine is usually the option of choice as it is the fastest to accomplish. Fortunately, in case of the human SARS coronavirus, the virus did grow well on Vero cells, was efficiently inactivated either by formol or β-propiolactone, and appeared to be very immunogenic in several animal models as well as in human beings. After 2003, no more human SARS cases were observed and the development of such vaccines has generally been put on hold for the present.

Recombinant Protein Vaccines

As discussed above, a further simple strategy, particularly when only antibodies are required, is to use recombinant DNA methods to express a single surface structural protein of the virus in a host–vector system such as *Escherichia coli* or one of several yeast species. This approach may be adopted when the virus cannot be propagated efficiently in culture, making a killed vaccine approach impossible, when the inactivation process may diminish immunogenicity, or when a focused immune response against a specific protein is required. This approach has proved very successful for hepatitis B, where the vaccine is composed of particulate complexes of the virus surface glycoprotein HBsAg produced in yeast. These particles mimic virus-like particles produced during natural infection and induce a highly protective and long-lasting immune response. This vaccine has been on the market since 1992 as a three-dose series of injection, each containing 5–20 μg HBsAg. A new version of this vaccine has recently been licensed with a formulation containing 20 μg HBsAg, adjuvanted with monophosphoryl lipid A (MPL) and alum, which reduces the incidence of nonresponders compared to a population vaccinated with the licensed vaccine.

The recently introduced human papilloma virus vaccines have also been developed using the recombinant protein approach (see below). But as with killed vaccine approaches, one size does not fit all, and for many viruses the approach of recombinant protein expression has not proved successful for a variety of reasons. For example, many viruses have a complex structure that cannot easily be reproduced in foreign hosts at high yield, particularly when the final structure is formed from several conformationally interdependent proteins. Incorrectly folded or immature proteins may not elicit functional, protective antibodies. Second, for some viruses, immune responses

to several proteins together may be necessary to provide complete protection against disease. Third, recombinant proteins administered conventionally are generally poor at providing cellular responses of a Th1 profile that may be necessary for protection against viruses such as HIV, hepatitis C virus (HCV), and members of the herpes family. Consequently, for many viruses, live-attenuated approaches or more complex production systems and/or methods of delivery are required as discussed below.

Live-Attenuated Vaccines

Live-attenuated vaccines are used to prevent diseases such as yellow fever, polio, mumps, measles, and rubella. They are based on viral strains that have lost their virulence, but are still capable of replicating sufficiently well to provoke a protective immune response. They cause infection but without inducing the clinical manifestations, eliciting a humoral as well as cellular immune response. Historically, the attenuation was obtained by passage in animals. The first demonstration of attenuation of a virus in cell culture was that of the yellow fever virus by Lloyd and Theiler. The attenuation resulted from a prolonged passage on cultures of chick embryo tissue. Another example is that of the development of the oral polio vaccines by Albert Sabin in the 1950s. Wild-type strains of each of the three serotypes were passaged in monkey testicular tissue both *in vitro* and *in vivo*, while testing in monkeys was performed by intracerebral inoculation at various stages of passage. Eventually, strains were selected that were unable to induce paralysis in animals. The number of passages and cloning steps required to achieve the desired level of attenuation varied between the serotypes. These vaccines are still routinely used in many countries of the world and have been the principal tool with which the WHO has pursued its campaign of global polio eradication.

The advent of genome sequencing and recombinant DNA techniques in the 1980s allowed the key mutations conferring attenuation, empirically introduced by Sabin's passages, to be identified. In addition to temperature sensitivity mutations affecting protein structure, all three attenuated strains had in common, point mutations in the 5′ noncoding regions which affected the stability of RNA secondary structure believed to be important for interaction with host factors and for internal entry of ribosomes.

Rotavirus

As discussed above, live-attenuated vaccines may also be based on a closely related animal virus. The rotavirus vaccine licensed by Merck in 2006 is based on the bovine WC3 rotavirus. The original monovalent bovine strain is naturally attenuated for human beings, but does not induce protective immunity. To improve the effectiveness of the strain for human use, reassortant strains were prepared which contained genes encoding capsid proteins from the most common human serotypes on a background of the bovine strain. The present vaccine contains five single gene reassortants, each containing a gene for a capsid protein from human serotypes G1, G2, G3, G4, and PIA (**Figure 1**). A three-dose regimen with $2.0–2.8 \times 10^6$ infectious units per reassortant, administered orally beginning at age 6–12 weeks with a 4–10 week interval between doses, provides 70% protection against both mild and severe rotavirus diarrhea.

Nowadays, for some viruses, attenuated viral vaccines can be designed on a more rational basis, by specifically targeting virulence factor functions that may not be essential for virus replication, especially in cell culture, but are necessary *in vivo* to counter host innate defense mechanisms. An example here is the NS1 gene of influenza A viruses. This protein is able to downregulate interferon production in the virus-infected cell and some deletion mutants of NS1 lack this function and are therefore much more easily controlled by the host interferon response and are thereby attenuated. The augmented interferon (IFN) response provoked by such viruses may also have the advantage of providing stronger immune stimulation resulting in increased immune responses. So far, such strains have only been tested in animal models but they offer promise as future influenza vaccine strains.

A drawback of many live-attenuated vaccines is that they require a cold chain from point of production to point of use and this may pose logistical difficulty, especially in developing countries. Also, the safety of live-attenuated viral vaccines is under constant scrutiny because of the risk, albeit small, that the mutations conferring attenuation will revert to wild type, allowing the virus to become virulent again. This is the reason why some live-attenuated vaccines are not recommended for immunosuppressed patients.

Viral Vectors

If neither the killed nor the attenuated vaccine approach is appropriate or feasible, one can consider the use of a viral vector. In this case, an attenuated virus is used as a backbone carrying immunogenic proteins of the virus of interest. In general, the viral proteins chosen are the membrane and/or envelope proteins as these proteins are presented on the outside of the virus particle and recognized by the immune system. An example of a viral vector is the yellow virus vaccine strain 17D. This vaccine strain was developed in the 1930s, since which time over 400 million people have been immunized with this vaccine. The strategy here is to use the 17D vaccine as a vector to deliver the two structural proteins, the premembrane PRE-M and envelope proteins from closely related

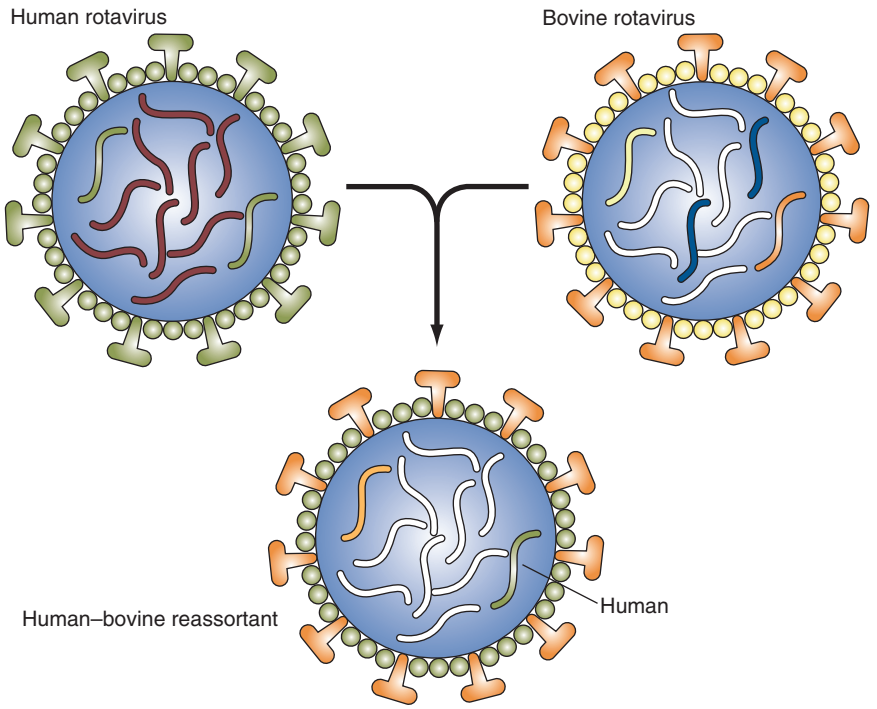


Figure 1 Rotavirus reassortant to generate oral live virus vaccine. RotaTeq is a polyvalent vaccine consisting of five human-bovine reassortants: four G serotypes (G1, G2, G3, G4) representing 80% of the G strains circulating worldwide, and one P serotype representing >75% of the P strains circulating worldwide. Reprinted by permission from Macmillan Publishers Ltd: *Nature Medicine* (Buckland BC (2005) The process development challenge for a new vaccine. *Nature Medicine* 11: S16–S19.), copyright (2005).

flaviviruses. The resulting chimeric virus needs to be viable and to replicate efficiently in an acceptable cell substrate for vaccine production. The chimerivax dengue virus approach (by Acambis in collaboration with sanofi pasteur) has been developed using the PRE-M and envelope genes of wild-type clinical isolates. The technology involved is illustrated in **Figure 2**. The yellow fever virus genome has been cloned as an infectious cDNA. This infectious cDNA is manipulated to remove the PRE-M and E-Genes of yellow fever virus and exchange them for the coat protein genes of each of the dengue virus serotypes. Thus four individual chimerivax cDNAs are constructed. Transcribing these cDNAs to RNA provides infectious RNA with which to transfect cells in culture. Thus, the resulting virus is a heterologous virus containing the immunizing antigens of dengue virus with the replicative engine of the yellow fever 17D vaccine. Chimeric dengue viruses are expected to mimic the biological properties of yellow fever 17D which has the excellent characteristics of providing minimal reactogenicity and lifelong immunity. The candidate vaccines have been characterized in preclinical models, neurovirulence in mice, viremia and immunogenicity in monkeys, and shown to have desirable characteristics. Moreover, when the four constructs are mixed and administered to monkeys, seroconversion against all four dengue serotypes appears to occur simultaneously in most infected animals. Moreover, the antibodies generated seem to be functional in that they neutralize dengue viruses in plaque reduction tests. The chimerivax dengue viruses grow well in culture and are well suited to industrial scaleup. In human volunteers, the chimeric viruses are safe and well tolerated and elicit specific immune responses against the different dengue serotypes. These strains therefore provide excellent candidates for further development.

DNA

Since the early 1990s, there has been considerable interest in the possible use of naked DNA as a vaccine delivery method. Naked DNA has the advantage that it can be taken up by cells and express the viral protein encoded. Depending on the conditions, this expression can be mid- to long term, thereby providing a substantial stimulation of the immune response. DNA vaccinology has apparently worked well in mice, but, so far, results in humans have been mainly disappointing requiring milligram amounts of DNA. Delivery of the DNA on colloidal gold, however, seems to offer a better prospect of success, as reported recently for hepatitis B virus and influenza. Regulatory issues concerning the use of DNA vaccines and its possible insertion integration into chromosomal genes are potential drawbacks to this type of approach, especially for use in prophylactic vaccination in infants. Further work is needed on safety issues before it can be seriously considered as a means to vaccinate populations.

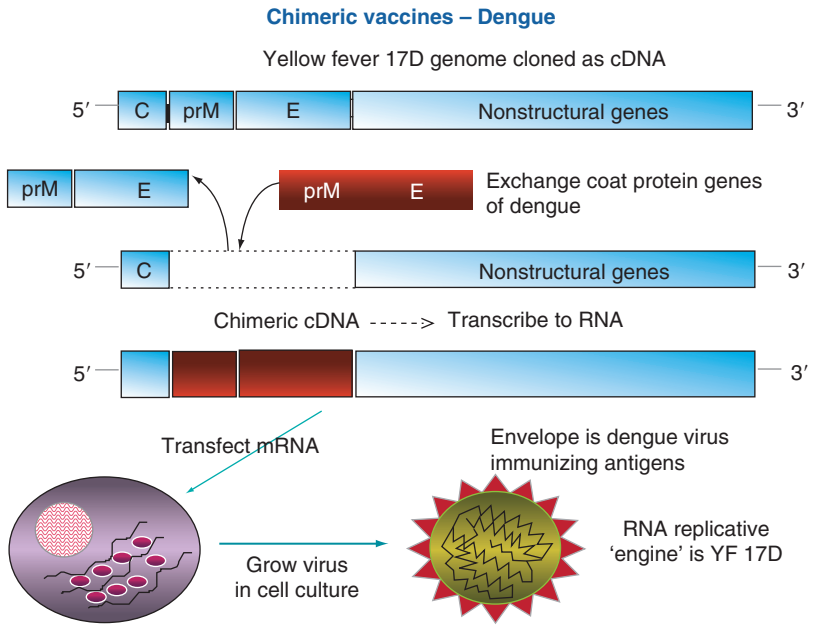


Figure 2 A DNA copy of the genome of yellow fever virus (blue) is manipulated to replace the prM and E genes by those of a related flavivirus such as Dengue (red). Transfection of mRNA transcribed from the resulting cDNA produces a 'chimeric virus' in cell culture.

The Challenge of Antigenic Variation

Antigenic Variation

Antigenic variation is displayed by a number of important pathogenic viruses and poses a particular problem for vaccine developers. The variation may be manifest in different ways depending on the virus' natural biology. Thus, for some viruses such as foot-and-mouth disease virus (FMDV), rhinoviruses, and HPV, multiple antigenic variants or serotypes co-circulate, sometimes with particular geographical patterns or ecological niches. Individual strains may show antigenic drift, presumably generating new serotypes over time. The rate of drift and the generation of new serotypes are not well understood for these viruses but may involve genetic recombination as well as cumulative mutational change. Other viruses may show a different pattern of antigenic variation: for example, influenza A viruses circulate as a limited number of subtypes (currently two in humans, H1 and H3), and each of these accumulate antigenic changes over several seasons (antigenic drift), escaping the most recently generated population-based immunity as they evolve. Occasionally, a new subtype may emerge (antigenic shift) either through genetic reassortment of a human strain with an avian strain, or possibly through direct evolution from an avian strain. Generally new subtypes displace existing subtypes as was the case when H2 emerged to displace H1 in 1957 and when H3 displaced H2 in 1968. For reasons that are not clear, this displacement did not occur when H1 reemerged in 1976 and since then there have been two influenza A subtypes co-circulating in the human population. This type of 'longitudinal' antigenic variation is clearly different from that of the multiple serotype viruses discussed above, and is generally more tractable in terms of vaccine development. Yet a different pattern is observed with HIV and to some extent HCV, where a limited number of genetic clades may contain very many different antigenic variants and where longitudinal variation to escape recently generated immune responses occurs within a single persistently infected individual. This type of natural biology generates a plethora of antigenic variants that can co-circulate. Providing immunological protection against all of these is an immense challenge for vaccine developers.

Multiple Serotype Vaccines

So what are the strategies available to develop vaccines against antigenically variable viruses? Most straightforwardly, one can generate simple killed vaccines against the currently circulating strains as discussed above and use these where the virus is prevalent. This strategy has had some success in the case of FMDV, where the geographical range of the virus may be (at least partially) restricted by regulations on movement of susceptible farm animals, and the vaccines are cheap and quick to prepare. However, even though simple killed vaccines have been shown to work for individual serotypes of rhinoviruses, it is difficult to imagine that this approach would be effective for this virus where, presumably because of widespread human contact and international travel, there seems to be a freer global circulation of multiple and

unpredictable serotypes each winter. Moreover, for most people, rhinovirus infections are relatively trivial and therefore the balance of medical need versus industrial feasibility/commercial attractiveness of preparing vaccines in advance against multiple strains does not favor such a strategy. It would perhaps be a different matter if it were possible to design a simple rhinovirus vaccine that provided cross-protection against all serotypes.

The case of HPV is more manageable because of the small number of serotypes associated with severe disease. Thus, in this case, a significant impact on disease can be made by vaccinating against just a few of the many different genotypes. Both currently available vaccines contain HPV types 16 and 18 to vaccinate against cervical cancer, and the Merck vaccine contains, in addition, HPV types 6 and 11 to vaccinate against genital warts (condylomata accuminata). The absence of efficient cell culture systems for papillomaviruses has required the development of eukaryotic expression systems to produce virus-like particles composed of the L1 capsid protein, which are highly immunogenic. Future, second-generation vaccines will likely incorporate one or more of the additional highly or moderately oncogenic serotypes, such as 31, 33, and 35.

Influenza Annual Vaccination

For influenza, the limited number of circulating subtypes in any particular season makes it possible to adopt a strategy of annual vaccination. Thus, current influenza vaccines are trivalent, containing strains of H1 and H3 of influenza A and an influenza B strain. The strategy of annual vaccination is not without risk and requires a high level of international cooperation on disease surveillance and strain isolation, construction of high-yielding seed viruses, preparation of reagents for formulation, and industrial production. Following strain selection and recommendation by WHO, vaccine production has to occur over a very tight time schedule to ensure that vaccine is ready for the following winter (**Figure 3**). Occasionally problems arise such as a mismatch between the selection of a particular vaccine strain and the virus that eventually circulates during the following winter. This may compromise the effectiveness of that particular component of the vaccine. Other potential problems include less than optimal growth of the high growth reassortant seed at the industrial scale, leading to less vaccine being produced, and the lateness of seeds or reagents impacting on prompt delivery of vaccine for the flu season. The vast majority of influenza vaccine used currently is partially purified, killed whole or split virus prepared in embryonated hen's eggs, formulated to 15 μg of hemagglutinin (HA) of each strain, and provides generally good protection that correlates well with the induction of virus neutralizing or hemagglutination-inhibiting antibodies. However, vaccines prepared using different technologies are arriving on the market or are in advanced stages of development. These include live-attenuated (cold-adapted) strains, licensed by MedImmune in the USA in 2003, influenza-recombinant surface protein (hemagglutinin) produced in a baculovirus expression system from Protein Sciences, and inactivated

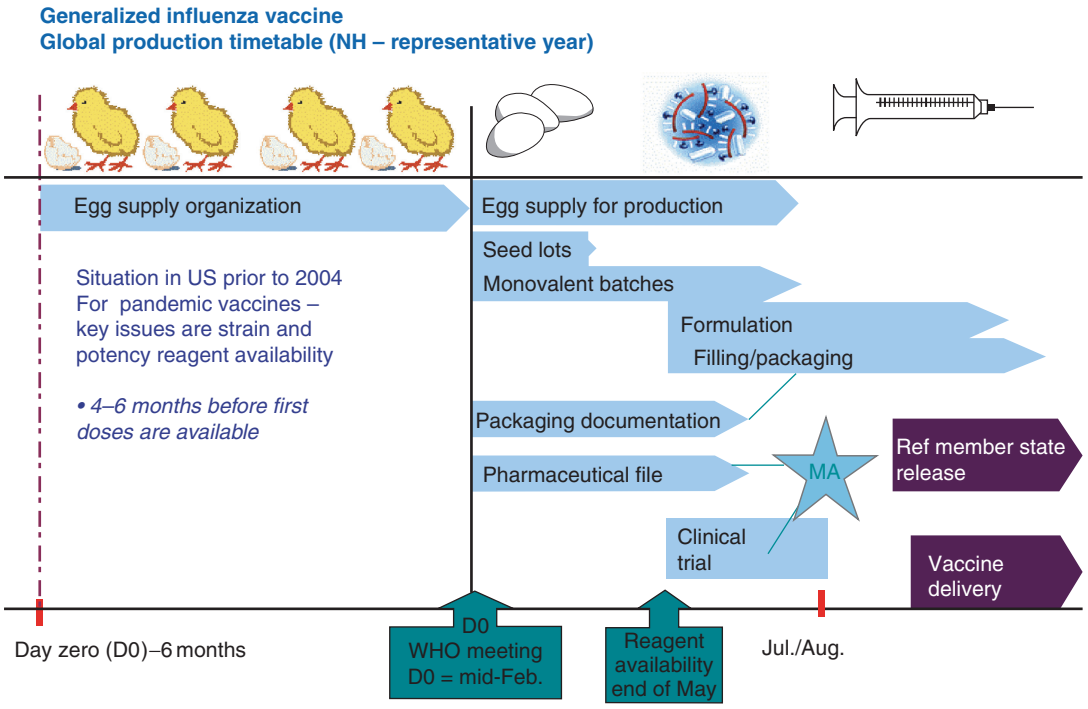


Figure 3 Approximate time schedule for the production of annual influenza vaccine from embryonated hens eggs.

virus vaccines prepared in cell cultures such as Vero, Madin–Darby canine kidney (MDCK), or PerC-6. This exploration of alternative technologies in recent years has been fueled by several criteria, perhaps the most important of which is greater and more flexible scaleup capability. The recent concerns over the possible emergence of an H5 pandemic has focused attention on the present worldwide limits in capacity, most notably in a situation of 'surge' demand, and governments have responded by providing the industry with incentives to increase capacity and diversify methods of production. A further response to this concern has been 'dose sparing' clinical studies on influenza vaccines adjuvanted with alum and other, proprietary adjuvants. These studies, using an H5N1 strain, suggest that it may be possible to reduce the vaccine dose from 2×90 μg HA nonadjuvanted, to 2×30 μg HA adjuvanted with aluminum hydroxide or even as low as 3.75 μg HA adjuvanted with new proprietary adjuvants. Moreover, there have been renewed suggestions that it may be possible to develop vaccines with a broader and perhaps multiseasonal protective effect by stimulating cellular immune responses, particularly against nucleoprotein (NP), a claim currently made for the live-attenuated approach, and even a universal flu vaccine, for example, based on the well-conserved M2 virus surface protein. Animal challenge experiments, especially using multiple arrays of the M2 protein, have been encouraging to date and suggest it may be possible to provoke a much stronger response against M2 than that induced by natural infection. Whether such a response will provide solid protection in humans however remains to be established.

HIV Approaches

The vast array of antigenic variants of HIV and HCV renders the approaches discussed above extremely difficult for these types of viruses. For HIV in particular, many strategies have been tried, so far without significant success. Early on in the AIDS pandemic the focus was on the use of simple recombinant surface glycoproteins, aimed at inducing neutralizing antibodies in the hope that even limited protection against homologous or closely related strains would provide a proof of concept that could be built upon. Unfortunately, this type of approach was not successful and a large phase III clinical trial carried out by Vaxgen using *E. coli*, which produced gp120, was not able to provide convincing evidence of protection even against strains closely related to that present in the vaccine formulation. Subsequently, there has been considerable effort on the induction of cellular responses, especially CD8 cytotoxic T lymphocytes, and more recently on a balanced cellular response to include CD4 effector mechanisms. The objective here is to provide the means for the immune system to launch an immediate attack on the first cells to become infected following exposure to the virus. Ideally, such an attack would prevent the primary viremia by eliminating the virus before it becomes established in the body. However, more realistically, there is evidence from primate studies that strong preexisting T-cell responses can control the primary viremia and reduce the viral set point during the asymptomatic phase. A low viral set point is associated with slow or no progression to AIDS. In addition, the HIV evades immune surveillance by actively downregulating the major histocompatibility complex I (MHCI) molecules on the surface of infected cells by the HIV nef protein. Vaccination strategies to produce cellular responses have mainly used vectors such as the vaccinia virus strains MVA and NYVAC, canarypox, adenoviruses, or naked DNA, either with multiple doses of a single type of construct or in heterologous prime-boost strategies. Antigens delivered have ranged from substantial regions of gag-pol-env of HIV to multiple copies in a 'string of beads' format of defined T-cell epitopes presented by common human leukocyte antigen (HLA) haplotypes. In general, these studies have not delivered T-cell responses of sufficient magnitude to be strongly encouraging, although one such strategy based on canarypox delivery of gag-pol-env antigens, followed by boosting with a recombinant env protein, has progressed to a clinical phase III study. Although many commentators have expressed doubt about whether this approach will show efficacy, it may generate useful information on the role of cellular responses in controlling HIV loads.

Most recently, HIV vaccine efforts have again turned to the induction of neutralizing antibodies, this time aimed specifically at epitopes that have been defined by studying unusual but highly informative broadly neutralizing monoclonal antibodies. The fact that such antibodies exists is highly encouraging from a vaccine perspective. The concepts here are based on the notion that certain conserved but crucial regions of gp120 or gp41 are naturally poorly immunogenic, either because they are relatively hidden in the conformationally folded protein or are shielded by strongly immunogenic noncritical domains or by glycosylation, or because they are only transiently exposed during the structural rearrangements that accompany cell binding and virus penetration. It is argued that because antibodies against these regions are neutralizing they will be protective if they can be generated prophylactically with sufficient avidity and at sufficient titer. This 'cryptic epitope' idea has been discussed for several viruses over many years and is akin to that mentioned above for influenza M2, in that the objective is to generate a far stronger response against a particular antigen or epitope than that resulting from natural infection. Such antibodies, once induced, will need to have the kinetic properties necessary to effectively neutralize the virus *in vivo*. So far there are no examples among virus vaccines that prove this concept. For HIV gp160, the particular

construction, presentation, and formulation of molecules able to raise high-titer antibodies against these conserved regions (many of which are imprecisely defined) are far from obvious. Nevertheless, the induction of prophylactic immune responses of this type is certainly worth detailed investigating in detail, given the magnitude of the HIV problem. The challenge of developing a vaccine against HIV however remains immense.

Conclusions and Perspectives

The easy viral vaccine targets of significant medical importance have been done. The viruses against which we do not have vaccines today are either of regionalized or sporadic importance medically and the incentives to develop them have not been sufficiently large or they are viruses that pose significant challenges in terms of their biological characteristics. Thus, HIV and HCV pose challenges because of their antigenic variation and the fact that natural immune responses are unable to protect and/or eliminate the virus. RSV poses challenges because of the immunopotentiation of pathogenesis that, for infants, must be avoided at all costs.

Nevertheless, there are grounds for optimism. A new generation of adjuvants, making it possible to selectively orientate immune responses toward Th1 or Th2 as necessary, promises the possibility of being able to 'improve on nature' in terms of immune response provoked by the viral antigens. New developments in vectors and virus 'chimeras' offer promise for vaccines such as dengue and perhaps RSV and parainfluenza viruses and targeted modification of immunomodulatory genes may offer prospects of new vaccines against herpes family viruses. Fundamental studies on virus pathogenesis, epidemiology, and immunobiology are greatly aided by new technologies such as genomics and proteomics and it is likely that the improved understanding will increase the technical and scientific feasibility of developing new viral vaccines in the years ahead.

See also: AIDS: Vaccine Development; Antigenic Variation; Antigenicity and Immunogenicity of Viral Proteins; DNA Vaccines; Immune Response to viruses: Antibody-Mediated Immunity; Neutralization of Infectivity; Vaccine Production in Plants; Vaccine Safety.

Further Reading

Buckland BC (2005) The process development challenge for a new vaccine. *Nature Medicine* 11: S16–S19.

Garber DA, Silvestri G, and Feinberg MB (2004) Prospects for an AIDS vaccine; Three big questions, no easy answers. *Lancet* 4: 379–413.

Koelle D and Corey L (2003) Recent progress in herpes simplex virus immunobiology and vaccine research. *Clinical Microbiological Reviews* 16(1): 69–113.

Plotkin SA, Rupprecht CE, and Koprowski H (2005) Rabies vaccine. In: Plotkin SA and Orenstein WA (eds.) *Vaccines,* 4th edn., pp. 1011–1038. Philadelphia: Saunders.

Vaccinia Virus

G L Smith, Imperial College London, London, UK

© 2008 Elsevier Ltd. All rights reserved.

The Origin of Vaccinia Virus

The origin and natural host of vaccinia virus (VACV) remain unknown and have been discussed in detail by Baxby. Although cowpox virus (CPXV) is believed to have been used by Jenner in 1796 for vaccination against smallpox, Downie demonstrated in 1939 that the smallpox vaccine strains in use in the twentieth century were biologically distinct from CPXV. They were called VACV after *vacca*, Latin for cow. Analysis of the genomes of many orthopoxviruses (OPVs) by restriction endonuclease digestion and sequencing confirmed that VACV is a distinct OPV species. Genome sequence analyses showed that VACV was not formed directly from CPXV and variola virus (VARV) by recombination or indirectly from either virus by passage and mutation.

So where did VACV come from? The most probable explanation is that VACV is an OPV that is either no longer endemic or present in a host species in which it is not recognized. Horsepox virus (HSPV) has been proposed, and several anecdotes are consistent with this theory. First, Jenner and other early vaccinators took vaccine from horses when the supply of cowpox (a rare disease) was short. Second, the VACV interferon gamma (IFN-γ)-binding protein binds equine IFN-γ (and IFN-γ from several other species). Third, the sequence of an OPV that caused an epidemic in horses in Mongolia is more closely related to VACV strains than to other OPVs. However, none of these observations proves that VACV has an equine origin. It is possible that VACV in horses is a zoonosis and that its natural host is elsewhere, rather as CPXV and monkeypox virus (MPXV) are

misnomers since their natural reservoirs are different types of rodent.

Currently, VACV-like viruses are circulating among buffaloes in northern India and cattle in South America, but it remains unclear whether these represent infections of animals with the smallpox vaccine that was transferred from man or whether they are natural reservoirs of VACV. VACV can infect many species and has a relatively broad host range, like CPXV and unlike VARV and camelpox virus (CMLV).

If one assumes that Jenner used CPXV for smallpox vaccination in 1796 (and this is uncertain because there is no virus isolate to study), sometime between 1796 and 1939 VACV replaced CPXV as the smallpox vaccine. It is not known when this happened, but anecdotal evidence suggests that it had occurred by the middle of the nineteenth century. First, the smallpox vaccine that was taken from England to the USA in 1856 and that became the New York City Board of Health vaccine is VACV and not CPXV. Second, the histological descriptions of cells infected by the smallpox vaccines in the late nineteenth century mentioned the cytoplasmic B-type inclusion bodies that are characteristic of OPV infections and made by both VACV and CPXV, but failed to report the much more obvious A-type inclusion bodies made by CPXV and not VACV. The failure to report A-type inclusions is consistent with the vaccine used at that time not being CPXV.

VACV: The Smallpox Vaccine

Widespread vaccination with VACV led to the control of smallpox in many countries by the 1970s, its removal from the Indian subcontinent by 1975, and its eradication by 1979. The last naturally occurring case was in Somalia in 1977, but the last person to die of smallpox was in Birmingham, UK, in 1978, following a laboratory escape of the virus. The eradication of smallpox remains the greatest triumph of the World Health Organisation (WHO) and a fine example of the benefit of cooperation among nations. Smallpox and its eradication are described beautifully in a monumental book published by the WHO in 1988.

Although VACV was used to achieve the global eradication of smallpox, it was a vaccine with an imperfect safety record. Several types of complication were noted. Generalized vaccinia was a systemic skin infection that usually resolved without sequelae. Progressive vaccinia was a grave condition that occurred in people with T-cell immunodeficiency. In these cases, the virus spread from the vaccination site without restriction and was often fatal. Eczema vaccinatum was a skin condition associated with infection of eczematous patients and usually resolved safely. There were also unpredictable neurological complications, such as encephalitis and encephalopathy, that were sometimes fatal. Finally, there were cases of myocarditis, and these have been evident again during recent vaccinations in the USA. All types of complication were more frequent in primary vaccinees than in those vaccinated previously. Large-scale studies of the complications of smallpox vaccination in the USA during the late 1960s recorded nine deaths after 14.2 million vaccinations. Without careful screening of vaccinees, it is probable that widespread vaccinations today with the old vaccines would induce a greater number of complications because of more widespread eczema and increased immunodeficiency due to infection with human immunodeficiency virus.

Attenuated VACV Strains

The complications of smallpox vaccination led investigators to develop safer candidate vaccines for smallpox. These were of two types: virus preparations from which infectivity had been removed by chemical or physical means, and virus strains that had diminished virulence. Trials with killed virus preparations failed to induce protection in models of OPV infection because these vaccines were composed largely of intracellular mature virus (IMV) and so lacked the antigens present in the outer envelope of the extracellular enveloped virus (EEV) (see the section titled 'Virion structure'). These antigens are important for the induction of protective immunity against OPV. The attenuated virus vaccines show more promise and several were produced, including modified virus Ankara (MVA) in southern Germany, LC16m8 in Japan, and NYVAC in USA. However, although these viruses are certainly less reactogenic in man, their ability to induce protective immunity is uncertain because they were never used in populations that were exposed to smallpox. They might induce protection against smallpox or they might not. While their utility as smallpox vaccines is uncertain, they do show considerable promise as recombinant vaccines for the delivery of antigens from other pathogens against which protective immunity is needed. In addition, these attenuated virus vaccines might be used for primary smallpox vaccination, followed by vaccination with the traditional vaccines known to protect against smallpox, in order to reduce adverse reactions. Indeed, this strategy was used with the MVA strain in Germany.

Taxonomy

Vaccinia virus is the most intensively studied species in the genus *Orthopoxvirus* of the family *Poxviridae*, and several VACV strains have been sequenced. The first strain sequenced was Copenhagen, which has been a reference for comparison with the sequences of additional VACV strains and other OPVs. Other commonly used, sequenced VACV

strains are Western Reserve (WR), Lister (and derivatives LC16m0 and LC16m8), and MVA. All VACV strains cluster closely together phylogenetically, as do all other OPV species except for CPXV, where two strains (GRI-90 and Brighton Red) are more divergent and might eventually be considered as separate species. HSPV appears to be the OPV most closely related to VACV.

Genome Structure

The VACV genome is a linear, double-stranded DNA (dsDNA) molecule of between 185 and 200 kbp with covalently closed termini that are linked by hairpin loops. There are inverted terminal repeats (ITRs) that vary in length between different VACV strains. These contain blocks of tandem repeats and a few genes that, consequently, are diploid. VACV genes are tightly packed so that there is little noncoding DNA, and mRNAs are not spliced. Overall, the genome may be divided broadly into three regions. The central region (about 100 kbp) encodes proteins that are conserved between VACV strains, other OPVs, and, indeed, all chordopoxviruses. Most (89) of these proteins are essential for some aspect of virus replication. In contrast, the left and right terminal regions encode proteins that are more variable between different VACV strains and other OPVs and are nonessential for virus replication. Instead, these genes affect virus host range, virulence, and interactions with the host immune system. Like other OPVs, the VACV genome is rich (67%) in adenine and thymine (A + T) residues.

Virion Structure

The structure of the VACV particle has been studied extensively and different models have been presented. There is agreement that there are two morphologically distinct forms of virus produced from each infected cell, and these have been called IMV and EEV. The terms mature virus (MV) and extracellular virus (EV) have been used by some authors recently. Each virion is brick-shaped, has dimensions of 250 nm × 350 nm, and is surrounded by one or more lipid membranes. The formation of IMV and EEV is considered later (see the section titled 'Assembly'). Studies in the early 1960s proposed that the IMV particle is surrounded by a single membrane, which is synthesized *de novo* in the cytoplasm and is not linked to cellular membranes. Subsequently, this view was challenged and it was proposed that the IMV form was surrounded by a double layer of membrane derived from and continuous with preexisting cellular membranes between the endoplasmic reticulum and the Golgi stack. Current evidence favors a single membrane covered by a proteinaceous layer. Inside this, there is a dumbbell-shaped virus core with lateral bodies in the concavities of the core. The core contains pores in its outer layer and is surrounded by a palisade of protein spikes. Within the core, the virus genome is packaged with structural proteins and enzymes that are needed to transcribe virus genes upon infection.

The EEV form comprises an IMV that is surrounded by an additional lipid membrane derived from the *trans*-Golgi network or early endosomes. The extra lipid envelope is fragile and contains antigens that are absent from the IMV particle.

Replication Cycle

Entry

The entry of VACV starts with the binding of either an IMV or EEV to the cell surface. The cell receptors for these virions have not been identified, but are different. The entry of IMV is considered first. IMV particles can bind glycosaminoglycans (GAGs) or laminins on the cell surface. Although several virus proteins (H3, D8, and A27) were reported to bind GAGs, these interactions seem nonessential because virions lacking these proteins individually retain infectivity. After binding to the cell, the IMV particle enters either by fusion at the cell surface in a pH-independent manner (**Figure 1**) or by endocytosis followed by fusion with the vesicle membrane. Unlike other enveloped viruses, which usually have a single protein mediating fusion of the virus and cellular membranes, the VACV fusion machine consists of a complex of at least nine proteins that are all essential for entry.

The entry of EEV is more complicated, owing to the need to remove both lipid envelopes before the core can be released into the cytoplasm. This is achieved by shedding the EEV outer envelope via a nonfusogenic mechanism that is triggered by interaction of EEV surface proteins with GAGs on the cell surface (**Figure 2**). This enables the IMV particle within the EEV envelope to bind to the cell surface and enter in the same way as a free IMV particle. Once a virus core is released into the cell, it is transported on microtubules deeper into the cell, and virus factories develop near the nucleus.

Transcription

Within minutes of infection, VACV mRNAs are synthesized by the virus DNA-dependent RNA polymerase, which is packaged in virions together with associated transcription factors and enzymes to cap and polyadenylate the mRNAs. Virus mRNAs are extruded from the core, presumably through the pores in the core wall. Early proteins include enzymes for synthesis of nucleoside triphosphate precursors and for DNA replication, and factors that block the innate immune response to infection. After early proteins have been expressed, the virus core is

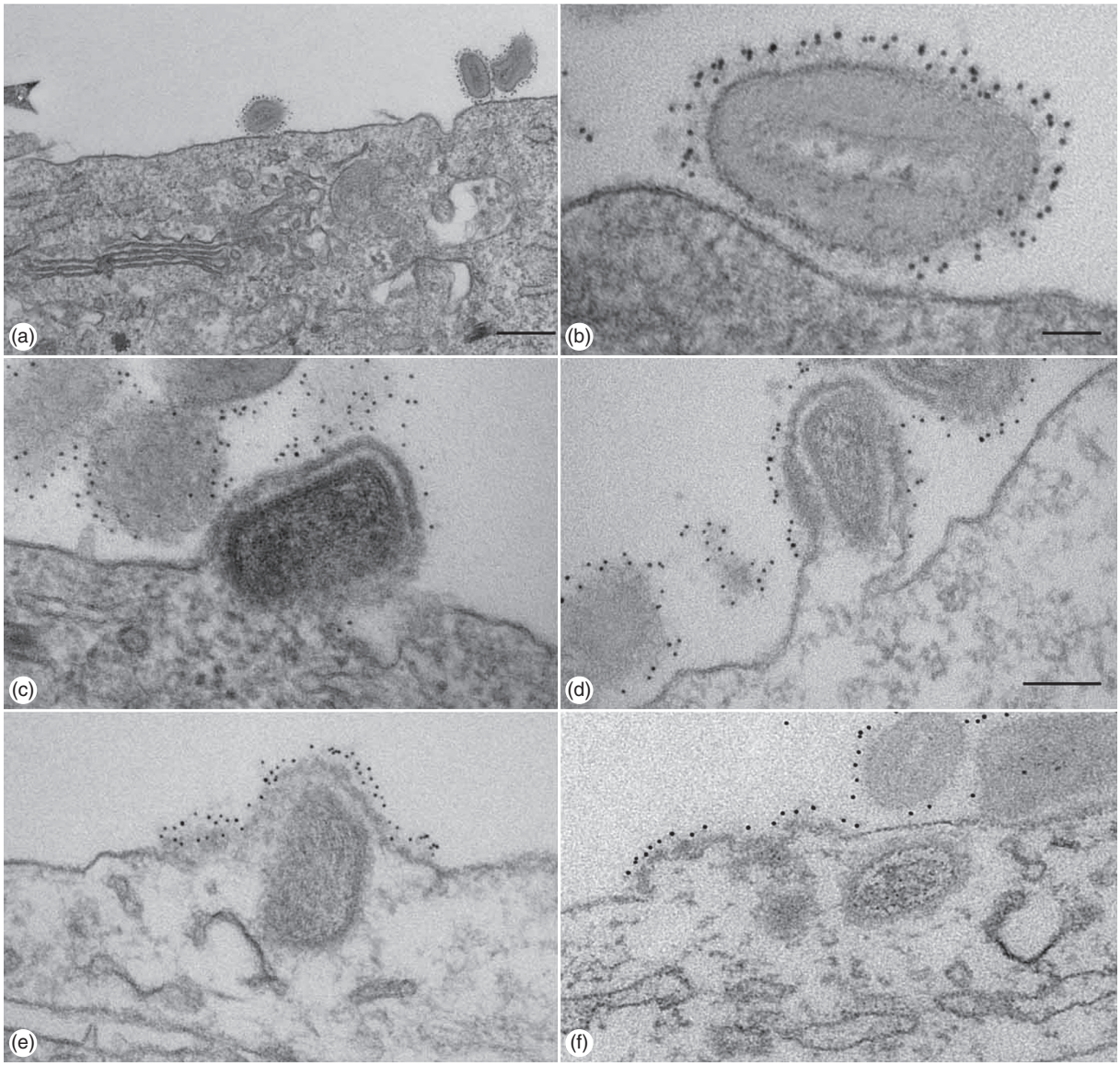


Figure 1 Electron micrographs showing the entry of VACV IMV by fusion at the cell surface. Purified VACV strain Western Reserve (WR) IMV (325 p.f.u./cell) was spinoculated onto PtK_2 cells to study the binding and entry of IMV, and samples were either retained on ice or incubated at 37 °C for 10 min. The IMV surface antigen D8 was labeled with D8-specific monoclonal antibody (mAb) for immunoelectron microscopy. Images show IMV particles bound to cells at 4 °C (a) and 37 °C (b), the IMV membrane fusing with the plasma membrane (c and d), the core entering into the cytosol (e), and the core leaving the site of entry (f). Scale = 200 nm (a), 50 nm (b), and 100 nm (d). Panels (c)–(f) are all shown at the same magnification. Reproduced from Carter GC, Law M, Hollinshead M, and Smith GL (2005) Entry of the vaccinia virus intracellular mature virion and its interactions with glycosaminoglycans. *Journal of General Virology* 86: 1279–1290, with permission from the Society for General Microbiology.

uncoated further and the genome is released for DNA replication. Once virus DNA replication has commenced, the pattern of transcription changes and intermediate genes are transcribed. Several intermediate genes encode late transcription factors needed for expression of late genes. Late genes encode the structural proteins that make up new virus particles, enzymes that may be packaged into these virions, and additional factors that influence the host response to infection. Host protein synthesis is shut off shortly after VACV infection, and this is mediated by the D10 protein, which removes the methylated cap structure of mRNAs. Virus and host mRNAs are both cleaved, and, since the former are more abundant, virus protein synthesis predominates.

DNA Replication

Virus DNA replication takes place in cytoplasmic factories from which most cellular organelles are excluded. The virus genome encodes many of the factors and

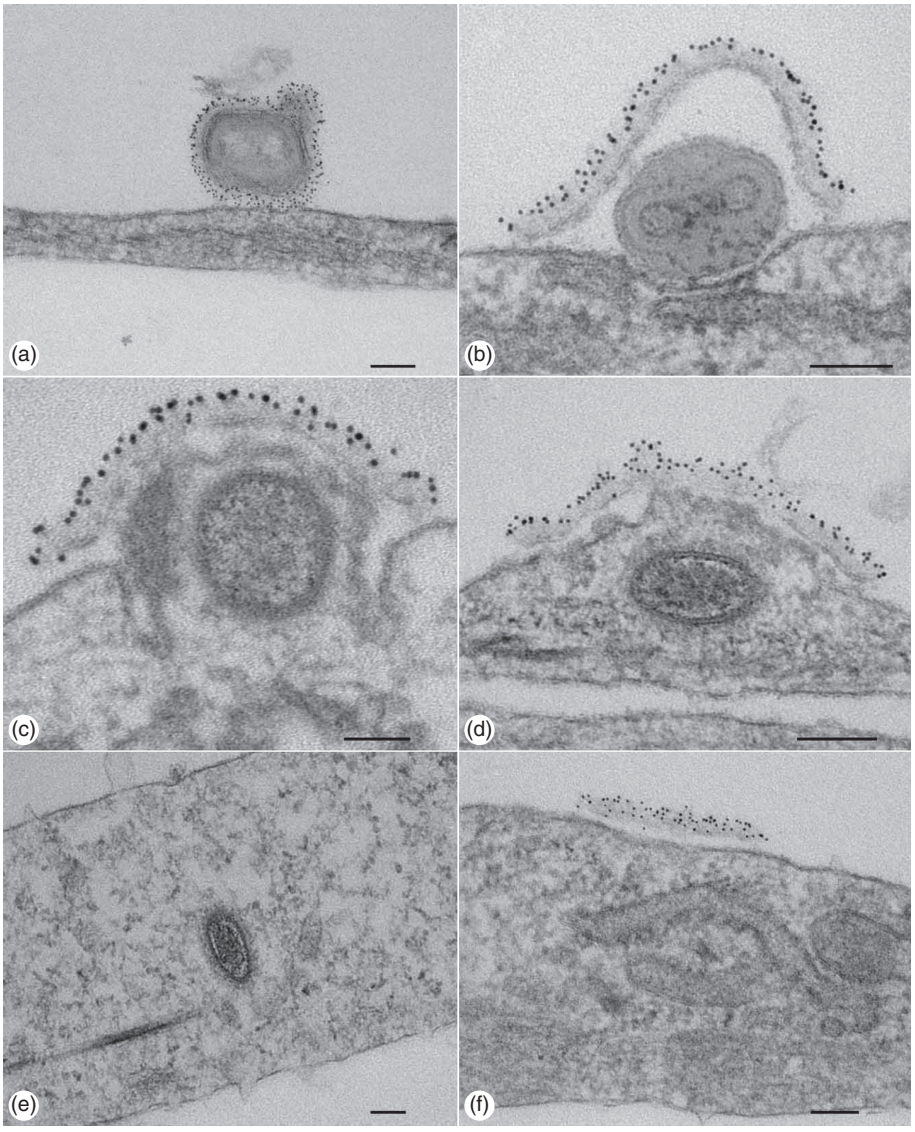


Figure 2 Electron micrographs showing the entry of EEV into cells by ligand-induced nonfusogenic dissolution of the EEV outer envelope followed by fusion of the IMV with the plasma membrane as in **Figure 1**. Fresh EEV of VACV strain WR was spinoculated onto PtK2 cells at 4 °C (a) and then incubated at 37 °C for 10 min (b–f). The EEV surface was labeled by rat anti-B5 monoclonal antibody (mAb) 19C2 (10), then by rabbit anti-rat IgG followed by 6 nm protein A–gold conjugate, and the samples were processed for electron microscopy. Scale = 100 nm (for all panels). Reproduced from Law M, Carter GC, Roberts KL, Hollinshead M, and Smith GL (2006) Ligand-induced and nonfusogenic dissolution of a viral membrane. *Proceedings of the National Academy of Sciences, USA* 103: 5989–5994, with permission from the National Academy of Sciences, USA.

enzymes needed for DNA replication, such as a DNA polymerase, nucleoside phosphohydrolase, a single-stranded DNA-binding protein, a processivity factor, and a protein kinase. In addition, the virus encodes a topoisomerase and DNA ligase, although the latter is (surprisingly) not essential for virus replication. DNA replication starts by the introduction of a nick into one DNA strand near the terminal hairpin loop. This enables the loop to unfold and serve as a template for DNA synthesis. After elongation to the end of the unfolded hairpin, the nascent and parent DNA strands separate, refold to create the hairpin, and thereby enable DNA replication to continue down the dsDNA genome by strand displacement. On reaching the distal end of the genome, the polymerase elongates round the hairpin and along the opposite DNA strand to produce concatemeric DNA molecules of greater than unit length. These are resolved into unit-length monomers by introduction of specific nicks near the terminal hairpins followed by re-ligation. Up to 10 000 copies of the virus genome may be produced from each infected cell, but only a fraction of these may be packaged into new virus particles.

Assembly

Virus assembly occurs in cytoplasmic factories in which the first visible structures are crescent shaped and are composed of virus protein and host lipid (**Figure 3**). The available evidence favors a model in which these structures contain a single lipid bilayer covered by a protein layer. The surface layer contains the D13 protein, which is essential for morphogenesis. Viruses lacking the D13 protein, or treated with the drug rifampicin, fail to form crescents. The D13 protein forms trimers that assemble into a lattice, or sheet, which appears to stabilize the formation of a nascent membrane. The crescents then grow into complete ovals or spheres into which the virus genome is packaged before the immature virion is sealed. Proteolytic cleavage of some of the capsid proteins and condensation of the virus core lead to the formation of an infectious IMV.

Most IMV particles remain within the cell until it lyses, but some are transported on microtubules to near the microtubule organizing center where they are wrapped by a double membrane (derived from either the *trans*-Golgi network or endosomes) that has been modified by the inclusion of several virus proteins (mostly glycosylated). The wrapping process produces a virion with three membranes that is called intracellular enveloped virus (IEV) (or wrapped virus (WV), using the nomenclature adopted recently by some authors), which is transported on microtubules to the cell surface. Upon reaching the cell periphery, the virus outer membrane fuses with the plasma membrane to externalize the virion by exocytosis. This surface virion is called cell-associated enveloped virus (CEV) and is surrounded by two membranes. CEV induces the formation of actin tails from beneath the plasma membrane where the CEV sits, and growth of these tails propels the virion away from the cell and into surrounding cells. VACV mutants unable to synthesize actin tails form small plaques, indicating the importance of this mechanism for cell-to-cell spread of virus. Alternatively, virions exposed on the cell surface may be released as EEV.

EEV are important for long-range spread of virus infection in cell culture and *in vivo*. The ability to spread in this way is conveniently studied by the comet assay, in which virus plaques are allowed to form on cell monolayers in liquid culture medium. Under these conditions, EEV particles spread from the original plaque in a unidirectional manner (caused by convection currents), giving rise to a comet-shaped plaque in which the primary plaque represents the head and secondary plaques form the tail. Antibodies directed against the EEV surface, but not IMV, inhibit comet formation. EEV particles not only are important for virus spread *in vivo* but are also important targets for antibodies that protect against OPV-induced disease.

The release of EEV from the cell surface or the retention of CEV is influenced by not only the cell type and virus proteins, primarily the A34 protein, but also the B5 and A33 EEV-specific envelope glycoproteins.

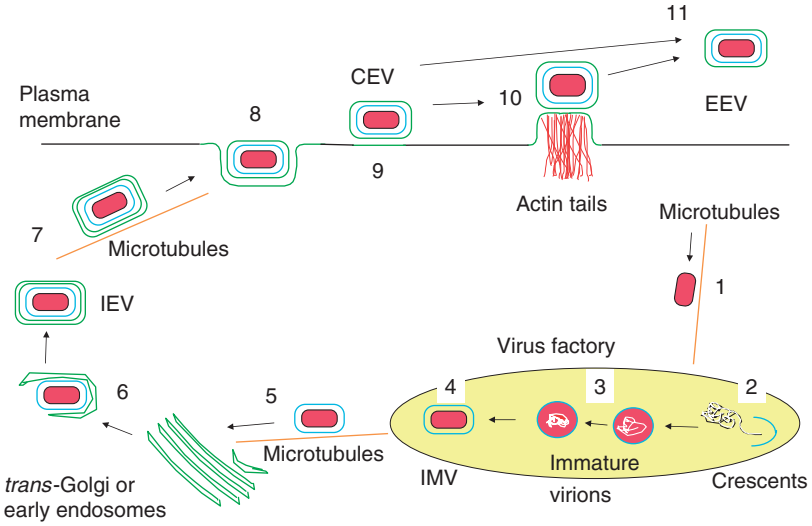


Figure 3 Schematic presentation of VACV morphogenesis. 1. After VACV entry, the virus core is transported on microtubules to a site near the nucleus where a virus factory develops. 2. A virus crescent forms and becomes associated with virus DNA associated with core proteins. 3. The virus membrane is sealed to form immature virions, which undergo condensation associated with proteolytic cleavage of several capsid proteins to form IMV (4). 5. IMV is transported on microtubules. 6. IMV is wrapped by modified membranes derived from the *trans*-Golgi network or endosomes to form intracellular enveloped virus (IEV). 7. IEV is transported on microtubules to the cell periphery. 8. IEV fuses with the plasma membrane to expose a cell-associated enveloped virus (CEV) on the cell surface by exocytosis. 9. CEV on the cell surface may induce the formation of actin tails (10) or dissociate from the cell to form EEV. 11. EEV may be formed by release of CEV from the cell surface before or after formation of actin tails. Modified from Smith GL, Vanderplasschen A, and Law M (2002) The formation and function of extracellular enveloped vaccinia virus. *Journal of General Virology* 83: 2915–2931, with permission from the Society for General Microbiology.

Virulence

VACV virulence and the mechanisms by which infection leads to disease (pathogenesis) have been studied in several animal models. The validity of any single model is uncertain because the natural host of VACV is unknown, but mice have been used most often and have been infected by dermal scarification or intranasal, intradermal, intraperitoneal, intracranial, or intravenous routes. The intranasal or intradermal routes are considered more physiologically relevant and have been used widely. Following intradermal infection, the virus causes a localized mild disease characterized by the formation of a lesion that heals in about 2 weeks. Virus does not spread from the site of infection and animals remain healthy. The intranasal model causes a more systemic illness in which the animals lose weight and the virus may spread to other tissues. The outcome of infection is influenced by the dose and strain of virus administered.

Many VACV proteins have been reported to affect the outcome of infection in one model or another. These proteins may be grouped into three types. The first group comprises enzymes (such as thymidine kinase, thymidylate kinase, and ribonucleotide reductase) that synthesize nucleoside precursors, or enzymes involved in DNA metabolism (e.g., DNA ligase). The second group comprises proteins that affect virus transport, release, and cell-to-cell spread. These include intracellular proteins F12 and A36, and the EEV proteins F13, A33, A34, and B5. The third and largest group comprises proteins that interfere with the host response to infection, which have been called immunomodulators.

VACV immunomodulators are numerous and target many aspects of the host immune system. They may be subdivided into those proteins that act within the infected cell and those that are secreted and act extracellularly. Intracellular proteins inhibit signaling pathways, such as those leading from Toll-like receptors (TLRs) or receptors for interleukins (ILs), tumor necrosis factor (TNF), or IFNs to the production of pro-inflammatory cytokines or IFNs. Such proteins include A46, A52, K1, M2, and N1. Other intracellular proteins (B13, F1, and N1) may block apoptosis, and yet others (K3 and E3) block the action of IFN-induced antiviral proteins. One intracellular protein contributes to virulence by synthesizing steroid hormones (A44). Extracellular proteins bind to complement factors (VCP), type I and type II IFNs (B8 and B18), cytokines IL-1β, IL-18, and TNF-α (B15, C12, and CrmC), and CC chemokines (vCKBP, B7, and A41). These VACV proteins function either by preventing soluble factors binding to their natural receptors on cells, or by preventing the establishment of chemokine concentration gradients, thereby diminishing the host response to infection. Collectively, these immunomodulatory proteins provide a substantial defense against the innate immune system. The contribution of individual proteins to virus virulence has been studied by using mutants lacking specific genes in several mouse models of infection. Loss of specific genes can either diminish, enhance, or have no effect on virulence, and the outcome can vary depending on the model of infection used.

The degree of virus attenuation derived from loss of proteins involved in virus transport and spread is in general much greater than that derived from removal of immunomodulators or enzymes synthesizing nucleoside triphosphate precursors.

Host Range

VACV infects a very broad range of cultured cells and only a few cell types, such as Chinese hamster ovary (CHO) cells, are nonpermissive. VACV also infects a broad range of host species, rather like CPXV and unlike the narrow host range of VARV and CMLV.

VACV Expression Vectors

VACV was developed as an expression vector in 1982. Since that time, this expression system has been refined to synthesize proteins in significant quantities in mammalian cells, and is employed widely as a laboratory tool by immunologists, virologists, and cell and molecular biologists. Recombinant VACV strains have been proposed as live vaccines for infectious diseases and cancer, and as oncolytic tools to target human cancers. The widespread use of VACV-based therapies has been hampered by their imperfect safety record. Therefore, attenuated strains are being utilized and engineered to improve immunogenicity by removal or modification of immunomodulatory proteins.

Antiviral Drugs

During the last 5 years, there has been a concerted effort to develop drugs against OPVs due to concerns about the possible use of VARV in bioterrorism. Given the highly conserved nature of OPV replication, the targets for these drugs are mostly conserved between OPVs and work against both VARV and VACV. Two particularly encouraging drugs are cidofovir (and acylated derivatives thereof) and ST-246. The former is a licensed drug for treating human cytomegalovirus infection and the latter is a new compound that targets morphogenesis by blocking formation of IEV particles by inhibiting wrapping of IMVs.

Immune Response to Vaccination

VACV infection in man and animals induces a robust antibody and T-cell response. It is clear that both are important in protection against VACV-induced disease,

and a T-cell response is essential for recovery from infection. The latter was evident historically from the life-threatening infections caused by vaccination of children suffering from T-cell immunodeficiency. The exact correlates of protection against smallpox are unknown, not least because smallpox was eradicated before much of modern immunology was developed. However, recent vaccination programs to defend against the perceived threat of bioterrorism with VARV have prompted detailed studies of the immune responses induced by VACV strains that protected against smallpox. These studies showed that both antibody and T-cell responses are very long-lived in humans (many decades) and that antibody responses were induced against a wide range of antigens. Several proteins on the surface of IMV are targets for neutralizing antibodies (including A27, D8, and H3), whereas the only antigen on the EEV surface that is a target for neutralizing antibody, in the absence of complement, is B5. B5 is therefore an important component of vaccines against OPV diseases, including smallpox. It is notable that one of the attenuated vaccines proposed as a safer vaccine against smallpox (LC16m8) lacks the B5 antigen.

Future Perspectives

VACV remains the only vaccine to have eradicated a human disease, smallpox. Despite the eradication of this disease 30 years ago, there remain many good reasons to continue to study VACV today. The ongoing development of this virus as a vaccine for infectious disease and cancer remains central, and its wide range of immunomodulatory proteins for interference with the innate immune system make it an excellent model for studying interactions between viruses and their host.

Acknowledgment

The author is a Wellcome Principal Research Fellow.

See also: Cowpox Virus; Poxviruses; Smallpox and Monkeypox Viruses.

Further Reading

Baxby D (1981) *Jenner's Smallpox Vaccine: The Riddle of the Origin of Vaccinia Virus.* London: Heinemann.
Broyles SS (2003) Vaccinia virus transcription. *Journal of General Virology* 84: 2293–2303.
Carter GC, Law M, Hollinshead M, and Smith GL (2005) Entry of the vaccinia virus intracellular mature virion and its interactions with glycosaminoglycans. *Journal of General Virology* 86: 1279–1290.
Condit RC, Moussatche N, and Traktman P (2006) In a nutshell: Structure and assembly of the vaccinia virion. *Advances in Virus Research* 66: 31–124.
Fenner F, Anderson DA, Arita I, Jezek Z, and Ladnyi ID (1988) *Smallpox and Its Eradication.* Geneva: World Health Organization.
Fenner F, Wittek R, and Dumbell KR (1989) *The Orthopoxviruses.* London: Academic Press.
Lane JM, Ruben FL, Neff JM, and Millar JD (1969) Complications of smallpox vaccination, 1968: National surveillance in the United States. *New England Journal of Medicine* 281: 1201–1208.
Law M, Carter GC, Roberts KL, Hollinshead M, and Smith GL (2006) Ligand-induced and nonfusogenic dissolution of a viral membrane. *Proceedings of the National Academy of Sciences, USA* 103: 5989–5994.
Moss B (1996) Genetically engineered poxviruses for recombinant gene expression, vaccination, and safety. *Proceedings of the National Academy of Sciences, USA* 93: 11341–11348.
Moss B (2006) Poxvirus entry and membrane fusion. *Virology* 344: 48–54.
Moss B (2007) *Poxviridae*: The viruses and their replication. In: Knipe DM (ed.) *Field's Virology,* 5th edn; vol. 2, pp. 2905–2946. Philadelphia, PA: Lippincott Williams and Wilkins.
Seet BT, Johnston JB, Brunetti CR, *et al.* (2003) Poxviruses and immune evasion. *Annual Review of Immunology* 21: 377–423.
Smith GL (2007) Genus *Orthopoxvirus*: Vaccinia virus. In: Mercer AA, Schmidt A, and Weber O (eds.) *Poxviruses,* pp. 1–45. Berlin: Springer.
Smith GL, Vanderplasschen A, and Law M (2002) The formation and function of extracellular enveloped vaccinia virus. *Journal of General Virology* 83: 2915–2931.

Relevant Website

http://www.poxvirus.org – Poxvirus Bioinformatics Resource Center.

Varicella-Zoster Virus: General Features

J I Cohen, National Institutes of Health, Bethesda, MD, USA

Published by Elsevier Ltd.

Glossary

Acantholysis Breakdown of a cell layer in the epidermis by separation of individual epidermal keratinocytes from their neighbors.
Dysesthesia Distortion of any sense, especially touch.

History

Descriptions of vesicular rashes characteristic of chickenpox (varicella) date back to the ninth century. In 1875, Steiner showed that chickenpox was an infectious agent by transmitting the disease from chickenpox vesicle fluid to previously uninfected people. Shingles (zoster) has

been recognized since ancient times. In 1909, Von Bokay suggested that chickenpox and shingles were related infections, an idea that was confirmed experimentally in the 1920s and 1930s when children inoculated with fluid from zoster vesicles were shown to contract chickenpox. In 1943, Garland suggested that zoster was due to reactivation of varicella virus that remains latent in sensory nerve ganglia, and, in 1954, Hope–Simpson reaffirmed this hypothesis.

The viral agents of varicella and zoster were first cultivated by Weller in 1952 and shown on morphologic, cytopathic, and serologic criteria to be identical. In 1984, Straus and colleagues showed that viruses isolated during sequential episodes of chickenpox and zoster from the same patient had identical restriction endonuclease patterns, proving the concept of prolonged latent carriage of the virus. In 1983, Gilden showed that varicella-zoster virus (VZV) DNA is latent in human sensory ganglia and, Hyman and colleagues showed that VZV RNA is present in human trigeminal ganglia. In 1974, Takahashi developed a live attenuated VZV vaccine for prevention of varicella, and, in 2005, Oxman and colleagues showed that a high potency formulation of this vaccine is effective in reducing rates of zoster and postherpetic neuralgia.

Taxonomy and Classification

VZV (species *Human herpesvirus 3*) is a member of genus *Varicellovirus* in subfamuly *Alphaherpesvirus* of the family *Herpesviridae*. Other alphaherpesviruses that infect humans include herpes simplex viruses 1 and 2 (HSV-1 and HSV-2), and, rarely, B virus (a macaque herpesvirus). All of these agents exhibit relatively short replicative cycles, destroy the infected cell, and establish latent infection in sensory ganglia.

Simian varicella virus (SVV) is the most closely related, well-characterized virus to VZV. Natural infection with SVV can cause a varicella-like illness in Old World monkeys; the virus establishes latency in trigeminal ganglia, and can spontaneously reactivate to cause a rash that can transmit the virus to naive animals. The complete sequence of SVV has been determined and the virus shares nearly an identical set of genes with VZV. SVV is not known to infect humans.

Geographic and Seasonal Distribution

Varicella and zoster infections occur worldwide. Over 90% of varicella occurs during childhood in industrialized countries located in the temperate zone, but infection is commonly delayed until adulthood in tropical regions. Zoster may occur less frequently in tropical areas, because of later acquisition of primary infection. Varicella infection is epidemic each winter and spring, while zoster occurs throughout the year, without a seasonal preference.

Host Range and Virus Propagation

The reservoir for VZV is limited to humans. The virus inherently grows poorly in nonhuman animals or cell lines. Myers and colleagues, however, developed a guinea pig animal model of VZV infection by adapting the virus for growth in guinea pig embryo cells *in vitro*. Inoculation of animals results in a self-limited viremic infection and the emergence of both humoral and cellular immunity. Latent VZV DNA has been demonstrated in dorsal root and trigeminal ganglia. Rats, cotton rats, and mice inoculated with VZV develop latent infection of dorsal root ganglia. Inoculation of VZV into fetal thymus–liver implants in severe combined immune deficiency (SCID) mice results in virus replication in T cells; inoculation of virus into subcutaneous fetal skin implants reproduces many of the histopathologic features of varicella. Intravenous inoculation of SCID mice with VZV-infected human T cells results in virus infection of human skin xenografts.

An alternative, but less ideal, animal model involves the common marmoset (*Callithrix jacchus*). VZV replicates in the lungs with a mild pneumonia and a subsequent humoral immune response. Inoculation of chimpanzees with VZV results in a transient rash containing viral DNA and evokes a modest humoral immune response. None of these animal models have, as yet, reproduced the disease pattern seen in humans, namely a vesicular rash and spontaneous reactivation from latency.

VZV is usually cultured in human fetal diploid lung cells in clinical laboratories. The virus has been cultivated in numerous other human cells including melanoma cells, primary human thyroid cells, astrocytes, Schwann cells, and neurons, and can be grown in some simian cells including primary African green monkey kidney cells and Vero cells, and in guinea pig embryo fibroblasts.

VZV is extremely cell associated. The titer of virus released into the cell culture supernatant is very low, and preparation of cell-free virus, by sonication or freeze–thawing cells, usually results in a marked drop in viral titer. Therefore, virus propagation is usually performed by passage of infected cells onto uninfected cell monolayers. VZV is detected by its cytopathic effect with refractile rounded cells that gradually detach from the monolayer, or by staining with fluorescein-labeled antibody.

Genetics

Several markers can be used to distinguish different strains of the virus. These include temperature sensitivity, plaque size, antiviral sensitivity, and restriction

endonuclease cleavage patterns. The molecular basis for most of these strain differences is unknown, but viruses that are resistant to acyclovir usually have mutations in their thymidine kinase gene; other resistant strains have mutations in the DNA polymerase gene.

The genome of the prototypical laboratory strain VZV Dumas consists of 124 884 bp. The identification of many viral genes was made by analogy to HSV-1 genes with similar sequences and by genetic-complementation studies in which cell lines expressing selected VZV proteins were used to support the growth of HSV-1 mutants. Cosmids and bacterial artificial chromosomes (BACs) derived from VZV have been developed to allow targeted deletion, insertion, or site-directed point mutations in individual viral gene products. Using these systems, 22 viral genes have been shown to be dispensable, and 5 genes have been shown to be required, for virus replication *in vitro*. Recombinant VZV containing Epstein–Barr virus (EBV), hepatitis B virus, HIV, or HSV genes have been constructed.

Evolution

Comparison of the nucleotide and predicted amino acid sequences of VZV with HSV-1 and HSV-2 indicates that these viruses originated from a common ancestor. They share similar gene arrangements and only five genes of VZV do not appear to have HSV counterparts.

VZV is more distantly related to all other human herpesviruses, but many of the nonstructural proteins involved in viral replication have conserved elements and activities. Comparison of VZV, for example, with EBV shows that the majority of VZV genes are homologous with EBV. Three large blocks of genes are conserved, although rearranged within the two genomes.

VZV isolates have been classified into three different clades, corresponding to Japanese, European, and mosaic genotypes (combination of European and Japanese). Other investigators have proposed two clades: a Singapore/Japanese and a North American/European genotype.

Serologic Relationships and Variability

There is only one serotype of VZV. Antibodies detected by the complement-fixation test and virus-specific immunoglobulin M (IgM) antibodies decline rapidly after convalescence from varicella. Other, more sensitive serologic tests, including immune adherence hemagglutination (IAHA), fluorescence antibody to membrane antigen (FAMA), and enzyme-linked immunosorbent assay (ELISA), recognize antibodies that persist for life. VZV-specific antibodies are boosted by both recrudescent infection (zoster) and exposure to others with varicella.

Variability of VZV strains has been shown primarily by differences in restriction endonuclease patterns. Passage of individual strains *in vitro* eventually results in minor changes in restriction endonuclease patterns, predominantly through deletion or reiteration of small repeated elements scattered throughout the genome. Other than these sites, the genome sequence is remarkably stable. For example, the sequence of the thymidine kinase gene has been determined for several epidemiologically unrelated wild-type and acyclovir-resistant strains and found to possess >99% nucleotide and amino acid sequence identity.

Epidemiology

Varicella may occur after exposure of susceptible persons to chickenpox or herpes zoster. Over 95% of primary infections result in symptomatic chickenpox. Over 90% of individuals in temperate countries are infected with VZV before age 15.

Zoster is due to reactivation of VZV in patients who have had prior chickenpox; some of these patients may not recall the primary infection. Zoster is not clearly related to exposure to chickenpox or to other cases of zoster. About 10–20% of individuals ultimately develop herpes zoster – the risk of which rises steadily with age (**Figure 1**). Severely immunocompromised patients, such as those with the acquired immunodeficiency syndrome (AIDS), have a particularly high incidence of zoster. Recurrent zoster is uncommon; less than 4% of patients experience a second episode. Asymptomatic viremia has been detected in bone marrow transplant recipients and has been followed by recovery of cell-mediated immunity.

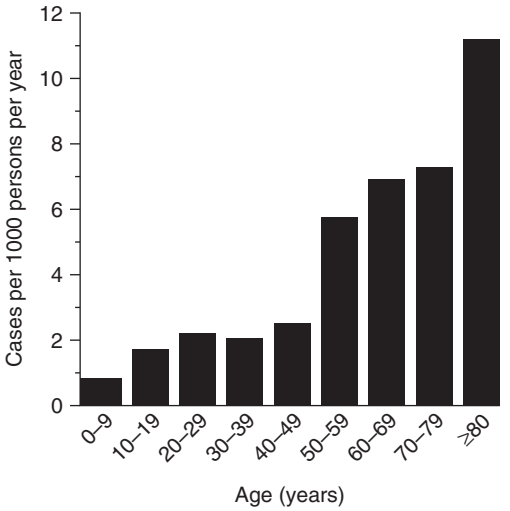


Figure 1 Incidence of herpes zoster. Reproduced from Kost RG and Straus SE (1996) Postherpetic neuralgia – pathogenesis, treatment, and prevention. *New England Journal of Medicine* 335: 32–42, with permission from Massachusetts Medical Society.

Transmission and Tissue Tropism

VZV is transmitted by the respiratory route. VZV has been detected by polymerase chain reaction (PCR) in room air from patients with varicella or zoster. Intimate, rather than casual, contact is important for transmission. Chickenpox is highly contagious; about 60–90% of susceptible household contacts become infected. Herpes zoster is less contagious than chickenpox. Only 20–30% of susceptible contacts develop varicella. Patients with varicella are infectious from two days before the onset of the rash until all the lesions have crusted.

Primary infection with VZV results in viral replication in the upper respiratory tract and oropharynx with lesions present on the respiratory mucosa. T cells subsequently becomes infected and transmit virus to the skin. Virus has been detected by PCR in the oropharynx and virus has been cultured from the blood early during varicella. Virus infection of epidermal cells in the skin is thought to be initially limited by interferon; later T cells trafficking in the skin may become infected and disseminate the virus to various organs throughout the body including the nervous system. During latency, VZV DNA can be detected in ganglia of the cranial nerves (e.g., trigeminal ganglia), dorsal roots (e.g., thoracic and trigeminal ganglia), and autonomic nervous system (e.g., celiac and vagus nerve) by PCR. Using *in situ* hybridization, VZV has been detected predominantly, if not exclusively, in neurons. During latency, only 6 of the 70 known viral genes are expressed.

Zoster is due to reactivation of virus in the sensory ganglia. The factors leading to its reactivation are not known, but are associated with neural injury and cellular immune impairment. Reactivated virus spreads down the sensory nerve to the skin, where the resulting vesicles are typically confined to a single dermatome. Viremia and subsequent cutaneous or visceral dissemination of lesions may occur in zoster, especially in immunocompromised patients.

Pathogenicity

Passage of wild-type VZV in cell culture by Takahashi in 1974 led to attenuation of the virus and changes in its temperature sensitivity and infectivity for certain cell lines. The resulting Oka vaccine strain has nucleotide polymorphisms at 31 sites. Comparison of the complete nucleotide sequence of the Oka vaccine strain with its parental virus shows 42 nucleotide and 20 amino acid differences. Multiple nucleotide changes in several genes are responsible for attenuation of the vaccine for growth in skin. The Oka vaccine strain can be distinguished from wild-type strains by differences in restriction endonuclease patterns.

Clinical Features of Infection

The incubation period for chickenpox is 2 weeks, with a range of 10–21 days. The disease begins with fever and malaise, followed by a generalized vesicular rash (**Figure 2(b)**). Lesions tend to appear first on the head and trunk and then spread to the extremities. New lesions usually follow viremic waves for 3–5 days and, in the normal host, most lesions are crusted and healed by 2 weeks. Lesions in different stages coexist in an individual. The disease is usually self-limited in the normal host.

Complications of varicella are more common in neonates, children with malnutrition, immunocompromised patients (e.g., malignancy or immunosuppressive therapy), pregnant women, and older adults. These complications include bacterial superinfection of the skin, pneumonia, hepatitis, encephalitis, thrombocytopenia, and purpura fulminans. Reye syndrome occurs in rare children who take aspirin to treat varicella fevers. Prior to the widespread use of the varicella vaccine in the USA, there were about 3–4 million cases of varicella each year with about 100 deaths; however, the number of cases has dropped sharply and death from varicella in the USA is very rare.

Zoster usually presents with pain and dysesthesias 1–4 days before the onset of the vesicular rash. The rash is usually painful and confined to a single dermatome (**Figure 2(c)**), but may involve several adjacent dermatomes. Fever and malaise often accompany the rash. Vesicles often are pustular by day 4 and become crusted by day 10 in the normal host.

Postherpetic neuralgia (PHN), manifested by pain lasting for weeks to several years in the area of the initial rash, is the most common and disconcerting complication of zoster in the normal host. Less common complications include encephalitis, myelitis, the Ramsay–Hunt syndrome (lesions in the ear canal, with auditory and facial nerve involvement), ophthalmoplegia, facial weakness, and pneumonitis. Immunocompromised patients with zoster are more likely to develop disseminated disease (**Figure 2(d)**) with neurologic, ocular, or visceral involvement. Patients with AIDS have a high frequency of zoster and may develop recurrent or chronic disease with verrucous, hyperkeratotic skin lesions.

Pathology and Histology

Varicella lesions are readily recognized in the skin and mucous membranes. However, similar lesions also occur in the mucosa of the respiratory and gastrointestinal tracts, liver, spleen, and any tissue, and remain unrecognized except in severe cases. With severe disease, there is inflammatory infiltration of the small vessels of most organs. Zoster causes inflammation and necrosis of the

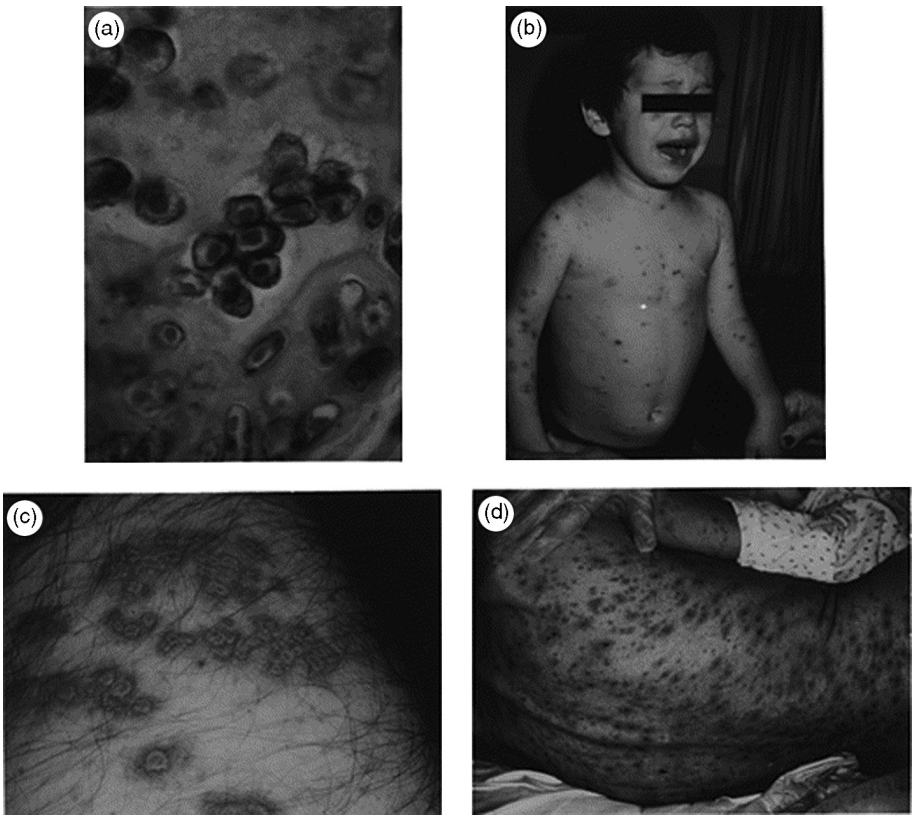


Figure 2 Histopathology and clinical findings of varicella-zoster virus infections. (a) Eosinophilic intranuclear inclusions from a skin biopsy in a patient with herpes zoster (original magnification ×400). (b) Chickenpox in a child. (c) Localized zoster in an adult. (d) Disseminated zoster in a patient with chronic lymphocytic leukemia. Reproduced from Straus SE, Ostrove JM, Inchauspe G, *et al*. (1988) Varicella-zoster virus infections: Biology, natural history, treatment, and prevention. *Annals of Internal Medicine* 108: 221–237, with permission from American College of Physicians.

sensory ganglia and its nerves, and skin lesions that are histopathologically identical to those seen with varicella.

Cutaneous lesions due to VZV begin with infection of capillary endothelial cells followed by direct spread to epidermal epithelial cells. The epidermis becomes edematous with acantholysis and vesicle formation. Mononuclear cells infiltrate the small vessels of the dermis. Initially, vesicles contain clear fluid with cell-free virus, but later the vesicles become cloudy and contain neutrophils, macrophages, interferon, and other cellular and humoral components of the inflammatory response pathways. Subsequently, the vesicles dry leaving a crust that heals usually without scarring.

Cells infected with VZV show eosinophilic intranuclear inclusions with multinucleate giant cell formation (**Figure 2(a)**). These changes are not specific for VZV, as they are seen with HSV and cytomegalovirus infections.

Immune Response

Infection with VZV elicits both a humoral and cellular immune response. The ability of VZV immune globulin (VZIG) to attenuate or prevent infection in exposed children (see the next section) indicates that virus-specific antibody is important in protection from primary infection. The presence of VZV-specific immunoglobulin G (IgG) does not correlate, however, with protection from zoster. Antibody to VZV is often present by the time the rash of varicella first appears. Virus-specific IgM, IgG, and IgA are present within 5 days of symptomatic disease; however, only IgG persists for life. Antibodies to viral glycoproteins gE, gB, gH, and the immediate-early 62 protein (IE62) have been detected during acute infection, and the titers of antibodies to these proteins are boosted during recurrent infection. The mere presence of antibody to VZV glycoproteins in children with leukemia who had received live varicella vaccine is not adequate to prevent breakthrough varicella or zoster.

Cellular immune responses to VZV are more important in recovery from acute varicella infection and for prevention of, and recovery from, zoster. The level of cellular immunity correlates with disease severity during acute varicella. Cytotoxic T cells that lyse virus-infected cells are present by 2–3 days after the onset of the rash of varicella. Cell-mediated immunity, as measured by

lymphocyte proliferative response, is directed against cells expressing glycoproteins gE, gB, gH, gI, and gC, the IE4, IE62, and IE63 proteins, and other viral proteins. Interferon is present in VZV vesicles.

Most varicella infections result in lifelong immunity to reinfection. Second episodes of varicella are rare; these individuals tend to have reduced humoral and cellular immunity to VZV at the time of the second infection. Zoster is associated with a reduction in cellular immunity to VZV that, in the normal host, is partially restored in response to this recurrent infection. Recurrent zoster is uncommon, except in severely immune-deficient patients, such as those with AIDS.

Prevention and Control

Prevention of varicella can be achieved by restricting exposure or by resorting to either immunoglobulin prophylaxis or vaccination with live, attenuated virus. If given within 4 days of exposure to the virus, VZIG prevents or attenuates varicella in seronegative persons. The preparation has no effect in modifying zoster. VZIG is recommended for individuals (1) with recent, close contact to patients with varicella or zoster, (2) who are susceptible to varicella, and (3) who fall in a high-risk category. The latter include premature or certain newborn infants, pregnant women, and patients with congenital or acquired cellular immunodeficiencies. Supplies of VZIG are currently very limited in the USA and VariZIG, prepared from high-titer human immune serum, has replaced VZIG.

The live, attenuated varicella vaccine (Oka strain) was licensed in the USA in 1995 and is recommended for vaccination of healthy children and adults. Most children develop adequate humoral and cellular immunity to varicella after a single dose of vaccine; an additional dose enhances the degree of immunity and is recommended for susceptible children and adults. A rash may follow vaccination. It is usually mild, but can be severe if the vaccine is given to patients experiencing periods of profound cellular immune impairment. The live vaccine virus establishes neural latency and can reactivate. Thus, zoster has been reported in vaccinees, especially those who are immunocompromised, but the rate appears to be no higher than that following natural infection. Vaccination may be used for postexposure prophylaxis.

A high-titer formulation of the Oka vaccine virus was licensed in the USA in 2006. It reduced the frequency of zoster and PHN by 51% and 67%, respectively, in healthy persons $\geq$60 years old. The most common side effects were mild injection-site reactions; no significant severe adverse reactions were attributed to the vaccine.

Patients with varicella or zoster should be isolated from susceptible persons until all lesions have crusted. This is particularly important for hospital workers and immune-deficient patients.

Acyclovir and leukocyte interferon have been used in the treatment of varicella and zoster in immunocompromised patients. Interferon proved to be an inadequate and impractical therapy. Acyclovir is the current treatment of choice for selected infections. It results in a shorter duration of symptoms and decreased visceral dissemination of varicella or zoster in the immunocompromised host. Acyclovir also prevents spread of trigeminal zoster to the eye and modestly shortens the duration of varicella and zoster symptoms in the normal host. Analogs of acyclovir, such as famciclovir and valaciclovir, result in higher levels of antiviral activity than oral acyclovir and have been licensed for oral therapy of zoster in the USA. Acyclovir-resistant strains of VZV have been reported in patients with AIDS; these infections are best treated with foscarnet. Corticosteroids, when used early during zoster, reduce acute pain. Herpes zoster, particularly in elderly patients, may lead to prolonged and severe PHN. Treatment of PHN is difficult and often unsatisfactory, but many patients experience improvement with gabapentin, pregabalin, or tricyclic antidepressant drugs like amitriptyline.

Future Perspectives

Widespread vaccination of children with the attenuated, live varicella vaccine has reduced the incidence and severity of varicella. Use of a high-titer live varicella vaccine was shown to lower the incidence of zoster and postherpetic neuralgia in the elderly. Further research is needed into the mechanisms of VZV latency and reactivation as well as identification of which viral proteins are critical for protection from varicella and zoster. Since the live varicella vaccine can reactivate to cause zoster and since breakthrough cases of varicella continue to occur in vaccinated persons, further knowledge of VZV latency and immunity should lead to safer and more effective vaccines against varicella and herpes zoster.

See also: Herpes Simplex Viruses: General Features; Herpes Simplex Viruses: Molecular Biology; Herpesviruses: Discovery; Herpesviruses: Latency; Varicella-Zoster Virus: Molecular Biology.

Further Reading

Cohen JI, Brunell PA, Straus SE, and Krause PR (1999) Recent advances in varicella-zoster virus infection. *Annals of Internal Medicine* 130: 922–932.

Cohen JI, Straus SE, and Arvin AM (2007) Varicella-zoster virus: Replication, pathogenesis, and management. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn, pp. 2773–2818. Philadelphia, PA: Lippincott Williams and Wilkins.

Gershon A and Arvin A (2000) *Varicella-Zoster Virus*. Cambridge: Cambridge University Press.
Gilden DH, Cohrs RJ, and Mahalingham R (2003) Clinical and molecular pathogenesis of varicella-zoster virus infection. *Viral Immunology* 16: 243–258.
Hambleton S and Gershon AA (2005) Preventing varicella-zoster disease. *Clinical Microbiology Reviews* 18: 70–80.
Heininger U and Seward JF (2006) Varicella. *Lancet* 368: 1365–1376.
Kimberlin D and Whitley R (2007) Varicella-zoster vaccine for the prevention of zoster. *New England Journal of Medicine* 356: 1338–1343.
Kost RG and Straus SE (1996) Postherpetic neuralgia – pathogenesis, treatment, and prevention. *New England Journal of Medicine* 335: 32–42.
Ku CC, Besser J, Abendroth A, Grose C, and Arvin AM (2005) Varicella-zoster virus pathogenesis and immunobiology: New concepts emerging from investigations with the SCIDhu mouse model. *Journal of Virology* 79: 2651–2658.
Mitchell BM, Bloom DC, Cohrs RJ, Gilden DH, and Kennedy PG (2003) Herpes simplex virus and varicella zoster latency in ganglia. *Journal of Neurovirology* 9: 194–204.
Oxman MN, Levin MJ, Johnson GR, *et al.* (2005) A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. *New England Journal of Medicine* 352: 2271–2284.
Straus SE, Ostrove JM, Inchauspe G, *et al.* (1988) Varicella-zoster virus infections: Biology, natural history, treatment, and prevention. *Annals of Internal Medicine* 108: 221–237.

Varicella-Zoster Virus: Molecular Biology

W T Ruyechan and J Hay, The State University of New York, Buffalo, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Virion and Genome Structure

The Virion

The virion of varicella-zoster virus (VZV), like those of all herpesviruses, is enveloped and approximately spherical, with a diameter of 100–300 nm. A minimum of 30 proteins ranging in size from ~250 to 17 kDa have been identified as components of the virion. The viral double-stranded DNA genome is contained in an icosahedral nucleocapsid 100 nm in diameter that has 5:3:2 axial symmetry and is embedded within a structure known as the tegument. The tegument is composed of virus-encoded proteins, several of which are involved in expression of the viral genome (see below). The thickness of the tegument can vary, even within a single virion. The lipid envelope of the virus, which is derived from cellular membranes, surrounds the tegument and contains a minimum of eight viral glycoproteins.

The Genome

The VZV genome is a linear, double-stranded DNA molecule of *c.* 125 kbp with a G + C content of 46%. The genome consists of two covalently joined segments, the unique long (U_L) and unique short (U_S) regions, each bounded by a terminal and internal inverted repeat (TR_L/IR_L (88 bp) and TR_S/IR_S (7.3 kbp) respectively). The repeats allow structural rearrangements of the unique regions to occur during viral DNA replication and, as a result, encapsidated genomes exist as a mixture of four isomers. Two isomers are found predominantly in viral particles and are termed P (prototype) and I_S (inverted U_S). Two other isomers, which contain U_L inverted with respect to U_S in the P and I_S configurations, are designated I_L and I_{SL}. These represent 2–5% of the virion DNA. A small amount of full-length closed circular DNA (representing 0.1–5% of the total) has also been isolated from virions. The function of these molecules in relation to the viral life cycle remains unknown.

Analysis of the complete sequence of the VZV Dumas strain revealed the protein-coding potential of a total of 71 open reading frames (ORFs) that were named sequentially according to location in the genome (ORF1–ORF71, with ORF33.5 overlapping ORF33 and ORF62–ORF64 in IR_S duplicated as ORF69–ORF71 in TR_S). Two other genes (ORFS/L and ORF9A) were added subsequently. The genome map thus contains 71 unique genes, the first of which (ORFS/L) is located at the left end of the genome and the last (ORF68) at the right end of U_S, with ORF62–ORF64 in IR_S duplicated as ORF69–ORF71 in TR_S. The VZV ORFs encode proteins involved in regulation of viral gene expression, viral DNA replication, and regulation of nucleotide pools, and structural proteins including those required for DNA packaging and capsid assembly, as well as the viral glycoproteins (**Table 1**).

Understanding of the roles of the VZV gene products was long hampered owing to the highly cell-associated nature of the virus, which precluded plaque purification, and to the lack of a good animal model of VZV infection. The development of a cosmid transfection system and, more recently, a VZV bacterial artificial chromosome (BAC), coupled with a severe combined immunodeficiency (SCID) mouse/human (hu) xenograft model of pathogenesis, has resulted in considerable progress in the delineation of VZV gene function.

Productive Infection

Productive VZV infection begins with fusion of the virus envelope with the membrane of a susceptible cell. This occurs via interactions between one or more VZV

Table 1 Functions of VZV genes

Gene	*HSV*	*Pred. mol. wt. (Da)*	*Properties*	*Function*
1	*** [a]	12 103	Hydrophobic C-terminus	Unknown
2	***	25 983		Unknown
3	UL55	10 149		Unknown
4	UL54	51 540	Hydrophilic dimer	(IE) Transcription regulation
5	UL53	38 575	Hydrophobic/membrane	Glycoprotein K
6	UL52	122 541		DNA primase[b]
7	UL51	28 245		Tegument protein
8	UL50	44 816		dUTPase
9	UL49	32 845	Interacts with IE62 in cytoplasm/tegument component	Tegument/trafficking
9A	UL49.5	9800	Present in cell membrane	Glycoprotein N
10	UL48	46 573		Tegument/transactivator
11	UL47	91 825	Hydrophilic/acid N-terminus	Tegument[b]
12	UL46	74 269		Tegument
13	***	34 531		Thymidylate synthase
14	UL44	61 350	N-terminal repeats	Glycoprotein C
15	UL43	44 522	Hydrophobic	Membrane?
16	UL42	46 078	DNA-binding, polymerase processivity	DNA polymerase processivity subunit[b]
17	UL41	51 365		Virion host shutoff/tegument
18	UL40	35 395		Ribonucleotide reductase (small subunit)
19	UL39	86 823		Ribonucletide reductase (large subunit)
20	UL38	53 969		Capsid
21	UL37	115 774		Tegument[b]
22	UL36	306 325		Tegument
23	UL35	24 416	Hydrophilic/STQ rich	Capsid/hexon tip[b]
24	UL34	30 451	Hydrophobic C-terminus	Phosphoprotein
25	UL33	17 460	Hydrophilic N-terminus	DNA packaging[b]
26	UL32	65 692		DNA packaging[b]
27	UL31	38 234	Hydrophilic/basic N-terminus	Nuclear matrix associated[b]
28	UL30	130 041	Hydrophilic	DNA polymerase catalytic subunit
29	UL29	132 133	Hydrophilic	Major ssDNA binding protein
30	UL28	86 968		DNA packaging[b]
31	UL27	98 026		Glycoprotein B
32	***	15 980	Hydrophilic/acidic	Unknown
33	UL26	66 043		Protease/capsid maturation[b]
33.5	UL26.5	34 000		Capsid assembly[b]
34	UL25	65 182		DNA packaging/capsid[b]
35	UL24	28 973	Basic/nuclear	Important for replication in skin and T cells
36	UL23	93 646	Hydrophilic	Pyrimidine deoxynucleoside kinase
37	UL22	93 646	Hydrophobic	Glycoprotein H
38	UL21	60 395		Tegument[b]
39	UL20	27 078	Hydrophobic/membrane	Virion egress[b]
40	UL19	154 971		Major capsid protein
41	UL18	34 387		Capsid
42/45	UL15	82 752	Spliced gene product	DNA packaging/DNA terminase?[b]
43	UL17	73 905		DNA packaging/tegument[b]
44	UL16	40 243		Tegument[b]
46	UL14	22 544		Tegument[b]
47	UL13	54 347		Protein kinase/tegument
48	UL12	61 268		Deoxyribonuclease
49	UL11	8907	Hydrophilic	Virion component[b]
50	UL10	48 669	Hydrphobic	Glycoprotein M
51	UL9	94 370		DNA origin binding protein/helicase
52	UL8	86 343		Helicase/primase complex accessory protein[b]
53	UL7	37 417		Unknown
54	UL6	86 776		Virion component[b]
55	UL5	98 844		DNA helicase[b]
56	UL4	27 166	S- and T-rich	Unknown
57	***	8079	Hydrophilic/basic	Tegument/virion egress[b]

Continued

Table 1 Continued

Gene	*HSV*	*Pred. mol. wt. (Da)*	*Properties*	*Function*
58	UL3	25 093	Hydrophilic/basic	Unknown
59	UL2	34 375		Uracil-DNA glycosylase
60	UL1	17 616	Acidic	Glycoprotein L
61	IE110	50 913	Zinc ring finger	Transcriptional regulator
62/71	IE175	139 989	Dimer/phosphoprotein	(IE) major transcriptional activator/tegument
63/70	US1	30 494	Hydrophilic	(IE) transcriptional regulator/tegument
64/69	US10	19 868		Virion component
65	US9	11 436	Hydrophobic	Tegument[b]
66	US3	43 677		Protein kinase
67	US7	39 362	Required for growth in skin and T cells	Glycoprotein I
68	US8	69 953	Required for virus growth	Glycoprotein E
S/L	***	24 640	Spans junction of the genome termini	Plaque morphology/cell adhesion

[a]Asterisks indicate genes that do not have herpes simplex virus (HSV) counterparts.
[b]Function inferred from data with HSV.
The predicted molecular weights are based on the sequence of the Dumas strain of VZV.

glycoproteins and as yet unidentified receptors on the cell surface. Upon entry and uncoating, the capsid is translocated to the nuclear membrane and the viral genome enters the nucleus, presumably through a nuclear pore. Viral tegument proteins also enter the nucleus, including several that regulate viral gene expression. Transcription and viral DNA replication are then believed to occur in a regulated temporal cascade, as is typical of all herpesviruses. Both viral and cellular proteins are involved in these processes. Progeny viral DNA molecules in the form of concatamers are presumably generated via a rolling-circle mechanism, and then cleaved and packaged into preassembled capsids. Tegument assembly is believed to occur at least in part in the nucleus. The infectious virion is believed to be formed following the de-envelopment/re-envelopment model currently extant for herpesvirus morphogenesis (see below). The virus grows slowly in tissue culture and is highly cell associated, and purified virions exhibit low levels of infectivity. The cell-associated nature of the virus has also led to speculation that, during infection of specific cell types, infectious spread occurs primarily via cell-to-cell fusion, whereas for other cell types intact cell-released virions are required.

Genome Replication

Replication of the VZV genome has not been subject to extensive biochemical analysis, primarily due to the difficulty in obtaining sufficient numbers of synchronously infected cells. Consideration of the relative proportions of the isomeric genome forms and the frequency of novel junctions in DNA isolated from virions and from infected cells have led to the following model. Upon entry into the nucleus, the viral genome circularizes and undergoes a limited number of rounds of bidirectional replication. This initiates at one or both copies of the origin of DNA replication (ori_S), which is located in TR_S and IR_S. The minimal ori_S sequence consists of a series of 16 AT repeats and an upstream consensus binding sequence (5′-CGTTCGCACTT-3′) for the origin-binding protein encoded by ORF51. During this phase, inversion of U_L and U_S is believed to occur via intramolecular recombination mediated by the inverted repeats. Replication then shifts to a rolling-circle mechanism, generating head-to-tail concatamers, and this phase accounts for the bulk of viral DNA synthesis. Finally, the newly synthesized DNA is cleaved into unit-length molecules and packaged into preassembled capsids in the nucleus. The unequal proportions of isomeric genome forms within particles are postulated to result from preferential cleavage of concatemers at the novel junction between TR_L and TR_S generated by the initial circularization event, rather than at the normal junction between IR_L and IR_S.

The full complement of viral and cellular proteins required for complete replication of the VZV genome has not yet been enumerated. However, orthologs of the seven genes required for origin-dependent replication of herpes simplex virus (HSV-1) DNA have been identified. These include the proteins encoded by ORF6, ORF16, ORF28, ORF29, ORF51, ORF52, and ORF55. ORF6, ORF55, and ORF52 encode the primase, helicase, and an accessory factor, respectively, which are believed to form a heterotrimeric complex by analogy with HSV-1. ORF28 encodes the catalytic subunit of the viral DNA polymerase and is the target of the antiviral drugs acyclovir and valacyclovir. ORF16 encodes the polymerase processivity factor that interacts directly with the catalytic subunit. The ORF29 protein is the major single-strand DNA-binding protein (SSB) and, as indicated above, the ORF51 protein binds to ori_S in a site-specific manner and also contains a helicase activity. Of these seven factors, only the DNA polymerase, the origin-binding protein, and SSB have been characterized at a biochemical level.

Overview of Gene Expression

Upon entry into the cell nucleus, the VZV genome is transcribed by the host cell's general transcription apparatus, including RNA polymerase II (RNA Pol II). By analogy with other alphaherpesviruses, the ORFs are believed to be expressed in three distinct kinetic classes: immediate early (IE), early (E), and late (L). However, clear evidence for this temporal cascade has been difficult to obtain owing to the low titers and cell-associated nature of VZV grown in tissue culture. Only three proteins have been shown to be expressed under IE conditions. These are IE4 (encoded by ORF4), IE62 (ORF62 and its counterpart in TRs, ORF71), and IE63 (ORF63 and its counterpart, ORF70) the latter two of which are expressed from ORF62 and ORF63 in IR_S (and their duplicates in TR_S, ORF70 and ORF71).

VZV promoters have been shown in general to be essentially quiescent in the absence of virus-encoded transcriptional activators. Several VZV-encoded proteins have been shown in transient transfection assays to be capable of transactivating or transrepressing specific viral promoters, promoters from heterologous virus families, and cellular promoters. These include the IE62, IE4, IE63, ORF10, and ORF61 proteins. However, IE62 is considered to be the primary viral transactivator during the lytic phase of VZV replication. It is known to activate expression of genes from all three putative kinetic classes of VZV genes and can act independently of all other viral factors. Efficient activation of VZV promoters by IE62 usually occurs via synergistic interactions between this viral protein and ubiquitous cellular transcription factors whose consensus binding sites are found within VZV promoters. Predominant among these factors are specificity factor-1 (Sp1) and upstream factor (USF), which are predicted to be involved in regulating the expression of over half of the viral genome. When other viral factors are present, they have been shown to be capable of modulating the level of IE62 activation, even in some cases where the promoter in question appeared responsive only to IE62.

Promoter Structure

Results from analysis of a small number of VZV promoters, and predictions of the positions of putative promoters, indicate they appear to be relatively typical RNA Pol II promoters with TATA-like elements and binding sites for ubiquitous cellular transcription factors upstream of the TATA elements. Due to the compact nature of the VZV genome, many promoters are predicted to lie completely or partially within the coding sequences of genes immediately upstream. VZV promoters are also characterized by the fact that the TATA elements required or predicted to be positioned correctly for expression are frequently atypical and therefore less likely to be bound with high affinity by the TATA-binding protein (TBP). Thus far, no TATA-less VZV promoters have been identified and no promoters showing dependence on the presence of an initiator element (INR) at or just downstream of the start site of transcription are known. This most likely reflects the fact that only a few promoters have been analyzed in detail at the functional level, rather than any specific absence of these alternatives.

Potential VZV promoters can be separated into three general categories: A, B, and C type. This classification is preliminary as it is based on a meager amount of functional data. A-type promoters contain only a TATA element. B-type promoters contain a TATA element and one or more functional upstream binding sites for ubiquitous cellular transcription factors with which IE62 can interact physically or functionally. Examples of such promoters are that for ORF67 (encoding a glycoprotein, gI) and the regulatory element controlling divergent expression of ORF28 (DNA polymerase) and ORF29 (SSB). C-type promoters contain a TATA element, binding sites for factors with which IE62 can interact, and binding sites for factors that function to activate the promoter independently of IE62. The only known example of this type of promoter is that driving expression of the gene (ORF62) that encodes IE62 itself. A fourth class of promoter, at this time hypothetical, is one that is activated in the absence of IE62 by cellular or other viral proteins.

IE Gene Expression

Examination of the putative promoter regions of the three IE genes reveals, as noted above, that only the promoter for ORF62 (and its duplicate, ORF71), which encode IE62, contains binding sites for the cellular factor Oct-1. The presence of the Oct-1 recognition sequence and the existence of ORF10, the ortholog of HSV-1 VP16, indicate that IE62 can be expressed via the same mechanism as the HSV-1 IE genes, whereas IE4 and IE63 might be expressed via mechanisms possibly novel to VZV. By analogy with the HSV-1 IE expression mechanism, ORF10 would interact with Oct-1 bound to the promoter and the cellular transcription factor host cell factor-1 (HCF-1) to activate IE62 expression (**Figure 1**). This does appear to be one mechanism by which IE62 is expressed, since the existence of Oct-1/ORF10/HCF-1 complexes interacting with the IE62 promoter has been demonstrated, and depletion of HCF-1 by small interfering RNA (SiRNA) significantly reduces ORF10-mediated transactivation of the IE62 core promoter.

IE62 has also been shown to regulate expression of its own promoter and, unlike HSV-1 ICP4, is capable of positive as well as negative regulation. An alternative pathway for initial expression of the three IE genes involves the direct action of IE62 that enters the infected

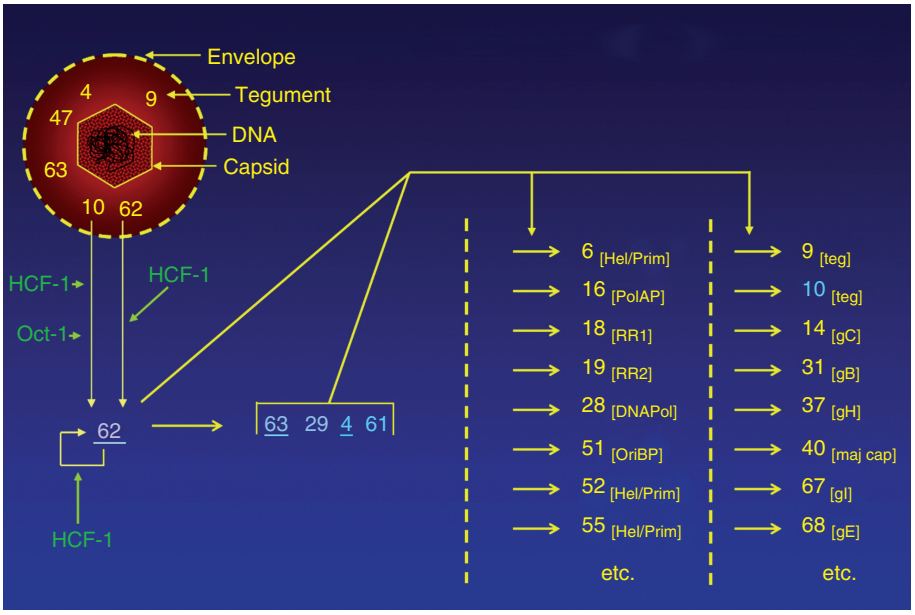


Figure 1 Schematic of the VZV virion and data on lytic phase gene expression. Numbers refer to the ORFs within the VZV genome. The IE genes are underlined. VZV genes whose protein products are known to be directly involved in viral gene expression are shown in blue. Cellular factors involved in IE gene expression are shown in green. Cellular factors such as Sp1 and USF known to be involved in non-IE gene expression are not shown. The vertical dashed lines delineate ORFs whose gene products are primarily involved in DNA replication and nucleotide metabolism or in the structure of the virion.

cell as part of the virion tegument. This pathway involves the cellular factor HCF-1, which has been shown to interact functionally with IE62. IE62 and HCF-1 cooperate in activating the IE62 promoter, resulting in a positive feedback loop allowing for efficient IE62 expression and IE62-mediated activation of the IE4 and IE63 promoters. This mechanism appears to be unique among the alphaherpesviruses to VZV.

Non-IE Gene Expression

Expression of the majority of VZV genes homologous to HSV-1 E and L genes is believed to be mediated primarily through the action of IE62 in conjunction with other viral proteins (the IE4, IE63, ORF61, and ORF29 proteins) and cellular factors. Information regarding expression of these genes is limited owing to the small number of promoters that have been analyzed. It is currently believed that IE62 is capable of transactivating the great majority of VZV promoters in the absence of other viral gene products. However, this rule is not hard and fast since the responsiveness to IE62 of promoters expressing structural proteins such as gI appears to be much less than that observed with IE promoters and the promoters controlling expression of DNA polymerase and SSB. Based on their ability to interact directly with IE62 and the frequent presence of their binding sites in VZV promoters, the ubiquitous cellular factors USF and Sp1 appear to play a significant role in IE62-mediated expression of VZV genes. They are believed to stabilize TBP binding, and both have been shown to interact directly with IE62. The roles of these transcription factors in IE62-mediated activation of specific VZV promoters have been validated in the SCID/hu mouse model of VZV pathogenesis.

These results represent only the beginnings of our understanding of the full spectrum of VZV gene regulation. As indicated above, IE4 and the ORF61 protein can also activate some non-IE promoters in the absence of IE62. Moreover, in the case of low levels of IE62 in the infected cell, expression of the ORF47 kinase and IE4 remains at essentially wild-type levels, in contrast to other known IE62-responsive genes, thus suggesting that there may be alternative non-IE62-mediated mechanisms for expression of some VZV genes. Other cellular transcription factors are also likely to be involved in VZV gene expression, including those in the CREB family (AP-1 and ATF-2). Finally, it is not known whether HCF-1 is required for activation of all IE62-responsive promoters, or whether this factor is important only for IE gene expression.

Protein Translation

Little is known about the details of translation in VZV-infected cells. It is assumed that mRNAs are translated in a fashion similar to that in HSV-1- and other alphaherpesvirus-infected cells, and that translation occurs on both free and membrane-bound polysomes.

Following translation, viral proteins are efficiently transported to specific cellular organelles (e.g., the nucleus and *trans*-Golgi network), where they perform their roles in viral replication and particle assembly. It has been shown recently that IE63 increases expression of the cellular factor EF-1α, which is involved in the initiation of translation. Thus, VZV infection may increase the efficiency of translation.

Post-Translational Processing

The most extensively studied post-translational processes involving VZV proteins are glycosylation and phosphorylation. Glycosylation is mediated by cellular genes, whereas phosphorylation involves the kinases encoded by ORF47 and ORF66, as well as cellular kinases. The VZV glycoproteins are all N-linked glycosylated and the majority show additional modifications such as O-linked glycosylation, sulfation, and phosphorylation. For example, gE contains both O-linked and N-linked glycans and is heavily sulfated and phosphorylated, with phosphorylation occurring at the level of both the polypeptide chain and glycosyl residues. The phosphorylated amino acids are serine and threonine but not tyrosine, and the enzymes involved are probably cellular casein kinases I and II. The enzyme responsible for phosphorylation of the complex oligosaccharides has not been identified, and roles for the specific phosphorylated or sulfated residues are unknown.

Viral proteins that are modified primarily by phosphorylation include the IE62, ORF61, and IE63 proteins. Phosphorylation of IE62 is known to involve the ORF66 and ORF47 kinases, as well as cellular kinases such as casein kinases I and II and protein kinase C. The function of the majority of these phosphorylation events is unknown, but phosphorylation by the ORF66 kinase plays a role in intracellular localization of IE62. Specifically, upon phosphorylation by the ORF66 kinase, IE62 is excluded from the infected cell nucleus, resulting in a diminution of viral gene expression and incorporation of IE62 into the virion particle. The ORF47 and ORF66 kinases also play roles in viral cell tropism and pathogenesis, since viruses lacking these functions are deficient for growth in human T cells and primary skin cells.

Assembly, Release, and Cytopathology

Models for assembly and release of VZV virions are derived from extensive electron microscopic studies and from analysis of the synthesis and trafficking of VZV glycoproteins in infected cells. However, no consensus model exists. Fully assembled capsids containing unit-length genomes representative of all four possible isomeric forms appear to be released from the nucleus, having acquired a portion of the tegument and a lipid coat from the nuclear lamella. These particles are then deenveloped and bud into the Golgi apparatus, where additional tegument proteins are acquired. The mature viral envelope derived from Golgi membranes and containing the fully processed viral glycoproteins is then added. The particles are either released from the cell by fusion of virus-containing vesicles with the plasma membrane or of vesicles containing enveloped particles with lysosomes, in which case complete or partial degradation of the virions may take place. The involvement of lysosomes is proposed to rest on their mannose-6-phosphate receptors, which are capable of binding this sugar present on the surface of VZV particles. This process does not occur in the skin of the human host since the cells in the superficial layers of the skin lack lysosomal vesicles. This may explain why virus isolated from vesicle fluid is more infectious than that obtained in tissue culture and why it exhibits a higher and more stable titer.

In tissue culture, cells infected with VZV initially show increased refractility followed by rounding and swelling. Multinucleated cells are observed late in infection, although the extent of syncytium formation is cell type dependent and usually is less than that seen with syncytial HSV-1 strains. The infected cells eventually detach from the support surface. The nuclei of infected cells are larger than those of uninfected cells, with marginally located chromatin and peripherally located nucleoli.

Gene Expression during Latency

There is evidence that, during VZV latency, a small number of lytic genes are expressed at both the RNA and protein levels. These include ORF4, ORF21, ORF29, ORF62, and ORF66. These proteins play unknown roles during latency, and are made even more intriguing in that they are localized primarily in the cytoplasm rather than the nucleus. The relative frequencies of detection of expression of these genes varies considerably, with the two most frequently detected being the products of ORF63 (IE63) and ORF29 (SSB). Therefore, the promoters regulating expression of these two genes appear to be the best candidates in which to find clues relating to the mechanisms of latent gene expression.

The putative IE63 promoter borders on ori_S, which is present in TR_S and IR_S. Little work has been published concerning the promoter-regulating expression of IE63. A putative TATA element has been identified by computer analysis 28 nt upstream from the transcriptional start site, as have several potential binding sites for Sp1 and CAAT. None of these elements has yet been authenticated by site-specific mutation.

More information is available concerning expression of ORF29. The promoter lies within an intergenic region situated between the divergently oriented ORF28 and ORF29 protein-coding regions, and shares a required USF site with the ORF28 promoter. ORF28 and ORF29 can be expressed either coordinately or independently, and the observed expression of only ORF29 during latency may involve neuron-specific cellular factors that recognize sequences within the ORF29 promoter and lead to its activation.

An alternative regulatory mechanism could involve variations in the chromatin structures of the promoter regions of the latently expressed genes. Like the HSV-1 genome, the VZV genome is episomal during latency and is likely to be bound by histones. This raises the possibility that, during latency, the promoters and coding regions of the putative latency-associated genes, particularly ORF63 and ORF29, could be histone-free (euchromatic) and non-repressed. How and why these specific regulatory elements could be singled out remains to be established.

HCF-1 may also play a role in reactivation of VZV from latency. In contrast to the situation in other tissues, HCF-1 is not detected in the nuclei of sensory ganglia but rather in the cytoplasm. However, experimental conditions that induce reactivation of HSV-1 result in rapid nuclear localization of HCF-1. Thus, redistribution of HCF-1 may trigger expression of IE62, ultimately resulting in the production of infectious virus.

Subjects for Future Investigation

Molecular Mechanism of Vaccine Attenuation

The live-attenuated vaccine for prevention of varicella has been in use in Japan for over two decades and was approved for use in the USA in 1995, where coverage is now approaching 95% of the susceptible pediatric population. This vaccine has also recently been shown to be efficacious for the prevention of zoster and for mitigation of postherpetic neuralgia. This being said, the vaccine represents a mixture of strains and retains the capacity to establish a latent infection and reactivate. Moreover, the molecular mechanism of attenuation remains unknown. A large number of mutations are present in vaccine virus IE62, but it is clear that the cause of attenuation extends beyond IE62 and is multifactorial. An important area of future study would involve definition of the mechanism of attenuation and production of a better characterized, and therefore safer, second-generation vaccine.

Molecular Basis of Post-Herpetic Neuralgia

One of the problems facing the current aging population in the USA and the rest of the world is the morbidity associated with post-herpetic neuralgia resulting from episodes of zoster. Nothing is known about the roles that viral genes play in this process. Investigation of the effects of specific VZV proteins on the expression or elaboration of neuropeptides and neuronal processes will be important in elucidating the molecular mechanisms responsible for post-herpetic neuralgia. This may aid the development of drugs specific for amelioration of this syndrome. Particular focus would be appropriate on the IE63 and ORF29 proteins, which have been detected in human ganglia.

VZV and Cellular Processes

There is now a considerable and continuously expanding body of information concerning the interaction of various VZV gene products with host cell components. Furthermore, recently reported gene array analyses indicate that the expression of numerous cellular genes is affected during infection. However, little information is available on the specific VZV factors involved in these changes and in what ways these changes are important for infection and pathogenesis. VZV-induced alteration of host cell function is known to differ to some extent from that observed with HSV-1, since VZV infection, unlike HSV-1 infection, inhibits the cellular nuclear factor kappa B (NFκB) pathway. Examination of these questions at the molecular level in the context of the various cell types infected by VZV will reveal important information, which will not only provide insights into VZV infection but also into fundamental cellular processes.

See also: DNA Vaccines; Herpes Simplex Viruses: General Features; Herpes Simplex Viruses: Molecular Biology; Herpesviruses: General Features; Herpesviruses: Latency; Persistent and Latent Viral Infection; Pseudorabies Virus; Varicella-Zoster Virus: General Features.

Further Reading

Cohen JI and Straus SE (2001) Varicella zoster virus and its replication. In: Fields BM, Knipe DM, and Howley PM (eds.) *Virology*, pp. 2707–2730. Philadelphia, PA: Lippincott-Raven.

Gomi Y, Sunamachi H, Mori Y, Nagaike K, Takahashi M, and Yamanishi K (2002) Comparison of the complete DNA sequences of the varicella vaccine and its parental virus. *Journal of Virology* 76: 11447–11459.

Grose C, Maresova L, Medighesi G, Scott GK, and Thomas G (2006) Endocytosis of varicella zoster virus glycoproteins: Virion envelopment and egress. In: Sandri-Goldin RM (ed.) *Alpha Herpesviruses: Molecular and Cellular Biology*, pp. 157–174. Norfolk, UK: Caister Academic Press.

Kenyon TK, Homan E, Storlie J, Ikoma M, and Grose C (2003) Comparison of varicella zoster virus ORF47 protein kinase and casein kinase II and their substrates. *Journal of Medical Virology* 70: 95–102.

Kinchington PR and Cohen JI (2000) Viral proteins. In: Arvin AM and Gershon AA (eds.) *Varicella Zoster Virus: Virology and Clinical Management*, pp. 74–104. Cambridge: Cambridge University Press.

Ku C-C, Besser J, Abendroth A, Grose C, and Arvin AM (2005) Varicella-zoster virus pathogenesis and immunobiology: New concepts emerging from investigations with the SCIDhu mouse model. *Journal of Virology* 79: 2651–2658.
Mitchell BM, Bloom DC, Cohrs RJ, Gilden DH, and Kennedy PGH (2003) Herpes simplex virus-1 and varicella-zoster virus latency in ganglia. *Journal of Neurovirology* 9: 194–204.
Nagaike K, Mori Y, Gomi Y, *et al.* (2004) Cloning of the varicella-zoster virus genome as an infectious bacterial artificial chromosome in *Escherichia coli*. *Vaccine* 22: 4069–4074.
Narayanan A, Nogueira ML, Ruyechan WT, and Kristie TM (2005) Combinatorial transcription of the HSV and VZV IE genes is strictly determined by the cellular coactivator HCF-1. *Journal of Biological Chemistry* 280: 1369–1375.
Oxman MN, Levin MJ, Johnson GR, *et al.* (2005) A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. *New England Journal of Medicine* 325: 2271–2284.
Ruyechan WT (2006) Varicella zoster virus transcriptional regulation and the roles of VZV IE proteins. In: Sandri-Goldin RM (ed.) *Alpha Herpesviruses: Molecular and Cellular Biology*, pp. 1–20. Norfolk, UK: Caister Academic Press.
Ruyechan WT and Hay J (2000) DNA Replication. In: Arvin AM and Gershon AA (eds.) *Varicella Zoster Virus: Virology and Clinical Management*, pp. 51–73. Cambridge: Cambridge University Press.
Silverstein S and Straus SE (2000) Pathogenesis of latency and reactivation. In: Arvin AM and Gershon AA (eds.) *Varicella Zoster Virus: Virology and Clinical Management*, pp. 123–141. Cambridge: Cambridge University Press.

Varicosavirus

T Sasaya, National Agricultural Research Center, Ibaraki, Japan

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Big-vein disease of lettuce A lettuce disease that is characterized by abnormal dilation or enlargement of a vein or artery in a lettuce leaf, decreasing market value and reducing the proportion of marketable lettuce plants. The name 'big-vein disease' refers to the appearance of the major symptoms.
L protein A main subunit of the polymerase complex, responsible for most of the functions required for transcription and replication: RNA-dependent RNA polymerase, mRNA 5′ capping, 3′ poly(A) synthesis, and protein kinase activities.
Olpidium brassicae An obligate parasitic root-infecting fungus and a member of the *Chytridiomycetes* that seldom appears to be deleterious to the host plants but acts as vector of plant viruses.
Stop-and-start model A model for RNA synthesis of nonsegmented negative-sense RNA viruses that the viral polymerase complex transcribes a gene, polyadenylates and releases mRNA, and reinitiates transcription of the next gene under the direction of the gene-junction sequence.

History

Big-vein disease of lettuce (*Lactuca sativa* L.) was first reported in 1934. The causal agent was postulated to be a root-infecting virus, but the virus particles remained unidentified for more than half a century. In 1983, a rod-shaped virus was found in field-grown lettuce showing big-vein symptoms. Because the rod-shaped virus is always associated with big-vein-affected lettuce, it was regarded as the agent that induced big-vein disease, although this virus alone had not been shown to actually induce big-vein symptoms in lettuce. The rod-shaped virus was named *Lettuce big-vein virus* and was classified as the type species of the genus *Varicosavirus*, which had been established by the International Committee on the Taxonomy of Viruses (ICTV) in 2000.

Since 2000, however, a second virus assigned to the genus *Ophiovirus* and named mirafiori lettuce virus (MiLV; also referred to as mirafiori lettuce big-vein virus), has been consistently isolated by various researchers from field-grown lettuce showing big-vein symptoms. Mechanical inoculation of partially purified MiLV preparations and transmission experiments using MiLV- or lettuce big-vein associated virus (LBVaV)-carrying fungal vectors independently (and conclusively) showed that MiLV, but not LBVaV, is the causal agent of big-vein disease. Therefore, lettuce big-vein virus was renamed lettuce big-vein associated virus by ICTV in 2005. However, the etiology of big-vein disease is still not fully understood, because big-vein affected plants serologically negative for MiLV but positive for LBVaV were reported in a field experiment in Italy. A transgenic line carrying the coat protein (CP) gene of LBVaV in antisense orientation showed resistance both to LBVaV infection and to MiLV infection, and was thus resistant to big-vein disease.

Stunt disease of tobacco (*Nicotiana tabacum* L.) reported only from Japan was first recorded in 1931 as a seed-bed disorder. Stunt disease seriously damaged tobacco leaf production in Japan until the 1960s, when the disease was found to be transmitted by an obligate parasitic soil-borne fungus, and tobacco growers paid more attention to tobacco

seed-bed sanitation. Because tobacco with stunt symptoms invariably has a varicosavirus and inoculation of tobacco seedlings with partially purified virion preparation produces typical tobacco stunting, the virus is regarded as the causal agent of tobacco stunt disease. Because of marked differences in the host reaction between the varicosavirus and LBVaV, the virus was initially considered to be a distinct species, rather than a strain of LBVaV, and named tobacco stunt virus (TStV). LBVaV and TStV are serologically indistinguishable and a recent comparison of nucleotide and amino acid sequences for LBVaV and TStV confirmed that TStV is a strain, a tobacco strain, of LBVaV.

In 2000, ICTV listed camellia yellow mottle virus and freesia leaf necrosis virus as tentative varicosaviruses. However, in 2005 ICTV dropped the two viruses from the genus *Varicosavirus* due to the lack of reliable information on their biological and virion properties. LBVaV is thus the only confirmed virus species of the genus *Varicosavirus*.

LBVaV was initially believed to have a divided genome consisting of two components of double-stranded (ds) RNA, approximately 7.0 and 6.5 kbp in size. Once LBVaV purification was established and the complete nucleotide sequence of LBVaV determined, a precise reinvestigation of LBVaV genome components showed that LBVaV is not a dsRNA virus but a single-stranded RNA virus with a bipartite genome. Negative-sense and positive-sense RNAs are separately encapsidated in the virions, with the negative-sense RNA being predominant.

Taxonomy and Classification

LBVaV particles are fragile, nonenveloped rods about 18 nm in diameter with a dominant particle length of 360 nm. Virus particles have a central canal about 3 nm in diameter and an obvious helix with a pitch of about 5 nm, resembling the inner striated nucleocapsid core of the rhabdovirus. Virus particles are very unstable *in vitro*; so the helix of particles, especially those in purified preparations, tends to loosen and come partially uncoiled, even if fixed with glutaraldehyde.

Criteria for membership in the genus are nonenveloped rod-shaped particles, a two-segmented negative-sense RNA genome, and transmission through moist soil by motile zoospores of an obligate parasitic soil-borne fungus. The fungal vector is reported to be *Olpidium brassicae*, but the taxonomy and nomenclature of the fungus needs to be reevaluated.

Genetics and Evolution

Nucleotide sequence and genome-mapping studies of LBVaV showed that the LBVaV genome consists of 12 878 nucleotides (nt) and is divided into two segments (**Figure 1**). The larger LBVaV genome segment (LBVaV RNA1) contains antisense information for a small gene and a large gene that encodes a large protein (L protein)

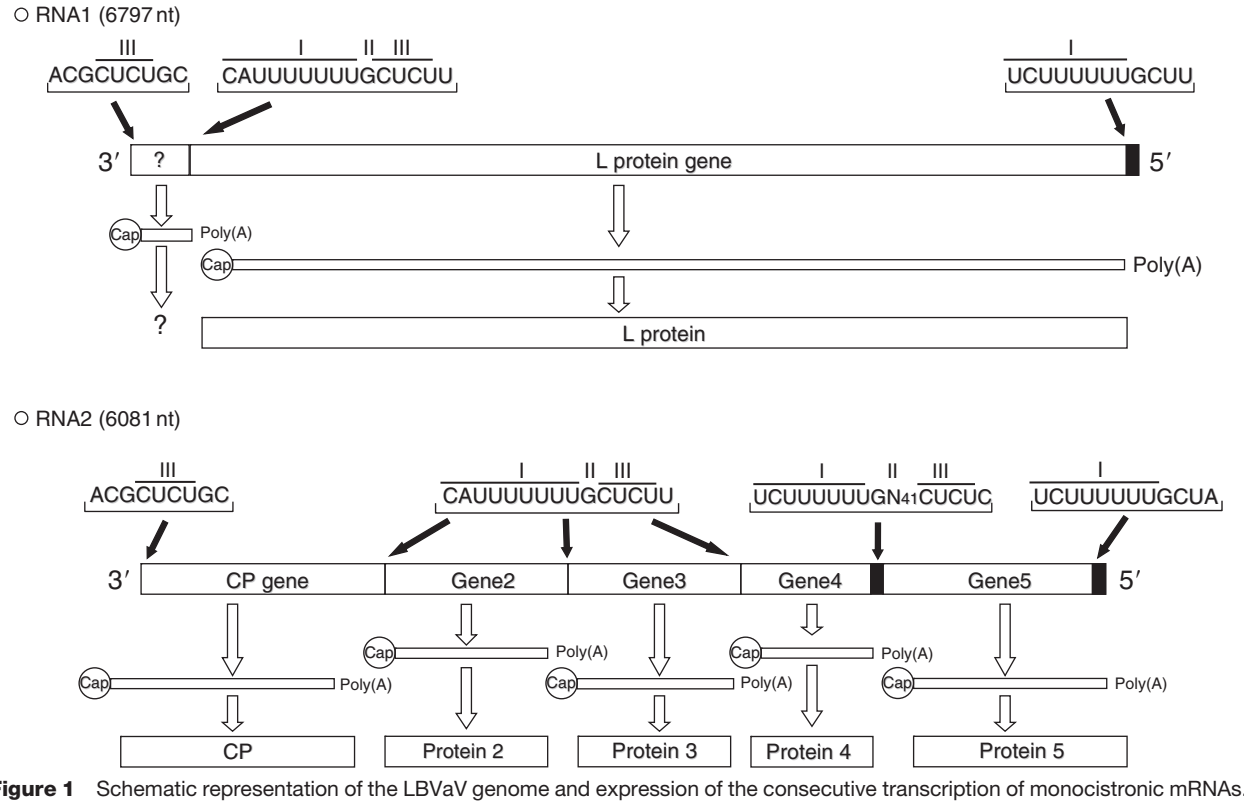


Figure 1 Schematic representation of the LBVaV genome and expression of the consecutive transcription of monocistronic mRNAs. Gene-junction regions, including a gene-end sequence (I), an intergenic sequence (II), and a gene-start sequence (III), are shown above negative-sense genomic RNAs.

of 2040 amino acids. The smaller LBVaV genome segment (LBVaV RNA2) contains antisense information for five major genes, which have coding capacities of 397, 333, 290, 164, and 368 amino acids, with the gene at the 3′ end of the LBVaV RNA2 encoding the CP. FASTA and BLAST analyses of the LBVaV CP and L protein revealed the amino acid sequence similarities to the nucleocapsid (N) proteins and the L proteins of rhabdoviruses, respectively. However, the translated sequences from the other four LBVaV genes showed no striking similarity to other known viral sequences.

Even though LBVaV is a two-segmented negative-sense RNA virus with a fragile nonenveloped rod shape, LBVaV shares several features with a rhabdovirus, which has a linear nonsegmented negative-sense RNA genome and large enveloped particles with a prominent fringe of peplomers.

L Protein Encoded on RNA1

As with other negative-sense RNA viruses, the L protein of LBVaV is positively charged and contains polymerase and RNA binding domains. Alignment of the LBVaV L protein sequence with those of several other negative-sense RNA viruses shows the conservation of functional motifs of an RNA-dependent RNA polymerase (**Figure 2**). The L protein of LBVaV contains a conserved premotif A, which presumably plays an important role in RNA template binding and positioning, and four motifs that correspond to motifs A, B, C, and D, thought to comprise the palm and finger regions of the polymerase active site in negative-sense RNA viruses. A putative ATP binding motif, $Kx_{17}GxGxG$, proposed to be involved in ATP binding associated with polyadenylation or protein kinase activity, is present at position 1643–1665, and LBVaV actually transcribes 3′ polyadenylated mRNAs.

The L protein is the only region sufficiently conserved to establish evolutionary relationships among negative-sense RNA viruses. Even though the LBVaV genome is segmented, most of the conserved motifs previously identified in the L proteins of nonsegmented negative-sense RNA viruses are conserved in the L protein of LBVaV (**Figure 2**), particularly the conserved sequence GDN in motif C, which likely constitutes the evolutionary and functional equivalent of the sequence GDD in polymerases of positive-sense RNA viruses. This is in striking contrast to the segmented genome of negative-sense RNA viruses whose polymerase has the conserved sequence SDD. Amino acids G at position 662 and W at position 671 in motif B, specific to the nonsegmented viruses, are also conserved in the L protein of LBVaV. Tetrapeptide E(F/Y)xS, located downstream from motif D, which is specifically conserved in polymerases of segmented negative-sense RNA viruses, is not found in the L protein of LBVaV.

Phylogenetic data and pair-wise amino acid sequence comparisons of the partial LBVaV L protein with those of nonsegmented negative-sense RNA viruses also prove that LBVaV is more closely related to viruses of the family

```
                    Premotif A                      Motif A
LBVaV   518 KEREIKVAARMYSLMTERMRYYFVLTEGL <39> NINIDFSKWNTNMR <54>
LNYV    490 KEREMNPTPRMFALMSHLMRVYVVITESM <48> CMSLDFEKWNGHMR <55>
NCMV    544 KEREMNPVARMFALMTLKMRSYVVITENM <42> CINMDFEKWNLNMR <56>
SYNV    567 KEREMKTKARFFSLMSYKLRMYVTSTEEL <41> SMNIDFSKWNQNMR <54>
RYSV    537 KERELKIMARFFALLSFKMRLYFTATEEL <41> VINMDFVKWNQQMR <55>
VSIV    530 KERELKLAGRFFSLMSWKLREYFVITEYL <42> ANHIDYEKWNNHQR <56>
RABV    543 KERELKIEGRFFALMSWNLRLYFVITEKL <42> AFHLDYEKWNNHQR <58>
BEFV    556 KERELKEEGRFFSLMSYELRDYFVSTEYL <42> ANNIDYEKWNNYQR <56>
IHNV    494 KEMELKIKGRGFGLMTFMPRLLQVLRESI <40> NKSLDINKFCTSQR <67>
TSWV   1286 KMQRTKT-DREIYLMSMKVKMMLYFIEHT <49> FLSADQSKWSASGL <69>
RSV    1416 KNQHGG--LREIYVLNIFERIMQKTVEDF <41> STSDDASKWNQGHY <75>

                    Motif B                 Motif C          Motif D
LBVaV       MCYRGHLGGFEGLRQKGWTVATVCLL <12> LMGQGDNQII <64> LDGRQLPQWYKKT
LNYV        YSFTGHKGGQEGLRQKGWTIFTVVCL <12> IMGMGDNQVL <64> LKGVPLSMDLKKI
NCMV        KSYEGHIRGFEGLRQKGWTVFTVVLI <12> LMGQGDNQVL <64> YKGVPLCSSLKRI
SYNV        WSRTGDESGKEGLRQKGWTITTVCDI <12> LIGGGDNQVL <63> YSGVPLRGRLKVI
RYSV        VCWIDDGAGKEGIRQKAWTIMTVCDI <12> LVGGGDNQVL <64> YKGVPLRSPLKQV
VSIV        VCWQGQEGGLEGLRQKGWTILNLLVI <12> VLAQGDNQVI <63> FRGVIRGLETKRW
RABV        TCWNGQDGGLEGLRQKGWSLVSLLMI <12> VLAQGDNQVL <63> FRGNILVPESKRW
BEFV        VCWEGQKGGLEGLRQKGWSILNYLMI <12> ILAQGDNQTI <63> IEGTIKGLPTKRW
IHNV        GVFSGLKGGIEGLCQYVWTICLLLRV <12> ILAQGDNVII <62> HCPQHLTLAIKKA
TSWV        NTYPVSMNWLQGNLNYLSSVYHSCAM <18> WIVHSDDNAT <32> KSFCITLNPKKSY
RSV         SYIETETGMMQGILHYTSSLFHAIFL <31> NMESSDDSSF <31> GTYLGIYKSPKST
```

Figure 2 Comparison of L protein motifs conserved among LBVaV, eight rhabdoviruses (lettuce necrotic yellows virus (LNYV), northern cereal mosaic virus (NCMV), sonchus yellow net virus (SYNV), rice yellow stunt virus (RYSV), vesicular stomatitis Indiana virus (VSIV), rabies virus (RABV), bovine ephemeral fever virus (BEFV), and infectious hematopoietic necrosis virus (IHNV)), and tomato spotted wilt virus (TSWV) and rice stripe virus (RSV), two segmented negative-sense RNA viruses of plants. Numbers at the beginning of lines indicate the position of the first displayed amino acid. Numbers within brackets indicate the numbers of amino acids not represented in the figure. Conserved residues recognized previously for L proteins of negative-sense RNA viruses are shown in bold letters and residues characteristic of the division between nonsegmented and segmented negative-sense RNA viruses are underlined.

Rhabdoviridae than to those of the family *Bornaviridae*, *Filoviridae*, and *Paramyxoviridae*. Phylogenetic analysis of the partial L proteins between LBVaV and rhabdoviruses shows that the L protein of LBVaV clusters with viruses of the family *Rhabdoviridae*, and that the L protein of LBVaV is more distantly related to those of plant rhabdoviruses (genera *Cytorhabdovirus* and *Nucleorhabdovirus*) than they are to each other. On the other hand, the L protein of LBVaV is more closely related to those of plant rhabdoviruses than those of other rhabdoviruses (**Figure 3**).

Proteins Encoded on RNA2

The first gene at the 3′ end of genomic RNA encodes the CP which shows low but significant similarity to the N protein of rhabdoviruses. The other genes encode proteins of unknown functions, while the second and fifth encoded proteins (proteins 2 and 5) have features slightly similar to corresponding proteins of rhabdoviruses.

Gene Bank Database Homology searches using the amino acid sequence of the LBVaV CP consistently retrieve N proteins of rhabdoviruses. Phylogenetic analysis of the LBVaV CP and N proteins of rhabdoviruses shows that the LBVaV clusters with viruses of the family *Rhabdoviridae*, and that LBVaV CP is most closely related to the N protein of infectious hematopoietic necrosis virus (IHNV) a fish rhabdovirus (genus *Novirhabdovirus*), and to a lesser extent with plant rhabdoviruses. In a

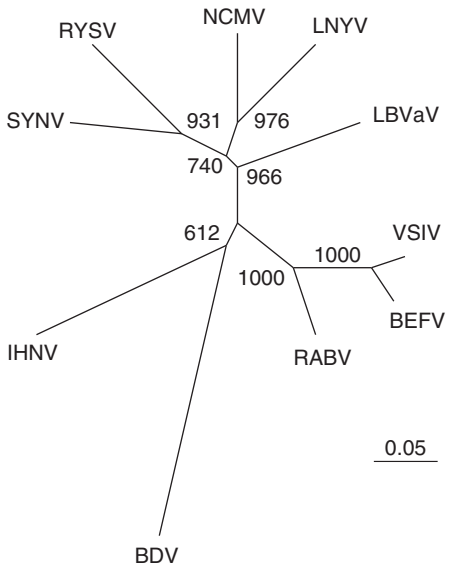


Figure 3 Computer-generated tree illustrating phylogenetic relationships between LBVaV and selected rhabdoviruses on the basis of aligned amino acid sequences of the partial L protein between premotif A and motif D. The tree is constructed using neighbor joining, and numbers at nodes are confidence values for 1000 bootstrap replicates; bootstrap values below 500 are not shown. Borna disease virus (BDV) is used as an out-group. See **Figure 2** for abbreviations of virus names.

PSI-BLAST search against the ProDom protein domain database, the carboxy-terminal part of the LBVaV CP shows similarity to two domains; one is the homologous domain among N proteins of plant rhabdoviruses and another is the homologous domain among those of fish rhabdoviruses.

The LBVaV protein 2 is encoded by the second gene that corresponds in position to a gene coding for the phosphoprotein (P protein) of rhabdoviruses, which interacts with the N protein and the L protein and constitutes the active polymerase complex. However, the 333 amino acid protein encoded by the second gene of RNA2 has no significant amino acid sequence relatedness to P proteins of rhabdoviruses. The overall structure of the LBVaV protein 2 and P proteins, assessed by amino acid composition and by relative hydropathicity distribution, is similar. Coiled-coil regions of P proteins of rhabdoviruses have been used to predict the regions for their oligomerization and interaction. Analysis using the COILS2 program predicts that the LBVaV protein 2 contains three possible coiled-coil regions at the amino-terminal (aa 29–48), central (aa 122–135), and carboxy-terminal (aa 239–253) domains that are similar in position, to those in the P protein of sonchus yellow net virus (SYNV). In site-directed mutagenesis experiments using the bacterially expressed P protein of vesicular stomatitis Indiana virus (VSIV), detailed analysis of phosphorylation sites for cellular casein kinase II in the P protein confirmed that five of ten potential phosphorylation sites of the P protein, which may regulate transcription and replication, are required to form the active polymerase complex. The LBVaV protein 2 also has ten potential phosphorylation sites, and the five essential phosphorylation sites for the P protein of VSIV are conserved in positions similar to those of the LBVaV protein 2. A predominantly basic 20-amino acid-long region near the carboxy terminus of the P protein of VSIV, which may act as a site for binding to the L protein, appears to be highly conserved in the carboxy-terminal region of the LBVaV protein 2.

The LBVaV protein 5, which corresponds in position to a gene coding for the glycoprotein (G protein) of rhabdoviruses, also has little direct relatedness to the envelope-associated glycoproteins of rhabdoviruses. The LBVaV protein 5 does not contain any canonical sites for N-linked glycosylation, and does not contain the N terminal signal peptide or carboxy-terminal hydrophobic transmembrane anchor domains, which are common among G proteins of rhabdoviruses. Conservation of cysteine residues is another common feature of G proteins of rhabdoviruses. Alignment using the Clustal W program for G proteins with plant rhabdoviruses shows that 11 cysteine residues are situated at conserved positions. In the case of LBVaV, six of ten cysteine residues are located at similar conserved positions in G proteins of plant rhabdoviruses. The LBVaV protein 5 may thus result from the degeneration of G proteins of plant

rhabdoviruses. The six cysteine residues in the LBVaV protein 5 that may play an important role in activities, such as forming the secondary structure of G proteins of plant rhabdoviruses appear to have been stringently conserved among the LBVaV protein 5 and G proteins of plant rhabdoviruses. The other five cysteine residues, however, appear to have evolved extensively to accommodate diverse host requirements and vector adaptations.

Transcription Termination/Initiation Strategy

From the LBVaV genome, capped and polyadenylated monocistronic mRNAs are transcribed. In the gene-junction regions of LBVaV, transcription termination/polyadenylation and initiation signal sequences comparable to those of rhabdoviruses especially cytorhabdoviruses are recognized (**Figure 4**) suggesting that LBVaV and rhabdoviruses may use a similar mechanism of transcription, a stop-and-start model, to differentially express individual genes from a contiguous virus genome.

Gene-junction regions of LBVaV, and likewise rhabdoviruses, are grouped into three elements: a gene-end sequence (element I) at the 3′ end of each gene on the genomic template, which is required for transcription termination/polyadenylation; a short nontranscribed intergenic sequence (element II), which is not represented in mRNAs and separates each gene; and a gene-start sequence (element III) located at the beginning of each subsequent gene, which plays an important role in transcription initiation. Gene-end sequences of rhabdoviruses consist of an AU-rich region, a cytidylate, and a poly(U) tract and the poly(U) tract is thought to be a template for the poly(A) tail of mRNA due to reiterative transcription or slippage. The gene-end sequences of LBVaV are either 3′-UAUNCAUUUUUUU-5′ (type A) or 3′-AUAAUCUUUUUU-5′ (type B), reminiscent of those observed in rhabdoviruses. When the gene-end sequence of LBVaV is type A, transcription of the next gene is initiated immediately after a single nucleotide G, while for type B, the two genes (genes 5 and L) were located at the 5′ most ends of each RNA (negative-sense) or transcription is reinitiated after a long 42 nt intergenic sequence located between genes 4 and 5, suggesting that the two variations of the gene-end sequences may influence signaling initiation of downstream mRNA synthesis as reported for VSIV in which the gene-end sequence plays an important role both in transcription termination/polyadenylation and in reinitiating transcription of downstream genes.

		I	II	III
LBVaV	TypeA	UN**CAUUUUUU**UU	**G**(N)$_X$	C**UCU**
	TypeB	**AAUCUUUUUU**		
LNYV		**AUUCUUUU**	**G**(N)$_X$	C**UCU**
NCMV		**AUUCUUUUU**	**G**A	C**UCU**
SYNV		**AUUCUUUUU**	**G**G	UU**G**W
RYSV		**AUUAUUUUU**	**G**GG	UU**GU**
VSIV		**AUACUUUUUU**UU	**G**A	UU**GU**
RABV		GYA**CUUUUUU**UU	**G**(N)$_X$	UU**GU**
IHNV		UR**UCUUUUUU**UU	**G**/A	CCR**W**

Figure 4 Comparison of gene-junction regions between LBVaV and selected rhabdoviruses. Gene-junction regions are separated into three elements: element I is a gene-end sequence that constitutes the poly(U) tract at the 3′ end of each gene on negative-sense genomic RNAs and plays the role of a transcription termination/polyadenylation signal; element II is a short intergenic sequence that is not transcribed during mRNA synthesis; and element III is a gene-start sequence that constitutes the initiation site for transcription of each mRNA. Bold type in viral sequences indicates consensus nucleotides, R and W indicate A/G and A/U, and (N)*x* corresponds to a variable number of nucleotides. See **Figure 2** for abbreviations of virus names.

The short nontranscribed intergenic sequence in most rhabdoviruses generally consists of a few nucleotides. Although intergenic sequences of rhabdoviruses are highly conserved in sequence and length in the genera *Vesiculovirus* and *Nucleorhabdovirus* but not in the genera *Lyssavirus*, *Ephemerovirus*, and *Cytorhabdovirus*, the first residue of the intergenic sequence is G. Site-directed mutagenesis experiments in the intergenic sequence using the infectious cDNA of VSIV indicated that the G residue is required for efficient termination of the upstream transcript. In the case of LBVaV, the intergenic sequence, except between genes 4 and 5, is highly conserved, consisting of a single nucleotide, G. Intergenic sequences of fish rhabdoviruses in the genus *Novirhabdovirus* also consist of a single nucleotide, G or A.

In contrast to most rhabdoviruses, including nucleorhabdoviruses whose gene-start sequence in gene-junction regions is 3′-UUGU-5′, the conserved gene-start sequence of LBVaV is 3′-CUCU-5′. Sequence 3′-CUCU-5′ is also conserved in gene-junction regions of lettuce necrotic yellows virus (LNYV) and northern cereal mosaic virus (NCMV) in the genus *Cytorhabdovirus*, suggesting that sequence 3′-CUCU-5′ may be important in acting as a transcription initiation signal for both LBVaV and cytorhabdoviruses, which replicate in the cytoplasm of plant cells.

Host Range and Geographic Distribution

LBVaV is extremely unstable *in vitro* making a mechanical transmission difficult, time-consuming, and inefficient. Since LBVaV inocula are frequently contaminated with MiLV and specific detection of LBVaV and MiLV has become feasible only very recently, available information on the symptomatology and experimental host range of LBVaV needs to be verified. We recently confirmed through fungal vector transmission experiment, however, that LBVaV systemically infected seven species in four families, and the infected plants including lettuce did not show any symptoms. In contrast, the experimental host range of the tobacco strain of LBVaV encompasses 41 species in nine dicotyledonous families

following mechanical inoculation and 35 plant species in 13 families after fungal vector transmission. The tobacco strain does not infect lettuce, but induces vein clearing, vein necrosis, mottling, and stunting in many *Nicotiana* species.

LBVaV is widespread in cool to temperate regions and several parts of subtropical regions in Europe, Asia, and North and South America, but the tobacco strain probably occurs only in Japan.

Transmission and Vectors

In nature, LBVaV is transmitted through motile zoospores of an obligate parasitic soil-inhabiting fungus. LBVaV is very stable in soil within long-lived, resting spores of the fungus for 20 years or more. It remains unclear whether the virus replicates inside fungal spores.

The natural vector for LBVaV is reported to be *Olpidium brassicae* (Wor.) Dang. *O. brassicae* was first found in a cabbage (*Brassica oleracea*) root in 1878 and the species name of the fungus is derived from the genus name of cabbage. Many strains of *O. brassicae* differing significantly in host specificity, morphology, and vector capacity have been recognized. In particular, a crucifer strain, which readily multiplies in the roots of crucifer plants such as cabbage and mustard, is differentiated from a noncrucifer strain that can not multiply in the roots of crucifer plants. The crucifer strain is host-specific and the noncrucifer strain highly plurivorous. Morphologically, zoosporangia and exit tubes of the crucifer strain differ slightly from those of the noncrucifer strain. The noncrucifer strain is generally a vector of MiLV and tobacco necrosis virus but the noncrucifer strain fails to transmit these viruses. Furthermore, the crucifer strain shows sexual reproductivity, although the noncrucifer strain does not reproduce sexually. Zoospores from a single-sporangium isolate of the crucifer strain do not develop a resting spore, but after mating between different mating (sexual) types, a resting spore forms. In contrast, zoospores from the noncrucifer strain develop a resting spore without mating. Based on these differences, the crucifer and noncrucifer strains have been divided into two distinct species, but this proposal has long been ignored. Recent rDNA-ITS analysis of *O. brassicae* strongly supported the notion that crucifer and noncrucifer strains are separate species rather than strains of the same *Olpidium* species, and a new species name, *O. virulentus*, has been proposed for the noncrucifer strain. The noncrucifer strain was confirmed to be a vector of LBVaV, whereas there is no reliable evidence that the crucifer strain (*O. brassicae*) is capable of transmitting LBVaV.

See also: Ophiovirus; Plant Rhabdoviruses; Rabies Virus; Vector Transmission of Plant Viruses.

Further Reading

Banerjee AK, Barik S, and De BP (1991) Gene expression of nonsegmented negative strand RNA viruses. *Pharmacology and Therapeutics* 51: 47.

Das T, Gupta AK, Sims PW, Gelfand CA, Jentoft JE, and Banerjee AK (1995) Role of cellular casein kinase II in the function of the phosphoprotein (P) subunit of RNA polymerase of vesicular stomatitis virus. *Journal of Biological Chemistry* 270: 24100–24107.

Einer-Jensen K, Krogh TN, Roepstorff P, and Lorenzen N (1998) Characterization of intramolecular disulfide bonds and secondary modifications of the glycoprotein from viral hemorrhagic septicemia virus, a fish rhabdovirus. *Journal of Virology* 72: 10189–10196.

Lot H, Campbell RN, Souche S, Milne RG, and Roggero P (2002) Transmission by *Olpidium brassicae* of *Mirafiori lettuce virus* and *Lettuce big-vein virus*, and their roles in lettuce big-vein etiology. *Phytopathology* 92: 288–293.

Rose JK and Whitt MA (2001) *Rhabdoviridae*: The viruses and their replication. In: Knipe M and Howley PM (eds.) *Field Virology,* 4th edn., pp. 1221–1224. New York: Raven Press.

Sasaya T, Ishikawa K, and Koganezawa H (2002) The nucleotide sequence of RNA1 of *Lettuce big-vein virus*, genus *Varicosavirus*, reveals its relation to nonsegmented negative-strand RNA viruses. *Virology* 297: 289.

Sasaya T and Koganezawa H (2006) Molecular analysis and virus transmission tests place *Olpidium virulentus*, a vector of *Mirafiori lettuce big-vein virus* and tobacco stunt virus, as a distinct species rather than a strain of *Olpidium brassicae*. *Journal of General Plant Pathology* 72: 20–25.

Sasaya T, Kusaba S, Ishikawa K, and Koganezawa H (2004) Nucleotide sequence of RNA2 of *Lettuce big-vein virus* and evidence for a possible transcription termination/initiation strategy similar to that of rhabdoviruses. *Journal of General Virology* 85: 2709–2717.

Tordo N, Haan PD, Goldbach R, and Poch O (1992) Evolution of negative-stranded RNA genomes. *Seminars in Virology* 3: 341–357.

Vector Transmission of Animal Viruses

W K Reisen, University of California, Davis, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Anthroponosis Virus transmitted among human hosts.
Arbovirus Virus transmitted by blood-feeding arthropods among vertebrates.
Diapause Arthropod hibernation.
Gonotrophic cycle Cycle of blood feeding and egg deposition.
Transmission Distribution of virus usually by the bite of an infectious vector.

Vector Animal (usually an arthropod) that distributes viruses among hosts.
Vector competence Ability of vector to become infected with and transmit a virus.
Vectorial capacity Mathematical measure of case distribution or the force of transmission.
Viremia The presence of viruses within the vertebrate host peripheral circulatory system.
Zoonosis Virus transmitted mainly among animal hosts with incidental transmission to humans.

Vector Transmission

This article describes how vectors (usually arthropods and especially insects) distribute or transmit viruses among vertebrate hosts. Although occasional instances of mechanical transmission by contaminated mouthparts of the feeding arthropod have been documented and nonviremic transmission among blood-feeding vectors has been demonstrated in the laboratory and occurs in nature, virus transmission generally is biological and propagative (**Figure 1**). This type of transmission demonstrates that the virus infects and multiplies within the tissues of both arthropod and vertebrate hosts before transmission can occur. Viruses transmitted by arthropods have been lumped into a loose assemblage termed 'arboviruses' that includes viruses within the families *Bunyaviridae*, *Flaviviridae*, *Rhabdoviridae*, and *Togaviridae*. Transmission rates and therefore the numbers of cases typically remain at low levels, unless there is a disruption in system balance such as a climate anomaly, virus mutation to a virulent form, or an ecological disturbance, or large-scale host movement that enables virus amplification to epidemic levels.

Horizontal Transmission

Arthropod vectors, such as mosquitoes, feed on vertebrates to obtain blood which is used as a dietary supplement to stimulate and support egg development. If the vertebrate host is circulating a sufficient quantity of virus within its peripheral blood (i.e., is viremic) and if the arthropod is susceptible to infection, then the arthropod may become infected soon after blood feeding. After the blood meal is digested and the eggs are laid, completing the gonotrophic cycle, the surviving arthropods will then seek a subsequent blood meal to initiate the maturation of the next batch of eggs. The duration of the gonotrophic cycle essentially delineates the frequency of vector–host contact and therefore the number of opportunities for transmission by an arthropod during its lifetime. Horizontal arbovirus transmission occurs after the arthropod becomes infectious and is capable of expectorating virus when it feeds to obtain the next and subsequent blood meals. All arthropods expectorate salivary secretions while blood feeding, and this provides a mechanism to deliver the virus to the host thus completing horizontal transmission. After becoming infectious, most arthropods continue transmitting virus throughout the remainder of their lifetime. West Nile virus (WNV) is an example of a virus maintained by horizontal transmission from bird to mosquito to bird.

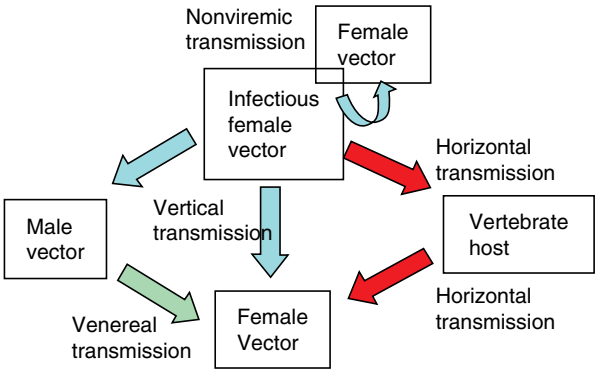


Figure 1 Types of arbovirus transmission within arthropod vectors and between arthropod vectors and vertebrate hosts. Vertical transmission can be trans-stadial, as in ticks, or transgenerational, as in mosquitoes.

Vertical Transmission

Some arboviruses acquired by the arthropod host are transmitted vertically to the next blood-feeding life stage. In ticks that feed once per life stage, transmission is trans-stadial (across life stages) from the larvae or nymphs that acquire the virus, molt, and then pass the virus to nymphs and/or adults that transmit their acquired infection to vertebrate hosts during the next blood meal. Tick-borne encephalitis virus (TBEV) is an example of a virus that is vertically maintained and transmitted within populations of the tick vectors, *Ixodes persulcatus* or *Ixodes ricinus*. In Diptera, acquisition of virus by the host-seeking female may be followed by passage of virus through the egg, larval, and pupal stages to adults in the next generation when transmission to vertebrate hosts is facilitated by infectious females. LaCrosse virus (LACV) is an example of a virus that is maintained vertically by efficient vertical transmission by its primary mosquito host, *Aedes triseriatus*, but is also transmitted horizontally to chipmunk reservoir hosts and occasionally to humans. In contrast, vertical passage of WNV occurs occasionally, but still may be important in virus amplification and persistence. In most arthropods vertical transmission is not 100% efficient, and therefore for a virus to be maintained, amplification by horizontal transmission is necessary to increase the number of infected individuals within the vector population.

Venereal Transmission

Although male mosquitoes and other arthropods may become infected vertically, they usually do not blood-feed and therefore generally are 'dead end' hosts for the viruses that infect them. However, in some instances infected males are able to transmit virus venereally to uninfected females during mating. These females may become infective and then transmit virus horizontally as well as vertically. LACV is also an example of a virus that may be transmitted venereally and then horizontally and vertically by infected females. In contrast, all male tick life stages require a blood meal for molting and therefore can become infected orally. In some instances these males, orally infected as immatures, can transmit virus venereally.

Nonviremic Transmission

When multiple vectors feed in close proximity, virus may be transmitted from infectious to uninfected arthropods without the host developing a viremia. This method of transmission initially was described for TBEV among *Ixodes* ticks that often attach near to one another on the host and require 4–5 days to blood-feed. Recently, however, nonviremic transmission has also been demonstrated for *Culicoides* flies as well as mosquitoes in laboratory experiments.

Types of Transmission Cycles

Epidemiologists have classified virus transmission cycles by the types of vertebrate hosts, with zoonoses maintained by transmission among animal hosts with occasional tangential involvement of humans, and anthroponoses maintained by direct transmission among humans (**Figure 2**).

Zoonoses

Many arboviruses are zoonoses that are maintained and amplified in transmission cycles involving one or more vectors and a variety of animal hosts. When amplification progresses to elevated levels, transmission may spill over to tangentially include domestic animals and/or humans. St. Louis encephalitis virus is an example of a zoonosis that is maintained and amplified by horizontal transmission by mosquitoes among birds, but spills over to tangentially infect humans (**Figure 2**). In this instance, birds serve as maintenance reservoir and/or amplification hosts, because they are susceptible and capable of producing viremias of sufficient titer to infect blood-feeding mosquitoes. Humans are 'dead end' hosts, because they do not produce sufficient viremias to infect additional mosquitoes. Vectors that participate in the basic zoonotic cycle are termed maintenance vectors, whereas those that carry the virus from the enzootic cycle (among animals) to domestic animals or humans are termed 'bridge' vectors. Bridge vectors may not be able to maintain transmission in the absence of more susceptible and effective maintenance vectors. Although in many zoonoses humans are 'dead end hosts', humans still become infected and may develop serious disease.

Anthroponoses

In some circumstances, humans produce a viremia sufficiently elevated to infect vectors and maintain a vector–human transmission cycle independent of animal hosts. Dengue viruses (DENVs) are examples of anthroponotic viruses maintained by human–mosquito–human transmission (**Figure 2**). Although there have been reports of horizontal enzootic and vertical transmission of DENV, these events rarely have been associated with or have led

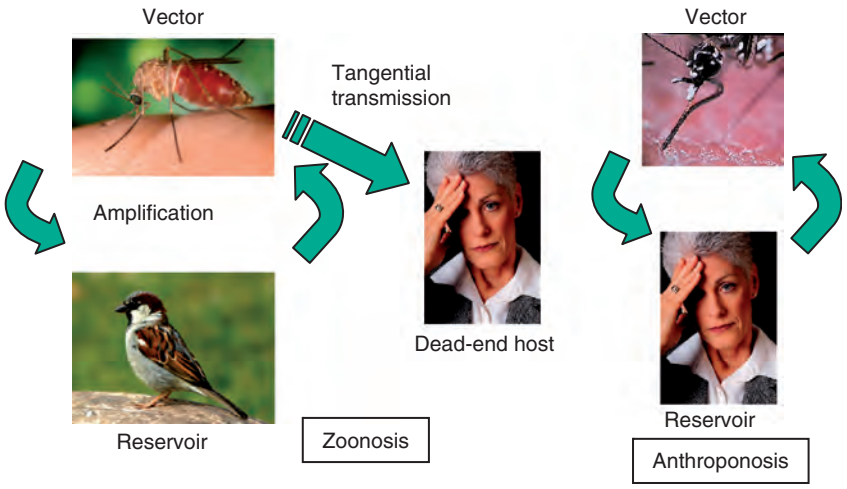


Figure 2 Comparison between a bird-maintained zoonotic virus (such as the St. Louis encephalitis virus) and an anthroponotic virus (such as dengue virus). Activities to decrease or interrupt virus amplification and thereby prevent human cases.

to major epidemics (transmission to or among humans). The distinction between zoonoses and anthroponoses becomes blurred with viruses such as yellow fever virus, in which there is a distinct enzootic primate–mosquito–primate maintenance cycle in jungle habitats and then an epidemic human–mosquito–human cycle in urban habitats.

Factors Enabling Transmission

The efficiency of arbovirus transmission depends upon genetic factors that encode for susceptibility to infection and for biological traits that enable vector survival and repeated susceptible host contact. These properties may be summarized, respectively, as vector competence and vectorial capacity.

Vector Competence

The innate ability of an arthropod to acquire and transmit an arbovirus is termed vector competence. Infection occurs when the ingested virus binds to receptor sites in the lumen of the midgut of the vector and then enters the midgut cells. Susceptibility to infection typically relates to the number of receptor sites and to the affinity of the infecting virus to bind with these sites. Many arthropods lack suitable binding sites, do not get infected, and have what is termed a midgut infection barrier. Others allow midgut infection and perhaps low-level virus replication, but the infection remains midgut-limited and does not disseminate to infect the salivary glands. Post-transcriptional gene silencing by RNA interference (RNAi) is a natural antiviral response in mosquitoes. As a countermeasure, some arboviruses evade RNAi or produce RNAi suppressors that limit antiviral defense. These viruses replicate to high levels and escape the midgut; however, not all infect the salivary glands or they infect the salivary glands but are not transmitted. These salivary gland barriers may be similar to midgut infection and escape barriers and are referred to as salivary gland infection and escape barriers. Still other less-known vector attributes may downregulate infections after or during dissemination, also limiting transmission. In these vectors, transmission peaks soon after infection, but then decreases over time, so that older vectors have lower transmission rates than younger vectors. In general, however, competent vectors frequently become infected and efficiently transmit co-evolved viruses. The risk of infection and transmission by competent vectors increases as a function of vector–host contact which may be measured by the vector's reproductive age.

Vectorial Capacity

In general, vector competence defines the ability of a vector to become infected with and transmit an arbovirus, usually under laboratory conditions. By comparison, vectorial capacity (C) is an epidemiological measure of the force of transmission or the daily case dissemination rate, and combines ecological factors relating to the frequency of reservoir host contact (ma, daily vector density in relation to host abundance, squared to account for refeeding), daily vector survival (P), the duration of the extrinsic incubation period (e), and vector competence (V):

$$C = \frac{ma^2 V(P^e)}{-\ln P}$$

Although originally developed to describe the rate of malaria transmission, this formula has been used to describe arbovirus transmission dynamics or to compare the importance of different vector species. For a zoonosis such as WNV, ma^2 becomes bites per bird per night. Frequently, measures of vector abundance (m, originally the man-biting rate) are combined with measures of vector host selection (a, the anthropophagic index or frequency of feeding on humans) to estimate bites per host per day. For zoonoses these terms must be changed to reflect the maintenance host species and the frequency with which they are fed upon by maintenance vector species. Vector blood-meal host-selection patterns determine the vertebrate hosts most frequently infected, and their susceptibility and viremia response then determines the effectiveness of transmission. In general, vectors with a narrow host range that includes susceptible host species will more effectively and rapidly amplify viruses than those with a broad host range that includes both susceptible and nonsusceptible host species. The latter vector species may be important as bridge vectors; for example, *A. aegypti* is a very effective vector of DENV because it feeds almost entirely on humans, whereas *A. albopictus* is less efficient because of its broad host range.

Daily survival (P) typically is measured by examination of host-seeking female reproductive age or from mark–release–recapture studies. In the current formula, P is considered to be constant throughout the life of the vector; however, some studies indicate that mortality rates may actually decrease as a function of vector age, thereby increasing vectorial capacity. The extrinsic incubation period (e) is discussed in detail below, but essentially is the time from infection to transmission by the vector. Vector competence (V) is the same as defined above and measures the ability of the vector to become infected and transmit virus.

Extrinsic Incubation Period

Early in arthropod-transmitted viral epidemics, there are distinct time periods between episodes of new cases that delineate the 'extrinsic incubation period' or virus development outside of the vertebrate or human host. In reality,

this measure of transmission dynamics is related to the time from virus acquisition by the vector until the transmission leads to new cases. Because arthropod body temperature approximates ambient conditions and because virus replication is both temperature limited and dependent, the duration of the extrinsic incubation period changes with latitude and as a function of climate variation, being shortest during summer. Degree–day models can be generated in the laboratory by incubating infected females at different temperatures and measuring the time until they are capable of transmission. Transmission is temperature limited, because viruses have threshold below which they will not replicate. This lower threshold delineates viral distribution in time and space. Theoretically, viruses with a lower (i.e., cool) temperature threshold are expected to have a wider geographic distribution and can be transmitted more effectively at northern latitudes than those with a higher (warm) threshold. Above this lower limit, the rate of viral replication within the vector is dependent upon the temperature of the vector environment and typically increases as an exponential function of ambient temperature. Vector behavior may modify the temperature environment in comparison with ambient conditions, especially during cool periods of the year or day. Species that rest in houses, for example, have a more ameliorated temperature than those that rest outdoors. Nocturnal species that rest in burrows during the day and come out to seek blood-meal hosts at night always enter a warmer environment and therefore their body temperatures may slightly exceed ambient conditions. Both examples of insect behavioral change of body temperature can alter virus replication, transmission rates, and therefore virus amplification.

Phases of Transmission

Transmission dynamics vary over time and space and may be grouped into three phases: introduction or maintenance, amplification or epidemic, and subidence.

Maintenance

Seasonal changes in vector abundance or the autumnal cessation of blood-feeding behavior limit or interrupt arbovirus maintenance transmission. In general, viruses either persist within host populations or become regionally extinct and require reintroduction. At southern latitudes with sufficient vector abundance and warm temperatures, viruses may be transmitted continually during winter. In southern areas with alternating dry and wet seasons, vector abundance changes related to precipitation also dictate the extent and timing of the transmission season. At northern latitudes mechanisms for local maintenance may include persistent infections within either vector or vertebrate host populations that are acquired during fall and then relapse during the following spring. Alternatively, viruses may become regionally extinct and are reintroduced the following spring. Unfortunately, data supporting most overwintering mechanisms are fairly fragmentary despite years of investigation. Recent studies of the molecular genetics of multiple viral isolations made over time and space show patterns of change indicating mutation or extinction, followed by the introduction and persistence of new genotypes. Often, rather small changes in the viral genome can result in marked changes in virulence that affect fitness and therefore amplification. The changes enhancing virulence may be associated with increased host and geographical range. WNV is an example of a virus that has changed genetically, extended its distribution markedly, and has caused serious epidemics throughout northern latitudes in Europe and North America.

Amplification

After maintenance or introduction at low levels, arboviruses undergo seasonal amplification (or increase in the numbers of infected hosts) during favorable weather periods. The rate of amplification is dependent upon vector competence and vectorial capacity factors, but may be limited by vertebrate host immunity and climate. Acquired immunity or depopulation of the primary vertebrate host population during the previous season or seasons may affect transmission during the subsequent season, because a large percentage of infected vectors transmit virus to immune hosts that do not become infective to other vectors. In short-lived vertebrate hosts, such as house sparrows, population turnover rates are high and elevated herd immunity levels short-lived. However, elevated immunity rates in long-lived hosts, such as humans, may limit infection to younger, nonimmune cohorts. A second factor that may constrain transmission is the effect of climate on vector abundance. Arthropod populations tend to vary markedly in response to climate variation. During years when climate is unfavorable, abundance may remain low and below thresholds necessary to effectively amplify virus. During these years maintenance transmission remains at very low levels or virus may disappear during the current and subsequent seasons. In contrast, rapid vector population increases and longer survivorship during favorable weather periods frequently are accompanied by concurrent increases in virus amplification to epidemic levels.

Subsidence

Epizootic and/or epidemic-level transmission may be relatively short-lived and generally is followed by subsidence. Typically, the amplification curve attains asymptotic epidemic levels and then begins to subside due to decreasing numbers of susceptible hosts, seasonal changes

in day length or climate, and/or focused intervention measures. In general, the more intensive and extensive the epidemic transmission, the faster and more widespread the seroconversion or depopulation rates within the primary host population, and the more rapid the focal subsidence. During years with low-level transmission, sufficient naive hosts may remain and provide a source for viral amplification during the following season. Eventually, the numbers of susceptible hosts decrease, slowing the force of transmission and decreasing the numbers of new cases.

Seasonal changes in day length and weather can produce comparable dramatic decreases in epidemic transmission without exhausting the number of available hosts which will remain available for amplification during the following season. At temperate latitudes, the onset of cold weather and shortening photoperiod may induce diapause in the primary vector(s), terminate blood feeding, and interrupt transmission, leading to rapid subsidence. At lower latitudes, comparable seasonality of transmission can be induced by variation in rainfall. Amplification the following season may be dependent upon the intensity of transmission during the end of the previous season and the amount of virus present in the overwintering host populations.

Risk Factors

The risk of infection and disease in vertebrates is related directly to the degree of exposure to host-seeking infectious arthropods and to the vertebrate hosts' immune response to infection.

Residence

Undoubtedly, place of residence markedly affects the risk of infection. Mostly due to the ecology of the arthropod vectors and vertebrate hosts, most arboviruses tend to be focal in their distribution and associated with particular landscapes. Humans often become infected by zoonotic viruses when housing areas are constructed within transmission foci or when human alteration of the landscape creates environments that bring vector and maintenance host together. Rural housing within or adjacent to wooded areas supporting the mosquito *A. triseriatus* has been found to be a critical risk factor for LACV infection in children. In contrast, urban *Culex* populations produced in municipal wastewater systems interfacing with periurban communally roosting American crow populations have led the spatial aggregation of WNV infection risk in humans.

Climate

Climate variation affects temperature and precipitation patterns, mosquito abundance and survival, and therefore arbovirus transmission patterns. These changes often vary at different temporal scales. At temperate latitudes seasonal changes in temperature and photoperiod drive transmission cycles, whereas at tropical latitudes seasonal changes may relate more to wet and dry seasons. The magnitude of annual temperature and precipitation changes may have decadal or shorter cycles based on changes in sea surface temperature, such as the El Niño/southern oscillation change in the Pacific sea surface temperature that markedly alters precipitation and temperature patterns in parts of the Americas. These cycles alter storm tracks that affect mosquito and avian abundance, the intensity and frequency of rainfall events, and groundwater depth.

Age

In the absence of acquired immunity, different viruses seem to exhibit a predilection to cause disease in different host age classes. This may be related to differential biting rates by vector mosquitoes on different host age groups or due to age-related differences in innate immunity. Host-seeking mosquitoes seem to feed more readily on adult than on nestling birds of the same species. With regard to illness, for example, western equine encephalomyelitis virus (WEEV) tends to cause more frequent and severe disease in infants (<1 year), whereas SLEV causes more severe illness in the elderly (>65 years). For endemic viruses, the prevalence of previous infection increases as a function of age, and therefore older cohorts tend to be protected by a greater prevalence of acquired immunity than younger cohorts. This age-related pattern of acquired immunity can change the apparent-to-inapparent ratio of clinical disease for endemic viruses.

Occupation

Different occupations place workers in different environments at different times, and therefore vary their risk for mosquito and arbovirus exposure. For example, farm workers who pick and pack vegetables outdoors at night in southern California were found to have an 11% seroprevalence rate against SLEV, whereas persons residing within Los Angeles were found to have a lower 1.7% exposure rate. In contrast, persons sitting at home in late afternoon in Thailand have a high attack rate by *A. aegypti* and a high exposure rate to dengue viruses, compared to persons working outdoors from morning to night in agricultural professions.

Socioeconomic Status

Historically, socioeconomic status has been related closely to the distribution of cases during urban epidemics. Homes and municipal drainage systems frequently are not well maintained in low-income neighborhoods, and this has shown to be related to the distribution of human cases. In the tropics, lack of a municipal water system and domestic

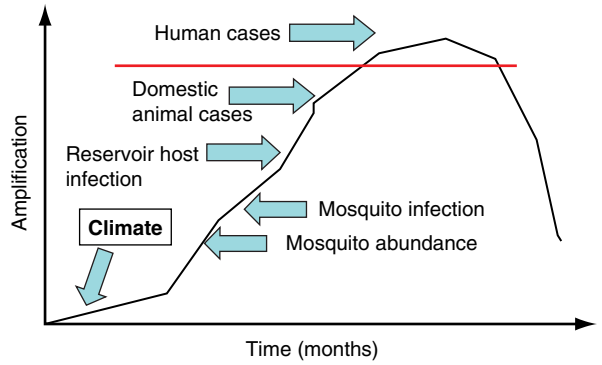


Figure 3 Surveillance indicators used to measure enzootic virus amplification and forecast the risk of human infection. Climate variation can provide the earliest indication of pending risk and may drive the rate of viral amplification.

water storage has been linked strongly to *A. aegypti* abundance and to disease risk.

Forecasting Risk

The cascade of different surveillance measures becoming positive over time as virus amplifies can be used to forecast the risk of human infection, especially for endemic zoonoses. **Figure 3** shows the approximate relative times when surveillance measures might become positive along the enzootic amplification curve for a virus such as WEEV. Usually, human cases are the last factor to be detected and tangential transmission occurs only after considerable WEEV amplification in the mosquito–bird cycle. These surveillance data can be used to focus on mosquito control.

See also: Animal Rhabdoviruses; Epidemiology of Human and Animal Viral Diseases.

Further Reading

Black WC, IV and Moore CG (2006) Population biology as a tool for studying vector-borne diseases. In: Beaty BJ and Marquardt WC (eds.) *The Biology of Disease Vectors*, pp. 393–416. Niwot, CO: University Press of Colorado.

Edman JD (2000) Arthropod transmission of vertebrate parasites. In: Eldridge BF and Edman JD (eds.) *Medical Entomology*, pp. 151–164. Dordrecht, The Netherlands: Kluwer.

Eldridge BF (2000) The epidemiology of arthropodborne diseases. In: Eldridge BF and Edman JD (eds.) *Medical Entomology*, pp. 165–186. Dordrecht, The Netherlands: Kluwer.

Hardy JL, Houk EJ, Kramer LD, and Reeves WC (1983) Intrinsic factors affecting vector competence of mosquitoes for arboviruses. *Annual Review of Entomology* 28: 229–262.

Reisen WK (2002) Epidemiology of vector-borne diseases. In: Mullen GR and Durden LA (eds.) *Medical and Veterinary Entomology*, pp. 16–27. New York, NY: Academic Press.

Vector Transmission of Plant Viruses

S Blanc, INRA–CIRAD–AgroM, Montpellier, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Fitness The relative ability of an individual (or population) to survive and reproduce in a given environment.
Helper component (HC)-transcomplementation An HC encoded by a viral genome X mediates the vector transmission of a virus particle containing a viral genome Y.
Horizontal transmission The transmission of a virus, parasite, or other pathogen from one individual to another within the same generation, as opposed to vertical transmission.
Pierce-sucking insects Insects adapted to sap or blood feeding, with the mouthparts transformed into long chitin needles that can pierce and penetrate tissues and allow pumping up their content.
Quasispecies Ensemble of mutant viral genomes constituting a viral population.
Vector Organism acquiring a pathogen on an infected host and inoculating it in a new healthy one.
Vertical transmission The transmission of a pathogen from the parent(s) to the offspring, usually through the germline.

Introduction

Viruses are intracellular parasites diverting the host cellular machinery for their own replication and offspring particles production. As such, they most often negatively affect the hosting cells, sometimes even killing them, and hence repeatedly and unavoidably face the problem of moving on and colonizing new healthy and potent 'territories'. Within a single host, viruses can both diffuse from cell to cell and be transported on longer distances by the vascular system. While animal viruses use membrane

fusions (if enveloped) or membrane receptors to penetrate healthy cells, plant virus entry during the host invasion is always resulting from a passage through 'tunnels' traversing the cell wall, called plasmodesmata, and ensuring a cytoplasm continuity between adjacent cells. Any viral population can grow this way only until the physical limits of the host are reached. Then, a critical passage in the 'outside world' separating two compatible hosts has to be successfully achieved. Because animals are motile and often come in contact, some associated viruses can directly access either blood or permissive tissues of a healthy host and operate a cell entry resembling that involved during invasion of single hosts. However, a most frequently adopted strategy relies on additional organisms, capable of sampling the virus population within an infected host, transporting, disseminating, and efficiently inoculating infectious forms of this virus within host population. Such organisms are designated vectors, giving rise to the term vector transmission. Vector transmission is found frequently in animal viruses and, presumably due to stable hosts and to the need of covering considerable distances between them, has been adopted by the vast majority of plant viruses. Each virus species is submitted to different ecological conditions; hence, an impressive complexity of host–virus–vector interactions has been unraveled over a century of research efforts. The object of this chapter is to synthesize the knowledge available at present in the field of vector transmission of viruses, with a special emphasis on plant viruses, where a great diversity of strategies have been discovered and documented. Indeed, the numerous patterns of vector transmission described for plant viruses include all those reported in animal viruses and many more.

Plant Virus Vectors

Any organism that is creating a break into the cell wall, either for penetrating a plant or simply for feeding on it, and that is capable of covering the distance between two separated plants, can possibly be used as a vector by viruses, for traveling through space and time. Vectors have been described in groups of organisms as diverse as parasitic fungi, nematodes, mites, and most importantly insects (**Table 1**). The pattern of virus uptake, preservation, transport, dissemination, and inoculation can be very

Table 1 Vectors and mode of transmission in families of plant viruses

Family[a]	*Vector*	*Mode of vector-transmission*[b]
Bromoviridae genus *Alfamovirus*	Aphids	Noncirculative capsid strategy
Bromoviridae genus *Cucumovirus*	Aphids	Noncirculative capsid strategy
Bromoviridae genus *Ilarvirus*	Thrips	?
Bromoviridae genus *Oleavirus*	?	?
Bromoviridae genus *Bromovirus*	Beetle	?
Bunyaviridae	Thrips, planthopper	Circulative propagative
Caulimoviridae	Aphid, mealybug, leafhopper	Noncirculative helper strategy
Circoviridae	Aphid	Circulative nonpropagative
Closteroviridae	Aphid, whitefly, mealybug	Noncirculative
Comoviridae genus *Comovirus*	Beetle	?
Comoviridae genus *Fabavirus*	Aphid	Noncirculative
Comoviridae genus *Nepovirus*	Nematode	Noncirculative capsid strategy
Geminiviridae	Leafhopper, whitefly	Circulative nonpropagative[c]
Luteoviridae	Aphid	Circulative nonpropagative
Partiviridae	?	?
Potyviridae genus *Potyvirus*	Aphid	Noncirculative helper strategy
Potyviridae genus *Ipomovirus*	Whitefly	Noncirculative
Potyviridae genus *Macluravirus*	Aphid	Noncirculative
Potyviridae genus *Rymovirus*	Mite	Noncirculative
Potyviridae genus *Tritimovirus*	Mite	Noncirculative
Potyviridae genus *Bymovirus*	Fungus	Circulative
Reoviridae	Planthopper, leafhopper	Circulative propagative
Rhabdoviridae	Leafhopper, aphid	Circulative propagative
Sequiviridae	Aphid, leafhopper	Noncirculative helper strategy
Tombusviridae	Fungus	Noncirculative

[a]The families are broken down to the genus level when they contain genera with totally different vectors and mode of transmission.
[b]The helper or capsid strategies (see **Table 2**) are mentioned when experimentally demonstrated for at least one of the member species. When no complement is added to either circulative or noncirculative, it reflects the lack of further information.
[c]For at least one member species (*Tomato yellow leaf curl virus*, TYLCV), replication within the vector is still being debated.
The noncirculative viruses, or assimilated as discussed in the text, are in blue. The circulative viruses, or assimilated as described in the text, are in green.

different, due to the specific biology of all three (plant, virus, and vector) partners. However, viruses transmitted by 'pierce-sucking' insects are quantitatively predominant, and the classification established for their various modes of transmission is widely used as a reference for comparison with others. For this reason, hemipteran insect transmission will be described first in details and succinctly compared later on with that by other types of vectors.

Transmission of Plant Viruses by Insects

History of the Classification of the Different Modalities of Transmission

The transmission of plant viruses has been investigated for over a century with the most common vectors being sap-feeding insects with pierce-sucking mouth parts, particularly aphids, and also whiteflies, leafhoppers, planthoppers, and mealybugs. Pioneer studies have demonstrated the complexity and diversity of the interactions between plant viruses and their insect vectors. Even as late as the 1950s, scientists, using the tools at hand, were merely measuring quantitative traits such as the time required for virus acquisition on infected plants and the time during which the virus remained infectious within the vector. Three categories were then defined: (1) the nonpersistent viruses, acquired within seconds and retained only a few minutes by their vectors; (2) the semipersistent viruses, acquired within minutes to hours and retained during several hours; and (3) the persistent viruses that require minutes to hours for acquisition, and can be retained for very long periods, often until the death of the vector. It is important to note that, though the classification and terminology have changed in the last decades, these categories are still used by a number of authors, and thus often encountered in the literature.

In an early study on 'nonpersistent' viruses, the transmission of potato virus Y was abolished by chemical (formaldehyde) or ultraviolet (UV) treatments of the extremity of the stylets of live viruliferous aphids, demonstrating that infectious virus particles were retained there. It was first believed that the transmission of 'nonpersistent' viruses could be assimilated to mechanical transmission, stemming from nonspecific contamination of the stylets, the vector acting simply as a 'flying needle'. Consistent with this was the repeated demonstration that 'nonpersistent' viruses are lost upon moulting of the viruliferous vectors. Later on, the hypothesis of virus uptake during sap ingestion and inoculation during putative regurgitation led to a change from vectors as flying needles to vectors as 'flying syringes', the virus–vector relationship still being considered as nonspecific (**Figure 1**). It is interesting that, while in plant viruses recent data unequivocally convinced the scientific community that the situation is much more complex, likely involving specific receptors in vectors for specific virus species (see the next section), in animal viruses very few studies are available at present and this mode of transmission is still referred to as 'mechanical vector transmission'. The prime conclusion from these experiments is that the virus–vector association occurs externally, on the cuticle lining the food or salivary canal in the insect stylets. Because semipersistent viruses are also lost upon vector moulting, their association with the vector was also proposed to be external, likely in the stylets, though a

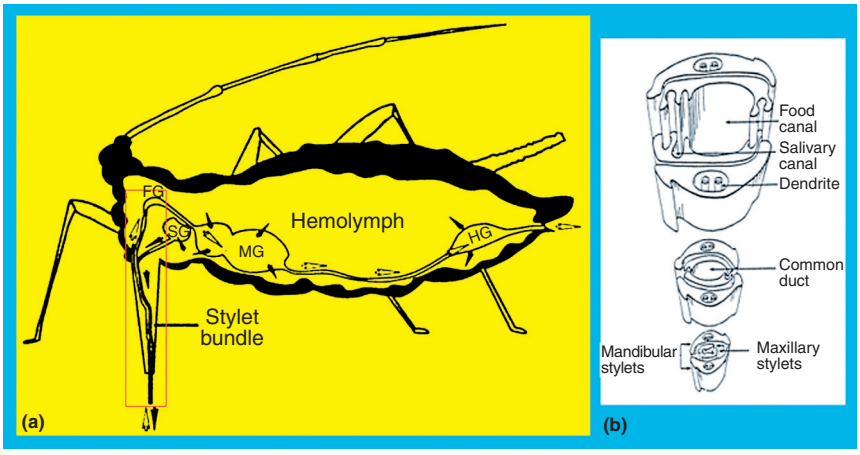


Figure 1 Different routes of plant viruses in their aphid vectors. (a) The white arrows represent the ingestion of circulative viruses, whereas the black arrows materialize their cycle within the aphid body, and inoculation in a new host plant. The red square area indicates the region of the anterior feeding system, where noncirculative viruses are retained in their vectors. (b) Cross sections of the stylet bundle illustrating the inner architecture of maxillary stylets which defines interlocking structures, food canal and salivary canal, fused at the distal extremity into a single common duct, where most noncirculative viruses are thought to be retained (see text). Adapted from Taylor CE and Robertson WM (1974) Electron microscopy evidence for the association of tobacco severe etch virus with the Maxillae in Myzuspersical (Sulz.). *Journal of Phytopathology* 80: 257–266.

possible location 'upstream', on the cuticle lining the anterior gut of the insect, was also proposed in some cases.

In sharp contrast, many persistent viruses were observed within the vector body by electron microscopy, in various organs and tissues, indicating an internal association with the vectors. Such viruses were shown to pass through the gut epithelium into the hemolymph and join the salivary glands to be ejected together with saliva (**Figure 1**). A latent period of hours to days after acquisition, during which the virus cannot be efficiently inoculated, is consistent with the time needed for completing this cycle within the vector body. Moreover, microinjection of purified persistent viruses within the insect hemolymph subsequently resulted in efficient transmission to new healthy plants, proving that virus within the vector body can get out and be inoculated to host plants.

Altogether, these results prompted a revision of the classification of the modes of transmission in the late 1970s, based on qualitative criteria and still valid today (**Table 2**). The non- and semipersistent viruses were grouped in a new category designated 'noncirculative viruses', and the persistent viruses were named 'circulative viruses'. While circulative animal viruses (arboviruses) in fact infect their vectors where they efficiently replicate, some circulative plant viruses can seemingly operate their cycle in the vector body without any cell infection and replication. Hence, the category 'circulative' has been broken down into the two subcategories: 'propagative' and 'nonpropagative'. The various families and/or genera of plant viruses and their associated vectors and modes of transmission are listed in **Table 1**.

During the last decades, the implementation of molecular and cellular biology has provided invaluable tools for studying the molecular mechanisms of virus–vector interaction. The data currently available for each category are summarized in the following subsections.

Circulative Transmission

Logically, circulative viruses are ingested by vectors, while feeding on infected plants. Some viruses are limited to phloem tissues, which the insect vector can reach within minutes to hours depending on the species, which explains the long feeding period required for their acquisition. As schematized in **Figure 1(a)**, the viruses cross the mid- or hindgut epithelium, are released into the hemolymph, and can then adopt various pathways to traverse the salivary glands, and be released in their lumen, wherefrom they will be inoculated upon salivation into healthy hosts. During this basic cycle , the virus encounters and must overcome diverse cellular barriers, where the existence of specific virus–vector interaction has long been established experimentally, though specific receptors have not been identified so far.

Propagative transmission

Propagative transmission of plant viruses is the homolog of that of arboviruses in vertebrates. Members of the virus families *Rhabdoviridae*, *Reoviridae*, *Bunyaviridae*, and the genus *Marafivirus* are transmitted this way. In compatible virus–vector associations, after infecting the gut epithelium, virus particles are released in the hemocoel cavity and colonize various organs and tissues of the vector, including, ultimately, the salivary glands. The viruses can either diffuse in the hemolymph and concomitantly infect different organs, or follow a constant pattern of spread from organ to organ, as demonstrated for rhabdoviruses which move in (and spread from) the central

Table 2 Different modes of plant virus transmission by insects with pierce-sucking mouth parts

	Circulative		*Noncirculative*	
Transmission modes[a]	*Propagative*	*Nonpropagative*	*Capsid strategy*	*Helper strategy*
Acquisition time[b]	Minutes to hours	Minutes to hours	Seconds to hours	Seconds to hours
Retention time[c]	Days to months	Days to months	Minutes to hours	Minutes to hours
Inoculation time[d]	Minutes to hours	Minutes to hours	Seconds to minutes	Seconds to minutes
Association with vectors[e]	Internal	Internal	External	External
Replication in vectors	Yes	No	No	No
Requirement of an HC[f]	No	No	No	Yes

[a]These modes of transmission were established and are widely accepted for virus transmission by pierce-sucking insects. As discussed in the text, they sometimes also apply to other types of vector.
[b]The length of time required for a vector to efficiently acquire virus particles upon feeding on an infected plant.
[c]The length of time during which the virus remains infectious within its vector, after acquisition.
[d]The length of time required for a vector to efficiently inoculate infectious virus particles to a new healthy plant.
[e]Internal means that the virus enters the inner body of its vector, passing through cellular barriers. External means that the virus binds the cuticle of the vector and never passes through cellular barriers.
[f]A helper component (HC) is involved in cases where the virus particles do not directly recognize vectors, acting as a molecular bridge between the two.

nervous system. Within the vector, all these cases are very similar to genuine infection of an insect host; hence, it is difficult to decide whether insects are proper vectors of propagative viruses, or should rather be considered as alternative hosts. Apart from the genus *Marafivirus*, all propagative viruses are in families that comprise viruses that infect animals, suggesting that they might have evolved from insect viruses by secondarily acquiring the capacity to replicate in plants.

Nonpropagative transmission

This particular association between insect vectors and plant viruses is reasonably well understood only for members of some plant virus species in the family *Luteoviridae*. Such viruses have developed original mechanisms of viral transport, both when passing through gut and salivary gland barriers, and when traveling into the hemocoel cavity.

The cycle of luteoviruses within their vector body involves specific ligand-receptor-like recognition at the cell entry of both the gut epithelium and the salivary glands. While viral ligands are known to be structural proteins of the coat and extension thereof, very little is known about the putative counterpart receptors on the cell membranes of the vectors. Despite this lack of full understanding of the molecular process, many electron microscopy and molecular studies have determined in detail the route of luteovirus particles within the vector, across cellular layers (**Figure 2**). Once the virus reaches either the apical membrane of the gut epithelium, or the basal membrane of the accessory salivary gland cells, and attaches to the specific receptors, it provokes invagination of the plasmalemma, forming small coated virus-containing vesicles. Soon after budding, the coated vesicles deliver the virus particles to a larger uncoated membrane endosomal compartment. Interestingly, luteoviruses mostly escape the route of degradation of internalized material ending in lysosomes. Instead, the virus particles become concentrated in the endosomes, and *de novo* elongated uncoated vesicles are repacked, transporting the viruses to the basal or apical membrane, in gut and accessory salivary gland cells, respectively. The elongated vesicles, which contain rows of virions (**Figure 2**), finally fuse with plasma membranes and release the virus either into the hemocoel cavity or into the lumen of the salivary ducts. Despite these extensive searches of luteovirus particles in their insect vectors, by several independent groups of scientists, no particles have been observed in any organ other than the gut or the accessory salivary glands. Furthermore, classical monitoring of the viral titers within the vector, for assessing viral replication, failed to demonstrate an increase over time. Consequently, it is generally accepted that virus particles, either included in membrane vesicles or suspended in the hemolymph, never come in contact with the cell cytoplasm, thus precluding any possibility of viral replication.

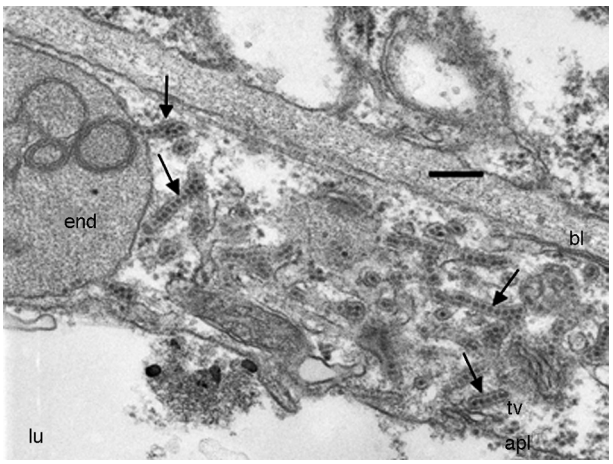


Figure 2 Transcytosis of cucurbit aphid-born yellows virus (family *Luteoviridae*) in hindgut cell of the aphid vector *Myzus persicae*. Luteovirus particles present in the gut lumen (lu) are internalized from the apical plasmalemma (apl) and transported to the basal lamina (bl) in a complex pattern involving different vesicular structures, described in the text. A network of uncoated tubular vesicles is visible (tv indicated by arrows), sometimes connected to the endosome (end). The bar represents 100 nm. The photograph is provided by Catherine Reinbold and Véronique Brault (INRA, Colmar, France). Reproduced from Blanc S (2007) Virus transmission – getting out and in. In: Waigmann E and Heinlein M (eds.) *Viral Transport in Plants*, pp. 1–28. Berlin: Springer, with kind permission of Springer Science and Business Media.

Virus transfer into the hemolymph is believed to occur by passive diffusion. However, the possible impact of the insect immune system at this step of the virus life cycle is often discussed. A study demonstrated that a major protein of the aphid hemolymph, the symbionin, was mandatory for efficient luteovirus transmission. The symbionin is a homolog of the *Escherichia coli* chaperone GroEL, secreted in aphids by endosymbiotic bacteria of the genus *Buchnera*. Eliminating symbiotic bacteria, and thus symbionin, by antibiotic treatments significantly reduced the aphid efficiency as a vector. Consistently, direct evidence of a physical interaction between symbionin and luteovirus particles has been detected in several viral species, and virus mutants deficient in symbionin binding are poorly transmitted. Two hypotheses can explain the positive action of symbionin and are still debated, since no direct proof could be experimentally obtained: (1) it exhibits protective properties, masking the virus to the immune system and maintaining its integrity during transfer through the hostile hemolymph environment, or (2) its putative chaperon activity ensures correct folding facilitating transfer into the salivary glands.

A similar phenomenon was later demonstrated for other circulative nonpropagative viruses (in the family *Geminiviridae*) by other vectors (whiteflies), suggesting

that symbionin participation may be a general phenomenon in this mode of transmission.

Noncirculative Transmission of Plant Viruses

This mode of transmission is by far the most frequently encountered in plant viruses and concerns over 50% of the viral species described to date. Noncirculative viruses do not enter the body of their vectors. They simply attach to receptor sites located externally on the cuticle lining the anterior part of the digestive tract, most often the alimentary/salivary canals within the stylets or the foregut region, and wait until the vector has moved to another plant, where they contrive to be released to initiate a new infection. It has often been proposed that difference between non- and semipersistent viruses, in acquisition and retention time within the vector, was due to a differential location of the binding sites, the former being retained in the stylets and the latter higher up in the foregut. This distinction, however, is not experimentally supported at present, and these two categories should be considered with caution (they are no longer included in **Table 2**). Because noncirculative viruses are adsorbed on the vector cuticle during sap ingestion, and released in many cases during salivation, the favored but still hypothetical location for the retention sites is at the distal extremity of the maxillary stylets, where a single common salivary/food canal is serving for both sap uptake and saliva ejection (**Figure 1(b)**). The best-studied cases of noncirculative transmission are those of viruses in the genera *Cucumovirus*, *Potyvirus*, and *Caulimovirus*, transmitted by various aphid species. Mutagenesis studies of the viral proteins interacting with the vector's mouthparts have clearly indicated that some single-amino-acid substitutions can specifically abolish the virus transmissibility by certain vector species but not by others. Such results, obtained both with cucumber mosaic virus and cauliflower mosaic virus, demonstrate a very specific virus–vector recognition, and strongly suggest the existence of a receptor in the insect mouthparts. Unfortunately, here again and despite the quantitative importance of noncirculative transmission in plant viruses, the viral ligands are well characterized (see below), but the receptors on the insect cuticle stand as the major black box in this field of research, their chemical nature and even their precise location remaining largely hypothetical.

The viral protein motifs directly involved in the attachment to vector putative receptors have been characterized in a number of cases. The frequent occurrence of both transmissible and nontransmissible isolates in the same virus species has greatly facilitated the identification of viral gene regions involved in vector transmission, by simple sequencing of viral genomes, and reverse genetic approaches have been successfully used for definitive confirmation. These investigations clearly revealed two distinct viral strategies for controlling the molecular mechanisms of virus–vector association (**Figure 3**), which represent the currently preferred subcategories in noncirculative transmission (**Table 2**).

The helper strategy

The development of artificial feeding of insect vectors, through stretched parafilm membranes, made it possible to assess the transmission of purified virus particles. The primary striking result was that, in most cases, purified virions are not transmissible, suggesting the requirement of an additional component that is probably eliminated during purification. Sequential feeding on plants infected with transmissible and nontransmissible isolates of such virus species showed that the former produced a compound that could be acquired by the vector, and subsequently mediated the transmission of the nontransmissible isolate. This compound, first described in potyviruses and caulimoviruses, was designated helper component (HC). In both cases, the HC was later purified and demonstrated to be a viral nonstructural protein produced upon plant cell infection, named P2 in caulimoviruses and HC-Pro in potyviruses. P2 and HC-Pro are responsible for specific recognition of the vector. They can be acquired on their own by the aphid and bind to putative receptors within its mouthparts. They exhibit a separated domain, which specifically binds homologous virus particles, thus creating a molecular bridge between virus and vector (**Figure 3**). It is important to realize that HCs can be acquired on their own by the vector, subsequently scavenging virions from homologous species acquired in other locations of the same plant, or even in other plants. This phenomenon extending the assistance between related viral genomes during vector transmission has been termed HC-transcomplementation (**Figure 3**), and may have some important implications in the population genetics and evolution of a virus species (discussed below).

Capsid Strategy

However, for some viruses transmitted by aphids, particularly in the genera *Cucumovirus*, *Alfamovirus*, and *Carlavirus*, purified viral particles are readily acquired and transmitted by the vector. This indicated unequivocally that the coat protein of members of the species must be capable of direct attachment to vector receptors (**Figure 3**). An experiment confirming this conclusion was the demonstration that tobacco mosaic virus, which is not transmitted by any vector, can be transmitted by aphids when its RNA genome is encapsidated *in vitro* into the coat protein of an aphid-transmissible cucumovirus. Recently, the amino acid positions in the coat protein of cucumber mosaic virus, involved in binding to the putative receptors of aphids, have been identified. Their substitution differentially affects the efficiency of transmission by distinct

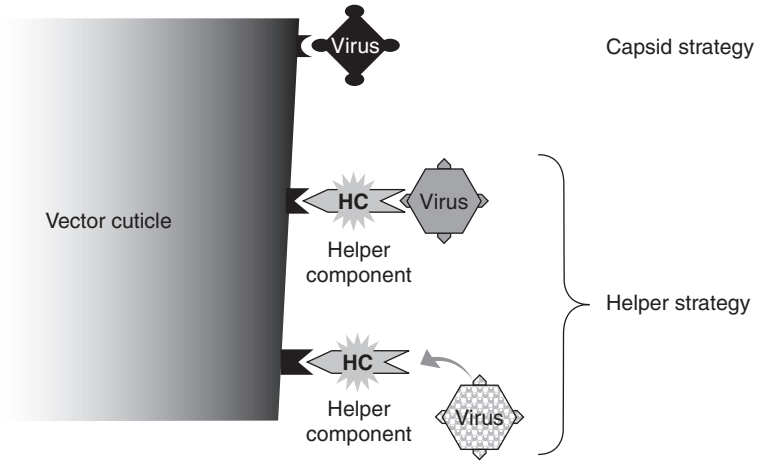


Figure 3 Two molecular strategies for virus–vector interaction in noncirculative transmission of plant viruses. Both strategies allow the retention of virus particles in the vector mouth parts or foregut on putative receptors located at the surface of the cuticular lining. In the capsid strategy, a motif of the coat protein is able to directly bind to the vector's receptor. In the helper strategy, virus–vector binding is mediated by a virus-encoded nonstructural protein (HC), which creates a reversible 'molecular bridge' between the two. HC can be acquired alone, prior to the virion, and thereby allows HC-transcomplementation. In this case, an HC encoded by a genome X (for instance that encapsidated in the gray virion) can subsequently assist in the transmission of a genome Y of the same population, encapsidated in the dotted virion. This possible sequential acquisition of HC and virion is symbolized by the arrow. Reproduced from Froissart R, Michalakis Y, and Blanc S (2002) Helper component-transcomplementation in the vector transmission of plant viruses. *Phytopathology* 92: 576–579, with permission from The American Phytopathological Society.

aphid species, definitely confirming that no HC is required for virus/vector interaction.

Transmission by Beetles

The only cases of insect transmission diverting clearly from one of the patterns detailed above is that described in insect with 'biting-chewing' mouthparts, the best documented examples being beetles, though other insects have a similar feeding behavior.

In contrast to insect with 'pierce-sucking' mouthparts, the beetle feeding is damaging tissues and killing cells, implying an obligate translocation of deposited viruses toward adjacent live cells where they can initiate infection. Beetles have long been reported to acquire and retain viruses from numerous virus species, usually those with highly stable virus particles, and to release them together with the regurgitation of disrupted plant material, lubricating the mouthparts during feeding on new host plants. Surprisingly, however, only some rare species are actually successfully transmitted. A high amount of RNase activity has been found in the beetle regurgitation liquid, and shown to block infection by non-beetle-transmissible viruses. Hence, those viral species that are efficiently transmitted are likely capable of translocation in the vascular system, and/or transfer to unwounded cells, away from the RNase activity. Some virus particles have been detected within the hemolymph of their beetle vector, only seconds after acquisition on infected plants. No correlation between the presence of virions in the hemolymph and success of transmission has yet been established, precluding a possible comparison with the circulative or noncirculative transmission described above.

Transmission by Noninsect Vectors

Although quantitatively less important, noninsect vectors have been identified in several plant virus species. Some are arthropod mites with a morphology distantly related to that of insects, others are totally unrelated organisms such as nematodes or even fungi. However, in many instances, a comparison with the mode of transmission defined in **Table 2** remains possible, demonstrating that this classification can be used as a general basis applicable to all cases of vector transmission.

Transmission by Mites

The transmission of plant viruses by mites has been far less studied, and the molecular and cellular mechanisms of virus–vector relationships remain poorly understood. Several different viral species have been studied and the results suggest the existence of different types of interactions with their respective vectors. This variety is illustrated by two examples: (1) the presence of massive amounts of bromegrass mosaic virus (BMV, family *Bromoviridae*) particles within cells of the gut epithelium of the insect *Eriophyes tulipae* seems to indicate that the virus replicates within the vector, resembling circulative-propagative transmission; (2) wheat streak mosaic virus (WSMV, family *Potyviridae*) appears to be noncirculative. WSMV was

recently demonstrated to interact with its mite vectors through an HC-Pro homolog, thus likely suggesting a helper strategy. Further investigations on additional viral species are required to evaluate the diversity of the modes of transmission found with mite vectors, but it seems likely that they will be closely related to those described for insect vectors in **Table 2**.

Transmission by Nematodes

The transmission by nematode vectors is particularly interesting. Such vectors are moving very slowly in the soil (one or a few meters per year), and cannot disseminate the transmitted virus over long distances. Instead, they retain virus particles, for several months or even years, and usually transmit them in the same location but from plants of a growing season n to plants of the growing season $n + 1$. Consequently, nematode transmission is resulting in a time travel for the virus, rather than a space travel.

Nematodes feed by piercing root cells with protractile stylets, constituting the anterior part of their feeding apparatus, and by ingesting the cell content together with viruses when host plants are infected. In all cases described in the literature, the virus particles are adsorbed on the cuticle lining the foregut and are presumably released from there when inoculated in a new host plant. Since viruses are lost upon moulting of the nematode, the virus–vector association is external and this mode of transmission can be assimilated to the noncirculative transmission. Interestingly, the two subcategories of noncirculative transmission also appear to be represented in nematode transmission. Indeed, among the viruses belonging to two viral genera using nematodes as vectors, *Nepovirus* and *Tobravirus*, viruses in the former genus have been shown to directly attach to the retention sites through their coat protein (capsid strategy), whereas in the latter genus, viruses produce a nonstructural protein which allows transcomplementation (see the helper strategy in **Figure 3**). As in other virus–vector associations, the viral proteins involved in vector recognition were identified, but the putative receptor in the nematodes remains unknown.

Transmission by Fungi

Parasitic fungi are coming in contact with their host plants under the form of motile zoospores, which can digest the root cell wall and penetrate into the cytoplasm, from where they will colonize the whole plant. Two different patterns of virus transmission exist. Some viruses, for instance in the genus *Bymovirus*, are present within the fungus cytoplasm early during formation of the future zoospores in infected plant cells. They will remain inside the zoospore until its cytoplasm is injected in the next host cell. In other cases, for instance in cucumber necrotic virus (genus *Tombusvirus*), the best-studied example of a fungus-transmitted virus, virions are specifically retained at the surface of the zoospore envelope, and inoculated into the plant upon cell wall digestion and fungal penetration. In this case, the receptors of the vector were partially characterized. They have been shown to be distributed at the surface of the zoospore of *Olpidium bornovanus*, and their chemical nature was identified as a glycoprotein.

Altogether, the transmission of plant viruses by fungi can tentatively be regarded as homologous to the categories noncirculative, for those viruses associated to the external coat of the zoospore, and circulative for those internalized in its cytoplasm. Unfortunately, not enough information is available to definitely decide whether viral replication is effective within the fungus, and hence whether both propagative and nonpropagative subcategories are present.

Transmission and Evolution

One aspect of vector transmission that is rarely considered in the literature is its evident impact on the population genetics and evolution of viruses. Indeed, while vector transmission is always regarded as an excellent means of maintenance and spread within host populations, it also implies that the virus is able to solve certain problems. First of all, one must bear in mind that a virus population rapidly accumulates very numerous mutations and develops a swarm of genome variants generally designated 'quasispecies'. In such a genetic context, the virus sample collected, transported, and inoculated by the vector will be highly variable. This sample, which gives rise to a new population after each round of vector transmission, will obviously condition the composition of the viral lineage, and thus its evolution. In this regard, two key parameters must be considered:

1. The distribution of viral variants within the host plant determines what is actually accessible for sampling by the vector. If variants are homogeneously distributed within the whole plant, several can be acquired together by the vector. In contrast, if they are physically isolated in different cells, organs, or tissues, only one or few will be collected.
2. The pattern of the virus acquisition by the vector can dramatically change the composition of the transmitted viral sample. When a given vector collects the virus all at once in a single location, the diversity of genome variants transmitted tends to be restricted. On the other hand, collecting the viral sample bits by bits upon successive probing in different locations of the infected host will increase the genetic diversity of the pool of viral genomes transmitted.

It has been repeatedly demonstrated experimentally that successive genetic bottlenecks imposed in viral lineages are

drastically decreasing the mean fitness of RNA virus populations, sometimes even driving them toward extinction. One can then easily conceive that viruses will develop strategies to avoid or compensate for genetic bottlenecks during their life cycle. It is highly probable that different strategies of vector transmission found in plant viruses have different impacts on the fitness and evolution of viruses, through the different pools of genome variants that are collected and inoculated. For instance, in the helper strategy, it has been suggested that HC-transcomplementation (**Figure 3**) allows the vector to sample viruses in several steps and in several locations of the host, thus opening the genetic bottleneck, and perhaps attenuating its detrimental effect.

Future Prospects

From a mechanistic standpoint, future advances will mainly concern the characterization of the vector receptors that are used by viruses to ensure successful transmission. Indeed, none of these molecules have been identified so far, in any vector species and category of transmission. Apart from the example of the fungus-transmitted CNV described above, not even their chemical nature and precise location have been established.

From an evolutionary standpoint, two aspects have been mostly neglected in spite of their undeniable importance: (1) the size of the virus sample transmitted by a given vector remains largely unknown in most cases, (2) the distribution of the genome variants, constituting the swarm of mutants of the viral quasispecies, within different cells, organs, and tissues of the host plant is largely unresolved, precluding any evaluation of what genetic diversity from a given viral population is actually accessible to the vectors.

See also: Vector Transmission of Animal Viruses.

Further Reading

Blanc S (2007) Virus transmission – getting out and in. In: Waigmann E and Heinlein M (eds.) *Viral Transport in Plants*, pp. 1–28. Berlin: Springer.

Froissart R, Michalakis Y, and Blanc S (2002) Helper component-transcomplementation in the vector transmission of plant viruses. *Phytopathology* 92: 576–579.

Gray S and Gildow FE (2003) Luteovirus–aphid interactions. *Annual Review of Phytopathology* 41: 539–566.

Ng JC and Falk BW (2006) Virus–vector interactions mediating non-persistent and semi-persistent transmission of plant viruses. *Annual Review of Phytopatholy* 44: 183–212.

Power AG (2000) Insect transmission of plant viruses: A constraint on virus variability. *Current Opinion in Plant Biology* 3(4): 336–340.

Rochon D, Kakani K, Robbins M, and Reade R (2004) Molecular aspects of plant virus transmission by olpidium and plasmodiophorid vectors. *Annual Review of Phytopathology* 42: 211–241.

Taylor CE and Robertson WM (1974) Electron microscopy evidence for the association of tobacco severe etch virus with the Maxillae in Myzuspersical (Sulz.). *Journal of Phytopathology* 80: 257–266.

Whitfield AE, Ullman DE, and German TL (2005) Tospovirus–thrips interactions. *Annual Review of Phytopathology* 43: 459–489.

Vegetable Viruses

P Caciagli, Istituto di Virologia Vegetale – CNR, Turin, Italy

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Incubation The time period from the initial infection (inoculation) to the manifestation of the disease (symptoms). For virus diseases of vegetables, incubation can vary between 2 and 40 days, most commonly between 1 and 3 weeks.

Meristem A tissue in plants consisting of undifferentiated cells, found in zones of the plant where growth (cell division) can take place (meristematic dome). The term 'meristem tip' is used to denote the meristem dome together with 1–2 primordial leaves and measuring between 0.1 and 0.5 cm in height.

Roguing The practice of removing diseased (or abnormal) plants from a crop.

Vector An organism capable of transmitting a pathogen from one plant host to another by establishing specific relationships with the pathogen itself.

Vegetables

The plant species/subspecies cultivated as vegetables are close to 200 and belong to more than 30 botanical families, representing, with fruits, about 35% of the total

food derived from plants (including cereals, vegetable oils, and sugar crops).

Viruses

According to different estimates, 3–5% of overall vegetable production is lost to virus infections, but losses can be occasionally very high, and are normally higher where pest control is lower, for example, in developing countries.

Keeping in mind that viruses of potato, sweet potato, cassava, and legumes are not considered in this article, as they are dealt with elsewhere in this encyclopedia, a total of about 200 viruses (excluding cryptic viruses, satellites, and viroids) have been reported as naturally infecting 67 of the 129 vegetables considered here. These viruses belong to 30 genera in 15 families and to nine genera unassigned to families; eight viruses are in three families, but have not been assigned to genera, and three more viruses have not been assigned to any taxa (**Table 1**).

Table 1 Taxonomic assignment of vegetable viruses, their numbers per genus, and their vectors

Genome type	*Family*	*Genus*	*No. of virus species*	*Vectors*
Single-stranded DNA	*Geminiviridae*	*Begomovirus*	35	Whiteflies
		Curtovirus	2	Leafhoppers
		Mastrevirus	1	Leafhoppers
	Nanoviridae	*Babuvirus*	1	Aphids
Double-stranded DNA RT	*Caulimoviridae*	*Badnavirus*	4	Mealybugs
		Caulimovirus	1	Aphids
Double-stranded RNA	*Reoviridae*	*Fijivirus*	1	Planthoppers
Single-stranded, negative-sense RNA	*Rhabdoviridae*	*Cytorhabdovirus*	2	Aphids
		Nucleorhabdovirus	2	Aphids
			1	Leafhoppers
		Unassigned in the family	2	Aphids
			1	Leafhoppers
			2	Not known
	Bunyaviridae	*Tospovirus*	3	Thrips
	Unassigned	*Ophiovirus*	2	Fungi
		Varicosavirus	1	Fungi
Single-stranded, positive-sense RNA	*Sequiviridae*	*Sequivirus*	2	Aphids
	Comoviridae	*Comovirus*	3	Beetles
		Fabavirus	1	Aphids
		Nepovirus	11	Nematodes
	Potyviridae	*Ipomovirus*	1	Whiteflies
		Potyvirus	40	Aphids
		Unassigned in the family	1	Aphids
	Luteoviridae	*Polerovirus*	3	Aphids
		Unassigned	2	Aphids
	Tombusviridae	*Aureusvirus*	1	Fungi
		Carmovirus	3	Fungi
		Necrovirus	1	Fungi
		Tombusvirus	5	Fungi
	Bromoviridae	*Alfamovirus*	1	Aphids
		Cucumovirus	2	Aphids
		Ilarvirus	5	(Thrips)
	Tymoviridae	*Tymovirus*	4	Beetles
	Closteroviridae	*Closterovirus*	3	Aphids
		Crinivirus	5	Whiteflies
	Flexiviridae	*Allexivirus*	6	Mites
		Carlavirus	8	Aphids
		Potexvirus	9	None known
	Unassigned	*Benyvirus*	1	Plasmodiophorids
		Ourmiavirus	2	None known
		Pomovirus	1	Plasmodiophorids
		Sobemovirus	1	None known
		Tobamovirus	7	None known
		Tobravirus	2	Nematodes
		Umbravirus	3	Aphids
	Unassigned family	Unassigned genus	1	Plasmodiophorids
			2	Not known

As tomatoes, watermelons, cabbages, onions, cucumbers, and eggplants make up almost 50% of the total vegetable production (legumes, potatoes, sweet potatoes, and cassava excluded), it is no wonder that more than 90 viruses have been found in these crops, with about 50 viruses in tomatoes, the most cultivated vegetable of all (about 13% of the total) (**Table 2**).

Ecology and Epidemiology

Factors normally considered in epidemiological studies are hosts, pathogens, and environment. To these, we can add vectors and human action.

Host

Among possible reactions of a plant to virus attack, only susceptibility and tolerance (plant systemically infected with very mild or no visible effects) favor the spread of viruses. It is evident, from both the number of viruses isolated and the number of vegetables found naturally infected, that most vegetables escape virus infections, for a number of different reasons, including resistance, possibly as a consequence of centuries of human selection for good-looking products. In fact, only 38 vegetables are infected, under natural conditions, by more than one virus, and only 25 by more than two viruses.

Table 2 The top vegetables in the world (excluding potato, sweet potato, cassava, and legumes), their production, and the number of viruses found in each crop in natural infection

Vegetable	*Family*	*Production*[a]	*Viruses*[b]
Tomato	Solanaceae	126.1	53
Watermelon	Cucurbitaceae	97.1	14
Cabbages	Brassicaceae	66.7	2
Onion	Alliaceae	62.1	4
Yam	Dioscoreaceae	46.7	3
Cucumber	Cucurbitaceae	41.6	20
Plantain banana	Musaceae	33.0	2
Eggplant	Solanaceae	30.4	8
Pepper	Solanaceae	27.2	28
Carrot (and turnip)	Apiaceae	23.8	13
Lettuce and chicory	Asteraceae	21.9	21
Squash, pumpkin	Cucurbitaceae	20.4	3
Cauliflower and broccoli	Brassicaceae	17.7	3
Garlic	Alliaceae	14.2	9
Spinach	Chenopodiaceae	12.8	10
Taro	Araceae	11.3	3
Asparagus	Asparagaceae	6.6	6
Okra	Malvaceae	5.3	2
Leek	Alliaceae	1.8	3
Artichoke	Asteraceae	1.4	14
Total		881	193[c]

[a]Data, expressed in millions of tons, refer to year 2004; from data made available by the Food and Agriculture Organization, United Nations.
[b]Number of viruses detected from natural infections; the same virus may infect more than one vegetable.
[c]Total of different virus species detected in vegetables.

Pathogen

The main virus features that affect epidemiology are variability, host range, and transmission pathways. The higher the variability of a virus, the higher is its chance to adapt to new hosts and environments. Most vegetable viruses (as most plant viruses) have a single-stranded RNA (ssRNA) genome, the genome most prone to errors during replication, as it lacks the proofreading mechanisms that are present in DNA replication. Although it has some drawbacks, this feature ensures a high level of variability to viruses with a single genome component. Viruses with a divided genome can also count on natural reassortment of multiple-component genomes to increase their variability. This can strongly influence the host range of the virus. Out of 76 viruses infecting more than one vegetable crop, only 12 naturally infect more than five crops, with eight of them having an ssRNA divided genome (viz., alfalfa mosaic virus, arabis mosaic virus, cucumber mosaic virus, lettuce infectious yellows virus, radish mosaic virus, squash mosaic virus, tobacco rattle virus, and tomato black ring virus); six of these have vegetable hosts in more than one botanic family. Viruses of vegetables with a divided genome are only 30% of the total. The analysis of the host-range done for this work does not include all cultivated plants and, furthermore, the host range of viruses is not usually restricted to cultivated plants, but nevertheless the number of crops naturally infected is a good indicator of the host-range width. The host range is also strictly interconnected to the ways viruses are transmitted. Viruses, even those infecting centenary trees, need to be able to pass from one susceptible host to another. The means by which viruses move from host to host can be summarized as (1) vectors (flagellate protists: plasmodiophorids, fungi, nematodes, and arthropods); (2) seed and pollen; (3) vegetative propagation; and (4) mechanical transmission.

1. Vectors of the viruses infecting vegetables are shown in **Table 1** (column 5). Transmission by vectors involves all epidemiological factors: the virus, the vector, the host plant, the environment, in both the biotic and abiotic aspects, and man-action, in the form of movement of vectors, viruses, and host plants around the world.
2. Out of the 134 viruses that infect vegetables and have been studied for seed transmission, only 33 are seed transmitted in some plants. However, only 15 (listed

in **Table 3**) are seed-transmitted in vegetables. Nevertheless, seed transmission is of paramount importance, as it can start virus infection in a newly planted crop. All viruses known to be seed-transmitted in vegetables have single-stranded, positive-sense RNA genome.

3. Viruses that are vegetatively propagated are listed in **Table 4** together with the number of viruses reported from natural infection in each crop. With the exception of artichoke, the number of virus detected is linked to the distribution of crops in terms of both the total production and cultivation areas outside the center of origin.

4. Mechanical transmission is particularly important for viruses (for instance, tobamo- and potexviruses) that reach high concentration in their vegetable hosts, particularly in the epidermis and hairs, and maintain infectivity for a long time in the environment. The risk is particularly high for crops frequently manipulated during the growing season, as man and his tools can act as carriers. Mechanical transmission due to repeated cuts for harvesting are known for zucchini yellow mosaic virus in zucchini squash, and for radish mosaic virus in rocket (eruca).

The case of tobacco mosaic virus (TMV) is worth a special mention, as an example of a 'seed-mechanical' transmission: TMV can contaminate the tegument of a tomato seed and from here infect the seedling during its growth. A special case is also the pollen-mediated transmission of tobacco streak virus and pelargonium zonate spot virus. These viruses can be mechanically inoculated by thrips that rub contaminated pollen on the leaf surface while moving on a host plant.

Environment

The environment has an influence on virus spread through both biotic and abiotic factors. Among biotic factors, the most important is wild vegetation that can act as reservoir for viruses, and fauna that may include potential vectors. Among abiotic factors, soil has an influence on vectors of soil-borne viruses. Light, sandy soils favor the spread of nematode-borne viruses, while heavy, water-retaining soils favor the spread of plasmodiophorid- and fungal-borne viruses. Rain and humidity, wind, and, most of all, temperature has a strong influence on virus epidemiology, because of their effects on arthropod vectors. High humidity and moderate temperatures favor multiplication of aphids, while strong winds and rains impair their long-distance movements. Higher temperatures are more suited for whiteflies and leafhoppers. So, it is not by chance that whitefly-borne viruses are more common in areas with subtropical climate, and

Table 3 Viruses transmitted through vegetable seeds

Family	*Virus (genus)*	*Vegetable*	*Species*	*Family*
Comoviridae	Squash mosaic virus (*Comovirus*)	Watermelon	*Citrullus lanatus (C. vulgaris)*	Cucurbitaceae
		Squash, pumpkin	*Cucurbita maxima*	Cucurbitaceae
	Chicory yellow mottle virus (*Nepovirus*)	Parsley	*Petroselinum crispum* subsp. *tuberosum*	Apiaceae
		Chicory	*Cichorium intybus*	Asteraceae
	Beet ringspotvirus (*Nepovirus*)	Mangolds (beet roots, fodder beet)	*Beta vulgaris*	Chenopodiaceae
		Swiss chard	*Beta vulgaris* var. *cicla*	Chenopodiaceae
	Tobacco ringspot virus (*Nepovirus*)	Lettuce	*Lactuca sativa*	Asteraceae
	Tomato black ring virus (*Nepovirus*)	Celery	*Apium graveolens*	Apiaceae
		Lettuce	*Lactuca sativa*	Asteraceae
		Tomato	*Solanum lycopersicon*	Solanaceae
Potyviridae	Celery latent virus (*Potyvirus?*)	Celery	*Apium graveolens*	Apiaceae
	Lettuce mosaic virus (*Potyvirus*)	Lettuce	*Lactuca sativa*	Asteraceae
Tombusviridae	Tomato bushy stunt virus (*Tombusvirus*)	Pepper	*Capsicum annuum*	Solanaceae
		Tomato	*Solanum lycopersicon*	Solanaceae
	Cucumber leaf spot virus (*Aureusvirus*)	Cucumber	*Cucumis sativus*	Cucurbitaceae
Bromoviridae	Alfalfa mosaicvirus (*Alfamovirus*)	Pepper	*Capsicum annuum*	Solanaceae
	Spinach latent virus (*Ilarvirus*)	Spinach	*Spinacia oleracea*	Chenopodiaceae
Unassigned to family	Cucumber green mottle mosaic virus (*Tobamovirus*)	Squash, pumpkin	*Cucurbita maxima*	Cucurbitaceae
		Watermelon	*Citrullus lanatus (C. vulgaris)*	Cucurbitaceae
	Pepper mild mottle virus[a] (*Tobamovirus*)	Pepper	*Capsicum annuum*	Solanaceae
	Tobacco mosaic virus[a] (*Tobamovirus*)	Pepper	*Capsicum annuum*	Solanaceae
		Tomato	*Solanum lycopersicon*	Solanaceae
	Tomato mosaic virus[a] (*Tobamovirus*)	Tomato	*Solanum lycopersicon*	Solanaceae

[a]Transmission of tobamoviruses is not a true seed transmission as a result of embryo infection, but a 'seed-mechanical' transmission; TMV can contaminate the tegument of a seed and from here infect the seedling during its growth.

Table 4 Vegetatively propagated vegetables from which viruses have been reported, and the number of viruses reported in natural infections

Species	*Common name*	*Family*	*No. of viruses reported*
Cynara scolimus	Artichoke	Asteraceae	14
Allium sativum	Garlic	Alliaceae	9
Asparagus officinalis	Asparagus	Asparagaceae	6
Allium ascalonicum	Shallot	Alliaceae	5
Colocasia esculenta or *C. antiquorum*	Taro	Araceae	4
Ullucus tuberosus	Olluco	Basellaceae	4
Canna edulis	Achira	Cannaceae	3
Dioscorea spp.	Yam	Dioscoreaceae	3
Arracacia xanthorrhiza	Arracacha	Apiaceae	2
Musa spp.	Plantain banana	Musaceae	2
Tropaeolum tuberosum	Mashua	Tropaeolaceae	2
Rorippa (Nasturtium) officinale or *R. officinale* × *R. microphyllum*	Water cress	Brassicaceae	1
Bambuseae (tribe)	Bamboo shoot	Poaceae	1

that the global warming is accompanied by an increase of their importance and diffusion areas.

Control Measures

Control of virus and virus diseases in vegetables, as in most crops, is indirect and based upon a number of strategies for preventing virus infection. Seeds should be as virus-free as possible, using certified seeds whenever possible, disinfecting seeds of crops susceptible to tobamoviruses by a number of simple, but effective chemical treatments, when certified seed is not available. Multiplication material of vegetatively propagated species (**Table 4**) need to be selected and virus-free vegetative stock prepared and maintained. For freeing plant material from viruses one can use (1) heating of dormant plant parts (thermotherapy) and growing small shoot tips removed immediately after heat treatment; (2) meristem tip culture, particularly efficient for eliminating viruses from plants of the family Araceae, for example, may be preceded by or associated with heat treatment (very effective for garlic, for example); (3) chemical treatment with antiviral substances, like ribavirin, either alone or with DHT; usually disappointing, it may work in association with the other two methods. Seedlings should be protected before and soon after transplanting as plants are usually very susceptible at this stage. After transplanting, it is important to prevent virus spread from outside and within the crop. Contamination from the soil can be avoided by removing debris from previous crop (particularly for tobamo-, tombus-, and carmoviruses) or by crop rotation, alternating crops susceptible to different viruses; crop rotation may help also in controlling vectors of limited mobility (nematodes, fungi, plasmodiophorids). Crops can be protected against airborne vectors by physical means, either excluding the vectors (nets and covers of various types, wind-breakers) or repelling them (mulching with repellent effect, e.g., UV-reflective silver plastic), or by chemical treatments. Biologic control of vectors is not very efficient, particularly against introduction of viruses into the crop, as efficient vectors can be occasional visitors of the crop rather than regular colonizers. Control of weeds and volunteer plants from the previous crop is also very important in reducing early infections. When a vegetable is grown on a wide territory crop after crop, a 'crop-free period' can be very efficient in preventing virus infections (e.g., carrot virus Y in carrots in southern Australia). As for avoiding the introduction of viruses in areas (islands, countries, continents) where they are not present yet, most agriculturally advanced countries set quarantine rules aimed at excluding specific viruses (or their vectors) or limiting their further spread; setting and applying quarantine measures can be quite complex, but it may be worth particularly for viruses transmitted through seed or in dormant vegetative parts. In order to control the spread of viruses within the crops, one needs to reduce the movement of viruses. For vector-borne viruses, a number of chemical treatments are available for controlling the different vectors. Hygiene of operator and implements (by washing with a solution of 3% trisodium orthophosphate, for example) is particularly important for viruses readily mechanically transmissible (tobamo-, potex-, carmoviruses, but also comoviruses) in crops that need frequent tending. Roguing can be effective only if virus incidence is still low (e.g., less than 10%), the incubation is short, and the movement of the virus from plant to plant, by whatever means, is slow. When roguing plants hosting vectors, care should be taken not to disperse them while removing plants. A further possibility for protecting a vegetable crop from virus damage is artificial infection of the plants with a strain of the virus causing only mild disease symptoms (cross-protection) or associating a satellite to a virus to the same aim (e.g., cross-protection is used for protecting cucurbits from damages caused by zucchini yellow mosaic virus and tomatoes from tomato mosaic virus; co-infection with a satellite can be used to reduce damages by cucumber mosaic virus in pepper and tomato). Both methods have a number of objections, based on ecological and practical considerations, so they are not recommended as general practice, but still can reduce virus

damages at least for some time in some crops. Another method of choice to avoid virus infection of vegetables is resistance, to vectors in few instances (aphids, whiteflies, thrips) and to viruses in many cases. For analysis of resistance to viruses, either natural or engineered, readers are referred to specific articles of this encyclopedia. **Table 5** lists some of the crops, and their viruses, for which natural resistance is commercially available. Transgenic lines of vegetables have been developed that resist to very damaging viruses, but they are not listed here because, for a number of reasons, transgenic seeds are not available to the majority of growers yet.

A Few Special Cases

A few viruses infecting vegetables and the symptoms they cause in their hosts are hereafter described, chosen as examples among the many vegetable virus diseases. For details on the structure and properties of the viruses mentioned, the reader is referred to the specific articles of this encyclopedia.

Cucumber Mosaic Virus

Cucumber mosaic virus (CMV) is a positive-sense ssRNA virus with a tripartite genome encapsidated in isometric particles measuring about 30 nm in diameter. CMV has a very wide host range (more than 800 species in 70 botanic families). Natural infections have been reported from at least 10 vegetable species and in a large number of weeds, the most important virus reservoir. In many of these weeds CMV is not associated to any symptoms and is efficiently seed-transmitted. The virus is transmitted by several aphid species in a nonpersistent manner: infectivity is retained by vectors for a few hours and is lost with molt. Symptoms of CMV in vegetables like tomato plants infected at an early stage are chlorotic local lesions between the secondary leaf vein. Necrosis, first in the form of brown leaf areas, and later as brown lines along petioles and stems, proceeds toward the bottom of the plant, which can die in a few weeks after infection. Fruits, if present, are misshaped, sun-burnt, and necrotic. Plants infected at a mature stage normally show growth reduction, bushy appearance, and leaf deformation (shoestring-like leaves). Fruits have reduced size and do not ripen. Depending on a number of factors, including the presence of satellite RNAs, plants may have normal-looking leaves but show necrosis of fruits (losses are quite reduced in this case) or show typical, but stronger symptoms (in this case, losses can be very high). Symptoms of CMV infection in 'pepper' plants depend on the virus strain and on the plant age at the moment of infection. In young pepper plants CMV causes leaf yellowing and narrowing, and necrotic symptoms, on both the foliage and the fruit. If older plants are infected, CMV symptoms can be mild and appear on lower, yellowing leaves as green ring spots or oak-leaf patterns, but also as a mild mosaic, with a general dull appearance. Cucurbits infected by CMV, particularly zucchini, squash, and melons, show strong mosaic

Table 5 Vegetables and viruses for which natural resistance of different degrees has been incorporated in commercially available cultivars

Species	*Common names*	*No. of viruses reported*	*Genetic (natural) resistance available to*
Lactuca sativa	Lettuce	20	Lettuce mosaic virus (*Potyvirus*)
Brassica oleracea var. *capitata*	Cabbage	2	Turnip mosaic virus (*Potyvirus*)
Spinacia oleracea	Spinach	10	Cucumber mosaic virus (*Cucumovirus*)
Cucumis sativus	Cucumber	20	Cucumber mosaic virus (*Cucumovirus*), papaya ringspot virus (*Potyvirus*), watermelon mosaic virus (*Potyvirus*), zucchini yellow mosaic virus (*Potyvirus*)
Cucurbita maxima	Squash, pumpkin	3	Cucumber mosaic virus (*Cucumovirus*), papaya ringspot virus (*Potyvirus*), watermelon mosaic virus (*Potyvirus*), zucchini yellow mosaic virus (*Potyvirus*)
Cucurbita pepo	Zucchini, marrows, pumpkin, squash	14	Cucumber mosaic virus (*Cucumovirus*), papaya ringspot virus (*Potyvirus*), watermelon mosaic virus (*Potyvirus*), zucchini yellow mosaic virus (*Potyvirus*)
Capsicum annuum	Pepper	27	Cucumber mosaic virus (*Cucumovirus*), pepper mottle virus (*Potyvirus*), potato virus Y (*Potyvirus*), tobacco etch virus (*Potyvirus*), tobacco mild green mosaic virus (*Tobamovirus*), tobacco mosaic virus (*Tobamovirus*), tomato spotted wilt virus (*Tospovirus*)
Solanum lycopersicon	Tomato	53	Tobacco mosaic virus (*Tobamovirus*), tomato mosaic virus (*Tobamovirus*), tomato spotted wilt virus (*Tospovirus*)
Solanum melongena	Eggplant (aubergine)	8	Cucumber mosaic virus (*Cucumovirus*), tomato mosaic virus (*Tobamovirus*)

symptoms and leaf narrowing, and distortion (**Figure 1**). Fruits are pitted and misshapen, unmarketable. To worsen things, mixed infections with other viruses are common. Celery and parsley infected by CMV show leaf yellowing and necrosis. 'Lettuce' infected by CMV shows intense mosaic, vein chlorosis and, frequently, vein browning and necrosis when temperature drops below 13 °C. CMV infection of spinach causes a variety of symptoms including stunting, yellowing, and mottling of the older leaves and malformation of the younger leaves.

Garlic Viruses

Because of exclusive vegetative propagation, by cloves or, in some cultivars, by inflorescence bulbils, garlic is particularly prone to accumulate complex virus infections. The most known virus-associated syndrome in garlic is mosaic. Symptoms include various degrees of leaf discolorations, most pronounced in the younger leaves, chlorotic mottling, striping, and streaking. Plants are also stunted compared to healthy looking plants under the same conditions. Most probably, two potyviruses are responsible for mosaic in garlic: one is a garlic strain of onion yellow dwarf virus (OYDV-G), the other a specific strain of leek yellow stripe virus (LYSV-G). Garlic clones, first freed from OYDV and LYSV and then mechanically inoculated with either virus, have been shown to suffer yield reduction from 17% to 60% depending on the virus–cultivar combinations. Yield losses are worse in case of mixed infections or chronic infections (vegetatively propagated material originally infected). Two aphid-borne carlaviruses, garlic common latent virus (GarCLV) and shallot latent virus (SLV), also known as garlic latent virus (GarLV), have also been identified as parts of the garlic virus complex. A number of allexiviruses (genus *Allexivirus*; family *Flexiviridae*) have been identified in recent years in garlic. Although none of them has been associated with any particular symptom, some of them cause yield effects, particularly in mixed infections with OYDV and LYSV. Garlic dwarf virus (GDV) (family *Reoviridae*; genus *Fijivirus*), originally isolated in France, causes well-defined symptoms of dwarfing and leaf thickening.



Figure 1 Symptoms of cucumber mosaic virus infection on zucchini leaves. Courtesy of V. Lisa.

Tobamoviruses

Tobamoviruses are possibly the most-studied viruses of plants. Tobamoviruses have rod-shaped particles 300 × 18 nm, each containing a single molecule of positive-sense, ssRNA. Particles are very stable. No biological vector is known, but because of high stability and high concentration in plant tissues, tobamoviruses are readily transmitted mechanically during crop tending. They are also seed-transmitted but, as mentioned before, in a manner very different from true seed transmission. Tobacco mosaic virus (TMV) (from which the name of the genus is derived) and tomato mosaic virus (ToMV) are the most known, but other viruses, pepper mild mottle virus (PMMoV) and tobacco mild green mosaic virus (TMGMV), infect vegetables in the family Solanaceae and can be differentiated by biological and serological tests. TMV, ToMV, and PMMoV commonly infect peppers, causing chlorotic mosaic, leaf distortion, sometimes systemic necrosis, and defoliation, depending on the usual factors: plant cultivar and age, virus strain, light intensity, and temperature. Fruits are disfigured, with discolored or necrotic areas. PMMoV usually causes milder symptoms on leaves but is more severe on fruits. TMGMV has been found occasionally in pepper. Tomato plants are mostly infected by ToMV, but occasionally also by TMV. The most characteristic symptoms are mottled areas of light and dark green on the leaves. Plants infected at an early stage of growth are yellowish and stunted. Leaves may also be malformed, narrowed, although not as much as with CMV, or showing enations (outgrowths) on the lower leaf lamina. High temperature can mask leaf symptoms. Fruits can be from almost normal to misshapen and be reduced in size and number, showing uneven ripening, corky or necrotic rings, internal browning. TMV and ToMV can infect eggplant occasionally, causing mild symptoms on both leaves and fruits.

Tomato Spotted Wilt Virus

Tomato spotted wilt virus (TSWV) is the type member of the genus *Tospovirus* in the family *Bunyaviridae*. Virus particles are roughly isometric, 80–90 nm in diameter, enveloped in a lipoprotein membrane, contain three single-stranded linear RNAs, one of negative and two of ambisense polarity, associated with a nucleoprotein to form the nucleocapsid. TSWV is transmitted by thrips in a circulative, propagative manner. It can infect hundreds of species in 70 botanical families. The first symptoms of TSWV infection in tomato are chlorotic

spots, 3–4 mm in diameter, on apical leaves, rapidly turning to bronze and dark-brown (necrosis). If infected when young, the plant will eventually die. If infected later, dark brown streaks also appear on stems and leaf petioles; growing tips are greatly stunted, and usually severely affected with systemic necrosis. Fruits, mostly reduced in size, will display characteristic symptoms – immature fruit have mottled, light green rings with raised centers that develop to orange and red discoloration patterns on mature fruits (**Figure 2**). TSWV-infected pepper shows symptoms similar to those described for tomatoes. Plants infected at a very early stage are usually severely stunted and yield no fruit. Plants infected later show chlorotic or necrotic rings on leaves and stems. Some cultivars react with flower and leaf drop. Fruits develop necrotic or discolored spots or rings. Lettuce and endive infected when young turn yellow, collapse, and die. Older plants develop marginal wilting, yellowing, and necrosis of the leaves. Typical is the bending of midribs as a consequence of symptoms appearing only on one half of the leaf. The plant may also look twisted. Endive appears to be more susceptible than lettuce. Chicory infected by TSWV has a stunted, bushy, yellowish look, with chlorotic necrotic spots along the midribs and secondary veins. Eggplants and artichokes can also be naturally infected by TSWV, but only occasionally.

Turnip Mosaic Virus

Turnip mosaic virus (TuMV) is a typical member of the genus *Potyvirus* in the family *Potyviridae*. It has nonenveloped, filamentous virions, usually flexuous, with a modal length of 720 nm, containing a monopartite, positive-sense ssRNA genome. It is transmitted by several species of aphids in a nonpersistent manner. It has a relatively wide host range; naturally, infections occur mainly in the family Brassicaceae and in few weeds outside this family. All *Brassica* species are susceptible to TuMV. The virus also infects lettuce, watercress, radish, and rocket, and it is present all over the world. Young leaves of infected cauliflower plants show chlorotic ringspots followed, as the leaf ages, by yellow or brownish spots surrounded by circular or irregular necrotic rings. Generally, these occur in the vicinity of the leaf veins. Leaf blight appears in infected sections when many lesions coalesce. Outer leaves of infected cabbages may show ringspot pattern or appear more uniformly necrotic, but, as the leaves age, a chlorotic color almost replaces the normal green tissue. In white cabbage, the occurrence of internal necrotic lesions, sunken and coalescent, has been correlated with the presence of field symptoms of turnip mosaic virus. This postharvest disorder may result in the loss of large quantities of stored cabbage. Symptom expression is temperature sensitive, with most pronounced symptoms at temperatures ranging from 22 to 30 °C, and is very much masked below 17 °C. Other *Brassica* species, like turnip, broccoli, and Chinese cabbage, react to TuMV infection with different degrees of mosaic, necrosis, leaf distortion, and reduced growth.



Figure 2 Symptoms of tomato spotted wilt virus infection on tomato fruits. Courtesy of the late P. Roggero.

Tomato Yellow Leaf Curl Virus and Related Begomoviruses

Tomato yellow leaf curl virus (TYLCV) is a member of the genus *Begomovirus* in the family *Geminiviridae*. It has geminate particles measuring 20 × 30 nm and encapsidating a monopartite genome of circular, ssDNA 2.8 kbp in size. The virus is transmitted by the whitefly *Bemisia tabaci* in a circulative manner, but it is not mechanically transmissible. Seed transmission has not been reported. TYLCV has a relatively narrow natural host range. Infected tomato plants are stunted, with branches and petioles tending to assume an erect position. Leaflets of the infected plants are smaller than those of healthy ones and upward curled with margins more or less yellow (**Figure 3**). Flowers have a normal appearance, but fruit production is strongly reduced. TYLCV was first described in Israel and it is now known to be present in the Mediterranean basin, in Egypt and Sudan, in Iran, and in the Caribbean basin. A number of monopartite begomoviruses, transmitted by *B. tabaci*, inducing very similar symptoms on tomato plants, with some differences in the host range and significant differences in their sequences, have been described in recent years and classified as distinct species of the genus *Begomovirus* (e.g., *Tomato yellow leaf curl Sardinia virus*). Similar symptoms on tomato plants are induced by other begomoviruses, named in the same way, but are actually bipartite viruses (e.g., tomato yellow leaf curl China, -Iran, -Thailand virus); some of these viruses may be mechanically transmissible with difficulties. A few other begomoviruses, named tomato leaf curl someplace virus, where someplace is the name of the area (state, country) of origin, may induce some yellowing of tomato leaves, despite the name. Molecular diagnostics are therefore the only tools for identification of begomoviruses inducing yellow leaf curl symptoms in tomato plants.



Figure 3 Leaves of a tomato plant infected by tomato yellow leaf curl virus (right) compared to leaves of a healthy plant (left).



Figure 4 Symptoms of zucchini yellow mosaic virus infection on zucchini fruits. Courtesy of the late P. Roggero.

Zucchini Yellow Mosaic Virus

Zucchini yellow mosaic virus (ZYMV) is a typical member of the genus *Potyvirus* (family *Potyviridae*) (see TuMV). It is transmitted by several species of aphids in a nonpersistent manner and its natural host range is essentially limited to members of the family Cucurbitaceae. ZYMV is a 'recent' virus – it was first detected in 1973 in southern Europe, the first epidemics were reported in 1979–80, and in 1996 it was already present practically in all the areas where cucurbits are grown. We still lack an acceptable explanation for the rapid worldwide diffusion of the virus. Early symptoms in infected zucchini appear as vein clearing of fine leaf veins, followed by a general yellowing of the leaf with dark green areas. Later on, the mosaic becomes stronger, and vein banding appears. Leaves can be deformed to shoestring appearance, but the most affected are fruits, that are severely malformed and often develop longitudinal cracks (**Figure 4**). Similarly, squash and pumpkin plants develop knobby areas on the fruits resulting in prominent deformations. In watermelons, as in melons, ZYMV induces yellow mosaic, severe malformations of leaves and fruits, that often display radial or longitudinal cracks. On cucumber, symptoms of ZYMV infection are less severe than on the other cucurbit crops; mosaic of variable intensity, followed by dark green vein banding and discoloration of fruits.

See also: Alphacryptovirus and *Betacryptovirus*; Cucumber Mosaic Virus; Diagnostic Techniques: Microarrays; Diagnostic Techniques: Plant Viruses; Flexiviruses; Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Geminiviruses; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes; Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Plant Virus Diseases: Economic Aspects; Potyviruses; *Tobamovirus*; Tomato Spotted Wilt Virus; Tomato Yellow Leaf Curl Virus; *Tospovirus*; Vector Transmission of Plant Viruses.

Further Reading

Conti M, Gallitelli D, Lisa V, *et al.* (1996) *I principali Virus delle Piante Ortive.* Milan: Bayer.

Jones JB, Jones JP, Stall RE, and Zitter TA (eds.) (1991) *Compendium of Tomato Diseases.* St. Paul, MN: The American Phytopathological Society.

Pernezny K, Roberts PD, Murphy JF, and Goldberg NP (eds.) (2003) *Compendium of Pepper Diseases.* St. Paul, MN: The American Phytopathological Society.

Ryder EJ (1999) *Lettuce, Endive and Chicory.* Cambridge: CABI Publishing.

Thresh JM (ed.) (2006) *Advances in Virus Research 67: Plant Virus Epidemiology.* San Diego, CA: Academic Press.

Zitter AT, Hopkins DL, and Thomas CE (eds.) (1996) *Compendium of Cucurbit Diseases.* St. Paul, MN: The American Phytopathological Society.

Relevant Website

http://vegetablemdonline.ppath.cornell.edu – Cornell Plant Pathology Vegetable Disease Web Page.

Vesicular Stomatitis Virus

S P J Whelan, Harvard Medical School, Boston, MA, USA

© 2008 Elsevier Ltd. All rights reserved.

Historical Perspective

Vesicular stomatitis viruses (VSVs) are transmitted naturally by arthropods to a broad range of animal species. A clinically significant acute disease is manifest in domesticated animals, notably cattle, horses, and pigs, and it is characterized by fever and the appearance of vesicular lesions in the mouth, tongue, udder teats, and hoof coronary bands. Symptoms are therefore similar to those following infection with the apthovirus, foot-and-mouth disease virus (FMDV) and, consequently, rapid diagnosis is important in livestock. VSV infection was first described in the USA in 1916, following an epidemic in cattle and horses. However, reports from 1862 describe a clinically similar disease in army horses during the American Civil War. Today the virus is distributed throughout the Americas and is enzootic in Central America. In Panama, estimates suggest that up to 29% of the human population has been exposed to the virus as judged by the presence of neutralizing antibodies. Infection of humans can result in a mild febrile illness, but is generally asymptomatic. VSVs have been described rarely outside the Western Hemisphere.

Taxonomy and Classification

VSVs have a nonsegmented negative-sense RNA genome and are assigned to the order *Mononegavirales*. Within this order, they are assigned to the family *Rhabdoviridae,* based upon their characteristic 'bullet' shape. Within the rhabdovirus family, they are further assigned to the genus *Vesiculovirus*. A list of the nine currently recognized species assigned to the genus is provided in **Table 1**, along with their geographic distribution and sources of the virus in nature. The *Eighth Report of the International Committee on the Taxonomy of Viruses* lists an additional 19 members tentatively assigned to this genus. The type species is *Vesicular stomatitis Indiana virus*. Vesicular stomatitis Indiana virus (VSIV) has been widely studied in laboratories as a prototype of all the *Mononegavirales* and will be the primary focus of this article. Much is known about the replication and molecular biology of VSIV. VSIV was isolated following an outbreak of a vesicular disease of cattle in Richmond, Indiana, in 1925. The infectious agent was maintained by serial passage in animals and eventually became the Indiana serotype of VSV. *Vesicular stomatitis New Jersey virus* is also classified as a species in the genus *Vesiculovirus*. It was isolated following an outbreak in cattle in 1926 and is serologically and genetically distinct from VSIV. Vesicular stomatitis Alagoas virus (VSAV) was isolated from domesticated animals in Alagoas Brazil during an outbreak of VSV.

Structure of VSV

Particles

A schematic of VSIV is shown in **Figure 1** along with an electron micrograph showing virus particles. The particles appear bullet-shaped and are approximately 180 nm long and 70 nm in diameter. The particles comprise 74% protein, 20% lipid, 3% RNA, and 3% carbohydrate. The virus possesses a lipid envelope that is decorated with trimeric spikes of the 67 kDa attachment glycoprotein (G). The interior of the particle contains a ribonucleoprotein (RNP) core of the genomic RNA complexed with the viral nucleocapsid (N) protein. This N-RNA core is associated with the RNA-dependent RNA polymerase (RdRp), the viral components of which are a 241 kDa large (L) protein and a tetramer of a 29 kDa accessory

Table 1 Geographic distribution and sources of natural isolation of viruses representing the nine recognized species in the genus *Vesiculovirus*

Virus	*Geographic distribution*	*Source*
Carajas virus (CJSV)	Brazil	Phlebotomine sandflies
Chandipura virus (CHPV)	India, Nigeria	Mammals, sandflies
Cocal virus (COCV)	Argentina, Brazil, Trinidad	Mammals, mosquitoes, mites
Isfahan virus (ISFV)	Iran, Turkmenistan	Sandflies, ticks
Maraba virus (MARAV)	Brazil	Phlebotomine sandflies
Piry virus (PIRYV)	Brazil	Mammals
Vesicular stomatitis Alagoas virus (VSAV)	Brazil, Columbia	Mammals, sandflies
Vesicular stomatitis Indiana virus (VSIV)	Americas	Mammals, mosquitoes, sandflies
Vesicular stomatitis New Jersey virus (VSNJV)	Americas	Mammals, mosquitoes, midges, blackflies, houseflies

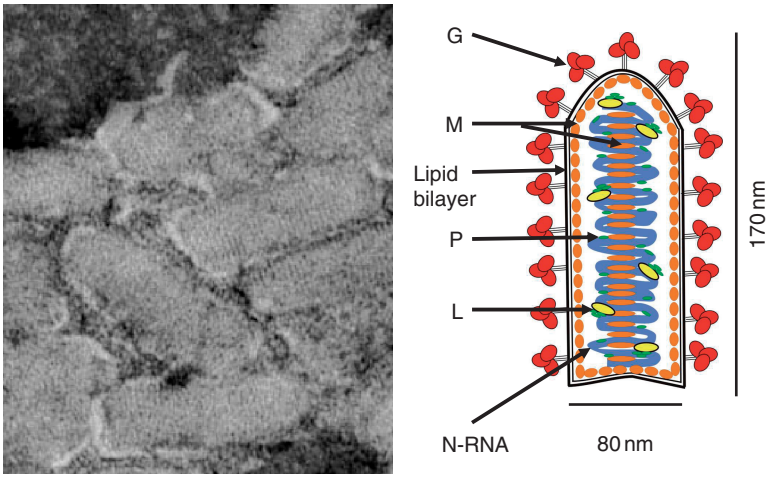


Figure 1 Electron micrograph of VSIV particles and a schematic illustration of the virion. At left, a negative-stained image of a group of virus particles is shown. At right, a schematic illustration of the virion is shown along with the dimensions of a viral particle. N-RNA, nucleocapsid protein coated RNA; P, phosphoprotein; M, matrix protein; G, attachment glycoprotein; L, large polymerase subunit. Kindly provided by David Cureton, Harvard Medical School.

phosphoprotein (P). Together, these form the internal helical nucleocapsid. Another major structural component of the virus particles is the 26 kDa matrix (M) protein, which is located below the membrane and associated with the nucleocapsid. The approximate composition of the particle is one molecule of RNA, 1200 copies of N, 500 copies of P, 1800 copies of M, 1200 copies of G, and 50 copies of L. The role of these proteins in viral replication is described in more detail below.

Genome

VSIV has a nonsegmented, negative-sense RNA genome of 11 161 nt. The genome comprises a 50 nt 3′ leader region (le), five genes that encode in order the N, P, M, G, and L proteins, and a 59 nt 5′ trailer region (**Figure 2**). The 5′ and 3′ ends of the genome are not modified and contain a 5′ triphosphate and 3′ hydroxyl. A key feature of all mononegaviruses is that the RNA genome is not found naked within infected cells. Instead, it is present as a ribonucleoprotein complex, in which it is completely covered by the viral nucleocapsid protein. This N-RNA template, associated with the viral L and P proteins, comprises the transcription-competent core of the virus, and delivery of this complex is required to initiate the infectious cycle. Consequently, in contrast to positive-sense RNA viruses, the naked RNA is not infectious.

Viral Replication Cycle

The replication cycle (**Figure 3**) should be considered a continuum of events. However, it is convenient to divide the cycle into the following three stages.

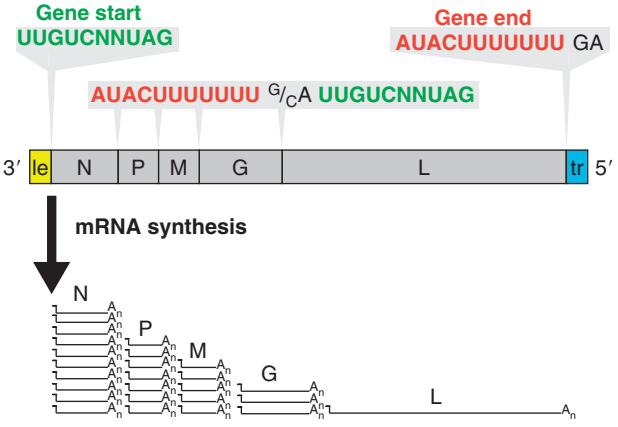


Figure 2 A schematic illustration of the VSIV genome highlighting the polymerase regulatory elements. The genome is represented 3′–5′ as a series of boxes, comprising le = leader region, N = nucleocapsid, P = phospho, M = matrix, G = glyco, L = large polymerase, and tr = trailer region. The conserved *cis*-acting elements that regulate polymerase activity during mRNA synthesis are shown for emphasis. Colors: green, the conserved residues of the gene-start sequence; red, the conserved residues of the gene-end sequence; and black, the nontranscribed residues of the gene junction. The leader and trailer regions also contain key elements that regulate polymerase activity and serve as promoters as described in the text.

Attachment, Entry, and Uncoating

Attachment of VSV to host cells is mediated by the glycoprotein which binds to the surface of cells. Given that VSV G can mediate infection of almost all cells in culture, either the receptor for VSV must be widely distributed, or the virus may be able to utilize multiple surface molecules for attachment. Phosphatidylserine (PS) was long thought to be the receptor for VSV but

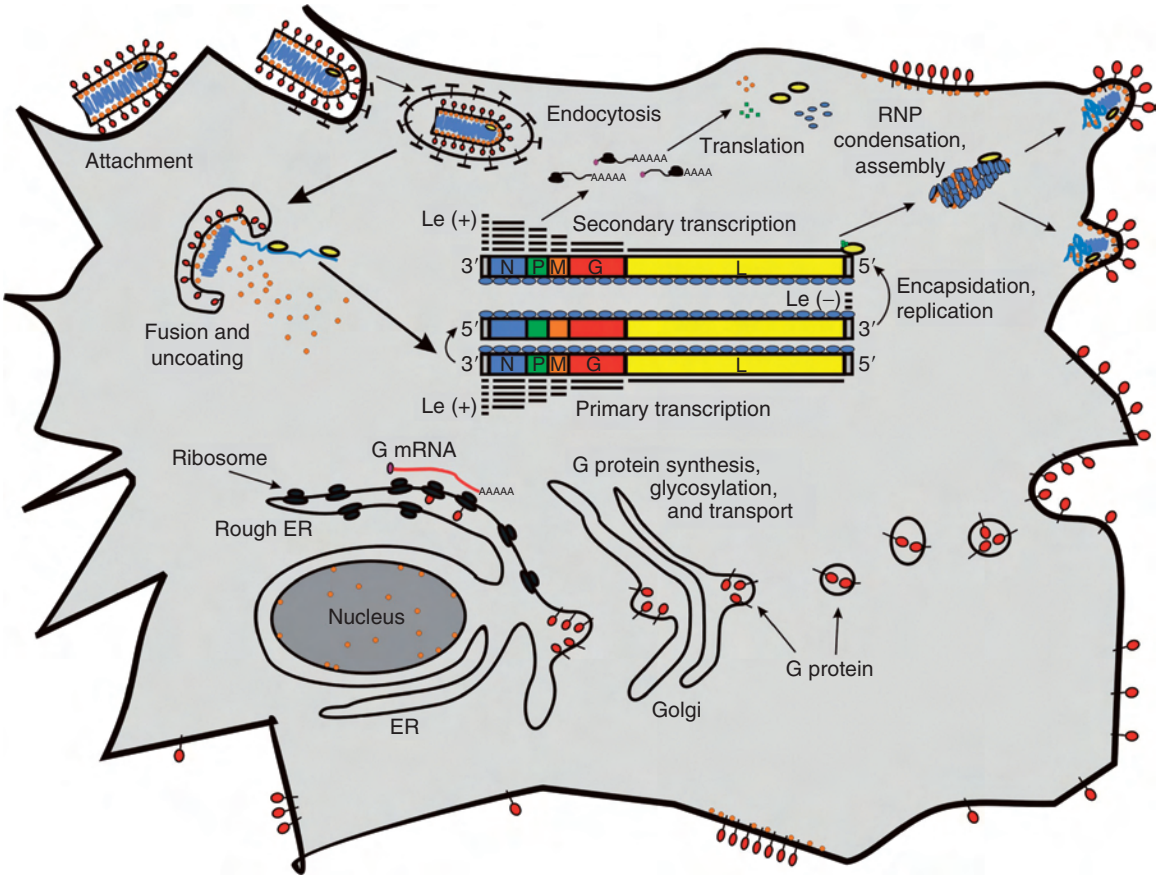


Figure 3 A schematic illustration of the replication cycle of VSV. The replication cycle (described in the text) is depicted showing attachment of virus to the cell, internalization, release of the viral core into the cytoplasm, primary viral mRNA synthesis, mRNA translation, genomic replication, secondary viral mRNA synthesis, assembly, and budding of infectious particles. Kindly provided by David Cureton, Harvard Medical School.

recent studies have questioned this finding. Importantly, the PS binding site in VSV G is internal to the trimer in its prefusion form. Following attachment to the cell, the virus is internalized via clathrin-dependent endocytosis and delivered to an early endosome. The pH threshold necessary to trigger the conformational alterations in VSIV G that promote fusion of the viral and cellular membranes is approximately 6.2. This pH is reached in the early endosome, and viral and cellular membranes fuse to release the transcription-competent RNP core into the cytoplasm of the cell. Recent work has called into question this conventional view of the VSV entry pathway and has posited a new model for viral entry. In this model, fusion and RNP release are spatially and temporally separated. Specifically, the low pH encountered during endocytic transport triggers fusion and the delivery of the RNP core into an intralumenal vesicle within an endosomal carrier vesicle. A subsequent (cell-mediated) fusion event is then required to fuse the membrane of the intralumenal vesicle with the limiting membrane of the cell and deliver the RNP into the cytoplasm to initiate the infectious process. Irrespective of the precise route by which virus enters the cell, the end result is the delivery of the transcription-competent core into the cytoplasm. This is accompanied by the release of M protein, which can migrate to the nucleus where it plays a role in inhibiting host gene expression.

Gene Expression

Following the delivery of the transcription-competent core into the cytoplasm, RNA synthesis can begin. The replication cycle of VSV is entirely cytoplasmic, occurring efficiently in enucleated cells. The demonstration that purified VSV particles contain a functional RdRp that is active *in vitro* has led to major advances in our understanding of viral gene expression. During RNA synthesis, the polymerase uses the encapsidated genomic RNA as template in two distinct reactions: (1) transcription of five mRNAs that encode the N, P, M, G, and L proteins; and (2) replication to yield full-length antigenomic, and then genomic RNA strands. Our current

understanding of gene expression is summarized as follows. In response to a specific promoter element that is provided by sequences within the 3′ leader region and the conserved residues of the first (N) gene-start (3′-UUGUCNNUAG-5′) sequence, the RdRp initiates mRNA synthesis. Synthesis commences at the N gene-start sequence and generates an mRNA that is capped, methylated, and polyadenylated, each of these reactions occurring co-transcriptionally. Termination at the end of the N gene is achieved by the polymerase recognizing a highly conserved sequence element referred to as the gene-end 3′-AUACUUUUUUUG/C-5′. This sequence signals the polymerase to stutter on the U_7 tract to generate the polyA tail, and leads to termination of mRNA synthesis. Termination at the end of the N gene is essential for the polymerase to be able to transcribe the P gene. A poorly understood event that is localized to a short region at the N–P gene junction results in the synthesis of approximately 30% less P mRNA than N mRNA. This sequential and polar synthesis of the viral mRNAs continues through the entire genome and provides a gradient of viral mRNA synthesis such that N > P > M > G > L. These products of mRNA synthesis are illustrated in **Figure 2**.

Among the notable steps of mRNA synthesis are the unusual mechanisms by which the 5′ and 3′ ends of the RNA are formed. Each stage of mRNA cap formation is distinct from those employed in other systems. Specifically, the 5′ end of the pppApApCpApG mRNA is capped by an unusual ribonucleotidyltransferase activity that transfers the monophosphate RNA onto GDP derived from GTP to form the *G*pp*p*ApApCpApG mRNA cap structure. In contrast, all other capping reactions are catalyzed by an RNA guanylytransferase that transfers GMP onto the 5′ end of a diphosphate RNA through a reaction that involves a covalent enzyme GMP intermediate. The VSV L protein is responsible for this novel reaction, which appears to involve a covalent intermediate between L and the viral mRNA. The resulting *Gpp*pApApCpApG cap structure is then methylated at guanine N-7 and ribose 2′-O positions to yield 7^{m}G*pp*pA^{m}pApCpApG. These activities are also provided by the L protein, and again differ from conventional mRNA cap methylation reactions. For VSIV, the two enzymatic activities have been shown to share a single binding site for the methyl donor, *S*-adenosyl-L-methionine (SAM). In contrast, other cap methylation reactions are normally executed by two distinct proteins with separate binding sites for the methyl donor SAM. Formation of the 3′ end of the RNA is also unusual. Specifically, polyadenylation occurs in a pseudo-templated fashion in which the polymerase complex reiteratively transcribes the conserved U tract present at the end of each VSV gene.

The viral mRNAs are efficiently translated by the host translation machinery, but how they compete with cellular mRNAs for translation is not well understood. Viral protein synthesis is essential for replication of the genomic RNA. Ongoing translation provides a continuous supply of soluble N protein that drives the encapsidation of the nascent RNA chain. This process is intimately linked to genomic RNA replication which first results in the production of a full-length encapsidated complementary antigenome RNA. This antigenome can then serve as template to produce more progeny genomic RNAs for use as templates for further mRNA synthesis in a process referred to as secondary transcription.

Precisely how the different polymerase activities are regulated in infected cells remains poorly understood. Two functionally distinct pools of polymerase have been purified from cells. One initiates internally at the N gene-start sequence and functions as the viral transcriptase. A second complex initiates at the 3′ end of the genome and functions as the viral replicase. These complexes are reported to differ in their composition such that the transcriptase comprises the viral P and L proteins, together with several cellular proteins including translation elongation factor-1α, heat shock protein 60, and the host cell RNA guanylyltransferase. In contrast, the replicase is reported to comprise the viral N, P, and L proteins.

In addition to the species of RNA described above, two short leader RNAs are generated during RNA synthesis: a 47 nt Le+ from the 3′ end of the genomic RNA and a 45 nt Le– from the 3′ end of the antigenomic RNA. The function of these RNAs is poorly understood, although a role for the Le+ in the shutoff of host gene expression has been described. A long-standing model for the regulation of RNA synthesis in VSV postulates that polymerase initiates all RNA synthesis at position 1 of the genome, and during synthesis of Le+ a crucial regulatory decision is made to either terminate leader and initiate mRNA synthesis at the N gene start, or alternatively to read through the leader–N gene junction and synthesize the full-length antigenome. The obligatory requirement for protein synthesis to provide a source of N protein to encapsidate the nascent RNA during genome replication led to the suggestion that N protein availability switches polymerase activity from transcriptase to replicase.

In recent years, there has been an accumulation of evidence that conflicts with this model. Specifically, a VSV mutant containing a single amino acid change in the template-associated N protein produces an excess of N mRNA over Le+ *in vitro*, suggesting that polymerase can synthesize N independently of Le+. In another series of experiments, recombinant VSVs, containing a 60 nt gene inserted between the leader region and the N gene, were employed. The recombinant viruses were examined to determine the effect of altering the potential number of

ultraviolet (UV)-induced dimers between adjacent uracil residues. Such dimers block progression of the polymerase. These experiments showed that changing the UV sensitivity of the Le+ had no effect on the sensitivity of the 60 nt mRNA in infected cells, suggesting that polymerase can initiate synthesis internally at the first gene start. In addition, two separate pools of polymerase can be isolated from infected cells, one that initiates internally at the N gene start and the second that initiates at the 3′ end of the genome. These findings support the hypothesis that mRNA synthesis can initiate independently of leader synthesis, and show that polymerase function is not simply switched by N protein levels. However, viral gene expression is controlled, it results in the exponential amplification of the input genomic RNA, yielding progeny genomes that can be assembled into infectious particles, the next phase of the viral replication cycle.

Assembly and Budding

Assembly of infectious virus particles involves many critical interactions. Our current understanding of this intricate process is that the matrix (M) protein complexes with the RNP core and represses transcription of viral mRNAs. This condensed RNP complex acquires a lipid envelope that has been modified by the insertion of an externally oriented glycoprotein. Details of how the RNP is transported to the site of budding and how the matrix interacts with and condenses the RNP are poorly understood. The M protein is only found associated with RNPs at sites of viral budding, and how genomic RNPs (rather than antigenomic RNPs) are specifically selected for budding is unclear. The M protein contains two 'late domains' (PTAP and PPPY motifs) that appear to be critical in the late phase of the assembly-release pathway. Amino acid substitutions in the PPPY motif result in the accumulation of bullet-shaped virions that are stalled at a late stage of virus budding. This motif, and similar motifs in proteins from other enveloped viruses, target virus for budding through interaction with components of the endosomal sorting complex required for transport or ESCRT pathway. The release of infectious particles completes the replication cycle and provides progeny virions for infection of the next cell.

The kinetics of the viral replication cycle are rapid. In mammalian cells in culture, one infectious particle can produce 10 000 infectious progeny within 8 h. While much remains to be explored about the biology of VSV, it is one of the best understood animal viruses. Studies on VSV should continue to prove informative in understanding how enveloped viruses enter and bud from their host cells, and how nonsegmented negative-strand RNA viruses (mononegaviruses) express their genetic information.

Functions of the Viral Proteins

Nucleocapsid protein

The N protein coats the viral genomic RNA and the positive-sense antigenomic replicative intermediate to form ribonucleoprotein (RNP) complexes. The interaction with the N protein renders the RNA resistant to cleavage by ribonucleases. It is in this form that the RNA is presented to the polymerase to serve as template. The N protein comprises 422 amino acids and has a molecular weight of approximately 48 kDa. The crystal structure of a complex of 10 molecules of N protein bound to 90 nt of RNA has been solved, revealing that N has a bilobed structure with RNA bound between the lobes. The structure indicates that N protein must be either transiently displaced or substantially remodeled during copying of the RNA genome by the viral polymerase.

Phosphoprotein

P is a multiply phosphorylated acidic protein of 265 amino acids that functions as an essential polymerase cofactor and plays an additional role in maintaining N protein in a soluble form necessary for RNA encapsidation. Sequence analysis has identified three domains of P. An acidic N-terminal domain (domain I) of 150 amino acids contains phosphorylation sites at Ser 60, Thr 62, and Ser 64. Phosphorylation of these residues by the host casein kinase II leads to the oligomerization of P protein and together with the large polymerase protein L, assembly of the polymerase complex. Domain I is separated from domain II by a highly variable hinge region comprising residues 150–210. Domain II (residues 210–244) contains additional phosphorylation sites at Ser 226, Ser 227, and Ser 233 that appear to be important for RNA replication. Domain III is basic and comprises the C-terminal 21 amino acids. A crystal structure of a fragment comprising amino acids 107–177 of the P protein has been solved, providing evidence that P protein functions as a tetramer. In addition to P, two proteins (C and C′) are produced from the P gene. These proteins are small (55 and 65 amino acids, respectively), are highly basic, and have not been detected in virus particles. Recombinant viruses that are unable to produce C and C′ replicate normally in cultured mammalian cells. The role of these proteins is unclear but may be important for infections in insects and/or mammalian hosts *in vivo*.

Matrix protein

The matrix (M) protein is the major structural component of virus particles. M is a small 229-amino-acid multifunctional protein. It condenses viral RNPs and drives budding of virus particles from the host plasma membrane. In addition, M downregulates host gene expression by directly interacting with Rae 1, thus inhibiting nuclear transport. The crystal structure of amino acids 48–229 of

the matrix protein has been solved and reveals regions of M that may be required for membrane association. Two N-terminal truncations of M are generated in infected cells by initiation of translation at methionines 33 and 51. A recombinant virus engineered to ablate the expression of these two alternate forms of M shows a modest reduction in cytopathic effect in culture cells.

Glycoprotein

The attachment glycoprotein (G) is a trimer present on the surface of the virion. It is responsible for attachment of virus to cells and promotes fusion of the viral and cellular membranes during endocytosis. The protein is synthesized as a 511-amino-acid polypeptide that is co-translationally inserted into membranes in the endoplasmic reticulum (ER). An N-terminal 16-amino-acid signal sequence is cleaved from the nascent polypeptide upon insertion. A 20-amino-acid hydrophobic sequence acts as the membrane anchor and serves as a stop-transfer signal during ER translocation. The remaining 29 amino acids of G remain as a cytoplasmic tail. Co- and post-translational modifications result in N-glycosylation of two asparagine residues in the ectodomain, and palmitoylation of a cysteine in the cytoplasmic domain. The role of palmitoylation in infection is unclear as some VSV strains lack this modification. However, glycosylation permits association of G with calnexin, an ER-resident chaperone, and mutations that prevent glycosylation result in G protein aggregation. Mature G protein is selectively transported to the basolateral surface of polarized epithelial cells. At the plasma membrane, VSV G clusters in microdomains that are the sites of virus assembly and budding. The determination of the crystal structure of a fragment of the G protein in both pre- and postfusion conformations has provided new insight into the entry process. The structures show that each G protein monomer contains two hydrophobic fusion loops that insert into the target cell membrane. Substantial rearrangements of the G protein that are driven by the low pH environment encountered in the endocytic pathway drive fusion of the viral and cellular membranes.

Large polymerase protein

The large polymerase protein (L) comprises 2109 amino acids. This 241 kDa multifunctional protein is responsible for template binding, ribonucleotide polymerization, and the co-transcriptional modification of the 5′ and 3′ terminus of the viral mRNAs so that they are capped and methylated. To date, structures are not available for any portion of the L protein. Amino acid sequence alignments of the L genes of representative members of the families *Rhabdoviridae*, *Paramyxoviridae*, and *Filoviridae* have led to the identification of six regions of sequence conservation (CRI–CRVI), separated by regions of no or low sequence homology. These conserved regions are thought to represent the functional domains of L protein. CRIII contains clearly identifiable motifs found in all polymerases and in which alterations to a universally conserved aspartic acid residue eliminate polymerase activity in reconstructed RNA synthesis assays. CRVI functions as a messenger RNA cap-modifying enzyme. Amino acid substitutions to this region disrupt mRNA cap methylation at both the guanine N-7 and ribose 2′-O positions of the cap structure. The regions of L involved in other modifications have not yet been assigned. As described above, L protein has been reported to associate with a number of host proteins, including translation elongation factor-1α, heat shock protein 60, and the host RNA guanylyltransferase. However, the functional significance of these interactions is not yet certain.

Host Range and Transmission

In nature, VSVs can infect a broad range of animals including mammals and invertebrates. Overt disease is typically only seen in cattle, horses, and swine, but there is evidence of natural infection in a range of wild ruminants, ungulates, carnivores, marsupials, and rodents. Infection can be transmitted directly from animal to animal, but this requires an abrasion or a means of introducing the virus below the skin. Humans can also be infected by contact with vesicular lesions or the saliva of infected animals, resulting in an influenza-like illness. Aerosol transmission has also been reported in humans. Insect vectors appear to be important in natural transmission of the virus. VSVs have been isolated on more than 40 occasions from hematophagous insects including mosquitoes, biting midges (*Culicoides* spp.), phlebotomine sand flies (*Lutzomyia* spp.), and black flies (*Simulium* spp.). In addition, VSV has been experimentally transmitted to mice from infected mosquitoes and between insects through co-feeding. Susceptible insect hosts are also capable of transovarial transmission and these infected progeny have been shown to infect mammalian hosts. Recovery of vesicular stomatitis New Jersey virus (VSNJV) from non-biting flies suggests that mechanical transmission may occur. There is also evidence that grasshoppers (*Melanoplus sanguinipes*) can be infected experimentally, and cattle that ingest infected grasshoppers can develop disease.

Consistent with the ability to infect a broad range of experimental animals, VSV replicates efficiently in a variety of cell lines of vertebrate and invertebrate origin. The Syrian hamster kidney cell line, BHK-21, is commonly used to generate viral stocks of high titer (10^9–10^{10} pfu ml^{-1}). VSV can also infect and replicate to high titers in cultured cells derived from insects, reptiles, and fish, and replication can occur in several insect cell lines. Infection of mammalian, avian, and some insect cells results in a cytopathic effect (CPE). In fibroblasts, this is

seen as an increase of membrane blebbing followed by extensive cell rounding. The M protein is responsible for these changes in infected cells. A noncytopathic infection occurs in some insect cells including lines derived from *Aedes aegypti*, *Aedes albopictus* (C6/36), and *Drosophila melanogaster*. Experimentally, the virus has been shown to express its genetic information in spheroblasts derived from the yeast, *Saccharamoyces cerevisae*, and to produce infectious virions in embryonic cells derived from the nematode *Caenorhabditis elegans*. Thus, the host requirements for viral replication are provided by an extremely broad range of eukaryotes.

Evolution

Several principles of evolutionary biology have been explored using VSV as a model system. The viral polymerase has an error rate of 10^{-3} to 10^{-5} per nucleotide per round of replication. Given the genome size is 11 161 nt, each time the genome is copied, on average, at least one nucleotide change is introduced. These mutations are typically deleterious, others will be neutral, and some may offer a selective advantage. Consequently, this high mutation rate contributes to VSV's ability to rapidly evolve and adapt to environmental changes. The high mutation rate also begs the question as to why VSV does not mutate itself out of existence. Rather than existing as a single defined sequence, the virus exists as a swarm of mutant sequences around a consensus sequence or a viral quasispecies. Critically, it is this quasispecies that is the biologically relevant target for selection.

Experiments in which the diversity of the viral population is artificially restricted by performing serial plaque-to-plaque transfers (thus restricting the infectious virus population to 1 on each transfer), rapidly result in fitness losses. Such an accumulation of deleterious mutations in populations of asexual organisms lacking compensatory mechanisms such as sex or recombination was predicted by Muller in a concept referred to as Muller's ratchet. This principle has been nicely illustrated in studies of VSV, which is not known to undergo homologous recombination. Studies with VSV have also illustrated other fundamental principles of population genetics. Work by the laboratory of John Holland demonstrated that VSVs of equal fitness could coexist for several generations, but one population would then rapidly outgrow the other. This demonstrated that populations accumulate mutations and, infrequently, mutations provide a selective advantage which leads to its outgrowth. Importantly, the mutation rates in both populations were the same, reflecting an intrinsic property of the viral polymerase. This is referred to as the Red Queen effect. This is a hypothesis proposed by the evolutionary biologist Leigh van Valen in reference to Lewis Carroll's book *Through the Looking Glass*, in which the Red Queen comments to Alice that "it takes all the running you can do, to keep in the same place."

Despite the intrinsically high polymerase error rate, VSVs isolated in nature can show a remarkable genetic stability. For example, sequence analysis of viruses from different hosts and different years has shown that those isolated from multiple hosts within the same region over a period of decades were more closely related than viruses isolated in the same year, but from a different ecological zone. This suggests that ecological factors are a major driving force in the evolution of VSV. Consistent with this, viruses from similar ecological zones 800 km apart were found to be more closely related than viruses from different ecological zones 25 km apart. VSV evolution is unusual in this apparent lack of a 'molecular clock' in which the genome sequence does not show a clear relationship with year of isolation.

The evolutionary origins of VSVs are uncertain but the conservation of gene order and sequence similarity to other members of the *Mononegavirales* indicate they diverged from a common ancestor. Although the number of genes differ, their order is maintained across the *Mononegavirales* as 3′ N–P–M–G–L 5′. The significance of this gene order has been elegantly tested in engineered VSIV recombinants by shuffling the order of the three central genes (P, M, and G), and by moving the N and G genes. Remarkably, all gene orders yield infectious virus, and the replication kinetics of several recombinant viruses in which the P, M, and G genes were shuffled were similar to those of the wild-type parent virus. Consistent with its critical role in driving RNA encapsidation during genome replication, moving the N gene from its promoter proximal position diminished viral replication. These experiments suggest that the conserved gene order may reflect an ancestral gene order that has remained frozen because of the lack of a mechanism for homologous recombination in the *Mononegavirales*.

Geographic and Seasonal Distribution

VSVs are typically restricted to the Americas. Within this region, VSIV and VSNJV are the two most commonly isolated serotypes and have the broadest geographic distribution ranging from Peru to Canada. Vesicular stomatitis Alagoas virus (VSAV) is the major serotype that is isolated in Brazil. Both VSIV and VSNJV are seen in the United States although most outbreaks are associated with VSNJV. In the US, the last outbreak was from 2004 to 2006. In this outbreak, viral infection was reported in Texas, New Mexico, and Colorado in 2004, and the outbreak spread to include Arizona, Utah, Wyoming, Nebraska, Montana, and Idaho in 2005, with a small number of cases reported in Wyoming in 2006. Overall some 1000 animals were affected, representing the most significant outbreak

of disease in the US for almost 10 years. Vesicular stomatitis is endemic in many Latin American countries. In 2002, VSNJV and VSIV were reported in Panama, Costa Rica, Nicaragua, El Salvador, and Mexico, and VSNJV was reported in Honduras, Guatemala, and Belize. In 2004, VSNJV and VSIV were isolated in Columbia, Venezuela, Ecuador, and Peru, and VSIV in Brazil and Bolivia. Although infection is reported throughout the Americas, the highest incidence usually is in tropical regions with more sporadic outbreaks in temperate regions. In temperate regions, outbreaks typically peak in late summer and end by the first frost. In tropical regions, peak incidence typically coincides with the end of the rainy season. These peaks correlate with high insect population levels and, consistent with the role of insect vectors in disease spread, outbreaks tend to spread along waterways. Although, on occasion, VSV has been reported in parts of Europe and Africa, such reports are rare and are probably linked to importation of infected animals.

Pathogenesis and Pathology

Following exposure of animals to VSV, the incubation period is usually 2–4 days. Prior to the development of lesions, animals can show signs of depression, lameness, a fever of up to 41.5 °C, and excessive salivation. In infected cattle, swine and horses, pink to white papules appear in the mouth, lips, gums, nose, teats, and feet. In these regions, the epithelium separates from the basal layer forming a vesicle that fills with clear yellowish fluid that contains very high titers of infectious virus. These vesicles combine and readily rupture. In some cases, lesions appear at a secondary site relative to the initial point of inoculation. The development of secondary lesions is suggestive of a viremia, but virus has not been isolated from the blood of experimentally inoculated natural hosts. Infection results in significant losses for the livestock industry as weight loss can be significant (up to 135 kg in beef cattle), and dairy cattle usually cease milk production. In the absence of secondary infections, the vesicular lesions typically heal in 1–2 weeks and the animals start to regain weight. Viral RNA has been isolated from animals several months after infection although infectious virus has not been recovered.

Immune Response

Our understanding of the immune response to VSV in natural hosts is limited. However, in experimental animals, notably mice, the immune response to VSV has been characterized. Interferon plays an important role in the resistance of older mice to VSV infection and, consistent with this, adult mice that are unable to produce interferon succumb to infection. Different strains of VSV induce different levels of interferon, but there is no clear correlation between levels of interferon *in vitro* and pathogenesis in animals. Neutralizing antibody has also been shown to play a role in the defense against VSV infection in experimentally infected swine and mice. The target of these neutralizing antibodies is the viral attachment protein G, and adoptive transfer experiments have shown that they are sufficient to protect young mice against infection. In endemic areas, susceptible hosts often have detectable neutralizing antibody responses to VSV. However, these responses are not protective, perhaps reflecting the fact that viral replication is largely confined to the epithelium. Experimental infection of mice with VSV also induces a strong cytotoxic T-lymphocyte (CTL) response. The N protein is the predominant antigen recognized and the resulting CTLs are not serotype specific. However, the functional significance of these CTLs in protection against infection is uncertain as mice incapable of mounting such a response survive VSV infection.

Diagnosis, Prevention, and Control

The similarity of symptoms between VSV and FMDV make rapid diagnosis important, especially in countries that are free of FMDV. Diagnosis is achieved typically by analysis of samples of fluid from vesicular lesions using reverse transcription-polymerase chain reaction (RT-PCR), enzyme-linked immunosorbent assay (ELISA), or growth of virus in cell culture. VSV is listed as a notifiable disease by the World Organisation for Animal Health (OIE) and a positive diagnosis results in quarantine of the affected area. To control spread of disease, farm equipment is disinfected using a 2% bleach solution to interrupt animal-to-animal spread, and insect control measures can be introduced. Vaccines against VSIV and VSNJV have been used to control outbreaks in Central and South America. Although vaccination is not routinely practiced throughout the Americas, experiments demonstrate a substantive decrease in clinical infections in vaccinated animals.

Future Perspectives

VSVs will continue to serve as an important prototype of the nonsegmented negative-strand RNA viruses. The advantages of VSV as a model for these viruses are: (1) its relative safety, in that the virus is not a significant human pathogen; (2) abundant viral replication in a broad range of cultured cells yielding up to 10 000 pfu $cell^{-1}$ 8–12 h post inoculation of mammalian cells; (3) a robust reverse genetic system which permits the generation of helper-dependent viruses; (4) crystal structures of the N-RNA template, a portion of the P and M proteins,

and the pre- and postfusion forms of the G protein; and (5) an *in vitro* system for viral mRNA synthesis using purified recombinant polymerase. Capitalizing on these advantages will continue to provide new mechanistic insights into the molecular details of viral entry, gene expression, and assembly. Among the key questions to address are understanding: (1) how and where the viral RNP core enters a cell; (2) how and where the N-RNA serves as template for gene expression; (3) how the large multifunctional polymerase serves to initiate, cap, methylate, polyadenylate, and terminate mRNA synthesis in response to specific sequence elements; (4) how the activities of the polymerase are controlled between mRNA synthesis and genome replication; and (5) how the nucleocapsid templates are selected for assembly and budding from the cell. In addition to the fundamental questions regarding the molecular biology of these viruses, the future holds promise for unraveling the significance of the different host species and vectors in the biology of infection. Genomic approaches will likely yield clues as to how VSV interacts with host cells of insect and mammalian origin, and how these cells respond to infection. Many questions remain to be answered regarding the ecology of the virus including the maintenance of the virus, its transmission, and its relative genetic stability in nature. Such studies are not only warranted in understanding VSV as a model virus, but also for the prospects of using VSV as an oncolytic virus and a live-attenuated vaccine vector for human disease. These potential applications should only add to the urgency with which studies on this prototypic virus are pursued.

See also: Chandipura Virus; Rabies Virus; Quasispecies.

Further Reading

Eigen M (1993) Viral quasispecies. *Scientific American* 269: 42–49.
Gaudier M, Gaudin Y, and Knossow M (2002) Crystal structure of the vesicular stomatitis virus matrix protein. *EMBO Journal* 21: 2886–2892.
Green TJ, Zhang X, Wertz GW, and Luo M (2006) Structure of the vesicular stomatitis virus nucleoprotein-RNA complex. *Science* 313: 357–360.
Lyles DS and Rupprecht CE (2006) In: Knipe DM, Howley PM, Griffin DE, Lamb RA, and Martin MA (eds.) *Fields Virology,* 5th edn., p. 1364. Philadelphia: Lippincott Williams and Wilkins.
Novella IS (2003) Contributions of vesicular stomatitis virus to the understanding of RNA virus evolution. *Current Opinion in Microbiology* 6: 399–405.
Ogino T and Banerjee AK (2007) Unconventional mechanism of mRNA capping by the RNA-dependent RNA polymerase of vesicular stomatitis virus. *Molecular Cell* 25: 85–97.
Roche S, Bressanelli S, Rey FA, and Gaudin Y (2006) Crystal structure of the low-pH form of the vesicular stomatitis virus glycoprotein G. *Science* 313: 187–191.
Roche S, Rey FA, Gaudin Y, and Bressanelli S (2007) Structure of the prefusion form of the vesicular stomatitis virus glycoprotein G. *Science* 315: 843–848.
Rodriguez LL, Fitch WM, and Nichol ST (1996) Ecological factors rather than temporal factors dominate the evolution of vesicular stomatitis virus. *Proceedings of the National Academy of Sciences, USA* 93: 13030–13035.
Wertz GW, Perepelista VP, and Ball LA (1998) Gene rearrangement attenuates expression and lethality of a nonsegmented negative strand RNA virus. *Proceedings of the National Academy of Sciences, USA* 95: 3501.
Whelan SPJ, Barr JN, and Wertz GW (2004) Transcription and replication of non-segmented negative-strand RNA viruses. *Current Topics in Microbiology and Immunology* 283: 61–119.

Viral Killer Toxins

M J Schmitt, University of the Saarland, Saarbrücken, Germany

© 2008 Elsevier Ltd. All rights reserved.

Glossary

ER-associated degradation (**ERAD**) A cellular quality control mechanism ensuring efficient removal of misfolded and/or unassembled proteins from the endoplasmic reticulum (ER) lumen and their subsequent elimination by the cytoplasmic ubiquitin–proteasome system.
Heterokaryon Coexistence of two or more genetically different nuclei in a common cytoplasm.
Importins A family of proteins that transport macromolecules into the eukaryotic nucleus.
Killer virus system A killer virus system consists of a helper totivirus and a satellite double-stranded (ds) RNA encoding the unprocessed precursor of a secreted protein toxin that also gives functional immunity.
Preprotoxin Toxin precursor in the cytoplasm of a killer yeast which is post-translationally imported into the secretory pathway for toxin processing, maturation, and secretion.
Satellite dsRNA An encapsidated dsRNA that is dependent on a helper virus for encapsidation and replication.
Spheroplasts A yeast cell whose cell wall has been enzymatically removed.

Introduction

Although double-stranded (ds) RNA viruses had previously been identified in filamentous fungi, dsRNA viruses in yeast were discovered as determinants of the killer phenomenon in *Saccharomyces cerevisiae* and are now known to be associated with the presence of cytoplasmically inherited members of the totivirus family, which are frequently found in different yeast genera. Among these, the killers of *S. cerevisiae*, *Zygosaccharomyces bailii*, *Hanseniaspora uvarum*, and *Ustilago maydis* – the latter being the cause of corn smut – are best characterized. Characteristic for all killers is the secretion of protein toxins that are lethal to sensitive strains of different species and genera. Cell killing is usually achieved in a receptor-mediated process, requiring initial toxin binding to components of the outer yeast cell surface (such as β-1,6-D-glucans, α-1,3-mannoproteins, or chitin) and subsequent toxin transfer to a secondary plasma membrane receptor. Depending on the toxin, final lethality can be caused by plasma membrane damage, G1 or S phase cell-cycle arrest, and/or by rapid inhibition of DNA synthesis.

In the yeasts *S. cerevisiae*, *Z. bailii*, and *H. uvarum* as well as in the maize smut fungus *U. maydis*, the killer phenotype is cytoplasmically inherited and caused by an infection with dsRNA viruses of the family *Totiviridae* that are widely distributed among yeast and higher fungi. Since the majority of fungal mycoviruses are noninfectious and symptomless in the corresponding host, they are often classified as cryptic viruses or virus-like particles (VLPs). All known fungal viruses spread vertically by cell–cell mating and/or heterokaryon formation. In *S. cerevisiae*, diploids formed by mating of a killer with a sensitive strain are likewise killers, as are all haploid progeny of subsequent meiosis. In contrast, virus-free strains are usually sensitive nonkillers, while those containing ScV-L-A and a toxin-encoding M-dsRNA are killers (see below). Sensitive strains survive mating with killers, and cytoplasmic mixing of the multiple M-dsRNA copies during zygosis accounts for the inheritance pattern during meiosis. Extracellular spread of virions is generally hampered by the rigid yeast and fungal cell-wall barrier, and fungal viruses have adopted a strategy of transmission via mating and hyphal fusion (which occurs frequently in nature) making an extracellular route of spread dispensable. While some of these viruses can be associated with adverse phenotypic effects on the fungus (like La France disease in *Agaricus bisporus*, plaque formation in *Penicillium*, and hypovirulence in *Cryphonectria*), dsRNA viruses and their associated satellite dsRNAs in *S. cerevisiae*, *U. maydis*, *H. uvarum*, and *Z. bailii* are responsible for a killer phenotype which is based on the secretion of a polypeptide toxin (killer toxin) that is lethal to a variety of sensitive yeast and fungal strains. With the exception of toxin-secreting strains of *Z. bailii*, killer toxin production is usually associated with specific immunity, protecting the corresponding killer yeast against its own viral toxin.

dsRNA Viruses and Killer Phenotype Expression in *S. cerevisiae*

On the basis of killing profiles and the lack of cross-immunity, three major killer types (K1, K2, K28) have so far been identified in *S. cerevisiae*. Each of them produces a specific killer toxin and a self-protecting immunity component. Killer phenotype expression correlates with the presence of two types of dsRNA species stably persisting in the cytoplasm of the infected host: the genomic dsRNA of the helper virus, ScV-L-A, and one of three toxin-coding satellite dsRNAs that have been recognized so far (ScV-M1, ScV-M2, or ScV-M28). The ScV-L-A and M-dsRNAs are separately encapsidated into capsids encoded by ScV-L-A dsRNA and are present in high copy number in the yeast cell cytoplasm. *In vivo*, ScV-L-A does not confer a phenotype nor does it lead to host-cell lysis or slower cell growth. While the killer phenotype can be transmitted to sensitive yeast cell spheroplasts (harboring ScV-L-A) by transfection with an ScV-M preparation (either during mating or cotransformation with a dsDNA plasmid), extracellular transmission occurs rarely, if at all, in nature. The survival strategy adopted by these dsRNA viruses appears to be a balanced host interaction resulting in stable maintenance, little if any growth disadvantage, and vertical transmission. Mechanisms of exiting and entering the host cell through its tough and rigid cell wall are rendered unnecessary by relatively efficient horizontal transmission during the frequent zygosis events in yeast. Acquisition of a toxin-encoding M satellite dsRNA provides positive selection for both this dsRNA and ScV-L-A, since virus-free segregants are killed.

As summarized in **Table 1**, the linear dsRNA genome of ScV-L-A contains two open reading frames (ORFs) on its plus-strand RNA: ORF1 encodes the major capsid protein Gag necessary for encapsidation and viral particle structure, the second gene (ORF2) represents the RNA-dependent RNA polymerase Pol which is *in vivo* expressed as a Gag–Pol fusion protein by a –1 ribosomal frameshift event. In contrast to L-A, each M-dsRNA genome contains a single ORF coding for a preprotoxin (pptox) representing the unprocessed precursor of the mature and secreted toxin that also gives functional immunity. Since each toxin-coding ScV-M dsRNA depends on the coexistance of ScV-L-A for stable maintenance and replication, the killer viruses resemble classical satellites of ScV-L-A. Although the presence of all three M-dsRNAs with different killer specificities in a single cell is excluded at the replicative level of the M genomes, this limitation can be by-passed by introducing cDNA copies of the K2 and K28 pptox genes into a

Table 1 Members of the family *Totiviridae* involved in killer phenotype expression

Totivirus/satellite dsRNA	*Virus host*	*Function of virus*	*dsRNA (kb)*	*Encoded protein(s)*
ScV-L-A	*Saccharomyces cerevisiae*	Helper virus	L_A (4.6)	Gag, major capsid protein; Gag-Pol, RDRP[a]
ScV-M1	*Saccharomyces cerevisiae*	Satellite dsRNA	M_1 (1.6)	K1 preprotoxin
ScV-M2	*Saccharomyces cerevisiae*	Satellite dsRNA	M_2 (1.5)	K2 preprotoxin
ScV-M28	*Saccharomyces cerevisiae*	Satellite dsRNA	M_{28} (1.8)	K28 preprotoxin
UmV-H	*Ustilago maydis*	Helper virus	H (6.1)	Gag, major capsid protein; Gag-Pol, RDRP[a]
UmV-P1	*Ustilago maydis*	Satellite dsRNA	M_{P1}(1.4)	KP1 preprotoxin
UmV-P4	*Ustilago maydis*	Satellite dsRNA	M_{P4} (1.0)	KP4 toxin
UmV-P6	*Ustilago maydis*	Satellite dsRNA	M_{P6} (1.2)	KP6 preprotoxin
HuV-L	*Hanseniaspora uvarum*	Helper virus	L_A (4.6)	Gag, major capsid protein; Gag-Pol, RDRP[a]
HuV-M	*Hanseniaspora uvarum*	Satellite dsRNA	M_{Hu} (1.0)	KT470 toxin precursor
ZbV-L	*Zygosaccharomyces bailii*	Helper virus	L_{Zb} (4.6)	Gag, major capsid protein; Gag-Pol, RDRP[a]
ZbV-M	*Zygosaccharomyces bailii*	Satellite dsRNA	M_{Zb} (2.1)	prepro-zygocin

[a]RDRP, RNA-dependent RNA polymerase.
Adapted from Schmitt MJ and Breinig F (2002) The viral killer system in yeast: From molecular biology to application. *FEMS Microbiology Reviews* 26: 257–276, with permission from Blackwell Publishing.

natural K1 killer, resulting in stable triple-killers producing three different virus toxins at a time and simultaneoulsy expressing triple-toxin immunity.

As typical for many dsRNA viruses, the replication cycles of ScV-L-A and M-dsRNAs depend on the presence of specific packaging signals at or near the 3′ end of the viral single-stranded RNA (ssRNA) transcript. These RNA regions function as viral binding sites (VBSs) and consist of a stem–loop structure whose stem is interrupted by an unpaired protruding A residue (**Figure 1**). For replication of L-A, two sequence elements are essential: an internal replication enhancer (IRE), which is essentially indistinguishable from the VBS, and a small stem–loop structure 5 bp from the 3′-terminus (3′-TRE). While L-A contains just a single VBS element, the toxin-coding transcripts of M1, M28, and M_{Zb} (encoding the *Z. bailii* viral toxin zygocin) each have two such VBS domains. *In vivo*, these VBS elements are *cis*-active sequences that are recognized by Gag–Pol and subsequently packaged into new viral particles. The replicase reaction on the (+)ssRNA template takes place *in viro* (i.e., within the viral capsid) and requires a correct 3′-end sequence and structure. Within the intact and mature virion, conservative transcription of the plus-strand from the dsRNA template requires recognition of its very 5′-terminal sequence by Gag–Pol. In M28 (as in other dsRNAs), the plus-strand initiates with 5′-GAAAA(A); since there are little additional conserved sequences immediately downstream, this terminal recognition element (5′-TRE) may be all that is necessary for transcription initiation (**Figure 1**).

Viral Replication Cycle

Yeast ScV-L-A virions are noninfectious icosahedral particles 39 nm in diameter that show certain similarities to mammalian reoviruses and rotaviruses. Each L-A virus consists of a single copy of the 4.6 kb L-A dsRNA genome which is encapsidated by 60 asymmetric dimers of the 76 kDa coat protein Gag and two copies of the 171 kDa Gag–Pol fusion protein. During conservative replication, the single-stranded plus-strand RNA (L-A(+)ssRNA) is transcribed within the viral particle (*in viro*) and subsequently extruded into the cytoplasm where it serves as (1) messenger RNA for translation into the viral proteins Gag and Gag–Pol and (2) RNA template which is packaged into new viral particles. Once this coat assembly is completed, Gag–Pol functions as replicase, synthesizes a minus-strand, and generates the dsRNA genome of the mature virus. The replication cycle of the toxin-coding M satellite dsRNA resembles that of L-A with the exception that each M virion can accept two copies of the smaller M-dsRNA genome before the ssRNA transcript is extruded into the cytoplasm; in analogy to certain DNA bacteriophages, this phenomenon has been named 'headful packaging'.

Viral Preprotoxin Processing and Toxin Maturation

In totivirus infected killer yeasts, the toxin-encoding M(+)ssRNA transcript is translated on free cytosolic ribosomes into a pptox precursor which is post-translationally imported into the secretory pathway for further processing, maturation, and toxin secretion. Interestingly, intracellular pptox processing in killer strains of either *S. cerevisiae* (K1, K2, K28), *Z. bailii* (zygocin), or *U. maydis* (KP4) is mechanistically conserved, resulting in the secretion of a biologically active monomeric or α/β heterodimeric virus toxin (**Figure 2**). In case of K28 killer strains containing ScV-M28, pptox is processed into a heterodimer whose α- and β-subunit is covalently linked by a single disulfide bond. Since the unprocessed toxin precursor resembles a secretory protein, it contains a hydrophobic

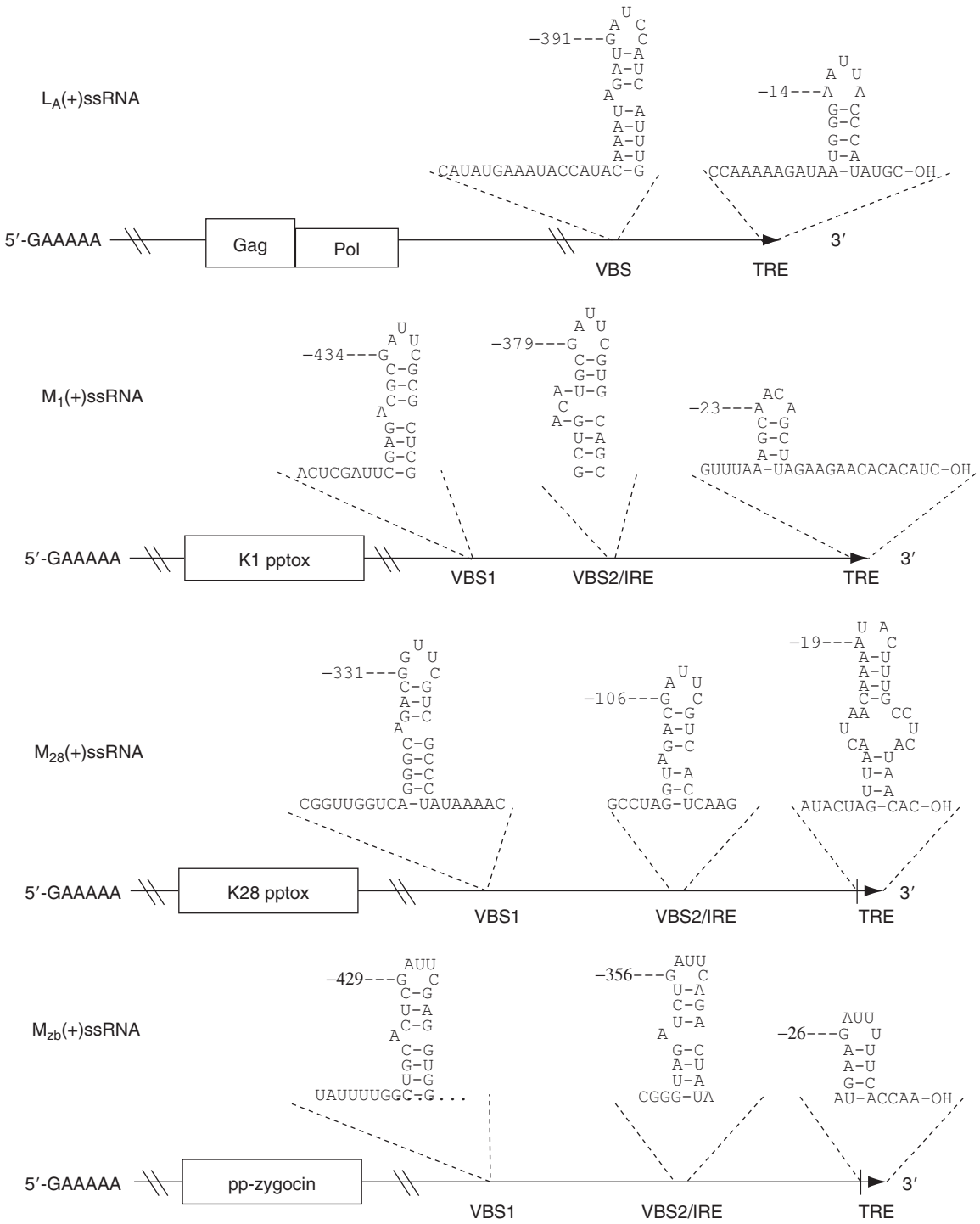


Figure 1 Comparison of the 5′- and 3′-ssRNA sequences in ScV-L-A and in the toxin-coding transcripts of the satellite ScV-M1, ScV-M28, and ZbV-M dsRNAs. Potential *cis*-active sequences within the 3′-termini are indicated (VBS, viral binding site; IRE, internal replication enhancer; TRE, 3′-terminal recognition element). Numbers are shown for distance from the 3′ end of each (+)ssRNA.

signal sequence for pptox import into the ER lumen followed by the toxin subunits α (10.5 kDa) and β (11.0 kDa) which are separated from each other by a potentially N-glycosylated γ-sequence (**Figure 2**). During passage through the yeast secretory pathway the K28 toxin precursor is enzymatically processed to the biologically active heterodimer in a way that is highly homologous to prohormone conversion in mammalian cells. In a late Golgi compartment, the N-glycosylated γ-sequence is removed by the action of the furin-like endopeptidase Kex2p, the

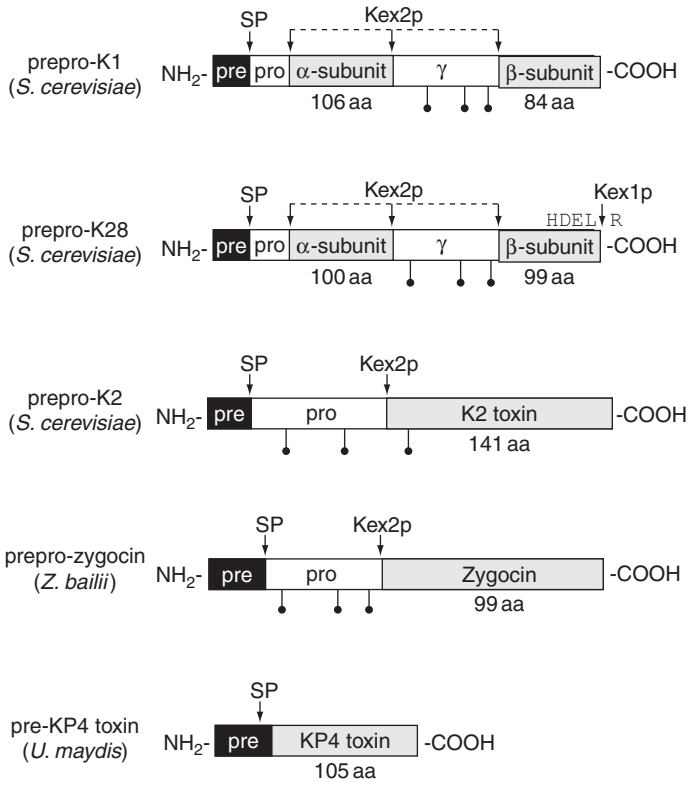


Figure 2 Analogy of toxin precursor processing in totivirus-infected killers of *S. cerevisiae*, *Z. bailii*, and *U. maydis*. Schematic outline of the precursor proteins (preprotoxins) encoding either a heterodimeric α/β toxin (K1, K28) or a monomeric virus toxin (K2, zygocin, KP4). Signal peptidase (SP) cleavage and Kex2p endopeptidase processing sites are indicated. Potential *N*-glycosylation sites are indicated with black circles (aa, amino acids).

C-terminus of β is trimmed by carboxypeptidase Kex1p cleavage, and the biologically active protein is secreted as a 21 kDa α/β heterodimer whose β-C-terminus carries a four-amino-acid epitope (HDEL) that represents a classical ER retention signal. Since this signal is initially masked by a terminal arginine residue (HDEL*R*), ER retention of the toxin precursor is prevented until the protoxin enters a late Golgi compartment where Kex1p cleavage uncovers the HDEL signal of the toxin (**Figure 3**).

Endocytosis and Intracellular Transport of the K28 Virus Toxin

In contrast to most virally encoded yeast killer toxins, which do not enter their host but rather kill a sensitive cell by disrupting plasma membrane function, K28 is taken up by receptor-mediated endocytosis and subsequently targeted to the secretory pathway. Once it has reached an early endosomal compartment, the toxin travels the secretion pathway in reverse and translocates into the cytosol. Responsible and essential for this retrograde transport is a short-amino-acid motif at the carboxyterminus of the K28 β-subunit (HDEL) which functions as ER targeting/retention signal that is normally present at the C-terminus of resident chaperones in the ER lumen such as Kar2p/BiP and Pdi1p, the protein disulfide isomerase. In yeast and higher eukaryotes, H/KDEL-carrying proteins are recognized by a membrane-bound H/KDEL receptor ensuring efficient recycling of H/KDEL-proteins from an early Golgi compartment back to the ER. In case of the K28 virus toxin, this sequence allows retrograde transport from early endosomes (via Golgi) to the ER from where the toxin enters the cytoplasm and transduces its lethal signal into the nucleus. Endocytotic uptake and retrograde transport is a common strategy realized in certain prototypes of bacterial toxins such as *Pseudomonas* exotoxin A, *Escherichia coli* heat-labile toxin (HLT), or even Shiga toxin. All these protein toxins are family members of microbial A/B toxins, which are usually internalized by receptor-mediated endocytosis, followed by reverse secretion via Golgi and ER. Interestingly, many of these toxins contain putative ER retention signals at their C-termini, and H/KDEL-dependent mechanisms have, therefore, been postulated to be of major importance for toxin entry into mammalian cells. In this respect, a major difference between the yeast K28 virus toxin and bacterial A/B toxins is that K28 itself

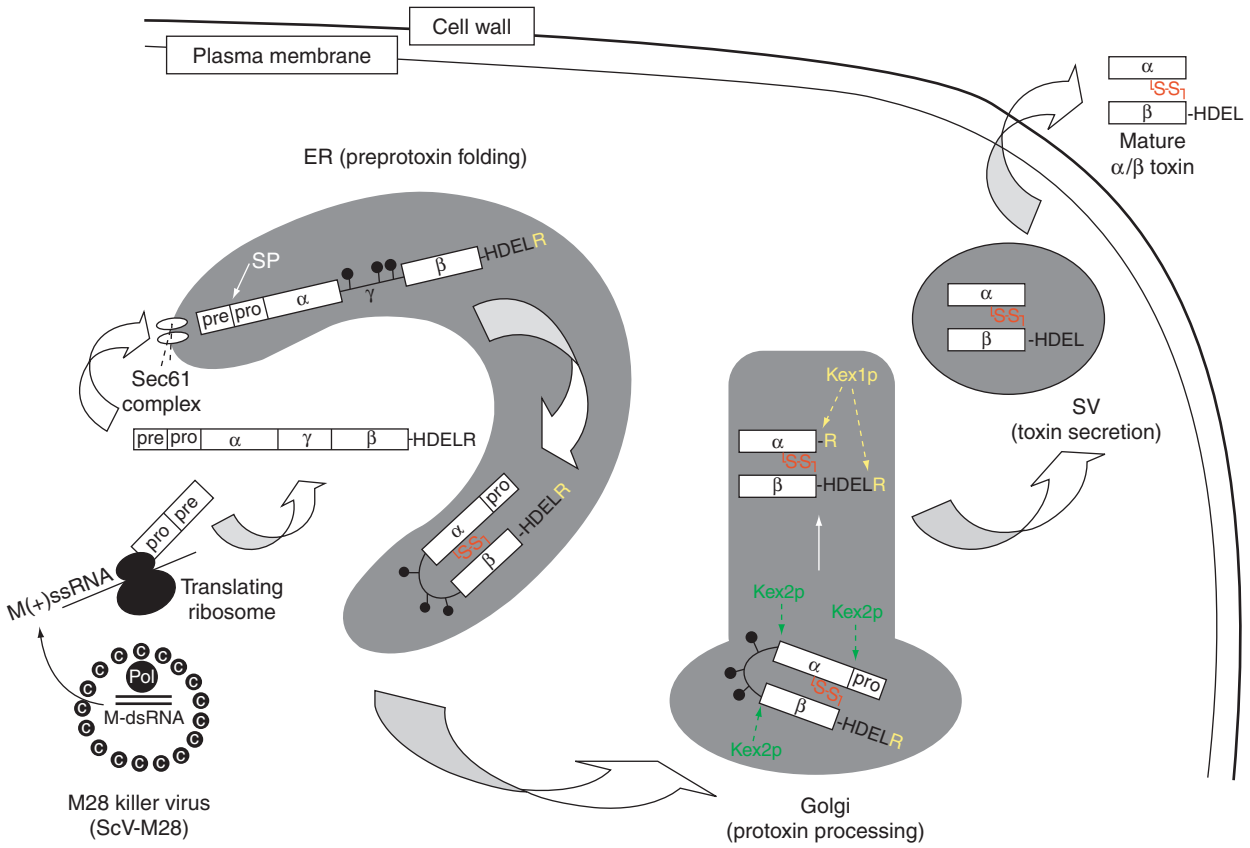


Figure 3 Preprotoxin processing and toxin secretion in an ScV-M28-infected killer yeast. After *in vivo* translation of the preprotoxin coding killer virus transcript, the toxin precursor is post-translationally imported into the endoplasmic reticulum (ER) through the Sec61 complex. Signal peptidase (SP) cleavage in the ER lumen removes the N-terminal signal sequence (pre-region) and protoxin folding is mediated by lumenal ER chaperones. The intervening γ-sequence is N-glycosylated and a single disulfide bond between α and β is generated. In a late Golgi compartment, Kex2p endopeptidase cleaves the pro-region, removes the γ-sequence, and carboxypeptidase Kex1p cleavage trimms the C-termini of both subunits, leading to the secretion of mature α/β toxin whose β-C-terminal HDEL motif is uncovered and thus accessible for interaction with the HDEL receptor of the target cell. SV, secretory vesicle. Adapted from Schmitt MJ and Breinig F (2002) The viral killer system in yeast: From molecular biology to application. *FEMS Microbiology Reviews* 26: 257–276, with permission from Blackwell Publishing.

is produced and secreted by a eukaryotic (yeast) cell, and therefore the C-terminal ER-targeting signal in the toxin precursor is initially masked by a terminal arginine residue which ensures successful pptox passage through the early secretory pathway. Once the toxin has reached a late Golgi compartment, Kex1p cleavage removes the β-C-terminal arginine residue and thereby uncovers the ER targeting signal of the virus toxin. To ensure proper access of the ER targeting signal to the K/HDEL receptor of the sensitive target cell, many A/B toxins (including the yeast K28 virus toxin) contain a unique disulfide bond at or near the C-terminus. Consequently, mutant toxin variants with altered inter- and/or intra-subunit disulfide bonding are nontoxic *in vivo* due to the incapability to reach their intracellular target. Thus, disulfide bond formation in microbial and viral A/B toxins is of major importance to ensure interaction competence of the toxins with the K/HDEL-receptor of the target cell.

ER Exit and Nuclear Entry of the K28 Virus Toxin

During host-cell penetration, K28 retrotranslocates from the ER into the cytosol and dissociates into its subunit components. The β-subunit is subsequently polyubiquitinated and proteasomally degraded while the cytotoxic α-subunit enters the nucleus and causes cell death (**Figure 4**). ER exit of the α/β heterodimeric toxin is mediated by the Sec61 complex, termed translocon, which functions as major transport channel in the ER membrane of yeast and higher eukaryotes. In yeast, each translocon resembles a core heterotrimeric complex consisting of the transmembrane protein Sec61p and the two smaller subunits Sbh1p and Sss1p. Besides being the major channel for co- and post-translational protein import into the ER, Sec61p is also involved in the export and removal of malfolded and/or misassembled proteins from the secretory pathway to initiate their proteasomal

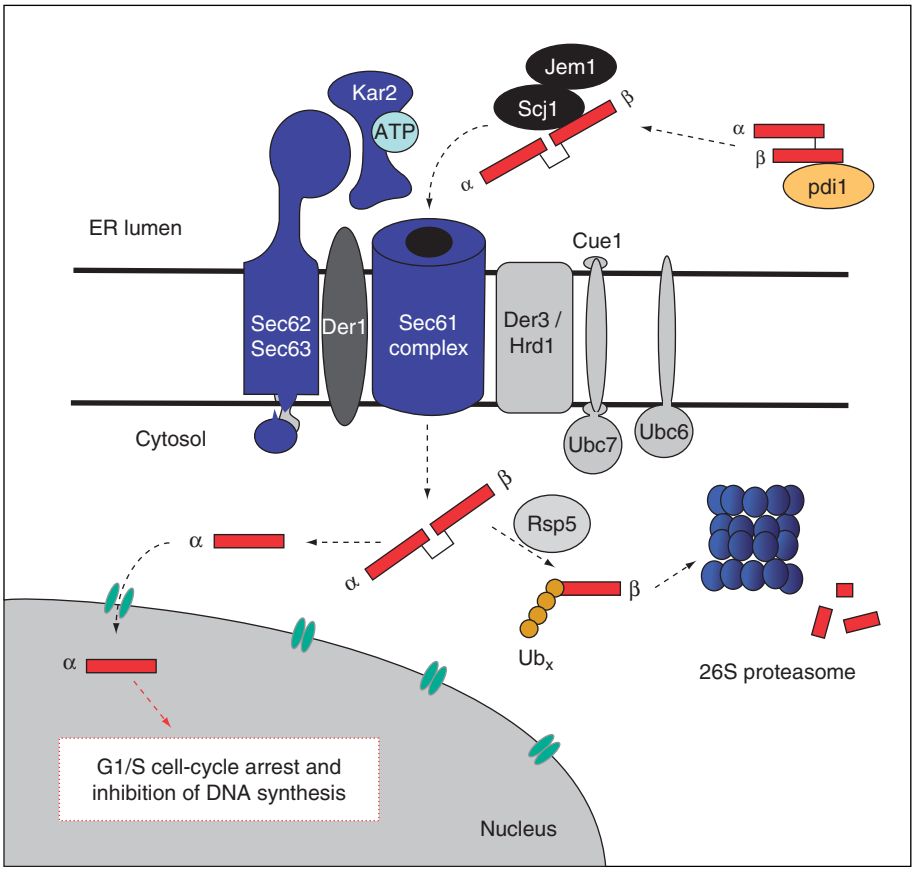


Figure 4 Retrotranslocation of the K28 virus toxin from the ER and its lethal effect in the nucleus. After endocytotic uptake and retrograde transport via Golgi and ER, the toxin is gated through the Sec61p export channel by the help of lumenal ER chaperones such as Pdi1p, Kar2p, Jem1p, and Scj1p. Within the cytosol, β is ubiquitinated and proteasomally degraded while α enters the nucleus and causes cell death. The α-toxin interacts with essential host proteins involved in eukaryotic cell-cycle control and causes cell death through G1/S cell-cycle arrest and inhibition of DNA synthesis. Cellular components of the ER quality control system ERAD (such as Cue1p, Ubc7p, Der3p/Hrd1p, Ubc6p, and Der1p) are not involved in ER-to-cytosol export of the K28 virus toxin (ERAD components are shown in gray color). Reproduced from Leis S, Spindler J, Reiter J, Breinig F, and Schmitt MJ (2005) *Saccharmyces cerevisiae* K28 toxin – A secreted virus toxin of the A/B family of protein toxins. In: Schmitt MJ and Schaffrath R (eds.) *Topics in Current Genetics 11: Microbial Protein Toxins*, pp. 111–132. Berlin, Heidelberg: Springer, with kind permission of Springer Science and Buisness Media.

degradation in the cytosol. In addition to its central function in protein quality control in the ER, Sec61 is also responsible for ER retrotranslocation of certain plant, microbial and viral A/B toxins such as ricin, cholera toxin, *Pseudomonas* exotoxin A, and the yeast K28 virus toxin. In contrast to microbial and plant A/B toxins, however, retrotranslocation of K28 from the ER lumen is independent of ubiquitination and proteasome activity and classical components normally involved in ER-associated protein degradation (ERAD) are not required for ER exit of this virus toxin. In K28 intoxicated cells, toxin translocation competence in the ER strongly depends on the activity of lumenal Hsp70 chaperones (such as Kar2p/BiP and Pdi1p), Hsp40 cochaperones (such as Scj1p and Jem1p), and additionally requires proper maintenance of calcium homeostasis in the ER (**Figure 4**). So far it is not known what cellular component within or near the ER membrane is responsible for toxin exit, but K28 might be a fruitful tool to identify a novel transport pathway for protein transport across the ER membrane.

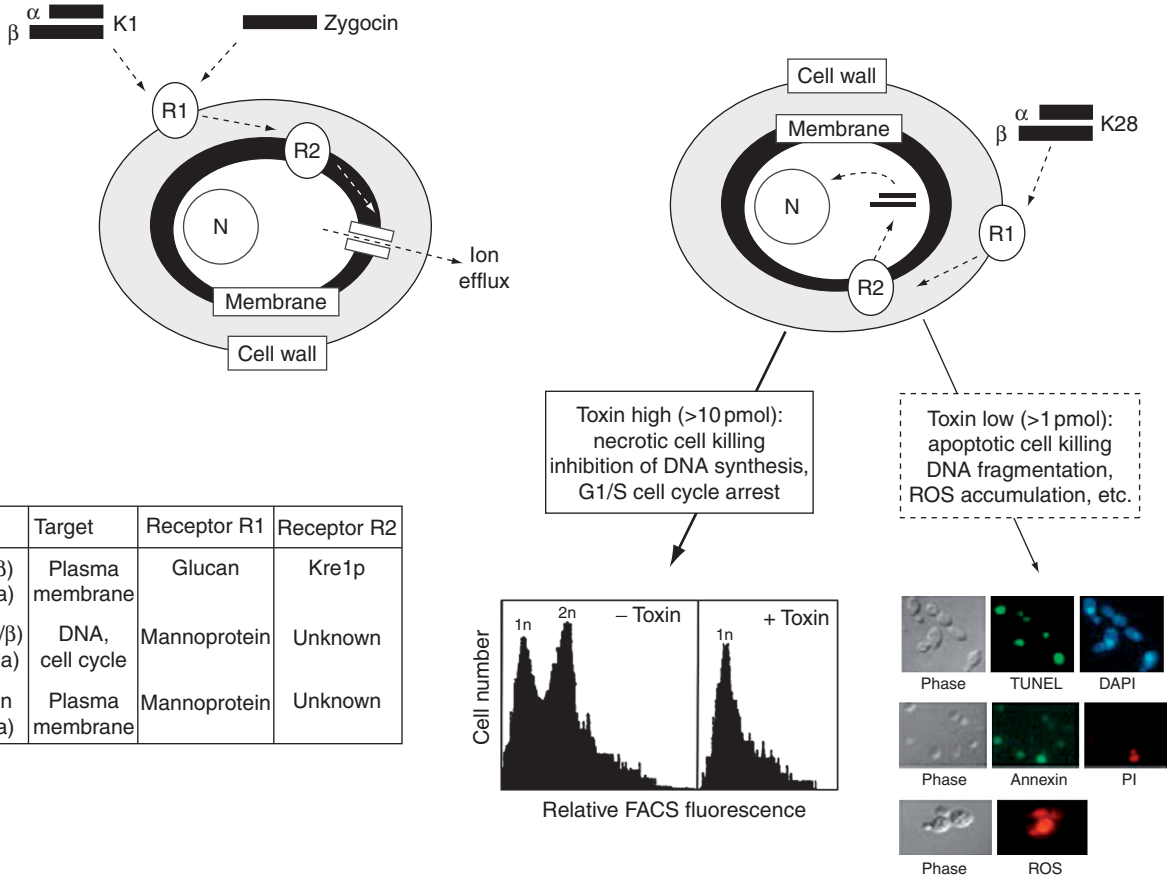
K28 Affects DNA Synthesis, Cell-Cycle Progression, and Induces Apoptosis

Although the cytotoxic α-component of K28 (10.5 kDa) can enter the nucleus by passive diffusion, extension of α by a classical nuclear localization sequence (NLS) significantly enhances its *in vivo* toxicity due to faster and more efficient nuclear import mediated by α/β importins of the host. Within the nucleus, α interacts with host proteins of essential function in eukaryotic cell cycle control and initiation of DNA synthesis. Thus, as the virus toxin targets evolutionary highly conserved proteins with basic and central function, toxin resistance mechanisms based on mutations in essential chromosomal host genes hardly

occur *in vivo*, indicating that the toxin has developed a very efficient strategy to penetrate and kill its target cell. Most interestingly, while high toxin concentrations (10 pmol or higher) cause necrotic cell killing via cell-cycle arrest and inhibition of DNA synthesis, treatment with low doses of viral killer toxins (<1 pmol) results in an apoptotic host-cell response triggered by the accumulation of reactive oxygen species, ROS (**Figure 5**). Since toxin concentration is usually low in the natural environment of a killer yeast, toxin-induced apoptosis is probably an important prerequisite for efficient cell killing. Furthermore, since apoptosis is also important in the pathogenesis of virus infections in mammals, it is not surprising that toxin-encoding yeast killer viruses (as has been shown for ScV-M1 and ScV-M2) can also induce a programmed suicide pathway in noninfected yeast. Although viral killer toxins were shown to be primarily responsible for this phenomenon, yeast killer viruses are not solely responsible for triggering a cell death pathway in yeast.

Lethality of Membrane Damaging Viral Killer Toxins

Yeast viral killer toxins kill sensitive cells in a receptor-mediated fashion by interacting with receptors at the level of the cell wall and the cytoplasmic membrane (**Figure 5**). The initial step involves rapid toxin binding to a primary receptor R1 which is localized within the mannoprotein or β-1,6-glucan fraction of the cell wall. In the second step the toxin translocates to the plasma membrane and interacts with a secondary receptor R2 (**Figure 5**). To date, only the membrane receptor for the K1 virus toxin has been identified: Kre1p, an O-glycosylated cell surface protein initially GPI-anchored to the plasma membrane and involved in β-1,6-glucan biosynthesis and K1 cell wall receptor assembly. Once bound to the plasma membrane, ionophoric virus toxins (such as K1 and zygocin) disrupt cytoplasmic membrane function by forming cation-selective ion channels, while K28 enters the cell and acts in the nucleus: DNA



Toxin	Target	Receptor R1	Receptor R2
K1 (α/β) (19 kDa)	Plasma membrane	Glucan	Kre1p
K28 (α/β) (21 kDa)	DNA, cell cycle	Mannoprotein	Unknown
Zygocin (10 kDa)	Plasma membrane	Mannoprotein	Unknown

Figure 5 Receptor-mediated toxicity of the viral killer toxins K1, zygocin, and K28. Killing of a sensitive yeast is envisaged in a two-step process involving initial toxin binding to receptors within the cell wall (R1) and the cytoplasmic membrane (R2). After interaction with the plasma membrane, ionophoric toxins such as K1 and zygocin disrupt cytoplasmic membrane function, while K28 enters the cell by endocytosis and diffuses into the nucleus to cause cell death (note that the cell surface receptors R1 and R2 are different in all three toxins; see also table inset). At high toxin doses (>10 pmol) sensitive cells arrest in the cell cycle with pre-replicated DNA (1n; left panel), while cells treated with K28 in low concentrations (<1 pmol) respond with apoptosis as shown by typical apoptotic markers such as chromosomal DNA fragmentation (TUNEL-positive cells), accumulation of reactive oxygen species (ROS), and phosphatidylserin exposure at the external surface of the plasma membrane detected by annexin-V staining (right panel).

synthesis is rapidly inhibited and cells arrest at the G1/S boundary of the cell cycle (**Figure 5**).

Ion channel formation in yeast membranes induced by the K1 virus toxin was initially reported using patch-clamping techniques as a result from direct toxin action. However, this observation is inconsistent with the complete resistance seen in immune yeast cell spheroplasts, and so far receptor-independent channels have not been observed, neither in yeast membranes nor in *Xenopus laevis* oocytes. Similar to K1 (and probably K2 as well), zygocin represents a membrane-damaging virus toxin which is produced and secreted by ZbV-M-infected killer strains of the osmotolerant spoilage yeast *Z. bailii*. Zygocin itself is a monomeric, nonglycosylated protein toxin with an unusual broad killing spectrum, being equally active against phytopathogenic as well as human pathogenic yeasts including *Candida albicans*, *C. glabrata*, *C. tropicalis*, and *Sporothrix schenkii*. Since even filamentous fungi such as *Fusarium oxysporum* and *Colletotrichum graminicola* are effectively killed by the toxin, zygocin represents a virus toxin with significant antimycotic potential. Similar to K1 but significantly more efficient, zygocin disrupts plasma membrane integrity and causes rapid cell killing. Its ionophoric mode of action has been reinforced by *in silico* sequence analysis, identifying a stretch of potential α-helical conformation that forms an amphipathic structure characteristic for membrane-disturbing antimicrobial peptides such as alamethicin, melittin, and dermaseptin. In addition, this feature is accompanied by a transmembrane helix at the C-terminus of zygocin which is predicted to favor a membrane permeabilizing potential, not by activating native ion channels but rather by establishing pores by itself after toxin oligomerization. It is therefore assumed that the hydrophobic part in zygocins' amphipathic α-helix is responsible for toxin binding to the target cell. The postulated model of zygocin action resembles that of human α-defensins. In analogy to alamethicin, toxicity of zygocin is probably mediated by incorporation of its transmembrane helix into the plasma membrane, a process solely driven by the natural transmembrane potential of the energized yeast and fungal plasma membrane. Thus, zygocins' mode of action portrays the lethal mechanism of antimicrobial peptides that are produced by virtually all higher eukaryotes. Mechanisms of resistance against antimicrobial peptides are rare and often limited to changes in the composition of the cytoplasmic membrane. In major contrast to mammalian cells, the outer leaflet of yeast and fungal membranes is enriched in negatively charged lipids. Due to the cationic net charge of antimicrobial peptides (including zygocin), an affinity to these lipids facilitates toxin adsorption to the target membrane. Consistent with that, deletion of chromosomal genes whose gene products affect plasma membrane lipid composition (such as *PDR16* and *PDR17*) causes a dramatic decrease in zygocin sensitivity because toxin binding to the plasma membrane is largely prevented in the genetic background of a yeast *pdr16/17* mutant. In contrast to K1, a zygocin-specific membrane receptor is not required for its *in vivo* toxicity as the physicochemical properties of zygocin allow efficient plasma membrane interaction independent of any membrane receptor or docking protein.

Self-Protection in Killer Virus-Infected Yeast – Toxin Immunity

For many decades it was unknown how a killer virus-infected yeast protects itself against its own secreted toxin. In killer yeast, functional immunity is essential for survival since the toxins often target and inhibit central eukaryotic cell functions. This is in major contrast to bacterial toxins such as cholera toxin and Shiga toxins which selectively kill eukaryotes, thus making immunity dispensable in a prokaryotic host. Recently, the mechanism of protecting immunity against the K28 virus toxin has been elucidated. It is now known that immune cells take up external toxin (either produced by itself or by other K28 killers) and translocate it back to the cytosol where the reinternalized α/β toxin rapidly forms a complex with the pptox precursor that has not yet been imported into the ER. Within this complex, the K28 heterodimer is selectively ubiquitinated and proteasomally degraded while the pptox moiety of the complex is in part released to be either imported into the ER (to give active virus toxin) or to complex a newly internalized K28 heterodimer. In this process, the amount of cytosolic ubiquitin is critical for immunity and overexpression of mutant ubiquitin (blocked in polyubiquitin chain formation) results in a significant decrease in toxin secretion and a suicidal phenotype based on nonfunctional immunity. Alternatively, decreasing cytosolic ubiquitin causes an increase in toxin secretion, while immunity is not impaired as sufficient pptox is available for K28 complex formation. This simple and highly efficient mechanism ensures that a toxin-producing killer yeast is fully protected against the lethal action of its own toxin. In contrast to K28 immunity, the precise mechanism(s) of self-protection against the ionophoric virus toxins K1 and zygocin is still obscure and remains largely unknown.

See also: Fungal Viruses; Totiviruses; Ustilago Maydis Viruses; Yeast L-A Virus.

Further Reading

Breinig F, Sendzik T, Eisfeld K, and Schmitt MJ (2006) Dissecting toxin immunity in virus-infected killer yeast uncovers an intrinsic strategy of self-protection. *Proceedings of the National Academy of Sciences, USA* 103: 3810–3815.

Bruenn JA (2002) The double-stranded RNA viruses of *Ustilago maydis* and their killer toxins. In: Tavantzis SM (ed.) *dsRNA Genetic Elements: Concepts and Applications in Agriculture, Forestry, and Medicine*, pp. 109–124. Boca Raton, FL: CRC Press.

Bruenn JA (2005) The *Ustilago maydis* killer toxins. In: Schmitt MJ and Schaffrath R (eds.) *Microbial Protein Toxins*, pp. 157–174. Berlin, Heidelberg: Springer.

El-Sherbeini M and Bostian KA (1987) Viruses in fungi: Infection of yeast with the K1 and K2 killer virus. *Proceedings of the National Academy of Sciences, USA* 84: 4293–4297.

Ivanovska J and Hardwick JM (2005) Viruses activate a genetically conserved cell death pathway in a unicellular organism. *Journal of Cell Biology* 170: 391–399.

Leis S, Spindler J, Reiter J, Breinig F, and Schmitt MJ (2005) *S. cerevisiae* K28 toxin – a secreted virus toxin of the A/B family of protein toxins. In: Schmitt MJ and Schaffrath R (eds.) *Microbial Protein Toxins*, pp. 111–132. Berlin, Heidelberg: Springer.

Reiter J, Herker E, Madeo F, and Schmitt MJ (2005) Viral killer toxins induce caspase-mediated apoptosis in yeast. *Journal of Cell Biology* 168: 353–358.

Schmitt MJ and Breinig F (2002) The viral killer system in yeast: From molecular biology to application. *FEMS Microbiology Reviews* 26: 257–276.

Schmitt MJ and Breinig F (2006) Yeast viral killer toxins: Lethality and self-protection. *Nature Reviews Microbiology* 4: 212–221.

Schmitt MJ and Neuhausen F (1994) Killer toxin-secreting double-stranded RNA mycoviruses in the yeasts *Hanseniaspora uvarum* and *Zygosaccharomyces bailii*. *Journal of Virology* 68: 1765–1772.

Weiler F and Schmitt MJ (2005) Zygocin – a monomeric protein toxin secreted by virus-infected *Zygosaccharomyces bailii*. In: Schmitt MJ and Schaffrath R (eds.) *Microbial Protein Toxins*, pp. 175–187. Berlin Heidelberg: Springer.

Wickner RB (1996) Double-stranded RNA viruses of *Saccharomyces cerevisiae*. *Microbiology Reviews* 60: 250–265.

Viral Membranes

J Lenard, University of Medicine and Dentistry of New Jersey (UMDNJ), Piscataway, NJ, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Viruses of many kinds possess lipids as integral components of their structure. Lipid-containing, or enveloped, viruses include: *Baculo-*, *Bunya-*, *Corona-*, *Filo-*, *Herpes-*, *Lenti-*, *Orthomyxo-*, *Paramyxo-*, *Pox-*, *Retro-*, *Rhabdo-*, and *Togaviridae*. Despite the great diversity of these viruses in regard to structure, replicative strategy, host range and pathogenicity, the function of the lipids in each case is the same – to form a membrane surrounding the encapsidated viral RNA or DNA genome. The lipids of the viral envelope form a continuous bilayer that functions as a permeability barrier protecting the viral nucleocapsid from the external milieu. Embedded in this bilayer are numerous copies of a limited number of virally encoded transmembrane proteins (often just one or two) that are required for virus entry into a potential host cell. These proteins mediate two essential functions: attachment of the virion to the cell surface; and fusion of the viral envelope with a cell membrane, resulting in accession of the viral nucleocapsid containing the genome to the cellular cytoplasm.

The membrane is acquired during viral assembly within an infected cell. This generally occurs by the budding of the previously assembled viral nucleocapsid through a cell membrane, incorporating host cell lipids and viral membrane proteins, but excluding virtually all cell membrane proteins. The particular cell membrane through which viral budding occurs is characteristic for each virus. Many viruses bud through the plasma membrane, but bunyaviruses bud through the Golgi apparatus, coronaviruses chiefly through the endoplasmic reticulum, and herpesviruses through the nuclear membrane. Poxviruses, which are among the largest and most complex animal viruses, are unique in acquiring two discrete membranes through a series of interactions with different cellular organelles, in a process that is not well understood.

Viral membranes have been extensively studied for many years, and for many reasons. The budded viral envelopes themselves represent isolated, readily purifiable subdomains of discrete cellular membrane bilayers. The envelope proteins that mediate entry of viruses into infectible cells are regarded as prototypes of membrane fusion machines. The initial binding of virions to potential host cells is mediated by prototypical ligand–receptor interactions in which viral proteins (ligands) bind to cell surface proteins (receptors). These properties of viral envelopes represent potential targets for antiviral therapy; they also provide models for important cellular phenomena.

Viral Bilayers

For many non-pathogenic viruses that can be readily purified in sufficient quantities in the laboratory, the bilayer arrangement of lipids in the viral envelope has been directly demonstrated using physical methods. It is assumed that the lipids in all viral envelopes are similarly arranged in a bilayer, based on those observations, and on the fact that the host cell membranes from which the viruses bud all contain bilayers, and also because it is the only physically reasonable arrangement of lipids that could supply the required protection from environmental stresses. Intact virions are impermeant to proteases and other enzymes.

Indeed, virions can swell and shrink in response to changes in osmolarity, showing that viral envelopes are impermeant to small molecules and ions as well as large proteins, and thus must consist of intact bilayers that completely surround the encapsidated viral genome.

The lipid composition of various viruses grown under different conditions, and in different cell types, has been studied in detail. These have shown that wide variations in lipid composition are tolerated in many viruses; they have provided no evidence that any substantial fraction of envelope lipids is bound to viral envelope proteins specifically, or exists in a nonbilayer conformation.

Recent studies have shown that many viruses bud from specialized regions of the plasma membrane known as 'rafts'. Rafts are regions of the plasma membrane characterized by high concentrations of sphingolipids (sphingomyelin and sphingoglycolipids) and cholesterol. These components participate in the formation of separate, partially miscible phases, distinguishable from the more fluid phase(s) which are relatively enriched in unsaturated phospholipids. Raft and non-raft phases co-exist as contiguous bilayers, but diffusion is relatively restricted within raft phases, and exchange of molecules between phases occurs more slowly than diffusion within a single phase. Although the existence of local heterogeneity in lipid and protein structure has been demonstrated in cell membranes using a variety of physical and detergent extraction techniques, a precise definition of rafts has not been achieved. There is little agreement regarding their size, or whether they are nucleated by lipids or by proteins. Despite these uncertainties, certain membrane constituents are clearly concentrated in specialized regions rich in sphingolipids and cholesterol, and these have been identified as the sites of important cellular signaling and transport functions, and of assembly and budding for many enveloped viruses. Detailed lipid analyses of purified budded virions have confirmed their origin in rafts, and have in turn helped to define the lipid composition of virus-associated rafts.

Viral Membrane Proteins

The proteins of viral membranes, like those of other membranes, may be classified as either integral or peripheral. Integral proteins are those that cross the membrane bilayer at least once, and thus cannot be solubilized without disrupting the bilayer, that is, without using detergents. Peripheral proteins are also membrane associated, but they do not cross the membrane and they can be removed from it by treatment with aqueous salts, high pH or chaotropic agents, which leave intact the hydrophobic interactions that stabilize the bilayer.

Most integral membrane proteins of enveloped viruses span the bilayer only once, although exceptions exist. Each transmembrane (anchoring) domain is a sequence of 18–27 predominantly hydrophobic amino acid residues. Because transmembrane sequences are inherently insoluble in water, integral membrane proteins require detergents for extraction from the bilayer and solubilization. When detergents and lipids are both removed from purified viral proteins, they tend to aggregate into rosettes, forming a kind of protein micelle, with the transmembrane sequences clustered together at their centers in order to maximize hydrophobic interactions and minimize contact with water. Purified viral membrane proteins can be reinserted into lipid bilayers of defined composition by mixing the detergent-solubilized protein with lipids, then removing the detergent by dialysis. These reconstituted viral membranes ('virosomes') often possess the native receptor binding and fusion activities.

The functions of the major integral viral membrane proteins are: first, to attach the virus to the uninfected host cell; and second, to effect penetration of the genome into the host cell cytoplasm through membrane fusion of the viral envelope with a host cell membrane. As much as 90% of each viral receptor binding/fusion protein is external to the viral membrane and thus accessible to removal and/or degradation by added proteases. Viral membrane proteins are often morphologically identifiable in electron micrographs as 'spikes' on the outer surface of membrane particles. Under favorable conditions, nearly the entire external domain may be rendered soluble and recovered intact and correctly folded after proteolytic removal from the virion, facilitating crystallization and structural analysis. These domains possess oligosaccharide side chains identical to those of cellular integral proteins in structure and attachment sites, and often possess disulfide bonds as well, reflecting the viral proteins' normal procession through the cell's endoplasmic reticulum–Golgi system (**Figure 3**).

While these proteins constitute the major fraction (>95%) of viral integral membrane proteins, there is an additional class of small integral proteins that oligomerize within the bilayer to form channels that facilitate the transport of ions or small molecules. These proteins have been called 'viroporins'. They include the M2 protein of influenza, the 6K protein of alphaviruses, and Vpu of HIV-1. They are thought to function in various ways to facilitate the assembly and release of new viral particles from the infected cell.

Peripheral membrane proteins are attached to the viral membrane by a combination of hydrophobic and electrostatic interactions. They may penetrate the bilayer to some extent, but they do not cross it as integral proteins do. Viral peripheral membrane proteins include M1 of influenza, M of paramyxoviruses, and MA of retroviruses. All enveloped viruses except the togaviruses encode an M-like peripheral protein that functions to bring together the envelope and nucleocapsid components during viral assembly.

Cellular Virus Receptors: Virus Membranes as Ligands

The first step in infection, attachment of the virus to the outer surface of the host cell, is performed by specific membrane proteins of enveloped viruses. One or more unique cellular 'receptors' are recognized by each species or strain of virus. The presence of a specific cellular receptor is often the major factor determining the susceptibility of a particular species to infection; it also determines the infectibility of different tissues or cells within infected individuals.

Different viruses may bind to any of a large number of different cell surface proteins, carbohydrates, or lipids. Binding serves several purposes. Most generally, it attaches the virus to the uninfected cell, maintaining proximity, and increasing effective viral concentration on the cell surface. More specifically, interaction of viral spikes with specific cell surface proteins may initiate conformational changes that activate the viral proteins for fusion. Binding to certain cell surface proteins may also promote endocytosis of the virus, by any of several cellular pathways. Endocytosis introduces the viral envelope into the lower pH of the endosome, which is required for activation of some viral fusion proteins. Activation of certain cell surface receptors by virus binding may also initiate specific signaling cascades within the cell, which may be useful to the virus during subsequent steps of infection.

Each enveloped virus exhibits unique binding specificities of its membrane proteins with particular cell surface features, resulting in a unique combination of these effects. For example, the HIV-1 recognition protein gp120 exhibits a near total specificity for binding to the CD4 receptor on immune cells. Further activation of the virus' fusion protein gp40 occurs by interaction with a co-receptor, either the chemokine receptor CCR5 or CXCR4. In contrast, orthomyxo- and many paramyxoviruses have much broader specificity. Their recognition proteins (HA or HN, respectively) bind to sialic acid residues attached to various cell surface proteins or lipids. Different strains show preference for sialic acid in different covalent linkages. Rhabdoviruses such as vesicular stomatitis or rabies virus are still less specific, binding indiscriminately to negative charge clusters, whether created by lipids, proteins, oligosaccharides, or surface-bound polyanions. This nonspecific binding property helps to account for the broad host range of these viruses, although some have also been reported to bind specifically to acetylcholine receptors, which may explain their neurotropism.

Viral Fusion

Fusion of the viral envelope with a cell membrane is facilitated by integral viral membrane proteins. The best studied viruses (HIV-1, orthomyxo-, paramyxo-, retro-, toga-, and rhabdoviruses) each possess a single fusogenic glycoprotein, but herpes- and poxviruses may possess several that work together. Virosomes consisting only of a purified viral fusion protein reconstituted into a lipid bilayer vesicle fuse readily with protein-free lipid bilayers, suggesting that the fusion proteins can act on host cell lipids and do not require participation by host cell proteins.

Because of their ready availability and ease of purification, viral fusion proteins have served as prototypes for understanding biological fusion reactions. Several general principles have emerged, which have been found to apply to at least one major class of cellular fusion reactions (those mediated by proteins called SNAREs) as well as all characterized viral fusions.

First, both viral and cellular fusion proteins act directly on the lipid bilayer to facilitate rearrangements identical to those that occur during protein-free lipid bilayer fusion. Fusion is thought to occur through a series of steps, constituting the so-called 'stalk–pore' pathway (**Figure 1**). The two closely apposed bilayers (**Figure 1**(**a**)) dimple

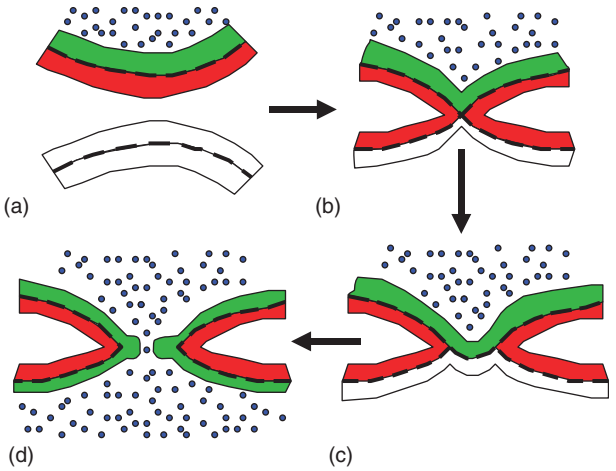


Figure 1 The stalk–pore mechanism of membrane bilayer fusion. (a) Initial pre-fusion state. The two leaflets of the cell membrane bilayer are colored green and red. The cytoplasm is indicated by blue dots. The viral membrane is colorless. (b) The stalk structure. In this state, continuity is established between the outer leaflets of the two membrane bilayers, allowing lipid mixing. (c) The fusion diaphragm constitutes a single bilayer separating the two aqueous compartments, comprising the inner leaflets of the cellular and viral membrane bilayers. The outer leaflets have already fused, and their lipids have mixed. (d) The fusion pore arises from rearrangement of the limited-area fusion diaphragm, perhaps facilitated by fusion proteins. Expansion of the fusion pore allows complete mixing of aqueous compartments and completes fusion. Fusion can occur even between protein-free lipid bilayers under certain conditions, but fusion proteins increase efficiency, probably by acting at each step in this pathway. Reproduced from Chernomordik LVand Kozlov MM (2005) Membrane hemifusion: Crossing a charm in two leaps. *Cell* 123: 375–382.

towards each other to form the 'stalk' (**Figure 1(b)**). This thins to form a 'hemifusion diaphragm' (**Figure 1(c)**), which now separates the two aqueous compartments by a single membrane bilayer in place of the two that separated them in the pre-fusion state. The hemifusion bilayer consists of the inner leaflets of the two original bilayers, the outer leaflets having already fused with consequent mixing of their lipid components. The hemifusion diaphragm can then rearrange to form a 'fusion pore' (**Figure 1(d)**), which must stabilize and widen to allow aqueous mixing, and thus complete the fusion reaction.

Precisely how viral membrane proteins promote fusion via the stalk–pore mechanism remains a subject of active research. Several properties of viral fusion proteins are known to be essential for activity, however. All viral and cellular fusion proteins are oligomeric, usually trimeric for virus fusion proteins. In most cases, several (probably 5–7) trimers must act cooperatively in order to complete the fusion reaction. One attractive idea is that the several fusion protein trimers encircle a limited area of bilayer, into which the fusion proteins can then transfer the energy released by their ensuing conformational transitions (**Figure 2**) in order to effect the lipid rearrangements required for fusion. Viral fusion proteins might potentiate any or all of the fusion steps: initial stalk formation, hemifusion diaphragm formation from the stalk, fusion pore formation from the hemifusion diaphragm, expansion of the initial fusion pore to complete fusion.

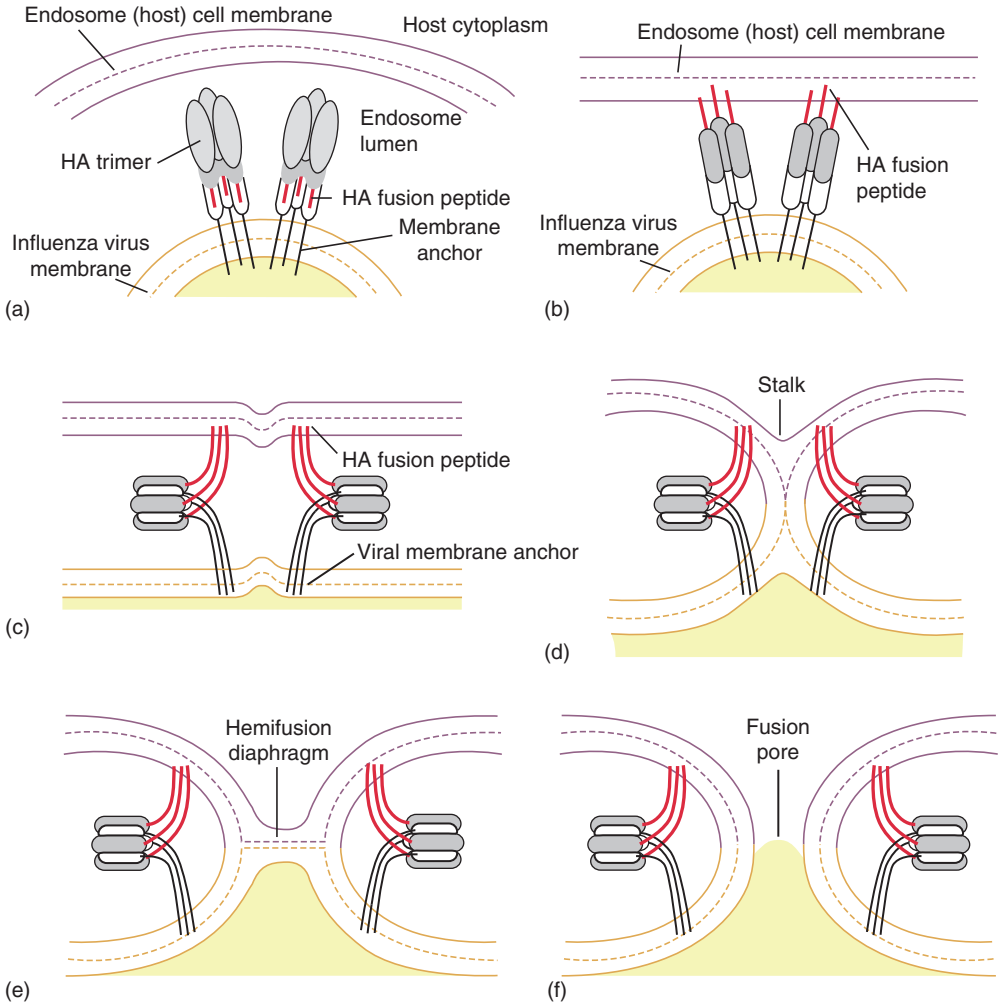


Figure 2 Proposed stalk–pore mechanism for membrane fusion mediated by a class I virus fusion protein, influenza hemagglutinin, HA. (a) The influenza virus has been internalized into the host cell endosome by receptor-mediated endocytosis (not shown). (b) Acidification of the endosome causes a conformational change in HA, which causes the fusion peptide (red) to become exposed; it subsequently inserts into the endosomal membrane. (c) Further refolding and clustering of HA trimers leads to bending of the two membranes toward each other. The resulting stalk (d) rearranges into the hemifusion diaphragm (e), where the virus interior is separated from the cytoplasm by a single bilayer, composed of the internal leaflets of the original membranes. This eventually rearranges, resulting in the formation of the fusion pore (f), which initially flickers and then dilates to complete the fusion reaction (not shown). Intra-leaflet lipid mixing, illustrated in **Figure 1**, is not shown. From Cross KJ, Burleigh LM, and Steinhauer DA (2001) Mechanism of cell entry by influenza virus. *Expert Reviews in Molecular Medicine* 6: 1–18, Cambridge University Press.

Within these general principles two distinct classes of viral fusion proteins have been recognized, possessing radically different architecture. Type I viruses include orthomyxo-, paramyxo-, retro-, and coronaviruses. Type II viruses include toga- and flaviviruses. Despite their different structures, these two classes of proteins facilitate the same lipid modifications during fusion.

All virus fusion proteins must remain inactive during biosynthesis and assembly so as to prevent premature, indiscriminate, counterproductive fusion within the infected cell. Both type I and type II fusion proteins are activated by a two-step process potentiated by interactions with a host cell. The inactive type I precursor protein is first cleaved at a specific site by limited proteolysis during assembly, generating a metastable, active form (**Figure 2**(**a**)). This then undergoes a conformational change mediated either by interaction with a specific cellular co-receptor (HIV-1), or by interaction with viral recognition proteins (paramyxoviruses) or by the low pH inside an endosome (orthomyxoviruses). Type II viral fusion proteins acquire their active conformation by an incompletely understood rearrangement of viral envelope proteins to form fusion protein trimers followed by interactions with specific lipids, notably cholesterol.

Activated fusion proteins of either type possess the following three structural features, which are required for complete fusion (**Figure 2**):

1. *Transmembrane domain.* This is the helical hydrophobic sequence (around 20 residues) that defines the fusion protein as an integral protein, and fixes it irreversibly in the viral bilayer. The transmembrane domain is inserted into the membrane bilayer during synthesis on membrane-bound ribosomes and appears not to rearrange during subsequent processing, activation or fusion. Type I and type II fusion proteins possess similar transmembrane domains. Not all transmembrane domains can participate in fusion reactions; a certain amount of conformational flexibility is required.

The transmembrane domain is required to complete the fusion reaction. Constructs in which the external, fusogenic domain of the influenza HA protein was attached only to the outer leaflet of the viral bilayer by a covalent bond with a lipid were capable of inducing hemifusion only, but were unable to complete the reaction.

2. *Fusion peptide.* This is a second relatively hydrophobic sequence that inserts into the cell membrane bilayer, and thus serves to bind the virus and cell membranes together. Exposure of the fusion peptide, enabling it to penetrate the target cell membrane, is an essential aspect of fusion protein activation, and requires a conformational change from a precursor form (**Figure 2**(**b**)). In type I proteins, this results from proteolytic activation; the influenza fusion peptides, for example, comprise the newly created N termini. Type II fusion peptides are located in a protruding loop of the protein structure, which is exposed by a poorly understood interaction with lipids, notably cholesterol. As with the transmembrane domain, a certain amount of conformational flexibility is required in the fusion peptide.

3. *A rigid, oligomeric rod-like structure connecting the fusion peptide with the transmembrane domain.* This consists of a helical coiled-coil in type I proteins (**Figure 2**(**b**)), and an arrangement of β-sheet domains in type II proteins. Once the fusion peptide has inserted into the target membrane, fusion is completed by the rearrangement of this metastable structure to its lowest free energy form (**Figures 2**(**d**)–**2**(**e**)). In order to assume this form, the rigid oligomer folds back upon itself, forming a 'hairpin' (**Figures 2**(**d**)–**2**(**e**)), thus dragging the cell membrane, tethered by the fusion peptide, toward the viral membrane, tethered by the transmembrane domain. The free energy released by this rearrangement is transferred to the lipid bilayers, providing the energy necessary to complete the fusion reaction. In the final fused product, the fusion peptide and the transmembrane domain are adjacent to each other in the same membrane, held in proximity by the fully stable, rigid hairpin (**Figure 2**(**f**)).

Membrane Synthesis and Viral Assembly

Viruses generally use the housekeeping mechanisms already operating in the infected cell in order to make maximal use of their limited genomes. Hence, viral membrane protein synthesis is carried out on host cell membrane-bound ribosomes, which inserts them into the endoplasmic reticulum membrane in the correct orientation (**Figure 3**). There they are glycosylated by the host cell machinery and assembled into appropriate oligomers, as directed by their own primary amino acid sequence. Most viral glycoproteins are then passed on to the Golgi by cellular mechanisms, where they are further glycosylated by host cell enzymes. For this reason, the envelope proteins of vesicular stomatitis virus (VSV), influenza, and a few other enveloped viruses have provided valuable tools to study the glycosylation and transport of membrane proteins through the cellular endoplasmic reticulum–Golgi–plasma membrane system. Because host cell protein synthesis is often inhibited by infection with these viruses (by a variety of cytopathic mechanisms), large amounts of a single viral membrane protein are produced and correctly processed in infected cells, without competition by cellular proteins.

Likewise, viral proteins are targeted to specific cellular locations by cellular processes. The viral proteins display the same amino acid sequence 'addresses' as host cell proteins, which are recognized by the host cell glycosylation and transport machinery. For example, the single VSV glycoprotein, named G, is glycosylated in the endoplasmic

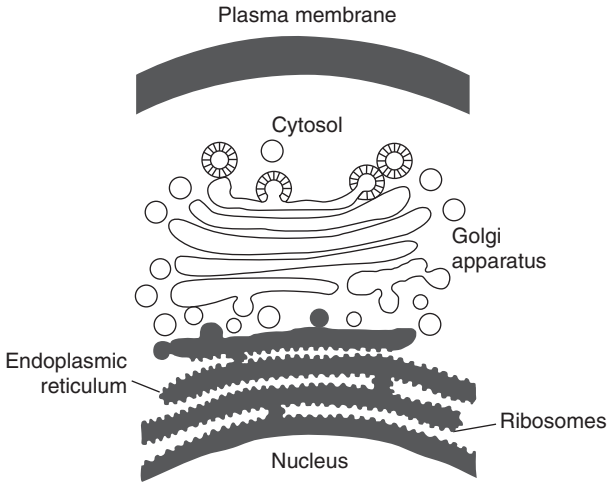


Figure 3 The endoplasmic reticulum–Golgi–plasma membrane system of a cell. All viral and cellular integral membrane proteins are synthesized by ribosomes bound to the endoplasmic reticulum membrane. Proteins destined for the plasma membrane are transported first to the proximal region of the Golgi (the *cis* face), then sequentially through the Golgi cisternae, to the *trans* face and out to the plasma membrane. In polarized cells, targeting to the apical or basolateral surface of the cell occurs from the *trans* face of the Golgi. Assembly and budding of different enveloped viruses occurs at characteristic points within this membrane system.

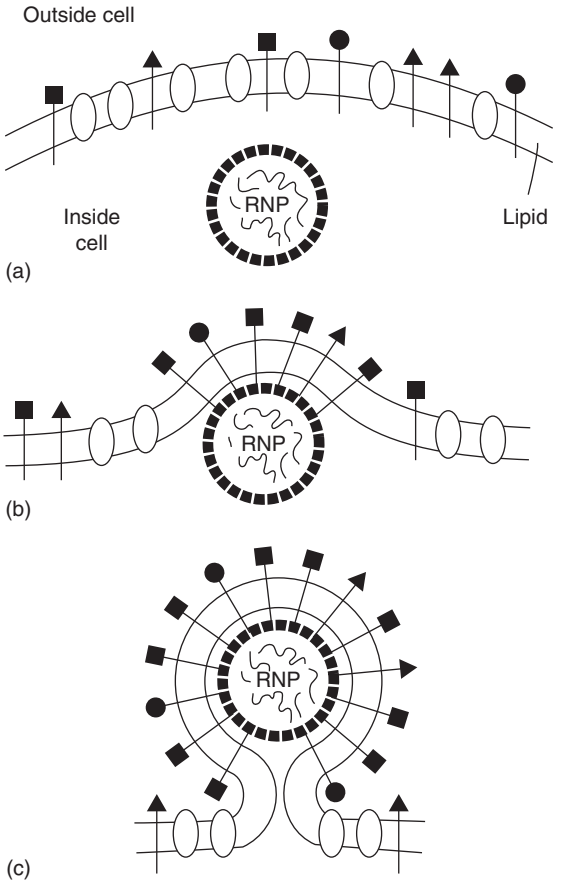


Figure 4 One kind of virus budding. Viral glycoproteins, inserted into the cellular membrane at the endoplasmic reticulum and processed through the Golgi to the plasma membrane (see **Figure 3**), associate with the assembled viral nucleocapsid. The direct association pictured here is characteristic of togaviruses. For other viruses, possessing helical nucleocapsids, the association is mediated by a peripheral membrane protein. Cellular membrane proteins are excluded from the envelope of the mature virion. This may occur during assembly, as pictures, or by prior formation of a viral membrane patch (or raft), before the nucleocapsid arrives at the membrane.

reticulum, the oligosaccharide is modified in the Golgi, and the mature protein is targeted to the basolateral plasma membrane of polarized cells after entry into the late Golgi. The influenza HA protein, on the other hand, is glycosylated and delivered to the apical plasma membrane of the same polarized cells after passage through the intracellular membrane system. The retention of coronavirus glycoproteins by the endoplasmic reticulum, and of bunyavirus glycoproteins by the Golgi, reflects the operation of the same cellular mechanisms that retain resident cellular proteins in these organelles. The localization of viral membrane proteins in turn, determines the location of viral assembly and budding.

The budding process consists of the wrapping of a viral glycoprotein-enriched piece of membrane around the previously assembled nucleocapsid, which contains the viral genome (**Figure 4**). Remarkably, the completed viral envelope contains viral proteins and host cell lipids, with host cell membrane proteins being almost completely excluded. There is much less discrimination, however, between different viral proteins than between viral and cellular proteins, since viral envelope proteins of one kind can assemble with nucleocapsids of another, resulting in the formation of pseudotype virions. Pseudotypes have proven useful in redirecting specific viral genomes to alternate host cells since membrane attachment proteins are major determinants of host cell specificity (see above). It has been suggested that the discrimination between viral and cellular membrane proteins may arise from the exclusion of cellular proteins from virus-associated raft-like lipid phases.

Different viruses have been described, that bud at every stage in the endoplasmic reticulum–Golgi–plasma membrane pathway (**Figure 3**). While paramyxo-, orthomyxo-, rhabdo-, and togaviruses (and many others) generally bud from the plasma membrane, they have also been shown to bud intracellularly under certain conditions. Some retroviruses assemble at the plasma membrane, while others do not; this has provided a classical basis for distinguishing between different types of retroviruses. Other viruses normally bud intracellularly, from the endoplasmic reticulum or Golgi apparatus, for example, coronaviruses and bunyaviruses respectively, but these have also

been observed to bud further down the pathway. In these cases, the nucleocapsid assembles on the cytoplasmic face of the membrane, and then buds into the intracellular organelle. The newly formed virion may then be secreted out of the cell through the normal secretory pathway, although this does not always occur efficiently.

In all enveloped viruses except togaviruses, budding is mediated by a peripheral membrane protein, usually called M or MA, which links the glycoprotein-containing patch of lipids with the viral nucleocapsid, containing the viral genome. The M proteins interact specifically with nucleocapsids of their own viral species, but they do not always interact specifically with the corresponding viral glycoproteins. Instead, they may concentrate on the cytoplasmic side of the raft-like lipid phases that accumulate various viral glycoproteins. This could provide the structural basis for the formation of pseudotype virions, and might explain why many viruses contain widely varying ratios of glycoproteins to M or nucleocapsid proteins.

In contrast, togaviruses, which lack any M protein, possess an icosahedral nucleocapsid, which interacts directly with the cytoplasmic domain of the viral membrane protein. Completed virions contain an equal number of nucleocapsid and membrane protein molecules. Both are in a similar geometric arrangement, mediated by specific protein–protein interactions between them.

As described above, the lipids of the viral membrane are taken from the host cell membrane during budding. No new lipids are specifically synthesized in response to viral infection. Alterations in cellular lipid metabolism have been reported to result from some viral infections in cultured cells, but these are probably secondary to other cytopathic effects; there is no indication that they play an important role in the progress of infection.

See also: Baculoviruses: Molecular Biology of Nucleopolyhedroviruses; Bunyaviruses: General Features; Coronaviruses: General Features; Filoviruses; Herpesviruses: General Features; Orthomyxoviruses: Molecular Biology; Reticuloendotheliosis Viruses; Togaviruses: General Features.

Further Reading

Alberts B, Johnson A, Lewis J, Raff M, Roberts K, and Walter P (2002) *Molecular Biology of the Cell,* 4th edn., chs. 10–13. New York: Garland Science (Taylor and Francis Group).

Briggs JAG, Wilk T, and Fuller SD (2003) Do lipid rafts mediate virus assembly and pseudotyping? *Journal of General Virology* 84: 757–768.

Chernomordik LV and Kozlov MM (2005) Membrane hemifusion: Crossing a chasm in two leaps. *Cell* 123: 375–382.

Cross KJ, Burleigh LM, and Steinhauer DA (2001) Mechanism of cell entry by influenza virus. *Expert Reviews in Molecular Medicine* 6: 1–18, Cambridge University Press.

Gonzalez ME and Carrasco L (2003) Viroporins. *FEBS Letters* 552: 28–34.

Harrison SC (2006) Principles of virus structure. In: Knipe DM, Howley PM, Griffin DE, *et al.* (eds.) *Fields Virology,* 5th edn, pp. 59–98. Philadelphia: Lippincott Williams and Wilkins.

Jardetzky TS and Lamb RA (2004) A class act. *Nature* 427: 307–308.

Kielian M and Rey FA (2006) Virus membrane fusion proteins: More than one way to make a hairpin. *Nature Reviews Microbiology* 4: 67–76.

Simons K and Vaz WLC (2004) Model systems, lipid rafts and cell membranes. *Annual Review of Biophysics and Biomolecular Structure* 33: 269–295.

Smith AE and Helenius A (2004) How viruses enter animal cells. *Science* 304: 237–242.

Viral Pathogenesis

N Nathanson, University of Pennsylvania, Philadelphia, PA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Viral pathogenesis deals with the interaction between a virus and its host. Included within the scope of pathogenesis are the stepwise progression of infection from virus entry through dissemination to shedding, the defensive responses of the host, and the mechanisms of virus clearance or persistence. Pathogenesis also encompasses the disease processes that result from infection, variations in viral pathogenicity, and the genetic basis of host resistance to infection or disease. A subject this broad cannot be treated in a single entry, and this article focuses on the dissemination of viruses and their pathogenicity.

Sequential Steps in Viral Infection

One of the cardinal differences between viral infection of a simple cell culture and infection of an animal host is the structural complexity of the multicellular organism. The virus must overcome a number of barriers to accomplish the stepwise infection of the host, beginning with entry,

followed by dissemination, localization in a few target tissues, shedding, and transmission to other hosts.

Entry

Some viruses (such as papillomaviruses, poxviruses, and herpes simplex viruses) will replicate in cells of the skin or mucous membranes. Following infection of skin, viruses may spread further by passage of intact virions or virus-infected macrophages and dendritic cells to the regional lymph nodes. A considerable number of viruses cause sexually transmitted infection, which is usually acquired by either by penetration through breaks in the skin or by direct invasion of the superficial epithelium of the mucous membranes. Contaminated blood, semen, or secretions may introduce hepatitis B virus directly into the circulation via breaks in skin and membranes, while human immunodeficiency virus (HIV) may be trapped on the surface of dendritic cells (Langerhans cells) in the epidermis and then transported to draining lymph nodes. Although the skin is a formidable barrier, mechanical injection that breaches the barrier is a 'natural' route of entry for many viruses. More than 500 individual arboviruses are maintained in nature by a cycle that involves a vector and a vertebrate host. When the infected vector takes a blood meal, virus contained in the salivary gland is injected. Also, virus may be transmitted accidentally by contaminated needles, injection of a virus-contaminated therapeutic, transfusion with virus-contaminated blood or blood products, or tattooing.

The oropharynx and gastrointestinal tract are important portals of entry for many viruses, and enteric viruses may invade the host at a variety of sites from the oral cavity to the colon. Some viruses produce localized infections that remain confined to the gastrointestinal tract whereas others disseminate to produce systemic infection. Most enteric viruses, such as rotaviruses, infect the epithelium of the small or large intestine, but some enteroviruses, such as poliovirus, replicate in the lymphoid tissue of the gut and nasopharynx. There are numerous barriers to infection via the enteric route. First, much of the ingested inoculum will remain trapped in the luminal contents and never reach the wall of the gut. Second, the lumen constitutes a hostile environment because of the acidity of the stomach, the alkalinity of the small intestine, the digestive enzymes found in saliva and pancreatic secretions, and the lipolytic action of bile. Third, the mucus that lines the intestinal epithelium presents a physical barrier protecting the intestinal surface. Fourth, phagocytic scavenger cells and secreted antibodies in the lumen can reduce the titer of infectious virus. Viruses that are successful in using the enteric portal tend to be resistant to acid pH, proteolytic attack, and bile, and some may actually exploit the hostile environment to enhance their infectivity. The importance of anal intercourse as a risk factor for hepatitis B and HIV infection has led to the recognition that some viruses can gain entry through the lower gastrointestinal tract, but the exact mechanism of HIV infection through the anocolonic portal remains to be determined.

Respiratory infection may be initiated either by virus contained in aerosols that are inhaled by the recipient host, or by virus that is contained in nasopharyngeal fluids and is transmitted by hand-to-hand contact. Aerosolized droplets are deposited at different levels in the respiratory tract depending upon their size: those over 10 μm diameter are deposited in the nose, those 5–10 μm in the airways, and those <5 μm in the alveoli. Once deposited, the virus must bypass several effective barriers, including phagocytic cells in the respiratory lumen, a covering layer of mucus, and ciliated epithelial cells that clear the respiratory tree of foreign particles. The temperature of the respiratory tract varies from 33 °C in the nasal passages to 37 °C (core body temperature) in the alveoli. Viruses (such as rhinoviruses) that can replicate well at 33 °C but not at 37 °C are limited to the upper respiratory tract and, conversely, viruses that replicate well at 37 °C but not at 33 °C (such as influenza virus) mainly infect the lower respiratory tract. Most respiratory viruses initiate infection by replicating in epithelial cells lining the alveoli or the respiratory tree, but some viruses will also replicate in phagocytic cells located either in the respiratory lumen or in subepithelial tissues.

Spread

Once infection is established at the site of entry, most viruses will spread locally by cell-to-cell transmission of infection. Some viruses remain localized near their site of entry while others disseminate widely. Viruses that are released only at the apical surface of infected cells tend to remain localized, while those that are released at the basal surface tend to disseminate. Usually, the first step in dissemination of the infection is the transport of virus via the efferent lymphatic drainage from the initial site of infection to the regional lymph nodes, either as free virions or in virus-infected phagocytic cells.

The single most important route of dissemination is the circulation, which can potentially carry viruses to any site in the body. There are several sources of viremia. Virus may enter with the efferent lymphatic flow, or may be shed from infected endothelial cells or circulating mononuclear leukocytes. Viruses can circulate either free in the plasma phase of the blood ('plasma' viremia) or associated with formed elements ('cell-associated' viremia), and these two types of viremia have quite different characteristics. An 'active' viremia is caused by the active replication of virus in the host, which occurs after a lag

period required for tissue replication and shedding into the circulation. Termination of viremia is often quite abrupt and coincides with the appearance of neutralizing antibody in the serum.

Plasma viremia reflects a dynamic process in which virus continually enters the circulation and is removed. The rate of turnover of virus within the plasma compartment is best expressed as transit time, the average duration of a virion in the blood compartment. Typically, transit times range from 5 to 60 min and tend to decrease as the size of the virions increases. Circulating viruses are removed by the phagocytic cells of the reticuloendothelial system, principally in the liver (Kupffer cells), and to a lesser extent in the lung, spleen, and lymph nodes. Once the host has developed circulating antibody, plasma virus is very rapidly neutralized in the circulation and transit time is reduced by several fold. Plasma viremias are usually short-lived (about a week), but there are notable exceptions.

A number of viruses replicate in cells that are found in the circulation, particularly monocytes, B or T lymphocytes, or (rarely) erythrocytes, and produce a 'cell-associated' viremia. Cell-associated viremia may be of short duration, as in the case of ectromelia and other poxviruses. However, in many instances, cell-associated virus titers are low, and viremia persists for the life of the host rather than terminating when neutralizing antibody appears.

There are several ways in which a virus might cross the vascular wall, but the precise mechanism for penetrating the blood–tissue barrier is unknown in most instances. Potential routes into tissues include passage between endothelial cells at sites where there are no tight junctions, translocation of virus-containing endosomes through endothelial cells, replication within endothelial cells, and tissue invasion of virus-infected lymphocytes or monocytes.

Some viruses can disseminate by spreading through the axons of peripheral nerves. Although less important than viremia, neural spread plays an essential role for certain viruses (such as rabies viruses and several herpesviruses) while other viruses (such as poliovirus and reovirus) can utilize both mechanisms of spread.

Localization or Tropism

One of the salient features that distinguishes viruses is their localization or tropism within the animal host. The names of individual viral diseases often reflects the organs or tissues that are involved; thus, smallpox, poliomyelitis, and hepatitis each have their characteristic features. Tropism is regulated both by viral dissemination and cellular susceptibility. Disease localization may not correspond to the distribution of infection since it reflects both the spread of the virus and the host response to infection. The localization of a virus is determined at several phases of infection, including the portal of entry, systemic spread by viremia or the neural route, and the invasion of local organs or tissues.

Most viruses are quite selective in the cell types that are infected *in vivo* and this selectivity plays a significant role in localization. One major determinant of cellular susceptibility is the presence of viral receptors, namely, molecules on the cellular surface that act as receptors for the specific virus, usually by binding a protein on the virion surface (the viral attachment protein). For instance, poliovirus can only replicate in primate tissues, because the poliovirus receptor is expressed only by primate cells. Transgenic mice expressing the the poliovirus receptor can be infected and develop typical paralytic poliomyelitis after intracerebral injection.

Cellular susceptibility also may be determined at post-entry steps in the replication cycle. An example is Newcastle disease virus, a paramyxovirus of birds. Virulent isolates of Newcastle disease virus encode a fusion protein that is readily cleaved by furin, a proteolytic enzyme present in the Golgi apparatus, so that the virion is activated during maturation prior to reaching the cell surface, and before budding of nascent virions. This makes it possible for virulent strains of the virus to infect many avian cell types, thereby increasing its tissue host range, and causing systemic infections that are often lethal. Avirulent strains of Newcastle disease virus cannot be cleaved by furin and depend upon extracellular proteases, which restricts the spread of the virus beyond the respiratory tract.

For oncogenic viruses, the tumors may represent a distant echo of virus localization. Epstein–Barr virus (EBV) infects epithelial cells and B cells. Latently infected B cells are immortalized, and, rarely, these B cells undergo further transformation into lymphomas by virtue of additional genetic events such as chromosomal transposition (t8:14).

Shedding and Transmission

Acute viral infections are characterized by brief periods (days to a few weeks) of intensive virus shedding into respiratory aerosols, feces, urine, or other bodily secretions or fluids. Persistent viruses are often shed at relatively low titers, but this may be adequate for transmission over the prolonged duration of infection (months to years). Although many viruses replicate in the skin, relatively few are spread from skin lesions. Several viruses are shed in the semen, including hepatitis B and human immunodeficiency virus. A number of viruses are excreted in colostrum and milk, including cytomegalovirus, mumps, rubella, and HIV. Blood is also an important source of transmitted virus, particularly for those viruses that produce persistent plasma or cell-associated viremias, such as

hepatitis B, C, and D viruses, HIV, HTLV-I, HTLV-II, and cytomegalovirus, and which can be transmitted by contaminated needles and transfusions. A number of viruses have been isolated from the urine but viruria is probably not important for transmission of most viruses.

Once shed, there are a several means by which a virus is transmitted from host to host in a propagated chain of infection. Probably the most important mechanism is contamination of the hands of the infected transmitter from feces, oral fluids, or respiratory secretions expelled during coughing or sneezing. The virus is then passed by hand-to-hand contact leading to oral, gastrointestinal, or respiratory infection. A second common route is inhalation of aerosolized virus. A third significant mechanism involves direct person-to-person contact (oral–oral, genital–genital, oral–genital, or skin–skin). Finally, indirect person-to-person transmission can occur via blood or contaminated needles. Common source transmissions are less frequent but can produce dramatic outbreaks, via contaminated water, food products, or biologicals such as blood products and vaccines.

Viral Virulence

Virulence refers to the ability of a virus to cause illness or death in an infected host, relative to other isolates or variants of the same agent. The study of virulence variants can provide important insights into pathogenesis, and carries the potential for development of attenuated live virus variants that can be used as vaccines. One approach to virulence is to map the genetic determinants that underlie the virulence phenotype; the other approach is a study of pathogenesis, which describes the differences in infections with viral variants of different virulence. Measures of virulence may be quantitative, or qualitative, since variants of a single virus may differ markedly in their cellular tropism, their mode of dissemination in the infected host, or the disease phenotype that they produce.

Viral Genetic Determinants of Virulence

Viral virulence is encoded in the viral genome and expressed through structural proteins, nonstructural proteins, or noncoding sequences. A large body of information about virus variants has established several principles.

1. The use of mutants has made it possible to identify the role of individual genes and proteins as determinants of the biological behavior of many viral variants.
2. There is no 'master' gene or protein that determines virulence, and attenuation may be associated with changes in any of the structural or nonstructural proteins, or in the noncoding regions of the genome.
3. The virulence phenotype can be altered by very small changes in the genome, if they occur at 'critical sites'. At such sites, a single point mutation leading to the substitution of a specific amino acid or base is often sufficient to alter virulence. For most viruses with small genomes (<20 kb), only a few discrete 'critical sites' have been discovered, usually fewer than 10 per genome.
4. It is possible to create variants with attenuating mutations at several critical sites and these may be more attenuated than single point mutants, and less prone to reversion.
5. Attenuating mutations are often host range mutations that affect replication in some cells but not in others.
6. Although many attenuating mutations have been sequenced, relatively few have been characterized at a biochemical or structural level as to their mechanism of action.

Genetic determinants of viral virulence and attenuation may be illustrated with examples from a few selected virus groups. The attenuation of the vaccine strains of poliovirus is due to critical sites, both in the 5′ nontranslated region (NTR) upstream from the long open reading frame and in selected structural and nonstructural proteins. Each of the three strains of oral polio vaccine (OPV) carry attenuating point mutations in the 5′ NTR (at positions 480, 481, or 472, in types 1, 2, and 3, respectively). Attenuating mutations at these sites are associated with reduced neurovirulence in monkeys and reduced ability to replicate in the central nervous system of transgenic mice bearing the poliovirus receptor. Each of the OPV strains carries at least one mutation that is associated with an alteration in a viral structural or nonstructural protein and confers temperature sensitivity and reduced neurovirulence, and these mutations involve different proteins for the three OPV strains. It has been suggested that mutations in the structural proteins produce attenuation via structural transitions that occur either during virion uncoating or assembly.

Bunyaviruses are negative stranded RNA viruses with a trisegmented genome. The large (L) RNA segment encodes the viral polymerase; the middle (M) RNA segment encodes two glycoproteins (G1 and G2) and a nonstructural protein, Ns_m; the small (S) segment encodes the nucleoprotein (N) and a nonstructural protein, Ns_s. Attenuation can involve either the ability of the virus to replicate in the central nervous system (neurovirulence) or its ability to cause viremia and reach the central nervous system (neuroinvasiveness). Reduced neuroinvasiveness of one bunyavirus virus strain is associated with reduced ability to replicate in striated muscle and consequent low viremogenicity. Attenuation maps to the M RNA segment encoding the viral glycoproteins suggesting that there is an alteration in the infection of myocytes (but not of neurons). Another attenuated bunyavirus showed a

striking reduction in its ability to replicate in the central nervous system of adult mice, and attenuation maps to the L RNA segment. The attenuated virus replicates poorly in C1300 NA neuroblastoma and other murine cell lines, and presumably has a mutant polymerase that restricts viral replication in neurons and certain other cell types.

Reoviruses are double-stranded 10-segmented RNA viruses. Most of the virulence determinants represent qualitative differences between type 1 Lang and type 3 Dearing in tissue and organ tropism rather than quantitative differences in disease severity

1. Reovirus type 1 Lang disseminates through the blood and causes an ependymitis in the brain while type 3 Dearing disseminates through the neural route and causes a neuronotropic encephalitis in the brain. The S1 segment encodes the σ1 protein, which is the viral attachment protein, and is the major determinant of dissemination and brain cell tropism.
2. The M1 segment encoding the μ2 protein influences replication in cardiac myocytes and myocarditis.
3. The M2 segment encoding the μ1 protein affects the protease sensitivity of the virion, and quantitative neurovirulence.
4. The L1 segment encoding λ3 protein influences replication in cardiac myocytes and myocarditis.
5. The L2 segment encoding λ2 protein influences replication levels in the intestine, titers of shed virus, and horizontal transmission between mice.

A new class of virus-encoded proteins has been recognized that contribute to the virulence of viruses by mimicking normal cellular proteins. This group of 'cell-derived' genes has been identified primarily within the genomes of large DNA viruses that have a greater capacity to maintain accessory genes than do viruses with small genomes. 'Virokines' are secreted from virus-infected cells and mimic cytokines, thereby perturbing normal host responses. 'Viroceptors' resemble cellular receptors for cytokines (including antibodies or complement components) that are thereby diverted from their normal cellular targets. Some virus-encoded proteins interfere with antigen presentation and immune induction, while others prevent apoptosis, or interrupt intracellular signaling initiated by cytokines or interferons.

Pathogenic Mechanisms of Virulence

There are many sequential steps in a viral infection and a difference in the comparative replication of two virus variants at any one of these steps can account for their relative degrees of virulence. Attenuated variants usually will replicate or spread less briskly in one or several tissues associated with the pathogenic process. A reduction in viremia can be an important mechanism for the reduction in virulence. For instance, the Mahoney strain of type 1 poliovirus causes a much higher viremia and paralytic rate than several other poliovirus strains that are less viremogenic. Different variants of a single virus can exhibit differences in their tropism for organs, tissues, and cell types, which confers a multidimensional character upon virulence. The three attenuated strains in the oral poliovirus vaccine provide an example. Both attenuated and wild-type polioviruses are able to replicate in the gastrointestinal tract to similar levels, judging from the shedding of virus in the feces, but the attenuated strains show a reduction of about 10 000-fold (relative to the wild-type strains) in their ability to replicate in the spinal cord after intraspinal injection. In this instance, virulence for the target organ (the spinal cord), rather than viremogenicity, appears to be the most important determinant of virulence.

Host Determinants of Susceptibility and Resistance

Studies with inbred animals have documented that the outcome of infections often varies in different strains of mice, and genetic analyses have been conducted to determine the form of inheritance of susceptibility, and to identify the responsible gene. Several generalizations may be made:

1. Most genetic loci control susceptibility to a specific family of viruses and not to all viruses.
2. Susceptibility can often be mapped to a single autosomal locus, but multiple loci have been identified in some instances.
3. Susceptibility may be either dominant or recessive.
4. Where loci have been mapped, they usually are distant from the major histocompatibility locus, and in most such instances, the mechanism of susceptibility is not immunological.
5. The exact mechanism of susceptibility has not been well defined in most instances, although there are some examples that map to the major histocompatibility complex (MHC), implying an immunological explanation.

It is difficult to investigate genetic determinants of host susceptibility in outbred populations of animals or humans. However, recent studies of HIV-1 have provided substantial evidence of a number of human genetic determinants of susceptibility to infection or to the rate of progression to overt AIDS. The most striking of these is a polymorphism in the gene that encodes the chemokine receptor, CCR5, which is the major co-receptor for HIV-1. Some individuals have a deletion in the gene (the Δ32 deletion) such that no CCR5 protein is expressed. Those who are homozygous for the Δ32 mutation are at very low risk of HIV infection, and those who are heterozygous for

the Δ32 deletion can be infected but have prolonged incubation periods to onset of AIDS by comparison with CCR5+ homozygous individuals.

The host response to a viral infection may also be influenced by a variety of physiological variables such as age, sex, stress, and pregnancy, all of which have been shown to influence the outcome of infection with some viruses. In general, very young animals are more susceptible than adult hosts to acute viral infections, and are more likely to undergo severe or fatal illness. The innate high susceptibility of infants born of nonimmune mothers is revealed only in those rare instances where a virus has disappeared from an isolated community so that all age groups lack immune protection. A classical example is measles, which can be a devastating disease with a mortality of up to 25% in nonimmune infants. Less commonly, advanced age can also be associated with increased susceptibility to viral infection. St. Louis encephalitis and West Nile encephalitis are examples of this phenomenon.

See also: Innate Immunity: Defeating; Innate Immunity: Introduction; Persistent and Latent Viral Infection.

Further Reading

Barber DL, Wherry EJ, Casopust D, *et al.* (2006) Restoring function in exhausted CD8 T cells during chronic viral infection. *Nature* 439: 682–687.

Martin MP and Carrington M (2005) Immunogenetics of viral infections. *Current Opinion in Immunology* 17: 510–516.

Nathanson N, Ahmed R, Brinton MA, *et al.* (eds.) (2007) *Viral Pathogenesis and Immunity,* 2nd edn. London: Academic Press.

Nathanson N and Tyler K (2005) The pathogenesis of viral infections. In: Mahy BWJ and ter Meulen V (eds.) *Topley and Wilson's Microbiology*, pp. 236–269. London: Hodder Arnold.

Racaniello VR (2006) One hundred years of poliovirus pathogenesis. *Virology* 344: 9–16.

Tumpey TM, Basler CF, Aguilar PV, *et al.* (2005) Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 310: 77–80.

Viral Receptors

D J Evans, University of Warwick, Coventry, UK

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The majority of viruses have an extracellular phase in the life cycle, which is a necessary part of virus transmission and dissemination. To initiate the replication cycle for the production of progeny virions, the virus must first enter a cell. Cell entry is mediated by the specific interaction of the virus with molecules on the cell surface – the receptor – resulting in attachment, a process that leads to internalization of either part or all of the virus particle, or the genome. A cell that expresses the cognate cell-surface receptor for a virus is termed susceptible. Not all susceptible cells to which the virus binds are capable of supporting infection and replication, perhaps due to the absence of necessary intracellular components. Cells that support virus binding, entry, replication, and progeny virion release are termed permissive.

With certain notable exceptions, such as viruses of fungi that exhibit no extracellular stage in the life cycle or plant viruses introduced mechanically by insect vectors, the virus receptor therefore forms the primary determinant of virus tropism. The identification and characterization of the receptor therefore provides important insights into the early stages of the virus replication cycle, and has been a major research focus of molecular virologists worldwide.

A Definition of Virus Receptor and Co-Receptors

Our enhanced understanding of virus–receptor interactions and the early events in cell entry have demonstrated that this process is, in many cases, considerably more complicated than originally thought, involving multiple cell-surface or intracellular molecules with different roles in the entry events. A classical definition of a virus receptor would be the cell-surface molecule that mediates virus attachment and cell entry. Our current understanding of these processes demonstrates that many viruses use different molecules to mediate attachment and post-attachment entry events. For these reasons the term co-receptor is often used to indicate an accessory molecule implicated in cell entry. Both receptors and co-receptors will be considered within the scope of this article. Rather than aim to provide a complete catalog of viruses and receptors, the focus of this article is to use key examples to illustrate specific types of virus–receptor interactions and introduce the general concepts involved in receptor identification and characterization.

The Identification of Virus Receptors

Since a working definition of a receptor is a specific cell-surface molecule involved in virus attachment, the

identification of the receptor requires a means of detecting virus binding, and a requirement to demonstrate the specificity of the process.

Direct detection of a virus bound to the cell requires some form of labeling – whether metabolic labeling with a radioisotope or labeling with a conjugated fluorescent marker – and subsequent purification of the virus particle, often by density gradient centrifugation. Indirect methods of detecting virus can also be used, for example, the hemagglutination phenotype demonstrated by sialic acid binding viruses such as influenza A virus, or by detection of a bound virus with antibodies to the virus particle.

The specificity of the observed binding event typically requires the inhibition of the process using a defined block. The routine availability of monoclonal antibodies has hugely facilitated this approach to receptor identification, though alternate methods such as the use of enzymatic pretreatment of cells with heparinase or neuraminidase (NA) have also been used to demonstrate interactions with nonprotein cell-surface molecules.

Finally, in cases where the receptor is a single protein molecule, the specificity of the virus interaction can be further demonstrated by transformation of a nonsusceptible cell with DNA encoding the protein. Following expression at the cell surface, the acquisition of a virus binding phenotype is tested, perhaps combined with the demonstration of specificity by the inhibition with monoclonal antibodies. For example, the molecular cloning of the poliovirus receptor (PVR) involved expression of the protein in murine fibroblasts, which conferred susceptibility to an already permissive cell type.

Typically, a combination of the approaches outlined above is necessary to unequivocally demonstrate the identity of a virus receptor. Multiple confirmatory approaches are required as each technique has inherent uncertainties. For example, monoclonal antibodies may cross-react with closely related membrane proteins, or transformation of DNA may induce receptor expression, rather than encode the receptor *per se*. Similar techniques, and the associated caveats, are subsequently used in the initial stages of characterization of the virus–receptor interaction, for example, the receptor protein domain(s) to which the virus attaches, or the cell or tissue specificity of attachment.

Specific Examples of Virus Receptors

The examples used below illustrate a range of different types of virus–receptor interactions – those involving a single type of target molecule on the cell surface, either proteins or carbohydrate moieties, and those in which multiple receptors and co-receptors have been implicated. Due to their pivotal role in determining tissue and host tropism, and hence influence on pathogenesis, particular effort has been made in identifying receptors for important human and animal virus pathogens. However, the broad concepts illustrated below apply equally to the cell-surface proteins of bacteria used by bacteriophages – where the exquisite specificity of the interaction is used in classification and typing of *Salmonella*.

Sialic Acid

Influenza A is an enveloped virus that causes epidemic and pandemic respiratory tract infections in humans, but is naturally an enteric pathogen of aquatic birds. Both of the external virus glycoproteins are involved in interaction with the cellular receptor, sialic acid, the first identified receptor for a human virus. The trimeric hemagglutinin (HA) molecule, consisting of a stalk and globular head, carries a conserved cleft or pit at the membrane-distal end into which the sialic acid binds. Sialic acids are a family of negatively charged monosaccharides derived from neuraminic acid; those used as virus receptors are usually attached to a penultimate galactose by either an (α2,3)- or (α2,6)-linkage. The structure of an HA–sialyllactose complex has been determined at atomic resolution and provides insight into the specificity of binding, which in human strains involves (α2,6)-linked sialic acid in contrast to the binding of avian strains which exclusively interact with (α2,3)-linked sialic acid. Interaction of influenza with sialic acid is a low-affinity process, attachment being facilitated by multimeric binding to many HA molecules. The tetrameric NA virus glycoprotein has enzymatic activity that hydrolyses the glycosidic linkage of the receptor sialic acid, so enabling the release of progeny virus from the cell surface. NA-inhibitors have been developed as successful antiviral therapies.

Although influenza A is the best-characterized sialic acid-binding virus, there are many other examples, including human parainfluenza virus (hPIV) types 1–3 (and the murine hPIV analog Sendai virus), picornaviruses (rhinovirus type 67, enterovirus type 70), polyomaviruses (JC and BK virus), type 3 reoviruses, group C rotaviruses and adeno-associated virus. As with influenza A virus, predominant bias to either (α2,6)- or (α2,3)-linked sialic acid has been detected in each of these examples.

Heparan Sulfate and Other Glycosaminoglycans

Glycosaminoglycans (GAGs) (including heparan, chondroitin, and dermatan sulfate) are negatively charged sulfated sugars which are widely implicated in attachment of a range of viruses as diverse as herpes simplex virus (HSV), enteroviruses, flaviviruses, and respiratory syncytial virus. However, despite the isolation of clinical

virus isolates with GAG-binding capacity, definitive evidence that GAGs have a role in the cell infection process *in vivo* is yet to be obtained. The relevance of GAGs has been further questioned by the observation that foot and mouth disease virus (FMDV), a picornavirus that binds $\alpha_v\beta_3$ integrins *in vivo*, can readily adapt to use GAGs in cell culture – this process is discussed further below.

GAG binding is routinely demonstrated by inhibiting virus binding to the cell surface, or virus infection, with soluble analogs such as heparin sulfate, by heparinase pretreatment of cells, or by analyzing binding to cells deficient in GAG synthesis. The interaction of FMDV and heparin has also been determined crystallographically and shown to involve a region of the capsid distinct from the surface-exposed loop that binds the *in vivo* receptor, $\alpha_v\beta_3$ integrin. GAG-binding domains are usually linear or conformational regions of the virus surface that are rich in basic residues, and adaptation to GAG binding often involves the acquisition of basic (lysine or arginine) amino acid substitutions.

Most viruses shown to bind GAGs are also known to interact with at least one additional cell surface receptor. Glycoprotein C (gC) of HSV-1 and HSV-2 binds HS, and the gD interaction with certain forms of GAGs appears necessary for postbinding prefusion events in cell infection. However, gD also interacts with HVEM (herpes virus entry mediator, a member of the tumor necrosis factor receptor family) and nectins 1 and 2, suggesting that HSV may either exhibit considerable redundancy in cell-surface interactions or, interestingly, that the virus carries a number of binding activities tailored to particular cell or tissue types. Similarly, GAG-binding echoviruses appear to also require the primary receptor for entry, decay accelerating factor (DAF) (Williams and Evans, unpublished data).

The cell–receptor interactions of human immunodeficiency virus (HIV) have received particular attention. In addition to the primary receptor, CD4, and the chemokine co-receptors (see below), syndecans with GAG chains have been implicated in macrophage infection by HIV. Similarly, infection of CD4-negative microvascular endothelial cells, a probable route via which the virus gains access to the central nervous system (CNS), has been shown to be a lipid raft-independent process involving cell-surface proteoglycans. Again, as with HSV, the complexity of interaction with multiple cell receptors, and the *in vivo* function, is yet to be satisfactorily dissected.

Histo-Group Antigens as Receptors

The wide range of nonprotein molecules used for attachment by viruses also includes the histo-blood group antigens (HBGAs), which serve as receptors for the noroviruses, positive-strand RNA viruses that cause acute gastroenteritis. HBGAs are complex carbohydrates present both as free oligosaccharides and attached to the surface of mucosal epithelia. The HBGA phenotypes of humans are polymorphic and complex, and are associated with multiple gene families. This was consistent with the demonstration that norovirus infection was influenced by both prior acquired immunity and the genetic background – patients with blood group O were significantly more susceptible to infection than those with blood group B. Subsequent binding studies with norovirus virus-like particles (VLPs) – which retain the receptor-binding capacity of the virus which cannot be cultured *in vitro* – and specific antibodies to blood-group antigens, soluble blood-group antigens or glycosidases, have confirmed a role for these molecules in virus attachment.

Immunoglobulin Superfamily Proteins

Our understanding of the range of cell-surface proteins that function as virus receptors is influenced by the methods utilized to identify the protein. Since these methods are predominantly geared to isolating single-gene traits, for example, cDNA library screening, it is unsurprising that the majority of proteins definitively identified as receptors are single proteins, rather than one component of a multiprotein complex. Perhaps reflecting their relative abundance at the cell surface, proteins belonging to the immunoglobulin superfamily are implicated in the attachment of many viruses.

Poliovirus, the causative agent of paralytic poliomyelitis, binds the membrane-distal Ig-like domain of CD155, the PVR. Receptor binding initiates a series of irreversible conformational changes in the virus particle, which results in the externalization of capsid proteins usually hidden in the mature infectious particle, and the delivery of the virus genome to the cell cytoplasm. Data are accumulating to suggest that this process involves formation of a pore traversed by the genome, though other models exist. That PVR is sufficient to mediate these processes has been elegantly demonstrated by several methods; PVR expression in murine cells renders them fully permissive for poliovirus infection, and is the basis for the generation of PVR-transgenic mouse lines for neurovirulence studies, the conformational changes observed at the cell surface can be recapitulated using poliovirus and soluble PVR (sPVR) *in vitro*, and – most recently – the bound virus–receptor complex has been visualized by crystallography and, using cryo-EM, studied at the surface of PVR-loaded membrane vesicles.

Human rhinoviruses are very closely related to members of the enterovirus genus to which poliovirus belongs. The rhinoviruses can be divided into two predominant groups based upon their receptor usage; of these, the major receptor group bind to the membrane-distal Ig-like domain of intracellular adhesion molecule type 1 (ICAM-1). Like poliovirus, receptor binding initiates

conformational changes in the rhinovirus particle that are considered essential for infection. A further similarity is the region of the capsid involved in the receptor interaction. In both poliovirus and the major group rhinoviruses, the receptor binds to a canyon or cleft surrounding the fivefold axis of icosahedral particle symmetry. Two decades ago, Rossmann proposed that this canyon is too narrow to allow the binding of virus neutralizing antibodies, thereby allowing the virus to retain a relatively invariant receptor-binding domain in the presence of immunoselection. More recent crystallographic studies have demonstrated that some neutralizing antibodies penetrate deep into the rhinovirus canyon. This suggests that the canyon has less to do with evasion of immune surveillance, but has instead evolved in response to receptor binding requirements and to accommodate the structural changes that occur post-binding.

The Ig-like protein that has received some of the most intense scrutiny is CD4, the primary receptor for HIV. The identification of CD4 as a receptor for HIV went some way to explain the tropism and pathogenesis of the virus; CD4+ T cells were often depleted in HIV-infected individuals. Antibodies to CD4 blocked virus attachment, fusion and infection, and expression of CD4 in murine cells permitted virus binding. Detailed analysis using monoclonal antibodies specific for each Ig-like domain of CD4, and subsequent site-directed mutagenesis of the receptor, demonstrated that the attachment protein of HIV (the virus surface glycoprotein gp120) binds to a surface-exposed loop of the most membrane-distal of the four Ig-like domains of CD4. Gp120 carries the conserved CD4 attachment site which is protected, though not completely hidden, from interaction with neutralizing antibodies by the diversity, conformational flexibility, and heterogeneous glycosylation of the surrounding protein. One characteristic exhibited by HIV-infected long-term nonprogressors is the presence of a potent neutralizing antibody response to the CD4 binding site – because the virus must retain a largely invariant receptor-binding domain, these antibodies are active against divergent virus isolates, and significant effort has been expended in generating vaccine candidates capable of inducing a similar immune response. Other therapeutic approaches based upon the conserved interaction of gp120 and CD4 have been investigated. Although soluble CD4 receptors, lacking the transmembrane domains, block HIV infection *in vitro*, they have been largely ineffective in clinical trials. These disappointing results have been attributed to the need to maintain high serum concentrations of a protein with a short half-life, and the ability of the virus to spread directly from cell to cell by fusion, rather than by extracellular virus. Similarly, approaches involving toxins fused to soluble CD4 that are used to target gp120 expressed at the surface of infected cells have only been promising in *in vitro* assays.

Complement Control Proteins as Virus Receptors

A number of viruses have been shown to bind to complement control proteins, also termed the regulators of complement activity (RCA) family proteins. These proteins, characterized by a two-disulfide protein fold termed a short consensus repeat (SCR), are involved in protecting autologous cells from complement-mediated lysis.

Epstein–Barr virus (EBV) attachment to B lymphocytes occurs by the interaction of the virus gp350/220 envelope glycoprotein with the cell-surface C3dg receptor designated CR2 (CD21). CR2 was identified as the receptor following the observed similarity between gp350/220 sequences and C3dg, the complement protein target for CR2 activity. Further supporting evidence included the demonstration that multimerized peptides of the proposed gp350/220 or C3dg binding domains inhibited attachment, and the receptor identity was confirmed by expression studies in CR2-deficient target cells. CR2 possesses 16 extracellular SCR domains, the two most membrane-distal being the site of gp350/220 binding, as demonstrated by the construction of murine-human CR2 chimeric receptors, and using SCR-specific monoclonal antibodies in blocking studies.

Laboratory-adapted measles virus binds another RCA family protein member, CD46, a four-SCR domain protein that acts as a cofactor in the proteolytic inactivation (and hence cell protection) of C3b/C4b. Antisera to CD46 blocks virus binding and infection, and transfection of rodent cells with CD46 renders them susceptible to infection by the Edmonston and Hallé strains of measles virus. Further studies demonstrated that clinical isolates of measles cultured in human B-cell lines exhibited a cell tropism inconsistent with the use of CD46 as the receptor – further details are provided in the section on laboratory adaptation.

DAF, a 55 kDa RCA-family protein, is the receptor for a significant number of human enteroviruses, including the coxsackie B viruses and many echoviruses. In contrast to many of the other examples presented here, where binding is to the membrane-distal domain of the receptor, the majority of the known DAF-binding enteroviruses interacts with the third SCR, together with the second and/or fourth SCR domains. Recent completion of the atomic structure of DAF shows it to be a rod-shaped protein, with the four SCR domains projecting in a broadly linear fashion from the top of a heavily glycosylated Ser/Thr-rich stalk, which distances the complement-control functions from the cell membrane. Unsurprisingly, considering the receptor structure, interaction of DAF with echoviruses does not involve the canyon on the virus surface, but instead occurs across the twofold axis of symmetry, with the receptor interface occupying a surface-exposed region of the capsid. In the case of the coxsackieviruses, DAF binding serves both to

attach the virus to the cell surface and initiate a cascade of signaling events which results in the transport of the virus to the co-receptor for infection – CAR, the coxsackie and adenovirus receptor, which is located at tight junctions and not normally accessible to the virus.

Co-Receptors for Infection

The distinction between a receptor and a co-receptor for infection is largely semantic. As our understanding of virus binding and entry improves, it is clear that this is often a multistage process involving a cascade of interactions between the virus and cell-surface proteins. The initial interaction is usually defined as being to the receptor, with subsequent interactions being mediated by co-receptors. This order can be reversed if the first interaction is a relatively nonspecific event – for example, the binding of HSV to HS – in which case co-receptor binding may be considered to temporally precede interaction with a more specific molecule that perhaps defines the cell or tissue tropism of the virus. Therefore, the discrimination between nonspecific attachment molecules, receptors, and co-receptors – in which all are sometimes required for infection – is open to interpretation. What is clearly important for many viruses is the requirement for a range of interactions between the virus and the cell surface. Indeed, as our understanding of virus infection improves, the number of examples in which a single receptor is implicated – such as poliovirus – is dwindling, and may represent the exception, rather than the rule.

Although the coxsackie B viruses bind DAF at the cell surface, infection also requires CAR, an Ig-superfamily protein with two extracellular domains, which interacts with the canyon of coxsackie B viruses or the fiber of adenovirus types 2 and 5. Like the poliovirus–PVR interaction, binding of coxsackie B viruses to CAR – at the cell surface or in solution – triggers the irreversible conformational changes associated with particle uncoating. However, CAR is naturally located in tight junctions where it mediates homotypic cell adhesion, and is therefore not immediately accessible for virus attachment at the apical cell surface. Recent elegant studies have demonstrated that virus binding to DAF at the latter site results in Abl-kinase mediated signaling events which cause actin rearrangements that allow virus movement to the tight junctions where uncoating occurs. DAF binding also activates Fyn-kinase, which results in the phosphorylation of caveolin and subsequent virus entry in caveolin vesicles. The coxsackie B viruses therefore exploit the signaling capacity of their low-affinity receptor (DAF) to traffic to the high-affinity, uncoating receptor (CAR) and thereby cross the epithelial barrier.

Like poliovirus and rhinovirus, binding of HIV to CD4 initiates conformational changes in the trimeric gp120 that are a prerequisite for the subsequent fusion events. However, unlike the two picornavirus examples, HIV must also interact with a co-receptor as a necessary precursor to the fusion of viral and cellular membranes. This requirement for a co-receptor explains why HIV can bind to human CD4 expressed on murine cells – which lack the co-receptor – without undergoing membrane fusion. A mouse-CD4 cell line was transformed with a human cDNA library and used to functionally screen for additional proteins which would enable gp120-mediated membrane fusion. This, or analogous, screens were used to define the co-receptor for HIV as one of a related series of receptors for chemotactic cytokines such as RANTES, MIP-1α and MIP-1β. The chemokine receptors identified, CXCR4 or CCR5, belong to the seven-transmembrane-domain G-protein-coupled receptor superfamily. Co-receptor specificity appears to be determined by the third hypervariable domain (V3) of gp120 – a target for potently neutralizing antibodies *in vitro* – with an additional conserved domain of gp120 contributing to generic features of the chemokine receptor attachment. The critical conserved residues of the latter domain remain hidden from immune surveillance until exposed by the structural changes in gp120 that occur as a consequence of CD4 binding. The importance of the chemokine co-receptor in HIV infection is supported by the observed genetic resistance to HIV of individuals with a deletion and frameshift within CCR5. Similarly, co-receptor identification provided an explanation for the observed suppression of HIV infection by the chemotactic cytokines released by CD8+ T cells. Finally, the identification of chemokine receptors as critical co-receptors of HIV infection has also contributed to our understanding of virus tropism and evolution *in vivo*. CCR5-tropic strains of HIV are predominant for transmission and during the asymptomatic phase of virus infection. CCR5 is expressed at high levels in activated/memory cells and in gut-associated lymphoid tissue, the primary site of HIV replication. As infection progresses, CXCR4-tropic viruses evolve, able to exploit the wider tissue expression of the CXCR4 receptor. The, perhaps logical, assumption that this broadening of cell and tissue tropism is the cause of disease progression is contradicted by the failure to observe a switch to CXCR4-tropic viruses in a significant proportion of patients with acquired immune deficiency syndrome (AIDS).

Laboratory Adaptation and Receptor Usage by Clinical Virus Isolates

Due to the possession of error-prone replication strategies, many viruses exhibit an exquisite ability to adapt to the environment in which they are grown – for example, by the generation of mutations that confer resistance to

neutralizing antibodies. The same variation can confound the correct identification of virus receptors in laboratory studies, due to the adaptation to alternate receptors for cell attachment and entry.

The example of heparan sulfate binding by FMDV has already been used to illustrate this process. HS-adapted FMDV isolates are apathogenic in animals, presumably reflecting the evolutionary adaptability of the virus to an environment in which receptor usage is not a rate-limiting process in infection, transmission, or dissemination. The virus interacts with the *in vivo* receptor ($\alpha_v\beta_3$ integrin) using a conserved Arg-Gly-Asp amino acid triplet (RGD motif) on a surface-exposed loop of the VP1 capsid protein. Laboratory-adapted HS-binding FMDV strains can dispense with $\alpha_v\beta_3$ integrin binding altogether, as demonstrated by the use of reverse genetics to mutate the RGD motif to another sequence. Being acid-labile, it is likely that uncoating of FMDV is mediated by entry of the virus–receptor complex into an endosome. This is supported by the further demonstration that it is possible to engineer anti-FMDV antibodies onto the cell surface to which the virus is bound, and via which infection can occur in the absence of either HS or integrin binding.

CD46, like most other complement-control proteins, is expressed ubiquitously on serum-exposed cells. Clinical isolates of measles virus, isolated in either B95a cells or human B-cell lines, do not infect all CD46-expressing cell lines, suggesting that they may use an alternate receptor. Subsequent functional expression cloning studies resulted in the isolation of a cDNA encoding human SLAM (signaling lymphocyte-activation molecule; also known as CDW150) that conferred susceptibility to B95a-cell cultured measles virus. SLAM is an Ig-superfamily protein expressed on a range of immune system cells (macrophages, dendritic cells, some B and T cells) that, when liganded to SLAM on an adjacent cell, transduces a signal resulting in T_H2 cytokine (IL13 and 4) production. All measles virus isolates tested can infect cells via SLAM, though some – including the lab-adapted Edmonston strain – can also use CD46. However, studies suggest that CD46 binding is irrelevant *in vivo* and arises upon culture by the substitution of as few as a single amino acid in the HA protein of the virus.

Summary

Virus receptors are key components of the early events involved in cell infection. The examples outlined above should illustrate that the definition of receptors acting solely as attachment molecules is overly simplistic. In addition to attachment, receptors also actively contribute to entry by initiating conformational changes in the virus that lead to uncoating. In addition, they provide mechanisms for internalization – which increasingly appear to involve signaling events that occur upon receptor binding – in which the virus may subvert a natural receptor cycling process. Furthermore, the identification of virus receptors contributes significantly to our understanding of host, tissue, and cell tropism, and helps explain aspects of virus pathogenesis. The selected bibliography provides further examples illustrating aspects of virus receptor identification and function.

See also: Cytokines and Chemokines; Foot and Mouth Disease Viruses; Influenza; Measles Virus; Viral Pathogenesis.

Further Reading

Belnap DM, McDermott BMJ, Filman DJ, *et al.* (2000) Three-dimensional structure of poliovirus receptor bound to poliovirus. *Proceedings of the National Academy of Sciences, USA* 97: 73–78.

Bergelson JM, Cunningham JA, Droguett G, *et al.* (1997) Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science* 275: 1320–1323.

Coyne CB and Bergelson JM (2006) Virus-induced Abl and Fyn kinase signals permit coxsackievirus entry through epithelial tight junctions. *Cell* 124: 119–131.

Evans D and Almond J (1998) Cell receptors for picornaviruses as determinants of cell tropism and pathogenesis. *Trends in Microbiology* 6: 198–202.

Hogle JM (2002) Poliovirus cell entry: Common structural themes in viral cell entry pathways. *Annual Review of Microbiology* 56: 677–702.

Lindahl G, Sjobring U, and Johnsson E (2000) Human complement regulators: A major target for pathogenic microorganisms. *Current Opinion in Immunology* 12: 44–51.

Lusso P (2006) HIV and the chemokine system: 10 years later. *EMBO Journal* 25: 447–456.

Maddon PJ, Dalgleish AG, McDougal JS, Clapham PR, Weiss RA, and Axel R (1986) The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* 47: 333–348.

Mendelsohn CL, Wimmer E, and Racaniello VR (1989) Cellular receptor for poliovirus: Molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. *Cell* 56: 855–865.

Olofsson S and Bergstrom T (2005) Glycoconjugate glycans as viral receptors. *Annals of Medicine* 37: 154–172.

Pettigrew DM, Williams DT, Kerrigan D, Evans DJ, Lea SM, and Bhella D (2006) Structural and functional insights into the interaction of echoviruses and decay-accelerating factor. *Journal of Biological Chemistry* 281: 5169–5177.

Rossmann MG (1989) The canyon hypothesis. Hiding the host cell receptor attachment site on a viral surface from immune surveillance. *Journal of Biological Chemistry* 264: 14587–14590.

Tan M and Jiang X (2005) Norovirus and its histo-blood group antigen receptors: An answer to a historical puzzle. *Trends in Microbiology* 13: 285–293.

Ward T, Pipkin PA, Clarkson NA, Stone DM, Minor PD, and Almond JW (1994) Decay accelerating factor (CD55) identified as the receptor for echovirus 7 using CELICS, a rapid immuno-focal cloning method. *EMBO Journal* 13: 5070–5074.

Yanagi Y, Takeda M, and Ohno S (2006) Measles virus: Cellular receptors, tropism and pathogenesis. *Journal of General Virology* 87: 2767–2779.

Viral Suppressors of Gene Silencing

J Verchot-Lubicz, Oklahoma State University, Stillwater, OK, USA
J P Carr, University of Cambridge, Cambridge, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Dicer, or Dicer-like (DCL) enzymes RNAse III or RNAse-III-like enzymes responsible for digesting the noncoding regions of mRNAs to produce 21–24 nt single-strand RNAs known as miRNAs and siRNAs.
Green fluorescent protein (GFP) This is derived from jellyfish and fluorescence green. Excitation wavelength is 488 nm and emission is above 520 nm. Fusions involving GFP are often used to study protein subcellular targeting or distribution in tissues.
MicroRNAs (miRNAs) Single-strand RNAs that are 21–24 nt in length are found in eukaryotes and arise from noncoding regions of transcripts. These are produced by nucleolytic processing by DICER, and RNAse-III-like enzyme. These are crucial components of the RNAi pathway.
RNA interference (RNAi) Similar to post-transcriptional gene silencing. More specifically, cellular or synthetic small RNA molecules can target homologous mRNA for degradation thereby preventing gene expression.
RNA silencing or post-transcriptional gene silencing (PTGS) Mechanism regulating gene expression by regulating RNA accumulation after transcription. Mechanism involves RNA degradation machinery to shut off gene expression.
Short interfering RNAs (siRNAs) Double-strand RNAs that are 21–24 nt in length which are generated by DICER or Dicer-like enzymes. SiRNAs can spread systemically in *C. elegans* and may cause silencing in distal organs. Some single-strand RNAs are made double-strand by RNA-dependent RNA polymerases. These double-stranded products are then cleaved by DICER.
Transcriptional gene silencing (TGS) Silencing of genes in the nucleus. A small RNA molecule triggers *de novo* DNA methylation thereby blocking transcription. Small RNA typically is homologous to the target gene.
Virus-induced gene silencing (VIGS) Viral RNAs can trigger for PTGS similar to small RNAs. Several plant viruses have been engineered as vectors for use in experiments shutting off gene expression by PTGS. Fragments of genes, antisense RNAs, small RNAs can be introduced into the viral vector and silencing is induced upon inoculation with the recombinant virus.

Introduction

RNA silencing, also known as post-transcriptional gene silencing (PTGS) or RNA interference (RNAi) is a mechanism regulating gene expression in a wide range of eukaryotes. RNA silencing is a mechanism in which small RNAs block gene expression by targeting homologous mRNAs without impacting nuclear DNA. Andrew Hamilton and David Baulcombe first showed that short antisense RNAs of 20–25 nt which share homology with target mRNAs are produced in silenced tissues. Since 1999 additional short RNAs have been identified, including microRNAs and siRNAs, which target homologous RNA sequences either for sequence specific degradation or, in some instances, function to repress mRNA translation.

PTGS is induced by double-stranded RNAs in most eukaryotic systems. Since most RNA viruses form double-stranded RNA replication intermediates, replicating viruses often trigger PTGS, which subsequently degrades all homologous RNAs within the cell. This has led researchers to suggest that PTGS may have originally evolved as an antiviral defense mechanism. The ability of PTGS to target viral RNAs for degradation was demonstrated in the early 1990s when transgenic plants expressing untranslatable transcripts of the viral coat protein or replicase gene were found to be resistant to infection by the homologous virus while remaining susceptible to unrelated viruses. Virus resistance was also reported in experiments using transgenic plants which failed to accumulate detectable levels of the transgenically expressed coat protein RNA.

The laboratory of William Dougherty provided the first reports of transgenic plants recovering from virus infection. A set of transgenic tobacco plants expressing a nontranslatable tobacco etch virus (TEV) coat protein RNA were initially susceptible to virus infection but then recovered and became highly resistant to secondary inoculation. Viral RNA was undetectable and transgene RNAs showed lower steady-state level accumulation in recovered leaves indicating that an RNA degradation mechanism was triggered. Contemporary studies by Rob Goldbach's laboratory showed that nontranslatable transcripts for the tomato spotted wilt virus N protein-protected transgenic plants from infection by the virus. The idea that viruses trigger a cellular antiviral defense pathway which degrades homologous RNAs was further supported by experiments in the laboratory of

David Baulcombe showing that recovery can occur during infection of nontransgenic tobacco plants. *Nicotiana clevelandii* inoculated with the nepovirus tomato black ring virus (TBRV) strain W22 initially showed clear virus symptoms but later recovered. Plants were resistant to secondary inoculation with the same W22 strain but were susceptible to inoculation with the heterologous nepovirus, tobacco ringspot virus (TRSV), and showed partial protection to secondary infection with the TBRV strain BUK. Insertion of a fragment of the TBRV genome into the potato virus X (PVX) genome was sufficient to block infection of PVX in recovered plants indicating that plants contain an inducible sequence-specific degradation mechanism that may be a component of the plant immune response to RNA virus infection.

This same PTGS mechanism also provides cross-protection against secondary virus infection seen in the TBRV and PVX experiments described above and in experiments showing that plants recovered from infection with tobacco rattle virus containing GFP (TRV-GFP) were resistant to secondary infection with PVX-GFP. Technology based on PTGS has been developed to engineer virus resistance in transgenic plants.

Viral-induced gene silencing (VIGS) in transgenic plants can result in methylation of the cognate transgene or nuclear gene. It has been suggested that *de novo* DNA methylation triggered by transcriptional gene silencing (TGS) or PTGS is driven by siRNAs imported into the nucleus, which recruit DNA methyltransferases to similar target sequences. Thus, RNA viruses, such as PVX or tobacco rattle virus (TRV), have been engineered as vectors used for knocking out host gene expression. These are termed VIGS vectors. Entire genes or fragments of genes can be inserted into the viral vector and following inoculation, induce silencing of the cognate endogenous gene. One of the earliest examples of VIGS targeting an endogenous gene was insertion of the phytoene desaturase gene (PDS) into PVX. PDS is involved in carotenoid biosynthesis and affects plants' susceptibility to photobleaching. Virus-induced PDS silencing causes leaves to lose all green color and bleach white under normal lighting conditions. The most popular research tools to study TGS and PTGS are transgenic plants expressing the green fluorescent protein (GFP) and PVX or TRV vectors containing fragments of the GFP coding sequence. GFP expression can be monitored using a UV lamp and over time GFP expression disappears throughout the entire plant. In 1997, Olivier Voinnet and David Baulcombe first reported GFP-transgene silencing by an RNA virus and also demonstrated the silencing signal can spread systemically to suppress gene expression in distal regions. Thus, the use of VIGS to suppress expression of endogenous genes has become an important tool for analysis of gene function.

DNA viruses can also be used as gene silencing vector. The chalcone synthase gene, which is involved in flower pigmentation in *Petunia hybrida* flowers, was inserted into tobacco yellow dwarf virus genome and flower pigmentation was completely altered in virus infected petunia. Cabbage leaf curl virus (CabLCV), a member of the genus *Begomovorus* of the family *Geminiviridae*, has been engineered to express any endogenous targeted gene of *Arabidopsis* and proved to be an efficient tool for scientists studying gene expression in this model plant. Similarly, the African cassava mosaic virus (ACMV), another begomovirus, modified to be a silencing vector, was demonstrated to be able to silence a variety of endogenous genes in cassava, thereby providing a useful tool to breeders for that crop.

Viral-Derived Short Interfering RNAs

Small RNAs of approximately 21–24 nt are found in all eukaryotes and belong to two general classes: microRNAs (miRNAs) and short interfering RNAs (siRNAs). miRNAs are 21–24 nt ssRNAs and arise from nonprotein coding regions of transcripts which are nucleolytically processed by an RNAse-III-like enzyme called DICER in animals and *Caenorhabditis elegans*, or DICER-like (DCL) in *Arabidopsis*. siRNAs similar to miRNAs range in size from 21 to 24 nt, but are dsRNAs derived from longer double-stranded RNA (dsRNA), including RNAs containing inverted repeats or replicative forms of RNA viruses.

Animals and *C. elegans* encode only a single DICER while *Arabidopsis* encodes four DCL proteins. While studies in *Drosophila* and plants show that DICER plays a role in antiviral defense, currently there is no direct evidence that RNA silencing acts as a natural antiviral defense mechanism in vertebrates. Dicer 2 mutants in *Drosophila* are hypersusceptible to virus infection. All four DCL proteins in *Arabidopsis* are involved in generating siRNAs from DNA and RNA viruses. DCL1 and DCL4 proteins produce 21 nt siRNAs, DCL2 produces 22 nt siRNAs, and DCL3 produces 24 nt siRNAs. While reports show all four DCL proteins contribute to the production of siRNAs from geminiviruses (CaLCuV) and pararetroviruses (cauliflower mosaic virus; CaMV) in plants, DCL2 and DCL4 generate siRNAs for defense against RNA viruses.

Cellular RNA-dependent RNA polymerases (RDR) also contribute to the formation of siRNAs. Single-stranded RNAs are made double stranded by cellular RDRs and then are cleaved by DICER to produce siRNAs. *HEN1* encodes a methyltransferase which acts alongside the DCL proteins to methylate the 3′-terminal nt protecting siRNAs from degradation. siRNAs then guide sequence-specific RNA-induced silencing complexes (RISCs) to target sequences for degradation. The RISC is comprised of several proteins including *ARGONAUTE* (*AGO*), which bind siRNAs or target sequences. RDR proteins use the siRNAs as primers for

synthesis of dsRNAs from target viral RNAs. *Arabidopsis* encodes three *RDR* genes named *RDR1*, *RDR2*, and *RDR6*. *RDR1* is salicylic acid inducible. *Arabidopsis* plants showing defects in RDR1 show increase susceptibility to TMV and TRV. *RDR6* is also known as *SDE1* or *SGS2* and is required for transgene-generated short RNAs. *SDE5* is a factor recently identified which acts with *RDR6* to generate dsRNAs. *SDE5* may function in the nuclear transport of dsRNAs produced by *RDR6*. Mutant plants deficient in *RDR6* show increased susceptibility to cucumber mosaic virus (CMV) but not to other viruses.

Research in plants and in *C. elegans* first showed that a systemic silencing signal which spreads to distal organs is likely to be an siRNA or dsRNA. Research has shown that 21 nt siRNAs can spread 10–15 cells in tobacco and *Arabidopsis* plants. For more extensive movement, additional rounds of signal amplification are needed to produce a second generation of siRNAs. A model of transitive RNA silencing was proposed by Voinnet and colleagues in 2003 in which dsRNAs are synthesized *de novo* by RDR6, which are then cleaved by DICER to generate the second generation of 21 nt siRNAs. Cycles of siRNA propagation and movement into neighboring tissues lead to general silencing throughout surrounding tissues. Systemic silencing spread was proposed to rely on vascular transport of longer 25 nt siRNAs.

RNAi-Based Antiviral Therapies

Viral siRNAs accumulate to significant levels in plants and insects but have not been characterized in human cells infected with any RNA viruses. However, synthesized siRNAs have been shown to block replication and accumulation of a wide range of animal RNA and DNA viruses in cell cultures and vertebrate systems. There are examples of siRNAs targeted to specific sequences in the genomes of viruses including poliovirus (PV), foot and mouth disease virus (FMDV), hepatitis virus A (HVA), influenza virus, SARS-COV, HIV, and hepatitis virus B (HVB) which reduce virus titer and inhibit replication in cell cultures and in mice. Researchers using synthesized RNAs targeting different viral RNA sequences reported this strategy to be a successful form of antiviral therapy. For monopartite RNA viruses such as PV, FMDV, HVA, short RNAs targeting conserved sequences corresponding to genes encoding structural proteins or the viral replicase have been successful.

One of the important issues in developing RNAi therapeutics has been the pressure on target sequences to mutate which causes the virus to escape the suppressive activity of the siRNA molecule. To address this concern researchers have relied on bioinformatics tools and GenBank database to search entries of virus sequences to identify highly conserved nucleic acid elements ranging from 21 to 25 nt in length. Comparisons among virus isolates have been crucial for determining the most conserved regions. Among viruses, which have high mutation rates, therapies combining synthesized short RNA molecules targeting several conserved sequences have been most effective at reducing the occurrence of escape viruses.

The polyomavirus Simian virus 40 (SV40) as well as members of the family *Herpesviridae* including Epstein–Barr virus (EBV), herpes simplex virus 1 (HSV-1), Kaposi's sarcoma-associated virus (KSHV), and were found to encode miRNAs. At least 23 miRNAs have been identified in EBV-infected lymphocytes, many of which map to the BART gene. All BART gene miRNAs accumulate mainly during latency suggesting that they likely play a role in this stage of infection. KSHV is also associated with lymphomas and 12 miRNAs have been identified in latent infected cells. For KSHV a different set of miRNAs are seen during lytic infection suggesting that specific miRNAs are expressed during different stages of the viral life cycle. Viral-encoded miRNAs function to regulate viral gene expression and to downregulate host transcription. Since many of the herpesviral miRNAs associate with latency, they likely play a role in enabling the virus to evade the host immune system for many years. Further research is needed to find out if viral miRNAs affect tumorogenesis and if RNAi technology can be used to alter the onset of cancer.

Viral Suppressors of RNA Silencing Counter Cellular Defenses to Promote Infection

Plant and insect viruses encode silencing suppressor proteins which inhibit one or more steps in the miRNA or siRNA degradation pathway, thus countering the antiviral defense machinery. Many silencing suppressors bind dsRNA, siRNAs, and miRNAs. The potyvirus HC-Pro blocks the RISC from acting on target RNAs (**Figure 1**). HC-Pro was the first identified suppressor of RNA silencing in plants and was discovered by researchers studying viral synergistic diseases. Synergism is a phenomenon in which one virus shows increased titer and symptom induction due to the presence of a second but unrelated virus. Many, but not all, examples of synergy involve co-infection in which one of the viral partners is a potyvirus. Building on the early studies of PVX/PVY synergy, Vicki Vance, James Carrington, and their colleagues carried out further investigations of PVX/PVY synergy at the molecular level. They examined virus-specific RNA production in doubly infected plants and used PVX-derived vectors and transgenic plants to express segments of PVY and other potyviral genomes. Transgenic plants expressing the potyviral P1/HC-Pro sequence developed the same synergistic response when inoculated with unrelated

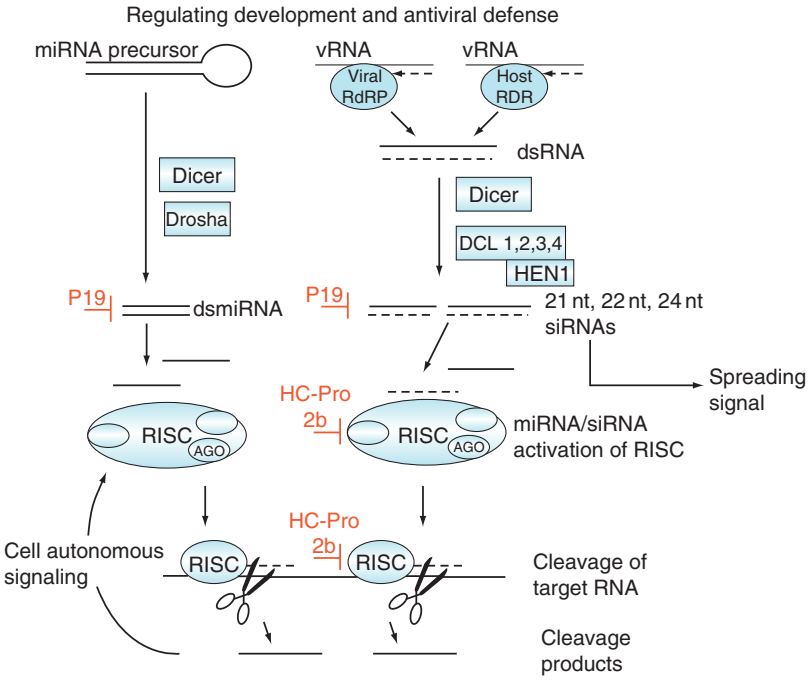


Figure 1 Model for RNA silencing updated from Himber C, Dunoyer P, Moissiard G, Ritzenthaler C, and Voinnet O (2003) Transitivity-dependent and -independent cell-to-cell movement of RNA silencing. *EMBO Journal* 22(17): 4523–4533; Matzke MA and Matzke AJ (2004) Planting the seeds of a new paradigm. *PLoS Biology* 2: E133; Blevins T, Rajeswaran R, Shivaprasad PV, *et al.* (2006) Four plant Dicers mediate viral small RNA biogenesis and DNA virus induced silencing. *Nucleic Acids Research* 34(21): 6233–6246. Double-stranded RNAs (dsRNAs) are produced either by hairpin interactions or by viral and host RNA-dependent RNA polymerases which synthesize complementary RNAs using viral RNA (vRNA) or mRNA templates. Dicer or Dicer-like proteins cleave the dsRNAs to smaller miRNAs or siRNAs which then guide the RISC complex to the target RNAs. Target RNAs are cleaved into short RNAs which can interact with the RISC complex to continue the cycle of cell autonomous RNA degradation. Some siRNAs are diverted prior to the RISC complex and move cell-to-cell and long distance to perpetuate silencing in surrounding and distal tissues. Examples of viral silencing suppressors are identified in red which block silencing by acting on the siRNAs or RISC complex.

viruses such as PVX, TMV, and CMV. P1/HC-Pro silencing suppression was demonstrated by crossing the transgenic plants with transgenic plants that were silencing for the GUS gene. The progeny lines showed restored GUS expression indicating that P1/HC-Pro suppressed transgene silencing. In a second set of experiments the GFP transgene was silencing by PVX vector containing GFP. When P1/HC-Pro was added, GFP expression was restored. Contemporaneously with this work, it was shown that CMV is also able to counteract RNA silencing and that this is mediated by the CMV 2b protein.

A reversal of silencing assay was developed for identification of viral silencing suppressor proteins. This assay uses the GFP expressing *Nicotiana benthamiana* line 16c which is susceptible to GFP silencing by infiltrating leaves with a suspension of *Agrobacterium* carrying the GFP gene. This induces silencing of GFP throughout the entire plant. GFP expression is restored to silenced plants following infection with PVX containing a gene for a silencing suppressor. Variations on this approach include grafting of transgenic plants that are silenced for reporter gene expression to scions that are transgenic for the same reporter gene and for the candidate silencing suppressor protein. This approach was used to demonstrate that the CMV 2b protein inhibits systemic silencing in plants. Using this approach a wide number of viral silencing suppressor proteins have been identified (**Table 1**). Remarkably, some viruses encode more than one silencing suppressor. For example, citrus tristeza virus encodes at least three proteins with silencing suppressor activity, which can confound attempts by the host at generating resistance to the virus.

In general, silencing suppressor proteins inhibit production of siRNAs. Many silencing suppressor proteins bind siRNAs preventing their incorporation into the RISC. The tombusvirus p19 is one example whose crystal structure was recently described. *In vitro* assays show that p19 binds 21 nt siRNAs. The potyvirus P1/HC-Pro acts to block the RISC reducing siRNA accumulation while enhancing miRNA accumulation. The cucumovirus 2b inhibits the spread of the silencing signal but also accumulates in the nucleus where it interferes with silencing-induced DNA methylation.

Several viral silencing suppressor proteins have cross-kingdom activity, that is, they are able to suppress RNA silencing in both insect and plant cells. In a seminal

Table 1 Viral silencing suppressors (April 2007)

Host	*Genome*	*Virus genus*	*Virus name(s)*	*Suppressor(s)*	*Other name/function(s)*
Plant	DNA	*Begomovirus*	Tomato leaf curl virus	C2	Transcriptional activator
			Tomato yellow leaf curl virus	C2	Adenosine kinase inhibitor
			African cassava mosaic virus	AC2, AC4	
			Mungbean yellow mosaic virus	AC2	
			Tomato golden mosaic virus	AL2	
		Curtovirus	Beet curly top virus	L2	
	(+) ssRNA	*Aureusvirus*	Pothos latent virus	P14	
		Benyvirus	Beet necrotic yellow vein virus	P14	Regulate RNA2, CP accumulation
				P31	Enhance P14 activity in roots
		Carmovirus	Turnip crinkle virus	P38	CP
			Hibiscus chlorotic ringspot virus	CP	
		Closterovirus	Beet yellows virus	P21	
			Beet yellow stunt virus	P22	
			Citrus tristeza virus	P20	
				P23	
				CP	
			Grapevine leafroll-associated virus-2	P24	
		Crinivirus	Sweet potato chlorotic stunt virus	P22	
				RNAse 3	
		Comovirus	Cowpea mosaic virus	Small CP	
		Cucumovirus	Cucumber mosaic virus	2b	Systemic movement virulence
			Tomato aspermy virus	2b	
		Furovirus	Soil-borne wheat mosaic virus	19K	
		Hordeivirus	Barley stripe mosaic virus	γb	
			Poa semilatent virus		
		Ipomovirus	Cucumber vein yellowing virus	P1	Protease
		Pecluvirus	Peanut clump virus	P15	
		Polerovirus	Beet western yellows virus		
			Cucurbit aphid-borne yellows virus		
			Potato leafroll virus		
		Potexvirus	Potato virus X	TGBp1	Intercellular movement
		Potyvirus	Potato virus Y	HC-Pro	Systemic movement, protease, transmission
			Tobacco etch virus		
			Turnip mosaic virus		
			Zucchini yellow mosaic virus		
		Sobemovirus	Rice yellow mottle virus	P1	Systemic movement
			Cocksfoot mottle virus		
		Tobamovirus	Tobacco mosaic virus	126K	Replicase component
			Tomato mosaic virus		
		Tobravirus	Tobacco rattle virus	16K	Seed transmission
		Tombusvirus	Artichoke mottled crinkle virus	P19	Virulence, movement
			Carnation Italian ringspot virus		
			Cymbidium ringspot virus		
			Tomato bushy stunt virus		
		Tymovirus	Turnip yellow mosaic virus	P69	Virulence, movement
		Vitivirus	Grapevine virus A	P10	
	(−) ssRNA	*Tenuivirus*	Rice hoja blanca virus	NS3	
		Tospovirus	Tomato spotted wilt virus	NS_S	
	dsRNA	*Phytoreovirus*	Rice dwarf virus	Pns10	
	Viroid	*Pospiviroid*	Potato spindle tuber viroid	RNA secondary structure	
		Avsunviroid	Avocado sunblotch viroid		
Fungus	dsRNA	*Hypovirus*	Cryphonectria hypovirus 1-EP713	p29	Protease

Continued

Table 1 Continued

Host	*Genome*	*Virus genus*	*Virus name(s)*	*Suppressor(s)*	*Other name/function(s)*
Animal	DNA	*Adenovirus*	Adenovirus	VA1 RNA	
		Poxvirus	Vaccinia	E3L	Interferon antagonist
	(+) ssRNA	*Flavivirus*	Hepatitis C virus	Core protein	
		Nodavirus	Flock house virus Nodamura virus Striped jack nervous necrosis virus Greasy grouper nervous necrosis virus	B2	
		Picorna-like	Cricket paralysis virus	N-terminal domain of non-structural protein	
	(−) ssRNA	*Orthomyxovirus*	Influenza A, B, C viruses	NS1	Interferon antagonist
		Orthobunyavirus	La Crosse virus	NS_S	
	Retrovirus	*Lentivirus*	Human immunodeficiency virus-1	Tat	
		Spumavirus	Primate foamy virus type 1	Tas	
	dsRNA	*Orthoreovirus*		σ3	Outer shell protein
	Viroid-like	*Deltavirus*	Hepatitis delta virus	RNA secondary structure	

Updated from tables collated by Bucher E, Lohuis D, van Poppel PM, Geerts-Dimitriadou C, Goldbach R, and Prins M (2006) Multiple virus resistance at a high frequency using a single transgene construct. *Journal of General Virology* 87: 3697–3701; Li F and Ding SW (2006) Virus counterdefense: Diverse strategies for evading the RNA-silencing immunity. *Annual Review of Microbiology* 60: 503–531; Palukaitis and MacFarlane (2006); and Silhavy D and Burgyan J (2004) Effects and side-effects of viral RNA silencing suppressors on short RNAs. *Trends in Plant Science* 9: 76–83.

study, the group of S.W. Ding used both transgenic plants harboring silenced reporter genes and cultured insect cells to demonstrate that the B2 protein of flock house virus (FHV), an insect-infecting virus, was a silencing suppressor. Since then, a variety of insect and vertebrate-infecting, as well as fungus-infecting, viruses have been shown to encode proteins with RNA silencing activity under various assay conditions (**Table 1**). However, the results obtained by assaying viral proteins for silencing suppression in nonhost systems need to be interpreted with caution. For example, the influenza virus NS1 protein, which inhibits the interferon system in human cells, also inhibits RNA silencing in plants and in drosophila cells but not in HeLa cell cultures.

Recently, Deleris and colleagues created lines of *Arabidopsis thaliana* plants carrying single or multiple mutant alleles of the *DCL* genes. These plants offer an additional and less ambiguous method for identifying silencing suppressors, at least for plant viruses. This is illustrated by experiments using a turnip crinkle virus (TCV) derivative in which the coat protein gene, which also functions as a silencing suppressor, was replaced by a *GFP* gene. Compared to wild-type TCV, this modified virus is compromised in movement and symptom induction. However, symptom induction and systemic movement were restored in double *dcl2–dcl4* mutant plants; to the same extent seen in plants constitutively expressing a transgene encoding TCV coat protein.

Viroids are infectious small circular RNAs which do not encode a protein and rely on host DNA-dependent RNA polymerases for replication. Biao Ding's laboratory detected small 21 nt RNAs that are seen in PSTVd-infected plants and are active in RISC-mediated cleavage of target RNAs containing the GFP coding sequence fused to a homologous RNA segment, but the small RNAs do not impact PSTVd accumulation. While further experiments found no indication that PSTVd RNA suppresses silencing, experiments showed that PSTVd secondary structure blocks RISC-mediated cleavage. Thus, instead of suppressing RNA silencing, secondary structure of the PSTVd genome provides protection against degradation by the silencing machinery.

Silencing Suppressors Affect Plant Development

Many plant viral suppressors of RNA silencing have the ability to cause disease by altering the normal course of plant development. This was revealed by analysis of mutant viruses. For example, naturally occurring mutations in the gene for the TMV 126 kDa replicase protein result in a masked (symptomless) phenotype which relates to decreased viral silencing suppression activity and rate of systemic movement. Site-specific mutations introduced into the tobacco vein mottling virus (a potyvirus) HC-Pro gene also altered symptom expression. More drastic forms of mutagenesis, such as complete deletion of the 2b protein gene, created CMV strains that did not induce symptoms.

Transgenic plants expressing known or candidate viral silencing suppressor genes have been used to characterize the effects of the protein on plant gene expression, metabolism, and development, in the absence of virus infection. In most cases, stable expression of RNA silencing suppressors such as potyviral P1/HC-Pro proteins, tombusviral P19 proteins, beet yellows virus P21 protein, TCV CP and ACMV or SLCMV AC4 proteins, strongly disrupted plant development. This was most often seen as stunting of the plants and deformation of the stem, petioles, leaves, and flowers.

In the case of the CMV 2b protein, the strength of the altered phenotype in 2b-transgenic *Arabidopsis* plants corresponded with the severity of the symptoms induced by the strain from which the gene had been obtained. Transgenic expression of a 2b protein from a severe strain strongly inhibited growth of primary roots while both mild and severe strain 2b proteins enhanced the elongation of lateral roots. Overall, the effects of severe strain 2b proteins expressed as transgenes mimicked the phenotypes resulting from mutations in genes regulating the auxin response pathway and in *ago1* mutant plants.

Viral Suppression of the miRNA Pathway

It has been assumed that silencing suppressors alter plant development because they target elements that are common to, or highly similar between, the antiviral siRNA pathway and miRNA-directed regulation of host mRNA accumulation and translation (**Figure 1**). Since miRNAs negatively regulate the mRNA levels of several factors with roles in development (e.g., scarecrow-like factors, auxin response factors etc.), silencing suppressors which have the ability to interfere with components of small RNA-directed pathways can trigger or inhibit aspects of plant development.

Northern analysis has been used to study changes in the levels of miRNAs and target mRNAs accumulation in transgenic *Arabidopsis* plants expressing viral silencing suppressors. This method of analysis has provided evidence that some viral silencing suppressors disrupt of miRNA-regulated gene expression. The technique has also been used to monitor of levels of longer dsRNA species, miRNA precursor transcripts, miRNA duplexes, as well as the mRNA targets and their breakdown products (**Figure 1**). In combination with *in vivo* or *in vitro* studies of RNA or protein binding by silencing suppressor proteins, comparisons of steady-state levels of these various RNA species in nontransgenic versus transgenic plants expressing the silencing suppressors have helped reveal the target(s) of silencing suppressors within the miRNA pathway.

Is interference with miRNA-regulated gene expression entirely due to a case of mistaken identity, in which silencing suppressors accidentally or incidentally inhibit common targets or similar steps within the miRNA and siRNA-directed pathways? In some cases, for example where the mode of action of a silencing suppressor is to bind dsRNAs in a relatively nonsize selective manner, such as the TCV CP or the aureusvirus P14 protein, there would be a greater potential for cross-inhibition between the two pathways. In cases where the silencing suppressor acts by size-selective RNA binding, or can bind selectively to protein components of the silencing pathways (e.g., binding only to specific members of the AGO or DCL protein families), there is the possibility that silencing suppressors discriminate between components of the two RNA silencing pathways (**Figure 1**). Speculatively, this ability to discriminate suggests the possibility that natural selection operates on the genes for silencing suppressor to produce factors that minimize damage to host plants, or produce developmental changes that in some way favor the replication, spread, or transmission of the virus.

A recent breakthrough in animal virus research revealed that HIV-1 infection suppresses the microRNA pathway in a manner that promotes HIV-1 infection, but it is not evident whether this phenomenon depends on a viral silencing suppressor protein. Knockdown of *Dicer* and *Drosha* in HIV-1 infected cells showed that these two RNAse III enzymes contribute to suppression of HIV-1 infection. Microarray experiments identified miRNAs that were upregulated or induced only in HIV-1 infected cells. miR-17/92 is a polycistronic miRNA cluster which is downregulated during HIV-1 replication. miR-17/92 cluster includes miR17–5p and miR20a which target the histone acetylase PCAF, a cofactor for Tat in HIV-1. Thus, the miRNAs do not target HIV-1 but cellular factors necessary for HIV-1 gene expression. Thus, HIV-1 suppression of miR-17/92 cluster ensures a necessary supply of PCAF for virus replication.

See also: Plant Resistance to Viruses: Natural Resistance Associated with Recessive Genes; Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes; Virus Induced Gene Silencing (VIGS).

Further Reading

Blevins T, Rajeswaran R, Shivaprasad PV, *et al.* (2006) Four plant Dicers mediate viral small RNA biogenesis and DNA virus induced silencing. *Nucleic Acids Research* 34(21): 6233–6246.

Bucher E, Lohuis D, van Poppel PM, Geerts-Dimitriadou C, Goldbach R, and Prins M (2006) Multiple virus resistance at a high frequency using a single transgene construct. *Journal of General Virology* 87: 3697–3701.

Cai X, Schafer A, Lu S, *et al.* (2006) Epstein–Barr virus microRNAs are evolutionarily conserved and differentially expressed. *PLoS Pathogens* 2(3): e23.

Deleris A, Gallego-Bartolome J, Bao JS, Kasschau KD, Carrington JC, and Voinnet O (2006) Hierarchical action and inhibition of plant Dicer-like proteins in anti-viral defense. *Science* 313(5783): 68–71.
Galiana-Arnoux D, Dostert C, Schneemann A, Hoffmann JA, and Imler JL (2006) Essential function *in vivo* for Dicer-2 in host defense against RNA viruses in drosophila. *Nature Immunology* 7(6): 590–597.
Gitlin L, Stone JK, and Andino R (2005) Poliovirus escape from RNA interference: Short interfering RNA-target recognition and implications for therapeutic approaches. *Journal of Virology* 79(2): 1027–1035.
Hamilton A, Voinnet O, Chappell L, and Baulcombe D (2002) Two classes of short interfering RNA in RNA silencing. *EMBO Journal* 21 (17): 4671–4679.
Hamilton AJ and Baulcombe DC (1999) A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* 286(5441): 950–952.
Himber C, Dunoyer P, Moissiard G, Ritzenthaler C, and Voinnet O (2003) Transitivity-dependent and -independent cell-to-cell movement of RNA silencing. *EMBO Journal* 22(17): 4523–4533.
Li F and Ding SW (2006) Virus counterdefense: Diverse strategies for evading the RNA-silencing immunity. *Annual Review of Microbiology* 60: 503–531.
Li HW, Li WX, and Ding SW (2002) Induction and suppression of RNA silencing by an animal virus. *Science* 296(5571): 1319–1321.
Marathe R, Anandalakshmi R, Smith TH, Pruss GJ, and Vance VB (2000) RNA viruses as inducers, suppressors and targets of post-transcriptional gene silencing. *Plant Molecular Biology* 43(2–3): 295–306.
Matzke MA and Matzke AJ (2004) Planting the seeds of a new paradigm. *PLOS Biology* 2: E133.
Pfeffer S, Sewer A, Lagos-Quintana M, *et al.* (2005) Identification of microRNAs of the herpesvirus family. *Nature Methods* 2(4): 269–276.
Pfeffer S and Voinnet O (2006) Viruses, microRNAs and cancer. *Oncogene* 25(46): 6211–6219.
Segers GC, van Wezel R, Zhang XM, Hong YG, and Nuss DL (2006) Hypovirus papain-like protease p29 suppresses RNA silencing in the natural fungal host and in a heterologous plant system. *Eukaryotic Cell* 5(6): 896–904.
Silhavy D and Burgyan J (2004) Effects and side-effects of viral RNA silencing suppressors on short RNAs. *Trends in Plant Science* 9: 76–83.

Viroids

R Flores, Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Valencia, Spain
R A Owens, Beltsville Agricultural Research Center, Beltsville, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Catalytic RNA RNA molecules that are able to catalyze, in a protein-free medium, specific reactions involving the formation or breakage of covalent bonds. In nature, these reactions are usually transesterifications (self-cleavage and ligation) affecting the catalytic RNA itself.
Hammerhead structure The conserved secondary/tertiary structure shared by the smallest class of natural ribozymes. Most have been found in one or both strands of certain viroid and viroid-like satellite RNAs where they mediate self-cleavage of multimeric intermediates arising from replication through a rolling-circle mechanism.
Ribozyme RNA motif responsible for the catalytic activity of certain RNA molecules. In nature, they are found embedded within catalytic RNAs.

Introduction

Viroids are the smallest known agents of infectious disease – small (246–401 nt), highly structured, circular, single-stranded RNAs that lack detectable messenger RNA activity. While viruses have been described as 'obligate parasites of the cell's translational system' and supply some or most of the genetic information required for their replication, viroids can be regarded as 'obligate parasites of the cell's transcriptional machinery'. Thus far, viroids are known to infect only plants.

The first viroid disease to be studied by plant pathologists was potato spindle tuber. In 1923, its infectious nature and ability to spread in the field led Schultz and Folsom to group potato spindle tuber disease with several other 'degeneration diseases' of potatoes. Nearly 50 years were to elapse before Diener's demonstration in 1971 that the molecular properties of its causal agent, potato spindle tuber viroid (PSTVd), were fundamentally different than those of conventional plant viruses.

Genome Structure

Efforts to understand how viroids replicate and cause disease without the assistance of any viroid-encoded polypeptides have prompted detailed analysis of their structure. Viroids possess rather unusual properties for single-stranded RNAs (e.g., a pronounced resistance to digestion by ribonuclease and a highly cooperative thermal denaturation profile), leading to an early realization that they might have an unusual higher-order structure.

To date, the complete sequences of 29 distinct viroid species plus a large number of sequence variants have

been determined (**Table 1**). All are single-stranded circular RNAs containing 246–401 unmodified nucleotides. Theoretical calculations and physicochemical studies indicate that PSTVd and related viroids assume a highly base-paired, rod-like conformation *in vitro* (**Figure 1**). Pairwise sequence comparisons suggest that the series of short double helices and small internal loops that comprise this so-called 'native' structure are organized into five domains whose boundaries are defined by sharp differences in sequence similarity.

The 'central domain' is the most highly conserved viroid domain and contains the site where multimeric PSTVd RNAs are cleaved and ligated to form circular progeny. The 'pathogenicity domain' contains one or more structural elements which modulate symptom expression, and the relatively small 'variable domain' exhibits the greatest sequence variability between otherwise closely related viroids. The two 'terminal domains' appear to play an important role in viroid replication and evolution. Although these five domains were first identified in PSTVd, apple scar skin viroid (ASSVd) and related viroids also contain a similar domain arrangement. Certain viroids such as *Columnea* latent viroid (CLVd), Australian grapevine viroid (AGVd), and *Coleus blumei* viroid 2 (CbVd 2) appear to be 'mosaic molecules' formed by exchange of domains between two or more viroids infecting the same cell. RNA rearrangement/recombination can also occur within individual domains, leading, in coconut cadang-cadang (CCCVd) and citrus exocortis (CEVd) viroids, to duplications of the right terminal domain plus part of the variable domain. This domain model is not shared by avocado sunblotch (ASBVd) and related viroids.

Much less is known about viroid tertiary structure, especially *in vivo* where these molecules almost certainly accumulate as ribonucleoprotein particles. UV-induced

Table 1 Classification of viroids of known nucleotide sequence

Family[a]	*Genus*[a]	*Name*	*Abbreviation*	*Nucleotides*[b]
Pospiviroidae	*Pospiviroid*	Chrysanthemum stunt	CSVd	354–356
		Citrus exocortis	CEVd	368–375 (463–467)
		Columnea latent	CLVd	370–373
		Iresine	IrVd	370
		Mexican papita	MPVd	359–360
		Potato spindle tuber	PSTVd	356–361 (341)
		Tomato apical stunt	TASVd	360–363
		Tomato chlorotic dwarf	TCDVd	360
		Tomato planta macho	TPMVd	359–360
	Cocadviroid	Citrus viroid IV	CVd-IV	284
		Coconut cadang-cadang	CCCVd	246–247 (287–301)
		Coconut tinangaja	CTiVd	254
		Hop latent	HLVd	256
	Hostuviroid	Hop stunt[c]	HSVd	294–303
	Apscaviroid	Apple dimple fruit	ADFVd	306,307
		Apple scar skin[d]	ASSVd	329–334
		Australian grapevine	AGVd	369
		Citrus bent leaf	CBLVd	315,318
		Citrus dwarfing	CDVd	294,297
		Grapevine yellow speckle 1	GVYSVd 1	366–368
		Grapevine yellow speckle 2[e]	GYSVd 2	363
		Pear blister canker	PBCVd	315,316
	Coleviroid	*Coleus blumei* 1	CbVd 1	248–251
		Coleus blumei 2	CbVd 2	301,302
		Coleus blumei 3	CbVd 3	361–364
Avsunviroidae	*Avsunviroid*	Avocado sunblotch	ASBVd	246–251
	Pelamoviroid	Chrysanthemum chlorotic mottle	CChMVd	398–401
		Peach latent mosaic	PLMVd	335–351
	Elaviroid	Eggplant latent	ELVd	332–335

[a]Classification follows scheme proposed by Flores *et al*. (see VIII Report of the International Committee on Taxonomy of Viruses) with minor modifications. The nucleotide sequences of blueberry mosaic, burdock stunt, *Nicotiana glutinosa* stunt, pigeon pea mosaic mottle, and tomato bunchy top viroids are currently unknown; consequently, these viroids have not been assigned to specific genera. Whether apple fruit crinkle and citrus viroid original source should be considered variants or new viroid species of genus *Apscaviroid* is pending.
[b]Sizes of variants containing insertions or deletions arising *in vivo* are shown in parentheses.
[c]Includes cucumber pale fruit, citrus cachexia, peach dapple, and plum dapple viroids.
[d]Includes pear rusty skin and dapple apple viroids.
[e]Formerly termed grapevine viroid 1B.

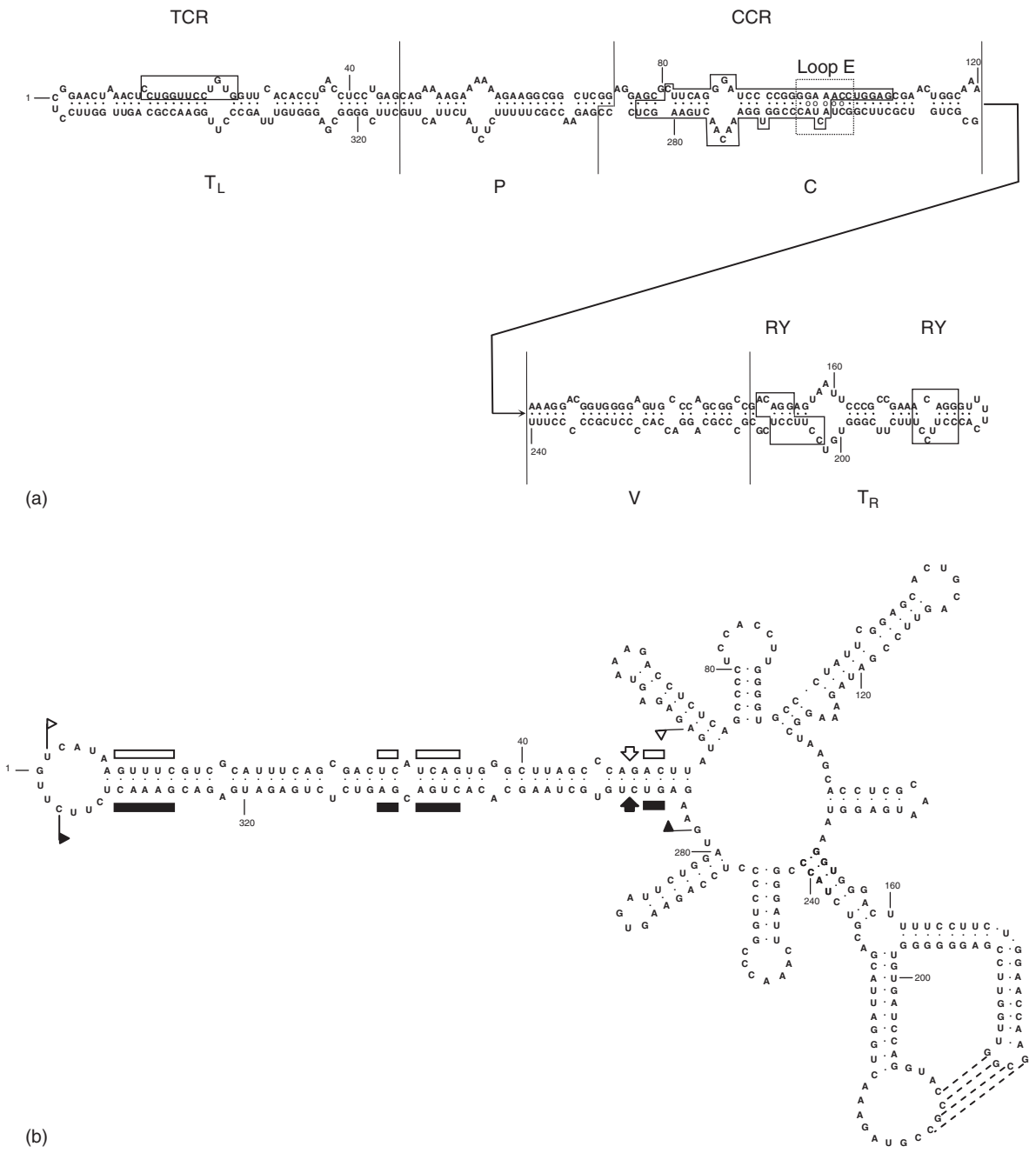


Figure 1 (a) The rod-like secondary structure of PSTVd (intermediate strain) showing the five domains characteristic of members of the family *Pospiviroidae:* the terminal left (T_L), pathogenicity (P), central (C), variable (V), and terminal right (T_R). The central conserved region (CCR) is located within the C domain and contains a UV-sensitive loop E motif with noncanonical base pairs (denoted by circles). The T_L domains of genera *Pospiviroid* and *Apscaviroid* contain a terminal conserved region (TCR), while those of genera *Hostuviroid* and *Cocadviroid* contain a terminal conserved hairpin (not shown). The T_R may also contain 1–2 copies of a protein-binding RY motif. (b) The branched secondary structure of PLMVd (reference variant). Plus and minus self-cleavage domains are indicated by flags, nucleotides conserved in most natural hammerhead structures by bars, and the self-cleavage sites by arrows. Black and white symbols refer to plus and minus polarities, respectively. Nucleotides involved in a pseudoknot are indicated by broken lines. Redrawn with modifications from Gross HJ, Domdey H, Lossow C, *et al*. (1978) Nucleotide sequence and secondary structure of potato spindle tuber viroid. *Nature* 273: 203–208; Hernández C and Flores R (1992) Plus and minus RNAs of peach latent mosaic viroid self-cleave *in vitro* through hammerhead structures. *Proceedings of the National Academy of Sciences, USA* 89: 3711–3715; Bussière F, Ouellet J, Côté F, Lévesque D, and Perreault JP (2000) Mapping in solution shows the peach latent mosaic viroid to possess a new pseudoknot in a complex, branched secondary structure. *Journal of Virology* 74: 2647–2654.

cross-linking of two nucleotides within a loop E motif in the central domain of PSTVd provided the first definitive evidence for such tertiary interactions. Similar UV-sensitive structural elements have also been discovered in a number of other RNAs including 5S eukaryotic rRNA, adenovirus VAI RNA, and the viroid-like domain of the hepatitis delta virus genome. Loop E forms during the conversion of multimeric PSTVd RNAs into monomers. The ability of ASBVd-related RNAs to undergo spontaneous self-cleavage mediated by hammerhead ribozymes as well as the presence of pseudoknots critical for infectivity in some other members of the *Avsunviroidae* (**Figure 1**) provide additional evidence for the functional importance of viroid tertiary structure.

Classification

Based upon differences in the structural and functional properties of their genomes, viroids species are assigned to one of two taxonomic families (see **Table 1**). Members of the family *Pospiviroidae* (type member PSTVd) have a rod-like secondary structure that contains five structural–functional domains and several conserved motifs. Most members of the family *Avsunviroidae* (type member ASBVd), in contrast, appear to adopt a branched conformation, and multimeric RNAs of all family members behave as catalytic RNAs and undergo spontaneous self-cleavage (**Figure 1**). Differences in their sites of replication also support this classification scheme; that is, PSTVd and ASBVd replicate in the nucleus and the chloroplast, respectively, and the same appears to occur for other members of each family. Each family is subdivided into genera according to certain demarcating criteria. Groups of sequence variants that show >90% sequence identity in pairwise comparisons and share some common biological property are arbitrarily defined as viroid species. *In vivo*, each viroid species is actually a 'quasispecies', that is, a collection of closely related sequences subject to a continuous process of variation, competition, and selection. There is phylogenetic evidence for an evolutionary link between viroids and other viroid-like subviral RNAs (**Figure 2**).

Host Range and Transmission

All viroids are mechanically transmissible, and most are naturally transmitted from plant to plant by man and his tools. Individual viroids vary greatly in their ability to infect different plant species. PSTVd can replicate in about 160 primarily solanaceous hosts, while only two members of the family Lauraceae are known to support ASBVd replication. HSVd has a particularly wide host range that includes herbaceous species as well as woody perennials. Many natural hosts are either vegetatively propagated or crops that are subjected to repeated grafting or pruning operations. PSTVd, ASBVd, and CbVd1 are vertically transmitted through pollen and/or true seed, but the significance of this mode of transmission in the natural spread of disease is unclear. PSTVd can be encapsidated by the coat protein of potato leafroll virus (PLRV, a polerovirus) as well as velvet tobacco mottle virus (VTMoV, a sobemovirus), and epidemiological surveys suggest that PLRV facilitates viroid spread under field conditions.

Commonly used techniques for the experimental transmission of viroids include the standard leaf abrasion methods developed for conventional viruses, 'razor slashing' methods in which phloem tissue in the stem or petiole is inoculated via cuts made with a razor blade previously dipped into the inoculum, and, in the case of CCCVd, high-pressure injection into folded apical leaves. Viroids can also be transmitted by either plant transformation or 'agroinoculation' during which a modified *Agrobacterium tumefaciens* Ti plasmid is used to introduce full-length viroid-complementary DNA into the potential host cell. Either technique can overcome the marked resistance of some hosts to mechanical inoculation. Identification of the molecular mechanism(s) that determine viroid host range remains an important research goal.

Symptomatology

Viroids and conventional plant viruses induce a very similar range of macroscopic symptoms. Symptom expression is usually optimal at the same relatively high temperatures (30–33 °C) that promote viroid replication. Stunting and leaf epinasty (a downward curling of the leaf lamina resulting from unbalanced growth within the various cell layers) are considered the classic symptoms of viroid infection. Other commonly observed symptoms include vein clearing, veinal discoloration or necrosis, and the appearance of localized chlorotic/necrotic spots or mottling in the foliage. Symptoms may also be expressed in flowers and bark, and fruits or tubers from viroid-infected plants may be abnormally shaped or discolored. Viroid infection of certain citrus rootstock/scion combinations may result in tree dwarfing (**Figure 3**). Viroid infections are often latent and rarely kill the host.

Viroid infections are also accompanied by a number of cytopathic effects – chloroplast and cell wall abnormalities, the formation of membranous structures in the cytoplasm, and the accumulation of electron-dense deposits in both chloroplasts and cytoplasm. Metabolic changes include dramatic alterations in growth regulator levels.

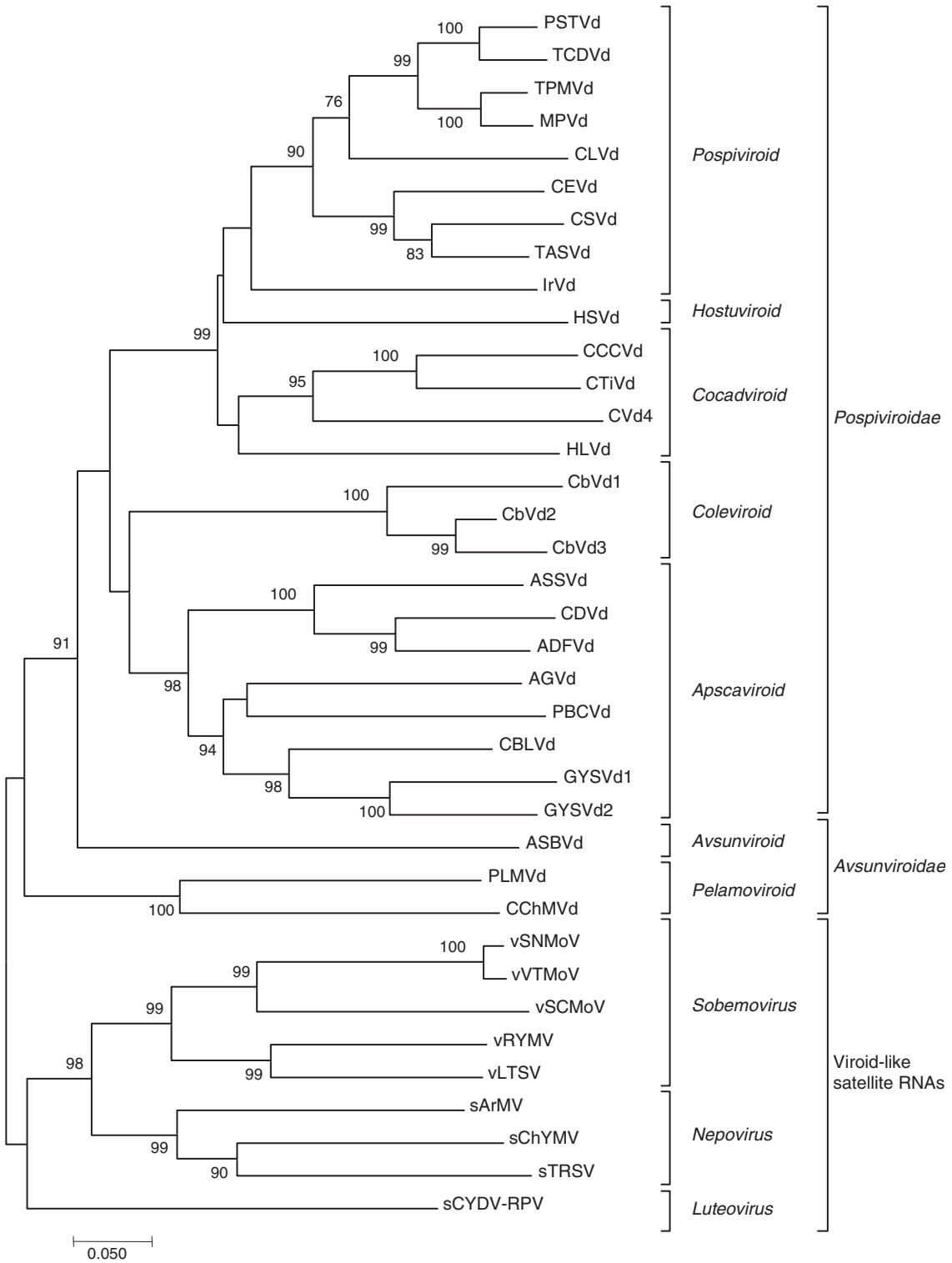


Figure 2 Neighbor-joining phylogenetic tree obtained from an alignment manually adjusted to take into account local similarities, insertions/deletions, and duplications/rearrangements described in the literature for viroid and viroid-like satellite RNAs. Bootstrap values were based on 1000 random replicates (only values >70% are shown). Viroid abbreviations are those used in **Table 1**. Viroid-like satellite RNAs: lucerne transient streak virus (sLTSV); rice yellow mottle virus (sRYMV); subterranean clover mottle virus (sSCMoV); *Solanum nodiflorum* mottle virus (sSNMoV)); velvet tobacco mottle virus (sVTMoV); tobacco ringspot virus (sTRSV); *Arabis* mosaic virus (sArMV); chicory yellow mottle virus (sChYMV); cereal yellow dwarf virus-RPV (sCYDV-RPV). Adapted from Elena SF, Dopazo J, de la Peña M, Flores R, Diener TO, and Moya A (2001) Phylogenetic analysis of viroid and viroid-like satellite RNAs from plants: A reassessment. *Journal of Molecular Evolution* 53: 155–159.

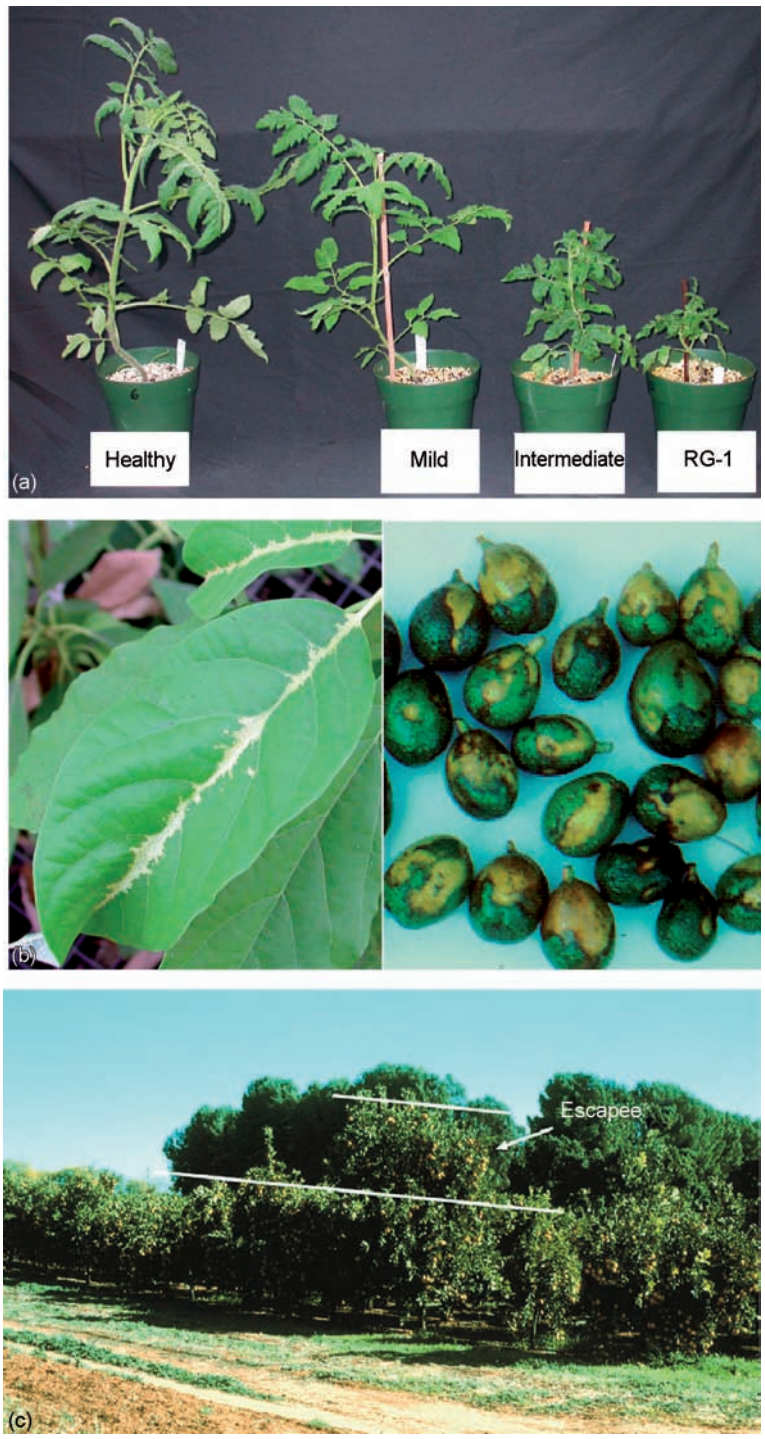


Figure 3 (a) Symptoms of PSTVd infection in Rutgers tomato approximately 4 weeks after inoculation of cotyledons with PSTVd strains causing mild, intermediate, and severe symptoms. (b) Symptoms of ASBVd infection in avocado fruits and leaves. (c) Viroid-induced dwarfing of citrus growing on susceptible rootstocks: All trees in the block were graft-inoculated with CDVd shortly after transfer to the field; only one tree (right foreground) escaped infection. Note the difference in height.

Geographic Distribution

Although PSTVd, HSVd, CEVd, and ASBVd are widely distributed throughout the world, other viroids have never been detected outside the areas where they were first reported. Several factors may contribute to this variation in distribution pattern. Among the crops most affected by viroid diseases are a number of valuable woody perennials such as grapes, citrus, various pome and stone fruits, and hops. Propagation and distribution of improved cultivars is

highly commercialized, with the result that many cultivars are now grown worldwide. The international exchange of plant germplasm also continues to increase at a rapid rate. In both instances, the large number of latent (asymptomatic) hosts facilitates viroid spread.

Epidemiology and Control

Viroid diseases pose a potential threat to agriculture, and several are of considerable economic importance. Ready transmission of PSTVd by vegetative propagation, foliar contact, and true seed or pollen continues to pose a serious threat to potato germplasm collections and breeding programs. Coconut cadang-cadang has killed over 30 million palms in the Philippines since it was first recognized in the early 1930s. While many viroids were first detected in ornamental or crop plants, most viroid diseases are thought to result from chance transfer from endemically infected wild species to susceptible cultivars. Several lines of circumstantial evidence are consistent with this hypothesis:

1. The experimental host ranges of several viroids include many wild species, and these wild species often tolerate viroid replication without the appearance of recognizable disease symptoms.
2. Although co-evolution of host and pathogen is often accompanied by appearance of gene-for-gene vertical resistance, no useful sources of resistance to PSTVd infection have been identified in the cultivated potato.
3. Viroids and/or viroid-related RNAs closely related to TPMVd and CCCVd have been detected in weeds and other wild vegetation growing near fields containing viroid-infected plants.

Growers and plant pathologists are unlikely to have simply overlooked diseases with symptoms as severe as those of chrysanthemum stunt or cucumber pale fruit, two diseases first reported after World War II. Large-scale monoculture of genetically identical crops and the commercial propagation/distribution of many cultivars are two comparatively modern developments which would facilitate the development of serious disease problems following the chance transfer of viroids from wild hosts to cultivated plants. Viroid diseases may also arise by transfer between cultivated crop species. For example, pears provide a latent reservoir for ASSVd; likewise, while HSVd infections of grapes are often symptomless, this viroid causes severe disease in hops. In both instances, the two crops are often grown in close proximity.

Because no useful sources of natural resistance to viroid disease are known, diagnostic tests continue to play a key role in efforts to control viroid diseases. Since viroids lack a protein capsid, the antibody-based techniques used to detect many plant viruses are not applicable. Tests based upon their unique molecular properties have largely supplanted biological assays for viroid detection. Problems with viroid bioassays include the length of time required for completion (weeks to years) and difficulties in detecting mild or latent strains. Several rapid (1–2 day) protocols involving polyacrylamide gel electrophoresis (PAGE) under denaturing conditions take advantage of the circular nature of viroids. Using these protocols, nanogram amounts of viroid can be unambiguously detected without the use of radioactive isotopes. In recent years, diagnostic procedures based upon nucleic acid hybridization or the polymerase chain reaction (PCR) are being widely used. The simplest methods involve the hybridization of a nonradioactively labeled viroid-complementary DNA or RNA probe to viroid samples that have been bound to a solid support followed by colorimetric or chemiluminescent detection of the resulting DNA–RNA or RNA–RNA hybrids. Such conventional 'dot blot' assays can detect picogram amounts of viroids using clarified plant sap or tissue prints rather than purified nucleic acid as the viroid source. PCR-based protocols are finding increasing acceptance in those cases where either this level of sensitivity is inadequate or a number of closely related viroids are present in the same sample.

Molecular Biology

Although devoid of messenger RNA activity, viroids replicate autonomously and cause disease in a wide variety of plants. Much has been learned about the molecular biology of viroids and viroid–host interaction over the past 25 years, but the precise nature of the molecular signals involved remains elusive. A series of questions first posed by Diener summarizes the many gaps in our current understanding of the biological properties of these unusual molecules:

1. What molecular signals do viroids possess (and cellular RNAs evidently lack) that induce certain DNA-dependent RNA polymerases to accept them as templates for the synthesis of complementary RNA molecules?
2. What are the molecular mechanisms responsible for viroid replication? Are these mechanisms operative in uninfected cells? If so, what are their functions?
3. How do viroids induce disease? In the absence of viroid-specified proteins, disease must arise from direct interaction(s) of viroids (or viroid-derived RNA molecules) with host-cell constituents. Infections by PSTVd and ASBVd induce RNA silencing (see below).
4. What determines viroid host range? Are viroids restricted to higher plants, or do they have counterparts in animals?
5. How did viroids originate?

Replication

A variety of multimeric plus- and minus-strand RNAs have been detected by nucleic acid hybridization in viroid-infected tissues. Based on their analysis, viroid replication has been proposed to proceed via a 'rolling circle' mechanism that involves reiterative transcription of the incoming plus circular RNA to produce a minus-strand RNA template. ASBVd and related viroids utilize a symmetric replication cycle in which the multimeric minus strand is cleaved to unit-length molecules and circularized before serving as template for the synthesis of multimeric plus strands. PSTVd and related viroids utilize an asymmetric cycle in which the multimeric minus strand is directly transcribed into multimeric plus strands. In both cases, the multimeric plus strands are cleaved to unit-length molecules and circularized.

A diversity of host-encoded enzymes have been implicated in viroid replication. Low concentrations of α-amanitin specifically inhibit the synthesis of both PSTVd plus and minus strands in nuclei isolated from infected tomato, strongly suggesting the involvement of DNA-dependent RNA polymerase II, transcribing an RNA template, in the replication of PSTVd and related viroids. In nuclear extracts, transcription of the PSTV plus strand by RNA polymerase II starts in the left terminal loop; furthermore, incubation of active replication complexes containing CEVd with a monoclonal antibody directed against the carboxy-terminal domain of RNA polymerase II results in the immunoprecipitation of both CEVd plus- and minus-strand RNAs. Mature PSTVd plus strands accumulate in the nucleolus and the nucleoplasm, while *in situ* hybridization indicates that minus-strand RNAs are confined to the nucleoplasm. The identity of the polymerase(s) responsible for replication of members of the family *Avsunviroidae* in the chloroplast is less certain. ASBVd synthesis is resistant to tagetitoxin, strongly indicating the involvement of a nuclear-encoded chloroplastic RNA polymerase. Initiation sites for both ASBVd plus- and minus-strand synthesis have been mapped to the AU-rich terminal loops of their respective native structures.

In vitro evidence indicates that specific cleavage of multimeric PSTVd plus-strand RNAs requires (1) rearrangement of the conserved central region to form a branched structure containing a GNRA tetraloop and (2) the action of one or more host-encoded nucleases. Other less-efficient processing sites can also be used *in vivo*. Plus- and minus-strand RNAs of ASBVd and related viroids, in contrast, undergo spontaneous self-cleavage through hammerhead ribozymes to form linear monomers (**Figure 4**). Addition of certain chloroplast proteins acting as RNA chaperones facilitates this hammerhead ribozyme-mediated self-cleavage reaction. The final step in viroid replication is the ligation of linear monomers to form mature circular progeny. Plant cells are known to contain RNA ligase activities which can act upon the 5′ hydroxyl and 2′,3′ cyclic phosphate termini formed by either cleavage pathway.

Movement

Upon entering a potential host cell, viroids must move to either the nucleus (*Pospiviroidae*) or chloroplast (*Avsunviroidae*) before beginning replication. Available data suggest that PSTVd enters the nucleus as a ribonucleoprotein complex formed by the interaction of cellular proteins with specific viroid sequence or structural motifs. VirP1, a bromodomain-containing protein isolated from tomato, has a nuclear localization signal and binds to the terminal right domain of PSTVd. Proteins such as TFIIIA and ribosomal protein L5 that bind to the loop E motif may also be involved in viroid transport into the nucleus. How ASBVd or other members of the family *Avsunviroidae* enter and exit the chloroplast is currently unknown.

To establish a systemic infection, viroids leave the initially infected cell – moving first from cell to cell and then long distances through the host vasculature. Upon injection into symplasmically isolated guard cell in a mature tomato leaf, fluorescently labeled PSTVd RNA does not move. Injection into interconnected mesophyll cells, in contrast, is followed by rapid cell-to-cell movement through the plasmodesmata. Long-distance movement of viroids, like that of nearly all plant viruses, occurs in the phloem where it follows the typical source-to-sink pattern of photoassimilate transport. Viroid movement in the phloem almost certainly requires formation of a ribonucleoprotein complex, possibly involving a dimeric lectin known as phloem protein 2 (PP2), the most abundant protein in phloem exudate. Movement of PSTVd in the phloem appears to be sustained by replication in supporting cells and is tightly regulated by developmental and cellular factors. For example, *in situ* hybridization reveals the presence of PSTVd in vascular tissues underlying the shoot apical meristem of infected tomato, but entry into the shoot apical meristem itself appears to be blocked. Another important control point for PSTVd trafficking is the bundle sheath–mesophyll boundary in the leaf. By disrupting normal pattern of viroid movement, it may be possible to create a plant that is resistant/immune to viroid infection.

Pathogenicity

Sequence comparisons of naturally occurring PSTVd and CEVd variants as well as infectivity studies with chimeric viroids, constructed by exchanging the pathogenicity domains of mild and severe strains of CEVd, have clearly shown that the pathogenicity domain in the family *Pospiviroidae* contains important determinants of symptom expression. Symptom expression is also affected by the

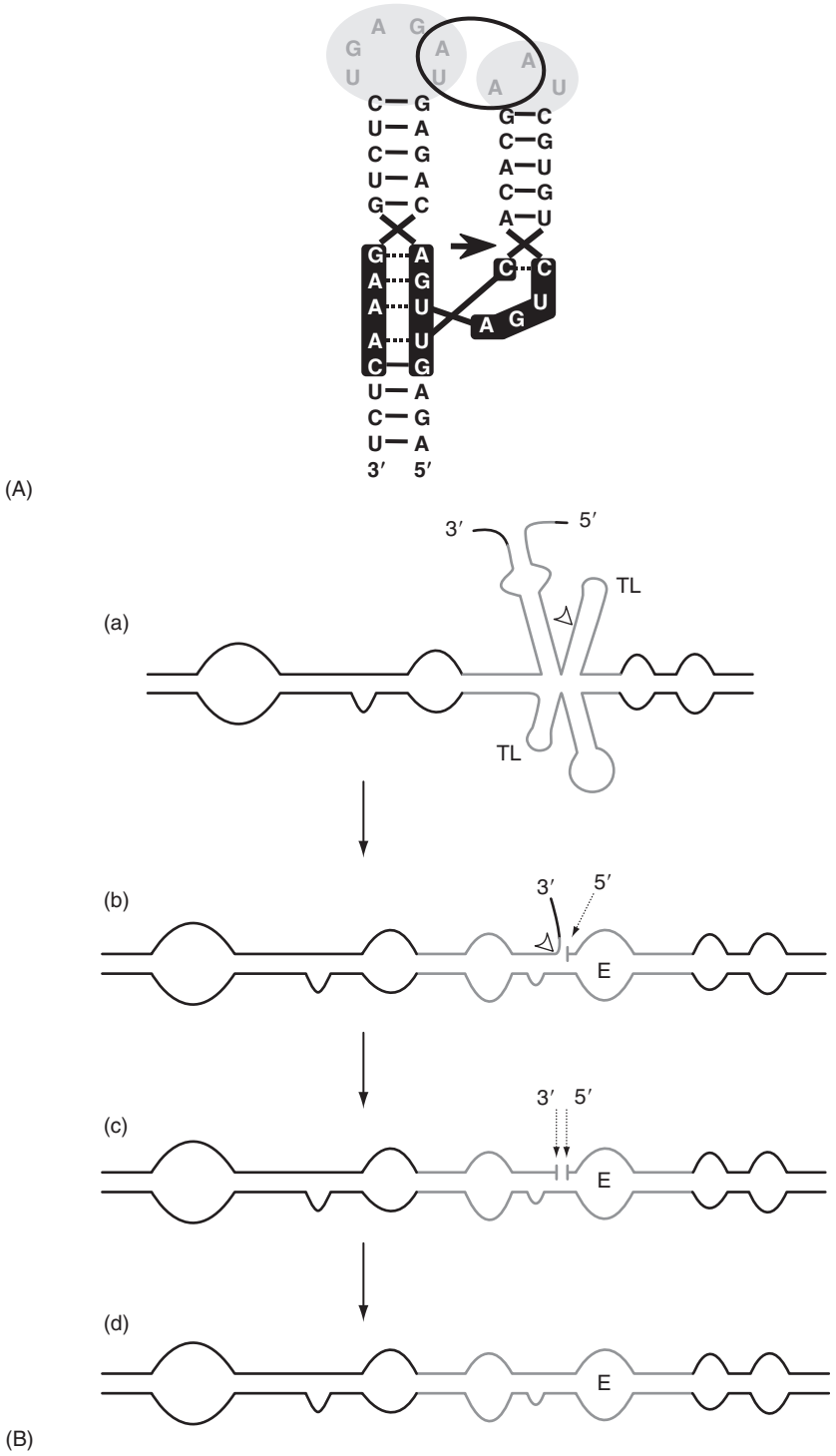


Figure 4 Cleavage of multimeric viroid RNAs requires rearrangement of the native structure. (A) During transcription, the strands of both polarities of members of the family *Avsunviroidae* can fold into hammerhead structures (here illustrated for the hammerhead of the PLMVd plus RNA) and self-cleave accordingly. Nucleotides conserved in most natural hammerhead structures are on a black background, and the self-cleavage site is denoted by an arrow. A circle delimits the presumed tertiary interaction between terminal loops enhancing the catalytic activity. Watson-Crick and noncanonical base pairs are represented by continuous and discontinuous lines, respectively. After self-cleavage, the RNA adopts a new conformation favoring ligation (or self-ligation). (B) Processing of a longer-than-unit-length plus PSTVd RNA transcript in a potato nuclear extract. The central conserved region of the substrate for the first cleavage reaction (a) contains a tetraloop (denoted TL). After dissociation of the 5′ segment from the cleavage site, the new 5′ end refolds and is stabilized by formation of a UV-sensitive loop E (b), while the 3′ end partially base-pairs with the lower strand. Single-stranded nucleotides at the 3′ end are then cleaved between positions 95 and 96 (c), and ligation of the 5′ and 3′ termini (d) results in formation of mature circular progeny. From Baumstark T, Schröder ARW, and Riesner D (1997) Viroid processing: Switch from cleavage to ligation is driven by a change from a tetraloop to a loop E conformation. *EMBO Journal* 16: 599–610.

rate of viroid replication, and sequence changes in the variable domain have been shown to regulate progeny titers in infected plants. Studies with TASVd revealed the presence of a third pathogenicity determinant in the left terminal loop. Also, a single U/A change position 257 in the central domain of PSTVd results in the appearance of severe stunting and a 'flat top' phenotype. In the family *Avsunviroidae,* determinants of pathogenicity have been mapped to either a tetraloop capping a hairpin stem in chrysanthemum chlorotic mottle viroid (CChMVd) or an insertion that folds into a hairpin also capped by a tetraloop in peach latent mosaic viroid (PLMVd).

The ability of novel viroid chimeras to replicate and move normally from cell to cell implies certain basic similarities between their structures *in vitro* and *in vivo* but provides no information about the nature of the molecular interactions responsible for symptom development. Until recently, it was widely assumed that the mature viroid RNA was the direct pathogenic effector. Just like viruses, however, viroid replication is also accompanied by the production of a variety of small (21–26 nt) RNA molecules. The role of these small interfering RNAs (siRNAs) in viroid pathogenicity is not yet clear, but the inverse relationship between accumulation levels of the mature viroid RNAs and the corresponding siRNAs for members of the family *Avsunviroidae* suggest that the latter may regulate the titer of the former. Also, recovery of tomato plants from the symptoms of severe PSTVd infections is preceded by the accumulation of PSTVd-specific siRNA.

Viroid infections are accompanied by quantitative changes in a variety of host-encoded proteins. Certain of these are 'pathogenesis-related' proteins whose synthesis or activation is part of a general host reaction to biotic or abiotic stress, but others appear to be more specific. In tobacco, PSTVd infection results in the preferential phosphorylation of a host-encoded 68 kDa protein that is immunologically related to an interferon-inducible, dsRNA-dependent mammalian protein kinase of similar size. The human kinase is differentially activated by PSTVd strains of varying pathogenicity *in vitro*, while infection of tomato by intermediate or severe strains of PSTVd induces the synthesis of PKV, a dual-specificity, serine/threonine protein kinase. Broad changes in host gene expression following PSTVd infection have been detected by complementary DNA macroarray analysis.

Host Range

Possibly as a result of its involvement in the cleavage/ligation of progeny RNA, nucleotides in the central domain of PSTVd and related viroids appear to play an important role in determining host range. For example, a single nucleotide substitution in the loop E motif results in a dramatic increase in the rate of PSTVd replication in tobacco. The biological properties of CLVd also suggest that this domain contains one or more host-range determinants. CLVd appears to be a natural mosaic of sequences present in other viroids; phylogenetic analysis (see **Figure 2**) suggests that it can be considered to be a PSTVd-related viroid whose conserved central domain has been replaced by that of HSVd. Like HSVd (but not PSTVd or related viroids), CLVd can replicate and cause disease in cucumber.

Origin and Evolution

Much of the early speculation about viroid origin involved their possible origin as 'escaped introns' (i.e., descent from normal host RNAs). More recently, however, viroids have been proposed to represent 'living fossils' of a precellular RNA world that assumed an intracellular mode of existence sometime after the evolution of cellular organisms. The presence of ribozymes in members of the *Avsunviroidae* strongly supports this view.

The inherent stability of viroids and viroid-like satellite RNAs (structurally similar to viroids but functionally dependent on helper viruses) which arises from their small size and circularity would have enhanced the probability of their survival in primitive, error-prone RNA self-replicating systems and assured their complete replication without the need for initiation or termination signals. Most viroids (but not satellite RNAs or random sequences of the same base composition) also display structural periodicities with repeat units of 12, 60, or 80 nt. The high error rate of prebiotic replication systems may have favored the evolution of polyploid genomes, and the mechanism of viroid replication (i.e., rolling-circle transcription of a circular template) provides an effective means of genome duplication.

Viroids and viroid-like satellite RNAs all possess efficient mechanisms for the precise cleavage of their oligomeric replication intermediates to form monomeric progeny. PSTVd and related viroids appear to require proteinaceous host factor(s) for cleavage, but others (members of the family *Avsunviroidae* and viroid-like satellite RNAs) contain ribozymes far smaller and simpler than those derived from introns. Thus, ASBVd and the other self-cleaving viroids may represent an evolutionary link between viroids and viroid-like satellite RNAs. No viroid is known to code for protein, a fact that is consistent with the possibility that viroids are phylogenetically older than introns.

Phylogenetic evidence for an evolutionary link between viroids and other viroid-like subviral RNAs has been presented by Elena *et al.* (see **Figure 2**). Among several subviral RNAs possibly related to viroids is carnation small viroid-like RNA, a 275 nt circular molecule with self-cleaving hammerhead structures in both its plus and minus strands that has a DNA counterpart. This novel retroviroid-like element shares certain features with both viroids and a small RNA transcript from newt.

See also: Hepatitis Delta Virus; Origin of Viruses; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes; Quasispecies; Ribozymes; Satellite Nucleic Acids and Viruses.

Further Reading

Baumstark T, Schröder ARW, and Riesner D (1997) Viroid processing: Switch from cleavage to ligation is driven by a change from a tetraloop to a loop E conformation. *EMBO Journal* 16: 599–610.

Bussière F, Ouellet J, Côté F, Lévesque D, and Perreault JP (2000) Mapping in solution shows the peach latent mosaic viroid to possess a new pseudoknot in a complex, branched secondary structure. *Journal of Virology* 74: 2647–2654.

Diener TO (1979) *Viroids and Viroid Diseases.* New York: Wiley-Interscience.

Ding B, Itaya A, and Zhong X (2005) Viroid trafficking: A small RNA makes a big move. *Current Opinion in Plant Biology* 8: 606–612.

Elena SF, Dopazo J, de la Peña M, Flores R, Diener TO, and Moya A (2001) Phylogenetic analysis of viroid and viroid-like satellite RNAs from plants: A reassessment. *Journal of Molecular Evolution* 53: 155–159.

Flores R, Hernandez C, Martinez de Alba AE, Daros JA, and DiSerio F (2005) Viroids and viroid–host interactions. *Annual Review of Phytopathology* 43: 117–139.

Flores R, Randles JW, Owens RA, Bar-Joseph M, and Diener TO (2005) Viroids. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball AL (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 1145–1159. San Diego, CA: Elsevier Academic Press.

Gross HJ, Domdey H, Lossow C, *et al*. (1978) Nucleotide sequence and secondary structure of potato spindle tuber viroid. *Nature* 273: 203–208.

Hadidi A, Flores R, Randles JW, and Semancik JS (eds.) (2003) *Viroids.* Collingwood: CSIRO Publishing.

Hernández C and Flores R (1992) Plus and minus RNAs of peach latent mosaic viroid self-cleave *in vitro* through hammerhead structures. *Proceedings of the National Academy of Sciences, USA* 89: 3711–3715.

Hull R (2002) *Matthews' Plant Virology,* 4th edn. New York: Academic Press.

Virus Classification by Pairwise Sequence Comparison (PASC)

Y Bao, Y Kapustin, and T Tatusova, National Institutes of Health, Bethesda, MD, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Demarcation A mapping of ranges of pairwise distances into taxonomic categories.

classification method that has drawn more and more attention from virologists is pairwise sequence comparison (PASC). In this article, we briefly describe various sequence comparison methods, introduce the PASC tool, and compare it with other methods.

Introduction

Virus classification is very important for virus research. It is also an extremely difficult task for many virus families. Traditionally, virus classification relied on properties such as virion morphology, genome organization, replication mechanism, serology, natural host range, mode of transmission, and pathogenicity. Yet viruses sharing the above properties can reveal tremendous differences at the genome level. For example, classification of many phages is currently based on presence, structure, and length of a tail, and this approach has been shown not to correlate with genomic information, leading to a very difficult situation and hundreds of unclassified phages.

Molecular virus classification based on virus sequences has been used increasingly in recent years, thanks to the growing number of viral sequences available in the public sequence databases. The most commonly used sequence comparison methods include multiple sequence alignment and phylogenetic analysis. Another molecular

Sequence Comparison Methods

A universal approach to compare biological sequences, in a sense of producing meaningful results at various levels of divergence, is in the realm of sequence alignment. An alignment is an arrangement of residues of two or more sequences in a way that reveals their possible relatedness, with space characters inserted into the sequences to indicate single-residue insertions and deletions. A variety of algorithms and programs are available to suit a wide range of problems requiring sequence alignments as parts of their solutions. Depending on the specifics of a problem, different types of algorithms or their combinations may work best. Most alignment algorithms can be broadly categorized by the scope of their application on sequences (local vs. global), or by the number of sequences involved (pairwise vs. multiple).

Each pairwise alignment can be viewed as an array of per-residue operations transforming one sequence to the other. These operations are substitutions (called matches

and mismatches in nucleotide alignments), insertions, and deletions. A generalization of this concept to multiple alignments is possible. Alignments are scored using a scoring scheme appropriate for a biological context. The widely used affine scheme assigns substitution scores to substitutions and a penalty to each space, and an additional penalty to each gap defined as a maximal consecutive run of spaces. Given a set of sequences, an alignment is called optimal if it has the maximal score over all possible alignments. Optimal alignments are not necessarily unique; two or more alignments can be tied with the same score.

Local algorithms are capable of detecting similarities between arbitrary parts of sequences. Applications involving local alignments are numerous, including search for orthologous genes or conserved protein domains. Alignments produced with local algorithms are tractable to mathematical analysis, which allowed the building of tools that evaluate statistical significance of the alignments. The algorithm for computing optimal local alignments is known as Smith–Waterman and it runs in time proportional to the product of the sequences' lengths. Since this is too slow for large-scale searches, many heuristic methods (with BLAST being the most popular) have been developed, allowing matching typical queries against gigabase-sized archives of sequence in a matter of seconds.

Fast as they are, algorithms like BLAST are not suitable for all applications. Although they are capable of picking out segments of high similarity, no segment of input sequences is guaranteed to be a part of the resulting alignments, and some segments may belong to more than one individual alignment. Additional post-processing steps are often required in order to produce consistent sets of local alignments. This complicates the use of local alignments in applications involving uniform computing of identities.

When sequences in the set are expected to align end-to-end, global alignment algorithms are applicable. The strict algorithm for computing an optimal global alignment is known as Needleman–Wunsch. With the running time estimate being the same as in the Smith–Waterman, global alignments allow straightforward evaluation of identities as they provide unambiguous mapping for every residue. An important end-space free variant is used when one of the sequences is expected to align in the interior of another. Global algorithms are normally not suitable for applications aiming to capture rearrangement events.

Multiple sequence alignments can technically be viewed as a generalization of the pairwise case. However, they often serve different goals, such as a detection of weak and/or dispersed similarities over a set of sequences known to share a common function or structure. Computing an optimal multiple sequence alignment is a computationally costly task, and most implementations use various heuristics to approximate the alignment in a reasonable time. Note that even when the optimal multiple alignment is available, pairwise alignments inferred from it are not guaranteed to be optimal. There are many multiple sequence alignment tools available: CLUSTALW, DIALIGN, MAFFT, MUSCLE, PROBCONS, ProDA, and T-COFFEE, etc.

Phylogenetic analysis is probably the most frequently used molecular virus classification tool. A phylogeny or evolutionary tree is a mathematical structure which is used to model the historical relationships between groups of organisms or sequences. Main types of methods used to construct phylogenetic trees include distance-based methods (such as neighbor-joining), parsimony, maximum likelihood, and other probabilistic inference techniques. The most common distance-based methods utilize multiple sequence alignments to estimate the evolutionary distance between each pair of sequences and reconstruct the tree from the distances. Either protein sequences or DNA sequences can be used.

Phylogenetic analysis was used in the vast majority of virus families described in the Eighth Report of the International Committee on the Taxonomy of Viruses (ICTV) to support their classification. It has also been applied to the classification of a large group of distantly related viruses. For example, phages consist of many different families. Therefore, the conventional phylogenetic analysis that uses genomic sequences or individual protein sequences would not work for the classification of phages as a whole group. A 'phage proteomic tree' was developed to classify phages by the overall similarities of all protein sequences present in the phage genomes.

Although phylogenetic analysis is well established as a tool for virus classification, it is usually computationally intensive, and requires expertise to perform the analysis and to interpret the results. A more robust method is preferred so that researchers without an advanced computer system and advanced knowledge about the phylogenetic analysis can also use it. Also, as discussed below, despite the fact that some of the sequence alignment methods (such as BLAST) are very fast and easy to carry out, their results will not reveal the taxonomic relationships between the two viruses. A method that can place a new virus in the appropriated taxonomic position is desired. PASC is a good combination of the two methods.

The Principle of PASC

In the PASC system, pairwise global alignment is performed on complete genomes or particular protein sequences for each viral family, and their percentage of identity is calculated. The number of virus pairs at each percentage is plotted. The distribution of the identities is not evenly spread, but rather clustered into groups of peaks for viruses at strain, species, genus, and subfamily levels. The percentage range of each peak serves as a good reference for

taxonomic classification based on sequence similarities. This method has been applied to polioviruses using the protein and nucleotide sequences of the VP1 gene, as well as the whole genome sequence, coronaviruses using the protein sequences of the polymerase and helicase, potyviruses using the protein and nucleotide sequences of the complete ORF and the coat protein gene, geminiviruses using the complete sequences of DNA-A, flexiviruses using the protein and nucleotide sequences of the three major viral gene products (replication protein, triple gene block and coat protein), papillomaviruses using the nucleotide sequences of the L1 gene, and poxviruses using the protein sequences of the DNA polymerase. There is an increasing interest to expand PASC to other virus families.

In order to apply PASC to a larger number of virus families and be used by a wide range of virologists, the following should be considered:

1. The same algorithm and sequence dataset should be used to determine the demarcations and to place new viruses in the right taxonomic position.
2. The sequence dataset used for demarcation determination and the virus taxonomy database should be updated frequently to reflect the most recent status.
3. The algorithm should be robust and fast enough for large sequence sets.
4. The system should be readily accessible to researchers worldwide and easy to use.

PASC Implementation at NCBI

The National Center for Biotechnology Information (NCBI) has developed a web-based PASC system that meets all of the criteria mentioned above. In this implementation, complete viral genomes are organized into groups corresponding to broad taxonomic entities such as family or floating genus. Within each group, alignments are pre-computed and stored in a database for each pair of the genomes. The alignments are used to evaluate identities, defined as the ratio of matching residues over the total alignment length. The alignments are computed using the pairwise global algorithm with the affine scoring scheme assigning one to matches and minus one to mismatches and nonterminal spaces and gaps. Since the genomes vary in lengths, terminal spaces are not penalized during the alignment computing but taken into account when computing the identity.

PASC interface is built around a histogram of pairwise identities. The primary feature of the interface is the comparison of an external sequence such as a newly sequenced viral genome, with genomes in a user-selected group. After the sequence is submitted, PASC will start computing the alignments, or extracting them from the database if the query is a member of the group. At the end of the process, a user is presented with a list of closest matches. Matches can be selected to visualize their positions on the identity distribution chart.

The PASC system at NCBI not only reproduced results for virus families for which PASC had been applied to, but also generated data with well-separated identity distributions that can be used as taxonomy demarcations for other virus families such as *Caliciviridae*, *Flaviviridae*, and *Togaviridae* among others.

Applications of PASC

PASC can be used to define taxonomy demarcations for many viral families. Two examples are shown here. In the first example, 45 complete genomes from the family *Luteoviridae* were used to construct the distribution of the pairwise identities among the genomes (**Figure 1**). Pairs located at 88% and up are all from different strains in the same species; pairs between 53% and 85% are mostly from different species in the same genus; and pairs located at 52% and lower are all from different genera. These percentages can therefore serve as demarcations for classification of luteoviruses. In the second example, the pairwise identities of 38 RNA-dependent RNA polymerase (RdRp) gene segment of viruses in the family *Reoviridae* were calculated and plotted (**Figure 2**). Similar to the above mentioned example, 81% and up, between 59% and 76%, and 57% and below can be used as boundaries for strains, species, and genera of reoviruses based on the identities between their polymerase genes. It should be noted that the determination of demarcation using PASC is not always straightforward. For some virus families, phenotypic characteristics of the viruses are required to be taken into account.

PASC can place newly sequenced viruses into the correct taxonomy group. For example, a genomic sequence of a luteovirus (GenBank accession number AY956384) appeared recently in the international sequence databases with the name chickpea chlorotic stunt virus, which is not an official ICTV species name. When this sequence was tested with the luteoviruses in the PASC system, it was found that the virus with the highest sequence similarity to it is cucurbit aphid-borne yellows virus in the genus *Polerovirus*. The similarity is 61.7%, which is in the demarcation of different species in the same genus. It can thus be suggested that this virus is a member of a new species in the genus *Polerovirus*.

Finally, PASC can identify possible questionable classifications in the existing groups when the peaks on the graph are very well separated. **Table 1** lists the pairs of the RdRp segment of reoviruses whose identities are between 54% and 54.5% in **Figure 2**. From the description above, this region represents pairs of viruses from different genera.

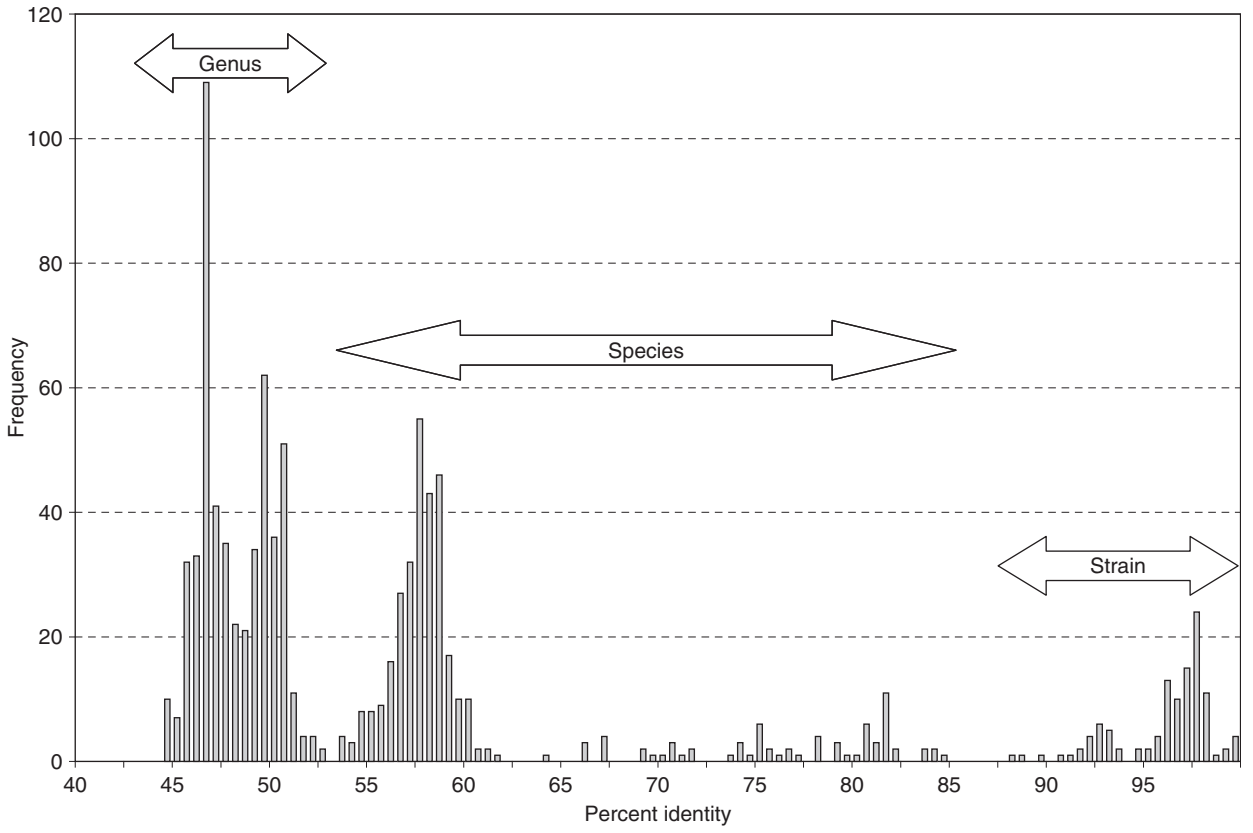


Figure 1 Frequency distribution of pairwise identities from the complete nucleotide sequence comparison of 45 luteoviruses.

This is true for most of the pairs in the table. However, the group also includes a pair containing St. Croix river virus and palyam virus, which both currently belong to the genus *Orbivirus*. Further investigation using PASC revealed that palyam virus has identities higher than 59% with many other viruses in the family, and therefore is indeed an orbivirus. The highest identity of St. Croix river virus with other viruses is only about 54%, which is lower than the demarcation for species in the same genus. This suggests that the species *St. Croix river virus* should probably be placed in a new genus in the *Reoviridae* family.

Advantages of PASC Compared to Other Methods

Unlike other classification methods based on the phenotypic properties of viruses, PASC is a quantitative tool. For those virus families that are suitable for PASC analyses, demarcations can be easily determined and new viruses can be clearly placed into the correct taxonomy. However, there are times when PASC alone cannot give a definite classification, and other viral properties have to be considered.

Compared with another quantitative approach, phylogenetic analysis, PASC is less computationally intensive and can be easily updated with new sequence data. In addition, PASC results are relatively easier to interpret, which can be potentially done by a computer program without human intervention. It would therefore be possible to set up an automatic system for high throughput classification.

Many researchers use BLAST to search sequence databases to find best matches for viral sequences of interest. Although BLAST is readily available and easy to run, it is not the best tool for virus classification. This is because BLAST is a local alignment program, and, as discussed above, it may not take highly variable regions into account when calculating identities. Even when an output from BLAST covers the whole sequence as a single alignment, information about the taxonomy relationship between the query virus and the virus closest to it is not immediately available. For example, an identity of 75% could be within the range of the same species in one viral family, but in the range of different species in another family. On the contrary, PASC suggests explicitly whether the query virus is in the same species as some existing viruses, or if it should be assigned within a new species or genus in the family.

The total number of virus sequences in the GenBank/EMBL/DDBJ databases is more than 4 times now than 5 years ago (from about 109 000 to about 446 000 in

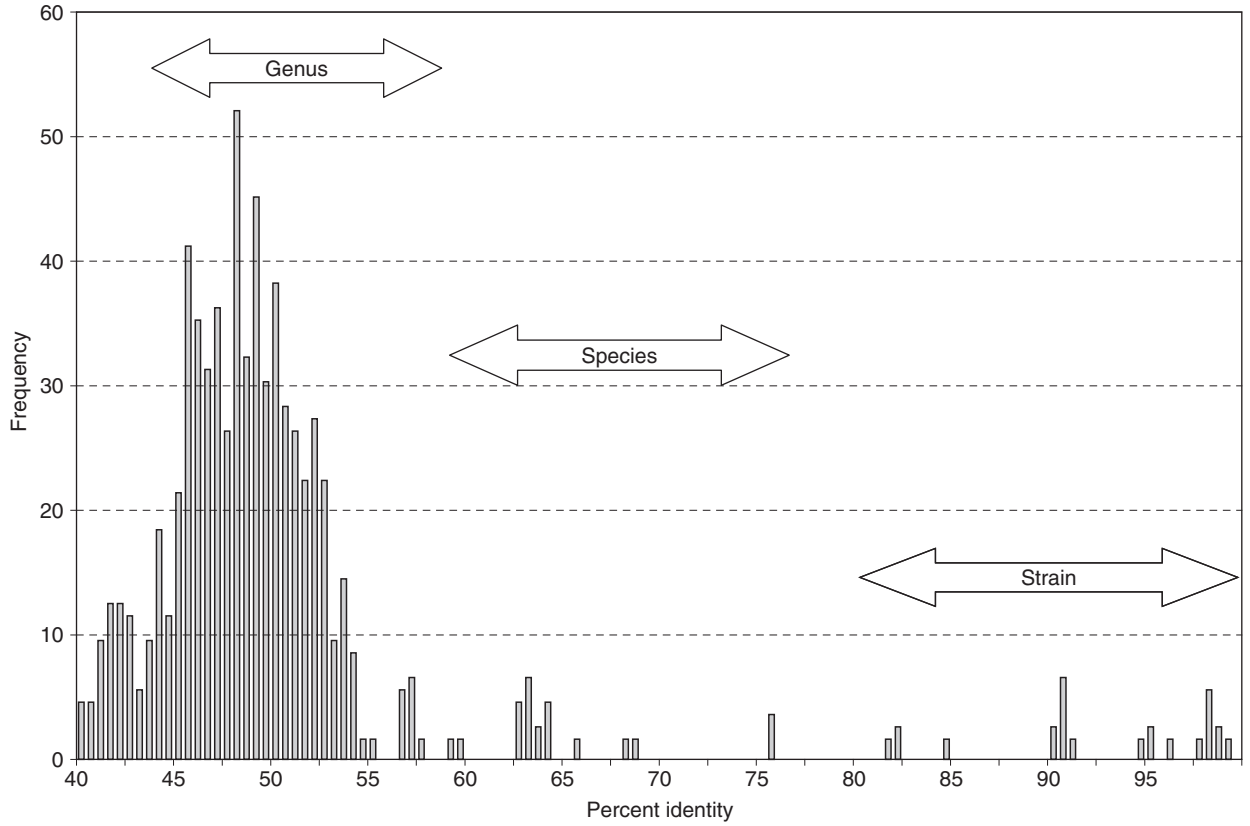


Figure 2 Frequency distribution of pairwise identities from the nucleotide sequence comparison of the RdRp segments of 38 reoviruses.

Table 1 Pairs of the RdRp segment of the reoviruses with identities between 54% and 54.5%

Identity	*Same genus?*	*Same species?*	*Genome 1*[a]	*Genome 2*
0.544895	No	No	20279540 Coltivirus|Eyach virus	37514915 Mycoreovirus|Mycoreovirus 3
0.542353	No	No	8574569 Seadornavirus| Banna virus	24286507 Cypovirus|Cypovirus 1|Dendrolimus punctatus cypovirus 1
0.541812	No	No	8574569 Seadornavirus| Banna virus	32470626 Orthoreovirus|Mammalian orthoreovirus| Mammalian orthoreovirus 3
0.541744	No	No	8574569 Seadornavirus| Banna virus	25808995 Orbivirus|Bluetongue virus|Bluetongue virus 2|Corsican bluetongue virus
0.541257	Yes	No	50253405 Orbivirus|St. Croix River virus	50261332 Orbivirus|Palyam virus
0.541096	No	No	22960700 Coltivirus|Colorado tick fever virus	32349409 Mycoreovirus|Mycoreovirus 1| Cryphonectria parasitica mycoreovirus-1 (9B21)
0.540999	No	No	8574569 Seadornavirus| Banna virus	14993633 Cypovirus|Cypovirus 1
0.54089	No	No	14993610 Cypovirus| Cypovirus 14	20177438 Fijivirus|Nilaparvata lugens reovirus

[a]The numbers correspond to sequences in GenBank. The taxonomy lineages from the genus level of the viruses are also shown.

October 2006). It is possible to test the PASC system on many virus families now. New sequencing technology makes it possible to generate large amounts of virus sequences from environmental samples without the need to isolate and purify virus particles. In such cases, a molecular based method is the only way to classify the viral sequences, and PASC can be very useful for this purpose.

Limitations of PASC

Although PASC has been applied successfully to several virus families, the approach has some limitations.

First of all, PASC is not suitable for virus families whose current classification is largely based on virus morphologies, such as phages in the families *Siphoviridae* and *Podoviridae*. The whole genome PASC may not work well for virus families with highly diverse sequences. This includes viruses with low overall sequence similarities or large differences in genome sizes and organization. For example, in the family *Herpesviridae*, the percentage of identity between different species in the genus *Varicellovirus* ranges from 39% (between cercopithecine herpesvirus 9 and suid herpesvirus 1) to 83% (between bovine herpesvirus 1 and bovine herpesvirus 5), while the percentage of identity between a virus in the genus *Simplexvirus* and one in the genus *Varicellovirus* could be as high as 54% (between cercopithecine herpesvirus 2 and bovine herpesvirus 5). The overlap of such identities makes it impossible to determine the species and genus demarcations for herpesviruses. In the family *Poxviridae*, the largest genome (canarypox virus) is almost 3 times as big as the smallest one (bovine papular stomatitis virus). The huge differences in the genomes sizes will introduce large artifacts when calculating the identities. In such cases, single gene or a cluster of genes needs to be used in PASC instead of whole genomes. The polymerase protein sequences of poxviruses have been used to perform PASC and a good result was obtained. However, not every single gene can be used for PASC. The genes must be present in all viruses of a family and be very conserved. They need to be tested extensively and accepted by the research community as a useful taxonomic criterion before being applied to the PASC system.

Second, for those families whose PASC were constructed with whole genomes, the query sequences to be tested on PASC to determine their taxonomic positions have to be complete genome sequences as well in order to get an accurate prediction. Although a percentage of identity can be obtained when a partial genome is used, the value will be smaller than what it really should be if a complete genome were used because of the way percentage of identity is calculated in this system. In addition, this value obtained with a partial sequence may not reflect the real taxonomic position of this virus, if, for example, recombination is frequent and important for this virus. This will reduce the number of sequences that can be tested by PASC.

Last, it is almost impossible to get identical PASC results when different methods are used. As mentioned above, there are many pairwise sequence alignment programs available. Even within a single program, variation of parameters can affect alignments. After an alignment is obtained, there can be various ways to calculate the distance. For demarcations computed using different types of distances, it may be difficult to choose a rational reason to privilege one demarcation threshold over another. Moreover, once a demarcation is adopted and a researcher uses a different definition to measure the distance between a new virus and an existing one, the comparison with the histogram will sometimes be misleading. These issues can be overcome by using a centralized PASC system where the alignment identities of new viruses are computed using the same algorithm and the same parameter set that were used to compute identities within the family and to create the demarcation.

Conclusion

PASC is a molecular classification tool for many virus families. It calculates the pairwise identities of virus sequences within a virus family and displays their distributions, and can help determine the demarcations at strains, species, genera, and subfamilies level. PASC has many advantages over conventional virus classification methods. The tool has been successfully applied to many virus families, although it may not work well for virus families with highly diverse sequences. The PASC tool at NCBI established distributions of identity for a number of virus families. A new virus sequence can be tested with this system within a few minutes to suggest the taxonomic position of the virus in these families. This system eliminates the potential discrepancies in the results caused by different algorithms and/or different data used by the virology community. Data in the system can be updated automatically to reflect changes in virus taxonomy and additions of new virus sequences to the public database. The web interface of the tool makes it easy to navigate and perform analyses.

See also: Taxonomy, Classification and Nomenclature of Viruses; Virus Species; Virus Databases.

Further Reading

Edgar RC and Batzoglou S (2006) Multiple sequence alignment. *Current Opinion in Structural Biology* 16: 368–373.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy, Classification and Nomenclature of Viruses: Eighth Report of the International Committee on the Taxonomy of Viruses.* London: Academic Press.

Felsenstein J (2004) *Inferring Phylogeny.* Sunderland, MA: Sinauer Associates.

Gusfield D (1997) *Algorithms on Strings, Trees, and Sequences: Computer Science and Computational Biology.* Cambridge: Cambridge University Press.

Page RD and Holmes EC (1998) *Molecular Evolution: A Phylogenetic Approach.* Oxford: Blackwell Science.

van Regenmortel MHV (2007) Virus species and virus identification: Past and Current Controversies. *Infection, Genetics and Evolution* 7: 133–144.
van Regenmortel MHV, Bishop DH, Fauquet CM, *et al.* (1997) Guidelines to the demarcation of virus species. *Archives of Virology* 142: 1505–1518.

Relevant Website

http://www.ncbi.nlm.nih.gov – NCBI, PASC.

Virus Databases

E J Lefkowitz and M R Odom, University of Alabama at Birmingham, Birmingham, AL, USA
C Upton, University of Victoria, Victoria, BC, Canada

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Annotation The process of adding descriptive information to a data set and the individual data elements that comprise that data set. May include both computational analyses and manual curation.
Curation The process of manually adding descriptive information or explanatory material to a data set usually based on a review of the existing literature.
Database A collection of information, usually stored electronically on a computer.
Database, hierarchical (XML) Extensible markup language. A formally defined hierarchical organization of data.
Database management system (DBMS) A database software package designed to operate on a particular computer platform and operating system.
Database schema A map of the database structure, including all of the fields, tables, and relationships that comprise the framework for storage of information within that database.
Database, relational A database structure that provides the ability to define formal relationships between all data elements stored within the database.
Ontology A controlled vocabulary used to formally describe (for our purposes) a biological process or entity.
SQL Structured Query Language. A computer language used to query relational databases for information.

Introduction

In 1955, Niu and Frankel-Conrat published the C-terminal amino acid sequence of tobacco mosaic virus capsid protein. The complete 158-amino-acid sequence of this protein was published in 1960. The first completely sequenced viral genome published was that of bacteriophage MS2 in 1976 (GenBank accession number V00642). Sanger used DNA from bacteriophage phiX174 (J02482) in developing the dideoxy sequencing method, while the first animal viral genome, SV40 (J02400), was sequenced using the Maxam and Gilbert method and published in 1978. Viruses therefore played a pivotal role in the development of modern-day sequencing methods, and viral sequence information (both protein and nucleotide) formed a substantial subset of the earliest available biological databases. In 1965, Margaret O. Dayhoff published the first publicly available database of biological sequence information. This *Atlas of Protein Sequence and Structure* was available only in printed form and contained the sequences of approximately 50 proteins. Establishment of a database of nucleic acid sequences began in 1979 through the efforts of Walter Goad at the US Department of Energy's Los Alamos National Laboratory (LANL) and separately at the European Molecular Biology Laboratories (EMBL) in the early 1980s. In 1982, the LANL database received funding from the National Institutes of Health (NIH) and was christened GenBank. In December of 1981, the Los Alamos Sequence Library contained 263 sequences of which 50 were from eukaryotic viruses and 12 were from bacteriophages. By its tenth release in 1983, GenBank contained 1865 sequences (1 827 214 nucleotides) of which 449 (457 721 nucleotides) were viral. In August of 2006, GenBank (release 154) contained approximately 59 000 000 records, including 367 000 viral sequences.

The number of available sequences has increased exponentially as sequencing technology has improved. In addition, other high-throughput technologies have been developed in recent years, such as those for gene expression and proteomic studies. All of these technologies generate enormous new data sets at ever-increasing rates. The challenge, therefore, has been to provide computational systems that support the storage, retrieval, analysis, and display of this information so that the research scientist can take advantage of this wealth of

resources to ask and answer questions relevant to his or her work. Every article in this encyclopedia contains knowledge that has been derived in part from the analysis of large data sets. The ability to effectively and efficiently utilize these data sets is directly dependent on the databases that have been developed to support storage of this information. Fortunately, the continual development and improvement of data-intensive biological technologies has been matched by the development and improvement of computational technologies. This work, which includes both the development and utilization of databases as well as tools for storage and analysis of biological information, forms a very important part of the bioinformatics field. This article provides an overview of database structure and how that structure supports the storage of biological information. The different types of data associated with the analysis of viruses are discussed, followed by a review of some of the various online databases that store general biological information as well as virus-specific information.

Databases

Definition

A database is simply a collection of information, including the means to store, manipulate, retrieve, and share that information. For many of us, lab notebook fulfilled our initial need for a 'database'. However, this information storage vehicle did not prove to be an ideal place to archive our data. Backups were difficult, and retrieval more so. The advent of computers – especially the desktop computer – provided a new solution to the problem of data storage. Though initially this innovation took the form of spreadsheets and electronic notebooks, the subsequent development of both personal and large-scale database systems provided a much more robust solution to the problems of data storage, retrieval, and manipulation. The computer program supplying this functionality is called a 'database management system' (DBMS). Such systems provide at least four things: (1) the necessary computer code to guide a user through the process of database design; (2) a computer language that can be used to insert, manipulate, and query the data; (3) tools that allow the data to be exported in a variety of formats for sharing and distribution; and (4) the administrative functions necessary to ensure data integrity, security, and backup. However, regardless of the sophistication and diverse functions available in a typical modern DBMS, it is still up to the user to provide the proper context for data storage. The database must be properly designed to ensure that it supports the structure of the data being stored and also supports the types of queries and manipulations necessary to fully understand and efficiently analyze the properties of the data.

Data

The development of a database begins with a description of the data to be stored, all of the parameters associated with the data, and frequently a diagram of the format that will be used. The format used to store the data is called the database schema. The schema provides a detailed picture of the internal format of the database that includes specific containers to store each individual piece of data. While databases can store data in any number of different formats, the design of the particular schema used for a project is dependent on the data and the needs and expertise of the individuals creating, maintaining, and using the database. As an example, we will explore some of the possible formats for storing viral sequence data and provide examples of the database schema that could be used for such a project.

Figure 1(**a**) provides an example of a GenBank sequence record that is familiar to most biologists. These records are provided in a 'flat file' format in which all of the information associated with this particular sequence is provided in a human-readable form and in which all of the information is connected in some manner to the original sequence. In this format, the relationships between each piece of information and every other piece of information are only implicitly defined, that is, each line starts with a label that describes the information in the rest of the line, but it is up to the investigator reading the record to make all of the proper connections between each of the data fields (lines). The proper connections are not explicitly defined in this record. As trained scientists, we are able to read the record in **Figure 1**(**a**) and discern that this particular amino acid sequence is derived from a strain of Ebola virus that was studied by a group in Germany, and that this sequence codes for a protein that functions as the virus RNA polymerase. The format of this record was carefully designed to allow us, or a computer, to pull out each individual type of information. However as trained scientists, we already understand the proper connections between the different information fields in this file. The computer does not. Therefore, to analyze the data using a computer, a custom software program must be written to provide access to the data.

Extensible markup language (XML) is another widely used format for storing database information. **Figure 1**(**b**) shows an example of part of the XML record for the Ebola virus polymerase protein. In this format, each data field can be many lines long; the start and end of a data record contained within a particular field are indicated by tags made of a label between two brackets ('<label>...</label>'). Unlike the lines in the GenBank record in **Figure 1**(**a**), a field in an XML record can be placed inside of another, defining a structure and a relationship between them. For example, the *TSeq_orgname* is placed inside of the *TSeq* record to show that this

GenBank record

```
LOCUS       NP_066251               2212 aa            linear   VRL 30-MAR-2006
DEFINITION  polymerase [Zaire ebolavirus].
ACCESSION   NP_066251
VERSION     NP_066251.1  GI:10313999
DBSOURCE    REFSEQ: accession NC_002549.1
KEYWORDS    .
SOURCE      Zaire ebolavirus (ZEBOV)
  ORGANISM  Zaire ebolavirus
            Viruses; ssRNA negative-strand viruses; Mononegavirales;
            Filoviridae; Ebola-like viruses.
REFERENCE   1  (residues 1 to 2212)
  AUTHORS   Volchkov,V.E., Volchkova,V.A., Chepurnov,A.A., Blinov,V.M.,
            Dolnik,O., Netesov,S.V. and Feldmann,H.
  TITLE     Characterization of the L gene and 5' trailer region of Ebola virus
  JOURNAL   J. Gen. Virol. 80 (Pt 2), 355-362 (1999)
   PUBMED   10073695
...
REFERENCE   8  (residues 1 to 2212)
  AUTHORS   Volchkov,V.E.
  TITLE     Direct Submission
  JOURNAL   Submitted (20-AUG-1998) Institute of Virology, Philipps-University
            Marburg, Robert-Koch-Str. 17, Marburg 35037, Germany
COMMENT     PROVISIONAL REFSEQ: This record has not yet been subject to final
            NCBI review. The reference sequence was derived from AAD14589.
            Method: conceptual translation.
FEATURES             Location/Qualifiers
     source          1..2212
                     /organism="Zaire ebolavirus"
                     /strain="Mayinga"
                     /db_xref="taxon:186538"
     Protein         1..2212
                     /product="polymerase"
                     /function="synthesis of viral RNAs; transcriptional RNA
                     editing"
                     /note="L"
                     /calculated_mol_wt=252595
     CDS             1..2212
                     /gene="L"
                     /locus_tag="ZEBOVgp7"
                     /coded_by="NC_002549.1:11581..18219"
                     /citation=[1]
                     /db_xref="GeneID:911824"
ORIGIN
        1 matqhtqypd arlsspivld qcdlvtracg lyssyslnpq lrncklpkhi yrlkydvtvt
       61 kflsdvpvat lpidfivpvl lkalsgngfc pveprcqqfl deiikytmqd alflkyylkn
...
     2101 ndynqqrqsr tqtyhfirta kgritklvnd ylkfflivqa lkhngtwqae fkklpelisv
     2161 cnrfyhirdc nceerflvqt lylhrmqdse vklierltgl lslfpdglyr fd
//
```

(a)

XML record

```
<?xml version="1.0"?>
<!DOCTYPE TSeqSet PUBLIC "NCBI_TSeq.dtd">
<TSeqSet>
<TSeq>
  <TSeq_seqtype value="protein"/>
  <TSeq_gi>10313999</TSeq_gi>
  <TSeq_accver>NP_066251.1</TSeq_accver>
  <TSeq_taxid>186538</TSeq_taxid>
  <TSeq_orgname>Zaire ebolavirus</TSeq_orgname>
  <TSeq_defline>polymerase [Zaire ebolavirus]</TSeq_defline>
  <TSeq_length>2212</TSeq_length>
  <TSeq_sequence>
  MATQHTQYPDARLSSPIVLDQCDLVTRACGLYSSYSLNPQLRNCKLPKHIYRLKYDVTVTKFLSDVPVAT
  LPIDFIVPVLLKALSGNGFCPVEPRCQQFLDEIIKYTMQDALFLKYYLKNVGAQEDCVDEHFQEKILSSI
  ...
  QLQIQRSPYWLSHLTQYADCELHLSYIRLGFPSLEKVLYHRYNLVDSKRGPLVSITQHLAHLRAEIRELT
  NDYNQQRQSRTQTYHFIRTAKGRITKLVNDYLKFFLIVQALKHNGTWQAEFKKLPELISVCNRFYHIRDC
  NCEERFLVQTLYLHRMQDSEVKLIERLTGLLSLFPDGLYRFD
  </TSeq_sequence>
</TSeq>
</TSeqSet>
```

(b)

Figure 1 Data formats. Examples of two different formats for organizing sequence data are shown. (a) An example of a GenBank flat file sequence record. (b) Part of the same record using a hierarchical XML format.

organism name applies only to that sequence record. If the file contained multiple sequences, each *TSeq* field would have its own *TSeq_orgname* subfield, and the relationship between them would be very clear. This self-describing hierarchical structure makes XML very powerful for expressing many types of data that are hard to express in a single table, such as that used in a spreadsheet. However, in order to find any piece of information in the XML file,

a user (with an appropriate search program) needs to traverse the whole file in order to pull out the particular items of data that are of interest. Therefore, while an XML file may be an excellent format for defining and exchanging data, it is often not the best vehicle for efficiently storing and querying that data. That is still the realm of the relational database.

'Relational database management systems' (RDBMSs) are designed to do two things extremely well: (1) store and update structured data with high integrity, and (2) provide powerful tools to search, summarize, and analyze the data. The format used for storing the data is to divide it into several tables, each of which is equivalent to a single spreadsheet. The relationships between the data in the tables are then defined, and the RDBMS ensures that all data follow the rules laid out by this design. This set of tables and relationships is called the schema. An example diagram of a relational database schema is provided in **Figure 2**. This Viral Genome Database (VGD) schema is an idealized version of a database used to store viral genome sequences, their associated gene sequences, and associated descriptive and analytical information. Each box in **Figure 2** represents a single object or concept, such as a genome, gene, or virus, about which we want to store data and is contained in a single table in the RDBMS. The names listed in the box are the columns of that table, which hold the various types of data about the object. The 'gene' table therefore contains columns holding data such as the name of the gene, its coding strand, and a description of its function. The RDBMS is

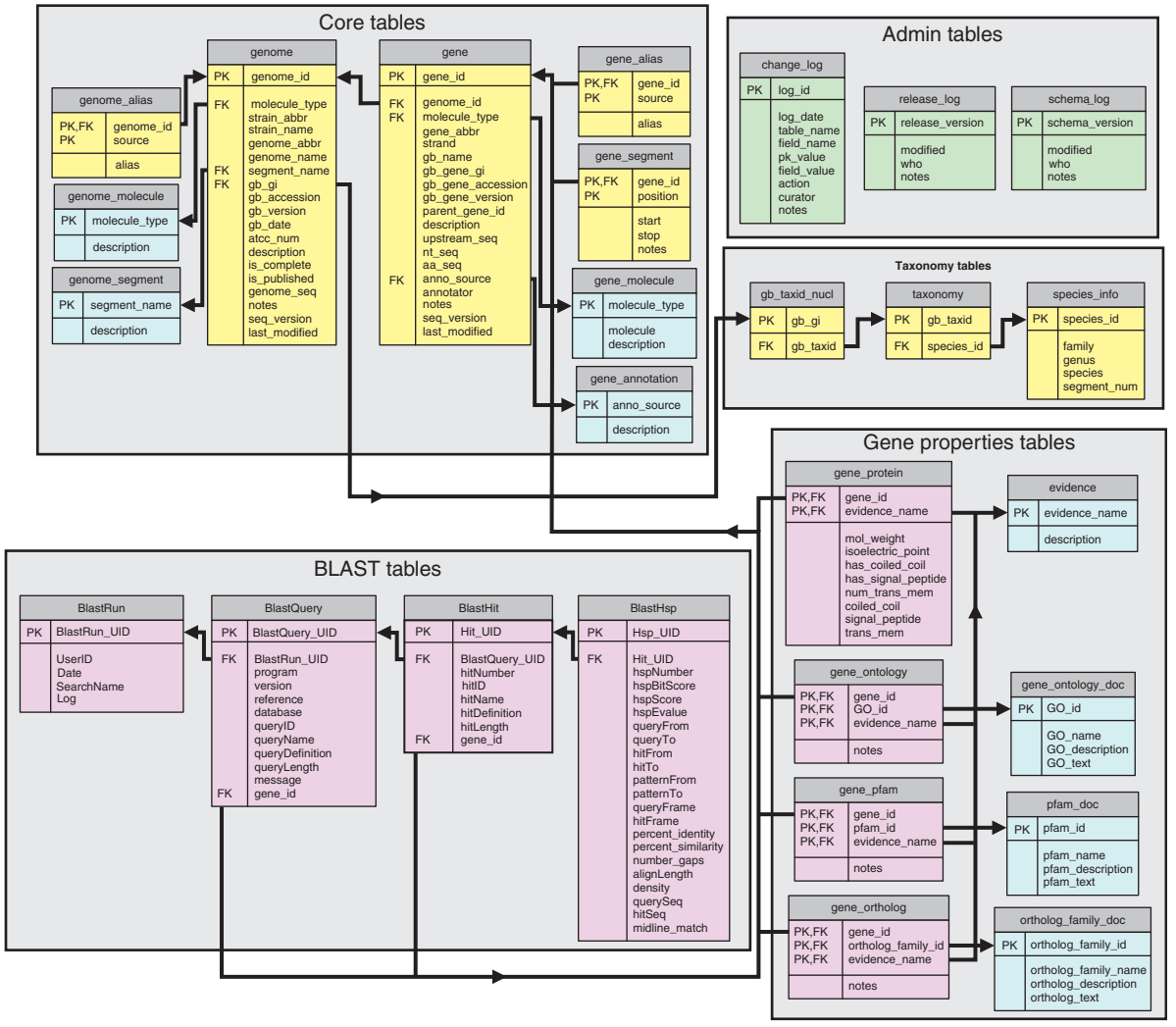


Figure 2 VGD Database schema. Underlying structure of a Viral Genome Sequence Database (VGD). Data tables are grouped according to the type of information contained in each set of tables. Each table contains a set of fields that hold a particular type of data. Lines and arrows display the relationships between fields as defined by the foreign key (FK) and primary key (PK) that connect two tables. (Each arrow points to the table containing the primary key.) Tables are color-coded according to the source of the information they contain: yellow, data obtained from the original GenBank sequence record and the ICTV Eighth Report; pink, data obtained from automated annotation or manual curation; blue, controlled vocabularies to ensure data consistency; green, administrative data.

able to enforce a series of rules for tables that are linked by defining relationships that ensure data integrity and accuracy. These relationships are defined by a foreign key in one table that links to corresponding data in another table defined by a primary key. In this example, the RDMS can check that every gene in the 'gene' table refers to an existing genome in the 'genome' table, by ensuring that each of these tables contains a matching 'genome_id'. Since any one genome can code for many genes, many genes may contain the same 'genome_id'. This defines what is called a one-to-many relationship between the 'genome' and 'gene' tables. All of these relationships are identified in **Figure 2** by arrows connecting the tables.

Because viruses have evolved a variety of alternative coding strategies such as splicing and RNA editing; it is necessary to design the database so that these processes can be formally described. The 'gene_segment' table specifies the genomic location of the nucleotides that code for each gene. If a gene is coded in the traditional manner – one ORF, one protein – then that gene would have one record in the 'gene_segment' table. However, as described above, if a gene is translated from a spliced transcript, it would be represented in the 'gene_segment' table by two or more records, each of which specifies the location of a single exon. If an RNA transcript is edited by stuttering of the polymerase at a particular run of nucleotides, resulting in the addition of one or more nontemplated nucleotides, then that gene will also have at least two records in the 'gene_segment' table. In this case, the second 'gene_segment' record may overlap the last base of the first record for that gene. In this manner, an extra, nontemplated base becomes part of the final gene transcript. Other more complex coding schemes can also be identified using this, or similar, database structures.

The tables in **Figure 2** are grouped according to the type of information they contain. Though the database itself does not formally group tables in this manner, database schema diagrams are created to benefit database designers and users by enhancing their ability to understand the structure of the database. These diagrams make it easier to both populate the database with data and query the database for information. The core tables hold basic biological information about each viral strain and its genomic sequence (or sequences if the virus contains segmented genomes) as well as the genes coded for by each genome. The taxonomy tables provide the taxonomic classification of each virus. Taxonomic designations are taken directly from the *Eighth Report of the International Committee on Taxonomy of Viruses* (ICTV). The 'gene properties' tables provide information related to the properties of each gene in the database. Gene properties may be generated from computational analyses such as calculations of molecular weight and isoelectric point (pI) that are derived from the amino acid sequence. Gene properties may also be derived from a manual curation process in which an investigator might identify, for example, functional attributes of a sequence based on evidence provided from a literature search. Assignment of 'gene ontology' terms (see below) is another example of information provided during manual curation. The BLAST tables store the results of similarity searches of every gene and genome in the VGD searched against a variety of sequence databases using the National Center for Biotechnology Information (NCBI) BLAST program. Examples of search databases might include the complete GenBank nonredundant protein database and/or a database comprised of all the protein sequences in the VGD itself. While most of us store our BLAST search results as files on our desktop computers, it is useful to store this information within the database to provide rapid access to similarity results for comparative purposes; to use these results to assign genes to orthologous families of related sequences; and to use these results in applications that analyze data in the database and, for example, display the results of an analysis between two or more types of viruses showing shared sets of common genes. Finally, the 'admin' tables provide information on each new data release, an archive of old data records that have been subsequently updated, and a log detailing updates to the database schema itself.

It is useful for database designers, managers, and data submitters to understand the types of information that each table contains and the source of that information. Therefore, the database schema provided in **Figure 2** is color-coded according to the type and source of information each table provides. Yellow tables contain basic biological data obtained either directly from the GenBank record or from other sources such as the ICTV. Pink tables contain data obtained as the result of either computational analyses (BLAST searches, calculations of molecular weight, functional motif similarities, etc.) or from manual curation. Blue tables provide a controlled vocabulary that is used to populate fields in other tables. This ensures that a descriptive term used to describe some property of a virus has been approved for use by a human curator, is spelled correctly, and when multiple terms or aliases exist for the same descriptor, the same one is always chosen.

While the use of a controlled vocabulary may appear trivial, in fact, misuse of terms, or even misspellings, can result in severe problems in computer-based databases. The computer does not know that the terms 'negative-sense RNA virus' and 'negative-strand RNA virus' may both be referring to the same type of virus. The provision and use of a controlled vocabulary increases the likelihood that these terms will be used properly, and ensures that the fields containing these terms will be easily comparable. For example, the 'genome_molecule' table contains the following permissible values for 'molecule_type': 'ambisense ssRNA', 'dsRNA', 'negative-sense ssRNA', 'positive-sense ssRNA', 'ssDNA', and 'dsDNA'. A particular viral genome must then have one of these values entered into the 'molecule_type' field

of the 'genome' table, since this field is a foreign key to the 'molecule_type' primary key of the 'genome_molecule' table. Entering 'double-stranded DNA' would not be permissible.

Annotation

Raw data obtained directly from high-throughput analytical techniques such as automated sequencing, protein interaction, or microarray experiments contain little-to-no information as to the content or meaning. The process of adding value to the raw data to increase the knowledge content is known as annotation and curation. As an example, the results of a microarray experiment may provide an indication that individual genes are up- or downregulated under certain experimental conditions. By annotating the properties of those genes, we are able to see that certain sets of genes showing coordinated regulation are a part of common biological pathways. An important pattern then emerges that was not discernable solely by inspection of the original data. The annotation process consists of a semiautomated analysis of the information content of the data and provides a variety of descriptive features that aid the process of assigning meaning to the data. The investigator is then able to use this analytical information to more closely inspect the data during a manual curation process that might support the reconstruction of gene expression or protein interaction pathways, or allow for the inference of functional attributes of each identified gene. All of this curated information can then be stored back in the database and associated with each particular gene.

For each piece of information associated with a gene (or other biological entity) during the process of annotation and curation, it is always important to provide the evidence used to support each assignment. This evidence may be described in a Standard Operating Procedure (SOP) document which, much like an experimental protocol, details the annotation process and includes a description of the computer algorithms, programs, and analysis pipelines that were used to compile that information. Each piece of information annotated by the use of this pipeline might then be coded, for example, 'IEA: Inferred from Electronic Annotation'. For information obtained from the literature during manual curation, the literature reference from which the information was obtained should always be provided along with a code that describes the source of the information. Some of the possible evidence codes include 'IDA: Inferred from Direct Assay', 'IGI: Inferred from Genetic Interaction', 'IMP: Inferred from Mutant Phenotype', or 'ISS: Inferred from Sequence or Structural Similarity'. These evidence codes are taken from a list provided by the Gene Ontology (GO) Consortium (see below) and as such represent a controlled vocabulary that any data curator can use and that will be understood by anyone familiar with the GO database. This controlled evidence vocabulary is stored in the 'evidence' table, and each record in every one of the gene properties tables is assigned an evidence code noting the source of the annotation/curation data.

As indicated above, the use of controlled vocabularies (ontologies) to describe the attributes of biological data is extremely important. It is only through the use of these controlled vocabularies that a consistent, documented approach can be taken during the annotation/curation process. And while there may be instances where creating your own ontology may be necessary, the use of already available, community-developed ontologies ensures that the ontological descriptions assigned to your database will be understood by anyone familiar with the public ontology. Use of these public ontologies also ensures that they support comparative analyses with other available databases that also make use of the same ontological descriptions. The GO Consortium provides one of the most extensive and widely used controlled vocabularies available for biological systems. GO describes biological systems in terms of their biological processes, cellular components, and molecular functions. The GO effort is community-driven, and any scientist can participate in the development and refinement of the GO vocabulary. Currently, GO contains a number of terms specific to viral processes, but these tend to be oriented toward particular viral families, and may not necessarily be the same terms used by investigators in other areas of virology. Therefore it is important that work continues in the virus community to expand the availability and use of GO terms relevant to all viruses. GO is not intended to cover all things biological. Therefore, other ontologies exist and are actively being developed to support the description of many other biological processes and entities. For example, GO does not describe disease-related processes or mutants; it does not cover protein structure or protein interactions; and it does not cover evolutionary processes. A complementary effort is under way to better organize existing ontologies, and to provide tools and mechanisms to develop and catalog new ontologies. This work is being undertaken by the National Center for Biomedical Ontologies, located at Stanford University, with participants worldwide.

Access (Searching for Information)

The most comprehensive, well-designed database is useless if no method has been provided to access that database, or if access is difficult due to a poorly designed application. Therefore, providing a search interface that meets the needs of intended users is critical to fully realizing the potential of any effort at developing a comprehensive database. Access can be provided using a number of different methods ranging from direct query of the database using the relatively standardized 'structured query language' (SQL), to customized applications designed to provide the ability to ask sophisticated questions regarding the data

contained in the database and mine the data for meaningful patterns. Web pages may be designed to provide simple-to-use forms to access and query data stored in an RDBMS.

Using the VGD schema as a data source, one example of an SQL query might be to find the gene_id and name of all the proteins in the database that have a molecular weight between 20 000 and 30 000, and also have at least one transmembrane region.

Many database providers also provide users with the ability to download copies of the database so that these users may analyze the data using their own set of analytical tools.

Output (Utilizing Information)

When a user queries a database using any of the available access methods, the results of that query are generally provided in the form of a table where columns represent fields in the database and the rows represent the data from individual database records. Tabular output can be easily imported into spreadsheet applications, sorted, manipulated, and reformatted for use in other applications. But while extremely flexible, tabular output is not always the best format to use to fully understand the underlying data and the biological implications. Therefore, many applications that connect to databases provide a variety of visualization tools that display the data graphically, showing patterns in the data that may be difficult to discern using text-based output. An example of one such visual display is provided in **Figure 3** and shows conservation of synteny between the genes of two different poxvirus species. The information used to generate this figure comes directly from the data provided in the VGD. Every gene in the two viruses (in this case crocodilepox virus and molluscum contagiosum virus) has been compared to every other gene using the BLAST search program. The results of this search are stored in the BLAST tables of the VGD. In addition, the location of each gene within its respective genomic sequence is stored in the 'gene_segment' table. This information, once extracted from the database server, is initially text but it is then submitted to a program running on the server that reformats the data and creates a graph. In this manner, it is much easier to visualize the series of points formed along a diagonal when there are a series of similar genes with similar genomic locations present in each of the two viruses. These data sets may contain gene synteny patterns that display deletion, insertion, or recombination events during the course of viral evolution. These patterns can be difficult to detect with text-based tables, but are easy to discern using visual displays of the data.

Information provided to a user as the result of a database query may contain data derived from a combination of sources, and displayed using both visual and textual

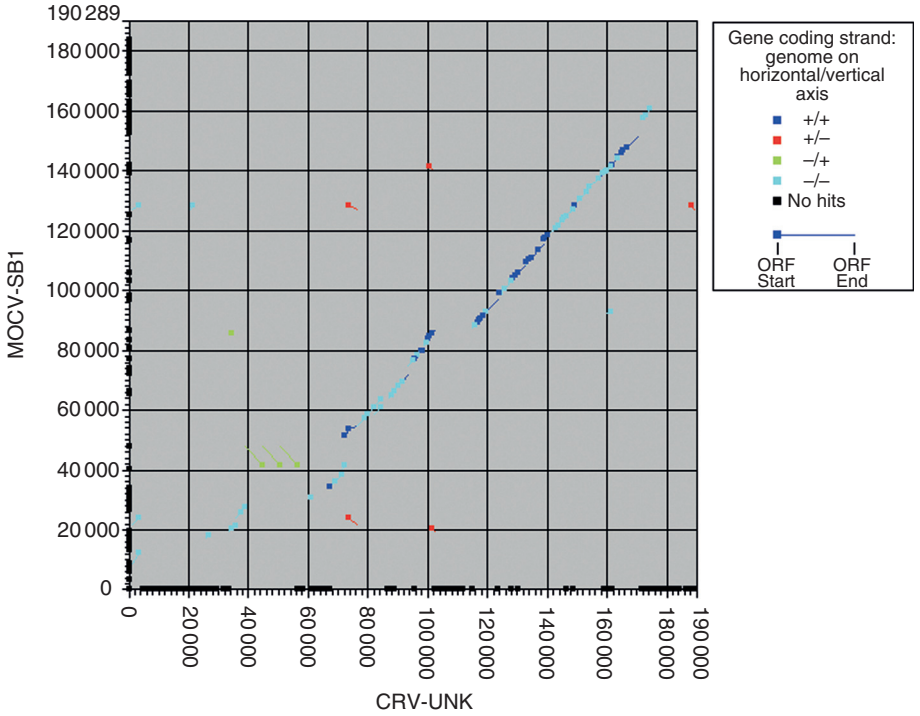


Figure 3 Gene synteny plot. A comparison of gene sequence and genomic position conservation between crocodilepox virus (horizontal axis) and molluscum contagiosum virus (vertical axis). All predicted proteins encoded by each virus were compared to each other using BLASTP. Each pair of proteins showing some measure of similarity, as determined by a BLAST expect (*E*) value <0.00001, was plotted according to the location of each gene on each respective genome. The color of the points reflects the identity of the coding strand of each gene. Black points along either of the two axes represent proteins unique to that genome.

feedback. **Figure 4** shows the web-based output of a query designed to display information related to a particular virus gene. The top of this web page displays the location of the gene on the genome visually showing surrounding genes on a partial map of the viral genome. Basic gene information such as genome coordinates, gene name, and the nucleotide and amino acid sequence are also provided. This information was originally obtained from the original GenBank record and then stored in the VGD database. Data added as the result of an automated annotation pipeline are also displayed. This includes calculated values for molecular weight and pI; amino acid composition; functional motifs; BLAST similarity searches; and predicted protein structural properties such as transmembrane domains, coiled-coil regions, and signal sequences. Finally, information obtained from a manual curation of the gene through an extensive literature search is also displayed. Curated information includes a mini review of gene function; experimentally determined gene properties such as molecular weight, pI, and protein structure; alternative names and aliases used in the literature; assignment of ontological terms describing gene function; the availability of reagents such as antibodies and clones; and also, as available, information on the functional effects of mutations. All of the information to construct the web page for this gene is directly provided as the result of a single database query. (The tables storing the manually curated gene information are not shown in **Figure 2**.) Obviously, compiling the data and entering it into the database required a substantial amount of effort, both computationally and manually; however, the information is now much more available and useful to the research scientist.

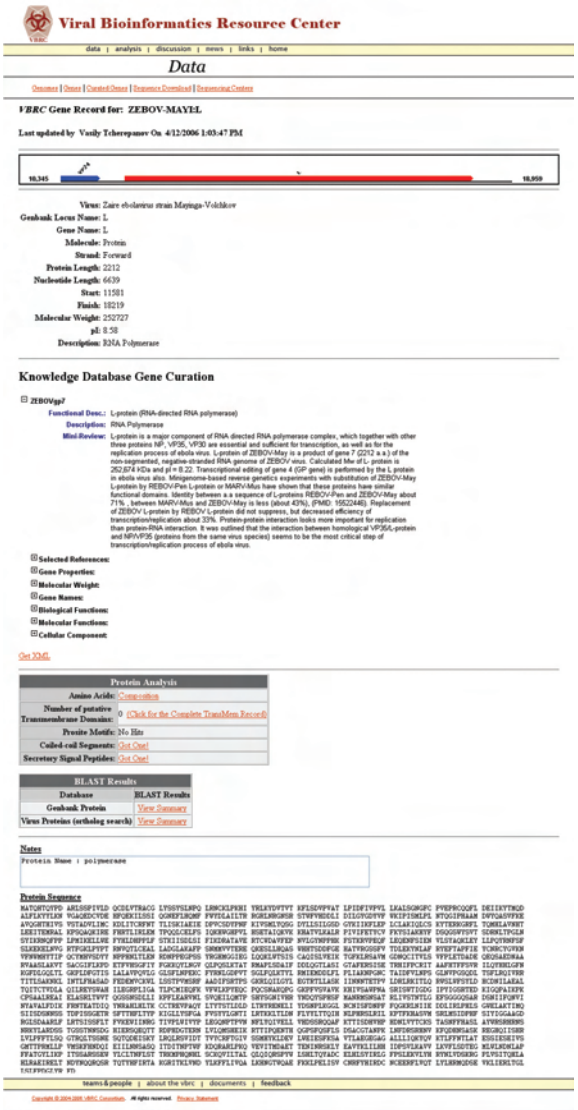


Figure 4 Gene record. An example of a gene record derived from data in the Viral Genome Database. The record contains a map showing the gene's location; basic descriptive information; analytical data; manually curated descriptive information; links to protein sequence analyses and BLAST similarity data; and the gene sequence itself.

Errors

No discussion of databases would be complete without considering errors. As in any other scientific endeavor, the data we generate, the knowledge we derive from the data, and the inferences we make as a result of the analysis of the data are all subject to error. These errors can be introduced at many points in the analytical chain. The original data may be faulty: using sequence data as one example, nucleotides in a DNA sequence may have been misread or miscalled, or someone may even have mistyped the sequence. The database may have been poorly designed; a field in a table designed to hold sequence information may have been set to hold only 2000 characters, whereas the sequences imported into that field may be longer than 2000 nucleotides. The sequences would have then been automatically truncated to 2000 characters, resulting in the loss of data. The curator may have mistyped an Enzyme Commission (EC) number for an RNA polymerase, or may have incorrectly assigned a genomic sequence to the wrong taxonomic classification. Or even more insidious, the curator may have been using annotations provided by other groups that had justified their own annotations on the basis of matches to annotations provided by yet another group. Such chains of evidence may extend far back, and the chance of propagating an early error increases with time. Such error propagation can be widespread indeed, affecting the work of multiple sequencing centers and database creators and providers. This is especially true given the dependencies of genomic sequence annotations on previously published annotations. The possible sources of errors are numerous, and it is the responsibility of both the database provider and the user to be aware of, and on the lookout for, errors. The database provider can, with careful database and

application design, apply error-checking routines to many aspects of the data storage and analysis pipeline. The code can check for truncated sequences, interrupted open reading frames, and nonsense data, as well as data annotations that do not match a provided controlled vocabulary. But the user should always approach any database or the output of any application with a little healthy skepticism. The user is the final arbiter of the accuracy of the information, and it is their responsibility to look out for inconsistent or erroneous results that may indicate either a random or systemic error at some point in the process of data collection and analysis.

Virus Databases

It is not feasible to provide a comprehensive and current list of all available databases that contain virus-related information or information of use to virus researchers. New databases appear on a regular basis; existing databases either disappear or become stagnant and outdated; or databases may change focus and domains of interest. Any resource published in book format attempting to provide an up-to-date list would be out-of-date on the day of publication. Even web-based lists of database resources quickly become out-of-date due to the rapidity with which available resources change, and the difficulty and extensive effort required to keep an online list current and inclusive. Therefore, our approach in this article is to provide an overview of the types of data that are obtainable from available biological databases, and to list some of the more important database resources that have been available for extended periods of time and, importantly, remain current through a process of continual updating and refinement. We should also emphasize that the use of web-based search tools such as Google, various web logs (Blogs), and news groups, can provide some of the best means of locating existing and newly available web-based information sources. Information contained in databases can be used to address a wide variety of problems. A sampling of the areas of research facilitated by virus databases includes

- taxonomy and classification;
- host range, distribution, and ecology;
- evolutionary biology;
- pathogenesis;
- host–pathogen interaction;
- epidemiology;
- disease surveillance;
- detection;
- prevention;
- prophylaxis;
- diagnosis; and
- treatment.

Addressing these problems involves mining the data in an appropriate database in order to detect patterns that allow certain associations, generalizations, cause–effect relationships, or structure–function relationships to be discerned. **Table 1** provides a list of some of the more useful and stable database resources of possible interest to virus researchers. Below, we expand on some of this information and provide a brief discussion concerning the sources and intended uses of these data sets.

Data

Major repositories of biological information

The two major, overarching collections of biological databases are at the NCBI, supported by the National Library of Medicine at the NIH, and the EMBL, part of the European Bioinformatics Institute. These large data repositories try to be all-inclusive, acting as the primary source of publicly available molecular biological data for the scientific community. In fact, most journals require that, prior to publication, investigators submit their original sequence data to one of these repositories. In addition to sequence data, NCBI and EMBL (along with many other data repositories) include a large variety of other data types, such as that obtained from gene-expression experiments and studies investigating biological structures. Journals are also extending the requirement for data deposition to some of these other data types. Note that while much of the data available from these repositories is raw data obtained directly as the result of experimental investigation in the laboratory, a variety of 'value-added' secondary databases are also available that take primary data records and manipulate or annotate them in some fashion in order to derive additional useful information.

When an investigator is unsure about the existence or source of some biological data, the NCBI and EMBL websites should serve as the starting point for locating such information. The NCBI Entrez Search Engine provides a powerful interface to access all information contained in the various NCBI databases, including all available sequence records. A search engine such as Google might also be used if NCBI and EMBL fail to locate the desired information. Of course PubMed, the repository of literature citations maintained at NCBI, also represents a major reference site for locating biological information. Finally, the journal *Nucleic Acids Research* (NAR) publishes an annual 'database' issue and an annual 'web server' issue that are excellent references for finding new biological databases and websites. And while the most recent NAR database or web server issue may contain articles on a variety of new and interesting databases and websites, be sure to also look at issues from previous years. Older issues contain articles on many existing sites that may not necessarily be represented in the latest

Table 1 Virus databases and other related information

Information resource name	*Sponsor*	*URL*	*Description*
Indices of databases			
All the Virology on the WWW	David M. Sander; Tulane University	http://www.virology.net/	Collection of virology information on the Internet
Nucleic Acids Research 2006 Database Issue	Journal: Nucleic Acids Research	http://nar.oxfordjournals.org/content/vol34/suppl_1/index.dtl	Issue describing molecular biology databases
Nucleic Acids Research 2006 Web Server Issue	Journal: Nucleic Acids Research	http://nar.oxfordjournals.org/content/vol34/suppl_2/index.dtl	Issue describing molecular biology websites
General sites			
NCBI	National Center for Biotechnology Information (NCBI), National Library of Medicine, National Institutes of Health (NIH)	http://www.ncbi.nlm.nih.gov/	Resource for molecular biology information
PubMed	National Library of Medicine, NIH	http://www.pubmed.gov/	Biomedical literature database
GenBank Sequence Database	NCBI	http://www.ncbi.nih.gov/Genbank/	Annotated collection of publicly available nucleotide sequences
NCBI Trace Archive	NCBI	http://www.ncbi.nlm.nih.gov/Traces/	NCBI repository of sequence trace files
NCBI Assembly Archive	NCBI	http://www.ncbi.nlm.nih.gov/Traces/assembly/	NCBI repository of sequence assembly files
EMBL Sequence Database	European Molecular Biology Laboratory (EMBL)	http://www.ebi.ac.uk/embl/	Annotated collection of publicly available nucleotide sequences
DDBJ Sequence Database	DNA Data Bank of Japan	http://www.ddbj.nig.ac.jp/	Annotated collection of publicly available nucleotide sequences
UniProt (Universal Protein Resource)	EMBL; Protein Information Resource, Georgetown University; Swiss Institute of Bioinformatics	http://www.ebi.ac.uk/uniprot/	Protein sequence repository
Taxonomy			
ICTVnet	International Committee on Taxonomy of Viruses (ICTV)	http://www.danforthcenter.org/iltab/ictvnet/	ICTV official website
ICTVdb	ICTV	http://www.ncbi.nlm.nih.gov/ICTVdb	ICTV database of virus taxonomy
NCBI Taxonomy Browser	NCBI	http://www.ncbi.nlm.nih.gov/Taxonomy/	Taxonomy-based retrieval of sequence data
Virus-oriented			
NCBI Viral Genomes Resource	NCBI	http://www.ncbi.nlm.nih.gov/genomes/VIRUSES/viruses.html	NCBI resources for the study of viruses
ASV	American Society for Virology (ASV)	http://www.asv.org/	Society to promote the exchange of information and stimulate discussion and collaboration among scientists active in all aspects of virology
IUMS-Virology	International Union of Microbiological Societies	http://www.iums.org/divisions/divisions-virology.html	Society to promote the study of microbiological sciences internationally

Continued

Table 1 Continued

Information resource name	*Sponsor*	*URL*	*Description*
Viruses: From Structure to Biology	ASV; Washington University School of Medicine	http://medicine.wustl.edu/~virology/	Historical overview of virus research
BRCs (Bioinformatics Resource Centers for Biodefense and Emerging or Re-Emerging Infectious Diseases)	National Institute of Allergy and Infectious Diseases (NIAID), NIH	http://www.brc-central.org/	NIAID/NIH-funded Bioinformatics Resource Centers providing web-based genomics resources to the scientific community on viruses in category A, B, and C priority pathogens
VBRC (Viral Bioinformatics Resource Center)	University of Alabama at Birmingham; NIAID	http://www.vbrc.org/	Bioinformatics resources directed at *Arenaviridae, Bunyaviridae, Flaviviridae, Filoviridae, Paramyxoviridae, Poxviridae*, and *Togaviridae*
Viral Bioinformatics-Canada	University of Victoria, Canada	http://www.virology.ca/	Viral genomics resources
PATRIC (PathoSystems Resource Integration Center)	Virginia Bioinformatics Institute; NIAID	http://patric.vbi.vt.edu/	Bioinformatics resources directed at *Caliciviridae, Coronaviridae*, hepatitis A virus, hepatitis E virus, *Lyssaviridae*
VirOligo	Oklahoma State University	http://viroligo.okstate.edu/	Virus-specific oligonucleotides for PCR and hybridization
VirGen	Bioinformatics Centre, University of Pune, India	http://bioinfo.ernet.in/virgen/	Annotated and curated database for complete viral genome sequences
VIDE (Virus Identification Data Exchange)	Australian Centre for International Agricultural Research; University of Idaho	http://image.fs.uidaho.edu/vide/	Online descriptions and lists of plant viruses
RNAs and Proteins of dsRNA Viruses	Institute for Animal Health, UK Biotechnology and Biological Sciences Research Council	http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/	Databases for the study of *Reoviridae, Cystoviridae, Birnaviridae, Totiviridae*
euHCVdb (European HCV Database)	Institute for the Biology and Chemistry of Proteins, CNRS and Lyon University	http://euhcvdb.ibcp.fr/	European hepatitis C virus database
HCV Database Project	Los Alamos National Laboratories; NIAID	http://hcv.lanl.gov/	HCV sequence and immunology database
Hepatitis Virus Database	National Institute of Genetics (Japan)	http://s2as02.genes.nig.ac.jp/	Database for the study of hepatitis B, C, and E
HERVd (Human Endogenous Retrovirus Database)	Institute of Molecular Genetics, Academy of Sciences of The Czech Republic	http://herv.img.cas.cz/	Human endogenous retrovirus database
HIV Drug Resistance Database	Stanford University	http://hivdb.stanford.edu/	HIV drug resistance database
HIV Sequence Database	Los Alamos National Laboratories; NIAID	http://www.hiv.lanl.gov/	HIV sequence, resistance, immunology, and vaccine trials databases
HIV-1, Human Protein Interaction Database	Southern Research Institute, Birmingham, Alabama; NCBI	http://www.ncbi.nlm.nih.gov/RefSeq/HIVInteractions/	Interactions of HIV-1 proteins with those of the host cell

BioHealthBase (Influenza)	University of Texas Southwestern Medical Center; NIAID	http://www.biohealthbase.org/	Bioinformatics resources directed at influenza
Influenza Sequence Database	Los Alamos National Laboratories	http://www.flu.lanl.gov/	Influenza sequence database
Influenza Virus Resource	NCBI	http://www.ncbi.nlm.nih.gov/genomes/FLU/	Influenza sequence database
IVDB (Influenza Virus Database)	Beijing Genomics Institute	http://influenza.genomics.org.cn/	Integrated information resource and analysis platform for influenza virus
Influenza Genome Sequencing Project	NIAID	http://www.niaid.nih.gov/dmid/genomes/mscs/influenza.htm	NIAID influenza sequencing project
Poxvirus Bioinformatics Resource Center	University of Alabama at Birmingham; NIAID	http://www.poxvirus.org/	Poxvirus genomic sequences, gene annotations, and analysis
SARS-CoV RNA SSS Database	Center for Modern Biology, School of Life Sciences, Yunnan University, Kunming, China	http://www.liuweibo.com/sarsdb/	SARS Secondary Structural Sequence database
ACLAME (A Classification of Genetic Mobile Elements)	Department of Macromolecular Conformation and Bioinformatics, Free University of Brussels, Belgium	http://aclame.ulb.ac.be/	Database of mobile genetic elements
Subviral RNA Database	University of Ottawa	http://subviral.med.uottawa.ca/	Viroids and viroid-like RNAs
Genomes of the T4-like phages	Department of Biochemistry, Tulane University	http://phage.bioc.tulane.edu/	Sequences of T4-like bacteriophages
DPVweb (Descriptions of plant viruses)	Association of Applied Biologists (UK)	http://www.dpvweb.net/	Plant virus database
ILTAB (International Laboratory for Tropical Agricultural Biotechnology)	Danforth Plant Sciences Center, St. Louis, Missouri	http://www.danforthcenter.org/iltab/	*Caulimoviridae, Geminiviridae*, and *Potyviridae* database
Functional motifs			
InterPro	European Bioinformatics Institute (EBI)	http://www.ebi.ac.uk/interpro/	Database of protein families, domains, and functional sites
CDD (Conserved Domain Database)	NCBI	http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtml	Conserved Domain Database and search service
PFAM (Protein families)	Washington University in St. Louis	http://pfam.wustl.edu/	Database of protein families and Hidden Markov models
PRINTS (Protein fingerprints)	University of Manchester, England	http://www.bioinf.man.ac.uk/dbbrowser/PRINTS/	Conserved motifs used to characterize a protein family
PROSITE	Swiss Institute of Bioinformatics	http://ca.expasy.org/prosite/	Database of protein families and domains
VIDA (Virus database of homologous protein families)	Wohl Virion Centre, University College London and Genome Informatics Research Lab, Universitat Pompeu Fabra, Barcelona	http://www.biochem.ucl.ac.uk/bsm/virus_database/	Homologous protein families from complete and partial virus genomes
VOCs (Viral Orthologous Clusters)	University of Victoria, Canada	http://athena.bioc.uvic.ca/workbench.php?tool=vocs	Viral orthologous clusters database

Continued

Table 1 Continued

Information resource name	*Sponsor*	*URL*	*Description*
VOGs (Viral Clusters of Orthologous Groups)	NCBI	http://www.ncbi.nlm.nih.gov/genomes/VIRUSES/vog.html	Viral clusters of orthologous groups database
Structural			
PDB (Protein Data Bank)	Research Collaboratory for Structural Bioinformatics	http://www.rcsb.org/	Three-dimensional structure database
Macromolecular Structure Database	EBI	http://www.ebi.ac.uk/msd/	Macromolecular structure database
Dali	Institute of Biotechnology, University of Helsinki, Finland	http://ekhidna.biocenter.helsinki.fi/dali/	Database of 3D structure comparisons
The Big Picture Book of Viruses	David M. Sander; Tulane University	http://www.virology.net/Big_Virology/	Catalog of virus pictures on the Internet
VIPERdb (Virus particle explorer)	Scripps Research Institute Structural Biology	http://viperdb.scripps.edu/	Icosahedral virus capsid structures
Virus World	Institute for Molecular Virology, University of Wisconsin-Madison	http://virology.wisc.edu/virusworld/	Images of virus capsids from X-ray crystallography and cryo-EM data
Pathway			
Reactome	Cold Spring Harbor Laboratory, EBI, Gene Ontology Consortium	http://www.reactome.org/	Curated knowledgebase of biological pathways
BioCyc	SRI International, Menlo Park, California	http://www.biocyc.org/	Collection of databases describing genome and metabolic pathways
KEGG (Kyoto Encyclopedia of Genes and Genomes)	Kyoto University and the Human Genome Center at University of Tokyo	http://www.genome.jp/kegg/	Database of biological pathways and genes
Other			
GO (Gene Ontology Database)	Gene Ontology Consortium	http://www.geneontology.org/	Development of controlled vocabularies (ontologies) that describe gene products in terms of their associated biological processes, cellular components, and molecular functions
National Center for Biomedical Ontology	Stanford University	http://bioontology.org/	Development of innovative technologies and methods that allow scientists to create, disseminate, and manage biomedical ontologies
GEO (Gene Expression Omnibus)	NCBI	http://www.ncbi.nlm.nih.gov/geo/	A gene expression/molecular abundance data repository

IEDB (Immune Epitope Database and Analysis Resource)	La Jolla Institute for Allergy and Immunology	http://www.immuneepitope.org/	Dissemination of immune epitope information
IntAct (Protein interactions)	EBI	http://www.ebi.ac.uk/intact/	Database and analysis system for protein interactions
Analytical tools			
ExPASy	Swiss Institute of Bioinformatics (SIB)	http://ca.expasy.org/	Tools for the analysis of protein sequences, structures, etc.
PASC (Pairwise Sequence Comparison)	NCBI	http://www.ncbi.nlm.nih.gov/sutils/pasc/	Analysis of pairwise identity distribution within viral families
NCBI Viral Genotyping Tool	NCBI	http://www.ncbi.nlm.nih.gov/projects/genotyping/	Identification of viral sequence genotypes
Medically oriented			
AIDSinfo	US Department of Health and Human Services	http://aidsinfo.nih.gov/	Information on HIV/AIDS treatment, prevention, and research
AMA Infectious diseases	American Medical Association	http://www.ama-assn.org/ama/pub/category/1797.html	Online information resources
CDC NCID (National Center for Infectious Diseases)	US Centers for Disease Control and Prevention (CDC)	http://www.cdc.gov/ncidod/diseases/	Centers for Disease Control and Prevention (USA) disease index
CIPHI Fact Sheets	Canadian Institute of Public Health Inspectors	http://action.web.ca/home/ciphiont/readingroom.shtml	Information pages for public health, including viral diseases
CSEI (Center for the Study of Emerging Infections)	Institute for BioSecurity, St Louis University, School of Public Health	http://www.emerginginfections.slu.edu/	Information on emerging infections
Diseases & Conditions	New York State Department of Health	http://www.health.state.ny.us/diseases/	General public information
ENIVD (European Network for Diagnostics of ''Imported'' Viral Diseases)	European Commission	http://www.enivd.org/	European Network for Diagnostics of 'Imported' Viral Diseases
EPR (Epidemic and Pandemic Alert and Response)	World Health Organization (WHO)	http://www.who.int/csr/disease/en/	WHO integrated global alert and response system for epidemics and other public health emergencies
European Centre for Disease Prevention and Control	The European Union	http://www.ecdc.eu.int/	European Centre for Disease Prevention and Control
MedScape	WebMD	http://www.medscape.com/	Information and educational tools for specialists
Merck Manual of Diagnosis and Therapy, Infectious Diseases	Merck & Co., Inc.	http://www.merck.com/mrkshared/mmanual/section13/sec13.jsp	Online reference of infectious diseases
MMWR (Morbidity and Mortality Weekly Report)	CDC	http://www.cdc.gov/mmwr/	CDC Morbidity and Mortality Weekly Report
NIH Vaccine Research Center	Dale and Betty Bumpers' Vaccine Research Center, NIAID, NIH	http://www.vrc.nih.gov/	NIH vaccine research

journal publication, but are nevertheless still available and current.

There are several websites that serve to provide general virus-specific information and links of use to virus researchers. One of these is the NCBI Viral Genomes Project, which provides an overview of all virus-related NCBI resources including taxonomy, sequence, and reference information. Links to other sources of viral data are provided, as well as a number of analytical tools that have been developed to support viral taxonomic classification and sequence clustering. Another useful site is the All the Virology on the WWW website. This site provides numerous links to other virus-specific websites, databases, information, news, and analytical resources. It is updated on a regular basis and is therefore as current as any site of this scope can be.

Taxonomy and classification

One of the strengths of storing information within a database is that information derived from different sources or different data sets can be compared so that important common and distinguishing features can be recognized. Such comparative analyses are greatly aided by having a rigorous classification scheme for the information being studied. The International Union of Microbiological Societies has designated the International Committee on Taxonomy of Viruses (ICTV) as the official body that determines taxonomic classifications for viruses. Through a series of subcommittees and associated study groups, scientists with expertise on each viral species participate in the establishment of new taxonomic groups, assignment of new isolates to existing or newly established taxonomic groups, and reassessment of existing assignments as additional research data become available. The ICTV uses more than 2600 individual characteristics for classification, though sequence homology has gained increasing importance over the years as one of the major classifiers of taxonomic position. Currently, as described in its Eighth Report, the ICTV recognizes 3 orders, 73 families, 287 genera, and 1950 species of viruses. The ICTV officially classifies viral isolates only to the species level. Divisions within species, such as clades, subgroups, strains, isolates, types, etc., are left to others. The ICTV classifications are available in book form as well as from an online database. This database, the ICTVdb, contains the complete taxonomic hierarchy, and assigns each known viral isolate to its appropriate place in that hierarchy. Descriptive information on each viral species is also available. The NCBI also provides a web-based taxonomy browser for access to taxonomically specified sets of sequence records. NCBI's viral taxonomy is not completely congruent with that of ICTV, but efforts have been under way to ensure congruency with the official ICTV classification.

Nucleotide sequence data

The primary repositories of existing sequence information come from the three organizations that comprise the International Nucleotide Sequence Database Collaboration. These three sites are GenBank (maintained at NCBI), EMBL, and the DNA Data Bank of Japan (DDBJ). Because all sequence information submitted to any one of these entities is shared with the others, a researcher need query only one of these sites to get the most up-to-date set of available sequences. GenBank stores all publicly available nucleotide sequences for all organisms, as well as viruses. This includes whole-genome sequences as well as partial-genome and individual coding sequences. Sequences are also available from large-scale sequencing projects, such as those from shotgun sequencing of environmental samples (including viruses), and high-throughput low- and high-coverage genomic sequencing projects. NCBI provides separate database divisions for access to these sequence datasets. The sequence provided in each GenBank record is the distillation of the raw data generated by (in most cases these days) automated sequencing machines. The trace files and base calls provided by the sequencers are then assembled into a collection of contiguous sequences (contigs) until the final sequence has been assembled. In recognition of the fact that there is useful information contained in these trace files and sequence assemblies (especially if one would like to look for possible sequencing errors or polymorphisms), NCBI now provides separate Trace File and Assembly Archives for GenBank sequences when the laboratory responsible for generating the sequence submits these files. Currently, the only viruses represented in these archives are influenza A, chlorella, and a few bacteriophages.

An important caveat in using data obtained from GenBank or other sources is that no sequence data can be considered to be 100% accurate. Furthermore, the annotation associated with the sequence, as provided in the GenBank record, may also contain inaccuracies or be out-of-date. GenBank records are provided and maintained by the group originally submitting the sequence to GenBank. GenBank may review these records for obvious errors and formatting mistakes (such as the lack of an open reading frame where one is indicated), but given the large numbers of sequences being submitted, it is impossible to verify all of the information in these records. In addition, the submitter of a sequence essentially 'owns' that sequence record and is thus responsible for all updates and corrections. NCBI generally will not change any of the information in the GenBank record unless the sequence submitter provides the changes. In some cases, sequence annotations will be updated and expanded, but many, if not most, records never change following their initial submission. (These facts emphasize the responsibility that submitters of sequence data have to ensure the accuracy of their

original submission and to update their sequence data and annotations as necessary.) Therefore, the user of the information has the responsibility to ensure, to the extent possible, its accuracy is sufficient to support any conclusions derived from that information. In recognition of these problems, NCBI established the Reference Sequence (RefSeq) database project, which attempts to provide reference sequences for genomes, genes, mRNAs, proteins, and RNA sequences that can be used, in NCBI's words, as "a stable reference for gene characterization, mutation analysis, expression studies, and polymorphism discovery". RefSeq records are manually curated by NCBI staff, and therefore should provide more current (and hopefully more accurate) sequence annotations to support the needs of the research community. For viruses, RefSeq provides a complete genomic sequence and annotation for one representative isolate of each viral species. NCBI solicits members of the research community to participate as advisors for each viral family represented in RefSeq, in an effort to ensure the accuracy of the RefSeq effort.

Protein sequence data

In addition to the nucleotide sequence databases mentioned above, UniProt provides a general, all-inclusive protein sequence database that adds value through annotation and analysis of all the available protein sequences. UniProt represents a collaborative effort of three groups that previously maintained separate protein databases (PIR, SwissProt, and TrEMBL). These groups, the National Biomedical Research Foundation at Georgetown University, the Swiss Institute of Bioinformatics, and the European Bioinformatics Institute, formed a consortium in 2002 to merge each of their individual databases into one comprehensive database, UniProt. UniProt data can be queried by searching for similarity to a query sequence, or by identifying useful records based on the text annotations. Sequences are also grouped into clusters based on sequence similarity. Similarity of a query sequence to a particular cluster may be useful in assigning functional characteristics to sequences of unknown function. NCBI also provides a protein sequence database (with corresponding RefSeq records) consisting of all protein-coding sequences that have been annotated within all GenBank nucleotide sequence records.

Virus-specific sequence databases

The above-mentioned sequence databases are not limited to viral data, but rather store sequence information for all biological organisms. In many cases, access to nonviral sequences is necessary for comparative purposes, or to study virus–host interactions. But it is frequently easier to use virus-specific databases when they exist, to provide a more focused view of the data that may simplify many of the analyses of interest. **Table 1** lists many of these virus-specific sites. Sites of note include the NIH-supported Bioinformatics Resource Centers for Biodefense and Emerging and Reemerging Infectious Diseases (BRCs). The BRCs concentrate on providing databases, annotations, and analytical resources on NIH priority pathogens, a list that includes many viruses. In addition, the LANL has developed a variety of viral databases and analytical resources including databases focusing on HIV and influenza. For plant virologists, the Descriptions of Plant Viruses (DPV) website contains a comprehensive database of sequence and other information on plant viruses.

Structural information

The three-dimensional structures for quite a few viral proteins and virion particles have been determined. These structures are available in the primary database for experimentally determined structures, the Protein Data Bank (PDB). The PDB currently contains the structures for more than 650 viral proteins and viral protein complexes out of 38 000 total structures. Several virus-specific structure databases also exist. These include the VIPERdb database of icosahedral viral capsid structures, which provides analytical and visualization tools for the study of viral capsid structures; Virus World at the Institute for Molecular Virology at the University of Wisconsin, which contains a variety of structural images of viruses; and the Big Picture Book of Viruses, which provides a catalog of images of viruses, along with descriptive information.

Functional motifs and orthologous clusters

Ultimately, the biology of viruses is determined by genomic sequence (with a little help from the host and the environment). Nucleotide sequences may be structural, functional, regulatory, or protein coding. Protein sequences may be structural, functional, and/or regulatory, as well. Patterns specified in nucleotide or amino acid sequences can be identified and associated with many of these biological roles. Both general and virus-specific databases exist that map these roles to specific sequence motifs. Most also provide tools that allow investigators to search their own sequences for the presence of particular patterns or motifs characteristic of function. General databases include the NCBI Conserved Domain Database; the Pfam (protein family) database of multiple sequence alignments and hidden Markov models; and the PROSITE database of protein families and domains. Each of these databases and associated search algorithms differ in how they detect a particular search motif or define a particular protein family. It can therefore be useful to employ multiple databases and search methods when analyzing a new sequence (though in many cases they will each detect a similar set of putative functional motifs). InterPro is a database of protein families, domains, and functional sites that combines many other existing motif databases. InterPro

provides a search tool, InterProScan, which is able to utilize several different search algorithms dependent on the database to be searched. It allows users to choose which of the available databases and search tools to use when analyzing their own sequences of interest. A comprehensive report is provided that not only summarizes the results of the search, but also provides a comprehensive annotation derived from similarities to known functional domains. All of the above databases define functional attributes based on similarities in amino acid sequence. These amino acid similarities can be used to classify proteins into functional families. Placing proteins into common functional families is also frequently performed by grouping the proteins into orthologous families based on the overall similarity of their amino acid sequence as determined by pairwise BLAST comparisons. Two virus-specific databases of orthologous gene families are the Viral Clusters of Orthologous Groups database (VOGs) at NCBI, and the Viral Orthologous Clusters database (VOCs) at the Viral Bioinformatics Resource Center and Viral Bioinformatics, Canada.

Other information

Many other types of useful information, both general and virus-specific, have been collected into databases that are available to researchers. These include databases of gene-expression experiments (NCBI Gene Expression Omnibus – GEO); protein–protein interaction databases, such as the NCBI HIV Protein-Interaction Database; The Immune Epitope Database and Analysis Resource (IEDB) at the La Jolla Institute for Allergy and Immunology; and databases and resources for defining and visualizing biological pathways, such as metabolic, regulatory, and signaling pathways. These pathway databases include Reactome at the Cold Spring Harbor Laboratory, New York; BioCyc at SRI International, Menlo Park, California; and the Kyoto Encyclopedia of Genes and Genomes (KEGG) at Kyoto University in Japan.

Analytical Tools

As indicated above, the information contained in a database is useless unless there is some way to retrieve that information from the database. In addition, having access to all of the information in every existing database would be meaningless unless tools are available that allow one to process and understand the data contained within those databases. Therefore, a discussion of virus databases would not be complete without at least a passing reference to the tools that are available for analysis. To populate a database such as the VGD with sequence and analytical information, and to utilize this information for subsequent analyses, requires a variety of analytical tools including programs for

- sequence record reformatting,
- database import and export,
- sequence similarity comparison,
- gene prediction and identification,
- detection of functional motifs,
- comparative analysis,
- multiple sequence alignment,
- phylogenetic inference,
- structural prediction, and
- visualization.

Sources for some of these tools have already been mentioned, and many other tools are available from the same websites that provide many of the databases listed in **Table 1**. The goal of all of these sites that make available data and analytical tools is to provide – or enable the discovery of – knowledge, rather than simply providing access to data. Only in this manner can the ultimate goal of biological understanding be fully realized.

See also: Evolution of Viruses; Phylogeny of Viruses; Taxonomy, Classification and Nomenclature of Viruses; Virus Classification by Pairwise Sequence Comparison (PASC).

Further Reading

Ashburner M, Ball CA, Blake JA, *et al.* (2000) Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. *Nature Genetics* 25(1): 25–29.

Bao Y, Federhen S, Leipe D, *et al.* (2004) National Center for Biotechnology Information Viral Genomes Project. *Journal of Virology* 78(14): 7291–7298.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (2005) *Virus Taxonomy: Classification and Nomenclature of Viruses. Eighth Report of the International Committee on Taxonomy of Viruses.* San Diego, CA: Elsevier Academic Press.

Galperin MY (2006) The Molecular Biology Database Collection: 2006 update. *Nucleic Acids Research* 34(database issue): D3–D5.

Joshi-Tope G, Gillespie M, Vastrik I, *et al.* (2005) Reactome: A knowledgebase of biological pathways. *Nucleic Acids Research* 33(database issue): D428–D432.

Kellam P and Alba MM (2002) Virus bioinformatics: Databases and recent applications. *Applied Bioinformatics* 1(1): 37–42.

Korber B, LaBute M, and Yusim K (2006) Immunoinformatics comes of age. *PLoS Computational Biology* 2(6): e71.

Kuiken C, Korber B, and Shafer RW (2003) HIV sequence databases. *AIDS Reviews* 5(1): 52–61.

Kuiken C, Mizokami M, Deleage G, *et al.* (2006) Hepatitis C databases, principles and utility to researchers. *Hepatology* 43(5): 1157–1165.

Lefkowitz EJ, Upton C, Changayil SS, *et al.* (2005) Poxvirus bioinformatics resource center: A comprehensive *Poxviridae* informational and analytical resource. *Nucleic Acids Research* 33(database issue): D311–D336.

Lesk AM (2005) *Database Annotation in Molecular Biology.* Chichester, UK: Wiley.

Lindler LE, Lebeda FJ, and Korch G (2005) *Biological Weapons Defense: Infectious Diseases and Counterbioterrorism.* Totowa, NJ: Humana Press.

Natarajan P, Lander GC, Shepherd CM, *et al.* (2005) Exploring icosahedral virus structures with VIPER. *Nature Reviews Microbiology* 3(10): 809–817.

Rubin DL, Lewis SE, Mungall CJ, *et al.* (2006) National Center for Biomedical Ontology: Advancing biomedicine through structured organization of scientific knowledge. *Omics* 10(2): 185–198.

Yusim K, Richardson R, Tao N, *et al.* (2005) Los Alamos Hepatitis C Immunology Database. *Applied Bioinformatics* 4(4): 217–225.

Virus Entry to Bacterial Cells

M M Poranen and A Domanska, University of Helsinki, Helsinki, Finland

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Adsorption Initial interaction between a virus particle and a cellular receptor molecule.
Bacteriophage (or phage) A virus that infects bacteria.
Capsid The protective protein coat of a virus particle.
Cell envelope Plasma membrane and cellular structures located outside the plasma membrane.
Cell wall Cellular structures located outside the plasma membrane.
Lipopolysaccharide A unique glycolipid of the outer membrane of Gram-negative bacteria.
Peptidoglycan A polymer consisting of long glycan chains cross-linked via peptide bridges and forming homogenous layer outside the plasma membrane of eubacteria component of the cell wall.
Receptor A specific molecule or molecular assembly exposed on the surface of a cell to which a virus entering the cell attaches.
Receptor-binding protein A virion protein responsible for the interaction of a virion with a specific cellular receptor molecule.
Vertex Fivefold symmetry position of the icosahedra; one icosahedral particle has 12 fivefold symmetry positions.
Viral envelope An outer lipid–protein bilayer of a virus.
Virion A virus particle, the extracellular form of a virus.

Introduction

Viruses are intracellular parasites that are dependent on the metabolic apparatus of the cell. Unlike other parasitic self-replicating systems, like plasmids and viroids, viruses possess an extracellular phase that allows spread from one infected cell or organism to another. Consequently, viruses have to have means to infect new host cells. The entry into a suitable host cell is a key event for the viral reproduction and survival.

Host Cell Barriers

The nature of the host cell wall has a great influence on the viral entry strategy. Gram-positive bacteria have a single internal lipid bilayer and a thick cell wall made of peptidoglycan while Gram-negative cells are covered by an internal membrane, a thin layer of peptidoglycan, and an outer membrane. In addition, bacterial cells may secrete polysaccharides that make a protective extracellular capsule on the surface of the cell.

The relatively strong and inert cell wall of eubacteria efficiently restricts the passage of macromolecules. In addition, bacterial cells do not have endocytic-like uptake systems, which are commonly utilized by eukaryotic viruses to gain access into the host cell. These features of the host strongly influence the mechanism employed by bacterial viruses to gain access into the host cytoplasm. In fact, the capsids of most bacteriophages are never internalized into the host cell; only the viral genome with some necessary protein factors is delivered across the host envelope.

Virions as a Genome Delivery Devise

Virions represent an extracellular form of the virus. It is a vehicle, which allows the virus to resist the harsh environment outside the cell. In addition to its protective nature, the main task of the virion is to recognize the host and to deliver the viral genome with necessary accessory factors to the new host cell. The mechanism of the genome delivery is typically reflected in the structure of the capsid (see below).

The nature of the viral genome influences the mechanisms of virus entry. Viruses that have genomes which cannot be expressed using the enzymatic apparatuses of the host, need to also bring viral polymerases into the host cell. This applies to all viruses having dsRNA or negative-sense ssRNA genomes. Regardless of the type of the genome, many viruses deliver some accessory protein factors inside the host. These are required in the early stage of the infection, prior to the viral genome expression, either to complete the entry process or for successful genome replication and expression.

Host Recognition

Host cell recognition by viruses is a highly specific process. Basically, all bacteriophages have, on their exterior surface, some protein that binds to a receptor molecule exposed on the surface of a susceptible cell. The receptor-binding proteins are often localized in the vertices of the

icosahedral virions or the tips of the helical virions. In tailed bacteriophages the initial recognition is carried out by the fibers that are connected to the distal end of the tail (**Figure 1**(**a**)). The specific recognition leads to irreversible structural rearrangements in the virion components; the viral receptor-binding complexes overcome an energy barrier and fold into a minimal energy state. These conformational changes lead to more tight attachment and eventually trigger the entry process. The rigid structure of the virion is destabilized so that the genome delivery can be accomplished. The icosahedral capsids of bacteriophages seldom disassemble completely; only the structures at the vertices become labile making openings for genome release.

Many bacteriophages, including icosahedral ssRNA bacteriophages (e.g., MS2, Qβ), filamentous ssDNA bacteriophages (e.g., Ff, as M13, fd, and fl), and enveloped dsRNA phage (ϕ6), utilize a bacterial pilus as their primary receptor. The pilus enables efficient capture of the virion at a distance from the cell surface, and the retraction of the pilus translocates the bound phages to the host envelope (**Figure 1**(**b**)). This allows phages to get access to the cell surface regardless of the polysaccharide capsule, which could restrict the easy access to the cell surface. Some phages (e.g., K1-5, K5, K1E), however, may also use the polysaccharide capsule as the initial site of recognition and binding. Typically, these phages have enzymatic activities associated with the virion for capsule degradation.

Other receptor sites for bacteriophages are lipopolysaccharides, various cell envelope and flagellar proteins, as well as cell-wall carbohydrates. The tailed phages infecting Gram-negative bacteria use either the lipopolysaccharide moieties (e.g., T2, T4, T7) or envelope proteins, such as porins and transporters (e.g., PP01, T1, T5, λ) as their receptors, while phages infecting Gram-positive bacteria typically attach to the cell-wall teichoic acids. Membrane proteins or peptidoglycan moieties rarely are used as attachment sites for bacteriophages infecting Gram-positive bacteria.

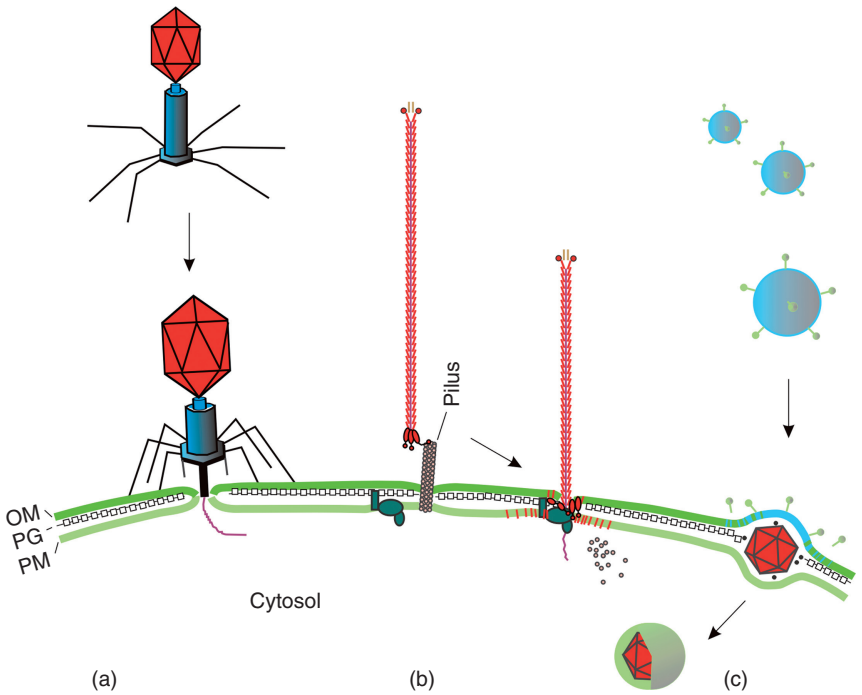


Figure 1 Schematic presentation of the main entry strategies utilized by bacterial viruses. OM, outer membrane; PG, peptidoglycan; PM, plasma membrane. (a) Genome delivery through an icosahedral vertex; given example is a phage with long-contractile tail from the family *Myoviridae* (e.g., T4). Primary interaction between the phage and the host cell is mediated by the tail fibers (black). Contraction of the tail sheath (blue) facilitates the penetration of the cell envelope. The viral genome (purple) is released from the head (red) of the phage virion through the tail tube (black) into the host cytosol. The protein capsid remains outside the cell. (b) Dissociation of filamentous phage capsid at the cell envelope. The receptor binding protein located at the tip of the helical virion interacts with the bacterial pilus. Pilus retraction brings the virion on the cell surface enabling interaction with the co-receptor molecule (green). The viral genome (purple) is released into the host cytosol as the virion proteins are inserted into the plasma membrane. (c) Penetration of the nucleoprotein complex of an enveloped dsRNA virion. The viral spike proteins (green) on the virion surface mediate the interaction between the host and the virion. Fusion between the host outer membrane and phage envelope (blue) takes place leading to the mixing of host and viral lipids. The nucleoprotein assembly (nucleocapsid; red) released into the bacterial periplasm penetrates the peptidoglycan network with the aid of a lytic enzyme (black) located on the nucleocapsid surface. Subsequently, the host plasma membrane is penetrated via an endocytic-like process.

Cell-Wall Penetration

The virions of many bacteriophages contain peptidoglycan-hydrolyzing enzymes. These are specialized proteins that locally and temporarily disrupt the peptidoglycan network, thus allowing the penetration of the cell wall. Similar enzymes are also involved in the release of progeny phages from the host cell at the end of the infection cycle. However, when assembled into virions, they likely have an active and crucial role in the entry process.

The lytic activity of virion-associated peptidoglycan-hydrolyzing enzymes is stringently controlled to ensure localized openings in the cell wall. This allows maintaining cell integrity, which is essential for successful production of progeny viruses during the infection cycle. For this reason such enzymes are tightly incorporated into the virus particle. The peptidoglycan-hydrolyzing enzymes of tailed bacterial viruses are often associated with the tail structures or, in the case of some short-tailed phages (e.g., T7), with internal head proteins that are ejected at the beginning of the infection cycle. Icosahedral dsDNA phages with internal membrane and no tail (e.g., PRD1, Bam35) as well as the enveloped dsRNA phage $\phi 6$ also possess peptidoglycan-hydrolyzing enzymes in their virions (see below; **Figure 1**(**c**)).

The most common cell-wall-degrading enzymes found in bacteriophage virions are lysozymes and lytic transglycosylases. Both lysozymes and lytic transglycosylases cleave the same glycosidic bond between *N*-acetylmuramic acid and *N*-acetylglucosamine units of the glycan strands. The end product of lysozyme is a disaccharide with reducing end of *N*-acetylmuramic acid, whereas that of lytic transglycosylase is a disaccharide containing a ring structure (anhydromuramic acid).

Not all phages have specialized enzymes in their virions for the penetration of the host cell wall during entry. These phages rely on preexisting channels within the host envelope. This applies to bacterial viruses with ssDNA and ssRNA genomes. For example, infection of filamentous ssDNA phages absolutely depends on a protein complex assembled in the bacterial plasma membrane spanning the peptidoglycan layer (**Figure1**(**b**)).

Genome Delivery Mechanisms of Phages

In general, entry strategies of bacterial viruses fall into three main categories: (1) genome delivery through an icosahedral vertex, (2) virion dissociation at the cell envelope, and (3) virion penetration via membrane fusion and endocytic-like event (**Figure 1**). All known tailed dsDNA phages (e.g., T4, T5, T7), dsDNA phages with an internal membrane (tectiviruses), as well as icosahedral ssDNA and ssRNA phages deliver their genome through a genome delivery apparatus located at one of the capsid vertices (**Figure 1**(**a**)). Capsids of filamentous phages and membrane-containing phage PM2 disassemble completely at the cell envelope (**Figure 1**(**b**)), whereas the enveloped dsRNA bacterial viruses utilize a unique membrane fusion-type uncoating at the outer membrane and virus subparticle internalization through the plasma membrane (**Figure 1**(**c**)).

Icosahedral Tailed dsDNA Bacterial Viruses

The tailed dsDNA bacteriophages use the tail structure as a genome delivery devise (**Figure 1**(**a**)). The tail is a protein tube of various length and complexity attached at one of the vertices of the icosahedral head. During the entry process the viral genome enclosed within the head travels through the tail into the host cell. Despite the numerous types of tailed bacterial viruses, they all possess one of the following three types of tails: long contractile, long noncontractile, or short noncontractile tail. The type of tail serves as the main criterion for classification of tailed bacteriophages and also influences the genome delivery mechanism.

The contractile tail which is common for members of the family *Myoviridae* is a complex, long tube assembly surrounded by a sheath that is terminated by a base plate typically decorated with terminal tail fibers (**Figure 1**(**a**)). The tail fibers act as sensor to detect the host bacterium. Interaction between the tail fibers and their surface receptors leads to conformational changes in the base plate and subsequent irreversible adsorption of the virus on bacterial cells. This triggers contraction of the tail sheath, which then drags the base plate along the tail tube making the tube protrude from the base plate (**Figure 1**(**a**)). Subsequently, the tail tube penetrates the cell envelope and phage DNA is released into the cell through a transmembrane channel made from viral proteins, host proteins, or a combination of both. The force required for penetration is associated with the contraction of the tail sheath. The peptidoglycan-hydrolyzing activity present in the tail tube facilitates the penetration of the peptidoglycan layer. Some viral proteins enter the cell with the phage DNA in order to protect it from host exonuclease activities and are implicated in the early stages of phage genome transcription.

The bacteriophages of the family *Syphoviridae* have long noncontractile tails. The noncontractile tail is composed of a relatively flexible tube ending with a tip (or straight fiber) and few tail fibers attached to the tube. In contrast to myoviruses, tails of syphoviruses do not possess a contractile sheath and therefore do not contract during DNA ejection. Nevertheless, the long noncontractile tail undergoes considerable conformational changes, which occur after primary interaction with the host cell. These conformational changes mostly concern the distal part of the tail (straight fiber). The straight fiber

penetrates the cell envelope and concomitantly shrinks in length while it increases in diameter. This signals the DNA to be released from the head of the virus. The straight fiber of the tail provides the channel for DNA penetration through the host cell envelope. Alternatively, viral DNA travels through a pre-existing pore within the host envelope, as many bacteriophages with noncontractile tails adsorb to channel forming protein complexes such as porins and transporters.

Members of the family *Podoviridae* have short noncontractile tails decorated with tail fibers. Tail fibers are responsible for host recognition and binding. Most of these viruses bind to the lipopolysaccharide of the outer membrane of Gram-negative bacteria. In contrast to the long tails of myo- and syphoviruses, the tails of podoviruses are too short to span the host cell envelope. After tail fiber attachment to lipopolysaccharide, several proteins are ejected from the virion. In the intact virion these proteins either form the internal core structure of the head or are located in the tail. Proteins ejected from the virion are involved in the formation of the channel across the cell envelope, thus extending the short tail and allowing the phage genome to travel from the virion into the cytoplasm.

Icosahedral dsDNA Bacterial Viruses with an Internal Membrane

The internal membrane of membrane containing dsDNA bacteriophages plays an important role during the entry process. According to the genome, the icosahedral membrane containing dsDNA phages fall into two families, *Tectiviridae* with the type member PRD1 and *Corticoviridae* with the only member PM2. The genome of tectiviruses is a linear dsDNA molecule with terminal proteins attached to the 5′-end, whereas the PM2 genome is a highly supercoiled circular dsDNA molecule. Obviously, these viruses use different strategies to deliver their genomes into the host cell.

Icosahedral dsDNA bacterial viruses with an internal membrane from the family *Tectiviridae* (e.g., PRD1) attach to the host cell receptor via a spike protein. The receptor structure for tectiviruses infecting Gram-negative cells is encoded by a multiple drug resistance conjugative plasmid. Specific interactions between the phage and the host induce dissociation of the spike complex from one of the capsid vertices and subsequent formation of a pore in the viral capsid. The internal membrane is transformed into a tubular tail-like structure, which protrudes from the capsid through the pore and penetrates the outer layers of the host cell. The rigid protein capsid stays intact outside the cell. Two lytic enzymes are associated with the virion (membrane) and function during the peptidoglycan penetration. A current model suggests that the viral integral membrane fuses with the host plasma membrane, thus connecting the cytoplasm with the interior of the viral membrane leading to delivery of the viral genome into the host cytoplasm. The members of the family *Tectiviridae* that infect Gram-positive bacteria (e.g., Bam35) attach to the host cell peptidoglycan structure. The internal membrane of these viruses also transforms into a tubular structure suggesting a similar mechanism for DNA entry into the host cell. The membrane tube of tectiviruses forms a channel for DNA translocation and thus is a counterpart to the tails of dsDNA bacteriophages discussed above.

Bacteriophage PM2 (*Corticoviridae*) infects Gram-negative marine bacteria from the genus *Pseudoalteromonas*. It has a circular dsDNA genome. The internal membrane of PM2 does not transform into a tubular structure in contrast to the internal membranes of tectiviruses. Instead, the capsid of the virus completely dissociates on the cell surface and the lipid core faces the host's outer membrane. The viral membrane most likely fuses with the bacterial outer membrane delivering the highly supercoiled dsDNA genome into the periplasm, from where it travels to the host cytosol.

Pleomorphic dsDNA Bacterial Viruses

The quasi-spherical virions of pleomorphic dsDNA bacterial viruses (*Plasmaviridae*) are nucleoprotein complexes within a lipid-protein membrane. The only classified member of the family *Plasmaviridae* is Acholeplasma phage L2. Acholeplasma are wall-less bacteria; thus, the phage adsorbs directly on the plasma membrane. It is assumed that adsorption leads to fusion of viral and host cell membranes resulting in entry of the nucleoprotein complexes into the cell.

Icosahedral ssDNA Bacterial Viruses

The icosahedral ssDNA bacterial viruses of the family *Microviridae* infect either free-living enterobacteria (e.g., ϕX174, G4, and α3) or obligate intracellular parasites lacking a cell wall, like chlamydia (e.g., Chp2) and spiroplasma (SpV4).

Icosahedral ssDNA bacterial viruses infecting free-living enterobacteria contain large spike complexes at the vertices of the virion. The spike complex is composed of five major spike proteins (G in ϕX174) and one minor spike protein (H in ϕX174) located at the spike apex. These spikes are responsible for the phage binding to the host cell, as well as for ejection of the ssDNA genome into the host cytoplasm. Bacteriophages infecting free-living enterobacteria adsorb to the lipopolysaccharide structures of the cell's outer membrane. Although microviruses are tail-less, they may follow an entry pathway similar to that of tailed phages (e.g., T4). Initially, ϕX174-like viruses adsorb reversibly to the cells. This is

analogous to the interaction of the tail fibers of tailed phages with the host cell. After reversible adsorption, which allows detection of the host bacterium, follows the irreversible adsorption. Virus binding to a suitable lipopolysaccharide induces conformational changes in the spike proteins and leads to DNA release from the viral capsid. Similarly to the tail of tailed phages, the major spike proteins constitute a channel through which the ssDNA travels into the host cytosol. The minor spike protein penetrates through the host plasma membrane along with the viral DNA. The viral capsid lacking DNA and one of 12 minor spike proteins remains outside the cell.

Large spike complexes are not present in the virions of phages that infect parasitic bacteria. Instead, they have elaborate viral coat protein protrusions on the threefold axis of symmetry not seen in the ϕX174-like viruses. These protrusions are likely responsible for the receptor recognition. Bacteriophages infecting parasites mostly use protein receptors present in the plasma membrane of wall-less bacteria.

Filamentous ssDNA Bacterial Viruses

Filamentous ssDNA bacterial viruses (family *Inoviridae*) mostly infect Gram-negative bacteria. Usually, they utilize the sites of bacterial envelope where outer and inner membranes are in close proximity. The protein complexes functioning as co-receptors are preferentially localized at these sites of the cell envelope.

The receptor-binding protein of filamentous bacteriophages is a minor coat protein (pIII in Ff phages) located at one end of the extended phage particle (**Figure 1(b)**). At the beginning of the infection process this protein binds to the tip of the bacterial pilus. Bacterial pilus is a complex assembly immobilized in the bacterial plasma membrane that spans the periplasmic space and protrudes through the outer membrane (**Figure 1(b)**). Pili are able to retract when they bind to the solid surface and are used for bacterial motility. Upon phage adsorption the pilus rapidly retracts bringing the phage particle through the outer membrane close to its co-receptor, a protein complex (TolQRA for Ff phages) located in the inner membrane and periplasm of the bacterial cell envelope (**Figure 1(b)**). Virus adsorption to the primary receptor (pilus) uncovers the co-receptor-binding domain in the receptor-binding protein of the virion. This allows the interaction between the receptor-binding protein of the virus and the co-receptor to proceed, thereby anchoring the virion to the bacterial plasma membrane. This triggers insertion of adjacent major coat proteins of the filamentous phage particle into the bacterial plasma membrane leading to uncoating of the virion and ssDNA translocation into the host cytoplasm (**Figure 1(b)**). The uncoating of the virion and genome translocation both require the functional co-receptor complex, which is involved in the formation of the channel for DNA traveling through the peptidoglycan and the plasma membrane.

Icosahedral Enveloped dsRNA Bacterial Viruses

The dsRNA bacteriophages (family *Cystoviridae*) have a unique entry mechanism among bacteriophages. As other dsRNA viruses, bacteriophages with a dsRNA genome deliver their genome within a large protein capsid, which protects the enclosed dsRNA genome throughout the infection cycle. Internalization of large nucleoprotein complexes into bacterial cells is rare. The protein capsid of dsRNA bacterial viruses, however, penetrates the host plasma membrane via a mechanism similar to endocytic entry of animal viruses (**Figure 1(c)**).

The virion of dsRNA bacteriophages contains three structural layers that sequentially assist in the penetration of the host outer membrane, the peptidoglycan layer, and the plasma membrane. The spikes protruding from the virion surface are involved in the receptor recognition and binding (**Figure 1(c)**). The dsRNA bacteriophages adsorb either to a bacterial pilus (e.g., ϕ6), which then retracts, or to polysaccharide (e.g., ϕ13). After phage adsorption at the outer membrane, the viral envelope fuses with the host membrane, thus uncoating the virion and placing the resulting viral nucleoprotein complex (or nucleocapsid) into the periplasm (**Figure 1(c)**). The fusion between the viral envelope and bacterial outer membrane is driven by the transmembrane proteins of the viral envelope whose fusogenic properties are activated after virus adsorption. The lytic enzyme of the virus located between the envelope and nucleocapsid locally digests the host cell-wall peptidoglycan, thereby allowing the nucleocapsid to reach the plasma membrane (**Figure 1(c)**). Eventually, the nucleocapsid penetrates the plasma membrane via an endocytic-like route. This involves the formation of the plasma membrane curvature at the contact site resulting in an intracellular vesicle, which then pinches off from the plasma membrane (**Figure 1(c)**). The transcriptionally active viral particle is released from the vesicle into the host cytosol.

Icosahedral ssRNA Bacterial Viruses

All of the known icosahedral ssRNA phages (e.g., MS2, Qβ, GA, family *Leviviridae*) are pilus specific and utilize bacterial pili as attachment site. The virions of icosahedral ssRNA phages contain a single attachment protein (or maturation protein), which carries out the initial interaction with the host pilus. The attachment protein is covalently linked to both ends of the genomic ssRNA. In addition, this protein is partially exposed on the capsid surface likely forming one of the capsid vertices. Virus adsorption initiates the cleavage of the attachment protein

into two fragments and later dissociation of those fragments from the viral capsid. As the pilus retracts into the cell, the attachment protein fragments together with the genomic ssRNA are pulled inside the host cell. The empty capsid remains outside the cell.

Bacteriophages with ssRNA genomes do not possess the peptidoglycan-hydrolyzing enzymes present in many other bacterial viruses which facilitate the peptidoglycan penetration during the entry process. Instead, ssRNA phages rely on the host cell pilus assembly to reach the cytoplasm.

Energetics

The viral genome transport across the host membrane is not a passive process. The force required for cell envelope penetration comes from different sources. As discussed earlier, phages with long contractile tails gain energy from the contraction of the tail sheath. This powerful process allows viral DNA to pass the host membrane in a very short time (e.g., 30 s for T4). Another type of energy comes from the pressure inside the viral capsid, which often is very high: In the capsids of dsDNA bacteriophages, the pressure may reach 50 atm (~5MPa)! This internal pressure of the virion, built up during DNA packaging, likely facilitates DNA ejection from the capsid during the initial stages of genome delivery.

The viral genome translocation may also be dependent on different host cell activities. The genome of T7 bacteriophage is pulled into the cytoplasm first by the host RNA polymerase and then by the RNA polymerase encoded from the phage genome. The retraction of bacterial pili and the transfer of the virus particles along the pili also depend on the cellular energy. Also, in many cases viral genome translocation requires membrane potential, which might provide energy to move DNA or RNA, stabilize a transmembrane channel, or regulate the genome translocation process.

See also: Icosahedral Enveloped dsRNA Bacterial Viruses; Icosahedral ssRNA Bacterial Viruses; Icosahedral Tailed dsDNA Bacterial Viruses.

Further Reading

Bennett NJ and Rakonjac J (2006) Unlocking of the filamentous bacteriophage virion during infection is mediated by the C domain of pIII. *Journal of Molecular Biology* 356: 266–273.

Fane BA, Brentlinger KL, Burch AD, *et al.* (2006) ϕX174 *et al*., the *Microviridae*. In: Calendar R (ed.) *The Bacteriophages,* 2nd edn., pp. 129–145. New York: Oxford University Press.

Grahn AM, Daugelavicius R, and Bamford DH (2002) Sequential model of phage PRD1 DNA delivery: Active involvement of the viral membrane. *Molecular Microbiology* 46: 1199–1209.

Kivela HM, Daugelavicius R, Hankkio RH, Bamford JKH, and Bamford DH (2004) Penetration of membrane-containing double-stranded-DNA bacteriophage PM2 into *Pseudoalteromonas* hosts. *Journal of Bacteriology* 186: 5342–5354.

Letellier L, Boulanger P, de Frutos M, and Jacquot P (2003) Channeling phage DNA through membranes: From *in vivo* to *in vitro*. *Research in Microbiology* 154: 283–287.

Ponchon L, Mangenot S, Boulanger P, and Letellier L (2005) Encapsidation and transfer of phage DNA into host cells: From *in vivo* to single particles studies. *Biochimica et Biophysica Acta* 1724: 255–261.

Poranen MM, Daugelavicius R, and Bamford DH (2002) Common principles in viral entry. *Annual Review of Microbiology* 56: 521–538.

Rydman PS and Bamford DH (2002) Phage enzymes digest peptidoglycan to deliver DNA. *ASM News* 68: 330–335.

Van Duin J and Tsareva N (2006) Single-stranded RNA phages. In: Calendar R (ed.) *The Bacteriophages,* 2nd edn., pp. 175–196. New York: Oxford University Press.

Virus Evolution: Bacterial Viruses

R W Hendrix, University of Pittsburgh, Pittsburgh, PA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Chromosome The physical DNA or RNA molecule present in the virion and containing the information of the genome. The chromosome can differ from the genome by, for example, having a terminal repetition of part of the genomic sequence or being circularly permuted relative to other chromosomes in the population.

Genome The totality of the genetic complement of a virus. The genome is a conceptual object usually expressed as a sequence of nucleotides.

Homologous Having common ancestry. Homology of two sequences is often inferred on the basis of a high percentage of identity between the sequences, but homology itself is either present or not and is not expressed as a percentage.

Novel joint A novel juxtaposition of sequences in a genome resulting from a nonhomologous recombination event.

Bacteriophage Evolution

Bacterial viruses ('bacteriophages' or 'phages' for short) have probably been evolving for 4 billion years or more, but it is only in recent years that we have come to a relatively detailed view of the genetic mechanisms that underlie phage evolution. Phages do not leave fossils in the conventional sense, but the sequences of phage genomes contain more detailed information about how the phages have evolved than any conventional fossil has. Because of the importance of sequence, our understanding of phage evolution has increased dramatically as more phage sequences have become available over the past few years. Even more important than the individual sequences, however, is the fact that we have multiple genome sequences that we can compare to each other. It is in such comparisons of different genomes that we see the unmistakable signatures of past evolutionary events, which would be completely invisible in an examination of a single genome alone.

Most of the work on phage evolution has addressed evolution of the double-stranded DNA (dsDNA) tailed phages, the members of the order *Caudovirales*, and most of the discussion here deals with that group. Toward the end of the article, we will consider evolution of other groups of phages as well as recent evidence suggesting deep evolutionary connections between some of the bacteriophage groups and viruses that infect members of the Eukarya and Archaea.

The Tailed Phage Population

Nothing in phage evolution makes sense except in the light of what we have learned about the nature of the global population of tailed phages. The most remarkable fact is that the size of the population is almost incomprehensibly large – a current estimate is that there are about 10^{31} individual phage particles on the planet. To give a feeling for the magnitude of this number, if these phages were laid end to end, they would reach into space a distance of ~200 million light years. This is apparently a very dynamic population; ecological studies of marine viruses suggest that the entire population turns over every few days. To replenish the population would then require about 10^{24} productive infections per second on a global scale. Each such infection is an opportunity for evolutionary change, either by mutation during replication or by recombination with the DNA in the cell, which will almost always include DNA of other phages in the form of resident prophages. At a lower frequency, cells may be co-infected by two different phages, affording a different opportunity for genetic exchange. As described below, the evolutionary events that are inferred from genome sequence comparisons include extremely large numbers of improbable events, and the only way these events could have given rise to the existing phage population is if they have had an extremely large number of opportunities to occur.

Evolutionary Mechanisms in the dsDNA Tailed Phages

The Nature of the Genomes

The genomes of the dsDNA tailed phages use DNA very efficiently, with typically about 95% of the sequence devoted to encoding proteins. Spaces between genes can often be identified as containing expression signals such as transcription promoters or terminators. Genes are typically arranged in groups that are transcribed together. The specific types of genes in a genome, their numbers, and how they are arranged along the DNA are all highly variable among phages. Genome size is also variable, with the smallest known phages in this group having genomes of about 19 kbp and about 30 genes and the largest consisting genomes of 500 kbp and nearly 700 genes.

Types of Evolutionary Changes

Comparisons of genome sequences between phages reveal the sorts of changes in the genome sequence that in aggregate constitute phage evolution. The first of these is point mutation, in which one base pair is substituted for another. The number of point mutational differences seen between two homologous sequences varies from none to so many that our ability to detect any residual similarity of the sequence, often measured at the more sensitive level of the encoded amino acid sequence, has vanished. The amount of difference between two sequences due to point mutation is a function of how long it has been since they diverged from a common ancestral sequence, because such differences accumulate progressively over time. In practice, however, it is an unreliable measure of time for a number of reasons: we do not know the mutational rates for phages in a natural setting, we do not know that those rates have been constant over evolutionary time, and different genes accumulate changes at different rates because any mutations that are detrimental to the function of the encoded protein will be lost from the population by natural selection, and different proteins have different tolerances for mutational changes in their amino acid sequences.

The other major type of evolutionary change to a genome is DNA recombination. Particularly important is 'illegitimate' or 'nonhomologous' recombination, in which two different DNA sequences are joined together to form an association of sequences that did not exist before, known as a 'novel joint'. Nonhomologous recombination can join parts of two or more genomes into one

new genome; it can also cause deletion or inversion of a sequence within a genome by mediating recombination between two sequences in the genome. Nonhomologous recombination can in principle – and, it appears, also in practice – produce virtually any novel joint that can be imagined. The results of laboratory experiments have shown that homologous recombination happens many orders of magnitude more frequently than nonhomologous recombination. Unlike nonhomologous recombination, homologous recombination does not leave a novel joint that can be detected in analysis of the sequence, but it has the potential to reassort any flanking novel joints, bringing them and their associated sequences together in new combinations and providing a mechanism for them to move rapidly through the population.

Genome Comparisons

There is typically nothing in a single phage genome sequence, viewed in isolation, that reveals the presence of changes in the phage's evolutionary past, of either mutational or recombinational origin. When multiple genomes are compared, a wealth of differences corresponding to such changes is revealed. **Figure 1** illustrates a comparison for a group of four rather similar genomes of phages infecting enteric bacterial hosts. The physical gene map shown is that of *Escherichia coli* phage HK97, and the histograms indicate the locations of sequences in the other phages that match HK97. It is apparent that the genome sequences match each other in a patchwork fashion, and the parts of the sequence that match the HK97 sequence are different for the three phages. In other words, the genomes are genetic mosaics with respect to each other. In a pairwise comparison of genomes, the transitions between where sequences match and where they do not are abrupt, even when they are examined at the level of the nucleotide sequence. This implies that there have been nonhomologous recombination events in the ancestry of the phages, creating novel joints in the resulting recombinant phages. These novel joints are detected when they are compared with a sequence that did not suffer that particular recombination event. In the comparisons shown in **Figure 1**, there is evidence for about 75 ancestral nonhomologous recombination events, occurring in either the ancestry of HK97 or in that of one of the three phages being compared. It is worth noting that this probably underestimates the number of such events, because any novel joints that lie in the common ancestry of all these phages would not have been detected.

A striking feature of the novel joints in the sequence revealed by such genome sequence comparisons is that they fall predominantly at gene boundaries. While some are precisely at the gene boundaries, some also fall in the middle of spaces between genes or even a few codons into the upstream or downstream gene's coding region. More rarely, we can see novel joints in the interior of a gene's coding region. In the case of some such genes for which the encoded proteins are well characterized, the novel joints in the DNA sequence fall at a position corresponding to a domain boundary of the protein. Another feature of the genome comparisons that extends across all the groups of phages examined to date is that there are clusters of genes that are never, or rarely, separated by nonhomologous recombination. These are typically genes whose protein products are known to function together, such as the genes encoding the structural proteins of the head.

A frequent consequence of nonhomologous recombination among viruses is the transfer of genetic material from the genomes of one viral lineage into the genomes of a different viral lineage, a process known as 'horizontal transfer' or 'lateral transfer' of genes. This is the process that gives rise to the mosaicism in the genomes discussed above. It also means that different parts of a genome may, and most often do, have different evolutionary histories. This last fact leads to an interesting and still unresolved difficulty for viral taxonomists. That is, attempts to represent the relationships among viruses by a hierarchical taxonomy, as is conventionally done, necessarily fail to capture the multiple different sets of hierarchical relationships displayed by the individual exchanging genetic modules, deriving from their different evolutionary histories. The viruses, of course, are unaware of human attempts to classify them and so are unaffected by the resulting controversies.

Evolutionary Mechanisms

When the mosaicism of phage genomes was first seen in DNA heteroduplex mapping experiments in the late 1960s, it was proposed that there might be special sites in the DNA, possibly at gene boundaries, that served as points of high-frequency nonhomologous recombination, either through short stretches of homology or through a site-specific recombination mechanism. This view was formulated as the 'modular evolution' model of phage evolution, in which it was proposed that exchange between genomes took place repeatedly at these special sites, leading to the observed mosaic relationship between genomes.

With the current availability of many more genomes and data at the level of nucleotide sequence, it has become clear that, while the mosaic results of phage evolution are much as described nearly 40 years ago, the mechanisms by which that state is achieved are fundamentally different than what had been proposed. That is, the observations are best explained by a model of rampant nonhomologous recombination among phage genomic sequences, with the sites of recombination being distributed, to a first approximation, randomly across the sequence with no regard for gene boundaries or other features of the sequence. Most recombinants produced this way will be nonfunctional

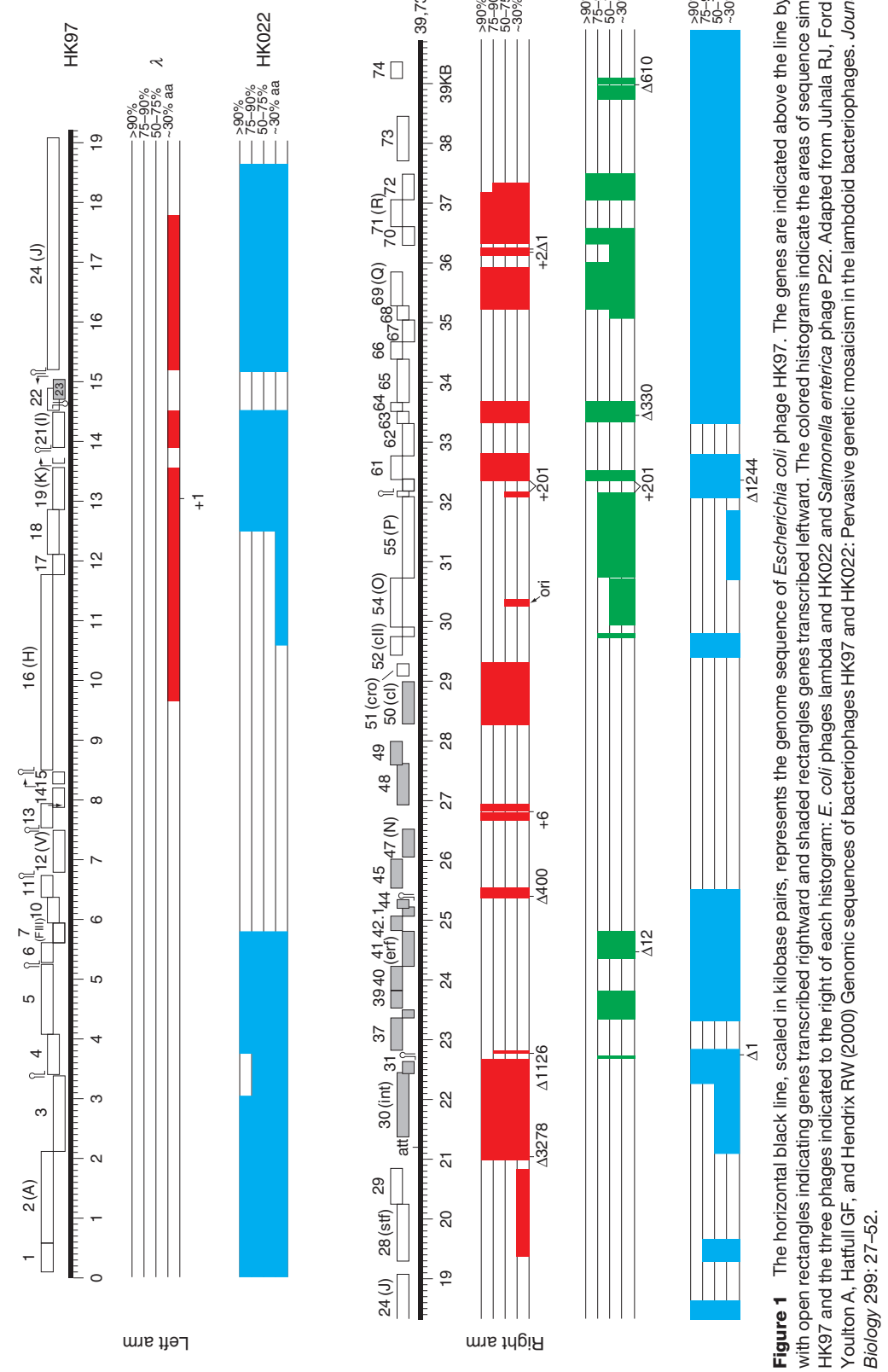


Figure 1 The horizontal black line, scaled in kilobase pairs, represents the genome sequence of *Escherichia coli* phage HK97. The genes are indicated above the line by rectangles, with open rectangles indicating genes transcribed rightward and shaded rectangles genes transcribed leftward. The colored histograms indicate the areas of sequence similarity between HK97 and the three phages indicated to the right of each histogram: *E. coli* phages lambda and HK022 and *Salmonella enterica* phage P22. Adapted from Juhala RJ, Ford ME, Duda RL, Youlton A, Hatfull GF, and Hendrix RW (2000) Genomic sequences of bacteriophages HK97 and HK022: Pervasive genetic mosaicism in the lambdoid bacteriophages. *Journal of Molecular Biology* 299: 27–52.

because, for example, they have glued together parts of two encoded functional proteins into a nonfunctional chimera. The tiny fraction of such recombinants that are fully functional and competitive in the natural environment will be those that have recombination joints where they do no harm, like at gene boundaries; the rest will be rapidly eliminated from the population by natural selection. We would also expect that two recombining genomes would not typically be lined up in register, and this would most often lead to nonfunctional recombinants. There are a few examples of genomes with short quasi-duplications that can be explained by a slightly out of register recombination event, supporting this supposition.

Nonhomologous recombination also has the potential to bring novel genes into a genome and to mediate large-scale reorganizations of genomes, as seen for example in *E. coli* phage N15 which has head and tail structural genes homologous to those of phage lambda and, in the other half of the genome, genes from a very different, apparently plasmid-like, source. Another example is the *Shigella* phage SfV, which has head genes in the phage HK97 sequence family and tail genes like those of phage Mu.

The entire process described above can be viewed as a classical Darwinian scenario in which tremendous diversity is generated in the population by a combination of point mutations and homologous and nonhomologous recombination, and the stringent sieve of natural selection acts to eliminate all but the fittest recombinants. The survivors have largely, though not entirely, lost the appearance of having been generated by what is essentially a random mutational process.

Evolution of Other Phage Types

Although most of the genome comparisons that have led to our current understanding of phage evolution have been carried out with the tailed phages, there has been some work done with other groups. The most extensive has been done with members of the families *Microviridae* and the *Inoviridae*, phages with small, circular single-stranded DNA (ssDNA) chromosomes, typified by the well-studied *E. coli* phages ϕX174 and M13. For both of these groups there is evidence for horizontal exchange of sequences, as in the tailed phages, but it is quantitatively much less prominent than what is seen in the tailed phages. There are several differences between the tailed phages and these two groups of smaller phages, some or all of which may contribute to the differences in evolutionary outcomes seen between them. These include 10- to 100-fold differences in the sizes of the genomes and the fact that the members of the *Microviridae* and *Inoviridae* do not encode recombination functions, among others.

Another study looked at evidence for evolutionary change among members of the family *Cystoviridae*, a group of phages with a double-stranded RNA (dsRNA) genome, divided into three physical segments. Comparisons among environmentally derived genome sequences showed no evidence for recombinational exchange within the segments but clear indications of reassortment of the segments. Thus it appears that evolution in this group of viruses achieves the same general outcome as evolution of the tailed phages, namely horizontal exchange of genetic modules, but by a very different mechanism. Analogous horizontal exchange by reassortment of the segments of a segmented genome has been extensively characterized in the influenza viruses, a group of animal viruses with a single-stranded RNA (ssRNA) genome of eight segments.

Deep Evolutionary Connections

Although there are some genes that are found in all tailed phages – for example, genes encoding the major capsid protein – these are very diverse in sequence. This diversity is sufficiently great that it is not possible, on the basis of their sequences, to group them into a single sequence family, at either the DNA or the protein sequence levels. Thus the subsets of capsid protein sequences that share demonstrable sequence similarity may represent independent nonhomologous lineages of capsid proteins, or alternatively they may all be part of the same lineage but have diverged in sequence to the point that in many cases no surviving sequence similarity can be demonstrated. However, recent structural work on the polypeptide folds of capsid proteins appears to be extending our analytical reach farther back in evolutionary time and giving a resolution to this question. High-resolution X-ray structures of the capsid proteins of phages HK97 and T4 demonstrate that they have a common fold which might indicate common ancestry despite the absence of any surviving sequence similarity. Cryoelectron microscopic structures of phages representing two more capsid protein sequence groups, P22 and ϕ29, make a less rigorous but still convincing case that these capsid proteins also share in a common polypeptide fold and so are most likely part of the same lineage. Remarkably, a similar experiment on (the animal virus) herpes simplex virus argues that the shell-forming domain of its major capsid protein also shares the tailed phage capsid protein fold and so may be part of the same lineage.

A similar and more completely documented case for a virus lineage extending across the cellular domains of the hosts they infect can be made for a group that includes bacteriophage PRD1 (a member of the family *Tectiviridae*), archaeal virus STIV, and eukaryotic viruses adenovirus, PBCV1 (*Phycodnaviridae*), and mimivirus. This is again based on shared capsid protein folds, in this case an unusual double 'jellyroll' fold. Finally, there is compelling

structural similarity between the cystoviruses, the group of phages with segmented dsRNA chromosomes, and a characteristic double protein shell discussed above, and the reoviruses of plant and animal hosts.

The simplest interpretation of these observations, though not the only possible interpretation, is that there were already viruses resembling these different types of contemporary viruses prior to the divergence of cellular life into the three current domains. The different types of viruses in this view would have co-evolved with their hosts as the hosts divided into domains and descended to the present time, with in the end nothing to indicate their common origins except the shared structure of their capsid proteins. Whatever the truth of this matter, an important caveat to such an interpretation is that the evidence for common viral ancestries across host domains is at present based primarily on conserved properties of capsid proteins, and so any conclusions about common lineages, strictly speaking, only apply to capsid protein lineages. However, despite such reservations, the data make an intriguing though still tentative case that viruses resembling at least three groups of contemporary viruses had already evolved into forms something like those of contemporary viruses before the three domains of cellular life had begun to separate, ~3.5 billion years ago.

See also: Evolution of Viruses; Origin of Viruses; Phylogeny of Viruses; Quasispecies.

Further Reading

Brüssow H and Hendrix RW (2002) Phage genomics: Small is beautiful. *Cell* 108: 13–16.

Casjens SR (2005) Comparative genomics and evolution of the tailed-bacteriophages. *Current Opinion in Microbiology* 8: 451–458.

Fuhrman JA (1999) Marine viruses and their biogeochemical and ecological effects. *Nature* 399: 541–548.

Hatfull GF, Pedulla ML, Jacobs-Sera D, *et al.* (2006) Exploring the mycobacteriophage metaproteome: Phage genomics as an educational platform. *PLoS Genetics* 2: e92. http://genetics.plosjournals.org/perlserv/?request=get-document&doi=10.1371%2Fjournal.pgen.0020092 (accessed June 2007).

Juhala RJ, Ford ME, Duda RL, Youlton A, Hatfull GF, and Hendrix RW (2000) Genomic sequences of bacteriophages HK97 and HK022: Pervasive genetic mosaicism in the lambdoid bacteriophages. *Journal of Molecular Biology* 299: 27–52.

Nolan JM, Petrov V, Bertrand C, Krisch HM, and Karam JD (2006) Genetic diversity among five T4-like bacteriophages. *Virology Journal* 3: 30–44.

Suttle CA (2005) Viruses in the sea. *Nature* 437: 356–361.

Virus-Induced Gene Silencing (VIGS)

M S Padmanabhan and S P Dinesh-Kumar, Yale University, New Haven, CT, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Functional genomics The study of genes with respect to the role they play within biological processes.

High-throughput screening A method to efficiently test a large number of putative genes to identify candidates that may regulate a specific biological process.

Hypersensitive response A defense response in plants that initiates cell death to restrict the growth of pathogen.

Knockdown Reduction in the expression of a gene.

Knockout Complete inhibition of gene expression.

Reverse genetics Approaches used to define the function of a gene or sequence of DNA within the context of the organism.

T-DNA Transfer DNA from *Agrobacterium tumefaciens*, a vector routinely used for transforming plants.

Introduction

Virus-induced gene silencing (VIGS) is an RNA silencing-based technique used for the targeted downregulation of a host gene through the use of a recombinant virus. The term VIGS was originally coined to describe the phenomenon of recovery of a host plant from viral infection. Today, its usage is predominantly in reference to a tool for turning down host gene expression, especially in plants. In principle, a plant gene of interest can be silenced by infecting the plant with a viral vector that has been modified to express a nucleic acid sequence homologous to the host gene. Viral infection and synthesis of double-stranded RNA (dsRNA) viral replication intermediates initiate the innate RNA silencing or post-transcriptional gene silencing (PTGS) pathway, ultimately leading to the degradation of host transcript. The potency, specificity, and speed of PTGS have thus been harnessed to create an efficient gene knockout system. The ability of VIGS to rapidly initiate silencing and generate mutant phenotypes without the use of laborious transgenic approaches has

made it the tool of choice for characterizing genes, especially in plant species where conventional analytical techniques have had limited success.

PTGS and the Principle behind VIGS

The term virus-induced gene silencing was first used by A. van Kammen to describe the process by which some plants were able to recover from viral infection, coincident with the loss of viral RNA from the infected tissue. The recovery process was soon understood to be associated with the plant inherent RNA-silencing mechanism which is an evolutionarily conserved antiviral system. Most plant viruses are RNA viruses and produce dsRNA intermediates during replication. In contrast to host messenger RNA, the viral dsRNA is made up of relatively long stretches of complementary RNA strands. The host identifies these as foreign and triggers the RNA-silencing pathway. The plant recovery is thus the successful outcome of the actions of this surveillance system.

The phenomenon of RNA silencing, initially observed in plants where it was called PTGS, has since been known to operate in almost all eukaryotic species including fungi, worms, flies, and mammals. In fungi, the process is called quelling while in animal systems it is known as RNAi (RNA interference). In plants and other eukaryotes, the mechanism of RNA silencing of viruses is highly conserved. The dsRNA which is generally synthesized by a viral polymerase or in some cases a host-encoded RNA-dependent RNA polymerase is recognized and cleaved by Dicer, a ribonuclease III (RNase III)-like enzyme. This results in the production of 21–24 nt duplex molecules called short-interfering RNAs (siRNA's). In an ATP-dependent manner, the siRNA's are denatured and one strand is exclusively incorporated into a multi-subunit nuclease complex called the RNA-induced silencing complex (RISC). Within the RISC, siRNA serves as a guide to recognize and base pair with the homologous RNA (in this case the viral RNA), eventually leading to degradation of the target RNA. This occurs via an RNase H-like cleavage mechanism directed by the RISC. One of the key factors that make PTGS such a potent system of host defense is that once initiated, it can maintain the silencing effect by relaying a diffusible silencing signal throughout the plant. As the virus moves within the host tissue, those cells that have received the silencing signal are primed to recognize the viral RNA and initiate its degradation.

PTGS and its efficiency against viral genomes can be exploited to create recombinant viral vectors that serve as tools for gene silencing. Viruses carrying segments of host genes, when used to infect plants, would produce siRNA's specific to the host mRNA. RISC-mediated degradation of target host mRNAs would ultimately lead to downregulation of gene expression. The infected plant would thus have a phenotype similar to a loss-of-function mutant of the gene of interest. The efficiency of viruses in initiating silencing was first shown in plants infected with a recombinant tobacco mosaic virus (TMV) carrying a fragment of the plant gene *phytoene desaturase* (*PDS*), a regulator of carotenoid biosynthesis. Infected *Nicotiana benthamiana* plants showed degradation of the host PDS mRNA and resultant alterations in the pigment synthesis pathway led to significant photobleaching in the leaves. The potential of this gene knockout system was immediately recognized and has since been applied to more than ten plant species. The *PDS* gene now serves as the conventional gene used for testing the efficacy of a virus in inducing silencing (**Figure 1**).

The Development of VIGS as a Tool for Functional Genomics

Several VIGS vectors have been developed over the past 8 years (**Table 1**). The choice of VIGS vector plays a key role in efficient silencing and there are many factors to be considered when choosing the virus to be used for VIGS. Ideally, the virus must produce little or no symptoms during infection, thereby facilitating easy visualization and interpretation of the mutant phenotype. It must induce persistent silencing. Viruses with strong silencing suppressors are avoided since they can interfere with the establishment of silencing. It is advantageous to have infectious cDNA clones of the virus for cloning purposes and the virus must retain infectivity after insertion of foreign DNA. The virus should also show uniform spread, infect most cell types including the meristem, and preferably show a broad host range.

RNA Virus-Based Vectors

TMV, an RNA virus belonging to the genus *Tobamovirus*, was the earliest viral vector to be used for VIGS but the severity of symptoms in susceptible tissue and inability to invade the meristem limited its application. A PVX (potato virus X)-based vector produced milder infection symptoms but displayed a narrower host range and did not infect the growing points of infected plants. Tobacco rattle virus (TRV) was shown to overcome all these disadvantages and currently is the most widely used VIGS vector, especially in solanaceous hosts. It can infect all cell types including the meristem and so can be used to investigate the role of genes in early developmental processes. The silencing is more persistent when compared to other viral vectors and it is documented to infect a wide range of hosts.

TRV is a plus-sense RNA virus with a bipartite genome. RNA-1 encodes the viral replicase and movement

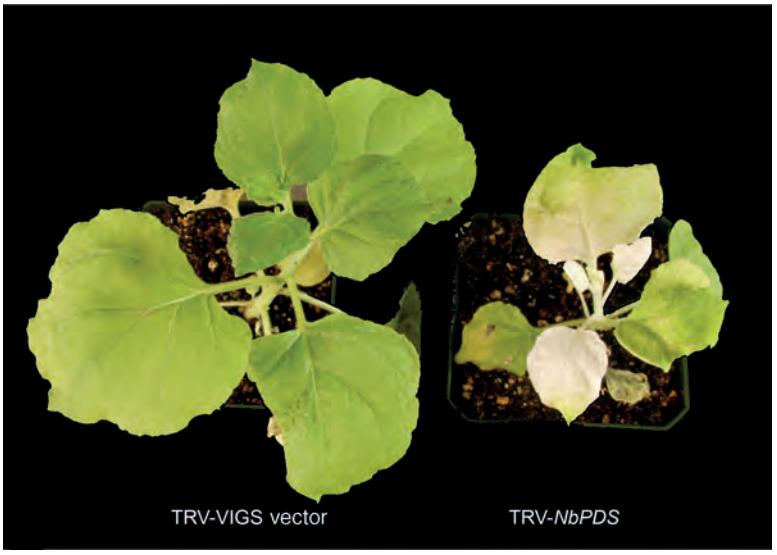


Figure 1 Silencing of the *PDS* gene (phytoene desaturase) in *N. benthamiana* using TRV-based VIGS system. *Nicotiana benthamiana* plants infected with the empty vector TRV-VIGS do not have a noticeable phenotype while TRV-*NbPDS*-infected plants show photobleaching, a hallmark of PDS silencing.

Table 1 Selected list of plant viruses used as VIGS vectors for gene silencing

Virus	*Genus*	*Host*	*Method of infection*
Tobacco mosaic virus	*Tobamovirus*	*N. benthamiana*	Inoculation of infectious viral RNA
Potato virus X	*Potexvirus*	*N. benthamiana,* potato	Inoculation of infectious viral RNA; agro-inoculation
Tobacco rattle virus	*Tobravirus*	*N. benthamiana, Arabidopsis thaliana,* tomato, pepper, petunia, potato, poppy	Agro-inoculation
Pea early browning virus	*Tobravirus*	Pea	Agro-inoculation
Satellite Tobacco mosaic virus (with helper TMV)	*Tobamovirus*	Tobacco	Inoculation of infectious viral RNA
Bean pod mottle virus	*Comovirus*	Soybean	Inoculation of infectious viral RNA
Tomato golden mosaic virus (TGMV)	*Geminivirus*	*N. benthamiana*	Microprojectile bombardment
Cabbage leaf curl virus (CaLCuV)	*Geminivirus*	*A. thaliana*	Microprojectile bombardment
African cassava mosaic virus (ACMV)	*Geminivirus*	Cassava	Microprojectile bombardment
Barley stripe mosaic virus	Hordeivirus	Barley, wheat	Inoculation of infectious viral RNA
Fescue strain of Brome mosaic virus (F-BMV)	Bromovirus	Rice, maize, barley	Inoculation of infectious viral RNA

proteins, while RNA-2 contains genes for the coat protein and two nonstructural proteins, one of which is needed for vector transmission. Since the latter two genes are dispensable for viral replication and spread in plants, RNA-2 could be modified to replace the nonstructural genes with a multiple cloning site for insertion of host-derived gene sequences. The host sequence to be inserted into the viral vector could theoretically be as small as 23 nt (the size of an siRNA) but in practice the ideal size range is between 300 and 1500 bp.

There are many ways of introducing the virus into the plant system and these include rubbing the plants with infectious viral transcripts or biolistic delivery. However, the most effective means appear to be incorporation of the viral cDNA into *Agrobacterium tumefaciens*-based plant transformation vectors (T-DNA vectors) under the control of constitutive promoters followed by infiltration of the *Agrobacterium* cultures into the host. This ensures not just ease in the inoculation procedure but also efficient transformation and high levels of viral RNA production within plant cells. For the TRV system, VIGS can be induced by simply mixing individual *Agrobacterium* cultures containing RNA-1 and modified RNA-2 and then co-infiltrating into young plants using a needle-less syringe. Alternatively, the cultures can also be vacuum-infiltrated or sprayed onto the plant.

The appearance of VIGS-related phenotype depends on a number of factors including the plant species used, the age of the plant, and environmental factors like temperature and humidity. Under optimal conditions in *N. benthamiana* plants, TRV-induced silencing phenotypes can be visualized as early as 1 week. TRV is also one of the few viruses that have been modified into a highly efficient cloning and expression system for use in large-scale functional genomics screens. One of the most useful features of TRV vectors is their ability to induce VIGS in a number of solanaceous hosts like *N. benthamiana*, tomato, potato, pepper, petunia, poppy (Eudicot species), and the model system *Arabidopsis thaliana* (family Brassicaceae).

A number of other viruses have been adopted for VIGS in species where TRV has not been efficacious (**Table 1**). For tobacco plants, researchers have developed the satellite virus-induced silencing system (SVISS), which is a two-component system that uses a modified satellite TMV (that acts as the inducer of silencing) and a helper TMV-U2 virus (that promotes viral replication). Most of the VIGS vectors thus far described have been designed for dicotyledonous species though a majority of the economically important food crops such as rice, maize, wheat, and barley are monocots. The RNAi machinery has been shown to function in monocots and hence research in recent years has focused on finding ideal monocot VIGS vectors. The hordeivirus barley stripe mosaic virus (BSMV) was the first virus adapted for VIGS in barley and subsequently applied to wheat. A specific strain of brome mosaic virus isolated from tall fescue (F-BMV) was cloned, characterized, and modified for use as a VIGS vector. F-BMV-based vectors were shown to effectively silence genes from rice, barley, and some cultivars of maize. The successful adaptation of VIGS technology in monocots will, in the future, aid in the functional characterization of genes in these otherwise recalcitrant systems.

DNA Virus-Based Vectors

DNA viruses belonging to the family *Geminiviridae* like tobacco golden mosaic virus (TGMV) and cabbage leaf curl virus (CaLCuV) have been successfully employed for silencing and serve as good alternatives to RNA viruses. CaLCuV was also the first VIGS vector to be used to knockdown gene expression in *Arabidopsis*. Geminiviruses are highly conserved in their genetic sequence and so, in theory, these viruses can be quickly adapted for VIGS based on information from one virus. Other beneficial properties of geminiviruses are that they are genetically more stable than RNA-based vectors, can invade meristematic tissue, and infect a wide range of plants including economically important crops. In fact, begomoviruses like African cassava mosaic virus (ACMV) and pepper huasteco yellow vein virus (PHYVV) have been successfully employed as VIGS vectors in cassava and pepper. As a caveat, it must be noted that most of the geminiviruses used so far appear to induce some symptoms of infection and thus care must be taken when interpreting VIGS phenotypes.

Applications of VIGS in Aiding Plant Gene Characterization

There have been numerous examples validating VIGS as an effective method for loss-of-function studies in plant systems. The preeminent use of this technique has been in studying plant–pathogen interactions, especially in deciphering plant defense pathways. Resistance against pathogens in many plants is dictated by the presence of specific *R* genes and each gene exclusively initiates defense against one race of a given pathogen. Numerous studies have used VIGS to study genes that are activated or regulated in *R* gene pathways. These include *N* gene-mediated resistance to TMV, *Rx1*-mediated resistance to PVX, *Mla13*-mediated resistance to powdery mildew, *Cf-4* regulated resistance to *Cladosporium fulvum*, and *Pto*-mediated resistance to *Pseudomonas syringae* in tomato. In some of these studies, cDNA libraries inserted into VIGS vectors were screened to identify genes that were essential for the *R* gene-activated hypersensitive response (HR) which is a hallmark of plant defense. These studies in parallel identified common components in disease resistance like the chaperone protein HSP90, mitogen-activated protein (MAP) kinase pathway proteins, regulators of the protein degradation pathway, and TGA and WRKY transcription factors. The use of VIGS has also aided in the identification of novel pathways that play a role in defense or pathogenesis. For instance, autophagy, an evolutionarily conserved programmed cell death response, was found to be important for limiting cell death induced during viral infection. Similarly, $P58^{IPK}$, a plant ortholog of the inhibitor of mammalian dsRNA-activated protein kinase (PKR), was shown to be recruited by TMV to maintain infectivity. While the function of PKR in defense has been well characterized in animal systems, this was the first study showing a role for this protein in plant viral pathogenesis.

Meristem invasion by viruses like TRV make them ideal for use in silencing genes that may be important for cell proliferation, tissue differentiation, and flower development. Studies on floral genes have mainly been limited to model systems like *Arabidopsis* and petunia, but with the advent of VIGS it is now possible to test floral homeotic genes in a wide range of hosts and analyze conservation or divergence of gene functions between species. As a case study, in solanaceous plants, the ortholog of *Arabidopsis* and *Antirrhinum* floral homeotic gene *AP3/DEFICIENS* was silenced and found to produce a phenotype similar to that seen in *Arabidopsis*. The TRV system was also used to

initiate VIGS in tomato fruits showing its potential in studying fruit development. An *N. benthamiana* cystein protease, calpain, was shown to play a key role in cell differentiation and organogenesis using the TRV-VIGS system. Similarly, the role of proliferating cell nuclear antigen (PCNA) was investigated with geminivirus-derived vectors. The primary advantage of using VIGS for such studies is not just that it expands the study of plant growth and differentiation into nonhost systems, but also that it overcomes problems of embryo lethality or sterility which would be unavoidable if true mutants of these genes were being studied.

Among its other applications, VIGS has been adopted for analyzing biosynthetic pathways (sterol synthesis and pigment synthesis), characterizing genes involved in stress response or hormone response, organelle biogenesis, and to look at transport of proteins within the cell (especially nuclear transport mediated by different importins).

Advantages of Using VIGS as a Tool for Functional Genomics

In plants, the conventional methodology for gene function analysis has been to use insertional mutagenesis to shut down expression of the gene of interest and study the consequence of its loss to the plant. The T-DNA plasmid of *Agrobacterium*, which is capable of inserting into the plant genome, has for a long time been one of the tools of choice for disrupting plant genes. An alternative approach has been to use transposons. While being powerful tools for reverse genetics, they came with a number of limitations. Laborious and time-consuming screens had to be carried out to search for T-DNA insertions or transposon tagged lines. Loss of genes essential in the early developmental stages led to embryo lethality while functionally redundant genes rarely showed a discernible phenotype when knocked out and so these genes could not be identified in screens. These techniques were also not amenable for genomic-scale studies because of the difficulty of achieving genome saturation and problems with multiple insertions. The advent of RNA-silencing technology meant that it was possible to disrupt the function of a specific gene by the introduction of gene-specific self-complementary RNA, also known as hairpin RNA (hpRNA). This process still required plant transformation and screening to identify silenced plants. Many of the constraints imposed by these methodologies have been overcome with the discovery of VIGS and its subsequent use a tool.

The biggest advantage with this technology is the short time needed to go from gene sequence to functional characterization of its knockdown. While screening for a potential T-DNA knockout would take at least a few months, with VIGS it can be scaled down to 2 weeks. Thus relationships between genes and phenotypes/functions can be established quickly. This factor alone has catapulted VIGS into one of the most widely accepted and popular tools for gene analysis. VIGS is not as labor intensive as the other techniques since it does not involve transformation of plant tissue. Application of the virus and induction of VIGS are easy, especially after incorporation of viral sequences into intermediate plant transformation vectors. VIGS can, in general, be induced at any stage during a plant's life cycle – thus it is still possible to characterize genes that may otherwise be lethal when knocked out during early stages of development. One can overcome redundancy by carefully choosing the gene sequence to be inserted into the viral vector so as to maximize (or minimize) the silencing of related genes. Many VIGS vectors can be used on multiple host species and this can prove useful for testing gene functions against different genetic backgrounds. These vectors have accelerated the characterization of genes in many nonmodel hosts like *N. benthamiana*, petunias, potato, soybean, and even poplars where conventional techniques achieved very limited success. Finally, as has been shown with TRV and PVX, VIGS vectors can be adapted for use in high-throughput functional genomic screens.

Limitations of VIGS

It must be noted that, like all other techniques, VIGS also has its limitations. Above all, it can only induce a transient silencing response. In almost all cases, we observe an eventual recovery of the host from the viral infection and subsequent replenishment of gene transcript levels. Due to the nature of the silencing phenomenon, even during the peak silencing stages, one cannot be assured of complete knockout of transcript levels. In spite of using weak or attenuated viruses, it is not possible to avoid some of the host gene expression changes that are associated with the viral infection and these factors must be taken into consideration while interpreting the results. Ideally, therefore, VIGS should be used in conjunction with or validated by other techniques of gene function analysis. The use of recombinant viruses also requires greater precautionary measures and care must be taken to avoid accidental transmission of the modified or infectious virus.

Conclusions

With the sequencing of numerous genomes completed or near completion, we have in our hands a vast amount of genetic data that is to be deciphered, genes that need to be characterized, and pathways that are to be elucidated. The need of the hour is for powerful functional analysis tools and in the last few years VIGS has proven to be the

breakthrough technology that aids in rapid and robust gene characterization. Plant gene analysis has traditionally been carried out in few model systems whose genomes have been sequenced and are also easily amenable to transformation techniques. VIGS is one of the few tools that can be applied on a broad spectrum of plants, since most plants are susceptible to viral infections. VIGS will therefore play a crucial role in promoting gene analysis in many nonmodel systems including those that have duplicated genomes or do not have a sequenced genome. A plethora of new viruses are being added to the list of VIGS vectors and it is exciting to note the addition of vectors for monocots like rice, wheat, and maize. Small grain cereals are among the world's most important food crops but gene discovery and annotation in these systems have been carried out on only a small scale mainly due to the lack of tools for reverse genetics. The adaptation of VIGS in monocots will therefore play an important role in gene analysis and annotation in these economically important crops.

Within the animal kingdom, RNA-silencing technology has been harnessed to carry out genomewide silencing screens. In human cells, *Drosophila*, and *Caenorhabditis elegans*, dsRNA or hpRNA's designed to systematically target a majority of predicted genes have been screened to identify novel players in cell growth, proliferation, and cell death. We now have all the tools necessary to carry out similar whole genome functional discovery studies in plants. The successful application of VIGS in two completed plant genome sequences, *Arabidopsis* and rice, opens up the possibility of carrying out systematic loss-of-function studies in these systems. Many plant genomes have been partially annotated in the form of expressed sequenced tags (EST) libraries, which serve as inventories of expressed genes. These EST libraries can also serve as ideal resources for use in VIGS-mediated high-throughput screening. In the postgenomics era, VIGS will prove to be a technique that will help accelerate the conversion of genomic data to functionally relevant information and contribute to our understanding of the molecular processes occurring within plants.

See also: Plant Antiviral Defense: Gene Silencing Pathway.

Further Reading

Baulcombe DC (1999) Fast forward genetics based on virus-induced gene silencing. *Current Opinion in Plant Biology* 2: 109–113.

Burch-Smith T, Miller J, and Dinesh-Kumar SP (2003) PTGS Approaches to large-scale functional genomics in plants. In: Hannon G (ed.) *RNAi: A Guide to Gene Silencing*, p. 243. New York: Cold Spring Harbor Laboratory Press.

Burch-Smith TM, Anderson JC, Martin GB, and Dinesh-Kumar SP (2004) Applications and advantages of virus-induced gene silencing for gene function studies in plants. *Plant Journal* 39: 734–746.

Carrillo-Tripp J, Shimada-Beltran H, and Rivera-Bustamante R (2006) Use of geminiviral vectors for functional genomics. *Current Opinion in Plant Biology* 9: 209–215.

Dinesh-Kumar SP, Anandalakshmi R, Marathe R, Schiff M, and Liu Y (2003) Virus-induced gene silencing. *Methods in Molecular Biology* 236: 287–294.

Ding XS, Rao CS, and Nelson RS (2007) Analysis of gene function in rice through virus-induced gene silencing. *Methods in Molecular Biology* 354: 145–160.

Lu R, Martin-Hernandez AM, Peart JR, Malcuit I, and Baulcombe DC (2003) Virus-induced gene silencing in plants. *Methods* 30: 296–303.

Robertson D (2004) VIGS vectors for gene silencing: Many targets, many tools. *Annual Review of Plant Biology* 55: 495–519.

Virus Particle Structure: Nonenveloped Viruses

J A Speir and J E Johnson, The Scripps Research Institute, La Jolla, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Nonenveloped viruses provide model systems for high-resolution structural study of whole virus capsids (and their components) and the principles of large-scale nucleoprotein tertiary and quaternary interactions. In many cases, they are relatively small, stable, and highly symmetric particles that can be purified as homogeneous samples in large quantities. These properties make them suitable for the techniques of X-ray crystallography, X-ray fiber diffraction, and cryoelectron microscopy (cryo-EM) combined with image reconstruction. Enveloped virus particles generally have greater flexibility and asymmetry, two features that inhibit application of high-resolution techniques.

The first crystal structures of intact virus capsids published in the late 1970s and early 1980s are examples of the simplest forms of replicating organisms. They were small, spherical, positive-sense single-stranded (ss) RNA plant viruses about 170–350 Å in diameter with $T = 1$ or $T = 3$ symmetry, which were easy to purify in gram quantities. By the mid- to late 1980s, the structures of small ssRNA animal viruses (poliovirus and the common cold virus, rhinovirus) and the intact, 3000 Å-long ssRNA helical tobacco mosaic virus (TMV) were also completed. The range of virus types, sizes, and complexity addressed by X-ray crystallography has grown significantly with advances in X-ray technology and computing. Detailed polypeptide and nucleic acid models have now been fitted

into data between 4–1.8 Å resolution. Crystal structures of 800 Å diameter virus capsids with $T = 25$ icosahedral symmetry have now been determined and show quaternary polypeptide interactions as intricate and varied as their biology.

Spherical particles larger than 800 Å in dimension have been studied by cryo-EM providing detailed images of some of the most complex structures ever visualized at high resolution. This technique is also readily applied to smaller viruses like those described above. Typical cryo-EM studies produce 15–30 Å resolution image reconstructions, but under ideal conditions sub-nanometer resolution can be obtained. The upper diameter limit for applying the cryo-EM technique to viruses is yet to be hit; however, with increased sample thickness (e.g., particle diameter), comes an increase in data collection and analysis restrictions. Currently, the two largest spherical virus structures determined with cryo-EM are the 2200 Å diameter PpV01 algae virus, that has an icosahedral protein shell with $T = 219$ quasi-symmetry, and the 5000 Å diameter mimivirus, found in amoebae, that has an icosahedral protein shell with estimated $T = 1179$ quasi-symmetry. While the PpV01 structure was determined at 30 Å resolution, the thickness of ice needed to embed mimivirus (7500 Å for shell + attached fibers) limited the image reconstruction to 75 Å resolution, which is the reason for the estimated T number. Thus, cryo-EM can provide detailed images of symmetrical virus particles of almost any size and is an increasingly valuable tool in virus structural studies.

In cases where capsid size or other factors prohibit obtaining near-atomic resolution data, structures of the individual proteins or their assembly products can be determined by nuclear magnetic resonance (NMR) and/or X-ray crystallography. Capsid subunit folds, nucleic acid-binding sites, and some details of quaternary structure can be gathered in this way. Exciting leaps in understanding virus capsid assembly, interactions, and dynamics have come from combining individual structures with cryo-EM particle images to build complete pseudo-atomic models for the larger, more-complex virus capsids. The combined approach generates discoveries that could not have been obtained from either method alone. This article highlights some of the important features of the more than 200 high-resolution nonenveloped virus structures determined to date.

The Building Blocks

The β-Barrel Fold

The earliest spherical virus capsid structures, both of animal and plant viruses, revealed a strikingly common fold for the structural subunits that continues to be found in new virus structures: the antiparallel β-barrel (**Figure 1**). It has been found mainly in icosahedral particles, but is also known to form the bacilliform particles of alfalfa mosaic virus (AMV). The barrel is formed by two back-to-back antiparallel β-sheets with a jelly-roll topology defining a protein shell approximately 30 Å thick. The β-barrels position in the capsid shells in a variety of orientations with the insertions between β-strands having a great range of sizes, giving each virus the ability to evolve a distinct structure, function, and antigenic identity. Indeed, the structure of nudaurelia capensis omega virus (NωV), an insect tetravirus, revealed an entire 133-residue immunoglobin-like domain inserted between strands E and F that forms a major portion of the particle surface. Just as prevalent, the very N- and C-termini of the capsid proteins are often extended polypeptides that do not form part of the barrel. Those portions of the extended termini close to the barrel can have ordered structure to various degrees depending on the local (quasi-)symmetry environment (see **Figure 1**), and tend to engage in varied types of interactions, such as binding one or more nearby subunits and/or nucleic acid, that play critical roles in particle assembly, stability, and nucleic acid packaging.

Other Folds

While the β-barrel remains a common element in a wide range of virus structures, other capsid subunit folds have been discovered in both enveloped (not shown) and nonenveloped particles (**Figure 2**). The nonenveloped subunits also form icosahedral shells as well as helical rods. The structure determination of the $T = 3$, ssRNA bacteriophage MS2 in 1990 revealed the first new fold for an icosahedral virus capsid subunit. It consists of a five-stranded β-sheet that faces the inside of the particle, with a hairpin loop and two helices facing the exterior. The only structural similarity to the particles formed by β-barrels was their assembly into a $T = 3$ quasi-icosahedral shell. Just 10 years later, the head (capsid) structure of the $T = 7$, dsDNA tailed bacteriophage HK97 showed another new capsid subunit fold, and the same fold has also been found in bacteriophage T4 capsids. Other than the high probability that a capsid subunit with a β-barrel is going to form an icosahedral particle, no other general themes can be derived from the variety of subunit folds to predict particle size, quasi-symmetry, or host specificity.

Completely Symmetric Capsids

$T = 1$ Icosahedral Particles

The simplest of the spherical particles, the $T = 1$ capsids, have only one protein subunit in each of the 60 positions related by icosahedral symmetry. This has been the symmetry found in three of the four known encapsulated satellite viruses, one of which is the 180 Å diameter,

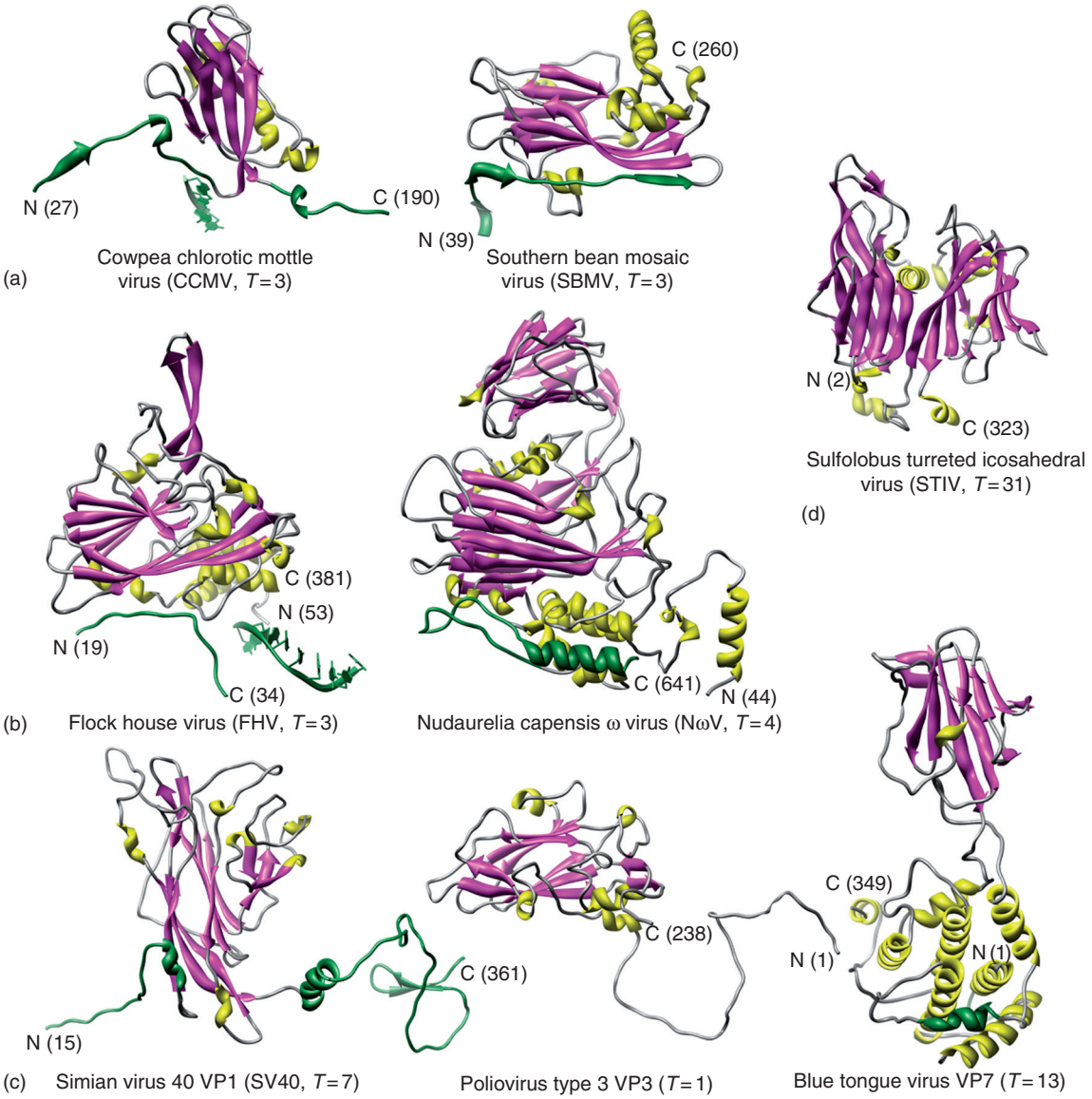


Figure 1 Structure of (a) plant, (b) insect, (c) vertebrate, and (d) archaea virus protein subunits that assemble into icosahedral shells. The name of the virus appears below the corresponding protein subunit along with the capsid triangulation number. The N- and C-termini are labeled with the residue numbers in brackets. The N-termini (and sometimes the C-termini) usually consist of highly charged residues associated with the interior of the virus particle and/or the packaged nucleic acid. As such, they do not follow icosahedral symmetry and are regularly disordered (invisible) in the final structures. Many virus subunit structures determined to near atomic resolution have the β-barrel fold and/or insertions with nearly all β-structure (colored magenta). Multiple copies (from 180 to 900) of the single subunit shown for each virus, except for that of poliovirus, form the entire icosahedral protein shell. Assembly of icosahedral virus particles with more than 60 subunits requires quasi-symmetric interactions often involving subtle to extensive differences in structure at the subunit N- and C-termini. The subunit regions involved in quasi-symmetric interactions critical to virion structure and assembly are colored green (only a single variation is shown for each virus; not yet known for STIV). The 'switch' in structure between identical subunits is a response to differences in the local chemical environment, defined by the number of subunits forming the icosahedral shell, in order to maintain similar bonding between neighboring subunits. The structural variations include the presence or absence of highly ordered RNA structure (green stick models) in FHV and CCMV. Poliovirus utilizes multiple copies of two additional subunits highly similar to VP3 to form a complete virion. Thus, there is no quasi-symmetry in poliovirus (note the absence of any green highlights) since neighboring subunits are different proteins.

ssRNA satellite tobacco mosaic virus (STMV) particle (**Figures 3(a)** and **3(b)**). The small, compact STMV capsid subunits (159 a.a.) have the β-barrel fold with the β-strands running roughly tangential to the particle curvature with the B–C, D–E, F–G, and H–I turns pointing toward the fivefold axes. This is a common subunit orientation and will be simply called 'tangential' going forward. The turns between strands are all short (no insertions),

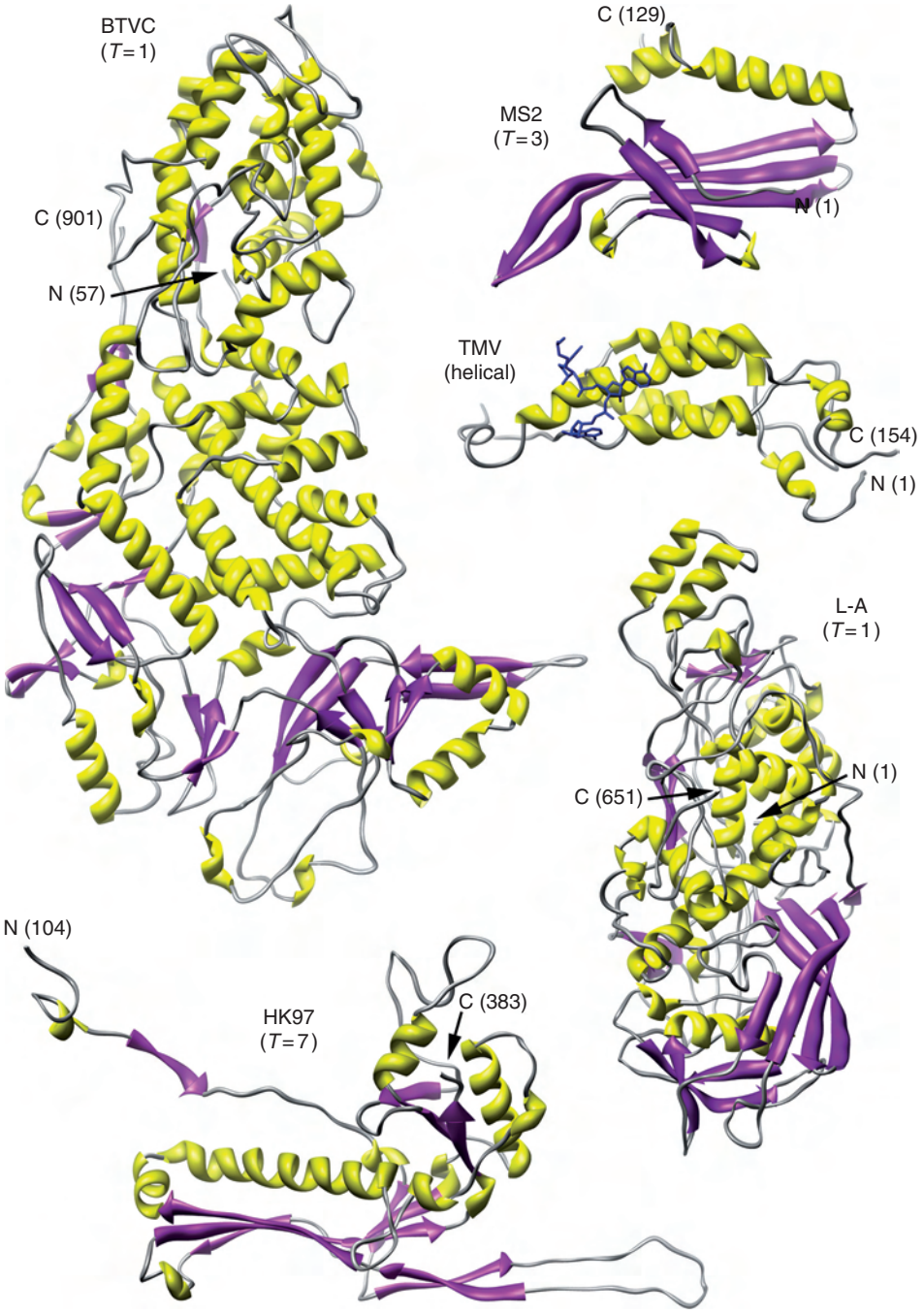


Figure 2 Structure of capsid subunits with folds other than the β-barrel. The name of the virus appears next to the corresponding protein subunit along with the capsid triangulation number (if applicable). The N- and C-termini are labeled with the residue numbers in brackets. With the exception of MS2, helical structure is dominant, with some β-sheet formations mixed into the folds. The subunit of the helical TMV rod is mostly composed of a four antiparallel α-helix bundle that forms the core of the rods, but has a small β-sheet away from the axis that forms the rod surface. The three bound nucleotides per TMV subunit are shown as a blue stick model (see the section titled 'Helical particles'). Both the BTVC (inner shell) and L–A subunits form $T = 1$ particles with two copies of the subunit in the icosahedral asymmetric unit (discussed in text), but do not share any detectable structural homology. The BTVC inner shell subunit is the largest capsid subunit determined to near-atomic resolution.

with only an extended N-terminus jutting 60 Å from the barrel that helps form both the threefold and twofold symmetry related interactions (trimers and dimers) via hydrogen bonds and extension of antiparallel β-sheets. The strongest inter-subunit contacts occur at the capsid subunit dimers, where a large number of both hydrophobic and hydrophilic interactions form across a large buried surface between the subunits. The interior surface of the

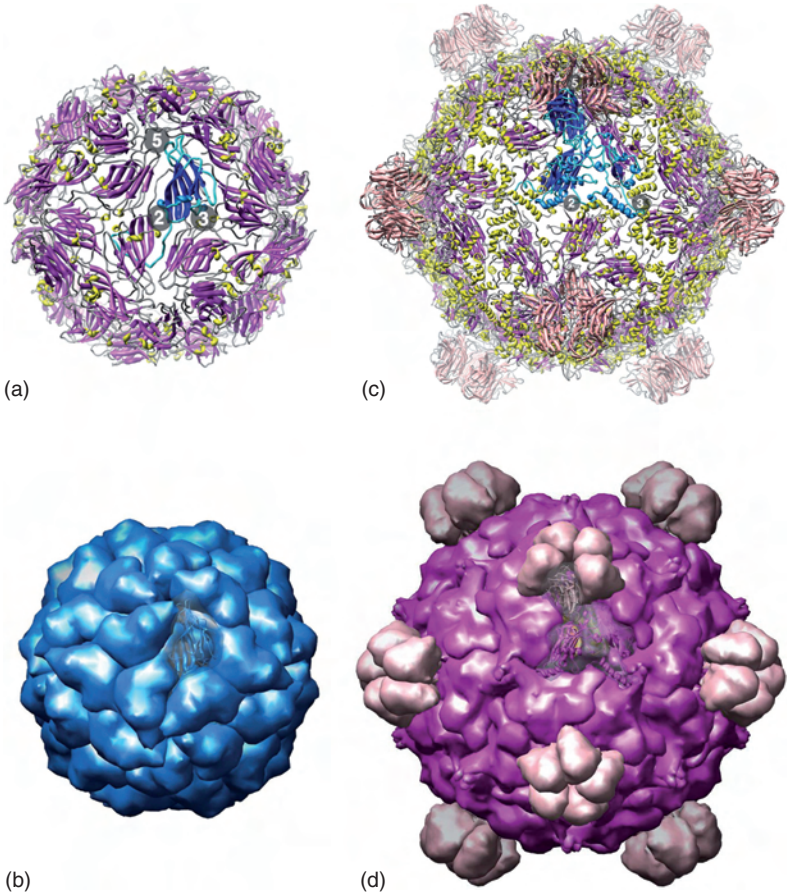


Figure 3 Structures of two $T=1$ particles viewed down an icosahedral twofold rotation axis (approximately to scale). STMV is displayed in (a) and (b), and ΦX174 in (c) and (d). The top images show all 60 capsid subunits rendered as ribbon drawings (β-strands – magenta or pink; helices – yellow), with the icosahedral asymmetric unit (IAU) shown all in blue. The fivefold, threefold, and twofold icosahedral axes are labeled with semitransparent gray circles containing the appropriate rotation number in white. The images are depth-queued such that features further away fade into the white background. The back of the particles are purposely faded (invisible) to make a clearer representation. The bottom images are surface representations of the same structures above, with the IAU shown as a transparent surface to show the ribbon drawing of the capsid subunits. Note the more compact look of STMV due to the close approach of complementary surfaces on the β-barrels vs. the more spread-out look of ΦX174 due to its use of extended loops to create a contiguous capsid. The exterior of the ΦX174 shell includes the G-protein (pink subunits), which surrounds hydrophilic channels at the fivefold axes, and has a β-barrel fold that is oriented nearly parallel to the fivefold axes such that they form 12 broad spikes on the particle surface. This will be called the 'axial' orientation. This increases the particle dimensions at these axes by 60 Å but does not contribute to the enclosed volume as they sit atop an essentially complete protein shell. (b, d) Courtesy of the Viperdb web site.

STMV capsid dimers binds a large section of ordered double-helical RNA. Two additional symmetry-related subunits interact with the RNA via their extended N-termini. The protein–RNA interactions are split between direct side-chain bonds to the ribose sugars, and water-mediated hydrogen bonds by residues of both the local subunit dimer β-barrels and N-termini. Altogether the ordered RNA accounts for about 59% of the packaged nucleic acid. Although STMV is one of the simplest icosahedral viruses, its features demonstrate many of the basic protein–protein and protein–RNA bonding schemes that have been observed in larger and more complex particles.

While an advantage of the $T=1$ capsid is its equivalent subunit environments, its major limitation is the small particle volume. To create larger shells, the subunits either have to be larger or there have to be more of them. The former is seen in the crystal structure of the ssDNA bacteriophage ΦX174 capsid (**Figures 3(c)** and **3(d)**). Its 426 a.a. F proteins form a 340 Å diameter $T=1$ icosahedral particle, which has nearly fourfold greater volume compared to the satellite virus capsids. Each subunit has the β-barrel fold, but it accounts for only one-third of the F protein. The remainder of the capsid subunit is composed of long loops inserted between the β-strands that make up the majority of inter-subunit interactions and capsid surface. ΦX174 has additional capsid proteins, but the contiguous protein shell is made up of only the F protein. While a larger subunit can create a viable amount of enclosed volume, native $T=1$ particles are uncommon. This may be due to

inefficiencies in dealing with the larger amount of genome dedicated to the major capsid protein and the more complex structural requirements of a single large subunit to build a stable and functional particle.

Helical Particles

The only high-resolution structures of helical viruses are from three members of the genus *Tobamovirus*, whose type member is TMV. The tobamoviruses form rod-shaped particles 3000 Å long and 180 Å in diameter, with a central hole about 40 Å across. The particles are built from over 2100 identical capsid protein subunits that are 158 a.a. in size, and arranged in a right-handed helix with 49 subunits in every three turns and a pitch of 23 Å. The subunit has a narrow, elongated structure composed of a four-helix bundle on one end, and a small β-sheet on the other (**Figure 2**). The subunits sit perpendicular to the particle axis, with the helix end of the subunit nearest the center and the β-sheet end forming the outer surface of the rod. Neighboring subunits stack upon one another in the particle helix such that two helices of each protein interact with two others from the neighboring subunit, forming another four-helix bundle to build the rod.

The structure of the TMV particle determined in the late 1980s revealed the first details of virus capsid protein interactions with nucleic acid. A long loop inserted between two of the subunit helices forms the interior channel of the particle that includes a continuous RNA binding site at the edge of the helices. The entire RNA genome is well ordered in the TMV structure by conforming to the helical symmetry of the coat protein stacking. There are three nucleotides bound to each protein subunit (**Figure 2**) over the entire length of the rod, which adds subunits during assembly until the entire genome is encapsidated. The RNA is bound at its phosphate groups by direct, calcium-mediated, and probably water-mediated interactions with nearby arginine and aspartic acid residues, and at its sugars and bases by hydrophobic complementary, polar interactions, and a few hydrogen bonds from local arginine and aspartic acid residues. This mode of nucleic acid binding showed that, since the viral genome sequences do not share the symmetry of their enclosing particles, interactions with the coat protein are complementary to the nucleic acid, but non-base-specific to ensure that the entire genome is encapsidated. The same general principles continue to be found in the ordered protein–nucleic acid interactions observed in icosahedral particle structures.

Quasi-Symmetric Capsids

T = 3 Icosahedral Particles

Icosahedral particles built from multiples of 60 identical capsid subunits exist in great numbers, and are arranged with many variations on the quasi-symmetry first predicted by Caspar and Klug in 1962. In one of the closest matches to their predictions, 90 coat protein dimers (180 total coat proteins) of the ssRNA plant cowpea chlorotic mottle virus (CCMV) assemble with nearly ideal $T = 3$ quasi-symmetry into the shape of a 280 Å diameter truncated icosahedron, which is defined by planar pentamer (formed by A subunits) and hexamer (formed by B and C subunits) morphological units (capsomers) at the fivefold and threefold (quasi-sixfold) axes, respectively (**Figure 4**, top). The 190 a.a. capsid subunit folds into a small β-barrel with short loops between strands, but has both extended N- and C-termini (**Figure 4**, bottom left). All 180 subunits have the axial orientation with the N-termini extending toward the center of the pentamers and hexamers, and the C-termini extending outward into neighboring capsomers by reaching across the twofold axes. The very ends of the C-termini are clamped in between the β-barrel and N-terminal extension of the twofold related subunit, forming the largest number of interactions between capsid subunits and tying the capsomers together. This occurs at both the twofold and quasi-twofold axes with high fidelity. The dimer interactions also define the dihedral angles between the planar capsomers; the angles between hexamer–hexamer (twofold) and pentamer–hexamer (quasi-twofold) interfaces only differ by 4° (**Figure 4**, bottom right), producing a smooth particle curvature. A triangular unit of A, B, and C subunits defines the icosahedral asymmetric unit (IAU), and also a high-fidelity quasi-threefold axis. Calcium and RNA binding sites are preserved at all three interfaces (calcium site exterior, RNA site interior, and under the calcium site) with nearly equivalent positions of the coordinating side chains.

The pentamer and hexamer units show more differentiation. Besides subtle contact changes between the β-barrels, the N-termini of the hexamer subunits form a nearly prefect sixfold symmetric parallel β-tube about the threefold axes in an impressive display of quasi-sixfold interactions. The N-termini of the pentamer subunits extend a bit toward the fivefold axes but form no structure due to disorder after residue 42. Interestingly, deletion of the β-hexamer in one study did not prevent hexamers from forming, but did destabilize the particle. Other than the differences seen at the capsomer centers, the remainder of the interactions between the A, B, and C subunits are subtly changed to accommodate the differences in chemical environments, rather than switched from one form to another, and may explain the highly pleomorphic properties of the CCMV capsid subunit.

T = 4 Icosahedral Particles

The ssRNA insect tetraviruses remain the only known viruses that have a nonenveloped, $T = 4$ capsid. The crystal structure of the 400 Å diameter NωV capsid in

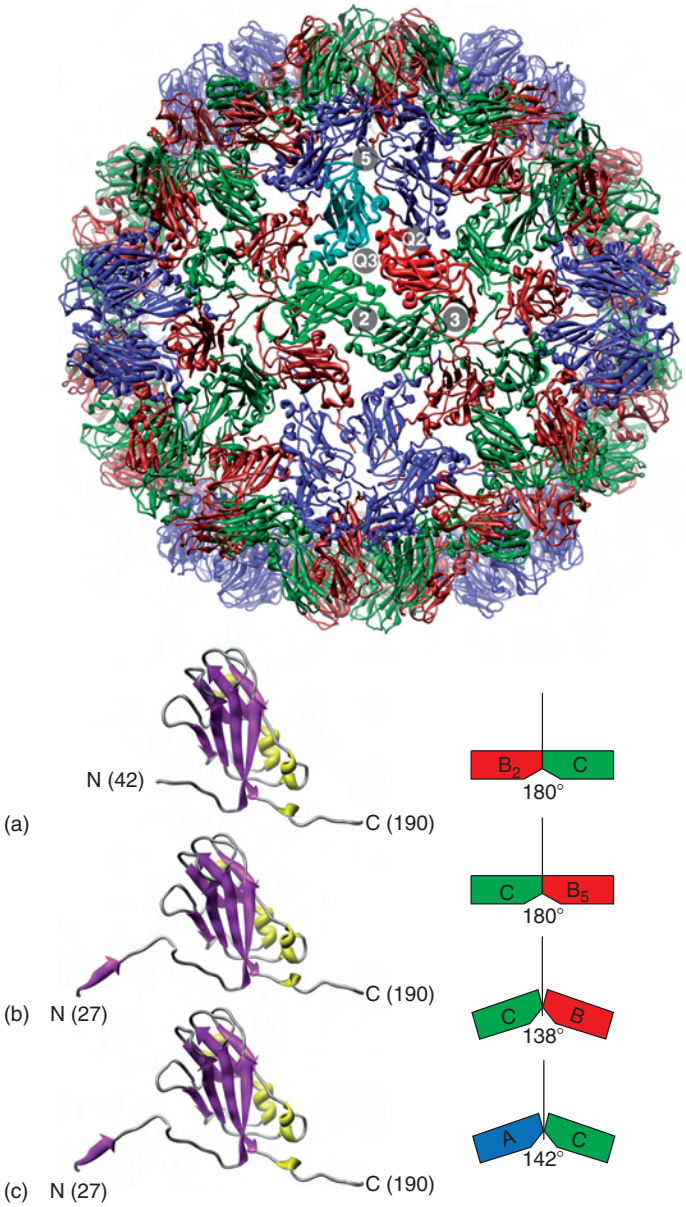


Figure 4 Structure of the $T = 3$ CCMV particle viewed down an icosahedral twofold axis. Icosahedral and quasi-symmetry (Q) axes are labeled as in **Figure 3**. The quasi-sixfold axes are not labeled as they coincide with the icosahedral threefold axes. (top) The entire CCMV particle with A-subunits colored blue, B colored red, and C colored green. An IAU is shown above the twofold axis in brighter colors and larger ribbons. The calcium sites between quasi-threefold related subunits are strongly implicated in CCMV particle expansion, which occurs in the absence of divalent metal ions at high pH. The CCMV crystal structure shows that under these conditions the charged residues of the calcium-binding site would cause a repulsion between quasi-threefold related subunits, and a cryo-EM structure of the swollen form of CCMV confirmed the opening of large holes at the quasi-threefolds. Indeed, swollen CCMV breaks apart into subunit dimers and RNA in high salt concentrations, and under various solution conditions, can reassemble into a number of structures including $T = 1$, $T = 3$, and $T = 7$ particles, long tubes, multi-shelled particles and tubes, and laminar plates. It is one of the most pleomorphic viral subunits ever studied, but amazingly only makes $T = 3$ particles in plant infections. (Bottom left) Individual structures of the proteins in the A, B, and C subunit environments colored as in **Figure 2**. Note they are closely identical except for the lack of extended N-terminal structure in A, and slightly varying directions taken by the C-termini away from the β-barrels (not readily visible in these views). (Bottom right) Diagrammatic representation of the flat and bent subunit/capsomer interfaces. Individual blocks represent the subunits as viewed tangentially to the particle curvature. Colors and letters designate the subunit type with the dihedral angle between them given underneath.

1996 showed the 644 a.a. capsid subunit has three domains: an exterior Ig-like fold, a central β-barrel in tangential orientation, and an interior helix bundle (**Figure 5**, right side). The helix bundle is created by the N- and C-termini of the polypeptide before and after the β-barrel fold, and the Ig-like domain is an insert between the E and F β-strands. All 240 of the NωV capsid subunits undergo autoproteolysis at residue 571 after assembly,

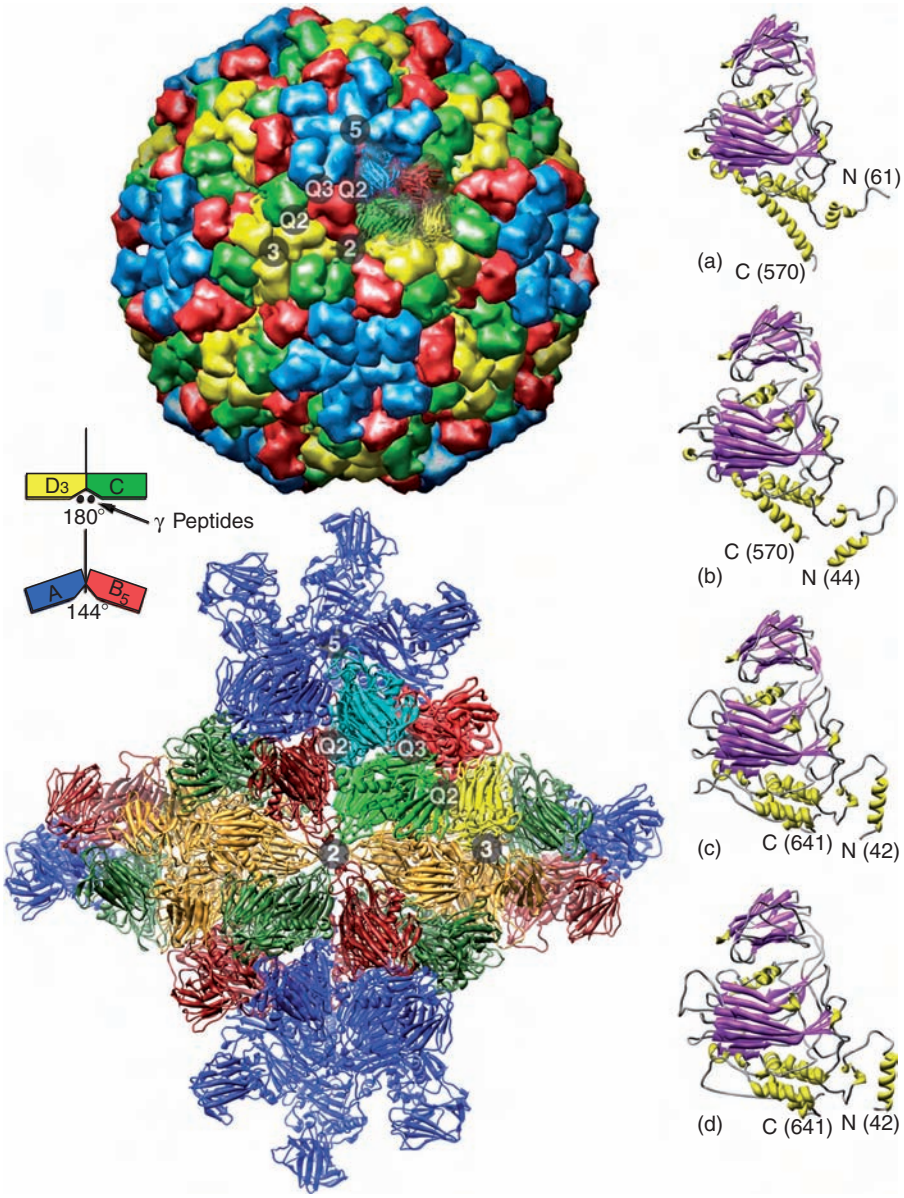


Figure 5 Structure of the $T = 4$ NωV particle viewed down an icosahedral twofold axis. Icosahedral and quasi-symmetry (Q) axes are labeled as in **Figure 3**. The low-fidelity quasi-sixfold axes are not labeled as they coincide with the icosahedral twofold axes. (Right) Individual structures of the proteins in the A, B, C, and D subunit environments colored as in **Figure 2**. While the basic folds and domains are similar, they differ considerably at both the N- and C-termini, in loop structure at one end of the barrel, and slightly in position and orientation of the Ig domains. In particular, the C- and D-subunits have additional ordered polypeptide in the helical domain that dictates switching between bent and flat contacts in the capsid architecture (see below and text). (Left) Surface representation of the entire $T = 4$ particle (as in **Figure 1**) and a more detailed ribbon representation of the area between the four fivefold axes surrounding the center twofold axis. A diagrammatic representation of the subunit/capsomer interfaces is also shown as in **Figure 3**. Complete pentamers are only shown on the top and bottom of the ribbon image. The A-, B-, and C-subunits are colored as in **Figure 3**, and the D-subunits are colored yellow. An asymmetric unit is shown above and to the right of the twofold axis in brighter colors and larger ribbons (also shown in surface representation above as ribbons). The $T = 4$ arrangement can be thought of as two sets of triangles: one set is formed by the quasi-threefold related A-, B-, and C-subunits, and the other is formed by the icoshedral threefold related D-subunits. The dihedral angles of the interfaces between two ABC units (144°), and between ABC and DDD units (180°; both are quasi-twofold related contacts) creates flat triangles with fivefold axes at the corners, and bending between them along the lines from fivefold to twofold axes. Surface image courtesy of the Viperdb web site.

leaving the cleaved portion of the C-terminus, called the γ-peptide, associated with the capsid. The fold and tangential orientation of the β-barrel together with the autoproteolysis revealed a strong relationship between tetraviruses and the $T = 3$ insect nodaviruses. Indeed, the folds and catalytic sites superimpose with little variation, and a γ-peptide also exists in the nodaviruses. Unlike in CCMV, the quasi-symmetry of these insect particles is

obviously controlled by a molecular switch (**Figure 5**, left side). In NωV, a segment of the γ-peptide (residues 608–641) is only ordered in the C and D subunits, and functions as a wedge between the ABC and DDD units to prevent curvature and create a flat contact. The interface between two ABC units is bent partly due to the lack of ordered γ-peptide. A similar situation exists in the nodaviruses, except that the ordered polypeptide between twofold related C-subunits in the $T=3$ particle comes from the A-subunit N-terminus, and ordered duplex RNA also fills the interface. The structural information from the tetravirus group has defined important relationships and some common themes in the biology of the insect viruses.

T = 7 Icosahedral Particles

All three $T=7$ capsid structures determined at near atomic resolution are dsDNA viruses with unique features: two mammalian polyomaviruses, SV40 and polyoma virus, and the head of the λ-like tailed bacteriophage HK97 from the family *Siphoviridae*. The 500 Å diameter SV40 capsid is constructed of 72 pentamers arranged with $T=7d$ quasi-symmetry, not 12 pentamers and 60 hexamers as predicted by quasi-equivalence theory. Thus, 60 of the SV40 pentamers are actually in hexavalent environments as they are located in positions that are occupied by hexamers in an ideal $T=7$ lattice. As the valency of a true $T=7$ lattice is present, it was a surprise to discover it could be achieved without the expected number of identical subunits or domains (i.e., five subunits instead of six). The pentamers contain five copies of the 364 a.a. VP1 protein, which have the axial β-barrel orientation and extended C-terminal arms. Interlocking secondary structures of the G-strands and DE loops with clockwise neighbors form the intimately associated pentamers. The C-arms of all five subunits in each pentamer reach outward to one of three specific types of neighboring pentamers depending on the local environment (threefold and two types of twofold clusters), and form another β-strand between the N-terminus and BIDG sheet of the neighboring subunit (a similar clamp to that of CCMV). This ties the neighboring pentamers together in a general way determined by the position and valency of each pentamer. Remarkably, the VP1 proteins and clamp structures in the pentamer cores are structurally identical, and only the direction and ordered structure of the C-terminal arms are changing as they switch contacts between the nonequivalent pentamers.

By comparison, the 650 Å diameter HK97 capsid is a true $T=7l$ icosahedron, composed of 420 subunits arranged in 12 concave pentamers and 60 planar hexamers. Strikingly, the 385 a.a. protein subunit has a new virus fold (**Figure 6**). Each subunit forms two covalent isopeptide bonds (cross-links) with neighboring subunits on opposite sides of the protein to create topologically linked protein rings arranged with icosahedral symmetry, also a new find in protein structure studies. Notably, the cross-links are formed between subunits that encircle the pentamers and hexamers (one from each neighboring capsomer), not between the subunits forming them. This defines how their complex interweaving occurs at the particle threefold and quasi-threefold axes (**Figure 6**). The fact that this is the final mature state of the HK97 head particle, and that the cross-links are not yet formed in particles at the early to mid-stages of the capsid maturation process, has led to informative studies on capsid dynamics using HK97 as a model system. The final step of HK97 maturation involves formation of the final 60 cross-links in the transition from expansion intermediate IV (EI-IV) to the mature capsid (head II). The pentons appear to be dynamic, oscillating up and down relative to the contiguous capsid shell, until cross-linked. See the article 'Principles of Virus Structure' for animations of the entire transition from the prohead state through head II.

T = 13 Icosahedral Particles

The 700 Å diameter dsRNA blue tongue virus core (BTVC) particle remains the largest molecular structure ever determined by X-ray crystallography to near-atomic resolution. The core particle has two shells: an inner $T=1$ particle, composed of 120 large (901 a.a.) VP3 proteins (two in each icosahedral asymmetric unit), which packages a few RNA polymerases and 10 strands of dsRNA, and an outer $T=13l$ particle composed of 780 VP7 proteins (349 a.a.), for a total of 900 capsid proteins and a molecular weight of over 54 000 000 Da. The VP7 subunits form a trimer in solution. The trimer structure was determined by X-ray crystallography and combined with cryo-EM images of BTVC to determine the entire BTVC crystal structure. The VP7 subunit has two domains, an outer β-barrel and an inner helix bundle (**Figure 1**), both of which have extensive contacts in the trimer. In an interesting twist, the β-barrel is actually an insert between two helices in the linear VP7 sequence, and the helix bundle is responsible for the majority of interactions between trimers in the assembled shell. The VP7 trimers are postulated to 'crystallize' or assemble on the inner shell's surface into the 12 pentamers and 120 hexamers, which follow quasi-symmetry with high fidelity having very little differences in their intercapsomer interactions. In contrast, the inner $T=1$ shell breaks from quasi-equivalence theory by using large-scale flexing of the two huge VP3 proteins to generate similar shapes of interactions in the absence of similar contacts, and build a nearly featureless hydrophobic surface for interaction with VP7. Together, these shells present a dazzling array

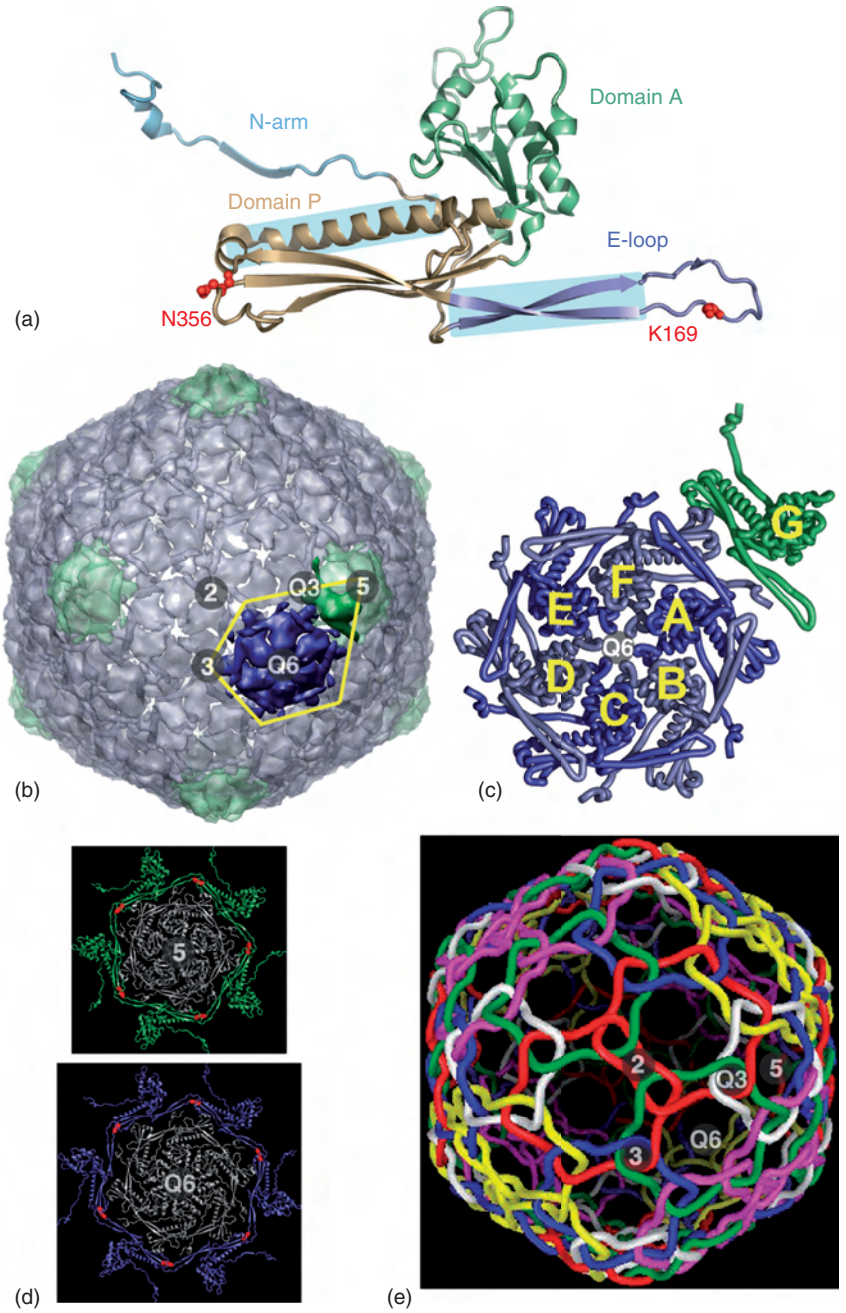


Figure 6 Structure of the $T = 7$ HK97 particle. Icosahedral and quasi-symmetry (Q) axes are labeled as in **Figure 3**. (a) HK97 subunit fold. The small HK97 subunits have mixed α/β structure, forming two domains in the shape of an L that have a continuous hydrophobic core, and placing the two residues involved in cross-linking other subunits (red) on opposite sides of the protein. (b) Surface representation of the entire HK97 capsid viewed down an icosahedral twofold axis, with pentamers colored separately from hexamers. The IAU is outlined in yellow. Unlike the $T = 3$ and $T = 4$ structures, none of the hexamers sit on icosahedral axes. (c) Ribbon diagram of the seven subunits in the IAU showing how they align such that the P-domains and E-loops overlap to define the sides of the local capsomers, but place their tips (with the cross-linking residues) outside the capsomer to be accessible to neighboring subunits. (d) The two types of cross-linked subunit 'circles' or 'rings' in HK97. The surrounding cross-linked subunits (one from each neighboring capsomer) are shown in green (5-circle) or blue (Q6-circle), with the cross-link shown in red. (e) Diagrammatic representation of the interlocking cross-links in HK97. The 5- or Q6-circles, now with subunit and cross-links just represented by tubes, are colored separately showing how they form molecular chainmail by interweaving at the icosahedral and quasi-threefold axes. This molecular chainmail stabilizes the unusually thin 18-Å-wide protein shell (only half the width usually formed by capsids composed of β-barrel subunits), which can be described as a protein balloon. The capsids are also stabilized by other extensive inter-subunit interactions with up to nine other coat proteins. The N-arm, which is not involved in the cross-links, extends 67 Å away from the subunit to contact subunits in neighboring capsomers. (a–d) Courtesy of Dr. Lu Gan. (e) Courtesy of Gabe Lander.

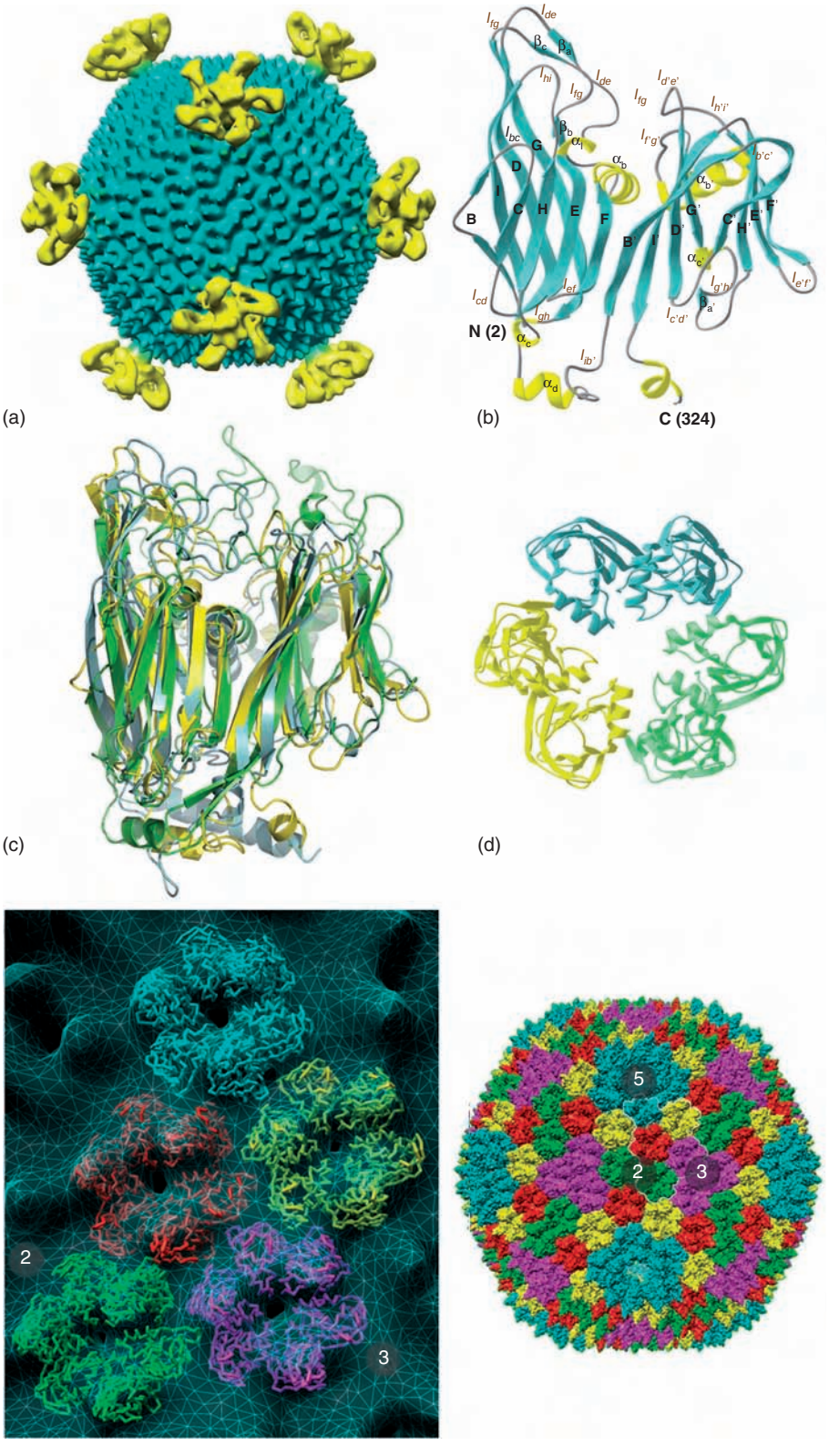


Figure 7 Continued

of protein interactions that go from near perfect conformity with quasi-equivalent theory to defining a new category of viral subunit interactions in the $T=1$ particle.

T = 25 and Larger Icosahedral Particles: The Double-Barrel Subunit

Virus capsids having to assemble with $T=25$ quasi-symmetry or higher have avoided the enormous challenge of accurately placing 1500 or more individual proteins in a shell by reducing the problem to just a few related hexon locations. The crystal structure of the hexon from the dsDNA, $T=25$ adenovirus capsid revealed it is a trimer of three identical 967 a.a. proteins. Each protein has two axially oriented β-barrel domains. In the trimer, the barrels are almost precisely related by a pseudo-sixfold axis such that each forms a corner of the hexon base. The hexon is the building block for the particle and is extremely stable due to interpenetrating polypeptides in the trimer. Thus, a stable trimer satisfies the hexavalent lattice positions in $T=25$ adenovirus as the stable pentamers do in $T=7$ SV40. Combined with cryo-EM images of the capsid, these structures revealed that there are only four different hexon-binding sites in adenovirus (pentons have a different subunit), and that much larger hexon facets can be constructed in this manner. In a recent series of groundbreaking discoveries that surprised virus researchers, a combination of both cryo-EM and crystallography methods revealed that the hexon structures of large dsDNA viruses in all domains of life have similar 'double-barrel trimers' forming pseudo-hexagonal shapes. These include adenovirus (eukarya), $T=25$ PRD1 (bacteria), $T=169$ paramecium bursaria chlorella 1 virus (PBCV-1, green algae), and, most recently, $T=31$ sulfolobus turreted icosahedral virus (STIV, archaea) (**Figure 7**). When the double-barrel subunits from all four viruses are superimposed, over 46% of their polypeptides are within 2.2 Å r.m.s.d. of each other. For the first time, these apparently complicated and unrelated viruses infecting quite different hosts have been solidly linked by their structures, supporting the hypothesis that at least one virus lineage originated before life separated into three domains.

Bacilliform Particles

The ssRNA plant alfalfa mosaic virus (AMV), a member of the family *Bromoviridae* that includes CCMV, forms four types of bacilliform particles composed of one of the four genomic RNAs and a single 220 a.a. capsid subunit. The particles are 180 Å in diameter and range in length from 300 to 570 Å. The spherical ends have $T=1$ symmetry, while the cylindrical bodies have a repeating sixfold symmetry with large holes at the hexamer centers. After a mild trypsin treatment and removal of RNA, the capsid subunit readily forms $T=1$ particles that crystallize and their structure has been determined. The $T=1$ particle is 220 Å in diameter with large holes 35 Å across at the fivefold axes. The subunit is a simple β-barrel in the axial orientation with extended termini at the base. The C-termini reach across the twofold axes to hook between the N-terminus and β-barrel of the symmetry-related subunit in a strikingly similar fashion to the structure of the CCMV particle. Thus, this structure of the AMV endcaps helps demonstrate how the highly pleomorphic capsid proteins of bromoviruses can assemble into several particle forms via strong but flexible dimer contacts depending on solution conditions.

Psuedo-Symmetric Capsids

P = 3 Icosahedral Particles

Instead of having three copies of a single protein in slightly different chemical environments, a number of viruses have evolved with two or three different proteins in each environment that have unique primary structures but similar folds. The coat protein arrangements are similar to that proposed for $T=3$ lattices such that they are given the $P=3$ (pseudo $T=3$) designation. Unlike the single protein in T capsid arrangements, the different

Figure 7 Structure of the $T=31$ STIV capsid and major coat protein (MCP). (a) The cryo-EM image reconstruction of the entire STIV capsid viewed down an icosahedral twofold axis. The shell formed by 900 copies (300 trimers) of the MCP is shown in blue, and the 200 Å tall turrets that compose the fivefold axes are shown in yellow. (b) Crystal structure of the MCP, with β-strands colored cyan and helices yellow. Like other large dsDNA viruses, the protein folds into two covalently linked β-barrels, one shown on the left and the other on the right. Interestingly, the large capsid subunit of the plant comoviruses is the only one outside the large dsDNA virus families with the double-barrel fold. (c) Superposition of the major coat proteins from STIV (yellow), PRD1 (cyan), and PBCV-1 (green) in approximately the same orientation as (b), showing the closely similar double-barrel folds. (d) Model of the STIV MCP trimer, based on that seen in the other dsDNA viruses. Each protein is colored separately, and each barrel makes the corner of one of the five unique hexon units seen in the assembled $T=31$ virus. (e) Top view of the STIV asymmetric unit from the cryo-EM structure (shown as a blue lattice) with Cα traces of the MCP trimer fitted in the hexon positions. Icosahedral axes are labeled as in previous figures, with the blue hexon just below the fivefold axis. The highly correlated fit between the atomic model and cryo-EM reconstruction reveals possible inter-subunit interactions, quasi-symmetry, and how the main protein shell is constructed, shown in (f). (f) Pseudo-atomic model of the complete MCP shell using the same color scheme as in (e), with the asymmetric unit outlined in white. Note the modularity of the hexons, which theoretically can be exploited to form capsids of extremely large size such as the 5000 Å diameter mimivirus. Courtesy of Dr. Reza Khayat *et al*., as published in Khayat R, Tang L, Larson ET, *et al*. (2005) Structure of an archaeal virus capsid protein reveals a common ancestry to eukaryotic and bacterial viruses. *Proceedings of the National Academy of Sciences, USA* 102: 18944–18949.

capsid proteins in P arrangements are free to mutate, function, and form interactions independent of one another but more of the genome must be used to encode multiple capsid proteins. Two well-studied families with $P = 3$ capsids are the ssRNA animal picornaviruses and plant comoviruses, which have a common genome organization. The structures of several human rhinovirus (HRV) serotypes (common cold virus) are 320 Å diameter particles with the VP1, VP2, and VP3 capsid subunits in a $T = 3$-like IAU (**Figure 8**). These subunits all have the β-barrel fold oriented tangentially and similar overall structure except for some loops and extended protein termini. The exterior of the particle has a deep cleft circling the fivefold axes (viral canyon) that binds the ICAM receptor on the surface of cells and which is also bound by neutralizing antibodies.

The capsid of the plant bean-pod mottle virus (BPMV) is also 320 Å in diameter, but has only two subunits, small and large. Both fold into β-barrel domains: one in the small subunit and two that remain covalently attached in the large subunit. The subunits assemble into a $T = 3$-like shell such that the small subunit is equivalent to VP1, and the large subunit to VP2 and VP3 in the HRV capsids. The BPMV structure had the first ordered protein–RNA interactions seen in an icoshedral virus. Seven ribonucleotides in a single RNA strand were visible inside the capsid shell near the icosahedral threefold axis in a shallow pocket formed by nine different segments from both domains of the large capsid protein. A few specific interactions and a larger number of nonbonded contacts bind the ordered RNA segment.

Other Icosahedral Particles with Uncommon Architectures

While shell arrangements other than T lattices are possible with multiple single or unique capsid proteins in the IAU, these are so far rare. The inner $T = 1$ shell of the BTVC is described above in the $T = 13$ virus section. A very similar $T = 1$ shell has been described for the 440 Å diameter dsRNA yeast L–A virus capsid structure, which also has two copies of the single 680 a.a. in the IAU. This arrangement is emerging as a common structure among the inner protein shells of dsRNA viruses that transcribe mRNA inside the particle and extrude the nascent strands through holes in the shell.

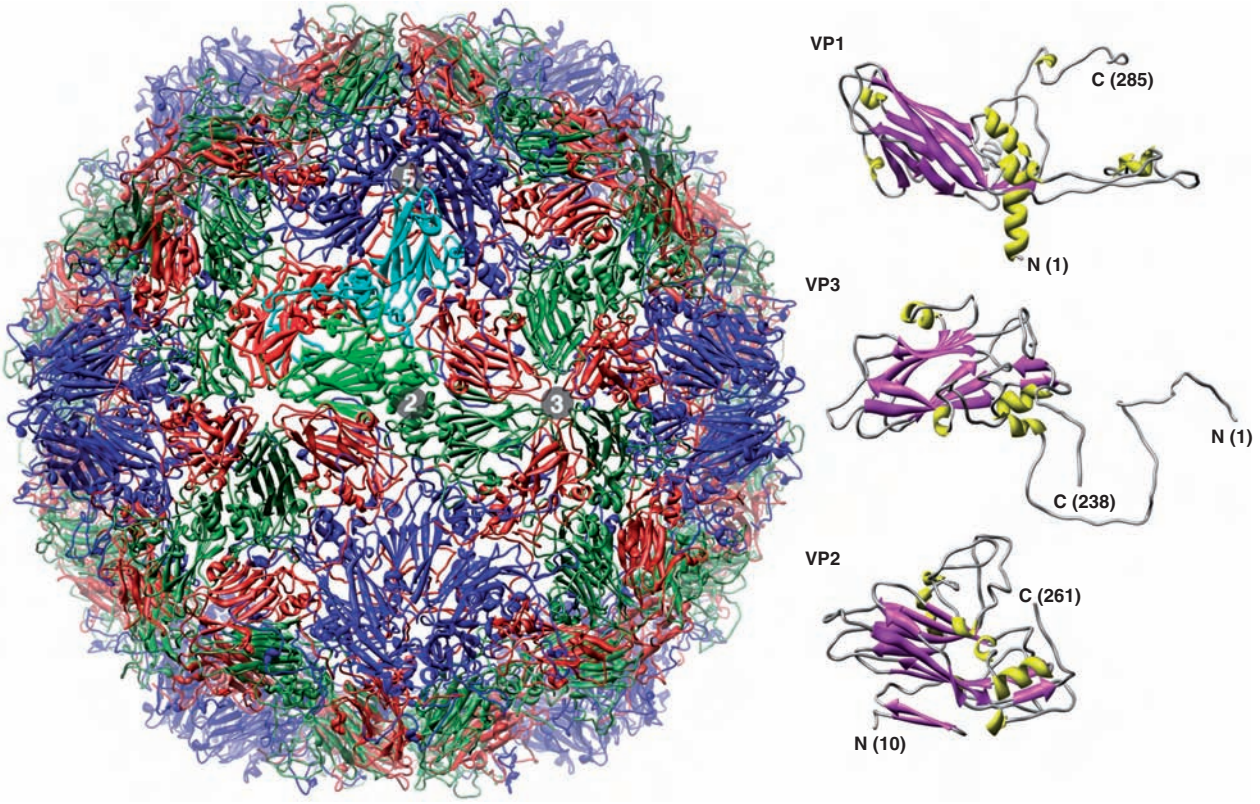


Figure 8 Structure of the $P = 3$ HRV-16 capsid viewed down an icosahedral twofold axis. Icosahedral axes are labeled as in **Figure 3**, but note there are is no quasi-symmetry since the proteins have unique primary structures. The VP1 proteins are colored blue, VP2 green, and VP3 red. An asymmetric unit is shown above and to the left of the twofold axis in brighter colors and larger ribbons (note the color and asymmetric unit definitions are a bit different than in $T = 3$ particles). Structures of the three different proteins are shown to the right and colored as in **Figure 2**. Note the slight tilt of the VP1 subunits that helps to create the canyon surrounding the pentamers.

Concluding Remarks

A great deal of our early knowledge of virus structure came from the nonenveloped viruses, and they continue to provide valuable data in understanding the virus life cycle. The important role of viruses in disease makes it imperative we understand the chemistry and biology of their capsids in ever greater detail. While some overall themes emerge, such as the general nature of capsid protein–protein interactions, protein–RNA interactions, and protein shell organizations, their remarkable variation and complexity defies attempts to form a comprehensive understanding of how they assemble and function. Recent structural evidence pointing to the existence of a primordial virus may lead to important new insights as virus structural studies address even more complex particles at an accelerated rate.

See also: Capsid Assembly: Bacterial Virus Structure and Assembly; Cryo-Electron Microscopy; Electron Microscopy of Viruses; Tobacco Mosaic Virus; Virus Particle Structure: Principles.

Further Reading

Burnett RM (2006) More barrels from the viral tree of life. *Proceedings of the National Academy of Sciences, USA* 103: 3–4.

Chiu W and Johnson JE (eds.) (2003) *Advances in Protein Chemistry, Vol. 64: Virus Structure.* San Diego: Academic Press.

Freddolino PL, Arkhipov AS, Larson SB, McPherson A, and Schulten K (2006) Molecular dynamics simulations of the complete satellite tobacco mosaic virus. *Structure* 14: 437–449.

Khayat R, Tang L, Larson ET, *et al.* (2005) Structure of an archaeal virus capsid protein reveals a common ancestry to eukaryotic and bacterial viruses. *Proceedings of the National Academy of Sciences, USA* 102: 18944–18949.

Rossmann MG, Morais MC, Leiman PG, and Zhang W (2005) Combining X-ray crystallography and electron microscopy. *Structure* 13: 355–362.

Roy P, Maramorosch K, and Shatkin AJ (eds.) (2005) *Advances in Virus Research, Vol. 64: Virus Structure and Assembly.* San Diego: Academic Press.

Relevant Website

http://viperdb.scripps.edu/ – Viperdb Web Site (A Database of Virus Structures with Many Visualization and Analysis Tools).

Virus Particle Structure: Principles

J E Johnson and J A Speir, The Scripps Research Institute, La Jolla, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The virion is a nucleoprotein particle designed to move the viral genome between susceptible cells of a host and between susceptible hosts. An important limitation on the size of the viral genome is its container, the protein capsid. The virion has a variety of functions during the virus life cycle (**Table 1**); however, the principles dictating its architecture result from the need to provide a container of maximum size derived from a minimum amount of genetic information. The universal strategy evolved for the packaging of viral nucleic acid employs multiple copies of one or more protein subunit types arranged about the genome. In most cases the subunits are arranged with well-defined symmetry, but there are examples where assembly intermediates and sometimes mature particles are not globally symmetric. The assembly of subunits into nucleoprotein particles is, in many cases, a spontaneous process that results in a minimum free energy structure under intracellular conditions. The two broad classes of symmetric virions are helical rods and spherical particles.

Helical Symmetry

The nucleoprotein helix can, in principle, package a genome of any size. Extensive studies of tobacco mosaic virus (TMV) show that protein subunits will continue to add to the extending rod as long as there is exposed RNA. Protein transitions required to form the TMV helix from various aggregates of subunits are now understood at the atomic level. It is clear that subunits forming the helix display significant polymorphism in the course of assembly; however, excluding the two ends of the rod, all subunits are in identical environments in the mature helical virion. This is the ideal protein context for a minimum free energy structure. In spite of these packaging and structural attributes the helical virion must be deficient in functional requirements that are common for animal viruses because they are found only among plant, bacterial, and archeal viruses. Even among plant viruses, only 7 of the 25 recognized groups are helical. The large majority of all viruses are roughly spherical in shape.

Icosahedral Symmetry

The architectural principles for constructing a 'spherical' virus were first articulated by Crick and Watson in 1956. They suggested that identical subunits were probably distributed with the symmetry of Platonic polyhedra (the tetrahedron, 12 equivalent positions; the octahedron, 24 equivalent positions; or the icosahedron, 60 equivalent positions). Subunits distributed with the symmetry of the icosahedron (**Figure 1(a)**) provide the maximum sized particle, for a given sized subunit, in which all copies of a subunit lie in identical positions. The repeated interaction of chemically complementary surfaces at the subunit interfaces leads naturally to such a symmetric particle. The 'instructions' required for assembly are contained in the tertiary structure of the subunit (**Figure 1(b)**). The actual assembly of the protein capsids is a remarkably accurate process. The use of subunits for the construction of organized complexes places strict control on the process and will naturally eliminate defective units. The reversible formation of noncovalent bonds between properly folded subunits leads naturally to error-free assembly and a minimum free energy structure.

Table 1 Functions of the virus capsid in simple RNA viruses

Assembly	Subunits must assemble to form a protective shell for the RNA
Package	Subunits specifically package the viral RNA
Binding to receptors	The capsid may actively participate in virus infection processes binding to receptors and mediating cell entry (animal) and disassembly
Transport	Virion transport within the host (plant)
Mutation	Capsid protein mutation to avoid the immune system
RNA replication	Some capsid proteins function as a primer for viral RNA replication

Virus Structures

Crystallographic studies of nearly 90 unique, icosahedral viruses have demonstrated that there are a limited

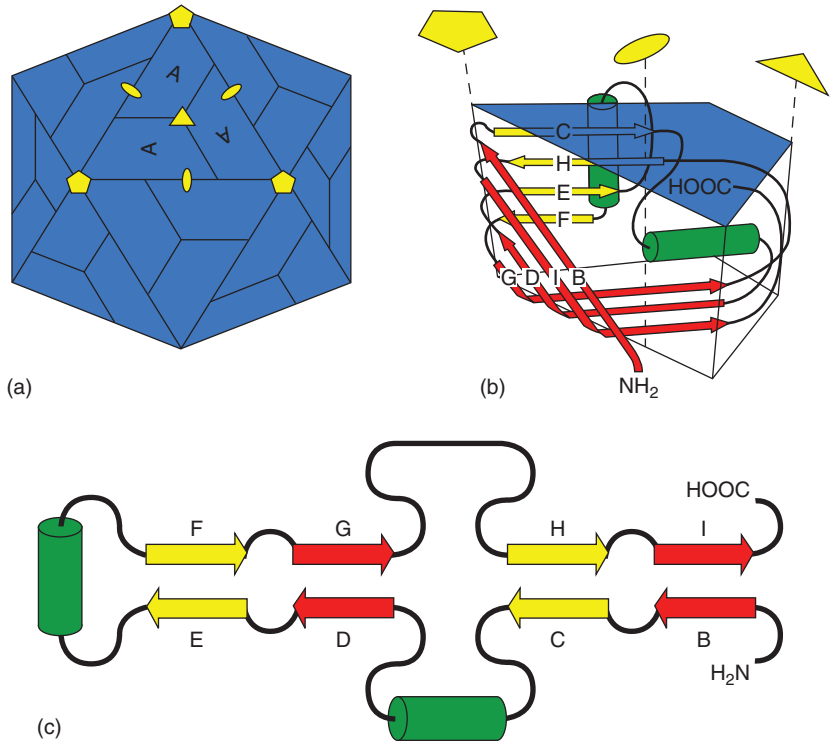


Figure 1 (a) The icosahedral capsid contains 60 identical copies of the protein subunit (blue) labeled A. These are related by fivefold (yellow pentagons at vertices), threefold (yellow triangles in faces), and twofold (yellow ellipses at edges) symmetry elements. For a given sized subunit this point group symmetry generates the largest possible assembly (60 subunits) in which every protein lies in an identical environment. (b) A schematic representation of the subunit building block found in many RNA and some DNA viral structures. Such subunits have complementary interfacial surfaces which, when they repeatedly interact, lead to the symmetry of the icosahedron. The tertiary structure of the subunit is an eight-stranded β-barrel with the topology of the jellyroll (see c-, β-strand and helix coloring is identical to b). Subunit sizes generally range between 20 and 40 kDa with variation among different viruses occurring at the N- and C-termini and in the size of insertions between strands of the β-sheet. These insertions generally do not occur at the narrow end of the wedge (B–C, H–I, D–E, and F–G turns). (c) The topology of viral β-barrel showing the connections between strands of the sheets (represented by yellow or red arrows) and positions of the insertions between strands. The green cylinders represent helices that are usually conserved. The C–D, E–F, and G–H loops often contain large insertions.

number of folds utilized in forming viral capsids. By far the most common fold is the eight-stranded antiparallel β-sandwich shown schematically in **Figure 1**(**b**). The details of all the folds observed in nonenveloped virus capsids and their distribution are discussed elsewhere in this encyclopedia.

Early ideas explaining spherical virus architecture were extended on the basis of physical studies of small spherical RNA plant viruses. The large yields and ease of preparation made them ideal subjects for investigations requiring substantial quantities of material. Protein subunits forming virus capsids of this type are usually 20–40 kDa. An example of a virus consistent with the Crick and Watson hypothesis is satellite tobacco necrosis virus (STNV) which is formed from 60 identical 25 kDa subunits. The particle's outer radius is 80 Å and the radius of the internal cavity is 60 Å providing a volume of 9×10^5 Å^3 for packaging RNA. A single hydrated ribonucleotide in a virion will occupy on average roughly 600–700 Å^3. Thus, the STNV volume is adequate to package a genome of only 1200–1300 nt. STNV is a satellite virus and the packaged genome codes for only the coat protein. The 'helper virus', tobacco necrosis virus, supplies proteins required for RNA replication. Most simple ribovirus genomes contain coding capacity for at least two proteins; roughly, 1200 nt for the capsid protein and 2500 nt for an RNA-directed RNA polymerase. The inner radius required to package such a minimal genome is 90 Å. Consistent with this requirement were experimental studies showing that the vast majority of simple spherical viruses had outer radii of at least 125 Å, which corresponds to inner radii of roughly 100 Å for a typical shell thickness for a 400 residue subunit. Such particles had to be formed from more than 60 subunits, yet X-ray diffraction patterns of crystalline tomato bushy stunt virus (TBSV) and turnip yellow mosaic virus (TYMV) were consistent with icosahedral symmetry.

Quasi-Equivalent Virus Capsids

Although a number of investigators developed hypotheses explaining the apparent inconsistent observations, Caspar and Klug, in 1962, derived a general method for the construction of icosahedral capsids that contained multiples of 60 subunits. The method systematically enumerates all possible quasi-equivalent structures and is similar to that derived by Buckmeister Fuller to construct geodesic domes. The quasi-equivalent theory of Caspar and Klug explained the distribution of morphological units (features identifiable at low resolution by electron microscopy often corresponding to hexamer, pentamer, trimer, or dimer aggregates of the subunits) on all structures observed to date, but the results from high-resolution crystallographic studies have shown some remarkable inconsistencies with the microscopic principles upon which the theory is based.

Quasi-equivalence is best visualized graphically. Formally, subunits forming quasi-equivalent structures must be capable of assembling into both hexamers (which are conceptually viewed as planar) and pentamers (which are convex because one subunit has been removed from the planar hexamer and yet similar (quasi-equivalent) contacts are maintained). Caspar and Klug proposed that, logically, atomic interactions of fivefold-related and sixfold-related subunits could be closely similar, as only the dihedral angle between subunits would change. They argued that this difference could be accommodated by allowed variations in noncovalent bond angles between residues stabilizing the oligomers. This prediction was correct for some virus capsids, as illustrated below for cowpea chlorotic mottle virus (CCMV), but in many viruses there is an explicit molecular switch that changes the oligomeric state. If subunits assembled as all hexamers the result would be a sheet of hexamers and a closed shell could not form (**Figure 2**(**a**)). The rules of quasi-equivalence described a systematic procedure for inserting pentamers into the hexagonal net in such a way as to form a closed shell with exact icosahedral symmetry. **Figure 2** illustrates this principle and the selection rules for inserting pentamers. **Figure 3** illustrates how the morphogenesis of such an assembly may occur using the crystallographic structure of CCMV as inspiration. CCMV was the first virus structure determined that agreed in detail with the predictions of Caspar and Klug.

The quasi-equivalence theory has been universally successful in describing surface morphology of spherical viruses observed in the electron microscope and, prior to the first high-resolution crystallographic structure of a virus, it was assumed that the underlying assumptions of Caspar and Klug were essentially correct. The structure of TBSV determined at 2.9 Å resolution revealed an unexpected variation from the concept of quasi-equivalence which was defined as "any small, non random, variation in a regular bonding pattern that leads to a more stable structure than does strictly equivalent bonding." Unlike CCMV, the structure of TBSV showed that differences occurring between pentamer interactions and hexamer interactions were not small variations in bonding patterns, but almost totally different bonding patterns. **Figure 4** shows diagrammatically the subunit interactions in the shell of TBSV, SBMV, BBV, and TCV. These high-resolution structures revealed that the mathematical concept of quasi-equivalence predicted surface lattices accurately, but not for the reasons expected. Bonding contacts between quasi-threefold-related subunits are maintained with little deviation from exact symmetry while quasi-twofold contacts and icosahedral twofold contacts (which are predicted to be very similar) are quite different. The hexamer quasi-symmetry is better described as a trimer of dimers in the TBSV and related structures. Unlike the conceptual model and the CCMV capsid, the particle curvature in TBSV results from both pentamers and hexamers.

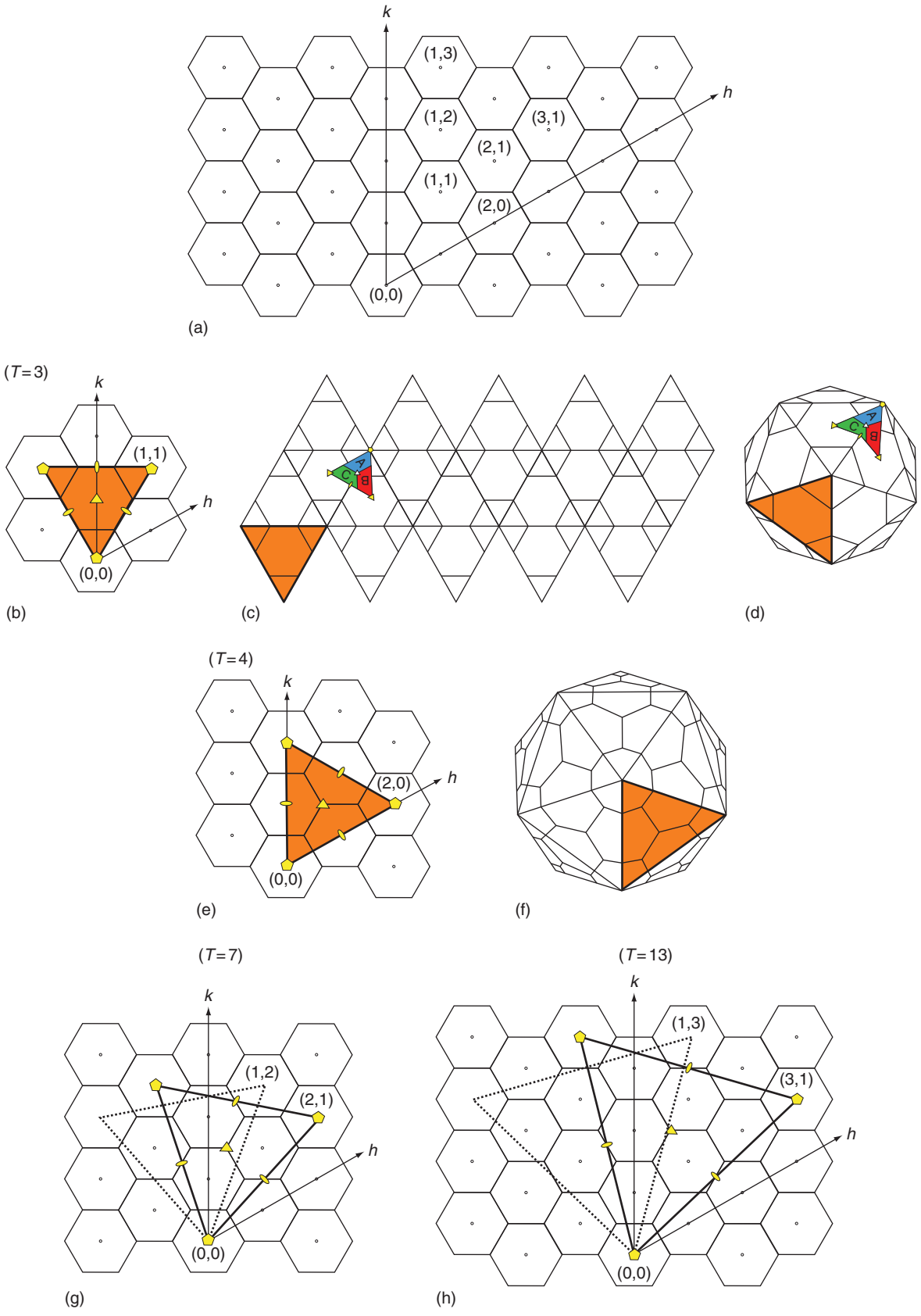


Figure 2 Continued

The high-resolution $T = 3$ structures showed that the overall features of the quasi-equivalent theory were correct, but the underlying concepts of quasi-equivalent bonding had to be revised. The first low-resolution structure (22.5 Å) of a $T = 7$ virus required an even greater conceptual adjustment to the underlying principles of quasi-equivalence. Rayment, Baker, and Caspar reported, in 1982, that the polyomavirus capsid contained 72 capsomers as previously reported from electron microscopy studies, but that all the capsomers were pentamers of protein subunits even when they were located at hexavalent lattice points. The $T = 7$ surface lattice predicts 12 pentamers and 60 hexamers; thus, the prediction of the number and position of the morphological units is correct, but the fine structure of the morphological units is incorrect. Although the result was highly controversial when first reported, additional electron microscopy studies and the 3.5 Å resolution X-ray structure of polyomavirus have fully confirmed the all-pentamer structure. This result clearly shows the limits of theory in predicting virus structure and indicates that further understanding of capsid structure will come only from experimental studies. The structure of the polyomavirus and its relatives illustrates an important concept when considering surface lattice formation. The feature of greatest importance is not the symmetry of the morphological unit positioned on the hexamer sites, but only its ability to accommodate six neighbors (it is a hexavalent position). Normally morphological units with sixfold symmetry accomplish this, but here, rather acrobatic molecular switching has permitted a pentamer of subunits to accommodate six neighbors. Although the all pentamer capsid has been observed for the $T = 7$ structure of papilloma viruses, cauliflower mosaic virus appears to have the hexamer/pentamer distribution predicted by quasi-equivalent theory as do the $T = 7$ capsids of the lambda-like bacteriophage, HK97. A substantial number of complex virus structures have been determined by cryo-electron microscopy and the surface lattices agree well with the predictions of quasi-equivalence. Thus there is considerable confidence in the lattice assignments, but the capsomer structure and therefore number of subunits must be carefully confirmed.

Figure 2 Geometric principles for generating icosahedral quasi-equivalent surface lattices. These four constructions show the relation between icosahedral symmetry axes and quasi-equivalent symmetry axes. The latter are symmetry elements that hold only in a local environment. (a) It is assumed in quasi-equivalence theory that hexamers and pentamers (a hexamer contains six units and a pentamer contains five units) can be interchanged at a particular position in the surface lattice. Hexamers are initially considered planar (an array of hexamers forms a flat sheet as shown) and pentamers are considered convex, introducing curvature in the sheet of hexamers when they are inserted. Inserting 12 pentamers at appropriate positions in the hexamer net generates the closed icosahedral shell, composed of hexamers and pentamers. The positions at which hexamers are replaced by pentamers are defined by the indices h and k measured along the labeled axes. The values of (h,k) used in the following examples are labeled. To construct a model of a particular quasi-equivalent lattice, one face of an icosahedron (equilateral triangles colored orange in (b–f)) is generated in the hexagonal net. The origin (0,0) is replaced with a pentamer, and the (h,k) hexamer is replaced by a pentamer. The third replaced hexamer is identified by threefold symmetry (i.e., complete the equilateral triangle). Each quasi-equivalent lattice is identified by a number $T = h^2 + hk + k^2$ where h and k are the indices used above. T indicates the number of quasi-equivalent units in the icosahedral asymmetric unit. For the purpose of these constructions it is convenient to choose the icosahedral asymmetric unit as 1/3 of an icosahedral face defined by the triangle connecting a threefold axis to two adjacent fivefold axes. Other asymmetric units can be chosen such as the triangle connecting two adjacent threefold axes and an adjacent fivefold axis (see (c) and **Figure 4**). The total number of units in the particle is 60T, given the symmetry of the icosahedron. The number of pentamers must be 12 and the number of hexamers is $(60T - 60)/6 = 10(T - 1)$. (b) One face of the icosahedron for a $T = 3$ surface lattice is identified by the orange triangle with the bold outline. The yellow symmetry labels are the same as those defined in **Figure 1**. The hexamer replaced has coordinates $h = 1$, $k = 1$. The icosahedral asymmetric unit is 1/3 of this face and it contains three quasi-equivalent units (two units from the hexamer coincident with the threefold axis and one unit from the pentamer). (c) Arranging 20 identical faces of the icosahedron as shown can generate the three-dimensional model of the quasi-equivalent lattice. Three quasi-equivalent units labeled A (blue), B (red), and C (green) are shown. These correspond to the three quasi-equivalent units defined in **Figures 3** and **4** rather than the alternative definition used in (a) and (b). (d) The folded icosahedron is shown with hexamers and pentamers outlined. The orange face represents the triangle originally generated from the hexagonal net. The $T = 3$ surface lattice represented in this construction has the appearance of a soccer ball. The trapezoids labeled A, B, and C identify quasi-equivalent units in one icosahedral asymmetric unit of the rhombic triacontahedron discussed in **Figure 4**. (e) An example of a $T = 4$ icosahedral face ($h = 2$, $k = 0$). In this case the hexamers are coincident with icosahedral twofold axes. (f) A folded $T = 4$ icosahedron with the orange face corresponding to the face outlined in the hexagonal net. Note that folding the $T = 4$ lattice has required that the hexamers have the curvature of the icosahedral edges. (g) A single icosahedral face generated from the hexagonal net for a $T = 7$ lattice. Note that there are two different $T = 7$ lattices ($h = 2$, $k = 1$ in bold outline; and $h = 1$, $k = 2$ in dashed outline). These lattices are the mirror images of each other. To fully define such a lattice, the arrangement of hexamers and pentamers must be established as well as the enantiomorph of the lattice. (h) A single icosahedral face for a T=13 lattice is shown. The two enantiomorphs of the quasi-equivalent lattice ($h = 3$, $k = 1$ – bold; and $h = 1$, $k = 3$ – dashed) are outlined. The procedure for generating quasi-equivalent models described here does not exactly correspond to the one described by Caspar and Klug in 1962. Caspar and Klug distinguish between different icosadeltahedra by a number $P = h^2 + hk + k^2$ where h and k are integers that contain no common factors but 1. The deltahedra are triangulated to different degrees described by an integer f that can take on any value. In their definition $T = Pf^2$. The description in this figure has no restrictions on common factors between h and k; thus, $T = h^2 + hk + k^2$ for all positive integers. The final models are identical to those described by Caspar and Klug.

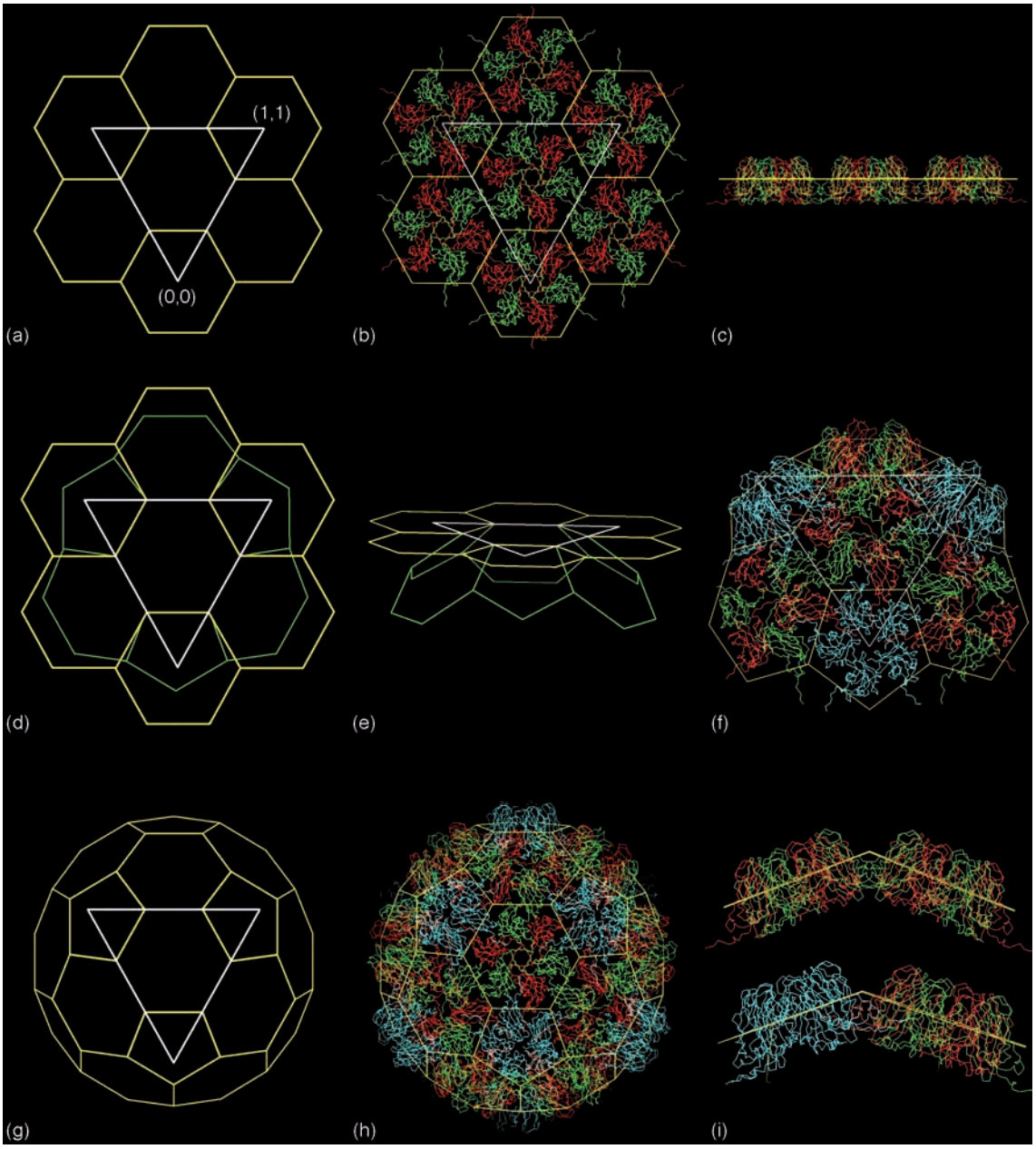


Figure 3 Molecular graphics construction of a $T = 3$ quasi-equivalent icosahedron. (a) Hexagonal sheet overlaid with the triangular coordinates (white) for a theoretical $T = 3$ quasi-equivalent icosahedron ($h = 1$, $k = 1$, see **Figure 2**(**b**)). The sheet has true sixfold rotational symmetry about axes passing through the hexamer centers, which are normal to the sheet. (b) Copies of the hexamer coordinates from the CCMV X-ray structure (colored by asymmetric unit position, see **Figures 2**(**c**)–**2**(**d**) and **4**) can be positioned in the sheet by simple translations. (c) A side view of the modeled sheet demonstrates its planarity. (d) Hexamers at the corners of the white ($h = 1$, $k = 1$) triangle become pentamers. The planar sheet (yellow model) takes on curvature to maintain contacts between the polygons (green model). (e) The magnitude of the pentamer-induced curvature is displayed in the side view of the partial polyhedron. (f) Coordinates of the CCMV X-ray structure fit this construction without any manipulation. (g) A completed $T = 3$ icosahedral model. The 12 pentamers generate curvature that closes the structure. This cage (a truncated icosahedron) accurately describes the geometric morphology of CCMV (h) which is composed of modular, planar, pentamers (12) and hexamers (20). Angular pentamer–hexamer and hexamer–hexamer interfaces (i) stabilize curvature in the absence of convex pentamers used to construct the soccer ball of **Figure 3**(**d**) (see also **Figure 4**).

Picorna-Like Virus Capsids

A number of viral capsids are constructed with pseudo $T = 3$ symmetry. These structures contain β-barrel subunits (**Figure 1**(**b**)) in the quasi-equivalent environments formed in $T = 3$ structures, but each of the three β-barrels in the asymmetric unit has a unique amino acid sequence. Rather than 180 identical subunits, the $P = 3$ particles contain 60 copies each of three different subunits (**Figure 5**). These structures do not require quasi-equivalent bonding

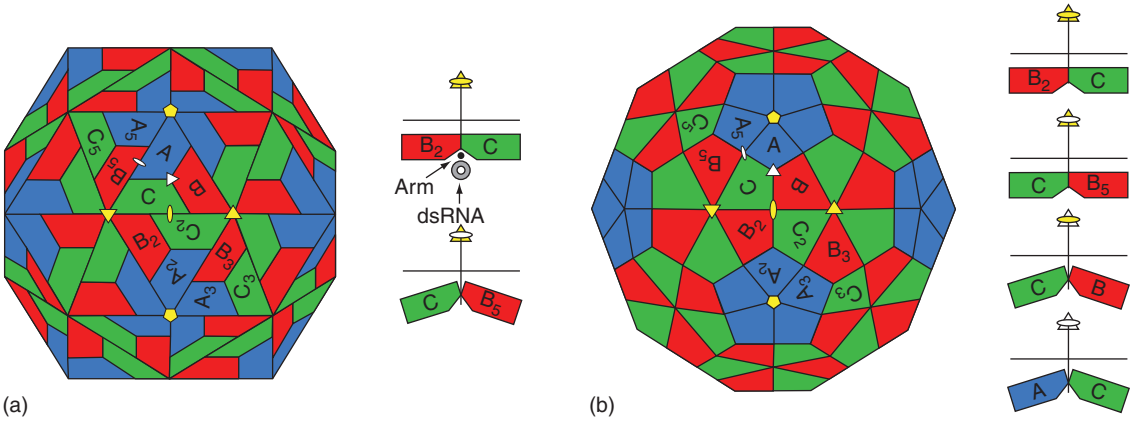


Figure 4 Although quasi-equivalence theory can predict, on geometrical principles, the organization of hexamers and pentamers in a viral capsid, the detailed arrangement of subunits can only be established empirically. High-resolution X-ray structures of $T = 3$ plant and insect viruses show that the particles are organized like the icosahedral rhombic triacontahedron or truncated icosahedron. A convenient definition of the icosahedral asymmetric unit for both geometrical shapes is the wedge defined by icosahedral threefold axes left and right of the particle center and an icosahedral fivefold axis at the top. The icosahedral asymmetric unit contains three subunits labeled A (blue), B (red), and C (green) (see **Figures 2(c)–2(d)**). The asymmetric unit polygons represent chemically identical protein subunits that occupy slightly different geometrical (chemical) environments as indicated by differences in their coloring. Polygons with subscripts are related to A, B, and C by icosahedral symmetry (i.e., A to A_5 by fivefold rotation). The shapes of the $T = 3$ soccer ball model in **Figure 2(d)**, truncated icosahedron in **Figure 3**, and rhombic triacontahedron are all different; however, the quasi-symmetric axes are in the same positions relative to the icosahedral symmetry axes for all three models. Quasi-threefold and quasi-twofold axes are represented by the white symbols. The quasi-sixfold axes are coincident with the icosahedral threefold axes in $T = 3$ particles as shown in **Figures 2(b)–2(d)** and **3**. (a) The rhombic triacontahedron is constructed by placing rhombic faces perpendicular to icosahedral twofold symmetry axes (yellow ellipse). Thus, the A, B, and C polygons are co-planar within each asymmetric unit. The shape of the subunit in $T = 3$ plant and insect viruses is nearly identical to the shape of the subunit in the $T = 1$ virus and they pack in a very similar fashion. The $T = 1$ subunits in one face (**Figure 1(a)**) are related by an icosahedral threefold axis while the $T = 3$ subunits in one face are related by a quasi-threefold axis. The dihedral angle between subunits C and B_5 (juxtaposed across quasi-twofold axes) is 144° and is referred to as a bent contact (bottom right image), while the dihedral angle between subunits C and B_2 (juxtaposed across icosahedral twofold axes) is 180° and is referred to as a flat contact (top-right image). Two dramatically different contacts between subunits with identical amino acid sequences are generated by the insertion of an extra polypeptide from the N-terminal portion of the A or C subunits into the groove formed at the flat contact. This polypeptide is called an 'arm'. The flat contact can also be upheld by insertion of nucleic acid structure into the same groove. Neither N-terminal arms nor nucleic acid structure has been observed in the groove across the quasi-twofold axis; thus, C and B_5 are in direct contact as in, for example, the X-ray structure of flock house virus (FHV). (b) A truncated icosahedron achieves curvature at different interfaces compared to the rhombic triacontahedron. Interactions between B_2–C and between C–B_5 polygons are both defined by 180° dihedral angles (side view at top right), whereas bends similar in magnitude occur within the asymmetric unit at the B–C and C–A polygon interfaces (138° and 142°, respectively; side view at bottom right). This creates the planar pentamer and hexamer morphological units characteristic of the truncated icosahedron and the CCMV X-ray structure (**Figure 3(h)**).

because each unique interface will have different amino acids interacting, rather than the same subunits forming different contacts. The animal picornaviruses have capsids of this type. Animal virus capsids undergo rapid mutation which avoids recognition by the circulating immune system. Capsids composed of three subunit types could mutate in one subunit without affecting the other two. This would be less likely to affect assembly or other functions of the particle in $P = 3$ shells than it would in $T = 3$ shells. At least one plant virus group displays $P = 3$ shells, the comoviruses. An interesting variation occurs in these capsids when compared with the picornaviruses. Two of the domains forming the shell are contained in a single polypeptide chain. This phenomenon is readily understood in the context of the synthesis of the subunits in picorna and comoviruses. In both cases the proteins are synthesized as a polyprotein that is subsequently cleaved into functional proteins by a virally encoded protease. Clearly one of the cleavage sites in picornaviruses is missing in the comoviruses, resulting in these two domains being still a 'polyprotein'. Tobacco ringspot virus (TRV) is an example where all three subunits are linked in a single polypeptide chain like 'beads on a string'. It is interesting to note that the X-ray structure of TRV showed that the linkage of subunits in the capsid is the same as that predicted for the precursor polyprotein of picornaviruses based on the their X-ray structures, supporting the divergent evolution of the $P = 3$ capsids from plant viruses to animal viruses.

Virus Particle Maturation

While $T = 3$ and $P = 3$ animal viruses undergo maturation processes that confer infectivity, there is little change in their particle morphology during this process.

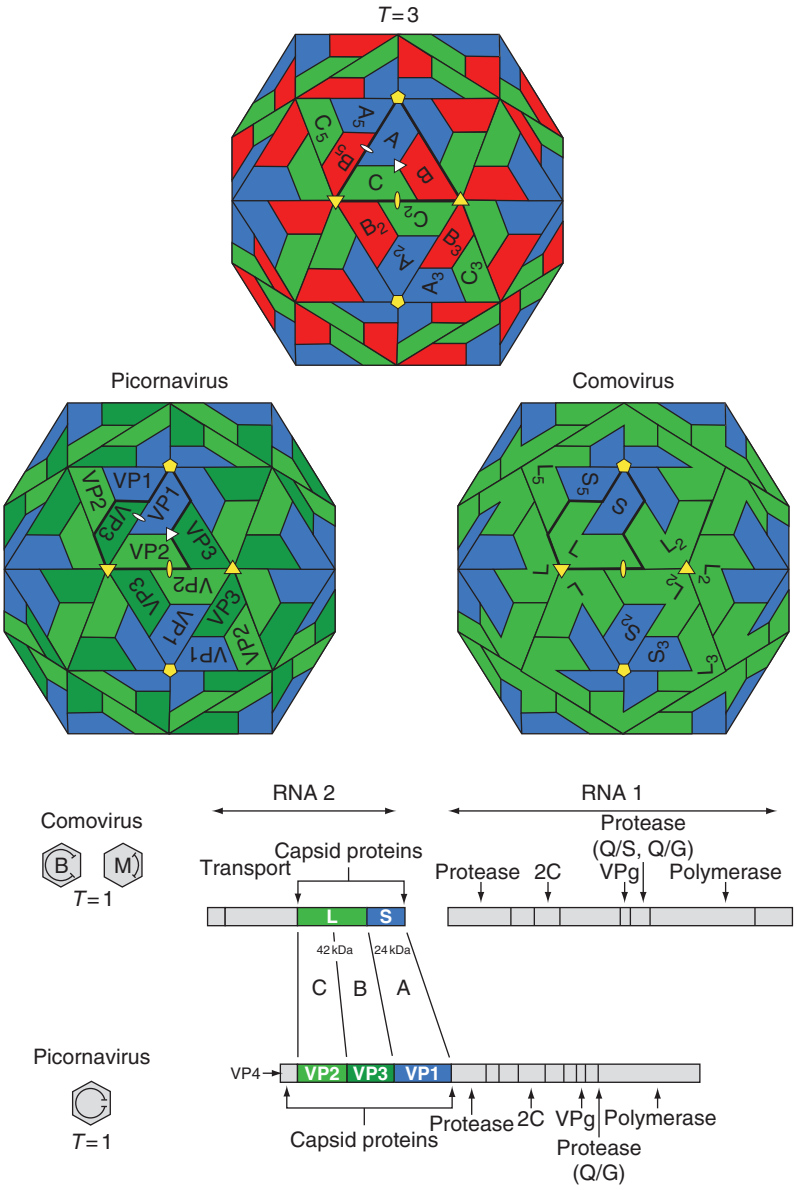


Figure 5 A comparison of $T = 3$, picornavirus, and comovirus capsids. In each case, one trapezoid represents a β-barrel and the icosahedral asymmetric units are outlined in bold. The icosahedral asymmetric unit of the $T = 3$ shell contains three identical subunits labeled A, B, and C (see **Figure 4**). The asymmetric unit of the picornavirus capsid contains three β-barrels, but each has a characteristic amino acid sequence labeled VP1, VP2, and VP3. The comovirus capsid is similar to the picornavirus capsid except that two of the β-barrels (corresponding to the green VP2 and VP3 units) are covalently linked to form a single polypeptide, the large protein subunit (L), while the small protein subunit (S) corresponds to VP1 (note the similar color shading). The individual subunits of the comovirus and picornavirus capsids are in identical geometrical (chemical) environments (e.g., VP1 and S are always pentamers) technically making these $T = 1$ capsids, but they are often referred to as pseudo $T = 3$, or $P = 3$ capsids. Comoviruses and picornaviruses have a similar gene order, and the nonstructural 2C and polymerase genes display significant sequence homology. The relationship between the capsid subunit positions in these viruses and their location in the genes is indicated by color-coding and the labels A, B, and C in the gene diagram.

In contrast, bacteriophages, such as HK97, display dramatic subunit reorganization during maturation. (The head (capsid) of the HK97 bacteriophage first assembles as a metastable prohead (prohead I and II), and then undergoes a complex maturation process that involves large-scale macromolecular transitions that expand the protein shell. There are three well-defined expansion intermediates (EI-I/II, EI-III, EI-IV) that end with the EI-IV state, which is the final step before head maturation is complete. The crosslinks between the 420 subunits are formed during these steps, except for the last 60 situated around the 12 pentamers. Formation of the final 60 crosslinks is the final step that creates head II, the mature capsid.) The X-ray structure of mature HK97 showed that each subunit in the $T = 7l$ capsid makes contact with nine other subunits, displaying molecular

promiscuity at an unprecedented level. These interactions, together with covalent cross-links between subunits create an exceptionally stable particle. It is clear, however, that such a structure cannot be achieved in a single step. It is too complicated. The solution is to assemble in stages. The initial assembly product is called a procapsid. This is formed by three gene products: 415 copies of the capsid protein, 12 copies of the portal subunit, and about 60 copies of a virally encoded protease that is inside the 500 Å diameter particle. This is an equilibrium assembly that is similar to the assembly of simpler viruses. Subunits are confined to discrete volumes and contact only immediate neighbors, again, as observed with simple viruses. Completion of assembly signals protease activation, and 103 residues are cut from each capsid protein and the protease auto-digests, leaving an empty particle that is a substrate for DNA packaging enzymes. When the terminase complex, also virally encoded, attaches to the double-stranded DNA viral genome and the portal, DNA packaging ensues and after ~10% of the DNA is packaged, the particle undergoes an extraordinary reorganization involving 40° rotations of subunits and 40 Å translations, totally remodeling the contacts resulting in the highly interlocked structure in the mature virion. Maturation of this type is entirely programmed into the original tertiary and quaternary structures and is an excellent example of a molecular machine.

Concluding Remarks

This overview of virus structure and assembly illustrates the remarkable level of sophistication associated with one or a few gene products acting in concert. The requirement for maximum utility from minimal genetic information has led to the evolution of proteins with exceptionally high functional density that are studied to develop better understanding of virus-associated disease and as accessible paradigms for cellular function.

See also: Capsid Assembly: Bacterial Virus Structure and Assembly; Tobacco Mosaic Virus; Virus Particle Structure: Nonenveloped Viruses.

Further Reading

Caspar DLD (1980) Movement and self-control in protein assemblies. Quasi-equivalence revisited. *Biophysics Journal* 32: 103–138.

Caspar DLD and Klug A (1962) Physical principles in the construction of regular viruses. *Cold Spring Harbor Symposia on Quantitative Biology* 27: 1–24.

Crick FHC and Watson JD (1956) Structure of small viruses. *Nature* 177: 473–475.

Johnson JE and Speir JA (1997) Quasi-equivalent viruses: A paradigm for protein assemblies. *Journal of Molecular Biology* 269: 665–675.

Natarajan P, Lander GC, Shepherd CM, Reddy VS, Brooks CL, III, and Johnson JE (2005) Exploring icosahedral virus structure with VIPER. *Nature Reviews Microbiology* 3: 809–817.

Speir JA, Bothner B, Qu C, Willits DA, Young MJ, and Johnson JE (2006) Enhanced local symmetry interactions globally stabilize a mutant virus capsid that maintains infectivity and capsid dynamics. *Journal of Virology* 80: 3582–3591.

Tang L, Johnson KN, Ball LA, Lin T, Yeager M, and Johnson JE (2001) The structure of pariacoto virus reveals a dodecahedral cage of duplex RNA. *Nature Structural Biology* 8: 77–83.

Zandi R, Reguera D, Bruinsma RF, Gelbart WM, Rudnick J, and Reiss H (2001) Origin of icosahedral symmetry in viruses. *Proceedings of the National Academy of Sciences, USA* 101: 15556–15560.

Zlotnick A (2005) Theoretical aspects of virus capsid assembly. *Journal of Molecular Recognition* 18: 479–490.

Virus Species

M H V Van Regenmortel, CNRS, Illkirch, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Ecological niche The ecological niche of a virus refers to certain biological properties of the virus, such as host range, tissue tropism in the host, and type of vector, rather than a geographical space or a particular environment. In the absence of the virus, its ecological niche property is also absent and the notion of a vacant niche is thus meaningless.

Polythetic class A polythetic class consists of members that exhibit overall similarity and have a large number of common properties. However, the members of a polythetic class do not all share a common character that constitutes a single defining property of the class. In contrast, the members of a monothetic class, such as those of the order *Mononegavirales*, possess such a single defining property, that is, the presence of a negative-sense, single-stranded RNA (ssRNA) genome.

Introduction

The question of what is a virus species is related to the general problem of how the world of viruses should be partitioned and ordered for achieving a coherent scheme of distinct and easily recognizable viral entities. In view of the variability of viruses arising from the error-prone process of genomic replication, it is often difficult to decide whether a newly encountered virus is the same as one seen previously, for this requires that we give an answer to the vexing question: "How different must two virus isolates be in order to be considered different viruses, rather than the same virus?" Virologists usually have no difficulty in distinguishing pathogenic variants of a virus, while still recognizing these variants as the same kind of virus. Even without realizing it, they pass judgment on the significance of the observed differences between individual virus isolates and, if the extent of difference appears small enough, they will consider the variant to be the same virus. In taxonomic parlance, they will say that the variant belongs to the same virus species.

'Species' is the universally accepted term for the lowest taxonomic clustering of living organisms. Although viruses are not organisms, they are considered to be biological entities because they possess some of the properties of biological agents, such as having a genome and being able to adapt to particular hosts and changing environments. The classification system of viruses therefore uses the same hierarchical ranks of order, family, genus, and species used in all biological classifications.

Although the species category is the most fundamental one in any biological classification, it took many years before virologists started to assign viruses to separate species. Virus genera and families were readily accepted but there was considerable opposition to the idea that the species concept could be applied to viruses. One reason for this reluctance was the lack of agreement among biologists about what a species actually is. It is in fact remarkable that a century and a half after the appearance of Charles Darwin's *The Origin of Species*, there is still no general agreement about what constitutes a plant, animal, or microbial species. In different branches of biology, no less than 22 different species concepts have been applied.

Distinguishing between Abstract Classes and Real Objects

A basic requirement for clear thinking is the ability to distinguish between real, tangible objects, such as viruses, and the abstract classes used in taxonomy, such as families and species, which are mental constructs that exist only in the minds of people who think about them. Although a taxonomic class is defined by properties possessed by concrete objects, it is only an abstract thought that has no real existence outside of human minds. For this reason, viral species, genera, and families cannot cause diseases and cannot be purified, sequenced, or seen through electron microscopes. Only the viruses themselves can be studied in this manner. Classes used in biological classification have a hierarchical structure. A class, such as a particular species, can belong only to one higher rank immediately above it such as a particular genus and that genus, in turn, can only belong to one family. The relationship known as 'class membership' is the relationship between an abstract class or thought and the members of that class which are real, concrete objects located in space and time. Class membership is thus able to bridge two different logical categories, the abstract and the concrete. It is important to be able to establish a certain link between these two logical categories, as it is impossible for an object such as a virus to be 'part' of an abstraction, such as a species. Similarly, an abstract thought cannot be 'part of' a concrete object. The correct statement is to say that the viruses that are handled by virologists are members of certain abstract constructs, such as species or genera. These constructs are invented by taxonomists for the purpose of introducing some order to the bewildering variety of viruses.

A universal class, also known as an Aristotelian class, is defined by properties that are constant and immutable. This allows members of such a class to be recognized with absolute certainty, because one or more property is necessarily present in every member of the class. Virus families, for instance, are universal classes because they consist of members, all of which share a number of defining properties that are both necessary and sufficient for class membership. Allocating a virus to a family is thus an easy task, because a few structural or chemical attributes will suffice to allocate the virus to a particular family. For instance, all the members of the family *Adenoviridae* are nonenveloped viruses that have an icosahedral particle and double-stranded DNA, with projecting fibers at the vertices of the protein shell.

Unfortunately, not all properties of members of classes correspond to unambiguous and stable properties such as the presence or absence of a DNA genome or of a particular particle morphology. Many qualitative properties of concrete objects are inherently vague and do not possess precise borderlines. For instance, in the description of viruses, properties used for recognizing members of species, such as the degree of genome sequence similarity between virus isolates or the nature of the symptoms induced by a virus in its host, whether mild or severe, tend to be inherently imprecise and fuzzy. As a result the species classes that can be conceptualized on the basis of such properties will themselves be fuzzy and membership in the class will then be a matter of convention or stipulation. This explains some of the difficulties one encounters when dealing with the species level in any biological classification.

When fuzziness is accepted as an unavoidable characteristic of species, it becomes possible to describe species in terms of continuums with hazy boundaries. In a similar way, colors can be distinguished conceptually in spite of the continuous nature of the spectrum of electromagnetic waves, and mountain peaks are given names in spite of the absence of sharp boundaries in geological rock formations.

Species in Biology

The traditional view of species is that they correspond to groups of similar organisms that can breed among themselves and produce fertile offspring. The classical definition of so-called 'biological species' states that "species are groups of interbreeding natural populations which are reproductively isolated from other such groups." The reproductive isolation often simply reflects a geographic isolation or a behavioral incompatibility. This definition is only applicable to organisms that reproduce sexually and it has limited value in the plant kingdom because of the high frequency of hybridization between different species of plants. Even in animals, the criterion of reproductive isolation does not always hold, as shown by the ability of dogs, wolves, and coyotes to interbreed although they are members of different species of the same genus.

In order to make the definition of biological species applicable to asexual organisms, it was later modified as follows: "a species is a reproductive community of populations, reproductively isolated from others, that occupies a specific niche in nature." Some authors reject the view that asexual organisms can form biological species but most biologists disagree with that view, as this would render the species concept inapplicable to a large portion of the biological realm.

Another species concept is that of evolutionary species, which has been defined as "a single lineage of ancestor–descendant populations which maintains its identity from other such lineages and which has its own evolutionary tendencies and historical fate." Such a concept does not provide any guidance on how far back in time a species can be traced. Life on Earth is a biological and historical continuum and it is as difficult to demarcate boundaries in time that would separate individual evolutionary species as it is to define clear-cut breeding discontinuities that would separate different biological species. These difficulties led to the view that species are similar to fuzzy sets with unclear boundaries and that it is impossible to draw sharp boundaries between them, as is done with universal classes such as genera and families. In practice this means that it is not possible to rely on a single defining property for differentiating between two species. This led to the proposal that species are so-called 'polythetic classes' defined by a combination of properties, each of which might occur outside any given class yet could be absent in a member of the particular class (**Figure 1**).

The members of a polythetic class exhibit overall similarity and have a large number of characters in common. However, in contrast to the members of a monothetic or universal class, they need not all share a common character that could be used as a defining property of the class. A polythetic class is actually a cluster concept based on the concept of family resemblance introduced in philosophy by Ludwig Wittgenstein.

What Is a Virus Species?

In the past, many virologists were opposed to the introduction of species in virus classification because they assumed that the only legitimate species concept was that of biological species defined by sexual reproduction, gene pools, and reproductive isolation. Such a concept is obviously not applicable to viruses that are replicated as clones. Another reason for the reluctance to adopt the species category in virus classification was the absence of a satisfactory definition of a virus species. Various definitions of virus species had been proposed over the years but none gained general acceptance. One definition, for instance, stated that "virus species are strains whose properties are so similar that there seems little value in giving them separate names." Such a definition is not very helpful as it simply replaces undefined species by undefined strains and suggests that attributing names to viruses is the same activity as constructing a taxonomy.

In 1991, the International Committee on Taxonomy of Viruses (ICTV), which is the body established by the International Union of Microbiological Societies to make decisions on matters of virus classification and

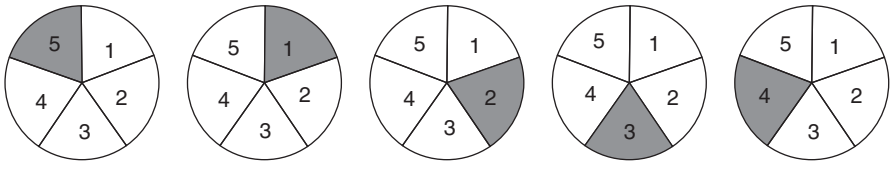


Figure 1 Schematic representation of the distribution of properties (1 to 5) in five members of a polythetic class. Each member possesses several of these properties (4 out of 5) but no single property is present in all the members of the class. The missing property in each case is represented by the gray section.

nomenclature, endorsed the following definition which was proposed by the author: "a virus species is a polythetic class of viruses that constitute a replicating lineage and occupy a particular ecological niche." This definition is not only based on phenetic similarities among members of species but also stresses the internal cohesion present in biological lineages that share a common biotic niche. Another important aspect of the definition is that a virus species is defined as a polythetic class rather than as a traditional universal class (see **Figure 1**). The ICTV has been using the concept of polythetic class for the creation of separate species taxa.

The total DNA or RNA sequence found in a virion corresponds to the viral genome and is part of the viral phenotype because it corresponds to a portion of the virion's chemical structure. The phenome of the virus corresponds to its observable physical properties, including the morphology and molecular constitution of the virion, as well as the biochemical activities and relational properties of the virus. The phenotype has a temporal dimension as it changes over time, for instance during replication.

A classification based on genome sequences is actually a phenotypic classification with the characters being molecular, rather than morphological or relational. Although putative phylogenetic relationships can be inferred from sequences, they remain tentative and depend on many tacit assumptions. There is little justification for the common assumption that when species are demarcated on the basis of a proposed phylogeny, this produces a classification that is necessarily more correct or useful than a classification based on other phenotypic characters.

Defining Species as a Taxonomic Class Is Not the Same As Demarcating Individual Viral Species

As a definition is the explanation of the meaning of a word or concept, it is possible to give a definition of the concept species. In contrast, it is not possible to give a definition of an object such as a particular virus, as objects can only be described by enumerating their properties and cannot be 'defined'. When viruses or any other object are given proper names, their names do not have a meaning that can be captured in a definition. When we define the name of an abstract species class by means of defining properties, we state the conditions for that name to apply to any given member of the species.

Two types of species definitions must be distinguished. One definition concerns the taxonomic species category corresponding to the lowest level in a hierarchical system of classification. The definition of virus species accepted by the ICTV is a definition of that type and it was introduced because many virologists thought that the species concept was not applicable to viruses. However, such a definition of the category species should not be confused with the definitions or demarcations of each of the 1950 species classes listed in the Eighth ICTV Report published in 2005. Confusing the two meanings of species, that is, as a particular class which has certain viruses as members and as a taxonomic category, respectively, would amount to confuse the concept of chemical element (the class of all elements) with the class corresponding to a single element, such as gold defined by its atomic number 79.

It must be emphasized that once the taxonomic category of virus species had been accepted by virologists, this did not do away with the difficult task of demarcating and defining the hundreds of individual species classes that had to be created on the basis of different combinations of properties for each polythetic species class. In order to demarcate individual species, it was necessary to rely on properties that were not present in all the members of the genus to which the species belongs, because such properties obviously would not permit individual species to be differentiated. Characteristics such as virion morphology, genome organization, method of replication, and number and size of viral proteins are shared by all the members of a genus and are thus not useful for distinguishing individual species in a genus. The properties that are useful for discriminating between individual species within a genus are the natural host range, cell and tissue tropism, pathogenicity, mode of transmission, certain physicochemical and antigenic properties of virions, and small differences in genome sequence. Unfortunately, these properties can be altered by only one or a few mutations and they may therefore vary in different members of the same species. This is one of the reasons species demarcation is not an easy task and often requires drawing boundaries across a continuous range of genomic and phenotypic variability. Taxonomic decisions at the level of species are very much a matter of opinion and adjudication rather than of logical necessity. There is indeed no precise degree of genome difference that could be used as a cutoff point to differentiate between two species nor is there a simple quantitative relationship between the extent of genome similarity and the similarity in phenotypic and biological characteristics of a virus.

There is also a strong subjective element in virus classification, as the practical need to distinguish between individual viruses is not the same in all areas of virology. From a human perspective, not all infected hosts are equally relevant. Human pathogens or pathogens that infect animals and plants of economic importance tend to be studied more intensively than the very many viruses that infect insects or marine organisms. As a result fine distinctions based on minor differences in host range or pathogenicity may be emphasized in the case of viruses of particular interest to humans and the criteria used for differentiating individual species may thus depend on

the type of host that is infected. There is, of course, no reason the criteria used for distinguishing between species should be the same in all the virus genera and families, because the need to make certain distinctions are not the same in all fields of virology.

Virus Identification

In order to identify a virus as a member of a species, use is made of so-called diagnostic properties that allow the virus to be recognized as being similar to other viruses. This, of course, means that the species had to be created and defined by taxonomists beforehand. Only when the properties of many members of an established species are compared, is it possible to discover which diagnostic property or set of properties will discriminate between the members of that species and other species, thereby allowing viruses to be identified. A single diagnostic character may sometimes suffice for virus identification but this character should not be confused with the collective set of combined properties which has been used initially to demarcate and define the species as a polythetic class. Species are not defined by means of one or more diagnostic properties used for virus identification but are defined by taxonomists, who stipulate which covariant sets of shared properties have to be present in most members of the species. It is the frequent, combined occurrence of these properties in individual members of a species that allows one to predict many of the properties of a newly discovered virus once it has been identified as a member of a particular species. If a species could be defined monothetically instead of polythetically, that is, by a single diagnostic character and nothing else, the identification of a virus as a member of a species would not be very informative. A single diagnostic character, such as a nucleotide motif or the reactivity with a monoclonal antibody, may suffice to identify a virus but such a property should not be mistaken for a single defining characteristic of the species.

This apparent contradiction between the need for many characters to define and delineate a species and the fact that a single property may suffice to identify a virus disappears when it is realized that defining an abstraction, such as a species, is a task different from identifying a concrete object, such as a virus. Providing a definition of an abstract taxonomic class and identifying concrete objects are not equivalent tasks and they abide by different logical rules.

Species Names and How to Write Them

After the category species was accepted as the lowest taxonomic class to be used in viral taxonomy, the ICTV decided in 1998 that the existing English common names of viruses were to become the official species names and that, to denote the difference, species names should be written in italics with a capital initial letter. This typography, which is similar to that used for other taxa such as virus genera and families, makes it possible to distinguish virus species officially recognized by the ICTV and written in italics from other viral entities such as tentative species and virus strains written in Roman characters.

When ICTV introduced italicized virus species names, it did not intend that the existing names of viruses written in Roman characters should be abandoned. In their writings, virologists need to refer continuously to the viruses they study and because these viruses are not taxonomic abstractions, their names should be written in Roman characters.

In most cases it is necessary only once in a scientific paper to draw attention to the taxonomic position of the virus and this can be done by mentioning that the virus under study, for example, measles virus, is a member of the species *Measles virus*, genus *Morbillivirus*, and family *Paramyxoviridae*. In publications written in languages other than English, the common names of the virus remain those used in that language. It will suffice, for instance, to say once: "le virus de la rougeole est un membre de l'espèce *Measles virus*."

It is correct to say that a virus, a strain, or an isolate of the species *Cucumber mosaic virus* has been sequenced but incorrect to say the species itself has been sequenced. Unfortunately, some authors confuse viruses with species and write that the species *Cucumber mosaic virus* (instead of the virus cucumber mosaic virus) has been isolated from a tobacco plant, is transmitted by an aphid vector, is the causal agent of a mosaic disease, and has been sequenced. This is of course incorrect because virus species are man-made taxonomic classes and do not have hosts, vectors, or sequences. For the same reason, the genus *Cucumovirus* or the family *Paramyxoviridae* cannot be isolated, visualized in an electron microscope, or sequenced.

At a time when English is more widely understood than Latin, the use of italicized English instead of italicized Latin for the names of virus species is more practical and is in line with the emergence of English as the modern language of international scientific communication. Most virologists are opposed to the Latinization of species names and welcomed the introduction of well-known English species names. They certainly preferred this to the creation of an entirely new set of Latin names for the 1950 different virus species that are recognized at present.

Binomial Names for Virus Species

A problem with the current way of naming virus species is that the name of the species (for instance *Measles virus*) differs only in typography from the name of the virus (measles virus). One way to facilitate the distinction

between virus names and species names would be to change the current names of virus species into nonlatinized binomial names. Such a system, which has been advocated by plant virologists for many years, consists in replacing the word '*virus*' appearing at the end of the existing species name by the genus name which also ends in '-*virus*'. *Measles virus* then becomes *Measles morbillivirus*, *Hepatitis A virus* becomes *Hepatitis A hepatovirus,* and *Tobacco mosaic virus* becomes *Tobacco mosaic tobamovirus*. The advantage of such a system, which could be implemented without problems for about 98% of all virus species names, is that inclusion of the genus name in the species name provides additional information about the properties of the virus. A changeover to binomial species names would not affect the common names of viruses in English or other languages since names such as measles virus or 'virus de la rougeole' would remain the same. The ICTV is currently debating the possibility of introducing binomial species names and some decision is likely to be made in the near future.

Given that the common names of viruses are used repeatedly in scientific texts there is a need for abbreviating them and the ICTV has published several lists of recommended acronyms for virus names. Since the names of virus species are used only very seldom in publications, there is no need to abbreviate them. If binomial names of virus species were introduced in the future, the abbreviations of common names of viruses will of course not be affected.

See also: Nature of Viruses; Phylogeny of Viruses; Quasispecies; Taxonomy, Classification and Nomenclature of Viruses.

Further Reading

Calisher CH and Mahy BWJ (2003) Taxonomy: Get it right or leave it alone. *American Journal of Tropical Medicine and Hygiene* 68: 505–506.

Claridge MF, Duwah HA, and Wilson MR (eds.) (1997) *Species: The Units of Biodiversity.* London: Chapman and Hall.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy, Classification and Nomenclature of Viruses: Eighth Report of the International Committee on Taxonomy of Viruses*, San Diego, CA: Elsevier Academic Press.

Lewontin RC (1992) Genotype and phenotype. In: Keller EF and Lloyd EA (eds.) *Keywords in Evolutionary Biology*, pp. 137–144. Cambridge, MA: Harvard University Press.

Pigliucci M (2003) Species as family resemblance concepts: The (dis-)solution of the species problem? *Bioessays* 25: 596–602.

Van Regenmortel MHV (1990) Virus species, a much overlooked but essential concept in virus classification. *Intervirology* 31: 241–254.

Van Regenmortel MHV (2003) Viruses are real, virus species are man-made taxonomic constructions. *Archives of Virology* 148: 2481–2488.

Van Regenmortal MHV (2006) Virologists, taxonomy and the demands of logic. *Archives of Virology* 151: 1251–1255.

Van Regenmortel MHV (2007) Virus species and virus identification: Past and current controversies. *Infection, Genetics and Evolution* 7: 133–144.

Van Regenmortel MHV and Mahy BWJ (2004) Emerging issues in virus taxonomy. *Emerging Infectious Diseases* 10: 8–13.

Viruses and Bioterrorism

R F Meyer and S A Morse, Centers for Disease Control and Prevention, Atlanta, GA, USA

Published by Elsevier Ltd.

Introduction

Man has known that biological organisms and toxins were useful as weapons of war long before the germ theory of disease was understood. However, as the twentieth century came to a close, the perceived difficulties in production, weaponization, and deployment of these biological weapons as well as a belief that moral restraints would preclude the use of these weapons gave many a false sense of security. Recently, a number of events have served to focus attention on the threat of terrorism and the potential for the use of biological, chemical, or nuclear weapons against the military, civilian populations, or agriculture for the purpose of causing illness, death, or economic loss. This possibility became a reality in October 2001 when someone sent spores of *Bacillus anthracis* to media companies in New York City and Boca Raton, Florida, resulting in five deaths, considerable panic throughout the United States and other countries, and raised the awareness of our vulnerability.

There are more than 1400 species of infectious organisms that are known to be pathogenic for humans; many additional organisms are capable of causing disease in animals or plants. Realistically, only a few of these infectious agents pose serious problems or are capable of affecting human, animal, or plant health on a large scale. Even fewer of these agents are viruses. Viruses that could be used as weapons against humans, animals, or plants generally possess traits including ease of production and dissemination, transmissibility, environmental stability, and high morbidity and mortality rates.

Definitions

The use of biological agents is often characterized by the manner in which they are used. For the purposes of this article 'biological warfare' is defined as a special type of warfare conducted by a government against a target; 'bioterrorism' is defined as the threat or use of a biological agent (or toxin) against humans, animals, or plants by individuals or groups motivated by political, religious, ecological, or other ideological objectives. Furthermore, terrorists can be distinguished from other types of criminals by their motivation and objective; however, criminals may also be driven by psychological pathologies and may use biological agents. When criminals use biological agents for murder, extortion, or revenge, it is called a 'biocrime'.

Historical Perspective

The use of viral agents for biological warfare has a long history, which predates their recognition and isolation by culture. Their early use is consistent with what, at the time, was known about infectious diseases, particularly smallpox. In the sixteenth century, the Spanish explorer, Francisco Pizarro, presented the indigenous peoples of South America with variola-contaminated clothing, which resulted in widespread epidemics of smallpox. During the French and Indian War (1745–67), Sir Jeffrey Amherst, commander of the British forces in North America, suggested the deliberate use of smallpox to 'reduce' Native American tribes hostile to the British. Captain Ecuyer (one of Amherst's subordinates), fearing an attack on Ft. Pitt from Native Americans, acquired two variola-contaminated blankets and a handkerchief from a smallpox hospital and, in a gesture of good will, distributed them to the Native Americans. As a result, several outbreaks of smallpox occurred in various tribes in the Ohio River valley. In 1775, during the Revolutionary War, the British attempted to spread smallpox among the Continental forces by inoculating (variolation) civilians fleeing Boston. In the South, there is evidence that the British were going to distribute slaves who had escaped during hostilities, and were sick with smallpox, back to the rebel plantations in order to spread the disease.

The use of viruses other than Variola major is a more recent phenomenon and reflects an increased knowledge of how to grow and stabilize viruses for delivery purposes. Allegations have been made by the government of Cuba that the CIA was responsible for the massive outbreaks of swine fever in 1971 and dengue fever in 1980 that ravaged the country. However, subsequent investigations have failed to find substantive proof of CIA involvement in these outbreaks. The Aum Shinrikyo, a religious cult responsible for the 1995 release of sarin gas in the Tokyo subway system, was also involved in biological warfare activity and sent a team of 40 people to Zaire to acquire Ebola virus. Fortunately, they were unsuccessful in this endeavor. In 1997, unknown farmers in New Zealand deliberately and illegally introduced rabbit hemorrhagic disease virus (a calicivirus) onto the south island as an animal control tool to kill feral rabbits.

Over the past two decades, the human immunodeficiency virus (HIV) has been involved in a number of biocrimes. This most likely reflects the availability of HIV-contaminated blood as a source of this virus. For example, in 1990, Graham Farlow, an asymptomatic HIV-positive inmate at a prison in New South Wales, Australia, injected a guard with HIV-contaminated blood. The guard became infected with HIV; Farlow subsequently died of AIDS. In 1992, Brian T. Stewart, a phlebotomist at a St. Louis, MO hospital, injected his 11-month-old son with HIV-contaminated blood during a fight over payment of child support. In 1993, Iwan E. injected his former girlfriend with 2.5 ml of HIV-contaminated blood after she broke up with him. In 1994, Dr. Richard J. Schmidt, a married Louisiana gastroenterologist, injected a former lover with HIV-contaminated blood. Molecular typing of the HIV strains demonstrated that she contracted the same strain of HIV as found in one of Dr. Schmidt's patients. In perhaps the most famous case, Dr. David Acer, a Florida dentist infected with HIV, transmitted the disease to six of his patients between 1987 and 1990. The intentional infection of these patients is a possibility although there is no direct evidence. In spite of these incidents, HIV has not been included on lists of threat agents for public health bioterrorism preparedness. However, some contend that HIV has great weapon potential if the goal is to destabilize a society.

Viruses have also been involved in suspected incidents or hoaxes. In 1999, an article appeared suggesting that the CIA was investigating whether Iraq was responsible for causing the outbreak of West Nile fever in the New York City area. The story relied heavily on a previous story written by an Iraqi defector, claiming that Saddam Hussein planned to use West Nile virus strain SV 1417 to mount an attack. The investigation indicated that there was no known evidence of bioterrorism involved in the spread of West Nile virus. A fictional 'virus' was also involved in one of the largest bioterrorism hoaxes in 2000. According to e-mail messages widely circulated on the Internet, an organization known as the Klingerman Foundation was mailing blue envelopes containing sponges contaminated with a fictional pathogen called the 'Klingerman virus'. According to the e-mail alert, 23 people had been infected with the virus, including 7 who died.

Viruses as Bioweapons

Advances in viral culture and virus stabilization made during the second half of the twentieth century facilitated

large-scale production of viral agents for aerosol dissemination. A report for the United Nations on chemical and biological weapons and the effects of their possible use gave estimates on the numbers of casualties produced by a hypothetical biological attack (**Table 1**). Three viruses (Rift Valley fever virus, Tick-borne encephalitis virus, and Venezuelan Equine Encephalomyelitis (VEE) virus) were evaluated in a scenario in which 50 kg of the agent was released by aircraft along a 2 km line upwind of a population center of 500 000. The viral agents produced fewer casualties and impacted a smaller area when compared with the bacterial agents used in this hypothetical model. Of note, smallpox was apparently not evaluated because it had not yet been eradicated and level of vaccine-induced immunity in the population was high.

Viral agents were part of the biological weapons arsenal of both the Soviet Union and the United States (**Table 2**). VEE virus was stockpiled by both countries as an incapacitating agent; Variola major and Marburg viruses were stockpiled as lethal agents by the Soviet Union. The Soviet Union reportedly conducted a live field test of Variola major virus on Vozrozhdeniye Island in the Aral Sea in the 1970s, in which 400 g of the virus was released into the atmosphere by explosion. Unfortunately, a laboratory technician who was collecting plankton samples from an oceanographic research vessel 15 km from the island became infected. It was reported that after returning home to Aralsk, she transmitted the infection to several people including children. All those infected died. A number of other viruses that infect humans (e.g., Ebola virus, Lassa fever virus, enterovirus 70) or livestock (e.g., foot and mouth disease virus, rinderpest, Newcastle disease virus) have also been studied for their offensive capabilities or for the development of medical and veterinary countermeasures.

Today, with the increased level of concern, a number of viruses have been cited as possible weapons for use against humans or animals (**Table 2**). The requirements for an ideal biological warfare agent include availability, ease of production, stability after production, a susceptible population, absence of specific treatment, ability to incapacitate or kill the host, appropriate particle size in aerosol so that the virus can be carried long distances by prevailing winds and inhaled deeply into the lungs of unsuspecting victims, ability to be disseminated via food or water, and the availability of a vaccine to protect certain groups. Other factors such as the economic and psychological impact of an attack on animal agriculture with a viral agent must also be considered.

Table 1 Estimates of casualties produced by hypothetical biological attack

	Downwind		
Agent	*Reach (km)*	*Dead*	*Incapacitated*
Rift Valley fever	1	400	35 000
Tick-borne encephalitis	1	9500	35 000
VEE	1	200	19 800
Francisella tularensis	>20	30 000	125 000
Bacillus anthracis	>20	95 000	125 000

Note. These estimates are based on the following scenario: release of 50 kg of agent by aircraft along a 2 km line upwind of a population center of 500 000.

Variola major is considered to be the major viral threat agent for humans. Thus, considerable effort has been expended toward preparing the public health and medical communities for the possibility that this agent will be employed by a terrorist. Variola major is considered to be an ideal terrorist weapon because it is highly transmissible by the aerosol route from infected to susceptible persons; the civilian populations of most countries contain a high proportion of susceptible persons; the disease is associated with a high morbidity and about 30% mortality; initially, the diagnosis of a disease that has not been seen for almost 30 years would be difficult; and, other than the vaccine, which may be effective in the first few days post infection, there is no proven treatment available.

Alphaviruses (**Table 2**) are also of concern because they can be produced in large amounts in inexpensive and unsophisticated systems; they are relatively stable and highly infectious for humans as aerosols, and strains are available that produce incapacitating (e.g., VEE) or lethal infections (EEE case fatality rates range from 50–75%). Furthermore, the existence of multiple serotypes of VEE and EEE viruses, as well as the inherent difficulties of inducing efficient mucosal immunity, make defensive vaccine development difficult.

The filoviruses and arenaviruses that cause hemorrhagic fever have also been considered as agents that might be used by terrorists because of their high virulence and capacity for causing fear and anxiety. The filoviruses, Ebola and Marburg, can also be highly infectious by the airborne route. Humans are generally susceptible to infection with these viruses with fatality rates greater than 80%, and infection can be transmitted between humans through direct contact with virus-containing body fluids. There are five species of arenaviruses (Lassa fever, Junin, Machupo, Guanarito, and Sabia) that can cause viral hemorrhagic fevers with a case fatality rate of about 20%. Large quantities of these viruses can be produced by propagation in cell culture. Infection occurs via the respiratory pathway suggesting that dissemination via aerosol might be used by a terrorist. Human to human transmission has also been reported with aerosol transmission the most likely route for at least some of the secondary cases. The filoviruses and arenaviruses discussed above are BSL-4

Table 2 Classification of viral agents that are considered to be of concern for bioterrorism and biowarfare and those that have been weaponized or studied for offensive or defensive purposes as part of former or current national biological weapons programs

Nucleic acid	*Family*	*Genus*	*Species*
Negative-sense single-stranded RNA	Arenaviridae	Arenaviruses	Lassa fever[a,b]
			Junin[a,b]
			Machupo[a,b]
			Sabia
			Guanarito
	Bunyaviridae	Phlebovirus	Rift Valley fever[b]
		Nairovirus	Crimean-Congo HF
		Hantavirus	Hantaan and related viruses[b]
			Sin Nombre
	Orthomyxoviridae	Influenzaviruses	Influenza A[b]
	Filoviridae	Filovirus	Ebola[a]
			Marburg[c]
	Paramyxoviridae	Henipavirus	Nipah virus
		Morbillivirus	Rinderpest[a,b,d,e,f]
		Avulavirus	Newcastle disease virus[b]
Positive-sense single-stranded RNA	Flaviviridae	Flavivirus	Yellow fever[a,b,d]
			Dengue[b]
			Tick-borne encephalitis virus[a]
			Japanese encephalitis virus[a]
			Omsk hemorrhagic fever virus
	Togaviridae	Alphavirus	Venezuelan equine encephalomyelitis virus[c,g]
			Eastern equine encephalomyelitis virus[b]
			Western equine encephalomyelitis virus[b]
			Chikungunya virus[b]
	Picornaviridae	Enterovirus	Enterovirus 70[h]
		Hepatovirus	Hepatitis A virus
		Apthovirus	Foot and mouth disease virus[f,i]
Double-stranded DNA	Poxviridae	Orthopoxvirus	Variola major[c,b,j]
			Camelpox[h]
	Asfarviridae	Asfivirus	African swine fever virus[a]

[a]Studied by the Soviet Union BW program.
[b]Studied by the U.S. BW program.
[c]Weaponized by the Soviet Union BW program.
[d]Studied by the Canada BW program.
[e]Studied by the France BW program.
[f]Studied by the Germany BW program.
[g]Weaponized by the U.S. BW program.
[h]Studied by the Iraq BW program.
[i]Studied by the Iran BW program.
[j]Studied by the North Korea BW program.

agents and diagnostic capacities for infections caused by these viruses are limited.

Impact of Biotechnology

Because the nucleic acid of many viruses, including some that are currently not threats, can be manipulated in the laboratory, the potential for genetic engineering remains a serious threat. Biotechnology, which has had a tremendous impact on the development of medicines, vaccines, and in the technologies needed to counter the threat of naturally occurring disease, can also be used to modify viruses with unintended consequences or even for the development of novel biological agents. Several examples involving viruses are presented below.

Mousepox Virus

An Australian research group was investigating virally vectored immunocontraceptive vaccines based on ectromelia virus, the causative agent of the disease termed mousepox. They created a recombinant virus, which expressed the mouse cytokine IL-4 in order to enhance the antibody-mediated response to other recombinant antigens carried on the virus vector. Instead, the ectromelia virus vector expressing IL-4 altered the host's immune response to this virus resulting in lethal infections in normally genetically

resistant mice (e.g., C57BL/6). Additionally, this virus also caused lethal infections in mice previously immunized against infection with ectromelia virus. The creation of this 'supermousepox' virus led to speculation that similar genetic engineering could be performed on Variola major leading to a biological weapon that would be effective against an immunized population.

Pandemic Influenza

The influenza pandemic of 1918–19, which followed World War I, was uniquely severe, causing an estimated 20–40 million deaths globally. This pandemic happened before the advent of viral culture and very little was known about the virus until the discovery of the polymerase chain reaction (PCR). Recently, the complete coding sequences of all eight viral RNA segments has been determined by using reverse transcription-PCR (RT-PCR) to amplify the viral RNA sequences from formalin-fixed and frozen tissue samples from individuals who died during this pandemic in an effort to shed light on both the reasons for its extraordinary virulence and evolutionary origin. More recently, researchers reconstructed the 1918 Spanish influenza pandemic virus using reverse genetics and observed that this reconstructed virus exhibited exceptional virulence in the model systems examined and that the 1918 hemaglutinin and polymerase genes were essential for optimal virulence.

Synthetic Genomes

A full-length poliovirus complementary DNA (cDNA) (*c.* 7500 bp) has been synthesized in the laboratory by assembling oligonucleotides of plus- and minus-strand polarity. The synthetic poliovirus cDNA was transcribed by RNA polymerase into viral RNA, which translated and replicated in a cytoplasmic extract of uninfected HeLa S3 cells, resulting in the *de novo* synthesis of infectious poliovirus. The publication of this research raised concerns that more complicated viruses (e.g., Variola major or Ebola) could be synthesized from scratch based on publicly available sequences, or that viruses could be created that do not exist in the wild.

Recognition, Response, and Deterrence

An effective defense requires a comprehensive approach that includes: prevention of access to viral stocks; improved means of detecting deliberately induced disease outbreaks; rapid medical recognition of specific syndromes (e.g., hemorrhagic fever syndrome); rapid laboratory identification of viruses in patient specimens; prevention of person–person transmission; reliable decontamination procedures; development of effective vaccines; and development of effective antiviral therapy.

Rapid and accurate detection of biological threat agents is the basis of an effective public health response to bioterrorism. In order to address this issue, CDC in collaboration with other partners established a national network of laboratories called the Laboratory Response Network (LRN), which was provided with the tools to accomplish this mission. Rapid assays utilizing advanced molecular and immunological technologies for detection of agents such as variola virus, as well as emerging public health threats such as SARS coronavirus and H5N1 influenza virus, were distributed to member laboratories. Equipment, training, and proficiency testing are elements of the LRN and contribute to a uniform operational plan. The importance of high-quality standardized testing for detection of these agents is exemplified by the rapid need for medical countermeasures to protect or treat civilian populations. Accurate laboratory analysis is a major element in the decision process for deployment of the Federal Government's Strategic National Stockpile (SNS) of medical countermeasures.

As part of the effort to deter biological terrorism and strengthen the law enforcement response to such an act, the US recently established a microbial forensic laboratory known as the National Bioforensics Analysis Center that operates in partnership with the Federal Bureau of Investigation. Scientists are already developing methods for the forensic investigation of incidents involving viruses.

Summary

For the terrorist, the use of a viral agent would pose a challenge due to problems associated with acquisition, cultivation, and dissemination. The target for an attack with a viral agent can range from humans to animals and plants. Therefore, agricultural targets are also a major concern. Nature has provided many challenges to combating viral diseases. Viral agents are much more prone to genetic variation and mutation, and can be manipulated or created in the laboratory to take on desired characteristics. Differentiating between natural and intentional viral disease outbreaks can be challenging. Unlike bacterial diseases, many of which are treatable, there are fewer medical countermeasures to employ when dealing with viral infections. Laboratory diagnostic methods and reagents must continuously be refined to account for genetic changes and variants. Thus, the challenge of developing bioterrorism countermeasures is significant. Fortunately, this effort contributes to combating natural disease events more effectively, which has global benefits.

See also: AIDS: Disease Manifestation; AIDS: Global Epidemiology; AIDS: Vaccine Development.

Further Reading

Bray M (2003) Defense against filoviruses used as biological weapons. *Antiviral Research* 57: 53–60.

Breeze RG, Budowle B, and Schutzer SE (eds.) (2005) *Microbial Forensics.* Burlington, MA: Academic Press.

Carus WS (2002) *Bioterrorism and Biocrimes. The Illicit Use of Biological Agents since 1900.* Amsterdam, The Netherlands: Fredonia Books.

Cello J, Paul AV, and Wimmer E (2002) Chemical synthesis of poliovirus cDNA: Generation of infectious virus in the absence of natural template. *Science* 297: 1016–1018.

Charrel RN and de Lamballerie X (2003) Arena viruses other than Lassa virus. *Antiviral Research* 57: 89–100.

Enserink M (2002) Did bioweapons test cause a deadly smallpox outbreak? *Science* 296: 2116–2117.

Henderson DA, Inglesby TV, Gartlett JG, *et al.* (1999) Smallpox as a biological weapon. *JAMA* 281: 2127–2137.

Hopkins DR (1983) *Princes and Peasants. Smallpox in History.* Chicago, IL: University of Chicago Press.

Jackson RJ, Ramsay AJ, Christensen SD, Beaton S, Hall DF, and Ramshaw IA (2001) Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. *Journal of Virology* 75: 1205–1210.

Lanciotti RS, Roehrig JT, Duebel V, *et al.* (1999) Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286: 2333–2337.

Metzker ML, Mindell DP, Liu X, Ptak RG, Gibbs RA, and Hillis DM (2002) Molecular evidence of HIV-1 transmission in a criminal case. *Proceedings of the National Academy of Science, USA* 99: 14292–14297.

Rotz LD, Khan AS, Lillibridge SR, Ostroff SM, and Hughes JM (2002) Public health assessment of potential biological terrorism agents. *Emerging Infectious Diseases* 8: 225–229.

Sidwell RW and Smee DF (2003) Viruses of the Bunya- and Togaviridae families: Potential as bioterrorism agents and means of control. *Antiviral Research* 57: 101–111.

Tumpey TM, Basler CF, Aguilar PV, *et al.* (2005) Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* 310: 77–80.

World Health Organization(1970) *Health Aspects of Chemical and Biological Weapons.* Geneva: WHO.

Viruses Infecting Euryarchaea

K Porter, B E Russ, A N Thorburn, and M L Dyall-Smith, The University of Melbourne, Parkville, VIC, Australia

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Alkaliphilic Having a requirement for an environment with a high pH.

Burst size The number of infectious virus particles released per cell.

Carrier state Persistent infection of a host cell by a virus, with the surviving host persistently carrying and continually producing virus without entering a lysogenic state.

Circular permutation A change in the sequence of the linear DNA termini that does not alter the relative sequence (e.g., circular permutation of ABCDEFGH could generate BCDEFGHA, CDEFGHAB, etc).

Concatamer Two or more DNA molecules that are linked together to form a long, linear DNA molecule.

Cured A host cell that was once a lysogen, but no longer carries viral DNA in any form.

Halophilic Having a requirement for an environment with a high salt concentration.

Headful packaging The mechanism of packaging viral DNA based on the size of the virus head, rather than the length of the viral genome.

Hyperthermophile Having a requirement for an environment with a high temperature ($>80\,^{\circ}C$).

Insertion sequences Repetitive sequences of DNA that can move from one site to another within the viral DNA.

Integrase/recombinase An enzyme which can integrate viral DNA into the genome of its host cell.

Lysogen A host cell that has been infected by a virus that remains dormant, despite the presence of viral DNA.

Lytic virus A virus that is able to infect a host, replicate, and subsequently leave the host cell by rupturing (lysing) the host cell.

Methanogenic Having the ability to produce methane.

Monovalent A virus that has a host range limited to one species.

Prophage A virus that is dormant within the host cell.

Protein-primed replication Replication of DNA via the interaction of the DNA polymerase with specific proteins, rather than DNA or RNA primers.

Temperate virus A virus that is able to infect a host, but remain dormant within the host cell.

Terminal redundancy Linear DNA with the same sequence at each end.

Transduction The transfer of host DNA from one host cell to another by a virus.

Transfection The introduction of pure viral genomic DNA into a host cell, producing viable virus.

Introduction

In comparison with the viruses infecting Bacteria and Eukarya, our understanding of viruses that infect the third domain of life, the Archaea, is still in the early stages. The first archaeal virus was accidentally discovered in 1974, 3 years before the recognition of Archaea as a separate domain. Twenty-two years later, a total of only ~35 archaeal viruses and virus-like particles (VLPs) had been reported, the vast majority of which displayed head-and-tail morphologies and possessed linear double-stranded DNA (dsDNA) genomes. By 2006, at least 63 viruses and VLPs had been described, which infect members of the two major archaeal kingdoms, Crenarchaeota, including the extremely thermophilic sulfur-metabolizers, and Euryarchaeota, including the anaerobic methanogens, the extreme halophiles, and some hyperthermophiles. All have dsDNA genomes and while the total number of archaeal viruses is still miniscule compared with the >5100 published bacteriophages, they display a wide diversity of morphologies and characteristics. This article focuses on virus representatives infecting the members of the kingdom Euryarchaeota, a group for which the diversity of known representatives has blossomed in recent years.

Early Euryarchaeal Viruses and VLPs

In the early days of euryarchaeal virus research (1974–93), the range of virus and VLP representatives of the Euryarchaeota was limited. Attempts to isolate these particles were rare and many of the published representatives were discovered accidentally, for example, as contaminants in 'pure' preparations of archaeal flagella. In hindsight, the inability to culture the environmentally dominant Archaea restricted the isolation of viruses to those that infected easily grown laboratory strains, for example, *Halobacterium* for the haloarchaea and *Methanothermobacter* and *Methanobrevibacter* for the methanogens. Except for one oblate VLP with a circular dsDNA genome observed in cultures of *Methanococcus voltae* strain A3, all representatives possessed head-and-tail morphologies with complex protein profiles and genomes of linear dsDNA, similar to the 'classical' bacteriophages of the families *Myoviridae* (head-and-tail viruses with contractile tails) and *Siphoviridae* (head-and-tail viruses with noncontractile tails).

Like their hosts, the viruses of the extremely halophilic Archaea generally required high levels of NaCl or $MgSO_4$ for stability, although halobacterium salinarum viruses Hh-1 and ΦN were relatively stable in low salt, with the latter virus retaining infectivity even after prolonged incubation in distilled water. Many of the extremely halophilic viruses (or haloviruses) were lytic, with latent periods ranging from 6 to 17 h and burst sizes between 60 and 1300 PFU/cell. These latent periods and burst sizes appeared to be regulated by external salt concentrations, a phenomenon proposed to be significant for virus replication during changing salinity conditions in the environment. Some haloviruses such as halobacterium salinarum virus ΦH were truly temperate, while others such as halobacterium salinarum virus S45 persistently infected their host cells.

The viruses of the methanogens were lytic (by definition, no infectivity could be shown for VLP A3), with latent periods ranging from 4 to 9 h and burst sizes between 6 and 20 PFU/cell. However, some controversy still exists regarding the virus–host relationship for ψM1 and relatives (see below).

In general, the characterization of the early euryarchaeal virus and VLP isolates did not progress to the molecular level and it is likely that most of the early isolates are now lost. The exceptions were ΦH, which is one of the most thoroughly studied archaeal viruses, and methanothermobacter marburgensis virus ψM1, and relatives ψM2 and ψM100 (see below). ΦH was isolated after spontaneous lysis of its host culture and was able to infect a range of *Hbt. salinarum* strains. The virion morphologically resembled members of the family *Myoviridae* and contained a linear dsDNA genome of *c.* 59 kbp, which was packaged by a headful mechanism. The genome was partially sequenced and was shown to be transcribed in early, middle, and late phases. Although the virus particles contained linear DNA, ΦH was temperate and circular viral genomes (plasmids) could be isolated from infected cells. Genomic DNA was able to be transfected into *Hbt. salinarium* and *Haloferax volcanii* cells, producing viable virus particles. This was the first demonstration of transfection in Archaea, and allowed the optimization of plasmid transformation. However, ΦH was highly unstable, due to the activity of insertion sequences and inversion of the 'L-fragment', which could act as an autonomous plasmid. For this reason, work on ΦH was discontinued in the late 1990s.

Current Euryarchaeal Viruses and VLPs

The published viruses and VLPs infecting members of the kingdom Euryarchaeota in the readily accessible literature are listed in **Table 1**. This table excludes nine uncharacterized head-and-tail viruses with *c.* 50 nm diameter heads, isolated in 1977 from the Great Salt Lake in Utah, USA, on uncharacterized *Halobacterium* isolates. The remainder of this article focuses on detailing the advances in research for euryarchaeal viruses and VLPs that have been studied since 2001.

ψM1, ψM2, and ψM100; Viruses of the Methanogenic Archaea

A monovalent virus of *M. marburgensis*, ψM1 was isolated several times from an anaerobic sludge-bed reactor in

Table 1 Viruses and virus-like particles of the kingdom Euryarchaeota

Virus	*Publication date*	*Isolating host*	*Isolated from*	*Infection pathway*	*Genome size (kbp)*	*GC content (%)*	*Particle size (nm)*	*Number of proteins; size (kDa)*	*Lipids*
Order *Caudovirales*, family *Myoviridae*									
Viruses with an isometric head, contractile tail, and linear, double-stranded DNA genome									
ΦCh1	1997	*Natrialba magadii* DSM 3394	*Natrialba magadii*	Lytic, temperate	58.5[a,b] AF440695	61.9	70 (head); 130 (tail)	9; 15–80	nd[c]
ΦH	1982	*Halobacterium salinarum* ATCC 29341	*Halobacterium salinarum*	Temperate	59[d]	65	64 (head); 170 (tail)	>3; 22–80	nd
HF1	1993	*Haloferax lucentense* NCIMB 13854	Cheetham, VIC, Australia	Lytic, carrier state	75.7[b] AY190604	55.8	58 (head); 94 (tail)	>4; 20–55	nd
HF2	1993	*Halorubrum coriense* ACAM 3911	Cheetham, VIC, Australia	Lytic, carrier state	77.7[b]AF222060	55.8	58 (head); 94 (tail)	>4; 20–55	nd
Hs1	1974	*Halobacterium salinarum* strain 1	*Halobacterium salinarum*	Lytic, carrier state	nd	nd	50 (head); 120 (tail)	nd	nd
Ja.1	1975	*Halobacterium salinarum* NRC 34001	The Salt Ponds of Yallahs, Jamaica	Lytic	230	nd	90 (head); 150 (tail)	nd	nd
S41	1998	*Halobacterium salinarum* NRC 34001	Little Salt Pond in Yallahs, Jamaica	Lytic, carrier state	nd	nd	89 (head); 141 (tail)	nd	nd
S50.2	1998	*Halobacterium salinarum* NRC 34001	Little Salt Pond in Yallahs, Jamaica	Lytic, carrier state	nd	nd	63 (head); 78 (tail)	nd	nd
S4100	1998	*Halobacterium salinarum* NRC 34001	Little Salt Pond in Yallahs, Jamaica	Lytic, carrier state	nd	nd	56 (head); 85 (tail)	nd	nd
S5100	1990	*Halobacterium salinarum* NRC 34001	Little Salt Pond in Yallahs, Jamaica	Lytic, carrier state	nd	nd	65 (head); 76 (tail)	nd	nd
Order *Caudovirales*, family *Siphoviridae*									
Viruses with an isometric head, noncontractile tail, and linear, double-stranded DNA genome									
ΦF1	1993	*Methanobacterium thermoformicium* FF1	Anaerobic sludge-bed reactor	Lytic	85	nd	70 (head); 160 (tail)	nd	nd
ΦF3	1993	*Methanobacterium thermoformicium* FF1	Anaerobic sludge-bed reactor	Lytic	36	nd	55 (head); 230 (tail)	nd	nd
ΦN	1988	*Halobacterium salinarum* NRL/JW	*Halobacterium salinarum*	nd	56[e]	70	55 (head); 85 (tail)	>1; 53	nd
ψM1	1989	*Methanothermobacter marburgensis* DSM 2133	Experimental anaerobic sludge digester	Lytic, possible integration	26.8[b] AF065411, AF065412	46.3	55 (head); 210 (tail)	3; 10–35	nd
ψM2	1989	Methanothermobacter marburgensis DSM 2133	Spontaneous deletion mutant of ψM1	Lytic, possible integration	26.1[b] AF065411	46.3	55 (head); 210 (tail)	3; 10–35	nd
ψM100	2001	*Methanothermobacter wolfeii*	Defective provirus in host chromosome	Defective provirus	28.8[b] AF301375	45.4	nd	nd	nd
B10	1982	*Halobacterium* sp. B10	nd	Lytic	nd	nd	nd	nd	nd
Hh-1	1982	*Halobacterium salinarum* ATCC 29341	Fermented anchovy sauce	No lysis during release, carrier state	37.2	67.05	60 (head); 100 (tail)	>1; 35	nd

Continued

Table 1 Continued

Virus	*Publication date*	*Isolating host*	*Isolated from*	*Infection pathway*	*Genome size (kbp)*	*GC content (%)*	*Particle size (nm)*	*Number of proteins; size (kDa)*	*Lipids*
Hh-3	1982	*Halobacterium salinarum* ATCC 29341	Fermented anchovy sauce	Lytic, carrier state	29.4	62.15	75 (head); 50 (tail)	lt; 1; 47	nd
PG	1986	*Methanobrevibacter smithii* strain G	Rumen fluid samples	Lytic	50	nd	nd	nd	nd
PMS1	1986	*Methanobrevibacter smithii*	nd	Lytic	35	nd	nd	nd	nd
S45	1984	*Halobacterium salinarum* NRC 34001	The Salt Ponds of Yallahs, Jamaica	No lysis, persistent infection	nd	nd	40 (head); 70 (tail)	nd	nd
VTA	1999	*Methanococcus voltae* PS	*Methanococcus voltae*	nd	4.4	nd	40 (head); 61 (tail)	nd	nd
Genus *Salterprovirus*									
Viruses that are spindle-shaped, with a tail and a linear, double-stranded DNA genome, with terminal-bound proteins.									
His1	1998	*Haloarcula hispanica* ATCC 36930	Avalon saltern, VIC, Australia	Lytic, carrier state	14.9[b] AF191796	40	74 × 44 (body); 7 (tail)	nd	Possible
His2	2006	*Haloarcula hispanica* ATCC 36930	Pink Lakes, VIC, Australia	Lytic, carrier state	16.1[b] AF191797	40	67 × 44 (body); nd (tail)	>4; 21–62	Possible
Unclassified									
Viruses that are spherical, with a linear, double-stranded DNA genome									
SH1	2005	*Haloarcula hispanica* ATCC 36930	Serpentine Lake, WA, Australia	Lytic, carrier state	30.9[b] AY950802	68.4	70	15; 4–185	Yes
Unclassified									
Virus-like particles that are polyhedral									
CWP	1988	*Pyrococcus woesei*	nd	nd	No nucleic acid	nd	nd	3	nd
Unclassified									
Virus-like particles that are oblate, with a circular, double-stranded DNA genome									
VLPA3	1989	*Methanococcus voltae* A3	*Methanococcus voltae* A3	Temperate	23	nd	52 × 70	1 (and 3 minor); 13	nd
Unclassified									
Virus-like particles that are lemon-shaped, with a tail, and a circular, double-stranded DNA genome									
VLP PAV1	2003	'*Pyrococcus abysii*' GE23	'*Pyrococcus abysii*'	Nonlytic, persistent infection	17.5[b]	nd	120 × 80 (body); 15 (tail)	>3; 6–36	Possible

[a]Genome completely sequenced.
[b]Also packages host derived RNA. Some genome adenine residues are methylated.
[c]nd, not determined.
[d]Some sequence data available: 405323, 405325, AH004327, S63933, S63992, S63994, X00805, X80161, X80162, X80163, X80164, X52504.
[e]All genome cytosine residues are methylated.

1989. The particles resemble bacteriophages of the family *Siphoviridae*, with a head diameter of *c.* 55 nm and a tail of *c.* 210 nm × 10 nm, composed of individual segments and an enlarged terminal segment (**Figure 1**). The ψM1 virion contains a 26.8 kbp genome and three major structural proteins. The proteins appear to be encoded by two open reading frames (ORFs), designated 13 and 18, whose products are post-translationally modified to produce the three proteins. To form the larger protein, the products of the ORFs are covalently cross-linked. The smaller proteins are then formed by processing of this protein at the N- and C-termini. The product of ORF 13 is similar to proteins found in Gram-positive Bacteria and bacteriophages; for example, it shows 28% identity over 330 amino acids to prophage PBSX protein XkdG in *Bacillus subtilis*.

The linear dsDNA genome of ψM1 is terminally redundant and circularly permuted, and packaged via a headful packing mechanism. Upon passage in laboratory cultures a spontaneous ψM1 deletion mutant designated ψM2, which lacked a 0.7 kbp segment (DR1), was found to become dominant. The 26.1 kbp ψM2 genome (AF065411) (**Figure 2**) and the DR1 ψM1 segment (AF065412) were sequenced. The ψM2 genome has a guanine–cytosine (GC) content of 46.3% and encodes 31 ORFs, seven of which have been assigned putative functions, again similar to those in Gram-positive Bacteria and bacteriophages. This suggests some gene exchange between bacterial and archaeal viruses, or a common ancestor of the bacterial and archaeal viruses. DR1 encodes ORF A, a duplication of ψM2 ORF 27. Although this element affects the stability of ψM1, the insertion and deletion of DR1 does not appear to interrupt any downstream ORFs.

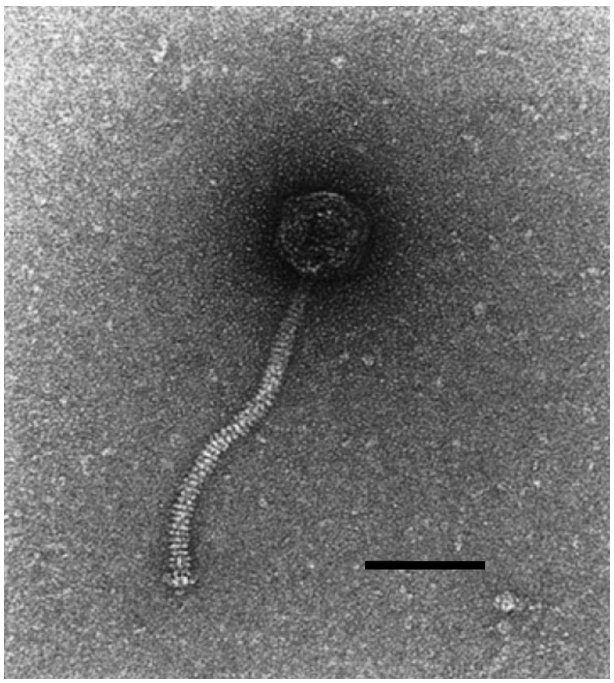


Figure 1 Electron micrograph of ψM1, negatively stained with uranyl acetate. Scale = 70 nm. Adapted from *Archives of Microbiology*, 152, 1989, p. 106, Characterization of ψM1, a virulent phage of *Methanobacterium thermoautotrophicum* Marburg, Meile L, Jenal U, Studer D, Jordan M, and Leisinger T, figure 1, © Springer-Verlag 1989, with kind permission from Springer Science and Business Media.

Under laboratory conditions, ψM1 is a lytic virus, with no evidence of lysogen formation. The virus produces a pseudomurein endoisopeptidase, a lytic enzyme that cleaves host pseudomurein, encoded by *peiP* (ORF 28). Unexpectedly, ORF 29 encodes a putative site-specific integrase/recombinase, suggesting that ψM1 and ψM2 are actually temperate. This theory is supported by the absence of a viral DNA polymerase, which is usually found in lytic viruses, and the capacity of ψM1 particles to mediate transduction of resistance and biosynthesis markers, an ability as yet unobserved in other archaeal viruses. In addition, ψM1-resistant cultures of *Methanothermobacter wolfeii* spontaneously lyse and carry a chromosomal prophage, known as ψM100, which is homologous to ψM1 and ψM2. ψM100 appears to be a defective virus, as no VLPs are observed in autolysates but pseudomurein endoisopeptidase PeiW, encoded by ψM100 ORF 28, is present. The 28.8 kbp ψM100 sequence (AF301375) (**Figure 2**) has a GC content of 45.4%. It contains a 2.8 kbp fragment, IRFa, which has a GC content of 33.4% and apparently originates from a source not homologous to ψM1 and ψM2. The remaining 26.0 kbp of the ψM100 genome is 70.8% identical to ψM2. The lytic/temperate relationship between ψM1-like viruses and *M. marburgensis* remains unresolved.

Viruses of the Extremely Halophilic Archaea

Head-and-Tail Viruses HF1 and HF2

In 1993, a deliberate search for haloviruses infecting a wider range of haloarchaea resulted in the isolation of HF1 and HF2 from Cheetham Saltworks in Victoria, Australia. Both viruses are lytic, but can enter unstable carrier states in laboratory cultures. They have mutually exclusive host ranges, HF1 infecting a wide range of hosts from the genera *Haloarcula*, *Halobacterium*, and *Haloferax*, while HF2 strictly infects *Halorubrum* species. HF2 is sensitive to chloroform exposure, but HF1 is relatively insensitive to this solvent. In aspects other than host range and chloroform sensitivity, HF1 and HF2 are very similar. Their particles have identical morphologies, resembling bacteriophages of the family *Myoviridae*, with heads of *c.* 58 nm in diameter and tails of *c.* 94 nm in length (**Figure 3**). The structural proteins of HF1 and HF2 are very similar, with four major and several minor proteins. They also share similarly sized genomes of linear dsDNA.

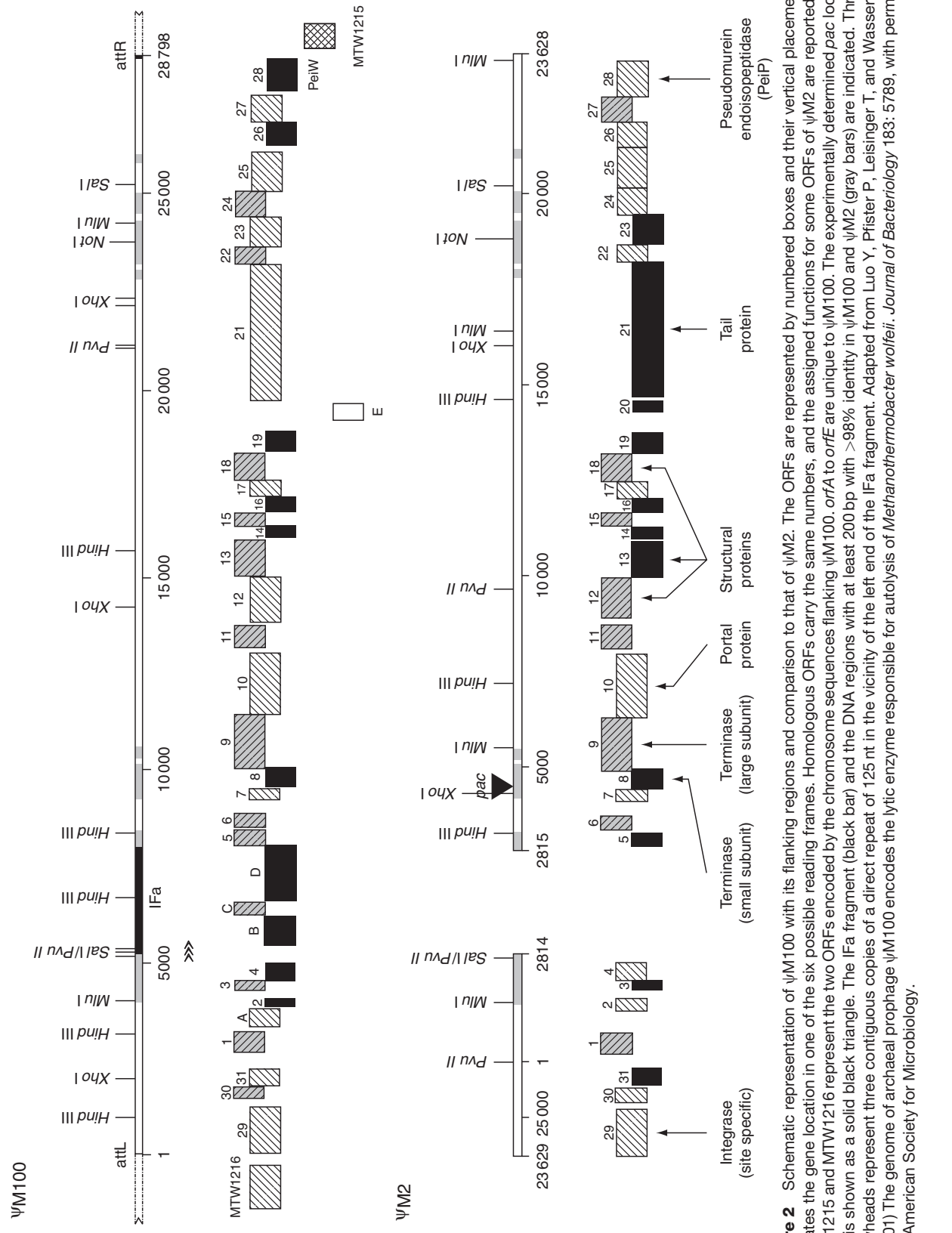


Figure 2 Schematic representation of ψM100 with its flanking regions and comparison to that of ψM2. The ORFs are represented by numbered boxes and their vertical placement indicates the gene location in one of the six possible reading frames. Homologous ORFs carry the same numbers, and the assigned functions for some ORFs of ψM2 are reported. MTW1215 and MTW1216 represent the two ORFs encoded by the chromosome sequences flanking ψM100. *orfA* to *orfE* are unique to ψM100. The experimentally determined *pac* locus for ψM2 is shown as a solid black triangle. The IFa fragment (black bar) and the DNA regions with at least 200 bp with >98% identity in ψM100 and ψM2 (gray bars) are indicated. Three arrowheads represent three contiguous copies of a direct repeat of 125 nt in the vicinity of the left end of the IFa fragment. Adapted from Luo Y, Pfister P, Leisinger T, and Wasserfallen A (2001) The genome of archaeal prophage ψM100 encodes the lytic enzyme responsible for autolysis of *Methanothermobacter wolfeii*. *Journal of Bacteriology* 183: 5789, with permission from American Society for Microbiology.

The genome of HF2 (AF222060) replicates via concatamer formation. It is 77.7 kbp in length (**Figure 4**), has a GC content of 55.8%, and contains 121 closely spaced ORFs. Approximately 12% of ORFs show similarity to known sequences. The HF2 ORFs are transcribed as operons, with distinct early, middle, and late transcripts. The ORFs are likely to be strategically organized into three groups: (1) genes involved in establishing infection; (2) genes involved in DNA synthesis, modification, and replication; (3) and genes involved in virus assembly and release. There are long intergenic repeats throughout the genome, which appear to be linked to transcription regulation. The genomic arrangement suggests that such viruses mediate genetic exchange across a range of hosts, resulting in mosaic virus genomes (see below).

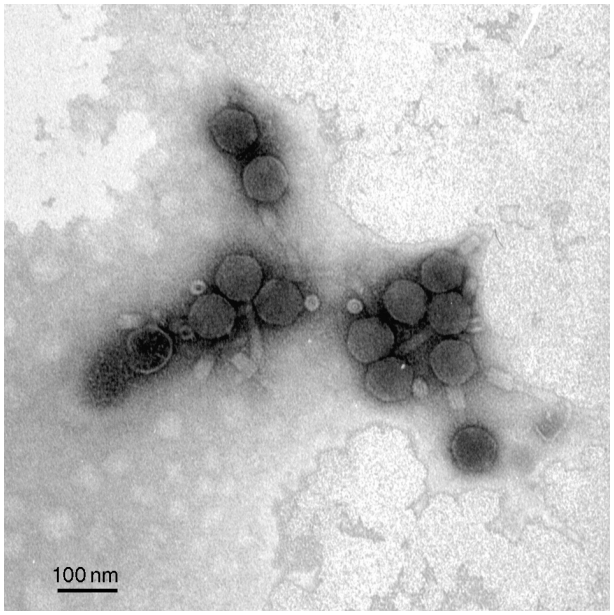


Figure 3 Electron micrograph of HF2, negatively stained with uranyl acetate.

The HF1 genome (AY190604) is 75.9 kbp in length (**Figure 4**), has a GC content of 55.8%, and encodes 117 ORFs, of which 13% show similarity to known sequences (excluding those of HF2). The first 48 kbp of the HF1 genome is identical to that of HF2, apart from one silent base change, and the remaining sequence is 87% identical. In the latter region, HF2 contains two unique ORFs, two pairs of ORFs that are joined in HF1, and several ORFs with less than 70% amino acid identity to those in HF1. Divergence in this region of the genome probably explains the wide differences in viral host range and chloroform sensitivities of the two viruses. The path of sequence divergence also indicates that a recent recombination event exchanged the end of one virus for that of a third, unknown but related, virus.

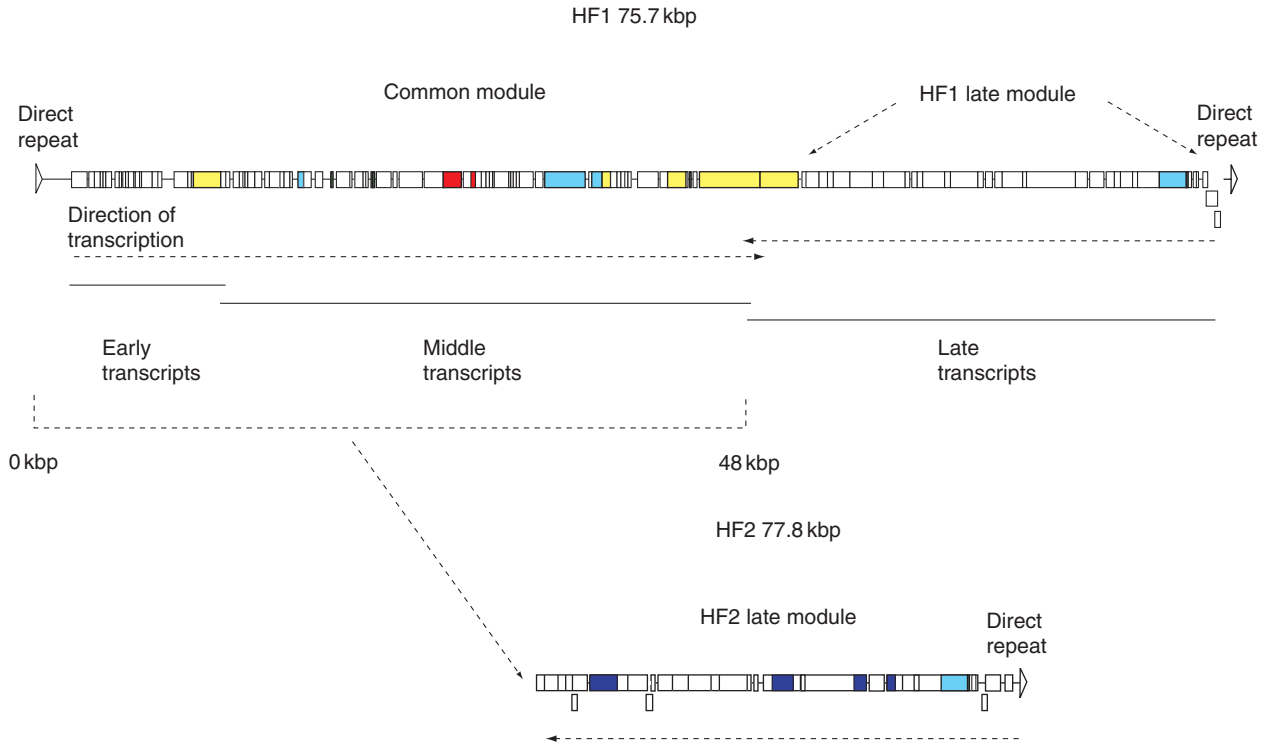


Figure 4 Closely related haloviruses HF1 (75.898 kbp) and HF2 (77.670 kbp) have genomes that are identical from 0 to 48 kbp, after which there is significantly lower homology. Direction of transcription is indicated below the genes in the diagram, as is the pattern of gene expression. Genes are drawn to scale. Genes highlighted in red are involved in transcription; dark blue – virion structure; yellow–nucleic acid modification; light blue – replication and repair.

The Haloalkaliphilic Virus, ΦCh1

Although most extremely halophilic Archaea live at a neutral pH, there is a group that is not only extremely halophilic, but is also alkaliphilic. In 1997, a haloalkaliphilic virus, ΦCh1, was isolated after spontaneous lysis of a culture of the haloalkaliphilic archaeon *Natrialba magadii*. ΦCh1 is monovalent, producing plaques on lawns of ΦCh1-cured *Nab. magadii* cells. The virus is also temperate, with lysogens containing genomic ΦCh1 DNA integrated into the *Nab. magadii* chromosome. Virus integration is potentially mediated by putative site-specific recombinases, Int1 and Int2, encoded by ORFs 35 (*int1*, which has been shown to mediate inversion and excision reactions, resulting in small, circular molecules) and 45 (*int2*).

ΦCh1 particles resemble the family *Myoviridae*, with a head of *c.* 70 nm in diameter and a contractile tail *c.* 130 nm × 20 nm (**Figure 5**). The ΦCh1 virion contains at least nine structural proteins, designated A to I in decreasing size order. Proteins A and H are encoded by ORF 19 and protein E is encoded by ORF 11 (gene *E*). Gene *E* is transcribed late in cell infection and protein E is found to be associated with the host membrane, suggesting a role in DNA packaging or assembly of coat proteins during viral exit.

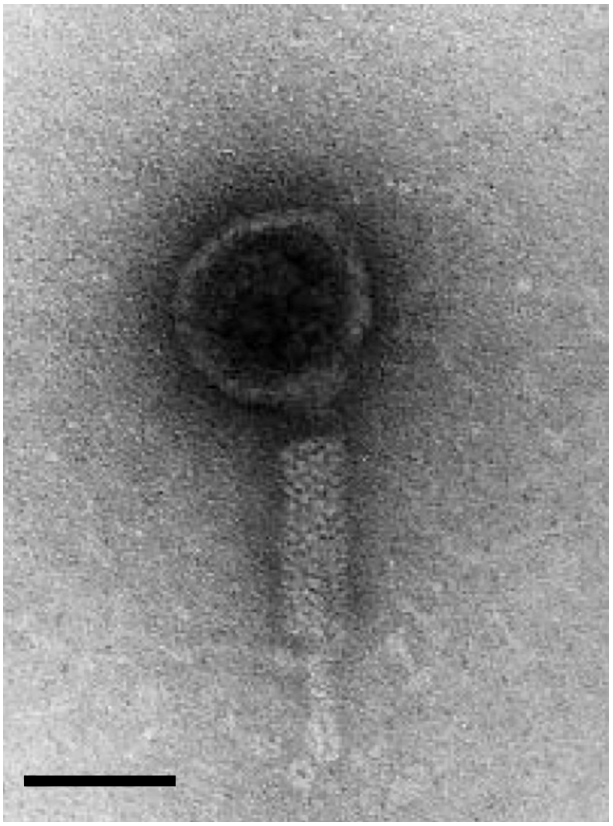


Figure 5 Electron micrograph of ΦCh1, negatively stained with phosphotungstate. A contracted tail and the anchor structure between head and tail are visible. Scale = 50 nm. Adapted from Witte A, Baranyi U, Klein R, *et al*. (1997) Characterization of *Natronobacterium magadii* phage ΦCh1, a unique archaeal phage containing DNA and RNA. *Molecular Microbiology* 23: 605, with permission from Blackwell Science Ltd.

ΦCh1 virus particles contain both linear dsDNA and host-derived RNA species. Genomic ΦCh1 DNA is modified at adenine residues within various sequences, including 5′-GATC-3′. Although DNA methylation is a common viral mechanism for avoiding host-mediated restriction, it was unexpected in ΦCh1, as *Nab. magadii* does not have methylated DNA. An N^6-adenine methyltransferase, encoded by ORF 94 of ΦCh1, is transcribed and expressed late in cell infection. These factors suggest that ΦCh1 may infect other hosts.

The ΦCh1 genome has a circular replicative form and is circularly permuted and terminally redundant, suggesting a headful packing mechanism. The 58.5 kbp genome (AF440695) has a GC content of 61.9% and is predicted to contain 98 ORFs (**Figure 6**). In a similar manner to head-and-tail bacteriophages, the ORFs appear to be organized into three transcriptional units encoding (1) structural and morphogenesis proteins; (2) replication, stabilization, and gene regulation proteins; and (3) proteins of mostly unknown functions. Around half of the ORFs show similarity to sequences in the databases, although most of these are proteins of unknown function. The genome sequence of ΦCh1 shows strong similarity to the incomplete genome of ΦH, which is surprising given that the two viruses were isolated from distinctly different environments and hosts. This unexpected relationship between supposedly diverse viruses suggests that similar viruses may be widely distributed throughout various hypersaline environments across the planet.

His1 and His2: Spindle-Shaped Haloviruses of the Genus *Salterprovirus*

From 1974 to 1998, all of the viruses described for the kingdom Euryarchaeota were of head-and-tail morphology. Nevertheless, in waters such as the Dead Sea, the most abundant VLPs observed by direct electron microscopy were spindle shaped. In a study of a Spanish saltern, spindle-shaped VLPs were found to increase in abundance with increasing salinity. In 1998, a spindle-shaped halovirus, His1, was reported. Isolated from the Avalon saltern in Victoria, Australia, on a lawn of *Haloarcula hispanica*, the His1 virion is *c.* 74 nm × 44 nm, with a short 7 nm tail (**Figure 7(a)**). His1 resembles crenarchaeal virus SSV1 and due to the morphological similarities and comparable genome sizes of these two viruses, His1 was initially, and mistakenly, placed in the *Fuselloviridae* family of archaeal viruses (type species, SSV1).

In 2006, a second spindle-shaped virus named His2 was described. It was isolated from the Pink Lakes in Victoria, Australia, and also plaques on *Har. hispanica*. By negative-stain electron microscopy, His2 particles are

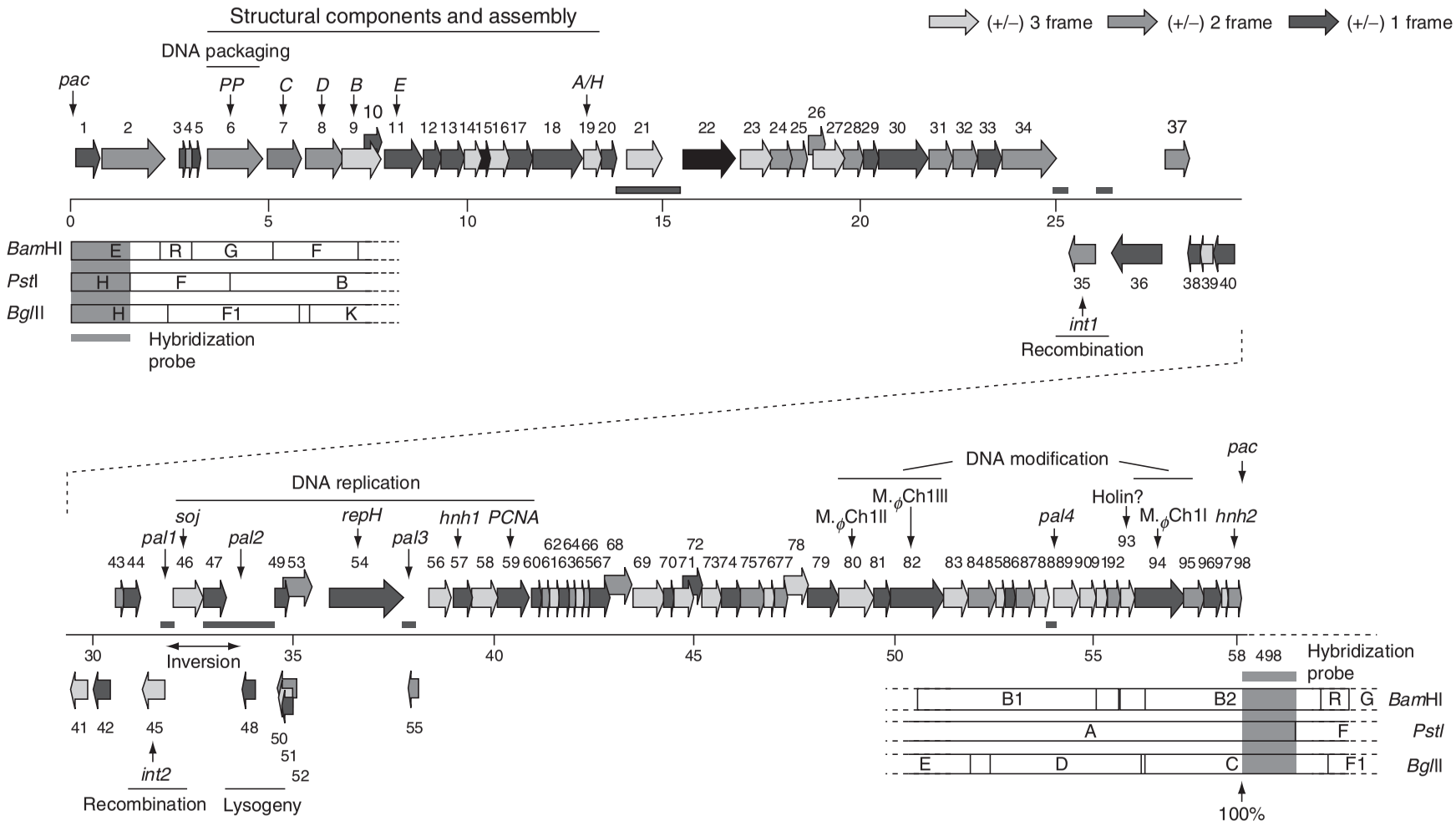


Figure 6 Linear representation of the 58 498 bp ΦCh1 genome, starting at the *pac* site. ORFs are represented by arrows and numbered. Putative and verified functions of predicted gene products are indicated. The shades of the arrows indicate the frames of the ORFs: light gray, third reading frame; mid-gray, second reading frame; and dark gray, first reading frame for both orientations. A partial restriction map showing the region embedding the *pac* site and the fragments that are created during the cut at *pac* is given beneath the genome map. Both maps are correlated with each other. The binding region for the hybridization probe that was used to map the *pac* site is shaded. Low G+C regions are indicated by black bars, and the segment that is inverted in pΦHL with respect to ΦCh1 is indicated by an arrow. The putative binding sites for site-specific recombinases, *pal1* to *pal4*, are also indicated. Adapted from Klein R, Baranyi U, Rössler N, Greineder B, Scholz H, and Witte A (2002) *Natrialba magadii* virus ΦCh1: First complete nucleotide sequence and functional organization of a virus infecting a haloalkaliphilic archaeon. *Molecular Microbiology* 45: 852, with permission from Blackwell Science Ltd.

more flexible than His1 particles, with the virions being *c.* 67 nm × 44 nm, with a short tail (**Figure 7(b)**). The His2 virion contains at least four structural proteins, designated viral protein (VP) 1 (62 kDa) to VP 4 (21 kDa). To date, the ORFs encoding VPs 2 and 3 have not been determined. VP 1 is encoded by ORF 29 and predicted to be exported to the cell surface and glycosylated. ORF 29, and the downstream ORFs 30, 31, and 33, have closely similar sequences in at least four species of extremely halophilic Archaea, although the segments of similarity do not appear to be part of provirus genomes. The functions of these archaeal homologs have not yet been determined.

Although both monovalent haloviruses are virulent, they appear to enter a carrier state and have the capacity to exit cells without causing cell lysis. His1 and His2 are relatively stable in the pH range 3–9; however, His1 is more resistant than His2 to exposure to both increased temperature and lowered ionic environments. Both His1 and His2 are sensitive to chloroform and have low buoyant densities and flexible virus particles. These factors suggest that the virions contain lipids.

The genomes of haloviruses His1 (AF191796) and His2 (AF191797) are linear dsDNA of 14.5 and 16.1 kbp, respectively (**Figure 8**). The genomes have a GC content of *c.* 40%, about 23% lower than *Har. hispanica*, suggesting that the haloviruses are not well equipped to replicate in *Haloarcula*. Moreover, no tRNA genes have been detected in the genomes of these viruses.

The His1 and His2 genomes each contain 35 ORFs and show almost no sequence similarity to one another or to known sequences. The notable exceptions are the putative DNA polymerases of the two viruses, which are weakly similar to one another at the nucleotide level and show 42% identity at the protein level. The DNA polymerases appear to be members of family B DNA polymerases that use the protein-priming mechanism. The His1 and His2 genomes have inverted terminal repeat (ITR) sequences at each end (105 and 525 nt, respectively), and the 5′ end of each is linked to a terminal protein (TP), which is essential for DNA replication. These characteristics are typical of systems that replicate DNA via protein priming.

At the molecular level, His1 and His2 are distantly related to one another; however, they do not appear to be related to fuselloviruses at all, with distinctly different genome structures, replication strategies, virus–host relationships, and genome sequences. Consequently, haloviruses His1 and His2 have been placed in an independent virus genus, *Salterprovirus*.

Spherical Halovirus SH1

In addition to the spindle-shaped and head-and-tail VLPs observed in hypersaline waters such as the Dead Sea, isometric particles were also present in high numbers. These could have been classical head-and-tail VLPs, which had lost their tails through the negative-stain treatment, which tends to disrupt halophilic virus particles. However, in 2003, a spherical halovirus named SH1 that resembled these isometric particles was first noted. It was isolated from Serpentine Lake, Rottnest Island, Western Australia, on *Har. hispanica*. The SH1 virion is *c.* 70 nm in diameter and displays an outer capsid layer with a compact core particle of *c.* 50 nm in diameter (**Figure 9**). Morphologically, it resembles viruses such as the bacteriophage PRD1, human adenovirus, and archaeal virus STIV, which all share common architecture.

SH1 is virulent, infecting both *Har. hispanica* and an uncharacterized isolate of the genus *Halorubrum*. The virion is stable to exposure to temperatures of 50 °C and in a pH range of 6–9, but is sensitive to exposure to both chloroform and lowered ionic environments. SH1 particles contain a lipid layer and 15 structural proteins,

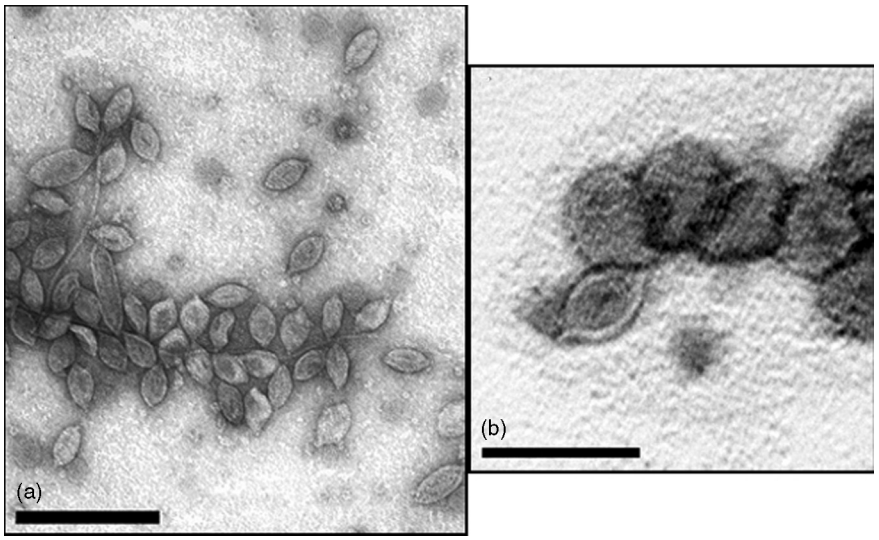


Figure 7 Electron micrograph of (a) His1 and (b) His2, negatively stained with uranyl acetate. Scale = 200 nm (a); 100 nm (b).

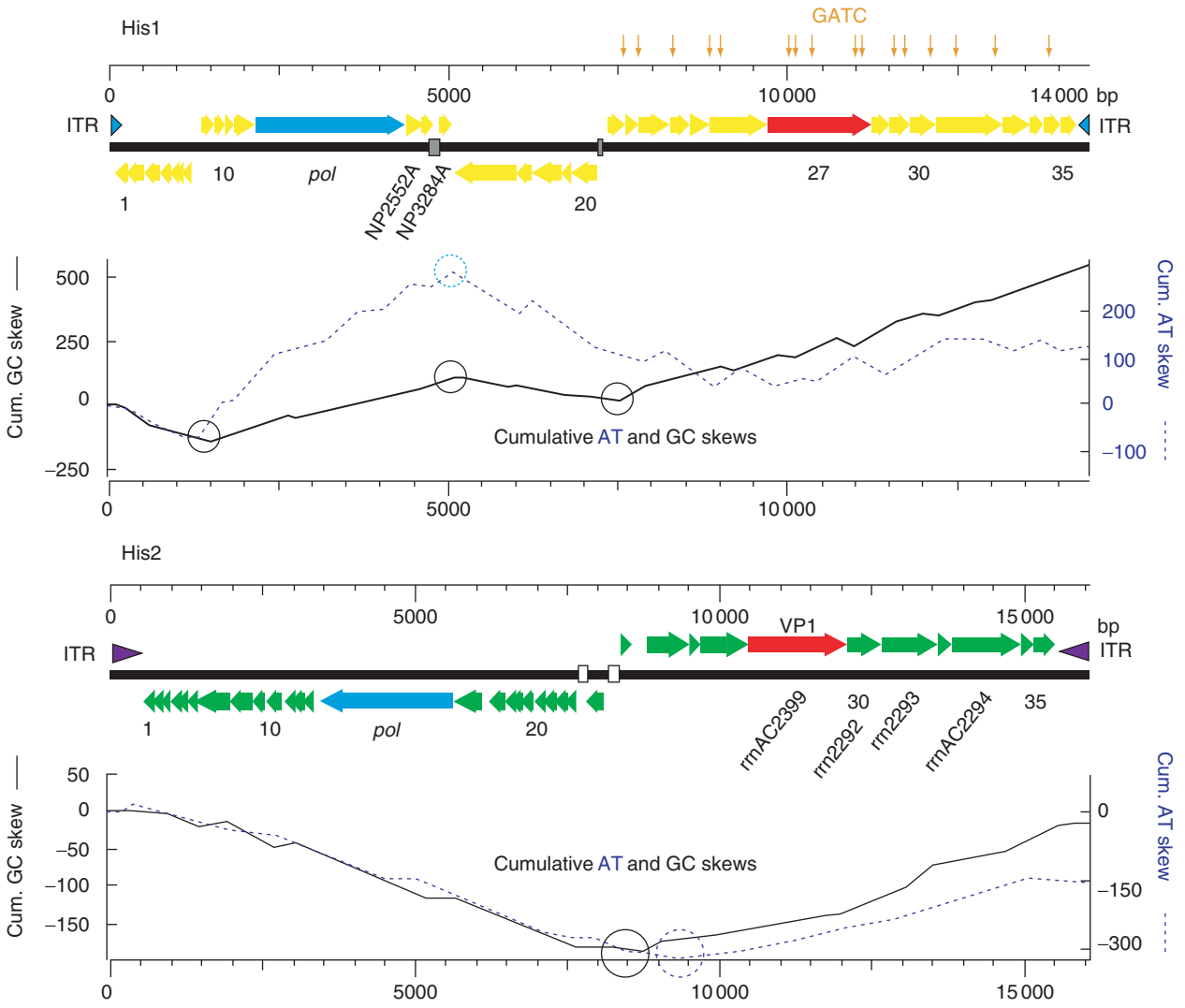


Figure 8 Diagram of the His1 and His2 genomes. The major features are shown, including the predicted ORFs (yellow and green arrows), putative DNA polymerases (blue arrows), VP1 and its His1 homolog (red arrows), terminal inverted repeat sequences (purple arrowheads), and regions of nucleotide sequence repeats (gray boxes). Scale bars, in bp, are shown above each genome diagram. ORFs are numbered (left to right), and some have been named (*pol*, DNA polymerase; VP1, major capsid protein) or the accession numbers of closely related sequences given. The template strand for each ORF is indicated by the direction of arrows and their position above or below the black line. The vertical arrows about the His1 genome scale bar show positions of the sequence GATC. Plots of cumulative GC skew (solid black line) and AT skew (dashed blue line) are shown below each genome diagram, with inflection points indicated by circles. Adapted from Bath C, Cukalac T, Porter K, and Dyall-Smith ML (2006) His1 and His2 are distantly related, spindle-shaped haloviruses belonging to the novel virus group, *Salterprovirus*, *Virology* 350: 233, with permission from Elsevier.

designated VP 1 to VP 15 in decreasing size order. Details on the locations and possible functions of the SH1 virion proteins are available.

The genome of SH1 (AY950802) is linear dsDNA of 30.9 kbp (**Figure 10**), with a GC content of 68.4%. The viral genome contains 56 ORFs, which show very little sequence similarity to known sequences. It contains 309 bp ITRs. The molecular features of SH1 indicate that it is a member of a novel virus group. Based on the evidence from electron microscopy surveys, haloviruses similar to SH1 are likely to be widespread and dominant members of hypersaline systems.

PAV1, A VLP of the Hyperthermophilic Order Thermococcales

No virus has ever been reported to infect the hyperthermophilic Euryarchaeota. However, two major groups of VLPs, rod-shaped particles and spindle-shaped particles, have been observed in enrichment cultures of samples obtained from deep-sea hydrothermal vents. In 2003, such spindle-shaped VLPs were discovered in supernatant of a '*Pyrococcus abysii*' culture and were designated '*P. abysii*' virus 1 (PAV1). PAV1 is continuously released, but does not cause lysis of host cells and cannot be

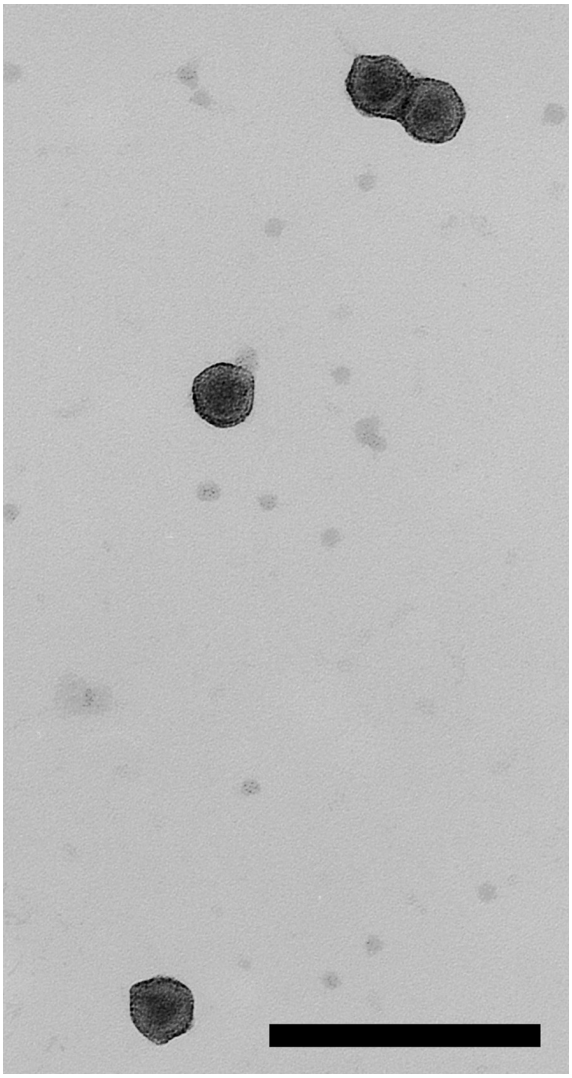


Figure 9 Electron micrograph of SH1, stained with uranyl acetate. Scale = 200 nm.

induced to infectivity using ultraviolet or γ irradiation, mitomycin C, or heat or pressure shock. No viral genomes integrated into the host chromosome have been detected.

PAV1 particles are flexible and similar in appearance to both the fuselloviruses and the salterproviruses, being *c.* 120 nm × 80 nm, with a tail of *c.* 15 nm and tail fibers (**Figure 11**). The virion is composed of at least three structural proteins, of *c.* 6, 13, and 36 kDa. PAV1 is sensitive to exposure to chloroform, Triton X-100, proteinase K, and sodium dodecyl sulfate. Combined with its low buoyant density and flexible particles, this suggests that the particles contain lipid.

The genome of PAV1 is circular dsDNA of 17.5 kbp. It contains 24 putative ORFs, which show no similarity to sequences in the databases. A detailed description of the PAV1 genome should be published soon.

Conclusion

At first glance, it may appear that the virus representatives of the kingdom Euryarchaeota resemble the more thoroughly studied bacterial and eukaryal viruses, or the viruses of the kingdom Crenarchaeota. However, on a molecular level, the euryarchaeal viruses are clearly distinct and radically different from any other known viruses. Many of the representatives, such as PAV1 and SH1, show almost no similarity to any sequences in the databases and even the head-and-tail representatives, which appear to resemble the 'classical' bacteriophages in both morphology and genome structure, encode many previously unobserved genes.

Fortunately, it appears that we are now in a good position to culture euryarchaeal viruses. Whether all the dominant morphological forms of euryarchaeal viruses have been isolated is still uncertain; however, the current

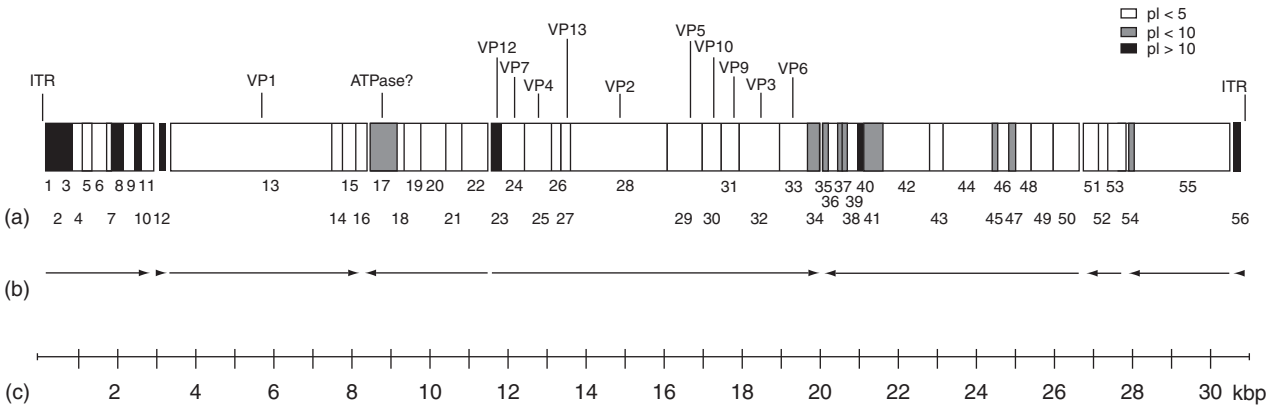


Figure 10 Genome organization of SH1. (a) Predicted ORFs are numbered 1–56 from left to right and shaded according to the calculated isoelectric point of the predicted gene products. Structural virion components (VP 1 to VP 14) determined by protein chemistry methods are marked. (b) Direction of transcription is depicted by arrows. (c) Scale bar. Adapted from Bamford DH, Ravantti JJ, Rönnholm G, *et al*. (2005) Constituents of SH1, a novel, lipid-containing virus infecting the halophilic euryarchaeon *Haloarcula hispanica*. *Journal of Virology* 79: 9102, with permission from American Society for Microbiology.

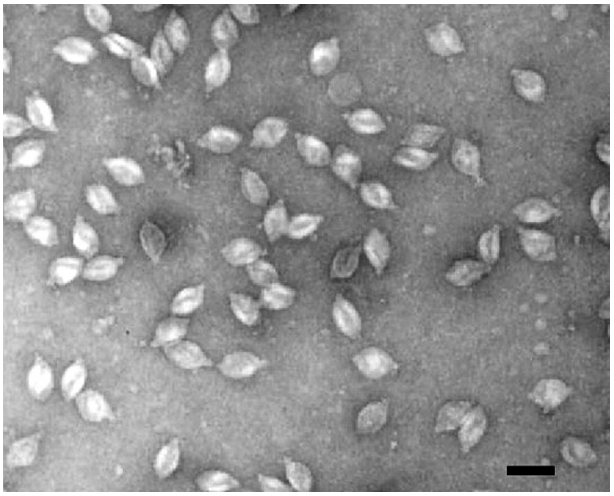


Figure 11 Electron micrograph of PAV1, negatively stained with 2% uranyl acetate. Scale = 100 nm. Adapted from Geslin C, Le Romancer M, Erauso G, Gaillard M, Perrot G, and Prieur D (2003) PAV1, the first virus-like particle isolated from a hyperthermophilic euryarchaeote, ''*Pyrococcus abyssi*''. *Journal of Bacteriology* 185: 3890, with permission from American Society for Microbiology.

cultivation techniques appear to have isolated all of the dominant morphological forms of haloviruses (head-and-tail, spindle shaped, and spherical) that have been observed in hypersaline waters. The current representatives suggest that there may be relatively few dominant virus families infecting the kingdom Euryarchaeota, and that these virus families may be widespread. This view may change radically when the metagenomic studies of archaeal environments, such as those performed by the Venter Institute program, begin to bear fruit. In any case, the major goal now is to develop genetic systems to analyze the viruses already isolated, in order to explore and understand their genetic properties, ecological significance, and evolutionary impact.

See also: Crenarchaeal Viruses: Morphotypes and Genomes; Fuselloviruses of Archaea.

Further Reading

Bamford DH, Ravantti JJ, Rönnholm G, *et al.* (2005) Constituents of SH1, a novel, lipid-containing virus infecting the halophilic euryarchaeon *Haloarcula hispanica*. *Journal of Virology* 79: 9097–9107.

Bath C, Cukalac T, Porter K, and Dyall-Smith ML (2006) His1 and His2 are distantly related, spindle-shaped haloviruses belonging to the novel virus group, *Salterprovirus*. *Virology* 350: 228–239.

Bath C and Dyall-Smith ML (1998) His1, an archaeal virus of the. *Fuselloviridae* family that infects *Haloarcula hispanica. Journal of Virology* 72: 9392–9395.

Dyall-Smith M, Tang S-L, and Bath C (2003) Haloarchaeal viruses: How diverse are they? *Research in Microbiology* 154: 309–313.

Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) (2005) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses.* San Diego, CA: Elsevier Academic Press.

Geslin C, Le Romancer M, Erauso G, Gaillard M, Perrot G, and Prieur D (2003) PAV1, the first virus-like particle isolated from a hyperthermophilic euryarchaeote, ''*Pyrococcus abyssi*''. *Journal of Bacteriology* 185: 3888–3894.

Klein R, Baranyi U, Rössler N, Greineder B, Scholz H, and Witte A (2002) *Natrialba magadii* virus ΦCh1: First complete nucleotide sequence and functional organization of a virus infecting a haloalkaliphilic archaeon. *Molecular Microbiology* 45: 851–863.

Luo Y, Pfister P, Leisinger T, and Wasserfallen A (2001) The genome of archaeal prophage ψM100 encodes the lytic enzyme responsible for autolysis of *Methanothermobacter wolfeii*. *Journal of Bacteriology* 183: 5788–5792.

Meile L, Jenal U, Studer D, Jordan M, and Leisinger T (1989) Characterization of ψM1, a virulent phage of *Methanobacterium thermoautotrophicum* Marburg. *Archives of Microbiology* 152: 105–110.

Pfister P, Wasserfallen A, Stettler R, and Leisinger T (1998) Molecular analysis of. *Methanobacterium* phage ψM2. *Molecular Microbiology* 30: 233–244.

Porter K, Kukkaro P, Bamford JKH, *et al.* (2005) SH1: A novel, spherical halovirus isolated from an Australian hypersaline lake. *Virology* 335: 22–33.

Rössler N, Klein R, Scholz H, and Witte A (2004) Inversion within the haloalkaliphilic virus ΦCh1 DNA results in differential expression of structural proteins. *Molecular Microbiology* 52: 413–426.

Tang S-L, Nuttall S, and Dyall-Smith M (2004) Haloviruses HF1 and HF2: Evidence for a recent and large recombination event. *Journal of Bacteriology* 186: 2810–2817.

Tang S-L, Nuttall S, Ngui K, Fisher C, Lopez P, and Dyall-Smith M (2002) HF2: A double-stranded DNA tailed haloarchaeal virus with a mosaic genome. *Molecular Microbiology* 44: 283–296.

Witte A, Baranyi U, Klein R, *et al.* (1997) Characterization of *Natronobacterium magadii* phage √Ch1, a unique archaeal phage containing DNA and RNA. *Molecular Microbiology* 23: 603–616.

Visna-Maedi Viruses

B A Blacklaws, University of Cambridge, Cambridge, UK

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Dyspnea Shortness of breath.
Endocytosis Where a particle is enveloped by the cell membrane and internalized into a vacuole, an endosome.
Germinal center Area where B cells undergo proliferation after encountering their specific antigen with helper T lymphocytes.
Glomerulonephritis Inflammation of the glomeruli.
Hyperplasia Increase in the number of cells.

Leukoencephalitis Inflammation of the brain caused by leukocytes.
Lymphoid follicle Organized lymphocyte clusters.
Pneumonitis Inflammation of the lung.

Introduction

Visna-maedi virus (VMV) is a retrovirus in the genus *Lentivirus*. Other lentiviruses are human (HIV), simian (SIV), feline (FIV), and bovine (BIV) immunodeficiency viruses, caprine arthritis encephalitis virus (CAEV), and equine infectious anemia virus (EIAV). VMV causes a persistent infection of sheep leading to pneumonitis, demyelinating leukoencephalitis, mastitis, and arthritis, eventually killing the host. VMV originally came to prominence during an epidemic of lung and a separate wasting disease in sheep in Iceland from the 1930s to the 1950s. It was isolated from cases of interstitial pneumonitis (*maedi* = shortness of breath in Icelandic) and demyelinating leukoencephalomyelitis (causes *visna* = wasting in Icelandic) and was the first lentivirus isolated in 1957. Study of the Icelandic epidemic, with scrapie and ovine pulmonary adenomatosis (caused by another retrovirus, Jaagsiekte sheep retrovirus), allowed Björn Sigurdsson to introduce the concept of slow viral infections in 1954. These infections are typified by long incubation periods with pathology developing slowly and progressively, eventually leading to death of the host. The derivation of the genus name, *lenti* (Latin for slow), comes from this.

Clinical lung disease typical of maedi, and in many cases now known to be caused by VMV viruses, has been described since the early 1900s in other countries. Maedi is therefore also known as ovine progressive pneumonia or Montana sheep disease (USA), Graaf Reinet disease (South Africa), zwoegerziekte (the Netherlands), and la bouhite (France). VMV (and/or CAEV) is present in most countries worldwide apart from Iceland (eradicated during the 1940s and the 1950s), Australia, and New Zealand. Where infections are present, economic losses due to loss of milk production, failure to thrive, early culling, and death of animals are seen.

The major target cells for VMV are macrophages and dendritic cells. These are also common target cells for all the other lentiviruses. Aspects of lentivirus infection such as early infection and sites of persistence in accessory cells of the immune system may therefore be studied with VMV without the complication of lymphocyte infection.

Small Ruminant Lentiviruses

Historically, when a lentivirus was isolated from sheep, it was classified as VMV and from goats as CAEV. Although it was known that experimentally VMV could infect goats and CAEV infect sheep, it was not until recently that the natural interspecies transmission of the viruses was shown by molecular epidemiology. Sequencing data from geographically distant countries and from incidences of interspecies transmission into previously uninfected populations shows that VMV and CAEV are able to infect either species as well as other wild small ruminant species. The viruses have therefore now been grouped together as the small ruminant lentiviruses (SRLVs) although the terms VMV and CAEV are also in common use. This article focuses on studies using 'classical' VMV strains in sheep but also draws on data from CAEV and goats.

Visna-Maedi Virus

Virion and Genome

The virion is enveloped with a spherical or coffin-shaped core like other lentiviruses. The virion packages a diploid RNA genome of 9189–9256 bp. Several strains and molecular clones have been fully sequenced. It is a complex retrovirus with three structural genes, *gag*, *pol*, and *env*, and three auxilliary genes, *vif*, *tat* (or *vpr*), and *rev* (**Figure 1**). The functions of the different gene products have been deduced by direct study of VMV and CAEV and also by comparison to HIV and other lentiviruses (**Table 1**).

Replication Cycle

Replication of the virus occurs in a manner similar to that of other retroviruses. Virus binds directly to the cellular receptor or via Fc receptors if coated in antibody. The virion core is released into the cytoplasm by fusion of the envelope with the plasma membrane or, if the particle has been taken up by endocytosis, with the endosomal membrane. The RNA genome is reverse-transcribed to a double-stranded DNA (dsDNA) intermediate (provirus). The process of DNA replication duplicates the RNA termini forming the long terminal repeats (LTRs) of the provirus which contain the virus promoter, mRNA start site, and polyadenylation signals. The provirus integrates into the host cell genome with no apparent preferred site for integration. It is this integrated provirus that serves as template for viral mRNA and genome production. VMV has a complex mRNA expression pattern. *In vitro*, during productive replication of fibroblast cells, there is early synthesis and transport of *tat*/*vpr* and *rev* doubly spliced mRNA to the cytoplasm. Once REV is expressed, late synthesis and transport of *gag*, *pol*, *env*, and *vif* single or unspliced mRNAs or genomic RNA are mediated. This allows expression of the viral structural proteins, virion assembly (by budding through the plasma membrane or into vacuoles), and thus productive replication.

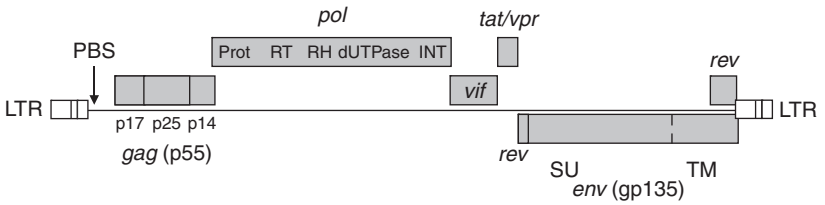


Figure 1 Proviral map of VMV. The provirus structure of VMV is shown with the position of open reading frames indicated. The proviral long terminal repeats (LTRs) contain the U3, R, and U5 regions (in this order) made during reverse transcription. The viral RNA genome contains R/U5 and U3/R at the 5′ and 3′ termini, respectively, where R is a short direct repeat. The primer binding site (PBS) for initiation of reverse transcription is indicated (binds tRNA lysine). The open reading frames are *gag*, *pol*, *vif*, *tat/vpr*, *env*, and *rev*, which contains two exons.

Table 1 Gene products of VMV

ORF	*mRNA*	*Products*	*Function*
gag	Full-length unspliced	Precursor Gag p55 cleaved to:	Major core proteins of virion
		Matrix p17	
		Capsid p25	
		Nucleoprotein p14	
pol	Full-length unspliced	Precursor Gag/Pol polyprotein made by ribosomal frameshift, cleaved to:	
		Protease	Aspartic protease cleaves Gag and Pol
		Reverse transcriptase	Synthesizes proviral cDNA
		RNase H	Degrades genomic RNA to allow second-strand cDNA synthesis
		dUTPase	Reduces dUTP incorporation and so A-to-G mutation in second-strand synthesis. Mutants attenuated *in vitro* and *in vivo*
		Integrase	Integrates provirus into host genome
env	Singly spliced	Precursor Env gp135 cleaved to:	
		Surface subunit, SU gp110	Binds to cellular receptor
		Transmembrane subunit, TM gp41	Fuses viral envelope to cellular membrane (plasma or endosomal) to release virion core into cytoplasm
vif	Singly spliced	VIF	Assumed to be like HIV-1 VIF: inhibits incorporation of cytidine deaminases into virion and so stops new cDNA degradation or G-to-A mutations. Mutants attenuated *in vitro* and *in vivo*
tat/vpr	Doubly spliced	TAT/VPR	Historically called TAT but little evidence to support TAT function, more similar to VPR: LTR has no TAR element, high promoter activity without TAT, and TAT increases promoter activity by two- to threefold; cellular location nuclear; not secreted; packaged in virion; arrests cells in G2 phase of cell cycle. Mutants viable *in vivo* and *in vitro*
rev	Doubly spliced	REV	Interacts with rev-responsive element (RRE) in *env* coding region to allow export of unspliced and singly spliced mRNA species from the nucleus

Target Cells

Monocyte/macrophages and dendritic cells are the major target cell type infected *in vivo* although other cell types have also been shown to either harbor viral nucleic acid or express viral protein, for example, lung, gut, and mammary gland epithelial cells. The frequency of infected cells *in vivo* is very low with often less than 1% of the macrophage population being infected. There is no productive infection of lymphocytes unlike the primate lentiviruses. The range of cells that VMV infect *in vitro* is wider than macrophages with fibroblasts, an important resource for growing virus in the laboratory, and epithelial, endothelial, and smooth muscle cells all reported to replicate virus.

Replication in the monocyte/macrophage lineage is tightly linked to the maturation stage of the macrophage. Three replication states have been suggested: latent,

restricted, and productive. Latent is where cells contain provirus but do not express viral products. In productive infection *in vitro* and *in vivo*, cells express approximately 5000 copies of viral RNA per cell, contain viral proteins, and produce virions. *In vivo* however, most infected cells express low quantities (approximately 5–200 copies) of viral RNA but no protein. This is called restricted replication and is seen in immature macrophages, for example, blood monocytes. It is only when macrophages mature in tissues that productive viral replication is found. Thus macrophages have been called 'Trojan horses' in SRLV infection in that they silently deliver the virus to tissues where virus replication can occur.

The cellular receptors for SRLV have not been identified although VMV strains K1514 and EV1 use a receptor(s) widely expressed on different cells and species. For K1514 this has been mapped to sheep chromosome 3p and the syntenic region of human chromosome 2 (2p25 > q13), and for EV1 to mouse chromosomes 2 and/or 4. If a one-component receptor is assumed, this excludes ovine major histocompatibility complex (MHC) class II (previously identified as a possible receptor by Env binding) and many of the cellular proteins used as co-receptors by other lentiviruses, for example, CXCR4 and CCR5. Studies using viral binding to cellular blots have identified possible candidate receptor proteins that have not yet been fully identified although a membrane proteoglycan is implicated. CAEV strains 63 or Cork may use a receptor which is much more restricted in expression in different species and although it has not been identified, is known, from interference and inhibition assays, to be different from the receptor for VMV strain K1514.

The cellular tropism of VMV is also affected by the ability of the virus to express the provirus. Different promoter activities are the consequence of LTR sequence and its ability to bind different transcription factors. Unlike the primate lentiviruses, SRLV LTRs have no NF-κB binding sites, but interact with AP-1 (only K1514), AP-4, and the AML/PEBP2/CBF family of transcription factors. Nothing is known about the changes that occur in maturing macrophages to allow viral expression although replication is known to be dependent on activation of the extracellular signal-related kinase/p38 mitogen-activated protein (ERK/MAP) kinase pathways.

Transmission

There are two important routes of transmission for VMV. The first is respiratory by aerosolized lung exudates between animals that are closely housed and/or in contact for long periods of time. The second is oral by colostrum/milk from infected dams to offspring.

As VMV is usually cell associated *in vivo* (there are few reports of cell-free virus), it has commonly been thought that the infective moiety in lung exudates and colostrum/milk was virus-infected macrophages or epithelial cells. However, recent data suggest that cell-free virus may be important in respiratory transmission. The presence of cell-free virus in the lining fluid of lungs with severe lesions has been shown and the lower lung is a more efficient point of infection than the trachea. Aerosolized particles small enough to reach the lower lung could contain free virus but infected cells would be too large. Once in the lower lung, a variety of possible target cells are present: macrophages (both alveolar and interstitial), dendritic cells, and lung epithelial cells, all of which may allow virus replication.

It is less clear in studies looking at oral transmission of VMV to lambs using colostrum/milk whether cell-free virus or virus-infected cells are important. Induction of lactation corresponds to virus expression in mammary tissue and colostrum contains cell-free virus as well as infected macrophages and epithelial cells. In a transmission study in lambs infected naturally by colostrum, ileal epithelial cells at the tips of the villi were important in early viral replication with virus antigen expression also seen in mononuclear cells in the lamina propria (macrophages), Peyer's patches, and mesenteric lymph nodes (macrophages and dendritic cells). Cell-free virus in colostrum may infect epithelial cells, but these cells could also transcytose viral particles. Similarly infected cells, especially macrophages in colostrum/milk, could transcytose into the lamina propria or beyond to infect the lamb. The appearance of infected mononuclear cells in the lamina propria within 10 h suggests that this route of infection also occurs. Therefore both cell-free and cell-associated virus could infect lambs via colostrum.

Immunity

Acute Infection Cannulation Model

Immune responses to VMV are slow to be detected in blood after natural infection. Seroconversion may take 6 months to 2 years to occur and sporadic T-cell reactivities in peripheral blood lymphocytes are often reported. However, when infection is studied in an acute infection cannulation model (**Figure 2**), immune responses are detected much earlier. The induction of antibody specific for Gag p25 can be shown within 4 days by enzyme-linked immunosorbent assay (ELISA) and neutralizing antibody (presumably specific for Env) within 10 days in efferent lymph. T-cell reactivity (by proliferation to Gag antigen and VMV-specific cytotoxic T-lymphocyte precursors (pCTLs)) is detected within 7–15 days. A low percentage of sheep also show directly active cytotoxic T lymphocytes (CTLs) in efferent lymphocytes. Therefore the kinetics of the immune response to VMV are within normal ranges seen to other viruses. However, it takes

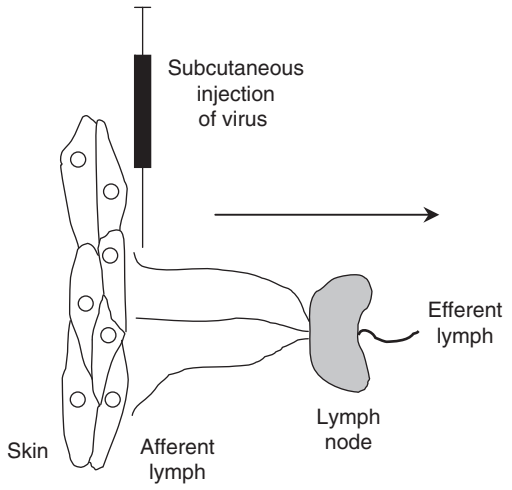


Figure 2 Lymphatic drainage diagram. The acute infection cannulation model for VMV infection of sheep uses subcutaneous injection of virus. The cellular drainage from the site of infection may be collected by cannulation of the lymphatic vessels draining the area. The lymph node directly draining the site of infection may also be biopsied. Lymphatic drainage flows from the tissue through the afferent lymph to the lymph node and then to the efferent lymph (indicated by an arrow). Virus in lymph cells (detectable by 1–4 days) or plasma (none detected) may be quantified, cells carrying virus identified (macrophages and dendritic cells), and immune reactivity monitored (see text). Even using this model, infected cells are at very low frequency, for example, 1 in 10^5–10^6 cells, although increased levels of infected cells rise to a peak by approximately 10–14 days and then decrease to low persistent levels.

much more time for this immune reactivity to be detected systemically. The slow replication rate of VMV and low frequencies of virus-infected cells may be responsible for this weak systemic immune response.

Antibody Responses

In blood, the antibody response is first seen to Gag antigens, in particular capsid antigen p25 (usually 1 month after experimental infection). The anti-Env response develops slowly, but as disease develops it eventually predominates. Other viral proteins also induce antibody responses. Neutralizing antibody responses are targeted to Env and develop very slowly (usually 2–3 months after experimental infection). Virus neutralization causes inhibition of viral binding to the cell (using fibroblasts as target cells) or inhibition of viral reverse transcription and/or integration (using macrophages as target cells). At least five epitopes have been defined in Env by monoclonal antibody reactivity and one immunodominant region is found in transmembrane (TM) Env. There are regions of hypervariability in Env sequences linked to antibody reactivity and escape (see below). VMV-specific antibodies are also detectable in colostrum and milk, cerebrospinal fluid (CSF), and synovial fluid.

Good IgM and IgG1 responses to VMV are induced but in natural infection, sheep do not make an IgG2-isotype response to viral proteins. Persistently infected sheep are able to make an IgG2 response to viral proteins immunized in adjuvant, and it is therefore thought that there may be abnormalities in the antigen-presenting function or T-cell help to B cells during natural infection. This isotype restriction is not seen in goats infected with CAEV; indeed, the predominant isotype induced is an indicator of whether pathology may develop, with strong IgG1 responses to Env SU associated with development of arthritis. The antiviral antibodies induced precipitate viral antigen in agar gel immunodiffusion tests and activate complement but the lack of an IgG2 response is associated with the lack of measurable antiviral antibody-dependent cellular cytotoxicity (ADCC) reactivity in antibody from infected sheep.

T-Lymphocyte Responses

Peripheral blood T-lymphocyte (PBL) proliferative responses to virus antigens are often intermittent with reactivity induced by 1–6 weeks post infection or later, depending on route and amount of virus challenge. Proliferating cells respond to Gag and Env although other viral antigens have not been tested. The response to Gag is mediated by CD4+ T cells. Proliferative T-cell responses to VMV have been detected in CSF by 3 weeks post infection and this reactivity coincides with the initiation of lesion development. Directly active CD8+ CTLs are seen in a low proportion of infected sheep (<20%) but all sheep develop pCTLs (CD8+ T lymphocytes) in their PBL by 3 months after experimental infection. These are induced by day 12 in lymph nodes draining the site of infection and are detectable in lymph nodes and efferent lymph of persistently infected sheep. Infected macrophages can present antigen via MHC class II to stimulate CD4+ T lymphocytes and MHC class I to act as targets for CD8+ CTL as well as to stimulate effector CTL function. Infected macrophages in lesions may therefore present antigen to lymphocytes.

Protective Immune Responses

Protective immune mechanisms have not been defined against VMV. After experimental infection, viral-infected cell frequencies peak, usually 1–2 weeks post infection, and then drop to levels maintained during persistent infection, suggesting that immune mechanisms are induced which control the level of infection. However, experiments to define protective T-lymphocyte subset(s) by *in vivo* antibody depletion of lymphocytes have not been successful at defining protective cell types. Indeed the experiments showed that CD4+ T lymphocytes are required for efficient infection of sheep by

VMV although this lymphocyte subset is not infected. In contrast to the immunodeficiency viruses, no role was detected for CD8+ T cells in control of virus replication. However, the low levels of VMV replication and the return of CD8+ T lymphocytes to the peripheral circulation within 14 days of the start of depletion may mean that sufficient CTL function remained to control the infection. Further work therefore needs to be carried out to investigate protective immune mechanisms against VMV.

Immune Abnormalities

There is no gross immunodeficiency after infection by SRLV; however, there are conflicting reports of immune abnormalities in SRLV-infected animals including changes in total immunoglobulin levels, CD4/CD8 T-cell ratios, decreased suppressor cell activity, and reduced delayed-type hypersensitivity (DTH) responses in skin and increased responses to certain bacterial infections which may be traced to macrophage dysfunction. The specific clinical state of animals is hard to normalize between studies and this may lead to the differing results.

Pathogenesis

Viral Entry

Cell-free virus or virus-infected cells enter the body where they may infect dendritic cells or macrophages in the primary infection site. These cells carry virus to the lymph node where infection is established and then macrophages leaving the lymph node disseminate virus throughout the body leading to a persistent infection. An important tissue for persistent infection of the animal is the bone marrow. Small foci of cells showing restricted replication of VMV have been identified which also express macrophage markers. These are probably myeloid stem cells although no study has defined these well. Infected stem cells may act as a continual source of infected monocyte/macrophages and/or dendritic cells which constantly seed the periphery and tissues. Where the cells mature and express viral antigen, the immune response to the infected cells then causes pathology. Recent molecular data question this theory as very few bone marrow samples from CAEV-infected goats assayed by polymerase chain reaction (PCR) for both proviral DNA and RNA were positive for virus. However, the levels of infection in bone marrow have not been defined well and may be below the detection limit of the assays used. Further work must be done to clarify this point. If bone marrow is not a constant source of infected cells, the virus must be maintained in infected tissues, perhaps lymph nodes and spleen. *In viv*o VMV may be isolated from many tissues including lung, mammary gland, joints, CNS, lymph nodes, spleen, and bone marrow. However, Kupffer cells of the liver are not permissive for virus infection and liver is not involved in pathology of the infection.

Clinical Signs

Infection by VMV does not cause obvious clinical signs for many months and sometimes years (2–5 years). Once clinical signs occur there is a progressive increase in the severity of the clinical signs with time. The diseases caused by SRLV are all typified by chronic active inflammatory processes. Within tissues, there is lymphocyte infiltration and proliferation, and the formation of organized lymphoid follicles and germinal centers. Lesions consist mostly of lymphocytes, macrophages, and plasma cells, often with CD8+ T lymphocytes predominating. This is overlaid with tissue-specific pathology (see **Table 2** for affected organs). Rarely, more acute disease may be seen in younger animals, usually leukoencephalitis, arthritis, or pneumonitis with high mortality.

Viral Cytopathic Effect or Immunopathology?

What causes the gradual buildup of pathology is not clear but it has two contributing factors: the effect of virus infection on infected cell and tissue function and the immune response to the virus. Little is known about the direct effect of virus on tissues although peptide analogs of the *tat/vpr* gene product are neurotoxic in rats and mice transgenic for the *tat/vpr* gene show lymphoproliferative disorders. Indeed, the number of virus-infected cells in lesions is low, so direct viral damage may be minimal.

There is a major immunopathological component to lesion formation. Early studies using immunosuppression reduced the number of CNS lesions seen in sheep infected intracerebrally, although the extent of infection within the CNS was the same, suggesting that direct viral toxicity was not a mediator of pathology. Similarly immunization with viral antigen before or after infection or superinfection with virus often increases the level of pathology seen. There is a positive correlation between virus load and pathology, and it is thought the immune response to viral antigen drives lesion formation. This is difficult to comprehend in early lesions where little virus antigen is detectable but once started, a positive-feedback loop of inflammation and increased virus expression drives progressive damage.

Both lymphocytes and macrophages taken from tissues with pathology show an activated phenotype: for example, they upregulate MHC class I and II, and adhesion molecule expression. However, there is variability in findings with functional assays and cytokine expression as to whether cells are activated or inhibited. Alveolar macrophages from lungs with pneumonitis show increased production of granulocyte monocyte colony stimulating

Table 2 Diseases caused by SRLV

Clinical sign (name)	*Age of animal infected*	*Pathology*	*Infected cells*
Dyspnea/ pneumonia (maedi)	Adult sheep, 2–5 years old Goats, 1–6 months old	Interstitial pneumonitis with perivascular infiltrates; lymphofollicular hyperplasia (some with germinal centers) around vessels, bronchi, and bronchioles; smooth muscle hyperplasia near terminal bronchioles and alveolar ducts	Type I and II pneumocytes, interstitial and alveolar macrophages, endothelial cells and fibroblast-like cells Cell-free virus in lung lining fluid
Wasting and ascending paralysis (visna)	Adult sheep, 2–5 years old Goats 1–6 months old	Periventricular encephalitis (white matter) with perivascular cuffing and infiltration of the parenchyma by mononuclear cells; focal demyelination in brain and spinal cord; meningitis; areas of necrosis; raised cell numbers in CSF; intrathecal antibody production	Interstitial macrophages, microglial cells, rare endothelial, choroidal epithelial cells and fibroblasts of choroid plexus, astrocytes Cell-free virus in CSF rare
Enlarged, hardened udder ('hard bag')	Adult ewes and does	Indurative mastitis with lymphoid hyperplasia, and plasma cells in glandular interstitium, occasional infiltrates into ductal walls and lumens; periductal proliferation and follicle formation; gradual loss of epithelium; diffuse fibrosis; increased numbers of cells and cellular debris in lumen causing increased somatic cell count in milk	Epithelial cells, macrophages, endothelial cells and fibroblast-like cells infected Cell-free virus in colostrum/milk
Swollen joints, lameness ('big knee')	Goats, 1–2 years old Rare in sheep	Chronic arthritis with hyperplastic synovial membrane; subsynovial mononuclear cell infiltrates (follicle formation with germinal centers); increased synovial fluid with raised numbers of lymphocytes (some macrophages and synovial cells) and IgG1 levels (produced *in situ*); angiogenesis in villi and subintima; areas of cellular necrosis; frequent mineralization; increase in fibrous connective tissue around synovial membranes	Synovial membrane cells, macrophages, fibroblasts, endothelial cells. Cell-free virus in synovial fluid
Other affected organs			
Lymphadenopathy		Cortical hyperplasia (increased T cells); germinal center expansions and increased B-lymphocyte areas	Macrophages (and dendritic cells)
Kidney		Glomerulonephritis and interstitial nephritis; medulla and corticomedullary junction lesions; proliferation of mesangial and kidney endothelial cells; lymphatic clusters and follicles	Tubular epithelial cells, macrophages
Third eyelid		Lymphoproliferative inflammation	Macrophages, and glandular, ductal and surface epithelia
Skin		Decreased DTH responses	

factor (GM-CSF), interleukin (IL)-8, and fibronectin but not IL-6, IL-10, or transforming growth factor (TGF) β. Phenotypic changes seen *in vivo* may be caused by immune activation during the inflammatory reaction; however, some studies on infected macrophages *in vitro* suggest direct viral effects on cytokine expression and function. VMV infection of macrophages causes increased expression of IL-8 mRNA but decreased phagocytic and chemotactic activity. MHC class I and II, and LFA-1 and -3 expression are unaffected by infection. CAEV-infected macrophages show a decreased response to bacterial products: inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF)-α, IL-1β, IL-6, and IL-12p40 induction are all affected. Infection of cells has also been shown to lead to cell death via apoptosis. Infection of macrophages has a complex effect on function which is also altered by the presence of other cells. Co-culture of VMV-infected macrophages with autologous lymphocytes induces the secretion of lentivirus-induced interferon (lentiferon). This is probably a mixture of interferon (IFN)-α and IFN-γ. Effects of lentiferon include inhibition of proliferation and maturation of monocytes and

of virus assembly in infected cells, thus decreasing virus replication, but it also increases MHC class II expression on macrophages which may contribute to lymphoproliferative responses.

The cytokines important in upregulating versus inhibiting VMV expression are poorly understood, partly due to the lack of purified ovine cytokines. Only GM-CSF has been shown to increase VMV transcription and antigen production in macrophages. GM-CSF and TNF-α treatment of cells increases CAEV LTR promoter activity and so may cause increased virus replication. However, there is mixed evidence as to the role that type I IFN plays against VMV infection: pretreatment of choroid plexus cells with a source of ovine type I IFN does not inhibit VMV replication, while pretreatment of cells with human IFN-α does block replication. *In vivo* treatment of lambs early in infection with IFN-τ reduces virus replication and disease development. Although not a cytokine, NO is also known to inhibit virus replication in macrophages.

Autoimmunity

The lesions caused by SRLV in the CNS and joints are very similar to those seen in multiple sclerosis and rheumatoid arthritis of humans. These have links to autoimmunity, and indeed autoimmunity would explain the chronic development of pathology seen in these infections. However, there is very little evidence for this as a mechanism of lesion formation with VMV. There is no response to myelin basic protein (lymphocytes or antibody) or lipid antigen (antibody) in sheep with visna although elevated levels of autoimmune antibody (specific for rheumatoid factor, single-stranded DNA (ssDNA), and cardiolipin) in serum have been detected in infected sheep.

Role of Lymphocytes

CD4+ T lymphocytes are important in promoting infection and lesion formation. At the time of acute infection, CD4+ T lymphocytes are necessary for efficient establishment of infection and later lymphocytes are necessary for lesion development. Therefore, immune responses to unrelated antigens, whether secondary infections or experimental immunizations or vaccinations, may cause increased inflammatory reactions that accelerate lesion development.

Role of the Host and Virus Genetics

Breed resistance to infection and disease development has been documented in sheep and goats. In sheep, the Icelandic breed was particularly susceptible to VMV infection and development of visna, while the Karakul flock, from which infected animals introduced the disease to Iceland, never showed signs of disease. Border Leicester rams are resistant to visna but develop arthritis. This and agricultural practice (e.g., are animals milked?) may explain, in part, the geographical distribution of clinical signs of VMV infection; for example, Iceland commonly saw pneumonitis and encephalomyelitis, UK usually notes pneumonitis, and Spain has documented pneumonitis and mastitis. With interspecies transmission of SRLV, it is not clear whether strains more closely resembling VMV in goats will cause increased incidences of pneumonic disease or whether it is goat genetics which determines joints as a major site of lesion development. In USA, the VMV strains isolated are more closely related to CAEV than European VMV strains and yet pneumonia is still commonly seen in sheep. In goats with CAEV, where only $\sim$30% of animals develop arthritis, linkages to certain MHC class I alleles have been associated with development of disease.

Strains of virus that can be differentiated phenotypically *in vitro* can cause different patterns of lesion development. Usually strains growing to high titer and causing cytopathic effects in macrophages are the most virulent *in vivo*. Strains selected for increased neurovirulence grow better in sheep choroid plexus cells than those that cause pneumonia. This is linked to LTR sequence variation. Therefore, both host and viral genetics are important elements in disease progression.

Immune Evasion

SRLVs persist in the host despite an immune response that includes antibody, CD4+, and CD8+ T lymphocytes. Several mechanisms of viral immune evasion have been noted that may allow this to occur. First, integration into the host genome allows latent infection and persistence in cells without expression of antigen. Similarly, gene expression is tightly regulated with no expression of antigen in immature macrophages. Thus these cells are invisible to the immune system. Second, low-titer neutralizing antibody responses producing low-affinity antibody mean that virus may be able to dissociate from antibody and infect cells. Third, neutralization escape mutants have been shown to emerge during infection. Mutants arise by point mutation in Env, one of the first documented incidences of antigenic drift in viruses. Later, the neutralizing antibody response may broaden to include these mutants. It is thought these mutants have a selective advantage and help the virus to persist in the host. Whether escape mutants are important in VMV pathogenesis is debatable as the original parental virus may be isolated late after infection, showing it has not been cleared from animals and replaced by the mutants. Similarly, neutralization mutants arise when antibody is not present, and their presence is unrelated to severity of pathology. Fourth, the ability of VMV to cause cell fusion means that

cell-free virus is not required (and very rarely seen *in vivo*) as virus may pass from cell to cell directly and so is not exposed to antibody. Fifth, antibody-mediated enhancement of infection of macrophages has been shown in which antibody-coated virus is taken up into macrophages via Fc receptors, thus increasing the effective viral dose. Sixth, there is no ADCC response to the virus. Seventh, although technically not immune evasion, the virus uses the CD4+ T-cell response to help replication, either by activation of macrophages or attraction of macrophages to the relevant tissue.

Control

Because of its insidious nature, control of VMV infection is difficult. Due to the economic losses and animal welfare problems that the disease causes, control programs are in place in many countries. These are based on serological tests, usually ELISAs (often to capsid p25 and TM Env) or agar gel immunodiffusion tests. However, because of the length of time taken for induction of a detectable antibody response in blood, and the possibility of fluctuations in the response, testing regimes involve repeated tests to maintain accreditation of SRLV-free status. PCR has not been used routinely for VMV diagnosis due to the strain variation that is present within SRLV and the low provirus load in blood of persistently infected animals.

Several options are open to try to derive SRLV-free flocks. Iceland eradicated the disease in the 1940s and the 1950s by culling all infected flocks and restocking from SRLV-clean animals. Others have used removal of serologically positive animals (and their progeny) from the flock after each test round until serologically free status is achieved. In the Netherlands, there was a successful policy of re-deriving flocks from 'snatched' lambs. These are lambs taken at birth from their mothers before they suckle infected colostrum. The lambs are then hand-reared on either heat-inactivated or bovine colostrum. Once VMV-free flocks are achieved, careful control of importation of animals into flocks/countries is necessary to maintain this status, as it is importation of infected asymptomatic animals that then live in close contact with the host flock that often leads to spread of infection into previously free areas, for example, Iceland (1933) and Finland (1981). With proof now of interspecies transmission, SRLV-free status will only be maintained if both sheep and goats are kept separate or both species kept SRLV free.

Treatment and Vaccination

At present, there is no treatment against infection although certain anti-retroviral drugs do have activity against VMV. There have now been several vaccination studies using inactivated virus preparations, live-attenuated viruses, and subunit vaccines administered by a variety of vectors, for example, protein, plasmid, and vaccinia virus, using a variety of routes and different adjuvants or cytokine immunomodulators. None has produced sterilizing immunity and some have increased the levels of pathology seen. Induction of neutralizing antibody by immunization with recombinant Env has proven problematic using baculo- and vaccinia virus-expressed antigen in sheep. The best result has been with bacterial-expressed Env gp70 but only low titers of antibody were seen using this immunogen. Some vaccines have reduced virus load early in infection but pathology has still developed, although in some cases this is less severe than control animals. The most promising result has come from CAEV in goats using DNA prime and then protein boost with Env in Freund's incomplete adjuvant. There was decreased viral replication in lymph node and synovium, and reduced development of severe arthritis for more than 18 months using this vaccine.

The possibility of inducing antibody that enhances the ability of virus to infect macrophages, the induction of CD4+ T lymphocytes responses that may increase the efficiency of the primary infection, as well as increasing the pathological response are all aspects of the immune response to VMV that needs much further study before a successful vaccine will be released to the field.

Conclusions

VMV has many features in common with the other lentiviruses including infection of macrophages and dendritic cells. There is now an understanding of the type of lesion VMV induces in many tissues. However, there is still much work to be done with VMV to answer questions on sites of persistence of the virus, triggers of lesion development, protective immune responses, and vaccine control of the infection.

See also: Equine Infectious Anemia Virus; Human Immunodeficiency Viruses: Molecular Biology; Human Immunodeficiency Viruses: Pathogenesis; Immune Response to viruses: Antibody-Mediated Immunity; Simian Immunodeficiency Virus: Animal Models of Disease; Simian Immunodeficiency Virus: General Features; Viral Pathogenesis.

Further Reading

Blacklaws B, Bird P, and McConnell I (1995) Early events in infection of lymphoid tissue by a lentivirus, maedi-visna. *Trends in Microbiology* 3: 434–440.

Blacklaws BA, Berriatua E, Torsteinsdottir S, *et al.* (2004) Transmission of small ruminant lentiviruses. *Veterinary Microbiology* 101: 199–208.

de Andres D, Klein D, Watt NJ, *et al.* (2005) Diagnostic tests for small ruminant lentiviruses. *Veterinary Microbiology* 107: 49–62.

Eriksson K, McInnes E, Ryan S, *et al.* (1999) CD4(+) T-cells are required for the establishment of maedi-visna virus infection in macrophages but not dendritic cells *in vivo*. *Virology* 258: 355–364.

Eriksson K, McInnes E, Ryan S, *et al.* (1999) *In vivo* depletion of CD8+ cells does not affect primary maedi visna virus infection in sheep. *Veterinary Immunology and Immunopathology* 70: 173–187.

Gendelman HE, Narayan O, Kennedy-Stoskopf S, *et al.* (1986) Tropism of sheep lentiviruses for monocytes: Susceptibility to infection and virus gene expression increase during maturation of monocytes to macrophages. *Journal of Virology* 58: 67–74.

Gendelman HE, Narayan O, Molineaux S, *et al.* (1985) Slow, persistent replication of lentiviruses: Role of tissue macrophages and macrophage precursors in bone marrow. *Proceedings of the National Academy of Sciences, USA* 82: 7086–7090.

Georgsson G, Pálsson PA, and Pétursson G (1990) Some comparative aspects of visna and AIDS. In: Racz P, Haase AT, and Gluckman JC (eds.) *Modern Pathology of AIDS and Other Retroviral Infections*, pp. 82–98. Basel: Karger.

Haase AT (1986) Pathogenesis of lentivirus infections. *Nature* 322: 130–136.

Peterhans E, Greenland T, Badiola J, *et al.* (2004) Routes of transmission and consequences of small ruminant lentiviruses (SRLVs) infection and eradication schemes. *Veterinary Research* 35: 257–274.

Ravazzolo AP, Nenci C, Vogt HR, *et al.* (2006) Viral load, organ distribution, histopathological lesions, and cytokine mRNA expression in goats infected with a molecular clone of the caprine arthritis encephalitis virus. *Virology* 350: 116–127.

Ryan S, Tiley L, McConnell I, and Blacklaws B (2000) Infection of dendritic cells by the maedi-visna lentivirus. *Journal of Virology* 74: 10096–10103.

Sigurdsson B (1954) Mædi, a slow progressive pneumonia of sheep: An epizoological and a pathological study. *British Veterinary Journal* 110: 255–270.

Sigurdsson B (1954) Rida: A chronic encephalitis of sheep. With general remarks on infections which develop slowly and some of their special characteristics. *British Veterinary Journal* 110: 341–354.

Thormar H (2005) Maedi-visna virus and its relationship to human immunodeficiency virus. *AIDS Reviews* 7: 233–245.

W

Watermelon Mosaic Virus and Zucchini Yellow Mosaic Virus

H Lecoq and C Desbiez, Institut National de la Recherche Agronomique (INRA), Station de Pathologie Végétale, Montfavet, France

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Mild-strain cross-protection A plant systemically infected by a mild virus strain will not develop additional symptoms when inoculated by a severe strain of the same virus. Most often, the severe strain does not multiply in the cross-protected plant.
Filiformy A leaf deformation symptom in which the leaf blade is drastically reduced but not the veins, giving a shoestrings aspect to the leaf.
Transmission propensity A measure of vector importance quantifying the natural ability of a species to inoculate a plant with a virus under conditions that allow vectors to move and feed freely.

History and Taxonomy

Watermelon Mosaic Virus

Mosaic diseases of cucurbit crops were first reported in the 1920s and the early literature contains a diversity of names for viruses or virus diseases that were only partially characterized. In 1940, Milbrath reported a severe watermelon (*Citrullus lanatus*) mosaic disease in California, but the nomenclature of the causal agent remained controversial until 1979. In 1965, Webb and Scott compared ten isolates from southern USA of what was then called the watermelon mosaic virus (WMV) complex. Based on cross-protection experiments, serological relationships and host range reactions they divided them into two groups: WMV1 and WMV2. WMV1 and WMV2 were considered as different viruses, also different from a watermelon mosaic isolate reported from South Africa in 1960 by van Regenmortel. Further work by Milne and Grogan in 1969 increased the confusion by concluding that WMV1 and WMV2 were strains of the same virus. In 1979, Purcifull and Hiebert definitively clarified the situation by demonstrating that WMV1 and WMV2 were indeed serologically distinct entities, and that WMV1 was closely related to papaya ringspot virus (PRSV). Today, WMV1 is considered as the W strain of PRSV, while WMV2 is referred to as watermelon mosaic virus (WMV). In addition, the same authors showed that a watermelon mosaic virus isolate from Morocco was a third serological entity. This isolate is considered as a distinct virus, Moroccan watermelon mosaic virus (MWMV), to which probably also belongs the South African isolate.

So, from the initial watermelon mosaic virus complex emerged three different virus species: *Watermelon mosaic virus*, *Papaya ringspot virus*, and *Moroccan watermelon mosaic virus*.

Zucchini Yellow Mosaic Virus

An apparently new cucurbit virus was isolated in 1973 from a zucchini squash (*Cucurbita pepo*) plant in Northern Italy, and Lisa *et al.* described this virus as belonging to a new potyvirus species, *Zucchini yellow mosaic virus*, in 1981. In 1979, many melon (*Cucumis melo*) crops were devastated in southwestern France by an apparently new virus disease, whose causal agent was tentatively named muskmelon yellow stunt virus; very rapidly, it appeared that it was a strain of zucchini yellow mosaic virus (ZYMV). Within a few years, ZYMV was reported in many countries on the five continents, and in this regard, ZYMV appears as a typical example of an emerging plant virus.

Classification

Based on particle morphology, aphid transmissibility, serological relationships, ability to induce pinwheel cytoplasmic inclusions in host cells, genome organization, and nucleotide sequences, WMV and ZYMV were identified as members of the genus *Potyvirus*, family *Potyviridae*. Several other potyviruses have been shown to infect

cucurbit crops including PRSV-W, MWMV, zucchini yellow fleck virus (ZYFV), melon vein banding mosaic virus (MVBMV), telfairia mosaic virus (TeMV), turnip mosaic virus (TuMV), clover yellow vein virus (ClYVV), and bean yellow mosaic virus (BYMV). However, these viruses have either only limited geographic distribution or minor economical incidence.

Molecular analyses based on the coat protein (CP) coding sequence revealed that cucurbit-infecting potyviruses belong to several 'clusters' of closely related species: ZYMV and WMV belong to a cluster that also contains mostly legume-infecting potyviruses, whereas PRSV, MWMV, and ZYFV are grouped in a 'PRSV-like' cluster containing mostly cucurbit-restricted viruses (**Figure 1**).

Symptoms

Watermelon Mosaic Virus

WMV induces a diversity of symptoms according to the isolate and the host cultivar. On leaves, symptoms are mosaics, vein banding, more or less severe leaf deformations, and filiformy. Some isolates induce discoloration and deformation on fruits of zucchini squash susceptible cultivars while other isolates do not affect fruit and yield quality. Mosaic and discoloration are also observed on leaves and fruits of some melon cultivars (**Figure 2**).

Zucchini Yellow Mosaic Virus

Since its first detection, ZYMV was recognized as a virus causing extremely severe symptoms leading to complete yield losses in the case of early contamination. This severity of symptoms was probably an important factor for the rapid identification of ZYMV soon after its first outbreaks, in many countries. In melon, leaf symptoms include vein clearing, yellow mosaic, leaf deformation, occasionally with blisters and enations. There is often a severe plant stunting. Some ZYMV isolates can induce a rapid and complete wilt in cultivars possessing the *Fn* gene. On fruits, a diversity of symptoms are observed: external mosaic or necrotic cracks, internal marbling, and hardening of the

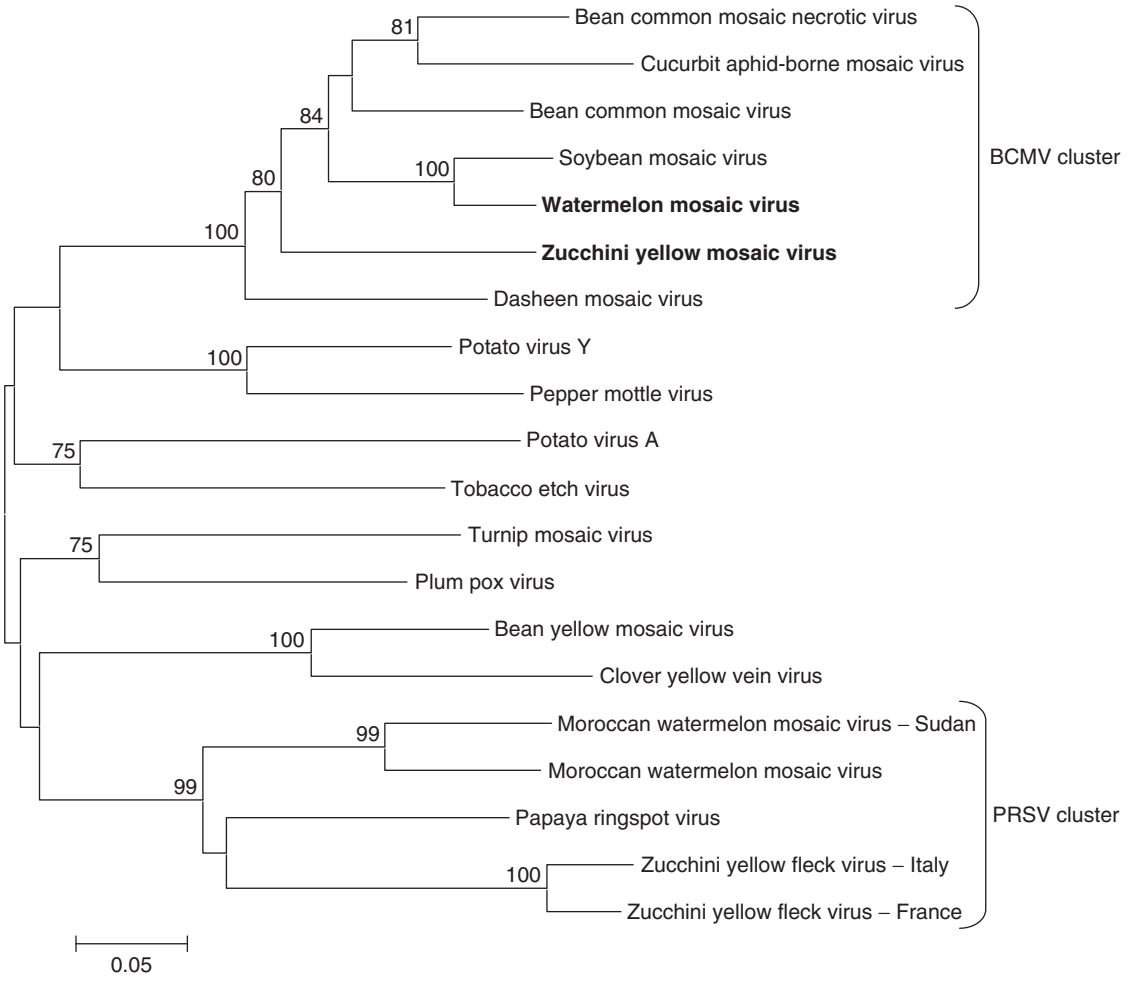


Figure 1 Relationships based on CP amino acid sequences, of cucurbit-infecting potyviruses. Branch lengths indicate the molecular divergence of viruses (the scale bar represents 0.05 mutations per residue). Figures at some nodes represent bootstrap values (in %), indicating the robustness of each node. Only values above 75% are indicated.



Figure 2 Mosaic symptoms on a leaf and fruit of a melon plant infected by a moderately severe WMV isolate.



Figure 3 Severe mosaic and deformations on leaves and fruits of a zucchini squash plant infected by ZYMV. Reproduced from Astier S, Albouy J, Maury Y, and Lecoq H (2001) *Principes de virologie végétale: Génome, pouvoir pathogène, écologie*. Paris: INRA Editions, with permission from QUAE.

flesh. Seeds are occasionally severely deformed and have poor germination rates. In zucchini squash, symptoms are very severe on leaves with mosaic, yellowing, leaf distortion and sometimes very severe filiformy. Fruits are generally severely misshaped with prominent knobs and are of course unmarketable (**Figure 3**). In cucumber (*Cucumis sativus*) and watermelon, mosaic and deformations are generally observed on leaves and fruits.

Synergism

Synergism has been observed between WMV or ZYMV and other viruses infecting cucurbits which can be expressed either by increase in virus multiplication rates or by more severe symptoms. Significant increase in cucumber mosaic virus (CMV) multiplication rate was observed in cucumber plants co-infected by ZYMV. CMV could also partially overcome a resistance in cucumber when in mixed infection with ZYMV. In grafted cucumbers, double infection by CMV and ZYMV induces a severe and rapid wilting reaction which is not observed with infections by CMV or ZYMV alone. When WMV or ZYMV are in mixed infection with the polerovirus, cucurbit aphid-borne yellows virus (CABYV), CABYV multiplication rate and symptom intensity are increased. As for other potyviruses, these synergetic effects could be related to the strong potyvirus gene silencing suppressor.

Geographic Distribution

Both WMV and ZYMV are now widely distributed in the major cucurbit production areas worldwide. Their geographic distributions are broadly overlapping and frequent mixed infections are observed in the fields.

For unknown reasons, WMV appears to be rare or absent in cucurbits in subtropical or tropical areas. For instance, in exhaustive surveys conducted in Nepal, Sudan, and French West Indies, no WMV was detected, although ZYMV was relatively abundant. In Florida, WMV is frequent in northern and central counties but is not detected in southern counties, whereas ZYMV can be found throughout the state. This cannot be related to a lack of potential WMV vectors or reservoirs because both are abundant in tropical or subtropical areas.

ZYMV is present worldwide in almost all countries where cucurbits are grown, under temperate, Mediterranean, subtropical, and tropical climatic conditions. It affects highly mechanized cropping systems (such a glasshouse crop production in Northern Europe) as well as more traditional agroecosystems (such as flood-irrigated crops on the Nile banks). ZYMV has been reported in very remote areas including semi-desertic regions or islands.

Host Range

WMV has a relatively wide experimental host range for a potyvirus. It infects over 170 species in 26 mono- or dicotyledonous families. Besides cucurbits, WMV causes mosaic diseases in legumes (pea, broad bean) and orchids (vanilla, *Habenaria radiata*) and infects many weeds that can serve as alternative hosts. Generally, naturally infected weeds do not present evident symptoms of viral infection.

ZYMV has a relatively narrow host range. In natural conditions, it infects mostly cultivated or wild cucurbits but also a few flower species (*Delphinium*, *Althea*) or weeds.

Diagnostic Method

The confusion that was prevalent in the early descriptions of watermelon mosaic diseases was mainly due to

the convergence of symptoms caused by WMV, PRSV, and MWMV in cucurbits and to the lack of proper diagnostic tools. Symptoms and virus particle morphology were clearly insufficient to differentiate these viruses. The production of specific polyclonal antisera, and the development of simple serological tests, such as the gel double-diffusion test in agar containing sodium dodecyl sulfate and sodium azide (SDS-ID), brought a major contribution to the proper diagnosis of cucurbit potyviruses. In particular, this method contributed to the rapid and unequivocal identification of ZYMV in several countries soon after its first observation. Now, double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) is generalized and commercial kits are available for WMV and ZYMV. Recently, dipstick serological tests based on the lateral flow technique have been developed that allow an easy and rapid diagnosis of ZYMV in the fields.

Monoclonal antibodies (MAbs) have been produced for ZYMV and WMV. They proved to be very useful to study the serological variability and to differentiate ZYMV and WMV subgroups. They have also been used successfully to analyze virus interactions, and in particular cross-protection efficiency and specificity.

Many partial nucleotide sequences are now available for ZYMV and WMV, particularly in the CP coding region, which allowed the development of specific primers for each virus. However, in routine testing, DAS-ELISA seems to be more reliable than RT-PCR.

Vector Relationships

WMV is transmitted by at least 35 aphid species in 19 genera. Fewer aphid species were tested for their ability to transmit ZYMV, and 11 were identified as ZYMV vectors. *Aphis craccivora*, *Aphis gossypii*, *Macrosiphum euphorbiae*, and *Myzus persicae* are efficient WMV and ZYMV vectors. Some aphid species were shown to be poor or nonvectors of WMV and ZYMV, which suggests some level of specificity in the virus–vector interaction.

WMV and ZYMV are transmitted on the nonpersistent mode: they are acquired and transmitted during very short probes (a few seconds to minutes), and their retention period in the vector is relatively short (a few hours).

WMV and ZYMV as typical potyviruses require the presence of a virus-encoded helper component (HC-Pro) protein for transmission. HC-Pro from WMV and ZYMV are interchangeable and both mediate efficiently the transmission of purified virions of both species.

Several ZYMV isolates that have lost aphid transmissibility have been characterized, and a unique feature for this virus is that single amino acid mutants have been identified in the three domains important for transmission. ZYMV-NAT has an A to T substitution in the DAG motif in the CP, ZYMV-PAT a T to A substitution in the PTK motif of HC-Pro, and ZYMV-R1A a K to E substitution in the KLSC motif of HC-Pro. These mutants led to the identification of an interaction between the HC-Pro and CP through their PTK and DAG domains.

The nontransmissible isolate ZYMV-NAT (having the DTG motif in the CP) could be transmitted by aphids from plants infected concomitantly by a transmissible isolate of PRSV. This occurred through heteroencapsidation, a phenomenon by which ZYMV RNA is completely or partially encapsidated by PRSV CP, which is functional in aphid transmission. An aphid nontransmissible isolate deficient for HC-Pro can also be transmitted by aphids when in mixed infection with an isolate that has a functional HC-Pro. The transmissible isolate provides its functional HC-Pro to mediate the transmission of the deficient isolate. These two mechanisms can contribute to the maintenance, in natural conditions, of variants which have lost their vector transmissibility.

An interesting interaction has been observed between ZYMV and *Aphis gossypii*, an aphid vector colonizing cucurbit crops. *Aphis gossypii* lives longer and produces more offspring on ZYMV-infected than on noninfected plants. In addition, more alatae are produced on infected plants, which may stimulate the spread of ZYMV. These phenomena might be related to the observed changes in phloem exudates' composition (free amino acids, sugars) in virus-infected plants.

Epidemiology

Virus Sources

WMV has not been described as seed borne in cucurbits or other crops, but may be transmitted through vegetative propagation in vanilla. ZYMV seed transmission remains controversial. It has been reported in squash but other studies repeatedly failed to observe seed transmission. This is an important issue, since ZYMV seed transmission would be the simplest explanation for the rapid ZYMV dissemination throughout the world in the 1980s. If ZYMV seed transmission does occur, it is at a low rate and may be only for some strains or hosts. Another possible way for long-distance dissemination of ZYMV is through the globalization of vegetable production and trade. It has been shown that ZYMV-infected fruits imported from Central America into Europe could be very efficient virus sources for aphids.

Virus sources from which epidemics will initiate could be overwintering weeds or crops infected during the previous crop season. In tropical and subtropical regions, cucurbit crops or weeds grow all year round, and viruses could easily move from an old infected crop or cucurbit weed to a young planting. In more temperate regions,

non-cucurbit weeds were found to be efficient reservoirs for WMV but not for ZYMV. Winter protected-crops could contribute to ZYMV overwintering in Mediterranean regions and residential gardens were found to be important sources of ZYMV in California.

Efficient Vectors

Many potential aphid vector species have been identified for WMV and ZYMV. A study was conducted to compare the vector capacity of two aphid species, one colonizing cucurbits (*A. gossypii*), the other not (*A. craccivora*). Two parameters were used. Transmission efficiency was measured in the laboratory with single aphids exposed in sequence to an infected plant and then to four healthy plants. Transmission propensity was measured by arena tests (more representative of natural conditions) in which aphids could move between plants and feed without interference. It was shown that *A. craccivora* had both a higher efficiency and propensity to disseminate ZYMV than *A. gossypii*. This highlights the importance of noncolonizing transient vector species in the epidemiology of ZYMV.

Pattern of Spread

The same general pattern is observed for WMV and ZYMV. The first contaminations occur generally shortly after planting, depending upon the availability of virus sources in the environment. The secondary virus spread then occurs when aphid flights, particularly of noncolonizing species, spread the virus from the primary infection foci to the rest of the crop. The diseased plants are often distributed in large patches that rapidly extend and join each other, leading to the complete contamination of the crop within a few weeks. Simultaneously, the infected crop will serve as a source of virus to contaminate weeds or nearby young plantings. Epidemic development curves have an overall S-shape, generally fitting well with the logistic model.

Control Methods

Prophylactic Measures

Prophylactic measures are intended to prevent or limit the contact of viruliferous aphids with cultivated plants. They are not specific for a particular virus and are generally efficient for all aphid-borne viruses. These include careful weeding near plantings and avoiding overlapping crops in the same area to reduce virus and aphid sources near new plantings. Plastic mulches have a repelling action on aphids and significantly delay WMV and ZYMV spread. However, they confer only a temporary protection that is limited to the early stages of the crop, because their efficiency decreases when plant growth covers their surface. Row covers of different types (unwoven, perforated plastics, etc.) can also be used; they physically prevent winged aphids from reaching the plants, but they must be removed to allow insect pollination necessary for cucurbits. Both methods have a major drawback: they require a lot of plastic material that farmers must dispose in an environmentally sound way after the crop cycle. Insecticides applications have generally been found inefficient in limiting WMV and ZYMV spread. This is to relate to the large number of winged aphids that land on the plants and to the rapidity of the transmission process. Oil applications can delay virus spread when inoculum pressure is moderate. When applicable, a 1 month crop-free period has been shown to be efficient in limiting ZYMV virus spread.

Cross-Protection

Although the mechanism of cross-protection has not been fully elucidated (it probably relies on the gene silencing machinery), this method has been developed at a commercial level to protect cucurbit crops against ZYMV. The principle is simple (**Figure 4**): when a mild virus isolate (i.e., that has no significant impact on commercial yield) is inoculated to young seedlings, it protects the plant from subsequent contaminations by severe isolates of the same virus. The mild strain ZYMV-WK is a natural variant of a severe aphid nontransmissible isolate. Although efficient against most ZYMV isolates, ZYMV-WK does not protect against very divergent isolates such as those from Réunion Island, indicating some specificity in the protection. A single amino acid change (R to I) in the FRNK conserved domain of the HC-Pro is responsible for symptom attenuation of ZYMV-WK. A complete technological package (mild strain production, quality control protocols, inoculation machines) has been developed to implement commercially ZYMV cross-protection. Mild WMV isolates have been reported that could also have a potential for cross-protection.

Resistant Cultivars

The use of virus-resistant cultivars is probably the easiest and cheapest way to control plant viral diseases at the farmer's level. Breeding for resistance still relies mainly upon searching for resistance characters in germplasm collections and introgression of the resistance gene(s) into commercially acceptable cultivars. Considerable efforts have been made to look for resistance to WMV and ZYMV in genetic resources, and some WMV- or ZYMV-resistant commercial cultivars are now available. Some resistance genes confer complete and durable resistance (such as the *zym* gene in cucumber), while others confer only partial resistances or may be overcome by virus evolution (such as the *Zym* gene in melon). An

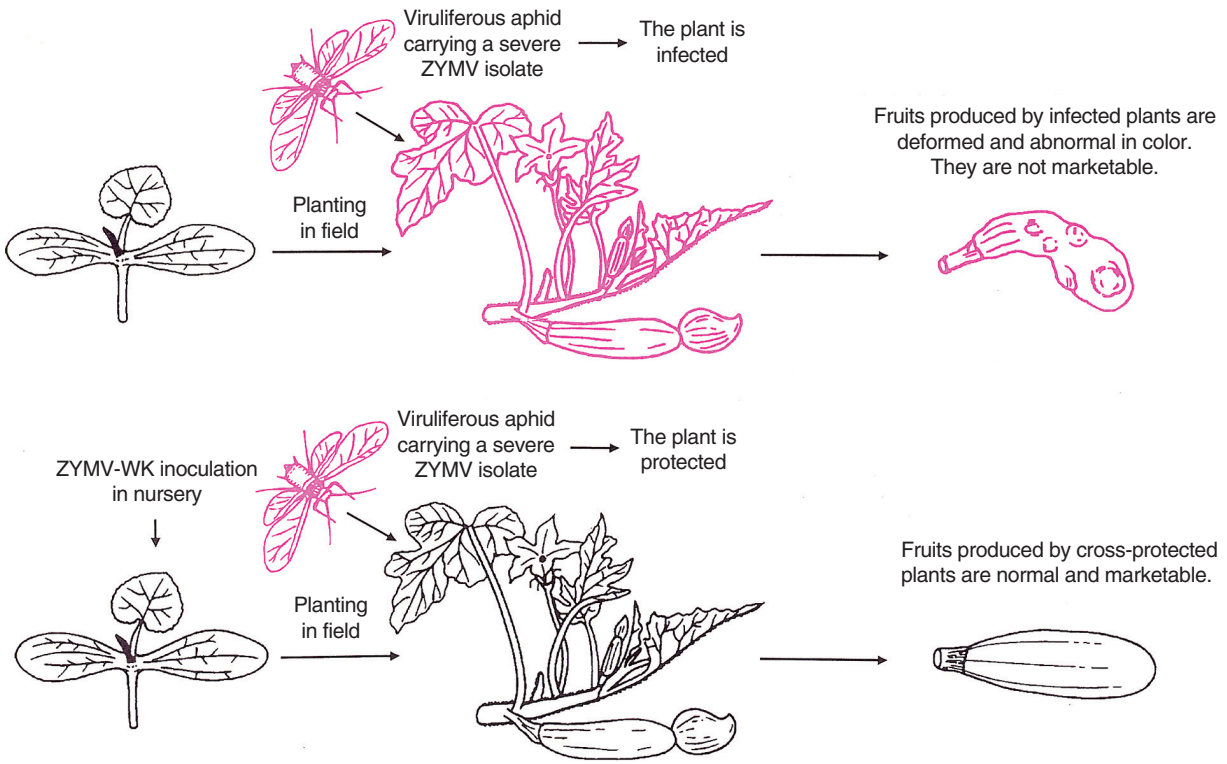


Figure 4 Representation of mild strain cross-protection as applied with ZYMV-WK in zucchini squash. Reproduced from Astier S, Albouy J, Maury Y, and Lecoq H (2001) *Principes de virologie végétale: Génome, pouvoir pathogène, écologie*. Paris: INRA Editions, with permission from QUAE.

interesting situation is observed for ZYMV resistance in squash. Although the resistance level was high in the original accession of *Cucurbita moschata* in which the resistance was identified, when transferred through interspecific crosses to zucchini squash (*C. pepo*), the resistance phenotype was different: ZYMV multiplies but the plants present only very mild symptoms (a phenomenon called tolerance). However, tolerance appears not to be stable since aggressive variants of the virus (i.e., causing severe symptoms in tolerant plants) may emerge in these plants. A single amino acid change in the P3 gene is sufficient to confer this aggressive phenotype. However, the aggressive variants are counter-selected when in competition with common ZYMV isolates in susceptible cucurbits. This genetic load associated with aggressiveness could be a factor that will make the tolerance durable. In melon, a resistance to WMV and ZYMV transmission by *A. gossypii* was found to be governed by the single dominant gene *Vat*. This gene is present in many commercial cultivars, but confers only limited protection in the fields, probably because WMV and ZYMV are also transmitted by many other aphid species in natural conditions.

In the last two decades, attempts were made to obtain WMV and ZYMV transgenic resistant plants using the pathogen-derived resistance approach. Different constructs were tested to obtain resistant WMV or ZYMV plants and the best results were obtained with the full-length CP gene and with ribozymes. Freedom II, a transgenic squash hybrid containing the WMV and ZYMV CP genes, was released in the USA in 1995, as the first virus-resistant transgenic crop to be commercially cultivated in the world. It proved to have a very efficient resistance to WMV and ZYMV in field conditions. Similar biotech crops are presently grown mainly in southeastern USA, particularly during late summer and fall when WMV and ZYMV inoculum pressure is high.

Variability

Only limited biological variability has been reported for WMV. This concerns mainly differences in symptom intensity, host range, or aphid transmissibility. In contrast, from its first description, ZYMV appeared to have a very important biological diversity. When collections of field isolates were compared, an important variability was revealed in host range and symptoms on susceptible hosts, with isolates producing mild or atypical mosaic symptoms, necrosis, or wilting reactions. When these isolates were inoculated to melon or squash varieties possessing resistance genes, different pathotypes could be differentiated. Important variability has also been observed in aphid transmissibility and several aphid nontransmissible or poorly transmissible isolates have been described.

Limited serological variability was observed in WMV and ZYMV when polyclonal antibodies were used. However, the development of monoclonal antibodies against WMV and ZYMV allowed the characterization of serotypes closely correlated to the molecular variability.

The molecular variability of ZYMV and WMV was assessed mostly on the CP region, particularly the N-terminal part of the CP that is known for potyviruses to be highly variable and is frequently used for molecular studies. More than 200 partial CP sequences are now available for ZYMV. Strains from some regions, for example, Réunion Island and Singapore, are highly divergent molecularly, whereas isolates from other regions are more closely related, and fall into three main clusters without geographic structure; more recent sequence data available for Asian strains (China, Korea, Taiwan) indicate that some of those strains tend to locate between previously described groups or clusters. Molecular analyses have allowed, in a few cases, tracking the putative origins of ZYMV strains emerging in a new area.

Genetics and Evolution

WMV and ZYMV genome organization is very similar to that of other potyviruses sequenced so far. The single-stranded, positive-sense RNA genome (9.6 kbp for ZYMV, 10 kbp for WMV excluding the polyadenylated 3′ extremity) is translated as a single polyprotein that is self-cleaved in 10 functional proteins.

The full-length sequence revealed that WMV is related to the legume-infecting soybean mosaic virus (SMV); however, the P1 protein is 135 amino acids longer than that of SMV, and the N-terminal half of P1 shows no relation to SMV but is 85% identical to another legume-infecting potyvirus, bean common mosaic virus (BCMV). This suggests that WMV has emerged through an ancestral recombination event between an SMV-like and a BCMV-like potyvirus. SMV and BCMV have narrow host ranges, mostly restricted to legumes, while WMV is one of the potyviruses with the broadest host range, including monocots and dicots. The impact of its recombinant nature on WMV biological properties remains unknown and speculative.

Partial sequence data also indicate the presence of intraspecific recombinants in WMV: the molecular variability was structured in three groups based on the CP sequence; when other parts of the genome were considered, several isolates switched groups, indicating that recombination may have taken place. By sequencing the full-length genome of putative recombinants, recombination points were characterized in different parts of the genome.

In the case of ZYMV, sequence analysis softwares suggest that recombination might have taken place, but the situation is not as clear-cut as for WMV.

Besides recombination, viral genomes also evolve by mutations that take place during replication. In the case of ZYMV, the biological consequences of several point mutations that emerged in natural conditions were assessed by molecular studies: sequences of closely related strains with different biological properties were compared, and the mutations observed – particularly non-silent mutations located in conserved domains – were introduced by site-directed mutagenesis in an infectious cDNA of ZYMV in order to check if the mutation alone is sufficient to induce the difference in biological properties.

Molecular studies have thus shown that single point mutations in HC-Pro or P3 of ZYMV were important for symptoms. Similarly, single mutations in HC-Pro or CP greatly affect aphid transmissibility. For unknown reasons, some ZYMV isolates such as ZYMV-E15 seem to be particularly prone to mutations and many variants (mild, aphid nontransmissible, aggressive, or virulent) have been derived from this isolate.

Biotechnological Application

The development of infectious cDNA clones of ZYMV brought the possibility of using ZYMV as a biotechnological tool to produce proteins of pharmaceutical or crop protection interest. The gene coding for the protein of interest can be inserted in the ZYMV genome either between the P1 and HC-Pro domains or between the NIb and CP domains. For optimal protein production, there is the possibility to use mild clones that have the mutation in the FRNK motif of HC-Pro so that they produce mild symptoms and do not affect plant growth. The protein may be produced in the edible cucurbit fruits, and therefore used directly for oral administration. Several molecules of pharmaceutical interest have been efficiently produced through this technology: the human interferon-alpha 2, antiviral and antitumor proteins MAP30 and GAP31, and a mite allergen. This technology also provided a way to produce large amounts of nucleocapsid proteins of five tospoviruses that could be used for immunization and production of specific antibodies. Finally, the *bar* gene coding for a phosphinothricin acetyltransferase that confers resistance to herbicides based on glufosinate ammonium has been inserted into the mild ZYMV expression vector. In weed-infested plots, this construct efficiently protected inoculated zucchini squash plants from damage caused by a herbicide treatment that completely destroyed the weeds. In addition, these plants were protected against severe ZYMV isolates. It is interesting to see that ZYMV, which emerged as the most damaging cucurbit virus in the last decades, can be manipulated and used for the benefit of human health and agriculture.

See also: Bean Common Mosaic Virus and Bean Common Mosaic Necrosis Virus; Diagnostic Techniques: Serological and Molecular approaches; Papaya Ringspot Virus; Plant Antiviral Defense: Gene Silencing Pathway; Plant Resistance to Viruses: Engineered Resistance; Plant Resistance to Viruses: Natural Resistance Associated with Dominant Genes; Plant Virus Diseases: Economic Aspects; Plant Virus Vectors (Gene Expression Systems); Polyomaviruses of Mice; Potyviruses; Recombination; Ribozymes; Vector Transmission of Plant Viruses; Vegetable Viruses.

Further Reading

Arazi T, Slutsky SG, Shiboleth YM, *et al.* (2001) Engineering zucchini yellow mosaic potyvirus as a non-pathogenic vector for expression of heterologous proteins in cucurbits. *Journal of Biotechnology* 87: 67–82.

Astier S, Albouy J, Maury Y, and Lecoq H (2001) *Principes de virologie végétale: Génome*, *pouvoir pathogène*, écologie. Paris: INRA Editions.

Desbiez C and Lecoq H (1997) Zucchini yellow mosaic virus. *Plant Pathology* 46: 809–829.

Desbiez C and Lecoq H (2004) The nucleotide sequence of watermelon mosaic virus (WMV, *Potyvirus*) reveals interspecific recombination between two related potyviruses in the 5′ part of the genome. *Archives of Virology* 149: 1619–1632.

Fuchs M, Ferreira S, and Gonsalves D (1997) Management of virus diseases by classical and engineered protection. *Molecular Plant Pathology On-Line*. http://www.bspp.org.uk/mppol/1997/0116fuchs (accessed October 2007).

Lecoq H (1998) Control of plant virus diseases by cross protection. In: Hadidi A, Kheterpal RK, and Koganezawa H (eds.) *Plant Virus Disease Control*, pp. 33–40. St. Paul, MN: APS Press.

Lecoq H (2003) Cucurbits. In: Loebenstein G and Thottapilly G (eds.) *Viruses and Virus-Like Diseases of Major Crops in Developing Countries*, pp. 665–687. Dordrecht: Kluwer Academic Publishers.

Lisa V and Lecoq H (1984) *Zucchini Yellow Mosaic Virus. CMI/AAB Descriptions of Plant Viruses No. 282.* Kew, UK: Commonwealth Mycological Institute.

Purcifull DE and Hiebert E (1979) Serological distinction of watermelon mosaic virus isolates. *Phytopathology* 69: 112–116.

Purcifull D, Hiebert E, and Edwardson J (1984) *Watermelon Mosaic Virus 2. CMI/AAB Descriptions of Plant Viruses No. 293.* Kew, UK: Commonwealth Mycological Institute.

West Nile Virus

L D Kramer, Wadsworth Center, New York State Department of Health, Albany, NY, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Bridge vector Competent mosquito vector that becomes infected following feeding on vertebrate hosts within the enzootic transmission cycle, and subsequently infects humans and other incidental hosts.

Enzootic A pathogen that is constantly present in an animal population, but usually only affects a small proportion of animals at any one time.

Epidemic Severe outbreak within a region or a group of humans.

Epizootic An outbreak of disease affecting many animals of one kind at the same time.

Extrinsic incubation period Period of time required for mosquito to transmit virus after ingesting infectious blood meal.

Neuroinvasive disease Disease caused by pathogen that infects nerve cells.

Nonviremic transmission Viral transmission to uninfected arthropod in the absence of detectable replication of virus in the vertebrate host.

Pathogenesis Origin and development of disease; more specifically the cellular events and reactions and other pathologic mechanisms occurring in the development of disease.

Phylogenetic analysis A method developed by biological systematists to reconstruct evolutionary genealogies of species based on nucleotide sequence relatedness.

Vertical transmission Viral transmission from adult female arthropod to her immediate progeny.

Viremia The presence of virus in the blood.

Zoonosis Also called zoonotic disease; refers to pathogens that can be transmitted from animals, whether wild or domesticated, to humans.

Classification

West Nile virus (WNV) is a member of the family *Flaviviridae*, genus *Flavivirus*, which is composed of approximately 70 members classified into 12 antigenic subgroups. The family *Flaviviridae* includes two additional genera,

Pestivirus (including veterinary pathogens such as bovine viral diarrhea viruses) and *Hepacivirus* (including the human pathogen hepatitis C virus). WNV belongs to the Japanese encephalitis (JE) antigenic complex, which includes the human pathogens St. Louis encephalitis, Rocio, and Ilheus viruses in the Americas, Japanese encephalitis virus in Asia, Murray Valley encephalitis virus in Australia, and other viruses, most of them not associated with encephalitis.

History and Geographic Distribution

WNV was first isolated in 1937 from the blood of a febrile woman in the West Nile district of Uganda. The next evidence of activity occurred when WNV was isolated from mosquitoes and birds and identified as the etiologic agent in sick children in North Africa and the Middle East in the 1950s. West Nile neuroinvasive disease (WNND) was first recognized during an outbreak in elderly patients in Israel in 1957. In the 1960s, WN encephalitis in equines was recognized in Egypt and France. The largest outbreak of WN fever occurred in 1974 in Cape Province, South Africa, leading to approximately 10 000 cases. Increasing frequency of severe outbreaks began occurring in 1996 in humans and horses, largely in the Mediterranean Basin. The range of WNV expanded suddenly in 1999 with the introduction of the virus into the New York City area, although the mode of introduction is unknown. The virus became successfully established as it expanded its range westward to encompass the contiguous United States, northward into Canada in 2001, and southward beginning in 2002 into Mexico, Central America, the Caribbean and parts of South America. From 1999 to 2006, approximately 22 790 cases of WN disease were reported in the US. The largest epidemics of neuroinvasive disease caused by WNV in North America occurred in 2002 and 2003 in the US, when 2946 and 2866 cases, respectively, of WNND were identified. WNV disease also has been noted in Cuba and Argentina in addition to the US and Canada in the Western Hemisphere, but scant evidence of morbidity and mortality has been observed in tropical America. Possible reasons for the lack of overt disease in tropical America are cross-protection from other flaviviruses circulating in tropical regions, less virulent strains of WNV, less-competent arthropod and avian hosts than in temperate regions, and greater diversity of host species in the tropics.

Molecular Epidemiology

WNV is the most widely distributed flavivirus in the world (**Figure 1**). Isolates comprise two main lineages based on nucleotide sequence homology (**Figure 2**). Lineage 1 includes three clades: (1a) isolates from Africa, Asia, Europe, Middle East, North America, and Russia; (1b) isolates from Australia (Kunjin); (1c) most Indian isolates. The strain of WNV introduced into the US in 1999 has 99.7% homology in both nucleic acid and amino acid sequence with a 1998 Israeli goose isolate. Lineage 2 contains strains isolated in sub-Saharan Africa and Madagascar, and includes the prototype 1937 Ugandan isolate. Severe WNND in humans has been associated only with lineage 1 strains. The strain responsible for the South African WN fever outbreak in 1974 is in lineage 2. Comparison of isolates from lineage 1 indicates differences in virulence between North American and Afro-European strains, but the degree of difference varies with the specific avian host. Enhanced virulence of the North American strains appears to be partially correlated with envelope (E) protein glycosylation. Strains within lineage 2, while not associated with overt human outbreaks, also vary phenotypically with respect to virulence in animal models. Two potential new lineages have been identified recently; a lineage 3 virus was isolated from *Culex pipiens* in the Czech Republic (Rabensburg virus) in 1997 and 1999, and a lineage 4 virus isolated from *Dermacentor marginatus* ticks in Russia in 1998 (LEIV-Krnd88–190). Rabensburg virus showed 77–78% identity to lineage 1 and 2 WNV strains, 77% identity to strain LEIV-Krnd88–190, and 71–76% identity to other representatives of the JE antigenic complex.

Phylogenetic analyses of WNV isolates from the US indicate that the virus remains highly conserved genetically. A single conserved amino acid change in the E gene (V159A), first detected in 2001, occurred with increasing frequency from 2002 through 2004 throughout the US (**Figure 3**), and became the dominant genetic variant circulating throughout North America. This genotype, 'North American dominant', probably resulted from a random mutation that became fixed in the viral RNA of the originally introduced genotype, 'Eastern US' strain. The dominant genotype disseminates 2–4 days earlier following feeding, in *Culex* spp. mosquito vectors, effectively reducing the extrinsic incubation period and thereby increasing the reproductive ratio or R_0 of WNV. This reproductive advantage, and a higher viremia observed in some avian species, may have contributed to displacement of the Eastern US genotype. Temporal and spatial clustering of isolates suggest the occurrence in some cases of localized virus spread by mosquitoes or resident birds, and in others long-distance dispersal by migratory birds or possibly by human transport. Nonetheless, it is clear from the phylogenetic evidence based on US isolates that there was a single introduction of WNV into North America from the Old World. This is in contrast to WNV in Israel, where both lineage 1 and 2 strains are frequently introduced, most likely during bird migration.

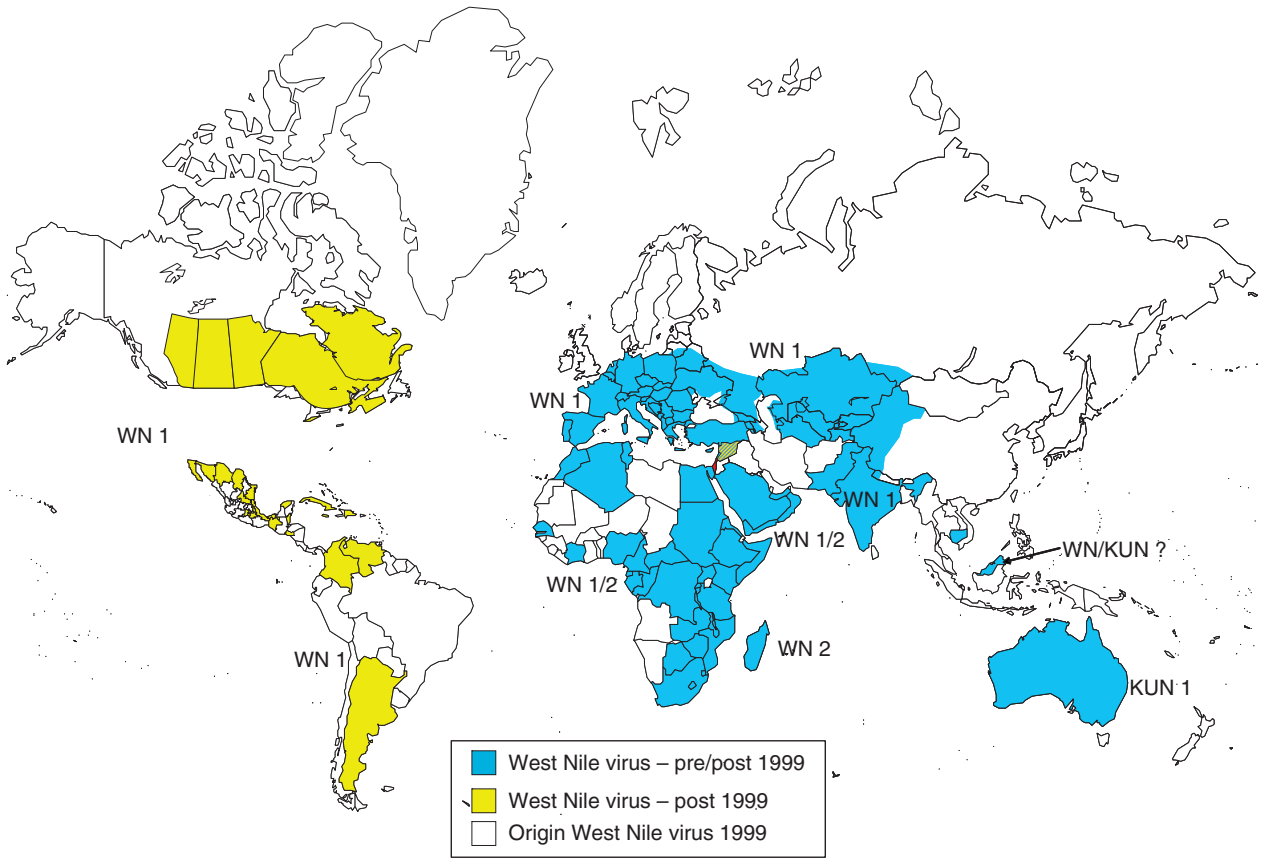


Figure 1 Global distribution of West Nile virus lineages 1 and 2. Areas in yellow have evidence of West Nile virus in the years (to 2006) following introduction of a lineage I (WN 1) virus strain in New York in 1999. This viral strain most likely originated in Israel, colored blue with yellow stripes. WN 1 viruses have been isolated from Africa, the Middle East, Europe, Russia, India, and North America. Isolations of lineage 2 WN viruses (WN 2) are found in Africa and the Middle East. Kunjin virus (KUN 1) is a subtype of lineage 1 isolated in Australia. A WN/KUN-like virus with a unique genotype (WN/KUN?) has been isolated from Sarawak, Malaysia. Modified from Scherret JH, Mackenzie JS, Hall RA, Duebel V, and Gould EA (2002) Phylogeny and molecular epidemiology of West Nile and Kunjin viruses. In: Mackenzie JS, Barrett ADT, and Duebel V (eds.) *Japanese Encephalitis and West Nile Viruses*, 379pp. Berlin, Heidelberg, and New York: Springer, with kind permission of Springer Science and Business Media.

The Virion and the Viral Genome

The West Nile virion is spherical, enveloped, approximately 50 nm in diameter, with relatively smooth surfaces and icosahedral symmetry (**Figure 4**). It contains a host-derived lipid bilayer surrounding a nucleocapsid that consists of the viral RNA complexed with multiple copies of the capsid protein. The viral genome is linear, positive-sense, single-stranded RNA, 11 029 bases in length. The 5′ noncoding region of WNV has a methylated type 1 cap (m^7GpppAmp); the 3′ noncoding region lacks a polyadenylated tail and in its place has a 5′-CU_{OH}-3′. These two regions contain conserved secondary structures critical to viral replication. The viral genome encodes three structural and seven nonstructural proteins in a single long open reading frame of 10 299 nt (nt 97–10 395), which is cleaved co- and post-translationally. The structural proteins (capsid (C), membrane (prM/M), and envelope (E)) are encoded at the 5′ end of the genome and the nonstructural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) at the 3′ end. The E protein, the predominant protein of the virion, plays a major role in viral assembly, receptor binding, and membrane fusion, and is the principal target for neutralizing antibody. The NS proteins are responsible for viral RNA replication but also may function in viral assembly and in evasion of the host immune response. The putative function and nucleotide positions of the viral proteins are listed in **Table 1**.

Replication

WNV is able to replicate in a great variety of vertebrate cells. Replication is a complex process mediated in a controlled fashion through sequential interactions among viral RNA, proteins and host factors (**Figure 5**). The virion initially binds to the cell receptor and then enters via receptor-mediated endocytosis. Glycosaminoglycans have

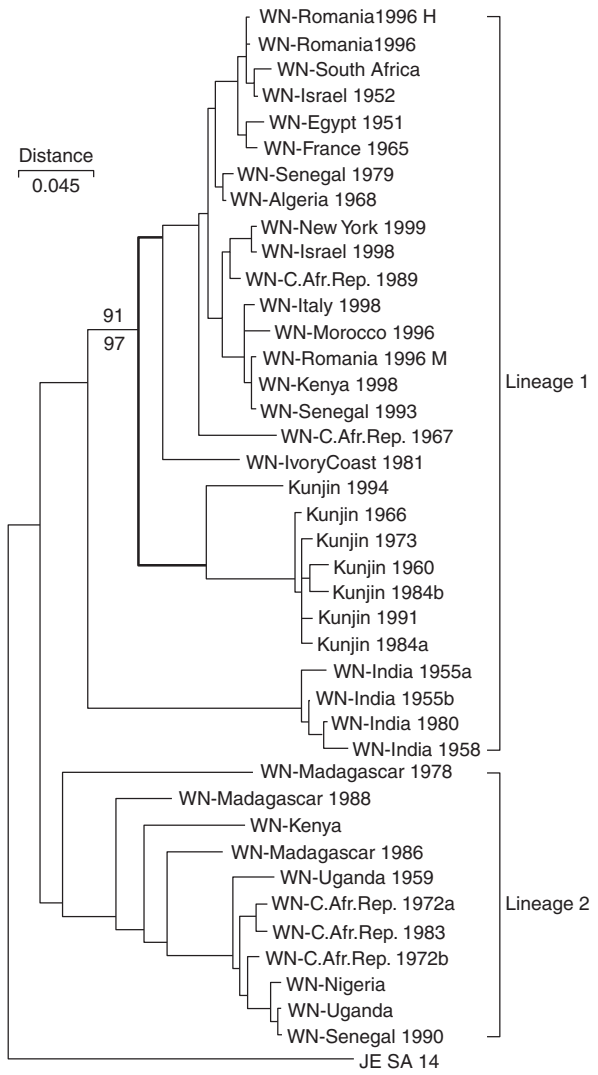


Figure 2 Phylogenetic tree based on E-glycoprotein nucleic acid sequence data (255 base pairs). The tree was constructed with the program MEGA by neighbor-joining with Kimura two-parameter distance (scale bar). Bootstrap confidence level (500 replicates) and a confidence probability value based on the standard error test were calculated using MEGA and are included on the tree (top and bottom values, respectively), illustrating support for the division between the lineage 1 WN virus group (not including the India isolates) and the KUN virus group. The best estimated length of the segment (bold line) separating these groups, in units of expected nucleotide substitutions per site, is 0.06928 and is statistically significantly positive ($P < 0.01$) by the likelihood ratio test (fastDNAml maximum likelihood program). An approximate 95% confidence interval for the true length of this segment is 0.03347, 0.10737. GenBank accession numbers can be found in Lanciotti RS, Roehrig JT, Deubel V, *et al.* (1999) Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286: 2333–2337, reproduced with permission.

been proposed as the putative receptors, although definitive proof is lacking. Following release of the nucleocapsid into the cytoplasm, the viral RNA is uncoated and translation proceeds, forming a single polyprotein which is cleaved into the ten viral proteins. Genomic plus-sense RNA is transcribed into complementary minus-sense RNA at the endoplasmic reticulum membranes, forming replication complexes, which in turn serve as templates for the synthesis of new plus-sense RNA. The process is asymmetric, yielding 10- to 100-fold excess plus strands. The plus-sense RNA is packaged by viral C protein to form the nucleocapsid that is enclosed in an envelope consisting of a host-derived lipid bilayer and viral prM/M and E proteins. prM is cleaved in the *trans*-Golgi network, while M remains inserted in the envelope of the virion. Mature virions are released from the infected cell by exocytosis beginning 10–12 h after infection.

Ecology

Vectors

WNV is a zoonosis maintained in an enzootic cycle, transmitted primarily between avian hosts and ornithophilic mosquito vectors (**Figure 6**). Mosquitoes of the genus *Culex* are the predominant vectors in the enzootic cycle throughout the range of the virus' distribution, although the particular species of *Culex* varies according to geographic location. In the northeast, *Culex pipiens* and *C. restuans* may be important enzootic catalysts in the spring, while the former, an urban species that reaches high densities in midsummer, also may be the critical vector to humans in the northeastern and north central US as well as in recent outbreaks in Europe and Israel. *C. quinquefasciatus*, a member of the *C. pipiens* complex, is the primary vector in the southern US and, presumably, Latin America. Another important vector in these locations is *C. nigripalpus,* while *C. tarsalis* is the predominant vector in rural areas of western states in the US. Other *Culex* spp., such as *C. univitattus* and *C. antennatus* (Europe and Africa), *C. vishnui complex* (India), and *C. annulirostris* (Australia) have been implicated in the transmission cycle.

Vector competence varies between species and within populations of individual species. The *C. pipiens* complex contains two genetically distinct forms, that is, *C. pipiens* form 'pipiens' and form 'molestus'. The two forms differ in physiology and behavior with obvious implications to their epidemiological importance. Form 'pipiens' is thought to be exclusively ornithophilic while the urban form 'molestus' will feed on mammals. The two forms have been shown to not interbreed in northern Europe, in contrast to US populations, which contain individuals with hybrid genetic signatures (*pipiens* × *molestus*). Such hybridization, which also has been noted in southern Europe, may generate bridge vectors, disposed to feed on both birds and mammals. Indeed, US populations of *C. pipiens*, as well as *C. nigripalpus* and *C. tarsalis*, have been demonstrated to shift their feeding from birds to mammals in the late summer and early fall, and therefore may act as bridge vectors to infect equid and human hosts. It is

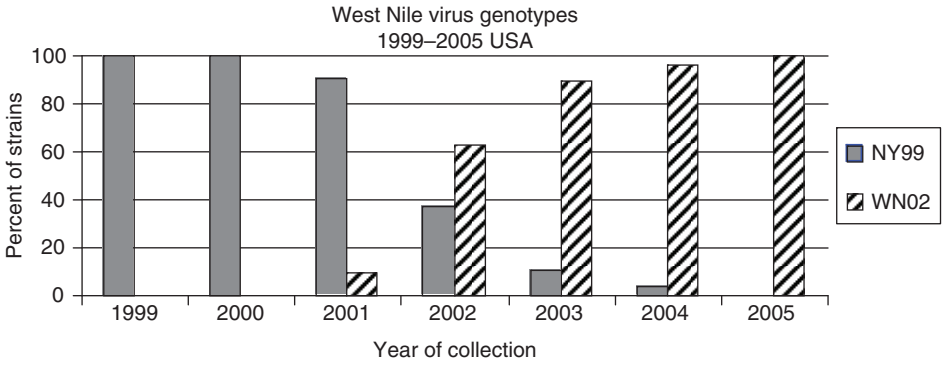


Figure 3 Prevalence of West Nile virus isolates in the United States characterized as Eastern US genotype (shaded box) and North American dominant genotype (striped box) from 2000 to 2005. Reproduced from Snapinn KW, Holmes EC, Young DS, Bernard KA, Kramer LD, and Ebel GD (2007) Declining growth rate of West Nile virus in North America. *Journal of Virology* 81(5): 2531–2534, with permission from American Society for Microbiology.

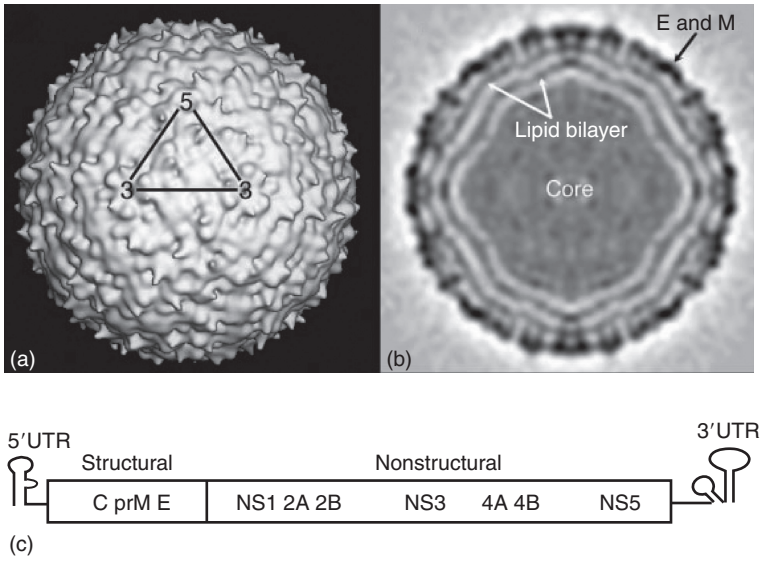


Figure 4 West Nile virion (a, b) and genome (c). WNV structure as reconstructed by cryo-EM. (a) A surface-shaded view with one asymmetric unit of the icosahedron indicated by the triangle. (b) Central section of the reconstruction, showing the concentric layers of mass density. (c) WNV genome, single-stranded positive-sense RNA, approximately 11 kb in length, consisting of a 5′ untranslated region (UTR), a single long open reading frame (ORF), and a 3′ UTR. The ORF encodes three structural and seven nonstructural proteins. (a, b) Modified from Mukhopadhyay S, Kim BS, Chipman PR, Rossmann MG, and Kuhn RJ (2003) Structure of West Nile virus. *Science* 302(5643): 248, as published in Kramer LD, Li J, and Shi PY (2007) West Nile virus: Highlights of recent advances. *Lancet Neurology* 6: 171–181, with permission from Science and Elsevier.

likely that other species of bridge vectors also become involved since virus has been isolated from mosquitoes of approximately 75 species of 10 genera worldwide; but the relative importance of each species in transmission to birds or humans must take into account factors of vectorial capacity, including field infection rates and population density. Many mosquito species actually may be incidental vectors of little epidemiologic significance, but further research is required to determine their importance in the ecology and epidemiology of WNV in North America.

The mechanism(s) of WNV perpetuation over adverse seasons and years may vary by region and country, but possible mechanisms include continuous low-level virus transmission, reinitiation after reintroduction of virus by migratory birds from locations where virus is active year-round, vertical transmission to females about to enter reproductive diapause in winter, and recrudescence of low levels of virus in chronically infected birds when mosquitoes are active (**Figure 6**). Viral RNA and infectious virus have been detected in experimentally infected wild birds more than 6 weeks after inoculation. Virus

Table 1 Functions and nucleotide positions of West Nile virus proteins

Viral protein	*Function*	*Position in genome*
Structural proteins		
C	Regulation of viral replication	97–465
(Pr)M	E folding and function	(466–7421) 742–966
E	Receptor binding	967–2469
	Membrane fusion	
	Viral assembly	
Nonstructural proteins (all NS proteins are required for viral replication)		
NS1	Early replication events (interaction with NS4A essential for replication)	2470–3525
NS2A[a]	Replication complex Interferon antagonist	3526–4218
NS2B[a]	Cofactor for viral protease Interferon antagonist assembly and release of virions	4219–4611
NS3	Serine protease	4612–6468
	NTPase and RTPase activity	
	RNA helicase Interferon antagonist	
NS4A[a]	Replication complex Interferon antagonist	6469–6915
NS4B[a]	Interferon antagonist Virion assembly	6916–7680
NS5	Methyl transferase	7681–10 395
	RNA dependent RNA polymerase Interferon antagonist	

[a]Functions not well characterized.

has been isolated from diapausing adult *C. pipiens* in the eastern US in the winter, from vertically infected male and female *C. univitattus* in Kenya, and from *C. quinquefasciatus* larvae in California during the summer transmission season. Vertical transmission has been demonstrated in the laboratory at rates varying with the population of mosquitoes tested.

Alternate modes of vector transmission have been observed in the laboratory and/or field. Nonviremic transmission has been demonstrated in the laboratory, with low rates of infection in co-feeding mosquitoes. This mode of transmission may expand the enzootic transmission cycle to involve mammals and birds that generally mount viremias too low to infect feeding mosquitoes. WNV has been isolated repeatedly in Russia from soft ticks (*Argasidae*), but their role in the transmission cycle is not clear. In addition, soft ticks have been demonstrated to transmit virus in the laboratory, and nonviremic transmission has been demonstrated. *Ixodidae* (hard ticks) allow the virus to pass transstadially, but are incompetent vectors. Other arthropods have been suggested as alternative vectors, including dermanyssoid mites, swallow bugs, and hippoboscid flies.

Vertebrates: Birds

Birds of more than 300 species in North America have been reported infected with WNV, as determined by virus isolation or antibody, confirming their role as the primary vertebrate in the enzootic cycle. Feeding by infected mosquitoes is the most common route of infection, but transmission to birds also has been demonstrated by direct contact, presumably via the fecal–oral route since there is significant shedding of virus, and by ingestion of infected mosquitoes or of carrion by omnivorous birds such as corvids and raptors. Experimental studies indicate that birds vary significantly in susceptibility and response to infection. The hallmark of WNV activity in North America is the significant morbidity and mortality in a wide range of species, although bird deaths also have been noted in the Middle East and recently in eastern Europe. An Egyptian strain isolated in the 1950s from a sick pigeon (*Columba livia*) is virulent in experimentally infected hooded crows (*Corvus corone*) and house sparrows (*Passer domesticus*). Corvids, especially crows, magpies, and jays, appear to be the most highly susceptible to disease, with 100% of American crows (*Corvus brachyrhynchos*) becoming sick and dying after infection with low doses of WNV. Nonetheless, corvids do not appear to be the primary amplifying hosts in the enzootic transmission cycle. In the northeastern and mid-Atlantic US, the American robin (*Turdus migratorius*) appears to be highly preferred by the predominant vector *C. pipiens*. Disproportionate numbers of mosquito blood meals are taken from robins and they have high levels of IgG antibody to WNV, indicating their importance in the enzootic cycle. In the laboratory, robins have average host competence determined by susceptibility, number of days infectious, and level of viremia or mean infectiousness. High levels of WNV IgG have been detected in other birds as well, including cardinals and wrens, and in some locations, house sparrows.

Vertebrates: Other

Thirty species of mammals and occasionally other vertebrates including reptiles and amphibians have been found

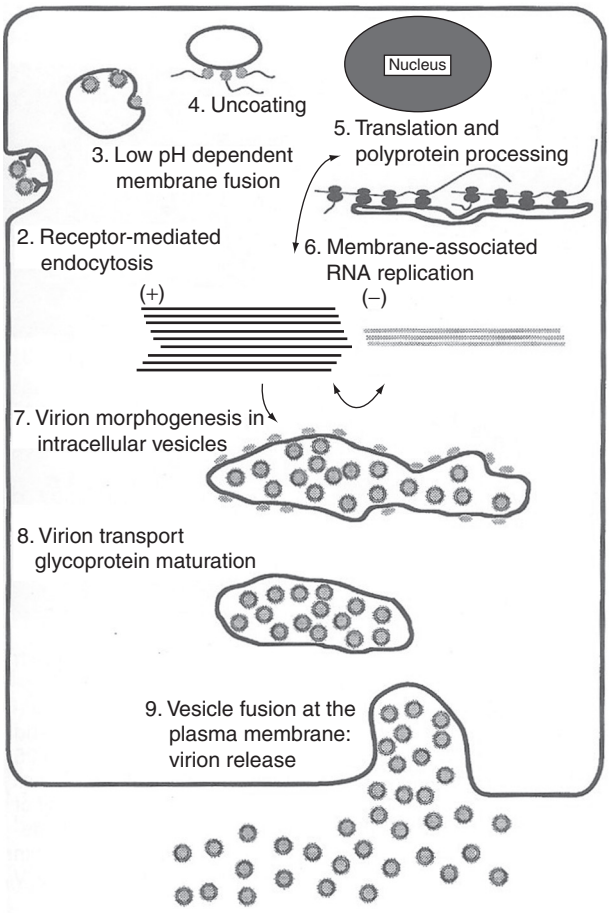


Figure 5 Flavivirus replication cycle. Highlights of the flaviviral replication cycle are diagrammed. Reproduced from Rice CM (1996) *Flaviviridae*: The viruses and their replication. In: Fields BN, Knipe DM, Howley PM, *et al*. (eds.) *Fields Virology*, 3rd edn., pp. 931–959. Philadelphia: Lippincott-Raven Publishers, with permission from Lippincott Williams and Wilkins.

infected with WNV. Their role in the transmission cycle is less significant than that of birds because of their generally low levels of viremia, but some small mammals, such as rabbits and chipmunks, have been demonstrated in the laboratory to mount sufficiently high levels of virus in the blood to infect a small proportion of feeding *Culex* spp. mosquitoes. In the US and Mexico, farmed alligators raised at high temperatures in crowded conditions demonstrate significant mortality and mount high viremias. Transmission appears to occur directly between alligators, as well as through ingestion of uncooked infected horse meat. Enzootics in equines have occurred in the US, France, Italy, Morocco, and Israel. Unvaccinated equines develop infections ranging from asymptomatic to encephalitic disease, and demonstrate a case–fatality rate of approximately 25%. Because of their low viremias, they likely are incidental hosts in the transmission cycle.

Low levels of WNV antibody in domestic pets have been detected in serosurveys in the US and in the Highveld region of South Africa. In the laboratory, dogs and cats are readily infected with WNV, but dogs do not develop detectable viremia and peak titers in cats are too low to infect most mosquitoes. Cats also may become infected by the oral route following feeding on dead infected mice. Sera from wild-caught vertebrates after intensive activity in 2003 in the US also demonstrated high levels of infection in raccoons, Virginia opossums, fox squirrels, and eastern gray squirrels. It is unclear how much morbidity and mortality is associated with WNV in wild mammals, but occasional cases of overt disease have been reported. Some small mammals, such as chipmunks and rabbits, have been shown experimentally to mount viremias sufficient to infect mosquitoes.

Vertebrate Pathogenesis

Infected mosquitoes expectorate virus with their saliva mostly intradermally but also intravascularly while probing and feeding, and their salivary secretions contain potent pharmacologic compounds that affect viral pathogenesis. The initial site of viral replication in the vertebrate is thought to be subdural Langerhans' dendritic cells at the site of inoculation. Activated dendritic cells migrate to draining lymph nodes where the virus replicates further, antigen processing begins, and an early immune response may become evident. Virus enters the blood by way of the efferent lymphatics and thoracic duct, resulting in a viremia that carries the virus to the visceral organs of the body, and possibly facilitates virus crossing the blood–brain barrier. However, the mechanism for the latter is unknown and may occur via infected inflammatory cells, retrograde transport along peripheral nerve axons, replication in epithelial cells, or by another route. Neurons, the main target cell in the central nervous system, suffer pathology, as do bystander nerve cells. In addition, there is immune-mediated tissue damage.

Factors such as age, immune status, and genetic susceptibility of the host, strain of virus, dose of inoculum, and route of infection, affect pathogenesis of WNV. In the mouse, variations in a single gene, Flv, on chromosome 5, determine the rodent's susceptibility to WN disease, but not infection. Susceptibility has been mapped to the gene encoding the L1 isoform of the interferon-inducible, antiviral effector enzyme 2′–5′-oligoadenylate synthetase. An intact immune system is critical to prevention of disease, and both humoral and cellular factors play a role in protection against disease. Neutralizing antibody is the principal means of viral clearance. Interferon-dependent innate immune responses are essential in limiting infection of WNV, as is the adaptive immune response. Complement, dendritic cells and chemokines probably also play roles in viral clearance. The mechanism by which infection is cleared from neurons appears to involve CD8+ T cells in

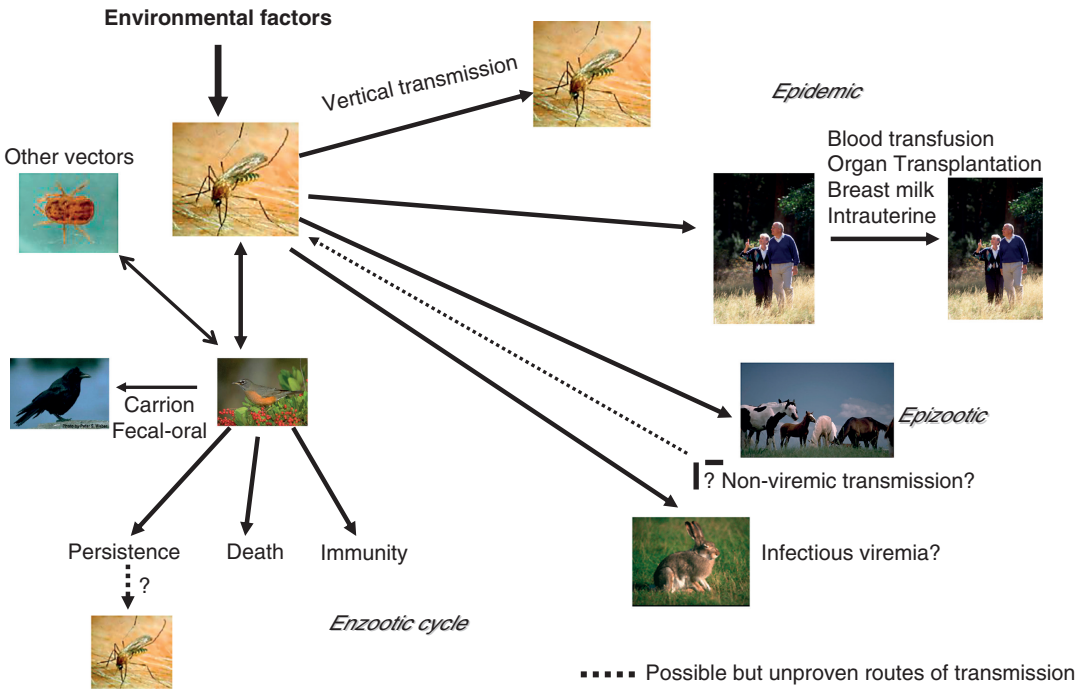


Figure 6 West Nile virus transmission cycle. The enzootic cycle is illustrated, as well as epidemic and epizootic hosts. Solid arrows indicate confirmed transmission pathways; dotted lines proposed pathways that have not been confirmed in nature.

the perforin-dependent class I major histocompatibility complex (MHC)-restricted system. Persistence of virus has been noted to occur in some vertebrates following natural and experimental infection, even in the presence of neutralizing antibody. Hamsters shed WNV in their urine for ≥ 8 months and virus can be isolated from their brains as long as 53 days after infection.

Clinical Disease

The range of symptoms associated with WNV infection extends from uncomplicated febrile illness to meningitis, neuropathies, muscle weakness, paralysis, and encephalitis. Symptoms generally become apparent 2–14 days after infection by mosquito bite, but 80% of infected individuals are asymptomatic. The majority of symptomatic patients present with flu-like symptoms, termed WN fever, which in the US has been associated with substantial morbidity. The duration of symptoms may be from days to months, and the patient may require hospitalization. WNND includes meningitis, encephalitis, and acute flaccid paralysis (AFP). From 1999 to 2007, 27 083 cases of WN disease were reported in the US, of which 57.3% were WN fever and 40.3% were WNND resulting in 1054 fatalities (3.9% of all, 9.7% of WNND) (**Table 2**). Given the proportion of symptomatic cases (~20%), approximately 135 415 individuals were infected with WNV over the 8-year period, and 8.1% of all infections were WNND. Males were more commonly infected than were females. Movement disorders, that is, dyskinesias, frequently characterized as parkinsonism are common. The constellation of fever, headache, and signs of meningeal irritation, cannot be easily discriminated from other flaviviral encephalitides. Patients with AFP experience loss of spinal anterior horn cells and have accompanying asymmetric weakness. Risk factors for WNND include age, diabetes, and possibly a history of hypertension or cardiovascular disease. The median age of WNND cases in the US since 1999 is 57 years (46 years for WNF), but there have been reports of WNND in children and AFP in young adults. Infection may persist in the central nervous system of immunologically compromised patients, leading to extended neurologic illness and sequelae. The mortality rate following WN encephalitis is greater than following WN meningitis. Immunosuppression, chronic renal disease, and hepatitis C infection have been recognized as risk factors for death after adjustment for age. Rare cases of hemorrhagic WNV disease have been noted.

The vast majority of human cases are transmitted by the bite of an infected mosquito. However, human-to-human transmission after blood transfusions, organ transplantation, and through mothers' milk have occurred in the US and there has been one instance of intrauterine transplacental transmission. High-throughput nucleic acid detection assays were developed and implemented by blood banks in response to transfusion transmission in order to protect the blood supply.

Table 2 Human West Nile virus disease cases, United States, 1999–2005

Year	*Total cases*	*WNND cases (% of total)*	*WNF cases (% of total)*	*Other clinical/unspecified*	*Fatalities (% of WNND cases)*
1999	62	59	3	0	7
2000	21	19	2	0	2
2001	66	64	2	0	9
2002	4 156	2 946	1 160	50	284
2003	9 856	2 860	6 830	166	264
2004	2 539	1 142	1 269	128	100
2005	3 000	1 294	1 607	99	119
2006	4 269	1 459	2 616	194	177
2007	3 114	1 059	2 026	29	92
Total	27 083	10 902 (40.3%)	15 515 (57.3%)	666	1054 (3.9%/9.7%)

WNND, West Nile neurologic disease; WNF, West Nile fever.
Reported to CDC, as of 06 November 2007.

Diagnostics

Human

Studies of infected blood donors indicated the presence of WNV IgM and IgA at the earliest on day 3 and in all cases by day 8–9 after RNA was detected; IgG antibody appeared about 1 day later (**Figure 7**). The detection of IgM antibody in the cerebrospinal fluid (CSF) by a monoclonal antibody (MAb) capture enzyme-linked immunosorbent assay (MAC ELISA) in conjunction with evidence of neurologic symptoms has been accepted as diagnostic of WNV disease. Presence of IgM antibody in the serum alone is strongly suggestive of recent infection but not definitive due to persistence for at least 16 months (199 days in the CSF) in patients with WNND, and to some cross-reactivity with antibody to other flaviviruses. The plaque reduction neutralization test (PRNT) in Vero cell culture remains the gold standard in diagnosis of flavivirus infections because of the extensive cross-reactions complicating diagnosis. A fourfold rise in neutralizing antibody titer between paired acute-phase and convalescent-phase sera confirms WNV infection, as does a fourfold or greater titer to WNV compared to related flaviviruses. However, interpretation is complicated in individuals who have had prior infection with another flavivirus. Microsphere immuno assay (MIA) using recombinant E protein has equivalent sensitivity to ELISA. In primary flavivirus infection, the specificity of the MIA procedure is high (>90%) compared to PRNT. However, as with PRNTs, the interpretation of WNV-E MIA is confounded when WNV is a secondary flavivirus infection. Sera from dengue (flavivirus) patients are highly likely to be positive in MIA procedures using recombinant WN E protein. Addition of WNV NS5 to a multiplex test panel increases specificity, since sera from patients with other past flavivirus infections are likely to be negative to WNV NS5 but reactive to WNV E. Serologic assays are more sensitive than detection of viral nucleic acid by reverse transcription-polymerase chain reaction (RT-PCR) once symptoms are evident because of the rapid clearance of virus by most individuals; however, detection of viral RNA is confirmatory.

Clinical findings also aid diagnosis. Neurologic symptoms indicate neuroinvasive disease. Electrophysiologic tests and magnetic resonance imaging (MRI) may be useful in cases of acute flaccid paralysis. Elevated lymphocyte counts and protein levels may be present in CSF during WNV infection. Epidemiological data also may be critical for diagnostic consideration because other flavivirus infections cause similar symptoms.

Surveillance Specimens

Submission of dead birds, particularly American crows, has provided an excellent sentinel system for WNV activity in the US, but depends upon participation by the public. In addition, over time this may not remain a reliable indicator of virus activity if selection for resistance to disease occurs. Tissues or swabs from dead birds and pooled mosquitoes are tested by real-time RT-PCR for viral nucleic acid, by cell culture procedures that detect live virus followed by confirmation using specific assays, and/or by procedures that detect viral antigen. High-throughput virus-specific molecular assays, such as real-time RT-PCR, have been developed to accommodate testing of large numbers of specimens in a timely manner.Rapid assays utilizing dip sticks to detect viral antigen also have been adopted for surveillance, but have low sensitivity. Detection of antibody in sera of live sentinel captive birds or wild-caught birds using an indirect or a competitive blocking ELISA, with confirmation by PRNT, generally has proven less useful as an early warning indicator of viral activity.

Prevention and Control

Vaccines and Therapeutics

Currently, the only available treatment for disease caused by WNV is supportive, given that no therapeutic

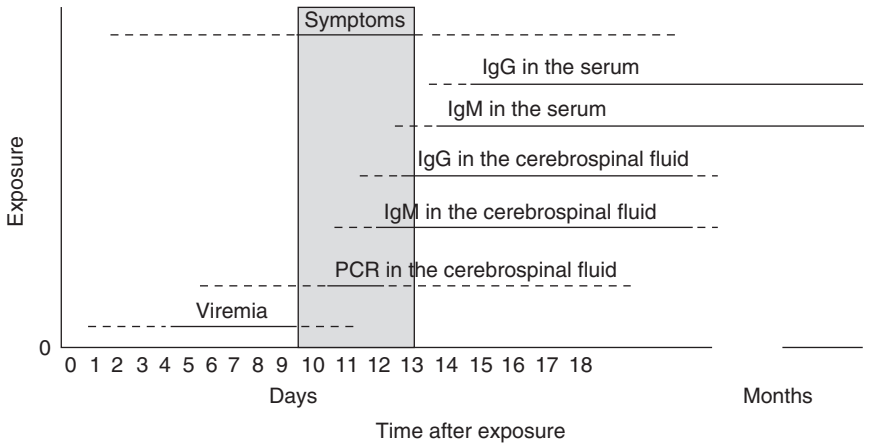


Figure 7 Schematic of virologic and serologic tests in West Nile virus encephalitis. Solid lines represent the more common results; broken lines represent reported ranges. The shaded box is an example of a typical patient. Reproduced from Gea-Banacloche J, Johnson RT, Bagic A, Butman JA, Murray PR, and Agrawal AG (2004) West Nile virus: Pathogenesis and therapeutic options. *Annals of Internal Medicine* 140: 545–553, with permission from American College of Physicians.

treatment has shown consistent clinical efficacy. Therapeutic interventions include passive administration of high-titer immune immunoglobulin. MAb, in particular humanized MAb against WNV E protein, also has shown some benefit. Interferon offers promise based on animal models but may not be helpful in treating the late-stage central nervous system disease. Finally, antiviral therapy using small sequence-specific nucleic-acid-based molecules to suppress WNV replication in tissue culture is being evaluated but the timing and high dose required remain issues of concern. Targets of intervention include viral RNA polymerase, RNA helicase, or other viral replication enzymes. WNV replicon-based high-throughput screening assays have been developed to aid in discovery of compounds with potential antiviral activity.

Advances also have been made in development and implementation of effective vaccines. Protection has been demonstrated in equids following immunization with inactivated cell culture-derived virus (Ft. Dodge). Recombinant canarypox virus containing the prM and E genes of WNV also has been licensed for use in equids. Two recombinant live virus vaccines are in clinical trial in humans. One uses a yellow fever 17D strain infectious clone, with the original yellow fever virus prM and E genes replaced by attenuated WNV genes (Acambis' Chimerivax-WN), and another uses attenuated dengue virus 4 with deletions in the 3′ end as the backbone (NIH/Macrogenics). Both live, attenuated and chimeric vaccines have the advantage of eliciting humoral and cellular responses in the host after a single dose. Other approaches include DNA vaccines that encode prM, E, or C; subunit vaccines of purified recombinant prM and E; and cross-reactive flaviviruses or WNV variants.

Public Health Measures

Reduction of WNV transmission currently relies on reduction of mosquito populations or minimizing contact between vectors and humans. Mosquito control agencies in the US have undertaken extensive larviciding to kill immature stages of mosquito vectors in the aquatic environment. Source reduction, that is, removal of standing water, also has proven helpful. Personal protection measures, for example, wearing loose clothing, use of repellents, particularly 'DEET' (*N*,*N*-diethyl-m-toluamide or *N*,*N*-diethly-3-methylbenamide), or use of window screens, reduce direct contact with mosquitoes. Community action groups have worked to increase awareness and educate the public.

Summary

The introduction of WNV to the US and its subsequent spread and successful establishment in the Western Hemisphere alerted the world to the impact of globalization on pathogen dissemination. The number of WNND cases in 2002 and 2003 represent the largest outbreak of neurologic diseases in North America. Future research undoubtedly will address development of efficacious therapeutics, effective vaccines, novel methods to control mosquitoes, and accurate risk prediction. Each of these is dependent upon solid basic research on WNV ecology, pathogenesis, and immunology.

See also: Classical Swine Fever Virus; Dengue Viruses; Flaviviruses: General Features; Flaviviruses of Veterinary Importance; Japanese Encephalitis Virus; Yellow Fever Virus.

Further Reading

Beasley DW (2005) Recent advances in the molecular biology of West Nile virus. *Current Molecular Medicine* 5: 835–850.

Brinton MA (2002) The molecular biology of West Nile Virus: A new invader of the Western Hemisphere. *Annual Review of Microbiology* 56: 371–402.

Davis CT, Ebel GD, Lanciotti RS, *et al.* (2005) Phylogenetic analysis of North American West Nile virus isolates, 2001–2004: Evidence for the emergence of a dominant genotype. *Virology* 25: 252–265.

Diamond MS (2005) Development of effective therapies against West Nile virus infection. *Expert Review of Anti-Infective Therapy* 3: 931–944.

Gea-Banacloche J, Johnson RT, Bagic A, Butman JA, Murray PR, and Agrawal AG (2004) West Nile virus: Pathogenesis and therapeutic options. *Annals of Internal Medicine* 140: 545–553.

Hayes CG (1989) West Nile fever. In: Monath TP (ed.) *The Arboviruses: Epidemiology and Ecology,* vol. V, ch. 49, pp. 59–88. Boca Raton, FL: CRC Press.

Hayes EB and Gubler DJ (2006) West Nile virus: Epidemiology and clinical features of an emerging epidemic in the United States. *Annual Review of Medicine* 57: 181–194.

Kramer LD, Li J, and Shi PY (2007) West Nile virus: Highlights of recent advances. *Lancet Neurology* 6(2): 171–181.

Lanciotti RS, Roehrig JT, Deubel V, *et al.* (1999) Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 286: 2333–2337.

Monath TP and Heinz FX (1996) Flavivirus. In: Fields BN, Knipe DM, Howley PM, *et al.* (eds.) *Fields Virology,* 3rd edn., pp. 961–1034. Philadelphia: Lippincott-Raven Publishers.

Mukhopadhyay S, Kim BS, Chipman PR, Rossmann MG, and Kuhn RJ (2003) Structure of West Nile virus. *Science* 302(5643): 248.

Rice CM (1996) *Flaviviridae*: The viruses and their replication. In: Fields BN, Knipe DM, Howley PM, *et al.* (eds.) *Fields Virology,* 3rd edn., pp. 931–959. Philadelphia: Lippincott-Raven Publishers.

Scherret JH, Mackenzie JS, Hall RA, Duebel V, and Gould EA (2002) Phylogeny and molecular epidemiology of West Nile and Kunjin viruses. In: Mackenzie JS, Barrett ADT, and Duebel V (eds.) *Japanese Encephalitis and West Nile Viruses*, 379pp. Berlin, Heidelberg, and New York: Springer.

Shi PY and Wong SJ (2003) Serologic diagnosis of West Nile virus infection. *Expert Review Molecular Diagnostics* 3: 733–741.

Smithburn KC, Hughes TP, Burke AW, *et al.* (1940) A neurotropic virus isolated from the blood of a native Ugandan. *American Journal of Tropical Medicine* 20: 471–492.

Snapinn KW, Holmes EC, Young DS, Bernard KA, Kramer LD, and Ebel GD (2007) Declining growth rate of West Nile virus in North America. *Journal of Virology* 81(5): 2531–2534.

White Spot Syndrome Virus

J-H Leu, J-M Tsai, and C-F Lo, National Taiwan University, Taipei, Republic of China

© 2008 Elsevier Ltd. All rights reserved.

Glossary

***In situ* hybridization (ISH)** A technique that uses a labeled complementary DNA or RNA probe to hybridize and detect a specific DNA or RNA sequence in a section of tissue (*in situ*).

Microarray A DNA microarray is a collection of microscopic dsDNA or oligonucleotide spots that are deposited on a solid glass slide at high density. Each spot commonly represents a single gene. DNA microarrays use DNA–DNA or DNA–RNA hybridization to perform simultaneous large-scale analyses of the expression levels of the corresponding genes.

RNAi (RNA interference) RNAi refers to the introduction of homologous double-stranded RNA to specifically interfere with the target gene's expression in the cells/organisms. It was originally discovered in *C. elegans*, and is now widely used in many organisms for null mutation. Recent studies suggest that RNAi can be applied in shrimp as well through intramuscular injection of dsRNA.

Introduction

White spot disease (WSD) is a highly contagious viral disease of penaeid shrimp. Its onset is rapid, and high levels of mortality can result within just a few days. Following the first outbreak of WSD in China and Taiwan in 1992–93, this viral disease spread quickly to other shrimp-farming areas, including Japan (1993), Thailand (1993), the United States (1995), Central and South America (1999), France (2002), and Iran (2002). Nowadays, WSD is considered endemic in almost all shrimp-producing countries in Asia and the Americas, causing serious economic damage to the shrimp culture industry. The causative agent of WSD is white spot syndrome virus (WSSV), which is a large (80–120 × 250–380 nm), non-occluded, rod-shaped to elliptical, double-stranded (ds) DNA virus. The virus has a remarkably broad host range among crustaceans, including many species of shrimp, crayfish, crab, and lobster. WSSV replication and virion assembly occur in the hypertrophied nuclei of infected cells without the production of occlusion bodies. The WSSV virion consists of a rod-shaped nucleocapsid surrounded by a trilaminar envelope and a unique, tail-like extension at one end. The virions contain a circular,

supercoiled, dsDNA genome of about 300 kilobase pairs (kbp). Complete genome sequence analyses have shown that most of the WSSV open reading frames (ORFs) encode proteins bearing no homology to known proteins, while some identifiable genes are more homologous to eukaryotic genes than to viral genes. Based on these genetic analyses and on its unique morphological features, WSSV has been classified as the sole member of a new virus family.

Taxonomy

After its initial discovery, WSSV was classified as the genus *nonoccluded Baculovirus* (*NOB*) of the subfamily *Nudibaculovirinae* of family *Baculoviridae*. However, based on its unique morphological features, on its genomic structure and composition, and on phylogenetic analyses, *White spot syndrome virus 1* was reassigned by the International Committee on Taxonomy of Viruses in 2004 as the type species of the genus *Whispovirus* and the sole member within the new virus family *Nimaviridae*. The family name refers to the thread-like, polar extension on the virus particle (*nima* is Latin for 'thread'). Today, there is still only a single species within the genus *Whispovirus*. However, various geographical isolates with genotypic variability (variants) have been identified within this species. Other names that have been used in the literature for WSSV include: hypodermal and hematopoietic necrosis baculovirus (HHNBV), rod-shaped nuclear virus of *Penaeus japonicus* (RV-PJ), systemic ectodermal and mesodermal baculovirus (SEMBV), and white spot baculovirus (WSBV).

Transmission, Host Range, and Epidemiology Studies of WSSV

WSSV is contagious and highly virulent in penaeid shrimp. In cultured shrimp, WSSV infection can cause a cumulative mortality of up to 100% within 3–10 days of the first signs of disease. WSSV can be transmitted horizontally, either *per os* when the shrimp feed on diseased individuals, contaminated food or infected carcasses, or else through exposure to virus particles in the water, in which case the route of infection is primarily through the gills or other body surfaces. The virus is also transmitted vertically from brooder to offspring. However, transmission is not transovarial because the virus appears to attack only young developing oocytes, which die before reaching maturation. It is therefore more likely that vertical transmission is caused by contamination of the egg mass. Penaeid shrimp are highly susceptible to WSSV. Although there is evidence of resistance during the larval and early (younger than PL6) postlarval stages, WSSV can cause disease in shrimp at any growth stage.

WSSV has a remarkably broad host range. Almost every species of penaeid shrimp is susceptible to WSSV infection. Moreover, the virus can infect other marine, brackish, and freshwater crustaceans, including crayfish, crabs, and spiny lobsters. However, in contrast to penaeid shrimp, infection is often not lethal in these species, and consequently they may serve as reservoirs and carriers of the virus. Furthermore, at least one insect, the shore fly (a member of the family Ephyridae), as well as copepods collected from WSSV-affected farms, have been diagnosed as WSSV-positive by PCR, suggesting that they are also possible reservoir hosts.

In epidemiological studies of WSSV, variations in the number of the 54 bp tandem repeat located between the *rr1* and *rr2* genes (see the section titled 'Identification through homology comparison') have been used as a strain-specific, genetic marker. The number of repeats varies greatly when infected shrimps are collected from different ponds or from the same ponds at different times, but in almost every outbreak of WSD, shrimps from the same pond usually have the same number of repeats. This suggests that in each pond, a single WSSV isolate is the causative agent. High variations in the number of 54 bp repeats have been reported in strains of WSSV from Thailand, India, and Vietnam. In Vietnam, a particular repeat pattern was observed in shrimps from outbreak ponds, but no repeat pattern was found to be useful as a prediction of pathogenicity or geographical origin in any of these different strains. On the other hand, cultured shrimps and wild crustaceans from the same WSD outbreak pond had different 54 bp repeat patterns, suggesting that the wild crustaceans may not have been responsible for infecting the cultured shrimp. However, this interpretation has been questioned by a recent study showing that the number of the 54 bp repeats as well as the pathogenicity changed when a given WSSV isolate was passaged through different crustacean hosts. This study further implies that the variations in repeat number could result from host selection rather than geographical isolation.

Clinical Features and Pathology

The most commonly observed clinical sign of WSD in shrimp is white spots in the exoskeleton and epidermis. These may range in size from minute spots to disks several millimeters in diameter which may coalesce into larger plates. The spots may result from abnormal deposits of calcium salts by the cuticular epidermis or result from disruption to the transfer of exudates from the epithelial cells to the cuticle. Infected animals are lethargic, reduce their food consumption, and display a reddish to pink body discoloration due to expansion of cuticular chromatophores. Moribund shrimp exhibit systemic destruction of

target tissues with many infected cells showing homogeneous hypertrophied nuclei. At advanced stages of infection, numerous virus particles are released into the hemolymph from the lesions, causing a general viremia.

It should be noted that although the white spots are a typical and characteristic clinical sign of WSD, white spots on the carapace of shrimp can also be caused by other environmental stress factors, such as high alkalinity or a bacterial shell disease. Conversely, moribund shrimp with WSD may have few, if any, white spots.

To date, no species of penaeid shrimp is known to show significant resistance to WSD.

Histopathology

The prime targets for WSSV replication are tissues of ectodermal (cuticular epidermis, fore- and hindgut, gills, and nervous tissue) and mesodermal (lymphoid organ, antennal gland, connective tissue, and hematopoietic tissue) origin. Tissues of endodermal origin (hepatopancreas and midgut) are not affected by the virus. Histopathological observation reveals similar cellular changes upon WSSV infection in all target tissues. In the early stage of infection, affected cells display nuclear hypertrophy (**Figure 1**), nucleoli dissolution, and chromatin margination, and the central area changes into a homogeneous eosinophilic region. The infected cells then proceed to develop an intranuclear eosinophilic Cowdry A-type inclusion, which subsequently becomes a light basophilic, denser inclusion separated by a transparent zone from the marginated chromatin. During this time, the cytoplasm becomes less dense and more lucent. In the late stage of infection, the nuclear membrane is disrupted, causing the intranuclear transparent zone to fuse with the lucent cytoplasm. At the end of cellular degeneration, the nucleus or whole cell disintegrates, leading to loss of cellular architecture. In moribund shrimp, most tissues and organs are heavily infected with the virus, and they exhibit severe multifocal necrosis.

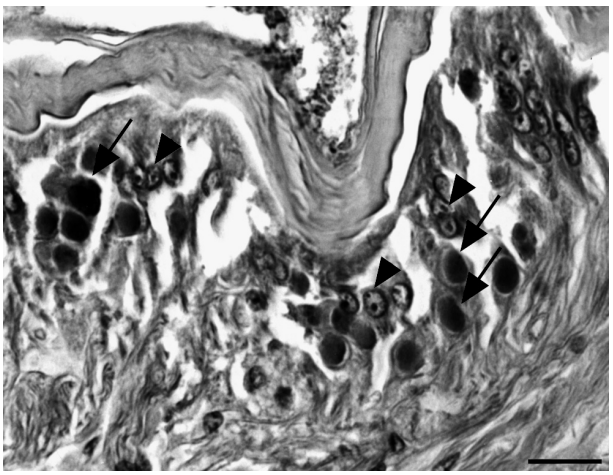


Figure 1 Hematoxylin and eosin (HE) staining of tissue section from *Penaeus monodon* cuticular epithelium of eyestalk infected with WSSV. Degenerated cells characterized by hypertrophied nuclei (black arrows) are readily seen. The cells with normal nuclei are also indicated with arrowheads. Scale = 20 μm.

Temporal studies coupled with *in situ* hybridization (**Figure 2**) have shown that the first WSSV-positive signals are detected in the stomach, gills, cuticular epidermis, and connective tissue of the hepatopancreas. At later stages of infection, the lymphoid organ, antennal gland, muscle tissue, hematopoietic tissue, heart, midgut, and hindgut also become positive. As infection proceeds, the stomach, gill, hematopoietic tissue, lymphoid organ, antennal gland, and cuticular epidermis become heavily infected with WSSV, which leads to serious damage and necrosis (and/or apoptotsis; see the section titled 'Viral–host relationships').

Viral Morphology and Morphogenesis

The WSSV virion is a nonoccluded particle with a trilaminar envelope. It is elliptical to bacilliform (olive-like) in shape and measures about 270 × 120 nm. A long, tail-like projection is often seen extending from the narrow end of the purified virion (**Figure 3**(**a**)). The cylindrical, rod-shaped nucleocapsid is about 300 × 65 nm, which is longer than the intact virion. The nucleocapsid displays a very distinctive pattern consisting of a stacked series of rings (about 16 in total) that run perpendicular to the longitudinal axis of the capsid. The thickness of these rings is quite constant, at about 20 nm. Each ring consists of two rows of 12–14 globular subunits, each approximately 10 nm in diameter (**Figure 3**(**b**)).

The entire WSSV replication cycle takes place in the nucleus and, in an acutely infected shrimp, can be completed within 24 h post infection. Initial signs of WSSV replication in the nucleus include nuclear hypertrophy and chromatin margination. Viral morphogenesis begins with *de novo* formation of the viral envelope, which can be seen at first as fibrillar fragments in the nucleoplasm. As infection progresses, these fragments form into the membranous and/or vesicular precursors of the viral envelope. The development of nucleocapsids begins with the formation of long empty tubules (**Figure 4**(**a**)) which have a diameter similar to that of empty nucleocapsids and a segmentation similar to that of nucleocapsids. They can exist individually or may be laterally aligned in groups of two or three to form a larger structure. The assembled tubules break into fragments of 12–14 rings to form empty naked capsids (**Figure 4**(**b**)) which are then surrounded by envelopes, leaving an opening at one end. Nucleoproteins, which have a filamentous appearance, enter the capsid through this open end, while

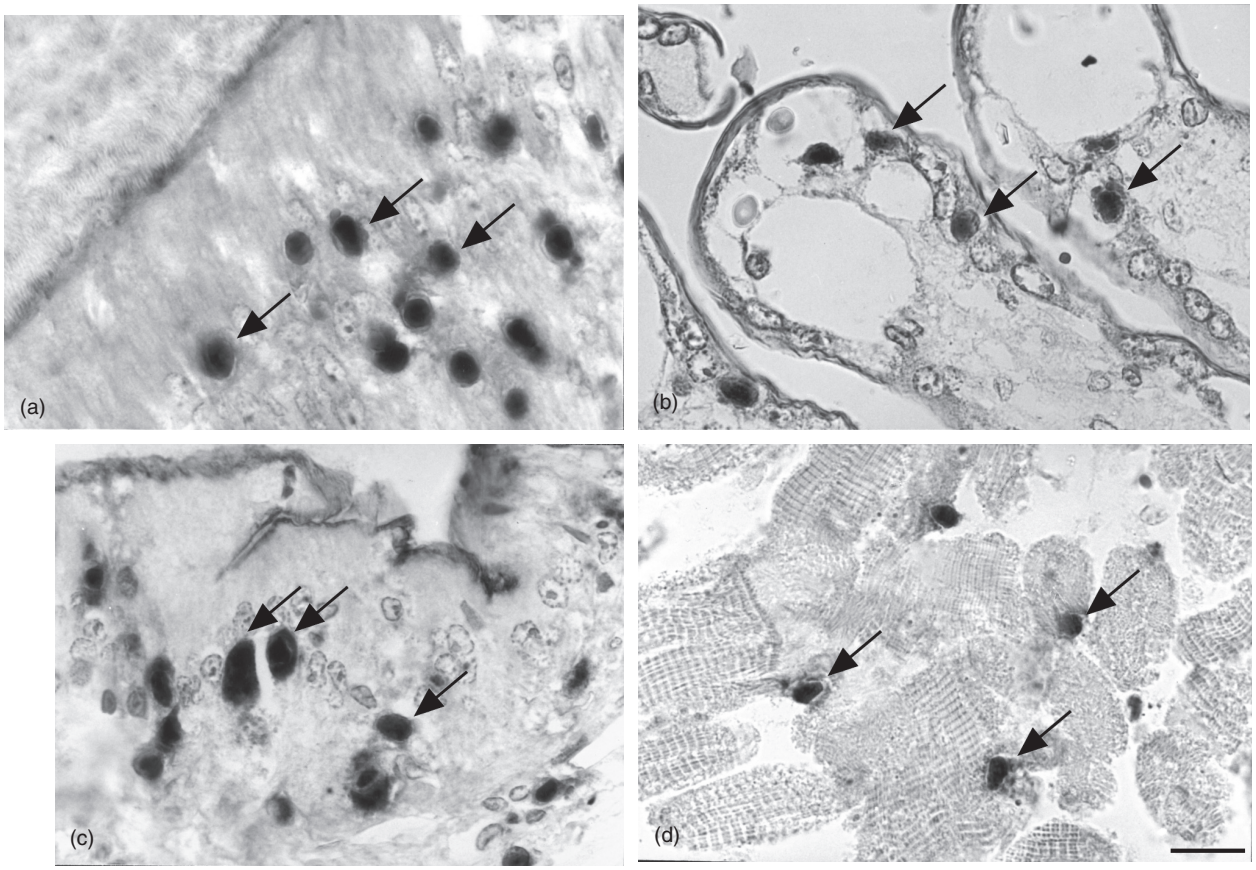


Figure 2 *In situ* hybridization analysis of WSSV positive cells (arrows) in (a) the integument, (b) the gill, (c) the stomach, and (d) the heart from experimentally infected *Penaeus monodon* at 60 hpi. The infected cells shown here in the heart are connective tissue cell; muscle cells are not usually targeted by WSSV. Scale = 20 μm.

simultaneously increasing the diameter of the virion. Finally, the open end of the nucleocapsid is closed, and the envelope narrows at the open end to form the apical tail of the mature virion, which has now obtained its characteristic olive-like shape.

Virion Structure and Composition

WSSV virions have a complex SDS-PAGE protein profile (**Figure 5(a)**). Early structural protein studies used SDS-PAGE coupled with Western blot analyses and/or N-terminal sequencing of proteins to identify at least six structural proteins, including VP35, VP28, VP26, VP24, VP19, and VP15. More recent studies have used mass spectrometry to analyze the protein profiles of purified WSSV virions separated by SDS-PAGE and 2-D electrophoresis, and more than 40 structural proteins have now been identified. The major structural proteins are VP664, VP28, VP26, VP24, VP19, and VP15 (**Figures 5** and **6**). A biochemical fractionation study using differential concentrations of salt and detergent has tentatively classified the WSSV structural proteins into envelope, tegument, and capsid proteins. VP15 is a basic capsid protein with *in vitro* DNA-binding activity, and it has high homology to the lysine- and arginine-rich DNA-binding proteins of the insect baculoviruses. This protein is therefore thought to be responsible for packing the WSSV DNA into the nucleocapsid. VP26, a tegument protein, can interact with actin, but the importance of this interaction remains unknown. VP19, VP24, and VP28 are envelope proteins, and an *in vitro* physical interaction between VP24 and VP28 has been reported. VP664, the major capsid protein, takes its name from its calculated molecular weight of 664 kDa, and it is not only the largest protein encoded by the WSSV genome, but also the largest viral structural protein ever found. This protein is encoded by the intron-less giant ORF (*wssv419*) of 18234 nt. Immunoelectron microscopy of purified virions has shown that VP664 forms the globular subunits that are visible in the nucleocapsid ring structures (**Figure 6**). In addition to VP15 and VP664, the capsid contains at least four other minor proteins: VP160A (WSSV344), VP160B (WSSV94), VP60B (WSSV474), and VP51C (WSSV364) (**Figure 5**).

A number of viruses use the Arg-Gly-Asp (RGD) motif to bind to cellular integrins during infection and

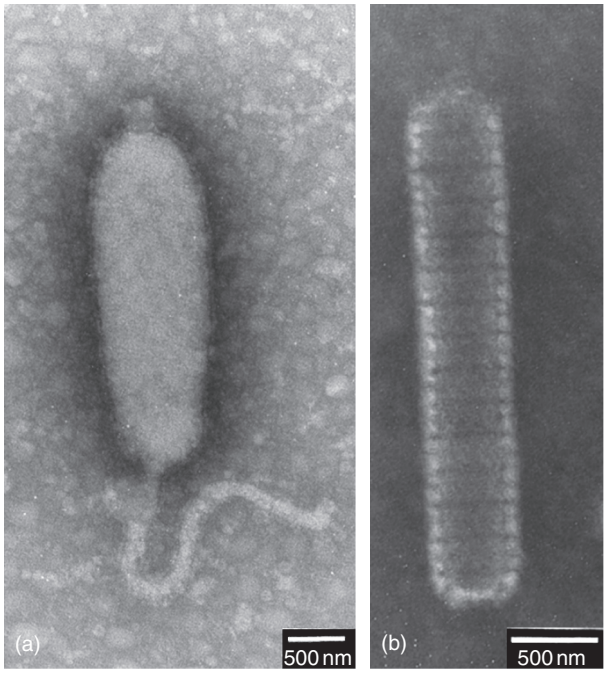


Figure 3 Morphology of the WSSV virions. (a) Intact WSSV virion showing the tail-like extension on one end. (b) A WSSV nucleocapsid showing the stacked ring structures, which are made up of two rows of globular subunits.

this cell attachment site signature can be identified in at least six WSSV structural proteins. It has been shown that one of these six structural proteins, VP110 (WSV035), can attach to shrimp host cells, and that this adhesion can be blocked by synthetic RGDT peptides, suggesting that the RGD motif in VP110 may play a role in WSSV infection. VP28 may also bind to host cells even though it lacks the RGD motif, and virus neutralization tests originally suggested that VP28 was critical for systemic WSSV infection. However, recent studies have shown that the observed phenomenon was, in fact, a nonspecific inhibitory effect of rabbit serum. Thus, to date, although SDS-PAGE indicates that VP28 is the most abundant virion protein, its role remains elusive. In addition to VP28, virus neutralization tests have been used to identify other structural proteins in the envelope and determine their role in WSSV infection. However, considering what is now known for VP28, the results of neutralization tests should be interpreted with care.

None of the five small major WSSV structural proteins (VP28, VP26, VP24, VP19, and VP15) appears to be glycosylated. However, increased migration in SDS-PAGE after N-linked glycosidase F treatment of structural protein VP180 (encoded by *wsv001*) suggests that this large collagen-like protein is N-glycosylated. This is the first reported evidence of an intact collagen gene in a virus genome.

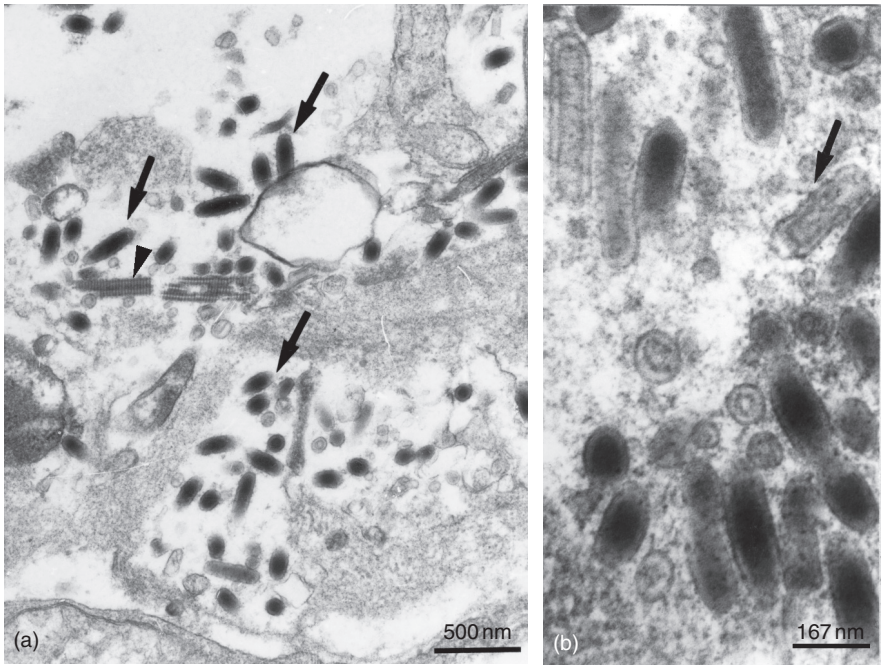


Figure 4 Transmission electron micrograph of WSSV-infected tissues from beneath the cephalothoracic exoskeletoal cuticle. (a) The viral particles spread among the necrotic area. The complete viral particles are indicated with arrows and the long empty tubules with an arrowhead. (b) High magnification of virus particles with rod-shaped morphology. A viral particle with an empty nucleocapsid is indicated with an arrow.

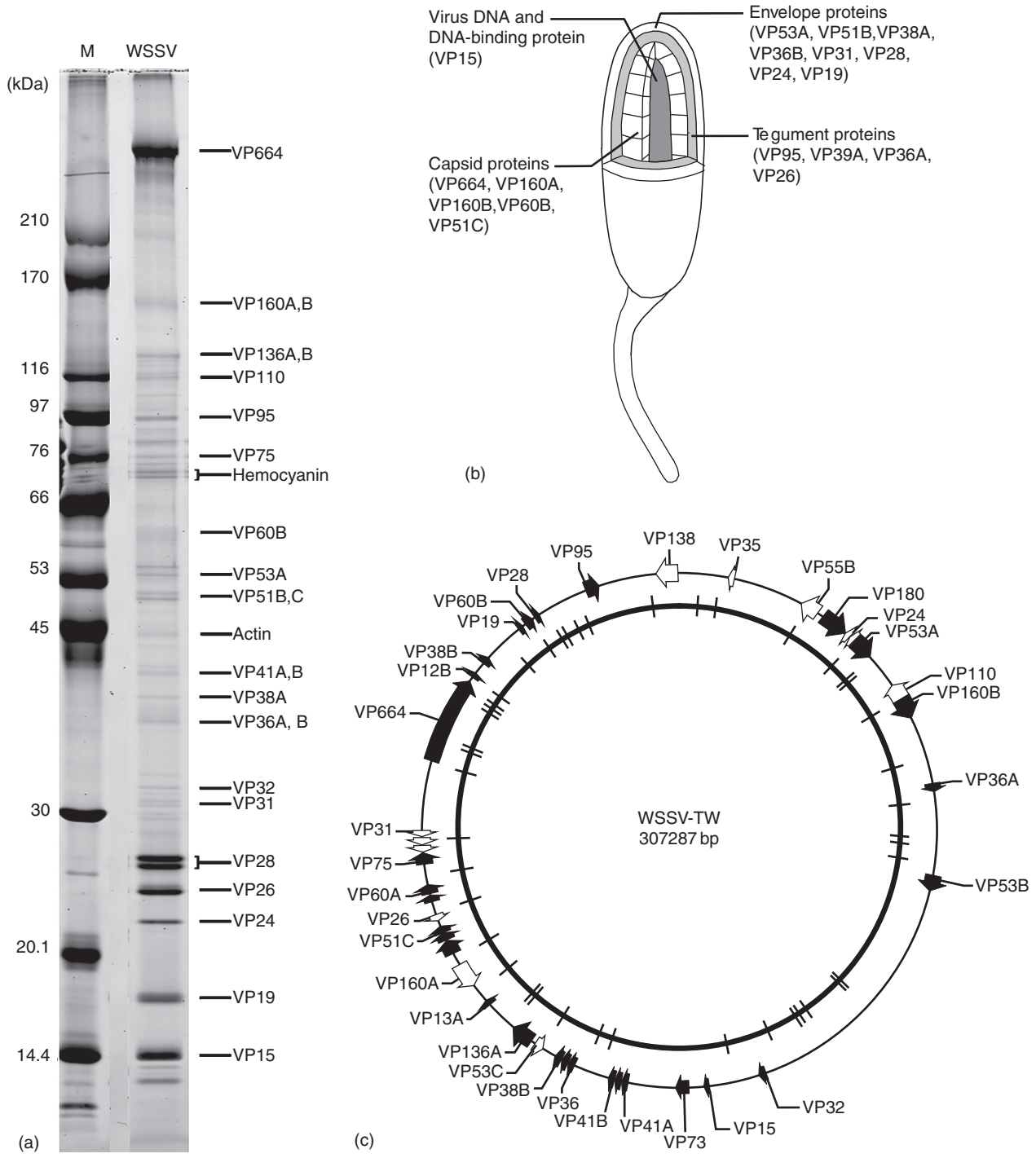


Figure 5 WSSV structural proteins. (a) SDS-PAGE profile of purified WSSV virions. The six major and some minor structural protein bands are indicated. The bands corresponding to two host proteins co-purified with the virion are also indicated. (b) A proposed schematic diagram to show the WSSV virion structure and the possible location of WSSV structural proteins and its DNA. (c) Distribution of 41 WSSV structural protein genes in the WSSV-TW genome. The inner circle shows predicted HindIII restriction enzyme cutting sites.

WSSV Genome Structure

The WSSV genome is a large ds circular DNA of about 300 kbp. The complete genome sequences of three isolates originating from China (WSSV-CN), Taiwan (WSSV-TW), and Thailand (WSSV-TH) indicated sizes of 305, 307, and 297 kbp, respectively. Genetic variations among these isolates include two major polymorphic loci

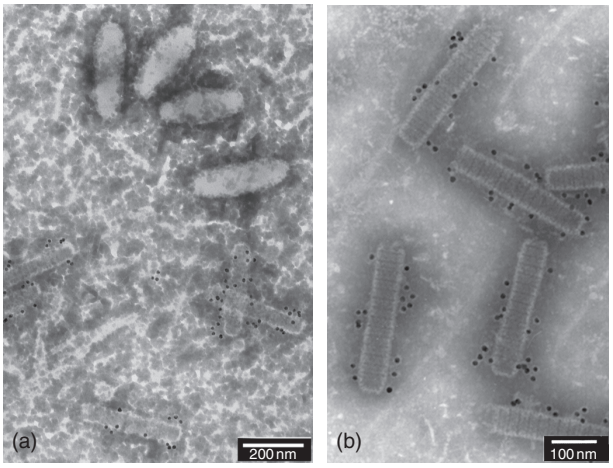


Figure 6 Immunoelectron microscopy analysis of purified WSSV virions detected with VP664 antibody followed by gold-labeled secondary antibody. (a) The VP664 antibody specifically binds to the nucleocapsid and not to the viral envelope. (b) Most of the gold particles are localized at the perimeter of the nucleocapsid.

(a 13 kbp deletion in WSSV-TH and a variable region prone to recombination), a transposase sequence that was only present in WSSV-TW, and variation in the number of repeats and single nucleotide mutations. Not including the two major polymorphic loci and the transposase sequence, these three isolates share an overall nucleotide sequence identity of 99.32%. It has been shown that differences in the major polymorphic 13 kbp deletion locus are related to differences in virulence of WSSV isolates.

The WSSV genome has a total G + C content of 41% uniformly distributed over the genome. Although most WSSV genome sequences are unique, 3% of the genome consists of highly repetitive sequences, which are organized into nine homologous regions (hrs). These are distributed throughout the genome, and are located largely in intergenic regions. The presence of hrs is a feature of many baculovirus genomes. Baculovirus hrs function as enhancers of early gene transcription and as initiation sites for DNA replication. They have also been implicated as sites of DNA recombination. It is likely that WSSV hrs will be shown to have similar functions.

A total of 532 putative ORFs consisting of a minimum of 60 codons have been identified by sequence analysis of the WSSV-TW isolate. Of these, 140 ORFs have a potential downstream polyadenylation site (AATAAA). The size of proteins encoded by these predicted ORFs ranges from 60 to 6077 amino acids. Only 30% of the predicted ORFs encode proteins homologous to any other known proteins or motifs. As about 40 structural proteins have been identified, approximately 8% of the ORFs encode structural proteins. Some of the predicted ORFs encode proteins that share significant similarities (40% or even higher) to each other and can therefore be classified into the same gene family. There are ten putative gene families in the WSSV genome, and it is possible that these families arose from gene duplication.

WSSV Genes

Identification through Homology Comparison

The WSSV genome contains several genes that can be identified unambiguously through homology searches. These genes are involved in nucleotide metabolism and DNA replication, and encode *thymidylate synthase*, *dUTPase*, *ribonucleotide reductases* (*rr1* and *rr2*; two separate ORFs encoding the two subunits), chimeric *thymidylate/thymidine kinase* (*TK-TMK*), and *DNA polymerase*. The chimeric *thymidylate/thymidine kinase* gene is a unique feature of WSSV, as these genes in other large DNA viruses are encoded in separate ORFs. The enzymatic activities of dUTPase, ribonucleotide reductase, and TK/TMK have been demonstrated by using purified recombinant proteins.

Some proteins from predicted ORFs show weak similarity to known proteins, including nonspecific nuclease (WSV191), TATA-box binding protein (TBP; WSV303), CREB-binding protein (CBP; WSV100), and helicase (WSV447). The activity of the nonspecific nuclease has been demonstrated using a purified recombinant protein produced in *Escherichia coli*.

Some proteins contain well-defined domains or motifs, and therefore their possible functions can be inferred. Two protein kinases, PK1 (WSSV482) and PK2 (ORF61), have been identified because they each contain the catalytic domain of serine/threonine protein kinase. Two proteins containing the EF-hand, calcium-binding motif have also been identified (WSV079 and WSV427; WSV427 is a latency-related gene), suggesting that WSSV might modulate calcium levels in infected cells. Four proteins with RING-H2 finger motifs have been annotated (WSV199, WSV222, WSV249, and WSV403). This motif has been implicated in ubiquitin-conjugating enzyme (E2)-dependent ubiquitination; many proteins containing a RING finger play a key role in the ubiquitination pathway. Ubiquitination activity has been demonstrated for WSV222 and WSV249, and their interacting host proteins have been identified using yeast-two hybrid screening. Some proteins contain conserved zinc finger or leucine zipper motifs. These motifs have been shown to be involved in DNA-protein interactions and in regulation of transcriptional activation. Of these, *wssv126* is one of three immediate early genes that have been identified to date.

Identification through Functional Studies

As most WSSV gene products show no significant homology to known proteins, *ab initio* research is required to

identify their functions. For large dsDNA viruses, gene expression is usually categorized sequentially as immediate early, early, and late. Immediate early genes are expressed in the absence of *de novo* viral protein synthesis, early genes are involved in DNA synthesis, and late genes are expressed following viral DNA replication and encode the viral structural proteins and proteins required for assembly, maturation, and release of viral particles. Three immediate early genes, *ie1* (*wssv126*), *ie2* (*wssv418*), and *ie3* (*wssv242*), have been identified in WSSV-infected shrimp by showing that their transcription is not inhibited by cycloheximide. IE1 contains the Cys2/His2-type zinc finger motif, which is implicated in DNA-binding activity, suggesting that IE1 might function as a transcription factor. The gene products of *ie2* and *ie3* contain no recognizable functional motifs, and their functions remain unknown. Three latency–related genes have been identified: *wsv151*, *wsv427,* and *wsv 366.* These genes have been shown to be relatively highly expressed in asymptomatic carrier shrimp that tested negative for WSSV using a commercial PCR detection kit. When expressed as recombinant proteins in Sf9 insect cells, WSV151 is a nuclear protein of approximately 165 kDa, while WSV427 interacts *in vitro* with a novel shrimp serine/threonine protein phosphatase. A novel anti-apoptosis protein (ORF390) has also been identified. WSSV infection induces bystander, noninfected cells to undergo apoptosis, whereas WSSV-infected cells are not apoptotic. ORF390 has been shown to inhibit apoptosis induced either by infection with a mutant baculovirus or by treatment with actinomycin D. The protein has two putative caspase9 cleavage sites and a caspase3 cleavage site, and therefore might function like the AcMNPV P35 protein by directly binding to and inhibiting the activity of caspase9 and/or caspase3.

WSSV Gene Expression and Regulation

Since no suitable shrimp cell line is available, studies of WSSV gene expression have long been carried out *in vivo* in penaeid shrimp or crayfish by using RT-PCR. In the black tiger shrimp, *P. monodon*, WSSV immediate early and early genes (e.g., *rr1*, *rr2*, *pk1*, *tk-tmk*, *dnapol*, *ie1*, *ie2,* and *ie3*) are detected as early as 2–4 hpi (hours post infection) and their transcription levels either steadily and slowly increase till the end of the study (48 or 60 hpi) or else reach a plateau phase at 12–18 hpi. For late genes (the major viral structural protein genes), although very low levels could be detected as early as 2–4 hpi, transcription levels surged at 12–18 hpi and steadily increased thereafter. In crayfish, expression of the late genes was not observed until at least 18–24 hpi.

WSSV gene expression has been globally analyzed using microarrays. In one study, the results revealed that about 23.5% of WSSV genes began to express at 2 hpi, 4.2% at 6 hpi, 17.7% at 12 hpi, and 47.9% at 24 hpi. Most of the WSSV structural protein genes were expressed either at 12 hpi (9 WSSV structural protein genes) or at 24 hpi (14 WSSV structural protein genes). However, several structural protein genes were expressed at 2 hpi, suggesting that these proteins might also have important roles in the early infection stage. In another microarray study, clustering of the transcription profiles of the individual WSSV genes during infection showed two major classes of genes; the first class reached maximal expression at 20 hpi and the second class at 2 days post infection. Since most of the known WSSV early genes were found in the first class, the other genes in this class were also thought to be early genes. Conversely, the genes that clustered with the structural protein genes in the second class were likewise thought to be late genes. Therefore, RT-PCR and microarray studies suggest that, as for other large dsDNA viruses, the expression of WSSV genes is regulated in a coordinated series of cascades.

The promoter regions of WSSV early and late genes have been analyzed. The upstream region of WSSV early genes contains a TATA box and an initiator. This is similar to the *Drosophila* RNA polymerase II core promoter sequences, suggesting that the WSSV early genes are under the control of cellular transcription machinery. Alignment of the regions upstream of all the major structural protein genes has identified a degenerate motif (ATNAC) that could be involved in WSSV late gene transcription, while only one of these genes was found to contain a functional TATA box. These differences between the promoter regions of the early and late genes are sufficient to account for the differential transcription patterns between early and late genes, and they also suggest that WSSV uses at least two different classes of transcription machinery for gene expression. Since the WSSV genome contains no homologs of RNA polymerase subunits, at least one novel transcription factor (e.g., IE1, IE2, or IE3) would need to be induced at the early infection stage. These factors, which are presumably able to recognize a late gene-specific motif (such as the 'ATNAC' motif), could then recruit the host cellular transcription machinery to transcribe the late genes.

Viral–Host Relationships

Apoptosis

Apoptosis is a cell suicide program that enables multicellular organisms to direct and control cell numbers in tissues and to eliminate cells, including virus-infected cells that may be harmful to the survival of the organism. If apoptosis occurs during the early stage of infection, virus production is severely limited; because it inhibits the spread of progeny virus in the host, apoptosis is recognized as an antiviral defense. In consequence, most animal viruses

have evolved strategies to evade or delay an early apoptosis response. The classical signs of apoptosis can be identified in WSSV-infected shrimp, including nuclear disassembly, fragmentation of DNA into a ladder, and increased caspase3 activity. Furthermore, there is a positive correlation between the severity of WSSV infection and the number of apoptotic cells. Among the WSSV target tissues, the subcuticlar and abdominal epithelia are the most seriously damaged, and these epithelial tissues exhibit the highest incidence of apoptotic cells. However, it is significant that cells displaying apoptotic characteristics do not contain virions, whereas those containing WSSV virions are not apoptotic. It is therefore reasonable to suppose that apoptosis is employed by shrimp as a protective response to prevent the spread of WSSV, while in the infected cells, the anti-apoptosis protein ORF390 blocks apoptosis and thus facilitates multiplication of the virus.

Hemocyte Responses to WSSV Infection

In crustaceans, the hemocytes (blood cells) mediate many defense-related activities that are essential for the nonspecific immunity, including phagocytosis, melanization, encapsulation, cytotoxicity, and clotting. They also produce a vast array of antimicrobial proteins, such as agglutinins, antimicrobial peptides, and lysozymes. Hemocyte responses to WSSV have been investigated thoroughly in penaeid shrimp and crayfish. WSSV infection decreases the total hemocyte count (THC) in shrimp but not in crayfish. The reduced THC in shrimp is probably due to lysis by the virus of infected hemocytes as well as due to virus-induced apoptosis in both circulating hemocytes and hematopoietic tissue. In addition, circulating hemocytes migrate to tissues that have a high number of virus-infected cells, although this is probably a general defensive response rather than a specific antiviral response. WSSV infection also induces apoptosis in crayfish hemocytes but the percentage of apoptotic hemocytes is much lower in crayfish (1.5%) than in shrimp (20%).

WSSV infection differentially affects the three morphologically and functionally distinct hemocyte types that have been identified in crustacean hemolymph, commonly referred to as semigranular cells (SGCs), granular cells (GCs), and hyaline cells (HCs). In shrimp and crayfish, both SGCs and GCs can be infected with WSSV but SGCs are the preferential target. This suggests that SGCs are more susceptible to WSSV and that the virus replicates more rapidly in SGCs than in GCs. However, the melanization activity of GCs in WSSV-infected crayfish is reduced. The third type of hemocyte (HC) is refractory to WSSV infection in shrimp but their role in antiviral defense is still not clear. On the other hand, GCs and SGCs each play a major defensive role and WSSV infection of these cell types is likely to weaken shrimp defenses and reduce shrimp health. Since hemocytes are also necessary for clotting, the low THC also accounts for the phenomenon that hemolymph withdrawn from WSSV-infected shrimp and crayfish always has a delayed (or sometimes completely absent) clotting reaction.

Protection of Shrimp Against WSSV Infection Using Vaccination and RNAi Strategies

Since the earliest outbreaks of WSD, several advances have been made in our understanding of how shrimp might be protected against WSSV infection. For example, although it is generally thought that shrimps lack the immunoglobulin-based adaptive immune system, recent studies have shown that when *Penaeus japonicus* shrimps survive either natural or experimental WSSV infections, they sometimes show resistance to subsequent challenge with WSSV. This 'quasi-immune response' suggests that shrimps may have an innate immune system that includes specific memory. Further research into this phenomenon has shown that intramuscular injection or oral administration of either inactivated WSSV virions or recombinant structural protein VP28 similarly provide shrimps and crayfish with some degree of protection against WSSV infection. Another potential means of limiting WSSV infection is to take advantage of the *in vivo* roles of RNA interference induced by dsRNA. In *Litopenaeus vannamei* shrimp, whereas long dsRNA was shown to induce both sequence-dependent and sequence–independent antiviral responses, WSSV gene-specific dsRNAs produce strong anti-WSSV activity. Similar results have also been reported for the gene-specific dsRNAs of another shrimp virus, yellow head virus. However, the anti-WSSV activity of WSSV gene-specific dsRNAs varies greatly from one WSSV gene to another, and the effectiveness of each candidate needs to be tested empirically. Further, the antiviral activity of WSSV gene-specific small interfering RNA (21–23 bp dsRNA) has not yet been conclusively demonstrated.

See also: Apoptosis and Virus Infection; Shrimp Viruses.

Further Reading

Leu JH, Tsai JM, Wang HC, *et al.* (2005) The unique stacked rings in the nucleocapsid of the white spot syndrome virus virion are formed by the major structural protein VP664, the largest viral structural protein ever found. *Journal of Virology* 79: 140–149.

Lo CF, Wu JL, Chang YS, *et al.* (2004) Molecular characterization and pathogenicity of white spot syndrome virus. In: Leung KY (ed.) *Molecular Aspects of Fish and Marine Biology, Vol. 3: Current Trends in the Study of Bacterial and Viral Fish and Shrimp Diseases*, pp. 155–188. Singapore: World Scientific.

Marks H, Ren XY, Sandbrink H, *et al.* (2006) *In silico* identification of putative promoter motifs of white spot syndrome virus. *BMC Bioinformatics* 7: 309–322.

Robalino J, Bartlett T, Shepard E, *et al.* (2005) Double-stranded RNA induces sequence-specific antiviral silencing in addition to nonspecific immunity in a marine shrimp: Convergence of RNA

interference and innate immunity in the invertebrate antiviral response? *Journal of Virology* 79: 13561–13571.

Tsai JM, Wang HC, Leu JH, *et al.* (2004) Genomic and proteomic analysis of thirty-nine structural proteins of shrimp white spot syndrome virus. *Journal of Virology* 78: 11360–11370.

Tsai JM, Wang HC, Leu JH, *et al.* (2006) Identification of the nucleocapsid, tegument, and envelope proteins of the shrimp white spot syndrome virus virion. *Journal of Virology* 80: 3021–3029.

van Hulten MCW, Witteveldt J, Peters S, *et al.* (2001) The white spot syndrome virus DNA genome sequence. *Virology* 286: 7–22.

Witteveldt J, Cifuentes CC, Vlak JM, *et al.* (2004) Protection of *Penaeus monodon* against white spot syndrome virus by oral vaccination. *Journal of Virology* 78: 2057–2061.

Wongteerasupaya C, Pungchai P, Withyachumnarnkul B, *et al.* (2003) High variation in repetitive DNA fragment length for white spot syndrome virus (WSSV) isolates in Thailand. *Diseases of Aquatic Organisms* 54: 253–257.

Yang F, He J, Lin X, *et al.* (2001) Complete genome sequence of the shrimp white spot bacilliform virus. *Journal of Virology* 75: 11811–11820.

Zhang X, Huang C, and Hew CL (2004) Use of genomics and proteomics to study white spot syndrome virus. In: Leung KY (ed.) *Molecular Aspects of Fish and Marine Biology, Vol. 3: Current Trends in the Study of Bacterial and Viral Fish and Shrimp Diseases*, pp. 204–236. Singapore: World Scientific.

Zuidema D, van Hulte MCW, Marks H, *et al.* (2004) Virus–host interaction of white spot syndrome virus. In: Leung KY (ed.) *Molecular Aspects of Fish and Marine Biology, Vol. 3: Current Trends in the Study of Bacterial and Viral Fish and Shrimp Diseases*, pp. 237–255. Singapore: World Scientific.

Y

Yatapoxviruses

J W Barrett and G McFadden, The University of Western Ontario, London, ON, Canada

© 2008 Elsevier Ltd. All rights reserved.

Introduction

The most recently accepted ICTV designation for the *Poxviridae* includes a new primate genus in the subfamily *Chordopoxvirinae*. The genus *Yatapoxvirus* contains two recognized members: the type species, *Yaba monkey tumor virus* (YMTV) and *Tanapox virus* (TPV).

History

Poxvirus members of the genus *Yatapoxvirus* have been identified relatively recently following infection of monkeys at primate centers in Nigeria (1957) and in several states of the USA (1967), and infection of native African populations of equatorial Africa (late 1950s and early 1960s). YMTV infection produces benign histiocytomas in man and monkeys that rapidly proliferate, leading to large subcutaneous masses that are considered benign tumors. TPV infection causes localized, dermal lesions which are located on the extremities and which resolve slowly. The yatapoxviruses have been shown to infect humans and other primates only (**Table 1**). Based on genomic sequence data and morphology studies, the yatapoxviruses are structurally similar to other members of the *Poxviridae*.

YMTV was first observed following a spontaneous outbreak of subcutaneous tumors in a colony of captive rhesus monkeys housed in open-air pens in June 1957 at Yaba, near Lagos, Nigeria. The superficial growths were first observed in a single rhesus monkey and subsequently spread to 20 of 35 rhesus monkeys from that colony over the following several weeks. Although this primate center housed several other species in close proximity, only a single other species of primate (dog-faced baboon) was affected during this outbreak. The lesions in all cases eventually regressed.

TPV was first identified (1957) following two outbreaks among local people living in villages along the Tana River of Kenya. The illness was characterized by acute febrile reaction and associated pox-like skin lesions. A more widespread illness of 50 cases occurred in 1962 that included both genders and a spread of ages. The observation that the infection occurred during years of dramatic floods and increased mosquito activity, and the fact that the lesions appeared only on areas of exposed skin suggested that a biting insect, most likely a mosquito, was the vector of transmission. Virus isolated from infected humans could only be grown *in vitro* on various human and monkey cell lines. In addition, further transmission of infection by the inoculation virus was successful only in man and monkey. Therefore, most likely, an undefined local monkey species acts as the reservoir species for TPV.

Supporting these conclusions was the occurrence of mild poxvirus infections in three primate centers in the USA in 1966. The affected monkeys, mainly rhesus macaques, exhibited lesions that were similar in appearance and histology to those of the native Kenyans infected with tanapox. The lesions were hypertropic, slow in developing, with little evidence of vesiculation, and these characteristics were quite different from the lesions produced by a similarly appearing nodule that arises from monkeypox infection of man. In addition, at least two handlers at these centers contracted an infection which produced symptoms and lesions that clinically and histologically resembled tanapox infection.

Taxonomy and Classification

The prefix 'yata' is a contraction from the names of the two recognized members of the yatapoxviruses: *ya*ba monkey tumor virus and *ta*napox virus. The members of the yatapoxviruses are closely related by sequence similarity, serology, and immunodiffusion tests. This genus is comprised of only two recognized members (YMTV and TPV) that so far have only been shown to infect primates, including humans. The members of this genus are immunologically distinct from members of the seven other *Chordopoxvirinae* genera. Vaccination by poxviruses from other genera, including vaccinia virus, will not protect from infection by

Table 1 Members of the *Yatapoxvirus* genus

Member	*Abbreviation*	*Natural host*	*Major arthropod vector*	*Natural host disease*	*Length of infection*
Yaba monkey tumor virus	YMTV	Monkeys of Africa and Malaysia	Probably mosquito	Large, multicellular masses (2–5 cm)	Spontaneously regresses 6–12 weeks
Tanapox	TPV	Humans	Probably mosquito	Individual, round, raised nodules	Resolution in 3–4 weeks
Yaba-like disease	YLDV	Captive primates/ human handlers	Probably mosquito in monkeys, possibly scratches or bites to human handlers	Individual-few raised nodules	Resolution in 3–4 weeks

the yatapoxviruses and, conversely, a previous infection with a yatapoxvirus will not block other poxvirus infections.

A third member, yaba-like disease virus (YLDV), considered a strain of TPV, has been isolated from monkeys and their human handlers at several primate centers in the USA. Although ICTV does not recognize YLDV, the complete genomic sequence has been deposited in GenBank and provides scientists with genetic information about the closely related TPV.

Properties of the Virion

Morphologically, the yatapoxvirus viruses resemble typical oval-shaped poxvirus virions. YMTV and TPV particles contain a lipid outer membrane, inner membrane, and enclosed dumbbell-shaped core and lateral bodies. The virus measures between 250 and 300 nm on the long axis.

DNA Replication, Transcription, and Translation

Generally, viral DNA replication and protein synthesis of the yatapoxviruses is similar to, albeit slower than, the prototypical poxvirus, vaccinia virus. Yatapoxvirus DNA synthesis occurs in the cytoplasm of infected cells in 'virus factories'. Sequencing has confirmed that the genomes of the yatapoxviruses encode the standard suite of conserved poxvirus housekeeping genes, including transcription factors and enzymes required for viral replication, transcription, and translation.

One consistent difference between YMTV and other poxviruses has been the observation that YMTV replicates more efficiently *in vitro* at 35 °C rather than 37 °C. The reason for this temperature sensitivity is undefined; however, it may be the result of a required viral-encoded enzyme that is temperature sensitive or prolonged passage in a poikilothermic, nonmammalian host. Regardless, the cycle of replication follows standard poxvirus early gene expression, DNA synthesis, late gene expression, and morphogenesis. Viral DNA synthesis is detected as early as 3 h post infection (p.i.). Progeny virus first appears at 24 h p.i. *in vitro* and plateaus at 72 h p.i. This replication cycle is six to ten times longer than for vaccinia virus. YMTV has a narrow host range in tissue culture. In addition, *in vitro* infection involves a prolonged replication cycle and generally low virus yield. Continuous cells from cercopithecus monkey kidney cells (CV-1) are susceptible to YMTV infection; however, cell lines derived from other monkeys (BGMK, OMK) and other species (e.g., HeLa, RK13, and CEFs) do not support productive infection.

In contrast, TPV replication is most efficient at 37 °C. TPV replication follows the typical cascade of poxvirus DNA synthesis and production of infectious progeny but exhibits slower kinetics when compared to vaccinia virus, although it is much faster than YMTV. The eclipse period ranges from 24 to 48 h p.i.; however, this variation is dependent on the multiplicity of infection.

Transient expression of native yatapoxvirus genes from standard mammalian expression vectors results in low-to-no protein expression. However, if the viral coding sequence is optimized to favor the use of more commonly used human codons, the result is generally much higher protein expression levels from transfected human cells.

Properties of the Genome

The members of the yatapoxviruses encode the smallest characterized genomes within the *Poxviridae* (**Table 2**). Complete genomic sequencing information is now available for all yatapoxviruses. The genomes of the yatapoxvirus members are very A+T- rich, a characteristic shared by other members of the *Poxviridae* including members of the ortho-, sui-, capri-, and avipoxviruses. The YMTV genome is 70% A+T, 134.7 kbp long, and encodes for 140 genes. In contrast, TPV is 144.6 kbp, 73% A+T, and encodes for 155 genes (**Table 2**). Comparison between the complete YLDV sequence and the sequenced genomes of TPV indicates a high degree of sequence identity of approximately 98%, supporting the claim that TPV and YLDV are strains of the

Table 2 Features of the yatapoxvirus genomes

Member	*Genome size (bp)*	*Single copy genes*	*Duplicated genes*	*Terminal inverted repeat (bp)*	*% A + T*
TPV	144 565	155	1	1868	73
YMTV	134 721	139	1	1962	70.2
YLDV	144 575	150	1	1883	73

same virus. Comparison of the genomic sequences of YMTV and TPV indicates 78% identity. All of the genes identified in YMTV are also encoded by TPV; however, YMTV has lost 13 open reading frames (ORFs) found in TPV. The yatapoxviruses encode many of the same structural and housekeeping genes that are found in other poxviruses. The immunomodulatory genes of the yatapoxviruses also include a handful of novel genes predicted to be involved in regulation of immune response that are unique to the yatapoxvirus or are found in only a few other poxviruses. These include a new inhibitor of human tumor necrosis factor (TNF α (2L), virally encoded versions of chemokine receptors (7L and 145R), and TPV/YLDV (but not YMTV) encodes a viral IL-10 homolog (134R).

Immunity

YMTV and TPV show minimal to moderate cross-reactivity in collected sera. However, YMTV infection can protect nonhuman primates from TPV challenge. Monkeys infected with TPV and then challenged with YMTV exhibit reduced tumors and delayed symptoms. Comparison between YMTV and TPV infection identified both type-common and type-specific antigens. Circulating neutralizing antibodies, although present in the sera of several species of monkeys, are ineffective in preventing growth of YMTV tumors or reinfection. Immunity to superinfection is observed as long as tumors are present or regressing; however, following total tumor regression a new infection results in new tumor formation. Complement-fixing and complement-fixing-inhibiting antibodies are present in clinical and convalescent stages, respectively, of rhesus monkeys infected with either TPV or YMTV. The persistence of complement-fixing antibodies in monkeys infected with TPV is 10–12 weeks. Complement-fixing antibody was detected up to 35 weeks p.i. in monkeys infected with YMTV.

Tropism and Transmission – Humans

Naturally occurring YMTV infections have only been identified in nonhuman primates. However, accidental and volunteer infections have been established in humans. Infected humans develop lesions similar to those observed in monkeys although the proliferative responses are less pronounced and regression occurs earlier than in monkeys. Recovery of infectious virus from human lesions followed by serial passage and titration confirmed that YMTV was able to replicate productively in primary human tissue. Inoculation of rabbits, guinea pigs, hamsters, rats, mice, and dogs failed to produce proliferative lesions. In addition, no lesions were observed following inoculation of embryonated eggs. Testing of nonhuman primates suggests that rhesus and cynomolgus species are most susceptible as hosts.

TPV infections have only been identified in native populations of equatorial Africa or visitors to that region. The fact that infections appeared during years of extensive flooding, and that lesions are observed only on exposed areas of skin and occur on the extremities, suggests that transmission is via a biting arthropod vector (e.g., mosquitoes).

YLDV infection of monkey handlers at primate centers of the US exhibited a brief fever followed by production of a few raised, necrotic nodules that completely resolved.

Tropism – Nonhuman Primates

YMTV and YLDV have been identified from monkeys kept as research subjects at primate centers. Monkeys infected with YMTV were kept in open-air pens and infection spread from a single individual to other segregated monkeys, suggesting biting arthropods as the likely transmission vector. Research groups have screened sera collected from monkeys of various parts of the world for antibodies against either YMTV or TPV. Generally, antibodies against YMTV and TPV were identified from various monkey species from Africa, as well as cynomolgus monkeys from Malaysia, suggesting that these species suffer from infection, possibly subclinical, with TPV or YMTV. No antibodies to either virus were detected in Indian rhesus monkeys or from any of the monkey species tested from South America. Since rhesus monkeys are highly susceptible to clinical infection by both yatapoxviruses, it is surprising that none of the collected samples confirmed any previous infection. Although natural infection must be rare, the geographical location of the Indian primate species that are located between the African and Malaysian species (both of which were YMTV/TPV positive) suggests that negative serology results from primates in India may be misleading.

Pathogenesis

Infection of susceptible primates by YMTV targets histiocytes which rapidly divide and produce tumors. Histiocytes are cells, of either macrophage or Langerhans cell lineage, that migrate to areas of cellular disturbance. Following YMTV inoculation, histiocytes migrate to the site of virus inoculation during the first 48 h. The infiltrating histiocytes begin to exhibit cellular alterations, including enlargement of the nucleus and the prominence of nucleoli, by 72 h p.i. By day 5, the altered histiocytes become spindle shaped and exhibit mitotic activity. Also by day 5, inclusion bodies begin to become visible. Actively dividing cells begin to assume spiral forms between days 7 and 9 and acquire well-defined reticular patterns. By week 2, many degenerating cells along with other actively dividing cells become apparent. This scenario continues for the next 4–6 weeks. Eventually, the number of degenerating cells starts increasing and the number of dividing cells diminishes. Host immune response to infection is generally insignificant until regression of the lesion is nearly complete. At this point, there is evidence of lesion infiltration with host mononuclear cells. Necrosis of the center of the lesion is generally not part of the regression process; however, the tumor will exhibit surface ulceration. The beginning of degeneration is characterized by eosinophilic cytoplasmic mass, cytoplasmic lipid accumulation, vacuolization, and eventual cell dissolution. Tumor regression is not correlated to serum antibody titers. It is postulated that tumor regression is due to *in vivo* cytopathic effects rather than an innate immune response. However, the cytopathogenic effect of the virus eventually kills the cells and the tumor regresses. Natural regression can take up to several months, after which the individual is susceptible to reinfection.

TPV infection begins with a short febrile illness lasting several days that may include severe headaches, general fatigue, and body aches. This is followed by the appearance of a single, or a few, pock-like lesions that initially resemble those of variola. Tanapox lesions begin as a papule and develop into a hard, raised nodule and are normally located on the extremities. Nodules normally regress after 3–4 weeks.

Geographic and Seasonal Distribution

Naturally occurring yatapoxvirus infections have been extremely rare and geographically limited. TPV infections in human populations have only been observed in the equatorial belt of Africa between Zaire and Kenya. Recent TPV infections of humans have been limited to very few cases. Infection of human handlers with YLDV at primate centers in the US was effectively stopped by appropriate changes to biological safety conditions. YMTV infections have only been observed in nonhuman primates in Africa primate centers. Although a worldwide survey of primate sera has identified antibodies against YMTV in monkeys in both Africa and Malaysia, and TPV-neutralizing antibodies in native populations of equatorial Africa, the numbers of observed cases are rare.

The yatapoxviruses are probably transmitted by biting arthropod vectors (mosquitoes?) and therefore environmental conditions such as rainy seasons, excessive flooding, and natural occurrences that bring humans into closer contact with infected nonhuman primates will lead to increased observation of infections.

Immune Modulation

TPV is a self-limiting infection in humans and natural infection for YMTV has not been observed in humans. Only a small amount of research has been done on the molecular aspects of a TPV infection and none has been undertaken with YMTV. It is known that TPV-infected cells secrete a 38 kDa glycoprotein that has been shown to bind human interferon-γ, human IL-2, and human IL-5. In addition, the secreted, early protein 2L of TPV represents a new class of virally encoded tumor necrosis factor (TNF)-binding proteins found in the members of the yatapoxviruses, swinepox, and the unclassified deerpox virus. This 2L protein binds human TNFα with high affinity but is unable to bind other members of the human TNF superfamily. In addition, TPV 2L inhibits human TNFα from binding to TNF receptors I and II, as well as blocking TNF-induced cytolysis. However, 2L protein was shown to be unable to bind IFN-γ, IL-2, or IL-5 under experimental conditions.

TPV has been isolated from humans living in (or visiting) equatorial Africa and YLDV from workers at primate centers in the USA. The complete genomic sequence is presently available from TPV, YLDV and YMTV. Based on these sequences, it is clear that the TPV and YLDV genomes are 98% identical, which is clearly on the level of genetic similarity between strains of vaccinia virus. Based on the sequencing, a large number of host-modulating viral genes were predicted but only a handful have been studied. These include a new viral homolog of CCR8, a novel TNF inhibitor, and a viral-encoded member of the IL-10 family.

Epidemiology

YMTV is likely endemic in African monkeys. Neutralizing antibodies have been detected in monkeys from Africa and Malaysia, suggesting that YMTV possibly represents a latent infection in several monkey species. If this is true, it is surprising that monkeys from India have not yet been

reported to contain the same neutralizing antibodies. Infection in man has only occurred through injection of isolated YMTV.

The reservoir host for TPV is thought to be wild monkeys in equatorial Africa, from which natives can be infected by mosquito transmission. TPV infection is more common in years of heavy flooding when high water forces monkeys and man into closer proximity, while at the same time mosquito populations are at their zenith.

Prevention and Control

Control of YMTV and YLDV infection in man requires normal biological safety protocols to be exercised by animal handlers, including the wearing of protective clothing. TPV infection in equatorial Africa will require mosquito control measures. Separation of Asian and African monkey species at primate centers and markets and protective clothing for merchants and animal handlers may also help.

Future Perspectives

The reservoir status of primates with the yatapoxviruses is not a major human health concern. However, the features of infection, including YMTV targeting of histiocytes and tumor formation, the lack of long-term protection following regression of the initial infection, the observation that early TPV infection is difficult to distinguish from monkeypox infection, and the observation that members of this genus of poxviruses do in fact infect humans without eliciting an effective immune response may offer some unique insights into the mechanism of human zoonotic infections. Given the current concerns for early diagnoses of any and all human poxvirus infections, research on this group of poxviruses is likely to continue.

See also: Poxviruses; Smallpox and Monkeypox Viruses; Vaccinia Virus; Zoonoses.

Further Reading

Brunetti CR, Amano H, Uedo Y, *et al.* (2003) Complete genomic sequence and comparative analysis of the tumorigenic poxvirus yaba monkey tumor virus. *Journal of Virology* 77: 13335–13347.

Brunetti CR, Paulose-Murphy M, Singh R, *et al.* (2003) A secreted high-affinity inhibitor of human TNF from tanapox virus. *Proceedings of the National Academy of Sciences, USA* 100: 4831–4836.

Downie AW and Espana C (1973) A comparative study of tanapox and yaba viruses. *Journal of General Virology* 19: 37–49.

Knight JC, Novembre FJ, Brown DR, Goldsmith CS, and Esposito JJ (1989) Studies on tanapox virus. *Virology* 172: 116–124.

Lee HJ, Essani K, and Smith GL (2001) The genome sequence of yaba-like disease virus. *Virology* 281: 170–192.

Nazarian SH, Barrett JW, Frace AM, *et al.* (2007) Comparative genetic analysis of genomic DNA sequences of two human isolates of *Tanapox virus*. *Virus Research* 129: 11–25.

Relevant Website

http://www.poxvirus.org – Poxvirus Bioinformatics Resource Center.

Yeast L-A Virus

R B Wickner, National Institutes of Health, Bethesda, MD, USA
T Fujimura and R Esteban, Instituto de Microbiología Bioquímica CSIC/University of Salamanca, Salamanca, Spain

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Decapping Removal of the methylated GMP in 5′–5′ linkage at the 5′ end of most eukaryotic mRNAs.

Ribosomal frameshifting Ribosomes changing reading frame on an mRNA, in response to a special mRNA structure, to synthesize a protein from two overlapping reading frames.

Introduction

The L-A virus of bakers/brewers yeast *Saccharomyces cerevisiae* is one of several RNA viruses infecting this organism, each of which spreads via the cell–cell fusion of mating, rather than by the extracellular route. The totivirus L-A, like members of the very similar L-BC family of viruses, is a single-segment 4.6 kbp double-stranded RNA (dsRNA) virus encapsidated in icosahedral particles with a single major coat protein called Gag.

The 20S and 23S RNA replicons are naked cytoplasmic single-stranded RNA (ssRNA) replicons except for their bound RNA-dependent RNA polymerases.

The L-A virus serves as the helper virus for any of several smaller satellite dsRNAs, called M dsRNAs, each encoding a secreted protein toxin and immunity to that toxin, producing the 'killer' phenomenon. Killer strains can eliminate some of the competition by this means, although only about 10% of wild strains harbor a killer dsRNA, suggesting there are costs to carrying this replicon. The killer phenotype was used to study the genetics of M dsRNAs and the helper L-A. Several functional variants of L-A were defined based on their interactions with different M dsRNAs and with the host, and host mutants affected in virus expression or propagation were also examined.

History

In 1963, Makower and Bevan reported that some yeast strains secrete a toxin that kills other yeasts This led to the identification by Bevan and by Fink of cellular dsRNAs and later viral particles correlated with the killer phenomenon. Studies of the chromosomal genes affecting the killer system revealed that the Kex2 protease, identified by its requirement for toxin secretion, was the long-sought proinsulin-processing enzyme. The Mak3 *N*-acetyltransferase, whose acetylation of Gag is needed for viral assembly, established consensus sequences for such enzymes. The loss of the L-A virus in *mak3* mutants revealed a second dsRNA species of the same size, called L-BC, and unrelated to the killer system.

Virion Structure

L-A virions have icosahedral symmetry, but contain 120 Gag monomers per particle, in apparent violation of the rules of quasi-equivalence. In fact, each virion is composed of 60 asymmetric dimers of Gag (**Figure 1**), a feature that is common to the cores of all dsRNA viruses that have been characterized. One type of Gag molecule makes contact with the icosahedral fivefold and twofold axes, but not with the threefold axes. The second type of Gag finds itself in contact with the threefold axis, but not with either the five- or twofold axes (**Figure 1**). The two environments of Gag lead to two distinct conformations, suggesting that Gag may be more flexible than some other coat proteins. The L-A virion has more volume per nucleotide than do ssRNA or dsDNA viruses. These facts may reflect the requirement that the dsRNA moves inside the particle and is transcribed by the RNA-dependent RNA polymerase that is fixed to the inner virion wall (see below). Pores at the fivefold axes are assumed to allow entry of nucleotides and exit of (+) strand transcripts, but retention of the dsRNA genome. A trench on the outside contains His154, the central active site residue of the mRNA-decapping activity to which the 7-methyl-GMP structure becomes covalently attached (**Figure 2**). A

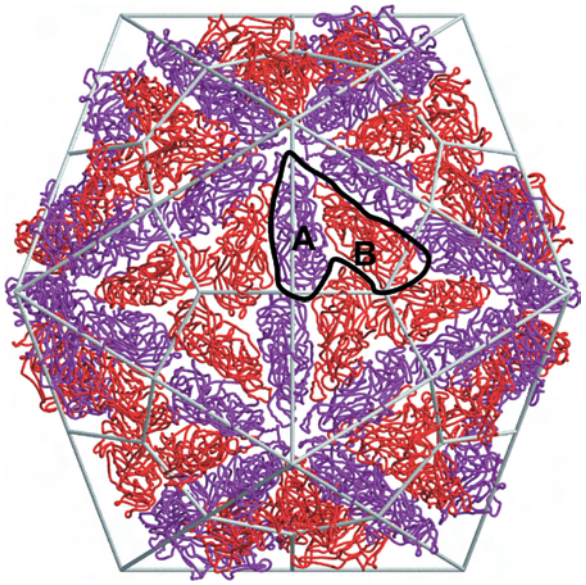


Figure 1 Wire diagram of the L-A virus capsid. The major coat protein, Gag, is found in two nonequivalent positions: 'A' molecules contact the fivefold and twofold axes, while 'B' molecules contact the threefold axes. Reproduced from Naitow I, Tang J, Canady M, Wickner RB, and Johnson JE (2002) L-A virus at 3.4 Å resolution reveals particle architecture and mRNA decapping mechanism. *Nature Structural Biology* 9: 725–728, with permission from Nature Publishing Group.

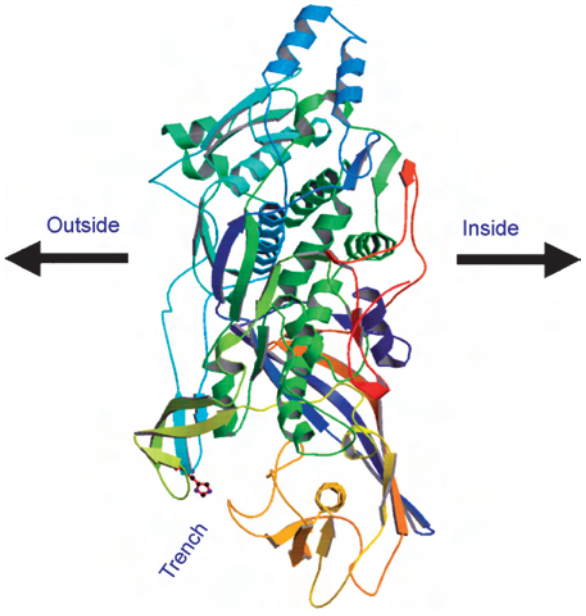


Figure 2 Ribbon diagram of a single Gag molecule. The trench on the outer surface includes His154, the Gag residue to which 7meGMP is covalently attached by the decapping activity.

layered density observed for the viral dsRNA may reflect the dsRNA's rigid structure and the fact that it is forced to press against the inner capsid wall.

Genome Organization

The single segment of the L-A genome is a 4.6 kbp dsRNA with two long open reading frames (ORFs) (**Figure 3**). The 5′ ORF encodes the major coat protein, called Gag in analogy with retroviruses, while the longer 3′ ORF, called Pol, encodes the RNA-dependent RNA polymerase, and has homology with similar enzymes of other ssRNA and dsRNA viruses. Pol is expressed only as a fusion protein whose amino end is a nearly complete Gag molecule. The fusion is carried out by a −1 ribosomal frameshift event in the region of overlap of the two ORFs. An RNA pseudoknot, in the region of overlap of the ORFs, slows ribosome progression at a point on the mRNA (of the form X XXY YYZ (0 frame indicated)) where bonding of the A-site and P-site tRNAs to the mRNA is nearly as good in the −1 frame as in the 0 frame. About 1% of ribosomes slip into the −1 frame, and, continuing translation, make the Gag–Pol fusion protein.

Replication Cycle

Transcription

Viral particles have an RNA-dependent RNA polymerase that is due to the Pol part of the Gag–Pol fusion protein. Transcription is a conservative reaction, meaning that the parental strands remain together after the synthesis of a new (+) strand. The L-A (+) strand transcripts are extruded from the particles into the cytoplasm where they serve as both mRNA and as the species that is packaged by coat proteins to make new viral particles. However, (+) strand transcripts of the smaller M dsRNA or of deletion mutants of L-A are often retained within the particle where they may be replicated. This is called 'headful replication' because the volume of the particle seems to be the determinant of how many dsRNA molecules may accumulate in each particle.

RNA Packaging

The RNA packaging site (**Figure 3**) is a stem–loop structure about 400 nucleotides from the 3′ end of the L-A (+) strand. This stem–loop has an essential A residue protruding on the 5′ side of the stem. The loop sequence is also important, but the stem sequence is not critical, as long as the stem structure can form. The packaging site on the RNA is recognized by the proximal part of the Pol domain of the Gag–Pol fusion protein. The Gag part of Gag–Pol is part of the capsid structure, so the Gag–Pol fusion protein structure assures packaging of viral (+) strands in new particles.

RNA Replication

The (−) strand synthesis reaction is called replication (**Figure 4**). This reaction involves recognition of the

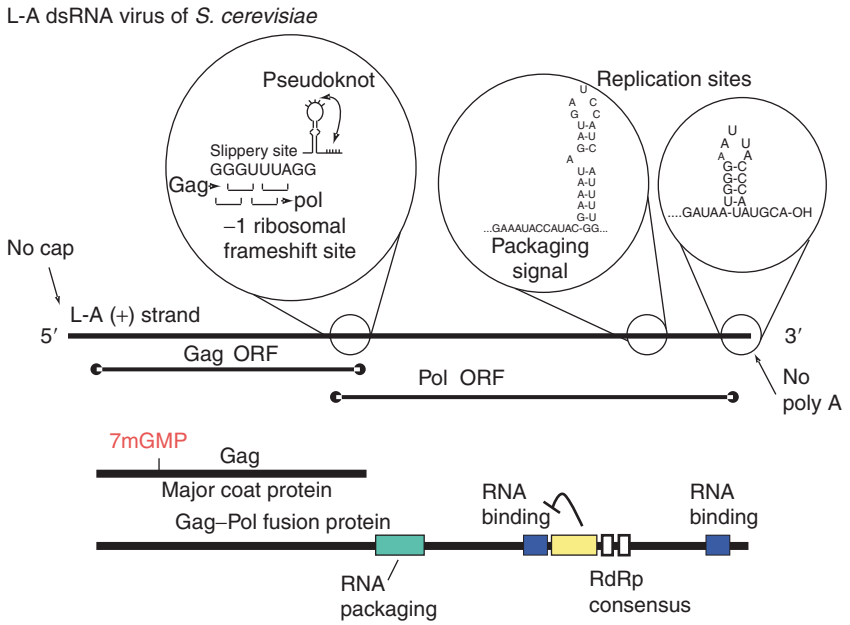


Figure 3 Genome organization. The L-A (+) strand encodes Gag and Pol in overlapping reading frames. The mRNA lacks 5′ cap and 3′ polyA structures. The location of the packaging site on the RNA and the region of the Pol protein segment (green box) that recognizes the packaging site are indicated. The RNA sites for replication, and the parts of Pol with homology to other RNA-dependent RNA polymerases (RdRp) are indicated. The cryptic *in vitro* RNA binding site (blue box) is inhibited by the area shown by the yellow box.

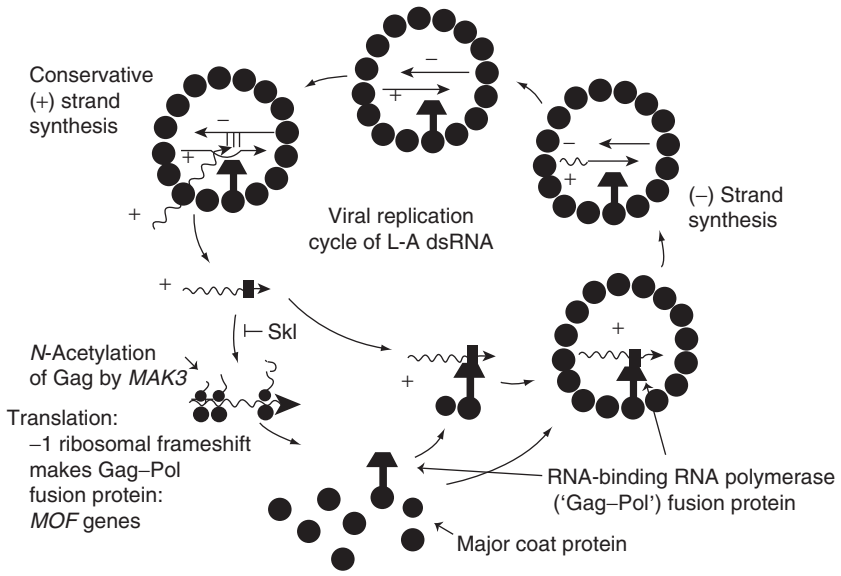


Figure 4 Replication cycle of the L-A virus. Both (+) and (–) RNA strand synthesis occur within the viral particles, but at different stages of the cycle. RNA (+) strands (transcripts) are extruded from the particles and serve as both mRNA and the species packaged to make new particles. Translation of viral mRNA is blocked by Ski proteins. N-terminal acetylation of Gag by Mak3p is necessary for viral assembly.

internal binding site on the L-A (+) strands by Pol, followed by interaction with the now nearby 3′ end and initiation of new RNA (–) chains. Multiple rounds of RNA synthesis can proceed within the viral particle in the 'headful replication' process discussed above.

Viral Translation

The translation apparatus is the battleground of RNA viruses with their hosts. Poliovirus protease cleaves eIF4G so that host-capped mRNAs cannot be translated but its own IRES-containing mRNAs are used. Influenza virus steals caps from host mRNAs.

L-A (+) strand transcripts lack both the 5′ cap and 3′ polyA structures typical of eukaryotic mRNAs, and so are at a distinct disadvantage. Mutations resulting in a relative deficiency of 60S ribosomal subunits selectively lose the ability to express cap-minus, polyA-minus mRNAs such as those of L-A. The *SKI* genes encode a series of proteins whose function is to specifically block the expression of non-polyA mRNAs. The Ski proteins have effects on both translatability of non-polyA mRNAs and on their stability. The *SKI1/XRN1* gene encodes a 5′→3′ exoribonuclease specific for uncapped mRNA. As a defense against its degradation of viral mRNA, L-A's Gag protein has a decapping activity that produces, from cellular mRNAs, 'decapitated decoys', uncapped RNAs that serve as alternative targets for the exonuclease action. In the absence of this decapping activity, viral mRNAs are not expressed, but deletion of the *SKI1/XRN1* gene restores viral mRNA expression.

L-A Genetics

Several natural variants of L-A have been described. The ability of an L-A variant to support M dsRNA replication was first called [HOK] (=helper of killer) and then H when it was found to be a property of L-As such as L-A-H, L-A-HN, or L-A-HNB. Making several chromosomal *MAK* genes dispensable for M propagation was named [B] (=bypass) and is found on L-A-HNB. Ability to exclude L-A-H was named [EXL] (=exclusion) and then just E on L-A-E. Insensitivity to the action of [EXL] was called [NEX] (=nonexcludable), and then shortened to N as in L-A-HN or L-A-HNB. The molecular basis of these interactions has resisted study because of the current inability to obtain L-A from a cDNA clone. M dsRNA can be supported from a cDNA clone of L-A, but the L-A virus has not been shown to be regenerated from these transcripts.

Other RNA Replicons in Yeast: L-BC, 20S RNA, 23S RNA

20S RNA was discovered as an RNA species which appeared when cells were placed under conditions that induce meiosis and spore formation, namely near-starvation for nitrogen and provision of acetate as a carbon source. It was shown that 20S RNA is an independent replicon, and can be made independent of the sporulation or meiosis processes. 20S RNA encodes its own RNA-dependent RNA polymerase which is bound to the otherwise naked cytoplasmic RNA. The mechanism of

20S RNA replication control by culture conditions remains to be elucidated.

23S RNA is a related, but independent yeast replicon which was found as a dsRNA form (called T). 23S RNA also encodes its RNA-dependent RNA polymerase. Both 20S RNA and 23S RNA can be launched from cDNA clones to form replicating virus.

L-BC is a totivirus, related to L-A but independent of it. Its dsRNA is essentially the same size as that of L-A although its copy number is usually about tenfold lower. Thus it was not detected until chromosomal mutants that lose L-A were examined. L-BC does not interact with the killer system, and confers no obvious phenotype, so its genetics has not been extensively explored.

See also: Narnaviruses; Totiviruses; Viral Killer Toxins.

Further Reading

Bevan EA, Herring AJ, and Mitchell DJ (1973) Preliminary characterization of two species of dsRNA in yeast and their relationship to the 'killer' character. *Nature* 245: 81–86.

Bostian KA, Elliott Q, Bussey H, Burn V, Smith A, and Tipper DJ (1984) Sequence of the preprotoxin dsRNA gene of type I killer yeast: Multiple processing events produce a two-component toxin. *Cell* 36: 741–751.

Dinman JD, Icho T, and Wickner RB (1991) A –1 ribosomal frameshift in a double-stranded RNA virus of yeast forms a gag-pol fusion protein. *Proceedings of the National Academy of Sciences, USA* 174–178.

Esteban R, Fujimura T, and Wickner RB (1989) Internal and terminal cis-acting sites are necessary for *in vitro* replication of the L-A double-stranded RNA virus of yeast. *EMBO Journal* 8: 947–954.

Fujimura T, Esteban R, Esteban LM, and Wickner RB (1990) Portable encapsidation signal of the L-A double-stranded RNA virus of *S. cerevisiae*. *Cell* 62: 819–828.

Fujimura T, Ribas JC, Makhov AM, and Wickner RB (1992) Pol of gag-pol fusion protein required for encapsidation of viral RNA of yeast L-A virus. *Nature* 359: 746–749.

Fuller RS, Brake A, and Thorner J (1989) Intracellular targeting and strructural conservation of a prohormone-processing endoprotease. *Science* 246: 482–486.

Icho T and Wickner RB (1989) The double-stranded RNA genome of yeast virus L-A encodes its own putative RNA polymerase by fusing two open reading frames. *Journal of Biological Chemistry* 264: 6716–6723.

Leibowitz MJ and Wickner RB (1976) A chromosomal gene required for killer plasmid expression, mating, and spore maturation in *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences, USA* 73: 2061–2065.

Masison DC, Blanc A, Ribas JC, Carroll K, Sonenberg N, and Wickner RB (1995) Decoying the cap-mRNA degradation system by a dsRNA virus and poly(A)-mRNA surveillance by a yeast antiviral system. *Molecular and Cellular Biology* 15: 2763–2771.

Naitow H, Canady MA, Wickner RB, and Johnson JE (2002) L-A dsRNA virus at 3.4 angstroms resolution reveals particle architecture and mRNA decapping mechanism. *Nature, Structural Biology* 9: 725–728.

Sommer SS and Wickner RB (1982) Yeast L dsRNA consists of at least three distinct RNAs; evidence that the non-Mendelian genes [HOK], [NEX] and [EXL] are on one of these dsRNAs. *Cell* 31: 429–441.

Tercero JC and Wickner RB (1992) *MAK3* encodes an N-acetyltransferase whose modification of the L-A *gag* N-terminus is necessary for virus particle assembly. *Journal of Biological Chemistry* 267: 20277–20281.

Vodkin MH, Katterman F, and Fink GR (1974) Yeast killer mutants with altered double-stranded ribonucleic acid. *Journal of Bacteriology* 117: 681–686.

Wickner RB (2006) Viruses and Prions of Yeast, Fungi and Parasitic Microorganisms. In: Knipe DM and Howley PM (eds.) *Fields Virology,* 5th edn. Philadelphia, PA: Lippincott Williams and Wilkins.

Yellow Fever Virus

A A Marfin, Centers for Disease Control and Prevention, Atlanta, GA, USA
T P Monath, Kleiner Perkins Caufield and Byers, Menlo Park, CA, USA

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Aedes A genus of mosquitoes strongly involved in the transmission of yellow fever virus. *Aedes (Ae.) aegypti* is a species belonging to the genus that breeds in close association with humans and is often responsible for epidemics of yellow fever.

Flavivirus A virus in the genus *Flavivirus*.

Genotype Genetic composition of an individual (virus), used to delineate relatedness of individuals.

Host A vertebrate species (including humans) infected by virus. An effective host is one that developes viremia sufficient to infect vectors and thus contributes to the virus transmission cycle.

Pathogenesis The process whereby virus infection proceeds in the host, leading to spread of the infection to vital organs and the development of damage to cells and tissues.

Transmission cycle The sequential infection of blood-feeding vectors and vertebrate hosts

responsible for maintaining and amplifying virus in nature.
Vector An arthropod (e.g., mosquito or tick) capable of becoming actively infected by blood feeding and of subsequently transmitting virus to a vertebrate host.

Introduction

Yellow fever virus (YFV) is the prototype species of the virus family *Flaviviridae* (and of the genus *Flavivirus*), which includes approximately 70 single-stranded RNA viruses, most of which are transmitted by mosquitoes or ticks. Other viruses in this family include the four dengue viruses, West Nile virus, Japanese encephalitis virus, and tick-borne encephalitis viruses.

History

Yellow fever (YF) is the original 'viral hemorrhagic fever', a systemic illness characterized by high viremia; hepatic, renal, and myocardial injury; hemorrhage; and high lethality. Genetic sequence analysis reveals that YFV diverged from the ancestral flaviviral lineage, roughly 3000 years ago, earlier than did other mosquito-borne flaviviruses. The first description of epidemic hematemesis occurred in Mexico in 1648, which suggests that the virus and its mosquito vector, *Aedes aegypti*, were introduced from Africa with the slave trade. Furthermore, monkeys of New World species are more susceptible to lethal infection than are African monkeys, also suggesting that YFV was recently introduced. The West African and South American genotypes of YFV are more closely related to each other than to other genotypes, indicating the probable source of introduction. During the eighteenth and nineteenth centuries, YF epidemics affected coastal cities in the Americas and Europe; the disease was likely introduced via ships infested with YFV-infected mosquitoes. In the mid- and late nineteenth century, physicians, most notably Carlos Findlay, suggested that YFV was transmitted by mosquitoes. In 1900, Walter Reed and colleagues demonstrated that the agent was a filterable virus transmitted by *Ae. aegypti* mosquitoes. In 1927, YFV was isolated from a Ghanaian man; efforts to establish continuous direct passage in animals resulted in establishment of the Asibi strain. Isolation of the Asibi strain and a contemporary ('French') strain isolated in Dakar, Senegal, enabled development of quantitative methods that allowed more precise scientific studies, as well as two live, attenuated vaccines.

Taxonomy, Classification, and Variation

YFV is antigenically distinguished from all other flaviviruses by neutralization test, and all strains of YFV belong to a single serotype. Among flaviviruses, YFV is more closely related to Wesselsbron, Sepik, Edge Hill, Bouboui, Uganda S, Banzi, Jugra, Saboya, and Potiskum viruses.

Wild-type YFV is genetically stable compared to other RNA viruses, likely due to restrictions imposed by replication in vertebrate and invertebrate hosts and maintained by a high-fidelity RNA-dependent RNA polymerase. Despite this stability, genomic sequence analyses of structural and nonstructural genes and of YFV-specific repeat sequences have identified at least seven distinct genotypes. Analyses indicate YFV arose in Africa and divided into West and East African genotypes prior to introduction into the Americas. Currently, five African genotypes are recognized – West Africa I, West Africa II, East/Central Africa, East Africa, and Angola. South American YFV can be divided into two genotypes – South America I (in Brazil) and South America II (in the western part of the continent). These genotypes demonstrate a high degree of homology but can frequently be clustered by time periods, geography, and by predominant transmission patterns.

Properties of the Virion

YFV virions are small (40–60 nm diameter), spherical, and have short surface projections. The icosohedral nucleocapsid contains the single-stranded RNA genome and a single core protein (C protein), and is surrounded by a lipid bilayer. Viral infectivity is rapidly inactivated by heat (56 °C for 30 min), ultraviolet radiation, and lipid detergents.

Properties of the Genome

The YFV genome is comprised of a single strand of plus-sense (i.e., infectious) RNA that contains 10 862 nt and is composed of a 118-nt 5′ noncoding region (NCR) preceded by a type 1 cap structure, a single open reading frame (ORF) of 10 233 nt that encodes 11 viral proteins, and a 511-nt 3′ NCR without a polyadenylated tail. The 5′ and 3′ NCRs have conformational structure and complementary sequences that are important in cyclization of the viral genome during encapsidation and replication and which function as promoters during replication. Mutations or deletions in these regions affect replication and virulence. The 3′ NCR contains conserved consensus sequences which pair with 5′ NCR sequences during cyclization and serve as the common recognition site for the viral polymerase. Directly upstream from these

sequences, the 3′ NCR forms a 3′ terminal hairpin structure and pseudoknots to serve as promoters for genomic replication.

Viral Proteins

The ORF encodes three structural proteins at the 5′ end (capsid (C), pre-membrane (preM), and envelope (E) proteins), followed downstream by 7 nonstructural (NS) proteins (NS1–NS2A–NS2B–NS3–NS4A–NS4B–NS5). The mature virion includes these three structural proteins, while the NS proteins are responsible for replication and polyprotein processing including cleavage of the polyprotein into proteins.

The C protein (MW ~ 11 kDa) interacts with genomic RNA to form the virion nucleocapsid, anchors the nucleocapsid to the endoplamic reticulum, and provides signal sequence to the preM protein. The preM glycoprotein (~27 kDa) forms an intracellular heterodimer to stabilize the E protein during exocytosis. During exocytosis, the preM protein is processed by a furin-like protease leaving a small M structural protein (~8 kDa) anchored in the virus envelope of the extracellular virion. The M protein spans the viral membrane and has exposed antigenic domains that may induce a minor immunologic response. Abnormal preM/M cleavage may result in incorporation of preM into mature virions that later affect E protein conformation, inhibit fusion, and reduce infectivity.

The E protein (~50 kDa) is the major surface structure glycoprotein. The three-dimensional configuration of the E protein forms three domains, is determined by disulfide bonding, and is critical for its role in cell tropism, membrane fusion, virulence, and immunity. Strain-, type-, and flavivirus group-specific epitopes are present in this glycoprotein. The hydrophobic C-terminus of this protein is anchored to the lipid bilayer of the viral envelope by a 170-Å-long rod. The C-terminus is connected by a flexible region to domain I, the central part of the molecule with up-and-down topology having eight antiparallel β-strands and containing the N-terminus. Inhibition of this flexible region results in decreased infectivity. Two long loops in domain II extend laterally and are responsible for dimerization. A conserved stretch of 14 amino acids in these loops is the fusion domain that allows internalization of the nucleocapsid into the infected cell. Domain III contains sites involved in binding cell receptors. Conformational neutralization determinants are scattered on the outer surface of all three domains.

The NS1 glycoprotein is involved in RNA replication through its interaction with NS4A and is both expressed on the cell surface and released extracellularly. The extracellular form contains virus-specific and cross-reactive epitopes. Antibodies to NS1 do not neutralize virus infectivity but do provide protective immunity by complement-mediated lysis and rapid clearance of cells with NS1 on the surface. NS1 is highly conserved across all YF strains. NS2A is a small protein (~22 kDa) that interacts with NS3, NS5, and 3′ NCR sequences and plays an important role in RNA replication and in the assembly or release of virions. NS3 (~70 kDa) and NS2B (~14 kDa) interact to form a complex with important enzymatic functions, including serine protease (responsible for post-translational cleavage of virus polyprotein), RNA helicase, and RNA triphosphatase activities. Because of its critical functions, the NS3 gene sequence is also highly conserved. NS3 is also present in cell membranes, contains virus-specific T-cell epitopes that are targets for cytotoxic T-cells. The NS4A (~16 kDa) and NS4B (~27 kDa) proteins are membrane associated and play a role in regulating RNA replication. The NS5 protein (~103 kDa) is a highly conserved RNA-dependent RNA polymerase in virus replication and methyltransferase during 5′ cap methylation.

Assembly and Replication

Assembly of these proteins into viral particles and subsequent exocytosis of mature virions occur in close association with the endoplasmic reticulum of secretory cells (e.g., mosquito salivary gland and various mammal exocrine and endocrine glands). Virion assembly occurs in concert with host cell secretory components and virions are released in secretory granules without significantly disturbing host cellular function or macromolecular synthesis.

After release, virions enter uninfected host cells by attaching to still-undefined receptors and are taken up in clathrin-coated vesicles. Following an acid-mediated change in the E protein that allows fusion of the virus with the host's endosomal membrane, nucleocapsids are released from the vesicles into the cytoplasm. The full-genome-length, plus-sense RNA is translated to make complementary minus strands that serve as templates for progeny plus strands. These plus strands serve as mRNAs for translation of structural and NS proteins including enzymes required for continued virus production and post-translational cleavage of the polyprotein; as templates for the transcription of new minus-sense strands; and as genomes of newly produced mature virions.

Molecular Determinants of Virulence

The entire genomes of the 17D and Asibi strains and of the French neurotropic vaccine and French viscerotropic strains have been sequenced and compared. Comparing sequences and different biological properties of these

pairs allows insight into molecular determinants of virulence. Although some potentially important specific sequences have been identified, virulence is clearly multigenic and determined by nonstructural and structural genes. Most studies on these molecular determinants have employed mouse models. Because mice manifest neurotropism and not viscerotropism, such studies are of limited value in dissecting pathogenesis. More recent studies in hamsters susceptible to lethal hepatic dysfunction and necrosis resembling wild-type YFV may allow identifying genetic sequences associated with viscerotropism, but YFV strains must be adapted by serial passage in hamsters before they elicit disease. Nonhuman primates are susceptible to wild-type YFV strains and develop a syndrome closely resembling that in humans.

The 17D and Asibi strains differ at four nucleotides in the 3′ NCR and 20 amino acids in the coding region – eight in the E gene; four in the NS2A gene; two in the NS5 gene; and one each in the M, NS1, NS2B, NS3, NS4A, and NS4B genes.

Given the functional importance of E protein in attachment of YFV to and entry into the cell, one or more of the amino acid differences in this protein likely plays a role in attenuation. Mutations in three areas of E protein alter virulence properties – the tip of the fusion domain in domain II, the molecular hinge between domains I and II, and the portion of domain III containing the putative receptor ligand. Of the eight amino acids that distinguish E proteins of Asibi and 17D viruses four are nonconservative, suggesting that one or more may be responsible for attenuation. Three of these nonconservative changes occur in the hinge region and likely alter the acid-dependent change in E protein conformation required for virus entry. Of nonconservative mutations outside of the hinge region, two (E305 and E380) are located in domain III, which contains determinants involved in tropism and cell attachment. The mutation at amino acid E380 of YF 17D occurs in the putative integrin cell receptor. Studies of this region in other flaviviruses also suggest that mutations in the putative cell receptor-binding region of domain III play a role in determining neurovirulence.

Other studies emphasize the multigenic nature of virulence and suggest that one or more of the 11 amino acid changes in NS proteins, or base changes in the 3′ NCR, may contribute to attenuation. Studies with other flaviviruses have shown that mutations in the NS proteins reduce neurovirulence, probably because NS proteins are critical to the virus replication mechanism. Eleven amino acid changes in the NS proteins of Asibi strain viruses occurred during derivation of the 17D vaccine strain. These changes in NS proteins may affect assembly or release of YFV particles, changes in the RNA helicase and triphosphatase enzymes for unwinding RNA during replication, and activity of the RNA-dependent RNA polymerase. Similarly, the 3′ NCR terminal region is involved in folding of the stem–loop structure which serves as a critical promoter during replication. Changes in this sequence or the number of repeat sequences may alter the stem–loop region, interfere with virus replication, and contribute to attenuation.

Little is known about the determinants of YFV viscerotropism (the ability of YFV to replicate and damage non-neural tissue, such as the liver) principally because assessing this property in nonhuman primates is difficult. Because neuro- and viscerotropic properties may reside in distinct regions, one cannot conclude that attenuation of one correlates with attenuation of the other. However, the hinge region of the E protein contains residues implicated in both neurotropism and viscerotropism. Studies in golden hamsters, which develop disease resembling human YFV, suggest that domain III sites implicated as cell receptor ligands are responsible for hepatotropism.

Host Range and Virus Propagation

A highly conserved region in the domain III of the E protein contains an arginine-glycine-aspartic acid (RGD) motif and is the likely site of virus-cell attachment. Because of the broad host range of flaviviruses, the receptors are highly conserved structures found in chordate and arthropod phyla. YFV replicates and produces cytopathic changes and plaque formation in a wide variety of cell types including primary chick and duck embryo cells; continuous porcine, hamster, rabbit, and monkey kidney cell lines; and cells of human origin such as HeLa, KB, Chang liver, and SW-13. The virus replicates in Fc receptor-bearing macrophages and macrophage cell lines; replication is enhanced by antibody. Mosquito cell lines, especially AP-16 cells, are highly susceptible and often used for primary isolation or efficient laboratory propagation.

Virus isolation is accomplished by intrathoracic inoculation of *Toxorhynchites* or *Ae. aegypti* mosquitoes, inoculation of mosquito cell line cultures (i.e., AP61 and C6/36 cells), intracerebral inoculation of suckling mice or inoculation of specific nonhuman primates. Intrathoracic inoculation of mosquitoes is the most sensitive method. Because infected mosquitoes show no signs of infection, virus is demonstrated by immunofluorescence, subsequent passage to a susceptible host, or amplification of YFV RNA sequences by reverse transcriptase-polymerase chain reaction (RT-PCR). AP61 cells are more sensitive than other *in vitro* methods for primary isolation and show cytopathic effects within 5–7 days of inoculation. Immunofluorescence assays to identify viral antigens and RT-PCR to identify RNA sequences are positive before development of cytopathic effects.

Susceptible vertebrate hosts that show signs of infection include infant mice, which develop fatal encephalitis when

infected by peripheral or intracerebral routes; mice greater than 8 days old develop fatal encephalitis only following intracerebral challenge. Nonhuman primates of many species develop fatal hepatitis resembling human disease. The only nonprimate species that develop lethal hepatitis following infection are the European hedgehog and the golden hamster. Antibodies to YFV have been found in a wide variety of field-collected wild vertebrates and wild animals such as rodents and bats. With the exception of opossums in South America, there is no support for a role of nonprimate species in the transmission cycle.

Geographic and Seasonal Distribution

YF occurs in tropical South America and sub-Saharan Africa, where the enzootic transmission cycle involves tree-hole-breeding mosquitoes and nonhuman primates. *Aedes aegypti*-infested regions of Central and North America, the Caribbean, and Southern Europe had periodic outbreaks through the early 1900s and are considered at risk, should the virus be reintroduced. Despite the prevalence of *Ae. aegypti* in tropical Asia, YFV has not appeared. Cross-protection by immunity to dengue viruses may decrease the probability that YFV can be transported from inaccessible, endemic parts of Africa and South America. Low vector competence of Asian populations of *Ae. aegypti* may be responsible for diminishing the risk of virus transmission. Areas at highest risk for reintroduction of virus and secondary spread by re-emerging *Ae. aegypti* include coastal regions and interior towns throughout equatorial South America, Panama, Central America, the West Indies, Mexico, and the southern United States. All are areas affected by YF in the past.

Breeding of many mosquito species that transmit YFV occurs in tree holes and is dependent on rainfall; consequently, transmission increases during tropical rainy seasons and decreases or stops during dry seasons. The peridomestic vector *Ae. aegypti* breeds in receptacles used by humans for water storage, and is less dependent on rainfall. Where this mosquito is involved in virus transmission, YF may occur in the dry season in both rural and urban areas. Temperature also influences YFV transmission rates; a few degrees increase may shorten the extrinsic incubation period (a period of viral replication in a recently infected mosquito prior to the mosquito being capable of transmitting virus to a host) by days, resulting in a significantly increased rate of transmission. Warm temperature also increases biting and reproductive rates of *Ae. aegypti.*

Disease Incidence

Official notifications of disease likely underestimate disease incidence and case–fatality rate, although underreporting occurs to variable degree. During the 20-year period ending in 2004, 28 264 cases and 7880 deaths were reported to the World Health Organization (WHO). In Africa, which accounted for 24 684 cases (87%), the annual incidence varied between 1 and 5104 cases, suggesting highly variable reporting. Despite inconsistent reporting, enzootic and endemic transmission was known to occur continuously in affected regions. During this period, Africa reported 5815 or 74% of the worldwide deaths due to YF, with a case–fatality rate of 24%. The frequency and intensity of epidemics in Africa are due to interhuman transmission by mosquito vectors present in high-density, high-human populations, and low immunization coverage. West Africa appears to be at highest risk of YF emergences, probably reflecting the role of domestic *Ae. aegypti* mosquito vectors. In South America, YF occurs in the Amazon region and contiguous areas. Between 1985 and 2004, 3580 cases and 2074 deaths (case–fatality rate 58%) were reported to WHO by South American countries. The annual incidence varies by country due to fluctuating epizootic activity. The incidence of YF in South America is roughly 20% of that of Africa, due to transmission by enzootic as opposed to epidemic vectors, lower densities of vectors, monkeys and human hosts, and relatively high vaccination coverage. The higher case–fatality rate in South America probably reflects the reporting of more severe cases there, and the fact that surveillance there is based on death reports and postmortem liver examination, although it remains possible that the South American genotype(s) is more virulent than those in Africa.

Epidemiology and Transmission Cycles

YFV is present at high titers in the blood of infected, susceptible hosts (humans and nonhuman primates) for several days, during which mosquito vectors collecting a blood meal may become infected. Virus replicates sequentially in the midgut epithelium, body, and salivary glands of the mosquito. The period between the infecting blood meal and the point at which sufficient replication allows transmission to a vertebrate host is the 'extrinsic incubation period'. This is a temperature-dependent process and takes roughly a week to complete.

Transmission cycles differ according to the hosts and vectors involved. Although the resulting clinical disease does not differ, recognizing the type of cycle is important for disease control. The 'urban cycle' involves humans as the viremic host and *Ae. aegypti* breeding in peridomestic, man-made containers that hold unpolluted water. The 'sylvatic cycle', the predominant pattern in equatorial rain forests of Africa and South America, involves transmission between nonhuman primates and tree-hole-breeding mosquito vectors. In Africa, a third cycle, the

'savanna cycle', is recognized in moist grasslands bordering the rain forests where *Aedes* mosquitoes other than *Ae. aegypti* feed on both humans and nonhuman primates. In all of these settings, the rate of transmission varies widely depending on the density of vectors and the presence of susceptible primates. Primates of many nonhuman species are susceptible to YFV infection. Most African primates produce viremias sufficient to infect mosquitoes without developing illness, whereas many South American primates develop lethal infections. Depletion of vertebrate hosts through natural immunization and death during epizootic waves is a factor in the cyclic appearance of YF activity. In many areas, with environmental pressures markedly reducing monkey populations, human beings serve as hosts in the YFV transmission cycle. No nonprimate species are involved in enzootic transmission.

The risk of nonimmune persons becoming infected is determined by geographic location, season, activities that lead to exposure, duration of exposure to mosquito bites, and the intensity of YFV transmission. Although reported cases of human disease are an important guide to YFV activity, reported numbers may be low due to a high level of immunity in the population or to insensitive surveillance. In areas where vaccination is widely practiced, YFV may circulate between monkeys and mosquitoes, with few human cases occurring.

The means of survival of YFV across long dry seasons, when sylvatic mosquito vectors are absent, remains in question. Transovarial transmission to *Aedes* and *Haemagogus* mosquito eggs, which survive desiccation in tree holes and hatch when the rains return, likely play a major role. Low-level horizontal transmission by drought-resistant vectors, and alternative horizontal and vertical transmission cycles involving ticks, are theorized mechanisms. Although persistent infection of experimentally infected nonhuman primates has been documented, infections in these hosts generally are not accompanied by viremia sufficiently high to infect vectors.

The most alarming future prospect involves the reemergence of urban transmission in South America and the spread to heavily populated, *Ae. aegypti*-infested areas currently free from disease, such as the coastal regions of South America, the Caribbean, and North America, and regions where the virus has never become established but which have susceptible human populations and competent mosquito vectors, such as the Middle East, coastal East Africa, the Indian subcontinent, Asia, and Australia. Alterations in human demography and behavior, in virus activity, and in the distribution of *Ae. aegypti* underlie the potential for epidemiological change. Such alterations include: (1) increasing urban populations; (2) changes in commerce, transportation, and communication that allow human population expansion into previously remote endemic zones; (3) reinvasion of South America by the peridomestic *Ae. aegypti*; (4) presence of efficient jungle vectors in degraded periurban habitats; (5) introduction of new competent *Aedes* species (e.g., *Ae. albopictus*) that may bridge the jungle and urban transmission cycles; (6) relaxation of regulations and of enforcement of vaccination certification for travelers; and (7) possibly climate change.

Laboratory-acquired infections were common in the pre-vaccine era and remain of concern today, particularly where unvaccinated clinical laboratory personnel encounter blood from patients in early stages of illness. Some laboratory infections were probably acquired via bites of experimentally infected mosquitoes or of wild mosquitoes infected after feeding on experimental animals. Others resulted from direct contact with blood or aerosols of dried virus. The stability of YFV permits transmission within a short period after generation of an infectious aerosol.

Clinical Features

The broad clinical spectrum of YF includes abortive infection with nonspecific flu-like illness, and 'classic' YF – potentially lethal pansystemic disease with fever, jaundice, renal failure, and hemorrhage. Serosurveys to identify persons with asymptomatic infections estimate that only about 15–25% of infected persons develop classic YF. Diagnosis of sporadic cases without classic symptoms is difficult; as a result, often only cases of classic YF are notified, and deaths may be more frequently notified than cases. These features lead to an underestimate of morbidity and overestimate of case–fatality rate. The variability in clinical response to infection is multifactoral and includes intrinsic and acquired host resistance factors and differences in pathogenicity of virus strains.

Classic YF is a triphasic disease that begins with abrupt onset of fever, headache, myalgia, anorexia, and nausea 3–6 days after infection. This 'period of infection' is clinically nonspecific but corresponds to a viremic phase when a person is infectious to feeding mosquitoes. Fatal cases appear to have a longer duration of viremia than do survivors. After 3–4 days, a 'period of remission', lasting up to 48 h, begins with abatement of fever and symptoms. Cases of abortive infection simply recover at this stage. Because such cases remain anicteric with nonspecific symptoms, clinical diagnosis of YF is not possible. Roughly 15–25% of persons infected with YFV enter a 'period of intoxication', typically 3–5 days after onset, characterized by a moderate to severe disease characterized by jaundice, the return of fever, relative bradycardia, oliguria, hemetemesis, and other hemorrhagic diatheses. Virus disappears from blood and antibodies appear 7–10 days after infection. The subsequent course reflects dysfunction of the hepatic, renal, and cardiovascular systems. Central nervous system (CNS) signs include delirium, convulsions, and coma. In severe cases, the cerebrospinal fluid is under increased pressure and may

contain elevated protein but no cells. No inflammatory changes have been found in brains of persons with severe illness; cerebral edema or metabolic factors apparently cause CNS signs. Between the fifth and tenth day of illness, the patient either dies or recovers rapidly. Terminal events are characterized by hypotension, shock, and respiratory distress.

Pathology and Pathogenesis

Two biological properties are inherent to all wild-type YFV strains: 'viscerotropism', the ability to cause viremia and subsequently to infect and damage liver, heart, and kidneys, and 'neurotropism', the ability to infect the brain parenchyma causing encephalitis. Wild-type YFV strains are predominantly viscerotropic in humans and in nonhuman primates. True YF viral encephalitis is rare; even after intracerebral inoculation of YFV, susceptible nonhuman primates die from hepatitis rather than from encephalitis.

The course of infection following inoculation of wild-type YFV has been partially investigated using nonhuman primates. Virus replicates at the site of skin inoculation and spreads to draining lymph nodes, then to central lymph nodes, and subsequently to visceral organs. Lymphoid cells are early viral replication sites, and in the liver Kupffer cells in the sinusoids are the gateway to subsequent hepatocyte infection. Results of prior research, primarily of dengue infections, makes clear that dendritic cells (DCs) play a primary role in the early stages of infection, and that these cells not only process viral antigens into immunogenic peptides and present them to T-cells, but also secrete a cascade of immunoregulatory cytokines that shape innate and adaptive immune responses.

In humans with viscerotropic disease, degeneration of hepatocytes occurs in the last phase of infection. In fatal cases, up to 100% of hepatocytes undergo coagulative necrosis characteristic of apoptosis. The mid-zone of the liver lobule is principally affected, with sparing of cells bordering the central vein and portal tracts. Viral antigen and viral RNA are demonstrable in cells undergoing pathological changes, suggesting that cytopathology is mediated by direct injury from virus replication and from accumulation of virions and viral protein in the endoplasmic reticulum. The reason for this peculiar distribution of hepatic injury is unknown; mid-zonal necrosis has been described in low-flow hypoxia, ATP depletion and oxidative stress of marginally oxygenated cells at the border between anoxic and normoxic cells. Eosinophilic degeneration with condensed nuclear chromatin (Councilman bodies), indicative of apoptosis, characterizes hepatocyte injury rather than the ballooning and rarefaction necrosis seen in hepatitis caused by other viruses. Inflammatory changes are minimal and persons who survive YF do not develop scarring or cirrhosis due to this infection. Eosinophilic degeneration without inflammation, along with fatty change of renal tubular epithelium, also characterizes renal pathology. Focal degeneration may be present in myocardial cells. Lymphoid follicles of lymph nodes and spleen show necrosis. The brain shows edema and petechial hemorrhage but viral invasion and encephalitis are rare. Decreased hepatic synthesis of clotting factors causes hemorrhagic manifestations.

The mediators of the profound shock of YF are unknown but cytokine dysregulation (systemic inflammatory response syndrome, SIRS) likely mediates this terminal stage as in other sepsis syndromes. The appearance of antibody and cellular responses coincides with onset of the 'period of intoxication', suggesting that immune clearance may be, in part, responsible for the cytokine storm events. Persons with fatal YF have elevated pro-inflammatory cytokines compared to persons with nonfatal cases of YF, resembling the profile seen in bacterial sepsis. Overall, these findings suggest that high levels of pro-inflammatory cytokines contribute to lethality.

Immune Responses

Prior to appearance of specific cytotoxic T-cells and immunoglobulins, innate immunity, comprised of natural killer (NK) cells, interferons (IFNs), and other pro-inflammatory cytokines, play a significant role in limiting viral replication during early infection. NK cells recognize and lyse cells displaying viral antigens; secrete IFN-α and pro-inflammatory cytokines that have direct antiviral activity; activate DCs and macrophages; and promote a Th1 adaptive immune response. YFV antigens directly activate DCs via type I IFN signal transduction and Toll-like receptors. Through Toll-like receptor activation, pro-inflammatory molecules (e.g., interleukins IL1-β, IL2, IL6, IL8, IL12) are expressed and further activate NK cells. This robust innate response to YFV likely underlies the rapid, strong, and durable adaptive responses to this virus. Flaviviruses have evolved weak mechanisms for avoiding these innate responses, including blocking the IFN signal transducer pathways by NS2A and NS4B. Those patients with a poor outcome may develop signs of a dysregulated pro-inflammatory cytokine response (SIRS).

Seven to ten days after infection, a specific immune response is detectable when immunoglobulin M (IgM; enzyme-linked immunosorbent assay, ELISA), hemagglutination-inhibiting, and neutralizing antibodies begin to appear. IgM antibodies peak during the second week and usually decline rapidly over the next 30–60 days while neutralizing antibody continues to increase. When YFV represents the patient's first flavivirus infection, the magnitude of the IgM response is significantly greater than in persons with prior flavivirus exposure. Although little is known about cytolytic antibodies against viral proteins on the surface of infected cells, antibody-dependent cell-mediated cytotoxicity (ADCC), and cytotoxic T-cells,

these mechanisms, in addition to neutralizing antibody, presumably mediate clearance of primary infection. Based on immunologic studies of YFV vaccine recipients, neutralizing antibody generally peaks 4–6 weeks after infection but high titers of neutralizing antibodies persist for more than 10 years and provide complete protection against disease on reexposure to the virus. No documented case of a second clinical YFV infection has ever been reported. Although initial antibody response to infection is YFV antigen specific, with affinity maturation, specificity declines and cross-reactions with other flaviviruses develop during the subsequent several week of the immune response. Persons with prior heterologous flavivirus immunity develop broadly cross-reactive antibody responses during YFV infections. Previous infection with flaviviruses, such as Zika, dengue, or Wesselsbron viruses, provides partial cross-protection against YFV and may ameliorate the clinical severity of YF.

Prevention and Control

Domestic control of *Ae. aegypti* mosquitoes remains important but is difficult to sustain. Currently, the most effective approach to control of YF is by immunizing persons living in or traveling to endemic areas. 17D vaccine is the only strain currently used for human immunization against YFV. It is a live, attenuated vaccine produced in embryonated chicken eggs. 17D vaccines are not biologically cloned and are heterogeneous mixtures of multiple virion subpopulations ('genetic swarms'); differences in plaque size, oligonucleotide fingerprints, and nucleotide sequences have been found but do not appear to affect safety or efficacy. Currently, manufacturers in seven countries market YFV 17D vaccine. Three manufacturers in Brazil, France, and Senegal produce large amounts of vaccine for the Expanded Programme of Immunization and for mass vaccination campaigns. As of 2006, annual global vaccine production was approximately 60 million doses. Monath *et al.* thoroughly review the development, immune response, and efficacy of the 17D vaccine strain.

As of 2006, over 400 million persons have been immunized with YFV vaccines. Over roughly 70 years of use, 17D vaccines have been acknowledged as one of the safest and most effective live vaccines in use. Recently, however, close scrutiny has been brought to bear as clinical and histopathological evidence has emerged linking 17D vaccines to severe and previously unrecognized adverse events, including viscerotropic disease closely resembling that caused by wild-type YFV. Although rare mutational events in 17D vaccine virus during replication in the host can alter pathogenicity, these recently reported serious adverse events were not associated with either mutations that change virulence or tropism of the virus or selection of virulent variants *in vivo*. Investigations suggest that host susceptibility, rather than a change in the virus, is responsible for these serious, adverse events. Advanced age and thymic disease appear to be risk factors for development of vaccine-associated viscerotropic disease. The incidence of this complication, which carries a case–fatality rate of approximately 50%, is believed to be 1:400 000.

See also: Yellow Head Virus.

Further Reading

Barrett ADT and Monath TP (2003) Epidemiology and ecology of yellow fever. *Advances in Virus Research* 61: 291–317.

Carter HR (1931) *Yellow Fever: An Epidemiological and Historical Study of Its Place of Origin.* Baltimore, MD: Williams and Wilkins.

Cordellier R (1991) The epidemiology of yellow fever in western Africa. *Bulletin of the World Health Organization* 69(1): 73–84.

Monath TP and Barrett ADT (2003) Pathogenesis and pathophysiology of yellow fever. *Advances in Virus Research* 60: 343–397.

Monath TP, Teuwen D, and Cetron M (in press) Yellow fever vaccine. In: Plotkin SA, Orenstein WA, and Offit PA (eds.) *Vaccines Expert Consult*, 5th edn. Philadelphia, PA: Saunders.

Strode GK (ed.) (1951) *Yellow Fever,* pp. 385–426. New York: McGraw-Hill.

Yellow Head Virus

P J Walker, CSIRO Australian Animal Health Laboratory, Geelong, VIC, Australia
N Sittidilokratna, Centex Shrimp and Center for Genetic Engineering and Biotechnology, Bangkok, Thailand

© 2008 Elsevier Ltd. All rights reserved.

Glossary

Antennal gland Complex excretory glands located behind the eyes on antenna on the head of decapods.

Hepatopancreas An organ of the digestive tract of arthropods and fish that provides the functions which are performed separately by the liver and pancreas in mammals.

Lymphoid organ Also known as the Oka organ, a component of the hematopoeitic system of penaeid shrimp consisting of lymphoid cells around the two subgastric arteries.
Pseudoknot A complex folded structure in an RNA molecule.
Slippery sequence A nucleotide sequence at which ribosomal slippage can occur during translation to cause a change in the reading frame.

Introduction

Yellow head virus (YHV) is a pathogen of the black tiger shrimp (prawn), *Penaeus monodon*, which is one of the world's major aquaculture species. Yellow head disease was first reported in central Thailand in 1990 from which it spread rapidly along the eastern and western coasts of the Gulf of Thailand to southern farming regions. Outbreaks of yellow head disease have since been reported from most of the major shrimp farming countries in Asia. It is suspected that the YHV (rather than monodon baculovirus, which is not usually pathogenic for juvenile shrimp) may have previously caused the crash of the shrimp farming industry in Taiwan during the late 1980s. Mortalities usually occur during the mid-late stages of grow-out in ponds with complete crop loss commonly occurring within 3 days of the first signs of disease. YHV is one genotype in a complex of closely related viruses infecting black tiger shrimp. Other genotypes include gill-associated virus (GAV) which has been associated with relatively less severe forms of disease in farmed shrimp in Australia, and at least four other genotypes for which no disease association has yet been established. YHV and the other genotypes are endemic throughout the Indo-Pacific region, occurring commonly as low-level chronic infections in healthy shrimp.

Taxonomy and Classification

YHV is a positive-sense single-stranded RNA (ssRNA) virus that shares aspects of genome organization, replication, and transcription with coronaviruses, toroviruses, and arteriviruses with which it is classified in the order *Nidovirales*. In 2002, the International Committee on Taxonomy of Viruses (ICTV) established the genus O*kavirus* in the new family *Roniviridae* to accommodate YHV and closely related GAV. *Okavirus* is derived from the Oka or lymphoid organ of penaeid shrimp in which the virus is commonly detected; *Roniviridae* is derived from the sigla rod-shaped nidovirus. *Gill-associated virus* was assigned as the type species of the genus because its biological and molecular characterization were more complete. YHV is currently classified as a member of the species *Gill-associated virus*. No virus other than those described in the yellow head complex is currently assigned to the *Roniviridae* but several viruses with similar morphology have been reported in crabs and fish. Roniviruses are the only members of the order *Nidovirales* that are currently known to infect invertebrates.

Virion Structure and Morphology

YHV virions are rod-shaped, enveloped particles (~50 nm × ~175 nm) with prominent diffuse spikes (~8 nm × ~11 nm) projecting from the surface (**Figure 1(a)**). Internal helical nucleocapsids are approximately 25 nm in diameter and have a periodicity of 5–7 nm. Filamentous nucleocapsid precursors, approximately 15 nm in diameter and of variable length (~80–450 nm), are observed in the cytoplasm, sometimes densely packed in paracrystalline arrays (**Figure 1(b)**). Nucleocapsids acquire trilamellar lipid envelopes by budding through membranes into intracytoplasmic vesicles or at the cell surface (**Figure 1(c)**). It has been reported that long nucleocapsid precursors generate elongated, enveloped structures that subsequently fragment into mature virions. The morphology of GAV virions is indistinguishable from that of YHV.

YHV virions contain a polyadenylated 26.6 kDa (+) ssRNA genome and three structural proteins. The nucleoprotein (p20) is a highly hydrophilic, basic protein that complexes with the genomic RNA in nucleocapsids. Transmembrane glycoproteins gp64 and gp116 are components of the envelope that form the visible projections on the virion surface. YHV infectivity can be at least partially neutralized by antibody to gp116 but not by antibody to gp64. It is reported that gp116 docks with a 65 kDa cell membrane protein (pmYRP65) that mediates YHV entry into susceptible shrimp cells. Knockdown of pmYRP65 expression has been reported to totally abrogate susceptibility of shrimp cells to YHV infection.

Genome Organization and Transcription Strategy

The 26 662 nt YHV genome comprises four long open reading frames (ORFs) designated ORF1a, ORF1b, ORF2, and ORF3 (**Figure 2**). ORF1a (12 216 nt) and ORF1b (7887 nt) encode all of the elements of a large replicase complex. ORF1a encodes a 4072 aa polyprotein (pp1a) that contains a 3C-like cysteine protease catalytic domain flanked by putative transmembrane domains. The pp1a protease has autolytic activity and appears to be involved in processing the replicase polyproteins. ORF1b overlaps ORF1a by 37 nt. Expression of ORF1b requires

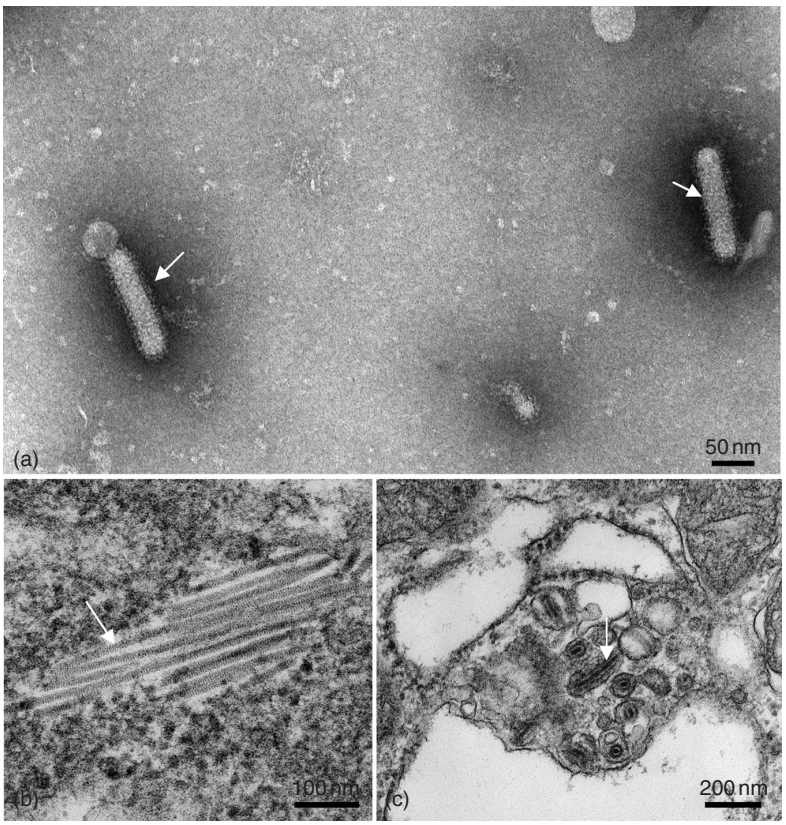


Figure 1 Transmission electron micrographs of YHV. (a) Image of virions stained with heavy metal salts showing the external appearance of the enveloped particles (arrows). (b) Image of an ultrathin section of helical nucleocapsids (arrow) within the gill of an infected shrimp. (c) Image of an ultrathin section of virions (arrow) within the gill of an infected shrimp. Kindly provided by Dr. Alex Hyatt, CSIRO, Australian Animal Health Laboratory, Geelong, Australia.

a −1 ribosomal frameshift at a slippery sequence (AAAUUUU) near a complex pseudoknot structure in the mRNA. The extended 6688 aa polyprotein (pp1ab) contains RNA-dependent RNA polymerase (RdRp), multinuclear zinc-binding (ZBD), helicase (HEL), 3′-5′ exoribonuclease (ExoN), uridylate-specific endoribonuclease (NendoU), ribose-2′-*O*-methyltransferase (O-MT) catalytic domains, and other cysteine/histidine-rich domains that are conserved in pp1ab of other nidoviruses. ORF2 encodes the 146 aa nucleocapsid protein (p20). Roniviruses are unique among known nidoviruses in that the nucleocapsid protein gene is located upstream rather than downstream of the glycoprotein genes. ORF3 encodes a 1666 aa polyglycoprotein that is processed to generate virion envelope glycoproteins gp64 and gp116. Proteolytic cleavage of ORF3 occurs at two [Ala-X-Ala] motifs immediately following predicted transmembrane domains that appear to function as signal peptides. The cleavage also generates a 228 aa (~22 kDa) protein that contains triple membrane-spanning domains and resembles M-proteins in coronaviruses. The YHV M-like protein appears to be present in infected cells at relatively low levels. The YHV genome also features significant noncoding regions, including a 71 nt untranslated region (UTR) at the 5′-terminus, a 352 nt UTR between ORF1b and ORF2, and a 54 nt UTR between ORF2 and ORF3. The 677 nt region between ORF3 and the 3′-poly[A] tail contains no long ORFs (>65 nt) and so also appears to be a long UTR (**Figure 2**).

Much of our understanding of YHV molecular biology has been obtained by comparison with closely related GAV. The 26 235 nt GAV genome is similar in structural organization to YHV, varying principally in the size and structure of the UTRs. In GAV, the ORF1b–ORF2 UTR comprises only 93 nt. The 638 nt region downstream of GAV ORF3 encodes a 252 nt ORF (ORF4) that has potential to express an unidentified 83 aa polypeptide with a deduced molecular weight ~9.2 kDa. A short ORF in the corresponding region of the YHV genome is truncated with a termination codon after only 20 aa and is unlikely to be expressed. Like other nidoviruses, the GAV genome is transcribed as a nested set of 3′-co-terminal mRNAs comprising the full-length genome and two subgenomic messenger RNAs (sg mRNAs) that initiate at conserved

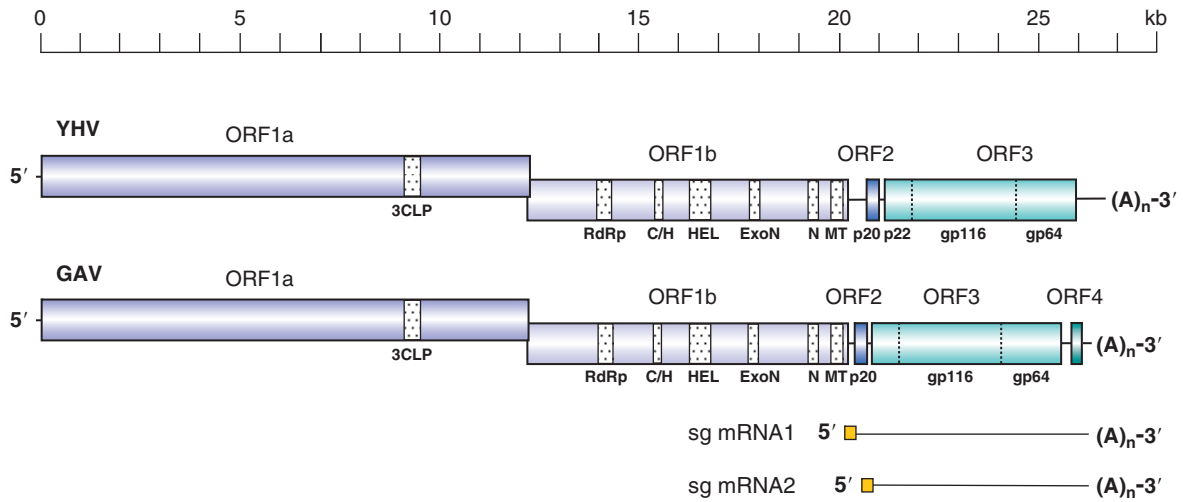


Figure 2 Genome organization of YHV and GAV indicating the locations of subgenomic mRNAs (sg mRNA1 and sg mRNA2) and functional domains in ORF1a (3CLP) and ORF1b (RdRp, C/H, HEL, ExoN, N, and MT). Also indicated are proteolytic cleavage sites in the ORF3 polyprotein that is processed post-translation to generate triple-membrane-spanning protein (p22) and transmembrane glycoproteins (gp116 and gp64).

transcription-regulating sequences (TRSs) in noncoding regions immediately upstream of ORF2 and ORF3. However, unlike coronaviruses and arteriviruses, conserved GAV (and YHV) TRSs are not present in the 5′-UTR of genomic RNA and so do not mediate splicing of common 5′-leader sequences on to the sg mRNAs. Sequences with partial identity to the conserved ORF2 and ORF3 TRSs occur upstream of GAV ORF4 (and the truncated YHV ORF4) but these do not appear to be functional.

Geographic Distribution and Host Range

Surveys for the presence of viral genomic RNA have indicated that YHV and other genotypes in the complex are endemic in black tiger shrimp populations across its natural geographic range throughout the Indo-Pacific. Yellow head disease has been reported in farmed tiger shrimp from Thailand, Taiwan, China, the Philippines, Vietnam, Malaysia, Indonesia, India, Sri Lanka, and Madagascar. Although natural infection and disease have been reported only in black tiger shrimp and kuruma shrimp (*Marsupenaeus japonicus*), YHV can cause high rates of mortality following experimental infection of most other farmed marine shrimp species, including Pacific white shrimp (*Litopenaeus vannamei*), Pacific blue shrimp (*Litopenaeus stylirostris*), brown tiger shrimp (*Penaeus esculentus*), white banana shrimp (*Fenneropenaeus merguiensis*), white shrimp (*Litopenaeus setiferus*), brown shrimp (*Farfantepenaeus aztecus*), hopper and brown-spotted shrimp (*Farfantepenaeus duorarum*), red endeavour prawn (*Metapenaeus ensis*), and Jungas shrimp (*Metapenaeus affinis*). Some species of palemonid shrimp and krill are also susceptible to experimental infection. Crabs appear to be refractory to YHV infection and disease.

GAV has been associated with a less-aggressive disease of juvenile black tiger shrimp in Australia called mid-crop mortality syndrome. However, several other viruses have also been detected in shrimp with this condition and the etiology remains uncertain. GAV does cause disease and mortalities following experimental infection of several farmed shrimp species, including black tiger, brown tiger, and kuruma shrimp. GAV and other genotypes in the YHV complex have also been detected in healthy black tiger shrimp from Taiwan, the Philippines, Malaysia, Brunei, Indonesia, Vietnam, Thailand, India, Mozambique, and Fiji. A very high prevalence of GAV infection has been reported in healthy black tiger shrimp from eastern Australia. Evidence of GAV infection has also been detected in mud crab (*Scylla serrata*) in an experimental aquaculture facility.

Pathology

Shrimp are susceptible to YHV infection from late postlarval stages but mass mortality in ponds usually occurs in early-to-late juvenile stages. Disease and mortalities usually occur within 2–4 days of a period of exceptionally high feeding activity followed by an abrupt cessation of feeding. Moribund shrimp congregate at pond edges near the surface and may exhibit a bleached overall appearance and discoloration of the cephalothorax caused by yellowing of the underlying hepatopancreas.

YHV infects tissues of ectodermal and mesodermal origin, including lymphoid organ, hemocytes, hematopoeitic tissue, gill lamellae, and spongy connective tissue of the subcutis, gut, antennal gland, gonads, nerve tracts, and ganglia. In severe infections, there is a generalized cell degeneration with prominent nuclear condensation, pyknosis and karyorrhexis, and basophilic, perinuclear cytoplasmic inclusions in affected tissues. There is evidence of apoptosis, including chromatin condensation and DNA fragmentation, in hemocytes, lymphoid organ, and gill tissues and it has been suggested that widespread apoptosis rather than necrosis is the cause of disease and mortalities.

YHV, GAV, and other viruses in the yellow head complex can also occur as low-level chronic infections in apparently healthy shrimp. Chronic infections have been observed in shrimp of all life stages collected from hatcheries and farms, and in the survivors of experimental infection. For GAV, the progression of infection following experimental challenge has been shown to be dose related. Shrimp infected with a high dose of GAV progress rapidly to disease with high viral loads and typical pathology leading to mortalities. Shrimp infected with a low dose do not develop disease and the virus persists as a low-level infection for at least 60 days. There is also evidence that stress can lead to rapid increases in viral load. For YHV, the onset of disease has been associated with the stress of molting. During chronic infections, there is little histopathology other than the accumulation of partitioned foci of cells with hypertrophic nuclei (spheroid bodies) in the lymphoid organ. Spheroid bodies appear to form in shrimp as part of a nonspecific defense mechanism for clearance of infectious agents and other foreign bodies.

Host Response to Infection

As invertebrates, shrimp lack antibodies, cytokines, T-lymphocytes, and other powerful components of the vertebrate immune system that allow a specific adaptive response to viral infection, clearance of virus and infected cells, and long-term immunological memory. There is also no evidence in shrimp of interferon, natural killer (NK) cells, or other key components of the vertebrate natural immune system that allow an immediate nonspecific defense against viruses. Nevertheless, shrimp do appear to have a capacity to respond to viral infection and highly pathogenic viruses are commonly present as low-level chronic infections in apparently healthy shrimp. For YHV, there is no evidence of an inflammatory response at the primary sites of infection. However, YHV accumulates in spheroid bodies in the lymphoid organ during chronic persistent infections, and it is thought that the lymphoid organ has an important role in filtering granulated hemocytes and the clearance of viruses from infected shrimp. It has been reported that cells within lymphoid organ spheroids become apoptotic during infection and may be cleared during molting. Apoptotic cells have been observed in lymphoid organs, hemocytes, and gills during acute YHV infections in what appears to be a fundamental host defensive reaction. It has also been reported that double-stranded RNA (dsRNA) corresponding to sequences in viral replicase and glycoprotein genes specifically inhibits YHV infection *in vitro* and *in vivo*, suggesting that RNA interference may play a role in the host response to infection.

Transmission

The natural transmission cycle of YHV has not been studied in detail. Experimentally, YHV infection and disease can be transmitted horizontally by injection, ingestion of infected tissue, immersion in membrane-filtered tissue extracts, or by cohabitation with infected shrimp. Transmission of disease by ingestion has been demonstrated from the late postlarval stages onward. Transmission has also been demonstrated by injection of black tiger shrimp with extracts of paste shrimp (*Acetes* sp.) and mysid shrimp (*Palaemon styliferus*) collected from infected ponds. For GAV, there is evidence that horizontal transmission can occur from chronically infected shrimp in the absence of disease.

There is no direct evidence of vertical transmission of YHV but it can be detected as a chronic infection in broodstock prior to spawning, and polymerase chain reaction (PCR) screening to eliminate infected broodstock and seed is increasingly being used to reduce risks of yellow head disease in ponds. GAV has been detected in spermatophores and mature ovarian tissue of broodstock, and in fertilized eggs and nauplii spawned from infected females. Examination by electron microscopy has revealed virions in seminal fluid but not in sperm cells. Artificial insemination of infected broodstock has shown that vertical transmission occurs efficiently from both male and female parents. Transmission is probably by surface contamination or infection of tissue surrounding the fertilized egg. The high prevalence of yellow head complex viruses in postlarvae collected from hatcheries in Australia and several Asian countries supports the view that vertical transmission has an important role in the infection cycle of all genotypes, particularly during propagation for aquaculture.

Genetic Diversity

YHV is one of several closely related genotypes that have been detected in black tiger shrimp in the Indo-Pacific region. Analysis of nucleotide and deduced amino

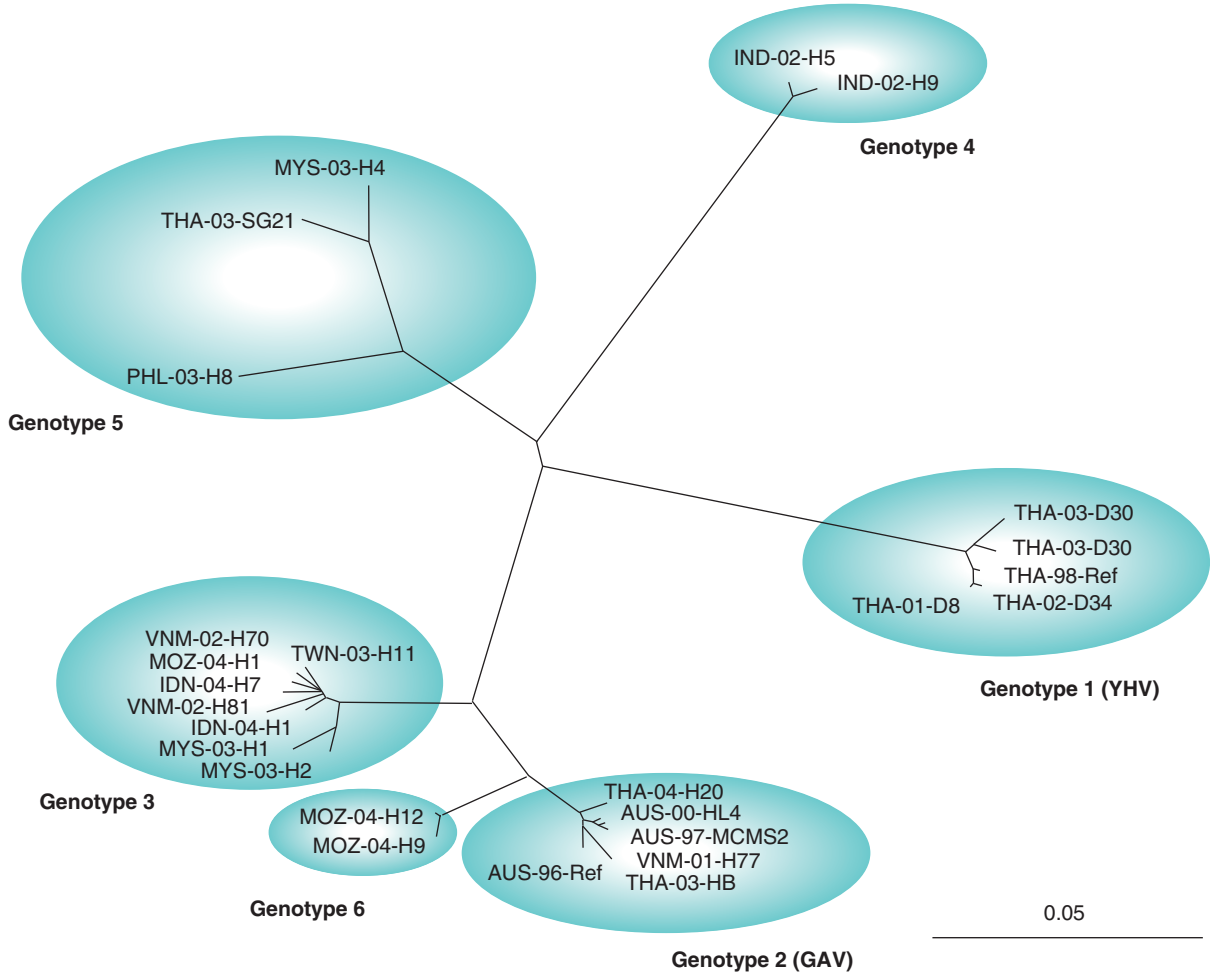


Figure 3 Phylogenetic tree constructed from an alignment of nucleotide sequences obtained from 26 viruses detected in *Penaeus monodon* shrimp from the Indo-Pacific region. The sequences correspond to a 671 nucleotide region of the ORF1b gene encompassing elements of the HEL domain. The alignment illustrates the six known genotypes in the yellow head complex including YHV (genotype 1), GAV (genotype 2), and four other genotypes (genotypes 3, 4, 5, and 6) that have been detected only in healthy shrimp. The viruses were obtained from shrimp collected in India (IND), Malaysia (MYS), Thailand (THA), the Philippines (PHL), Australia (AUS), Vietnam (VNM), Mozambique (MOZ), Indonesia (IDN), and Taiwan (TWN) between 1997 and 2004. Sequences kindly provided by Mrs Priyanjalie Wijegoonawardane, CSIRO Livestock Industries, St. Lucia, Australia.

acid sequences in a relatively conserved region of the ORF1b gene has identified at least six distinct genetic lineages in the complex (**Figure 3**). In pairwise alignments, nucleotide sequence identity between consensus sequences representing each genotype ranges from 80.3% to 96.5%. Variation within genotypes is generally low, with nucleotide sequence identities between isolates in the range 97.1–100%, except genotype 5 for which three available isolates have been reported to share 93.0–97.1% identity.

YHV (genotype 1) is the only genotype that has been detected in shrimp with typical signs of yellow head disease. Although the disease has been reported from many sites in Asia, isolates are currently available only from Thailand and Taiwan. Genotype 1 is the most distantly related to other lineages and appears to occur less commonly than other genotypes in healthy shrimp. GAV (genotype 2) is the only other lineage known to be associated with disease of any form. Analysis of complete genome sequences of prototype strains of YHV and GAV indicates similar nucleotide sequence identities for ORF1a (79.7%), ORF1b (82.3%), ORF2 (81.0%), and a slightly lower level of identity for ORF3 (73.2%). Amino-acid-sequence identities between YHV and GAV proteins are similar for the replicase pp1ab (84.9%), nucleoprotein p20 (84.4%), and glycoprotein gp64 (83.9%), and lower for the M-like protein p22 (74.8%) and glycoprotein gp116 (71.7%).

GAV has been detected in black tiger shrimp from Australia, Vietnam, and Thailand. Phylogenetic analysis

of sequences analyzed to date suggests that all isolates may have originated from translocated Australian shrimp. Genotype 3 has been detected to date in Taiwan, Vietnam, Indonesia, Malaysia, Thailand, and Mozambique. It appears to be the most widely distributed and most frequently detected genotype. Genotype 4 has been detected only in India. Genotype 5 has been detected in the Philippines, Malaysia, and Thailand. As indicated above, genotype 5 is genetically the most diverse genotype and may be split into three distinct lineages as more isolates become available. Genotype 6 has been detected only in Mozambique.

Assignment of these genotypes has been based primarily on comparisons of sequences in a conserved region of the ORF1b gene. Analysis of nucleotide sequences in the 5′-terminal region of the ORF3 polyglycoprotein gene indicates more genetic variability and suggests that genetic recombination is contributing to diversity in the complex. Of 24 isolates examined recently, almost one-third were assigned to different genotypes in comparative phylogenetic analyses of nucleotide sequences in the ORF1b and ORF3 regions. Genetic recombination is a phenomenon known to occur commonly in other nidoviruses. It appears that the vast international trade in live shrimp broodstock and seed for aquaculture is providing adequate opportunities for recombination and diversification of the gene pool. This appears to confound the assignment of coherent genetic lineages and may have significant consequences for both the emergence and definitive diagnosis of disease.

Diagnosis and Disease Management

Gross clinical signs of YHV infection including yellowing of the carapace and erratic swimming behavior are not observed consistently and are not sufficiently pathognonomic to be useful for disease diagnosis. Histologically, moderate to large numbers of basophilic, spherical, cytoplasmic inclusions in tissues of ectodermal and mesodermal origin are indications of YHV infection and can be used for presumptive diagnosis. Confirmatory diagnosis of yellow head disease requires the use of electron microscopy or molecular methods such as the reverse transcriptase-polymerase chain reaction (RT-PCR) or *in situ* hybridization assays. Antibody-based tests such as western blotting and dot-blot nitrocellulose enzyme immunoassay (NC-EIA) are also available. Low-level chronic infections with YHV and other genotypes can be detected by nested RT-PCR or other highly sensitive molecular genetic tests such as real-time PCR or loop-mediated isothermal amplification (LAMP). Accurate genotype assignment can only be achieved by PCR, sequence analysis, and comparison with sequences of other known genotypes.

No effective vaccines or therapeutics are currently available for the control of YHV and no genetically resistant shrimp stocks have been reported yet. Disease management is primarily through pathogen exclusion by PCR screening of broodstock and/or seed, the application of on-farm biosecurity and sanitary measures, and stress reduction by careful management of water quality during grow-out.

Current Status

Key aspects of the biology of YHV infection are yet to be resolved and yellow head continues to be a disease of concern to aquaculture farmers. No direct link has been demonstrated between the presence of virus in infected broodstock and the appearance of disease on farms. Assumptions about vertical transmission come by analogy with GAV and may well be accurate. However, the prevalence of YHV in healthy shrimp appears to be far lower than for GAV and other genotypes, and it is unclear how it maintains a cycle of natural infection. It is possible that YHV is commonly introduced to ponds in healthy wild shrimp or other carrier crustaceans but surveys to date have not revealed a likely source. The host–viral interaction during the chronic phase of infection, the transition from chronic to acute phases, and the role of stress in disease emergence are also poorly understood, and there is little understanding of the molecular basis of virulence variations between YHV and other genotypes. A more comprehensive study of the sources of YHV infection and host and/or environmental factors leading to emergence of yellow head disease should be conducted. Emerging capabilities in shrimp genomics and proteomics will greatly facilitate this work.

There is an emerging understanding of RNA interference (RNAi) as a potentially powerful mechanism for the control of viral diseases. Inhibition of YHV infection in primary lymphoid organ cell culture has been demonstrated by treatment with dsRNA corresponding to YHV protease, polymerase, and helicase domains. Injection of shrimp with protease domain dsRNA has also been shown to inhibit YHV replication and mortalities. Knockdown of the shrimp dicer-1 endoribonuclease gene expression has demonstrated that the antiviral effects of dsRNA are caused by RNAi. RNAi technology has useful applications in studies of the molecular biology of YHV infection and, if delivered cost-effectively, could potentially find commercial application in the management of yellow head disease.

Roniviruses are also seen as important links in understanding the evolutionary biology of (+) ssRNA viruses. Considerations of virion structure and the size, complexity and structural organization of the genome suggest that roniviruses form a genetic lineage ancestral to

coronaviruses and toroviruses. Studies of the ronivirus 3C-like cysteine protease encoded in ORF1a have also revealed structural similarities to coronaviruses in the catalytic site but substrate specificity and binding sites are more similar to those of potyviruses, suggesting that they bridge the gap between these distantly related proteases. A pseudoknot structure and slippery sequence at the ribosomal frameshift site is also distinct from the H-type structures characteristic of many vertebrate nidoviruses. Further molecular studies of ronivirus structure and function should provide insights into the evolution of these unusual viruses.

See also: Barley Yellow Dwarf Viruses; Tomato Yellow Leaf Curl Virus.

Further Reading

Assavalapsakul W, Smith DR, and Panyim S (2006) Identification and characterization of a *Penaeus monodon* lymphoid cell-expressed receptor for yellow head virus. *Journal of Virology* 80: 262–269.

Chantanachookin C, Boonyaratpalin S, Kasornchandra J, *et al.* (1993) Histology and ultrastructure reveal a new granulosis-like virus in *Penaeus monodon* affected by 'yellow-head' disease. *Diseases of Aquatic Organisms* 17: 145–157.

Cowley JA, Cadogan LC, Spann KM, Sittidilokratna N, and Walker PJ (2004) The gene encoding the nucleocapsid protein of gill-associated nidovirus of *Penaeus monodon* prawns is located upstream of the glycoprotein gene. *Journal of Virology* 78: 8935–8941.

Cowley JA, Dimmock CM, Spann KM, and Walker PJ (2000) Gill-associated virus of *Penaeus monodon* shrimp: An invertebrate virus with ORF1a and ORF1b genes related to arteri- and coronaviruses. *Journal of General Virology* 81: 1473–1484.

Cowley JA, Dimmock CM, and Walker PJ (2001) Gill-associated nidovirus of *Penaeus monodon* prawns transcribes 3′-coterminal subgenomic RNAs that do not possess 5′-leader sequences. *Journal of General Virology* 83: 927–935.

Cowley JA, Hall MR, Cadogan LC, Spann KM, and Walker PJ (2002) Vertical transmission of covert gill-associated virus (GAV) infections in *Penaeus monodon*. *Diseases of Aquatic Organisms* 50: 95–104.

Cowley JA and Walker PJ (2002) The complete genome sequence of gill-associated virus of *Penaeus monodon* prawns indicates a gene organisation unique among nidoviruses. *Archives of Virology* 147: 1977–1987.

Dhar AK, Cowley JA, Hasson KW, and Walker PJ (2004) Genomic organization, biology and diagnosis of Taura syndrome virus (TSV) and yellowhead virus (YHV) of penaeid shrimp. *Advances in Virus Research* 63: 353–421.

Gorbalenya A, Enjuanes L, Ziebuhr J, and Snijder EJ (2006) *Nidovirales*: Evolving the largest RNA virus genome. *Virus Research* 117: 17–37.

Jitrapakdee S, Unajak S, Sittidilokratna N, *et al.* (2003) Identification and analysis of gp116 and gp64 structural glycoproteins of yellow head nidovirus of *Penaeus monodon* shrimp. *Journal of General Virology* 84: 863–873.

Sittidilokratna N, Hodgson RAJ, Cowley JA, *et al.* (2002) Complete ORF1b-gene sequence indicates yellow head virus is an invertebrate nidovirus. *Diseases of Aquatic Organisms* 50: 87–93.

Sittidilokratna N, Phetchampai N, Boonsaeng V, and Walker PJ (2006) Structural and antigenic analysis of the yellow head virus nucleocapsid protein p20. *Virus Research* 116: 21–29.

Walker PJ, Bonami JR, Boonsaeng V, *et al.* (2005) *Roniviridae*. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, and Ball LA (eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses*, pp. 973–977. San Diego, CA: Elsevier Academic Press.

Walker PJ, Cowley JA, Spann KM, *et al.* (2001) Yellow head complex viruses: Transmission cycles and topographical distribution in the Asia-Pacific region. In: Browdy CL and Jory DE (eds.) *The New Wave: Proceedings of the Special Session on Sustainable Shrimp Culture, Aquaculture*, pp. 292–302. Baton Rouge: World Aquaculture Society.

Ziebuhr J, Bayer S, Cowley JA, and Gorbalenya AE (2003) The 3C-like proteinase of an invertebrate nidovirus links coronavirus and potyvirus homologs. *Journal of Virology* 77: 1415–1426.

Z

Zoonoses

J E Osorio and T M Yuill, University of Wisconsin, Madison, WI, USA

© 2008 Elsevier Ltd. All rights reserved.

Introduction

Zoonoses are diseases transmissible from vertebrate animals, other than humans, to people. Mammals, birds, reptiles, and probably amphibians are reservoirs or amplifier hosts for these viral zoonoses. Frequently, these viruses cause little or no overt disease in their nonhuman vertebrate hosts. Some zoonotic viruses have very limited host ranges; others may infect a wide range of vertebrates. Human infection may vary from unapparent to fatal disease. Both new and old viral zoonoses are especially important in emerging and re-emerging virus diseases. Transmission of zoonotic viruses may occur by a variety of routes. They include: 'direct' (rabies) or 'indirect' (hantavirus) contact; 'nosocomial' (arenavirus and filovirus); 'aerosol transmission' (SARS coronavirus); 'vertical' (*in utero*) (arenaviruses); and 'vector- or arthropod-borne' (yellow fever, YF). Viral zoonotic diseases occur on every continent except, perhaps Antarctica. Some are found around the world, in a variety of ecological settings. Others are found only in very limited ecologic and geographic foci. Although hundreds of viruses are zoonotic, the importance of many of these viruses has not yet been established. Some of the more important viral zoonoses will be discussed briefly.

Rabies Virus

Rabies is one of the oldest reported zoonoses. Rabies virus infection causes nervous system disease that ends in death. Animals can become infected without nervous system disease, develop antibodies, and survive, but play no role in transmission. Classical rabies is found all around the world except in Antarctica, Britain, the Hawaiian Islands, Australia, and New Zealand. Transmission occurs by the bite of an infected animal. Aerosol (droplet) transmission is rare. Dogs and cats are the main reservoirs in tropical developing countries where more than 99% of all human cases occur. In industrialized countries, wild mammals are the main reservoirs and the species involved vary from region to region. The principal species are as follows: in North America, skunks, raccoons, and foxes; in Europe, foxes; and in the Caribbean, mongooses. Bats in all enzootic regions harbor rabies with vampire bats especially important in the Neotropics, where they transmit rabies to cattle, horses, and other domestic animals, and, occasionally, to humans. Rabies virus is classified in the genus *Lyssavirus* of the family *Rhabdoviridae*. Genetic relationships between rabies isolates from different species and geographic areas have been established by genomic sequence analysis (**Table 1**).

Diagnosis is based on characteristic altered behavior of infected mammals, confirmed by either isolation of virus; demonstration of intracellular antigen by immunofluorescence; or of virus genomic sequences. Postexposure treatment is accomplished by thorough washing of the bite wound, administration of hyperimmune serum or globulin, and administration of antirabies vaccine. Dogs and cats in enzootic areas should be vaccinated. Other domestic animals and humans at high risk should also be vaccinated. Vaccination campaigns of free-ranging red fox populations in Europe and raccoons and coyotes in the USA have been carried out by oral administration of recombinant vaccinia-vectored vaccines in bait.

Hantavirus Hemorrhagic Fevers and Pulmonary Syndrome Viruses

Hantaviruses belong to the genus *Hantavirus* of the family *Bunyaviridae*. In the Americas, hantavirus can cause hantavirus pulmonary syndrome (HPS), an infectious disease typically characterized by fever, myalgia, and headache and followed by dyspnea, noncardiogenic pulmonary edema, hypotension, and shock. HPS has also been reported and confirmed in seven countries in South America: Argentina, Bolivia, Brazil, Chile, Paraguay, Uruguay, and Panama (**Table 2**). Hantaviruses are harbored by wild rodents which often live in close association with humans. Virus

Table 1 Rabies and related lyssaviruses

Virus name	*Lyssavirus genotype*	*Location*	*Host*
Rabies	1	Worldwide	Many wild and domestic mammals
Lagos bat	2	Africa	Bats, water mongoose (but no human disease)
Mokola	3	Africa	Several terrestrial mammals
Duvenhage	4	Africa	Bats
European bat-1	5	Europe	Bats
European bat-2	6	Europe	Bats
Australian bat	7	Australia	Bats
Aravan	New, proposed	Kyrgystan	Bats
Khujand	New, proposed	Tajikistan	Bats
Irkut	New, proposed	Russia	Bats
West Caucasian bat	New, proposed	Russia	Bats

Table 2 Hantaviruses that cause human disease

Virus name	*Distribution*	*Rodent host*	*Human disease*
Hantaan	Asia, Europe	*Apodemus agrarius*	Hemorrhagic fever with renal syndrome
Seoul	Worldwide	*Rattus* spp.	Hemorrhagic fever with renal syndrome
Dobrava-Belgrade	Europe, Middle East	*Apodemus flavicollis*	Hemorrhagic fever with renal syndrome
Puumala	Europe, Asia	*Clethrionomys glareolus*	Hemorrhagic fever with renal syndrome
Sin Nombre	North America	*Peromyscus maniculatus*	Pulmonary syndrome
New York	North America	*Peromyscus leucopus*	Pulmonary syndrome
Bayou	North America	*Oryzomys palustris*	Pulmonary syndrome
Black Creek Canal	North America	*Sigmodon hispidus*	Pulmonary syndrome
Andes	South America	*Oligoryzomys longicaudatus*	Pulmonary syndrome
Hu39694	South America	Unknown	Pulmonary syndrome
Juquitiba	South America	Unknown	Pulmonary syndrome
Laguna Negra	South America	*Calomys laucha*	Pulmonary syndrome
Lechiguanas	South America	*Calomys laucha*	Pulmonary sybdrome
Oran	South America	*Oligoryzomys longicaudatus*	Pulmonary syndrome
Choclo	Panama	*Zygodontomys brevicauda*	Pulmonary syndrome
Monogahela	North America	*Peromyscus maniculatus nubiterrae*	Pulmonary syndrome
Bermejo	South America	*Oligoryzomys chacoensis*	Pulmonary syndrome
Central Plata	South America	*Oligoryzomys flavescens*	Pulmonary syndrome
Araraquara	South America	*Bolomys lasiurus*	Pulmonary syndrome

is shed in urine and other excreta. Outbreaks of HPS have been associated with ecological changes and invasion of human habitations by expanding rodent populations. Diagnosis has been complicated by the lack of efficient and sensitive isolation and serological methods. Rodent control and avoidance of exposure to rodent excreta, especially in dust, are the only methods available currently for prevention of transmission to humans.

Arenavirus Hemorrhagic Fever Viruses

Arenaviruses are transmitted by the same kind of rodents that carry hantaviruses. They can also cause hemorrhagic fevers and, even though the prototype of this family has been long known (lymphocytic choriomeningitis virus, LCMV), viruses within this family are still being discovered. They produce human diseases in the Old World (Lassa fever in Africa) and New World (Junin, Machupo, and, later on, Guanarito and Sabia in South America). There are about 22 different arenaviruses in the Americas, but only four are associated with significant human disease. These pathogenic arenaviruses establish persistent infection in their rodent hosts, and the virus is shed in urine, infecting humans who live in close contact with these contaminated environments. Lassa fever is also transmitted nosocomially in rural hospitals to other people in contact with blood from viremic patients. Control of these diseases is attempted mainly by reduction of rodent populations. A live, attenuated vaccine has been developed for Argentine hemorrhagic fever, and a vaccinia-vectored vaccine has been developed for Lassa fever. Ribavirin is effective for treating arenavirus infection if administered early in the course of infection.

YF Virus

YF is a flavivirus that causes hemorrhagic disease with severe liver damage and death in up to half of the most acute cases. Humans or primates transport the virus from its sylvan cycle in forested areas to rural or urban areas, where other vector mosquitoes transmit it. YF remains a disease of significant public health importance, with an estimated 200 000 cases and 30 000 deaths annually. The disease is endemic in tropical regions of Africa and South America; nearly 90% of YF cases and deaths occur in Africa. It is a significant hazard to unvaccinated travelers to these endemic areas. Re-establishment of the major urban vector, *Aedes aegypti*, the recent spread of the Asian tiger mosquito (*Ae. albopictus*) as well as the rise in air travel has increased the risk of introduction and spread of the disease. YF is an acute infectious disease characterized by sudden onset with a two-phase development, separated by a short period of remission. The clinical spectrum of YF varies from very mild, nonspecific, febrile illness to a fulminating, sometimes fatal disease with pathognomic features. In severe cases, jaundice, bleeding diathesis, with hepatorenal involvement is common. There is no specific treatment for YF, making the management of YF patients extremely problematic. YF can be diagnosed by virus isolation, detection of circulating antigens, demonstration of a significant rise in specific YF virus antibodies, and microscopic detection of viral inclusion bodies or antigen in tissues taken at postmortem examination. Insecticide spraying and elimination of breeding sites in homes can be used for vector control in epidemic situations. Disease can be prevented in humans by vaccination.

West Nile Virus

West Nile virus (WNV) is an arthropod-borne RNA flavivirus that causes a mild infection to acute febrile disease with rash, and occasional encephalitis (mainly in the elderly) is produced in humans. The virus is widely spread, occurring from India and Pakistan westward through the Middle East and into Africa, and northward into Europe, the republics of the former USSR, and in the Western Hemisphere. WNV first appeared in North America in 1999 with an outbreak in New York City producing high mortality in crows and other birds. Subsequently, the virus has spread rapidly throughout North America, the Caribbean, Mexico, and into South America. WNV is now present in every state except Hawaii, and Alaska. It is believed that the NY99 virus has remained stable and unchanged. The ecology of WNV involves maintenance in a bird–mosquito–bird cycle and occasional infection of humans and horses. *Culex* spp. are the main vectors, and birds are the vertebrate hosts. There is no vaccine currently available for humans, although there is one for equine animals.

Chikungunya Virus

Chikungunya (CHIK) is an alphavirus of the family *Togaviridae* that has been responsible for acute febrile disease with rash and severe arthralgia in people in Africa and Asia. An outbreak of CHIK was reported on Reunion Island in March 2005 that resulted in >3500 confirmed cases and an estimated 250 000 suspected cases, affecting >25% of the island's inhabitants. CHIK virus is maintained in sylvan or savanna cycles involving wild primates and arboreal *Aedes* mosquitoes. In both Africa and Asia, the virus also has an urban cycle involving humans and *Ae. aegypti* mosquitoes that is more important from a public health standpoint.

Sindbis Virus

Sindbis (SIN) virus is one of the most widely distributed arthropod-borne viruses in the world, being found in Africa, Europe, Asia, and Australia. Disease in humans is usually mild, and is characterized by acute fever, with arthralgia, myalgia, and rash. There are periodic epidemics in Finland, where it is termed Podosta disease. SIN virus is maintained in wild bird populations, with transmission by *Culex* spp. mosquitoes. In Africa and the Middle East, SIN is often found in the same ecosystems where WN virus is being transmitted. The virus is an alphavirus of the *Togaviridae*. Phylogenetic analysis indicates that there is one major genetic cluster of western SIN virus strains in Africa and another in Australia and Asia. There is evidence of some geographic mixing of western strains of SIN virus that suggest long-distance transport via migrating birds. There is no vaccine available. Since many of the mosquito vectors breed in extensive rice fields, large-scale control would be expensive.

Crimean-Congo Hemorrhagic Fever Virus

Crimean-Congo hemorrhagic fever (CCHF) virus is very widely distributed, and is found from eastern Europe and the Crimean, eastward through the Middle East to western China, and southward through Africa to South Africa. CCHF is characterized by severe hemorrhagic fever with hepatitis, with case mortality of 10–50%. Maintenance of CCHF virus involves horizontal transmission from *Hyalomma* ticks to mammals, and vertical transmission in ticks through the eggs. *Hyalomma* ticks have also been found on birds migrating between Europe and Africa – a

mechanism for long-distance dispersal of the virus. Human CCHF cases have occurred in workers handling livestock and their products in Saudi Arabia and the United Arab Emirates which have been attributed to importation of infected cattle and their ticks from Somalia and the Sudan. There are no vaccine or tick control measures available. CCHF virus belongs to the genus *Nairovirus* of the *Bunyaviridae*. Genetic analysis indicates that reassortment and recombination occur in nature.

Sandfly Fever Viruses

Sandfly fever (Sicilian, Naples, and Toscana) viruses are endemic in the Mediterranean area. They cause acute febrile disease in humans, with occasional aseptic meningitis. In central Italy, Toscana virus (TOSV) caused one-third of previously undiagnosed cases of aseptic meningitis. There are at least two genetic lineages of TOSV – Spanish and Italian. The viruses are members of the genus *Phlebovirus* of the *Bunyaviridae*. They are transovarially and horizontally transmitted by phlebotomine sandflies. Wild mammals are presumed reservoirs.

Viruses Occurring in the Americas

Encephalitis Viruses

Venezuelan equine encephalitis (VEE) viruses are made up of a closely related complex of subtypes with several varieties, which have differing epidemiology, geographic distributions, and disease importance. The epizootic/epidemic (VEE, IAB, and IC) virus variants are of greatest concern. In equine animals, the virus causes acute encephalitis, and case fatality may approach 80%. Survivors may have serious neurological deficits. Although the case–fatality rate in humans is low (less than 1%), the large numbers of acutely infected people that occur during an epidemic may completely overwhelm the local health-care system. VEE, IAB, and IC viruses are maintained in northern South America, where they have periodically swept through Venezuela and Colombia in epidemic waves, with occasional extensions into Ecuador and massively through Central America into Mexico and South Texas. Epidemic spread depends on the availability of susceptible equine populations (the amplifying host) and abundant mosquito vectors of several species. The interepidemic maintenance systems remain undefined. There is evidence that the epizootic strains may arise by mutation of subtype ID enzootic virus. The enzootic strains are maintained in limited foci involving rodents and *Culex* (*Melanconion*) spp. mosquitoes from Florida to Argentina. With the exception of subtype IE, which has caused epizootics in horses in Mexico, these enzootic virus strains do not cause disease in equine animals, but can cause acute febrile illness in humans. The VEE complex viruses are in the genus *Alphavirus* of the *Togaviridae*. There is an effective live, attenuated vaccine for both human and equine use. Because the maintenance of equine herd immunity is costly, most animal health agencies do not carry out ongoing, intensive vaccination campaigns. Thus, the risk of reoccurrence of explosive outbreaks remains.

Eastern (EEE) and western (WEE) equine encephalitis viruses occur in epidemic form in North America, but have also been found in Central and South America. Generally, EEE is maintained in eastern North America but has caused scattered epizootics and cases in the Caribbean, and in Central and South America. EEE virus can be divided into a North American-Caribbean clade, an Amazon Basin clade, and a Trinidad, Venezuela, Guyana, Ecuador, and Argentina clade. During the past several years, there have been modest increases in several US states in the number of reported human cases of EEE. In North America, WEE occurs in western and prairie states and provinces and along the west coast. WEE has caused sporadic cases of encephalitis in equine animals, but not humans, in Argentina and Uruguay. Both involve wild birds and mosquito vectors, with spillover into equine population and humans, causing clinical encephalitis and death. Central nervous sequelae may occur among survivors. Effective vaccines are available commercially for equine animals, and experimental vaccines are used for laboratory personnel. Effective mosquito abatement to control vector populations has been carried out in the West for many years. Insecticide application is used for vector control in epidemic situations.

St. Louis encephalitis (SLE) virus occurs from Canada to Argentina and causes sporadic but extensive epidemics in the USA, with most epidemics occurring in the West, down the Ohio and Mississippi valleys into Texas, and in Florida. Wild passerine birds are amplifying hosts in North America, but in the Southeastern USA and the Neotropics mammals may play an epidemiologic role in virus maintenance and transmission. SLE virus is transmitted by *Culex* spp. mosquitoes in the USA. SLE virus is a flavivirus of the *Togaviridae*, and is closely related to Japanese encephalitis virus. In humans, SLE is characterized by febrile disease, with subsequent encephalitis or aseptic meningitis, and strikes older people more often than the young. Since no vaccine is available, SLE prevention and control relies on surveillance, vector control, and screening of dwelling windows and doors.

Powassan (POW) virus is a North American member of the flavivirus tick-borne encephalitis (TBE) complex. Although POW virus is widely distributed across the USA and Canada, and westward into far eastern Russia, disease (febrile, with encephalitis) has only been detected in the eastern states and provinces of North America. The transmission cycle involves small mammals and *Ixodes* ticks.

La Crosse (LAC) and other California serogroup encephalides are human pathogens in North America. Prior to the arrival of WNV, LACV was the most important cause of endemic arboviral encephalitis in the US, causing an estimated average of 80 cases per year, affecting mainly preschool-aged children. It is endemic in the Upper Midwest, but occasional cases occur elsewhere. Although fatality is uncommon, the disease is severe enough to cause prolonged hospitalization. LAC virus is maintained transovarially in treehole breeding by *Ochlerotatus* (formerly *Aedes*) *triseriatus* mosquitoes with horizontal transmission to small forest mammal reservoirs and to humans. The other California group viruses affecting people have similar epidemiologies, but do not cause disease as commonly. California encephalitis virus was isolated in California, and has occasionally caused human disease there. Snowshoe hare (SSH) virus occurs in the Northern USA and across Canada, and has caused human encephalitis in the eastern provinces. Jamestown Canyon (JC) virus is widely distributed across the USA, and has been shown to cause human disease, mainly in adults in the Midwest, and to infect deer. Like LAC virus, these other viruses have the same close epidemiological relationship with their *Ochlerotatus* vectors. These viruses are members of the California serogroup of the genus *Bunyavirus* of the family *Bunyaviridae*. SSH virus is an antigenic variant of LAC virus.

Colorado Tick Fever Virus

Colorado tick fever (CTF) is endemic in sagebrush–pine–juniper habitats of the higher elevations (over 1200 m) in the mountains of the western states and provinces of North America. Although seldom fatal, CTF can cause serious disease in humans (fever, chills, headache, retroorbital pain, photophobia, myalgia, abdominal pain, and generalized malaise) with prolonged convalescence. CTF may present as hemorrhagic or central nervous system disease, and is most severe in preadolescent children. Males are infected over twice as frequently as are females. The virus is transmitted by and overwinters in *Dermacentor andersoni*. Wild rodents are the vertebrate hosts, and develop a prolonged viremia. CTF virus is classified in the genus *Coltivirus* of the family *Reoviridae* and is serologically related to Eyach virus from Germany. Avoidance of tick bites is the main preventive measure available, but control of rodents and the ticks that inhabit their burrows can be applied in foci of virus maintenance in the field.

Vesicular Stomatitis Virus

Vesicular stomatitis (VS) virus is endemic in Central and northern South America and in the southeastern USA, causing an acute, febrile vesicular disease in cattle, horses, and pigs. Sporadic VS epidemics occur in the southwestern states of the USA. Both of the major serotypes, VS-Indiana and VS-New Jersey, cause influenza-like illness in humans and are an occupational hazard to people handling cattle. The VS viruses comprise a complex of related serotypes and subtypes in the Americas, with related vesiculoviruses (family *Rhabdoviridae*) in Africa and Asia. Many of these viruses are transmitted horizontally and transovarially by phlebotomine sandflies, with evidence for infection of wild rodents and other small mammals. However, the role of these mammals in the epidemiology of VS viruses is unclear because they do not develop viremia. Grasshoppers have been shown to be susceptible experimentally, but their role as reservoirs or amplifiers in nature remains to be established.

Other Neotropical Viruses

Oropouche virus, a Simbu serogroup bunyavirus, causes epidemics, occasionally severe, or acute febrile disease with arthralgia and occasional aseptic meningitis in humans in the Brazilian and Peruvian Amazon as well as Surinam and Panama. During rainy season epidemics, the virus is transmitted by *Culicoides paraensis* biting midges. Enzootic maintenance cycles are believed to involve forest mammals and arboreal mosquitoes.

Mayaro (MAY) virus occurs epidemically in the Brazilian and Bolivian Amazon Basin, and has also been associated with human febrile disease in Surinam and Trinidad. In humans, the acute, nonfatal, febrile disease with rash is clinically similar to CHIK, an alphavirus to which it is antigenically and taxonomically related. MAY virus appears to be maintained in nature in a cycle similar to that of YF, with arboreal mosquito vectors and primate hosts, but also involving other mammals and birds.

Una virus is a close relative of MAY virus and causes human febrile disease also, but its natural history is not known. Una virus has been isolated from several mosquito species, and has been found at scattered sites from northern South America to Argentina. Antibodies have been found in humans, horses, and birds. Genetic analysis suggests that Una virus is maintained in discrete foci.

Rocio virus was first isolated from fatal human encephalitis cases during an explosive outbreak of acute febrile disease in coastal Sao Paulo State, Brazil in 1975, after which sporadic outbreaks have continued. This virus is an ungrouped flavivirus in the *Togaviridae* and is serologically related to Murray Valley encephalitis virus from Australia. The epidemiology is unclear but probably involves wild birds, and several mosquito species are suspected vectors.

Cowpox-Like Viruses

Cantagalo and related viruses are orthopoxviruses newly reported in Brazil. They can cause vesiculopustular lesions on the hands, arms, forearms, and face of dairy milkers.

Virus particles can be detected by either direct electron microscopy (DEM) in vesicular fluids and scab specimens or isolated in cell culture and embryonated chicken eggs. The epidemiological significance of these new vaccinia viral strains and their origins remains unknown.

Viruses Occurring in Europe

Tahyna (TAH) virus is widely distributed in Europe and has been reported in Africa. TAH virus produces an influenza-like febrile disease, with occasional central nervous system involvement. The virus is a bunyavirus of the California serogroup, in the *Bunyaviridae*. Like LAC virus, small forest mammals are TAH virus reservoirs, and the virus is horizontally and transovarially transmitted by *Ochlerotatus* mosquitoes. There are no effective control measures.

Omsk hemorrhagic fever occurs in a localized area of western Siberia. Disease can be severe, with up to 3% case fatality, and sequelae are common. This virus is a member of the TBE complex of the flaviviruses. The virus is epizootic in wild muskrats, which had been introduced into the area, and is associated with ixodid ticks. Muskrat handlers are at highest risk of infection. Water voles and other rodents are also vertebrate hosts of the virus. TBE virus vaccine is used in high-risk individuals to provide protection.

TBE virus is also a member of the TBE complex of flaviviruses. TBE virus has been classified into three subtypes: European, far eastern, and Siberian. Because recreation in wooded areas has increased in recent years, TBE has become the most frequent arthropod-borne disease in Europe. The virus occurs in deciduous forests in Western Europe from the Mediterranean countries, westward to France, northward to the Scandinavian countries, and eastward to Siberia. It is maintained in a transmission cycle involving small mammals and *Ixodes* spp. ticks. Human infection also occurs through the consumption of unpasteurized milk from infected cows and goats. Infection can be prevented by an inactivated vaccine and avoidance of tick bites.

Cowpox virus is an orthopoxvirus in the *Poxviridae*. It has a wide host range. Domestic cats are the most important source of human infection, transmitting the virus from wild rodent reservoirs to people. In addition to cattle, this virus has produced severe, generalized infections in a variety of incidental animal hosts in zoos and circuses, including elephants and large cats, which may die. Humans develop typical poxvirus lesions (vesicle and pustule formation), usually on the hands. Laboratory diagnosis (characterization of isolated virus) is required to differentiate cowpox from other nodule-forming zoonotic poxviruses such as orf virus, bovine papular stomatitis virus, and pseudocowpox virus, which are worldwide in distribution.

Viruses Occurring in Africa and the Middle East

Rift Valley Fever Virus

Rift Valley fever virus (RVF) is among the most serious arbovirus infections in Africa today. Repeated RVF epidemics in sub-Saharan Africa cause serious disease in small ruminant animals and humans. RVF disease has expanded its historical geographic range in the livestock-raising areas of eastern and southern Africa and into the Middle East (Saudi Arabia and Yemen) over the past 25 years, causing massive epidemics in Egypt, along the Mauritania–Senegal border and in Madagascar. A major outbreak in East Africa began in 2006 in northeastern Kenya, and spread into southern Somalia and Tanzania. Cattle, sheep, and humans are affected. Abortion storms with febrile disease and bloody diarrhea occur in ruminant animals, and mortality may be heavy in young stock. Most infected humans develop febrile disease, with prolonged convalescence. A few individuals develop more severe disease, with liver necrosis, hemorrhagic pneumonia, meningoencephalitis, and retinitis with vision loss. The human case–fatality rate is less than 1%. RVF virus is in the genus *Phlebovirus* of the *Bunyaviridae*. In sub-Saharan Africa, RVF virus is closely tied to its *Aedes* mosquito vectors. RVF vectors transmit the virus transovarially and horizontally. The virus persists in mosquito eggs laid around seasonally flooded pools and depressions. When these pools flood, the eggs hatch and infected mosquitoes emerge and begin transmission. The vertebrate reservoir hosts of RVF virus are unknown. Field and laboratory workers need to exercise caution to avoid becoming infected by exposure to the virus during postmortem examination of animals or processing materials in the laboratory. Both live, attenuated and inactivated vaccines are available for animals, but the unpredictability of scattered, sporadic RVF outbreaks across sub-Saharan Africa is a major obstacle for implementation of extensive, cost-effective vaccination programs.

Marburg and Ebola Viruses

The reappearance of epidemic Ebola disease in Kikwit, Democratic Republic of the Congo (formerly Zaire) in 1995 and Makokou, Gabon in 1996 again focused international attention on this hemorrhagic disease. Marburg and Ebola viruses have sporadically caused severe hemorrhagic fever in humans. Marburg virus, although of African origin, first appeared in laboratory workers in Germany who had handled cell cultures originating from African primates. Later, epidemics of severe hemorrhagic fever occurred in the Sudan and in Zaire, and Ebola virus was isolated. The first nonlaboratory epidemic of Marburg virus occurred in the Democratic Republic of the Congo from 1998 to 2000. A second, and more severe, outbreak

occurred in Uige Province of Angola from 2004 to 2005, when 329 of 374 infected people died (case–fatality rate of 88%). These viruses produce hemorrhagic shock syndrome and visceral organ necrosis, and have the highest case–fatality rate (30–90%) of the hemorrhagic fevers. These viruses, with their bizarre filamentous, pleomorphic morphology, belong to the family *Filoviridae*. They are presumed to be zoonotic, but their hosts in nature and mechanisms of transmission in the field have not been determined. Most of the Makokou, Gabon patients had very recently butchered chimpanzees. A variant of Ebola virus has been isolated from chimpanzees from Côte D'Ivoire, but since wild primates suffer severe disease, they are unlikely to be maintenance reservoirs. Nosocomial transmission of Marburg and Ebola viruses has occurred frequently; a high level of patient isolation and biosafety containment are essential to avoid hospital- and laboratory-acquired infection. Serologic diagnosis is accomplished by means of indirect immunofluorescence or enzyme-linked immunosorbent assay (ELISA) test, with antigen specificity confirmed by western blot. No vaccines or control measures are available.

Monkeypox Virus

Human monkeypox is a severe, smallpox-like illness. Monkeypox belongs to the genus *Orthopoxvirus* of the *Poxviridae*. Monkeypox virus (MPXV) is endemic in rodents in West and Central Africa, with the occurrence of sporadic human cases. The case–fatality rate in humans appears to be higher in Central Africa than in West Africa, raising questions about possible difference in virulence in these two large geographic areas. The largest epidemic of human monkeypox ever documented occurred in the Katako-Kombe area of the Democratic Republic of the Congo (formerly Zaire) in 1996–97, with over 500 people becoming ill and five deaths. Rodent-to-human transmission occurred, as did subsequent secondary human-to-human spread. Vaccinia is protective against infection, but its use has been discontinued with the eradication of smallpox. In 2003, monkeypox emerged for the first time in the Western Hemisphere and caused an outbreak in the United States (Midwestern states) affecting 37 people exposed to ill prairie dogs purchased from pet stores. The virus entered the US upon the importation of exotic rodents from Ghana (West Africa). Recent nucleotide sequence analysis demonstrated the existence of two genetically distinct variants of the virus, called the West African and Congo Basin clades. The strain that caused the US outbreak belonged to the West African clade.

Semliki Forest Virus

Semliki Forest (SF) virus caused an extensive epidemic of human disease in Bangui, Central African Republic, in 1987. SF virus is an alphavirus in the *Togaviridae*. It occurs across East, Central, and West Africa, and has been isolated from various mosquitoes and from wild birds. Antibodies have also been found in wild mammals. The SF virus maintenance cycle probably involves *Ae. africanus* mosquitoes and vervet monkeys.

Orungo Virus

Orungo (ORU) virus caused mild epidemic disease (fever, nausea, headache, and rash) in Nigeria. The virus occurs in a band across Africa from Uganda to Sierra Leone. It is probably mosquito transmitted, but the species that transmit it in nature are not known. Although the vertebrate reservoir hosts are unknown, wild primates have antibody and are suspected to be involved in virus maintenance.

Alkhurma Virus

Alkhurma virus (a variant of Kysanur Forest disease virus, family *Flaviviridae*, genus *Flavivirus*) is an emerging pathogen responsible for hemorrhagic fever in the Middle East. This virus was isolated from hemorrhagic fever patients in Saudi Arabia in 1995. Transmission can occur from tick bites, handling carcasses of infected animals, or drinking unpasteurized milk. The case–fatality rate is 25%.

Viruses Occurring in Asia

Influenza Viruses

Influenza viruses belong to the family *Orthomyxoviridae,* which consists of five genera: influenza A, influenza B, influenza C, Isavirus, and Thogoto viruses. Influenza A viruses are widely distributed in nature and can infect a wide variety of birds and mammals. Influenza A virus subtypes are classified on the basis of the antigenicity of their surface glycoproteins hemagglutinin (HA) and neuraminidase (NA). To date, 16 HA and 9 NA genes are known to exist. Of these genes, only three HA (H1, H2, and H3) and two NA (N1 and N2) subtypes have circulated in the human population in the twentieth century. During the last 100 years, the most catastrophic impact of influenza was the pandemic of 1918, also known as the Spanish flu (H1N1), which resulted in the loss of more than 500 000 lives in the United States and caused about 40 million deaths worldwide.

The ability of influenza viruses to undergo antigenic changes is the cause of ongoing significant public health concern. New subtypes emerge when human virus captures genes from animal influenza viruses via reassortment; an event that can occur when both virus types simultaneously infect a host (antigenic shift). The threat imposed by influenza virus has been further elevated with the recent introductions of avian influenza viruses into

the human population. Avian influenza viruses were initially considered nonpathogenic for humans. However, this perception has changed since 1997, when 18 Hong Kong residents were infected by an avian influenza virus of the H5N1 subtype that resulted in six deaths. Over the next few years, several other cases of direct avian-to-human transmission were reported, including the ongoing outbreak of highly pathogenic H5N1 influenza viruses in several Asian, African, and European countries. Migratory waterfowl – most notably wild ducks – are the natural reservoir of avian influenza viruses, and these birds are also the most resistant to infection. Domestic poultry, including chickens and turkeys, are particularly susceptible to epidemics of rapidly fatal influenza. Direct or indirect contact of domestic flocks with wild migratory waterfowl has been implicated as a frequent cause of epidemics. Live bird markets have also played an important role in the spread of epidemics. Viruses of low pathogenicity can, after circulation for sometimes short periods in a poultry population, mutate into highly pathogenic viruses. Quarantine of infected farms and destruction of infected or potentially exposed flocks are standard control measures aimed at preventing spread to other farms and eventual establishment of the virus in a country's poultry population.

Severe Acute Respiratory Syndrome

In February 2003, a new and previously unknown disease, severe acute respiratory syndrome (SARS), was reported to the World Health Organization (WHO). SARS originated in the province of Guangdong in southern China in November 2002 where it initially was thought to cause atypical pneumonia. However, within a short time the virus spread to Hong Kong, Singapore, Vietnam, Canada, the United States, Taiwan, and several European countries. A novel coronavirus (CoV) was identified as the etiological agent. The SARS-CoV affected more than 8000 individuals worldwide and was responsible for over 700 deaths during the first outbreak in 2002–03. For reasons unknown the SARS virus is less severe and the clinical progression a great deal milder in children younger than 12 years of age. In contrast, the mortality rate was highest among patients >65 years and can exceed 50% for persons at or above the age of 60 years. Farmed masked palm civets (*Paguma larvata*) and two other mammals in live animal markets in China were sources of SARS-CoV human infection. Three species of horseshoe bats (*Rhinolophus* spp.) are probable wildlife reservoirs in China.

Kyasanur Forest Disease

Kyasanur Forest disease (KFD) was first recognized in India in 1957, when an acute hemorrhagic disease appeared in wild monkeys and people frequenting forested areas. KFD has been slowly spreading in India. Human cases have increased from 1999 to 2005, with peak incidence in January and February. The cause of this increase is unknown. KFD virus is a member of the TBE complex of flaviviruses. The basic virus maintenance cycle involves forest mammals (primates, rodents, bats, and insectivores) and ixodid ticks, mainly *Haemaphysalis spinigera*. The virus can be isolated in mice and cell cultures, including tick cells. An inactivated vaccine provides some protection to people at risk of infection.

Japanese Encephalitis Virus

Japanese encephalitis (JE) virus is found in a broad area from far eastern Russia, northeastern Asia through China and Southeast Asia to Papua New Guinea and the Torres Strait Islands of Australia and westward into India. JE virus causes the greatest number of clinical human cases, thousands annually, predominantly in children. It produces encephalitis in humans and horses, and acute febrile disease with abortion in swine, an amplifying host. Herons and egrets are wildlife amplifying hosts. The virus is transmitted by *Culex* spp. mosquitoes. The over-wintering mechanism in temperate Asia is unknown. JE virus is a member of a complex of four related flaviviruses in the family *Flaviviridae*. Prevention of disease is mainly through vaccination of humans, horses, and swine. Insecticides and integrated pest control measures that include natural compounds (*Bacillus thurengiensis* toxins), larvicidal fish, and larval habitat modification have been successfully used in China. Use of pyrethroid-impregnated bed netting can also prevent transmission.

Chandipura

Chandipura virus is ubiquitous across the Indian subcontinent. It is a *Vesiculovirus* in the *Rhabdoviridae*. Chandipura has caused epidemics of febrile diseases, sometimes with encephalopathy. An outbreak occurred in 2004 with a case–fatality rate of 78.3% in children in India (Gujarat State). The virus is transmitted by *Phlebotomus* spp. Sergentomine sandflies and infects a variety of mammals. The virus has also been isolated in West Africa.

Nipah

Nipah virus (NiV) was first recognized in peninsular Malaysia in 1998, where it caused encephalitis and respiratory disease in commercially raised pigs, with transmission to humans in contact with them, with 40–76% case fatality. The virus was found in five species of giant fruit bats (*Pteropus* spp.) there and NiV was

isolated from partially eaten fruit. Subsequently, there were five NiV outbreaks recognized in Bangladesh, also associated with *Pteropus* bats. Transmission in Bangladesh was directly from bats, via contaminated fruit and date palm sap.

Viruses Occurring in Australia

Murray Valley Encephalitis Virus and Kunjin Virus

Murray Valley encephalitis (MVE) virus and the closely related Kunjin virus are flaviviruses that cause encephalitis, although Kunjin virus more commonly produces a nonencephalitic illness with polyarthralgia. MVE virus is endemic in avian species and is found in humans in northern Western Australia, the Northern Territory, and Queensland. MVE virus is endemic in northern areas of Western and Northern Australia, and in New Guinea. Kunjin virus occurs over a much wider area, including most of tropical Australia, Sarawak, Borneo, Papua New Guinea, and Saibai Island in the Torres Strait. There is some evidence that infection with these viruses is increasing in incidence. In northern Australia, MVE cases occur predominantly between February and July, corresponding to the end of the monsoon season, when the mosquito vector (*Culex annulirostris*) proliferates in flooded environments. MVE and Kunjin viruses are flaviviruses, family *Flaviviridae*, of the Japanese encephalitis complex. Kunjin is a subtype of WNV. RNA sequencing indicates that the Australian strains of MVE virus are similar to, but different from Papua New Guinea isolates. No vaccine is available. Control is achieved through application of larvicides.

Ross River Virus

Ross River (RR) virus has caused annual epidemics of febrile disease with polyarthritis and rash with most cases occurring in November through April. It is the most commonly reported arthropod-borne virus disease in Australia. RR virus occurs in all Australian states and territories, but is most commonly found in the northern states and coastal areas. Within the past two decades, RR virus has spread through several Pacific Islands in epidemic form and appears to have become endemic in New Caledonia. Convalescence can be long. RR virus is an alphavirus of the family *Togaviridae*. The enzootic maintenance cycles of RR virus in Australia are not well defined, but wild and domestic mammals appear to be the reservoir hosts, and the principal mosquito vectors are salt marsh *Aedes* spp. and freshwater *Culex* spp. In the Pacific Islands outbreaks, the virus was probably transmitted from person to person by *Aedes* mosquitoes.

Barmah Forest Virus

Barmah Forest virus is the second most common mosquito-borne disease in Australia. It causes subclinical and clinical infections in humans, including fever, myalgia, polyarthralgia, and rash. It is an alphavirus in the *Togaviridae*. The virus appears to be endemic in eastern Australia. It has been isolated from 25 different mosquito species in five genera. *Ochlerotatus vigilax* (previously known as *Aedes vigilax*), is considered a major vector. Its vertebrate hosts have not been established, although marsupials are suspected.

Control

On-going disease surveillance supported by rapid, reliable diagnosis is critical for recognition and control of zoonotic diseases. Serological diagnosis by means of ELISA tests, virus neutralization, and other tests, coupled with detection of the virus itself by virus isolation, immunohistochemistry, immunofluoresence, or PCR techniques are standard laboratory approaches, but are not available in all countries. Timely laboratory results must reach clinicians treating infected individuals and become incorporated into epidemiological databases and early warning systems to assure rapid response by public health authorities to control outbreaks appropriately.

Control of zoonotic virus diseases is accomplished by breaking the cycle of transmission. This is usually achieved by eliminating or immunizing vertebrate hosts, and reducing vector populations. Reduction of reservoir host populations is usually not accomplished because it is too expensive, not environmentally safe, and not technically or logistically feasible. However, there have been some notable exceptions. Bolivian hemorrhagic fever, caused by Machupo virus, was controlled by reduction of its rodent hosts through intensive rodenticiding. The principal vampire bat reservoir of rabies, *Desmodus rotundus*, is being controlled by the application of warfarin-type anticoagulants. Control programs like these have to be continuous to be effective. Their reduction or discontinuation results in host population recovery through reproduction and immigration, which may result in re-emergence of disease sweeping through the increasing, susceptible cohort.

Immunization of hosts is another control approach. Safe and effective rabies vaccines are being used for immunization of humans, domesticated animals, and some wildlife species. The human diploid cell vaccine is extremely effective, free of adverse effects, and widely available but at a cost too high for use in many developing countries. Safe, effective animal vaccines of cell culture origin are on the market. After some initial public resistance, raccoon populations in the eastern USA and wild foxes in Europe are being successfully immunized by means of an oral, vaccinia-vectored recombinant vaccine.

This experience illustrates the need for public understanding, in order to counteract fear of dispersal of a genetically engineered virus. However, vaccines will not be developed for many zoonotic viral diseases that affect relatively few people and are of very limited concern geographically.

Vector control is another promising but difficult area of zoonoses reduction or elimination. Insecticide application has become more problematic because both vectors themselves, as well as public opinion, have become more resistant to their use. Integrated pest management techniques, well developed for the control of many crop insects, along with the use of natural pesticides such as *Bacillus thurengiensis* toxin, offers promise for the effective, environmentally safe control of dipterous vectors. Control of tick vectors is likely to remain a problem for some time to come.

Emerging and Re-emerging Zoonoses

Ecological Change

Human disturbance has become a feature of nearly every part of the planet. All too often these disturbances create habitats that favor increases in populations of key hosts and vectors, with subsequent increased transmission of viral zoonoses. Nowhere are ecological changes happening more rapidly and profoundly than in the world's tropics. Conversion of tropical forests to agricultural ecosystems simplifies diverse ecosystems and provides either native or introduced host or vector species the conditions necessary to become more abundant, and sustain intensified virus transmission in areas where people live and work. In Africa, recent YF epidemics have been increasing dramatically in agricultural areas. Some agricultural irrigation development projects have created extensive vector breeding habitats, with an increase in mosquito-transmitted disease. The extensive dams constructed in Senegal were followed by epidemics of RVF, with numerous cases of disease in humans and small ruminant animals. The public health consequences of development projects must never be overlooked.

Global climate change will also bring ecological changes and shifts of human populations that will affect the occurrence of viral zoonoses. There is general consensus that changes in the global climate will happen with unprecedented speed. With those changes will come alterations in the geography of natural and agricultural ecosystems, with corresponding changes in the distribution of zoonotic diseases and the intensity of their transmission. It is clear that El Niño southern oscillation phenomena have increased rainfall, with resulting increases in rodent populations and occurrence of HPS in the Southwestern USA, and increased breeding sites for mosquito vectors of RVF virus in Africa. While it is not possible to predict accurately what the world will be like in 100 years or what zoonotic diseases are likely to be most troublesome, it is certain that things will be different, and constant surveillance will be essential to avoid serious problems or deal promptly and effectively with the ones that arise.

Movement of zoonotic viruses can result from the displacement of infected animals, contaminated animal products, and virus-carrying arthropod vectors. Pets, sport, laboratory, and agricultural animals are moving around the world as never before. Although international and national regulations have been established to prevent movement of infected individuals, it is not possible to test for all possible zoonotic viruses, and prevent them from crossing international boundaries. Moreover, significant numbers of animals of high commercial value move illegally. The importation of highly virulent Newcastle disease (ND) virus has been occasionally linked to smuggled birds.

Zoonotic viruses may be transported by movement of arthropod vectors, too. Just as the YF mosquito, *Ae. aegypti*, moved around the world in water casks aboard sailing vessels, mosquitoes are transported around the world in international commerce. Ships still transport mosquito vectors. The Asian tiger mosquito, *Ae. albopictus*, has become established in the Western Hemisphere after multiple introductions in eggs deposited in used tires. This mosquito is capable of transmitting YF, VEE, JC, and LAC encephalitis viruses. Perhaps of greater concern, modern transport aircraft have been shown to move vector mosquitoes internationally.

Human activity alters animal populations, contact between wild and domestic animals, and human–animal interactions, changing the occurrence of zoonotic diseases and the risk of infection to humans. For example, emergence of new influenza strains is related to the interaction of populations of people, pigs, and aquatic birds.

Social Change

Increasing human populations place great demands on the public health and other government services, especially in developing countries where needs for zoonoses diagnosis, control, and prevention are greatest and resources are most limited. Some preventive measures could be implemented by the people who live in the affected areas themselves, and at minimal costs, if they knew why and how they needed to do it. Public education and information is essential for control and prevention of zoonotic diseases; however, it takes more than civic action to deal with them. Delivery of public education, disease surveillance and diagnosis, and the technical materials and logistical support for control or preventive programs depend on national or international scientific and financial support. International technical cooperation and financial support are imperative.

Summary

Zoonoses are diseases transmissible from animals, other than humans, to people. Both new and old viral zoonoses are important in emerging and re-emerging virus diseases. Some zoonotic viruses occur worldwide, in a variety of ecological settings. Others are found only in limited ecologic and geographic foci. Important worldwide zoonotic viruses include rabies, hantaviruses, arenaviruses, yellow fever virus, chikungunya virus, Sindbis virus, Crimean-Congo hemorrhagic fever virus, and the sandfly fever viruses. In the Americas, common zoonotic viruses include the encephalitis viruses, Colorado tick fever virus, vesicular stomatitis, and others. Zoonotic viruses in Europe include Tahyna virus, the tick-borne encephalitis viruses, and cowpox virus. There are several zoonotic viruses in Africa and the Middle East, including Rift Valley fever virus, Marburg and Ebola filoviruses, monkeypox virus, Semliki Forest virus, Orungo virus, and Alkhurma virus. Zoonotic viruses occurring in Asia include the influenza viruses, SARS coronavirus, Kyasanur Forest virus, Japanese encephalitis virus, Chandipura virus, and Nipah virus. Zoonotic viruses occurring in Australia include Murray Valley encephalitis virus, Kunjin virus, Ross River virus, and Barmah virus. The human and animal health importance of these viruses and their control are discussed. Because rapid ecological change is occurring worldwide, additional zoonotic viruses will emerge.

See also: Bunyaviruses: General Features; Emerging and Reemerging Virus Diseases of Vertebrates; Fish Rhabdoviruses; Hantaviruses; Lassa, Junin, Machupo and Guanarito Viruses; Marburg Virus.

Further Reading

Daszek P, Cunningham AA, and Hyatt AD (2000) Emerging infectious diseases of wildlife – Threats to biodiversity and human health. *Science* 287: 443–449.

Gritsun TT, Nuttall PA, and Gould EA (2003) Tick-borne flaviviruses. *Advances in Virus Research* 61: 317–371.

Karesh WB, Cook RA, and Newcomb J (2005) Wildlife trade and global disease emergence. *Emerging Infectious Diseases* 11: 1000–1002.

World Health Organization. *Avian Influenza: Assessing the Pandemic Threat.* Geneva: WHO/CDS/2005.29.

TAXONOMIC INDEX

A

Adenoviridae **1:1–9**, **1:9–16**, **1:17–23**, 2:120
Alfamovirus **3:46–56**
Allexivirus **1:96–98**
Alloherpesviridae 2:431
Alphacryptovirus **1:98–104**
Alphaherpesvirinae 1:363, 2:405–406, 2:431, **4:581–585**
Alphavirus **5:96–101**, 5:107
Amdovirus 4:95
Anellovirus **1:104–111**
Aphthovirus **2:265–274**
Aquabirnaviridae 3:83–84
Aquareovirus **1:163–169**
Arteriviridae **1:176–186**
Ascoviridae **1:186–193**
Ascovirus 1:188*t*
Asfarviridae 1:50
Astroviridae **1:204–210**, 2:121
Astrovirus 1:205
Atadenovirus 2:120
Aviadenovirus 1:27
Avsunviroidae 5:335
Avulavirus 4:41*t*

B

Baculoviridae **1:211–219**, **1:219–225**, **1:247–254**, **1:254–265**, 1:265, 3:497–498
Barnaviridae **1:286–288**
Benyvirus **1:308–314**
Betacryptovirus **1:98–104**
Betaherpesvirinae 1:635–636, 2:431
Birnaviridae 1:321
Bocavirus 4:95
Bornaviridae **1:341–347**, 3:324
Bornavirus 1:341
Brevidensovirus 4:83, 4:96
Bromoviridae **1:386–390**, 2:255
Bunyaviridae 1:391, **1:399–401**, 3:479

C

Caliciviridae **1:410–419**, 2:117
Capillovirus 1:420
Capripoxvirus **1:427–432**
Cardiovirus **1:440–448**
Carlavirus **1:448–453**
Carmovirus **1:453–457**
Caudovirales 5:11*t*
Caulimoviridae **1:457–464**, **1:464–469**
Caulimovirus 1:464
Chloriridovirus 3:155
Chordopoxvirinae 4:323
Chrysoviridae **1:503–513**
Chrysovirus 1:503–504
Circoviridae **1:513–520**
Closteroviridae 2:255
Coltivirus **1:533–541**, 4:536
Comoviridae 1:569
Coronaviridae **1:549–554**, **1:554–563**, 2:122, 3:419–420, 5:151–152
Corticoviridae 3:1
Coxsackievirus **1:580–587**
Cucumovirus **1:614–620**
Curtovirus **1:301–307**
Cystoviridae 3:6
Cytomegalovirus **1:624–634**, **1:634–642**, **2:474–485**

D

Deltavirus 2:375
Densovirinae 4:76, 4:96
Densovirus 4:96
Dependovirus 4:93
Dicistroviridae **2:37–44**
Dinovernavirus 3:134

E

Eholavirus 2:57
Endornavirus **2:109–116**
Enterovirus 2:65–66
Entomopoxvirinae **2:136–140**
Erythrovirus 4:95

F

Fijivirus 4:150
Filoviridae **2:198–205**, 3:324
Flaviviridae 2:6, **2:234–241**, **2:241–253**
Flavivirus 2:6, 2:242
Flexiviridae **2:253–259**
Foveavirus 1:422
Furovirus **2:291–296**
Fuselloviridae **2:296–300**

G

Gammaherpesvirinae 1:363, 2:431, **4:585–594**
Geminiviridae **2:97–105**, **4:164–170**
Giardiavirus **2:312–316**
Granulovirus **1:211–219**, 1:247, 1:265

H

Hantavirus **2:317–321**
Henipavirus **2:321–327**
Hepacivirus 2:249, 2:367
Hepadnaviridae **2:327–335**, **2:335–343**
Hepatovirus 2:343
Herpesviridae **1:362–368**, **2:205–212**, **2:383–397**, **2:405–411**, 2:431, **2:498–505**
Hordeivirus **2:459–467**
Hypoviridae 2:288, **2:580–585**

I

Ictalurivirus 2:431
Idaeovirus **3:37–42**
Iflavirus 3:42
Ilarvirus **3:46–56**
Influenzavirus A 3:96
Influenzavirus B 3:96
Influenzavirus C 3:96
Inoviridae 2:191
Inovirus 2:191, 3:117–118
Iridoviridae 1:50, **3:155–161**, **3:161–167**
Iridovirus 3:155, 3:167
Isavirus 3:96
Iteravirus 4:83, 4:96

L

Lentivirus 4:594, 4:603
Leporipoxvirus **3:225–231**
Levivirus **1:381–386**
Luteoviridae **3:231–238**
Lymphocystivirus 3:155

M

Machlomovirus **3:259–263**
Maculavirus 5:206
Marafivirus 5:205
Marnaviridae **3:280–285**
Mastadenovirus 1:26–27
Megalocystivirus 3:155
Metapneumovirus 4:41*t*
Metaviridae **3:301–311**
Mimiviridae **3:311–319**
Mimivirus 3:312
Mononegavirales **3:324–334**, 5:11*t*
Morbillivirus **3:285–292**, 4:41*t*
Mycoreovirus **3:378–383**
Myoviridae **3:30–37**

N

Nanoviridae **3:385–391**
Narnaviridae **3:392–398**
Narnavirus 3:392
Necrovirus **3:403–405**
Nepovirus **3:405–413**
Nidovirales **3:419–430**, 5:11*t*
Nodaviridae **3:430–438**
Norovirus 2:117, 3:439
Novirhabdovirus 2:221–222
Nucleopolyhedrovirus 1:247, 1:254, 1:265

O

Ophiovirus 3:447
Orbivirus **3:454–466**
Orthobunyavirus **3:479–483**
Orthomyxoviridae 3:96, **3:483–489**
Orthoreovirus 4:383
Oryzavirus 4:150
Ourmiavirus **3:500–501**

P

Papillomaviridae **4:8–18**, **4:26–34**, **4:34–40**
Paramyxoviridae 2:321, 3:285, 3:324, **4:40–47**, **4:52–57**
Paramyxovirinae 2:321
Parapoxvirus **4:57–63**
Parechovirus 2:122
Partitiviridae **4:63–68**, **4:68–76**
Partitivirus 4:63, 4:68–69
Parvoviridae **4:76–85**, **4:85–90**, **4:90–97**
Parvovirinae **4:90–97**
Parvovirus 4:92
Pecluvirus **4:97–103**
Pefudensovirus 4:96
Pestivirus 2:246
Phlebovirus 4:490–491
Phycodnaviridae **4:116–125**
Phytoreovirus 4:150
Picobirnaviridae 1:327
Picornaviridae 1:542, 2:122, **4:129–140**, 4:243
Pneumovirus **1:614–620**, 4:41*t*
Podoviridae 3:31
Polerovirus **3:231–238**
Poliovirus 4:243
Polydnaviridae **4:256–261**
Polyomaviridae 4:34–35, 4:271, 4:277
Pomovirus **4:282–287**
Pospiviroidae 5:335
Potexvirus **4:310–313**
Potyviridae **4:313–322**
Poxviridae 4:323
Pseudoviridae **4:352–357**

R

Ranavirus 3:155
Reoviridae 3:133–134, **4:149–156**, **4:382–390**
Respirovirus 4:41*t*, 4:47
Retroviridae **2:105–109**, 4:446, **4:459–467**

Rhabdoviridae **1:111–121**, **2:221–227**, 2:228, 3:324, **4:187–197**, **4:576–581**
Rhadinovirus 3:190
Rhinovirus 1:542, **4:467–475**
Roniviridae 3:419–420
Rotavirus 2:118
Rubivirus 5:107
Rubulavirus 4:19, 4:41*t*

S

Sadwavirus **4:523–526**
Sapovirus 3:439
Seadornavirus **4:535–546**
Sequiviridae **4:546–551**
Sequivirus **4:546–551**
Sobemovirus **4:644–652**
Suipoxvirus **3:225–231**

T

Tectiviridae 3:1
Tenuivirus **5:24–27**
Teschovirus 2:126–127
Tetraviridae **5:27–37**
Thogotovirus 3:96
Tobamovirus **5:68–72**
Tobravirus **5:72–76**
Togaviridae 1:174, 2:255, **5:76–83**, **5:91–96**, **5:107–116**
Tombusviridae 1:453, **5:145–151**
Torovirus **5:151–157**
Tospovirus **5:157–163**
Totiviridae **5:163–174**
Totivirus **5:163–174**
Trichovirus 1:424
Tymoviridae 2:255, **5:199–207**

U

Umbravirus **5:209–214**

V

Varicosavirus **5:263–268**
Vesiculovirus 1:497
Vitivirus 1:425

W

Waikavirus 4:547

Y

Yatapoxvirus **5:461–465**

SUBJECT INDEX

Notes

Cross-reference terms in italics are general cross-references, or refer to subentry terms within the main entry (the main entry is not repeated to save space). Readers are also advised to refer to the end of each article for additional cross-references - not all of these cross-references have been included in the index cross-references.

The index is arranged in set-out style with a maximum of three levels of heading. Major discussion of a subject is indicated by bold page numbers. Page numbers suffixed by T and F refer to Tables and Figures respectively. *vs.* indicates a comparison.

This index is in letter-by -letter order, whereby hyphens and spaces within index headings are ignored in the alphabetization. Prefixes and terms in parentheses are excluded from the initial alphabetization.

To save space in the index the following abbreviations have been used

CJD - Creutzfeldt–Jakob disease

CMV - cytomegalovirus

EBV - Epstein–Barr virus

HCMV - human cytomegalovirus

HHV - human herpesvirus

HIV - human immunodeficiency virus

HPV - human papillomaviruses

HSV - herpes simplex virus

HTLV - human T-cell leukemia viruses

KSHV - Kaposi's sarcoma-associated herpesvirus

RdRp - RNA-dependent RNA polymerase

RNP - ribonucleoprotein

SARS - severe acute respiratory syndrome

TMEV - Theiler's murine encephalomyelitis virus

For consistency within the index, the term "bacteriophage" has been used rather than the term "bacterial virus."

A

A_2 gene (bacteriophage Qβ), single gene-mediated host cell lysis 3:257–258
A151R polyprotein, African swine fever virus replication 1:46–47
A238L protein, African swine fever virus infection 1:47
A/Aichi/68 HA1 (influenza virus), antigenic drift 1:131–132
AalDNV *see* Aedes albopictus densovirus type 2 (AalDNV)
Abaca bunchy top virus (ABTV) 3:389*t*
Abacavir 2:510
Abiotic transmission, rice yellow mottle virus 4:488
Abomasal, definition 1:427
Abortion, equid herpesvirus 1 infection *see* Equid herpesvirus 1 infection
Abortive infection 4:110–111
Abortive transformation, murine polyomavirus infection 4:273
ABPV *see* Acute bee paralysis virus (ABPV)
A/B toxin(s), K28 virus toxin *vs.* 5:303–304
Abutilon mosaic virus (AbMV), DNA β satellite origin 1:320–321
AbV-1 *see* Agaricus bisporus virus 1 (AbV-1)
Abystoma tigrinum virus (ATV), genome sequencing 2:233
Ac32 gene, granuloviruses 1:217
Acanthamoeba polyphaga mimvirus 3:311–312
Acantholysis, definition 5:250
Acanthoma
  definition 3:319
  molluscum contagiosum virus infection 3:322
Acaricides, Crimean-Congo hemorrhagic fever prevention 1:602–603
Accessory protease(s) (PL^{pro}), nidoviruses, proteolytic processing 3:424
Accidental hosts, cowpox virus 1:577
AC genes, bean golden mosaic virus 1:298
Acherontia atropas virus (AaV) 5:28*t*
Acheta domesticus densovirus (AdDNV), infection 4:79
Acibenzolar-*S*-methyl, tomato spotted wilt virus management 5:137, 5:137*f*
Acidianus bottle-shaped virus (ABV) 1:590, 1:591*f*
Acidianus filamentous virus(es) (AFV), virion structure 1:589–590
  core 1:590
Acidianus filamentous virus 1 (AFV1) 1:593
Acidianus filamentous virus 2 (AFV2) 1:593
Acidianus two-tailed virus (ATVc)
  genome 1:593
  genome integration 1:591–592
    integrase 1:592–593
  host cell lysis 1:591
  virion morphology 1:587, 1:589*f*
    tail structure 1:587–589
Acid sphingomyelinase (ASM)
  definition 1:541
  rhinovirus infection prevention 1:548
ACLAME (A Classification of Genetic Mobile Elements) 5:357*t*
ACLSV *see* Apple chlorotic leaf spot virus (ACLSV)

AcMNPV *see* Autographa californica multiple nucleopolyhedrovirus (AcMNPV)
ACMV *see* African cassava mosaic virus (ACMV)
ACMVUG *see* African cassava mosaic virus (ACMV)
Acquired immunodeficiency syndrome (AIDS) *see* AIDS
Acquisition access period (AAP), mungbean yellow mosaic virus transmission 3:368
Acute bee paralysis virus (ABPV)
  host range 2:40
  IRES structure 2:41*f*
Acute coryza *see* Common cold
Acute flaccid paralysis (AFP) 2:130
Acute hemorrhagic conjunctivitis *see* Conjunctivitis
Acute infections
  cannulation model, Visna-Maedi virus infection 5:426
  persistent *vs.* 4:109
  see also specific viral infections
Acute respiratory disease (ARD) 2:554–555
Acute-T-cell lymphomas, Herpesvirus Ateles 4:586–587
Acyclovir 2:436–437, 2:557
  channel catfish virus treatment 2:211
  chronic suppressive therapy 1:145–146
  combination therapy 1:152
  EBV infection 2:165–166
  history 1:145–146
  HSV infection 2:394–395
    mode of action 2:394–395
    resistance 2:394–395
  Pacheco's disease 2:410
  selectivity 1:146
  varicella-zoster virus infection 5:255
    resistance 5:255
Ad5 E1A proteins 1:10*f*
Ad9, adenovirus malignant transformation 1:16
Ad12 E1A proteins 1:12–13
AD169 strain, HCMV 1:636, 2:486
Adansonian system, classification 5:19
Adaptation, bacteriophage population ecology 2:75–76
Adaptive immune system 3:60
  definition 1:121
  henipavirus infections 2:326
  hepatitis C 2:373
  persistent infection 4:112
  principal effector molecules 3:60–61
  T lymphocytes 3:78–79
  see also Cell-mediated immune response; *specific components*
Adaptive landscape, definition 4:359
Adaptor proteins, apoptosis 1:232
ADAR1 *see* Adenosine deaminase 1 (ADAR 1)
ADE *see* Antibody-dependent enhancement (ADE)
Adefovir 1:143*t*
  HAART-associated renal disease 1:56–57
  hepatitis B treatment 2:359, 2:365–366
    resistance development 2:359
Adelaide river virus (ARV) 4:184*t*
  genome 1:358*f*, 1:360
    α1 ORF 1:360
    structural protein genes 1:360
  transcription 1:360
    β gene 1:360
    β-L gene junction 1:360
Adeno-associated virus vectors 2:303
  dependovirus replication 4:93
  genome 4:93
    episomes 4:94–95
    recombinant genomes 4:94–95
  replication 2:303, 4:93, 4:94*f*
  structure 2:303
  see also Recombinant adeno-associated virus (rAAV)
Adenocarcinoma
  Lucke´ tumor herpesvirus 2:206
Adenosine deaminase 1 (ADAR 1)
  interferon-induced antiviral response 3:115–116
  murine polyomavirus 4:273
Adenosine kinase, beet curly top virus pathogenesis 1:306
Adenoviridae 1:1, 1:2*t*, 1:10*t*, 1:17, 1:24, 2:120, 5:11*t*
Adenovirus(es) **1:1–9**, **1:17–23**
  antigenic properties 1:5
    genus-specific antigens 1:5
    neutralization assays 1:5
  apoptosis inhibition 1:157–160, 1:158*t*
  biological properties 1:5
  bovine 1:26
  characteristics 2:117*t*
  co-evolution 1:1
  DNA replication 1:19, 1:21*f*
    origin of replication 1:20
    proteins associated 1:19–20
  early studies 2:457
  equine 1:26
  fiber genes 1:3, 1:6
    genome organization 1:3
  gene expression 1:19
  gene therapy vectors 2:303
    helper dependent vectors 2:303
    recombinant 2:303
    serotypes 2:303
  genome 1:3, 1:18
    fiber genes *see above*
    nucleotide composition 1:3, 1:4*f*
    splicing 1:3
  history 1:1, 1:17
  human *see* Human adenovirus(es)
  infection *see* Adenovirus infections
  morphology 2:554–555
  phylogeny 1:7*f*, 1:8
  proteins 1:4
  see also specific proteins
  replication 1:3, 4:409–410
    assembly 1:21
    DNA replication *see above*
    host cell attachment 1:18, 1:18*f*
    host cell entry 1:3–4, 1:18
    rate 1:22
    release 1:21
    transcription 1:3–4
    VAI RNA 1:3–4, 1:161
  serotypes 1:9
  simian 1:26
  stability 1:3, 1:25
  structure 5:391
  transmission 1:5
  vaccines/vaccination 1:29
  vectors 1:23, 2:303
    helper dependent vectors 2:303
    recombinant 2:303
    serotypes 2:303
  virions 1:3, 1:17, 2:78*f*
    capsid 1:17–18
    capsomers 1:3, 2:78
    electron microscopy 2:78, 2:85–86
    fiber unit 1:17–18
    penton base 1:17–18
    viral core 1:18
  see also specific viruses
Adenovirus(es), enteric (EAd) 2:120
  classification 2:120
  evolution 2:120
  genetics 2:120
  geographical distribution 2:120
  history 2:120
  infection
    clinical features 2:121
    diagnosis 2:120
    nonseasonal epidemics 2:121
    pathogenesis 2:121
  morphology 2:120*f*
  seasonal distribution 2:120
  see also specific viruses
Adenovirus death protein (ADP) 1:22
Adenovirus infections **1:24–30**
  acute hemorrhagic conjunctivitis 2:495
  cytopathic effects 1:22
  detection 1:28
  epidemic keratoconjunctivitis 2:495, 2:495*f*
  epidemiology 1:25
    HAdV-A 1:25
    HAdV-B1 1:25
    HAdV-B2 1:25
    HAdV-C 1:25
    HAdV-D 1:25
    HAdV-E 1:26
    HAdV-F 1:26
    tissue tropism 1:25
  epizootiology 1:26
    bovine 1:26
    canine 1:26
    equine 1:26
    frog 1:28
    ovine 1:26
    porcine 1:26
    reptilian 1:27
    simian 1:26
  history 1:24
  identification 1:28
  immune system evasion 1:22–23, 4:114*t*
  pathology 1:5, 1:22
  persistence 4:109*t*
  prevalence 1:25
  prevention 1:29
  simple follicular conjunctivitis 2:495
  therapy 1:29
  tissue tropism 1:22, 1:22*f*
  transmission 1:25
Adjunctive therapy, organ transplantation 3:471
Adjuvant(s) 3:68
  definition 1:69, 5:226
  HIV vaccine development *see* HIV vaccines/vaccination
Admin table(s), virus databases 5:352
Adoxophyes orana granulovirus (AdorGV) 1:212
ADP-ribose-1″-phosphatase (ADRP), nidoviruses 3:425
Adsorption, definition 5:365
Adsorption electron microscopy 2:19, 2:21*f*
Adult T-cell leukemia (ATLL) 2:561, 2:565, 5:198
  classification 2:565
    subtypes 2:565, 2:566*t*, 2:567*t*
  clinical features 2:561, 2:566, 2:567
    acute 2:561, 2:566
    chronic 2:561, 2:566
    hematopoietic precursor cell differentiation 2:567–568
    hypercalcemia 2:567–568
    onset 2:561
    skin lesions 2:568, 2:568*f*
    smoldering 2:561
  complications 2:567
    opportunistic infection 2:568, 2:569–570
    pulmonary 2:568
  cytological features 2:566, 2:567*f*
    cytogenetic abnormalities 2:566
    lymph node histology 2:566
  diagnosis 2:565, 2:566*t*
  epidemiology 2:564, 2:565
    incidence 2:565
  etiology 2:561
    carriers 2:561
  immuno-virological features 2:566
  pathogenesis 2:568, 2:569*f*
    leukemogenesis 2:561, 2:563
    tax protein 2:562, 2:568–569
  prognosis 2:569
  therapy 2:569
Adult T-cell lymphoma 2:565
  EBV associated 2:155
  HTLV-1 associated *see* Human T-cell leukemia virus 1 (HTLV-1)
Adventitious agents, vaccine safety 5:226, 5:228
Adverse event following immunization (AEFI) 5:226, 5:227–228, 5:230*t*, 5:231*t*, 5:232
  benefits 5:232, 5:232*t*
  classification 5:232
  definition 5:232
  evaluation 5:231
  public perceptions 5:230

vaccine safety assessment 5:232
benefits 5:232, 5:232*t*
public perceptions 5:230
Aedes
breeding cycle 5:473
definition 5:469
see also Mosquito(es)
Aedes aegypti
Chikungunya virus transmission 5:100
Dengue virus transmission 2:5, 2:7, 2:9–10
defective viruses 1:173
infection control, Aedes aegypti densovirus 3:128, 4:79
larval habitats 2:9–10
RNAi in disease control 3:153
O'nyong-nyong virus 3:154
Venezuelan equine encephalitic virus transmission 5:99–100, 5:104
yellow fever virus transmission 2:455
Aedes aegypti densovirus (AeDNV) 3:128, 4:79
Aedes albopictus
Dengue virus transmission 2:5, 2:7
eastern equine encephalitic virus transmission 5:103–104
Aedes albopictus densovirus type 2 (AalDNV) 4:83
virion structure 4:78
Aedes polynesiensis 2:5
Dengue virus transmission 2:7
Aedes pseudoscutellaris dinovernavirus (ApDNV-1) 3:135, 3:136, 3:136*f*
genome 3:139*t*, 3:140–141
infection 3:140
infectivity 3:140
protein functions 3:141, 3:141*t*
Aedes sollicitans, eastern equine encephalitic virus transmission 5:103–104
Aedes taeniorhynchus, Venezuelan equine encephalitic virus transmission 5:104
Aedes vexans arabiensis, Rift Valley fever virus transmission 4:491
AeMV *see* Arabis mosaic virus (ArMV)
Aerosol transmission
common cold 4:472
nepovirus transmission 3:411
SARS CoV 4:557
tobacco ringspot virus transmission 3:411
Aerosol vaccines, safety 5:229
Affinity maturation (immunoglobulins) 3:61
AFMoV *see* Angelonia flower mottle virus (AFMoV)
Africa
banana bunchy top virus 1:277
cotton leaf curl disease 1:563
African cassava mosaic disease **1:30–37**
control 1:34, 2:101
biological control 1:36
crop isolation 1:35
cultural methods 1:35
East African cassava mosaic virus infection 1:36
infected plant removal 1:35
whitefly resistance 1:36
economic importance 1:30, 1:34
yield losses 1:34
field-level epidemiology 1:33
future work 1:36
history 1:30
host responses 1:33
post-transcriptional gene silencing 1:33
infection mechanisms 1:33
pandemics 1:32, 2:100
crop yield losses 2:101
emergence mechanisms 2:104
epidemiology 2:101
geographical distribution 2:100, 2:100*f*
symptoms 2:101
transmission mechanisms 2:101
plant resistance 1:34
plant breeding 1:34–35
regional epidemiology 1:33
transgenic resistance 1:35
DNA interference 1:35
nonviral protein expression 1:35
RNA interference 1:35
viral protein expression 1:35
African cassava mosaic virus (ACMV) 1:31–32
classification 2:101
distribution 1:32*f*
epidemiology 2:101
infection *see* African cassava mosaic disease
regional epidemiology 1:33–34
transmission 2:104
Ugandan strain 2:100–101
epidemiology 2:101
geographical distribution 2:100–101
infection symptoms 2:101, 2:101*f*
transmission 2:101
virus-induced gene silencing 5:326
African green monkeys
simian immunodeficiency virus infection *see* Simian immunodeficiency virus infection
African green monkey cytomegaloviruses (AgmCMV)
genome 1:636–637
infectivity 1:639*f*
isolates 1:636
African green monkey kidney cells, SV40 infection 4:630
African horse sickness (AHS)
cardiac (subacute) form 1:40
macrolesions 1:41
clinical signs 1:40
cardiac (subacute) form 1:40
horse sickness fever 1:40
mixed form 1:40
pulmonary form 1:40
control 1:42
movement restriction 1:42
diagnosis 1:41
clinical signs 1:41–42
conformation 1:42
differential diagnosis 1:42
equine viral arteritis *vs.* 1:42
purpura hemorrhagica *vs.* 1:42
geographical distribution 1:37
historical aspects 1:37
macrolesions 1:41
cardiac (subacute) form 1:41
mixed form 1:41
pulmonary interlobular edema 1:41
microlesions 1:41
mixed form 1:40
mortality rate 3:458
as notifiable disease 1:37
pathogenesis 1:40
cardio-vascular system 1:40–41
lymphatic system 1:40–41
regional lymph nodes 1:40
viral organ accumulation 1:40
viremia 1:40
pathology 1:41
pulmonary form 1:40
treatment 1:42
African horse sickness virus (AHSV) **1:37–43**, 2:96
capsid 3:462–463
epidemiology 1:41
genome 1:37, 1:39*f*
heat resistance 1:38–40
history 3:457
host range 1:40, 3:457
infection *see* African horse sickness (AHS)
replication 1:39*f*
serotypes 1:37, 1:38
taxonomy 1:37
transmission 1:41, 3:457
Culicoides 1:41
virion 1:37, 1:38*f*
nonstructural protein 1 1:37, 1:39*f*
nonstructural protein 2 1:37
nonstructural protein 3 1:38, 1:39*f*
nonstructural protein 3a 1:38, 1:39*f*
VP1 1:37
VP3 1:37
VP4 1:37
VP6 1:37
VP7 trimers 1:37, 1:38*f*
African peanut clump virus *see* Peanut clump virus (PCV)
African swine fever virus (ASFV) **1:43–51**, 2:96
assembly 1:46
characteristics 1:171*t*
classification 1:50
distribution 1:43
genome
gene functions 1:49*t*
isolate diversity 1:43
structure 1:45, 1:48*f*, 1:170–172
history 1:43
infection *see below*
multigene families 1:48
replication 1:45, 1:46–47, 1:46*f*
A151R polyprotein 1:46–47
aggresomes 1:46–47
DNA replication 1:46
mRNA transcription 1:46
virus entry into macrophages 1:45
transmission 1:43, 1:44*f*
virus assembly 1:46
virus-encoded proteins 1:47, 1:49*t*
A238L protein 1:47
AP endonuclease 1:47
DNA replication and repair 1:47
immune system evasion 1:47
mRNA transcription 1:47
virus structure 1:45, 1:45*f*
capsid 1:45
stability 1:170–172
African swine fever virus infection
apoptosis 1:44–45, 1:48
characteristics 1:44
host range 1:43
immune evasion 1:47
A238L protein 1:47
CD2v protein 1:47–48
macrophages 1:47
NL-S protein 1:47–48
macrophages 1:44
immune system evasion 1:47
replication 1:44
virus entry 1:44, 1:45
pathogenesis 1:44
pigs 1:44
AFV *see* Acidianus filamentous virus(es) (AFV)
AG7088 1:548
Agalactia, definition 1:427
Agar double diffusion assays, plum pox virus strains/groups 4:239
Agaricus bisporus virus 1 (AbV-1) 1:510, 2:286–288
genome 1:510
virion structure 1:510
Agenerase 2:513
Ageratum yellow vein virus (AYVV)
βC1 1:318
begomovirus satellite identification 1:315
Age-relations
Japanese encephalitis 3:184
neural cell infection 1:473
St Louis encephalitis virus transmission 5:273
vector transmission, animal viruses 5:273
western equine encephalomyelitis virus transmission 5:273
zoster (shingles) 5:252, 5:252*f*
Aggresome(s), African swine fever virus replication 1:46–47
AgmCMV *see* African green monkey cytomegaloviruses (AgmCMV)
AgMNPV *see* Anticarsia gemmatalis multiple nucleopolyhedrovirus (AgMNPV)
Agnathans, immunoglobulin phylogeny 3:58, 3:60*f*
Agnoprotein (LP1), SV40 infection 4:634
AGO1, Argonaute proteins 3:151
AGO2, Argonaute proteins 3:151
Agonadal (AG) tissue, Hz-2V infection 3:144–145
Agraulis vanillae virus (AvV) 5:28*t*
Agriculture/farming
bovine enteroviruses 2:123–124
capripoxviruses 1:429

Agriculture/farming (*continued*)
classical swine fever 1:529–530
fish 2:207
hepatitis E virus infection 2:382
yellow fever virus epidemics 5:494
Agrobacterium tumefaciens-based expression system (T-DNA) 4:232*f*, 4:233
definition 5:375
Helicoverpa armigera stunt virus 5:36
heterooligomeric protein expression 4:233
plant-produced vaccines 5:224
platforms 4:237*f*
recombination 4:232*f*, 4:233
virus-induced gene silencing 5:377
limitations 5:379
Agroinfection 4:233
definition 3:263
Agroinfiltration 1:81
Agroinoculation
definition 1:457, 5:138
viroid transmission 5:335
Agropyron mosaic virus (AgMV) 1:490
AHF *see* Argentine hemorrhagic fever (AHF)
AHS *see* African horse sickness (AHS)
AHSV *see* African horse sickness virus (AHSV)
Aichi virus, gastroenteritis 2:122
AIDS
clinical features 4:599–600
definition 1:69
CD4+ T lymphocyte count 1:52
etiology 2:143–144
life expectancy 1:51–52
medical achievements 2:535*t*
ocular complications 2:496
classification of 2:496
pandemics 2:534
pathogenesis 2:534–535, 2:535*f*, 2:538*f*
related infections
HCMV infection 2:439
JCV reactivation 4:265–266
KSHV infection *see* Kaposi's sarcoma-associated herpesvirus (KSHV)
prevalence 2:505
vaccine development *see* HIV vaccines/vaccination
AIDSinfo database 5:357*t*
AIDSVAX 1:70
AILV *see* Artichoke Italian latent virus (AILV)
Aino virus, Akabane virus *vs.* 1:80
Airborne transmission 2:140, 2:145–146
AK602 2:507*t*
Akabane virus **1:76–81**, 1:391*t*, 3:480
Aino virus *vs.* 1:80
classification 1:76
epidemiology 1:78
genome 1:76
geographical distribution 1:76, 3:480–481
historical aspects 1:76, 3:480
taxonomy 1:76
tissue tropism 1:78
transmission 1:78
vaccines/vaccination 1:80
vectors 3:480
control 1:80
virion properties 1:76
stability 1:77
Akabane virus infection
arthrogryposis 1:77
epidemics 1:77–78
calves
clinical signs 1:77
pathogenesis 1:78
pathology 1:78
clinical signs 1:77
congenital defects 1:76, 1:77, 3:480–481
control 1:80
diagnosis 1:79
differential diagnosis 1:80
associated viruses 1:80
noninfectious causes 1:80
hydraencephaly 1:77
epidemics 1:77–78
fetal infection stages 1:78
pathology 1:78
immunocompetence 1:78
isolation 1:79
management strategies 1:80
pathogenesis 1:78
fetal infection stages 1:78
incubation period 1:78
pathology 1:78
prophylaxis 1:80
seasonal distribution 1:79
vertebrate hosts 1:77
Alcelaphine herpesvirus 1 (AIHV-1) 1:364*t*
species barriers 2:428
Alcephaline herpesvirus 1 (AIHV1) 1:364*t*
Alcephaline herpesvirus 2 (AIHV2) 1:364*t*
Aleutian mink disease virus (AMDV) 4:95
genome 4:94*f*, 4:95
infection 4:90, 4:95
virion structure 4:92*f*
Alfalfa cryptic virus (ACV), genome 1:99–100
Alfalfa mosaic virus (AMV) **1:81–87**, 5:63
Bacopa virus infections 4:215
characteristics 5:63*t*
classification 1:81
coat protein 1:81, 1:85
crystallization 1:83
dependent infectivity 3:47
eIF4G binding 1:84*f*, 1:85
monomer numbers 1:81–82
movement 1:85
RNA interaction 1:82–83, 1:85–86
RNA replication 1:84–85
RNA translation 1:83–84, 1:85
structure 1:81–82
control 1:86
coat protein transformation 1:86
reservoir hosts 1:86
virus-free seeds 1:86
encapsidation 1:85
epidemiology 1:86
genome 1:81, 1:82, 1:82*f*, 5:63–64
CP binding site 1:82–83
movement protein 1:82–83
replicase protein P1 1:82–83
replicase protein P2 1:82–83
historical aspects 1:81
infection *see below*
movement 1:85
coat protein 1:85
movement protein 1:85
promoter 3:54
replication 3:49
coat protein–RNA complex dissociation 3:54
reservoir hosts 1:86
RNA replication 1:84
coat protein 1:84–85
minus-strand synthesis 1:84
minus to positive strand switch 1:86
P1 protein 1:85
P2 protein 1:85
plus-strand synthesis 1:84–85
in tobacco 1:84
RNA translation 1:83, 1:84*f*
coat protein role 1:83–84
poly(A) binding protein (PABP) 1:83–84
structure 1:81, 1:82*f*, 5:391
β-barrel fold 5:381, 5:382*f*
bottom component 1:81–82
coat protein *see above*
middle component 1:81–82
top component *a* 1:81–82
top component *b* 1:81–82
taxonomy 1:81
transmission 1:86
aphid vector 1:86
seeds 1:86
Alfalfa mosaic virus (AMV) infection
economic significance 1:81
host range 1:81
Impatiens 4:215
petunia 4:213, 4:214*f*
potato 4:300*t*
tobacco *see below*
tobacco 5:63
symptoms 5:63
virus–host relationships 1:86
symptoms 1:86
Alfamovirus 1:81, 1:387, 3:46, 3:47
Algal blooms, termination 4:124
Algal virus(es) **1:87–95**, 1:88*t*
associated families 1:87
definition 1:87
double-stranded RNA viruses 1:90
ecological implications 1:94
phylogeny 1:93*f*
maximum likelihood trees 1:90*f*
ssDNA viruses 1:92
ssRNA viruses 1:87
see also specific viruses
Alignment algorithms (classification) 5:342
BLAST 5:343
global 5:343
local 5:343
multiple sequence 5:343
pairwise sequence comparison *see* Pairwise sequence comparison (PASC)
phylogenetic analysis 5:343
scoring schemes 5:342–343
Alkaliphile 5:411
Alkhurma virus 5:53
zoonoses 5:491
Allele(s) 4:177
dominant 4:177
recessive 4:178
Allelolevivirdae 3:21
Allexivirus **1:96–98**
genome 1:97, 1:97*f*
coat protein 1:97–98
open reading frames 1:97–98
subgenomic RNA 1:98
TGB protein 1:97–98
geographical distribution 1:98
history 1:96
replication 1:97
RNA polymerase 1:97–98
transmission 1:97
viral composition 1:96
nucleic acid 1:97
physical properties 1:97
physicochemical properties 1:97
proteins 1:97
viral structure 1:96, 1:97*f*
morphological types 1:96
Allexivirus 1:96, 1:96*t*, 1:98, 1:426*f*, 2:255
Alligator(s), West Nile virus infection 5:445–446
Allium virus X (AVX) 4:311*t*
Allograft transplantation, HCMV infection 2:483–484
Alloherpesviridae 2:431
Almpiwar Group, animal Rhabdoviruses 1:116, 1:119*t*
Almpiwar virus 4:184*t*
Alovudine 2:507*t*
Alphacryptovirus 1:98, 1:100*t*, 2:109–110
Alphacryptovirus(es) **1:98–104**, 1:100*t*
classification 1:99
evolutionary relationships 1:103
genome 1:100
history 1:98
molecular biology 1:100
replication 1:100
taxonomy 1:99
transmission 1:99, 1:102
virion properties 1:99
virus–host relationships 1:102
see also specific viruses
Alpha-dystroglycan (aDG), arenavirus cell attachment/entry 3:245
Alphaentomopoxvirus 4:323, 4:323*t*
Alphaentomopoxvirus 2:136
characteristics 2:137*t*
host range 4:323
structure 4:325

Alphaentomopoxvirus 4:323
Alphaherpesvirinae 1:364*t*, 2:383, 2:405–406, 2:431, 2:432*t*, 4:341, 4:583, 5:11*t*, 5:251
Alphaherpesvirus 2:432, 5:251
Alphaherpesvirus(es)
  avian viruses *see* Avian alphaherpesvirus(es)
  discovery history 2:420
  equine viruses 2:412
  genital disease 1:366*f*
  host range 1:365
  latent infections 2:392*f*
  replication 1:363
  simian *see* Simian alphaherpesvirus(es)
  transmission 1:365
  see also Bovine herpesvirus(es); Herpes simplex virus(es) (HSV); Varicella-zoster virus (VZV); *specific viruses*
Alphanodavirus 3:430
  capsids 3:432, 3:432*f*
    alpha protein structure 3:432
    promoters 3:432
    quaternary structure 3:432*f*, 3:433
  geographical distribution 3:431–432
  infection 3:437
    insects 3:126*t*
  RNA packaging 3:432, 3:433
    molecular determinants 3:433
    random cellular RNA 3:434
  RNA replication 3:435–436
  virion assembly 3:433
  virion maturation 3:433
Alphanodavirus 3:431
Alphapapillomavirus
  carcinoma associated 4:15
  evolution 4:12–13
Alphapapillomavirus 4:9, 4:35
Alpha protein structure, alphanodavirus capsids 3:432
Alpharetrovirus 4:460
Alpharetrovirus(es)
  assembly 2:518
  cell-derived sequences 4:461
  classification 4:460
  morphology 4:461–462
  taxonomy 4:446
  see also specific viruses
Alphavirus 2:230, 4:514, 5:83, 5:91–92, 5:96, 5:101, 5:107, 5:108–110, 5:109*t*, 5:117
Alphavirus(es) 5:77, 5:83, **5:96–101**, 5:97*t*, 5:108–110, 5:109*t*
  antigenic groups 5:83
  assembly 1:195, 1:197*f*
    capsid dimerization 1:196
    genome encapsidation 1:196
    glycoproteins 1:197
    nucleocapsid 1:196–197
    in vitro assembly system 1:196
  bioterrorism 5:408
  budding 1:200
  definition 5:107
  epidemiology 5:100
  evolution
    arbovirus relationships 1:174
    relationships 1:174, 5:96, 5:98*f*
    RNA sequences 5:110–111
  genetics 5:103
  genome 5:98, 5:119*f*
  geographical distribution 5:77, 5:84*f*
  history 5:96
  infection *see below*
  life cycle 1:195, 5:102–103
    E1 glycoprotein function 1:195
    E2 glycoprotein function 1:195
    E3 glycoprotein function 1:195
    nucleocapsid release 1:195
    replication *see below*
  propagation 5:87
  replication 5:98, 5:120*f*
    assembly 1:196
    budding 1:196, 5:121
    host cell attachment 5:98, 5:118
    host cell entry 5:118–119
    nonstructural proteins 5:98, 5:119, 5:120
    protein processing 5:99*f*
    replicase complex 5:119
    rubella virus *vs.* 5:121
    sites 5:112–113
    structural protein maturation 5:121
  replicon systems 5:121–122
  RNA recombination 4:377
  species 5:93*t*
  transmission 5:92, 5:99, 5:111
    mosquito-borne 5:99
    non-mosquito-borne 5:99
    transovarial 5:111
    zoonotic 5:111–112
  type species 5:91–92
    Sindbis virus 5:91–92
  virion structure 1:195, 1:196*f*, 5:98
    E1 glycoprotein 1:195–196
    E2 glycoprotein 1:195–196
    nucleocapsid 5:98
  see also specific viruses
Alphavirus infections
  animal models 5:85
  arthritis 5:85, 5:87*t*
    treatment 5:88
  associated diseases 5:117
  clinical manifestations 5:86*t*
  diagnosis 5:87, 5:115
  disease control 5:116
  hosts 5:111
  pathogenesis 5:115
  pathology
    cell morphological changes 5:103–104
    cytopathic effects 5:103–104
    disease patterns 5:96
    molecular changes 5:103–104
  treatment 5:88
  zoonoses 5:111–112
  see also specific viruses
Alstroemeria virus X (AlsVX) 4:311*t*
Alternanthera mosaic virus (AltMV) 4:311*t*
Alzheimer's disease
  HSV associated 2:391
  viral etiology 1:475
AMA infectious diseases database 5:357*t*
α-Amanitin, viroid replication 5:339
Amantadine
  history 1:145
  influenza treatment 2:556, 3:102
    resistant variants 1:148
Amasya cherry disease (ACD) 1:509–510
Amasya cherry disease associated chrysovirus (ACDACV) 1:504*t*, 1:505–506
Ambisense genome(s)
  definition 1:390, 1:482, 5:157
  tenuivirus 5:25–26, 5:25*f*
Amdovirus 4:95
Amdovirus(es) 4:95
  classification 4:92*t*
  genome 4:94*f*, 4:95
  see also specific viruses
AMDV *see* Aleutian mink disease virus (AMDV)
American oyster reovirus (13p2RV) 1:164*t*
AMEV *see* Amsacta moorei entomopoxvirus (AMEV)
Amidase(s), endolysin function 3:251
Amino acid sequences/sequencing
  aquareovirus genetic diversity 1:167
  barley yellow dwarf viruses 1:279–280
  EBV 2:158–159
  seadornavirus 4:541–542, 4:541*f*
Amino acid substitutions, eukaryotic initiation factor-4E- mediated resistance 4:183–184
Amino acid transport protein
  Mason–Pfizer monkey virus cell receptor 4:627
  Simian retrovirus D cell receptor 4:627
Aminoacyl-tRNA synthetase(s)
  definition 3:311
  mimvirus gene content 3:316
Aminothiazole derivatives 1:149
Ammonium molybdate 2:81
Amphibian herpesvirus(es) **2:205–212**
  classification 2:206
  distribution 2:207
  evolution 2:209
  genetics 2:209
    genome characteristics 2:210*t*
  growth properties 2:209
  history 2:206
  infection
    clinical features 2:207
    control 2:211
    immune response 2:211
    pathogenesis 2:207
    pathology 2:207
    prevention 2:211
  see also specific viruses
Amplicons
  definition 2:93
  gene therapy 2:304, 2:396
Amprenavir 2:513, 2:514*t*
Amsacta moorei entomopoxvirus (AMEV) 2:136, 2:137*f*
  DNA ligase 2:139
  genome 2:138
  poly(A) polymerase 2:139
AMV *see* Alfalfa mosaic virus (AMV)
Amyloid
  prion formation
    self-propagating 4:337
    transfection with 4:339
  structure 4:338*f*, 4:339
Amyotrophic lateral sclerosis
  echoviruses 2:69
  viral etiology 1:475
Analgesics 5:83
Analytical tools, virus databases 5:357*t*, 5:364
Anamnesis 3:319
Anaphase-promoting complex (APC) 1:11
Anaphylaxis 5:226
Anastomosis
  definition 2:580
  hypoviruses 2:584
Ancestral retrotransposons 4:436
Ancillary viral protein(s) 3:348, 3:349
Andean potato latent virus (APLV) 4:298*t*
Andean potato mottle virus (APMV) 4:298*t*
Anellovirus 1:105, 1:106
Anellovirus(es) **1:104–111**
  distribution 1:107
  epidemiology 1:107
    global distribution 1:107
    mixed infections 1:109
    non-human primate infection 1:109
    prevalence rates 1:107
    risk exposure 1:107
  genome 1:105, 1:106*f*
  history 1:105
  infection *see below*
  phylogeny 1:106, 1:108*f*
    open reading frame 1 1:107
    sequence homology 1:107, 1:109*f*
  replication 1:105
  small
    history 1:105
    phylogeny 1:106, 1:107, 1:109*f*
  transmission 1:107
  virion properties 1:105
  see also specific viruses
Anellovirus infections
  clinical significance 1:110
    diseases associated 1:110
    hepatitis 1:110
    viral load *vs.* host immune status 1:110
  detection approaches 1:109
  isolation from biological samples 1:109
  mixed 1:109
  viral load 1:109
Anemia
  definition 3:155
  equine infectious anemia virus infection 2:173

Angel fish aquareovirus (AFRV) 1:164*t*
Angelonia flower break virus (AnFBV) 4:217, 4:220*f*
Angelonia flower mottle virus (AFMoV)
  host ranges 4:208
  verbenaceae infection 4:218, 4:220*f*, 4:221*f*
Angelonia virus infections 4:215
  see also specific viruses
Angiotensin converting enzyme 2 (ACE-2), SARS CoV host cell binding 4:555–557
Angola, Marburg virus outbreak 2:200, 3:272, 3:275, 3:276*f*
Anguilla herpesvirus (AngHV-1) 2:233
Animal contact, human monkeypox virus infections 4:642
Animal domestication, arbovirus transmission 1:176
Animal importation, infection control 5:431
Animal model(s)
  defective interfering viruses 2:3
  Eastern equine encephalitic virus 5:79
  Ebola viruses infection 2:202–203
  equine encephalitis 5:103
  filovirus infection 2:202–203
  hepatitis C virus 2:370
  herpes simplex viruses latency 2:437
  Kaposi's sarcoma 3:193
  Marburg virus infection 2:202–203
  multiple sclerosis 5:43–44
  seadornavirus infections 4:545
  severe acute respiratory syndrome 4:558
  simian immunodeficiency virus *see* Simian immunodeficiency virus (SIV)
  togovirus infection 5:103
  transgenic plant vaccine production 5:221
  varicella-zoster virus infection 5:251
Animal reservoir(s)
  definition 2:377
  orthopoxviruses 1:579–580
Annihilator mutant, definition 1:231
Annotation *see* Database(s)
Anogenital warts *see* Human papillomavirus infections
ANS (1-anilo-8-naphthalene sulfonate) 5:35
Antagonistic epistasis 4:359
Antagonistic pleiotropy 4:359
Antennal gland 5:476
Anterograde transport, CNS invasion pathways 1:470
Antheraea eucalypti virus (AeV) 5:28*t*
Anthoxanthum latent blanching virus (ALBV) 2:459
Anthroponoses 5:270, 632:270
  Dengue viruses 5:270–271
Anthroponosis 5:268
Anti-apoptotic proteins
  adenovirus E1B-19K gene/protein 1:13*f*
  HCMV immune system evasion 1:637
Antibodies **3:56–70**
  antigen binding *see* Antibody–antigen binding
  antigenicity 1:138
  biological functions 3:62
  definition 3:78
  history 3:56, 3:57*t*
  immunoglobulin phylogeny 3:58
  inactivation, common cold 4:472–473
  infections
    henipavirus 2:326
    HSV 2:393
    mammalian reovirus 4:389
    murine CMV 1:630*t*, 1:631
    nervous system viruses 1:470
    persistent infection 4:112
    pseudorabies virus 4:349
    rotavirus 4:512
    togavirus 5:87–88
  mutational resistance 5:323–324
  neonatal immunization 2:53
  neutralizing *see* Neutralizing antibodies (NAbs)
  phage display function 2:198
  plant virus vectors, expression 3:64
  prevalence 2:343
  protective 3:64
  structure, complementarity-determining regions 1:138
  T-cells *vs.* 3:71
  therapeutic, Marburg virus infection 3:279
  vaccine development strategies 5:236–237
    AIDS *see* HIV vaccines/vaccination
  variation, HIV 1:132
  see also Immunoglobulin(s)
Antibody–antigen binding 1:138, 1:139
  antibody concentration 3:414
  antibody-dependent disease enhancement 3:66
  conserved epitope binding 3:417–418
  dissociation constant 3:414–415
  envelope glycoproteins 3:414–415
  infection neutralization *see* Infection neutralization
  occupancy 3:414
  see also Immune complexes
Antibody-dependent cellular cytotoxicity (ADCC), yellow fever 5:475–476
Antibody-dependent enhancement (ADE) 3:66
  disease association 3:66
Antibody phage display 2:448–449
  antibody selection 2:198
  bacteriophage M13 2:197–198
  biomedical applications 2:198
  potato virus Y 4:290
Anticarsia gemmatalis multiple nucleopolyhedrovirus (AgMNPV) 3:130–131
  limitations 3:131
Antigen(s) **1:137–142**
  antibody binding *see* Antibody–antigen binding; Immune complexes
  antigenicity, viral proteins 1:137
  associated antibodies 1:138
  epitopes 1:137
    continuous 1:138, 1:139*f*
    definition 3:413, 4:594
    detection, T-cells 3:73
    discontinuous 1:138, 1:139*f*, 1:141
    immune system evasion 3:77
    reactivity level 1:139, 3:73
    size 1:139
    structure 1:139
    see also B-cell epitope(s)
  exogenous 3:72–73
  histo-blood groups 3:444
  immune response 1:137, 3:62–64
  infection neutralization 3:414
  molecular parameters 1:140
  nonenveloped viruses, assembly 1:204
  processing 3:72
    interference 3:77
  recognition 3:65*f*
  variation *see* Antigenic variation;
  see also specific viruses
Antigenemia, definition 4:507
Antigenic drift
  definition 3:95, 3:483
  vaccine development 5:240
    drift rate 5:240
  see also specific viruses
Antigenic shift
  definition 3:95, 3:483
  vaccine development 5:240
  see also specific viruses
Antigenic variation **1:127–137**, 2:146–147
  definition 1:127
  manifestations 1:127
  mutational mechanisms 1:127
  vaccines 1:127–128
    development strategies 5:240
    human papilloma viruses 1:127–128
  viral serotypes 1:128
  see also specific viruses
Antigenome
  definition 2:375, 3:324, 4:52
  hepatitis delta virus replication 2:376*f*
  mononegavirus replication 3:330–331
Antigen presentation **1:121–126**, 3:72
  definition 3:70
  pathways 1:123
    MHC-I 1:123, 3:72
    MHC-II 1:124, 3:72
  viral subversion 1:125
  see also Antigen-presenting cells (APCs)
Antigen-presenting cells (APCs) 1:122, 1:122*f*, 1:620, 3:72
  definition 1:69
  maturation 1:123, 1:126
  types 1:122–123
  see also specific types
Anti-G protein human monoclonal antibodies 2:326
Anti-HBsAg antibodies 2:357
Antiholin(s)
  definition 3:248, 3:250
  S107 3:253
    holin S105 *vs.* 3:253–254
    lysis timing 3:253
  strategy diversity 3:255
Anti-poxvirus chemical agents 3:230
Antiretroviral therapy 1:143*t*, 1:146, 1:153, 1:353, **2:505–517**, 2:518
  combination therapy 1:151, 2:516
    acyclovir 1:152
    regimens 2:516
    see also Highly active antiretroviral therapies (HAART)
  in development 2:507*t*
  drug resistance 1:148–149
  entry inhibitors 2:506
  HAART *see* Highly active antiretroviral therapies (HAART)
  integrase inhibitors 2:507*t*, 2:510
  maturation inhibitors 2:516
    mode of action 2:516
  nucleoside analogs *see* Nucleoside analogs
  nucleotide analogs *see* Nucleotide analogs
  protease inhibitors *see* Protease inhibitors
  reverse transcriptase inhibitors 2:509
    non-nucleoside reverse transcriptase inhibitors *see* Non-nucleoside reverse transcriptase inhibitors (NNRTIs)
    nucleoside reverse transcriptase inhibitors *see* Nucleoside reverse transcriptase inhibitors (NRTIs)
  see also specific drugs
Antisense binding, mRNA 1:148
Antiviral drug(s) **1:142–154**, 1:143*t*
  action spectrum 1:147
    broad spectrum 1:147, 1:154
  aminothiazole derivatives 1:149
  combination therapy 1:151
  compound classes 1:148
    antisense binding, mRNA 1:148
    helicase inhibitors 1:149
    neuraminidase inhibitors 1:149
    polymerase inhibitors 1:149
    protease inhibitors 1:148
  compound toxicity 1:147
    long-term therapy 1:147
    nephrotoxicity 1:147
    tests 1:147
  development 1:146
    coronaviruses 1:561
  drug interactions 1:147
  drug resistance 1:150, 1:151
    genetic barrier 1:150
  history 1:145
    acyclovir *see* Acyclovir
    drug development 1:146
  mechanism of action 1:148*f*, 1:151
  mutational potential 1:150
  nonenveloped viruses, assembly 1:204
  organ transplantation 3:469–470
    adjunctive therapy 3:471
    drug toxicity 3:471
    duration 3:471
    post-transplant prophylaxis 3:471
    prophylaxis 3:471
  prodrugs 1:150
  selective activity index 1:146
    assays 1:146
    CC_{50} 1:146–147
    definition 1:146
    EC_{50} 1:146
    EC_{99} 1:146

IC_{50} 1:146
therapeutic ratio 1:147
target classes 1:148
niche targets 1:153
virus assembly 1:149
virus attachment 1:148
toxicity 1:147
tests 1:147
virus fitness 1:151
virus latency 1:152
see also specific drug classes
see also specific drugs
ANV *see* Avian nephritis virus (ANV)
APCs *see* Antigen-presenting cells (APCs)
ApDNV-1 *see* Aedes pseudoscutellaris dinovernavirus (ApDNV-1)
AP endonuclease, African swine fever virus 1:47
Aphid(s)
alfalfa mosaic virus transmission 1:86
banana bunchy top virus transmission 1:277
barley yellow dwarf virus transmission 1:279, 1:282–283
bean common mosaic virus transmission 1:291
bean common necrosis virus transmission 1:291
caulimovirus transmission 1:463
citrus tristeza virus transmission 1:521, 1:524
cucumber mosaic virus transmission 1:618, 1:619, 5:287–288
luteovirus transmission 3:235–236, 3:237
nanovirus transmission 3:386
papaya ringspot virus transmission 4:1, 4:4
plant rhabdovirus transmission 4:195
see also specific viruses
plum pox virus transmission 4:241
potato virus transmission 4:302
potato virus Y transmission 4:289, 4:293, 4:303
potyvirus transmission, proteins associated 4:320
sowthistle yellow vein virus transmission 4:195–196
umbravirus transmission 5:213
watermelon mosaic virus transmission 5:436
zucchini yellow mosaic virus transmission 5:436
Aphid transmission factor (ATF), caulimovirus transmission 1:465
Aphis gossypii, zucchini yellow mosaic virus transmission 5:436
Aphthovirus 2:266
Apipoxvirus 4:324*t*
Aplastic anemia 2:343
APOBEC3 (cytidine deaminases) 3:109
APOBEC3G gene/protein
evolution 2:471–472
HIV replication 2:521
hypermutation 2:471–472, 3:109
nonsynonymous polymorphisms 2:471–472
retrovirus infection resistance 2:471
APOBECs, definition 2:467
Apodemus (woodmice), cowpox virus hosts 1:577, 1:578
Apoptosis **1:154–162**, 1:156*f,* 1:232, 1:252
adaptor proteins 1:232
as antiviral response 1:236
cell culture 1:237
biochemical changes associated 1:155
biochemical pathways 1:232
caspase-activated DNase (CAD) 1:252
caspases 1:232*f*
cellular death pathways 1:155
cross-talk 1:155–157
DARK 1:232
death receptor signaling 1:157
FLIP proteins 1:160
viral caspase regulators 1:160
viral regulators 1:157
definition 1:104, 1:186, 1:231–232, 1:247, 2:534, 3:111, 5:116
DIAP1 1:232
DRONC 1:232
infections
adenoviruses 1:157–160
African swine fever virus 1:44–45, 1:48
ascovirus 1:191, 1:191*f*
HCMV 1:637
HSV 2:391–392
iridovirus 3:173
KSHV 3:200
mammalian reoviruses 4:387
nidovirus 3:429
pseudorabies virus 4:349, 4:350
white spot syndrome virus 5:457
yeast viruses 1:162
inhibition
adenoviruses 1:157–160
baculovirus *see* Baculovirus apoptosis inhibition
KSHV 3:200
IPNV induced 3:86
mitochondrial pathway, viral regulators 1:161
Bcl-2 family proteins 1:155–157, 1:156*f,* 1:161–162
morphological changes associated 1:155
persistent infection 4:113
regulation 1:232
regulatory viral RNAs 1:161
virus-encoded miRNA 1:161
RNA-binding proteins 1:161
pathogen recognition receptors 1:161
SINV glycoproteins 5:122
Apoptosome 1:231
Apoptotic bodies, definition 1:186, 3:111
Apple chlorotic leaf spot virus (ACLSV) 4:204
associated diseases 1:424, 4:205
associated species 1:424
genome 1:424
movement protein 1:424
open reading frames 1:424
hosts 1:424
particle structure 1:421*f*
Apple mosaic virus (ApMV) 4:205
Apple stem grooving virus (ASGV) 1:420*t,* 4:202*f,* 4:206
affected hosts 1:420
genome 1:420–421
coat protein 1:421
open reading frames 1:420–421
occurrence 1:420
particle structure 1:420, 1:421*f*
transmission 1:420
Apple stem pitting virus (ASPV) 4:205
genome 1:423
analysis 1:423*f,* 1:424
open reading frames 1:423
particle structure 1:421*f*
Apple topworking disease 1:422
Apricitabine 2:507*t,* 2:510
Apricot latent ringspot virus (ALRSV) 3:406*t*
Apricots, Sharka disease 4:239
Aptivus® 2:516
Apurinic/apyrimidinic
definition 4:436
distribution 4:442*t*
Aquabirnavirus 1:321–322, 1:322*t,* 2:232, 3:83, 3:84
Aquaculture, definition 4:560
Aquareovirus 1:163, 2:231, 4:536*t*
Aquareovirus(es) **1:163–169**
genetic diversity 1:164*t,* 1:167
amino acid sequences 1:167
genogroups 1:167
RNA electrophoresis 1:167
RNA–RNA hybridization 1:167
genome 1:165
open reading frame 1:165
sequencing 1:165, 2:232
geographical distribution 1:166
aquareovirus A 1:166
aquareovirus B 1:166
isolates 1:166
host range 1:166
morphogenesis 1:163
proteins 1:165
nonstructural proteins 1:165
NS1 1:165–166
NS2 1:165–166
NS4 1:165–166
structural proteins 1:165
VP1 1:165–166
VP2 1:165–166
VP3 1:165–166
VP4 1:165–166
VP5 1:165–166
VP6 1:165–166
VP7 1:165–166
replication 1:163
species 2:232*t*
isolation 1:169
type species 4:536*t*
taxonomy/classification 1:163, 1:164*t,* 1:169
antibody-based cross neutralization 1:163
RNA cross-hybridization 1:163
species demarcation criteria 1:163
transmission 1:167
virion 1:163, 1:165*f*
capsid 1:163
see also specific viruses
Aquareovirus A 1:164*t,* 1:166, 4:536*t*
Aquareovirus B 1:164*t,* 1:166
Aquareovirus C 1:164*t*
Aquareovirus D 1:164*t*
Aquareovirus E 1:164*t*
Arabidopsis thaliana
beet curly top virus 1:307
eIF4E-mediated resistance 4:183, 4:184–185
gene silencing 4:141, 4:142
Dicer-like proteins 4:145
natural *cis*-antisense transcript-derived siRNA pathway 4:142
RDR genes 5:326–327
RdRp 6 4:146–147
siRNA accumulation 4:145–146
infection resistance 1:456
HRT gene 4:174–175
RCY1 gene 4:175
transposons 3:309
Arabis mosaic virus (ArMV) 1:490, 3:406*t*
diseases 3:411
infectious DNA clones 3:408–409
satellites 3:411, 4:531
Aracacha virus B-oca strain 4:298*t*
Arbovirus(es) **1:170–176**
classification 1:170
definition 1:170, 1:399, 2:5, 3:454, 3:479, 4:490, 5:83, 5:101, 5:107, 5:268, 5:269
evolutionary relationships 1:173
alphaviruses *vs.* 1:174
flaviviruses *vs.* 1:173
genome 1:170
geographical distribution 1:170
infection 1:170, 1:175
in animals 1:175
CNS associated 1:175
encephalitis 1:474
febrile illness 1:175
hemorrhagic fever 1:175
neural cell infection 1:473
persistence mechanisms 1:397–398, 2:235
taxonomy 1:170, 1:171*t*
transmission cycles 1:170, 1:176
animal domestication 1:176
host feeding range 1:176
human reservoirs 1:176
virion composition 1:170
virion properties 1:170
see also specific viruses
Archaea
definition 3:472
lipid structure, related to evolution 1:400
origin of 3:473
Arenaviridae 3:203, 3:239, 3:247–248
Arenavirus(es) **3:203–212**, 3:204*t,* **3:243–248**
assembly/budding 3:247
matrix protein 3:247
Z protein 3:247
bioterrorism 5:408–409
cell attachment/entry 3:245
alpha-dystroglycan 3:245
GP-2 glycoprotein 3:245

Arenavirus(es) (*continued*)
classification 3:203, 3:204*t*
epidemiology 3:205
evolution 3:205, 3:240
gene products 3:244
see also specific proteins
genetics 3:205
B-cell epitope variation 3:205
genome 3:243
intergenic regions 3:243, 3:244, 3:244*f*
terminal nucleotide sequences 3:243
geographical distribution 3:204
glycoproteins 3:244
GP-1 3:244
GP-2 3:244
posttranslational processing 3:244
precursor 3:243
signal peptide 3:244
history 3:203
host range 3:204
infections *see below*
life cycle 3:245
L polymerase 3:244
RNA replication/transcription 3:245
sequence analysis 3:245
structure 3:244–245
molecular phylogeny 3:247
nucleoprotein 3:243, 3:244
RNA replication/transcription 3:245, 3:246
propagation 3:204, 3:205
RdRp 3:243
reverse genetics 3:247
RNA replication/transcription 3:245
genomic promoters 3:246–247
homotypic viral interference 3:246
L polymerase 3:245
nontemplated nucleotides 3:247
nucleoprotein 3:245, 3:246
regulation 3:246, 3:246*f*
Z protein 3:245–246
Z RING domain 3:245–246
seasonal distribution 3:204
tissue tropism 3:207
transmission 3:207
vaccines/vaccination 3:210
virion properties 3:203
genome 3:203–204
glycoprotein precursor gene 3:203–204
morphology 3:203–204
nuclear ribosomal protein 3:203–204
nucleoprotein gene 3:203–204
virion structure 3:244
Z protein 3:245
RNA replication/transcription 3:245–246
see also specific viruses
Arenavirus infections
clinical features 3:208
control 3:210
drug prophylaxis 3:211
rodents 3:210
therapy 3:211
immune response 3:210
Lassa fever *see* Lassa fever
pathogenesis 3:207
prevention 3:210
vaccines 3:210
rodents 3:204
coevolution 3:205
control 3:210
transmission 3:204, 3:206
zoonoses 2:95
hemorrhagic fever 5:486
hosts 5:486
transmission 5:486
see also specific diseases/disorders
Argasids, definition 2:234
Argas pusillus, Keterah virus 1:400
Argentine hemorrhagic fever (AHF)
clinical features 3:209
epidemiology 3:206
histopathology 3:209
pathogenesis 3:208
capillary leakage 3:208
hypovolemic shock 3:208
proteinuria 3:208
pathology 3:209
therapy 3:211
Arginine-glycine-aspartic acid (RGD) motif 2:67
Argonaute (AGO) proteins 3:151, 4:141, 4:143*t*
AGO1 3:151, 4:142
AGO2 3:151
PAZ domain 3:151
Arracacha virus A (AVA) 3:406*t*
diseases 3:411
Arteriviridae 1:177, 3:419–420, 5:11*t*, 5:152
Arterivirus 1:177
Arterivirus(es) **1:176–186**
classification 1:177
envelope protein 3:423
M protein 3:423
S protein 3:423
evolution 1:182
RNA recombination 1:182
serologic relationships 1:182
serologic variability 1:182
genetics 1:181
genome 1:177, 1:179, 1:179*f*, 3:424*f*
frameshifting 1:179–180
open reading frames 1:179–180
proteolytic processing 3:424
replicase 1:179–180, 3:423–424, 3:425*f*
transcription regulation sequences 1:179–180, 1:180*f*, 3:423–424, 3:428
geographical distribution 1:177
history 1:176
host range 1:177
infection 3:421
clinical features 1:183
control 1:185
histopathology 1:183
immune response 1:184
immune system evasion 1:185
pathogenicity 1:183
pathology 1:181, 1:183
prevention 1:185
tropism 1:181, 1:182
propagation 1:177
protein properties 1:180
nonstructural proteins 1:179–180, 1:179*f*, 1:181
nucleocapsid 1:180–181
pp1a 1:178*f*, 1:180
pp1ab 1:180
replication 1:180*f*, 1:181
cytoplasmic double-membrane vesicle formation 1:181
genome encapsidation 1:181
sites 1:181
subgenomic RNA production 1:180*f*, 1:181
taxonomy 1:177
transmission 1:182
virion properties 1:177
morphology 1:177–179
stability 1:177–179
virion structure 1:178*f*
capsid 1:179
envelope proteins 1:179, 3:423
M protein 1:180–181, 3:423
S protein 3:423
see also specific viruses
Arthralgia 1:399
Ross River virus infection 5:114
Arthritis
alphavirus infection *see* Alphavirus infections
caprine arthritis encephalitis virus infection 5:430
definition 5:83
rubella virus infection 4:518–519
Arthrogryposis
Akabane virus infections *see* Akabane virus
definition 1:76, 1:328
Arthropod parvovirus(es) *see* Parvovirus(es), arthropod
Arthropods, definition 2:234
Arthroponoses 5:270, 5:270*f*
Artichoke Aegean ringspot virus (AARSV) 3:406*t*
Artichoke curly dwarf virus (ACDV) 4:311*t*
Artichoke Italian latent virus (AILV) 3:406*t*
diseases 3:411
Artichoke yellow ringspot virus (AYRSV) 3:406*t*
tobacco disease 5:66
Artiodactyl(s) 1:440
ARV *see* Adelaide river virus (ARV)
ARV1 *(Rudivirus),* morphology 1:589, 1:589*f*
ASBVd *see* Avocado sunblotch viroid (ASBVd)
Ascites 1:37
Ascomycete(s) 2:574
Ascomycota 4:419
retrotransposons 4:423, 4:424*t*
Ascoviridae 1:186, 1:187, 1:188*t*, 5:11*t*
Ascovirus 1:188*t*
Ascovirus(es) **1:186–193**
ecology 1:189
evolution 1:192
genome 1:189, 1:190*f*
geographical distribution 1:187
history 1:187
host range 1:189
infection
cell biology 1:191
cytopathology 1:187*f*, 1:188*f*, 1:191
disease symptoms 1:191
insects 3:126*t*
pathogenesis 1:191
pathology 1:191
rate 1:189
tissue tropism 1:192
virion-containing vesicles 1:191–192
morphology 1:186
origin 1:192
replication 1:192
virion assembly 1:188*f*, 1:192
taxonomy 1:187, 1:188*t*
variants 1:187
transmission 1:186, 1:189
virion composition 1:189
lipid membranes 1:189
virion structure 1:188*f*, 1:189
see also specific viruses
Aseptic meningitis 4:389, 4:490
coxsackieviruses 1:585, 1:585*t*
disease surveillance 2:46
notifications 2:45
HIV infection 1:57
sandfly fever virus infection 5:488
Asexual organisms, species classification 5:403
Asexual sporulation 2:576–577
Asfarviridae 1:50, 1:170, 5:11*t*
Asfarvirus(es)
apoptosis inhibition 1:158*t*
see also specific viruses
Asfivirus, virion structure 1:170–172
ASFV *see* African swine fever virus (ASFV)
ASGV *see* Apple stem grooving virus (ASGV)
AsiA, bacteriophage transcription 5:177
Asialoglycoprotein receptor (ASGPR), hepatitis A virus infection 2:12
Asian macaque, SIV disease model 2:539–540, 4:599
Asian tiger mosquito 5:494
Asparagus virus 3 (AV-3) 4:311*t*
Aspergillus ochraceous virus (AoV), antigenic properties 4:65
ASPV *see* Apple stem pitting virus (ASPV)
Assemblin, HCMV capsid formation 2:487
Assembly
definition 4:406
origin of *see* Origin of assembly
Assembly protein, HCMV capsid formation 2:487
Asthma, rhinovirus infection 1:546
Astrocyte(s), JCV infection 4:281–282
Astroviridae 1:205, 2:121, 5:11*t*
Astrovirus 1:205

Astrovirus(es) **1:204–210**
  characteristics 2:117*t*
  classification 1:205, 2:121
    nomenclature 1:205
  detection 1:204–205
  epidemiology 1:205
    animals 1:206
    co-pathogens 1:206
    distribution 1:206
    infection rates 1:205
    serotypes 1:206
  evolution 1:208, 2:121
    interspecies transmission 1:208
    mutation rates 1:208
  genetics 2:121
  history 1:205, 2:121
  host range 2:121
  infections *see below*
  molecular biology 1:207
    cell entry 1:207
    genome organization 1:207*f*
    open reading frames 1:207
    RdRp, expression 1:207–208
  morphology 1:204–205, 1:205*f*, 2:121*f*
  propagation 1:206
  protein expression 1:207
  stability 1:205, 1:210
  transmission 1:205
  see also specific viruses
Astrovirus infections 1:204–205
  clinical features
    avian 1:209
    mammalian 1:208
  control 1:210
  immune response 1:209
    cellular 1:209
    humoral 1:209
    turkey model 1:209–210
  pathogenesis 2:121
    avian 1:209
    experimental models 2:121
    mammalian 1:208
  pathology
    avian 1:209
    mammalian 1:208
  prevalence 1:204–205, 2:121
  prevention 1:210
  treatment 1:210
ASV database 5:357*t*
Aswan Dam project, Rift Valley fever virus epidemic 4:494
Atadenovirus 1:1, 1:2*t*, 1:17, 1:26–27, 1:27–28, 2:120
Atadenovirus(es) 1:6
  bovine 1:8
  genome
    LH region 1:8
    nucleotide composition 1:6–8
    organization 1:18
    RH region 1:8
  host range 1:6–8
  host switches 1:5, 1:8–9
  nomenclature 1:1
  phylogeny 1:7*f*, 1:8
  see also specific viruses
Atazanavir 2:513, 2:514*t*
Ateline herpesvirus 2 *see* Herpesvirus Ateles (HVA)
Ateline herpesvirus 3 *see* Herpesvirus Ateles (HVA)
Atherosclerosis, rat cytomegalovirus 1:633
Atkinsonella hypxylon virus (AhV-1) 4:69, 4:71*f*
Atlantic salmon aquareovirus (ASRV) 1:164*t*
Atlantic salmon paramyxovirus (ASPV) 2:229
ATLL *see* Adult T-cell leukemia (ATLL)
ATPases
  dsDNA bacteriophage genome packaging 2:310–311
  P-loop, Crenarchaeal viruses 1:594
ATP-dependent transporters-associated antigen - processing (TAP) 1:124
ATP-DNA complex, dsDNA bacteriophage genome packaging 2:309–310
Attachment
  baculoviruses 1:250
  definition 4:407
  see also specific viruses
Attachment glycoproteins, henipavirus 2:322–323
Attenuated virus cross-protection, Sharka disease control 4:242
Attenuation (vaccines) *see* Live-attenuated vaccines
A-type inclusions (ATIs)
  avipoxvirus 2:275
  cowpox virus *see* Cowpox virus (CPXV)
Aubian wheat mosaic virus (AWMV) 1:490
Aujeszky's disease (AD) 4:341, 4:349
  immunology 4:349
  see also Pseudorabies virus (PrV)
Aum Shinrikyo, bioterrorism 5:407
Aura virus (AURAV) 5:109*t*
Aureusvirus 5:145–146
Australia
  banana bunchy top virus epidemics 1:277
  Hendra virus 2:324
  rabbit hemorrhagic fever virus 1:411
Australian antigen *see* Hepatitis B surface antigen (HBsAg)
Australian bat lyssavirus 4:184*t*
Autism, vaccines safety 5:233
Autoantibodies
  EBV infection, response to 2:153
  see also specific antibodies
Auto-disable syringe
  definition 5:226
  vaccine safety 5:227, 5:229
Autographa californica multiple nucleopolyhedrovirus (AcMNPV) 1:238, 1:247
  apoptosis inhibition 1:233*f*
  BV envelope composition 1:263
  DNA replication 1:250
  evolution 2:182
  expression vector function 3:131, 3:132
  genome 1:256, 1:259*f*
    homologous repeat regions 1:218, 1:256–257
  host range 3:129
  host range determinants 1:252–253
  ODV envelope composition 1:263
  replication cycle 1:239*f*
    budded virus 1:238
    occlusion-derived virus 1:238
  virion structure 1:223
Autoimmunity, Visna-Maedi virus infection 5:430
Autolysis 3:249
Autonomous retrotransposons 4:436
Autophagy 5:378
  MHC-II antigen presentation 1:125
AVA *see* Arracacha virus A (AVA)
Avenavirus 5:145–146
Aviadenovirus 1:1, 1:2*t*, 1:17, 1:27, 2:120
Aviadenovirus(es) 1:6
  disease patterns 1:6
  fiber genes 1:6
  genome 1:6, 1:18
    dUTP pyrophosphatase 1:6
  host range 1:6
  see also specific viruses
Avian adenovirus(es) 1:27
  infections 1:27
    fiber genes 1:6
    GAM-1 protein 1:6
  see also specific viruses
Avian alphaherpesvirus(es) 2:405–406
  genetics 2:408
  see also specific viruses
Avian hepadnavirus(es) **2:327–335**
  classification 2:327
  genome 2:329
  history 2:334
  host range 2:329
  infection 2:341
    disease control 2:332
    hepatocytes 2:327, 2:329, 2:330*f*, 2:332, 2:334*f*
    pathogenesis 2:332
    sites 2:327
    tissue tropism 2:329, 2:334*f*
  phylogeny 2:327, 2:332, 2:333*f*
    viruses associated 2:332
  replication 2:327, 2:329
    budding 2:330*f*, 2:331
    cccDNA 2:327, 2:329–330, 2:331
    DNA synthesis 2:330
    DR1 2:330–331
    DR1* 2:330–331
    DR2 2:330–331
    genome integration 2:331–332
    nuclear transport 2:331
    pregenome packaging 2:330, 2:331*f*
    receptor binding 2:329
    sites 2:327, 2:329
    transcription 2:328*f*, 2:330, 2:330*f*
  structure 2:328
  transmission 2:327
  see also specific viruses
Avian hepatitis E virus 2:377–378, 2:378*f*, 2:380
  hepatitis–splenomegaly syndrome 2:380
Avian herpesvirus(es) **2:405–411**
  genetics 2:408
  host range 2:406, 2:407
  infections
    control 2:410
    pathobiology 2:409
    prevention 2:410
  morphology 2:408
  phylogeny 2:407*f*
  virus propagation 2:407
  see also specific viruses
Avian influenza virus(es)
  classification 3:101
  evolution 3:98–99
  H5N1 pandemic 3:100, 3:493, 5:491
  transmission 3:96
  see also specific viruses
Avian leukemia virus 4:466–467
Avian leukosis virus(es) (ALV)
  classification 4:455
  infection
    host range 4:455
    oncogenes/oncogenicity 4:457
  replication 4:456
  see also specific viruses
Avian metapneumovirus 4:45
  genome 3:327*f*
Avian nephritis virus (ANV) 1:209
  pathogenesis 1:209
Avian poxvirus(es) 4:326–327
  see also specific viruses
Avian reovirus(es) 4:383
  see also specific viruses
Avian retrovirus(es) **4:455–459**
  cellular transformation 4:457
  classification 4:455
  distribution 4:455
  endogenous viruses 4:457
  evolution 4:458
  genetics 4:457
  genome 4:455
    modifications 4:455–456
    noncoding regions 4:456
    oncogenes 4:455–456
  glycoproteins 4:456
  history 4:455
  host range 4:455
  infections
    control 4:458
    immune response 4:458
    oncogenes/oncogenicity 4:448
    pathogenicity 4:458
    prevention 4:458
  physical properties 4:456
  propagation 4:455
  protein properties 4:456
    Env precursor 4:456
    Gag-Pro precursor 4:456

Avian retrovirus(es) (*continued*)
glycoproteins 4:456
Pol reading frame 4:456
replication 4:456
particle release 4:457
provirus transcription 4:457
receptors 4:456
reverse transcription 4:456–457
translation 4:457
viral DNA integration 4:457
taxonomy 4:455
tissue tropism 4:458
transmission 4:458
virion properties 4:455
morphology 4:455
see also specific viruses
Avihirnavirus 1:322*t*, 2:232
Avihepadnavirus 2:327, 2:351–352
Avipoxvirus 2:274
Avipoxvirus(es) **2:274–284**
antigens 2:275
classification 4:324*t*
epizootiology 2:278
extracellular enveloped virus 2:276–277, 2:277*f*
transmission 2:279
gene expression 2:275
protein homologs 2:275
genome 2:275
infection *see below*
intracellular mature virus 2:276–277, 2:277*f*
transmission 2:279
isolation 2:279
molecular phylogenetics 2:277, 2:278*f*
molluscum contagiosum virus *vs.* 2:276
replication 2:276
genetic reactivation 2:276
host range restriction 2:276
lipid metabolism 2:277
virion morphogenesis 2:276
survival factors 2:275
A-type inclusion protein 2:275
glutathione peroxidase 2:275
immunomodulators 2:276
photolyases 2:275
taxonomy 2:274
transmission 2:279
vaccines/vaccination
infection prevention 2:281
recombinant vaccines 2:283
virion structure 2:274
virus propagation 2:277
see also specific viruses
Avipoxvirus infections
clinical manifestations 2:279
control 2:281
detection 2:279
inter-strain differentiation 2:281
intra-species differentiation 2:281
monoclonal antibodies 2:280
PCR-based analysis 2:280
restriction enzyme digestion 2:280
serology 2:279
Southern blotting 2:280
diagnosis 2:279
histology 2:279
host range 2:278
prevention 2:281
vaccination 2:281, 2:283
reintroduction programs, birds 2:279
treatment 2:283
variants 2:283
antigenic 2:283
integrated reticuloendotheliosis sequences 2:283
tumorigenic 2:283
Avirulence genes *(Avr)*
definition 4:170
plant natural resistance 4:171, 4:172*t*, 4:173
mutation 4:173
recessive resistance 4:178
recognition evasion 4:176
Avirulence proteins
definition 4:173
recognition 4:175
Avirulent strains, cacao swollen shoot virus infection 1:403
Avocado sunblotch viroid (ASBVd)
host range 5:335
replication 5:339, 5:340*f*
Avsunviroidae 5:11*t*, 5:333*t*, 5:335
Avsunviroids
monomer circularization mechanism 4:479–480
pathogenicity determinants 5:339–341
replication 4:476, 4:478*f*
see also specific viruses
Avulavirus 4:41*t*
Avulavirus(es)
classification 4:41*t*
genome 3:327*f*
see also specific viruses
Axoplasmic flow, neural cell infection 1:473
AYRSV *see* Artichoke yellow ringspot virus (AYRSV)
AYVV *see* Ageratum yellow vein virus (AYVV)
5′Azacytidine, EBV infection control 2:155–156
AZT *see* Zidovudine (AZT)

B

B2 protein, RNAi suppression 3:152
B5 antigen, VACV 5:249–250
B10 virus 5:413*t*
B119L polyprotein 1:46–47
B602L protein 1:46–47
Baboon cytomegalovirus (BaCMV) 1:635*t*, 1:639*f*
Baboon reovirus (BRV) 4:383
Babuvirus
characteristics 3:389*t*
genome 3:387*f*
Babuvirus 1:274, 3:385
BAC *see* Bacterial artificial chromosome (BAC)
Bacilliform particles 5:391
Bacillus anthracis, bioterrorism 5:406, 5:408*t*
Bacillus subtilis, associated phages 4:401, 4:402–403
Back mutations
definition 4:359
quasispecies 4:361–362
BacMam *see* Baculovirus expression vector(s)
Bacmid shuttle baculovirus expression vectors, Bac-to-Bac 1:241
Bacopa virus infections 4:215, 4:218*f*
see also specific viruses
BacPAK6 DNA 1:240, 1:240*f*
Bacteria
detection, bacteriophage use 2:448–449
filamentous phage pathogens 3:123
superinfection, molluscum contagiosum virus 3:322
Bacterial artificial chromosome (BAC)
definition 1:237
*flash*BAC expression vector system 1:244
varicella-zoster virus 5:256
Bacterial cell wall
carbohydrates 5:366
definition 5:365
penetration *see* Bacteriophage(s), bacterial cell entry
Bacterial viruses *see* Bacteriophage(s)
Bacteriophage(s) **2:442–450**
capsid assembly *see* Bacteriophage(s), capsid assembly
characteristics 2:444–445
classification 5:342, 5:347
'bacteriophage proteomic tree' 5:343
coat protein
folding 1:436
procapsid maturation 1:436, 1:437*f*
conversion 2:445–446
definition 2:442, 5:365
double stranded DNA *see* Double stranded DNA (dsDNA) bacteriophages
double stranded RNA *see* Double stranded RNA (dsRNA) bacteriophages
dsDNA *see* Double stranded DNA (dsDNA) bacteriophages
dsRNA *see* Double stranded RNA (dsRNA) bacteriophages
ecology *see* Bacteriophage(s), ecology
evolution **5:370–375**
capsid proteins 5:374
co-infections 5:371
evolutionary connections 5:374
genome comparisons 5:372
genome organization 5:371
host cell co-evolution 5:374–375
mechanisms 5:371, 5:372
filamentous ssDNA *see* Filamentous ssDNA bacteriophages
genome packaging **2:306–312**, 2:307*t*
capsid assembly 2:306
genome replication 2:306
genome *vs.* chromosome 2:307
nucleotide selection 2:307
packaging motor complex 2:307
precursor capsid assembly 2:307
historical aspects 3:21
host cell infection *see* Bacteriophage(s), bacterial cell entry
host cell lysis *see* Bacteriophage(s), host cell lysis
host cell receptors 4:407–408
icosahedral dsDNA *see* Icosahedral dsDNA bacteriophages
icosahedral ssDNA *see* Icosahedral ssDNA bacteriophages
library 2:190
lysogeny 5:181
Lwoff, Andre´ experiments 2:445
research history 2:445
in molecular biology 3:21–22
nomenclature 2:442–443
nonhomologous recombination 5:371–372, 5:372–374, 5:373*f*
genome comparisons 5:372
horizontal transfer 5:372
novel gene acquisition 5:374
novel sequence joints 5:372
populations 5:371
prevalence 2:444
productive infections 5:371
recombination 4:374
replication **4:399–406**
research history 2:443, 2:447*t*
applications 2:448, 2:449*t*
bacteriophage therapy 2:444, 2:449*t*
bacteriophage typing 2:444
classification 2:446
discovery 2:443, 2:444*t*, 2:445*t*
diversification 2:446
ecology 2:444, 2:449
genome properties 2:446
genome sequencing 2:446, 2:448*t*
lysogeny 2:445
molecular biology tools 2:444
viral nature 2:444
'single-gene lysis' 3:256
gene A_2 3:257
gene E 3:256–257
gene L 3:258
single-stranded RNA *see* Single-stranded RNA bacteriophages
ssRNA *see* Single-stranded RNA bacteriophages
structure 2:448*f*
T4-even phages 4:401
T4-like phages *see* T4-like bacteriophages
T7-like phages *see* T7-like bacteriophages
tailed *see* Caudovirus(es); Tailed bacteriophages
therapy 2:444, 2:448–449, 2:449*t*
transcription regulation **5:174–186**
AsiA 5:177
elongation 5:175–176
host RNAP-mediated elongation 5:175
initiation 5:175–176
isomerization 5:175–176
promoter binding 5:175
RNAP structure 5:175
see also specific bacteriophages

typing 2:444, 2:446
bacteriophage research history 2:444
definition 2:442
see also specific types
Bacteriophage(s), bacterial cell entry **5:365–370**
cell wall penetration 5:367
lysozymes 5:367
lytic transglycosylases 5:367
peptidoglycan-hydrolyzing enzymes 5:366*f*, 5:367
pre-existing channels 5:366*f*, 5:367
energetics 5:370
host cell activity 5:370
sources 5:370
genome delivery 5:367
icosahedral vertex 5:367
virion dissociation 5:367
virion penetration 5:367
see also specific viruses
host cell barriers 5:365
eubacteria 5:365
Gram-negative bacteria 5:365
Gram-positive bacteria 5:365
host recognition 5:365, 5:366*f*
bacterial pilus 5:366
cell wall carbohydrates 5:366
flagellar proteins 5:366
lipopolysaccharides 5:366
receptor-binding proteins 5:365–366
virion structure 5:365
viral genome effects 5:365
Bacteriophage(s), capsid assembly **1:432–440**, 1:434*f*, 2:306
assembly pathways 1:434
protein conformational changes 1:434–435
catalysis 1:438
chaperonin assembly 1:438
enzyme assembly 1:438
jigs 1:438
scaffolds 1:438
templates 1:438
covalent protein modifications 1:439
cross-linking 1:439
lipid membrane acquisition 1:439
proteolytic cleavage 1:439
history 1:433
pathways 1:434
strategies/mechanisms 1:435
branched pathways 1:435
genome types 1:434
nucleic acid encapsidation 1:439
quality control 1:435
subassemblies 1:435
symmetrical protein arrays 1:436
helical arrays 1:437
icosahedral array maturation 1:436, 1:437*f*
symmetry disruption 1:437
symmetry mismatches 1:437
types 1:435*t*
Bacteriophage(s), ecology **2:71–78**
bacteria-like *vs.* virion like existence 2:73
favorable growth conditions 2:73–74
infecting states 2:73–74
community ecology 2:76
bacterial physiological burdens 2:76
bacterial versatility 2:76
bacteriophage-mediated genetic transduction 2:76–77
bacteriophage-mediated population crashes 2:76
bacteriophage resistance 2:76
principles 2:76
ecosystem ecology 2:77
bacteriophage-induced bacterial lysis 2:77
nutritional movement impacts 2:77
existence within environments 2:72
bacteriophage environmental diversity 2:72
bacteriophage number determination 2:72, 2:72*f*
landscape ecology 2:73
organismal ecology 2:73
growth parameters 2:73
life cycle variations 2:73, 2:73*f*
spatially structured environments 2:73
population ecology 2:74
adaptation 2:75–76
bacterial density 2:75
bacteriophage decay rate 2:74–75
broth culture 2:74
burst size 2:75
competition 2:75
decay rate 2:74–75
environmental conditions 2:74
extracellular search 2:74–75
growth inhibition 2:75
selective pressures 2:75
Bacteriophage(s), host cell lysis **3:248–258**
antiholins 3:255
endolysins 3:251
extrusion 3:249
holin–endolysin lysis *see* Holin–endolysin lysis
lysis modes 3:250
progeny virion release 3:249
rotting *vs.* 3:249
'single-gene lysis' 3:256
gene A_2 3:257
gene E 3:257
gene L 3:258
Bacteriophage 21 3:254–255
Bacteriophage Bam35
genome 3:5
host range 3:3
Bacteriophage Bxbl 5:185
Bacteriophage Cp-1 4:402–403
Bacteriophage CTXΦ
genome 3:121
virulence factors 3:123
Bacteriophage ϕ29
evolution 5:374
genome size 3:36
replication 4:402
DNA polymerase 4:403, 4:404*f*
membrane-localized proteins 4:404
replication forks 4:403–404, 4:404*f*
sliding back mechanism 4:403
Bacteriophage fd 2:194
composition 2:193–194
genome packaging 2:193–194, 2:193*f*
pIII 2:194
pVIII
central region 2:194
composition 2:193–194, 2:193*f*
N-terminal region 2:194
pentapeptide motifs 2:194, 2:194*t*
structure 2:192*t*, 2:193–194
Bacteriophage Ff 3:117, 3:118*f*
assembly pathway 2:191
capsid helical symmetry 3:121
DNA conformation 3:121
DNA synthesis 2:196
host-encoded polymerases 2:196
pII 2:196
genome 2:192*f*, 3:120
genome packaging 2:191
infection 2:194
F pilus 2:195–196
Tol–Pal system 2:195–196, 2:195*f*
plaque formation 3:118
post-transcriptional modification 2:196
protein synthesis 2:196
host-encoded polymerases 2:196
pIII maturation 2:196
pIV 2:196–197
pVIII 2:196
structure 3:119–120, 3:120*f*
gp7/9 3:119–120
transcription 2:196
translation 2:196
virus assembly 2:197
pIV channel conformations 2:197
progeny elongation 2:197
pV-coated genome 2:197
sites 2:197
Bacteriophage fOg44 3:254–255
Bacteriophage HK022 5:185
Bacteriophage HK97
coat protein folding 1:436
cross-linking 1:439
evolution 5:374
procapsid maturation 1:436, 1:437*f*
evolution 5:372, 5:373*f*
phylogeny 3:36
structure 5:388, 5:389*f*
Bacteriophage ϕ6 3:6
structure 3:7*f*
Bacteriophage L2 1:439
Bacteriophage L5 5:185
Bacteriophage λ
BaculoDirect expression vector system *see* Baculovirus expression vector(s)
CII accumulation 5:183
genome 4:400–401
genome packaging 2:309, 2:309*f*
integrase host factor 2:309
holin–endolysin lysis 3:252
antiholin S107 3:253
cell energy metabolism 3:252
dual start motif 3:252*f*
holin S105 3:253
lambda holin S105 3:253
lysis cassette 3:252–253, 3:252*f*
promoter activation 3:252–253
Rz–Rz1 genes 3:254
life cycle 5:182*f*
lysis 5:181
lysogeny 5:181, 5:182, 5:182*f*
lytic growth 5:184
persistence 5:184
suppression mechanisms 5:185
terminator role 5:184
transcription regulation 5:184
promoters 5:182–183, 5:182*f*
P_{aQ} 5:183
P_{Int} 5:183
P_L 5:182–183, 5:184
P_R 4:400–401, 5:182–183
P_{RE} 5:182–183
prophage excision 5:182*f*, 5:184
Fis protein 5:184
Int protein 5:184
Xis protein 5:184
RecF recombination 4:374
regulatory strategies 5:181
replication 4:400–401
host proteins 4:400–401
protein O 4:400–401
site-specific prophage insertion 5:181–182
structure 3:30–31, 3:31*f*, 3:32
tail shaft assembly 1:438
tape measure protein 1:438
Bacteriophage M13
cloning vector function 2:197
genome 2:192, 2:192*f*
gene expression 2:192
intergenic region 2:192
phage display 2:197–198
Bacteriophage 'morons' 2:76
Bacteriophage Mu
genome 4:404–405
replication 4:404
DnaB 4:404–405
protein PriA 4:404–405
transposome formation 4:404–405
Bacteriophage N4 5:181
regulatory strategies 5:181
transcription
early genes 5:181
late genes 5:181
middle genes 5:181
promoters 5:181
RNAPII 5:181
vRNAP 5:181
Bacteriophage P2 5:185
antiholin gene 3:255
associated proteins 3:250
host cell lysis 3:250, 3:255

Bacteriophage P22
  evolution 5:374
  procapsid structure 1:611–612
  structure 1:434*f*
    scaffold proteins 1:438
Bacteriophage Pf1
  capsid helical symmetry 3:121
  DNA conformation 3:121
  plaque formation 3:118
Bacteriophage Pf2 1:201–202
Bacteriophage Pf3
  capsid helical symmetry 3:121
  DNA conformation 3:121
Bacteriophage Pf4 3:123–124
Bacteriophage phi6 4:379–380
Bacteriophage ϕX174
  DNA packaging 3:19
  DNA replication 3:16, 3:17*f*
  evolution 3:20
  gene E 3:256–257
    MraY resistance 3:257
    Rz–Rz1 vs 3:257
    single gene lysis 3:256–257
  genetic map 3:15*f*
  genome packaging 2:311
  history 3:13
  host cell attachment 3:15–16
  host cell lysis 3:256–257
  host cell penetration 3:16
  morphogenesis 3:19
  nomenclature 3:13
  replication 4:405
    GpC protein 4:405
    preprimosome assembly 4:405
    protein A 4:405
    SSB protein 4:405
    stage I 4:405
    stage II 4:405
    stage III 4:405
  structure 1:434*f*, 3:14*f*, 5:384–385, 5:384*f*
    procapsid 3:18*f*
Bacteriophage PM2, genome
    delivery 5:368
Bacteriophage PRD1
  genome 3:5
    delivery 5:368
    encoded proteins 3:2*t*
    organization 3:5, 3:5*f*
  host range 3:3
  inverted terminal repeats 4:402–403
  life cycle 3:3
    DNA entry 3:4
    host cell lysis 3:4–5
    receptor recognition 3:3–4
    replication initiation 3:4, 4:403
  structural motif conservation 1:610
  structure 1:434*f*, 1:438, 3:1, 3:3*f*
    membrane 3:2–3, 3:2*t*
    vertices 3:4*f*
    X-ray crystallography 3:3*f*
'Bacteriophage proteomic tree' 5:343
Bacteriophage Qbeta 4:380
  A_2 gene 3:257–258
Bacteriophage SPP1
  genome 4:401
  replication 4:401
    associated host proteins 4:401
    helicase 4:401
    primase 4:401
Bacteriophage T2
  history 1:433
  structure 2:448*f*
Bacteriophage T3 5:178
  coupled transcription 5:179
  DNA entry 5:179
  early transcription 5:179
    promoters 5:179
    termination 5:179
  late transcription 5:178*f*, 5:179
    class III genes 5:179
    termination 5:179
  middle transcription 5:179
    class II genes 5:179
    promoters 5:178*f*, 5:179
  regulatory strategies 5:178
    coupled transcription 5:179
    DNA entry 5:179
    early transcription 5:179
    late transcription 5:179
    middle transcription 5:179
    temporal gene expression 5:179
    transcription regulation 5:180
  temporal gene expression 5:179
    early genes 5:179
    early gene transcription 5:179
  transcription regulation 5:180
Bacteriophage T4 3:30–31, 3:31*f*, 5:176
  assembly
    gene products 1:438–439
    jig fiber 1:438
  DNA injection 3:33–34
  early transcription
    Alt protein 5:176–177
    early promoters 5:176
    σ^{70} 5:176
  evolution 2:181
  genome map 5:176*f*
  genome size 3:36
  host cell lysis
    lysozyme E 3:251
    RI protein 3:255
  late transcription 5:177
    gp33 5:178
    gp45 5:178
    gp55 5:178
  middle transcription 5:177
    ADP-ribosylation 5:177
    promoters 5:177
    RNA synthesis 5:176*f*, 5:177
  protein injection 3:34
  regulatory strategies 5:176
    early transcription 5:176
    late transcription 5:177
    middle transcription 5:177
  replication 4:402*f*
  replication forks 4:401
  structure 1:433, 1:434*f*, 3:32, 3:33*f*
    baseplate 1:608, 1:609*f*
    tails/tail fibers 3:32
Bacteriophage T5 3:33–34
Bacteriophage T7 5:178
  coupled transcription 5:179
  DNA entry 5:179
  early transcription 5:179
    promoters 5:179
    termination 5:179
  genetic map 5:178*f*
  late transcription 5:178*f*, 5:179
    class III genes 5:179
    termination 5:179
  middle transcription 5:179
    class II genes 5:179
    promoters 5:178*f*, 5:179
  mutants 1:612
  open reading frames 5:178
  regulatory strategies 5:178
    coupled transcription 5:179
    DNA entry 5:179
    early transcription 5:179
    late transcription 5:179
    middle transcription 5:179
    RNA polymerase 5:180
    RNA processing 5:180
    temporal gene expression 5:179
    transcription regulation 5:180
  RNA polymerase 5:180
  RNA processing 5:180
  temporal gene expression 5:179
    early gene transcription 5:179
  transcription regulation 5:180
    class II genes 5:180
    late genes 5:180
    RNAP phosphorylation 5:180
    T7 DNA host cell entry 5:180
Bacteriophage Xf1 3:121
Bac-to-Bac *see* Baculovirus expression vector(s)
BaculoDirect *see* Baculovirus expression vector(s)
Baculoviridae 1:211, 1:220, 1:247, 1:254, 1:265, 3:129, 3:497–498, 4:567, 5:11*t*
Baculovirus(es) **1:247–254**
  apoptosis inhibition *see below*
  budded viruses 1:247
  classification 1:247, 3:129
    genome sequencing 1:247–248
  envelope fusion protein 4:451, 4:454*f*
  errantiviruses 4:453
  evolution 1:249
  expression vectors *see* Baculovirus expression vector(s)
  F protein 4:454
    cleavage 4:453, 4:454*f*
    definition 4:451
  genomes 1:249
    core genes 1:249
    multiple homologous repeat regions 1:249
    transcription 1:249
  historical perspective 1:247
  host specificity 1:252, 1:265, 3:129–130
  infection *see* Baculovirus infection
  insect pest control 1:253, 3:129, 3:130*t*
    infectivity 3:131
    limitations 3:129–130
  morphology 1:247
  mosquito *see* Mosquito baculovirus(es)
  nomenclature 1:247
    host species 1:248
  nucleocapsids 1:248–249
  occlusion bodies 2:137, 4:570
    formation 1:265–266
    function 2:137
  phenotypes 1:265
  phylogeny 1:220, 3:497–498
  replication cycle 1:250
    attachment 1:250
    budding nucleocapsids 1:251
    cathepsin 1:251–252
    cell culture 1:250
    cell entry 1:250
    chitinase 1:251–252
    DNA replication 1:250
    early gene expression 1:250
    late gene expression 1:251
    morphogenesis 1:251
    p10 gene/protein 1:251
    polyhedrin gene/protein 1:251
    promoters 1:251
    release 1:251
    structural protein expression 1:251
    transcription regulation 1:250
    uncoating 1:250
    virus dissemination 1:251
    virus-enhancing factor (VEF) 1:250
  replication levels 3:129
  spindle production 2:138
  structure 1:265
  taxonomy 1:247
  transmission 3:310
  virion structure 2:137
    budded 3:129
    occlusion-derived 3:129
  see also specific viruses
Baculovirus apoptosis inhibition 1:158*t*
  host specificity 1:253
  inhibitor of apoptosis proteins 1:252
    BIR domain 1:236*f*
    distribution 1:235
    gene homology 1:235–236
    mechanism 1:235–236
  nonpermissive/semipermissive infections 1:234
  P35 1:252
    caspase inhibition 1:160–161
    general apoptosis inhibition 1:234
    gene transcription effects 1:235
    structure 1:234*f*

timing 1:234
viral cell entry 1:234
Baculovirus expression vector(s) 1:253
BacMam 1:245
advantages for use 1:245–246
cell lines 1:245
complement-mediated inactivation 1:246
entry mechanism 1:245–246
gene therapy use 1:246
hybrid promoters 1:245
transduction efficiency 1:245
BacPAK6 DNA 1:240, 1:240*f*
Bac-to-Bac 1:241
advantages 1:241
bacmid shuttle 1:241
E. coli transformation 1:241
insect cell transfection 1:241, 1:242*f*
site-specific transposition 1:241
Tn7 transposon 1:241
viral DNA extraction 1:241, 1:242*f*
BaculoDirect 1:241
bacteriophage λ 1:241
entry clone generation 1:243–244
Gateway cloning vectors 1:241
recombinant virus purification 1:244
recombination reaction 1:243–244
definition 1:238
disadvantages 1:246
*flash*BAC 1:244
advantages for use 1:244–245
automated high-throughput screening 1:245
bacterial artificial chromosome utilization 1:244
compatibility 1:244
homologous recombination 1:244
gene delivery, mammalian cells 1:245
insect cell culture 1:238
cell lines 1:238
culture media 1:238
plaque purification 1:240–241
polyhedrin promoter 1:253
recombinant viruses 3:131
efficacy improvements 1:240
isolation 1:240
systems 1:238
see also specific systems
transfer vectors 1:239–240
Baculovirus infection
env-mediated 3:310
host defence 1:267
developmental residence 1:267
F proteins 1:267
GP64 1:267
infected midgut cell shedding 1:267
M trait 1:267
host defence modulation 1:252
apoptosis inhibition *see* Baculovirus apoptosis inhibition
ecdysteroid glucosyltransferase 1:252
molting inhibition 1:252
manifestations 3:129, 3:129*f*
pathogenesis **1:265–272**
in vitro *see below*
secondary 1:265–266, 1:266*f*
systemic infection 1:267
terminal disease 1:269, 1:269*f*
pupation 1:269
V-CATH 1:269
virulence 3:129
in vitro pathogenesis 1:269
BRO proteins 1:271
F-actin cables 1:270
GV-specific cell lines 1:270
host nuclear structure 1:270, 1:271*f*
host RNA polymerase II 1:270, 1:270*f*
late-stage infection 1:270
microtubules 1:270, 1:271*f*
NPV-specific cell lines 1:270
nuclear F-actin 1:270–271
nuclear structural change 1:271
nucleus infection 1:270
P6.9–DNA interactions 1:271
P78/83 1:271
very late factor-1 1:270–271
very late infection phase 1:272
wandering behavior 1:269
Badnavirus 1:403, 1:459*t*
Badnavirus(es) 1:459*t*
host chromosome integration 1:462
open reading frames 1:466*f*
ornamental plant species infection 1:403
species 1:403
transmission 1:463
virion structure 1:458–460
see also specific viruses
Bafinivirus(es)
genome 3:424*f*
replicase genes 3:425*f*
see also specific viruses
BAG-1 protein, JCV 4:264
Bahia Grand Group, animal rhabdoviruses 1:116
Bahia Grand virus (BGV) 1:116
Bak protein(s), apoptosis 1:155–157
BALB/c mice, MHV-68 pathogenesis 3:377
Balsaminaceae virus infections 4:214
see also individual species
Bam35 bacteriophage *see* Bacteriophage Bam35
Bamboo mosaic virus (BaMV) 4:311*t*
RNA satellites 4:310, 4:531
expression 4:535
variation/evolution 4:534
*Bam*HI-A rightward transcripts (BARTs) *see* Epstein–Barr virus (EBV)
BaMMV *see* Barley mild mosaic virus (BaMMV)
BaMV *see* Bamboo mosaic virus (BaMV)
Banana bunchy top virus (BBTV) **1:272–279**
characteristics 3:389*t*
coat protein 1:274
detection assays 1:278
ELISA 1:278
PCR 1:273, 1:278
genome 1:274, 1:275*f*, 3:387–388, 3:387*f*
coat protein 1:274
intergenic regions 1:274
movement protein 1:274
nuclear shuttle protein 1:274
Rep 1:274
geographical distribution 1:274–275, 1:276
Africa 1:277
current 1:277
historical aspects 1:276
historical aspects 1:274
geographical distribution 1:276
host range 1:273, 3:386
infection *see below*
phylogenetics 1:274, 1:276*f*
resistance 1:278
transgenic plants 1:278
taxonomy 1:274
transmission 1:277, 3:386
aphid vectors 1:277
circulative transmission 1:274
vegetative propagation 1:277
viral particles 1:274, 1:274*f*
Banana bunchy top virus infection
control 1:277
genetically-modified resistant plants 1:278
infection eradication 1:277–278
isolation 1:277–278
economic importance 3:386
epidemiology 1:277
Australian epidemics 1:277
Fijian epidemics 1:277
Pakistan epidemic 1:277
eradication 1:277–278
symptoms 1:273, 1:273*f*, 3:386
histology 1:273
phloem 1:273
Banana streak virus (BSV) 1:403
host genome integration 1:462
Bangladesh, Nipah virus 2:324
Banna virus (BAV) 4:536*t*
antigenic relationships 4:541
capsid structure 4:538, 4:539*f*
characteristics 1:171*t*
diagnostic assays
molecular assays 4:544–545
serology 4:544–545
epidemiology 4:537
functional studies 4:542
vaccine development 4:542
VP9 expression 4:542
genome 4:538, 4:540*f*
conserved sequences 4:541–542
sequencing 4:539
host range 4:537
infections
clinical features 4:544
as public health problem 4:537–538
phylogenetics 4:540–541
proteins, electrophoretic profiles 4:538*f*, 4:539
rotaviruses *vs.*
evolutionary relationships 4:542
structural features 4:542, 4:545*f*
transmission 4:537
as type species 4:536
BARE1 element 4:435
Barfin flounder nervous necrosis virus (BFNNV) 2:229–230
Barley *(Hordeum vulgare)*
dsRNAs 2:112
protoplasts, Brome mosaic virus replication 1:382
resistance genes, loci 4:182
viruses **1:490–497**, 1:491*t*
see also specific viruses
Barley mild mosaic virus (BaMMV) 1:492
infection 1:493*f*
control 1:493
Barley stripe mosaic virus (BSMV) 1:490, 2:459
coat proteins 2:459, 2:461–462
genome 2:459–460, 2:460*f*
infection
chlorophyll affects 2:463–464
chloroplast morphological change 2:463–464
virion accumulation 2:463–464
pathogenesis 2:464
ND18 strain 2:464, 2:466
proteins 2:463, 2:464–466
replicase proteins 2:461
replication, gRNAs 2:461
TGB1/RNP complex 2:462
TGB3 movement protein 2:464
Barley virus B1 (BarV-B1) 4:311*t*
Barley yellow dwarf virus (BYDV) 1:279, 1:496
disease 1:476
resistance 1:496
symptoms 1:476, 1:496
genetically-modified resistant plants 1:285–286
genome 1:280
coat protein 1:282
noncoding regions 1:281
open reading frames 1:280
ORF4 mutations 1:282
MAV strain 1:279
epidemiology 1:285
genome 1:280
replication 1:283–284
species-specificity 1:283
PAS strain 1:280
PAV strain
epidemiology 1:285
genome 1:280, 1:281–282
host range 1:282–283
replication 1:283–284
species-specificity 1:283
RMV strain 1:279
replication 1:283–284
SGV strain 1:279
replication 1:283–284
transmission 1:496

Barley yellow dwarf virus(es) **1:279–286**
  classification 1:279
    strains 1:279, 1:280*f*
    see also specific strains
  control 1:285
    insecticide applications 1:285–286
    insecticide-treated seeds 1:285–286
    planting times 1:286
    resistant strains 1:285–286
    transgenic plants 1:285–286
  diagnosis 1:285
    control strategies 1:285
  effects of 1:279
  epidemiology 1:285
  genome 1:280
    coat protein 1:280, 1:282
    'leaky scanning' 1:281
    long-distance frame shift element 1:281–282
    noncoding regions 1:281
    open reading fame 1:280
    ORF4 mutations 1:282
    protein functions 1:282
    VPgs 1:282
  historical aspects 1:279
  host range 1:282
  RdRp 1:279–280, 1:280*f*
  replication 1:283
    phloem 1:283–284, 1:284*f*
  taxonomy 1:279
    amino acid sequences 1:279–280
    RdRps 1:279–280, 1:280*f*
  transmission 1:282
    aphids 1:279, 1:282–283
    circulative strain-specific manner 1:283
    species-specificity 1:283
  virion particle 1:280, 1:280*f*
    coat proteins 1:280
    VPg 1:280
  virus–host relations 1:284
    chlorosis 1:284–285
    stunting 1:284–285
    yield losses 1:284–285
  see also specific viruses
Barley yellow mosaic virus (BaYMV) 1:492
  infection
    control 1:493
    symptoms 1:492, 1:492*f*
    yield losses 1:492
Barley yellow streak mosaic virus (BaYSMV) 1:490
  structure 1:490
  transmission 1:492
Barley yellow striate mosaic virus (BYSMV) 1:492
  infection 1:492
  replication 4:193
  transmission 1:492
Barmah Forest virus (BFV) 5:88
  characteristics 5:109*t*
  geographical distribution 5:84*f*, 5:88
  host range 5:88
  infection
    clinical features 5:83–84, 5:86*t*, 5:87*t*, 5:88
    zoonosis 5:493
Barnard, J E, Ectromelia virus 3:342
Barnaviridae 1:287, 2:288, 5:11*t*
Barnavirus 1:287, 2:288
Barnavirus(es) **1:286–288**
  evolutionary relationships 1:287, 1:288*f*
  expression 1:287
  genome 1:287
    open reading frames 1:287, 1:287*f*
  historical aspects 1:286
  host range 1:288
  infections, fungi 2:287*t*
  structure 1:287
  transmission 1:288
  type virus 1:287
  virion properties 1:287
  virion structure 1:287*f*
  see also specific viruses
Barrier control methods, potato virus Y
    infections 4:295
BART gene, micro RNAs 5:327
Barur virus (BARV) 1:116
Basal cell(s)
  division 4:20, 4:21
  HPV *see* Human papillomavirus(es) (HPVs)
  papillomavirus life cycle 4:28–29
Basella alba (Malabar spinach), dsRNAs 2:112
Basidiomycota 4:419
  retrotransposons 4:423, 4:424*t*
Basidiospore(s), fungal virus transmission 2:285–286
Bat(s)
  filovirus host 2:200
  Hendra virus host 2:95
  paramyxovirus host 4:40–42, 4:45–46, 4:56
  rabies virus host 4:370–371, 5:485
  SARS CoV host 1:555, 4:555
BAV *see* Banna virus (BAV)
Bawden, Frederick, tobacco mosaic virus 3:399
Bax protein(s), apoptotic role 1:155–157
BaYMN *see* Barley yellow mosaic virus (BaYMV)
BaYSMV *see* Barley yellow streak mosaic virus (BaYSMV)
BBMV *see* Broad bean mottle virus (BBMV)
BBNV *see* Broad bean necrosis virus (BBNV)
BBSV *see* Beet black scorch virus (BBSV)
BBTV *see* Banana bunchy top virus (BBTV)
BBWW
  Bacopa virus infections 4:215
  nonspecific infections 4:219, 4:221*f*
βC1, Ageratum yellow vein virus 1:318
BC1 protein, mungbean yellow mosaic viruses 3:367
bc-3 resistance gene *see* Bean common mosaic virus (BCMV); Bean common necrosis virus (BCMNV)
B cell(s)
  activation 3:65*f*
  attributes 3:61
  clonal selection 3:61
  definition 3:78
  development 1:621
    Burkitt's lymphoma 5:195
  differentiation 1:121, 3:61
  function 1:122–123
  Lassa fever infection 3:210
  localization 1:122–123
  persistent infection 4:112
  transformation, lymphocryptovirus 4:592–593
  virus latency 2:440–441
    antigen receptor 2:441
  see also Antibodies; Immunoglobulin(s); *specific viral infections*
B-cell antigen receptor (BCR), EBV reactivation 2:441
B-cell epitope(s)
  definition 1:137
  variation 3:205
B-cell malignancies 5:196
  lymphomas, reticuloendotheliosis virus infection 4:416
  multicentric Castleman's disease 5:196
Bcl-2 family proteins
  adenovirus E1B-19K gene/protein *vs.* 1:13–14
  apoptosis 1:155–157, 1:156*f*, 1:161–162
  definition 1:154
BCMNV *see* Bean common necrosis virus (BCMNV)
BCMV *see* Bean common mosaic virus (BCMV)
BCTV *see* Beet curly top virus (BCTV)
BCV *see* Beet cryptic virus (BCV)
BdMV *see* Burdock mottle virus (BdMV)
BDV *see* Border disease virus (BDV)
Bean *(Phaseolus vulgaris)*
  dsRNAs 2:111–112
    molecular evolution 2:115
  as food crop 1:296
  resistance genes, loci 4:182
Bean calico mosaic virus (BaCMV)
  identification/naming 1:298
  infection 4:167*f*
    ELISA diagnosis 1:299
Bean common mosaic virus (BCMV) **1:288–295**
  classification 1:289
  epidemiology 3:217
  genome 1:292
    composition 1:291
    encoded proteins 1:292, 1:292*f*
    post-translational proteolytic processing 1:292
    recombination 1:293, 1:294
    variability 1:293
  geographical distribution 1:289
    origin 1:289–291
    peanut stripe isolates 1:289–291
  history 1:289, 1:290*f*, 1:292*f*, 3:215
  host range 1:291, 1:294
    experimental hosts 1:291
    peanut stripe isolates 1:291
    soybean isolates 1:291
  replication 1:292
  serological relationships 1:291
    broad-spectrum monoclonal antibodies 1:292
  serotypes 1:289
  taxonomy 1:289
    phylogenetic analysis 1:290*f*
    subgroups 1:289, 1:289*t*
  transmission 1:291
    aphids 1:291, 3:214
    seeds 1:291, 3:213*f*, 3:214, 3:215–216
  virion properties 1:291
Bean common mosaic virus infection
  control 1:294
  diagnosis 1:294
    RT-PCR 1:294
    serological techniques 1:294
  host resistance genes 1:292
    bc-3 resistance gene 1:292–293
    I gene 1:292, 1:293
    peanut stripe isolates 1:293
    transgenic resistance 1:293
  pathogenicity 1:292
    determinants 1:292, 3:215
    seed color-related susceptibility 1:293
  pathology 1:292
  prevention 1:294
  symptomatology 1:294
    cytoplasmic inclusion morphology 1:294
Bean common necrosis virus (BCMNV) **1:288–295**
  classification 1:289
  genome 1:292
    composition 1:291
    encoded proteins 1:292, 1:292*f*
    P1 gene variability 1:294
    post-translational proteolytic processing 1:292
    recombination 1:293, 1:294
  geographical distribution 1:289
    origin 1:291
  history 1:289
  host range 1:291, 1:294
  NL-3 K strain 1:293
  recombination 1:293
    NL-3 K strain 1:293
    occurrence 1:294
  replication 1:292
  serological relationships 1:291
    broad-spectrum monoclonal antibodies 1:292
  taxonomy 1:289
  transmission 1:291
    aphids 1:291
    Dendrobium mosaic virus 1:291
    seeds 1:291
  virion properties 1:291
Bean common necrosis virus infection
  control 1:294
  diagnosis 1:294
    RT-PCR 1:294
    serological techniques 1:294
  host resistance genes 1:292
    bc-3 resistance gene 1:292–293
    I gene 1:292, 1:293
    introgression 1:293
  pathogenicity 1:292
    determinants 1:292
    hypersensitivity 1:292

pathology 1:292
prevention 1:294
symptomatology 1:294
cytoplasmic inclusion morphology 1:294
Bean dwarf mosaic begomovirus (BDMV) 3:218
Bean golden mosaic virus (BGMV) **1:295–301**
genome
AC genes 1:298
bean golden yellow mosaic virus *vs.* 1:298–299
common region 1:298
nuclear shuttle protein 1:298
open reading frames 1:298
identification/naming 1:298
infection *see* Bean golden/yellow mosaic disease
molecular characteristics 1:298
vectors 1:296
Bean golden/yellow mosaic disease
diagnosis 1:299
ELISA 1:299
PCR 1:299
disease resistance 1:299
economic importance 1:296
etiology 1:297
DNA probes 1:297–298
electron microscopy 1:297
first isolation 1:297
PCR 1:297–298
history 1:296
host species 1:299–300
management 1:300
resistant cultivars 1:300–301
vector control 1:300–301
pathogenicity 1:299
nucleolus hypertrophy 1:299
resistance
dwf gene 1:300
plant breeding 1:299–300
soybean infections 3:217, 3:218
symptoms 1:296
vector control 1:300–301
Bean golden yellow mosaic virus (BGYMV) 4:165
genome 1:298–299
identification/naming 1:298
infection *see* Bean golden/yellow mosaic disease
molecular characteristics 1:297
Bean-pod mottle virus (BPMV) 5:392
Bean yellow dwarf virus 4:236
Bean yellow mosaic virus (BYMV)
infectivity 3:214
CMV interactions 3:214–215
transmission 3:216*f*
seeds 3:215–216
Bebaru virus (BEBV) 5:109*t*
Bechhold, Heinrich 2:455
Bee(s)
iflavirus infection 3:42
transmission 3:45
ilarvirus transmission 3:55
control 3:55
Beet black scorch virus (BBSV) 3:403
satellite virus 3:404
Beet cryptic virus (BCV) 1:98
BCV1 *vs.* BCV2 1:102
mixed infections 1:102
phylogeny 4:74–75
transmission 1:102
cross-pollination 1:102
Beet curly top virus (BCTV) **1:301–307**, 4:165
C1 protein 1:303
C2 protein 1:303
C3 protein 1:303
C4 protein 1:303
control 1:306
defective interfering DNAs 1:307
plant breeding 1:307
vector physical barriers 1:307
genome 1:303, 1:303*f*
open reading frames 1:303
geographical distribution 1:302
history 1:301
host range 1:306
Arabidopsis thaliana 1:307
infection 4:167*f*
potatoes 4:300*t*
pathogenesis 1:306
adenosine kinase 1:306
C2 proteins 1:306
C4 proteins 1:306
leaf curl 1:305*f*, 1:306
transcriptional gene silencing 1:306
phylogeny 1:302
Rep 1:303–304
retinoblastoma-related tumor-suppressor protein binding 1:307
replication 1:303
deletions 1:304
recombination-dependent replication 1:303–304
rolling circle replication 1:303–304
subgenomic viral DNAs 1:304
seasonal distribution 1:302
Circulifer tenellus 1:302–303
transmission 1:304
Circulifer tenellus 1:301–302, 1:302–303, 1:305, 1:305*f*
phloem limitation 1:306
as type species 1:302
V1 protein 1:303
V2 protein 1:303
V3 protein 1:303
virus movement 1:304
Beetle(s)
legume virus transmission 3:216–217
maize chlorotic mottle virus transmission 3:262
plant virus transmission 5:280
population management, oryctes virus 3:146
Rice yellow mottle virus transmission 1:488
Beet leaf curl virus (BLCV), vector relationships 4:195
Beet mild curly top virus (BMCTV) 1:302
geographical distribution 1:302–303
host range 1:306
replication 1:304
Beet necrotic yellow vein virus (BNYVV) 1:308
antigenic properties 1:310
control
genetic modification 1:310
resistant varieties 1:309–310
cytoplasmic localization 1:310
genome 1:311*f*, 1:312
RdRp 1:312
subgenomic RNAs 1:311*f*, 1:312
geographical distribution 1:309
infection (rhizomania) 1:308
protein dependence 1:312–313
yield losses 1:308
particle properties 1:310
RNA satellites
expression 4:535
large single-stranded RNA satellites 4:531
satellite-like single-stranded RNAs 4:532
as type species 1:308
Beet ringspot virus (BRSV) 3:406*t*
diseases 3:411
genome 3:407–408
sequence identity 3:408
infectious DNA clones 3:408–409
Beet severe curly top virus (BSCTV) 1:302
geographical distribution 1:302–303
host range 1:306
replication 1:304
Beet soil-borne mosaic virus (BSBMV)
antigenic properties 1:310
genome 1:312
geographical distribution 1:309
infection 1:308
transmission 1:308
Beet soil-borne virus (BSBV) 4:283
CP proteins 4:284
genome, RNA2 4:284
geographical distribution 4:285–286
RT proteins 4:284
serological relationship 4:285*f*, 4:286
TGB protein 4:283–284
virus–host interactions 4:284
Beet virus Q (BVQ) 4:283
CP proteins 4:284
genome, RNA2 4:284
geographical distribution 4:285–286
RT proteins 4:284
serological relationship 4:285*f*, 4:286
TGB protein 4:283–284
virus–host interactions 4:284
Beet western yellows virus, associated RNAs 4:533
BEFV *see* Bovine ephemeral fever virus (BEFV)
Begomovirus 1:30, 1:298, 1:302, 2:98, 4:165
Begomovirus(es) 4:164, 4:198
β ssDNA satellites 1:315, 1:316*f*
cotton leaf curl disease 2:102
function 1:318
functions 1:318
identification 1:315
interaction complexity 1:320
diversification, rapid evolution 4:166
DNA satellites 1:315, 4:165, 4:528*t*, 4:530
β ssDNA satellites *see above*
'helper' virus infection 4:166
evolution 4:166
genome 4:165
infection *see below*
resistance to 4:178–182
transmission 2:98, 4:165
whitefly 2:98
type species 4:165
virus-induced gene silencing vectors 5:378
see also specific viruses
Begomovirus infections 4:166, 5:65, 5:289
βC1 pathogenicity 1:318
gene silencing suppression 2:102
helper begomoviruses 1:318
nuclear localization 1:318
sweetpotato 4:661*t*, 4:666
geographical distribution 4:666
host range 4:666
infection symptoms 4:666
phylogeny 4:666
symptoms 4:166
tobacco 5:65
geographical distribution 5:65
Beijerinck, Martinus W 2:450–451, 5:54
virus discovery 3:399
BEL, LTR retrotransposons 4:438
Bell's palsy 5:234
Bemisia tabaci see Whitefly
Benyvirus 1:308, 4:283
Benyvirus(es) **1:308–314**
antigenic properties 1:310
control 1:309
chemical control 1:309–310
genetic modification 1:310
resistant varieties 1:309–310
differentiation of 1:312
epidemiology 1:309
genome 1:311*f*, 1:312, 1:313
geographical distribution 1:309
host range 1:308
infections 1:308
diagnosis 1:313
morphology 1:313
nucleic acid properties 1:312
other taxa *vs.* 1:313
particle properties 1:310, 1:310*f*
transmission 1:309
Polymyxa zoospores 1:309
type species 1:308
see also specific viruses
Berne virus *see* Equine torovirus (EToV)
Berrimah virus 4:184*t*
β-amyloid plaque visualization, bacteriophage display 2:198
β-barrel fold
alfalfa mosaic virus structure 5:381, 5:382*f*
nonenveloped virus structure 5:381, 5:382*f*

β-barrel fold (*continued*)
nudaurelia capensis omega virus structure 5:381
spherical viruses *see* Spherical virus(es)
βC1
β capsid protein, Providence virus 5:33
β-sheet(s)
prion propagation 4:340
prion structure 4:339
β ssDNA satellite(s) **1:314–321**
comparisons, Indian tomato leaf curl viruses *vs.* 5:130
cotton leaf curl disease *see* Cotton leaf curl disease (CLCuD)
diversity 1:318
geographical distribution 1:318
host adaptation 1:319–320
origin 1:317*f*, 1:319*f*, 1:320
replication 2:102
structure 1:316, 1:317–318, 1:317*f*
satellite conserved region 1:317–318
trans-replication 1:320
Betacryptovirus 1:99, 1:100*t*, 2:109–110
Betacryptovirus(es) **1:98–104**, 1:100*t*
evolutionary relationships 1:103
genome 1:100
history 1:98
molecular biology 1:100
replication 1:100
transmission 1:99, 1:102
virion properties 1:99
virus–host relationships 1:102
see also specific viruses
Betaentomopoxvirus 4:323, 4:323*t*
Betaentomopoxvirus 2:136
characteristics 2:137*t*
structure 4:325
Betaherpesvirinae 1:625, 1:635–636, 1:635*t*, 2:431, 2:432*t*, 2:474, 5:11*t*
Betaherpesvirus(es)
associated genera 1:635–636, 1:635*t*
bigenic amplification 2:426*f*
classification criteria 2:432
evolution 2:478–482
genome 2:475, 2:476*f*
alphaherpesviruses *vs.* 1:636
gammaherpesviruses *vs.* 1:636
sequences 1:636
latency 2:439
pathogenicity 2:427–428
see also specific viruses
Betanodavirus 3:431
Betanodavirus(es) 3:430
capsids 3:433
genotypes 3:431
host range 3:432
infection
fish 2:229
symptoms 3:437–438
transmission 3:437–438
type species 2:229
see also specific viruses
Betapapillomavirus 4:9, 4:10*f*
Betapapillomavirus, carcinoma associated-infection 4:15
Betaretrovirus 3:175, 3:335, 4:460, 4:624
Betaretrovirus(es)
assembly 2:518, 4:462
genome 4:624, 4:624*f*
morphology 4:461–462
ruminants **3:175–182**
see also specific viruses
Betatetravirus(es)
insect infections 3:126*t*
see also specific viruses
Bet gene/protein *see* Foamy viruses
Bevirimat 2:507*t*, 2:516
BFV *see* Barmah Forest virus (BFV)
BGMV *see* Bean golden mosaic virus (BGMV)
BGYMV *see* Bean golden yellow mosaic virus (BGYMV)
Bhanja virus 1:399
distribution 1:399
Haemaphysalis intermedia 1:399
BHF *see* Bolivian hemorrhagic fever (BHF)
BHV1 *see* Bovine herpesvirus 1 (BHV1)
BHV2 *see* Bovine herpesvirus 2 (BHV2)
Bicistronic, definition 3:263
Big liver and spleen disease virus (BLSV) 2:380
The Big Picture Book of Viruses 5:357*t*
Big-vein disease of lettuce *see* Lettuce big-vein disease
BILR 355 BS 2:507*t*
Binomial names 5:405
Bioassay(s)
luteoviruses 3:237
Taura syndrome virus 5:7–8
Bioavailability 4:552
Biocides, pecluvirus infection control 4:102
Biocrime
definition 5:407
see also Bioterrorism
BioCyc database 5:357*t*
Biodissemination, oryctes rhinoceros virus transmission 3:499
Biodistribution, definition 2:301
BioHealth Database 5:357*t*
Bioinformatics, microarrays and 2:16
Bioinformatics Resource Centers (BRCs) 5:363
Biological control 3:125
African cassava mosaic disease 1:36
agents 3:125
baculoviruses *see* Baculovirus(es)
densoviruses *see* Densovirus(es)
strategies 3:125
see also Insect pest control; *specific viruses*
Biological fitness 4:359
Biological warfare
definition 5:407
see also Bioterrorism
Biopesticides, tetraviruses 5:36
Biosystem 3:400
Biotechnology
bacteriophages, filamentous ssDNA 2:197
bacteriophage use 2:448–449
watermelon mosaic virus 5:439–440
zucchini yellow mosaic virus 5:439–440
Bioterrorism **5:406–411**
alphaviruses 5:408
Aum Shinrikyo 5:407
Bacillus anthracis 5:406, 5:408*t*
biotechnology 5:409
mousepox virus 5:409
pandemic influenza 5:410
synthetic genomes 5:410
definitions 5:407
deterrence 5:410
history 5:407
recognition 5:410
response 5:410
virus classification 5:409*t*
Biotic transmission, rice yellow mottle virus epidemiology 4:488
Biotype(s)
bovine viral diarrhea viruses 1:376
definition 1:374
Bioweapon(s) 5:407
associated factors 5:408
hypothetical attack 5:407–408, 5:408*t*
see also Bioterrorism
Bipartite genomes
definition 1:295
potyviruses 4:316–317
Bipartite nuclear targeting sequence, definition 3:447
Bipartite virus, definition 4:164
Biphasic milk fever, tick-borne encephalitis virus infection 5:53
Bird(s)
Crimean-Congo hemorrhagic fever virus 1:597
see also individual species
see also individual viruses
'Bird flu' pandemic *see* Influenza
BIR domain, inhibitor of apoptosis proteins 1:236*f*
Birnaviridae 1:321, 1:322*t*, 2:232, 5:11*t*
Birnavirus(es) **1:321–328**
characteristics 5:62*t*
evolutionary relationships 1:326
VPg-linked genome replication strategy 1:326
fish *see* Fish birnavirus(es)
gene expression 1:322
genera 2:232
genome 1:322, 1:323*f*
open reading frames 1:322
history 1:321
nomenclature 1:321
RdRp 1:322
catalytic motifs 1:322–323
palm domain 1:322–323
replication 1:325
morphogenesis 1:326
transcription 1:325–326
VLP formation 1:326
shellfish 4:566
virion composition 1:322
virion proteins 1:322
capsid protein VP2 1:322, 1:324
internal protein VP3 1:325
nonstructural protein VP5 1:325
protease VP4 1:325, 1:325*f*
pVP2 peptides 1:324
RdRp VP1 1:322
virion structure 1:322, 1:323*f*
virus assembly 1:325
see also specific viruses
Birth defects, definition 4:514
Bison, bovine herpesvirus 2 1:367
Bivalves, definition 4:560
BIV_{R29} infections (cattle)
experimental infection 1:352
serology 1:350
BK virus(es) (BKV) **4:261–271**
associated disorders 4:269
cytoplasmic entry
erythrocyte membrane binding 4:281
sialic acid receptors 4:280–281
genome oncogenicity
experimental animals 4:269
human tumors 4:270
mechanisms of 4:269
strain potency 4:269
host cell attachment
phospholipase A2 activity 4:281
receptors 4:280
infection 4:269
organ transplantation syndromes 3:470
phospholipid bilayer role 4:281
transplant-associated 3:468
reactivation 4:269
replication 4:279
structural organization 4:269
transmission 4:269
see also specific viruses
Blackcurrant reversion virus (BRV) 3:406*t*
diseases 3:411
transmission 3:411
Black queen-cell virus (BQCV)
host range 2:40
infection 2:42
transmission 2:42
Black raspberry necrosis virus (BRNV) 3:37, 4:523, 4:524*t*
genome sequencing 4:524–525
host range 4:525
transmission 4:525
vector control 4:525–526
Black-seeded bean cultivars, infection resistance 1:293
'Black spot' 1:515
BLAST 5:352
output 5:354
pairwise sequence comparison *vs.* 5:345
sequence comparison methods 5:343
viral evolution study 2:179–180
Blebbing, definition 1:231
Blepharoconjunctivitis 2:491

Blindness
  HCMV 2:496
  measles 2:494–495
BLMoV *see* Blueberry leaf mottle virus (BLMoV)
Blood, virus shedding 5:316–317
Blood-borne virus(es)
  CNS invasion pathways 1:470, 1:472*f*
  see also specific viruses
Blood–brain barrier
  CNS invasion pathways 1:471
  nervous system viruses 1:469–470
  SIV infection 4:600
  TMEV infection 1:447
  West Nile virus infection 5:446
Blood transfusions
  Crimean-Congo hemorrhagic fever treatment 1:603
  HCV infection 2:336
  hepadnavirus transmission 2:336
  hepatitis E virus transmission 2:336
  HTLV-1 transmission 2:559
  KSHV transmission 3:191
  vCJD risk 1:373
Blueberry leaf mottle virus (BLMoV) 3:406*t*
  diseases 3:411
  genome 3:408
Blueberry shoestring virus (BSSV) 4:646*t*
Bluetongue virus(es) (BTV) **1:328–335**
  capsid assembly 1:333
    VP3 1:333
    VP7 1:333
  capsid structure 3:459, 3:459*f*, 3:460*f*
    modifications 3:459–460
    outer capsid proteins 1:329
    VP2 3:459, 3:461, 3:462–463, 3:463–464
    VP5 3:459, 3:462–463, 3:463–464
    VP7 3:459–460, 3:461
  cell egress 1:333
    calpactin light chain 1:333
    NS3/NS3A proteins 1:333
    Tsg101 1:333
  core particle 1:329, 1:330*f*, 5:382*f*, 5:388–391, 5:392
    trimeric proteins 1:329–330
    X-ray crystallography 5:388–391
  epidemiology 1:334
  gene expression control 4:153–154
  genome 1:331, 1:331*t*, 3:460, 3:461*f*, 3:462*f*
    noncoding regions 1:331
    sequence conservation 1:331
    terminal sequences 1:331
  geographical distribution 3:454–457, 3:458
  history 1:328, 3:454
  host cell attachment 1:331
    VP2 1:331–332
    VP5 1:332
  host cell entry 1:331
  infection
    clinical features 1:333–334, 3:458
    control 1:334, 3:458–459
    economic losses 3:458–459
    host range 3:457
    immunity 1:334
    outbreaks, Europe 3:458
    pathogenesis 1:333
    prevention 1:334
    sheep 1:333–334
    tissue tropism 3:458
  morphology 1:329, 1:329*f*
  mRNA production 4:152–153
  protein synthesis 1:332
    NS1 1:332
    NS2 1:332, 1:333
    phosphorylation 1:332–333
  replicase/transcription complex 4:153–154
  replication 1:331, 4:152–153
  seasonal distribution 3:458
  serotypes 3:454–457, 3:455*t*, 3:459, 3:464*f*
    BTV-8 3:458
  stability 3:459
  structure 1:329, 1:329*f*, 3:459, 3:459*f*, 5:382*f*
    capsid *see above*
    core particle *see above*
    NS3 3:464
  transcription 1:332
    VP4 1:332
    VP6 1:332
  transmission 1:333, 1:334
    temperature effects 3:457
    vector competence 3:457
  as type species 4:536*t*
  vaccines/vaccination 1:334
    inactivated 1:334–335
    live attenuated 1:334
  viral core channels 4:152–153
  virion properties 1:329
  see also specific viruses
BMCTV *see* Beet mild curly top virus (BMCTV)
BmDNV-1 *see* Bombyx mori densovirus type 1 (BmDNV-1)
BmNPV infection, wandering behavior 1:269
BMS-378806 2:507*t*
BMV *see* Brome mosaic virus (BMV)
BNYVV *see* Beet necrotic yellow vein virus (BNYVV)
Bocavirus 4:95
Bocavirus(es) 4:95
  classification 4:92*t*
  definition 2:93
  genome 4:94*f*, 4:95–96
  see also specific viruses
Bohle iridovirus (BIV), host range 3:156
Boletus virus X (BolVX) 4:311*t*
Bolivian hemorrhagic fever (BHF)
  clinical features 3:209
  epidemiology 3:206
  histopathology 3:209
  pathogenesis 3:208
  pathology 3:209
  therapy 3:211
Bombyx mori cypovirus 1 (BmCPV-1) 3:136
Bombyx mori densovirus type 1 (BmDNV-1) 4:79
  genome 4:83
  infection 4:79
Bombyx mori densovirus type 2 (BmDNV-2) 4:76
Bone marrow
  transplantation, human herpesvirus infection 2:498–499, 2:502
  Visna-Maedi virus infection 5:428
Booster vaccination 2:54
Bootstrap analysis 4:127
Border disease 2:247
  control 1:340
    vaccines/vaccination 1:340
  detection 1:340
    at birth 1:340
    enzyme-linked immunosorbent assay 1:340
    reverse transcriptase-polymerase chain reaction 1:340
  fetal infection 1:337
    immune-mediated damage 1:337
  historical aspects 1:336
  pathogenesis 1:336, 1:337*f*, 2:248
    cell culture 1:337–338
    fleece coarseness 1:336
    immune-mediated damage 1:337
    neurological signs 1:336
  persistent viremia 1:336
    late-onset disease 1:337
    myelin deficiency 1:336–337
  symptoms 1:337
Border disease virus (BDV) **1:335–341**, 1:526–527, 2:246
  genogroups 1:338
  genome 1:338
    internal ribosomal entry site 1:339
    open reading frame 1:338
    sequence identity 1:339
    sequencing 1:338
  heat inactivation 1:338
  historical aspects 1:336
    isolation 1:336
  infection *see* Border disease
  molecular biology 1:338
  nonstructural proteins 1:339
    N^{pro} 1:339–340
    NS2-3 1:339–340
    NS3 protease 1:340
    NS5A 1:340
  particle size/morphology 1:338
  physico-chemical properties 1:338
  proteins 1:339, 1:339*f*
  replication 1:340
    attachment 1:340
    clathrin-dependent endocytosis 1:340
  structural proteins 1:339, 1:339*f*
  transmission 2:247
Borna disease virus (BDV) **1:341–347**
  detection 1:346–347
  epidemiology 1:344
    virus reservoirs 1:344
  genome 1:341, 1:342*f*, 3:326*f*
    open reading frames 1:341
  geographical distribution 1:342
  history 1:341
  infection *see below*
  life cycle 1:342
  propagation 1:342
  protein properties 1:341
    open reading frames 1:341–342
  replication 1:342
    efficiency 1:342, 3:331
    mechanisms 1:342
    splicing 3:330
  synonyms 1:341
  variability 1:344
  virion properties 1:341
    nucleocapsid protein structure 3:331
    phosphoprotein 3:331
    stability 1:341
Borna disease virus infection
  clinical features 1:345
    disease characterization 1:345
    incubation time 1:345
  control 1:346
  histopathology 1:345–346, 1:346*f*
  host range 1:342
    experimental hosts 1:344
  immune response 1:345
  pathogenicity 1:345
  pathology 1:345
  prevention 1:346
  rats 1:344
    clinical features 1:345
    immune response 1:345
    pathology 1:345–346
  serology 1:344
  tissue tropism 1:342, 1:344
    neurotropism 1:344–345
  transmission 1:344
Bornaviridae 3:324, 5:11*t*
Bornavirus 1:321–322
Bornavirus(es)
  characteristics 5:62*t*
  genome 3:326*f*
  P mRNA transcripts 3:331
  transcription 3:330
  see also specific viruses
Bos, Lute 5:55
Botrytis virus F (BVF), classification 2:288
Botrytis virus X 2:255
Bottle-shaped virus(es) 1:590
  see also specific viruses
Bottom component (B), alfalfa mosaic virus 1:81–82
BoTV *see* Bovine torovirus (BoTV)
Bovine adenovirus(es) 1:26
  see also specific viruses
Bovine atadenovirus(es) 1:8
  see also specific viruses
Bovine enteric calicivirus(es) 1:411
  see also specific viruses
Bovine enterovirus(es) (BEV) 2:123
  classification 2:123
  genome 2:126

Bovine enterovirus(es) (BEV) (*continued*)
virion structure 2:128, 2:128*f*
see also specific viruses
Bovine enzootic fever *see* Bovine ephemeral fever virus (BEFV)
Bovine ephemeral fever virus (BEFV) 4:184*t*
antigenic variation 1:360
site G1 1:360–361
site G2 1:360–361
site G3 1:360–361
site G4 1:360–361
epizootiology 1:355
vectors 1:355
genome 1:357, 1:358*f*
geographical distribution 1:355
history 1:354–355
infection
immune response 1:361
pathogenesis 1:356
pathology 1:356
taxonomy 1:355, 1:356*f*
transcription 1:359
α coding region 1:359–360
β coding region 1:359–360
γ coding region 1:359–360
polyadenylation signal 1:359–360
transmission 1:115
vaccines/vaccination 1:361, 1:362
virion morphogenesis 1:356
virion structure 1:356, 1:357*f*
G protein 1:356–357
nucleocapsid 1:356–357
virus encoded proteins 1:357
G_{NS} protein 1:359
G protein 1:358, 1:361*f*
L protein 1:359
M protein 1:358
N protein 1:357
P protein 1:358
small nonstructural proteins 1:359
Bovine ephemerovirus, genome 3:326*f*
Bovine gammaherpesvirus(es)
distribution 1:365
history 1:363
transmission 1:365
Bovine herpesvirus(es) **1:362–368**, 1:364*t*
antigenic relationships 1:365
classification 1:363, 1:364*t*
epidemiology 1:365
geographical distribution 1:365
history 1:362
infection
clinical disease 1:366
control 1:367
immune response 1:367
latency 1:365
pathogenesis 1:365
prevention 1:367
replication 1:363
structure 1:363
see also specific viruses
Bovine herpesvirus 1 (BHV1) 1:364*t*
genital disease control 1:367
history 1:362–363
latency 1:365
vaccines/vaccination 1:367
Bovine herpesvirus 2 (BHV2) 1:364*t*
history 1:363
mammilitis 1:366
Bovine herpesvirus 3 (BHV3) 1:363
Bovine herpesvirus 4 (BHV4) 1:363, 1:364*t*
Bovine herpesvirus 5 (BHV5) 1:364*t*
Bovine herpesvirus 6 (BHV6) 1:364*t*
Bovine immunodeficiency virus(es) (BIV) **1:347–354**
classification 1:347
clinical features 1:351
economic impact 1:353
epidemiology 1:351
evolution 1:350
genetics 1:350
variability 1:350
genome 1:349
open reading frames 1:349–350
geographical distribution 1:348, 1:348*f*
prevalence 1:348
history 1:347
host range 1:350
infection
clinical features 1:351
control 1:353
histopathology 1:352
immune response 1:352
pathology 1:352
prevention 1:353
secondary diseases 1:352
propagation 1:350
associated cell types 1:350
serology 1:350
BIV_{R29}-infected cattle 1:350
taxonomy 1:347
tissue tropism 1:351
transmission 1:351
iatrogenic 1:351
PCR detection 1:351
variability 1:350
virion structure 1:349, 1:349*f*
viral core 1:349
viral enzymes 1:349
see also specific viruses
Bovine influenza *see* Bovine ephemeral fever virus (BEFV)
Bovine malignant catarrhal fever (MCF)
causative agents 1:363, 1:365, 1:366–367
clinical features 1:366–367
control 1:367
history 1:363
hosts 1:363, 1:365
pathogenesis 1:365
prevention 1:367
Bovine papillomavirus 1 (BPV-1) 4:32
genome 4:31*f*
pathology 4:29–30
research history 4:26
structure 4:32*f*
viral protein functions 4:31–32
E1 4:22
E2 4:22, 4:36
E4 4:32
E5 4:23
E6 4:31–32
E7 4:31–32
L1 4:32
L2 4:32
Bovine papillomavirus 4 (BPV-4) 4:32–33
Bovine papular stomatitis virus (BPSV) 4:57–58
host range 4:58
Bovine pestivirus(es) 2:4
see also specific viruses
Bovine respiratory syncytial virus
genome 4:42, 4:43*f*
reverse genetics 4:44–45
Bovine rhinovirus(es) (BRVs)
classification 4:468
see also specific viruses
Bovine spongiform encephalopathy (BSE) **1:368–374**, 5:189
associated cases 1:368, 1:369–370, 5:189, 5:189*f*
disease characteristics 5:189
epidemiology 1:369, 1:370*f*
mathematical models 1:369–370
geographical distribution 1:370, 1:371*f*, 5:190
history 1:368, 5:189
detection 5:189
incubation period 1:368–369
novel forms 1:370–371
origin 1:368, 5:189
prevention 5:190
active testing 1:369, 1:370
legislation 1:368
passive surveillance 1:369, 1:370
specified bovine offal ban 1:369
species barrier/prion variant 4:332, 4:339
tissue distribution 1:368–369
transmission 1:370
vaccine safety concerns 5:228
see also Creutzfeldt–Jakob disease (CJD)
Bovine torovirus (BoTV) 5:151–152
antigenic properties 5:155
attachment/cell entry 5:153
buoyant densities 5:153
epidemiology 5:155
genotypes 5:155
historical aspects 5:151
host range 5:154
infection
diarrhea 5:155–156
pathogenesis 5:155–156
tissue tropism 5:155
Bovine viral diarrhea virus(es) **1:374–381**, 1:526–527
characteristics 1:378
virion inactivation 1:378
virion structure 1:378
genome recombination 2:248
history 1:375, 2:246
biotypes 1:376
early reports 1:375
genotypes 1:377
isolation 1:376
modified live virus release 1:376
postvaccinal mucosal disease 1:376
reduction and eradication programs 1:378
infection *see below*
molecular biology 1:379
genome 1:377*f*, 1:378, 1:379
viral proteins 1:379
viral replication 1:380
vaccines/vaccination 2:248–249
see also specific viruses
Bovine viral diarrhea virus 1 (BVDV-1)
antigenic/genetic relationships 1:338
classification 1:336
type 2 *vs.* 1:378, 1:379, 2:246
Bovine viral diarrhea virus 2 (BVDV-2)
antigenic/genetic relationships 1:338
classification 1:336
prevalence 2:246
type 1 *vs.* 1:378, 1:379, 2:246
Bovine viral diarrhea virus infection
clinical features 1:375, 1:375*f*, 2:248
cytopathic type 1:375*f*, 1:376
noncytopathic type 1:375*f*, 1:378
control 1:380
detection 1:380, 2:248
hemorrhagic syndrome 1:377–378
BVDV1 *vs.* BVDV2 1:379
clinical symptoms 1:377–378
mucosal disease
disease characteristics 1:375
etiology 1:376
pathogenesis 2:248
persistent 1:376
pregnancy 2:248
BPSV *see* Bovine papular stomatitis virus (BPSV)
BPV-1 *see* Bovine papillomavirus 1 (BPV-1)
BQVC *see* Black queen-cell virus (BQCV)
Bracovirus(es) (BV)
definition 4:256
gene families 4:250–251
immunoevasive activity 4:255–256
immunosuppressive effects 4:254
infections 4:253
insects 3:126*t*
phylogeny 4:252*f*
replication 4:260–261
virion structure 4:257, 4:257*f*
nucleocapsid 4:257
see also specific viruses
Bradford coccus 3:312
Brain
human herpesvirus infection 2:503
JCV infection 4:262
pseudorabies virus infection 4:349
see also Central nervous system (CNS)

Branch length, phylogenetic trees 4:126
Brassica napus, cauliflower mosaic virus silencing suppression 1:468–469
Brazilian wheat spike virus (BWSpV) 1:492, 5:24
BRCs database 5:357*t*
Breakthrough infection 1:69
Breast cancer
  mouse mammary tumor virus model 4:274
  Simian retrovirus D 4:625, 4:629
Breastfeeding, HIV infection prevention 1:66
Breda virus 2:122
  diarrhea 2:122
Brevidensovirus 4:76, 4:83, 4:94*t*, 4:96
Brevidensovirus(es) 4:76, 4:96
  classification 4:94*t*
  genome 4:76–77, 4:83, 4:95*f*, 4:96–97
    monosense genomes 4:83
  virion structure 4:78
  see also specific viruses
Bridge vector(s) 4:652
  definition 5:440
  vector transmission, animal viruses 5:270
  West Nile virus transmission 5:443–444
BRNV *see* Black raspberry necrosis virus (BRNV)
Broad bean mottle virus (BBMV)
  defective-interfering RNAs 1:389, 4:380
  recombination 4:380
Broad bean necrosis virus (BBNV) 4:283
  CP proteins 4:284
  genome
    RNA2 4:284
    untranslated regions 4:283
  geographical distribution 4:285
  particles 4:283
  RT proteins 4:284
  small open reading frame 4:284
Broad spectrum antiviral therapy 1:147, 1:154
Brome mosaic virus (BMV) **1:381–386**, 1:492
  alphavirus-like superfamily 1:381
  coat protein translation 1:381
    RNA3 1:384
  foreign gene expression 1:381
    reverse genetics 1:382
  gene expression 1:381
  genome 1:381, 1:383*f*
    cis-signals 1:383
    noncoding regions 1:383
    promoter 3:54
    RdRp enzyme 1:389
    RNA1 1:381, 1:388*f*
    RNA2 1:381, 1:388*f*
    RNA3 1:381, 1:383–384, 1:388*f*, 1:389
    RNA4 1:381, 1:384, 1:388*f*
  host factors 1:384
  infectious *in vitro* transcripts 1:381
  as a model 1:381
  protein characterization 1:381
  RdRp 1:382, 1:389
    promoter interactions 1:384
  recombination, template switching 1:385
  replication 1:382, 1:383*f*
    associated proteins 1:382
    conserved proteins 1:382
    host factors 1:384
    protein localization 1:382
    recombination 1:389
  RNA encapsidation 1:385, 1:389
  RNA-protein interactions 1:383
  RNA recombination 1:385, 4:377–378
    homologous recombination 4:378, 4:378*f*
    host factors 4:379
    replicases 4:378–379
  structure 1:387, 1:388*f*
  subgenomic mRNA synthesis 1:384, 1:389
    core promoter 1:384
    pathway elucidation 1:384
  transmission 1:390, 5:280–281
  virion
    assembly polymorphism 1:385
    classes 1:381
    size 1:385
    structure 1:385
Brome mosaic virus infection
  host range 1:385
  spread of 1:385
    essential proteins 1:385
    systemic spread alterations 1:385–386
5′-Bromo-4-chloro-3-indolylphosphate (BCIP) 2:24
Bromoviridae 1:81, 1:381, 1:387, 2:255, 3:46, 5:11*t*
Bromovirus 1:81, 1:381, 1:387, 3:46
Bromovirus(es) **1:386–390**
  cucumovirus *vs.* 1:387
  gene expression 1:388
  genome 1:387*t*, 1:388
  homologous recombination 1:389
  infections *see below*
  nonhomologous recombination 1:389
  phylogenetics 1:387
  RNA replication 1:389
  taxonomy 1:387, 1:387*t*
  transmission 1:390
  tRNA-like structure 1:81
  virion structure 1:387
  virus–host relationship 1:390
  see also specific viruses
Bromovirus infections
  associated host factors 1:389
  resistance genes 4:179*t*
  tobacco disease, economic importance 5:63
    alfalfa mosaic virus 5:63
    cucumber mosaic virus 5:63
    disease outbreaks 5:63
    tobacco streak virus 5:64
Bronchiolitis
  definition 2:551
  human respiratory syncytial virus infection 2:548
Bronchopneumonia, morbillivirus infection 4:504
BRO proteins, baculovirus 1:271
Broth culture, bacteriophages 2:74
Brown citrus aphid *(Toxoptera citricida),* citrus tristeza virus transmission 1:521, 1:524
Brown trout reovirus 1:164*t*
BRSV *see* Beet ringspot virus (BRSV)
BSBMV *see* Beet soil-borne mosaic virus (BSBMV)
BSBV *see* Beet soil-borne virus (BSBV)
BSCTV *see* Beet severe curly top virus (BSCTV)
BSE *see* Bovine spongiform encephalopathy (BSE)
BSMV *see* Barley stripe mosaic virus (BSMV)
*Bsu*361 restriction enzyme 1:240
BSV *see* Banana streak virus (BSV)
Bubaline herpesvirus 1 (BuHV1) 1:364*t*
Budded virion(s) (BV)
  baculoviruses 3:129
  definition 1:254
  nucleopolyhedrovirus structure 1:255–256, 1:263
    AcMNPV 1:263
    GP64 protein 1:263
Budding
  alphaviruses 1:196, 1:200, 5:121
  avian hepadnavirus 2:330*f*, 2:331
  Chandipura virus 1:503
  duck hepatitis B virus 2:330*f*, 2:331
  Ebola viruses 2:59–60
  equine infectious anemia virus 2:169–170
  flaviviruses 1:199
  foamy virus 2:264
  HTLV-1 2:561
  maize mosaic virus 4:193, 4:196
  mononegaviruses 3:333
  mumps virus replication 3:359–360
  nucleorhabdoviruses 3:333, 3:334
  paramyxoviruses 3:333–334
  plant rhabdoviruses 4:194–195
  Rift Valley fever virus 1:397
  rubella virus 5:121
  vesicular stomatitis virus 5:295
  virus membranes 5:313, 5:313*f*
    M proteins 5:314
Buffalo, capripoxvirus infection 1:428
Bu gene 3:41
Buggy Creek virus 5:93*t*
Bunyamweravirus 3:480
Bunyamwera virus (BUNV)
  genome 1:394*f*
  replication 1:397
Bunyaviridae 1:170, 1:391, 1:399, 2:317–318, 3:479, 4:490–491, 5:11*t*, 5:27, 5:133, 5:157–158
Bunyavirus(es) **1:390–399**
  attenuation 5:317–318
  definition 1:390
  genomes 1:172, 3:482
    coding strategies 1:393, 1:394*f*
    reassortment 1:395, 1:396*f*
    RNA segments 1:392, 2:317–318
    S segment 1:395
    terminal complementarity 1:392–393
  infections 1:391*t*
    apoptosis inhibition 1:158*t*, 1:161
    human respiratory infection 2:555
    persistent 1:397
    vectors 1:392
  replication cycle 1:395
    host cell attachment 1:395
    host cell entry 1:395
    RNA replication 1:396*f*, 1:397, 1:398*f*
    transcription 1:395, 1:396*f*
    translation 1:396–397
    uncoating 1:395
    virion assembly 1:397
    virion release 1:397
  reverse genetics 1:398
  transmission 1:391, 1:392*f*
    vector competence 1:392
  'unassigned' **1:399–401**, 1:400*t*
    definition 1:399
  virion proteins 1:392, 1:393*f*
    glycoproteins 1:393
    L protein 1:393
    nucleocapsid 1:392
  virion structure 1:172, 1:393*f*
    morphology 1:393*f*
  see also specific viruses
Burdock mottle virus (BdMV) 1:308–309
  genome 1:312
  transmission 1:309
Burkitt's lymphoma
  chromosome translocations 2:154
  EBV infection 2:154, 2:154*f*, 2:158–159, 4:115, 5:194
    B-cell development 5:195
    endemic 2:154, 5:194
    epidemiology 5:195
    seroepidemiological studies 2:154
    sporadic 5:194
  history 2:149
  incidence 2:154
  Simian retrovirus D 4:629
Burley tobacco 5:61
  virus resistance 5:64
Burnett, F M, Ectromelia virus 3:342
Burst size
  bacteriophage population ecology 2:75
  definition 2:71, 5:411
  haloviruses 5:412
  methanogenic Archaea-infecting viruses 5:412
Bushmeat
  HIV origin 2:532, 4:604
  SIV cross-species transmission 2:532–533
Bushpig *(Potamochoerus porcus),* African swine fever virus 1:43
BVDV-1 *see* Bovine viral diarrhea virus 1 (BVDV-1)
BVDV-2 *see* Bovine viral diarrhea virus 2 (BVDV-2)
BVQ *see* Beet virus Q (BVQ)
Bxbl bacteriophage 5:185
BYDV *see* Barley yellow dwarf virus (BYDV)
Bymovirus 4:315*t*
Bymovirus(es) 1:492, 1:493
  genome 1:492
  infection
    control 1:493
    plant resistance genes 4:179*t*
    symptoms 1:492, 2:292
  P2-2 protein 2:293–294, 4:317

Bymovirus(es) (*continued*)
transmission 5:281
variants 1:492–493
see also specific viruses
BYMV *see* Bean yellow mosaic virus (BYMV)
BYSMV *see* Barley yellow striate mosaic virus (BYSMV)

C

C1 proteins, beet curly top virus 1:303
C2 proteins, beet curly top virus 1:303, 1:306
C3 proteins, beet curly top virus 1:303
C4 proteins, beet curly top virus 1:303, 1:306
C-6 virus, sweetpotato infections 4:661*t*
Cabassou virus (CABV) 5:92, 5:93*t*
characteristics 5:109*t*
Cabbage, turnip mosaic virus infection 5:289
Cabbage leaf curl virus (CabLCV) 5:326
CABV *see* Cabassou virus (CABV)
Cacao swollen shoot virus (CSSV) **1:403–410**, 4:200
classification 1:403
genome 1:405, 1:406*f*
dsDNA 1:404–405
N-terminal coat protein 1:405
open reading frames 1:405
ORF1 1:405
ORF2 1:405
ORF3 1:405–406
ORF alignments 1:405, 1:407*t*
variation 1:405
viral homology 1:406, 1:408*f*
geographical distribution 1:404
host range 1:403
infection *see below*
molecular characterization 1:404
molecular epidemiology 1:408
phylogenetic tree 1:405–406, 1:408*f*
transmission 1:403, 1:404, 4:200
Pseudococcidae mealybugs 1:404, 1:404*f*
reservoir hosts 1:404
variability 1:405, 1:407*t*
PCR diagnostic tests 1:405
virion 1:404
Cacao swollen shoot virus (CSSV) infection
control 1:404, 1:409, 4:200
'cutting-out' campaigns 1:404, 1:409
eradication policies 4:200
isolation 1:409
PCR diagnosis 1:409
resistance breeding 1:409
strain protection 1:409
diagnosis 1:408
see also specific methods
economic importance 1:403, 1:404
geographical distribution 1:404
yield losses 4:200
PCR diagnosis 1:409
ORF3 1:409
primers 1:409
sequence alignment 1:409
reservoir hosts 1:404
serology 1:408
ELISA 1:408–409
immunocapture polymerase chain reaction 1:408
strain variation 1:408–409
variation 1:405
virobacterial agglutination test 1:408
symptoms 1:403, 1:404*f*
avirulent strains 1:403
defoliation 1:403
red veins 1:403
stem swellings 1:403
Cache valley virus 1:391*t*
Cactus virus X (CVX) 4:311*t*
Cadmium-induced glycine-rich protein (cdiGRP) 3:355
Caecum, definition 1:37
Caenorhabditis elegans, RNA interference assays 3:149
FHV infection 3:153
CAEV *see* Caprine arthritis encephalitis virus (CAEV)
CaGMV *see* Cassava green mottle virus (CaGMV)
Caladium virus X (CalX) 4:311*t*
Calcineurin-dependent pathways, African swine fever virus immune system evasion 1:47
Calcium channels, KP4 toxin action 5:216
Calf serum, vaccine safety concerns 5:228
Calibrachoa mottle virus (CbMV)
Calibrachoa infection 4:214, 4:214*f*
petunia infection 4:213
Calibrachoa virus infections 4:214
see also specific viruses
Caliciviridae 1:90*f*, 1:410, 1:411, 1:411*t*, 2:117, 2:230, 2:378, 3:439, 3:439*t*, 3:440*f*, 5:11*t*
Calicivirus(es) (CaCV) **1:410–419**, 2:116
characteristics 2:117*t*
epidemiology 1:415
fish *see* Fish calicivirus(es)
genetics 2:118
genome 1:412–413, 1:413*f*, 3:440–441
NS proteins 3:440–441, 3:442*t*
geographical distribution 1:413, 2:117
history 1:410, 2:116
infections 3:439*t*
clinical signs 1:417
control 1:418, 2:118
host range 1:414, 2:116–117
immune response 2:118
immunity 1:417
pathogenesis 1:416, 2:118
prevalence 2:118
prevention 1:418, 2:118
symptoms 1:515
veterinary pathogens 3:439
morphology 1:411–412, 2:116–117, 2:117*f*
phylogeny 1:413*f*
propagation 1:414
properties 1:411
nonstructural proteins 1:413
seasonal distribution 2:117
serologic relationships 1:415
structure 1:411–412, 1:412*f*, 1:414*f*, 2:117*f*
capsid 2:118
transmission 2:117–118
see also specific viruses
Calimorpha quadripuntata virus (CqV) 5:28*t*
Callitrichine herpesvirus 3 (CalHV-3) 4:587*t*
Calomys
arenavirus transmission 3:204
Junin virus infection 3:204–205
Calves
astrovirus pathogenesis model 1:208
see also specific virus infections
Calyx cell, definition 4:256
Camelpox virus (CMPV), infection 4:329
Campoletis sonorensis ichnovirus (CsIV)
genome 4:258, 4:258*f*
coding density 4:258–259
cys-motif 4:255, 4:258–259
open reading frames 4:258–259
rep genes 4:258–259
segment nesting 4:258–259
transposable elements 4:257–258
vankyrin gene family 4:258–259
vinnexin gene family 4:258–259
host immune response abrogation 4:253–254
ankyrin genes 4:255
encapsulation 4:255
hemocyte adhesion 4:255
innexin expression 4:255
melanization 4:255
Campyloganthia, definition 1:328
CaMV *see* Cauliflower mosaic virus (CaMV)
Canada, HIV epidemiology 1:65
Canarypox-like virus(es) 2:278
see also specific viruses
Canarypox virus (CNPV) 2:275
Cancer
Mason–Pfizer monkey virus 4:629
see also specific cancers
Cancer therapy, echoviruses 2:70
Candlestick syndrome, African cassava mosaic virus 2:101
Canine adenovirus infection 1:5
Canine distemper virus (CDV) 4:502
cell entry 3:285
evolution 4:502
geographical distribution 4:498
host cell binding 4:500
host range 4:499–500
infection 4:498
clinical features 4:504
vaccines/vaccination 4:506
Canine oral papillomavirus (COPV) 4:33
Canine parvovirus infection
control 4:90
pathogenesis 2:122, 4:89
Canker
chestnut blight 2:575
definition 2:574
Cannibalism, Taura syndrome virus transmission 5:7
Cantalago virus 5:489–490
Canyon hypothesis, picornavirus gene expression 4:131
Cap genes, porcine circoviruses 1:516
Capillary fragility, Crimean-Congo hemorrhagic fever 1:601–602
Capillary endothelia, CNS invasion pathways 1:471–472
Capillary leakage, Argentine hemorrhagic fever 3:208
Capillovirus **1:419–427**
associated species 1:420, 1:420*t*
biological properties 1:420
classification 1:420*t*
genome 1:420
heterogeneity 1:422
particle structure 1:420, 1:421*f*
phylogeny 1:426*f*
replication 1:420
serology 1:421
strains 1:422
Capillovirus 1:420, 1:420*t*, 1:426*f*
Cap-independent RNA translation 4:133, 4:133*f*
rhinoviruses 4:470
Cap protein(s), porcine circoviruses 1:517, 1:519
Caprine arthritis encephalitis virus (CAEV) 5:424
infection 5:430
macrophage infection 5:428–430
vaccines/vaccination 5:431
Caprine herpesvirus 1 1:364*t*, 1:366
Caprine herpesvirus 2 1:364*t*, 1:367
Capripoxvirus 1:427
Capripoxvirus(es) **1:427–432**
classification 1:427, 4:324*t*
distribution 1:428
evolution 1:429
genetics 1:428
recombination 1:428
sequence similarity 1:428, 1:432
stability 1:428
strain identification 1:428
history 1:427
infections *see below*
isolation 1:428
propagation 1:428
serologic relationships 1:429
taxonomy 1:427
viability 1:430
see also specific viruses
Capripoxvirus infections
clinical features 1:430
papules 1:430
epizootiology 1:429
morbidity rates 1:429
mortality rates 1:429
histopathology 1:431
host range 1:428
immune response 1:431
incubation period 1:430
pathogenicity 1:430
pathology 1:431
prevention and control 1:431
climate 1:431–432
vaccination 1:431–432
tissue tropism 1:429
transmission 1:429–430
papules 1:429–430
Capsicum see Pepper *(Capsicum)*

Capsid(s)
  architecture 5:365
  β-barrel folding motif 1:202*f*
  definition 4:407
  evolutionary connections 5:374
  helical 1:200–201
  icosahedral 1:200–201
    definition 4:514
  picornaviruses 1:201, 1:201*f*
  structural polymorphism 1:611–612
  symmetry 1:433
    helical 1:200–201
    icosahedral 1:200–201, 4:68
    picornaviruses 1:201, 1:201*f*
    T=2, definition 4:68
  see also specific viruses
Capsid-associated transcriptase, Ustilago maydis viruses 5:217
Capsid protein (CP)
  see specific viruses
Cap-snatching
  bunyavirus transcription 1:395–396
  definition 1:390, 3:483
  infectious salmon anemia virus 3:93
  influenza virus transcription 3:487
  tenuivirus replication 5:24, 5:25*f*, 5:26
Capsomers
  adenovirus virion morphology 1:3, 2:78
  definition 3:264
Carboxypeptidase D, avian hepadnavirus replication 2:329
Carcinogenesis
  HPV infection *see* Human papillomavirus(es) (HPVs)
  SV40 infection 4:631, 4:636, 4:637–638
  see also Oncogene(s)
Carcinoma
  alphapapillomavirus associated 4:15
  HPV associated 4:14–15
Cardamine chlorotic fleck virus (CCFV) 1:454
Cardamom bushy dwarf virus (CBDV), characteristics 3:389*t*
Cardiac disease
  coxsackieviruses 1:586
  HIV infection *see* HIV infection
Cardio-vascular system, African horse sickness 1:40–41
Cardiovirus 1:441, 1:442, 5:38
Cardiovirus(es) **1:440–448**
  classification 1:442
  encephalomyocarditis virus
    see Encephalomyocarditis virus (EMCV)
  epidemiology 1:444
  evolution 1:443
  genome 1:442
  geographical distribution 1:444
  history 1:441
  host range 1:444
  infection
    clinical features 1:445
    pathogenicity 1:445
    pathology 1:445
    prevention 1:447
  propagation 1:442
  proteins 1:442, 1:442*f*
    leader 2:126
  replication 1:443
  taxonomy 1:441*t*, 1:442, 1:443*f*, 1:444*f*
  transmission 1:444
  virion properties 1:442
  see also specific viruses
Caribbean, HIV epidemiology 1:64
Carlavirus 1:426*f*, 1:442, 1:448, 1:449*t*, 1:451*f*, 4:306
Carlavirus(es) **1:448–453**
  classification 1:448, 1:451*f*
  gene expression 1:450
  genome 1:450, 1:451*f*
    open reading frames 1:451
  geographical distribution 1:452
  host range 1:452
  infections
    control 1:452
    cytopathology 1:452
    epidemiology 1:452
    sweetpotato 4:661*t*
    symptomatology 1:452
  physicochemical properties 1:450
  serology 1:452
  structure 1:450
  taxonomy 1:448, 1:451*f*
  transmission 1:449*t*, 1:452
  see also specific viruses
Carmovirus 1:453, 1:454*t*, 5:145–146
Carmovirus(es) **1:453–457**, 1:454*t*
  defective-interfering RNAs 1:456, 5:150
  distribution 1:453
  evolutionary relationships 1:453
  gene expression 1:455
  genome 1:454*f*, 1:455
    open reading frames 1:455
  host range 1:453
  infections
    economic significance 1:453
    resistance genes 4:179*t*
  replication 1:455
    3′ coterminal subgenomic RNAs 1:455
    associated structural elements 1:456
  satellite viruses 1:456
  transmission 1:453
  virion assembly 1:454
  virion structure 1:453, 1:454
  virus–host interactions 1:456
  see also specific viruses
Carnation cryptic virus (CarCV) 1:99
Carnation latent virus 1:448–450
Carnation mottle virus (CarMV) 5:145–146
Carnation mottle virus (CarMV), genome 5:147*f*
Carnation ringspot virus (CRSV) 5:145–146
  genome 5:147*f*
Carp pox herpesvirus 2:233
Carp pox herpesvirus infection 2:208
Carriers
  definition 3:6, 5:411
  icosahedral enveloped dsDNA bacteriophages 3:12
  infectious pancreatic necrosis virus 3:87
  Simian retrovirus D 4:627–628
Carrot red leaf virus (CRLV), associated RNAs 4:533
Case-control studies 2:143
  definition 2:140
  retrospective 2:143
Case-fatality rate, definition 2:44, 2:142
Caspase(s) 1:155, 1:156*f*
  apoptosis 1:232*f*
  definition 1:154, 1:186, 1:231, 1:247
  inhibition, P35 1:235
  regulators, death receptor signaling 1:160
Caspase-3 1:155
Caspase-8 1:155
  activation 1:156*f*, 1:160
Caspase-9 1:155–157
Casphalia extranea, biological control 3:128
Casphalia extranea densovirus (CeDNV) 4:79
Cassava (*Manihot esculenta*) 1:30
Cassava American latent virus (CsALV) 3:406*t*
Cassava common mosaic virus (CsCMV) 4:311*t*
  genome 4:312
Cassava green mottle virus (CaGMV) 3:406*t*
  diseases 3:411
Cassava mosaic disease (CMD) 4:198
  control 4:198
  detection 4:198
  symptoms 4:198
  transmission 4:198
  yield losses 4:197, 4:198
Cassava mosaic geminivirus(es) (CMGs) **1:30–37**
  distribution 1:31, 1:32*f*
    diagnostic tests 1:31–32
  genome 1:30
    open reading frames 1:30–31
  recombination 1:32
  replication 1:31
  structure 1:30
  transmission 1:32
    vegetative cuttings 1:32–33
    whitefly vector 1:32–33
  see also specific viruses
Cassava virus C (CsVC) 3:500
  taxonomy 3:500
Cassava virus X (CsVX) 4:311*t*
Cat(s)
  West Nile virus infection 5:446
  see also under Feline
  see also specific viruses
Catalytic RNA 5:332
Catarrhines 4:611
Caterpillars, ascovirus infection 1:189
Cathepsin 1:215*t*, 1:216
  baculovirus expression vectors 1:251–252
Cattle
  Akabane virus infection 1:78
  bluetongue virus infection 1:334
  capripoxviruses 2:124
  import, virus panzootics 4:497
  parapoxviruses *see* Parapoxvirus(es) (PPVs)
  rinderpest viruses *see* Rinderpest virus(es) (RPVs); *see also under Bovine*
Cattle feed, BSE 5:189, 5:190
Cattle plague 4:497
Caudovirales 3:30–31, 3:32*t*, 5:11*t*, 5:23, 5:413*t*
Caudovirus(es) 3:30–31
  assembly
    procapsid assembly 1:435–436
    protein cleavage 1:439
    protein conformational changes 1:434–435
    protein synthesis 1:434–435
    symmetry disruptions 1:437
    tail shaft assembly 1:438
  classification 5:11*t*, 5:23
  DNA injection 3:33–34
  evolution 3:474–475
    cystoviruses *vs.* 5:374
    genome comparisons 5:372
    genome organization 5:371
    inoviruses *vs.* 5:374
    mechanisms 5:371, 5:372
    microviruses *vs.* 5:374
    populations 5:371
    types 5:371
  genome packaging 2:306
  protein injection 3:34
  structure 3:31
  see also Bacteriophage(s); Icosahedral tailed dsDNA bacteriophages; *specific viruses*
Cauliflower mosaic virus (CaMV)
  DNA recombination mechanisms 4:376
  gene map 1:461*f*, 1:467*f*
  genome 1:467*f*
    discontinuity 1:460
    ORF I 1:460, 1:468
    ORF II 1:468
    ORF III 1:468
    ORF IV 1:466
    ORF V 1:467
    ORF VI 1:468
    size 1:460
  history 1:457
  inclusion bodies 1:458*f*, 1:464
  movement within plants, virion-associated protein 3:353
  pathogenicity determinants 1:463–464
  precapsid protein 1:466
  replication 1:457
    terminal redundancy 1:461
  ribosomal shunt mechanism 1:460–461
  RNase H 1:467
    replication 1:462
  35S promoter 1:457–458
  vector recognition 5:279
  virus stability 1:467
  as a virus vector 1:457–458
Caulimoviridae 1:403, 1:457, 1:458, 1:459*t*, 1:464–465, 4:430, 4:451, 4:482, 5:11*t*
Caulimovirus 1:459*t*, 1:464–465, 1:465*t*

Caulimovirus(es) **1:457–464**, **1:464–469**, 1:465*t*
  bacilliform virions 1:458, 1:464
  capsid properties 1:465, 1:468
  DNA recombination mechanisms 4:376
  genome 1:460, 1:465
    discontinuity 1:460
    DNA structure 1:457
    replication 1:467
    reverse transcriptase 1:460
    RNA processing 1:468
    transcription 1:467
    translation mechanism 1:468
  host range 1:463, 1:464–465
  icosahedral virions 1:458, 1:464
  inclusion bodies 1:458*f*, 1:464, 1:465, 1:466*f*
  open reading frames 1:465, 1:466*f*
  protein properties 1:465
    aphid transmission factor 1:465
    capsid protein 1:466
    movement protein 1:465
    POL polyprotein and cleavage products 1:467
    processing 1:468
    transactivator of translation/viroplasmin 1:467
    virion-associated protein 1:466
  replication 1:461, 1:462*f*
  silencing and silencing suppression 1:468
  sweetpotato infections 4:661*t*
  transmission 1:463
    aphids 1:463
    aphid transmission factor 1:465
  virion properties 1:465
  virion structure 1:458
  virus stability 1:467
  see also specific viruses
Causality assessment, definition 5:226
CAV *see* Chicken anemia virus (CAV)
Cavalon IR, EHV-4 treatment 2:419
Cavemovirus 1:459*t*
Cavemovirus(es) 1:459*t*
  host chromosome integration 1:462
  open reading frames 1:466*f*
  transmission 1:463
  virion structure 1:458–460
  see also specific viruses
CbMV *see* Calibrachoa mottle virus (CbMV)
CBP/p300 1:11
CcBV *see* Cotesia congregata bracovirus (CcBV)
CC chemokine(s) 1:623
CCHF *see* Crimean-Congo hemorrhagic fever (CCHF)
CCHFV *see* Crimean-Congo hemorrhagic fever virus (CCHFV)
CCMV *see* Cowpea chlorotic mottle virus (CCMV)
CC-NB-LRR protein, natural resistance 4:171
CCR2b receptor, SIV cell attachment 4:606–607, 4:618
CCR5 receptor
  definition 2:534
  inhibitors 2:507*t*
  see also HIV infection; Simian immunodeficiency virus (SIV)
CCR7 receptor 1:624
CCRS *see* Cherry chlorotic rusty spot disease (CCRS)
CCRV *see* Channel catfish reovirus (CCRV)
CCSV *see* Cynodon chlorotic streak virus (CCSV)
CCV *see* Channel catfish virus (CCV)
CD2v protein, African swine fever virus 1:47–48
CD4 receptor, HIV infection 2:506, 4:408
CD4+ T-cells 3:75
  antiviral memory 3:80
  CD8+ T cells *vs.* 3:72–73, 3:72*f*
  circulation 3:75
  cytokine secretion 3:80
  definition 1:69, 2:534
  Dengue fever 2:13
  HIV infection *see* HIV infection
  immunopathology 3:77
  murine CMV infection 1:630*t*
  persistent infection 4:112
  regulatory function 3:75
  SIV infection *see* Simian immunodeficiency virus (SIV)
  subtypes 3:80
  TMEV infection, response 5:43
  Visna-Maedi virus infection 5:430–431
  see also T helper cell(s)
CD8+ T-cells 3:74, 3:74*f*
  antigen processing 3:72
  antiviral memory 3:80, 3:116
  CD4 T cells *vs.* 3:72–73, 3:72*f*
  clonal expansion 3:73*f*, 3:74, 3:75*f*, 3:116
  cytokine/chemokine production 3:74–75
  Dengue fever 2:13
  HIV infection *see* HIV infection
  mechanism of action 3:80
  murine CMV infection 1:630*t*
  persistent infection 4:112
  SIV infection 4:600–601, 4:609, 4:621
  Visna-Maedi virus infection 5:427
  see also Cytotoxic T-cells (CTLs)
CD46, virus-host cell binding 5:324
CDC NCID (National Center for Infectious Diseases) 5:357*t*
CDD (Conserved Domain Database) 5:357*t*
CDV *see* Canine distemper virus (CDV)
Cebus cytomegalovirus (CeCMV) 1:635*t*, 1:639*f*
CeHV-17 *see* Ceropithecine herpesvirus 17 (CeHV-17)
Cell-associated virus (CEV), vaccinia virus assembly 5:248
Cell attachment/entry
  endocytosis 4:409
    process 4:409
  fusion 4:409
  replication 4:407
  translocation 4:409
  virus membranes 5:310
    binding specificity 5:310
  see also Receptor(s); *specific viruses*
Cell barriers, bacterial cell viral entry
  see Bacteriophage(s), bacterial cell entry
Cell–cell fusion mating, yeast L–A virus transmission 5:465–466
Cell culture
  aquareovirus infection diagnosis 1:167–168
  baculoviruses 1:250
  Border disease 1:337–338
  Herpesvirus Saimiri 4:586
  varicella-zoster virus propagation 5:251
Cell-cycle link protein, definition 1:272
'Cell-derived' genes, virulence 5:318
Cell envelope, definition 5:365
Cell fusion, Visna-Maedi virus infection 5:430–431
Cell line immortalization, EBV-encoded nuclear antigen 3 family 2:162
Cell lysis
  acidianus two-tailed virus 1:591
  icosahedral enveloped dsDNA bacteriophages 3:11
Cell-mediated immune response **3:70–77**
  antigen presentation 3:72
  antigen processing 3:72
  common cold 4:473
  Ectromelia virus infection 3:345
  human papillomavirus infection 4:17
  immune evasion 3:77
  immunodominance 3:73, 3:73*f*
    pattern changes 3:73
  immunopathology 3:76
  induction 3:73, 3:74*f*
  persistent infections 3:76
  T-cell memory 3:75
  varicella-zoster virus infection 5:254–255
  see also specific components
Cell membranes
  BEFV proliferation 1:357
  icosahedral enveloped dsDNA bacteriophages 3:11
Cell(s),origin
  parasitic RNA, evolution of 3:476
  viral roles 3:478
  virus-first hypothesis 3:475
Cell receptors, measles virus 3:287
Cell signaling, murine polyomavirus middle T 4:274, 4:274*f*, 4:275
Cell surface markers, rhadinovirus human T-cell transformation 4:591
Cell-to-cell transmission
  cowpea mosaic virus proteins 1:573
  grapevine fanleaf virus 3:410
  maize streak virus 3:268
  nepovirus 3:407*f*, 3:410
  neural cell infection 1:473
Cell tropism
  definition 1:554
  host cell attachment 4:408
Cellular cytidine deaminases *see* Retrovirus infections, resistance to
Cellular DNA synthesis, murine polyomavirus large T 4:276
CeMV *see* Cetacean morbillivirus (CeMV)
Centers for Disease Control and prevention (CDC) 2:94
Central Africa, HIV infection 1:63
Central America, HIV infection 1:65
Central nervous system (CNS)
  arbovirus infection 1:175
  coxsackievirus infection 1:585
  equid herpesvirus 1 infection 2:417–418
  equine encephalitic togavirus infection 5:106
  flavivirus infection 2:245
  HSV infection 2:304
  JC virus infection 4:262
  lactate dehydrogenase-elevating virus infection 1:184
  MHV-68 infection 3:376
  rabies virus infection 4:370
  yellow fever 5:474–475
  see also Nervous system virus(es)
Centrosema mosaic virus (CenMV) 4:311*t*
Cephalothorax, definition 4:567
Ceratitis capitata idnoreovirus 5 (CcIRV-5) 3:140
Cereal virus(es) 1:475-482, 1:490-497
  see specific viruses
Cereal yellow dwarf disease 1:476
Cereal yellow dwarf virus (CYDV) 1:279, 1:496
  genome
    coat protein 1:282
    noncoding regions 1:281
    open reading frames 1:280
    ORF4 mutations 1:282
    VPg 1:282
  RNA satellites
    replication 4:533, 4:534
    structure 4:534
  RPS strain 1:279
  RPV strain 1:279
    epidemiology 1:285
    genome 1:279
    host range 1:282–283
    replication 1:283–284
    structure 3:233
  transmission 1:496
Cerebrospinal fluid (CSF)
  alphavirus encephalitis 5:106
  echovirus detection 2:69
Ceropithecine herpesvirus 12 (CeHV-12) 4:587*t*
Ceropithecine herpesvirus 14 (CeHV-14) 4:587*t*
Ceropithecine herpesvirus 15 (CeHV-15) 4:587*t*
Ceropithecine herpesvirus 17 (CeHV-17) 4:587*t*
  discovery 4:586
Certification schemes, infection control measures 1:524
Cervical cancer
  Gardasil vaccine 5:224
  HSV-2 associated 2:394
  papillomavirus infection *see* Human papillomavirus infections
Cervical intraepithelial neoplasia (CIN)
  detection 4:19
  HPV types associated 4:15
Cervid herpesvirus 1 (CerHV1) 1:364*t*
Cervid herpesvirus 2 (CerHV2) 1:364*t*
Cesium chloride gradient purification
  cowpea mosaic virus purification 1:569–570
  rhinoviruses 4:469

Cetacean morbillivirus (CeMV) 4:498
  epizootiology 4:504
  evolution 4:502
CfMV *see* Cocksfoot mottle virus (CfMV)
cg4715 protein 4:452*f*, 4:454
Chaetoceros salsugineum, viral infection of 1:87
Chaetoceros salsugineum nuclear inclusion virus (CsNIV) 1:88*t*, 1:92, 4:116
  algal infections 1:92
  burst size 1:92–94
  genome 1:92–94, 1:94*f*
  host features 1:92–94
  phylogeny 1:93*f*
  virion structure 1:94*f*
Chalcone synthase gene, virus-induced gene silencing 5:326
Challenge models, infectious pancreatic necrosis virus infection 3:88
Chandipura virus (CPHV) **1:497–503**
  budding 1:503
  classification 1:497
  composition 1:499
  gene expression 1:500
    mRNA modifications 1:501
    viral RdRp 1:500, 1:502
  gene structure 1:499
  genome 1:498*f*, 1:499
  genome encapsidation 1:502
    model 1:502–503
    N protein 1:502–503
  history 1:497
  infection *see below*
  life cycle 1:499, 1:500*f*
    glycoprotein G role 1:500
    matrix M protein role 1:500
    viral invasion 1:499
  phosphoprotein P 1:501
    dimerization 1:501
    phosphorylation 1:501
    unphosphorylated 1:501, 1:502
  replication 1:502
    model 1:502
    transcription–replication switch 1:500*f*, 1:502
  structure 1:498*f*, 1:499
  taxonomy 1:497
  transcription 1:500
    stop–start model 1:500, 1:501*f*
  transmission 1:498–499
  viral assembly 1:503
    matrix protein 1:503
Chandipura virus infection
  acute encephalitis 1:497–498
    fatality rate 1:497–498
    symptoms 1:498
  epidemiology 1:497
  zoonoses 5:492
Changuinola virus 3:464–465
Channel catfish reovirus (CCRV) 1:164*t*
  genome 1:165
Channel catfish virus (CCV)
  capsid structure 2:207*f*
  genetics
    gene families 2:209–211
    genome characteristics 2:210*t*
    genome structure 2:209
    koi herpesvirus *vs.* 2:211
    open reading frames 2:209–211, 2:210*f*
  growth properties 2:209
    protein expression 2:209
  history 2:206–207
  infection
    acyclovir treatment 2:211
    clinical features 2:208
    detection 2:208
    histopathology 2:208
    immune response 2:211
    occurrence 2:207
    prevention and control 2:211
  transmission 2:208
  virulence 2:208
Chapare virus, geographical distribution 3:204
Chaperone(s)
  plant virus movement 3:353
  prions and 4:337, 4:338*f*
    specificity 4:337–338
Chaperonin(s), bacteriophage assembly 1:438
Charge-coupled device (CCD) camera 1:605
Charleville virus (CHVV) 4:184*t*
Chelonus, BV-mediated immunosuppression 4:254–255
Chemical control, benyvirus 1:309–310
Chemical platforms, tetraviruses 5:36
Chemical tags, Nudaurelia ω virus 5:36–37
Chemokine(s) **1:620–624**
  α family 1:623
  β family 1:623–624
    receptors 4:408
  definition 1:620, 3:70
  Dengue fever 2:13
  families 1:623
  function 1:623
  homologs, KSHV immune evasion 3:200
  ligands, HIV-1 cell entry inhibitors 2:468–469
  receptors 1:624
    definition 4:611
    HHV immune evasion 2:501
    HIV-host cell interactions 5:323
    see also specific receptors
  regulation 1:620
  rhinovirus infection 1:546
Chemosis, definition 1:580
Chenopodium mosaic virus X (ChMVX) 4:311*t*
Chenopodium necrosis virus (ChNV) 3:403
Cheravirus 3:405–407
Cherries
  Sharka disease 4:239
  see also specific viruses
Cherry chlorotic rusty spot disease (CCRS) 1:509–510, 1:511
  etiology 1:511
Cherry chlorotic rusty spot disease associated chrysovirus (CCRSDACV), genome 1:505–506
Cherry leafroll virus (CLRV) 3:406*t*
  diseases 3:411
  population structures 3:412
  transmission 4:205
  walnut tree infection 4:206
Cherry mottle leaf virus (CMLV) 4:204
  genome 1:425
  hosts 1:424
  replication 1:425
Cherry virus A 1:420–421
Chestnut blight
  biocontrol 2:584
  Cryphonectria parasitica 2:575, 2:580
  epidemic 2:580–581
  history 2:575
Chick embryo, animal virus cultivation 2:456
Chicken anemia virus (CAV)
  associated disease symptoms 1:514
  genome 1:516–517
    anellovirus *vs.* 1:107
  rolling circle replication 1:517
  taxonomy 1:513–514, 1:514*f*
  tissue tropism 1:514
  virion structure 1:514
Chicken influenza virus 2:4
Chicken inhibitor-of-apoptosis protein (ch-IAP1) 4:418
Chickenpox
  clinical features 5:253, 5:254*f*
    lesions 5:253
  complications 5:253
    Reye syndrome 5:253
  historical aspects 5:250–251
  incubation period 5:253
Chickpea chlorotic stunt virus 5:344
Chicory yellow mottle virus (ChYMV) 3:406*t*
Chikungunya virus (CHIKV) 5:88, 5:113
  characteristics 1:171*t*
  evolutionary relationships 1:175
  genetic lineages 5:88
  geographical distribution 5:84*f*, 5:88, 5:113
  infection *see below*
  transmission 5:88, 5:100, 5:111–112, 5:113
    Aedes aegypti 5:100
    mosquitoes 5:111–112
    sylvan 5:113
Chikungunya virus infection
  acute illness 5:89
  clinical features 5:86*t*, 5:87*t*, 5:88
    hemorrhagic manifestations 5:89
  Dengue virus infection *vs.* 2:5
  disease outbreaks 5:89, 5:113, 5:487
  disease surveillance 5:89, 5:487
    global programs 2:50
  morbidity rates 5:100
  mortality rates 5:89
  pregnancy 5:87
  zoonoses 5:487
CHIKV0 *see* Chikungunya virus (CHIKV)
Chilo iridescent virus (CIV) *see* Invertebrate iridescent virus-6 (IIV-6)
Chimera(s), definition 1:513
Chimeric transcript(s)
  definition 4:445
  retrovirus recombination 4:446–447
Chimeric virus(es)
  definition 1:410
  see also specific viruses
Chimerivax Dengue virus, vaccine development 5:238–239, 5:240*f*
Chimpanzee cytomegalovirus (CzCMV) 1:635*t*, 1:639*f*
Chimpanzee rhadinovirus 4:587*t*
Chinese hamster ovary (CHO) cells, cowpox virus growth 1:576
Chinese mitten crab reovirus 1:164*t*
Chinese wheat mosaic virus (CWMV) 1:493
  host range 2:292
  transmission 1:493
Chinook salmon aquareovirus (DRCRV) 1:164*t*
Chinook salmon reovirus (SCRV) 1:164*t*
Chironomus luridus entomopoxivirus 2:136
Chitinase, baculoviruses 1:251–252
Chlamydia
  icosahedral ssDNA bacteriophages 5:368
  viruses *vs.* 3:476
Chlorella virus(es) 4:123
  history 4:116
  see also specific viruses
Chloridovirus(es)
  insect infections 4:126
  see also specific viruses
Chloris striate mosaic virus (CSMV) 1:476
Chlorophyll defects, plant diseases, economic aspects 3:218
Chloroplast(s)
  rice yellow mottle virus 4:487–488
  tymovirus replication site 5:203
  vaccine production in plants 5:223
Chlorosis
  barley yellow dwarf viruses 1:284–285
  cucumber mosaic virus infection 1:617–618
  definition 1:614, 4:207
Chlorovirus 4:117*t*, 4:118
Chlorovirus(es)
  insect infections 3:126*t*
  see also specific viruses
Chlorpromazine, JCV 4:280
Chordopoxvirinae 1:427, 2:136, 3:226, 4:58, 4:323–325, 4:324*t*, 5:11*t*, 5:461–462
Chordopoxvirus(es) (CV) 2:136
  bacopa virus infections 4:215
  entomopoxviruses *vs.* 2:137
  genome 4:325–326
  phylogeny 4:326–327
  see also Entomopoxvirus(es); *specific viruses*
Chorioretinitis 2:388–390
Choristoneura fumiferana cypovirus 16 (CfCPV-16) 3:138, 3:139*t*
Choristoneura fumiferana multiple embedded nucleopolyhedrovirus (CfMNPV) 1:248
  host range determinants 1:252–253
Choroid plexus

Choroid plexus (*continued*)
CNS invasion pathways 1:471–472
definition 1:37
Chromosomes
definition 2:306
fungal retrotransposons translocation breakpoints 4:426
hepatitis B virus integration 2:366
icosahedral tailed dsDNA bacteriophages 3:35
Chronic bee-paralysis virus (CBPV) 4:527*t*, 4:530
Chronic bee-paralysis virus-associated satellite virus (CBVA) 4:527*t*
Chronic infections 4:114
immune control 4:112
initiation 4:109–110
pathogenesis 4:111
reactivation 4:111
virus–host co-evolution 4:111
yellow head virus infection 5:480
see also Persistent infections; *specific infections*
Chronic suppressive therapy, acyclovir 1:145–146
Chronic wasting disease (CWD) 5:188
clinical characteristics 5:188
geographical distribution 5:188
host range 5:188
prevention 5:189
transmission 5:188
experimental 5:188
Chrysanthemum stunt viroid (CSVd) 4:223, 4:223*f*
Chrysanthemum virus B 4:223
Chrysanthemum virus infections 4:221
see also specific viruses
Chrysoviridae 2:111, 2:286, 5:11*t*, 5:214
Chrysovirus 1:503–504
Chrysovirus(es) **1:503–513**
biological properties 1:509
chryso-P3 1:509*t*
functions 1:511
'phytoreo S7 domain' 1:507
chryso-P4 1:508
functions 1:511
classification 1:503–504, 1:504*t*
evolution 1:508*f*, 1:510, 1:512*f*
gene expression 1:507
capsid proteins 1:504*t*, 1:507
RdRp 1:504*t*, 1:507, 1:508*f*
genome 1:504, 1:505*f*, 1:507*t*
5′ untranslated regions 1:506–507, 1:506*f*
CAA repeats 1:506–507
dsRNA segments 1:504–505, 1:506*f*
infections
fungi 2:287*t*
mixed infection 1:509
occurrence 1:511
replication 1:508
transmission 1:509, 1:510–511
virion properties 1:504
RdRp activity 1:504
virion structure 1:508, 1:509*f*
capsid 1:508–509, 1:510*f*
see also specific viruses
Chub reovirus (CHRV) 1:164*t*
Chum salmon aquareovirus (CSRV) 1:164*t*
CHV1 *see* Cryphonectria parasitica virus (CHV1)
CHV-EP713 virus 2:581
field trials 2:584
genetic organization 2:581, 2:583*f*
p29 expression 2:583–584
phenotypic change determinants 2:583
CHV-Euro7 virus, phenotypic change determinants 2:583
CHV-NB58 virus, genetic organization 2:582
Chytrid fungi
legume virus transmission 3:217
tombusvirus transmission 5:149–150
Chytridiomycota 4:419
retrotransposons 4:423, 4:424*t*
Cicadellid leafhoppers, phytoreovirus transmission 4:154–155
CID *see* Cytomegalic inclusion disease (CID)
Cidofovir 2:557
adenovirus infection treatment 1:29
HSV treatment 2:395
mode of action 2:395
CiMV *see* Citrus mosaic virus (CiMV)
CIN *see* Cervical intraepithelial neoplasia (CIN)
CIPHI Fact Sheets 5:357*t*
CI protein, potyvirus protein properties 4:317
Circadulina, definition 3:264
Circoviridae 1:107, 1:513, 5:11*t*
Circovirus(es) **1:513–520**
genome 1:516
associated proteins 1:517
open reading frames 1:517
infection
associated diseases 1:514
diagnosis 1:516
immune system interactions 1:516
pathogenesis 1:514
nanoviruses *vs.* 3:391
PCV transcription 1:517
replication 1:518
taxonomy 1:513, 1:514*f*
transmission 1:514
virion structure 1:514
VP1 gene/protein
antibody induction 1:517
replication 1:517
see also specific viruses
Circular dichroism (CD), inovirus structure 3:120–121
Circular genome replication, dsDNA bacteriophage genome packaging 2:308–309
Circular permutation
definition 3:161, 5:411
invertebrate iridoviruses 3:163–164
Circulating recombinant forms, definition 2:525
Circulating vaccine-derived poliovirus 4:246–248
Circulative strain-specific manner, barley yellow dwarf virus transmission 1:283
Circulative transmission
banana bunchy top virus transmission 1:274
definition 1:272
Circulative virus(es)
definition 1:482
see also specific viruses
Circulifer tenellus, beet curly top virus 1:301–302, 1:302–303, 1:305, 1:305*f*
Circumcision, HIV infection 1:60, 1:66
Cirrhosis
definition 2:241
HCV infection 2:249
cis-acting replicating elements *(cre)*
definition 4:129
narnavirus *see* Narnavirus
picornavirus *see* Picornavirus(es)
RNP complexes *see* Ribonucleoprotein (RNP)
cis-acting siRNA pathway, plant gene silencing 4:142
Citrus mosaic bacilliform virus (CMBV) 1:403
Citrus mosaic virus (CiMV) 4:523, 4:524*t*
genome sequencing 4:524–525
host range 4:525
transmission 4:525
Citrus production 1:520
Citrus psorosis virus (CPsV) 3:447, 3:448*t*
coat protein 3:450
serology 3:450
control 3:453
genome 3:449, 3:450*t*
RNA2 3:449
sequence 3:448–449
pathogenicity 3:450–451
phylogenetic analysis 3:447
transmission 3:453
Citrus root disease 1:520
Citrus sudden death (CSD) 2:91, 5:206
causal agents 2:92
classification 2:92
disease symptoms 2:92
epidemiology 2:92
geographical distribution 2:91
infection control 2:92
transmission 2:92
yield losses 2:92
Citrus sudden-death-associated virus (CSDaV) 2:92
classification 2:92
genome 2:92, 5:205
Citrus tristeza virus (CTV) **1:520–525**, 2:92, 4:197
classification 1:521
genome 1:522
defective RNAs 1:523, 1:523*f*
functions 1:522
nontranslated regions 1:522, 1:522*f*
open reading frames 1:522–523, 1:522*f*
subgenomic RNAs 1:523
geographical distribution 1:520, 1:521, 4:197–198
history 1:520
infection *see below*
replication 1:523
strains 1:521, 4:198
taxonomy 1:521
transmission 1:521, 1:524, 4:197–198
aphids 1:521, 1:524
experimental 1:520
virion structure 1:521
thread-like particles 1:520
Citrus tristeza virus infection 1:521
control measures 1:524, 4:198
certification schemes 1:524
cross-protection 1:524, 4:198
eradication 1:524, 4:198
genetically-modified resistant plants 1:525
grafting 1:524
resistance 1:524
cytopathology 1:521
diagnosis 1:524
economic impact 1:524
crop yield losses 4:197–198
host range 1:521
CIYMV *see* Clover yellow mosaic virus (CIYMV)
CJD *see* Creutzfeldt–Jakob disease (CJD)
C-Jun protein, JCV gene expression 4:264
Clades
definition 4:436
varicella-zoster virus 5:252
Classical swine fever virus (CSFV) **1:525–533**
antigenic relationships 1:338, 1:527
classification 1:336, 1:526
cytopathic isolates 1:526
epidemiology 2:247
genome 1:526
history 2:246
phylogeny 1:526–527, 1:527*f*
properties 1:526
protein expression 1:526
replication 1:526
tissue location 1:529
virion assembly 1:526
taxonomy 1:526
transmission 1:528
experimental 1:528
vaccines/vaccination 1:531, 2:248–249
booster vaccines 1:531–532
marker vaccines 1:531–532
Classical swine fever virus infection 1:528, 2:247
acute 1:528
antibodies, neutralization 1:531
chronic 1:528
clinical manifestations 2:248
diagnosis 1:530, 2:248
antibody detection 1:531
differential diagnosis 1:530
direct antigen detection 1:530
isolation in cell culture 1:530
nucleic acid detection 1:530
economic impact 1:526, 1:532
epizootiology 1:529
histopathology 1:529
history 1:526
host range 1:528
immune response 1:531
incubation period 1:528

late onset 1:528
pathology 1:529
pigs 1:528
prenatal 1:528
prevention and control 1:532
secondary infections 1:528
severity 1:528
virulence 1:528
Classification **5:9–24**
Adansonian system 5:19
databases 5:362
definition 5:9
demarcation criteria 5:19, 5:20*t*
hierarchical 5:10, 5:22
Linnaean system 5:19
order of presentation 5:11*t*, 5:21
criteria 5:21
phylogeny 4:128
polythetic 5:19
sequence comparisons 5:19, 5:20, 5:342
alignment algorithms 5:342
BLAST 5:343
global 5:343
local 5:343
multiple sequence 5:343
pairwise sequence comparison *see* Pairwise sequence comparison (PASC)
phylogenetic analysis 5:343
scoring schemes 5:342–343
species **5:401–406**
abstract classes *vs.* real objects 5:402
Aristotelian class 5:402
binomial names 5:405
classical 5:403
criteria associated 5:403
definitions 5:9, 5:21, 5:402, 5:403
demarcation 5:404
diagnostic properties 5:405
evolutionary concept 5:403
nomenclature 5:405
phenotypic concept 5:404
phylogenetic analysis 4:126–127
polythetic classes 5:401
qualitative properties 5:402
taxonomic 5:404
taxonomic classes *vs.* 5:404
typography 5:405
universal system 5:22
virus identification 5:405
universal system 5:11*t*, 5:22
families 5:23
genera 5:22
orders 5:23
species *see above*
virus properties 5:342, 5:347
see also Nomenclature; Taxonomy
Class-switching *see* Immunoglobulin(s)
Clathrin-dependent endocytosis
Border disease virus 1:340
yellow fever virus 5:471
CLCuD *see* Cotton leaf curl disease (CLCuD)
CLCuMV *see* Cotton leaf curl Multan virus (CLCuMV)
Cleave site mutations
Nudaurelia ω virus 5:34*f*, 5:35–36
tetraviruses 5:35
Clethrionomys, cowpox virus hosts 1:577, 1:578
Clevudine, hepatitis B treatment 1:152–153
Climate, vector transmission 5:273
Climate change, zoonotic effects 5:494
Clinical lung disease, Visna-Maedi viruses 5:424
Clinical trials, gene therapy *see* Gene therapy
Clonal selection, B-cells 3:61
Cloning vector(s)
bacteriophage M13 2:197
definition 2:190
Closed circular DNA, avian hepadnavirus 2:327
Closteroviridae 1:521, 2:255, 5:11*t*
Closterovirus 1:521
Clouds, quasispecies 2:178–179
Clover yellow mosaic virus (CIYMV) 3:447, 4:311*t*
verbenaceae infection 4:218
CLRV *see* Cherry leafroll virus (CLRV)
'Clump' disease, pecluvirus infections 4:97–98
CMD *see* Cassava mosaic disease (CMD)
CMLV *see* Cherry mottle leaf virus (CMLV)
CMV *see* Cucumber mosaic virus (CMV)
c-myc, reticuloendotheliosis virus infection 4:416
CO_2 (carbon dioxide) symptom *see* Sigma virus (SIGV)
Coagulopathy
Ebola virus infection 2:64, 2:203
Marburg virus infection 2:203, 3:277
Coastal Plains virus 4:184*t*
Coat protein (CP) 3:354
definition 1:432
plant engineered resistance 4:156–157, 4:157–160, 4:162, 5:439
RNA complexes 3:24
see also specific viruses
Cocksfoot mild mosaic virus (CMMV) 4:646*t*
Cocksfoot mottle virus (CfMV) 4:646*t*
infection resistance 4:651–652
virion structure 4:650*f*
Cocoa necrosis virus (CNV) 3:406*t*
Coding capacity, replication 4:407
Coding sequences, pecluvirus genome 4:99
Codon 129 polymorphism 1:371, 1:372, 4:330
CJD 4:331–332
prion strain propagation 4:335
Codons, definition 4:623
Co-evolution
adenoviruses 1:1
chronic infection 4:111
definition 2:317
Cohabitation experiments
definition 5:91
salmon pancreas disease virus 5:94
Cohort studies 2:143
definition 2:140
Coho salmon aquareovirus (CSRV) 1:164*t*
Coho salmon reovirus (CSRV) 1:164*t*
protective effects 1:167
Coiled-coil motif, definition 4:97
Coital exanthema 1:362–363, 1:366
Coleus blumei viroid 1 (CbVd1), transmission 5:335
Coliphage(s)
bacteriophage conversion 2:445–446
propagation 2:444–445
Colorado tick fever (CTF) 1:533–534
clinical features 1:538–539
cytopathic effects 1:536
diagnosis 1:538–539
distribution 1:534, 1:534*f*
incidence 1:533–534
male *vs.* females 5:489
neurological disorders 1:538–539
symptoms 5:489
Colorado tick fever virus (CTFV) 1:533, 4:536, 5:489
characteristics 1:171*t*
cross-reactivity 1:536–537
distribution 1:534, 1:534*f*
genome 1:536, 1:537*f*
geographical distribution 5:489
history 1:533–534
host range 1:534–535, 5:489
infection *see above*
serotypes 1:533
transmission 1:535
blood transfusion 1:535
as type species 4:536*t*
vectors 1:534
zoonoses 5:489
Color-coded table(s), virus databases 5:352
Colostrum-deprived (CD) calves, definition 5:151
Coltivirus 1:533, 1:539*f*, 4:536, 4:536*t*
Coltivirus(es) **1:533–541**, 1:539*f*
antigenic relationships 1:536
classification 1:533, 1:539*f*
distribution 1:534
epidemiology 1:534
genetic relationships 1:536
genome 1:535, 1:535*f*
dsRNA segments 1:536
history 1:533
host range 1:535
infection
clinical features 1:538
diagnostic assays 1:538
immunity 1:540
prevention 1:540
treatment 1:540
prototype species 4:536
replication 1:535
transmission 1:535
vectors 1:535
type species 4:536*t*
virion properties 1:535
stability 1:535–536
virion structure 1:172, 1:535*f*
see also specific viruses
Comet assay, vaccinia virus spread 5:248
Commelina virus X (ComVX) 4:311*t*
Commelina yellow mottle virus (CoMYV), genome 1:458
discontinuity 1:458, 1:461
gene map 1:461*f*
open reading frames 1:461
size 1:461
Commercialization, transgenic plants 4:5
Common ancestry, icosahedral tailed dsDNA bacteriophages 3:36
Common cold 4:468
clinical features 4:472
lower respiratory tract 4:472, 4:474*f*
sleep pattern disruption 4:472
coronavirus infection 1:554–555
definition 4:552
epidemiology 4:471
immune response 4:472
antibody-mediated virus inactivation 4:472–473
cellular immunity 4:473
immunoglobulin A 4:472–473
immunoglobulin G 4:472–473
incubation period 4:472
National Center for Health Statistics 1:541
pathogenesis 4:472
proinflammatory mediators 4:472, 4:473*f*
prevention/control 4:473
antiviral therapy 4:473–475
vaccine development 4:473–475
rhinovirus infection *see* Rhinovirus infections
symptoms 1:541
transmission 1:541, 4:472
aerosol inhalation 4:472
hand-to-hand contact 4:472
Common region (CR)
bean golden mosaic virus 1:298
mungbean yellow mosaic viruses 3:366
Common vehicle transmission, definition 2:140, 2:145
Common warts, papillomavirus associated 4:16
Community ecology
bacteriophages *see* Bacteriophage(s), ecology
Comoviridae 1:90*f*, 1:569, 1:573, 3:405–407, 5:11*t*
Comovirus 1:569
Comovirus(es)
plant resistance genes 4:179*t*
see also specific viruses
structure 5:391–392
see also specific viruses
Compacted genomes, RNA virus experimental evolution 4:363
Comparative genome analysis, Infectious salmon anemia virus 2:229
Competition, bacteriophage population ecology 2:75
Complement 3:110, 3:112
activation 3:79–80, 3:79*f*, 3:112
antiviral properties 3:110
control proteins
HIV-1 infection 3:110
receptor role 5:322
definition 3:78, 3:104, 3:112

Complement (*continued*)
infection neutralization role 3:414
KSHV immune evasion 3:199–200
opsonization 3:110
pathways 3:110, 3:112
regulation 3:110
Complementarity-determining region(s) (CDRs)
antibody footprint 1:138
IgG paratopes 1:138
Complementary sense, definition 1:295
Complementary sense genes, maize streak virus 3:267
Complement fixation assay(s)
coltivirus infection diagnosis 1:540
Japanese encephalitis diagnosis 3:186
orthobunyavirus 3:482
principles 2:31
Compositae virus infections 4:221
see also individual species
see also specific viruses
CoMYV *see* Commelina yellow mottle virus (CoMYV)
Concatamer(s)
definition 2:306, 4:475, 5:411
replication 2:308–309
Confocal laser scan microscopy (CLSM) 2:26–27
Conformational selection model 4:330
Congenital infections/disease
Akabane virus 1:76
definition 4:514
HCMV infection 2:483
herpesviruses 2:146
rubella virus *see* Congenital rubella syndrome (CRS)
Congenital malformations, Akabane virus 3:480–481
Congenital rubella syndrome (CRS) 4:514, 4:518
clinical manifestations 4:519
diagnosis 4:520
histopathology 4:519
neuropathology 4:519
transmission 5:111
virus persistence 4:519
Conjunctiva 2:491
infection 2:491
Conjunctival hyperemia, definition 1:580
Conjunctivitis
acute hemorrhagic 2:495
coxsackievirus infections *see* Coxsackievirus(es)
definition 2:130
enterovirus 70 2:132
human adenoviruses 2:495
human enterovirus D 2:124
adenoviruses *see* Human adenovirus(es)
associated RNA viruses 2:492
EBV 2:496
Connecticut virus (CNTV) 1:116
Connochaetes gnu (wildebeest), bovine malignant catarrhal fever 1:363
Consensus sequence, definition 4:164
Conservation (insect pest control) 3:125
Conserved consensus sequences, geminivirus genome 4:168
Conserved epitope binding, antigen–antibody complexes 3:417–418
Conserved functional domains, three-hybrid assay 3:28–29
Conserved genes
Herpesvirus Ateles 4:588
Herpesvirus Saimiri 4:588
tailed bacteriophage evolution 2:182*f*
cis-acting Conserved sequence elements (CSEs) 5:120–121
Constitutive transport element (CTE), Simian retrovirus D 4:625
Continuous cropping, pecluvirus infection control 4:102
Continuous epitopes 1:138, 1:139*f*
Contractile tails, definition 3:30
Contrast transfer function (CTF), cryo-electron microscopy 1:605–606
Convalescence stage, Crimean-Congo hemorrhagic fever 1:601
Conventional dendritic cell(s) (cDC)
activation 3:106
definition 3:104
Convergence model, epidemiology 2:141, 2:142*f*
Convergent evolution, definition 4:256
Convolvulaceae 4:659–660
Coprophagy, definition 3:89
Coquillettidia perturbans, eastern equine encephalitic virus transmission 5:103–104
Co-receptor(s)
definition 5:319
infection role 5:323
Ab-mediated neutralization 3:415
receptor *vs.* 5:323
specificity 5:323
see also specific viruses
Core genes, baculoviruses 1:249
C-*ori,* definition 3:264
Corky ringspot (potato disease) 4:307, 4:307*f*
Cornea 2:491
immunology 2:492
infection 2:491
Corn leaf necrosis disease complex 1:482
Corn lethal necrosis (CLN) 3:259
Corn stunt disease complex 1:482
symptoms 1:482
transmission 1:482
Coronaviridae 1:177, 1:549, 1:554–555, 2:122, 3:419–420, 4:553–554, 5:111*t*, 5:151–152
Coronavirus 1:549, 2:230
Coronavirus(es) **1:549–554**, **1:554–563**, 2:122
classification 1:549
evolution 1:550*t*, 1:552, 1:553*f*
gene expression 1:550
genera 1:549, 2:230
genome 1:550, 1:555–556, 1:559–560, 1:559*t*, 3:424*f*
accessory genes 3:426–427
nonstructural proteins 3:423–424
nucleotide cyclic phosphodiesterase 3:425–426
open reading frames 1:550–551, 1:555–556
proteolytic processing 3:424
replicase genes 3:425*f*
replication–transcription complex 1:551, 3:423–424, 3:428
size 1:550–551
human 1:553
infection *see below*
nomenclature 1:554–555
recombination 1:552, 1:558–559, 1:560
replication 1:551, 1:552, 1:557, 1:558*f*
glycoprotein synthesis 1:551–552
host cell attachment 1:551–552, 1:556–557
host cell fusion 1:557
ORF1b translation 1:557
replicase function 1:557
template switching 1:557–558
transcription 1:557–558, 1:559*f*
virus particle formation 1:552
virus particle release 1:552
reverse genetics 1:560
RNA recombination 4:377, 4:379, 4:379*f*
SARS CoV *see* Severe acute respiratory syndrome coronavirus (SARS CoV)
taxonomy 1:549, 3:420–421
virion properties 1:549
virion structure 1:549, 1:555–556, 1:555*f*, 2:122*f*
accessory proteins 1:559
envelope protein 3:423
membrane protein 1:557
nucleocapsid protein 1:557, 3:421–423
spike glycoprotein 1:556–557, 1:556*f*
S protein 3:423
see also specific viruses
Coronavirus infections
antiviral drug development 1:561
associated diseases 1:553
co-infection 1:553
persistent infection 1:553
associated species 1:550*t*
cellular effects 3:429
common cold 1:554–555
host range 1:553
pigs 1:553
respiratory infection 2:555
target tissues 1:549
transmission 2:122
vaccines/vaccination 1:553
development 1:561
virus–host associations 4:128
Corticoviridae 3:1, 5:11*t*
Corticovirus(es)
genome delivery 5:368
see also Icosahedral dsDNA bacteriophages; *specific viruses*
Corticovirus PM2 3:1, 3:2*t*
genome 3:5
encoded proteins 3:2*t*
structure 3:2
membrane 3:3
Corvids, West Nile virus vectors 5:445
Coryza *see* Common cold
Cosmid transfection system, varicella-zoster virus 5:256
Cote d'Ivoire, HIV infection 1:63
Cote d'Ivoire Ebola virus (CIEBOV) 2:57
Cotesia congregata bracovirus (CcBV)
BV-mediated immunosuppression 4:254–255
C-type lectins 4:255
gene expression 4:250–251
genome 4:260
Cotesia kariyai, polydnavirus immunoevasive activity 4:255–256
Co-transfection, definition 1:237
Cotton, begomovirus resistance 2:104
Cotton leaf curl Alabad virus (CLCuAV) 1:566
Cotton leaf curl Burewala virus (CLCuBV) 1:566
Cotton leaf curl disease (CLCuD) 2:102
begomovirus satellite identification 1:315–316
causal agents 2:102
see also β ssDNA satellite(s)
control 1:568, 2:102
crop yield losses 2:102
DNA-1 component 1:565
definition 1:565
nanovirus Rep-1 satellites *vs.* 1:565
Rep protein 2:102
transmission 2:102
DNA β components 1:564, 1:565*f*, 1:566
begomovirus association 1:566
identification 1:564–565
replication 1:566
engineered resistance 1:568
post-transcriptional gene silencing 1:568
transcriptional gene silencing 1:568
epidemiology 2:102
etiology 1:564
elucidation 1:564
geographical distribution 1:563, 2:102, 2:103*f*
historical aspects 1:563, 2:102
in Africa 1:563
etiology elucidation 1:564
in Southern Asia 1:563
monopartite begomoviruses 1:566, 1:567*f*
replication 1:566
natural resistance 1:568
breakdown of 1:568
species 1:568
symptoms 1:563, 1:564*f*, 2:102, 2:104*f*
transmission 2:102–104
trans-replication 1:320
Cotton leaf curl Gezira virus 1:566–568, 1:567*f*
Cotton leaf curl Kokhran virus (CLCuKV) 1:566
Cotton leaf curl Multan virus (CLCuMV) 1:564
begomovirus satellite identification 1:315
β ssDNA functions 1:318
Cotton leaf curl Rajasthan virus (CLCuRV) 1:566
Cotton leaf curl virus (CLCuV) **1:563–569**
classification 2:102
DNA satellites 4:530

Cottontail rabbit *(Sylvilagus floridanus),* Shope fibroma virus infection 3:225
Cottontail rabbit papillomavirus (CRPV) 4:33
  pathology 4:29–30
  vaccination 4:33
Councilman bodies, yellow fever 5:475
Counterterrorism, mathematic modeling in epidemiology 2:147–148
Courtois, Ghislaine, Crimean-Congo hemorrhagic fever 1:597
Covalently closed circular DNA (cccDNA)
  avian hepadnavirus replication 2:327, 2:329–330, 2:331
  hepatitis B virus genome 2:360–361
Covert infection, definition 3:161
Cowpea chlorotic mottle virus (CCMV)
  Brome mosaic virion *vs.* 1:385
  structure 1:387, 1:388*f*, 5:382*f*, 5:385, 5:386*f*, 5:391
    capsid 1:388
    hexamer units 5:385
    icosahedral asymmetric unit 5:385
    pentamer units 5:385
    pH-dependent structural transitions 1:388
  transmission 1:390
Cowpea mosaic virus (CPMV) **1:569–574**
  in biotechnology 1:573
    plant expression vectors 1:573–574, 1:573*f*
  genome 1:570
    in vitro translation 1:571
    open reading frame 1:571
    RNA-1 1:571, 1:572*f*
    RNA-2 1:571, 1:572*f*
    sequencing 1:570–571
  historical aspects 1:569
  movement in plants 3:352
  particle properties 1:569
    electrophoresis 1:570
    nonenveloped particles 1:569
    RNA sedimentation behavior 1:569, 1:570*f*
  phylogeny 1:573
  proteins 1:571
    cell-to-cell transmission 1:573
    electrophoresis 1:570
    RdRp 1:572–573, 1:572*f*
    RNA-1 encoded 1:572, 1:572*f*
    RNA-2 encoded 1:573
    small basic protein 1:570
  purification 1:569
    cesium chloride gradients 1:569–570
    sucrose density gradients 1:569
  replication 1:573
    membrane association 1:573
  structure 1:570
    X-ray crystallography 1:570
  transmission 1:569
  as type member 1:569
  vectors, transmission 1:569
Cowpea stunt 3:218
Cowpox
  Apodemus 1:577, 1:578
  cats 1:577
    clinical features 1:578–579
    tissue tropism 1:578, 1:579
    virus transmission 1:578
  clinical features 1:578
  histopathology 1:579
  human infections 1:578, 1:579
  immune response 1:579
  pathology 1:579
  prevention and control 1:579
  rodents
    clinical features 1:579
    epidemics 1:576
    as a host 1:577
    tissue tropism 1:577
  smallpox vaccination 4:640
Cowpox-like virus(es)
  zoonoses 5:489
  see also specific viruses
Cowpox virus (CPXV) **1:574–580**
  accidental hosts 1:577
  A-type inclusions 1:574–575, 1:575*f*, 1:576
    associated proteins 1:576
  Brighton Red strain 1:575
    evolution 1:577
    genome size 1:575
    protein coding 1:576
  classification 1:574
  distribution 1:576
  epidemiology 1:577
  evolution 1:577, 2:182
  genetics 1:577
  genome 1:575
    stability 1:575
  history 1:574
  host range 1:577
  infection *see* Cowpox
  physical properties 1:576
    inactivation 1:576
    rodent epidemics 1:576
  propagation 1:577
  protein properties 1:576
    immune modulation 1:576
  replication 1:576
  serology 1:577
  smallpox vaccination 5:243
  taxonomy 1:574
  tissue tropism 1:578
  transmission 1:578
  virion properties 1:575, 1:575*f*
  virulence 1:578
  zoonoses 5:490
Coxsackie-adenovirus receptor (CAR) 1:18–19, 1:22, 2:129, 5:323
Coxsackie A virus infection, clinical manifestations 1:582
Coxsackie B virus
  capsid gene sequences 1:583
  host cell binding 5:322–323
  infection
    clinical manifestations 1:582
    myocarditis 2:68–69
    neonates 1:585
  RNA replication 4:136
Coxsackievirus 1:581*t*
Coxsackievirus(es) **1:580–587**
  classification 1:581
    typing systems 1:581
  distribution 1:582
  epidemiology 1:582
    common serotypes 1:582
  evolution 1:583
    nucleotide substitutions 1:583
  genetics 1:583
    diversity 1:583
    genome organization 1:584*f*
  history 1:580
  host cell attachment 1:543–544, 1:582
  host range 1:581
  infections *see below*
  propagation 1:581
    cell systems 1:582
    clinical specimens 1:582
  replication 2:67
  taxonomy 1:581, 1:581*t*
  transmission 1:582
  virion structure 1:582, 1:583*f*
  see also specific viruses
Coxsackievirus infections
  acute hemorrhagic conjunctivitis 1:585, 1:585*t*
    disease characterization 1:585
    incubation period 1:585
  clinical manifestations 1:584, 1:585*t*
    acute hemorrhagic conjunctivitis *see above*
    cardiac disease 1:586
    central nervous system disease 1:585
    diabetes 1:586
    febrile rash 1:585
    neonatal disease 1:585
    perinatal disease 1:585
    pleurodynia 1:585
    prenatal disease 1:585
    respiratory disease 2:552
  immunity 1:583
    heterotypic response 1:583–584
  pathogenesis 1:582, 1:583
    disseminated infection 1:583
    primary infection site 1:583
  prevention 1:586
  treatment 1:586
Cp-1 bacteriophage 4:402–403
CP binding site (CPB), alfalfa mosaic virus 1:82–83
CP gene/protein
  transgenic plants 4:5
CpGV *see* Cydia pomonella granulovirus (CpGV)
CPHV *see* Chandipura virus (CPHV)
CPMV *see* Cowpea mosaic virus (CPMV)
C (capsid) protein
  measles virus 3:287
  yellow fever virus 5:471
CPsV *see* Citrus psorosis virus (CPsV)
CPV-1 *see* Cypovirus 1 (CPV-1)
CPXV *see* Cowpox virus (CPXV)
CR2 (cell surface receptor)
  EBV binding 5:322
  structure 5:322
Cranefly *(Tipula paludosa),* larval infection 3:161
cre see cis-acting replicating elements *(cre)*
Crenarchaeal virus(es) **1:587–596**
  diversity 1:588*t*
    classification 1:587
    evolutionary relationships 1:594–595
  gene functions 1:594
    archaeal homologs 1:594
    dUTPase 1:594
    glycotransferases 1:594
    P-loop ATPases 1:594
    transcription regulators 1:594
  genomes
    gene transcription 1:593
    inverted terminal repeats 1:593
    linear genomes 1:593
    orthologous genes 1:594
    replication 1:593
    structure 1:593
  genomics 1:592*f*, 1:594
  history 1:587
  morphotypes 1:587
    bottle-shaped viruses 1:590
    droplet-shaped viruses 1:590
    filamentous viruses 1:589
    rod-shaped viruses 1:589
    spherical viruses 1:590
    spindle-shaped viruses 1:587
  virion composition 1:590
    proteins 1:590–591
  virus–host interactions 1:591
    optimum growth temperatures 1:591
  see also specific viruses
Crenarchaeota 5:412
Creutzfeldt–Jakob disease (CJD) 1:474, 4:340, 5:190
  characterization 5:190–191
  diagnosis 4:334, 5:190–191
  familial 5:191, 5:192
  iatrogenic 4:332, 5:191
  mortality 5:190–191
  progression 5:190–191
  PrP^{SC} tissue distribution 4:334
  sporadic 4:331–332, 5:191
    subtypes 5:191
  variant *see* Variant Creutzfeldt–Jakob disease (vCJD)
Cricket paralysis virus (CrPV)
  gene expression 5:5
  genetic diversity 5:6
  geographical distribution 2:43
  host range 2:40–41
  IRES structure 2:41*f*
  structure 2:39*f*
Crimean-Congo hemorrhagic fever (CCHF)
  at-risk groups 1:602
  clinical features 1:601

Crimean-Congo hemorrhagic fever (CCHF) (*continued*)
convalescence stage 1:601
hemorrhagic manifestations 1:601, 1:601*f*
hemorrhagic stage 1:601
incubation stage 1:601
neuropsychiatric changes 1:601
prehemorrhagic stage 1:601
control 1:602
diagnosis 1:602
ELISA 1:602
immunofluorescent assays 1:602
RT-PCR 1:602
virus isolation 1:602
differential diagnosis 1:602
historical aspects 1:596–597
Courtois, Ghislaine 1:597
mortality 1:601
pathogenesis 1:601
capillary fragility 1:601–602
host-induced mechanisms 1:602
thrombocytopenia 1:601–602
virus localization 1:601–602
prevention 1:602
acaricides 1:602–603
exposure minimization 1:602–603
insect repellents 1:602–603
tick control 1:602–603
symptoms 1:596
treatment 1:603
blood transfusions 1:603
fluid replacement 1:603
ribavirin 1:603
supportive 1:603
zoonoses 5:487
Crimean-Congo hemorrhagic fever virus (CCHFV) 1:391*t*, **1:596–603**
characteristics 1:171*t*
classification 1:597, 1:598*t*
ecology 1:597
birds 1:597
reservoir species 1:597
epidemiology 1:597
seasonal aspects 1:597–598
genome 1:394*f*, 1:598
L segment 1:599
M segment 1:599
S segment 1:599
geographical distribution 1:597, 5:487–488
history 1:596
infection *see* Crimean-Congo hemorrhagic fever (CCHF)
L RNA segment 1:599
phylogenetics 1:600–601
phylogenetics 1:599, 1:600*f*
L RNA segment sequence 1:600–601
M RNA segment sequence 1:600–601
S RNA segment sequence 1:600–601
replication 1:599
reservoir hosts 1:597
taxonomy 1:597
transmission 5:487–488
Hyalomma ticks 1:596, 1:597–598, 1:599*f*
virion structure 1:598, 1:599*f*
envelope glycoproteins 1:598–599
large polypeptide 1:598–599
nucleocapsid protein 1:598–599
Crimean hemorrhagic fever (CHF) *see* Crimean-Congo hemorrhagic fever (CCHF)
Crimson clover latent virus (CCLV) 3:406*t*
Crinivirus 2:87
Crinivirus(es) 2:88
infection symptoms 2:88
sweetpotato infections 4:661*t*
transmission 2:88
see also specific viruses
Cripavirus 2:37, 2:38*t*
Cripavirus, insect infections 3:126*t*
Crixivan *see* Indinavir
crmA gene 1:576
Crocodile poxvirus(es) 4:326–327
Crop(s)
disease resistance
barley yellow dwarf virus control 1:285–286
bean golden/yellow mosaic disease management 1:300–301
beet necrotic yellow vein virus control 1:309–310
benyvirus control 1:309–310
maize chlorotic mottle virus 3:263
maize streak virus control 3:270
pomovirus control 4:287
see also Plant(s), natural disease resistance
engineered resistance 2:454, 4:157, 4:159*t*
pest-induced losses
studies 4:197
vegetatively propagation 4:197
viroid infections 5:337–338
transfer from wild species 5:338
see also specific crops
see also specific viruses
Cro protein 5:184
Cross-breeding, ornamental plant species virus infections 4:208, 4:226–229
Cross-linking, bacteriophage assembly 1:439
Cross-protection 2:454, 5:438*f*
citrus tristeza virus infection control 1:524
cucumber mosaic virus 1:615
definition 1:520, 1:614, 3:46, 5:433
papaya ringspot virus 4:5
plant gene silencing 4:145
vegetable viruses 5:286–287
watermelon mosaic virus 5:437
zucchini yellow mosaic virus 5:437
Cross-reactivity
antibodies 5:87–88
antigenic variation 1:127
psittacinepox virus 2:278–279
Cross-species transmission
hepatitis E virus 2:381
HIV *see* HIV
paramyxoviruses 4:40–42
SIV *see* Simian immunodeficiency virus (SIV)
Cross-talk, apoptosis 1:155–157
Croup, definition 2:551
CRPV *see* Cottontail rabbit papillomavirus (CRPV)
CRS *see* Congenital rubella syndrome (CRS)
CRSV *see* Carnation ringspot virus (CRSV)
'Crumbly fruit' Raspberry bushy dwarf virus infection 3:38, 3:38*f*
Crustaceans, aquareovirus infections 1:167
Cryo-electron microscopy (cryoEM) **1:603–614**
advantages 2:81*f*, 2:82
biological insights 1:610
definition 1:193, 4:68
history 1:603–604
icosahedral reconstruction 1:605, 1:605*f*, 1:608, 1:608*f*
algorithms 1:606
contrast transfer function 1:605–606
deconvolution 1:606
power spectrum 1:605–606, 1:606*f*
procedure steps 1:605–606, 1:605*f*
signal-to-noise ratio 1:605–606
image analysis 2:84–85
resolution 2:84–85
limitations 2:82
radiation damage 2:82
low-dose cryo-tomographic imaging 1:608, 1:609*f*
resolution 1:609–610
low-dose single particle imaging 1:604
defocus values 1:605
electron dose 1:605
recording 1:605
sample storage 1:604–605
nonenveloped virus structure 5:380, 5:381
sample preparation 1:604, 2:81, 2:81*f*
concentration 2:81–82
temperature 2:81–82
vitrification 1:604, 2:84
single-particle density maps 1:606, 1:607*f*
structural motif conservation 1:610, 1:611*f*
structural polymorphism in maturation 1:611, 1:612*f*
technological advances 2:82
viral genome 1:612, 1:613*f*
virus–antibody complex 1:612
virus–cell receptor complex 1:612
virus particle modeling, density map constraints 1:608
docking 1:608
see also specific viruses
Cryo-sectioning 2:83
vitrification 2:83–84
methods 2:83–84
Cryo-tomographic imaging (cryo-ET)
analysis 2:84–85
resolution 2:84–85
CEMOVIS 2:84
cryo-electron microscopy 1:604, 1:608
sample preparation 2:82
Cryparin 2:578, 2:578*f*
Cryphonecria parasitica virus (CHV1)
infection
chestnut blight, 2:580
transcriptional profiling studies, 2:583
Cryphonectria hypovirus 1 - EP713 (CHV-1/EP713)
host range, 2:285
infection, 3:379
MyRV-1/Cp9B21 double infection, 3:381
Cryphonectria parasitica, 3:378
CHV-1, biological control, 2:285
mycoreovirus infection, 3:379f
Cryphonectria parasitica virus (CHV1) 2:289, 2:576
classification 2:576
gene expression 2:577–578
genome 2:576, 2:576*f*
history 2:576
hypovirulence *see* Hypovirulence
infection
chestnut blight 2:575, 2:575*f*, 2:580
fungal mating, effects 2:578
signal transduction 2:577
slow growth mutations 2:577
symptoms 2:575*f*, 2:577
vesicle accumulation 2:577, 2:577*f*
protein expression studies 2:578, 2:579*f*
processing signals 2:578
protease p29 2:579
vesicle accumulation 2:579
structure 2:576
transcriptional profiling studies 2:582–583
transmission 2:576
viruses associated 2:575–576
Cryphonectria hypovirus 1 - EP713 (CHV-1/EP713)
host range 2:285
infection 3:383
MyRV-1/Cp9B21 double infection 3:381
Cryphonectria parasitica 3:378
CHV-1, biological control 2:291
mycoreovirus infection 3:379*f*
'Cryptic epitopes' HIV vaccine development 5:242
Cryptotope(s)
definition 1:137–138
location 1:137–138
TMV antigenicity 5:58
Cryptovirus(es) 4:68–69
associated plant hosts 1:99, 1:100*t*
infection, viral concentrations 4:69
mixed infections 1:102
RdRp 1:99
molecular mass 1:101
phylogeny 1:103*f*
replication 1:101–102
sequences 1:101
translation 1:101
serology 1:102
symptoms of infection 1:102
transmission 4:69
see also Alphacryptovirus(es); Betacryptovirus(es); *specific viruses*
Crystallization, alfalfa mosaic virus coat protein 1:83
CsCMV *see* Cassava common mosaic virus (CsCMV)
CSDaV *see* Citrus sudden-death-associated virus (CSDaV)

CSEI (Center for the Study of Emerging Infections) 5:357*t*
CSFV *see* Classical swine fever virus (CSFV)
CSL binding
  EBV-encoded nuclear antigen 2 2:161
  EBV-encoded nuclear antigen 3 family 2:162
CslV *see* Campoletis sonorensis ichnovirus (CsIV)
CSSV *see* Cacao swollen shoot virus (CSSV)
CsVC *see* Cassava virus C (CsVC)
C-terminal domain, *TRIM5* gene/protein 2:469–470
CTF *see* Colorado tick fever (CTF)
CTFV *see* Colorado tick fever virus (CTFV)
'CTL exhaustion' viral titer effects 3:82
CTLs *see* Cytotoxic T-cells (CTLs)
CTV *see* Citrus tristeza virus (CTV)
CTXΦ bacteriophage *see* Bacteriophage CTXΦ
Cucumber mosaic virus (CMV) **1:614–620**, 5:287
  bacopa virus infections 4:215
  characteristics 5:63*t*
  classification 1:615
  control, vector control 1:619
  ecology 1:618
  epidemiology 1:618
  evolution 1:619
  genetics 1:617
    2b protein 1:617
    3a protein 1:617
    3b protein 1:617
    hypersensitivity response 1:617
    RNA functions 1:617
    variation 1:619
  genome 1:616, 5:63–64
    nontranslated regions 1:616
    RNA species 1:616
  geographical distribution 1:615
    subgroup IB 1:615
    subgroup II 1:615
  history 1:615
  infection
    chlorosis 1:617–618
    control 1:619
    cucurbits 5:287–288, 5:288*f*
    cytopathology 1:618
    dahlia 4:223, 4:223*f*
    economic impact 1:618–619
    genetically-modified resistant plants 1:619
    host range 1:615, 4:208
    Impatiens 4:215, 4:217*f*
    mixed 5:287–288
    necrosis 5:287–288
    Osteospermum 4:221, 4:222*f*
    pathology 1:617
    pelargonium 4:211, 4:211*f*
    pepper plants 5:287–288
    petunia 4:213, 4:213*f*
    potato 4:300*t*
    sweetpotato 4:661*t*, 4:668
    tobacco 5:63
  movement 1:617
    2b protein 1:617
    3a protein 1:617
  propagation 1:615
  replication 1:617
    1a protein 1:617
    2a protein 1:617
    initiation 1:617
    virion assembly 1:617
  RNA satellites 1:616
    control 1:619
  small, linear single stranded RNA satellites 4:531–532
    origin 4:534
    replication 4:533
    structure 4:534
    variation/evolution 4:534
  synergism 5:435
  taxonomy 1:615
  transgenic plants 4:157, 4:158*f*
    gene silencing suppression 5:331
  transmission 1:617, 1:618
    aphids 1:618, 5:287–288
    efficiency 1:618
    horizontal 1:618
    seed 1:618
  vector recognition 5:279
  virus properties 1:616
    stability 1:616
  virus structure 1:616
Cucumber soil-borne virus (CSBV) 1:453
Cucumber vein yellowing virus (CVYV) 2:90
  classification 2:90
  epidemiology 2:90
  genome 2:90
  geographical distribution 2:90
  host range 2:90–91
  infection
    control 2:91
    disease symptoms 2:90
    yield losses 2:90
  P1 protein 4:317
  transmission 2:90
Cucumis melo (melon), dsRNAs 2:112
Cucumovirus 1:81, 1:387, 1:615, 3:46
Cucumovirus(es)
  disease resistance genes 4:179*t*
  petunia infection 4:213, 4:213*f*
  sweetpotato infections 4:661*t*
  tRNA-like structure 1:81
  see also specific viruses
Cucurbit aphid-borne yellows virus
  classification 5:344
  synergism 5:435
Cucurbit yellow stunting disorder virus (CYSDV) 2:88
  classification 2:89
  divergence 2:89
  epidemiology 2:89
  genome 2:89
    encoded proteins 2:89
    open reading frames 2:89
  geographical distribution 2:88
  infection
    control 2:89
    disease symptoms 2:88
    host range 2:89
    yield losses 2:88
  transmission 2:89
  virion structure 2:89
Culex
  EEEV transmission 5:99–100
  Saint Louis encephalitis virus transmission 4:655, 4:657–658
  West Nile virus transmission 5:443–444
Culex molestus, West Nile virus transmission 5:443–444
Culex nigripalpus, West Nile virus transmission 5:443
Culex nigripalpus nucleopolyhedrovirus (CuniNPV) 1:220
  auxiliary functions, associated genes 1:224
  DNA replication 1:223
    bro proteins 1:223
    essential genes 1:223
  genome 1:222
    homology 1:222
  infection 1:222
    histopathology 1:221*f*
    pathology 1:220–221
    rate 1:222
  life cycle 1:221
  phylogeny 1:224–225, 1:224*f*
  RNA transcription 1:222–223
    species-specific differences 1:222–223
    transcription factors 1:222–223
  transmission 1:221–222
    salt effects 1:221–222
  virion functions 1:223–224
    phenotypes 1:223
  virion occlusion 1:223–224
    associated genes 1:223–224
    occlusion body morphology 1:223–224
Culex pipiens, West Nile virus transmission 5:443
Culex pipiens densovirus (CpDNV) 4:82–83
Culex taeniopus, eastern equine encephalitic virus transmission 5:103–104
Culex tarsalis
  Saint Louis encephalitis virus transmission 4:655
  western equine encephalitic virus transmission 5:104
  West Nile virus transmission 5:443
Culicoides
  African horse sickness virus transmission 1:41
  definition 1:37, 3:454
  orbivirus transmission 3:457
Culicoides brevitarsis, Akabane virus transmission 1:78–79
Culicoides paraensis, Oropouche virus vector 3:482
Culicoid midges, definition 2:234
Culiseta melanura
  eastern equine encephalitic virus transmission 5:103–104
  EEEV transmission 5:99–100
  highlands J virus transmission 5:103–104
Cullin 5-based E3 ubiquitin ligase, in malignant transformation 1:14*f*, 1:15
Cultivar decline, definition 4:659
Cultural practices
  maize streak virus 3:270
  mungbean yellow mosaic virus management 3:369–370
  ornamental plant species virus infections 4:208
  pecluvirus infection control 4:102
CuniNPV *see* Culex nigripalpus nucleopolyhedrovirus (CuniNPV)
Curation, definition 5:348
Curtovirus 1:302, 2:98, 4:165
Curtovirus infections
  resistance genes 4:179*t*
  symptomatology 4:166
Curvularia protuberata, tropical panic grass association 2:290
Curvularia thermal tolerance virus (CThTV) 2:290
CUS-1 prophage 3:123
CUS-2 prophage 3:123
Cutaneous adult T-cell lymphoma (CTCL) 2:568, 2:568*f*
Cutaneous human papilloma virus(es)
  detection 4:14
  distribution 4:14
  transmission 4:10
Cutaneous lesions, varicella-zoster virus infection 5:254
Cut-like protein1/CCAT displacement protein 3:340
'Cutting-out' campaigns, cacao swollen shoot virus infection 1:404, 1:409
CVYV *see* Cucumber vein yellowing virus (CVYV)
CWD *see* Chronic wasting disease (CWD)
CWMV *see* Chinese wheat mosaic virus (CWMV)
CWP virus 5:413*t*
CXC chemokine(s) 1:623
CXCR4 chemokine receptor
  HIV infection 2:506, 2:518–519, 2:535–536, 5:323
  SIV-host cell interactions 4:618
CXCR5 chemokine receptor 1:624
Cyanosis, definition 1:37
Cycas necrotic shunt virus (CNSV) 3:406*t*
Cyclins, retroviral 2:214–215
Cyclosporine A (CsA)
  HCV replication 2:373
  HIV infection resistance 2:470
Cydia pomonella granulovirus (CpGV) 1:247
  gene content 1:214, 1:215*f*, 1:215*t*
    imperfect palindromes 1:218
  inhibitors of apoptosis 1:216–217
  insect pest control 3:130–131
CYDV *see* Cereal yellow dwarf virus (CYDV)
Cylindrical inclusions, definition 2:18
Cymbidium mosaic virus (CYMMV) 4:225–226, 4:225*f*, 4:226*f*, 4:227*f*, 4:311*t*
Cynodon chlorotic streak virus (CCSV) 1:477
  infection, symptoms 1:477, 1:478*f*
  transmission 1:477
Cynomolgus EBV 4:587*t*
Cynosurus mottle virus (CnMoV) 4:646*t*
Cypovirus 3:134, 3:134*t*, 4:536*t*
Cypovirus(es)
  antigenic relationships 3:141

Cypovirus(es) (*continued*)
see also specific viruses
genetic relationships 3:141
genome 3:136
segments 3:138, 3:139*t*
size distribution 3:138
termini 3:138, 3:139*t*
history 3:135
host range 3:135
infections *see below*
infectivity 3:137
insect pest control 3:126*t*
nomenclature 3:141
polyhedra 3:136, 3:137–138
replication 3:136
assembly 3:139
uptake 3:138–139
transmission 3:135
virion properties 3:134, 3:136
capsid 3:136
proteins 3:136
stability 3:137–138
see also specific viruses
Cypovirus 1 (CPV-1) 3:141
as type species 4:536*t*
Cypovirus 12 (CPV-12) 3:141
Cypovirus 14 (CPV-14) 3:141
Cypovirus infections 3:135
host range 3:135
insect pest control 3:127
polyhedra 3:135
composition 3:138
infectivity 3:137–138
pupae 3:135
symptoms 3:127, 3:135
tissues associated 3:135
Cyprinid herpesvirus 1 (CyHV-1) *see* Carp pox herpesvirus
Cyprinid herpesvirus 3 *see* Koi herpesvirus (KHV)
Cyprus, sandfly fever viruses 4:495
CYSDV *see* Cucurbit yellow stunting disorder virus (CYSDV)
Cys-motif, Campoletis sonorensis ichnovirus 4:258–259
Cysteine protease(s) 1:155
Cystic fibrosis transmembrane conductance regulator (CFTR), definition 4:507
Cystoviridae 3:7, 5:11*t*
Cystovirus(es)
evolution 5:374
see also specific viruses
genome delivery 5:369
genome packaging 2:306, 2:307*t*, 2:311
nucleotide selection 2:307
historical aspects 3:6
replication 4:406
L segment mRNA 4:406
pac sequence 4:406
see also Icosahedral enveloped dsDNA bacteriophages; *specific viruses*
Cytidine deaminases (APOBEC)
definition 4:611
innate immunity 3:109
SIV infection 4:622
Cytochrome *c*, apoptosis 1:155–157
Cytogenetic abnormalities, adult T-cell leukemia 2:566
Cytokine(s) **1:620–624**
definition 1:620, 3:70, 3:78, 3:104, 3:112
Ectromelia virus infection 3:345
effects 1:620
families 1:621
function 1:620
interferon type I 5:136
murine CMV infection 1:630*t*, 1:632
networks 1:620
parapoxvirus infection 4:61
persistent infections 4:112
pro-inflammatory
common cold 4:473*f*
yellow fever 5:475
receptors 1:620
regulation 1:620
rhinovirus infection 1:546
yellow fever 5:475
see also specific cytokines
'Cytokine storm' 3:99
Cytomegalia 1:624, 2:490
Cytomegalic inclusion disease (CID) 2:474
cytopathology 2:474
diagnosis 2:474
Cytomegalovirus 1:625, 1:625*t*, 1:635–636, 1:635*t*, 2:432*t*, 2:474, 2:485–486
Cytomegalovirus(es) (CMV)
genomic comparisons 1:636–637
infections
chronic disease 4:114–115
measles *vs.* 3:289–290
replication
egress 1:628*f*, 1:629
genome packaging 1:628–629
virion morphology 1:625
virion structure 1:625
see also specific viruses
Cytopathic effect(s)
Colorado tick fever virus infection 1:536
definition 1:335, 1:374, 1:580, 2:212, 2:221, 3:161, 4:497, 5:116
togaviruses 5:122
Cytopathic vacuoles (CPVs), togavirus replication sites 5:121
Cytopathology
potato virus Y *see* Potato virus Y (PVY)
potexvirus 4:312
sadwavirus infection 4:525
tenuivirus infection 5:26
varicella-zoster virus infection 5:261
Cytoplasmic exchange, Cryphonectria parasitica virus transmission 2:576
Cytoplasmic life cycle, RNA viruses 4:377
Cytoplasmic male sterility (CMS), definition 2:109
Cytoplasmic polyhedrosis, cypovirus infection 3:135
Cytoplasmic spherical bodies, edornaviruses 2:110
Cytoplasmic targeting/retention signal (CTRS), retroviral replication 4:466
Cytorhabdovirus 1:119*t*, 3:160*f*, 4:187, 4:188*t*
Cytorhabdovirus(es)
classification 1:119*t*
see also specific viruses
genome 3:326*f*
host cell entry 3:333
replication 4:193, 4:194*f*
virus-encoded proteins 3:332–333
see also specific viruses
Cytoskeleton, intracellular movement, role 3:352
50% Cytotoxic concentration (CC_{50}), antiviral therapy 1:146–147
Cytotoxic T-cells (CTLs)
death receptor activation 3:345
definition 4:382
Ectromelia virus infection 3:345
granule exocytosis pathway 3:345
hepatitis A virus infection 2:12
HIV infection *see* HIV infection
mechanism of action 3:80
murine CMV infection 1:630*t*, 1:631, 1:632*f*
varicella-zoster virus infection 5:254–255
VSV infection 5:298
yellow fever 5:475
Cytotoxic test(s), antiviral therapy 1:147

D

D13 protein, vaccinia virus 5:248
Dacryoadenitis 2:491, 2:495
DAdV-1 *see* Duck adenovirus 1 (DAdV-1)
DAF *see* Decay-accelerating factor (DAF)
Dahlia, virus infections 4:221
see also specific viruses
Dahlia mosaic virus (DMV) 4:221, 4:222*f*
Daily survival *(P),* vectorial capacity 5:271
Dairy industry, economic losses due to phages 2:448–449
Dali 5:357*t*
Dama trima virus (DtV) 5:28*t*
Damom bushy dwarf virus (CBDV), characteristics 3:389*t*
Dane particles 2:335–336
hepatitis B virus 2:352
Daphne virus X (DVX) 4:311*t*
DARK, apoptosis 1:232
Darunavir 1:143*t*
Dasychira pudibunda virus (DpV) 5:28*t*
Database(s) **5:348–365**, 5:357*t*
access 5:353
analytical tools 5:357*t*, 5:364
annotation 5:353
definition 5:348
Inferred from Electronic Annotation 5:353
Standard Operating Procedures 5:353
data associated 5:349
classification 5:362
functional motifs 5:363
major repositories 5:356
nucleotide sequence data 5:362
orthologous clusters 5:363
protein sequence data 5:363
structural information 5:363
taxonomy 5:362
virus-specific sequence data 5:363
definition 5:348
errors 5:355
formats 5:349, 5:350*f*
output 5:354, 5:354*f*
BLAST 5:354
web-based 5:354–355
search tools, web-based 5:356
tables
admin 5:352
BLAST 5:352
color-coding 5:352
gene properties 5:352
gene segments 5:352
genome_molecule 5:352–353
taxonomy 5:352
Database management system(s) (DBMS) 5:349
definition 5:348
Database schema, definition 5:348
Data protection act(s), HIV/AIDS surveillance 2:46
DB52 protein, HCMV 2:487
DCL1 protein, short interfering RNAs 5:326
DCL2 protein, short interfering RNAs 5:326
DCL3-dependent RNA silencing 4:145–146
DCL3 protein, short interfering RNAs 5:326
DCL4 protein 4:147–148, 5:326
DCV *see* Drosophila C virus (DCV)
DDBJ Sequence Database 5:357*t*
DDE transposases 4:436
Death certification, disease surveillance 2:45
Death-inducing signaling complex (DISC) 1:157
'Death rafts' lambda holin S105 3:253
Death receptor signaling 1:157
FLIP proteins 1:154, 1:160
apoptosis 1:160
viral caspase regulators 1:160
viral regulators 1:157
Decay-accelerating factor (DAF)
echovirus replication 2:67
enterovirus 70 2:132
enterovirus binding 2:129
virus binding 5:322–323
coxsackie-adenovirus receptor 5:323
coxsackievirus 1:543–544
enteroviruses 5:322–323
DED1 protein 1:384–385
Deer tick virus 2:239
DE expression, nucleopolyhedrovirus 1:262
Defective interfering DNAs (DI DNA) 2:1
beet curly top virus control 1:307
recombination *see* Recombination
Defective interfering particles
definition 1:211
equine herpesviruses 2:416
granuloviruses 1:218
Defective interfering RNAs (DI RNA) 2:1
definition 1:520
geminiviruses 1:315–316
hepatitis A virus 2:4

hepatitis B virus 2:4
production 2:2*f*
tombusviruses *see* Tombusvirus(es)
tospoviruses 5:159
Defective interfering virus(es) **2:1–4**
assays 2:3
biological effects 2:3
cyclic variations 2:2, 2:3*f*
defectiveness 2:1
defective viruses *vs.* 2:2
experimental animal models 2:3
genomes 2:1
history 2:1
interference 2:2
matrix protein role 2:3
natural infections 2:3
detection 2:3–4
structure 2:1
see also specific viruses
Defective viruses, defective interfering viruses *vs.* 2:2
Defoliation, cacao swollen shoot virus infection 1:403
Deformations, definition 4:207
Deformed wing virus (DWV) 3:42
genome 3:45
tissue tropism 3:45
Degenerate primers, PCR *see* Polymerase chain reaction (PCR)
Degenerative quasispecies 4:359
Delavirdine 2:510, 2:511*t*
Delayed type hypersensitivity (DTH), TMEV infection 5:43
Delbrück, Max, bacteriophage infection kinetics 2:444–445
Delphacid planthoppers *see* Planthoppers
Delta antigen 2:375
Deltabaculovirus 1:220
Delta protein(s) 2:376
Deltaretroviridae 2:558
Deltaretrovirus 4:460
Deltavirus *see* Hepatitis delta virus (HDV)
Deltavirus 2:375
Dementia
HIV-associated *see* HIV infection, dementia
nervous system viruses 1:474
Democratic Republic of Congo
Ebola virus outbreaks 2:199
Marburg virus outbreaks 2:200, 3:272
monkeypox virus outbreak 5:491
Demyelination
definition 5:37
see also specific infections
Dendritic cells (DC)
conventional *see* Conventional dendritic cell(s) (cDC)
Dengue fever 2:13
differentiation 3:112
Ebola virus infection 2:202–203
efficiency 1:123
function 1:122–123
HIV pathogenesis 2:538–539, 2:539*f*
induction 3:73, 3:74*f*
innate immunity 3:112
localization 1:122–123
maturation 3:109
migration 1:125
murine CMV infection 1:630*t*
parapoxvirus infections 4:61
persistent infections 4:112
subsets 1:123
types 3:112
Visna-Maedi virus infection 5:425
West Nile virus infection 5:446
Dendrobium mosaic virus, transmission 1:291
bean common necrosis virus 1:291
Dendrograms, definition 2:174
Dendrolimus punctatus tetravirus (DpTV) 5:28*t*
capsid protein precursors 5:31–32, 5:32*t*
discovery 5:28
genomes 5:31
open reading frames 5:31
RNA2 length 5:31
replicases 5:31
Dendrolimus spectabilis, biological control 3:127
Dengue fever 2:5
bioterrorism 5:407
clinical diagnosis 2:12
clinical features 2:11
adults 2:11
children 2:11
Dengue hemorrhagic fever/Dengue shock syndrome *see below*
hemorrhagic fever 2:245
control/prevention 2:13
vaccines/vaccination 2:13
diagnosis
see specific methods
disease severity 2:9
epidemics 2:5, 2:6
severity 2:9
histopathology 2:12
immune response 2:12
immunoglobulin G diagnosis 2:12
cross reactivity 2:12
persistence 2:12
secondary infection 2:12
incidence 2:5–6, 2:6*f*
incubation period 2:11
pathology 2:12
immunopathogenic mechanisms 2:13
prevalence 2:243
prognosis 2:12
rate 2:243
serology
serologic relationships 2:8
serotypes 2:5
subcomplexes 2:6
variability 2:8
treatment 2:12
Dengue hemorrhagic fever/Dengue shock syndrome (DHF/DSS) 2:5, 2:11, 2:245
characterization 2:10, 2:11
control 2:13
critical stage of disease 2:11
epidemiology 2:9, 2:9*f*
history 2:5
infection prevention 2:13
pathogenetic mechanism 2:9*f*, 2:10
Dengue virus(es) (DENVs) **2:5–14**, 2:96
anthroponoses 5:270–271
antigenic variation, yellow fever virus *vs.* 1:127
associated proteins 2:6
characteristics 1:171*t*
classification 2:6
cross reactivity 2:12
DENV-1 serotype 2:5
associated epidemics 2:10–11
DENV-2 serotype 2:5
American 2:7–8
antigenic variation 2:9
Cuba 2:10–11
Southeast Asia 2:7–8
vector modifications 3:153
Vietnam 2:7–8
DENV-3 serotype 2:5
antigenic variation 2:9
isolation 2:7
DENV-4 serotype 2:5
antigenic variation 2:9
DNA vaccination 2:53
epidemiology 2:9
maintenance cycles 2:8*f*, 2:9
etiology 2:5
evolution 2:8, 2:8*f*
theories 2:8
genetics 2:7
diversity 2:7
genetic drift 2:8
intraserotypic recombination 2:8
partial nucleotide sequencing 2:7–8
genetic variation studies 2:7
partial nucleotide sequencing 2:7–8
genome 2:244*f*
geographical distribution 2:6
history 2:5
host range 2:7
life cycle 2:9–10
pathogenicity 2:10, 2:11*f*
replication sites 2:10
seasonal distribution 2:6
influencing factors 2:7
serotypes 2:6
see also specific serotype
size 2:6
structure 2:243, 2:244*f*
taxonomy 2:6
tissue tropism 2:10
transmission 2:5, 2:6, 2:10, 2:243
Aedes aegypti 1:173, 2:5, 2:7, 2:10
Aedes albopictus 2:5, 2:7
Aedes polynesiensis 2:5, 2:7
defective virus 1:173
peak transmission 2:7
vectorial capacity 5:271
virus propagation 2:7
in vitro studies 2:10–11
see also specific viruses
Density gradient centrifugation 2:452
Densovirinae 4:76, 4:77*t*, 4:86, 4:92*t*, 4:94*t*, 4:96, 5:11*t*
Densovirus 4:94*t*, 4:96
Densovirus(es)
ambisense genomes 4:77, 4:77*t*, 4:81
subgroup A 4:81, 4:81*f*
subgroup B 4:81
subgroup C 4:81*f*, 4:82
see also specific viruses
biological control 4:79
biological properties 4:78
capsid proteins 1:228
classification 4:76, 4:92*t*, 4:94*t*
gene cassettes 4:95*f*, 4:96
gene expression 4:79
nonstructural proteins 4:81
structural proteins 4:81
genome 4:76–77, 4:79, 4:81*f*, 4:95*f*, 4:96
ambisense *see above*
leaky scanning 4:79–81
monosense genomes 4:83
phospholipase A2 motif 4:81
sequence motif conservation 4:82*f*
host range 4:79
infection
insects 3:126*t*
pathology 4:79
symptoms 4:79
tissue tropism 4:79
insect pest control 3:126*t*, 3:127, 4:79
phylogenetic comparisons 4:82*f*, 4:84
nonstructural motifs 4:84, 4:84*f*
unclassified 4:83
virion structure 4:78
capsid 4:78
see also specific viruses
Deoxythymidine monophosphate (dTMP) 3:477–478
Deoxyuridine triphosphatase 1:47
Dependovirus 4:93
Dependovirus(es)
classification 4:92*t*
see also specific viruses
genome 4:94*f*
infection 4:94–95
replication 4:93
see also specific viruses
Dera Ghazi Khan virus 1:598*t*
Dermacentor andersoni (tick), Colorado tick fever virus transmission 1:534, 1:535
Dermacentor variabilis (dog tick), Colorado tick fever virus transmission 1:534
Dermatitis, contagious pustular 4:57–58
Desomotubule(s), plant virus movement 3:352
Desquamation 4:552
Deubiquitinating enzyme(s) (DUB), SARS CoV 1:562
Developed countries, KSHV transmission 3:190
Developmental resistance, baculovirus 1:267
Development perturbation, hypovirulence 2:575, 2:577
Dexmethasone, BHV1 genital disease 1:367

DHBcAg (duck hepatitis B virus c antigen) 2:328–329
DHBeAg (duck hepatitis B virus e antigen) 2:328–329
DHBsAg (duck hepatitis B virus surface antigens) 2:328
DHBV *see* Duck hepatitis B virus (DHBV)
D'Herelle, Félix Hubert 2:443
  bacteriophage therapy 2:443
Diabetes mellitus, coxsackieviruses 1:586
Diabetes mellitus, type I, human enterovirus B 2:69
Diadromus pulchellus ascovirus (DpAV) 1:189
Diadromus pulchellus idnoreovirus (DpIRV-1) 3:135
  genome segments 3:140
  proteins 3:140, 3:140*t*
Diadromus pulchellus reovirus 4:536*t*
Diagnostic techniques
  equine infectious anemia virus 2:173
  molecular approaches **2:29–37**
  multianalyte methods 2:35–36
  neglected methods 2:28
  new methods 2:14, 2:28
  organ transplantation, infections 3:470
  viroid infection 5:338
  see also specific methods
  see also specific viruses
Dianthovirus 5:145–146
Dianthovirus(es)
  genome 5:148
  see also specific viruses
  geographical distribution 5:150
  taxonomy 5:145–146
  tombusvirus taxonomy 5:145–146
  see also specific viruses
DIAP1, apoptosis 1:232
Diapause 4:652, 5:268
Diaporthe RNA virus (DRV) 2:288–289
Diarrhea
  adenovirus-induced 2:121
  astrovirus-induced 2:121
  bovine torovirus 5:155–156
  Breda virus 2:122
  coronavirus-induced 2:122
  human torovirus infection 5:156
  torovirus-induced 2:122
  see also specific virtal infections
Diascia, virus infections 4:215
  see also specific viruses
Diatom(s), viral infection 1:95
Dicer(s) 3:149
  definition 5:325
  domains 3:149–150
  gene evolution 3:154–155
  miRNA biogenesis 3:150, 5:326
  nodaviruses 3:436–437
  organismal distribution 3:149–150
Dicer-2 3:149–150
  R2D2 protein interactions 3:150–151
Dicer-like proteins
  engineered resistance in plants 4:161
  plant gene silencing 4:143*t*, 4:145
    antiviral defense 4:145
    Arabidopsis thaliana 4:145
    DCL3-dependent RNA silencing 4:145–146
Diceromyia, Dengue virus transmission 2:7
Dichlorodiphenyltrichloroethane (DDT), Dengue virus control 2:97
Dicistronic mRNA, definition 3:46
Dicistroviridae 1:90*f*, 2:37, 4:567, 5:11*t*
Dicistrovirus(es) **2:37–44**
  biophysical properties 2:38, 2:39*f*
    morphology 2:38
    structural proteins 2:38, 2:40*f*
  classification 2:37
  gene expression 2:39
  genome 2:38, 2:39*t*
    intergenic regions 2:41*f*
    open reading frames 2:38–39
    untranslated regions 2:38–39
  geographical distribution 2:43
  history 2:37
  host range 2:40
  infection
    insects 3:126*t*
    pathology 2:42
  marnaviruses *vs.* 3:281, 3:281*f*
  phylogeny 2:43, 2:43*f*
  replication 2:39, 2:42*t*
    IRES elements 2:39, 2:41*f*
    translation initiation 2:39
  strain variation 2:43
  transmission 2:42
  see also specific viruses
Dicotyledon(s)
  definition 1:533
  recessive resistance genes 4:178
Didanosine 2:510
Diet, prion diseases 1:372, 4:332
Differential hosts, definition 4:659
Differentiating infected from vaccinated animals (DIVA), pseudorabies virus infection 4:350–351
Diffusion, lipid rafts 5:309
Dihydrofolate reductase, murine polyomavirus large T 4:276
Dimorphotheca virus infections 4:221
  see also specific viruses
Dinovernavirus 3:134, 3:134*t*
Dinovernavirus(es)
  antigenic relationships 3:142
  see also specific viruses
  distribution 3:136
  genetic relationships 3:142, 3:142*f*
  genome 3:140
  host range 3:136
  infection 3:136
  replication 3:140
  transmission 3:136
  virion properties 3:134, 3:140
  see also specific viruses
Dioscorea alata bacilliform virus (DaBV) 1:403
Diptera 1:111, 2:37, 3:133
Direct chemical control, ornamental plant species virus infections 4:226–229
Direct contact transmission 2:141, 2:145
Directed evolution, first-generation plant virus vectors 4:230
DIRS1 *see* LTR-retrotransposons
DIRS retrotransposons 4:433
Discistroviridae 4:567
Discorea latent virus (DLV) 4:311*t*
Disease(s)
  antibody-dependent enhancement 3:66
  causation criteria 2:144*t*
  definition 2:44
  prevention
    eradication by vaccination 2:51
    implications 2:148
  see also specific diseases
Diseases Conditions database 5:357*t*
Disease surveillance **2:44–51**
  data analysis 2:48
    age distribution 2:48
    occupational groups 2:48
    person 2:48
    place 2:49
    seasonal trends 2:48
    secular trends 2:48
    time 2:48
    travel history 2:48
  data collection 2:45
    accuracy 2:47
    completeness 2:47
    consistency 2:48
    death certification 2:45
    environmental sources 2:46
    general practitioner systems 2:45
    hospital administrations 2:46
    laboratory data 2:45, 2:46
    mass control programs 2:47*f*
    nonhuman sources 2:46
    notifications 2:45
    outbreaks 2:46
    purpose 2:44–45
    representativeness 2:47
    serological surveillance 2:45
    sickness absence 2:46
    survey *vs.* 2:45
    system attributes 2:47
    system evaluation 2:50
    timeliness 2:47
  data interpretation 2:49
    data origin 2:49
    infection stage 2:49
  feedback 2:49
  food poisoning 2:46
  global programs 2:50
  international programs 2:50
  see also specific diseases
Disseminated intravascular coagulation (DIC) 2:198
  filovirus infection 2:203
Dissociation constant 3:413
  antibody–antigen binding 3:414–415
Distal symmetric sensory polyneuropathy *see* HIV infection
Distemper
  clinical features 4:504
  control 4:505
  diagnosis 4:506
  histopathology 4:504
  immune response 4:505
  pathology 4:504
  prevention 4:505
  serology 4:502
  vaccination 4:506
Distemper virus(es) **4:497–507**
  classification 4:498
  epizootiology 4:502
  evolution 4:501
  genome 4:500
  geographical distribution 4:498
  history 4:497
  host range 4:499
  infection *see* Distemper
  propagation 4:500
  taxonomy 4:498
  transmission 4:504
  see also specific viruses
Distinct genes, phylogenetic analysis 4:127
DmGypV *see* Drosophila melanogaster Gypsy virus (DmGypV)
DNA
  binding proteins
    nucleopolyhedroviruses 1:257–260
    SV40 T-ag 4:633
  concatamers 3:35–36
  injection
    bacteriophage T4 3:33–34
    icosahedral tailed dsDNA bacteriophages 3:33
  origin of 3:477
  packaging 1:432
  reassortment 4:166
  repeat regions 1:229
  replication 3:478
    African swine fever virus 1:47
    baculoviruses 1:250
  restriction digestion 2:456
  shuffling
    first-generation plant virus vectors 4:230
    recombinant adeno-associated virus 2:304
  substrate recruitment 2:309
  translocation 2:311
DNA-1 component *see* Cotton leaf curl disease (CLCuD)
DNA-A satellite(s), cotton leaf curl disease transmission 2:102
DNA bacteriophages 3:117
DNA β-components *see* β ssDNA satellite(s)
DNA β satellite, abutilon mosaic virus 1:320–321
DNA-B satellite(s), Indian tomato leaf curl viruses 5:130
DNA chromosome assembly, dsDNA bacteriophages 2:308–309
DNA-dependent DNA polymerase, DNA virus evolution 2:182
DNA encapsidation 1:432
DNA helicase
  African swine fever virus 1:47
  origin of 3:478
DNA interference, African cassava mosaic disease 1:35

DNA ligase
  Amsacta moorei entomopoxivirus 2:139
  Melanoplus sanguinipes entomopoxivirus 2:139
DNA microarrays *see* Microarrays
DNA polymerase(s)
  African swine fever virus 1:47
  bacteriophage ϕX174 4:405
  DNA satellite replication 4:533
  HSV replication 2:401
  origin 3:478
DNA primase, African swine fever virus 1:47
DNA probes 1:295
  bean golden/yellow mosaic disease 1:297–298
DNA translocase(s), bacteriophage assembly 1:439
DNA transposon(s) 4:428
DNA vaccine(s) **2:51–55**
  advantages 2:52
  antigen expression 2:52
  clinical applications 2:54
  composition 2:52
    EMCV IRES promoter insertion 2:53
    two gene expression cassettes 2:53
  concept 2:52
  delivery 2:52–53
  development strategies 5:236
    HIV 1:75
  improvements 2:54–55
    DNA uptake 2:54–55
  limitations 2:54
    transfection efficiency 2:54
  malaria 2:53
  multi-component 2:53
  neonatal immunization 2:53
    effectiveness 2:53
    memory induction 2:53
  plasmids 2:52
    co-administration of immune stimulators 2:54
    integration into cells 2:52–53
    interference 2:53
    production 2:52
    purification 2:52
  production 2:52
    timescale 2:52
  safety issues 2:52
DNA virus(es)
  antigenic variation 1:128
  evolution of 2:181
    DNA-dependent DNA polymerase comparisons 2:182
  fish viruses 2:227
  genome 1:612
  insect pest control 3:127
  nucleotide sequence analysis 2:21
  ocular disease 2:495
  recombination 4:374
    cellular machinery 4:374
    eukaryotic viruses 4:375
    functional marker mutations 4:374–375
    host genome acquisition 4:374
    host methods *vs.* 4:375*f*
    plant viruses 4:376
    prokaryotic viruses 4:374
    recA 4:374
    three-factor cross method 4:374–375
    viral DNA-repair systems 4:374
    virally-encoded systems 4:374
    see also specific viruses
  replication 4:407
    double-stranded DNA viruses 4:410
    RNA intermediates 4:411
    single-stranded DNA 4:410
  RNA viruses *vs.* 3:477
  see also specific viruses
dNTP-binding site, Maize streak virus *rep* gene 3:268
Doctor fish virus 16 (DFV-16) 2:233
Dogs
  rabies virus 4:372
  see also under Canine
Dolichos yellow mosaic virus 3:370–371
Domestic animals
  rabies virus vaccination 4:373
  see also specific animals
Dopamine receptor(s), JCV host cell attachment 4:280
'Dose sparing' vaccine development strategies 5:241–242
Double membrane vesicles (DMV)
  definition 1:554
  nidovirus replication 3:426
Double stranded DNA (dsDNA) 3:148
Double stranded DNA (dsDNA) bacteriophages
  genome packaging 2:308*f*
    ATPase complex 2:310–311
    ATP-DNA complex 2:309–310
    capsid architecture 2:307–308
    circular genome replication 2:308–309
    concatamer replication 2:308–309
    DNA chromosome assembly 2:308–309
    DNA substrate recruitment 2:309
    DNA translocation 2:311
    energy dependency 2:310*f*
    integrated segment replication 2:308–309
    linear DNA replication 2:308–309
    nucleotide selection 2:307
    precapsid assembly 2:307
    'riddle-of-the-ends' 2:308
    termination 2:310
  replication 4:400
    5′ DNA ended terminal protein phages 4:402
    bacteriophage Mu 4:404
    bacteriophage SPP1 4:401
    ϕX174 phages 4:405
    lambdoid phages 4:400
    T4-like phages 4:401
    T7-like phages 4:401
  see also specific viruses
Double-stranded DNA (dsDNA) virus(es)
  classification 5:11*t*
  replication 4:410
    cytoplasmic 4:410
    nuclear 4:410
  RNA intermediates, replication 4:411
  transcription 4:410
    icosahedral enveloped bacteriophages 3:11
    RNA intermediate 4:411
  see also specific viruses
Double stranded RNA (dsRNA) bacteriophages
  genome packaging 2:306
  replication 4:405
  see also specific viruses
Double-stranded RNA (dsRNA) virus(es)
  classification 5:11*t*
  replication 4:410
  see also specific viruses
Double-subgenomic promoter virus(es) (DP-virus)
  infectious clone technology 5:121–122
  see also specific viruses
DpIRV-1 *see* Diadromus pulchellus idnoreovirus (DpIRV-1)
DpTV *see* Dendrolimus punctatus tetravirus (DpTV)
DPVweb (Descriptors of Plant Viruses) 5:357*t*
DR1, avian hepadnavirus 2:330–331
DR2, avian hepadnavirus 2:330–331
Dragon grouper nervous necrosis virus (DGNNV) 3:434
DRONC, apoptosis 1:232
Droplet-shaped virus(es) 1:590
  see also specific viruses
Drosophila C virus (DCV)
  gene expression 5:5
  geographical distribution 2:43
  host range 2:40–41
  transmission 2:42
Drosophila melanogaster
  gypsy retrotransposon 3:306
    env-mediated infection 3:310
    follicle cell, expression 3:302–304
    open reading frames 1:466*f*
    reverse transcription priming 3:306
    target site specificity 3:309
    transposition rates 3:302–304
  immune response
    Dicer gene evolution 3:154–155
    parasitoid attack 4:252–253
    protein tyrosine phosphatase function 4:252–253
    siRNA 3:149
  retroelements associated 3:302
    flamenco 3:302–304
    gypsy *see above*
    transposition levels 3:302–304
Drosophila melanogaster Gypsy virus (DmGypV) 4:451
  cg4715 protein 4:454
  env protein cleavage 4:453
  monoclonal antibodies, infectivity 4:452
Drosophila melanogaster idnoreovirus-5 (DmIRV-5) 3:140
Drosophila melanogaster zam virus (DmZamV) 4:452–453
Drosophila S virus 3:135
Drug(s)
  antiretroviral *see* Antiretroviral therapy
  prophylaxis, arenavirus infection control 3:211
  resistance 1:151
    genetic barrier 1:150
    hepatitis B virus *see* Hepatitis B virus (HBV) replication 1:151
Dry eye, HIV infection 2:144
Dual-start motif 3:248
  antiholin diversity 3:252*f*
Duck adenovirus 1 (DAdV-1)
  classification 1:27
  egg drop syndrome 1:27
Duck hepatitis B virus (DHBV) 2:336
  classification 2:327
  genome 2:328*f*
    open reading frames 2:329
  infection 2:330*f*
    congenital 2:332
    histology 2:334*f*
    persistent 2:334*f*
    tissue tropism 2:332
  replication 2:327
    budding 2:330*f*
    cccDNA 2:327
    DNA synthesis 2:330
    DR1 2:330–331
    DR1* 2:330–331
    DR2 2:330–331
    nuclear transport 2:331
    pregenome packaging 2:331*f*
    receptors 2:329
    reverse transcription 2:328*f*
    sites 2:327
    transcription 2:328*f*
  structure 2:328
    antigens 2:328
    envelope 2:328
    nucleocapsid 2:328*f*
    polymerase 2:328
Ducklings, astrovirus infection 1:209
Duck virus enteritis (DVE) 2:406
Dugbe virus 1:597
dUTPase 1:594
dUTP pyrophosphatase 1:6
Duvenhage virus 4:184*t*
dwf gene 1:300
DWV *see* Deformed wing virus (DWV)
Dysesthesia, definition 5:250
Dysplasia 2:212
Dyspnea 5:423

E

E1A gene/protein *see* Adenovirus infections
E1B-19K gene/protein *see* Adenovirus infections
E1B-55K, adenovirus E4orf6 gene/protein interactions 1:14–15
E1B-AP5 gene/protein, adenovirus malignant transformation 1:15
E1B gene/protein, adenovirus malignant transformation 1:13
E1E4 *see* Human papillomavirus(es) (HPVs)
E1 glycoprotein function, alphavirus life cycle 1:195
E1 proteins
  alphavirus replication 5:99
  HPV *see* Human papillomavirus(es) (HPVs)
E2F/DP complex, HPV replication 4:25

E2 glycoprotein function, alphavirus life cycle 1:195
E2 protein
  alphavirus replication 5:99
  bovine viral diarrhea viruses 1:379
  HPV *see* Human papillomavirus(es) (HPVs)
E3 gene, siadenoviruses 1:8
E3 glycoprotein function, alphavirus life cycle 1:195
E4 gene/protein
  adenovirus malignant transformation 1:13
  adenovirus MRE11-RAD50-NSB1 complex 1:15
  orf6 *see* Adenovirus infections
  papillomaviruses 4:36
E5 gene/protein, HPV 4:23
E6 gene/protein *see* Human papillomavirus(es) (HPVs)
E6 promoter, papillomavirus gene expression 4:37–38
E7 gene/protein *see* Human papillomavirus(es) (HPVs)
E120R polyprotein, African swine fever virus replication 1:47
EACMCV *see* East African cassava mosaic Cameroon virus (EACMCV)
EACMKV *see* East African cassava mosaic Kenya virus (EACMKV)
EACMV *see* East African cassava mosaic virus (EACMV)
EACMZV *see* East African cassava mosaic Zanzibar virus (EACMZV)
Early genes
  baculoviruses 1:250
  Herpesvirus Saimiri 4:588–589
  icosahedral tailed dsDNA bacteriophages 3:34
Early proteins, murine polyomavirus 4:273
East Africa, HIV infection 1:62*f*
East African cassava mosaic Cameroon virus (EACMCV) 1:31–32
  distribution 1:32*f*
East African cassava mosaic Kenya virus (EACMKV) 1:31–32
  distribution 1:32*f*
East African cassava mosaic Malawi virus (EACMMV) 1:31–32
  distribution 1:32*f*
East African cassava mosaic virus (EACMV) 1:31–32
  regional epidemiology 1:33–34
East African cassava mosaic Zanzibar virus (EACMZV) 1:31–32
  distribution 1:32*f*
Eastern equine encephalitic virus (EEEV) 5:113
  animal models 5:79
  characteristics 5:109*t*
  epidemiology 5:104*f*
  evolutionary relationships 1:174
  geographical distribution 5:102
  host range 5:102–103
    North America 5:102–103
  infection
    case–fatality rates 5:105
    clinical features 5:105
    diagnosis 5:105
    encephalitis 5:78
    epidemic 5:101
    epizootics 5:103–104
    incidence 5:103–104
    incubation period 5:78
    pathogenesis 5:115
    pathology 5:106
    recovery 5:105
    treatment 5:82
    vaccines 5:488
  pathogenicity 5:105
  replication 5:105
  subtypes 5:101
  tissue tropism 5:104
  transmission 5:104
    vector control 5:111–112
    vectors 5:103–104
  virulence 5:105
Eastern Europe, HIV infection 1:64
EAV *see* Equine arteritis virus (EAV)
EBNA *see* Epstein–Barr virus (EBV)
EBNA-1 *see* Epstein–Barr virus (EBV)
EBNA-2 *see* Epstein–Barr virus (EBV)
EBNA-3 *see* Epstein–Barr virus (EBV)
EBNA-LP *see* Epstein–Barr virus (EBV)
Ebola hemorrhagic fever (EHF) 2:57
  diagnosis 2:63
  history 2:57
Ebolavirus 2:57, 2:199
Ebola virus(es) (EBOV) **2:57–65**
  bioterrorism 5:407
    fatality rates 5:408–409
    transmission 5:408–409
  cell tropism 2:61
  classification 2:57
  disease surveillance 2:199
  genetic stability 2:179
  genome 3:327*f*
    Marburg virus *vs.* 2:201–202
  history 5:490–491
  immune evasion 3:108
  inactivation 2:57–58
  life cycle 2:61*f*
    mRNA transcripts 2:61–62
    replication 2:61–62
    transcription 2:61–62
    virus assembly 2:62
  matrix proteins 2:59*f*
    L protein 2:59–60
    NP 2:59–60
    VP24 2:59–60
    VP30 2:59–60
    VP35 2:59–60
    VP40 2:59–60
  proteins 2:60*t*
    budding 2:59–60
    fusion protein biosynthesis 3:293
    glycoproteins 3:332
    matrix proteins *see above*
    RNP complex 2:59
  structure 3:329*f*
  taxonomy 2:57
  transmission 5:490–491
  viral receptors 2:61
  virion properties
    morphology 2:59*f*
    nucleocapsid 2:57–58
  see also specific viruses
Ebola virus infection
  animal models 2:202–203
  clinical features 2:62
  coagulopathy 2:203
  Democratic Republic of Congo 2:199
  dendritic cells 2:202–203
  diagnosis 5:490–491
  endothelial cell activation 2:203
  fatality rates 2:57
  immune response 2:203
    adaptive 3:108–109
    evasion 3:108
  incubation period 2:202
  mortality rate 2:199
  nonhuman primates 2:203
  pathogenesis 2:63
    Marburg virus *vs.* 3:278
  pathogenicity 2:63*f*
  symptoms 2:62
  treatment 2:204
    antiviral therapy 2:64
    experimental strategies 2:64
  vaccines 2:65
  viremia 2:62
  zoonoses 5:490
  see also Ebola hemorrhagic fever (EHF)
EBV *see* Epstein–Barr virus (EBV)
Ecdysteroid, definition 4:256
Ecdysteroid glucosyltransferase, baculovirus infection 1:252
Echinochloa hoja blanca virus (EHBV) 1:487, 5:24
  phylogenetics 5:26*f*
  species discrimination 5:26
Echinochloa ragged stunt virus (ERSV), characteristics 4:150*t*
Echovirus(es) **2:65–71**
  cancer therapy 2:70
  classification 2:65
  epidemiology 2:69
  genome 2:66–67
    5′ noncoding region 2:67
  history 2:65
  infections *see below*
  laboratory diagnosis 2:69
  molecular characteristics 2:66
    capsid 2:66–67
    structural studies 2:67
  mutation rate 2:68
    genetic lineages 2:70
    recombination 2:70
  replication 2:66
    associated proteins 2:67–68
    cellular effects 2:68
    cycle 2:67
    translation 2:67
  serotypes 2:65–66
    3D structures 2:66–67
  transmission 2:69
    children 2:69–70
  see also specific viruses
Echovirus 4, disease surveillance 2:45
Echovirus infections
  amyotrophic lateral sclerosis 2:69
  clinical manifestations 2:68
    respiratory disease 2:552
  human respiratory infection 2:552
  vaccines/vaccination 2:70
Ecological niche(s), definition 5:401
Ecology
  bacteriophages *see* Bacteriophage(s), ecology
  definition 3:212
  HIV cross-species transmission 2:532–533
  zoonoses 5:494
Economic importance
  alfalfa mosaic virus 1:81
  fish viruses 2:227
  infectious hematopoietic necrosis virus 2:228
  ornamental plant species virus infections 4:208
  potato mop-top virus infections 4:286–287
  viral hemorrhagic septicemia virus 2:227
Ecosystem, definition 2:71
Ectocarpus virus(es) (EsV) 4:116
  genome 4:119
    open reading frames 4:122*f*
  replication 4:122
  see also specific viruses
Ectothermic, definition 3:155
Ectromelia virus 3:342
  biotechnology *vs.* bioterrorism 5:409
  enzootic 3:343
  epidemiology 3:342
    inoculation routes 3:342–343
  evolution 2:182
  future work 3:346
  genetics 3:342
  geographical distribution 3:342
  historical aspects 3:342
    Barnard, J E 3:342
    Burnett, F M 3:342
    Marchal, J 3:342
  host range 3:342
  infection *see below*
  mouse strain susceptibility 3:343
  propagation 3:342
  protein p28 3:346
  seasonal distribution 3:342
Ectromelia virus infection
  clinical features 3:344*f*
    age effects 3:343
  future work 3:346
  histopathology 3:343
  immune response 3:345
    cell-mediated 3:345
    host-response modulation 3:346
    humoral immunity 3:345
  intracytoplasmic inclusion bodies 3:344*f*

lesions
intranasal inoculation 3:345
intraperitoneal inoculation 3:345
kidneys 3:345
liver lesions 3:344
lymph nodes 3:345
skin lesions 3:344
spleen lesions 3:345
mousepox 4:329
pathogenesis 3:343
incubation period 3:343
as model system 3:343
pathology 3:343
Ectropis obliqua picorna-like virus (EoPV) 3:45
Edible vaccine(s) 5:221
polio vaccine *see* Polio vaccines/vaccination
proof of concept 5:222–223
rabies virus vaccination 4:373
Edornavirus(es) **2:109–116**
cytoplasmic spherical bodies 2:110
genome 2:110*f*
open reading frames 2:110–111
RdRp 2:112
historical aspects 2:110
large dsRNAs 2:110
members 2:113*t*
molecular evolution 2:115
host species dependence 2:115
vertical transmission 2:115
nonplant 2:111
genomes 2:110*f*
members 2:111
Phytophthora 2:111
phylogenetic trees 2:112*f*
proteins 2:112
uridine diphosphate glycosyltransferase 2:112–113
RdRp 2:110–111
replication 2:115
rice 2:110
RNA helicase 2:110–111
site-specific coding strand nicks 2:110*f*
tentative members 2:111
phylogenetic trees 2:112*f*
vertical transmission 2:113
molecular evolution 2:115
see also specific viruses
EDS1 protein, plants natural resistance 4:176
EEEV *see* Eastern equine encephalitic virus (EEEV)
Eel herpesvirus 2:233
Eel virus American (EVA) 2:228
Eel virus Europe X (EVEX) 2:228
EEV *see* Extracellular enveloped virus (EEV)
Efavirenz 2:510
50% Effective concentration (EC_{50}), antiviral therapy 1:146
Effective population size 4:359
Effector memory T-cell(s) 3:70
Effector T-cell(s) 1:620–621
definition 3:70
population expansion 3:74
Efuviritide 1:143*t*
Egf gene family 4:254
EGFR *see* Epidermal growth factor receptor (EGFR)
Egg drop syndrome (EDS) 1:27
Eggplant mosaic virus-tobacco strain (EMV-T) 5:67
characteristics 5:63*t*
Eggplant mottled dwarf virus (EMDV) 5:67
associated disease symptoms 5:67
characteristics 5:63*t*
incidence 5:67
potato infections 4:300*t*
EHDV *see* Epizootic hemorrhagic disease virus (EHDV)
EHF *see* Ebola hemorrhagic fever (EHF)
EHNV *see* Epizootic hematopoietic necrosis virus (EHNV)
EHV-1 *see* Equid herpesvirus 1 (EHV-1)
EHV-2 *see* Equid herpesvirus 2 (EHV-2)
EHV-3 *see* Equid herpesvirus 3 (EHV-3)
EHV-4 *see* Equid herpesvirus 4 (EHV-4)
EHV-6 *see* Equid herpesvirus 5 (EHV-5)
EHV-9 *see* Equid herpesvirus 9 (EHV-9)
EIA *see* Equine infectious anemia (EIA)
EIAV *see* Equine infectious anemia virus (EIAV)
EICP0 protein, equid herpesvirus 1 replication 2:415
eIF4G, alfalfa mosaic virus coat protein binding 1:84*f*
Eigen, Manfred, quasispecies 2:177–178, 4:359–360
Eighth ICTV Report on Virus Taxonomy 5:10
species demarcation criteria 5:21
Eimeria brunetti RNA virus 1 (EbRV-1) 5:173–174
ELDKWAS peptide *see* HIV vaccines/vaccination
Electron(s), penetrating power 2:78
Electron density
single-particle density maps 1:607*f*
resolution 1:607*f*
virus particle modeling 1:607*f*
Electron microscopy (EM) **2:78–86**
adenovirus virion morphology 2:78
adsorption EM 2:21*f*
cell preparation 2:82
antibody labelling 2:83
classical thin sectioning 2:84*f*
cryo-EM, vitreous sections 2:84
cryo-sectioning *see* Cryo-sectioning
cryo-transmission 4:68
hepatitis B virus 2:351
icosahedral tailed dsDNA bacteriophages 3:31
image analysis 2:84
immunoelectron microscopical decoration 2:21*f*
immunosorbent EM 2:21*f*
large particle preparation 2:82
plant virus detection 2:27
bean golden/yellow mosaic disease 1:297
cytological alterations 2:27*f*
tobacco mosaic virus 2:452
virus particle aggregates 2:27*f*
single particle imaging 2:78
cryo-electron microscopy *see* Cryo-electron microscopy (cryoEM)
cryo-electron tomography *see* Cryo-tomographic imaging (cryo-ET)
metal shadowing *see* Metal shadowing
negative staining *see* Negative staining
sample contamination 2:78–79
taxonomy 5:9–10
ultrastructural studies 2:27
varicella-zoster virus assembly 5:261
Electroporation
definition 2:51
DNA vaccine delivery 2:55
Elford, William, virus discovery 3:399
ELISA *see* Enzyme-linked immunosorbent assay (ELISA)
Elk herpesvirus 1 (EIHV1) 1:364*t*
Elongation terminator(s) 5:176
Elvucitabine 2:507*t*
EMBL Sequence Database 5:357*t*
EMCV *see* Encephalomyocarditis virus (EMCV)
EMDV *see* Eggplant mottled dwarf virus (EMDV)
Emerging viral diseases **2:93–97**
contributing factors 2:93
definition 4:40
ecological factors 2:96
history 2:93
human behavior 2:94
human demographics 2:94
recognition of 2:93
vector control 2:97
zoonoses 2:95
see also specific viruses
Emiliania huxleyi virus (EhV) 4:116
genome 4:119
open reading frames 4:122*f*
RNA polymerase 4:122
Emtricitabine 2:510
EMV-T *see* Eggplant mosaic virus-tobacco strain (EMV-T)
Enamovirus 3:231, 3:232*f*, 3:232*t*
Enanthem, definition 4:639
Enation, definition 1:301
Encapsidated RNA2, pomovirus genome 4:284
Encapsidated RNA3, pomovirus genome 4:284
Encapsidation
alfalfa mosaic virus 1:85
bacteriophages 1:439
Brome mosaic virus assembly 1:385
DNA 1:432
foamy viruses life cycle 2:264
genome 1:196
heterologous *see* Hetero-encapsidation
Encephalitis 5:101
Akabane virus infection 1:77
alphavirus infection 5:115
arbovirus infection 1:474
bovine herpes virus 1 infection 1:366
bovine herpes virus 5 infection 1:365
bovine immunodeficiency viruses infection 1:352
coxsackievirus infection 1:585*t*
definition 1:474
equid herpesvirus-9 infection 2:418
HIV infection 1:57
HSV infection 1:474
human herpesvirus-6 infection 2:503
Japanese encephalitis virus infection 2:236
Langat virus infection 5:53
mumps 3:361
Powassan virus infection 2:239
rabies 3:466
St. Louis encephalitis virus infection 2:236
TMEV infection 5:41–42
zoonoses 5:488
see also specific viruses
Encephalomyelitis
definition 1:474
TMEV infection 5:41–42
Encephalomyocarditis virus (EMCV)
history 1:441
infection 1:446*f*
clinical features 1:445
clinical outbreaks 1:441–442
diagnosis 1:447
histopathology 1:446*f*
human 1:445
pathogenicity 1:445
pathology 1:445
persistent 1:445–446
pigs 1:445–446
prevention 1:447–448
route 1:445
tissue tropism 1:445
vaccines/vaccination 1:447–448
internal ribosome entry sites 4:134–135
Encephalomyocarditis virus (EMCV) 1:443*f*
Encephalopathy
betanodavirus fish infections 2:229–230
definition 5:186
Dengue virus infection 2:10
Endemic(s) 2:5
definition 2:142
Enders, John Franklin, animal virus cultivation 2:456
Endive, tomato spotted wilt virus infection 5:288–289
Endocytosis
definition 5:423
host cell entry 4:409
process 4:409
virus membranes 5:310
virus uncoating 4:409
Endogenous pararetroviral sequences (EPRVs) 4:430
Endogenous retrovirus(es) (ERV) **2:105–109**
classification 2:107
definition 2:105
discovery 2:106
distribution 2:106
evolution 2:106–107
host benefits 2:107
nomenclature 2:108
origins 2:107
mechanisms 2:107
recombination 2:106
structure 2:106*f*
taxonomy 2:108*f*
see also Provirus(es)

Endolysins 3:251
  amidases 3:251
  definition 3:248
  icosahedral tailed dsDNA bacteriophages 3:35
  see also Holin–endolysin lysis
Endoparasitic wasp(s)
  ascovirus infection 1:189
  definition 1:186
Endopeptidase(s), endolysin function 3:251
Endoplasmic reticulum (ER)
  Brome mosaic virus replication in yeast 1:383
  rotavirus replication 4:510
  yellow fever virus assembly 5:471
Endoplasmic reticulum-associated degradation (ERAD)
  definition 5:299
  K28 virus toxin translocation 5:304–305
Endoreduplication, definition 1:301
Endornavirus 2:288
Endosomes
  MHC-II antigen presentation 1:124–125
  proteases 1:124–125
Endothelial cell(s)
  Ebola virus infection 2:203
  Lassa fever pathogenesis 3:207
  Marburg virus infection 2:203
Endothelial syncytial cells, measles 3:289
Endotheliotropic elephant herpesvirus 2:428
Energetics, bacterial cell viral entry *see* Bacteriophage (s), bacterial cell entry
Energy dependency, dsDNA bacteriophage genome packaging 2:310*f*
Enfuvirtide 2:506
Engineered resistance, crops 2:454
Enhancin 1:256
Entecavir 1:143*t*
Enteric cytopathogenic human orphan viruses *see* Echovirus(es)
Enteric infection(s)
  astroviruses 1:204–205
  barriers against 5:315
Enteric virus(es) **2:116–123**
  acute gastroenteritis 2:116
  see also specific viruses
Enterobacteria, icosahedral ssDNA bacteriophages 5:368
Enterobacteria phage M13 2:192*t*
  pVIII 2:193
Enterovirus 1:581, 1:581*t*, 2:65–66, 2:130, 4:243
Enterovirus(es) 2:130
  bovine *see* Bovine enterovirus(es) (BEV)
  classification 2:123
  genome 2:125*f*
    2A protein 2:126
    5′ nontranslated region 2:126
    leader protein 2:126
  host cell entry 5:315
  human *see* Human enterovirus(es)
  occurrence 2:70
  porcine 2:124
  replication 2:127
  simian *see* Simian enteroviruses (SEV)
  stability 2:124
  swine vesicular disease virus 2:124
  vaccines/vaccination 2:70
  virion properties 2:128
    stability 2:128
  virion structure 2:128*f*
    capsid 2:128
  see also Human enterovirus(es)
Enterovirus 68 2:130
  cellular interactions 2:131
  history 2:130
  infection
    clinical presentation 2:131
    epidemiology 2:131
    infectivity, loss 2:131
    pathogenesis 2:131
  transmission 2:131
Enterovirus 69 2:131
Enterovirus 70 2:132
  cellular interactions 2:132
  history 2:132
  infection
    acute hemorrhagic conjunctivitis 2:132
    clinical presentation 2:132
    control 2:133
    diagnosis 2:12
    epidemiology 2:132
    eye infection 2:132
    infectivity 2:133
    neurological symptoms 2:133
    pandemics 2:132
    pathogenesis 2:132
    pathology 2:132
    prevention 2:133
    treatment 2:133
  origin 2:132
  transmission 2:132
Enterovirus 71 2:133
  cellular interactions 2:133
  genetic characteristics 2:133
    genogroups 2:133
    subgroups 2:133
  history 2:133
  infection
    Asian epidemics 2:133
    clinical presentation 2:134
    control 2:134
    diagnosis 2:134
    epidemiology 2:133
    immune response 2:133
    pathogenesis 2:133
    pathology 2:134
    prevention 2:134
    treatment 2:134
  novel types 2:134
Enterovirus 94 2:135
  genome sequence comparisons 2:135
Entomobirnavirus 1:321–322, 1:322*t*, 2:232
Entomopathogenic nematodes, iridovirus infection 3:166
Entomopoxvirinae 2:136, 4:323, 5:11*t*
Entomopoxvirus(es) **2:136–140**
  fusolin/spindles 2:138
  genome 2:138
    gene content 4:326
    genome size 2:138
  infections 2:137
    insects 3:126*t*
  insect pest control 3:128
    occlusion bodies 3:128
  molecular biology 2:139
    inhibitor of apoptosis genes 2:139–140
    proteolytic processing 2:139
    RNA polymerase complex 2:139
    transcription factor homologs 2:139
  phylogeny 2:136
    unclassified viruses 2:136
  replication 2:138
    life cycle 2:138–139
    RNA production 2:139
  virion structure 2:137*f*
    chordopoxviruses *vs.* 2:137
    poxviruses *vs.* 3:128–129
  see also specific viruses
ENTV *see* Enzootic nasal tumor virus (ENTV)
Envelope, definition 4:407
Enveloped virus(es)
  antiviral therapy 1:147
  see also specific viruses
  assembly *see below*
  families associated 5:308
  host cell attachment 5:308
  lipid composition 5:308
  membrane fusion 3:292
  membrane proteins 5:309
  replication
    assembly 4:411
    release 4:412
  see also Virus membrane(s)
Enveloped virus(es), assembly **1:193–200**
  envelope 1:194
    acquisition 1:194
    glycoproteins 1:194
    origin 1:194
  host factor utilization 1:200
  icosahedral viruses 1:195
    alphaviruses 1:195
    flaviviruses 1:195
  see also specific viruses
Envelope fusion protein, insect retroviruses 4:454*f*
Envelope glycoprotein(s)
  antigen–antibody complexes 3:414–415
  Crimean-Congo hemorrhagic fever virus 1:598–599
  definition 1:127
  flaviviruses 1:198
  yellow head virus 5:477–478
  see also specific viruses
Envelope (env) protein
  definition 5:157
  see also specific viruses
Envelope (E) proteins
  Japanese encephalitis virus 3:183
  see also specific proteins
env gene/protein
  see specific viruses
ENVID (European Network for Diagnostics of "Imported" Viral Diseases) 5:357*t*
Environment, maize streak virus 3:270
Env-like domains (retrotransposons) 4:451
  definition 4:428
  LTR-retrotransposons 4:435
env-like gene
  definition 4:352
  functions 4:434
  metaviruses 3:309–310
  pseudoviruses 4:355
  SIRE1 virus 4:355
Env precursor protein, avian retroviruses 4:456
Enzootic disease
  definition 2:377
  fowlpox 2:274
Enzootic nasal tumor virus (ENTV) 3:181
  cell transformation 3:181–182
  classification 3:175
  infection 3:181
  Jaagsiekte sheep retrovirus *vs.* 3:181–182
Enzootic viruses
  see specific viruses
Enzyme(s), prions as 4:340
Enzyme immunoassay(s) (EIA) 2:32*f*
  definition 2:29
Enzyme-linked immunosorbent assay (ELISA)
  banana bunchy top virus 1:278
  bean calico mosaic virus infection 1:299
  bean golden mosaic virus infection 1:299
  bean golden yellow mosaic virus infection 1:299
  benyvirus infection diagnosis 1:313
  Border disease detection 1:340
  cacao swollen shoot virus infection 1:408
  citrus tristeza virus detection 4:198
  classical swine fever virus detection 1:530
  Crimean-Congo hemorrhagic fever 1:602
  definition 1:295
  HCV detection 2:374
  henipavirus infections 2:326
  Japanese encephalitis diagnosis 3:186
  Marburg virus detection 3:278–279
  measles diagnosis 3:289–290
  mungbean yellow mosaic viruses 3:364–365
  orthobunyavirus infection diagnosis 3:482–483
  papillomavirus infection 4:17
  plant reovirus detection 4:155
  plant viruses 2:24*f*, 2:453
  see also specific viruses
  potato virus Y 4:290
  pseudorabies virus detection 4:350*f*
  rhinovirus detection 4:471
  rice yellow mottle virus diagnosis 4:488
  squash leaf curl virus infection 1:299
  Taura syndrome virus, infection diagnosis 5:8
  varicella-zoster virus 5:252

virus isolation 4:494
VLP-based 4:17
VSV detection 5:298
Eorf6, adenovirus E1B-55K gene/protein interaction 1:14–15
Eosinophilic degeneration, yellow fever 5:475
Eosinophilic inclusions, varicella-zoster virus infection 5:254*f*
EpCV *see* Epirus cherry virus (EpCV)
Ependymal cells, nervous system viruses 1:470
Ephemerovirus 1:355
Ephemerovirus(es)
classification 1:119*t*
see also specific viruses
genome 3:326*f*
see also specific viruses
Ephrin B2, henipavirus tissue tropism 2:322–323
Epicrisis, definition 3:319
Epidemic(s) 2:525
Chikungunya virus 5:89
concentrated 1:58
definition 2:141
generalized 1:58
groundnut rosette, Nigeria 3:217
keratoconjunctivitis, adenovirus infection 2:495*f*
SARS 4:552
viral adaptation 2:533
Visna-Maedi virus infection 5:424
see also specific infections
'Epidemic curves' 2:147
Epidemic-level transmission, subsidence 5:272–273
Epidemiology **2:140–148**
convergence model 2:142*f*
definition 2:141
disease occurrence assessment 2:141
associated attributes 2:142
disease prevention implications 2:148
Henle-Koch postulates 2:143
history 2:141
mathematical modeling 2:147
definition 2:141
uses 2:148
studies 2:142
case-control studies 2:143
cohort studies 2:143
etiologic studies 2:143
molecular epidemiologic studies 2:143
parameters 2:142–143
sentinel studies 2:144
seroepidemiologic studies 2:144
vaccine trials 2:144
virus transmission 2:146
among individuals 2:144
arthropod transmission cycles 2:147
common patterns 2:146
host clinical status 2:146
host population immunity 2:146
modes 2:145
population size 2:147
virus virulence 2:146
zoonotic transmission cycles 2:147
see also specific diseases/disorders
Epidermal growth factor receptor (EGFR)
activation by HPV E5 4:23
HCMV-host cell binding 1:626
molluscum contagiosum virus infection 3:320
Epidermal hyperplasia, fish adenoviruses 2:234
Epidermodysplasia verruciformis (EV) 4:16
epithelial restriction barriers 4:35–36
HPV infection 4:20
Epifluorescent microscopy
bacteriophage counts 2:72
definition 2:71
Epirus cherry virus (EpCV) 3:500
taxonomy 3:500
Episomes
definition 2:301
EBV infection 2:158*f*
Epistasis, definition 4:359
Epitheliotropic 3:155
Epithelium
morbillivirus infection 4:504–505
papillomavirus infection 4:32
Epitope mapping, plant virus infection diagnosis 2:26
Epitopes *see* Antigen(s)
Epivir® *see* Lamivudine
Epizootic hematopoietic necrosis, fish iridoviruses 2:233
Epizootic hematopoietic necrosis virus (EHNV) 2:233, 3:155–156
infection 3:158–159
Epizootic hemorrhagic disease virus (EHDV)
capsid 3:462–463
history 3:457
Epizootic infections 4:490
avian adenoviruses 1:27
avipoxviruses 2:278
bluetongue virus 3:457
definition 4:497, 4:567
lumpy skin disease virus 1:427
shellfish herpesviruses 4:563
Epizootic transmission, subsidence 5:272–273
EPR (Epidemic and Pandemic Alert and Response) 5:357*t*
E (envelope) proteins, yellow fever virus 5:471
Epsilonretrovirus 2:231
Epsilonretrovirus(es) 2:220*f*
see also specific viruses
Epsilonretroviruses 2:219, 4:460
Epstein–Barr virus (EBV) **2:148–157**
*Bam*HI-A rightward transcripts 2:157–158
expression patterns 2:165
Notch signaling pathway 2:163–164
open reading frames 2:159*f*
defective interfering viruses 2:4
distribution 2:149
EBV-encoded nuclear antigens 2:157–158
latency 2:440–441
see also specific types
EBV-encoded nuclear antigen 1 2:161
latency 2:160
EBV-encoded nuclear antigen 2 2:161
CSL targeting 2:161
EBV-encoded nuclear antigen 3 , regulation by 2:162
EBV-encoded nuclear antigen-LP, regulation by 2:162
gene expression effects 2:161
latency 2:160–161
PU-1 targeting 2:162
transcription transactivator 2:161
EBV-encoded nuclear antigen 3 2:162
cell line immortalization 2:162
CSL binding 2:162
EBV-encoded nuclear antigen 2 regulation 2:162
gene duplication 2:162
transactivation domains 2:162
EBV-encoded nuclear antigen-LP 2:161
EBV-encoded nuclear antigen 2 regulation 2:162
latency 2:160–161
EBV-encoded small RNA 2:164
expression patterns 2:165
RNA-protein complexes 2:164
structure 2:164
tumorigenic potential 2:164
evolution 2:151
genetic diversity 2:158
amino acid sequence identity 2:158–159
sequence polymorphisms 2:159
strains 2:158–159
genetics 2:156*f*
diversity *see above*
intertypic recombination 2:150
genome 2:148
coding potential 2:157
linearity 2:157
open reading frames 2:157
organization 2:157
terminal repeats 2:157
see also specific proteins
historical aspects 2:149
infection *see below*
latent membrane proteins 2:157–158
oncogenesis 5:194
see also specific types
latent membrane protein 1 2:162
latency 2:160–161
phenotypic changes 2:162
signaling 2:162–163
structure 2:162–163
TRAF signaling 2:163
transformation effector sites 2:162–163
latent membrane protein 2A 2:163
Hodgkin lymphoma 2:163
latency 2:160–161
signaling 2:163
structure 2:163
latent membrane protein 2B 2:163
signaling 2:163
structure 2:163
latent membrane protein 3 2:160–161
miRNAs 2:164
molecular biology **2:157–167**
DNA recombination mechanisms 4:375
persistence 2:152*f*
propagation 2:150
proteins 2:157
see also specific proteins
replication 2:160
cell entry 2:160
induction efficiency 2:160
strain variation 2:150
LMP1 variant 2:150
P3HR-1 virus 2:150
prototype strain 2:150
strain 1 2:158–159
strain 2 2:158–159
taxonomy 2:148
vaccines/vaccination 2:155–156
virion properties 2:157
morphology 2:157
virion structure 2:149*f*
Epstein–Barr virus-encoded nuclear antigen 1 (EBNA-1) *see* Epstein–Barr virus (EBV)
Epstein–Barr virus-encoded nuclear antigen 2 (EBNA-2) *see* Epstein–Barr virus (EBV)
Epstein–Barr virus-encoded nuclear antigen 3 (EBNA-3) *see* Epstein–Barr virus (EBV)
Epstein–Barr virus-encoded nuclear antigen-LP (EBNA-LP) *see* Epstein–Barr virus (EBV)
Epstein–Barr virus-encoded small RNA (EBERs) *see* Epstein–Barr virus (EBV)
Epstein–Barr virus infection 2:164
apoptosis inhibition 1:157–160
associated carcinomas 5:195
nasopharyngeal carcinoma 5:195
B cells 2:151
persistence 2:151–152
propagation 2:150
Burkitt's lymphoma *see* Burkitt's lymphoma
carcinomas 5:195
gastric 2:155
nasopharyngeal *see below*
conjunctivitis 2:496
control 2:155
5′ azacytidine 2:155–156
gene expression patterns 2:165
Hodgkin's lymphoma 2:154*f*
disease characterization 5:194
incidence 2:154–155
viral gene expression 5:194
host range 2:150
immune response 2:153
infectious mononucleosis 2:153
latency 2:157–158
associated genes/proteins 5:194
associated tumors 2:441
EBV-encoded nuclear antigen 1 2:160
EBV-encoded nuclear antigen 2 2:160–161
EBV-encoded nuclear antigen-LP 2:160–161
EBV nuclear antigens 2:440–441
episome generation 2:158*f*
gene expression 2:160

Epstein–Barr virus infection (*continued*)
latent membrane protein 1 2:160–161
latent membrane protein 2A 2:160–161
latent membrane protein 3 2:160–161
latent membrane proteins 2:151–152
reactivation 2:165–166
site 2:151
lymphoproliferative malignancies 5:194
adult T-cell lymphoma 2:155
Burkitt's lymphoma *see* Burkitt's lymphoma
Hodgkin's lymphoma *see above*
in immunosuppressed 2:153
lymphocyte transformation *in vitro* 5:194
natural killer-cell lymphoma 2:155
natural killer cells 5:195
post-transplantation lymphoma 2:152*f*
T cell 2:155
molecular biology 2:159
nasopharyngeal carcinoma 2:155
viral gene expression 2:155
oncogenesis 5:316
persistent 2:165
resting memory B-cells 2:166*f*
prevention 2:155
primary infection 2:152*f*
reactivation 2:441*f*
replication role 2:165
acyclovir treatment experiments 2:165–166
host, effects on 2:166–167
latent infection reactivation 2:165–166
Rta protein 2:166
Zta protein 2:166
Epstein–Barr virus (EBV) mononucleosis syndrome 2:555
Equid herpesvirus 1 (EHV-1)
defective interfering particles 2:416
DNA structure 2:416
open reading frame 2:416
sequence conservation 2:416
distribution 2:412
DNA homology 2:413
epidemiology 2:417
experimental hosts 2:413
gene cloning 2:413
genome 2:413–414
functional analyses 2:414
gp2 2:414
IR genes 2:414
regulatory genes 2:414*t*
histopathology 2:418
history 2:411–412
infection *see below*
replication
defective mutants 2:416
early proteins 2:415
entry receptor 2:414
IE gene 2:415
initiation 2:415
IR3 gene 2:415
late proteins 2:415
transcription regulation 2:415
tissue tropism 2:413
virion structure 2:416
capsid proteins 2:416
Equid herpesvirus 1 infection
clinical features 2:411–412
incubation period 2:417
pregnancy 2:417
recovery 2:417
pathology 2:418
experimental infections 2:418
prevention and control 2:419
bacterial complications 2:419
quarantine 2:419
vaccination 2:419
spontaneous abortion 2:411–412
Equid herpesvirus 2 (EHV-2)
distribution 2:412
epidemiology 2:417
history 2:412
infection 2:418
Equid herpesvirus 3 (EHV-3)
distribution 2:412
DNA homology 2:413
epidemiology 2:417
history 2:412
infection 2:418
histopathology 2:418
Equid herpesvirus 4 (EHV-4)
DNA homology 2:413
epidemiology 2:417
history 2:412
infection 2:417
pathology 2:418
Equid herpesvirus 5 (EHV-5)
epidemiology 2:417
infection 2:418
Equid herpesvirus 9 (EHV-9)
DNA homology 2:413
history 2:412
infection 2:418
Equine adenovirus infection 1:26
Equine arteritis virus (EAV)
genome 1:179–180
geographical distribution 1:177
history 1:176
host range 1:177
infection
clinical features 1:183
focal myometritis 1:183
neutralizing antibodies 1:184
pathogenicity 1:183
pathology 1:183
pregnancy 1:183
tissue tropism 1:182–183
vaccination 1:185
structure 1:178*f*
Equine encephalitic togavirus(es) **5:101–107**
classification 5:101
conserved proteins 5:103
distribution 5:102
epidemiology 5:103
evolution 5:103
genetics 5:103
genome 5:101
history 5:101
infection
brain examination 5:106
cellular immunity 5:106
clinical features 5:105
control 5:106
horses 5:106
host range 5:102
immune response 5:106
pathogenicity 5:105
pathology 5:106
prevention 5:106
laboratory animals, infection 5:103
life cycle 5:102–103
nomenclature 5:101
propagation 5:102
protein properties 5:102
replication 5:102
cell attachment 5:102
protein transport 5:102
taxonomy 5:101
tissue tropism 5:104
transmission 5:104
vaccines/vaccination 5:106
virion properties 5:101
sensitivity 5:101
size 5:101
virion structure 5:101
in vitro propagation 5:103
see also specific viruses
Equine herpesvirus(es) **2:411–420**
classification 2:412
defective interfering particles 2:416
distribution 2:412
epidemiology 2:417
evolution 2:417
genetics 2:413
genome 2:413
functional analyses 2:414
gp2 2:414
IR genes 2:414
history 2:411
host range 2:413
infection
clinical features 2:417
histopathology 2:418
immune response 2:418
pathogenicity 2:417
pathology 2:418
persistent infection 2:416
prevention and control 2:419
morphology 2:412
propagation 2:413
replication 2:414
serology 2:417
taxonomy 2:412
tissue tropism 2:417
transmission 2:417
vaccines/vaccination 2:419
pregnancy 2:419
side effects 2:419
see also specific viruses
Equine infectious anemia (EIA) 2:167
acute 2:172
chronic 2:172
clinical features 2:172*f*
control 2:173
histopathology 2:172
hepatic necrosis 2:172–173
lymphocytic infiltrations 2:172–173
immune response 2:173
inapparent carriers 2:172
pathogenicity 2:171
pathology 2:172
prevention 2:173
rate 2:170
tissue tropism 2:171
Equine infectious anemia virus (EIAV) **2:167–174**
classification 2:167
diagnostic assays 2:173
epidemiology 2:170
incidence 2:169–170
evolution 2:171*f*
envelope variants 2:171
genetics 2:170
reverse transcription 2:170
genome 2:169*f*
env gene 2:168
gag gene 2:168
pol gene 2:168
rev gene 2:168
tat gene 2:168
geographical distribution 2:170
history 2:167
host range 2:170
infection *see* Equine infectious anemia (EIA)
infectivity 2:171
propagation 2:170
replication 2:169*f*
assembly 2:169–170
budding 2:169–170
genome integration 2:169
host cell attachment 2:169
polyprotein processing 2:169
transcription 2:169
seasonal distribution 2:170
serological relationships 2:171
transmission 2:170
control 2:173
vaccines/vaccination 2:173–174
viral protein properties 2:168*f*
capsid 2:168
envelope glycoproteins 2:168
RNP complex 2:168
virion properties 2:167
morphology 2:168*f*
Equine influenza virus(es) 3:101
see also specific viruses

Equine torovirus (EToV) 5:151–152
  antigenic properties 5:155
  buoyant densities 5:153
  genotypes 5:155
  geographical distribution 5:154
  historical aspects 5:151
  host range 5:154
  infection, seroconversion 5:155–156
  replication
    attachment/cell entry 5:153
    transcription 5:153–154
Equine viral arteritis, African horse sickness *vs.* 1:42
Equine virus(es)
  see specific viruses
ER *see* Endoplasmic reticulum (ER)
ERAD *see* Endoplasmic reticulum-associated degradation (ERAD)
Eradication programs, rabies virus infection 4:372
ERCC4-like nuclease 1:47
E^{rns} protein
  bovine viral diarrhea viruses 1:378
  classical swine fever virus 1:526
Errantivirus 3:301–302, 4:451
Errantivirus(es) 4:453
  baculovirus *vs.* 4:453
  distribution 4:452
  envelope fusion protein 4:453
    cleavage 4:453
  infectivity 4:451
    env role 4:452
  retrotransposons 4:433
  see also specific viruses
Error(s), virus databases 5:355
Error catastrophe *see* Evolution (of viruses)
Error-prone replication 4:360
  tailed bacteriophage evolution 2:181
  viral evolution 2:177
Error threshold 4:359
Erythema infections 4:95
Erythrocyte(s)
  BK virus membrane binding 4:281
  human B19 parvovirus 4:89
Erythrocyte necrosis virus 3:155
Erythrovirus 4:95
Erythrovirus(es)
  classification 4:92*t*
  see also specific viruses
  genome 4:94*f*
  see also specific viruses
Escape hypothesis, viral origins 3:476*f*
  bacteriophages 3:473
  virus-encoded proteins 3:473–474
Escape mutants
  definition 1:127
  HIV 1:74–75
  passive immunization 3:67
  rate 3:66–67
  Visna-Maedi virus infection 5:430–431
Escherichia coli
  associated phages 4:400–401
    bacteriophage T4 5:175*f*
    ssRNA bacteriophage 4:405
  DNA vaccine production 2:52
  transcription, bacteriophage N4
    gyrase role 5:181
    SSB role 5:181
  transcription elongation terminators 5:176
  transformation, baculovirus expression vectors 1:241
Escherichia coli 018:K1:H7, virulence factors 3:123
Ethiopia, HIV infection 1:63
Ethylene, plant resistance to viruses 4:176–177
Etiologic studies, epidemiology 2:143
Etiology, definition 1:301
EToV *see* Equine torovirus (EToV)
Etravirine 2:510
Eubacteria, bacterial cell viral entry 5:365
Eucocytis meeki virus (EmV) 5:28*t*
euHCVdb 5:357*t*
Eukaryotic initiation factor-2α (eIF2α) 3:173
Eukaryotic initiation factor-3 (eIF-3) 1:384
Eukaryotic initiation factor-4E (eIF4E) 4:183*f*
  amino acid substitutions 4:183–184
  natural resistance *vs.* 4:184
  role 4:183*f*
  subfamily 4:183
  VPg interactions 4:185
Eukaryotic initiation factor-4F (eIF-4F)
  picornavirus replication 4:133
  plant resistance genes 4:185
  role 4:183*f*
Eukaryotic initiation factor-4G (eIF-4G)
  enterovirus translation 2:126
  foot-and-mouth disease virus 2:269
  picornavirus 2:269
  plant resistance genes 4:185
Eukaryotic virus(es)
  see specific viruses
Euploea corea virus (EcV) 5:28*t*
European bat lyssavirus 4:184*t*
European catfish virus (ECV) 2:233
European Center for Disease Prevention and Control 5:357*t*
European Molecular Biology Laboratory (EMBL) 5:356
European rabbit *(Oryctolagus cuniculus),* calicivirus infection 1:410
European sheatfish virus (ESV) 2:233
European spruce sawfly *(Gilpinia hercyniae)*
  biological control 3:125
  history 1:225–226
European wheat striate mosaic virus (EWSMV) 5:24
Euryarchaea-infecting virus(es) **5:411–423**
  extreme halophilic Archaea-infecting viruses 5:415
    haloalkaliphilic virus 5:418
    head-and-tail viruses 5:415
    salterprovirus 5:418
    spherical halovirus SH1 5:420
  methanogenic Archaea-infecting viruses 5:412
    burst size 5:412
    latent period 5:412
  research 5:412
    history 5:412
  thermococcales-infecting virus (PAV1) 5:421
    genome 5:422
    virion composition 5:422
  see also specific viruses
EV *see* Epidermodysplasia verruciformis (EV)
Everglades virus (EVEV) 5:109*t*
Everted DNA
  definition 3:117
  inovirus DNA conformation 3:121
Evolution (of viruses) **2:174–184**
  as basic science 2:175
  cellular *vs.* acellular 3:475
    metabolism 3:475
  continuous 2:180*f*
    genetic stability *vs.* 2:179
  error catastrophe 2:178
    definition 2:178–179, 4:359
    genome size limitation 2:178–179
    quasispecies *see* Quasispecies
  error prone replication 2:177
  experiments in *see below*
  historical aspects 2:177
    fitness definition 2:177
    mutation rates 2:177
    non-species definitions 2:177
  host evolution *vs.* 2:176
    non-extinction 2:176
    polyphyletic origins 2:176
    species jumping 2:176
  modular 5:145
  mutations 4:125
  quasispecies 2:177
    clouds 2:178–179
    definition 2:177–178
    Eigen, Manfred 2:177–178
    Shuster, Peter 2:177–178
    swarms 2:178–179
  in real time 2:175
  RNA stability 2:179
  RNA virus–host interactions 3:154
  sequence space 2:178
    fitness landscape 2:178*f*
    genetic bottlenecks 2:179
  similarity tools 2:179
    BLAST 2:180–181
    Markov chain Monte Carlo method 2:180–181
  species classification 5:403
  species jumping 2:175
  specific features 2:175
  virulence variation 2:175
  virus–host congruence 2:179
  see also Origin(s) of viruses; *specific viruses*
Evolution (of viruses), RNA virus experiments 4:362
  evolutionary properties 4:363
    evolutionary convergencies 4:364
    evolutionary parallelisms 4:364
    fitness tradeoffs 4:364
    transmission bottlenecks 4:364
  parameters 4:363
    compacted genomes 4:363
    high per-base mutation rate 4:363
    rapid growth 4:363
  quasispecies theory relevance 4:364
    error catastrophe *vs.* lethal mutagenesis 4:365
    robustness evolution 4:364
Evolutionary convergencies, RNA virus experimental evolution 4:364
Evolutionary parallelisms, RNA virus experimental evolution 4:364
Exanthem, definition 1:580
Exanthema subitum 2:498
Exaptation, definition 4:436
Exogenous antigen(s) 3:72–73
3′-5′ Exonuclease (ExoN) 5:477–478
Exophthalmia, definition 3:89
3′–5′ Exoribonuclease 3:424–425
Expanded Programme Immunization (EPI), hepatitis B virus vaccination 2:364
ExPASy 5:357*t*
Exposed-uninfected (EU) individual(s), definition 1:69
Extensible markup language (XML), virus database formats 5:350*f*
Extracellular antibody neutralization, neural cell infection 1:473
Extracellular enveloped virions, variola virus replication 4:639
Extracellular enveloped virus (EEV)
  African horse sickness *vs.* 1:42
  cowpox virion types 1:575
  see also specific viruses
Extracellular search
  bacteriophage population ecology 2:74–75
  definition 2:71
Extrahepatic biliary atresia (EHBA) 4:382
Extra small virus (XSV) 4:527*t*
  genome 4:574
  taxonomy 4:575
Extreme halophilic Archaea-infecting virus(es) *see* Euryarchaea-infecting virus(es); *see specific viruses*
Extreme resistance (ER) (plants) 4:174
Extrinsic incubation period *see* Vector(s)
Extrinsic pathway (apoptosis) 1:155
Eyach virus (EYAV) 1:533
  cross-reactivity 1:536–537
  distribution 1:534*f*
  evolution 1:537
  genome 1:540*f*
Eye
  anatomy 2:492*f*
  infections *see* Eye infection(s)
  lens 2:491
  physiology 2:491
  see also entries beginning ocular
Eye infection(s) **2:491–498**
  adenoviruses 2:495
  associated viruses 2:68
  see also specific viruses
  hepatitis C virus 2:491–492
  molluscum contagiosum virus 2:496

Eye infection(s) (*continued*)
necrotizing inflammation 2:492
rubella virus 2:492–494
transmission 2:491
varicella zoster virus 2:496
see also entries beginning ocular

F

Faba bean necrotic stunt virus (FBNSV) 3:386
characteristics 3:389*t*
Faba bean necrotic yellows virus (FBNYV) 3:218*f*
characteristics 3:389*t*
epidemic, Egypt 3:218
genome 3:387*f*
integral genome segments 3:388
satellite-like rep DNA 3:388–390
host range 3:386
infection
agroinoculation experiments 3:386
economic importance 3:386
transmission 3:386
aphids 3:386
Fabavirus 1:573
F-actin cables, baculovirus in vitro
pathogenesis 1:270
Famciclovir 1:150
HSV infection 2:394–395
Families 5:9
Famvir® 1:143*t*
Fancy carp reovirus 1:164*t*
Farming *see* Agriculture/farming
Farr, William 2:141
Fascia 5:83
togavirus infection 5:84
Fascial 1:37
Fascitis 5:83
togavirus infection 5:84
Fas ligand(s)
apoptotic role 1:157
Fas interactions 3:80
Fatal familial insomnia (FFI) 5:192
associated genetic mutation 5:192–193
clinical characteristics 5:192–193
Fatal hemorrhagic disease 1:636
FBL-2 protein 2:373
FBNSV *see* Faba bean necrotic stunt virus (FBNSV)
FBNYV *see* Faba bean necrotic yellows virus (FBNYV)
fd bacteriophage *see* Bacteriophage fd
FDV *see* Fiji disease virus (FDV)
Febrile rash, coxsackieviruses 1:585
Fecal–oral cycle
hepatitis E virus 2:380
virus transmission patterns 2:146
Feldmannia virus(es) (FsV) 4:116
see also specific viruses
Feline calicivirus(es) 1:410
antigenic variation 1:415
geographical distribution 1:413–414
host range 1:414–415
infection
clinical features 1:417
immunity 1:417
propagation 1:415
transmission 1:416
vaccines/vaccination 1:418
see also specific viruses
Feline immunodeficiency virus (FIV) **1:347–354**
classification 1:347
diagnostics 1:353–354
epidemiology 1:351
dual infections 2:187
evolution 1:350
pathogenic 1:353–354
genetics 1:350
subtypes 1:350
variability 1:350
genome 1:349
open reading frames 1:349–350
geographical distribution 1:348*f*
prevalence 1:348–349
history 1:347
host range 1:350
infection *see below*
propagation 1:350
serology 1:350
cross-reactivity 1:350
taxonomy 1:347
subtypes 1:348
tissue tropism 1:351
transmission 1:351
transplacental 1:351
variability 1:350
virion structure 1:349*f*
viral core 1:349
viral enzymes 1:349
Feline immunodeficiency virus infection
clinical features 1:351
CNS disease 1:352
stages 1:351–352
viral load 1:351–352
histopathology 1:352
immune response 1:352
pathology 1:352
neurological abnormalities 1:352
prevention and control 1:353
antiretroviral drugs 1:353
vaccine trials 1:353
Feline leukemia virus (FeLV) 2:107, **2:185–190**
classification 2:185
control 2:189
defective interfering viruses 2:4
envelope gene variation 2:188
FeLV-A 2:188
FeLV-B 2:188
FeLV-C 2:188
epidemiology 2:186
viral reactivation 2:186
evolution 2:188
genome 2:185
geographical distribution 2:186
history 2:185
host range 2:187
infection *see below*
pathogenicity 2:188
replication 2:185
life cycle 2:186*f*
virion assembly 2:186
subgroups 2:187
taxonomy 2:185
transmission 2:189
age-related resistance 2:189
vaccines/vaccination 2:189
virion structure 2:185
Feline leukemia virus infection
clinical features 2:187
mortality 2:187
neoplasms 2:187–188
dual infections 2:187
immune response 2:189
immunosuppression 2:188
oncogenesis 2:188
pathology 2:187
prevention 2:189
transient infection 2:186
Feline panleukopenia virus (FPV) 4:85
infection pathogenesis 4:89
Feline parvovirus(es) (CPV) 4:90
see also specific viruses
Feline sarcoma virus(es) (FeSV) **2:185–190**
classification 2:185
envelope gene variation 2:188
epidemiology 2:186
genome 2:185
geographical distribution 2:186
history 2:185
infection
clinical features 2:187
control 2:189
immune response 2:189
pathology 2:187
prevention 2:189
vaccines/vaccination 2:189
pathogenicity 2:188
recombination 2:185
replication 2:185
taxonomy 2:185
transmission 2:189
virion structure 2:185
see also specific viruses
Ferret(s), influenza virus infection 3:101
FeSV *see* Feline sarcoma virus(es) (FeSV)
Fetal infection(s)
see specific infections
Ff bacteriophage *see* Bacteriophage Ff
FFI *see* Fatal familial insomnia (FFI)
FG loops, translational repression/assembly initiation complex 3:28
F glycoprotein, measles virus 3:287–288
FHV *see* Flock house virus (FHV)
Fiber genes
adenoviruses 1:3
aviadenoviruses 1:6
fowl adenoviruses 1:6
Fibrillarin 5:209
umbravirus infection 5:212
Fibrin degradation products (FDP) 2:199
Fibroblast(s), CMV infection 1:639*f*, 3:321–322
Fibroblast growth factors (FGF), granuloviruses 1:217
Fibropapilloma-associated turtle herpesvirus (FPTHV) 2:428
Fibropapillomatosis 2:428
Fibropapillomavirus(es)
life cycle 4:20
see also specific viruses
tissue tropism 4:30*f*
see also specific viruses
Field-level epidemiology, African cassava mosaic disease 1:33
Field trials, papaya ringspot virus resistance 4:5
Fiji, banana bunchy top virus epidemics 1:277
Fiji disease virus (FDV) 4:149–150
characteristics 4:150*t*
genome 4:151*f*
as type species 4:536*t*
Fiji leaf gall disease 4:149–150
Fijivirus 1:484, 4:150, 4:536*t*
Fijivirus(es)
characteristics 4:150*t*
classification 4:150
characteristics 4:150
genome 4:150
dsRNA segments 4:151*f*
terminal sequences 4:151*t*
host range 4:154
transmission 4:154
virion structure 4:151
capsid 4:151
see also specific viruses
Filamentous brown algae, associated viruses 4:123
ecological impact 4:124
Filamentous ssDNA bacteriophages **2:190–198**, 3:117–118, 3:119*t*
bacterial hosts 3:123
biotechnology 2:197
classification 2:191
composition 2:193
genome 2:192*f*
genome delivery 5:369
pili 5:369
receptor-binding proteins 5:369
Gram-negative bacteria infection 3:117–118, 5:369
life cycle 2:195
DNA synthesis 2:196
infection 2:195
protein synthesis 2:196
virus assembly 2:191–192
phage display 3:124
physical properties 2:191
receptor complexes 3:118
structure 2:191
pVIII 2:193*f*
taxonomy 2:191
Filiformism 1:614
Filoviridae 2:57, 2:199, 3:273, 3:276*f*, 3:324

Filovirus(es) **2:198–205**
  bioterrorism 5:408–409
  characteristics 5:62*t*
  gene expression 2:201
  genome 2:202*f*
  history 2:199
  hosts 2:200
  transmission 2:200
    amplification 2:200
  vaccines/vaccination 2:204
  virion composition 2:200
  virion structure 2:201*f*
    glycoproteins 2:200–201
    receptors, putative 2:200–201
    RNP complex 2:201
    VP24 2:201
    VP30 2:201
    VP35 2:201
    VP40 2:201
  see also specific viruses
Filovirus infections
  animal models 2:202
  apoptosis inhibition 1:158*t*
  clinical features/presentation 2:202
  disseminated intravascular coagulation 2:203
  host responses 2:203
  incubation period 2:202
  prevention 2:204
    antisense technology 2:204–205
    candidate vaccines 2:204
    RNA interference 2:204–205
  treatment 2:204
    interferon type I 2:205
    recombinant nematode anticoagulant protein c2 2:204–205
Filtration experiments, virus discovery 3:398–399
Fingerlings 3:83
Finlaya, Dengue virus transmission 2:7
First-generation vectors *see* Plant virus(es)
Fish adenovirus(es) 1:28
  infections 1:28, 2:234
    epidermal hyperplasia 2:234
  see also specific viruses
Fish birnavirus(es) 2:232
  transmission 2:232
  vaccines 2:232
  vaccines/vaccination 2:232
  see also specific viruses
Fish calicivirus(es) 2:230
  phylogenetic analysis 2:230–231
  see also specific viruses
Fish herpesvirus(es) **2:205–212**
  classification 2:206
  distribution 2:207
  evolution 2:209
  genetics 2:209
  genomes 2:210*t*
  growth properties 2:209
  history 2:206
  infection 2:233–234
    clinical features 2:207
    host species 2:206
    immune response 2:211
    pathogenesis 2:207
    pathology 2:207
    prevention and control 2:211
  see also specific viruses
Fish iridovirus(es) 2:233
  epizootic hematopoietic necrosis 2:233
  genome sequencing 2:233
  virion morphology 2:233
  see also specific viruses
Fish nodavirus(es) 2:229
  affected species 2:229–230
Fish paramyxovirus(es) 2:229
  historical aspects 2:229
  see also specific viruses
Fish reovirus(es) 2:231
Fish retrovirus(es) **2:212–221**, 2:231
  historical aspects 2:231
  nucleotide sequences 2:231
  phylogeny 2:220*f*
  see also specific viruses
Fish rhabdovirus(es) **2:221–227**, 2:228
  affected species 2:228
  classification 2:223*f*
  phylogenetic analysis 2:228
  reverse genetic analysis 2:228
  transmission 2:221
  see also specific viruses
Fish rhabdovirus infection
  challenge models 2:221
  control 2:225
    management practices 2:226
    transmission 2:226
    vaccination 2:226
  cytopathic effects 2:221
  detection 2:225
    serological methods 2:225
  mortality 2:221
  symptoms 2:221
  vaccines/vaccination 2:226
Fish virus(es) **2:227–234**
  DNA viruses 2:227
  economic impacts 2:227
  as reportable diseases 2:227
  RNA viruses 2:227
  see also specific viruses
Fitness 5:274
  viral evolution 2:177
Fitness landscape
  definition 2:175
  sequence space 2:178*f*
Fitness tradeoffs, RNA viruses 4:364
5′-nontranslated region, Pepino mosaic virus 4:105
Flagellar proteins, bacterial cell viral entry 5:366
flamenco (transcription factor) 3:302–304
Flanders virus (FLANV) 4:184*t*
*flash*BAC *see* Baculovirus expression vector(s)
Flat-type smallpox 4:640
Flaviviridae 1:170, 1:526–527, 2:6, 2:235, 2:242, 2:367, 3:183, 5:11*t*, 5:45, 5:440–441
Flavivirus 2:6, 2:242, 5:45, 5:108, 5:440–441
Flavivirus(es) **2:241–253**
  antigenic determinants 2:8–9
  apoptosis inhibition 1:158*t*
  assembly 1:198
    capsid 1:199
    glycoproteins 1:198
    pre-membrane protein cleavage 1:199
    vesicle packets 1:199
  budding 1:199
  classification 2:242
    re-classification 5:20
  definition 5:469
  epidemiology 2:243
    human activity 2:243
  evolution 1:174
    arboviruses *vs.* 1:173
    mutation frequency 1:173–174
  fusion proteins 3:297
  genome 1:172
    encoded proteins 2:235
  geographical distribution 1:174
  global distribution 2:243
  history 2:242
  host cell exit strategies 1:200
  human pathogens 1:198
  infections *see below*
  life cycle 1:198
  maturation 2:243–245
  physical properties 2:243
    stability 1:172
  polyprotein processing 1:198
  replication 2:243
  structure 2:242
  taxonomy 2:242*t*
  transmission 1:170
    mosquito-borne *vs.* tick-borne 1:173–174
    resistance 2:245
    tick-borne 2:6
  veterinary important **2:234–241**
    mosquito-borne 2:236
    tick-borne 2:239
  virion morphology 2:235
  virion structure 1:172
    capsid 1:198
    envelope 1:198
    immature virion 1:198
    mature virion 1:198
    nucleocapsid 1:199
    pre-membrane protein 1:198
    proteins 2:243–245
  see also specific viruses
Flavivirus infections
  animal disease 1:175
  cell tropism 2:245
  central nervous system 2:245
  clinical disease 2:243
  diagnosis 2:245
    immunoglobulin G 2:245–246
  host cell entry 2:242
    candidate receptors 2:243
  immunomodulation 4:114*t*
  pathogenesis 2:245
  persistence 4:109*t*
  prevention 2:245
  secondary infection 2:245
Fleck disease complex 4:203
  tymoviruses 4:203
Fleece coarseness, Border disease 1:336
Flexiviridae 1:448–450, 2:254*t*, 2:255, 2:288
Flexivirus(es)
  biological properties 2:254
  classification 2:253*t*
  genome 2:255*f*
  phylogenetic relationships 1:426*f*
    analysis limitations 2:257–258
    coat protein 2:258*f*
    movement protein 2:256
    replication protein 2:256*f*
    RNA-binding protein 2:258*f*
    TGB protein 2:257*f*
  replication 2:253
    translation 2:253
  transmission 2:254
  virion morphology 2:253
  see also specific viruses
FLIP proteins *see* Death receptor signalling
Flock house virus (FHV)
  host range 3:431–432
  RNAi suppression 3:152
  RNA replication
    cis-acting signals 3:436
    long-distance base paring 3:436
  structure 5:382*f*
Floral homeotic gene(s), virus-induced gene silencing 5:378–379
Flow cytometry 2:71
Flower breaking, definition 4:207
Fluid replacement, Crimean-Congo hemorrhagic fever treatment 1:603
Fluorescence antibody to membrane antigen (FAMA), varicella-zoster virus 5:252
6-Fluoro-3-hydroxy-3-pyrazine carboxamide (T-705) 1:153
Flv gene, West Nile virus 5:446–447
FMV *see* Fort Morgan virus (FMV)
Foamy viruses (FV) **2:259–265**
  associated hosts 2:259
  Bet gene/protein 2:471
    overexpression 2:263
    synthesis 2:263
  classification 2:260
  DNA integration
    life cycle 2:264
    vectors for gene therapy 2:264–265
  env gene/protein 2:262
  epidemiology 2:259
    at risk populations 2:259–260
  evolution 2:264

Foamy viruses (FV) (*continued*)
gag gene/protein 2:262
synthesis 2:262
gene therapy vectors 2:264
DNA integration 2:264–265
genetics 2:264
gene expression 2:262
see also specific genes
genome 2:262*f*
infection 2:260
tissue cultures 2:261*f*
life cycle 2:263*f*
assembly 2:264
budding 2:264
DNA integration 2:264
encapsidation 2:264
host cell entry 2:263–264
packaging 2:264
reverse transcription 2:264
transcription 2:264
translation 2:264
uncoating 2:264
morphology 2:261*f*
nomenclature 2:259
pol proteins 2:262
enzymatic activity 2:262–263
synthesis 2:262
protein organization 2:262
Bet 2:263
env 2:262
gag 2:262
pol 2:262
Tas 2:263
taxonomy 2:260
transmission 2:259–260
virion properties 2:261
see also specific viruses
Focal adhesion kinase (FAK), KSHV host cell entry 3:197
Focal myometritis 1:183
Foetal bovine serum, insect cell culture 1:238
fOg44 bacteriophage 3:254–255
Foliage, Sharka disease 4:238
Follicle cell(s), gypsy RNA transcripts, expression 3:302–304
Follicular dendritic cell(s) (FDC), prion replication 4:334
Fomite(s) 2:141
Fomite transmission, respiratory viruses 2:556
Food hygiene, norovirus infection 1:418
Food poisoning, disease surveillance systems 2:46
Foorkey disease 1:275–276
Foot-and-mouth disease
clinical features 2:273*f*
control 2:273
immune response 2:273
pathogenicity 2:272
pigs 2:270–271
vaccination 2:273
prevention 2:273
tissue tropism 2:272
Foot-and-mouth disease virus(es) (FMDV) **2:265–274**
animal enterovirus associated 2:124
classification 2:266
epidemiology 2:272
evolution 2:272*f*
genetics 2:271
genome 2:267*f*
internal ribosome entry sites 2:268*f*
sequencing 2:143
untranslated region 2:267
geographical distribution 2:270*f*
nonendemic areas 2:272
history 2:266
host range 2:270
neutralization escape mutants 3:418
propagation 2:270
proteins 2:267
antigenic sites 2:271
$3C^{pro}$ 2:268
$3D^{pol}$ 2:268
leader 2:268
P1 region 2:268
P2 region 2:268
P3 region 2:268
structural 2:266
truncations 2:266–267
VP1 2:266–267
VPg 2:267
receptor binding 5:321
replication
host cell attachment 2:270
host cell entry 2:270
post-translational processing 2:267*f*
RNA replication 2:268
translation 2:269
virus assembly 2:269
virus release 2:269
serological relationships 2:272*f*
taxonomy 2:266
transmission 2:272
vaccines/vaccination 2:273
formalin inactivation 2:273
virion properties 2:266
structure 2:266*f*
see also specific viruses
Foreign gene delivery, giardiavirus vectors 2:314
Fort Morgan virus (FMV) 5:92
characteristics 5:109*t*
Fosamprenavir 1:143*t*
Foscarnet 1:143*t*
Foscavir® 1:143*t*
Foveavirus 1:420*t*, 1:422, 1:426*f*
Foveavirus(es) **1:419–427**
associated species 1:422
biological properties 1:422
classification 1:420*t*
genome 1:423
heterogeneity 1:424
open reading frames 1:423
particle structure 1:421*f*
phylogeny 1:426*f*
replication 1:423
strains 1:424
tissue tropism 1:423–424
see also specific viruses
Fowlpox
cutaneous form 2:280*f*
detection
inter-strain differentiation 2:281
intra-species differentiation 2:281
monoclonal antibodies 2:280
PCR 2:282*f*
diphtheritic form 2:281*f*
histology 2:279
variants 2:283
Fowlpox-like virus(es) 2:278
see also specific viruses
Fowlpox virus (FWPV) **2:274–284**
antigens 2:275
history 2:274
infection *see* Fowlpox
lipid metabolism, host effects 2:277
survival factors 2:275–276
taxonomy 2:274
transmission 2:279
vaccines/vaccination 2:282*f*
contamination 2:283
virus propagation 2:277
variants
antigenic 2:283
tumorigenic 2:283
Foxtail mosaic virus (FoMV) 4:311*t*
F-pilus, filamentous ssDNA bacteriophage infection 2:191
F proteins
baculovirus infection resistance 1:267
henipavirus 2:322–323
measles virus 3:286
nucleopolyhedrovirus 1:263
paramyxovirus classification 3:285
FPV *see* Feline panleukopenia virus (FPV)
FpV-1 *see* Fusarium poae virus (FpV-1)
Fractalkine 2:542
FrAdV-1 *see* Frog adenovirus 1 (FrAdV-1)
Fragaria chiloensis latent virus (FCiLV) 3:47
Fragaria vesca, Raspberry bushy dwarf virus infection 3:39
Frameshifts, mouse mammary tumor virus replication 3:337*f*
Francisella tularensis, bioterrorism evaluation 5:408*t*
Franklinella occidentalis, tomato spotted wilt virus association 5:136
Fraser's hypotheses, plant recessive resistance genes, mechanisms 4:182
Freesia sneak virus (FSV) 3:448
coat protein 3:450
geographical distribution 3:452–453
transmission 3:453
Freeze-dried vaccine(s), safety 5:229
Freund's incomplete adjuvant, Visna-Maedi virus vaccination 5:431
Frog adenovirus(es)
infection pathogenesis 1:28
see also specific viruses
Frog adenovirus 1 (FrAdV-1) 1:28
Frog herpesvirus(es)
genetics 2:211
see also specific viruses
Frog virus 3 (FV3) 2:233
replication cycle 3:171*f*
cellular receptor, host cell entry 3:170
DNA methylation 3:170
DNA synthesis 3:170
RNA synthesis, early 3:170
RNA synthesis, late 3:170
virion assembly 3:171
sites of 3:172*f*
Frog virus 4 (FV4), genome 2:209
Frosch, Paul 5:55
virus discovery 3:399
Fruit drop, Sharka disease 4:239
Fruit trees, diseases **4:201–207**
pome fruits 4:205
causal agents 4:206
control 4:206
geographical distribution 4:206
symptoms 4:205
transmission 4:206
yield losses 4:205
stone fruits
causal agents 4:205
control 4:205
geographical distribution 4:205
nematode-borne 4:198
Sharka 4:201
symptoms 4:204
transmission 4:205
yield losses 4:204
Fry 3:83
FSV *see* Freesia sneak virus (FSV)
Fukuoka virus (FUKAV) 4:184*t*
Full length genome sequencing, plum pox virus 4:240
'Full virus vector' strategy *see* Plant virus(es)
Fulminant hepatitis 2:357
Functional genomics 5:375
Functional marker mutations, DNA virus recombination 4:374–375
Functional motifs, virus databases 5:363
Fungal virus(es) **2:284–291**
classification 2:287*t*
double-stranded DNA viruses 2:286
genomes 2:286
morphology 2:286
replication 2:289
gene expression 2:289
genomes 2:574
ssRNA viruses 2:288
unassigned ssRNA viruses 2:288
historical aspects 2:284
host range 2:285
experimental 2:285
hypovirulence *see* Hypovirulence

infections
host defenses 2:289
latency 2:285
mixed 2:286
plant–fungal mutualistic associations 2:291
symptom expression 2:285
replication 2:289
yeast as a model host 2:290
taxonomy 2:286
transmission 2:285
virological technical advances 2:289
see also Mycovirus(es)
Fungi
classification 4:419
definition 4:419
filamentous, killer phenomenon 5:164
infections
biocontrol of 2:580
symptom classification 2:574
see also specific infections
plant virus transmission 5:281
retrotransposons *see* Retrotransposons, fungi
Furin
definition 2:542
henipavirus 2:322–323
Furin-like cleavage, baculovirus F protein 4:454*f*
Furovirus 1:308, 2:291–292, 2:295, 4:283
Furovirus(es) **2:291–296**
classification 4:283
definition 2:291
genome 2:294*f*
geographical distribution 2:292
history 2:291
infections
control 2:292
diagnosis 2:295
diseases associated 2:292
host range 2:292
symptoms 2:295
movement, long distance 2:292
phylogeny 2:294*f*
proteins 2:293
coat protein 2:293–294
movement 2:292
transmission 2:292
virus differentiation 2:293
virus properties 2:293
antigenic 2:293
nucleic acids 2:293
structure 2:293*f*
see also specific viruses
Furovus 1:308
Fusarium poae virus (FpV-1) 4:73
morphology 4:73
Fuselloviridae 1:587, 2:296–297, 5:11*t*
Fusellovirus(es) **2:296–300**
biochemical characterization 2:297
Crenarchaea infections 1:588*t*
culture-independent studies 2:299
definition 2:296–297
distribution 2:297
genomes 1:593
conservation 1:593
history 2:297
integrases 2:299
genome integration 2:299
tRNA genes 2:299
in vitro studies 2:299
isolates 2:298
plasmid virus hybrids 2:299
structural genomics 2:300
vectors 2:299
virion morphology 1:587
virion production 2:296–297
see also specific viruses
'Fusiform' virus(es) 2:300
see also specific viruses
Fusion, host cell entry 4:409
Fusion-associated small transmembrane (FAST) proteins 4:383–384
Fusion inhibitors, HIV antiviral therapy 1:148
Fusion loop(s), membrane fusion 3:300
Fusion peptide(s), membrane fusion 3:296
Fusion pore(s) 3:292
formation 3:294*f*
Fusion protein(s)
class I 3:293
biosynthesis 3:293
cooperativity 3:296
general fusion model 3:297*f*
hemagglutinin 3:293
see also Hemagglutinin (HA)
membrane anchors 3:295–296
refolding 3:297*f*
class II 3:297
activated structure 3:297
biosynthesis 3:297
cooperativity 3:299
native structure 3:297
trimerization 3:299*f*
class III 3:300*f*
biosynthesis 3:299
fusion loops 3:300
definition 4:407
Fusogenic orthoreovirus(es) 4:383
see also specific viruses
Fusolin/spindles, entomopoxviruses 2:138
FV *see* Foamy viruses (FV)
Fv1 (Friend virus susceptibility factor 1) 2:469
saturation of 2:469
strain specificity 2:469
FV3 *see* Frog virus 3 (FV3)
Fv4, receptor-envelope interaction 2:468–469
FWPV *see* Fowlpox virus (FWPV)

G

Gag (group-specific antigen)
LTR retrotransposons 4:438–439
Visna-Maedi virus infection, immune response 5:427
yeast L–A virus virion structure 5:466*f*
gag gene/protein
definition 4:428
LTR retrotransposons 4:420
pseudovirus organization 4:353
structural similarities 2:520
see also specific viruses
Gag-Pro precursor protein 4:456
Gait spasticity, TMEV infection 5:42
Galleria mellonella (wax moth), parvovirus infection 4:76
Galleria mellonella densovirus (GmDNV) 4:78
Gallid herpesvirus 1 (GaHV-1) 2:406
Gallid herpesvirus 2 (GaHV-2) 2:406
GALT *see* Gut-associated lymphoid tissue (GALT)
GAM-1 protein, fowl adenoviruses 1:6
Gammaentomopoxvirinae 4:323
Gammaentomopoxvirus
characteristics 2:137*t*
structure 4:325
Gammaentomopoxvirus 2:136, 4:323
Gammaherpesvirinae 1:364*t*, 2:431, 5:11*t*
Gammaherpesvirus(es)
bigenic amplification 2:426*f*
bovine *see* Bovine gammaherpesvirus(es)
classification 2:432
equine viruses 2:412
latency 2:440
lymphoproliferative disease 2:428
pathogenicity 2:427–428
simian **4:585–594**
future work 4:593
members 4:587*t*
virus-encoded Bcl-2 proteins 1:162
see also specific viruses
Gammapapillomavirus 4:9
see also specific viruses
Gammaretrovirus 4:460
Gammaretrovirus(es)
classification 4:460
detection 2:17
morphology 4:461–462
taxonomy 4:446
see also specific viruses
Ganciclovir (GCV) 2:557
BaculoDirect expression vector system 1:244
history 1:146
prodrug 1:146
Gan Gan virus 1:400
Ganglia 2:436
Ganglioside(s), Gt1b 4:280
Ganjam virus 1:597*f*
Gardasil anti-HPV L1 major capsid protein VLP vaccine 5:224
yield 5:224
Garlic common latent virus (GarCLV) 5:288
Garlic dwarf virus (GDV) 4:150*t*
Garlic virus(es) 5:288
infection 5:288
Gastric acid, host defence 4:512
Gastric carcinoma(s)
distribution 2:155
EBV associated 2:155
Gastroenteritis
associated viruses 2:116
see also specific viruses
deaths 2:116
definition 3:438
symptoms 2:116
Gastrointestinal tract
adult T-cell leukemia 2:568
enteric viruses 2:116
MMR vaccine safety 5:233
mouse mammary tumor virus transmission 3:337
SIV replication 4:608
togavirus infection 5:84
villi 3:443
virus entry 5:315
Gastrointestinal virus(es)
organ transplantation 3:470
see also specific viruses
Gateway cloning vectors, BaculoDirect expression system 1:241
GAV *see* Gill-associated virus (GAV)
gB gene, herpesvirus 2:426–427
GBLV *see* Grapevine Bulgarian latent virus (GBLV)
GBP-i protein, JCV 4:264
GB virus(es) 2:252
structure 2:252
see also specific viruses
GCMV *see* Grapevine chrome mosaic virus (GCMV)
GCRV *see* Grass carp aquareovirus (GCRV)
Geminiviridae 1:298, 1:302, 2:98, 4:165, 4:169*f*, 5:11*t*
Geminivirus(es) **4:164–170**
defective interfering RNAs 1:315–316
definition 2:97
diversification 4:166
DNA reassortment 4:166
rapid evolution 4:166
DNA recombination mechanisms 4:376
DNA satellites 4:530
economic importance 4:166
distribution effects 4:167
yield losses 4:166–167
emerging **2:97–105**
evolution 4:166
genera 4:165
discrimination 4:168
genome 4:165
conserved consensus sequences 4:168
monopartite 4:165
host shifts 4:166
infections *see below*
molecular diagnosis 4:167
PCR 4:167
phylogenetic analysis 4:169*f*
replicase-associated protein 4:168
serology 4:167
viral coat protein DNA sequence 4:168
movement 3:350
phylogenetics 4:169*f*
nanoviruses *vs.* 3:391
proteins 4:165
replication 1:317–318
satellite viruses 4:534

Geminivirus(es) (*continued*)
structure 4:165*f*
transmission 4:165
virus-induced gene silencing vectors 5:378
see also specific viruses
Geminivirus infections 4:167*f*
management 4:168
variant detection 4:169
vector population reduction 4:168–169
sweetpotatoes 4:661*t*
symptomatology 4:167*f*
tobacco diseases 5:65
GenBank 5:350*f*
sequence data 5:19*f*
accuracy 5:362–363
taxonomic data 5:19*f*
Gene(s)
delivery, baculovirus expression vectors 1:245
diversity, retrovirus evolution 2:183–184
duplication, EBV-encoded nuclear antigen 3 2:162
nonchromosomal, definition 4:336
Gene A_2, bacteriophage-mediated host cell lysis 3:257
Gene E, bacteriophage-mediated host cell lysis 3:257
Gene expression
definition 4:407
varicella-zoster virus latency 5:261
Gene expression profiling 2:14–15
probes 2:15
sequence specificity 2:15
Gene expression systems
limitations 4:229
plant virus vectors *see* Plant virus(es)
Gene-for-gene resistance 4:170
history 4:171
Gene guns, DNA vaccine delivery 2:54–55
Gene L, bacteriophage-mediated host cell lysis 3:258
Gene Ontology (GO) Consortium 5:353
Gene parity plots 1:225
Gene property tables 5:352
Genera 5:9
General fusion model, class I fusion proteins 3:295–296
General practitioners (GPs), disease surveillance systems 2:45
Generative propagation, definition 4:207
Gene regulation
varicella-zoster virus latency 5:262
Gene segment table(s) 5:352
Gene silencing **4:141–149**
cis-acting siRNA pathway 4:142
core reactions 4:141
definition 1:296
environmental influences 4:148
heterochromatin siRNA pathway 4:142
natural *cis*-antisense transcript-derived siRNA pathway 4:142
plant disease resistance *see* Genetically-modified plants, disease resistance
plants *see* Plant(s)
RdRp 6 4:146
trans-acting siRNA pathway 4:142
virus-inducced *see* Virus-induced gene silencing (VIGS)
see also MicroRNAs (miRNAs)
Gene tagging, fungal retrotransposons 4:427
Gene therapy **2:301–306**
biodistribution
adeno-associated virus vectors 2:304
clinical trials 2:305
clinical trials 2:302
administration route 2:304–305
HSV 2:304
lentivirus 2:304–305
onco-retrovirus 2:304–305
phases 2:305
preclinical safety 2:305
recombinant adeno-associated virus 2:303–304
recombinant adenovirus 2:304–305
special considerations 2:305
tumors 2:304–305
vector genome biodistribution 2:305
considerations 2:301
definition 2:301
requirements 2:301
vectors
design 2:302
effectivity 2:301
host range 2:302
toxicity 2:301
types 2:302
see also specific viruses
Genetically modified organism (GMO), definition 1:296
Genetically-modified plants
antibody expression 3:64
disease resistance *see below*
exogenous protein expression 4:235
bean yellow dwarf virus 4:236
chemical induction 4:235
estrogen-inducible promoters 4:236
ethanol induction 4:236
hybridization 4:235
inducing treatments 4:235
magnifection 4:234
strategies 4:235
transgenic systems 4:235
gene silencing *see below*
heterooligomeric protein expression 4:233
'noncompeting viral vectors' 4:233
transient systems 4:233
yields 4:233
industrial-scale protein expression 4:234
Agrobacterium-mediated delivery 4:232*f*, 4:234
genome modifications 4:234
recombinant protein yield 4:234–235, 4:235*f*, 4:236*f*
vaccine production *see below*
virus vectors *see below*
Genetically-modified plants, disease resistance **4:156–164**, 4:159*t*
associated species 4:157, 4:159*t*
benefits of 4:160
broad spectrum resistance 4:163
commercial release 4:160–161, 4:163
disease control 4:157, 4:162
economic impact 4:160–161, 4:163
environmental safety 4:161
evaluation 4:157
field evaluation 4:163
gene flow 4:162
gene silencing 4:145, 4:147*f*, 5:331
cross-protection 4:145
HEN1 4:146
plant recovery 4:145
RISC 4:146
SDE3 4:147
global availability 4:157–160
heterologous encapsidation 4:162
mechanisms 4:161
antiviral factors 4:157
gene silencing *see above*
post-transcriptional silencing 4:161
protein-mediated 4:161
RNA silencing 4:161, 4:162, 5:64
plant development 4:156
associated antiviral factors 4:157
benefits of 4:160
history 4:156
transgenic-wild type hybrids 4:162
vectors *see* Genetically-modified plants, virus vectors
watermelon mosaic virus 4:162
zucchini yellow mosaic virus 4:162
pollen 4:162
recombination 4:162
risks, real *vs.* perceived 4:163
RNA interference 3:153
strategies 4:156
target traits 4:157
see also Virus-induced gene silencing (VIGS)
Genetically-modified plants, vaccine production **5:221–225**
animal models 5:221
chloroplast expression 5:223
contamination, vaccine safety 5:221
cost 5:221
'guinea pig' argument 5:224
gut-associated lymphoid tissue 5:222
history 5:222
HPV 5:223
limitations 5:223–224
maize 5:223–224
mucosa-associated lymphoid tissue 5:222
Newcastle disease virus 5:224
Norwalk virus 5:223
obstacles 5:224
plant tissues used 5:223
ProdiGene 5:223–224
public perceptions 5:221
soybean plants 5:223–224
technological advances 5:222
Genetically-modified plants, virus vectors **4:229–238**, **5:274–282**, 5:275*t*
applications 4:233
nanoscale material manufacture 4:236
replication cycle manipulation 4:229–230
research tools 4:233
classification 5:276*f*, 5:277*t*
'non-persistent' viruses 5:276–277
persistent viruses 5:277
semipersistent viruses 5:276–277
cowpea mosaic virus 1:573–574, 1:573*f*
first-generation vectors 4:230
CP fusions 4:230, 4:232*f*
directed evolution 4:230
DNA shuffling 4:230
'full virus' strategy 4:230
host improvement 4:230
large scale production 4:230
limitations 4:230
N-glycosylation 4:231–232
post-transcriptional silencing *see* Post-transcriptional gene silencing (PTGS)
recombinant protein yields 4:230
replicon delivery 4:230
TMV-based vectors 4:230
viral promoters 4:230
second-generation vectors 4:232
viral constituents 4:232
Genetic bottlenecks, viral evolution 2:179
Genetic diversity
gill-associated virus 5:481*f*
potato virus Y tomato infections 4:295
Genetic drift
definition 4:359
Dengue viruses 2:8
hantaviruses 2:318
Genetic reactivation, avipoxvirus replication 2:276
Genetic shift, hantaviruses 2:318
Gene transfer
Herpesvirus Saimiri 4:591
rhadinovirus human T-cell transformation 4:591
Genital infection(s)
alphaherpesviruses 1:366*f*
alphaherpesviruses, bovine 1:366*f*
herpesviruses 2:436–437
human papillomaviruses *see* Human papillomavirus (es) (HPVs)
Genitourinary medicine clinic(s) (GUM) 2:44
Genogroups
aquareovirus 1:167
Border disease virus 1:338
definition 2:221
Genome(s)
bacterial cell viral entry *see* Bacteriophage(s), bacterial cell entry
co-packaging 1:133
definition 1:520
integration 2:331–332
retrotransposons, effects of *see* Retrotransposons
size
error catastrophe limitation 2:178–179
retrotransposon content 4:443
see also specific viruses
Genome molecule tables, virus databases 5:352–353

Genome replication, definition 4:407
Genomes of the T4-like Phages database 5:357*t*
Genomic precursor 3:6
Genomics, phylogenetic analysis 4:127
Genomic segment 3:6
Genotypes/genotyping 5:469
  definition 4:507
  see also specific viruses
GEO (Gene Expression Omnibus) 5:357*t*
Geoduck clam aquareovirus (CLV) 1:164*t*
Geraniums
  see under Pelargonium
German measles *see* Rubella
Germline(s), endogenous retroviral origins 2:107
Germplasm, infection control 3:219
Gerstmann–Straüssler–Scheinker syndrome (GSS) 5:192
  clinical characteristics 5:192
  genetic mutations 5:192
  geographical distribution 5:192
  illness duration 5:192
Getah virus (GETV) 5:92
  characteristics 5:109*t*
GETV *see* Getah virus (GETV)
GFkV *see* Grapevine fleck virus (GFkV)
GFLV *see* Grapevine fanleaf virus (GFLV)
GFP *see* Green fluorescent protein (GFP)
gH/gL complex(es), human herpes virus replication 2:501
Giant cell disease, definition 4:611
Giant cell pneumonia, measles 3:289
Giant mimivirus, evolution 3:477
Giardia lamblia 2:312
Giardiasis 2:315
Giardiavirus 2:286, 2:313, 5:164, 5:165*t*
Giardiavirus(es) (GLV) **2:312–316**
  biochemical characteristics 2:313
  classification 2:313
  evolution 2:315
  as gene therapy vector 2:314
  genome 2:314*f*, 5:167*f*
    mRNA structure 2:314
    mRNA translation 5:167
    open reading frames 2:313
    untranslated regions 2:314
  geographical distribution 2:313
  history 2:312
  host range 2:313
  infection 2:315*f*, 5:169
  physical characteristics 2:313*f*
    RdRp activity 2:313
  replication 2:314
  taxonomy 2:313
    ScV *vs.* 2:315
  see also specific viruses
Giardivirus 5:164
Gill-associated virus (GAV) 5:477
  classification 4:567
  genetic diversity 5:481*f*
  genome 5:478–479
    open reading frame 5:478–479
    transcription-regulating sequences 5:478–479
  infection
    chronic infections 5:480
    mortality 5:479
  transmission 5:480
  see also Yellow head virus (YHV)
Gill disease (fish), KHV infection 2:208
Gill necrosis disease 4:561
  histopathology 4:562
  manifestations 4:561–562
Gills, definition 4:560
Gilpinia hercyniae (European spruce sawfly)
  biological control 3:125
  history 1:225–226
Gilthead seabream reovirus 1:164*t*
Ginger chlorotic flack virus (GCFV) 4:646*t*
GINV *see* Grapevine berry inner necrosis virus (GINV)
Gladiavirus 5:165*t*
Glc1.8 (mucin), BV-mediated immunosuppression 4:254
Global Advisory Committee on Vaccine Safety (GACVS) 5:232
Global Rinderpest Eradication Programme (GREP) 4:505
Global vaccine coverage, measles 3:290*f*
Glomeromycota 4:419
  retrotransposons 4:423
Glomerulonephritis
  definition 5:423
  immune complex deposition 3:79*f*
GLRaV-2 *see* Grapevine leafroll associated virus-2 (GLRaV-2)
Glucocorticoids, virus transcriptional regulation 3:340
Glut-1 receptor, HTLV-1 infection 2:559
Glutathione peroxidase, avipoxvirus survival factors 2:275
GLV *see* Giardiavirus(es) (GLV)
Glycan shields, infection neutralization 3:66–67
Glycine decarboxylase, avian hepadnavirus replication 2:329
Glycine max see Soybean *(Glycine max)*
Glycoprotein(s)
  enveloped viruses 3:292
  membrane fusion
    biosynthesis 3:293
    class I 3:293
    class II 3:297
    class III 3:299
  see also specific glycoproteins
Glycoprotein G role, Chandipura virus life cycle 1:500
Glycosaminoglycan(s) 2:542
Glycosaminoglycan(s) (GAGs)
  associated viruses 5:320–321
  binding domains 5:321
  receptor role 5:320
  see also Heparan sulfate (HS)
  VACV-host cell entry 5:245
Glycosidase(s), endolysin function 3:251
Glycosylation
  HCMC modifications 2:489–490
  plant virus vectors 4:231–232
  varicella-zoster virus proteins 5:261
Glycotransferases 1:594
Gnasthostome immunoglobulins
  diversity 3:58
  phylogeny 3:60*f*
Gnotobiotic (Gn) calves 5:151
GO (Gene Ontology Database) 5:357*t*
Goatpox 1:427
  epidemiology 1:427
Goatpox virus (SPPV), geographical distribution 4:329
Goatpox virus (SPPV), transmission 4:329
Gokushovirus(es)
  genetic map 3:15*f*
  genome content 3:14–15
  microviruses *vs.* 3:20
  morphogenesis 3:19
  virion morphology 3:14–15
    procapsid assembly 3:15
  see also specific viruses
Gokushovirus ϕMH2K 3:20
Golden ide reovirus (GIRV) 1:164*t*
Golden shiner reovirus (GSRV) 2:231
  genome 1:165
  infection 1:166
Gold-handed tamarin lymphocryptovirus (SmiLHV1) 4:587*t*
Golgi apparatus
  bunyavirus assembly 1:397
  varicella-zoster virus assembly 5:261
Gonads, definition 3:495
Gonad-specific virus *see* Helicoverpa zea-2 virus (Hz-2V)
Gonotrophic cycles 4:652
  definition 5:268
  horizontal transmission 5:269
Gorilla(s) 2:529, 2:530*f*, 2:533
Gorilla rhadinovirus 4:587*t*
GP-1 glycoprotein, arenavirus 3:244
GP-2 glycoprotein, arenavirus 3:244, 3:245
gp41 gene/protein
  definition 1:127
  nucleopolyhedrovirus 1:264
GP64 protein
  baculovirus infection resistance 1:267
  nucleopolyhedroviruses 1:263, 1:264
    BV envelope structure 1:263
gp120 *see* HIV
GPCMV *see* Guinea pig cytomegalovirus (GPCMV)
GpC protein, bacteriophage ϕX174 4:405
G protein
  Hendra virus 2:323
  henipavirus 2:326
G protein-coupled receptor(s) (GPCR), HCMV immune system evasion 1:637, 1:638*f*
Grafting 4:201–202
  grapevine diseases *see* Grapevine(s), diseases
  infection control measures 1:524
Gramineae
  fijivirus hosts 4:154
  oryzavirus hosts 4:154
  phytoreovirus hosts 4:154–155
Gram-negative bacteria
  bacterial cell viral entry 5:365
  filamentous ssDNA bacteriophages 5:369
Gram-positive bacteria, bacterial cell viral entry 5:365
Gram staining, mimivirus structure 3:312*f*, 3:316–317
Granulin, definition 1:247
granulin gene, granulovirus 1:265
Granulocyte-macrophage colony-stimulating factor inhibitory factor (GM-CSF)
  parapoxviruses 4:62
  Visna-Maedi virus infection 5:428–430
Granulocytes, innate immunity 3:112
Granulovirus 1:211, 1:220, 1:247, 1:254, 1:265
Granulovirus(es) **1:211–219**
  A-T content 1:216
  classification 1:211, 1:220
  fast-killing 1:212
  gene expression 1:216
    early genes 1:216
    fibroblast growth factors 1:217
    inhibitors of apoptosis 1:214, 1:216
    late genes 1:216
    metalloproteinase 1:216
    polyhedron envelope/calyx protein 1:217
    very late genes 1:216
  genome 1:213, 1:213*f*, 1:213*t*, 1:214*f*
  host range 1:265
  infections
    cycle 1:211
    insects 3:126*t*
  morphology 1:212*f*
  nucleocapsids 1:249
  occlusion bodies 1:211
  packaging 1:265
    granulin gene 1:265
  pathogenesis 1:268
  replication 1:212*f*, 1:214
    origins of 1:218
  slow-killing 1:212
  taxonomy 1:211
  virion structure 1:212*f*
    budded virions 1:212*f*
    occlusion-derived virions 1:211, 1:212*f*
  see also specific viruses
Granzymes 3:74–75
Grapevine(s), diseases **4:201–207**
  causal agents 4:203
    classification 4:203
    see also specific viruses
  control 4:204
  geographical distribution 4:203
  graft incompatibility 2:91
    causal agents 2:91
    see also specific viruses
    disease symptoms 2:91
    geographical distribution 2:91
    infection control 2:91
    syrah decline 2:91

Grapevine(s), diseases (*continued*)
transmission 2:91
yield losses 2:91
infectious decline 4:203
transmission 4:203
infectious degradation 4:203
symptoms 4:202, 4:202*f*
transmission 4:203
yield losses 4:202
see also specific viruses
Grapevine berry inner necrosis virus (GINV) 1:424
genome 1:424
Grapevine Bulgarian latent virus (GBLV) 3:406*t*
diseases 3:411
Grapevine chrome mosaic virus (GCMV) 3:406*t*
diseases 3:411
genome 3:408
Grapevine fanleaf virus (GFLV) 3:406*t*
cell-to-cell movement 3:410
control, transgenic plants 3:412
diseases 3:411
genome 3:407–408
infectious DNA clones 3:408–409
large single-stranded RNA satellites 4:531
movement 3:352
population structures 3:412
regulated polyprotein processing 3:409
replication 3:409
satellites 3:410–411
transmission, soil nematodes 3:411
Grapevine fleck virus (GFkV)
characteristics 5:206
genome 5:202*f*, 5:206
coat protein 5:206
replication polyprotein 5:206
Grapevine leafroll associated virus-2 (GLRaV-2) 2:91
molecular variants 2:91
transmission 2:91
Grapevine rootstock stem lesion-associated virus (GRSLaV) 2:91
Grapevine rupestris stem pitting-associated virus (GRSPaV) 2:91
Grapevine Tunisian ringspot virus (GTRSV) 3:406*t*
diseases 3:411
Grapevine virus A (GVA)
genome 1:425–426
particle structure 1:421*f*
replication 1:426
Grapevine virus B (GVB)
genome 1:425–426
replication 1:426
Grass carp aquareovirus (GCRV) 1:164*t*
genome 1:165
infection 1:166
vaccine 1:167, 1:168–169
Grass carp virus (GCV) 2:231
Grasses, maize streak virus 3:265, 3:271
Great island virus 3:465
Green fluorescent protein (GFP)
definition 5:325
plant virus distribution 2:26–27
plant virus movement 3:349, 3:354
Green fluorescent protein-transgene silencing, virus-induced gene silencing 5:326
Groundnut rosette 5:209–210
causative agents 3:214–215
Nigerian epidemic 3:217, 3:218
Groundnut rosette assistor virus (GRAV) 5:211
Groundnut rosette virus (GRV)
cell-to-cell movement 5:212, 5:212–213, 5:212*f*
infection *see* Groundnut rosette
ORF3 5:212*f*
ORF4 5:210*f*
phloem-dependent long-distance movement 5:210*f*
resistance 5:213
satellite-like single-stranded RNAs 4:532
transmission, RNA satellites 5:211
Group C virus(es), orthobunyavirus reassortment 3:482
see also specific viruses
GRV *see* Groundnut rosette virus (GRV)
GS-9137 2:510–513
GS9148 (antiviral prodrug) 1:150
GSK-364735 2:507*t*
GSRV *see* Golden shiner reovirus (GSRV)
GSS *see* Gerstmann–Stra¨ussler–Scheinker syndrome (GSS)
Gt1b ganglioside(s), JCV infection 4:280
GTRSV *see* Grapevine Tunisian ringspot virus (GTRSV)
Guanarito virus 2:95
geographical distribution 3:204
history 3:203
Guanidine, prion inhibition 4:337–338
Guanidine hydrochloride (Gu-HCl), picornavirus replication 4:136–137
Guanyltransferase, mycoreovirus structure–function relationships 3:378–379
Guard hypothesis, plant natural resistance 4:175
Guenon, definition 4:611
Guillain–Barré syndrome
definition 5:226
HIV infection 1:474–475
swine flu vaccine safety 5:233
Guinea pig(s)
foot-and-mouth disease virus infection 2:271
Marburg virus infection 3:277–278
varicella-zoster virus infection 5:251
Zaire Ebola virus infection 2:62
Guinea pig cytomegalovirus (GPCMV) 1:633
genome 1:633
infection
associated tissues 1:634
transplacental infection 1:634
vaccines 1:634
Gumboro disease *see* Infectious bursal disease virus (IBDV)
Guppy aquareovirus (GRV) 1:164*t*
Guppy virus 6 (GV-6) 2:233
Gut-associated lymphoid tissue (GALT)
edible vaccine development 5:222
HIV infection 1:71
resident memory CD4 T cells 1:71
Guttaviridae 1:587, 5:11*t*
GVA *see* Grapevine virus A (GVA)
GVB *see* Grapevine virus B (GVB)
GV-specific cell lines, baculovirus in vitro pathogenesis 1:270
Gypcheck, insect pest control 3:130
gypsy retrotranspon *see* Drosophila melanogaster
Gyrovirus(es)
associated diseases 1:514
see also specific viruses

H

HA *see* Hemagglutinin (HA)
HAART *see* Highly active antiretroviral therapies (HAART)
Haddock reovirus 1:164*t*
Haemaphysalis intermedia, Bhanja virus 1:399
Hairpin ribozyme(s) 4:476
ligation mechanism 4:479
structural dynamics 4:479
rate of folding 4:476
schematic 4:478*f*
virus-induced gene silencing 5:379
Haldane–Muller principle, quasispecies 4:361
Halibut reovirus 1:164*t*
Haloalkaliphilic virus(es) 5:418
see also specific viruses
Haloarcula hispanica, SH1 virus infection 5:420
Halobacterium salinarum, associated viruses 5:412
Halophile, definition 5:411
Halovirus(es) 5:415
burst size 5:412
haloalkaliphilic viruses 5:418
head-and-tail viruses 5:415
latent period 5:412
salterprovirus 5:418
spherical halovirus SH1 5:420
see also specific viruses
HAM *see* Tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM)
Hammerhead ribozyme(s) 4:476
classification 4:476
definition 5:332
ligation mechanism 4:479
structural dynamics 4:479
structure and folding 4:476
catalytic core 4:476–478
magnesium ions 4:478
secondary structure 4:480*f*
Hand, foot and mouth disease (HFMD)
coxsackieviruses 1:585
enterovirus 71 infection 2:134
neurological complications 2:134
symptoms 2:134
Hand-to-hand contact, common cold 4:472
Hanseniaspora uvarum, killer phenomenon 5:300
Hantaan virus 1:391*t*
genomes 1:394*f*
Hantavirus 1:391, 1:391*t*, 2:317–318, 3:480, 4:490–491
Hantavirus(es) 1:399, **2:317–321**
diagnostics 2:320
ecology 2:318
European pathogens 2:318
epidemiology 2:318
genome 2:317
history 2:317
incidence 2:317
infections *see below*
phylogeny 2:318–319
replication, host cell entry 1:395
seroprevalence 2:320
transmission 1:391, 2:318
types 2:319*t*
vaccines/vaccination 2:320
virus structure 2:317
see also specific viruses
Hantavirus infections
clinical features 2:319
emerging diseases 2:95
hemorrhagic fever with renal syndrome 2:317, 5:485
associated species 2:317, 2:318
incidence 2:317, 2:318
pathogenesis 2:320
risk factors 2:318
humans 2:317
respiratory infection 2:555
pathogenesis 2:319
prevention 2:320
vaccines 2:320
zoonoses 5:486*t*
geographical distribution 5:485–486
hemorrhagic fever 5:485
rodents 2:318
Hantavirus pulmonary syndrome (HPS) 2:317, 2:555
pathogenesis 2:320
zoonoses 5:485
Hare fibroma virus (HFV) 3:225
characteristics 3:225*t*
infection 3:229–230
HaRNAV *see* Heterosigma akashiwo RNA virus (HaRNAV)
HaRNAV virus 4:116
Harrisinia brillians granulovirus (HabrGV) 1:269
Hart Park Group, animal rhabdoviruses 1:115, 1:119*t*
Hart Park virus 4:184*t*
HASTY, microRNA pathway in gene silencing 4:141
Hatchery, definition 4:560
HAV *see* Hepatitis A virus (HAV)
Hazelnut trees(s), diseases 4:206
causal agents 4:207
classification 4:207
geographical distribution 4:206
symptoms 4:206
transmission 4:207
yield losses 4:206
see also specific viruses
HBeAg *see* Hepatitis B e antigen (HBeAg)
HBoV *see* Human bocavirus (HBoV)
HBsAg *see* Hepatitis B surface antigen (HBsAg)

HBV *see* Hepatitis B virus (HBV)
HCC *see* Hepatocellular carcinoma (HCC)
HCMV *see* Human cytomegalovirus (HCMV)
HcRNAV *see* Heterocapsa circularisquama RNA virus (HcRNAV)
HCV *see* Hepatitis C virus (HCV)
HCV Database Project 5:357*t*
HCV pseudoparticles (HCVpp) 2:372
H DNA 4:588
H (heavy) dsRNAs, Ustilago maydis viruses 5:215
HDV *see* Hepatitis delta virus (HDV)
Head, definition 3:30
Head-and-tail virus(es) 5:415
  morphology 5:412
Headful packaging 5:411
Heat inactivation, Border disease virus 1:338
Heat resistance, African horse sickness virus 1:38–40
Heat-shock experiments, Sigma virus replication 4:579
Heat shock protein 70 homolog (Hsp70h) 3:353
HEF *see* Hemagglutinin-esterase-fusion (HEF)
HeLa cells, JC virus infection 4:280
Helical arrays
  bacteriophage assembly 1:437
  bacteriophages 1:437
  inovirus protein coat assembly 1:438
  myovirus 1:437–438
Helical particles 5:383*f*, 5:385
Helical symmetry 5:393
Helical virus(es) 1:201
  see also specific viruses
Helicase(s)
  RNA virus evolution 2:180–181
  yellow head virus 5:477–478
  see also DNA helicase
Helicase inhibitors 1:149
Helicoverpa amigera stunt virus (HaSV) 5:28*t*
  Agrobacterium-mediated transgenesis 5:36
  capsid 5:33
    assembly 5:33
    maturation 5:34–35
    morphology 5:31*f*
    protein precursors 5:31–32
  discovery 5:28
  genomes 5:29, 5:31
    RNA2 length 5:31
  infection 5:36
    histopathology 5:36
  insect pest control 3:127
  replicases 5:31
Helicoverpa zea virus(es), oryctes rhinoceros virus *vs.* 3:496
  see also specific viruses
Heliothis zea-1 virus (Hz-1V)
  genome 3:145*f*
  history 3:144
  morphology 3:145
  replication 3:146
    stages 3:146
    transcription 3:146
Heliothis zea-2 virus (Hz-2V)
  genome 3:145*f*
  history 3:145*f*
  infection
    agonadal tissue 3:144–145
    pathology 3:146–147
    symptoms 3:147
  morphology 3:145
    envelope 3:148*f*
  replication 3:147
Helminthosporium victoriae 145S virus (Hv145SV) 1:503–504
  classification 1:503–504
  genome 1:505–506
  infection
    disease characteristics 5:169–170, 5:169*f*
    transformation vector construction 5:169–170
  structure, coat protein 1:509
  transmission 5:169–170
Helminthosporium victoriae 190S virus (Hv190SV) 2:285, 5:164, 5:165*t*, 5:166
  capsids 5:166
  genome 5:165*t*
    open reading frames, overlap 5:166–167, 5:167*f*
    RdRp 5:167
    structural features 5:167
  Hv-p68 gene/protein 5:171*f*
  infection
    disease characteristics 5:169–170, 5:169*f*, 5:171
    symptom severity 5:169–170, 5:169*f*, 5:170*f*
    transformation vector construction 5:169–170
  replication 5:168*f*
    phosphorylation 5:168–169, 5:168*f*, 5:171
    proteolytic processing 5:168–169, 5:168*f*, 5:171
    RNA polymerase activity 5:167–168
  taxonomy 5:164, 5:165*t*
  transmission 5:169–170
  yeast L-A virus *vs.* 5:165
Helper adenovirus(es) *see* Adeno-associated virus vectors
Helper component protein (HC-Pro) 2:579
  definition 4:546
  potato virus Y 4:288
  potyviruses *see* Potyvirus(es)
  RNAi suppression 3:152
  virus transmission 5:280*f*
    watermelon mosaic virus 5:436
    zucchini yellow mosaic virus 5:436
Helper component (HC)-transcomplementation
  definition 5:274
  plant virus transmission 5:281–282
Helper-dependent mini-replicons, human respiratory syncytial virus 2:547
Helper T-cells *see* T helper cell(s)
Helper virus(es)
  begomovirus infection 4:166
  see also specific viruses
  β ssDNA satellites 1:314–315
  definition 4:526
  yeast L–A virus 5:465–466
  see also specific viruses
Hemadsorption 1:43
Hemagglutination-inhibition (HAI) test(s)
  cowpox virus infection 1:579
  influenza virus 1:129–131, 2:31
  Japanese encephalitis diagnosis 3:186
  orthobunyavirus reassortment 3:482
  principles 2:31
  togavirus detection 5:107–108
Hemagglutination-neuraminidase protein (HN) *see* Paramyxovirus(es)
Hemagglutinin (HA)
  definition 1:127, 4:497
  HA2
    chain reversal 3:294, 3:295*f*
    loop region 3:294
    thermostability 3:294–295
  henipavirus 2:322–323
  human parainfluenza viruses 4:47–48
  influenza viruses *see* Influenza virus(es)
  Japanese encephalitis virus 3:183
  mumps virus 3:356
Hemagglutinin-esterase-fusion (HEF) 1:559–560, 3:492
  definition 3:89
  function 3:485
  infectious salmon anemia virus 3:93
  structure 3:492
Hemagglutinin-neuraminidase (HN), henipavirus 2:322–323
Hematophagous arthropod vectors
  definition 1:111, 1:170
  orbivirus transmission 3:457
Hematopoietic necrosis herpesvirus 2:208, 2:233
Hematopoietic precursor cells, adult T-cell leukemia 2:567–568
Hemifusion 3:292
Hemifusion diaphragm 5:311*f*
Hemiptera 2:37, 4:164
Hemivirus 4:353*t*
Hemivirus(es) 4:352
  see also specific viruses
Hemocoel 4:250
  definition 3:231
Hemoconcentration 1:328
Hemocyte(s)
  definition 4:250, 4:560
  insect immune responses
    adhesion factors 4:252–253
    differentiation 4:251–252, 4:253*f*
    proliferation 4:251–252, 4:253*f*
  types 4:252
  white spot syndrome virus infection *see* White spot disease (WSD)
Hemocytic infection virus disease 4:561
Hemolymph 1:301
  definition 3:231
  plant virus transmission 5:278
Hemolysis
  equine infectious anemia virus infectious 2:173
  mumps virus infection 3:356
Hemorrhage
  acute CSFV infection 1:528
  Chikungunya virus infection 5:89
  Marburg virus infection 3:278
Hemorrhagic fever
  African swine fever virus infection 1:44
  arbovirus infection 1:175
  arenavirus infection 5:486
  bioweapons 5:408–409
  see also specific hemorrhagic fevers
Hemorrhagic fever with renal syndrome (HFRS) *see* Hantavirus infections
Hemorrhagic kidney syndrome (HKS) 3:94
Hemorrhagic pock(s), cowpox 1:577, 1:579
Hemorrhagic smallpox 4:640
Hemorrhagic syndrome
  bovine viral diarrhea viruses *see* Bovine viral diarrhea virus infection
  definition 1:374
HEN1, plant gene silencing 4:146
Henderson–Patterson bodies *see* Molluscum lesion
Hendra virus (HeV) 4:40, 4:52
  Australia 2:324
  fusion proteins, proteolytic processing 4:54
  genetic stability 2:179
  genome 3:327*f*
  global distribution 4:45
  historical aspects 2:94, 2:321
  infection 2:554
    clinical features 2:325
    direct transmission 2:324
    G protein 2:323
    host cell attachment 4:44, 4:53–54
    host range 2:554
    pathology 2:325
    zoonoses 2:95
Henipavirus 2:321, 4:41*t*
Henipavirus(es) **2:321–327**, 2:322*f*
  cell attachment
    attachment glycoproteins 2:322–323
    ephrin B2 2:322–323
    glycoprotein attachment proteins 2:322–323
    hemagglutinin 2:322–323
    hemagglutinin-neuraminidase 2:322–323
    sialic acid-independent mechanisms 2:322–323
  classification 4:41*t*
  epidemiology 2:324
  evolution 2:323
  future work 2:326
  genetics 2:323
  genome 3:327*f*
  geographical distribution 2:323
  history 2:321
  infections *see below*
  molecular biology 2:322
    F protein 2:322–323, 2:326
    furin 2:322–323
    G protein 2:326
    interferon inhibition 2:323, 2:326
    P gene 2:323, 2:326

Henipavirus(es) (*continued*)
V protein 2:323, 2:326
W protein 2:323
molecular features 2:321–322
propagation 2:323
transmission 2:324
direct *vs.* indirect transmission 2:324
see also specific viruses
Henipavirus infections
clinical features 2:325
neurological 2:325
prolonged infection 2:325
respiratory problems 2:325
control 2:326
ant-G protein human monoclonal antibodies 2:326
ribavirin 2:326
ELISA 2:326
histopathology 2:325
host range 2:323
immune responses 2:326
adaptive 2:326
antibodies to 2:326
innate 2:326
morbidity 2:324–325
mortality 2:324–325
pathogenicity 2:324
pathology 2:325
natural hosts 2:326
prevention 2:326
serology 2:323
tissue tropism 2:324
ephrin B2 expression 2:324
vaccines/vaccination 2:326
see also specific viruses
Henivirus 4:352
Henle-Koch postulates, epidemiology 2:143, 2:144*t*
Hepacivirus 2:242, 2:249, 2:367, 5:440–441
Hepacivirus(es) 2:249
history 2:249
infection
clinical disease 2:249, 2:251
diagnosis 2:252
epidemiology 2:249
pathogenesis 2:251
treatment 2:252
replication 2:249
structure 2:249
see also specific viruses
Hepadnaviridae 2:327, 2:351–352, 2:360, 4:460, 5:11*t*
Hepadnavirus(es) **2:335–343**
avian *see* Avian hepadnavirus(es)
epidemiology 2:337
evolution 3:477
genetic variation 2:337, 2:339*f*, 2:340*f*
common determinants 2:337–338
open reading frames 2:338
subtypes 2:337–338
genome 2:336, 2:338*f*
open reading frames 2:336
geographical distribution 2:338
infection
apoptosis inhibition 1:158*t*
avian *see* Avian hepadnavirus(es)
clinical features 2:340
immunomodulation 4:114*t*
pathology 2:340
persistent 4:109*t*
prevention 2:341
rodents 2:341
treatment 1:143*t*, 2:341
LTR retrotransposons 4:438
phylogeny 2:337*f*
structure 2:336
surface proteins 2:336, 2:338*f*
transmission 2:335
parenteral 2:335
post-transfusion 2:336
see also specific viruses
Heparan sulfate (HS), receptor role 5:320
alphavirus 5:118
foot-and-mouth disease virus 5:324
porcine respiratory and reproductive syndrome virus 1:181
pseudorabies virus 4:346
tick-borne encephalitis viruses 5:46
Hepatic necrosis, equine infectious anemia virus infection 2:172–173
Hepatitis 1:104
Hepatitis A
asialoglycoprotein receptor 2:12
clinical features 2:347
biochemical parameters 2:347
children 2:347
extrahepatic manifestations 2:348
control 2:350
disease surveillance 2:50
diagnosis 2:349
immunoglobulin G 2:12
epidemics 2:343
epidemiology 2:349
at risk individuals 2:350
seasonal distribution 2:350
seroprevalence 2:349–350
fulminant 2:348
fatality rate 2:348
host range 2:346
immune response 2:349
adaptive 2:349
innate 2:349
infection course 2:348*f*
incubation period 2:347
reconvalescence period 2:347
pandemics 2:343
pathology 2:347
prevention 2:350
prolonged 2:348
relapsing 2:348
frequency 2:348
pathogenesis hypotheses 2:348
tissue tropism 2:346
Hepatitis A virus (HAV) **2:343–350**
cell culture 2:347
cytopathic variants 2:347
defective interfering RNAs 2:4
gene expression 2:344
proteolytic processing 2:344, 2:346*f*
RNA transcription 2:344
translation 2:344
genome 2:344, 2:345*t*
internal ribosome entry site 4:130, 4:134–135
growth characteristics 2:347
history 2:343
infection *see* Hepatitis A
morphogenesis 2:344, 2:346*f*
morphology 2:344
physicochemical properties 2:344
replication cycle 2:347
stability 2:344, 2:350
taxonomy 2:343
genotypes 2:343
transmission 2:343, 2:346
vaccines/vaccination 2:350
development 5:236–237
Hepatitis B 2:333–334
acute infection 2:357
antiviral therapy 1:143*t*, 1:152
clevudine 1:152–153
drug resistance 1:151, 2:359, 2:365
genotype effects 2:355–357
lamivudine 1:152, 2:359, 2:365
nucleoside analogs 2:359
nucleotide analogs 2:359
telbivudine 1:152
children 2:340
chronic infection 2:357
course of 2:357, 2:358*f*
HBeAg negative group 2:358
immune clearance phase 2:357–358
mutant viruses 2:358
nonreplicative phase 2:357–358
reactivation phase 2:357–358
treatment 2:364
drug resistance 2:342
epidemiology 2:352
prevalence 2:352
hepatitis delta virus co-infection 2:342, 2:375, 2:376
hepatocarcinogenesis 5:197
hepatocellular carcinoma *see* Hepatocellular carcinoma (HCC)
infection markers 2:357
anti-HBsAg antibodies 2:357
natural history 2:357
fulminant hepatitis 2:357
organ transplantation 3:469
persistent 4:109*t*
treatment 2:359
antiviral therapy *see above*
HBeAg-negative patients 2:359
HBeAg-positive patients 2:359
immunomodulators 2:359
protracted 2:359
Hepatitis B core antigen (HBcAg) 2:360
Hepatitis B e antigen (HBeAg) 2:352–353, 2:364
mutation 2:364
negative patients, treatment 2:359
positive patients, treatment 2:359
seroconversion 2:364
tolerogenic effects 2:352–353
transmission, placental 2:474
Hepatitis B immune globulin (HBIG) 2:364
Hepatitis B surface antigen (HBsAg) 2:360, 2:362
antibody response 2:362
infection marker 2:357
discovery 2:350–351
hepatitis delta virus co-infection 2:342
phylogenetic tree 2:351*f*
research history 2:335–336
structure 2:362, 2:363*f*
transgenic plant vaccine production 5:223
limitations 5:223–224
virus yield 5:223
vaccination programs 2:363, 5:197
virion structure 2:352
Hepatitis B virus (HBV) **2:350–360**, **2:360–367**
antiviral therapy 1:152
chromosomal integration 2:366
defective interfering RNAs 2:4
distribution 2:352
drug resistance 2:342, 2:359
molecular biology 2:359
duck hepatitis B virus *vs.* 2:334
epidemiology 2:352
prevalence 2:352
genome 2:329, 2:336, 2:338*f*, 2:352, 2:353*f*, 2:360–361, 2:361*f*
cccDNA 2:360–361
nucleocapsid protein 2:352–353
open reading frames 2:336–337, 2:361
pre-core/core open reading frames 2:352–353
pre-core mRNA 2:352–353
pregenomic RNA 2:352–353, 2:353*f*
genotypes 2:355, 2:356*t*
antiviral response effects 2:355–357
geographical distribution 2:355
pathogenesis effects 2:355–357
geographical distribution 2:340, 2:341*f*, 2:355
hepatitis B e antigen *see* Hepatitis B e antigen (HBeAg)
hepatitis B surface antigen *see* Hepatitis B surface antigen (HBsAg)
hepatitis delta virus association 4:532–533
historical aspects 2:350
discovery 2:350–351
electron microscopy 2:351
hepatocellular carcinoma connection 2:351
PCR 2:351
plasma-derived vaccine development 2:351
recombinant vaccine development 2:351
history 2:336
infection *see* Hepatitis B
phylogenetic trees 2:351*f*

proteins
L (large) protein 2:352
M (middle) protein 2:352
S (small) protein 2:352
replication 2:337, 2:353, 2:354*f*, 2:360, 2:362*f*
direct repeats 2:361
hepatocyte receptors 2:353
host cell entry 2:360
polymerase 2:364–365, 2:365*f*
pregenomic RNA 2:353–354, 2:355*f*, 2:361, 2:362*f*, 2:365*f*
RNase H 2:353
templates 2:353
transcription 2:361
subtypes 2:354, 2:356*t*
S protein sequence 2:354–355
taxonomy/classification 2:351
phylogenetic tree 2:351*f*
transmission 2:334, 2:335–336
vaccines/vaccination 2:51–52, 2:341–342, 2:358, 2:363, 4:108–109
beneficial aspects 2:358–359
composition 5:237
efficacy 2:358–359
'plasma-derived' vaccine 2:363–364
reactions to 5:230*t*, 5:231*t*
recombinant vaccines 2:358
safety 5:233
vaccine escape mutants 2:359
variants 2:357
evolution 2:355–357
virion structure 2:352, 2:352*f*, 2:360, 2:362*f*, 2:375
Dane particles 2:352
lipid bilayer 2:352
virus entry 5:315
Hepatitis C
acute 2:251
chronic 2:251
clinical manifestation 2:251, 2:368, 4:115
disease progression 2:368, 2:368*f*
extrahepatic symptoms 2:368
incubation period 2:368
viremia 2:368
diagnosis 2:93, 2:251, 2:374
eye 2:491–492
histopathology 2:368
host range 2:370
immune response
adaptive 2:373
innate 2:373
T-cell responses 3:76
immune system evasion 3:108
liver 2:341
biopsy 2:252
cirrhosis 2:249
disease mechanism 2:251–252
hepatocarcinogenesis 5:197
organ transplantation 3:469
pathogenesis 2:251
pathology 2:368
persistent 2:251
risk factors 2:368
screening 2:368, 2:374
transmission 2:368
treatment 2:252, 2:374
antiviral therapy 1:153
Hepatitis C virus (HCV) **2:367–375**
evolution 2:177*f*
experimental systems 2:370
animal models 2:370
cell culture 2:372, 2:372*f*
genome 2:249–250, 2:250*f*, 2:369, 2:369*f*
core+1 proteins 2:370
IRES structure 2:249–250
nonstructural proteins 2:369–370
genotypes 2:249, 2:367–368
geographical distribution 2:367
history 2:249, 2:367
host cell entry 2:251
infection *see* Hepatitis C
prevalence 2:249, 5:198
protein functions 5:198
cellular proteins 2:250–251
E1 2:250
E2 2:250
NS5A 2:251
p7 2:250
proteases 2:250–251
RNA-binding core protein 2:250
replication cycle 2:370, 2:371*f*
host cell binding 2:370
rate 1:150
replicon system 1:153
virus release 2:370
taxonomy 2:367
tissue tropism 2:370
virion properties 2:249–250, 2:370
polyprotein processing 2:250
virion structure 2:371*f*
pseudoparticles 2:372
virus-host interactions 2:373
replication factors 2:373
Hepatitis delta virus (HDV) 2:342, **2:375–377**, 4:532
classification 2:375
epidemiology 2:376
genome 2:342, 2:375–376
infection
clinical features 2:377
control 2:377
hepatitis B co-infection 2:342, 2:375, 2:376
properties 2:375
replication 2:375, 2:376*f*, 4:530
antigenome 2:376*f*
mechanism 2:376
site 2:376
ribozyme 4:480
replication 4:480
structure 4:481*f*
satellite viruses 2:375
structure 2:375
transmission 2:336, 2:376
Hepatitis E virus (HEV) **2:377–383**
avian *see* Avian hepatitis E virus
classification 2:378
phylogeny applications 4:128
gene expression 2:378
genome 2:378, 2:379*f*
ORF3 2:380
transcription 2:380
translation 2:380
history 2:377–378
infection *see* Hepatitis E virus infection
natural history 2:382
taxonomy 2:378, 2:379*f*
transmission 2:377–378, 2:380, 2:382
intravenous infection 2:382
vaccines/vaccination 2:382–383
virion properties 2:378
sedimentation coefficient 2:378
stability 2:378
virion structure 2:378
capsid 2:378–380
Hepatitis E virus infection
cross-species 2:381
diagnosis, immunoglobulin G 2:382
epidemiology 2:380
mortality rate 2:380, 2:381
seroprevalence 2:380
host range 2:381
immunity 2:382
pathogenesis 2:381
prevention and control 2:382
rodents 2:381
tissue tropism 2:381
zoonoses 2:381
swine 2:380
Hepatitis–splenomegaly (HS) syndrome, avian hepatitis E virus 2:380
Hepatitis Virus Database 5:357*t*
Hepatocellular carcinoma (HCC) 2:341, 5:197
hepatitis B 2:351, 2:366, 5:197
immunization 2:341–342
hepatitis C 5:197
transcription transactivation 5:197
Hepatocellular necrosis, Marburg virus infection 3:278
Hepatocyte(s)
avian hepadnavirus infection *see* Avian hepadnavirus(es)
degeneration, yellow fever 5:475
hepatitis B 2:341, 2:353
hepatitis C 2:368
Hepatopancreas 4:567, 5:476
Hepatopancreatic parvovirus (HPV)
genome 4:572, 4:572*f*
geographical distribution 4:572
host range 4:572
infection 4:571
taxonomy 4:572
Hepatovirus 2:343
Herd immunity 2:141, 2:146–147
L'He´ritie'r, P, Sigma virus discovery 4:576
Heron hepatitis B virus 2:327
Herpangina, coxsackieviruses 1:585, 1:585*t*
Herpes folliculitis 2:388–390
Herpes simplex virus(es) (HSV) **2:383–397**, **2:397–405**, 4:582, 4:582*f*
associated proteins 2:399
cytopathological involvement 2:403
DNA replication 2:401
nomenclature 2:399, 2:402*t*
pathogenesis involvement 2:403
regulatory 2:399
structural 2:401
see also specific proteins
binding, glycoproteins 5:321
classification 2:383
DNA recombination mechanisms 4:375
envelope glycoproteins
function 2:397, 2:403
structure 2:397
epidemiology 2:387
gene expression 2:399
enhancers 2:399
phases 2:398
promoters 2:399, 2:401*f*
transcript properties 2:399
gene therapy vectors 2:396
brain tumors 2:304
clinical trials 2:304
genome 2:304
infected cell types 2:304
therapeutic genes 2:396
genome 2:383, 2:398, 2:400*f*
inversions 2:398
map 2:400*f*
open reading frames 2:399
regions 2:398
history 2:383
host cell entry 2:398
molecular epidemiological studies 2:143
oncolytic viruses 2:396
replication 2:384, 2:385*f*, 2:398
assembly 2:386, 2:399
DNA 2:385–386, 2:398
egress 2:386, 2:399
glycoproteins 2:398
host cell effects 2:386
host cell entry 2:384
post-translational processing 2:398
release 2:399
RNA export 2:398
transcription 2:385, 2:398
translation 2:398
research 2:404
transmission 2:387
vaccines/vaccination 2:395–396
clinical trails 2:396
virion structure 2:383, 2:384*f*, 2:397
capsid 2:207*f*, 2:383–384, 2:397, 2:399
envelope 2:397
tegument 2:397
see also specific viruses

Herpes simplex virus 1 (HSV-1)
  apoptosis activation 2:388
  evolution 2:181
  genome, varicella-zoster virus *vs.* 5:252
  host cell entry 2:388
  infection
    children 2:388–390
    encephalitis 2:387
    manifestations 2:390*f*
    pathogenicity 2:389*f*
    persistence 4:109*t*
    rhinitis 2:388–390
    seroprevalence 2:387
  reactivation 2:388–390, 2:405*f*
Herpes simplex virus 2 (HSV-2)
  acquisition, influencing factors 2:387
  animal studies 2:388
  genome, varicella-zoster virus *vs.* 5:252
  HIV infection association 1:66
    clinical trials 1:66
    pre-exposure prophylaxis 1:66
  infection 2:390–391, 2:390*f*
    clinical features 2:390–391
    females 2:390–391
    fetal risks 2:387
    males 2:390–391
    pathogenicity 2:389*f*
    persistent 4:109*t*
    pregnancy 2:387
    proliferative lesions 2:395*f*
    seroprevalence 2:387
    squamous cervical cancer 2:394
    treatment 2:395*t*
  replication 2:386*f*
  transmission 2:388
    children 2:388
    neonatal 2:388
Herpes simplex virus-associated erythema multiforme (hem) 2:391
Herpes simplex virus infections
  antibodies 2:393
  apoptosis inhibition 1:157–160
  associated Alzheimer's disease 2:391
  associated risk 2:387
  clinical features 2:388, 4:115
    associated disease 2:388
    children 2:388–390, 2:555
    immunocompromised patients 2:391
    incubation period 2:388
  diagnosis 2:393
  encephalitis 1:474, 2:387
    neonatal mortality 2:388–390
    transmission 2:388
  eye 2:384, 2:388–390, 2:491–492, 2:495–496
    neonates 2:495–496
  host range 2:384
  immune evasion 1:125, 2:144–145, 2:393–394, 2:394*f*, 3:108–109
  immune response 2:393
    children 2:393
    naturally acquired immunity 2:393–394
  latency 2:391, 2:392*f*, 2:404, **2:436–442**, 2:439*f*
    animal models 2:437
    establishment of 2:437
    gene expression 2:438
    immunological studies 2:438
    latent phase transcripts 2:404
    maintenance of 2:438
    mechanism 2:391–392
  neonatal/fetal disease 2:393
    mortality rates 2:393
  neoplasia 2:394
    Ras/MEK/ERK pathway 2:394
  nervous system infection 1:469
  organ transplantation 3:469
  pathogenicity 2:388, 2:389*f*
  pathology 2:393
  pharyngitis 2:555
  pregnancy 2:393
  prevention 2:394
  reactivation 2:386, 2:405*f*, 2:438, 2:439*f*
    associated stimuli 2:391
    transplant-associated 3:467
  serology 2:387
  therapy 2:394
    acyclovir *see* Acyclovir
    foscarnet 2:395
    helicase inhibitors 1:149
    long-term daily suppressive therapy 2:394–395
  tissue tropism 2:387
Herpestidae, definition 4:367
Herpesviridae 1:363, 1:635–636, 2:148–149, 2:206, 2:233, 2:405–406, 2:412, 2:423, 2:424*t*, 2:431, 2:432*t*, 2:474, 2:498, 4:341, 5:11*t*, 5:251, 5:347
Herpesvirus(es) 2:383, **2:430–436**, 2:474
  amphibian *see* Amphibian herpesvirus(es)
  avian *see* Avian herpesvirus(es)
  bovine *see* Bovine herpesvirus(es)
  caudoviruses *vs.* 1:610–611
  classification 2:423, 2:431, 2:432*t*
    biological 2:432
    criteria 2:423
    genera 2:233, 5:251
    genomic 2:432
    host specificity 2:423–424
    morphological 2:431
    phylogenetic trees 2:425*f*, 2:426–427
    sequence 2:432, 2:433*f*
    serological 2:432
    subfamilies 1:363, 1:635–636, 2:423, 2:424*t*, 2:431, 4:581–582
  definition 2:431
  discovery **2:420–430**
    degenerate primer PCR 2:420
    detection methods 2:420
    history 2:420
    numbers discovered 2:421, 2:424*t*
    pathogenic impact 2:427
  DNA polymerase gene 2:423
  equine *see* Equine herpesvirus(es)
  evolution 2:435
    co-adaptation 2:435
    timescale 2:435
  fish *see* Fish herpesvirus(es)
  gB structure 3:300
  gene content 2:434
    arrangement 2:434–435
    complements 2:434
    core genes 2:435
    sequence comparisons 2:435
    transcript functions 2:434–435
  gene therapy vectors 2:304, 2:396
    structure 2:304
  genome 2:433, 2:434*f*
    5′ CG deficiency 2:433
    comparisons 1:636
    structure types 2:433–434
  genotypes 2:378
  percentage identity 5:347
  replication 2:430, 4:409–410
    DNA replication 2:431
    expression 2:431
    host cell attachment 3:310
    host cell entry 2:430
    maturation 2:431
    release 2:431
  virion structure 2:430, 2:431*f*
    capsid 2:430
    core 2:430
    envelope 2:430
    tegument 2:430
  see also specific viruses
Herpesvirus 8 (HHV-8) *see* Kaposi's sarcoma-associated herpesvirus (KSHV)
Herpesvirus Ateles (HVA) 4:586, 4:587*t*
  classification 4:585–586
  gene content 4:588
    conserved genes 4:588
  genome 4:588
    H DNA 4:588
    L DNA 4:588
  historical aspects 4:586
  infection
    acute-T-cell lymphomas 4:586–587
    affected species 4:586
    host range 4:586
    pathology 4:586
    transfected T-cell lines 4:587–588
  isolation 4:586
  replication 4:588
  strain classification 4:588
  transmission 4:586
Herpes virus entry mediator (HVEM), viral replication 2:398
Herpesvirus infections
  antiviral therapy 1:143*t*, 1:146, 1:152
    acyclovir *see* Acyclovir
    drug resistance 1:151
    HSV *see* Herpes simplex virus(es) (HSV)
  apoptosis inhibition 1:158*t*
  congenital disease 2:146
  HCMV coinfection 2:485–486
  hosts 2:421–422, 2:424*t*
  human respiratory infection 2:555
  immune evasion 1:125
  immunomodulation 4:114*t*
  latency **2:436–442**
  organ transplantation 3:467, 3:469
  pathogenic impact, species barriers 2:428
  persistence 4:109*t*
  RNAi therapeutics 5:327
  shellfish *see* Shellfish virus(es)
Herpesvirus papio 2 (HVP-2), detection 4:582–583
Herpesvirus Saimiri (HVS) 2:428, 4:586, 4:587*t*
  affected species 4:586
  cell culture 4:586
  classification 4:585–586
  gene content 4:588
    conserved genes 4:588
    early infection genes 4:588–589
    host nucleus localization 4:589
  genome 4:588
    H DNA 4:588
    L DNA 4:588
  historical aspects 4:586
    discovery 4:586
  host range 4:586
  human T-cell transformation 4:593
    gene transfer 4:591
  oncogenic signaling 4:589
  pathology 4:586
  replication 4:588
  sequestered cellular genes 4:589
  Stp oncoproteins 4:589
    human T-cell transformation 4:591
    sequence homology 4:589
    signaling 4:589
    T-cell leukemia 4:589
    transcription 4:589
  strain classification 4:586, 4:588, 4:588*f*
  Tio oncoprotein 4:590
    Lck phosphorylation 4:590
    Src phosphorylation 4:590
  Tip oncoproteins 4:589
    human T-cell transformation 4:591
    Lck binding 4:590
    signaling 4:590
    Src kinase homology 4:589–590
    T-cell proliferation 4:589–590
  transformation 4:589
  transmission 4:586
Herpes zoster ophthalmicus, HIV infection 2:497
Herpetic whitlow 2:388–390
Herring aquareovirus (HRV) 1:164*t*
HERVd (Human Endogenous Retrovirus Database) 5:357*t*
Heterobasidion annosum, partitivirus transmission 4:67
Heterocapsa circularisquama, viral infection of 1:87

Heterocapsa circularisquama RNA virus (HcRNAV) 1:88*t*, 1:89
  ecotypes 1:89, 1:90
  genome 1:89, 1:91*f*
    open reading frames 1:89, 1:89*f*
  host range 1:89–90
  host-virus dynamics 1:95
  phylogeny 1:89, 1:93*f*
  virion structure 1:89, 1:91*f*
Heterochromatin protein 1 (HP1) 2:439
Heterochromatin siRNA pathway 4:142
Hetero-encapsidation 4:97
  zucchini yellow mosaic virus transmission 5:436
Heterogeneous nuclear ribonucleoprotein (hnRNP), nidovirus replication 3:427
Heterokaryon 5:299
Heterooligomeric protein expression, transgenic plants 4:233
Heterosigma akashiwo 3:280
  infection
    cytopathology 3:280
    susceptibility 3:280
Heterosigma akashiwo RNA virus (HaRNAV) 3:280, 3:281
  genome 3:280–281, 3:281*f*
  phylogenetic analyses
    polymorphisms 3:283, 3:284*t*
    RdRp domains 3:281, 3:283
  virion structure 3:280, 3:280*f*
    proteins associated 3:281, 3:281*f*
Heterotelomeric parvovirus(es), replication 4:90–91
  see also specific viruses
Heterotypic immune response, coxsackieviruses 1:583–584
Het-S prion 4:337, 4:340
HeV *see* Hendra virus (HeV)
HEV-A *see* Human enterovirus(es) (HEV)
HEV-B *see* Human enterovirus B (HEV-B)
HEV replicon system 2:380
Hexamer units, cowpea chlorotic mottle virus structure 5:385
Hexons
  adenovirus antigens 1:5
  HSV capsid structure 2:397
HF1 virus 5:413*t*, 5:415
  genome 5:417, 5:417*f*
  host range 5:415
HF2 virus 5:413*t*, 5:415
  genome 5:417, 5:417*f*
  structure 5:417*f*
HFMD *see* Hand, foot and mouth disease (HFMD)
H glycoprotein, measles virus 3:287–288
HGS004 2:507*t*
Hh-1 virus 5:413*t*
Hh-3 virus 5:413*t*
HHV-4 *see* Epstein–Barr virus (EBV)
HHV-6 *see* Human herpesvirus-6 (HHV-6)
HHV-7 *see* Human herpesvirus-7 (HHV-7)
HHV-8 *see* Kaposi's sarcoma-associated herpesvirus (KSHV)
Hibiscus chlorotic ringspot virus (HCRSV) 1:453
Hibiscus-infecting tobamovirus(es) 5:69
  see also specific viruses
Hibiscus latent ringspot virus (HLRSV) 3:406*t*
High-affinity laminin receptor (HALR), Sindbis virus 5:118
Highlands J virus 5:81
  characteristics 5:109*t*
  evolutionary relationships 1:174
  geographical distribution 5:102
  infection
    encephalitis 5:81
    epidemiology 5:81–82
    host range 5:102–103
  transmission, *Culiseta melanura* 5:103–104
Highly active antiretroviral therapies (HAART) 1:66–68, 1:353, 2:506
  associated renal disease 1:56–57
  drug resistance 2:516, 2:518
  HCMV infection 2:484
  HIV infection *see* HIV infection
  JCV reactivation 4:265–266
  KSHV infection 3:192, 3:194
Highly exposed seronegative individual(s) (ES) *see* HIV infection
Highly polymorphic region, infectious salmon anemia virus 3:93
High pathogenicity avian influenza (HPAI) virus(es) 3:101
  HA cleavage sites 3:101–102
  see also specific viruses
High per-base mutation rate, RNA virus 4:363
High Plains virus (HPV) 1:477
  infection 1:477
High-pressure freezing, cryo-electron microscopy 2:84
High-throughput screening 5:375
Hind III enzyme, cowpox virus genome digestion 1:574, 1:575
Hippocampus sclerosis, Borna disease virus associated 1:342–344, 1:343*f*
Hirame rhabdovirus (HIRRV) 2:221–222, 2:228, 4:184*t*
Hirst, George, influenza virus 2:456
His1 virus 5:413*t*, 5:418
  cell exit 5:420
  DNA polymerase 5:420
  genome 5:420, 5:421*f*
  structure 5:418, 5:420*f*
His2 virus 5:413*t*, 5:418
  cell exit 5:420
  DNA polymerase 5:420
  genome 5:420, 5:421*f*
    open reading frames 5:418–420
  structure 5:418–420, 5:420*f*
Histiocytes, Yaba monkey tumor virus infection 5:464
Histo-blood groups 1:410, 3:444
Histo-group antigen(s) (HBGAs), receptor role 5:321
Histone(s)
  acetylation 1:11
  EBV latency, role 2:442
Histopathology
  Helicoverpa armigera stunt virus infection 5:36
  henipavirus infections 2:325
  Providence virus 5:36
  rabbitpox 3:347
  tetravirus infections 5:36
'Hit and run' transformation, adenovirus malignant transformation 1:16
HIV **2:517–525**
  antibody variation 1:132
  antigenic variation 1:128, 1:132
    circulating recombinant forms 1:59, 1:132–133
    envelope glycoprotein *see below*
    genome co-packaging 1:133
    global variation 1:132
    intra-patient variation 1:132
    population genetics 1:133
    replicase 1:133, 1:133*f*
    unique recombinant forms 1:59
  biocrime 5:407
  classification 1:132–133, 2:526
    serogroups 1:59, 2:526
    subtypes 1:59, 1:60*f*, 2:524, 2:526
  cross-species transmission 2:525–526, 2:529, 2:536–537, 4:604–606
    ecological change 2:532–533
    evidence 4:612
    geographic location 2:529
    HIV-1 2:529
    HIV-2 2:531
    method 2:532
    origins *see below*
    pathogenic potential 2:533
    risks 2:532
    timeline 2:532
  defective interfering viruses 2:4
  envelope glycoproteins 1:132, 1:134
    antibody recognition 1:136
    binding 5:322
    CD4 binding site 1:136
    glycosylation 1:136
    structure 1:134
    see also specific types
env gene/protein
  CD4 T-cell response 1:71
  consensus sequence 1:73–74
  immunogenicity 1:75
  infection pathogenesis 2:538*f*, 2:540
  membrane fusion 3:296–297
  neutralizing antibodies 1:72–73
  retrovirus organization 2:519–520
  sequence evolution 1:73
escape mutants 2:541
etiology 2:143–144
evolution 2:175, 2:176*f*, 2:527
  HIV-1/SIV_{cpz} 2:528, 2:530*f*
  HIV-2/SIV_{gor} 2:529, 2:530*f*
  HIV-2/SIV_{smm} 2:527
  ZR59 2:532
expression regulation 3:304–306
gag gene/protein
  expression 1:73–74
  retrovirus organization 2:519–520
  vaccine developments 1:73–74
genome 2:518, 2:519*f*, 2:526, 2:528*f*
gp41
  antigenic variation 1:136
  host cell entry 2:506
gp120 2:519–520, 2:538, 5:310, 5:323
  antigenic variation 1:134, 1:135–136
  definition 1:127
  glycosylation 1:136
  host cell attachment/entry 2:506, 2:538, 4:408, 5:323
  hypervariable loops 1:135–136
gp160
  neotopes 1:137–138
  neutralizing antibodies 1:137–138
historical aspects 2:517–518, 2:525, 2:534, 4:460
  discovery 4:625
host cell attachment, receptors 4:408
infection *see* HIV infection
life cycle 2:505–506
neutralizing antibodies 1:72–73, 1:137–138
nomenclature 4:594–595
origin 1:59, **2:525–534**, 4:595*f*, 4:611
  bushmeat 2:532
  chimpanzees 2:528, 2:530*f*
  evolution *see above*
  gorillas 2:533
  polio vaccine, human-induced theory 2:530*f*
  subspecies identification 2:528, 2:529
  vertical transmission 2:528–529
regulatory proteins 2:521
  Nef 2:523
  Rev 2:522
  Tat 2:522
  Vif 2:523, 4:598–599
  Vpr 2:522
  Vpu 2:523
replication 2:518, 2:520*f*
  activation 2:539–540
  APOBEC3G 2:521, 3:109, 4:598–599
  cellular factors, role 2:521
  control 2:535
  co-receptors 1:134–135, 2:518–519
  early 2:537
  gp41 2:519–520
  host cell entry 1:134–135, 2:506, 2:519
  primary receptor 2:518–519
  rate 1:150
  transcription 2:519
  translation 2:519–520
  viral release 2:520–521
  virus entry 5:315
  virus shedding 5:316–317
Rev protein 2:522
  binding activity 2:522
  splicing 2:522
sensitivity, *TRIM5* gene/protein 2:470
taxonomy 2:526
transmission 1:59, 1:60, 1:60*t*, 1:73, 2:94, 4:601, 4:602
  cross-species *see above*
  determinants 2:537

HIV (*continued*)
influencing factors 2:538
risk factors 1:60
vaccines/vaccination *see* HIV vaccines/vaccination
see also AIDS
HIV-1
cell entry
CCR5 co-receptor 2:468
chemokine ligands as inhibitors 2:468–469
origin 2:528, 2:530*f*
subspecies identification 2:528, 2:529
vertical transmission 2:528–529
vaccines/vaccination
AIDSVAX trials 1:70
ELDKWAS peptide 1:141
virus protein U 2:523
definition 2:467
functions 2:523
lentiviruses 2:473
structure 2:523
ZR59 2:532
HIV-1, Human Protein Interaction Database 5:357*t*
HIV-2
associated serogroups 2:533
cross-species transmission 2:531, 2:531*f*
evolution 2:527
geographical distribution 1:59
origin 2:531, 2:531*f*
geographic location 2:529
timeline 2:532
prevalence 1:59, 2:524
HIV Drug Resistance Database 5:357*t*
Hivid® 2:510
HIV infection **1:51–58**, 3:195
acute renal failure 1:55
causes 1:55–56
incidence 1:55–56
antiretroviral therapy 1:146, 1:153
fusion inhibitors 1:148
see also below highly-active antiretroviral therapy
associated infections 2:94
HPV 4:14
human respiratory infections 2:556
Japanese encephalitis concurrent infection 3:184
JCV reactivation 4:262, 4:265–266
Kaposi's sarcoma *see* Kaposi's sarcoma (KS)
KSHV *see* Kaposi's sarcoma-associated herpesvirus (KSHV)
measles 3:289
associated malignancies 1:52
cases 1:51–52
CCR5 receptor 2:468
Δ32 genetic mutation 2:506, 2:537, 5:318–319
HHV co-infection 2:503
host-cell interactions 2:506, 2:518–519, 5:318–319, 5:323
pathogenesis 2:535–536
CD4+ T cells 1:52, 2:534–535
attachment 1:136, 2:518–519, 5:322
bystander activation 2:540–541
cell count 1:52
co-receptors 2:535–536
depletion 2:535, 2:535*f*
downregulation 1:72, 2:524
gp120 conformation 2:540, 5:323
MALT-associated 2:537*t*, 2:539–540
proliferation 2:535
CD8+ (cytotoxic) T-cells 1:71
acute-to-chronic phase transition 2:539*f*, 2:540
bystander activation 2:540–541
highly exposed seronegative individuals 1:70–71
vaccination strategies 1:69–70
dementia 1:57, 1:474
disease progression 1:57
manifestations 1:57
disease surveillance
data collection sources 2:45, 2:46
data interpretation 2:49
data protection acts 2:46
death certification 2:46
risk factors 2:46
screening systems 2:47
distal symmetric sensory polyneuropathy 1:58
disease characterization 1:58
opportunistic infection treatment effects 1:58
prevalence rate 1:58
eye 2:144, 2:146
herpes zoster ophthalmicus 2:497
retinopathy 2:146
gp160, neutralizing antibodies 1:137–138
Guillain–Barre´ syndrome 1:474–475
highly active antiretroviral therapies
associated renal disease 1:56, 1:57
immune reconstitution inflammatory syndrome *see* Immune reconstitution inflammatory syndrome (IRIS)
mortality rate 1:51–52
neurological manifestations 1:57
highly exposed seronegative individuals 1:70–71
CTL responses 1:70–71
seroconversion 1:71
history 1:59, 2:505
HLA effects
progression indicator 1:70–71, 2:538
vaccine development 1:74–75
host cell interactions 4:598, 5:321
immune complex disease 1:56
categories 1:56
clinical presentation 1:56
immune evasion 1:72*t*, 1:74*t*
Ab-mediated neutralization 2:540, 3:418, 3:418*f*
Ab-mediated neutralization mutants 3:418, 3:418*f*
APOBEC3G 3:109
virus infectivity factor 3:109
immune response
Ab-dependent disease enhancement 3:66
CD4+ T cells *see above*
CD8+ (cytotoxic) T-cells *see above*
immune evasion *see above*
monoclonal antibodies 3:64–65
neutralizing antibodies *see below*
T-cell responses 3:76
intravenous drug abuse 1:60, 1:64
treatment 1:66
kidney disease 1:54
acute renal failure 1:55
chronic renal failure 1:56
mortality rates 1:54–55
nephropathy *see below*
thrombotic microangiopathy 1:56
lipodystrophy 1:53
fat accumulation sites 1:53, 1:54*f*, 1:55*f*
management 1:53–54
thymidine nucleoside analog treatment 1:53
metabolic syndrome 1:53, 1:53*t*
cardiovascular morbidity 1:53
cardiovascular mortality 1:53
exacerbation 1:53
management 1:53
manifestations 1:53
prevalence 1:53
mortality rates, influenza *vs.* 3:96
nephropathy 1:56
ethnicity 1:56
microalbuminuria 1:56
prevalence 1:56
treatment 1:56
neurological complications 1:57, 1:472*f*, 1:473–474, 1:474–475
encephalitis 1:57
neutralizing antibodies 1:132, 3:64–65
acute-to-chronic phase transition 2:540
env protein 1:72–73
gp160 1:137–138
neutralizing monoclonal antibodies 1:69–70, 1:71
properties 1:73*t*
opportunistic infections 1:52–53, 1:52*t*, 1:68
human herpesviruses 2:503
Pneumocystis jirovecii 1:52
organ transplantation, risks 3:470
pathogenesis **2:534–542**, 2:538*f*, 4:115
activation-induced apoptosis 2:536
acute phase 2:537, 2:539
acute-to-chronic transition 2:539*f*, 2:540
bystander activation 2:540–541
bystander cell death 2:534, 2:536
cell turnover rate 2:536
chronic phase 2:537, 2:539*f*, 2:540
CTL-mediated response 2:540
dendritic cells 2:538–539, 2:539*f*
disease progression, markers 2:535
env protein 2:538*f*, 2:540
immunopathogenesis 2:536, 4:115
mucosa-associated lymphoid tissue, role 2:538*f*, 2:539–540
progression duration 2:536
replication control 2:535
therapy implications 2:541
patient care 1:66
persistence 4:109*t*
prevention 1:59, 1:65
see also Antiviral drug(s)
primary isolates 1:69
protease inhibitors 1:148
drug resistance 1:148–149
resistance to, cyclosporine A 2:470
as sexually transmitted disease 1:60, 4:601
risk factors 1:60
SIV *vs.* 4:601
susceptibility, Δ32 mutation 2:506, 2:537, 5:318–319
treatment 1:66, 1:67*t*
highly active antiretroviral therapies *see above*
protease inhibitors *see above*
zoonoses 2:95
HIV infection epidemiology **1:58–68**, 1:60*f*, 1:61*f*
Asia 1:63, 1:64
prevalence 1:63
Southeast 1:63–64
Brazil
prevalence 1:64–65
risk factors 1:64–65
treatment 1:66–68
Caribbean 1:64
China
IV drug abuse 1:64
plasma donation 1:64
prevalence 1:64
transmission 1:64
developing countries 2:534
Eastern Europe 1:64
IV drug abuse 1:64
global prevalence 1:61
global response 1:65
HIV-2 *see* HIV-2
Latin America 1:64
Middle East 1:65
molecular 1:59
new infections 1:61
North Africa 1:65
North America 1:65
Canada 1:65
new infections 1:65
risk factors 1:65
Oceania 1:65
sub-Saharan Africa 1:61, 1:62, 1:62*f*
new infections 1:62–63
prevalence 1:61–62, 1:62*f*
risk factors 1:62
treatment 1:66–68
transmission 1:60, 1:60*t*
risk factors 1:60
Western Europe 1:65
see also specific countries
HIV Sequence Database 5:357*t*
HIV vaccines/vaccination 1:66, **1:69–76**, 5:242
adjuvants 1:75
co-administration 1:75
delivery systems 1:75
immune potentiators 1:75
AIDSVAX 1:70

antibodies 1:72
antigen optimization 1:73
env evolution 1:72, 1:72*t*
in vivo role 1:72
antigenic drift 5:240
drift rate 5:240
antigenic shift 5:240
antigens 5:242
Banna virus 4:542
cell-mediated immune response 1:74
antigen target selection 1:74
cellular response induction 5:242
clinical trials 1:70
CTL responses 1:70
tat protein neutralization 1:72
common cold 4:473–475
coronaviruses 1:561
delivery methods 1:75
ELDKWAS peptide 1:141
envelope glycoproteins 1:134, 1:134*f*, 1:135*f*, 1:136
env gene/protein 1:72–73
glycoproteins 5:242
history 1:69–70
immune response induction 1:75
adjuvants 1:75
HLA genotype 1:70–71
immunogen class 1:75
mutation rate 1:70
implications 2:541
CTL-based vaccines 2:542
neutralizing antibodies 1:141
HIV gp41 1:141
induction 2:542, 5:242
protection correlates 1:70
SIV studies 1:71
safety 5:234
disease course alterations 5:234
immune response alterations 5:234
transmission 5:234
studies 1:70
killed microorganisms 1:70
live-attenuated microorganisms 1:70
subunit variations 1:70
VaxGen 1:70
vectors 1:75, 5:242
HK022 bacteriophage 5:185
HK97 bacteriophage *see* Bacteriophage HK97
HMPV *see* Human metapneumovirus
Hodgkin's and Reed-Sternberg (HRS) cells 2:154–155
Hodgkin's lymphoma
EBV associated *see* Epstein–Barr virus infection
latent membrane protein 2A 2:163
Hog cholera virus *see* Classical swine fever virus (CSFV)
Holin–endolysin lysis 3:250
bacteriophage fOg44 3:254–255
bacteriophage λ *see* Bacteriophage λ
definition 3:248
pinholins 3:254
Rz–Rz1 genes 3:252
definition 3:249
gene diversity 3:254
gene product function 3:254
reading frames 3:254
SAR endolysins 3:254
signal sequences 3:254
Holins 3:250
definition 3:248, 3:250
homology 3:250, 3:251*f*
icosahedral tailed dsDNA bacteriophages 3:35
lambda holin S105 3:253
mutational effects 3:249–250
S^{21} function 3:254–255
topology 3:250
transmembrane helical domains 3:250, 3:251*f*
triggering 3:248
Homologous recombination
bacteriophages 4:402*f*
evolution 5:371–372
baculovirus expression vectors 1:239–240, 1:244
*flash*BAC 1:244
brome mosaic virus 4:378, 4:378*f*
bromoviruses 1:389
definition 1:238
hantaviruses 2:318
phylogenetic analysis 4:127
RNA viruses 4:377–378, 4:378*f*
Homologous repeat region(s)
definition 1:254
granuloviruses 1:218
nucleopolyhedroviruses 1:256–257
Homoplasty 4:436
Homopterans, definition 1:111
Homotelomeric parvovirus(es)
replication 4:90–91
see also specific viruses
Homotypic viral interference, arenavirus replication 3:246
Hong Kong influenza (1968) pandemic 3:100
genome reassortment 3:99
Hop stunt viroid (HSVd), host range 5:335
Hordeivirus(es) **2:459–467**
cereal genomic analysis applications 2:466
coat protein 2:461–462
cytopathology 2:463
gene expression 2:459
genome 2:459
gRNA-encoded proteins 2:460–461
gRNAs 2:460
sgRNAβ1 2:461
sgRNAβ2 2:461
host range 2:459
infection
chloroplast morphological changes 2:463–464
TGB movement protein role 2:464
pathogenesis 2:464
proteins 2:461
coat proteins 2:461
pathogenesis proteins 2:463
replicase 2:461
TGB movement proteins *see below*
RdRp 2:461
replication 2:463, 2:464, 2:465*f*
TGB movement proteins 2:462
chloroplasts, effects on 2:464
expression 2:461
TGB1 2:462
TGB2 2:462
TGB3 2:463
transmission 2:464
see also specific viruses
Hordeum vulgare see Barley *(Hordeum vulgare)*
Horizontal gene transfer
bacteriophage evolution 5:372
definition 3:30
icosahedral tailed dsDNA bacteriophages 3:36
partitivirus evolution 4:74–75
Horizontal transmission 5:274
definition 2:141
mouse mammary tumor virus 3:337
Hormone-regulated gene expression, virus transcriptional regulation 3:338*f*, 3:340
Hormone response elements (HREs), definition 3:334
Horse(s)
West Nile virus infection 2:237
see also under equine
Horsegram yellow mosaic virus 3:370–371
Horsepox, smallpox vaccination 4:640
Horsepox virus (HPXV), vaccinia virus origin 5:243–244
Horseradish curly top virus (HrCTV) 1:302
geographical distribution 1:302–303
host range 1:306
Horse sickness fever 1:40
Hospital administrations, disease surveillance 2:46
Host(s)
clinical status related to epidemiology 2:146
definition 5:469
genome
DNA virus recombination 4:374
mouse mammary tumor virus replication 3:336
resistance
genes 4:156
RNA silencing 4:161, 4:162
see also specific viruses
Hosta virus X (HVX) 4:311*t*
Host cell(s) 2:505
Host cell factor-1 (HCF-1), varicella-zoster virus gene expression 5:259
Host specificity, baculoviruses *see* Baculovirus(es)
House sparrows, Saint Louis encephalitis virus transmission 4:655
HPIVs *see* Human parainfluenza virus(es) (hPIVs)
HPS *see* Hantavirus pulmonary syndrome (HPS)
HPV *see* Hepatopancreatic parvovirus (HPV); High Plains virus (HPV)
HPV16 *see* Human papilloma virus 16 (HPV16)
HPV31 *see* Human papilloma virus 31 (HPV31)
HrCTV *see* Horseradish curly top virus (HrCTV)
HRSV *see* Human respiratory syncytial virus (HRSV)
HRT gene, *Arabidopsis thaliana* 4:174–175
HRV2 *see* Human rhinovirus 2 (HRV2)
HRV16 *see* Human rhinovirus 16 (HRV16)
Hs1 virus 5:413*t*
HSV-1 *see* Herpes simplex virus 1 (HSV-1)
HTLV-1 *see* Human T-cell leukemia virus 1 (HTLV-1)
HTLV-1 associated myelopathy (HAM) *see* Tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM)
HTLV-1-associated myelopathy (HAM) *see* Tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM)
HTLV-2 *see* Human T-cell leukemia virus 2 (HTLV-2)
HToV *see* Human torovirus (HToV)
Hughes virus 1:598*t*
Human adenovirus(es)
classification, serotypes 1:17
see also specific viruses
epidemiology
HAdV-A 1:25
HAdV-B1 1:25
HAdV-B2 1:25
HAdV-C 1:25
HAdV-D 1:25
HAdV-E 1:26
HAdV-F 1:26
gene expression 1:20*f*
coding potential 1:19
MLTU coding potential 1:19
genome 1:18, 1:18*f*
infections *see below*
taxonomy 1:1
Human adenovirus infections 1:26, 1:28
acute hemorrhagic conjunctivitis 2:495
classification 1:24, 1:24*t*
epidemic keratoconjunctivitis 2:495, 2:495*f*
experimental infection 1:28
human respiratory infection 2:554
vaccines 2:557
immune response 1:28
immunocompromised patients 1:28
incubation period 1:28
malignant transformation *see below*
pathogenesis 1:28
classification 1:24, 1:24*t*
experimental infection 1:28
immune response 1:28
immunocompromised patients 1:28
incubation period 1:28
infection sites 1:28
pathology 1:28
serotypes 1:24, 1:24*t*
pathology 1:28
serotypes 1:24, 1:24*t*
simple follicular conjunctivitis 2:495
Human adenovirus infections, malignant transformation **1:9–16**
Ad9 1:16
anaphase-promoting complex 1:11

Human adenovirus infections, malignant
transformation (*continued*)
clinical manifestations 1:9
E1A gene/protein 1:10, 1:10*f*, 1:12, 1:19
Ad5 E1A proteins 1:10*f*
Ad12 E1A proteins 1:12–13
CBP/p300 1:11
exon 2 1:12
expression 1:10–11
MHC downregulation 1:12–13
N-terminal region 1:11–12
oncogenicity of 1:12
p400 binding 1:11–12
PML-containing nuclear oncogenic bodies interactions 1:12
pRB interactions 1:12
promyelocytic leukemia interactions 1:12
protein expression 1:10*f*
transforming properties of 1:10
TRRAP binding 1:11–12
E1B-19K gene/protein 1:13
anti-apoptotic signaling role 1:13*f*
BCL-2 *vs.* 1:13–14
transforming properties of 1:13
E1B-55K gene/protein 1:13, 1:14, 1:14*f*
Eorf6 interactions 1:14–15
E1B-AP5 gene/protein 1:15
E1B gene/protein 1:13
E4 gene/protein 1:13
E4orf1 gene/protein 1:16
E4orf3 gene/protein 1:14, 1:15
E4orf6 gene/protein 1:14
C-terminal region 1:14–15
E1B-55K interactions 1:14–15
histone acetylation 1:11
history 1:9
'hit and run' transformation 1:16
major histocompatibility complex downregulation 1:12–13
MRE11-RAD50-NSB1 complex 1:14, 1:15
E4orf3 1:15
NFκB 1:12–13
nuclear export signals 1:14
properties 1:9
transfection studies 1:9–10
tumorigenic potential 1:9
Human B19 parvovirus(es) 4:85–86
tissue tropism 4:89
see also specific viruses
Human behavior, emerging viral diseases 2:94
Human bocavirus (HBoV) 2:94
infection 2:555
Human coronavirus(es) 1:553
Human cytomegalovirus (HCMV) **2:474–485**, **2:485–491**
classification 2:474
evolution 2:476*f*, 2:478
genetics 2:475, 2:479*t*
sequence identity 2:477
variants 2:475
genome 1:637, 2:476*f*, 2:486
HSV-1 *vs.* 2:486
origin of DNA replication 2:486
variable genes 1:637
geographical distribution 2:475
glycoproteins 2:487–488
history 2:474
laboratory strains 1:639
NS5A protein 2:369–370
physical properties 2:489
infectivity 2:489
prevalence 2:475
propagation 2:475
proteins 2:486
early 2:486
glycosylation 2:489–490
immediate early 2:486
late 2:486
nonvirion 2:486
phosphorylation 2:489–490
posttranslational modification 2:489
proteolytic cleavage 2:489–490
virion 2:487, 2:488*t*
replication 1:626, 2:475, 2:478*f*, 2:489
DE phase 2:477
DNA replication 2:489
egress 2:477–478, 2:478*f*
encapsidation 2:477–478
host cell entry 1:627–628, 2:477
IE phase 1:627–628, 2:477
L phase 1:628, 2:477
metabolic changes in infected cells 2:489
regulation 2:489
tethering 1:626
transcription characterization 2:489
translation characterization 2:489
seasonal distribution 2:475
transmission 2:439, 2:482
horizontal 2:482
placental 2:474
transplacental 2:474, 2:482–483
virion properties 2:475–477, 2:485
capsid 2:485–486, 2:487, 2:488*t*, 2:490
capsomeres 2:485–486
envelope 2:485–486, 2:487–488, 2:488*t*, 2:490
nucleocapsid 2:475–477
tegument 2:475–477, 2:485–486, 2:487, 2:488*t*
Human cytomegalovirus infection 2:474
AIDS 2:439
antiviral drugs 2:474, 2:484
HAART 2:484
blindness 2:496
clinical features 2:483, 4:114–115
chronic disease 4:114–115
congenital disease 2:483
control 2:484
cytopathology 2:490
HCMV release 2:490
diagnostic tests 3:471
host range 2:475
cell tropism 2:475
immune evasion 1:125, 1:637, 2:484, 2:487, 3:110
anti-apoptotic proteins 1:637
apoptosis 1:637
G protein-coupled receptors 1:637, 1:638*f*
immunocompromised hosts 2:474
infectious retinitis 2:496, 2:496*f*, 2:497
latency 2:439, 2:440*f*, 2:482
chromatin structure 2:439
genome 2:440
major immediate early promoter 2:439
newborns 2:483
pathogenesis 2:482
pathology 2:483
persistence 4:109*t*
pregnancy 2:482–483, 4:114–115
prevention 2:484
reactivation 2:439, 2:440, 2:440*f*, 2:483
JCV/HIV infection 4:266
transplant-associated 3:467–468, 3:468*t*
transplantation 2:483–484, 3:469
allograft 2:483–484
solid organ 2:484
universal prophylaxis 3:471
virus shedding 2:482
Human demographics, emerging viral diseases 2:94
Human diploid cell vaccine 5:493–494
Human endogenous retrovirus(es) (HERV) 2:108
see also specific viruses
Human enterovirus(es) (HEV) **2:130–136**
classification 1:581, 2:130
features 2:130
structure 2:130
history 2:130
infection
disease surveillance 2:46
respiratory disease 2:552
novel types 2:131*t*, 2:134
history 2:134
transmission 2:130
see also specific viruses
Human enterovirus B (HEV-B) 2:65–66
enterovirus 69 2:131
infection
clinical manifestations 2:68
diabetes, type I 2:69
neonates 2:68
novel types 2:135
Human enterovirus D (HEV-D) 2:124
Human extrahepatic biliary atresia (EHBA), mammalian reovirus infection 4:389
Human herpesvirus(es)
see specific viruses
Human herpesvirus 4 (HHV-4) *see* Epstein–Barr virus (EBV)
Human herpes virus 5 *see* Human cytomegalovirus (HCMV)
Human herpesvirus-6 (HHV-6) **2:498–505**
classification 2:498
genome 2:499, 2:500*f*
geographical distribution 2:498
HHV-6A 2:498, 2:502
HHV-6B 2:498, 2:502
history 2:498
infection *see below*
laboratory culture 2:499
replication 2:501
transmission 2:502
Human herpesvirus-6 infection
antiviral therapy 2:504–505
cell tropism 2:499
congenital 2:502
control 2:504
diagnosis 2:504
disease associations 2:502
immune response 2:504
protective immunity 2:504
immunocompromised patients 2:502
bone marrow transplantation 2:498–499, 2:502
solid organ transplantation 2:498–499, 2:502, 3:469
immunomodulation 2:501
infant fever 2:502
latency 2:499
neuroinflammatory disease 2:503
multiple sclerosis 2:504
pathogenesis 2:502
persistence, brain 2:503
Human herpesvirus-7 (HHV-7) **2:498–505**
classification 2:498
genome 2:499
geographical distribution 2:498
history 2:498
infection *see below*
laboratory culture 2:499
replication 2:501
transmission 2:502
Human herpesvirus-7 infection
antiviral therapy 2:504–505
cell tropism 2:499
control 2:504
cytopathic effects 2:499
diagnosis 2:504
disease associations 2:502
immune response 2:504
protective immunity 2:504
immunocompromised patients 2:502
bone marrow transplantation 2:502
solid organ transplantation 2:502
immunomodulation 2:501
infant fever 2:502
latency 2:499
neuroinflammatory disease 2:503
multiple sclerosis 2:504
pathogenesis 2:502
persistence, brain 2:503
Human herpesvirus-8 (HHV-8) *see* Kaposi's sarcoma-associated herpesvirus (KSHV)
Human herpesvirus infections
immunocompromised patients
bone marrow transplantation 2:502
HIV/AIDS 2:503

solid organ transplantation 2:502
persistence 4:109*t*
Human immunodeficiency virus *see HIV*
Humanized antibodies 2:543
Human lymphocyte antigen (HLA), HIV infection *see* HIV infection
Human metapneumovirus infection 2:553
clinical features 2:553
pediatric 2:553
Human monkeypox virus (MPXV) 4:329
Human papillomavirus(es) (HPVs) **4:8–18**, **4:18–26**
capsid structure 4:25–26
classification 4:8
E1^E4 gene/protein 4:22
cornified cell envelope association 4:23
expression 4:22–23
motifs 4:23
overexpression 4:23
role 4:22
E1 gene/protein 4:21
associated cellular factors 4:21–22
role 4:21, 4:36
E2 gene/protein 4:22
binding sites 4:21–22, 4:36
Brd4 binding disruption 4:22
role 4:22
transcriptional regulation 4:22
E5 gene/protein 4:23
E6 gene/protein 4:24
cellular targets 4:37
immortalization 4:24
motifs 4:24
protein interactions 4:24
role 4:24, 4:37
E7 gene/protein 4:25
associated cellular factors 4:25
pocket protein-binding motif 4:25
pRB interactions 4:25
role 4:25, 4:37
structure 4:25
evolution 4:10*f*, 4:12
mechanisms 4:13
gene functions 4:21
see also specific genes
genome 4:8, 4:19
early region 4:8
late region 4:8
oncogenes 4:19
open reading frames 4:8
post-transcriptional modifications 4:19
regions 4:19, 4:19*f*
transcription 4:19
upstream regulatory region 4:8
genotypes 4:8–9, 4:18–19
genital high-risk types 4:17
variants 4:9–10
history 4:8
L1 4:13, 4:25
L2 4:25
life cycle 4:20, 4:20*f*
basal cell 4:20
cellular location 4:20
DNA integration 5:196
host cell entry 4:20
nonproductive infection establishment 4:20
nonproductive infection maintenance 4:21
productive infection 4:10–12, 4:21
productive stage 4:21
progeny production 4:21
suprabasal cell 4:20
replication 4:10
gene expression 4:10–12
genome integration 4:12
host cell entry 4:10–12
release 4:10–12, 4:12*f*
vegetative 4:10–12
taxonomy 4:8, 4:10*f*
transmission 4:10, 4:38
cutaneous types 4:10
genital infections 4:38
genital types 4:10
risk factors 4:38
vaccines/vaccination 2:51–52, 4:19, 4:39, 5:223, 5:225
antigenic variation 1:127–128
bivalent vaccine 4:18
development 5:235
strategies 4:17, 5:241
tetravalent vaccine 4:18
virion structure 4:8, 4:9*f*
capsids 4:13
see also specific viruses
Human papillomavirus infections
anogenital warts 4:14–15, 4:16
intraepithelial neoplasia 4:16
carcinogenesis/neoplasia 4:8, 4:10–12, 4:38–39
anogenital cancer 4:19
anogenital warts *see above*
cervical cancer *see below*
conjunctival tumors 2:496
diagnosis 4:39
squamous cell carcinoma 2:492*f*, 2:496, 4:20, 5:197
cervical cancer 4:8, 4:18–19, 4:38–39, 5:196
causative mechanism 4:15
Papanicolaou test 4:16, 4:17, 4:39
risk factors 4:15–16
transformation zone 4:38–39
clinical features 4:16
control 4:17
diagnosis 4:39
as emerging disease 2:93
epidemiology 4:14
Hybrid Capture 2 test 4:14
epidermodysplasia verruciformis 4:20, 5:197
eye infections 2:491–492
genital infections
anogenital warts *see above*
cell immortalization 4:12
clinical features 4:16
detection 4:14
distribution 4:14
immune response 4:17
incidence 4:14
transformation zone 4:10
transmission 4:10
histopathology 4:14, 4:16
host range 4:10
immortalization potential 4:21
E5 4:23
immune response 4:16, 4:39
immunodeficiency 4:17
immunomodulation 4:114*t*
infection sites 5:197
pathogenesis 4:19, 5:196
pathogenicity 4:11*t*, 4:14, 4:38
pathology 4:16
persistent 4:109*t*, 4:115
prevention 4:17
renal transplant recipients 3:470
serological relationships 4:13
tissue tropism 4:10
treatment 4:17, 4:39
vaccines/vaccination 4:19, 4:108–109
warts 4:19
anogenital *see above*
common 4:16
experimental transmission 4:8, 4:26
genital 4:16
incidence 4:16
palmoplantar myrmecia 4:16
pathogenesis 4:19
pathogenicity 4:14–15
treatment 4:39
Human papilloma virus 5 infection, epidermodysplasia verruciformis 4:8
Human papilloma virus 6 infection, anogenital warts 4:14–15
Human papilloma virus 8 (HPV8), carcinogenic potential 5:197
Human papilloma virus 11 infection, anogenital warts 4:14–15
Human papilloma virus 16 (HPV16) 4:14
gene expression 5:196
E2 binding sites 4:36
E5 role 4:23–24
E6 role 4:24–25
E7 role 4:25
genome 4:35*f*
infection *see below*
taxonomy 4:35
Human papilloma virus 16 infection
cancer 4:16, 4:115
epithelial restriction barriers 4:35–36
related histological abnormalities 5:196
tumor development 4:10
immune system evasion 4:17
nonproductive infection 4:20–21
Human papilloma virus 18 (HPV18), E7 role 4:25, 4:115
Human papilloma virus 31 (HPV31)
E5 role 4:23–24
E6 role 4:24–25
E7 role 4:25
L2 role 4:26
Human parainfluenza virus(es) (hPIVs) **4:47–52**
classification 4:47
evolution 4:50
genetics 4:50
genome 4:47
geographical distribution 4:49
hemagglutinin–neuraminidase 4:47–48
cleavage 4:48
morphology 4:48–49
historical aspects 4:47
infection 2:553
clinical features 4:50*t*, 4:51
control 4:51
diagnosis 4:51
epidemiology 4:50, 4:50*t*
host range 4:50
immune response 4:51
pathogenicity 4:50
pathology 4:51
prevention 4:51
transmission 4:50
propagation 4:50
proteins 4:47
attachment protein 4:48
fusion protein 4:49
hemagglutinin–neuraminidase *see above*
matrix protein 4:47–48, 4:49
nucleocapsid 4:47–48
P protein 4:47–48
structural proteins 4:47–48
replication 4:49
serology 4:50
taxonomy 4:47
transcription 4:49
translation 4:49
types 4:47
virion structure 4:47, 4:48*f*
see also specific viruses
Human polyomavirus *see BK virus*; *JC virus*; *KI virus*; *WU virus*
Human parainfluenza virus-4 2:17
Human reservoirs
see specific viruses
Human respiratory syncytial virus (HRSV) **2:542–551**
antigens 2:547
dimorphism 2:553
monoclonal antibodies against 2:547
subgroups 2:547
classification 2:543
genetics 2:547
recombinant virus 2:547
genome 2:545*f*, 2:546
history 2:543
infection *see below*
replication 2:546
antigenome 2:546
host cell attachment 2:547
progeny release 2:546
transcription 2:546

Human respiratory syncytial virus (HRSV) (*continued*)
stability 2:547
transmission 2:548
vaccines/vaccination 2:550
disease enhancement 2:550, 2:557
virion proteins 2:543
F glycoprotein 2:543, 2:544
G glycoprotein 2:543
L protein 2:543
M2-2 protein 2:545
M protein 2:545
NS1 protein 2:545–546
NS2 protein 2:545–546
nucleocapsid 2:543
nucleocapsid phosphoprotein 2:543
small hydrophobic protein 2:543, 2:544–545
transcriptional processivity factor 2:543
virion structure 2:543, 2:545*f*
Human respiratory syncytial virus infection 2:553
cell culture 2:546
children 2:553
clinical features 2:548
complications 2:548
diagnosis 2:548
elderly patients 2:548
epidemiology 2:548, 2:549*f*, 2:553
incidence 2:548
experimental animals 2:546
immunity 2:549
immunoprophylaxis 2:549
incubation period 2:548
pathogenesis 2:548*f*, 2:549
re-infection 2:548
risk factors 2:548
treatment 2:549
Human respiratory tract 2:551
Human respiratory virus(es) **2:551–558**
antiviral drugs 2:556
co-infections 2:556
transmission 2:556
disease syndromes 2:551
immunocompetent hosts 2:551
immunocompromised hosts 2:552
vaccines 2:557
see also specific viruses
Human rhinovirus(es) 5:391–392, 5:392*f*
see also specific viruses
Human rhinovirus 2 (HRV2)
antigenicity 1:545
host cell attachment 1:543–544
Human rhinovirus 14 (HRV14) 1:545
Human rhinovirus 16 (HRV16)
infection 1:546
macrophage activation 1:547
Human rhinovirus 87 (HRV87) 1:545–546
Human rhinovirus A (HRV-A) 4:468
Human rhinovirus B (HRV-B) 4:468
Human subacute sclerosing panencephalitis (SSPE) 2:4
Human T-cell leukemia virus 1 (HTLV-1) **2:558–564**, 5:198
basic leucine zipper protein 2:563
classification 2:558
evolution 2:561
genetics 2:560
clonal integration 2:566–567, 2:567*f*
stability 2:565
transcriptional regulation 2:213–214
genome 2:559, 2:560*f*
pX region 2:558, 2:560
regulatory factors 2:560
variability 2:561, 2:565
history 2:558, 2:564
receptor 2:559
replication 2:559, 2:560*f*
budding 2:561
integration 2:560
maturation 2:561
reverse transcription 2:560
splicing 2:560
transcription 2:560
Tax protein 2:562
cell cycle checkpoint attenuation 2:562
transcriptional activation 2:562
transcriptional repression 2:562
tumor suppressor protein inactivation 2:562
transmission 2:559
across species 2:561
blood transfusion 2:559, 2:564–565
breastfeeding 2:559, 2:563, 2:564–565
clustering 2:559
sexual transmission 2:559, 2:564–565
zoonosis 2:559
Human T-cell leukemia virus 1 infection 2:558, 2:559, **2:564–574**, 2:565*t*
adult T-cell leukemia 5:198
adult T-cell lymphoma 2:565, 5:198
cell morphology 5:198–199
characterization 5:198–199
classification 2:565
clinical features 2:566, 2:567
complications 2:567
cytological features 2:566
diagnosis criteria 2:565
epidemiology 2:565
immuno-virological features 2:566
pathogenesis 2:568
symptoms 5:198–199
therapy 2:569
associated cell types 2:559
associated myelopathy *see* Tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM)
cell transformation 2:559
T-cell transformation 5:198
control 2:563
epidemiology 2:564
incidence 2:564–565
geographical distribution 2:559
age-dependent infection 2:559
clustering 2:559
endemic areas 2:564
familial aggregation 2:559
host range 2:559
infective dermatitis 2:571
children 2:572
differential diagnosis 2:572
disease characterization 2:572, 2:572*f*
geographical distribution 2:571–572
history 2:571–572
occurrence 2:572
pathology 2:572
treatment 2:572
kerato-conjunctivitis 2:573
myositis 2:572
disease characterization 2:572
histology 2:572–573
musculo-skeletal symptoms 2:572–573
sporadic inclusion-myositis 2:573
Tax protein 2:572–573
pathogenicity 2:561
persistence 4:109*t*
prevention 2:563
tropical spastic paraparesis *see* Tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM)
uveitis 2:573
prognosis 2:573
symptoms 2:573
therapy 2:573
zoonoses 2:559
Human T-cell leukemia virus 2 (HTLV-2) 2:559
genetic stability 2:179
infection, persistence 4:109*t*
Human torovirus (HToV) 5:151–152
antigenic properties 5:155
buoyant densities 5:153
genotypes 5:155
geographical distribution 5:154
historical aspects 5:151
infection, diarrhea 5:156
Humoral immune system
Ectromelia virus infection 3:345
varicella-zoster virus infection 5:254
see also Antibodies; B cell(s); Cytokine(s); Immunoglobulin(s)
Humpty Doo virus 4:184*t*
Humulus japonicus latent virus (HJLV) 3:47
Hv145SV *see* Helminthosporium victoriae 145S virus (Hv145SV)
Hv190SV *see* Helminthosporium victoriae 190S virus (Hv190SV)
HVA *see* Herpesvirus Ateles (HVA)
Hv-p68 gene/protein
activity 5:171–172
overexpression 5:171, 5:171*f*
upregulation 5:171, 5:171*f*
HVS *see* Herpesvirus Saimiri (HVS)
Hyalomma marginatum, Wanowrie virus 1:400
Hyalomma ticks, Crimean-Congo hemorrhagic fever virus transmission 1:596, 1:597–598, 1:599*f*
Hybrid Capture 2 test, human papillomavirus screening 4:14
Hybridization
industrial-scale protein production 4:235
microarray design 2:16
Hybrid promoters, BacMam baculovirus expression vectors 1:245
Hydraencephaly (HE), definition 1:76, 1:328
Hydrangea ringspot virus (HdRSV) 4:311*t*
Hydropericardium 1:37, 1:328
Hydrophobin(s) 2:578
Hydropic, definition 1:427
Hydrothorax 1:37, 1:328
Hydroxyurea, EBV infection control 2:155–156
Hymenoptera 1:225–226
definition 2:37, 3:133
iflavirus infection 3:42
Hymenopteran parasitoids, iridovirus infection 3:166
Hypercalcemia, adult T-cell leukemia 2:567–568
Hyperendemicity 2:5
Dengue virus transmission 2:5, 2:10–11, 2:11*f*
Hypergammaglobulinemia
definition 4:594
SIV infection 4:601
Hypermutation, APOBEC3G 3:109
Hyperplasia
definition 1:301, 2:212, 3:155, 4:594, 5:423
papillomavirus-induced 4:31–32
Hypersensitivity reactions 4:173–174
Arabidopsis 4:174–175
bean common necrosis virus infection 1:292
cucumber mosaic virus 1:617
definition 4:170, 4:173, 5:375
potato virus Y potato infections 4:291, 4:292*f*
Rx genes 4:174
Sharka disease control 4:242
tobacco mosaic virus 4:173, 4:174*f*
Hyperthermophiles
definition 1:587, 5:411
optimum growth temperatures 1:591
Hypertrophy 1:301
Hyphae 2:574
Hyphal anastomosis
Cryphonectria parasitica virus transmission 2:576
definition 2:284, 4:68
Hv145SV transmission 5:169–170
Hv190SV transmission 5:169–170
mycoreovirus movement 3:380
Hypocritae jacobeae virus (HjV) 5:28*t*
Hypogammaglobulinemia, post-transplant 3:471
Hyposoter exiguae ichnovirus 4:257*f*
Hyposoter exiguae idnoreovirus 2 (HeIRV-2) 3:140
Hypotension 1:328
Hypothetical taxonomy units (HTUs) 4:126
Hypotonic hyporesponsive episode 5:226
Hypoviridae 2:111, 2:288, 2:581, 5:11*t*
Hypovirulence **2:574–580**
Cryphonectria parasitica virus 2:576
definition 2:284, 2:574–575, 2:580

development perturbation 2:575, 2:577
fungal viruses 2:285, 2:574
virulence perturbation 2:575
Hypovirus(es) **2:580–585**
anastomosis 2:584
biological control 2:584
transgenic hypovirulent strains 2:584
double stranded RNA satellites 4:531
fungal dsRNA viruses 2:111
gene expression 2:581
genetic organization 2:581, 2:582*f*
host range 2:585
infections, fungi 2:287*t*
replication 2:289
taxonomy 2:581
virulence attenuation 2:583
virus–host interactions 2:582
phenotypic changes 2:582
replication element mapping 2:583
symptom determinants 2:583
see also specific viruses
Hypovirus p29, RNA silencing 2:290
Hypovolemic shock, Argentine hemorrhagic fever 3:208
Hz-1V *see* Helicoverpa zea-1 virus (Hz-1V)
HZ-2v *see* Helicoverpa zea-2 virus (Hz-2V)

I

I-309 1:623–624
Iatrogenic transmission, definition 2:141, 2:146
ICAM-1, rhinovirus cell entry 4:470, 4:471
Ichneumonoid wasp(s), polydnavirus phylogeny 4:261
Ichnovirus(es) (IV)
definition 4:256
see also specific viruses
gene families 4:250–251
infection
host tissue 4:253
immunoevasive activity 4:255–256
immunosuppressive effects 4:255
insects 3:126*t*
phylogeny 1:192, 4:252*f*
replication 4:260–261
virion structure 4:257, 4:257*f*
see also specific viruses
Icosahedral, definition 1:533
Icosahedral array maturation 1:436, 1:437*f*
Icosahedral asymmetric unit (IAU), cowpea chlorotic mottle virus 5:385
Icosahedral dsDNA bacteriophages **3:1–6**
classification 3:1
enveloped *see* Icosahedral enveloped dsDNA bacteriophages
genomes 3:5
genomics 3:5
life cycle 3:3
DNA entry 3:4
genome replication 3:4
host cell lysis 3:4
particle assembly 3:4
receptor recognition 3:3
virion properties 3:1
virion structure 3:1
DNA 3:2
membrane 3:2
Icosahedral enveloped dsDNA bacteriophages **3:6–13**
applications 3:12
polymerase 3:12
carrier state 3:12
classification 3:7
genetic tools 3:12
reverse genetics 3:12
genome 3:9, 3:10*f*
nonstructural proteins 3:10
sequence identity 3:10
structural proteins 3:9
structure 3:6
genome delivery 5:366*f*, 5:369
lipopolysaccharide binding 5:369
pilus binding 5:369
spike proteins 5:369
historical aspects 3:6
host range 3:7
with internal membrane 5:368
nucleocapsid 3:7
assembly 3:8
physico-chemical properties 3:7
polymerase complex 3:7–8, 3:8*f*
conformational changes 3:8
RdRp 3:6, 3:7–8, 3:8*f*
replication 3:10, 3:10*f*
host cell attachment 3:11
host cell lysis 3:11
membrane acquisition 3:11
nucleocapsid maturation 3:11
outer membrane fusion 3:11
packaging 3:11
plasma membrane penetration 3:11
procapsid assembly 3:11
recombination 3:11
transcription 3:11
structural proteins 3:8, 3:9
P1 protein 3:7–8, 3:8*f*, 3:9*f*
P2 protein 3:8, 3:9*f*
P4 protein 3:7–8, 3:9*f*
P7 protein 3:7–8
P8 protein 3:8, 3:9*f*
structure 3:7, 3:7*f*
membrane envelope 3:8
in vitro assembly/packaging/replication 3:7, 3:12
see also specific viruses
Icosahedral particles
cryo-electron microscopy 1:605, 1:605*f*
P=3 5:391, 5:392*f*
T=1 5:381, 5:384*f*
T=3 5:385, 5:386*f*
T=4 5:385, 5:387*f*
T=7 icosahedral particles 5:388, 5:389*f*
T=13 5:382*f*, 5:388
T=25 5:390*f*, 5:391
Icosahedral ssDNA bacteriophages **3:13–20**
cell lysis 3:19
protein E 3:19
DNA
binding protein 3:19
packaging 3:19
replication 3:16
evolution 3:19
studies 3:19
gene expression 3:14*t*, 3:16
genome content 3:14
genome delivery 5:368
lipopolysaccharide-binding 5:368–369
spike complexes 5:368–369
history 3:13
host cell
attachment 3:15
penetration 3:15
recognition 3:15
hosts 5:368
morphogenesis 3:17
termination of 3:19
virion morphology 3:14, 3:14*f*
see also specific bacteriophages
see also specific viruses
Icosahedral ssRNA bacteriophages **3:21–30**
applications 3:28
capsid 3:22, 3:23*f*
conformations 3:22–24
maturation protein 3:24
gene expression control 3:24, 3:25*f*
maturation process 3:26
Min Jou sequence 3:26
RdRp 3:26
replicase 3:25*f*, 3:26
genome delivery 5:369
pilus binding 5:369–370
life cycle 3:22
assembly initiation 3:24
cell entry 3:24
coat protein–RNA complexes 3:24
RdRp 3:24
RdRp 3:24, 3:26
three-hybrid assay 3:28, 3:29*f*
conserved functional domains 3:28–29
nicotine vaccine 3:28–29
non-bacteriophage protein encapsidation 3:29
vaccine identification 3:28–29
translational repression/assembly initiation complex 3:23*f*, 3:26, 3:27*f*
coinfection 3:28
FG loops 3:28
mechanism 3:28
RNA-binding site 3:28
two-hybrid assay 3:29
Icosahedral symmetry 5:394
cryo-electron microscopy 1:604, 1:606
Icosahedral tailed dsDNA bacteriophages **3:30–37**
capsids 3:31, 3:31*f*, 3:33*f*, 3:35
stability 3:31–32
DNA injection 3:33
gene expression 3:34
early genes 3:34
gene classes 3:34
late genes 3:34
RNA polymerase 3:34
genomes 3:35
chromosome delivery 3:35
common ancestry 3:36
common structural themes 3:36
delivery 5:367
diversity 3:36
DNA concatamers 3:35–36
horizontal exchange 3:36
replication 3:35
historical aspects 3:30–31
host interaction 3:34
lysis 3:35
endolysin 3:35
Holin proteins 3:35
protein injection 3:34
replication 3:35
structure 3:31, 3:31*f*, 3:32*t*
electron microscopy 3:31
tails/tail fibers 3:32, 3:33*f*
binding to bacterium 3:32
long contractile tails 3:31, 3:31*f*, 5:366*f*, 5:367
long noncontractile tails 3:31, 3:31*f*, 5:367–368
short tails 3:31, 5:367, 5:368
temperate *vs.* virulent 3:33
virion assembly 3:34
capsid 3:35
pathways 3:34
procapsids 3:35
tail 3:34
Icosahedral vertex, bacterial cell viral entry 5:367
ICP0, HSV 2:385, 2:401
ICP22, HSV 2:385, 2:403–404
ICP27, HSV 2:400–401
Ictalurid herpesvirus 1 *see* Channel catfish virus (CCV)
Ictalurivirus 2:233, 2:431, 2:432*t*
Icterus, definition 2:350
ICTVdb 5:357*t*
ICTVNet 5:357*t*
Idaeovirus 3:37
Idaeovirus(es) **3:37–42**
geographical distribution 3:37
black raspberry isolates 3:37
resistance-breaking isolates 3:37
Scottish isolates 3:37
infection *see below*
nucleotide sequences 3:39
protein sequences 3:40
39kDa protein 3:40
190kDa protein 3:40
coat protein 3:40
transmission 3:38
viral relationships 3:41
virion composition 3:39
RNA 3:39
virion properties 3:39, 3:39*f*
see also specific viruses
Idaeovirus infections
control 3:41
detection 3:41

Idaeovirus infections (*continued*)
disease symptoms 3:38
experimentally infected plants 3:39
naturally infected plants 3:38
resistance genes 4:179*t*
therapy 3:41
Idnoreovirus 3:134, 3:134*t*, 4:536*t*
Idnoreovirus(es)
antigenic relationships 3:142
see also specific viruses
distribution 3:135
genetic relationships 3:142
genome 3:139, 3:139*t*
segments 3:139
host range 3:135
infection 3:135
replication 3:139
transmission 3:135
virion properties 3:134, 3:139
stability 3:139
structure 3:139
see also specific viruses
IE-1/IE-0 complex 1:261–262
IE1 protein, HCMV 1:640, 2:486
IE2 protein, HCMV 1:640, 2:486
IE62, varicella-zoster virus 5:259
IE63, varicella-zoster virus 5:261
IEDB (Immune Epitope Database and Analysis Resource) 5:357*t*
If1 bacteriophage 3:121
Iflavirus 1:90*f*, 3:42, 3:44, 3:44*f*
Iflavirus(es) **3:42–46**, 3:44*f*
capsid proteins 3:42, 3:43*f*
dicistroviruses *vs.* 3:44
host cell entry, role 3:43
maturation 3:43
VP4 3:43
characteristics 3:42, 3:42*f*
detection methods 3:45
genome 3:43, 3:43*t*
nonstructural proteins 3:43
polyprotein cleavage 3:43, 3:43*f*
infection
pathology 3:44
tissue tropism 3:44
replication 3:42*f*, 3:45
IRES function 3:45
translation 3:45
untranslated regions 3:44*f*, 3:45
VPg protein 3:45
transmission 3:44
see also specific viruses
Igbo-Ora virus 5:89
IKe bacteriophage, capsid helical symmetry 3:121
Ilarvirus 1:81, 1:387, 3:46, 4:205
Ilarvirus(es) **3:46–56**
coat protein
functional domain 3:52
genome activation role 3:49–52
RNA binding 3:52–53, 3:53*f*
virus structure 3:47
genome 3:47
host range 3:47, 3:55
infections *see below*
replication 3:49
conserved motifs 3:49–52, 3:52*f*
genome activation 3:49
poly A tail 3:52
RNA–coat protein complex 3:52
transcription 3:53–54
taxonomy 3:46
genus-distinguishing features 3:46
strains 3:48*t*
subgroups 3:47, 3:48*t*, 3:50*f*
transmission 3:54, 4:205
pollen-borne 3:54–55
virus structure 3:47, 3:50*f*
see also specific viruses
Ilarvirus infections
control 3:54
epidemiology 3:54
vegetative propagation 3:54
shock phase 3:47
sweetpotatoes 4:661*t*
ILTAB (International Laboratory for Tropical Agricultural Biotechnology) 5:357*t*
Iltovirus 2:406, 2:432*t*
Iltovirus infections 2:409
prevention 2:410
Imaging techniques
see specific techniques
Immediate early proteins (IE1), sawfly baculovirus 1:228
Immune adherence hemagglutination (IAHA), varicella-zoster virus 5:252
Immune clearance phase, chronic hepatitis B 2:357–358
Immune complexes 1:139
antibody concentration 3:414
conserved epitope binding 3:417–418
definition 3:78
dissociation constant 3:414–415
envelope glycoproteins 3:414–415
formation 3:79–80
occupancy 3:414
Immune evasion 2:144–145, 3:60, 3:66, 3:77
antigen processing interference 3:77
insects 4:255–256
interferons 3:107, 3:109
adaptive immune response consequences 3:109
invertebrate **4:250–256**
parasitoid attack 4:251
iridovirids 3:168–170
pattern recognition receptors 3:108
RIG-I pathway 3:108
RNA dependent protein kinase 3:108–109
T-cell epitope mutation 3:77
virus-mediated, RNA dependent protein kinase 3:108–109
see also specific viruses
Immune globulin, varicella-zoster virus 5:254
Immune homeostasis 3:71
Immune-mediated damage, Border disease 1:337
Immune potentiators, HIV vaccine development 1:75
Immune reconstitution inflammatory syndrome (IRIS) 1:52–53
opportunistic infections 1:52–53
Immune response 2:457
adaptive *see* Adaptive immune system
antibody-mediated *see* Antibodies; Humoral immune system
antigenicity, viral proteins 1:137, 3:62–64
cell-mediated *see* Cell-mediated immune response
definition 1:296
DNA vaccines 2:54
evasion *see* Immune evasion
gene therapy 2:303
heterotypic 1:583–584
innate *see* Innate immune system
insects 4:251, 4:255
modulation
antigen presentation 1:125
cowpox virus 1:576
KSHV latency 3:199
persistent infection 4:112
dysregulation 4:113
pathogen–host interactions 4:112
regulation 4:113
virus clearance *vs.* pathology 3:78, 3:79*f*
see also specific diseases/viral infections
Immune stimulator molecules, co-administration in DNA vaccination rephrase 2:54
Immunization
definition 5:226
hepatitis A 2:350
hepatitis B 2:341–342
hepatitis D virus coinfection 2:377
neonates 2:53
effectiveness 2:53
passive
definition 3:414
Ebola virus infection treatment 2:64
immunoglobulins 3:67
mucosal applications 3:67
post-exposure 3:67
pre-exposure 3:67
SARS 4:559
T-cell response 3:77
see also Inoculation; Vaccines/vaccination
Immunoassay(s)
principles 2:32
enzyme labels 2:32
solid-phase 2:32, 2:32*f*
sensitivity 2:33
specificity 2:33
see also specific assays
Immunoblotting, principles 2:33
Immunocapture polymerase chain reaction (IC-PCR) 1:408
Immunodeficiency
human papillomavirus infection 4:17
rhadinovirus human T-cell transformation 4:590
rotavirus infection 4:512
see also Immunosuppression; *specific diseases/disorders*
Immunodeficient vaccine-derived poliovirus (IVDPV) 4:248–249
Immunodominance
cell-mediated immunity 3:73, 3:73*f*
definition 3:71
pattern changes 3:73
Immunoelectron microscopy, benyvirus infection diagnosis 1:313
Immunoevasive ovarial protein(s) (IEP) 4:255–256
Immunofluorescent assays
Crimean-Congo hemorrhagic fever 1:602
Dengue virus 2:8–9
Japanese encephalitis diagnosis 3:186
principles 2:32*f*, 2:33
Immunogenicity **1:137–142**
definition 1:140
peptide conformation 1:140–141
Immunoglobulin(s)
affinity maturation 3:61
assembly 3:61
adaptive immunity 3:61
class switching
phylogeny 3:58
role 3:66*f*
complement activation 3:79–80, 3:79*f*
expression 3:79–80
hypermutation 3:61
immunomodulatory role 3:67
isotypes 3:63*f*
switching 3:66*f*
passive immunization 3:67
in clinical use 3:67
isolation 3:67
purification 3:67
phylogeny 3:58, 3:59*f*, 3:60*f*
agnathans 3:58, 3:60*f*
antibody-mediated immunity 3:58
somatic variation 3:58
production 1:621
sites 3:61
prophylaxis, varicella-zoster virus infection 5:255
receptor role 5:321
structure 3:62*f*
virus neutralization 3:79–80
antigen recognition 3:79–80
see also Antibodies; *specific types*
Immunoglobulin A (IgA)
common cold 4:472–473
distribution 3:64*f*
functions 3:64*f*
hepatitis A virus infection 2:12
mumps virus infection 3:362–363
rotavirus infection 4:512
structural organization 3:63*f*, 3:64*f*
subclasses 3:61
Immunoglobulin D (IgD)
distribution 3:64*f*
function 3:64*f*
structural organization 3:63*f*

Immunoglobulin E (IgE)
  distribution 3:64*f*
  function 3:64*f*
  structural organization 3:63*f*
Immunoglobulin G (IgG)
  common cold 4:472–473
  measles 3:289
  serological assays 2:30
    distribution 3:64*f*
    function 3:64*f*
    paratopes 1:138
    structural organization 3:63*f*
    see also specific viruses/infections
  SIV infection 4:600–601
Immunoglobulin M (IgM)
  alphavirus infection 5:87
  Dengue fever 2:12
  distribution 3:64*f*
  equine encephalitic togaviruses 5:82, 5:106
  function 3:64*f*
  hepatitis A 2:12
  measles 3:289
  rubella 4:520
  serological assays 2:30, 2:144
    complement fixation 2:31
    see also specific viruses/infections
  structural organization 3:63*f*
  West Nile virus 5:448
  yellow fever 5:475–476
Immunoglobulin Y (IgY) 3:58
Immunogold labeling 2:20
Immunohistochemistry
  emerging viral disease detection 2:94
  human respiratory syncytial virus infection 2:549
Immunological memory
  B cells 3:61
  definition 3:70, 3:71
  DNA vaccines 2:53
  latency 4:109–110
  persistent infection 4:109–110, 4:111*f*
  regulation 4:113
Immunomodulation
  avipoxvirus survival factors 2:276
  hepatitis B treatment 2:359
  persistent infection 4:113, 4:114*t*
    apoptosis 4:113
    cell stress 4:113
    TLR signaling 4:113
Immunopathology **3:78–83**
  associated host factors 3:81
    age 3:81
    genetics 3:82
    site of infection 3:82
  associated virus characteristics 3:81
    genetics 3:82
    viral titer 3:82
    virus strain 3:82
  cell-mediated immunity 3:76
  definition 3:71, 3:78
  infection 4:113
  Visna-Maedi virus infection 5:428
  see also specific viral infections
Immunoprecipitation
  papaya ringspot virus proteins 4:2
  RNP complexes 3:396
Immunosorbent electron microscopy (ISEM)
  definition 4:103
  plant virus infection diagnosis 2:19, 2:21*f*, 2:23*f*
Immunosorbent techniques, definition 2:18
Immunosuppression
  KSHV infection 3:190
  lymphoma 5:194
  measles 3:288
  organ transplantation, infections 3:466
  persistent infection 4:115
  reticuloendotheliosis virus infection 4:415–416
  TMEV infection 5:44
  see also Immunodeficiency; *specific viral infections*
IMNV *see* Infectious myonecrosis virus (IMNV)
Impatiens infections 4:214
  unknown 4:215, 4:218*f*
  see also specific viruses
Impatiens necrotic spot virus (INSV)
  Bacopa virus infections 4:215
  chrysanthemum infections 4:224
  dahlia infections 4:221–222
  geographical distribution 5:158
  host ranges 4:208
  Impatiens infections 4:214–215, 4:215*f*
  Osteospermum infection 4:221
Importins 5:299
INCB9471 2:507*t*
Incidence rate 1:59, 2:141, 2:142
Inclusion bodies 1:482
Inclusion body myositis, mumps 3:363
Inclusions
  potato virus Y 4:291
  tenuivirus infection 5:24, 5:25*f*
Incubation, Crimean-Congo hemorrhagic fever 1:601
Incubation, extrinsic 2:5
Incubation period
  chickenpox 5:253
  common cold 4:472
  definition 1:482
  Ectromelia virus 3:343
  hepatitis C 2:368
  Japanese encephalitis 3:185–186
Indexing, definition 4:659
India, HIV infection 1:64
Indian cassava mosaic virus (ICMV) 1:31–32
Indian peanut clump virus (IPCV) 1:494, 4:98
  genome 4:99
    open reading frames 4:101, 4:101*t*
    peanut clump virus *vs.* 4:101
    RNA2 4:101
    sequence comparisons 4:101
  geographical distribution 4:98
  infection
    diagnosis 4:98
    serological diagnosis 4:98–99
  reservoirs 4:98
  transmission 4:98–99
  virus particles 4:99
Indinavir 1:143*t*
  HAART-associated renal disease 1:56–57
Indirect contact transmission 2:145
Inducible NADPH-dependent nitric oxide synthase 4:387–388
Infected midgut cell shedding, baculovirus infection resistance 1:267
Infection(s)
  abortive 4:110–111
  acute
    definition 2:146
    diagnosis 2:30, 2:30*f*
    T-cell memory 3:75*f*, 3:76
  control 5:493
  enteric 2:146
  immune response phases 3:61*f*
  immune system regulation 4:113
  immunomodulation 4:113
  immunopathology 4:113
  localization 5:316
    cellular susceptibility 5:316
    regulation 5:316
  organ transplantation *see* Organ transplantation, infection risks
  persistent
    adaptive immunity 4:112
    antibodies 4:112
    cell-mediated immunity 3:73, 3:73*f*, 3:75*f*, 3:76
    definition 1:374, 4:514
    virus perpetuation 2:146
  risk 2:141
  spread 5:315
    blood–tissue barrier 5:316
    neural 5:316
  subclinical 2:146
  systemic 4:514
  see also specific infections
  see also specific viruses
Infection neutralization **3:413–419**
  antibody–antigen binding 3:414
    antibody concentration 3:414
    dissociation constant 3:414–415
    envelope glycoproteins 3:414–415
    occupancy 3:414
  antigens 3:414
  definition 3:413–414
  experimental measurement 3:414
  glycan shields 3:418–419
  kinetics 3:416
  mechanism 3:64–65, 3:415
  molecularity 3:416
  resistance 3:417
  tissue culture 3:418
  virion epitope occupancy 3:414
  virion surface area–Ab relationship 3:417
  virus escape 3:417
Infectious bronchitis virus (IBV)
  gene expression 1:551*f*
  infection
    associated disease 1:553
    host range 1:553
    persistent infection 1:553
    tissue targets 1:553
  structure 1:550*f*
  virion structure 1:549, 1:550*f*
Infectious bursal disease virus (IBDV)
  infection 1:326
    immunosuppression 1:326–327
    mortality rate 1:326–327
    very virulent strains 1:326–327
  structure 1:323*f*
    pVP2 1:324–325
    VP2 1:324
    VP3 1:325
    VP4 1:325
    VP5 1:325
  VP2 gene/protein
    base domain 1:324
    projection domain 1:324
    shell domain 1:324
Infectious clone(s)
  definition 1:549, 4:514
  genetically modified coronaviruses 1:553
Infectious degradation, grapevine disease, transmission 4:203
Infectious DNA clones
  Grapevine fanleaf virus 3:408–409
  nepovirus 3:408–409
Infectious flacherie virus (IFV)
  classification 5:2–3
  genome 3:45, 5:3
Infectious hematopoietic necrosis virus (IHNV) 2:228, 4:184*t*
  economic effects 2:228
  genome 2:223–224, 2:224*f*, 3:326*f*
  reverse genetic analysis 2:228
  salmonid infection 2:221–222
Infectious hypodermal and hematopoietic necrosis virus (IHHNV)
  genome 4:571–572, 4:572*f*
  geographical distribution 4:572
  host range 4:572
  infection 4:571
  taxonomy 4:572
  virion structure 4:78–79, 4:571
Infectious laryngotracheitis (ILT)
  antibody detection 2:410
  clinical features 2:406, 2:409
    incubation period 2:409
    symptom severity 2:409
  pathogenesis 2:409
  pathology 2:409
Infectious laryngotracheitis (ILT) virus
  cytopathology 2:407
  genetics 2:408
  history 2:406–407

Infectious laryngotracheitis (ILT) virus (*continued*)
host range 2:407
latency 2:409
morphology 2:408
propagation 2:407
reactivation 2:409
vaccination 2:406
Infectious mononucleosis (IM) 2:151, 2:153
immune response 2:153
lymphocytosis 2:153
incidence 2:153
measles *vs.* 3:289–290
see also Epstein–Barr virus infection
Infectious myonecrosis virus (IMNV) 4:572
genome 4:573, 4:573*f*, 5:167
geographical distribution 4:573
host range 4:573
infection 5:164, 5:169
properties 4:572
taxonomy 4:573, 5:164
Infectious pancreatic necrosis virus (IPNV) 2:232, **3:83–89**, 3:85*f*
genome 3:84, 3:86*f*
segment A 3:84
segment B 3:84, 3:86*f*
host cell attachment/entry 3:84–86
infection *see below*
phylogenetic analysis 3:84, 3:85*f*
prevalence 3:88
propagation 3:84
structure 3:84, 3:86*f*
antigens 3:84, 3:86*t*
neutralization sites 3:84
VP1 3:84–86
VP2 1:324, 3:84
VP3 3:84
VP4 1:325, 3:84
VP5 1:325
transmission 2:232, 3:88
vaccines/vaccination 2:233, 3:88
virulence determinants 3:87, 3:88*t*
Infectious pancreatic necrosis virus infection 1:327
acute 3:86
characteristics 3:86
histopathology 3:86
challenge models 3:88
clinical manifestations 1:327, 3:83
control
breeding 3:89
management 3:88
vaccination 3:88
cytopathic effects 3:84
disease patterns 3:83
epidemiology 3:88
mortality 1:327, 3:83, 3:87
persistent 3:86
carrier fish 3:87
virus location 3:87
serological 3:84
serotype Sp 3:87
Infectious pseudo-recombinants 1:296
Infectious pustular balanoposthitis 1:362–363
Infectious pustular vulvovaginitis (IPV) 1:362–363
Infectious salmon anemia virus (ISAV) 2:229, **3:89–95**
detection 3:91
economic impacts 2:227
genetics 3:91, 3:92*t*
cap-stealing 3:93
groups 3:93
hemagglutinin-esterase gene 3:93
highly polymorphic region 3:93
reassortment 3:93
transcription 3:93
virulence 3:93
genome 2:229, 3:90
comparative analysis 2:229
encoded proteins 3:91, 3:92*f*
overlapping reading frames 3:91
geographical distribution 3:90
history 3:90
host range 3:90
reservoirs 3:91
infection 2:229
clinical features 3:90, 3:94
control 3:95
diagnosis 3:95
disease course 3:93
hemorrhagic kidney syndrome 3:94
histopathology 3:94
immune response 3:95
incubation period 3:93
mortality 3:90, 3:93
pathogenesis 3:93
pathology 3:94
prevention 3:95
shedding 3:91
target cells 3:94
infectivity 3:91
replication 3:90
structure 3:90, 3:90*f*
surface glycoproteins 3:90
target cells 3:90
transmission 3:91
vertical 3:91
vaccines/vaccination 3:95
vectors 3:91
sea louse 3:91
Infectious spleen and kidney necrosis virus (ISKNV) 2:233
Infective dermatitis, HTLV-1 associated *see* Human T-cell leukemia virus 1 infection
Infectivity
Aedes pseudoscutellaris dinovernavirus 3:140
seadornaviruses 4:538–539
Infectivity factors, nucleopolyhedrovirus primary infection 1:266–267
Inferred from Electronic Annotation (IEA) 5:353
Inflammasome(s) 1:620, 1:621–622
Inflammation
HSV infection 2:393
measles 3:289
MHV-68 infection 3:375
necrotizing, eye infections 2:492
yellow head virus infection 5:480
Influenza 2:554
antiviral therapy 1:145, 1:153, 3:102
amantadine 3:102
channel-blocking compounds 1:148
resistant variants 1:148
Asian pandemic (1957) 3:100
mortality 3:100
control 5:491
disease 3:96
epidemics 3:96
HIV *vs.* 3:96
symptoms 3:96
disease surveillance 2:50
H5N1 pandemic 2:554, 3:100
avian influenza viruses 3:100, 3:493, 5:491
human infections 3:100
mortality rate 3:100
transmission 3:100
vaccination development 5:241–242
hemagglutination inhibition tests 2:31
human 3:100
replication 3:101
immunity 3:101
ocular diseases 2:492
pandemics 2:97, 3:100
associated pig infections 3:101
avian 3:493
bioterrorism 5:410
H1N1 pandemic 3:100, 5:491
H2N2 pandemic 3:100
H3N2 pandemic 3:100
H5N1 *see above*
pigs 3:101
symptoms 2:554
zoonoses 5:491
Influenza A virus
antigen structure variation 1:128
antigenic drift 3:100
sequence identity 3:96–97, 3:493
subtypes 1:128, 3:96, 3:489, 3:493, 5:491
birds 3:101
replication 3:101
evolution 2:180*f*
genome 3:99*f*, 3:483–484, 3:485
associated genes/proteins 3:485*t*
hemagglutinin 3:485, 3:490
function 3:485
neuraminidase ratio 3:97
post-translational modifications 3:485
host range 3:96–97
natural host 3:101
immune evasion, NS1 protein 3:109
infection 2:554
pandemics 3:96, 3:100
matrix proteins 3:485
neuraminidase
hemagglutinin ratio 3:97
inhibitors of 3:493
structure 3:493
replication
maturation 4:411–412
uncoating 4:409
sialic acid 5:320
vaccines/vaccination 3:103
development 5:241
development strategies 5:241
NS1 gene 5:238
see also Influenza
Influenza B virus 2:554
antigenic variation 3:96–97
genome 3:483–484
hemagglutinin 3:490
NB protein 3:97–98
neuraminidase 3:492
vaccination 3:103
Influenza C virus
antigenic variation 3:96–97
genome 3:483–484
noncoding sequences 3:484
RNP complex 3:484
hemagglutinin-esterase-fusion 3:484, 3:492
matrix proteins 3:485
Influenza Genome Sequencing Project 5:357*t*
Influenza Sequence Database 5:357*t*
Influenza vaccine(s) 2:458, 2:557, 3:103
antigenic variation, H2 displacement 5:240
inactivated 3:103
manufacture 3:103
intranasal, Bell's palsy *vs.* 5:234
live-attenuated virus 3:103
cold-adapted 3:103, 5:241–242
efficacy 3:103
production timescale 5:241–242, 5:241*f*
reactions to 5:230*t*
safety 5:228
strain selection 5:241–242
Influenza virus(es) **3:483–489**
antibody binding 3:493
antigenic drift 1:128, 1:131, 1:131*f*, 3:100, 3:493
A/Aichi/68 HA1 1:131–132
antigenic sites 1:131*f*
mechanism 1:128, 1:131
neuraminidase 1:128, 1:132, 1:132*f*
Type A influenza viruses 1:128
antigenic shift 1:128, 1:129*f*, 3:101
Type A influenza viruses 1:128, 1:129*f*
antigenic variation 1:128, 1:131*f*, 3:493
antigenic drift *see above*
antigenic shift *see above*
epitopes 1:129, 1:131*f*
H3 variants 1:131–132
neuraminidase 1:128, 1:132*f*
neutralization mechanism 1:129
antigens **3:489–495**
see also Hemagglutinin (HA); Neuraminidase (NA)
apoptosis inhibition 1:158*t*

avian *see* Avian influenza virus(es)
classification 3:483, 3:489
evolution 3:98
mutation rates 1:150, 3:98–99
genetic reassortment 3:99
genome 3:97–98, 3:484
RNP 3:97
H5N1 3:491
H7N7 2:554
hemagglutinin *see below*
host range
animal 3:101
molecular determinants 3:101
isolation 3:96
matrix proteins 3:485
neuraminidase *see below*
neutralization 1:129
epitopes 1:129, 1:131*f*
mechanism 1:129
nomenclature 3:96, 3:483, 3:489–490
NP protein 3:485
NS1 protein 3:102
function 3:102
immune system evasion 3:109
viral pathogenicity 3:102
NS proteins 3:485–486
PB2 protein 3:102
mRNA cap binding 3:102
virulence determinance 3:102
physical properties 3:484
propagation 3:96
protein properties 3:484
recombination 3:99
release 3:487
NA function 3:488
replication 3:486
assembly 3:487
host cell attachment 3:486, 3:486*f*
host cell entry 3:486
RNA synthesis 3:487
sites 3:96
transcription 3:487
reverse genetics 3:99, 3:488, 3:488*f*
vaccines 3:488
virus reconstruction 3:488
structure 3:96, 3:97*f*, 3:98*f*
matrix protein function 3:97–98
nonstructural proteins 3:97–98
receptor binding site 1:203
taxonomy 3:96
tissue tropism 3:96
transmission 3:96
virion composition 3:484
virion packaging 3:487
associated proteins 3:487
random incorporation model 3:487–488
selective incorporation model 3:487–488
virion structure 3:484, 3:484*f*
see also individual types
Influenzavirus A 3:96
Influenzavirus B 3:96
Influenzavirus C 3:96
Influenza virus hemagglutinin
Ab-mediated neutralization 3:415
antigenic variation 1:139*f*, 3:418, 3:493
amino acid substitutions 3:493
glycosylation 3:494
H3 HA subtype 1:128, 3:493
cleavage 3:101
definition 3:489
evolution 3:98–99
function 3:485, 3:490
influenza A virus 3:490
receptor binding activity 3:97–98
influenza B virus 3:490
mutation rates 3:98–99, 3:100
receptor specificity 3:102
structure 3:293, 3:490, 3:491*f*, 3:493
antibody complexes 3:494, 3:494*f*
HA0 3:294, 3:294*f*, 3:491
HA1 3:101–102, 3:293
HA2 3:101–102, 3:294, 3:295*f*, 3:491, 3:492*f*
pH effects 3:491, 3:492*f*
receptor binding domain 3:490
sialic acid 3:486, 3:490–491
synthesis 3:490
post-translational modifications 3:485, 3:490
Influenza virus neuraminidase 3:102, 3:485, 4:412
active site 3:492
antigenic drift 1:128, 1:132, 1:132*f*
definition 3:489
enzymatic activity 3:488, 5:320
influenza A virus 3:492
receptor destroying activity 3:97–98
influenza B virus 3:492
inhibitors 1:149, 3:102, 3:488
resistant variants 3:103
mutation rates 3:100
structure 3:492, 3:493*f*
antibody complexes 3:494*f*
sialic acid 3:492
synthesis 3:492
WSN strain 3:102
Influenza Virus Resources 5:357*t*
'In-group' phylogenetic analysis 4:127
inhibitor of apoptosis (iap) genes 2:139–140
Inhibitors of apoptosis (IAPs) 1:160–161
baculovirus *see* Baculovirus apoptosis inhibition
chicken 4:418
definition 1:154
granulovirus gene expression 1:214, 1:216–217
sawfly baculovirus genome 1:226
50% Inhibitory concentration (IC_{50}), antiviral therapy 1:146
Initiator caspases, definition 1:231
Innate immune system 3:58, **3:104–111**, **3:111–117**
antiviral effector protein targeting 3:108, 3:109
cellular responses 3:112
dendritic cells 3:112
granulocytes 3:112
macrophages 3:112
monocytes 3:112
natural killer cells 3:110, 3:112
clearance 4:114
complement *see* Complement
control genes 3:286
cytidine deaminases 3:109
definition 1:121, 3:78, 3:111
henipavirus infections 2:326
hepatitis C 2:373
interferons *see* Interferon(s)
pathogen-associated molecular patterns 3:106
pattern-recognition receptors 3:106, 3:107
persistent infection 4:112
PRR component targeting 3:108
RNA interference *see* RNA interference (RNAi)
Innovator, EHV-4 treatment 2:419
Inoculation
definition 1:296
routes 3:342–343
see also Immunization; Vaccines/vaccination
Inoculation access period (IAP) 3:368
Inoculative release (insect pest control) 3:125
Inoviridae 2:191, 3:117–118, 5:11*t*
Inovirus 2:191, 2:192*t*, 3:117–118
Inovirus(es) **3:117–125**
capsid helical symmetry 3:121
definition 3:117
discovery 3:117
DNA conformations 3:118*f*, 3:121
ecology 3:117
evolution 5:374
genome 3:121
delivery 5:369
packaging 2:306
host range 3:117
life cycles 3:122
assembly 3:123
bacteriophage attachment 3:122
DNA penetration 3:122
DNA replication 3:122
export 3:123
gene expression 3:122
prophage carrier states 3:123
virion extrusion 3:122
morphology 3:118, 3:118*f*
flexibility 3:118–119
length 3:118–119
protein subunit conformation 3:120
structure 3:119
coat proteins 3:119
DNA content 3:119
helical arrays 1:438
virion stoichiometry 3:121
see also Filamentous ssDNA bacteriophages
Insect(s)
associated viruses 3:126
control of *see* Insect pest control
crop losses 3:125
infections, insects 3:126*t*
innate immune response 3:149
parasitoid attack
immune response 4:251
polydnavirus immune evasion 4:255
pierce-sucking 5:274, 5:275–276
polydnavirus infection 4:253
virus-mediated immune abrogation 4:253
RNA viruses 5:3
vectors *see* Insect vectors
viruses
see specific viruses
Insect cell culture
culture media 1:238
growth temperature range 1:238
Insecticides 2:454
barley yellow dwarf virus control 1:285–286
biological control *vs.* 3:129–130
cucumber mosaic virus control 1:619
Indian tomato leaf curl viruses 5:133
maize streak virus control 1:481, 3:270
Rift Valley fever virus control 4:496
watermelon mosaic virus infection 5:437
zucchini yellow mosaic virus infection 5:437
Insect pest control **3:125–133**
Anticarsia gemmatalis multiple nucleopolyhedrovirus 3:130–131
chemical *vs.* biological control 3:129–130
DNA viruses 3:127
baculoviruses *see* Baculovirus(es)
densoviruses 3:127, 4:79
entomopoxviruses 3:128
Oryctes rhinoceros virus 3:127
host specificity 3:125–126, 3:129
RNA viruses 3:127
reoviruses 3:127
tetraviruses 3:127
strategies 3:125
biological control 3:125
conservation 3:125
inoculative release 3:125
inundation 3:125–126
Insect reovirus(es) **3:133–144**
antigenic relationships 3:141
distribution 3:135
genetic relationships 3:141
genome 3:136
history 3:135
host range 3:135
infection 3:135
replication 3:136
taxonomy 3:134*t*
transmission 3:135
virion properties 3:136
see also specific viruses
Insect repellents, Crimean-Congo hemorrhagic fever prevention 1:602–603
Insect retrovirus(es) **4:451–455**
envelope fusion protein 4:451
cellular homolog 4:454
LTR retroelements, classification 4:451
see also LTR-retroelements

Insect retrovirus(es) (*continued*)
origin 4:451, 4:452*f*
retroelements 4:451
see also specific viruses
Insect vectors 2:458
capripoxvirus 1:429–430, 1:432
hematophagous
definition 1:170
orbivirus transmission 3:457
luteovirus 3:237–238
potato virus Y transmission 4:289, 4:289*f*
role in viral zoonoses 2:147
virus replication 2:147
see also individual species
Insect virus(es)
nonoccluded *see* Nonoccluded insect virus(es) (NOIV), unassigned;
see specific viruses
Insertional mutagenesis
feline leukemia viruses 2:188, 2:189*t*
fungal retrotransposons 4:427
gene therapy 2:302
mouse mammary tumor virus infection 3:339
retroviral integration 2:105
Insertion sequences, definition 5:411
Instar, definition 2:234
Institute of Medicine of the National Academy of Sciences, USA 2:93
INSV *see* Impatiens necrotic spot virus (INSV)
IntAct (Protein Interactions) 5:357*t*
Integrase(s) 5:411
attachment site, fuselloviruses 2:299
definition 4:428, 5:411
genome integration, host cell 1:592–593, 2:510
inhibitors 2:507*t*, 2:510
LTR retrotransposons 4:438–439
metaviruses 3:306, 3:307–309
mouse mammary tumor virus 3:335
pseudoviruses 4:354–355
Simian retrovirus D 4:627
Integrase host factor, bacteriophage λ genome packaging 2:309
Integrated pest management (IPM) schemes 3:125
Integrated reticuloendotheliosis sequences, avipoxvirus variants 2:283
Integrated segment replication, dsDNA bacteriophage genome packaging 2:308–309
Integration 3:301
Integration sites, mouse mammary tumor virus infection 3:339–340
Integrative bacteriophage, definition 3:117
Integrin(s), viral cell entry
hantaviruses 1:395
HPV 4:20
KSHV 3:197
rotaviruses 4:510
see also specific types
Integrin $\alpha_2\beta_1$, echovirus 1 replication 2:67
Integrin $\alpha_3\beta_1$, KSHV host cell entry 3:197
Integrin $\alpha_6\beta_4$, HPV replication 4:20
Integrin $\alpha_v\beta_3$, foot-and-mouth disease virus-host cell interactions 5:321, 5:324
Integrin β_1, mammalian reoviruses, host cell entry 4:387
Intelence® 2:510
Intentional infection
HIV 5:407
see also Bioterrorism
Intercellular adhesion molecule 1 (ICAM-1)
coxsackievirus attachment 1:543–544
rhinovirus infection 1:546–547
Intercellular movement (plant virus(es)) 3:348
Interference, antigen processing 3:77
Interfering RNAs *see* RNA interference (RNAi)
Interferon(s) 3:105, 3:105*f*
activation 3:105–106
adenosine deaminase 1 3:115–116
antiviral protein induction 3:107
definition 3:104, 3:111, 5:117
interference/evasion 3:107, 3:109
component targeting 3:108, 3:109
henipavirus 2:323, 2:326
influenza infection 3:102
paramyxovirus 3:108
porcine circovirus 1:516
mammalian reovirus infection 4:390
murine CMV infection 1:632
persistent infection 4:112
production signals 3:107
signaling 3:107
therapy
filovirus infection 2:205
hepatitis B 2:342, 2:364
hepatitis delta virus treatment 2:377
phlebovirus infection 4:495–496
rhinovirus infection 2:556–557
side effects 3:116
treatable diseases 3:116
togaviruses 5:123
type I *see below*
type II 3:429–430
varicella-zoster virus infection 5:255
VSV infection 5:298
see also specific types
Interferon(s), type I 3:112
arterivirus infection 3:429–430
coronavirus infection 3:429–430
definition 3:104
direct antiviral effects 3:115
filovirus infection treatment 2:205
indirect antiviral effects 3:116
induction 3:113, 3:113*f*
IRF-7 3:114–115
pattern recognition receptors 3:113
rhinovirus infection, immune response 1:547–548
signaling 3:115
defects 3:112
JAK–STAT pathway 3:115
Visna-Maedi infection 5:430
see also Interferon-α (IFN-α)
Interferon-α (IFN-α)
lymphocytic choriomeningitis virus infection 3:242
togavirus infection 5:80, 5:123
Interferon-β (IFN-β)
gene expression 3:114–115, 3:114*f*
hepatitis A 2:349
hepatitis C 2:373
togavirus infection 5:80, 5:123
Interferon-γ (IFN-γ) 1:621
active form 1:621
arterivirus infection 3:430
Ectromelia virus infection 3:345–346
function 1:621
hepatitis A 2:349
receptors 1:621
Interferon regulatory factor-3 (IRF-3) 3:107
Interferon regulatory factor-4 (IRF-4) 4:417–418
Interferon regulatory factor-7 (IRF-7) 3:107, 3:114–115
Interferon stimulated gene(s) (ISG)
definition 5:117
togavirus infection 5:123
Intergenic region(s) (IGR)
arenavirus genome 3:243, 3:244, 3:244*f*
banana bunchy top virus 1:274
definition 2:37, 5:157
dicistrovirus genome 2:41*f*
Pepino mosaic virus 4:105, 4:106
Interleukin-1 (IL-1) 1:621–622
receptors 1:621–622
Interleukin-2 (IL-2) 1:622
receptors 1:622
signal transduction 1:622
Interleukin-4 (IL-4) 1:622
cellular effects 1:622
Ectromelia virus infection 3:345–346
Interleukin-5 (IL-5) 1:622
Interleukin-6 (IL-6) 1:622
KSHV homolog 3:193–194, 3:200
Interleukin-7 (IL-7) 1:622
receptor 1:622
Interleukin-8 (IL-8), Dengue fever 2:13
Interleukin-10 (IL-10) 1:622–623
parapoxviruses 4:62
receptor 1:622–623
Interleukin-12 (IL-12) 1:623
function 1:620
receptor 1:623
Interleukin-13 (IL-13), cellular effects 1:622
Interleukin-15 (IL-15) 1:623
Interleukin-17 (IL-17) 1:623
Interleukin-18 (IL-18) 1:622
Interleukin-26 (IL-26) 4:591
Intermediate subviral particles (ISVPs) 4:386
Internal ribosome entry sites (IRES)
Border disease virus 1:339
classical swine fever virus 1:526
definition 1:541, 3:42, 4:129, 5:1
echoviruses 2:67
enteroviruses 2:126
picornaviruses *see* Picornavirus(es)
rhinovirus genome 4:469
swine vesicular disease virus 2:127
Internal sequence (INT), LTR retrotransposons 4:438
International Agricultural Research Institutes (IARCs) 3:219
International Catalog of Arboviruses Including Certain Viruses of Vertebrates 1:170
International Code for Virus Classification and Nomenclature 5:10–19
International Committee on Taxonomy of Viruses (ICTV) 5:9, 5:362
database 5:10–19
Eighth ICTV Report on Virus Taxonomy 5:10
historical aspects 5:10
organization 5:10
species, definition 5:403–404
structure 5:10
subcommittees 5:10–19
taxonomic proposals 5:10
International Working Group on Legume Viruses (IWGLV) 3:213
International Working Group on Vegetable Viruses, merger 3:213–214
InterPro 5:357*t*
Intracellular enveloped virus (IEV) 4:328
Intracellular mature virus (IMV)
avipoxviruses *see* Avipoxvirus(es)
cowpox virion types 1:575
vaccinia virus *see* Vaccinia virus
variola virus replication 4:639
Intracellular movement
cytoskeleton role 3:352
definition 3:348
Intracytoplasmic A particles, mouse mammary tumor virus replication 3:336–337
Intracytoplasmic inclusion bodies, Ectromelia virus infection 3:343, 3:344*f*
Intraepithelial neoplasia, papillomaviruses 4:16
Intranasal infection, Borna disease virus 1:344–345
Intranasal inoculation, Ectromelia virus infection lesions 3:345
Intraserotypic recombination, Dengue viruses 2:8
Intraocular infection 2:491
Intraperitoneal inoculation, Ectromelia virus infection lesions 3:345
Intraspecies barrier(s), fungal virus recognition 2:289
Intra-species differentiation, avipoxviruses 2:281
Introgression 5:138
Introns, group I
catalytic core 4:478–479
mimivirus gene content 3:317
ribozyme function 4:475
structure 4:475
Intussusception, definition 4:507
Inundation (insect pest control) 3:125–126
Invertebrate iridescent virus(es) (IIVs) 3:161
taxonomy 3:168
Invertebrate iridescent virus-1 (IIV-1) 3:161

Invertebrate iridescent virus 3 (IIV-3)
genome 3:164
unique genes 3:164
infection 3:166*f*
structure 3:161
Invertebrate iridescent virus-6 (IIV-6) 3:161
genome 3:164, 3:165*f*
ribonucleotide reductase 3:164
genome sequencing 2:233
infection 3:165
structure 3:162–163, 3:163*f*
Invertebrate iridovirus(es) **3:161–167**
classification 3:161, 3:162*t*
tentative species 3:161
ecology 3:166
genotypic variation 3:166
seasonal transmission 3:166
evolution 3:164
genome 3:163, 3:165*f*
circular permutation 3:163–164
core genes 3:164
methylation levels 3:164
organization 3:165*f*
putative genes 3:164
repetitive DNA 3:164
geographical distribution 3:161
history 3:161
infections *see below*
propagation 3:162
replication 3:164
structure 3:161, 3:162–163, 3:163*f*
capsid 3:162–163
capsomers 3:162–163
core 3:162–163
transmission 3:166
virion properties 3:162
see also specific viruses
Invertebrate iridovirus infections 3:166*f*
cell–cell fusion 3:166
covert infection 3:161
detection 3:161
insects affected 3:165
disease characteristics 3:164
disease signs 3:164
economic importance 3:167
host range 3:162
patent disease 3:161, 3:166
pathology 3:165
route 3:162
tissue tropism 3:165–166
Inverted terminal repeats (ITRs), Crenarchaeal viruses 1:593
Invirase® 1:143*t*
in vitro assembly system, alphavirus 1:196
in vitro cell tropism, Simian retrovirus D 4:628
in vitro evolution, gene therapy viral vectors 2:304
In vitro polymerase assays, leishmaniaviruses 3:221
in vitro replication, Providence virus 5:32
in vitro translation, cowpea mosaic virus genome 1:571
in vivo cell tropism, Simian retrovirus D 4:626*f*, 4:628
Iontophoresis, definition 2:436
IPCV *see* Indian peanut clump virus (IPCV)
IPNV induced apoptosis 3:86
Ipomoea batacus (sweetpotato(es)) *see* Sweetpotato *(Ipomoea)*
Ipomoea crinkle leaf curl virus (ICLCV) 4:666
Ipomoea vein mosaic virus 4:665
Ipomoea yellow vein virus (IYVV) 4:666
Ipomovirus 4:315*t*
IR2 protein, equid herpesvirus 1 replication 2:415
IR3 gene, equid herpesvirus 1 replication 2:415
IR4 protein, equid herpesvirus 1 replication 2:415
Iranian wheat stripe virus (IWSV) 5:24
I resistance gene
bean common mosaic virus 1:292
bean common necrosis virus 1:293
IRF-3, innate immunity 3:107
IRF-7, innate immunity 3:107
IR gene(s), equid herpesvirus 1 2:414
Iridocyclitis 2:491
Irido-like virus(es)
shellfish viruses *see* Shellfish virus(es); *see specific viruses*
Iridovirid(s)
associated proteins 3:168–170
immune evasion 3:168–170
genome 3:168
coding potential 3:169*t*
DNA repeats 3:170
inversions 3:168–170
open reading frames 3:168–170
size 3:168–170
host range 3:170
replication **3:167–174**
taxonomy 3:167
MCP analyses 3:170
phylogenetic trees 3:169*f*
virion structure 3:167
Iridoviridae 1:50, 2:233, 3:158*t*, 3:167, 3:168*t*
Iridovirus 2:233, 3:155, 3:161, 3:168*t*
Iridovirus(es) **3:155–161**
apoptosis, effects on 3:173
apoptosis inhibition 1:158*t*
control 3:159
emerging pathogens 3:159
fish *see* Fish iridovirus(es)
genome 3:168
geographical distribution 3:156
historical aspects 3:155–156
host range 3:156
infections
clinical features 3:156
disease characteristics 3:164–165
effects on host cell 3:173
insects 3:126*t*
pathology 3:156
invertebrates *see* Invertebrate iridovirus(es)
phylogeny 1:192
replication **3:167–174**
DNA methylation 3:170
DNA synthesis 3:170
RNA synthesis, early 3:170
RNA synthesis, late 3:171
virion assembly 3:172
virus entry 3:170
structure 3:157*f*
transmission 3:159
virion structure 3:167
capsid 3:167
see also specific viruses
Iris diaphragm 2:491
IR-PTGS 4:142–145
post-transcriptional gene silencing 4:142–145
Isavirus 3:96
ISEM *see* Immunosorbent electron microscopy (ISEM)
Isogenic line, definition 2:109
Isolates, potato virus Y potato infections 4:291
Isolation
banana bunchy top virus 1:277
cacao swollen shoot virus infection 1:409
varicella-zoster virus infection 5:255
Isometric, definition 1:296
Israel turkey meningoencephalomyelitis virus 2:237
mosquito-borne 2:237
Iteravirus 4:76, 4:83, 4:96
Iteravirus(es)
classification 4:94*t*
see also specific viruses
genome 4:76–77, 4:95*f*
monosense genomes 4:83
virion structure 4:78
see also specific viruses
IUMS-Virology 5:357*t*
Ivanovsky, Dmitri 5:54
virus discovery 3:398–399
IVDB (Influenza Virus Database) 5:357*t*
Ivory Coast Ebola virus (ICEBOV) 2:199
Ixodid, definition 2:234

J

Ja.1 virus 5:413*t*
Jaagsiekte sheep retrovirus (JSRV) **3:175–182**
cell transformation 3:180
Env-induced transformation 3:181
oncogene activation 3:180–181
phosphatidylinositol 3-kinase docking 3:181
proto-oncogene transduction 3:180–181
classification 3:175
endogenous 3:178
Env protein 3:179
expression regulation 3:178–179
JSRV homology 3:178
long terminal repeats 3:179
replication defects 3:179
tissue tropism 3:178
genome 3:177*f*
env gene 3:176
gag gene 3:175–176
noncoding regions 3:176
orf-x 3:176
pol gene 3:175–176
pro gene 3:175–176
sequence variability 3:176
history 3:175
oncogenesis 2:219
ovine pulmonary adenocarcinoma *see* Ovine pulmonary adenocarcinoma (OPA)
particle assembly interference 2:473
replication cycle 3:176
assembly 3:178
expression 3:177
hyaluronidase 2 cellular receptor 3:176
long terminal repeats 3:176
virion proteins 3:175
capsid protein 3:175–176
matrix protein 3:175–176
reverse transcriptase 3:175–176
JAK–STAT pathway, interferon type I signaling 3:115
Jamestown Canyon (JC) virus 5:489
Japan, adult T-cell leukemia incidence 2:565
Japanese eel reovirus 1:164*t*
Japanese encephalitis
affected species 3:185
case fatality rates 2:236
clinical symptoms 3:185
incubation period 3:185–186
control 3:187
vector control 3:187
definition 3:182–183
diagnosis 3:186
complement fixation 3:186
enzyme-linked immunosorbent assay 3:186
hemagglutination inhibition 3:186
immunofluorescence 3:186
epidemiology 3:184
age-related 3:184
HIV concurrent infection 3:184
mosquito vectors 3:184
seasonality 3:184
zoonoses 3:184–185
livestock 3:182–183
pathogenesis 2:245
prevention 5:492
treatment 3:186
vaccines 3:187
adverse events 3:187
live vaccine 3:187
protective immunity mechanisms 3:187
recommendations 3:187
success of 3:187
zoonoses 3:184–185
Japanese encephalitis (JE) antigenic complex, West Nile virus classification 5:440–441
Japanese encephalitis virus (JEV) **3:182–188**
characteristics 1:171*t*
epidemiology 2:243
genotypes/genotyping 3:185
geographical distribution 3:184

Japanese encephalitis virus (JEV) (*continued*)
historical aspects 3:182–183
host range 2:236
infection *see* Japanese encephalitis
molecular epidemiology 3:185
antigenic groups 3:185
genotypes 3:185
strain variation 3:185
studies 3:185
pathogenicity 3:185
host determinants 3:185
neuroinvasiveness variation 3:185
viral determinants 3:185
physical properties 3:183
hemagglutinin 3:183
soluble complement-fixing antigen 3:183
virion morphology 3:183
RdRp 3:183–184
replication 3:183
nonstructural proteins 3:183–184
RdRp 3:183–184
receptor-mediated endocytosis 3:183–184
RNA synthesis 3:183–184
virion assembly 3:184
structural proteins 3:183
capsid protein 3:183
envelope protein 3:183
membrane protein 3:183
transmission 3:186
mosquito vectors 3:186
vaccine, reactions to 5:231*t*
virulence 3:185
Jaundice
hepadnaviruses 2:340
hepatitis A infection 2:343
JC virus(es) (JCV) **4:261–271**
cellular transcription factors 4:263
classification 4:261–262
genome 4:263*f*
coding regions 4:262
regulatory regions 4:264*f*
history 4:262
infection *see below*
latency 4:261–262
life cycle 4:265*f*
capsid proteins 4:265
LT-Ag role 4:265
LT-Ag protein
gene expression 4:263–264
life cycle 4:265
oncogenic role 4:268
Mad-1 strain
genome organization 4:262
oncogenic potential 4:267–268
regulatory region 4:264*f*
sequence repeats 4:263
oligodendrocyte infection 1:473
phylogenetics 4:128
reactivation 4:265
HIV infection 2:94
receptors 4:280
replication
agnoprotein 4:278
chlorpromazine 4:280
cytoplasmic entry 4:280
nuclear entry 4:280
serotonergic receptors 4:280
sialic acid receptor function 4:280
tissue tropism 4:261–262
viral interactions 4:266
BK viruses 4:267
HHV-6 4:266
human CMV 4:266
virion structure 4:261–262
JC virus infections
associated cells 4:262
astrocytes, host cell susceptibility 4:281–282
demyelination 4:267
glial cells 4:280
Gt1b gangliosides 4:280
HeLa cells 4:280
host cell attachment 4:280
host cell susceptibility
astrocytes 4:281–282
NF1X expression 4:281
lytic infection *vs.* tumor induction 4:267
oncogenic potential 4:262
PML 4:267
persistence 4:109*t*
prevalence 4:261–262
renal allograft recipients 3:470
at risk patients 4:262
JDV *see* Jembrana disease virus (JDV)
Jembrana disease virus (JDV)
genome 1:349
open reading frames 1:349–350
geographical distribution 1:348*f*
infection 1:351
taxonomy 1:348
virion structure 1:349
Jenner, Edward 1:574
smallpox 4:639
Jeryl Lynn strain (mumps virus), vaccination 3:363
Jet injection, DNA vaccine delivery 2:55
JEV *see* Japanese encephalitis virus (JEV)
JFH-1 HCV isolate 2:372–373
JGMV *see* Johnson grass mosaic virus (JGMV)
Jigs, bacteriophage assembly 1:438
Johnson grass mosaic virus (JGMV) 1:477
host range 1:477
infection
control 1:477
resistance 1:477
symptoms 1:477
'Join-cut-copy recombination' 4:401
Joinjakaka virus 1:118
J protein(s), microvirus DNA packaging 3:19
JSRV *see* Jaagsiekte sheep retrovirus (JSRV)
Junction adhesion molecule-A (JAM-A), mammalian reoviruses 4:387
Junin virus
geographical distribution 3:204
history 3:203
host range 3:206
infection *see* Argentine hemorrhagic fever (AHF)
transmission 3:204–205
vaccine against 3:210–211

K

K1 virus toxin 5:306–307
K28 virus toxin
A/B toxins *vs.* 5:303–304
α-component 5:305–306
β-subunit 5:303–304
cell binding 5:306–307
cellular effects
apoptosis 5:305
cell cycle progression 5:305
DNA synthesis 5:305
immunity 5:307
lethality 5:306*f*
resistance mechanisms 5:305–306
concentration effects 5:306*f*
endocytosis 5:303
endoplasmic reticulum exit 5:305*f*
Sec61 complex 5:304–305
intracellular transport 5:303
nuclear entry 5:304
preprotoxin processing 5:301–303
Kex1 cleavage 5:303–304
Kadam virus group 2:239
Kadipiro virus (KAD) 4:536
diagnostic assays 4:544–545
epidemiology 4:537
genome
conserved sequences 4:541–542
sequencing 4:539
host range 4:537
phylogenetics 4:540–541
proteins 4:538*f*
transmission 4:537
Kakugo virus (KV) 3:45
Kamese virus (KAMV) 4:184*t*
Kaplan criteria, norovirus infection diagnosis 3:444
Kaposin A, KSHV latency 3:199
Kaposi's sarcoma 5:195
Kaposi's sarcoma (KS) 5:195
animal model 3:193
antiherpesvirus drugs 3:193
antiviral drugs 3:193
cellular immune status 3:193
clinical subtypes 5:195–196
definition 2:93
history 3:195
KSHV detection 3:193
latent genes 3:200–201
see also specific genes
ocular associated 2:497
pathology 3:193
terminal repeat lengths 3:200–201
tumor growth 3:193
tumor morphology 5:195–196
viral gene expression 5:195–196
Kaposi's sarcoma-associated herpesvirus (KSHV) 4:587*t*
apoptosis inhibition 3:200
characterization 3:189–190
classification 4:585–586
evolution 3:190
lineages 3:190*f*
recombination 3:197
subtypes 3:197
genetics 3:191
LUR-encoded proteins 3:191
Rhesus rhadinovirus *vs.* 4:592
terminal repeats 3:191
genome 3:196*f*
gene functions 3:196
miRNAs 3:195–196
open reading frames 3:195–196
origins of replication 3:196
geographical distribution 3:189
history 3:189
host cell entry 3:197
associated cellular signal activation 3:197
gB glycoprotein 3:197
receptors 3:191
infection *see* Kaposi's sarcoma; *see below*
inhibitory proteins 3:189–190
kaposin B
immune evasion role 3:200
latency 3:199
molecular piracy 3:192
viral cyclins 3:192
replication 3:197
lytic 3:191
sequestered cellular genes 4:589
taxonomy 3:190
tissue tropism 3:197
associated cell lines 3:197
transmission 3:190
blood transfusion 3:191
casual transmission 3:190–191
developed countries 3:190
immunosuppression 3:190
maternal-child 3:190–191
transplantation 3:191
vIRF proteins
apoptosis inhibition 3:200
latency 3:199
primary effusion lymphoma 3:201
virion structure 3:195
Kaposi's sarcoma-associated herpesvirus (KSHV) complement-control protein (KCP) 3:199–200
Kaposi's sarcoma-associated herpesvirus infection 3:200
associated autoimmune disease 3:194
associated malignant disease *see* Kaposi's sarcoma (KS)
B-lymphoid malignancies 5:196
diagnosis 3:194
epidemiology 3:190
host range 3:191
immune evasion 3:199
chemokine homologs 3:200
complement control protein 3:199–200

interleukin-6 homolog 3:200
kaposin B 3:200
latency 3:199
modulator of immune recognition 3:199
immune response 3:192
incidence 3:190
incubation period 3:193
latent persistence 3:197, 3:198*f*
associated genes 3:198*f*
associated promoters 3:198*f*
organ transplantation 3:469
prevention 3:194
primary effusion lymphoma 3:193
apoptosis inhibition 3:200
latency 3:197–198
reactivation 3:469
treatment 3:194
Karolysis, definition 3:155
Karyorrhexis, definition 3:155
Kashmir bee virus 2:40
Kasokero virus 1:400
infection 1:400
transmission 1:400
Kawasaki disease 2:93
KEGG (Kyoto Encyclopedia of Genes and Genomes) 5:357*t*
Kelp fly virus (KFV) 3:44*f*
Kemerovo virus
geographical distribution 3:465
infection 3:465
Kenya, HIV infection 1:63
Keratinocyte(s)
human papillomavirus replication 4:10–12
immortalization 4:23
MCV infection 3:321
parapoxvirus infection 4:61
Kerato-conjunctivitis, HTLV-1 associated 2:573
Kern Canyon Group, animal rhabdoviruses 1:116
Kern Canyon virus 1:116
Keterah virus 1:400
Argas pusillus 1:400
global distribution 1:400
histological studies 1:400
Scotophilus temmencki 1:400
Keuraliba virus 4:184*t*
Kex2 processing, Cryphonectria parasitica virus 2:579*f*
KFDV *see* Kyasanur Forest disease virus (KFDV)
KHV *see* Koi herpesvirus (KHV)
Kidney(s)
Ectromelia virus infection lesions 3:345
HIV infection *see* HIV infection
infectious salmon anemia virus infection 3:94
see also under Renal
Killed vaccines
development 5:236
neonates 2:53
safety issues 5:228
Simian retrovirus D 4:629
Killer toxin(s) **5:299–308**
adsorption 5:307
dsRNA viruses 5:300
immunity 5:307
lethality 5:306
Killer virus system 5:299
Kimberley virus 4:184*t*
Kinetic neutralization curves 3:416
KI polyomavirus (KIPyV) 4:262
Koch's postulates 1:314
Koi herpesvirus (KHV)
detection 2:208
distribution 2:207
genome 2:209, 2:210*t*
growth properties 2:209
history 2:207
infection 2:208
mortality 2:207
Kolongo Group, animal rhabdoviruses 1:118
Kolongo virus (KOLV) 1:118
Kontonkan virus 1:112–114
Koolpinyah virus (KOOLV) 1:118
Koplik's spots, measles 3:289–290
Korean hemorrhagic fever 2:317
KP1 toxins, *Ustilago maydis* viruses 5:215–216
KP4 toxin, *Ustilago maydis* viruses 5:216*f*
KP6 toxin, *Ustilago maydis* viruses 5:217*f*
KP1461 2:507*t*
Kre1p toxin 5:306–307
Kakugo virus (KV) 3:45
KS *see* Kaposi's sarcoma (KS)
KSHV *see* Kaposi's sarcoma-associated herpesvirus (KSHV)
Kunjin virus, zoonoses 5:493
geographical distribution 5:493
Kununurra virus 1:118
Kuru 1:474
codon polymorphism 5:190
disease transmission 4:332
experimental 5:190
history 5:190
incubation period 4:332
Kuruma shrimp *(Penaeus japonicus),* infection 4:568
Kyasanur Forest disease 5:53
human cases 2:239
zoonoses 5:492
Kyasanur Forest disease virus (KFDV) 2:239
characteristics 1:171*t*
tick transmission 2:239
vectors 2:239
Kyphosis 1:76

L

L1 endonuclease, LINE-like retrotransposons 4:439–440
L1 proteins
HPV 4:25
HPV, vaccination 4:39
LINE-like retrotransposons 4:439–440
L2 bacteriophage 1:439
L2 proteins, HPV 4:25
L5 bacteriophage 5:185
Laboratory-acquired infections, yellow fever 5:474
Laboratory Response Network (LRN) 5:410
Laccase(s) 2:578
La Crosse (LAC) virus 5:489
characteristics 1:171*t*
classification 3:480
infections 5:489
mortality 3:480
morphology 1:393*f*
prevalence 3:480
reassortment 3:482
transmission 5:489
venereal transmission 5:269
vertical transmission 5:269
Lactase deficiency, rotavirus-induced diarrhea 4:511
Lactate dehydrogenase-elevating virus (LDV)
genome 1:179–180
geographical distribution 1:177
history 1:176
host range 1:177
infection *see below*
LDV-C 1:181
replication 1:181
host cell entry 1:181
transmission 1:182
Lactate dehydrogenase-elevating virus infection
cell tropism 1:177
clinical features 1:183
histopathology 1:184
immune response 1:184–185
cytotoxic T cells 1:185
neutralizing antibodies 1:184
immune system evasion 1:185
pathogenicity 1:183
pathology 1:184
lacZ gene/protein, baculovirus expression vector systems 1:240*f*
La France disease 1:510
historical aspects 1:286
La France infectious virus (LFIV) 1:286
La France isometric virus (LFIV) *see* Agaricus bisporus virus 1 (AbV-1)
Lagging strand 4:399
Lagomorph 1:533
Lagos bat virus 4:184*t*
Lagovirus 1:411*t,* 3:439
Lagovirus(es)
history 1:410
infections 3:439*t*
nonstructural proteins 1:412–413
see also specific viruses
Lake Victoria marburgvirus 3:273
Lambda holin S105 3:253
antiholin S107 *vs.* 3:253–254
membrane potential collapse 3:253
missense mutations 3:253
topology 3:253
λ1 protein, reovirus structure 4:394*f*
λ2 protein, reovirus structure 4:393
λ3 protein, reovirus structure 4:394*f*
λ bacteriophage *see* Bacteriophage λ
Lambda R, bacteriophage-mediated cell lysis 3:251
Lambdoid phages *see* Bacteriophage λ
Lamivudine 1:143*t*
hepatitis B therapy 1:152
LANA-1 (latency-associated nuclear antigen) 3:196
binding sites 3:197–198
KSHV infection, immune response 3:192
RTA inhibition 3:198
Lancisi, Giovanni, rinderpest epizootics 4:505
Landlocked salmon virus 1:164*t*
Langat virus
encephalitis 5:53
vaccines/vaccination 5:53
Laodelphax striatellus, rice black streaked dwarf virus transmission 1:484
Large dsRNAs, edornaviruses 2:110
Largemouth bass iridovirus (LMBV) 2:233
Large polypeptide (L), Crimean-Congo hemorrhagic fever virus 1:598–599
Large retrotransposon derivatives (LARDs), LTR-retrotransposons, unclassified 4:434
Large T *see* Polyomavirus(es)
Larvae
Aedes aegypti 2:9–10
definition 4:560
virus control
nucleopolyhedrovirus 1:266
Saint Louis encephalitis virus 4:659
Lassa fever
clinical features 3:208
children 3:208
complications 3:209
proteinuria 3:208–209
white blood cell count 3:208–209
control 5:486
epidemiology 3:206
histopathology 3:209
pathogenesis 3:207
endothelial cell dysfunction 3:207
incubation period 3:207
organ damage 3:207
pathology 3:209
risk factors 3:206
therapy 3:211
transmission 3:206
vaccination 3:210–211
Lassa virus
genetics 3:205
geographical distribution 3:204
history 3:203
host range 3:205
infection *see* Lassa fever
lymphocytic choriomeningitis virus cross-reactivity 3:240
tissue tropism 3:207
Last universal common ancestor (LUCA)
definition 3:473
viral origins 3:474
Late gene expression, baculoviruses 1:251
Latency **2:436–442**
antiviral therapy 1:152
characteristics 2:436
common themes 2:442
dynamic model 2:436

Latency (*continued*)
establishment 2:404
gene expression 2:438
detection 2:438
immunological memory 4:109–110
immunological studies 2:438
maintenance 2:404
persistent *vs.* 4:110*f*
post-transplantation reactivation 3:467
antirejection therapy 3:467
associated viruses 3:468
cellular pathways 3:467
herpesviruses 3:467
risk factors 3:467
reactivation 2:405*f*
respiratory tract infection 2:552
static model 2:436
see also specific viral infections
Latency-associated nuclear antigen *see* LANA-1 (latency-associated nuclear antigen)
Latency-associated transcript (LAT) 1:152
function 2:391–392
HSV detection 2:437
HSV infection 2:391–392
pseudorabies virus infection 4:343*f*
structure 2:404*f*
Latent membrane protein 1 (LMP-1) *see* Epstein–Barr virus (EBV)
Latent membrane protein 2A (LMP-2A) *see* Epstein–Barr virus (EBV)
Latent membrane protein 2B (LMP-2B) *see* Epstein–Barr virus (EBV)
Latent membrane proteins (LMPs) *see* Epstein–Barr virus (EBV)
Lateral flow tests, principles 2:33
Latex tests, principles 2:33
Latin America
HIV epidemiology 1:64
potato virus infection 4:297
see also individual countries
Latinized binomials, nomenclature 5:22
Lck
phosphorylation, Tio oncoprotein 4:590
Tip oncoprotein binding 4:590
L (light) dsRNAs, *Ustilago maydis* viruses 5:215
Le+, VSV replication 5:294
Leader proteins
apthoviruses 2:126
enteroviruses 2:126
Leading strand, definition 4:399
Leaf curl, beet curly top virus pathogenesis 1:305*f*
Leafhopper(s)
Maize streak virus transmission 3:264*f*
plant rhabdovirus transmission 4:195
rice tungro disease transmission 4:485
Leafroll disease
grapevines 4:202*f*
causal agents 4:203
transmission 4:203–204
potato virus infection 4:297
Leaky scanning 4:644
barley yellow dwarf viruses 1:281–282
definition 4:97
densovirus genome 4:79–81
Leaves
Maize streak virus 3:270
potato virus Y 4:291
Lectins, C-type
alphavirus host cell attachment 5:118
BV-mediated immunosuppression 4:255
Le Dantec virus (LDV) 4:184*t*
Leek white stripe virus (LWSV) 3:403
geographical distribution 3:404
Leek yellow stripe virus 5:288
LEF-3 protein 1:257–260
LEF11 protein 1:257–260
Legumes 3:212
associated viruses *see* Legume virus(es)
definition 3:212–213
seeds 3:212–213
see also Crop(s)
Legume virus(es) **3:212–220**
ecology 3:215
infection sources 3:215
economic importance 3:218
epidemiology 3:218*f*
disease development 3:218*f*
genetically-modified resistant plants 3:217, 3:219
history 3:213*f*
host range 3:213
resistant cultivars 3:217
seed transmission
infection control 3:219
transmission 3:215–216
transmission 3:215
direct contact 3:216
fungi 3:217
human 3:217
living organisms 3:216
nematodes 3:217
noncirculative 3:216–217
nonpersistent 3:216–217
persistent 3:214*f*
seed-borne *see above*
semipersistent 3:216–217
variation 3:215
vectors 3:216
infection control 3:219
see also specific viruses
Legume virus infections
control 3:219
germplasm 3:219
resistance 3:219
vectors 3:219
economic aspects
early infections 3:218
late infections 3:214*f*, 3:218
symptoms 3:214*f*
Leiomyosarcoma, definition 2:212
Leishmania RNA virus (LRV)
evolution 5:173–174
LRV1-4, endoribonuclease activity 3:222
taxonomy 3:220
viral persistence
5‘ UTR function 3:223
transcript cleavage 3:223
Leishmaniavirus 2:286, 3:220, 5:164, 5:165*t*
Leishmaniavirus(es) **3:220–225**
endoribonuclease activity 3:222
5‘ UTR role 3:222
genome 3:222*f*
open reading frames 3:222*f*
history 3:220
RNA protection assays 3:221
In vitro polymerase assays 3:221
polyprotein processing 3:222
infection maintenance, role 3:222
properties 3:220
RdRp 3:221
replication 3:221
model 3:224*f*
RdRp activity 3:221
structure 3:221*f*
viral persistence mechanisms 3:223
5‘ UTR functions 3:223
LRV1-4 transcript cleavage 3:223
ribosomal RNA 3:223
translation 3:223
see also specific viruses
Lembombo virus 3:465
Lens, eye *see* Eye
Lentiferon (lentivirus-induced interferon) 5:428–430
Lentivirus 1:347–348, 2:167, 2:526, 4:460, 4:594, 4:603, 5:424
Lentivirus(es)
classification 5:424
sequence homologies 5:21
evolution 4:595*f*
error-prone replication 4:596–597
recombination 4:596–597
gene therapy
psychology 2:302–303
vectors 2:302
genome 4:605*f*
group A 4:595–596
group B 4:595–596
group C 4:595–596
morphology 4:603
nomenclature 4:597*f*
phylogeny 4:605*f*
post-cell entry resistance 2:469
taxonomy 4:595
transmission 4:594
vif gene/protein 2:471
Vpu protein 2:473
see also specific viruses
Lentivirus protein R (Vpr) 2:522
G2 cell cycle arrest 2:522–523
PIC translocation function 2:522–523
Lentivrus-induced interferon (lentiferon) 5:428–430
Lepeophtheirus salmonis (sea louse), infectious salmon anemia virus vector 3:91
Lepidoptera
ascovirus infection *see* Ascovirus(es)
definition 2:37
granulovirus infection 1:211–212
iflavirus infection 3:42
nucleopolyhedrovirus infection 1:258*f*
polydnavirus life cycle 4:260–261
Lepidopteran nucleopolyhedrovirus(es) (NPV) 1:219–220
classification 1:220
occlusion bodies 1:220
phylogeny 1:260*f*
Leporipoxvirus 3:225*t*, 3:226
Leporipoxvirus(es) **3:225–231**
classification 3:226
DNA properties 3:226
evolution 3:228
genetic variability 3:228
geographical distribution 3:227
history 3:225
host range 3:227
infection
clinical features 3:228
control 3:230
cutaneous tumors 3:228–229
histopathology 3:229
immune response 3:229
pathogenesis 3:227
pathology 3:229
prevention 3:230
subcutaneous tumors 3:229
propagation 3:227
protein properties 3:226
replication
DNA replication 3:226
sites 3:227–228
transcription 3:226
translation 3:226
seasonal distribution 3:227
taxonomy 3:226
tissue tropism 3:228
transmission 3:228
efficiency 3:228
vectors associated 3:228
virion properties 3:226
morphology 3:226
see also Myxoma viruses (MYX)
Lepus europaenus, lagovirus infection 1:410
Lesions
bovine herpes viruses 1:365
chickenpox 5:253
cowpox infections 1:579
parapoxvirus infections 4:57
Lettuce, tomato spotted wilt virus infection 5:288–289
see also specific viruses
Lettuce big-vein disease
definition 5:263
historical aspects 5:263
Lettuce big-vein virus (LBVaV)
coat protein 5:266
phylogenetics 5:266
evolution 5:265*f*

genome 5:264*f*
geographical distribution 3:452*f*
historical aspects 5:263
host range 5:267
L protein 5:265
evolution 5:265*f*
phylogenetics 5:265–266
phylogenetics 5:265–266
protein 5 5:266–267
RdRp 5:265
transcription 5:267
transmission 5:264
Olpidium brassicae vector 5:264
virus particles 5:264
Lettuce necrotic yellows virus (LYNV) 4:193
genome 3:326*f*
Lettuce ring necrosis virus (LRNV) 3:447
coat protein 3:450
control, soil sterilization 3:453
genome 3:449
RNA4 3:449
geographical distribution 3:452
transmission, *Olpidium brassicae* 3:453
Leucania separata nucleopolyhedrovirus (LeseNPV) 1:256
Leukemic cells, HTLV-1 infection 2:561
Leukemogenesis *see* T-cell leukemia
Leukoencephalitis 5:424
Leukosis virus(es) (ALV), avian *see* Avian leukosis virus(es) (ALV)
Leviviridae 2:288, 3:21, 5:11*t*
Levivirus(es)
gene L 3:257*f*
see also specific viruses
see also specific viruses
Lexiva® 1:143*t*
L'Héritier, P, Sigma virus discovery 4:576
Liao ning virus (LNV) 4:536
epidemiology 4:537
genome
conserved sequences 4:541–542
sequencing 4:539
host range 4:537
phylogenetics 4:540–541
proteins 4:538*f*
replication
mammalian cell lines 4:539
mosquito cell lines 4:539
transmission 4:537
Lice, definition 5:91
Life, definition 3:399
Life expectancy, AIDS 1:51–52
Life-long immunity, varicella-zoster virus infection 5:254–255
Ligases *see* DNA ligase
Liliaceae infections 4:224
see also individual species
Lilium infections 4:224
see also specific viruses
Lily mottle virus (LMoV) 4:225*f*
Lily symptomless virus (LSV) 4:225*f*
Lily virus X (LVX) 4:225
LINE-1 clade 4:439
Lineage-specific retrotransposon 4:436
Linear DNA replication 2:308–309
LINE-like retrotransposons
mechanism of action 4:440*f*
L1 proteins 4:439–440
open reading frames 4:439
origins 4:441
structure 4:439
untranslated region 4:439
Linnaean system, classification 5:19
Lipid bilayers/membranes 5:308
acquisition, bacteriophage assembly 1:439
bacteriophages, assembly 1:439
composition 5:308
enveloped viruses
assembly 1:194
membrane fusion 3:292
SFV E1 insertion 3:299
function 5:308
hepatitis B virus virion 2:352
lipid arrangements 5:308–309
lipid composition 5:309
measles virus 3:286
Lipid metabolism, avipoxviruses 2:277
Lipid rafts
definition 3:292
Ebola virus assembly 2:62
measles virus assembly 3:333
membrane fusion 3:296–297
replication 4:411
virus membranes
composition 5:309
diffusion 5:309
Lipodystrophy
antiviral therapy 1:147
HIV infection *see* HIV infection
Lipopolysaccharide (LPS)
bacterial cell viral entry 5:366
bacteriophage ϕX174 cell attachment 3:15–16
definition 5:365
icosahedral enveloped dsDNA bacteriophages 5:369
icosahedral ssDNA bacteriophages 5:368–369
Liposomes
cypovirus uptake 3:138–139
DNA vaccine delivery 2:54
Lipothrixviridae 1:587, 5:11*t*
Lipothrixvirus(es)
Crenarchaea infections 1:588*t*
see also specific viruses
envelope composition 1:591
genomes 1:593
virion structure 1:589
see also specific viruses
Lipovnik virus
geographical distribution 3:465
infection 3:465
Liquid arrays, plant virus detection 2:28
Live-attenuated vaccines 5:236
definition 4:514
development 5:236
history 5:238
Japanese encephalitis 3:187
neonates 2:53
safety issues 2:52–53
Liver
anellovirus infection 1:110
avian hepadnavirus infection 2:327
cancer 5:197
hepatitis A virus infection 2:346
hepatitis B virus infection 5:197
hepatitis C virus infection 2:368
infectious salmon anemia virus infection 3:94
Marburg virus infection 3:278
transplantation 3:469
see also under Hepatic
Liver damage, Lassa fever 3:209
Liver lesions, ectromelia virus infection lesions 3:344
Loeffler, Friedrich 5:55
virus discovery 2:455
Long contractile tails, icosahedral tailed dsDNA bacteriophages 5:366*f*
Long-distance frame shift element (LDFE) 1:281–282
Long-distance movement
maize streak virus 3:268
nepovirus 3:410
Long distance RNA/RNA interactions, RNP complexes 3:397*f*
Long intergenic region (LIR), definition 3:264
Long interspersed nuclear elements (LINEs) 4:439
definition 4:437
LINE-1 clade 4:439
retrotransposons 4:429–430
see also LINE-like retrotransposons
Long noncontractile tails, icosahedral tailed dsDNA bacteriophages 5:367
Long terminal repeats (LTRs)
definition 4:428
mouse mammary tumor virus 3:335–336
pseudovirus genome 4:355
retrovirus evolution 2:183–184
Simian retrovirus D 4:625
Long-term nonprogressors (LTNP)
definition 1:69
HIV infection 1:71
Long-term therapy 1:147
Loop-mediated isothermal amplification (RT-LAMP) 2:25
Lopinavir 2:513
Louping ill virus 2:240
infection 2:240–241
clinical manifestations 5:52–53
transmission 2:240
ticks 2:240
Low density lipoprotein (LDL) receptors, coxsackievirus attachment 1:543–544
Lower respiratory tract
common cold 4:474*f*
definition 2:551
infection causes 2:551–552
structures associated 2:551
Low pathogenicity avian influenza (LPAI) virus(es) 3:101
HA cleavage sites 3:101–102
L polymerase
arenaviruses *see* Arenavirus(es)
rhabdovirus phylogeny 1:112–114
L protein
definition 5:263
Lettuce big-vein virus *see* Lettuce big-vein virus (LBVaV)
L RNA segment *see* Crimean-Congo hemorrhagic fever virus (CCHFV)
LRV1-4 *see* Leishmania RNA virus
LSM1–7/PAT/DHH1 protein complex 1:384–385
LT-Ag protein, BKV 4:269
LTR-retroelements
classification 4:451
definition 3:301
reverse transcriptase 4:452*f*
LTR-retrotransposons 4:421*f*
BEL 4:438
classification 4:429–430
definition 4:420
DIRS1 4:438
genome distribution 4:443
discovery 3:302
distribution 4:442*t*
evolution 4:441
quasispecies-like populations 4:432
genera 4:433
metaviruses 4:433
pseudoviruses 4:433
genes, effects on 4:444
hepadnaviruses 4:438
history 4:437
life cycle 4:429*f*
amplification 4:432*f*
LC8 proteins 4:430–431
lineage 3:305*t*
mechanism of action 4:439
members 4:437
open reading frames 4:420
origins, acquisition 4:441
phylogenetic studies 4:441
replication 4:424
gag gene 4:420
pol gene 4:420
primer-binding site 4:425
reverse transcription 4:425
variations 4:425
retroelements *vs.* 4:451
retroviruses *vs.* 4:431–432
reverse transcription 3:307*f*
structural diversity 4:429*f*
structure 4:438*f*
Gag 4:438–439
integrase 4:438–439
internal sequence 4:438
mutations 4:439

LTR-retrotransposons (*continued*)
open reading frames 4:438–439
Pol 4:438–439
primer binding site 4:439
reverse transcriptase 4:438–439
ribonuclease H 4:438–439
Tnt1 elements 4:432
transcription 4:430
Ty3/*gypsy see* Ty3/*gypsy* retrotransposon(s)
unclassified 4:434
large retrotransposon derivatives 4:434
terminal-repeat retrotransposons in miniature 4:434
Lucerne Australian latent virus (LALV) 3:406*t*
Lucerne Australian symptomless virus (LASV) 4:523
geographical distribution 4:523
host range 4:525
Lucerne transient streak virus (LTSV) 4:647
Lucke´ tumor herpesvirus (LTHV)
adenocarcinoma 2:206
cellular morphology 2:206*f*
distribution 2:207
genome 2:209
history 2:206
tumor induction 2:209
Lumpy skin disease (LSD) virus 1:427
epidemiology 1:429
Lungs
MHV-68 infection 3:375
SARS infection 4:557–558
see also under Pulmonary
Luteoviridae 1:279, 3:231, 3:232*f*, 3:232*t*, 5:209
see also specific viruses
Luteovirus 3:231
Luteovirus(es) **3:231–238**
biological properties 3:231
epidemiology 3:237
evolutionary relationships 3:235
gene expression 3:233
post-transcriptional gene silencing 3:234–235
strategies 3:233
VPg 3:235
genome 3:234*f*
open reading frames 3:231
subgenomic RNAs 3:233–234
geographical distribution 3:237
history 3:231
host range 3:235
infection
co-infection 3:237
control 3:237
crop yield losses 3:231
diagnosis 3:237
host–virus relationships 3:236
plant resistance genes 4:179*t*
symptoms 3:236–237
phylogeny 3:232*f*
replication 3:236
taxonomy 3:231
tobacco disease, economic importance 5:66
transmission 3:235
aphids 3:235–236
capsid RTD function 3:236
circulative 3:235
initiation 3:236*f*
nonpropagative 5:278*f*
virion composition 3:233
virion properties 3:231
stability 3:231–233
virion structure 3:233*f*
capsids 3:233
see also specific viruses
Luteovirus-associated RNAs 4:533
Lwoff, André, bacteriophage lysogeny experiments 2:445
Lychnis ringspot virus (LRSV) 2:459
coat proteins 2:459
genome 2:459–460
Lychnis symptomless virus (LycSLV) 4:311*t*
LydA holin, inhibition 3:255–256
Lymantria dispar multiple nucleopolyhedrovirus (LdMNPV) 1:217
virion structure 1:223
Lymantria ninayi virus (LnV) 5:28*t*
Lymphatic system, African horse sickness 1:40–41
Lymph nodes
adult T-cell leukemia histology 2:566
bovine immunodeficiency virus infection 1:352
dendritic cell activation 3:73–74
Ectromelia virus infection lesions 3:345
Lymphadenopathy
MHV-68 infection 3:375–376
SIV infection 4:601
Lymphoblastoid cell lines (LCLs)
EBV evolution 2:151
EBV propagation 2:150
Lymphocryptovirus 2:148–149, 2:432*t*
Lymphocryptovirus (LCV) 4:592
B-cell transformation 4:592–593
classification 4:585
distribution 4:593
genome sequence 4:592
phylogenetic trees 2:427*f*
taxonomy 2:426–427
EBV 2:148–149
Lymphocystis disease virus 1 (LCDV-1) 2:233
Lymphocystivirus 2:233, 3:155, 3:168*t*
Lymphocystivirus(es)
as emerging pathogen 3:159
see also specific viruses
geographical distribution 3:156
host range 3:156
infections, fish 3:156–158
phylogeny 3:160*f*
transmission 3:159–160
see also specific viruses
Lymphocyte(s) 3:78–79
apoptosis in African swine fever infection 1:44–45
apoptosis in filovirus infection 2:203
infiltrations, equine infectious anemia virus infection 2:172–173
in vitro transformation 5:194
see also B cell(s); CD4+ T-cells; CD8+ T-cells
Lymphocytic choriomeningitis virus (LCMV) **3:238–243**
classification 3:239
distribution 3:239
epidemiology 3:205–206
evolution 3:240
genetics 3:240
polymerase-binding sites 3:240
recombination 3:240
variants 3:240
genome, intergenic regions 3:244
history 3:238
host range 3:204–205
infection *see below*
phylogenetics 3:247–248
propagation 3:239
replication, respiratory syncytial virus *vs.* 3:246
reverse genetics 3:247
RNA replication/transcription 3:245–246
serology 3:240
strains 3:238
pathogenicity 3:241
structure 3:81
taxonomy 3:239
tissue tropism 3:239–240
transmission 3:239–240
transplant-related transmission 3:466–467
Z protein 3:245–246
promyelocytic leukemia protein interaction 3:245
RNA replication/transcription 3:245–246
Lymphocytic choriomeningitis virus infection
animal models 3:239*f*
adult infection 3:81
CNS infection 3:82
host genetics 3:82
infection site 3:82
neonatal infection 3:81
survival-determining factors 3:81
clinical features 3:241
grippe-like form 3:241
incubation period 3:241
meningeal form 3:241
meningoencephalomyelitic form 3:241
mortality 3:241
cytotoxic T-cells 3:238–239
initial site of infection 3:82
histopathology 3:241
immune evasion 1:125
immune response 3:242
early stage 3:242
late stage 3:242
survival-determining factors 3:81
T cells *see above*
pathogenesis 3:239*f*
pathogenicity 3:241
persistent infection 3:241
strain variant differences 3:241
pathology 3:241
persistent, terminal genomic deletions 3:247
prevention and control 3:242
Lymphocytosis, SIV infection 4:601
Lymphoid follicles
definition 5:424
yellow fever 5:475
Lymphoid organs, definition 5:477
Lymphoid tissue, SIV infection 4:601
Lymphomas
feline immunodeficiency viruses 1:351–352
fish retroviruses 2:213
immunosuppressed patients, EBV infections 5:194
Pacheco's disease 2:410
SIV infection 4:601
Lymphopenia 4:552
Lymphotoxins 1:620
Lyon-4 virus, antigenic properties 5:155
Lysis
definition 3:248
icosahedral tailed dsDNA bacteriophages *see* Icosahedral tailed dsDNA bacteriophages
rotting *vs.* 3:249
triggers 3:249–250
Lysogen 5:411
Lysogenic coliphage λ 2:445–446
Lysogeny 2:445
definition 2:442
Lysosomal protease(s) 1:157
Lysozymes, bacterial cell viral entry 5:367
Lyssavirus(es) 4:367–368
genome 3:326*f*
zoonoses 5:486*t*
see also specific viruses
Lytic transglycosylases, bacterial cell viral entry 5:367
Lytic virus(es) 5:411
replication 4:412
see also specific viruses

M

M1 virus, apoptosis 1:162
M2 gene
B cell specificity 3:375
latency 3:376–377
M2 ion channel blocker(s), influenza treatment 3:102
M2 virus, apoptosis 1:162
M13 bacteriophage *see* Bacteriophage M13
MAb 2F5, HIV-1 vaccine development 1:141
Macaca fascicularis gamma virus 4:587*t*
Macaca mulatta rhadinovirus *see* Ceropithecine herpesvirus 17 (CeHV-17)
Macaca nemestrina rhadinovirus 2 (MnRRV) 4:587*t*
Macaques *see* Rhesus macaques
Machlomovirus 5:145–146
evolution 3:263
genetics 3:261
genome 3:259
geographical distribution 3:261
historical aspects 3:259
infections
control 3:263
epidemiology 3:262
histopathology 3:262
host range 3:261

pathogenicity 3:262
pathology 3:262
prevention 3:263
propagation 3:261
replication 3:260
seasonal distribution 3:261
serotypes 3:261
transmission 3:262
variation 3:261
virion structure 3:259
see also specific viruses
Machlomovirus 3:259
Machupo virus
geographical distribution 3:204
history 3:203
host range 3:204–205
infection 3:210
Macluravirus 4:315*t*
Macrobrachium rosenbergii nodavirus (MrNV)
extra small virus complex 4:573
genome organization 4:573
geographical distribution 4:575
host range 4:575
infection *see* White tail disease
properties 4:574*f*
taxonomy 4:575
virus relationships 4:575
genome 4:574*f*
taxonomy 4:575
Macrobranchium rosenbergii nodavirus (MrNV) 4:527*t*
Macrolesions, African horse sickness *see* African horse sickness (AHS)
Macromolecular Structure Database 5:357*t*
Macrophages
African swine fever virus *see* African swine fever virus
astrovirus infection, role 1:209–210
Ebola virus infection 2:63–64
filovirus infection 2:203
function 1:122–123
immune control 4:112
innate immunity 3:112
JCV infection 4:267
localization 1:122–123
murine CMV infection 1:630*t*
rhinoviral infections 1:547
Maculavirus 5:200, 5:200*t*, 5:206
Maculavirus(es) 5:206
characteristics 5:206
properties 5:206
see also specific viruses
Maculopapular rash
definition 5:83
togavirus infection 5:84
Mad-1 strain *see* JC virus
Mad-4 strain (JC virus), genome 4:262
Mad cow disease *see* Bovine spongiform encephalopathy (BSE)
Maedi–Visna virus (MV) 1:126
Magnesium ion(s)
CuniNPV transmission 1:221–222
ribozyme folding 4:475–476
Main protease (M^{pro}), nidoviruses 3:424
Maintenance of killer genes (MAK), *Saccharomyces cerevisiae* 5:170–171
Maintenance transmission 5:272
seasonal changes 5:272
West Nile virus 5:272
Maize *(Zea mays)*
barley yellow dwarf disease 1:476
cereal yellow dwarf disease 1:476
corn stunt complex disease 1:482
diseases associated 1:476
vaccine production 5:223–224
virus infections **1:475–482**
control 1:475–476
crop yields 1:475–476
symptoms 1:476
transmission 1:475–476
see also specific viruses
Maize chlorotic dwarf virus (MCDV) 4:546–547
genome 4:548
geographical distribution 1:477
isolate variation 4:550–551
protein properties
leader protein 4:549
proteases 4:548–549
RdRp 4:549
Maize chlorotic mottle virus (MCMV) 3:259
discovery 3:259
epidemiology 3:262
genetics 3:261
RdRp 3:261
genome 5:147*f*
geographical distribution 3:261
infection *see* Maize mottle chlorotic stunt disease
pathogenicity 3:262
synergistic relationships 3:262
yield reduction 3:262
RdRp 3:261
replication 3:260–261
RNA strand templates 3:260–261
resistant plant lines 3:263
seasonal distribution 3:261
serotypes 3:261–262
structure 3:260*f*
transmission 3:262
beetles 3:262
soil 3:262
water 3:262
Maize dwarf mosaic virus A (MDMV-A) 1:479
infection
resistance 1:479
symptoms 1:479
yield losses 1:479
synergistic relationships, maize chlorotic mottle virus 3:262
transmission 1:479
Maize fine streak virus (MFSV) 1:479
infection, symptoms 1:478*f*
transmission 1:479
Maize Indian Fiji-like virus 1:479
maize infections 1:479
Maize Iranian mosaic virus (MIMV) 1:479
host range 1:479
Maize mosaic virus (MMV) 1:479
geographical distribution 1:479
infection
resistance 1:479
symptoms 1:479
replication, budding 4:193
transmission 4:195*f*
insect tissues associated 4:195–196
Maize mottle chlorotic stunt disease 1:479
geographical distribution 1:479–480
symptoms 1:479–480, 3:262–263
transmission 1:479–480
yield losses 1:478
Maize necrotic streak virus (MNeSV) 1:480
Maize rayado fino virus (MRFV) 1:480
geographical distribution 1:480
infection
control 1:480
cytological symptoms 5:205–206
incidence 1:480
replication, sites 5:205–206
transmission 5:205–206
Maize rough dwarf virus (MRDV) 1:480
characteristics 4:150*t*
geographical distribution 1:480
infection
control 1:480
symptoms 1:478*f*
transmission 1:480
Maize sterile stunt virus (MSSV) 1:480
Maize streak virus (MSV) 4:165
B strain 1:494
coat protein 3:266
diversity 3:265
genome sequence 3:265–266
relation to other mastreviruses 3:265*f*
evolution 3:265
future work 3:271
genome 3:266
coat protein 3:266
complementary sense genes 3:267
historical aspects 3:264
infection *see below*
long intergenic region 3:266
origin of replication 3:266
molecular biology 3:268
movement 3:270
cell-to-cell 3:268
leaves 3:270
long-distance movement 3:268
shoot apex 3:270
movement proteins 3:266
posttranslational modifications 3:268
particle assembly 3:268
particle structure 3:264*f*
plant retinoblastoma-related protein 3:267
repA (regulatory protein) 3:266
transactivation domain 3:268
translation 3:267
rep gene 3:266
dNTP-binding site 3:268
replication 3:268
rolling-circle replication 3:269*f*
short intergenic region 3:265*f*
complementary strand synthesis 3:266–267
strains 3:265*f*
transmission 1:481
leafhopper vectors 3:264*f*
virion sense genes 3:268
Maize streak virus infection 4:167*f*
control 1:481
insecticides 3:270
resistant crops 3:270
epidemiology 3:270
cultural practices 3:270
environment 3:270
host range 3:265
grasses 3:265
symptoms 1:481
yield losses 3:265
Maize stripe virus (MSpV) 5:24
host range 1:481
infection, symptoms 1:481
species discrimination, rice stripe virus 5:26
transmission 1:481
Maize white line mosaic virus (MWLMV) 4:527*t*
Major histocompatibility complex (MHC)
Ab-mediated immunity 3:56
allelic variation 1:123
assembly 1:123
co-stimulatory molecules 1:122
definition 3:78
downregulation
adenovirus infection 1:12–13
KSHV infection 3:193
expression 1:122
HCV infection response 2:373–374
host susceptibility to infection 5:318
restriction 3:72*f*
T-cell receptors 3:71–72
SIV infection 4:600–601
translocation 1:123
Major histocompatibility complex (MHC) class I, antigen presentation 1:620–621
cross-presentation 1:124
inflammatory form 1:123–124
loading complex 1:124
molecular mechanism 1:124
peptide binding 1:123–124
peptide degradation 1:123–124
structure 1:123
Major histocompatibility complex (MHC) class II
antigen presentation 1:620–621
autophagy 1:125
endosome proteases 1:124–125
peptide editing 1:124–125

Major histocompatibility complex (MHC) class II (*continued*)
invariant chain association 1:124
pseudo-peptide 1:124
mouse mammary tumor virus transmission 3:337
structure 1:124
peptide binding cleft 1:124
Malabaricus grouper nervous necrosis virus (MGNNV) 3:433*f*
capsid 3:433
Malarial catarrhal fever *see* Bluetongue virus(es) (BTV)
Malaysia, Nipah virus 2:323–324
Mal de Rio Cuarto virus (MRCV) 4:150*t*
host range 1:481
infection
control 1:481
symptoms 1:478*f*
transmission 1:481
Malignant rabbit fibroma virus (MRV) 3:225–226
Malignant transformation
adenoviruses *see* Adenovirus(es)
avian leukosis virus 4:458
HIV associated 1:52
reticuloendotheliosis viruses *see* Reticuloendotheliosis viruses (REV)
Malpighian cell(s) 2:212
Malva veinal necrosis virus (MVNV) 4:311*t*
Mamestra brassicae multiple nucleopolyhedrovirus (MbMNPV) 3:129
Mammalian cells
B-tropic mouse leukemia virus infection 2:469
Liao ning virus replication 4:539
N-tropic mouse leukemia virus infection 2:469
Mammalian orthoreovirus 4:392
Mammalian orthovirus 4:536*t*
Mammalian reovirus(es) 4:384
apoptosis 4:387
distribution 4:385
epidemiology 4:385
age-related susceptibility 4:385
genome 4:384*t*
reassortants 4:385
S1 segment 4:385*f*
host cell attachment 4:387
junction adhesion molecule-A 4:387
σ1 protein function 4:387
infection *see below*
intermediate subviral particles 4:386
phylogeny 4:385*f*
serotypes 4:384
strains 4:384
transmission 4:386*f*
L2 gene function 4:385–386
S1 gene function 4:385–386
virus–cell interactions 4:387
apoptosis 4:388*f*
cellular signaling pathways associated 4:387
inducible NADPH-dependent nitric oxide synthase 4:387–388
M2 gene function 4:387
S1 gene function 4:387
see also specific viruses
Mammalian reovirus infections
clinical features 4:388
aseptic meningitis 4:389
children 4:388–389
human extrahepatic biliary atresia 4:389
host range 4:385
immune response 4:389
antibodies 4:389
interferon 4:390
passive protection 4:389–390
T cells 4:389
pathogenesis 4:386
spread 4:386
tissue tropism 4:386*f*
Mammary adenocarcinomas, mouse mammary tumor virus infection 3:338–339
Mammary gland enhancer (MGE), mouse mammary tumor virus 3:341
Mandarivirus 1:426*f*, 2:255
Mansonia dubitans, Venezuelan equine encephalitic virus transmission 5:104
Mantle, definition 4:561
Marafivirus 5:200, 5:200*t*, 5:205
Marafivirus(es) 5:205
characteristics 5:205
genome 5:205
marafibox 5:205
infection 5:201
phylogenetic relationships 5:204*f*
properties 5:205
polyprotein processing 5:205
replication cycle 5:205
transmission 5:205
virion structure 5:205
coat proteins 5:205
see also specific viruses
Maraviroc 2:506
Marburg hemorrhagic fever (MHF) 3:272, 3:277
genome 3:327*f*
Marburg virus **3:272–280**
bioweapons 5:408
classification 3:273
composition 3:273
evolutionary relationships 3:275
Ebola *vs.* 3:275
phylogenetic analysis 3:276*f*
gene expression 3:273
genetic stability 2:179
genome 3:274*f*
Ebola virus *vs.* 2:201–202
polymerase complex 3:274–275
history 5:490–491
inactivation 3:273
infection *see below*
infectivity 3:273
occurrence 3:273*f*
replication 3:275
structure 3:274*f*
glycoprotein spikes 3:275
L protein 3:274–275
membrane-anchored spikes 3:273
RNP complex 3:273
VP24 3:275
VP30 3:274
VP40 3:275
taxonomy 3:273
transmission 3:276
Marburgvirus 2:199, 3:273
Marburg virus infection
animal models 2:202–203
clinical features 3:277*f*
coagulopathy 2:203
Democratic Republic of Congo 2:200
diagnosis 5:490–491
enzyme-linked immunosorbent assay 3:278–279
RT-PCR 3:278–279
disease outbreaks 3:272
disease surveillance 2:199
Democratic Republic of Congo 3:272
Ui´ge Province, Angola 3:276*f*
endothelial cell activation 2:203
fatality rate 3:272
hepatocellular necrosis 3:278
host range 3:276
animal reservoir 3:276–277
immune response 2:203
incubation period 2:202
liver function impairment 3:278
neutralization antibodies 3:279
nonfatal cases 3:277*f*
pathogenesis 3:277
Ebola virus *vs.* 3:278
guinea pigs 3:277–278
pathology 3:277
hepatocellular necrosis 3:278
parenchymal cells 3:278
prevention 3:279
transmission 5:490–491
treatment 2:204
supportive therapy 3:279
therapeutic antibodies 3:279
Ui´ge Province, Angola 2:200
vaccines/vaccination 3:279
zoonoses 5:490
Marchal, J, Ectromelia virus 3:342
Mardivirus
genetics 2:409
nomenclature 2:406
Mardivirus 2:432*t*
Marek's disease
pathobiology 2:409
prevention 2:411
Marek's disease virus (MDV)
genetics 2:408–409
genome integration 2:409–410
history 2:407
infection
associated T-cell lymphoproliferative syndromes 2:406
clinical features 2:409
control 2:411
differential diagnosis 2:410
economic impact 2:411
host range 2:407–408
latency 2:409–410
pathobiology 2:411
pathogenesis 2:409
pathology 2:409
vaccination 2:406
morphology 2:408
propagation 2:407–408
replication 2:409–410
transmission 2:409–410
Marine bacteriophage(s), ecology 4:124
Marine birnavirus (MABV) 4:566
Markov chain Monte Carlo method, viral evolution 2:180–181
Marmoset animal model, varicella-zoster virus infection 5:251
Marnaviridae 1:87, 1:90*f*, 3:280, 3:281
Marnavirus(es) **3:280–285**
phylogenetic analyses
capsid proteins 3:284*f*
RdRp domains 3:282*f*
see also specific viruses
Maruca vitrata nucleopolyhedrovirus (MaviNPV) 1:256
MAS, definition 1:296
Mason–Pfizer monkey virus (MPMV)
cell receptor, amino acid transport protein 4:627
classification 4:624
historical aspects 4:624–625
human infections 4:629
cancer 4:629
schizophrenia 4:629
serological surveys 4:629
natural hosts 4:627
pathogenesis 4:628
transmission 4:624
viral assembly 2:264
Mass vaccination programs, disease surveillance 2:47*f*
Mastadenovirus 1:1, 1:17, 1:26–27, 2:120
Mastadenovirus(es) 1:17
biological properties 1:5
genome 1:6
infectivity 1:5–6
proteins 1:6
bovine 1:27
genome 1:18
host cell entry 1:6
replication 1:4–5
virion morphology 1:3
see also specific viruses

Master sequence 4:359
Mastitis, MuV infection 3:361
Mastomys, Lassa fever epidemiology 3:206
Mastrevirus 1:296, 2:98, 4:165
Mastrevirus(es)
  infections 5:65
    monocots 4:166
  Rep proteins 1:518
  tobacco diseases, economic importance 5:65
  type species 4:165
  see also specific viruses
Maternal immunity, vertebrate parvoviruses 4:89
Maternally derived antibodies, neonatal immunization 2:53
Maternal milk, mouse mammary tumor virus transmission 3:337
Maternal transmission 1:376
Matrix metalloproteinase(s) (MMPs) 1:216
Matrix protein, definition 4:407
Matrix (M) proteins
  arenavirus assembly/budding 3:247
  Chandipura virus 1:503
  Ebola virus *see* Ebola virus(es) (EBOV)
  influenza A virus 3:485
  influenza C virus 3:485
  influenza viruses 3:485
  influenza viruses structure 3:97
  mononegaviruses 3:331
  orthoretroviruses 4:463–464
  paramyxoviruses 3:333
  vesicular stomatitis viruses 5:295
Matsukemin, *Dendrolimus spectabilis* control 3:127
Maturation
  definition 4:407
  icosahedral ssRNA bacteriophages 3:26
  replication 4:411
    proteases 4:411–412
    protein cleavage 4:411
Maturation inhibitors, antiretroviral therapy 2:516
Maturation (A) protein 3:24
Maus Elberfeld (ME) virus 1:441–442
MAV strain *see* Barley yellow dwarf virus (BYDV)
Maximum likelihood (ML), phylogenetic analysis 4:126–127
Maximum parsimony (MP), phylogenetic analysis 4:126–127
Mayaro virus 5:489
  characteristics 1:171*t*
  geographical distribution 5:113
  infection 5:113–114
    clinical features 5:87*t*
  transmission 5:89
Mayer, Adolf 5:54
MCF *see* Bovine malignant catarrhal fever (MCF)
MDA-5 helicases 3:59–60
  structural rearrangement 3:107
M-dsRNA, *Saccharomyces cerevisiae* L-A virus 5:300
M (medium) dsRNAs, Ustilago maydis viruses 5:215
M (middle) dsRNAs, Ustilago maydis viruses 5:217–218
Mealie blight *see* Maize streak virus (MSV)
Mealie yellow *see* Maize streak virus (MSV)
Mealybug(s), badnavirus transmission 1:463
Measles
  blindness 2:494–495
  clinical features 3:288
    Koplik's spots 3:289–290
  complications 3:288
    giant cell pneumonia 3:289
    HIV infection 3:289
    immunosuppression 3:288
    latent tuberculosis reactivation 3:288
    measles inclusion body encephalitis 3:289
    subacute sclerosing panencephalitis 3:289
  control 3:289
  diagnosis 3:289
    enzyme-linked immunosorbent assay 3:289–290
    reverse transcription PCR 3:289–290
  differential diagnosis 3:289–290
    cytomegalovirus infections *vs.* 3:289–290
    infectious mononucleosis *vs.* 3:289–290
    rubella *vs.* 3:289–290
    scarlet fever *vs.* 3:289–290
    toxoplasmosis *vs.* 3:289–290
    typhus *vs.* 3:289–290
  disease surveillance 2:47*f*
    age distribution 2:48
  elimination 5:232
  fatality 3:288
  histopathology 3:289
    endothelial syncytial cells 3:289
    Warthin–Finkeldy giant cells 3:289
  historical aspects 3:288
  immune response 3:289
    immunoglobulin G 3:289
    immunoglobulin M 3:289
    T-cell responses 3:289
  keratopathy 2:494–495
  mortality rate 4:52
  ocular complications 2:494–495
  pathology 3:289
    inflammation 3:289
    necrosis 3:289
    secondary infections 3:289
  persistent infection 2:147
  prevention 3:289
    global vaccine coverage 3:290*f*
    vaccination 3:290
  protective immunity 3:288
  secondary infections 3:289
  transmission 3:288
  vaccines/vaccination 3:290
    atypical measles 3:289
Measles inclusion body encephalitis 3:289
Measles/mumps/rubella (MMR) vaccine 4:57
  reactions to 5:230*t*
  safety 5:228
    autism *vs.* 5:233
    bowel disease *vs.* 5:233
    public perceptions 5:231
Measles virus **3:285–292**
  assembly 3:333
  cellular receptors 3:287
    membrane cofactor protein 3:288
    signaling lymphocyte activation molecule 3:285
  classification 3:285
  C protein 3:287
  envelope proteins 3:287
  F glycoprotein 3:287–288
  F protein spike 3:286
    particle structure 3:286
  genome 3:287*f*
    innate immunity control genes 3:286
    nucleocapsid protein 3:286
  host cell binding 5:322
  H protein 3:287–288
    host cell binding 4:53–54
  human respiratory infection 2:553
  human subacute sclerosing panencephalitis association 2:4
  infection *see* Measles
  L protein 3:286
    particle structure 3:286
  M protein 3:287
  multivalent vaccines 3:291
    hepatitis B surface antigen 3:291
  nucleocapsid protein 3:286
  oncolysis 3:291
  particle structure 3:286*f*
    lipid bilayer 3:286
    nucleocapsid core 3:286
    nucleocapsid protein 3:286
    polymerase cofactor 3:286
  P protein 3:286
    function 3:287
  RdRp 3:287
    stop–start mechanism 3:287
  replication 3:286
  RNP complex 3:287
  transmission 2:553–554
  vaccines/vaccination
    Ab-dependent disease enhancement 5:231
    definition 5:226
    efficacy *vs.* potency 5:228
    multivalent *see above*
    see also Measles/mumps/rubella (MMR) vaccine
  virus perpetuation 2:147
Mechanical transmission
  Pepino mosaic virus 4:108
  Taura syndrome virus 5:7
  vegetable viruses 5:284–285
Mediastinal lymph node (MLN), MHV-68 infection 3:375
Mediterranean crab *(Portunus depurator)*, shrimp virus infection 4:568
Mediterranean shorecrab reovirus (RC84) 1:164*t*
Mediterranean swimming crab reovirus (P) 1:164*t*
MedScape 5:357*t*
Megalocystivirus 2:233, 3:155, 3:167–168, 3:168*t*
Megalocystivirus(es)
  geographical distribution 3:156
  see also specific viruses
  host range 3:156
  infections, fish 3:156–158
  phylogeny 3:160*f*
  transmission 3:159–160
  vaccines/vaccination 3:160
Meibomian gland 2:491
Melaka virus 2:95
Melanization
  IV-mediated immunosuppression 4:255
  parasitoids 4:253*f*
Melanoplus sanguinipes entomopoxvirus (MSEV) 2:136
  DNA ligase 2:139
  genome 2:138
  insect pest control 3:128*f*
Melon, resistance genes 4:182
  quantitative resistance 4:182
Melon necrotic spot virus (MNSV)
  eIF-4E-mediated resistance to 4:186
  transmission 1:453
Membrane cofactor protein (MCP: CD46), measles virus cell entry 3:288
Membrane fusion **3:292–301**
  fusion loops 3:300
  fusion peptide 3:296
  fusion protein
    activated structure 3:297
    cooperativity 3:296
    native structure 3:297
  glycoproteins
    biosynthesis 3:293
    class I 3:293
    class II 3:297
    class III 3:299
    HA0 3:294
    HA2, low pH-activated conformation 3:294
    hemagglutinin structure 3:293
    herpesvirus gB structure, low pH-activated conformation 3:300
    VSV G structure, low pH-activated conformation 3:300
  lipid role 3:296
  protein cooperativity 3:296
  sites 3:292
  stalk hypothesis 3:292
  see also PH-mediated fusion
Membrane fusion protein, sawfly baculovirus genome 1:228
Membrane proliferation, nepovirus 3:409
Membrane proteins 5:310*f*
  aggregation 5:309
  enveloped viruses 5:309
  functions 5:308
  integral 5:309
    composition 5:309
    functions 5:309
  Japanese encephalitis virus 3:183
  peripheral 5:309
  viroporins 5:309
  see also specific viruses

Memory B-cell(s), EBV persistence 2:166*f*
Memory T-cell(s) 3:80
  acute infection, response 3:76
  classification 3:75–76
Menangle virus 2:95
Mengo virus 1:443–444
  virion structure 5:38–39
Meningitis
  aseptic *see* Aseptic meningitis
  echovirus 30 2:70
  human enterovirus B associated 2:68
  lymphocytic choriomeningitis virus associated 3:238
  Toscana virus associated 4:495
Meningococcal disease-associated prophage (MDA phage) 3:123
Meningoencephalitis, lymphocytic choriomeningitis virus associated 3:238–239
Merck Manual of Diagnosis and Therapy, Infectious Diseases 5:357*t*
Meristem
  definition 5:282
  virus-induced gene silencing 5:378–379
Meristem tip culture 2:454
Messenger RNA (mRNA)
  definition 4:407
  giardiaviruses 2:314
  monocistronic 1:513
  narnavirus host persistence 3:397*f*
  polycistronic 1:513
Metabolic syndrome, HIV infection *see* HIV infection
Metabolism
  cellular *vs.* acellular evolution 3:475
  thiomersal safety in vaccines 5:233
Metalloproteinase, granulovirus gene expression 1:216
Metal shadowing
  helix handedness determination 2:82
  sample preparation 2:83*f*
Metapneumovirus
  avian 4:45
  classification 4:41*t*
  genome 3:327*f*
Metapneumovirus 4:41*t*
Metaviridae 3:301, 4:430, 4:451, 5:11*t*
Metavirus(es) **3:301–311**
  env gene/protein 3:309
    homology 3:301–302
    infection 3:309
  expression 3:302
    transposition levels 3:302–304
  genome 4:429*f*
  host range 3:302
  infections, fungi 2:287*t*
  integration 3:301
    target site specificity 3:309
  LTR-retrotransposons 4:433
  reverse transcriptase homology 3:301
  reverse transcription 3:307*f*
    genome circularization 3:306
    priming 3:306
    RNase H activity 3:306
  transposition
    flamenco, transcription factor 3:302–304
    levels 3:302–304
  see also specific viruses
Metazoan, definition 2:105
Methanococcus voltae, 'fusiform' viruses 2:300
Methanogenic Archaea-infecting virus(es)
  see Euryarchaea-infecting virus(es)
Methanogens, definition 5:411
Methanothermobacter marburgensis virus (ψM1) 5:412
  genome 5:415
    open reading frames 5:412–415
  pseudomurein endoisopeptidase 5:415
  related viruses 5:412
  structure 5:415*f*
Methionine homozygotes (PRNP) *see* Codon 129 polymorphism
Methyl-β-cyclodextrin
  definition 1:541
  HRV2 uptake 1:543–544
Methyl-transferase(s), gene silencing 4:142
mf–1 gene, *Cryphonectria parasitica* mating 2:578
mf–2 gene, *Cryphonectria parasitica* mating 2:578
M glycoprotein, toroviruses 5:153
MHC *see* Major histocompatibility complex (MHC)
MHV-68 *see* Murine gammaherpesvirus 68 (MHV-68)
MIANS (maleimide-1-anilo-8-naphthalene sulfonate) 5:35
Mice *see* Mouse/mice
Microangiopathy-associated renal disease 1:57
Microarrays 2:35–36
  bioinformatics 2:16
  clinical applications 2:17
    DFA testing *vs.* 2:17
    panviral arrays 2:17
  definition 5:450
  design, viral detection 2:15
    parameters 2:15
  diagnostic techniques 2:35–36
  DNA 2:14
  implications
    public health 2:18
    scientific 2:18
  limitations 2:17
  plant virus detection 2:26
  research applications 2:17
  sample amplification 2:15
    panviral 2:16
    sample complexity 2:16
  sample processing 2:15
    sample origin 2:15–16
Microfilaments
  definition 3:348
  plant virus movement 3:352
Microhabitat, definition 2:234
Microlesions, African horse sickness 1:41
Micromonas pusilla, viral infection of 1:87
Micromonas pusilla reovirus (MpRV) 1:90
  biological properties 1:90–91
    virion structure 1:92*f*
  classification 1:90*f*
  genome 1:92*f*
  host features 1:95
  phylogeny 1:93*f*
  viral co-existence 1:95
Microplitis demolitor bracovirus (MdBV)
  genome 4:259*f*
    coding density 4:259–260
    egf-motif genes 4:259–260
    glc gene family 4:259–260
    ptp family 4:259–260
    segment O gene family 4:259–260
    transposable elements 4:257–258
    vankyrin gene family 4:259–260
  host immune abrogation 4:254
    gene families 4:254
Micropropagation, definition 4:207
MicroRNAs (miRNAs) 4:141
  apoptosis 1:161
  BART gene 5:327
  biogenesis, Dicers 3:150
  definition 1:154
  EBV 2:164
  plant gene silencing 4:141
    methylation 4:141
  viral suppression 5:328*f*
  virus interactions 3:152
    SV40 3:152–153
Microsphere immuno assay (MIA), West Nile virus detection 5:448
Microsporidia 4:419
  retrotransposons 4:423
Microtubules
  baculovirus in vitro pathogenesis 1:271*f*
  definition 3:348
  plant virus movement 3:350–352
Microtus, cowpox virus hosts 1:577
Microviridae 5:11*t*
Microvirus(es) 3:13
  cell lysis 3:19
    protein E 3:19
  DNA packaging 3:19
    J proteins 3:19
  evolution 5:374
    caudoviruses *vs.* 5:374
    Gokushoviruses *vs.* 3:20
  gene expression 3:16
    mRNA stability 3:16–17
  genome content 3:14
  genome packaging 2:306
    nucleotide selection 2:307
  morphogenesis 3:17
    termination of 3:19
  structure 1:434*f*
    scaffold proteins 1:438
  virion morphology 3:14*f*
  see also Bacteriophage ϕX174
Middleburg virus (MIDV) 5:92
  characteristics 5:109*t*
  evolutionary relationships 5:97
Middle component (M), alfalfa mosaic virus 1:81–82
Middle East, HIV epidemiology 1:65
Midgut accessibility, nucleopolyhedrovirus primary infection 1:266
MIE transcription units, cytomegaloviruses 1:640
  promoter/enhancer regions 1:641*f*
Migration
  Dengue virus transmission 2:8
  rabies virus host range 4:372–373
Mild virus strains, papaya ringspot virus 4:4
Milk vetch dwarf virus (MDV)
  characteristics 3:389*t*
  genome 3:387*f*
  host range 3:386
  transmission 3:386
Milot-strain cross-pollination, definition 5:433
Milt, definition 3:83
Mimiviridae 3:312
Mimivirus 3:312
Mimivirus(es) **3:311–319**
  amino acid composition 3:313–314
  classification 3:312
  evolution 3:318*f*
    gene homologs 3:318
  gene content 3:313–314
    duplications 3:314–315
    NCLDV-associated core genes 3:315
  genome 3:314*f*
    DNA repair-related genes 3:316
    DNA synthesis genes 3:316
    host signaling interfering pathway genes 3:316
    intein domains 3:317
    introns 3:317
    metabolic pathway-associated genes 3:316
    promoter structure 3:315
    translation-related genes 3:316
  history 3:312
  host range 3:312
  pathogenicity 3:312
  replication cycle 3:313
    infection scenario 3:313*f*
    transcription 3:313–314
  virion structure 3:317
    Gram staining 3:312*f*
    morphology 3:312*f*
    particle-associated mRNAs 3:318
    proteomics 3:317
  see also specific viruses
Mimotopes 1:140
  cross-reactivity 1:140
  definition 1:137
  screening 1:140
Min Jou sequence, icosahedral ssRNA bacteriophages 3:26
Mint virus X (MVX) 4:311*t*
Minus-strand synthesis, alfalfa mosaic virus RNA replication 1:84
Minus to positive strand switch, alfalfa mosaic virus RNA replication 1:86
Mirafiori lettuce virus (MiLV) 3:447
  coat protein 3:450
    phylogenetics 3:450

control, soil sterilization 3:453
genome 3:449
RNA2 3:449
RNA4 3:449
sequence 3:448–449
transmission, *Olpidium brassicae* 3:453
miRNAs *see* MicroRNAs (miRNAs)
Mites
mycoreovirus transmission 3:382–383
plant virus transmission 5:280
Mitochondria, nodavirus infection 3:436*f*
Mitochondrial apoptosis pathway
caspase activation 1:155–157
viral regulators 1:161
Bcl-2 family proteins 1:156*f*
Mitovirus 2:288
Mixed infections
anellovirus 1:109
aquareovirus 1:166
Pepino mosaic virus 4:107
see also specific infections
MK-0518 2:510
M-like protein, yellow head virus 5:477–478
MM virus 1:441–442
MMWR (Morbidity and Mortality Weekly report) 5:357*t*
ModA/ModB, bacteriophage T4 transcription 5:177
Modified live virus (MLV) vaccine
bovine viral diarrhea viruses 1:376
classical swine fever virus 1:531
infectious laryngotracheitis virus 2:406
Modular evolution model, bacteriophage evolution 5:372
Modulator of immune recognition (MIR), KSHV immune evasion 3:200
Mokola virus 4:184*t*
Molecular assays
Kadipiro virus 4:544–545
pecluvirus infections 4:99
seadornavirus infections 4:544–545
Molecular biology, techniques 2:456
see also specific methoods
Molecular chaperones 3:348
Molecular clock models
HIV origin 2:532
phylogenetic analysis 4:127
phylogeny 4:125
Molecular epidemiologic studies 2:143
definition 2:143
Molecularity
definition 3:413
infection neutralization 3:416
stoichiometry *vs.* 3:416
multihit 3:417
Poisson distribution 3:417*f*
Molecular piracy, KSHV 3:192
Molecular strain typing, definition 4:330
Molluscipoxvirus 2:136, 4:326–327
Molluscs, definition 4:561
Molluscum contagiosum virus (MOCV) **3:319–324**
avipoxviruses *vs.* 2:276
classification 3:319
epidermal growth factor receptor expression 3:320
evolution 3:320
genetic subtypes 3:320
genome 3:321*f*
poxvirus homology 3:320
history 3:319
infection
acanthoma 3:322
bacterial superinfection 3:322
clinical features 3:322
diagnosis 3:323
differential diagnosis 3:323
eye 2:496
host range 3:321
immune response 3:323
incidence 3:322
outbreaks 3:322
pathology 3:322
prevention 3:323
transmission 3:322
treatment 3:323
nonstructural proteins 3:320
propagation 3:321
survival factors, glutathione peroxidase 2:275–276
variola virus *vs.* 2:276
virion morphology 3:320*f*
Molluscum lesion 3:319
appearance 3:322
composition 3:322
persistence 3:322
Molting inhibition, baculovirus infection 1:252
Mongooses, rabies virus host range 4:373
Monitoring, phylogeny 4:127–128
Monkey(s)
SV40 hosts 4:636
see also specific types
Monkeypox virus (MPXV) 4:641
classification 4:641
ecology 4:643
evolution 4:643
future work 4:644
genetics 4:643
geographic expansion 4:643
Sudan 4:643
USA 4:643
historical aspects 4:641
infection *see below*
transmission 5:491
virion structure 4:642*f*
Monkeypox virus infection
disease surveillance 5:491
epidemics 5:491
fatality rate 5:491
host range 4:643
new hosts 4:643
humans 4:641
animal contact 4:642
clinical features 4:641
diagnostics 4:644
epidemiology 4:641
misdiagnosis 4:641–642
person-to-person transmission 4:642*f*
smallpox *vs.* 4:641
treatment 4:643
zoonoses 5:491
Monocistronic mRNA, definition 4:623
Monoclonal antibodies (MAbs)
avipoxvirus detection 2:280
Monoclonal antibodies, bean common mosaic virus 1:292
definition 4:623
Ebola virus infection treatment 2:64
HIV-1 vaccine development 1:141
passive immunization 3:67
potato virus Y 4:290
TMV structure elucidation 5:58
transgenic plant expression 4:232*f*
TMV utilization 4:236–237
use in epidemiology 2:143
watermelon mosaic virus 5:436
zucchini yellow mosaic virus 5:436
Monocotyledons
definition 1:533
mastrevirus infections 4:166
resistance genes, recessive 4:178
Monocyte chemotactic protein(s) (MCP) 1:623–624
Monocytes
Ebola virus infection 2:202–203
innate immunity 3:112
murine CMV infection 1:630*t*
rhinoviral infections 1:547
Monomer circularization mechanism, avsunviroids 4:479–480
Mononegavirales 3:325, 4:40, 5:291
Mononegavirus(es) 4:47
assembly 3:333
budding 3:333
definition 3:447
genome 3:329
gene end signals 3:329
gene start signals 3:329
intergenic regions 3:330
promoter sequences 3:329
glycoproteins 3:332
host cell entry 3:333
mRNA splicing 3:330
phylogenetic tree 3:328*f*
replication 3:330–331
RNA replication 3:330
transcription 3:330
initiation 3:330
polymerase 3:330
termination 3:330
viral proteins 3:331
glycoproteins 3:332
L protein 3:332
matrix proteins 3:331
nonstructural proteins 3:332
nucleocapsid 3:331
phosphoprotein 3:331
P mRNA encoded proteins 3:331
virus structure 3:329*f*
nucleocapsid complex 3:325–329
see also specific viruses
Mononuclear phagocytes, Dengue virus infection 2:10
Monopartite genomes
geminiviruses 4:165
potyviruses 4:314, 4:316–317
Monopartite virus(es) 4:164
see also specific viruses
Monophyletic group, definition 2:105
Mopeia virus 3:244
Morbillivirus 3:285, 4:41*t*, 4:498
Morbillivirus(es) 3:285, 4:47, 4:498
classification 4:41*t*
evolution 4:501–502, 4:501*f*
genome 3:327*f*, 4:500–501, 4:500*f*
encoded proteins 4:42–43, 4:500–501
genome length requirement 4:501
mRNA synthesis 4:500–501
nonstructural proteins 4:501
host cell receptors 4:500
infection
cytopathic effects 4:500
incubation period 4:504
route 4:45–46
sites 4:45–46
phylogeny 4:45
propagation 4:40–42, 4:500
replication, host cell attachment 4:44
serology, H protein 4:502
signaling lymphocyte activation molecule 3:285
transmission 4:45–46
see also specific viruses
Morphogenesis, baculoviruses 1:251
Mosquito(es) 2:235–236
cell lines 4:539
cg4715 protein, lineage 4:454
disease control methods 5:116
larval control 2:13
vertical transmission 2:8
Mosquito(es), as vectors
Akabane virus 1:77, 1:78–79
alphavirus 5:99, 5:111–112
bovine ephemeral fever virus 1:355
Chikungunya virus 5:111–112
control 2:13, 5:449
Dengue virus 2:5, 2:7
eastern equine encephalitic virus 5:78, 5:101
Eastern equine encephalitis virus 5:111–112
equine encephalitic togaviruses 5:102, 5:105, 5:106
flaviviruses 2:236
Israel turkey meningoencephalomyelitis virus 2:237
Japanese encephalitis virus 2:236, 3:184, 3:186
Saint Louis encephalitis virus 4:656
seadornavirus 4:537
Sitiawan virus 2:237
St. Louis encephalitis virus 2:236
Tataguine virus 1:400
Venezuelan equine encephalitic virus 5:111–112, 5:112*f*

Mosquito(es), as vectors (*continued*)
  Wesselsbron virus 2:237
  western equine encephalitic virus 5:77–78, 5:101
  West Nile virus 2:236, 5:443–444, 5:445
  yellow fever virus 2:238
  zoonoses 5:494
  see also individual species
Mosquito baculovirus(es)
  auxiliary functions 1:224
  see also specific viruses
  classification 1:220, 1:220*t*
  DNA replication 1:223
  epizootiology 1:222
  genome 1:222
  histopathology 1:220
  history 1:219–220
  host range 1:221
  life cycle 1:221
  occlusion bodies 1:220
    properties 1:220
    structure 1:221*f*
  pathology 1:220
  phylogeny 1:224, 1:224*f*
  RNA transcription 1:222
  structural functions 1:223
  transmission 1:221
  virion functions 1:223
  virion occlusion 1:223
  virion properties 1:220
Mosso das Pedras virus (MDPV) 5:109*t*
Mossuril virus (MOSV) 4:184*t*
Most recent common ancestor (MRCA), phylogenetic trees 4:126
MotA, bacteriophage transcription role 5:177
Moth(s), Hz-2V infection 3:147
Mother-to-child transmission, HIV infection 1:60–61
Mount Elgon Bat Virus (MEBV) 1:118
Mouse/mice
  lactate dehydrogenase-elevating virus infection 1:183
  models
    coxsackievirus isolation 1:582
    Ebola virus infection 2:62
    rhabdovirus isolation 1:111
    SARS infection 4:558
    VEEV infection 5:115
  strains, Ectromelia virus susceptibility 3:343
  transgenic *see* Transgenic mice;
  see also under Murine
Mouse encephalomyelitis virus(es) *see* Theiler's murine encephalomyelitis virus (TMEV)
Mouse-encephalomyelitis virus (ME virus) 1:441–442
Mouse hepatitis virus (MHV)
  replication 3:427
  RNA recombination 1:560
Mouse leukemia virus (MLV)
  mammalian cell infection 2:469
  mammalian cell resistance 2:469
  resistance 2:469
Mouse mammary tumor virus (MMTV) **3:334–342**
  capsid protein 3:335
  cell entry, transferrin receptor 1 3:336
  classification 3:335
  endoribonuclease activity 3:222
  future work 3:341
  genetic experimentation 3:339
  genetics 3:338
  genome 3:335, 3:335*f*
    long terminal repeats 3:335–336
    precursor polypeptides 3:335–336
    primer-binding site 3:335–336
    repeat regions 3:335–336
    splice acceptor 3:335–336
    splice donor 3:335–336
  history 3:335
  infection *see below*
  integrase 3:335
  middle T, breast cancer model 4:274
  nomenclature 3:335
  nucleocapsid protein 3:335
  replication 3:336
    frameshifts 3:336–337, 3:337*f*
    host genome integration 3:336
    intracytoplasmic A particles 3:336–337
    long terminal repeats 3:336
    precursor protein translation 3:336–337
    preintegration complex 3:336
    Rem protein 3:336–337
    reverse transcriptase 3:336
    RNA polymerase II 3:335*f*, 3:336
    sag gene 3:335*f*, 3:336
    termination 3:336
  reverse transcriptase 3:335
  RNP 3:335
  susceptibility 3:338
  taxonomy 3:335
  tissue tropism 3:337
    Cut-like protein1/CCAT displacement protein 3:340
    mammary gland enhancer 3:341
    negative regulatory elements 3:340
    special AT-rich binding protein 1 3:340
  transcriptional regulation 3:340
    glucocorticoids 3:340
    hormone-regulated gene expression 3:338*f*, 3:340
    transcription factor IID 3:340
  transmission 3:337, 3:338*f*
    horizontal 3:337
    intestinal tract 3:337
    maternal milk 3:337
    MHC class II 3:337
    T-cell receptor 3:337
    vertical 3:337
  virion 3:335
    capsid protein 3:335
Mouse mammary tumor virus infection
  immune response 3:341, 3:341*f*
    Sag proteins 3:341, 3:341*f*
    T cells 3:341
  mammary adenocarcinomas 3:338–339
  pathogenicity 3:339
    insertional mutagenesis 3:339
    integration sites 3:339–340
    T-cell lymphomas 3:339–340
  resistance to 3:339
    neutralizing antibodies 3:339
    Toll-like receptor 4 signaling 3:339
  susceptibility 3:338
    cellular factors 3:339
    host factors 3:339
    MHC class II subtypes 3:339
    mouse strains 3:338–339
Mouse minute virus (MMV), evolution 2:182–183
Mousepox virus *see* Ectromelia virus
Movement disorders, West Nile virus infection 5:447
Movement proteins (MPs) 3:349, 3:350*t*, 5:145
  alfalfa mosaic virus 1:82–83, 1:85
  banana bunchy top virus 1:274
  caulimovirus 1:465
  definition 3:264, 3:348
  flexivirus phylogenetics 2:256
  functional characteristics 3:349
  furovirus 2:292
  Maize streak virus *see* Maize streak virus (MSV)
  potexvirus 4:312
  tombusvirus replication 5:148–149
  tospoviruses 5:134–135
Movement restriction, African horse sickness control 1:42
MP-12 vaccine, Rift Valley fever virus 4:496
M protein
  measles virus 3:287
  Sigma virus structure 4:576–577
MPyV, receptors 4:279
MRC-5 vaccine, Rift Valley fever virus 4:496
MRE11-RAD50-NSB1 (MRN) complex *see* Adenovirus infections
M RNA segment sequence, Crimean-Congo hemorrhagic fever virus 1:599, 1:600–601
M trait, baculovirus infection resistance 1:267
Mu bacteriophage *see* Bacteriophage Mu
Mucambo virus (MUCV), characteristics 5:109*t*
Mucins
  definition 2:543
  Glc1.8 4:254
Mucosa-associated lymphoid tissue (MALT)
  definition 2:534
  edible vaccine development 5:222
  HIV infection, role 2:538*f*, 2:539–540, 2:541
Mucosal disease (MD) 1:374
Mucosal edema 1:328
Mucosal immunity, vaccination 5:223
Mulberry ringspot virus (MRSV) 3:406*t*
Muller's ratchet 2:179, 4:359
  definition 2:175
  VSV evolution 5:297
Multianalyte diagnostic techniques 2:35–36
Multicentric Castleman's disease (MCD) 3:194, 3:195, 3:201, 5:196
Multicentric fibrocarcinoma(s), feline leukemia viruses 2:188
Multi-dose vials, vaccine safety 5:229
Multigene families 1:43
  African swine fever virus 1:48
Multinuclear zinc-binding protein (ZBD), yellow head virus 5:477–478
Multiorgan failure, yellow fever vaccine 5:234
Multiple DNA-B components, mungbean yellow mosaic viruses 3:366
Multiple embedded nucleopolyhedrovirus (MNPV) 1:248
Multiple homologous repeat regions, baculoviruses 1:249
Multiple nucleocapsid per envelope (MNPVs), nucleopolyhedrovirus packaging 1:265, 1:266*f*
Multiple sclerosis (MS)
  animal model 5:43–44
  hepatitis B *vs.* 5:233
  human herpesvirus infection 2:504
  viral etiology 1:475
Multiple serotype vaccines 5:240
Multiple transcription initiation sites, mungbean yellow mosaic viruses 3:367
Multiplicative landscape
  definition 4:359
  error catastrophe 4:362
  quasispecies 4:362
Multiplying (M-type) infection, tobraviruses 5:73
Multivalent vaccines, measles virus *see* Measles virus
μ1 protein, reovirus structure, outer capsid 4:394*f*, 4:396
μ2 protein, reovirus structure, inner capsid 4:393–394
Mumps
  clinical features 3:356, 3:361
    associated organs 3:362*t*
    incubation period 3:361
    prognosis 3:362*t*
  disease surveillance 2:45
    data collection sources 2:45
  epidemiology 3:361
    acquired immunity 3:361
    genotypes 3:361
    history 3:361
    incidence 3:362*t*
  histopathology 3:357*f*, 3:361
  host range 3:360
    experimental 3:360
  immune response 3:362
    cell-mediated 3:362–363
    humoral 3:362–363
  nervous system infection 1:469
  ocular complications 2:495
  pathogenicity 3:362
    experimental infection 3:362
    neurovirulence 3:362
  pathology 3:361, 3:362*t*
    isolation 3:361
  prevention and control 3:363
  serology 3:360
Mumps virus (MuV) **3:356–363**
  chemical sensitivity 3:356
  classification 3:356
  distribution 3:360
  evolution 3:360

genetics 3:360
reverse 3:363
genome 3:327*f*, 3:357, 3:358*f*
history 3:356
epidemiology 3:361
inclusion body myositis and 3:363
infection *see* Mumps
propagation 3:360
cytopathic effect 3:360
protein properties 3:357, 3:358*t*
small hydrophobic protein 3:357, 3:360
RdRp 3:358–359, 3:359–360
replication 3:357
budding 3:359–360
co-transcriptional editing 3:359
host cell attachment 3:357–358
Ig sequences 3:359, 3:359*f*
site of 3:361
transcription 3:358–359
translation 3:359
SH protein 4:55
taxonomy 3:356
tissue tropism 3:361
transmission 3:361
vaccines/vaccination 3:361, 3:363
Jeryl Lynn strain 3:363
see also Measles/mumps/rubella (MMR) vaccine
virion properties 3:356
physical 3:357
structure 3:356, 3:357*f*
Mungbean yellow mosaic India virus (MYMIV) 3:364
DNA β component associated 1:320
genome 3:365–366, 3:365*f*
phylogenetic relationships 3:370–371
replication, Rep protein 3:366
transmission 3:368
as type species 3:364
Mungbean yellow mosaic virus(es) **3:364–372**
classification 3:364
coat protein 3:364–365
ecology 3:368
ELISA 3:364–365
epidemiology 3:368
gene expression regulation 3:366
BC1 protein 3:367
multiple transcription initiation sites 3:367
Rep protein 3:367
genome 3:365–366, 3:365*f*
common region 3:366
multiple DNA-B components 3:366
open reading frames 3:365–366
geographical distribution 3:364
infection *see below*
infectivity 3:367
Agrobacterium tumifaciens experiments 3:367
phylogenetic relationships 3:368*t*, 3:370–371
nucleotide sequence 3:369*t*, 3:371
phylogenetic tree 3:371, 3:371*f*
phylogenetic trees 3:371*f*
replication 3:366
Rep protein 3:366
rolling circle replication 3:366
taxonomy 3:364
transcript analysis 3:366–367
RT-PCR 3:366–367
transmission 3:368
acquisition access period 3:368
inoculation access period 3:368
vector lifecycle 3:368
whitefly 3:366, 3:368
as type species 3:364
virion structure 3:364
coat protein 3:364–365
see also specific viruses
Mungbean yellow mosaic virus infections
host range 3:367
management 3:369
cultural practices 3:369–370
disease resistance 3:369
RNAi 3:370
symptoms 3:364, 3:365*f*
Mupapillomavirus 4:9, 4:10*f*, 4:11*t*
MurA
inhibition 3:256–257, 3:256*f*, 3:257–258
missense mutation 3:257–258
Murein, synthesis inhibition 3:256–257, 3:256*f*
Murine cytomegalovirus(es) (MCMV) **1:624–634**
classification 1:635–636, 1:635*t*
genome 1:626, 1:627*f*, 1:636–637
Smith strain 1:626
laboratory strains 1:639
latency 1:629, 2:440
transcription regulation 1:629
reactivation 1:629
replication 1:627–628, 1:628*f*
capsid formation 1:628–629
origin of replication 1:627*f*, 1:628
transcription regulation 1:629
virion morphology 1:625
virion structure 1:625
capsid 1:625
envelope 1:626
glycoproteins 1:626*t*
tegument 1:625–626
Murine cytomegalovirus infections
antibodies 1:631
associated diseases 1:629
affecting factors 1:629
fibroblasts 3:321–322
host immune response 1:629, 1:630*t*
associated genes 1:630
immune system evasion 1:629, 1:630*t*
pathogenesis 1:629
seroprevalence 1:629
Murine gammaherpesvirus 68 (MHV-68) **3:372–378**
ecology 3:372
egress 3:374
genome 3:373, 3:373*f*
open reading frames 3:373
host cell entry 3:375
infection *see below*
origins 3:372
replication 3:374
essential genes 3:374
nonessential gene expression 3:374
transcription 3:374
spread 3:375, 3:376
strains 3:373
structure 3:373
unique viral genes, expression 3:373, 3:374
clusters 3:373–374
M1 3:374–375
M2 3:374–375
M3 3:374–375
M4 3:374–375
vtRNA 3:374–375
Murine gammaherpesvirus 68 infection 3:374
associated cell types 3:375
B cells 3:374
lymphomas 3:377
M2 gene 3:375
mediastinal lymph node 3:375
S11 cell line 3:377
inflammatory response 3:375
influencing host factors 3:376
latency 3:376
associated cell types 3:376
M2 gene 3:376–377
NS0 cell line 3:374
ORF73 3:376–377
pathogenesis 3:376*f*, 3:377
lymphoma 3:377
splenomegaly 3:377
Murine hepatitis virus (MHV) 1:553
Murine leukemia virus(es) (MLVs)
defective interfering viruses 2:4
evolution 2:183–184
xenotropic behavior 2:468
Murine norovirus(es) 1:418
see also specific viruses
Murine polyomaviruses *see* Polyomavirus(es), murine
Muromegalovirus 1:635–636, 2:432*t*
Murray Valley encephalitis (MVE) virus
characteristics 1:171*t*
geographical distribution 5:493
seasonal distribution 5:493
zoonoses 5:493
Muscle
Akabane virus infection 1:78
togavirus infection 5:83–84
Mushroom bacilliform virus (MBV) 1:287, 1:510, 2:288
evolutionary relationships 1:287, 1:288*f*
host range 1:288
phylogeny 4:652
transmission 1:288
virion properties 1:287
virion structure 1:287, 1:287*f*
Muskrats, Omsk hemorrhagic fever virus 2:239–240
Mutagenesis, insertional *see* Insertional mutagenesis
Mutagens, fungal retrotransposons 4:426
Mutation(s)
antiviral therapy 1:150
chronic hepatitis B 2:358
evolution 4:125
LTR retrotransposons 4:439
Mutational resistance, antibodies 5:323–324
Mutational robustness 4:359
quasispecies *see* Quasispecies
Mutation rates
rhinoviruses 4:470
RNA viruses 4:377
viral evolution 2:177
Mutation–selection balance 4:359
Mx proteins, interferon-induced antiviral response 3:107, 3:115–116
Myalgia 1:399
definition 1:580
Mycoreovirus 2:286, 3:378, 3:379*f*, 3:380, 4:536*t*
Mycoreovirus(es) **3:378–383**
gene expression, fungal 3:383
genome 3:380
encoded proteins, functions 3:380
segments 3:380, 3:381*f*
genomic relationships 3:380
infection 3:379–380
asexual spores 3:380
egress 3:379–380
μ1 protein homologs 3:379–380
mixed 3:380
movement, hyphal anastomosis 3:380
nomenclature 3:380
structure–function relationships 3:378
guanyltransferase 3:378–379
taxonomy 3:378, 3:379*f*, 3:380
transmission 3:380, 3:382–383
see also specific viruses
Mycoreovirus-1/Cp9B21 2:285, 3:381
double infection 3:381
gene product expression 3:381
host, effects 3:383
transmission 3:381
as type species 4:536*t*
virulence 3:381
Mycoreovirus-2/CpC18 3:382
host, effects 3:383
infected culture phenotype 3:379*f*, 3:382
MyRV-1/Cp9B21 *vs.* 3:382
Mycoreovirus-3/RnW370 3:382
composition 3:382
transmission 3:382–383
white rot control 3:382
Mycovirus(es) 4:63, 4:68–69
classification 4:63
definition 2:109, 2:284, 4:68
dsRNA genomes 2:286
infectivity 2:290
ssRNA genomes 2:288
transfection 4:66
unassigned ssRNA viruses, genomes 2:288
see also Chrysovirus(es); *specific viruses*
Myelin deficiency, Border disease 1:336–337
Myeloid dendritic cells (mDC) 3:112
type I interferon induction 3:115

Myiasis, definition 1:427
Myocardiotropism 1:440
Myocarditis
  coxsackie B virus 2:68–69
  definition 1:440, 1:533
Myositis *see* Human T-cell leukemia virus 1 infection
Myoviridae 3:30–31, 5:11*t*, 5:413*t*
Myovirus(es) 3:32*t*
  Euryarchaeota infections 5:413*t*
  genome packaging 2:306, 2:307*t*
  long contractile tails 5:367
  structure 1:434*f*, 3:31
    baseplates 1:437–438
    tails 1:437–438
  tails/tail fibers 3:32
  see also specific viruses
Myristic acid, capsid stabilization 2:128
Myrobalan latent ringspot virus (MLRSV) 3:406*t*
Myxomatosis 4:329
  clinical features 3:228–229
    primary tumors 3:228–229
    secondary tumors 3:229
  mortality rates 3:225, 3:228
  pathogenicity 3:228
  rabbit eradication programs 3:228
Myxoma viruses (MYX) 4:329
  characteristics 3:225*t*
  history 3:225
  infection
    apoptosis inhibition 1:161
    immune modulation 3:227
    immune response 3:230
    viral migration 3:229
    see also Myxomatosis
  virulence 2:146, 3:228

N

N1a gene/protein 4:3
N1b gene/protein 4:3
N4 bacteriophage *see* Bacteriophage N4
NADL strain, bovine viral diarrhea viruses 1:376–377
Nairobi sheep disease virus (NSDV) 1:391*t*, 1:596
  historical aspects 1:596, 1:597
  Rhipicephalus appendiculatus tick vector 1:598
Nairovirus 1:391, 1:391*t*, **1:596–603**, 3:480, 4:490–491
Nairovirus(es) 1:399
  history 1:596
  M segment-encoded polyprotein 1:395
  species 1:596, 1:598*t*
Nanoscale material manufacture, plant virus vector applications 4:236
Nanoviridae 1:274, 3:385, 5:11*t*
Nanovirus 3:385
Nanovirus(es) **3:385–391**
  aphid transmission 3:386
  associated DNAs 4:533
  biological properties 3:386
    host range 3:386
    tissue tropism 3:386
  characteristics 3:389*t*
  economic importance 3:386
  genome 1:315–316, 3:387, 3:387*f*
    DNA structure 3:387
    DNA types 3:388, 3:389*t*
    integral genome segments 3:388
    open reading frames 3:390
    satellite-like rep proteins 3:388, 3:390*f*
  geographical distribution 3:385
  history 3:385
  phylogenetic relationships 3:391
    circoviruses *vs.* 3:391
    geminiviruses *vs.* 3:391
  proteins 3:387*f*, 3:389*t*, 3:390
    DNA-C 3:390
    DNA-M 3:390
    DNA-N 3:390
    functions 3:387
    nonstructural 3:390
    nuclear shuttle protein 3:390
    rep 3:390
    Rep-1 satellites, DNA-1 component *vs.* 1:565
  replication 3:390–391
    DNA replication 3:390–391
    dsDNA synthesis 3:390–391
    mRNA transcription 3:390–391
  transmission 3:386
    aphids 3:386
  trans-replication 1:315–316
  virion properties 3:385
    serology 3:386
    structure 3:386*f*
  see also specific viruses
Narcissus mosaic virus (NMV) 4:311*t*
  genome 4:312
Narnaviridae 2:288, 3:392, 5:11*t*
Narnavirus 2:288, 3:392
Narnavirus(es) **3:392–398**
  cis-acting replication signals 3:394
    negative strands 3:395–396
    positive strands 3:394–395, 3:395*f*
  double stranded RNA satellites 4:531
  gene expression 2:289
  genome 2:288, 3:392, 3:393*f*
  historical background 3:392
  infections *see below*
  in vivo generation 3:394
  proteins 3:393, 3:393*f*
  replication intermediates 3:394
    RdRp 3:394
  virion lack 3:392
  see also Ribonucleoprotein (RNP)
Narnavirus infections
  fungi 2:287*t*
  host persistence 3:392, 3:396
    mRNA degradation 3:396, 3:397*f*
    'top-half' domain 3:395*f*, 3:398
Nasal infection route 5:155
Nasal T-cell lymphoma 5:195
Nasopharyngeal carcinoma 5:195
  anaplastic 5:195
  incidence 2:155, 5:195
  tumor characterization 2:155
  see also specific virus infections
National Bioforensics Analysis Center 5:410
National Center for Biomedical Ontology 5:357*t*
National Center for Biotechnology Information (NCBI) 5:356
  Assembly Archive 5:357*t*
  database 5:357*t*
  Entrez search engine 5:356–362
  pairwise sequence comparison, implementation 5:344
    PASC interface 5:344
  PubMed search engine 5:356–362
  Reference Sequence database 5:362–363
  Trace Archive 5:357*t*
  Viral Genome Resource 5:357*t*
  Viral Genomes Project 5:362
  Viral Genotyping Tool 5:357*t*
National Center for Health Statistics, common cold 1:541
National Immunization Days (NIDs), polio vaccination 4:245–246
Natrialba magadii, ϕCh1 infection 5:418
Natsudaidai dwarf virus (NDV) 4:523, 4:524*t*
  genome sequencing 4:524–525
  host range 4:525
  transmission 4:525
Natural *cis*-antisense transcript-derived siRNA pathway 4:142
Natural killer (NK) cells 3:110, 3:112
  definition 3:78
  EBV-associated lymphoma 2:155, 5:195
  function 3:110
  lymphocytic choriomeningitis virus infection 3:242
  murine CMV infection 1:630–631, 1:630*t*
  regulation 3:110
  yellow fever 5:475
Natural resistance, vegetable viruses 5:286–287, 5:287*t*
Natural selection 3:402
Navel orange infectious mottling virus (NIMV) 4:523, 4:524*t*
  genome sequencing 4:524–525
  host range 4:525
  transmission 4:525
NB-LRR protein, definition 4:170
NCBI *see* National Center for Biotechnology Information (NCBI)
ND10/POD domains, KSHV replication 3:199
Ndumu virus (NDUV) 5:109*t*
Necrosis
  apoptosis *vs.* 1:157
  definition 1:154–155, 4:207
  measles 3:289
Necrovirus 3:403, 5:145–146
Necrovirus(es) **3:403–405**
  genome 3:403, 3:403*f*
    open reading frames 3:403
    positive-sense RNA strand 3:403
  geographical distribution 3:404
  infection, host range 3:404
  interspecific relationships 3:404
    serology 3:404
  necrovirus, tobacco disease 5:66
  satellite viruses 3:404, 4:527*t*
  structure 3:403
  transmission 3:404
    seeds 3:404
    soil 3:404
    vectors 3:404
  type species 3:403
  virion particles 3:403
  virus–host relationships 3:404
  see also specific viruses
Needleman–Wunsch algorithms (classification) 5:343
nef gene, simian immunodeficiency virus 4:622
Nef protein (HIV) 2:523
  expression 2:524
  functions 2:523–524
Negative regulatory elements, mouse mammary tumor virus 3:340
Negative sense genome, definition 5:157
Negative staining 2:79, 2:80*f*
  advantages 2:81
  heavy metal salts 2:79
  image analysis 2:85–86
  limitations 2:81
  positive staining *vs.* 2:79
  sample preparation 2:79, 2:80*f*
    concentration 2:79
  stains 2:79
    ammonium molybdate 2:81
    phosphotungstic acid 2:81
    sodium silicotungstate 2:79, 2:80*f*
    uranyl acetate 2:79, 2:80*f*
  support films 2:79
Negative-strand RNA virus(es)
  recombination 4:380
  see also specific viruses
Negishi virus 2:241
Negro coffee mosaic virus (NeCMV) 4:311*t*
Neighbor-joining methods
  definition 2:525
  phylogenetic analysis 4:126–127
Neisseria meningitidis, virulence factors 3:123
Nelfinavir 2:514*t*
Nelson Bay virus (NBV) 4:383
Nematicides, nepovirus control 3:412
Nematodes, plant virus transmission 5:280*f*, 5:281
  grapevine disease 4:203
  legume virus 3:217
  pea-early browning virus 3:217, 3:218
  tobacco rattle virus 4:308
Nemesia ring necrosis virus (NeRNV) 4:215, 4:219*f*, 5:200*t*, 5:204
  nemesia infections 4:215–217, 4:220*f*
  verbenaceae infection 4:218
Nemesia virus infections 4:215
  see also specific viruses
NendoU, nidovirus genome 3:425
Neocheck-S, insect pest control 3:130

Neodiprion abietis nucleopolyhedrovirus (NeabNPV) 1:225–226
Neodiprion lecontei nucleopolyhedrovirus (NeleNPV) 1:226
  gene parity plots 1:228, 1:229*f*
  genome 1:226, 1:227*f*
    auxiliary genes 1:228
    DNA repeat regions 1:229
    inhibitors of apoptosis 1:226
    membrane fusion proteins 1:228
    nonsyntenic region 1:226, 1:227*f*
    regulator of chromosome condensation 1:228
    trypsin-like serine protease 1:226–227
    zinc finger protein 1:228
  phylogeny 1:229
Neodiprion sertifer nucleopolyhedrovirus (NeseNPV) 1:226
  gene parity plots 1:228, 1:229*f*
  genome 1:226
    auxiliary genes 1:228
    DNA repeat regions 1:229
    inhibitors of apoptosis 1:226
    membrane fusion proteins 1:228
    nonsyntenic region 1:226, 1:227*f*
    regulator of chromosome condensation 1:228
    trypsin-like serine protease 1:226–227
    zinc finger protein 1:228
  phylogeny 1:229
Neonatal disease(s)
  coxsackieviruses 1:585
  HCMV 2:483
  see also specific infections
Neonatal immunization, maternally derived antibodies 2:53
Neoplasia
  definition 2:212
  reticuloendotheliosis virus infection 4:416
  simian immunodeficiency virus infection 4:608–609
  see also Cancer; Carcinogenesis
Neotopes
  definition 1:137–138
  TMV antigenicity 5:58
Neotropical virus(es) 5:489
Nephotettix cincticeps, rice dwarf virus transmission 1:483
Nephotettix impicticeps, rice tungro virus transmission 1:485
Nephropathia epidemica (NE) 2:317, 2:318
  clinical features 2:319–320
Nephropathy, polyomavirus-associated 4:269
Nephrotoxicity
  antiretroviral treatment 1:54–55
  antiviral therapy 1:147
Nepovirus 1:573, 3:405, 4:523
Nepovirus(es) **3:405–413**
  cell-to-cell movement 3:407*f*, 3:410
  future research 3:412
  genera 3:406*t*
  genome 3:407, 3:408*f*
    sequence identity 3:408, 3:408*f*
    untranslated regions 3:408
    viral protein linkage 3:407–408
  historical perspective 3:405
  infections *see below*
  long distance movement 3:410
  particle structure 3:407, 3:407*f*
  population structures 3:412
    sequence diversity 3:412
  regulated polyprotein processing 3:409
    NTB domain 3:409
    proteinases 3:409
  replication 3:409
    membrane proliferation 3:409
    NTB protein 3:409–410
    VPg domain 3:409–410
  satellites 3:410
    type D 3:411
    VPg links 3:410–411
  transmission 3:411
    aerial vectors 3:411
    seed transmission 3:411
    soil nematodes 3:411
    vector species specificity 3:411
  see also specific viruses
Nepovirus infections 3:411
  control 3:412
    infected plant removal 3:412
    nematicides 3:412
    transgenic plants 3:412
  economic impact 3:411
  host range 3:410
  infectious DNA clones 3:408–409
  petunia infection 4:213
  plant post-transcriptional gene silencing 3:410
  symptomatology 3:410
  tobacco disease 5:65
Nerine virus X (NVX) 4:311*t*
Nerve terminals, CNS invasion pathways 1:470
Nervous system virus(es) **1:469–475**
  acute infections 1:474
  see also Encephalitis
  anatomic considerations 1:469
    blood–brain barrier 1:469–470
    ependymal cells 1:470
    extracellular environment 1:469–470
    neuron membranes 1:470
  cell damage mechanisms 1:473
    immune responses 1:473
    lysis 1:473
  clinical features 1:474
  CNS invasion pathways 1:470, 1:471*f*
    along nerves 1:470
    anterograde transport 1:470
    blood-bourne 1:470, 1:472*f*
    blood–brain barrier 1:471
    capillary endothelia 1:471–472
    choroid plexus 1:471–472
    experimental systems 1:470
    nerve terminals 1:470
    olfactory spread 1:470–471
    particle size *vs.* 1:471
    reticuloendothelial cells 1:471
  neural cell infection 1:473
    age-relation 1:473
    axoplasmic flow 1:473
    cell-to-cell spread 1:473
    extracellular antibody neutralization 1:473
    oligodendrocytes 1:473
    vascular endothelial cells 1:473
  physiologic considerations 1:469
    antibodies 1:470
    T cells 1:470
  slow infections 1:474
    dementia 1:474
  see also Neuroinvasive disease; *specific viruses*
Neuraminidase (NA)
  definition 1:127
  function 1:149
  human parainfluenza viruses 4:47–48
  infection neutralization 3:413–414
  influenza viruses *see* Influenza virus neuraminidase
  mumps virus 3:356
Neuraminidase inhibitors 1:149
Neurodegenerative disease, JCV infection 4:262
Neuroinvasive disease 4:652
  definition 1:469, 5:440
  Japanese encephalitis virus 3:185
  West Nile virus 3:466
Neurological disease
  Border disease 1:336
  equid herpesvirus 1 infection 2:417–418
  henipavirus infections 2:325
  Nipah virus infection 2:325
Neuronal cell death, prions 4:336
Neuronal inclusions, rabies virus 4:371
Neuropsychiatric changes, Crimean-Congo hemorrhagic fever 1:601
Neurospora crassa VS ribozyme 4:480
Neurotropic virus(es)
  definition 1:441, 1:469
  see also specific viruses
Neurovirulence test(s)
  definition 5:226
  mumps virus pathogenicity 3:362
Neurovirulent virus(es)
  definition 1:469
  see also specific viruses
Neutralization *see Infection Neutralization*
Neutralization assays
  adenovirus 1:5
  antigen production measurements 3:414
  defective viruses 3:414
  echovirus 2:69
  human papillomavirus 4:17
  orthobunyavirus 3:482
  quantal assays 3:414
  steps 3:414
  West Nile virus 5:448
  yellow fever virus 5:470
Neutralization escape 3:417
  foot-and-mouth disease virus 3:418
  HIV-1 3:418
Neutralization resistance *see* Resistance
Neutralizing antibodies (NAbs) 3:64
  avian retroviruses 4:458
  classical swine fever virus 1:531
  definition 1:127, 2:534
  effectiveness 3:64
  experimental measurement 3:414
  flavivirus infection 3:66
  HIV infection *see* HIV infection
  human herpesvirus infection 2:504
  human papillomavirus infection 4:13–14
  immunoglobulin isotypes 3:65–66
  influenza viruses 1:129–131
  Marburg virus infection 3:279
  mechanism, replication blocking 3:415
  mouse mammary tumor virus infection 3:339
  picornaviruses 1:138
  residual infectivity 3:415
    'persistent fraction' 3:415
  Tat protein 1:72
  tests for
    principles 2:31
    residual infectivity 2:31
  vaccine development 1:141
    HIV gp41 1:141
  virus binding 3:64–65
Neutralizing monoclonal antibodies (NmAbs), definition 1:69
Nevirapine 1:143*t*
  limitations 2:510
Newcastle disease virus 5:316
  genome 3:327*f*
  infection 4:45–46
  tissue tropism 4:46
  vaccine development
    glycoproteins 5:224
    plant-produced vaccine 5:224
New England Primate Research Center (NEPRC) 4:603
New Minto virus (NMV) 1:116
NF-1 protein 4:264, 4:281
NF-1X protein 4:281
NFκB
  adenovirus malignant transformation 1:12–13
  apoptotic role 1:156*f*, 1:157–160
  BV-mediated immunosuppression 4:254, 4:255
  definition 1:154
  JCV gene expression 4:264
  KSHV latency 3:198–199
  oncogenicity role 4:417
Ngaingan virus 4:184*t*
Ngari virus
  genome segment reassortment 1:395
  strain reassortment 3:482
N gene(s)
  plant resistance 4:173
  tobacco resistance 4:176
NIa protease 4:321
NIa protein 4:317–319
NIb protein 4:319
Nicotiana benthamiana
  satellite virus pathogenicity 1:318
  virus-induced gene silencing 5:376, 5:378
    gene silencing 4:146–147, 4:148
  virus vector research 4:231

Nicotiana glutinosa 5:68
Nicotiana rustica 5:60
Nicotiana tabacum see Tobacco *(Nicotiana tabacum)*
Nicotiania glutinosa, viral assays 5:68
Nicotine vaccine, three-hybrid assay 3:28–29
Nidovirales 1:177, 1:549, 2:230, 3:419, 3:420*f*, 5:11*t*, 5:23, 5:152
Nidovirus(es) 1:177, **3:419–430**, 3:420*f*, 5:11*t*, 5:23
  definition 1:549
  evolution 1:182
  families 1:549, 4:553–554
  fish 2:230
  gene expression 3:423
  gene transcription 3:428
    sgRNAs 3:428
    transcription–regulation sequences 3:428
  genome 3:419–420, 3:423, 3:424*f*
    accessory protein genes 3:426
    ADP-ribose-1″-phosphatase 3:425
  infection
    apoptosis 3:429
    disease associated 3:421
    host cell affects 3:429
  nucleocapsid protein 3:421–423
  origin 3:428
  phylogeny 3:419, 3:421*f*
  proteolytic processing 3:423
    3C-like protease 3:424
    accessory proteases 3:424
  replicase 3:423, 3:425*f*
    3′–5′ exoribonuclease 3:424–425
    ADP-ribose-1″-phosphatase 3:425
    NendoU 3:425
    nucleotide cyclic phosphodiesterase 3:425
    ribose-2′-*O*-methyltransferase 3:424–425
  replication 3:427
    cis-acting sequences 3:427
    double membrane vesicles 3:426
    host factors associated 3:427
  structural protein genes 3:426
  virus structure 3:421, 3:422*f*
    structural proteins 3:423
  see also specific viruses
Niemann–Pick disease, definition 1:541
NIH Vaccine Research Center 5:357*t*
Nilaparvarta lugens reovirus (NLRV)
  genome 4:151, 4:152*t*
  pathogenicity 4:155
Nilaparvata bakeri, rice ragged stunt virus transmission 1:485
Nilaparvata lugens, rice ragged stunt virus transmission 1:485
Nilaparvata lugens reovirus (NLRV) 4:150*t*
Nimaviridae 1:247, 4:567, 5:11*t*, 5:451
Nipah virus (NiV) 4:40, 4:52
  epidemiology 2:324
  evolution
    Bangladesh 2:324
    Malaysia 2:323–324
  fusion proteins 4:54
  genetic stability 2:179
  global distribution 4:45
  historical aspects 2:321
    classification 4:128
  host cell attachment 4:44, 4:53–54
  host range 2:554
  infection *see below*
  transmission 2:324
Nipah virus infection
  clinical features 2:325, 4:56–57
    neurological 2:325
    prolonged infection 2:325
  human respiratory infection 2:554
  immune system evasion, IFN targeting 3:108
  mortality rate 4:56–57
  pathology 2:325
  risk factors 2:324
  serology 2:323
    antibodies to 2:326
  zoonoses 2:95, 5:492
Nitasoxanide 4:512
Nitrocellulose enzyme immunoassay (NC-EIA) 5:482
4-Nitrotetrazoliumchloride blue (NTB) 2:24
Nkolbisson virus (NKOV) 1:116
NKV virus(es) 2:235
NL-S protein 1:47–48
Nodamura virus (NoV) 3:431
  host range 3:431–432
  pathogenesis 3:437
Nodaviridae 2:229, 3:430, 3:431, 3:431*t*, 4:567, 5:11*t*
Nodavirus(es) **3:430–438**
  apoptosis inhibition 1:158*t*
  B2 protein 3:152
  capsids 3:432
  gene expression regulation 3:435
    protein synthesis 3:435
    RNA3 3:435
    RNA synthesis 3:435
  genetics 3:436
    recombination 3:436
  genome 3:430, 3:434, 3:434*f*
    packaging 3:433
    RNA1 3:430, 3:434
    RNA2 3:430, 3:434
    RNA3 3:434–435
  geographical distribution 3:431, 3:431*t*
  infections
    epidemiology 3:437
    host range 3:431, 3:431*t*
    insects 3:126*t*
    mitochondrial changes 3:436*f*, 3:437
    pathogenesis 3:437
    virus–host interactions 3:437
  protein B2 3:152, 3:436, 3:437*f*
  RdRp 3:436
  RNA recombination 4:379
  RNA replication 3:435
    cis-acting sequences 3:436
    protein A 3:435
    replication complexes 3:435, 3:436*f*
    RNA1 3:435
  RNA silencing suppression 3:152, 3:436, 3:437*f*
  satellite viruses 4:527*t*
  taxonomy 3:431, 3:431*t*
  virion assembly 3:433
  virion properties 3:432
  see also specific viruses
NOD-like receptor(s) (NLRs) 1:155
Nomenclature **5:9–24**
  definition 5:9
  latinized binomials 5:22
  nonlatinized binomials 5:22
  orthography 5:9, 5:21–22
  retrotransposons 4:437
  uniform system 5:21
  see also Classification
Non-β-barrel fold 5:381, 5:383*f*
Nonchromosomal gene(s), definition 4:336
Noncoding regions (NCRs)
  see specific viruses
Noncoding RNA, RNA satellites replication 4:533–534
'Noncompeting viral vectors' 4:233
Nondefective 'parent' virus(es) (St particles) 2:1, 2:3
Nonenveloped virus(es) 2:301
Nonenveloped virus assembly **1:200–204**
  antigenic sites 1:204
  antiviral agents 1:204
  architecture 1:200
    capsid 1:200–201, 1:201*f*
    genome size 1:200–201
  atomic structure 1:201
  evolution 1:203
  host receptor recognition site 1:203
  nucleic acid–protein interaction 1:202
  spherical viruses, atomic structure 1:202
  structure determination methods 1:201
Nonenveloped virus structure **5:380–393**
  β-barrel fold 5:381, 5:382*f*
  cryo-electron microscopy 5:380, 5:381
  historical aspects 5:380–381
  non-β-barrel fold 5:381, 5:383*f*
  nuclear magnetic resonance 5:381
  pseudo-symmetric particles 5:391
    P=3 icosahedral particles 5:391, 5:392*f*
  quasi-symmetric particles 5:385
    bacilliform particles 5:391
    T=3 icosahedral particles 5:385, 5:386*f*
    T=4 icosahedral particles 5:385, 5:387*f*
    T=7 icosahedral particles 5:388, 5:389*f*
    T=13 icosahedral particles 5:382*f*, 5:388
    T=25 icosahedral particles 5:390*f*, 5:391
  symmetric capsules 5:381
    helical particles 5:383*f*, 5:385
    T=1 icosahedral particles 5:381, 5:384*f*
  X-ray crystallography 5:380, 5:381
  X-ray fiber diffraction 5:380
Non-extinction, viral evolution 2:176
Non-Hodgkin's lymphoma
  incidence in AIDS patients 2:153–154
  ocular associated 2:497
Nonhomologous recombination
  bacteriophages *see* Bacteriophage(s)
  bromoviruses 1:389
  definition 2:301
  RNA viruses 4:377–378
Nonhuman primates *see* Primates, nonhuman
Nonlatinized binomials, nomenclature 5:22
Non-LTR retrotransposons 4:428–429, 4:438*f*, 4:439
  distribution 4:442*t*
  members 4:439
  phylogenetic studies 4:441
  see also Long interspersed nuclear elements (LINEs); Penelope-like elements (PLEs); Short interspersed repetitive elements (SINEs); SVA retrotransposons; Target-primed retrotransposons
Nonmultiplying (NM-type) infection, tobraviruses 5:73
Non-nucleoside reverse transcriptase inhibitors (NNRTIs) 1:149, 2:507*t*, 2:509
  see also specific drugs
  drug structure 2:510
  genetic barriers 1:150–151
  limitations 2:510
Nonoccluded insect virus(es) (NOIV), unassigned **3:144–148**
  genome 3:145
  history 3:144
  infection
    pathology 3:146
    persistence 3:147
  physical properties 3:145
  replication 3:145
  transmission 3:146
Nonpermissive cells, rhinoviruses 4:471
Non-phage protein encapsidation, three-hybrid assay 3:29
Nonplant edornavirus(es) *see* Edornavirus(es)
Nonprimate cytomegalovirus(es) **1:624–634**
Nonpropagative transmission, definition 1:301
Nonreplicative phase, chronic hepatitis B 2:357–358
Nonsegmented genomes, single-stranded negative sense RNA virus replication 4:410
Non-species definitions, viral evolution 2:177
Nonspecific flu-like illness, yellow fever 5:474
Nonsteroidal anti-inflammatory agents (NSAIDs), definition 5:83
Nonstructural (NS) protein(s)
  see specific viruses
Nonsynonymous polymorphisms, *APOBEC3G* 2:471–472
Nontemplated nucleotides, arenavirus replication 3:247
Nontranslated regions, satellite viruses 4:530
Nonviral protein expression, African cassava mosaic disease 1:35
Nonviremic transmission 5:270
  definition 5:440
Norovirus 1:411–412, 1:411*t*, 2:117, 3:439, 3:439*t*
Norovirus(es) **3:438–445**
  antigenic variation 1:415
  characteristics 2:117*t*
  classification 3:438–439, 3:440*f*, 3:441*t*

composition 3:440
culture 1:411
evolution 3:442
genetic recombination 3:442–443
gene expression 3:440
genetics 2:118
genogroups 2:117
genome 2:117, 3:440, 3:442*f*
open reading frames 3:440
geographical distribution 1:414
history 1:411
infections *see below*
prevalence 2:118
recombination 1:415
replication 3:441, 3:443*f*
receptors associated 1:416–417
site 3:443
structure 1:411–412, 2:117*f*, 3:440, 3:442*f*
VP1 3:440
VP2 3:440
VPg 3:440
taxonomy 3:439
transmission 1:416, 2:117–118, 3:443
vaccines/vaccination 1:418, 3:444
see also specific viruses
Norovirus infection 3:439*t*
clinical features 1:417, 3:443
gastroenteritis 1:411, 2:117–118, 3:438–439, 3:443
complications 1:418
control 1:418, 3:444
diagnosis 3:444
disease surveillance 2:46
data collection sources 2:46
gastroenteritis 2:46
food hygiene 1:418
host range 1:414–415, 3:443
immune response 1:417–418, 3:444
histo-blood groups 3:444
Norwalk virus 1:411
pathogenesis 1:416
incubation 1:416
pathogenicity 3:443
murine virus 3:443
prevention 3:444
treatment 3:443–444
North Africa, HIV epidemiology 1:65
North America, ophiovirus 3:450–451
Northern cereal mosaic virus (NCMV) 1:494, 4:184*t*
host range 1:494
infection, yield losses 1:494
Norvir® 1:151
Norvirhabdovirus, genome 1:112
'Norwalk agent' 2:117
Norwalk virus
cDNA clones 1:415
detection 1:411
gastroenteritis 1:411
history 3:438
plant-produced vaccine 5:223
structure 1:412*f*
Norwegian salmonid alphavirus (NSAV/SAV-3) 2:230, 5:93*t*, 5:94, 5:95
Nosocomial transmission, definition 1:59, 2:141, 2:146
Nosology, definition 1:533
Notch signaling pathway, *Bam*HI-A rightward transcripts 2:163–164
Notifications, disease surveillance
data collection, sources 2:45
meningitis, aseptic 2:45
Novirhabdovirus 1:119*t*, 2:221–222, 2:222*t*, 2:228, 3:160*f*
Novirhabdovirus(es) 2:221
classification 1:119*t*, 2:221–222, 2:222*t*
definition 2:221
genetic diversity 2:224
genome 2:223–224, 2:224*f*, 3:326*f*
transcription 2:223–224
virion structure 2:222–223, 2:224*f*
glycoprotein 2:222–223
matrix protein 2:222–223
nonvirion protein 2:222–223
nucleocapsid 2:222–223
phosphoprotein 2:222–223
polymerase 2:222–223
see also specific viruses
N-pilus, filamentous ssDNA bacteriophage infection 2:191
N^{pro} protein
Border disease virus 1:339–340
bovine viral diarrhea viruses 1:378, 1:379
classical swine fever virus 1:526
N protein
bacteriophage lambda 5:184
Chandipura virus genome encapsidation 1:502–503
NPV-specific cell lines, baculovirus in vitro pathogenesis 1:270
NS1 protein
aquareovirus 1:165–166
flaviviruses 3:66
influenza viruses *see* Influenza virus(es)
parvoviruses 4:88
NS2-3 protein
Border disease virus 1:339–340
bovine viral diarrhea virus biotypes 1:376–377, 1:377*f*, 1:379–380
NS2 protein
aquareovirus 1:165–166
parvoviruses 4:88
NS3/4A protease, apoptosis inhibition 1:161
NS3 protease, Border disease virus 1:340
NS3 protein, bovine viral diarrhea virus biotypes 1:376–377, 1:377*f*
NS4A protein, bovine viral diarrhea virus 1:379–380
NS4B protein, bovine viral diarrhea virus 1:379–380
NS4 protein, aquareovirus 1:165–166
NS5A protein, Border disease virus 1:340
NS5B protein, HCMV 2:369–370
NSDV *see* Nairobi sheep disease virus (NSDV)
NSm protein 5:134–135
nsP1 5:98
nsP2 5:98
nsP3 5:98
nsP4
alphavirus replication 5:98
viral enterotoxic role 4:511
NTB domain, nepovirus 3:409
NTB protein, nepovirus 3:409–410
N-terminal coat protein, cacao swollen shoot virus 1:405
Nuclear export signals (NES)
adenovirus malignant transformation 1:14
Borna disease virus replication 1:342
umbravirus infection 5:212
Nuclear F-actin, baculovirus in vitro pathogenesis 1:270–271
Nuclear hypertrophy
definition 3:495
Penaeus duorarum single nuclear polyhedrosis virus 4:571
Nuclear localization signals (NLS)
Borna disease virus replication 1:342
ophiovirus 3:450
porcine circoviruses 1:519
umbravirus infection 5:212
Nuclear magnetic resonance (NMR)
inovirus structure 3:120–121
nonenveloped virus structure 5:381
Nuclear shuttle protein (NSP)
banana bunchy top virus 1:274
bean golden mosaic virus 1:298
definition 1:272
Nuclear transport, avian hepadnavirus replication 2:331
Nuclease(s), polymerase chain reaction 2:35
Nucleic acid amplification techniques *see* Polymerase chain reaction (PCR)
Nucleic acid detection 2:33
Nucleic acid probes, pecluvirus 4:99
Nucleic Acid Research (NAR) 5:356–362, 5:357*t*
Nucleocapsid(s)
alphavirus assembly 1:196–197
baculoviruses 1:248–249
definition 4:407
maturation, icosahedral enveloped dsDNA bacteriophages 3:11
nucleopolyhedrovirus primary infection 1:267
potexvirus 4:310
varicella-zoster virus 5:256
yellow head virus 5:477
Nucleocapsid core
alphavirus assembly 1:195
definition 1:194
measles virus 3:286
orthoretrovirus structure 4:463–464
Nucleocapsid (N) protein
Crimean-Congo hemorrhagic fever virus 1:598–599
hepatitis B virus 2:352–353
measles virus 3:286, 3:287
mouse mammary tumor virus 3:335
tenuivirus 5:25
toroviruses 5:153
Nucleocytoplasmic large DNA virus (NCLDV) superfamily 1:50
associated core genes 3:315
classification 3:315, 3:315*f*
mimiviruses 3:312, 3:315
phycodnavirus 4:118
definition 3:311
evolution 3:474–475, 3:478
Nucleolus
definition 5:209
hypertrophy, bean golden/yellow mosaic disease 1:299
umbravirus infection 5:212
Nucleolytic ribozyme(s)
chemical reaction 4:475–476, 4:477*f*
definition 4:475
Nucleopolyhedrovirus 1:220, 1:247, 1:254, 1:260*f*, 1:265
Nucleopolyhedrovirus(es) (NPVs) 1:225–226, **1:254–265**
classification 1:254
DNA replication 1:250–251, 1:257
genes 1:257–260, 1:259*f*, 1:261*t*, 1:264
DE expression 1:262
IE-1/IE-0 complex 1:261–262
late expression factors 1:262
oral infectivity 1:264
genome 1:255, 1:256
group I 1:263–264
group II 1:263
history 1:225–226
infection *see below*
life cycle 1:255
host cell entry 1:256
morphogenesis 1:262
nucleocapsids 1:249
occlusion bodies 1:264
occlusion-derived virions 1:255, 1:255*f*, 1:263
*Ac*MNPV replication 1:238
function 1:223
life cycle 1:221
packaging 1:265
multiple nucleocapsid per envelope 1:265
polyhedrin gene 1:265
single nucleocapsid per envelope 1:265, 1:266*f*
proteins 1:257–260
F proteins 1:263
GP41 1:264
GP64 1:263, 1:264
P6.9 1:262–263
VP39 1:262–263
transcription 1:256
early gene expression 1:261
late gene expression 1:262
temporal regulation 1:261
virus structure
BV envelopes 1:255–256, 1:257*f*, 1:263
nucleocapsids 1:255, 1:255*f*, 1:257*f*, 1:262
ODV envelopes 1:255–256, 1:255*f*, 1:263
polyhedra 1:264
tegument proteins 1:264
see also Lepidopteran nucleopolyhedrovirus(es) (NPV); *specific viruses*
Nucleopolyhedrovirus infections
DNA infectivity 1:261
host range 1:265

Nucleopolyhedrovirus infections (*continued*)
infection cycle 1:256, 1:258*f*
insects 3:126*t*
pathogenesis 1:266
primary infection 1:266
infectivity factors 1:266–267
larval protection strategies 1:266
midgut accessibility 1:266
nucleocapsids 1:267
occlusion derived virus 1:266–267
receptor binding 1:266–267
secondary infection 1:267
tracheal cells 1:267
Nucleoprotein (NP)
arenaviruses 3:243, 3:244
yellow head virus 5:477
Nucleorhabdovirus 1:119*t*, 1:488, 3:160*f*, 4:187, 4:188*t*
Nucleorhabdovirus(es)
budding 3:333, 3:334
classification 1:119*t*
genome 3:326*f*
host cell entry 3:333
infection model 4:193–194, 4:194*f*
tobacco diseases, economic importance 5:67
virus-encoded proteins 3:332–333
nucleocapsid 3:331
phosphoprotein 3:331
see also specific viruses
Nucleoside analogs 1:149
channel catfish virus treatment 2:211
hepatitis B treatment 2:342, 2:359, 2:365
history 1:145
see also specific drugs
Nucleoside diphosphate kinase (NDK) 3:316
Nucleoside reverse transcriptase inhibitors (NRTIs) 2:507*t*, 2:509, 2:511*t*
drug resistance 1:150–151
side effects 2:509
Nucleotide analogs 1:149
hepatitis B treatment 2:359
see also specific drugs
Nucleotide cyclic phosphodiesterase (CPD), nidoviruses 3:425
Nucleotide selection
bacteriophage genome packaging 2:307
cystovirus genome packaging 2:307
dsDNA bacteriophage genome packaging 2:307
Nucleotide sequences
fish retroviruses 2:231
mungbean yellow mosaic viruses 3:369*t*, 3:371
virus databases 5:362
Nucleotide substitution rate, definition 4:436
Nuclear pyknosis, definition 1:580
Nucleus infection, baculovirus in vitro pathogenesis 1:270
Nudaurelia capensis β virus (NβV) 5:28*t*
capsid morphology 5:31*f*
capsid protein precursors 5:29, 5:32*t*
discovery 5:27–28
genomes 5:29
infection 5:36
Nudaurelia capensis omega virus (NωV) 5:28*t*
capsid assembly 5:33
capsid maturation 5:34–35
capsid morphology 5:31*f*
capsid protein precursors 5:31–32, 5:32*t*
capsid structure 5:33, 5:34*f*
chemical tags 5:36–37
cleave site mutations 5:34*f*, 5:35–36
discovery 5:28
genomes 5:31
RNA2 length 5:31
structure 5:382*f*, 5:385–388, 5:387*f*
β-barrel fold 5:381
Nudaurelia ϵ virus (NϵV) 5:28*t*
Nupapillomavirus 4:9, 4:10*f*, 4:11*t*
Nupapilomavirus 4:9
Nursery, definition 4:561
Nus factors, bacteriophage lambda 5:184
Nymph, definition 1:533
Nystagmus, definition 1:76

O

Oak Vale virus 4:184*t*
Oat blue dwarf virus (OBDV)
infection 5:205–206
replication 5:205–206
Oat chlorotic stunt virus (OCSV) 5:145–146
genome 5:147*f*
Oat golden stripe virus (OGSV) 2:292
Oat sterile dwarf virus (OSDV) 4:150*t*
OBDV *see* Oat blue dwarf virus (OBDV)
Obligate intracellular parasites, icosahedral ssDNA bacteriophages 5:368, 5:369
Obodhang virus 4:184*t*
Obuda pepper virus (ObPV) 4:173
Occlusion bodies (OB)
baculoviurus *see* Baculovirus(es)
definition 1:211, 1:219, 1:254
insect pest control 3:128
nucleopolyhedrovirus 1:264
Occlusion-derived virion(s) (ODV)
baculoviruses 3:129
definition 1:254
nucleopolyhedroviruses *see* Nucleopolyhedrovirus(es) (NPVs)
Occupancy, antigen–antibody complexes 3:414
Occupations
St Louis encephalitis virus 5:273
vector transmission 5:273
Oceania, HIV epidemiology 1:65
Ockelbo disease 5:90
seasonal distribution 5:90
Oct-1 recognition sequence, varicella-zoster virus 5:259
Ocular adnexae 2:491
Ocular diseases
AIDS *see* AIDS
DNA viruses 2:494*t*, 2:495
influenza virus 2:492
mumps virus 2:495
RNA viruses 2:492, 2:493*t*
uveitis 2:573
Ocular immunology 2:492
tears 2:492
Odonstyle, definition 3:405
Odontoglossum ringspot virus (ORSV) 4:225–226, 4:228*f*
Odontophore, definition 3:405
Office Internationale des Epizooties (OIE) 5:1
Ogyia pseudosugata multiple nucleopolyhedrovirus (MNPV) 3:125
OHMV *see* Omsk hemorrhagic fever virus (OHFV)
Oil palm(s), infection control 3:128, 4:79
Oka vaccine strain, varicella-zoster virus 5:253, 5:255
Okavirus 4:567, 5:477
Okavirus(es)
classification 4:567
genome 3:424*f*
replicase genes 3:425*f*
Okazaki fragment 4:400
OLE1 protein 1:384–385
Oleavirus 1:81, 1:387, 3:46
2′–5′ Oligoadenylate (2–5 OAS), interferon-induced antiviral response 3:107, 3:115–116
Oligodendrocytes
demyelination in JCV infection 4:267
neural cell infection 1:473
TMEV infection 5:42–43
Oligonucleotides, use in microarrays 2:15
Olpidium brassicae
Lettuce big-vein virus 5:264, 5:268
Lettuce ring necrosis virus transmission 3:453
Mirafiori lettuce virus transmission 3:453
Ophiovirus transmission 3:453
Tulip mild mottle mosaic virus transmission 3:453
Olive latent ringspot virus (OLRSV) 3:406*t*
Olive latent virus 1 (OLV-1) 3:403
geographical distribution 3:404
virus–host relationship 3:404–405
Olive mild mosaic virus (OMMV) 3:403
geographical distribution 3:404
OLV-1 *see* Olive latent virus 1 (OLV-1)
Omegatetravirus(es)
capsid protein precursors 5:31–32, 5:31*f*, 5:32*t*
insect infections 3:126*t*
Omsk hemorrhagic fever 5:53, 5:490
Omsk hemorrhagic fever virus (OHFV) 2:239, 5:53
muskrats 2:239–240
vector 2:239–240, 5:53
Oncogene(s)
adenoviral 1:10–11, 1:13
see also Adenovirus infections
definition 2:213
HPV 4:19
retroviral *see* Retrovirus(es)
Oncogenesis 2:457, 5:316
hepatitis B 2:366
Herpesvirus Saimiri 4:589
HPV E6 4:24
Jaagsiekte sheep retrovirus 2:219
Oncolysis, measles virus 3:291
Oncolytic virotherapy 3:285, 3:291
One-step growth 2:442
Onion yellow dwarf virus (OYDV) 5:288
ONNV *see* O'nyong nyong virus (ONNV)
Ontology, definition 5:348
O'nyong nyong virus (ONNV) 5:89, 5:114
characteristics 1:171*t*
evolutionary relationships 1:175
geographical distribution 5:84*f*, 5:89, 5:114
infection
clinical features 5:86*t*, 5:87*t*, 5:89
epidemic 5:89
RNAi role 3:154
transmission 5:89, 5:100, 5:114
Oomycetes 2:288
Open reading frames (ORFs)
aquareovirus 1:165
*Bam*HI-A rightward transcripts 2:159*f*, 2:163–164
barley yellow dwarf viruses 1:280
barnaviruses 1:287, 1:287*f*
bean golden mosaic virus 1:298
beet curly top virus 1:303
Border disease virus 1:338
cacao swollen shoot virus 1:405, 1:407*t*
cassava mosaic geminiviruses 1:30–31
cereal yellow dwarf virus 1:280
cowpea mosaic virus genome 1:571
definition 1:296, 1:482, 1:533, 5:199
Dendrolimus punctatus tetravirus 5:31
EBV 2:157
edornaviruses 2:110–111, 2:112, 2:113
giardiavirus 2:313
gill-associated virus 5:478–479
hepatitis E virus 2:378
Indian peanut clump virus 4:101
LINE-like retrotransposons 4:439
LTR retrotransposons 4:420, 4:438–439
mungbean yellow mosaic viruses 3:365–366
necrovirus 3:403
omega tetraviruses 5:31
ophiovirus 3:449
Peanut clump virus 4:101*t*
pecluvirus 4:99–100, 4:101*t*
Pepino mosaic virus 4:105
pomovirus 4:283
retrotransposons 4:437
rice yellow mottle virus 4:486
sadwaviruses 4:524, 4:525*f*
target-primed retrotransposons 4:420–421, 4:425
tetraviruses 5:29
toroviruses 5:152–153
yeast L–A virus 5:467
yellow fever virus genome 5:470–471
yellow head virus 5:477–478, 5:482
see also under ORF
Operational taxonomy units (OTUs) 4:126
Ophidium brassicae 5:263
Ophioviridae 3:447
Ophiovirus 3:447, 5:263

Ophiovirus(es) **3:447–454**
  Asia 3:450–451
  classification 3:447, 3:449*f*
    coat protein sequences 3:447
    RT-PCR 3:448
    serology 3:448
    species demarcation 3:447
  genome 3:449, 3:449*f*, 3:450*t*
    nuclear localization sequence 3:450
    open reading frames 3:449
    RNA2 3:449
    RNA4 3:449
    subgenomic RNAs 3:449–450
  geographical distribution 3:450
    Asia 3:450–451
    Mediterranean 3:450–451
    North America 3:450–451
    South America 3:450–451
  host ranges 3:453
  infection
    control 3:453
    cytopathology 3:453
    pathogenicity 3:450, 3:451*f*
    prevention 3:453
    symptoms 3:453
  members 3:448*t*
  proteins 3:450
    coat protein 3:450
  RdRp 3:447
  taxonomy 3:447
    RdRp 3:447
  transmission 3:453
    Olpidium brassicae 3:453
  virion particles 3:448
  see also specific viruses
Opportunistic infections
  adult T-cell leukemia complications 2:568, 2:569–570
  HIV infection *see* HIV infection
Opsonization 3:110
  definition 3:104
Opt-out HIV testing 1:66
Opuntia virus X (OPX) 4:311*t*
Or-1V *see* Oryctes virus (Or-1V)
Oral bioavailability, antiviral prodrugs 1:150
Oral focal epithelial hyperplasia 4:16
Oral infection route 5:155
Oral infectivity, nucleopolyhedrovirus genes 1:264
Oral vaccination *see* Edible vaccine(s)
Orbivirus 3:454, 3:455*t*, 4:536, 4:536*t*
Orbivirus(es)
  animal pathogens associated 3:454
  classification 3:454, 3:455*t*
  evolutionary relationships 3:461, 3:463*f*
  genome 1:172, 3:460, 3:462*t*
    open reading frames 3:460
    untranslated regions 3:460
  history 3:454
  host range 3:457
  infection 3:458
    epidemiology 3:458
    human 3:464
  replication 3:460
    cell tropism 3:461
  transmission 3:454, 3:457
  type species 4:536*t*
  viral RNAs 3:459
  virion properties 3:459
  virion proteins 3:459
  virion structure 1:172, 3:459
  see also specific viruses
Orchidaceae virus infections 4:225
  see also specific viruses
Orchitis 1:533
Order(s) 5:10
  definition 5:9
  universal system 5:23
ORF1, cacao swollen shoot virus 1:405
ORF2, cacao swollen shoot virus 1:405
ORF3, cacao swollen shoot virus 1:405–406
ORF4 mutations
  barley yellow dwarf virus 1:282
  cereal yellow dwarf virus 1:282
ORF29, varicella-zoster virus latency 5:262
ORF1629, baculovirus expression vectors 1:244
ORF d63, fusellovirus protein structure 2:300
ORF f93, fusellovirus protein structure 2:300
ORFV *see* orf virus (ORFV)
orf virus (ORFV) 4:57–58
  genome 4:59, 4:60*f*
    pathogenesis-related genes 4:59–60
  host range 4:58
  inactivation 4:58
  infection
    clinical features 4:58
    immune response 4:61
    lesion progression 4:58
    reinfection 4:58, 4:61
    teat lesions 4:59
  morphology 4:59*f*
  synonyms 4:58
  vaccines/vaccination 4:62–63
  virus shedding 4:58
Organism(s), definition 3:400
  cluster concept 3:400
  properties 3:400
  reproductive lineage 3:400
Organismal ecology
  bacteriophages *see* Bacteriophage(s), ecology
Organotypic raft(s) 4:32
Organ transplantation, infection risks **3:466–472**
  antiviral therapy 3:469–470
    adjunctive therapy 3:471
    drug toxicity 3:471
    duration 3:471
    post-transplant prophylaxis 3:471
    prophylaxis 3:471
  associated viruses 3:466, 3:466*t*
    HCMV infection 2:483–484
    KSHV transmission 3:191
    rabies virus 4:370
  clinical consequences 3:466
  diagnostic assays 3:470
  donor-derived 3:466
  effects 3:468, 3:468*t*
  immunocompromised patients 3:466
  latent infection reactivation 3:467
    antirejection therapy 3:467
    associated viruses 3:468
    cellular pathways 3:467
    herpesviruses 3:467
    Kaposi's sarcoma 3:469
    risk factors 3:467
  novel infections 3:468
  screening 3:466, 3:467
  therapy 3:471
  vaccination 3:471, 3:472*t*
  virus-specific syndromes 3:469
  see also specific transplants
Organ transplantation rejection 3:468–469
Oriental tobacco 5:61
Origin of assembly sequence (OAS)
  definition 5:68, 5:145
  pecluvirus 4:101–102
Origin of replication (ori)
  definition 1:211
  HSV virus vectors 2:396
  maize streak virus 3:266
Origin(s) of viruses 3:402, **3:472–479**, 3:474*f*
  ancient origin 3:474
  cellular *vs.* acellular evolution 3:475
  classical view 3:473
    consequences of 3:473
  DNA, origin of 3:477
  escape hypothesis 3:473, 3:476, 3:476*f*
    bacteriophages 3:473
    virus-encoded proteins 3:473–474
  genome plasticity 3:477–478
  modern cells, relationship with 3:473
  phylogenetic analysis 3:402
  reduction hypothesis 3:473, 3:476, 3:476*f*
  RNA *vs.* DNA viruses 3:477
  trinity concept 3:473–474
  viral eukaryogenesis hypothesis 3:478
  virus-first hypothesis 3:473, 3:475, 3:476*f*
    acellular machinery, coevolution of 3:475
  see also Evolution (of viruses)
ori-Lyt, HCMV 1:640, 2:486
Ornamental plant species virus infections **4:207–229**, 4:216*t*
  control 4:226
    cross-breeding 4:226–229
    direct chemical control 4:226–229
    guidelines 4:226–229
  cross-breeding 4:208
  cultural practice 4:208
  economic importance 4:208
  host ranges 4:208
  risk of 4:208
  species introduction 4:208
  vector introduction 4:208
  worldwide trade 4:208
  see also specific species
Ornithodoros moubata (soft tick vector) 1:43, 1:44*f*
Oropouche virus 1:391*t*, 3:481, 5:489
  vector 3:482
Orthobunyavirus 1:76, 1:391, 1:391*t*, 3:480, 4:490–491
Orthobunyavirus(es) 1:399, **3:479–483**
  definition 3:479
  genome segment reassortment 1:393*f*, 1:395
  historical aspects 3:479
  host species 3:480
  infection 3:480
    diagnosis 3:482–483
    treatment 3:482
  morphology 1:393*f*
  reassortment 3:482
    complement fixation tests 3:482
    group C viruses 3:482
    hemagglutination inhibition tests 3:482
    neutralization tests 3:482
  replication, host cell entry 1:395
  RNA structure–function relationships 3:482
  species 3:480, 3:481*t*
  transmission 1:392, 1:392*f*, 3:480
  vectors 3:480
  virion proteins
    NSm protein 1:393
    NSs protein 1:395
  see also specific viruses
Orthography, nomenclature 5:9, 5:21–22
Orthohepadnavirus 2:351–352
Orthohepadnavirus, species infected 2:351–352
Orthologous clusters, virus databases 5:363
Orthomyxoviridae 1:170, 3:96, 3:483–484, 3:489, 5:11*t*
Orthomyxovirus(es)
  fish 2:229
  genera 3:96, 3:483–484, 3:489, 3:490
  human respiratory infection 2:554
  virion structure 1:172
  see also specific viruses
Orthopoxvirus 3:342, 4:324*t*, 4:639, 4:641, 5:244–245
Orthopoxvirus(es) 5:489–490
  animal reservoirs 1:579–580
  classification 4:324*t*
  conserved genes 2:138
  molluscipoxvirus 2:136
  phylogeny 4:326–327
  see also specific viruses
Orthoptera, definition 1:111, 2:37
Orthoreovirus 4:383, 4:391, 4:392, 4:536*t*
Orthoreovirus(es) 3:378–379
  fusogenic 4:383
    FAST proteins 4:383–384
  genome 4:383, 4:384*t*
  infection
    clinical features 4:388
    immune response 4:389
  type species 4:536*t*
  see also specific viruses
Orthoretrovirinae 2:231, 2:260*t*, 4:412–413, 4:430, 4:460, 5:11*t*
Orthoretrovirus(es)
  assembly 2:518
  classification 4:460

Orthoretrovirus(es) (*continued*)
life cycle 2:263
viral proteins 4:463–464
see also specific viruses
Orungo virus 3:465
zoonoses 5:491
OrV *see* Oryctes rhinoceros virus (OrV)
Oryctalagus, myxoma infection *see* Myxoma viruses (MYX)
Oryctes rhinoceros virus (OrV) **3:495–500**
assembly 3:495
biocontrol 3:499
genome 3:497
genetic variation 3:497
restriction map 3:497, 3:497*f*
size 3:497
history 3:495
infection *see below*
insect pest control 3:127, 3:132
nomenclature 3:495
phylogeny 3:497, 3:498*f*
replication 3:495
cell entry 3:495
taxonomy 3:497
transmission 3:132, 3:499
adult beetles 3:499
larvae 3:499
virion properties 3:496, 3:496*f*
associated proteins 3:496
size 3:496
tail-like appendage 3:496
Oryctes rhinoceros virus infection 3:495, 3:496*f*
insect pest control 3:132
midgut epithelium 3:497*f*
pathology 3:495
adult beetles 3:495
larval morphology 3:495
Oryctes virus (Or-1V) 3:144
beetle population management 3:146
infection 3:144
beetle population management 3:146
pathology 3:146
persistence 3:147
replication 3:146
symptoms 3:146
transmission 3:146
morphology 3:145, 3:147*f*
replication 3:145–146
structural proteins 3:146
virion assembly 3:145–146
Orygia psuedotsugata multiple neucleopolyhedrovirus (MNPV) 3:125
Oryza rufipogon endornavirus (OrEV) 2:110–111
genome 2:110*f*
Oryza sativa see Rice *(Oryza sativa)*
Oryza sativa endornavirus (OsEV) 2:110–111
copy number regulation 2:114
in vitro *vs.* in vivo 2:114
pollen grains 2:114
genome 2:110*f*
vertical transmission 2:113–114
host species 2:114
Oryzavirus 4:150–151, 4:536*t*
Oryzavirus(es)
characteristics 4:150*t*
classification 4:150
genome 4:151
dsRNA segments 4:151–152, 4:152*f*, 4:153*t*
size 4:150–151
host range 4:154
transmission 4:154
type species 4:536*t*
virion structure 4:151
capsid 4:151–152
see also specific viruses
Oseltamivir 3:102–103, 3:492, 3:493*f*
influenza virus infection 2:556
OsEV *see* Oryza sativa endornavirus (OsEV)
Osloss strain, bovine viral diarrhea viruses 1:376–377
O-some 4:400
Ostelmavir 1:143*t*
Osteospermum virus infections 4:221
see also specific viruses
Ostreid herpesvirus 1 (OsHV-1) 2:429, 4:563, 4:564*f*
genome 4:564*f*
genes 4:564–565
ORFs 4:564–565
taxonomy 4:565
Ourmia melon virus 3:500
pathogenicity 3:501
taxonomy 3:500
Ourmiavirus 3:500
Ourmiavirus(es) **3:500–501**
control 3:501
cytopathology 3:501
genome 3:500
RNA-1 3:500
RNA-2 3:500
RNA-3 3:500
taxonomy 3:500
geographical distribution 3:501
morphology 3:500
other viruses *vs.* 3:500
particles 3:500
pathogenicity 3:501
prevention 3:501
proteins 3:501
transmission 3:501
see also specific viruses
Outbreak control, phylogeny 4:127
Outer membrane fusion, icosahedral enveloped dsDNA bacteriophages 3:11
'Outgroup' phylogenetic analysis 4:127
Output (information utilization), virus databases 5:354, 5:354*f*
BLAST 5:354
OvHV-2 *see* Ovine herpesvirus 2 (OvHV-2)
Ovine adenovirus(es) 1:26
Ovine herpesvirus 1 (OvHV-1) 1:364*t*
Ovine herpesvirus 2 (OvHV-2) 1:364*t*
Ovine herpesvirus 2 (OvHV-2) infection
clinical features 1:367
species barriers 2:428
Ovine pulmonary adenocarcinoma (OPA) 3:179
disease characterization 3:179–180, 3:180*f*
experimental model 3:180
Oviposition, definition 3:264
Oyster(s), norovirus infection 1:418
Oyster velar virus (OVV) 4:561
Oyster velar virus disease (OVVD)
histopathology 4:562
manifestations 4:561–562

P

P1 gene/protein
alfalfa mosaic virus 1:85
bean common necrosis virus 1:294
icosahedral enveloped dsDNA bacteriophages 3:7–8, 3:8*f*, 3:9*f*
potyvirus 4:317
rhinovirus 4:469
rice yellow mottle virus 4:486–487
tobacco etch virus 4:317
P2 bacteriophage *see* Bacteriophage P2
P2 gene/protein
alfalfa mosaic virus 1:85
bacteriophage PRD1 3:1–2
caulimovirus 5:279
icosahedral enveloped dsDNA bacteriophages 3:8, 3:9*f*
rhinovirus 4:469
P3-6K1 protein, tobacco etch virus 4:317
P3 gene, zucchini yellow mosaic virus 5:437–438
P3 gene/protein
rhinovirus 4:469
watermelon mosaic virus 5:437–438
P4 gene/protein
icosahedral enveloped dsDNA bacteriophages 3:7–8, 3:9*f*
ssDNA bacteriophages 2:311–312
P5CDH gene, gene silencing 4:142
P6.9–DNA interactions, baculoviruses 1:271
P7 gene/protein, icosahedral enveloped dsDNA bacteriophages 3:7–8
P8 gene/protein, icosahedral enveloped dsDNA bacteriophages 3:8, 3:9*f*
p10 gene/protein, baculoviruses 1:251
p19 gene/protein, RNAi suppression 3:152
P22 bacteriophage *see* Bacteriophage P22
P23 protein, alphavirus 5:98
p29 protease, CHV-EP713 virus 2:583–584
P35 *see* Baculovirus apoptosis inhibition
p37 protein, cauliflower mosaic virus 1:466
p44 protein, cauliflower mosaic virus 1:466
p53 gene/protein
apoptosis, small T protein murine polyomavirus 4:275
HPV E6 4:24
HTLV-1 2:562
tax protein function *see* Tax protein
mutation rates 5:198
papillomavirus 4:37
p54 gene/protein, African swine fever virus 1:46–47
p72 gene/protein, African swine fever virus 1:46–47
P78/83, baculoviruses 1:271
P90 gene/protein, togavirus 5:119
P123 gene/protein, alphavirus 5:98
P150 gene/protein, togavirus gene/protein 5:119
p220 polyprotein, African swine fever virus 1:46–47
p400 gene/protein, adenovirus E1A 1:11–12
P1234 gene/protein
alphavirus 5:98
togavirus 5:120
Pacheco's disease 2:406
acyclovir 2:410
clinical signs 2:409
history 2:406–407
immune prophylaxis 2:410
incubation period 2:409
psittacid herpesvirus 1 2:406
Packaging
definition 3:6
icosahedral enveloped dsDNA bacteriophages 3:11
Packaging ATPase, definition 2:306
Packaging motor complex, bacteriophages 2:307
Pac site 3:6
PAD4 protein, plant natural resistance 4:176
Padlock probes, plant virus detection 2:28, 2:28*f*
Pairwise sequence comparison (PASC) 5:19, **5:342–348**, 5:357*t*
applications 5:344
boundaries 5:344
taxonomy demarcations 5:344, 5:345*f*
BLAST *vs.* 5:345
considerations 5:344
identity distribution 5:343–344
limitations 5:347
parameter variability 5:347
programs 5:347
NCBI implementation 5:344
PASC interface 5:344
principles 5:343
Pakistan, banana bunchy top virus epidemic 1:277
Palindromes 1:211
Palivizumab 2:550, 2:556
Palm civets, SARS CoV transmission 1:555, 4:555, 4:559–560
Palmoplantar myrmecia, papillomavirus associated warts 4:16
PAMPs *see* Pathogen-specific molecular patterns (PAMPs)
Pandemics 2:525
definition 1:30, 1:59, 2:44, 2:97, 2:142, 2:517, 2:525, 3:95
see also specific infections
see also specific viruses
Panencephalitis, subacute sclerosing (SSPE) 2:494–495
Pangola stunt virus (PaSV) 4:150*t*
Panicavirus 5:145–146, 5:147*f*
Panicovirus 5:145–146
Panicum mosaic satellite virus (PMSV) 4:527*t*
host virus accumulation interference 4:530
variation/evolution 4:534
virion structure 4:530

Panicum mosaic virus (PMV) 5:145–146, 5:147*f*
  genome 5:147*f*
Panleukopenia 4:85
Panviral microarrays
  bioinformatics 2:16
  clinical applications 2:17
  sample amplification 2:16
Papanicolaou test ('pap test'), cervical papillomavirus infection 4:16, 4:17, 4:39
Papaya, engineered resistance 4:160–161
Papaya leaf curl virus (PaLCuV) 1:566
Papaya leaf distortion mosaic virus (PLDMV), papaya ringspot virus *vs.* 4:1
Papaya lethal yellowing virus (PLYV) 4:645
  infection 4:651
Papaya mosaic virus (PapMV) 4:311*t*
Papaya ringspot virus (PRSV) **4:1–8**
  classification 4:1
  CP gene/protein 4:2–3, 4:4
  evolution 4:2
  genome 4:1, 4:2*f*, 4:3*t*
  geographical distribution 4:3
  historical aspects 4:1
  host range specificity determinants 4:3
  infection *see below*
  papaya leaf distortion mosaic virus *vs.* 4:1
  particle size 4:1
  phylogenetics 4:3
  proteins 4:3*t*
    immunoprecipitation 4:2
    proteinases 4:2
    see also specific proteins
  sequence diversity 4:2
    CP gene/protein 4:2–3
    isolates 4:3
    management proteins 4:2–3
    phylogenetics 4:3
  suppression of gene silencing 4:4
  transmission 4:4
    aphid vectors 4:1
Papaya ringspot virus infection
  host range 4:1
    P-type 4:1, 4:3
    W-type 4:1, 4:3
  local lesion formation determinants 4:4
  pathogen-derived resistance 4:4, 4:6*f*
    cross-protection 4:5
    mild virus strains 4:4
    tolerant plant breeding 4:5
  symptoms 4:1, 4:2*f*
  symptom severity determinants 4:4
    CP gene/protein 4:4
    strain-specific crossprotection 4:4
  transgenic resistance 4:5, 4:157–160, 4:321
    commercialization 4:5
    CP gene 4:5
    field trials 4:5
    'parasite-derived protection' 4:5
    resistance to 4:6*f*, 4:7
Papillomatosis 4:16
Papillomaviridae 4:8–9, 4:27, 4:34
Papillomavirus(es) **4:26–34**
  apoptosis inhibition 1:158*t*
  classification 4:27
  evolution 4:28*f*
    L1 nucleotide sequences 4:35
  gene expression 4:30
    chromatin structure 4:38
    E6 promoter 4:37–38
    early region 4:30
    late region 4:30
  genome 4:32*f*
    open reading frames 4:34
  history 4:26
  infections *see below*
  propagation 4:32
  proteins 4:31
    E1 4:31
    E2 4:31
    E2TA 4:31
    E4 4:32
    E4 ORF 4:36
    E5 4:31
    E6 4:31–32
    E7 4:31–32
    E8 4:30–31
    L1 4:32
    L2 4:32
  replication 4:30
    cell surface receptors 4:30
    mutation rates 4:27
    virion assembly 4:30
  taxonomy 4:27
  tissue tropism 4:30*f*
  transmission 4:27
  types 4:34
  virion properties 4:32
  virion structure 4:32*f*
  see also specific viruses
Papillomavirus infections
  clinical features 4:27
  control 4:33
  detection 4:33
  diagnosis 4:33
  epidemiology 4:38
  host range 4:27
    species specificity 4:27
  immune response 4:33
    humoral 4:33
    regression 4:33
  pathogenicity 4:27
  pathology 4:30*f*
  prevention 4:33
Papova-like virus(es), shellfish viruses 4:566
Papovaviridae 4:277
Papovavirus(es)
  human respiratory infection 2:556
  infections, persistence 4:109*t*
  see also specific viruses
Papua New Guinea, HIV epidemiology 1:65
Papules
  definition 4:639
  smallpox 4:639–640
Parainfluenza virus(es) **4:40–47**
  classification 4:40
  epidemiology 4:45
  genetic relationships 4:45
  genome 4:43*f*
    leader region 4:42
    transcriptional control signals 4:42
  host range 4:40
  infection 2:553
    control 4:45
    diseases 4:45
    immune response 4:45
    pathogenesis 4:45
    prevention 4:45
  propagation 4:40
  proteins 4:42
  replication 4:44
    host cell attachment 4:44
    M protein 4:44
    RNA synthesis 4:44
    transcription 4:44
    virus assembly 4:44
  reverse genetics 4:44
  serologic relationships 4:45
  taxonomy 4:40
  transmission 4:45
  virion properties 4:42*f*
Paralogs
  cytomegaloviruses 1:638*f*
  definition 1:634
  mimivirus genome 3:314–315
Paralysis
  coxsackieviruses 1:580–581
  poliovirus 4:242–243
Paralytic rabies *see* Rabies virus (RABV)
Paramecium bursaria, chlorella virus infection 4:116
Paramecium bursaria chlorella 1 virus (PBCV-1), structure 5:391
Paramyxoviridae 2:321, 3:285, 3:324, 3:356, 4:40, 4:41*t*, 4:47, 4:52
Paramyxovirinae 2:321, 2:543, 2:544*f*, 4:40, 4:41*t*, 4:52, 5:11*t*
Paramyxovirus(es) 3:285
  animal pathogens 4:41*t*
  apoptosis inhibition 1:158*t*
  assembly 3:333
  attachment 4:42–43
    sialic acid 4:53–54
  budding 3:333–334
  characteristics 5:62*t*
  classification 4:52
    F protein 3:285
    subfamilies 4:40
  fish *see* Fish paramyxovirus(es)
  F protein 3:285
  fusion 4:42–43
    conformational changes 4:54
    proteolytic processing 4:54
  genome 4:42
    overlapping reading frames 4:52–53
    P gene 4:44
  glycoprotein 4:42–43
  glycoproteins 3:332
    hemagglutination activity 3:332
  hemagglutination-neuraminidase protein
    function 3:358*t*
    proteolytic cleavage 3:359
    structure 3:356
  infection *see below*
  nucleocapsid 4:54
    structure 3:356
  P mRNA transcripts 3:331
  polymerase 4:54
    abundance 4:54–55
    P–L protein complex 4:54–55
  proteins 4:42
    matrix 4:54
    phosphoprotein 4:54
    small hydrophobic proteins 4:42–43
    viral degradation complex 4:55
    V protein 4:44
    see also specific types
  replication 4:55*f*
    assembly 4:55–56
    glycoprotein modifications 4:55–56
    transcription 4:55–56
    translation 4:55–56
  reverse genetics 4:56
  ribonucleoprotein 3:356
  rinderpest virus relationship 4:497
  taxonomy 2:321
  transmission 4:56
  as vaccine vectors 4:44–45
  viral degradation complex 4:55
  virion structure 4:498
    nucleocapsid 3:356
    ribonucleoprotein structure 3:356
  virus structure 3:329*f*
  see also specific viruses
Paramyxovirus infections 4:56
  host range 4:56
  human respiratory infection 2:553
  immune response
    evasion 3:108
    MDA-5 3:106
  persistence 4:109*t*
  prevention 4:46
    vaccination 4:46
  treatment 4:56
  vaccines/vaccination 4:46–47
    live attenuated *vs.* inactivated 4:46
Parapoxvirus 4:58, 4:324*t*
Parapoxvirus(es) (PPVs) **4:57–63**
  classification 4:58
  genomes 4:59
    recombination 4:60
    transcription 4:60
  history 4:57–58
  host range 4:58

Parapoxvirus(es) (PPVs) (*continued*)
inections *see below*
physical properties 4:59
propagation 4:58
protein properties 4:60
structural proteins 4:60
replication 4:60
taxonomy 4:58
transmission 4:59
vectors 4:62
virion properties 4:59*f*
see also specific viruses
Parapoxvirus infections
clinical features 4:58
epidemiology 4:58
immune response 4:61
immunomodulation 4:61
chemokine-binding protein 4:62
granulocyte-macrophage colony-stimulating factor inhibitory factor 4:62
interferon resistance protein 4:62
viral interleukin-10 4:62
viral vascular endothelial growth factor 4:61
pathogenesis 4:61
serologic relationships 4:58
Pararetroviridae 4:430
Pararetroviruses, plants *see* Plant pararetrovirus(es)
'Parasite-derived protection' transgenic plants 4:5
Parasitoids
definition 4:250
insect immune responses 4:251
encapsulation 4:253*f*
hemocyte differentiation 4:251–252
hemocyte proliferation 4:251–252
polydnavirus immune evasion 4:255
signaling pathways 4:252–253
life cycle 4:251*f*
wasps 4:250
Paratopes
definition 1:137
IgG 1:138
infection neutralization 3:416
Parechovirus 2:122
Parechovirus, respiratory disease 2:553
Parenchyma, rice yellow mottle virus 4:487–488
Parenchymal cells, Marburg virus infection 3:278
Pariacoto virus (PaV)
host range 3:431–432
nucleic acid–protein interaction 1:203*f*
RNA packaging 3:432*f*
structure 3:432*f*
Paris polyphylla virus X (PPVX) 4:311*t*
Parkinson's disease, viral etiology 1:475
Parotitis, MuV infection 3:356
Parr 3:83
Parrots, Pacheco's disease 2:406
Parry's Creek virus (PCV)
morphology 1:112*f*
Parsley virus 5 (PaV-5) 4:311*t*
Parsnip virus 3 (ParV-3) 4:311*t*
Parsnip virus 5 (ParV-5) 4:311*t*
Parsnip yellow fleck virus (PYFV)
genome 4:548*f*
isolate variation 4:550
transmission 4:551
Partial genome sequencing, epidemiology 2:143
Particle assembly
maize streak virus 3:268
retrovirus infection resistance 2:473
Particle maturation, virus structure 5:399
Particle purification, seadornavirus 4:538
Particles **5:393–401**
maize streak virus 3:264*f*
Simian retrovirus D 4:626*f*
Partitiviridae 1:99, 1:103*f*, 1:104*f*, 1:503–504, 2:109–110, 2:286, 4:68–69, 5:11*t*, 5:214
Partitivirus 4:63, 4:64*t*, 4:68–69
Partitivirus(es) 1:100*t*
biological properties 4:69
classification 2:109–110
fungal dsRNA viruses 2:111
double stranded RNA satellites 4:531
genera 1:99
genome 1:100
infections
fungi 2:287*t*
host range 4:75
viral concentrations 4:69
phylogeny 1:103*f*
CP cluster 4:74–75
horizontal transfer 4:74–75
RdRp cluster 4:74–75
RdRp 4:63, 4:66–67, 4:69–71
replicase activity 4:69–71
transcriptase activity 4:69–71
replication 4:71*f*
taxonomy 4:74
transmission 4:69
virion properties 4:69
virion structure 4:72
capsid 4:72
virus characterization 1:99
see also specific viruses
Partitivirus(es), fungal **4:63–68**
antigenic properties 4:65
classification 4:63
dsRNA viruses 2:111
evolution 4:65
gene expression 4:65
genome 4:65
dsRNA1 4:65
dsRNA2 4:65
infections
mixed 4:69
pathogenesis 4:67
infectivity assays 4:75
multiplication 4:66*f*
phylogenetic relationships 4:67*f*
taxonomy 4:63
tentative members 1:100*t*
transfection 4:66
transmission 4:67
asexual spores 4:69
virion properties 4:64
virion structure 4:64
Parvoviridae 2:555, 4:76, 4:86, 4:92, 4:92*t*, 4:567, 4:572, 5:11*t*
Parvovirinae 4:86, 4:92
Parvovirus 4:92
Parvovirus(es) 2:122
arthropod **4:76–85**
capsid structure 4:76
classification 4:76
history 4:76
capsid 4:86
assembly 4:86
cell receptor binding 4:87
genome packaging 4:86
role 4:86
structure 4:87*f*
classification 4:92*t*
subfamilies 4:92
control 4:89
evolution 4:88
rate 4:88–89
genome 4:94*f*
DNA replication initiator proteins 4:90
gene cassettes 4:90
hairpins 4:90
inverted terminal repeats 4:88*f*
NS1 protein 4:88
NS2 protein 4:88
open reading frames 4:87–88
packaging 4:86
promoters 4:87–88
sequence variation 4:88
geographical distribution 4:86
history 4:85
infections *see below*
propagation 4:86
proteins
antigenic sites 4:91–92
polypeptides 4:91
SF3 helicases 4:91–92
VP1 4:91
replication 4:90
DNA amplification 4:90–91
heterotelomeric parvoviruses 4:90–91
homotelomeric parvoviruses 4:90–91
phases 4:90
site-specific single-strand nicks 4:91
seasonal distribution 4:86
sequence motif conservation 4:82*f*
shrimp 4:571
genome organization 4:571
geographical distribution 4:572
host range 4:572
taxonomy 4:572
taxonomy 4:86
tissue tropism 4:86
B19 virus 4:86
transmission 4:86
vaccines/vaccination 4:89
virion structure 4:92*f*
see also specific viruses
Parvovirus B19 4:95
aplastic anemia 4:115
transplant-associated transmission 3:468
diagnostic assays 3:470–471
syndromes associated 3:470
Parvovirus infections 2:555
age-related disease 4:86
diagnosis 4:89
endosome role 4:87
host range 4:86
immune response 4:89
maternal immunity 4:89
insects 3:126*t*
nuclear entry 4:87
pathogenesis 4:89
persistence 4:109*t*
prevention 4:89
PASC *see* Pairwise sequence comparison (PASC)
Pasteur, Louis, animal virus cultivation 2:456
Patas monkeys, simian hemorrhagic fever virus infection 1:183
Patchouli virus X (PatVX) 4:311*t*
Patent infection 3:161
NPV pathology 1:220
Pathogen-associated molecular patterns (PAMPs) 1:123
definition 3:104
Pathogen-derived resistance (PDR) 4:156
definition 1:563
history 4:156
papaya ringspot virus *see* Papaya ringspot virus (PRSV)
RNAi role 3:152
Pathogenesis (of viruses) **5:314–319**
definition 5:314
entry 5:315
infection 5:314
localization 5:316
resistance 5:318
shedding 5:316
spread 5:315
susceptibility 5:318
genetic loci 5:318
mapping 5:318
tissue tropism 5:316
transmission 5:315, 5:316
virulence 5:317
genetic determinants 5:317
measurement of 5:317
pathogenic mechanisms 5:318
see also specific infections
see also specific viruses
Pathogenesis proteins (γb), hordeiviruses 2:463
Pathogen exclusion
aquareovirus infection management 1:168–169
yellow head virus infection management 5:482

Pathogenicity
see also specific infections
Pathogenicity determinants, avsunviroids 5:339–341
Pathogen-specific molecular patterns (PAMPs) 5:119*f*
dsDNA 3:114
dsRNA 3:113
envelope proteins 3:114
ssRNA 3:113–114
Patois group viruses, reassortment 3:482
PATRIC 5:357*t*
Pattern-recognition receptors (PRRs) 3:106
apoptosis 1:161
innate immunity 3:107
interferon type I 5:119*f*
virus-mediated immune evasion 3:108
PAV1 virus 5:421
genome 5:422
virion composition 5:422
PAV strain *see* Barley yellow dwarf virus (BYDV)
PAZ domain, Argonaute proteins 3:151
PB2 protein *see* Influenza virus(es)
PBCV-1 virion
genome 4:120*f*
methylation 4:119
open reading frames 4:122*f*
polyamine biosynthesis 4:124
replication 4:119
assembly 4:119–122
DNA synthesis 4:119–122
host cell attachment 4:119
host cell entry 4:119
release 4:119–122
transcription 4:119
structure 4:118*f*
capsid 4:118
glycoproteins 4:118–119
lipid membrane 4:118
PBFDV *see* Psittacine beak and feather disease virus (PBFDV)
PCPV *see* Pseudocowpox virus (PCPV)
PDB (Protein DataBank) 5:363
PDV *see* Prune dwarf virus (PDV)
PDZ domain(s), HPV E6 interactions 4:24
PE2 polyprotein, alphavirus assembly 1:197
Pea *(Pisum),* resistance genes, loci 4:182
Peaches *(Prunus persica),* Sharka disease 4:239
Peach mosaic virus (PcMV) 4:204
Peach rosette mosaic virus (PRMV) 3:406*t*
Pea early-browning virus (PEBV)
disease recurrence 3:217
movement studies 5:73
transmission 5:75
2b protein, role 5:75
2c protein role 5:75
nematodes 3:217
seeds 5:74–75
Pea enation mosaic disease, prevention 5:213
Pea enation mosaic virus-1 (PEMV-1), transmission 3:236
Pea enation mosaic virus-2 (PEMV-2)
movement 5:212–213
ORF3 5:212
RNA satellites function 5:211
Peanut clump virus (PCV) 4:98
genome 4:100*f*
Indian peanut clump virus *vs.* 4:101
intergenic regions 3:244
open reading frames 4:101*t*
RNA2 4:101
geographical distribution 4:98
infection
diagnosis 4:98
economic impact 4:98
symptoms 4:98
serological diagnosis 4:98–99
transmission 4:98–99
virus particles 4:99
Pea seed-borne mosaic potyvirus (PSbMV) 3:215–216
transmission 3:216
Pecluvirus 1:308, 4:98, 4:283
Pecluvirus(es) **4:97–103**
biological properties 4:98
furovirus *vs.* 2:295–296
future work 4:103
genome 4:99
coding sequences 4:99
noncoding sequences 4:100*f*
open reading frames 4:99–100
post-transcriptional gene silencing 4:99–100
RNA1 4:99–100
RNA2 4:99–100
sequence comparisons 4:100
t-RNA-like structure 4:100
geographical distribution 4:98
historical aspects 1:308
infections *see below*
molecular diagnosis 4:99
nucleic acid probes 4:99
reverse transcription PCR 4:99
molecular properties 4:99
particle assembly 4:101
reservoirs 4:98
seasonal distribution 4:98
transmission 4:98
Polymyxa vectors 4:98–99
sap 4:98–99
seeds 4:98–99
virus-like particles 4:102
virus particles 4:99
origin of assembly sequence 4:101–102
see also specific viruses
Pecluvirus infections
'clump' disease 4:97–98
control 4:102
biocides 4:102
continuous cropping 4:102
cultural practices 4:102
host plant resistance 4:102
epidemiology 4:101
host life cycle 4:102
host range 4:98
molecular diagnosis 4:99
serological diagnosis 4:99
symptoms 4:98
Pefudensovirus 4:76, 4:96
Pefudensovirus(es)
classification 4:94*t*
genome 4:95*f*
Pegylated interferon α-2, hepatitis B treatment 2:359
Pelargonium chlorotic ring pattern virus (PCRPV) 4:210*f*
Pelargonium flower break virus (PFBV) 4:209*f*
host ranges 4:208
Pelargonium leaf curl virus 4:210*f*
Pelargonium line pattern virus (PLPV) 4:209*f*
Pelargonium ring spot virus (PelRSV) 4:209
Pelargonium vein clearing virus (PelVCV) 4:210
Pelargonium virus infections 4:208
nonspecific 4:210
specific 4:208
yellow net vein disease 4:210*f*
see also specific viruses
Pelargonium zonate spot virus (PZSV) 4:210
Penaeid shrimps 2:37
Penaeus duorarum single nuclear polyhedrosis virus (PdSNPV)
genome 4:570
infection cycle 4:571
nuclear hypertrophy 4:571
Penaeus japonicus (kuruma shrimp), infection 4:568
Penaeus monodon densovirus (PmDNV) 4:83–84
Penaeus monodon-type baculovirus (MBV) 4:570
geographical distribution 4:571
host range 4:571
transmission 4:571
Penaeus vannamei singular nucleopolyhedrosis virus (PvSNPV) 4:570
Penelope-like elements (PLEs) 4:421–423
distribution 4:442*t*
history 4:437
origins 4:441
replication 4:426
structure 4:440
transposition 4:441
Penetration, definition 4:407
Penicillium chrysogenum virus (PcV) 1:503–504
chryso-P3 protein 1:507
evolutionary relationships 1:511
genome 1:505*f*
5′ untranslated regions 1:506*f*
virion structure 1:509*f*
capsid 1:510*f*
coat proteins 1:509
Penicillium stoloniferum virus F (PsV-F)
genome 4:73
virion morphology 4:73
Penicillium stoloniferum virus S (PsV-S)
genome 4:72–73
replication 4:66*f*
virion morphology 4:72*f*
capsid 4:73*f*
dimeric protrusions 4:72–73
Pennisetum mosaic virus (PenMV) 1:481
Pennisetum mosaic virus, maize infections 1:481
Pentamer units, cowpea chlorotic mottle virus 5:385
Penton bases
adenoviruses 1:17–18
HSV capsid structure 2:397
Pepino mosaic virus (PepMV) 2:87
antiserum 4:104–105
capsid protein 4:104
classification 2:87
coat protein 4:106
genome 2:87
coat protein 4:106
5′-nontranslated region 4:105
intergenic region 4:105
open reading frames 2:87
TGB protein 1 4:105
TGB protein 2 4:106
TGB protein 3 4:106
3′-nontranslated region 4:106
triple gene block 4:105
geographical distribution 2:87
historical aspects 4:104
infection *see below*
isolates/strains 4:106
full-length sequences 4:106
relationships between 4:107
restriction fragment length polymorphism 4:106–107
RT-PCR 4:106
sequence data 4:106
single-stranded conformation polymorphism pattern 4:106
phylogeny 2:88*f*
structure 2:87
transmission 2:87
mechanical transmission 4:108
seed transmission 4:108
viability 2:87
virions 4:104
capsid protein 4:104
stability 4:104–105
Pepino mosaic virus infection
control 2:88
damage 4:107
environmental conditions 4:108
epidemiology 2:87
geographical distribution 4:107
mixed infections 4:107
population studies 4:107
strain dependency 4:107
host range 2:87
alternative hosts 2:87
experimental 2:87
natural hosts 4:104
weed hosts 4:104
mixed infections 4:108
symptoms 2:87
inoculation studies 4:104

Pepino mosaic virus infection (*continued*)
tomato 2:87
symptoms 2:87
yield losses 2:87
Pepper *(Capsicum)*
cucumber mosaic virus infection 5:287–288
potato virus Y infections *see* Potato virus Y (PVY)
resistance genes, loci 4:182
Pepper mild mottle virus (PMMoV) 5:288
Pepper ringspot virus (PeRSV)
geographical distribution 5:76
host range 5:76
Pepper veinal mottle virus (PVMV), resistance to 4:184
Pepper vein banding virus (PVBV), nuclear inclusion a protein 4:317–319
Pepsin, host defence against rotaviruses 4:512
Peptide editing, MHC-II antigen presentation 1:124–125
Peptidoglycan A 5:365
Peptidoglycan-hydrolyzing enzymes, bacterial cell viral entry 5:366*f*
Peptidyl-prolyl cis transferase-isomerase (PPIase), role in cell lysis 3:19
Perforin(s) 3:80
Pericarditis 1:533
Perina nuda virus (PnV) 3:45
Perinatal disease, coxsackieviruses 1:585
Perinet virus 4:184*t*
Periplaneta fuliginosa densovirus (PfDNV)
classification 4:77
infection 4:79
Persistent infections **4:108–116**
acute *vs.* 4:110*f*
apoptosis 4:113
chronic disease manifestations 4:114
pregnancy 4:114–115
concepts 4:108
definition 1:335
immune control 4:112
adaptive response 4:112
cytokines 4:112
dendritic cells 4:112
dysregulation 4:113
immunological memory 4:111*f*
interferons 4:112
macrophages 4:112
pathogen–host interactions 4:112
immunocompromised patients 4:115
immunomodulation 4:113
apoptosis 4:113
cell stress 4:113
TLR signaling
immunopathology 4:113
latent *vs.* 4:109
pathogenesis 4:111*f*
reactivation 4:111
vaccines/vaccination 4:108–109
booster 4:109–110
viruses associated 4:112
Persistently associated transcript (PAT1), Hz-1V replication 3:146
Person-to-person transmission, monkeypox virus 4:642*f*
Pertussis vaccine, safety 5:228
Peste des petits ruminants virus (PPRV)
epidemiology 4:45
epizootiology 4:503*f*
geographical distribution 4:499*f*
host range 4:499
propagation 4:500
Pestivirus 1:336, 2:242, 2:246, 5:108, 5:440–441
Pestivirus(es) 2:246
antigenic relationships 1:338
bovine viral diarrhea viruses 1:378
classification 1:336
E1 protein 2:247–248
E2 protein 2:247–248
genetic relationships 1:338
genome 2:247*f*
ORF processing 2:247–248
history 2:246
infection 2:246
control 2:248
diagnosis 2:248
disease manifestations 2:246–247
epidemiology 2:246
host range 2:246
pathogenesis 2:248
persistence 2:246
prevention 2:248
physical properties 2:247
replication 2:247
structure 2:247
transmission 2:246
see also specific viruses
Petechiae, definition 3:454
Petunia vein banding virus (PetVBV) 4:211
Petunia vein clearing virus (PVCV) 4:212*f*
transmission 1:462
host genome integration 1:462
Petunia virus infections 4:211
nonspecific 4:211
specific 4:211
see also specific viruses
Petuvirus
host chromosome integration 1:462
open reading frames 1:466*f*
virion structure 1:458–460
Petuvirus 1:459*t*
PEV *see* Porcine enterovirus(es) (PEV)
Peyer's patches, poliovirus infection 4:243
Pf1 bacteriophage *see* Bacteriophage Pf1
Pf2 bacteriophage 1:201–202
Pf3 bacteriophage *see* Bacteriophage Pf3
Pf4 bacteriophage 3:123–124
PFAM database 5:357*t*
P gene, henipavirus 2:323
PG virus 5:413*t*
PHABULOSA (PHB) 4:142
PHABVOLUTA (PHV) 4:142
Phacochoerus aethiopicus (warthog), African swine fever virus 1:44*f*
Phaeovirus 4:117*t*
Phage *see* Bacteriophage(s)
Phage display *see* Antibody phage display
Pharyngoconjunctival fever 2:554–555
Phaseolus vulgaris see Bean *(Phaseolus vulgaris)*
Phase transitions 4:359
Phenotypes 4:40
species classification 5:404
Phi6 bacteriophage 4:379–380
Φ29 bacteriophage *see* Bacteriophage ϕ29
ϕCh1 virus 5:418
genome 5:419*f*
methylation 5:418
open reading frames 5:418
structure 5:418*f*
ϕF1 virus 5:413*t*
ϕF3 virus 5:413*t*
ϕH virus 5:413*t*
genome 5:412
morphology 5:412
transfection 5:412
ϕN virus 5:412
ϕX174 bacteriophage *see* Bacteriophage ϕX174
Philosamia cynthia X ricini virus (PxV) 5:28*t*
Φ_L promoter 5:179
Φ_M promoter 5:179
Phlebotomine flies, definition 2:234
Phlebotomus fever virus(es) 4:490
see also specific viruses
Phlebotomus perniciosus, Toscana virus transmission 4:491
Phlebovirus 1:391, 3:480, 4:490–491, 5:27
Phlebovirus(es) **4:490–497**
control 4:496
definition 4:490
G2 proteins 3:310
genome 4:492*f*
history 4:491
infections *see below*
NSs protein 1:395
species 4:493*t*
structure 4:490–491
transmission prevention 4:496
vaccines/vaccination 4:496
see also specific viruses
Phlebovirus infections
diagnostic procedures 4:494
isolate identification 4:494
serologic tests 4:494
virus detection 4:494
virus recovery 4:494
epidemiology 4:494
prevention 4:496
therapy 4:495
Phloem
banana bunchy top virus 1:273
barley yellow dwarf virus replication 1:284*f*
definition 5:209
pH-mediated fusion
herpesvirus gB structure 3:300
influenza-host cell entry 3:486*f*
VSV G structure 3:300
Phocine distemper virus (PDV)
epizootiology 4:502–504
geographical distribution 4:498–499
history 4:498
propagation 4:500
Phosphatidylserine, VSV-host cell attachment 5:292–293
Phosphoenolpyruvate carboxykinase, reticuloendotheliosis virus 4:416
Phospholipase A2
BK virus-host cell attachment 4:281
densovirus 4:81
parvovirus 4:78
Phospholipid bilayer, BK virus infection, role 4:281
Phosphoprotein P *see* Chandipura virus
Phosphoproteins
HCMC 2:489–490
murine polyomavirus middle T 4:274–275
VSV 5:295
Phosphorylation, varicella-zoster virus proteins 5:261
Phosphotransferase, sawfly baculovirus genome 1:227
Phosphotungstic acid, negative staining 2:81
Photolyases, avipoxvirus survival factors 2:275
Phthorinaea operculella granulovirus (PhopGV) 1:214*f*
Phycodnaviridae 1:87, 4:118
Phycodnavirus(es) **4:116–125**
composition 4:118
ecology 4:124
evolution 2:181–182
genomes 4:119
history 4:116
replication 4:119
assembly 4:119–122
DNA synthesis 4:119–122
host cell attachment 4:119
host cell entry 4:119
release 4:119–122
transcription 4:119
structure 4:118
viral encoded enzymes 4:123
sugar metabolism 4:123
see also specific viruses
Phylogenetics 4:126
alignment algorithms 5:343
bootstrap analysis 4:127
definition 2:130
distinct genes 4:127
fungal retrotransposons 4:422*f*
genomics 4:127
homologous recombination 4:127
'in-group' 4:127
LTR retrotransposons 4:441
maximum likelihood 4:126–127
maximum parsimony 4:126–127
molecular clock models 4:127
neighbor-joining methods 4:126–127
non-LTR retrotransposons 4:441

'outgroup' 4:127
satellite viruses 4:534
sequence alignment 4:126, 5:343
species differences 4:126–127
unweighted pair group method with arithmetic means 4:126–127
see also specific viruses
Phylogenetic trees 4:126
branch length 4:126
definition 2:105
hypothetical taxonomy units 4:126
most recent common ancestor 4:126
operational taxonomy units 4:126
topology 4:126
see also specific viruses
Phylogeny **4:125–129**
antibody-mediated immunity 3:58
applications 4:127
classification 4:128
definition 1:441
diversity 4:125–126
geographical distribution 4:128
goals of 4:125
identification 4:127
molecular clock model 4:125
monitoring 4:127–128
new virus identification 4:128
outbreak control 4:127
virus–host associations 4:128
zoonotic reservoir identification 4:128
see also specific viruses
Physical contact, Simian retrovirus D transmission 4:627–628
Phytoene desaturase (PDS), virus-induced gene silencing role 2:466
Phytophthora 2:288
edornaviruses 2:111
Phytophthora endornavirus 1 (PEV1) 2:111
Phytoplankton
ecological dynamics 1:94–95
viral infection of 1:87
Phytoreovirus 1:483, 1:484, 4:150–151, 4:536*t*
Phytoreovirus(es) 4:536*t*
characteristics 4:150*t*
classification 4:150
genome 4:152
dsRNA segments 4:153*f*
size 4:150–151
host range 4:154
transmission 4:154
type species 4:536*t*
virion structure 4:152
capsid 4:152
see also specific viruses
Phytoreovirus P7 proteins 1:507
Phytosanitation, cassava mosaic disease control 4:198
PI-3 kinase, Jaagsiekte sheep retrovirus infection 3:181
Picobirnavirus(es) **1:321–328**
definition 2:93
detection 1:327
genome 1:327
HIV co-infection 2:94
mutation 1:327
RdRp 1:327
RdRp structure 1:327
virion structure 1:327
see also specific viruses
Picorna-like capsids 5:398
Picorna-like virus(es) 2:37
shellfish viruses 4:565
see also specific viruses
Picornaviridae 1:90*f*, 1:442, 1:542, 1:573, 1:580–581, 2:65–66, 2:266, 2:343, **4:129–140**, 4:130*t*, 4:243–244, 4:244*t*, 4:468, 5:2–3, 5:11*t*, 5:20, 5:38
Picornavirus(es) 2:65–66
apoptosis inhibition 1:158*t*
capsid 4:132*f*
canyon region 4:131
VP1 4:131
VP2 4:131
VP3 4:131
VP4 4:131
capsid structure 1:201*f*
classification 4:130*t*
$3D^{pol}$ 4:137
fish 2:229
gene expression 4:131
genomes 4:131*f*
internal ribosome entry sites 4:134*f*
noncoding regions 4:130
infections
antibody-mediated neutralization 3:416
insects 3:126*t*
respiratory infection 2:552
internal ribosome entry sites 4:130, 4:133, 4:133*f*, 4:138–139
hepatitis A virus replication 2:344
hepatitis C virus replication 2:370
PABP interactions 4:138–139
PCBP interactions 4:135
PTB interactions 4:134*f*
replication 4:133
type I 4:133
type II 4:133
type *vs.* type II 4:134–135
neutralizing antibodies 1:138
polyprotein processing 4:130
process 5:20
RdRp 4:137
replication 2:127
cis-acting replicating elements 4:139
host cell entry 4:131
internal ribosome entry sites *see above*
polyprotein processing 4:136*f*
RNA replication 4:138*f*
RNA synthesis 4:139
translation 4:132
uncoating 4:409
VPg role 4:132–133
RNA recombination 4:377
structure 5:391–392
viral proteinase substrates 4:135
capsid proteins 4:135
protein cleavage shutoff 4:137
RNA replication proteins 4:136
translation inhibition 4:137
see also specific viruses
Picornavirus-like superfamily 3:44
Pig(s)
lymphoproliferative disease 2:428
post-weaning multisystemic wasting syndrome 1:515–516
vesicular exanthema 1:410
see also under Porcine
Pigeon pea sterility mosaic virus (PPSMV) 3:216–217
Pigeonpox virus(es) 2:278
pIII, bacteriophage fd 2:194
Pike fry rhabdoviruses (PFRV) 2:228
Pili 3:21
filamentous ssDNA bacteriophage infection 5:369
F-type 2:191
N-type 2:191
type IV 2:191
icosahedral enveloped dsDNA bacteriophages 5:369
icosahedral ssRNA bacteriophages 5:369–370
viral entry 5:366
Pilin, filamentous ssDNA bacteriophage infection 2:191
Pineapple bacilliform virus (PBV) 1:403
Pinholins
definition 3:249
holin–endolysin lysis 3:254
Pinniped calicivirus infection, pigs 1:414–415
Pinnipeds
calicivirus infection 1:414–415
definition 1:410
Pinwheel, definition 1:483
Pirie, Norman, tobacco mosaic virus 3:399
Pixuna virus (PIXV), characteristics 5:109*t*
Plant(s)
development 5:330
post-transcriptional gene silencing 3:410
removal, disease control 1:35, 3:412
Plant(s), natural disease resistance (dominant genes) **4:170–177**
avirulence genes 4:173
NB-LRR protein 4:171, 4:172*t*, 4:175
coiled-coil structure 4:171
divergence 4:171
TIR-NB-LRR 4:171
R gene-*Avr* recognition mechanisms 4:175
guard hypothesis 4:175
products 4:171
responses to viruses 4:173
R genes
durability 4:175–176
polymorphism 4:171
products 4:171
responses to viruses 4:173
signaling mechanisms 4:176
Sw-5 gene 4:174
Plant(s), natural disease resistance (recessive genes) **4:177–187**
eukaryotic initiation factor-4E *see* Eukaryotic initiation factor-4E (eIF4E)
gene types 4:178
mechanisms 4:182
Fraser's hypotheses 4:182
translation initiation factors, roles 4:183
molecular structure 4:182
pathotypes 4:182
phenotypes 4:182
prevalence 4:178, 4:179*t*
VPg role 4:178–182
Plantago asiatica mosaic virus (PlAMV) 4:311*t*
Plantago severe mottle virus (PlSMoV) 4:311*t*
Plantain virus X (PlVX) 4:311*t*
Plant breeding
bean golden/yellow mosaic disease resistance 1:299–300
beet curly top virus resistance 1:307
papaya ringspot virus resistance 4:5
potato virus Y resistance 4:293, 4:294*f*
Sharka disease resistance 4:242
Plant chrysovirus(es)
occurrence 1:511
see also specific viruses
Plant–fungal mutualistic associations 2:291
Planthoppers
fijivirus transmission 4:150*t*, 4:154
oryzavirus transmission 4:150*t*, 4:154
rice ragged stunt virus transmission 1:485
tenuivirus transmission 5:26
Planting times, barley yellow dwarf virus control 1:286
Plant pararetrovirus(es)
endogenous 1:464–465
genera 1:464–465
see also specific viruses
Plant-produced vaccines *see* Vaccines/vaccination
Plant reovirus(es) **4:149–156**, 4:150*t*
classification 4:150
distribution 4:154
gene expression 4:152
genome 4:151
host range 4:150, 4:154
infection 4:149–150
control 4:155
diagnosis 4:155
symptoms 4:155
pathogenicity 4:155
replication 4:152
taxonomy 4:150
transmission 4:150*t*, 4:154
virion structure 4:151
see also specific viruses
Plant retinoblastoma-related protein (pRBR), maize streak virus 3:267
Plant rhabdovirus, transmission 4:195
Plant viroids/virusoids, ribozymes of 4:476

Plant virus(es)
  history **2:450–455**
    biochemical age 2:451
    biological age 2:451
    biophysical age 2:451
    crop resistance 2:454
    enzyme-linked immunosorbent assays 2:453
    heat treatments 2:454
    meristem tip culture 2:454
    molecular biology age 2:453
    nucleotide sequence information 2:453
    prehistory 2:450
    purification 2:453
    replication 2:453
    vectors 2:453–454
    viral entity recognition 2:450
    yeast systems 2:453
  host cell receptors 4:408
  infections *see below*
  morphologies 2:22*f*, 2:23*f*
  recombination 4:376
  replication 2:453
  vectors *see* Plant virus vectors;
  see also specific viruses
Plant virus infections
  diagnosis **2:18–29**
    applications 2:19
    functional studies 2:26
    host plant reactions 2:21, 2:24*f*
    immunoelectron microscopy *see below*
    see also specific techniques
  economic aspects 4:156, **4:197–201**
    chlorophyll defects 3:218
    climatic regions 4:197
    faba bean necrotic yellows nanovirus infection 3:218
    transmission 4:197
    yield losses 4:197
  emerging/re-emerging viruses **2:86–93**
  immunoelectron microscopy 2:20, 2:21*f*, 2:23*f*
    antibody reactivities 2:20–21
    mixed infections 2:20–21
  movement **3:348–356**
    host factors 3:349
    intercellular 3:348, 3:352
    intracellular 3:348, 3:349, 3:351*f*
    RNA viruses 3:350
    systemic 3:348, 3:353
    transport/accumulation regulation 3:354
    tubule forming viruses 3:353
    see also Movement proteins (MPs)
  nucleotide sequence analysis 2:21
    DNA viruses 2:21
    RNA viruses 2:21
  physical studies 2:26
  primary diagnosis 2:19
    symptom inspection 2:19, 2:20*f*
  routine detection 2:23
  structural property studies 2:26
  vectors *see below*
  virus-encoded proteins 3:332
  see also specific viruses
Plant virus vectors
  associated viruses 4:229
  deconstruction 4:229, 4:230
  definition 2:18, 5:275–276
  evolution 5:281
    viral variant distribution 5:281
    virus acquisition patterns 5:281
  history 4:230
  insects *see below*
  non-insect vectors associated 5:277*t*
    fungi 5:281
    mites 5:280
    nematodes 5:281
  vegetable virus transmission 5:284–285
    control 5:286–287
  see also specific vectors
Plant virus vectors, insects 5:276
  beetle-mediated transmission 5:280
  circulative transmission 5:276*f*, 5:277, 5:277*t*
    nonpropagative 5:278*f*
    propagative 5:277
  classification 5:276
  noncirculative transmission 5:276*f*
    capsid strategy 5:280*f*
    helper strategy 5:279
    see also Helper component
    host cell attachment 5:279
    retention sites 5:279
  see also specific insects
Plaque assays 2:72*f*
  fusellovirus detection 2:297–298
  history 2:456
Plaque-forming unit (Pfu) 3:21
Plaque reduction (PRNT) 4:494
Plasmacytoid dendritic cells (pDC) 3:112
Plasmalemma, ascovirus infection 1:191
Plasmaviridae 5:11*t*
Plasmavirus(es), genome delivery 5:368
Plasmids, DNA vaccines *see* DNA vaccine(s)
Plasmodesmata
  caulimovirus infection 1:464
  definition 1:457
  plant virus movement 3:348
  TMV-associated changes 5:71
Plasmodiophorid fungi *see Polymyxa* (plasmodiophorid fungi)
Plasmodiophorid fungi *(Polymyxa graminis)*
  furovirus transmission 2:292
  legume virus transmission 3:217
  pomovirus transmission 4:285*f*
  rice necrosis mosaic virus transmission 1:489
  rice stripe necrosis virus transmission 1:489
Plastic mulches, infection control 5:437
Plastochron 3:264
Platelet-derived growth factor receptor β (PDGFβ), HPV E5 4:23
Platforms, *Agrobacterium tumefaciens*-based expression system 4:237*f*
Plautia stali intestine virus (PSIV), gene expression 5:5
Pleconaril 2:70
  rhinovirus infection 2:556–557
Pleiotropic mutations 4:177
Pleomorphic dsDNA bacteriophages, genome delivery 5:368
Pleurodynia
  coxsackieviruses 1:585
  definition 1:580
'Plowright vaccine' rinderpest viruses 4:506
Plum *(Prunus)*
  raspberry bushy dwarf virus infection 3:39
  Sharka disease 4:239
  see also entries under Prunus
  see also specific viruses
Plum pox virus (PPV) 4:201
  genome 4:239
    noncoding region 4:317
  geographical distribution 4:238
  historical aspects 4:201
  infections *see* Sharka disease
  strains/groups 4:240*f*
    agar double diffusion assays 4:239
    evolution 4:240–241
    full length genome sequencing 4:240
    PAGE 4:239
    PPV-C (cherry) 4:240
    PPV-D *(Dideron)* 4:240
    PPV-EA (El Amar) 4:240
    PPV-M *(Marcus)* 4:239–240
    PPV-Rec *(recombinase)* 4:240
    PPV-W (Winona) 4:240
    RFLP 4:241
    serology 4:241
  symptoms 4:201
  transmission 4:238
    aphid vectors 4:241
    efficiency 4:241
    nonpersistent infections 4:241
    seed transmission 4:241
  virion 4:239
Plus-strand synthesis, alfalfa mosaic virus 1:84–85
PM2 (corticovirus) *see* Corticovirus PM2
PM2 bacteriophage, genome delivery 5:368
PML-containing nuclear oncogenic bodies, adenovirus E1A gene/protein interactions 1:12
PMS1 virus 5:413*t*
Pneumabort K 2:419
Pneumocystis jirovecii
  definition 4:623
  HIV-associated opportunistic infection 1:52
Pneumonia
  cats, cowpox virus infection 1:579
  definition 2:551
  HSV associated 2:391
  human respiratory syncytial virus infection 2:548
  mimivirus infection 3:312
  SARS 4:552
Pneumonitis 5:424
Pneumovirinae 4:40, 4:41*t*, 4:47, 4:52
Pneumovirus 4:41*t*
Pneumovirus(es)
  classification 4:41*t*
  genome 3:327*f*
    leader region 4:42
  M2 gene 3:332
  nonstructural proteins 3:332
  nucleocapsid 3:325–329
  propagation 4:40–42
  see also specific viruses
Poa semilatent virus (PSLV) 2:459
  coat proteins 2:459
  genome 2:459–460
  pathogenesis proteins 2:463
  TGB3 movement protein 2:464
Pock 4:639
Pocket factor, enterovirus capsid stabilization 2:128
Pocket protein-binding motif, HPV E7 4:25
Podospora anserina, prion 4:337
Podosta disease *see* Sindbis virus (SINV)
Podoviridae 3:31, 3:32*t*, 5:11*t*
Podovirus(es)
  DNA injection 3:33–34
  genome packaging 2:306
  short tails 5:368
  structure 1:434*f*
Pogosta disease, seasonal distribution 5:90
Poinsettia latent mottle virus (PnLMoV) 4:651*f*
Poinsettia mosaic virus (PnMV) 5:204*f*
  characteristics 5:206
  genome 5:202*f*
  host range 5:206
  properties 5:206
  transmission 5:206
Point mutations, bacteriophage evolution 5:371
Point-of-care tests (POC), principles 2:33
Poisson distribution, infection neutralization 3:416–417
Pol (polymerase), LTR retrotransposons 4:438–439
Polerovirus 3:231, 3:232*f*, 3:232*t*
pol gene/protein
  LTR retrotransposons 4:420
  Simian retrovirus D 4:627
  SIV serological relationships 4:607–608
polh promoter, baculovirus 1:238
  *flash*BAC 1:244
Poliomyelitis 2:65–66
  acute 1:474
  disease surveillance 2:48*f*
    data collection sources 2:48*f*
  immune system evasion 3:108
  pathogenesis 4:242
  Peyer's patches 4:243
  systemic infection 4:243
  TMEV infection 5:42
  transmission 4:243
  vaccine-derived 4:246
Polio vaccines/vaccination 2:458
  disease eradication 5:232
    developing countries 4:245–246
    Northern Europe 4:245–246
    tropical climates 4:245–246
  HIV-1 origin, human-induced theory 2:530*f*

inactivated 4:244
monovalent 4:245–246
National Immunization Days 4:245–246
Nigeria
circulating vaccine-derived poliovirus 4:246–248
disease eradication 4:246
National Immunization Days 4:246
oral 4:244
post-vaccination virus excretion 4:245
Sabin strains 4:244
mutation 5:317
type 1 4:244–245
type 2 4:244–245
type 3 4:245
safety
adverse reactions 5:230*t*
SV40 contamination 4:630
vaccine-associated paralytic poliomyelitis 4:246
vaccination program cessation 4:249
Poliovirus 4:129
Ab-mediated neutralization 3:416
cell surface binding 2:129
classification 4:129
genome 4:243
noncoding regions 4:139
sequencing 2:143
geographical clustering 4:244
host cell receptor 4:131–132
infection *see* Poliomyelitis
neural cell infection 1:473
protein 3A 2:579–580
proteinase substrates
protein 2C 4:136
structure 4:136–137
protein 3AB 4:137
proteolytic processing 2:127
RNA translation 2:457
serotypes 4:243
vaccines/vaccination *see* Polio vaccines/vaccination
viremia 4:243
virulence 5:318
vaccine reversion 4:246
virus structure 4:132*f*
Poliovirus 4:243
Poliovirus type 3, structure 5:382*f*
Pollen
gene flow in transgenic plants 4:162
Oryza sativa endornavirus 2:114
POL polyprotein, caulimoviruses 1:467
pol proteins
see specific viruses
Pol reading frame, avian retroviruses 4:456
Polyacrylamide gel electrophoresis (PAGE), plum pox virus strains/groups 4:239
Polyactide-$_L$-glycolide (PLG), DNA vaccine delivery 2:54
Polyadenylation
cauliflower mosaic virus transcription 1:467–468
definition 1:296
Poly-A retrotransposons *see* Target-primed retrotransposons
Polyarthritis 1:399
Poly(A) binding protein (PABP)
alfalfa mosaic virus coat protein 1:83–84
picornaviruses 4:138–139
Polycistronic mRNA, single-stranded positive sense RNA viruses 4:410
Polycistronic RNA 5:145
Polyclonal antiserum 1:296
Polydnaviridae 1:247, 4:256–257, 5:11*t*
Polydnavirus(es) (PDV) **4:256–261**
apoptosis inhibition 1:158*t*
classification 4:256
definition 4:256
genome 4:257
gene expression 4:258
sequence analysis 4:257–258
transposable elements 4:257–258
infection *see below*
life cycle 4:260*f*
assembly 4:260–261
virion accumulation 4:260–261
phylogeny 4:261
replication 4:260
gene expression 4:260–261
taxonomy 4:256
virion structure 4:257
brachovirus 4:257
bracovirus 4:257
ichnovirus 4:257
virulence genes 4:250
see also specific viruses
Polydnavirus infections 4:253
immune evasion 4:253
virulence genes 4:250
insects 3:126*t*
virus-mediated immune abrogation 4:253
wasp–virus mutualism 4:250
Polyhedra *see* Occlusion bodies
Polyhedrin 1:238
baculoviruses 1:251
definition 1:254
gene 1:265
molecular arrangement 3:136
structure 3:136
Polyhedron envelope/calyx protein (PEP/P10) 1:217
Polylactosaminoglycan 2:543
Polymerase chain reaction (PCR) 2:34*f*
advantages 2:35
definition 2:29
degenerate primers 2:420, 2:421*f*
detection spectrum 2:423
method variations 2:421
primer binding sites 2:422*f*
detection/diagnosis
adenovirus 1:29
anellovirus 1:109
avipoxviruses 2:280
banana bunchy top virus 1:273
bean golden mosaic virus infection 1:299
bean golden/yellow mosaic disease 1:297–298
bean golden yellow mosaic virus 1:299
benyvirus 1:313
cacao swollen shoot virus 1:405
Dengue virus 2:8–9
emerging viral diseases 2:93
fusellovirus 2:299
geminiviruses 4:167
hepatitis B virus 2:351
herpesviruses 2:421*f*
orthobunyavirus 3:482–483
plant viruses 2:25*f*
potyvirus 4:316
pseudorabies virus 4:350
rabies virus 4:372
rhinovirus 4:471
Taura syndrome virus 5:8
VSV 5:298
internal controls 2:35
limitations 2:33–34
multiplexing 2:25
principles 2:33–34
amplification 2:34
product visualization 2:34*f*
sequencing 2:35
reverse transcription *see* Reverse transcription polymerase chain reaction (RT-PCR)
vaccine safety 5:228
Polymerase complex (PC)
definition 3:6
icosahedral enveloped dsDNA bacteriophages 3:8*f*
Polymerase inhibitors 1:149
Polymerases
discovery 2:456–457
icosahedral enveloped dsDNA bacteriophages 3:12
large 5:296
conserved domains 5:296
see also specific types
Polymerase stuttering, mumps virus 3:358–359
Polymorphisms
definition 5:186
nonsynonymous 2:471–472
Polymyxa (plasmodiophorid fungi)
benyvirus transmission 1:309
furovirus transmission 2:292
legume virus transmission 3:217
pecluvirus transmission 4:98–99
rice necrosis mosaic virus transmission 1:489
rice stripe necrosis virus transmission 1:489
Polymyxa graminis see Plasmodiophorid fungi *(Polymyxa graminis)*
Polyoma enhancer, polyomavirus 4:272
Polyomaviridae 4:34–35, 4:277, 4:631
Polyomavirus 4:631
Polyomavirus(es) **4:277–282**
apoptosis inhibition 1:158*t*
classification 4:277
papillomaviruses *vs.* 4:277
evolution 2:182–183
genome 4:278
functional regions 4:278
host cell susceptibility 4:281
DNA-binding proteins 4:281
non-coding region hypervariability 4:281
infections
associated nephropathy 4:269
respiratory 2:556
murine *see below*
physical properties 4:277
stability 4:278
physicochemical properties 4:277
virion assembly 4:278
proteins 4:278
agnoprotein 4:278
capsid 4:278
T proteins 4:278
replication 4:409–410
control 4:281
cytoplasmic entry 4:279
nuclear entry 4:279
receptors 4:279
structure 5:388
taxonomy 4:34–35
virion morphology 4:277
capsomers 4:277
structural proteins 4:277
see also specific viruses
Polyomavirus(es), murine **4:271–277**
cytoplasmic entry 4:279
endocytosis 4:279
host cell receptor binding 4:279
large plaque isolates 4:279
small plaque isolates 4:279
genome 4:272*f*
genetic analysis 4:272
polyoma enhancer 4:272
T antigens 4:271–272
see also specific antigens
historical aspects 4:271
infection 4:273
abortive transformation 4:273
entry processes 4:273
kinetics of 4:273
stable transformation 4:273
large T 4:275
cellular DNA synthesis 4:276
dihydrofolate reductase activation 4:276
infection kinetics 4:273
replication 4:273
retinoblastoma gene product binding 4:276
structure 4:276*f*
thymidine kinase activation 4:276
transcription 4:273
transformation 4:276
middle T 4:274
cell signaling 4:274*f*
membrane association 4:274
phosphorylation 4:274–275
PTK complex 4:274
small T complementation 4:275
small T *vs.* 4:273–274
src family tyrosine kinases 4:274
transcription 4:273

Polyomavirus(es), murine (*continued*)
transformation 4:274
transgenic models 4:274
as models 4:271
particle structure 4:271
VP1 structural protein 4:271
VP2 structural protein 4:271
VP3 structural protein 4:271
replication 4:272
transcription 4:272
RNA transcription 4:273
ADAR enzyme 4:273
early proteins 4:273
T proteins 4:273
small T 4:275
cellular distribution 4:275
cellular functions 4:275
middle T complementation 4:275
middle T *vs.* 4:273–274
p53-induced apoptosis 4:275
transcription 4:273
transformation role 4:275
transformation 4:273
host effects 4:277
tumorigenesis 4:271
Polyphyletic origins, viral evolution 2:176
Poly(A) polymerase, Amsacta moorei entomopoxivirus 2:139
6K Polyprotein, alphavirus 1:197
Polyprotein(s) 4:129
alphavirus assembly 1:197
definition 2:343, 4:407
leishmaniaviruses 3:222
rice yellow mottle virus 4:487
Polypurine tract (PPT) 4:428
Polypyrimidine tract binding protein (PTB), IRES interactions 4:134*f*
Polythetic class(es) (classification)
definition 5:401
Pome fruits, viral infections *see* Fruit trees, diseases; *see specific viruses*
Pomovirus 1:308, 4:283
Pomovirus(es) **4:282–287**
classification 4:283
control 4:286
genetic modification 4:287
resistant cultivars 4:287
CP gene/protein 4:284
genome 4:283
encapsidated RNA2 4:284
encapsidated RNA3 4:284
open reading frames 4:283
RT proteins 4:284
TGB proteins 4:283–284
untranslated regions 4:283
virus movement proteins 4:283–284
geographical distribution 4:284
historical aspects 1:308
infection
control 4:286
diagnosis 4:286
host range 4:284
virus–host interactions 4:284
movement 4:285*f*
particles 4:283
reverse-phase PCR 4:286
serological relationship 4:285*f*
taxonomy 4:283
transmission 4:284
plasmodiophorid vectors 4:285*f*
see also specific viruses
Pongine herpesvirus 1 (PoHV-1) 4:587*t*
Pongine herpesvirus 2 (PoHV-2) 4:587*t*
Pongine herpesvirus 3 (PoHV-3) 4:587*t*
Population ecology, bacteriophages *see* Bacteriophage(s), ecology
Population genetics
fungal retrotransposons 4:427
HIV antigenic variation 1:133
Population studies, Pepino mosaic virus 4:107
Porcine adenovirus(es) 1:26
Porcine circovirus (PCV)
chimeras 1:516
genome 1:515*f*
conserved regions 1:516
open reading frames 1:517
proteins, functional analyses 1:518
DNA binding 1:518
localization 1:519
P_{rep} repression 1:518–519
protein–protein interactions 1:519
replication 1:518
replication 1:518
conserved elements 1:518
origin of replication 1:518
structure 1:514*f*
transcription 1:517
P_{cap} 1:517–518
P_{rep} 1:517–518
type 1 1:516
type 2 1:516
host cell attachment 1:517–518
immune system interactions 1:516
post-weaning multisystemic wasting syndrome 1:515–516
tissue tropism 1:518
Porcine enterovirus(es) (PEV) 2:124
genome 2:126
Porcine lymphotropic gammaherpesvirus 1 (PLHV-1) 2:428
Porcine lymphotropic herpesvirus 3 (PLHV-3) 2:421
Porcine parvovirus(es) (PPV) 4:89
Porcine respiratory and reproductive syndrome virus (PRRSV) 1:176–177
geographical distribution 1:177
infection *see below*
structure 1:178*f*
Porcine respiratory and reproductive syndrome virus infection
cell tropism 1:182–183
clinical features 1:183
host range 1:177
immune response 1:184
neutralizing antibodies 1:184
pathogenicity 1:183
vaccination 1:185
Porcine torovirus (PToV) 5:151–152
antigenic properties 5:155
classification 5:151–152
genotypes 5:155
Porencephaly, definition 1:76
por mutation (MurA) 3:257–258
Portal 3:30
Portal protein 1:433
bacteriophage structure 1:437
Porthos latent virus (PLV), genome 5:147*f*
Portunus depurator (Mediterranean crab), shrimp virus infection 4:568
Positive-sense RNA strand, necrovirus 3:403
Positive staining, negative staining *vs.* 2:79
Positive strand RNA 4:129
Pospiviroidae 5:11*t*, 5:333*t*, 5:335
Pospiviroids
classification 5:335
monomer circularization mechanism 4:479–480
replication 4:478*f*
see also specific viruses
Post-attachment neutralization (PAN) 3:415
kinetics 3:416
Postexposure treatment, rabies virus 4:373
Postherpetic neuralgia (PHN) 5:253, 5:262
Postlarvae, definition 4:567
Postmarketing surveillance (vaccine safety) 5:231
Post-transcriptional gene silencing (PTGS) 5:375–376
African cassava mosaic disease 1:33
βC1 role 1:318
cis-acting siRNA pathway 4:142–145
IR-PTGS 4:142–145
S-PTGS 4:142–145
cotton leaf curl disease 1:568
definition 4:97
exploitation of 5:376
luteoviruses 3:234–235
pecluvirus 4:99–100
potato virus Y helper component protein 4:288
resistance, engineered 4:161
tombusviruses 5:148–149
vector competence 5:271
VIGS system development 5:375–376
Post-transcriptional modifications
arenavirus glycoproteins 3:244
toroviruses 5:154
Post-transplantation lymphomas (PTLs), EBV associated 2:152*f*
Post-weaning multisystemic wasting syndrome (PMWS) 1:515–516
diagnostic criteria 1:516
experimental disease reproduction 1:515–516
mortality rate 1:515–516
Potamochoerus porcus (bushpig), as an African swine fever virus host 1:43
Potato *(Solanum),* infection resistance
breeding 4:297–302
extreme resistance 4:303*f*
gene duplication 4:172–173
N genes 4:303
R genes 4:303
types 4:297–302
Potato 14R virus (P14V) 4:298*t*
Potato aucuba mosaic virus (PAMV) 4:300*t*, 4:311*t*
transmission 4:310
Potato black ringspot virus (PBRSC) 3:406*t*
diseases 3:411
Potato deforming mosaic virus (PDMV) 4:298*t*
Potato latent virus (PotLV) 4:300*t*
Potato leafroll virus (PLRV) 4:297*t*
economic impact 4:199
engineered resistance 4:157–160
transmission 4:302
Potato mop-top virus (PMTV) 4:283
CP gene/protein 4:284
geographical distribution 4:286
infection
control 4:308
detection 4:308
economic importance 4:286–287
symptoms 4:286*f*
movement in plants 4:285*f*
particles 4:283
RNA3 genome 4:284
RT proteins 4:284
serological relationship 4:285*f*
small open reading frame 4:284
TGB protein 4:283–284
tissue distribution 4:286
transmission 4:287
virus–host interactions 4:284
Potato rough dwarf virus (PRDV) 4:298*t*
Potato spindle tuber disease (PSTVd) 5:332
epidemiology 5:338
gene silencing suppression 5:330
host range 5:335
propagation 5:338
replication 5:339
RNA cleavage 5:339
symptoms 5:337*f*
tobacco 5:341
viral movement 5:339
phloem 5:339
Potato virus(es) 4:199
control 4:199
genetically-modified resistant plants 4:303
measures used 4:302
quarantine 4:303
resistance 4:297–302
seed potatoes 4:302
disease traits 4:302
damage associated 4:302
symptoms 4:302
yield losses 4:302
history 4:297

pathogens 4:303
transmission 4:302
aphid 4:302
see also specific viruses
Potato virus A (PVA) 4:306
geographical distribution 4:306
host range 4:306
infection
diagnosis 4:306
symptoms 4:306
yield losses 4:306
propagation 4:306
serogroups 4:306
Potato virus M (PVM) 4:307*f*
genome 1:451*f*
Potato virus P (PVP) 4:298*t*
Potato virus S (PVS) 4:297*t*
genome variability 4:306
host range 4:306–307
infection 4:306*f*
transmission 4:302
Potato virus T (PVT) 4:298*t*
Potato virus U (PVU) 3:406*t*
Potato virus V (PVV) 4:300*t*
Potato virus X (PVX) 4:297*t*
Avr gene expression 4:172–173
genome 4:312*f*
history 2:451
infection
control 4:306
diagnosis 4:305–306
Rx gene resistance 4:174
transgenic resistance 5:325–326
yield losses 4:199
strains 4:305–306
transmission 4:302
as type species 4:310
virulence 4:305–306
virus-induced gene silencing 5:327–328
Potato virus Y (PVY) 4:297*t*
characteristics 5:63*t*
coat protein 4:288
control 4:287
evolution 4:296
genome 4:288*f*
coat protein 4:288
historical aspects 4:287
Smith, Kenneth Manley 2:451
infection *see below*
phylogenetics, potyvirus relationships 4:291
RNA synthesis 4:288
serology 4:290
ELISA 4:290
monoclonal antibodies 4:290
phage display antibody technology 4:290
strain discrimination 4:290–291
significance 4:287
strains 4:303
taxonomy 4:296
transmission 4:199
aphid 4:303
aphid vectors 4:289
determination 4:289
insect vectors 4:289*f*
vegetative propagation 4:288–289
viral particle 4:288
helper component protein 4:288
viral genome linked protein 4:288
virus-induced gene silencing 5:327–328
Potato virus Y infection 4:215
cytopathy 4:291
inclusions 4:291
leaves 4:291
disease symptoms 5:64
economic impact, yield losses 4:199
genetically-modified resistant plants 4:157–160
host plants 4:289
specificity 4:289
weeds 4:289
see also individual species
peppers 4:289
barrier control methods 4:295
control 4:293–294
isolate effects 4:293
pvr^2 gene 4:294
pvr^4 gene 4:294–295
resistance genes 4:293–294
see also specific genes
symptoms 4:294*f*
yield effects 4:293
petunia 4:213*f*
potato 4:289, 4:291
aphid transmission 4:293
breeding 4:294*f*
control methods 4:293
hypersensitivity reactions 4:292*f*
isolates 4:291
quarantine 4:293
strains 4:291
symptoms 4:291*f*
unclassified isolates 4:291–292
yield reduction 4:291
tobacco 4:289, 4:295
as diagnostic species 4:290*f*
economic importance 5:64
resistance breeding 4:295
strains 4:295
symptoms 4:295*f*
yield losses 4:295
tomato 4:289, 4:295
economic impact 5:64
genetic diversity 4:295
resistance 4:295
strains 4:295
Potato yellow dwarf virus (PYDV) 4:196
Potato yellowing virus (PYV) 4:298*t*
Potato yellow mosaic virus (PYMV) 4:298*t*
Potato yellow vein virus (PYVV) 4:298*t*
Potexvirus 1:426*f*, 2:255, **4:310–313**, 4:311*t*
Potexvirus(es) **4:310–313**
CP gene/protein 4:312
degradation 4:312
cytopathology 4:312
genome 4:312
CP 4:312
movement proteins 4:312
replicase 4:312
sequences 4:311*t*
untranslated region 4:312*f*
geographical distribution 4:313
infections *see below*
phylogenetics 4:310
physicochemistry 4:310
CP degradation 4:312
sedimentation coefficients 4:310–312
ultraviolet absorbance spectra 4:310–312
serology 4:313
structure 4:310
nucleocapsids 4:310
virions 4:310
symptomatology 4:313
taxonomy/classification 4:310
phylogenetic analysis 4:310
species demarcation 4:310
transmission 4:310
type species 4:310
see also specific viruses
Potexvirus infections 4:310
control 4:313
transgenic resistant plants 4:313
epidemiology 4:313
host range 4:310
Potyviridae 1:289, 1:573, 4:314, 4:315*f*, 4:315*t*, 5:11*t*
Potyvirus 1:289, 4:313, 4:314, 4:314*f*, 4:315*f*, 4:315*t*, 5:433–434, 5:434*f*
Potyvirus(es) **4:313–322**
biotechnological applications 4:321
vectors 4:321
classification 4:313
evolution 4:320
speciation events 4:321
genomes 4:316
5′ noncoding region 4:317
bipartite structure 4:316–317
monopartite structure 4:314
helper component protein
functions 4:317
pathogenicity 4:320
protein properties 4:317
RNAi suppression 3:152
transmission 5:279
infections *see below*
legume viruses 3:215
propagation 4:319
proteins 4:317
CI protein 4:317
coat proteins 4:316
CP gene/protein 4:319
helper component 4:317
NIa protein 4:317
NIb protein 4:319
P1 protein 4:317
pathogenicity 4:320
proteolytic processing 4:318*f*
VPg protein 4:317–319
replication 4:319*f*
host-virus interactions 4:320
RdRp 4:319
replication complex 4:319–320
RNA replication 4:320
siRNA accumulation 4:319–320
virus transport 4:320
RNA recombination 4:377
serology 4:316
CP core 4:316
taxonomy 4:314
criteria 4:313
phylogenetic analysis 4:315*f*
standards 4:314*f*
transmission 4:320
aphids 4:320
vectors 4:321
virion properties 4:316
structure 4:316
virus-induced gene silencing, viral synergism 5:327–328
see also specific viruses
Potyvirus infections
control 4:321
diagnostics 4:316
RT-PCR 4:316
epidemiology 4:321
host range 4:313–314
see also individual species
outbreak severity 4:321
pathogenicity 4:320
plants
orchids 4:225–226
petunia 4:213
sweetpotato 4:663
tobacco 5:64
resistance to 4:178–182
genes 4:179*t*
symptoms 4:313
Powassan virus (POWV) 5:488
encephalitis 2:239
host 2:239
tick-borne 2:239
vector 2:239
Poxviridae 1:427, 1:574–575, 3:226, 3:342, 4:323, 5:11*t*, 5:244–245, 5:347
Poxvirus(es) **4:322–330**
apoptosis inhibition 1:158*t*
avian 4:326–327
classification 4:323
genome size 5:347
DNA recombination mechanisms 4:375
entomopoxvirus *vs.* 3:128–129
gene content 4:326
DNA replication associated genes 4:326
nucleotide composition 4:326
transcription associated genes 4:326

Poxvirus(es) (*continued*)
genome 4:325
central region 4:325–326
inverted terminal repeats 4:325–326
molluscum contagiosum virus *vs.* 3:320
size 5:347
immunomodulation 4:114*t*
infections *see below*
membrane development 5:308
phylogeny 4:326*f*
replication 4:407
DNA replication 4:327
gene expression 4:327
host cell entry 4:327
morphogenesis 4:322–323
transcription 4:326
uncoating 4:409
virion structure 4:325
see also specific viruses
Poxvirus Bioinformatics Resource Center 5:357*t*
Poxvirus infections
disease associated 4:322–323
immune evasion 4:329
cellular protein mimics 4:329–330
insects 3:126*t*
pathogenesis 4:328
persistence 4:109*t*
prevention 3:230
Pecluvirus, genome 4:100*f*
PPL-100 2:507*t*
P protein, Sigma virus structure 4:576–577
PPV-C (cherry), plum pox virus strains/groups 4:240
PPV-D *(Dideron),* plum pox virus strains/groups 4:240
PPV-EA (El Amar), plum pox virus strains/groups 4:240
PPV-M *(Marcus),* plum pox virus strains/groups 4:239–240
PPV-Rec *(recombinase),* plum pox virus strains/groups 4:240
PPV-W (Winona), plum pox virus strains/groups 4:240
Pradimicin A (PRM-A) 1:153
Prasinovirus 4:117*t*
PRD1 bacteriophage *see* Bacteriophage PRD1
Precapsid assembly, dsDNA bacteriophage genome packaging 2:307
Precursor capsid assembly, bacteriophages 2:307
Precursor polypeptides, mouse mammary tumor virus 3:335–336
Precursor protein translation, mouse mammary tumor virus replication 3:336–337
Pre-existing channels, bacterial cell viral entry 5:366*f*
Pregenome packaging, avian hepadnavirus 2:331*f*
Pregenomic RNA (pgRNA)
hepatitis B virus 2:353*f*
hepatitis B virus replication 2:355*f*
Pregnancy
Akabane virus infection 1:77
bovine viral diarrhea virus infection 2:248
chronic infections 4:114–115
enterovirus 71-induced hand, foot and mouth disease 2:134
HCMV infection 2:482–483
rubella virus infection 4:518
Prehemorrhagic stage, Crimean-Congo hemorrhagic fever 1:601
Preintegration complex (PIC)
HIV 2:519
retroviral replication 4:465
PRE-M gene(s), vaccine vectors 5:238–239
PreM (pre-membrane) protein, yellow fever virus 5:471
Prenatal disease, coxsackieviruses 1:585
Preprimosome
bacteriophage ϕX174 4:405
definition 4:400
Preprotoxin
apoptotic role 1:162
definition 5:299
processing 5:303*f*
Prestige 2:419
Prezista 1:143*t*
Prevaccinol 2:419
Prevalence rate, definition 2:141
Prevention of mother-to-child transmission (PMTCT), HIV 1:66
Primary effusion lymphoma (PEL) 5:196
KSHV *see* Kaposi's sarcoma-associated herpesvirus (KSHV)
Primary host defence, baculovirus infection *see* Baculovirus infection
Primates, nonhuman
anellovirus infection 1:109
Dengue virus transmission 2:7
Ebola virus infection 2:203
filoviruses 2:200
hepatitis B virus 2:338
HIV cross-species transmission 4:612
influenza virus infection 3:101
KSHV-like viruses 3:190
SIV infection 4:612–617, 4:620
SV40 hosts 4:636
yellow fever 5:475
see also individual species
see also specific viruses
Primer-binding site (PBS)
definition 4:428
LTR retrotransposons 4:425, 4:430, 4:439
mouse mammary tumor virus 3:335–336
Primitive forest cycle, Dengue virus 2:8*f,* 2:9
Primosome 4:400
Principle effector molecules, adaptive immunity 3:60–61
PRINTS database 5:357*t*
Prion(s) 2:457, **4:330–336**
classification 5:11*t*
composition 5:187
conformational selection model 4:330
definition 4:330, 4:337, 5:186
diseases *see* Prion diseases
domains 4:338*f*
shuffling 4:339
formation, amyloid 4:337, 4:339
function 5:187
history 4:331
infectivity 1:369
molecular strain typing 4:330
nomenclature 4:331, 4:335
prion protein (PrP^{C})
conformational states 4:331
definition 4:330, 5:186
depletion 4:336
expression 4:332–333
knock out mice 4:333
polymorphism 4:340
structure and function 4:331, 4:332
PRNP gene *see PRNP* gene
protein-only hypothesis 4:332, 4:334
C-terminal domain 4:333
N-terminal domain 4:333
PrP conformational states 4:332
PrP^{L}, neurodegeneration 4:336
replication 4:334
scrapie isoform (PrP^{SC})
detection 1:369
protein conformation 4:333–334
proteolysis 4:333, 4:333*f*
structure and function 4:333
strains 4:334
biological properties 4:334
detection 4:335
structure and function 4:332
transmission 4:332
barrier 4:331
Prion(s), yeast and fungal **4:336–341**
amyloid structure 4:339
biological role 4:340
chaperones and 4:338*f*
domain shuffling 4:339
enzymes as 4:340
formation 4:339
generation 4:339
genetic signature 4:337, 4:337*f*
history 4:337
self-propagating amyloid 4:337
species barriers, variants and 4:338
transfection with amyloid 4:339
Prion diseases 4:337
chronic wasting disease 5:188
diagnosis 4:334
etiological agent 5:186
genetic mutation associated 5:192
familial CJD 5:192
fatal familial insomnia 5:192
Gerstmann–Sträussler–Scheinker syndrome 5:192
genetic susceptibility 4:331–332
host range 5:188*t*
incubation period 4:330, 4:332, 4:334, 5:186
neuropathology 5:186
pathogenesis 4:334
histopathological changes 4:334, 4:335*f*
neuronal cell death 4:336
tissue distribution 4:334
PrP^{C} conversion 5:187
subclinical infection 4:330
see also Bovine spongiform encephalopathy (BSE); Creutzfeldt–Jakob disease (CJD); Kuru; Scrapie; Variant Creutzfeldt–Jakob disease (vCJD)
PRNP gene 4:330
codon 129 polymorphism 1:371, 1:372, 4:330
prion strain propagation 4:335
sporadic CJD 4:331–332
strain propagation 4:335
variant CJD 4:331–332
knock-out mice 4:331, 4:333
pathogenic mutations 4:332
PRO140 2:507*t*
Proboscivirus 1:636
classification 1:635*t*
Procapsids
definition 1:433, 2:306, 3:6, 3:13, 3:30
icosahedral enveloped dsDNA bacteriophages 3:11
icosahedral tailed dsDNA bacteriophages 3:35
maturation 1:436, 1:437*f*
Processive polymerization 4:400
Processivity factor 4:400
ProdiGene 5:223–224
Prodrugs, antiviral therapy 1:150
pro gene/protein, Simian retrovirus D 4:624*f,* 4:625
Prognathia 1:328
Programmed cell death *see* Apoptosis
Programmed cell death-1 (PD-1) 1:126
Progressive multifocal leukoencephalopathy (PML)
associated immunosuppressive conditions 4:262
clinical signs 4:267
histopathology 4:267*f*
JCV infection *vs.* tumor induction 4:267
Prohead 3:30
Pro-inflammatory cytokines *see* Cytokine(s)
Pro-integration complex (PIC), mouse mammary tumor virus 3:336
Projection mapping, icosahedral particles 1:608
Promoters, baculoviruses 1:251
Promyelocytic leukemia (PML)-containing nuclear oncogenic bodies (PODs) 1:12
Promyelocytic leukemia (PML) protein 3:245
Propagative infection 1:483
Prophages
bacteriophages 2:445
definition 2:442, 5:411
Pro-phenoloxidases (pro-POs) 4:251–252, 4:252–253, 4:254
Prophylaxis, organ transplantation 3:471
Pro proteins, retroviruses 4:446, 4:461
PROSITE 5:357*t*
Protapanteles paleacrita brachovirus 4:257*f*
Prot, definition 4:428
Protease(s)
$2A^{pro}$ 1:542
arteriviruses 1:180
mode of action 2:513

nidoviruses 3:424
pseudovirus gene expression 4:354–355
SARS CoV therapeutic targets 1:561–562, 1:561*f*
virus maturation 4:411–412
see also specific types
Protease inhibitors 1:148, 2:507*t*, 2:513, 2:514*t*
HCV therapy 1:153
Protease p29, Cryphonectria parasitica virus 2:579
Protective antibodies 3:64
Protective immunity
definition 5:226
Japanese encephalitis vaccines 3:187
measles 3:288
Protein(s)
cross-linking, bacteriophage assembly 1:439
evolution of 3:478
sequence data, virus databases 5:363
see also specific proteins
Protein 5, lettuce big-vein virus 5:266–267
Protein A, bacteriophage ϕX174 4:405
Protein antibiotics 3:249, 3:256*f*
Protein arrays, symmetrical 1:436
helical arrays 1:437
icosahedral array maturation 1:436, 1:437*f*
symmetry disruption 1:437
symmetry mismatches 1:437
Proteinases
nepovirus 3:409
papaya ringspot virus 4:2
see also specific types
Protein DataBank (PDB) 5:363
Protein kinases, plant resistance 4:176–177
Protein methylesterase (PME) 3:355
Protein priming replication 5:411
Protein tyrosine phosphatase(s) (PTPs)
immunosuppressive effects 4:254, 4:255
Microplitis demolitor bracovirus 4:259–260
Proteinuria, Argentine hemorrhagic fever 3:208
Proteolytic cleavage
arterivirus genome 3:424
bacteriophage assembly 1:439
fusion protein biosynthesis 3:293
Hendra virus fusion proteins 4:54
Nipah virus fusion proteins 4:54
posttranslational modifications in HCMC 2:489–490
PrP^{SC} 4:333, 4:333*f*
Sendai virus fusion proteins 4:54
Proteomics 3:311
Protomer, definition 1:580
Proto-oncogenes 2:213
definition 2:105
Protoplasts 5:157
Brome mosaic virus replication 1:382
fungal virus transfer 2:290
Providence virus (PrV) 5:28*t*
assembly 5:33
β capsid protein 5:33
capsid structure 5:31*f*, 5:33
genome 5:29
infection, histopathology 5:36
in vitro replication 5:32
morphology 5:31*f*
protein precursors 5:29–31, 5:30*f*
transmission electromicroscopy 5:33
Provirus(es)
definition 2:505
feline leukemia viruses 2:185–186
primers 2:105–106
retroviral integration 2:105, 4:446–447
selective forces 2:107
structure 2:105–106
PrP^{C} *see* Prion(s)
PrP^{SC} *see* Prion(s), scrapie isoform; Scrapie (PrP^{SC})
PRRs *see* Pattern-recognition receptors (PRRs)
PRRSV *see* Porcine respiratory and reproductive syndrome virus (PRRSV)
Prune dwarf virus (PDV) 4:204
mixed infections 4:239
Prunus necrotic ringspot virus (PNRSV) 4:204
coat protein 3:53, 3:53*f*
mixed infections 4:239
transmission 3:55
Pruritus, pseudorabies virus infection 4:348
PrV *see* Providence virus (PrV)
Prymnesiovirus 4:117*t*
Pseudococcidae (mealybugs), cacao swollen shoot virus transmission 1:404, 1:404*f*
Pseudocowpox virus (PCPV) 4:57–58
host range 4:58
spread 4:58
synonyms 4:58
Pseudogenes 2:360
Pseudoknots
definition 5:68
hepatitis delta virus 4:480
VS RNA 4:480
yellow head virus 5:477–478
Pseudolysogeny 2:71
Pseudomonas aeruginosa, virulence factors 3:123–124
Pseudomurein endoisopeptidase, Methanothermobacter marburgensis virus 5:415
Pseudoparticles, hepatitis C virus 2:372
Pseudoplusia includens virus (PiV) 5:28*t*
Pseudorabies virus (PrV) **4:341–352**
Bartha strain 4:351
capsid 4:342
assembly 4:346
replicative role 4:346
UL17 4:342
UL25 4:342
envelope 4:346
glycoproteins 4:344*t*, 4:346
nonglycosylated membrane proteins 4:346
genome 4:342, 4:343*f*
gene functions 4:344*t*
genes 4:344*t*
inversions 4:342
history 4:341
infection *see below*
infectivity 4:348
morphology 4:341, 4:342*f*
neurobiological tool function 4:351
replication cycle 4:346, 4:347*f*
capsid assembly 4:346
DNA replication 4:346
egress 4:346–347
host cell attachment 4:346
host cell entry 4:346
IE180 function 4:346
nucleotide metabolism 4:346
transcription 4:346
UL3 protein kinase 4:346–347
UL31/UL34 complex formation 4:346–347
virion morphogenesis 4:347–348
taxonomy 4:341
tegument 4:342
capsid-proximal tegument 4:342–346
envelope-proximal tegument 4:342–346
formation 4:347–348
UL41 4:342–346
UL48 4:342–346, 4:347–348
tissue tropism 4:349
transmission 4:348–349
Pseudorabies virus infection
antibodies 4:349
apoptosis 4:349, 4:350
clinical features 4:341, 4:348
control 4:350
ELISA 4:350, 4:350*f*
screening 4:350
vaccination 4:349, 4:350
epidemiology 4:350
histology 4:349
host range 4:341, 4:348
immune system evasion 4:349
immunology 4:349
antibodies 4:349
infected *vs.* vaccinated animals 4:350–351, 4:350*f*
latency 4:343*f*, 4:349
neural 4:348–349
pathology 4:348
pigs 4:348*f*
mortality rate 4:348
neonates 4:348
pregnancy 4:348
vaccines/vaccination 4:349, 4:350
Pseudorecombination
definition 1:30, 1:615, 5:124
Indian tomato leaf curl viruses 5:128–129, 5:131–132
Pseudotype virions, virus membranes 5:313
Pseudoviridae 4:352, 4:430, 4:451, 5:11*t*
Pseudovirus 4:352, 4:353*t*
Pseudovirus(es) 4:12, **4:352–357**
classification 4:353*t*
env-like gene 4:355
evolutionary relationships 4:355
phylogenetic tree 4:356*f*
reverse transcriptase 4:355–356
gene expression 4:353
integrase 4:354–355
protease 4:354–355
genome 4:353, 4:354*f*
gag/pol ratio 4:353–354
long terminal repeats 4:355
pol coding region 4:354–355
primer binding sites 4:355
hemiviruses *vs.* 4:433
infections, fungi 2:287*t*
LTR-retrotransposons 4:433, 4:451
organization 4:433
phylogeny, genome sequencing 4:357
replication cycle 4:355
gag translation 4:355
LTR regions 4:355
pol translation 4:355
viral cDNA integration 4:355
viral cDNA synthesis 4:355
virion properties 4:352
virus-like intermediate 4:352–353
virus-like particles 4:352–353
see also specific viruses
PSI-inducibility (PIN) prion 4:337, 4:339
ϕ6 bacteriophage *see* Bacteriophage ϕ6
PSI prion 4:337
isogenic strains 4:340
species barrier 4:338
Psittacid herpesvirus 1 (PsHV-1)
genetics 2:408
host range 2:407
infection *see* Pacheco's disease
propagation 2:407
replication sites 2:409
transmission 2:409
virus shedding 2:409
Psittacine beak and feather disease virus (PBFDV)
disease symptoms 1:514–515
virion structure 1:514
Psittacinepox virus(es) 2:278, 2:278*f*
antigenic cross-reactivity 2:278–279
ψM1 virus 5:412
genome 5:415
open reading frames 5:412–415
pseudomurein endoisopeptidase 5:415
related viruses 5:412
structure 5:412–415, 5:415*f*
ψM2 virus 5:412
genome 5:415, 5:416*f*
ψM100 virus 5:412
genome 5:416*f*
homology 5:415
pseudomurein endoisopeptidase 5:415
Psorophora confinnis, Venezuelan equine encephalitic virus transmission 5:104
PSV *see* Pyrobaculum spherical virus (PSV)
PTK complex, murine polyomavirus middle T 4:274
PToV *see* Porcine torovirus (PToV)
PU-1 targeting, EBV-encoded nuclear antigen 2 2:162
PubMed 5:356–362, 5:357*t*

Pulau virus 2:95
Pulmonary complications, adult T-cell leukemia 2:568
Pulmonary edema
  definition 1:328
  enterovirus 71 infection 2:134
Pulmonary interlobular edema, African horse sickness 1:41
Pulsed-field electrophoresis 2:71
Pupation, baculovirus terminal disease 1:269
Purpura hemorrhagica 1:37
  African horse sickness *vs.* 1:42
Pustules 4:639
Puumala virus 1:391*t*
pVII *see* Bacteriophage fd
pVIII gene/protein
  bacteriophages, filamentous ssDNA structure 2:193, 2:193*f*
  structure 2:193, 2:193*f*
pvr^2 gene/protein
  eIF4E-mediated resistance, plants 4:184
  potato virus Y infections 4:294
pvr^4 gene/protein, potato virus Y infections 4:294–295
pvr6 gene/protein, eIF4E-mediated resistance, plants 4:184
Pyknosis 3:342
Pyrobaculum spherical virus (PSV)
  envelope composition 1:591
  morphology 1:590, 1:590*f*
  protein composition 1:591
Pyrococcus abysii
  'fusiform' viruses 2:300
  PAV1 infection 5:421–422

Q

Qalyub virus 1:598*t*
Qβ RNA polymerase, quasispecies 2:177–178
Q protein, bacteriophage lambda 5:184, 5:185
Quantitative real-time PCR, rhinovirus detection 4:471
Quantitative taxonomy *see* Taxonomy
Quantitative trait loci (QTL)
  definition 4:178
  polygenic resistance in plants 4:178
    gene mapping 4:182
Quarantine
  equid herpesvirus 1 infection 2:419
  potato virus infection control 4:303
  potato virus Y potato infections 4:293
  Sharka disease control 4:241
Quasi-equivalence
  definition 1:433, 5:145
  icosahedral array assembly 1:433
  tombusviruses 5:146
  virus structure 5:395
Quasi-equivalent conformers, definition 3:21
Quasispecies **4:359–365**
  common assumptions 4:361
    relaxation of 4:361
  definition 5:274
  error catastrophe 4:362
    multiplicative landscape 4:362
  fundamental model 4:360
    equation solutions 4:360
    error-prone replication 4:360
  historical context 4:359
    Eigen, Manfred 4:359–360
  HIV 1:128, 1:133
  mutation robustness, development of 4:361
    back mutations 4:361–362
    Haldane–Muller principle 4:361
    secondary mutations 4:361–362
    'survival of the flatest' 4:362, 4:361–362
  relevant predictions 4:361
  scientific context 4:360
    as population genetics 4:360
    variability 4:360, 4:360*t*
  Taura syndrome virus 5:6
  viral evolution *see* Evolution (of viruses)

R

R2D2 protein (RNA interference) 3:150
  Dicer-2 interactions 3:150–151
Rabbit(s)
  CSFV vaccine production 1:528
  economic impact 1:411
  HSV reactivation 2:437
  see also specific infections
Rabbit fibroma virus (SFV) 3:225*t*
Rabbit hemorrhagic disease 1:416
  clinical features 1:417
  control 1:418
  vaccines/vaccination 1:417, 1:418
Rabbit hemorrhagic fever virus 1:410
  antigenic variation 1:415
  Australia 1:411
  geographical distribution 1:413–414
  host range 1:414–415
  infection *see above*
  replication, receptors associated 1:417
  stability 1:412
  structure 1:412*f*
  transmission 1:416
Rabbitpox
  clinical features 3:347, 3:347*f*
  future work 3:347
  histopathology 3:347
  immune response 3:347
  pathogenesis 3:346
  pathology 3:347
Rabbitpox virus 3:346
  classification 3:346
  epidemiology 3:346
  genetics 3:346
  history 3:346
Rabies virus (RABV) 2:96, 4:184*t*, **4:367–373**
  cell tropism 4:370
  classification 4:367–368, 5:485
  epidemiology 4:372
  evolution 4:368
  genome 3:326*f*, 4:367, 4:368*f*
    G gene 4:367–368
    M gene 4:367–368
    P gene 4:367–368
  geographical distribution 2:96
  infection *see below*
  infectivity 4:367–368
  morphology 4:367, 4:368*f*
  phosphoprotein 3:331
  phylogeny 4:368, 4:369*f*
    Arctic lineage 4:368, 4:372
    'cosmopolitan' lineage 4:368, 4:372
    mutations 4:368
  proteins 4:367–368
  replication 4:370
  transmission 4:370
  vaccines/vaccination 2:96, 4:373, 5:237, 5:485, 5:493–494
    domestic animals 4:373
    humans 4:373
    potency 4:373, 5:237
    wild animals 4:373
Rabies virus infection
  acute 1:474
  clinical spectrum 4:371
    encephalitic form 4:371
    incubation period 4:371–372
    neural cell infection 1:473
    paralytic form 4:371
    symptoms 4:371
  control 4:373
  diagnosis 4:371, 5:485
    cell culture 4:371–372
    neuronal inclusions 4:371
    PCR 4:372
    virus isolation 4:371–372
  eradication 4:372
  host range 2:96, 4:370–371, 4:372
    bats 4:370–371, 5:485
    dogs 4:372
    migratory species 4:372–373
    mongooses 4:373
    see also Zoonoses, below
  mortality 2:96
  organ transplantation risks 4:370
    encephalitis 3:466
  pathogenesis 4:370
    neural involvement 4:370
  postexposure treatment 4:373, 5:485
  prevention 4:373
  survival 4:370–371
  zoonoses 5:485, 5:486*t*
    animal reservoirs 5:485
    geographical distribution 5:485
    transmission 5:485
Racecadotril 4:511, 4:512
Racivir 2:507*t*
Radiological abnormalities 4:552
Ralstonia solanacearum, resistance in rice 4:175
Raltegravir 2:510
Raman spectroscopy 2:190
Ramsay–Hunt syndrome, zoster (shingles) 5:253
Rana catesbeiana virus Z (RCV-Z) 3:172*f*
Rana pipiens, Lucke´ tumor herpesvirus infection 2:209
Ranavirus 2:233, 3:155, 3:168*t*
Ranavirus(es)
  geographical distribution 3:156
  host range 3:156
  infection 3:158–159
  phylogeny 3:160*f*
  transmission 3:159–160
Ranid herpesvirus 1 *see* Lucke´ tumor herpesvirus (LTHV)
RANTES 1:623–624
Ranunculus white mottle virus (RWMV) 3:447, 3:448*t*
  coat protein 3:450
  genome 3:449
  geographical distribution 3:451
  phylogenetic analysis 3:447
  transmission 3:453
Raphidovirus 4:117*t*
Rapid growth, RNA virus experimental evolution 4:363
Rashes, smallpox 4:639–640
Raspberry bushy dwarf virus (RBDV) 3:37, 3:41
  geographical distribution 3:37
    black raspberry isolates 3:37
    resistance-breaking isolates 3:37
    Scottish isolates 3:37
  ilarvirus *vs.* 3:41
  infection
    control 3:41
    detection 3:41
    disease symptoms 3:38, 3:38*f*
    naturally infected plants 3:38
  nucleotide sequences 3:39, 3:39*f*
    cucumber mosaic virus 2b gene *vs.* 3:40
    non-coding sequences 3:40
    RNA-1 3:40
    RNA-2 3:39–40
  resistant cultivars 3:41
  virion composition 3:39
  virion properties 3:39, 3:39*f*
Raspberry ringspot virus (RpRSV) 3:406*t*, 4:204
  diseases 3:411
  infectious DNA clones 3:408–409
  transmission 3:411
Rat1 mutation (MurA) 3:257–258
Rat cytomegalovirus (RCMV) 1:632
  atherosclerosis 1:633
  infection
    atherosclerosis model 1:633
    immune system evasion 1:633
    immunocompromised animals 1:633
    pathogenesis 1:633
    reactivation 1:633
  Maastricht strain 1:632–633
    genome 1:633
Rategravir 2:510
RDR genes, *Arabidopsis thaliana* 5:326–327
Reactivation 2:405*f*
  antigen detection 2:33
  chronic hepatitis B 2:357–358
  chronic infection pathogenesis 4:111

HCMV infection 2:483
HHV-6 2:502–503
HHV-7 2:502
latency 4:110–111
persistent infection pathogenesis 4:112
Reactome database 5:357*t*
Readthrough domain(s) (RTD), luteovirus transmission 3:236
Reassortment
cucumber mosaic variation 1:619
definition 1:390, 3:479, 4:164, 4:391
Ngari virus strains 3:482
orthobunyavirus *see* Orthobunyavirus(es)
Patois group viruses 3:482
reovirus genome 4:392
recA, DNA virus recombination 4:374
Receptor(s) 5:310, **5:319–324**
clinical isolates, laboratory adaptation 5:323
antibody resistance 5:323–324
competition, retrovirus infection resistance 2:468–469
complement control proteins 5:322
definition 4:407
glycosaminoglycan 5:320
heparan sulfate 5:320
histo-group antigens 5:321
host cell attachment 4:407–408, 5:319
binding specificity 4:408–409
cell tropism 4:408
plant viruses 4:408
identification 5:319
direct 5:320
indirect 5:320
immunoglobulin superfamily proteins 5:321
inactivation 2:468
infection neutralization role 3:416
sialic acid 5:320
specificity 5:320
see also specific receptors
see also specific viruses
Receptor activated pathway (apoptosis) 1:155
Receptor-binding proteins
bacterial cell viral entry 5:365–366
definition 5:365
filamentous ssDNA bacteriophages 5:369
Recombinant virus(es)
definition 3:95, 3:483, 5:124
HIV vaccine development 1:75
see also specific viruses
Recombinant adeno-associated virus (rAAV), gene therapy vector 2:303
DNA shuffling 2:304
peptide insert size 2:304
serotypes 2:304
in vitro evolution 2:304
Recombinant adenovirus (rAd), gene therapy vector 2:303
first generation 2:303
second generation 2:303
Recombinant herpes simplex virus, gene therapy vector 2:303
Recombinant nematode anticoagulant protein c2
Ebola virus infection treatment 2:64, 2:204–205
Marburg virus infection treatment 2:204–205
Recombinant vaccines
avipoxviruses 2:283
development 5:237
immune response 5:237–238
virus structure 5:237–238
hepatitis B virus 2:351, 2:358
TMV 5:59–60
vaccinia virus vaccines 4:629
Recombinase *see* Integrase(s)
Recombination **4:374–382**
Agrobacterium tumefaciens-based expression system 4:232*f*, 4:233
bacteriophage evolution 5:371–372
baculovirus expression vectors 1:243–244
Brome mosaic virus 1:389
Campoletis sonorensis ichnovirus 4:258–259
capripoxviruses 1:428
coronaviruses 1:552, 1:558–559, 1:560
cucumber mosaic virus 1:619
defective-interfering RNAs 4:380
historical aspects 4:380
negative-strand RNA viruses 4:380
single internal deletions 4:380
definition 1:410, 4:164
echoviruses 2:70
endogenous retroviruses 2:106
enteroviruses 2:127–128
feline sarcoma viruses 2:185
heterologous encapsidation 4:162
homologous *see* Homologous recombination
icosahedral enveloped dsDNA bacteriophages 3:11
influenza viruses 3:99
intertypic 2:150
intraserotypic 2:8
KSHV 3:197
nodaviruses 3:436
nonhomologous *see* Nonhomologous recombination
noroviruses 1:415, 3:442–443
retroviruses 4:446–447
RNA viruses 4:377
sapoviruses 3:442–443
T-cell receptors 3:71–72
tomato leaf curl viruses, Indian 5:131
watermelon mosaic virus 5:439
zucchini yellow mosaic virus 5:439
Recombination-dependent replication, beet curly top virus 1:303–304
Red clover mottle comovirus (RCMV) 3:216
Red clover necrotic mosaic dianthovirus (RCNMV) 3:216
Red clover necrotic mottle virus 3:354–355
Red grouper reovirus 1:164*t*
Red grouse, louping ill virus infection 2:240–241
Red Queen hypothesis 2:175, 2:179, 5:297
Red raspberry *(Rubus idaeus),* idaeovirus infection 3:37
Red sea bream iridovirus (RSIV), infected cell morphology 3:173
Red-spotted grouper nervous necrosis virus (RGNNV) 2:229–230
Reduction hypothesis, viral origins 3:473, 3:476, 3:476*f*
Red veins, cacao swollen shoot virus infection 1:403
Reed, Walter, Yellow Fever Virus 2:455
Reemerging diseases
ecological factors 2:96
history 2:93
recognition of 2:93
vertebrate **2:93–97**
contributing factors 2:93
human behavior 2:94
human demographics 2:94
zoonoses 2:95, 5:494
see also specific infections
ref genes/proteins 4:579–580, 4:580*f*
RegB endoribonuclease, bacteriophage T4 5:177
Regional lymph nodes, African horse sickness 1:40
Regulated polyprotein processing
Grapevine fanleaf virus 3:409
nepovirus *see* Nepovirus
Regulator of chromosome condensation (RCC1) protein 1:228
Regulator of complement activity (RCA) 5:322
γHV68 3:110
Regulator of expression of virion proteins (Rev) *see* HIV
Regulator of transcriptional activation (RTA), KSHV 3:195, 3:199
Regulatory viral RNAs, apoptosis 1:161
Reintroduction program(s) (birds), poxvirus infection 2:279
'Relational database management systems' (RDBMSs) 5:351–352
Release
baculoviruses 1:251
definition 4:407
Relenza *see* Zanamivir
Relenza® 1:143*t*
Rem A 3:334
Rem protein, mouse mammary tumor virus replication 3:336–337
Renal failure, chronic, HIV infection 1:56
Renal transplantation
immunosuppression 3:471
JC polyomavirus infection 3:470
papillomavirus infection 3:470
therapy 3:471
Reniform 1:186
Reo-like virus(es), shellfish viruses 4:566
Reoviridae 1:93*f*, 1:163, 1:170, 2:118–119, 2:231, 2:286, 3:133–134, 3:134*t*, 3:454, 4:149–150, 4:383, 4:384*t*, 4:391, 4:392*t*, 4:509, 4:536, 5:11*t*, 5:344–345, 5:346*f*, 5:346*t*
Reovirus(es) 3:454, 4:149–150, **4:382–390**, **4:390–399**, 4:392*t*
characteristics 2:117*t*
classification 4:384*t*
fungal dsRNA viruses 2:111
fusogenic orthoreoviruses 4:383
genera 2:231, 4:536, 4:536*t*
genome 1:163, 4:391, 4:391*t*, 4:394*f*
large segments 4:391–392
medium segments 4:391–392
nontranslated region 4:391–392, 4:394*f*
RdRp conservation 1:537
reassortants 3:454, 4:392
small segments 4:391–392
history 4:383
infections
apoptosis inhibition 1:158*t*
clinical features 4:388
fish 2:231
fungi 2:287*t*
immune response 4:389
insects 3:126*t*
respiratory 2:556
inner capsid 4:392, 4:393*f*
λ1 protein 4:393, 4:394*f*, 4:395*t*
λ2 protein 4:393, 4:394*f*
λ3 protein 4:393, 4:394*f*
μ2 protein 4:393–394
σ2 protein 4:393*f*, 4:394
insect pest control 3:127
mammalian *see* Mammalian reovirus(es)
outer capsid 4:394
μ1 protein 4:394*f*, 4:396
σ1 protein 4:396
σ3 protein 4:396
σ3 protein cleavage 4:396–398
plant *see* Plant reovirus(es)
RdRp
classification 5:344–345, 5:346*f*, 5:346*t*
gene conservation 1:537, 1:538*t*, 1:539*f*
sequence comparison 5:344–345, 5:346*f*, 5:346*t*
replication 4:396, 4:397*f*
assembly 4:398
assortment process 4:398
cap formation 4:398
host cell binding 4:396–398, 4:397*f*
host cell entry 4:393*f*, 4:396–398
λ2 proteins 4:398–399, 4:399*t*
σ3 protein 4:398
σNS protein 4:398
tissues associated 4:396
transcription 4:397*f*, 4:398
strain nomenclature 4:383
taxonomy/classification 1:163, 3:454, 4:383
virulence determinants 5:318
virus structure 4:383, 4:391
see also specific viruses
Reovirus type 1 Lang, virulence determinants 5:318
REP 9, antiviral therapy, enveloped viruses 1:147
RepA, definition 3:264
repA (regulatory protein), maize streak virus *see* Maize streak virus (MSV)
Repeat (R) regions, mouse mammary tumor virus 3:335–336
Repetitive DNA, iridoviruses 3:164
rep gene/protein
adeno-associated virus vectors 2:303
bacteriophage ϕX174 3:16
banana bunchy top virus 1:274
beet curly top virus *see* Beet curly top virus (BCTV)

rep gene/protein (*continued*)
Campoletis sonorensis ichnovirus 4:258–259
definition 3:264, 3:385
Maize streak virus *see* Maize streak virus (MSV)
mungbean yellow mosaic India virus replication 3:366
mungbean yellow mosaic viruses 3:366, 3:367
nanoviruses 3:388, 3:390, 3:391
porcine circoviruses 1:516, 1:517–518, 1:518–519
tomato leaf curl viruses Indian replication 5:132
tomato yellow leaf curl virus 5:144
Replicase(s)
alfalfa mosaic virus 1:82–83
arteriviruses 3:423–424, 3:425*f*
brome mosaic virus 4:378–379
coronaviruses 1:552, 1:552*f*
Dendrolimus punctatus tetravirus 5:31
engineered resistance in plants 4:156–157
Helicoverpa armigera stunt virus 5:31
hordeiviruses 2:461
icosahedral ssRNA bacteriophages 3:25*f*, 3:26
omega tetraviruses 5:31
potexvirus 4:312
RNA virus evolution 2:180–181
Thosea asigra virus 5:29
tobravirus replication 5:74–75
Replicase-associated protein (REP), geminiviruses 4:168
Replication 1:147, **4:406–412**, 4:408*f*
antiviral therapy *see* Antiviral drug(s)
assembly 4:411
sites 4:411
blocking, Ab-mediated neutralization 3:415
coding capacity 4:407
definition 1:296
double-stranded DNA viruses *see* Double stranded DNA (dsDNA) bacteriophages
host cell attachment 4:407
receptors *see* Receptor(s)
maturation *see* Maturation
release 4:412
stages 4:407, 4:408*f*
temperature effect 5:315
transcription *see* Transcription
uncoating 4:409
endocytic pathway 4:409
see also specific viruses
Replication competent, definition 2:301
Replication competent retrovirus (RCR) 2:302
'Replication complex' ribonucleoprotein 3:393–394
Replication initiator proteins (rep), nanoviruses 3:388, 3:390, 3:391
Replication protein, flexiviruses 2:255, 2:256*f*
Replication–transcription complex, arterivirus genome 3:423–424
Replicon systems
alphaviruses 5:121–122
definition 5:117
HCV 1:153
hepatitis E virus 2:380
industrial-scale protein production 4:235
togaviruses 5:121–122
Replisome 4:400
Reportable diseases, fish viruses 2:227, 2:228*t*
Reptilian adenovirus(es) 1:27
Rescriptor® 2:510
Resequin, EHV-4 treatment 2:419
Reservoir hosts
see specific viruses
Residence, vector transmission 5:273
Residual infectivity, neutralizing antibody tests 2:31
Resins
antibody labelling 2:83
classical thin sectioning 2:83
Resistance
antibody-mediated infection neutralization 3:417
conserved epitope binding 3:417–418
mutations 3:417
breeding
cacao swollen shoot virus infection 1:409
potato virus Y tobacco infections 4:295
rice yellow mottle virus control 4:488–489
definition 1:296
gene-for-gene
definition 4:170, 4:171
history 4:171
partial 4:178
potato virus Y tomato infections 4:295
virus-induced gene silencing 5:378
Visna-Maedi virus infection 5:430
watermelon mosaic virus 5:437–438
zucchini yellow mosaic virus 5:437–438
see also Plant(s), natural disease resistance (dominant genes); Plant(s), natural disease resistance (recessive genes)
Resistance genes, definition 4:170
Respiratory distress syndrome, hantavirus infection 2:317
Respiratory infections
adenoviruses 1:25
anelloviruses 1:110
barriers against 5:315
disease surveillance 2:46
echoviruses 2:68
EHV-4 2:418
equid herpesvirus 1 2:418
henipavirus infections 2:325
human respiratory syncytial virus *see* Human respiratory syncytial virus (HRSV)
lymphocytic choriomeningitis virus *see* Lymphocytic choriomeningitis virus infection
organ transplantation 3:469–470
virus entry 5:315
see also specific infections
Respiratory syncytial virus 3:246
genome 3:327*f*
Respiratory virus(es)
see specific viruses
Respirovirus 4:41*t*, 4:47
Respirovirus(es)
classification 4:41*t*
genome 3:327*f*
'Resting complex' ribonucleoproteins 3:393–394
Resting memory B-cells, EBV infection 2:165, 2:166*f*
Reston Ebola virus (REBOV)
genome 2:58–59
geographical distribution 2:62
history 2:57
Restriction enzyme digests 2:456
avipoxvirus detection 2:280
varicella-zoster virus 5:252
Restriction factor 2:467
Restriction fragment length polymorphisms (RFLPs)
apple stem pitting virus strains 1:424
definition 1:301, 4:103
Pepino mosaic virus 4:106–107
plum pox virus strains/groups 4:241
Restrictive infection, Visna-Maedi virus infection 5:425–426
Reticulated evolution, definition 2:175
Reticuloendothelial cells, CNS invasion pathways 1:471
Reticuloendotheliosis viruses (REV) **4:412–419**
classification 4:412
genome 4:413, 4:414*f*
geographical distribution 4:415
history 4:412
infection *see below*
protein properties 4:413, 4:414*f*
envelope protein 4:413
v-Rel 4:413
replication 4:413
genome integration 4:415
nucleocapsid assembly 4:415
RNA transcription 4:414
subgenomic RNAs 4:414–415
viral maturation 4:415
taxonomy 4:412
transmission 4:415
vaccine contamination 2:283
virion properties 4:413
morphology 4:413
v-Rel complexes
mutations 4:417, 4:417*f*
target genes 4:417
transformation 4:417, 4:418*t*
see also specific viruses
Reticuloendotheliosis virus infections
host range 4:415
immune response 4:415
pathogenesis 4:415
Gag proteins 4:416
neoplastic diseases 4:416
non-neoplastic diseases 4:415
transformation by REV-T 4:416
v-Rel complexes *see* Reticuloendotheliosis viruses
runting syndrome 4:416
Retina 2:491
acute necrosis 2:497
betanodavirus fish infections 2:229–230
infection 2:491
Retinitis, HCMV infection 2:496, 2:496*f*, 2:497
Retinoblastoma (RB) cell cycle regulator
function 4:37
murine polyomavirus large T 4:276
Retinoblastoma-related tumor-suppressor protein (pRBR) 1:307
Retinochoroiditis 2:491
HCMV 2:496
Retinoic acid inducible protein (RIG-I) pathway 3:59–60, 3:106
viral evasion 3:108
Retrales 4:430
Retriposons *see* Target-primed retrotransposons
Retroelements 4:451
definition 4:430
LTR retrotransposons *vs.* 4:451
Retroperitoneal fibromatosis-associated herpesvirus 4:587*t*
Retroperitoneal fibromatosis herpesvirus (RFHV) 4:628–629
Retroperitoneal fibrosis, rhadinovirus infection 4:592
Retrotransposineae 4:430
Retrotransposition, definition 4:419
Retrotransposons 4:352, **4:436–445**
definition 4:352, 4:419, 4:437
errantiviruses 4:433
evolutionary features 4:441
functions 4:437
fungi *see below*
genome effects 4:443
content 4:443
genes, effects on 4:444
genomic distribution 4:443
mammalian 4:443
history 4:437
whole genome sequencing 4:437
host responses 4:444
life cycle 3:304*f*
nomenclature 4:437
origins 4:441
as phylogenetic markers 4:442
plants *see below*
retroviruses *vs.* 4:352
structure 4:437, 4:438*f*
open reading frames 4:437
target-primed *see* Target-primed (TP) retrotransposons
see also LINE-like retrotransposons*see also* LTR-retroelements*see also* Metavirus(es)*see also* Non-LTR retrotransposons*see also* Penelope-like elements (PLEs) *see also* SINE retrotransposons
Retrotransposons, fungi **4:419–427**
distribution 4:423
diversity 4:423–424
genome effects 4:426
chromosomal translocation breakpoints 4:426
mutagens 4:426
proportion from retrotransposons 4:426
host effects 4:426–427
RIP 4:426–427

phylogenetic analysis 4:420, 4:422*f*
replication 4:424
types 4:420, 4:421*f*, 4:422*f*
uses of 4:427
gene tagging 4:427
insertional mutagens 4:427
population genetics 4:427
variation 4:427
see also specific types
Retrotransposons, plants **4:428–436**
classification 4:428, 4:431*t*
env-like functions 4:434
LTR-retrotransposons *see* LTR-retroelements
nomenclature 4:435
non-LTR-retrotransposons 4:428–429
classification 4:429–430
structural variants 4:428
transmission 4:428
Retrovir® *see* Zidovudine (AZT)
Retrovirales 4:430
see also specific viruses
Retroviridae 1:347–348, 2:107, 2:108*f*, 2:185, 2:213, 2:231, 2:260, 2:260*t*, 2:518, 2:526, 3:175, 3:335, 4:412–413, 4:430, 4:446, 4:451, 4:459, 4:460, 4:461*f*, 4:603, 4:604*t*, 4:624, 5:11*t*
Retrovirus(es) **4:459–467**
acute transforming 4:466
apoptosis inhibition 1:158*t*
avian *see* Avian retrovirus(es)
classification 2:213, 2:260*t*, 4:459, 4:460
endogenous *see* Endogenous retrovirus(es) (ERV)
env gene/protein 4:446
deletions 4:447
surface protein 4:463
transmembrane protein 4:463
evolution 2:183
gene diversity 2:183–184
long terminal repeats 2:183–184
fish *see* Fish retrovirus(es)
gag gene/protein 4:446, 4:461
deletions 4:447
fusions 4:447–448
virion assembly 4:466
gene therapy vectors 2:302
life cycle 2:302
replication competent 2:302
types 2:302
genome 2:213, 4:446, 4:459, 4:460, 4:462*f*, 4:594
history 4:459
infection *see below*
life cycle 2:213, 2:467–468, 2:468*f*
infection initiation 2:213
LTR-retrotransposons *vs.* 4:431–432
morphology 4:461, 4:463*f*, 4:594
pol proteins 4:446
deletions 4:447
proteins 4:463
receptors 4:464
replication 4:464
assembly 4:466
attachment 4:464
genomic mRNA transcription 4:465
integration 4:465
maturation 4:411–412
penetration 4:464
polyprotein processing 2:213, 4:466
reverse transcription 4:464
translation 4:465–466
virion release 4:466
retrotransposons *vs.* 4:352
RNA recombination 4:380
structure 2:213
taxonomy 4:446, 4:460, 4:461*f*
transporters 4:464
see also specific viruses
Retrovirus infections
antiviral therapy 1:143*t*
immunomodulation 4:114*t*
integration 3:307–309
proviruses 2:105, 4:446–447
selective forces 2:107
oncogenes/oncogenicity 2:213, 2:457, **4:445–450**
cellular sequence acquisition 4:446, 4:447*f*
classes of 4:449*t*
co-infection 4:447
cooperation of 4:448
defective replication 4:447, 4:466–467
functional classes of 4:448, 4:449*t*
fusion proteins 4:447
gain of function 4:448
historical perspective 4:446
overexpression 4:448
taxonomy 4:446
pathogenesis 4:466
acute transforming viruses 4:466
persistence 4:109*t*
resistance to *see below*
Retrovirus infections, resistance to **2:467–474**
acquisition 2:467–468
cellular cytidine deaminases 2:471, 2:472*f*
APOBEC3G 2:471
specificity 2:471
future work 2:473
post-entry 2:469
see also Fv1 (Friend virus susceptibility factor 1); *TRIM5* gene/protein
specificity 2:469
virus entry 2:468
receptor competition 2:468–469
receptor-envelope interaction 2:468–469
receptor inactivation 2:468
route importance 2:471
xenotropism 2:468
virus generation 2:471
particle assembly 2:473
zinc finger antiviral protein 2:473
Reverse genetics
arenaviruses 3:247
Brome mosaic virus BMV 1:382
bunyaviruses 1:398
coronaviruses 1:560
definition 1:390, 3:6, 3:96, 3:483, 4:40, 5:375
fish rhabdoviruses 2:228
icosahedral enveloped dsDNA bacteriophages 3:12
Infectious hematopoietic necrosis virus 2:228
influenza viruses 3:99, 3:488
lymphocytic choriomeningitis virus 3:247
parainfluenza viruses 4:44–45
paramyxovirus characterization 4:40
RNAi 3:151–152
Viral hemorrhagic septicemia virus 2:228
Reverse-phase polymerase chain reaction, pomovirus 4:286
Reverse transcriptase(s)
active site 4:464–465
antiretroviral drug mechanism of action 2:509
definition 1:457, 4:419, 4:428
HIV antigenic variation 1:133
inhibitors 2:509
LTR-retrotransposons 3:301, 4:438–439
mouse mammary tumor virus 3:335, 3:336
pseudovirus evolution 4:355–356
retrotransposons 4:428–429
Reverse transcriptase-endonuclease, Simian retrovirus D 4:627
Reverse transcription 3:307*f*, 4:409–410, 4:464, 4:465*f*
double-stranded DNA virus replication 4:411
equine infectious anemia virus genetics 2:170
LTR retrotransposons 4:425
priming
gypsy retrotransposon 3:306
Tf1 retrotransposon 3:306–307
Ty3 retrotransposon 3:306
single-stranded RNA virus replication 4:410–411
virus classification 5:11*t*
Reverse transcription polymerase chain reaction (RT-PCR)
aquareovirus infection diagnosis 1:167–168
Border disease diagnosis 1:340
classical swine fever virus diagnosis 1:530–531
Crimean-Congo hemorrhagic fever diagnosis 1:602
definition 2:29, 4:103
echovirus infection diagnosis 2:69
furovirus infection diagnosis 2:295
iflavirus infection diagnosis 3:45
Marburg virus infection diagnosis 3:278–279
measles diagnosis 3:289–290
monkeypox virus infection diagnosis 4:644
mungbean yellow mosaic virus infection diagnosis 3:366–367
ophiovirus classification 3:448
pecluvirus infection diagnosis 4:99
Pepino mosaic virus infection diagnosis 4:106
phlebovirus infection diagnosis 4:494
plant virus infection diagnosis 2:21–23, 2:26
principles 2:35*f*
rice yellow mottle virus diagnosis 4:488
tospovirus infection diagnosis 5:162
yellow fever diagnosis 5:472
yellow head virus infection diagnosis 5:482
Rev protein *see* HIV
REV-T-mediated transformation, reticuloendotheliosis virus infection 4:416
Rex protein, HTLV-1 2:560–561
Reyataz® 2:513
Reye syndrome, chickenpox 5:253
Rhabdoviridae 1:111, 1:170, 1:488, 1:497, 2:228, 3:324, 4:187, 4:367–368, 4:576, 5:11*t*, 5:291
Rhabdovirus(es) 1:111, 3:324
animal *see below*
apoptosis inhibition 1:158*t*
associated genera 1:497
characteristics 5:62*t*
genome 1:173, 3:326*f*
glycoproteins 3:332
fusion protein biosynthesis 3:300
host cell entry 3:333
morphology 1:497
plant *see below*
virion structure 1:173
virus structure 3:325, 3:329*f*
see also specific viruses
Rhabdovirus(es), animal **1:111–121**
Almpiwar Group 1:116, 1:119*t*
classification 1:111, 1:119*t*
immune reagents 1:111
evolutionary relationships 1:112, 1:114*f*
L polymerase, block III 1:112–114
fish *see* Fish rhabdovirus(es)
genetics 1:112
genome 1:112, 1:113*f*
open reading frames 1:112
history 1:111
host range 1:115
infection
clinical features 1:115
insects 3:126*t*
pathology 1:115
isolate CS1056 1:118
isolate CSIRO75 1:118
isolate DP1163 1:118
Kolongo Group 1:118
morphology 1:112, 1:112*f*
Mount Elgon Bat Virus Group 1:118
nonassigned serogroups 1:113*t*, 1:115
Bahia Grand Group 1:116
Hart Park Group 1:115, 1:119*t*
Kern Canyon Group 1:116, 1:119*t*
Le Dantec Group 1:116, 1:119*t*
Sawgrass Group 1:116
Timbo Group 1:116
propagation 1:111, 1:115
Sandjimba Group 1:118
Tibrogargan Group 1:117, 1:119*t*
transmission 1:115
virion properties 1:112
Rhabdovirus(es), plant **4:187–197**
classification 4:187
distribution 4:195
evolution 4:195, 4:196
genome 4:189, 4:190*f*
gene-junction sequences 4:189
noncoding sequences 4:189

Rhabdovirus(es), plant (*continued*)
open reading frames 4:189
structure 4:189
host range 4:196
specificity determinants 4:196
infections
barriers 4:196
cytopathology 4:193
orchid infections 4:225–226
matrix protein 4:188–189
nucleocapsid core 4:188–189
nucleocapsid protein 4:189*f*, 4:190
nuclear localization signals 4:190
pathogens associated 4:187
polymerase 4:193
activity 4:193
proteins 4:189
glycoprotein 4:189*f*, 4:192
matrix 4:192
phosphoprotein 4:190
position X proteins 4:192
position Y orfs 4:189*f*, 4:192
replication 4:193
see also specific proteins
replication 4:193
nuclear entry 4:194–195
protein interactions 4:194–195
virion-associated polymerase 4:193–194
viroplasm formation 4:194–195
taxonomy 4:187, 4:188*t*
transmission 4:187
vector relationships 4:195
virion composition 4:188, 4:189
lipids 4:189
matrix protein 4:188–189
nucleocapsid core 4:188–189
virion morphology 4:188, 4:189*f*
Rhadinovirus 2:432*t*, 3:190, 3:195, 4:585–586
Rhadinovirus(es) 3:190, 3:195, 4:585–586, 4:587*t*
distribution 4:593
evolution 3:197
gene vectors 4:593
human T-cell transformation 4:585–586, 4:590, 4:593
cell surface markers 4:591
generation 4:590
gene transfer 4:591
immunodeficiency research 4:590
interleukin-26 discovery 4:591
phenotypes 4:590
molecular piracy 3:192
murine gammaherpesvirus 68 3:372
retroperitoneal fibrosis 4:592
sequestered cellular genes 4:589
subgroups 2:424–426
see also specific viruses
Rhesus macaque cytomegalovirus (RhCMV) 1:635*t*, 1:639*f*
Rhesus macaque rhadinovirus *see* Ceropithecine herpesvirus 17 (CeHV-17)
Rhesus macaques
simian hemorrhagic fever virus infection 1:183
SIV infection *see* Simian immunodeficiency virus infection
yatapoxvirus infection 5:461
Rhesus papillomavirus 1 (RhPV) 4:33
Rhesus rhadinovirus (RRV) 2:428, 4:591
discovery 4:586
genome 4:592
KSHV *vs.* 4:592
historical aspects 4:591–592
natural occurrence 4:591
pathology 4:591
replication 4:592
Rhinitis 1:104
HSV-1 associated 2:388–390
Rhinomune 2:419
Rhinopneumonitis, EHV infections 2:417
clinical management 2:419
Rhinorrhea 2:543
Rhinovirus 1:542
Rhinovirus(es) **4:467–475**
antigenicity 1:204, 1:545
antigenic variability 4:471
cross-reactivity 4:471
assembly 4:470
capsid composition 1:542
capsid proteins 4:468–469
cell entry 4:470
ICAM-1 4:470, 4:471
cesium chloride gradient purification 4:469
classification 4:468
detection 4:471
enzyme-linked immunosorbent assays 4:471
PCR 4:471
quantitative real-time PCR 4:471
serology 4:471
genome 1:542, 1:542*f*, 2:125, 4:469
5′ nontranslated region 2:126, 4:139
internal ribosome entry sites 4:469
IRES sequence 1:542
P1 4:469
P2 4:469
P3 4:469
history 4:468
host cell attachment 1:543, 1:543*f*, 1:544*f*, 5:321–322
antirhinoviral strategies 1:547–548, 1:547*t*
canyon hypothesis 1:544, 1:545*f*, 1:545*t*, 1:612–613, 5:321–322
fatty acid function 1:544–545
HRV2 1:543–544
ICAM-1 1:546
LDL receptors 1:546
methyl-β-cyclodextrin effects 1:543–544
VCAM-1 1:546
host cell entry 1:543
infection *see* Common cold; *see below*
life cycle 4:470
phylogeny 1:545
propagation 4:471
proteins 4:469
VPg protein 4:469–470
RdRp 4:470
replication 1:543, 4:470
cap-independent translation 4:470
capsid maturation 1:542
mutation rate 4:470
polyprotein processing 1:542
polyprotein translation 1:542
RdRp 4:470
VPg 3AB priming 1:543
serotypes 4:468, 4:468*t*, 4:471
survival features 4:469
taxonomy 1:542, 4:468
transmission 1:541
virion 4:468, 4:469*f*
structure 1:544
see also specific viruses
Rhinovirus infections 2:552, 4:129
antirhinoviral strategies 1:547
acid sphingomyelinase 1:548
cross-reacting antibodies 1:548
vaccine development 5:240–241
asthma 1:546
COPD 1:546
cycle 1:543–544
detection 1:546
host cell reactions 1:546
cytokine production 1:546
host range 4:471
nonpermissive cells 4:471
in tissue culture 4:471
lower respiratory tract illness 2:552
models 1:546
PI-3 kinase
host cell reactions 1:546–547
inhibition 1:547
prevention, acid sphingomyelinase 1:548
in serious illness 4:468
serology 4:471
symptoms, molecular origin 1:546
treatment 2:556–557
see also Common cold
Rhizidiovirus, fungal infections 2:287*t*
Rhizoctonia solani virus 717 (RhsV 717)
antigenic properties 4:65
genome 4:65*t*
homology 4:65*f*
structure 4:64*f*
Rhizomania *see* Beet necrotic yellow vein virus (BNYVV)
Rhizosolenia setigera, viral infections 1:87
Rhizosolenia setigera RNA virus (RsRNAV) 1:87, 1:88*t*
biological properties 1:87
genome 1:87–88
phylogenetic analysis 3:281–282, 3:283*t*
RdRp domains 3:283
phylogeny 1:93*f*
propagation 1:87–88
virion structure 1:89*f*
Rhododendron necrotic ringspot virus (RoNRSV) 4:311*t*
Rhubarb virus 1 (RV-1) 4:311*t*
Ribavirin 1:143*t*
arenaviruses 3:211
Crimean-Congo hemorrhagic fever treatment 1:603
definition 2:543
henipavirus infection treatment 2:326
human respiratory syncytial virus 2:549–550
Lassa fever treatment 3:211
phleboviruses 4:495
respiratory virus infection treatment 2:556
Ribonuclease H, LTR retrotransposons 4:438–439
Ribonuclease P (RNaseP), structure 4:475
Ribonucleoprotein (RNP) 3:393
cis-acting signals 3:396
immunoprecipitation 3:396
long distance RNA/RNA interactions 3:396, 3:397*f*
definition 4:129, 5:157
mouse mammary tumor virus 3:335
RdRps 3:393–394
'replication complex' 3:393
'resting complex' 3:393
see also specific viruses
Ribonucleotide reductase
African swine fever virus 1:47
Invertebrate iridescent virus-6 3:164
Ribose-2′-*O*-methyltransferase (O-MT)
nidoviruses genome 3:424–425
yellow head virus 5:477–478
Ribosomes
frameshifting 1:549, 3:220, 4:623, 4:644
Simian retrovirus D 4:627
Ustilago maydis viruses 5:214–215
RNA, evolution of 3:478
RNA 23S, ribozyme function 4:475
shunt
cauliflower mosaic virus 1:460–461
definition 1:457
Ribozyme(s) **4:475–481**
chemical reactions 4:475
ligation mechanism 4:479
monomer circularization mechanism 4:479
definition 2:375, 4:475, 5:332
group I introns 4:475
hammerhead *see* Hammerhead ribozyme(s)
Neurospora crassa 4:480
nucleolytic 4:475
chemical reaction 4:475–476, 4:477*f*
plant viroids/virusoids 4:476
classification 4:477*f*
hairpin *see* Hairpin ribozyme(s)
replication 4:476, 4:478*f*
satellite viruses 4:480
classification 4:477*f*
Varkud satellite 4:475
Rice *(Oryza sativa)* **1:482–489**
edornaviruses 2:110
infection 1:483
production 1:483

viruses
see under Oryza
see specific viruses
Rice black streaked dwarf virus (RBSDV) 1:481, 1:484
characteristics 4:150*t*
classification 1:484
genome 1:484
history 1:484
host range 1:484
infection 1:484
maize 1:481
resistance 1:484
proteins 1:484
transmission 1:484
Rice dwarf virus (RDV) 1:483, 4:149–150
characteristics 4:150*t*
disease 1:483, 4:149–150
symptoms 1:483–484
genome 4:152, 4:153*f*, 4:154*t*
history 1:483
host range 1:483–484
transmission 1:483
vectors 1:483
Rice gall dwarf virus (RGDV) 1:484
characteristics 4:150*t*
classification 1:484
disease 1:484
symptoms 1:484
host range 1:484
tissue tropism 1:484
transmission 1:484
vectors 1:484
Rice grassy stunt virus (RGSV) 5:24
disease 1:486
resistance 1:487
symptoms 1:487
genome 1:486–487
history 1:486–487
transmission 1:487
Rice hoja blanca virus (RHBV) 1:487, 5:24, 5:26, 5:26*f*
classification 1:487
genome 1:487
geographical distribution 1:487
history 1:487
infection 1:487, 5:26
epidemics 5:24
host range 1:487–488
incubation within vector 1:487
resistance 1:487–488
symptoms 1:487–488
transmission 1:487
Rice necrosis mosaic virus (RNMV)
classification 1:489
disease 1:489
transmission 1:489
Rice ragged stunt virus (RRSV) 1:484–485, 4:149–150
characteristics 4:150*t*
classification 1:484–485
genome 4:152*f*, 4:153*t*
history 1:484–485
infection 1:484
host range 1:485
pathogenicity 4:155
resistance 1:485
structure 1:484–485
transmission 1:485
as type species 4:536*t*
Rice stripe necrosis virus (RSNV) 1:308, 1:488–489
antigenic properties 1:310
genome 1:312, 1:489
geographical distribution 1:309, 1:489
history 1:488–489
infection 1:308, 1:488
resistance 1:489
symptoms 1:488–489
structure 1:488–489
transmission 1:488–489
soil 1:309
Rice stripe virus (RSV) 1:486
classification 1:486
genome 1:486
geographical distribution 1:486
history 1:486
infection 1:486
epidemics 5:24
host range 1:486
resistance 1:486
symptoms 1:486
species discrimination, maize stripe virus 5:26
transmission 1:486
as type species 5:24
vectors 1:486
Rice tungro bacilliform virus (RTBV) 4:482
classification 4:482
genome 1:485, 4:482, 4:484*f*
transcription 4:482
host range 1:485–486
infection *see below*
rice tungro spherical virus coupling 1:458, 4:551
transmission 1:463
strains 4:482–484
structure 4:484*f*
transmission 4:484*f*
vectors 1:485
Rice tungro disease 1:458, 1:485, **4:481–485**
control 4:485
geographical distribution 4:482, 4:483*f*
history 1:485
plant resistance 1:486, 4:485
severity 4:482
subgroups 1:485
symptoms 1:485–486, 4:482, 4:483*f*
transmission 4:482, 4:483*f*, 4:551
vectors associated 4:482
Rice tungro spherical virus (RTSV) 4:484, 4:546–547
classification 4:484
genome 1:485, 4:485, 4:548
polyprotein processing 4:485
structure 4:548*f*
host range 1:485–486, 4:482
isolate variations 4:550
plant resistance 1:486
proteins
coat proteins 4:549–550
leader protein 4:549
proteases 4:548–549
RdRp 4:549
rice tungro bacilliform virus coupling 1:458, 4:551
transmission 1:463, 1:485
structure 4:484, 4:484*f*, 4:547*f*
capsid 4:484–485
transmission 4:484*f*
vectors 1:485
Rice wilted stunt virus 5:24
Rice yellow mottle virus (RYMV) 4:646*t*
biological properties 4:487
chloroplasts 4:487–488
cytology 4:487–488
host range 4:487
parenchyma 4:487–488
plant, distribution in 4:487
vector transmission 4:488
xylem 4:487–488
coat protein 4:486, 4:487
diversity 4:489
evolution 4:489
genome 1:488, 4:486, 4:487*f*
coat protein 4:487
open reading frames 4:486
P1 protein 4:486–487
polyproteins 4:487
RNA satellites 4:486
VPg 4:487
historical aspects 1:488, 4:485–486
identification 4:488
inffection *see below*
phylogenetic relationships 4:487
RNA satellites 4:486
transmission 1:488
abiotic transmission 4:488
biotic transmission 4:488
vectors 4:488
control 4:488
virion particle 4:486, 4:486*f*
coat protein 4:486
X-ray crystallography 4:486
virion structure 4:649, 4:650*f*
P1 protein 4:647
Rice yellow mottle virus infection 1:488
control
genetically-modified resistant plants 1:488
resistance breeding 4:488–489
transgenic plants 4:488–489
vector control 4:488
crop yield losses 4:651
diagnosis 4:488
ELISA 4:488
RT-PCR 4:488
symptoms 4:488
epidemiology 4:488, 4:489*f*
abiotic transmission 4:488
biotic transmission 4:488
as emergent disease 4:488
symptoms 4:488
host range 1:488
pathology 4:650–651
resistance 1:488, 4:651–652
symptoms 1:488
Rice yellow stunt virus (RYSV) 1:488, 4:184*t*
disease 1:488
resistance 1:488
genome 1:488
history 1:488
movement hypothesis, P3 protein 4:192
P6 protein 4:192
transmission 1:488
Rickettsia, neural cell infection 1:473
'Riddle-of-the-ends' dsDNA bacteriophage genome packaging 2:308–309
Rift Valley fever **4:490–497**
affected species 4:492
bioterrorism evaluation 5:407–408, 5:408*t*
clinical features 4:491, 4:492–493
disease manifestations 5:490
epidemics
Aswan Dam project 4:494
Egypt 4:492–493
insecticides 4:496
epidemiology 4:494
epizootics 4:494, 5:490
incidence 4:492
mortality 4:492–493, 4:494
zoonoses 5:490
Rift Valley fever virus (RVFV) 1:391*t*
characteristics 1:171*t*
genome 4:491
genomes 1:394*f*
infection *see above*
isolation 4:491
maturation 4:492
morphology 4:491
protein translation 4:491
replication strategy 4:491
ambisense replication 4:492
budding 1:397
transmission 4:491, 4:492, 5:490
Aedes vexans arabiensis 4:491
prevention 4:496
vaccines/vaccination 4:496
formalin-inactivated 4:496
inactivated 4:496
live vaccine candidates 4:496
virion structure 4:491
RIG-like helicase(s) 1:155
Rilpivirine 2:507*t*
Rimantadine
history 1:145
influenza treatment 3:102
resistance 1:148, 3:102
RIN4 protein, plant viral resistance 4:175

Rinderpest virus(es) (RPVs) **4:497–507**
  associated animal pathogens 4:497
  classification 4:498
  epizootiology 4:502
  evolution 4:501
  genome 4:500
  geographical distribution 4:498
  history 4:497
    cattle imports 4:497
    panzootics 4:497
  host cell binding 4:500
  host range 4:499
    susceptibility 4:499*t*
  infection
    clinical features 4:504
    control 4:505
    diagnosis 4:506
    histopathology 4:504
    immune response 4:505
    pathology 4:504
    prevention 4:505
    serology 4:502
    vaccination 4:506
  propagation 4:500
  signaling lymphocyte activation molecule 3:285
  taxonomy 4:497, 4:498
  transmission 4:504
  vaccines/vaccination 4:506
    egg-adapted vaccines 4:506
    'Plowright vaccine' 4:506
  see also specific viruses
RING domain, definition 1:231
Rio Negro virus (RNV), characteristics 5:109*t*
RIP (repeat-induced point mutation), fungal retrotransposons 4:426–427
RI protein, T4 bacteriophage host cell lysis 3:255
RIRIS *see* Immune reconstitution inflammatory syndrome (IRIS)
Ritonavir 1:151, 2:513, 2:514*t*
Rmcf, receptor-envelope interaction 2:468–469
RMV strain, barley yellow dwarf virus *see* Barley yellow dwarf virus (BYDV)
RNA
  chemical modification in evolution 3:477–478
  folding 4:475–476
  hypoviruses 2:581
  influenza virus replication 3:487
  microarray use 2:15–16
  packaging, yeast L–A virus 5:467, 5:467*f*
  poliovirus translation 2:457
  synthesis
    Japanese encephalitis virus replication 3:183–184
    potato virus Y 4:288
RNA-1
  cowpea mosaic virus genome 1:571, 1:572*f*
  ourmiavirus 3:500
  pecluvirus genome 4:99–100
RNA-2
  beet soil-borne virus genome 4:284
  beet virus Q genome 4:284
  broad bean necrosis virus genome 4:284
  citrus psorosis virus 3:449
  cowpea mosaic virus 1:571, 1:572*f*
  Dendrolimus punctatus tetravirus genomes 5:31
  Helicoverpa armigera stunt virus genomes 5:31
  Indian peanut clump virus 4:101
  mirafiori lettuce virus 3:449
  Nudaurelia ω virus 5:31
  omega tetraviruses 5:31
  ophiovirus 3:449
  ourmiavirus 3:500
  peanut clump virus 4:101
  pecluvirus genome 4:99–100
RNA-3
  ourmiavirus 3:500
  potato mop-top virus genome 4:284
RNA-4
  lettuce ring necrosis virus 3:449
  mirafiori lettuce virus 3:449
  ophiovirus 3:449
RNA-binding proteins
  apoptosis 1:161
  flexiviruses phylogenetics 2:257, 2:258*f*
  pathogen recognition receptors 1:161
RNA-binding site, translational repression/assembly initiation complex 3:28
RNA cross-hybridization, aquareovirus taxonomy/classification 1:163
dsRNA dependent protein kinase (PKR)
  HSV infection 2:403
  interferon-induced antiviral effects 3:107, 3:115–116
  iridovirus infection 3:173
  togavirus infection 5:123
  virus-mediated immune system evasion 3:108–109
RNA-dependent RNA polymerase (RdRp) 1:99
  cellular 5:326–327
  definition 4:52, 5:117
  gene silencing 4:141, 4:142
  see also specific viruses
RNA-dependent RNA polymerase 6 4:146
RNA-directed DNA methylation (RdDM) 4:141
RNA editing
  definition 2:375, 3:285
  hepatitis delta virus replication 2:376
RNA electrophoresis, aquareovirus genetic diversity 1:167
RNA genome(s)
  positive sense 5:117
  see also specific viruses
RNA helicase 2:110–111
RNA-induced silencing complex (RISC) 5:376
  associated proteins 3:149, 5:326–327
  definition 3:148
  nodavirus RNA silencing 3:436–437
  plant gene silencing 4:146
  resistance engineering in plants 4:161
  tombusviruses replication 5:148–149
RNA interference (RNAi) **3:148–155**, 3:150*f*, 5:327
  Argonaute protein interactions 3:151
  components 3:149
  definition 1:154, 2:199, 3:149, 4:310, 5:325, 5:450
  Dicers *see* Dicer(s)
  disease control 3:153
  function 3:149
  mechanism of action 3:149
    effector phase 3:149
    initiator phase 3:149
  micro RNAs *see* Micro RNAs (miRNAs)
  properties 3:151
    animals 3:151
    plants 3:151
  R2D2 protein interactions 3:150
  small interfering RNA *see* Small-interfering RNAs (siRNAs)
  transitive RNAi 3:151
  vector competence 5:271
  viral encoded suppressors 3:152
  virus interactions 3:152
    African cassava mosaic disease 1:35
    B2 protein, Flock house virus 3:152
    filovirus infection prevention 2:204–205
    model systems 3:153
    mosquitoes–arbovirus interactions 3:154
    mungbean yellow mosaic virus management 3:370
    RNA virus–host evolution 3:154
    white spot disease 5:458
    yellow head virus infection management 5:482
    see also specific viruses
  see also Post-transcriptional gene silencing (PTGS)
RNA polymerase
  antigenic variation, role 1:127
  elongation 5:175–176
  elongation termination 5:176
  entomopoxviruses 2:139
  icosahedral tailed dsDNA bacteriophages 3:34
  isomerization 5:175–176
  modifications in T4 infection 5:176–177
  origin of 3:478
  promoter binding 5:175
  structure 5:175
  transcription initiation 5:175–176
RNA polymerase II
  coxsackievirus 1:583
  HSV transcription 2:398, 2:399
  influenza virus replication 3:487, 3:493
  iridovirus 3:170, 3:171–172
  mouse mammary tumor virus 3:335*f*, 3:336
RNA protection assays, leishmaniaviruses 3:221
RNA-protein complexes, EBV-encoded small RNA 2:164
RNA pseudoknot, yeast L–A virus 5:467
RNA recombination
  arterivirus evolution 1:182
  mouse hepatitis virus 1:560
RNA replication
  alfalfa mosaic virus *see* Alfalfa mosaic virus (AMV)
  yeast L–A virus 5:467, 5:468*f*
RNA–RNA crossover events, RNA virus recombination 4:377
RNA–RNA hybridization
  aquareovirus genetic diversity 1:167
  aquareovirus infection diagnosis 1:167–168
RNAs and Proteins of dsDNA Viruses database 5:357*t*
RNA sedimentation behavior, cowpea mosaic virus 1:569, 1:570*f*
RNase H
  hepatitis B virus replication 2:353
  metavirus 3:306
  see also specific viruses
RNase III
  RNA interference 3:149–150
  T7 RNA processing 5:180
RNase protection assays, plant virus detection 2:29
RNA silencing 5:376
  DNA viruses 4:146
  engineered plant resistance 4:161, 4:162
  fungal virus infection defence 2:290
  hordeiviruses 2:463
  nodaviruses 3:436
  see also RNA interference (RNAi); Virus-induced gene silencing (VIGS)
RNA-specific adenosine deaminase 1 (ADAR 1) *see* Adenosine deaminase 1 (ADAR 1)
RNA stability, viral evolution 2:179
RNA strand templates, maize chlorotic mottle virus 3:260–261
RNA structure–function relationships, orthobunyavirus 3:482
RNA translation, alfalfa mosaic virus *see* Alfalfa mosaic virus (AMV)
RNA virus(es)
  antiviral therapy 1:143*t*
  DNA viruses *vs.* 3:477
  evolution of 2:180
    helicases 2:180–181
    reduction hypothesis 3:476
    replicases 2:180–181
  fish viruses 2:227, 2:228
  fusion proteins 3:293*t*, 3:297
  genome 1:612
  insect pest control 3:127
  nucleotide sequence analysis 2:21
  ocular disease 2:492, 2:493*t*
  positive strand 2:378
    replication 1:382
  recombination 4:377
    cytoplasmic life cycle 4:377
    homologous recombination 4:377–378, 4:378*f*
    with host-derived sequence 4:377
    molecular mechanisms 4:377
    mutation rates 4:377
    nonhomologous recombination 4:377–378
    recombination by reassortment 4:377
    RNA–RNA crossover events 4:377
  replication 4:407
    double-stranded RNA 4:410
    single-stranded negative sense RNA 4:410
    single-stranded positive sense RNA 4:410
    single-stranded RNA viruses, DNA intermediate 4:410
  research history 2:452
  see also specific viruses

'RNA world' 3:473
  virus evolution 3:475
Rnq1 amyloid, prion generation 4:339
Robovirus(es) 2:317
Rochambeau virus (RBUV) 1:118
Rocio virus 5:489
  characteristics 1:171*t*
Rodents
  cardiovirus infection 1:444–445
  Saint Louis encephalitis virus transmission 4:656
  see also specific viruses
Roguing 5:282
  vegetable viruses 5:286–287
Rolling-circle replication (RCR)
  beet curly top virus 1:303–304
  circoviruses 1:517
  definition 1:314, 1:513, 2:375, 5:138
  equine herpesviruses 2:415
  hepatitis delta virus 2:376
  KSHV 3:199
  maize streak virus 3:268, 3:269*f*
  mungbean yellow mosaic virus replication 3:366
  ribozymes 4:476, 4:478*f*
  RNA satellites replication 4:534
  varicella-zoster virus 5:258
Rolling-hairpin replication (RHR) 4:90
Roniviridae 3:419–420, 4:567, 5:11*t*, 5:152, 5:477
Ronivirus(es)
  genome 3:424*f*
    accessory proteins 3:426
    structural proteins 3:426
  infection 3:421
  structure 3:421–423
  see also specific viruses
Root beardness *see* Beet necrotic yellow vein virus (BNYVV)
Root madness *see* Beet necrotic yellow vein virus (BNYVV)
Rosellinia necatrix, Mycoreovirus 3 infection 3:380
Rosellinia necatrix virus (RnV-1)
  infectivity assays 4:75
  latency 4:67–68
Roseola infatum 2:502
Roseolovirus 1:635–636, 2:432*t*
Roseolovirus(es)
  classification 1:635–636, 1:635*t*
  genome
    direct repeats 2:499
    origin of lytic replication 2:499–500
  infection 2:498
  see also specific viruses
Ross River virus (RRV) 5:89, 5:114
  characteristics 1:171*t*
  genotypes 5:89
  geographical distribution 5:84*f*, 5:89
  host range 5:90
  infection *see below*
  seasonal distribution 5:90
  transmission 5:90, 5:100, 5:114
Ross River virus infection
  clinical features 5:87*t*
  clinical manifestations 5:83–84, 5:86*t*, 5:114
    arthralgia 5:114
    arthritis 5:85, 5:114
    gastrointestinal 5:84
    pregnancy 5:87
    rash 5:84
  host factors 5:87
  incidence 5:90
  zoonoses 5:493
    geographical distribution 5:493
Rotarix 4:513
RotaShield 4:513
RotaTeq 4:513
Rotavirus 2:118–119, 4:509, 4:536*t*
Rotavirus(es) 2:118, **4:507–513**
  characteristics 2:117*t*
  classification 2:118, 4:509
    genotypes 2:118–119, 4:509
    serotypes 2:118–119, 4:509
    strain variability 4:509
  genetics 2:119
  genome 4:509
    functional analysis 4:510
    genetic diversity mechanisms 4:510
  geographical distribution 2:119
  global distribution 4:509
  group A, genome reassortment 4:509
  history 2:118, 4:507
  infections *see below*
  morphology 4:508, 4:508*f*
    associated proteins 4:508
  mutation rate 4:510
  replication 4:509
    host cell entry 4:510
    nonstructural proteins 4:510
    sites of 4:510–511
  transmission 4:511
  vaccines/vaccination 2:120, 4:512–513
    development 5:235, 5:238
    dose regimen 5:238
    efficacy *vs.* potency 5:228
    reassortant strains 5:238
Rotavirus A 4:536*t*
Rotavirus infections
  acute gastroenteritis 2:118, 2:119–120, 4:507, 4:512
  antibodies 4:512
  children 2:118, 2:119–120, 4:507, 4:512
  clinical characteristics 2:119, 4:511
    diarrhea *see below*
    immunodeficiency 4:512
    virus excretion 4:511
  control 2:120
  diagnosis 4:511
    ELISA 4:511
    RNA analysis 4:511
  diarrhea 2:118, 4:507
    children 2:119–120, 4:511
    mechanisms 2:119, 4:511, 4:511*t*
    pigs 4:511
  epidemiology 4:509
    temperate zones 4:509
  host range 2:119, 4:509
  human pathogens 4:509
  immune response 2:120, 4:512
    antibodies 4:512
    children 4:512
    neonates 4:512
  mortality 2:118, 4:507, 4:511–512
    children 4:507
  pathogenesis 2:119, 4:510, 4:511*t*
  prevalence 4:507, 4:508*t*, 4:509, 4:511–512
  prevention 2:120
  re-infection 4:508*t*, 4:512–513
  respiratory 2:555
  seasonal distribution 2:119
  treatment 2:119–120, 4:511
  virus-host interactions 4:507
Rottboelia yellow mottle virus (RoMoV) 4:646*t*
Rous sarcoma virus (RSV)
  expression regulation, frameshifts 3:304–306
  oncogenesis studies 4:446, 4:459–460
  propagation 4:446
  reverse transcription priming 3:308*f*
  transformation-defective 4:446
Route importance, retrovirus infection resistance 2:471
Row covers, infection control 5:437
RPV strain, cereal yellow dwarf virus *see* Cereal yellow dwarf virus (CYDV)
RSP2 protein 4:175
Rta protein, EBV infection 2:166
RT proteins 4:284
Rubella 5:117
  affected organs 4:519
  clinical features 4:518
    arthritis 4:518–519
    eyes 2:492–494
    rash 4:518–519
    rashes 4:518–519
    vascular lesions 4:519
  complications 5:111
  congenital *see* Congenital rubella syndrome (CRS)
  control 4:520
  cytopathology 4:517, 5:103–104
  diagnosis 4:520
    immunoglobulin M 4:520
    serodiagnosis 4:519
  epidemic 4:514
  fetal 4:518, 4:519, 4:520
  fetal infections 4:518, 4:519, 4:520
  histopathology 4:519
  host range 4:514
  immune response 4:519
  incubation period 4:518
  measles *vs.* 3:289–290
  pathogenesis 4:519
  pathology 4:519
  persistence 4:519
  pregnancy 4:518, 4:520
  prevention 4:520
  re-infection 4:518
Rubella virus (RUBV) **4:514–522**, 5:83
  classification 4:514, 5:83
  epidemiology 4:518
    vaccination programs 4:518
  evolution 4:517, 5:111
  genetics 4:517
  genome 4:515, 4:516*f*, 5:117, 5:119*f*
    infectious clone development 4:515
    open reading frames 4:515
    P150 protein 4:515
    proteolytic cleavage 4:515
  infection *see* Rubella
  propagation 4:514
  protein processing, alphavirus *vs.* 5:121
  replication 4:516
    budding 5:121
    capsid morphogenesis 4:517
    cellular site 4:517, 4:518
    glycan modifications 4:517
    host cell attachment 5:118
    host cell entry 4:516, 5:118–119
    regulation 4:517
    replication complex 5:119
    RNA synthesis 4:516–517
    structural protein maturation 5:121
    translation 4:516–517
  replicon systems 5:121–122
  serological relationships 4:518
  taxonomy 5:117
  tissue tropism 4:518
  transmission 4:518, 5:113
  vaccines/vaccination 4:520
    arthritis risk 4:520–521
    developing countries 4:521
    history 4:521
    modifications 4:521
    pregnancy 4:520–521
    programs 4:518
  virion properties 4:515, 4:515*f*
    C protein 4:515
    glycoprotein spikes 4:515
    stability 4:515, 4:517–518
  virion structure 4:514, 5:108
  virus perpetuation 2:146
Rubivirus 2:230, 4:514, 5:91–92, 5:107, 5:108, 5:117
Rubulavirus 4:41*t*, 4:47
  associated species 3:356
  classification 4:41*t*
  genome 3:327*f*
    P gene 4:44
  glycoproteins 3:332
  immune evasion, STAT protein targets 3:108
Rubulavirus 4:19, 4:41*t*
Rubus Chinese seed-borne virus (RCSV) 4:523, 4:524*t*
  geographical distribution 4:523
  host range 4:525
Rubus idaeus (red raspberry)
  idaeovirus infection 3:37
  Raspberry bushy dwarf virus infection 3:38
Rudiviridae 1:587, 5:11*t*

Rudivirus(es) 1:587
  Crenarchaea infections 1:588*t*
  DNA-binding protein 1:591
  gene functions 1:594
  genome 1:593
  morphology 1:589
Rugose mosaic disease 3:55
Rugose wood disease complex 4:202–203, 4:202*f*
  causal agents 4:203
  transmission 4:203–204
Runting syndrome, reticuloendotheliosis virus infection 4:416
Rupestris stem pitting-associated virus (RSPaV) 1:423
Ruprintivir 1:548
Rural cycle, Dengue virus 2:8*f*, 2:9
Ruska, Helmut, physical studies of viruses 2:455–456
Russian influenza (1977) pandemic 3:100
Rv-cyclin(s) 2:214–215
  localization 2:216
  transcription activation 2:216
  transcription inhibition 2:216
Rx2 gene, plant resistance 4:174
Rx genes
  hypersensitive response 4:174
  plant resistance 4:174
    signaling mechanisms 4:176
Ryegrass mottle virus (RGMoV) 4:646*t*, 4:650–651
Rymovirus 4:315*t*
Rz-Rz1 genes *see* Holin–endolysin lysis

S

S41 virus 5:413*t*
S45 virus 5:413*t*
S50.2 virus 5:413*t*
S107 antiholin 3:253
  holin S105 *vs.* 3:253–254
  lysis timing 3:253
S4100 virus 5:413*t*
S5100 virus 5:413*t*
Sabia virus 2:95
  history 3:203
Sabin, Albert Bruce 4:383
Sacbrood virus (SBV) 3:42
  classification 5:2–3
  genome 3:45, 5:3
Saccharomyces cerevisiae
  Brome mosaic virus replication studies 1:382
  killer phenomenon 5:164, 5:170–171, 5:300
    maintenance of killer genes 5:170–171
    phenotype inheritance 5:300
    preprotoxin processing 5:301–303
    superkiller genes 5:170–171
  PIN prion 4:337
  retrotransposons 4:420
  Ty elements 4:420
  vacuolar protease B 4:340
  viral replication model 2:290
  virus transmission 5:300
Saccharomyces cerevisiae L-A virus (ScV-L-A) *see* Yeast L–A virus (ScV-L-A)
Saccharomyces cerevisiae M virus(es) (ScV-M)
  killer phenomenon 5:300–301
  M28 virus 5:301
    preprotoxin processing 5:304*f*
  replication 5:301
Sadwavirus 3:405–407, 4:523, 4:524*t*
Sadwavirus(es) **4:523–526**
  epidemiology 4:525
  genome 4:524
    open reading frame 4:524, 4:525*f*
  geographical distribution 4:523
  host range 4:525
  infections *see below*
  phylogenetics 4:524
  satellites 4:525
  sequence comparisons 4:524
  strains 4:523
  taxonomy/classification 4:523, 4:524*t*
  type species 4:523
  virion 4:523, 4:524*f*
    sedimentation rates 4:523–524
    size 4:523–524
  virus propagation 4:525
  see also specific viruses
Sadwavirus infections
  cytopathology 4:525
  prevention/control 4:525
    detection 4:525–526
    vector control 4:525–526
'Safety box(es)', vaccine safety 5:229–230
sag gene/protein, mouse mammary tumor virus 3:335*f*, 3:336, 3:341, 3:341*f*
Saimirine herpesvirus 2 *see* Herpesvirus Saimiri (HVS)
Saint Louis encephalitis virus (SLEV) 2:236, **4:652–659**
  classification 4:653
  distribution 2:236, 4:653, 4:654*f*
  epidemiology 4:653, 4:654*f*, 4:656
    overwintering 4:656
    virus reintroduction 4:657
  geographical distribution 5:488
  geographic variation 4:653–655
    clusters 4:653–655
  history 4:653
  infection *see below*
  lineages 4:653–655
  multiple strain circulation 4:653–655
  pathogenicity 4:656
  replication 4:656
    conditions 4:657–658
  structure 4:653–655
  subsidence 4:658
  transmission 2:236, 4:657–658, 5:488
    cycles 4:658
  virulence changes 4:653–655
  virus amplification 4:657
Saint Louis encephalitis virus infection
  asymptomatic 2:236
  case fatality rates 2:236
  clinical features 4:656, 5:488
  control 4:659
  epidemics 4:653
    seroprevalence rates 4:658
  host range 4:655
    arthropods 4:655
    birds 4:655
    domestic animals 4:655
    humans 4:655
    wild mammals 4:656
  persistence 4:657
  prevention 4:659
  reintroduction via migration 4:657
  risk factors
    age 4:658
    occupation 4:658
    residence 4:657*f*, 4:658
    socioeconomic status 4:658
    weather 4:659
Sakhalin virus 1:598*t*
Salicylic acid (SA)
  definition 4:170
  plant signaling mechanisms 4:176–177
Saliva, West Nile virus transmission 5:446
Salivary gland virus(es) *see* Human cytomegalovirus (HCMV)
Salmonid alphavirus(es) (SAVs) 2:230, 5:92
  see also specific viruses
Salmonid herpesvirus(es) 2:208
  genetics 2:211
  growth properties 2:209
Salmonid herpesvirus 1 (SalHV-1) 2:233
Salmonid herpesvirus 2 (SalHV-2) 2:233
Salmon pancreas disease virus (SPDV) 2:230, 5:93*t*, 5:94
  characteristics 5:109*t*
  cohabitation experiments 5:94
  disease 5:94
  genome 5:94
  identification 5:94
  infection 2:230
Salmon swimbladder sarcoma-associated retrovirus (SSSV) 2:217
  genome 2:217–218, 2:218*f*
    central DNA flap 2:217–218
  infection
    mortality 2:219
    symptoms 2:217, 2:218*f*
  isolation 2:217
  pathogenesis 2:219
    insertional activation 2:219
  phylogeny 2:219–220, 2:220*f*
  prevalence 2:218
  seasonality 2:218
  sequence comparisons 2:218
  transmission 2:218
Salterprovirus 5:418
  Euryarchaeota infections 5:413*t*
  'fusiform' viruses 2:300
Salterprovirus 5:413*t*
Sandfly 4:490
Sandfly fever virus(es) 4:490, 4:493, 1:391
  epidemiology 4:495
  infection
    aseptic meningitis 5:488
    clinical features 4:493
  isolation 4:491
  transmission 5:488
  zoonoses 5:488
  see also specific viruses
Sandjimba Group, animal rhabdoviruses 1:118
Sandjimba virus (SJAV) 1:118
San Miguel sea lion virus (SMSV) 1:410, 2:230
  geographical distribution 1:413–414
  structure 1:412*f*
S-antigen, Borna disease virus 1:341–342
Sap, pecluvirus transmission 4:98–99
Sapelovirus 2:126–127
Sapovirus 1:411, 1:411*t*, 2:117, 3:438–439, 3:439*t*, 3:440*f*, 3:441*t*
Sapovirus(es) **3:438–445**
  antigenic variation 1:415
  characteristics 2:117*t*
  classification 3:438–439, 3:440*f*, 3:441*t*
  composition 3:440
  evolution 3:442
    genetic recombination 3:442–443
  gene expression 3:440
  genome 3:440, 3:442*f*
    open reading frames 3:440
  history 1:411
  infection *see below*
  nonstructural proteins 1:412–413
  replication 3:441, 3:443*f*
  structure 3:440, 3:442*f*
    VP1 3:440
    VP2 3:440
    VPg 3:440
  taxonomy 3:439
  transmission 1:416, 3:443
  vaccines/vaccination 3:444
  see also specific viruses
Sapovirus infections 3:439*t*
  clinical features 3:443
    pediatric gastroenteritis 3:439
  complications 1:418
  control 1:418, 3:444
  diagnosis 3:444
  gastroenteritis, clinical signs 1:417
  host range 1:414–415, 3:443
  immune response 1:417–418, 3:444
    histo-blood groups, antigens 3:444
  pathogenicity 3:443
  prevention 3:444
Saquinavir 1:143*t*
Sarcolemma 1:533
SARS *see* Severe acute respiratory syndrome (SARS)
SARS-CoV RNA SSS Database 5:357*t*
Satellite(s)
  Arabis mosaic virus 3:411
  classification 5:11*t*
  definition 1:314, 2:97, 4:164, 4:526

Satellite DNAs **4:526–535**
  definition 4:526
  replication 4:533
    host DNA polymerase 4:533
  single-stranded DNA satellites 4:530
  see also β ssDNA satellite(s)
  structure 4:533
    sequence comparisons 4:533
  see also specific types
Satellite dsRNA, definition 5:299
Satellite-like RNA
  classification 4:529*t*
  definition 4:526
Satellite maize white line mosaic virus (SMWLMV) 4:527*t*
Satellite nucleic acids
  classification 4:527, 4:528*t*
  definition 4:526
  evolution 4:534
  geographical distribution 4:527
  historical aspects 4:527
  sequence expression 4:535
  sequence variation 4:534
  viruses, control of 4:535
  see also Satellite DNAs; Satellite RNAs
Satellite Panicum mosaic virus (SPMV) 4:527*t*
Satellite-related nucleic acids 4:529*t*, 4:532
  satellite-like ssRNAs 4:532
  virus-associated DNAs 4:529*t*, 4:533
  virus-associated nucleic acids 4:532
Satellite RNAs **4:526–535**
  definition 1:615, 4:526, 4:644
  double stranded 4:528*t*, 4:531
  replication 4:526, 4:533
    helper virus *see* Helper virus(es)
    host virus replication machinery 4:533
    noncoding RNA 4:533–534
    rolling-circle mechanism 4:534
  single stranded 4:531
    large 4:528*t*, 4:531
    small circular 4:528*t*, 4:532
    small linear 4:528*t*, 4:531
  structure 4:533
    in vitro models 4:534
  see also specific viruses
Satellite tobacco mosaic virus (STMV) 4:527*t*
  structure 5:381–384, 5:384*f*
Satellite viruses 4:480, **4:526–535**
  β ssDNA satellites *see* β ssDNA satellite(s)
  classification 4:527, 4:527*t*
  definition 3:385, 4:526
  evolution 4:534
  geographical distribution 4:527
  historical aspects 4:527
  host virus accumulation interference 4:530
  nontranslated regions 4:530
  particle structure 4:530
  sequence variation 4:534
    phylogenetic analysis 4:534
  structure 1:314–315
  viruses, control of 4:535
  see also specific viruses
Satellite virus-induced gene silencing (VIGS) *see* Virus-induced gene silencing (VIGS)
Satsuma dwarf virus (SDV) 4:524*t*
  genome sequencing 4:524–525
  geographical distribution 4:523
  host range 4:525
  transmission 4:525
  as type species 4:523
  vector control 4:525–526
'Savanna type' yellow fever transmission 5:473–474
Sawflies 1:225–226, 1:229–230, 1:230*f*
Sawfly baculovirus(es) **1:225–231**
  gene content 1:226
  gene parities 1:228
  genome 1:226
    auxiliary genes 1:228
    densovirus capsid protein 1:228
    DNA repeat regions 1:229
    immediate early proteins 1:228
    inhibitors of apoptosis 1:226
    membrane fusion protein 1:228
    open reading frames 1:226
    phosphotransferase 1:227
    regulator of chromosome condensation protein 1:228
    trypsin-like serine protease 1:226
    zinc finger protein 1:228
  infection 1:229–230
  phylogeny 1:228
  tissue tropism 1:229–230
Sawgrass Group, Rhabdoviruses, animal 1:116
SBV *see* Sacbrood virus (SBV)
Scab material, orf virus shedding 4:58, 4:63
Scaffold proteins
  bacteriophage assembly 1:438
  capsid formation in HCMV 2:487
  definition 1:433, 1:438, 3:13
  microviruses
    functions 3:15
    morphogenesis role 3:17–18
Scallion virus X (ScaVX) 4:311*t*
Scarlet fever, measles *vs.* 3:289–290
Schistosoma mansoni, ribozyme structure 4:478–479
Schizophrenia
  Borna disease virus associated 1:342–344
  Mason–Pfizer monkey virus 4:629
  viral etiology 1:475
Schlumbergera virus X (SCX) 4:311*t*
SCID mice, definition 4:382
Sclera 2:491
Sclerophthora macrospora virus A (SmV A) 2:289
Sclerophthora macrospora virus B (SmV B) 2:289
Sclerotinia sclerotiorum debilitation-associated, replicase 2:288
Scoliosis 1:76
Scoring schemes, alignment algorithms 5:342–343
Scotophilus temmencki, Keterah virus 1:400
Scrapie (PrP^{SC}) 1:474, 4:338, 5:187
  history 5:186
  occurrence 5:187–188
  prion function 4:333
  prion properties 5:186–187
  prion structure 4:333–334
  susceptibility 5:188
  transmission 5:187
  see also Bovine spongiform encephalopathy (BSE)
Scrophulariaceae virus infections 4:215
  see also specific viruses
ScV, giardiavirus *vs.* 2:315
ScV-L-A *see* Yeast L–A virus (ScV-L-A)
SDE3, antiviral defense 4:147, 4:147*f*
SDE5, virus-induced gene silencing function 5:326–327
SDI RNA *see* Defective interfering RNAs (DI RNA)
SDV *see* Satsuma dwarf virus (SDV)
Seadornavirus 1:533, 4:536, 4:536*t*, 4:540, 4:540*t*
Seadornavirus(es) **4:535–546**, 4:536*t*
  antigenic relationships 4:540
    amino acid-sequencing 4:541–542, 4:541*f*
    conserved sequences 4:542
    RdRp 4:542, 4:543*t*, 4:544*f*
    VP7 4:541–542
  capsid structure 4:538, 4:538*f*
    electron microscopy 4:539*f*
  definition 4:536
  distribution 4:537, 4:537*f*
  epidemiology 4:537
  genome 1:172, 4:538
    sequencing 4:539
  historical overview 4:536
  infections *see below*
  infectivity 4:538–539
  nomenclature 4:536
  particle purification 4:538
  phylogenetic relationships 4:540, 4:540*t*
  proteins 4:538
    electrophoresis 4:538*f*, 4:539
    electrophoretic profiles 4:538*f*, 4:539
    VP1 4:538
    VP2 4:538
    VP3 4:538
    VP4 4:538
    VP8 4:538
    VP9 4:538
  RdRp 4:542, 4:543*t*, 4:544*f*
  replication 4:538, 4:539
    mosquito cell lines 4:539
  transmission 4:537
    mosquito vector 4:537
  type species 4:536, 4:536*t*
  vectors 4:537
  virion properties 4:538
  see also specific viruses
Seadornavirus infections
  animal models 4:545
  clinical features 4:544
  diagnostic assays 4:544
    molecular assays 4:544–545
    serology 4:544–545
  host range 4:537
  immunity 4:546
  treatment 4:546
    vaccines 4:546
  vaccines/vaccination 4:546
Seagulls, Taura syndrome virus transmission 5:7
Sea louse *(Lepeophtheirus salmonis),* infectious salmon anemia virus vector 3:91
Seals
  canine distemper virus 4:498
  phocine distemper virus 4:498
Seasonal trends
  disease surveillance 2:48
  maintenance transmission 5:272
  subsidence 5:273
  see also specific viral infections
Sec61 complex, K28 virus toxin 5:304–305
Secondary infections
  measles 3:289
  nucleopolyhedrovirus 1:267
Secondary mutations, quasispecies 4:361–362
Second-generation vectors, plant viruses 4:232
Secretory cells, yellow fever virus assembly 5:471
Secretory component (SC), adaptive immunity role 3:61
Secular trends, disease surveillance 2:48
Seed(s), viral transmission 5:284–285
  alfalfa mosaic virus 1:86
  bean common mosaic virus 1:291, 1:293
  bean common necrosis virus 1:291
  control 5:285*t*, 5:286–287
  cucumber mosaic virus 1:618
  necrovirus 3:404
  nepovirus 3:411
  pecluvirus 4:98–99
  Pepino mosaic virus 4:108
  plum pox virus 4:241
  zucchini yellow mosaic virus 5:436
Seed coats, infection susceptibility 1:293
Seedling-yellow reaction, citrus tristeza virus infection 1:521
Seed potato production 4:297–302
  certification schemes 4:199
Seed programs (plants), infection control 1:295, 3:219
Segmented genomes
  double-stranded RNA virus replication 4:410
  single-stranded negative sense RNA virus replication 4:410
Segment nesting, Campoletis sonorensis ichnovirus 4:258–259
Selection coefficient 4:359
Selective activity index *see* Antiviral drug(s)
Self antigens 1:124
Selonopsis invicta virus-1 (SINV-1) 2:40
Semipersistent transmission 1:457, 1:483, 4:546
Semliki Forest virus (SFV) 5:90
  antigenic group 5:83
  characteristics 5:109*t*
  geographical distribution 5:84*f*, 5:90
  host protein synthesis shut-off 5:123
  replication 5:118
  zoonoses 5:491
Semotivirus 4:451

Semotivirus(es)
  classification 4:451
  distribution 4:452*f*, 4:453
  see also specific viruses
Sendai virus 4:54
  genome 3:327*f*
Sentinel studies, epidemiology 2:144
Seoul virus 1:391*t*
  associated disease 2:318, 2:319–320
Sequence(s) 1:296
  alignment, phylogenetic analysis 4:126
  alignment algorithms *see* Alignment algorithms (classification)
  analysis, arenavirus L polymerase 3:245
  Blueberry leaf mottle virus 3:408
  comparisons, pecluvirus genome 4:100
  Grapevine chrome mosaic virus 3:408
  icosahedral enveloped dsDNA bacteriophages 3:10
  nepovirus 3:408, 3:408*f*, 3:412
Sequence homology, Stp oncoproteins 4:589
Sequence polymorphisms, EBV 2:159
Sequence space 4:359
  definition 2:175
  viral evolution *see* Evolution (of viruses)
Sequestered cellular genes
  Herpesvirus Saimiri 4:589
  Human herpesvirus 8 4:589
  rhadinovirus 4:589
Sequiviridae 1:90*f*, 4:546–547, 4:547*t*, 5:11*t*
Sequivirus 4:547, 4:550
Sequivirus(es) **4:546–551**
  genome 4:548, 4:548*f*
  infection, tissue tropism 4:547
  isolate/strain variation 4:550
  phylogenetic relationships 4:550
    coat proteins 4:550
    NTP-binding domains 4:550
    RdRp 4:550
  protein properties 4:548
    proteases 4:548
    proteolytic processing 4:548
  taxonomy 4:547
  viral interactions 4:551
  virion properties 4:547
  see also specific viruses
Serine-like protease 2:343
Seroconversion, equine torovirus 5:155–156
Seroepidemiologic studies 2:144
Serological assay(s)
  applications 2:31
  clinical specimens used 2:31
  coltivirus infection 1:540
  definition 4:207, 4:514
  limitations 2:31
  principles 2:30, 2:31
    complement fixation tests 2:31
    hemagglutination inhibition tests 2:31
    immunoassays 2:32
    immunoblotting 2:33
    isotype-specific markers 2:30
    lateral flow tests 2:33
    latex tests 2:33
    neutralizing antibody tests 2:31
    point-of-care tests 2:33
  rubella virus infection 4:519
Serological differentiation index (SDI) 5:59
Serology
  avipoxviruses 2:279
  citrus psorosis virus 3:450
  disease surveillance 2:45, 2:46
  geminivirus molecular diagnosis 4:167
  Indian peanut clump virus 4:98–99
  Mason–Pfizer monkey virus 4:629
  ophiovirus classification 3:448
  orthobunyavirus infection diagnosis 3:482–483
  peanut clump virus 4:98–99
  pecluvirus infections 4:99
  plum pox virus strains/groups 4:241
  potato virus Y *see* Potato virus Y (PVY)
  rhinovirus detection 4:471
  rhinoviruses 4:468, 4:468*t*, 4:471
  seadornavirus infections 4:544–545
  Simian retrovirus D 4:629–630, 4:629*f*
Seroprevalence
  epidemiology 2:142
  simian alphaherpesviruses infection 4:582, 4:584
Serosal hemorrhage, definition 1:328
Serotonin receptor 2A ($5HT_{2A}$), JCV cell attachment 4:265, 4:280
Serotypes, definition 4:507
Serp1 protein 3:227
Serpentine chemokine receptor(s) 2:276
Seryl–threonyl protein kinase 5:180
Sesbania mosaic virus (SeMV) 4:646*t*, 4:649, 4:650*f*
Severe acute respiratory syndrome (SARS) 1:549, 1:553, 2:94, **4:552–560**
  animal models 4:558
  cellular effects 3:429
  classification 4:553–554
  clinical features 2:555, 4:557
    children 4:557
    extrapulmonary manifestations 4:557
  diagnosis 2:17, 4:558
    sero-conversion 4:558
    viral load 4:558
  disease surveillance 2:50, 4:552
    data collection sources 2:50
  epidemic 1:555, 2:96, 4:552, 4:553*t*
  geographical distribution 5:492
  history 4:552
  host range 1:553, 2:94
    zoonoses 1:555, 4:555, 5:492
  immune response 4:559
  incubation period 4:557
  mortality rate 4:552, 4:557, 5:492
  organ transplantation 3:470
    diagnosis 3:470
  pathogenesis 4:558
  pathology 4:557–558
  re-emergence 4:559
  treatment 4:558
    antiviral drugs 4:558
    antiviral therapy 4:558
    neutralizing monoclonal antibodies 1:561
    virus targets 1:561–562, 1:561*f*
  virus–host associations 4:128
Severe acute respiratory syndrome coronavirus (SARS CoV) 2:555, 4:553
  ecology 4:555
  endolysins, definition 3:249
  genome 3:426*f*, 4:554, 4:554*f*
    accessory protein production 4:555
    open reading frames 4:554
    polyprotein production 4:554–555
  historical aspects, classification 4:128
  holin–endolysin lysis 3:254
    membrane depolarization 3:255
    RI protein 3:255
  host cell binding, angiotensin converting enzyme 2 4:555–557
  infection *see* Severe acute respiratory syndrome (SARS)
  origin 5:492
  phylogeny 4:555, 4:556*f*
  receptors 4:555
  reverse genetics 1:560–561
  structure 4:553–554
  transmission 4:557
    aerosol transmission 4:557
    host factors 4:557
    interspecies 4:560
    zoonosis 5:492
  Urbani strain 4:559
  vaccines/vaccination 1:561, 4:559, 5:237
    passive immunization 4:559
    timescale 5:237
  virulence, protein 6 1:559–560
Sexually transmitted disease(s)
  HCMV infection 2:482
  HPV infection 4:19, 4:38
  HTLV-1 2:559
  see also Venereal transmission; *specific infections*
SFV E1, membrane fusion 3:297, 3:298*f*, 3:299
SGV strain *see* Barley yellow dwarf virus (BYDV)
SH1 virus 5:413*t*, 5:420
  genome 5:421, 5:422*f*
  stability 5:420–421
  structure 5:420, 5:422*f*
SH3BGRL protein 4:418
Shallot latent virus (SLV) 5:288
Shallot virus X (ShVX) 1:96
Sharka disease 4:200, 4:201, 4:202*f*, 4:204, 4:321
  control 4:241
    attenuated virus cross-protection 4:242
    hypersensitive response development 4:242
    plant breeding programs 4:242
    quarantine measures 4:241
    tolerant varieties 4:241
    transgenic plants 4:242
  distribution 4:239
  ecology 4:241
  economic importance 4:238
    crop yield losses 4:201
    stone fruit crops 4:238
  host range 4:238
    Prunus 4:238
  mixed infections 4:239
    prune dwarf virus 4:239
    prunus necrotic ringspot virus 4:239
  symptomatology 4:238
    affecting factors 4:238–239
    apricots 4:239
    cherries 4:239
    foliage 4:238
    fruit drop 4:239
    ornamental trees 4:239
    peach 4:239
    plum 4:239
Shedding 2:144
  animal viruses 5:316
  persistent viruses 5:316–317
Sheep
  astrovirus pathogenesis model 1:208
  capripoxvirus transmission 1:429
  Jaagsiekte sheep retrovirus infection *see* Jaagsiekte sheep retrovirus (JSRV)
  louping ill virus infection 2:240–241
  ovine pulmonary adenocarcinoma *see* Ovine pulmonary adenocarcinoma (OPA)
  parapoxvirus infection *see* Parapoxvirus(es) (PPVs)
  prion transmission 4:338
  see also under Ovine
Sheeppox 1:427
  clinical features 1:430*f*, 1:431*f*
  epidemiology 1:427, 1:429
  epizootiology 1:429
  eradication of 1:428
Sheeppox virus (SPPV)
  geographical distribution 4:329
  infection *see above*
  transmission 4:329
Sheep red cells, complement fixation tests 2:31
Shellfish
  cultivation 4:561
  definition 4:561
Shellfish virus(es) **4:560–567**
  birnaviruses 4:566
  herpesviruses 4:563
    diagnosis 4:565
    epidemiological surveys 4:565
    epizootiology 4:563
    evolution 4:565
    genome 4:563
    hosts 4:563
    infections 4:563
    locations 4:563
    taxonomy 4:565
    vertebrate viruses *vs.* 4:563–564
    virus ultrastructure 4:563
  historical aspects 4:561
  information lack 4:561
  irido-like viruses 4:561
    epizootiology 4:561

histopathology 4:562
hosts 4:561
infections 4:561
locations 4:561
morphology 4:562*f*
see also Gill necrosis disease
nonspecific viral infections 4:561
papova-like viruses 4:566
picorna-like viruses 4:565
reo-like viruses 4:566
Shine–Dalgarno sequence 3:220
Shingles *see* Varicella-zoster virus (VZV)
Shoot apex, maize streak virus 3:270
Shope fibroma virus (SFV) infection
cottontail rabbit 3:225
immune response 3:229–230
pathogenicity 3:228
tumor development 3:228
Short intergenic region (SIR)
definition 3:264
maize streak virus *see* Maize streak virus (MSV)
Short interspersed repetitive elements (SINEs) 4:439
definition 4:437
distribution 4:442*t*
genes, effects on 4:444
origins 4:441
retrotransposons 4:429–430
Short inverted terminal repeats, bacteriophages 4:402–403
Short tails, icosahedral tailed dsDNA bacteriophages 3:31, 5:367, 5:368
Shrimp baculovirus(es) 4:569
characteristics 4:570
morphology 4:570
occlusion bodies 4:570
geographical distribution 4:571
host range 4:571
infection cycle 4:571
polyhedra 4:570, 4:570*f*
Shrimps, penaeid
definition 2:37
infection *see* Shrimp virus(es)
Shrimp virus(es) **4:567–576**, 4:568*t*
baculoviruses *see* Shrimp baculovirus(es)
disease control 4:575
disease emergence, factors associated 4:569
farmed shrimp production, impacts 4:569
annual production losses 4:569
history 4:567
infectious myonecrosis virus infection *see* Infectious myonecrosis virus (IMNV)
Taura syndrome disease *see* Taura syndrome disease (TSD)
white spot syndrome virus *see* White spot syndrome virus;
see also specific viruses
Shuster, Peter, quasispecies 2:177–178
Siadenovirus 1:1, 1:2*t*, 1:17
Siadenovirus(es) 1:1, 1:8
evolutionary history 1:8
genome 1:8, 1:18
E3 gene 1:8
host switches 1:5, 1:8–9
see also specific viruses
Sialic acid
BK viruses 4:280–281
influenza viruses 1:149
paramyxovirus–host cell binding 4:53–54
porcine respiratory and reproductive syndrome 1:181
α-2,3-Sialic acid
BK virus-host cell binding 4:280–281
influenza virus-host cell binding 3:486
α-2,6-Sialic acid
associated viruses 5:320
influenza virus-host cell binding 3:102
JCV cell attachment 4:265, 4:280
receptor role 5:320
structure 5:320
Sialic acid-independent mechanisms (SLAM), henipavirus cell attachment 2:322–323
Sialoadhesin 1:181
Sibine fusca, biological control 3:128
Sibine fusca densovirus. (SfDNV) 4:79
Sickness absence, disease surveillance 2:46
Sida yellow mosaic virus 1:318–319, 1:319*f*
Sieve-tube cells 1:296
σ1 protein
function 4:387
outer capsid 4:396
σ2 protein, inner capsid 4:393*f*, 4:394
σ3 protein, outer capsid 4:396–398
σ^{70} 5:175
bacteriophage λ 5:185
bacteriophage T4 5:177
AsiA binding 5:177
σ-factor(s) 5:175
bacteriophage λ 4:400–401
definition 4:400
see also specific factors
Sigma virus (SIGV) 1:118, **4:576–581**
classification 4:576
phylogenetic tree 4:576, 4:577*f*
definition 4:576
discovery 4:576
L'He´ritie'r, P 4:576
Tessier, G 4:576
genome 4:576, 4:577*f*
infection *see below*
phylogenetic trees 4:577*f*
replication 4:579
early mutants 4:579
heat-shock experiments 4:579
late phase mutants 4:579
middle-stage mutants 4:579
ts mutants 4:579
transmission 4:576, 4:578
efficiency 4:579
females 4:579
males 4:579
vertical 4:578
virion structure 4:576, 4:577*f*
M protein 4:576–577
P protein 4:576–577
Sigma virus infection
CO_2 sensitivity 4:576, 4:578
specificity 4:578
thoracic ganglia invasion 4:578
detection 4:578
host immunity 4:579
ref genes/proteins 4:579–580, 4:580*f*
host range 4:578
Sigmodontinae 2:93
Signaling lymphocyte activation molecule (SLAM), cell entry
canine distemper virus 3:285
measles virus 3:285, 3:288
morbillivirus 3:285
rinderpest virus 3:285
Signal peptide (SP), arenavirus glycoproteins 3:244
Signal transducers and activators of transcription (STAT), henipavirus 2:326
Silencing-suppressor proteins
definition 5:145
resistance engineering in plants 4:161
Simian adenovirus(es)
pathogenesis 1:26
see also specific viruses
Simian agent 8 (SA8) 4:582–583
Simian alphaherpesvirus(es) **4:581–585**
classification 4:583, 4:583*t*
epidemiology 4:584
evolution 4:583
history 4:581
infection 4:584
cross-reactivity 4:582–583
cytopathic effects 4:582*f*, 4:584
immune response 4:584
reactivation 4:584
seroprevalence 4:582, 4:584
physical properties 4:584
replication 4:584
transcription 4:584
translation 4:584
transmission 4:584
see also specific viruses
Simian cytomegalovirus(es) **1:634–642**
classification 1:635
epidemiology 1:638
evolution 1:635
gene expression control 1:640
genome 1:636
infection, persistent 1:638
physical properties 1:638
replication strategies 1:638
virion structure 1:636
see also specific viruses
Simian enteroviruses (SEV) 2:124
A-2 plaque virus 2:124
genome 2:127
see also specific viruses
Simian foamy virus (SFV)
genetic stability 2:179
human infection 2:532
Simian hemorrhagic fever virus (SHFV) 1:177
genome 1:179–180
geographical distribution 1:177
host range 1:177
infection
clinical features 1:183
immune response 1:185
pathogenicity 1:183
Simian immunodeficiency virus (SIV) **4:603–611**
agm 4:621
infectivity 4:621
classification 2:526, 2:527*t*, 4:603, 4:604*t*, 4:612, 4:613*t*
host dependent 2:526
serology 2:526
coreceptor usage 4:618
cpz (chimpanzee) 2:528, 2:530*f*, 2:533
CCR5 receptor expression 4:620
cross-species transmission 2:533, 4:611, 4:612
evolutionary history 2:528
geographical foci 4:618
hunting 2:533
origin 2:530*f*, 2:533
subspecies identification 2:528
vertical transmission 2:528–529
cross-species transmission 4:618
bushmeat 2:532–533, 4:604
risks 2:532
SIVagm 4:618
SIVcpz origin 2:533
SIVsmm 4:611
definition 2:534
discovery 4:594
epidemiology 4:608
evolution 4:596, 4:605*f*, 4:607
primate hosts 2:533
genetics 4:607
diversity 4:607
DNA synthesis 4:607
genome composition 4:617
genome 4:595, 4:605*f*, 4:617
accessory genes 4:607, 4:617
auxiliary genes 4:606*t*, 4:607
open reading frames 4:607
patterns 4:617, 4:617*f*
recombination 4:596, 4:617
structural genes 4:607, 4:617
geographical distribution 4:604
gor (gorilla) 2:530*f*
history 4:603, 4:604*t*
infection *see below*
mac (macaque)
evolution 2:527–528
history 4:594, 4:612
nef gene 4:622
origin 4:596
molecular biology 4:597
host cell receptors 4:598, 4:618
life cycle 4:597–598
propagation 4:598

Simian immunodeficiency virus (SIV) (*continued*)
nomenclature 4:594, 4:597*f*
origin 4:595*f*
phylogenetic relationships 4:612, 4:615*t*, 4:616*f*
propagation 4:598, 4:606, 4:618, 4:619*t*
receptors associated 4:605*f*, 4:606, 4:606*t*
co-receptors 4:606–607
replication
natural host 4:620, 4:621*f*
replicative capacity 4:599
sites 4:608
serologic relationships 4:607
Simian retrovirus D *vs.* 4:628
spike protein structure 1:609–610, 1:610*f*
taxonomy 4:594, 4:603, 4:612
transmission 4:599, 4:608, 4:620
blood 4:601
cross-species *see above*
experimental 4:601–602
interspecies 4:595*f*, 4:596–597, 4:597*f*
mother–infant 4:602
sexual 4:601
variants 4:600
virulence 4:622
Simian immunodeficiency virus infection **4:611–623**
African green monkeys
immune response 4:622
infectivity 4:621
animal models **4:594–603**
clinical features 4:601
dissemination 4:599
histopathology 4:601
immune response 4:600
pathogenesis 4:599
pathology 4:601
vaccine testing 4:602
blood–brain barrier 4:600
CCR5 receptor
host-cell interactions 4:606–607, 4:618
receptor expression 4:620
natural hosts 4:620
CD4+ T cells 4:599–600, 4:603–604
host cell attachment 4:598, 4:603–604, 4:618
receptor expression, natural hosts 4:620
CD8+ T cells 4:600–601
cell tropism 4:608–609, 4:620
clinical features 4:601, 4:608, 4:622
control 4:610
diagnosis 4:618
antibody detection 4:618
cell culture, assay 4:618
propagation 4:618
dissemination 4:599, 4:599*f*
dual/multiple infections 4:597
epidemiology 4:618
histopathology 4:601
history 4:612
HIV infection animal model 4:601
HIV *vs.* 4:601
host range 2:525–526, 2:536–537, 4:600, 4:604, 4:618
AIDS resistance 2:537–538
genetic resistance 4:622
immune response 4:600, 4:609, 4:610*f*, 4:620
antibodies 4:620–621
CD4+ T-cell depletion 4:620
CD8+ lymphocytes 4:609
hypergammaglobulinemia 4:601
IL-7 role 4:620
immunoglobulin G 4:600–601
natural host 4:620
T-cell regenerative capacity 4:622
lymphadenopathy 4:601
lymphocytosis 4:601
lymphoid tissues 4:601
lymphomas 4:601
major histocompatibility complex 4:600–601
opportunistic infections 4:608–609
pathogenesis 2:536–537, 2:537*t*, 4:599, 4:620, 4:621*f*
pathology 4:601, 4:620, 4:622
persistence 4:609, 4:620
natural host 4:620
prevalence 4:618
prevention 4:610
vaccines/vaccination 4:610–611
rhesus macaques 4:612
clinical presentation 4:608, 4:622
history 4:603
virulence 4:622
terminal stages 4:608–609
tissue tropism 4:620
transmission 4:618
viral load 4:608–609
Simian retrovirus D **4:623–630**
capsid structure 4:626*f*, 4:627
cell receptor 4:627
classification 4:624
env gene/protein 4:627
gag gene/protein 4:625
genome 4:624*f*
long terminal repeats 4:625
historical aspects 4:624
discovery 4:625
human infections 4:629
breast cancer association 4:625, 4:629
Burkitt's lymphoma 4:629
serological surveys 4:629–630, 4:629*f*
tissue culture 4:629
in vitro cell tropism 4:628
in vivo cell tropism 4:626*f*, 4:628
natural hosts 4:627
pathogenesis 4:628
replication 4:625
constitutive transport element 4:625
env gene/protein 4:627
gag gene/protein 4:625
integrase 4:627
particles 4:626*f*
pol gene/protein 4:627
pro gene/protein 4:624*f*, 4:625
reverse transcriptase-endonuclease 4:627
ribosomal frameshifts 4:627
spike protein 4:627
surface membrane receptor 4:627
transmembrane protein 4:627
serotypes 4:624
simian immunodeficiency virus *vs.* 4:628
transmission 4:624, 4:627
carriers 4:627–628
physical contact 4:627–628
vaccines 4:629
killed-virus 4:629
recombinant vaccinia virus 4:629
Simian T-cell leukemia virus(es) (STLV), isolation 2:558
Simian T-cell leukemia virus III (STLV-III) 4:603
Simian varicella virus (SVV) 4:584, 5:251
Simian virus 5 (SV5)
isolation 3:356
SH protein 4:55
Simian virus 40 (SV40) **4:630–638**
classification 4:631
distribution 4:636
DNA recombination mechanisms 4:375–376
epidemiology 4:636
genetics 4:636
restriction map 4:632, 4:632*f*
strain origins 4:637*t*
genome 4:631, 4:632*f*
history 4:630
host range 4:636
infection
agnoprotein LP1 4:634
carcinogenesis 4:631, 4:636, 4:637–638
immune response 4:638
pathogenicity 4:637
pathology 4:637
prevention and control 4:638
microRNA 3:152–153, 5:327
oligodendrocyte infection 1:473
poliovirus vaccine 4:630, 4:637
safety events 5:232
propagation 4:636
proteins 4:633
capsid 4:634
T-antigens 4:633, 4:634–635
receptors 4:279
replication 4:634
cytoplasmic entry 4:635
MHC class I receptor 4:633
microRNA 4:635
nucleic acid replication 4:635
post-translational processing 4:635
time course 4:634–635
transcription 4:635
virion uptake and release 4:635
serology 4:636, 4:637, 4:638
structure 5:382*f*, 5:388, 5:391
T-antigens
cellular protein targets 4:633–634
functional domains 4:634*f*
properties/functions 4:633, 4:633*t*
replication role 4:634–635
transcription role 4:635
variable domain 4:634
taxonomy 4:631
tissue tropism 4:637
transmission 4:637
virion properties 4:631, 4:631*t*
DNA packaging 4:631, 4:632
maturation 4:636
stability 4:631
Simple follicular conjunctivitis, adenovirus infection 2:495
Simplexvirus 2:432*t*
Sindbis virus (SINV) 5:90
antigenic group 5:83
characteristics 1:171*t*
genetic lineages 5:90
geographical distribution 5:90
glycoproteins, apoptosis 5:122
host range 5:90
infection *see below*
replication 5:118
high-affinity laminin receptor 5:118
structure 5:118*f*
transcriptional downregulation 5:122
transmission 5:90
as type species 5:91–92
Sindbis virus infection
apoptosis 5:122
cellular responses 5:122
IFN-mediated antiviral response 5:123–124
clinical features 5:86*t*, 5:87*t*
disease characterization 5:487
epidemics 5:90
zoonoses 5:487
strain mixing 5:487
SINE retrotransposons
mechanism of action 4:440
structure 4:440
Singapore grouper iridovirus (SGIV) 2:233
Single-dose vials, vaccine safety 5:229
'Single-gene lysis'
definition 3:249
see also Bacteriophage(s)
Single internal deletions, defective-interfering RNAs 4:380
Single nucleocapsid per envelope (SNPVs), nucleopolyhedrovirus 1:265, 1:266*f*
Single-step growth
bacteriophage organismal ecology 2:73
definition 2:71
Single-stranded conformation polymorphism (SSCP) pattern 4:106
Single-stranded DNA satellites *see* β ssDNA satellite(s)
Single-stranded DNA virus(es)
algal viruses 1:92
classification 5:11*t*
filamentous *see* Filamentous ssDNA bacteriophages

genome packaging 2:306, 2:307*t*, 2:312*f*
P4 enzyme 2:311–312
translocating NTPase 2:311–312
replication 4:410
Single-stranded genome 1:483
Single-stranded RNA bacteriophages
genome 1:439, 2:306, 2:311, 4:405
external protein components 2:306
replication 4:405
replicase composition 4:405–406
Single-stranded RNA virus(es)
algal viruses 1:87
fungal viruses 2:288
negative sense
classification 5:11*t*
replication 4:410
positive sense
classification 5:11*t*
replication 4:410
replication 4:410
see also specific viruses
Sin Nombre virus 1:391*t*
detection 2:93–94
transmission 2:95
SINV *see* Sindbis virus (SINV)
Siphoviridae 3:30–31, 5:11*t*, 5:181, 5:413*t*
Siphovirus(es)
DNA injection 3:33–34
Euryarchaeota infections 5:413*t*
genome packaging 2:306, 2:307*t*
protein cross-linking 1:439
structure 3:31
tail shaft assembly 1:438
see also specific viruses
SIRE1 virus, *env*-like gene 4:355
Sirevirus 4:352, 4:353*t*
Sirevirus(es) 4:431*t*, 4:433
genome 4:354
see also specific viruses
siRNA *see* Small-interfering RNAs (siRNAs)
Site-specific coding strand nicks, edornaviruses 2:110*f*, 2:113
Sitiawan virus 2:237
SIV *see* Simian immunodeficiency virus (SIV)
Sixth disease 2:498
Skin
tumors, fish 2:213
viral infection 5:315
barrier against 5:315
papillomaviruses 4:27–28, 4:30*f*
see also Epithelium
Skin lesions
adult T-cell leukemia 2:568
clinical features 2:568, 2:568*f*
cowpox infections 1:579
Ectromelia virus infection lesions 3:344
Ski proteins, yeast L–A virus 5:468
SLAM protein, virus-host cell binding 5:324
morbillivirus replication 4:44, 4:500
Sleeping disease virus (SDV) 5:93*t*, 5:94
characteristics 5:109*t*
classification 5:95
genotypes/genotyping 5:95
genotyping 5:95
glycoproteins 5:95
historical aspects 5:94
isolation 5:95
Sleep pattern disruption, common cold 4:472
Sliding back mechanism, definition 4:399
Slippery sequence, definition 5:477
slyD gene 3:257
Small-angle X-ray scattering (SAXS) 5:34–35
Small basic protein (VPg), cowpea mosaic virus 1:570
Small-interfering RNAs (siRNAs) 4:141, 5:326, 5:376
Arabidopsis thaliana gene silencing 4:145–146
definition 3:149, 5:325
engineered resistance in plants 4:161
mammalian cells 3:153
production 3:149
RNA silencing role 5:376
signal amplification 3:151
viroid pathogenicity 5:341
Smallpox
Ab-mediated immunity 3:56
antiviral drugs 5:249
bioterrorism 5:407, 5:408
clinical features 4:639
eye infections 2:496
flat-type 4:640
hemorrhagic 4:640
papules 4:639–640
rash 4:639–640
vesicles 4:639–640
deaths 1:574
eradication 2:458, 4:640, 5:244
World Health Organization 4:640
historical aspects 4:639, 5:244
Jenner, Edward 4:639, 4:640
variola minor 4:639
human monkeypox virus infections *vs.* 4:641
mortality rate 4:329
pathogenesis 4:329
transmission 4:640
vaccines/vaccination 1:574, 1:579, 2:458, 4:640, 5:244
B5 antigen 5:249–250
complications 4:640, 5:244
cowpox 4:640
horsepox 4:640
immune response 5:249
variolation 4:640
disease impact 4:640
Small ruminant lentiviruses (SRLV) 5:424
Small T, murine polyomavirus *see* Polyomavirus(es)
Smear test(s) 4:16
Smelt aquareovirus (SRV) 1:164*t*
Smith, Kenneth Manley, potato viruses 2:451
Smithiantha latent virus (SmiLV) 4:311*t*
Smith–Waterman algorithms (classification) 5:343
SMK toxin, Ustilago maydis viruses 5:215–216, 5:216*f*
Smoldering adult T-cell leukemia 2:561
Smolts, definition 3:83, 3:90, 5:91
SMSV *see* San Miguel sea lion virus (SMSV)
Smut killer toxins 2:285
Snakehead reovirus (SKRV) 1:164*t*
Snakehead retrovirus (SnRV) 2:216, 2:231
genome 2:217, 2:217*f*
open reading frames 2:217
nucleotide sequence 2:231
phylogeny 2:219, 2:220*f*
Snakehead rhabdovirus (SHRV) 2:221–222, 2:228, 4:184*t*
reverse genetic analysis 2:228
Snow, John, epidemiology 2:141
Snowshoe hare (SSH) virus 5:489
classification 3:480
Sobemovirus 1:488, 4:645
Sobemovirus(es) **4:644–652**
classification 4:645, 4:646*t*
genome 4:645
open reading frames 4:645, 4:648*f*
satRNA 4:645
sgRNA 4:645
geographical distribution 4:646*t*
host range 4:645
infection
economic importance 4:650
pathology 4:650
resistance 4:179*t*, 4:650
symptoms 4:650–651
movement within host 4:649
tissues associated 4:649
phylogenetic relationships 4:651*f*, 4:652
protein functions 4:647
P1 4:647
polyprotein 4:648
serine protease 4:648
VPg 4:648
replication 4:645
mutation rates 4:647
RdRp expression 4:645, 4:648
satRNA 4:647
translation 4:645
satellite viruses 4:527*t*
stability 4:649
subcellular localization 4:649
transmission 4:645, 4:646*t*
virion structure 4:649, 4:650*f*
capsid 4:649
coat protein 4:649
see also specific viruses
Social change, zoonoses 5:494
Socioeconomic status, vector transmission, animal viruses 5:273
Sodium silicotungstate, negative staining 2:79, 2:80*f*
Soft tick vector *(Ornithodoros moubata),* African swine fever virus host 1:43, 1:44*f*
Soil
maize chlorotic mottle virus transmission 3:262
necrovirus transmission 3:404
rice stripe necrosis virus 1:309
sterilization
infection control measures 5:286–287
lettuce ring necrosis virus control 3:453
mirafiori lettuce virus control 3:453
tombusvirus transmission 5:149–150
Soil-borne cereal mosaic virus (SBCMV) 1:493, 1:494*f*
geographical distribution 1:493
infection, control 1:493
Soil-borne wheat mosaic virus (SBWMV) 1:308, 1:493, 2:291–292
genome 2:293
geographical distribution 1:493
infection 2:292
resistance 1:493
movement, via xylem 2:292
properties
antigenic 2:293
structure 2:293*f*
strains 2:292
transmission 1:309
zoospores 2:292
Soil nematodes
Grapevine fanleaf virus transmission 3:411
nepovirus transmission 3:411
tobacco ringspot virus transmission 3:411
tomato ringspot virus transmission 3:411
Solanaceae
Tnt1 elements 4:432
virus infections 4:211
see also Potato *(Solanum)*; Tomato *(Solanum esculentum)*
Solanum apical leaf curl virus (SALCV) 4:298*t*
Solanum nodiflorum mottle virus (SNMoV) 4:646*t*
Solenopsis invicta virus-1 (SINV-1) 2:40
Solid organ transplantation, human herpesvirus infection 2:498–499, 2:502
Solid-phase immunoassay(s) 2:32, 2:32*f*
Soluble complement-fixing antigen, Japanese encephalitis virus 3:183
Sonchus yellow net virus (SYNV) 4:184*t*
genome 3:326*f*
glycoprotein function 4:192–193
nucleocapsid protein expression 4:190, 4:191*f*
phosphoprotein function 4:190
complex formation 4:190–191
importin binding 4:191
nuclear export signals 4:191
phosphorylation sites 4:190
polymerase protein 4:193
Sooty mangabeys, HIV-2/SIVsmm evolution 2:527, 4:604–606
Sorghum chlorotic spot virus (SrCSV) 2:292
Sour orange rootstock, citrus tristeza virus pandemic 1:520
South African cassava mosaic virus (SACMV) 1:31–32
distribution 1:32*f*
South America, ophiovirus 3:450–451
Southern Asia, cotton leaf curl disease 1:563

Southern bean mosaic virus (SBMV) 4:646*t*
Southern blotting, avipoxviruses 2:280
Southern cowpea mosaic virus (SCPMV) 4:646*t*, 4:649, 4:650*f*
  P1 protein 4:647
Southern elephant seal virus (SESV) 5:92, 5:93*t*
  characteristics 5:109*t*
  genetics 5:92–94
  isolation 5:92–94
  tick transmission 5:92–94
Soviet Union, bioweapons 5:408
Sowbane mosaic virus (SoMV) 4:646*t*
Sowthistle yellow vein virus 4:195–196
Soybean *(Glycine max)*
  bean golden mosaic virus infection 3:217
  infection resistance 4:175
    I gene 4:175
  vaccine production 5:223–224
  see also specific viruses
Soybean mosaic virus (SMV)
  epidemiology 3:217
  pathogenicity 4:320
  resistance evasion 4:175
  transmission 3:214
    seeds 3:215–216
Soymovirus
  open reading frames 1:466*f*
  transmission 1:463
  virion structure 1:458–460
Soymovirus 1:459*t*
Spanin, definition 3:249, 3:250
Spanish influenza pandemic (1918-1919) 3:100, 5:491
  bioterrorism 3:99
  mortality rate 3:100
  virus pathogenicity 3:99
Spatial structure, definition 2:71
Spawner mortality virus (SMV) 4:571
SPCFV *see* Sweetpotato chlorotic fleck virus (SPCFV)
SPCSV *see* Sweetpotato chlorotic stunt virus (SPCSV)
SPDV *see* Salmon pancreas disease virus (SPDV)
Special AT-rich binding protein 1 (SATB1) 3:340
Species barriers
  alcelaphine herpesvirus 1 2:428
  prion variants and 4:338
Species definition *see* Classification
Species jumping
  definition 2:141
  viral evolution 2:175
Specific pathogen free (SPF) lines
  definition 5:1
  Taura syndrome virus 5:3
Spectroscopy, inovirus structure 3:120–121
SPFMV *see* Sweetpotato feathery mottle virus (SPFMV)
Spherical halovirus SH1 5:420
  genome 5:421, 5:422*f*
  stability 5:420–421
  structure 5:420, 5:422*f*
Spherical virus(es) 1:202
  β-barrel fold 1:202, 1:202*f*
    drug-induced modifications 1:204
    evolution 1:203
  capsid protein structure 1:202
    β-barrel folding motif *see above*
  Crenarchaeal virus morphotypes 1:590
  see also specific viruses
Spheroidin 2:137
Spheroplast, definition 5:299
Spherules, Brome mosaic virus replication in yeast 1:383
Spike complexes, icosahedral ssDNA bacteriophages 5:368–369
Spike (S) proteins
  coronavirus structure 1:556–557
    conformational change 1:557
    therapeutic developments 1:557
  icosahedral enveloped dsDNA bacteriophages 5:369
  Simian retrovirus D 4:627
  toroviruses 5:153, 5:154
Spill-back events, definition 2:221
Spill-over events, definition 2:221
Spinach curly top virus (SCTV) 1:302
  geographical distribution 1:302–303
  host range 1:306
  replication 1:304
Spinal leptomeninges, inflammation 5:42
Spindle-shaped sulfolobus virus(es) (SSVs), morphology 1:587, 1:589*f*
Spindle-shaped sulfolobus virus 1 (SSV1)
  replication induction 1:592–593
  transcription 1:593
    open reading frames 1:593–594
    promoters 1:594
Spindle-shaped virus(es)
  Crenarchaeal viruses 1:587
  see also specific viruses
SpLCV *see* Sweetpotato leaf curl virus (SpLCV)
Spleen lesions, Ectromelia virus infection lesions 3:345
Splenomegaly
  definition 1:410
  lactate dehydrogenase-elevating virus infection 1:184
  MHV-68 infection 3:375, 3:377
  SIV infection 4:601
Splice acceptor (SA) 3:335–336
Splice donor (SD) 3:335–336
Splicing
  adenoviruses 1:3
  influenza virus 3:487
Spodoptera frugiperda, insect cell culture 1:238
Sporadic Creutzfeldt–Jakob disease (sCJD) 1:371
Sporadic inclusion-myositis (sIBM) 2:571*f*, 2:573
Spore balls 4:282
Spores, asexual, partitivirus transmission 4:69
SPP1 bacteriophage *see* Bacteriophage SPP1
SPPV *see* Goatpox virus (SPPV)
Spraing (potato disease) 4:282, 4:307, 4:307*f*
Spring viremia of carp virus (SVCV) 2:224–225, 2:228, 4:184*t*
  phylogeny 2:223*f*, 2:224–225
Sprot, definition 4:428
S protein sequence, hepatitis B virus subtypes 2:354–355
S-PTGS, post-transcriptional gene silencing 4:142–145
Spumaretrovirinae 2:231, 2:260*t*, 4:460, 5:11*t*
Spumavirus 4:460
Spumavirus(es) 4:460
  classification 4:460
  gag protein 4:464
  gene therapy vectors 2:302–303
  replication characteristics 4:460
  see also specific viruses
Squamous cell carcinoma (SCC)
  HSV-2 associated 2:394
  human papilloma virus infection 2:492*f*, 2:497
Squamous intraepithelial lesions (SIL), human papillomavirus infection 4:16
Squash leaf curl virus (SLCV)
  ELISA diagnosis 1:299
  identification/naming 1:298
Squash plants
  genetically-modified resistant plants 4:158*f*
    CP genes 4:157–160
    gene flow 4:162
  viral disease control 4:157, 4:158*f*
  see also specific viruses
Squirrel fibroma virus (SqFV) 3:225–226
  characteristics 3:225*t*
  infection 3:229–230
Squirrel monkey lymphocryptovirus (SscLHV1) 4:587*t*
src family tyrosine kinases
  murine polyomavirus 4:274
  phosphorylation, Tio oncoprotein 4:590
  Tip oncoprotein homology 4:589–590
Sri Lankan cassava mosaic virus (SLCMV) 1:31–32
  DNA satellites 4:530–531
S RNA segment sequence, Crimean-Congo hemorrhagic fever virus 1:599, 1:600–601
SRO5 gene silencing 4:142
SSB protein, bacteriophage ϕX174 replication 4:405
SSSV *see* Salmon swimbladder sarcoma-associated retrovirus (SSSV)
SSV-1
  detection 2:297, 2:297*f*
  fuselloviruses 2:297
  genome sequence 2:297, 2:298*f*
SSV-2, fuselloviruses 2:298
SSV-3, fuselloviruses 2:298
SSV-K1, fuselloviruses 2:298
SSV-RH, fuselloviruses 2:298
ST-246, antiviral function 1:149
Stable transformation, murine polyomavirus infection 4:273
Stalk hypothesis, membrane fusion 3:292
Stalk-pore pathway, virus fusion 5:310–311, 5:311*f*, 5:313*f*
Standard Operating Procedures (SOPs), database annotation 5:353
Stanley, Wendel 5:55
  tobacco mosaic virus 2:452, 3:399
Stavudine 2:510
Stealth virus 1:636
  definition 1:634
Stegomya, Dengue virus transmission 2:7
Stem cell(s), HSV infection 2:388
Stem pitting 4:204
  citrus tristeza virus infection 1:521
Stem swellings, cacao swollen shoot virus infection 1:403
Stiffsiekte *see* Bovine ephemeral fever virus (BEFV)
St Louis encephalitis virus
  transmission
    age 5:273
    mosquito-borne 2:236
    occupation 5:273
  as zoonosis 5:270, 5:270*f*
STMV *see* Satellite tobacco mosaic virus (STMV)
Stocrin® 2:510
Stoichiometry, definition 3:413
Stone fruits, viral infections *see* Fruit trees, diseases; *see specific viruses*
Stop-and-start model 5:263
St particles (nondefective 'parent' virus(es)) 2:1, 2:3
Stp oncoproteins *see* Herpesvirus Saimiri (HVS)
Strains
  cacao swollen shoot virus infection 1:408–409
  Japanese encephalitis virus 3:185
  potato virus Y 4:290–291, 4:292–293
    tobacco infections 4:295
  varicella-zoster virus 5:251–252
Stratified squamous epithelia, HPV life cycle 4:20
Strawberry crinkle virus 4:184*t*
Strawberry latent ringspot virus (SLRSV) 4:523, 4:524*t*
  genome sequencing 4:524–525
  geographical distribution 4:523
  host range 4:525
  RNA satellites 4:525
  transmission 4:525
  vector control 4:525–526
Strawberry mild yellow edge virus (SMYEV) 4:311*t*
  genome 4:312
Strawberry mottle virus (SMoV) 4:523, 4:524*t*
  genome sequencing 4:524–525
  geographical distribution 4:523
  host range 4:525
  transmission 4:525
  vector control 4:525–526
Stress, viral reactivation 2:438
Stress kinases, togavirus infection response 5:123
Striped bass aquareovirus (SBRV) 1:164*t*
Striped jack nervous necrosis virus (SJNNV) 2:229–230
  genome 2:229
Striped leaf disease *see* Maize streak virus (MSV)
Stromal keratitis 2:388–390
Strongyloides stercoralis, HTLV-1 associated infection 2:568
Structural information, virus databases 5:363
Structural proteins
  see specific viruses

Structure determination methods, nonenveloped viruses 1:201
Structured query language (SQL) 5:353–354
Stunt disease of tobacco 5:263–264
  see also Lettuce big-vein virus (LBVaV)
Stunting, barley yellow dwarf viruses 1:284–285
Subacute sclerosing panencephalitis (SSPE) 1:474, 2:494–495, 3:289
  definition 3:285
Subclinical infection, definition 2:146
Subcutaneous fibromatosis 3:225–226
Subgenomic DNAs, beet curly top virus 1:304
Subgenomic RNA (sgRNA)
  beet necrotic yellow vein virus 1:311*f*, 1:312
  carmovirus 1:455
  definition 1:520, 3:46, 4:207, 5:145, 5:200
  nidoviruses 3:428
  ophiovirus 3:449–450
  tombusvirus 5:149
Sub-Saharan Africa, HIV epidemiology *see* HIV infection
Subsidence
  seasonal changes 5:273
  vector transmission 5:272
Substilisin-like protease(s), fusion protein synthesis 3:293
Subterranean clover mottle virus (SCMoV) 4:646*t*, 4:651
Subterranean clover stunt virus (SCSV)
  characteristics 3:389*t*
  economic importance 3:386
  genome 3:387*f*, 3:388
  host range 3:386
  transmission 3:386
    aphids 3:386
Subtractive hybridization
  definition 4:445
  Rous sarcoma virus, transformation-defective 4:446
Subunit vaccine(s) 2:52, 5:236
Subviral agents, classification 5:11*t*
Subviral RNA Database 5:357*t*
Suc-HSA 2:507*t*
Sucrose density gradients, cowpea mosaic virus purification 1:569
Sudan
  HIV epidemiology 1:65
  monkeypox virus 4:643
Sudan Ebola virus (SEBOV)
  fatality rates 2:57, 2:199
  genome 2:58–59
  host range 2:62
Sugarcane bacilliform virus (SCBV) 1:403
  host range 1:463
Sugarcane mosaic virus (SCMV) 1:481
  maize chlorotic mottle virus synergy 3:262
Suid herpesvirus 1 *see* Pseudorabies virus (PrV)
Suipoxvirus 3:225*t*, 3:226, 4:324*t*
Suipoxvirus(es) **3:225–231**
  classification 3:225*t*, 3:226, 4:324*t*
  DNA properties 3:226
  evolution 3:228
  genetic variability 3:228
  geographical distribution 3:227
  history 3:225
  infection
    clinical features 3:228
    control 3:230
    histopathology 3:229
    host range 3:227
    immune response 3:229
    pathogenesis 3:227
    pathogenicity 3:228
    pathology 3:229
    prevention 3:230
    tissue tropism 3:228
  propagation 3:227
  protein properties 3:226
  replication
    DNA replication 3:226
    sites 3:227–228
    transcription 3:226
    translation 3:226
  seasonal distribution 3:227
  taxonomy 3:226
  transmission 3:228
  virion properties 3:226
    morphology 3:226
  see also specific viruses
Sulfolobus, fuselloviruses 2:297
Sulfolobus islandicus filamentous virus (SIFV) 1:589–590
Sulfolobus islandicus rod-shaped virus(es) (SIRV)
  genome 1:593
  morphology 1:589, 1:589*f*
  replication 1:593
Sulfolobus neozealandicus droplet-shaped virus (SNDV)
  genome modifications 1:593
  morphology 1:590, 1:591*f*
Sulfolobus solfataricus, fuselloviruses 2:297–298
  vectors 2:299
Sulfolobus tengchongensis spindle-shaped virus 1 (STSV1)
  gene functions 1:594
  genome 1:593
  genome modifications 1:593
  morphology 1:589
Sulfolobus turreted icosahedral virus (STIV)
  morphology 1:590, 1:590*f*
  structure 5:382*f*, 5:390*f*, 5:391
Sup35p
  domain shuffling 4:339
  formation 4:337, 4:339
Superantigens, definition 3:334
Superkiller genes (SKI), *Saccharomyces cerevisiae* 5:170–171
Supportive therapy
  Crimean-Congo hemorrhagic fever 1:603
  Marburg virus infection 3:279
Suppression of gene silencing, papaya ringspot virus 4:4
Suppressor of virus-induced gene silencing 3:46
Suprabasal cell(s) *see* Human papillomavirus(es) (HPVs)
Supramolecular adhesion complex (SMAC) 1:122*f*
Supraorbital fossae 1:37
Surveillance measures, transmission risk forecasting 5:274
'Survival of the flatest' quasispecies 4:361–362, 4:362*f*
Sustiva® 2:510
SV40 *see* Simian virus 40 (SV40)
SVA retrotransposons 4:439
  genes, effects on 4:444
  mechanism of action 4:440
  structure 4:440
    variable number tandem repeat 4:440
Sw-5 gene, plant natural resistance 4:174
Swarms, quasispecies 2:178–179
Sweetpotato *(Ipomoea)* 4:659
  production 4:659–660, 4:660*f*
  vegetative propagation 4:660
Sweetpotato virus(es) **4:659–669**, 4:661*t*
  average yields 4:199
  begomoviruses 4:666
  control 4:200
    breeding programs 4:200
  diseases 4:199
    yield losses 4:200
  grafting 4:660, 4:664*f*
  infections 4:667, 4:668*f*, 4:669*f*
    multiple infections 4:667–668
  potyviruses 4:663, 4:665
    geographical distribution 4:665
    phylogeny 4:665, 4:665*f*
  propagation 4:200
  transmission 4:200
  see also specific viruses
Sweetpotato chlorotic fleck virus (SPCFV) 4:661*t*, 4:667
  genome 4:667
  host range 4:667
Sweetpotato chlorotic stunt virus (SPCSV) 4:200, 4:661*t*, 4:667
  genome 4:667
  geographical distribution 4:667
  host range 4:667
  infections
    chlorotic dwarf 4:668
    multiple infections 4:667–668
  serotypes 4:667
  structure 4:667
  transmission 4:667
Sweetpotato feathery mottle virus (SPFMV) 4:661*t*, 4:663
  host range 4:663–664
  infection rate 4:200
  multiple infections 4:667–668
  phylogeny 4:663–664, 4:665*f*
  structure 4:663–664
  transmission 4:663–664
Sweetpotato latent virus (SwPLV) 4:661*t*, 4:665
  infection rate 4:200
Sweetpotato leaf curl Georgia virus (SPLCGV) 4:661*t*, 4:666
Sweetpotato leaf curl virus (SpLCV) 1:302, 4:661*t*, 4:666
  phylogeny 4:666
Sweetpotato leaf speckling virus (SPLSV) 4:661*t*, 4:667
Sweetpotato mild mottle virus (SPMMV) 4:661*t*, 4:665–666
Sweetpotato mild speckling virus (SPMSV) 4:661*t*, 4:665
Sweetpotato severe mosaic disease (SPSMD) 4:668–669
Sweetpotato vein mosaic virus (SPVMV) 4:661*t*, 4:665
Sweetpotato virus 2 4:661*t*
Sweetpotato virus G (SPVG) 4:661*t*, 4:665
Sweetpotato virus Y (SPVY) 4:665
Sweetpotato yellow dwarf virus (SPYDV) 4:661*t*
Swimbladder sarcoma virus (SSSV) 2:231
Swine cytomegalovirus 1:634
Swine fever
  bioterrorism 5:407
  see also African swine fever virus (ASFV); Classical swine fever virus (CSFV)
Swine flu (vaccination) 5:233
Swine hepatitis E virus 2:377–378
Swine pestivirus(es) 2:4
Swinepox virus (SPV) 3:226
  characteristics 3:225*t*
Swine vesicular disease virus (SVDV) 2:124
  genome 2:127
  history 2:124
  structure 2:128–129
  virion structure 2:128–129
  virulence 2:127
SwPLV *see* Sweetpotato latent virus (SwPLV)
syk tyrosine kinase 1:546–547
Sylvatic circulation
  definition 5:83
  yellow fever virus 2:238–239, 5:473–474
Sylvilagus floridanus (cottontail rabbit)
  leporipoxvirus infection 3:229
  Shope fibroma virus infection 3:225
Symbionin, luteovirus transmission 5:278
Sympatric 4:611
Sympatrically 3:479
Syncitin protein, retroviral origin 2:107
Syncytia
  channel catfish virus infection 2:209
  definition 3:285
  foamy virus infection 2:260
Synergism
  definition 5:124
  Indian tomato leaf curl viruses 5:128–129
Synovial fluid 5:83
Synovial membranes 5:83
Synteny 1:225
Synthetic genome(s), bioterrorism 5:410
Syphovirus(es) 5:367–368
Systemic immune response syndrome (SIRS), yellow fever 5:475
Systemic movement (plant viruses), definition 3:348

T

T2 bacteriophage *see* Bacteriophage T2
T3 bacteriophage *see* Bacteriophage T3
T4 bacteriophage *see* Bacteriophage T4
T4-even phages 4:401
T4-like bacteriophages
  genome 4:401
  replication 4:401, 4:402*f*
    leading strand synthesis 4:401
    origin of replication 4:401
T5 bacteriophage 3:33–34
T7 bacteriophage *see* Bacteriophage T7
T7 DNA polymerase 4:401–402
T7 helicase/primase 4:401–402
T7-like bacteriophages
  genome 4:401–402
  replication 4:401
    bacteriophage-encoded proteins 4:401–402
    concatamer formation 4:402
    origin of replication 4:401–402
    promoters 4:401–402
    terminal repeat sequence duplication 4:402, 4:403*f*
T7 lysozyme 3:251
T7 RNA polymerase 4:401–402, 5:180
  consensus sequence 5:180
  efficiency 5:180
  primase initiated synthesis 4:402
  promoters 5:180
  reverse genetics 4:44–45
  termination efficiency 5:180
T7 ssDNA binding protein 4:401–402
T-705 (6-fluoro-3-hydroxy-3-pyrazine carboxamide) 1:153
Taastrup virus 4:184*t*
Tacaribe complex virus(es), classification 3:203
Tacaribe virus (TACV) 3:247–248
Tachykinin 2:543
TAdv-3 *see* Turkey adenovirus 3 (TAdV-3)
Tahyna virus 1:391*t*, 5:490
Tail, definition 3:30
Tailed bacteriophages 2:448*t*
  assembly 1:439
  evolution 2:181
    conserved genes 2:182*f*
    error prone replication 2:181
    genome comparisons 5:372
    genome organization 5:371
    types 5:371
  populations 5:371
    co-infections 5:371
    productive infections 5:371
  structure 1:433
    symmetry disruptions 1:437
  see also specific types
Tail fiber, definition 3:30
TAK-220 2:507*t*
TAK-652 2:507*t*
Tamarin, definition 1:104
Tamdy virus 1:400
Tamiflu *see* Oseltamivir; Oseltamivir
Tamiflu® 1:143*t*
Tamus red mosaic virus (TRMV) 4:311*t*
Tanapox virus (TPV) 5:462, 5:462*t*
  distribution 5:464
  genome 5:462–463
  history 5:461
  host range 5:465
  infection
    control 5:465
    immune modulation 5:464
    immunity 5:463
    pathogenesis 5:464
    prevention 5:465
  replication 5:462
  tropism
    humans 5:463
    nonhuman primates 5:463
T-antigens
  murine polyomavirus 4:271–272
  SV40 proteins *see* Simian virus 40 (SV40)
Tape measure protein, bacteriophage λ 1:438
TAP/NXF1 mRNA export pathway 2:398
TaqMan PCR 2:26
Targeting induced local lesions in genomes (TILLING) 4:186
Target-primed (TP) retrotransposons 4:421*f*
  distribution 4:423
  open reading frames 4:420–421
  protein coding regions 4:420–421
  replication 4:425
    open reading frame 4:425
Taro bacilliform virus (TaBV) 1:403
TAS genes, plant gene silencing 4:142
Tasmanian Atlantic salmon reovirus (TSRV) infection 1:168*f*
Tas protein, foamy virus 2:263
TATA-binding protein (TBP), varicella-zoster virus 5:259
TATA box
  HSV replication 2:401*f*
  JCV genome 4:264*f*
Tataguine virus 1:400
  global distribution 1:400
  infection 1:400
  transmission 1:400
Tat protein
  antibody neutralization 1:72
  expression 2:522
  function 2:522
  JCV reactivation 4:266
Taura syndrome disease (TSD) 5:1
  clinical signs 5:3*f*
  control 5:8
  diagnosis 5:7
    bioassay 5:7–8
    ELISA 5:8
    histology 5:8
    RT-PCR 5:8
  economic impact 5:1
  farming practices 4:569
  geographical distribution 5:7
  host range 2:40–41
  mortality rate 5:3–4
  pathogenesis 5:4
    acute phase 5:4
    chronic phase 5:4
    histopathology 5:3*f*
    target tissues 5:4
    transition phase 5:4
  prevention 5:8
  resistance 5:3
Taura syndrome virus (TSV) **5:1–9**
  classification 4:567
  epizootics 5:1
  evolution 5:6
    quasispecies 5:6
  as fungicides 5:1–2
  gene expression 5:5
    encoded proteins 5:5
    IGR region 5:5
    inhibition of apoptosis 5:5
    IRES 5:5
    protease motif 5:5
    transcription 5:5
  genetics 5:5
    Belize isolates 5:6
    diversity 5:7*f*
    Mexican isolates 5:5–6
    Texas isolates 5:6
  genetic variation 2:43
  genome 5:1
    open reading frames 5:5
  history 5:1
  infection *see* Taura syndrome disease (TSD); *see above*
  transmission 5:6
    cannibalism 5:7
    mechanical 5:7
    seagulls 5:7
  virion structure 2:38
TAV *see* Tomato aspermy virus (TAV)
TaV *see* Thosea asigra virus (TaV)
Taxa 5:19
  definition 1:399
Taxonomy **5:9–24**
  definition 5:9
  historical aspects 5:9
    electron microscopy 5:9–10
    pathogenic properties 5:9–10
  nomenclature 5:21
  PASC applications 5:344
  potato virus Y 4:296
  quantitative 5:20
  species classification vs.demarcation 5:404
  taxa descriptions 5:19
  viral species 5:21
  virus databases 5:352
  see also Classification; *specific viruses*
Tax protein 5:198
  adult T-cell leukemia pathogenesis 2:559–560
  HTLV-1 infection *see* Human T-cell leukemia virus 1 (HTLV-1)
  myositis 2:572–573
TBE E, membrane fusion role 3:298*f*
TBRV *see* Tomato black ring virus (TBRV)
TBSV *see* Tomato bushy stunt tombusvirus (TBSV)
TC-10 GTPase 4:418
TC-83 live attenuated vaccine, Venezuelan equine encephalitic virus 5:106
T cell(s) 3:61–62
  activation 1:122
    HIV infection 2:524
  antibodies *vs.* 3:71
  Borna disease virus infection 1:345
  classes 1:121
  cytotoxic *see* CD8+ T-cells
  definition 3:78
  Dengue fever 2:13
  downregulation 1:126
  exhaustion 3:76
    definition 3:71
  HCV infection 2:373–374
  HIV infection 3:76
  HSV infection 2:393
  HTLV-1 infection *see* Human T-cell leukemia virus 1 (HTLV-1)
  immunopathology role 3:80*f*
  mammalian reovirus infection 4:389
  measles 3:289
  memory *see* Memory T-cell(s)
  mouse mammary tumor virus infection 3:341
  nervous system viruses 1:470
  persistent infection 3:75*f*
  proliferation, Tip oncoproteins 4:589–590
  recognition 3:72*f*
  subpopulations 3:80
  tolerance 1:124
  transformation, HTLV-1-associated malignancies 5:198
  viral load, response 3:76
  Visna-Maedi virus infection 5:427
  see also CD4+ T-cells
T-cell immunoglobulin mucin 1 (TIM1), hepatitis a virus detection 2:346
T-cell leukemia 2:561
  adult *see* Adult T-cell leukemia (ATLL)
  HTLV basic leucine zipper protein 2:563
  Stp oncoproteins 4:589
  tax protein 2:562
T-cell lymphomas
  adult *see* Adult T-cell lymphoma
  EBV-associated 5:195
  mouse mammary tumor virus infection 3:339–340
  reticuloendotheliosis virus infection 4:416
T-cell lymphosarcoma, feline leukemia viruses 2:187–188
T-cell receptor (TCR) 3:71
  definition 3:71
  engagement 3:73*f*
  MHC-restriction 3:71–72
  mouse mammary tumor virus transmission 3:337
  recombination 3:71–72

$TCID_{50}$, definition 1:37
TCV *see* Turnip crinkle virus (TCV)
T-DNA *see Agrobacterium tumefaciens*-based expression system (T-DNA)
Tear(s), ocular immunology 2:492
Tectiviridae 1:1, 3:1, 5:11*t*
Tectivirus(es)
  genome delivery 5:368
  genome packaging 2:306
  structure 1:434*f*
    symmetry disruptions 1:437
  see also Icosahedral dsDNA bacteriophages; *specific viruses*
Tegument
  HCMV structure 2:485–486
  HSV structure 2:397
Telbivudine 1:152
  resistance development 2:359
Telomerase 4:418
Telomeric repeats, mardivirus 2:409
Temperate virus, definition 5:411
Temperature limits, extrinsic incubation period 5:271–272
Templates
  bacteriophage assembly 1:438
  Brome mosaic virus, RNA recombination 1:385
Tench reovirus (TNRV) 1:164*t*
Tenofovir 2:510
  HAART-associated renal disease 1:56–57
Tenosynovitis, definition 5:83
'Tentative Species Names' 5:21
Tenuivirus 5:24
Tenuivirus(es) **5:24–27**
  antigenic properties 5:26
  biological properties 5:26
  genome 5:25*f*
    ambisense arrangement 5:25*f*
    nucleocapsid protein 5:25
    RNPs 5:25
  historical aspects 5:24
  infection
    cytopathology 5:26
    host range 5:26
    inclusions 5:25*f*
    symptoms 5:26
  phylogenetics 5:26
  relation to other taxa 5:27
  replication 5:25
    cap-snatching 5:25*f*
  transcription 5:25*f*
  transmission 5:26
    plant hopper vector 5:26
  type species 5:24
  virion 5:24
    components 5:24
    see also specific components
    morphology 5:25*f*
    physical properties 5:24
  see also specific viruses
Teratogens, definition 3:479
Terminal nucleotide sequences, arenavirus genome 3:243
Terminal protein(s) (TP) 4:402
  classification 4:402–403
  TP–dAMP complex 4:403
Terminal redundancy
  cauliflower mosaic virus transcription 1:467–468
  definition 5:411
Terminal-repeat retrotransposons in miniature (TRIMs), LTR-retrotransposons, unclassified 4:434
Terminal repeats, EBV 2:157
Termination, dsDNA bacteriophage genome packaging 2:310
Teschovirus 2:126–127
Tessier, G, Sigma virus discovery 4:576
Tetraviridae 5:11*t*, 5:27, 5:28*t*
Tetravirus(es) **5:27–37**
  β-like genome 5:30*f*
    capsid protein precursor 5:31*f*
    omegatetraviruses *vs.* 5:29
    open reading frames 5:29
    RdRp 5:29
  biotechnology applications 5:36
    biopesticides 5:36
    chemical platforms 5:36
  capsid assembly 5:33
  capsid maturation 5:34
    labeling experiments 5:35
    small-angle X-ray scattering 5:34–35
  capsid morphology 5:31*f*
  capsid proteins 5:29
    precursors 5:31*f*
  capsid structure 5:34*f*
    cryo-electron microscopy 5:33
    high-resolution 5:33
    X-ray crystallography 5:33
  cleave site mutations 5:35
  definition 5:27
  genomes 5:29
    delivery 5:368
    two RNA species 5:29
    see also specific genome types
  historical aspects 5:27–28
  infections 5:36
    histopathology 5:36
    host range 5:36
    insects 3:126*t*
    pathogenicity 3:127
    sites 3:127
    symptoms 3:127
    transmission 5:36
    tropism 5:36
  insect pest control 3:127
  ω-like genome 5:31
    capsid protein precursors 5:31*f*
    open-reading frames 5:31
    replicases 5:31
    RNA2 5:31
  RdRp 5:29
  structure 5:387*f*
  see also specific viruses
TEV movement (RTM) proteins 3:355
Tf1 transposon (yeast)
  expression 3:306
  integrase 3:309
    target site specificity 3:309
  reverse transcription priming 3:307*f*
Tf2 transposon (yeast)
  frameshifts 3:304–306
  induction, stress 3:304
  translation 3:304–306
TFIIIB (transcription factor) 3:309
TFIIIC (transcription factor) 3:309
TGB movement protein(s) 3:352
  allexivirus genome 1:97–98
  beet soil-borne virus 4:283–284
  beet virus Q 4:283–284
  flexiviruses 2:257*f*
  hordeiviruses 2:462
    TGB1 *see* TGB1 movement protein
    TGB2 *see* TGB2 movement protein
    TGB3 *see* TGB3 movement protein
  pomovirus genome 4:283–284
  potato mop-top virus 4:283–284
  see also specific proteins
TGB1 movement protein 2:462
  expression 2:462
  Pepino mosaic virus 4:105
  RNA binding activity 2:462
  RNA helicase activity 2:462
  TGBp1 helicase 3:353
TGB2 movement protein 2:462
  Pepino mosaic virus 4:106
  TGBp2 3:352
TGB3 movement protein 2:463
  domains 2:463
  Pepino mosaic virus 4:106
  translation 2:463
Thailand, HIV infection 1:63–64
Theiler's murine encephalomyelitis virus (TMEV) **5:37–45**
  classification 5:38*f*
  final gene products 5:39
  genome 5:40*f*
    coding limits 5:39
  geographical distribution 5:37
  history 1:441
  infection *see below*
  physical properties 5:38
  polyprotein processing 5:40*f*
    capsid proteins 5:40
    cleavage sites 5:39
    L* protein 5:40
  RNA replication 5:40
    internal ribosome entry site 4:134–135
  serologic relationships 5:38
  taxonomy 1:443*f*
  tissue tropism 5:41
  transmission 5:41
  virion structure 5:39*f*
    capsid proteins 5:38–39
    Mengo virus *vs.* 5:38–39
Theiler's murine encephalomyelitis virus infection
  blood–brain barrier 1:447
  clinical features 1:446
    gait spasticity 5:42
    late disease 5:42
    poliomyelitis 5:42
    spinal leptomeninges, inflammation 5:43*f*
  demyelination 5:43*f*
    incidence 5:44
  diagnosis 1:447
  genomic determinants 5:41
  histopathology 1:447
  host range 5:37
  immune response 5:43
    demyelination 5:43*f*
    IgG response 5:43
    survival-determining factors 3:82
  macrophages
    pathogenesis 5:43*f*
    persistence 5:42–43
  neurovirulence 1:446–447
    capsid proteins 5:41
    L* protein 5:41
  pathogenesis 1:446
  pathology 1:446
  persistence sites 5:42
Theiler's virus *see* Theiler's murine encephalomyelitis virus (TMEV)
T helper cell(s)
  cytokine/chemokine production 1:621
  differentiation 1:621
  type 1
    definition 2:51
    immune response functions 3:80
  type 2
    cytokine production 1:621
    definition 2:51
  see also CD4+ T-cells
Therapeutic ratio, antiviral therapy 1:147
Thermococcales-infecting virus (PAV1)
  see Euryarchaea-infecting virus(es)
Thermoproteus tenax spherical virus 1 (TTSV1) 1:590
Thermoproteus tenax virus 1 (TTV1)
  host cell lysis 1:591
  virion core structure 1:590
θ replication mode
  bacteriophage λ 4:400–401
  definition 4:400
Thiafora virus 1:598*t*
Thiomersal 5:228
Thogoto virus
  characteristics 1:171*t*
  virion structure 1:172
Thogotovirus 3:96
Thoracic ganglia invasion, Sigma virus 4:578
Thosea asigra virus (TaV) 5:28*t*
  capsid assembly 5:33
  capsid protein precursor 5:29–31
  capsid protein precursors 5:29–31
  genome 5:29
  RdRp 5:29
  replicase 5:29

Thread-like particles, citrus tristeza virus 1:520
3′-nontranslated region, Pepino mosaic virus 4:106
Three-day sickness *see* Bovine ephemeral fever virus (BEFV)
Three-factor cross method, DNA virus recombination 4:374–375
Three-hybrid assay *see* Icosahedral ssRNA bacteriophages
Thrip vectors
  tobacco streak virus transmission 3:55
  Tomato spotted wilt virus *see* Tomato spotted wilt virus
  tospovius *see* Tospovirus(es)
Thrombocytopenia
  classical swine fever 1:529
  Crimean-Congo hemorrhagic fever 1:601–602
  definition 2:241
  Dengue fever symptoms 2:11–12
Thrombosis, definition 1:328
Thrombotic microangiopathy, HIV infection 1:56
Thymic lymphosarcoma(s), feline leukemia viruses 2:189
Thymidine kinase activation, murine polyomavirus large T 4:276
Thymidine nucleoside analogs 1:53
Thymidylate kinases, virus-encoded proteins in African swine fever virus 1:47
Tibrogargan virus (TIBV) 4:184*t*
Tick(s) 2:235
  as an African swine fever virus host 1:44*f*
  coltivirus transmission *see* Coltivirus(es), transmission
  control, Crimean-Congo hemorrhagic fever prevention 1:602–603
  flavivirus transmission 2:239
  life cycle 1:173–174
  vertical transmission 5:269
  West Nile virus transmission 5:445
Tick-borne encephalitis virus (TBEV) 2:240
  bioterrorism evaluation 5:407–408
  European subtype 2:240
  Far Eastern subtype 2:240
  geographical distribution 5:490
  host cell attachment 5:48*f*
  host cell entry 5:46
    cellular receptors 5:46
    E protein 5:46
  infectivity 5:45
  life cycle 5:46
  nomenclature 5:45
  nonviremic transmission 5:270
  phylogeny 5:46*f*
  protein processing 5:48
  RNA replication 5:49
    associated proteins 5:49–50
    replication complex formation 5:51*f*
    replicative intermediates 5:49
  RNA translation 5:50*f*
  Siberian subtype 2:240
  taxonomy 5:45
  vaccines/vaccination 5:53
  virion assembly 5:48*f*
  virion maturation 5:50
  virion release 5:50
  virion structure 5:45
    capsid 5:48*f*
    envelope glycoprotein 5:47*f*
      conformational changes 5:48*f*
    M protein 5:46
Tick-borne encephalitis virus infection
  biphasic milk fever 5:53
  clinical manifestations 5:52
  control 5:53
  encephalitis 2:240
    incidence 5:51
  incidence 5:51
  pathogenesis 5:51
  prevention 5:53
  transmission 5:490
    vertical transmission 5:269
  zoonoses 5:490
Tick-borne flavivirus(es) 2:239
  see also specific viruses
TICV *see* Tomato infectious chlorosis virus (TICV)
Tiger frog virus (TFV) 2:233
Tiger prawn reovirus 1:164*t*
Tiger puffer nervous necrosis virus (TPNNV) 2:229–230
Tiger shrimp reovirus 1:164*t*
Timbo Group, rhabdoviruses 1:116
Tind, fusellovirus promoter 2:299
Tioman virus 2:95
Tip oncoproteins *see* Herpesvirus Saimiri (HVS)
Tipranavir 2:516
Tipula paludosa (cranefly), larval infection 3:161
TIR-NB-LRR protein, plant natural resistance 4:171
Tissue culture
  neutralization sensitivity 3:418
  Simian retrovirus D 4:629
Tissue factor (TF) expression
  definition 2:199
  Ebola virus infection 2:64
Tissue print immunoblotting, plant virus infection diagnosis 2:25*f*
Tissue tropism
  definition 4:407
  see also specific viruses
TLC1 element 4:435
TLCNDV *see* Tomato leaf curl New Dehli virus (TLCNDV)
TLCV *see* Tobacco leaf curl virus (TLCV)
TLRs *see* Toll-like receptor(s) (TLRs)
T lymphocytes *see* T cell(s)
Tm-2 genes 4:176
TM Biocontrol-1 3:125
TME *see* Transmissible mink encephalopathy (TME)
TMEV *see* Theiler's murine encephalomyelitis virus (TMEV)
TMGMV *see* Tomato mild green mosaic virus (TMGMV)
TMMMV *see* Tulip mild mottle mosaic virus (TMMMV)
TMV *see* Tobacco mosaic virus (TMV)
Tn7 transposon, Bac-to-Bac expression vectors 1:241
TNSV *see* Tobacco necrosis satellite virus (TNSV)
Tnt1 elements 4:432
  U3 regions 4:432
T-number, definition 3:30
TNV *see* Tobacco necrosis virus (TNV)
TNV-A *see* Tobacco necrosis virus A (TNV-A)
TNV-D *see* Tobacco necrosis virus D (TNV-D)
TNX-355 2:507*t*
  HIV entry inhibitors 2:506
Tobacco *(Nicotiana tabacum)* 5:60
  history 5:60
  infections 5:61
    alfalfa mosaic virus 5:63
    artichoke yellow ringspot virus 5:66
    economic importance 5:61
    potato spindle tuber disease 5:341
    see also specific diseases/viruses
  plant virus movement 3:350–352, 3:351*f*, 3:353–354
  satellite virus-induced silencing system 5:378
  types 5:61
  virus propagation , associated species 5:61
  virus resistance 5:61
    TBRV-acquired immunity 5:325–326
    TRSV-acquired immunity 5:65
Tobacco apical stunt virus (TASV) 5:62*t*
Tobacco bushy top disease 5:67
Tobacco bushy top virus (TBTV) 5:62*t*
Tobacco curly shoot virus (TbCSV) 1:320
  characteristics 5:62*t*
Tobacco etch virus (TEV)
  characteristics 5:62*t*
  genome 5:64
  geographical distribution 5:64
  infection
    economic importance 5:64
    symptoms 5:64
  NIa protease 4:321
  P1 protein 4:317
  P3-6K1 protein 4:317
  transgenic resistance 5:64
  transmission 5:64
Tobacco leaf curl disease 5:65
Tobacco leaf curl-like virus(es) 5:65
  genome 5:65
  geographical distribution 5:65
Tobacco leaf curl virus (TLCV) 5:65
  characteristics 5:62*t*
  geographical distribution 5:65
Tobacco mild green mosaic virus (TMGMV) 5:61–63
  characteristics 5:62*t*
Tobacco mosaic satellite virus (TMSV) 4:535
  characteristics 5:63*t*
Tobacco mosaic virus (TMV) 5:62*t*
  antigenicity 5:58
  assembly 5:57*f*
    disk aggregate 5:56
    elongation 5:68–69
    initiation 5:56–57
  biotechnology applications 5:59
    engineered resistance 4:156–157
    experimental vaccines 5:223
    magnifection 4:234
    nanoscale material production 4:236
    promoter utilization 4:230
  capsid protein 4:236
    monoclonal antibody production 4:236–237
    protein fusion 4:236
  cell-cell movement 5:59
  characteristics 5:62*t*
  chemical properties 5:55
  coat proteins 5:68–69
    aggregation forms 1:201–202
    disks 1:201–202
    epitopes 5:58
    symmetry 1:201–202
  crystallization 3:399
  disassembly 5:57
  gene silencing 5:376
  genome 5:385
  history 5:61
  hypersensitive response 4:174*f*
  infection
    Impatiens infections 4:215
    nonspecific infections 4:222*f*
    petunia infection 4:212*f*
    potato 4:300*t*
    resistance to 4:173, 5:68
    sweetpotatoes 4:661*t*
  movement within plants 3:350–352
    movement protein 3:351*f*
  mutants 5:55–56
  physical properties 5:55
  replication 5:59
    translation 5:59
  satellite viruses 4:527*t*
  structure 5:68–69
    capsid protein–nucleic acid interactions 5:385
    historical aspects 5:380–381
    origin of assembly 5:69
    X-ray crystallography 5:380–381
  transient vector construction 5:61
  transmission 5:285
    capsid strategy 5:280*f*
Tobacco mottle virus (TMoV) 5:66–67
  characteristics 5:62*t*
Tobacco necrosis satellite virus (TNSV) 4:527
  characteristics 5:63*t*
  noncoding regions 4:530
  properties 4:530
  virion structure 4:530
Tobacco necrosis virus (TNV) 5:66
  characteristics 5:62*t*
  genome 5:147*f*
  legume infection 3:214*f*
  petunia infection 4:213
  satellite viruses 4:527
  transmission via chytrid fungi 3:214*f*

Tobacco necrosis virus A (TNV-A) 5:145–146
  geographical distribution 3:404
  satellite virus 3:404
  virus–host relationship 3:404–405
Tobacco necrosis virus D (TNV-D) 3:403
  geographical distribution 3:404
  satellite virus 3:404
  tulip infections 4:224
  virus–host relationship 3:404–405
Tobacco necrotic dwarf virus (TNDV) 5:66
  characteristics 5:62*t*
Tobacco rattle virus (TRV) 5:72
  associated vectors 5:66
  characteristics 5:62*t*
  genome 5:376–377
  geographical distribution 5:76
  host range 5:76
  infection
    detection 4:308
    petunia infection 4:213
    symptoms 5:76
    tulips 4:224
  morphology 4:307–308
  movement studies 5:73
    RNA1 5:73
    RNA2 5:73
  recombination 5:74
  transmission 4:307–308
    2b protein, role 5:75
  virus-induced gene silencing role 5:376
Tobacco ringspot virus (TRSV) 3:406*t*
  associated disease symptoms 5:65
  cell-to-cell movement 3:410
  characteristics 5:62*t*
  diseases 3:411
  infections
    pelargonium infections 4:210
    petunia infection 4:213
  infectious DNA clones 3:408–409
  particle structure 3:407
  ribozyme 4:476
    structural dynamics 4:479
  satellite viruses 3:411
    RNA satellites 4:534
  transmission 5:65
    aerial vectors 3:411
    soil nematodes 3:411
Tobacco rosette disease 5:66
Tobacco streak virus
  characteristics 5:62*t*
  economic importance 5:63
  genome 3:50*f*
    open reading frames 3:47
  infections
    dahlia 4:223
    host range 3:55
    Impatiens 4:215
    petunia 4:213
    potato 4:300*t*
    sweetpotato 4:661*t*
  transmission 3:55
Tobacco stunt virus (TSV) 5:66
  characteristics 5:62*t*
Tobacco vein clearing virus (TVCV), transmission 1:462
  host genome integration 1:462
Tobacco vein distorting virus (TVDV) 5:66–67
  characteristics 5:62*t*
Tobacco vein mottling virus (TVMV)
  characteristics 5:62*t*
  geographical distribution 5:64–65
  transmission 5:64
  virion structure 4:316
Tobacco virus(es) **5:60–68**
  associated satellite viruses 5:63*t*
  naturally occurring 5:63*t*
  satellite viruses 5:61
  see also specific viruses
Tobacco wilt virus (TWV) 5:62*t*
Tobacco yellow dwarf virus (TYDV) 5:65
Tobacco yellow vein assistor virus 5:62*t*
Tobacco yellow vein virus (TYVV) 5:67
Tobamovirus 5:59, 5:68
Tobamovirus(es) **5:68–72**
  antigenic relationships 5:59
  cis-acting sequences 5:69
  classification 5:68
  composition 5:68
  genome 5:70*f*
    hibiscus-infecting viruses 5:69
    open reading frames 5:69
  history 5:68
  infection
    Calibrachoa 4:214
    Impatiens 4:214–215
    petunia 4:213
    resistance 4:173
    symptoms 5:288
    tobacco 5:61
    virus-host interactions 5:70
  replication 5:71
    rate 5:71
    translational disassembly 5:71
  satellite TMV 5:71
  structure 5:68
  taxonomy 5:68
  transmission 5:288
  viral proteins 5:70
    coat protein 5:70
    movement protein 5:70
  see also specific viruses
Tobravirus 5:72
Tobravirus(es) **5:72–76**
  characteristics 5:72
  coat protein 5:72–73
    antigenic regions 5:72–73
  furovirus *vs.* 2:295–296
  gene expression 5:73
    RNA1 5:74
    RNA2 5:74
    subgenomic RNA 5:74
  gene silencing 5:75
  genome 5:73
  infection
    diseases associated 5:76
    M-type 5:73
    NM-type 5:73
    recovery 5:76
    resistance 5:76
    tobacco 5:66
  movement studies 5:73
  proteins encoded 5:74
    cell-to-cell movement 5:74–75
    coat proteins 5:75
    replicase 5:74–75
    RNA1 5:73
    RNA2 5:73
    subgenomic RNA 5:74
  RNA sequences 5:73*f*
    recombination 5:74
    RNA1 5:72–73
    RNA2 5:72–73
    subgenomic RNA 5:72–73
  satellite viruses 4:527*t*
  taxonomy 5:72
  transmission 5:72
  virion production 5:72
  virion structure 5:72*f*
  see also specific viruses
ToCGV *see* Tomato leaf curl Gujarat virus (ToCGV)
ToCV *see* Tomato chlorosis virus (ToCV)
Togaviridae 1:174, 1:195, 2:230, 2:255, 4:514, 5:11*t*, 5:77, 5:83, 5:91–92, 5:96, 5:101, 5:108, 5:117
Togavirus(es) **5:107–116**
  cell viability 5:122
  evolution 5:108
  fish 2:230
  genera 2:230
  genome 1:173
  history 5:107
  infections *see below*
  infectious clone technology 5:121
  pathogens associated 5:113
  see also specific viruses
  phylogenetic relationships 1:174
  RdRp 5:119
  replication 5:120*f*
    genome packaging 5:121
    host cell entry 5:118
    sites 5:121
    specificity 5:120–121
    structural protein maturation 5:121
    transcription 5:119
    translation 5:119
    uncoating 5:118
    virion egress 5:121
    virus attachment 5:118
  systematics 5:108
  transmission cycles 5:111
  vectors 5:121–122
  virion morphology 2:230
  virion structure 1:173
    E1 envelope glycoprotein 5:108
    envelope spikes 5:108
    glycoproteins 5:117
    nucleocapsid 5:108
    stability 1:173
  see also specific viruses
Togavirus infections 5:121
  2–5 A synthase/RNaseL pathway 5:123–124
  clinical manifestations 5:83
    acute 5:83
    children 5:83
    chronic illness 5:85
    encephalitis *see below*
    joint involvement *see below*
    pregnancy 5:85
    rashes 5:84
  cytopathic effects 5:122
  diagnosis 5:87
    antibodies 5:87–88
    hemagglutination inhibition tests 5:107–108
  diseases associated 5:111
    New World viruses 5:111
    Old World viruses 5:111
  double-subgenomic promoter viruses 5:121–122
  encephalitis **5:76–83**
    diagnosis 5:82
    epidemiology 5:80*f*
    geographical distribution 5:79*f*
    treatment 5:82
  host cell interactions 5:122
    protein shut-off 5:122
  hosts 5:111
  interferon-mediated antiviral response 5:123
  joints 5:83–84
    pattern of involvement 5:84
    swelling 5:84
  replicon systems 5:121–122
  treatment 5:88
ToLCD *see* Tomato leaf curl disease (ToLCD)
Toll-like receptor(s) (TLRs)
  apoptosis inhibition 1:161
  cellular distribution 3:106
  definition 3:104
  function 1:123
  innate immune response 3:149
    expression 3:58–59
    nucleic acid recognition 3:58–59
    signal cascades 3:58–59
    viral protein recognition 3:106
  research history 3:58–59
  signaling
    parasitoid attack 4:252–253
    persistent infection 4:113
Toll-like receptor 4 (TLR4)
  adapter proteins 3:107
  mouse mammary tumor virus infection 3:339
Tol QRA proteins, bacteriophages 3:122
Tomato *(Solanum esculentum)*
  Sw-5 gene 4:174
  see also specific viruses
Tomato apical stunt viroid (TASVd) 5:339–341

Tomato aspermy virus (TAV)
  chrysanthemum infections 4:224
  petunia infection 4:213
  small, linear single stranded RNA satellites 4:531–532
Tomato black ring virus (TBRV) 3:406*t*
  infections 3:411
    peach tree 4:198
    pelargonium 4:211*f*
    petunia 4:213
    potato 4:300*t*
  large single-stranded RNA satellites 4:531
  transgenic resistance 5:325–326
Tomato bushy stunt tombusvirus (TBSV) 3:216
  genome 5:147*f*
  RNA recombination 4:379
  structure 1:454–455
Tomato chlorosis virus (ToCV) 2:90
  epidemiology 2:90
  genome 2:90
  geographical distribution 2:90
  infection
    classification 2:90
    control 2:90
    disease symptoms 2:90
    yield losses 2:90
  virion morphology 2:90
Tomato infectious chlorosis virus (TICV) 2:89
  classification 2:89
  epidemiology 2:89
  genome 2:89
  geographical distribution 2:89
  host range 2:89
  infection
    control 2:90
    disease symptoms 2:89
    yield losses 2:89
  transmission 2:89–90
  virion morphology 2:89
Tomato leaf curl Bangalore virus (ToLCBV) 1:567*f*
Tomato leaf curl disease (ToLCD)
  epidemiology 5:125
    relative humidity 5:126
    vector movement 5:125–126
    virus persistence 5:126
    weather, effects 5:125–126
  history 5:125
  yield losses 5:125
Tomato leaf curl Gujarat virus (ToCGV) 5:127
  DNA satellites 5:127
Tomato leaf curl New Delhi virus (TLCNDV) 5:127
  DNA β component association 1:320
  DNA satellites 4:530–531
  infection 5:132
    movement protein 5:132
    nuclear shuttle protein 5:132
  replication 1:566
Tomato leaf curl virus (TLCV)
  DNA satellites 4:530
  satellite viruses 4:527
Tomato leaf curl viruses (Indian) **5:124–133**
  distribution 5:128*f*
  diversity 5:126
  DNA-A satellites
    comparisons 5:129
    pseudo-recombination 5:131–132
    recombination 5:131
  epidemiology 5:125
  genome 5:126
  infections
    host range 5:126
    management 5:133
    pathogenesis 5:132
    resistance 5:133
    symptoms 5:125*f*
  molecular relationships 5:129*f*
    common region sequence comparisons 5:130
    DNA-A sequence comparisons 5:129
    DNA-B sequence comparisons 5:130
    DNA-β sequence comparisons 5:130
  phylogeny 5:129*f*
  pseudo-recombination 5:128–129
    DNA-A 5:131–132
  recombination 5:131*f*
    DNA-A 5:131
  replication 5:132
    Rep protein 5:132
  synergism 5:128–129
  tomato leaf curl Karnataka virus 5:128
  tomato leaf curl Pune virus 5:128
  transmission 5:125
  see also specific viruses
Tomato leaf curl virus-satellite (ToLCV-sat) 1:315
Tomato mild green mosaic virus (TMGMV)
  Impatiens infections 4:217*f*
  petunia infection 4:213*f*
Tomato mosaic virus (ToMV) 5:61–63
  bacopa virus infections 4:215
  Calibrachoa infection 4:214*f*
  characteristics 5:63*t*
  petunia infection 4:211–212
  phloem-dependent long-distance movement 5:212
  potato infection 4:300*t*
Tomato pseudo-curly top virus (TPCTV) 4:165
  infections 4:166
Tomato ringspot virus (ToRSV) 3:406*t*
  diseases 3:411
  genome 3:407–408
    sequence identity 3:408
  infectious DNA clones 3:408–409
  pelargonium infections 4:210
  petunia infection 4:213
  regulated polyprotein processing 3:409
  replication 3:409
    NTB protein 3:409–410
  transmission, soil nematodes 3:411
Tomato spotted wilt virus (TSWV) **5:133–138**
  biology 5:133
  epidemiology 5:136
  genome 5:134*f*
    L RNA 5:134
    M RNA 5:134
    N gene variability 5:133–134
    RdRp 5:134
    ribonucleoproteins 5:134
    S RNA 5:134
  history 5:157
  infection 5:133–134
    acibenzolar-*S*-methyl 5:137*f*
    bacopa virus infections 4:215
    chrysanthemum infections 4:224*f*
    dahlia infections 4:223*f*
    diagnosis 5:136
    economic impact 5:136
    host ranges 5:133–134
    Impatiens infections 4:214
    management 5:137
    nemesia infections 4:220*f*
    NSm protein role 5:134–135
    Osteospermum infection 4:222*f*
    pelargonium infections 4:211*f*
    potato 4:308–309
    symptoms 5:134*f*
    tobacco 5:65
  molecular biology 5:134
  morphology 5:134
  RdRp 5:134
  resistance
    Tm-2 genes 4:176
    transgenic 5:325–326
  structure 5:288–289
  taxonomy 5:134*f*
    classification descriptors 5:133
  transmission *see below*
  virus structure 5:65
Tomato spotted wilt virus transmission 5:133–134
  thrips 5:157
    acquisition 5:159–160
    control 5:162–163
    infection effects 5:161
    management 5:137
Tomato yellow leaf curl China virus (TYLCCNV)
  βC1 role 1:318
  DNA satellites 4:535
Tomato yellow leaf curl disease 2:98
  crop yield losses 2:98
  geographical distribution 2:99*f*
  symptoms 2:98
Tomato yellow leaf curl Sardinia virus (TYLCSV)
  begomovirus satellite identification 1:315
  geographical distribution 5:140–141
Tomato yellow leaf curl virus (TYLCV) 5:289
  begomovirus satellite identification 1:315
    DNA β component associated 1:320
  classification 2:98
  epidemiology 2:98
  gene expression 2:100
  genome 5:289
    open reading frames 2:101
  geographical distribution 5:289
  history 5:138
  host range 5:139
  infection *see* Tomato yellow leaf curl disease; *see below*
  structure 5:289
  transmission 5:289
    disease incidence 5:139
    sexual 5:143
  vector relationships 5:141
    host fertility 5:143
    host life expectancy 5:143
    host tissues associated 5:141
    virus retention 5:141
    whitefly *see below*
  virus reservoir 2:98
  whitefly 5:139
    biotypes 5:139
    disease incidence 5:139
    fertility, host effects 5:143
    life expectancy, host effects 5:143
    sexual transmission 5:143
    tissues associated 5:141
    virus retention 5:141
Tomato yellow leaf curl virus infection 4:167*f*
  control 2:100
    breeding programs 4:200–201
    chemical 4:200–201
    genetic engineering 5:144
    physical barriers 4:200–201
  diagnosis 5:140–141
  genetically-modified resistant plants 5:144
    hybrid plants 5:143
    marker-assisted breeding 5:144
    Rep gene 5:144
  symptoms 5:290*f*
  tomato 5:139
    breeding programs, viral resistance 5:144*f*
    yield losses 4:200
  see also Tomato yellow leaf curl disease
Tombusviridae 1:453, 3:403, 5:145
Tombusvirus 5:145–146
Tombusvirus(es) 1:453
  defective interfering RNAs
    pathogenesis 5:150
    recombination 4:380
    replication 5:149
  epidemiology 5:149
  evolution 5:145
  genome 5:146
  geographical distribution 5:150
  host range 5:149
  infection *see below*
  phylogeny 5:145
  RdRp 1:453, 5:146, 5:148–149
  replication 5:148
    defective-interfering RNA 5:149
    movement protein expression 5:148–149
    post-transcriptional gene silencing 5:148–149
    termination 5:149
    translation 5:147*f*

RNA satellites
genome organization 5:148
pathogenesis 5:150
satellite viruses 5:148
stability 5:148
transmission 5:145
virion structure 5:148*f*
CP subunits 5:148*f*
P domain 5:146
R domain 5:146
S domains 5:146
see also specific viruses
Tombusvirus 19 5:328
Tombusvirus infections
cytopathology 5:150
pathogenesis 5:150
defective interfering RNAs 5:150
recombination 5:150
RNA satellites 5:150
resistance genes 4:179*t*
ToMV *see* Tomato mosaic virus (ToMV)
Tonate virus (TONV) 5:109*t*
Tonoplast, definition 1:615
Tonsil(s)
EBV replication 2:441
vCJD diagnosis 4:334
Top component *a* (Ta) 1:81–82
Top component *b* (Tb) 1:81–82
'Top-half' domain, narnavirus host persistence 3:395*f*
Topocuvirus 1:296, 2:98, 4:165
Topoisomerases, mimivirus gene content 3:316
Topology, phylogenetic trees 4:126
Torovirus 1:549, 2:230, 5:151
Torovirus(es) 2:122
antigenic properties 5:155
classification 5:151
envelope protein
M protein 3:423
S protein 3:423
epidemiology 5:155
gene expression, transcription 3:428
genetic relationships 5:155
genome 5:152*f*
nucleotide cyclic phosphodiesterase 3:425*f*
open reading frames 5:152–153
proteolytic processing 3:424
RdRp 5:152–153
replicase genes 3:425*f*
geographical distribution 5:154
history 5:151
infection
clinical features 5:155
gastroenteritis 3:421
histopathology 5:156
host range 5:154
immune response 5:156
pathogenesis 5:155
pathology 5:156
prevention/control 5:156
tissue tropism 5:155
transmission 2:122
physical properties 5:153
buoyant densities 5:153
proteins 5:153
envelope associations 5:153
M glycoprotein 5:153
nucleocapsid protein 5:153
spike protein 5:153
RdRp 5:152–153
replication 5:153
assembly 5:154
attachment 5:153
budding 5:154
egress 5:154
entry 5:153
maturation 5:154
post-translational processing 5:154
transcription 5:153
translation 5:154
uncoating 5:153
taxonomy 5:151
transmission 5:155
oral/nasal route 5:155
virions 5:152*f*
see also specific viruses
Torque teno mini virus (TTMV) 1:105, 1:108*f*
discovery 1:105
genome 1:105
open reading frame 2 1:106
infection
diseases associated 1:110
mixed 1:109
viral load *vs.* host immune status 1:110
morphology 1:105
Torque teno virus (TTV) 1:105, 1:108*f*
epidemiology 1:107
global distribution 1:107
prevalence rates 1:107
risk exposure 1:107
genome 1:106*f*
history 1:105
infection
hepatitis 1:110
mixed 1:109
viral load *vs.* host immune status 1:110
morphology 1:105
replication 1:105–106
open reading frame 1 1:106
untranslated region 1:105–106
sequence homology 1:109*f*
ToRSV *see* Tomato ringspot virus (ToRSV)
Torticollis, definition 1:76
Toscana virus (TOSV) 4:493
epidemiology 4:495
infection
clinical features 4:493
disease duration 4:494
seroprevalence studies 4:494
transmission 4:493
Phlebotomus perniciosus 4:491
Tospovirus 1:391, 4:490–491, 5:133, 5:157–158
Tospovirus(es) **5:157–163**
classification 5:157–158
definition 1:399
epidemiology 5:161
evolution 5:162
genetics 5:162
genetic drift 5:162
genome reassortment 5:162
haplotypes 5:162
genome 5:157–158
defective interfering RNA 5:159
IGRs 5:159
L RNA 5:160*f*
M RNA 5:160*f*
S RNA 5:160*f*
transcription termination 5:159
geographical distribution 5:158
history 5:157
infection
chrysanthemum infections 4:224
control 5:162
cytopathology 5:161
detection 5:162
diagnosis 5:162
economic aspects 5:158
host range 5:158
nemesia infections 4:220*f*
NSm protein role 1:393–395
Osteospermum infection 4:221
pathogenicity 5:161
petunia infection 4:213
prevention 5:162
resistance genes 4:179*t*
symptoms 5:161
propagation 5:158
protein properties 5:159
glycoprotein function 5:159–160
N protein 5:159–160
NSm protein 5:159–160
NSs protein 5:159–160
polyprotein cleavage 5:159–160
tubule induction 5:159–160
replication 5:160*f*
resistance 5:162
taxonomy 5:157
tobacco disease, economic importance 5:65
transmission 5:158
mechanical 5:162
seed/pollen 5:162
thrips 5:158
weeds 5:162
virion properties 5:159*f*
nucleocapsids 5:158
ribonucleoprotein 5:158
see also specific viruses
TOSV *see* Toscana virus (TOSV)
Totiviridae 2:286, 2:313, 3:220, 4:567, 5:164, 5:214
Totivirus 2:286, 5:164, 5:165*t*, 5:214
Totivirus(es) 2:111, 4:567
biological properties 5:169
classification 5:164
double stranded RNA satellites 4:531
evolution 4:65
evolutionary relationships 5:172
capsid sequences 5:173*f*
RdRp sequences 5:172*f*
gene expression 5:166
genera 5:214
genome 5:164
open reading frames 5:167*f*
RdRp expression 5:164
infection
fungi 2:287*t*
mixed 5:169
killer phenomenon 5:164
host genes associated 5:170–171
preprotoxin processing 5:301
toxin maturation 5:301
phylogenetic analysis 2:286
RdRp expression 2:286
replication cycle 5:167
RNA polymerase activity 5:167–168
taxonomy 5:164
transmission 5:169
virion composition 5:165
virion properties 5:164
virion structure 5:166*f*
capsids 5:165
virus–host relationships 5:170
see also specific viruses
Toxic shock syndrome, vaccine contamination 5:229
Toxin(s), bacterial, bacteriophage-mediated cell lysis 3:249–250
Toxoplasmosis, measles *vs.* 3:289–290
Toxoptera citricida (brown citrus aphid), citrus tristeza virus transmission 1:521
T phage(s), morphotypes 2:448*f*
TΦ promoter, T7 bacteriophage transcription 5:179
T proteins, murine polyomavirus 4:273
TPV *see* Tanapox virus (TPV)
Tracheal cells, nucleopolyhedrovirus 1:267
TRAF signaling, latent membrane protein 1 2:163
Trager duck spleen necrosis virus (TDSNV) 4:413–414
Tranosema rostrale ichnovirus (TrIV) 4:255
Trans-acting siRNA pathway, plant gene silencing 4:142
TAS genes 4:142
Transactivation domains
EBV-encoded nuclear antigen 3 family 2:162
maize streak virus 3:268
Transactivation response (TAR) element
JCV reactivation in HIV infection 4:266
RNA interference in HIV infection 1:161
Transactivator of translation/viroplasmin (TAV), cauliflower mosaic virus 1:467
Transcriptase, bunyavirus replication 1:396*f*
Transcription 4:410
discontinuous 1:554
double-stranded RNA viruses 4:410
immediate early 2:437

Transcription (*continued*)
single-stranded DNA viruses 4:410
single-stranded negative sense RNA 4:410
single-stranded positive sense RNA 4:410
single-stranded RNA viruses, DNA intermediate 4:410
Stp oncoproteins 4:589
see also specific viruses
Transcriptional gene silencing (TGS) 5:326
beet curly top virus pathogenesis 1:306
cotton leaf curl disease 1:568
definition 5:325
Transcriptional regulatory sequence(s) (TRSs) 1:554
Transcription factor IID (TFIID) 3:340
Transcription factors, plant resistance to viruses 4:176–177
Transcription-regulating sequences (TRSs)
arterivirus genome 1:180*f*
coronaviruses 1:551
crenarchaeal viruses 1:594
gill-associated virus 5:478–479
nidoviruses 3:428
Transcription transactivator, EBV-encoded nuclear antigen 2 2:161
Transcytosis, definition 4:507
Transduction
definition 5:411
feline leukemia viruses 2:188
Trans-envelope network, definition 2:191
Transesterification, definition 4:475
Transfected T-cell lines, Herpesvirus Ateles 4:587–588
Transfection, definition 5:411
Transfection studies, adenovirus malignant transformation 1:9–10
Transferrin receptor 1 (Tfr1), mouse mammary tumor virus cell entry 3:336
Transfer RNA (tRNA)
nonsense suppressor 4:337
pseudovirus taxonomy 4:352
reverse transcription priming 3:306
Transfer RNA-like structure
bromovirus 1:81
cucumovirus 1:81
definition 4:97
pecluvirus genome 4:100
Transfer RNA (Uracil-5)-methyltransferase 3:316
Transformation, malignant *see* Malignant transformation
Transformation effector sites (TES), latent membrane protein 1 2:162–163
Transgene(s)
definition 1:457
gene therapy 2:301
Transgenic mice
HCV infection 2:370–372
influenza virus infection 3:101
JCV oncogenic potential 4:268
SV40 pathogenicity 4:637–638
Transgenic pathogen-derived resistance, definition 1:520
Trans-Golgi vesicles, Cryphonectria parasitica virus infection 2:579
Translation 4:183*f*
maize streak virus 3:267
simian alphaherpesviruses 4:584
togavirus infection 5:122
Translational codons, definition 1:296
Translational repression
definition 3:21
icosahedral ssRNA bacteriophages *see* Icosahedral ssRNA bacteriophages
Translation enhancer elements, SINV infection 5:122
Translation initiation factors, mimivirus gene content 3:316
Translocating NTPase, ssDNA bacteriophage genome packaging 2:311–312
Translocation, host cell entry 4:409
Transmembrane glycoproteins, yellow head virus 5:477
Transmembrane (TM) protein, Simian retrovirus D 4:627
Transmissible gastroenteritis virus (TGEV) 1:550*t*
M glycoprotein 1:549–550
porcine 1:553
Transmissible mink encephalopathy (TME)
occurrence 5:187
prion strains 4:334–335
Transmissible spongiform encephalopathies (TSEs) *see* Prion diseases
Transmission 5:315
airborne 2:140
barriers 4:331
bottlenecks 4:364
definition 2:141
see also specific transmission modes
epidemiology 2:144
common patterns 2:146
modes 2:145
virus entry 2:144
virus shedding 2:144
horizontal *see* Horizontal transmission
maternal 1:376
nonviremic 5:440
routes 5:315
transovarial 1:170, 1:533, 5:111
definition 1:186
transplacental 1:351
definition 2:141
transstadial 1:533
vertical *see* Vertical transmission;
see also specific viruses
Transmission cycle, definition 5:469
Transmission electron microscopy, Providence virus 5:33
Transmission propensity, definition 5:433
Transovarial transmission *see* Transmission
Transplacental transmission *see* Transmissible gastroenteritis virus (TGEV)
Transposable elements, retrotransposons *see* Retrotransposons
Transposition, Bac-to-Bac expression vector 1:241
Transposons
Arabidopsis thaliana 3:309
definition 4:437
Transpososome, definition 4:400
Transpososomes, bacteriophage Mu 4:404–405
Transtadial infection, definition 1:483
Tree shrew herpesvirus 1:634
T regulatory cells (Tregs), HSV infection 2:393–394
Triangulation number
definition 1:433
icosahedral phage assembly 1:436
Triatoma virus (TrV) 2:42
Tribec virus
geographical distribution 3:465
infection 3:465
Trichomonas vaginalis virus(es) 5:172*f*
Trichoplusia ni, insect cell culture 1:238
Trichoplusia ni granulovirus (TnGV) 1:268–269
Trichoplusia ni TED virus (TniTedV) 4:453
definition 4:451
Trichoplusia ri virus (TrV) 5:28*t*
infection 5:36
Trichovirus 1:420*t*, 1:424, 1:426*f*
Trichovirus(es) **1:419–427**
associated species 1:424
biological properties 1:424
classification 1:420*t*
genome 1:424
open reading frames 1:424
particle structure 1:421*f*
phylogeny 1:426*f*
replication 1:424
serology 1:425
strains 1:425
transmission 1:424
see also specific viruses
Trigeminal ganglia, HSV latency 2:391
TRIM5α, HIV replication, role 2:521
TRIM5 gene/protein 2:469–470
evolution 2:470
HIV sensitivity 2:470
sequences 2:470
structure 2:470*f*
C-terminal domain 2:469–470
TRIM-Cyp, HIV inhibition 2:470
Triple gene block (TGB)
definition 4:310
Pepino mosaic virus 4:105
Triradiate lumen, definition 3:405
Triskelions, definition 1:328
Triticum mosaic virus (TriMV) 1:494
Tritimovirus 4:315*t*
tRNA *see* Transfer RNA (tRNA)
Trocara virus (TROV) 5:109*t*
Tropical spastic paraparesis/HTLV-1 associated myelopathy (TSP/HAM) 1:475
clinical features 2:563
diagnosis 2:570
disease characterization 2:571*f*
etiology 2:563
geographical distribution 2:570
incidence 2:563
incubation period 2:570
neurological features 2:570
pathogenesis 2:571
prognosis 2:571
therapy 2:571
TRRAP, adenovirus E1A gene/protein binding 1:11–12
TRSV *see* Tobacco ringspot virus (TRSV)
Trubanaman virus 1:400
TRV *see* Tobacco rattle virus (TRV)
Trypsin-like serine protease 1:226
TSD *see* Taura syndrome disease (TSD)
TSeq_orgname, virus database formats 5:349–351
ts mutants, Sigma virus replication 4:579
Tst-1 protein, JCV 4:264
TSV *see* Taura syndrome virus (TSV)
TTV *see* Torque teno virus (TTV)
Tuberculosis (TB), HIV related infection 1:68
Tubules, bluetongue virus replication 1:332
Tulip(s), infection history 2:450
Tulipa virus infections 4:224
see also specific viruses
Tulip breaking virus (TBV) 4:224*f*
Tulip mild mottle mosaic virus (TMMMV) 3:447
coat protein 3:450
disease-resistant cultivars 3:453
genome 3:449
geographical distribution 3:452*f*
Olpidium brassicae transmission 3:453
'Tulipomania' 2:450
Tulip virus X (TVX) 4:225
Tumor(s) 2:457
avipoxviruses infection 2:283
brain
BKV associated 4:270
JCV associated 4:268
EBV-encoded small RNA 2:164
FeLV associated 2:187–188
JCV infection 4:267
MHV-68 associated 3:377
murine polyomavirus 4:271
see also Cancer; Oncogene(s)
Tumor necrosis factor-α (TNF-α) 1:621
apoptotic role 1:157
function 1:621
Marburg virus infection 3:278
receptor binding 1:621
synthesis 1:621
Tumor necrosis factor binding protein (vTNF-BP) 1:157
Tumor necrosis factor receptor(s) (TNFR) 1:621
apoptotic role 1:157
Tumor suppressor(s)
adenoviral targets 1:9–10
E1A/pRB interactions 1:12
E7/pRB interactions 4:25
Tumor virus(es), human **5:193–199**
oncogenic virus features 5:193
see also specific viruses
Tungrovirus
open reading frames 1:466*f*
resistance genes 4:179*t*

virion structure 1:458–460
see also specific viruses
Tungrovirus 1:459*t*, 4:482
Tupaia, definition 1:104
Turbot aquareovirus (TRV) 1:164*t*
Turkey adenovirus 3 (TAdV-3)
classification 1:27
genome 1:4*f*
pathogenicity 1:8
Turkeypox virus 2:277
Turkeys, astrovirus infection 1:206
Turkish tobacco 5:61
Turnip crinkle virus (TCV) 1:454*f*
assembly 1:455
origin of 1:455
CP, role 1:456
defective-interfering RNAs 1:456
gene expression 1:455
gene silencing suppression 5:330
genome, intergenic regions 3:244
host range 1:454
resistance to 1:456
Arabidopsis thaliana 1:456
RNA recombination 4:379
RNA satellites 1:456
transmission 1:454
virion structure 1:454–455
CP subunit 1:454–455
Turnip mosaic virus (TuMV) 5:289
host range 5:289
Impatiens infections 4:218*f*
infection symptoms 5:289
petunia infection 4:213
structure 5:289
Turnip rosette virus (TRoV) 4:646*t*
Turnip vein clearing virus (TVCV)
Impatiens infections 4:214–215
petunia infection 4:211–212
Turnip yellow mosaic virus (TYMV) 5:200
TVCV *see* Tobacco vein clearing virus (TVCV)
TVL, host range 5:154
TVMV *see* Tobacco vein mottling virus (TVMV)
20S RNA 3:392
22S RNA 3:392
2A protein, enteroviruses 2:126
2B protein, picornavirus 4:136
2–5 A synthase/RNaseL pathway, togavirus infection 5:123–124
Twort, Frederick William, bacteriophage research 2:443
Ty1-Copia retrotransposon(s) 4:433
history 4:433
Ty1 gene/protein
replication cycle 4:355
virion structure 4:354*f*
Ty3/*gypsy* retrotransposon(s) 4:433
distribution 4:443
expression regulation 3:304
frameshifts 3:304–306
host genome integration
target site specificity 3:309
TFIIIB, transcription factor 3:309
TFIIIC, transcription factor 3:309
induction 3:304
reverse transcription priming 3:306
Ty elements, *Saccharomyces cerevisiae* 4:420
TYLCCNV *see* Tomato yellow leaf curl China virus (TYLCCNV)
Tymoviridae 2:255, 5:11*t*, 5:200
Tymovirus 5:200, 5:200*t*
Tymovirus(es) **5:199–207**
capsid structure 5:201*f*
empty capsids 5:201*f*
characteristics 5:201
classification 5:200*t*
fleck disease complex 4:203
genome 5:202*f*
coat protein 5:203
open reading frames 5:201
overlapping protein 5:203
replication polyprotein 5:203
tRNA-like structure 5:202–203
tymobox 5:203
infection 5:203
phylogenetic relationships 5:204*f*
properties 5:201
stability 5:201–202
replication 5:203
encapsidation 5:202
RNA replication 5:203
sites 5:201
vesicle formation 5:203
species demarcation 5:204
tobacco diseases 5:67
transmission 5:203
Typhus, measles *vs.* 3:289–290
Typography, species nomenclature 5:405
Tyrosine recombinase (YR) retrotransposons 4:421*f*
distribution 4:423
replication 4:426

U

U3 regions, Tnt1 elements 4:432
Ubiquitination, killer toxin immunity 5:307
Uganda, HIV infection 1:63
UHBV *see* Urochloa hoja blanca virus (UHBV)
Ukraine, HIV infection 1:64
UL5 protein 2:415
UL112 phosphoproteins 2:486–487
Ulcerative disease rhabdovirus (UDRV) 2:228
Ultraviolet absorbance spectra, potexvirus 4:310–312
Ultraviolet resonance Raman spectroscopy, definition 2:191
Umbravirus 5:209
Umbravirus(es) **5:209–214**
assistor virus interactions 5:212
groundnut rosette assistor virus 5:211
classification 5:209
gene expression 5:210
genome 5:211*f*
frameshifting 5:210–211
open reading frames 5:210–211
host range 5:213
infection 5:209–210
control 5:213
disease associations 5:209
fibrillarin 5:212
nucleolus involvement 5:212
prevention 5:213
resistance genes 4:179*t*
movement
cell-to-cell 5:211
phloem-dependent long-distance 5:212
nomenclature 5:209
replication 5:211
RNA satellites 5:211
tobacco disease, economic importance 5:66
transmission 5:209
aphids 5:213
virus properties 5:210*f*
Unassigned virus(es) 5:21–22
Una virus (UNAV) 5:489
Uncoating 4:409
baculoviruses 1:250
definition 4:407
endocytic pathway 4:409
products 4:409
replication 4:409
UniProt 5:363
United States of America (USA)
bioweapons 5:408
monkeypox virus 4:643
Universal tree of life 3:473
Untranslated regions (UTRs)
broad bean necrosis virus genome 4:283
giardiavirus genomes 2:314
LINE-like retrotransposons 4:439
nepovirus 3:408
pomovirus genome 4:283
potexvirus 4:312*f*
yellow head virus 5:479*f*
Unweighted pair group method with arithmetic means (UPGMA) 4:126–127
Upper respiratory tract, structure 2:551
Uranotaenia sapphirina nucleopolyhedrovirus (UrsaNPV) 1:220
occlusion bodies 1:220
transmission 1:221–222
Uranyl acetate stains 2:80*f*
limitations 2:79
Urbani, Carlo 4:552
Urbanization, Dengue virus 2:8*f*
'Urban type' yellow fever transmission 5:473–474
Ure2p prions
domain shuffling 4:339
essential sequences 4:339
formation 4:337
URE3 prion 4:337
Urethritis, HSV-2 infection 2:390–391
Uridine diphosphate glycosyltransferase (UGT) 2:112–113
Uridylate-specific endoribonuclease (NendoU) 5:477–478
Uridylylation
definition 4:129
picornaviruses 4:139–140
Urochloa hoja blanca virus (UHBV) 5:24
phylogenetics 5:26*f*
species discrimination 5:26
Urodeles, definition 3:155
UrsaNPV *see* Uranotaenia sapphirina nucleopolyhedrovirus (UrsaNPV)
Urticarial 5:83
Ustilago maydis virus(es) **5:214–219**
definition 5:214
genetic engineering 5:218
genome 5:214
H (heavy) dsRNAs 5:215
isolates 5:215
L (light) dsRNAs 5:215
M (medium) dsRNAs 5:215
ribosomal frameshifting 5:214–215
RdRp 5:214, 5:218
replication 5:217
capsid-associated transcriptase 5:217
L (light) dsRNAs 5:217
M (middle) dsRNAs 5:217–218
structure 5:218
taxonomy 5:214
toxins 5:215
KP1 5:215–216
KP4 5:216*f*
KP6 5:217*f*
SMK 5:216*f*
structure 5:216*f*
transcription 5:217
Ustilago maydis virus H1 (UmV-H1), genome 5:166
Uukuniemi virus, structure 4:490–491
Uveitis 2:573
HTLV-1 associated 2:573
prognosis 2:573
symptoms 2:573
therapy 2:573

V

V1 protein, beet curly top virus 1:303
V2 protein, beet curly top virus 1:303
V3 protein, beet curly top virus 1:303
Vaccine-associated paralytic poliomyelitis (VAPP) 4:246
Vaccines/vaccination 2:458
administration methods 3:68
aerosol 5:229
injection *see below*
intradermal *vs.* intramuscular 2:54
animal viruses 5:225
development strategies 5:235
antigenic variation 1:127–128
autogenous 1:440
components, reactions to 5:230
development **5:235–243**
animal models 5:236
antigenic variation 5:240
candidates 5:235–236

Vaccines/vaccination (*continued*)
DNA 5:239
multiple serotypes 5:240
principles 5:236
recommended cell cultures 5:237
technological advances 5:235–236
vectors 5:238
see also Vector(s)
viral epitopes 1:141
virus properties 5:235–236
diluent 5:226
history 2:51
human viral diseases 3:68
categories 3:68
effectiveness 3:68
injection 5:229
auto-disable syringe 5:229–230
unsafe practices 5:229
niche targets 1:153–154
organ transplantation, infections 3:471
persistent viral infections 4:108–109
plasma-derived 2:351
production in plants *see* Genetically-modified plants
safety *see below*
T-cell responses 3:76
technological advances 2:51–52
trials, epidemiology 2:144
types 5:236
see also specific types
vectors 4:44–45
veterinary *see* Veterinary vaccine(s)
Zaire Ebola virus infection 2:65
see also Inoculation
Vaccines/vaccination, safety **5:226–235**
administration 5:229
aerosol 5:229
auto-disable syringe 5:227
injectable 5:229
multidose vials 5:229
oral 5:229
single-dose vials 5:229
adventitious agent 5:226
adverse event following immunization *see* Adverse event following immunization (AEFI)
AEFI surveillance 5:232
aerosol 5:229
causality assessment 5:227–228
classical events 5:232
SV40 5:232
swine flu 5:233
contamination 5:229
plant produced vaccines 5:221
public perceptions 5:231
vial type 5:229
design 5:228
considerations 5:227*f*
functions 5:227
manufacture 5:228
killed 5:228
live 5:228
potency 5:228
monitoring and assessment 5:231
presentation 5:229
programmatic factors 5:227
freeze-dried 5:229
public perceptions 5:230
recipient reactions 5:230
common/mild 5:230
rare/severe 5:230
SV40 contamination 4:630, 4:637, 5:228
thermostability 5:230
thiomersal 5:233
vaccine-associated paralytic poliomyelitis 4:246
Vaccinia-like virus(es) 5:244
Vaccinia virus (VV) 4:325, **5:243–250**
antiviral therapy 5:249
apoptosis inhibition 1:161
attenuated strains 5:244
extracellular enveloped virus 5:244
intracellular mature virus 5:244
cell culture 5:248
DNA recombination mechanisms 4:375
expression vectors 5:249
genome 2:275, 5:245
host cell entry 5:245, 5:247*f*
host range 5:249
immune evasion 1:125
intracellular mature virus 4:325, 5:245
assembly 4:327, 5:248
attenuation 5:244
export pathways 4:328
proteinaceous bodies 4:327
structure 4:325
morphogenesis 4:328
origin 5:243
rabbits *see* Rabbitpox
replication 1:576, 5:245
assembly 4:327, 5:248, 5:248*f*
associated proteins 5:246–247
DNA replication 5:246–247
export pathways 4:328
host cell entry 4:327, 5:245
morphogenesis 4:327, 4:328*f*
transcription 5:245
taxonomy 5:244
strains 5:244–245
vectors 5:249
virion structure 4:325, 5:245
core 4:325, 5:245
extracellular enveloped virus 4:325
intracellular mature virus 4:325
morphogenesis 5:248*f*
virulence 5:249
animal models 5:249
associated proteins 5:249
attenuation 5:249
immunomodulators 5:249
intradermal infection 5:249
intranasal infection 5:249
Vacuolar protease B (Prb1p), prion function 4:340
Vacuoles, sobemovirus localization 4:649–650
Valaciclovir 1:143*t*
antiviral prodrugs 1:150
HSV infection 2:394–395
Valcyte® 1:143*t*
Valency, definition 3:413
Valganciclovir (VGCV) 1:143*t*, 1:146
Valtrex® 1:143*t*
Vankyrin gene family
Campoletis sonorensis ichnovirus 4:258–259
Microplitis demolitor bracovirus 4:259–260
phylogeny 4:261
Variable number tandem repeat (VNTR), SVA retrotransposons 4:440
Variant Creutzfeldt–Jakob disease (vCJD) 1:371, 4:331–332, 4:333*f*, 4:335*f*, 5:191
associated cases 1:372, 5:191
classical Creutzfeldt–Jakob disease *vs.* 5:191*t*
clinical and subclinical infection 1:371
characteristics 5:191*t*
diagnosis 5:191–192
mortality rate 1:372
prevalence 1:371
epidemiology 1:372
associated deaths 1:372, 1:372*f*
valine homozygotes 1:372
geographical distribution 1:372–373, 1:373*t*
history 1:368
infectivity 1:371
mortality rate 1:368
origin 1:371
pathogenesis 1:371
prevention and control 1:372
public health implications 1:373
blood transfusions 1:373
sporadic *vs.* 1:371
transfusion associated 4:334, 5:192
Varicella-zoster virus (VZV) **5:250–256**, 5:254*f*
assembly 5:261
electron microscopy 5:261
Golgi apparatus 5:261
bacterial artificial chromosome 5:256
classification 5:251
cosmid transfection system 5:256
cytopathology 5:261
epidemiology 5:252
geographical distribution 5:251
seasonal distribution 5:251
evolution 5:252
clades 5:252
future work 5:255
wide-spread vaccination 5:255
gene expression 5:259
host cell transcription apparatus 5:259
see also specific genes
genetics 5:251
strains 5:251–252
see also specific genes
genome 5:252, 5:256
HSV *vs.* 5:252
open reading frames 5:256
genome replication 5:258
bidirectional 5:258
rolling-circle mechanism 5:258
historical aspects 5:250–251
first cultivation 5:251
host range 5:251
immediate early (IE) gene expression 5:259
host cell factor-1 (HCF-1) 5:259
IE62 5:259
Oct-1 recognition sequence 5:259
infection *see* Chickenpox; Zoster (shingles); *see below*
molecular biology **5:256–263**
nucleocapsid 5:256
post-translational processing 5:261
glycosylation 5:261
phosphorylation 5:261
productive infections 5:256
cell attachment 5:256–258
promoters 5:259
classification 5:259
TATA-binding protein 5:259
propagation 5:251
cell culture 5:251
protein translation 5:260
release 5:261
tissue culture studies 5:261
reservoir 5:251
serology 5:252
enzyme-linked immunosorbent assay 5:252
fluorescence antibody to membrane antigen 5:252
immune adherence hemagglutination 5:252
taxonomy 5:251
transmission 5:253
vaccines/vaccination 4:108–109, 5:255
attenuation mechanisms 5:262
effects of 5:255
Oka vaccine strain 5:253, 5:255
variability 5:252
restriction enzyme digests 5:252
virion structure 5:256
morphology 5:256
Varicella-zoster virus (VZV), infection
animal models 5:251
eye 2:496
histology 5:253
cutaneous lesions 5:254
eosinophilic inclusions 5:254, 5:254*f*
immune response 5:254
cellular immune responses 5:254–255
cytotoxic T-cells 5:254–255
humoral immune responses 5:254
life-long immunity 5:254–255
latency 2:438, 5:253
gene expression 5:261
gene regulation 5:262
host cell factor-1 5:262
IE63 5:261
ORF29 5:262
management 5:255
immune globulin 5:254

organ transplantation 2:496
pathogenicity 5:253
pathology 5:253
prevention 5:255
acyclovir 5:255
exposure 5:255
immunoglobulin prophylaxis 5:255
isolation 5:255
leukocyte interferon 5:255
primary 5:253
tissue tropism 5:253
see also Chickenpox; Zoster (shingles)
Varicellovirus 5:251, 5:347
Varicosavirus 5:263, 5:264
Varicosavirus(es) **5:263–268**
evolution 5:264
genetics 5:264, 5:264*f*
geographical distribution 5:267
historical aspects 5:263
host range 5:267
tobacco disease, economic importance 5:66
transcription 5:267
transmission 5:268
vectors 5:268
see also Lettuce big-vein virus (LBVaV); *specific viruses*
Variolae vaccinae, history 1:574
Variola major, bioterrorism 5:407
biotechnological impacts 5:409–410
as a bioweapon 5:408
Variola minor 4:639
Variolation *see* Smallpox
Variola virus (VARV) 4:322–323, 4:639
classification 4:639
evolution 4:643
genetics 4:643
historical aspects 4:639
infection *see* Smallpox
molluscum contagiosum virus *vs.* 2:276
person-to-person transmission 4:643
replication
extracellular enveloped virions 4:639
intracellular mature virions 4:639
Varkud satellite (VS) ribozyme(s) 4:475
secondary structure 4:480*f*
VA RNA genes, adenovirus replication 1:3–4
immune system evasion 1:22–23
Varroa destructor virus 1 (VDV-1) 3:42
genome 3:45
tissue tropism 3:45
Varroa mite(s), iflavirus transmission 3:42, 3:45
Vascular choroid 2:491
Vascular dysfunction
Ebola virus infection 2:62, 2:63, 2:203
HCMV infection 2:474
Marburg virus infection 2:203, 3:278
Vascular endothelial cells, neural cell infection 1:473
Vascular endothelial growth factor (vEGF) 4:61–62
Vascular lesions, rubella virus infection 4:519
Vascular permeability, Dengue fever 2:12
VaxGen, HIV-1 vaccine trials 1:70
VBRC database 5:357*t*
V-CATH, baculovirus terminal disease 1:269
vcyv protein, KSHV latency 3:198
Vector(s)
animal viruses *see below*
bridge vectors *see* Bridge vector(s)
competence 4:653, 5:271
definition 1:390, 5:269
post-transcriptional gene silencing 5:271
RNA interference 5:271
definition 2:141, 2:146, 2:234, 2:301, 3:212, 4:164, 4:208, 5:60, 5:107, 5:269, 5:274, 5:282, 5:470
extrinsic incubation period 5:271–272
feeding 5:269
blood-meal host-selection patterns 5:271
gene silencing
DNA viruses 5:378
RNA viruses 5:376
infection control 1:295, 5:494
introduction 4:208
maintenance 4:652
plant viruses *see* Plant virus(es)
transient 5:60
vaccine development strategies 5:236, 5:238
see also specific vectors
Vectorial capacity 5:271
daily survival 5:271
definition 5:269
Dengue viruses 5:271
vector blood-meal host-selection patterns 5:271
zoonoses 5:271
Vectors, animal virus(es) **5:268–274**
bridge vectors 5:270
cycle types 5:270, 5:270*f*
arthroponoses 5:270, 5:270*f*
zoonoses 5:270
definition 5:269, 5:269*f*
enabling factors 5:271
vector competence 5:271
vectorial capacity 5:271
extrinsic incubation period 5:271
definition 5:271–272
temperature limits 5:271–272
vector behavior 5:271–272
forecasting risk 5:274, 5:274*f*
surveillance measures 5:274
horizontal 5:269
gonotrophic cycle 5:269
vector feeding 5:269
nonviremic transmission 5:270
phases of transmission 5:272
amplification 5:272
maintenance 5:272
subsidence 5:272
risk factors 5:273
age 5:273
climate 5:273
occupation 5:273
residence 5:273
socioeconomic status 5:273
venereal transmission 5:270
vertical 5:269
ticks 5:269
see also specific viruses
Vegetable(s) 5:282
losses 5:283
see also Crop(s); *individual species*
Vegetable virus(es) 2:87, **5:282–291**
ecology 5:284
epidemiology 5:284
garlic viruses 5:288
hosts 5:284–285
infection control measures 5:285*t*, 5:286
cross-protection 5:286–287
natural resistance 5:286–287, 5:287*t*
roguing 5:286–287
soil contamination 5:286–287
pathogenicity 5:284
genetic variability 5:284–285
taxonomy 5:283, 5:283*t*, 5:284*t*
transmission
control 5:286
environmental factors 5:285
mechanical 5:284–285
seeds *see* Seed(s), viral transmission
vectors 5:284–285
vegetative propagation 5:284–285, 5:286*t*
see also specific viruses
Vegetative incompatibility *(vic)* genes 2:576
definition 2:284, 2:580
Vegetative propagation
banana bunchy top virus transmission 1:277
cassava mosaic geminiviruses transmission 1:32–33
definition 4:208
Ilarviruses 3:54
potato virus Y transmission 4:288–289
vegetable virus transmission 5:286*t*
Vein-clearing, definition 5:200
Veins, plant virus movement 3:353–354, 3:354*f*
Velum, definition 4:561
Velvet tobacco mosaic virus (VTMoV) 4:646*t*
Venereal transmission
LaCrosse virus 5:269
vector transmission, animal viruses 5:270
see also Sexually transmitted disease(s)
Venezuelan equine encephalitic virus (VEEV) 5:79, 5:111–112, 5:112*f*, 5:114
bioterrorism evaluation 5:407–408, 5:408*t*
characteristics 1:171*t*
geographical distribution 5:77, 5:102, 5:488
host cell attachment 5:118
host range
enzootic 5:79–80, 5:103, 5:104
epizootic 5:79–80, 5:103, 5:104
structure 5:118*f*
subtypes 5:80
tissue tropism 5:104
transmission 5:79–80, 5:104, 5:111–112, 5:112*f*
Aedes 5:99–100, 5:104, 5:112
enzootic 5:80, 5:114
epizootic 5:114
minimum infectious dose 5:112
vaccines/vaccination 5:488
Venezuelan equine encephalitic virus (VEEV) infection 5:488
clinical features 5:105
adults 5:80
children 5:105
diagnosis 5:82
epidemic 5:80, 5:101, 5:488
epidemiology 5:104
experimental 5:105
fatality rates
horses 5:80, 5:488
humans 5:488
immune response 5:80–81
blood–brain barrier 5:81
cell-mediated cytotoxicity 5:81
γβ T-cell deficiency 5:81
vaccinated animals 5:81
murine model 5:80–81, 5:115
pathogenesis 5:115
pathogenicity 5:105
pathology 5:80
symptoms 5:114
treatment 5:82
Venezuelan hemorrhagic fever 3:207
clinical features 3:209
epidemiology 3:207
histopathology 3:210
pathology 3:210
Venturia canescens, polydnavirus immunosuppressive activity 4:255–256
Verbenaceae virus infections 4:218
nonspecific infections 4:219, 4:221*f*
see also specific viruses
Vero cells
definition 2:93
phlebovirus isolation 4:494
Vertebrates
Akabane virus infections 1:77
as viral reservoirs in zoonoses 2:147
Vertebrate virus(es) **2:455–459**
see also specific viruses
Vertex, definition 5:365
Vertical transmission 2:5
definition 2:37, 2:141, 2:146, 5:274, 5:440
edornaviruses 2:113, 2:115
LaCrosse virus 5:269
mouse mammary tumor virus 3:337
Oryza sativa endornavirus *see* Oryza sativa endornavirus (OsEV)
Sigma virus 4:578
tick-borne encephalitis virus 5:269
yellow head virus 5:480
Very late factor-1 (VLF-1) 1:270–271
Very late infection phase, baculovirus in vitro pathogenesis 1:272
Vesicle-associated membrane protein-associated protein A (VAP-A) 2:373
Vesicle packet(s), flaviviruses 1:199

Vesicles
  definition 4:639
  smallpox 4:639–640
Vesicular disease, definition 1:410
Vesicular exanthema 1:410
  clinical features 1:417
  control 1:418
  epidemiology 1:415–416
  incubation period 1:417
  pathogenesis 1:416
Vesicular exanthema virus infection *see* Vesicular exanthema
Vesicular lesions, foot-and-mouth disease virus 2:272
Vesicular stomatitis, disease history 1:497
Vesicular stomatitis virus(es) (VSV) **5:291–299**
  assembly 3:333
  classification 5:291
  diagnosis 5:298
  distribution 5:298
  evolution 5:297
    ecological factors 5:297
    mutations 5:297
    origins 5:297
  fusion loops 3:300
  genome 3:326*f*, 5:292, 5:292*f*
  glycoproteins
    function 5:292–293, 5:296
    glycoprotein G 3:299–300, 5:312–313
    structure 5:296
  history 5:291
  host range 5:296
  immune response 5:298
  infection
    incubation period 5:298
    pathogenesis 5:298
    pathology 5:298
    prevention and control 5:298
    zoonoses 5:489
  matrix (M) proteins 5:295
    domains 5:295
    function 5:295–296
    kinetics 5:295
    structure 5:295–296
  nucleocapsid core
    immune response involvement 5:298
    structure 5:295
  particle structure 5:291
    RNP core 5:291–292
  protein functions 5:295
    glycoprotein 5:292–293, 5:296
    large polymerase 5:296
    matrix protein 5:295
    nucleocapsid 5:295
    phosphoprotein 5:295
  replication 5:292, 5:293*f*
    assembly 5:295
    attachment 5:292
    budding 5:295
    cell culture 5:296–297
    gene expression 5:293
    host cell entry 5:292
    uncoating 5:292
  RNA polymerase II 5:293–294
    error rate 5:297
    functionally distinct pools of 5:294
    Le+ role 5:294
    mRNA cap formation 5:294
    mRNA synthesis 5:292*f*
  RNP core
    function 5:295
    structure 5:291–292
  structure 5:291
  taxonomy 5:291
  transmission 1:115, 5:291, 5:297
    experimental 5:296
  see also specific strains
Vesicular stomatitis Algoas virus (VSAV) 5:297–298
Vesicular stomatitis Indiana virus (VSIV) 4:184*t*
  characteristics 1:171*t*
  history 5:291
  structure 5:292*f*
Vesicular stomatitis New Jersey virus (VSNJV) 4:184*t*
  distribution 5:297–298
  history 5:291
  transmission 5:296
Vesiculovirus 1:497, 1:498*t*, 5:291, 5:291*t*
Vesiculovirus(es) 1:119*t*
  definition 2:221
  genome 3:326*f*
  geographical distribution 5:291*t*
Vesiculovirus-like virus(es) 2:222*t*, 2:224
  genome 2:224*f*, 2:225
    intergenic regions 2:225
  phylogeny 2:225
  virion structure 2:224*f*, 2:225
Vesivirus 1:411*t*, 3:439
Vesivirus(es)
  genome 1:413
  history 1:410
  infections 3:439*t*
  stability 1:412
  see also specific viruses
Veterinary vaccine(s) 3:68
  aims 3:68
  research and development 3:68
    development strategies 5:235
VFLIP
  apoptosis 1:160
  KSHV 3:198–199, 3:200
Vibrio parahaemolyticus 3:123–124
Vicia cryptic virus (VCV) 1:99
  cultivar analysis 1:103
  genome 1:100, 1:101*f*
    5′ nontranslated region 1:101, 1:101*f*
    open reading frames 1:100
Vicia faba endornavirus (VfEV) 2:110–111
  genome 2:110*f*
  molecular evolution 2:115
Vicriviroc 2:507*t*
  HIV entry inhibitors 2:506
VIDA database 5:357*t*
VIDE (Virus Identification Data Exchange) 5:357*t*
Videx® 2:510
Vietnam, DENV-2 2:7–8
vif gene/protein
  definition 2:467
  lentiviruses 2:471
Vinnexin gene family 4:258–259
Viola mottle virus (VMoV) 4:311*t*
VIPERdb database 5:357*t*, 5:363
VIPER-like elements 4:421–423
  replication 4:426
Viracept® 2:514*t*
Viral binding sites (VBS), Saccharomyces cerevisiae L-A virus replication 5:301, 5:302*f*
Viral Bioinformatics - Canada 5:357*t*
Viral chemokine-binding protein (vCBP), parapoxviruses 4:62
Viral chemokine receptors (vCCRs)
  HHV infection 2:501
  see also specific receptors
Viral 'cross-talk' transplant rejection 3:468–469
Viral degradation complex (VDC) 4:55
"Viral eukaryogenesis," 3:478
Viral Genome Database (VGD) 5:351–352, 5:351*f*, 5:355*f*
Viral genome linked protein (VPg) 4:288
Viral Genomes Project 5:362
Viral hemorrhagic septicemia virus (VHSV) 2:228, 4:184*t*
  economic effects 2:227, 2:228
  host range 2:228
  reverse genetic analysis 2:228
  salmonid infection 2:221–222
Viral inclusion bodies (VIBs) 1:332
Viral infectivity factor (Vif) 2:523, 4:598–599
  mechanism of action hypotheses 2:523
  SIV infection 4:622
Viral inhibitor of apoptosis (vIAP) 3:200
Viral interferon resistance protein (OVIFNR) 4:62
Viral load
  definition 2:505
  T cells, response effects 3:76
Viral mitochondria-localized inhibitor of apoptosis (vMIA) 1:158*t*, 1:162
Viral nervous necrosis 2:229–230
'Viral ocean' 3:474
Viral protein genome-linked (VPg) protein 2:125
  definition 2:37, 4:129, 4:644
  nepovirus 3:407–408
  picornavirus replication 4:132–133
  potyvirus protein properties 4:317–319
  recessive plant resistance genes 4:178–182
  replication strategy, birnavirus evolutionary relationships 1:326
  viral resistance 4:184
Viral vascular endothelial growth factor (vEGF) 4:61
Viramune® *see* Nevirapine
Viread® *see* Tenofovir
Viremia 1:399, 4:653
  active 5:315–316
  African horse sickness 1:40
  African swine fever virus 1:44
  cell-associated 5:316
  definition 4:40, 5:269, 5:440
  Dengue virus 2:9
  Ebola virus infection 2:62
  hepatitis C 2:368
  hepatitis C virus 2:368
  immunoglobulin role 3:79–80
  phleboviruses 4:494
  plasma 5:316
    transit time 5:316
  poliovirus 4:243
  termination 5:315–316
Viremogenicity 4:653
vIRF protein(s) *see* Kaposi's sarcoma-associated herpesvirus (KSHV)
Virginia tobacco 5:61
Virion(s)
  adsorption, bacteriophages 2:73
  cacao swollen shoot virus 1:404
  definition 1:433, 1:483, 2:234, 2:306, 2:505, 3:30, 4:407, 4:623, 5:157, 5:365
  dissociation, bacterial cell viral entry 5:367
  enveloped 4:514
  epitope occupancy
    definition 3:413
    infection neutralization 3:414
  immature 1:193
  iridoviruses 2:233
  Japanese encephalitis virus 3:183, 3:184
  lysis-induced release 3:249
  Maize streak virus 3:268
  necrovirus 3:403
  ophiovirus 3:448
  penetration 5:367
  potexvirus 4:310
  production 3:401–402
  receptor-binding site 3:401
  structure 2:457
    hepatitis B surface antigen 2:352
  togaviruses 2:230
  toroviruses 5:152, 5:152*f*
  virus *vs.* 3:400
Virion-associated transcriptional transactivator (VATT) 3:170
Virion-containing vesicle(s) 1:186
  see also specific viruses
Virion-induced transcriptional transactivator (VITT) 3:170
Virobacterial agglutination test (VBA) 1:408
Viroceptors 5:318
  leporipoxvirus infection 3:227
Viroid(s) **5:332–342**
  classification 5:11*t*, 5:333*t*, 5:335, 5:336*f*
  definition 2:375, 3:348, 5:332
  evolution 3:475, 5:336*f*, 5:341
  genome 5:332, 5:333*t*, 5:334*f*
    domains 5:333
    loop E motif 5:333–335

properties 5:332
ribozyme-mediated cleavage 5:333–335, 5:334*f*
geographical distribution 5:337
history 5:332
host range 5:335, 5:336*f*, 5:341
infections *see below*
movement 5:339
cell-cell 5:339
origin 5:341
replication 5:339
α-amanitin 5:339
error rate, evolution related 5:341
host-encoded enzymes associated 5:339
RNA cleavage 5:340*f*
structure
stability 5:341
tertiary 5:333–335
transmission 5:335
agroinoculation 5:335
experimental 5:335
wild species *vs.* crops 5:338
virus-induced gene silencing 5:330
Viroid infections
control 5:338
cytopathic effects 5:335
diagnostic tests 5:338
epidemiology 5:338
pathogenicity 5:339
small interfering RNA 5:341
symptomatology 5:335, 5:337*f*
Virokines 5:318
leporipoxvirus infection 3:227
see also Cytokine(s)
VirOligo database 5:357*t*
Viroplasm(s) 4:194–195, 4:194*f*
definition 2:18
Viroporin(s) 5:309
membrane proteins 5:309
picornavirus replication 2:127
Virosomes 5:310
VirP1 protein 5:339
Virucidal agent(s) *see* Antiviral drug(s)
Virulence 5:317
attenuating mutations, genetic determinants 5:317, 5:318
definition 4:178
genetic determinants 5:317
attenuating mutations 5:317, 5:318
'cell-derived' genes 5:318
leporipoxviruses 3:227
phenotype 5:317
principles 5:317
suipoxviruses 3:227
measurement of 5:317
pathogenic mechanisms 5:318
vaccine safety 5:228–229
vaccinia virus 5:249
variation, viral evolution 2:175
see also specific viruses
Virulence factors 3:123
Avr proteins 4:173
transmission 3:123
yellow fever virus *see* Yellow fever (YF) virus
Virulence gene(s) 4:170
Virulence perturbation, hypovirulence 2:575
Virulent-systemic (VS) feline calicivirus disease 1:416
Virus(es) **3:398–403**
antigen detection 2:33
assembly
antiviral therapy 1:149
virus membranes 5:312
associated nucleic acids 4:526
attachment 1:148
bacteria *vs.* 2:456
binding neutralization 3:64–65
biochemistry 2:456
caspase regulators 1:160
chemical composition 2:456
Chlamydia vs. 3:476
coat protein DNA sequence 4:168
core 3:6
'cross-talk' apoptosis 1:155–157
cultivation 2:456
definition 2:451, 2:455, 3:400
discovery 3:398
history 3:398
diseases 3:66
see also specific diseases
DNA-repair systems 4:374
DNA virus recombination 4:374
envelope 5:365
evolution *see* Evolution (of viruses)
fitness 3:402
antiviral therapy 1:151
definition 3:402
fusion 5:310
hemifusion diaphragm 5:310–311, 5:311*f*
stalk-pore pathway 5:310–311, 5:311*f*, 5:313*f*
virosomes 5:310
fusion proteins 5:310
activation 5:312, 5:313*f*
properties 5:311, 5:313*f*
structural features 5:312
genomes 2:457
evolution of 3:477–478
integration 3:401
historical aspects **2:455–459**
immune system evasion 1:637, 2:144–145, 2:487
induced cell transformation 3:180
infection neutralization 3:417
isolates 3:402
isolation 1:602
latency *vs.* active replication 3:401–402
life cycle 3:400
membranes *see* Virus membrane(s)
mitochondrial apoptosis 1:161
movement proteins 4:283–284
origins *see* Origin(s) of viruses
perpetuation in nature 2:146
arthropod transmission cycles 2:147
host clinical status 2:146
host population immunity 2:146
population size 2:147
virus virulence 2:146
zoonotic transmission cycles 2:147
progeny 3:402
proteins 3:401, 3:401*f*
escape hypothesis 3:473–474
expression 1:35
immunogenicity *see* Immunogenicity
purification
BaculoDirect expression vector system 1:241
X-gal 1:240–241
release 4:412
replication *see* Replication
serotypes 3:402
antigenic variation 1:128
species classification 5:405
specific sequence data 5:357*t*, 5:363
strains
characteristics 3:402
definition 3:402
structure **5:393–401**
helical symmetry 5:393
icosahedral symmetry 5:394
particle maturation 5:399
picorna-like capsids 5:398
quasi-equivalent capsids 5:395
structures **5:393–401**
subversion 1:125
transfer RNA 3:375
transmission *see* Transmission; *see specific transmission modes*
tropism 2:505
vaccines *see* Vaccines/vaccination
virion *vs.* 3:400
virus particle *vs.* 3:400
see also specific topics
see also specific viruses
Virus-associated interfering RNA (VAI RNA) 1:3–4, 1:161
Virus attachment protein, definition 4:407
Virus-enhancing factor (VEF), baculoviruses 1:250
Viruses: From Structure to Biology 5:357*t*
Virus-first hypothesis 3:473, 3:475, 3:476*f*
Virus-free seeds, alfalfa mosaic virus 1:86
Virus Identification Data Exchange project (VIDE) 3:213
Virus-indexed propagation program(s) 4:200
Virus-induced gene silencing (VIGS) 4:148, **5:325–332**, **5:375–380**
Agrobacterium tumefaciens-based transformation vectors 5:377, 5:379
Arabidopsis thaliana 5:326–327
barley stripe mosaic virus 2:466
definition 5:325, 5:375–376
food crops, vectors 5:378
functional genomics tool 5:376
advantages 5:379
DNA virus-based vectors 5:326, 5:377*t*, 5:378
limitations 5:379
RNA virus-based vectors 5:376, 5:377*t*
VIGS-related phenotype 5:378
gene characterization applications 5:378
resistance gene pathways 5:378
GFP-transgene silencing 5:326
history 5:325
induction 5:325
overexpression 4:148
phytoene desaturase gene 5:326
principles 5:376
solanaceous hosts 5:378
suppression **5:325–332**, 5:329*t*
cross-kingdom activity 5:328–330
HC-Pro 5:327–328, 5:328*f*, 5:330
immune evasion 5:327
miRNA pathway 5:328*f*, 5:331
plant development, affects 5:330
reversal of silencing assays 5:328
transgenic plant grafting 5:328
viral synergism 5:327–328
tobacco mosaic virus 5:376
transgenic resistance, plants
cross-protection 5:326
tobacco 5:325–326
tomato 5:325–326
viral-derived siRNAs 5:326
antiviral therapies 5:327
Virus infectivity factor (Vif)
HIV-1 infection 4:598–599
immune system evasion 3:109
SIV infection 4:622
Virus-like particles (VLPs) 1:98
algal infections 1:87
birnavirus replication 1:326
definition 4:97, 4:352, 4:428
LTR-retrotransposon life cycle 4:430
pecluvirus 4:102
pseudoviruses 4:352–353
salterprovirus 5:418
Virus membrane(s) **5:308–314**
acquisition 5:308
budding 5:313, 5:313*f*
M proteins 5:314
fusion *see below*
ligand role 5:310
endocytosis 5:310
host cell attachment 5:310
lipid bilayers 5:308
lipid arrangements 5:308–309
lipid composition 5:309
lipid rafts 5:309
proteins 5:309, 5:310*f*
aggregation 5:309
functions 5:308
integral 5:309
peripheral 5:309
viroporins 5:309
pseudotype virions 5:313
synthesis 5:310*f*, 5:312
glycosylation 5:312
viral assembly 5:312

Virus membrane fusion 5:310
  hemifusion diaphragm 5:310–311, 5:311*f*
  proteins 5:310
    activation 5:312, 5:313*f*
    properties 5:311, 5:313*f*
    structural features 5:312
  stalk-pore pathway 5:310–311, 5:311*f*, 5:313*f*
  virosomes 5:310
Virus nervous necrosis disease 3:432
Virus neutralization test (VNT) 1:531
Virus World 5:357*t*
Viscerotropism *see* Yellow fever
Visna 4:460
Visna-Maedi virus(es) (VMV) **5:423–432**
  animal importation 5:431
  cell tropism 5:424, 5:425
    cellular receptors 5:426
  env gene/protein 5:431
  genome 5:424, 5:425*f*, 5:425*t*
  geographical distribution 5:430
  history 5:424
  infections *see below*
  replication cycle 5:424
    macrophage infection 5:425–426
    provirus integration 5:424
  synonyms 5:424
  transmission 5:426
    oral 5:426
    respiratory 5:426
  vaccines/vaccination 5:431
    Freund's incomplete adjuvant 5:431
  virion structure 5:424
Visna-Maedi virus infection
  acute infection cannulation model 5:426
  clinical lung disease 5:424
  control 5:431
    animal importation 5:431
    eradication 5:431
    serological tests 5:431
  dendritic cells 5:425
  escape mutants 5:430–431
  granulocyte monocyte colony stimulating factor 5:428–430
  Icelandic epidemic 5:424
  immune response 5:426
    abnormalities 5:428
    acute infection cannulation model 5:426, 5:427*f*
    antibody response 5:427
    CD8+ T cells 5:427
    ENV antigens 5:427
    GAG antigens 5:427
    genetics, role 5:430
    macrophages *see below*
    neutralizing antibodies *see below*
    protective immune response 5:427
    seroconversion 5:426–427
    T-lymphocyte response 5:427
  immune system evasion 5:430
    CD4+ T cells 5:430–431
    cell fusion 5:430–431
    genome integration 5:430–431
  interferons, type I 5:430
  latency 5:425–426
  macrophages 5:425
    immune system evasion 5:430–431
    pathogenesis 5:428–430
    phenotypic changes 5:428–430
  neutralizing antibodies 5:430–431
    vaccination 5:431
  pathogenesis 5:428–430, 5:429*t*
    associated tissues 5:428
    autoimmunity 5:430
    clinical signs 5:428
    cytopathic effects 5:428
    host cell entry 5:428
    immunopathology 5:428
    lesion composition 5:428
    lymphocytes, role 5:430
  persistent 5:428
  resistance 5:430
  treatment 5:431
Visnia 1:474
Vitivirus 1:420*t*, 1:425, 1:426*f*
Vitivirus(es) **1:419–427**
  associated species 1:425
  biological properties 1:425
  classification 1:420*t*
  disease characterization 1:425
  genome 1:425
  hosts 1:425
  particle structure 1:421*f*, 1:425
  phylogeny 1:426, 1:426*f*
  replication 1:425
  serology 1:426
  transmission 1:425
  see also specific viruses
Vitrification, cryo-electron microscopy 1:604, 2:83–84
  CEMOVIS 2:84
  methods 2:83–84
  sample concentration 1:604
VLF-1 protein 1:257–260
VLPA3 virus 5:413*t*
VLP-based, papillomavirus infection 4:17
VOCs database 5:357*t*
VOGs database 5:357*t*
Voles
  cowpox virus hosts 1:577, 1:578
  murine gammaherpesvirus 68 3:372
Vomiting, rotaviruses 4:512
V-*ori* 3:264
VP1 gene/protein
  African horse sickness virus 1:37
  aquareovirus 1:165–166
  circovirus *see* Circovirus
  echovirus detection 2:69
  foot-and-mouth disease virus 2:266–267
  micromonas pusilla reovirus 1:91–92
  murine polyomavirus 4:271
  seadornavirus 4:538
  swine vesicular disease virus structure 2:128–129
VP2 gene/protein
  aquareovirus 1:165–166
  circoviruses 1:517
  infectious bursal disease virus *see* Infectious bursal disease virus
  murine polyomavirus 4:271
  parvovirus capsid structure 4:86
  poliovirus 2:129
  seadornavirus 4:538
  swine vesicular disease virus structure 2:129
VP3 gene/protein
  African horse sickness virus 1:37
  aquareovirus 1:165–166
  birnaviruses 1:325
  murine polyomavirus 4:271
  poliovirus 2:129
  seadornavirus proteins 4:538
  swine vesicular disease virus structure 2:129
VP4 gene/protein
  African horse sickness virus 1:37
  aquareovirus 1:165–166
  birnaviruses 1:325, 1:325*f*
  poliovirus 2:129
  seadornavirus proteins 4:538
  swine vesicular disease virus structure 2:129
VP5 gene/protein
  aquareovirus 1:165–166
  birnaviruses 1:325
VP6 gene/protein
  African horse sickness virus 1:37
  aquareovirus 1:165–166
VP7 gene/protein
  African horse sickness virus 1:37, 1:38*f*
  aquareovirus 1:165–166
  seadornavirus 4:541–542
VP8 gene/protein, seadornavirus proteins 4:538
VP9 gene/protein
  Banna virus 4:542
  seadornavirus proteins 4:538
VP16 gene/protein, HSV latency 2:437
VPg protein
  barley yellow dwarf viruses 1:280, 1:282
  cereal yellow dwarf virus 1:282
  nepovirus 3:409–410
  rhinoviruses 4:469–470
  rice yellow mottle virus 4:487
Vpr *see* Lentivirus protein R (Vpr)
V protein, henipavirus 2:323, 2:326
Vpu gene/protein *see* HIV-1
v-Rel complexes *see* Reticuloendotheliosis viruses
VTA virus 5:413*t*

W

Waikavirus
  genome 4:548
  tissue tropism 4:547
Waikavirus 4:547, 4:547*t*
Walleye dermal sarcoma (WDS) 2:214*f*, 2:215
  appearance 2:214
  incidence 2:214
  viral accessory protein roles 2:215–216
Walleye dermal sarcoma virus (WDSV) 2:231
  accessory proteins, pathogenic role 2:215
    OrfB protein 2:216
    OrfC protein 2:216
    origins 2:216
  disease cycle control 2:216
  genome
    alternative splicing 2:215*f*, 2:216
    open reading frames 2:214–215
  infection *see above*
  isolation 2:214, 2:215*f*
  molecular biology 2:231
  transmission 2:214
Walleye discrete epidermal hyperplasia (WEH) 2:214, 2:214*f*
  tumor incidence 2:214
Walleye epidermal hyperplasia virus(es) (WEHV)
  genome, open reading frames 2:214–215
  infection *see above*
  isolation 2:214, 2:215*f*
  transmission 2:214
Walleye epidermal hyperplasia virus type 1 (WEHV-1) 2:231
Walleye epidermal hyperplasia virus type 2 (WEHV-2) 2:231
Walleye fish, skin tumors 2:214
Walleye herpesvirus 2:208–209
Walnut blackline 4:206
Walnut trees(s), diseases 4:206
  causal agents 4:206
  control 4:206
  geographical distribution 4:206
  symptoms 4:206
  transmission 4:206
  yield losses 4:206
Wandering behavior
  baculovirus infection 1:269
  BmNPV infection 1:269
Wanowrie virus 1:400
  Hyalomma marginatum 1:400
Warthin–Finkeldy giant cells, measles 3:289
Warthog *(Phacochoerus aethiopicus)*, as an African swine fever virus host 1:43, 1:44*f*
Warts *see* Papillomavirus infections
Water
  hepatitis E virus transmission 2:380, 2:382
  Maize chlorotic mottle virus transmission 3:262
Watermelon mosaic virus (WMV) **5:433–440**
  biotechnological applications 5:439
  classification 5:433
  efficient vectors 5:437
  epidemiology 5:436
    virus sources 5:436
  evolution 5:439
  genetics 5:439
  geographical distribution 5:435
  history 5:433
  host range 5:435

infection *see below*
spread 5:437
strains 5:433
taxonomy 5:433, 5:434*f*
use in transgenic plants 4:157, 4:158*f*
heterologous encapsidation 4:162
variability 5:438
vector relationships 5:436
Watermelon mosaic virus infection
control methods 5:437
cross-protection 5:437, 5:438*f*
genetically-modified resistant plants 5:438
prophylaxis 5:437
resistant cultivars 5:437
diagnostic methods 5:435
symptoms 5:434, 5:435*f*
synergism 5:435
Watson, James
bacteriophage structure, research history 1:433
virus protein coats 2:452
Wax moth *(Galleria mellonella),* parvovirus infection 3:127–128, 4:76
symptoms 3:127–128
Weed(s)
cucumber mosaic virus transmission 1:618–619
control 1:619
potato virus Y 4:289
tobravirus transmission 5:76
zucchini yellow mosaic virus transmission 5:436–437
WEEV *see* Western equine encephalitis virus (WEEV)
Well boats 3:90
Wesselsbron virus 2:237
geographical distribution 2:238
infection 2:238
Rift Valley fever virus vaccine 2:237–238
transmission 2:238
mosquitoes 2:237
vaccine 2:237–238
West Africa, HIV infection 1:63
Western blotting
definition 4:623
plant virus detection 2:26
yellow head virus infection 5:482
Western equine encephalitis virus (WEEV) 5:77, 5:114
Ab-mediated neutralization 3:416
characteristics 1:171*t*
evolutionary relationships 1:174, 5:96–97
geographical distribution 5:77, 5:102, 5:114–115
infection *see below*
isolation 5:82
phylogeny 5:77–78
subtypes 5:101
tissue tropism 5:105
transmission 5:77–78, 5:99–100, 5:101, 5:115
age 5:273
forecasting risk 5:274
Western equine encephalitis virus infection 5:77, 5:82
case–fatality rates 5:77, 5:105
clinical features 5:77, 5:105, 5:115
encephalitis characterization 5:77
diagnosis 5:82
epidemiology 5:104, 5:104*f*
host range 5:102–103
incubation period 5:77
pathogenicity 5:105
pathology, brain examination 5:106
treatment 5:82
Western Europe, HIV infection 1:65
transmission 1:65
West Nile (WN) encephalitis 5:447
West Nile (WN) fever 5:441
outbreaks 5:441
symptoms 5:447
West Nile neuroinvasive disease (WNND) 5:441
epidemics 5:441
fatalities 5:447
risk factors 5:447
symptoms 5:447
virus lineage associated 5:441
West Nile virus (WNV) 2:96, 2:236, **5:440–450**
bioterrorism 5:407
bird vectors 5:445
American robin 5:445
corvids 5:445
morbidity/mortality rates 5:445
surveillance specimens 5:448
characteristics 1:171*t*
classification 5:440
ecology 5:443
epidemiology 2:243, 2:247*f*, 5:441
'North American dominant' genotype 5:441
genome 5:442
noncoding regions 5:442
nonstructural proteins 5:442
proteins encoded 5:445*t*
structural proteins 5:442
geographical distribution 4:128, 5:441, 5:443*f*, 5:487
lineages 2:237, 5:441
history 5:441
phylogenetic analysis 5:441, 5:443*f*
replication 5:442, 5:446*f*
host cell attachment 5:442–443
RNA synthesis 5:442–443
transcription 5:442–443
transmission 2:236, 5:447
nonviremic 5:445
perpetuation mechanisms 5:444–445
vectors *see below*
vertical 5:444–445, 5:447*f*
vectors 2:236, 5:443, 5:445
American robin 5:445
birds *see above*
competence 5:443–444
mosquitoes 2:236
vertebrates 5:445
virion structure 5:442, 5:444*f*
West Nile virus infection
alligators 5:445–446
clinical features 2:236, 5:447
movement disorders 5:447
control 5:448
public health measures 5:449
therapeutics 5:448
vaccination 5:448
vaccines/vaccination 5:448
diagnostics 5:448
human 5:448
surveillance specimens 5:448
disease surveillance 5:487
epidemics 2:236
horses 2:237
host range 2:237
immune response 5:446–447
immune system evasion 3:110
complement 3:110
incidence 5:448*t*
maintenance transmission 5:272
neurological 2:237, 5:448
organ transplantation, risks 3:466, 3:470
pathogenesis 5:446
host status 5:446–447
zoonosis 5:443, 5:447*f*, 5:487
see also West Nile neuroinvasive disease (WNND)
Whatroa virus (WHAV) 5:92, 5:93*t*
characteristics 5:109*t*
Wheat American striate mosaic virus (WASMV) 1:495
Wheat dwarf virus (WDV) 1:495
infection 1:495
strains 1:495
transmission 1:495
Wheat eqlid mosaic virus (EqMV) 1:495
Wheat spindle streak mosaic virus (WSSMV) 1:493, 1:493*f*
Wheat streak mosaic virus (WSMV) 1:495
host range 1:495
infection
prevention 1:495
resistance 1:495
symptoms 1:495, 1:496*f*
synergistic relationships, maize chlorotic mottle virus 3:262
transmission 1:495, 5:280–281
Wheat virus(es) **1:490–497**, 1:491*t*
see also specific viruses
Wheat yellow head virus (WYHV) 1:495
Wheat yellow leaf virus 1:495
Whispovirus 4:567, 5:451
White bream virus (WBV) 2:230
White clover cryptic virus(es) (WCCV) 1:99
phylogeny 4:67*f*
White clover mosaic virus (WClMV) 4:311*t*
genome 4:312
transmission 4:310, 4:312
White-faced saki lymphocryptovirus (PpiLHV1) 4:587*t*
Whitefly *(Bemisia tabaci)*
African cassava mosaic virus transmission 1:36, 2:101
cassava mosaic geminiviruses transmission 1:32–33
cotton leaf curl disease transmission 2:102–104
cucumber vein yellowing virus transmission 2:90
cucurbit yellow stunting disorder virus transmission 2:89
definition 5:138
mungbean yellow mosaic virus transmission 3:366, 3:368
population control 2:104
tomato infectious chlorosis virus transmission 2:89–90
tomato yellow leaf curl virus *see* Tomato yellow leaf curl virus
vilruliferous, definition 5:138
Whitefly-transmitted geminivirus(es) (tomato) (WTG) 4:200, 5:125
White rot control, mycoreovirus-3/RnW370 3:382
White spot disease (WSD) 5:450–451
clinical features 5:451
farming practices 4:569
geographical distribution 5:450–451
hemocytes
host responses 5:458
semigranular cells 5:458
histopathology 5:452
host range 5:450–451
mortality rate 5:451
pathology 5:451
RNAi strategies 5:458
shrimp production losses 4:569
vaccines/vaccination 5:458
White spot syndrome virus (WSSV) **5:450–459**
apoptosis 5:457
classification 4:567
clinical features 5:451
epidemiology 5:451
evolution 2:182
gene expression 5:457
early genes 5:457
immediate early genes 5:457
promoter regions 5:457
regulation 5:457
structural protein genes 5:457
genes 5:456
encoded proteins 5:456
expression *see above*
identification, comparative homology 5:456
identification, functional studies 5:456
immediate early 5:456–457
latency-related 5:456–457
genome 5:455
tandem repeats 5:451
geographical distribution 5:450–451
histopathology 5:452, 5:452*f*, 5:453*f*
host cell attachment 5:453–454
host range 5:451
host relationships 5:457
apoptosis 5:457
hemocyte responses 5:458
infection *see* White spot disease (WSD)
life cycle 5:452–453
morphogenesis 5:452, 5:454*f*

White spot syndrome virus (WSSV) (*continued*)
morphology 5:452, 5:454*f*
pathology 5:451
structural proteins 5:453, 5:455*f*, 5:456*f*
capsid 5:453
envelope 5:453–454
synonyms 5:451
taxonomy 5:451
transmission 5:451
virion assembly 5:452–453
virion composition 5:450–451, 5:453
virion structure 5:453, 5:455*f*
White sturgeon iridovirus (WSIV) 3:155
infection 3:156–158
White tail disease 4:573
affected tissues 4:573
geographical distribution 4:575
host range 4:575
Wild animals
rabies virus vaccination 4:373
see also specific animals
Wild boar
arterivirus evolution 1:182
classical swine fever 1:530, 1:532
Wildebeest *(Connochaetes gnu),* bovine malignant catarrhal fever 1:363
Wild potato mosaic virus (WPMV) 4:298*t*
Wild type, definition 4:359
WIN compounds, antirhinoviral strategies 1:547–548
Winter wheat mosaic virus 5:24
Woese, Carl 3:474
trinity concept of evolution 3:473–474
Wongabel virus (WONV) 4:184*t*
Woodchuck hepatitis virus (WHV) 2:329, 2:366–367
Woodmice *(Apodemus),* cowpox virus hosts 1:577, 1:578
World Health Organization (WHO)
Dengue hemorrhagic fever/Dengue shock syndrome diagnostic criteria 2:11–12, 2:12*t*
smallpox eradication 4:640
yellow fever incidence 5:473
Worldwide trade, ornamental plant species virus infections 4:208
Wound tumor virus (WTV) 3:382–383
characteristics 4:150*t*
genome 4:152, 4:153*f*
as type species 4:536*t*
virion structure 4:152
W protein, henipavirus 2:323
WSN influenza strain, neuraminidase 3:102
WU virus (WUV) 4:262

X

Xenotransplantation
papillomavirus propagation 4:32
retroviral expression 2:108–109
Xenotropic behavior
definition 2:467
murine leukemia viruses 2:468
Xenotropism, retrovirus infection resistance 2:468
Xf1 bacteriophage 3:121
X-gal, baculovirus expression vectors, plaque purification 1:240–241
X-linked lymphoproliferative syndrome (XLP) 2:153
mortality rate 2:153
XLP *see* X-linked lymphoproliferative syndrome (XLP)
X protein (Borna disease virus) 3:331
X-ray crystallography 1:603, 2:453
bluetongue virus core particle structure 5:388–391
cowpea mosaic virus 1:570
nonenveloped virus structure 5:380, 5:381
rice yellow mottle virus 4:486
tetravirus capsid structure 5:33
tobacco mosaic virus 2:452, 5:380–381
X-ray diffraction 1:201
nonenveloped virus structure 5:380
XSV *see* Extra small virus (XSV)
Xylem
definition 3:348
rice yellow mottle virus 4:487–488

Y

Yaba-like disease virus (YLDV) 5:462, 5:462*t*
distribution 5:464
Yaba monkey tumor virus (YMTV) 5:462*t*
distribution 5:464
genome 5:462–463
history 5:461
infection 5:461
control 5:465
immunity 5:463
pathogenesis 5:464
prevention 5:465
tumor regression 5:464
tropism
humans 5:463
nonhuman primates 5:463
virion properties 5:462
Yatapoxvirus 4:324*t*, 5:461, 5:462*t*
Yatapoxvirus(es) **5:461–465**, 5:462*t*
classification 4:324*t*
epidemiology 5:464
genome 5:462, 5:463*t*
geographical distribution 5:464
history 5:461
infection
control 5:465
immune modulation 5:464
immunity 5:463
pathogenesis 5:464
prevention 5:465
replication
DNA replication 5:462
transcription 5:462
translation 5:462
seasonal distribution 5:464
transmission 5:463
tropism
humans 5:463
non-human primates 5:463
virion properties 5:462
see also specific viruses
YD71 protein, Brome mosaic virus 1:384–385
Yeast(s)
killer phenomenon 2:285, 5:164
ion channel formation 5:307
maintenance of killer genes 5:170–171
superkiller genes 5:170–171
toxin immunity 5:307
prions *see* Prion(s), yeast and fungal
Tf1 transposon *see* Tf1 transposon (yeast)
Tf2 transposon *see* Tf2 transposon (yeast)
Ty3 retrotransposon *see* Ty3/*gypsy* retrotransposon(s)
viruses
apoptosis 1:162
replication models 2:290
see also specific viruses
see also under Saccharomyces
Yeast L–A virus (ScV-L-A) **5:465–469**
apoptosis 1:162
capsid structure 5:165
evolution 5:173–174
genetics 5:468
genome 5:467, 5:467*f*
open reading frame 5:467
open reading frames 5:166, 5:300–301
RNA pseudoknot 5:467
Helminthosporium victoriae 190S virus *vs.* 5:165
as helper virus 5:465–466
history 5:466
killer phenomenon 5:170–171
host genes associated 5:170–171
satellite dsRNAs associated 5:300
replication 5:301, 5:467
RNA packaging 5:467, 5:467*f*
RNA polymerase activity 5:167–168
RNA replication 5:467, 5:468*f*
transcription 5:467
viral binding sites 5:301, 5:302*f*
translation 5:468
positive strand 5:468
Ski proteins 5:468
transmission, cell–cell fusion mating 5:465–466
virion structure 5:466, 5:466*f*
Gag 5:466–467, 5:466*f*
Yellow dwarf virus(es)
infection
risk assessment 1:496
symptoms 1:496
transmission 1:496
see also specific viruses
Yellow fever
cases associated 5:487
clinical features 5:474
classic disease 5:474–475
CNS involvement 5:474–475
fatal cases 5:474–475
nonspecific flu-like illness 5:474
spectrum 2:243
diagnosis 2:243
epidemics 5:494
agriculture 5:494
epidemiology 2:243, 5:473
virus survival 5:474
geographical distribution 5:473
historical aspects 5:470
vector identification 5:470
immune response 5:475
antibody-dependent cellular cytotoxicity 5:475–476
cytotoxic T-cells 5:475
immunoglobulin M 5:475–476
natural killer cells 5:475
pro-inflammatory cytokines 5:475
incidence 5:473
WHO figures 5:473
mortality rate 5:487
neurotropism 5:475
pathogenesis 2:245, 5:475
cytokine dysregulation 5:475
primate models 5:475
systemic immune response syndrome 5:475
pathology 5:475
prevention/control 5:476
vaccines/vaccination *see below*
seasonal distribution 5:473
Aedes breeding cycle 5:473
transmission cycles 5:473
laboratory-acquired infections 5:474
'savanna type' 5:473
'sylvatic type' 5:473
'urban type' 5:473, 5:474
vaccines/vaccination 5:476
17D vaccine 5:476
safety 2:246, 5:234
vaccine/vaccination 2:239
reactions to 5:231*t*
safety 2:246, 5:234
strain 17D 5:238–239, 5:240*f*
vectors, identification 5:470
viscerotropism 5:475
Councilman bodies 5:475
eosinophilic degeneration 5:475
hepatocyte degeneration 5:475
lymphoid follicles 5:475
Yellow fever (YF) virus 2:238, **5:469–476**
assembly 5:471
endoplasmic reticulum 5:471
secretory cells 5:471
cell entry, clathrin-coated vesicles 5:471
characteristics 1:171*t*
circulation, sylvatic 2:238–239
classification 5:470
neutralization tests 5:470
RdRp 5:470
genome 5:470
noncoding region 5:470–471
open reading frames 5:470–471
reverse transcriptase-PCR 5:472

history 2:238, 2:242, 2:455
host range 2:238–239, 5:472
vertebrates 5:472–473
infection *see* Yellow fever
isolation 5:472
propagation 5:472
proteins 5:471
C (capsid) protein 5:471
E (envelope) proteins 5:471, 5:472
nonstructural proteins 5:471, 5:472
preM (pre-membrane) protein 5:471
RdRp 5:470
replication 5:471
taxonomy 5:470
transmission 2:238, 5:487
Aedes aegypti 2:455
mosquitoes 2:238
variation 5:470
Dengue virus *vs.* 1:127
genotypes 5:470
virion 5:470
virulence determinants 5:471
E protein 5:472
nonstructural proteins 5:472
sequences 5:471–472
viscerotropism 5:471–472
zoonoses 5:487
Yellow head virus (YHV) **5:476–483**
classification 4:567
genetic diversity 5:480, 5:481*f*
open reading frames 5:482
genome 5:477
open reading frames 5:477–478, 5:482
pseudoknot structure 5:477–478
untranslated region 5:477–478, 5:479*f*
genotypes 5:479
geographical distribution 5:479
historical aspects 5:477
host range 5:479
infection *see below*
proteins 5:477–478
RdRp 5:477–478
structural proteins 5:477
taxonomy/classification 5:477
transmission 5:480
vertical transmission 5:480
virion 5:477, 5:478*f*
nucleocapsids 5:477
nucleoprotein 5:477
structural proteins 5:477
transmembrane glycoproteins 5:477
see also Gill-associated virus (GAV)
Yellow head virus infection
cell entry 5:477
chronic infections 5:480
diagnosis 5:482
nitrocellulose enzyme immunoassay 5:482
RT-PCR 5:482
Western blotting 5:482
host response 5:480
inflammation 5:480
management 5:482
pathogen exclusion 5:482
RNA interference 5:482
mortality 5:479
pathology 5:479
tissue types 5:480
Yellow mosaic-affected vines 4:202, 4:202*f*
Yellows disease, raspberry bushy dwarf virus 3:38, 3:38*f*
Yersinia pestis, virulence factors 3:123
YGEV *see* Transmissible gastroenteritis virus (TGEV)
YHV *see* Yellow head virus (YHV)
Yield losses
African cassava mosaic disease 1:34
barley yellow dwarf viruses 1:284–285
beet necrotic yellow vein virus infection 1:308
geminiviruses 4:166–167
maize chlorotic mottle virus 3:262
potato virus Y potato infections 4:291
YLDV *see* Yaba-like disease virus (YLDV)
ψM1 virus 5:413*t*
ψM2 virus 5:413*t*
ψM100 virus 5:413*t*
YMTV *see* Yaba monkey tumor virus (YMTV)
Young pigeon disease syndrome (YPDS) 1:515
Y-transposase 4:436

Z

Zaire Ebola virus (ZEBOV)
classification 2:57
endemic 2:57
genome 2:58
geographical distribution 2:62
history 2:57
host range 2:62
experimental models 2:62
infection
animal models 2:202–203
clinical features 2:62
mortality rate 2:199
pathogenicity 2:63
treatment 2:64
life cycle 2:61
protein properties 2:59
glycoproteins 2:60
matrix proteins 2:59
RNP complex 2:59
taxonomy 2:57
vaccines 2:65
recombinant VSV 2:65
virion properties 2:57
Zalcitabine 2:510
ZAM elements 4:433
Zanamivir 1:143*t*
influenza virus infection treatment 1:153, 2:556, 3:102–103, 3:492
Zea mays see Maize *(Zea mays)*
ZEBOV *see* Zaire Ebola virus (ZEBOV)
Zebrafish endogenous retrovirus 2:219
phylogeny 2:219–220, 2:220*f*
Zerit® 2:510
Ziagen® 2:510
Zidovudine (AZT) 2:510, 2:511*t*
adult T-cell leukemia therapy 2:569–570
Zimbabwe, HIV epidemiology 1:65–66
Zinc finger antiviral protein (ZAP), retrovirus infection resistance 2:473
Zinc finger proteins
Ilarvirus replication 3:52–53
sawfly baculovirus genome 1:228
Zoo animals, cowpox infection 1:577
Zoonoses 5:270, **5:485–495**
arthropod vectors 2:147
Asian tiger mosquito 5:494
sandflies 5:488
control 5:493
definition 1:170, 2:141, 2:147, 2:241, 2:377, 2:517, 4:40, 4:52, 4:490, 4:624, 5:107, 5:269, 5:440
diagnosis 5:493
ecological change 5:494
as emerging diseases 2:95, 5:494
organ transplant infections 5:490
reservoir identification, phylogeny 4:128
social change 5:494
transmission 5:485
control 5:493
vectorial capacity 5:271
vector transport 5:494
see also specific diseases/disorders
see also specific viruses
Zoospore(s)
furovirus transmission 2:292
plant virus transmission 5:281
Zoster (shingles)
clinical features 5:253, 5:254*f*
complications 5:253
immunocompromised 5:253
postherpetic neuralgia 5:253
Ramsay–Hunt syndrome 5:253
epidemiology 5:252
age-relation 5:252, 5:252*f*
immunocompromised 5:252
etiology 5:252
virus reactivation 5:253
immunocompromised patients 5:252, 5:253
pathology 5:253–254
Zostera marina (seagrass), dsRNAs 2:112
Z protein
arenavirus assembly/budding 3:247
lymphocytic choriomeningitis virus replication/transcription 3:245–246
Z RING domain, arenavirus replication 3:245–246
Zta protein, EBV infection 2:166
Zucchini yellow mosaic virus (ZYMV) 5:290, **5:433–440**
biotechnological applications 5:439
classification 5:433
efficient vectors 5:437
epidemiology 5:436
spread 5:437
virus sources 5:436
evolution 5:439
genetics 5:439
geographical distribution 5:435
history 5:433
host range 5:435
infection *see below*
taxonomy 5:433, 5:434*f*
transmission 5:290
Aphis gossypii 5:436
via seed 5:436
weeds 5:436–437
ZYMV-NAT 5:436
use in transgenic plants 4:157, 4:158*f*
heterologous encapsidation 4:162
variability 5:438
vector relationships 5:436
Zucchini yellow mosaic virus (ZYMV) infection
control methods 5:437
cross-protection 5:437, 5:438*f*
genetically-modified resistant plants 5:438
prophylaxis 5:437
resistant cultivars 5:437
diagnostic methods 5:435
epidemics 5:290
symptoms 5:290, 5:434, 5:435*f*
synergism 5:435
Zygocactus symptomless virus (ZSLV) 4:311*t*
Zygocactus virus X (ZVX) 4:311*t*
Zygocin 5:307
structure 5:307
Zygomycota 4:419
retrotransposons 4:423, 4:424*t*
Zygosaccharomyces bailii
killer spectrum 5:307
phenotype inheritance 5:300
ZYMV *see* Zucchini yellow mosaic virus (ZYMV)